Title

A Dual-Color Genetically Engineered Mouse Model for Multispectral Imaging of the Pancreatic Microenvironment

Journal

Pancreas, 42(6)

ISSN

0885-3177

Authors

Snyder, Cynthia S
Harrington, Austin R
Kaushal, Sharmeela
et al.

Publication Date

2013-08-01

DOI

10.1097/mpa.0b013e31828643df

Peer reviewed

Published in final edited form as:
Pancreas. 2013 August ; 42(6): 952–958. doi:10.1097/MPA.0b013e31828643df.

A dual color, genetically engineered mouse model for multi-spectral imaging of the pancreatic microenvironment

Cynthia S. Snyder, MD,
UCSD Moores Cancer Center, University of California, San Diego, La Jolla, California

Austin R. Harrington,
UCSD Moores Cancer Center, University of California, San Diego, La Jolla, California

Sharmeela Kaushal, PhD,
UCSD Moores Cancer Center, University of California, San Diego, La Jolla, California

Evangeline Mose, BS,
UCSD Moores Cancer Center, University of California, San Diego, La Jolla, California

Andrew M. Lowy, MD,
Division of Surgical Oncology, Department of Surgery and UCSD Moores Cancer Center, University of California, San Diego, La Jolla, California

Robert M. Hoffman, PhD, and
Department of Surgery and UCSD Moores Cancer Center, University of California, San Diego, La Jolla, California and AntiCancer, Inc., San Diego, California

Michael Bouvet, MD
Division of Surgical Oncology, Department of Surgery and UCSD Moores Cancer Center, University of California, San Diego, La Jolla, California and VA Healthcare System, San Diego, California

Abstract

Objectives—To develop a mouse model for multi-spectral fluorescence imaging of the pancreas and pancreatic microenvironment.

Methods—Cre/*loxP* technology was used to develop this model. We crossed *mT/mG* indicator mice, engineered to constitutively express a conditional tdTomato transgene that converts to green fluorescent protein (GFP) expression following exposure to Cre recombinase, with *Pdx1-Cre* transgenic mice. To characterize this model for studies of pancreas biology, we performed bright light and fluorescence imaging of body cavities and intact organs and confocal microscopy of pancreata from offspring of *Pdx1-Cre* and *mT/mG* crosses.

Results—*Pdx1-Cre* - *mT/mG* mice demonstrated bright GFP expression within the pancreas and duodenum and intense tdTomato expression in all other organs. GFP expression was mosaic in *Pdx1-Cre* - *mT/mG* pancreata, with most showing extensive conversion from tdTomato to GFP expression within the epithelial-derived elements of the pancreatic parenchyma. Because both GFP and tdTomato are membrane-targeted, individual cell borders were clearly outlined in confocal images of *mT/mG* pancreata.

Conclusions—This mouse model enables multi-spectral fluorescence imaging of individual cells and cell processes at the microscopic level of the pancreatic microenvironment; it should

Address correspondence to: Michael Bouvet, MD, Department of Surgery, Moores UCSD Cancer Center, 3855 Health Science Drive #0987, La Jolla, CA 92093-0987, Phone: 858-822-6191, Fax: 858-822-6192, mbouvet@ucsd.edu.

prove valuable for a variety of fluorescence imaging studies, ranging from pancreatic development to pancreatic cancer biology.

Keywords

Pancreas; Cre recombinase; pdx1; conditional gene targeting; fluorescence imaging

INTRODUCTION

The use of fluorescent proteins for imaging of biological processes has yielded important information in many fields of research. The development of genetically engineered mice that express fluorescent proteins either constitutively or in a tissue-specific manner has been an important advance, allowing for the imaging of *in vivo* processes that range from normal vertebrate development to human cancer biology.[1–4]

Conditional gene targeting using site-specific recombinase systems (Cre/*loxP*, Flp/*FRT*) is a powerful genetic technology being applied to the study of gene function.[5] Primarily used for tissue-specific loss of function (gene knockout) studies in mice, conditional gene targeting technology has also been used to control the temporospatial expression of fluorescent protein-encoding reporter transgenes.[6,7] These Cre reporter mouse models are useful for characterizing and validating newly-generated Cre recombinase-expressing transgenic lines prior to beginning tissue-specific gene knockout studies. As fluorescence imaging technologies advance, allowing for improved multi-spectral imaging of biological processes, applications for the simultaneous use of multiple fluorophores in *in vivo* studies expand.[8,9]

Recently, a conditional fluorescent reporter mouse model (*mT/mG*) was developed in which transgene expression of tdTomato (a red fluorescent protein) converts to the expression of GFP (enhanced green fluorescent protein) following Cre recombinase-mediated intramolecular rearrangement of the fluorescent protein-encoding transgene.[10] We wished to explore and validate the use of *mT/mG* mice for fluorescence imaging studies of pancreas biology. *Pdx1-Cre* transgenic mice have been used extensively to induce genetic recombination within the murine pancreas. PDX-1, also known as IPF-1, IDX-1, and STF-1, was originally identified as a transcription factor that regulates expression of insulin and other hormones produced by islet cells of the adult pancreas.[11–13] PDX-1 is also a homeodomain protein expressed specifically during early stages of mouse embryonic development in tissues destined to become the pancreas, antral stomach, and rostral duodenum. PDX-1 is sometimes referred to as a master regulator of vertebrate pancreas development.[14] PDX-1-expressing cells of embryonic mice give rise to all three pancreatic epithelial cell types: exocrine acinar cells, endocrine islet cells, and duct cells.[15] In adult mice PDX-1 continues to be expressed at high levels by insulin-producing, beta cells of the pancreatic islets.[11] *Pdx1-Cre* mice express Cre recombinase within the developing foregut-derived pancreas and duodenum during embryogenesis,[15,16] recapitulating the expression pattern of the endogenous *pdx1* gene.

To generate mice in which the pancreatic parenchyma, expressing membrane-targeted GFP, is highlighted against the membrane-targeted red fluorescent backlight of stromal and non-epithelial-derived pancreatic tissues, we crossed *Pdx1-Cre* transgenic mice with *mT/mG* Cre reporter mice. To assess the suitability of this mouse model for imaging studies of the pancreas, we imaged offspring of *Pdx1-Cre* and *mT/mG* intercrosses at both the macroscopic and microscopic levels using bright light and fluorescence imaging modalities.

MATERIALS AND METHODS

Mice

Establishment of the *mT/mG* reporter line (hereafter referred to as mTmG) was previously described.[10] Founder mTmG mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). The mTmG reporter transgene is driven by a strong, ubiquitous promoter (ACTB) from the well-characterized *Gt(ROSA)26Sor* genomic locus. In the absence of Cre recombinase, mTmG mice constitutively express tdTomato, a non-oligomerizing DsRed variant with a 12-residue amino acid linker fusing two copies of the protein in a tandem dimer arrangement.[17] Following exposure to Cre recombinase and excision of the tdTomato expression cassette, the rearranged mTmG transgene converts to the expression of GFP (enhanced green fluorescent protein). Both tdTomato and GFP are membrane-targeted, allowing for delineation of single cells and cell processes using fluorescence microscopy. mTmG mice were maintained by crossing mTmG heterozygotes (mTmG/+) with wild type mice. Litters were screened for the mTmG transgene by PCR genotyping of tail DNA. *Pdx1-Cre* transgenic mice were obtained from Dr. Andrew Lowy and have been previously described.[18] These mice have been deposited with the Jackson Laboratory (JAX Stock No. 014647). *Pdx1-Cre* hemizygotes (PdxCre-positive, $PdxCre^{+}$) and mTmG indicator mice (mTmG-positive) are maintained as separate lines. Both lines are on a mixed C57BL/6 × 129 (129X1/SvJ × 129S1/Sv)F1 background, backcrossed several generations onto C57BL/6. Both lines show normal fertility and viability. *Pdx1-Cre* hemizygotes ($PdxCre^{+}$) were crossed with mTmG heterozygotes (mTmG/+) to produce the mice used in these studies. $PdxCre^{+}$ and mTmG/+ transgenic mice were identified by PCR genotyping using primer sets recommended by the Jackson Laboratory. Genotyping was performed on DNA isolated using standard protocols from tail snips obtained at or just prior to weaning of litters. All mice were maintained in accordance with institutional policies on the use of vertebrate animals in research following protocols approved by the Institutional Animal Care and Use Committee at the University of California San Diego.

Imaging of Mice

We imaged mice ranging in age from mid-gestation to late adulthood (greater than 1 year of age). Images shown herein were obtained from young adult mice (approximately six weeks of age), an age chosen arbitrarily in order to demonstrate the findings in healthy (young) mice during a relatively steady state developmental period (adulthood). All mice in these studies were euthanized prior to imaging by carbon dioxide asphyxiation followed by cervical dislocation. For imaging of animals at necropsy and for *ex vivo* imaging of mouse organs we used an OV100™ small animal imaging system (Olympus Corp, Tokyo, Japan) equipped with an MT-20 light source (Olympus) and a highly sensitive Hamamatsu Electron Multiplying DP70 CCD camera (Olympus). The OV100 uses a 100W halogen lamp for brightfield imaging and a 150W xenon lamp for fluorescence excitation. For imaging of tdTomato fluorescence (excitation and emission maxima at 554 nm and 581 nm, respectively), we used red fluorescence protein filter sets (excitation 545/30 and emission 598/55). For GFP imaging (excitation and emission maxima at 484 nm and 510 nm, respectively) we used a 475/40 excitation filter and either a 530/50 band pass emission filter or a 510 long pass emission filter that allows for simultaneous visualization of both GFP and tdTomato fluorescence. All GFP images shown in this manuscript were obtained using the long pass GFP emission filter. Fluorescence images of organs in Figure 2A were adjusted equally for brightness and contrast. All other OV100 images are shown without post-acquisition processing.

A Maestro™ EX *in vivo* imaging system (CRi, Woburn, MA, USA) was used for multi-spectral separation. The Maestro™ EX imaging module contains a high-resolution, CCD

camera, a solid-state liquid crystal wavelength tuning element, spectrally optimized optics, an emission filter assembly, a flexible fiber-optic lighting system, and an adjustable specimen stage. The illumination module includes a Cermax®-type 300W xenon light source and a fiber-optic delivery system. All images were assembled using PowerPoint software (Microsoft Corp, Redmond, WA, USA).

Confocal Microscopy

Fresh tissues were frozen in OCT (Tissue-Tek® OCT™, Sakura Finetek, CA, USA) and sectioned at twenty microns using a cryostat (Leica Microsystems, Wetzlar, Germany). Methanol-fixed cryosections were counterstained with DAPI prior to imaging. Confocal images were acquired using a FLUOVIEW FV1000 confocal laser scanning microscope (Olympus), a 20X/0.75 NA objective, and Olympus FLUOVIEW FV1000 image acquisition software.

Histology

Fresh tissues obtained at the time of necropsy were fixed in 10% buffered formalin or Bouins fixative. Fixed tissues were paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E) according to standard protocols. H&E-stained permanent sections were examined using an Olympus BX41 microscope (Olympus) equipped with a Micropublisher 3.3 RTV camera (QImaging, Surrey, BC, Canada). All images were acquired using QCapture software (QImaging) without post-acquisition processing.

RESULTS

Crosses of *Pdx1-Cre* mice with mTmG mice yielded litters with the expected ratios of genotypes. Mice obtained from these crosses fell into three phenotypic groups: mTmG-negative (either *Pdx1-Cre* -positive or *Pdx1-Cre* -negative, but non-fluorescent and therefore termed +/+ for imaging purposes); mTmG-positive, *Pdx1-Cre* -negative (mTmG); and mTmG-positive, *Pdx1-Cre* -positive (*Pdx1-Cre*/mTmG) double transgenic mice.

Pdx1-Cre/mTmG mice showed no apparent defects, bred normally, and had normal life expectancies (>1 year). For imaging studies, same-sex adult littermates from each of the three phenotypic groups were imaged simultaneously. Non-fluorescent littermates were imaged alongside their fluorescent (mTmG-positive) siblings to establish optimal exposure times and to control for auto-fluorescence, particularly of the gastrointestinal tract. Three 6-week old female littermates, imaged using an Olympus OV100 small animal imaging system (Figure 1A) and a CRi Maestro™ EX *in vivo* imaging system (Figure 1B) and either brightfield illumination or fluorescence (tdTomato or GFP) illumination settings, demonstrated bright, generalized expression of tdTomato and tissue-specific expression of GFP. Figure 1C shows genotyping data for the mice in Figure 1. In mTmG-positive mice, all tissues examined expressed the mTmG transgene and demonstrated bright tdTomato fluorescence in the absence of Cre expression (Figures 1 and 2 and Supplemental Figure 1). As expected, the *Pdx1-Cre* transgene showed tissue-specific expression. Imaging of all major organs from *Pdx1-Cre*/mTmG, mice demonstrated that expression of GFP was limited to the pancreas and the proximal small intestine (Figures 1 and 2, *Pdx1-Cre*/mTmG genotype). GFP expression in the proximal small intestine (duodenum) tapered off distally (Figure 2B). Images obtained from *Pdx1-Cre*/mTmG mice ranging in age from early post-natal life to late adulthood have established that the fluorescent phenotypes described above are established during embryogenesis and are stably maintained throughout murine adult life (data not shown).

The conversion of red (mT) to green (mG) fluorescence is extensive but not complete in *Pdx1-Cre*/mTmG pancreata, resulting in a mosaic pattern of fluorescence within the pancreatic parenchyma that varied from individual to individual. Pancreatic mosaicism of mT and mG expression is best demonstrated macroscopically in Figure 2C (Littermate 2 versus Littermate 1, both PdxCre^{+}, mTmG/+) and microscopically in Figure 3B. Figure 2C Littermate 2 was the most extreme example of genetic mosaicism seen in our studies. Most of the *Pdx1-Cre*/mTmG mice we examined more closely resembled the *Pdx1-Cre*/mTmG mouse shown in Figure 1 and Littermate 1 in Figure 2C, both of which demonstrate extensive pancreatic expression of mG. Confocal imaging of mTmG-positive pancreata demonstrated bright membrane staining and clear delineation of cell borders, especially in acinar cells (Figure 3). Islet cells of *Pdx1-Cre*/mTmG mice also demonstrated GFP expression. The intensity of membrane staining in islet cells was significantly less than in the surrounding acinar cells (Figure 3A). Small pancreatic ducts and individual pancreatic ductal cells were difficult to discriminate from the brightly fluorescent acinar cells in these unstained cryosections.

The vascular scaffolding, the most prominent component of the stromal matrix in normal pancreas, demonstrated bright red fluorescence in *Pdx1-Cre*/mTmG pancreata (Figure 3A, tdTomato panel). Although incomplete on a tissue basis, we found that conversion of mT to mG fluorescence appeared complete within individual pancreatic cells of *Pdx1-Cre*/mTmG mice. Co-expression of red and green fluorescence, which would appear as yellow fluorescence in images, was not seen within individual cells (Figure 3B).

To assess for unanticipated toxic effects of fluorescent protein (mT or mG) expression on the pancreas, we performed histological studies on pancreata from fluorescent (*Pdx1-Cre*/mTmG and mTmG) experimental mice and from non-fluorescent (mTmG-negative) control mice. The histological appearance of exocrine (acini and ducts) and endocrine (islets) elements was unremarkable in H&E-stained sections taken from *Pdx1-Cre*/mTmG (Figure 4) and mTmG (data not shown) mice and was comparable to the H&E findings seen in non-fluorescent, control pancreata.

DISCUSSION

Applications for the *in vivo* use of expressed fluorescent reporters to address biological questions are expanding rapidly, mirroring ongoing technological advancements in imaging and an ever increasing number and diversity of genetically-modified organisms available for use in these studies.[19,20] Fluorescent reporter mice designed for use in Cre/*loxP* conditional gene targeting studies are an example.[21–23] In the present study we used mTmG (*mT/mG*) fluorescent reporter mice and *Pdx1-Cre* transgenic mice to generate a dual-color mouse model suitable for use in studies of pancreas biology using fluorescence imaging technology.

There are now many Cre recombinase-expressing lines available for studies of pancreas biology. These include lines wherein Cre recombinase is expressed under control of the elastase (Ela), rat insulin II (RIP), acinar procarboxypeptidase (proCPA1), or pancreas-specific transcription factor 1a (Ptf1a, p48) promoters,[24–27] Each of these lines yields unique and characteristic spatial patterns of Cre expression *in vivo* and differing degrees of inter-individual variability of expression.[28] Some of these lines, including the *Pdx1-CreER* variant of the *Pdx1-Cre* line,[15] have been engineered to allow for temporal as well as spatial control of Cre expression. *Pdx1-Cre* mice are arguably the best characterized of these lines, yielding widespread, early, and comparatively consistent, Cre-expression throughout the pancreatic epithelial compartment. For these reasons, we chose to work with *Pdx1-Cre* mice in developing this model. For certain studies or to address specific biological questions, the

choice of a line with more spatially or temporally restrictive Cre expression may be more appropriate or may provide complementary information.[28]

We found that *Pdx1-Cre*/mTmG mice demonstrate intense, generalized, membrane-targeted tdTomato expression with bright, membrane-targeted GFP expression localized to the pancreas and duodenum. In mTmG mice lacking Cre recombinase, expression of membrane-targeted tdTomato (mT) fluorescent protein is particularly bright in the pancreas; this finding does not appear to be due to membrane-targeting of the mTmG transgene-encoded fluorescent protein product, as previous studies have also shown robust pancreatic expression of fluorescent proteins that were not membrane-targeted.[29]

The finding of mosaic GFP expression in *Pdx1-Cre*/mTmG pancreata is interesting and is consistent with previous studies demonstrating mosaic pancreatic expression of Cre recombinase under *pdx1* promoter control.[15,30] Mosaic expression of *pdx1* promoter-driven constructs is not unique to *Pdx1-Cre* transgenics. Expression constructs engineered using this same 4.3 kilobase *pdx1* promoter cassette to drive expression of proteins other than Cre recombinase have also yielded mosaic patterns of pancreatic expression.[31–33] Studies looking at endogenous PDX-1 expression during murine pancreatic morphogenesis have found that PDX-1 expression levels rise and fall at slightly different times in each of the embryonic pancreatic epithelial components: acini, ducts, and islets.[34] Owing to the variable timecourse of endogenous PDX-1 protein expression in different epithelial elements of the pancreas, snapshot studies of endogenous PDX-1 expression do not yield an answer as to whether or not the normal murine pancreas harbors isolated parenchymal cells that escaped a PDX-1-expressing heritage (endogenous mosaics). However, *pdx1* knockouts form only rudimentary pancreata in the absence of PDX-1 expression.[16] Expression of genomic fragments of the *pdx1* locus encompassing this approximately 4.3 kilobase *pdx1* promoter cassette have been shown to rescue the lethal, apancreatic phenotype seen in *pdx1*$^{-/-}$ knockout mice, suggesting that this promoter construct is sufficient to recapitulate endogenous levels of pancreatic PDX-1 expression *in vivo*.[32,35] Studies have shown significant variability in PDX-1 expression levels between adjacent cells of developing epithelial cell cords and acinar cell clusters in the embryonic murine pancreas.[36] It seems likely that the mosaicism of mT and mG expression seen in adult *Pdx1-Cre*/mTmG pancreata is the manifestation of a threshold effect of *Pdx1-Cre* expression on mTmG transgene rearrangement during embryonic pancreatic organogenesis. As such, the conversion from mT to mG expression is a digital readout of an analog system of *Pdx1-Cre* expression. Genetic variables such as strain background may influence expression of *Pdx1-Cre* or the process of Cre-mediated mT to mG conversion. However, the mosaicism demonstrated in Figure 2C occurred in *Pdx1-Cre*/mTmG littermates born to breeders that had been backcrossed onto C57Bl/6 for several generations. This finding argues that the genetic process of *Pdx1-Cre*-mediated conversion from mT to mG fluorescence, while largely deterministic at the whole organ level, is also influenced by stochastic variables acting on individual cells during pancreatic organogenesis.

We also observed mosaic expression of GFP in the small intestine of *Pdx1-Cre*/mTmG mice, although this expression pattern is probably more accurately characterized as a dilutional tapering off of GFP-expressing cell clusters as one moves distally from the duodenum, which shares its ontogeny with the pancreas, towards the more caudal regions of the small intestine that arise from embryonic tissues that fall outside the *pdx1* expression domain. Whereas, PDX-1 expression is required for development of the pancreas, PDX-1 expression is dispensable for morphogenesis of the duodenum.[37] Although the duodena of *pdx1* knockout mice are structurally normal at the macroscopic level, *pdx1* knockout mice have been reported to show functional deficits that correlate with cytologic abnormalities at the microscopic level, including morphological changes in the epithelium of the duodenal

villi, absence of Brunner's glands, and decreased numbers of duodenal enteroendocrine cells.[16,35] Subsequent studies looking at roles for PDX-1 in adult duodenum have documented mosaic expression of both upstream regulators[38] and putative downstream target genes[39] of *pdx1*/PDX-1 in the duodenum. This mosaicism has sometimes been a confounding variable in the interpretation of gene knockout studies of duodenal development and function. Future studies of duodenal biology involving conditional gene targeting may benefit from the multi-spectral lineage tracking enabled by use of mTmG reporter mice in combination with duodenal Cre-expression mice such as the *Pdx1-Cre* mice used in the present study.

Despite the mosaicism that occurs in the pancreas as a whole, conversion of red (mT) to green (mG) fluorescence appears to be complete within individual pancreatic cells of *Pdx1-Cre*/mTmG mice. Co-expression of mT and mG fluorescence, which would result in the appearance of a yellow fluorescence signal in superimposed images of red and green fluorescence emission, was not seen in individual cells of the adult pancreas. Muzumdar et al[10] documented an mT to mG transitional state in hepatocytes of tamoxifen-treated, mTmG mice crossed with *actin-CreER* mice, as well as in a variety of tissues when mTmG reporter mice were crossed with other Cre-expressing transgenic lines; the authors did not report findings specific to the pancreas. The absence of tdTomato perdurance within individual, Cre-recombined, GFP-expressing pancreatic cells in *Pdx1-Cre*/mTmG mice is testimony to the remote timing of initial *Pdx1-Cre* expression during ontogeny of the pancreas, allowing time for washout of mT fluorescent protein and replacement by newly-expressed mG fluorescent protein. PDX-1 expression by developing pancreatic acinar and duct cells decreases at mid-gestation, and it is expressed at low levels in adult pancreatic acini and ducts. By contrast, adult pancreatic islet β cells express high levels of PDX-1, and it is required for their maintenance and for insulin expression.[33,40,41] Confocal images of cryosectioned adult *Pdx1-Cre*/mTmG pancreata, acinar cells demonstrate bright GFP (mG) fluorescence, whereas mG fluorescence of pancreatic islet cells is comparatively weak. This seemingly contradictory finding may reflect lower ongoing levels of mTmG transgene expression from the *Gt(ROSA)26Sor* genomic locus in islet cells as compared to acinar cells. Alternatively, the finding may correlate with structural, functional, or dynamic differences between the cell membranes of islet and acinar cells.

This fluorescent mouse model demonstrates many valuable characteristics and overcomes a number of hurdles that can limit the utility of a fluorescent mouse model for experimental study. Genetic expression of mT and mG is stable, heritable, and non-diffusible from cell to cell; qualities important for studies that entail tracking of living cells and their progeny over time. As such, this model would prove ideal for a variety of lineage tracing experiments. Lineage tracing approaches, pioneered in the field of developmental biology, are now being used to address questions in the fields of stem cell biology and cancer biology, amongst others.[42–45] The use of multiple fluorescent protein markers, as in this binary fluorescent *Pdx1-Cre*/mTmG model, expands the types of questions that can be addressed in lineage tracing experiments. For example, multispectral approaches entailing the use of two, or more, fluorescent protein markers have recently been used to address questions of clonality in mouse models of neural and intestinal cancer biology.[8,46] Although toxicity sometimes limits the utility of fluorescent protein markers for *in vivo* studies of cell biology, we saw no evidence to suggest toxicity of mT or mG expression in this *Pdx1-Cre*/mTmG model. Low expression of genetically encoded fluorescent markers is another obstacle sometimes encountered. By contrast, the robust mT and mG fluorescence we observed in *Pdx1-Cre*/mTmG pancreata should make this mouse model useful for a variety of studies that entail intravital imaging.

In summary, this dual color, double transgenic mouse model is optimal for multi-spectral fluorescence imaging of the pancreas at the macroscopic level and for fluorescence imaging of individual cells and cell processes at the microscopic level of the pancreatic microenvironment. This model should prove to be a valuable tool for a variety of fluorescence imaging studies, ranging from pancreas development to pancreatic cancer biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Contract grant sponsor: NIH, Contract Grant Number: R01-CA132971 (to M.B. and AntiCancer, Inc)

Confocal microscopy was performed using equipment belonging to the UCSD Neuroscience Microscopy Shared Facility core, which is funding by shared facility grant P30 NS047101. Processing of tissues for histological review was performed by the UCSD Moores Cancer Center Histology Shared Facility core laboratory.

References

1. Hoffman RM. Recent advances on in vivo imaging with fluorescent proteins. Methods Cell Biol. 2008; 85:485–495. [PubMed: 18155476]
2. Okabe M, Ikawa M, Kominami K, et al. ‘Green mice’ as a source of ubiquitous green cells. FEBS Lett. 1997; 407:313–319. [PubMed: 9175875]
3. Vintersten K, Monetti C, Gertsenstein M, et al. Mouse in red: red fluorescent protein expression in mouse ES cells, embryos, and adult animals. Genesis. 2004; 40:241–246. [PubMed: 15593332]
4. Megason SG, Fraser SE. Digitizing life at the level of the cell: high-performance laser-scanning microscopy and image analysis for in toto imaging of development. Mech Dev. 2003; 120:1407–1420. [PubMed: 14623446]
5. Nagy A. Cre recombinase: the universal reagent for genome tailoring. Genesis. 2000; 26:99–109. [PubMed: 10686599]
6. Novak A, Guo C, Yang W, et al. Z/EG, a double reporter mouse line that expresses enhanced green fluorescent protein upon Cre-mediated excision. Genesis. 2000; 28:147–155. [PubMed: 11105057]
7. Srinivas S, Watanabe T, Lin CS, et al. Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC Dev Biol. 2001; 1:4. [PubMed: 11299042]
8. Schepers AG, Snippert HJ, Stange DE, et al. Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas. Science. 2012; 337:730–735. [PubMed: 22855427]
9. Kulesa PM, Teddy JM, Smith M, et al. Multispectral fingerprinting for improved in vivo cell dynamics analysis. BMC Dev Biol. 2010; 10:101. [PubMed: 20868502]
10. Muzumdar MD, Tasic B, Miyamichi K, et al. A global double-fluorescent Cre reporter mouse. Genesis. 2007; 45:593–605. [PubMed: 17868096]
11. Ohlsson H, Karlsson K, Edlund T. IPF1, a homeodomain-containing transactivator of the insulin gene. EMBO J. 1993; 12:4251–4259. [PubMed: 7901001]
12. Leonard J, Peers B, Johnson T, et al. Characterization of somatostatin transactivating factor-1, a novel homeobox factor that stimulates somatostatin expression in pancreatic islet cells. Mol Endocrinol. 1993; 7:1275–1283. [PubMed: 7505393]
13. Miller CP, McGehee RE Jr, Habener JF. IDX-1: a new homeodomain transcription factor expressed in rat pancreatic islets and duodenum that transactivates the somatostatin gene. EMBO J. 1994; 13:1145–1156. [PubMed: 7907546]
14. Murtaugh, LC.; Cassiano, J.; De La, OJ-P. Development of the endocrine and exocrine pancreas. In: Lowy, AM.; Leach, SD.; Philip, PA., editors. Pancreatic Cancer MD Anderson Solid Tumor Oncology Series. New York, NY, USA: Springer Science+Business Media, LLC; 2008. p. 117-135.

15. Gu G, Dubauskaite J, Melton DA. Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. Development. 2002; 129:2447–2457. [PubMed: 11973276]
16. Offield MF, Jetton TL, Labosky PA, et al. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development. 1996; 122:983–995. [PubMed: 8631275]
17. Shaner NC, Campbell RE, Steinbach PA, et al. Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. Nat Biotechnol. 2004; 22:1567–1572. [PubMed: 15558047]
18. Hingorani SR, Petricoin EF, Maitra A, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell. 2003; 4:437–450. [PubMed: 14706336]
19. Viotti M, Nowotschin S, Hadjantonakis AK. Afp::mCherry, a red fluorescent transgenic reporter of the mouse visceral endoderm. Genesis. 2011; 49:124–133. [PubMed: 21442721]
20. Fink D, Wohrer S, Pfeffer M, et al. Ubiquitous expression of the monomeric red fluorescent protein mCherry in transgenic mice. Genesis. 2010; 48:723–729. [PubMed: 20853428]
21. Abe T, Kiyonari H, Shioi G, et al. Establishment of conditional reporter mouse lines at ROSA26 locus for live cell imaging. Genesis. 2011; 49:579–590. [PubMed: 21445964]
22. Dieguez-Hurtado R, Martin J, Martinez-Corral I, et al. A Cre-reporter transgenic mouse expressing the far-red fluorescent protein Katushka. Genesis. 2011; 49:36–45. [PubMed: 21254335]
23. Shioi G, Kiyonari H, Abe T, et al. A mouse reporter line to conditionally mark nuclei and cell membranes for in vivo live-imaging. Genesis. 2011; 49:570–578. [PubMed: 21504045]
24. Habbe N, Shi G, Meguid RA, et al. Spontaneous induction of murine pancreatic intraepithelial neoplasia (mPanIN) by acinar cell targeting of oncogenic Kras in adult mice. Proc Natl Acad Sci U S A. 2008; 105:18913–18918. [PubMed: 19028870]
25. Kawaguchi Y, Cooper B, Gannon M, et al. The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. Nat Genet. 2002; 32:128–134. [PubMed: 12185368]
26. Postic C, Shiota M, Niswender KD, et al. Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase. J Biol Chem. 1999; 274:305–315. [PubMed: 9867845]
27. Zhou Q, Law AC, Rajagopal J, et al. A multipotent progenitor domain guides pancreatic organogenesis. Dev Cell. 2007; 13:103–114. [PubMed: 17609113]
28. Gidekel Friedlander SY, Chu GC, Snyder EL, et al. Context-dependent transformation of adult pancreatic cells by oncogenic K-Ras. Cancer Cell. 2009; 16:379–389. [PubMed: 19878870]
29. Tran Cao HS, Kimura H, Kaushal S, et al. The cyan fluorescent protein (CFP) transgenic mouse as a model for imaging pancreatic exocrine cells. JOP. 2009; 10:152–156. [PubMed: 19287108]
30. Gannon M, Herrera PL, Wright CV. Mosaic Cre-mediated recombination in pancreas using the pdx-1 enhancer/promoter. Genesis. 2000; 26:143–144. [PubMed: 10686611]
31. Nelson SB, Schaffer AE, Sander M. The transcription factors Nkx6.1 and Nkx6.2 possess equivalent activities in promoting beta-cell fate specification in Pdx1+ pancreatic progenitor cells. Development. 2007; 134:2491–2500. [PubMed: 17537793]
32. Gannon M, Gamer LW, Wright CV. Regulatory regions driving developmental and tissue-specific expression of the essential pancreatic gene pdx1. Dev Biol. 2001; 238:185–201. [PubMed: 11784003]
33. Wu KL, Gannon M, Peshavaria M, et al. Hepatocyte nuclear factor 3beta is involved in pancreatic beta-cell-specific transcription of the pdx-1 gene. Mol Cell Biol. 1997; 17:6002–6013. [PubMed: 9315659]
34. Guz Y, Montminy MR, Stein R, et al. Expression of murine STF-1, a putative insulin gene transcription factor, in beta cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. Development. 1995; 121:11–18. [PubMed: 7867492]
35. Boyer DF, Fujitani Y, Gannon M, et al. Complementation rescue of Pdx1 null phenotype demonstrates distinct roles of proximal and distal cis-regulatory sequences in pancreatic and duodenal expression. Dev Biol. 2006; 298:616–631. [PubMed: 16962573]

36. Fujitani Y, Fujitani S, Boyer DF, et al. Targeted deletion of a cis-regulatory region reveals differential gene dosage requirements for Pdx1 in foregut organ differentiation and pancreas formation. Genes Dev. 2006; 20:253–266. [PubMed: 16418487]
37. Jonsson J, Carlsson L, Edlund T, et al. Insulin-promoter-factor 1 is required for pancreas development in mice. Nature. 1994; 371:606–609. [PubMed: 7935793]
38. Gao N, LeLay J, Vatamaniuk MZ, et al. Dynamic regulation of Pdx1 enhancers by Foxa1 and Foxa2 is essential for pancreas development. Genes Dev. 2008; 22:3435–3448. [PubMed: 19141476]
39. Chen C, Fang R, Davis C, et al. Pdx1 inactivation restricted to the intestinal epithelium in mice alters duodenal gene expression in enterocytes and enteroendocrine cells. Am J Physiol Gastrointest Liver Physiol. 2009; 297:G1126–1137. [PubMed: 19808654]
40. Ahlgren U, Jonsson J, Jonsson L, et al. beta-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. Genes Dev. 1998; 12:1763–1768. [PubMed: 9637677]
41. Holland AM, Hale MA, Kagami H, et al. Experimental control of pancreatic development and maintenance. Proc Natl Acad Sci U S A. 2002; 99:12236–12241. [PubMed: 12221286]
42. Buckingham ME, Meilhac SM. Tracing cells for tracking cell lineage and clonal behavior. Dev Cell. 2011; 21:394–409. [PubMed: 21920310]
43. Kretzschmar K, Watt FM. Lineage tracing. Cell. 2012; 148:33–45. [PubMed: 22265400]
44. Talchai C, Xuan S, Lin HV, et al. Pancreatic beta Cell Dedifferentiation as a Mechanism of Diabetic beta Cell Failure. Cell. 2012; 150:1223–1234. [PubMed: 22980982]
45. Itzkovitz S, Blat IC, Jacks T, et al. Optimality in the development of intestinal crypts. Cell. 2012; 148:608–619. [PubMed: 22304925]
46. Liu C, Sage JC, Miller MR, et al. Mosaic analysis with double markers reveals tumor cell of origin in glioma. Cell. 2011; 146:209–221. [PubMed: 21737130]

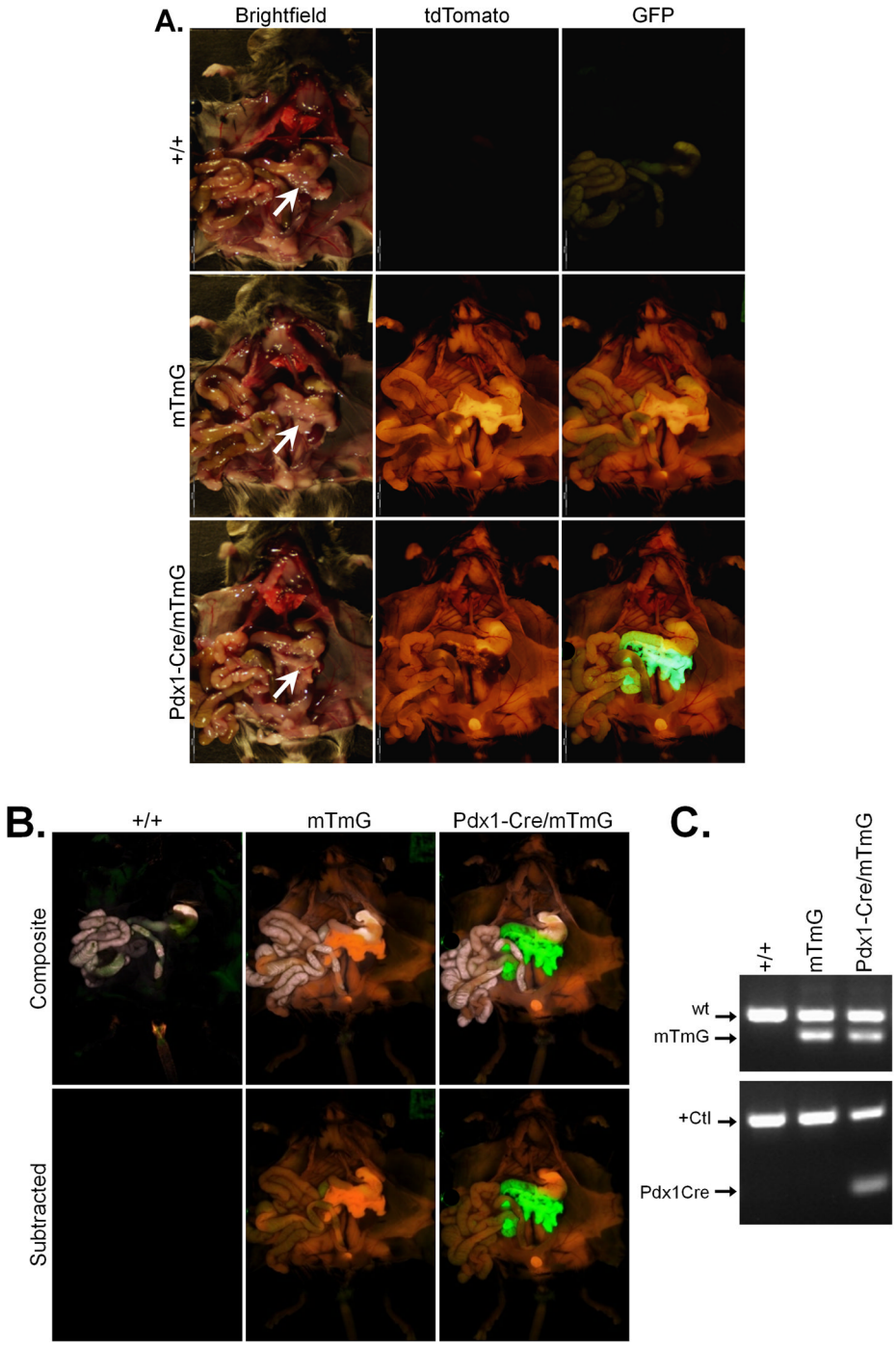
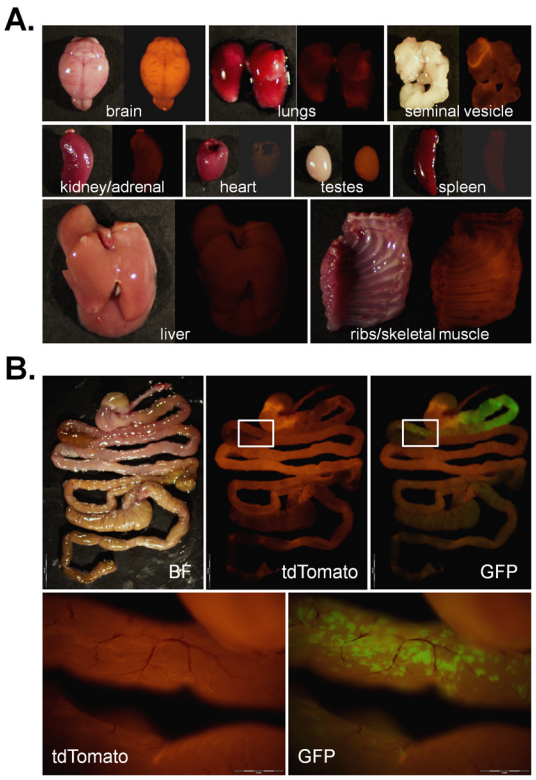


FIGURE 1. Ubiquitous expression of tdTomato and tissue-specific expression of GFP demonstrated in three female littermates from a *Pdx1-Cre* x mTmG cross at necropsy

Non-fluorescent, mTmG transgene-negative (+/+) and fluorescent, mTmG-positive (mTmG or *Pdx1-Cre*/mTmG) littermates were euthanized and imaged at 6 weeks of age. Livers and diaphragms were removed to better expose the stomach and pancreas. **A.** Images acquired using an Olympus OV100 small animal imaging system and either bright light (Brightfield) or fluorescence (tdTomato or GFP) illumination and instrument settings. All mice were imaged during a single imaging session using the same exposure times for all genotypes.

The pancreas of each mouse is indicated by a white arrow in Brightfield images. GFP expression is limited to the pancreas and proximal small bowel of the *Pdx1-Cre*/mTmG littermate (bottom row, right panel); whereas tdTomato is brightly expressed within the pancreas of the mTmG littermate lacking *Pdx1-Cre* (middle row, both tdTomato and GFP settings). Images of non-fluorescent (+/+) littermate are shown for comparison (top row). **B.** Images acquired using a CRi Maestro™ EX *in vivo* imaging system, which allows for unmixing of fluorescent emission spectra and subtraction of autofluorescence. Composite images (upper row) show total tissue fluorescence, consisting of auto-fluorescence and the fluorescence produced by independent fluorophores (tdTomato and GFP), for +/+, mTmG, and *Pdx1-Cre*/mTmG littermates. Subtracted images (lower row) show the appearance of tissues after autofluorescence is removed from images using Maestro™ software. **C.** Multiplex PCR genotyping of the mice shown in **A** and **B** for the presence of the mTmG and *Pdx1-Cre* transgenes versus wild type (wt, upper gel image) or internal positive control (+Ctl, lower gel image) sequences, respectively.



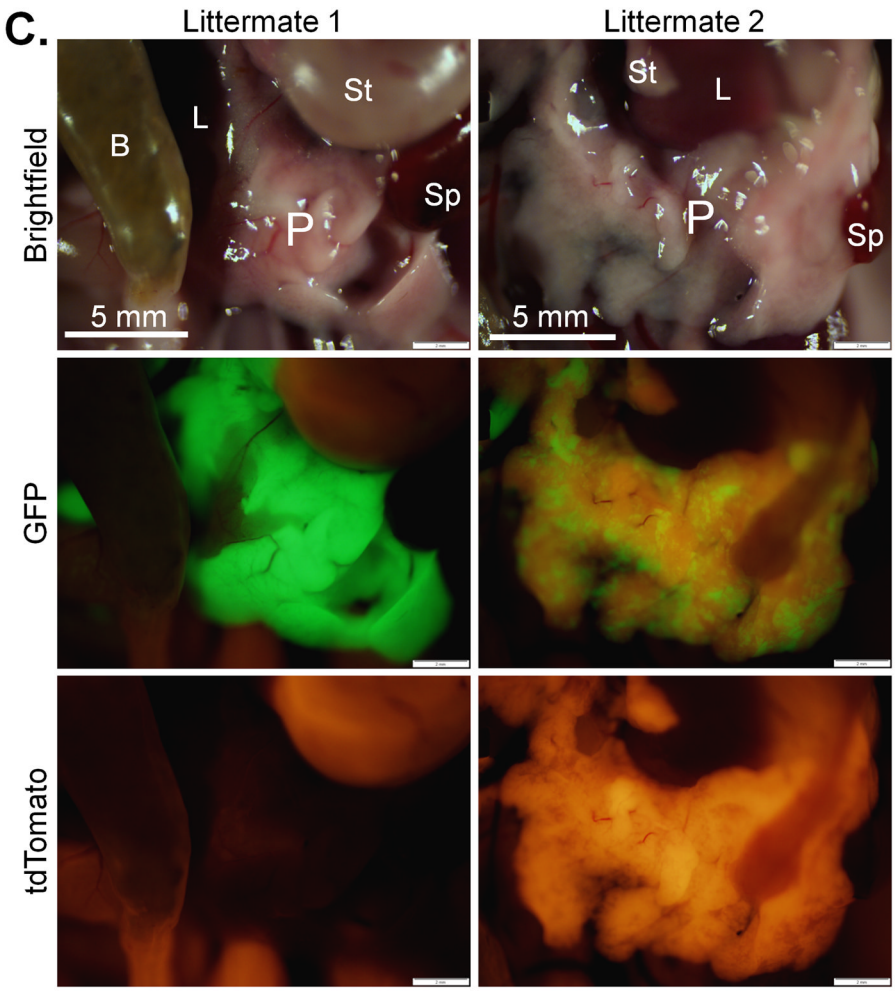
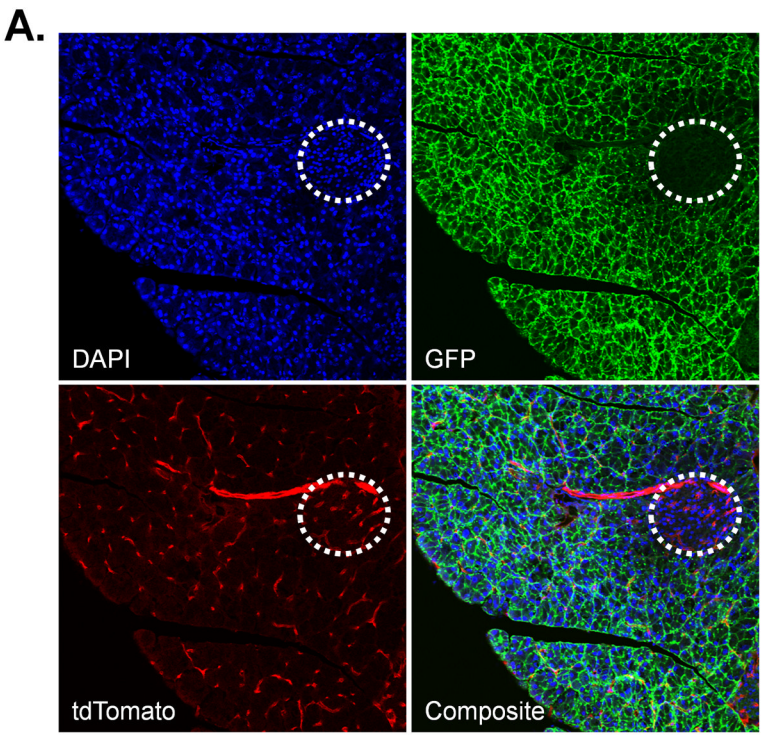


FIGURE 2. Expression of GFP in *Pdx1-Cre*/mTmG mice localizes to the pancreas and duodenum

Ex vivo imaging of dissected organs demonstrates the specificity and extent of *Pdx1-Cre*-mediated GFP expression in *Pdx1-Cre*/mTmG mice. **A.** Paired brightfield (left) and tdTomato fluorescence (right) images of major organs from a *Pdx1-Cre*/mTmG male adult; GFP expression was not detected in any of these organs (GFP data not shown). Exposure times used were the same for all organs shown; organs characterized by high circulatory blood volumes demonstrate significant hemoglobin-associated fluorescence quenching. **B.** Brightfield (BF) and fluorescence (tdTomato and GFP) images show the gastrointestinal tract, extending from the esophagus to the rectum, from a *Pdx-1Cre*/mTmG mouse (pancreas dissected away). *Pdx1-Cre*-mediated extra-pancreatic GFP expression is highest in the rostral duodenum; tapering off in the distal duodenum (white inset boxes in upper panel images; shown at greater magnification in lower panel images). **C.** Pancreata of two *Pdx1-Cre*/mTmG littermates at necropsy. Littermate 2 was very atypical and demonstrated an unusually high degree of mosaicism for *Pdx1-Cre*-mediated GFP expression within the pancreas. The extent of *Pdx1-Cre*-mediated conversion of tdTomato expression to GFP expression resembled Littermate 1 in the large majority of *Pdx1-Cre*/mTmG mice examined. (P, pancreas; St, stomach; Sp, spleen; L, liver; B, bowel)



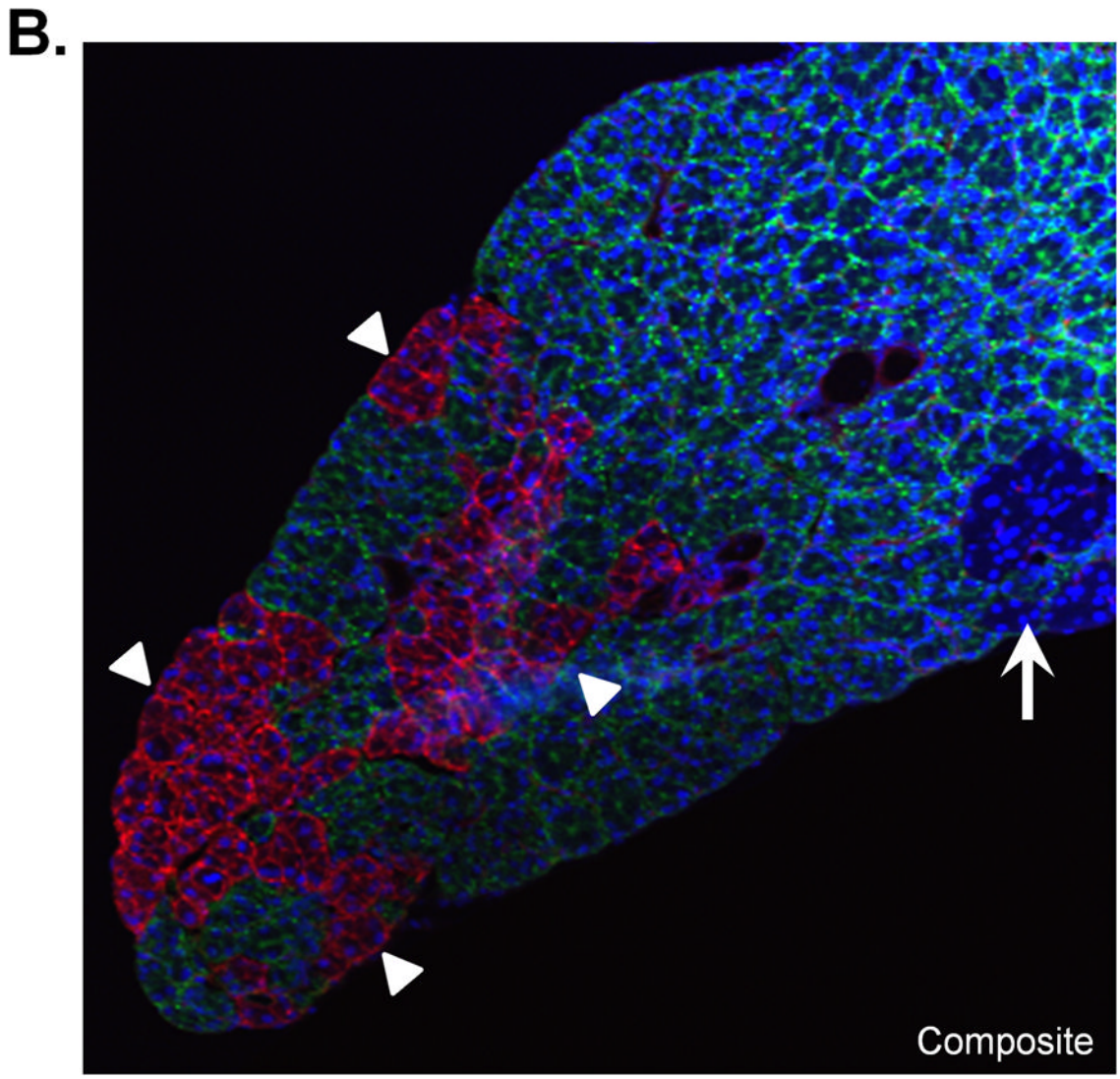


FIGURE 3. Detailing of the pancreatic microenvironment by membrane-targeted expression of tdTomato and GFP within the *Pdx1-Cre*/mTmG mouse pancreas

Membrane-targeted tdTomato or GFP proteins are expressed from the unrearranged or Cre-recombined mTmG transgene, respectively. **A.** Green pancreatic acinar cells (GFP) and red vasculature (tdTomato) are clearly outlined components of the pancreatic microenvironment in *Pdx1-Cre*/mTmG mice. A small pancreatic islet (outlined in white) contains GFP-expressing islet cells that are faint in comparison to the brightly GFP-positive acinar cells. DAPI was used to counterstain cell nuclei. A second islet (not outlined) is present at the extreme lower right edge of the image. **B.** Low leves of genetic mosaicism, characterized by the presence of focal irregular clusters of bright tdTomato-expressing acinar cells (white arrowheads) against a background of GFP-expressing pancreatic acini, was a common finding in *Pdx1-Cre*/mTmG pancreata. A small intra-pancreatic lymph node (white arrow), whose identity was determined by H&E-staining of serial tissue sections, also appears in this composite image.

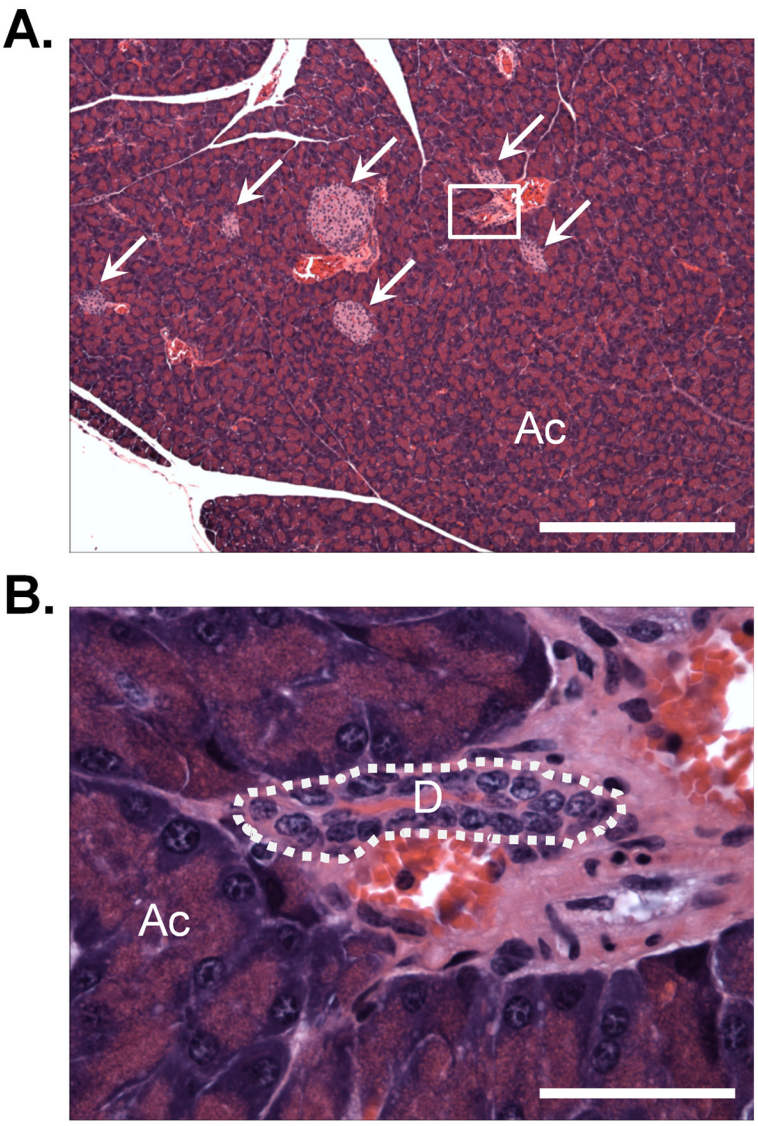


FIGURE 4. Normal histological appearance of *Pdx1-Cre*/mTmG mouse pancreas

H&E-stained section of formalin-fixed, paraffin-embedded pancreas from a *Pdx1-Cre*/mTmG mouse. **A.** Low power magnification view of the pancreas from a *Pdx1-Cre*/mTmG mouse demonstrates the normal predominance of pancreatic acinar tissue (Ac), with pancreatic islets (arrows) distributed throughout the acinar tissue background. The area within the white box is enlarged in panel B. (scale bar = 500 micron) **B.** High magnification view of a pancreatic duct (D, outlined in white) and adjacent vascular structures surrounded by acinar tissue. (scale bar = 50 micron)