Title
Heterogeneity of Pericytes after Ischemic Stroke

Author
Pham, Thanh Thuy Dan

Publication Date
2021

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Heterogeneity of Brain Pericytes after Ischemic Stroke

A dissertation submitted in partial satisfaction of

the requirements for the degree Doctor of Philosophy in Molecular, Cellular, and Integrative Physiology

by

Thanh Thuy Dan Pham

2021

ABSTRACT OF THE DISSERTATION

Heterogeneity of Brain Pericytes after Ischemic Stroke

by

Thanh Thuy Dan Pham

Doctor of Philosophy in Molecular, Cellular, and Integrative Physiology

University of California, Los Angeles, 2021

Professor S. Thomas Carmichael, Chair

Stroke is the number one leading cause of adult disability in the US. While medical intervention has significantly decreased mortality rate; stroke survivors still face devastating disability that affect their quality of life. With limited therapeutic interventions, promoting the brain repair and recovery remains an unmet need in medical sciences. While the brain has limited regeneration capability, its endogenous reactive and repair mechanism is not well-understood.

Pericytes, a less-well-understood member of brain, are a heterogenous population of perivascular cell that regulate development and maintenance of structural elements of the blood brain barrier, vascular stability, and angiogenesis. Recent studies have confirmed their remarkable contractile ability, their control of cerebral blood flow and crosstalk with other cell types after injury. Due to its lack of exclusive markers, its overlapping markers with other cell types in the brain, including a newly identified population of perivascular fibroblasts and its heterogeneity, findings of brain pericyte after injury have been controversial.

Through development of an array of novel molecular tools, coupled with the use of single cell RNA sequencing and novel pharmacological agents, this dissertation characterizes the post-stroke pericyte heterogeneity and its contribution to the brain repair processes. Pericyte population was characterized after stroke on the tissue level to understand their proliferative capacity and their

association with the regenerative neurovascular niche. Using two novel molecular lineage-tracing models, subpopulations of pericyte were labeled and lineage-traced to delineate its contribution to the fibrotic scar. Using single cell RNA seq, the finding was confirmed and further characterized the molecular mechanism of pericyte contribution to (1) the brain fibrotic scar and (2) its role in establishing the regenerative neurovascular niche. Using the RNAseq dataseq as a guiding foundation, three specific manipulation studies were carried out to further delineate the heterogenous role of pericyte after stroke: *Stat3* knockout in brain-specific pericyte, Loxl2/3 pathway inhibition by small molecule drugs and brain-specific pericyte ablation. These findings from these studies revealed the highly conserved nature of the brain's fibrotic scar formation processes and the essential contribution of pericyte to the scar formation and maintenance. Pericyte heterogeneity, especially the fibrotic-scar-formation population and the peri-infarct population that associate with the neurovascular niche, needs to be taken into consideration when developing therapeutic treatment.

Ultimately, these studies implicate the multi-faceted roles of pericyte in the brain repair and recovery processes, and provide further evidence to the growing body of research being done on the biology of pericyte in homeostasis and after injury

The dissertation of Thanh Pham is approved.

Luisa Iruela-Arispe

Arjun Deb

Michael Sofroniew

Weizhe Hong

Stanley Thomas Carmichael, Committee Chair

University of California, Los Angeles 2021

To my family, friends and my two kitties for their endless love and support.

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1:

Chapter 2

Chapter 3

Chapter 3

ACKNOWLEDGEMENTS

I'd first like to thank my parents and my sisters, because I would not have even started this journey if it wasn't for their support. I would also like to thank my extended Vietnamese family in Vietnam and in Houston, for being amazing supporters to support me through years of college. I'd like to thank my two kitties Gon and Cow for being the fluffiest floofs and endless source of cuteness through the past 5 years. Thank you all for everything and I love you.

I'd like to thank my My Trang family – the Sasine's! Thank you for welcoming me into your family and treated me as your own, making me feel so welcoming through one of the roughest patch of my graduate studies. I love you all!

I'd like to thank Dr. Tom Carmichael for giving me the opportunity to work in his lab, and have trained me into the scientist that I am today. Tom has been an amazing mentor for me, not only in science but beyond science: he has been a fountain of insightful advice about leadership, career and life. Those are incredible life lessons that I will carry with me to the rest of my career. Most importantly, I wouldn't be the entrepreneur that I am today without Tom's complete understanding of my personal and professional growth outside of the lab. I have learned and grown so much under his mentorship. Thank you for investing in me as a scientist and beyond! I would also like to thank the past and present members of the Carmichael Lab. Thank you Teena for being such a great source of support and calmness for me through all my stress. Thank you Junyu for putting up with me through all my antics. Thank you, Catherine, for being an amazing friend and a great shoulder for me to cry on. Thank you, Stacey, Martin and Irene, for being great bench mates and put up with all my antics and disorganization! Thank you, Nora and Jose, for being such great cat sitters and listening to me venting for hours about whatever!! Thank you, Amy, for putting up with me spamming you with texts when I was drunk at the Gordon Conferences! Thank you Kiro for hours of random conversation in the surgery room! Thank you, Liang, for getting me started on alternative career path in science! Thank you, Tiffani, for being an awesome friend and taught me all the techniques when I first started lab!

Thank you, Michal, for enabling a big chunk of my thesis with your virus! Thank you, Tatiana and Nora, for helping me manage the long list of animal colonies – couldn't have done any of these without you both! Thank you, Min, for being such a positive and encouraging person to my last year of PhD! The list could go on longer than the entire main text of this thesis, but I am grateful for all the memories and am blessed to have such a great group of friends and colleagues that had made me the person I am today! I wouldn't have survived those late-night experiments and that rough presential election without your supports!

I will always be thankful for the support I've received from the UCLA community. I'd like to thank my committee members Dr. Arispe, Dr. Deb, Dr. Hong and Dr. Sofroniew for their continuous guidance and only wish that I had reached out to them more throughout my time at UCLA. I want to specially extend my appreciation to Dr. Weizhe Hong and his lab, specially Emily who had spend time to teach me the technique of scRNAseq. I would like to thank Dr. Frye and Yesenia for being an amazing support network through it all! Yesenia, you are amazing!

I'd like to give special thanks to Felicia, Jessica, and Jeffery at the FACS core for all those hours making my experiments even possible. I would like to thank my MCIP cohort who has been the best friend group I have ever had! I will forever miss our Big Bear trip and all those happy hours! These are the great friendship I will make sure to maintain for years to come. Thank you for all the friends that I vent to, grab coffee with, go to happy hours with or just Blaze pizza together! My dissertation would not have been possible without any of you.

I would also like to give special thanks to all my friends and mentors in UCLA Anderson Business School, UCLA Business of Science Center and the UCLA Anderson Accelerator Program. Van, Trish, Alex, Janet, you have been such great mentors and role models for me through my entrepreneurship journey. I would like to also thank the friends I made in UCLA Law School, especially Katie and Mary for being one of the best friends that I could have and for all the free legal advices that you had given me

Last, but most importantly, I'd like to thank Josh for being an amazing partner with me through the last few years. Thank you for making sure I am warm and comfortable at all time with mountains of fuzzy blankets and pillows. Thank you for going on doumb adventures with me. #TeamGoosePlop forever!

The work I have completed has been supported by the NIH, the AHA, Dr. Jennifer Buchwald's Fellowship, Brain Repair Institute, the Adelson Foundation and Eli & Edythe Broad Center of Regenerative Medicine and Stem Cell Research Center at UCLA.

VITA/BIOGRAPHICAL SKETCH

EDUCATION

Ph.D Candidate – Molecular, Cellular, and Integrative Physiology **Sept. 2015 – present**
Current GPA: 4.0/4.000
University of California, Los Angeles (UCLA)

B.S. – Biomedical Sciences **Sept. 2012 - June 2015**
Texas A&M University – Corpus Christi
GPA: 3.93/4.000
(*summa cum laude)*

RESEARCH EXPERIENCE

Ph.D. Candidate, Molecular, Cellular, and Integrative Physiology **Sept. 2015 -present**
University of California, Los Angeles (UCLA)
Advisor: S.Thomas Carmichael, M.D., Ph.D.

July. 2014 – Dec. 2014

Undergraduate Researcher
University of Oxford & University of Bristol
Advisors: Craig McArdle Ph.D & Rajesh Thakker. Ph.D

Summer Undergraduate Researcher **May.2014 – Aug. 2014**
Baylor College of Medicine
Advisor: Qiang Tong, Ph.D.

Undergraduate Researcher **June.2013 - Dec. 2013**
University of California, San Diego
Advisor: Mark Lawson, Ph.D.

PUBLICATIONS

1. Kathryn L Garner, Rebecca M Perrett, Margaritis Voliotis, Clive Bowsher, George R Pope, **Thanh Pham**, Christopher Caunt, Krasimira Tsaneva-Atanasova, Craig A McArdle. *Information Transfer in Gonadotropin-Releasing Hormone (GnRH) Signaling: Extracellular Signal-Regulated Kinase (ERK)-Mediated Feedback Loops Control Hormone Sensing*. Journal of Biological Chemistry, 2016. Vol 291, 2246-2259
2. Kathryn L Garner, Margaritis Voliotis, Hussah Alobaid, Rebecca Perrett, **Thanh Pham**, Krasimira Tsaneva-Atanasova, Craig A McArdle. *Information transfer via gonadotropin-releasing hormone receptors to ERK and NFAT: Sensing GnRH and sensing dynamics*. Journal of the Endocrine Society, 2017. Vol 1, 260-277

PRESENTATIONS

AHA/UCLA Women's Heart Health **April 2019**
Poster: Pericyte Heterogeneity after Stroke

Cold Spring Harbor Conference – Blood Brain Barrier **April. 2019**
Poster: Pericyte Contribution to the Fibrotic Scar Formation

Society of Neuroscience Conference **Nov. 2018**
Poster: Heterogeneity of Pericytes after Stroke

Gordon Conference: Blood Brain Barrier **June. 2018**
Poster: Pericytes at the center of the ischemic neurovascular niche

Society of Neuroscience Conference **Nov. 2017**
Poster: Pericytes at the intersection of ischemic brain repair

Society of Neuroscience Conference **Nov. 2016**
Poster: Pericytes' proliferative capacity after ischemic injury

HONORS AND AWARDS

American Heart & Stroke Association Predoctoral Fellowship **2018-2020**

Gordon Conference: Blood Brain Barrier – Predoctoral Poster Award **2018**
Poster: Pericytes at the center of the ischemic neurovascular niche

Jennifer S Buchwald Graduate Fellowship in Physiology **2016-2017**

Brain Repair Institute Travel Award **2017,2018**

CHAPTER 1

INTRODUCTION

STROKE BIOLOGY

Clinical Challenges

Stroke is the second leading cause of death worldwide and a leading cause of adult disability in the United States, affecting more than 33 million people worldwide (Benjamin et al., 2018). In the United States, modern medical interventions have significantly reduced the mortality rate of stroke in the past few decades. However, for 6.6 million adult stroke survivors, the lifelong disabilities ranging from sensorimotor to cognitive to mood dysfunction are devastating and significantly reduce their quality of life (Benjamin et al., 2018; Mozaffarian et al., 2015). For these patients, current treatment options are limited in both efficacy, scope and duration. These epidemiological data emphasize a large unmet need in treatment to repair and regenerate the brain after ischemic injury.

Current therapeutic options and the majority of the current therapeutic treatments that are in the regulatory pipeline are focused solely in the acute phase of the stroke. Recombinant tissue plasminogen activator (tPA), which promotes reperfusion of blood to the stroke penumbra and inhibit cell deaths, is the sole approved therapy for stroke patients. With a limited administration window of the first 5 hours after stroke, it has limited clinical scope with only less than 5% of stroke patients were administered (DeMers et al., 2012). Mechanical thrombectomy is another procedure for stroke patients with large artery occlusion in the anterior circulation. However, with 24 hours clinical window of stroke onset, only 10% of acute ischemic stroke patients go through the procedure (Chia et al., 2016).

For stroke survivors in the chronic phase, physical and occupational therapies have been proven to have limited improvement in motor-related disabilities. However, neurological deficits such as cognitive deficits persist in ~50% of patients without effective treatments (Kelly-Hayes et al., 2003). With the aging population and the rise of other cardiovascular-related diseases in the US, incidence of stroke is increasing by 1% every year (Madsen et al., 2020). The jarring statistic

emphasizes the need for novel therapeutic interventions, starting with further understanding of stroke biology and the brain repair mechanisms

The Ischemic Brain

Ischemic stroke, characterized as the acute loss of blood flow to regions of the brain, accounts for 70-80% of all stroke cases (Shiber et al., 2010). Caused by a thrombus or embolus, the loss of blood flow leads to a damaging cascade of cellular events that directly affect neurons and non-neuronal cells (e.g., astrocytes, pericytes, endothelial cells) and leads to cell death, wound repair response and tissue reorganization. The complex multicellular responses can be split into three main phases: acute phase, subacute phase and chronic phase.

The acute phase occurs seconds to hours after injury, characterized by acute death of brain cells, axonal damage, disruption of vasculature and the integrity of the blood brain barriers (BBB). Endothelial cells in the local area of insult trigger platelet adhesion and activation. That leads to the coagulation and blood clot formation at the injury core (Carmichael, 2010, 2016; Shiber et al., 2010; Yau et al., 2015)**.** All these cellular actions initiate a secondary injury of local edema, cytotoxic products which leads to immune cell recruitment and activity in the damaged area. Microglia migrate towards the injury sites to initiate clearance and removal of debris (Bodhankar et al., 2015; Hu et al., 2012; L. Li et al., 2020; Mathys et al., 2017)**.** Peripheral immune cells such as neutrophils and macrophages also infiltrate the injured brain to begin the wound repair process. Brain-resident cells such as astrocytes become reactive and hypertrophic (Anderson et al., 2016; Barreto et al., 2011; Becerra-Calixto & Cardona-Gómez, 2017)**.** The tissue reperfusion exacerbates the excitotoxicity-related death of neurons and furthers the axonal damage(Nour et al., 2013). The secondary injury insult of inflammation, apoptosis and reperfusion injury is often worse than the damage caused by the initial insult (Carmichael, 2010; Fei et al., 2007; Vemuganti & Zhao, 2015).

The subacute phase of stroke is characterized by the proliferative and tissue reorganization phases after injury, occurring within the first two weeks after stroke (Carmichael, 2005, 2010,

2016). The boundaries between the infarcted region and the penumbra, peri-infarct region, are established within this phase by the placement of reactive astrocytes in the area immediately adjacent to the infarct and the fibrotic scar inside the infarcted core (Carmichael, 2016; Iihara et al., 1996). The burst of proliferation of endothelial cells and other progenitors initiates neovascularization of the injury site via angiogenesis (Ergul et al., 2012; Hatakeyama, n.d.; Ruan et al., 2015). These angiogenic vessels signal to other progenitor cells such as newly born neurons and oligodendrocyte precursor cells to localize to the peri-infarct tissue and create this regenerative neurovascular niche (Brumm & Carmichael, 2012). Neurons that lose axons will remodel to remap and reroute lost connections within the surviving populations (Benowitz & Carmichael, 2010; S. Li et al., 2015; Ruschel et al., 2015).

The chronic phase of stroke marked by the reestablishment of the BBB around the newly formed vessel and a tapering off the inflammatory response (Carmichael, 2016). The remodeling process of neural circuits and remyelination of axons continue during this period. With time, the remodeling of astrocytic and fibrotic scars continues through the dynamic changes of the extracellular matrix proteins (Dorrier et al., 2021; Vogelaar et al., 2015; Zbesko et al., 2018).

Regenerative Neurovascular Niche

Immature neurons, termed “neuroblasts”, migrate into peri-infarct tissue and tightly associate with angiogenic blood vessels. Angiogenesis is causally linked to neurogenesis, as blocking angiogenesis significantly reduces the localization of immature neurons to peri-infarct cortex (Ohab et al., 2006). These findings have identified a neurovascular niche for neural repair in stroke. This regenerative neurovascular niche is reminiscent of stem cell niches in the brain (SVZ) and peripheral organs, in which stem cells associate with a specialized microvasculature. In the SVZ, neural stem cells and their immediate downstream progeny associate with blood vessels(Jiang et al., 2001; Lindvall & Kokaia, 2015; Ohab et al., 2006; Ruan et al., 2015). Studies of the SVZ after stroke have identified signaling molecules that may stimulate this region, such as Notch, Hif1 and EphrinB (Carrica et al., 2019; Ernst et al., 2019; Xiao et al., 2009). Outside of

these studies of the SVZ, there has been little study of the molecular signaling systems in the regenerative neurovascular niche in the peri-infarct cortex. This is an important gap, because the neurovascular niche in the SVZ is very different from the regenerative neurovascular niche in peri-infarct cortex. Unlike the regenerative neurovascular niche in peri-infarct cortex, the neurovascular niche in the SVZ involves true neural stem cells and transit amplifying cells, which are not present in the peri-infarct region(Brumm & Carmichael, 2012).

The regenerative neurovascular niche in the periinfarct region include regenerative processes such as angiogenesis, neurogenesis, oligodendrocyte-genesis, and production of new connections via axonal sprouting. The cellular elements of this regenerative environment (the angiogenic blood vessels, pericyte, astrocytes, immature neurons, and sprouting axon, etc) display a high level of molecular communication to collectively reorganize and repair the injured brain(Brumm & Carmichael, 2012).

In addition to the cellular components of the fibrotic scar, the ECM plays crucial roles in serving as the physical substrate for cell adhesion, migration, and the regulation of cytokines, growth factors and chemokines molecules (de Winter et al., 2016; Schultz & Wysocki, 2009). Fibrotic scar, defined in details in the later section, seems to be inhibitory to neural regeneration. *In vitro* studies showed axons could not grow past the astrocyte-fibroblasts boundary (Shearer & Fawcett, 2001).

Reactive Astrocyte Barrier

Reactive astrocyte barrier formation, formerly confused as astrocytic scar, by reactive astrocytes surrounding the infarcted area occur within the first few weeks after injury, characterized by astrocyte hypertrophy. The hypertrophic astrocytes undergo massive restructuring that leads to the formation of compact astrocyte scars, which depends on IL-6 receptor, STAT3 and LKZ signaling (Anderson et al., 2016; Barreto et al., 2011; Becerra-Calixto & Cardona-Gómez, 2017). The compact glial scar is comprised of densely packed astrocytes with elongated processes directly interface and encapsulate the fibrotic lesion core.

Astrocytic scars are thought to inhibit regeneration through chondroitin sulfate proteoglycan (CSPGs)-production by astrocytes that inhibit the axonal outgrowth (Cafferty et al., 2007; Keough et al., 2016; Smith & Strunz, 2005). However, studies in the past decade have provided conflicting evidence, some challenging the prevailing dogma by suggesting that astrocytic processes aid instead of prevent the regenerative repair processes of the brain(Anderson et al., 2016; T. Yang et al., 2020).

Fibrotic Scar Formation

The fibrotic scar is a pathological wound healing process, characterized by the functional parenchymal tissue being replaced by the non-functional connective tissue with the excessive deposition of extracellular matrix (ECM) protein, secreted by activated fibroblasts as well as other cell types (Fernández-Klett & Priller, 2014). This wound healing process is often triggered by the recruitment of local and infiltrating immune cells into the central nervous system (CNS)(Schultz & Wysocki, 2009). The short-term function of this scar is thought to limit the inflamed secondary insult from the surrounding tissues, however, these cells do not withdraw entirely and are not replaced by regenerated tissue, as they persist chronically in the stroke lesion core (Carmichael, 2005; Ohab et al., 2006). Major components of this scar are endothelial cells, progenitors, inflammatory cells, stromal fibroblasts or fibroblast-like cells (Ali et al., 2014; Soderblom et al., 2013a). The prolonged presence of the matrix protein such as the ECM components, glycoproteins such as CSPG, HSPC, collagens, fibronectin and laminin is thought to delay CNS remodeling and regeneration (de Jong et al., 2012; Ray et al., 2013; Schultz & Wysocki, 2009). CNS fibrotic scar formation and persistence is not well-understood due to the different characters of scar formation in different animal models (Dias et al., 2020a; Dorrier et al., 2021; Fernández-Klett & Priller, 2014).

Dual scars interaction with dual roles

The glial-fibrotic interface of scars after stroke is not well understood in the CNS. Reactive astrocytes are known to interact and influence the fibroblasts and inflammatory cells (T. Yang et al., 2020; Zbesko et al., 2018). After spinal cord injury, astrocytes express ephrin-B2 ligand and communicate with EphB2-expressing fibroblasts, which leads to the establishment of the astroglial-fibrotic lesion border in the subacute phase (Bundesen et al., 2003, p. 2) . Type I collagen produced by fibroblasts in the lesion core has been shown to induce the reactive astrocyte barrier formation via the integrin-N-cadherin pathway (Neo & Tang, 2017, p. 1; Silver & Miller, 2004). Perivascular PDGFRb-expressing cells upregulate the expression of periostin, a component of the ECM after spinal cord injury and promote the upregulation of TNFa from infiltrating monocyte/macrophages to promote the scar formation after injury ((Yokota et al., 2017) Both astrocytic and fibrotic scars have been shown to be pivotal regulators of resident and infiltrating immune cells after CNS injury ((Ellison et al., 1998; Whetstone et al., 2003). An example of this is the release of cytokines and chemokines such as CCL2 and IL-6 by reactive astrocytes that triggers the polarization of microglia into a pro-inflammatory phenotype (Sofroniew, 2015). Microglia and other immune cells have been shown to also contribute to the production of ECM-associated protein such as osteopontin and promote the maturation of scar through the αvβ3 integrin on reactive astrocytes (Ellison et al., 1998)

As mentioned previously, there have been two conflicting bodies of evidence regarding the role of CNS scar: promoting tissue regeneration vs inhibiting the repair. While these need not be entirely mutually exclusive, conflict remains, especially regarding the overall contribution. In astrocytic scar, the axon growth inhibitory CSPGs produced solely by astrocyte has been regarded as the main contributor to the growth-inhibition of scar (Cafferty et al., 2007; Lang et al., 2015; Yiu & He, 2006). However, this dogma has been challenged in a series of studies recently (Anderson et al., 2016; Hara et al., 2017). The potential source of this discrepancy may arise from the heterogeneity of the scar forming astrocytes (Barnabé-Heider et al., 2010; Meletis et al., 2008).

For fibrotic scar, targeting fibrosis to aid repair has been evidenced in numerous studies. Targeting fibrosis for repair, especially in CNS has been reported such as using a neutralizing antibody against TGFb1, a small molecule inhibitor of TGFBR1and iron chelators (Hao et al., 2017; Hellal et al., 2011; Liu et al., 2011; Nguyen et al., 2010; Popovich et al., 2014; Ruschel et al., 2015; Walker et al., 2005). In rats, Deferoxamine, a FDA-approved iron chelator, reduces fibrosis and improves behavior recovery (Vogelaar et al., 2015).

The ability to run genetic ablation experiments to reduce fibrosis has brought new insight but also further confusion into the role of fibrosis after injury. In spinal cord injury, complete ablation of fibrosis worsened the behavioral outcome, while partial ablation improved functional recovery (Dias et al., 2018). Another study showed the complete prevention of fibrotic scar formation in contusive spinal cord injury worsens the integrity of the injury site and worsens the functional outcome (Hesp et al., 2018). In contrast, ablation of fibrosis in the EAE model of multiple sclerosis has been showed to improve motor recovery (Dorrier et al., 2021). These studies highlight the potential dual-role of fibrotic scar and inquire further investigation.

BRAIN PERICYTES

Brain pericytes make up less than 1% of the total cells in the brain (Attwell et al., 2016). Pericytes are a heterogeneous population of mural or stromal cells that surround micro vessels in various organs(Attwell et al., 2016). Although the term pericyte has an incomplete scientific definition, most studies refer to this cell-type based on its shape and close apposition to the blood vessel.

Brain Pericyte Functions

In homeostasis, pericytes are normally found integrated within the endothelial basal membrane of capillaries and post-capillary venules with their processes wrapping around the vessels (Attwell et al., 2016; Sweeney et al., 2016). This tight association with endothelial cells suggests pericytes function in the development and maintenance of the cerebral vasculature network and in the regulation of cerebral blood flow (Hall et al., 2014; Hill et al., 2015). Loss of pericyte-number in

viable pericyte-deficient mice leads to vascular dysfunction through diminished capillary perfusion, loss of blood flow responses to brain activation, and BBB breakdown through accumulation of neurotoxic serum molecules (Bell et al., 2010; Sweeney et al., 2016). Molecular signals from endothelial cells (ECs) such as angiopoietin 1, transforming growth factor beta (TGF-b) and platelet-derived growth factor-BB (PDGF-BB) help direct pericyte migration to aid the formation and maturation of vasculature and to stabilize the vascular wall and (Nikolakopoulou et al., 2019; Ribatti et al., 2011; Sweeney et al., 2016). In return, pericytes secrete proangiogenic factors such as vascular endothelial growth factor (VEGF), neurogenic locus notch homolog protein (NOTCH3) to activate the angiogenesis process in the CNS (Bergers & Song, 2005). They also control the proliferation of endothelial cells and the formation of the basement membrane (Ribatti et al., 2011). Overall, these various signaling pathways outline the importance of pericytes in maintaining vascular health.

Pericytes' contractile ability has been shown to regulate the change in cerebral blood flow (CBF), in response to localized changes in neuronal activity and after injury (Hall et al., 2014; Kisler et al., 2017; Mishra et al., 2016). This CBF-regulating function is not limited to pericyte subpopulations that possess contractile proteins such as alpha-smooth muscle actin (α-SMA), myosin or tropomyosin; it also occurs in pericytes associated with thin-strand venules. Non-contractile pericytes can regulate CBF via stiffening the vascular wall thereby changing the capillary transit time (Attwell et al., 2016; Jung et al., 2018).

Situated at the basement membrane, pericytes modulate and maintain the BBB through signaling factors to regulate the number of EC tight junctions and polarization of astrocyte endfeet (Armulik et al., 2005, 2011). Pericytes also control flow of macromolecules and substances between the brain parenchyma and blood stream (Ma et al., 2018).

Pericytes also perform neuroinflammatory functions, which are usually carried out by astrocytes, microglia and other infiltrating leukocytes (Jansson et al., 2014b). Pericytes have been shown to phagocytose, respond to inflammatory signals and even present antigens to immune cells

(Jansson et al., 2014a; Rustenhoven et al., 2017). Pericyte-mediated dysfunction via release of inflammatory signals and matrix metalloprotease 9 (MMP9) can lead to vascular dysfunction and BBB breakdown(Underly et al., 2017). Interestingly, Apolipoprotein E expression, a major genetic risk factor for Alzeimers' disease, can activate the proinflammatory pathway CypA-MMP9 in pericytes and lead to BBB breakdown and vascular dysfunction(Bell et al., 2010). Furthermore, in diseases, pericytes' recruitment of immune cells to the brain is crucial for the endogenous neuroinflammation response (S. Wang et al., 2012).

True to its mysterious nature, pericytes show mesenchymal properties both *in vitro* and *in vivo* by differentiating into multiple cell types including vascular cells, neurons, and microglia(Ahmed & El-Badri, 2018; Özen et al., 2014; Sakuma et al., 2016). However, this is still a topic of debate – current and future single cell RNA sequencing datasets will hopefully help delineate the distinct population and characteristics of brain pericytes (Vanlandewijck et al., 2018).

Pericyte Heterogeneity

Pericytes are one of the most challenging cells to study in the brain due to their elusive nature, lack of specific markers and overlapping markers with other cell types in the brain, including oligodendrocytes, smooth muscle cells and perivascular fibroblasts (Armulik et al., 2011). In previous studies, a variety of markers have been used to label brain pericytes, including chondroitin sulfate proteoglycan 4 (NG2), platelet-derived growth factor receptor-β (PDGFRβ), CD13, CD146, Vitronectin, and ATP binding cassette subfamily c member 9 (ABCC9) (Armulik et al., 2011; Attwell et al., 2016; Vanlandewijck et al., 2018). Morphologically and spatially, brain pericytes are differentiated not only by their location on the vasculature bed and their processes but also the variable expression of α-SMA (Hartmann et al., 2015). This heterogeneity, while exciting, presents a challenge in studying them in injury and diseases where there are dynamic molecular, spatial, temporal and morphological changes in a tissue. Recent findings have shown that only a subset of pericytes, mainly the ensheathing pericytes at the arteriolar end of the capillary bed, express high level of α-SMA compared to the thin-strand capillary pericytes

(Alarcon-Martinez et al., 2018). To date, there has not been a comprehensive study to understand the differentiated functions (if any), of different subpopulation of pericytes. Future studies on to interrogate the heterogeneity of pericytes are imperative to the complete understanding of this cell type and its function after stroke.

Pericytes in Ischemic Injury

After injury, the pericyte response can be characterized into two unique phases: a cell death and dysfunctional phase that occurs in the acute injury phase and the repair phase in the subacute phase. The acute phase of pericyte death and dysfunction could lead to worsening outcomes after injury. Ischemic pericytes have been shown to not only constrict the capillaries and worsen the injury, but also interrupts the tight junction and increase BBB permeability (Hall et al., 2014; Underly et al., 2017; Yemisci et al., 2009). The loss of pericyte coverage has been ascribed as the main contributor the dysfunctional phenotype after injury. The disruption of the Angpt-1 and TGFb1 in pericyte-endothelial cell cross talk creates less tight junction formation and increased permeability (Hori et al., 2004; Luissint et al., 2012).

In the subacute phase, pericytes participate actively in a regenerative phase. The most prominent role of pericytes after injury is their vital role in regulating angiogenesis (Birbrair et al., 2014; Ribatti et al., 2011). In mouse kidney injury models, TIMP3-expressing pericytes can mediate the activity of a class of metalloproteinases and stabilize the microvasculature (Schrimpf et al., 2012). Utilizing its role in angiogenesis, blocking CD146 expression can reduce pericyte-mediated-angiogenesis in tumor vessels, and hence, slow tumor progression in cancer (Lei et al., 2015, p. 146; Nollet et al., 2017). These findings position pericytes has a promising target for therapeutic treatment to promote revascularization after ischemic injury.

Besides their role in the vasculature, recent studies have suggested the essential role of pericyte crosstalk in other regenerative processes. Pericyte production of Laminin 2 has been suggested to promote oligodendrocyte precursor cell differentiation and maturation during CNS remyelination (De La Fuente et al., 2017). Pericyte-deficient mice have disrupted oligodendrocyte

differentiation and myelination (Montagne et al., 2018). However, it is unclear whether the observed pathologies originate directly from pericyte crosstalk or indirectly from the pericyte-induced BBB leakage. A pericyte' role in fibrosis, especially in the brain, has been another area of controversy. This controversy will be discussed in the next section.

Pericytes vs. Perivascular Fibroblasts – Origin of Fibrotic Scar

It is generally accepted that fibroblasts are the main regulatory cell type, across tissue systems, responsible for the deposition of the fibrotic ECM. In other tissue systems, fibroblasts are characterized by their αSMA marker, secretion of ECM protein (mainly collagen I and fibronectin), as well as high cell contractility (van Dijk et al., 2015). The source of scar-forming fibroblasts is still a topic of debate across different tissue systems. PDGFRb$^+$ and pericyte-like cells have been suggested to be the source of fibrosis in multiple organs (Alex & Frangogiannis, 2019; Kramann & Humphreys, 2014; Y.-C. Wang et al., 2019, p. 1). In the brain, the plotline is much more complex. Until recently, virtually all fibrotic scar after injury in the CNS used a penetrating lesion model that tears the meninges. The source of fibrotic scar in these models was suggested to be mainly meningeal fibroblasts that infiltrate into the injury site (Campbell & Windle, 1960). It was commonly believed that only penetrating injuries lead to fibrotic scarring and that it is largely absent in injuries with intact meninges (Fernandez & Pallini, 1985; Silver & Miller, 2004). However, this view has been largely refuted in studies using injury models with intact meninges (Soderblom et al., 2013b; Yahn et al., 2020). Using a GLAST-CreER transgenic line, pericytes differentiated into fibroblasts and contributed to the fibrotic scar after spinal cord injury (Dias et al., 2018; Göritz et al., 2011). Pericytes have been reported to be progenitor cells that transdifferentiate into fibroblast-like cells and contribute to fibrosis (Fernández-Klett et al., 2013; Iihara et al., 1996; Makihara et al., 2015). They also have demonstrated the ability to secrete pro-inflammatory factors that may drive fibrosis(Jansson et al., 2014b). Using Col1a1-GFP mice, perivascular fibroblasts, not pericytes, were suggested to be the main contributor of fibrotic scar (Dorrier et al., 2021; Guimarães-Camboa et al., 2017; Yahn et al., 2020). While these

independent studies confirmed the brain-resident perivascular origin of fibrotic scar, specifically the PDGFRb^{positive} cells' contribution to scar formation, the cell nomenclature, due to similar markers and spatial distribution of perivascular fibroblasts and pericytes, has led to significant confusion. Does the fibrotic scar consist of pericyte differentiating into fibroblasts, or does it consist of fibroblasts migrating to injury site, or both? Interestingly, with the current evidence, the third option is highly unlikely, as each study demonstrated that their "chosen" cell types are responsible for almost all the PDGFRb^{positive} cell in the fibrotic core.

The distinction between pericytes and perivascular fibroblasts are limited. Using data from single cell RNA seq, table 1 highlights the shared markers between perivascular fibroblasts and pericytes (Vanlandewijck et al., 2018). In addition to some unique markers, both cell types have a different anatomical location along the vasculature: pericytes mainly locate around the capillaries. Perivascular fibroblasts are mainly located in the non-capillary regions that were positive for LAMA1(Vanlandewijck et al., 2018). The heterogeneity of the pericyte thickens the plot further. In one study, clustering analysis of pericytes showed only one type of capillary pericyte, whereas perivascular fibroblasts had multiple subtypes (Vanlandewijck et al., 2018). However, another independent study showed three pericyte subtypes (Zeisel et al., 2018). Another difficulty in reconciling these studies is that many of the unique molecular marker genes, especially for fibroblasts, encode proteins that label the ECM rather than the cell itself (lumican, decorin, etc) (Dorrier et al., 2021).

THIS DISSERTATION

This dissertation was carried out with two main goals: (1) to develop novel molecular tools to aid studies of pericytes and (2) further the study of the role of pericytes after CNS injury. The work is divided into three separate chapters as such:

Chapter 2 – Characterization of two populations of pericytes: their association with the neurovascular niche and fibrotic scar contribution

This chapter looks specifically at periinfarct pericytes and how they respond after injury. Transgenic mice were utilized to characterize the proliferation and cellular association pattern of pericyte after photothrombotic stroke. Two novel molecular tools were developed to lineage traced pericyte, perivascular fibroblasts and meningeal fibroblasts after stroke. The results show that periinfarct pericytes proliferate and associate with the regenerative neurovascular niche. A subgroup of pericytes detaches from vasculature and migrates into the infarcted region to contribute to fibrotic scar. Interestingly, control studies suggest perivascular fibroblast, but not meningeal fibroblast, contribute to the fibrotic process.

Chapter 3 – Characterization of the entire heterogeneous pericyte cell population and their stroke-induced transcriptomic changes

This chapter outlines the molecular characterization of perivascular cells after stroke using single cell RNAseq (scRNAseq). This technique allows for characterization of the entire heterogeneous PDGFRb^{positive} cells after stroke and in sham. This data was used to (1) confirming the pericyte origin of fibrotic scar using pseudotime analysis and (2) identify molecular pathways that delineate pericyte-mediated mechanisms for neural repair after stroke.

Chapter 4 – Manipulation of post-stroke pericyte via cellular ablation, candidate gene loss-of-function and pharmacological agent targeting a specific pathway.

This chapter is comprised of several routes of pericyte manipulation to further understand their role after ischemic stroke. Firstly, brain pericyte ablation study was carried out using the novel

molecular tools developed in chapter 2. Ablated tissue showed increased infarcted size and reduction in angiogenesis and neurogenesis processes. Secondly, *Stat3* and *Loxl2/3* were identified as the candidate gene systems that is responsible for pericyte's contribution to the fibrosis process. Pericyte-specific *Stat3* was perturbed via loss-of-function studies while Lox2/3 pathway was inhibited using pharmacological small molecules delivered systematically. *Stat3*-knockout mice did not show any significant phenotype on tissue level while Loxl2/3 inhibitors showed significant reduction in infarcted size and increase in angiogenesis and neurogenesis. These data not only confirm post-stroke pericyte contribution to fibrotic scar but also emphasize the highly conserved nature of the brain scar formation process. Targeting the fibrotic scar is a potential therapeutic approach to aid repair and recovery after ischemic stroke.

Table 1

Molecular markers of brain pericytes and perivascular fibroblasts

Pericytes	**Pericyte and Fibroblasts**	**Fibroblasts**
Cspg4 (NG2)	Pdgfrb	Dcn
Abcc9	Anpep	Lum
Myl9		Lama1
Vtn		Pdgfra

REFERENCES

Ahmed, T. A., & El-Badri, N. (2018). Pericytes: The Role of Multipotent Stem Cells in Vascular Maintenance and Regenerative Medicine. *Advances in Experimental Medicine and Biology*, *1079*, 69–86. https://doi.org/10.1007/5584_2017_138

Alarcon-Martinez, L., Yilmaz-Ozcan, S., Yemisci, M., Schallek, J., Kılıç, K., Can, A., Di Polo, A., & Dalkara, T. (2018). Capillary pericytes express α-smooth muscle actin, which requires prevention of filamentous-actin depolymerization for detection. *ELife*, *7*, e34861. https://doi.org/10.7554/eLife.34861

Alex, L., & Frangogiannis, N. G. (2019). Pericytes in the infarcted heart. *Vascular Biology*, *1*(1), H23–H31. https://doi.org/10.1530/VB-19-0007

Alexa, A., & Rahnenfuhrer, J. (n.d.). *Gene set enrichment analysis with topGO*. 26.

Ali, S. R., Ranjbarvaziri, S., Talkhabi, M., Zhao, P., Subat, A., Hojjat, A., Kamran, P., Müller, A. M. S., Volz, K. S., Tang, Z., Red-Horse, K., & Ardehali, R. (2014). Developmental heterogeneity of cardiac fibroblasts does not predict pathological proliferation and activation. *Circulation Research*, *115*(7), 625–635. https://doi.org/10.1161/CIRCRESAHA.115.303794

Anders, S., & Huber, W. (2010). Differential expression analysis for sequence count data. *Nature Precedings*, 1–1. https://doi.org/10.1038/npre.2010.4282.1

Anderson, M. A., Burda, J. E., Ren, Y., Ao, Y., O'Shea, T. M., Kawaguchi, R., Coppola, G., Khakh, B. S., Deming, T. J., & Sofroniew, M. V. (2016). Astrocyte scar formation aids central nervous system axon regeneration. *Nature*, *532*(7598), 195–200. https://doi.org/10.1038/nature17623

Armulik, A., Abramsson, A., & Betsholtz, C. (2005). Endothelial/Pericyte Interactions. *Circulation Research*, *97*(6), 512–523. https://doi.org/10.1161/01.RES.0000182903.16652.d7

Armulik, A., Genové, G., & Betsholtz, C. (2011). Pericytes: Developmental, physiological, and pathological perspectives, problems, and promises. *Developmental Cell*, *21*(2), 193–215. https://doi.org/10.1016/j.devcel.2011.07.001

Attwell, D., Mishra, A., Hall, C. N., O'Farrell, F. M., & Dalkara, T. (2016). What is a pericyte? *Journal of Cerebral Blood Flow & Metabolism*, *36*(2), 451–455. https://doi.org/10.1177/0271678X15610340

Aumiller, V., Strobel, B., Romeike, M., Schuler, M., Stierstorfer, B. E., & Kreuz, S. (2017). Comparative analysis of lysyl oxidase (like) family members in pulmonary fibrosis. *Scientific Reports*, *7*(1), 149. https://doi.org/10.1038/s41598-017-00270-0

Barnabé-Heider, F., Göritz, C., Sabelström, H., Takebayashi, H., Pfrieger, F. W., Meletis, K., & Frisén, J. (2010). Origin of new glial cells in intact and injured adult spinal cord. *Cell Stem Cell*, *7*(4), 470–482. https://doi.org/10.1016/j.stem.2010.07.014

Barreto, G., White, R. E., Ouyang, Y., Xu, L., & Giffard, R. G. (2011). Astrocytes: Targets for Neuroprotection in Stroke. *Central Nervous System Agents in Medicinal Chemistry*, *11*(2), 164–173.

Becerra-Calixto, A., & Cardona-Gómez, G. P. (2017). The Role of Astrocytes in Neuroprotection after Brain Stroke: Potential in Cell Therapy. *Frontiers in Molecular Neuroscience*, *10*, 88. https://doi.org/10.3389/fnmol.2017.00088

Bell, R. D., Winkler, E. A., Sagare, A. P., Singh, I., LaRue, B., Deane, R., & Zlokovic, B. V. (2010). Pericytes Control Key Neurovascular Functions and Neuronal Phenotype in the Adult Brain and during Brain Aging. *Neuron*, *68*(3), 409–427. https://doi.org/10.1016/j.neuron.2010.09.043

Benjamin, E. J., Virani, S. S., Callaway, C. W., Chamberlain, A. M., Chang, A. R., Cheng, S., Chiuve, S. E., Cushman, M., Delling, F. N., Deo, R., de Ferranti, S. D., Ferguson, J. F., Fornage, M., Gillespie, C., Isasi, C. R., Jiménez, M. C., Jordan, L. C., Judd, S. E., Lackland, D., … Muntner, P. (2018). Heart Disease and Stroke Statistics—2018 Update: A Report From the American Heart Association. *Circulation*, *137*(12). https://doi.org/10.1161/CIR.0000000000000558

Benowitz, L. I., & Carmichael, S. T. (2010). Promoting axonal rewiring to improve outcome after stroke. *Neurobiology of Disease*, *37*(2), 259. https://doi.org/10.1016/j.nbd.2009.11.009

Bergers, G., & Song, S. (2005). The role of pericytes in blood-vessel formation and maintenance.

Neuro-Oncology, *7*(4), 452–464. https://doi.org/10.1215/S1152851705000232

Birbrair, A., Zhang, T., Wang, Z.-M., Messi, M. L., Olson, J. D., Mintz, A., & Delbono, O. (2014). Type-2 pericytes participate in normal and tumoral angiogenesis. *American Journal of Physiology. Cell Physiology*, *307*(1), C25-38. https://doi.org/10.1152/ajpcell.00084.2014

Bish, L. T., Morine, K., Sleeper, M. M., Sanmiguel, J., Wu, D., Gao, G., Wilson, J. M., & Sweeney, H. L. (2008). Adeno-associated virus (AAV) serotype 9 provides global cardiac gene transfer superior to AAV1, AAV6, AAV7, and AAV8 in the mouse and rat. *Human Gene Therapy*, *19*(12), 1359–1368. https://doi.org/10.1089/hum.2008.123

Bodhankar, S., Lapato, A., Chen, Y., Vandenbark, A. A., Saugstad, J. A., & Offner, H. (2015). Role for microglia in sex differences after ischemic stroke: Importance of M2. *Metabolic Brain Disease*, *30*(6), 1515–1529. https://doi.org/10.1007/s11011-015-9714-9

Brumm, A. J., & Carmichael, S. T. (2012). Not just a rush of blood to the head. *Nature Medicine*, *18*(11), 1609–1610. https://doi.org/10.1038/nm.2990

Bundesen, L. Q., Scheel, T. A., Bregman, B. S., & Kromer, L. F. (2003). Ephrin-B2 and EphB2 Regulation of Astrocyte-Meningeal Fibroblast Interactions in Response to Spinal Cord Lesions in Adult Rats. *The Journal of Neuroscience*, *23*(21), 7789–7800. https://doi.org/10.1523/JNEUROSCI.23-21-07789.2003

Butler, A., Hoffman, P., Smibert, P., Papalexi, E., & Satija, R. (2018). Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nature Biotechnology*, *36*(5), 411–420. https://doi.org/10.1038/nbt.4096

Cafferty, W. B. J., Yang, S.-H., Duffy, P. J., Li, S., & Strittmatter, S. M. (2007). Functional Axonal Regeneration through Astrocytic Scar Genetically Modified to Digest Chondroitin Sulfate Proteoglycans. *Journal of Neuroscience*, *27*(9), 2176–2185. https://doi.org/10.1523/JNEUROSCI.5176-06.2007

Campbell, J. B., & Windle, W. F. (1960). Relation of millipore to healing and regeneration in transected spinal cords of monkeys. *Neurology*, *10*, 306–311. https://doi.org/10.1212/wnl.10.3.306

Carmichael, S. T. (2005). Rodent models of focal stroke: Size, mechanism, and purpose. *NeuroRX*, *2*(3), 396–409. https://doi.org/10.1602/neurorx.2.3.396

Carmichael, S. T. (2010). Translating the frontiers of brain repair to treatments: Starting not to break the rules. *Neurobiology of Disease*, *37*(2), 237–242. https://doi.org/10.1016/j.nbd.2009.09.005

Carmichael, S. T. (2016). The 3 Rs of Stroke Biology: Radial, Relayed, and Regenerative. *Neurotherapeutics*, *13*(2), 348–359. https://doi.org/10.1007/s13311-015-0408-0

Carrica, L., Li, L., Newville, J., Kenton, J., Gustus, K., Brigman, J., & Cunningham, L. A. (2019). Genetic inactivation of hypoxia inducible factor 1-alpha (HIF-1α) in adult hippocampal progenitors impairs neurogenesis and pattern discrimination learning. *Neurobiology of Learning and Memory*, *157*, 79–85. https://doi.org/10.1016/j.nlm.2018.12.002

Cha, J., & Lee, I. (2020). Single-cell network biology for resolving cellular heterogeneity in human diseases. *Experimental & Molecular Medicine*, *52*(11), 1798–1808. https://doi.org/10.1038/s12276-020-00528-0

Chang, J., Lucas, M. C., Leonte, L. E., Garcia-Montolio, M., Singh, L. B., Findlay, A. D., Deodhar, M., Foot, J. S., Jarolimek, W., Timpson, P., Erler, J. T., & Cox, T. R. (2017). Pre-clinical evaluation of small molecule LOXL2 inhibitors in breast cancer. *Oncotarget*, *8*(16), 26066–26078. https://doi.org/10.18632/oncotarget.15257

Chen, Y.-T., Chang, F.-C., Wu, C.-F., Chou, Y.-H., Hsu, H.-L., Chiang, W.-C., Shen, J., Chen, Y.-M., Wu, K.-D., Tsai, T.-J., Duffield, J. S., & Lin, S.-L. (2011). Platelet-derived growth factor receptor signaling activates pericyte-myofibroblast transition in obstructive and post-ischemic kidney fibrosis. *Kidney International*, *80*(11), 1170–1181. https://doi.org/10.1038/ki.2011.208

Chia, N. H., Leyden, J. M., Newbury, J., Jannes, J., & Kleinig, T. J. (2016). Determining the Number of Ischemic Strokes Potentially Eligible for Endovascular Thrombectomy: A Population-Based Study. *Stroke*, *47*(5), 1377–1380. https://doi.org/10.1161/STROKEAHA.116.013165

Choi, Y. K., Maki, T., Mandeville, E. T., Koh, S.-H., Hayakawa, K., Arai, K., Kim, Y.-M., Whalen, M. J., Xing, C., Wang, X., Kim, K.-W., & Lo, E. H. (2016). Dual effects of carbon monoxide on

pericytes and neurogenesis in traumatic brain injury. *Nature Medicine*, *22*(11), 1335–1341. https://doi.org/10.1038/nm.4188

de Jong, S., van Veen, T. A. B., de Bakker, J. M. T., & van Rijen, H. V. M. (2012). Monitoring cardiac fibrosis: A technical challenge. *Netherlands Heart Journal*, *20*(1), 44–48. https://doi.org/10.1007/s12471-011-0226-x

De La Fuente, A. G., Lange, S., Silva, M. E., Gonzalez, G. A., Tempfer, H., van Wijngaarden, P., Zhao, C., Di Canio, L., Trost, A., Bieler, L., Zaunmair, P., Rotheneichner, P., O'Sullivan, A., Couillard-Despres, S., Errea, O., Mäe, M. A., Andrae, J., He, L., Keller, A., … Rivera, F. J. (2017). Pericytes Stimulate Oligodendrocyte Progenitor Cell Differentiation during CNS Remyelination. *Cell Reports*, *20*(8), 1755–1764. https://doi.org/10.1016/j.celrep.2017.08.007

de Winter, F., Kwok, J. C. F., Fawcett, J. W., Vo, T. T., Carulli, D., & Verhaagen, J. (2016). The Chemorepulsive Protein Semaphorin 3A and Perineuronal Net-Mediated Plasticity. *Neural Plasticity*, *2016*, 3679545. https://doi.org/10.1155/2016/3679545

DeMers, G., Meurer, W. J., Shih, R., Rosenbaum, S., & Vilke, G. M. (2012). Tissue plasminogen activator and stroke: Review of the literature for the clinician. *The Journal of Emergency Medicine*, *43*(6), 1149–1154. https://doi.org/10.1016/j.jemermed.2012.05.005

Dias, D. O., Kalkitsas, J., Kelahmetoglu, Y., Estrada, C. P., Tatarishvili, J., Ernst, A., Huttner, H. B., Kokaia, Z., Lindvall, O., Brundin, L., Frisén, J., & Göritz, C. (2020a). *Pericyte-derived fibrotic scarring is conserved across diverse central nervous system lesions* [Preprint]. Neuroscience. https://doi.org/10.1101/2020.04.30.068965

Dias, D. O., Kalkitsas, J., Kelahmetoglu, Y., Estrada, C. P., Tatarishvili, J., Ernst, A., Huttner, H. B., Kokaia, Z., Lindvall, O., Brundin, L., Frisén, J., & Göritz, C. (2020b). Pericyte-derived fibrotic scarring is conserved across diverse central nervous system lesions. *BioRxiv*, 2020.04.30.068965. https://doi.org/10.1101/2020.04.30.068965

Dias, D. O., Kim, H., Holl, D., Werne Solnestam, B., Lundeberg, J., Carlén, M., Göritz, C., & Frisén, J. (2018). Reducing Pericyte-Derived Scarring Promotes Recovery after Spinal Cord Injury. *Cell*,

173(1), 153-165.e22. https://doi.org/10.1016/j.cell.2018.02.004

Dorrier, C. E., Aran, D., Haenelt, E. A., Sheehy, R. N., Hoi, K. K., Pintarić, L., Chen, Y., Lizama, C. O., Cautivo, K. M., Weiner, G. A., Popko, B., Fancy, S. P. J., Arnold, T. D., & Daneman, R. (2021). CNS fibroblasts form a fibrotic scar in response to immune cell infiltration. *Nature Neuroscience*, *24*(2), 234–244. https://doi.org/10.1038/s41593-020-00770-9

Edelman, D. A., Jiang, Y., Tyburski, J. G., Wilson, R. F., & Steffes, C. P. (2007). Cytokine production in lipopolysaccharide-exposed rat lung pericytes. *The Journal of Trauma*, *62*(1), 89–93. https://doi.org/10.1097/TA.0b013e31802dd712

Ellison, J. A., Velier, J. J., Spera, P., Jonak, Z. L., Wang, X., Barone, F. C., & Feuerstein, G. Z. (1998). Osteopontin and its integrin receptor alpha(v)beta3 are upregulated during formation of the glial scar after focal stroke. *Stroke*, *29*(8), 1698–1706; discussion 1707. https://doi.org/10.1161/01.str.29.8.1698

Ergul, A., Alhusban, A., & Fagan, S. C. (2012). Angiogenesis: A Harmonized Target for Recovery after Stroke. *Stroke; a Journal of Cerebral Circulation*, *43*(8), 2270–2274. https://doi.org/10.1161/STROKEAHA.111.642710

Ernst, A.-S., Böhler, L.-I., Hagenston, A. M., Hoffmann, A., Heiland, S., Sticht, C., Bendszus, M., Hecker, M., Bading, H., Marti, H. H., Korff, T., & Kunze, R. (2019). EphB2-dependent signaling promotes neuronal excitotoxicity and inflammation in the acute phase of ischemic stroke. *Acta Neuropathologica Communications*, *7*(1), 15. https://doi.org/10.1186/s40478-019-0669-7

Fei, Z., Zhang, X., Bai, H., Jiang, X., Li, X., Zhang, W., & Hu, W. (2007). Posttraumatic secondary brain insults exacerbates neuronal injury by altering Metabotropic Glutamate Receptors. *BMC Neuroscience*, *8*(1), 96. https://doi.org/10.1186/1471-2202-8-96

Fernandez, E., & Pallini, R. (1985). Connective tissue scarring in experimental spinal cord lesions: Significance of dural continuity and role of epidural tissues. *Acta Neurochirurgica*, *76*(3), 145–148. https://doi.org/10.1007/BF01418478

Fernández-Klett, F., Potas, J. R., Hilpert, D., Blazej, K., Radke, J., Huck, J., Engel, O., Stenzel, W.,

Genové, G., & Priller, J. (2013). Early loss of pericytes and perivascular stromal cell-induced scar formation after stroke. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *33*(3), 428–439. https://doi.org/10.1038/jcbfm.2012.187

Fernández-Klett, F., & Priller, J. (2014). The fibrotic scar in neurological disorders. *Brain Pathology (Zurich, Switzerland)*, *24*(4), 404–413. https://doi.org/10.1111/bpa.12162

Göritz, C., Dias, D. O., Tomilin, N., Barbacid, M., Shupliakov, O., & Frisén, J. (2011). A Pericyte Origin of Spinal Cord Scar Tissue. *Science*, *333*(6039), 238–242. https://doi.org/10.1126/science.1203165

Guimarães-Camboa, N., Cattaneo, P., Sun, Y., Moore-Morris, T., Gu, Y., Dalton, N. D., Rockenstein, E., Masliah, E., Peterson, K. L., Stallcup, W. B., Chen, J., & Evans, S. M. (2017). Pericytes of Multiple Organs Do Not Behave as Mesenchymal Stem Cells In Vivo. *Cell Stem Cell*, *20*(3), 345-359.e5. https://doi.org/10.1016/j.stem.2016.12.006

Guo, J., Bertalan, G., Meierhofer, D., Klein, C., Schreyer, S., Steiner, B., Wang, S., Vieira da Silva, R., Infante-Duarte, C., Koch, S., Boehm-Sturm, P., Braun, J., & Sack, I. (2019). Brain maturation is associated with increasing tissue stiffness and decreasing tissue fluidity. *Acta Biomaterialia*, *99*, 433–442. https://doi.org/10.1016/j.actbio.2019.08.036

Hall, C. N., Reynell, C., Gesslein, B., Hamilton, N. B., Mishra, A., Sutherland, B. A., O'Farrell, F. M., Buchan, A. M., Lauritzen, M., & Attwell, D. (2014). Capillary pericytes regulate cerebral blood flow in health and disease. *Nature*, *508*(7494), 55–60. https://doi.org/10.1038/nature13165

Hao, J., Li, B., Duan, H.-Q., Zhao, C.-X., Zhang, Y., Sun, C., Pan, B., Liu, C., Kong, X.-H., Yao, X., & Feng, S.-Q. (2017). Mechanisms underlying the promotion of functional recovery by deferoxamine after spinal cord injury in rats. *Neural Regeneration Research*, *12*(6), 959–968. https://doi.org/10.4103/1673-5374.208591

Hara, M., Kobayakawa, K., Ohkawa, Y., Kumamaru, H., Yokota, K., Saito, T., Kijima, K., Yoshizaki, S., Harimaya, K., Nakashima, Y., & Okada, S. (2017). Interaction of reactive astrocytes with type I

collagen induces astrocytic scar formation through the integrin–N-cadherin pathway after spinal cord injury. *Nature Medicine*, *23*(7), 818–828. https://doi.org/10.1038/nm.4354

Hardcastle, T. J., & Kelly, K. A. (2010). baySeq: Empirical Bayesian methods for identifying differential expression in sequence count data. *BMC Bioinformatics*, *11*(1), 422. https://doi.org/10.1186/1471-2105-11-422

Hartmann, D. A., Underly, R. G., Grant, R. I., Watson, A. N., Lindner, V., & Shih, A. Y. (2015). Pericyte structure and distribution in the cerebral cortex revealed by high-resolution imaging of transgenic mice. *Neurophotonics*, *2*(4), 041402. https://doi.org/10.1117/1.NPh.2.4.041402

Hatakeyama. (n.d.). *Angiogenesis and neuronal remodeling after ischemic stroke*. Retrieved August 4, 2021, from https://www.nrronline.org/article.asp?issn=1673-5374;year=2020;volume=15;issue=1;spage=16;epage=19;aulast=Hatakeyama

Hellal, F., Hurtado, A., Ruschel, J., Flynn, K. C., Laskowski, C. J., Umlauf, M., Kapitein, L. C., Strikis, D., Lemmon, V., Bixby, J., Hoogenraad, C. C., & Bradke, F. (2011). Microtubule stabilization reduces scarring and causes axon regeneration after spinal cord injury. *Science (New York, N.Y.)*, *331*(6019), 928–931. https://doi.org/10.1126/science.1201148

Hesp, Z. C., Yoseph, R. Y., Suzuki, R., Jukkola, P., Wilson, C., Nishiyama, A., & McTigue, D. M. (2018). Proliferating NG2-Cell-Dependent Angiogenesis and Scar Formation Alter Axon Growth and Functional Recovery After Spinal Cord Injury in Mice. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *38*(6), 1366–1382. https://doi.org/10.1523/JNEUROSCI.3953-16.2017

Hill, R. A., Tong, L., Yuan, P., Murikinati, S., Gupta, S., & Grutzendler, J. (2015). Regional Blood Flow in the Normal and Ischemic Brain Is Controlled by Arteriolar Smooth Muscle Cell Contractility and Not by Capillary Pericytes. *Neuron*, *87*(1), 95–110. https://doi.org/10.1016/j.neuron.2015.06.001

Hori, S., Ohtsuki, S., Hosoya, K., Nakashima, E., & Terasaki, T. (2004). A pericyte-derived angiopoietin-1 multimeric complex induces occludin gene expression in brain capillary

endothelial cells through Tie-2 activation in vitro. *Journal of Neurochemistry*, *89*(2), 503–513. https://doi.org/10.1111/j.1471-4159.2004.02343.x

How, J., Liu, Y., Lombardi Story, J., Neuberg, D. S., Ravid, K., Jarolimek, W., Charlton, B., & Hobbs, G. S. (2020). Evaluation of a Pan-Lysyl Oxidase Inhibitor, Pxs-5505, in Myelofibrosis: A Phase I, Randomized, Placebo Controlled Double Blind Study in Healthy Adults. *Blood*, *136*(Supplement 1), 16–16. https://doi.org/10.1182/blood-2020-139223

Hu, X., Li, P., Guo, Y., Wang, H., Leak, R. K., Chen, S., Gao, Y., & Chen, J. (2012). Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke*, *43*(11), 3063–3070. https://doi.org/10.1161/STROKEAHA.112.659656

Hwang, B., Lee, J. H., & Bang, D. (2018). Single-cell RNA sequencing technologies and bioinformatics pipelines. *Experimental & Molecular Medicine*, *50*(8), 1–14. https://doi.org/10.1038/s12276-018-0071-8

Iihara, K., Sasahara, M., Hashimoto, N., & Hazama, F. (1996). Induction of platelet-derived growth factor beta-receptor in focal ischemia of rat brain. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *16*(5), 941–949. https://doi.org/10.1097/00004647-199609000-00018

Islam, S., Zeisel, A., Joost, S., La Manno, G., Zajac, P., Kasper, M., Lönnerberg, P., & Linnarsson, S. (2014). Quantitative single-cell RNA-seq with unique molecular identifiers. *Nature Methods*, *11*(2), 163–166. https://doi.org/10.1038/nmeth.2772

Jansson, D., Rustenhoven, J., Feng, S., Hurley, D., Oldfield, R. L., Bergin, P. S., Mee, E. W., Faull, R. L., & Dragunow, M. (2014a). A role for human brain pericytes in neuroinflammation. *Journal of Neuroinflammation*, *11*(1), 104. https://doi.org/10.1186/1742-2094-11-104

Jansson, D., Rustenhoven, J., Feng, S., Hurley, D., Oldfield, R. L., Bergin, P. S., Mee, E. W., Faull, R. L., & Dragunow, M. (2014b). A role for human brain pericytes in neuroinflammation. *Journal of Neuroinflammation*, *11*(1), 104. https://doi.org/10.1186/1742-2094-11-104

Ji, Z., & Ji, H. (2016). TSCAN: Pseudo-time reconstruction and evaluation in single-cell RNA-seq analysis. *Nucleic Acids Research*, *44*(13), e117. https://doi.org/10.1093/nar/gkw430

Jiang, W., Gu, W., Brännström, T., Rosqvist, R., & Wester, P. (2001). Cortical neurogenesis in adult rats after transient middle cerebral artery occlusion. *Stroke*, *32*(5), 1201–1207.

Jones, M. G., Andriotis, O. G., Roberts, J. J., Lunn, K., Tear, V. J., Cao, L., Ask, K., Smart, D. E., Bonfanti, A., Johnson, P., Alzetani, A., Conforti, F., Doherty, R., Lai, C. Y., Johnson, B., Bourdakos, K. N., Fletcher, S. V., Marshall, B. G., Jogai, S., … Davies, D. E. (2018). Nanoscale dysregulation of collagen structure-function disrupts mechano-homeostasis and mediates pulmonary fibrosis. *ELife*, *7*, e36354. https://doi.org/10.7554/eLife.36354

Jung, B., Arnold, T. D., Raschperger, E., Gaengel, K., & Betsholtz, C. (2018). Visualization of vascular mural cells in developing brain using genetically labeled transgenic reporter mice. *Journal of Cerebral Blood Flow & Metabolism*, *38*(3), 456–468. https://doi.org/10.1177/0271678X17697720

Kelly-Hayes, M., Beiser, A., Kase, C. S., Scaramucci, A., D'Agostino, R. B., & Wolf, P. A. (2003). The influence of gender and age on disability following ischemic stroke: The Framingham study. *Journal of Stroke and Cerebrovascular Diseases: The Official Journal of National Stroke Association*, *12*(3), 119–126. https://doi.org/10.1016/S1052-3057(03)00042-9

Keough, M. B., Rogers, J. A., Zhang, P., Jensen, S. K., Stephenson, E. L., Chen, T., Hurlbert, M. G., Lau, L. W., Rawji, K. S., Plemel, J. R., Koch, M., Ling, C.-C., & Yong, V. W. (2016). An inhibitor of chondroitin sulfate proteoglycan synthesis promotes central nervous system remyelination. *Nature Communications*, *7*(1), 11312. https://doi.org/10.1038/ncomms11312

Kharchenko, P. V., Silberstein, L., & Scadden, D. T. (2014). Bayesian approach to single-cell differential expression analysis. *Nature Methods*, *11*(7), 740–742. https://doi.org/10.1038/nmeth.2967

Kisler, K., Nelson, A. R., Rege, S. V., Ramanathan, A., Wang, Y., Ahuja, A., Lazic, D., Tsai, P. S., Zhao, Z., Zhou, Y., Boas, D. A., Sakadžić, S., & Zlokovic, B. V. (2017). Pericyte degeneration

leads to neurovascular uncoupling and limits oxygen supply to brain. *Nature Neuroscience*, *20*(3), 406–416. https://doi.org/10.1038/nn.4489

Kramann, R., & Humphreys, B. D. (2014). Kidney Pericytes: Roles in Regeneration and Fibrosis. *Seminars in Nephrology*, *34*(4), 374–383. https://doi.org/10.1016/j.semnephrol.2014.06.004

Lang, B. T., Cregg, J. M., DePaul, M. A., Tran, A. P., Xu, K., Dyck, S. M., Madalena, K. M., Brown, B. P., Weng, Y.-L., Li, S., Karimi-Abdolrezaee, S., Busch, S. A., Shen, Y., & Silver, J. (2015). Modulation of the proteoglycan receptor PTPσ promotes recovery after spinal cord injury. *Nature*, *518*(7539), 404–408. https://doi.org/10.1038/nature13974

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*(1), 559. https://doi.org/10.1186/1471-2105-9-559

Lei, X., Guan, C.-W., Song, Y., & Wang, H. (2015). The multifaceted role of CD146/MCAM in the promotion of melanoma progression. *Cancer Cell International*, *15*, 3. https://doi.org/10.1186/s12935-014-0147-z

Leiva, O., Matsuura, S., Findlay, A., Turner, C., Jarolimek, W., & Ravid, K. (2017). A Novel Lysyl Oxidase Inhibitor Attenuates Bone Marrow Fibrosis and Decreases Spleen Size in a Mouse Model of Primary Myelofibrosis. *Blood*, *130*(Supplement 1), 4214–4214. https://doi.org/10.1182/blood.V130.Suppl_1.4214.4214

Leng, N., Dawson, J. A., Thomson, J. A., Ruotti, V., Rissman, A. I., Smits, B. M. G., Haag, J. D., Gould, M. N., Stewart, R. M., & Kendziorski, C. (2013). EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics*, *29*(8), 1035–1043. https://doi.org/10.1093/bioinformatics/btt087

Li, L., Huang, Y., Yang, Z., Zhang, S., Han, Z., & Luo, Y. (2020). Potential microglia-based interventions for stroke. *CNS Neuroscience & Therapeutics*, *26*(3), 288–296. https://doi.org/10.1111/cns.13291

Li, S., Nie, E. H., Yin, Y., Benowitz, L. I., Tung, S., Vinters, H. V., Bahjat, F. R., Stenzel-Poore, M. P., Kawaguchi, R., Coppola, G., & Carmichael, S. T. (2015). GDF10 is a signal for axonal

sprouting and functional recovery after stroke. *Nature Neuroscience*, *18*(12), 1737–1745. https://doi.org/10.1038/nn.4146

Lindvall, O., & Kokaia, Z. (2015). Neurogenesis following Stroke Affecting the Adult Brain. *Cold Spring Harbor Perspectives in Biology*, *7*(11), a019034. https://doi.org/10.1101/cshperspect.a019034

Liu, J., Tang, T., & Yang, H. (2011). Protective effect of deferoxamine on experimental spinal cord injury in rat. *Injury*, *42*(8), 742–745. https://doi.org/10.1016/j.injury.2010.08.028

Luissint, A.-C., Artus, C., Glacial, F., Ganeshamoorthy, K., & Couraud, P.-O. (2012). Tight junctions at the blood brain barrier: Physiological architecture and disease-associated dysregulation. *Fluids and Barriers of the CNS*, *9*(1), 23. https://doi.org/10.1186/2045-8118-9-23

Ma, L., Zeng, Y., Wei, J., Yang, D., Ding, G., Liu, J., Shang, J., Kang, Y., & Ji, X. (2018). Knockdown of LOXL1 inhibits TGF-β1-induced proliferation and fibrogenesis of hepatic stellate cells by inhibition of Smad2/3 phosphorylation. *Biomedicine & Pharmacotherapy*, *107*, 1728–1735. https://doi.org/10.1016/j.biopha.2018.08.156

Ma, Q., Zhao, Z., Sagare, A. P., Wu, Y., Wang, M., Owens, N. C., Verghese, P. B., Herz, J., Holtzman, D. M., & Zlokovic, B. V. (2018). Blood-brain barrier-associated pericytes internalize and clear aggregated amyloid-β42 by LRP1-dependent apolipoprotein E isoform-specific mechanism. *Molecular Neurodegeneration*, *13*(1), 57. https://doi.org/10.1186/s13024-018-0286-0

Ma, Y., Sun, S., Shang, X., Keller, E. T., Chen, M., & Zhou, X. (2020). Integrative differential expression and gene set enrichment analysis using summary statistics for scRNA-seq studies. *Nature Communications*, *11*(1), 1585. https://doi.org/10.1038/s41467-020-15298-6

Madsen, T. E., Khoury, J. C., Leppert, M., Alwell, K., Moomaw, C. J., Sucharew, H., Woo, D., Ferioli, S., Martini, S., Adeoye, O., Khatri, P., Flaherty, M., De Los Rios La Rosa, F., Mackey, J., Mistry, E., Demel, S. L., Coleman, E., Jasne, A., Slavin, S. J., … Kleindorfer, D. O. (2020). Temporal Trends in Stroke Incidence Over Time by Sex and Age in the GCNKSS. *Stroke*, *51*(4), 1070–1076. https://doi.org/10.1161/STROKEAHA.120.028910

Makihara, N., Arimura, K., Ago, T., Tachibana, M., Nishimura, A., Nakamura, K., Matsuo, R.,

Wakisaka, Y., Kuroda, J., Sugimori, H., Kamouchi, M., & Kitazono, T. (2015). Involvement of platelet-derived growth factor receptor β in fibrosis through extracellular matrix protein production after ischemic stroke. *Experimental Neurology*, *264*, 127–134. https://doi.org/10.1016/j.expneurol.2014.12.007

Mathys, H., Adaikkan, C., Gao, F., Young, J. Z., Manet, E., Hemberg, M., De Jager, P. L., Ransohoff, R. M., Regev, A., & Tsai, L.-H. (2017). Temporal Tracking of Microglia Activation in Neurodegeneration at Single-Cell Resolution. *Cell Reports*, *21*(2), 366–380. https://doi.org/10.1016/j.celrep.2017.09.039

Matsuo, A., Tanida, R., Yanagi, S., Tsubouchi, H., Miura, A., Shigekusa, T., Matsumoto, N., & Nakazato, M. (2021). Significance of nuclear LOXL2 inhibition in fibroblasts and myofibroblasts in the fibrotic process of acute respiratory distress syndrome. *European Journal of Pharmacology*, *892*, 173754. https://doi.org/10.1016/j.ejphar.2020.173754

Meletis, K., Barnabé-Heider, F., Carlén, M., Evergren, E., Tomilin, N., Shupliakov, O., & Frisén, J. (2008). Spinal Cord Injury Reveals Multilineage Differentiation of Ependymal Cells. *PLOS Biology*, *6*(7), e182. https://doi.org/10.1371/journal.pbio.0060182

Mishra, A., Reynolds, J. P., Chen, Y., Gourine, A. V., Rusakov, D. A., & Attwell, D. (2016). Astrocytes mediate neurovascular signaling to capillary pericytes but not to arterioles. *Nature Neuroscience*, *19*(12), 1619–1627. https://doi.org/10.1038/nn.4428

Montagne, A., Nikolakopoulou, A. M., Zhao, Z., Sagare, A. P., Si, G., Lazic, D., Barnes, S. R., Daianu, M., Ramanathan, A., Go, A., Lawson, E. J., Wang, Y., Mack, W. J., Thompson, P. M., Schneider, J. A., Varkey, J., Langen, R., Mullins, E., Jacobs, R. E., & Zlokovic, B. V. (2018). Pericyte degeneration causes white matter dysfunction in the mouse central nervous system. *Nature Medicine*, *24*(3), 326–337. https://doi.org/10.1038/nm.4482

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., de Ferranti, S., Després, J.-P., Fullerton, H. J., Howard, V. J., Huffman, M. D., Judd, S. E., Kissela, B. M., Lackland, D. T., Lichtman, J. H., Lisabeth, L. D., Liu, S., Mackey, R. H., Matchar, D. B., …

Turner, M. B. (2015). Heart Disease and Stroke Statistics—2015 Update: A Report From the American Heart Association. *Circulation*, *131*(4). https://doi.org/10.1161/CIR.0000000000000152

Neo, S. H., & Tang, B. L. (2017). Collagen 1 signaling at the central nervous system injury site and astrogliosis. *Neural Regeneration Research*, *12*(10), 1600–1601. https://doi.org/10.4103/1673-5374.217323

Nguyen, D. T., Ding, C., Wilson, E., Marcus, G. M., & Olgin, J. E. (2010). Pirfenidone mitigates left ventricular fibrosis and dysfunction after myocardial infarction and reduces arrhythmias. *Heart Rhythm*, *7*(10), 1438–1445. https://doi.org/10.1016/j.hrthm.2010.04.030

Niclou, S. P., Franssen, E. H. P., Ehlert, E. M. E., Taniguchi, M., & Verhaagen, J. (2003). Meningeal cell-derived semaphorin 3A inhibits neurite outgrowth. *Molecular and Cellular Neuroscience*, *24*(4), 902–912. https://doi.org/10.1016/S1044-7431(03)00243-4

Nikolakopoulou, A. M., Montagne, A., Kisler, K., Dai, Z., Wang, Y., Huuskonen, M. T., Sagare, A. P., Lazic, D., Sweeney, M. D., Kong, P., Wang, M., Owens, N. C., Lawson, E. J., Xie, X., Zhao, Z., & Zlokovic, B. V. (2019). Pericyte loss leads to circulatory failure and pleiotrophin depletion causing neuron loss. *Nature Neuroscience*, *22*(7), 1089. https://doi.org/10.1038/s41593-019-0434-z

Nollet, M., Stalin, J., Moyon, A., Traboulsi, W., Essaadi, A., Robert, S., Malissen, N., Bachelier, R., Daniel, L., Foucault-Bertaud, A., Gaudy-Marqueste, C., Lacroix, R., Leroyer, A. S., Guillet, B., Bardin, N., Dignat-George, F., & Blot-Chabaud, M. (2017). A novel anti-CD146 antibody specifically targets cancer cells by internalizing the molecule. *Oncotarget*, *8*(68), 112283–112296. https://doi.org/10.18632/oncotarget.22736

Nour, M., Scalzo, F., & Liebeskind, D. S. (2013). Ischemia-Reperfusion Injury in Stroke. *Interventional Neurology*, *1*(3–4), 185–199. https://doi.org/10.1159/000353125

Ohab, J. J., Fleming, S., Blesch, A., & Carmichael, S. T. (2006). A Neurovascular Niche for Neurogenesis after Stroke. *Journal of Neuroscience*, *26*(50), 13007–13016.

https://doi.org/10.1523/JNEUROSCI.4323-06.2006

Özen, I., Deierborg, T., Miharada, K., Padel, T., Englund, E., Genové, G., & Paul, G. (2014). Brain pericytes acquire a microglial phenotype after stroke. *Acta Neuropathologica*, *128*(3), 381–396. https://doi.org/10.1007/s00401-014-1295-x

Pasterkamp, R. J., Giger, R. J., Ruitenberg, M. J., Holtmaat, A. J., De Wit, J., De Winter, F., & Verhaagen, J. (1999). Expression of the gene encoding the chemorepellent semaphorin III is induced in the fibroblast component of neural scar tissue formed following injuries of adult but not neonatal CNS. *Molecular and Cellular Neurosciences*, *13*(2), 143–166. https://doi.org/10.1006/mcne.1999.0738

Popovich, P. G., Tovar, C. A., Lemeshow, S., Yin, Q., & Jakeman, L. B. (2014). Independent evaluation of the anatomical and behavioral effects of Taxol in rat models of spinal cord injury. *Experimental Neurology*, *261*, 97–108. https://doi.org/10.1016/j.expneurol.2014.06.020

Puente, A., Fortea, J. I., Cabezas, J., Arias Loste, M. T., Iruzubieta, P., Llerena, S., Huelin, P., Fábrega, E., & Crespo, J. (2019). LOXL2—A New Target in Antifibrogenic Therapy? *International Journal of Molecular Sciences*, *20*(7), 1634. https://doi.org/10.3390/ijms20071634

Ray, S., Ju, X., Sun, H., Finnerty, C. C., Herndon, D. N., & Brasier, A. R. (2013). The IL-6 trans-signaling-STAT3 pathway mediates ECM and cellular proliferation in fibroblasts from hypertrophic scar. *The Journal of Investigative Dermatology*, *133*(5), 1212–1220. https://doi.org/10.1038/jid.2012.499

Ribatti, D., Nico, B., & Crivellato, E. (2011). The role of pericytes in angiogenesis. *International Journal of Developmental Biology*, *55*(3), 261–268. https://doi.org/10.1387/ijdb.103167dr

Roth, M., Enström, A., Aghabeick, C., Carlsson, R., Genové, G., & Paul, G. (2020). Parenchymal pericytes are not the major contributor of extracellular matrix in the fibrotic scar after stroke in male mice. *Journal of Neuroscience Research*, *98*(5), 826–842. https://doi.org/10.1002/jnr.24557

Ruan, L., Wang, B., ZhuGe, Q., & Jin, K. (2015). Coupling of neurogenesis and angiogenesis after

ischemic stroke. *Brain Research*, *1623*, 166–173. https://doi.org/10.1016/j.brainres.2015.02.042

Ruschel, J., Hellal, F., Flynn, K. C., Dupraz, S., Elliott, D. A., Tedeschi, A., Bates, M., Sliwinski, C., Brook, G., Dobrindt, K., Peitz, M., Brüstle, O., Norenberg, M. D., Blesch, A., Weidner, N., Bunge, M. B., Bixby, J. L., & Bradke, F. (2015). Axonal regeneration. Systemic administration of epothilone B promotes axon regeneration after spinal cord injury. *Science (New York, N.Y.)*, *348*(6232), 347–352. https://doi.org/10.1126/science.aaa2958

Rustenhoven, J., Jansson, D., Smyth, L. C., & Dragunow, M. (2017). Brain Pericytes As Mediators of Neuroinflammation. *Trends in Pharmacological Sciences*, *38*(3), 291–304. https://doi.org/10.1016/j.tips.2016.12.001

Saelens, W., Cannoodt, R., Todorov, H., & Saeys, Y. (2019). A comparison of single-cell trajectory inference methods. *Nature Biotechnology*, *37*(5), 547–554. https://doi.org/10.1038/s41587-019-0071-9

Sakuma, R., Kawahara, M., Nakano-Doi, A., Takahashi, A., Tanaka, Y., Narita, A., Kuwahara-Otani, S., Hayakawa, T., Yagi, H., Matsuyama, T., & Nakagomi, T. (2016). Brain pericytes serve as microglia-generating multipotent vascular stem cells following ischemic stroke. *Journal of Neuroinflammation*, *13*(1). https://doi.org/10.1186/s12974-016-0523-9

Schrimpf, C., Xin, C., Campanholle, G., Gill, S. E., Stallcup, W., Lin, S.-L., Davis, G. E., Gharib, S. A., Humphreys, B. D., & Duffield, J. S. (2012). Pericyte TIMP3 and ADAMTS1 Modulate Vascular Stability after Kidney Injury. *Journal of the American Society of Nephrology : JASN*, *23*(5), 868–883. https://doi.org/10.1681/ASN.2011080851

Schultz, G. S., & Wysocki, A. (2009). Interactions between extracellular matrix and growth factors in wound healing. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *17*(2), 153–162. https://doi.org/10.1111/j.1524-475X.2009.00466.x

Segel, M., Neumann, B., Hill, M. F., Weber, I., Viscomi, C., Zhao, C., Young, A., Agley, C. C.,

Thompson, A. J., Gonzalez, G., Sharma, A., Holmqvist, S., Rowitch, D. H., Franze, K., Franklin, R. J., & Chalut, K. J. (2019). Niche stiffness underlies the aging of central nervous system progenitor cells. *Nature*, *573*(7772), 130–134. https://doi.org/10.1038/s41586-019-1484-9

Shearer, M. C., & Fawcett, J. W. (2001). The astrocyte/meningeal cell interface—A barrier to successful nerve regeneration? *Cell and Tissue Research*, *305*(2), 267–273. https://doi.org/10.1007/s004410100384

Shiber, J. R., Fontane, E., & Adewale, A. (2010). Stroke registry: Hemorrhagic vs ischemic strokes. *The American Journal of Emergency Medicine*, *28*(3), 331–333. https://doi.org/10.1016/j.ajem.2008.10.026

Silver, J., & Miller, J. H. (2004). Regeneration beyond the glial scar. *Nature Reviews. Neuroscience*, *5*(2), 146–156. https://doi.org/10.1038/nrn1326

Smith, G. M., & Strunz, C. (2005). Growth factor and cytokine regulation of chondroitin sulfate proteoglycans by astrocytes. *Glia*, *52*(3), 209–218. https://doi.org/10.1002/glia.20236

Soderblom, C., Luo, X., Blumenthal, E., Bray, E., Lyapichev, K., Ramos, J., Krishnan, V., Lai-Hsu, C., Park, K. K., Tsoulfas, P., & Lee, J. K. (2013a). Perivascular fibroblasts form the fibrotic scar after contusive spinal cord injury. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *33*(34), 13882–13887. https://doi.org/10.1523/JNEUROSCI.2524-13.2013

Soderblom, C., Luo, X., Blumenthal, E., Bray, E., Lyapichev, K., Ramos, J., Krishnan, V., Lai-Hsu, C., Park, K. K., Tsoulfas, P., & Lee, J. K. (2013b). Perivascular Fibroblasts Form the Fibrotic Scar after Contusive Spinal Cord Injury. *Journal of Neuroscience*, *33*(34), 13882–13887. https://doi.org/10.1523/JNEUROSCI.2524-13.2013

Sofroniew, M. V. (2015). Astrogliosis. *Cold Spring Harbor Perspectives in Biology*, *7*(2), a020420. https://doi.org/10.1101/cshperspect.a020420

Sweeney, M. D., Ayyadurai, S., & Zlokovic, B. V. (2016). Pericytes of the neurovascular unit: Key

functions and signaling pathways. *Nature Neuroscience*, *19*(6), 771–783. https://doi.org/10.1038/nn.4288

Tirosh, I., Izar, B., Prakadan, S. M., Wadsworth, M. H., Treacy, D., Trombetta, J. J., Rotem, A., Rodman, C., Lian, C., Murphy, G., Fallahi-Sichani, M., Dutton-Regester, K., Lin, J.-R., Cohen, O., Shah, P., Lu, D., Genshaft, A. S., Hughes, T. K., Ziegler, C. G. K., … Garraway, L. A. (2016). Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science (New York, N.Y.)*, *352*(6282), 189–196. https://doi.org/10.1126/science.aad0501

Underly, R. G., Levy, M., Hartmann, D. A., Grant, R. I., Watson, A. N., & Shih, A. Y. (2017). Pericytes as Inducers of Rapid, Matrix Metalloproteinase-9-Dependent Capillary Damage during Ischemia. *The Journal of Neuroscience*, *37*(1), 129–140. https://doi.org/10.1523/JNEUROSCI.2891-16.2016

van Dijk, C. G. M., Nieuweboer, F. E., Pei, J. Y., Xu, Y. J., Burgisser, P., van Mulligen, E., el Azzouzi, H., Duncker, D. J., Verhaar, M. C., & Cheng, C. (2015). The complex mural cell: Pericyte function in health and disease. *International Journal of Cardiology*, *190*, 75–89. https://doi.org/10.1016/j.ijcard.2015.03.258

Vanlandewijck, M., He, L., Mäe, M. A., Andrae, J., Ando, K., Gaudio, F. D., Nahar, K., Lebouvier, T., Laviña, B., Gouveia, L., Sun, Y., Raschperger, E., Räsänen, M., Zarb, Y., Mochizuki, N., Keller, A., Lendahl, U., & Betsholtz, C. (2018). A molecular atlas of cell types and zonation in the brain vasculature. *Nature*, *554*(7693), 475–480. https://doi.org/10.1038/nature25739

Vemuganti, R., & Zhao, H. (2015). Mechanisms and therapies for acute CNS insults. *Metabolic Brain Disease*, *30*(2), 353. https://doi.org/10.1007/s11011-014-9585-5

Vogelaar, C. F., König, B., Krafft, S., Estrada, V., Brazda, N., Ziegler, B., Faissner, A., & Müller, H. W. (2015). Pharmacological Suppression of CNS Scarring by Deferoxamine Reduces Lesion Volume and Increases Regeneration in an In Vitro Model for Astroglial-Fibrotic Scarring and in Rat Spinal Cord Injury In Vivo. *PLoS ONE*, *10*(7), e0134371. https://doi.org/10.1371/journal.pone.0134371

Walker, J. E., Giri, S. N., & Margolin, S. B. (2005). A double-blind, randomized, controlled study of oral pirfenidone for treatment of secondary progressive multiple sclerosis. *Multiple Sclerosis (Houndmills, Basingstoke, England)*, *11*(2), 149–158. https://doi.org/10.1191/1352458505ms1134oa

Wang, S., Cao, C., Chen, Z., Bankaitis, V., Tzima, E., Sheibani, N., & Burridge, K. (2012). Pericytes Regulate Vascular Basement Membrane Remodeling and Govern Neutrophil Extravasation during Inflammation. *PLoS ONE*, *7*(9). https://doi.org/10.1371/journal.pone.0045499

Wang, T., Li, B., Nelson, C. E., & Nabavi, S. (2019). Comparative analysis of differential gene expression analysis tools for single-cell RNA sequencing data. *BMC Bioinformatics*, *20*(1), 40. https://doi.org/10.1186/s12859-019-2599-6

Wang, Y.-C., Chen, Q., Luo, J.-M., Nie, J., Meng, Q.-H., Shuai, W., Xie, H., Xia, J.-M., & Wang, H. (2019). Notch1 promotes the pericyte-myofibroblast transition in idiopathic pulmonary fibrosis through the PDGFR/ROCK1 signal pathway. *Experimental & Molecular Medicine*, *51*(3), 1–11. https://doi.org/10.1038/s12276-019-0228-0

Whetstone, W. D., Hsu, J.-Y. C., Eisenberg, M., Werb, Z., & Noble-Haeusslein, L. J. (2003). Blood-spinal cord barrier after spinal cord injury: Relation to revascularization and wound healing. *Journal of Neuroscience Research*, *74*(2), 227–239. https://doi.org/10.1002/jnr.10759

Xiao, M.-J., Han, Z., Shao, B., & Jin, K. (2009). Notch signaling and neurogenesis in normal and stroke brain. *International Journal of Physiology, Pathophysiology and Pharmacology*, *1*(2), 192–202.

Xu, X.-H., Jia, Y., Zhou, X., Xie, D., Huang, X., Jia, L., Zhou, Q., Zheng, Q., Zhou, X., Wang, K., & Jin, L.-P. (2019). Downregulation of lysyl oxidase and lysyl oxidase-like protein 2 suppressed the migration and invasion of trophoblasts by activating the TGF-β/collagen pathway in preeclampsia. *Experimental & Molecular Medicine*, *51*(2), 1–12. https://doi.org/10.1038/s12276-019-0211-9

Yahn, S. L., Li, J., Goo, I., Gao, H., Brambilla, R., & Lee, J. K. (2020). Fibrotic scar after experimental

autoimmune encephalomyelitis inhibits oligodendrocyte differentiation. *Neurobiology of Disease*, *134*, 104674. https://doi.org/10.1016/j.nbd.2019.104674

Yang, J., Savvatis, K., Kang, J. S., Fan, P., Zhong, H., Schwartz, K., Barry, V., Mikels-Vigdal, A., Karpinski, S., Kornyeyev, D., Adamkewicz, J., Feng, X., Zhou, Q., Shang, C., Kumar, P., Phan, D., Kasner, M., López, B., Diez, J., … Chang, C.-P. (2016). Targeting LOXL2 for cardiac interstitial fibrosis and heart failure treatment. *Nature Communications*, *7*(1), 13710. https://doi.org/10.1038/ncomms13710

Yang, T., Dai, Y., Chen, G., & Cui, S. (2020). Dissecting the Dual Role of the Glial Scar and Scar-Forming Astrocytes in Spinal Cord Injury. *Frontiers in Cellular Neuroscience*, *0*. https://doi.org/10.3389/fncel.2020.00078

Yau, J. W., Teoh, H., & Verma, S. (2015). Endothelial cell control of thrombosis. *BMC Cardiovascular Disorders*, *15*, 130. https://doi.org/10.1186/s12872-015-0124-z

Yemisci, M., Gursoy-Ozdemir, Y., Vural, A., Can, A., Topalkara, K., & Dalkara, T. (2009). Pericyte contraction induced by oxidative-nitrative stress impairs capillary reflow despite successful opening of an occluded cerebral artery. *Nature Medicine*, *15*(9), 1031–1037. https://doi.org/10.1038/nm.2022

Yiu, G., & He, Z. (2006). Glial inhibition of CNS axon regeneration. *Nature Reviews Neuroscience*, *7*(8), 617–627. https://doi.org/10.1038/nrn1956

Yokota, K., Kobayakawa, K., Saito, T., Hara, M., Kijima, K., Ohkawa, Y., Harada, A., Okazaki, K., Ishihara, K., Yoshida, S., Kudo, A., Iwamoto, Y., & Okada, S. (2017). Periostin Promotes Scar Formation through the Interaction between Pericytes and Infiltrating Monocytes/Macrophages after Spinal Cord Injury. *The American Journal of Pathology*, *187*(3), 639–653. https://doi.org/10.1016/j.ajpath.2016.11.010

Zbesko, J. C., Nguyen, T.-V. V., Yang, T., Frye, J. B., Hussain, O., Hayes, M., Chung, A., Day, W. A., Stepanovic, K., Krumberger, M., Mona, J., Longo, F. M., & Doyle, K. P. (2018). Glial scars are permeable to the neurotoxic environment of chronic stroke infarcts. *Neurobiology of Disease*,

112, 63–78. https://doi.org/10.1016/j.nbd.2018.01.007

Zeisel, A., Hochgerner, H., Lönnerberg, P., Johnsson, A., Memic, F., van der Zwan, J., Häring, M., Braun, E., Borm, L. E., La Manno, G., Codeluppi, S., Furlan, A., Lee, K., Skene, N., Harris, K. D., Hjerling-Leffler, J., Arenas, E., Ernfors, P., Marklund, U., & Linnarsson, S. (2018). Molecular Architecture of the Mouse Nervous System. *Cell*, *174*(4), 999-1014.e22. https://doi.org/10.1016/j.cell.2018.06.021

CHAPTER 2

Characterization of Post-Stroke Pericyte

INTRODUCTION

Stroke is the leading cause of adult disability (Benjamin et al., 2018). The brain has a limited regenerative capacity after stroke (Carmichael, 2016). One aspect of “regeneration” is the formation of new neurons, termed post-stroke neurogenesis(Ohab et al., 2006; Ruan et al., 2015). Post-stroke neurogenesis has been linked to functional improvement after stroke (Jiang et al., 2001; Lindvall & Kokaia, 2015; Ruan et al., 2015). Immature neurons (neuroblasts) from the subventricular zone (SVZ) migrate into a specific micro-environment adjacent to the stroke site, termed the neurovascular niche(Brumm & Carmichael, 2012; Ohab et al., 2006). In this niche, neuroblasts associate with angiogenic blood vessels (Ohab et al., 2006; Ruan et al., 2015). However, few of these neuroblasts survive to become mature adult neurons (Lindvall & Kokaia, 2015). A better understanding of the environment in the neurovascular unit in peri-infarct tissue may lead to approaches that promote survival of newly born neurons.

Pericytes, a less-well-understood member of the neurovascular unit, are a heterogeneous population of perivascular cell that regulates development and maintenance of structural elements of the blood brain barrier, vascular stability, and angiogenesis. Study of pericytes in tissue repair has been difficult due to the lack of exclusive markers and the cells’ complex heterogeneity (Attwell et al., 2016). Several studies have suggested that in response to ischemic stimuli in organs such as the liver, skin and kidney, pericytes coordinate extravasation of immune cells, actively participate in extracellular matrix production (ECM) and contribute to fibrosis (Dias et al., 2018; Fernández-Klett et al., 2013; Göritz et al., 2011). Additionally, genetic fate mapping studies have demonstrated that pericytes may be a source of myofibroblasts in animal models of renal and lung fibrosis (Chen et al., 2011; Edelman et al., 2007). However, direct evidence linking pericytes to fibroproliferation and ECM remodeling in the brain is lacking. Emerging evidence in spinal cord injury indicates that pericytes form the fibrotic scar that blocks axonal sprouting and recovery, and not astrocytes (Dias et al., 2020b; Roth et al., 2020). Other conflicting studies showed that perivascular fibroblasts, not pericytes, contribute to fibrotic scar formation (Dorrier et al., 2021; Guimarães-Camboa et al., 2017).

In this study, we hypothesized that pericytes respond to stroke and participate in the injury response. We utilized the PDGFRb^{Cre} mouse strain to characterize pericyte proliferation, association with other cell types after ischemic stroke. Periinfarct pericytes undergo a burst of proiliferation at 7-14 days after ischemic stroke. These proliferative pericytes closely associated with the proliferative vasculature and the immature neurons in the regenerative neurovascular niche. Using dual-promoter lineage tracing system, we identified a population of pericytes that migrate away from vasculature and accumulate in the infarct core, where they express ECM proteins. In control studies, we observed the migration of perivascular fibroblasts but not meningeal fibroblasts into the infarcted core.

RESULTS

Periinfarct pericytes proliferate in the subacute phase after ischemic injury

To observe the proliferative response of pericytes to ischemic stroke, we induced photothrombotic stroke at the motor cortex in young adult male (2-4 months old) *Pdgfrb$^{CRE/+}$;R26$^{tdT/+}$* mice. EdU (5-ethynyl-2'-deoxyuridine) was present in drinking water after stroke **(Figure 1A).** Animals were sacrificed and tissue were collected at multiple timepoints after stroke, covering the acute (3 days), subacute (7 and 14 days) and chronic phase (21 and 54 days) of injury. Immunohistochemistry for vasculature (CD31) and EdU were performed. *Pdgfrb$^{CRE/+}$;R26$^{tdT/+}$*mice label the entire pericyte population in the brain, but also label smooth muscle cells (SMCs), a limited number of neurons, perivascular fibroblasts and meningeal fibroblasts. An analysis pipeline using confocal imaging and high-throughput quantification was developed to systematically eliminate SMCs from the analysis and quantify proliferating pericytes (based on both spatial and morphological characteristics) **(Figure 1B).** The analysis of proliferating pericytes revealed a remarkable proliferating capacity across different timepoints after stroke **(Figure 1C-E)**. After stroke, overall periinfarct pericyte number were reduced in the acute phase of stroke, which is quickly compensated by the burst of proliferation which peaks at 7-14 days after injury (30% of pericyte were EdU^{+}). During the chronic phase, the proliferative rate returns to baseline **(Figure 1C-E).**

Proliferative pericytes closely associate with the regenerative neurovascular niche

Next, we set out to further investigate the spatial characteristics of proliferative pericytes in the periinfarct area, in particular the association with the regenerative neurovascular niche. Tissues were stained for Doublecortin (DCX) and Olig2 to label immature neurons ($DCX^{positive}Olig2^{negative}$); and CD31 for vasculature. Using Imaris, the average distance between the proliferative pericyte cell bodies to their closest neuroblasts and angiogenic vessels was measured. As pericytes possess long processes that wrap around vasculature that would complicate the analysis, only the pericyte cell body (~4um in diameter) was included in the analysis pipeline. We observed that ~30% of proliferative pericytes closely associated with a neuroblast (within 0-5um distance) **(Figure 2A-B).** Furthermore, true to its role in angiogenesis, ~60% of proliferative pericytes closely associated with an angiogenic vessel ($EdU^{positive}CD31^{positive}$) **(Figure 2C-D).** Our data demonstrates that proliferative pericytes are closely associated with the components of the regenerative neurovascular niche, suggesting its participation via cell-to-cell communication to aid the tissue repair and regeneration processes.

PDGFRb+ cell origin of fibrotic scar

In line with previously reported findings (Underly et al., 2017), in sham tissues, all the tdT^+ cells wrapped around blood vessels **(Figure 3A)**. However, at 3 days after stroke, we observed a population of tdT^+ cells (~18% of total tdT^+ population) that were not associated with the vasculature, and appeared to detach and be present in the brain parenchyma (termed, “floating cells”) **(Figure 3B-C).** These cells are mainly located within 100uM of the infarct border (identified by the $GFAP^+$ border), expressed the pericyte-specific marker CD13 and fibroblast marker Col1A1 **(Figure 3F)**. At 3 days post stroke, there was no tdT^+ population or fibrotic scar inside the infarct area **(Figure 3B-C)**. Interestingly, within 72 hours, this phenomenon rapidly changed with the accumulation of tdT^+ cells in the infarcted scar, as well as the reduction of the number of “floating pericytes” in the periinfarct region **(Figure 3D-E)**.

Further characterization of the tdT+ population inside the scar revealed its fibrotic scar phenotype.

The tdT+ cells inside the infarcted core express fibrotic markers such as COL1A1 and Fibronectin **(Figure 3F & Figure 4A)**. We also observed that the reactive astrocytic border ($GFAP^+$) encapsulated and closely associated with this fibrotic tdT^+ scar and together they defined the injury border **(Figure 4C)**. Interestingly, we observed a large number of neuroblasts present at this border, but mostly populated on the peri-infarcted side, not the infarct side **(Figure 4B).** Morphologically, the tdT^+ cells in the infarct were distinct from the peri-infarct pericytes. The infarct core tdT^+ cells had a larger cell body (~8-10um) compared to that of pericytes (~4-5um). They also had more a fibrous and thicker processes network that suggested a more complex ECM network compared to pericytes processes with thinner processes (**Figure 4D).** This, together with its lack of pericyte markers and its fibroblast marker expression, suggested that the infarct core tdT^+ cells possess a complete fibroblast phenotype.

This observation is aligned with the current findings in the literature about the $PDGFRb^+$ origin of the scar and begs the question of whether or not these fibrotic-scar forming cells in the infarct area originated from the periinfarct pericytes.

Development of dual-promoter pericyte lineage tracing systems

Fate-mapping pericytes after stroke has been a great challenge in the field due to lack of specific markers, animal models and molecular tools to specifically investigate the cells. To overcome this challenge, we combined transgenic animals and AAV virus with the FLEX labeling system; we developed two independent, novel molecular tools to specific label brain pericytes. The first molecular model is a dual-promoter system of AAV2-PDGFRb-Flex-GFP virus, injected transcranially into *NG2*$^{CreER/+}$;*Rosa26*$^{tdT/+}$ **(Figure 5A-B).** In these mice, tamoxifen administration will induce the Cre recombination in $NG2^+$ cells, resulting in not only the permanent labeling with tdTomato signal, but also the expression of GFP protein in cells that expresses PDGFRb **(Figure 5B-C)**. This allows for the GFP to be only expressed in PDGFRb+ and NG2+ cells, which yields specific labeling of pericytes **(Figure 5B). Might want to put in a reference about those two markers being sufficient for pericyte labeling.** A similar molecular system using transgenic mouse model

Tbx18$^{CreER/+}$;*Rosa26*$^{tdT/+}$ and AAV2-Myl9-Flex-GFP virus was also developed **(Figure 5D-E).** To date, there has not been any viral vector that could uniquely label pericytes. The development of these pericyte-specific molecular tools not only will set the stage for the following lineage tracing experiments but also allow for further interrogation of pericyte behavior in homeostasis, in disease, and the manipulation of this elusive cell population.

Pericytes contribute to the fibrotic scar after ischemic stroke

Using the novel molecular tools, we next set out to specifically lineage-trace pericytes after stroke. AAV was injected into the periinfarct region medially to the infarct area (motor cortex) of transgenic mice. After 2 weeks, animals received tamoxifen injections intraperitoneal injection at concentration of 20 mg/mL for 5 consecutive days **(Figure 6A)**. 1 week after the last day of tamoxifen (TM) injection, photothrombotic stroke or sham surgery was induced, allowing for a washout period to ensure no recombination after injury **(Figure 6A).** Animals were sacrificed and tissues were collected at various points after injury for further analysis **(Figure 6A)**.

At 7 days after stroke, we observed the presence of GFP$^+$ cells inside the infarct core from both lineage tracing systems **(Figure 6B-E)**. This indicated that pericytes in the periinfarct area migrated away from vasculature into the infarcted area and formed fibrotic scars after ischemic stroke. We quantified the number of GFP$^+$ cells present in the infarcted core and found that the number of GFP$^+$ cells in the fibrotic area increased as more days passed after injury **(Figure 6F).** These results are in agreement with previous findings in the spinal cord (Dias et al., 2018; Göritz et al., 2011), that brain-resident periinfarct pericytes migrated into the injury core and become fibrotic-scar-forming fibroblasts in the first 7 days after stroke.

Perivascular fibroblasts, not meningeal fibroblasts, also contribute to the fibrosis process

Next, we looked to reconcile our findings with previous studies that showed perivascular fibroblasts and meningeal fibroblasts contribute to the fibrotic scar after brain injury (Dorrier et al., 2021; Shearer & Fawcett, 2001) by setting up a series of control experiments to lineage-trace both of these cell populations after stroke. For perivascular fibroblasts, we developed a novel perivascular fibroblast

labeling system using a similar FLEX system as the one for pericytes previously discussed **(Figure 7A).** Perivascular fibroblasts are known for their LAMA1 expression, PDGFRb^{low} and PDGFRa^{high} expression (Vanlandewijck et al., 2018). Utilizing their TBX18 expression, we developed a dual-promoter system using *Tbx18$^{CreER/+}$;R26$^{tdT/+}$* mice and AAV2-Pdgfra-FLEX-GFP virus to specifically label perivascular fibroblasts **(Figure 7A-B).** Keeping the experimental timeline consistent with that of pericyte **(Figure 6A),** we found GFP^{+} cells inside the infarcted area 7 days after ischemic stroke, suggesting the contribution of perivascular fibroblasts in fibrotic scar formation **(Figure 6C).** The contribution of perivascular fibroblasts was highly variable between animals (**Figure 6D)**. This could be attributed to the variability in labeling perivascular fibroblasts due to variability in location, along with its scarcity along the vascular bed.

Next, for meningeal fibroblasts which express similar markers compared to periinfarct pericytes, we utilized a similar labeling system to that used on periinfarct pericyte (PDGFRb-NG2 labeling system) **(Figure 6C)**. The AAV virus was injected into the cisterna magna, allowing it to incorporate into the cerebrospinal fluid (CSF) and only label the meningeal population but not the cortical population **(Figure 7D-E).** After stroke, we did not see the presence of GFP+ cells inside the infarct core, indicating that meningeal fibroblasts do not contribute to the formation of fibrotic scar after stroke.

DISCUSSION

The role of pericytes in brain ischemic injury has been an underexplored topic in understanding the importance of these cells. We hypothesized that pericytes have dual functions after stroke: participation in the repair processes of the regenerative neurovascular niche and formation of fibrotic scar.

We observed the proliferative burst of pericytes that peaked around 7-14 days after ischemic injury. This window coincided with the peak of angiogenesis, neurogenesis and other regenerative processes in the brain (Carmichael, 2016). This, together with their close association with the angiogenic vessel and neuroblasts, suggested that proliferative pericytes play crucial roles in the establishment and continued restructuring of the neurovascular niche after injury. The close physical

association suggests potential cross-talk between pericytes and other members of the neurovascular niche. Our findings are in-line with the current literature about the role of pericytes in angiogenesis and neurogenesis. In fact, some of the molecular signaling with pericytes in the neurrovascuar niche has been documented in the literature. NOS-mediated pericyte and neuroblast crosstalk to promote neurogenesis has been suggested *in vitro* in a traumatic brain injury model (Choi et al., 2016). Oligodendrocyte-pericyte cross talk after injury has also been documented, which further positions pericytes as an important regulator of the regenerative events that happen in the subacute phase of stroke (De La Fuente et al., 2017).

Among the cellular events that happen within the first week after ischemic injury, fibrotic scar formation is one of the most understudied, due to the lack of molecular tools to understand both its role and cellular origin. Our initial immunohistochemistry confirmed the PDGFRb^{+} cell origin of the brain fibrosis. Post-mortem brain tissue from stroke patients also confirmed the PDGFRb+ cells within the lesion site (Fernández-Klett et al., 2013). Using multiple carefully designed labeling systems that were specifically developed to label pericytes, we were able to control the expression of GFP spatially and temporally in pericytes after stroke, allowing us to lineage trace the cell population after injury. Our results demonstrated that the periinfarct pericytes migrate into the infarct area, lose the pericyte fate and express prominent levels of COL1A1. These results highlight the trans-differentiation of pericyte into scar-forming fibroblasts after ischemic injury and suggest its multipotency that had previously been observed in spinal cord injury and various *in vitro* studies (Ahmed & El-Badri, 2018; Dias et al., 2018; Göritz et al., 2011; Sakuma et al., 2016). A recent study done by the Goritz group suggested that the pericyte-derived fibrotic scarring process is highly conserved across different central nervous system lesions, including traumatic brain injury, ischemic stroke, and a multiple sclerosis model (Dias et al., 2020b).

It is important to reconcile our data with previously reported findings that either refuted the role of pericytes in brain fibrosis or suggested that its contribution to the fibrotic scar, while present, was minimal (Dorrier et al., 2021; Guimarães-Camboa et al., 2017; Roth et al., 2020). Even in studies that

agreed with ours about pericyte contribution to the fibrosis process, the findings were at odds with each other about the characteristics of these pericyte scar-forming cells. Using a striatal stroke model, one study showed that while these pericytes expresses fibroblast markers, they remained associated with the vascular wall and rarely detached from the vasculature. Our study suggested the stromal location of the fibrotic scar-forming cells. (Dias et al., 2020b).

The first clear difference between these studies were the different use of injury models. Traumatic brain injury models used by some studies does not create the ischemic penumbra, therefore lacking the significant reorganization and regeneration after injury. The stab lesion's poor production of fibrotic tissue and low to no fibroblast-like cell after injury were also well-documented in other studies (Dias et al., 2020b). Using a clinically relevant model of ischemic injury along with the reevaluation of the contribution of Tbx18-expressing pericyte in the context of larger brain lesions, we confirmed the contribution of pericytes in the injury response. The discrepancy between the characteristics of our study that that observed in Dias et al., 2020 could be attributed to the heterogeneity between pericytes in the cortex vs striatal pericyte.

The second area that could explain the discrepancy is the difference in animal models used by various studies. Even in studies that use the same injury model such as experimental autoimmune encephalomyelitis (EAE), different findings were observed (Dias et al., 2020b; Dorrier et al., 2021). It is important to note that NG2-CreER used by some studies to lineage trace pericytes not only labels a subpopulation of pericytes, along with its contamination of oligodendrocyte lineage-cells, it is also not a good molecular model to fate-map pericytes after stroke. Given the discrepancies of these findings, our study was designed to improve upon these limitations and confounders by using conditional genetics and viral vectors, which gave us greater control of the spatial and temporal tracking of cells after injury.

Fibroblasts migrating from the damaged meningeal layer or from the perivascular space have long been considered the primary source of fibrotic scar tissue (Dorrier et al., 2021; Niclou et al., 2003; Pasterkamp et al., 1999; Shearer & Fawcett, 2001; Soderblom et al., 2013a). Our studies had showed

that in photothrombotic stroke in which the meningeal layer remains intact and limits the invasion of the dura mater-derived fibroblasts into the lesion, the extensive fibrotic generation still exists and did not originate from meningeal fibroblasts. Using dual-promoter control to label perivascular fibroblasts, we found the significant contribution of perivascular fibroblasts, particularly the population that existed prior to injury and not the population that derived from pericytes, to the fibrotic scar. However, due to the variability of distribution, we don't understand the exact contribution of pericytes and perivascular fibroblasts to the scar. Additional experiments are required to pinpoint both these cell type contributions.

Together, our study answered important questions left by previous studies and confirmed the pericyte contribution to the scar formation processes. By developing a novel toolkit, we hope to further the understanding of pericyte biology, and perivascular cell biology after ischemic injury.

METHODS

Mice

NG2$^{CreER/+}$ mice and *Rosa26*$^{tdT/+}$ mice were acquired from The Jackson Lab. Tbx18$^{Cre/+}$ were a gift from the Evans lab at the University of California, San Diego. *PDGFRb*$^{/+}$ mice were a gift from Dr. Volkhard Lindner (Maine Medical Center). For all experiments, young adult (2-4 months) C57Bl/6 male mice were used. All procedures were carried out with the approval of the UCLA Animal Research Committee.

Photothrombotic Stroke

Cortical stroke at the motor cortex was induced in mice using the stereotaxic surgery. Mice were initially anesthetized by mixture of oxygen and 5% isoflurane, then intubated at 2% for the duration of the operation. Body temperature was monitored and maintained at 37 degree Celcius +/- 0.5 degree by homeothermic heating pads. Rose Bangel, a photosensitive chemical was injected intraperitoneal at concentration of 10mg/mL. After 10 minutes to allow for systemic circulation of Rose Bangel, a cold light source KL1500 LCD (Zeiss) was placed on top of the exposed skull above the forelimb motor cortex (coordinates ML 1.5mm, AP 0.0mm). After 15 minutes, the wound was closed and animals were returned to cages for recovery. The photothrombotic stroke can reliably generate stroke size of 1-2 mm in diameter, dependent on the diameter and power of the light source (Carmichael, 2005)

Transcranial Viral Injection

Mice were prepared for surgery as previously described. After anesthesia, mice received injections in the medial intact peri-infarct cortex (Coordinates from Bregma: ML 0.5, AP 0.0mm) with the designated virus. A dental drill was used to create a small hole in the skull to allow for needle access, and a 33guage Hamilton needle, attached to a 25 ul was lowered into the cortex at 0.4mm coordinate from the brain surface. There, virus was infused at rate of 0.1 ul/min. Afterward, the needle was allowed to remain in the brain for 10 minutes to allow for additional infusion and prevent backflow before slowly retracted. The wound was closed with vetbond tissue adhesive (3M). Animal was

returned to the cage for recovery.

Cisterna Magna Viral Injection

Animals were prepared as previously described. After anesthesia, the skin at the base of the skull and neck muscle were carefully dissected to expose the brainstem and cisterna magna. Using a pulled glass pippet, attached to a 33-gauge syringe needle, virus was slowly infused into cerebrospinal fluid at the rate of 0.1ul/min. Afterward, the needle was allowed to remain in the brain for 10 minutes to allow for additional infusion and prevent backflow before slowly retracted. The wound was closed with vet bond tissue adhesive (3M). Animal was returned to the cage for recovery.

Tissue Collection

At indicated time, animals were sacrificed via transcardial perfusion with cold saline containing 2% Paraformaldehyde (PFA), 0.1M lysin, 0.01M NaO4, 0.175 NaP buffer. This mild fixation cocktail allows for preservation of many vasculature glycoprotein. Brains were dissected out and post-fixed in the same solution overnight at 4 degree Ceicius, then cryopreserved in 30% sucrose at 4 degree Celcius for an additional 48 hours. Afterwards, tissues were sectioned on cryostat (Lica CM 0530). 40uM sections were collected and stored in 50% glycerol antifreeze solution at -20 degree Celcius.

Immunohistochemistry

Immunohistochemistry was prepared according to standard laboratory protocol. Sections were removed from the antifreeze solution and washed 3 times in 0.02M KPBS for 5 minutes. Afterward, the sections went through incubations 30-minute-permeabilization step (1% Triton-X in KPBS). Dependent on the antibody used, antigen retrieval steps using Citric acid pH 6.0 at 80 degree Celcius for 30 minutes was added. Tissues were blocked in solution of 5% normal donkey serum (NDS), 0.1% Triton-X for 1 hour at room temperature. Tissues were then transferred directly into primary antibody cocktail that is prepared in 2% NDS, 0.1% Triton-X in KBPS for 24 hours at 4 degree Celcius. The antibody list and its respective concentration is detailed in supplemental table 1. Following the primary incubation, sections were washed 3 times in KPBS and incubated in the secondary antibody solution

that was prepared in 2% NDS, 0.1% Triton-X in KPBS for 1 hour at room temperature. Afterward, tissues were washed 3 times in KBPS for 10 minutes. EdU steps might be added at this stage dependent on the assay. Sections were mounted oonto triple-subbed slides and dehydrated with ethanol serial wash with increased concentration from 50-100% (1 minute each step), and xylene wash (5 minutes, twice). Next, slides were cover-slipped with the application of DPX mounting media.

EdU Administration and Immunohistochemistry Induction

EdU was administered in drinking water at the final concentration of 200ug/mL with antibiotic (cherry flavored for taste at the recommended dose. EdU was developed with a protocol modified from the Click-IT EdU imaging kit (Thermo Fisher). Briefly, sections were incubated for 30 minutes as the last step of immunohistochemistry for 30 minutes in 100mM TBS, pH 7.6 containing 100mM sodium ascorbate, 4mM CuSO4, and 2uM sulfo-Cy5 azide (Lumiprobe D3330). Sections were protected from light during this incubation.

Imaging and Imaris Analysis

Images were captured using a Nikon C2 confocal microscope at 40x magnification. Additional analysis were done on Imaris, as previously published (S. Li et al., 2015, p. 10)

AAV Virus Preparation

Appropriate promoters were developed and cloned into the AAV2-Flex virus backbone. Sequence were verified for insertion. Afterward, the vectors were packaged into AAV2 virus using previously published protocol (Bish et al., 2008). Briefly, the viral plasmid were transfected into HEKs cells using Lipofectamin 200 (Thermofisher). After 3 days of incubation, the cells were harvested into 150mMNaCl, 20mM Tris pH8.0 solution before being lyzed for 1 hour in the 10% sodium deoxycholate solution at 37 degree celcius. The cell debris were removed by centrifugation at 3000xg for 15 minutes and the rAAVs in supernation were harvested. Afterward, using the serial heparin column purification method, AAVs were purified then concentrated via the Amicon ultra-4 centrifugal filter units with 100,000 molecular weight cutoff. Virus is then tittered with HEK 293 cells before storing in -80 degree

Celcius.

Statistics

All quantification data are presented as mean ± standard error of the mean (SEM) and significance was determined by using Student's t-test or Two-way ANOVA. A p-value < 0.05 was considered statistically significant and data were analyzed using GraphPad Prism 8.

Figure 1

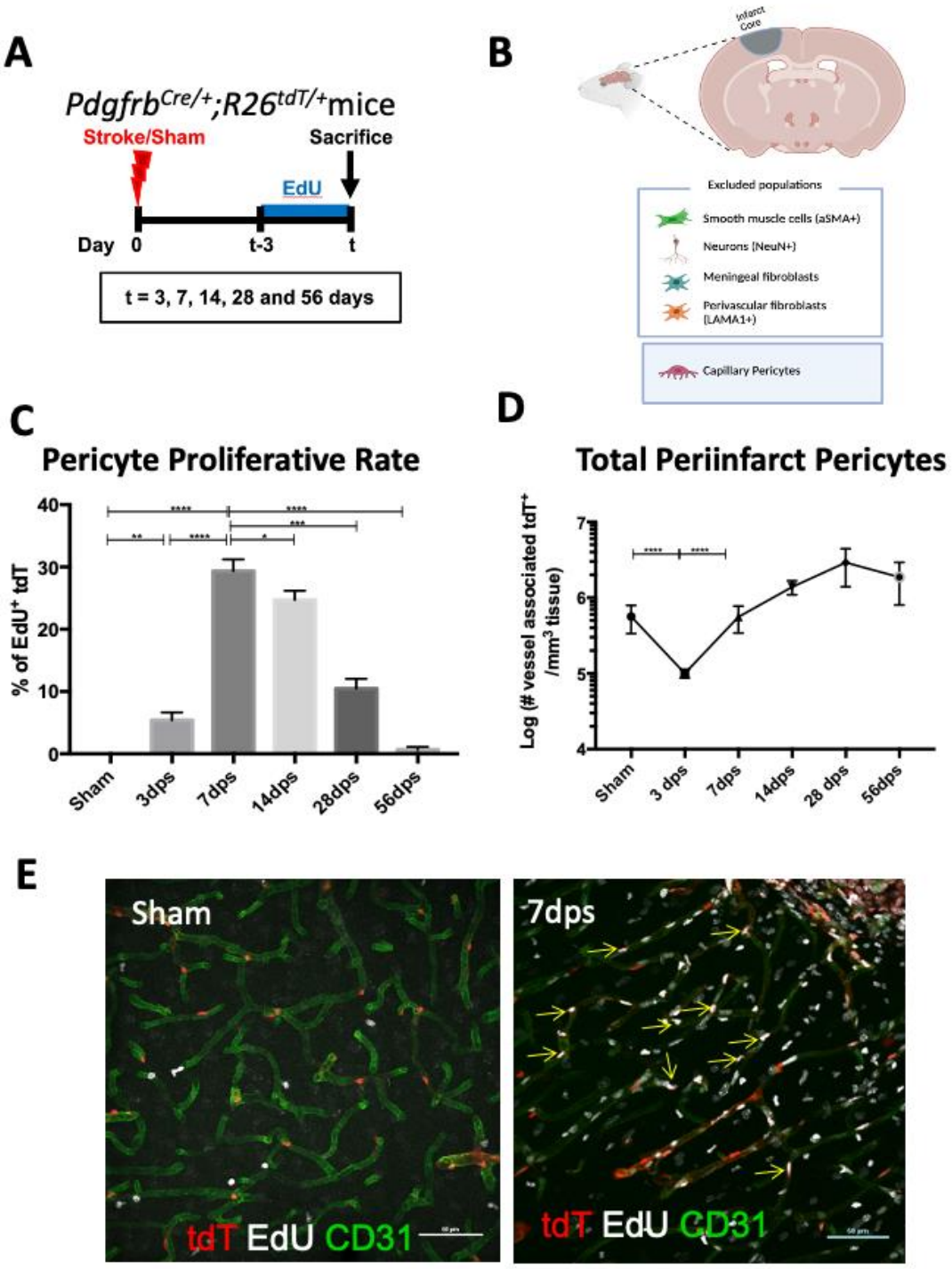

A
Pdgfrb^Cre/+;R26^tdT/+ mice
Stroke/Sham
Sacrifice
EdU
Day 0
t-3
t
t = 3, 7, 14, 28 and 56 days
B
Infarct Core
Excluded populations
Smooth muscle cells (aSMA+)
Neurons (NeuN+)
Meningeal fibroblasts
Perivascular fibroblasts (LAMA1+)
Capillary Pericytes
C
Pericyte Proliferative Rate
% of EdU+ tdT
Sham
3dps
7dps
14dps
28dps
56dps
D
Total Periinfarct Pericytes
Log (# vessel associated tdT+ /mm3 tissue)
Sham
3 dps
7dps
14dps
28 dps
56dps
E
Sham
tdT EdU CD31
7dps
tdT EdU CD31

Figure 1: PDGFRb $^{+}$ pericytes in the periinfarct proliferated robustly after stroke

(A) Experimental timeline **(B)** Schematic of excluded cell population in the analysis pipeline **(C)** Pericytes proliferative rate (% Edu+ pericytes) **(D)** Total periinfarct pericyte population (log of density pericyte over vasculature volume) **(E)** Representative image of proliferative pericyte after injury; yellow arrow= EdU+ pericytes. Scale bar: 50μm. *p-value < 0.05.

Figure 2

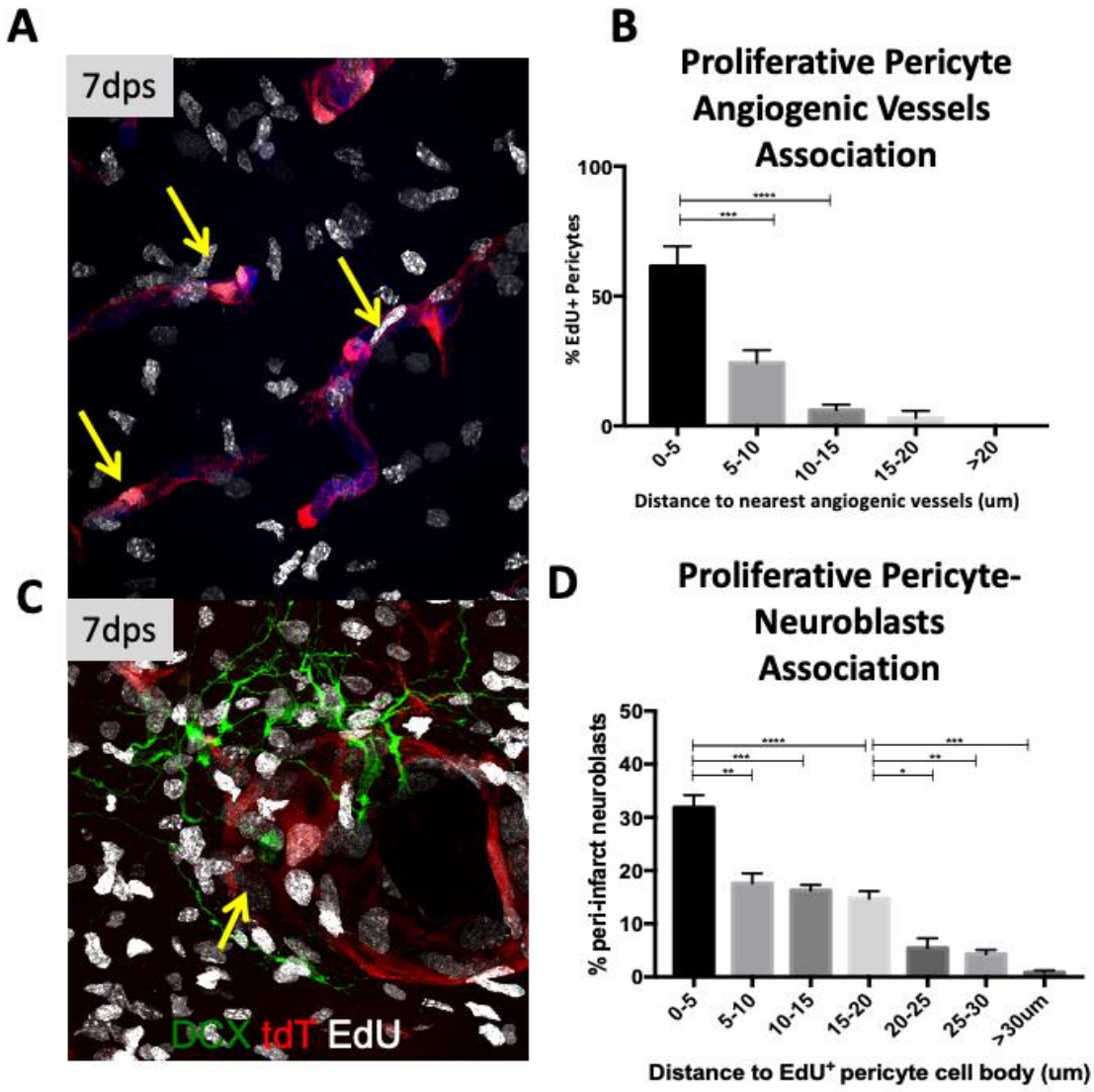
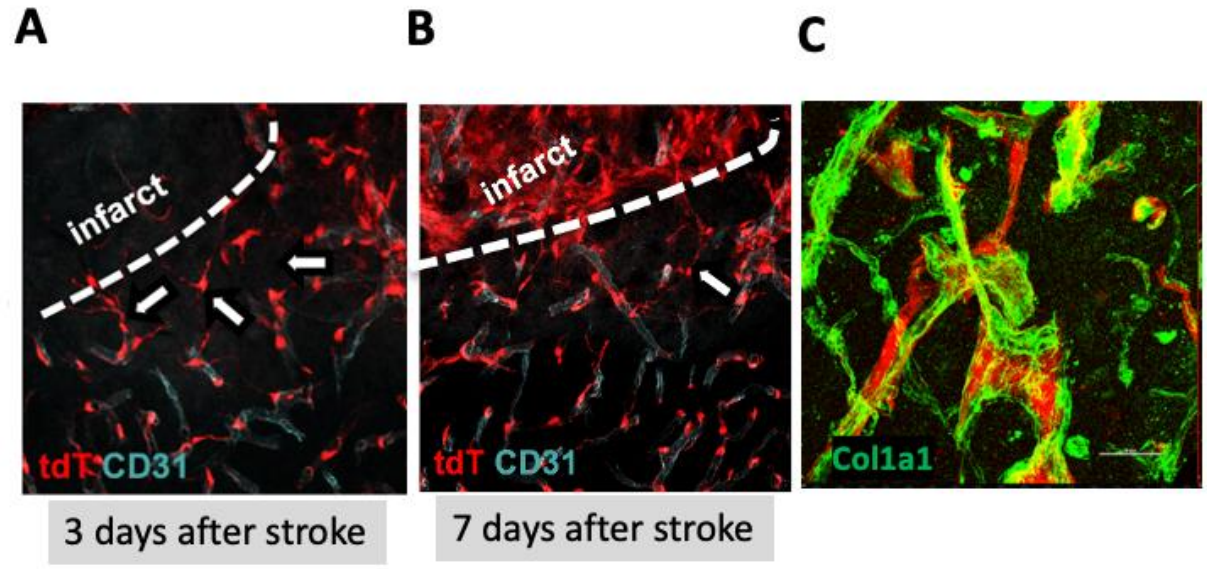
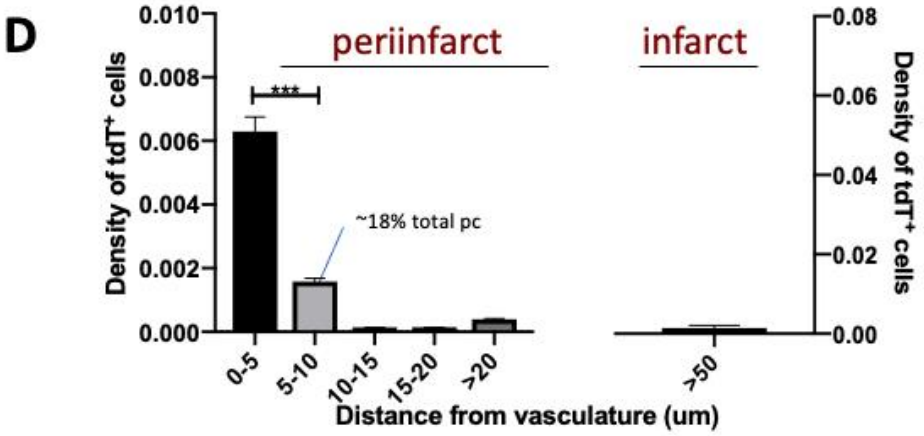

A
7dps
B
Proliferative Pericyte Angiogenic Vessels Association
% EdU+ Pericytes
100
50
0
****
***
0-5
5-10
10-15
15-20
>20
Distance to nearest angiogenic vessels (um)
C
7dps
DCX tdT EdU
D
Proliferative Pericyte-Neuroblasts Association
% peri-infarct neuroblasts
50
40
30
20
10
0
****
***
**
***
**
*
0-5
5-10
10-15
15-20
20-25
25-30
>30um
Distance to EdU+ pericyte cell body (um)

Figure 2: Proliferative periinfarct pericytes closely associated with the regenerative neurovascular niche

(A) Representative image of pericyte associated with angiogeneic vessel. Yellow arrow = angiogenic vessels. **(B)** Distances of proliferative pericytes to its nearest vasculature **(C)** Representative image of pericyte associated with neuroblasts (DCX= double cortin) **(D)** Distances of pericyte to its nearest neuroblasts. Scale bar: 50μm. *p-value < 0.05.

Figure 3


A
B
C
infarct
infarct
tdT CD31
tdT CD31
Col1a1
3 days after stroke
7 days after stroke


D
periinfarct
infarct
***
~18% total pc
Density of tdT+ cells
0.010
0.008
0.006
0.004
0.002
0.000
0.08
0.06
0.04
0.02
0.00
0-5
5-10
10-15
15-20
>20
>50
Distance from vasculature (um)

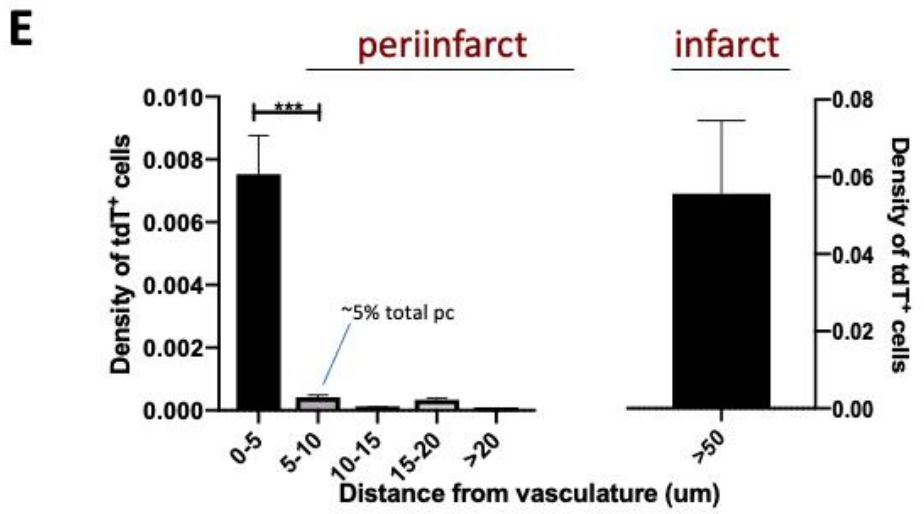

E
periinfarct
infarct
***
~5% total pc
Density of tdT+ cells
0.010
0.008
0.006
0.004
0.002
0.000
0.08
0.06
0.04
0.02
0.00
0-5
5-10
10-15
15-20
>20
>50
Distance from vasculature (um)

Figure 3: Pdgfrb+ cells accumulated the infarct area at the subacute phase after stroke

(A) tdTomato cells were observed away from vasculature in the 100um immediate region next to the infarct border at 3 days post stroke (dps**)**. **(B,C)** At 7 days after stroke, tdTomato cells accumulated the infarcted area and expressed COL1A1 **(D,E)** Quantification of the tdTomato cells in the periinfarct and infarct area, visualized by distances from vasculature. Scale bar: 100μm. *p-value < 0.05.

Figure 4

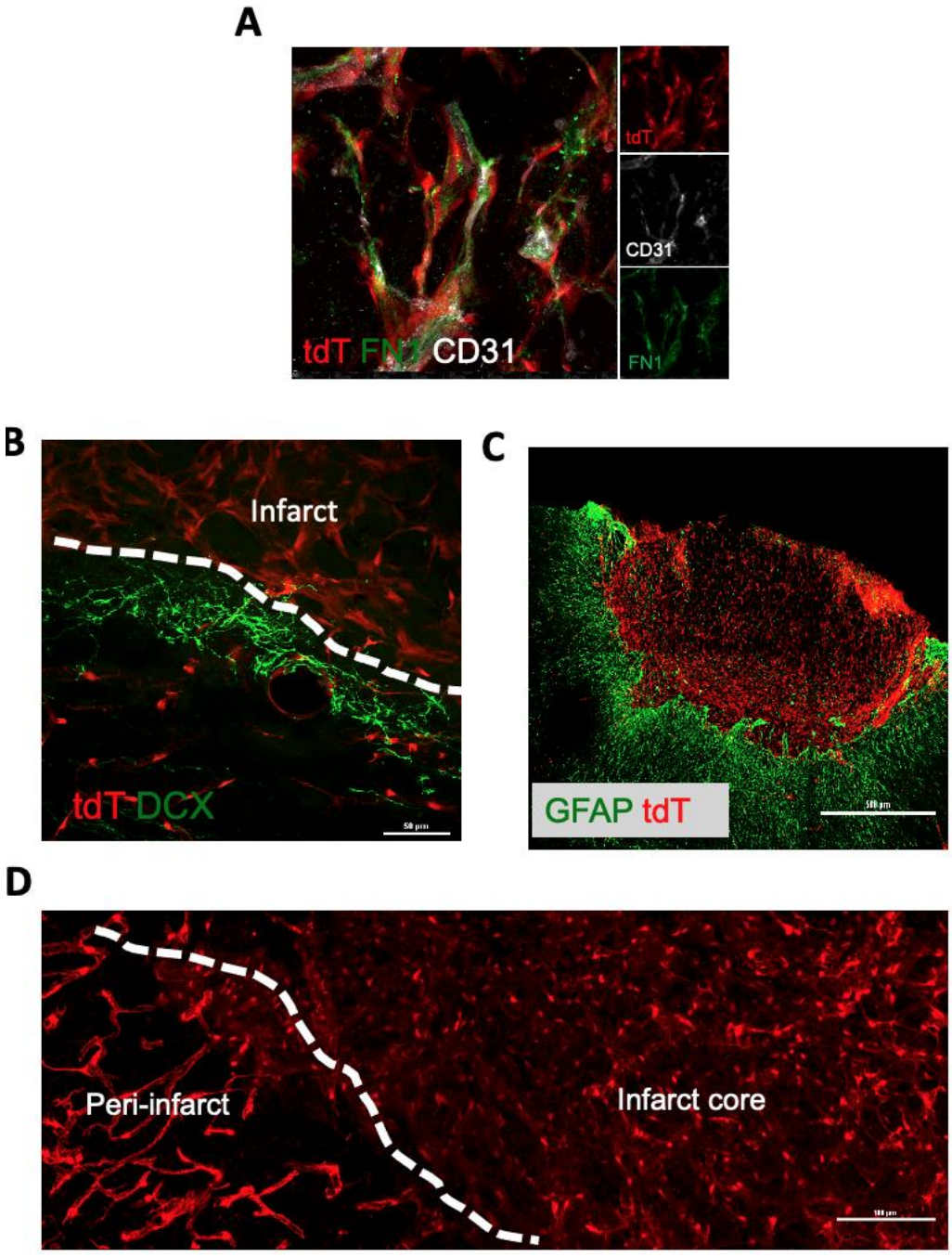

A
tdT FN1 CD31
tdT
CD31
FN1
B
Infarct
tdT DCX
C
GFAP tdT
D
Peri-infarct
Infarct core

Figure 4: PDGFRb+ cells contribution to scar

(A) PDGFRb+ cells expressed fibronectin markers in the core **(B-C)** PDGFRb+ cells formed a border against the DCX+ cells in the periinfarct cortex and the PDGFRb+ scar were encapsulated by the GFAP+ glia scar. **(D)** Morphologically, the periinfarct pericytes are distinctly different than the PDGFRb+ cells in the infarct core. Scale bar for panel A-C: 100um. Scale bar for panel D: 500um

Figure 5

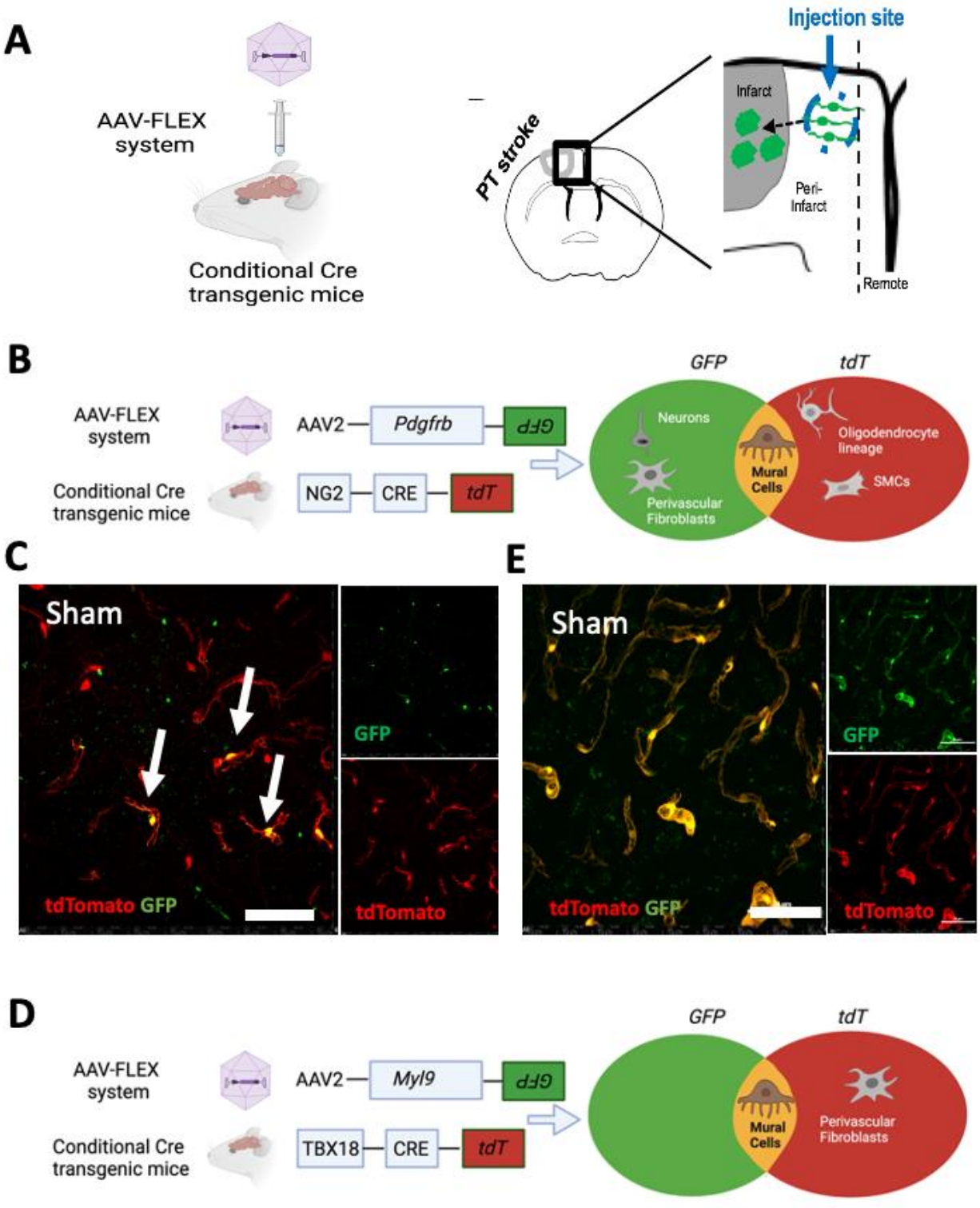

A
AAV-FLEX system
Conditional Cre transgenic mice
Injection site
Infarct
Peri-Infarct
Remote
PT stroke
B
AAV-FLEX system
AAV2
Pdgfrb
GFP
Conditional Cre transgenic mice
NG2
CRE
tdT
GFP
tdT
Neurons
Perivascular Fibroblasts
Mural Cells
Oligodendrocyte lineage
SMCs
C
Sham
tdTomato GFP
GFP
tdTomato
E
Sham
tdTomato GFP
GFP
tdTomato
D
AAV-FLEX system
AAV2
Myl9
GFP
Conditional Cre transgenic mice
TBX18
CRE
tdT
GFP
tdT
Mural Cells
Perivascular Fibroblasts

Figure 5: Development of dual-promoter-labeling systems to label pericytes

(A,B) Schematic of the first labeling system **(C)** Proof of concept for the labeling system that show the GFP expression specifically in pericytes **(D)** Schematic of the second labeling system **(E)** Proof of concept for the second labeling system. Scale bar: 50µm.

Figure 6

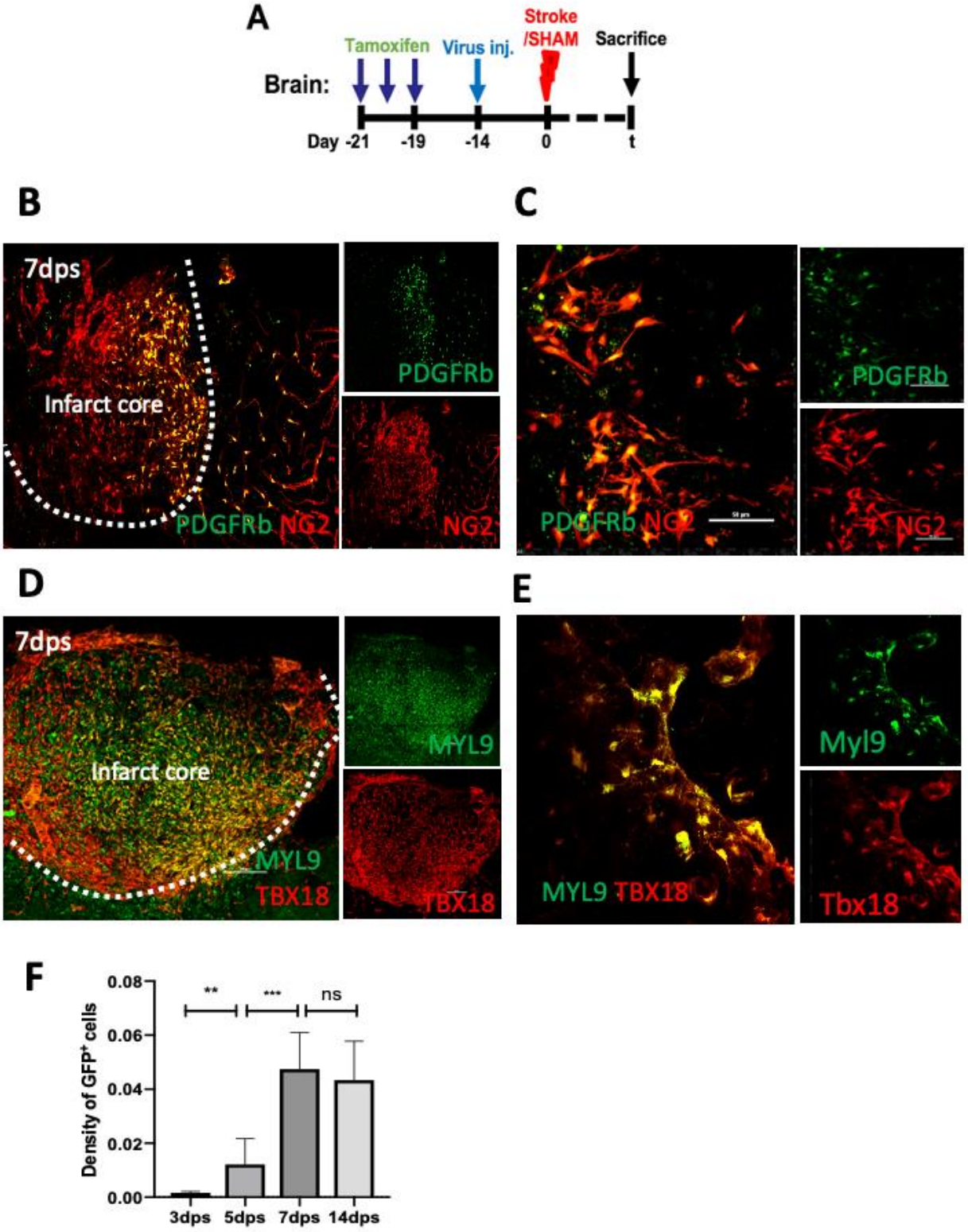

A
Stroke
/SHAM
Tamoxifen
Virus inj.
Sacrifice
Brain:
Day -21
-19
-14
0
t
B
7dps
Infarct core
PDGFRb NG2
PDGFRb
NG2
C
PDGFRb NG2
PDGFRb
NG2
D
7dps
Infarct core
MYL9
TBX18
MYL9
TBX18
E
MYL9 TBX18
Myl9
Tbx18
F
Density of GFP+ cells
0.08
0.06
0.04
0.02
0.00
**
***
ns
3dps
5dps
7dps
14dps

Figure 6: Post-stroke pericytes contributed to the fibrotic scar

(A) Experimental timeline **.** GFP+ cells were found inside the infarct area after the stroke in the first labeling system **(B,C)** and second labeling system **(D, E). (C)** and **(E)** are the higher magnification of **(C)** and **(E).** Scale bar: 100um for figure B &D. Scale bar : 50um for figure C & E.

Figure 7

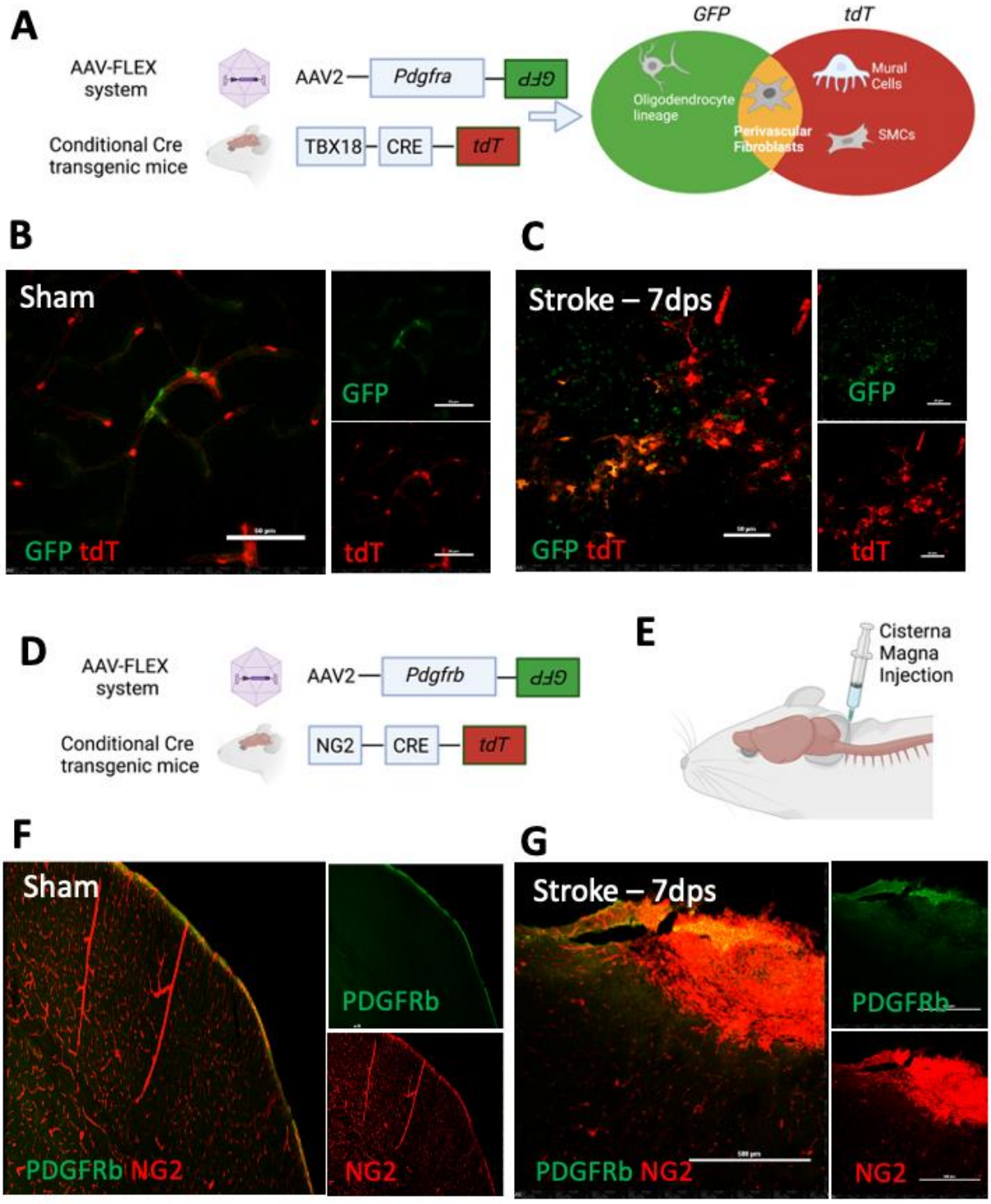

A
AAV-FLEX system
AAV2
Pdgfra
GFP
Conditional Cre transgenic mice
TBX18
CRE
tdT
GFP
tdT
Oligodendrocyte lineage
Mural Cells
Perivascular Fibroblasts
SMCs
B
Sham
GFP tdT
GFP
tdT
C
Stroke – 7dps
GFP tdT
GFP
tdT
D
AAV-FLEX system
AAV2
Pdgfrb
GFP
Conditional Cre transgenic mice
NG2
CRE
tdT
E
Cisterna Magna Injection
F
Sham
PDGFRb NG2
PDGFRb
NG2
G
Stroke – 7dps
PDGFRb NG2
PDGFRb
NG2

Figure 7: Perivascular fibroblasts, not meningeal fibroblasts, contributed to the fibrotic scar

(A) Schematic to label perivascular fibroblasts **(B,C)** Presence to GFP+ cells in the infarct core, suggesting the contribution of perivascular fibroblasts in the fibrotic scar after stroke. **(D)** Schematic for the meningeal fibroblasts labeling system. **(F, G)** There was no GFP+ cell inside the infarct core. Scale bar= 100um.

Supplementary Table 1 Immunofluorescence Staining Antibodies

Target Protein	Manufacturer (catalog no.)	Dilution
NG2	Millipore Sigma (AB5320)	1:50
CD146	Abcam (ab75769)	1:100
PDGFRβ	Abcam (ab32570)	1:100
PDGFRβ	eBioscience (14-1402-82)	1:50
VTN	ABClonal (A1667)	1:50

CHAPTER 3

Characterization of the entire heterogeneous pericyte cell population and their stroke-induced transcriptomic changes

INTRODUCTION

The rapid progression of next-generation sequencing (NGS) technologies in the last few years has provided many valuable insights and answered fundamental, outstanding questions in biology (Butler et al., 2018; Hwang et al., 2018; Islam et al., 2014). NGS-technologies, from genomics, transcriptomics and epigenomics, increasingly focused on and appreciated the heterogeneity of cell populations and the characterization of individual cells. These single cell analyses, especially single-cell RNA seq (scRNAseq), allow researchers to reexamine the previous scientific foundations that were laid forth by the traditional profiling of bulk populations to uncover new biological discoveries. Particularly in disease settings where cellular responses are rapidly changing through a pathological progression, delineating cell lineage relationships, subpopulations responses, or anomalous developmental progressions are crucial and highlight the potential application of scRNAseq

Stroke, the leading cause of adult disability and the second cause of death worldwide, remains one of the largest unmet medical needs for patients and clinicians(Benjamin et al., 2018). Due to the lack of therapeutic treatments, surviving patients face life-altering challenges and devastating disabilities that affect their motor, cognitive, and sensory functions (Kelly-Hayes et al., 2003). By understanding the biology of the brain after stroke, we hope to uncover pathways that could lay the foundations for therapeutic treatments to improve patient lives.

After stroke, the brain undergoes a rapid wave of tissue restructuring and reorganization through a plethora of proliferative, regenerative and scar-forming events that last weeks after injury (Carmichael, 2005, 2010, 2016). Pericytes, an elusive member of the regenerative neurovascular niche, are mural cells that wrap around vasculature and play crucial roles in angiogenesis, oligodendrocyte differentiation, BBB maintenance and fibrotic scar formation (Bell et al., 2010; Birbrair et al., 2014; De La Fuente et al., 2017; Dias et al., 2018; Göritz et al., 2011; Kisler et al., 2017; Sweeney et al., 2016). Studies of pericytes have been particularly challenging, and evidence for the role of pericytes in health and diseases have been conflicting, in part due to the inherent heterogeneity

of pericytes and the lack of precise molecular markers for labeling and animal models tools. With these characteristics, pericytes are a prime candidate for the application of scRNAseq technologies, especially in after ischemic injury.

Previous study has examined the transcriptome and subpopulations of perivascular and vascular populations in homeostasis (Vanlandewijck et al., 2018). Here, using scRNAseq, coupled with cutting-edge bioinformatics, we hope to capture the heterogeneity of pericytes that was suggested by morphological and spatial heterogeneity in health and after injury. We utilized the PDGFRb^{Cre} mouse strain for scRNAseq, to characterize not only the post-stroke pericyte population but also the perivascular fibroblast population. PDGFRb is a marker for both cell types (Vanlandewijck et al., 2018). Using cutting-edge bioinformatic analysis, we were able to confirm the diverse roles of pericyte after ischemic injury, including its participation in the neurogenesis and angiogenesis, along with its transdifferentiation into fibrotic scar. Via analysis of heart pericytes (in collaboration with the Ardehali Lab at UCLA), we also found that pericyte contribution to scar is conserved across multiple organs, including the heart. We identified several candidate gene systems that will be used for manipulated through gain and loss of function analyses to delineate pericyte functions after stroke in the next chapter.

RESULTS

Isolation of post-stroke PDGFRb^{+} cells for scRNAseq

To separate cell populations for scRNAseq, we isolated tdT+ cells from post-stroke and sham PDGFRb$^{CRE/+}$Rosa26$^{tdT/+}$ transgenic mice at 7 days after stroke via flow-activated cell sorting (FACS) **(Figure 1A).** We chose this FACS to enrich the pericyte population instead of subjecting the whole brain through the pipeline, because the pericyte component to most unselected scRNAseq is very small. Stroke tissue was separated into stroke core (based on tdTomato fluorescent signal) and stroke-periinfarct cortex **(Figure 1A)**. Cells were sorted on tdTomato positive, DAPI negative and Drag5 positive signals (**Figure B).** In order to assess the quality and reliability of the sorted cell

population **(Figure 1C),** we performed RT-qPCR on several cell populations including pericytes, endothelial cells, oligodendrocytes and microglia. The data confirmed our ability to FACS-isolated pericytes from the brain. Isolated cells were subjected to the microfluidic system of 10x Genomics for library preparation, then sequenced on Novaseq.

The pericyte population is distinctly separated from both activated fibroblasts in the stroke core and perivascular fibroblasts

The transcriptome underwent an extensive quality control process to filter out dead cells, mitochondrial contamination, and doublets **(Figure 2A)**. Variable gene analysis and clustering were carried out to reveal distinct clusters that were associated with vascular and perivascular cells **(Figure 2A-B).** We found that besides pericytes and fibroblast clusters, which made up 90% of the total sampled population, we had a moderate amount of endothelial cell contamination in our samples, along with the endothelial/pericyte doublet clusters (identified by expressing both markers of endothelial cells and pericytes) **(Figure 2A).** While we did not include these clusters in the subsequent analysis, we observed similar findings from a previous study of the subpopulation of the brain vasculature, including arterial (*Bmx, Efnb2,Vegfc)* and venous cells (*Nrf2f2)*(Vanlandewijck et al., 2018). Pericyte clusters were identified by a combination of pericyte markers including (*Pdgfrb, Vtn, Abcc9, Anpep, Des)*. Smooth muscle cells population markers were identified based on their *Acta2, Tagln* markers. Perivascular fibroblast markers that were used included *Pdgfra, Acta2, Cola1a2, Lama1*, as previously described (Vanlandewijck et al., 2018). We found that the sham and periinfarct samples held similar cellular distributions and patterns whereas the stroke core sample showed a distinct population of activated fibroblasts, which were characterized by *Col1a1, Fn1, Acta2, FSP1* clusters (**Figure 2E).** Further quality control analysis revealed that our clusters are well-represented by several samples and that they are not heavily biased by individual samples, which validated our clustering analysis **(Figure 2F).**

As stroke induces a highly proliferative state in most brain cells, especially at the 7-day post-injury

window, we used known cell cycle marker genes to compute cell cycle and predict cell cycle phase using a previously discussed method (Tirosh et al., 2016). We revealed that while the clusters are not biased by cell-cycle phase and most clusters were well-mixed for phases of the cell cycle **(Figure 2D)**. However, we found a heavy concentration of activated fibroblasts which were enriched in the G2M phase of cell cycle **(Figure 2D).**

Together, using scRNAseq analysis, we revealed distinct cellular distribution patterns of the stroke core tissue and stroke periinfarct tissues. We were able to distinguish between the stroke periinfarct pericyte population from the perivascular fibroblast populations, which laid the foundation for us to dive deeper into each of these clusters in subsequent analyses.

Periinfarct pericytes expresses angiogenetic and neurogenetic molecular signatures

Next, we performed differential gene expression analysis between sham and stroke samples using Monocle2 for each cluster to revealed stroke-induced changes of cellular expression profile (T. Wang et al., 2019). We identified 7792 differentially expressed genes, DEGs, **(Figure 3A).** We found that among the clusters that were analyzed, pericyte clusters have the highest number of DEGs, suggesting their dynamic stroke-induced change in the expression profile compared to other cell types **(Figure 3B).** Next, we performed the Gene Ontology (GO) enrichment analysis as previously reported (Alexa & Rahnenfuhrer, n.d.). In brief, for each cluster, we used the top 100 significant DEGs by average log fold change as the input into the EnrichR to query the GO Biological Process 2018 database **(Figure 3C)**. This analysis identified specific molecular pathways that were expressed by pericytes, fibroblasts and perivascular fibroblasts. Periinfarct pericyte clusters DEGs are distinct from that of perivascular fibroblasts and activated fibroblasts in the infarct core. Pericytes expressed a high level of angiogenic factors while activated fibroblasts expressed genes that regulate ECM and assembly **(Figure 3C).**

Next, we subjected the dataset through Weighted Correlation Network Analysis (WGCNA), which has been a tool for identifying the functional modules based on co-expression networks inferred from a large number of gene expression profiles. (Cha & Lee, 2020; Langfelder & Horvath, 2008). WGCNA

historically has been developed for bulk RNA sequencing datasets. Coupling WGCNA along with scRNAseq has proven to be a useful tool to unveil functional modules associated with disease states of cell types. Using our WCGNA analysis, we separated our dataset into four major groups **(Figure 3D).** Further analysis of the pericyte and fibroblast clusters identified distinct co-expression patterns **(Figure 3E)**. Periinfarct pericytes expressed high levels of angiogenesis pathways, along with cell proliferation while the fibroblast clusters showed high level of morphogenesis, migration, astrocyte and cell-to-cell interaction **(Figure 3F).**

Pseudo-time analysis confirmed pericyte-to-fibroblast-transition after injury

Next, we used pseudo-time analysis, TSCAN, to map out the pericyte-fibroblast transition that was identified in chapter 2. Pseudotime analysis or trajectory inference methods are analysis that align cell populations along a trajectory based on similarities in their single cell expression patterns (Saelens et al., 2019). First, we used TSCAN analysis on the pericyte and fibroblast clusters and reduced the dimension of the scRNAseq data set into clustering of cell states based on similar expression profile and revealed 12 distinct cell states **(Figure 4A).** Next, the algorithm constructed a minimum spanning tree to connect all the cluster centers using the shortest distance between each two clusters, as previously discussed (Ji & Ji, 2016). Here, our analysis identified distinct connection map between clusters 8, 1, 5, 4, 6 and 2 while clusters 3 and 7 were not connected to the main path as they diverged out from the branch **(Figure 4B & C).** Further analysis confirmed the transition between pericytes to activated fibroblasts via the reduction of a pericyte-specific marker (*Vtn)* and increased expression of a fibroblast marker *(Lum)* **(Figure 4D).** Distribution of samples in each of these cell states revealed that the pericyte cell fate exists predominantly in the sham sample; stroke periinfarct sample have most of the transitional states while the fibroblast cell fates are mostly from the stroke core sample **(Figure 4E).**

These observations further cemented the pericyte-fibroblast transition in the infarct core, in line with the tissue-level data we observed in chapter 2.

The pericyte contribution to the fibrotic process is conserved in the heart and brain

Previous studies had indicated that pericyte did not form fibrotic scar across multiple organs, which is at odds with our current findings about the contribution of pericyte in the fibrotic scar after stroke (Guimarães-Camboa et al., 2017). We asked whether the pericyte-fibrotic scar formation is conserved across multiple organs. With the collaboration with Dr. Reza Ardehali lab, we performed paralleled studies using similar animal models to previous studies.

Using the *Tbx18* $^{CRE/+}$ *Rosa26*$^{tdT/+}$ mouse model, we performed scRNAseq on tdTomato+ cells in the injury core and in the periinfarct region in both the brain and the heart. Clustering analysis revealed distinct clusters of the pericytes in both organs **(Figure 5A &B).** The isolated cells were then processed for single cell RNA sequencing (scRNA-seq), resulting in a transcriptomic dataset consisting of 37,001 cells from the heart and 15,353 cells from the brain **(Figure 5A)**. Other cell types in the heart (i.e., endothelial cells, cardiomyocytes, fibroblasts, smooth muscle cells, leukocytes) and the brain (i.e. endothelial cells, smooth muscle cells, microglia) were identified and excluded from our analysis. Pericytes were identified based on expression of known markers, such as *Rgs5, Mcam, Pdgfrb* and *Cspg4* for the heart and *Abcc9*, *Pdgfrb*, *Vtn*, *Cspg4* and *Anpep* for the brain **(Figure 5B).** The pericyte clusters for both systems were subjected to further downstream analysis **(Figure 5B).** Our scRNAseq analysis revealed a considerable number of common up-regulated genes and biological pathways related to fibrosis that were enriched in pericytes from the injured hearts and brains (compared to pericytes from uninjured organs), suggesting similar pro-fibrotic activity of pericytes in a common ischemic injury response. Gene Sets Enrichment Analysis (GSEA) identified a significant enrichment of key fibrosis pathways, including inflammation, immune response, and extracellular matrix (ECM) components **(Figure 5C)**

We next analyzed the gene expression profiles of cardiac and brain pericytes separately at a single cell resolution. Pericytes isolated from sham tissues of the heart and brain formed a distinct cluster from injured pericytes, suggesting a shift in their gene expression profile in response to injury **(Figure 5D).** We observed high expression of fibrosis-related genes in the pericytes located within the core

infarct regions and, to a lesser extent, in pericytes in the peri-infarct areas **(Figure 5D).** Conversely, genes associated with the pericyte-endothelium junction and vascular integrity were downregulated in infarct and peri-infarct pericytes compared to pericytes isolated from sham organs **(Figure 5D).** To validate these findings, we also performed unsupervised clustering to explore changes across the transcriptomes of pericytes from injured and healthy heart and brain tissue. This analysis confirmed that pathways associated with ECM assembly and collagen metabolism were downregulated in the sham setting and showed significant upregulation as cells transitioned to the infarct state.

We next performed immunohistochemistry to confirm the progression of pericytes to a fibrotic phenotype in MI and stroke and define their localization with respect to the ischemic regions. Stroke/MI and sham surgery were conducted on animals as described above. Immunohistochemistry for pericyte and fibrosis markers was performed on frozen sections and representative images of the scar and uninjured tissues were chosen **(Figure 5E)** We observed that in the absence of injury, pericytes maintain an intimal connection with the vascular bed and do not express detectable levels of fibrosis markers, such as periostin (POSTN) for the heart and COL1A1 for the brain **(Figure 5E).** However, in response to ischemic insult, we observed accumulation of pericytes in the infarct region in both organs many of them expressing POSTN and COL1A1 which was not seen in sham organs **(Figure 5E).**

DISCUSSION

The findings in this chapter have confirmed the heterogeneous response of pericyte after ischemic injury. By using the *Pdgfrb*$^{CRE/+}$ *Rosa26* $^{tdT/+}$, we captured the entire population of perivascular cells, along with scar-forming fibroblasts in the core. The 7-day post-stroke timepoint was chosen as this is the time of peak pericyte proliferation and regenerative activities in the periinfarct, as well as the peak of fibrotic scar formation in the infarct core. We aimed to capture the diverse response of pericyte during this time window through scRNAseq.

Firstly, in alignment with previous studies, we identified distinct clusters of pericyte and perivascular

fibroblasts in sham animals and in the periinfarct area where the tissue integrity and compositions are still intact compared to the infarct core (Vanlandewijck et al., 2018). Also in alignment with their studies and previously described morphological and spatial distinction of mural cell subgroups, the mural cell populations were clustered into arteriolar SMCs (*Acta2*^{high}, *Abcc9*^{negative}), and pre-capillary arteriolar SMC/pericyte hybrid population (*Acta2*^{positive}*, Abcc9*^{positive}*),* and capillary pericytes **(Figure 2B)** (Hartmann et al., 2015; Vanlandewijck et al., 2018). Interestingly, we did not observe the capillary pericyte subgroup. The perivascular fibroblasts were also distinctly clustered into two distinct clusters as in previous studies **(Figure 2B)** (Vanlandewijck et al., 2018).

Stroke induces a distinct phenotype for the pericytes in the infarcted population and the periinfarct population. Tools developed for differential gene expression analysis on bulk RNAseq can be applied to single cell data (Anders & Huber, 2010; Hardcastle & Kelly, 2010; Leng et al., 2013; T. Wang et al., 2019). However, scRNAseq data can have very different characteristics due to their small number and low capture efficiency of RNA molecules that lead to “drop-out” events (Kharchenko et al., 2014). This can lead to multimodality in expression values in different cells and lead to the heterogeneity of the cell-to-cell differential gene expression profile. Several methods have been developed to address these challenges. Here, we developed a novel WCGNA method that was designed specifically for single cell RNAseq (in collaboration with Dr. Vivek Swarup at University of California, Irvine). WCGNA, in combination with Gene Ontology Enrichment Analysis to further delineate the periinfarct pericyte expressed distinct molecular profile compared to that of sham **(Figure 2E, Figure 3)** (Y. Ma et al., 2020). Periinfarct pericytes expressed angiogenic and neurogenic molecular profiles, which suggested pericytes participate in the regenerative neurovascular niche **(Figure 3D-F).**

Interestingly, the WCGNA revealed the module pericyte-fibroblast transition that includes mesenchymal cell differentiation, morphogenesis, and migration **(Figure 3F).** In fact, this phenomenon is further cemented by the pseudotime analysis. Clear projection of periinfarct pericytes to the infarct core perivascular fibroblasts was seen **(Figure 4).** It is essential to note that TSCAN produced a linear inferable trajectory without any branches. How perivascular fibroblasts contribute

to the infarct core and fit into this trajectory would require additional analysis that use a more developed multifurcation such as PAGA or Monocle.

Lastly, using *Tbx18*$^{CRE/+}$ *RosaR26* $^{tdT/+}$ transgenic line (courtesy of Dr. Sylvia Evans, UCSD), we aim to replicate the previous study in clinically relevant models that produce robust fibrotic scar formation (Guimarães-Camboa et al., 2017). We observed the conserved role of pericytes in fibrotic scar formation in both the brain and the heart **(Figure 5).** The previous study used transaortic constriction in the heart and cortical stab wound in the brain, which have different pathological phenotypes from the models that were used in our studies. Transaortic constriction is a chronic form of cardiac injury that results in diffuse interstitial fibrosis across the heart. The stab wound injury does not create an ischemic penumbra, therefore lacking significant reorganization and regeneration upon injury. In our studies, we performed two clinically relevant pathological models of ischemic injury: MI, which leads to replacement fibrosis, and ischemic stroke, which results in discrete fibrotic scar enveloped by an astrocytic scar. Although different injury models could contribute to the observed differences, the clinically relevant models used in the current study highlight the important role of pericytes in cardiac and brain fibrosis. In summary, our data suggest that in the two most devastating ischemic injuries, the fibrotic response of pericytes and their contributions to ECM remodeling are highly conserved between the heart and brain.

Together, these bioinformatic analyses provide insights into the heterogenous response of pericytes after injury, further cement their contribution to the fibrotic scar and their regenerative role in the periinfarct cortex. These findings will lay the groundwork for manipulation study in chapter 4.

METHODS

Mice

Pdgfrb$^{Cre/+}$ mice were a gift from Dr. Volkhard Lindner (Maine Medical Center). *Tbx18*$^{CRE/+}$ *RosaR26* $^{tdT/+}$ mice were a gift from Dr. Sylvia Evans (UC San Diego). All procedures were carried out with the approval of the UCLA Animal Research Committee.

Photothrombotic Stroke

Cortical stroke at the motor cortex was induced in mice using the stereotaxic surgery. Mice were initially anesthetized by mixture of oxygen and 5% isoflurane, then intubated at 2% for the duration of the operation. Body temperature was monitored and maintained at 37 degree Celcius +/- 0.5 degree by homeothermic heating pads. Rose Bangel, a photosensitive chemical was injected intraperitoneal at concentration of 10mg/mL. After 10 minutes to allow for systemic circulation of Rose Bangel, a cold light source KL1500 LCD (Zeiss) was placed on top of the exposed skull above the forelimb motor cortex (coordinates ML 1.5mm, AP 0.0mm). After 15 minutes, the wound was closed and animals were returned to cages for recovery. The photothrombotic stroke can reliably generate stroke size of 1-2 mm in diameter, dependent on the diameter and power of the light source

Tissue dissociation and FACS Isolation

Mice were euthanized within 1 minutes using an a gas chamber of isoflurane. The brain were dissected and rinsed with 30mL of phosphate-buffered saline (PBS) with 10% Bovine Serum albumin (BSA) . The meningeal were carefully removed. Infarct core were dissected and separated from the periinfarct cortex. Using forceps, white matter was removed from the tissues. The tissues were processed using the Milteny Biotech Adult Brain dissociation kit (Catalog 130-107-677). In brief, tissues were enzymatically digested using the gentleMACS Octo Dissociator with Heater for 30 mins at 37 degree Celsius. Samples were centrifuged and washed with ice-cold PBS/0.1% BSA before filtering through a 70uM MACS SmartStrainer. Samples were collected and centrugiged again at 300xg, 10 minutes before DAPI and Drag5 (Thermofisher) were added immediately prior to FACS. Cells were analyzed or sorted using a BD FACSAria™ II cell sorter. All data was analyzed using FlowJo software.

scRNAseq

Sorted cells were resuspended and counted using a hemocytometer. Cells were prepared for scRNAseq using the 10x Genomics Chrominium controller and the Chromium Single Cell 3'GEM,

Library & Gel Bead Kit v3. Libraries were sequenced on a Novaseq Sequencer (Illuminia) with an average 150,000,000 reads sequenced per sample. Data was processed using 10x Cell Ranger pipeline and further analyzed using Seurat.

RNA Extraction and Reverse Transcription qPCR (RT-qPCR)

Sorted cells were sorted directly into the Trlzol LS Reagent (Thermo Fisher). RNA was extracted using the Trlzol™ LS Reagent and follow the manufacturer protocol. The extracted RNA was quantified using NanoDrop ™ according to the protocol. Conversion to complementary DNA (cDNA) was carried out using the iSript ™ cDNA Synthesis Kit (Bio-Rad) and following the manufacturer's instruction. RT-qPCR reactions were prepared with SYBR Green Master Mix (BioRad) and specific primers for target gene were used. The reactions were run on the CFX96 Touch ™ Real-Time PCR Detection System (Bio-Rad) and analysis was done with the double delta-CT method.

Single Cell RNA Analysis

Pseudo-time analysis (TSCAN) was performed according to previously published protocol (Ji & Ji, 2016). *Gene Sets Enrichment Analysis (GSEA) & Gene Ontology Analysis* was performed using previously published parameters (Y. Ma et al., 2020). WCGNA protocol was modified to befit scRNAseq analysis according to previous publication (Cha & Lee, 2020)

Statistics

All quantification data are presented as mean ± standard error of the mean (SEM) and significance was determined by using Student's t-test or Two-way ANOVA. A p-value < 0.05 was considered statistically significant and data were analyzed using GraphPad Prism 8. The heatmaps shown in our figure were created using the DoHeatmap function in Seurat, which visualizes the expression of selected genes after scaling the data. Based on these results, we found that there was a trend in the expression of fibrosis-related genes, suggesting a transition in the transcriptomes of pericytes between sham and injured hearts and brains. Many of these genes were associated with ECM assembly and the significance of this was further supported by our GSEA results (determination of significance is described below). However, as the DoHeatmap function itself does not indicate

significance, we have changed the manuscript to more accurately describe our findings.

To confirm the significance of the NES scores in our GSEA, we analyzed the false discovery rates (FDR) associated with our pathways of interest. A threshold of FDR <0.05 was set to determine significance in enrichment for each pathway. Below is a plot depicting the mean -log10(FDR) for each pathway across the pericyte clusters in both the heart and the brain. To avoid any confusion, we have changed the wording in our manuscript from “robust” to “significant” and have included the FDR threshold in the description of our GSEA.

Figure 1

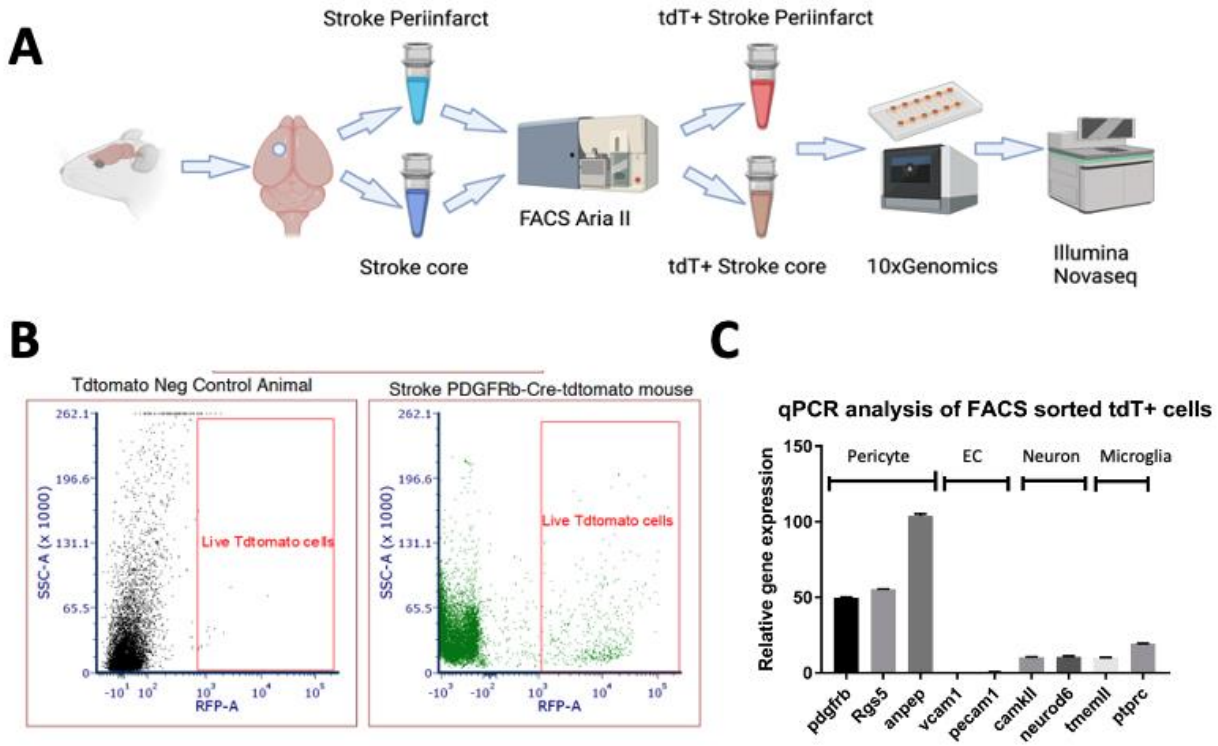
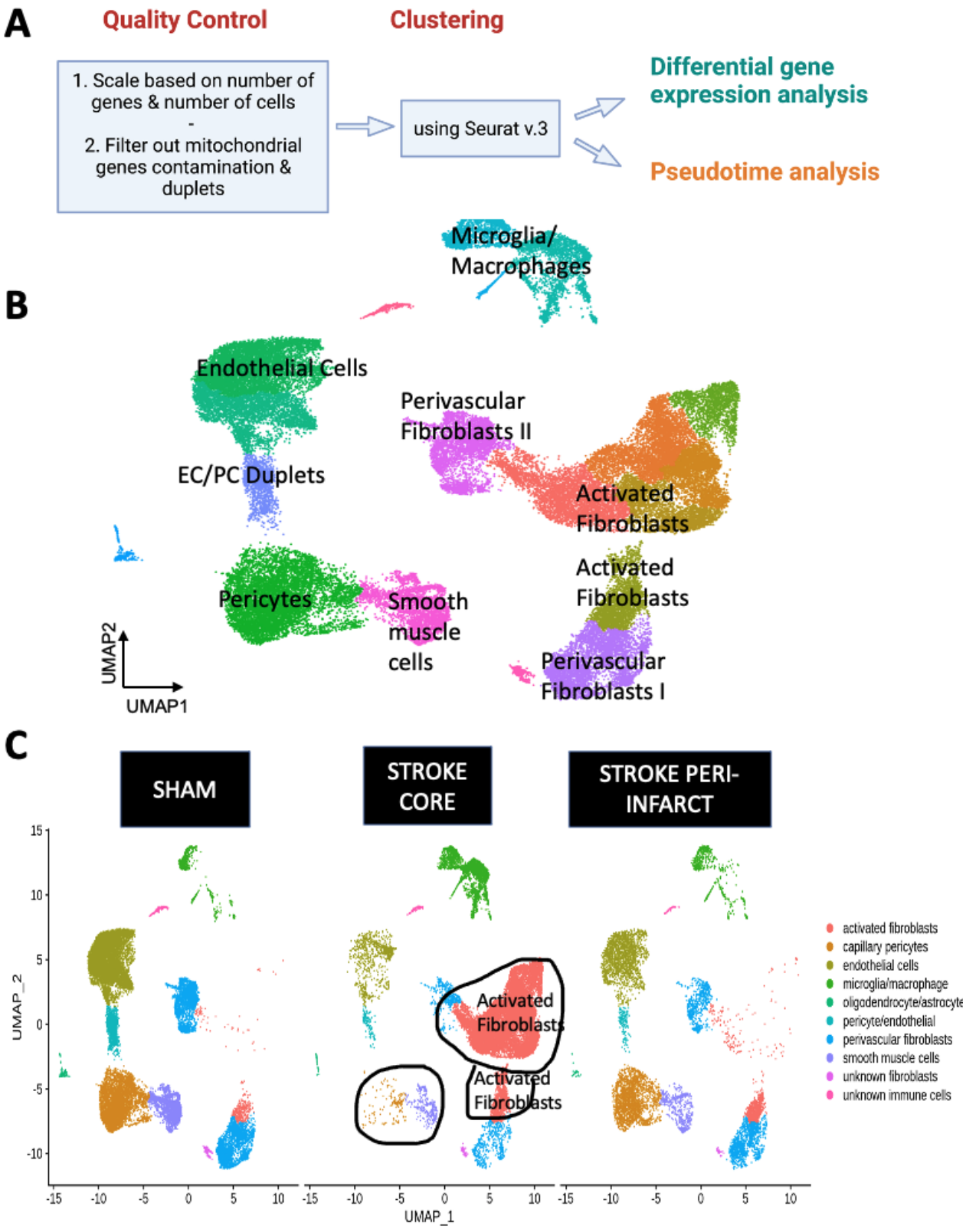

A
Stroke Periinfarct
tdT+ Stroke Periinfarct
FACS Aria II
Stroke core
tdT+ Stroke core
10xGenomics
Illumina
Novaseq
B
Tdtomato Neg Control Animal
Stroke PDGFRb-Cre-tdtomato mouse
SSC-A (x 1000)
RFP-A
Live Tdtomato cells
C
qPCR analysis of FACS sorted tdT+ cells
Relative gene expression
Pericyte
EC
Neuron
Microglia
pdgfrb
Rgs5
anpep
vcam1
pecam1
camkII
neurod6
tmemII
ptprc

Figure 1: scRNAseq pipeline

(A) Schematic of the single cell analysis pipeline **(B)** FACS sorted tdTomato cells **(C)** qPCR analysis confirms the high purity of sorted cells.

Figure 2


A
Quality Control
Clustering
1. Scale based on number of genes & number of cells
-
2. Filter out mitochondrial genes contamination & duplets
using Seurat v.3
Differential gene expression analysis
Pseudotime analysis
B
Microglia/ Macrophages
Endothelial Cells
Perivascular Fibroblasts II
EC/PC Duplets
Activated Fibroblasts
Activated Fibroblasts
Pericytes
Smooth muscle cells
Perivascular Fibroblasts I
UMAP2
UMAP1
C
SHAM
STROKE CORE
STROKE PERI-INFARCT
Activated Fibroblasts
Activated Fibroblasts
UMAP_2
UMAP_1
activated fibroblasts
capillary pericytes
endothelial cells
microglia/macrophage
oligodendrocyte/astrocyte
pericyte/endothelial
perivascular fibroblasts
smooth muscle cells
unknown fibroblasts
unknown immune cells

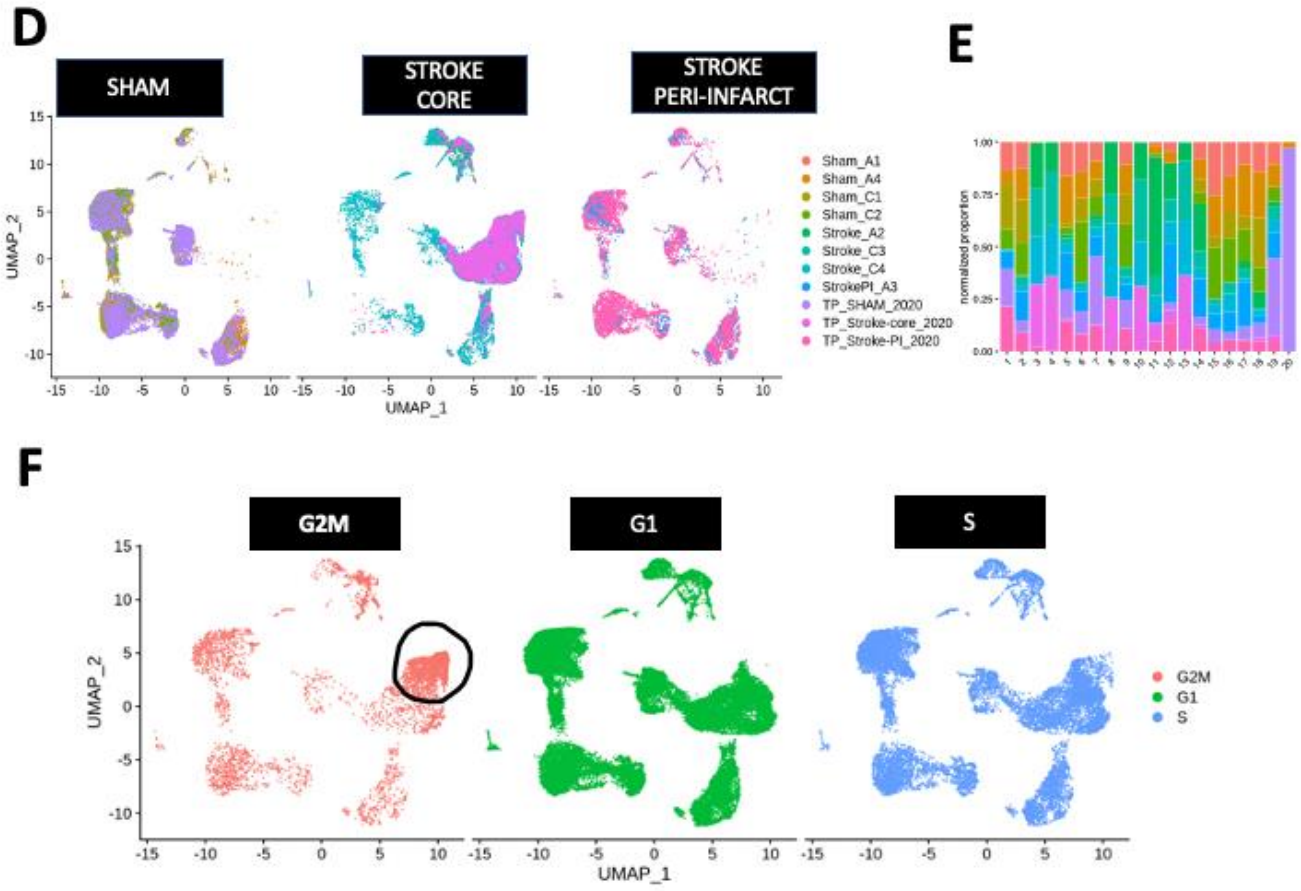
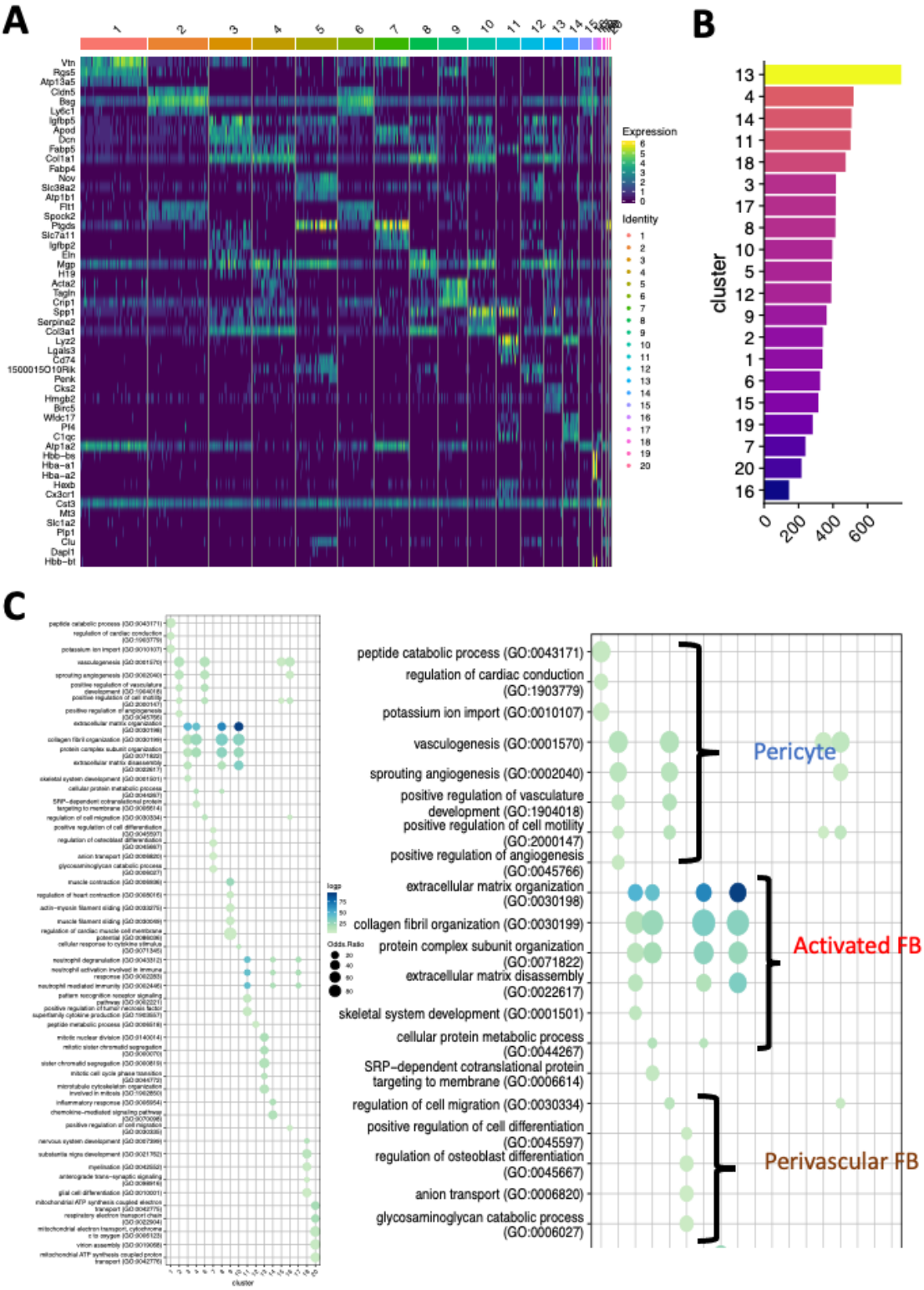

D
SHAM
STROKE CORE
STROKE PERI-INFARCT
UMAP_2
UMAP_1
Sham_A1
Sham_A4
Sham_C1
Sham_C2
Stroke_A2
Stroke_C3
Stroke_C4
StrokePI_A3
TP_SHAM_2020
TP_Stroke-core_2020
TP_Stroke-PI_2020
E
normalized proportion
F
G2M
G1
S
UMAP_2
UMAP_1
G2M
G1
S

Figure 2: Stroke core had distinct cellular types compared to sham and periinfarct.

(A) Schematic for bioinformatic analysis **(B)** UMAP of all cell types across sham, stroke core and stroke periinfarct sample **(C)** Cell clusters distribution across conditions revealed that sham and periinfarct samples have similar cell types components while stroke core samples contain of unique activated fibroblasts clusters **(D)** All clusters are well-distributed across sample conditions **(E)** Cell cycle analysis revealed that there is no bias of clustering due to cell cycle states. Stroke core activated fibroblast cluster (circle) have the most proliferating cells

Figure 3


A
B
C
cluster
Expression
Identity
Pericyte
Activated FB
Perivascular FB
peptide catabolic process (GO:0043171)
regulation of cardiac conduction (GO:1903779)
potassium ion import (GO:0010107)
vasculogenesis (GO:0001570)
sprouting angiogenesis (GO:0002040)
positive regulation of vasculature development (GO:1904018)
positive regulation of cell motility (GO:2000147)
positive regulation of angiogenesis (GO:0045766)
extracellular matrix organization (GO:0030198)
collagen fibril organization (GO:0030199)
protein complex subunit organization (GO:0071822)
extracellular matrix disassembly (GO:0022617)
skeletal system development (GO:0001501)
cellular protein metabolic process (GO:0044267)
SRP-dependent cotranslational protein targeting to membrane (GO:0006614)
regulation of cell migration (GO:0030334)
positive regulation of cell differentiation (GO:0045597)
regulation of osteoblast differentiation (GO:0045667)
anion transport (GO:0006820)
glycosaminoglycan catabolic process (GO:0006027)

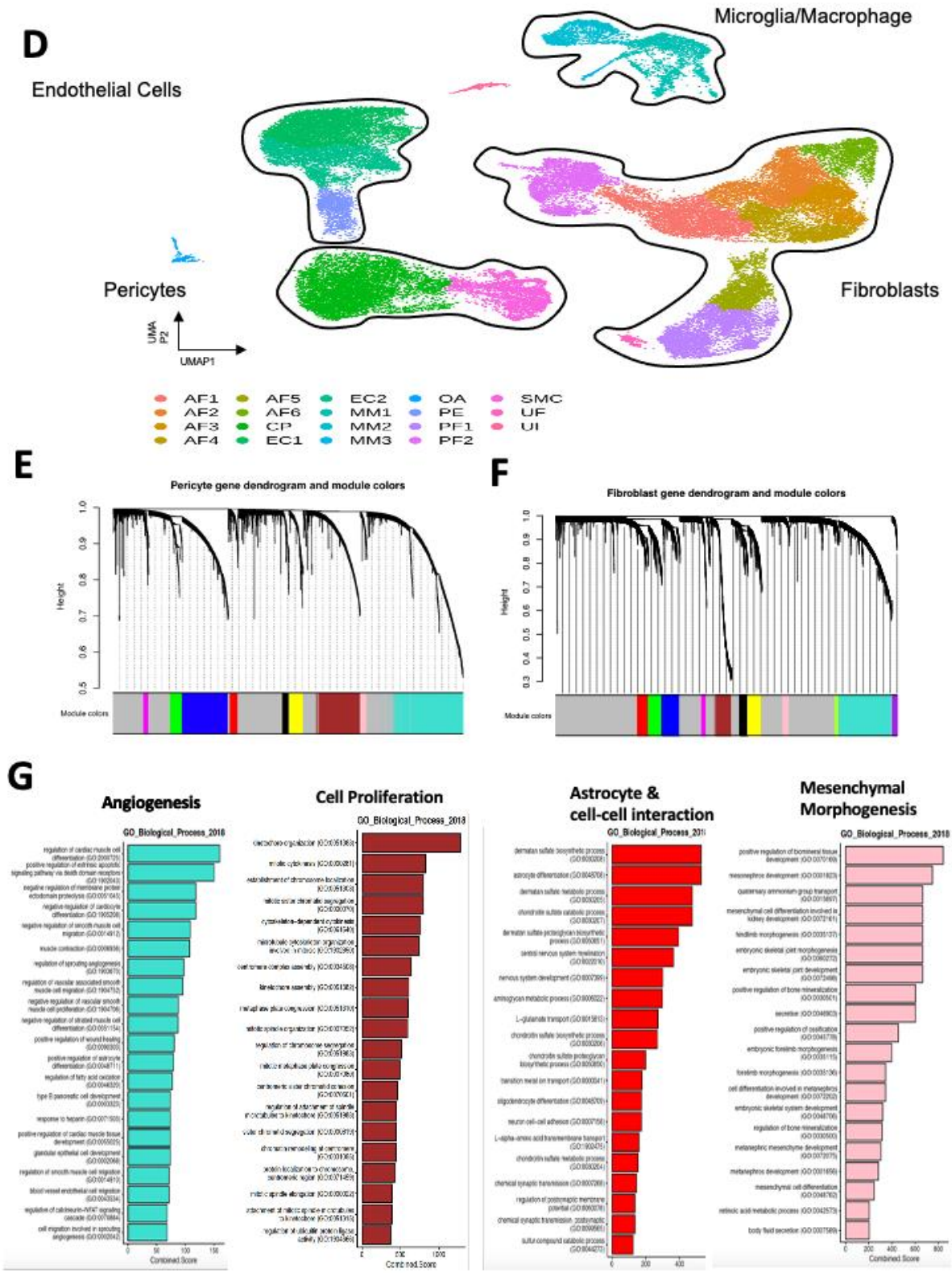
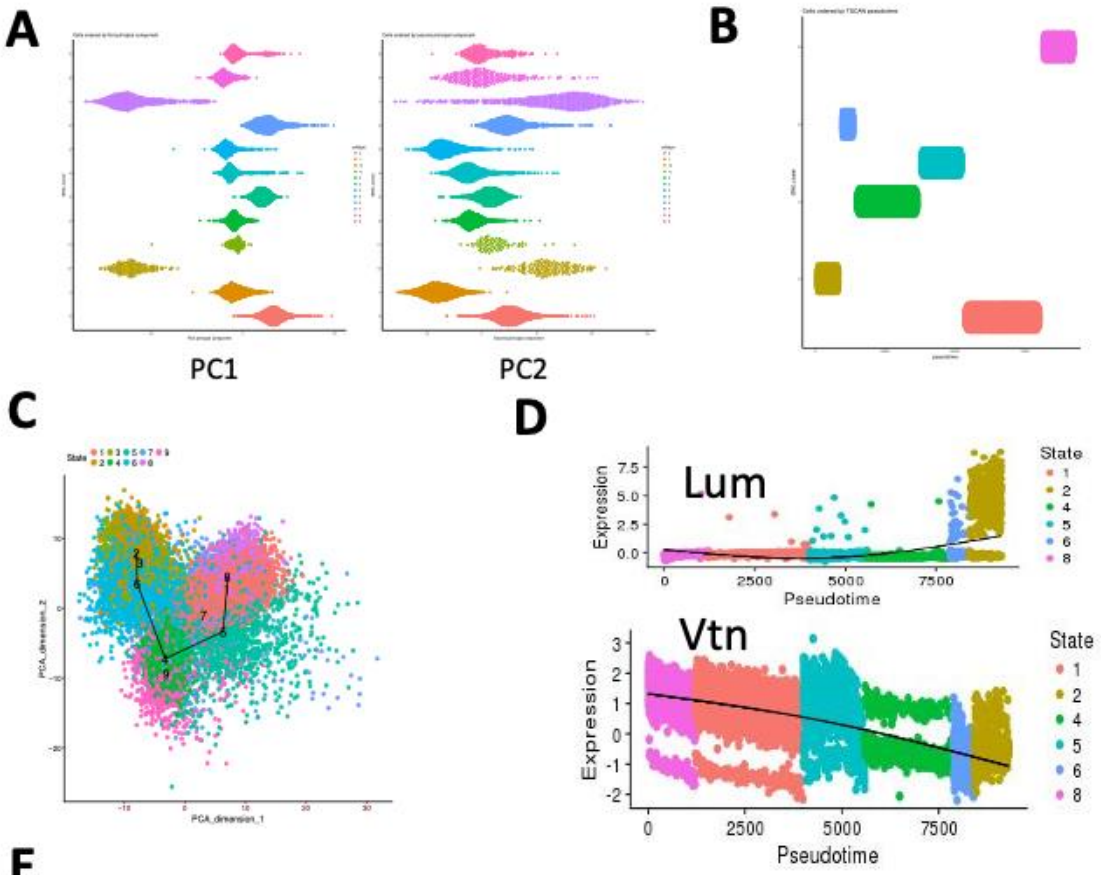

D
Microglia/Macrophage
Endothelial Cells
Pericytes
Fibroblasts
UMAP2
UMAP1
AF1
AF2
AF3
AF4
AF5
AF6
CP
EC1
EC2
MM1
MM2
MM3
OA
PE
PF1
PF2
SMC
UF
UI
E
Pericyte gene dendrogram and module colors
Height
Module colors
F
Fibroblast gene dendrogram and module colors
Height
Module colors
G
Angiogenesis
GO_Biological_Process_2018
Combined Score
Cell Proliferation
GO_Biological_Process_2018
Combined Score
Astrocyte &
cell-cell interaction
GO_Biological_Process_2018
Mesenchymal
Morphogenesis
GO_Biological_Process_2018
Combined Score

Figure 3: Heterogeneity of pericyte and fibroblast clusters

(**A)** Gene-set-enrichment-analysis (GSEA) revealed 7,734 differential gene expression across different clusters **(B)** Pericyte clusters (cluster 13) has the highest number of differential gene expression **(C)** Gene ontology analysis for single cell revealed distinct enrichment of molecular pathways for pericyte clusters ,activated fibroblasts and perivascular fibroblasts clusters **(D)** GWCNA analysis revealed 4 distinct clusters. Smaller clusters in the dataset (i.e., astrocytes, oligodendrocytes) were not included. **(E & F)** Dendogram showed distinct module between pericytes (E) and perivascular fibroblasts (F). **(G)** Close analysis of pericyte cluster reveal gene network associated with angiogenesis, cell proliferation, astrocyte interaction and mesenchymal morphogenesis.

Figure 4



E

Pseudo-time State

	8	1	5	4	6	2
Sham	85.16%	70.08%	27.22%	2.46%	4.18%	0.00%
StrokePI	14.84%	29.07%	69.09%	93.44%	53.53%	1.72%
Stroke core	0.00%	0.85%	3.70%	4.10%	42.29%	98.28%

Figure 4. Pseudotime analysis confirmed pericyte contribution to the fibrotic scar

TSCAN analysis use PCA to reduced dimensions of the dataset and revealed **(A)** 12 independent cell states . **(B&C)** Among these cell states, the algorithm created a “walk” through the most closely associated clusters and revealed 6 closely associated cell state, representing the transition between one clusters to the other. **(D)** Further analysis of the pseudotime revealed the increase expression of fibroblast marker (Lum) and reduction of pericyte marker (Vtn), which suggests the transition between pericyte to fibroblasts in the scRNAseq data. **(E)** We also confirmed that the sample distribution of pericytes is mostly in the sham and stroke periinfarct sample while stroke core is populated with the activated fibroblasts, which gave us the direction of the pseudotime

Figure 5

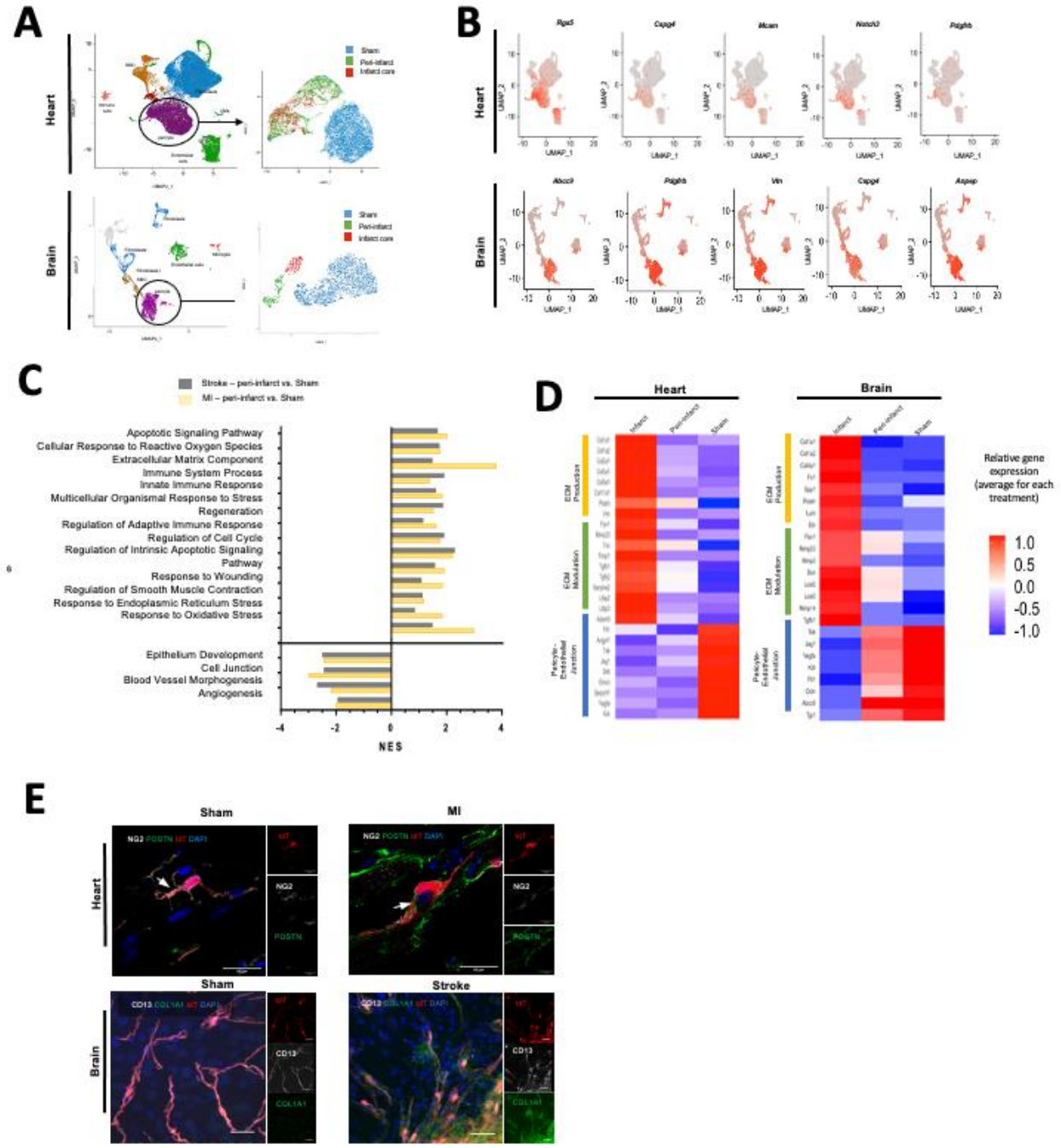

A
Heart
Brain
Sham
Peri-infarct
Infarct core
B
Heart
Brain
C
Stroke – peri-infarct vs. Sham
MI – peri-infarct vs. Sham
Apoptotic Signaling Pathway
Cellular Response to Reactive Oxygen Species
Extracellular Matrix Component
Immune System Process
Innate Immune Response
Multicellular Organismal Response to Stress
Regeneration
Regulation of Adaptive Immune Response
Regulation of Cell Cycle
Regulation of Intrinsic Apoptotic Signaling Pathway
Response to Wounding
Regulation of Smooth Muscle Contraction
Response to Endoplasmic Reticulum Stress
Response to Oxidative Stress
Epithelium Development
Cell Junction
Blood Vessel Morphogenesis
Angiogenesis
NES
D
Heart
Brain
Relative gene expression (average for each treatment)
1.0
0.5
0.0
-0.5
-1.0
E
Sham
MI
Heart
Sham
Stroke
Brain

Figure 5. Isolation and characterization of pericytes from brain and heart after ischemic injury.

A, Single cell RNA-seq analysis of lineage-traced TBX18-expressing cells from the injured and un-injured hearts and brains (n=3 biologically independent samples). UMAP analysis revealed presence of other cardiac and brain cell types (fibroblasts, endothelial cells, smooth muscle cells and immune cells). Feature plot of pericytes reveals a cluster of pericytes from the infarct and peri-infarct regions distinctly separated from uninjured pericytes (i.e. sham). SMC = Smooth Muscle Cells, CM = Cardiomyocytes. **B,** Feature plots of known pericyte markers in the heart and brain confirm the clustering and identification of pericytes. **C**, Gene Ontology categories reveals pathways that are similarly enriched in infarcted heart and brain pericytes when compared to uninjured pericytes. We observed parallel upregulation of pathways associated with ECM production, immune response and fibrosis. Conversely, pathways associated with blood vessel formation were downregulated, NES = Normalized Enrichment Score. **D,** Heatmap of genes associated with ECM production, ECM modulation, and pericyte-endothelium junction. While pro-fibrotic genes that regulate ECM remodeling were upregulated in cardiac pericytes isolated from the infarct core and to a lesser extent in the peri-infarct region, we observed a reduction in the expression of genes that regulate endothelial cell-pericyte interaction. Expression value on the color scale equates to log-2 fold change of gene expression. **E,** Confocal images show expression of POSTN (heart) and COL1A1(brain), markers associated with activated fibroblasts, in TBX18-expressing pericytes in the hearts and brains of animals that underwent sham, MI, or stroke. Images from MI and stroke were taken from the infarct core. Scale bar = 20μm. tdT = tdTomato.

REFERENCES

Ahmed, T. A., & El-Badri, N. (2018). Pericytes: The Role of Multipotent Stem Cells in Vascular Maintenance and Regenerative Medicine. *Advances in Experimental Medicine and Biology*, *1079*, 69–86. https://doi.org/10.1007/5584_2017_138

Alarcon-Martinez, L., Yilmaz-Ozcan, S., Yemisci, M., Schallek, J., Kılıç, K., Can, A., Di Polo, A., & Dalkara, T. (2018). Capillary pericytes express α-smooth muscle actin, which requires prevention of filamentous-actin depolymerization for detection. *ELife*, *7*, e34861. https://doi.org/10.7554/eLife.34861

Alex, L., & Frangogiannis, N. G. (2019). Pericytes in the infarcted heart. *Vascular Biology*, *1*(1), H23–H31. https://doi.org/10.1530/VB-19-0007

Alexa, A., & Rahnenfuhrer, J. (n.d.). *Gene set enrichment analysis with topGO*. 26.

Ali, S. R., Ranjbarvaziri, S., Talkhabi, M., Zhao, P., Subat, A., Hojjat, A., Kamran, P., Müller, A. M. S., Volz, K. S., Tang, Z., Red-Horse, K., & Ardehali, R. (2014). Developmental heterogeneity of cardiac fibroblasts does not predict pathological proliferation and activation. *Circulation Research*, *115*(7), 625–635. https://doi.org/10.1161/CIRCRESAHA.115.303794

Anders, S., & Huber, W. (2010). Differential expression analysis for sequence count data. *Nature Precedings*, 1–1. https://doi.org/10.1038/npre.2010.4282.1

Anderson, M. A., Burda, J. E., Ren, Y., Ao, Y., O'Shea, T. M., Kawaguchi, R., Coppola, G., Khakh, B. S., Deming, T. J., & Sofroniew, M. V. (2016). Astrocyte scar formation aids central nervous system axon regeneration. *Nature*, *532*(7598), 195–200. https://doi.org/10.1038/nature17623

Armulik, A., Abramsson, A., & Betsholtz, C. (2005). Endothelial/Pericyte Interactions. *Circulation Research*, *97*(6), 512–523. https://doi.org/10.1161/01.RES.0000182903.16652.d7

Armulik, A., Genové, G., & Betsholtz, C. (2011). Pericytes: Developmental, physiological, and pathological perspectives, problems, and promises. *Developmental Cell*, *21*(2), 193–215. https://doi.org/10.1016/j.devcel.2011.07.001

Attwell, D., Mishra, A., Hall, C. N., O'Farrell, F. M., & Dalkara, T. (2016). What is a pericyte? *Journal of Cerebral Blood Flow & Metabolism*, *36*(2), 451–455. https://doi.org/10.1177/0271678X15610340

Aumiller, V., Strobel, B., Romeike, M., Schuler, M., Stierstorfer, B. E., & Kreuz, S. (2017). Comparative analysis of lysyl oxidase (like) family members in pulmonary fibrosis. *Scientific Reports*, *7*(1), 149. https://doi.org/10.1038/s41598-017-00270-0

Barnabé-Heider, F., Göritz, C., Sabelström, H., Takebayashi, H., Pfrieger, F. W., Meletis, K., & Frisén, J. (2010). Origin of new glial cells in intact and injured adult spinal cord. *Cell Stem Cell*, *7*(4), 470–482. https://doi.org/10.1016/j.stem.2010.07.014

Barreto, G., White, R. E., Ouyang, Y., Xu, L., & Giffard, R. G. (2011). Astrocytes: Targets for Neuroprotection in Stroke. *Central Nervous System Agents in Medicinal Chemistry*, *11*(2), 164–173.

Becerra-Calixto, A., & Cardona-Gómez, G. P. (2017). The Role of Astrocytes in Neuroprotection after Brain Stroke: Potential in Cell Therapy. *Frontiers in Molecular Neuroscience*, *10*, 88. https://doi.org/10.3389/fnmol.2017.00088

Bell, R. D., Winkler, E. A., Sagare, A. P., Singh, I., LaRue, B., Deane, R., & Zlokovic, B. V. (2010). Pericytes Control Key Neurovascular Functions and Neuronal Phenotype in the Adult Brain and during Brain Aging. *Neuron*, *68*(3), 409–427. https://doi.org/10.1016/j.neuron.2010.09.043

Benjamin, E. J., Virani, S. S., Callaway, C. W., Chamberlain, A. M., Chang, A. R., Cheng, S., Chiuve, S. E., Cushman, M., Delling, F. N., Deo, R., de Ferranti, S. D., Ferguson, J. F., Fornage, M., Gillespie, C., Isasi, C. R., Jiménez, M. C., Jordan, L. C., Judd, S. E., Lackland, D., … Muntner, P. (2018). Heart Disease and Stroke Statistics—2018 Update: A Report From the American Heart Association. *Circulation*, *137*(12). https://doi.org/10.1161/CIR.0000000000000558

Benowitz, L. I., & Carmichael, S. T. (2010). Promoting axonal rewiring to improve outcome after stroke. *Neurobiology of Disease*, *37*(2), 259. https://doi.org/10.1016/j.nbd.2009.11.009

Bergers, G., & Song, S. (2005). The role of pericytes in blood-vessel formation and maintenance. *Neuro-Oncology*, *7*(4), 452–464. https://doi.org/10.1215/S1152851705000232

Birbrair, A., Zhang, T., Wang, Z.-M., Messi, M. L., Olson, J. D., Mintz, A., & Delbono, O. (2014). Type-2 pericytes participate in normal and tumoral angiogenesis. *American Journal of Physiology. Cell Physiology*, *307*(1), C25-38. https://doi.org/10.1152/ajpcell.00084.2014

Bish, L. T., Morine, K., Sleeper, M. M., Sanmiguel, J., Wu, D., Gao, G., Wilson, J. M., & Sweeney, H. L. (2008). Adeno-associated virus (AAV) serotype 9 provides global cardiac gene transfer superior to AAV1, AAV6, AAV7, and AAV8 in the mouse and rat. *Human Gene Therapy*, *19*(12), 1359–1368. https://doi.org/10.1089/hum.2008.123

Bodhankar, S., Lapato, A., Chen, Y., Vandenbark, A. A., Saugstad, J. A., & Offner, H. (2015). Role for microglia in sex differences after ischemic stroke: Importance of M2. *Metabolic Brain Disease*, *30*(6), 1515–1529. https://doi.org/10.1007/s11011-015-9714-9

Brumm, A. J., & Carmichael, S. T. (2012). Not just a rush of blood to the head. *Nature Medicine*, *18*(11), 1609–1610. https://doi.org/10.1038/nm.2990

Bundesen, L. Q., Scheel, T. A., Bregman, B. S., & Kromer, L. F. (2003). Ephrin-B2 and EphB2 Regulation of Astrocyte-Meningeal Fibroblast Interactions in Response to Spinal Cord Lesions in Adult Rats. *The Journal of Neuroscience*, *23*(21), 7789–7800. https://doi.org/10.1523/JNEUROSCI.23-21-07789.2003

Butler, A., Hoffman, P., Smibert, P., Papalexi, E., & Satija, R. (2018). Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nature Biotechnology*, *36*(5), 411–420. https://doi.org/10.1038/nbt.4096

Cafferty, W. B. J., Yang, S.-H., Duffy, P. J., Li, S., & Strittmatter, S. M. (2007). Functional Axonal Regeneration through Astrocytic Scar Genetically Modified to Digest Chondroitin Sulfate Proteoglycans. *Journal of Neuroscience*, *27*(9), 2176–2185. https://doi.org/10.1523/JNEUROSCI.5176-06.2007

Campbell, J. B., & Windle, W. F. (1960). Relation of millipore to healing and regeneration in transected spinal cords of monkeys. *Neurology*, *10*, 306–311. https://doi.org/10.1212/wnl.10.3.306

Carmichael, S. T. (2005). Rodent models of focal stroke: Size, mechanism, and purpose. *NeuroRX*,

2(3), 396–409. https://doi.org/10.1602/neurorx.2.3.396

Carmichael, S. T. (2010). Translating the frontiers of brain repair to treatments: Starting not to break the rules. *Neurobiology of Disease*, *37*(2), 237–242. https://doi.org/10.1016/j.nbd.2009.09.005

Carmichael, S. T. (2016). The 3 Rs of Stroke Biology: Radial, Relayed, and Regenerative. *Neurotherapeutics*, *13*(2), 348–359. https://doi.org/10.1007/s13311-015-0408-0

Carrica, L., Li, L., Newville, J., Kenton, J., Gustus, K., Brigman, J., & Cunningham, L. A. (2019). Genetic inactivation of hypoxia inducible factor 1-alpha (HIF-1α) in adult hippocampal progenitors impairs neurogenesis and pattern discrimination learning. *Neurobiology of Learning and Memory*, *157*, 79–85. https://doi.org/10.1016/j.nlm.2018.12.002

Cha, J., & Lee, I. (2020). Single-cell network biology for resolving cellular heterogeneity in human diseases. *Experimental & Molecular Medicine*, *52*(11), 1798–1808. https://doi.org/10.1038/s12276-020-00528-0

Chang, J., Lucas, M. C., Leonte, L. E., Garcia-Montolio, M., Singh, L. B., Findlay, A. D., Deodhar, M., Foot, J. S., Jarolimek, W., Timpson, P., Erler, J. T., & Cox, T. R. (2017). Pre-clinical evaluation of small molecule LOXL2 inhibitors in breast cancer. *Oncotarget*, *8*(16), 26066–26078. https://doi.org/10.18632/oncotarget.15257

Chen, Y.-T., Chang, F.-C., Wu, C.-F., Chou, Y.-H., Hsu, H.-L., Chiang, W.-C., Shen, J., Chen, Y.-M., Wu, K.-D., Tsai, T.-J., Duffield, J. S., & Lin, S.-L. (2011). Platelet-derived growth factor receptor signaling activates pericyte-myofibroblast transition in obstructive and post-ischemic kidney fibrosis. *Kidney International*, *80*(11), 1170–1181. https://doi.org/10.1038/ki.2011.208

Chia, N. H., Leyden, J. M., Newbury, J., Jannes, J., & Kleinig, T. J. (2016). Determining the Number of Ischemic Strokes Potentially Eligible for Endovascular Thrombectomy: A Population-Based Study. *Stroke*, *47*(5), 1377–1380. https://doi.org/10.1161/STROKEAHA.116.013165

Choi, Y. K., Maki, T., Mandeville, E. T., Koh, S.-H., Hayakawa, K., Arai, K., Kim, Y.-M., Whalen, M. J., Xing, C., Wang, X., Kim, K.-W., & Lo, E. H. (2016). Dual effects of carbon monoxide on pericytes and neurogenesis in traumatic brain injury. *Nature Medicine*, *22*(11), 1335–1341.

https://doi.org/10.1038/nm.4188

de Jong, S., van Veen, T. A. B., de Bakker, J. M. T., & van Rijen, H. V. M. (2012). Monitoring cardiac fibrosis: A technical challenge. *Netherlands Heart Journal*, *20*(1), 44–48. https://doi.org/10.1007/s12471-011-0226-x

De La Fuente, A. G., Lange, S., Silva, M. E., Gonzalez, G. A., Tempfer, H., van Wijngaarden, P., Zhao, C., Di Canio, L., Trost, A., Bieler, L., Zaunmair, P., Rotheneichner, P., O'Sullivan, A., Couillard-Despres, S., Errea, O., Mäe, M. A., Andrae, J., He, L., Keller, A., … Rivera, F. J. (2017). Pericytes Stimulate Oligodendrocyte Progenitor Cell Differentiation during CNS Remyelination. *Cell Reports*, *20*(8), 1755–1764. https://doi.org/10.1016/j.celrep.2017.08.007

de Winter, F., Kwok, J. C. F., Fawcett, J. W., Vo, T. T., Carulli, D., & Verhaagen, J. (2016). The Chemorepulsive Protein Semaphorin 3A and Perineuronal Net-Mediated Plasticity. *Neural Plasticity*, *2016*, 3679545. https://doi.org/10.1155/2016/3679545

DeMers, G., Meurer, W. J., Shih, R., Rosenbaum, S., & Vilke, G. M. (2012). Tissue plasminogen activator and stroke: Review of the literature for the clinician. *The Journal of Emergency Medicine*, *43*(6), 1149–1154. https://doi.org/10.1016/j.jemermed.2012.05.005

Dias, D. O., Kalkitsas, J., Kelahmetoglu, Y., Estrada, C. P., Tatarishvili, J., Ernst, A., Huttner, H. B., Kokaia, Z., Lindvall, O., Brundin, L., Frisén, J., & Göritz, C. (2020a). *Pericyte-derived fibrotic scarring is conserved across diverse central nervous system lesions* [Preprint]. Neuroscience. https://doi.org/10.1101/2020.04.30.068965

Dias, D. O., Kalkitsas, J., Kelahmetoglu, Y., Estrada, C. P., Tatarishvili, J., Ernst, A., Huttner, H. B., Kokaia, Z., Lindvall, O., Brundin, L., Frisén, J., & Göritz, C. (2020b). Pericyte-derived fibrotic scarring is conserved across diverse central nervous system lesions. *BioRxiv*, 2020.04.30.068965. https://doi.org/10.1101/2020.04.30.068965

Dias, D. O., Kim, H., Holl, D., Werne Solnestam, B., Lundeberg, J., Carlén, M., Göritz, C., & Frisén, J. (2018). Reducing Pericyte-Derived Scarring Promotes Recovery after Spinal Cord Injury. *Cell*, *173*(1), 153-165.e22. https://doi.org/10.1016/j.cell.2018.02.004

Dorrier, C. E., Aran, D., Haenelt, E. A., Sheehy, R. N., Hoi, K. K., Pintarić, L., Chen, Y., Lizama, C. O., Cautivo, K. M., Weiner, G. A., Popko, B., Fancy, S. P. J., Arnold, T. D., & Daneman, R. (2021). CNS fibroblasts form a fibrotic scar in response to immune cell infiltration. *Nature Neuroscience*, *24*(2), 234–244. https://doi.org/10.1038/s41593-020-00770-9

Edelman, D. A., Jiang, Y., Tyburski, J. G., Wilson, R. F., & Steffes, C. P. (2007). Cytokine production in lipopolysaccharide-exposed rat lung pericytes. *The Journal of Trauma*, *62*(1), 89–93. https://doi.org/10.1097/TA.0b013e31802dd712

Ellison, J. A., Velier, J. J., Spera, P., Jonak, Z. L., Wang, X., Barone, F. C., & Feuerstein, G. Z. (1998). Osteopontin and its integrin receptor alpha(v)beta3 are upregulated during formation of the glial scar after focal stroke. *Stroke*, *29*(8), 1698–1706; discussion 1707. https://doi.org/10.1161/01.str.29.8.1698

Ergul, A., Alhusban, A., & Fagan, S. C. (2012). Angiogenesis: A Harmonized Target for Recovery after Stroke. *Stroke; a Journal of Cerebral Circulation*, *43*(8), 2270–2274. https://doi.org/10.1161/STROKEAHA.111.642710

Ernst, A.-S., Böhler, L.-I., Hagenston, A. M., Hoffmann, A., Heiland, S., Sticht, C., Bendszus, M., Hecker, M., Bading, H., Marti, H. H., Korff, T., & Kunze, R. (2019). EphB2-dependent signaling promotes neuronal excitotoxicity and inflammation in the acute phase of ischemic stroke. *Acta Neuropathologica Communications*, *7*(1), 15. https://doi.org/10.1186/s40478-019-0669-7

Fei, Z., Zhang, X., Bai, H., Jiang, X., Li, X., Zhang, W., & Hu, W. (2007). Posttraumatic secondary brain insults exacerbates neuronal injury by altering Metabotropic Glutamate Receptors. *BMC Neuroscience*, *8*(1), 96. https://doi.org/10.1186/1471-2202-8-96

Fernandez, E., & Pallini, R. (1985). Connective tissue scarring in experimental spinal cord lesions: Significance of dural continuity and role of epidural tissues. *Acta Neurochirurgica*, *76*(3), 145–148. https://doi.org/10.1007/BF01418478

Fernández-Klett, F., Potas, J. R., Hilpert, D., Blazej, K., Radke, J., Huck, J., Engel, O., Stenzel, W., Genové, G., & Priller, J. (2013). Early loss of pericytes and perivascular stromal cell-induced

scar formation after stroke. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *33*(3), 428–439. https://doi.org/10.1038/jcbfm.2012.187

Fernández-Klett, F., & Priller, J. (2014). The fibrotic scar in neurological disorders. *Brain Pathology (Zurich, Switzerland)*, *24*(4), 404–413. https://doi.org/10.1111/bpa.12162

Göritz, C., Dias, D. O., Tomilin, N., Barbacid, M., Shupliakov, O., & Frisén, J. (2011). A Pericyte Origin of Spinal Cord Scar Tissue. *Science*, *333*(6039), 238–242. https://doi.org/10.1126/science.1203165

Guimarães-Camboa, N., Cattaneo, P., Sun, Y., Moore-Morris, T., Gu, Y., Dalton, N. D., Rockenstein, E., Masliah, E., Peterson, K. L., Stallcup, W. B., Chen, J., & Evans, S. M. (2017). Pericytes of Multiple Organs Do Not Behave as Mesenchymal Stem Cells In Vivo. *Cell Stem Cell*, *20*(3), 345-359.e5. https://doi.org/10.1016/j.stem.2016.12.006

Guo, J., Bertalan, G., Meierhofer, D., Klein, C., Schreyer, S., Steiner, B., Wang, S., Vieira da Silva, R., Infante-Duarte, C., Koch, S., Boehm-Sturm, P., Braun, J., & Sack, I. (2019). Brain maturation is associated with increasing tissue stiffness and decreasing tissue fluidity. *Acta Biomaterialia*, *99*, 433–442. https://doi.org/10.1016/j.actbio.2019.08.036

Hall, C. N., Reynell, C., Gesslein, B., Hamilton, N. B., Mishra, A., Sutherland, B. A., O'Farrell, F. M., Buchan, A. M., Lauritzen, M., & Attwell, D. (2014). Capillary pericytes regulate cerebral blood flow in health and disease. *Nature*, *508*(7494), 55–60. https://doi.org/10.1038/nature13165

Hao, J., Li, B., Duan, H.-Q., Zhao, C.-X., Zhang, Y., Sun, C., Pan, B., Liu, C., Kong, X.-H., Yao, X., & Feng, S.-Q. (2017). Mechanisms underlying the promotion of functional recovery by deferoxamine after spinal cord injury in rats. *Neural Regeneration Research*, *12*(6), 959–968. https://doi.org/10.4103/1673-5374.208591

Hara, M., Kobayakawa, K., Ohkawa, Y., Kumamaru, H., Yokota, K., Saito, T., Kijima, K., Yoshizaki, S., Harimaya, K., Nakashima, Y., & Okada, S. (2017). Interaction of reactive astrocytes with type I collagen induces astrocytic scar formation through the integrin–N-cadherin pathway after spinal

cord injury. *Nature Medicine*, *23*(7), 818–828. https://doi.org/10.1038/nm.4354

Hardcastle, T. J., & Kelly, K. A. (2010). baySeq: Empirical Bayesian methods for identifying differential expression in sequence count data. *BMC Bioinformatics*, *11*(1), 422. https://doi.org/10.1186/1471-2105-11-422

Hartmann, D. A., Underly, R. G., Grant, R. I., Watson, A. N., Lindner, V., & Shih, A. Y. (2015). Pericyte structure and distribution in the cerebral cortex revealed by high-resolution imaging of transgenic mice. *Neurophotonics*, *2*(4), 041402. https://doi.org/10.1117/1.NPh.2.4.041402

Hatakeyama. (n.d.). *Angiogenesis and neuronal remodeling after ischemic stroke*. Retrieved August 4, 2021, from https://www.nrronline.org/article.asp?issn=1673-5374;year=2020;volume=15;issue=1;spage=16;epage=19;aulast=Hatakeyama

Hellal, F., Hurtado, A., Ruschel, J., Flynn, K. C., Laskowski, C. J., Umlauf, M., Kapitein, L. C., Strikis, D., Lemmon, V., Bixby, J., Hoogenraad, C. C., & Bradke, F. (2011). Microtubule stabilization reduces scarring and causes axon regeneration after spinal cord injury. *Science (New York, N.Y.)*, *331*(6019), 928–931. https://doi.org/10.1126/science.1201148

Hesp, Z. C., Yoseph, R. Y., Suzuki, R., Jukkola, P., Wilson, C., Nishiyama, A., & McTigue, D. M. (2018). Proliferating NG2-Cell-Dependent Angiogenesis and Scar Formation Alter Axon Growth and Functional Recovery After Spinal Cord Injury in Mice. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *38*(6), 1366–1382. https://doi.org/10.1523/JNEUROSCI.3953-16.2017

Hill, R. A., Tong, L., Yuan, P., Murikinati, S., Gupta, S., & Grutzendler, J. (2015). Regional Blood Flow in the Normal and Ischemic Brain Is Controlled by Arteriolar Smooth Muscle Cell Contractility and Not by Capillary Pericytes. *Neuron*, *87*(1), 95–110. https://doi.org/10.1016/j.neuron.2015.06.001

Hori, S., Ohtsuki, S., Hosoya, K., Nakashima, E., & Terasaki, T. (2004). A pericyte-derived angiopoietin-1 multimeric complex induces occludin gene expression in brain capillary endothelial cells through Tie-2 activation in vitro. *Journal of Neurochemistry*, *89*(2), 503–513.

https://doi.org/10.1111/j.1471-4159.2004.02343.x

How, J., Liu, Y., Lombardi Story, J., Neuberg, D. S., Ravid, K., Jarolimek, W., Charlton, B., & Hobbs, G. S. (2020). Evaluation of a Pan-Lysyl Oxidase Inhibitor, Pxs-5505, in Myelofibrosis: A Phase I, Randomized, Placebo Controlled Double Blind Study in Healthy Adults. *Blood*, *136*(Supplement 1), 16–16. https://doi.org/10.1182/blood-2020-139223

Hu, X., Li, P., Guo, Y., Wang, H., Leak, R. K., Chen, S., Gao, Y., & Chen, J. (2012). Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke*, *43*(11), 3063–3070. https://doi.org/10.1161/STROKEAHA.112.659656

Hwang, B., Lee, J. H., & Bang, D. (2018). Single-cell RNA sequencing technologies and bioinformatics pipelines. *Experimental & Molecular Medicine*, *50*(8), 1–14. https://doi.org/10.1038/s12276-018-0071-8

Iihara, K., Sasahara, M., Hashimoto, N., & Hazama, F. (1996). Induction of platelet-derived growth factor beta-receptor in focal ischemia of rat brain. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *16*(5), 941–949. https://doi.org/10.1097/00004647-199609000-00018

Islam, S., Zeisel, A., Joost, S., La Manno, G., Zajac, P., Kasper, M., Lönnerberg, P., & Linnarsson, S. (2014). Quantitative single-cell RNA-seq with unique molecular identifiers. *Nature Methods*, *11*(2), 163–166. https://doi.org/10.1038/nmeth.2772

Jansson, D., Rustenhoven, J., Feng, S., Hurley, D., Oldfield, R. L., Bergin, P. S., Mee, E. W., Faull, R. L., & Dragunow, M. (2014a). A role for human brain pericytes in neuroinflammation. *Journal of Neuroinflammation*, *11*(1), 104. https://doi.org/10.1186/1742-2094-11-104

Jansson, D., Rustenhoven, J., Feng, S., Hurley, D., Oldfield, R. L., Bergin, P. S., Mee, E. W., Faull, R. L., & Dragunow, M. (2014b). A role for human brain pericytes in neuroinflammation. *Journal of Neuroinflammation*, *11*(1), 104. https://doi.org/10.1186/1742-2094-11-104

Ji, Z., & Ji, H. (2016). TSCAN: Pseudo-time reconstruction and evaluation in single-cell RNA-seq

analysis. *Nucleic Acids Research*, *44*(13), e117. https://doi.org/10.1093/nar/gkw430

Jiang, W., Gu, W., Brännström, T., Rosqvist, R., & Wester, P. (2001). Cortical neurogenesis in adult rats after transient middle cerebral artery occlusion. *Stroke*, *32*(5), 1201–1207.

Jones, M. G., Andriotis, O. G., Roberts, J. J., Lunn, K., Tear, V. J., Cao, L., Ask, K., Smart, D. E., Bonfanti, A., Johnson, P., Alzetani, A., Conforti, F., Doherty, R., Lai, C. Y., Johnson, B., Bourdakos, K. N., Fletcher, S. V., Marshall, B. G., Jogai, S., … Davies, D. E. (2018). Nanoscale dysregulation of collagen structure-function disrupts mechano-homeostasis and mediates pulmonary fibrosis. *ELife*, *7*, e36354. https://doi.org/10.7554/eLife.36354

Jung, B., Arnold, T. D., Raschperger, E., Gaengel, K., & Betsholtz, C. (2018). Visualization of vascular mural cells in developing brain using genetically labeled transgenic reporter mice. *Journal of Cerebral Blood Flow & Metabolism*, *38*(3), 456–468. https://doi.org/10.1177/0271678X17697720

Kelly-Hayes, M., Beiser, A., Kase, C. S., Scaramucci, A., D'Agostino, R. B., & Wolf, P. A. (2003). The influence of gender and age on disability following ischemic stroke: The Framingham study. *Journal of Stroke and Cerebrovascular Diseases: The Official Journal of National Stroke Association*, *12*(3), 119–126. https://doi.org/10.1016/S1052-3057(03)00042-9

Keough, M. B., Rogers, J. A., Zhang, P., Jensen, S. K., Stephenson, E. L., Chen, T., Hurlbert, M. G., Lau, L. W., Rawji, K. S., Plemel, J. R., Koch, M., Ling, C.-C., & Yong, V. W. (2016). An inhibitor of chondroitin sulfate proteoglycan synthesis promotes central nervous system remyelination. *Nature Communications*, *7*(1), 11312. https://doi.org/10.1038/ncomms11312

Kharchenko, P. V., Silberstein, L., & Scadden, D. T. (2014). Bayesian approach to single-cell differential expression analysis. *Nature Methods*, *11*(7), 740–742. https://doi.org/10.1038/nmeth.2967

Kisler, K., Nelson, A. R., Rege, S. V., Ramanathan, A., Wang, Y., Ahuja, A., Lazic, D., Tsai, P. S., Zhao, Z., Zhou, Y., Boas, D. A., Sakadžić, S., & Zlokovic, B. V. (2017). Pericyte degeneration leads to neurovascular uncoupling and limits oxygen supply to brain. *Nature Neuroscience*, *20*(3), 406–416. https://doi.org/10.1038/nn.4489

Kramann, R., & Humphreys, B. D. (2014). Kidney Pericytes: Roles in Regeneration and Fibrosis. *Seminars in Nephrology*, *34*(4), 374–383. https://doi.org/10.1016/j.semnephrol.2014.06.004

Lang, B. T., Cregg, J. M., DePaul, M. A., Tran, A. P., Xu, K., Dyck, S. M., Madalena, K. M., Brown, B. P., Weng, Y.-L., Li, S., Karimi-Abdolrezaee, S., Busch, S. A., Shen, Y., & Silver, J. (2015). Modulation of the proteoglycan receptor PTPσ promotes recovery after spinal cord injury. *Nature*, *518*(7539), 404–408. https://doi.org/10.1038/nature13974

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*(1), 559. https://doi.org/10.1186/1471-2105-9-559

Lei, X., Guan, C.-W., Song, Y., & Wang, H. (2015). The multifaceted role of CD146/MCAM in the promotion of melanoma progression. *Cancer Cell International*, *15*, 3. https://doi.org/10.1186/s12935-014-0147-z

Leiva, O., Matsuura, S., Findlay, A., Turner, C., Jarolimek, W., & Ravid, K. (2017). A Novel Lysyl Oxidase Inhibitor Attenuates Bone Marrow Fibrosis and Decreases Spleen Size in a Mouse Model of Primary Myelofibrosis. *Blood*, *130*(Supplement 1), 4214–4214. https://doi.org/10.1182/blood.V130.Suppl_1.4214.4214

Leng, N., Dawson, J. A., Thomson, J. A., Ruotti, V., Rissman, A. I., Smits, B. M. G., Haag, J. D., Gould, M. N., Stewart, R. M., & Kendziorski, C. (2013). EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics*, *29*(8), 1035–1043. https://doi.org/10.1093/bioinformatics/btt087

Li, L., Huang, Y., Yang, Z., Zhang, S., Han, Z., & Luo, Y. (2020). Potential microglia-based interventions for stroke. *CNS Neuroscience & Therapeutics*, *26*(3), 288–296. https://doi.org/10.1111/cns.13291

Li, S., Nie, E. H., Yin, Y., Benowitz, L. I., Tung, S., Vinters, H. V., Bahjat, F. R., Stenzel-Poore, M. P., Kawaguchi, R., Coppola, G., & Carmichael, S. T. (2015). GDF10 is a signal for axonal sprouting and functional recovery after stroke. *Nature Neuroscience*, *18*(12), 1737–1745. https://doi.org/10.1038/nn.4146

Lindvall, O., & Kokaia, Z. (2015). Neurogenesis following Stroke Affecting the Adult Brain. *Cold Spring Harbor Perspectives in Biology*, *7*(11), a019034. https://doi.org/10.1101/cshperspect.a019034

Liu, J., Tang, T., & Yang, H. (2011). Protective effect of deferoxamine on experimental spinal cord injury in rat. *Injury*, *42*(8), 742–745. https://doi.org/10.1016/j.injury.2010.08.028

Luissint, A.-C., Artus, C., Glacial, F., Ganeshamoorthy, K., & Couraud, P.-O. (2012). Tight junctions at the blood brain barrier: Physiological architecture and disease-associated dysregulation. *Fluids and Barriers of the CNS*, *9*(1), 23. https://doi.org/10.1186/2045-8118-9-23

Ma, L., Zeng, Y., Wei, J., Yang, D., Ding, G., Liu, J., Shang, J., Kang, Y., & Ji, X. (2018). Knockdown of LOXL1 inhibits TGF-β1-induced proliferation and fibrogenesis of hepatic stellate cells by inhibition of Smad2/3 phosphorylation. *Biomedicine & Pharmacotherapy*, *107*, 1728–1735. https://doi.org/10.1016/j.biopha.2018.08.156

Ma, Q., Zhao, Z., Sagare, A. P., Wu, Y., Wang, M., Owens, N. C., Verghese, P. B., Herz, J., Holtzman, D. M., & Zlokovic, B. V. (2018). Blood-brain barrier-associated pericytes internalize and clear aggregated amyloid-β42 by LRP1-dependent apolipoprotein E isoform-specific mechanism. *Molecular Neurodegeneration*, *13*(1), 57. https://doi.org/10.1186/s13024-018-0286-0

Ma, Y., Sun, S., Shang, X., Keller, E. T., Chen, M., & Zhou, X. (2020). Integrative differential expression and gene set enrichment analysis using summary statistics for scRNA-seq studies. *Nature Communications*, *11*(1), 1585. https://doi.org/10.1038/s41467-020-15298-6

Madsen, T. E., Khoury, J. C., Leppert, M., Alwell, K., Moomaw, C. J., Sucharew, H., Woo, D., Ferioli, S., Martini, S., Adeoye, O., Khatri, P., Flaherty, M., De Los Rios La Rosa, F., Mackey, J., Mistry, E., Demel, S. L., Coleman, E., Jasne, A., Slavin, S. J., … Kleindorfer, D. O. (2020). Temporal Trends in Stroke Incidence Over Time by Sex and Age in the GCNKSS. *Stroke*, *51*(4), 1070–1076. https://doi.org/10.1161/STROKEAHA.120.028910

Makihara, N., Arimura, K., Ago, T., Tachibana, M., Nishimura, A., Nakamura, K., Matsuo, R., Wakisaka, Y., Kuroda, J., Sugimori, H., Kamouchi, M., & Kitazono, T. (2015). Involvement of platelet-derived growth factor receptor β in fibrosis through extracellular matrix protein production after

ischemic stroke. *Experimental Neurology*, *264*, 127–134. https://doi.org/10.1016/j.expneurol.2014.12.007

Mathys, H., Adaikkan, C., Gao, F., Young, J. Z., Manet, E., Hemberg, M., De Jager, P. L., Ransohoff, R. M., Regev, A., & Tsai, L.-H. (2017). Temporal Tracking of Microglia Activation in Neurodegeneration at Single-Cell Resolution. *Cell Reports*, *21*(2), 366–380. https://doi.org/10.1016/j.celrep.2017.09.039

Matsuo, A., Tanida, R., Yanagi, S., Tsubouchi, H., Miura, A., Shigekusa, T., Matsumoto, N., & Nakazato, M. (2021). Significance of nuclear LOXL2 inhibition in fibroblasts and myofibroblasts in the fibrotic process of acute respiratory distress syndrome. *European Journal of Pharmacology*, *892*, 173754. https://doi.org/10.1016/j.ejphar.2020.173754

Meletis, K., Barnabé-Heider, F., Carlén, M., Evergren, E., Tomilin, N., Shupliakov, O., & Frisén, J. (2008). Spinal Cord Injury Reveals Multilineage Differentiation of Ependymal Cells. *PLOS Biology*, *6*(7), e182. https://doi.org/10.1371/journal.pbio.0060182

Mishra, A., Reynolds, J. P., Chen, Y., Gourine, A. V., Rusakov, D. A., & Attwell, D. (2016). Astrocytes mediate neurovascular signaling to capillary pericytes but not to arterioles. *Nature Neuroscience*, *19*(12), 1619–1627. https://doi.org/10.1038/nn.4428

Montagne, A., Nikolakopoulou, A. M., Zhao, Z., Sagare, A. P., Si, G., Lazic, D., Barnes, S. R., Daianu, M., Ramanathan, A., Go, A., Lawson, E. J., Wang, Y., Mack, W. J., Thompson, P. M., Schneider, J. A., Varkey, J., Langen, R., Mullins, E., Jacobs, R. E., & Zlokovic, B. V. (2018). Pericyte degeneration causes white matter dysfunction in the mouse central nervous system. *Nature Medicine*, *24*(3), 326–337. https://doi.org/10.1038/nm.4482

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., de Ferranti, S., Després, J.-P., Fullerton, H. J., Howard, V. J., Huffman, M. D., Judd, S. E., Kissela, B. M., Lackland, D. T., Lichtman, J. H., Lisabeth, L. D., Liu, S., Mackey, R. H., Matchar, D. B., … Turner, M. B. (2015). Heart Disease and Stroke Statistics—2015 Update: A Report From the American Heart Association. *Circulation*, *131*(4).

https://doi.org/10.1161/CIR.0000000000000152

Neo, S. H., & Tang, B. L. (2017). Collagen 1 signaling at the central nervous system injury site and astrogliosis. *Neural Regeneration Research*, *12*(10), 1600–1601. https://doi.org/10.4103/1673-5374.217323

Nguyen, D. T., Ding, C., Wilson, E., Marcus, G. M., & Olgin, J. E. (2010). Pirfenidone mitigates left ventricular fibrosis and dysfunction after myocardial infarction and reduces arrhythmias. *Heart Rhythm*, *7*(10), 1438–1445. https://doi.org/10.1016/j.hrthm.2010.04.030

Niclou, S. P., Franssen, E. H. P., Ehlert, E. M. E., Taniguchi, M., & Verhaagen, J. (2003). Meningeal cell-derived semaphorin 3A inhibits neurite outgrowth. *Molecular and Cellular Neuroscience*, *24*(4), 902–912. https://doi.org/10.1016/S1044-7431(03)00243-4

Nikolakopoulou, A. M., Montagne, A., Kisler, K., Dai, Z., Wang, Y., Huuskonen, M. T., Sagare, A. P., Lazic, D., Sweeney, M. D., Kong, P., Wang, M., Owens, N. C., Lawson, E. J., Xie, X., Zhao, Z., & Zlokovic, B. V. (2019). Pericyte loss leads to circulatory failure and pleiotrophin depletion causing neuron loss. *Nature Neuroscience*, *22*(7), 1089. https://doi.org/10.1038/s41593-019-0434-z

Nollet, M., Stalin, J., Moyon, A., Traboulsi, W., Essaadi, A., Robert, S., Malissen, N., Bachelier, R., Daniel, L., Foucault-Bertaud, A., Gaudy-Marqueste, C., Lacroix, R., Leroyer, A. S., Guillet, B., Bardin, N., Dignat-George, F., & Blot-Chabaud, M. (2017). A novel anti-CD146 antibody specifically targets cancer cells by internalizing the molecule. *Oncotarget*, *8*(68), 112283–112296. https://doi.org/10.18632/oncotarget.22736

Nour, M., Scalzo, F., & Liebeskind, D. S. (2013). Ischemia-Reperfusion Injury in Stroke. *Interventional Neurology*, *1*(3–4), 185–199. https://doi.org/10.1159/000353125

Ohab, J. J., Fleming, S., Blesch, A., & Carmichael, S. T. (2006). A Neurovascular Niche for Neurogenesis after Stroke. *Journal of Neuroscience*, *26*(50), 13007–13016. https://doi.org/10.1523/JNEUROSCI.4323-06.2006

Özen, I., Deierborg, T., Miharada, K., Padel, T., Englund, E., Genové, G., & Paul, G. (2014). Brain

pericytes acquire a microglial phenotype after stroke. *Acta Neuropathologica*, *128*(3), 381–396. https://doi.org/10.1007/s00401-014-1295-x

Pasterkamp, R. J., Giger, R. J., Ruitenberg, M. J., Holtmaat, A. J., De Wit, J., De Winter, F., & Verhaagen, J. (1999). Expression of the gene encoding the chemorepellent semaphorin III is induced in the fibroblast component of neural scar tissue formed following injuries of adult but not neonatal CNS. *Molecular and Cellular Neurosciences*, *13*(2), 143–166. https://doi.org/10.1006/mcne.1999.0738

Popovich, P. G., Tovar, C. A., Lemeshow, S., Yin, Q., & Jakeman, L. B. (2014). Independent evaluation of the anatomical and behavioral effects of Taxol in rat models of spinal cord injury. *Experimental Neurology*, *261*, 97–108. https://doi.org/10.1016/j.expneurol.2014.06.020

Puente, A., Fortea, J. I., Cabezas, J., Arias Loste, M. T., Iruzubieta, P., Llerena, S., Huelin, P., Fábrega, E., & Crespo, J. (2019). LOXL2—A New Target in Antifibrogenic Therapy? *International Journal of Molecular Sciences*, *20*(7), 1634. https://doi.org/10.3390/ijms20071634

Ray, S., Ju, X., Sun, H., Finnerty, C. C., Herndon, D. N., & Brasier, A. R. (2013). The IL-6 trans-signaling-STAT3 pathway mediates ECM and cellular proliferation in fibroblasts from hypertrophic scar. *The Journal of Investigative Dermatology*, *133*(5), 1212–1220. https://doi.org/10.1038/jid.2012.499

Ribatti, D., Nico, B., & Crivellato, E. (2011). The role of pericytes in angiogenesis. *International Journal of Developmental Biology*, *55*(3), 261–268. https://doi.org/10.1387/ijdb.103167dr

Roth, M., Enström, A., Aghabeick, C., Carlsson, R., Genové, G., & Paul, G. (2020). Parenchymal pericytes are not the major contributor of extracellular matrix in the fibrotic scar after stroke in male mice. *Journal of Neuroscience Research*, *98*(5), 826–842. https://doi.org/10.1002/jnr.24557

Ruan, L., Wang, B., ZhuGe, Q., & Jin, K. (2015). Coupling of neurogenesis and angiogenesis after ischemic stroke. *Brain Research*, *1623*, 166–173. https://doi.org/10.1016/j.brainres.2015.02.042

Ruschel, J., Hellal, F., Flynn, K. C., Dupraz, S., Elliott, D. A., Tedeschi, A., Bates, M., Sliwinski, C.,

Brook, G., Dobrindt, K., Peitz, M., Brüstle, O., Norenberg, M. D., Blesch, A., Weidner, N., Bunge, M. B., Bixby, J. L., & Bradke, F. (2015). Axonal regeneration. Systemic administration of epothilone B promotes axon regeneration after spinal cord injury. *Science (New York, N.Y.)*, *348*(6232), 347–352. https://doi.org/10.1126/science.aaa2958

Rustenhoven, J., Jansson, D., Smyth, L. C., & Dragunow, M. (2017). Brain Pericytes As Mediators of Neuroinflammation. *Trends in Pharmacological Sciences*, *38*(3), 291–304. https://doi.org/10.1016/j.tips.2016.12.001

Saelens, W., Cannoodt, R., Todorov, H., & Saeys, Y. (2019). A comparison of single-cell trajectory inference methods. *Nature Biotechnology*, *37*(5), 547–554. https://doi.org/10.1038/s41587-019-0071-9

Sakuma, R., Kawahara, M., Nakano-Doi, A., Takahashi, A., Tanaka, Y., Narita, A., Kuwahara-Otani, S., Hayakawa, T., Yagi, H., Matsuyama, T., & Nakagomi, T. (2016). Brain pericytes serve as microglia-generating multipotent vascular stem cells following ischemic stroke. *Journal of Neuroinflammation*, *13*(1). https://doi.org/10.1186/s12974-016-0523-9

Schrimpf, C., Xin, C., Campanholle, G., Gill, S. E., Stallcup, W., Lin, S.-L., Davis, G. E., Gharib, S. A., Humphreys, B. D., & Duffield, J. S. (2012). Pericyte TIMP3 and ADAMTS1 Modulate Vascular Stability after Kidney Injury. *Journal of the American Society of Nephrology : JASN*, *23*(5), 868–883. https://doi.org/10.1681/ASN.2011080851

Schultz, G. S., & Wysocki, A. (2009). Interactions between extracellular matrix and growth factors in wound healing. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *17*(2), 153–162. https://doi.org/10.1111/j.1524-475X.2009.00466.x

Segel, M., Neumann, B., Hill, M. F., Weber, I., Viscomi, C., Zhao, C., Young, A., Agley, C. C., Thompson, A. J., Gonzalez, G., Sharma, A., Holmqvist, S., Rowitch, D. H., Franze, K., Franklin, R. J., & Chalut, K. J. (2019). Niche stiffness underlies the aging of central nervous system progenitor cells. *Nature*, *573*(7772), 130–134. https://doi.org/10.1038/s41586-019-1484-9

Shearer, M. C., & Fawcett, J. W. (2001). The astrocyte/meningeal cell interface—A barrier to successful nerve regeneration? *Cell and Tissue Research*, *305*(2), 267–273. https://doi.org/10.1007/s004410100384

Shiber, J. R., Fontane, E., & Adewale, A. (2010). Stroke registry: Hemorrhagic vs ischemic strokes. *The American Journal of Emergency Medicine*, *28*(3), 331–333. https://doi.org/10.1016/j.ajem.2008.10.026

Silver, J., & Miller, J. H. (2004). Regeneration beyond the glial scar. *Nature Reviews. Neuroscience*, *5*(2), 146–156. https://doi.org/10.1038/nrn1326

Smith, G. M., & Strunz, C. (2005). Growth factor and cytokine regulation of chondroitin sulfate proteoglycans by astrocytes. *Glia*, *52*(3), 209–218. https://doi.org/10.1002/glia.20236

Soderblom, C., Luo, X., Blumenthal, E., Bray, E., Lyapichev, K., Ramos, J., Krishnan, V., Lai-Hsu, C., Park, K. K., Tsoulfas, P., & Lee, J. K. (2013a). Perivascular fibroblasts form the fibrotic scar after contusive spinal cord injury. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *33*(34), 13882–13887. https://doi.org/10.1523/JNEUROSCI.2524-13.2013

Soderblom, C., Luo, X., Blumenthal, E., Bray, E., Lyapichev, K., Ramos, J., Krishnan, V., Lai-Hsu, C., Park, K. K., Tsoulfas, P., & Lee, J. K. (2013b). Perivascular Fibroblasts Form the Fibrotic Scar after Contusive Spinal Cord Injury. *Journal of Neuroscience*, *33*(34), 13882–13887. https://doi.org/10.1523/JNEUROSCI.2524-13.2013

Sofroniew, M. V. (2015). Astrogliosis. *Cold Spring Harbor Perspectives in Biology*, *7*(2), a020420. https://doi.org/10.1101/cshperspect.a020420

Sweeney, M. D., Ayyadurai, S., & Zlokovic, B. V. (2016). Pericytes of the neurovascular unit: Key functions and signaling pathways. *Nature Neuroscience*, *19*(6), 771–783. https://doi.org/10.1038/nn.4288

Tirosh, I., Izar, B., Prakadan, S. M., Wadsworth, M. H., Treacy, D., Trombetta, J. J., Rotem, A., Rodman, C., Lian, C., Murphy, G., Fallahi-Sichani, M., Dutton-Regester, K., Lin, J.-R., Cohen,

O., Shah, P., Lu, D., Genshaft, A. S., Hughes, T. K., Ziegler, C. G. K., … Garraway, L. A. (2016). Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science (New York, N.Y.)*, *352*(6282), 189–196. https://doi.org/10.1126/science.aad0501

Underly, R. G., Levy, M., Hartmann, D. A., Grant, R. I., Watson, A. N., & Shih, A. Y. (2017). Pericytes as Inducers of Rapid, Matrix Metalloproteinase-9-Dependent Capillary Damage during Ischemia. *The Journal of Neuroscience*, *37*(1), 129–140. https://doi.org/10.1523/JNEUROSCI.2891-16.2016

van Dijk, C. G. M., Nieuweboer, F. E., Pei, J. Y., Xu, Y. J., Burgisser, P., van Mulligen, E., el Azzouzi, H., Duncker, D. J., Verhaar, M. C., & Cheng, C. (2015). The complex mural cell: Pericyte function in health and disease. *International Journal of Cardiology*, *190*, 75–89. https://doi.org/10.1016/j.ijcard.2015.03.258

Vanlandewijck, M., He, L., Mäe, M. A., Andrae, J., Ando, K., Gaudio, F. D., Nahar, K., Lebouvier, T., Laviña, B., Gouveia, L., Sun, Y., Raschperger, E., Räsänen, M., Zarb, Y., Mochizuki, N., Keller, A., Lendahl, U., & Betsholtz, C. (2018). A molecular atlas of cell types and zonation in the brain vasculature. *Nature*, *554*(7693), 475–480. https://doi.org/10.1038/nature25739

Vemuganti, R., & Zhao, H. (2015). Mechanisms and therapies for acute CNS insults. *Metabolic Brain Disease*, *30*(2), 353. https://doi.org/10.1007/s11011-014-9585-5

Vogelaar, C. F., König, B., Krafft, S., Estrada, V., Brazda, N., Ziegler, B., Faissner, A., & Müller, H. W. (2015). Pharmacological Suppression of CNS Scarring by Deferoxamine Reduces Lesion Volume and Increases Regeneration in an In Vitro Model for Astroglial-Fibrotic Scarring and in Rat Spinal Cord Injury In Vivo. *PLoS ONE*, *10*(7), e0134371. https://doi.org/10.1371/journal.pone.0134371

Walker, J. E., Giri, S. N., & Margolin, S. B. (2005). A double-blind, randomized, controlled study of oral pirfenidone for treatment of secondary progressive multiple sclerosis. *Multiple Sclerosis (Houndmills, Basingstoke, England)*, *11*(2), 149–158. https://doi.org/10.1191/1352458505ms1134oa

Wang, S., Cao, C., Chen, Z., Bankaitis, V., Tzima, E., Sheibani, N., & Burridge, K. (2012). Pericytes Regulate Vascular Basement Membrane Remodeling and Govern Neutrophil Extravasation during Inflammation. *PLoS ONE*, *7*(9). https://doi.org/10.1371/journal.pone.0045499

Wang, T., Li, B., Nelson, C. E., & Nabavi, S. (2019). Comparative analysis of differential gene expression analysis tools for single-cell RNA sequencing data. *BMC Bioinformatics*, *20*(1), 40. https://doi.org/10.1186/s12859-019-2599-6

Wang, Y.-C., Chen, Q., Luo, J.-M., Nie, J., Meng, Q.-H., Shuai, W., Xie, H., Xia, J.-M., & Wang, H. (2019). Notch1 promotes the pericyte-myofibroblast transition in idiopathic pulmonary fibrosis through the PDGFR/ROCK1 signal pathway. *Experimental & Molecular Medicine*, *51*(3), 1–11. https://doi.org/10.1038/s12276-019-0228-0

Whetstone, W. D., Hsu, J.-Y. C., Eisenberg, M., Werb, Z., & Noble-Haeusslein, L. J. (2003). Blood-spinal cord barrier after spinal cord injury: Relation to revascularization and wound healing. *Journal of Neuroscience Research*, *74*(2), 227–239. https://doi.org/10.1002/jnr.10759

Xiao, M.-J., Han, Z., Shao, B., & Jin, K. (2009). Notch signaling and neurogenesis in normal and stroke brain. *International Journal of Physiology, Pathophysiology and Pharmacology*, *1*(2), 192–202.

Xu, X.-H., Jia, Y., Zhou, X., Xie, D., Huang, X., Jia, L., Zhou, Q., Zheng, Q., Zhou, X., Wang, K., & Jin, L.-P. (2019). Downregulation of lysyl oxidase and lysyl oxidase-like protein 2 suppressed the migration and invasion of trophoblasts by activating the TGF-β/collagen pathway in preeclampsia. *Experimental & Molecular Medicine*, *51*(2), 1–12. https://doi.org/10.1038/s12276-019-0211-9

Yahn, S. L., Li, J., Goo, I., Gao, H., Brambilla, R., & Lee, J. K. (2020). Fibrotic scar after experimental autoimmune encephalomyelitis inhibits oligodendrocyte differentiation. *Neurobiology of Disease*, *134*, 104674. https://doi.org/10.1016/j.nbd.2019.104674

Yang, J., Savvatis, K., Kang, J. S., Fan, P., Zhong, H., Schwartz, K., Barry, V., Mikels-Vigdal, A., Karpinski, S., Kornyeyev, D., Adamkewicz, J., Feng, X., Zhou, Q., Shang, C., Kumar, P., Phan, D., Kasner, M., López, B., Diez, J., … Chang, C.-P. (2016). Targeting LOXL2 for cardiac

interstitial fibrosis and heart failure treatment. *Nature Communications*, *7*(1), 13710. https://doi.org/10.1038/ncomms13710

Yang, T., Dai, Y., Chen, G., & Cui, S. (2020). Dissecting the Dual Role of the Glial Scar and Scar-Forming Astrocytes in Spinal Cord Injury. *Frontiers in Cellular Neuroscience*, *0*. https://doi.org/10.3389/fncel.2020.00078

Yau, J. W., Teoh, H., & Verma, S. (2015). Endothelial cell control of thrombosis. *BMC Cardiovascular Disorders*, *15*, 130. https://doi.org/10.1186/s12872-015-0124-z

Yemisci, M., Gursoy-Ozdemir, Y., Vural, A., Can, A., Topalkara, K., & Dalkara, T. (2009). Pericyte contraction induced by oxidative-nitrative stress impairs capillary reflow despite successful opening of an occluded cerebral artery. *Nature Medicine*, *15*(9), 1031–1037. https://doi.org/10.1038/nm.2022

Yiu, G., & He, Z. (2006). Glial inhibition of CNS axon regeneration. *Nature Reviews Neuroscience*, *7*(8), 617–627. https://doi.org/10.1038/nrn1956

Yokota, K., Kobayakawa, K., Saito, T., Hara, M., Kijima, K., Ohkawa, Y., Harada, A., Okazaki, K., Ishihara, K., Yoshida, S., Kudo, A., Iwamoto, Y., & Okada, S. (2017). Periostin Promotes Scar Formation through the Interaction between Pericytes and Infiltrating Monocytes/Macrophages after Spinal Cord Injury. *The American Journal of Pathology*, *187*(3), 639–653. https://doi.org/10.1016/j.ajpath.2016.11.010

Zbesko, J. C., Nguyen, T.-V. V., Yang, T., Frye, J. B., Hussain, O., Hayes, M., Chung, A., Day, W. A., Stepanovic, K., Krumberger, M., Mona, J., Longo, F. M., & Doyle, K. P. (2018). Glial scars are permeable to the neurotoxic environment of chronic stroke infarcts. *Neurobiology of Disease*, *112*, 63–78. https://doi.org/10.1016/j.nbd.2018.01.007

Zeisel, A., Hochgerner, H., Lönnerberg, P., Johnsson, A., Memic, F., van der Zwan, J., Häring, M., Braun, E., Borm, L. E., La Manno, G., Codeluppi, S., Furlan, A., Lee, K., Skene, N., Harris, K. D., Hjerling-Leffler, J., Arenas, E., Ernfors, P., Marklund, U., & Linnarsson, S. (2018). Molecular Architecture of the Mouse Nervous System. *Cell*, *174*(4), 999-1014.e22.

https://doi.org/10.1016/j.cell.2018.06.021

CHAPTER 4

Manipulation of post-stroke pericyte via cellular ablation, candidate gene loss-of-function and pharmacological agent targeting a specific pathway

INTRODUCTION

Based on the bioinformatic analysis of the scRNAseq dataset described in Chapter 3, combined with the molecular tools developed in Chapter 2, we set out to further dissect the functional role of pericytes after stroke via three distinct methods of manipulation: complete ablation (via diptheria toxin, DT), molecular loss-of-function (*Stat3* knockdown) and pharmacological intervention (LOXL2/3 inhibition).

Ablation Study

Ablation studies in mural cells have been difficult, especially in the brain due to the essential role of pericyte in the BBB, vasculature development and maintenance (Armulik et al., 2011; van Dijk et al., 2015). Thus far, pericyte loss-of-function studies have been predominantly carried out in mice with *Pdgfrb* mutations or knockouts that lead to a reduction in pericyte numbers (Kisler et al., 2017; Montagne et al., 2018; Nikolakopoulou et al., 2019). These animals only have up to 50% pericyte coverage in the brain which leads to BBB leakage, developmental abnormalities, neurovascular coupling dysfunctions and other pathologies (Kisler et al., 2017; Montagne et al., 2018; Nikolakopoulou et al., 2019). These abnormalities and dysfunctions complicate study in the context of stroke, as it would be difficult to attribute the causal role of pericyte loss to the observed tissue phenotype. Alternatively, other studies have crossed the *Ras*-less mice with a pericyte-specific transgenic line (*Glast*^{CRE}) to inhibit the proliferation of pericytes after spinal cord injury (Dias et al., 2018; Göritz et al., 2011). This method, while elegantly executed, lacks the spatial control needed to dissect pericyte heterogeneity in the periinfarct region and in the infarct region.

Here, we crossed *Tbx18*$^{CRE/+}$*Rosa26*$^{tdT/}$ with i*DTR* animals (Jackson Laboratory, Cat 007900) and microdose DT was delivered transcranially into the periinfarct regions. We observed differential tissue phenotypes compared to control that suggest worsening tissue phenotype and functional outcome. Upon ablating the Tbx18+ cells on one side of the infarct (medial or lateral), while we

observed the loss of pericyte coverage in the injected site, we also saw the gradient formation of fibrotic scar as non-ablated pericytes migrate from the surrounding area into the infarcted area, suggesting that the process of pericytes' fibrotic scar formation is highly conserved.

Stat3 Knockdown Study

Stat3 was identified from the previous chapter as one of the top gene candidates (5.6 fold increase after stroke) that was responsible for the pericyte-fibroblasts transition. Previously, *Stat3* has been shown to play a role in astrogliosis (Anderson et al., 2016). Using viral knockdown of *Stat3*, we observed a reduction of pericyte density in the periinfarct regions while the infarct area volume and density remained unchanged. This either suggests *Stat3* plays little role in fibrotic scar formation or brain fibrosis is compensated by other pathways.

Study of LOXL2/3 Inhibitors

Loxl2 and *Loxl3* were other top candidates that were most differentiated in pericytes after stroke (5-6 fold expression increase compared to sham (FDR <0.01) . LOXL is a Cu-dependent amine oxidase that is involved in the post-transcriptional modification of type 1 collagen and elastin, which forms the crosslinks that stabilize the extracellular matrix (Puente et al., 2019, p. 2). Stabilization of ECM and increased tissue stiffness was found to be the main reason for persistent fibrosis and fibrotic associated-pathologies across multiple organs (Puente et al., 2019, p. 2). Previous studies had shown that increased LOXL2 or 3 correlates with the worsening outcomes in cardiac dysfunction, liver fibrosis, interstitial fibrosis, and pulmonary fibrosis, while inhibition of LOXL2 or 3 via antibody or genetic manipulation has improved functional outcomes (Aumiller et al., 2017; L. Ma et al., 2018; Matsuo et al., 2021, p. 2; Puente et al., 2019; Xu et al., 2019; J. Yang et al., 2016, p. 2). Interestingly, as LOXL2/3 inhibition will lead to softening of tissue, tissue stiffness in the brain correlates very well with its regenerative capability. Particularly, young mice with higher regenerative capability have softer brains compared to older mice (Guo et al., 2019). Moreover, the stiffer tissue in aged animals inhibits oligodendrocyte progenitor cell (OPC)

proliferation and differentiation (Segel et al., 2019).Together, this suggested the beneficial outcomes of LOXL2 and LOXL3 inhibition on functional recovery after stroke via attenuation of fibrosis and the promotion of regenerative events in the brain.

Here, systemic delivery of novel small molecule inhibitors of LOXL2/3 (Pharmaxis, Inc) showed significant reduction in infarcted area, along with robust regenerative events such as angiogenesis and neurogenesis. This positions LOXL2/3 as an attractive candidate to promote stroke recovery.

RESULTS

Ablating mural cells worsened tissue outcome after stroke

To further delineate the role of pericytes in the infarct core and the periinfarct regions, we used *Tbx18*$^{Cre/+}$*RosaR26*$^{tdTomato/+}$ mice, couple with the viral delivery of Diphtheria toxin receptor (DTR) and transcranial delivery of DT to achieve well-controlled ablation of mural cells **(Figure 1A).** We attempted at multiple strategies to achieve brain-pericyte-specific ablation and found that systemic injection of DT led to sicker animal with higher mortality, hence, confounded the study. We decided to go with well-control transcranial delivery of DT. Ablated animals had significantly larger infarct areas **(Figure 1B-C).** In the periinfarct area, we observed significantly lower pericyte coverage **(Figure 1D)**, vasculature density **(Figure 1E)** and neuroblast number **(Figure 1F).** Interestingly, we observed a lower intensity of GFAP in the astrocytic scar in these animals compared to sham at 10 days post injury **(Figure 1G),** suggesting disruption of the fibrotic-glial scar is dynamic.

Next, we injected a small dose of DT into either the lateral or the medial side of the cortex to ablate the mural cells on either side of the injury. We observed a reduction 30% reduction of pericyte cell number in the periinfarct compared to control. We see a migration and gradual formation from the fibrotic scar from the opposite side of the injury **(Figure 2),** which suggested the highly conserved process of pericyte-fibrotic scar formation. Interestingly, we saw the concentration of EdU+ cells which are mostly immune cells concentrated where the fibrotic scar are, suggesting the role of fibrotic scar in neuroimmune regulation. Futher studies are needed to examine the vascular tree,

angiogenesis and neurogenesis differences between the ablated side and non-ablated side.

PDGFRb+ cell-specific *Stat3* knockdown significantly attenuates the regenerative events in the periinfarct area

Stat3 is one of the top genes that came out of the scRNAseq dataset in chapter 3. We observed significantly increased expression of *Stat3* in both periinfarct pericytes and stroke core cells after stroke **(Figure 3A)**. Ingenuity Pathway Analysis (IPA) identified *Stat3* as one of the hub genes in our network of differential genes on IPA, which means it participated in multiple gene networks that are differentially expressed after stroke**(Figure 3B).** Using qPCR on FACS-sorted pericytes in sham and stroke, we confirmed the upregulation of *Stat3* in stroked tissue (3-fold-increase in periinfarct and 4-fold-increase in infarct tissue compare to sham) **(Figure 3C).** On the tissue level, we confirmed the increased expression of phosphorylated version of STAT3 (pSTAT3) at 3 days after stroke **(Figure 3D).** To knockdown *Stat3* in PDGFRb+ cells, we cloned the Cre-recombinase cassette into the AAV2-Pdgfrb backbone and injected the packaged AAV virus into the *Stat3* $^{Fl/Fl}$ mice (a gift from Dr. Michael Sofroniew at UCLA) **(Figure 4A)**. Virus was injected at two separate site, (1uL, titer 10^{13}) 0.5mm medial and lateral to the injury core, 4 weeks prior to surgery to allow for sufficient recombination and knockdown. The knockdown efficiency was confirmed via qPCR **(Figure 4B)** and IHC **(Figure 4C).** In the *Stat3* knockdown-animals, we observed significant disruption of the neurovascular niche. Pericyte coverage and vasculature density were significantly reduced **(Figure 4D-G).** Interestingly, we observed not only a reduction of neuroblast number **(Figure 4H)** but also the lack of neuroblast-pericyte association **(Figure 4I-J).** Interestingly, infarcted area volume remained unchanged **(Figure 4K)**.

LOXL2/3 inhibition promotes regeneration of the periinfarct cortex

Loxl2 and *Loxl3* are differentially upregulated in the stroke core and stroke periinfarct area (**Figure 5A).** To manipulate the LOXL2/3 pathways, we used a set of previously published small-molecule inhibitors to LOXL2 and LOXL3 that are currently in phase 1/2a clinical trial for thrombocythemia myelofibrosis (provided by Pharmaxis, Inc) (Chang et al., 2017; Jones et al., 2018; Leiva et al.,

2017). PXS-5382 is a LOXL2 inhibitor while PXS-5505 is a pan-LOXL2/3 inhibitor. Both were delivered to mice subdermal systemically via osmosis pump starting 3 days after injury **(Figure 5B).** At 10 days after injury, we observed a significant increase in proliferative events such as angiogenesis **(Figure 5C)**, pericyte proliferation **(Figure 5D)** and neuroblast number **(Figure 5E)** in the periinfarct regions in treated animals compared to the control group. Interestingly, while there was no difference in pericyte proliferation, PXS-5382-treated group showed increased vasculature density and neuroblast number compared to the PXS-5505 **(Figure 5C-E).**

LOXL2/3 inhibition led to significant reduction in infarct size and changed the glia-fibrotic scar border

In the infarct region, we observed overall remarkable tissue phenotypes. Infarcted volume is significantly reduced in the treated group compared to the non-treated group with smallest infarct in the PXS-5505-treated group **(Figure 6A & B).** Density of vasculature was significantly increased inside the infarct area in the treated group **(Figure 6C &D).** We next asked whether the vasculature network connected with the periinfarct vasculature network. By quantifying the "cross point" of vasculature into the infarct area, we found that the drug treated group had significantly more vessels crossing through the fibrotic-glial border to the infarct area compared the control group **(Figure 6E).** Interestingly, in contrast to the clean and precise fibrotic-glial border in control group, we observed significant overlapping and "invasion" of the reactive astrocyte boundary in the fibrotic scar area **(Figure 6F &G).**

DISCUSSION

To further understand the pericyte role after ischemic stroke, our studies have delineated the role of mural cell populations after injury. We found that the loss of pericytes led to significant enlargement of the infarct area, along with the breakdown of regenerative events such as angiogenesis in the periinfarct area **(Figure 1)**. As reduction of angiogenesis after stroke leads to worsening outcome, our tissue data suggested the functional outcomes post-stroke would be

expected to be worse in pericyte-ablated animals (Ruan et al., 2015),. This finding is line with previous studies in the spinal cord that complete ablation of pericytes led to worsening functional outcome (Dias et al., 2018; Göritz et al., 2011). *Tbx18*$^{CRE/+}$ *Rosa26*$^{tdT/+}$ express in not only pericytes but also perivascular fibroblasts, the ablation targeted the fibrotic-scar contribution from both populations. Our data confirmed that during the acute phase, fibrotic scar ablation seems to be detrimental to tissue regeneration, as reported previously (Dias et al., 2018; Göritz et al., 2011). Interestingly, by doing local ablation of TBX18+ cells, we found that the fibrotic scar forming process is highly conserved: if the process is disrupted in a specific area of the infarct, it is compensated by a fibrotic response from another,adjacent area (**Figure 2).** Further studies are needed to understand the extent of region-specific ablation that leads to specific delays in scar formation and whether the delay would affect functional outcomes. Furthermore, it would be of interest to understand the degree of contribution between perivascular fibroblasts and pericyte populations to the fibrotic scar and if one cell population is ablated or dysfunction, whether the other cell population would carry out the fibrotic scar formation responsibilities.

Our *Stat3* knockdown study suggested that the heterogeneous role of pericyte after stroke needs to be taken into consideration during pericyte-specific manipulation. In particular, we initially hypothesized that *Stat3* knockdown will lead to a partial reduction of fibrotic scar and potentially promote regeneration, in line with previous studies (Dias et al., 2018). However, our tissue data did not show a significant reduction in infarct scar, but demonstrated a worsening tissue outcome with the disruption of angiogenesis in the periinfarct regions **(Figure 3 & 4).** The lack of change in the infarcted area could be due to two particular reasons. The first and most obvious reason is that *Stat3* does not play an important role in fibrotic scar formation processes as our bioinformatic analysis has suggested. The second reason is that the scar-forming process is highly conserved and that manipulation of one gene or even one contributing cell population (i.e., pericytes or perivascular fibroblasts) would not make a significant impact in the total scar phenotype. Further studies are needed to clarify this.

Interestingly, our remarkable results in the LOXL2/3 studies, especially in the infarct area, showed that manipulation of the fibrotic scar is potentially beneficial for tissue regeneration and functional outcome. While we did not measure the tissue stiffness of the LOXL2/3 inhibitors treatment, previous studies had shown that PXS-5505 and 5382 had significantly soften fibrotic tissue in the lung and heart (How et al., 2020; J. Yang et al., 2016, p. 2). Softening tissue stiffness has been linked to increased regeneration (Guo et al., 2019; Segel et al., 2019) Previous studies showed that astrocytic boundary is beneficial to regeneration and functional outcomes in spinal cord injury (Anderson et al., 2016). With the increased angiogenesis, vasculature density and the intrusion of astrocytic scar, the tissue outcome hinted at the regenerative capacity and better outcomes of the treated animals. Further behavior studies are needed to provide concrete evidence of improved functional outcome in treatment groups. Since this is a pharmacologic study, it is possible that the inhibitors are acting on other cells which mediate the results.

The dual roles of pericytes are essential and targeting one of the roles can potentially lead to the disruption of the other processes to which the pericytes contribute, as we saw significant disruption of the periinfarct tissues with both *Stat3* knockdown and LOXL2/3 studies even though both studies were carried out to target fibrotic scars **(Figure 4 & 5).**

Together, our studies provide evidence of the essential role and highly conserved nature of the fibrotic scar formation process. Pericyte heterogeneity needs to be taken into consideration during therapeutic intervention and mechano-manipulation of the fibrotic scar via pharmacological agents could lead to improved tissue repair and regeneration after ischemic injury.

METHODS

Mice

Pdgfrb$^{Cre/+}$ mice were a gift from Dr. Volkhard Lindner (Maine Medical Center). *Tbx18*$^{CRE/+}$ *Rosa26* $^{tdT/+}$ mice were a gift from Dr. Sylvia Evans (UC San Diego). *Stat3 .* All procedures were carried out with the approval of the UCLA Animal Research Committee.

Photothrombotic Stroke

Cortical stroke at the motor cortex was induced in mice using the stereotaxic surgery. Mice were initially anesthetized by mixture of oxygen and 5% isoflurane, then intubated at 2% for the duration of the operation. Body temperature was monitored and maintained at 37 degree Celcius +/- 0.5 degree by homeothermic heating pads. Rose Bangel, a photosensitive chemical was injected intraperitoneal at concentration of 10mg/mL. After 10 minutes to allow for systemic circulation of Rose Bangel, a cold light source KL1500 LCD (Zeiss) was placed on top of the exposed skull above the forelimb motor cortex (coordinates ML 1.5mm, AP 0.0mm). After 15 minutes, the wound was closed and animals were returned to cages for recovery. The photothrombotic stroke can reliably generate stroke size of 1-2 mm in diameter, dependent on the diameter and power of the light source

Tissue dissociation and FACS Isolation

Mice were euthanized within 1 minutes using an a gas chamber of isoflurane. The brain were dissected and rinsed with 30mL of phosphate-buffered saline (PBS) with 10% Bovine Serum albumin (BSA) . The meningeal were carefully removed. Infarct core were dissected and separated from the periinfarct cortex. Using forceps, white matter was removed from the tissues. The tissues were processed using the Milteny Biotech Adult Brain dissociation kit (Catalog 130-107-677). In brief, tissues were enzymatically digested using the gentleMACS Octo Dissociator with Heater for 30 mins at 37 degree Celsius. Samples were centrifuged and washed with ice-cold PBS/0.1% BSA before filtering through a 70uM MACS SmartStrainer. Samples were collected and centrugiged again at 300xg, 10 minutes before DAPI and Drag5 (Thermofisher) were added immediately prior to FACS. Cells were analyzed or sorted using a BD FACSAria™ II cell sorter. All data was analyzed using FlowJo software.

Transcranial Injection

Mice were prepared for surgery as previously described. After anesthesia, mice received injections in the medial intact peri-infarct cortex (Coordinates from Bregma: ML 0.5, AP 0.0mm)

with the designated virus. A dental drill was used to create a small hole in the skull to allow for needle access, and a 33guage Hamilton needle, attached to a 25 ul was lowered into the cortex at 0.4mm coordinate from the brain surface. There, virus was infused at rate of 0.1 ul/min. Afterward, the needle was allowed to remain in the brain for 10 minutes to allow for additional infusion and prevent backflow before slowly retracted. The wound was closed with vetbond tissue adhesive (3M). Animal was returned to the cage for recovery.

RNA Extraction and Reverse Transcription qPCR (RT-qPCR)

Sorted cells were sorted directly into the Trlzol LS Reagent (Thermo Fisher). RNA was extracted using the Trlzol™ LS Reagent and follow the manufacturer protocol. The extracted RNA was quantified using NanoDrop ™ according to the protocol. Conversion to complementary DNA (cDNA) was carried out using the iSript ™ cDNA Synthesis Kit (Bio-Rad) and following the manufacturer's instruction. RT-qPCR reactions were prepared with SYBR Green Master Mix (BioRad) and specific primers for target gene were used. The reactions were run on the CFX96 Touch ™ Real-Time PCR Detection System (Bio-Rad) and analysis was done with the double delta-CT method.

Tissue Collection

At indicated time, animals were sacrificed via transcardial perfusion with cold saline containing 2% Paraformaldehyde (PFA), 0.1M lysin, 0.01M NaO4, 0.175 NaP buffer. This mild fixation cocktail allows for preservation of many vasculature glycoprotein. Brains were dissected out and post-fixed in the same solution overnight at 4 degree Ceicius, then cryopreserved in 30% sucrose at 4 degree Celcius for an additional 48 hours. Afterwards, tissues were sectioned on cryostat (Lica CM 0530). 40uM sections were collected and stored in 50% glycerol antifreeze solution at -20 degree Celcius.

Immunohistochemistry

Immunohistochemistry was prepared according to standard laboratory protocol. Sections were

removed from the antifreeze solution and washed 3 times in 0.02M KPBS for 5 minutes. Afterward, the sections went through incubations 30-minute-permeabilization step (1% Triton-X in KPBS). Dependent on the antibody used, antigen retrieval steps using Citric acid pH 6.0 at 80 degree Celcius for 30 minutes was added. Tissues were blocked in solution of 5% normal donkey serum (NDS), 0.1% Triton-X for 1 hour at room temperature. Tissues were then transferred directly into primary antibody cocktail that is prepared in 2% NDS, 0.1% Triton-X in KBPS for 24 hours at 4 degree Celcius. The antibody list and its respective concentration is detailed in supplemental table 1. Following the primary incubation, sections were washed 3 times in KPBS and incubated in the secondary antibody solution that was prepared in 2% NDS, 0.1% Triton-X in KPBS for 1 hour at room temperature. Afterward, tissues were washed 3 times in KBPS for 10 minutes. EdU steps might be added at this stage dependent on the assay. Sections were mounted oonto triple-subbed slides and dehydrated with ethanol serial wash with increased concentration from 50-100% (1 minute each step), and xylene wash (5 minutes, twice). Next, slides were cover-slipped with the application of DPX mounting media.

EdU Administration and Immunohistochemistry Induction

EdU was administered in drinking water at the final concentration of 200ug/mL with antibiotic (cherry flavored for taste at the recommended dose. EdU was developed with a protocol modified from the Click-IT EdU imaging kit (Thermo Fisher). Briefly, sections were incubated for 30 minutes as the last step of immunohistochemistry for 30 minutes in 100mM TBS, pH 7.6 containing 100mM sodium ascorbate, 4mM CuSO4, and 2uM sulfo-Cy5 azide (Lumiprobe D3330). Sections were protected from light during this incubation.

Imaging and Imaris Analysis

Images were captured using a Nikon C2 confocal microscope at 40x magnification. Additional analysis were done on Imaris, as previously published (S. Li et al., 2015, p. 10).

AAV Virus Preparation

Appropriate promoters were developed and cloned into the AAV2-Flex virus backbone. Sequence were verified for insertion. Afterward, the vectors were packaged into AAV2 virus using previously published protocol (Bish et al., 2008). Briefly, the viral plasmid were transfected into HEKs cells using Lipofectamin 200 (Thermofisher). After 3 days of incubation, the cells were harvested into 150mMNaCl, 20mM Tris pH8.0 solution before being lyzed for 1 hour in the 10% sodium deoxycholate solution at 37 degree celcius. The cell debris were removed by centrifugation at 3000xg for 15 minutes and the rAAVs in supernation were harvested. Afterward, using the serial heparin column purification method, AAVs were purified then concentrated via the Amicon ultra-4 centrifugal filter units with 100,000 molecular weight cutoff. Virus is then tittered with HEK 293 cells before storing in -80 degree Celcius.

Statistics

All quantification data are presented as mean ± standard error of the mean (SEM) and significance was determined by using Student's t-test or Two-way ANOVA. A p-value < 0.05 was considered statistically significant and data were analyzed using GraphPad Prism 8.

Figure 1

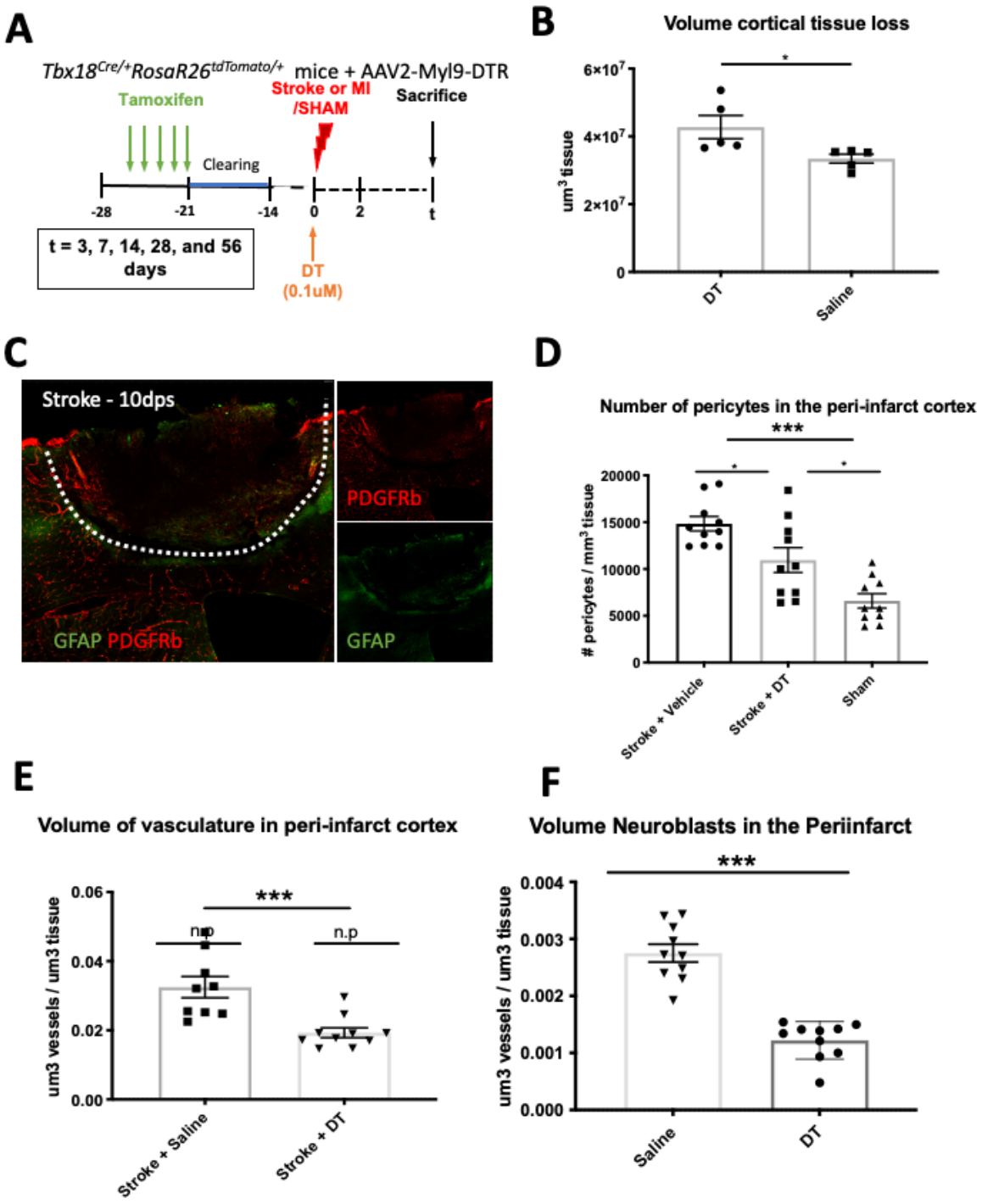

A
Tbx18Cre/+RosaR26tdTomato/+ mice + AAV2-Myl9-DTR
Tamoxifen
Stroke or MI /SHAM
Sacrifice
Clearing
-28
-21
-14
0
2
t
t = 3, 7, 14, 28, and 56 days
DT (0.1uM)
B
Volume cortical tissue loss
um3 tissue
6×10^7
4×10^7
2×10^7
0
DT
Saline
C
Stroke - 10dps
PDGFRb
GFAP PDGFRb
GFAP
D
Number of pericytes in the peri-infarct cortex
pericytes / mm3 tissue
20000
15000
10000
5000
0
Stroke + Vehicle
Stroke + DT
Sham
E
Volume of vasculature in peri-infarct cortex
um3 vessels / um3 tissue
0.06
0.04
0.02
0.00
n.p
n.p
Stroke + Saline
Stroke + DT
F
Volume Neuroblasts in the Periinfarct
um3 vessels / um3 tissue
0.004
0.003
0.002
0.001
0.000
Saline
DT

Figure 1: Ablation of TBX18 + cells enlarged the infarct and halted proliferation in the periinfarct

(A) Experimental timeline **(B)** Volume of infarct was calculated as the volume tissue by subtracting the volume of cortical tissue from the ipsilateral infarct. Ablated animals showed significant increased in infarct size. **(C)** Representative image of the lack of fibrotic tissue (PDGFRb staining) inside the infarct area. **(D-F)** Ablation of TBX18+ cells also affect the regenerative capability in the infarct area: **(D)** pericyte number **(E)** vasculature density and **(F)** number of neuroblasts in the periinfarct cortex Scale bar: 100μm. *p-value < 0.05.

Figure 2

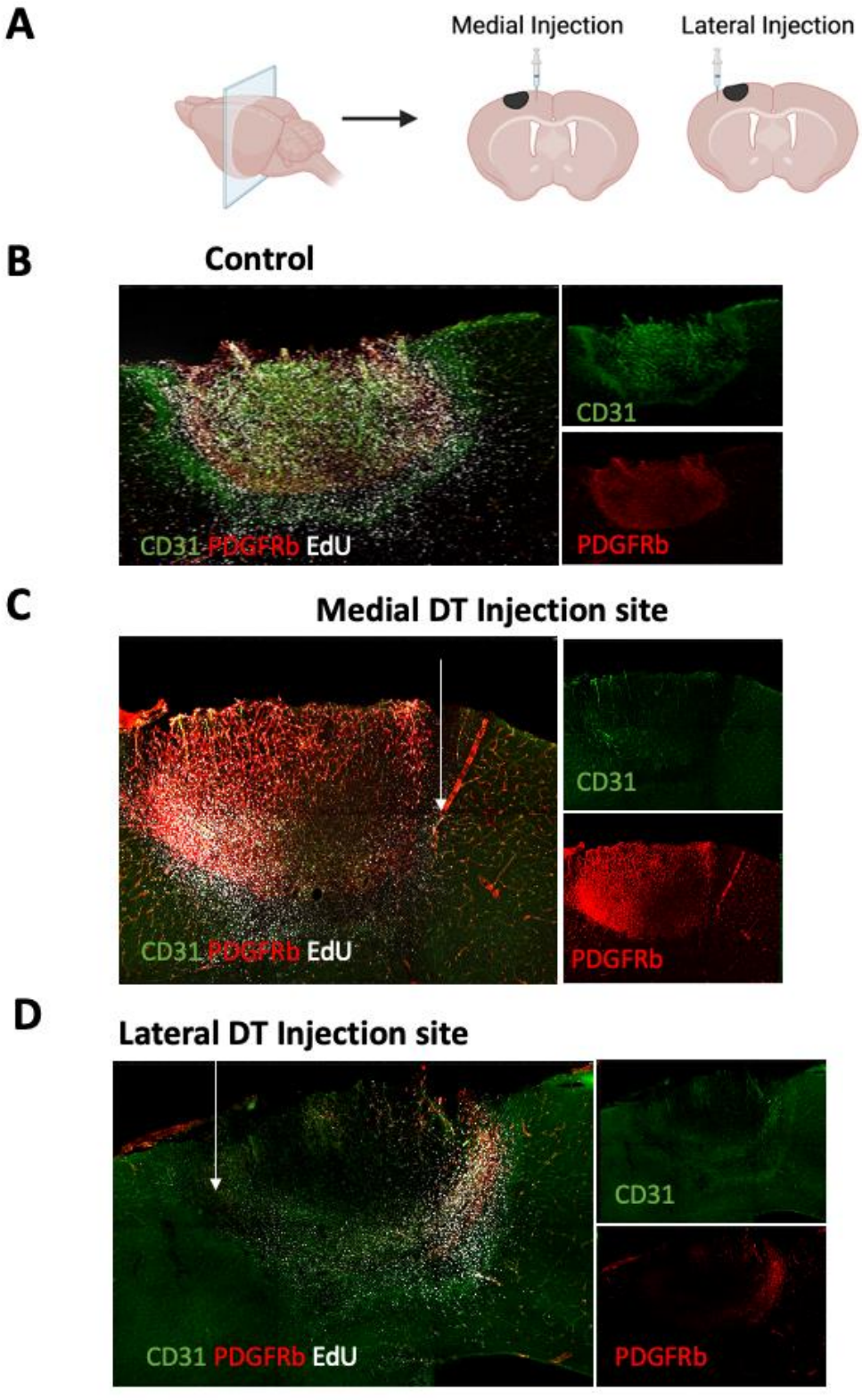

A
Medial Injection
Lateral Injection
B
Control
CD31 PDGFRb EdU
CD31
PDGFRb
C
Medial DT Injection site
CD31 PDGFRb EdU
CD31
PDGFRb
D
Lateral DT Injection site
CD31 PDGFRb EdU
CD31
PDGFRb

Figure 2: Spatial compensation of the fibrotic scar formation

(A) Schematic of the injection in the medial and lateral side of the infarct. **(B)** Sham animal showed equal distribution of the scar laterally. **(C)** Medial DT injection showed ablation occured in the medial side of the infarct and led to reduction in fibrotic scar (PDFRb+) on the medial side while the lateral scar formation was undisturbed. **(D)** Lateral DT injection showed ablation occurred in the lateral side of the infarct and led to reduction of fibrotic scar on the medial side lateral side while the medial side scar formation was undisturbed. Scale bar: 250μm. *p-value < 0.05.

Figure 3

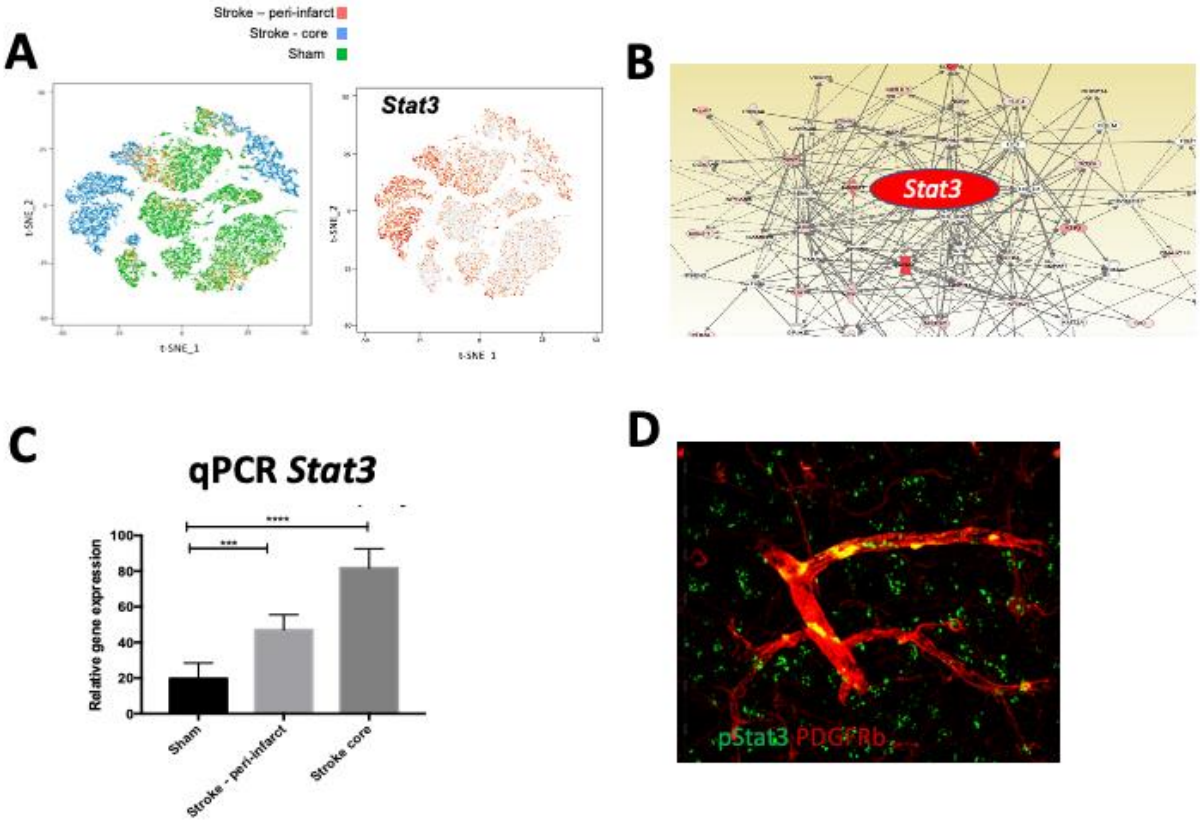

A
Stroke – peri-infarct
Stroke - core
Sham
Stat3
t-SNE_1
t-SNE_2
B
Stat3
C
qPCR Stat3
Relative gene expression
100
80
60
40
20
0
****
***
Sham
Stroke - peri-infarct
Stroke core
D
p5tat3 PDGFRb

Figure 3: *Stat3* is upregulated in pericytes after stroke

(A ,C) *Stat3* is upregulated after stroke, confirmed by the scRNAseq and qPCR of sorted PDGFRB+ cells from stroked PDGFRb $^{CRE/+}$ mice. **(D)** Phosphorylated STAT3 (pSTAT3) is increased after stroke. Scale bar: 50μm, $p<0.05$.

Figure 4

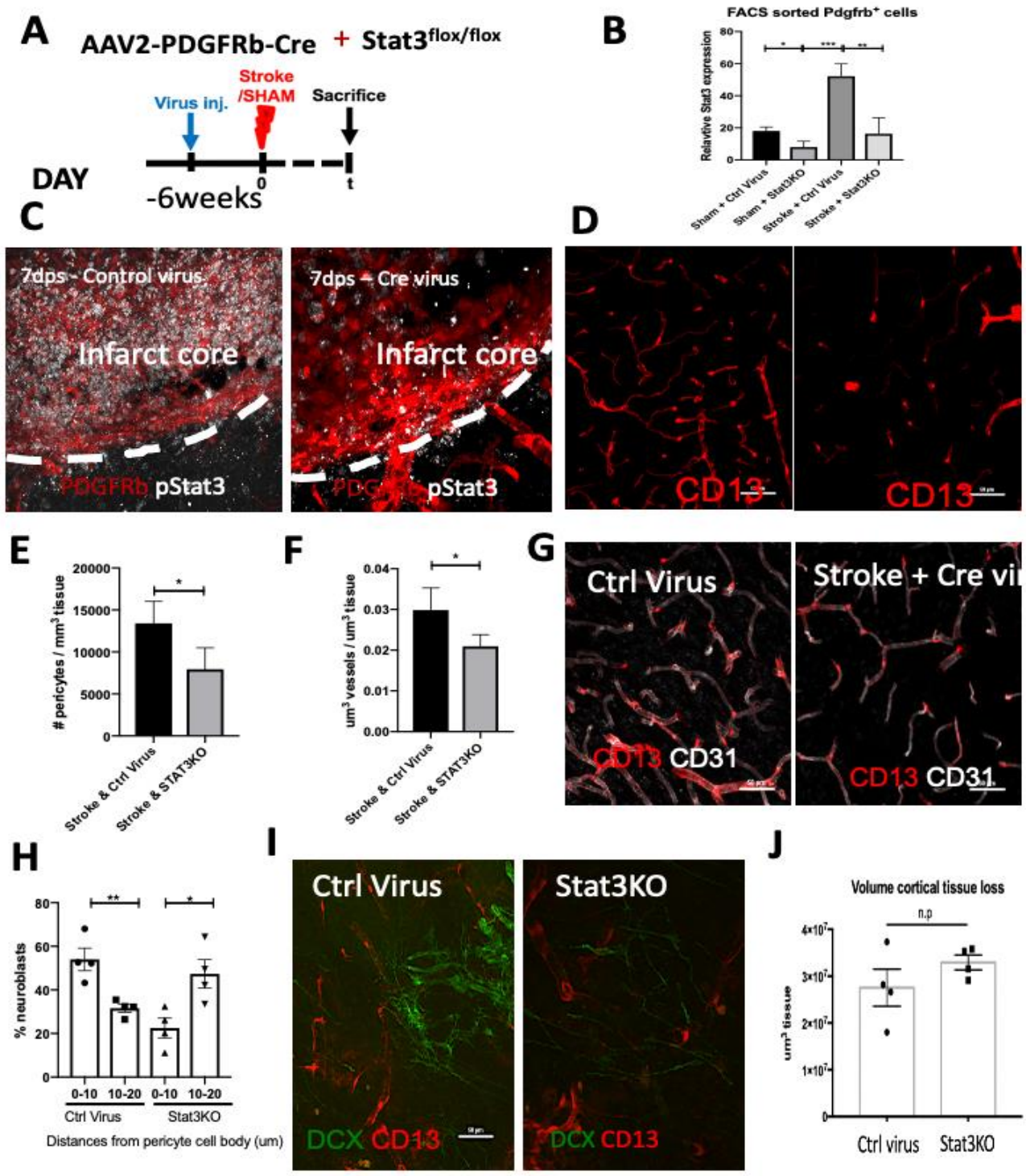
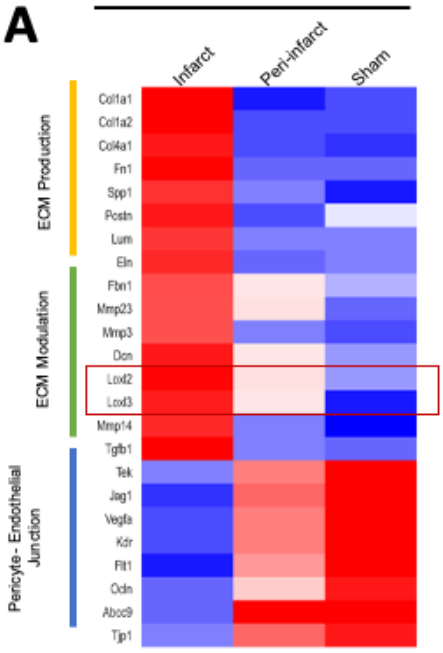
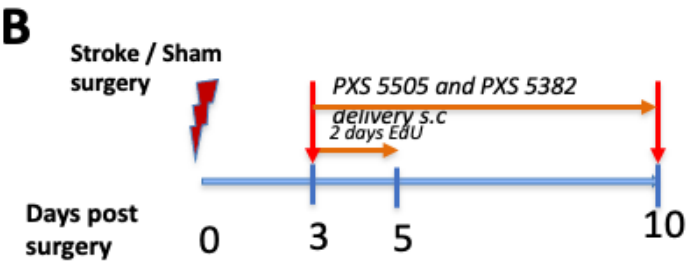

A
AAV2-PDGFRb-Cre + Stat3flox/flox
Virus inj.
Stroke /SHAM
Sacrifice
DAY
-6weeks
0
t
B
FACS sorted Pdgfrb+ cells
Relative Stat3 expression
Sham + Ctrl Virus
Sham + Stat3KO
Stroke + Ctrl Virus
Stroke + Stat3KO
C
7dps - Control virus
Infarct core
PDGFRb pStat3
7dps - Cre virus
Infarct core
PDGFRb pStat3
D
CD13
CD13
E
pericytes / mm3 tissue
Stroke & Ctrl Virus
Stroke & STAT3KO
F
um3 vessels / um3 tissue
Stroke & Ctrl Virus
Stroke & STAT3KO
G
Ctrl Virus
CD13 CD31
Stroke + Cre vi
CD13 CD31
H
% neuroblasts
0-10
10-20
0-10
10-20
Ctrl Virus
Stat3KO
Distances from pericyte cell body (um)
I
Ctrl Virus
DCX CD13
Stat3KO
DCX CD13
J
Volume cortical tissue loss
n.p
um3 tissue
Ctrl virus
Stat3KO

Figure 4: Stat3 knockdown decreased periinfarct neurovascular cells density and number.

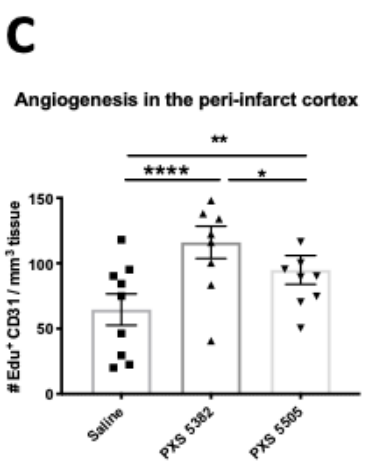
(A) Experimental timeline to knockdown Stat3. **(B)** qPCR of FACS-sorted PDGFRb+ cells from experimental animals and control animals confirmed the knockdown level of Stat3 after viral ablation. **(C)** pSTAT3 level was decreased after ablation. **(D-E)** *Stat3* knockdown animals showed reduction in pericyte numbers and (**F-G)** vascular density . **(H-I)** *Stat3* knockdown also reduced neuroblasts association in the neurovascular niche. **(J)** *Stat3* knockdown did not change the infarct size.

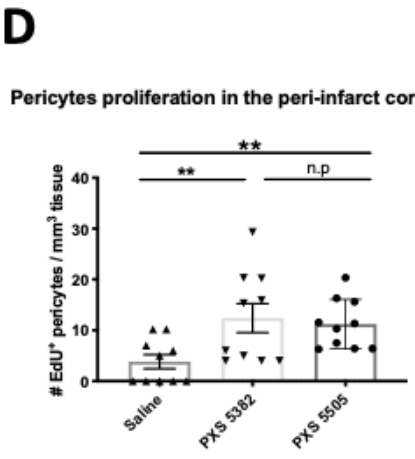
Figure 5





PXS-5382:
3mg/100uL – Alzet 1004 pump
PXS-5505:
15mg/100uL– Alzet 1007D [ump





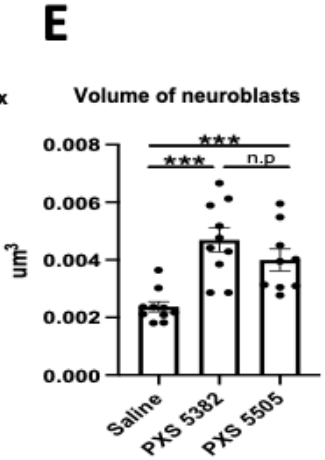


Figure 5. LOXL2/3 inhibitors promoted regeneration in the periinfarct tissue.

(A) Heatmap of differentially expressed genes aross sample that showed LOXL2 and LOXL3

were highly expressed in the infarct and periinfarct samples compared to sham. **(B)** Experimental timeline **(C-E)** LOX2/3 inhibitors-treated group showed significant increased in angiogenesis **(C),** pericytes proliferation **(D)** and neuroblasts number **(E)** in the periinfarct cortex after stroke

Figure 6

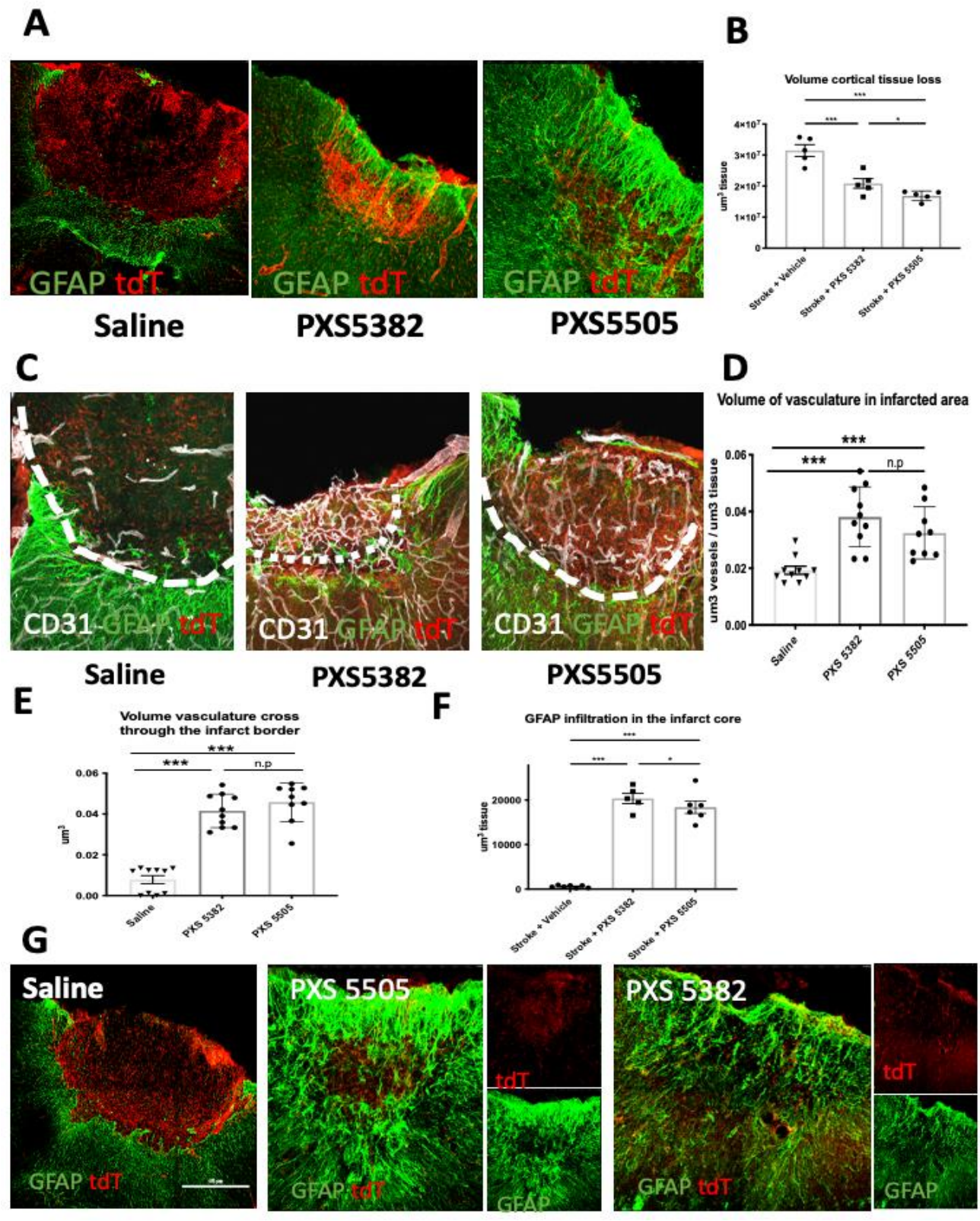

A
GFAP tdT
Saline
PXS5382
PXS5505
B
Volume cortical tissue loss
um³ tissue
Stroke + Vehicle
Stroke + PXS 5382
Stroke + PXS 5505
C
CD31 GFAP tdT
Saline
PXS5382
PXS5505
D
Volume of vasculature in infarcted area
um3 vessels / um3 tissue
n.p
Saline
PXS 5382
PXS 5505
E
Volume vasculature cross through the infarct border
um³
n.p
Saline
PXS 5382
PXS 5505
F
GFAP infiltration in the infarct core
um³ tissue
Stroke + Vehicle
Stroke + PXS 5382
Stroke + PXS 5505
G
Saline
PXS 5505
PXS 5382
tdT
GFAP

Figure 6. LOXL2/3 inhibitors significantly reduced the infarct size and promote regeneration after stroke.

(A -B) LOXL2/3 inhibitors decreased infarct size significantly. **(C-D)** LOXL2/3 inhibitors increased vasculature density inside the infarct **(E-G)** Treated animals significantly decrease the infarct size and **(F)** increased GFAP infiltration in the infarct core. Scale bar = 50μm. tdT = tdTomato.

REFERENCES

Ahmed, T. A., & El-Badri, N. (2018). Pericytes: The Role of Multipotent Stem Cells in Vascular Maintenance and Regenerative Medicine. *Advances in Experimental Medicine and Biology*, *1079*, 69–86. https://doi.org/10.1007/5584_2017_138

Alarcon-Martinez, L., Yilmaz-Ozcan, S., Yemisci, M., Schallek, J., Kılıç, K., Can, A., Di Polo, A., & Dalkara, T. (2018). Capillary pericytes express α-smooth muscle actin, which requires prevention of filamentous-actin depolymerization for detection. *ELife*, *7*, e34861. https://doi.org/10.7554/eLife.34861

Alex, L., & Frangogiannis, N. G. (2019). Pericytes in the infarcted heart. *Vascular Biology*, *1*(1), H23–H31. https://doi.org/10.1530/VB-19-0007

Alexa, A., & Rahnenfuhrer, J. (n.d.). *Gene set enrichment analysis with topGO*. 26.

Ali, S. R., Ranjbarvaziri, S., Talkhabi, M., Zhao, P., Subat, A., Hojjat, A., Kamran, P., Müller, A. M. S., Volz, K. S., Tang, Z., Red-Horse, K., & Ardehali, R. (2014). Developmental heterogeneity of cardiac fibroblasts does not predict pathological proliferation and activation. *Circulation Research*, *115*(7), 625–635. https://doi.org/10.1161/CIRCRESAHA.115.303794

Anders, S., & Huber, W. (2010). Differential expression analysis for sequence count data. *Nature Precedings*, 1–1. https://doi.org/10.1038/npre.2010.4282.1

Anderson, M. A., Burda, J. E., Ren, Y., Ao, Y., O'Shea, T. M., Kawaguchi, R., Coppola, G., Khakh, B. S., Deming, T. J., & Sofroniew, M. V. (2016). Astrocyte scar formation aids central nervous system axon regeneration. *Nature*, *532*(7598), 195–200. https://doi.org/10.1038/nature17623

Armulik, A., Abramsson, A., & Betsholtz, C. (2005). Endothelial/Pericyte Interactions. *Circulation Research*, *97*(6), 512–523. https://doi.org/10.1161/01.RES.0000182903.16652.d7

Armulik, A., Genové, G., & Betsholtz, C. (2011). Pericytes: Developmental, physiological, and pathological perspectives, problems, and promises. *Developmental Cell*, *21*(2), 193–215. https://doi.org/10.1016/j.devcel.2011.07.001

Attwell, D., Mishra, A., Hall, C. N., O'Farrell, F. M., & Dalkara, T. (2016). What is a pericyte? *Journal of*

Cerebral Blood Flow & Metabolism, *36*(2), 451–455. https://doi.org/10.1177/0271678X15610340

Aumiller, V., Strobel, B., Romeike, M., Schuler, M., Stierstorfer, B. E., & Kreuz, S. (2017). Comparative analysis of lysyl oxidase (like) family members in pulmonary fibrosis. *Scientific Reports*, *7*(1), 149. https://doi.org/10.1038/s41598-017-00270-0

Barnabé-Heider, F., Göritz, C., Sabelström, H., Takebayashi, H., Pfrieger, F. W., Meletis, K., & Frisén, J. (2010). Origin of new glial cells in intact and injured adult spinal cord. *Cell Stem Cell*, *7*(4), 470–482. https://doi.org/10.1016/j.stem.2010.07.014

Barreto, G., White, R. E., Ouyang, Y., Xu, L., & Giffard, R. G. (2011). Astrocytes: Targets for Neuroprotection in Stroke. *Central Nervous System Agents in Medicinal Chemistry*, *11*(2), 164–173.

Becerra-Calixto, A., & Cardona-Gómez, G. P. (2017). The Role of Astrocytes in Neuroprotection after Brain Stroke: Potential in Cell Therapy. *Frontiers in Molecular Neuroscience*, *10*, 88. https://doi.org/10.3389/fnmol.2017.00088

Bell, R. D., Winkler, E. A., Sagare, A. P., Singh, I., LaRue, B., Deane, R., & Zlokovic, B. V. (2010). Pericytes Control Key Neurovascular Functions and Neuronal Phenotype in the Adult Brain and during Brain Aging. *Neuron*, *68*(3), 409–427. https://doi.org/10.1016/j.neuron.2010.09.043

Benjamin, E. J., Virani, S. S., Callaway, C. W., Chamberlain, A. M., Chang, A. R., Cheng, S., Chiuve, S. E., Cushman, M., Delling, F. N., Deo, R., de Ferranti, S. D., Ferguson, J. F., Fornage, M., Gillespie, C., Isasi, C. R., Jiménez, M. C., Jordan, L. C., Judd, S. E., Lackland, D., … Muntner, P. (2018). Heart Disease and Stroke Statistics—2018 Update: A Report From the American Heart Association. *Circulation*, *137*(12). https://doi.org/10.1161/CIR.0000000000000558

Benowitz, L. I., & Carmichael, S. T. (2010). Promoting axonal rewiring to improve outcome after stroke. *Neurobiology of Disease*, *37*(2), 259. https://doi.org/10.1016/j.nbd.2009.11.009

Bergers, G., & Song, S. (2005). The role of pericytes in blood-vessel formation and maintenance. *Neuro-Oncology*, *7*(4), 452–464. https://doi.org/10.1215/S1152851705000232

Birbrair, A., Zhang, T., Wang, Z.-M., Messi, M. L., Olson, J. D., Mintz, A., & Delbono, O. (2014). Type-2

pericytes participate in normal and tumoral angiogenesis. *American Journal of Physiology. Cell Physiology*, *307*(1), C25-38. https://doi.org/10.1152/ajpcell.00084.2014

Bish, L. T., Morine, K., Sleeper, M. M., Sanmiguel, J., Wu, D., Gao, G., Wilson, J. M., & Sweeney, H. L. (2008). Adeno-associated virus (AAV) serotype 9 provides global cardiac gene transfer superior to AAV1, AAV6, AAV7, and AAV8 in the mouse and rat. *Human Gene Therapy*, *19*(12), 1359–1368. https://doi.org/10.1089/hum.2008.123

Bodhankar, S., Lapato, A., Chen, Y., Vandenbark, A. A., Saugstad, J. A., & Offner, H. (2015). Role for microglia in sex differences after ischemic stroke: Importance of M2. *Metabolic Brain Disease*, *30*(6), 1515–1529. https://doi.org/10.1007/s11011-015-9714-9

Brumm, A. J., & Carmichael, S. T. (2012). Not just a rush of blood to the head. *Nature Medicine*, *18*(11), 1609–1610. https://doi.org/10.1038/nm.2990

Bundesen, L. Q., Scheel, T. A., Bregman, B. S., & Kromer, L. F. (2003). Ephrin-B2 and EphB2 Regulation of Astrocyte-Meningeal Fibroblast Interactions in Response to Spinal Cord Lesions in Adult Rats. *The Journal of Neuroscience*, *23*(21), 7789–7800. https://doi.org/10.1523/JNEUROSCI.23-21-07789.2003

Butler, A., Hoffman, P., Smibert, P., Papalexi, E., & Satija, R. (2018). Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nature Biotechnology*, *36*(5), 411–420. https://doi.org/10.1038/nbt.4096

Cafferty, W. B. J., Yang, S.-H., Duffy, P. J., Li, S., & Strittmatter, S. M. (2007). Functional Axonal Regeneration through Astrocytic Scar Genetically Modified to Digest Chondroitin Sulfate Proteoglycans. *Journal of Neuroscience*, *27*(9), 2176–2185. https://doi.org/10.1523/JNEUROSCI.5176-06.2007

Campbell, J. B., & Windle, W. F. (1960). Relation of millipore to healing and regeneration in transected spinal cords of monkeys. *Neurology*, *10*, 306–311. https://doi.org/10.1212/wnl.10.3.306

Carmichael, S. T. (2005). Rodent models of focal stroke: Size, mechanism, and purpose. *NeuroRX*, *2*(3), 396–409. https://doi.org/10.1602/neurorx.2.3.396

Carmichael, S. T. (2010). Translating the frontiers of brain repair to treatments: Starting not to break the rules. *Neurobiology of Disease*, *37*(2), 237–242. https://doi.org/10.1016/j.nbd.2009.09.005

Carmichael, S. T. (2016). The 3 Rs of Stroke Biology: Radial, Relayed, and Regenerative. *Neurotherapeutics*, *13*(2), 348–359. https://doi.org/10.1007/s13311-015-0408-0

Carrica, L., Li, L., Newville, J., Kenton, J., Gustus, K., Brigman, J., & Cunningham, L. A. (2019). Genetic inactivation of hypoxia inducible factor 1-alpha (HIF-1α) in adult hippocampal progenitors impairs neurogenesis and pattern discrimination learning. *Neurobiology of Learning and Memory*, *157*, 79–85. https://doi.org/10.1016/j.nlm.2018.12.002

Cha, J., & Lee, I. (2020). Single-cell network biology for resolving cellular heterogeneity in human diseases. *Experimental & Molecular Medicine*, *52*(11), 1798–1808. https://doi.org/10.1038/s12276-020-00528-0

Chang, J., Lucas, M. C., Leonte, L. E., Garcia-Montolio, M., Singh, L. B., Findlay, A. D., Deodhar, M., Foot, J. S., Jarolimek, W., Timpson, P., Erler, J. T., & Cox, T. R. (2017). Pre-clinical evaluation of small molecule LOXL2 inhibitors in breast cancer. *Oncotarget*, *8*(16), 26066–26078. https://doi.org/10.18632/oncotarget.15257

Chen, Y.-T., Chang, F.-C., Wu, C.-F., Chou, Y.-H., Hsu, H.-L., Chiang, W.-C., Shen, J., Chen, Y.-M., Wu, K.-D., Tsai, T.-J., Duffield, J. S., & Lin, S.-L. (2011). Platelet-derived growth factor receptor signaling activates pericyte-myofibroblast transition in obstructive and post-ischemic kidney fibrosis. *Kidney International*, *80*(11), 1170–1181. https://doi.org/10.1038/ki.2011.208

Chia, N. H., Leyden, J. M., Newbury, J., Jannes, J., & Kleinig, T. J. (2016). Determining the Number of Ischemic Strokes Potentially Eligible for Endovascular Thrombectomy: A Population-Based Study. *Stroke*, *47*(5), 1377–1380. https://doi.org/10.1161/STROKEAHA.116.013165

Choi, Y. K., Maki, T., Mandeville, E. T., Koh, S.-H., Hayakawa, K., Arai, K., Kim, Y.-M., Whalen, M. J., Xing, C., Wang, X., Kim, K.-W., & Lo, E. H. (2016). Dual effects of carbon monoxide on pericytes and neurogenesis in traumatic brain injury. *Nature Medicine*, *22*(11), 1335–1341. https://doi.org/10.1038/nm.4188

de Jong, S., van Veen, T. A. B., de Bakker, J. M. T., & van Rijen, H. V. M. (2012). Monitoring cardiac fibrosis: A technical challenge. *Netherlands Heart Journal*, *20*(1), 44–48. https://doi.org/10.1007/s12471-011-0226-x

De La Fuente, A. G., Lange, S., Silva, M. E., Gonzalez, G. A., Tempfer, H., van Wijngaarden, P., Zhao, C., Di Canio, L., Trost, A., Bieler, L., Zaunmair, P., Rotheneichner, P., O'Sullivan, A., Couillard-Despres, S., Errea, O., Mäe, M. A., Andrae, J., He, L., Keller, A., … Rivera, F. J. (2017). Pericytes Stimulate Oligodendrocyte Progenitor Cell Differentiation during CNS Remyelination. *Cell Reports*, *20*(8), 1755–1764. https://doi.org/10.1016/j.celrep.2017.08.007

de Winter, F., Kwok, J. C. F., Fawcett, J. W., Vo, T. T., Carulli, D., & Verhaagen, J. (2016). The Chemorepulsive Protein Semaphorin 3A and Perineuronal Net-Mediated Plasticity. *Neural Plasticity*, *2016*, 3679545. https://doi.org/10.1155/2016/3679545

DeMers, G., Meurer, W. J., Shih, R., Rosenbaum, S., & Vilke, G. M. (2012). Tissue plasminogen activator and stroke: Review of the literature for the clinician. *The Journal of Emergency Medicine*, *43*(6), 1149–1154. https://doi.org/10.1016/j.jemermed.2012.05.005

Dias, D. O., Kalkitsas, J., Kelahmetoglu, Y., Estrada, C. P., Tatarishvili, J., Ernst, A., Huttner, H. B., Kokaia, Z., Lindvall, O., Brundin, L., Frisén, J., & Göritz, C. (2020a). *Pericyte-derived fibrotic scarring is conserved across diverse central nervous system lesions* [Preprint]. Neuroscience. https://doi.org/10.1101/2020.04.30.068965

Dias, D. O., Kalkitsas, J., Kelahmetoglu, Y., Estrada, C. P., Tatarishvili, J., Ernst, A., Huttner, H. B., Kokaia, Z., Lindvall, O., Brundin, L., Frisén, J., & Göritz, C. (2020b). Pericyte-derived fibrotic scarring is conserved across diverse central nervous system lesions. *BioRxiv*, 2020.04.30.068965. https://doi.org/10.1101/2020.04.30.068965

Dias, D. O., Kim, H., Holl, D., Werne Solnestam, B., Lundeberg, J., Carlén, M., Göritz, C., & Frisén, J. (2018). Reducing Pericyte-Derived Scarring Promotes Recovery after Spinal Cord Injury. *Cell*, *173*(1), 153-165.e22. https://doi.org/10.1016/j.cell.2018.02.004

Dorrier, C. E., Aran, D., Haenelt, E. A., Sheehy, R. N., Hoi, K. K., Pintarić, L., Chen, Y., Lizama, C. O.,

Cautivo, K. M., Weiner, G. A., Popko, B., Fancy, S. P. J., Arnold, T. D., & Daneman, R. (2021). CNS fibroblasts form a fibrotic scar in response to immune cell infiltration. *Nature Neuroscience*, *24*(2), 234–244. https://doi.org/10.1038/s41593-020-00770-9

Edelman, D. A., Jiang, Y., Tyburski, J. G., Wilson, R. F., & Steffes, C. P. (2007). Cytokine production in lipopolysaccharide-exposed rat lung pericytes. *The Journal of Trauma*, *62*(1), 89–93. https://doi.org/10.1097/TA.0b013e31802dd712

Ellison, J. A., Velier, J. J., Spera, P., Jonak, Z. L., Wang, X., Barone, F. C., & Feuerstein, G. Z. (1998). Osteopontin and its integrin receptor alpha(v)beta3 are upregulated during formation of the glial scar after focal stroke. *Stroke*, *29*(8), 1698–1706; discussion 1707. https://doi.org/10.1161/01.str.29.8.1698

Ergul, A., Alhusban, A., & Fagan, S. C. (2012). Angiogenesis: A Harmonized Target for Recovery after Stroke. *Stroke; a Journal of Cerebral Circulation*, *43*(8), 2270–2274. https://doi.org/10.1161/STROKEAHA.111.642710

Ernst, A.-S., Böhler, L.-I., Hagenston, A. M., Hoffmann, A., Heiland, S., Sticht, C., Bendszus, M., Hecker, M., Bading, H., Marti, H. H., Korff, T., & Kunze, R. (2019). EphB2-dependent signaling promotes neuronal excitotoxicity and inflammation in the acute phase of ischemic stroke. *Acta Neuropathologica Communications*, *7*(1), 15. https://doi.org/10.1186/s40478-019-0669-7

Fei, Z., Zhang, X., Bai, H., Jiang, X., Li, X., Zhang, W., & Hu, W. (2007). Posttraumatic secondary brain insults exacerbates neuronal injury by altering Metabotropic Glutamate Receptors. *BMC Neuroscience*, *8*(1), 96. https://doi.org/10.1186/1471-2202-8-96

Fernandez, E., & Pallini, R. (1985). Connective tissue scarring in experimental spinal cord lesions: Significance of dural continuity and role of epidural tissues. *Acta Neurochirurgica*, *76*(3), 145–148. https://doi.org/10.1007/BF01418478

Fernández-Klett, F., Potas, J. R., Hilpert, D., Blazej, K., Radke, J., Huck, J., Engel, O., Stenzel, W., Genové, G., & Priller, J. (2013). Early loss of pericytes and perivascular stromal cell-induced scar formation after stroke. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of*

the International Society of Cerebral Blood Flow and Metabolism, *33*(3), 428–439. https://doi.org/10.1038/jcbfm.2012.187

Fernández-Klett, F., & Priller, J. (2014). The fibrotic scar in neurological disorders. *Brain Pathology (Zurich, Switzerland)*, *24*(4), 404–413. https://doi.org/10.1111/bpa.12162

Göritz, C., Dias, D. O., Tomilin, N., Barbacid, M., Shupliakov, O., & Frisén, J. (2011). A Pericyte Origin of Spinal Cord Scar Tissue. *Science*, *333*(6039), 238–242. https://doi.org/10.1126/science.1203165

Guimarães-Camboa, N., Cattaneo, P., Sun, Y., Moore-Morris, T., Gu, Y., Dalton, N. D., Rockenstein, E., Masliah, E., Peterson, K. L., Stallcup, W. B., Chen, J., & Evans, S. M. (2017). Pericytes of Multiple Organs Do Not Behave as Mesenchymal Stem Cells In Vivo. *Cell Stem Cell*, *20*(3), 345-359.e5. https://doi.org/10.1016/j.stem.2016.12.006

Guo, J., Bertalan, G., Meierhofer, D., Klein, C., Schreyer, S., Steiner, B., Wang, S., Vieira da Silva, R., Infante-Duarte, C., Koch, S., Boehm-Sturm, P., Braun, J., & Sack, I. (2019). Brain maturation is associated with increasing tissue stiffness and decreasing tissue fluidity. *Acta Biomaterialia*, *99*, 433–442. https://doi.org/10.1016/j.actbio.2019.08.036

Hall, C. N., Reynell, C., Gesslein, B., Hamilton, N. B., Mishra, A., Sutherland, B. A., O'Farrell, F. M., Buchan, A. M., Lauritzen, M., & Attwell, D. (2014). Capillary pericytes regulate cerebral blood flow in health and disease. *Nature*, *508*(7494), 55–60. https://doi.org/10.1038/nature13165

Hao, J., Li, B., Duan, H.-Q., Zhao, C.-X., Zhang, Y., Sun, C., Pan, B., Liu, C., Kong, X.-H., Yao, X., & Feng, S.-Q. (2017). Mechanisms underlying the promotion of functional recovery by deferoxamine after spinal cord injury in rats. *Neural Regeneration Research*, *12*(6), 959–968. https://doi.org/10.4103/1673-5374.208591

Hara, M., Kobayakawa, K., Ohkawa, Y., Kumamaru, H., Yokota, K., Saito, T., Kijima, K., Yoshizaki, S., Harimaya, K., Nakashima, Y., & Okada, S. (2017). Interaction of reactive astrocytes with type I collagen induces astrocytic scar formation through the integrin–N-cadherin pathway after spinal cord injury. *Nature Medicine*, *23*(7), 818–828. https://doi.org/10.1038/nm.4354

Hardcastle, T. J., & Kelly, K. A. (2010). baySeq: Empirical Bayesian methods for identifying differential expression in sequence count data. *BMC Bioinformatics*, *11*(1), 422. https://doi.org/10.1186/1471-2105-11-422

Hartmann, D. A., Underly, R. G., Grant, R. I., Watson, A. N., Lindner, V., & Shih, A. Y. (2015). Pericyte structure and distribution in the cerebral cortex revealed by high-resolution imaging of transgenic mice. *Neurophotonics*, *2*(4), 041402. https://doi.org/10.1117/1.NPh.2.4.041402

Hatakeyama. (n.d.). *Angiogenesis and neuronal remodeling after ischemic stroke*. Retrieved August 4, 2021, from https://www.nrronline.org/article.asp?issn=1673-5374;year=2020;volume=15;issue=1;spage=16;epage=19;aulast=Hatakeyama

Hellal, F., Hurtado, A., Ruschel, J., Flynn, K. C., Laskowski, C. J., Umlauf, M., Kapitein, L. C., Strikis, D., Lemmon, V., Bixby, J., Hoogenraad, C. C., & Bradke, F. (2011). Microtubule stabilization reduces scarring and causes axon regeneration after spinal cord injury. *Science (New York, N.Y.)*, *331*(6019), 928–931. https://doi.org/10.1126/science.1201148

Hesp, Z. C., Yoseph, R. Y., Suzuki, R., Jukkola, P., Wilson, C., Nishiyama, A., & McTigue, D. M. (2018). Proliferating NG2-Cell-Dependent Angiogenesis and Scar Formation Alter Axon Growth and Functional Recovery After Spinal Cord Injury in Mice. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *38*(6), 1366–1382. https://doi.org/10.1523/JNEUROSCI.3953-16.2017

Hill, R. A., Tong, L., Yuan, P., Murikinati, S., Gupta, S., & Grutzendler, J. (2015). Regional Blood Flow in the Normal and Ischemic Brain Is Controlled by Arteriolar Smooth Muscle Cell Contractility and Not by Capillary Pericytes. *Neuron*, *87*(1), 95–110. https://doi.org/10.1016/j.neuron.2015.06.001

Hori, S., Ohtsuki, S., Hosoya, K., Nakashima, E., & Terasaki, T. (2004). A pericyte-derived angiopoietin-1 multimeric complex induces occludin gene expression in brain capillary endothelial cells through Tie-2 activation in vitro. *Journal of Neurochemistry*, *89*(2), 503–513. https://doi.org/10.1111/j.1471-4159.2004.02343.x

How, J., Liu, Y., Lombardi Story, J., Neuberg, D. S., Ravid, K., Jarolimek, W., Charlton, B., & Hobbs, G. S. (2020). Evaluation of a Pan-Lysyl Oxidase Inhibitor, Pxs-5505, in Myelofibrosis: A Phase I, Randomized, Placebo Controlled Double Blind Study in Healthy Adults. *Blood*, *136*(Supplement 1), 16–16. https://doi.org/10.1182/blood-2020-139223

Hu, X., Li, P., Guo, Y., Wang, H., Leak, R. K., Chen, S., Gao, Y., & Chen, J. (2012). Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke*, *43*(11), 3063–3070. https://doi.org/10.1161/STROKEAHA.112.659656

Hwang, B., Lee, J. H., & Bang, D. (2018). Single-cell RNA sequencing technologies and bioinformatics pipelines. *Experimental & Molecular Medicine*, *50*(8), 1–14. https://doi.org/10.1038/s12276-018-0071-8

Iihara, K., Sasahara, M., Hashimoto, N., & Hazama, F. (1996). Induction of platelet-derived growth factor beta-receptor in focal ischemia of rat brain. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *16*(5), 941–949. https://doi.org/10.1097/00004647-199609000-00018

Islam, S., Zeisel, A., Joost, S., La Manno, G., Zajac, P., Kasper, M., Lönnerberg, P., & Linnarsson, S. (2014). Quantitative single-cell RNA-seq with unique molecular identifiers. *Nature Methods*, *11*(2), 163–166. https://doi.org/10.1038/nmeth.2772

Jansson, D., Rustenhoven, J., Feng, S., Hurley, D., Oldfield, R. L., Bergin, P. S., Mee, E. W., Faull, R. L., & Dragunow, M. (2014a). A role for human brain pericytes in neuroinflammation. *Journal of Neuroinflammation*, *11*(1), 104. https://doi.org/10.1186/1742-2094-11-104

Jansson, D., Rustenhoven, J., Feng, S., Hurley, D., Oldfield, R. L., Bergin, P. S., Mee, E. W., Faull, R. L., & Dragunow, M. (2014b). A role for human brain pericytes in neuroinflammation. *Journal of Neuroinflammation*, *11*(1), 104. https://doi.org/10.1186/1742-2094-11-104

Ji, Z., & Ji, H. (2016). TSCAN: Pseudo-time reconstruction and evaluation in single-cell RNA-seq analysis. *Nucleic Acids Research*, *44*(13), e117. https://doi.org/10.1093/nar/gkw430

Jiang, W., Gu, W., Brännström, T., Rosqvist, R., & Wester, P. (2001). Cortical neurogenesis in adult rats after transient middle cerebral artery occlusion. *Stroke*, *32*(5), 1201–1207.

Jones, M. G., Andriotis, O. G., Roberts, J. J., Lunn, K., Tear, V. J., Cao, L., Ask, K., Smart, D. E., Bonfanti, A., Johnson, P., Alzetani, A., Conforti, F., Doherty, R., Lai, C. Y., Johnson, B., Bourdakos, K. N., Fletcher, S. V., Marshall, B. G., Jogai, S., … Davies, D. E. (2018). Nanoscale dysregulation of collagen structure-function disrupts mechano-homeostasis and mediates pulmonary fibrosis. *ELife*, *7*, e36354. https://doi.org/10.7554/eLife.36354

Jung, B., Arnold, T. D., Raschperger, E., Gaengel, K., & Betsholtz, C. (2018). Visualization of vascular mural cells in developing brain using genetically labeled transgenic reporter mice. *Journal of Cerebral Blood Flow & Metabolism*, *38*(3), 456–468. https://doi.org/10.1177/0271678X17697720

Kelly-Hayes, M., Beiser, A., Kase, C. S., Scaramucci, A., D'Agostino, R. B., & Wolf, P. A. (2003). The influence of gender and age on disability following ischemic stroke: The Framingham study. *Journal of Stroke and Cerebrovascular Diseases: The Official Journal of National Stroke Association*, *12*(3), 119–126. https://doi.org/10.1016/S1052-3057(03)00042-9

Keough, M. B., Rogers, J. A., Zhang, P., Jensen, S. K., Stephenson, E. L., Chen, T., Hurlbert, M. G., Lau, L. W., Rawji, K. S., Plemel, J. R., Koch, M., Ling, C.-C., & Yong, V. W. (2016). An inhibitor of chondroitin sulfate proteoglycan synthesis promotes central nervous system remyelination. *Nature Communications*, *7*(1), 11312. https://doi.org/10.1038/ncomms11312

Kharchenko, P. V., Silberstein, L., & Scadden, D. T. (2014). Bayesian approach to single-cell differential expression analysis. *Nature Methods*, *11*(7), 740–742. https://doi.org/10.1038/nmeth.2967

Kisler, K., Nelson, A. R., Rege, S. V., Ramanathan, A., Wang, Y., Ahuja, A., Lazic, D., Tsai, P. S., Zhao, Z., Zhou, Y., Boas, D. A., Sakadžić, S., & Zlokovic, B. V. (2017). Pericyte degeneration leads to neurovascular uncoupling and limits oxygen supply to brain. *Nature Neuroscience*, *20*(3), 406–416. https://doi.org/10.1038/nn.4489

Kramann, R., & Humphreys, B. D. (2014). Kidney Pericytes: Roles in Regeneration and Fibrosis.

Seminars in Nephrology, *34*(4), 374–383. https://doi.org/10.1016/j.semnephrol.2014.06.004

Lang, B. T., Cregg, J. M., DePaul, M. A., Tran, A. P., Xu, K., Dyck, S. M., Madalena, K. M., Brown, B. P., Weng, Y.-L., Li, S., Karimi-Abdolrezaee, S., Busch, S. A., Shen, Y., & Silver, J. (2015). Modulation of the proteoglycan receptor PTPσ promotes recovery after spinal cord injury. *Nature*, *518*(7539), 404–408. https://doi.org/10.1038/nature13974

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*(1), 559. https://doi.org/10.1186/1471-2105-9-559

Lei, X., Guan, C.-W., Song, Y., & Wang, H. (2015). The multifaceted role of CD146/MCAM in the promotion of melanoma progression. *Cancer Cell International*, *15*, 3. https://doi.org/10.1186/s12935-014-0147-z

Leiva, O., Matsuura, S., Findlay, A., Turner, C., Jarolimek, W., & Ravid, K. (2017). A Novel Lysyl Oxidase Inhibitor Attenuates Bone Marrow Fibrosis and Decreases Spleen Size in a Mouse Model of Primary Myelofibrosis. *Blood*, *130*(Supplement 1), 4214–4214. https://doi.org/10.1182/blood.V130.Suppl_1.4214.4214

Leng, N., Dawson, J. A., Thomson, J. A., Ruotti, V., Rissman, A. I., Smits, B. M. G., Haag, J. D., Gould, M. N., Stewart, R. M., & Kendziorski, C. (2013). EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics*, *29*(8), 1035–1043. https://doi.org/10.1093/bioinformatics/btt087

Li, L., Huang, Y., Yang, Z., Zhang, S., Han, Z., & Luo, Y. (2020). Potential microglia-based interventions for stroke. *CNS Neuroscience & Therapeutics*, *26*(3), 288–296. https://doi.org/10.1111/cns.13291

Li, S., Nie, E. H., Yin, Y., Benowitz, L. I., Tung, S., Vinters, H. V., Bahjat, F. R., Stenzel-Poore, M. P., Kawaguchi, R., Coppola, G., & Carmichael, S. T. (2015). GDF10 is a signal for axonal sprouting and functional recovery after stroke. *Nature Neuroscience*, *18*(12), 1737–1745. https://doi.org/10.1038/nn.4146

Lindvall, O., & Kokaia, Z. (2015). Neurogenesis following Stroke Affecting the Adult Brain. *Cold Spring*

Harbor Perspectives in Biology, *7*(11), a019034. https://doi.org/10.1101/cshperspect.a019034

Liu, J., Tang, T., & Yang, H. (2011). Protective effect of deferoxamine on experimental spinal cord injury in rat. *Injury*, *42*(8), 742–745. https://doi.org/10.1016/j.injury.2010.08.028

Luissint, A.-C., Artus, C., Glacial, F., Ganeshamoorthy, K., & Couraud, P.-O. (2012). Tight junctions at the blood brain barrier: Physiological architecture and disease-associated dysregulation. *Fluids and Barriers of the CNS*, *9*(1), 23. https://doi.org/10.1186/2045-8118-9-23

Ma, L., Zeng, Y., Wei, J., Yang, D., Ding, G., Liu, J., Shang, J., Kang, Y., & Ji, X. (2018). Knockdown of LOXL1 inhibits TGF-β1-induced proliferation and fibrogenesis of hepatic stellate cells by inhibition of Smad2/3 phosphorylation. *Biomedicine & Pharmacotherapy*, *107*, 1728–1735. https://doi.org/10.1016/j.biopha.2018.08.156

Ma, Q., Zhao, Z., Sagare, A. P., Wu, Y., Wang, M., Owens, N. C., Verghese, P. B., Herz, J., Holtzman, D. M., & Zlokovic, B. V. (2018). Blood-brain barrier-associated pericytes internalize and clear aggregated amyloid-β42 by LRP1-dependent apolipoprotein E isoform-specific mechanism. *Molecular Neurodegeneration*, *13*(1), 57. https://doi.org/10.1186/s13024-018-0286-0

Ma, Y., Sun, S., Shang, X., Keller, E. T., Chen, M., & Zhou, X. (2020). Integrative differential expression and gene set enrichment analysis using summary statistics for scRNA-seq studies. *Nature Communications*, *11*(1), 1585. https://doi.org/10.1038/s41467-020-15298-6

Madsen, T. E., Khoury, J. C., Leppert, M., Alwell, K., Moomaw, C. J., Sucharew, H., Woo, D., Ferioli, S., Martini, S., Adeoye, O., Khatri, P., Flaherty, M., De Los Rios La Rosa, F., Mackey, J., Mistry, E., Demel, S. L., Coleman, E., Jasne, A., Slavin, S. J., … Kleindorfer, D. O. (2020). Temporal Trends in Stroke Incidence Over Time by Sex and Age in the GCNKSS. *Stroke*, *51*(4), 1070–1076. https://doi.org/10.1161/STROKEAHA.120.028910

Makihara, N., Arimura, K., Ago, T., Tachibana, M., Nishimura, A., Nakamura, K., Matsuo, R., Wakisaka, Y., Kuroda, J., Sugimori, H., Kamouchi, M., & Kitazono, T. (2015). Involvement of platelet-derived growth factor receptor β in fibrosis through extracellular matrix protein production after ischemic stroke. *Experimental Neurology*, *264*, 127–134.

https://doi.org/10.1016/j.expneurol.2014.12.007

Mathys, H., Adaikkan, C., Gao, F., Young, J. Z., Manet, E., Hemberg, M., De Jager, P. L., Ransohoff, R. M., Regev, A., & Tsai, L.-H. (2017). Temporal Tracking of Microglia Activation in Neurodegeneration at Single-Cell Resolution. *Cell Reports*, *21*(2), 366–380. https://doi.org/10.1016/j.celrep.2017.09.039

Matsuo, A., Tanida, R., Yanagi, S., Tsubouchi, H., Miura, A., Shigekusa, T., Matsumoto, N., & Nakazato, M. (2021). Significance of nuclear LOXL2 inhibition in fibroblasts and myofibroblasts in the fibrotic process of acute respiratory distress syndrome. *European Journal of Pharmacology*, *892*, 173754. https://doi.org/10.1016/j.ejphar.2020.173754

Meletis, K., Barnabé-Heider, F., Carlén, M., Evergren, E., Tomilin, N., Shupliakov, O., & Frisén, J. (2008). Spinal Cord Injury Reveals Multilineage Differentiation of Ependymal Cells. *PLOS Biology*, *6*(7), e182. https://doi.org/10.1371/journal.pbio.0060182

Mishra, A., Reynolds, J. P., Chen, Y., Gourine, A. V., Rusakov, D. A., & Attwell, D. (2016). Astrocytes mediate neurovascular signaling to capillary pericytes but not to arterioles. *Nature Neuroscience*, *19*(12), 1619–1627. https://doi.org/10.1038/nn.4428

Montagne, A., Nikolakopoulou, A. M., Zhao, Z., Sagare, A. P., Si, G., Lazic, D., Barnes, S. R., Daianu, M., Ramanathan, A., Go, A., Lawson, E. J., Wang, Y., Mack, W. J., Thompson, P. M., Schneider, J. A., Varkey, J., Langen, R., Mullins, E., Jacobs, R. E., & Zlokovic, B. V. (2018). Pericyte degeneration causes white matter dysfunction in the mouse central nervous system. *Nature Medicine*, *24*(3), 326–337. https://doi.org/10.1038/nm.4482

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., de Ferranti, S., Després, J.-P., Fullerton, H. J., Howard, V. J., Huffman, M. D., Judd, S. E., Kissela, B. M., Lackland, D. T., Lichtman, J. H., Lisabeth, L. D., Liu, S., Mackey, R. H., Matchar, D. B., … Turner, M. B. (2015). Heart Disease and Stroke Statistics—2015 Update: A Report From the American Heart Association. *Circulation*, *131*(4). https://doi.org/10.1161/CIR.0000000000000152

Neo, S. H., & Tang, B. L. (2017). Collagen 1 signaling at the central nervous system injury site and astrogliosis. *Neural Regeneration Research*, *12*(10), 1600–1601. https://doi.org/10.4103/1673-5374.217323

Nguyen, D. T., Ding, C., Wilson, E., Marcus, G. M., & Olgin, J. E. (2010). Pirfenidone mitigates left ventricular fibrosis and dysfunction after myocardial infarction and reduces arrhythmias. *Heart Rhythm*, *7*(10), 1438–1445. https://doi.org/10.1016/j.hrthm.2010.04.030

Niclou, S. P., Franssen, E. H. P., Ehlert, E. M. E., Taniguchi, M., & Verhaagen, J. (2003). Meningeal cell-derived semaphorin 3A inhibits neurite outgrowth. *Molecular and Cellular Neuroscience*, *24*(4), 902–912. https://doi.org/10.1016/S1044-7431(03)00243-4

Nikolakopoulou, A. M., Montagne, A., Kisler, K., Dai, Z., Wang, Y., Huuskonen, M. T., Sagare, A. P., Lazic, D., Sweeney, M. D., Kong, P., Wang, M., Owens, N. C., Lawson, E. J., Xie, X., Zhao, Z., & Zlokovic, B. V. (2019). Pericyte loss leads to circulatory failure and pleiotrophin depletion causing neuron loss. *Nature Neuroscience*, *22*(7), 1089. https://doi.org/10.1038/s41593-019-0434-z

Nollet, M., Stalin, J., Moyon, A., Traboulsi, W., Essaadi, A., Robert, S., Malissen, N., Bachelier, R., Daniel, L., Foucault-Bertaud, A., Gaudy-Marqueste, C., Lacroix, R., Leroyer, A. S., Guillet, B., Bardin, N., Dignat-George, F., & Blot-Chabaud, M. (2017). A novel anti-CD146 antibody specifically targets cancer cells by internalizing the molecule. *Oncotarget*, *8*(68), 112283–112296. https://doi.org/10.18632/oncotarget.22736

Nour, M., Scalzo, F., & Liebeskind, D. S. (2013). Ischemia-Reperfusion Injury in Stroke. *Interventional Neurology*, *1*(3–4), 185–199. https://doi.org/10.1159/000353125

Ohab, J. J., Fleming, S., Blesch, A., & Carmichael, S. T. (2006). A Neurovascular Niche for Neurogenesis after Stroke. *Journal of Neuroscience*, *26*(50), 13007–13016. https://doi.org/10.1523/JNEUROSCI.4323-06.2006

Özen, I., Deierborg, T., Miharada, K., Padel, T., Englund, E., Genové, G., & Paul, G. (2014). Brain pericytes acquire a microglial phenotype after stroke. *Acta Neuropathologica*, *128*(3), 381–396.

https://doi.org/10.1007/s00401-014-1295-x

Pasterkamp, R. J., Giger, R. J., Ruitenberg, M. J., Holtmaat, A. J., De Wit, J., De Winter, F., & Verhaagen, J. (1999). Expression of the gene encoding the chemorepellent semaphorin III is induced in the fibroblast component of neural scar tissue formed following injuries of adult but not neonatal CNS. *Molecular and Cellular Neurosciences*, *13*(2), 143–166. https://doi.org/10.1006/mcne.1999.0738

Popovich, P. G., Tovar, C. A., Lemeshow, S., Yin, Q., & Jakeman, L. B. (2014). Independent evaluation of the anatomical and behavioral effects of Taxol in rat models of spinal cord injury. *Experimental Neurology*, *261*, 97–108. https://doi.org/10.1016/j.expneurol.2014.06.020

Puente, A., Fortea, J. I., Cabezas, J., Arias Loste, M. T., Iruzubieta, P., Llerena, S., Huelin, P., Fábrega, E., & Crespo, J. (2019). LOXL2—A New Target in Antifibrogenic Therapy? *International Journal of Molecular Sciences*, *20*(7), 1634. https://doi.org/10.3390/ijms20071634

Ray, S., Ju, X., Sun, H., Finnerty, C. C., Herndon, D. N., & Brasier, A. R. (2013). The IL-6 trans-signaling-STAT3 pathway mediates ECM and cellular proliferation in fibroblasts from hypertrophic scar. *The Journal of Investigative Dermatology*, *133*(5), 1212–1220. https://doi.org/10.1038/jid.2012.499

Ribatti, D., Nico, B., & Crivellato, E. (2011). The role of pericytes in angiogenesis. *International Journal of Developmental Biology*, *55*(3), 261–268. https://doi.org/10.1387/ijdb.103167dr

Roth, M., Enström, A., Aghabeick, C., Carlsson, R., Genové, G., & Paul, G. (2020). Parenchymal pericytes are not the major contributor of extracellular matrix in the fibrotic scar after stroke in male mice. *Journal of Neuroscience Research*, *98*(5), 826–842. https://doi.org/10.1002/jnr.24557

Ruan, L., Wang, B., ZhuGe, Q., & Jin, K. (2015). Coupling of neurogenesis and angiogenesis after ischemic stroke. *Brain Research*, *1623*, 166–173. https://doi.org/10.1016/j.brainres.2015.02.042

Ruschel, J., Hellal, F., Flynn, K. C., Dupraz, S., Elliott, D. A., Tedeschi, A., Bates, M., Sliwinski, C., Brook, G., Dobrindt, K., Peitz, M., Brüstle, O., Norenberg, M. D., Blesch, A., Weidner, N.,

Bunge, M. B., Bixby, J. L., & Bradke, F. (2015). Axonal regeneration. Systemic administration of epothilone B promotes axon regeneration after spinal cord injury. *Science (New York, N.Y.)*, *348*(6232), 347–352. https://doi.org/10.1126/science.aaa2958

Rustenhoven, J., Jansson, D., Smyth, L. C., & Dragunow, M. (2017). Brain Pericytes As Mediators of Neuroinflammation. *Trends in Pharmacological Sciences*, *38*(3), 291–304. https://doi.org/10.1016/j.tips.2016.12.001

Saelens, W., Cannoodt, R., Todorov, H., & Saeys, Y. (2019). A comparison of single-cell trajectory inference methods. *Nature Biotechnology*, *37*(5), 547–554. https://doi.org/10.1038/s41587-019-0071-9

Sakuma, R., Kawahara, M., Nakano-Doi, A., Takahashi, A., Tanaka, Y., Narita, A., Kuwahara-Otani, S., Hayakawa, T., Yagi, H., Matsuyama, T., & Nakagomi, T. (2016). Brain pericytes serve as microglia-generating multipotent vascular stem cells following ischemic stroke. *Journal of Neuroinflammation*, *13*(1). https://doi.org/10.1186/s12974-016-0523-9

Schrimpf, C., Xin, C., Campanholle, G., Gill, S. E., Stallcup, W., Lin, S.-L., Davis, G. E., Gharib, S. A., Humphreys, B. D., & Duffield, J. S. (2012). Pericyte TIMP3 and ADAMTS1 Modulate Vascular Stability after Kidney Injury. *Journal of the American Society of Nephrology : JASN*, *23*(5), 868–883. https://doi.org/10.1681/ASN.2011080851

Schultz, G. S., & Wysocki, A. (2009). Interactions between extracellular matrix and growth factors in wound healing. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *17*(2), 153–162. https://doi.org/10.1111/j.1524-475X.2009.00466.x

Segel, M., Neumann, B., Hill, M. F., Weber, I., Viscomi, C., Zhao, C., Young, A., Agley, C. C., Thompson, A. J., Gonzalez, G., Sharma, A., Holmqvist, S., Rowitch, D. H., Franze, K., Franklin, R. J., & Chalut, K. J. (2019). Niche stiffness underlies the aging of central nervous system progenitor cells. *Nature*, *573*(7772), 130–134. https://doi.org/10.1038/s41586-019-1484-9

Shearer, M. C., & Fawcett, J. W. (2001). The astrocyte/meningeal cell interface—A barrier to successful

nerve regeneration? *Cell and Tissue Research*, *305*(2), 267–273. https://doi.org/10.1007/s004410100384

Shiber, J. R., Fontane, E., & Adewale, A. (2010). Stroke registry: Hemorrhagic vs ischemic strokes. *The American Journal of Emergency Medicine*, *28*(3), 331–333. https://doi.org/10.1016/j.ajem.2008.10.026

Silver, J., & Miller, J. H. (2004). Regeneration beyond the glial scar. *Nature Reviews. Neuroscience*, *5*(2), 146–156. https://doi.org/10.1038/nrn1326

Smith, G. M., & Strunz, C. (2005). Growth factor and cytokine regulation of chondroitin sulfate proteoglycans by astrocytes. *Glia*, *52*(3), 209–218. https://doi.org/10.1002/glia.20236

Soderblom, C., Luo, X., Blumenthal, E., Bray, E., Lyapichev, K., Ramos, J., Krishnan, V., Lai-Hsu, C., Park, K. K., Tsoulfas, P., & Lee, J. K. (2013a). Perivascular fibroblasts form the fibrotic scar after contusive spinal cord injury. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *33*(34), 13882–13887. https://doi.org/10.1523/JNEUROSCI.2524-13.2013

Soderblom, C., Luo, X., Blumenthal, E., Bray, E., Lyapichev, K., Ramos, J., Krishnan, V., Lai-Hsu, C., Park, K. K., Tsoulfas, P., & Lee, J. K. (2013b). Perivascular Fibroblasts Form the Fibrotic Scar after Contusive Spinal Cord Injury. *Journal of Neuroscience*, *33*(34), 13882–13887. https://doi.org/10.1523/JNEUROSCI.2524-13.2013

Sofroniew, M. V. (2015). Astrogliosis. *Cold Spring Harbor Perspectives in Biology*, *7*(2), a020420. https://doi.org/10.1101/cshperspect.a020420

Sweeney, M. D., Ayyadurai, S., & Zlokovic, B. V. (2016). Pericytes of the neurovascular unit: Key functions and signaling pathways. *Nature Neuroscience*, *19*(6), 771–783. https://doi.org/10.1038/nn.4288

Tirosh, I., Izar, B., Prakadan, S. M., Wadsworth, M. H., Treacy, D., Trombetta, J. J., Rotem, A., Rodman, C., Lian, C., Murphy, G., Fallahi-Sichani, M., Dutton-Regester, K., Lin, J.-R., Cohen, O., Shah, P., Lu, D., Genshaft, A. S., Hughes, T. K., Ziegler, C. G. K., … Garraway, L. A.

(2016). Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science (New York, N.Y.)*, *352*(6282), 189–196. https://doi.org/10.1126/science.aad0501

Underly, R. G., Levy, M., Hartmann, D. A., Grant, R. I., Watson, A. N., & Shih, A. Y. (2017). Pericytes as Inducers of Rapid, Matrix Metalloproteinase-9-Dependent Capillary Damage during Ischemia. *The Journal of Neuroscience*, *37*(1), 129–140. https://doi.org/10.1523/JNEUROSCI.2891-16.2016

van Dijk, C. G. M., Nieuweboer, F. E., Pei, J. Y., Xu, Y. J., Burgisser, P., van Mulligen, E., el Azzouzi, H., Duncker, D. J., Verhaar, M. C., & Cheng, C. (2015). The complex mural cell: Pericyte function in health and disease. *International Journal of Cardiology*, *190*, 75–89. https://doi.org/10.1016/j.ijcard.2015.03.258

Vanlandewijck, M., He, L., Mäe, M. A., Andrae, J., Ando, K., Gaudio, F. D., Nahar, K., Lebouvier, T., Laviña, B., Gouveia, L., Sun, Y., Raschperger, E., Räsänen, M., Zarb, Y., Mochizuki, N., Keller, A., Lendahl, U., & Betsholtz, C. (2018). A molecular atlas of cell types and zonation in the brain vasculature. *Nature*, *554*(7693), 475–480. https://doi.org/10.1038/nature25739

Vemuganti, R., & Zhao, H. (2015). Mechanisms and therapies for acute CNS insults. *Metabolic Brain Disease*, *30*(2), 353. https://doi.org/10.1007/s11011-014-9585-5

Vogelaar, C. F., König, B., Krafft, S., Estrada, V., Brazda, N., Ziegler, B., Faissner, A., & Müller, H. W. (2015). Pharmacological Suppression of CNS Scarring by Deferoxamine Reduces Lesion Volume and Increases Regeneration in an In Vitro Model for Astroglial-Fibrotic Scarring and in Rat Spinal Cord Injury In Vivo. *PLoS ONE*, *10*(7), e0134371. https://doi.org/10.1371/journal.pone.0134371

Walker, J. E., Giri, S. N., & Margolin, S. B. (2005). A double-blind, randomized, controlled study of oral pirfenidone for treatment of secondary progressive multiple sclerosis. *Multiple Sclerosis (Houndmills, Basingstoke, England)*, *11*(2), 149–158. https://doi.org/10.1191/1352458505ms1134oa

Wang, S., Cao, C., Chen, Z., Bankaitis, V., Tzima, E., Sheibani, N., & Burridge, K. (2012). Pericytes

Regulate Vascular Basement Membrane Remodeling and Govern Neutrophil Extravasation during Inflammation. *PLoS ONE*, *7*(9). https://doi.org/10.1371/journal.pone.0045499

Wang, T., Li, B., Nelson, C. E., & Nabavi, S. (2019). Comparative analysis of differential gene expression analysis tools for single-cell RNA sequencing data. *BMC Bioinformatics*, *20*(1), 40. https://doi.org/10.1186/s12859-019-2599-6

Wang, Y.-C., Chen, Q., Luo, J.-M., Nie, J., Meng, Q.-H., Shuai, W., Xie, H., Xia, J.-M., & Wang, H. (2019). Notch1 promotes the pericyte-myofibroblast transition in idiopathic pulmonary fibrosis through the PDGFR/ROCK1 signal pathway. *Experimental & Molecular Medicine*, *51*(3), 1–11. https://doi.org/10.1038/s12276-019-0228-0

Whetstone, W. D., Hsu, J.-Y. C., Eisenberg, M., Werb, Z., & Noble-Haeusslein, L. J. (2003). Blood-spinal cord barrier after spinal cord injury: Relation to revascularization and wound healing. *Journal of Neuroscience Research*, *74*(2), 227–239. https://doi.org/10.1002/jnr.10759

Xiao, M.-J., Han, Z., Shao, B., & Jin, K. (2009). Notch signaling and neurogenesis in normal and stroke brain. *International Journal of Physiology, Pathophysiology and Pharmacology*, *1*(2), 192–202.

Xu, X.-H., Jia, Y., Zhou, X., Xie, D., Huang, X., Jia, L., Zhou, Q., Zheng, Q., Zhou, X., Wang, K., & Jin, L.-P. (2019). Downregulation of lysyl oxidase and lysyl oxidase-like protein 2 suppressed the migration and invasion of trophoblasts by activating the TGF-β/collagen pathway in preeclampsia. *Experimental & Molecular Medicine*, *51*(2), 1–12. https://doi.org/10.1038/s12276-019-0211-9

Yahn, S. L., Li, J., Goo, I., Gao, H., Brambilla, R., & Lee, J. K. (2020). Fibrotic scar after experimental autoimmune encephalomyelitis inhibits oligodendrocyte differentiation. *Neurobiology of Disease*, *134*, 104674. https://doi.org/10.1016/j.nbd.2019.104674

Yang, J., Savvatis, K., Kang, J. S., Fan, P., Zhong, H., Schwartz, K., Barry, V., Mikels-Vigdal, A., Karpinski, S., Kornyeyev, D., Adamkewicz, J., Feng, X., Zhou, Q., Shang, C., Kumar, P., Phan, D., Kasner, M., López, B., Diez, J., … Chang, C.-P. (2016). Targeting LOXL2 for cardiac interstitial fibrosis and heart failure treatment. *Nature Communications*, *7*(1), 13710.

https://doi.org/10.1038/ncomms13710

Yang, T., Dai, Y., Chen, G., & Cui, S. (2020). Dissecting the Dual Role of the Glial Scar and Scar-Forming Astrocytes in Spinal Cord Injury. *Frontiers in Cellular Neuroscience*, *0*. https://doi.org/10.3389/fncel.2020.00078

Yau, J. W., Teoh, H., & Verma, S. (2015). Endothelial cell control of thrombosis. *BMC Cardiovascular Disorders*, *15*, 130. https://doi.org/10.1186/s12872-015-0124-z

Yemisci, M., Gursoy-Ozdemir, Y., Vural, A., Can, A., Topalkara, K., & Dalkara, T. (2009). Pericyte contraction induced by oxidative-nitrative stress impairs capillary reflow despite successful opening of an occluded cerebral artery. *Nature Medicine*, *15*(9), 1031–1037. https://doi.org/10.1038/nm.2022

Yiu, G., & He, Z. (2006). Glial inhibition of CNS axon regeneration. *Nature Reviews Neuroscience*, *7*(8), 617–627. https://doi.org/10.1038/nrn1956

Yokota, K., Kobayakawa, K., Saito, T., Hara, M., Kijima, K., Ohkawa, Y., Harada, A., Okazaki, K., Ishihara, K., Yoshida, S., Kudo, A., Iwamoto, Y., & Okada, S. (2017). Periostin Promotes Scar Formation through the Interaction between Pericytes and Infiltrating Monocytes/Macrophages after Spinal Cord Injury. *The American Journal of Pathology*, *187*(3), 639–653. https://doi.org/10.1016/j.ajpath.2016.11.010

Zbesko, J. C., Nguyen, T.-V. V., Yang, T., Frye, J. B., Hussain, O., Hayes, M., Chung, A., Day, W. A., Stepanovic, K., Krumberger, M., Mona, J., Longo, F. M., & Doyle, K. P. (2018). Glial scars are permeable to the neurotoxic environment of chronic stroke infarcts. *Neurobiology of Disease*, *112*, 63–78. https://doi.org/10.1016/j.nbd.2018.01.007

Zeisel, A., Hochgerner, H., Lönnerberg, P., Johnsson, A., Memic, F., van der Zwan, J., Häring, M., Braun, E., Borm, L. E., La Manno, G., Codeluppi, S., Furlan, A., Lee, K., Skene, N., Harris, K. D., Hjerling-Leffler, J., Arenas, E., Ernfors, P., Marklund, U., & Linnarsson, S. (2018). Molecular Architecture of the Mouse Nervous System. *Cell*, *174*(4), 999-1014.e22. https://doi.org/10.1016/j.cell.2018.06.021