

# UC Davis

## UC Davis Previously Published Works

### Title

The Toxoplasma effector GRA28 promotes parasite dissemination by inducing dendritic cell-like migratory properties in infected macrophages

### Permalink

<https://escholarship.org/uc/item/1m72z6hm>

### Journal

Cell Host & Microbe, 30(11)

### ISSN

1931-3128

### Authors

Ten Hove, Arne L  
Braun, Laurence  
Rodriguez, Matias E  
[et al.](#)

### Publication Date

2022-11-01

### DOI

10.1016/j.chom.2022.10.001

Peer reviewed



Published in final edited form as:

*Cell Host Microbe*. 2022 November 09; 30(11): 1570–1588.e7. doi:10.1016/j.chom.2022.10.001.

## The *Toxoplasma* effector GRA28 promotes parasite dissemination by inducing dendritic cell-like migratory properties in infected macrophages

Arne L. ten Hoeve<sup>1,†</sup>, Laurence Braun<sup>2,†</sup>, Matias E. Rodriguez<sup>1</sup>, Gabriela C. Olivera<sup>1</sup>, Alexandre Bougdour<sup>2</sup>, Lucid Belmudes<sup>3</sup>, Yohann Couté<sup>3</sup>, Jeroen P. J. Saeij<sup>4</sup>, Mohamed-Ali Hakimi<sup>2,\*</sup>, Antonio Barragan<sup>1,5,\*</sup>

<sup>1</sup>Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, 106 91 Stockholm, Sweden

<sup>2</sup>Institute for Advanced Biosciences, INSERM U1209, CNRS UMR5309, Université Grenoble Alpes, Grenoble, France

<sup>3</sup>Univ. Grenoble Alpes, INSERM, CEA, UMR BioSanté U1292, CNRS, CEA, FR2048 38000, Grenoble, France.

<sup>4</sup>Department of Pathology, Microbiology, and Immunology, University of California Davis, Davis, CA 95616 California, United States of America

<sup>5</sup>Lead contact

### Summary

Upon pathogen detection, macrophages normally stay sessile in tissues while dendritic cells (DCs) migrate to secondary lymphoid tissues. The obligate intracellular protozoan *Toxoplasma gondii* exploits the trafficking of mononuclear phagocytes for dissemination via unclear mechanisms. We report that, upon *T. gondii* infection, macrophages initiate expression of transcription factors normally attributed to DCs, upregulate CCR7 expression with a chemotactic response, and perform systemic migration when adoptively transferred into mice. We show that parasite effector GRA28, released by the MYR1 secretory pathway, cooperates with host chromatin remodelers in the host cell nucleus to drive the chemotactic migration of parasitized macrophages. During in vivo challenge studies, bone marrow-derived macrophages infected with wildtype *T. gondii*

\*Corresponding authors antonio.barragan@su.se, mohamed-ali.hakimi@univ-grenoble-alpes.fr.

†These authors contributed equally to this work.

#### Author contributions

Experimentation: A.L.T.H., La.B., M.E.R., G.C.O., , Al.B., Lu.B.

Methodology: A.L.T.H., La.B., M.E.R., G.C.O., Y.C., Lu.B., J.P.J.S.

Data analysis and curation: A.L.T.H., An.B., La.B., Al.B., Y.C., M.A.H.,

Writing, review and editing: A.L.T.H., An.B., La.B., M.A.H., J.P.J.S.

Funding acquisition and supervision: An.B., M.A.H.

#### Declaration of interests

The authors declare no competing interests.

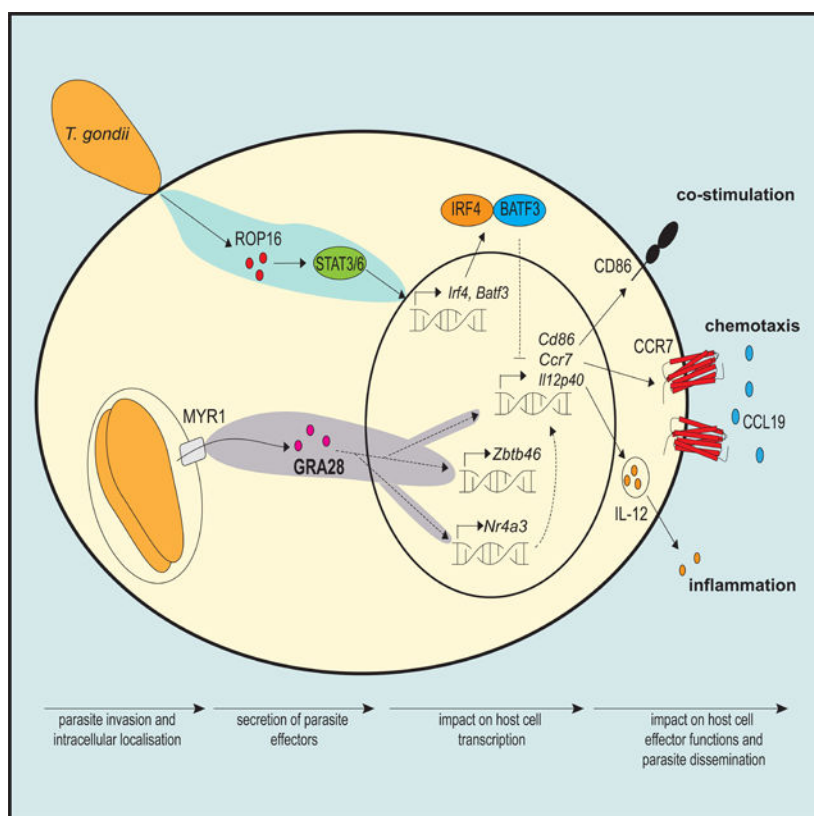
**Publisher's Disclaimer:** This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

outcompeted those challenged with MYR1- or GRA28- deficient strains in migrating and reaching secondary organs. This work reveals how an intracellular parasite hijacks chemotaxis in phagocytes and highlights a remarkable migratory plasticity in differentiated cells of the mononuclear phagocyte system.

## eTOC blurb

*Toxoplasma gondii* exploits the trafficking of phagocytes for dissemination. Ten Hoeve et al. show that parasitized macrophages acquire dendritic cell-like CCR7-dependent migratory properties via parasite effector GRA28-mediated transcriptional remodeling of the host cell, which facilitates *T. gondii* dissemination. This work highlights a remarkable migratory plasticity in differentiated mononuclear phagocytes.

## Graphical Abstract



## Keywords

Mononuclear phagocyte; Apicomplexa; chemotaxis; chemokine receptor 7; host-pathogen; intracellular parasitism

## Introduction

The mononuclear phagocyte system plays pivotal roles in immune responses and comprises principally monocytes, macrophages and dendritic cells (DCs) (Guilliams et al., 2014). The identification of DC-restricted precursors indicates ontological differences between DCs and macrophages (Sathe et al., 2014). While DCs arise from bone marrow-derived common DC precursors, various tissue-resident macrophages develop from yolk-sac or erythro-myeloid progenitors during fetal development (Guilliams et al., 2014). Macrophages and monocyte-derived DCs (Mo-DCs) also differentiate from monocytic precursors (Helft et al., 2015).

Ontology and differences in tissue localization are reflected in the transcriptomes of macrophages and DCs, which can be distinguished by transcriptional signatures (Miller et al., 2012). Marked by the expression of ZBTB46 (Satpathy et al., 2012), conventional DCs (cDCs) develop *in vivo* in response to STAT3-activating growth factor FLT3L (Laouar et al., 2003) and under the control of IRF8, BATF3 or IRF4 (Amon et al., 2019). Similarly, Mo-DCs depend on IRF4 for their differentiation while monocytes divert towards macrophages independently of IRF4 (Briseno et al., 2016). Together STAT3, BATF3, IRF4 and IRF8 form part of the regulatory network that controls cDC development (Lin et al., 2015).

The study of host-pathogen interactions provides a powerful approach to gain insight into cellular processes in immune cells. The protozoan *Toxoplasma gondii* commonly infects humans and other warm-blooded vertebrates, for example rodents. It is estimated that one third of the global human population encounters *T. gondii* during a life-time (Pappas et al., 2009). Following oral infection, *T. gondii* disseminates widely from the intestine to reach peripheral organs, such as the central nervous system. Although principally asymptomatic in healthy individuals, infection can cause life-threatening encephalitis in immune-compromised persons, severe neurological manifestations in the developing fetus, and recurrent ocular disease in immune-competent individuals (Montoya and Liesenfeld, 2004; Schluter and Barragan, 2019).

The disseminating stage of *T. gondii*-the tachyzoite- is obligate intracellular. Thus, active invasion of host cells, including leukocytes, is essential for parasite survival (Dobrowolski and Sibley, 1996). Invasion of host cells encompasses the discharge of secretory organelles, called rhoptries, into the cytosol and the secreted proteins (ROPs) modulate cellular responses of parasitized cells (Frickel and Hunter, 2021; Hakimi et al., 2017). The virulence-associated rhoptry kinase ROP16 activates STAT signaling in infected cells which results in inhibition of inflammatory cytokine secretion (Butcher et al., 2011; Saeij et al., 2007). Further, a secretory machinery (MYR) ensures transport of dense granule proteins (GRAs) across the intracellular parasitophorous vacuole where the parasite resides (Franco et al., 2016).

Shortly after oral infection, *T. gondii* crosses the intestinal epithelium (Barragan and Sibley, 2003; Dubey, 1997). Systemic spread from the intestine to peripheral organs via the blood circulation is largely mediated by parasitized CD11c<sup>+</sup> and CD11b<sup>+</sup> leukocytes (Courret et al., 2006). DCs can act as ‘Trojan horses’ for *T. gondii* dissemination in mice (Lambert et al., 2006) in a parasite genotype-related fashion (Lambert et al., 2009). Upon active invasion

by *T. gondii*, DCs are induced to migrate via activation of non-canonical GABAergic signaling and MAP kinase activation (Bhandage et al., 2020; Kanatani et al., 2017; Olafsson et al., 2019; Olafsson et al., 2020). This migratory activation, termed hypermigratory phenotype (Weidner and Barragan, 2014), implicates secreted parasite effectors (Drewry et al., 2019; Sangare et al., 2019; Weidner et al., 2016) and does not rely on chemotaxis (Fuks et al., 2012; Weidner et al., 2013).

Intestinal macrophages are present at higher densities along the villi and are located more closely to the epithelial cells than DCs (Schulz et al., 2009). Although macrophages and DCs share many functions, they differ in their migration under steady-state and inflammatory conditions. Upon inflammation, DCs migrate from peripheral tissues via lymphatic vessels to lymph nodes, for which the upregulation of the chemokine receptor CCR7 is indispensable (Alvarez et al., 2008; Forster et al., 1999). By and large, macrophages can be distinguished from DCs by their lack of *Ccr7* expression (Miller et al., 2012). In line with this, macrophages along the intestinal epithelium are sessile and do not normally migrate to draining lymph nodes upon Toll-like receptor (TLR) stimulation, unlike neighboring DCs (Schulz et al., 2009).

Here, we show that *T. gondii* imparts a DC-like transcriptional signature on macrophages and that parasitized macrophages upregulate CCR7 expression with the onset of chemotaxis. Our data highlight a mechanism by which *T. gondii* exploits the migratory plasticity of parasitized macrophages to potentiate its dissemination.

## Results

### ***T. gondii* induces expression of DC-associated transcription factors in parasitized macrophages**

The transcription factor IRF4 was found to be essential for the differentiation of monocytes to DCs but not macrophages (Briseno et al., 2016; Goudot et al., 2017). Because *T. gondii* encounters mononuclear phagocytes in tissues and modulates their migratory properties (Bhandage et al., 2020), we investigated the impact of infection on transcriptional signatures that define DCs and macrophages. Interestingly, upon challenge with live *T. gondii* tachyzoites (Figure 1A), an elevated expression of *Irf4*, similar to that in bone marrow-derived DCs (BMDCs), was measured in resident peritoneal macrophages (PEMs), macrophage-like PMA bone marrow-derived cells (PMA-BMCs) and bone marrow-derived macrophages (BMDMs) (Figure 1B). In contrast, neither challenge with tachyzoite lysate nor LPS altered *Irf4* expression. Flow cytometry analyses confirmed markedly elevated IRF4 protein expression in *T. gondii*-infected BMDMs but not in bystander BMDMs (Figure 1C). IRF4 expression in infected BMDMs reached levels comparable with expression in BMDCs (CD11c<sup>+</sup>MHCII<sup>hi</sup>) (Figure S1A), and with prominent nuclear staining (Figure S1B).

Additional DC signature genes include the transcription factors *Zbtb46*, *Batf3*, *Nr4a3* and *Runx3* (Miller et al., 2012) and ChIP data suggest that these genes are targeted by IRF4 (Figure S1C) (Boulet et al., 2019). In line with this, challenge with *T. gondii* resulted in a significant increase in *Zbtb46*, *Batf3*, *Nr4a3* and *Runx3* mRNA in macrophages, approaching or surpassing their relative expression in BMDCs (Figures 1D, 1E, 1F and S1D).

In contrast, expression was non-significantly altered by *T. gondii* lysate or LPS. Elevated ZBTB46 protein expression in *T. gondii*-challenged macrophages was confirmed by Western blotting (Figure 1G). Notably, challenge with *T. gondii* minorly impacted expression of the pDC-associated transcription factor *Spib*, receptor *Flt3* and Ly6C (Figures S1E, S1F and S1G), while expression of the M-CSF receptor CD115 was reduced on both BMDMs and PEMs, in the absence of a measurable by-stander effect (Figures 1H, 1I and S1H). We confirmed that *Zbtb46* and *Irf4* are induced by a variety of *T. gondii* strains (Figure S1I). Additionally, we could largely confirm induction of *Zbtb46*, *Batf3*, *Nr4a3* and *Irf4* by *T. gondii* in previously published transcriptomics data (Figure S2A and S2B). However, comparing *T. gondii*-induced transcriptional changes with Mo-DC differentiation and gene expression differences between BMDMs and BMDCs, we found no broad similarities. We conclude that challenge of different types of macrophages with *T. gondii* induces the expression of transcription factors associated with cDCs, but not with DC differentiation *per se*.

### ***T. gondii*-infected macrophages perform chemotaxis in response to CCL19**

Because *Zbtb46*, *Batf3*, *Nr4a3* and *Irf4* mediate DC development and differentiation, including associations to CCR7-mediated DC migration (Bajana et al., 2012), we addressed if infection impacted chemotaxis in macrophages. First, challenge with *T. gondii* tachyzoites induced a significantly superior transcriptional expression of *Ccr7*, related to LPS or MOI-equivalent doses of parasite lysate, and similar or superior to BMDCs (Figures 2A and S2C). BMDMs infected with *T. gondii* displayed elevated surface CCR7 expression over unchallenged macrophages (Figure 2B), all consistent with the relative expression profiles of DC-associated transcription factors (Figure 2C). Next, in chemotaxis assays with the CCR7-ligand chemokine CCL19, *T. gondii*-infected BMDM displayed a distinct migratory response towards the CCL19 source. Similarly, BMDCs chemotaxed (Figures 2D and 2E), as described (Helft et al., 2015), with undetectable chemotaxis by non-infected bystander BMDMs (Figure 2E) and LPS-challenged macrophages (Figure 2F). Additional control characterizations excluded chemokinetic or haptokinetic effects by CCL19 (Figures S2D and S2E). Of note, parasitized macrophages exhibited hypermotility, in line with previous results in monocytes and DCs (Bhandage et al., 2020), and this component of the migratory response was independent of CCL19 and was absent in bystander macrophages (Figures S2D and S2E). Further, we found that *T. gondii*-infected BMDMs expressed significantly higher levels of CD86 and MHCII, but not CD40 and CD80 (Figures 2G, 2H and S2F), consistent with the impact of *T. gondii* on macrophage phenotypical maturation, cytokine responses and proliferative responses in leukocytes (Figure S2G, S2H, S2I and S2J). While noting an increase in CD11c<sup>+</sup>MHCII<sup>+</sup>-expressing cells among resident CD11b<sup>hi</sup>CD19<sup>-</sup> peritoneal cells infected *in vitro* (Figure 2I), we also found increases of CD86 and MHCII on *T. gondii*-infected PEMs (Figure 2J). We conclude that challenge with *T. gondii* elevates the expression of CCR7 in parasitized macrophages with chemotactic responses to CCL19, in absence of a detectable bystander effect.

## The *T. gondii* MYR1 secretory pathway and ROP16-STAT signaling differently impact chemotaxis and migration-associated transcription in infected macrophages

Because the migratory responses indicated effects related to intracellularly located parasites, we assessed two main pathways utilized by *T. gondii* to modulate host cell responses and transcriptome: rhoptry (ROP) protein-mediated activation of STAT signaling (Saeij et al., 2007) and effector protein secretion via the MYR1 pathway (Franco et al., 2016; Naor et al., 2018). Importantly, chemotaxis was abrogated in macrophages infected with MYR1-deficient parasites (Tg *myr1*) but maintained upon challenge with ROP16-deficient parasites (Tg *rop16*) (Figure 3A). Consistently, *Ccr7* expression was reduced (Tg *myr1*) and elevated (Tg *rop16*), respectively, upon challenge with the mutant parasites (Figure 3B). In line with this, contrasting effects were observed on *Zbtb46*, *Irf4*/IRF4, *Batf3* and *Nr4a3* expression (Figures 3B, S3A and S3B). Moreover, the downregulation of CD115 was abrogated and partially reduced by MYR1 and ROP16 deficiency, respectively (Figure S3C). Consistent with the downmodulation of inflammatory cytokine responses by ROP16 (Jensen et al., 2013), macrophages challenged with Tg *rop16* expressed higher levels of *Il12p40* and CD86, and elicited higher *Ifng* expression in MLR (Figures 3C, 3D and S3D). STAT inhibition confirmed the implication of STATs in the observed transcriptional changes (Figure S3E). In sharp contrast, *Il12p40* and CD86 expression were significantly reduced upon challenge with Tg *myr1* parasites (Figures 3C and 3D).

To confirm that the observed phenotypic effects were linked to the MYR1 secretory pathway and not MYR1 expression itself, we assessed two additional mutants (Tg *rop17*, Tg *gra45*) associated with this secretory pathway (Panas et al., 2019; Wang et al., 2020) with similar effects on *Ccr7*, *Il12p40*, *Zbtb46* and *Irf4* expression (Figures 3E and S3F). In contrast, a mutant deficient in TgWIP, a rhoptry protein associated with non-chemotactic hypermotility of parasitized DCs (Sangare et al., 2019), maintained elevated expression of *Ccr7* and chemotaxis of macrophages, similar to wildtype (Figures 3E, 3F and S3F). Jointly, these data show that the expression of DC-associated transcripts and markers in *T. gondii*-challenged macrophages depend on both parasite-derived ROP16 and MYR1-mediated secretions, which partly have opposite effects. ROP16 inhibited phenotypical maturation of macrophages with reduction of *Ccr7* expression but with maintained CCL19-dependent chemotaxis. In contrast, the MYR1 secretion system heavily impacted proinflammatory cytokine response, elevated *Ccr7* expression and mediated CCL19-dependent chemotaxis.

## The MYR1-associated parasite effector GRA28 mediates CCR7-dependent chemotaxis of macrophages

Given the impact of the MYR1 secretion system on chemotaxis and transcription, we hypothesized that MYR1-secreted effector molecules that target the host cell nucleus mediated the observed phenotypes in macrophages. GRA28, a 200 kDa highly disordered dense granule protein, was initially suspected because the protein is exported to the host cell nuclei in a MYR1- and ASP5-dependent manner (Figures 4A and 4B) (Nadipuram et al., 2016) and, like other exported GRAs, contributes to the re-orchestration of host gene expression in macrophages but uniquely triggers CCR7 gene expression (Figures 4C, 4D, 4E, S4A and S4B). Comparing the *gra28* mutant to the parental strain, we found that the expression of a relatively small number of host genes was indeed upregulated

in a GRA28-dependent manner in human and murine macrophages, including CCL22 (Figures 4E, S4C and S4D), a previously described GRA28-regulated gene (Rudzki et al., 2021). Reintroduction of one copy of the gene into *gra28*-deficient parasites was sufficient to restore expression of these genes (Figures 4E, S4B, S4C and S4D). Although transcriptomics clearly identified GRA28 as a transcriptional activator in infected cells, it also showed that the protein triggers repression of a substantial fraction of host genes (Figures 4C and 4D). Quantitative reverse transcription PCR of mRNA from four of the repressed genes (i.e., *Cxcl3*, *Cxcl5*, *Vcam1*, and *Il1a*) documented a robust and persistent pattern of GRA28-mediated cytokine and *Vcam1* repression (Figure S4E).

Having identified GRA28 as a regulator of CCR7 expression, we investigated its role in the chemotaxis phenotype by comparing it with GRA24, another effector known to regulate the expression of chemokines and their receptors (Braun et al., 2013). Notably, while macrophages challenged with GRA24-deficient *T. gondii* (Tg *gra24*) maintained *Ccr7* expression and chemotaxis, challenge with GRA28-deficient tachyzoites (Tg *gra28*) dramatically reduced *Ccr7* expression, with abrogated chemotaxis to CCL19 (Figures 4F and 4G). Consistent with these results, ablation of GRA28, but not GRA24, abrogated the induction of *Il12p40* (Figure 4H) and the expression of CD86 (Figure 4I), compared with wild-type. In contrast, a downregulation of the M-CSF receptor CD115 (Figure 1H) was maintained for both mutants (Figure S4F). Finally, we confirmed the differential effects of GRA24- and GRA28-deficiency of transcription factors. In particular, the GRA24-dependent early growth response 1 (*Egr1*) upregulation (Braun et al., 2013; ten Hoeve et al., 2019) was maintained upon GRA28-deficiency (Figure 4J). Further, *Zbtb46* expression was reduced by both GRA24- and GRA28-deficiency compared with wild-type parasites, whereas *Irf4* expression was maintained or increased (Figure 4J). Consistently, induction of *Nr4a3* and *Runx3*, but not *Batf3*, was also partially dependent on GRA28 (Figure S4G). Taken together, these results show that GRA24 and GRA28 differently impact transcription and effector functions of macrophages and that the MYR1-mediated induction of *Ccr7* and chemotaxis in parasitized macrophages is dependent on GRA28 secretion.

### **GRA28 forms versatile complexes with chromatin modifiers with antagonistic activities**

GRA28 is an inherently unstructured protein (Hakimi et al., 2017) and, in this respect, has no discernible protein motifs or defined domains that might indicate specific interaction partners in the host cell nucleus, where it accumulates after infection. To gain functional insight into how GRA28 regulates host gene expression, we searched for host cell partners by applying conventional and affinity chromatography to extracts from murine macrophages infected with tachyzoites expressing a HAFlag-tagged version of GRA28 (Figures 5A and 5B). Silver staining analysis of the eluate showed that GRA28 binds to multiple proteins under very stringent wash conditions (0.5 M NaCl and 0.1% NP-40), forming two distinct complexes with apparent molecular weights of approximately 2 MDa (complex 1, fractions F14-F19) and 500–600 kDa (complex 2, fractions F20-F22) once resolved in size exclusion chromatography (Figure 5B). These partnerships were subsequently elucidated by proteomics based on mass spectrometry (Table S1) suggesting that GRA28 forms versatile complexes with *i)* the multi-subunit NuRD repressor complex, which includes chromatin remodeling ATPase (CHD4) and deacetylation enzymes (HDAC1,2) (Xue et al., 1998) and



ii) the SWI/SNF (BAF) chromatin remodeler, a 2 MDa multisubunit complex characterized by, among others, its core members SMARCB1, SMARCC1/SMARCC2, and SMARCA4 (also known as BRG1), which use energy from ATP hydrolysis to mobilize nucleosomes (Cairns et al., 1998; Kingston and Narlikar, 1999) (Figure 5C). Of note, GRA28 binds to a NuRD complex distinct from that of TgIST in that it does not contain STAT1, nor the transcriptional corepressors CtBP1 and CtBP2 (Figure S5A) (Gay et al., 2016).

These data suggest the intriguing possibility that once GRA28 enters the host cell nucleus, it cooperates with NuRD and SWI/SNF to repress and activate host gene expression, respectively. Accordingly, GRA28 is expected to bind to chromatin. ChIP analyses on samples of human fibroblasts infected with HAFlag knock-in tachyzoites showed that GRA28 was highly enriched in a specific manner near 1976 genes (10-fold enrichment, Figure S5B and Table S2) compared with control GRAX, which also accumulated in host nuclei after infection but was rarely bound to chromatin (137 genes, 10-fold enrichment) (Figure S5B and Table S2). GRA28 is bound to upstream and intergenic regions, and at the promoter level, the protein is enriched upstream and downstream of the transcription start site (TSS) (Figures 5D, 5E and 5C). Introns were also highly represented (33%) among GRA28-enriched regions, as reported for BRG1 in human cells (Attanasio et al., 2014). Of note, GRA28 was clearly enriched at the *CCR7* locus (Figure 5F), and its induction may be mediated by recruitment of SWI/SNF (BAF) by GRA28, a chromatin remodeler known to activate transcription by promoting nucleosome repositioning. Chromatin occupancy of GRA28 downstream and upstream of *CCR7*, sometimes far from TSS, suggests that GRA28 cooperates with chromatin remodelers to increase DNA accessibility by creating an open chromatin conformation over long distances that favors transcription factor binding to site-specific sequences and RNA polymerase progression (Figure 5F).

### Role of macrophage migratory activation on the dissemination of *T. gondii* *in vivo* in mice

To address the impact of *T. gondii*-induced migratory activation of BMDMs on dissemination *in vivo*, we designed separate approaches in mice. For the different conditions, equivalent numbers of pre-treated and pre-labelled cells were simultaneously adoptively transferred i.p. to mice in competition assays. Fourteen-18 h post-inoculation, organs were harvested and cells were characterized by flow cytometry (Figures 6A, S6A, S6B, S6C and S6D). First, unchallenged and *T. gondii*-challenged BMDMs were assessed for migration. Interestingly, challenged BMDMs (CMTMR<sup>+</sup>GFP<sup>+/-</sup>) migrated to the omentum, mesenteric lymph nodes (MLNs) and spleen at a higher rate than unchallenged BMDM (Deed Red<sup>+</sup>) and this difference was further accentuated in infected (CMTMR<sup>+</sup>GFP<sup>+</sup>) cells (Figure 6B). Next, when BMDMs challenged with wild-type or MYR1-deficient (Tg *myr1*) *T. gondii* were allowed to compete, relative lower numbers of *myr1*-infected BMDMs were retrieved in organs (Figure 6C). Similarly, BMDMs challenged with GRA28-deficient *T. gondii* (Tg *gra28*) migrated at a relative lower frequency compared to BMDMs challenged with wild-type *T. gondii* (Figure 6D), with a relative re-elevation of migration in BMDMs challenged with reconstituted parasites (Tg *gra28* + GRA28) (Figure 6E). Jointly, this indicated an implication of MYR1-associated GRA28 secretion in the migration of parasitized BMDMs.

These results, together with a previously observed enhanced migration of parasitized BMDCs (Bhandage et al., 2020; Lambert et al., 2006), motivated a comparison of the migratory behaviors of parasitized BMDCs and parasitized BMDMs. Both cell populations exhibited a comparable high migration rate, with BMDMs relatively enriched in spleen and omentum (Figure 6G), indicative of a migration capability by parasitized BMDMs. Finally, we found that the migration frequencies of CCR7-deficient BMDMs were reduced related to wild-type BMDMs (Figure 6G), confirming a relative dependency of parasitized BMDMs on CCR7 for migration. As in *in vitro* assays (Figure 3D), wild-type infected adoptively transferred BMDMs collected from spleen expressed significantly higher levels of CD86 (Figure 6H) and chemotaxis phenotypes were confirmed *in vitro* for all conditions (Figure S6E and S6F). Altogether, the data show that *T. gondii*-infected BMDMs can traffic to secondary lymphoid organs, with an impact of the MYR1 pathway, secreted effector GRA28 and homing receptor CCR7. We conclude that parasitized BMDMs exhibit enhanced systemic migration and can potentiate dissemination of intracellularly located *T. gondii*.

### Impact of *T. gondii* effectors on transcriptional activation and chemotaxis of human monocytes and macrophages

Because hypermigratory responses upon *T. gondii* challenge were also observed in infected human mononuclear phagocytes (Bhandage et al., 2020), we sought to confirm our key findings in human peripheral blood monocytes and monocyte-derived macrophages. Interestingly, parasitized human macrophages chemotaxed in a CCL19 gradient, unlike bystander macrophages (Figure 7A). Extending data in murine macrophages, Tg *myr1* parasites and LPS treatments failed to significantly induce chemotaxis (Figure 7A). Consistent with these findings, challenge with *T. gondii* wild-type induced *CCR7* expression in both monocytes and macrophages, in contrast with Tg *myr1* (Figures 7B). Despite undetectable chemotactic responses under these conditions, LPS challenge induced *CCR7* expression (Figures 7A, 7C and 7D). As in murine macrophages, we found that *IRF4* and *BATF3* were upregulated by *T. gondii* challenge in monocytes and macrophages, but not by tachyzoite lysates or LPS (Figures S7A, S7B and S7C). Furthermore, the induction of *IRF4* expression in human monocytes and macrophages was dependent on MYR1 (Figure S7D), while the induction of *BATF3* expression in human cells was in line with findings in murine macrophages (Figure S7E) and *ZBTB46* was not significantly affected (Figure S8F). Altogether, these data corroborate and extend our findings on the MYR1-dependent induction of chemotactic migratory activation to human monocytes and macrophages.

## Discussion

In peripheral tissues, the encounter of *T. gondii* with mononuclear phagocytes can result in neutralization of the parasite or in infection of the host cell. Here, we addressed the impact of infection on the migratory responses of macrophages. The data demonstrate that infection with *T. gondii* allows normally sessile macrophages to acquire migratory features typically attributed to DCs. First, infection led to transcriptional signatures consistent with the acquisition of DC-like migratory properties. Second, we identified the parasite effector GRA28 as the mediator of upregulated *CCR7* expression and chemotaxis in

parasitized macrophages. Third, the migratory responses of macrophages impacted parasite dissemination in mice.

We report that challenge of separate types of murine and human macrophages with *T. gondii* induces the expression of transcription factors typically associated with cDCs. Importantly, *T. gondii* infection, but not challenge with parasite lysates or LPS, induced expression of IRF4, ZBTB46, *Batf3* and *Nr4a3*. These responses coincided with CCR7-mediated chemotaxis by infected macrophages. Notably, most responses impacted infected cells but not non-infected bystander cells, indicating direct effects of parasitization. In DCs, BATF3 is essentially associated with the development of cDC1 (Grajales-Reyes et al., 2015), while IRF4 and NR4A3 are required for mo-DC differentiation and CCR7-mediated migration (Bajana et al., 2012; Boulet et al., 2019; Briseno et al., 2016). Moreover, ectopic expression of ZBTB46 rescues cDC differentiation from IRF8-deficient bone marrow cells (Satpathy et al., 2012). However, despite that the transcription factors induced are strongly associated with DCs, we found no evidence indicating differentiation of macrophages to DCs. Thus, rather than a global transcriptional reprogramming, intracellular *T. gondii* selectively targeted transcription, with alterations in migratory responses, activation and cytokine responses of macrophages. Moreover, the phenotypical effects of *T. gondii* infection were not limited to specific types of mouse macrophages, with generally a similar activation in human monocytes and monocyte-derived macrophages. Yet, in diverse cell types, host cell type-specific effects are to be expected (Swierzy et al., 2017).

We identify the secreted dense granule protein GRA28 as a mediator of CCR7 upregulation and chemotaxis of *T. gondii*-infected macrophages. GRA28 is exported to the host cell nucleus in a MYR1- and ASP5-dependent manner (Nadipuram et al., 2016)(Figure 4B) and was recently linked to transcriptional regulation of the placenta-associated chemokine CCL22 (Rudzki et al., 2021). GRA28 is a 200 kDa intrinsically disordered protein (IDR) expected to be structurally flexible and able to adopt multiple conformations to interact with SWI/SNF and NuRD, probably via independent short linear motifs (SLiMs), usually 3 to 11 residues, which remain to be defined. We can predict a prion-like structure (Zhang et al., 2015) in a central region of GRA28 that is known to phase-separate to form weaker multivalent interactions. In this regard, GRA28 can function as scaffold proteins to recruit chromatin remodelers and form membraneless condensates capable of dynamically exchanging components with opposing functions, i.e., SWI/SNF and NuRD, thereby selectively accelerating or inhibiting host gene expression.

GRA28 has in common with the dense granule protein TgIST that it binds to the NuRD repressor complex, but the nucleosome deacetylation and ATP-dependent chromatin remodeling functions of NuRD can be hijacked by both in different ways. TgIST may co-opt NuRD-associated HDACs that compete with HATs (e.g., p300/CBP) to prevent STAT1 acetylation and DNA dissociation, thereby impairing STAT1 recycling and IFN- $\gamma$  responsiveness (Gay et al., 2016; Olias and Sibley, 2016). TgIST was also shown to prolong STAT1 occupancy on chromatin and allosterically impairs its ability to recruit the acetyltransferase p300/CBP (Huang et al., 2022). GRA28 which binds to chromatin over long distances may use the ATP-dependent chromatin-remodeling enzymes CHD3 and CHD4 of NuRD to generate a nonpermissive chromatin state by altering the positioning of

nucleosomes, but this remains to be shown. In this regard, the diversification of effectors in *T. gondii*, as exemplified by the interaction of TgIST and GRA28 with the NuRD complex, is likely an evolutionary strategy of the parasite to adapt to multiple hosts and their different cell and organ types. Similarly, TgIST and TgNSM were shown to cooperate and to signal through chromatin by binding to their respective corepressors, NuRD and NCoR/SMRT (Gay et al., 2016; Olias and Sibley, 2016; Rosenberg and Sibley, 2021).

In sharp contrast to the effects of *myr1* and *gra28* mutants, challenge with a *rop16* mutant parasite conversely elevated *Ccr7* expression, with maintained chemotaxis. Because the secreted rophtry protein ROP16 is a known down-modulator of inflammatory cytokine responses by acting on STAT3/6 (Jensen et al., 2011; Saeij et al., 2007; Yamamoto et al., 2009), our findings phenotypically corroborate the recently described counteracting transcriptional effects of MYR1-dependent and MYR1-independent effectors (Naor et al., 2018; Rastogi et al., 2020). However, the data indicate that *Irf4*/IRF4 and *Batf3* induction are MYR1/GRA28-independent, while MYR1/GRA28-deficient parasites failed to upregulate *Ccr7* expression. This suggests that BATF3 and IRF4 may act in concert to remodel the chromatin in *T. gondii*-infected macrophages, jointly with MYR1-related factors, such as GRAs and *Egr1* (Braun et al., 2013; ten Hoeve et al., 2019), that ultimately enable transcription at the *Ccr7* locus. However, the precise contributions of IRF4 and BATF3 to the migratory phenotype remain to be addressed in future studies under IRF4- and BATF3-deficient conditions. Consistent with the above considerations, the IL-12 proinflammatory response and the expression of the costimulatory molecule CD86 generally coincided with CCR7 up- and downregulation. Jointly, the data demonstrate that *T. gondii* orchestrates the modulation of macrophage migration with both a pro-migratory pro-inflammatory effector axis (MYR1-dependent GRA secretion) and an alternative activation-mediating effector axis (ROP16) that downmodulates IL-12 responses. This establishes the CCR7-dependent pro-migratory axis as distinct from the M2-like signature driven by ROP16 (Jensen et al., 2011; Patil et al., 2014). Despite that CCR7 responses were observed for all strains tested, the existing polymorphisms of effectors between strains are likely of relevance, because of their link to virulence in type I/II strains and to dissemination (Barragan and Sibley, 2002; Hitziger et al., 2005; Sibley and Boothroyd, 1992). For example, type I ROP16 activates STAT3 more potently than type II ROP16, which may partly explain these differences (Yamamoto et al., 2009). Consequently, the pro-chemotactic effect of GRA28 is likely partly counteracted by ROP16 in a genotype-related fashion. Also, while GRA28 predominantly mediated CCR7 responses, it is likely that additional GRA proteins contribute to the equilibrium of other responses (Mercer et al., 2020; Mukhopadhyay et al., 2020). For example, the M1-associated effector GRA15 in type II strains (Jensen et al., 2011) modulates leukocyte adhesion in relation to hypermigration (Ross et al., 2022; Ross et al., 2021). Additionally, systemic responses mediated by PAMPs/PRRs are central for immunity in toxoplasmosis (Frickel and Hunter, 2021; Scanga et al., 2002) and MyD88-independent responses in infected cells also mediate protective immunity (Mercer et al., 2020; Mukhopadhyay et al., 2020). Here, GRA24 (type I) suppressed *Il12p40* expression, consistent with a previously reported IL-12-inhibiting role of p38 MAP kinase (Katholnig et al., 2013). This finding is however in contrast to previous findings on GRA24 in type II *T. gondii* (Braun et al., 2013; ten Hoeve et al., 2019). Similarly, GRA6-dependent

NFAT4 activation impacts immune responses in a strain-dependent manner (Ma et al., 2014). Altogether, this highlights the complex host-pathogen interplay mediated by polymorphic ROPs and GRAs in different parasite strains and host cells, leading to differences in CCR7 and IL-12 regulation.

We report a central role for the MYR1 secretory pathway in the chemotactic and proinflammatory responses of macrophages. This was further corroborated with mutants of GRA45 and ROP17, which are constituents of the MYR1 secretory pathway (Cygan et al., 2020). Interestingly, ROP17 has also been implicated in hypermigration of monocytic cells (Drewry et al., 2019), indicating dual or alternative modes of action by ROP17 on hypermigration. In contrast, chemotaxis and the IL-12 response were not abrogated upon deletion of the rhoptry protein TgWIP, implicated in the migratory activation of DCs (Sangare et al., 2019). However, the velocity of chemotaxing cells was diminished, indicating overlapping effects on cell locomotion. Indeed, hypermigration and chemotaxis jointly promote the migration of infected phagocytes *in vitro* (Fuks et al., 2012; Weidner et al., 2013) and, while the two migratory phenotypes are activated by separate signaling pathways, they commonly share a dependence on calcium signaling and MAP kinase signaling (Bhandage et al., 2020; Olafsson et al., 2020). Thus, based on the data at hand, rather than being antithetical, we postulate that both signaling cues cooperatively potentiate the migration of infected phagocytes and parasite dissemination. It is also noteworthy that, under the conditions applied, LPS treatment did not induce chemotaxis in human or mouse macrophages. This is in contrast to LPS responses by DCs (Fuks et al., 2012; Weidner et al., 2013) and indicative of a strong activation and rapid onset of chemotaxis upon *T. gondii* infection of macrophages.

Hyper migratory responses by myeloid mononuclear phagocytes (DCs, monocytes, macrophages, microglia) upon challenge with *T. gondii* appear to be a conserved feature across host species (human, mouse, bovine) and are also induced by the related coccidian *Neospora caninum* (Bhandage et al., 2020; Collantes-Fernandez et al., 2012; Garcia-Sanchez et al., 2019; Lambert et al., 2009). Yet, measurable differences exist between parasite strains/lineages in the magnitude of induction of the hyper migratory phenotype *in vitro* (Weidner and Barragan, 2014) and *in vivo* in mice (Lambert et al., 2009). Here, using an *in vivo* adoptive transfer setup (Fuks et al., 2012; Kanatani et al., 2017), we report that parasitized macrophages can potentiate the dissemination of *T. gondii* in a MYR1- and GRA28-dependent fashion. Consistent with these data, we previously showed that MYR1-deficient parasites had no defect in peritoneal survival but a defect in organ colonization (Sangare et al., 2019). Interestingly, adoptive transfer of CCR7-deficient infected macrophages significantly reduced but did not abolish migration to omentum, mesenteric lymph nodes and spleen, indicating a partial dependency on CCR7-mediated chemotaxis for systemic migration. Consistent with this, *in vitro*, CCR7-deficiency abolished chemotaxis of infected macrophages towards CCL19 while hypermotility was maintained. Thus, synergistically with the onset of GABA-mediated hypermigration (Bhandage et al., 2020; Lambert et al., 2011; Lambert et al., 2006), the acquired sensitivity of parasitized macrophages and other monocytic cells to CCL19/CCL21 gradients could carry replicating tachyzoites to lymph nodes and beyond, thereby potentiating systemic dissemination. Importantly, the MYR1-dependent CCR7 responses were corroborated in human macrophages and

monocytes, advocating for conserved signaling across species. Yet, differences in the transcriptional regulation were also noted, indicating that, despite the conserved migratory plasticity of macrophages, regulatory response differences exist between human and murine cells. Altogether, the data highlight the implication of host cell-related and parasite genotype-related components in ‘Trojan horse’-mediated dissemination, which require further investigation.

It is becoming increasingly clear that bacterial, viral and fungal microorganisms use elaborated strategies to thrive inside macrophages and other phagocytes (Mercer and Greber, 2013; Sansonetti and Di Santo, 2007; Tan and Russell, 2015). To do this, pathogens need to sense, respond and induce alterations in host cells that benefit their survival. Recent findings show that *Mycobacterium tuberculosis* manipulates alveolar macrophage trafficking for rapid localization to the lung interstitium (Lovey et al., 2022). Similarly, *Leishmania* parasites impact transcription and epigenetic regulation in the host macrophage, thereby modulating immune responses (Lecoeur et al., 2022), including the inhibition of macrophage motility (de Menezes et al., 2017). Following oral infection, *T. gondii* encounters sessile macrophages in the intestine and other peripheral tissues. Dissemination of *T. gondii* from the intestine is rapid, asymptomatic and in absence of generalized inflammation (Gregg et al., 2013). Our study provides a framework for how *T. gondii* manipulates the migration of mononuclear phagocytes to disseminate. The findings reveal a remarkable migratory plasticity (Friedl and Weigelin, 2008) in normally sessile macrophages, which can acquire migratory properties typically attributed to the more migratory DCs upon infectious challenge with *T. gondii*. The data also highlight a transcriptional plasticity linked to the migratory responses of differentiated monocytic cells, which obligate intracellular parasites may utilize to their advantage. Because macrophages and monocytes outnumber DCs in tissues, a migratory activation of these cells may favor dissemination of *T. gondii*. The findings unveil putative alternative pathways by which mononuclear phagocytes can be made migratory or activated, which could in extension be exploited, for example, in cell therapies.

## STAR methods

### RESOURCE AVAILABILITY

**Lead contact**—Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Antonio Barragan (antonio.barragan@su.se).

**Materials availability**—New plasmids and materials generated in this study are available from the lead contact.

### Data and code availability

- The MS proteomics data have been deposited to the ProteomeXchange Consortium through the PRIDE partner repository with the dataset identifier PXD032360. This paper analyzes existing, publicly available data. The NCBI GEO accession numbers for these datasets are GSE40727, GSE66899,

GSE64767, GSE99837 and GSE27972. All data reported in this paper will be shared by the lead contact upon request.

- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

**Mice**—Wild-type and CCR7-deficient C57BL/6 (6–10 week old, male and female) and wild-type BALB/c (6 week old, female) mice were used for the isolation of bone marrow. Bone marrow-derived macrophages (BMDMs), dendritic cells (BMDCs) and macrophage-like PMA bone marrow cells (PMA-BMC) were cultured from bone marrow with M-CSF-containing conditioned medium, recombinant GM-CSF or PMA as indicated in the Method details. Peritoneal cells were harvested from wild-type C57BL/6 mice (6–10 week old, male and female) via peritoneal lavage. C57BL/6 cells were cultured in RPMI 1640 with 10% fetal bovine serum (FBS), gentamicin (20 µg/mL) and glutamine (2 mM) and BALB/c cells in Dulbecco's modified Eagle's medium, high glucose, with 50 µM 2-mercaptoethanol, 1X non-essential amino acids and 2% penicillin/streptomycin-glutamine at 37°C/5% CO<sub>2</sub>. C57BL/6 mice (5–18 week old, male and female) were used for adoptive transfer of BMDMs and BMDCs. The Regional Animal Research Ethical Board, Stockholm, Sweden, approved experimental procedures and protocols involving extraction of cells from mice (permit numbers 9707/2018, 14458/2019 and N 78/16), following proceedings described in EU legislation (Council Directive 2010/63/EU).

**Primary human cells**—Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats obtained from healthy adult donors at the Karolinska University Hospital Blood Center. The sex or age of these donors are unknown to the authors due to privacy law restrictions. CD14<sup>+</sup> monocytes were obtained from PBMC via positive selection and used for generating macrophages with M-CSF. Cells were cultured at 37°C/5% CO<sub>2</sub> in RPMI 1640 with 10% fetal bovine serum (FBS), gentamicin (20 µg/mL) and glutamine (2 mM). The Regional Ethics Committee, Stockholm, Sweden, approved protocols involving human cells (application number 2006/116-31). All donors received written and oral information upon donation of blood at the Karolinska University Hospital Blood Center. Written consent was obtained for utilization of white blood cells for research purposes.

**Cell lines**—Human foreskin fibroblasts HFF-1 were cultured in Dulbecco's modified Eagle's medium, high glucose, (DMEM; Thermo Fisher scientific) with 10% fetal bovine serum (FBS; Sigma), gentamicin (20 µg/ml; Gibco or Sigma) and glutamine (2 mM; Gibco). Human THP-1 (male) and murine RAW 264.7 (male, BALB/c) cells were used for *in vitro* experiments and cultured at 37°C/5% CO<sub>2</sub>.

**T. gondii strains**—*T. gondii* tachyzoites were maintained by serial 2-day passages in human foreskin fibroblast HFF-1 monolayers. Freshly egressed tachyzoites were used for all infections. The different strains used are listed in the Key Resource Table. All cell cultures used were periodically tested for mycoplasma and found to be negative.

## METHOD DETAILS

**Mouse cell culture**—Cells from bone marrow of 6–10-week-old male or female wild-type or CCR7-deficient C57BL/6 mice (see Key Resource Table) were cultivated in RPMI 1640 with 10% fetal bovine serum (FBS), gentamicin (20 µg/mL) and glutamine (2 mM), referred to as complete medium (all reagents from Life Technologies), and supplemented with 20 ng/mL recombinant mouse GM-CSF. On day 6 of culture loosely adherent cells were removed and loosely adherent cells on day 7 were harvested as bone marrow-derived dendritic cells (BMDCs). Strongly adherent cells were harvested on day 7–8 as bone marrow-derived macrophages (BMDMs). Macrophage-like PMA bone marrow cells (PMA-BMCs) were generated from C57BL/6 bone marrow in complete medium supplemented with 20 ng/mL phorbol 12-myristate 13-acetate (PMA) and adherent cells were harvested after 6 days of differentiation. The predominant appearance of phagocytic monocyte-macrophages after PMA-supplemented bone marrow culture has been described previously (Stuart and Hamilton, 1980). For peritoneal macrophages (PEMs) C57BL/6 mice were euthanized and peritoneal lavage (10 ml PBS) was collected from the peritoneal cavity. After overnight culture in complete medium, loosely and non-adherent cells were removed by repeated washing and the adherent cells were used as PEM in experiments for RNA isolation. For flow cytometric analysis PEMs were identified phenotypically from peritoneal lavage cells that were challenged *in vitro* as indicated. BALB/c BMDMs were generated as described previously (He et al., 2018). Briefly, bone marrow was isolated by flushing femurs and tibias of six-week-old female BALB/c mice (Janvier Labs) and cultured for 1 week in complete macrophage medium (DMEM supplemented with 10% heat-inactivated FBS (Invitrogen), 20% conditioned medium from macrophage-colony stimulating factor-secreting L929 fibroblasts (Aziz et al., 2009), 50 µM 2-mercaptoethanol, 1X non-essential amino acids, and 2% penicillin/streptomycin-glutamine). After 6 days, the cells were washed with PBS to remove non-adherent cells, harvested in ice-cold PBS supplemented with 1 mM EDTA and replated for the assay. RAW264.7 (RRID:CVCL80493, ATCC, cat. No. TIB-71) cell line was cultured in DMEM (Invitrogen), supplemented with 10% heat-inactivated FBS (Invitrogen), 10 mM Hepes buffer, pH 7.2, 2 mM L-glutamine, and 50 µg/ml penicillin and streptomycin (Thermo Fisher Scientific). THP1 (ATCC, cat. No. TIB-202) cell line was cultured in RPMI (Invitrogen), supplemented with 10% heat-inactivated FBS (Invitrogen), 10 mM Hepes buffer, pH 7.2, 2 mM L-glutamine, 50 µM 2-mercaptoethanol and 50 µg/ml penicillin and streptomycin (Thermo Fisher Scientific). Cell purity was confirmed by microscopy and flow cytometry (Figure 1I, S1H and Data S1).

**Human cell culture**—Human CD14<sup>+</sup> monocytes were isolated from peripheral blood mononuclear cells (PBMC) after density gradient centrifugation on Lymphoprep with CD14 MicroBeads from buffy coats obtained from healthy donors at the Karolinska University Hospital Blood Center and cultured in complete medium. Macrophages were generated from CD14<sup>+</sup> monocytes through culture for 6–7 days in complete medium supplemented with 50 ng/mL human recombinant M-CSF.

**Infection challenges**—Carry-over from routine *T. gondii* culture to experiments was minimized by repeated washing of the freshly egressed tachyzoites before preparation of crude tachyzoites lysates (prepared by repeated freeze-thaw) or challenge with live



tachyzoites. For all qPCR and western blot experiments, C57BL/6 BMDMs, PEMs, PMA-BMCs and human monocytes and macrophages were challenged with freshly egressed *T. gondii* tachyzoites at MOI 2 for 18h. Tachyzoite lysates were used at amounts derived from equivalent tachyzoite numbers as indicated live parasites. LPS was used at a final concentration of 10 ng/mL. For qPCR experiments, BALB/c BMDMs, RAW and THP-1 cells were challenged with *T. gondii* tachyzoites for 24h as indicated. For flow cytometry assays, C57BL/6 BMDMs were challenged for 6h or 18h, as indicated, with GFP-expressing *T. gondii* tachyzoites (MOI 1) or with non-fluorescent protein-expressing tachyzoites (MOI 2). Peritoneal cells were challenged with GFP-expressing *T. gondii* type I tachyzoites (RH-LDM; MOI 0,5) for 18h for flow cytometry. Infection frequencies for GFP-expressing type I (RH) *T. gondii* tachyzoites (MOI 1 or 2) were determined by flow cytometry (Data S1). For chemotaxis experiments, cells were challenged for 12–14h with GFP-expressing or CMTMR-labeled *T. gondii* tachyzoites at MOI 1 before seeding in the chemotaxis chamber.

**Plasmid construction**—To construct vector pLIC-GRA28-HF, the coding sequence of GRA28 was amplified using the primers LIC-231960-F and LIC-231960-R and genomic DNA of Pru *ku80* strain of *T. gondii* as the DNA template. The resulting PCR product was cloned into the pLIC-HF-*dhfr* vector using the ligation independent cloning (LIC) method.

The plasmid pTOXO\_Cas9-CRISPR::sgGRA28 was generated as previously described (Curt-Varesano et al., 2016) to construct the *gra28* deletion in the RH *ku80* and Pru *ku80* strains. Briefly, primers GRA28-gRNA-Fwd and GRA28-gRNA-Rev containing the sgRNA targeting *GRA28* genomic sequence were phosphorylated, annealed and ligated into pTOXO\_Cas9-CRISPR plasmid linearized with BsaI, leading to pTOXO\_Cas9-CRISPR::sgGRA28.

To construct the pP<sub>GRA28</sub>-GRA28-HA vector, the promotor sequence of GRA28 and the *GRA28* coding sequence were amplified by PCR using the primers LICF-P<sub>GRA28</sub>\_F and LICR-GRA28\_R. The resulting PCR P<sub>GRA28</sub>-GRA28 DNA fragment was cloned into the plasmid pLIC-3HA-DHFR. The chimeric construct P<sub>GRA28</sub>-GRA28-3HA-3'DHFR was amplified using the primers UPRT-P<sub>GRA28</sub>\_F and UPRT-3'DHFR\_R and inserted within the UPRT locus.

***T. gondii* transfection**—Vectors were transfected into *T. gondii* strains by electroporation. Electroporation was conducted in a 2-mm cuvette at 1.100 V, 25 Ω and 25 μF. Stable integrants were selected in media containing 1 μM pyrimethamine or 10 μM 5-fluorodeoxyuracil and single-cloned in 96 wells plates by limiting dilution.

**Inhibitors**—When indicated, cells were treated with inhibitors TPCA-1 or AS1517499 at 3 μM or vehicle (DMSO) initiated 1h prior to challenge.

**Mixed leukocyte reaction (MLR)**—Syngeneic MLR was performed as described (Nussenzweig and Steinman, 1980). Briefly, BMDM were challenged for 6h as indicated, washed and co-cultured with syngeneic unfractionated splenocytes for 18h at 1:20 ratio. Splenocytes were freshly isolated from triturated spleens, filtered through a 40 μm cell strainer and subjected to red blood cell lysis (ammonium chloride).

**Quantitative polymerase chain reaction (qPCR)**—C57BL/6 BMDMs, PMA-BMCs and PEMs were cultured with complete medium or challenged with freshly egressed *T. gondii* tachyzoites of the indicated strains, lysate of egressed *T. gondii* tachyzoites (ME49) at MOI equivalents or LPS as indicated and lysed in TRI Reagent (Sigma-Aldrich). Total RNA was extracted according to the manufacturers' protocol using the Direct-zol RNA Miniprep kit and reverse transcribed with Maxima H Minus Reverse Transcriptase (Thermo Fisher). Real time qPCR was performed SYBR® green PCR master mix (KAPA biosystems), specific forward and reverse primers at target-dependent concentrations (100 or 200 nM) and cDNA (10–30 ng) in a QuantStudio 5 System (Thermo Fisher) with ROX as a passive reference. qPCR results were analyzed using the  $\Delta\Delta C_q$  method and displayed as fold change relative to unchallenged or as  $\Delta C_q$  and relative to Importin-8 and TATA-binding protein (TBP) as housekeeping genes. BALB/c BMDMs and RAW and THP-1 cells were either uninfected or *T. gondii*-infected (MOI = 6) for 24 h. Total RNA was isolated using TRIzol reagent (Thermo Fisher Scientific). Complementary DNA was synthesized with random hexamers using the High Capacity RNA-to-cDNA kit (Applied Biosystem). Samples were analysed by real-time quantitative PCR for *CCR7*, *CCL22*, *TNFAIP6*, *CCL24*, *CX3CL1*, *CXCL3*, *CXCL5*, *Vcam1* and *IL1a* using the TaqMan gene expression master mix (Applied Biosystem) according to the manufacturer's instructions. The internal control gene *TBP* was used for normalization. Primers are listed in the Key Resource Table.

**Flow cytometry**—C57BL/6 BMDMs or BMDCs were challenged as indicated and stained with anti-CD11c, CD11b, MHCII I-A/I-E, CD86, CD115, IRF4, Ly6C, IL-12p40 or isotype control antibodies and live/dead far red stain. For intracellular staining cells were fixed (2% PFA) and then permeabilized in either Tween-20 (1%) in FACS buffer (PBS/0,5% FBS/1mM EDTA; IL-12p40) or Triton X-100 (1%) in PBS/5% BSA (IRF4/isotype) supplemented with anti-CD16/CD32 antibody prior to staining. For IL-12p40 staining cells were cultured in the presence of Brefeldin A (eBioscience) for the last 4h of challenge. Extracellular staining was performed on fixed (2% PFA) or live cells, blocked with anti-CD16/CD32 antibody, in FACS buffer. For bead phagocytosis, PMA-BMCs were mixed with Nile Blue Fluorescent Particles (2.5–4.5  $\mu\text{m}$ , 10 particles/cell) and cultured for 1h on ice or at 37°, washed and fixed (2% PFA). PMA-BMCs kept on ice were <1% bead<sup>+</sup>. C57BL/6 peritoneal lavage cells were challenged *in vitro* as indicated, stained with LIVE/DEAD Far Red stain, fixed (2% PFA) and then stained with anti-CD11c, CD11b, CD19, MHCII I-A/I-E, CD86, CD115, Ly6C and F4/80 antibodies. PEMs were defined as CD19<sup>-</sup>CD11b<sup>hi</sup>CD11c<sup>-</sup> (Figure 1I and S1H). Flow cytometry was performed on a BD LSRFortessa flow cytometer (BD Biosciences) and analyzed with FlowJo X (FlowJo LLC).

**Western blot**—For Western blotting cells were challenged as indicated, harvested, washed with PBS and then lysed directly in Laemmli buffer for whole cell lysates. Proteins were separated using 8% or 10% SDS-PAGE, blotted onto a PVDF membrane and blocked (10% BSA in TBS/0,5% Tween-20) followed by incubation with primary and secondary antibodies: anti-TATA-binding protein, anti-ZBTB46, anti-mouse IgG-HRP or anti-rat IgG-HRP in 10% BSA/0,5% Tween-20 in TBS. Proteins were revealed by mean of enhanced chemiluminescence (GE Healthcare) in a BioRad ChemiDoc XRS+. Densitometry analysis was performed using ImageJ (NIH, MD, USA).

**Chemotaxis**—BMDCs, BMDMs or human macrophages were challenged as indicated, washed, resuspended in CM with 1 mg/mL bovine collagen type I (Sigma) and seeded into gelatin (1%)-coated or uncoated ibiTreat  $\mu$ -slide chemotaxis chambers (Ibidi, Martinsried, Germany). Collagen was allowed to polymerize for 45 minutes and media, inhibitors and 1,25  $\mu$ g/mL murine (BMDCs, BMDMs) or human recombinant CCL19 (human macrophages) were added as indicated conform the manufacturer's instructions (application note 23). Cells were then imaged every 2,5 (BMDCs) or 5 (BMDMs, human macrophages) min for 4h (BMDCs) or 8h (BMDMs, human macrophages; Zeiss Observer Z.1). Motility tracks for 35–60 cells per condition were analyzed using ImageJ software for each experiment.

**Immunofluorescence microscopy**—BMDMs were seeded on gelatin (1%)-coated glass coverslips, challenged freshly egressed *T. gondii* tachyzoites (RH1–1; MOI 2) for 18h and fixed with 2% PFA. Cells were then permeabilized with 0,1 or 1,0% Triton X-100 in PBS and stained with phalloidin Alexa Fluor 594 (Thermo Scientific) or anti-IRF4 and DAPI. Images were acquired on a Leica DMi8 with 63x objective.

**Adoptive transfers**—Adoptive transfers were performed as previously described (Bhandage et al., 2020). Briefly, BMDMs or BMDCs were stained with CellTracker CMTMR (2  $\mu$ M) or Deep Red (1  $\mu$ M) dyes ( $2,5 \times 10^6$  cells each), washed and challenged with freshly egressed *T. gondii* GFP-expressing or CFSE-stained RH tachyzoites (MOI 1,5) or left unchallenged in complete medium for 4h. Cells were then washed and injected i.p. into C57BL/6 mice. Mice were sacrificed 18h post-injection to collect spleens, mesenteric lymph nodes and omenta. The organs were triturated, filtered through 40  $\mu$ m cell strainer and fixed (4% PFA). Cells from the spleen were briefly subjected to red blood cell lysis (ammonium chloride) prior to fixation, blocked with anti-CD16/32 antibody in FACS buffer (PBS/0,5% FBS/1mM EDTA) and stained with CD11c antibody.

**Handling of publicly available datasets**—ChIP-seq data available from NCBI GEO series GSE40727 (IRF4), GSE66899 (H3K27ac), GSE64767 (H3K4me3), which are described elsewhere, was visualized in the USCS genome browser (Glasmacher et al., 2012; Grajales-Reyes et al., 2015; Lin et al., 2015). Changes in gene expression by 1 or 2 day treatment of sorted monocytes with GM-CSF and IL-4 were determined from RNA-seq read counts in NCBI GEO serie GSE99837 (Boulet et al., 2019). Microarray data from NCBI GEO series GSE27972 was analyzed with R packages oligo and limma to derive differentially expressed genes upon 6h *T. gondii* (RH) challenge of BMDMs (Morgado et al., 2011).

**RNA-seq**—For each biological assay,  $3 \times 10^6$  BMDMs were seeded per well in six-well tissue culture plates. Cells were left uninfected or infected with the Pru *ku80* and Pru *ku80 gra28* strains (MOI = 6) for 24 hr. Total RNAs were extracted and purified as described previously (Farhat et al., 2020). RNA-sequencing was performed by GENEWIZ (South Plainfield, NJ, USA) as described previously (Farhat et al., 2020).

**ChIP-seq assay**—HFF cells were infected (MOI = 6) for 24 h with RH *ku80* GRA28–HA–FLAG or GRAX–HA–FLAG. Cells were then cross-linked with 1% formaldehyde for

10 min before quenching with 125 mM glycine for 5 min. The ChIP assay was performed by using the Transcription Factor Chromatin Immunoprecipitation kit (Diagenode) according to the manufacturer's protocol. In brief, fixed cells were sonicated to shear the cross-linked chromatin into an average DNA fragment size of 200–600 bp. We used  $40 \times 10^6$  sorted nuclei in 300  $\mu$ l immunoprecipitation buffer supplemented with fresh proteinase inhibitors. By using a Diagenode Bioruptor precooled to 4 °C, shearing was achieved in 1.5-ml-low-binding tubes in the appropriate tube adaptor with 18 high-energy cycles of 30 sec on and 30 s off. The aforementioned antibodies were used for immunoprecipitation. After an overnight incubation, the DNA–protein–antibody complex was eluted. The crosslinks were reversed by heating the samples at 65 °C for 4 h. DNA was purified by using IPure kit (Diagenode) according to the manufacturer's protocol. 10 ng of your DNA samples were prepared for Illumina sequencing as the following steps: 1) DNA samples were blunt-ended; 2) A dA base was added to the 3' end of each strand; 3) Illumina's genomic adapters were ligated to the DNA fragments; 4) PCR amplification was performed to enrich ligated fragments; 5) Size selection of ~200–1500bp enriched product using AMPure XP beads. The completed libraries were quantified by Agilent 2100 Bioanalyzer. The libraries were denatured with 0.1 M NaOH to generate single-stranded DNA molecules, captured on Illumina flow cell, amplified in situ. The libraries were then sequenced on the Illumina NovaSeq 6000 following the NovaSeq 6000 S4 Reagent Kit (300 cycles) protocol. After the sequencing platform generated the sequencing images, the stages of image analysis and base calling were performed using Off-Line Basecaller software (OLB V1.8). Sequence quality was examined using the FastQC software. After passing Solexa CHASTITY quality filter, the clean reads were aligned to human genome (UCSC HG19) using BOWTIE software (V2.2.7). Aligned reads were used for peak calling of the ChIP regions using MACS V1.4.2. Statistically significant ChIP-enriched regions (peaks) were identified by IP, using a p-value threshold of  $10^{-4}$ . The peaks in samples were annotated by the nearest gene using the newest UCSC RefSeq database. The annotation of the peaks which were located within –2Kb to +2Kb around the corresponding gene TSS in samples can be found from the Peaks\_Promoter\_Annotation.xls file. The signal profile (at 10 bp resolution) with UCSC WIG file format was generated from ChIP-seq data, which can be visualized on UCSC genome browser or IGB browser (Integrated Genome Browser, Java Runtime Environment needed, <http://www.bioviz.org/igb/>). All wig files and a brief guide of visualizing the profiles can be found in Data\_Visualization folder.

**Chromatographic purification of GRA28-containing complexes**—Protein extracts from Raw cells infected with RH ku80 GRA28-HA-FLAG-tachyzoites were incubated with anti-FLAG M2 affinity gel (Sigma-Aldrich) for 1 hour at 4 °C. Beads were washed with ten column volumes of BC500 buffer (20 mM Tris-HCl, pH 8.0, 500 mM KCl, 20% glycerol, 1 mM EDTA, 1 mM dithiothreitol, 0.5% NP-40 and protease inhibitors). The bound polypeptides were eluted stepwise with 250  $\mu$ g ml<sup>-1</sup> FLAG peptide (Sigma-Aldrich) diluted in BC100 buffer. For size-exclusion chromatography, protein eluates were loaded onto a Superose 6 HR 10/30 column equilibrated with BC500. The flow rate was fixed at 0.35 ml min<sup>-1</sup> and 0.5-ml fractions were collected.

**Mass spectrometry-based proteomics**—Proteins contained in fractions from size-exclusion chromatography of GRA28-containing complexes purified by FLAG co-immunoprecipitation were solubilized in Laemmli buffer and stacked in the top of a 4–12% NuPAGE gel (Invitrogen). After staining with R-250 Coomassie Blue (Biorad), proteins were digested in-gel using trypsin (modified, sequencing purity, Promega), as previously described. The resulting peptides were analyzed by online nanoliquid chromatography coupled to MS/MS (Ultimate 3000 RSLCnano and Q-Exactive Plus, Thermo Fisher Scientific) using a 140 min gradient. For this purpose, the peptides were sampled on a precolumn (300  $\mu\text{m}$   $\times$  5 mm PepMap C18, Thermo Scientific) and separated in a 75  $\mu\text{m}$   $\times$  250 mm C18 column (Reprosil-Pur 120 C18-AQ, 1.9  $\mu\text{m}$ , Dr. Maisch). The MS and MS/MS data were acquired by Xcalibur (Thermo Fisher Scientific). Peptides and proteins were identified by Mascot (version 2.6.0, Matrix Science) through concomitant searches against the *T. gondii* database (ME49 taxonomy, v.30, downloaded from ToxoDB), the Uniprot database (Mus musculus taxonomy, August 2019 version), and a homemade database containing the sequences of classical contaminant proteins found in proteomic analyses (bovine albumin, keratins, trypsin, etc.). Trypsin/P was chosen as the enzyme and two missed cleavages were allowed. Precursor and fragment mass error tolerances were set at respectively at 10 ppm and 25 mmu. Peptide modifications allowed during the search were: Carbamidomethyl (C, fixed), Acetyl (Protein N-term, variable) and Oxidation (M, variable). The Proline software was used for the compilation, grouping, and filtering of the results (conservation of rank 1 peptides, peptide length  $\geq 7$ , PSM score  $\geq 25$ , false discovery rate of peptide-spectrum-match identifications  $< 1\%$  as calculated on peptide-spectrum-match scores by employing the reverse database strategy, and a minimum of 1 specific peptide per identified protein group). Proline was then used to perform a compilation, grouping and MS1 quantification, based on razor and specific peptides, of the identified protein groups. Proteins from the contaminant database were discarded from the final list of identified proteins. Proteins in each sample were further quantified through calculation of their corresponding intensity-based absolute quantification values (iBAQ). The MS proteomics data have been deposited to the ProteomeXchange Consortium through the PRIDE partner repository with the dataset identifier PXD032360.

## QUANTIFICATION AND STATISTICAL ANALYSIS

Western blot analysis was performed by band intensity quantification with ImageJ. qPCR results were analyzed using the  $C_q$  and  $\Delta C_q$  methods from  $C_q$  values provided by QuantStudio Design and Analysis Desktop Software. Cell tracks were obtained by manual tracking with ImageJ from image sequence files. Speeds and endpoints from individual cells were calculated and derived from these tracks with the Chemotaxis and Migration Tool and combined with track lengths calculated with R and RStudio. Flow cytometry data in FCS files was analyzed with FlowJo X. Quantification of RNA-seq and proteomics data was performed as described in the Method details. Details on the software used can be found in the Key Resource Table.

### Statistical analyses

Volcano plots, scatter plots, and histograms were generated with Prism. Statistical analyses were performed with R, RStudio and packages afex (repeated-measures ANOVA),

emmeans (Dunnett's and Holm-Bonferroni post-hoc), DAAG and rcompanion (one sample permutation). Statistical significance is defined as  $p < 0,05$ . The figure legends detail which hypothesis tests were employed, chosen based on experimental design, the hypothesis to be tested and data distribution, and which statistics are presented. All experiments were performed in biological replicates to allow for statistical analyses and in independent biological replicates as stated for each experiment in the manuscript. The number of  $n$  denotes the number of biological replicates (adoptive transfer experiments) or independent experiments.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

We are grateful to Dr. Reinhold Förster, Hannover University, Germany for valuable reagents. This work was funded by the Swedish Research Council (Vetenskapsrådet, 2018-02411 to An.B.) and the Olle Engkvist Byggmästare Foundation (193-609 to An.B.). The proteomic experiments were partially supported by Agence Nationale de la Recherche under projects ProFI (Proteomics French Infrastructure, ANR-10-INBS-08) and GRAL, a program from the Chemistry Biology Health (CBH) Graduate School of University Grenoble Alpes (ANR-17-EURE-0003). M.A.H., Al.B. and L.B. were supported by the Laboratoire d'Excellence (LabEx) ParaFrap [ANR-11-LABX-0024], the Agence Nationale pour la Recherche [Project HostQuest, ANR-18-CE15-0023], and Fondation pour la Recherche Médicale [FRM Equipe # EQU202103012571]. J.P.J.S. was funded by NIH R21AI151084.

## References

- Alvarez D, Vollmann EH, and von Andrian UH (2008). Mechanisms and consequences of dendritic cell migration. *Immunity* 29, 325–342. 10.1016/j.immuni.2008.08.006 [PubMed: 18799141]
- Amon L, Lehmann CHK, Baranska A, Schoen J, Heger L, and Dudziak D.(2019). Transcriptional control of dendritic cell development and functions. *International review of cell and molecular biology* 349, 55–151. 10.1016/bs.ircmb.2019.10.001 [PubMed: 31759434]
- Attanasio C, Nord AS, Zhu Y, Blow MJ, Biddie SC, Mendenhall EM, Dixon J, Wright C, Hosseini R, Akiyama JA, et al. (2014). Tissue-specific SMARCA4 binding at active and repressed regulatory elements during embryogenesis. *Genome Res* 24, 920–929. 10.1101/gr.168930.113 [PubMed: 24752179]
- Aziz A, Soucie E, Sarrazin S, and Sieweke MH (2009). MafB/c-Maf deficiency enables self-renewal of differentiated functional macrophages. *Science (New York, NY)* 326, 867–871. 10.1126/science.1176056
- Bajana S, Roach K, Turner S, Paul J, and Kovats S.(2012). IRF4 promotes cutaneous dendritic cell migration to lymph nodes during homeostasis and inflammation. *Journal of immunology (Baltimore, Md : 1950)* 189, 3368–3377. 10.4049/jimmunol.1102613 [PubMed: 22933627]
- Barragan A, and Sibley LD (2002). Transepithelial migration of *Toxoplasma gondii* is linked to parasite motility and virulence. *The Journal of experimental medicine* 195, 1625–1633. [PubMed: 12070289]
- Barragan A, and Sibley LD (2003). Migration of *Toxoplasma gondii* across biological barriers. *Trends Microbiol* 11, 426–430. [PubMed: 13678858]
- Bhandage AK, Olivera GC, Kanatani S, Thompson E, Loré K, Varas-Godoy M, and Barragan A. (2020). A motogenic GABAergic system of mononuclear phagocytes facilitates dissemination of coccidian parasites. *eLife* 9. 10.7554/eLife.60528
- Boulet S, Daudelin JF, Odagiu L, Pelletier AN, Yun TJ, Lesage S, Cheong C, and Labrecque N.(2019). The orphan nuclear receptor NR4A3 controls the differentiation of monocyte-derived dendritic cells following microbial stimulation. *Proceedings of the National Academy of Sciences of the United States of America* 116, 15150–15159. 10.1073/pnas.1821296116 [PubMed: 31285338]

- Boyle JP, Saeij JP, and Boothroyd JC (2007). *Toxoplasma gondii*: inconsistent dissemination patterns following oral infection in mice. *Experimental parasitology* 116, 302–305. 10.1016/j.exppara.2007.01.010 [PubMed: 17335814]
- Braun L, Brenier-Pinchart MP, Hammoudi PM, Cannella D, Kieffer-Jaquinod S, Vollaire J, Josserand V, Touquet B, Couté Y, Tardieux I, et al. (2019). The *Toxoplasma* effector TEEGR promotes parasite persistence by modulating NF- $\kappa$ B signalling via EZH2. *Nat Microbiol* 4, 1208–1220. 10.1038/s41564-019-0431-8 [PubMed: 31036909]
- Braun L, Brenier-Pinchart MP, Yogavel M, Curt-Varesano A, Curt-Bertini RL, Hussain T, Kieffer-Jaquinod S, Coute Y, Pelloux H, Tardieux I, et al. (2013). A *Toxoplasma dense granule* protein, GRA24, modulates the early immune response to infection by promoting a direct and sustained host p38 MAPK activation. *The Journal of experimental medicine* 210, 2071–2086. 10.1084/jem.20130103 [PubMed: 24043761]
- Briseno CG, Haldar M, Kretzer NM, Wu X, Theisen DJ, Kc W, Durai V, Grajales-Reyes GE, Iwata A, Bagadia P, et al. (2016). Distinct Transcriptional Programs Control Cross-Priming in Classical and Monocyte-Derived Dendritic Cells. *Cell reports* 15, 2462–2474. 10.1016/j.celrep.2016.05.025 [PubMed: 27264183]
- Butcher BA, Fox BA, Rommereim LM, Kim SG, Maurer KJ, Yarovinsky F, Herbert DR, Bzik DJ, and Denkers EY (2011). *Toxoplasma gondii* rhoptry kinase ROP16 activates STAT3 and STAT6 resulting in cytokine inhibition and arginase-1-dependent growth control. *PLoS pathogens* 7, e1002236. 10.1371/journal.ppat.1002236
- Cairns BR, Erdjument-Bromage H, Tempst P, Winston F, and Kornberg RD (1998). Two actin-related proteins are shared functional components of the chromatin-remodeling complexes RSC and SWI/SNF. *Mol Cell* 2, 639–651. 10.1016/s1097-2765(00)80162-8 [PubMed: 9844636]
- Collantes-Fernandez E, Arrighi RB, Alvarez-Garcia G, Weidner JM, Regidor-Cerrillo J, Boothroyd JC, Ortega-Mora LM, and Barragan A.(2012). Infected dendritic cells facilitate systemic dissemination and transplacental passage of the obligate intracellular parasite *Neospora caninum* in mice. *PloS one* 7, e32123. 10.1371/journal.pone.0032123 [PubMed: 22403627]
- Courret N, Darche S, Sonigo P, Milon G, Buzoni-Gatel D, and Tardieux I.(2006). CD11c- and CD11b-expressing mouse leukocytes transport single *Toxoplasma gondii* tachyzoites to the brain. *Blood* 107, 309–316. [PubMed: 16051744]
- Cygan AM, Theisen TC, Mendoza AG, Marino ND, Panas MW, and Boothroyd JC (2020). Coimmunoprecipitation with MYR1 Identifies Three Additional Proteins within the *Toxoplasma gondii* Parasitophorous Vacuole Required for Translocation of Dense Granule Effectors into Host Cells. *mSphere* 5. 10.1128/mSphere.00858-19
- de Menezes JP, Koushik A, Das S, Guven C, Siegel A, Laranjeira-Silva MF, Losert W, and Andrews NW (2017). *Leishmania* infection inhibits macrophage motility by altering F-actin dynamics and the expression of adhesion complex proteins. *Cellular microbiology* 19. 10.1111/cmi.12668
- Dobrowolski JM, and Sibley LD (1996). *Toxoplasma* invasion of mammalian cells is powered by the actin cytoskeleton of the parasite. *Cell* 84, 933–939. [PubMed: 8601316]
- Drewry LL, Jones NG, Wang Q, Onken MD, Miller MJ, and Sibley LD (2019). The secreted kinase ROP17 promotes *Toxoplasma gondii* dissemination by hijacking monocyte tissue migration. *Nat Microbiol* 4, 1951–1963. 10.1038/s41564-019-0504-8 [PubMed: 31332383]
- Dubey JP (1997). Bradyzoite-induced murine toxoplasmosis: stage conversion pathogenesis, and tissue cyst formation in mice fed bradyzoites of different strains of *Toxoplasma gondii*. *Journal of Eukaryotic Microbiology* 44, 592–602. [PubMed: 9435131]
- Farhat DC, Swale C, Dard C, Cannella D, Ortet P, Barakat M, Sindikubwabo F, Belmudes L, De Bock PJ, Couté Y, et al. (2020). A MORC-driven transcriptional switch controls *Toxoplasma* developmental trajectories and sexual commitment. *Nat Microbiol* 5, 570–583. 10.1038/s41564-020-0674-4 [PubMed: 32094587]
- Forster R, Schubel A, Breitfeld D, Kremmer E, Renner-Muller I, Wolf E, and Lipp M.(1999). CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 99, 23–33. 10.1016/s0092-8674(00)80059-8 [PubMed: 10520991]

- Fox BA, Ristuccia JG, Gigley JP, and Bzik DJ (2009). Efficient gene replacements in *Toxoplasma gondii* strains deficient for nonhomologous end joining. *Eukaryotic cell* 8, 520–529. 10.1128/EC.00357-08 [PubMed: 19218423]
- Franco M, Panas MW, Marino ND, Lee MC, Buchholz KR, Kelly FD, Bednarski JJ, Sleckman BP, Pourmand N, and Boothroyd JC (2016). A Novel Secreted Protein, MYR1, Is Central to *Toxoplasma*'s Manipulation of Host Cells. *mBio* 7, e02231–02215. 10.1128/mBio.02231-15 [PubMed: 26838724]
- Frickel EM, and Hunter CA (2021). Lessons from *Toxoplasma*: Host responses that mediate parasite control and the microbial effectors that subvert them. *The Journal of experimental medicine* 218. 10.1084/jem.20201314
- Friedl P, and Weigelin B.(2008). Interstitial leukocyte migration and immune function. *Nat Immunol* 9, 960–969. [PubMed: 18711433]
- Fuks JM, Arrighi RB, Weidner JM, Kumar Mendu S, Jin Z, Wallin RP, Rethi B, Birnir B, and Barragan A.(2012). GABAergic signaling is linked to a hypermigratory phenotype in dendritic cells infected by *Toxoplasma gondii*. *PLoS pathogens* 8, e1003051. 10.1371/journal.ppat.1003051
- Garcia-Sanchez M, Jimenez-Pelayo L, Horcajo P, Regidor-Cerrillo J, Olafsson EB, Bhandage AK, Barragan A, Werling D, Ortega-Mora LM, and Collantes-Fernandez E.(2019). Differential Responses of Bovine Monocyte-Derived Macrophages to Infection by *Neospora caninum* Isolates of High and Low Virulence. *Front Immunol* 10, 915. 10.3389/fimmu.2019.00915 [PubMed: 31114577]
- Gay G, Braun L, Brenier-Pinchart MP, Vollaire J, Jossierand V, Bertini RL, Varesano A, Touquet B, De Bock PJ, Coute Y, et al. (2016). *Toxoplasma gondii* TgIST co-opts host chromatin repressors dampening STAT1-dependent gene regulation and IFN-gamma-mediated host defenses. *The Journal of experimental medicine* 213, 1779–1798. 10.1084/jem.20160340 [PubMed: 27503074]
- Glasmacher E, Agrawal S, Chang AB, Murphy TL, Zeng W, Vander Lugt B, Khan AA, Ciofani M, Spooner CJ, Rutz S, et al. (2012). A genomic regulatory element that directs assembly and function of immune-specific AP-1-IRF complexes. *Science (New York, NY)* 338, 975–980. 10.1126/science.1228309
- Goudot C, Coillard A, Villani AC, Gueguen P, Cros A, Sarkizova S, Tang-Huau TL, Bohec M, Baulande S, Hacoheh N, et al. (2017). Aryl Hydrocarbon Receptor Controls Monocyte Differentiation into Dendritic Cells versus Macrophages. *Immunity* 47, 582–596.e586. 10.1016/j.immuni.2017.08.016 [PubMed: 28930664]
- Grajales-Reyes GE, Iwata A, Albring J, Wu X, Tussiwand R, Kc W, Kretzer NM, Briseno CG, Durai V, Bagadia P, et al. (2015). Batf3 maintains autoactivation of Irf8 for commitment of a CD8alpha(+) conventional DC clonogenic progenitor. *Nature immunology* 16, 708–717. 10.1038/ni.3197 [PubMed: 26054719]
- Gregg B, Taylor BC, John B, Tait-Wojno ED, Girgis NM, Miller N, Wagage S, Roos DS, and Hunter CA (2013). Replication and distribution of *Toxoplasma gondii* in the small intestine after oral infection with tissue cysts. *Infection and immunity* 81, 1635–1643. 10.1128/iai.01126-12 [PubMed: 23460516]
- Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, Segura E, Tussiwand R, and Yona S.(2014). Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* 14, 571–578. 10.1038/nri3712 [PubMed: 25033907]
- Hakimi MA, Olias P, and Sibley LD (2017). *Toxoplasma* Effectors Targeting Host Signaling and Transcription. *Clin Microbiol Rev* 30, 615–645. 10.1128/CMR.00005-17 [PubMed: 28404792]
- He H, Brenier-Pinchart MP, Braun L, Kraut A, Touquet B, Couté Y, Tardieux I, Hakimi MA, and Bougdour A.(2018). Characterization of a *Toxoplasma* effector uncovers an alternative GSK3/β-catenin- regulatory pathway of inflammation. *eLife* 7. 10.7554/eLife.39887
- Helft J, Bottcher J, Chakravarty P, Zelenay S, Huotari J, Schraml BU, Goubau D, and Reis e Sousa C.(2015). GM-CSF Mouse Bone Marrow Cultures Comprise a Heterogeneous Population of CD11c(+)MHCII(+) Macrophages and Dendritic Cells. *Immunity* 42, 1197–1211. 10.1016/j.immuni.2015.05.018 [PubMed: 26084029]
- Hitziger N, Dellacasa I, Albiger B, and Barragan A.(2005). Dissemination of *Toxoplasma gondii* to immunoprivileged organs and role of Toll/interleukin-1 receptor signalling for host resistance



- assessed by in vivo bioluminescence imaging. *Cellular microbiology* 7, 837–848. CMI517 [pii] 10.1111/j.1462-5822.2005.00517.x [PubMed: 15888086]
- Huang Z, Liu H, Nix J, Xu R, Knoverek CR, Bowman GR, Amarasinghe GK, and Sibley LD (2022). The intrinsically disordered protein TgIST from *Toxoplasma gondii* inhibits STAT1 signaling by blocking cofactor recruitment. *Nature communications* 13, 4047. 10.1038/s41467-022-31720-7
- Jensen KD, Hu K, Whitmarsh RJ, Hassan MA, Julien L, Lu D, Chen L, Hunter CA, and Saeij JP (2013). *Toxoplasma gondii* rhoptyr 16 kinase promotes host resistance to oral infection and intestinal inflammation only in the context of the dense granule protein GRA15. *Infection and immunity* 81, 2156–2167. 10.1128/iai.01185-12 [PubMed: 23545295]
- Jensen KD, Wang Y, Wojno ED, Shastri AJ, Hu K, Cornel L, Boedec E, Ong YC, Chien YH, Hunter CA, et al. (2011). *Toxoplasma* polymorphic effectors determine macrophage polarization and intestinal inflammation. *Cell host & microbe* 9, 472–483. 10.1016/j.chom.2011.04.015 [PubMed: 21669396]
- Kanatani S, Fuks JM, Olafsson EB, Westermarck L, Chambers B, Varas-Godoy M, Uhlen P, and Barragan A.(2017). Voltage-dependent calcium channel signaling mediates GABAA receptor-induced migratory activation of dendritic cells infected by *Toxoplasma gondii*. *PLoS pathogens* 13, e1006739. 10.1371/journal.ppat.1006739 [PubMed: 29216332]
- Katholnig K, Kaltenecker CC, Hayakawa H, Rosner M, Lassnig C, Zlabinger GJ, Gaestel M, Müller M, Hengstschläger M, Hörl WH, et al. (2013). p38 $\alpha$  senses environmental stress to control innate immune responses via mechanistic target of rapamycin. *Journal of immunology* (Baltimore, Md : 1950) 190, 1519–1527. 10.4049/jimmunol.1202683 [PubMed: 23315073]
- Kim K, Eaton MS, Schubert W, Wu S, and Tang J.(2001). Optimized expression of green fluorescent protein in *Toxoplasma gondii* using thermostable green fluorescent protein mutants. *Mol Biochem Parasitol* 113, 309–313. [PubMed: 11295185]
- Kingston RE, and Narlikar GJ (1999). ATP-dependent remodeling and acetylation as regulators of chromatin fluidity. *Genes Dev* 13, 2339–2352. 10.1101/gad.13.18.2339 [PubMed: 10500090]
- Lambert H, Dellacasa-Lindberg I, and Barragan A.(2011). Migratory responses of leukocytes infected with *Toxoplasma gondii*. *Microbes and infection / Institut Pasteur* 13, 96–102. 10.1016/j.micinf.2010.10.002
- Lambert H, Hitziger N, Dellacasa I, Svensson M, and Barragan A.(2006). Induction of dendritic cell migration upon *Toxoplasma gondii* infection potentiates parasite dissemination. *Cellular microbiology* 8, 1611–1623. CMI735 [pii] 10.1111/j.1462-5822.2006.00735.x [PubMed: 16984416]
- Lambert H, Vutova PP, Adams WC, Lore K, and Barragan A.(2009). The *Toxoplasma gondii*-shuttling function of dendritic cells is linked to the parasite genotype. *Infection and immunity* 77, 1679–1688. [PubMed: 19204091]
- Laouar Y, Welte T, Fu XY, and Flavell RA (2003). STAT3 is required for Flt3L-dependent dendritic cell differentiation. *Immunity* 19, 903–912. 10.1016/s1074-7613(03)00332-7 [PubMed: 14670306]
- Lecoeur H, Prina E, Gutierrez-Sanchez M, and Spath GF (2022). Going ballistic: *Leishmania* nuclear subversion of host cell plasticity. *Trends in parasitology* 38, 205–216. 10.1016/j.pt.2021.09.009 [PubMed: 34666937]
- Lin Q, Chauvistre H, Costa IG, Gusmao EG, Mitzka S, Hanzelmann S, Baying B, Klisch T, Moriggl R, Hennuy B, et al. (2015). Epigenetic program and transcription factor circuitry of dendritic cell development. *Nucleic acids research* 43, 9680–9693. 10.1093/nar/gkv1056 [PubMed: 26476451]
- Lovey A, Verma S, Kaipilyawar V, Ribeiro-Rodrigues R, Husain S, Palaci M, Dietze R, Ma S, Morrison RD, Sherman DR, et al. (2022). Early alveolar macrophage response and IL-1R-dependent T cell priming determine transmissibility of *Mycobacterium tuberculosis* strains. *Nature communications* 13, 884. 10.1038/s41467-022-28506-2
- Ma JS, Sasai M, Ohshima J, Lee Y, Bando H, Takeda K, and Yamamoto M.(2014). Selective and strain-specific NFAT4 activation by the *Toxoplasma gondii* polymorphic dense granule protein GRA6. *The Journal of experimental medicine* 211, 2013–2032. 10.1084/jem.20131272 [PubMed: 25225460]
- Mercer HL, Snyder LM, Doherty CM, Fox BA, Bzik DJ, and Denkers EY (2020). *Toxoplasma gondii* dense granule protein GRA24 drives MyD88-independent p38 MAPK activation, IL-12

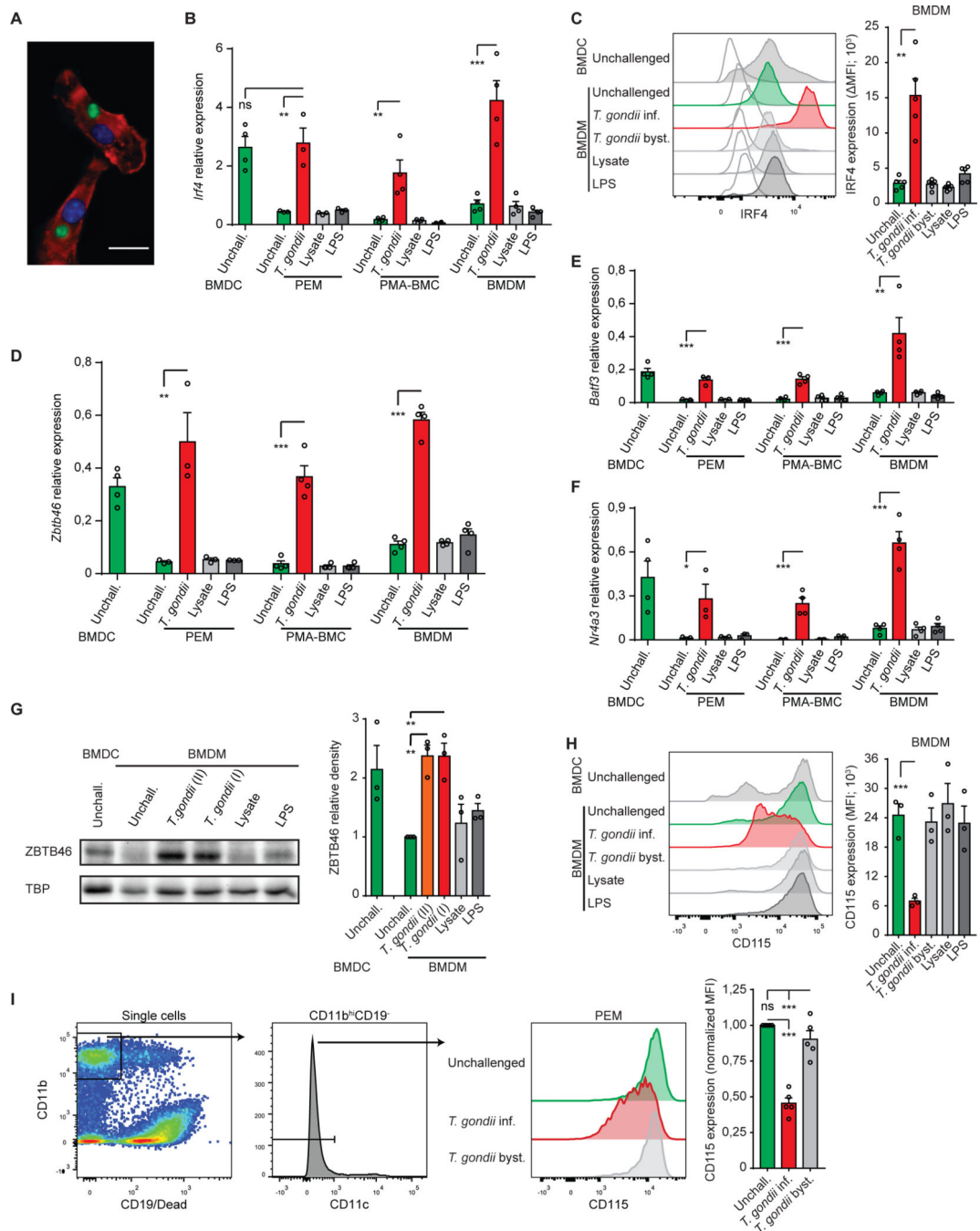
- production and induction of protective immunity. *PLoS pathogens* 16, e1008572. 10.1371/journal.ppat.1008572
- Mercer J, and Greber UF (2013). Virus interactions with endocytic pathways in macrophages and dendritic cells. *Trends Microbiol* 21, 380–388. 10.1016/j.tim.2013.06.001 [PubMed: 23830563]
- Miller JC, Brown BD, Shay T, Gautier EL, Jovic V, Cohain A, Pandey G, Leboeuf M, Elpek KG, Helft J, et al. (2012). Deciphering the transcriptional network of the dendritic cell lineage. *Nature immunology* 13, 888–899. 10.1038/ni.2370 [PubMed: 22797772]
- Montoya JG, and Liesenfeld O.(2004). Toxoplasmosis. *Lancet* 363, 1965–1976. [PubMed: 15194258]
- Morgado P, Ong YC, Boothroyd JC, and Lodoen MB (2011). *Toxoplasma gondii* induces B7–2 expression through activation of JNK signal transduction. *Infection and immunity* 79, 4401–4412. 10.1128/IAI.05562-11 [PubMed: 21911468]
- Mukhopadhyay D, Arranz-Solis D, and Saeij JPJ (2020). *Toxoplasma* GRA15 and GRA24 are important activators of the host innate immune response in the absence of TLR11. *PLoS pathogens* 16, e1008586. 10.1371/journal.ppat.1008586
- Nadipuram SM, Kim EW, Vashisht AA, Lin AH, Bell HN, Coppens I, Wohlschlegel JA, and Bradley PJ (2016). In Vivo Biotinylation of the *Toxoplasma* Parasitophorous Vacuole Reveals Novel Dense Granule Proteins Important for Parasite Growth and Pathogenesis. *mBio* 7. 10.1128/mBio.00808-16
- Naor A, Panas MW, Marino N, Coffey MJ, Tonkin CJ, and Boothroyd JC (2018). MYR1- Dependent Effectors Are the Major Drivers of a Host Cell's Early Response to *Toxoplasma*, Including Counteracting MYR1-Independent Effects. *mBio* 9. 10.1128/mBio.02401-17
- Nussenzweig MC, and Steinman RM (1980). Contribution of dendritic cells to stimulation of the murine syngeneic mixed leukocyte reaction. *The Journal of experimental medicine* 151, 1196–1212. 10.1084/jem.151.5.1196 [PubMed: 6445399]
- Olafsson EB, Ross EC, Varas-Godoy M, and Barragan A.(2019). TIMP-1 promotes hypermigration of *Toxoplasma*-infected primary dendritic cells via CD63-ITGB1-FAK signaling. *Journal of cell science* 132. 10.1242/jcs.225193
- Olafsson EB, Ten Hoeve AL, Li-Wang X, Westermark L, Varas-Godoy M, and Barragan A. (2020). Convergent Met and voltage-gated Ca(2+) channel signaling drives hypermigration of *Toxoplasma*-infected dendritic cells. *Journal of cell science* 134. 10.1242/jcs.241752
- Olias P, and Sibley LD (2016). Functional Analysis of the Role of *Toxoplasma gondii* Nucleoside Triphosphate Hydrolases I and II in Acute Mouse Virulence and Immune Suppression. *Infection and immunity* 84, 1994–2001. 10.1128/iai.00077-16 [PubMed: 27091930]
- Panas MW, Ferrel A, Naor A, Tenborg E, Lorenzi HA, and Boothroyd JC (2019). Translocation of Dense Granule Effectors across the Parasitophorous Vacuole Membrane in *Toxoplasma*-Infected Cells Requires the Activity of ROP17, a Rhoptry Protein Kinase. *mSphere* 4. 10.1128/mSphere.00276-19
- Pappas G, Roussos N, and Falagas ME (2009). Toxoplasmosis snapshots: global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. *International journal for parasitology* 39, 1385–1394. 10.1016/j.ijpara.2009.04.003 [PubMed: 19433092]
- Patil V, Zhao Y, Shah S, Fox BA, Rommereim LM, Bzik DJ, and Yap GS (2014). Co-existence of classical and alternative activation programs in macrophages responding to *Toxoplasma gondii*. *International journal for parasitology* 44, 161–164. 10.1016/j.ijpara.2013.08.003 [PubMed: 24083945]
- Rastogi S, Xue Y, Quake SR, and Boothroyd JC (2020). Differential Impacts on Host Transcription by ROP and GRA Effectors from the Intracellular Parasite *Toxoplasma gondii*. *mBio* 11. 10.1128/mBio.00182-20
- Rosenberg A, and Sibley LD (2021). *Toxoplasma gondii* secreted effectors co-opt host repressor complexes to inhibit necroptosis. *Cell host & microbe* 29, 1186–1198 e1188. 10.1016/j.chom.2021.04.016 [PubMed: 34043960]
- Ross EC, Hoeve ALT, Saeij JPJ, and Barragan A.(2022). *Toxoplasma* effector-induced ICAM-1 expression by infected dendritic cells potentiates transmigration across polarised endothelium. *Front Immunol* 13, 950914. 10.3389/fimmu.2022.950914 [PubMed: 35990682]

- Ross EC, Ten Hoeve AL, and Barragan A.(2021). Integrin-dependent migratory switches regulate the translocation of *Toxoplasma*-infected dendritic cells across brain endothelial monolayers. *Cellular and molecular life sciences : CMLS* 78, 5197–5212. 10.1007/s00018-021-03858-y [PubMed: 34023934]
- Rudzki EN, Ander SE, Coombs RS, Alrubaye HS, Cabo LF, Blank ML, Gutierrez-Melo N, Dubey JP, Coyne CB, and Boyle JP (2021). *Toxoplasma gondii* GRA28 Is Required for Placenta-Specific Induction of the Regulatory Chemokine CCL22 in Human and Mouse. *mBio*, e0159121. 10.1128/mBio.01591-21
- Saeij JP, Collier S, Boyle JP, Jerome ME, White MW, and Boothroyd JC (2007). *Toxoplasma* co-opts host gene expression by injection of a polymorphic kinase homologue. *Nature* 445, 324–327.10.1038/nature05395 [PubMed: 17183270]
- Sangare LO, Olafsson EB, Wang Y, Yang N, Julien L, Camejo A, Pesavento P, Sidik SM, Lourido S, Barragan A, et al. (2019). In Vivo CRISPR Screen Identifies TgWIP as a *Toxoplasma* Modulator of Dendritic Cell Migration. *Cell host & microbe* 26, 478–492 e478. 10.1016/j.chom.2019.09.008 [PubMed: 31600500]
- Sansonetti PJ, and Di Santo JP (2007). Debugging how bacteria manipulate the immune response. *Immunity* 26, 149–161. 10.1016/j.immuni.2007.02.004 [PubMed: 17307704]
- Sathe P, Metcalf D, Vremec D, Naik SH, Langdon WY, Huntington ND, Wu L, and Shortman K.(2014). Lymphoid tissue and plasmacytoid dendritic cells and macrophages do not share a common macrophage-dendritic cell-restricted progenitor. *Immunity* 41, 104–115. 10.1016/j.immuni.2014.05.020 [PubMed: 25035955]
- Satpathy AT, Kc W, Albring JC, Edelson BT, Kretzer NM, Bhattacharya D, Murphy TL, and Murphy KM (2012). *Zbtb46* expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. *The Journal of experimental medicine* 209, 1135–1152. 10.1084/jem.20120030 [PubMed: 22615127]
- Scanga CA, Aliberti J, Jankovic D, Tilloy F, Bennouna S, Denkers EY, Medzhitov R, and Sher A.(2002). Cutting edge: MyD88 is required for resistance to *Toxoplasma gondii* infection and regulates parasite-induced IL-12 production by dendritic cells. *J Immunol* 168, 5997–6001. [PubMed: 12055206]
- Schluter D, and Barragan A.(2019). Advances and Challenges in Understanding Cerebral Toxoplasmosis. *Frontiers in immunology* 10, 242. 10.3389/fimmu.2019.00242 [PubMed: 30873157]
- Schneider CA, Rasband WS, and Eliceiri KW (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature methods* 9, 671–675. [PubMed: 22930834]
- Schulz O, Jaensson E, Persson EK, Liu X, Worbs T, Agace WW, and Pabst O.(2009). Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *The Journal of experimental medicine* 206, 3101–3114. 10.1084/jem.20091925 [PubMed: 20008524]
- Sibley LD, and Boothroyd JC (1992). Virulent strains of *Toxoplasma gondii* comprise a single clonal lineage. *Nature (Lond)* 359, 82–85. [PubMed: 1355855]
- Stuart RK, and Hamilton JA (1980). Tumor-promoting phorbol esters stimulate hematopoietic colony formation in vitro. *Science (New York, NY)* 208, 402–404. 10.1126/science.6245446
- Swierzy IJ, Handel U, Kaever A, Jarek M, Scharfe M, Schluter D, and Luder CGK (2017). Divergent co-transcriptomes of different host cells infected with *Toxoplasma gondii* reveal cell type- specific host-parasite interactions. *Scientific reports* 7, 7229. 10.1038/s41598-017-07838-w [PubMed: 28775382]
- Tan S, and Russell DG (2015). Trans-species communication in the *Mycobacterium tuberculosis*-infected macrophage. *Immunological reviews* 264, 233–248. 10.1111/imr.12254 [PubMed: 25703563]
- ten Hoeve AL, Hakimi M-A, and Barragan A.(2019). Sustained Egr-1 Response via p38 MAP Kinase Signaling Modulates Early Immune Responses of Dendritic Cells Parasitized by *Toxoplasma gondii*. *Frontiers in Cellular and Infection Microbiology* 9. 10.3389/fcimb.2019.00349

- Wang Y, Cirelli KM, Barros PDC, Sangare LO, Butty V, Hassan MA, Pesavento P, Mete A, and Saeij JPJ (2019). Three *Toxoplasma gondii* Dense Granule Proteins Are Required for Induction of Lewis Rat Macrophage Pyroptosis. *mBio* 10. 10.1128/mBio.02388-18
- Wang Y, Sangare LO, Paredes-Santos TC, Hassan MA, Krishnamurthy S, Furuta AM, Markus BM, Lourido S, and Saeij JPJ (2020). Genome-wide screens identify *Toxoplasma gondii* determinants of parasite fitness in IFN $\gamma$ -activated murine macrophages. *Nat Commun* 11, 5258. 10.1038/s41467-020-18991-8 [PubMed: 33067458]
- Weidner JM, and Barragan A.(2014). Tightly regulated migratory subversion of immune cells promotes the dissemination of *Toxoplasma gondii*. *International journal for parasitology* 44, 85–90. 10.1016/j.ijpara.2013.09.006 [PubMed: 24184911]
- Weidner JM, Kanatani S, Hernandez-Castaneda MA, Fuks JM, Rethi B, Wallin RP, and Barragan A.(2013). Rapid cytoskeleton remodelling in dendritic cells following invasion by *Toxoplasma gondii* coincides with the onset of a hypermigratory phenotype. *Cellular microbiology* 15, 1735–1752. 10.1111/cmi.12145 [PubMed: 23534541]
- Weidner JM, Kanatani S, Uchtenhagen H, Varas-Godoy M, Schulte T, Engelberg K, Gubbels MJ, Sun HS, Harrison RE, Achour A, et al. (2016). Migratory activation of parasitized dendritic cells by the protozoan *Toxoplasma gondii* 14–3–3 protein. *Cellular microbiology* 18, 1537–1550. 10.1111/cmi.12595 [PubMed: 27018989]
- Xue Y, Wong J, Moreno GT, Young MK, Cote J, and Wang W.(1998). NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. *Mol Cell* 2, 851–861. 10.1016/s1097-2765(00)80299-3 [PubMed: 9885572]
- Yamamoto M, Standley DM, Takashima S, Saiga H, Okuyama M, Kayama H, Kubo E, Ito H, Takaura M, Matsuda T, et al. (2009). A single polymorphic amino acid on *Toxoplasma gondii* kinase ROP16 determines the direct and strain-specific activation of Stat3. *The Journal of experimental medicine* 206, 2747–2760. 10.1084/jem.20091703 [PubMed: 19901082]
- Zhang H, Elbaum-Garfinkle S, Langdon EM, Taylor N, Occhipinti P, Bridges AA, Brangwynne CP, and Gladfelter AS (2015). RNA Controls PolyQ Protein Phase Transitions. *Mol Cell* 60, 220–230. 10.1016/j.molcel.2015.09.017 [PubMed: 26474065]

**Highlights**

- *Toxoplasma* effector protein GRA28 drives a pro-migratory transcriptional remodeling
- In parasitized macrophages, GRA28 cooperates with host chromatin modifiers
- Parasitized macrophages upregulate CCR7 GRA28-dependently and chemotax
- GRA28 facilitates systemic migration of adoptively transferred macrophages in mice



**Figure 1. Expression of DC associated transcription factors in *T. gondii*-challenged macrophages**

**(A)** Representative micrograph shows primary bone marrow-derived macrophages (BMDMs) stained for F-actin (red) and nuclei (blue), with replicating intracellular GFP-expressing *T. gondii* tachyzoites (green) 18 h post-challenge. Scale bar = 20  $\mu$ m.

**(B)** qPCR analyses of *Irf4* cDNA from PEMs, PMA-BMCs and BMDMs challenged for 18 h with *T. gondii* type I tachyzoites (RH, MOI 2), tachyzoite lysate (MOI 2 equivalent) or LPS (10 ng/mL). For reference, macrophages and BMDCs were incubated in complete medium, unchallenged (unchall.).

(C) Flow cytometric analysis of IRF4 expression by BMDCs and BMDMs challenged as in (B) with MOI 1. *T. gondii*-infected and bystander cells are distinguished by GFP<sup>+/-</sup>. Bar graph depicts the differences between anti-IRF4 and isotype mean fluorescence intensity (MFI, mean+SE) from 5 independent experiments (n = 5).

(D, E, F) qPCR analyses of *Zbtb46* (D), *Batf3* (E) and *Nr4a3* (F) cDNA, respectively, in cells challenged as in (B).

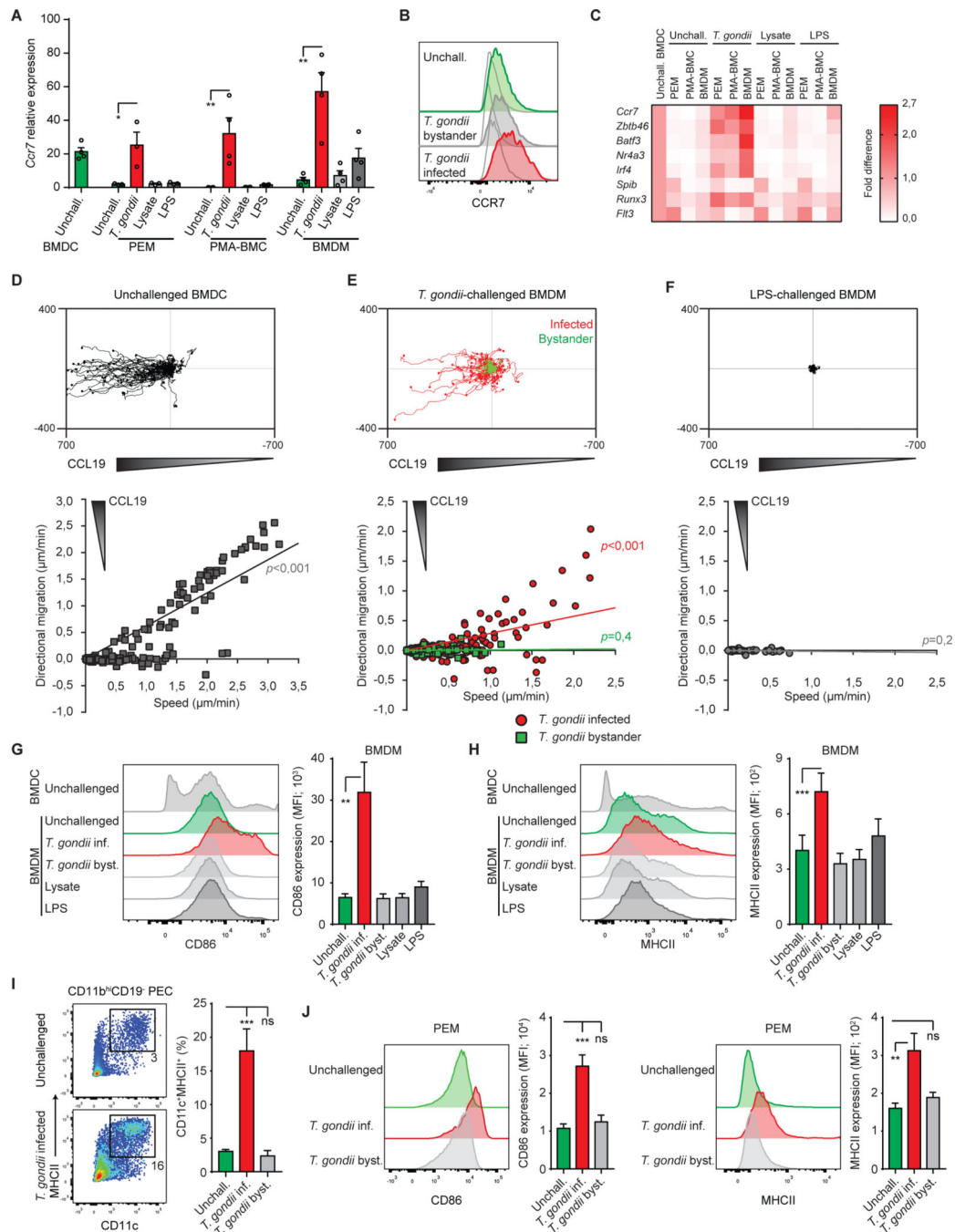
(G) Western blot analysis of ZBTB46 expression in BMDCs and BMDMs challenged for 18 h with *T. gondii* type I or type II tachyzoites, tachyzoite lysate or LPS. Bar graph depicts density (mean+SE) related to unchallenged BMDMs (=1, n = 3).

(H) Flow cytometry analysis of CD115 expression in BMDCs and BMDMs challenged as in (C). Bar graph depicts MFI (mean+SE) from 3 independent experiments.

(I) Flow cytometric analysis of CD115 expression on PEMs challenged as in (C) with *T. gondii* (MOI 0,5) or left unchallenged, gated from live peritoneal cells as indicated. Bar graph depicts MFI (mean+SE) related to unchallenged PEM (= 1, n = 5).

Relative expression ( $2^{-Cq}$ ) is displayed as mean+SE and individual measurements (n = 3–4). Statistical comparisons were made with ANOVA and Dunnett's (B, E, F, G and H) or Holm-Bonferroni (C, D and I) post-hoc tests (\* p 0,05, \*\* p 0,01, \*\*\* p 0,001, ns p > 0,05).

See also Figure S1.



**Figure 2. Expression of *Ccr7* and chemotaxis towards CCL19 by *T. gondii*-challenged macrophages**

(A) qPCR analyses of *Ccr7* cDNA from BMDCs, PEMs, PMA-BMCs and BMDMs challenged for 18 h with *T. gondii* type I tachyzoites (RH), tachyzoite lysate, LPS or left unchallenged (unchall.). Relative expression ( $2^{-Cq}$ ) is displayed as mean+SE and individual measurements (n = 3–4).



**(B)** Flow cytometric analysis of anti-CCR7 and isotype control stainings on BMDMs challenged for 18h with *T. gondii* type I tachyzoites or left unchallenged (unchall.). Representative of 2 independent experiments.

**(C)** Heat map depicting relative mRNA expression changes of indicated genes for different types of macrophages and challenges. Color scale indicates mean fold difference in expression related to unchallenged BMDCs (=1) from 3–4 independent experiments.

**(D, E, F)** Motility plots depict the displacement of unchallenged BMDCs and BMDMs challenged with *T. gondii* type I (RH) or LPS for 12 h in a collagen matrix with a CCL19 gradient as detailed in Methods details (scale in  $\mu\text{m}$ ). For each condition, directional migration ( $\mu\text{m}/\text{min}$ ) towards the CCL19 source and speed ( $\mu\text{m}/\text{min}$ ) of individual cells are displayed in graphs, with linear regression lines. For *T. gondii* challenged BMDMs (E), infected cells ( $\text{GFP}^+$ , red) and non-infected bystander cells ( $\text{GFP}^-$ , green) were analyzed. P- values indicate the directional migration compared to hypothetical zero directionality (one-sample permutation test). Data from 2 (BMDCs) or 3 independent experiments.

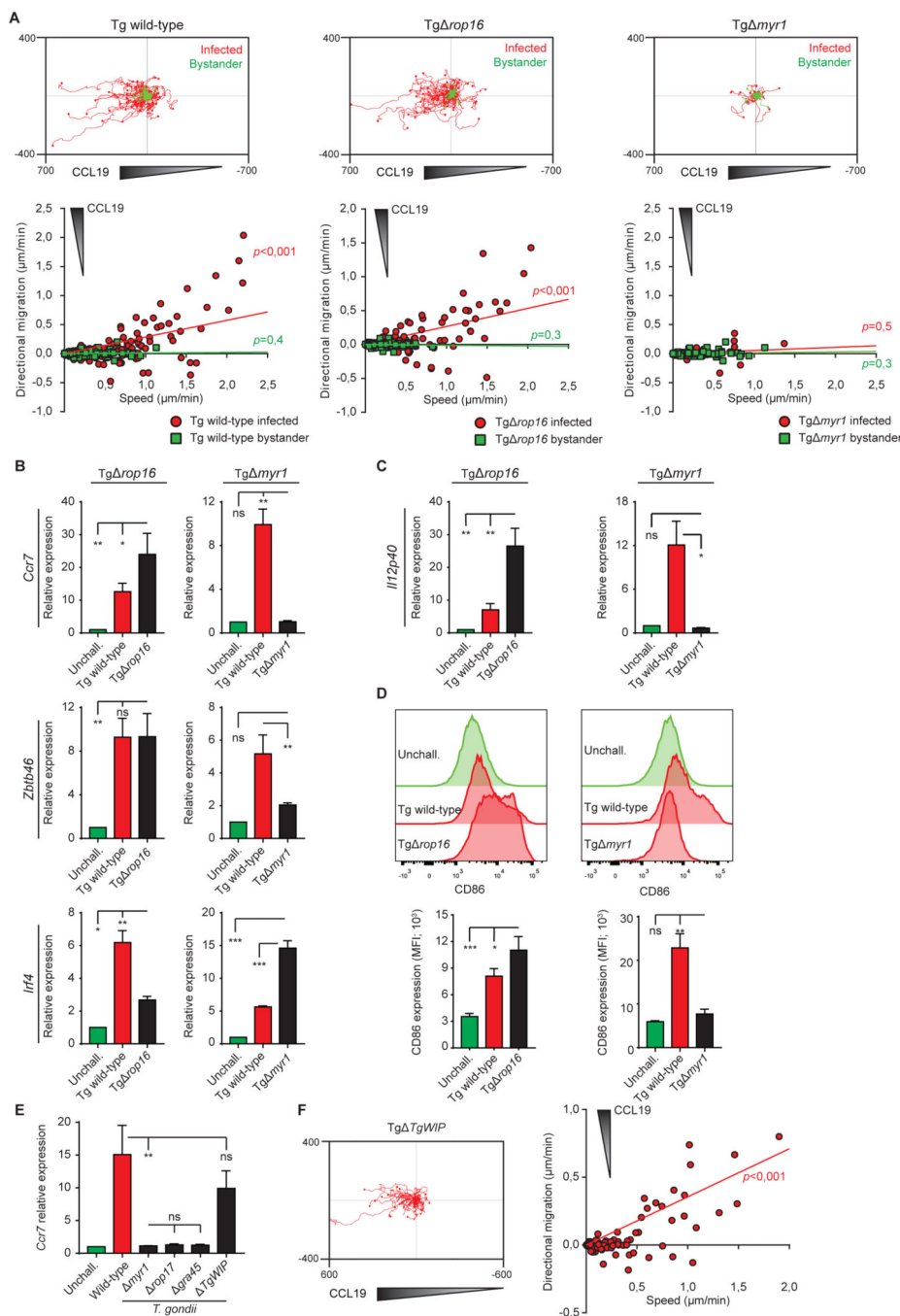
**(G, H)** Flow cytometric analysis of CD86 (G) and MHCII (H) expression in BMDCs and BMDMs challenged as in (A), with *T. gondii*-infected and bystander BMDMs distinguished by  $\text{GFP}^{+/-}$ . Bar graph depicts the MFI (mean+SE, n = 4).

**(I)** Flow cytometric analysis of CD11c and MHCII expression on  $\text{CD11b}^{\text{hi}}\text{CD19}^-$  peritoneal cells (PEC), challenged as in (C) with *T. gondii* or left unchallenged. Bar graph depicts the percentage of  $\text{CD11c}^+\text{MHCII}^+$  cells (mean+SE, n = 5).

**(J)** Flow cytometric analysis of CD86 and MHCII on PEMs ( $\text{CD11b}^{\text{hi}}\text{CD11c}^-\text{CD19}^-$ ), challenged as in (I). Bar graphs depict MFI (mean+SE, n= 5).

\* p 0,05, \*\* p 0,01, \*\*\* p 0,001, ns p > 0,05 (ANOVA and Holm-Bonferroni post-hoc test (A, G-J)).

See also Figure S2.



**Figure 3. Impact of parasite-derived secreted effectors on macrophage chemotaxis**  
**(A)** Motility plots depict displacement of BMDMs challenged with *T. gondii* type I (RH, Tg) wild-type, MYR1-deficient (*myr1*) or ROP16-deficient (*rop16*) tachyzoites for 12h in a collagen matrix with a CCL19 gradient (scale in μm). Directional migration (μm/min) towards the CCL19 source and speed (μm/min) of individual cells are displayed in graphs, with linear regression lines. Infected cells (red) and non-infected bystander cells (green) were analyzed. P-values indicate the directional migration compared to hypothetical zero directionality (one-sample permutation test).

**(B, C)** qPCR analysis of *Ccr7*, *Zbtb46* and *Irf4* (b) and *Iil2p40* (c) cDNA in BMDMs challenged for 18h with freshly egressed *T. gondii* wild-type, *myr1* or *rop16* tachyzoites or left unchallenged. Relative expression ( $2^{-Cq}$ ) is displayed as mean+SE related to unchallenged (= 1).

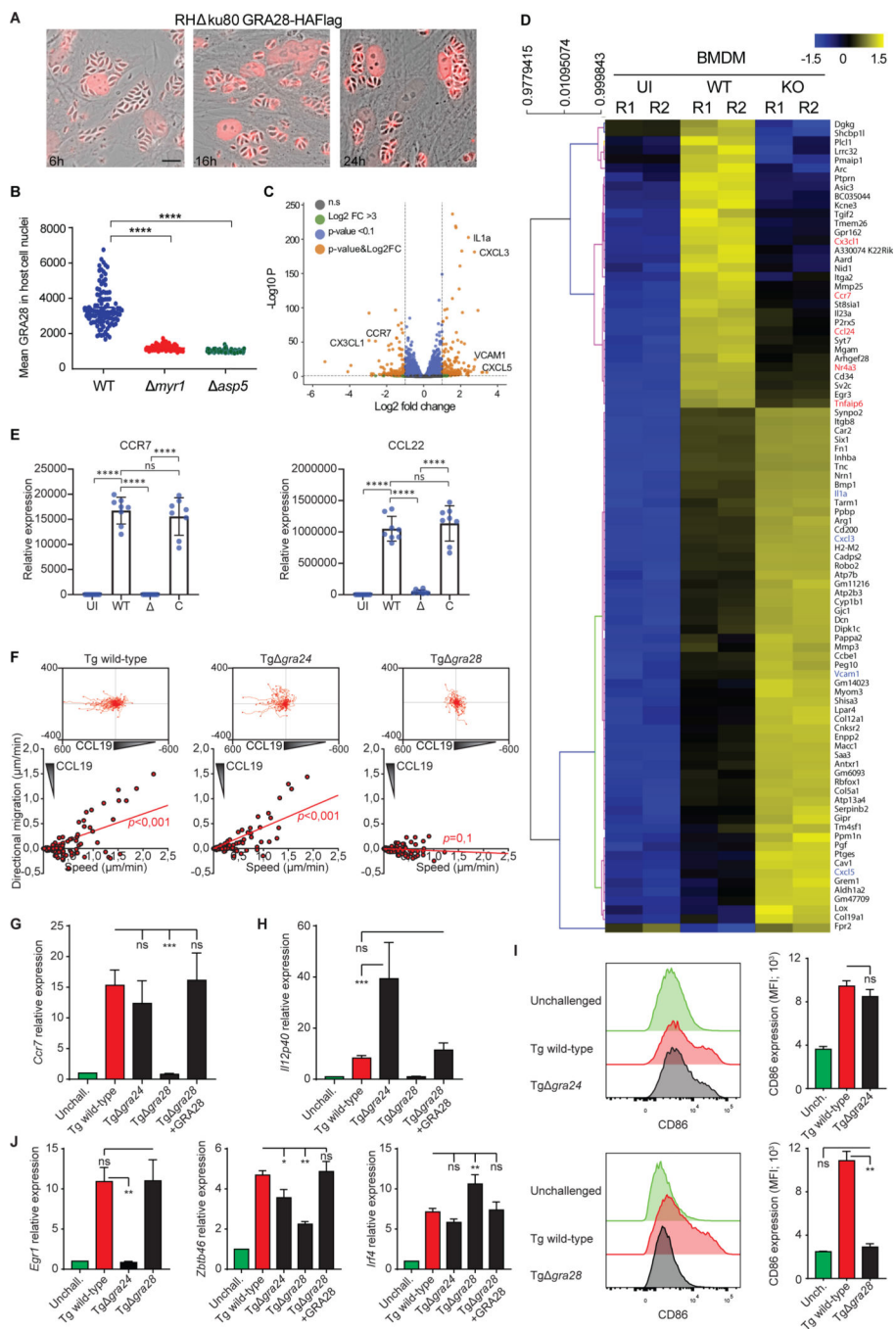
**(D)** Flow cytometric analyses of CD86 expression on BMDMs challenged as in (B). For wild-type/ *myr1* experiments, cells were gated for GFP<sup>+</sup>.

**(E)** qPCR analysis of *Ccr7* cDNA expression in BMDMs challenged with type I (Tg) wild-type, *myr1*, *rop17*, *gra45* and *TgWIP* tachyzoites and displayed as in (B).

**(F)** Motility plot shows displacement of BMDMs challenged with *TgWIP* tachyzoites in a CCL19 gradient and analysis as in (A).

All datasets are from of 3–5 independent experiments and displayed as mean+SE. \* p < 0,05, \*\* p < 0,01, \*\*\* p < 0,001, ns p > 0,05 (ANOVA and Dunnett's (B, C, D) or Holm-Bonferroni (E) post-hoc tests).

See also Figure S3.



**Figure 4. GRA28 is secreted in host cell nuclei in a Myr1 and Asp5-dependent manner to activate and repress gene expression in macrophages, with an impact on *Ccr7* expression and chemotaxis.** (A) Time course of GRA28 secretion and export to the host cell nucleus. HFFs were challenged with RH ku80 GRA28–HA–FLAG tachyzoites, stained with anti-HA antibodies (red) and visualized by epifluorescence and transmitted light microscopy at indicated timepoints (6, 12, 24 h). Scale bar, 10  $\mu$ m.

**(B)** RH *ku80* WT, *asp5* and *myr1* parasites were transiently transfected with pTUB8-GRA28-HAFlag. The amount of GRA28 in the nucleus was quantified in at least 100 host cells for each parasite strain.

**(C)** Genome-wide expression profiling of BALB/c BMDMs left uninfected or infected with WT and *gra28* type II (Pru) tachyzoites. The results of tests for differential expression between WT and *gra28* tachyzoites are presented in a volcano plot that plots the statistical significance against the fold change for each gene. The orange dots indicate transcripts that were significantly up and down regulated, using adjusted  $p < 0.1$  (Bonferroni-corrected) and  $\pm 3$ -fold change as the cut-off corresponding to each comparison. X-axis showing  $\log_2$  fold change, Y-axis showing  $-\log_{10}(p\text{-value})$ . Vertical dashed lines indicate threefold up- and down-regulation.

**(D)** Heat map representation of the differentially expressed mouse genes ( $>3$  fold, RPKM  $>5$  in at least one sample) between parental and *gra28*-infected cells. RPKM values were  $\log_2$  transformed, Gene/Row normalized, and mean centered using MeV.

**(E)** RAW macrophages were infected for 24 h with WT, *gra28* ( ) or Tg *gra28*+GRA28 (C), the complemented line of type I (RH) tachyzoites, or left unchallenged (UI). GOI and TBP mRNA levels were determined by RT-qPCR and GOI values were normalized to TBP. \*\*  $p < 0,01$ , \*\*\*  $p < 0,001$ , \*\*\*\*  $p < 0,0001$ , ns  $p > 0,05$  (One-way ANOVA and Tukey's multiple comparison test). Data are the mean  $\pm$  s.d. (n = 3).

**(F)** Motility plots and analyses of BMDMs challenged with type I (RH) wild-type, *gra24* or *gra28 T. gondii* tachyzoites (Tg) for 12h, and allowed to migrate in a CCL19 gradient. P-values indicate the directional migration compared to hypothetical zero directionality (one-sample permutation test).

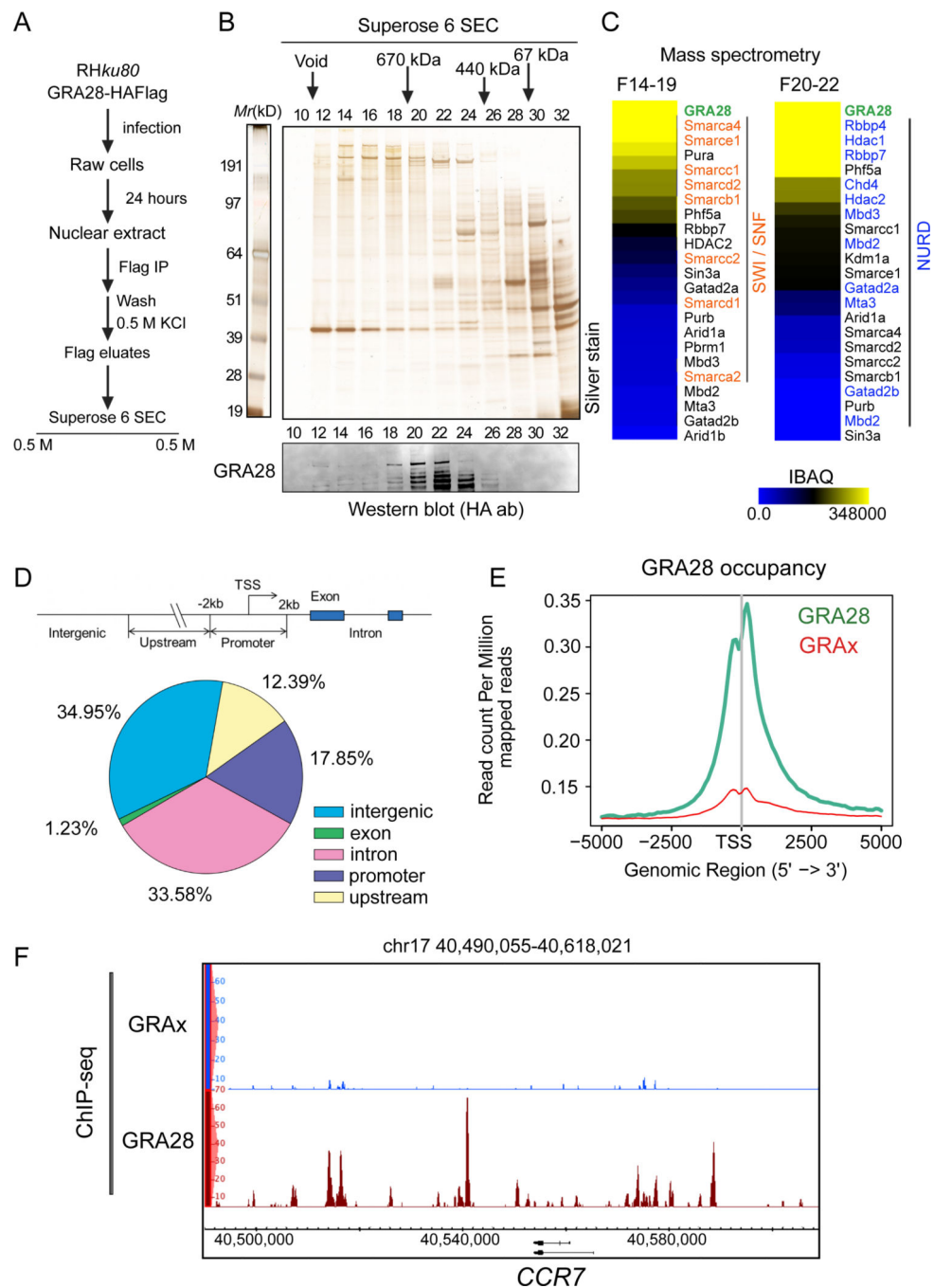
**(G, H)** qPCR analysis of *Ccr7* (G) and *IL12p40* (H) cDNA expression in BMDMs challenged with type I wild-type, *gra24*, *gra28* or *gra28*+GRA28 tachyzoites (Tg) for 18h (RH) or left unchallenged. Relative expression ( $2^{-Cq}$ ) is displayed as mean+SE related to unchallenged (=1; n = 4).

**(I)** Flow cytometric analyses of anti-CD86 staining on BMDMs challenged as in (B).

**(J)** qPCR analysis of *Egr1*, *Zbtb46* and *Irf4* in BMDMs challenged with type I wild-type (Tg), *gra24* or *gra28* or *gra28*+GRA28 tachyzoites or left unchallenged as in (G). Relative expression ( $2^{-Cq}$ ) related to unchallenged (=1; n = 3).

Datasets are from at least 3 independent experiments and displayed as mean+SE. \*  $p < 0,05$ , \*\*  $p < 0,01$ , \*\*\*  $p < 0,001$ , ns  $p > 0,05$  (ANOVA and Dunnett's (G, H, J) or Holm-Bonferroni (I) post-hoc test.

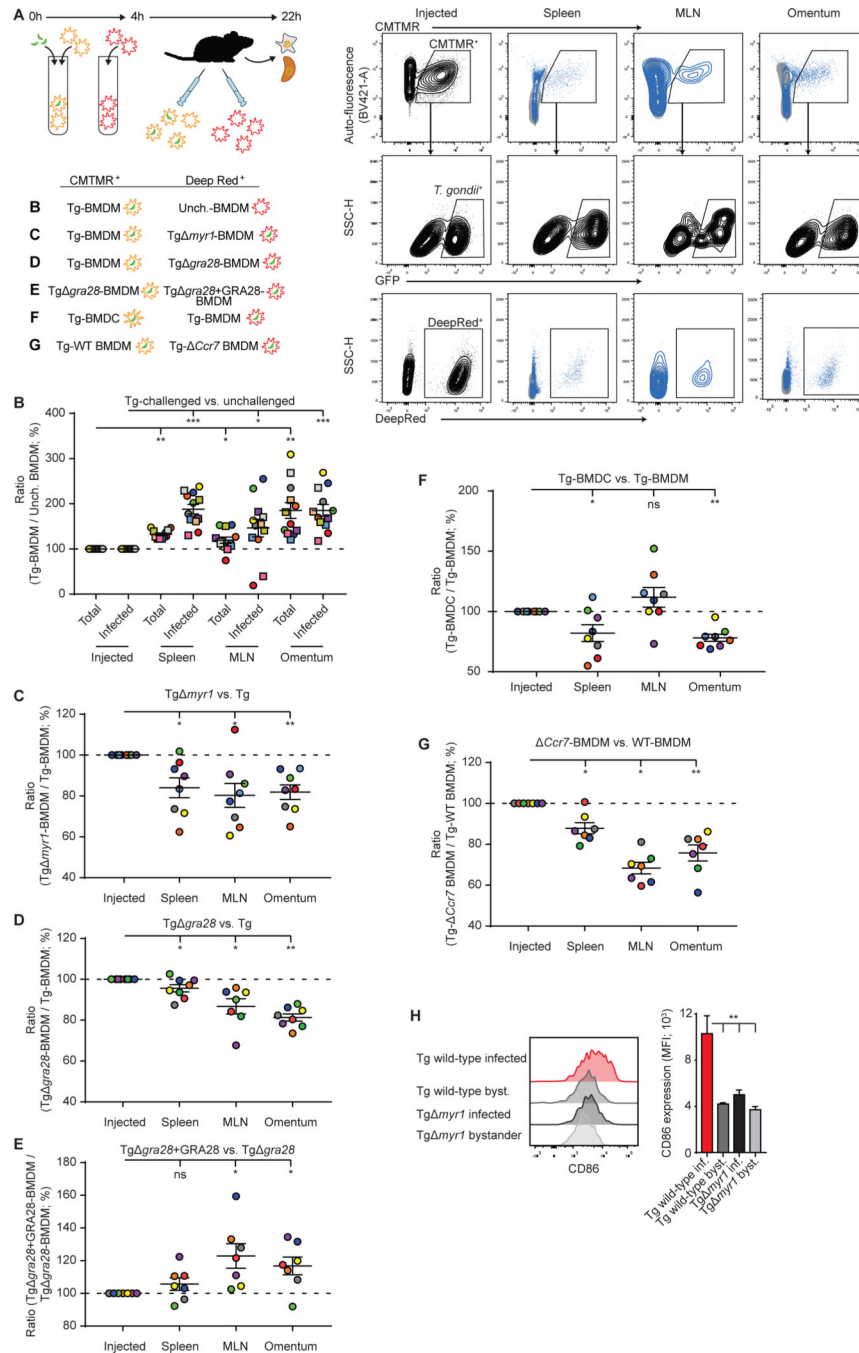
See also Figure S4.



**Figure 5. GRA28 binds to chromatin and partners with chromatin remodellers NuRD and SWI/SNF.**

(A, B) GRA28-associated proteins were purified by FLAG chromatography from protein extracts of the murine RAW macrophage line infected with RH *ku80* GRA28-HA-FLAG. Fractions from size-exclusion chromatography of GRA28-containing complexes after Flag-affinity selection were analyzed on gels by silver staining and then by mass spectrometry-based proteomics to detect GRA28 and the aforementioned partners.

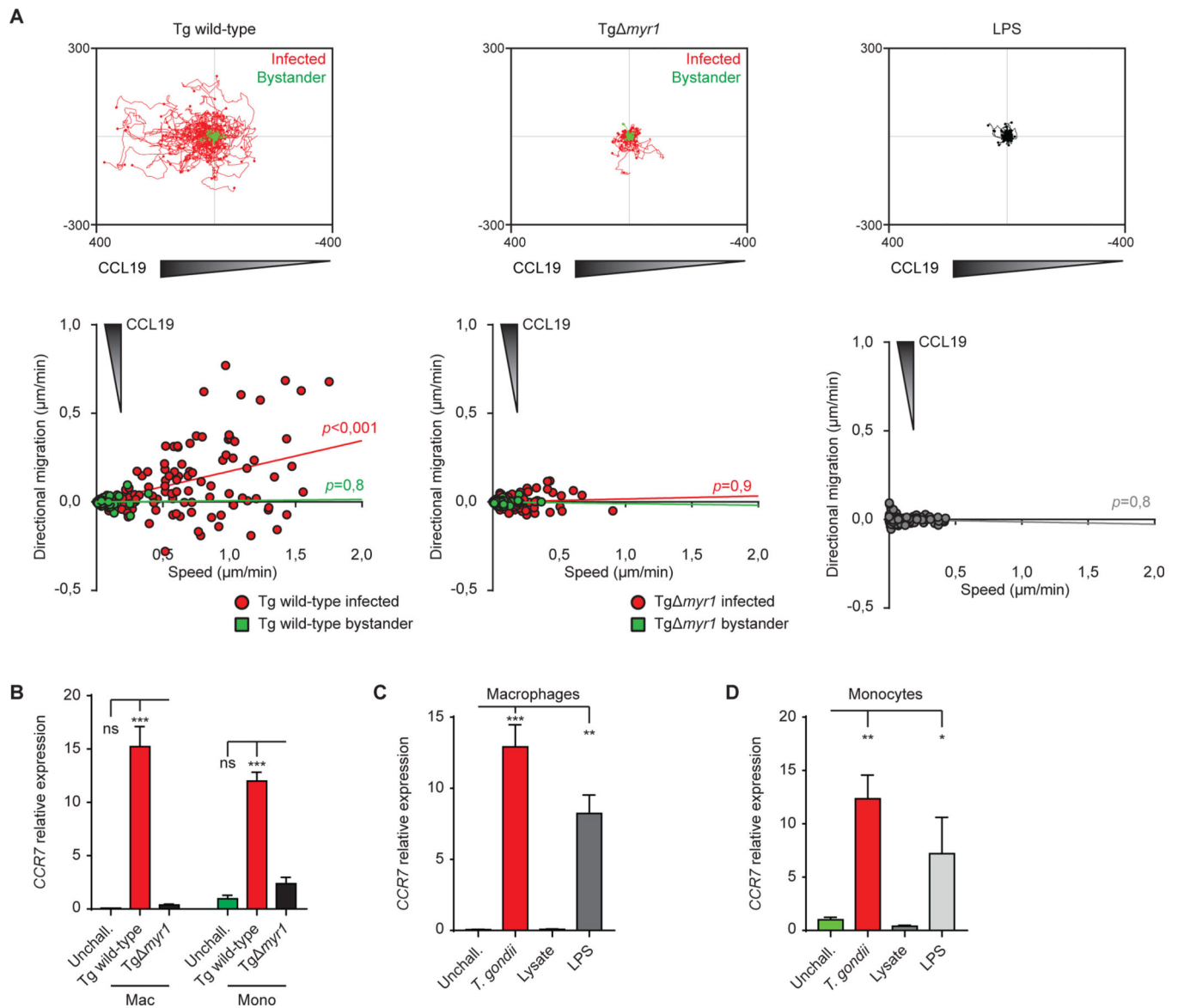
- (C) Heatmap showing the abundance ranks of each protein in each purified fraction derived from extracted iBAQ values. The identity of the proteins is indicated.
- (D) The pie chart shows the distribution of GRA28 peaks in the genome of human fibroblasts relative to gene features.
- (E) The average distribution of GRA28 and the control GRAX in gene promoters. Average-signal profiles of each protein were plotted over a region from – 5 kb to +5 kb relative to the TSS of each gene. Average tag count of the enrichment is shown on the y-axis.
- (F) IGB images of the gene *CCR7* and ChIP-seq signal peaks for GRA28 and GRAX. See also Figure S5.



**Figure 6. *In vivo* migration of *T. gondii*-challenged adoptively transferred BMDMs**  
**(A)** Illustration of experimental setup and conditions for co-adoptive transfers of *T. gondii* (RH)-challenged or unchallenged (unch.) BMDMs or BMDCs pre-labeled with CellTracker Deep Red or CMTMR dyes. **B, C, D, E, F, G** indicate, respectively, conditions and experimental setups for corresponding graphs below. Plots show flow cytometric detection of pre-labelled (CMTMR<sup>+</sup>/Deep red<sup>+</sup>) and parasitized cells (GFP<sup>+</sup>) extracted from organs 18h post-inoculation, as detailed under Methods. Events from organs are displayed in blue or black (inoculated) and in grey (non-inoculated).



- (B)** Flow cytometric analysis of *T. gondii*-challenged (Tg) and unchallenged (unch.) BMDMs in the spleen, MLNs and omentum 18h post-inoculation. Data is presented as the change in ratio between detected challenged CMTMR<sup>+</sup> cells and unchallenged Deep red<sup>+</sup> cells (total) or specified by infection (GFP<sup>+</sup>/GFP<sup>-</sup>) related to the inoculated ratio (normalized to 100%). Mean ratio change  $\pm$ SE and individual mice (n = 12) are displayed.
- (C)** Flow cytometric analysis of BMDMs treated as indicated in (A) in the spleen, MLNs and omentum. The change in ratio between Deep red<sup>+</sup>GFP<sup>+</sup> cells (Tg *myr1*-infected) and CMTMR<sup>+</sup>GFP<sup>+</sup> cells (Tg-infected) related to inoculated (=100%) is shown.
- (D)** Flow cytometric analysis of BMDMs treated as indicated in (A). The change in ratio between Deep red<sup>+</sup>CFSE<sup>+</sup> Tg *gra28*-infected cells and CMTMR<sup>+</sup>CFSE<sup>+</sup> Tg-infected cells related to inoculated (=100%) is shown.
- (E)** Flow cytometric analysis of BMDMs treated as indicated in (A). The change in ratio between Deep red<sup>+</sup>CFSE<sup>+</sup> Tg *gra28*+GRA28-infected cells and CMTMR<sup>+</sup>CFSE<sup>+</sup> Tg *gra28*-infected cells related to inoculated (=100%) is shown.
- (F)** Flow cytometric analysis of *T. gondii*-challenged (Tg) BMDM and BMDC in the spleen, MLNs and omentum 18h post-inoculation. Data is presented as the change in ratio between infected CMTMR<sup>+</sup>GFP<sup>+</sup> BMDCs and Deep red<sup>+</sup>GFP<sup>+</sup> BMDMs related to inoculated (=100%) is shown.
- (G)** Flow cytometric analysis of *T. gondii*-challenged (Tg) wild-type (WT) or CCR7-deficient (*Ccr7*) BMDM in the spleen, MLNs and omentum 18h post-inoculation. Data is presented as the change in ratio between infected CMTMR<sup>+</sup>GFP<sup>+</sup> BMDCs and Deep red<sup>+</sup>GFP<sup>+</sup> BMDMs related to inoculated (=100%) is shown.
- (H)** Flow cytometric analysis of anti-CD86 staining in BMDMs challenged as in (D) recovered from the spleen. Bar graph depicts MFI (mean $\pm$ SE) from 3 mice. Scatter plots display individual mice with symbols (n = 7–12) and mean $\pm$ SE (B-G). \* p 0,05, \*\* p 0,01, \*\*\* p 0,001, ns p > 0,05 (one-sample permutation (B-G) or ANOVA and Dunnett's (H) post-hoc tests (H)).
- See also Figure S6.



**Figure 7. Expression of DC-associated transcription factors and chemotaxis in *T. gondii*-challenged human monocytic cells**

(A) Motility plots and analyses ( $n = 3$ ) of human monocyte-derived macrophages challenged with *T. gondii* type I (RH, Tg) wild-type, Tg *myr1* or LPS for 12h in a CCL19 gradient as indicated under Methods details.

(B) qPCR analysis of *CCR7* cDNA from human macrophages and monocytes challenged for 18 h as in (A). Relative expression ( $2^{-Cq}$ ) is shown (mean+SE,  $n = 4$ ).

(C, D) qPCR analysis of *CCR7* cDNA from human macrophages (C) and human monocytes (D) challenged for 18 h with *T. gondii* type I tachyzoites (Tg), tachyzoite lysate, LPS. For reference cells were or left unchallenged (unchall.). Relative expression ( $2^{-Cq}$ ) is shown (mean+SE,  $n = 4-5$ ).

\*  $p < 0,05$ , \*\*  $p < 0,01$ , \*\*\*  $p < 0,001$ , ns  $p > 0,05$  (B-D; ANOVA, Dunnett's post-hoc test).

See also Figure S7.

## Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
PE anti-mouse CCR7 (clone 4B12)	eBioscience	Cat# 12-1971-82 RRID:AB_465905
PE-Cyanine7 anti-mouse CD11c (clone N418)	eBioscience	Cat# 25-0114-82 RRID:AB_469590
APC anti-mouse/human CD11b (clone M1/70)	BioLegend	Cat# 101211 RRID:AB_312794
anti-mouse CD16/CD32 (Mouse BD Fc Block™)	BD Biosciences	Cat# 553142 RRID:AB_394657
anti-mouse CD16/CD32 (clone 93)	eBioscience	Cat# 14-0161-82 RRID:AB_467133
Super Bright 780 anti-mouse CD40 (clone 1C10)	eBioscience	Cat# 78-0401-82 RRID:AB_2762674
Super Bright 645 anti-mouse CD80 (clone 16-10A1)	eBioscience	Cat# 64-0801-80 RRID:AB_2663120
Super Bright 600 anti-mouse CD86 (clone GL1)	eBioscience	Cat# 63-0862-80 RRID:AB_2662861
Brilliant Violet 605 anti-mouse CD115 (clone AFS98)	BioLegend	Cat# 135517 RRID:AB_2562760
Super Bright 702 anti-mouse MHCII I-A/I-E (clone M5/114)	eBioscience	Cat# 67-5321-82 RRID:AB_2717173
PE anti-mouse IRF4 (clone Q9-343)	BD Biosciences	Cat# 566649
PerCP-Cyanine5.5 anti-mouse IL-12/23 p40 (clone C17.8)	eBioscience	Cat# 45-7123-80 RRID:AB_1107021
PE anti-mouse Ly6C (clone HK1.4)	BioLegend	Cat# 128007 RRID:AB_1186133
PE anti-mouse ZBTB46 (clone U4-1374)	BD Biosciences	Cat# 565832 RRID:AB_2739372
APC/Fire 750 anti-mouse F4/80 (clone BM8)	BioLegend	Cat# 123151 RRID:AB_2616724
PE mouse IgG1 Kappa Isotype Control (clone MOPC-21)	BD Biosciences	Cat# 551436 RRID:AB_394195
PE rat IgG2a $\kappa$ Isotype Control (clone R35-95)	BD Biosciences	Cat# 553930 RRID:AB_479719
Anti-TATA binding protein	Abcam	Cat# ab51841 RRID:AB_945758
Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP	Thermo Fisher	Cat# 32430 RRID:AB_1185566
Chemicals, peptides, and recombinant proteins		
Brefeldin A Solution (1000x)	eBioscience	Cat# 00-4506-51
TPCA-1 STAT3 and IKK-2 inhibitor	MedChemExpress	Cat# HY-10074
AS1517499 STAT6 inhibitor	MedChemExpress	Cat# HY-100614
CellTracker Orange CMTMR Dye	Thermo Fisher	Cat# C2927
CellTracker Deep Red Dye	Thermo Fisher	Cat# C34565
CellTrace CFSE Cell Proliferation Kit	Thermo Fisher	Cat# C34554
LIVE/DEAD™ Fixable Far Red Dead Cell Stain Kit	Thermo Fisher	Cat# L34973

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
Nile Blue Fluorescent Particles, 2.5–4.5 $\mu\text{m}$	BD Biosciences	Cat # 556270
CD14 MicroBeads, human, 2 mL	Miltenyi Biotec	Cat # 130–050-201
MiniMACS Separation columns, type MS	Miltenyi Biotec	Cat # 130–042-201
Lymphoprep	Axis Shield Poc As	Cat# 1115754
Direct-zol RNA Miniprep kit	Zymo Research	Cat# R2052
Deposited data		
<a href="https://www.ebi.ac.uk/pride/login">https://www.ebi.ac.uk/pride/login</a>		N/A
MS proteomics	ProteomeXchange Consortium	PXD032360
Experimental models: Cell lines		
Human foreskin fibroblasts HFF-1	American Type Culture Collection	Cat# SCRC-1041 RRID:CVCL_3285
RAW 264.7	American Type Culture Collection	Cat# ATCC TIB-71 RRID:CVCL_0493
THP-1	American Type Culture Collection	Cat# ATCC TIB-202 RRID:CVCL_0006
Experimental models: Organisms/strains		
Mouse C57BL/6NCrl (wild-type)	Charles River Laboratories	Strain code 027 RRID:IMSR_CRL:027
Mouse B6.129P2(C)- <i>Ccr7<sup>m1Rfor</sup>/J</i> ( <i>CCR7<sup>-/-</sup></i> )	Jackson Laboratory	JAX stock #006621 RRID:IMSR_JAX:006621
<i>T. gondii</i> GT1 F3	American Type Culture Collection	Cat# 50838
<i>T. gondii</i> ME49-PTG GFPS65T	(Kim et al., 2001)	N/A
<i>T. gondii</i> ME49 RFP	(Wang et al., 2019a)	N/A
<i>T. gondii</i> RH-LDM GFPS65T	(Kim et al., 2001) (Barragan and Sibley, 2002)	N/A
<i>T. gondii</i> RH88 (RH-ERP)	American Type Culture Collection	Cat# 50838
<i>T. gondii</i> RH HPT	(Jensen et al., 2011)	N/A
<i>T. gondii</i> RH HPT rop16	(Jensen et al., 2011)	N/A
<i>T. gondii</i> RH1–1 cLuc GFP	(Boyle et al., 2007)	N/A
<i>T. gondii</i> RH myr1 cLuc GFP	(Wang et al., 2020)	N/A
<i>T. gondii</i> RH rop17 cLuc GFP	(Wang et al., 2019a)	N/A
<i>T. gondii</i> RH gra45 cLuc GFP	(Wang et al., 2020)	N/A
<i>T. gondii</i> RH Tgwip GFP	(Sangare et al., 2019)	N/A
<i>T. gondii</i> RH ku80	(Fox et al., 2009)	N/A
<i>T. gondii</i> RH ku80 gra24	(Braun et al., 2013)	N/A
<i>T. gondii</i> RH ku80 gra28	This manuscript	N/A
<i>T. gondii</i> RH ku80 gra28+GRA28	This manuscript	N/A
<i>T. gondii</i> Pru ku80	(Braun et al., 2019)	N/A
<i>T. gondii</i> Pru ku80 gra28	This manuscript	N/A
<i>T. gondii</i> Pru ku80 gra28+GRA28	This manuscript	N/A
Oligonucleotides		
See Table S3		N/A

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
ImageJ	(Schneider et al., 2012)	<a href="https://imagej.nih.gov/ij/">https://imagej.nih.gov/ij/</a>
R version 4.0.2	R Foundation for Statistical Computing	<a href="https://www.R-project.org/">https://www.R-project.org/</a>
RStudio version 1.3.1073	RStudio	<a href="https://www.rstudio.com">https://www.rstudio.com</a>
GraphPad Prism 7.0 / 8.0.2	GraphPad Software	<a href="http://www.graphpad.com">http://www.graphpad.com</a>
Chemotaxis and Migration Tool V2.0	Ibidi	<a href="http://www.ibidi.com">http://www.ibidi.com</a>
FlowJo X 10.0.7r2	Flowjo LLC	<a href="http://www.flowjo.com">http://www.flowjo.com</a>

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript