CD22 blockade restores homeostatic microglial phagocytosis in ageing brains

John V. Pluvinage1,2,3, Michael S. Haney3, Benjamin A. H. Smith1,4,5, Jerry Sun3, Tal Iram3, Liana Bonanno1,3, Lu Lin Li3, Davis P. Lee3, David W. Morgenstern6, Andrew C. Yang1,5, Steven R. Shuken3,7, David Gate3, Madeleine Scott1,8,9, Purvesh Khatri8,9, Jian Luo3,10, Carolyn R. Bertozzi4,5,7,11, Michael C. Bassik5,6 & Tony Wyss-Coray3,5,10,12,13

Microglia are among the longest-lived mammalian cell types, residing in the brains of mice for years1 and in humans for decades2. During this period, microglia peruse the brain parenchyma with ramified processes to sense perturbations3 and respond with specialized compensatory functions. One of the core microglial functions necessary for homeostasis in the central nervous system (CNS) is the recognition, engulfment and degradation of extracellular material via phagocytosis4. Whereas microglial phagocytosis is crucial throughout an organism’s lifespan, the targets of engulfment change depending on age. For example, complement-mediated synaptic pruning is essential for development of circumferential circuit refinement5,6, yet inappropriate activation of this pathway in neurodegenerative diseases may contribute to deleterious synaptic elimination7,8. During normal ageing and age-related disease, microglia encounter distinct perturbations in the CNS, including abundant myelin debris9 and protein aggregates. A complex system of extrinsic and intrinsic inhibitory molecules normally regulates microglial phagocytosis to enable efficient elimination of these targets without damaging surrounding tissue10. However, microglia in the aged brain are hypo-motile11, burdened with lysosomal cargo12,13 and chronically express pro-inflammatory signalling molecules14,15, indicative of impaired homeostatic function. We sought to understand the molecular mechanisms governing this impairment, and to determine the consequences of restoring homeostatic microglial phagocytosis on age-related brain dysfunction.

Screen for age-related modifiers of phagocytosis

Microglia undergo marked transcriptional changes with age16,17. However, the functional consequences of these molecular changes are not well understood. We aimed to identify genes that (1) modulate microglial phagocytosis, (2) are amenable to therapeutic intervention, and (3) are differentially expressed with age. To do so, we used CRISPR-Cas9 knockout screens to discover age-related genetic modifiers of microglial phagocytosis. These screens identified CD22, a canonical B cell receptor, as a negative regulator of phagocytosis that is upregulated on aged microglia. CD22 mediates the anti-phagocytic effect of α2,6-linked sialic acid, and inhibition of CD22 promotes the clearance of myelin debris, amyloid-β oligomers and α-synuclein fibrils in vivo. Long-term central nervous system delivery of an antibody that blocks CD22 function reprograms microglia towards a homeostatic transcriptional state and improves cognitive function in aged mice. These findings elucidate a mechanism of age-related microglial impairment and a strategy to restore homeostasis in the ageing brain.
Sialic acid inhibits phagocytosis via CD22

CD22 is canonically expressed on B cells, where it negatively regulates B cell receptor signalling by binding sialic acid and recruiting SHP-1 or SHP-1 via immunoreceptor tyrosine-based inhibitory motifs (ITIMs)\(^{38}\). To search for possible signalling partners of CD22 on microglia, we re-analysed our initial CRISPR–Cas9 screen for hits related to CD22 function. Surprisingly, CAMS, a key enzyme in sialic acid synthesis, and PTIPN6, which codes for SHP-1, were among the most significant hits (Fig. 2a). Time-lapse microscopy confirmed that knocking out CAMS (CAMS-KO) or PTIPN6 (PTIPN6-KO), or removal of sialic acid by treatment with sialidase or 3F AX-Neu5Ac, an inhibitor of sialic acid synthesis, robustly promotes phagocytosis (Fig. 2b, c; Extended Data Fig. 4a–e), phenocopying CD22 ablation. However, genetic or pharmacological inhibition of both CD22 and sialic acid simultaneously did not produce an additive effect (Fig. 2d, Extended Data Fig. 4f, g), suggesting that sialic acid is involved in CD22-mediated inhibition of phagocytosis.

To probe CD22–sialic acid receptor–ligand interactions, we used synthetic glycopolymers functionalized with lipid tails to introduce defined glycans onto the cell surface\(^{35}\) (Fig. 2e). We decorated CMAS-KO and PTIPN6-KO microglia with thiolated glycopolymers functionalized with lipid tails to introduce defined glycans onto the cell surface. CMAS-KO microglia showed significantly higher phagocytosis rates compared to PTIPN6-KO microglia, indicating that the loss of CAMS or PTIPN6 promotes phagocytosis, and removal of sialic acid by treatment with sialidase or 3F AX-Neu5Ac, an inhibitor of sialic acid synthesis, robustly promotes phagocytosis (Fig. 2b, c; Extended Data Fig. 4a–e). Phenocopying CD22 ablation. However, genetic or pharmacological inhibition of both CD22 and sialic acid simultaneously did not produce an additive effect (Fig. 2d, Extended Data Fig. 4f, g), suggesting that sialic acid is involved in CD22-mediated inhibition of phagocytosis.

To probe CD22–sialic acid receptor–ligand interactions, we used synthetic glycopolymers functionalized with lipid tails to introduce defined glycans onto the cell surface. CMAS-KO microglia showed significantly higher phagocytosis rates compared to PTIPN6-KO microglia, indicating that the loss of CAMS or PTIPN6 promotes phagocytosis, and removal of sialic acid by treatment with sialidase or 3F AX-Neu5Ac, an inhibitor of sialic acid synthesis, robustly promotes phagocytosis (Fig. 2b, c; Extended Data Fig. 4a–e). Phenocopying CD22 ablation. However, genetic or pharmacological inhibition of both CD22 and sialic acid simultaneously did not produce an additive effect (Fig. 2d, Extended Data Fig. 4f, g), suggesting that sialic acid is involved in CD22-mediated inhibition of phagocytosis.

To probe CD22–sialic acid receptor–ligand interactions, we used synthetic glycopolymers functionalized with lipid tails to introduce defined glycans onto the cell surface. CMAS-KO microglia showed significantly higher phagocytosis rates compared to PTIPN6-KO microglia, indicating that the loss of CAMS or PTIPN6 promotes phagocytosis, and removal of sialic acid by treatment with sialidase or 3F AX-Neu5Ac, an inhibitor of sialic acid synthesis, robustly promotes phagocytosis (Fig. 2b, c; Extended Data Fig. 4a–e). Phenocopying CD22 ablation. However, genetic or pharmacological inhibition of both CD22 and sialic acid simultaneously did not produce an additive effect (Fig. 2d, Extended Data Fig. 4f, g), suggesting that sialic acid is involved in CD22-mediated inhibition of phagocytosis.

To probe CD22–sialic acid receptor–ligand interactions, we used synthetic glycopolymers functionalized with lipid tails to introduce defined glycans onto the cell surface. CMAS-KO microglia showed significantly higher phagocytosis rates compared to PTIPN6-KO microglia, indicating that the loss of CAMS or PTIPN6 promotes phagocytosis, and removal of sialic acid by treatment with sialidase or 3F AX-Neu5Ac, an inhibitor of sialic acid synthesis, robustly promotes phagocytosis (Fig. 2b, c; Extended Data Fig. 4a–e). Phenocopying CD22 ablation. However, genetic or pharmacological inhibition of both CD22 and sialic acid simultaneously did not produce an additive effect (Fig. 2d, Extended Data Fig. 4f, g), suggesting that sialic acid is involved in CD22-mediated inhibition of phagocytosis.

To probe CD22–sialic acid receptor–ligand interactions, we used synthetic glycopolymers functionalized with lipid tails to introduce defined glycans onto the cell surface. CMAS-KO microglia showed significantly higher phagocytosis rates compared to PTIPN6-KO microglia, indicating that the loss of CAMS or PTIPN6 promotes phagocytosis, and removal of sialic acid by treatment with sialidase or 3F AX-Neu5Ac, an inhibitor of sialic acid synthesis, robustly promotes phagocytosis (Fig. 2b, c; Extended Data Fig. 4a–e). Phenocopying CD22 ablation. However, genetic or pharmacological inhibition of both CD22 and sialic acid simultaneously did not produce an additive effect (Fig. 2d, Extended Data Fig. 4f, g), suggesting that sialic acid is involved in CD22-mediated inhibition of phagocytosis.

To probe CD22–sialic acid receptor–ligand interactions, we used synthetic glycopolymers functionalized with lipid tails to introduce defined glycans onto the cell surface. CMAS-KO microglia showed significantly higher phagocytosis rates compared to PTIPN6-KO microglia, indicating that the loss of CAMS or PTIPN6 promotes phagocytosis, and removal of sialic acid by treatment with sialidase or 3F AX-Neu5Ac, an inhibitor of sialic acid synthesis, robustly promotes phagocytosis (Fig. 2b, c; Extended Data Fig. 4a–e). Phenocopying CD22 ablation. However, genetic or pharmacological inhibition of both CD22 and sialic acid simultaneously did not produce an additive effect (Fig. 2d, Extended Data Fig. 4f, g), suggesting that sialic acid is involved in CD22-mediated inhibition of phagocytosis.

To probe CD22–sialic acid receptor–ligand interactions, we used synthetic glycopolymers functionalized with lipid tails to introduce defined glycans onto the cell surface. CMAS-KO microglia showed significantly higher phagocytosis rates compared to PTIPN6-KO microglia, indicating that the loss of CAMS or PTIPN6 promotes phagocytosis, and removal of sialic acid by treatment with sialidase or 3F AX-Neu5Ac, an inhibitor of sialic acid synthesis, robustly promotes phagocytosis (Fig. 2b, c; Extended Data Fig. 4a–e). Phenocopying CD22 ablation. However, genetic or pharmacological inhibition of both CD22 and sialic acid simultaneously did not produce an additive effect (Fig. 2d, Extended Data Fig. 4f, g), suggesting that sialic acid is involved in CD22-mediated inhibition of phagocytosis.

To probe CD22–sialic acid receptor–ligand interactions, we used synthetic glycopolymers functionalized with lipid tails to introduce defined glycans onto the cell surface. CMAS-KO microglia showed significantly higher phagocytosis rates compared to PTIPN6-KO microglia, indicating that the loss of CAMS or PTIPN6 promotes phagocytosis, and removal of sialic acid by treatment with sialidase or 3F AX-Neu5Ac, an inhibitor of sialic acid synthesis, robustly promotes phagocytosis (Fig. 2b, c; Extended Data Fig. 4a–e). Phenocopying CD22 ablation. However, genetic or pharmacological inhibition of both CD22 and sialic acid simultaneously did not produce an additive effect (Fig. 2d, Extended Data Fig. 4f, g), suggesting that sialic acid is involved in CD22-mediated inhibition of phagocytosis.

To probe CD22–sialic acid receptor–ligand interactions, we used synthetic glycopolymers functionalized with lipid tails to introduce defined glycans onto the cell surface. CMAS-KO microglia showed significantly higher phagocytosis rates compared to PTIPN6-KO microglia, indicating that the loss of CAMS or PTIPN6 promotes phagocytosis, and removal of sialic acid by treatment with sialidase or 3F AX-Neu5Ac, an inhibitor of sialic acid synthesis, robustly promotes phagocytosis (Fig. 2b, c; Extended Data Fig. 4a–e). Phenocopying CD22 ablation. However, genetic or pharmacological inhibition of both CD22 and sialic acid simultaneously did not produce an additive effect (Fig. 2d, Extended Data Fig. 4f, g), suggesting that sialic acid is involved in CD22-mediated inhibition of phagocytosis.
anti-phagocytic effect of α2,6-linked sialic acid was abrogated in a CMAS/CD22 double-knockout background (Fig. 2g), demonstrating that α2,6-linked sialic acid inhibits phagocytosis in a CD22-dependent manner.

To assess inhibitory signalling downstream of CD22, we measured basal SHP-1 activity in BV2 cells and found that CD22-knockout (CD22-KO) cells express a reduced ratio of active phosphorylated SHP-1 to total SHP-1 protein, comparable to that found in CMAS-KO cells (Fig. 2h). Furthermore, treatment with a CD22 ligand-blocking antibody (Extended Data Fig. 4i) decreased SHP-1 activity in a dose-dependent manner (Extended Data Fig. 4k). Taken together, these data support a model in which CD22 binds an α2,6-sialylated ligand and promotes inhibitory SHP-1 signalling to restrain the phagocytic capacity of microglia.

**CD22 blockade clears debris from the ageing brain**

To assess the functional role of CD22 on aged microglia in vivo, we stereotypically co-injected myelin debris, a phagocytic substrate that accumulates in the ageing brain, with a CD22-blocking antibody or an IgG isotype control antibody into opposite brain hemispheres of the same aged mouse (Fig. 3a). We labelled the myelin debris with a control-ITIMs (Fig. 3b–d, g, h). After 48 h, treatment with CD22 antibody robustly promoted clearance of myelin debris compared to IgG (Fig. 3b, c). Because microglia appear to be the only cell type in the mouse CNS that express CD22 (Extended Data Fig. 3c), this effect is likely to be microglia-specific. Notably, CD22 blockade did not influence the recruitment of microglia to the injection site relative to IgG (Extended Data Fig. 5c) or a stab wound control (Extended Data Fig. 5d), and IgG itself did not influence myelin clearance (Extended Data Fig. 5f). Nearly all IBA1+ cells at the injection site were TEME11+= regardless of treatment (Extended Data Fig. 5g, h), suggesting that the pro-phagocytic effect of CD22 blockade is mainly mediated by resident microglia and not infiltrating peripheral macrophages. We confirmed the pro-phagocytic effect of anti-CD22 treatment ex vivo using freshly isolated microglia from aged mice and pH-sensitive fluorescent latex particles (Fig. 3d). To test this effect with an orthogonal approach, we injected labelled myelin debris into the brains of aged wild-type or Cd22−/− mice (Fig. 3e). We observed comparable clearance of myelin debris with genetic ablation of CD22 as with antibody-based blockade of CD22 (Fig. 3f). Furthermore, CD22 blockade (Fig. 3g) or genetic ablation (Extended Data Fig. 5i) had no significant effect in the young mouse CNS, where myelin debris is efficiently cleared at baseline and CD22 is minimally expressed.

Next, we evaluated the effect of CD22 blockade on microglial phagocytosis of oligomeric Aβ, a particularly neurotoxic protein aggregate that accumulates in the AD brain. Compared to IgG, anti-CD22 treatment efficiently cleared injected Aβ oligomers in vivo (Fig. 3h; Extended Data Fig. 5j–l). A larger percentage of residual Aβ in anti-CD22 treated hemispheres was contained in acidified lysosomes (Fig. 3i), suggesting that CD22 blockade promotes degradation of engulfed debris. In an analogous in vivo phagocytosis assay, we found that anti-CD22 treatment promotes the clearance of extracellular α-synuclein fibrils (Extended Data Fig. 5m–o), a pathological hallmark of Parkinson’s disease. Taken together, these data suggest that CD22 is a broad negative regulator of microglial phagocytosis in the ageing brain.

**CD22 blockade restores microglial homeostasis**

Ageing and disease overwhelm the homeostatic function of microglia, leading to a distinctive transcriptional state characterized by the downregulation of resting microglial genes and the upregulation of activated microglial genes. To assess the transcriptional effects of CD22 blockade, we implanted aged mice with osmotic pumps to continuously infuse a CD22 blocking antibody or an IgG control antibody directly into the cerebrospinal fluid for one month (Fig. 4a). As opposed to systemic antibody administration or Cd22−/− mice, this intervention enabled CNS-specific targeting of CD22 without B cell infiltration into the brain (Extended Data Fig. 6a) or substantial antibody leakage.
ARTICLE

RESEARCH

CD22 inhibition restores microglial phagocytosis in vivo.

Myelin debris labelled with Alexa Fluor 555 (AF555) was stereotactically co-injected with CD22 antibody or IgG into the cortex on opposite brain hemispheres of aged (14–16-month-old) mice. Representative images of AF555-labelled myelin (red, top row) overlaid with the myeloid marker IBA1 (green, bottom row) at the injection sites of IgG (left) or anti-CD22 (right) treated hemispheres of the same brain. Scale bars, 100 μm.

clearance of myelin debris in brain hemispheres of aged mice treated with IgG (black) or CD22 antibody (green), assessed 48 h after injection (n = 8, **P < 0.005, paired two-sided t-test).

d, Flow cytometry quantification of ex vivo phagocytosis of pH-sensitive beads by aged microglia pretreated with IgG or CD22 antibody (n = 6, **P < 0.005, paired two-sided t-test).

e, Labelled myelin debris was stereotactically injected into the cortices of aged wild-type (black) and Cd22−/− (blue) mice assessed 48 h after injection (n = 4, *P < 0.05, two-sided t-test, mean ± s.e.m.).

Discussion

Here, we show that CD22 is a negative regulator of phagocytosis that is upregulated on aged microglia. CD22 mediates the anti-phagocytic effect of α2,6-linked sialic acid, and inhibition of CD22, either via antibody blockade or genetic ablation, promotes the clearance of myelin debris, A3 oligomers and α-synuclein fibrils in vivo. Long-term CD22 blockade partially reverses the transcriptional signature of age- and disease-related microglia, increases markers of neuronal activation, and, ultimately, improves cognitive function in aged mice (Extended Data Fig. 10).

These findings raise several questions. First, what causes CD22 upregulation in the ageing brain? Recent studies have highlighted the importance of environmental cues in shaping microglial identity41–43. CD22 is enriched in a subpopulation of postnatal day 7 microglia (Extended Data Fig. 3d) that engulf apoptotic oligodendrocytes and resemble disease-associated microglia of ageing and neurodegenerating brains44. Therefore, it is possible that CD22 is upregulated as a negative feedback mechanism to restrain excessive phagocytosis in response to overwhelming cellular debris in the developing brain. During ageing, this protective developmental program might be inappropriately re-activated in response to increased myelin fragmentation and protein aggregation.

How might CD22 inhibition improve cognitive function in aged mice? Our RNA-seq analysis revealed that CD22 blockade reduces the expression of multiple homeostatic microglial genes (for example, Tgfbr1, P2ry13, Sall1, Il10ra and Me12a; Fig. 4d, Extended Data Figs. 7c, 8a, b) and the downregulation of activated and disease-associated microglial genes (for example, H2-K1, Tspo, Lgals3, Tnfsf13b and Clcl3; Fig. 4d, Extended Data Fig. 7d–h), partially reversing the transcriptional hallmarks of ageing and disease. We confirmed downregulation of Clcl3, a chemokine implicated in hippocampal impairment38, by ex vivo secretome profiling (Extended Data Fig. 8c). Notably, CD22 blockade abrogated Clcl3 secretion in the presence of oligomeric Aβ, but had no effect on basal levels.

To determine the effects of CD22 inhibition on age-related cognitive dysfunction, we assessed hippocampal–dependent learning and memory performance in aged wild-type and Cd22−/− mice. Surprisingly, Cd22−/− mice exhibited improved spatial memory in the forced-alternation Y-maze test (Fig. 4e) and improved associative memory in the contextual-fear-conditioning test (Fig. 4f). To more specifically assess the contribution of microglial CD22 to this behavioural phenotype, we performed the same tests in aged mice infused with IgG or anti-CD22 directly into the CNS via osmotic pump. Blocking CD22 specifically in the CNS phenocopied the learning and memory improvements seen in Cd22−/− mice (Fig. 4g, h), suggesting that microglial CD22 negatively and reversibly regulates cognitive function in the ageing brain. Notably, we did not observe a significant cognitive improvement in aged mice treated systemically with anti-CD22 via intraperitoneal injection, suggesting that peripheral B cells are unlikely to contribute to this effect (Extended Data Fig. 9a, b).

These hippocampal–dependent behavioural phenotypes were accompanied by an increase in the number of hippocampal dentate granule neurons expressing c-Fos, an immediate early gene induced by neuronal activation39 (Fig. 4i, j). In addition, anti-CD22 treatment promoted a modest increase in phosphorylated CREB (Extended Data Fig. 8d, e), a plasticity-related marker40, but did not alter hippocampal neurogenesis (Extended Data Fig. 8f). We observed no difference in C1q deposition (Extended Data Fig. 8g) or synaptic density, as assessed by pre- and post-synaptic markers, synaptophysin and PSD-95 (Extended Data Fig. 8h, i).

into the periphery (Extended Data Fig. 6b). We labelled the respective antibodies with a fluorescent dye to assess drug distribution within the brain parenchyma (Extended Data Fig. 6c) and target engagement with CD22 on microglia (Extended Data Fig. 6d).

After one month of continuous infusion, we performed RNA-seq on purified microglia from the brain hemispheres of these mice contralateral to the cannulation site to minimize injury-induced confounding factors. We found very few transcriptional differences between microglia from untreated and IgG-infused mice (Extended Data Fig. 7a). By contrast, we found 315 differentially expressed genes between microglia treated with IgG and those treated with CD22 antibody meeting a false discovery rate (FDR) cut-off of 10% (Supplementary Table 4; Extended Data Fig. 7b). We compared these genes to those that are differentially expressed between microglia from young and aged mice36, and between microglia from wild-type and end-stage 5xFAD AD-model mice37 (Fig. 4b, c). Anti-CD22 treatment promoted the upregulation of homeostatic microglial genes (for example, Tgfbr1, P2ry13, Sall1, Il10ra and Me12a; Fig. 4d, Extended Data Figs. 7c, 8a, b) and the downregulation of activated and disease-associated microglial genes (for example, H2-K1, Tspo, Lgals3, Tnfsf13b and Clcl3; Fig. 4d, Extended Data Fig. 7d–h), partially reversing the transcriptional hallmarks of ageing and disease. We confirmed downregulation of Clcl3, a chemokine implicated in hippocampal impairment38, by ex vivo secretome profiling (Extended Data Fig. 8c). Notably, CD22 blockade abrogated Clcl3 secretion in the presence of oligomeric Aβ, but had no effect on basal levels.

To determine the effects of CD22 inhibition on age-related cognitive dysfunction, we assessed hippocampal–dependent learning and memory performance in aged wild-type and Cd22−/− mice. Surprisingly, Cd22−/− mice exhibited improved spatial memory in the forced-alternation Y-maze test (Fig. 4e) and improved associative memory in the contextual-fear-conditioning test (Fig. 4f). To more specifically assess the contribution of microglial CD22 to this behavioural phenotype, we performed the same tests in aged mice infused with IgG or anti-CD22 directly into the CNS via osmotic pump. Blocking CD22 specifically in the CNS phenocopied the learning and memory improvements seen in Cd22−/− mice (Fig. 4g, h), suggesting that microglial CD22 negatively and reversibly regulates cognitive function in the ageing brain. Notably, we did not observe a significant cognitive improvement in aged mice treated systemically with anti-CD22 via intraperitoneal injection, suggesting that peripheral B cells are unlikely to contribute to this effect (Extended Data Fig. 9a, b).

These hippocampal–dependent behavioural phenotypes were accompanied by an increase in the number of hippocampal dentate granule neurons expressing c-Fos, an immediate early gene induced by neuronal activation39 (Fig. 4i, j). In addition, anti-CD22 treatment promoted a modest increase in phosphorylated CREB (Extended Data Fig. 8d, e), a plasticity-related marker40, but did not alter hippocampal neurogenesis (Extended Data Fig. 8f). We observed no difference in C1q deposition (Extended Data Fig. 8g) or synaptic density, as assessed by pre- and post-synaptic markers, synaptophysin and PSD-95 (Extended Data Fig. 8h, i).

Discussion

Here, we show that CD22 is a negative regulator of phagocytosis that is upregulated on aged microglia. CD22 mediates the anti-phagocytic effect of α2,6-linked sialic acid, and inhibition of CD22, either via antibody blockade or genetic ablation, promotes the clearance of myelin debris, A3 oligomers and α-synuclein fibrils in vivo. Long-term CD22 blockade partially reverses the transcriptional signature of age- and disease-related microglia, increases markers of neuronal activation, and, ultimately, improves cognitive function in aged mice (Extended Data Fig. 10).

These findings raise several questions. First, what causes CD22 upregulation in the ageing brain? Recent studies have highlighted the importance of environmental cues in shaping microglial identity41–43. CD22 is enriched in a subpopulation of postnatal day 7 microglia (Extended Data Fig. 3d) that engulf apoptotic oligodendrocytes and resemble disease-associated microglia of ageing and neurodegenerating brains44. Therefore, it is possible that CD22 is upregulated as a negative feedback mechanism to restrain excessive phagocytosis in response to overwhelming cellular debris in the developing brain. During ageing, this protective developmental program might be inappropriately re-activated in response to increased myelin fragmentation and protein aggregation.

How might CD22 inhibition improve cognitive function in aged mice? Our RNA-seq analysis revealed that CD22 blockade reduces the
microglial expression of various neuroregulatory pro-inflammatory molecules. Age-related impairments in perivascular lymphatic\(^4\) and meningeal lymphatic\(^4\) systems have been shown to affect cognitive function, and CD22-regulated phagocytosis may cooperate with these clearance pathways to control debris accumulation and local cytokine concentration in the ageing brain.

Similar to their dysfunction during normal ageing, microglia in the AD brain display signs of impaired phagocytosis, and allelic variants in microglia-specific AD-risk genes modify phagocytic function\(^2\). Several human RNA-seq datasets show that CD22 is upregulated in the AD brain\(^5\), enriched in subpopulations of microglia correlated with clinicopathological decline\(^2\), and, anomalously, expressed on human oligodendrocytes\(^3\). Furthermore, a variant in the INPP5D locus, which codes for the CD22 signalling partner SHIP-1, is associated with AD risk\(^4\). Determining whether CD22 function in the CNS is conserved between mice and humans will be an important step before translational studies are initiated.

In addition to AD, recent studies have found that CD22 is highly upregulated in mouse models of amyotrophic lateral sclerosis\(^4\), a motor neuron disease, and Niemann–Pick type C\(^5\), a lysosomal storage disease. Future investigations of CD22 on microglia may inform therapeutic strategies to treat or prevent these neurodegenerative diseases via restoration of homeostasis in the CNS.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-019-1088-4.

Received: 23 August 2018; Accepted: 1 March 2019; Published online 3 April 2019.

---

1. Füger, P. et al. Microglia turnover with aging and in an Alzheimer’s model via long-term in vivo single-cell imaging. Nat. Neurosci. 20, 1371–1376 (2017).
2. Réu, P. et al. The lifespan and turnover of microglia in the human brain. Cell Rep. 20, 779–784 (2017).
3. Nimmerjahn, A., Kirchhoff, F. & Helmchen, F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308, 1314–1318 (2005).
4. Arandjelovic, S. & Ravichandran, K. S. Phagocytosis of apoptotic cells in microglia. Science 312, 405–429 (2006).
5. Schafer, D. P. et al. Microglia sculpt postnatal neural circuits in an activity and context-dependent manner. Neuron 74, 691–705 (2012).
6. Paolicelli, R. C. et al. Synaptic pruning by microglia is necessary for normal brain development. Science 333, 1456–1458 (2011).
7. Hong, S. et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. Science 352, 712–716 (2016).
8. Lui, H. et al. Progranulin deficiency promotes circuit-specific synaptic pruning by microglia via complement activation. Cell 165, 921–935 (2016).
9. Safaiyan, S. et al. Age-related myelin degradation burdens the clearance function of microglia during aging. Nat. Neurosci. 19, 995–998 (2016).
10. Deczkowska, A., Amit, I. & Schwartz, M. Microglial immune checkpoint mechanisms. Nat. Neurosci. 21, 779–786 (2018).
11. Helendeihl, J. K. et al. Homeostatic and injury-induced microglia behavior in the aging brain. Aging Cell 13, 60–69 (2014).
12. Vaughan, D. W. & Peters, A. Neurological cells in the cerebral cortex of rats from young adulthood to old age: an electron microscopy study. J. Neurocytol. 3, 405–429 (1974).
13. Tremblay, M.-É., Zettel, M. L., Ison, J. R., Allen, P. D. & Majewska, A. K. Effects of aging and sensory loss on glial cells in mouse visual and auditory cortices. Glia 60, 541–558 (2012).
14. Sierra, A., Gottfried-Blackmore, A. C., McEwen, B. S. & Bulloch, K. Microglia derived from aging mice exhibit an altered inflammatory profile. Glia 55, 412–424 (2007).
15. Hickman, S. E., Allison, K. E. & El Khoury, J. Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer’s disease mice. J. Neurosci. 28, 8354–8360 (2008).
16. Hickman, S. E. et al. The microglial sensome revealed by direct RNA sequencing. Nat. Neurosci. 16, 1896–1905 (2013).
17. Grabert, K. et al. Microglial brain region-dependent diversity and selective regional sensitivities to aging. Nat. Neurosci. 19, 504–516 (2016).
18. Morgens, D. W. et al. Genome-scale measurement of off-target activity using Cas9 toxicity in high-throughput screens. Nat. Commun. 8, 15178 (2017).

19. Haney, M. S. et al. Identification of phagocytosis regulators using magnetic genome-wide CRISPR screens. Nat. Genet. 50, 1716–1727 (2018).

20. Böhlen, C. J., Bennett, F. C. & Bennett, M. L. Isolation and culture of microglia. Cell Reports 292, 793–797 (2017).

21. Böhlen, C. J. et al. Diverse requirements for microglial survival, specification, and function revealed by defined-microcultures. Neuron 94, 759–773 (2017).

22. Butovsky, O. et al. Identification of a unique TGF-β1-dependent molecular and functional signature in microglia. Nat. Neurosci. 17, 131–143 (2014).

23. Bennett, F. C., Deans, R. M., Li, A. & Bassik, M. C. Systematic comparison of CRISPR/Cas9 and RNAi screens for essential genes. Nat. Biotechnol. 34, 634–636 (2016).

24. Macauley, M. S., Crocker, P. R. & Paulson, J. C. Siglec-mediated regulation of immune cell function in disease. Nat. Rev. Immunol. 14, 653–666 (2014).

25. Nitschke, L., Carsetti, R., Ocker, B., Köhler, G. & Lamers, M. C. CD22 is a negative regulator of B-cell receptor signalling. Curr. Biol. 7, 133–147 (1997).

26. Li, Y.-Q., Sun, L. & Li. J. Macrophagy-dependent endocytosis of Japanese flounder IgM B cells and its regulation by CD22. Fishシェルフィ免疫. 183, 134–147 (2019).

27. Linnartz-Gerlach, B., Kopatz, J. & Neumann, H. Siglec functions of microglia. Glycobiology 24, 794–799 (2014).

28. Griciuc, A. et al. Alzheimer’s disease risk gene CD33 inhibits microglial uptake of amyloid beta. Neuron 78, 631–643 (2013).

29. Bennett, M. L. et al. New tools for studying microglia in the mouse and human CNS. Proc. Natl Acad. Sci. USA 113, E1738–E1746 (2016).

30. Zhang, Y. et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J. Neurosci. 34, 11929–11947 (2014).

31. Müller, J. et al. CD22 ligand-binding and signaling domains reciprocally regulate B-cell Ca2+ signaling. Proc. Natl Acad. Sci. USA 110, 12402–12407 (2013).

32. Hudak, J. E., Canham, S. M. & Bertozzi, C. R. Glycocalyx engineering reveals a Siglec-based mechanism for NK cell immunoevasion. Nat. Chem. Biol. 10, 69–75 (2014).

33. Ñenño-Orbea, J. et al. Molecular basis of human CD22 function and therapeutic targeting. Nat. Commun. 8, 764 (2017).

34. Dahlgren, K. N. et al. Oligodendroglial and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. J. Biol. Chem. 277, 32046–32053 (2002).

35. Deczkowska, A. et al. Disease-associated microglia: a universal immune sensor of neurodegeneration. Cell 173, 1073–1081 (2018).

36. Deczkowska, A. et al. Met2Gc restrains microglial inflammatory response and is lost in brain ageing in an FKN-deficient manner. Nat. Commun. 8, 717 (2017).

37. Wang, Y. et al. TREM2 lipid sensing maintains the microglial response in an Alzheimer’s disease model. Cell 160, 1061–1071 (2015).

38. Marciniak, E. et al. The chemokine MIP-1α/CCL3 impairs mouse hippocampal synaptic transmission, plasticity and memory. Sci. Rep. 5, 18562 (2015).

39. Cole, A. J., Saffen, D. W., Baraban, J. M. & Worley, P. F. Rapid increase of an NMDA receptor activation. Proc. Natl Acad. Sci. USA 110, 12402–12407 (2013).

40. Funikov, S. Y. et al. Flavonoid activity in different molecules and its regulation by CD22. Cell Reports 29, 784–799 (2019).
Animals. Aged C57BL/6 mice (18–24 months old) were obtained from the National Institute on Aging rodent colony. Young male C57BL/6 mice (2–4 months old) were obtained from Jackson Laboratories or Charles River Laboratories. Gd2+/− mice were originally generated by L. Nitschke (University of Erlangen) and provided by J. Paulson (Scripps Institute), and R. Ballet and E. Butcher (Stanford University). All experiments used male mice, except aged wild-type vs Gd2+/− behavioural experiments, which used balanced groups of male and female mice. All animal care and procedures complied with the Animal Welfare Act and were in accordance with institutional guidelines and approved by the V.A. Palo Alto Committee on Animal Research and the institutional administrative panel of laboratory animal care at Stanford University.

Cell culture. BV2 cells were originally obtained from E. Blasi (Università di Modena e Reggio Emilia) and expanded in DMEM supplemented with 10% FBS, penicillin/streptomycin, and GlutaMAX (Thermo Fisher Scientific). HEK293T cells (ATCC) were cultured in DMEM supplemented with 10% FBS, penicillin/streptomycin, and GlutaMAX. All cells were maintained in a humidified incubator containing 5% CO2 at 37 °C.

CRISPR-Cas9 screen. The 10-sgRNA-per-gene CRISPR-Cas9 deletion library was synthesized, cloned and infected into Cas9-expressing BV2 cells as previously described. In brief, −12 million (for membrane proteins’ sub-library) or −24 million (for drug targets, kinases, phosphatases’ sub-library) BV2 cells stably expressing EF1α-Cas9-BLAST were infected with the 10 guide/sgRNA sub-libraries (Addgene 1000000122 and 1000000124) at a multiplicity of infection <1. Infected cells underwent puromycin selection (1.5 μg/ml) for 5 days after which the puro- mycin was removed and cells were suspended in normal growth media without puromycin. After selection, sgRNA infection was confirmed by flow cytometry, which indicated that >90% of cells expressed the mCherry reporter. Sufficient sgRNA library representation was confirmed by deep sequencing after selection.

Cells were cultured and maintained at 1,000 × coverage for one week. Phagocytic screening prey were prepared by labelling 3 μm amino-coated polystyrene particles (Polysciences) with CyperSE pH-dependent fluorescent dye (GE Life Sciences). Cells were switched to serum-free medium 24 h before the screen, and incubated with serum-free medium containing phagocytic prey for 1 h before sorting. For the membrane proteins screen, 12.5 million cells were sorted per replicate (>1,000 × coverage) with 55.2% in the non-phagocytic gate and 11.2% in the phagocytic gate. For the drug target, kinase, phosphatase screen, 24 million cells were sorted per replicate (>1,000 × coverage) with 53.3% in the non-phagocytic and 19.4% in the phagocytic gate. At the end of each screen, genomic DNA was extracted for all populations separately using a QIAGEN Blood Midi Kit, sgRNA sequences were analysed for CD22 expression. A standard curve was constructed to correlate % of cells expressing the mCherry reporter. Sufficient sgRNA library representation was confirmed by deep sequencing after selection.

Cells were switched to serum-free medium 24 h before the screen, and incubated with serum-free medium containing phagocytic prey for 1 h before sorting. For the membrane proteins screen, 12.5 million cells were sorted per replicate (>1,000 × coverage) with 55.2% in the non-phagocytic gate and 11.2% in the phagocytic gate. For the drug target, kinase, phosphatase screen, 24 million cells were sorted per replicate (>1,000 × coverage) with 53.3% in the non-phagocytic and 19.4% in the phagocytic gate. At the end of each screen, genomic DNA was extracted for all populations separately using a QIAGEN Blood Midi Kit, sgRNA sequences were analysed for CD22 expression. A standard curve was constructed to correlate % of cells expressing the mCherry reporter. Sufficient sgRNA library representation was confirmed by deep sequencing after selection.

Quantitative flow cytometry. For RNA-seq, all populations separately using a QIAGEN Blood Midi Kit, sgRNA sequences were amplified by PCR using common flanking primers, and indices and adaptors were ligated to libraries and subsequently fragmented. Libraries were sequenced to a depth of >30 million reads per sample. Raw sequencing files were demultiplexed using bcftools, reads were aligned using STAR, the count matrix was generated using SummarizedExperiment, and differential expression analysis was performed using DESeq2 with standard settings.

In vitro phagocytosis assay. In vitro phagocytosis assays, BV2 cells were split into a 24-well plate 24 h before feeding to a density of 50,000 cells per well in serum-free medium. Following specific treatments, wells were washed twice with PBS and fed pHrodo red-labelled 3 μm amino-coated polystyrene particles (Polysciences) at a ratio of 10 particles per cell. Four phase and red-fluorescent images were per well were acquired every hour for 24 h using the Incucyte S3 live-cell analysis system (Essen Bioscience). For each time point, normalized phagocytosis was calculated using the following formula: (red object area)/(phase confluence). To combine data from independent experiments, we calculated phagocytosis relative to control by using the following formula: (red object area)/(phase confluence). To combine data from independent experiments, we calculated phagocytosis relative to control by using the following formula: (red object area)/(phase confluence).

After normalization and pooling, libraries were sequenced on a HiSeq 4000 (Illumina) using paired-end 100-bp reads. Libraries were sequenced to a depth of >30 million reads per sample. Raw sequencing files were demultiplexed using bcftools, reads were aligned using STAR, the count matrix was generated using SummarizedExperiment, and differential expression analysis was performed using DESeq2 with standard settings.

Flow cytometry. Antibodies to CD11b (clone M1/70, BioLegend) and CD45 (clone 30-F11, Biolegend) were used for microglia identification (CD11b+CD45+). For primary microglia immunophenotyping, the following leucins and antibodies were used: biotinylated Sambucus nigra agglutinin (SNA, Vector Labs), biotinylated Maackia amurensis agglutinins (MAA-1 and MAA-2, Vector Labs), biotinylated Erythrina cristagalli lectin (ECL, EY Labs), biotinylated wheat germ agglutinin (WGA, Vector), recombinant mouse Siglec-E (R&D Systems), recombinant mouse Siglec-H (clone SIG1, Biolegend) and CD33 (clone 9A11, eBioscience). For immunostaining, cells were passaged through a 100-μm strainer, blocked for 10 min on ice with mouse Fc-blocking reagent (BD Biosciences), and stained for 30 min on ice in PBS supplemented with 0.5% bovine serum albumin. When biotinylated antibodies (or lectins) were used for immunostaining, cells were stained with an anti-biotin Alexa Fluor 647 (Biolegend) for 15 min on ice following primary stain. When recombinant SiglecH were used, they were precomplexed with APC–Cy7-conjugated anti-human IgG Fc (Biolegend) on ice at 10 μg/ml each. The complex was used at a final staining concentration of 5 μg/ml. Live cells were identified using Sytox Blue viability dye. Flow cytometry analysis was performed on a BD LSRSort and sorting was performed on a BD FACSAria III. Data were analysed using FlowJo software (TreeStar).

RNAseq in situ hybridization. RNA in situ hybridization was performed on fresh frozen brain tissue using the Multiplex Fluorescence v2.1 kit (Advanced Cell Diagnostics) according to the manufacturer’s protocol. Probes for CD22 and TNEMI19 were commercially available from the manufacturer.

Lentivirus production and infection. HEK293T cells were transfected with the lentiviral packaging plasmids and sgRNA-containing plasmids. Supernatant from infected cells was harvested at 48 h and 72 h and concentrated with Lenti-X solution (Clontech). BV2 cells stably expressing Cas9 endonuclease under blasticidin (1 μg/ml) selection were infected with lentiviruses containing sgRNA plasmids with puromycin...
For sialidase treatment, cells were incubated with 400 nM Vibrio cholerae neuraminidase (MOPC21, BioXCell) for 1 h, followed by PBS washes and staining with mouse monoclonal anti-CD22 (Cy34, BioXCell) and rat anti-CD22 (OX-96, Bio-Rad). All antibodies were pre-labelled with IncuCyte FabFluor-pH Red (Essen Bioscience) according to the manufacturer's protocol. Nine phase and red fluorescent images per well were acquired every hour for 24 h using the IncuCyte S3 live-cell analysis system (Essen Bioscience). For each time point, we calculated antibody internalization using the following formula: (Red object area/Phase contrast object area) × 100. Technical triplicates for each experiment were averaged and reported.

**Immunohistochemistry.** Mice were euthanized with 2.5% (v/v) Avertin and transcardially perfused with ice-cold HBSS containing glucose and HEPES, or with 4% PFA. Hemibrains were post-fixed in 4% PFA at 4 °C overnight before preservation for 30% sucrose-substituted for 1-2 h. The hemibrains were sectioned into 40-μm coronal slices on a vibratome and stored in cryoprotective solution at −20 °C. Free-floating sections were permeabilized, blocked, and stained overnight at 4 °C with the following primary antibodies at the designated concentrations: goat anti-IBA1 (1:500, ab5076, Abcam), goat anti-P-ROX1 (1:500, AF2727, R&D), rabbit anti-c-Fos (1:400, 9F6, Cell Signaling), rabbit anti-αI3 (1:200, D54D2, Cell Signaling), rabbit anti-α-syntrophin (1:200, MFJF1, Abcam), goat anti-p-CREB (1:500, 6-019, Millipore), rabbit anti-synaptophysin (1:500, DS8F61H, Cell Signaling), rabbit anti-PSD95 (1:500, D27E11, Cell Signaling), rabbit anti-C1q (1:1200, ab182451, Abcam), goat anti-doublecortin (1:500, SC8066, Santa Cruz Biotech), rabbit anti-CD19 (1:500, D4V4B, Cell Signaling) and rabbit anti-TMEM119 (1:100, 28-3, Abcam). Sections were washed, stained with Alexa Fluor-conjugated secondary antibodies (1:250), mounted and set under a coverslip before imaging on a confocal laser-scanning microscope (Zeiss LSM880). Thioflavin S staining was performed after secondary antibody staining. In brief, sections were stained in a 0.1% (w/v) solution for 5 min, followed by 3 washes in 50% EtOH, and a 30 min rehydration in diH2O before mounting. All analyses were performed by a blinded observer.

**Preparation of fluorescent phagocytic material.** Purified CNS myelin was isolated as previously described 32. In brief, brains were harvested and their brains were homogenized. Crude brain homogenate was subjected to a series of sucrose gradients under ultracentrifugation followed by osmotic shocks in hypotonic buffers to remove myelin sheaths from severed axons. αI3 oligomers were prepared as previously described 35. In brief, αI3 (Anaspec) was treated with hexafluoroor sopanol to achieve a monomeric solution. Oligomerization was induced by diluting a 5 mM monomeric αI3 DMSO stock to 200 μM in ice-cold PBS and incubating overnight at 4 °C. Pre-formed α-syntrophin fibrils were prepared as previously described 35. Phagocytic material was conjugated to fluorescent dyes as previously described 21. For double-labelled myelin, 50 mg/ml myelin prep was incubated with equimolar concentrations of AlexaFluor555 NHS ester and CypherSE NHS ester in PBS with 0.1 M sodium bicarbonate for 45 min at room temperature. Myelin was washed 4 times with PBS and centrifugation to remove free dye. For αI3 and α-syntrophin, 100 μl of the 200 μM protein aggregates were incubated with 4 μl CypherSE NHS ester in PBS with 0.1 M sodium bicarbonate for 30 min at room temperature. Free dye was removed using BioSpin Micro P-6 desalting columns (BioRad).

**Ex vivo microglial phagocytosis assay.** Whole-brain myelin-depleted single-cell suspensions were prepared as described above. Next, a rough estimation of microglia counts from each brain was determined by strictly gating a volume-designated aliquot of the suspensions by forward scatter/ side scatter on a flow cytometer (BD Accuri C6). Each suspension was adjusted to contain equal microglia per unit volume. Then, single-cell brain suspensions were treated accordingly for 1 h in serum-free DMEM-F12 at 37 °C with periodic agitation. Treatments were washed out and cells were resuspended in FACS buffer containing pHero- or CypherSE-conjugated particles at a ratio of 50 particles per microglia. The cell–particle mixture was incubated for 1 h at 37 °C. The mixture was washed once and labeled using anti-CD11b–Cy5 at the ratio of 1:200 and AlexaFluor488 at the ratio of 1:500. Anti-CD11b fluorescence was stopped by transferring the suspensions to ice, where the cells were stained with antibodies to distinguish microglia. Microglial phagocytosis was assessed by flow cytometry by pre-gating live CD11b+CD45+ cells and assessing pH-dependent
On day 2, mice were subjected to two trials. In the first trial assessing contextual and 1,000-Hz tone followed by a 2-s foot shock (0.6 mA), with a 180-s interval. The labelled antibodies were loaded into 200-µl osmotic pumps (Alzet/Durect) with a 28-day infusion rate of 0.25 µl per h. An infusion rate was chosen to maintain a steady state concentration of 10 µg/ml, a dose shown to promote phagocytosis with this particular antibody ex vivo. Osmotic pumps were connected to a cannula (Brain infusion kit III, Alzet) inserted at +1 mm lateral, −0.3 mm anterior–posterior, and −3 mm deep relative to bregma in order to target the right lateral ventricle. The pump was placed subcutaneously and mice received post-surgical buprenorphine and Baytril. Mice were randomized to either the IgG or the anti-CD22 group.

**Quantification of antibody leakage into the periphery following intracerebroventricular administration.** One milligram of CD22 antibody was incubated with 30 × molar ratio of trans-cyclooctene (TCO) NHS ester (Click Chemistry Tools) overnight at 4 °C before being quenched with 1 M Tris pH 8.0 and desalted (Zeba, 40 kDa MWCO, Thermo). Mice were administered unlabelled or TCO-labelled CD22 antibody via osmotic pump intracerebroventricularly as detailed above, or with equimolar amounts intravenously (retro-oral) or intraperitoneally. Seven days later, blood was collected with EDTA anticoagulant by terminal intracardial bleeding. EDTA-plasma was isolated by centrifugation at 1,000 g for 15 min at 4 °C before aliquoting, flash freezing, and storage at −80 °C. Protein concentrations of plasma samples and unlabelled and TCO-labelled CD22 antibodies were measured with a BCA Protein Assay Kit (Pierce). Plasma (75 µg) was aliquoted, denatured in 1% SDS, and incubated for 90 min in the dark at room temperature with 6 µM SiR-tetrazine (Spirochrome) for subsequent click chemist-try detection by in gel fluorescence. Decreasing amounts (10 ×) of unlabelled and TCO-labelled CD22 antibody—from 1 µg to 0.1 ng—were processed similarly as a fluorescence standard. A 4 × stock solution of NuPAGE LDS (Thermo) was added to each sample before heating at 95 °C for 5 min. Proteins were briefly spun and separated by electrophoresis in 12% Bis–Tris polyacrylamide gels (Invitrogen). Gels were washed twice in distilled water for 15 min before SiR imaging in the 700-nm channel of an Odyssey CLx (LI-COR). To ensure accurate protein loading, gels were incubated with GelCode Blue Stain Reagent (Thermo) overnight before destaining in distilled water for at least 3 h and imaged in the 800-nm channel of an Odyssey CLx (LI-COR). CD22 fluorescence signals were quantified in accompanying Image Studio software (LI-COR).

**Behavioural assays.** The forced alternation Y-maze was performed as previously described with minor alterations. In brief, the test consisted of a 5-min training trial followed by a 5-min retrieval trial, with a 1-h inter-trial interval. For the training trial, one arm of the Y maze was blocked off and mice were allowed to explore the two open arms. One hour later, the mouse was again placed in the Y maze with all arms open and a black and white pattern placed at the end of the novel arm. Between mice and trials, the maze was wiped with ethanol to remove odour cues. For analysis, video was analysed by a blinded observer and both number of arm entries and time spent in each arm were quantified. Mice with less than two arm entries in the first minute of the retrieval trial were excluded from the analysis.

The fear-conditioning paradigm was performed as previously described. In brief, mice were trained to associate cage context or an audiovisual cue with an aversive stimulus (foot shock). The test was administered over two days. On day 1, mice were placed in a cage and exposed to two periods of 30 s of paired cue light and 1,000-Hz tone followed by a 2-s foot shock (0.6 mA), with a 180-s interval. On day 2, mice were subjected to two trials. In the first trial assessing contextual memory, mice were re-exposed to the same cage context, and freezing behaviour was measured during minute 1–3 using a FreezeScan tracking system (Cleversys). In the second trial measuring cued memory, mice were placed in a novel context and exposed to the same cue light and tone from day 1 after 2 min of exploration. Freezing behaviour was measured for 1–3 min following the cue. No significant differences in cued fear conditioning were observed between groups, consistent with previous rejuvenation studies from our laboratory.

**Assessment of the cognitive effects of systemically administered anti-CD22.** Aged (18-month-old) mice were intraperitoneally injected with 300 µg anti-CD22 (Cy3, BioXCell) or mouse IgG1 isotype control (MOPC21, BioXCell) twice weekly for one month, followed by cognitive assessment using the forced alternation Y-maze and contextual-fear-conditioning paradigms described above.

**Gene Ontology analysis.** For Gene Ontology analysis of CRISP–Cas9 screen hits, Panther was used to assess statistical overrepresentation of hits in the Reactome pathway database given a reference list containing all genes targeted by the sgRNA library. For analysis of RNA-seq data, Enrichr was used to assess enrichment of significantly up- or downregulated genes in KEGG, BioCarta, WikiPathways, and Reactome databases. Pre-ranked gene set enrichment analysis (GSEA) was performed with GSEA software v3 (Broad Institute) with default settings and signed log-normalized P values as a ranking metric.

**Ex vivo microglia secretome profiling.** Microglia from aged (20–24-month-old) mice were isolated as described above, with minor modifications. Namely, following myelin depletion, cells were incubated with CD11b-positive enrichment beads (Millipore), isolated by magnetic selection using LS-columns, and plated at 30,000 cells per well of a 96-well plate. Microglia were maintained in serum-free defined medium as previously described, and treated with either IgG (MOPC21, BioXCell, 20 µg/ml) or anti-CD22 (Cy3, BioXCell, 20 µg/ml) in the presence or absence of Aβ oligomers (5 µM) for 8 h. Following incubation, supernatant was collected, spun down to remove cells, and flash frozen on dry ice. A bead-based immunoassay for mouse cytokines and chemokines (Eve Technologies) was used for protein detection.

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

**Data availability** RNA-seq datasets have been deposited online in the Gene Expression Omnibus (GEO) under accession numbers GSE127542 and GSE127543.
Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | CRISPR screen for genetic modifiers of phagocytosis. a, Intersection and disjunction of 361 genes involved in phagocytosis expressed (fragments per kilobase of transcript per million mapped reads, FPKM >5) by BV2 cells (PRJNA407656) and primary microglia. b, Gating scheme for FACS separation of phagocytic and non-phagocytic BV2 cells, treated with vehicle (grey) or the actin-polymerization inhibitor, cytochalasin D (red). c, Time-lapse microscopy readout of phagocytosis by BV2 cells treated with vehicle (grey) or cytochalasin D (red) (n = 3, mean ± s.e.m.). d, e, Results from CRISPR–Cas9 screen targeting 954 membrane proteins (d) or 2,015 drug targets, kinases and phosphatases (e) in BV2 cells. Knockouts that promote phagocytosis (red) have a positive effect size and knockouts that inhibit phagocytosis (blue) have a negative effect size (screen performed in technical duplicate; dotted line, P = 0.05, two-sided t-test). f, g, Distributions of negative control sgRNAs (grey) and RAB9-targeting (f) or CMAS-targeting (g) sgRNAs (blue). Positive values indicate enrichment in the phagocytic fraction, and negative values indicate enrichment in the non-phagocytic fraction. h, Statistical overrepresentation test showing enrichment of Reactome pathway annotations within phagocytosis-promoting (red) and -inhibiting (blue) hits (Fisher’s exact test). i, CD22 expression in wild-type (blue), CD22-KO (green) and isotype-control stained (black) BV2 cells assessed by flow cytometry. j, Confluence of control (grey) and CD22-KO (green) BV2 cells during time-lapse microscopy phagocytosis assays (n = 3, mean ± s.e.m.). k, Number of beads ingested per cell were calculated in control and CD22-KO BV2 cells after 8 h of phagocytosis. While CD22-KO cells display enhanced phagocytosis at a population level (n = 3, *P < 0.05, two-sided t-test, mean ± s.e.m.), we observed no significant differences in the number of beads ingested per cell (two-way ANOVA). Red dot represents mean phagocytic index of the entire cell population. Data in b, c, i–k were replicated in at least two independent experiments.
Extended Data Fig. 2 | Immunophenotyping of sialic acid-related molecules on young and aged microglia. a–c, e, f. Flow cytometry analysis of young (red) and aged (blue) microglia for expression of fluorescence minus one (FMO) background fluorescence (a), plant-derived lectin ligands (b), conserved Siglecs (c), mouse-specific CD33-related Siglecs (e) and recombinant Siglec ligands (f). MFI shown on a biexponential scale. d. Microglia from wild-type or Cd22−/− aged mice were stained with the particular anti-CD22 clone (Ox97) used for immunophenotyping. Cd22−/− microglia show no staining relative to FMO. g. Gating strategy to immunophenotype microglia while minimizing autofluorescence. All data were replicated in at least two independent experiments.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Protein and transcript expression of CD22 in microglia. a, Flow cytometry gating scheme for analysis of CD22 expression in peripheral blood-derived myeloid cells (CD45⁺ CD11b⁺), immature B cells (CD45⁺ B220⁻ CD22lo) and mature B cells (CD45⁺ B220⁺ CD22hi). Quantibrite beads are shown in the top right panel. b, Quantification of flow cytometry analysis showing the number of CD22 molecules on various cell types, interpolated from the Quantibrite bead standard curve (n = 3, mean ± s.e.m.). c, CD22 expression in various cell types of the young mouse CNS, showing exclusive expression in microglia. Data from Barres laboratory RNA-seq (http://www.brainrnaseq.org/). d, t-distributed stochastic neighbor embedding (t-SNE) plot showing scRNA-seq of CD22 expression in microglia isolated from E14.5, P7 and adult mouse brains. CD22 is enriched in a subpopulation of P7 microglia. Data from ref. 44 (https://myeloidsca.appspot.com/). e, t-SNE plot showing scRNA-seq analysis of CD22 expression in cells from 20 different mouse tissues. CD22 is expressed in B cells and microglia, but is absent from non-myeloid brain cells (n = 7, young mice). Data from the Tabula Muris Consortium64. f, Violin plots of log-normalized CD22 counts per million reads (CPM) showing high expression in B cells from multiple organs and in microglia (n = 7, young mice). Data from the Tabula Muris Consortium. Data in a, b were replicated in at least 2 independent experiments.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Sialic acid-CD22-SHP1 signalling regulates phagocytosis. a, *S. nigra* agglutinin (SNA, recognizes α2,6-linked sialic acid) and *Maackia amurensis* agglutinin II (MAA II, recognizes α2,3-linked sialic acid) ligand expression in wild-type BV2 cells (orange), wild-type BV2 cells pretreated with sialidase (blue), and CMAS-KO cells (red) assessed by flow cytometry. Sialidase treatment and CMAS-KO reduce sialic acid ligands on the cell surface. b, Western blot showing SHP1 protein expression in wild-type and PTPN6-KO BV2 cells. For raw source image, see Supplementary Fig. 1. c, Confluence of control (grey), CMAS-KO (red), and PTPN6-KO (blue) BV2 cells during time-lapse microscopy phagocytosis assays (n = 3, mean ± s.e.m.). d, e, Phagocytosis of pH-sensitive fluorescent beads by untreated (black) and sialidase-treated (red) BV2 cells (d) or vehicle-treated and 3FAX-Neu5Ac-treated BV2 cells (e) before phagocytosis (n = 3, **P < 0.005, two-sided t-test; mean ± s.e.m.). f, Phagocytosis of pH-sensitive fluorescent beads by wild-type (black), wild-type + sialidase (red), CD22-KO (blue), or CD22-KO + sialidase (green) BV2 cells (n = 3, *P < 0.05, one-way ANOVA with Tukey’s multiple comparisons correction; mean ± s.e.m.). g, Microglia were acutely isolated from the brains of aged (18-month-old) wild-type (left) or Cd22<sup>−/−</sup> (right) mice, treated with or without sialidase, and incubated with pH-sensitive fluorescent latex beads. Microglia specific phagocytosis was measured using flow cytometry (n = 6, *P < 0.05, paired two-sided t-test). h, Representative images of BV2 cells coated with AlexaFluor 488-conjugated glycopolymer (green) and stained with a plasma-membrane-specific dye (CellMask, red) showing overlap (orange). Scale bars, 25 μm. i, Recombinant mouse CD22-human Fc fusion protein was pre-complexed with AF647 anti-human Fc secondary antibody, treated with various concentrations of IgG (black) or anti-CD22 (blue, red), and subsequently allowed to bind to ligands on the surface of BV2 cells or BV2 cells pretreated with sialidase (red). Binding was measured by flow cytometry. j, Internalization of IgG (black), function blocking anti-CD22 (clone Cy34.1, blue), and non-function-blocking anti-CD22 (clone OX96, green) conjugated to a pH-sensitive fluorescent dye by BV2 cells assessed by time-lapse microscopy (n = 3, mean ± s.e.m.). k, Western blot quantification of ratio of active p-SHP1 to total SHP1 protein in BV2 cells pretreated with various concentrations of anti-CD22. Blue line represents the fitted variable slope inhibitor-response curve. For raw source image, see Supplementary Fig. 1. All data were replicated in at least two independent experiments.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Validation of in vivo phagocytosis assay.

a, Representative images of myelin labelled with a pH-sensitive fluorescent dye (CypHer5E, white), a constitutively fluorescent dye (AF555, red) and stained for IBA1 (green). The majority of AF555 overlapping with IBA1 is also positive for CypHer5E, indicating localization to an acidified compartment. Scale bars, 100 μm. b, 3D reconstruction of a microglial cell (IBA1, green) with ingested myelin (CypHer5E and AF555, white and red, yellow arrow) near un-ingested myelin (AF555, red, white arrow). Scale bar, 5 μm. c, Microgliosis, as assessed by percentage of IBA1⁺ area at the injection site, was not altered by CD22 blockade (n = 8, paired two-sided t-test). d, Representative images of myelin (red) overlaid with the myeloid marker IBA1 (green) at the injection site of IgG (left) or PBS (middle) treated hemispheres of the same aged brain, or an image of a stab wound control (not injected with myelin). Scale bars, 100 μm. e, Microgliosis, as assessed by percentage of IBA1⁺ area at the injection site, was not altered by IgG compared to the stab wound control (n = 2, paired two-sided t-test). f, Clearance of myelin debris in the IgG (black) or PBS (blue) treated hemispheres assessed 48 h post-injection (n = 4, paired two-sided t-test). g, Representative images of IBA1 (grey), a macrophage marker, and TMEM119 (magenta), a microglia-specific marker, at the injection site in IgG (left) or anti-CD22 (right) treated hemispheres of the same aged brain. Scale bars, 100 μm. h, Percentage of IBA1⁺ phagocytes expressing TMEM119 at the injection site (n = 4, paired two-sided t-test). i, Clearance of myelin debris in young (2.5-month-old) wild-type (black) or Cd22⁻/⁻ (blue) mice was assessed 48 h after injection (n = 4, two-sided t-test; mean ± s.e.m.). j, Representative images of total Aβ (white), thioflavin S⁺ fibrillar Aβ (green) and IBA1 (red) in transgenic mice expressing human APP with Swedish and London familial AD mutations (left) or wild-type mice injected with Aβ oligomers 48 h before analysis (right). k, Representative images of Aβ (red, left column) and Aβ overlaid with the myeloid marker IBA1 (green, right column) at the injection site (±2 mm lateral, 0 mm A–P, −1.5mm D–V, relative to bregma) of IgG (top row) or anti-CD22 (bottom row) treated hemispheres of the same aged brain. Scale bars, 100 μm. l, Microgliosis, as assessed by percentage of IBA1⁺ area at the Aβ oligomer injection site, was not altered by CD22 blockade (n = 8, paired two-sided t-test). m, Representative images of α-synuclein and IBA1 at the injection site in IgG and anti-CD22 treated mice. Scale bars, 100 μm. n, Clearance of α-synuclein fibrils in the IgG (black) or anti-CD22 (green) treated hemispheres assessed 48 h post-injection (n = 7, *P < 0.05, paired two-sided t-test). o, Microgliosis, as assessed by percentage of IBA1⁺ area at the α-synuclein fibril injection site, was not altered by CD22 blockade (n = 7, paired two-sided t-test). All data were replicated in at least two independent experiments.
Extended Data Fig. 6 | Specificity and distribution of long-term, CNS-targeted antibody infusion via osmotic pump. a, Representative images of CD19⁺ B cells (red, top row), DAPI (blue, middle row), and merged (bottom row) in the spleen (left, positive control) and hippocampus (right) of a mouse treated with anti-CD22 via intracerebroventricular osmotic pump. b, Concentration of trans-cyclooctene-labelled anti-CD22 in the plasma seven days after administration of 200 μg anti-CD22 via intraperitoneal injection (n = 1) or intracerebroventricular osmotic pump infusion (n = 4), assessed by in-gel fluorescence and quantification based on a standard curve (mean ± s.e.m.). For raw source image, see Supplementary Fig. 1. c, Representative images of coronal brain sections of untreated (left column) and IgG treated (right column) mice. IgG was labelled with an Alexa Fluor 647–NHS ester (top row, white) to assess antibody distribution throughout the brain (bottom row, DAPI, blue). In addition to the para-ventricular areas, antibodies penetrated the thalamus and hippocampus. d, Flow cytometry analysis of Alexa Fluor 647-labelled antibody on microglia isolated from untreated (black), IgG (red) or anti-CD22 (blue) infused mice. Microglia from anti-CD22 treated mice display elevated Alexa Fluor 647 signal, indicative of antibody target engagement. Data in a, c and d were replicated in at least two independent experiments.
Extended Data Fig. 7  |  Anti-CD22 treatment partially reverses age- and disease-related microglia transcriptional signatures.  

**a**, Venn diagram showing the lack of any intersection among 315 genes differentially expressed between IgG \( (n = 7) \) and anti-CD22 \( (n = 7) \) treated microglia and 40 genes differentially expressed between untreated \( (n = 2) \) and IgG \( (n = 7) \) treated microglia at an FDR cutoff of 10% (Benjamini–Hochberg method). **b**, Hierarchical clustering of normalized read counts from IgG and anti-CD22 treated microglia, normalized by row mean. The top-100 differentially expressed genes are shown \( (n = 7) \). **c**, Enrichr Gene Ontology analysis of genes upregulated (red) and downregulated (blue) by anti-CD22 treatment (Fisher’s exact test, Benjamini–Hochberg FDR). **d**, GSEA showing normalized enrichment score for microglia genes modulated by anti-CD22 treatment within the gene signature for: ageing microglia (this study), disease-associate microglia\(^6\) (DAM), microglial neurodegenerative phenotype\(^6\) (MGnD), and microglia from lipopolysaccharide treated mice\(^2\) (LPS) (*FDR < 0.05"). **e–h**, GSEA showing enrichment distribution for microglia genes modulated by anti-CD22 treatment within the gene signature for ageing microglia (e), DAM (f), MGnD (g), and LPS-activated microglia (h).
Extended Data Fig. 8 | Protein-level assessment of long-term CD22 blockade in the hippocampus. a, Western blot for SALL1 and α-tubulin (loading control) in whole-hippocampus lysates from IgG and anti-CD22 treated mice. For raw source image, see Supplementary Fig. 1. b, Quantification of blot in a, showing upregulation of SALL1 protein in anti-CD22 hippocampi \((n = 3, \ast P < 0.05, \text{two-sided } t\text{-test})\), mean ± s.e.m.). c, Protein concentration of CCL3 in the supernatant of acutely isolated aged microglia treated for 8 h with IgG or anti-CD22 in the absence or presence of Aβ oligomers \((n = 4, \ast P < 0.05, \text{ANOVA with Sidak’s multiple hypothesis correction, mean ± s.e.m.)}\). d, Representative images of p-CREB expression (red) in the dentate gyrus of IgG (left) and anti-CD22 (right) treated mice. e, Quantification of p-CREB mean intensity in the dentate gyrus of IgG (black) and anti-CD22 (green) treated mice \((n = 7, \text{two-sided } t\text{-test, mean ± s.e.m.})\). f, Quantification of total doublecortin-positive cells in three equally-spaced dentate gyrus sections of IgG (black) and anti-CD22 (green) treated mice \((n = 3, \text{N.S.}\) not significant, two-sided \(t\text{-test, mean ± s.e.m.})\). g-i, Quantification of C1q mean intensity (g) and synaptophysin (h; pre-synaptic marker) or PSD-95 (i; post-synaptic marker) density in the hippocampus of IgG (black) and anti-CD22 (green) treated mice \((n = 3, \text{two-sided } t\text{-test, mean ± s.e.m.})\). All data were replicated in at least two independent experiments.
Extended Data Fig. 9 | Cognitive effects of systemically administered anti-CD22. a, Working memory and exploratory behaviour in aged (18-month-old) mice treated with IgG (black) or anti-CD22 (green) via intraperitoneal injection twice weekly for one month as assessed by percentage of time spent in the novel arm in a forced alternation Y-maze test (n = 6, two-sided t-test, mean ± s.e.m.). b, Contextual memory aged (18-month-old) mice treated with IgG (black) or anti-CD22 (green) via intraperitoneal injection twice weekly for one month as assessed by percentage of time displaying freezing behaviour in a contextual-fear-conditioning test (n = 6, two-sided t-test, mean ± s.e.m.).
Extended Data Fig. 10 | Graphical abstract. Microglial phagocytosis declines with age, accompanied by increased CD22 expression. CD22 blockade restores phagocytosis, promotes a homeostatic transcriptional state, and improves cognitive function in aged mice.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- n/a
- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- 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

- FACS Diva (BD)
- ZEN Black (Zeiss)
- Imaris (Bitplane)
- Incucyte S3 (Essen)
- ImageStudio (LI-COR)

Data analysis

- FlowJo (Treestar)
- Prism 7 (GraphPad)
- R (DESeq2, ggplot2, tidyverse)
- ImageJ
- GSEA v3 (Broad)
- Enrichr
- Panther
- casTLE

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

Raw sequencing data will be deposited in NCBI GEO. Raw CRISPR screen count data (Supp Table 1), analyzed CRISPR hits (Supp Table 2), RNA-seq (Supp Table 3 and 4) are provided.

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
Life sciences study design

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

Sample size
No power analyses were used to predetermine sample sizes. However, sample sizes were chosen based on prior literature using similar experimental paradigms.

Data exclusions
For long-term osmotic pump infusions, 4 mice were excluded and euthanized prior to experiment endpoint due to post-operative wound dehiscence. These exclusion criteria were predetermined as per relevant animal protocols.

Replication
For in vitro experiments, technical triplicates as well as independent experiments on separate days were performed to ensure reproducibility. For in vivo experiments, biological replicates as well as independent cohorts of mice were used. CRISPR screen data, RNA-seq data, and aged CD22−/− behavioral data were not replicated in independent experiments due to resource restrictions. All other data were replicated in at least two independent experiments as stated in figure legends.

Randomization
For long-term osmotic pump infusions, mice were randomized into 2 groups using a random list generator (random.org).

Blinding
All immunohistochemical and behavioral analyses were performed by a blinded observer.

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

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Antibodies            |
| ☑   | Eukaryotic cell lines |
| ☑   | Palaeontology         |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |
| ☑   | Clinical data         |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |

Antibodies

Antibodies used
Flow cytometry: CD11b (clone M1/70, BioLegend, cat. no. 101205, 1:100), CD45 (clone 30-F11, Biolegend, cat. no. 147708, 1:100), Siglec-1 (clone REA197, Miltenyi Biotec, cat. no. 130-105-004, 1:10), CD22 (clone OX-97, Biolegend, cat. no. 126112, 1:100), Siglec-E (clone M1304A01, Biolegend, cat. no. 677106, 1:100), Siglec-F (clone ES22-10D8, Miltenyi Biotec, cat. no. 130-102-241, 1:10), Siglec-G (clone SH1, BD Biosciences, cat. no. 563336, 1:100), Siglec-H (clone SS1, Biolegend, cat. no. 129612, 1:100), CD33 (clone 9A11, ebioScience, cat. no. 17-0331-82, 1:100)
IHC: goat anti-Iba1 (1:500, ab5076, Abcam), goat anti-Prox1 (1:500, AF2727, R&D), rabbit anti-c-Fos (1:400, 9F6, Cell Signaling), rabbit anti-amyloid beta (1:200, DS402, Cell Signaling), rabbit anti-alpha-synuclein (1:200, MF1R, Abcam), rabbit anti-p-CREB (1:500, 06-519, Millipore), rabbit anti-synaptophysin (1:500, D8F6H, Cell Signaling), rabbit anti-PSD95 (1:500, D27E11, Cell Signaling), rabbit anti-C1q (1:1200, ab182451, Abcam), goat anti-doublecortin (1:500, SC8066, Santa Cruz Biotech), anti-Tmem119 (1:200, 28-3, Abcam)
WB: mouse anti-a-tubulin (1:10,000, T9026, Sigma), rabbit anti-pSHP-1 (1:5,000, D11G5, Cell Signaling), anti-SHP-1 (1:10,000, C14H6, Cell Signaling), anti-Sall1 (1:1000, ab31526, Abcam)
In vivo: mouse IgG1 isotype control antibody (MOPC21, BioXCell, cat. no. BE0083, 1mg/mL) or anti-CD22 (Cy34, BioXCell, cat. no. BE0011, 1mg/mL)

Validation
All antibodies were validated for the indicated applications by the manufacturer. For the anti-CD22 flow cytometry antibody (Ox97), we confirmed specificity using a KO control. For the anti-CD22 therapeutic antibody (Cy34) used in vivo, we confirmed function-blocking activity in ligand binding, signaling, and internalization assays.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)
BV2 cells (E. Blasi), HEK293T (ATCC)

Authentication
Cell line authentication was performed by the supplier, but not independently authenticated in our lab.
Mycoplasma contamination  
Cell lines were tested for mycoplasma bi-annually.

Commonly misidentified lines  
(See ICLAC register)  
No commonly misidentified lines were used.

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

| Laboratory animals          | Mus musculus, male, C57Bl/6, aged (18-24 months from NIA rodent colony), young (2-4 months from Charles River or Jax), CD22/- from Scripps Research Institute (J. Paulson) |
| Wild animals                | This study did not involve wild animals. |
| Field-collected samples     | This study did not involve field-collected samples. |
| Ethics oversight            | Institutional Animal Care and Use Committee at Stanford University |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☑️ 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  
For immunostaining, cells were passed through a 100 micron strainer, blocked for 10 minutes on ice with mouse Fc-blocking reagent (BD), and stained for 30 minutes on ice in PBS supplemented with 0.5% bovine serum albumin.

Instrument  
FACS: BD FASCara III
Analysis: BD LSRII, Accuri C6

Software  
BD FACS Diva, FlowJo

Cell population abundance  
For immunophenotyping, at least 5,000 cells of the population of interest were analyzed.
For sorting for RNA-seq, at least 10,000 microglia were collected.

Gating strategy  
Positive and negative gates were set using fluorescence minus one (FMO) background intensity controls. Fluorophores were chosen to minimize spectral overlap.

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