Supplemental information

A genetic tool for the longitudinal study of a subset of post-inflammatory reactive astrocytes

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Figure S1. Validation of Lcn2 as an RA marker and design of the Lcn2CreERT2 mouse, related to Figure 1.
(A) mRNA expression of Lcn2, and the astrocytic marker Slc1a3 (top) or the endothelial cell marker Pecam1 (bottom) in animals given IP injections of LPS or saline detected by RNAscope. Expression of Lcn2 (red) was observed only in LPS treated animals, in both Pecam1 and Slc1a3 positive cells. Scale bars: Left and middle panels: 250 μm. Right: 15 μm. (B) Design of the Lcn2CreERT2 transgene. CreERT2 followed by a P2A signal was targeted downstream of the Lcn2 promoter and start codon, but upstream of the remainder of the first coding exon.
Figure S2. tdTomato labeling in Lcn2CreERT2;Ai9 mice after IP LPS injections, related to Figure 1. (A-C) tdTomato (red) immunolabeling of Lcn2CreERT2;Ai9 thalami and surrounding areas after single IP saline (A left panel) or single high-dose LPS injection (A right panel, B and C). Sections were co-labeled with DAPI and GFAP (A, green), lectin (B, green) or NeuN (D, green). tdTomato partially colocalizes with GFAP and lectin, but not NeuN. (D) Quantification of cells co-positive for tdTomato and GFAP or Lectin. (E) Coronal section through the forebrain of Lcn2CreERT2;Ai9 mice after low-dose LPS injections immunolabeled for tdTomato (red) and counterstained with DAPI (blue). Scale bars: (A, E): 500 μm; (B): 100 μm; (C): 1000 μm.
Figure S3. Lcn2CreERT2 expression in other disease models, related to Figures 2 and 3. (A) Representative images of intrathalamic saline and Kainic Acid injections (n=3). Brain sections from Lcn2CreERT2; Ai9 mice were immunostained for tdTomato and counterstained with DAPI. (B) Representative images of penetrating stab wound in Lcn2CreERT2; Ai9 brains immunostained for TdTomato (red), GFAP (green) and counterstained with DAPI (n=2). Left panel shows a low magnification image around the wound. Right panel displays a tdTomato+ and GFAP+ astrocyte in close proximity to the wound. (C) Lcn2CreERT2; Ai9; 5xFAD thalami at 6 months of age (n=3), immunostained for tdTomato (red), β-amyloid (green), GFAP (white) and counterstained with DAPI. Sparse tdTomato labeling was observed primarily in the thalamus and hypotalamus, but little to no labeling was found in the hippocampus. Right panel is a high magnification image of tdTomato+ cells in the vicinity of β-amyloid plaques. Scale bars: (A): 1000 μm; (B, left): 250 μm; (B, right): 50 μm; (C, left): 250 μm; (C, right): 30 μm.
Figure S4. Applications for the LcnCreERT2 line, related to Figure 4. (A) Isolation and sorting of reactive cells from the LcnCreERT2;A19 mouse. LcnCreERT2;A19 mice were treated with tamoxifen and saline or LPS dissolved in saline as described. Collected brains were prepared for FACS sorting through tissue dissociation, followed by Percoll gradients and filtration to size select for glia. Prepared cells were stained with DAPI in order to identify live vs dead cells. First bulk cells were identified from the sample on the basis of forward versus side scatter. From the bulk cell population, the DAPI negative population was identified to isolate live cells. From the DAPI negative population, cells were then selected that were positive for tdTomato, which were only identified in LPS-treated animals. (B-D) Combinatorial approaches for targeting reactive astrocytes. (B) Strategy overview. (C) Design of the AAV construct employed to deliver GCaMP7I to Lcn2CreERT2 expressing astrocytes. In the original non-recombined conformation, the GCaMP7i cassette is inverted with respect to the promoter (top). Following Cre-mediated recombination (bottom), GCaMP7i is flipped into the forward orientation, allowing for expression driven by the GFAP2.2 promoter. This results in expression only in astrocytes expressing active Cre. (D) Cre-dependent activation of the AAV-GFAP(long)-FLEX backbone. AAV (PHP.eB)-GFAP(long)-FLEX-GCaMP7 was injected stereotactically into the thalamus of Lcn2CreERT2 mice. Three weeks following injection, animals were gavaged with tamoxifen and injected with saline or LPS. Tissue was collected and immunostained for EGFP to reveal the expression of the GCaMP7i indicator, and counterstained with DAPI. (E) Higher magnification image of AAV-GFAP(long)-FLEX-GCaMP7+ astrocyte labeled with antibodies against EGFP and GFAP, counterstained with DAPI. Scale bars: (D): 250 μm; (E): 20 μm.
Figure S5. Expression of reactive astrocyte markers at different timepoints after LPS, related to Figure 5. (A) RNAscope for brain sections 24 hours after saline, 24 hours after LPS, or 1 month after LPS treatment to detect expression of the reactive markers Gfap and Lcn2, followed by immunostaining for tdTomato and counterstaining with DAPI. (B) Total integrated fluorescent density was quantified for Lcn2 and Gfap. *** p<0.001 by ANOVA followed by Tukeys HSD post-hoc. Scale bar: 50 μm.