A CRISPRi/a platform in human iPSC-derived microglia uncovers regulators of disease states

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Microglia are emerging as key drivers of neurological diseases. However, we lack a systematic understanding of the underlying mechanisms. Here, we present a screening platform to systematically elucidate functional consequences of genetic perturbations in human induced pluripotent stem cell-derived microglia. We developed an efficient 8-day protocol for the generation of microglia-like cells based on the inducible expression of six transcription factors. We established inducible CRISPR interference and activation in this system and conducted three screens targeting the ‘druggable genome’. These screens uncovered genes controlling microglia survival, activation and phagocytosis, including neurodegeneration-associated genes. A screen with single-cell RNA sequencing as the readout revealed that these microglia adopt a spectrum of states mirroring those observed in human brains and identified regulators of these states. A disease-associated state characterized by osteopontin (SPP1) expression was selectively depleted by colony-stimulating factor-1 (CSF1R) inhibition. Thus, our platform can systematically uncover regulators of microglial states, enabling their functional characterization and therapeutic targeting.

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neurons. By integrating inducible CRISPRi/a machinery into this cell line, we developed a genetic screening system that enables robust knockdown and overexpression of endogenous genes in human microglia. Using this platform, we conducted pooled CRISPRi and CRISPRa screens for modifiers of survival, phagocytosis and inflammatory activation, which uncovered microglia-specific genes controlling these phenotypes. A screen with single-cell RNA sequencing (scRNA-seq) as the readout revealed that these microglia adopt a spectrum of states mirroring those observed in human brains, and pinpointed regulators of specific states, which can enable the functional characterization and therapeutic targeting of these states.

Results

Rapid and scalable production of microglia-like cells. We set out to create a fast, robust and scalable differentiation protocol to differentiate iPSCs to microglia-like cells for use in CRISPR screens. To this end, we developed a strategy based on direct cell fate conversion by overexpression of transcription factors. Based on transcriptomic and developmental data\textsuperscript{20–22}, we selected six transcription factors highly expressed in human microglia: Hematopoietic Transcription Factor PU.1, MAF BZIP Transcription Factor B (MAFB), CCAAT Enhancer Binding Protein Alpha (CEBPα), CCAAT Enhancer Binding Protein Beta (CEBPβ), Interferon Regulatory Factor 5 (IRF5) and Interferon Regulatory Factor 8 (IRF8). We engineered an iPSC line with two integrated cassettes for the doxycycline-inducible expression of three transcription factors each in the Citrate Lyase Beta Like (CLYBL) and Adeno-Associated Virus Integration Site 1 (AAVS1) safe-harbor loci (Fig. 1a).

We established a simple three-step protocol to differentiate these iPSCs into microglia-like cells, which we will refer to as iTF-Microglia, in only 8 days (Fig. 1b). After doxycycline induction of transcription factor expression on day 0, medium was supplemented with cytokines granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-34 (IL-34) on day 2 to promote differentiation and survival. On day 4, the medium was additionally supplemented with the cytokines macrophage colony-stimulating factor (M-CSF) and transforming growth factor β (TGF-β). iTF-Microglia reached a fully ramified morphology on day 8 and maintained excellent viability for at least another 8 days (Fig. 1b).

We generally have continued doxycycline supplementation beyond day 8; however, this is not necessary for survival (Extended Data Fig. 1a,b). We confirmed robust inducible expression of the transgenic transcription factors (Fig. 1c).

The canonical microglia markers GPR34 and IBA1 were expressed in the iTF-Microglia at day 8 of differentiation (Fig. 1d). RNA sequencing (RNA-seq) confirmed downregulation of iPSC markers and induction of microglia markers in iTF-Microglia at day 9 and day 15 (Fig. 1e and Supplementary Table 1). Some markers, such as P2RY12, CSF1R, CYBB and CD14, slightly increased their expression from day 9 to day 15, indicating further incremental maturation from day 9 to day 15. While the transcriptomic signature of our microglia was distinct from primary human microglia, it was comparable to that of several other iPSC-derived microglia protocols (Fig. 1f and Extended Data Fig. 1c).

In conclusion, our results indicate robust expression of microglia markers in iTF-Microglia. Importantly, our differentiation strategy is compatible with large-scale pooled sgRNA screens, whereas classical protocols create population bottlenecks (Extended Data Fig. 1d).

Functional characterization of iTF-Microglia. Next, we asked whether iTF-Microglia recapitulated cellular functions of human microglia. iTF-Microglia robustly phagocytosed fluorescent beads (Extended Data Fig. 2a) and rat synaptosomes (Fig. 2a,b and Extended Data Fig. 2b). As expected, phagocytosis could be attenuated by the actin polymerization inhibitor Cytochalasin D, since phagocytosis depends on actin dynamics (Fig. 2a,b and Extended Data Fig. 2c).

To test the inflammatory response of iTF-Microglia to bacterial-derived lipopolysaccharide (LPS), we stimulated them with LPS for 24 h. LPS-stimulated iTF-Microglia were less ramified, and instead displayed the ameboid morphology characteristic of activated microglia (Fig. 2e and Extended Data Fig. 2d). Transcriptomic changes in response to LPS were substantially overlapping with those observed in iPSC-derived microglia we generated following an alternative, previously published\textsuperscript{18} protocol (Extended Data Fig. 2e and Supplementary Table 2). Transcriptomic changes in response to LPS were substantially overlapping with those observed in iPSC-derived microglia we generated following an alternative, previously published\textsuperscript{18} protocol (Extended Data Fig. 2e and Supplementary Table 2).

To examine cytokine secretion of iTF-Microglia, we measured the abundance of 36 cytokines secreted in standard culture conditions or following LPS stimulation. Control buffer-treated iTF-Microglia secreted most cytokines at low levels, but higher levels of CCL2 and CXCL1, suggesting the presence of activated cells under control conditions (Fig. 2e), consistent with previous reports suggesting that even primary microglia become partially activated when cultured\textsuperscript{23}. When stimulated with LPS, levels of most secreted cytokines increased, most prominently IL-6 with a 14-fold increase and IL-8 and CXCL10, both increased over fourfold (Fig. 2e).

We were able to coculture iTF-Microglia with iPSC-derived glutamatergic neurons (iNeurons) in medium optimized for survival and functionality of both cell types (Methods). Remarkably, cocultured iTF-Microglia displayed a pronounced ramified morphology (Fig. 2f). In conclusion, we show that iTF-Microglia effectively

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Fig. 1 | Rapid differentiation of iPSCs into microglia-like cells (iTF-Microglia) by transcription factor induction. a, Strategy for stable integration of six transcription factors in AAVS1 and CLYBL loci by TALEN-mediated integration: The doxycycline-inducible reverse transcriptional activator (rtTA3G) is driven by the constitutive CAG promoter. Human MAFB, CEBPα and IRF8 are driven by the tet response element (TRE3G) in the AAVS1 locus. Human PU.1, CEBPβ and IRF5 are driven by TRE3G in the CLYBL locus. All transcription factors are separated from each other via T2A ribosome skipping sequences. b, Overview of the differentiation process for generating iTF-Microglia. Top, timeline with media, cytokines and doxycycline (Dox); bottom, representative phase-contrast images of cells on the indicated days. Scale bar, 100 μm. c, Expression of six inducible transcription factors during iTF-Microglia differentiation. Transcript abundance (transcripts per million, TPM) of MAFB, CEBPα, IRF8 cassette and the PU.1, CEBPβ, IRF5 cassette at day 0, day 9 and day 15 of differentiation. Mean ± s.d., n = 3 biological replicates, P values from two-tailed Student’s t-test. d, Representative immunofluorescence micrographs of iTF-Microglia on day 8 of differentiation stained for microglia markers GPR34 and IBA1. Nuclei were labeled by Hoechst 33342. Scale bar, 100 μm. e, Expression of iPSC and microglia marker genes in iPSCs and derived iTF-Microglia on day 9 and day 15 of differentiation. The heatmap displays normalized and gene-centered TPM counts for selected genes (rows) for three biological replicates of timepoints (columns). iTF-Microglia express microglia homeostatic markers and activation markers, while losing their expression of iPSC markers. Asterisks highlight microglia-selective markers. f, Principal component analysis (PCA) on the expression of microglia marker genes of iTF-Microglia, human adult ex vivo microglia\textsuperscript{8}, fetal and adult microglia\textsuperscript{10}, human myeloid cells\textsuperscript{16}, other iPSC-microglia (iMG) / iPSC-microglia-like cells (iMGL)\textsuperscript{13,18} and iPSCs (this study and ref. \textsuperscript{18}). Each dot reflects an independent biological sample. Colors represent the different cell types.
a

AAVS1 locus
Exon 1
Exon 2
Exon 3
AAVS1 cut site

CLYBL locus
Exon 2
Exon 3
CLYBL cut site

Inducible
MAFB, CEBBPα, IRF8

Inducible
PU.1, CEBBPβ, IRF5

b

Stem flex
Essential 8
Advanced DMEM / F12
Media

Supplements
Cytokines

Day 0
Day 2
Day 4
Day 8
Day 15

P = 9.9 × 10–8
P = 1.5 × 10–5
P = 0.0001
P = 0.0002

100
1,000

Transcript abundance
( TPM )

P<0.0001

f

PC-1: 59% variance
PC-2: 12% variance

Primary human microglia (Guttikonda et al.60)
Human brain myeloid cells (Zhang et al.20)
In-house iMG (Brownjohn et al.18)
IMGL (Abud et al.13)
Adult microglia (Abud et al.13)
Fetal microglia (Abud et al.13)
IMG (Brownjohn et al.18)
IMGL (Chen et al.61)
N1/N2 IMGL (Chen et al.61)
In-house iPSCs
iTF-iPSCs
N2-hiPSCs (Chen et al.61)
iTF-Microglia Day 8
iTF-Microglia Day 15
iTF-Microglia Day 1

iPSC markers

MG markers homeostasis and activation

Relative transcript levels

iTF-iPSCs
iTF-Microglia

Day 9
Day 15

1151
Fig. 2 | Functional characterization of iTF-Microglia. a, Phagocytosis of pHrodo-Red-labeled rat brain-derived synaptosomes by iTF-Microglia. Representative images at 0 h and 12 h after synaptosome addition are shown. Treatment with 5 μM actin polymerization inhibitor Cytochalasin D decreases phagocytosis. Scale bar, 100 μm.

b, Phagocytosis of pHrodo-labeled rat brain-derived synaptosomes with or without Cytochalasin D treatment was quantified by flow cytometry at 0.5 h, 1.5 h and 2.5 h after synaptosome addition (mean ± s.d., n = 3 biological replicates; P values from two-tailed Student’s t-test).

c, Morphological changes of iTF-Microglia after LPS treatment are visualized by fluorescence microscopy. Samples were treated for 24 h with 100 ng ml⁻¹ LPS or buffer control and fixed samples were stained with Alexa Fluor 488-phalloidin for F-actin (green) and with Hoechst 33342 for nuclei (blue). Scale bar, 100 μm.

d, Transcriptomic changes caused by 50 ng ml⁻¹ LPS treatment in day 15 iTF-Microglia (n = 3 biological replicates). DEGs (P_adj < 0.05, two-tailed Student’s t-test) are labeled in black (increase). Other colors label genes associated with specific pathways that are discussed in the main text. e, Cytokines secreted by iTF-Microglia. Analysis of cytokine array signal (integrated density of dot blots) from supernatants of cultures treated with LPS or buffer control (mean ± s.d., n = 6 biological replicates; P values from two-tailed Student’s t-test). *GM-CSF is a component of the culture medium.

f, Coculture with iPSC-derived excitatory neurons promotes ramified morphology of iTF-Microglia. Representative fluorescence micrographs at low and high magnification of day 9 iTF-Microglia after 24 h in coculture. iTF-Microglia express membrane-localized Lck-mNeonGreen (green). Neurons are stained for the pre-synaptic marker synaptophysin (magenta). Nuclei are stained with Hoechst 33342 (blue). Scale bars, 100 μm. AU, arbitrary units; NS, not significant.

**Legend**: Brightfield pHrodo-synaptosomes.
Fig. 3 | Gene knockdown and overexpression by CRISPRi and CRISPRa in iTF-Microglia. a, Strategies for constitutive and inducible CRISPRi/CRISPRa in iTF-Microglia. Top, for constitutive CRISPRi, a dCas9-BFP-KRAB construct (catalytically dead Cas9 (dCas9) fused to BFP and the KRAB transcriptional repressor domain) is expressed from the constitutive CAG promotor integrated into the CLYBL safe-harbor locus. Middle, for inducible CRISPRi, dCas9-BFP-KRAB is tagged with ecDHFR degrons. Bottom, for inducible CRISPRa, CAG promotor-driven ecDHFR-dCas9-VPH was stably integrated into the CLYBL locus. VPH, activator domains containing 4× repeats of VP45, P65 and HSF1. Addition of TMP stabilizes the inducible CRISPRi/a machineries.

b, c, Functional validation of constitutive (b) or inducible (c) CRISPRi activity via flow cytometry of TFRC surface protein level stained iTF-Microglia expressing a TFRC-targeting sgRNA or an NTC sgRNA at different days of differentiation (mean ± s.d., n = 3 biological replicates; P values from two-tailed Student’s t-test). c, TMP was added to induce CRISPRi activity where indicated. d, Functional validation of inducible CRISPRi activity via TFRC immunofluorescence (IF) microscopy on day 8. Top row, NTC sgRNA. Bottom row, sgRNA targeting TFRC. TFRC, red; F-actin, green; nuclei, blue. Scale bar, 100 μm. e, Functional validation of inducible CRISPRa activity via flow cytometry of CXCR4 surface protein level staining in iTF-Microglia expressing CXCR4 sgRNA or NTC sgRNA (mean ± s.d., n = 3 biological replicates; P values from two-tailed Student’s t-test). TMP was added to induce CRISPRa activity where indicated.
phagocytose synaptosomes, respond to LPS and can be cocultured with iPSC-derived neurons.

**Gene perturbation by CRISPRi and CRISPRa in iTF-Microglia.**

Next, we established CRISPRi and CRISPRa in iTF-Microglia to enable robust knockdown and overexpression of endogenous genes, as well as large-scale loss- and gain-of-function genetic screens. Following the strategy we previously established in human iPSC-derived neurons11,12, we stably integrated constitutive CRISPRi machinery, inducible CRISPRi machinery or inducible CRISPRa machinery into safe-harbor loci of iPSCs also engineered with the inducible microgical transcription factors (Fig. 3a). In the constitutive CRISPRi line, the expression cassette contains a CAG promoter-driven dCas9-2FP-KRAB. In the inducible CRISPRi cassette, this CRISPRi machinery is flanked on both the N and the C termini with dihydrofolate reductase (DHFR) degrons. In the absence of the small molecule trimethoprim (TMP), DHFR degrons cause proteosomal degradation of fused proteins. Addition of TMP stabilizes the degron-tagged CRISPRi machinery. The inducible CRISPRa machinery consists of a DHFR-dCas9-VPH construct, which is similarly stabilized in the presence of TMP. Inducible CRISPRi/a systems enable flexible timing of the onset of gene perturbation in cells already expressing sgRNAs. This feature is particularly important for experiments in microglia: it enables lentiviral delivery of sgRNAs to occur in iPSCs, which are much more amenable to lentiviral infection than microglia, without prematurely affecting genes that may be relevant for differentiation. We confirmed a normal karyotype for the resulting monoclonal cell lines (Extended Data Fig. 3).

To validate CRISPRi activity, we transduced iPSCs with a lentiviral construct expressing an sgRNA targeting the transferrin receptor gene (TFRC) or a nontargeting control (NTC) sgRNA. In cells expressing the constitutive CRISPRi machinery, knockdown of TFRC was robust in iPSCs and iTF-Microglia both on the protein level (Fig. 3b and Extended Data Fig. 4a) and on the messenger RNA level (Extended Data Fig. 4c,e). In cells expressing the inducible CRISPRi machinery, TFRC knockdown was completely dependent on the presence of TMP, and effective on the mRNA and protein levels, albeit with reduced knockdown compared with the constitutive CRISPRi system (Fig. 3c,d and Extended Data Fig. 4b.d.f). For additional target genes we tested, we found examples of excellent knockdown (around 80%) with both the constitutive and the inducible systems for INPP5D (Extended Data Fig. 4g.h), but also an example of a gene (PICALM) that was effectively knocked down by 90% with the constitutive CRISPRi (Extended Data Fig. 4i), but not by inducible CRISPRi (Extended Data Fig. 4j). Despite these limitations of our current inducible CRISPRi system, we decided to use it for the studies presented in this study, since it enabled us to induce CRISPRi knockdown only upon differentiation, rather than in the iPSC state, thus reducing the likelihood of recovering phenotypes due to effects in iPSCs or on differentiation itself.

Next, we validated the functionality of the inducible CRISPRa machinery by testing the induction of the endogenous gene CXCR4. We observed a robust and tightly inducible increase of CXCR4 levels in iPSCs and iTF-Microglia on the mRNA level (Extended Data Fig. 4l,m) and the protein level (Fig. 3e and Extended Data Fig. 4k).

**CRISPRi screen for microglial survival and proliferation.**

Our first application of the inducible CRISPRi iTF-Microglia platform was to identify modifiers of microglia survival and proliferation in a pooled genetic screen (Fig. 4a). First, we transduced the iPSCs with our next-generation lentiviral CRISPRi sgRNA library targeting the 'druggable genome'24. This library consists of sgRNAs targeting 2,325 genes encoding kinases, phosphatases and other classes of druggable proteins, with five sgRNAs per gene and 500 NTC sgRNAs. After library transduction, iPSCs were differentiated into iTF-Microglia by addition of doxycline and TMP was added to induce CRISPRi activity. iTF-Microglia were collected before differentiation (day 0) and on day 15 post-induction. Frequencies of cells expressing each sgRNA were determined by next-generation sequencing to uncover genes for which sgRNAs showed significant changes in frequency, indicating a survival or proliferation phenotype (Fig. 4a and Supplementary Table 3).

We compared the results from the iTF-Microglia survival screen with our previously published11 CRISPRi survival screens in iPSC-derived neurons (Fig. 4b) and iPSCs (Extended Data Fig. 5a). We found that genes affecting microglial survival, neuronal survival and iPSC survival were largely distinct. Knockdown of cholesterol biogenesis enzymes and V-ATPase subunits drastically reduced neuronal but not microglial survival (Fig. 4b). Conversely, knockdown of members of the colony stimulating factor (CSF) receptor family (CSF1R, CSF2RB, CSF2RA) strongly reduced the survival of microglia but not neurons (Fig. 4b) or iPSCs (Extended Data Fig. 5a). In contrast, genes in the case of CDK8 knockdown even resulted in a very slight decrease in survival (Extended Data Fig. 5f). By contrast, knockdown of CSFR1 in day 8 iTF-Microglia reproduced the phenotype observed in the initial screen (Extended Data Fig. 5f).

**CRISPRi screen for modifiers of microglial activation.** In a second screen, we aimed to identify modifiers of inflammatory activation of microglia. For this screen, we chose cell-surface levels of cluster of differentiation 38 (CD38) as a readout for microglial activation. CD38, also known as cyclic ADP ribose hydrolase, is induced by LPS treatment in primary microglia32 and in our iTF-Microglia (Extended Data Figs. 2e and 5g). CD38 plays several roles in microglial activation, including in the secretion of proinflammatory cytokines33 and in activation-mediated cell death34. Altogether, these data suggest that CD38 is both a marker and an important effector for the activation of microglia and is therefore a suitable marker for the screening of inflammation modifiers.

The screen for modifiers of microglial activation was conducted as shown in Fig. 4d. Briefly, iPSCs expressing the inducible CRISPRi machinery were transduced with the pooled sgRNA library described above. The cells were then differentiated into iTF-Microglia, stained for cell-surface CD38 using a fluorescently tagged antibody and subjected to fluorescence-activated cell sorting (FACS) into CD38low and CD38high populations. Frequencies of cells expressing each sgRNA were quantified in these populations using next-generation sequencing.
Fig. 4 | Identification of modifiers of survival and inflammation by CRISPRi screens. a, Strategy. b, Comparison of Gene Scores from CRISPRi survival screens in iTF-Microglia (this study) versus iPSC-derived neurons. Each dot represents a gene; genes are color-coded by pathways. c, Validation of the phenotype of CSF1R knockdown. iTF-Microglia transduced with CSF1R-targeting or NTC sgRNAs were imaged on different days after differentiation, and live cells were quantified based on staining with Hoechst 33342. Data are shown as mean ± s.d., n = 3 wells per group; 7 fields were imaged for each well; P values from two-tailed Student’s t-test. d, Strategy for a CRISPRi screen to identify modifiers of the expression of CD38, a marker of reactive microglia. iPSCs expressing the inducible CRISPRi construct were transduced with the druggable genome sgRNA library. On day 0, doxycycline and cytokines were added to induce microglial differentiation, and TMP was added to induce CRISPRi activity. On day 8, iTF-Microglia were stained for cell-surface levels of CD38 and sorted by FACS into populations with low (bottom 30%) and high (top 30%) CD38 levels. Frequencies of iTF-Microglia expressing a given sgRNA were determined in each population by next-generation sequencing (NGS). e, Volcano plot indicating knockdown phenotype and statistical significance (two-sided Mann–Whitney U-test) for genes targeted in the CD38 level screen. Dashed line indicates the cut-off for hit genes (false discovery rate (FDR) = 0.1). Hit genes are shown in blue (knockdown decreases CD38 level) or red (knockdown increases CD38 level), nonhit genes are shown in orange and ‘quasi-genes’ generated from random samples of NTC sgRNAs are shown in gray. Hits of interest are labeled. f, Validation of the phenotype of MED1 and CDK12 knockdown. CD38 cell-surface levels measured by flow cytometry of day 8 iTF-Microglia targeting MED1, CDK12 compared with NTC sgRNA. Means ± s.d., n = 3 biological replicates; P values from two-tailed Student’s t-test. i1, i2 refer to independent CRISPRi sgRNAs targeting the indicated genes.
This CRISPRi screen identified several genes regulating cell-surface levels of CD38 (Supplementary Table 3). Knockdown of two transcriptional regulators, CDK12 and MED1, significantly increased CD38 surface levels in the screen (Fig. 4c) and in validation experiments (Fig. 4f). CDK12 is known to be involved not only in cell cycle progression but also in TNFα and noncanonical NF-kB signaling. While these previous reports may suggest a proinflammatory role of CDK12, our findings suggest that the role of CDK12 may be more nuanced or context-dependent, and we designated it for further investigation (see below). Another class of hits whose knockdown increased CD38 levels were members of the mitochondrial Complex I (NADH:ubiquinone oxidoreductase), NDUF4A8 and NDUF55 (Fig. 4e). Knockdown of components of this complex have previously been shown to promote an inflammatory state in macrophages45, validating our findings.

Taken together, our large-scale CRISPRi screens in iTF-Microglia uncovered microglia-specific survival modifiers and modulators of inflammatory activation, demonstrating the ability of the iTF-Microglia screening platform to identify microglia-specific biology.

Modifiers of synaptosome phagocytosis by microglia. Microglial phagocytosis is central to brain homeostasis from development through aging35. Dysfunctional or dysregulated phagocytosis has been implicated in neurodegenerative and psychiatric diseases46-49. To uncover regulators of microglial phagocytosis, we conducted parallel CRISPRi and CRISPRa screens in iTF-Microglia transduced with sgRNA libraries targeting the ‘druggable genome’. After 1.5 h of incubation with pHrodo-Red-labeled synaptosomes isolated from rat brains, iTF-Microglia were sorted via FACS based on the pHrodo-Red fluorescence signal (Fig. 5a), and screens were analyzed as described for the CD38 FACS-based screen.

There was little overlap between CRISPRi and CRISPRa hits (Extended Data Fig. 6a and Supplementary Table 3), confirming our previous findings from screens in diverse biological contexts that overexpression and knockdown screens can provide complementary insights41,46. A prominent exception was the actin-binding protein PFN1, coding mutations in which cause amyotrophic lateral sclerosis (ALS)46. PFN1 had opposing phenotypes on synaptosome phagocytosis upon CRISPRi repression and CRISPRa induction (Fig. 5b). Unexpectedly, knockdown of CSF1R increased phagocytosis (Fig. 5b), even though, as we had previously found (Fig. 4b), its knockdown decreased iTF-Microglia survival. Another remarkable hit was the AD risk factor INPP5D, knockdown of which slightly increased phagocytosis. Overexpression of CD209, a C-type lectin receptor present on the surface of macrophages and dendritic cells, greatly increased synaptosome phagocytosis (Fig. 5b). We validated these phenotypes from the primary CRISPRi screen individually in iTF-Microglia (Extended Data Fig. 6b) and in iPSC-derived microglia generated an alternative protocol51 (Extended Data Fig. 6c).

We further investigated the CRISPRa hits PFN1 and CD209. We validated upregulation of both genes by quantitative PCR (qPCR) (Extended Data Fig. 6e). Pattern-recognition receptor CD209 has previously been shown to regulate phagocytic capacity in macrophages45. To investigate substrate specificity of CD209, we monitored phagocytosis of two different substrates, pHrodo-Red-labeled synaptosomes and yellow-green (YG)-fluorescently labeled beads, either alone or in combination (Fig. 5c). Overexpression of CD209 increased phagocytosis of synaptosomes, but had a much smaller effect on bead phagocytosis (Fig. 5c), suggesting substrate specificity. However, when challenging iTF-Microglia with a mixture of beads and synaptosomes, bead phagocytosis was robustly increased, suggesting that synaptosomes might stimulate general phagocytosis via CD209.

PFN1 overexpression increased levels of F-actin in iTF-Microglia (Fig. 5d), consistent with previous finding that moderate overexpression of PFN1 induces long stress fiber-like actin cables49. This process could disturb orchestrated actin polymerization at the membrane and thus decrease phagocytosis. In addition to direct effects on the actin cytoskeleton, PFN1 knockdown has also been reported to result in anti-inflammatory changes52. Indeed, we observed transcriptional changes in immune-related genes and AD risk genes upon PFN1 overexpression in iTF-Microglia (Fig. 5e and Supplementary Table 4).

In conclusion, our complementary CRISPRi and CRISPRa screens identified known as well as novel phagocytosis modulators in microglia, which were validated in iPSC-derived microglia generated using an alternative protocol.

Distinct transcriptional states of iTF-Microglia. Several genes had CRISPRi phenotypes in more than one of the large-scale screens that we conducted (Extended Data Fig. 6f and Supplementary Table 3). We therefore hypothesized that some hit genes were not dedicated factors required for specific microglial processes, but rather regulators of distinct functional states. To test this hypothesis and gain more detailed insights into the mechanisms by which genes affect microglial functions, we selected 39 hit genes of interest, most of which had phenotypes in more than one of the large-scale primary screens (Extended Data Fig. 6f and Supplementary Table 3), for characterization in a CRISPR droplet sequencing (CROP-seq) screen, which couples CRISPRi perturbation to scRNA-seq. We introduced an sgRNA library targeting these genes (Supplementary Table 5) into iPSCs, induced iTF-Microglia differentiation and CRISPRi activity, and performed scRNA-seq of 58,302 iTF-Microglia on day 8 (Fig. 6a and Supplementary Table 6).

Unsupervised clustering and Uniform Manifold Approximation and Projection (UMAP) dimensional reduction of the single-cell transcriptomes uncovered distinct clusters (Fig. 6b). In one cluster, a high proportion of transcripts mapped to mitochondrial transcripts, suggesting damaged or dying cells; this cluster was...
removed from downstream analysis (Extended Data Fig. 7a). Two clusters exclusively contained cells expressing sgRNAs targeting CDK8 or TGFBR2 (Fig. 6b and Extended Data Fig. 7b). These cells expressed high levels of the pluripotency marker SOX2, but low levels of the microglia marker CSF1R (Extended Data Fig. 7c). Together with our previous experiments showing reduced IBA1 levels for iTF-Microglia targeting CDK8 and TGFBR2 (Extended Data Fig. 5b,c), these findings suggested disrupted microglial
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and late-response microglia in CK-p25 mouse models of neuro-

human brains. In the integrated UMAP, iTF-Microglia (left) with high biological replicates; values from two-tailed Student’s t-test (right) form a cluster (dashed outline). In brains from patients with AD, a higher fraction of microglia is in the SPP1-high cluster compared with control brains (data from Olah et al.; P value from two-sided Fisher’s exact test).

CROP-seq uncovers regulators of microglial cell states. We next identified the differentially expressed genes (DEGs) caused by CRISPRi knockdown of each gene targeted in the CROP-seq screen (Extended Data Fig. 8 and Supplementary Table 8). As expected, knockdown of functionally related genes resulted in shared DEG signatures. For example, knockdown of CSF1R, CSF2RA and CSF2RB resulted in an upregulation of genes encoding the major histocompatibility complex as well as CD36, CD74 and CD68 (Extended Data Fig. 8 and Supplementary Table 8), which are markers of phagocytic microglia and could explain the increased phagocytic capacity we observed in response to CSF1R knockdown (Fig. 5b and Extended Data Fig. 6b).

Given the surprising heterogeneity of iTF-Microglia, we investigated if CRISPRi knockdown of specific genes could control microglial cell states. Indeed, cells containing sgRNAs targeting genes such as CSF1R, CDK12 and MAPK14 were enriched or depleted from specific clusters (Fig. 7a), and more generally, knockdown of many genes specifically affected the frequency of cell states (Fig. 7b, Extended Data Fig. 9a and Supplementary Table 9).

Knockdown of CDK12 shifted cells into cluster 9 (CCL13+ chemokine) (Fig. 7a–c). To validate this phenotype, we used a flow cytometry approach in which secretion of CCL13 was inhibited with the transport inhibitor GolgiPlug. CCL13 levels were increased over twofold with knockdown of CDK12 (Fig. 7d), confirming our screen results. Next, we asked if the shift into cluster 9 might also have functional consequences for the iTF-Microglia. We measured differentiation. We removed those clusters from further analysis and retained the remaining cluster, which was characterized by high levels of CSF1R expression (Extended Data Fig. 7c). Importantly, 92.4% of cells expressing NTC sgRNAs were part of the cluster with high levels of CSF1R expression, confirming the high efficiency of our microglial differentiation protocol for unperturbed cells.

Unsupervised clustering and UMAP dimension reduction of the remaining 19,834 iTF-Microglia revealed nine transcriptionally distinct clusters (Fig. 6c and Extended Data Fig. 7d). Microglia heterogeneity in response to different environmental conditions in the brain has been extensively studied, but we were surprised to observe a wealth of distinct transcriptional states in the cultured iTF-Microglia. Importantly, NTC sgRNAs are represented in cells in every cluster (Extended Data Fig. 7d), suggesting the observed heterogeneity is an innate quality of the iTF-Microglia.

Principal component analysis identified two major biological axes broadly defining these states. The first principal component (PC1) corresponded to a polarized axis of inflammatory activation: starting from a central homeostatic state (cluster 6), one direction was defined by interferon-induced gene expression, whereas the other direction was defined by induction of chemokines (Fig. 6d and Extended Data Fig. 7e). The second principal component (PC2) captured markers of proliferation, mainly in cluster 7 (Fig. 6c and Extended Data Fig. 7f).

To further interpret each transcriptional microglial state, we performed differential gene expression analysis across the clusters (Supplementary Table 7) and named each cluster according to characteristic transcriptomic signatures (Fig. 6f).

Clusters 1 and 2 are both defined by high expression of interferon-induced genes and the complement gene C3. Cluster 1 is uniquely defined by high expression of chemokine CXCL10. Subsets of microglia characterized by upregulation of interferon response genes have been described in mouse models of neurodegeneration.

Cluster 3 is defined by the high expression of SPP1 (Fig. 6g), which encodes osteopontin. Importantly, SPP1 is upregulated in several disease-associated microglial states, including disease-associated microglia and activated response microglia in AD mouse models, and late-response microglia in CK-p25 mouse models of neurodegeneration. SPP1-positive microglial states are also enriched in patients with multiple sclerosis and mouse models characterized by expression of high levels of chemokines such as CCL2 and CCL3. Cluster 3 is defined uniquely by high expression of CCL13 (Fig. 6g). Such chemokine signatures have recently been found to be a hallmark of human microglia not observed in mice.

Taken together, scRNA-seq revealed that many important features of microglia diversity observed in human brains and in disease states are recapitulated in our iTF-Microglia in vitro model.

Fig. 6 | SCRNA-seq reveals distinct and disease-related microglia subclusters. a, Strategy for the CROP-seq screen. iPSCs expressing inducible CRISPRi machinery were transduced with a pooled library of 81 sgRNAs and CROP-seq vector pMK1334. iPSCs are differentiated to iTF-Microglia and subjected to scRNA-seq to obtain single-cell transcriptomes and to identify expressed sgRNAs. b, UMAP of the 28,905 cells in the post-quality control CROP-seq dataset. Cells are colored by sgRNA (CDK8, red; TGFBR2, orange) and cells with a high percentage of mitochondrial transcripts (blue). Microglia are labeled in green. Each dot represents a cell. c, UMAP depicting the 9 different clusters within the 19,834 microglia. Each dot represents a cell. The cells are color-coded based on their cluster membership. d, Ridge plots depicting iTF-Microglia clusters along PC1 (d) and PC2 (e). PC1 spans inflammation status (interferon activated–homeostatic–chemokine activated) while PC2 spans proliferation status. e, Heatmap of iTF-Microglia clusters along PC1 (d) and PC2 (e), are color-coded based on their cluster membership. Orange) and cells with a high percentage of mitochondrial transcripts (blue). Microglia are labeled in green. Each dot represents a cell. f, UMAP depicting the 9 different clusters within the 19,834 microglia. Each dot represents a cell. The cells are color-coded based on their cluster membership. g, UMAP of distinct marker expression of CCL13 (left) and SPP1 (right). Cluster 1 is a marker for cluster 9 and SPP1 is a marker for cluster 3. Cells are colored by the expression levels of the indicated gene. h, Phagocytic activity of iTF-Microglia in different states. Flow cytometry measurement of phagocytosis of pHrodo-Red-labeled synaptosomes (left), phagocytosis in CCL13-high and CCL13-low iTF-Microglia; right, phagocytosis in SPP1-high and SPP1-low iTF-Microglia). Values represent mean ± s.d. of n = 3 biological replicates; P values from two-tailed Student’s t-test. i, Integration of single-cell transcriptomes of iTF-Microglia and microglia from post-mortem human brains. In the integrated UMAP, iTF-Microglia (left) with high SPP1 expression and human brain-derived microglia with high SPP1 expression (right) form a cluster (dashed outline). j, In brains from patients with AD, a higher fraction of microglia is in the SPP1-high cluster compared with control brains (data from Olah et al.; P value from two-sided Fisher’s exact test).
iTF-Microglia Human microglia

**Figure a**: Transduce with sgRNA library

**Figure b**: Microglia differentiation

**Figure c**: Cluster analysis

**Figure d**: Gene expression analysis

**Figure e**: Transcriptome analysis

**Figure f**: Gene expression heatmap

**Figure g**: Relative expression analysis

**Figure h**: Synaptosome phagocytosis analysis

**Figure i**: Human microglia analysis

**Figure j**: Number of microglia in cluster analysis
Fig. 7 | CROP-seq reveals changes in cluster occupancy induced by gene knockdown. a, UMAP depicts cells with sgRNAs targeting MAPK14 (blue), CSF1R (red) and CDK12 (green), which are enriched in clusters 3, 6 and 9, respectively. Insert shows cluster 3. b, Changes in cluster distribution after CRISPRi knockdown of targeted genes in iTF-Microglia. Heatmap with hierarchical clustering of 37 target genes and NTC and their distribution in clusters 1–9. c, Proportion of cells in cluster 9 (CCL13+) expressing either sgRNAs targeting CDK12 or NTC. d, Validation of increased CCL13 in iTF-Microglia expressing sgRNAs targeting CDK12 compared with NTC. CCL13 levels were measured via flow cytometry after 5 h of GolgiPlug treatment. e, Decreased synaptosome phagocytosis of iTF-Microglia expressing sgRNAs targeting CDK12 compared with NTC. Phagocytosis is further reduced in the CCL13-high population of cells expressing sgRNAs targeting CDK12. Phagocytosis was measured via flow cytometry with additional staining for CCL13. f, Proportion of cells in cluster 3 (SPP1+) expressing either sgRNAs targeting MAPK14 or CSF1R, or NTC. g, h, Functional validation of altered percentage of SPP1+ cells in iTF-Microglia expressing sgRNAs targeting MAPK14 (g) or CSF1R (h) compared with NTC. SPP1 was measured via flow cytometry after treating cells for 5 h with GolgiPlug. i, Survival of iTF-Microglia after 24-h treatment with various concentrations of MAPK14 inhibitor Skepinone-L quantified by CellTiter-Glo assay. Mean ± s.d. of n = 12 biological replicates, analyzed by one-way analysis of variance (ANOVA). j, Percentage of SPP1-positive cells after 100 nM Skepinone-L treatment for 24 h or 36 h. SPP1 was measured via flow cytometry after an additional 5 h of GolgiPlug treatment. k, Survival of iTF-Microglia after 24-h treatment with various concentrations of CSF1R inhibitor PLX3397 quantified by CellTiter-Glo assay. Mean ± s.d. of n = 6 biological replicates. l, Percentage of SPP1+ cells after 24 h of PLX3397 treatment measured via flow cytometry after an additional 5 h of GolgiPlug treatment. In panels d, e, g, h, j, and l, values represent mean ± s.d. of n = 3 biological replicates; in all panels except i, P values are from the two-tailed Student’s t-test. i, i2 refer to independent CRISPRi sgRNAs targeting the indicated genes.
synaptosome phagocytosis in CCL13high cells (representative of the cells in cluster 9) and CCL13low cells (representative of other clusters) in CDK12 knockdown iTF-Microglia. As observed already in our phagocytosis screen (Fig. 5b), knockdown of CDK12 decreased synaptosome phagocytosis, in both the CCL13low and CCL13high populations (Fig. 7e). CDK12 knockdown caused transcriptional downregulation of phosphatidylserine recognition receptors in both CCL13low and CCL13high cells, which may contribute to the decreased phagocytic activity (Extended Data Fig. 9b). Phagocytosis was even further decreased in the CCL13high population (Fig. 7e), suggesting that microglia in the CCL13+ state have lower phagocytic capacity, as we showed previously (Fig. 6h). Knockdown of CDK12 also had some cluster-specific effects (Extended Data Fig. 9b), highlighting the complex effects of gene perturbation in both shifting occupancy of cells between defined functional states, and also affecting cellular pathways in both general and state-specific ways. Interestingly, MED1 had very similar knockdown phenotypes to CDK12, and both genes encode factors associated with general transcription by RNA polymerase II.

We next turned our attention to regulators of the disease-relevant SPP1-positive cluster 3. Knockdown of MAPK14 and CSFIR had dramatically opposing effects, increasing and decreasing occupancy in the SPP1 cluster, respectively (Fig. 7a,b). Using GolgiPlug treatment to block secretion of SPP1, we validated these phenotypes by flow cytometry: knockdown of MAPK14 increased the population of SPP1+ cells more than sixfold (Fig. 7g), whereas CSFIR knockdown greatly diminished the proportion of SPP1+ cells (Fig. 7h).

Based on these effects of genetic perturbations, we asked if pharmacological targeting of the same hits would similarly modulate the abundance of the SPP1+ state. Indeed, inhibition of MAPK14 with Skepinone-L increased the fraction of SPP1+ microglia in a time-dependent manner at nontoxic concentrations (Fig. 7i).

Given that pharmacological inhibition of CSFIR has shown beneficial effects in several neurodegenerative mouse models, and was observed by us and others to selectively affect subpopulations of microglia in mice56–59, we tested if pharmacological inhibition of CSFIR would reduce the proportion of SPP1+ microglia. While the CSFIR inhibitor PLX3397 showed dose-dependent toxicity in iTF-Microglia (Fig. 7k), low concentrations of CSFIR inhibitor that were nontoxic to bulk iTF-Microglia selectively depleted SPP1+ iTF-Microglia (Fig. 7l). Thus, both pharmacological and genetic inhibition of CSFIR can decrease the proportion of SPP1+ cells.

In conclusion, our CROP-seq screen enabled deep characterization of the hit genes that our primary screens identified and revealed the existence of a wealth of microglial cell states and their regulators. To enable the scientific community to further explore this large dataset, we implemented additional functionality in the CRISPRbrain data commons (https://www.crisprbrain.org/) we previously described11. Specifically, interactive three-dimensional UMAP representations and heatmaps enable the selective investigation of cells by expression levels of genes of interest, sgRNA identity and cluster membership.

Discussion
In this study, we described a platform for large-scale, multimodal CRISPRi/a-based genetic screens in human iPSC-derived microglia. We demonstrated the power of this platform in multiple large-scale screens. While CRISPR knockout strategies are commonly used for loss-of-function screens, the partial knockdown achieved by CRISPRi enables a more nuanced characterization of the function of essential genes. For example, we uncovered a selective vulnerability of microglia in the SPP1+ state to partial knockdown of the microglia-essential gene CSFIR. The use of human microglia (as opposed to mouse primary microglia) enabled us to recapitulate microglia features found in human but not mouse brain, such as a state characterized by a chemokine signature2,21. Notwithstanding, there are several areas for future optimization of our iTF-Microglia platform, as detailed in the Supplementary Discussion.

Our platform uncovered insights into microglial biology. We identified several genes associated with neurodegenerative diseases, including PFN1 and INPP5D, as modulators of phagocytosis in microglia (Fig. 5), thus pointing to a possible cellular mechanism by which variants in these genes contribute to disease (Supplementary Discussion).

scRNA-seq revealed that iTF-Microglia adopt a spectrum of states, including states mirroring those observed in human brains. Our CROP-seq screen identified genes controlling the distribution of iTF-Microglia across these states. We demonstrate that knockdown or pharmacological inhibition of MAPK14 or CSFIR promotes or depletes, respectively, the disease-relevant SPP1 state of microglia (Supplementary Discussion). This will make it possible to determine the role of SPP1+ microglia in different diseases, where they may play either beneficial or detrimental roles, and to manipulate this disease-associated microglial state for therapeutic benefit.

We anticipate that the screening platform we describe here can be broadly applied to screen for other microglia-related phenotypes, and to systematically identify regulators of different microglial states. Using iPSCs derived from patients with familial or sporadic diseases will enable the identification of potential therapeutic targets that can correct cellular phenotypes56. Introduction of microglia into cocultures or brain organoids can provide a screening platform to investigate their interactions with other brain cell types, such as synaptic pruning of neurons. Finally, transplantation of iTF-Microglia into postnatal, immune-deficient, humanized mice could result in microglia with an in vivo human microglial gene signature, including more homeostatic microglia, and enable the investigation of factors controlling the interaction of microglia with a model for diseased brain environment60–62.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41593-022-01131-4.

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Human iPSC culture. Human iPSCs (male WT1C11 background, Coriell Catalog (Cat.) No. GM25256) were cultured in StemFlex Basal Medium (Gibco; Cat. No. A3493-01) on BioLite Cell Culture Treated Dishes (Thermo Fisher Scientific; assorted Cat. Nos.) coated with Growth Factor Reduced, Phenol Red-Free, LDEV-Free Matrigel Basement Matrix (Corning; Cat. No. 356231) diluted 1:100 in Knockout DMEM (Gibco/Thermo Fisher Scientific; Cat. No. 10829-018). StemFlex was replaced every other day or every day once 50% confluent. When 70–80% confluent, cells were passaged by aspirating media, washing with DPBS (Gibco; Cat. No. 14190-144), incubating with StemPro Accutase Cell Dissociation Reagent (Gibco/Thermo Fisher Scientific; Cat. No. A1105-01) at 37°C for 7 min, diluting Accutase 1:5 in StemFlex, collecting cells in conicals, centrifuging at 220g for 5 min, aspirating supernatant, resuspending cell pellet in StemFlex supplemented with 10 nM 27632 dihydrochloride ROCK inhibitor (Tocris; Cat. No. 125410), counting and plating onto Matrigel-coated plates at the desired density. Human iPSCs (cultured in StemFlex medium with colonies at 60–80% confluency) were dissociated for experiments. For dissociation, iTF-Microglia were washed once with PBS before adding 100 µg/ml trypsin-EDTA (Gibco; Cat. No. 25200-056) for 5 min. Embryoid bodies were carefully collected and transferred into a 15–ml conical tube, and left to settle at 37°C for 30 min. Embryoid bodies were plated per well in 6-well plates and cultured in 3 ml of hematopoietic medium (2 mM GlutaMax, 10 ng/ml IL-34, 100 ng/ml M-CSF and 25 ng/ml Hum-IL-3 (Peprotech; Cat. No. 200-03) in X-Vivo 15 (Lonza; Cat. No. BE02-0640). 3 d later, the medium was exchanged every 3–4 d. Microglia progenitors were collected from suspension after 14–21 d and plated on PDL-coated plates in microglia maturation medium (2 mM GlutaMax, 10 ng/ml IL-34 and 10 ng/ml GM-CSF in Advanced RPMI-1640 (Gibco; Cat. No. 12630012). Microglia progenitors were further differentiated for 8 d with full medium change every 2–3 d before using them for experiments.

Differentiation and culture of iPSC-derived microglia following the protocol by Brownjohn and colleagues. Brownjohn iPSC Microglia (Brownjohn-iMG) were differentiated from dCas9-KRAB iPSCs (AICS-0090, Coriell Cat. No. AICS-0090-391) using the published protocol with minor modifications. In brief, iPSCs (cultured in StemFlex medium with colonies at 60–80% confluency) were dissociated to single cells with Accutase, collected and plated at 10,000 cells per well in 96-well ultra-low attachment, round bottom plates (Corning; Cat. No. 7819). Medium of embryoid bodies was exchanged for 2–4 d until the fraction of infected cells was described. Clones were selected using both neomycin and puromycin, thus generating the cell line we termed iTF-iPSCs. Next, iTF-iPSCs were transfected with pC13N-dCas9-BFP-KRAB (ref. 11), pt029-CYLBD-CAG-DHFR-dCas9-BFP-KRAB-NLS-DHFR (ref. 1) or pRT029-CYLBD-DDdCas9VP- GFP (ref. 1) to generate constitutive CRISPRi, inducible CRISPRi or inducible CRISPRa iTF-iPSC cell lines, respectively, in the CLYBL safe-harbor locus using the same TALEN-editing method. After transfection, BFP-positive (CRISPRi) or GFP-positive (CRISPRa) iTF-iPSCs were repeatedly enriched via FACs (BD FACSAria Fusion).

To generate monoclonal cell lines, 5,000 polyclonal CRISPR iTF-iPSCs were plated on 10–cm dishes to enable isolation of individual clones under direct visualization with an inverted microscope (Evo FL, Thermo Fisher Scientific) in a tissue culture hood via manual scraping. Monoclonal cell lines were tested for iTF-Microglia differentiation capability and CRISPRi/a activity. Human iPSC-derived iTF-Microglia cell culture and differentiation. iTF-iPSCs were grown in StemFlex until reaching at least 50% confluency and were grown for at least 24 h without ROCK inhibitor. They were dissociated and centrifuged as described above and pelleted cells were resuspended in day 0 differentiation medium containing the following: Essential 8 Basal Medium (Gibco; Cat. No. A11169-01) as a base, 10 nM ROCK inhibitor and 2 µg/ml doxycycline (Clontech; Cat. No. 631311). iTF-iPSCs were counted and seeded onto double-coated plates (Poly-d-Lysine precoated Bio plates (Corning, assorted Cat.) No. GM25256) were cultured in StemFlex basal medium (Gibco; Cat. No. 10829-018) with 0.5% BSA, 10% FCS, 100 ng/ml SCF (Peprotech; Cat. No. 300-07), 1 µg/ml EGF (Gibco; Cat. No. 100-03), 1 M-CSF and 25 ng/ml Human IL-34 (Gibco; Cat. No. 200-03) in X-Vivo 15 (Lonza; Cat. No. BE02-0640). Two-thirds of the medium was exchanged every 3–4 d. Microglia progenitors were collected from suspension after 14–21 d and plated on PDL-coated plates in microglia maturation medium (2 mM GlutaMax, 10 ng/ml IL-34 and 10 ng/ml GM-CSF in Advanced RPMI-1640 (Gibco; Cat. No. 1263012). Microglia progenitors were further differentiated for 8 d with full medium change every 2–3 d before using them for experiments.

iTF-Microglia coculture with neurons. iPS-cell derived neurons (Neurons) were differentiated from WTC11 iPSCs engineered to express NGN2 under a doxycycline-inducible system in the AAVS1 safe-harbor locus as previously described with minor modifications as follows: iPSCs were maintained and dissociated as described above and replated on Matrigel-coated dishes in N2 Pre-Differentiation Medium. After 3 d, hereafter day 0, the pre-differentiated neurons were dissociated to single cells with Accutase, collected and plated at 10,000 cells per well in PDL-coated 96-well plates in BrainPhys Neuronal Medium (BrainPhys ST(EM)CELL Technologies; Cat. No. 05790) as the base, 0.5 x N2 Supplement (Thermo Fisher; Cat. No. 17502-048), 0.5 x B27 Supplement (GIBCO/Thermo Fisher Scientific; Cat. No. 17504-044), 10 ng/ml NT-3 (PeproTech; Cat. No. 450-03), 10 ng/ml BDNF, 1 mg/ml Mouse laminin (Thermo Fisher; Cat. No. 23017-015) and 2 mg/ml doxycycline. On day 3, a full media change was performed. On day 7, half the medium was removed, and an equal volume of BrainPhys Neuronal Medium was added. On day 14, half the medium was removed and an equal volume of BrainPhys Neuronal Medium containing day 8 iTF-Microglia expressing Lck-mkNeonGreen and supplemented with 2X the cytokines of the iTF-Microglia medium was added. Then, 3,000 iTF-Microglia were added to each well and immunostaining experiments were performed after 1 d. Lenti transduction of iPSCs with sgRNA constructs. Individual or pooled sgRNAs were lentivirally packaged in HEK293T cells (ATCC Cat. No. CRL-3216) described, and introduced into CRISPRi or CRISPRa iPS cells via lentiviral delivery using TransIT-Lent Reagent (Mirus Bio; Cat. No. MR 6600) according to manufacturer’s protocol. Cells were selected with 2 µg/ml puromycin (Gibco; Cat. No. A11138-03) for 2–4 d until the fraction of infected cells was >0.9, as determined by flow cytometry of sgRNA-BFP fluorescence, after which cells were cultured for 2–4 d in the absence of puromycin to allow them to recover. sgRNA protopase sequences are provided in Supplementary Table 10.

qPCR. To quantify TFG, INPP5D or PICALM knockdown or CXCR4, CD209 or PFN1 overexpression, lysed cell pellets from human iPSCs or iTF-Microglia were thawed on ice, and total RNA was extracted using the Quick-RNA Miniprep Kit (Zymo; Cat. No. R1054). Complementary DNA was synthesized with the SensiFAST cDNA Synthesis Kit (Bioline; Cat. No. 650405). Samples were prepared for qPCR in technical triplicates in 5 µl reaction volumes using SensiFAST SYBR Lo-ROX 2X Master Mix (Bioline; Cat. No. BIO-94050), custom qPCR primers from Integrated DNA Technologies used at a final concentration of 0.2 µM and cDNA diluted at 1:3. qPCR was performed on an Applied Biosystems QuantStudio 6 Pro Real-Time PCR System using QuantStudio Real Time PCR software (v.1.3.) with the following Fast 2-Step protocol: (1) 95°C for 20 s; (2) 95°C for 5 s (denaturation); (3) 60°C for 20 s (annealing/extension); (4) repeat steps 2 and 3 for a total of 40 cycles; (5) 95°C for 1 s; (6) ramp 1.92°C s⁻¹ from 60°C to 95°C to establish melting curve. Expression fold changes were calculated using the ΔΔCT method, normalizing to housekeeping gene GAPDH. Primer sequences are provided in Supplementary Table 10.
Cell-surface protein staining for flow cytometry. Dissociated and resuspended iTF-Microglia were blocked for 15 min with 1:20 Human FC Block (BD Biosciences; Cat. No. 564220) and then stained with 1:66 PE-Cy7 anti-human CD14 (BioLegend; Cat. No. 3006-85) or CRISPRI validation at 1:66 PE-Cy7 anti-human CD71 (TFRC; BioLegend; Cat. No. 334112) for CRISPRI validation for 30 min in the dark. For the CD38 screen and validation experiments, iTF-Microglia were stained with 1:200 anti-hCD38 PE (R&D Systems; Cat. No. FAB2404P). Cells were washed twice with DPBS before analyzing them by flow cytometry using Cytometric Data File Viewer (PD-View; Fortessa X41) using PhRodo-Red-labeled synaptosomes at a concentration of 1 mg ml⁻¹ added per gram of brain tissue. Dounce homogenization was performed on ice and the centrifugation step was repeated. The supernatant was then centrifuged at 15,000 g for 20 min at 4 °C. The supernatant was removed and the wet pellet was washed two times with DPBS, and the centrifuged stained cells were normalized to non-stained control samples and data were plotted as fold change using Prism 8 (GraphPad, v.8.4.2).

Intracellular protein staining for flow cytometry. iTF-Microglia were treated for 6 h with 1:200 Golgiglue (BD; Cat. No. 555029) or dimethylsulfoxide as control before dissociating. Cells were fixed and permeabilized with the eBioscience Intracellular Fixation and Permeabilization Buffer Set (Invitrogen; Cat. No. 88-8824-00) according to the manufacturer’s instructions. Cells were stained with 1:75 Anti-Hu Osteopontin (SPP1) eFluor 660 (eBioscience; Cat. No. 50-0966-42) or 1:75 Human CCL13 488 (R&D Systems; Cat. No. IC327G) or its isotype controls Mouse IgG1 Control Alexa Fluor 488 conjugated (R&D Systems; Cat. No. IC002G) and Mouse IgGI kappa Isotype (eBioscience; Cat. No. 50-4714-82) overnight at 4 °C. Cells were washed twice with DPBS before analyzing them by flow cytometry using the BD Biosciences FACS Aria III and then stained with the BD FACSDiva (v.8.0.1.1) software. Flow cytometry data were analyzed using FlowJo (FlowJo, v.10.7.1); raw median fluorescence intensity values of CCL13, CD71 and C13 stained cells were normalized to isotype-control samples and data were plotted as fold change using Prism 8. The gating strategy used to determine the percentage of SPP1-positive cells is shown in Supplementary Fig. 1c.

Immunohistochemistry. iTF-Microglia monocytes and cocultures were differentiated in PDL-coated 96-well plates. They were fixed with 4% paraformaldehyde (Electron Microscopy Sciences; Cat. No. 15710) for 10 min at room temperature. After washing with DPBS three times, cells were permeabilized and blocked with 3% normal goat serum (Vector Laboratories; Cat. No. S-0002-20) with 0.01% Triton X-100 (TEKnova; Cat. No. T1-105) in PBS for 1 h at room temperature. Cells were then incubated with primary antibodies diluted in blocking buffer at 4 °C overnight. After that, cells were washed with DPBS three times and incubated with secondary antibodies diluted in blocking buffer for 1 h at room temperature. Cells were treated with DPBS three times and stained with 10 μg ml⁻¹ Hoechst 33342 (Thermo Fisher Scientific; Cat. No. H3570) for 10 min.

Synaptosome isolation and pHRodo-Red labeling. Synaptosomes were isolated from fresh Innovative Grade US Origin Rat Sprague Dawley Brain (Innovative Research; Cat. No. R37110) according to the manufacturer’s protocol. Synaptosome isolation and pHrodo-Red labeling.

Human cytokine array. Day 8 iTF-Microglia were treated with 100 ng ml⁻¹ LPS (Millipore Sigma; Cat. No. LP525) or DPBS control. After 24 h, the supernatant was collected and processed using the Proteome Profiler Human Cytokine Array Kit (R&D Systems; Cat. No. ARY005B), according to the manufacturer’s instructions. Data were acquired using Image Studio (v.5.2). For analysis of the signals, Fiji (v.2.0.0) was used to measure the integrated pixel density for each pair of duplicate dots representing a cytokine. Background signal was measured from negative control dots and then subtracted from each dot. The relative change in cytokine levels as a result of LPS treatment was obtained by comparing corresponding cytokine signals across multiple arrays performed in tandem.

Live-cell imaging. iTF-iPSCs transduced with individual sgRNAs as described above were passaged and differentiated into iTF-Microglia in the 96-well format described above. Starting on day 2 of differentiation, and continuing every 2 d until day 15, iTF-Microglia were stained with 10 μg ml⁻¹ Hoechst 33342 for 10 min at 37 °C, washed with PBS and imaged with the IN Cell Analyzer 6000 using IN Cell Analyzer 6000 Acquisition Software (v.4.0). Using the same 96-well format as described above, day 8 iTF-Microglia were stained for F-actin using 25 nM SiR-actin (Cytoskeleton; Cat. No. CY-SC001) probe diluted in iTF-Microglia medium, with a 4-h incubation at 37 °C. A full media change with iTF-Microglia medium was completed before imaging using the IN Cell Analyzer 6000.

CellTiter-Glo assay after pharmaceutical inhibition of CSF1R or MAPK4. For the survival screens, day 0 iPSCs and day 15 iTF-Microglia were lifted with TrypleE and then blocked and stained with anti-PE-CD38 as described above. Flow cytometry data were subjected to sample preparation for next-generation sequencing as described below.

For the CD38-activation screen, day 8 iTF-Microglia were dissociated with TrypleE and then blocked and stained with anti-PE-CD38 as described in the cell-surface staining section. Cells were sorted into high- and low-signal populations corresponding to the top 30% and the bottom 30% of the CD38-PE signal distribution (gating strategy shown in Supplementary Fig. 1a).

For the phagocytosis FACS screen, day 15 iTF-Microglia were incubated with pHRodo-Red-labeled synaptosomes as described in the phagocytosis section. Cells were then differentiated with TrypleE and sorted into high- and low-signal populations corresponding to the top 30% and the bottom 30% of the pHRodo-Red signal distribution (gating strategy shown in Supplementary Fig. 1b). Based on simulations, we previously found that this sorting strategy is optimal for hit detection in FACS-based screens. These data were subjected to sample preparation for next-generation sequencing as described previously. Briefly, for each screen sample, genomic DNA was isolated using a Macherey-Nagel Blood kit (Macherey-Nagel; Cat. No. 740954.20). sgRNA-encoding regions were amplified and sequenced on an Illumina HiSeq 4000.
QuantSeq. Cell culture medium was aspirated, cells were washed once with DPBS and RNA lysis buffer was added directly to wells containing day 0 IF-ITPsCs, day 15 ITF-Microglia × 50 ng/ml 24 h LPS treatment, Brownjohn-iMG × 100 ng/ml 24 h LPS treatment and day 15 ITF-Microglia. Microglia were differentiated using transgene effects after PNN overexpression, two different PNN sGRNs and NTG sGRNs were transduced into inducible CRISPRa PSCs and cells were differentiated to day 8 ITF-Microglia. Biological triplicates for each condition (approximately 0.15 million cells each) were pelleted, snap frozen and stored at −80°C. RNA was extracted using the RNeasy MiniPrep Kit (Zymo; Cat. No. R1055). Libraries were prepared from total RNA (250–473 ng per sample) using the QuantSeq 3’mRNA-Seq Library Prep Kit for Illumina (FWD) (Lexogen; Cat. No. 013UG009V0252) following the manufacturer’s instructions. Library amplification was performed with 14 total PCR cycles. mRNA-Seq library concentrations (mean of 1.13 ± 0.66 ng/μl) were measured with the Qubit dsDNA HS Assay Kit (Invitrogen; Cat. No. Q32851) on a Qubit 2.0. Library fragment-length distributions (mean of 287 ± 28 base pairs) were quantified with High Sensitivity D5000 Reagents (Agilent Technologies; Cat. No. 5067-5593) on the 4200 TapeStation System. The libraries were sequenced on an Illumina NextSeq 2000 instrument with single-end reads.

CROP-seq. A pooled sGRN library consisting of two sGRNs per targeted gene and four NTG sGRNs was designed to target 39 genes which were selected hit genes from ITF-Microglia survival and FACS-based screens (Supplementary Table 5; only one sGRN for gene DBF4 due to technical error). Briefly, top and bottom strands of sgRNA oligos were synthesized (Integrated DNA Technologies) and annealed in a buffer of formamide, pooled in equimolar amounts and ligated into our optimized CROP-seq vector, as previously described.13 Inducible CRISPRi ITF-ITPsCs were infected with the pooled sGRN library at <0.15 MOI and then selected for lentiviral integration. Next, ITF-ITPsCs were differentiated into ITF-Microglia and cultured with the addition of TMP. Day 8 ITF-Microglia were washed three times with DPBS, dissociated with DPBS and resuspended in nuclease-free water before loading onto four wells of the 10X Chromium Controller (10X Genomics, v3.1) according to the manufacturer’s protocol, with 35,000 cells recovered per sample as the target. Sample preparation was performed using the Chromium Next GEM Single Cell 3’ Reagent Kits v3.1 (10X Genomics, Cat. No. PN-1008121) according to the manufacturer’s protocol, reserving 10–30 ng of full-length cDNA to facilitate sGRN assignment by amplifying sGRN-containing transcripts using hemi-nested PCR reactions adapted from a previously published approach.14 cDNA fragment analysis was performed using the 4200 TapeStation System and sGRN enrichment libraries were separated and sequenced as spike-ins alongside the whole-transcriptome scRNA-seq libraries using a NovaSeq 6000 using the following configuration: Read 1: 28; Read 2: 91.

Computational and statistical analysis. Primary CRISPR screen analysis. Primary screens were analyzed using our previously published MAGeCK-INC bioinformatics pipeline,20 available at https://kimparzunlab.ucsf.edu/mageck-inc. Briefly, raw sequencing reads from next-generation sequencing were cropped and aligned to the reference using Bowtie v.0.12.9.25 to determine sGRN counts in each sample. The quality of each screen was assessed by plotting the log_2(counts) per sGRN on a rank order plot using ggplot2 v.3.3.3. Raw phenotypic score values were calculated for each screen following the reference method,25 as defined for ‘negative-control-quasi-genes’ that were generated by random sampling with replacement of five NTG sGRNs from all NTG sGRNs. The final phenotype score for each gene was calculated by subtracting the raw phenotype score by the median phenotype score of ‘negative-control-quasi-genes’ and then dividing by the standard deviation of raw phenotype scores of ‘negative-control-quasi-genes’. A ‘Gene Score’ was defined as the product of phenotype score and −log_(P-value). Hit genes were determined based on the Gene Score cut-off corresponding to an empirical false discovery rate of 10%. Volcano plots of Gene Scores were generated using ggplot2 v.3.3.3 (ref. 25).

RNA-seq analysis. Alignment and mapping were performed using Salmon v.1.4.0 (ref. 26) (the --noLengthCorrection flag was used for QuantSeq samples) and either the human reference genome GRCh38 (Gencode, release 73), or a custom GRCh38 reference genome containing the references for each 3TF gene-centric integration in ITF-ITPsCs, to obtain transcript abundance counts. Txipwing v.1.180 (ref. 72) was used to obtain gene level-count estimates. Genes with zero counts across all samples were removed from the analysis. To visualize differences in expression across samples, a list of gene symbols corresponding to microglia markers, microglia activation markers and iPSC markers was compiled from a previous publication;27 the normalized counts of each of these genes were then standardized across samples (that is, subtracting by the mean and dividing by the standard deviation), standardized using quantile-normalization using VST2.9.2 (ref. 28). To assess how ITF-Microglia compare to other ITPsCs-Microglia and primary microglia from a range of previous studies,29 raw eQTLs were obtained from the NCBI GEO database and subjected to the same analysis pipeline stated above. Then, principal component analysis was performed using microglial marker genes as input with DESeq2 v.1.30.1 (ref. 29). Differential gene expression analysis of LPS-treated ITF-Microglia samples and the PNN1 overexpression versus NTG ITF-Microglia, DESeq2 v.1.30.1 (ref. 30) was used to calculate the fold-log change and P values and to perform shrinkage of log-fold change for downstream visualization using ggplot2.20 To compare both LPS-treatment upregulated and downregulated DEGs in ITF-Microglia and Brownjohn-iMG, DEGs that were significant (P < 0.05) in at least one cell type were visualized using VennDiagram (v.1.6.20).
field of view, the total integrated intensity of the IBA1 stain was divided by the number of segmented nuclei based on Hoechst.

**Statistics and reproducibility.** No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications\(^{16}\). No randomization of samples was used since treatment group of cells were generally derived from the same population of cells. Data collection and analysis were not performed blind to the conditions of the experiments. No data points were excluded from analysis. Data distribution was assumed to be normal but this was not formally tested. For all imaging experiments (Figs. 1b,d, 2a,c,f and 3d and Extended Data Fig. 1a), similar results were observed in \(n=3\) independent experiments.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

All screen datasets and RNA transcriptomic datasets are publicly available in the CRISPRbrain data commons (http://crisprbrain.org/) (associated with Figs. 1, 2, 4, 5, 6, and 7 and Extended Data Figs. 1, 4, 5, 6 and 7). RNA-sequencing datasets reported in this paper are available on NCBI GEO, accession number GSE178317 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE178317). There are no restrictions on data availability.

**Code availability**

The MAGICck-iNC bioinformatics pipeline for analysis of pooled screens is available at https://kampmannlab.ucsf.edu/mageck-inc. The R notebooks for the RNA-seq and CROP-seq analyses are available at https://kampmannlab.ucsf.edu/article/microglia-analysis. The CellProfiler pipelines will be made available on request to the corresponding authors (M.K.), and will also be submitted to the CellProfiler depository of published pipelines (https://cellprofiler.org/examples/published_pipelines.html) upon publication.

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**Author contributions**

The iTF-Microglia differentiation strategy was developed and characterized by C.T.-L.H. and L.G. with contributions from C.D.C., L.Z., I.C.U., I.K., J.I. and M.E.W. Additionally, N.M.D., S.M.S., O.M.T., K.L., J.H. and M.K. contributed to the optimization and characterization of iTF-Microglia. CRISPR-based functional genomics studies were designed, conducted and analyzed by N.M.D., S.M.S. and M.K. with contributions from O.M.T., K.L., G.A. and J.H. S.M.S. led the computational analysis of all screens and RNA-seq experiments. S.H.L. and F.F. developed new features for the CRISPRbrain data commons with critical input from S.M.S., N.M.D. and M.K. and feedback from M.A.N. and A.B.S. N.M.D., S.M.S., O.M.T. and M.K. created the figures. N.M.D., S.M.S. and M.K. conceptualized and wrote the manuscript with input from the other authors. All authors reviewed and approved the final manuscript.

**Competing interests**

M.A.N. consults for Neuron23. M.A.N. and F.F. participated in this work in part due to a competitively awarded consulting contract between Data Tecnica International, LLC and the National Institutes of Health (USA). J.I. is a cofounder of AcuraStern, Inc. and Modulo Bio, and serves on the scientific advisory board of Spinogenix. L.G. is a founder of Arton Therapeutics. M.K. is an inventor on US patent 11,254,933 related to CRISPRi and CRISPRa screening; serves on the scientific advisory boards of Engine Bionsciences, Casma Therapeutics, Cajal Neuroscience and Alector; and is a consultant to Modulo Bio and Recursion Therapeutics. The remaining authors declare no competing interests.

**Additional information**

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Impact of Doxycycline removal on iTF-Microglia survival and sgRNA recovery in iPSC-derived microglia generated with different protocols. a, Comparison of iTF-Microglia viability after Day 8 with different protocols. Top: timeline with different doxycycline supplementation paradigms, bottom: representative phase-contrast images at Day 15 with the indicated doxycycline supplementation. Scale bar: 50 μm. b, Survival of iTF-Microglia at Day 15 after different doxycycline treatments indicated in a. Viable cells were quantified using the CellTiter-Glo assay. Values represent mean ± sd of n = 12 biological replicates; p values from two-tailed Student’s t-test. c, Principal component analysis (PCA) on the expression of microglia marker genes of iTF-Microglia, human adult ex-vivo microglia⁶⁰, fetal and adult microglia¹³, human myeloid cells²⁰, other iPSC-microglia¹³,¹⁸. No iPSC samples were included. Each dot reflects an independent biological sample. Colors represent the different cell types. d, sgRNA recovery after transduction with a pooled sgRNA library in iPSCs and differentiation with two different iPSC-Microglia protocols. Strategy for the infection of iPSCs with an sgRNA library with 13,025 elements and timepoint of sgRNA recovery in iPSC-Microglia with the actual recovered counts of sgRNAs after next-generation-sequencing (NGS) from the protocol from Brownjohn et al.¹⁸ (Top) and iTF-Microglia (Bottom).
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Phagocytosis capacity of iTF-Microglia and morphological changes after LPS treatment. a, b, Phagocytosis of yellow-green (YG) beads (a) or pHRodo-Red labeled synaptosomes (b) measured by flow cytometry. Histograms of YG-beads-FITC (a) and Synaptosome-PE (b) after 1.5 h of substrate exposure +/− 5 μM Cytochalasin D (CytoD) treatment. Controls are iTG-Microglia without substrate exposure. c, Phagocytosis of yellow-green (YG) beads at different timepoints. Flow cytometric quantification of the percentage of YG bead-positive cells at after 0.5 h, 1.5 h and 2.5 h of incubation with beads. Addition of 5 μM CytoD decreases the percentage of YG bead-positive cells. Means +/− sd, n = three individual biological replicates; p values from two-tailed Student’s t-test. d, Morphological changes of iTF-Microglia after LPS treatment. Swarm plots showing the automated quantification of microglia F-actin staining in area, shape factor and perimeter with explanation of the three parameters. Means +/− sd, n = 16 wells from 3 individual differentiations; p values from two-tailed Mann-Whitney test. e, Comparison of differentially expressed genes in response to LPS treatment in iTF-Microglia versus iPSC-derived microglia (iMG) differentiated following a previously published protocol by Brownjohn et al.18.
Extended Data Fig. 3 | Karyotyping of the monoclonal iTF-iPSC lines. A normal karyotype was confirmed for monoclonal lines a, iTF-iPSCs, b, constitutive CRISPRi iTF-iPSC, c, inducible CRISPRi iTF-iPSC, d, inducible CRISPRa iTF-iPSC lines.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Functional validation of CRISPRi/a activity in iPSCs and iTF-Microglia. a, b, Functional validation of constitutive (a) or inducible (b) CRISPRi activity via flow cytometry of TFRC surface protein level stained iPSCs expressing a TFRC-targeting sgRNA or a non-targeting control (NTC) sgRNA (mean + /− sd, n = 3 biological replicates; p values from two-tailed Student’s t-test). TMP was added to induce CRISPRi activity where indicated. c–d, Knockdown of TFRC in iPSCs with (a) the constitutive and (b) the inducible CRISPRi system. qPCR quantification of the relative fold change of TFRC mRNA levels in CRISPRi-iPSCs expressing a TFRC sgRNA as compared to a non-targeting control sgRNA in the presence or absence of trimethoprim (TMP). (mean + /− sd, n = 3 biological replicates; p values from two-tailed Student’s t-test). TFRC levels were normalized to the housekeeping gene GAPDH. e–j Knockdown of three different genes in iTF-Microglia with (e,g,i) constitutive CRISPRi and (f,h,j) inducible CRISPRi. qPCR quantification of the relative fold change of TFRC mRNA levels (e,f), INPP5D mRNA levels (g,h) or PICALM mRNA levels (I,j) in CRISPRi-iTF-Microglia expressing a TFRC sgRNA (e,f), INPP5D sgRNA (g,h) or PICALM sgRNA (I,j) compared to a non-targeting control sgRNA at different days of differentiation in the presence of TMP (mean + /− sd, n = 3 biological replicates, P values from two-sided Student’s t test). k, Functional validation of inducible CRISPRa activity via flow cytometry of CXCR4 surface protein level stained iPSCs expressing a CXCR4-targeting sgRNA or a non-targeting control (NTC) sgRNA (mean + /− sd, n = 3 biological replicates; p values from two-tailed Student’s t-test). TMP was added to induce CRISPRi activity where indicated. l–m, qPCR quantification of the relative fold change of CXCR4 mRNA levels in inducible CRISPRa-iPSCs expressing a CXCR4 sgRNA as compared to a non-targeting control sgRNA in the presence or absence of trimethoprim (TMP), which stabilizes the DHFR degron. (mean + /− sd, n = 3 biological replicates; p values from two-tailed Student’s t-test). CXCR4 levels were normalized to the housekeeping gene GAPDH.
Extended Data Fig. 5 | See next page for caption.
Knockdown of CDK8 and TGFBR2 induces proliferation and decreases microglia markers in iPSC-derived microglia generated with different protocols. 

a. Comparison of Gene Scores from CRISPRi survival/proliferation screens in iTF-Microglia (this study) vs. iPSCs. Each dot represents a gene. 
b-c, IBA1 staining in Day 8 CRISPRi iTF-Microglia containing sgRNAs targeting CDK8 or TGFBR2 compared to non-targeting control (NTC) sgRNAs. b, Representative images. Scale bar = 50 μm. c, Quantification. Mean +/-sd, n = 6 fields of view from 2 different wells for sgRNA; p values from two-tailed Student’s t-test. 
d-e, IBA1 staining in Day 8 iMGs generated by the protocol from Brownjohn et al., 2018 expressing sgRNAs targeting CDK8 compared to non-targeting control (NTC) sgRNAs. d, Representative images. Scale bar = 50 μm. e, Quantification. Mean +/-sd, n = 9 fields of view from 3 different wells for sgRNA; p values from two-tailed Student’s t-test. 
f, Relative change in live cells of iTF-Microglia at Day 8 (left) and Day 15 (right) containing sgRNAs targeting CDK8, CSF1R or TGFBR2 compared to non-targeting control sgRNAs. The inducible CRISPRi system was stabilized with TMP from Day 0 – Day 8 (left) or Day 8 – Day 15 (right). (mean +/-sd, n = 3 biological replicates; p values from two-tailed Student’s t-test. 
g, CD38 cell surface levels measured by flow cytometry in iTF-Microglia 24 h treatment with 100 ng/mL LPS or PBS control.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Validation of phagocytosis hits and overview of genes selected for the CROP-seq screen based on primary screens. 

**a**, Comparing Gene Scores for hits from phagocytosis CRISPRi and CRISPRa screens. Each dot represents a gene. 

**b–d**, Validation of (b,c) CRISPRi hits and (d) CRISPRa hits in (b,d) iTF-Microglia or (c) iPSC-derived microglia differentiated using an alternative protocol by Brownjohn et al. Phagocytosis of pHrodo-labelled synaptosomes by cells expressing either non-targeting control (NTC) sgRNAs or sgRNAs targeting CSF1R, INPP5D, PFN1 and PFN2 was quantified by flow cytometry. Values represent mean ± s.d of n = 3 biological replicates; p values from two-tailed Student’s t-test. 

**e**, Overexpression of CD209 (left) and PFN1 (right) with the inducible CRISPRa system in iTF-Microglia. QPCR quantification of the relative fold change of CD209 and PFN1 mRNA levels in iTF-Microglia expressing CD209 and PFN1 sgRNA as compared to a non-targeting control sgRNA in the presence of TMP (mean ± s.d, n = 3 biological replicates; p values from two-tailed Student’s t-test). CD209 and PFN1 levels were normalized to the housekeeping gene GAPDH. 

**f**, Binary heatmap of genes selected for the CROP-seq screen and their knockdown phenotype in the CRISPRi survival, phagocytosis and inflammation screens. Red: KD increases phenotype (positive hit). Blue: KD decreases phenotype (negative hit). Grey: not a significant hit, p > 0.1.
Extended Data Fig. 7 | Characterization of microglia cluster signatures. a-c, UMAP projection representing single-cell transcriptomes, with cells colored based on (a) the percentage of mitochondrial transcripts, (b) the expressed sgRNAs, with sgRNAs targeting CDK8 in orange, sgRNAs targeting TGFBR2 in blue, non-targeting control sgRNAs (NTC) in green, and all other sgRNAs in grey, or (c) expression levels of SOX2 (Left) or CSF1R (Right). d, Distribution of iTF-Microglia expressing non-targeting (NTC) sgRNAs across the 9 clusters described in Fig. 6. e, f, the top 40 genes with the highest embedding values for (e) the first principal component (PC-1) and (f) the second principal component (PC-2), displayed in ranking order.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | CROP-seq reveals transcriptomic changes in iTF-Microglia induced by gene knockdown. Changes in gene expression in response to CRISPRi knockdown of genes of interest in iTF-Microglia. Each column represents one CRISPRi-targeted gene. For each CRISPRi-targeted gene, cells with the strongest knockdown were selected and the top 20 differentially expressed genes in comparison to non-targeting control (NTC) sgRNA containing cells were selected. The merged set of these genes is represented by the rows. Rows and columns were clustered hierarchically based on Pearson correlation. Functionally related clusters of differentially expressed genes are labeled.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Transcriptomic changes in iTF-Microglia induced by CDK12 knockdown in cluster 9 and in all other clusters. 

**a**, Changes in cluster distribution after CRISPRi knockdown of targeted genes in iTF-Microglia. Distribution of cells according to the 37 targeted genes and non-targeting control (NTC) in clusters 1–9. 

**b**, Average differences of gene expression induced by CDK12 knockdown in cluster 9 compared to those in all other clusters. Genes encoding phosphatidylserine (PS) recognition receptors are labeled in magenta and Genes encoding MHC complex components are labeled in green.
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- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

For FACS data collection, we used BD FACSDiva (version 8.0.1.1). For microscopy data collection by InCell 6000, we used InCell Analyzer 6000 Acquisition Software (version 4.0). For qPCR data collection, we used Quant Studio Real Time PCR software (version 1.3). For cytokine array imaging, we used Image Studio (version 5.2). For Cell-TiterGlo luminescence data collection, we used SoftMax Pro 6.5.1.

Data analysis

For FACS analysis, we used FlowJo (version 10.7.1) and Prism 8 (8.4.2). For microscopy image analysis we used Fiji (version 2.0.0) and CellProfiler (version 4.1.3). For pooled CRISPR screen analysis, we used MAGeck-iNC (version 5.2) and Bowtie (version 0.12.9). For bulk RNA-seq analysis, we used Salmon (version 1.4.0), T foram (version 1.18.0), ggplot2 (version 3.3.3), Complex Heatmap (version 2.6.2), DESeq2 (version 1.30.1), and VennDiagram (version 1.6.20). For CROP-seq analysis, we used Cell Ranger (version 5.0.1), in R (version 4.0.3) we used DropletUtils (version 1.10.3) and Seurat (4.0.1).

The MAGeck-iNC bioinformatics pipeline for analysis of pooled screens available at https://kampmannlab.ucsf.edu/mageck-inc. The R notebooks for the RNA-seq and CROP-seq analysis are available at https://kampmannlab.ucsf.edu/article/microglia-analysis. The CellProfiler pipelines will be made available on request to the corresponding authors (MK), and will also be submitted to the CellProfiler depository of published pipelines (https://cellprofiler.org/examples/published_pipelines.html) upon publication.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All screen datasets and RNA-transcriptomic datasets are publicly available in the CRISPRbrain data commons (http://crisprbrain.org/) (associated with Figures 1, 2, 4,
RNA sequencing datasets reported in this paper are available on NCBI GEO, accession number GSE178317 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE178317). There are no restrictions on data availability.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample sizes were determined by existing studies in the field to enable statistical analyses and reproducibility, specifically our previous CRISPRi screens in iPSC-derived neurons (Tian et al. 2019, Neuron 104:239-255). |
| Data exclusions | No data was excluded from the relevant analyses. |
| Replication | Major findings were validated using independent samples and orthogonal approaches. Numbers of replicates are listed in each Figure. |
| Randomization | No randomization of samples was used since treatment group of cells were generally derived from the same population of cells. |
| Blinding | Blinding was not relevant to our study since no subjective rating of data was involved. Instead, we used computational quantification. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a | n/a |
| X Antibodies | X ChIP-seq |
| X Eukaryotic cell lines | X Flow cytometry |
| X Palaeontology | X MRI-based neuroimaging |
| Animals and other organisms | |
| Human research participants | |
| Clinical data | |

Antibodies

Primary antibodies: IBA-1 (1:1000, Wako; Cat. No. 019-19741), GPR34 (1:150, R&D Systems; Cat. No. MAB4617), TFRC (1:200, abcam; Cat. No. ab84036), Synaptophysin (1:1000, Synaptic Systems; Cat. No. 101004).

Secondary antibodies: Alexa Fluor 488 goat anti rabbit IgG (1:500, Invitrogen; Cat. No. A11034), Alexa Fluor 488 goat anti mouse IgG (1:500, Invitrogen; Cat. No. A11029), Alexa Fluor 568 goat anti rabbit IgG (1:500, Invitrogen; Cat. No. A11036), Alexa Fluor 647 goat anti chicken IgG (1:500 dilution; abcam Cat. No. ab 150171)

Coupled antibodies: PE-Cy7 anti human CD71 (TFRC) (1:66, BioLegend; Cat. No. 334112), PE/Cy7 anti human CD184 (CXCR4) (1:66, BioLegend; Cat. No. 306514), Human CCL13 488 (1.75, R&D Systems; Cat. No. IC327G), hCD38 PE (1:200, R&D Systems; Cat. No. FAB2404P), hOsteopontin eFluor 660 (1.75, eBioscience; Cat. No. 50-9096-42).

Validation

Antibodies were validated by the manufacturers as follows: Anti-IBA-1 (Wako; Cat. No. 019-19741) was validated by immunohistochemistry of microglia in rat cerebral cortex and mouse cerebellum. Anti-GPR34 (R&D Systems; Cat. No. MAB4617) has been validated by staining human GPR34 transfecants but not irrelevant transfecants. Anti-Synaptophysin (Synaptic Systems; Cat. No. 101004) was validated by western blotting of a synaptosomal fraction of rat brains and validated by immunostaining of hippocampal neurons. Anti-TFRC (abcam; Cat. No. ab84036) was validated by western blotting of various cell lysates, detecting a clear band of approximately 98 kDa (predicted molecular weight: 84 kDa).
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)
The human iPS line WTC11 was obtained from Coriell (GM25256). The human iPS line WTC11 was obtained with CRISPRi machinery was obtained from Coriell (AICS-0090-391). HEK293T cells were obtained from ATCC (CRL-3216).

Authentication
iPSCs were karyotyped (Extended Data Fig. 3) and characterized for maintaining capability to differentiate into iTF-Microglia. AICS-0090 cl.391 was authenticated by the Allen Institute for Cell Science. We did not authenticate the HEK293T cells.

Mycoplasma contamination
All lines were tested negative for mycoplasma using the Universal Mycoplasma Detection Kit (ATCC 30-1012KTM).

Commonly misidentified lines
No commonly misidentified lines were used.

Flow Cytometry

Plots

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
Cells were dissociated using Accutase (for iPSCs) or TrypLE (for iTF-Microglia) and analyzed or sorted by flow cytometry based on staining signals. For cell surface receptor stainings, dissociated and in DPBS resuspended iTF-Microglia were blocked for 15 min with 1:20 Human FC Block (BD Biosciences; Cat. No. 564220). For CRISPRi/a validation, cells were stained with 1:66 PE/Cy7 anti-human CD184 (CXCR4) (BioLegend; Cat. No. 306514) or 1:66 PE-Cy7 anti-human CD71 (TFRC) (BioLegend; Cat. No. 334112) for 30 min in the dark. Cells were washed twice with DPBS before analyzing them by flow cytometry. For the CD38 screen and validation experiments, iTF-Microglia were stained with 1:200 anti-hCD38 PE (R&D Systems; Cat. No. FAB2404P). Cells were washed twice with DPBS before analyzing them by flow cytometry.

For intracellular protein staining, dissociated iTF-Microglia were fixed and permeabilized with the eBioscience Intracellular Fixation and Permeabilization Buffer Set (Invitrogen; Cat. No. 88-8824-00) according to the manufacturer’s instructions. Cells were stained with 1:75 Anti-Hu Osteopontin (SPP1) eFluor 660 (eBioscience; Cat. No. 50-9096-42) or 1:75 Human CCL13 488 (R&D Systems; Cat. No. IC327G) or their isotype controls Mouse IgG1 Control Alexa Fluor 488 conjugated (R&D Systems; Cat. No. IC002G) and Mouse IGG1 kappa Isotype (eBioscience; Cat. No. 50-4714-82) overnight at 4°C. Cells were washed twice with DPBS before analyzing them by flow cytometry.

For analyzing phagocytosis rates and the phagocytosis screen, cells were washed twice with DPBS, dissociated, resuspended in ice-cold DPBS, and then analyzed via flow cytometry.

Cell population abundance
To analyze staining signals or uptake of fluorescent materials (beads or synaptosomes), cells were analyzed and not sorted. The starting populations were pure (either iPSCs or iTF-Microglia). For pooled screens, iTF-Microglia were sorted into high and low signal populations corresponding to the top 30% and the bottom 30% of the staining signal distribution.

Gating strategy
Preliminary FSC/SSC gates encompassed all live, single cells in the population. For pooled screens, these cells were sorted into high and low signal populations corresponding to the top 30% and the bottom 30% of the staining signal distribution.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.