Visceral obesity increases risk of cognitive decline in humans, but subcutaneous adiposity does not. Here, we report that beige adipocytes are indispensable for the neuroprotective and anti-inflammatory effects of subcutaneous fat. Mice lacking functional beige fat exhibit accelerated cognitive dysfunction and microglial activation with dietary obesity. Subcutaneous fat transplantation also protects against chronic obesity in wildtype mice via beige fat-dependent mechanisms. Beige adipocytes restore hippocampal synaptic plasticity following transplantation, and these effects require the anti-inflammatory cytokine interleukin-4 (IL4). After observing beige fat-mediated induction of IL4 in meningeal T-cells, we investigated the contributions of peripheral lymphocytes in donor fat. There was no sign of donor-derived lymphocyte trafficking between fat and brain, but recipient-derived lymphocytes were required for the effects of transplantation on cognition and microglial morphology. These findings indicate that beige adipocytes oppose obesity-induced cognitive impairment, with a potential role for IL4 in the relationship between beige fat and brain function.
The prevalence of obesity is increasing rapidly, and in the context of an aging population, the obesity epidemic has the potential to exacerbate projected increases in age-related cognitive decline and dementia. Visceral adiposity at midlife predicts subsequent rates of dementia independently of weight loss during the intervening decades, suggestive of a critical window for dysfunction in the adult brain. Fewer studies have focused on subcutaneous adiposity and cognitive decline, but cross-sectional data suggest that the “pear-shaped” distribution of body fat does not increase rates of mild cognitive impairment or dementia, and may be protective. Vulnerability to age-related cognitive decline with visceral or subcutaneous adiposity, therefore, follows the same pattern as risk for metabolic complications, but mechanism(s) linking subcutaneous adiposity with memory have yet to be identified.

Unlike visceral fat, which contains a homogeneous population of white adipocytes, subcutaneous fat contains both white adipocytes and “beige” adipocytes that expend energy in a manner analogous to brown fat. Beige adipocytes interact continuously with immune cells, and the acquisition of thermogenic features (beiging) requires induction of the anti-inflammatory cytokines interleukin-4 (IL4) by leukocytes in subcutaneous adipose tissue (SAT). Given the importance of IL4 signaling for allergic responses and autoimmunity, circulating concentrations of IL4 are tightly regulated. However, tissue-resident immune cells are capable of efflux and migration to other organs, including the brain and leptomeninges. Immune cell trafficking and local signaling at the blood–brain and blood–cerebrospinal fluid interfaces, therefore, enables inter-organ crosstalk independently of circulating factors or direct access to the brain parenchyma. Although peripheral macrophages gain access to the brain with chronic obesity, less is known about earlier neuroimmune interactions at the blood–brain and blood–cerebrospinal fluid interfaces. Moreover, despite emerging roles in other chronic inflammatory diseases, T-lymphocytes have received less attention with respect to their protective or pathogenic roles in the brain with obesity.

Here, we investigated immunoregulatory interactions between beige adipocytes and cognition through a series of dietary obesity and SAT transplantation experiments in male mice. The results indicate that beige adipocytes are indispensable for the neuroprotective and anti-inflammatory effects of subcutaneous fat, and implicate beige fat-stimulated IL4 production by meningeal lymphocytes in communication between SAT and the CNS.

Results
Beige adipocytes in subcutaneous fat oppose peripheral inflammation with dietary obesity. Expression of the transcription factor PRDM16 is required for beiging in subcutaneous fat. To investigate potential relationships between beige adipocytes and obesity-induced inflammation, transgenic mice lacking beige fat (Adiponectincre/Prdm16−/−, Tg) and non-transgenic littermate controls (nTg) were maintained on a low-fat or high-fat diet (LFD, HFD) for 1 month. Weight gain was similar in Tg and nTg mice over 4 wk (Fig. 1a), and the proportional weights of SAT, epididymal visceral adipose tissue (VAT), and interscapular brown adipose tissue (BAT) did not differ between genotypes (Fig. 1b). Beiging is accompanied by induction of thermogenic genes and formation of UCP1+ multinucleated adipocytes. These signatures are increased by cold exposure, but are also present at room temperature, which falls below thermoneutrality in mice. Anatomically, beige adipocytes are highly enriched in inguinal SAT, relative to the dorsolumbar region. To control for this anatomical gradient, tissue punches from paraffin-embedded inguinal SAT were used for gene expression endpoints after HNE staining on adjacent sections. qPCR analysis of thermogenic (Ucp1 and Ppargc1a) and brown fat differentiation genes (Cidea) revealed reduced expression in Tg mice, relative to nTg/LFD samples (Supplementary Fig. 1A). Reductions were comparable in Tg/LFD and Tg/HFD samples, and there was no loss of beiging-associated gene expression in samples from nTg/HFD mice (Supplementary Fig. 1A). Expression of Prdm16 was significantly reduced in Tg mice, irrespective of diet (Supplementary Fig. 1A), consistent with the original report characterizing this model. Downregulation of thermogenic genes was not attributable to global reductions in gene expression, as the adiocyte differentiation gene Pparg was similarly elevated in SAT from nTg/HFD mice and Tg/HFD mice (Supplementary Fig. 1A).

To examine histological signatures of beiging, we analyzed multinucleated adipocytes on HNE-stained sections from inguinal SAT (Supplementary Fig. 1B). Blinded visual inspection of (500–700) systematic random sampling fields from each animal revealed that multinucleated adipocytes were less frequent in Tg mice, irrespective of diet (Supplementary Fig. 1C, D; multinucleated adipocytes [% of fields], mean ± sem, n = 4/condition: nTg/LFD = 32.0 ± 2.9; nTg/HFD = 30.6 ± 2.5; Tg/LFD = 10.5 ± 1.6; Tg/HFD = 10.6 ± 0.9). Using the same approach, we observed that UCP1+ multinucleated adipocytes were less prevalent in inguinal SAT from Tg mice on either diet (Supplementary Fig. 1E, F; UCP1+ multinucleated adipocytes [%], mean ± sem, n = 4/condition: nTg/LFD = 17.1 ± 1.9; nTg/HFD = 17.8 ± 1.7; Tg/LFD = 7.5 ± 1.2; Tg/HFD = 6.3 ± 1.5). Inguinal SAT receives greater sympathetic innervation than dorsolumbar SAT in normal-weight WT mice, and sympathetic activity promotes beiging. For qualitative insight into innervation, we performed immunohistochemistry for tyrosine hydroxylase (TH) in inguinal SAT. TH-stained fibers were observed along the surface of inguinal SAT, with penetrating fibers interdigitating between adipocytes (Supplementary Fig. 1E). There was no overt effect of diet or genotype on the distribution of TH-labeled fibers (Supplementary Fig. 1E), consistent with the integrity of sympathetic innervation reported using 3-dimensional methods. Interscapular BAT also appeared normal, with no obvious differences in multinucleated adipocytes or UCP1 immunoreactivity (Supplementary Fig. 2A, B), as reported previously. Multinucleated adipocytes were not observed in VAT (Supplementary Fig. 2C), and UCP1 immunoreactivity was rare (Supplementary Fig. 2D). Taken together, these patterns are consistent with selective loss of beige adipocytes in SAT from Adiponectincre/Prdm16−/− mice.

Inflammation in obesity promotes metabolic pathology and contributes to obesity-induced cognitive dysfunction. While there was no effect of genotype or 4 wk HFD consumption on glucose tolerance (Fig. 1c), analysis of serum cytokines revealed significantly greater upregulation of interleukin-1β (IL1β) in Tg/HFD, relative to nTg/HFD (Fig. 1d). One month HFD consumption also increased circulating levels of TNFα, and this effect was comparable between genotypes (Fig. 1d). Ablation of beige adipocytes in SAT converts the tissue environment to a pro-inflammatory state reminiscent of visceral fat, based on induction of visceral-selective genes and increased macrophage infiltration with dietary obesity in Adiponectincre/Prdm16−/− mice with high-fat feeding. To determine whether similar changes occurred under the current experimental protocol, we quantified pro-inflammatory cytokine gene expression and visualized F4/80+ crown-like structures in SAT and VAT from Adiponectincre/Prdm16−/− mice and nTg littermates on HFD or LFD (Supplementary Fig. 3A). Crown-like structures were more prevalent in SAT from Tg/HFD mice, relative to nTg/HFD mice (Supplementary Fig. 3A; % fields with crown-like structures, mean ± sem, n = 4 mice/condition: nTg/LFD = 1.8 ± 0.3; nTg/HFD = 2.2 ± 0.3; Tg/LFD = 1.9 ± 0.6; Tg/HFD = 7.3 ± 1.2), and qPCR analysis
revealed significant increases in Ccl2, Il1b, and Tnfa expression (Supplemental Fig. 3D). Accumulation of crown-like structures was comparable in VAT from nTg/HFD and Tg/HFD mice (Supplemental Fig. 3C; mean ± sem, *n* = 4/condition: nTg/LFD = 1.8 ± 0.5; nTg/HFD = 8.6 ± 1.0; Tg/LFD = 2.2 ± 0.4; Tg/HFD = 9.1 ± 1.1), as was induction of pro-inflammatory cytokines (Supplemental Fig. 3D). Overall, these data are consistent with the acquisition of VAT-like features in SAT from mice lacking beige fat after HFD consumption.

Loss of beige adipose skews microglia toward pro-inflammatory activation with dietary obesity. To determine whether loss of beige adipocytes alters obesity-induced inflammation in the brain, we isolated forebrain mononuclear cells (FMCs) from Adiponectincre/Prdm16f/f mice and nTg littermates after 4 wk on HFD or LFD. The phenotype and activation state of FMCs was determined using isolated forebrain mononuclear cells (FMCs) from Adiponectincre/Prdm16f/f mice and nTg littermates after 4 wk on HFD or LFD. (Supplemental Fig. 3C; mean ± sem, *n* = 6 mice/condition). The same sampling scheme was applied for process complexity (right); symbols show group mean ± sem (n = 6 mice/condition).

Intraperitoneal glucose tolerance testing revealed no group differences (symbols show group mean ± sem, *n* = 6 mice/condition). Area under the curve (AUC) (inset; symbols represent individual mice). For all graphs, white circles represent nTg/LFD; black circles, nTg/HFD; blue squares, Tg/LFD; green diamonds, Tg/HFD. Data in a were analyzed using 2-way repeated-measures ANOVA; for (B-H), 2-way ANOVA. *p* < 0.05 relative to nTg/LFD; *p* < 0.05 relative to nTg/HFD, determined by Tukey’s multiple comparison test. For data, statistics, and exact *p*-values, see Source Data File 1.

These experiments revealed increases in obesity-induced microglial activation and macrophage infiltration in mice lacking beige fat (Fig. 1f, g). The CD45hi/Ly6Chi/CD169⁻/TMEM119⁻ population was significantly larger in Tg/HFD mice, relative to all other groups (Fig. 1f). This effect was specific to macrophages, as there were no group differences in the proportion of Ly6G⁻/CD11b⁻ neutrophils in these experiments (% of live cells, mean ± sem, *n* = 6/condition: nTg/LFD = 0.35 ± 0.06; nTg/HFD = 0.41 ± 0.12;
Susceptibility to obesity-induced hippocampal dysfunction in mice lacking beige fat. To investigate potential changes in cognition in mice lacking beige fat, Adiponectincre/Prdm16cre/fli (Tg) mice and nTg littermates were maintained on LFD or HFD for 4 wk, as shown (Fig. 1a). Non-overlapping cohorts of mice were tested in recognition memory paradigms before testing in the water maze or the Barnes maze, a spatial memory paradigm in which mice learn the location of an escape box located under one of twelve holes around the edge of a circular platform23. In the novel object preference task, Tg/HFD mice spent less time exploring the novel object, indicative of impaired memory for the familiar object presented 30 min earlier (Fig. 2a). This pattern was not explained by differences in total object exploration (% time with both objects, mean ± sem, n = 12/condition: nTg/LFD = 25.1 ± 2.3; nTg/HFD = 23.3 ± 2.5; Tg/LFD = 26.8 ± 1.8; Tg/HFD = 21.9 ± 2.5). Novel object preference testing was carried out after video tracking of exploratory behavior in an empty arena, as described13. There were no differences in total locomotor exploration (total distance in meters, mean ± sem, n = 12/condition: nTg/LFD = 6.5 ± 0.9; nTg/HFD = 6.3 ± 0.7; Tg/LFD = 6.5 ± 0.9). Given the absence of group differences in locomotor speed under these entry-to-criterion paradigm, reduced alternation in Tg/HFD mice likely reflects deficits in spatial recognition memory.

Subcutaneous fat transplantation rescues hippocampal function in mice with dietary obesity. We next considered whether increases in subcutaneous fat might offset the deleterious effects of obesogenic diets on memory and cognition in wild-type mice. To test this hypothesis and examine the potential role of beige adipocytes, male C57Bl6J mice were maintained on HFD for 10 wk before SAT transplantation from a lean nTg (TRANSnTg) or Adiponectincre/Prdm16cre/fli Tg donor (TRANSnTg, Fig. 3a). Parallel groups of mice were maintained on HFD or LFD before receiving

Tg/LFD = 0.31 ± 0.08; Tg/HFD = 0.39 ± 0.07). Macrophage infiltration was accompanied by pro-inflammatory activation of resident microglia, as determined by subsequent analysis of TRL4 and MHCII in CD45lo/Ly6Clo/CD169−/TMEoMIN19+ cells. Microglial induction of TRL4 was evident in nTg/HFD mice, relative to nTg/LFD mice, but these effects were amplified in Tg/HFD mice (Fig. 1g). Similar trends were observed for MHCII (Fig. 1g). Beige adipocytes in SAT interact with local immune cells, and these interactions are mediated in part by Th2 cytokines, such as IL423. Stimulation with IL4 upregulates microglial IL4 receptor-alpha (IL4Ra)24. To examine whether early obesity promotes Th2 cytokine signaling in microglia, we quantified IL4Ra and interleukin-10 receptor-alpha (IL10Ra) expression in CD45lo/Ly6Clo/CD169-/TMEoMIN19+ microglia. These data revealed increases in microglial IL4Ra expression in nTg/HFD, but not in Tg/HFD mice (Fig. 1g). There was no effect of diet or genotype on IL10Ra (Fig. 1g), but the stepwise induction of TRL4 and MHCII in microglia from nTg/HFD and Tg/HFD mice suggests that co-induction of IL4Ra might limit pro-inflammatory activation in obesity.

After observing a potential role for beige adipocytes in microglial polarization by flow cytometry, we quantified morphological and cellular indices of activation among IBA1+ cells in the hippocampal dentate molecular layer. At the 4 wk time point, microglia from nTg/HFD mice did not differ from nTg/LFD based on total process length (Fig. 1h, left) or Sholl analysis of process complexity (Fig. 1h, right). By contrast, IBA1-labeled microglia from Tg/HFD mice exhibited reductions in total length (Fig. 1h, left) and process complexity (Fig. 1h, right). Changes in microglial ramification in Tg/HFD mice were accompanied by accumulation of CD68+ microglia, indicative of vulnerability to obesity-induced microglial activation in mice lacking beige adipocytes.
sham surgery (HFD/SHAM, LFD/SHAM). Consumption of HFD increased body weights, but there was no effect of SAT transplantation on weight gain 2 wk after surgery (Fig. 3a). Dietary obesity also increased the proportional weights of epididymal VAT and resident SAT, but there was no effect of SAT transplantation on these parameters (Fig. 3b). Histological visualization of transplanted SAT revealed no relationship between donor genotype and rejection rates (Fig. 3b). Intrinsic features of SAT and extrinsic features of the subcutaneous environment contribute to depot-specific adipose tissue inflammation in obesity6,7. To determine whether beige adipocytes persist after transplantation into the peritoneal cavity, we visualized anatomical and gene expression signatures in multilocular adipocytes and UCP1 immunoreactivity were both present in transplants from nTg, but not Tg donors (Supplementary Fig. 5A, B), similar to the pattern observed in resident SAT from intact nTg and Tg mice (Supplementary Fig. 5D). However, expression of the adipocyte differentiation gene Pparg was unaffected by donor genotype (Supplementary Fig. 5D), consistent with the similarities in resident SAT from lean nTg and Tg mice (Supplementary Fig. 1A).

In the transplanted SAT, we observed that expression of beige adipocyte differentiation gene AdiponectinR1/PRDM16H/H mice (Tg) exhibit deficits in novel object preference (NOP) after 4 wk on a high-fat diet (HFD; n = 12 mice/condition). Symbol height shows data from individual mice and the line shows mean ± sem (applies to a, b). Mice lacking beige fat exhibit reduced spatial recognition memory in the Y-maze (n = 12 mice/condition). c, d Impaired performance during water maze acquisition training (c) and probe testing (d) in Tg/HFD mice. For c, symbols represent mean ± sem; for d, symbols represent individual mice and the line shows mean ± sem (n = 12 mice/condition). e, f Deficits in spatial memory acquisition in the Barnes maze (e) and inaccurate search behavior during the probe trial (f) in Tg/HFD mice. For e, symbols represent individual mice and line shows mean ± sem; for f symbols represent group mean ± sem (n = 12 mice/condition). Heatmaps (f, right) show search patterns during probe trial for the indicated groups. g Tg/HFD mice exhibit early-onset deficits in hippocampal long-term potentiation (for nTg/LFD and Tg/LFD, n = 7 slice recordings from 3 mice; nTg/HFD, n = 8 slice recordings from 4 mice; Tg/HFD, n = 8 slice recordings from 5 mice). Symbols show mean ± sem. h Deficits were eliminated in the presence of minocycline (for Tg/HFD, n = 7 slice recordings from 4 mice; all other groups, n = 7 slice recordings from 3 mice). Symbols show mean ± sem. i Group differences in field excitatory postsynaptic potential (fEPSP) slope 50–60 min after induction (symbols represent individual slice recordings and line shows mean ± sem). For all graphs, white circles represent nTg/LFD; black circles, nTg/HFD; blue squares, Tg/LFD; green diamonds, Tg/HFD. For a, c, e, 2-way repeated-measures ANOVA; for b, d, i, 2-way ANOVA; *p < 0.05 relative to nTg/LFD; #p < 0.05 relative to nTg/HFD, determined by Tukey’s multiple comparison test or Dunnett’s T3 for heterogeneity of variance. For data, statistics, and exact p-values, see Source Data File 2.

Transplantation did not restore gene expression for Prdm16, which was reduced in transplants from Tg donors (Supplementary Fig. 5D). Transplanted SAT from lean Tg donors also had lower levels of thermogenic (Ucp1, Pparc1a) and brown/beige adipocyte differentiation gene expression (Gide; Supplementary Fig. 5D). However, expression of the adipocyte differentiation gene Pparg was unaffected by donor genotype (Supplementary Fig. 5D), consistent with the similarities in resident SAT from lean nTg and Tg mice (Supplementary Fig. 1A).

We next evaluated the impact of SAT transplantation on glucose metabolism and circulating inflammatory cytokines. Intrapertitoneal glucose tolerance testing 2 wk post-surgery revealed no significant differences in glycemic control (Fig. 3c). Multiplex profiling of serum cytokines revealed significant increases in IL1b and IL2 in HFD/SHAM samples, relative to LFD/SHAM (Fig. 3d). Surgical increases in subcutaneous fat reduced circulating IL2 concentrations, but had no effect on IL1b (Fig. 3d). Circulating CXCL1 concentrations were numerically
higher in HFD mice, and this trend was statistically significant in TRANS\textsubscript{Tg} samples, relative to LFD/SHAM (Fig. 3d).

After investigating the consequences of acute SAT transplantation for systemic inflammation and metabolism, additional groups of Wt mice were maintained on LFD or HFD for 12 wk as shown (Fig. 3a), with cognitive testing 2 wk after SAT transplantation or sham surgery. In the object recognition paradigm, SAT transplantation restored novel object preference to levels that were identical to LFD/SHAM (Fig. 3g, left). Deficits in spatial memory acquisition were associated with impaired probe trial performance, as HFD/SHAM and TRANS\textsubscript{Tg} mice spent less time searching in the target quadrant than LFD/SHAM mice (Fig. 3g, right). By contrast, TRANS\textsubscript{Tg} mice did not differ from LFD/SHAM during acquisition training or in the probe trial (Fig. 3g). There were no group differences during visible platform training (distance [m], mean ± sem, n = 12/condition: LFD/SHAM = 5.7 ± 0.7; HFD/SHAM = 5.6 ± 0.5; TRANS\textsubscript{Tg} = 5.9 ± 0.7; TRANS\textsubscript{fl} = 6.1 ± 0.8), consistent with beige adipose-mediated restoration of memory and cognition following SAT transplantation.

SAT transplantation rescues hippocampal synaptic plasticity via IL4. To investigate the synaptic mechanisms for beige adipose-dependent mechanisms (Fig. 3f). In the water maze, HFD/SHAM and TRANS\textsubscript{Tg} mice exhibited significantly longer path lengths than LFD/SHAM during hidden platform training, indicative of deficits in spatial memory acquisition (Fig. 3g, left). Deficits in spatial memory acquisition were associated with impaired probe trial performance, as HFD/SHAM and TRANS\textsubscript{Tg} mice spent less time searching in the target quadrant than LFD/SHAM mice (Fig. 3g, right). By contrast, TRANS\textsubscript{Tg} mice did not differ from LFD/SHAM during acquisition training or in the probe trial (Fig. 3g). There were no group differences during visible platform training (distance [m], mean ± sem, n = 12/condition: LFD/SHAM = 5.7 ± 0.7; HFD/SHAM = 5.6 ± 0.5; TRANS\textsubscript{Tg} = 5.9 ± 0.7; TRANS\textsubscript{fl} = 6.1 ± 0.8), consistent with beige adipose-mediated restoration of memory and cognition following SAT transplantation.

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Beige adipocytes in subcutaneous fat alternatively activate microglia in obese mice. To determine whether increased SAT mass alters microglial activation in obesity and examine the potential role of beige fat in these effects, we isolated FMCs from LFD/SHAM, HFD/SHAM, TRANSnTg, and TRANS mice by density gradient centrifugation, as described. To focus on early-onset changes among resident microglia, mice were maintained on HFD or LFD for 2 wk before sham surgery or SAT transplantation, with cell isolation 2 wk after surgery, as shown (Fig. 4a). Cells were gated on size and granularity, followed by doublet discrimination and dead cell exclusion as shown (Fig. 1e). In our initial experiments, there was no evidence of macrophage infiltration in nTg mice maintained on HFD for 4 wk (Fig. 1f). However, we considered the possibility that surgery might unmask vulnerability to obesity-induced macrophage infiltration at the 4 wk time point. There was no effect of diet or SAT transplantation on the size of the CD45HI/Ly6CHI population (% of CD45HI/Ly6CHI population) (% of CD45HI/Ly6CHI population) (mean ± sem, n = 6/condition: LFD/SHAM = 8.5 ± 0.6; HFD/SHAM = 8.9 ± 0.7; TRANSnTg = 8.0 ± 1.1; TRANS = 7.9 ± 0.8).

After observing no apparent shift in cellular phenotype with SAT transplantation, resident microglia were gated as CD11b+ /CD45LO/Ly6CLO events for analysis of classical (M1) and alternative (M2) activation markers (Fig. 4b). Analysis of the M1 markers TLR4 and MHCII revealed that pro-inflammatory polarization persists in FMCs from SAT transplant recipients (Fig. 4b, c). However, subsequent analysis of M2 cytokine receptors revealed co-induction of interleukin-4 receptor-alpha (IL4Ra) in microglia from transplant recipients (Fig. 4b, d). Uptregulation of IL4Ra was observed in FMCs from TRANSnTg mice, but not in cells from TRANS mice (Fig. 4d). These effects were selective for IL4Ra, as there was no effect of diet or surgery on microglial IL10Ra (Fig. 4d).

Intracellular arginase-1 (Arg1) is a reliable downstream readout for IL4Ra-mediated anti-inflammatory activation. After observing the co-induction of IL4Ra and the classical activation markers MHCII and TLR4, we measured intracellular Arg1 in FMCs as a downstream marker of M2 polarization. SAT transplantation significantly increased intracellular Arg1 fluorescence, and this effect was dependent on beige adipocytes in the donor (Fig. 4e). To specifically look at hippocampal microglia, we visualized Arg1 using immunofluorescence. Analysis of Arg1 expression in IBA1+ cells revealed significantly more Arg1+ microglia in TRANSnTg, relative to HFD/SHAM and LFD/SHAM mice (Fig. 4f). In our first set of experiments, analysis of microglial process architecture revealed no effect of diet in nTg mice after 4 wk HFD (Fig. 1h). However, the underlying mechanisms for morphological responses to HFD over time remain poorly understood. To gain insight into heterogeneous morphological responses, we analyzed microglial morphology separately for the Arg1− and Arg1+ populations. Consistent with the anti-inflammatory M2 state, there was no effect of diet or surgery on the ramification of Arg1− microglia (Fig. 4g). By contrast, the subpopulation of Arg1+ microglia exhibited reduced anatomical complexity in HFD/SHAM mice, relative to LFD/SHAM (Fig. 4h). Anatomical simplification of Arg1− microglia was eliminated by SAT transplantation, but only when transplants were collected from an nTg donor (Fig. 4h). These results indicate that beige adipocytes promote M2 polarization, and that the subset of alternatively activated microglia that retains a highly ramified morphology. Taken together, these results strongly implicate SAT beige adipocytes as an anti-inflammatory stimulus, with a potential role in the resolution of obesity-associated neuroinflammation and cognitive decline.

Beige adipocytes promote IL4 induction in CNS T cells. After observing the upregulation of microglial IL4Ra in early obesity and a requirement for IL4 in synaptic rescue after SAT transplantation, we investigated potential sources of IL4 in the CNS and periphery. Mice were maintained on HFD or LFD for 4 wk, with SAT transplantation from nTg or Adiponectincre/Pdmd16/−/− (Tg) donors during week 2, as shown (Fig. 4a). Multiplex analysis of circulating cytokines revealed increases in IL1b and TNFa with early obesity (Fig. 5a). Increases in circulating pro-inflammatory cytokines occurred irrespective of surgery or donor genotype (Fig. 5a). IL4 is produced at very low levels in unstimulated animals, and its rapid turnover limits detectability by standard immunoassays. We, therefore, used an in vivo capture assay in which biotinylated antibodies were administered intraperitoneally for analysis of circulating IL4, or into the lateral cerebral ventricles for measures in CSF (Fig. 5b, c). Analysis of CSF collected from the cisterna magna 24 h later revealed increases in TRANSnTg, but not in TRANS mice (Fig. 5b). Increases in CSF IL4 were independent of circulating IL4 concentrations, which did not differ between diet and surgical conditions (Fig. 5c).

T cells in the meninges and choroid plexus are the primary source of IL4 under intact conditions. To visualize meningeal T cells, mice were transcardially perfused after CSF collection and decalcified skulls were sectioned for immunofluorescence. CD3ε+ T cells were quantified along alpha laminin-labeled basement membranes in the choroid plexus and meninges. These experiments revealed the accumulation of T cells in both compartments with dietary obesity (Fig. 5d, g). While increases in T-cell number were unaffected by SAT transplantation, streptavadin amplification of the biotinylated IL4 capture antibody revealed significant increases in IL4+ T cells in the choroid plexus and meninges (Fig. 5e, h). Induction of IL4 in meningeal T cells was observed in TRANSnTg mice, but not in TRANS mice (Fig. 5d–h), suggestive of responses to beige adipose-derived signals. We cannot exclude the possibility that other lymphocytes in addition to T cells might be generating IL4, but it should be noted that CD3ε−/IL4+ cells were rare (% of IL4+, mean ± sem from n = 5–6/condition: LFD/SHAM = 0 ± 0; HFD/SHAM = 1.5 ± 1.5; TRANSnTg = 1.8 ± 0.9; TRANS = 3.2 ± 2.1). CD3ε+/IL4+ and CD3ε−/IL4+ cells were localized to laminin-positive basement
membranes in the leptomeninges and choroid plexus, and were not observed in the CNS proper.

Mice used in the immunofluorescence experiments received intraventricular infusions of biotinylated antibodies against IL4. Of necessity, this approach involves some limited damage to the meninges during the stereotaxic injection. We, therefore, carried out flow cytometry experiments to measure IL4 in T cells from a non-overlapping cohort of LFD/SHAM, HFD/SHAM, TRANSnTg, and TRANSnTg during week 2. Symbols show group mean ± sem (n = 12 mice/condition).

Fig. 4 Beige adipocytes alternatively activate resident microglia in early obesity. a Weight gain in WT mice consuming a low-fat or high-fat diet (LFD, HFD) for 1 month, with a sham operation (SHAM) or transplantation of subcutaneous fat from a nontransgenic (TRANSnTg) or AdiponectinCre/PRDM16fl/fl transgenic donor (TRANSnTg) during week 2. Symbols show group mean ± sem (n = 12 mice/condition). b Co-induction of classical (TLR4) and alternative activation-associated markers (IL4Ra) in CD11b+ /CD45LO/Ly6CLO/CD169+/TMEM119+ microglia from mice in the indicated conditions. Numbers in the upper-right quadrant represent TLR4+ /IL4Ra+ events (% microglia). c Quantification of microglial MHCII and TLR4 revealed comparable pro-inflammatory polarization across all surgical conditions with dietary obesity. Symbols represent individual mice and bar height shows mean ± sem (n = 6 mice/condition; applies to c, d). d Beige adipose-dependent increases in microglial IL4Ra after SAT transplantation in HFD mice. e Left graph shows flow cytometric quantification of intracellular Arginase-1 (Arg1) mean fluorescence intensities (MFI) in gated microglia. Symbols represent individual mice and lines show mean ± sem (n = 6 mice/condition). Histogram (right) shows Arg1 MFI in gated microglia from representative samples in each condition (isotype shown in gray). f Quantification of Arg1 immunoreactivity in IBA1+ microglia on hippocampal sections. Symbols represent individual mice and lines show mean ± sem (n = 6 mice/condition). Micrographs to the right of panels f-h depict representative labeling in the dentate molecular layer for the indicated groups. g Sholl analysis of Arg1− microglia. Symbols represent mean ± sem from n = 6 mice/condition (5 Arg1− cells per mouse). h Sholl analysis of Arg1+ microglia reveals selective reductions in anatomical complexity that are alleviated by SAT transplantation from an nTg donor. Symbols represent mean ± sem from n = 6 mice/condition (5 Arg1+ cells per mouse). For a, repeated-measures ANOVA; for c–h, one-way ANOVA. *p < 0.05 relative to nTg-LFD; #p < 0.05 relative to nTg-HFD, determined by Tukey’s multiple comparison test or Dunnett’s T3 for heterogeneity of variance. For data, statistics, and exact p-values, see Source Data File 4.
the CNS T cells as a source for increases in CSF IL4 concentrations. Under normal conditions, parenchymal IL4 concentrations are low or undetectable.

**Fig. 5 Surgical increases in subcutaneous fat increase IL4 in CNS T cells via beige adipose-dependent mechanisms.**

- **a** Multiplex analysis of serum cytokines after 4 wk on a low-fat or high-fat diet (LFD, HFD), with the sham operation (SHAM) or SAT transplantation from a nontransgenic (TRANSnTg) or transgenic donor (TRANSTg) during week 2 (LFD/SHAM, n = 5; all other groups, n = 6). Symbols represent individual mice and lines show mean ± sem (applies to a–i). b Top panel shows schematic of in vivo antibody capture assay for detection of interleukin-4 (IL4) in cerebrospinal fluid (CSF). Graph (bottom) shows beige fat-dependent increases in CSF IL4 (n = 6 mice/condition). c Top panel shows in vivo capture approach for analysis of circulating IL4. Graph (bottom) shows quantification of serum IL4 by ELISA (n = 6 mice/condition). d Graph shows an increased T-cell number in the choroid plexus with dietary obesity (n = 6 mice/condition; applies to d–h). Micrographs (right) show representative labeling for laminin, CD3e, and biotinylated IL4 capture antibody in the choroid plexus (CP) following ICV delivery. Scale bar for anatomical reference micrograph (left) = 20 μm; for group comparison micrographs, scale bar = 10 μm. e Increased numbers of IL4+ T cells in the choroid plexus of TRANSnTg mice. f Meningeal CD3e+ cells were quantified along laminin-labeled basement membranes in the transverse sinus (TS) adjacent to the confluence of sinuses (COS) and posterior to the superior sagittal sinus (SSS). Scale bar for anatomical reference (left) = 20 μm; for group comparison micrographs, scale bar = 10 μm. g Meningeal T-cell number increases with obesity, irrespective of SAT transplantation. h Increased uptake of IL4 capture antibody in meningeal CD3e+ T cells after SAT transplantation from a nontransgenic (TRANSnTg) but not an Adiponectin(+/−)/PRDM16(+/−) transgenic donor (TRANSTg). Micrographs (right) show immunofluorescence detection of biotinylated anti-IL4 in CD3e+ lymphocytes. i Gating strategy for analysis of endogenous IL4 expression in CNS T cells (CD4+/CD3e+).
IL4 in the brain parenchyma has been reported in models of encephalitis and acute ischemic injury\textsuperscript{19,30}, we measured IL4 gene expression in primary astrocytes, microglia, and brain vascular endothelial cells (BVECs), as described\textsuperscript{15}. Cells were plated and maintained overnight in the presence or absence of the allergenic agent ovalbumin (OVA, 100 μg/mL). As reported previously for freshly isolated glia and vascular cells from the intact brain\textsuperscript{28,29}, we were unable to reliably amplify IL4 mRNA from unstimulated cells in these experiments (Supplementary Fig. 7A). Overnight exposure to OVA-induced robust IL4 mRNA expression in all cell types, but there was no effect of diet or surgery (Supplementary Fig. 7B). Stimulation with OVA induces expression of both anti- and pro-inflammatory cytokines, including interleukin-1beta (IL1b)\textsuperscript{31}. Ovalbumin-stimulated induction of IL1b was increased in microglia from mice with dietary obesity, relative to lean mice, but increases were comparable in cells from sham-operated mice and SAT transplant recipients (Supplementary Fig. 7C). There were no differences in cell viability after OVA, as determined by formazan cleavage assay (Supplementary Fig. 7D).

Recipient T cells are required for the effects of SAT transplantation on cognition and microglial activation. After observing increases in CSF IL4 and accumulation of CNS T cells in SAT transplant recipients, we considered the following scenarios: first, lymphocytes from the SAT donor could be trafficking into the CNS; and second, lymphocytes from the recipient could interact with beige adipocytes in a manner that enhances cognition and reduces neuroinflammation. To evaluate the first scenario, we performed SAT transplantation using donor mice with ubiquitous inducible expression of tdTomato (Fig. 6a). Reporter expression was driven by a hybrid CAGG promoter fused to creER (CAGG\textsuperscript{CreER}32). Consistent with published expression data from this model, widespread tdTomato fluorescence was evident 2 wk after tamoxifen administration (3 × 2.0 mg every 48 h, PO). To examine immune cell trafficking between fat and brain, mice were maintained on LFD or HFD for 10 wk before receiving SAT transplants from a lean CAGG\textsuperscript{CreER}/tdTomato\textsuperscript{0/2} donor. Two weeks after SAT transplantation, blood, stroma–vessel fraction (SVF), and FMCs were isolated and stained for flow cytometry. Cells were gated on size and granularity, followed by doublet discrimination and dead cell exclusion. T cells were gated as CD45<sup>-/CD3e<sup>+</sup>/CD4<sup>+</sup> events and monocytes were gated as CD45<sup>+</sup>/CD11b<sup>+</sup> as shown (Fig. 6b), tdTomato<sup>+</sup> events were quantified relative to total T cells or monocytes as a measure of lymphocyte trafficking. These experiments revealed no evidence of lymphocyte trafficking between transplanted SAT and the CNS (Fig. 6c). tdTomato<sup>+</sup> events comprised <0.5% of CNS T cells and <0.25% of CNS monocytes, and the proportion of tdTomato<sup>+</sup> cells in each gate was similar in LFD and HFD samples (Fig. 6c). Circulating tdTomato<sup>-</sup> lymphocytes were also scarce, and there was no effect of dietary obesity (Fig. 6c). Viable tdTomato<sup>+</sup> T cells and monocytes were present in SVF from the transplanted SAT (Fig. 6c), and infiltration of recipient (tdTomato<sup>-</sup>) lymphocytes into the transplant was unaffected by diet 2 wk after surgery (Fig. 6c). Relative to the CNS, lymphocyte trafficking between resident adipose depots and transplanted SAT was more frequent (Fig. 6c). Transplants were implanted into the peritoneal cavity, and interstitial continuity with resident epididymal fat likely accounted for the accumulation of tdTomato<sup>+</sup> T cells and monocytes in visceral fat. Dietary obesity was associated with increased numbers of tdTomato<sup>+</sup> T cells in resident VAT (Fig. 6c). In resident SAT, tdTomato<sup>+</sup> lymphocytes were less frequent than in VAT, and the proportions of tdTomato<sup>+</sup> T cells and monocytes were unaffected by diet (Fig. 6c). Taken together, these data suggest that trafficking of donor lymphocytes between transplanted SAT and the CNS is unlikely.

To determine whether recipient-derived lymphocytes might mediate communication between beige adipocytes in transplanted SAT and the CNS, we carried out additional experiments using scid mice on the C57Bl6 background (B6.scid). Homozygous B6. scid mutant mice were maintained on HFD for 10 wk before SAT transplantation or sham operation, with parallel groups of mice maintained on LFD before sham operation. Weight gain was increased with HFD consumption, but there was no effect of surgery (Fig. 6d). Analysis of circulating cytokines revealed increased serum IL1b with dietary obesity in B6.scid mice (Fig. 6e), consistent with the persistent pro-inflammatory effects of high-fat diet reported by other groups using this model\textsuperscript{33}.

Two weeks after surgery, mice were tested in the water maze before transcardial perfusion and immunofluorescence visualization of hippocampal microglia. Testing in the water maze revealed a requirement for recipient-derived lymphocytes in the effects of SAT transplantation on cognition (Fig. 6f). HFD/SHAM and HFD/TRANS scid mice had longer path lengths than LFD/SHAM scid mice during acquisition training (Fig. 6f; F<sub>2,33</sub> = 5.60, p < 0.01), and both groups of HFD mice spent less time swimming in the platform quadrant during the probe trial (Fig. 6f; F<sub>2,33</sub> = 12.17, p < 0.01). Quantification of microglial process architecture revealed a similar loss of responsiveness to SAT transplantation (Fig. 6g). Three months on HFD reduced microglial process length and complexity in B6.scid mice (F<sub>2,12</sub> = 4.97, p < 0.05), and reductions were comparable in HFD/SHAM and HFD/TRANS (Fig. 6g). Taken together, these data indicate that recipient-derived lymphocytes rescue cognition and restore microglial quiescence following SAT transplantation.

Discussion

This study has identified an immunoregulatory relationship between beige adipocytes in subcutaneous fat, peripheral lymphocytes, and parenchymal cells in the hippocampus. Mice lacking beige adipocytes exhibited stronger pro-inflammatory responses to a high-fat diet in the brain and periphery, and were more susceptible to cognitive deficits and hippocampal synaptic dysfunction. Subcutaneous fat transplantation restored hippocampus-dependent memory in WT recipients with dietary obesity and induced a unique microglial phenotype characterized by co-induction of pro- and anti-inflammatory markers. These effects were dependent on the presence of beige adipocytes in the donor, and electrophysiological recordings implicated IL4 signaling downstream of beige fat-mediated reinstatement of LTP. Beige adipocytes were required for induction of IL4 among lymphocytes in the meninges and choroid plexus, and recipient-derived lymphocytes were required for the cognitive effects of SAT transplantation. Lymphocyte interactions with beige adipocytes may therefore underlie the neuroprotective effects of subcutaneous adiposity, with a potential role for microglia as sensors and transducers in the CNS.

Visceral adiposity is a major risk factor for obesity-induced metabolic dysfunction, and mounting evidence supports similar associations with cognition\textsuperscript{4-6,15}. Subcutaneous adiposity is considered metabolically benign\textsuperscript{7}, but relative to visceral fat, less is known regarding relationships with neuroinflammation and memory. In the current report, we observed that SAT becomes more “visceral-like” in the absence of beige fat, based on induction of pro-inflammatory genes in SAT from Adiponectin<sup>creER</sup>/Pdmd1g<sup>fl/fl</sup> mice with dietary obesity. However, changes in peripheral inflammation were not required for cognitive rescue, as SAT transplantation normalized hippocampal function without attenuating obesity-induced elevations in circulating
pro-inflammatory cytokines. Co-induction of pro- and anti-inflammatory markers by resident microglia emerged as a signature of cognitive rescue in obese mice, and the dissociation between microglial activation and circulating cytokines suggested an alternative site for stimulation of this unique phenotype.

Cold-stimulating beiging in SAT is dependent on the anti-inflammatory cytokine IL4, and exercise-induced myokine release also promotes beiging via IL4-dependent mechanisms. In the current report, cognitive rescue in SAT transplant recipients was dependent upon the presence of beige adipocytes in the donor, with correlated changes in CSF (but not circulating) IL4. Saturable transporters at the blood–brain and blood-CSF barriers have been identified and characterized for other members of the interleukin family, but transport mechanisms governing the entry and exit of IL4 into the brain have yet to be identified. Induction of IL4 among cells of the brain parenchyma has been
and microbial infection37,38. In the current studies, we deter-
mine that enhance LTP, including Pi3K/AKT and ERK1/241. Activa-
tion of JAK1/STAT6 has not been directly linked with synaptic
plasticity, but other members of the JAK/STAT signaling pathway
and do not alter LTP42. In this report, we observed rapid rein-
statement of LTP1 in slices treated with minocycline, and rapid
elimination of protection in SAT transplant recipients following
incubation with an IL4 scavenging antibody. While temporal
kinetics do not demonstrate cell-type specificity, microglia
represent a more likely cellular substrate than astrocytes given
the different receptor complexes recruited by IL4 in the two cell types
and the distinct effects of downstream signaling cascades on LTP
and LTD. The overarching trend emerging from studies of adipose tissue
distribution and cognition appears to recapitulate patterns from
studies of insulin resistance and dysfunctional lipid metabolism,
with a protective role for subcutaneous fat and deleterious effects of
visceral adiposity. However, the relative inaccessibility of the
CNS for longitudinal studies is a barrier to identifying, under-
standing, and ultimately treating the underlying cellular interac-
tions that drive vulnerability to cognitive decline and dementia in
obesity. For example, microglial interactions with cortical
synapses and amyloid plaques have been visualized using long-
itudinal 2-photon imaging in lean mice34,45, but the pro-
flammatory systemic effects of obesity are likely to interact
with established methods for in vivo imaging46. Even the thin-
ness of the enzyme-mediated effect, and so less inflammation than cranial
window imaging in lean adult mice46,47, is likely to be complica-
ted by the disordered bone formation and turnover in the
caudate with chronic obesity48. In terms of translatable, MRI
PET and fMRI imaging techniques would enable direct comparison
of data from humans and rodents, but the resolution of MRI and
PET imaging and the small size of the mouse brain limits the
utility of this widely-used research model. As transgenic rats
become more widely available, longitudinal mechanistic obesity
studies will likely provide new insights into the pathogenesis of
obesity-induced cognitive dysfunction. Understanding the tem-
poral etiology of circuit dysfunction with obesity could potentially
uncover critical windows for noninvasive manipulation of neural
activity, with the goal of attenuating risk of age-related cognitive
decay and dementia in obese individuals.

Methods

Animals and diets. Animals were housed in a ventilator rack on Alpha-dry
bedding in a specific pathogen-free facility with food and water available ad libitum.
The colony room was maintained on a 12 h light-dark cycle (lights-on at 0600
h). Colony temperature was set at 22 °C and recorded temperatures ranged from 21
to 24 °C over the course of the experiments. Breeding pairs of Adiponectincre/
Prdm16fl mice46 were imported from the laboratory of Bruce Spiegelman and
bred in-house for these studies. All other mouse lines were purchased from Jackson
Labs (reporter line Ai14, strain #007914; CAGGcre mice, strain #004682; B6.scid,
strain #001913). Male transgenic mice and nTg littermates were maintained on
standard chow (Teklad). For transgene induction, mice received 2.0 mg
of tamoxifen by oral gavage every 48 h for 6 d. Body weights were determined weekly
for all experiments. All experimental procedures followed NIH guidelines and were
approved by the Institutional Animal Care and Use Committee at Augusta
University.

Adipose transplantation surgery, adipose histology, and adipose gene expression.
For SAT transplantation, recipients and donors were maintained under Isoflurane anesthetic as described in Supplementary Methods and as
reported99. Adipose tissue histology and gene expression assays are described in
Supplementary Methods and followed published protocols15,49. For a list of
probes used in gene expression assay, see Supplementary Table 1. For a
list of antibodies and concentrations used for immunohistochemistry (Supple-
mentary Table 2).

Behavioral tests. Cognitive testing in the water maze, novel object preference, and
Y-maze was carried out during the first half of the dark cycle (1800–2200 h), as
summarized in Supplementary Methods and as reported15. For the Barnes maze,
mice were tested on a white circular platform with 12 holes evenly spaced around
the perimeter. Testing was carried out over 7 d as described in Supplementary
Methods, and as shown (Supplementary Fig. 8).

Electrophysiology. Acute slices were prepared on a Vibratome and allowed to
recover for at least 1 h in carboxygenated artificial cerebrospinal fluid (ACSF)
before recording, as described in Supplementary Methods and as reported15,49.
For some experiments, slices were pre-incubated with minocycline (20 μM) or anti-IL4
neutralizing antibody (IL4 nAb, 5 μg/mL; rat anti-mouse IL4, clone 11B11; Invi-
rogen, Carlsbad, CA) for 30 min with continuous application throughout the
recording. For additional details, see Supplementary Methods.
Glucose metabolism and serum cytokines. Glucose tolerance testing was carried out as described in Supplementary Methods and as reported previously. Multiplex analysis of cytokines was performed using the Mesoscale Discovery platform (V-PLEX Pro-inflammatory Panel 1, Meso Scale Diagnostics, Rockville, MD) according to the manufacturer’s instructions. Serum samples were assayed in duplicate on a QuickPlex SQ120 reader at the Emory University multiplex immunoassay core facility. To quantify IL4 in serum and CSF, we used an in vivo capture assay modified from Finkelman and Morris. (details in Supplementary Methods).

Immunofluorescence and imaging. For immunofluorescence visualization of microglia, 40-micron coronal sections were cut as a 1:6 series using a freezing microtome (Leica), as reported in Supplementary Methods and as described. Immunofluorescence and morphological reconstruction procedures are outlined in Supplementary Methods. Protocols for decalcification, sectioning, and immunofluorescence analysis of meningeal T cells are also in Supplementary Methods. For a list of antibodies and dilutions, see Supplementary Table 2.

Cell isolation and flow cytometry. Cell isolation from brain and SVF was performed as described in Supplementary Methods and as reported. Flow cytometric data acquisition was carried out on a 5-laser BD LSR II in FACS Diva software version 8.1 (BD Biosciences), a Guava EasyCyte 5.0 with Incyte (version 2.6, Milipore), or on an Accuri C6 flow cytometer with Accuri C6 Plus software (version 3.4, BD Bioscience). For each combination of antibodies, compensation parameters were determined using unlabeled cells, single-labeled samples, and isotype controls. For a list of antibodies used in flow cytometry experiments, see Supplementary Table 3.

Ex vivo stimulation and qPCR. For ovalbumin stimulation, FMCs were plated (10⁵ cells/well) and stimulated with 100 micrograms/ml ovalbumin (Sigma–Aldrich; see Supplementary Methods). Methods for RNA extraction, cDNA synthesis, and qPCR are summarized in Supplementary Methods and followed published protocols. For ovalbumin stimulation, expression was determined by calculating ddCT, with the average dCT from LFD/SHAM cells as the reference group. For paraffin-embedded adipose tissues, RNA extraction was carried out as described, with modifications (Supplementary Methods). cDNA samples were amplified using Taqman probes, with the average dCT for nTg/LFD (Supplementary Figs. 1 and 3) or nTg donor samples (Supplementary Fig. 5) as the reference for calculating relative expression. See Supplementary Table 1 for a list of Taqman probes used in these experiments.

Statistics. Experimental data from Adiponectin(−/−)Prdm16(−/−) mice and littermate controls maintained on HFD or LFD were analyzed using 2 x 2 ANOVA or x 2 repeated-measures ANOVA with Tukey’s post hoc. For the SAT transplant experiments, the effects of diet, surgery, and donor genotype were analyzed using one-way ANOVA with repeated measures where appropriate, followed by Tukey’s post hoc. Heterogeneity of variance was addressed using the Geisser-Greenhouse correction or Welch’s correction, as appropriate. Lymphocyte trafficking was compared across LFD and HFD recipient using a bidirectional t-test for normally distributed data or a Mann–Whitney U-test for heterogeneity of variance. Statistical analyses were carried out in Graphpad Prism version 8.0 with statistical significance at p < 0.05.

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

Data availability
Detailed step-by-step protocols are available on request from the corresponding author. Source data are provided with this paper.

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References
1. Hales, C. M., Carroll, M. D., Fryar, C. D. & Ogden, C. L. Prevalence of obesity and severe obesity among adults: United States, 2017–2018. NCHS Data Brief. 360, 1–8 (2020).
2. Murphy, S. L., Xu, J., Kochanek, K. D. & Arias, E. Mortality in the United States, 2017. NCHS Data Brief. 328, 1–8 (2018).
3. Alzheimer’s Association. 2020 Alzheimer’s disease facts and figures. Alzheimers Dement. https://doi.org/10.1002/alz.12068 (2020).
4. Whitmer, R. A. et al. Central obesity and increased risk of dementia more than three decades later. Neurology 71, 1057–1064 (2008).
5. Kamogawa, K. et al. Abdominal fat, adipose-derived hormones and mild cognitive impairment: the J-SHIPP study. Dement. Geriatr. Cogn. Disord. 30, 432–439 (2010).
6. Després, J. P. & Lemieux, I. Abdominal obesity and metabolic syndrome. Nature 441, 881–886 (2006).
7. Cohen, P. & Spiegelman, B. M. Cell biology of fat storage. Mol. Biol. Cell 27, 2523–2527 (2016).
8. Rao, R. R. et al. Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. Cell 157, 1279–1291 (2014).
9. Qiu, Y. et al. Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. Cell 157, 1292–1308 (2014).
10. Wills-Karp, M. & Finkelman, F. D. Untangling the complex web of IL-4 and IL-13-mediated signaling pathways. Sci. Signal. 1, pe53 (2008).
11. Leonard, W. J., Lin J. X. & O’Shea J. J. The y-Chain of Cytokines: Basic Biology to Therapeutic Ramifications. Immunity. 50, 832–850 (2019).
12. Friedl, P. & Weigelin, B. Interstitial leukocyte migration and immune function. Nat. Immunol. 9, 960–969 (2008).
13. Filiano, A. J., Gadani, S. P. & Kipnis, J. How and why do T cells and their derived cytokines affect the injured and healthy brain? Nat. Rev. Neurosci. 18, 375–384 (2017).
14. Buckman, L. B. et al. Obesity induced by a high-fat diet is associated with increased immune cell entry into the central nervous system. Brain Behav. Immun. 35, 33–42 (2013).
15. Guo, D. H. et al. Visceral adipose NLRP3 impairns cognition in obesity via IL–1R1 on CX3CR1+ cells. J. Clin. Invest. 130, 1961–1976 (2020).
16. Cohen, P. et al. Ablation of Prdm16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. Cell 156, 304–316 (2014).
17. Chi, J. et al. Three-dimensional adipose tissue imaging reveals regional variation in beige fat biology and Prdm16-dependent sympathetic neurite density. Cell Metab. 77, 226–236.e3 (2018).
18. Wang, P. et al. A leptin–BDNF pathway regulating sympathetic innervation of adipose tissue. Nature 583, 839–844 (2020).
19. Ponomarev, E. D., Maresz, K., Tàn, Y. & Dittel, B. N. CNS-derived interleukin-4 is essential for the regulation of autoimmune inflammation and induces a state of alternative activation in microglial cells. J. Neurosci. 27, 10714–10727 (2007).
20. Butovsky, O. et al. Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. J. Clin. Invest. 122, 3063–3087 (2012).
21. Butovsky, O. et al. Identification of a unique TGF-β-dependent molecular and functional signature in microglia. Nat. Neurosci. 15, 157–160 (2012).
22. Barnes, C. A. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. J. Comp. Physiol. Psychol. 92, 174–104 (1979).
23. Rubinow, M. J., Arsenau, L. M., Beverly, J. L. & Juraska, J. M. Effect of the estrous cycle on water maze acquisition depends on the temperature of the water. Behav. Neurosci. 118, 863–868 (2004).
24. Kobayashi, K. et al. Monocytine selectively inhibits M1 polarization of microglia. Cell Death Dis. 4, e525 (2013).
25. Fenou, A. M., Hall, J. C., Genel, J. C., Popovich, P. G. & Godbout, J. P. IL-4 signaling drives a unique arginase+–IL–1β+ microglia phenotype and recruits macrophages to the inflammatory CNS: consequences of age-related deficits in IL–4Rα after traumatic spinal cord injury. J. Neurosci. 34, 8904–8917 (2014).
26. Finkelman, F. D. & Morris, S. C. Development of an assay to measure in vivo cytokine production in the mouse. Int. Immunol. 11, 1811–1818 (1999).
27. Alooisi, F., Ria, F., Penna, G. & Ardorini, L. Microglia are more efficient than astrocytes in antigen processing and in TH1 but not TH2 cell activation. J. Immunol. 160, 4671–4680 (1998).
28. 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).
29. Zhao, X. et al. Neuronal interleukin-4 as a modulator of microglial pathways and ischemic brain damage. J. Neurosci. 35, 11281–11291 (2015).
30. Hejen, J. F. et al. IL–1R3 blockade broadly attenuates the functions of six members of the IL–1 family, revealing their contribution to models of disease. Nat. Immunol. 20, 1138–1149 (2019).
31. Hayashi, S. & McAlonan, A. P. Efficient recombination in diverse tissues by a tamoxifen-inducible form of Cre: a tool for temporally regulated gene activation/inactivation in the mouse. Dev. Biol. 244, 305–318 (2002).
32. Ballak, D. B. et al. Combined B- and T-cell deficiency does not protect against obesity-induced glucose intolerance and inflammation. Cytokine 62, 96–103 (2013).
33. Derecki, N. C. et al. Regulation of learning and memory by meningeal immunity: a key role for IL–4. J Exp Med. 207, 1067–1080 (2010).
35. Louveau, A. et al. Structural and functional features of central nervous system lymphatic vessels. Nature 523, 337–341 (2015).
36. Schlüter, C. et al. Effector T-cell trafficking between the leptomeninges and the cerebrospinal fluid. Nature 530, 349–353 (2016).
37. Fitzpatrick, Z. et al. Gut-educated IgA plasma cells defend the meningeal venous sinuses. Nature 587, 472–476 (2020).
38. Benakis, C. et al. Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. Nat. Med. 22, 516–523 (2016).
39. Nolan, Y. et al. Role of interleukin-4 in regulation of age-related inflammatory changes in the hippocampus. J. Biol. Chem. 280, 9354–9362 (2005).
40. Barna, B. P. et al. Epidermal growth factor regulates astrocyte expression of the interleukin-4 receptor via a MAPK-independent pathway. Cell Immunol. 208, 18–24 (2001).
41. Malenka, R. C. & Bear, M. F. LTP and LTD: an embarrassment of riches. Neuron 44, 5–21 (2004).
42. Nicolas, C. S. et al. The Jak/STAT pathway is involved in synaptic plasticity. Neuron 73, 374–390 (2012).
43. Stowell, R. D. et al. Noradrenergic signaling in the wakeful state inhibits microglial surveillance and synaptic plasticity in the mouse visual cortex. Nat. Neurosci. 22, 1782–1792 (2019).
44. Meyer-Luehmann, M. et al. Rapid appearance and local toxicity of amyloid-β plaques in a mouse model of Alzheimer’s disease. Nature 451, 720–724 (2008).
45. Gyoneva, S. et al. Systemic inflammation regulates microglial responses to tissue damage in vivo. Glia 62, 1345–1360 (2014).
46. Markler, D. F., Tremblay, M. E., Lu, S. M., Majewska, A. K. & Gelbard, H. A. A thin-skull window technique for chronic two-photon in vivo imaging of murine microglia in models of neuroinflammation. J. Vis. Exp. 2010, 2059 (2010).
47. Nimmerjahn, A. Two-photon imaging of microglia in the mouse cortex in vivo. Cold Spring Harb. Protoc. 2012, pdb.prot069294 (2012).
48. Pirih, F. et al. Adverse effects of hyperlipidemia on bone regeneration and strength. J. Bone Miner. Res. 27, 309–318 (2012).
49. Erion, J. R. et al. Obesity elicits interleukin-1–mediated deficits in hippocampal synaptic plasticity. J Neurosci. 34, 2618–2631 (2014).
50. McGee-Lawrence, M. E. et al. Whole-body vibration mimics the metabolic effects of exercise in male leptin receptor-deficient mice. Endocrinology. 158, 1160–1171 (2017).

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Author contributions
All authors were directly involved in data acquisition and analysis. D.-H.G., B.B., and A.M.S. designed experiments. D.-H.G. and A.M.S. prepared the manuscript and all authors provided comments on subsequent drafts.

Competing interests
The authors declare no competing interests.

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