Supplementary Fig. 2

Saline  LPS

LCN2
Supplementary Fig 3

A

|       | Saline |                | LPS          |
|-------|--------|----------------|--------------|
|       | WT     | Lcn2^{-/-}     | WT           |
|       |        |                | Lcn2^{-/-}   |
| 8 hrs | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) | ![Image](image4.png) |

B

|       | Saline |                | LPS          |
|-------|--------|----------------|--------------|
|       | WT     | Lcn2^{-/-}     | WT           |
|       |        |                | Lcn2^{-/-}   |
| 24 hrs| ![Image](image5.png) | ![Image](image6.png) | ![Image](image7.png) | ![Image](image8.png) |
Supplementary Fig 4

A. Bcl2a1d

B. Hamp

C. Lif

D. Osm

E. Psors1c2

F. Timd4

G. Wfikkn1

H. Axl3

I. Fmo2

J. Ptgdr

K. Scarna2

RPKM

Saline LPS

WT

Lcn2−/−
**Supplementary Table I** Top 20 up-regulated brain transcripts after peripheral LPS challenge in WT mice by RNAseq

| Gene name | Mean RPKM<sup>a</sup> saline (log<sub>2</sub>) | Mean RPKM<sup>a</sup> LPS (log<sub>2</sub>) | FC<sup>b</sup> WT LPS/WT saline | Corrected P value |
|-----------|---------------------------------|---------------------------------|--------------------------------|------------------|
| *Saa3*   | -2.83                           | 6.81                            | 798.69                        | 4.66E-07         |
| *Lcn2*   | -3.21                           | 6.17                            | 666.42                        | 1.54E-08         |
| *Csf3*   | -4.42                           | 3.10                            | 182.93                        | 2.87E-10         |
| *Serpina3f* | -4.39                        | 2.03                            | 86.00                         | 1.63E-09         |
| *Ccl5*   | -2.53                           | 3.73                            | 76.52                         | 4.87E-06         |
| *Cxcl1*  | -2.88                           | 3.37                            | 76.42                         | 2.80E-07         |
| *Cxcl10* | -2.06                           | 3.72                            | 54.84                         | 3.10E-06         |
| *Slc10a6*| -4.75                           | 0.82                            | 47.52                         | 1.66E-07         |
| *Mmp3*   | -4.75                           | 0.79                            | 46.33                         | 1.95E-06         |
| *Ccl11*  | -3.83                           | 1.50                            | 40.14                         | 5.11E-07         |
| *Slfn4*  | -4.38                           | 0.90                            | 38.88                         | 1.08E-08         |
| *Saa2*   | -2.93                           | 2.22                            | 35.45                         | 2.21E-07         |
| *Cxcl2*  | -4.07                           | 1.03                            | 34.38                         | 5.82E-07         |
| *Saa1*   | -2.34                           | 2.76                            | 34.16                         | 5.47E-06         |
| *Steap4* | -2.32                           | 2.69                            | 32.34                         | 8.75E-08         |
| *Ch25h*  | -1.72                           | 3.25                            | 31.45                         | 1.25E-08         |
| *Timp1*  | -0.42                           | 4.51                            | 30.41                         | 2.11E-08         |
| *Plin4*  | -1.05                           | 3.66                            | 26.20                         | 6.14E-09         |
| *Zbp1*   | -3.99                           | 0.67                            | 25.18                         | 1.43E-09         |
| *Il1rn*  | -4.37                           | 0.01                            | 20.88                         | 4.06E-07         |

<sup>a</sup>RPKM Reads Per Kilobase of transcript per Million mapped reads  
<sup>b</sup>FC Fold Change
### Supplementary Table II: Metacore analysis pathway maps for LPS WT vs LPS Lcn2−/− transcriptomes

| Pathway Maps                                      | Corrected p value | FDR<sup>a</sup> | Network Objects                                                                 |
|---------------------------------------------------|-------------------|------------------|---------------------------------------------------------------------------------|
| Immune response: IL-10 signaling                  | 2.74E-06          | 1.01E-03         | IL-1 alpha, ICAM1, IL-6, Heme oxygenase 1, Fc gamma RI, IKBZ, SOCS3, G-CSF, CD14 |
| Immune response: Innate response to RNA viral infection | 1.32E-05          | 2.44E-03         | IRF7, MDA-5, Caspase-8, TLR3, RIPK1, LGP2                                        |
| SLE genetic marker specific pathways in antigen-presenting cells (APC) | 2.69E-05          | 3.30E-03         | A20, IRF1, IL-1 alpha, ICAM1, IRF8, IRF7, MDA-5, GRP78, CD40 (TNFRSF5)          |
| Immune response: CCL2 signaling                   | 1.18E-04          | 8.51E-03         | IL-1 alpha, ICAM1, IL-6, CCL2, VE-cadherin, MCPIP, Occludin                     |
| Role of cell adhesion in vaso-occlusion in Sickle cell disease | 1.90E-04          | 8.51E-03         | IL-1 alpha, ICAM1, CD44, Fc gamma RI, CD14, P-selectin                          |
| Immune response: IFN alpha/beta signaling pathway  | 1.96E-04          | 8.51E-03         | IRF1, ISG54, USP18, SOCS1, ISG15                                               |
| Signal transduction: NFκB activation              | 2.08E-04          | 8.51E-03         | IL-1 alpha, NF-κB2 (p100), NF-κB2 (p52), RelB (NF-κB subunit), RIPK1, TLR2, c-IAP2 |
| Development: Prolactin receptor signaling         | 2.08E-04          | 8.51E-03         | IRF1, NMI, SOCS3, OAS1, C/EBPbeta, c-Myc, SOCS1                                |
| Immune response: PKR in stress induced antiviral cell response | 2.08E-04          | 8.51E-03         | IRF1, IRF7, IL-6, Caspase-8, TLR3, TLR2, c-Myc                                 |
| Immune response: T regulatory cell mediated modulation of APC | 2.38E-04          | 8.78E-03         | IRF1, NF-κB2 (p100), ICAM1, IRF8, NF-κB2 (p52), CCL22                         |

<sup>a</sup>FDR False Discovery Rate
### Supplementary Table III

Metacore enrichment analysis by GO processes for LPS WT vs LPS Lcn2−/− transcriptomes

| Processes                              | Corrected p value | FDRa   | Network Objects                                                                 |
|----------------------------------------|-------------------|--------|---------------------------------------------------------------------------------|
| Defense response                       | 2.95E-38          | 6.37E-35 | UBCH8, GRO-3, IRF1, A20, OASL, ZC3HAV1, TBXA2R, Mx1, TCIRG1 (Atp6i), SPHK1, IL-1 alpha, CXCL16, NF-kB2 (p100), BDKRB1, PGES, ISG54, FAT10, ICAM1, CD44, IRF8, IRF7, IIGP, GRO-2, RSAD2, IFI56, NMI, ZBP1, IL18RAP, SP100, IL-6, MDA-5, BATF2, IL-19, CCL2, EFP, ISG20, PGD2R, TAP1 (PSF1), JMJD3, XAF1, Caspase-8, Heme oxygenase 1, Hepcidin, ICB-1, GBP6, PTX3, Fc gamma RI, CD40(TNFRSF5), IFI47, IFP 35, IKBZ, TLR3, SOCS3, Syntaxin 11, IFI16, UBE1L, OAS1, LRG-47, SAMHD1, SLAMF8, HA1B, RelB (NF-kB subunit), H2-M3, GBP7, IFIT1, RIPK1, I-TAC, TLR2, MIG, SP110, MxA, RIG-G, CCR7, Factor B, c-IAP2, CD14, C/EBPbeta, USP18, OAS3, IP10, Ro52, GBP5, Caspase-4, CCL13, GBP4, CCL22, PL scramblase 1, GBP1, GBP2, CCR2, TRIF (CARD15), SOCS1, CCL7, IRG1, BMX, Gbp8, P-selectin, ISG15, HLA-Cw3, RAGE, LGP2 |
| Response to other organism             | 3.25E-38          | 6.37E-35 | GRO-3, IRF1, A20, OASL, ZC3HAV1, TBXA2R, Mx1, C8FW (GIG2), IL-1 alpha, CXCL16, NF-kB2 (p100), BDKRB1, PGES, ISG54, ICAM1, P2Y2, IRF8, IRF7, IIGP, GRO-2, RSAD2, IFI56, ZBP1, IL-6, MDA-5, BATF2, CCL2, EFP, ISG20, MCPIP, Caspase-8, Hepcidin, GBP6, PTX3, Fc gamma RI, CD40(TNFRSF5), TLR3, SOCS3, IFI16, OAS1, PDE4B, LRG-47, SAMHD1, SLAMF8, HA1B, G-CSF, H2-M3, GBP7, IFIT1, I-TAC, TLR2, MIG, SP110, CMPK2, MxA, RIG-
| Response to external biotic stimulus | 4.24E-38 | 6.37E-35 |
|-------------------------------------|----------|----------|
|                                     | G, CCR7, Factor B, CD14, C/EBPbeta, OAS3, IP10, CCL13, GBP4, CCL22, PL scramblase 1, IFI44, GBP1, GBP2, Nod2 (CARD15), SOCS1, IRG1, P-selectin, ISG15, HLA-Cw3, LGP2 |

| Response to biotic stimulus         | 1.65E-37 | 1.85E-34 |
|-------------------------------------|----------|----------|
|                                     | GRO-3, IRF1, A20, OASL, ZC3HAV1, TBXA2R, Mx1, C8FW (GIG2), IL-1 alpha, CXCL16, NF-kB2 (p100), BDKRB1, PGES, ISG54, ICAM1, P2Y2, IRF8, IRF7, IIGP, GRO-2, RSAD2, IFI56, ZBP1, IL-6, MDA-5, BATF2, CCL2, EFP, ISG20, MCPIP, Caspase-8, Hepcidin, GBP6, PTX3, Fc gamma RI, CD40(TNFRSF5), TLR3, SOCS3, IFI16, OAS1, PDE4B, LRG-47, SAMHD1, SLAMF8, HA1B, G-CSF, H2-M3, GBP7, IFIT1, I-TAC, TLR2, MIG, SP110, CMPK2, MxA, RIG-G, CCR7, Factor B, CD14, C/EBPbeta, OAS3, IP10, CCL13, GBP4, CCL22, PL scramblase 1, IFI44, GBP1, GBP2, Nod2 (CARD15), SOCS1, IRG1, P-selectin, ISG15, HLA-Cw3, LGP2 |
| Category                                | Log2 Fold Change 1 | Log2 Fold Change 2 |
|-----------------------------------------|-------------------|-------------------|
| Immune response                         | 2.35E-37          | 2.12E-34          |
| Response to cytokine                    | 1.37E-36          | 1.03E-33          |
| Cellular response to cytokine stimulus  | 3.36E-35          | 2.16E-32          |

**Immune response**
- UBC8, GRO-3, CCL9, IRF1, A20, OASL, ZC3HAV1, Mx1, IL-2R gamma chain, IL-1 alpha, CXCL16, NF-kB2 (p100), ISG54, FAT10, ICAM1, CD44, IRF8, IRF7, IIGP, RT1-CE14, GRO-2, RSAD2, IFI56, ZBP1, IL18RAP, SP100, SBN02, IL-6, MDA-5, Oncostatin M, IL-19, CCL2, EFP, ISG20, JMJ3, XAF1, Caspase-8, Hepcidin, GBP6, PTX3, Fc gamma RI, PNPH, IFP 35, TLR3, SOCS3, Syntaxin 11, IFI16, UBE1L, OAS1, CRTH2, LRG-47, E4BP4, SAMHD1, HA1B, G-CSF, RelB (NF-kB subunit), H2-M3, GBP7, IFIT1, RIPK1, I-TAC, TLR2, Apo-2L(TNFSF10), MIG, SP110, MxA, RIG-G, CCR7, Factor B, c-IAP2, CD14, C/EBPbeta, USP18, LIF, OAS3, PD-L1, IP10, Ro52, GBP5, CCL13, GBP4, CCL22, PL scramblase 1, GBP1, GEM, GBP2, Nod2 (CARD15), SOCS1, CCL7, IRG1, BMX, Gbp8, ISG15, HLA-Cw3, RAGE, LDP2

**Response to cytokine**
- UBC8, GRO-3, IRF1, OASL, SPHK1, IL-2R gamma chain, IL-1 alpha, CXCL16, NF-kB2 (p100), PGES, ISG54, FAT10, ICAM1, Ifi205, CD44, P2Y2, IRF8, IRF7, IIGP, GRO-2, RSAD2, IFI56, IL18RAP, SP100, IL-6, GRP78, IFI203, Oncostatin M, CCL2, GPR17(Amida), EFP, ISG20, XAF1, Caspase-8, GBP6, Fc gamma RI, IFP 35, TLR3, SOCS3, IFI16, UBE1L, OAS1, OSMR, LRG-47, E4BP4, SAMHD1, G-CSF, RelB (NF-kB subunit), GBP7, IFIT1, RIPK1, I-TAC, Pyhin1, MIG, MxA, RIG-G, CCR7, CD14, USP18, OAS3, IP10, Ro52, GBP5, c-Myc, CCL13, GBP4, PL scramblase 1, GBP1, GBP2, CCRL2, SOCS1, IRG1, Gbp8, P-selectin, ISG15, HLA-Cw3

**Cellular response to cytokine stimulus**
- UBC8, GRO-3, IRF1, OASL, IL-2R gamma chain, IL-1
| **Defense response to other organism** | 4.25E-31 | 2.39E-28 |
|---------------------------------------|----------|----------|
| IRF1, OASL, ZC3HAV1, Mx1, ISG54, IRF8, IRF7, IIGP, RSAD2, IFI56, ZBP1, IL-6, MDA-5, BATF2, EFP, ISG20, Hepcidin, GBP6, Fc gamma RI, CD40(TNFRSF5), TLR3, IFI16, OAS1, LRG-47, SAMHD1, SLAMF8, HA1B, H2-M3, GBP7, IFIT1, TLR2, MIG, MxA, RIG-G, C/EBPbeta, OAS3, IP10, GBP4, PL scramblase 1, GBP1, GBP2, Nod2 (CARD15), P-selectin, ISG15, HLA-Cw3, LGP2 |

| **Immune system process** | 5.50E-31 | 2.75E-28 |
|--------------------------|----------|----------|
| UBCH8, GRO-3, CCL9, IRF1, A20, OASL, ZC3HAV1, Mx1, IL-2R gamma chain, IL-1 alpha, CXCL16, NF-kB2 (p100), ISG54, FAT10, ICAM1, CD44, IRF8, IRF7, H2-gs10, IIGP, RT1-CE14, GRO-2, RSAD2, IFI56, ZBP1, IL18RAP, SP100, SBN02, GPR109A, IL-6, MDA-5, BATF2, Oncostatin M, IL-19, CCL2, EFP, ISG20, TAP1 (PSF1), JMJD3, XAF1, Caspase-8, Heme oxygenase 1, Hepcidin, ICB-1, GBP6, PTX3, Bcl2a1d, Fc gamma RI, CD40(TNFRSF5), PNP, IFP 35, TLR3, SOCS3, Syntaxin 11, IFI16, UBE1L, OAS1, CRTH2, PDE4B, LRG-47, E4BP4, SAMHD1, HA1B, G-CSF, RelB (NF-kB subunit), H2-M3, GBP7, IFIT1, RIPK1, I-TAC, TLR2, Apo-2L(TNFFSF10), MIG, SP110, SAMD9L, MxA, RIG-G, |
| Cytokine mediated signaling pathway | 1.35E-28 | 6.06E-26 |
|-----------------------------------|---------|---------|

| CCR7, TPL2(MAP3K8), Factor B, c-IAP2, CD14, C/EBPbeta, USP18, TIPARP, LIF, OAS3, PD-L1, IP10, Ro52, GBP5, HHEX (PRH), CCL13, GBP4, CCL22, PL scramblase 1, GBP1, GEM, GBP2, Nod2 (CARD15), SOCS1, CCL7, Ferroportin 1, AML1 (RUNX1), IRG1, BMX, FAZF, Gbp8, TIE2, P-selectin, ISG15, HLA-Cw3, RAGE, BMP4, LGP2 |

UBCH8, GRO-3, IRF1, OASL, IL-2R gamma chain, IL-1 alpha, ISG54, ICAM1, CD44, IRF8, IRF7, IIGP, GRO-2, RSAD2, IFI56, SP100, IL-6, Oncostatin M, CCL2, GPR17(Amida), EFP, ISG20, XAF1, Caspase-8, Fc gamma RI, IFP 35, SOCS3, UBE1L, OAS1, OSMR, SAMHD1, G-CSF, RIPK1, I-TAC, MIG, MxA, RIG-G, CCR7, USP18, OAS3, IP10, Ro52, CCL13, GBP1, GBP2, CCRL2, SOCS1, ISG15, HLA-Cw3

\(^a\)FDR False Discovery Rate
**Supplementary Table IV** Metacore enrichment analysis by process networks for LPS WT vs LPS Lcn2−/− transcriptomes

| Networks                                      | Corrected p value | FDRᵃ | Network Objects                                                                 |
|-----------------------------------------------|-------------------|------|---------------------------------------------------------------------------------|
| Inflammation: interferon signaling            | 1.80E-23          | 2.31E-21 | IRF1, ISG54, ICAM1, IRF8, IRF7, IGI56, IL18RAP, CCL2, ISG20, TAP1 (PSF1), Caspase-8, Fc gamma RI, CD40(TNFRSF5), IFP 35, TLR3, SOCS3, I-TAC, Apo-2L(TNFSF10), MIG, MxA, C/EBPbeta, IFI44, GBP1, GBP2, SOCS1, CCL7, ISG15 |
| Immune response: Th17-derived cytokines       | 1.58E-09          | 1.01E-07 | PGES, ICAM1, IL-6, MMP-13, CCL2, IKBZ, SOCS3, G-CSF, I-TAC, MIG, C/EBPbeta, c-Myc, CCL7 |
| Chemotaxis                                    | 9.67E-09          | 4.12E-07 | GRO-3, IL-1 alpha, CXCL16, CD44, GRO-2, CCL2, I-TAC, MIG, CCR7, Cyr61, IP10, CCL13, CCL22, CCL7, AML1 (RUNX1) |
| Inflammation: IFN-gamma signaling            | 3.94E-07          | 1.04E-05 | IRF1, CXCL16, ICAM1, NFKBIE, Caspase-8, IFI16, MIG, Factor B, C/EBPbeta, IP10, c-Myc, SOCS1 |
| Inflammation: Innate inflammatory response    | 4.07E-07          | 1.04E-05 | IL-1 alpha, NF-kB2 (p100), PGES, IRF7, IL-6, NFKBIE, NF-kB2 (p52), TLR3, sCD14, RIPK1, I-TAC, TLR2, CD14, IP10 |
| Immune response: Innate immune response to RNA viral infection | 1.54E-06          | 3.29E-05 | IRF1, IRF7, IL-6, MDA-5, Caspase-8, TLR3, RIPK1, I-TAC, MxA, IP10 |
| Inflammation: IL-10 anti-inflammatory         | 3.24E-06          | 5.93E-05 | IL-1 alpha, NF-kB2 (p100), IRF8, IL-6, NFKBIE, MMP-13, |
| response                                                                 | p-value | q-value | Pathway and Genes                                                                                     |
|-------------------------------------------------------------------------|---------|---------|-----------------------------------------------------------------------------------------------------|
| Inflammation: Jak-STAT pathway                                           | 3.90E-05| 6.24E-04| IL-2R gamma chain, IL-6, Oncostatin M, IL-19, CCL2, CD40(TNFRSF5), SOCS3, OSMR, G-CSF, LIF, CCL13, SOCS1 |
| Inflammation: Amphoterin signaling                                      | 5.18E-05| 7.37E-04| IL-1 alpha, NF-kB2 (p100), ICAM1, IL-6, NFKBIE, MMP-13, CCL2, NF-kB2 (p52), TLR2, RAGE              |
| Cell adhesion: Platelet endothelium leukocyte interactions               | 9.98E-05| 1.28E-03| ICAM1, CD44, IL-6, NFKBIE, MMP-13, CCL2, TFPI-2, Occludin, CD40(TNFRSF5), G-CSF, Cyr61, P-selectin   |

\(^a\)FDR False Discovery Rate
Supplementary Table V Top 20 up-regulated brain transcripts in Lcn2−/− relative to WT mice after peripheral LPS challenge by RNAseq

| Gene name | Mean RPKM<sup>a</sup> Lcn2−/− LPS (log<sub>2</sub>) | Mean RPKM<sup>a</sup> WT LPS (log<sub>2</sub>) | FC<sup>b</sup> Lcn2−/− LPS/ WT LPS | Corrected P value |
|-----------|---------------------------------|---------------------------------|--------------------------------|-------------------|
| Cxcl11    | 0.76                           | -3.09                           | 14.4                           | 2.5E-04           |
| Timd4     | -1.20                          | -4.43                           | 9.4                            | 2.3E-04           |
| Bcl2a1d   | -1.44                          | -4.62                           | 9.1                            | 3.0E-03           |
| Cxcl10    | 6.66                           | 3.72                            | 7.7                            | 1.6E-03           |
| Gm5970    | 0.60                           | -2.32                           | 7.5                            | 7.0E-05           |
| Rsad2     | 4.45                           | 1.73                            | 6.6                            | 3.1E-04           |
| Ptx3      | 2.00                           | -0.71                           | 6.5                            | 7.7E-04           |
| Trim30c   | -1.90                          | -4.48                           | 6.0                            | 1.7E-03           |
| Ccl7      | 2.83                           | 0.33                            | 5.7                            | 5.8E-04           |
| Cxcl2     | 3.50                           | 1.03                            | 5.5                            | 7.6E-04           |
| Fam26f    | 1.54                           | -0.93                           | 5.5                            | 3.5E-04           |
| Hamp      | -1.64                          | -4.10                           | 5.5                            | 5.0E-03           |
| Irg1      | 0.69                           | -1.77                           | 5.5                            | 3.5E-04           |
| Mx1       | 2.50                           | 0.09                            | 5.3                            | 9.8E-04           |
| Lif       | -1.19                          | -3.57                           | 5.2                            | 7.5E-04           |
| Gm25206   | -0.55                          | -2.91                           | 5.1                            | 7.4E-03           |
| Cxcl9     | 2.88                           | 0.52                            | 5.1                            | 1.1E-04           |
| Mx2       | 2.19                           | -0.01                           | 4.9                            | 1.2E-04           |
| Ccr12     | 2.15                           | -0.12                           | 4.8                            | 1.6E-04           |
| Ccl2      | 3.72                           | 1.46                            | 4.8                            | 1.6E-04           |

<sup>a</sup>RPKM Reads Per Kilobase of transcript per Million mapped reads

<sup>b</sup>FC Fold Change
**Supplementary Table VI** Top 20 down-regulated brain transcripts in *Lcn2*−/− relative to WT mice after peripheral LPS challenge by RNAseq

| Gene name      | Mean RPKM \(^a\) *Lcn2*−/− LPS (log\(_2\)) | Mean RPKM \(^a\) WT LPS (log\(_2\)) | FC \(^b\) *Lcn2*−/− LPS/WT LPS | Corrected P value |
|----------------|---------------------------------------------|---------------------------------------|--------------------------------|--------------------|
| Slco1a4        | 2.23                                        | -0.10                                 | -5.1                           | 1.0E-04            |
| Nkx2-9         | -2.07                                       | -4.11                                 | -4.1                           | 8.1E-03            |
| Gm23497        | -1.23                                       | -3.22                                 | -4.0                           | 7.2E-03            |
| RP24-186G5.1   | -2.28                                       | -4.19                                 | -3.8                           | 9.3E-03            |
| Gm694          | 1.48                                        | -0.43                                 | -3.8                           | 1.3E-03            |
| Slc38a11       | -2.09                                       | -3.93                                 | -3.6                           | 3.8E-04            |
| Gm27353        | -0.38                                       | -2.15                                 | -3.4                           | 4.6E-03            |
| Ptgdr          | -1.83                                       | -3.57                                 | -3.4                           | 2.6E-03            |
| RP23-421C2.2   | 0.89                                        | 0.86                                  | -3.4                           | 6.9E-03            |
| Gbgt1          | -1.86                                       | -3.55                                 | -3.2                           | 3.0E-03            |
| Inmt           | -1.76                                       | -3.37                                 | -3.1                           | 1.2E-03            |
| Tm6sf2         | -1.68                                       | -3.29                                 | -3.0                           | 2.2E-03            |
| Gm17193        | -1.29                                       | -2.81                                 | -2.9                           | 4.0E-03            |
| Kpna2-ps       | -0.87                                       | -2.39                                 | -2.9                           | 3.2E-03            |
| Gm16044        | -0.42                                       | -1.92                                 | -2.8                           | 3.4E-03            |
| Fmo2           | -1.18                                       | -2.65                                 | -2.8                           | 9.3E-03            |
| Foxc2          | -1.23                                       | -2.68                                 | -2.7                           | 1.2E-03            |
| Gm10615        | -0.005                                      | -1.40                                 | -2.6                           | 2.2E-03            |
| Sema3g         | 0.16                                        | -1.19                                 | -2.6                           | 5.2E-05            |
| Zfp626         | -0.11                                       | -1.42                                 | -2.5                           | 4.2E-04            |

\(^a\)RPKM Reads Per Kilobase of transcript per Million mapped reads

\(^b\)FC Fold Change
Supplementary Fig 1. Peripheral LPS injection alters the proteome profile of the CNS. WT mice were injected with either saline or 2μg/g LPS and soluble TBSE lysates from hemi-brains of PBS perfused animals were assayed using a cytokine proteome array. Of the abundant proteins, A, LCN2 was one of the most highly induced by LPS challenge. B, Examination of down-regulated proteins that were abundant in the control group showed very few candidate proteins. Examination of lower abundance proteins, quantitated using a longer exposure, revealed additional candidate proteins that are C, induced or D, decreased in the CNS after peripheral LPS challenge.

Supplementary Figure 2. LCN2 is dramatically induced in the CSF following systemic LPS challenge. Western blot for LCN2 from cerebrospinal fluid (CSF) obtained from WT mice 24 hours post i.p. injection with saline or 2μg/g LPS. Data shown is a representative blot from N=3 experiments.

Supplementary Figure 3. Absence of LCN2 results in decreased mobility after LPS challenge. WT or Lcn2−/− mice were injected with either saline or 2μg/g LPS i.p. and assessed for total mobility using the open field assay (OFA). Shown are representative track plots from WT and Lcn2−/− mice at A, 8 or B, 24 hours post injection. Shown are representative track plots from N=9 (8 hour time point) or N=12 (24 hour time point) per condition from 2 independent experiments.
**Supplementary Figure 4.** LCN2 uniquely regulates select CNS transcripts following peripheral LPS challenge. RNAseq was conducted from hemi-brain tissue of PBS perfused WT or Lcn2−/− mice 8 hours post i.p. injection with 2μg/g LPS or saline. RPKM values from RNAseq of select transcripts that are uniquely A-H, increased or I-L decreased in LPS-Lcn2−/− mice relative to all other conditions are shown with corrected p values. (ANOVA, corrected p values, *p≤0.05, **p≤0.01, ***p≤0.001).
Supplementary Materials and Methods

Open field assay

Open field assay (OFA) was assessed between 9:00 A.M. and 5:00 P.M. Animals were acclimated to the room for an hour prior to the onset of testing at either 8 or 24 hours post injection. The apparatus was thoroughly cleaned with 30% ethanol and allowed to dry between test mice to remove any residual odors in the equipment. Mice were placed in a 40x40 cm black Plexiglas box with a brightly lit center. Behavioral activity was recorded by overhead Fujinon cameras for 15 minutes and was tracked using Anymaze software (Stoelting Co.; Wood Dale, IL). An imaginary 13cm x 13cm center region in the box and a perimeter region were digitally defined using Anymaze software. Side mounted photobeams located 7.6 cm from the floor of the box were used to monitor rearing activity.

Tissue processing

Animals were deeply anesthetized with pentobarbital prior to cardiac perfusion with phosphate-buffered saline (PBS) to expunge blood from the cerebrovasculature. For biochemical analysis, hemi-brain tissues were quickly frozen on dry ice and stored at -80°C until further processing. Tissues were briefly sonicated in Tris buffered saline with EDTA (TBSE) (50mM Tris pH=7.5, 150mM NaCl, 1mM EDTA) with 1x protease and phosphatase inhibitors (Thermo Scientific, Waltham, MA). An aliquot of the tissue suspension was immediately placed into Trizol for RNA isolation using the Direct-zol RNA kit (Zymo Research, Irvine, CA). Another aliquot was centrifuged for 15 min at 14,000 rpm at 4°C to isolate the
soluble TBSE fraction for cytokine assessment. TBSE tissue protein levels were assessed using a BCA kit (Thermo Scientific).

_Cytokine proteome profile array_

WT mice were injected with either saline or 2μg/g LPS i.p. and hemi-brains were harvested from PBS perfused animals 24 hours post challenge. TBSE hemi-brain fractions were pooled together from N=2 animals, for a total of 200μg of protein, from either saline or LPS injected animals and run on a Mouse XL Cytokine Array according to manufacturers instructions (R&D Systems, Minneapolis, MN). Briefly, membranes were blocked for 1 hour prior to addition of pooled TBSE samples (N=1 membrane for pooled saline samples, N=2 membranes for pooled LPS samples) and incubated overnight at 4°C. Membranes were washed 3 times in wash buffer and then incubated with cytokine detection cocktail for 1 hour, rocking at room temperature. Following an additional 3 washes, streptavidin-HRP was added for 30 minutes. Membranes were washed 3 times and then developed with enhanced chemiluminescent substrate (Thermo Scientific).

_ELISA and Western blotting_

Tissue cytokine levels were measured from hemi-brain TBSE tissue fractions using IL-1β, IL-6, TNFα (Biolegend, San Diego, CA), and LCN2 (R&D Systems) ELISA kits according to manufacturer’s instructions with values expressed as pg/mg of total protein. Western blots were probed with 1:500 dilution of biotinylated rat anti-LCN2 (cat # AF1857, R&D Systems, Minneapolis, MN), followed by horseradish
peroxidase (HRP) conjugated mouse anti-biotin secondary antibody. Blots for GFAP were probed with 1:1000 mouse anti-GFAP (cat #3670X, Cell Signaling Technology, Danvers, MA) followed by a HRP conjugated goat anti-mouse secondary. Membranes were stripped and re-probed with 1:10,000 dilution of mouse anti-GAPDH (cat# 2-RGM2, clone 6C5, Advanced Immunochemicals, Long Beach, CA)

**Immunohistochemistry**

PBS perfused hemi-brains were drop fixed into 10% neutral buffered formalin (Fisher Scientific, Waltham, MA) overnight at 4°C. Tissue was then placed in 30% sucrose (Sigma, Saint Louis, MO) dissolved in PBS overnight at 4°C. 50μ sagittal brain sections were cut on a freezing-sliding microtome and stored in cryoprotectant at -20°C until staining. Sections were blocked for endogenous peroxidase activity and permeabilized with 0.6% H2O2, 0.1% NaN3 in PBS-X (1X PBS containing 0.3% Triton-X) for 30 minutes at RT. Sections were blocked with 1% milk in PBS-X followed by incubation with rabbit anti-IBA1 at 1:8000 (cat# 019-9741, Wako, Richmond, VA) in 0.5% milk PBS-X for 2 days at 4°C. Sections were then incubated with the Vectastain kit anti-rabbit IgG (Vector Labs, Burlingame, CA) overnight at 4°C followed by ABC component for 4hrs and developed using the DAB kit (Vector Labs) according to manufacturer's instructions. Images were acquired using an Aperio XT Scanner (Aperio, Vista, CA) at a 20x magnification.

**qPCR**
Total RNA was isolated from sonicated tissues using a Direct-zol total RNA isolation kit according to manufacturer’s instructions with in-column DNase I treatment. Random-primed reverse transcription was performed according to manufacturer protocols (Invitrogen-Life Technologies, Grand Island, NY). cDNA was added to a reaction mix (10μL final volume) containing 300nM gene-specific primers and Universal SYBR green supermix (Biorad, Hercules, CA). All samples were run in triplicate and were analyzed on an ABI 7900 HT Fast Real Time PCR instrument (Applied Biosystems-Life Technologies). Relative gene expression was normalized to GAPDH controls and assessed using the 2^{-ΔΔCT} method. Primer sequences are as follows (5’ to 3’): GAPDH F: CTGCACCACCAACTGCTTAG, GAPDH R: ACAGTCTTCTGGTGCGCA GT, Aif1(Iba1) F: GGATTTGCAGGGAAAAG, Aif1(Iba1) R: TGGGATCATCGAGGAATTG.

In vivo microdialysis

In vivo microdialysis of awake, freely moving mice was performed as described\textsuperscript{21}. Mice were provided acetaminophen 48 hours before surgery. Mice were anesthetized using 1.5%-2.5% isoflurane, the head shaved and skin transected along the midline to expose the skull. Body temperature was maintained during the procedure using a heating blanket set at 37°C. A bore hole (0.75mm) was made above the left hippocampus (coordinates bregma -3.1mm, 2.5mm lateral) using a small animal stereotaxic device. A second bore hole was made into the right, posterior skull quadrant for an anchoring bone screw. The AtmosL Guide Cannula (PEG-X, Eicom) was then stereotactically inserted into the hippocampus (12° angle,
dura mater -1.2 mm) and cemented into place with dental cement. An AtmosLM Dummy probe (PED-X, Eicom) was inserted into the guide cannula and the wound was closed with surgical adhesive glue. Mice were injected with antibiotics (ampicillin; 100mg/kg, i.m) and placed into a clean cage and provided with access to food and water ad libitum. Mice were allowed to awaken and recover overnight. To sample large molecules from the extracellular space, microdialysis MegaProbes (1000-kilodalton MWCO membrane, Eicom) were used with a peristaltic push-pull pump (SciPro). Microdialysis perfusion buffer was aCSF (1.3 mM CaCl₂, 1.2 mM MgSO₄, 3 mM KCl, 0.4 mM KH₂PO₄, 25 mM NaHCO₃, and 122 mM NaCl, pH 7.35) containing 0.15% BSA (Sigma) that was filtered through a 0.1 μM membrane. Mice were kept under constant light conditions for the remainder of the experiment. LPS (2mg/kg) was injected (i.p.) 10 hrs post probe insertion. Microdialysis was performed at a flow rate of 1 μL/min and interstitial fluid (ISF) samples were collected hourly using a refrigerated fraction collector (SciPro).

Cytometric bead array

ISF samples were pooled into 2-hour fractions and assayed for pro-inflammatory cytokines using a a highly quantitative mouse inflammatory cytokine bead array kit according to manufacturer’s instructions (BD Bioscience, San Jose, CA). Samples were collected on a FACS Calibur (BD Bioscience, San Jose, CA) and analyzed with FlowJo software (Tree Star, Inc).

Illumina RNA sequencing and pathway analysis
A total of 16 mRNA samples were sequenced at Mayo Clinic using Illumina HiSeq 2000. Reads were mapped to the mouse genome mm10. Raw exon and gene read counts, along with sequencing QC, were generated using Mayo Clinic-developed RNAseq analytic pipeline: MAP-RSeq Version 2.0\textsuperscript{42}. Conditional Quantile Normalization (CQN) was applied to raw gene counts to correct for GC bias and technical variations\textsuperscript{43}. Based on the bi-modal distribution of the CQN normalized and log2 transformed RPKM (reads per kb per million) gene expression values, genes with average log2 RPKM < -2 across all experimental groups were considered to be too lowly expressed and were excluded from further analysis. Differential expression, principal component analysis (PCA), and hierarchical clustering were performed using Partek Genomics Suite (Partek Inc., St. Louis, MO) with ANOVA and multiple comparison adjustment of p values using Benjamini–Hochberg correction. Pathway analyses was performed in order to obtain a thorough understanding of gene interactions and associated networks. We used Fisher's Exact Test based MetaCore pathway analysis (Thomson Reuters, Version 6.25) with differentially expressed genes generated based on thresholds of fold change $\geq 1.5$ and adjusted p values <0.01.