

Supplementary Figure 1. Microglial morphology 6 hours after vehicle, LPS, or LPS+EP4 agonist stimulation. 400X representative confocal images are shown of hippocampal CA1 microglia stained with microglial markers Iba1 and CD68 6 hours after administration of vehicle, LPS, or LPS+AE1-329. Iba 1 labels the cytoskeleton, and detects cell soma and processes; CD68 labeling is punctate, as it is localized to endosomes and lysosomes, and is detected prominently in microglial processes. LPS stimulation results in increased Iba1 staining with some reduction in the number of Iba-1 positive ramified processes. Co-administration of EP4 agonist modestly reduces Iba1 staining as compared to LPS alone. Scale bar=25µm.

Supplementary Figure 2. Microglial morphology 24 hours after LPS in Cd11bCre:EP4+/+ and Cd11bCre:EP4f/f hippocampus does not show significant difference. 630X representative confocal images of hippocampal CA1 microglia are shown 24 hours after treatment with vehicle and LPS in Cd11bCre and Cd11bCre:EP4 f/f mice. Morphological assessment of microglia shows a modest increase in Iba1 immunoreactivity and thickening of processes in LPS versus vehicle treated Cd11bCre mice. Although at this time point of 24 hours there is significant upregulation of pro-inflammatory gene expression and increased lipid peroxidation, there is no clear difference in overall morphology between Cd11bCre and Cd11bCre:EP4 f/f mice. Scale bar= 25µm.

Supplementary Figure 3. Density centrifugation +/- Cd11b immunomagnetic purification yields >90% Cd11b-expressing microglial cells. (A) Flow cytometry of cells from density centrifugation yields 90.39% Cd11b-positive microglia, consistent with published data (36). (B) Immunomagnetic purification yields 97.62% Cd11b-positive microglia.

Supplemental Table 1 Primer Table

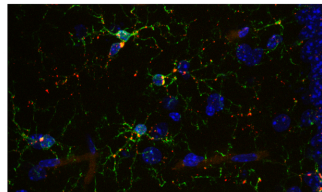
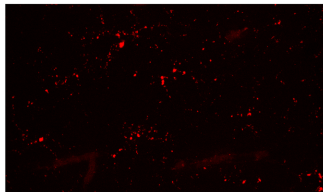
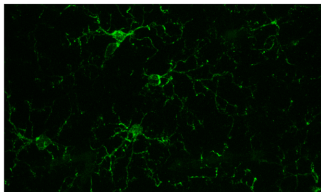
	Sense	Anti-sense	Accession Number in NCBI Entrez Nucleotide Database
18S	5'-CGGCTACCACATCCAAGGAA -3'	5'- GCTGGAATTACCGCGGCT -3'	AY248756
EP4	5'- AGACACCACCTCGCTGAGAAGCTTT -3'	5'- AACCTCATCCACCAACAGGACACT -3'	NM_008965
p67phox	5'- GCCGGAGACGCCAGAAGAGCTA -3'	5'- GGGGCTGCGACTGAGGGTGAA -3'	NM_010877
gp91phox	5'- CCAACTGGGATAACGAGTTCA -3'	5'- GAGAGTTTCAGCCAAGGCTTC-3'	NM_007807
P47phox	5'- TACAGCAAAGGACAGGACTGGGTT-3'	5'- GAGGCACTTGGCTTTCTGCAAAC-3'	NM_010876
iNOS	5'- TGACGGCAAACATGACTTCAG -3'	5'- GCCATCGGGCATCTGGTA-3'	MMU43428
COX-2	5'- TGCAAGATCCACAGCCTACC-3'	5'- GCTCAGTTGAACGCCTTTTG-3'	NM_011198
IL-10	5'- GGGTTGCCAAGCCTTATCGGAAAT -3'	5'- TCTTCAGCTTCTCACCCAGGGAAT -3'	NM_010548
TNF α	5'- GATCTCAAAGACAACCAACATGTG -3'	5'- CTCCAGCTGGAAGACTCCTCCCAG -3'	NM_013693
IL-1 β	5'- CCAGGATGAGGACATGAGCACC -3'	5'- TTCTCTGCAGACTCAAACCTCCAC -3'	NM_008361
IL-6	5'- CATAGCTACCTGGAGTACATGA -3'	5'- CATTTCATATTGTCAGTTCTTCG -3'	NM_031168

Iba1

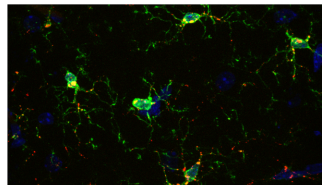
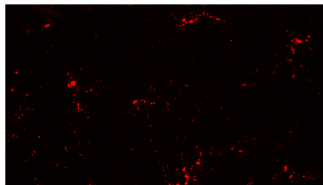
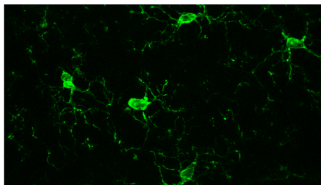
CD68

merge

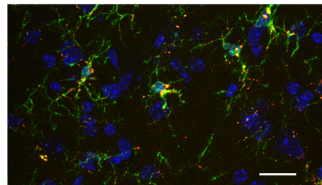
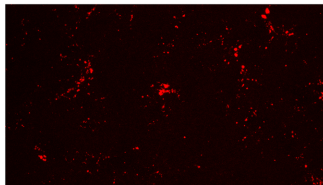
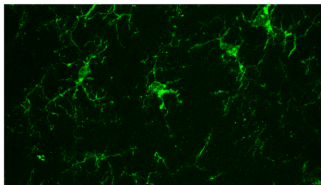
vehicle
6hr



LPS
6 hr



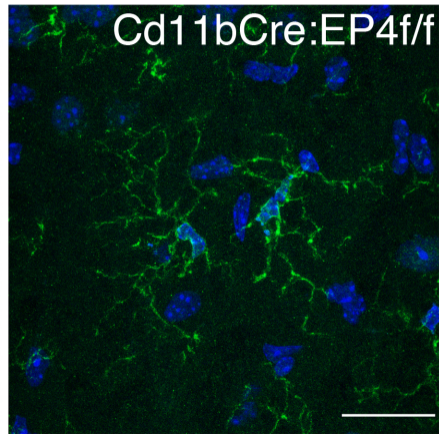
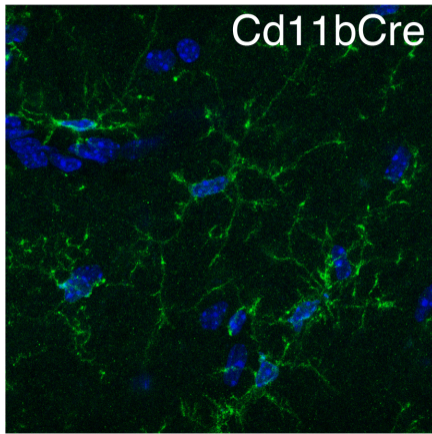
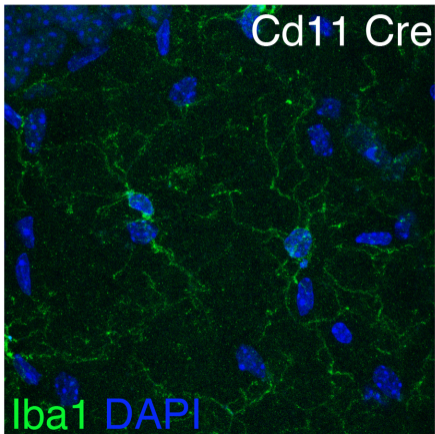
LPS+
AE1
6 hr

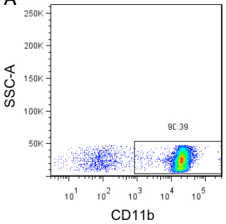


vehicle 24hr

LPS 24hr

LPS 24hr



A**B**