

## Visceral adipose NLRP3 impairs cognition in obesity via IL1R1 on Cx3cr1<sup>+</sup> cells

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Induction of the inflammasome protein cryopyrin (NLRP3) in visceral adipose tissue (VAT) promotes release of the pro-inflammatory cytokine interleukin-1 $\beta$  (IL1 $\beta$ ) in obesity. While this mechanism contributes to peripheral metabolic dysfunction, effects on the brain remain unexplored. These studies investigated whether visceral adipose NLRP3 impairs cognition by activating microglial interleukin-1 receptor 1 (IL1R1). After observing protection against obesity-induced neuroinflammation and cognitive impairment in NLRP3KO mice, we transplanted VAT from obese WT or NLRP3KO donors into lean recipients. Transplantation of VAT from a WT donor (TRANS<sub>WT</sub>) increased hippocampal IL1 $\beta$  and impaired cognition, but VAT transplants from comparably obese NLRP3KO donors (TRANS<sub>KO</sub>) had no effect. Visceral adipose NLRP3 was required for deficits in long-term potentiation (LTP) in transplant recipients, and LTP impairment in TRANS<sub>WT</sub> mice was IL1-dependent. Flow cytometric and gene expression analyses revealed that VAT transplantation recapitulated the effects of obesity on microglial activation and IL1 $\beta$  gene expression, and visualization of hippocampal microglia revealed similar effects in vivo. Inducible ablation of IL1R1 in CX3CR1-expressing cells eliminated cognitive impairment in mice with dietary obesity and in transplant recipients and restored immunoquiescence in hippocampal microglia. These results indicate that visceral adipose NLRP3 impairs memory via IL1-mediated microglial activation, and suggest that NLRP3-IL1 $\beta$  signaling may underlie correlations between visceral adiposity and cognitive impairment in humans.

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## 2 **Visceral adipose NLRP3 impairs cognition in obesity** 3 **via IL1R1 on Cx3cr1+ cells**

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**1 Abstract**

2 Induction of the inflammasome protein cryopyrin (NLRP3) in visceral adipose tissue (VAT)  
3 promotes release of the pro-inflammatory cytokine interleukin-1 $\beta$  (IL1 $\beta$ ) in obesity. While this  
4 mechanism contributes to peripheral metabolic dysfunction, effects on the brain remain  
5 unexplored. These studies investigated whether visceral adipose NLRP3 impairs cognition by  
6 activating microglial interleukin-1 receptor 1 (IL1R1). After observing protection against obesity-  
7 induced neuroinflammation and cognitive impairment in NLRP3KO mice, we transplanted VAT  
8 from obese WT or NLRP3KO donors into lean recipients. Transplantation of VAT from a WT  
9 donor (TRANS<sub>WT</sub>) increased hippocampal IL1 $\beta$  and impaired cognition, but VAT transplants from  
10 comparably obese NLRP3KO donors (TRANS<sub>KO</sub>) had no effect. Visceral adipose NLRP3 was  
11 required for deficits in long-term potentiation (LTP) in transplant recipients, and LTP impairment  
12 in TRANS<sub>WT</sub> mice was IL1-dependent. Flow cytometric and gene expression analyses revealed  
13 that VAT transplantation recapitulated the effects of obesity on microglial activation and IL1 $\beta$   
14 gene expression, and visualization of hippocampal microglia revealed similar effects in vivo.  
15 Inducible ablation of IL1R1 in CX3CR1-expressing cells eliminated cognitive impairment in mice  
16 with dietary obesity and in transplant recipients and restored immunoquiescence in hippocampal  
17 microglia. These results indicate that visceral adipose NLRP3 impairs memory via IL1-mediated  
18 microglial activation, and suggest that NLRP3-IL1 $\beta$  signaling may underlie correlations between  
19 visceral adiposity and cognitive impairment in humans.

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1 Obesity is a major public health issue, and while some overweight individuals are  
2 physiologically healthy, many develop serious pathological conditions. Obesity has a deleterious  
3 effect on the central nervous system (CNS), and these effects are not limited to classical  
4 metabolic circuits in the brain. Several studies have reported increased rates of age-related  
5 cognitive decline in human obesity (1-5) and atrophy of medial temporal lobe regions involved  
6 in memory, including the hippocampus (6-8). However, there are also negative reports that did  
7 not detect cognitive impairment or brain atrophy in obesity (9-11). Unresolved controversies  
8 surrounding obesity-induced cognitive impairment may be due to the use of weight/height ratio  
9 criteria that do not reflect differences in adipose tissue distribution. Individuals with the 'apple-  
10 shaped' distribution of body fat are at increased risk of developing diabetes (12). Visceral  
11 adiposity, as reflected by waist-to-hip ratio, is a stronger predictor of age-related cognitive  
12 impairment than body mass index (BMI) in humans (4, 13-14). Although consensus is emerging  
13 from human studies of dementia risk in obesity, work in animal models has yet to elucidate the  
14 specific mechanism linking visceral adiposity with cognitive impairment.

15 Deposition of visceral white adipose tissue (VAT) induces systemic inflammation and  
16 promotes the development of metabolic complications in obesity (15-16). Chronic lipid overload  
17 in visceral adipocytes is accompanied by release of damage-associated molecular patterns that  
18 attract monocyte precursors and induce their differentiation into adipose tissue macrophages  
19 (17). The inflammatory VAT microenvironment leads to formation of 'inflammasome' complexes  
20 that amplify innate immune responses in adipose tissue (18-19). NOD-like receptor family, pyrin  
21 domain containing 3 (NLRP3) is a core component of the inflammasome complex and visceral  
22 adipose NLRP3 induction promotes synthesis and release of the pro-inflammatory cytokine  
23 interleukin-1 $\beta$  (IL1 $\beta$ ) in obesity (18). Whole-body *Nlrp3*<sup>-/-</sup> mice develop obesity but are protected

1 against high-fat diet-induced adipose tissue inflammation and insulin resistance (18-19). The  
2 *Nlrp3* null mutation also protects against cognitive deficits in aged mice and in mouse models of  
3 Alzheimer's disease (20-21), but the consequences of tissue-specific NLRP3 induction for  
4 neuroplasticity and neuroinflammation have yet to be elucidated.

5 We hypothesized that NLRP3 induction in visceral adipose tissue initiates microglial  
6 activation and cognitive impairment by increasing IL1 $\beta$ . Peripheral IL1 $\beta$  enters the CNS via  
7 saturable transporters at the blood-brain barrier (BBB; 22), and we reasoned that microglial  
8 interleukin-1 receptor 1 (*Il1r1*) activation would initiate an autocrine amplification loop similar to  
9 that previously reported in other disease models (23). This hypothesis was tested in a series of  
10 dietary obesity and visceral adipose tissue (VAT) transplantation experiments using *Nlrp3*<sup>-/-</sup>  
11 mutant mice and transgenic mice with inducible deletion of *Il1r1* in CX3CR1-expressing cells.  
12 Collectively, these data suggest that CNS immune cells detect and amplify peripheral IL1 $\beta$   
13 generated following visceral adipose inflammasome activation, and that increases in CNS IL1 $\beta$   
14 downstream of this cascade impair hippocampal synaptic plasticity and cognition in obesity. Our  
15 findings add to the growing literature on dynamic interactions between the brain and peripheral  
16 tissues, and provide further support for reinterpretation of immune privilege in the CNS.

17

## 18 **Results**

### 19 *Resistance to obesity-induced neuroinflammation and cognitive dysfunction in NLRP3KO mice*

20 Obesity promotes formation of inflammasome complexes in multiple tissues, including the  
21 brain (24), but the link between NLRP3 and obesity-induced cognitive impairment remains  
22 correlative at present. To determine whether the inflammasome protein NLRP3 is required for  
23 obesity-induced microglial activation and cognitive dysfunction, we maintained *Nlrp3*<sup>-/-</sup> mice

1 (KO) and Wt littermates on high-fat or low-fat diet (HFD, LFD). Consistent with previous reports  
2 (18-19), Wt and NLRP3KO mice gained comparable amounts of weight during the 12wk period  
3 (Figure 1A). The weight of the visceral, subcutaneous, and interscapular fat pads was also  
4 unaffected by genotype (Figure 1B). However, NLRP3KO mice were protected against increases  
5 in IL1 $\beta$  in VAT with HFD consumption (Figure 1C;  $F_{1,12}=16.83$ ,  $p=0.002$ ). Wt/HFD mice exhibited  
6 significant increases in circulating IL1 $\beta$  ( $F_{1,12}=8.05$ ,  $p=0.02$ ), but NLRP3KO/HFD mice did not  
7 differ from Wt/LFD (Figure 1C). Protection against IL1 $\beta$  accumulation was also observed in  
8 hippocampal lysates from NLRP3KO/HFD mice (Figure 1C;  $F_{1,12}=6.8$ ,  $p=0.02$ ), suggesting that  
9 resistance to obesity-induced peripheral inflammation in NLRP3KO mice extends to the CNS.

10         Microglia continuously sense and respond to molecular patterns in the local environment.  
11 Because microglial process retraction is a well-characterized response to inflammation, we  
12 visualized IBA1+ cells in the hippocampal dentate gyrus and analyzed their morphology.  
13 Microglia from Wt/HFD mice exhibited significant reductions in process length and complexity,  
14 indicated by lower numbers of intersections at 1-micron intervals around the soma (Figure 1D,  
15  $F_{1,14}=6.61$ ,  $p=0.02$ ). Process length and complexity were unaffected in NLRP3KO/HFD mice,  
16 which did not differ from Wt/LFD (Figure 1D). There were no differences between Wt/LFD and  
17 NLRP3KO/LFD mice, indicating that NLRP3KO mice are resistant to HFD-induced microglial  
18 process retraction. Parallel visualization of the lysosomal marker CD68 revealed significant  
19 accumulation in IBA1+ microglia Wt/HFD mice, but not NLRP3KO/HFD mice (Figure 1D).  
20 Microglial CD68 accumulation was evident both qualitatively, and quantitatively, as determined  
21 by analysis of CD68+ puncta within regions of interest delineated by IBA1 labeling  
22 (Supplemental Figure 1A-B;  $F_{1,14}=13.47$ ,  $p<0.001$ ). Microglial CD68 accumulation occurs  
23 following phagocytosis, which could reflect protective or pathological responses (25). To

1 interpret changes in CD68 immunoreactivity, we performed immunofluorescence labeling for  
2 IBA1 and the classical activation marker MHCII. Consistent with previous studies (26),  
3 IBA1/MHCII double-positive cells were more frequent in Wt/HFD mice, relative to Wt/LFD  
4 (Supplemental Figure 1C-D;  $F_{1,14}=17.84$ ,  $p<0.001$ ). Colabeling for IBA1 and MHCII was rare in  
5 NLRP3KO/HFD mice, which did not differ from Wt/LFD (Supplemental Figure 1C-D).

6 To examine the role of NLRP3 in obesity-induced cognitive dysfunction, Wt and  
7 NLRP3KO mice were tested in the water maze after 12wk HFD or LFD. Wt/HFD mice had longer  
8 swim paths during acquisition training and exhibited deficits during the probe test, relative to  
9 Wt/LFD mice (Figure 1E; for acquisition,  $F_{1,36}=7.03$ ,  $p=0.01$ ; for probe,  $F_{1,60}=4.11$ ,  $p=0.04$ ). By  
10 contrast, NLRP3KO/HFD mice did not differ from Wt/LFD mice (Figure 1E). There was no effect  
11 of genotype in LFD mice, and all groups of mice performed similarly when swimming towards a  
12 visible platform (distance [m], mean $\pm$ sem: Wt/LFD=5.8 $\pm$ 1.1; Wt/HFD=5.9 $\pm$ 0.7; KO/LFD=5.4 $\pm$ 0.8;  
13 KO/HFD=5.3 $\pm$ 1.0). After observing NLRP3-dependent deficits in learning and memory, we  
14 investigated changes in hippocampal synaptic plasticity using extracellular recordings in brain  
15 slices. Stimulation of medial perforant path afferents to the dentate gyrus revealed significant  
16 reductions in long-term potentiation (LTP) in Wt/HFD mice (Figure 1F;  $F_{1,39}=6.88$ ,  $p=0.01$ ).  
17 Deficits in LTP were mediated by NLRP3, as slices from NLRP3KO/HFD mice exhibited LTP  
18 that was comparable to Wt/LFD (Figure 1F), indicating that whole-body ablation of NLRP3  
19 protects against obesity-induced microglial activation and maintains hippocampal plasticity in  
20 dietary obesity.

21

22 *Visceral adipose NLRP3 increases hippocampal interleukin-1 $\beta$*

1 To determine whether NLRP3 induction in visceral fat regulates hippocampal IL1 $\beta$ , we  
2 transplanted VAT from Wt/HFD or NLRP3KO/HFD donors into lean Wt recipients (Figure 2A).  
3 Mice that received transplants from a Wt donor (TRANS<sub>WT</sub>) or an NLRP3KO donor (TRANS<sub>KO</sub>)  
4 were compared with sham-operated Wt mice maintained on LFD (LFD/SHAM) or HFD  
5 (HFD/SHAM). Visceral fat transplantation had no effect on body weight or glycemic control in  
6 recipients 2wk after surgery (Figure 2A-B), and there were no differences in transplant viability  
7 between Wt and NLRP3KO donors (Supplemental Figure 2A; n rejections: TRANS<sub>WT</sub>, n=1;  
8 TRANS<sub>KO</sub>, n=2). There was also no evidence of compensatory atrophy in resident fat pads from  
9 transplant recipients (Supplemental Figure 2B). Cleavage and release of IL1 $\beta$  is a prominent  
10 consequence of visceral adipose NLRP3 induction (18). Quantification of IL1 $\beta$  in hippocampus,  
11 VAT, and serum revealed parallel increases in TRANS<sub>WT</sub> and HFD/SHAM mice (Figure 2C).  
12 Increases in hippocampal IL1 $\beta$  were dependent on NLRP3, as TRANS<sub>KO</sub> mice had lower levels  
13 of IL1 $\beta$  than TRANS<sub>WT</sub> and did not differ from LFD/SHAM (Figure 2C;  $F_{3,20}=6.42$ ,  $p=0.003$ ). In  
14 the transplanted VAT, IL1 $\beta$  concentrations were significantly higher in transplants from  
15 TRANS<sub>WT</sub>, relative to TRANS<sub>KO</sub> mice (Figure 2C;  $t_{10}=7.34$ ,  $p<0.001$ ). Dietary obesity increased  
16 IL1 $\beta$  concentrations in resident VAT, but these increases were not recapitulated by VAT  
17 transplantation (Figure 2C). qPCR analysis of *Il1b* mRNA in resident and transplanted VAT  
18 revealed similar trends, with increased expression in resident VAT from HFD/SHAM and in  
19 transplanted VAT from TRANS<sub>WT</sub> mice (Supplemental Figure 2C). Hippocampal *Il1b* induction  
20 was only observed following VAT transplantation, as mice that received subcutaneous adipose  
21 tissue (SAT) transplants from a Wt/HFD donor did not exhibit changes in *Il1b* mRNA  
22 (Supplemental Figure 2D). *Il1b* gene expression in SAT transplants did not differ from resident  
23 SAT in sham-operated mice (Supplemental Figure 2E), consistent with the relative

1 immunoquiescence reported in SAT, compared to VAT (16). Collectively, these data suggest  
2 that visceral adipose NLRP3 induction is required for increases in hippocampal IL1 $\beta$  after VAT  
3 transplantation.

4  
5 *Visceral adipose NLRP3 induction impairs hippocampal function*

6 For analysis of NLRP3-mediated hippocampal dysfunction after VAT transplantation,  
7 groups of LFD mice received transplants from Wt or NLRP3KO donors with dietary obesity, as  
8 shown (Figure 2A). Two weeks after surgery, transplant recipients and sham-operated controls  
9 were tested in the water maze, Y-maze, and novel object recognition tasks. In the water maze,  
10 HFD/SHAM and TRANS<sub>WT</sub> mice had longer path lengths during acquisition training and spent  
11 less time in the target quadrant during the probe trial (Figure 2D;  $F_{3,38}=4.68$ ,  $p=0.002$ ). The  
12 effects of VAT transplantation were NLRP3-dependent, as TRANS<sub>KO</sub> mice had shorter path  
13 lengths than TRANS<sub>WT</sub> mice and did not differ from LFD/SHAM (Figure 2D). Changes in  
14 performance were not attributable to deficits in visuomotor navigation, as there were no  
15 differences when swimming toward a visible platform (distance [m], mean $\pm$ sem:  
16 LFD/SHAM=7.29 $\pm$ 0.74; HFD/SHAM=6.97 $\pm$ 0.98; LFD/TRANS<sub>WT</sub>=6.85 $\pm$ 0.45; LFD/TRANS<sub>KO</sub>=  
17 6.47 $\pm$ 0.99). Similar patterns were observed in the Y-maze, where HFD/SHAM and TRANS<sub>WT</sub>  
18 mice alternated less frequently than LFD/SHAM (Figure 2E;  $F_{3,38}=6.59$ ,  $p=0.001$ ). Alternation  
19 deficits were not observed in TRANS<sub>KO</sub> mice, which did not differ from LFD/SHAM (Figure 2E).  
20 In the object recognition test, HFD/SHAM and TRANS<sub>WT</sub> mice exhibited comparable reductions  
21 in novel object preference 30min after training with two identical objects (Figure 2F;  $F_{3,44}=6.46$ ,  
22  $p=0.01$ ). Although within-subject reductions in novel object recognition over time were evident  
23 in all groups, TRANS<sub>KO</sub> mice spent more time exploring the novel object than TRANS<sub>WT</sub> mice,

1 and did not differ from LFD/SHAM (Figure 2F). Given that there were no differences in total  
2 object exploration (time with both objects [sec], mean $\pm$ sem: LFD/SHAM=90.88 $\pm$ 3.97;  
3 HFD/SHAM=81.50 $\pm$ 4.71; LFD/TRANS<sub>WT</sub>=90.28 $\pm$ 3.94; LFD/TRANS<sub>KO</sub>=85.03 $\pm$ 4.99), the  
4 collective outcome of these experiments is consistent with a requirement for NLRP3 in VAT  
5 transplantation-induced memory deficits.

6 After behavioral testing, mice were euthanized for slice preparation and extracellular  
7 recording of dentate gyrus LTP. Slice preparations from HFD/SHAM and TRANS<sub>WT</sub> mice  
8 exhibited smaller increases in the field excitatory postsynaptic potential (fEPSP) one hour after  
9 tetanic stimulation (Figure 2G;  $F_{3,41}=6.41$ ,  $p=0.001$ ). By contrast, LTP in TRANS<sub>KO</sub> slices was  
10 significantly greater than TRANS<sub>WT</sub>, and did not differ from LFD/SHAM (Figure 2G). There were  
11 no effects of diet or VAT transplantation on presynaptic paired-pulse plasticity or on the  
12 input/output ratio across a range of stimulation intensities (data not shown). To examine the role  
13 of hippocampal IL1 in LTP deficits, additional recordings were conducted in the presence of  
14 recombinant interleukin 1 receptor antagonist (IL1RA; 100 $\mu$ g/mL). Preincubation with IL1RA  
15 eliminated LTP deficits in HFD/SHAM and TRANS<sub>WT</sub> mice without influencing LTP in LFD/SHAM  
16 and TRANS<sub>KO</sub> mice (Figure 2H). Taken together, these results indicate that NLRP3 induction in  
17 VAT impairs cognition and suppresses LTP in an IL1-dependent manner.

18

### 19 *Visceral adipose NLRP3 increases penetration of peripheral IL1 $\beta$ across the BBB*

20 Coincident elevation of IL1 $\beta$  in VAT, serum, and hippocampus could reflect changes in  
21 blood-brain barrier (BBB) permeability, increased transport of peripheral IL1 $\beta$  into the CNS, or a  
22 signaling mechanism transduced by cerebrovascular cell populations. To determine whether  
23 dietary obesity and VAT transplantation regulate CNS exposure to peripheral IL1 $\beta$ , mice were

1 injected with 6xHistidine-tagged IL1 $\beta$  (6xHis-IL1 $\beta$ ; 10 micrograms, IV) and the fluorescent tracer  
2 sodium fluorescein (NaFI, 10mg/kg IP; Supplemental Figure 3A). Hippocampal lysates from  
3 HFD/SHAM and TRANS<sub>WT</sub> mice exhibited greater penetration of 6xHis-IL1 $\beta$ , relative to  
4 LFD/SHAM (Supplemental Figure 3B;  $F_{3,12}=9.96$ ,  $p=0.005$ ). Increases in 6xHis-IL1 $\beta$  were  
5 NLRP3-dependent, as lysates from TRANS<sub>KO</sub> mice did not differ from LFD/SHAM (Supplemental  
6 Figure 3B). Changes in CNS penetration of exogenous IL1 $\beta$  were region-specific, as no group  
7 differences were observed in cortical, hypothalamic, or cerebellar lysates (Supplemental Figure  
8 3C-E). These patterns are consistent with increased hippocampal exposure to peripheral IL1 $\beta$ ,  
9 but could arise due to BBB breakdown or changes in transport. Obesity increases BBB  
10 permeability (27-28), and quantification of NaFI in hippocampal lysates from HFD/SHAM mice  
11 replicated this observation (Supplemental Figure 3F;  $F_{3,12}=10.94$ ,  $p<0.001$ ). However, increases  
12 in BBB permeability were not recapitulated by VAT transplantation (Supplemental Figure 3F),  
13 suggesting that CNS exposure to peripheral IL1 $\beta$  in transplant recipients may arise from changes  
14 in transport. Collectively, these data indicate that dietary obesity and VAT transplantation  
15 promote CNS exposure to peripheral IL1 $\beta$  via distinct mechanisms.

### 16

### 17 *Cell type-specific responses to obesity and VAT transplantation*

18 Neuroinflammation encompasses interactions between neurons, microglia, astroglia, and  
19 cerebrovascular cell populations. To gain insight into the cell type-specific effects of obesity and  
20 VAT transplantation, we isolated forebrain mononuclear cells (FMCs), astrocytes, and brain  
21 vascular endothelial cells (BVECs) for flow cytometry and analysis of gene expression. Because  
22 macrophage infiltration has been reported in both genetic and dietary obesity (29-30), we initially  
23 conducted immunophenotyping experiments in FMCs to determine their identity (Figure 3A). In

1 FMCs from HFD/SHAM mice, we observed significant increases in the proportion of  
2 CD45<sup>hi</sup>/Ly6Chi/CD11b<sup>+</sup> cells, suggestive of macrophage infiltration (Figure 3B-C;  $F_{3,16}=7.75$ ,  
3  $p=0.001$ ). However, this increase was not present in FMCs from VAT transplant recipients  
4 (Figure 3B-C). After observing that obesity, but not VAT transplantation, promotes macrophage  
5 infiltration into the brain parenchyma, we examined markers of classical activation in  
6 CD45<sup>low</sup>/Ly6Clow/CD11b<sup>+</sup> microglia. Analysis of MHCII and TLR4 revealed evidence of  
7 microglial polarization in HFD/SHAM and TRANS<sub>WT</sub> samples (Figure 3D-E; for MHCII,  
8  $F_{3,16}=4.94$ ,  $p=0.02$ ; for TLR4,  $F_{3,20}=5.95$ ,  $p=0.02$ ). Microglial induction of MHCII and TLR4 was  
9 not observed in TRANS<sub>KO</sub> mice, which did not differ from LFD/SHAM (Figure 3D-E), consistent  
10 with a requirement for NLRP3 in VAT transplantation-induced microglial activation.

11 Analysis of gene expression in FMCs, astrocytes, and BVECs revealed cell type-specific  
12 responses to obesity and VAT transplantation. Increases in *I11b* mRNA were detected in FMCs  
13 and whole-hippocampal cDNA from HFD/SHAM and TRANS<sub>WT</sub> mice, relative to LFD/SHAM  
14 (Supplemental Figure 4A; for FMCs,  $F_{3,28}=7.75$ ,  $p=0.003$ ; for hippocampus,  $F_{3,28}=5.73$ ,  $p=0.004$ ).  
15 These effects were dependent on NLRP3, as TRANS<sub>KO</sub> samples exhibited reduced *I11b* relative  
16 to TRANS<sub>WT</sub> and did not differ from LFD/SHAM (Supplemental Figure 4A). Quantification of  
17 additional pro-inflammatory cytokines revealed distinct responses to HFD or VAT  
18 transplantation. Specifically, *I16* mRNA was increased in astrocytes and FMCs from HFD/SHAM  
19 mice, relative to LFD/SHAM (Supplemental Figure 4B; for FMCs,  $F_{3,28}=5.57$ ,  $p=0.003$ ; for  
20 astrocytes,  $F_{3,28}=3.65$ ,  $p=0.03$ ). Obesity also upregulated *Mcp1* expression in all cell types and  
21 in whole-hippocampal cDNA (Supplemental Figure 4C; for FMCs,  $F_{3,28}=3.51$ ,  $p=0.03$ ; for  
22 astrocytes,  $F_{3,32}=3.20$ ,  $p=0.04$ ; for BVECs,  $F_{3,28}=4.31$ ,  $p=0.01$ ; for hippocampus,  $F_{3,28}=5.02$ ,  
23  $p=0.006$ ).

1 Transcriptomic studies of microglia and other CNS cell populations report low but  
2 detectable expression of *Il1r1* under normal physiological conditions (31-32). Upregulation of  
3 microglial *Il1r1* has also been reported in disease models and after chemogenetic depletion (33-  
4 34). We examined cellular patterns of *Il1r1* expression and immunoreactivity using  
5 immunofluorescence and qPCR (Supplemental Figure 4D-F). Paraformaldehyde-fixed  
6 microglia, astrocytes, and BVECs from normal mice were immunoreactive for IL1R1 and  
7 phenotype-specific antigens (Supplemental Figure 4D). Quantification of *Il1r1* mRNA revealed  
8 significant increases in FMCs from HFD/SHAM and TRANS<sub>WT</sub> mice (Supplemental Figure 4E-  
9 F;  $F_{3,28}=9.77$ ,  $p=0.001$ ). Taken together, these results implicate microglia as early detectors and  
10 potential amplifiers of visceral adipose-derived IL1 $\beta$ .

11

### 12 *Obesity and VAT transplantation amplify microglial responses to IL1 $\beta$*

13 Peripheral macrophages and resident microglia exhibit sensitization in response to pro-  
14 inflammatory stimuli, including IL1 $\beta$ , which primes cells for autocrine amplification (33, 35;  
15 reviewed in 23). To examine whether microglia might amplify CNS responses to exogenous IL1 $\beta$   
16 in obesity, we measured media cytokines and gene expression in FMCs from LFD/SHAM,  
17 HFD/SHAM, TRANS<sub>WT</sub>, and TRANS<sub>KO</sub> mice. Cells from HFD/SHAM and TRANS<sub>WT</sub> mice had  
18 higher levels of media TNF $\alpha$  after stimulation with increasing concentrations of IL1 $\beta$ , relative to  
19 cells from LFD/SHAM mice (Figure 3F;  $F_{3,48}=2.98$ ,  $p=0.006$ ), indicative of sensitization. Visceral  
20 adipose NLRP3 was required for sensitization, as cells from TRANS<sub>KO</sub> mice did not differ from  
21 LFD/SHAM (Figure 3F). Sensitization of responses to IL1 $\beta$  were not explained by differences in  
22 viability, as there were no differences in cell survival after stimulation (Figure 3F). Priming of  
23 IL1 $\beta$ -stimulated gene expression was also evident based on lower thresholds for induction of

1 *IL1b* mRNA in FMCs from HFD/SHAM and TRANS<sub>WT</sub> mice (Figure 3G;  $F_{3,48}=9.18$ ,  $p<0.001$ ). By  
2 contrast, cells from TRANS<sub>KO</sub> mice exhibited IL1 $\beta$ -stimulated gene expression profiles that were  
3 comparable to LFD/SHAM (Figure 3G). When interpreted with the flow cytometry dataset (Figure  
4 3B), these outcomes suggest that the mixed population of microglia and infiltrating macrophages  
5 in FMCs from HFD/SHAM mice and the relatively homogeneous population of microglia in FMCs  
6 from TRANS<sub>WT</sub> mice exhibit comparable sensitization and priming in response to IL1 $\beta$ .  
7 Moreover, the lack of sensitization and priming in primary microglia from TRANS<sub>KO</sub> mice is  
8 consistent with a requirement for NLRP3 in these effects.

9  
10 *Visceral adipose NLRP3 induction activates microglia in vivo*

11 To investigate the consequences of VAT transplantation for microglia in the intact  
12 hippocampus, LFD/SHAM, HFD/SHAM, TRANS<sub>WT</sub>, and TRANS<sub>KO</sub> mice were perfused 2wk after  
13 surgery for immunohistochemical visualization of the microglial marker IBA1 and the activation  
14 marker MHCII. Unbiased stereological quantification of total microglial number in the dentate  
15 molecular layer revealed no evidence of proliferation or cell loss with dietary obesity or VAT  
16 transplantation (Figure 4A). Activated microglia typically retract their processes, and we used  
17 process number as an indicator of activation state in these experiments. Dietary obesity and  
18 VAT transplantation were accompanied by increases in the number of 'Simple' microglia with  $\leq 2$   
19 primary processes ( $F_{3,24}=7.14$ ,  $p=0.005$ ; Figure 4A), relative to LFD/SHAM. Microglial  
20 simplification was NLRP3-dependent, as counts of simple and complex ramified microglia did  
21 not differ between LFD/SHAM and TRANS<sub>KO</sub> mice (Figure 4A). 3D Reconstruction of IBA1+ cells  
22 revealed comparable reductions in process length and complexity in TRANS<sub>WT</sub> and HFD/SHAM  
23 mice (Figure 4B,  $F_{3,16}=9.04$ ,  $p<0.001$ ). Microglial process retraction was mediated by visceral

1 adipose NLRP3, as TRANS<sub>KO</sub> mice exhibited process lengths and Sholl profiles that did not differ  
2 from LFD/SHAM (Figure 4B). The effects of obesity and VAT transplantation were not limited to  
3 morphology, as visualization of the activation marker MHCII in IBA1+ cells revealed similar  
4 increases in colocalization in TRANS<sub>WT</sub> and HFD/SHAM mice (Figure 4C;  $F_{3,16}=12.84$ ,  $p=0.002$ ).  
5 By contrast, TRANS<sub>KO</sub> mice had significantly fewer IBA1/MHCII positive cells than TRANS<sub>WT</sub>,  
6 and did not differ from LFD/SHAM, consistent with NLRP3-mediated microglial activation in vivo  
7 with obesity and VAT transplantation.

8

### 9 *Generation and characterization of CX3CR1<sup>creERT</sup>/IL1R1<sup>fl/fl</sup> transgenic mice*

10 After observing correlated increases in IL1 $\beta$ , ex vivo sensitization, and microglial  
11 activation, we hypothesized that microglial IL1R1 activation might initiate these responses in  
12 dietary obesity. To address this question, we bred transgenic mice with inducible deletion of *Il1r1*  
13 under the *Cx3cr1* promoter (34). Although CX3CR1 is expressed by multiple monocyte lineages,  
14 differential turnover in resident microglia and peripheral myeloid cells enables selective  
15 manipulation of gene expression in the CNS >4wk after transgene induction (36). For additional  
16 insight into tissue-specific patterns of *Il1r1* expression in this model, groups of  
17 CX3CR1<sup>creERT</sup>/IL1R1<sup>fl/fl</sup> mice (Tg) and nontransgenic littermates (nTg) were maintained on  
18 standard diet from weaning, with tamoxifen induction at 10wk old and tissue collection 1wk or  
19 4wk post-induction (Supplemental Figure 5A). Amplification of genomic DNA from FMCs, spleen,  
20 VAT, and subcutaneous adipose tissue (SAT) using primers targeting the *Il1r1* deleted sequence  
21 revealed excision 1wk after tamoxifen in all tissues (Supplemental Figure 5B). However, at 4wk  
22 post-induction, the deleted sequence was only detectable in FMCs (Supplemental Figure 5B).  
23 qPCR analysis of *Il1r1* mRNA in the above tissues revealed parallel fluctuations in gene

1 expression (Supplemental Figure 5C-F). In FMCs from Tg mice, *Il1r1* mRNA was low or  
2 undetectable after >40 cycles of amplification (Supplemental Figure 5C). In spleen, VAT, and  
3 SAT, transient reductions in *Il1r1* mRNA were evident 1wk after induction, with complete  
4 recovery by 4wk (Supplemental Figure 5D-F), indicative of selective recombination in the brain  
5 one month after tamoxifen administration.

6

### 7 *Brain-specific resistance to obesity-induced inflammation in CX3CR1<sup>creERT</sup>/IL1R1<sup>fl/fl</sup> mice*

8 To induce obesity, Tg mice and nTg littermates were maintained on HFD or LFD for 12wk,  
9 with induction during week 5 (Figure 5A). Tg and nTg mice gained similar amounts of weight  
10 and did not exhibit differences in glycemic control (Figure 5A-B). Obesogenic diet consumption  
11 increased the weight of the visceral and subcutaneous fat pads, but these effects were  
12 comparable in Tg and nTg mice (Figure 5C). Quantification of IL1 $\beta$  concentrations in  
13 hippocampus, VAT, and serum revealed brain-specific resistance to IL1 $\beta$  accumulation in  
14 Tg/HFD mice (Figure 5D). nTg/HFD mice exhibited increases in hippocampal IL1 $\beta$  ( $F_{1,12}=20.83$ ,  
15  $p<0.001$ ), but hippocampal IL1 $\beta$  concentrations in Tg/HFD mice were comparable to nTg/LFD  
16 (Figure 5D). There was no effect of genotype on hippocampal IL1 $\beta$  in LFD mice, and HFD mice  
17 from both genotypes exhibited comparable increases in VAT and serum IL1 $\beta$  (Figure 5D; for  
18 serum,  $F_{1,12}=27.53$ ,  $p<0.001$ ; for VAT,  $F_{1,12}=23.66$ ,  $p<0.001$ ).

19 Increases in IL1 $\beta$  promote transmigration of peripheral monocytes into the CNS in other  
20 disease models (37), and after observing IL1R1-mediated increases in hippocampal IL1 $\beta$ , we  
21 examined whether Tg/HFD mice might be protected against obesity-induced macrophage  
22 infiltration (Figure 5E). Analysis of cell-surface IL1R1 expression in the  
23 SSc<sup>low</sup>/FSC<sup>mid</sup>/CD11b<sup>+</sup>/CD45<sup>+</sup> population from FMCs and lysed whole blood upheld the

1 brain-specific recombination observed during characterization of the mouse line (Supplemental  
2 Figure 5). Both Tg/LFD and Tg/HFD mice exhibited comparable reductions in IL1R1 expression,  
3 relative to nTg mice (Figure 5F). Flow cytometric immunophenotyping of FMCs revealed  
4 accumulation of CD45<sup>hi</sup>/Ly6Chi cells in nTg/HFD mice ( $F_{1,26}=5.78$ ,  $p=0.02$ ). Increases were not  
5 observed in FMCs from Tg/HFD mice (Figure 5G), suggestive of a requirement for IL1R1 in  
6 obesity-induced macrophage infiltration.

7         The CD11b<sup>+</sup>/CD45<sup>hi</sup>/Ly6Chi population predominantly contains infiltrating macrophages  
8 (38-39), but the parent population of CD11b<sup>+</sup>/CD45<sup>hi</sup> cells is heterogeneous in its ontogeny and  
9 origin. The meninges, choroid plexus, and perivascular space contain populations of CNS  
10 border-associated macrophages (BAMs) generated during embryonic development (39-40). In  
11 the *Cx3cr1<sup>creERT</sup>* line, long-lived BAMs exhibit persistent recombination >4wk post-induction (40),  
12 and to investigate responses in an analogous population, we analyzed intracellular IL1 $\beta$   
13 fluorescence in the CD11b<sup>+</sup>/CD45<sup>hi</sup>/Ly6C<sup>low</sup> cells. Reductions in IL1 $\beta$  mean fluorescence  
14 intensity (MFI) were evident in CD11b<sup>+</sup>/CD45<sup>hi</sup>/Ly6C<sup>low</sup> cells from Tg mice (Figure 5H;  
15  $F_{1,8}=31.3$ ,  $p<0.001$ ). Reductions were comparable in Tg/LFD and Tg/HFD mice, suggestive of  
16 diet-independent regulation (Figure 5H). Quantification of IL1 $\beta$  MFI in  
17 CD11b<sup>+</sup>/CD45<sup>low</sup>/Ly6C<sup>low</sup> microglia identified non-overlapping responses to obesity and IL1R1  
18 deletion. In nTg mice, IL1 $\beta$  fluorescence was weaker in microglia, relative to  
19 CD11b<sup>+</sup>/CD45<sup>hi</sup>/Ly6C<sup>low</sup> cells, irrespective of diet (Figure 5H). Dietary obesity increased  
20 microglial IL1 $\beta$  MFI in nTg mice (Figure 5H;  $F_{1,8}=11.31$ ,  $p=0.01$ ), but not in Tg mice, suggestive  
21 of IL1R1-dependent mechanisms.

22         To determine whether differential regulation of IL1 $\beta$  might reflect differences in activation,  
23 we quantified cell-surface MHCII and TLR4 in CD11b<sup>+</sup>/CD45<sup>low</sup>/Ly6C<sup>low</sup> microglia and in the

1 CD11b<sup>+</sup>/CD45<sup>hi</sup>/Ly6C<sup>low</sup> population, which includes BAMs (38-40). In microglia, IL1R1 was  
2 required for obesity-induced inflammatory polarization (Figure 5I; for MHCII,  $F_{1,14}=17.83$ ,  
3  $p=0.008$ ; for TLR4,  $F_{1,14}=15.18$ ,  $p<0.001$ ). Among CD45<sup>hi</sup>/Ly6C<sup>low</sup> cells, IL1R1 deletion  
4 reduced MHCII and TLR4 expression in a diet-independent manner (Supplemental Figure 6A-  
5 B; for MHCII,  $F_{1,14}=5.67$ ,  $p=0.03$ ; for TLR4,  $F_{1,14}=11.28$ ,  $p=0.005$ ). Basal expression of MHCII  
6 and TLR4 was also higher among CD45<sup>hi</sup>/Ly6C<sup>low</sup> cells, relative to microglia (Figure 5I and  
7 Supplemental Figure 6A-B). While these data do not capture the potential for dynamic  
8 interactions between the two cell populations over time, the outcome is suggestive of distinct  
9 responses to dietary obesity and IL1R1 deletion in microglia and brain macrophages.

10 We next visualized IBA1<sup>+</sup> cells in the hippocampal dentate gyrus to examine the role of  
11 IL1R1 in obesity-induced microglial reactivity under intact conditions. Based on their morphology  
12 and location in the brain parenchyma, the IBA1<sup>+</sup> cells sampled in these experiments likely  
13 represent resident microglia. Total process length and complexity were significantly reduced in  
14 nTg/HFD mice, but cells from Tg/HFD mice were indistinguishable from nTg/LFD controls  
15 (Figure 5J;  $F_{1,12}=5.56$ ,  $p=0.03$ ). Inducible ablation of IL1R1 also eliminated microglial  
16 accumulation of CD68<sup>+</sup> puncta with dietary obesity (Figure 5J). Microglia from Tg/LFD and  
17 nTg/LFD mice exhibited comparable morphology and CD68 immunoreactivity (Figure 5J),  
18 consistent with a requirement for IL1R1 in obesity-induced microglial activation.

19

#### 20 *Activation of IL1R1 on CX3CR1-expressing cells underlies hippocampal dysfunction in obesity*

21 After observing that IL1R1 was required for microglial IL1 $\beta$  activation, we examined the  
22 consequences of this cascade for hippocampal function. Transgenic mice and nTg littermates  
23 were maintained on HFD or LFD as shown (Figure 5A), with behavioral testing during weeks 10-

1 12. In the water maze, Tg/HFD mice were resistant to obesity-induced learning deficits, based  
2 on shorter path lengths relative to nTg/HFD mice, and intact performance during the probe trial  
3 (Figure 6A; for acquisition,  $F_{1,44}=7.22$ ,  $p=0.01$ ; for probe,  $F_{1,44}=12.62$ ,  $p<0.001$ ). There were no  
4 differences between nTg/LFD and Tg/LFD mice, and all groups performed comparably in the  
5 visible platform test (distance [m], mean $\pm$ sem: nTg/LFD=5.87 $\pm$ 0.4; nTg/HFD=5.62 $\pm$ 0.7;  
6 Tg/LFD=6.20 $\pm$ 0.5; Tg/HFD=5.2 $\pm$ 0.6). Similar patterns were observed in the novel object  
7 preference paradigm, where Tg/HFD mice were protected against deficits in recognition memory  
8 30min after training (Figure 6B;  $F_{1,24}=5.54$ ,  $p=0.03$ ). Differences in novel object preference were  
9 not explained by changes in total object exploration (time with both objects [sec], mean $\pm$ sem:  
10 nTg/LFD = 83.5 $\pm$ 5.4; nTg/HFD = 76.3 $\pm$ 5.6; Tg/LFD = 87.1 $\pm$ 4.9; Tg/HFD = 72.7 $\pm$ 4.5), and all  
11 groups of mice exhibited within-subject decrements in object recognition over time (Figure 6B).  
12 In the Y-maze, IL1R1 expression was required for deficits in spatial recognition, based on  
13 reductions in alternation behavior in nTg/HFD, but not Tg/HFD mice (Figure 6C;  $F_{1,25}=5.44$ ,  
14  $p=0.005$ ). In all paradigms, Tg/LFD mice did not differ from nTg/LFD controls, suggesting that  
15 microglial IL1R1 may be dispensable for hippocampus-dependent memory under basal  
16 conditions.

17 To examine the consequences of IL1R1-mediated neuroinflammation for hippocampal  
18 synaptic plasticity, we performed extracellular field potential recordings in hippocampal slices  
19 from Tg mice and nTg littermates after HFD or LFD. Induction of LTP revealed a pivotal role for  
20 IL1R1 in obesity-induced plasticity deficits (Figure 6D). nTg/HFD mice exhibited significant  
21 reductions in LTP magnitude, but Tg mice on HFD exhibited LTP that was comparable to  
22 nTg/LFD mice ( $F_{1,44}=8.86$ ,  $p=0.008$ ). Changes in LTP were not explained by alterations in the

1 input/output relationship (Figure 6E), or by changes in presynaptic paired-pulse depression  
2 (Figure 6F), suggestive of postsynaptic deficits following microglial IL1R1 activation.

3 While there is consensus that immune activation-induced elevations in IL1 $\beta$  impair LTP  
4 (41), the cellular targets for these effects remain poorly understood. To test the hypothesis that  
5 microglial IL1R1 activation underlies IL1 $\beta$ -mediated suppression of LTP, slices from nTg/LFD  
6 and Tg/LFD mice were pre-incubated with exogenous IL1 $\beta$  (1.0ng/mL) for 20min, with continued  
7 superperfusion of IL1 $\beta$  throughout recording (Figure 6G). Analysis of dendritic field potentials  
8 1hr after tetanic stimulation revealed significant impairment of LTP in nTg/LFD slices ( $t_{14}=5.77$ ,  
9  $p<0.001$ ). LTP deficits were not observed in slices from Tg/LFD mice, implicating microglial  
10 IL1R1 activation as a mechanism for IL1 $\beta$ -mediated LTP impairment. In support of this  
11 interpretation, co-application of the microglial inhibitor minocycline (20 $\mu$ M) and IL1 $\beta$  blocked LTP  
12 deficits in slices from nTg/LFD mice without influencing LTP in Tg/LFD slices (Figure 6G). Taken  
13 together, these results are consistent with an obligatory role for IL1R1 signaling among  
14 CX3CR1-expressing cells in IL1 $\beta$ -mediated synaptic dysfunction.

15

#### 16 *Direct evidence for autocrine amplification of IL1 $\beta$ after VAT transplantation*

17 To investigate the role of IL1R1 in neuroinflammation after VAT transplantation, Tg mice  
18 and nTg littermates received tamoxifen one month before sham operation or VAT transplantation  
19 surgery (Figure 7A). Two weeks after surgery, mice were fasted for IPGTT before being  
20 euthanized for isolation of FMCs or immunohistological analysis of IBA1+ microglia. There was  
21 no effect of genotype on weight gain after surgery (Figure 7A), and there were no changes in  
22 glycemic control in Tg mice (Figure 7B). Analysis of IL1 $\beta$  concentrations in hippocampus, serum,  
23 and VAT revealed brain-specific protection in Tg mice that received VAT transplants

1 (Tg/TRANS). Hippocampal IL1 $\beta$  concentrations were elevated in nTg/TRANS mice, but  
2 Tg/TRANS mice were indistinguishable from sham-operated mice (Figure 7C;  $F_{1,20}=6.38$ ,  
3  $p=0.02$ ). Transplant recipients had increased serum IL1 $\beta$ , relative to sham-operated mice, but  
4 increases in circulating IL1 $\beta$  were unaffected by genotype (Figure 7C). Concentrations of IL1 $\beta$   
5 were significantly higher in the transplanted VAT, relative to resident VAT, but there was no  
6 effect of recipient genotype on IL1 $\beta$  in either set of samples (Figure 7C). Fluctuations in adipose  
7 IL1 $\beta$  protein concentrations were paralleled by changes in *Il1b* gene expression, which was  
8 significantly elevated in transplanted VAT independently of recipient genotype (Figure 7D). After  
9 observing protection against hippocampal IL1 $\beta$  accumulation in Tg/TRANS mice, we analyzed  
10 microglial morphology and MHCII induction. VAT transplantation increased the number of  
11 IBA1/MHCII double-positive cells in nTg mice, but not in Tg mice (Figure 7E;  $F_{1,16}=24.52$ ,  
12  $p<0.001$ ). Changes in MHCII immunoreactivity were accompanied by IL1R1-mediated  
13 reductions in microglial process length and complexity (Figure 7E;  $F_{1,16}=8.85$ ,  $p=0.008$ ).  
14 Collectively, these results indicate that IL1R1 expression among CX3CR1-expressing cells is  
15 required for neuroinflammation following surgical increases in visceral fat.

16 After observing an obligatory role for IL1R1 in hippocampal IL1 $\beta$  accumulation, we  
17 investigated the potential requirement for IL1R1 in autocrine amplification of IL1 $\beta$  ex vivo.  
18 Analysis of gene expression in FMCs after stimulation with increasing concentrations of IL1 $\beta$   
19 revealed IL1R1-mediated priming in transplant recipients (Figure 7F). Cells from nTg/TRANS  
20 mice had lower thresholds for induction of *Il1b* mRNA, but cells from Tg/TRANS mice were  
21 comparable to nTg/SHAM (Figure 7F;  $F_{1,20}=48.28$ ,  $p<0.01$ ). Priming of gene expression was  
22 accompanied by IL1R1-mediated sensitization, based on increases in media TNF $\alpha$  in cells from  
23 nTg/TRANS, but not Tg/TRANS mice (Figure 7G;  $F_{1,20}=19.63$ ,  $p=0.002$ ). Changes in

1 sensitization and priming were not attributable to differential cell survival, as there were no group  
2 differences based on formazan cleavage assay (Figure 7G). The effect of genotype was only  
3 evident in transplant recipients, as cells from Tg/SHAM mice did not differ from nTg/SHAM  
4 (Figure 7F-G). Taken together, these patterns are consistent with IL1R1-mediated autocrine  
5 amplification of *IL1b* expression following VAT transplantation.

6

7 *Protection against obesity-induced microglial activation in CX3CR1<sup>creER</sup>/IL1R1<sup>fl/fl</sup> mice*

8 Microglia interact with neurons via receptor-mediated signaling, alone or in concert with  
9 physical interactions with synaptic terminals (25, 42-43). We previously reported that dietary  
10 obesity disrupts the organization of microglial processes around hippocampal synaptic terminals  
11 (26). To investigate whether VAT transplantation might recapitulate these effects and examine  
12 the potential contributions of IL1R1, Tg mice were crossed with the Thy1-eGFP(S) line to  
13 generate CX3CR1<sup>creERT</sup>/IL1R1<sup>fl/fl</sup> with endogenous fluorescence in dentate granule neurons  
14 (Figure 8A-B). Semi-automated detection and quantification of dendritic spines revealed  
15 reductions in total dendritic spine density after VAT transplantation (Figure 8A;  $F_{1,21}=10.92$ ,  
16  $p=0.003$ ). These effects were primarily attributable to loss of thin spines and were mediated by  
17 IL1R1 activation among CX3CR1-expressing cells (Figure 8A-B;  $F_{1,21}=9.01$ ,  $p=0.007$ ). Parallel  
18 visualization of IBA1 revealed preferential localization of microglial processes at mushroom  
19 spines, relative to other morphologies, in all groups of mice (Figure 8B; # spines sampled per  
20 animal,  $\text{mean} \pm \text{sem} = 551 \pm 75.2$ ). This pattern was further amplified by VAT transplantation in  
21 nTg/TRANS mice ( $F_{1,21}=10.08$ ,  $p=0.004$ ). The effects of VAT transplantation on microglial  
22 apposition at mushroom spines were IL1R1-dependent, as Tg/TRANS mice had similar  
23 proportions of mushroom spines with perisynaptic microglial processes, relative to nTg/SHAM

1 (Figure 8B). IL1R1-mediated perturbation of microglia-synapse relationships was only evident  
2 after VAT transplantation, as Tg/SHAM mice did not differ from nTg/SHAM in terms of spine  
3 density or microglial localization (Figure 8A-B). Collectively, these results are consistent with a  
4 multifaceted role for IL1R1 in regulating microglia/synapse interactions following surgical  
5 increases in visceral fat.

6 To examine the functional consequences of IL1R1-mediated changes in  
7 microglia/synapse relationships, we performed extracellular recordings in brain slices. Dentate  
8 gyrus LTP was significantly reduced in slices from nTg/TRANS, but not in Tg/TRANS mice  
9 (Figure 8C;  $F_{1,40}=10.56$ ,  $p=0.003$ ). Presynaptic paired-pulse plasticity was unaffected at  
10 interpulse intervals ranging from 50msec to 1sec (Figure 8D), and comparison of the input/output  
11 curve over a range of stimulus intensities revealed no effect of genotype or surgery (Figure 8E).  
12 Analysis of hippocampus-dependent memory in the water maze revealed that IL1R1 was  
13 required for memory deficits after VAT transplantation (Figure 8F). Tg/TRANS mice had shorter  
14 swim paths than nTg/TRANS mice during acquisition training and performed on par with  
15 nTg/SHAM during the probe trial (Figure 8F; for acquisition,  $F_{3,56}=9.09$ ,  $p=0.003$ ; for probe,  
16  $F_{1,56}=5.55$ ,  $p=0.03$ ). There were no differences between nTg/SHAM and Tg/SHAM during  
17 acquisition training, and there was no effect of genotype or VAT transplantation on navigation  
18 toward the visible platform (distance [m]; mean $\pm$ sem, nTg/SHAM =  $6.78 \pm 1.25$ ; nTg/TRANS =  
19  $7.27 \pm 0.98$ ; Tg/SHAM =  $6.46 \pm 1.49$ ; Tg/TRANS =  $7.21 \pm 0.77$ ). Ablation of IL1R1 also eliminated  
20 deficits in spontaneous alternation in the Y-maze after VAT transplantation (Figure 8G;  
21  $F_{1,38}=4.55$ ,  $p=0.04$ ). There were no differences in alternation between nTg/SHAM and Tg/SHAM  
22 mice (Figure 8G). In the object recognition task, Tg/TRANS mice performed significantly better  
23 than nTg/TRANS mice at the 30min post-training timepoint (Figure 8H;  $F_{3,43}=9.52$ ,  $p=0.005$ ).

1 There were no differences in object recognition between nTg/SHAM and Tg/SHAM, and all  
2 groups of mice exhibited within-subject reductions in novel object preference over time (Figure  
3 8H). Taken together, these data indicate that IL1R1 activation among CX3CR1-expressing cells  
4 underlies VAT transplantation-induced deficits in hippocampus-dependent memory.

5

## 6 **Discussion**

7 While there is consensus that obesity elicits pro-inflammatory responses in peripheral  
8 tissues, interpretation of similar changes in the brain is complicated by longstanding  
9 assumptions regarding immune privilege in the nervous system. The current studies add to a  
10 growing body of work challenging these assumptions by demonstrating that NLRP3  
11 inflammasome activation in visceral adipose tissue impairs cognition in obesity. These effects  
12 were mediated by activation of IL1R1 among CX3CR1-expressing cells, which detect and  
13 amplify IL1 $\beta$  in the brain. Given that aging, AD, and obesity are associated with NLRP3 induction  
14 and local inflammation in multiple tissues, including the CNS (20-21, 24), it is surprising that  
15 tissue-specific requirements for NLRP3 remain poorly understood. To our knowledge, the VAT  
16 transplantation studies in this report represent the first regionally selective manipulation of  
17 NLRP3 to examine tissue-specific regulation of synaptic plasticity and cognition.

18 Visceral adipose NLRP3 induction promotes caspase 1-mediated cleavage of pro-IL1 $\beta$ ,  
19 leading to release of mature IL1 $\beta$  from adipose tissue macrophages (18, 44-45). Multiple lines  
20 of evidence in this report suggest that exposure to peripherally generated IL1 $\beta$  initiates local  
21 amplification by brain-resident immune cells, similar to that reported following  
22 intracerebroventricular IL1 $\beta$  injections and in experimental autoimmune encephalomyelitis (33,  
23 46). However, it is also possible that CNS border-associated macrophages (BAMs) in the

1 meninges and perivascular space could instigate or oppose parenchymal responses to IL1 $\beta$ .  
2 Long-lived BAMs exhibit recombination at extended timepoints after induction in the  
3 CX3CR1<sup>creERT</sup> line (40), and interactions between BAMs and CSF immune cells were previously  
4 shown to regulate cognition (47). In light of these patterns, it is possible that obesity-induced  
5 cognitive deficits could arise due to IL1R1 activation on BAMs, alone or in concert with responses  
6 among resident microglia. Under one potential scenario, IL1R1 activation would promote  
7 synthesis and release of IL1 $\beta$  by BAMs, which would reach the hippocampus directly by volume  
8 transmission, or indirectly via paracrine signaling interactions with adjacent cells (46, 48).  
9 Although the indirect hypothesis cannot be ruled out, the timeframe for changes in hippocampal  
10 synaptic plasticity following *ex vivo* manipulation of IL1 signaling suggests a more proximal  
11 interaction between CX3CR1-expressing cells and synaptic terminals. Perivascular  
12 macrophages represent the most likely subpopulation of BAMs for these effects, given their  
13 parenchymal location and instigatory role in neuroinflammation following peripheral infection (49-  
14 50). However, in obesity peripheral inflammation develops more slowly than in models of acute  
15 infection, and the contributions of perivascular macrophages to chronic (neuro)inflammation are  
16 less certain. The difficult questions surrounding the origins of neuroinflammation in obesity are  
17 emblematic of larger gaps in knowledge related to CNS immune privilege and the degree of  
18 interaction between parenchymal cells and circulating factors. Although the subpopulation of  
19 CX3CR1-expressing cells that initiates obesity-induced cognitive dysfunction remains to be  
20 determined, the current report clearly implicates IL1R1 activation in brain-resident immune cells  
21 as a link between visceral adipose NLRP3 induction and cognitive impairment.

22         Within the CNS, IL1R1 is highly expressed among vascular endothelial cells, with lower  
23 but detectable expression in microglia, astrocytes, and neurons (31-34, 51-53). Microglial IL1R1

1 expression is upregulated following chemogenetic depletion and in animal models of chronic  
2 inflammation (33-34), consistent with a central role for IL1 signaling in repopulation and  
3 resolution of neuroinflammation. The downstream effects of IL1R1 activation in the brain are  
4 determined by cell type-specific expression of splice variants for the IL1 receptor accessory  
5 protein (AcP). The AcP variant expressed by glia is identical to the co-receptor expressed in  
6 peripheral tissues, while the AcPb variant is exclusively expressed by neurons (54-55). Both  
7 receptors have homologous extracellular regions, but the AcPb variant is unable to recruit the  
8 adapter protein MyD88, which is required for downstream induction of pro-inflammatory  
9 signaling cascades (23). Consistent with this, exogenous IL1 $\beta$  recruits NF $\kappa$ b-mediated  
10 transcriptional responses in glial cultures, but not in primary neurons (52). Interestingly, aging  
11 is accompanied by neuronal induction of the pro-inflammatory AcPb variant (56), suggesting that  
12 chronic exposure to low-level inflammation might reprogram neuronal IL1RAcP expression in  
13 other disease states. While the potential contributions of neuronal ILRAcP reconfiguration  
14 remain unexplored in obesity, it is tempting to speculate that a switch in AcP splice variant  
15 expression might occur downstream of local amplification of IL1 $\beta$  among CX3CR1-expressing  
16 cells in these experiments.

17 An extensive body of work indicates that exposure to levels of IL1 $\beta$  seen in chronic  
18 inflammatory diseases impairs hippocampal synaptic plasticity and cognition (57-59; reviewed  
19 in ref.41). The relationship between IL1 $\beta$  and hippocampal synaptic function is biphasic, with  
20 enhancement of synaptic plasticity following picomolar stimulation and deficits occurring after  
21 exposure to higher concentrations (54, 58, 60). Consistent with a facilitative role for low-level  
22 IL1R1 activation in synaptic physiology, whole-body IL1R1 knockout mice exhibit deficits in  
23 hippocampus-dependent memory and LTP (61). The dose-dependent effects of IL1 $\beta$  on

1 hippocampal synaptic plasticity have been interpreted as reflecting direct actions on neurons,  
2 but this assumption has yet to be adequately tested with modern cell type-specific approaches.  
3 In this report, we observed that ablation of IL1R1 among CX3CR1-expressing cells protects  
4 against IL1 $\beta$ -mediated deficits in LTP. The effects observed in normal-weight IL1R1Tg mice  
5 were similar to the protective effects of preincubation with minocycline, a broad-spectrum  
6 tetracycline antibiotic used experimentally to inhibit microglial activation (62-63). Taken together,  
7 these results suggest that the biphasic relationship between IL1 $\beta$  and hippocampal synaptic  
8 plasticity may reflect activation of IL1R1 on different cell types, with opposing consequences for  
9 memory and cognition.

10         Microglia regulate neuronal function indirectly by clearing dead cells and extracellular  
11 debris, and directly by releasing signaling molecules that support or suppress neuroplasticity  
12 (36). Interactions between microglia and neurons may also involve physical interposition of  
13 microglial processes between pre- and post-synaptic structures, alone or in concert with  
14 microglial internalization of synaptic terminals (25, 42). It is unlikely that microglial localization at  
15 mushroom spines in this report reflects synaptic stripping, given that a recent large-scale  
16 analysis of hippocampal dendritic spines using correlated light and electron microscopy revealed  
17 no evidence of postsynaptic internalization (43). VAT transplantation was associated with IL1R1-  
18 mediated loss of thin spines in these studies, consistent with our previously published  
19 ultrastructural studies in genetically obese db/db mice (64). However, any potential relationship  
20 between loss of thin spines and IL1R1-mediated microglial activation remains speculative at  
21 present. Understanding and manipulating glial interactions with synapses and circuits is a  
22 formidable challenge with the potential to uncover novel strategies for prevention and treatment  
23 of neurological disease.

1           In humans, there is consensus that insulin resistance is accompanied by increased risk  
2 of cognitive decline (65). By contrast, data from studies of BMI and cognition are often conflicting,  
3 with reports of increased risk (1-5), decreased risk (9), or negative findings (10-11, 66). The  
4 results of the current study could potentially underlie the reported associations between visceral  
5 adiposity and cognitive decline in human studies (4, 13-14). Given that a growing literature from  
6 longitudinal and twin studies supports a potential link between visceral adiposity and age-related  
7 cognitive decline, intervention studies may be warranted to prevent or attenuate risk. While there  
8 is a clear positive effect of lifestyle interventions, including the Mediterranean diet and exercise  
9 (67-68), long-term compliance can be difficult to achieve for most individuals. Surgical  
10 interventions such as Roux-en-Y gastric bypass, vertical banded gastroplasty, and laparoscopic  
11 adjustable gastric banding have begun to receive greater attention with respect to postoperative  
12 changes in memory and cognition. In a recent meta-analysis, significant improvements in  
13 attention, mood, and executive function were detected after bariatric surgery in morbidly obese  
14 patients (69). Cognitive improvements were independent of reductions in BMI, while reductions  
15 in circulating markers of inflammation were correlated with cognitive change in some, but not all  
16 studies (70-71). Bariatric surgery is sometimes performed together with surgical removal of fat  
17 from the visceral omentum (omentectomy). Although most studies did not report any metabolic  
18 improvements following omentectomy alone or with concurrent gastric bypass surgery (72-73;  
19 reviewed in ref.74), omentectomy has not been studied with respect to the regulation of cognition  
20 in humans. For mild to moderate obesity, surgical interventions will generate more risk than  
21 reward, even in the context of long-term vulnerability to age-related cognitive decline. However,  
22 studying brain circuits that respond to weight loss surgery could uncover novel targets for

1 noninvasive modulation using transcranial magnetic stimulation and other emerging  
2 technologies.

3

#### 4 **Materials and Methods**

##### 5 *Animals and diets*

6 To induce obesity, male mice were maintained on high-fat diet (HFD; Research Diets  
7 D12492) or low-fat diet (LFD; D12450) beginning at 8wk old. Wt and NLRP3KO mice (Jax strain  
8 #021302) were obtained from Jackson Labs or bred in-house for these experiments. Mice with  
9 inducible ablation of interleukin 1 receptor 1 in CX3CR1-expressing cells (CX3CR1<sup>creER</sup>/IL1R1<sup>fl/fl</sup>)  
10 were generated by crossing IL1R1flox mice (ref.34; kindly provided by Dr. Ari Waisman,  
11 University of Mainz, Mainz, Germany) with CX3CR1<sup>creER</sup> mice purchased from Jackson Labs  
12 (strain #021160). For some experiments, CX3CR1<sup>creER</sup>/IL1R1<sup>fl/fl</sup> transgenic mice were crossed  
13 with the Thy1-eGFP(S) line (Jackson labs strain #011070; breeding pairs donated by Dr. Lin  
14 Mei, Augusta University, Augusta, GA USA) to generate Tg mice with endogenous fluorescence  
15 in the dendritic arbor of dentate granule neurons. Transgenic mice and nTg littermates were  
16 administered tamoxifen (2mg in 0.2mL corn oil, PO) 3x every 48hr. For additional description of  
17 housing conditions, genotyping, and animal care, please see Supplemental Experimental  
18 Procedures.

19

##### 20 *Fat transplantation and determination of transplant viability*

21 For WAT transplantation, the epididymal or inguinal fat pads were removed and trimmed  
22 to 300mg before transplantation into the peritoneal cavity of recipients, as reported previously

1 (75). Transplants were collected at euthanasia for histological verification of transplant viability  
2 as described in Supplemental Experimental Procedures.

3

#### 4 *Behavioral testing*

5 Testing in the Y-maze and novel object preference tasks was carried out as described  
6 (26, 29). Hippocampus-dependent memory was assessed using the water maze, as reported  
7 previously (76-77). Data acquisition was carried out blind to experimental condition, with the  
8 exception of the visually evident phenotype in HFD mice. Data analysis was carried out blind in  
9 Anymaze software.

10

#### 11 *BBB permeability and IL1 $\beta$ transport*

12 Mice were injected with 6xHistidine-tagged recombinant IL1 $\beta$  (1.0 micrograms, IV; USBio)  
13 and the fluorescent tracer sodium fluorescein (NaFI; 10mg/kg, IP) before being euthanized by  
14 transcardial perfusion with saline, as described (28). The dose and route of administration for  
15 His-IL1 $\beta$  was selected based on the absence of acute IL1-mediated effects on BBB permeability  
16 (78). Detection and quantification of NaFI followed published methodology (28).

17

#### 18 *Enzyme-linked immunosorbent assay and western blotting*

19 Protein extraction and quantification of IL1 $\beta$  and TNF $\alpha$  by ELISA followed published  
20 protocols (79; see Supplemental Experimental Procedures). Methods for SDS-PAGE and  
21 western blotting were modified from published protocols (28, 75) and are described in  
22 Supplemental Experimental Procedures.

23

1 *Immunofluorescence, confocal microscopy, and morphological analysis*

2 For details regarding immunofluorescence reactions, see Supplemental Experimental  
3 Procedures. Images were acquired on a Zeiss 780 multiphoton microscope and analyzed by a  
4 blinded experimenter. For cell sampling and analysis, see Supplemental Experimental  
5 Procedures.

6

7 *Cell isolation and ex vivo stimulation*

8 Cells were isolated according to published protocols (28) before labeling with conjugated  
9 antibodies for flow cytometry or frozen for gene expression endpoints (see Supplemental  
10 Experimental Procedures). Methods for ex vivo stimulation experiments were modified from  
11 published studies (75, 79) and are described in Supplemental Experimental Procedures.

12

13 *Flow cytometry*

14 For cell-surface markers, immunophenotyping was carried out as described (29; see  
15 Supplemental Experimental Procedures). For intracellular flow cytometry, live cells were  
16 incubated with conjugated antibodies against CD45, Ly6C, and CD11b before fixation and  
17 permeabilization using commercial reagents (Affymetrix eBioscience). Cells were then washed  
18 and incubated with primary antibodies for intracellular detection of IL1 $\beta$  (Santa Cruz  
19 Biotechnology). Viable cells were visibly differentiated from debris by size gating and positivity  
20 for specific antibodies using a BD LSRII flow cytometer (BD Biosciences). Single stains were  
21 used to set compensation and isotype controls were used to determine the level of nonspecific  
22 binding. Analysis was carried out in FlowJo version 11.0 (BD Bioscience) or InCyte software  
23 (Millipore, Temecula, CA).

1

## 2 *RNA isolation and qPCR*

3 Total RNA extraction and cDNA synthesis followed published methodology (28, 64; see  
4 Supplemental Experimental Procedures).

5

## 6 *Hippocampal slice preparation and electrophysiology*

7 Hippocampal slice preparation and extracellular recording were carried out as described  
8 (28-29, 75; see Supplemental Experimental Procedures).

9

## 10 *Statistics*

11 For comparisons between LFD/SHAM, HFD/SHAM, TRANS<sub>WT</sub>, and TRANS<sub>KO</sub> mice, data  
12 were analyzed with one-way ANOVA or one-way repeated measures ANOVA followed by  
13 Tukey's HSD post hoc. For repeated-measures endpoints, data were analyzed using repeated-  
14 measures ANOVA and Tukey's HSD post hoc. For experiments comparing the effects of HFD  
15 or VAT transplantation in CX3CR1<sup>creERT2</sup>/IL1R1<sup>fl/fl</sup> transgenic mice (Tg) and nontransgenic  
16 littermates, single-endpoint datasets were analyzed using 2x2 ANOVA (diet x genotype or  
17 surgery x genotype) with Tukey's HSD post hoc. For repeated-measures endpoints, data were  
18 analyzed using 2x2 repeated-measures ANOVA with Tukey's HSD post hoc. Where appropriate,  
19 the Greenhouse-Geisser correction was applied for heterogeneity of variance. For all analyses,  
20 statistical significance was carried out in Graphpad version 8.0 (Prism, Carlsbad, CA) with  
21 significance at p<0.05.

22

## 23 *Study Approval*

1 All experiments followed NIH guidelines and were approved by the Animal Care and Use  
2 Committee at the Medical College of Georgia.

3  
4 **Author contributions:** AMS and DHG designed research studies; all authors were directly  
5 involved in conducting experiments, acquiring data, analyzing data, and writing the manuscript.

6  
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11 paraffin-embedded samples were carried out with assistance from the Genomics Core facility at  
12 Augusta University.

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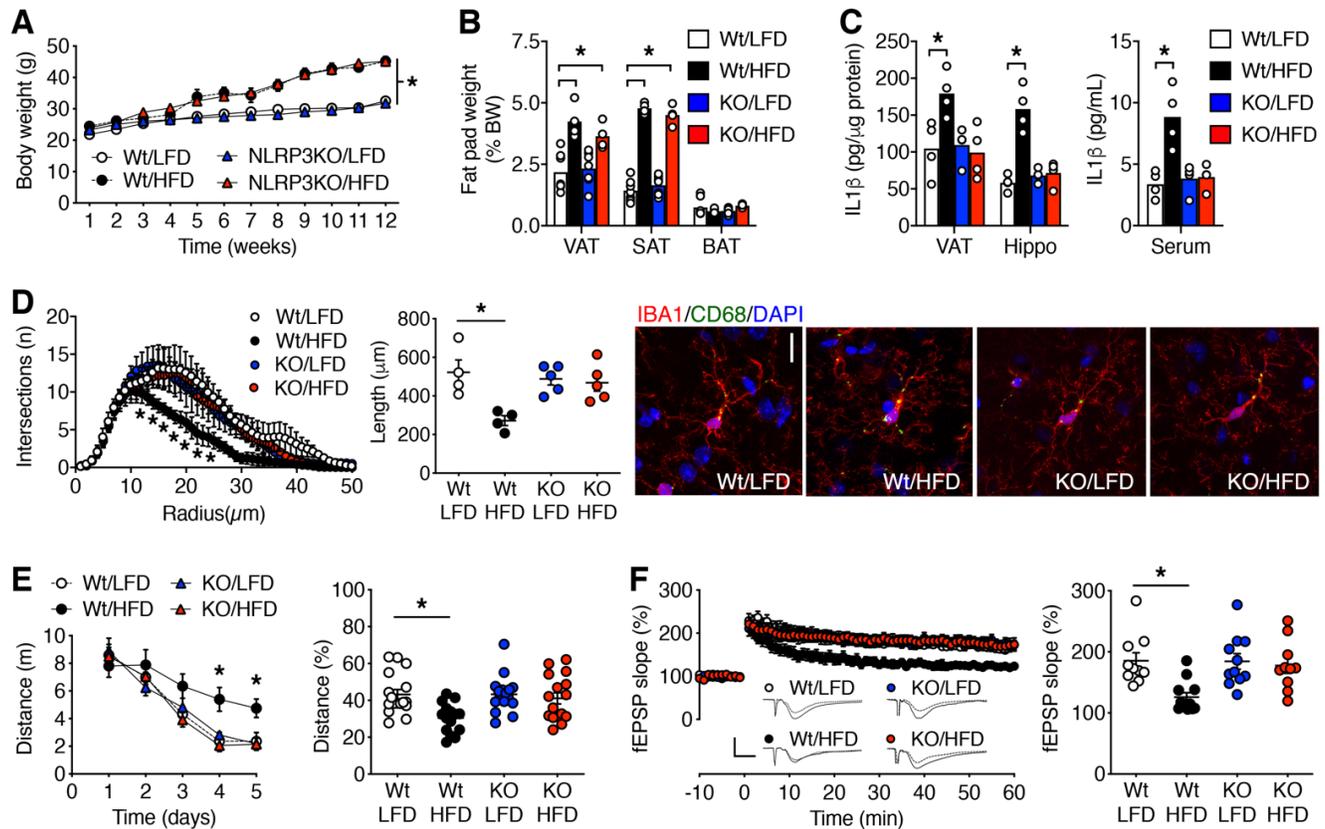
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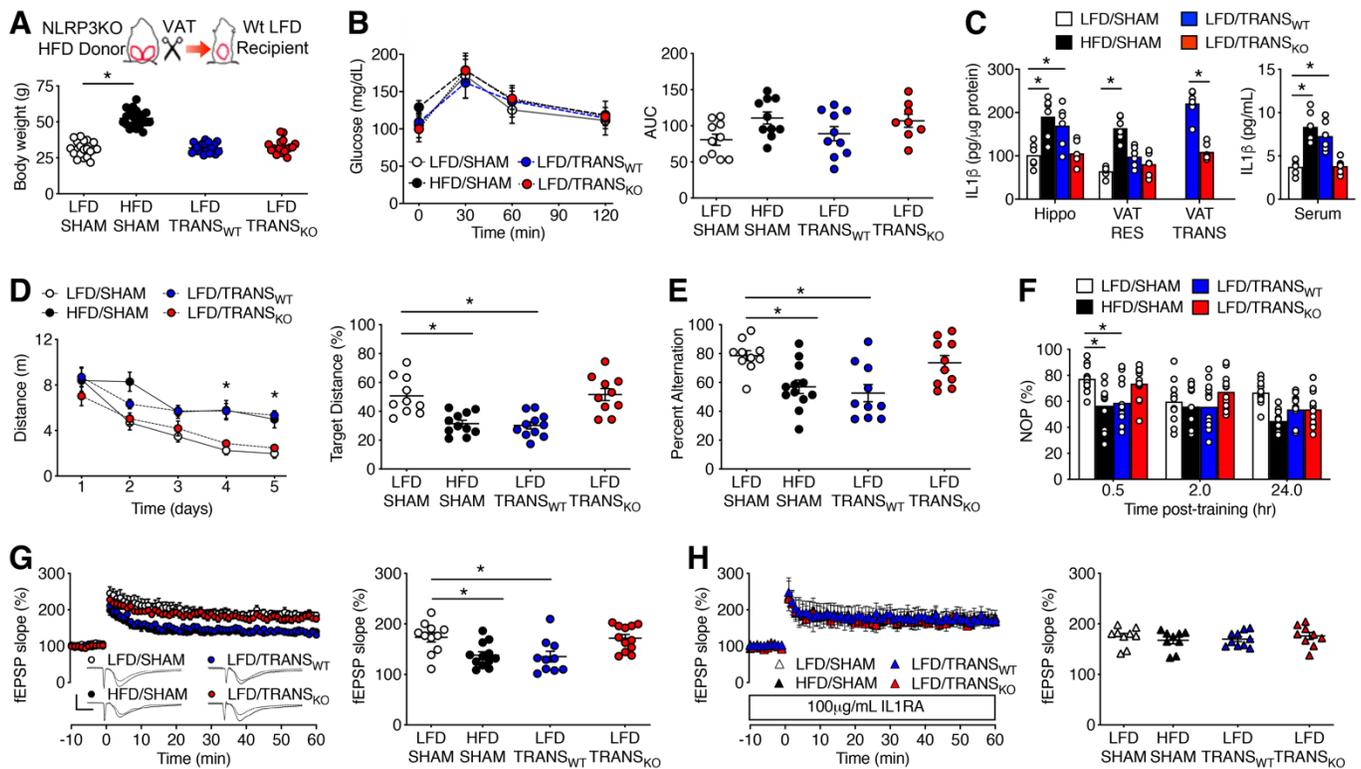
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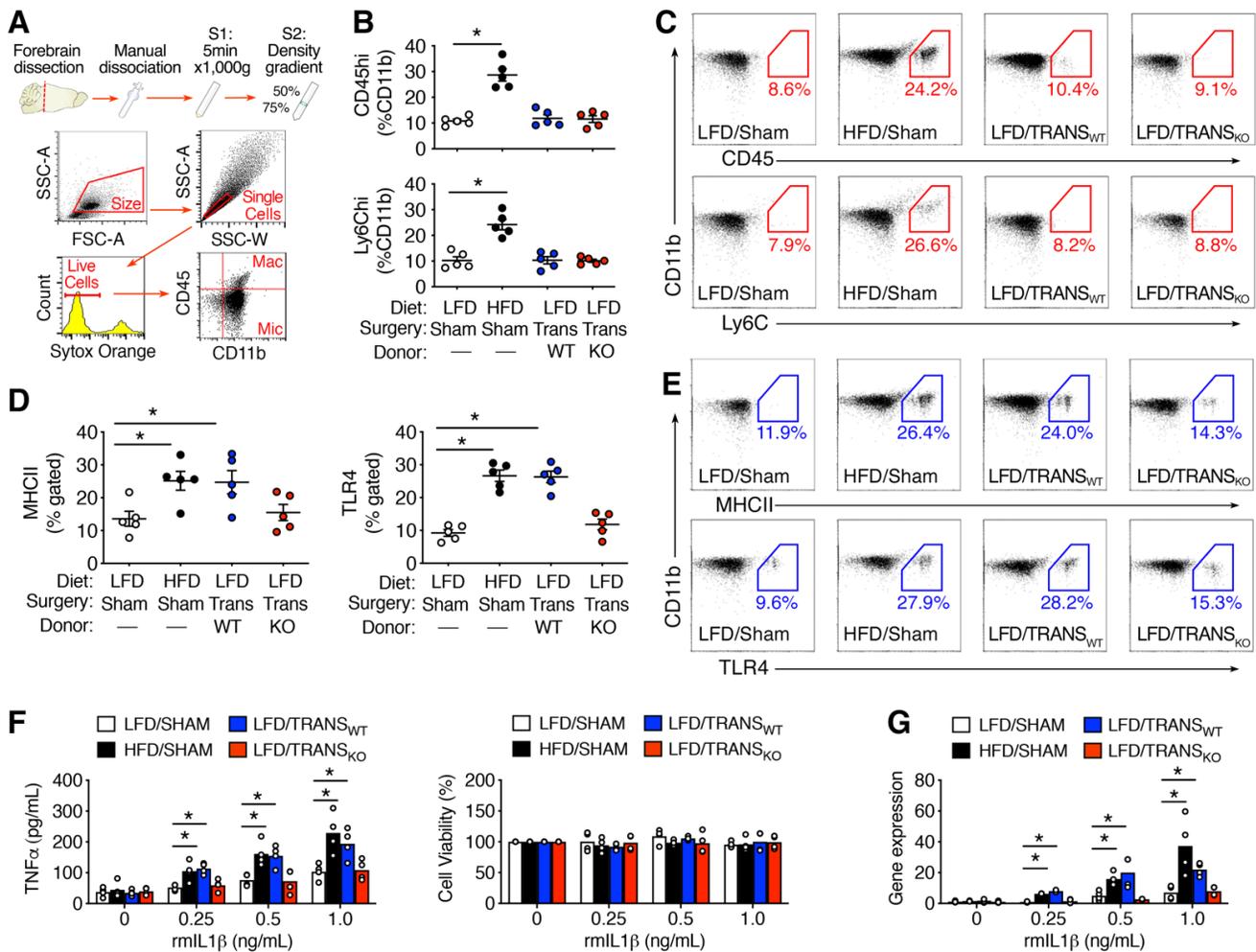
## Figures and Figure Legends



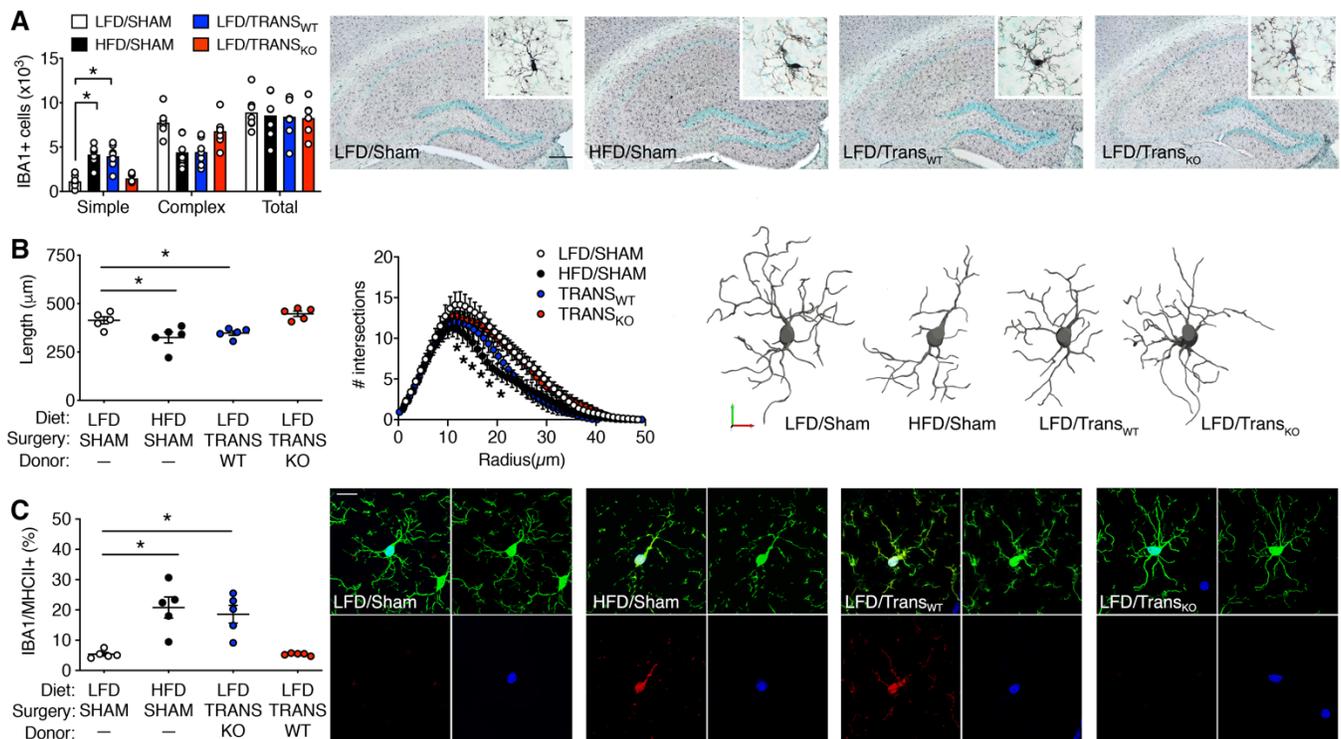
**Figure 1. Protection against obesity-induced hippocampal dysfunction in mice lacking inflammasome protein NLRP3.** (A) Male Wt and NLRP3 knockout (KO) mice gain weight similarly over time (n=20). (B) Adipose tissue hypertrophy is comparable in Wt/HFD and KO/HFD mice (n=6). (C) KO/HFD mice are protected against increases in IL1 $\beta$  in VAT, hippocampus, and serum (n=4). (D) NLRP3-dependent reductions in microglial process complexity (left) and total length (right) with dietary obesity (n=4). Micrographs (right; scale bar = 10 microns, applies to all panels) show increased CD68 immunoreactivity in Wt, but not KO/HFD mice (see Supplemental Figure 1). (E) NLRP3 is required for obesity-induced memory impairment in the water maze during acquisition training (left) and probe test (right; n=16). (F) Dentate gyrus long-term potentiation (LTP) was significantly reduced in an NLRP3-dependent manner with dietary obesity (n=10-12 slices, n=4-6 mice). For traces (inset, left), scalebar x=1msec, y=1mV. For all graphs, bar or line height represents the mean and error bars represent SEM. Asterisk (\*) denotes statistical significance at p<0.05 by 2-way repeated-measures ANOVA (A) or 2-way ANOVA (B-F) with Tukey's HSD post hoc.



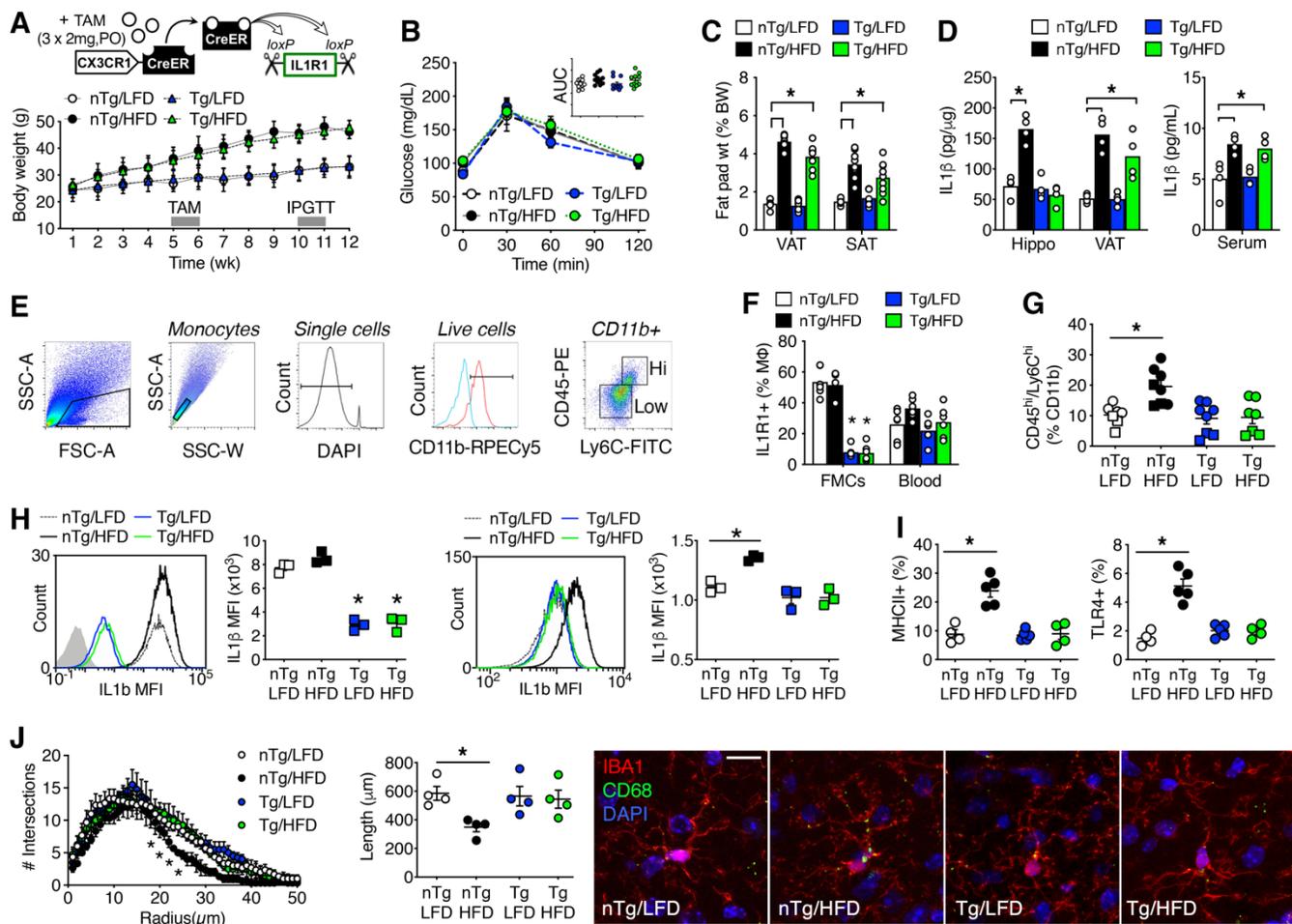
**Figure 2. Visceral adipose NLRP3 induction impairs hippocampus-dependent memory and synaptic plasticity.** (A) Top panel shows experiment schematic. Bottom graph shows body weights in Wt mice maintained on HFD or LFD for 10wk before sham operation (SHAM) or VAT transplantation from a Wt/HFD (TRANS<sub>WT</sub>) or NLRP3KO/HFD donor (TRANS<sub>KO</sub>) (n=20). (B) No effect of VAT transplantation on glycemic control, as determined by IPGTT (left) and analysis of the area under the curve (AUC, right; n=8-10). (C) Increases in hippocampal and serum IL1 $\beta$  in HFD/SHAM and TRANS<sub>WT</sub> mice, but not TRANS<sub>KO</sub> mice (n=6). (D) Visceral adipose NLRP3 impairs spatial memory acquisition (left) and probe trial performance (right; n=10-11). (E) NLRP3-mediated deficits in the Y-maze following VAT transplantation (n=10-12). (F) NLRP3-dependent reductions in novel object preference (NOP) (n=12). (G) TRANS<sub>WT</sub> mice recapitulate obesity-induced LTP deficits in an NLRP3-dependent manner (left), based on comparison of fEPSP slopes 60min after high-frequency stimulation (n=9-10 slices, n=4-5 mice). For traces (left inset), scalebar x=1msec, y=1mV. (H) Recombinant interleukin-1 receptor antagonist (IL1RA) eliminated LTP deficits in TRANS<sub>WT</sub> mice (n=9-10 slices, n=4-5 mice). For all graphs, bar or line height represents the mean and error bars represent SEM. Asterisk (\*) denotes statistical significance at p<0.05 by one-way ANOVA with Tukey's HSD post hoc.



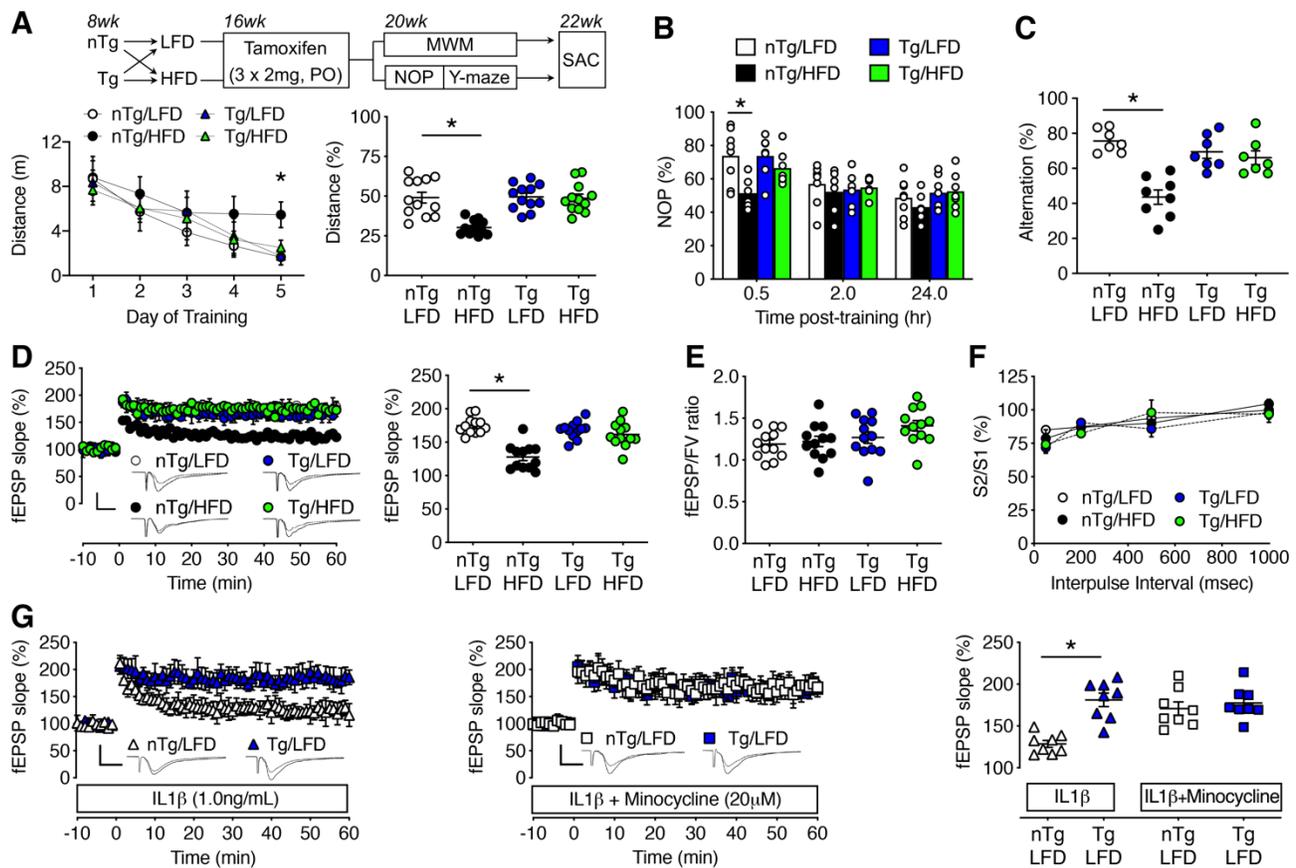
**Figure 3. NLRP3-mediated effects of visceral fat transplantation on forebrain microglia.** (A) Top panel shows schematic of cell isolation; bottom panel shows gating. (B) Visceral fat transplantation does not recapitulate obesity-induced macrophage infiltration (n=5). (C) Representative scatterplots of CD11b<sup>+</sup>/CD45<sup>hi</sup> cells (top) and CD11b<sup>+</sup>/Ly6Chi cells (bottom). Boxed area (red) shows gated events. (D) VAT transplantation recapitulates the effect of obesity on microglial induction of MHCII (left) and TLR4 (right) via NLRP3 (n=5). (E) Representative scatterplots of MHCII (top) and TLR4 (bottom) in CD11b<sup>+</sup> cells. Boxed area (blue) shows gated events. For all scatterplots (C, E) XY axis (log scale) min=10<sup>0</sup>, max=10<sup>5</sup>. (F) Visceral adipose NLRP3 increases sensitivity to exogenous IL1 $\beta$  in forebrain mononuclear cells (left) without influencing cell viability (right; n=4). (G) NLRP3-mediated priming of IL1 $\beta$ -stimulated gene expression with dietary obesity or VAT transplantation (n=4). For all graphs, bar or line height represents the mean and error bars represent SEM. Asterisk (\*) denotes statistical significance at p<0.05 by one-way ANOVA with Tukey's HSD post hoc.



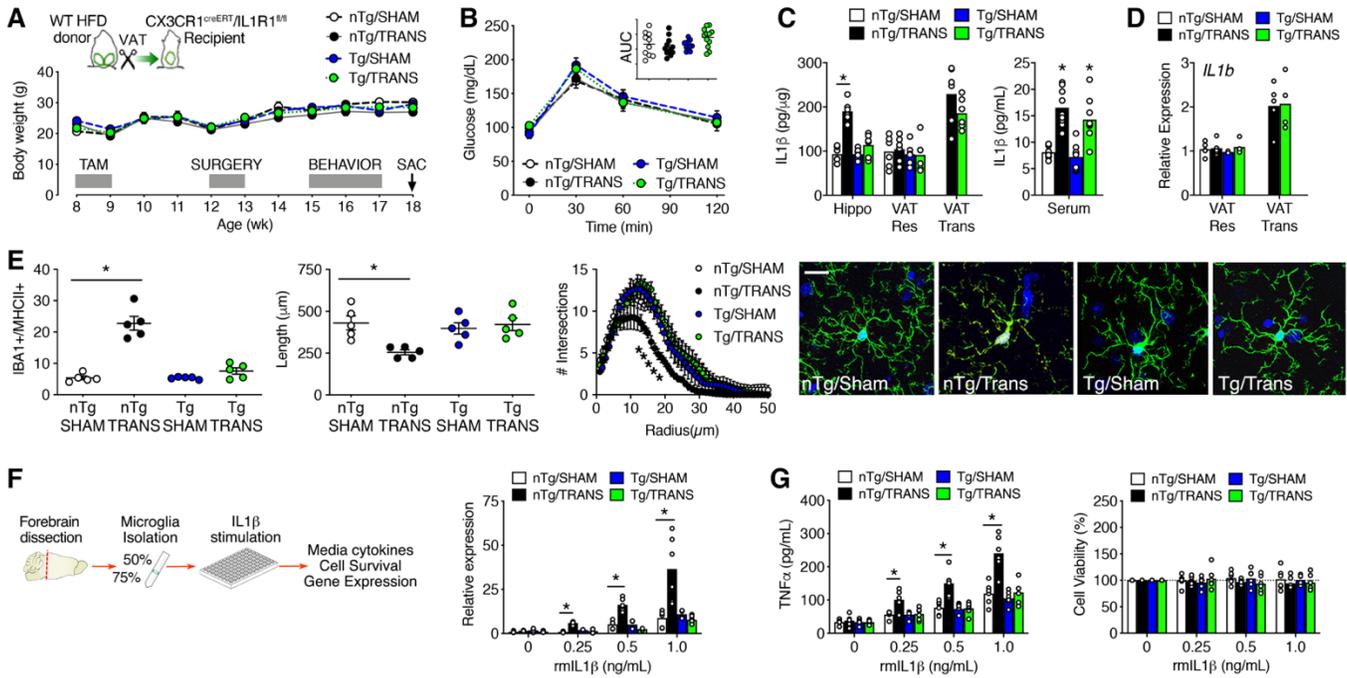
**Figure 4. Visceral adipose NLRP3 induction activates hippocampal microglia in the intact brain.** (A) Stereological quantification of IBA1+ microglia with  $\leq 2$  primary processes ('Simple'),  $> 2$  primary processes ('Complex'), and total microglial number ( $n=7$ ). Micrographs (right; scale bar = 5mm; for inset, scale bar = 10 microns) show peroxidase detection of IBA1. (B) Quantification of total process length (left) and complexity (right) revealed NLRP3-dependent simplification of IBA1+ microglia after VAT transplantation ( $n=5$ ). For tracings (right), scalebar for LFD/SHAM applies to all tracings ( $xyz=5$  micron). (C) VAT transplantation recapitulated the effects of obesity on microglial MHCII induction via NLRP3 ( $n=5$ ). Micrographs (right; scale bar = 10 microns) show double-labeling for IBA1 and MHCII in the hippocampal dentate gyrus. For micrographs, scale bar shown for LFD/SHAM applies to all panels. For all graphs, bar or line height represents the mean and error bars represent SEM. Asterisk (\*) denotes statistical significance at  $p < 0.05$  by one-way ANOVA with Tukey's post hoc.



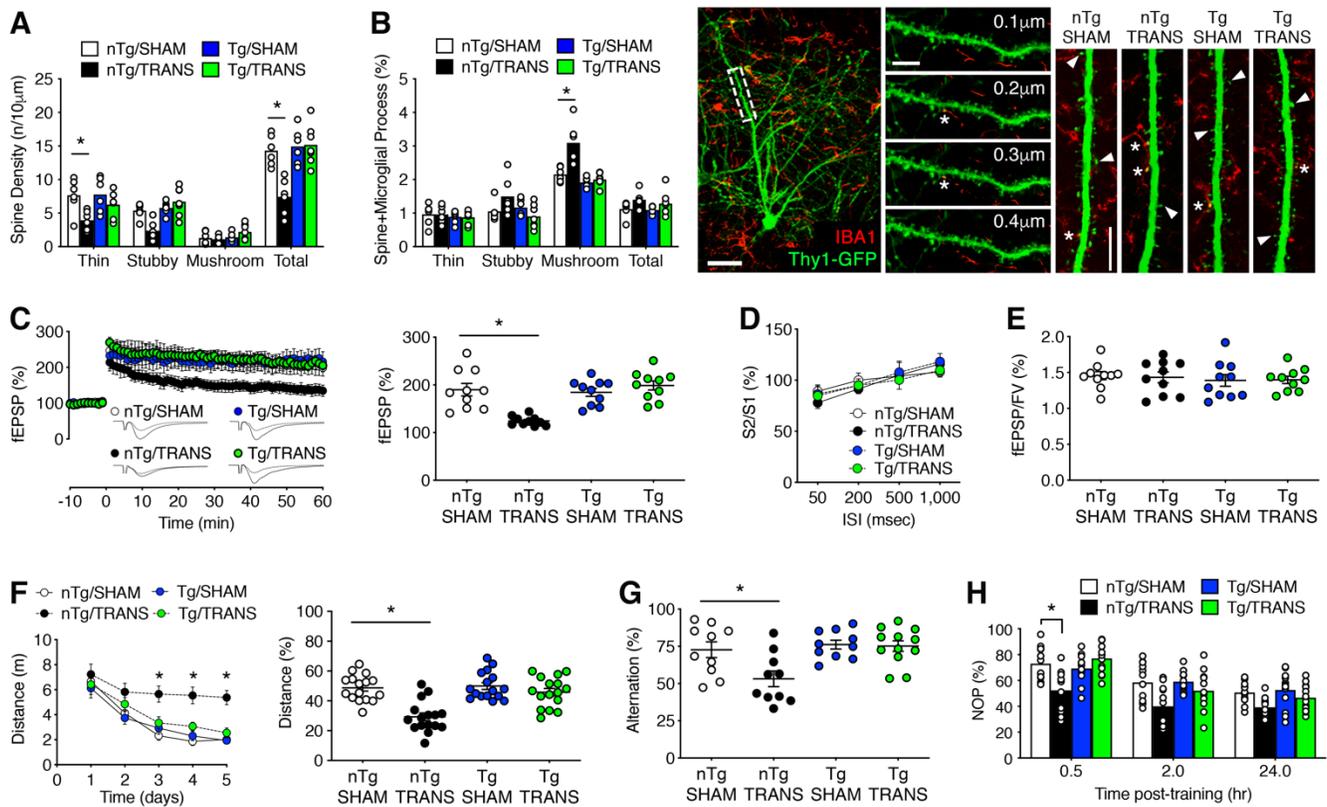
**Figure 5. Ablation of IL1R1 in CX3CR1-expressing cells confers resistance to obesity-induced neuroinflammation.** (A) Top panel shows design for CX3CR1<sup>cre/ERT2</sup>/IL1R1<sup>fl/fl</sup> transgenic mice. Weight gain (bottom) is similar in Tg and nTg littermates (n=24). (B) No effect of genotype on glycemic control (n=11-12). (C) Adipose tissue hypertrophy is unaffected by genotype (n=6-8). (D) Tg/HFD mice did not exhibit increases in hippocampal IL1 $\beta$ , despite comparable increases in VAT and serum IL1 $\beta$  (n=4). (E) Gating strategy for analysis of macrophage infiltration and microglial activation (text above plots indicates parent gate). (F) Flow cytometric validation of CNS-specific reductions in cell-surface IL1R1 expression (n=6). (G) Tg mice are resistant to CNS infiltration of CD11b<sup>+</sup>/CD45<sup>hi</sup>/Ly6C<sup>hi</sup> macrophages with dietary obesity (n=7-8; circles show data from cell-surface detection in live cells (n=4-5) and squares represent data from fixed cells (n=3)). (H) Nonoverlapping effects of obesity and IL1R1 deletion on intracellular IL1 $\beta$  in the CD11b<sup>+</sup>/CD45<sup>hi</sup>/Ly6C<sup>low</sup> population, which includes BAMs (right; gray region shows isotype) and in CD11b<sup>+</sup>/CD45<sup>low</sup>/Ly6C<sup>low</sup> microglia (left; n=3). (I) IL1R1 is required for obesity-induced microglial polarization, based on cell-surface detection of MHCII (left) and TLR4 (right) in CD11b<sup>+</sup>/CD45<sup>low</sup>/Ly6C<sup>low</sup> cells (n=4-5). (J) IL1R1-dependent anatomical simplification (left) and process retraction (right) with obesity (n=4). Micrographs show IL1R1-mediated accumulation of CD68 among IBA1<sup>+</sup> microglia (scale bar=10 microns, applies to all). For all graphs, bar or line height represents the mean and error bars show SEM. Asterisk (\*) denotes statistical significance at p<0.05 by 2-way ANOVA with Tukey's post hoc.



**Figure 6. Protection against obesity-induced hippocampal dysfunction in *CX3CR1<sup>creERT</sup>/IL1R1<sup>fl/fl</sup>* mice.** (A) Top panel shows experiment schematic (*italics* indicate age of mice). Bottom graphs show distance during water maze acquisition (left) and probe test (right;  $n=12$ ). (B) IL1R1 activation in CX3CR1-expressing cells impairs object recognition in obesity ( $n=6-8$ ). (C) Ablation of IL1R1 in CX3CR1-expressing cells eliminates deficits in the Y-maze with dietary obesity ( $n=7-8$ ). (D) Activation of IL1R1 in CX3CR1-expressing cells underlies obesity-induced LTP deficits ( $n=12$  slices,  $n=5-6$  mice; applies to panels D-F). For traces (inset, left), scalebar  $x=1$  msec,  $y=1$  mV. (E) No effect of diet or genotype on input/output ratios. (F) No effect of diet or genotype on paired-pulse depression. (G) Inducible ablation of IL1R1 among CX3CR1-expressing cells protects against IL1 $\beta$ -induced LTP deficits (left). Preincubation with minocycline blocks reductions in LTP with exposure to IL1 $\beta$  in slices from nTg mice (middle graph). Right graph shows normalized fEPSP slopes 60min after high-frequency stimulation ( $n=8$  slices from  $n=4$  mice per condition). For all graphs, bar or line height represents the mean and error bars show SEM. Asterisk (\*) denotes significance at  $p<0.05$  by 2-way ANOVA with Tukey's HSD post hoc.



**Figure 7. Inducible ablation of IL1R1 in CX3CR1-expressing cells prevents microglial activation and sensitization following surgical increases in visceral fat.** (A) Top panel shows experiment schematic. Graph (bottom) shows weight gain after VAT transplantation (TRANS) or sham operation (SHAM) in CX3CR1<sup>cre/ERT2</sup>/IL1R1<sup>fl/fl</sup> transgenic mice (Tg) and nTg littermates (n=32). (B) No effect of genotype or surgery on glycemic control (n=12). (C) Visceral fat transplantation increases hippocampal IL1β in nTg mice, but not in Tg mice, despite comparable elevations in serum IL1β (n=6). (D) qPCR analysis of IL1b demonstrating localized increases in the transplanted VAT (n=4-5). (E) IL1R1-dependent MHCII induction in IBA1+ cells after VAT transplantation (left). IL1R1-mediated reductions in total process length (middle) and complexity (right) in VAT transplant recipients (n=5). Micrographs show MHCII expression in IBA1+ cells (scale bar=10 microns, applies to all). (F) Schematic (left) shows experimental design for analysis of priming and sensitization (n=6). Graph (right) shows IL1R1-mediated autocrine amplification of IL1b gene expression in FMCs after VAT transplantation. (G) FMCs from VAT transplant recipients exhibit IL1R1-dependent sensitization, based on lower thresholds for increases in media TNFα (left). Sensitization and priming were not attributable to differences in cell survival (right; n=6). For all graphs, bar or line height represents the mean, error bars show SEM, and n-sizes represent number of mice. Asterisk (\*) denotes statistical significance at p<0.05 by 2-way ANOVA with Tukey's HSD post hoc.



**Figure 8. Visceral fat transplantation disrupts microglial organization at dendritic spines and impairs cognition by activating IL1R1 on microglia and brain macrophages.** (A) CX3CR1<sup>cre/ERT</sup>/IL1R1<sup>fl/fl</sup> transgenic mice were crossed with Thy-eGFP(S) mice for quantification of dendritic spine density and morphology. Loss of thin spines was associated with reductions in total dendritic spine density in nTg/TRANS mice (n=6-7, applies to A-B). (B) Analysis of IBA1+ microglial processes at different spine morphologies revealed preferential localization at mushroom spines. VAT transplantation increased the proportion of mushroom spines with microglial processes, and increases were IL1R1-dependent. For micrographs, left panel shows z-projection of Thy1-GFP and IBA1 (scalebar = 20 microns); middle panels show individual z-planes with asterisk (\*) indicating contact between IBA1+ processes and dendritic spines (scalebar = 10 microns). Far right panels show representative z-projection images from each condition with arrowheads indicating spines and asterisks indicating IBA1 contact at spine head (scalebar = 10 microns). (C) Protection against VAT transplantation-induced LTP deficits in Tg/TRANS (left), based on comparison of fEPSP slopes 60min after high-frequency stimulation (right; n=10 slices, n=4-5 mice, applies to D-E). (D) No change in presynaptic paired-pulse plasticity. (E) No effect of genotype or surgery on input/output ratios. (F) VAT transplantation impairs water maze acquisition (left) and probe trial performance (right) in nTg/TRANS, but not Tg/TRANS mice (n=16). (G-H) Ablation of IL1R1 in CX3CR1-expressing cells eliminates VAT transplantation-induced deficits in the Y-maze (G) and maintains object recognition memory (H; n=10-12, applies to G-H). For all graphs, bar or line height represents the mean and error bars show SEM. Asterisk (\*) denotes statistical significance at p<0.05 by 2-way ANOVA with Tukey's HSD post hoc.