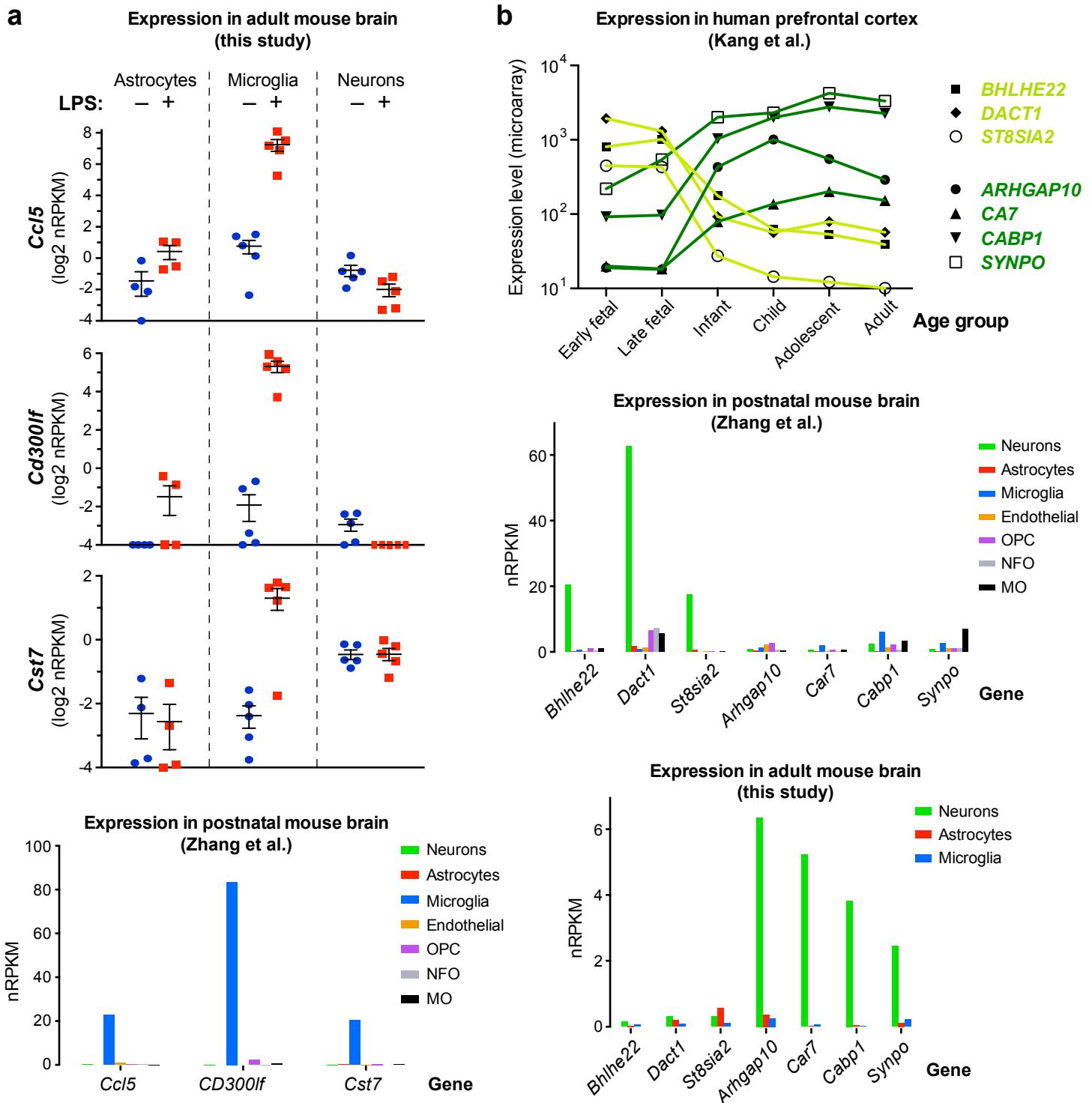
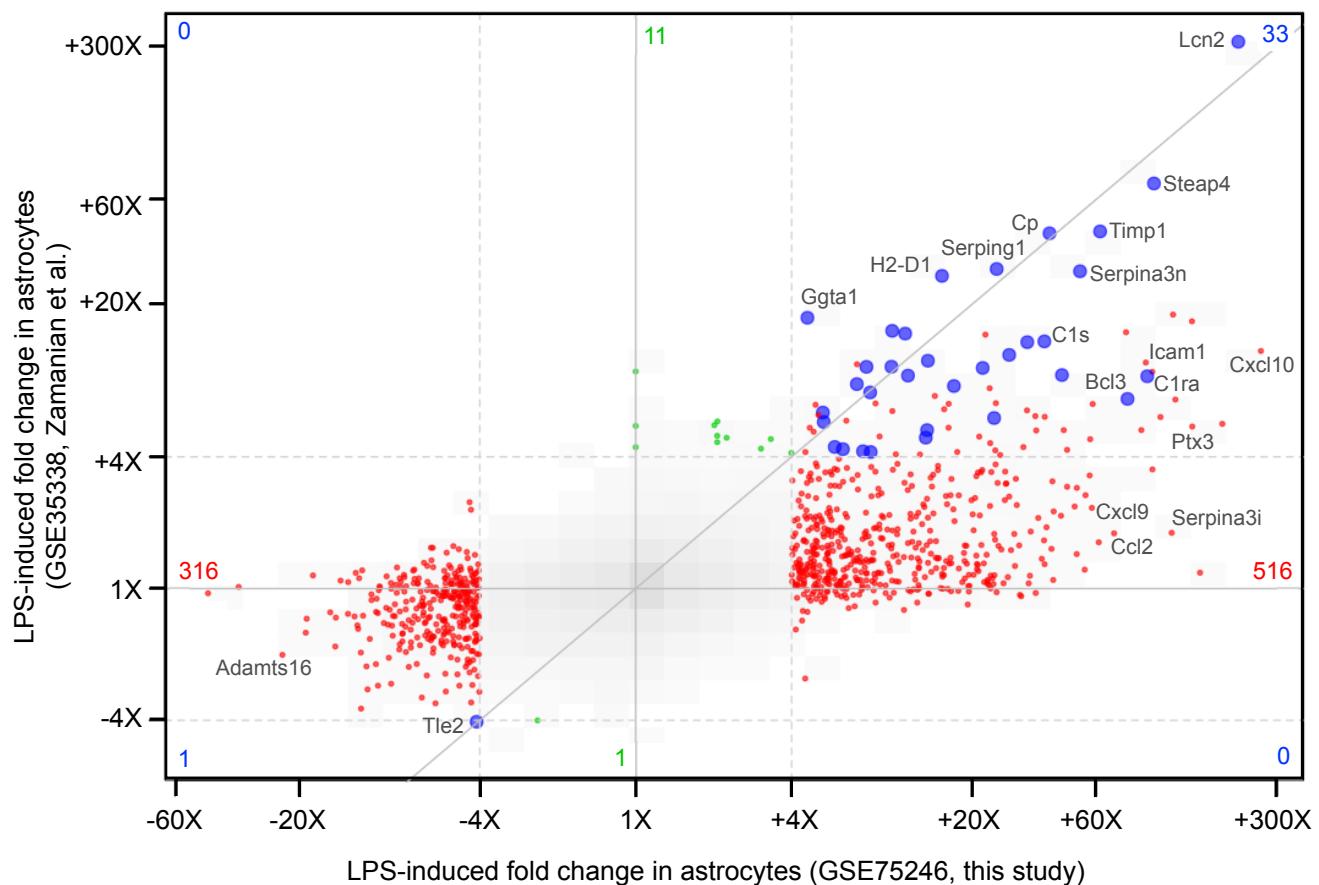


**Supplementary Figure 1 | Cell type marker expression in FACS-isolated cells from adult mouse cortex (qPCR).**

(a) RNA from cells isolated using anti-NeuN (*Rbfox3*, neurons), anti-GFAP (astrocytes), or anti-CD11b (*Itgam*, microglia) was tested by RT-qPCR for expression of the same markers used for sorting. Markers were normalized to *Gapdh* expression, and bars represent mean  $\pm$  s.d. (Prism). (b) Additional markers expressed by neurons (top row), astrocytes (middle row), and microglia (bottom row) were tested by RT-qPCR for expression level in NeuN<sup>+</sup>, GFAP<sup>+</sup>, and CD11b<sup>+</sup> sorted cell populations.

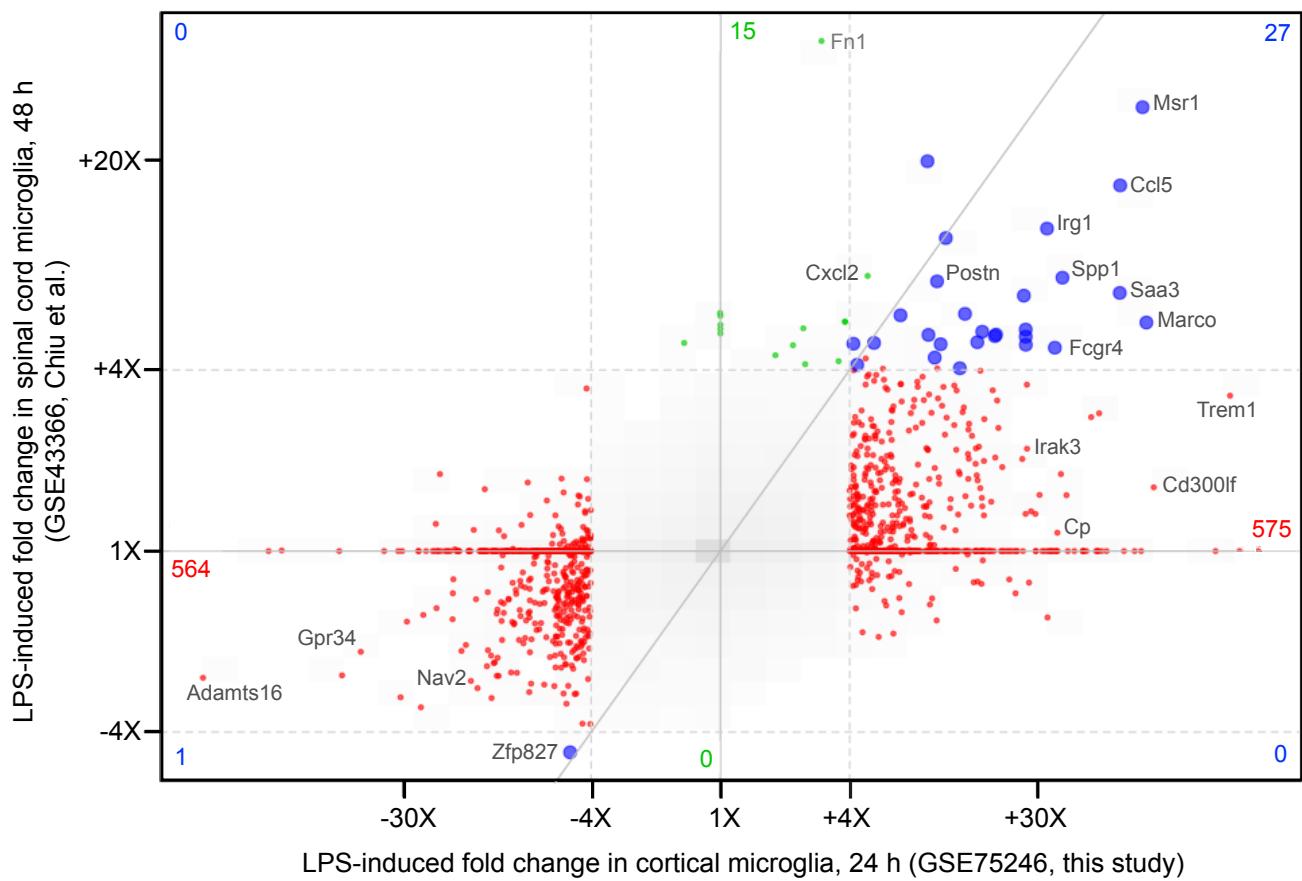


**Supplementary Figure 2 | Our method yields expression profiles for microglia and neurons in their unperturbed, mature states.** (a) Certain genes reported by others (Zhang et al., GSE52564) to be highly expressed in microglia (median values plotted,  $n=2$  per cell type) were not observed in our data (GSE75246) unless the animals were somehow challenged as with LPS injection ( $n=4-5$  samples per cell type plotted individually, bars = mean  $\pm$  s.e.m. (Prism)). (b) Certain genes expressed in postnatal (GSE52564,  $n=2$  per cell type) but not adult (GSE75246,  $n=4-5$  per cell type) mouse neurons were robustly detected in fetal but not adult human prefrontal cortex (Kang et al., GSE25219). Conversely, certain genes expressed in adult but not postnatal mouse neurons were expressed more highly in adult than fetal human prefrontal cortex. Median values plotted. Early fetal = 8-17 weeks post-conception ( $n=88$  tissue specimens), late fetal = 19-37 weeks post-conception ( $n=64$ ), infant = < 1 year after birth ( $n=28$ ), child = 1-9 years ( $n=32$ ), adolescent = 11-19 years ( $n=28$ ), and adult = 21-64 years ( $n=91$ ). \*Note: nRPKM values cannot be directly compared between GSE52564 and GSE75246 due to different library preparation methods. However, relative nRPKM differences among cell types within a study should be comparable between studies.



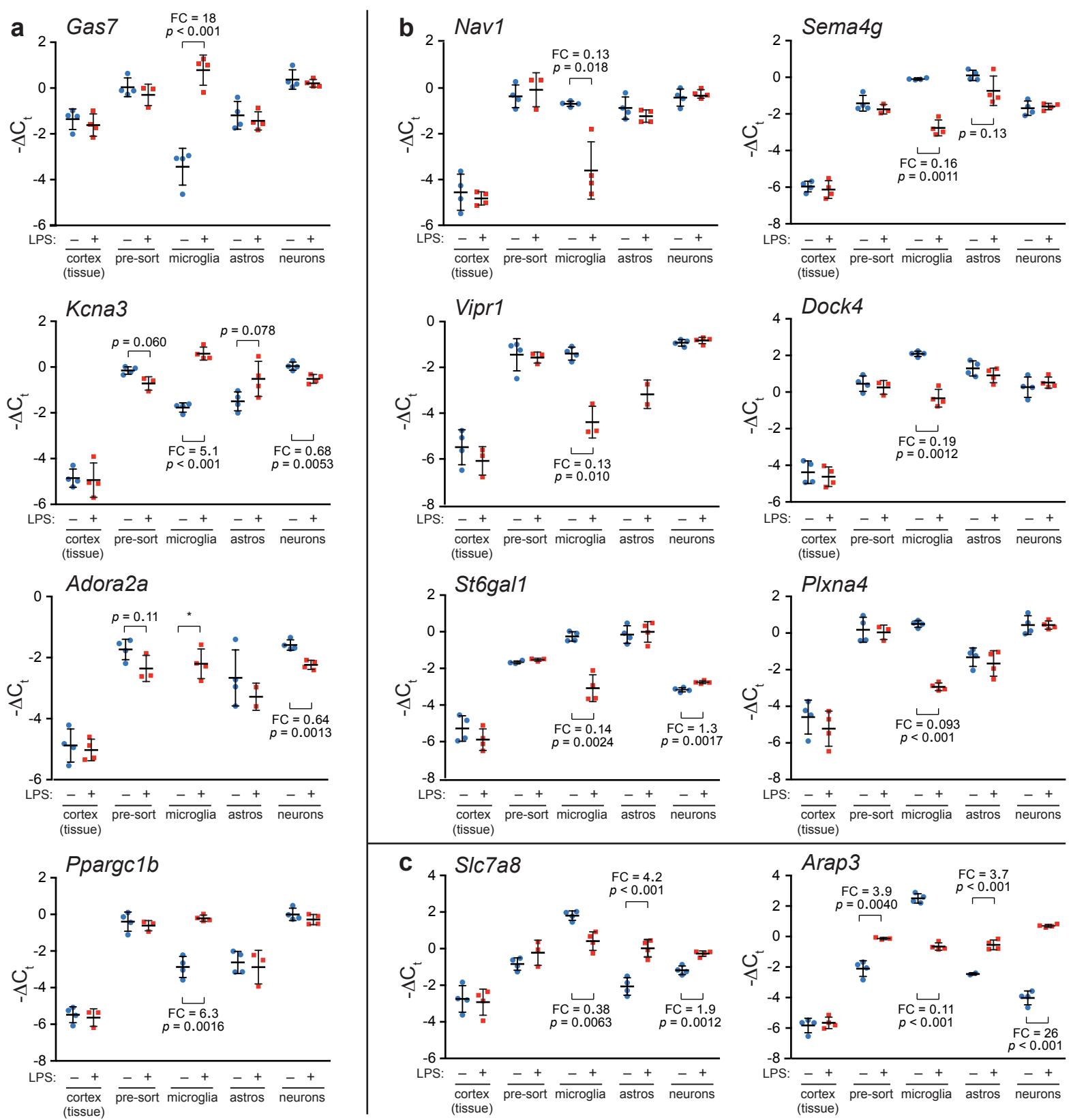
### Supplementary Figure 3 | Comparison of expression profiles for astrocytic response to endotoxemia.

This genome-wide "4-way" plot compares LPS-induced changes in astrocytic gene expression in this study (GSE75246) with those reported previously (GSE35338, Zamanian et al.). Each point represents a gene with  $\geq 4$ -fold change (adjusted  $p \leq 0.05$ ) in either study, with the fold-change between astrocytes from LPS-injected vs. control mice in our study plotted on the x-axis and that from GSE35338 on the y-axis. Blue points meet the cutoffs in both studies, red points only in our study, and green points only in GSE35338. The gray color indicates the distribution of fold-change values for all genes. (Genes for which no microarray data were available in GSE35338 are not plotted.) Possible reasons for the increased detection of LPS-induced genes in our study include the sensitivity of RNA sequencing compared to microarrays, the dose and strain of LPS used, and different methods for dissociating, labeling, and purifying astrocytes from intact mouse brain. See Supplementary Data 2 for interactive report of LPS-induced genes in our study.

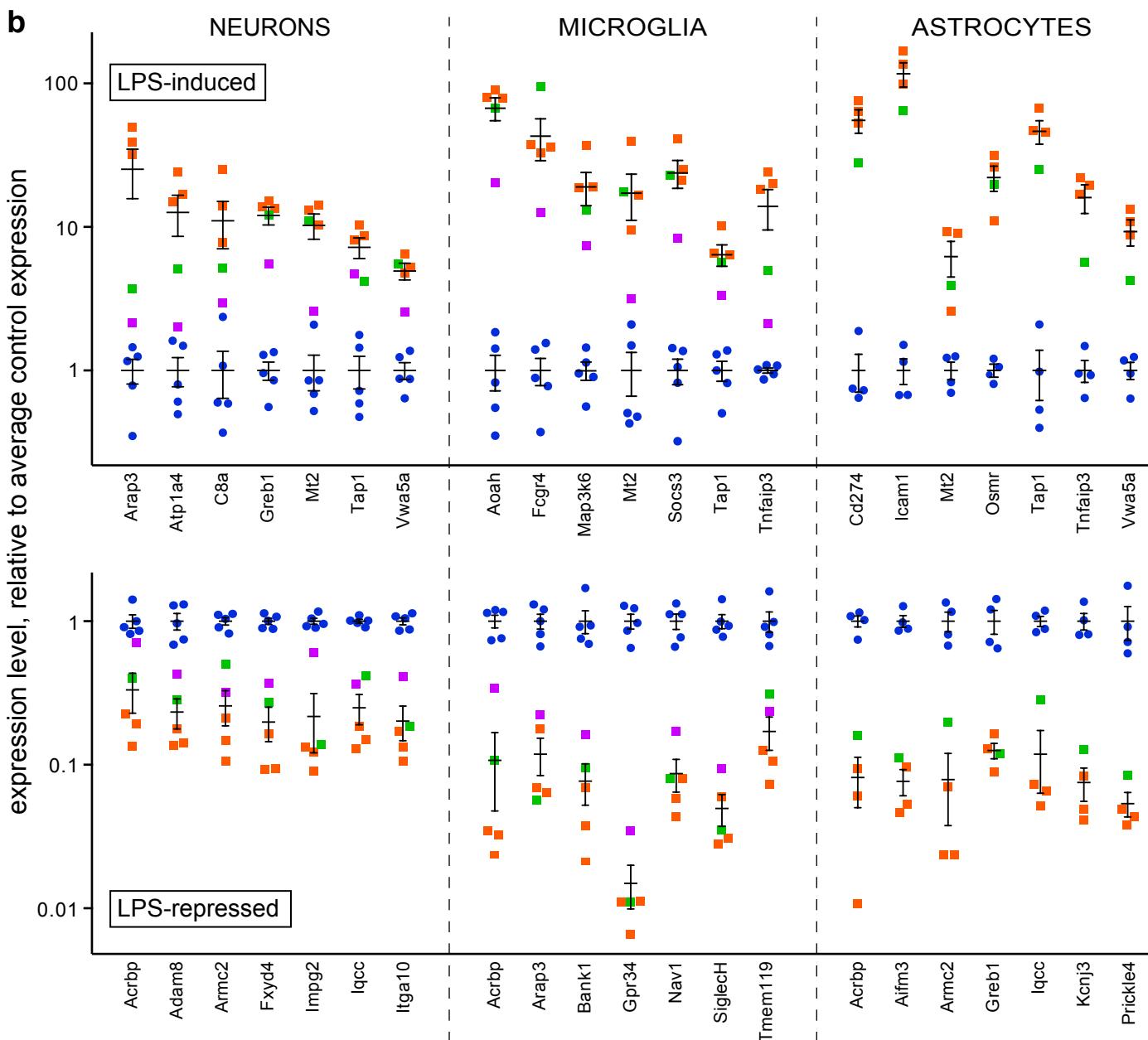
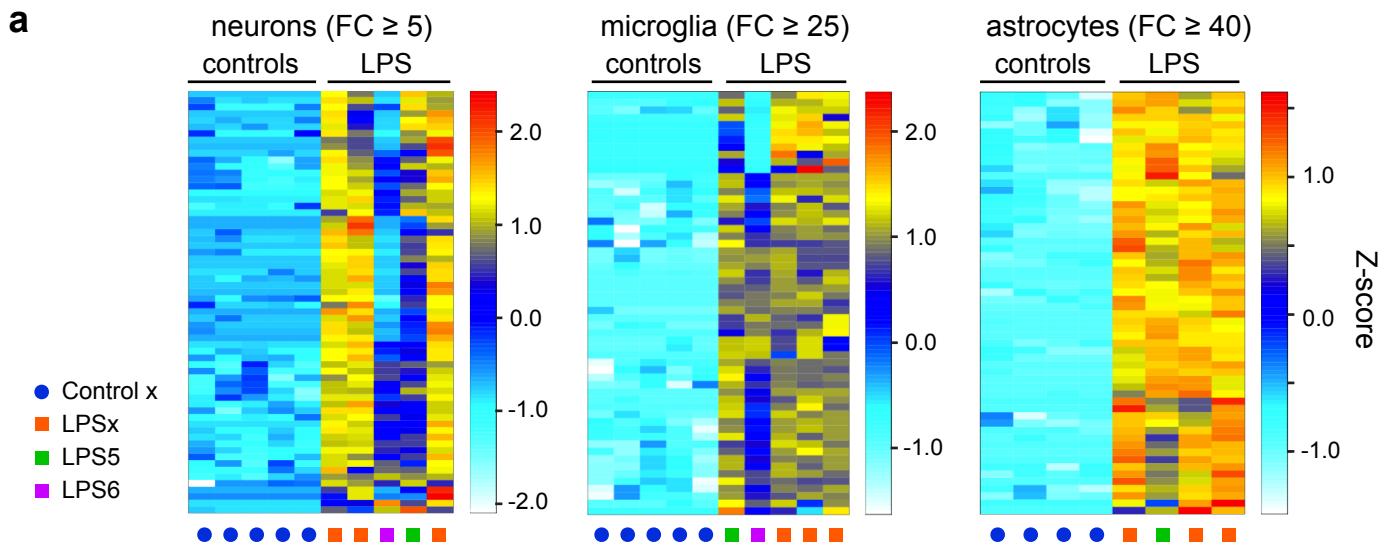


#### Supplementary Figure 4 | Comparison of expression profiles for microglial response to endotoxemia.

This genome-wide "4-way" plot compares LPS-induced changes in microglial gene expression in this study (GSE75246) with those reported previously (GSE43366, Chiu et al.). Each point represents a gene with  $\geq 4$ -fold change (adjusted  $p \leq 0.05$ ) in either study, with the fold-change between microglia from LPS-injected vs. control mice in our study plotted on the x-axis and that from GSE43366 on the y-axis. Blue points meet the cutoffs in both studies, red points only in our study, and green points only in GSE43366. The gray color indicates the distribution of fold-change values for all genes. Possible reasons for the increased detection of LPS-induced genes in our study measuring the transcriptional response at an earlier time point post-injection, the dose and strain of LPS used, the larger sample size in our study, differences between brain and spinal cord, and the different methods for dissociating and purifying microglia from intact CNS tissue. See Supplementary Data 2 for interactive report of LPS-induced genes in our study.



**Supplementary Figure 5 | Our method allows detection of differentially expressed genes in purified cell types that cannot be detected using whole tissue RNA samples.** 24 h after peripheral injections with LPS or saline ( $n=4/\text{group}$ ), perfused mouse brains were processed using one cortical hemisphere for whole tissue RNA and the other for dissociating and purifying individual cell types, followed by RT-qPCR tests for LPS-induced changes. Genes such as *Gas7*, *Kcna3*, *Adora2a*, and *Ppargc1b* were upregulated (a) in microglia from LPS-injected mice, while genes such as *Nav1*, *Vipr1*, *St6gal1*, *Sema4g*, *Dock4*, and *Plxna4* were downregulated (b). These changes were not observed in RNA from whole tissue or from dissociated, DAPI<sup>+</sup> cells ("pre-sort") due to constitutive expression in other CNS cell types. *Slc7a8* and *Arap3* were LPS-induced in astrocytes and neurons, but these changes were not observed in whole tissue RNA due to offsetting decreases in microglial expression (c). Bars represent mean  $\pm$  s.d., with fold change (FC) and unadjusted p-values (two-tailed t-test, unequal variance, unadjusted) shown.  $C_t$  values were normalized against *Gapdh* and *Hprt*. Note: For sorted cells, the fixation step affects RNA recovery for each gene differently, but consistently across samples for a given gene. Thus, although  $-\Delta C_t$  values from whole brain (unfixed) are frequently different than pre-sort (fixed),  $\Delta\Delta C_t$  values (fold changes) are preserved when comparing across treatments or disease states. Data points and calculations for fold change and p-value are absent when the target gene was undetected. One LPS "pre-sort" sample failed to amplify in all assays.



**Supplementary Figure 6 | Overall LPS response is consistent between cell types of a single animal.**

(a) For each cell type, a short list of the top ~60 LPS-induced genes was selected at  $p \leq 0.05$  and the indicated fold-change (FC). Heat maps show the Z-score-normalized  $\log_2(n\text{RPKMs})$  for these genes (rows) within the corresponding cell types, with each column representing that cell type from a single animal injected with either saline (blue dots) or LPS (colored squares). The LPS6 animal (magenta square) consistently showed a very weak transcriptional response in both neurons and microglia. (The astrocytes from this animal were excluded from the study due to neuronal contamination.) The LPS5 animal (green square) was also somewhat attenuated relative to the remaining three LPS-injected animals (orange squares). (b) Examples of LPS-induced (top chart) and LPS-repressed (bottom chart) gene expression from RNA-Seq data in each cell type demonstrate the overall trend that the transcriptional response in the LPS6 animal was severely attenuated in multiple cell types, and the response in the LPS5 animal was, overall, modestly attenuated in each cell type. Bars represent mean  $\pm$  s.e.m. (Prism).

Gene	Event name	Event Type	Assay	ForwardOligo	ReverseOligo	DetectionOligo
Adarb1	110532_3_2/2_A5SS	A5SS	const	TCACCATGTCCTCGAGTGCAC	ACGAAATGCTGAGCAGGGAA	ACGGTGAACGTGGGGCATCCAGGGG
Adarb1	110532_3_2/2_A5SS	A5SS	incl	CCAGACTCTCACCAAGCTGAC	TCCCCCTGCCAGACTCTATT	CCGAATCGCAAAGCAAGGGGACAGCTACGGACG
Adarb1	110532_3_2/2_A5SS	A5SS	skip	CGTCTAGAGAACAGCAG	GACTGGAGATCGGTGACACAG	CCGAATCGCAAAGCAAGGGGACAGCTACGGACG
Agap1	347722_1_2/2_SE	SE	const	GAGGACCACTGTAGAGGTGC	GACTGGAGATCGGTGACACAG	AAGAGGCCGCCAGGCCAGCAGCTCG
Agap1	347722_1_2/2_SE	SE	incl	CGAGTTACACATCTCACAAA	AAGGAGACCAGGCTGTAGC	TGTCAGTAGTGCTGGGCTACATTCA
Agap1	347722_1_2/2_SE	SE	skip	CAAATTAGCACACGGGGCTG	GGTGGGTGAGCTTGGGG	TCGGTGTGTTCCAGCCAAGTATCTCACAGCA
Akt3	23797_3_2/2_SE	SE	const	AGAGTGGACGGAAAGCTATCC	TGTTGAGTGGGCTACATTCA	CGTAGCCGACGGATTGAGAGGAAGGAGG
Akt3	23797_3_2/2_SE	SE	incl	AAATCACCTTGGACTTAAAT	ACAATTACATCTCTCTCTCT	CCAGCGTAGCCGACGATTGAGAGCA
Akt3	23797_3_2/2_SE	SE	skip	GGAAAAGAAGAAGTGGACCGGA	TGGGTCACAAATTCTCTCTCC	CCAGCGTAGCCGACGATTGAGAGCA
Bag6	224727_6_2/2_SE	SE	const	GACATTAGAGCCAGCGGAA	TTTCGTCCTCTGGCAGGCT	ACCTAGCCGCCCTGAGTGTGCTACCT
Bag6	224727_6_2/2_SE	SE	incl	GTGATGCTCACCTCTAGGGC	GCCTCTGGAGAGTCCAGCAGT	CCGAAGACGAAAGACAAATGCAAGGGTGAAGGGCCC
Bag6	224727_6_2/2_SE	SE	skip	GACGAAAGCTCCGCTGTAT	CCCGATGGCATTGGGAAG	ACTCAGGAAAGATCCAACTACAGCCCCAGCG
Bap1	104416_1_2/2_SE	SE	const	AGCTCGAAGGGTCATCATGG	TGCCATCAGGTGAAGCGAA	AGCGAATCGGCTTGACTCGAGGGAGGCC
Bap1	104416_1_2/2_SE	SE	incl	GGTAGACCGAACACAGCTCC	GTGAGCTGAATCATGCTCC	TGGAGGCCCTGAGCAGCTGAGAATCCAGC
Bap1	104416_1_2/2_SE	SE	skip	TGAGACAGCTCATGTACTCTG	CACCTCTCTGCACCATCTG	AGGTGACCCACAAACAGTCTGGAGGCCCTGC
Brpf1	78783_1_2/2_A5SS	A5SS	const	CAAGCTCTCTAGACCGGG	CAGGTAACCTGGTCCCG	ACCGGGCGAAAGTGGCTATAACGCCA
Brpf1	78783_1_2/2_A5SS	A5SS	incl	AGGGCAAGAGTCACCAAAAGCT	CTGCACTCTGGTCCCG	CGGAAAGCGGAAGGTGCTTGGGAATTGGCG
Brpf1	78783_1_2/2_A5SS	A5SS	skip	GGGAAAGCGGAAGATGTGAC	GGCTTAGTGGCAGGCAAAAT	TGGGGTGTGACTTGTGAGCAGGACCTTCTGCCA
Caprin1	53872_1_2/2_SE	SE	const	ACTGTGATGATTACCAAGGAACGA	TCCAAATATTGTGACTCTCTGGT	AGGGGAAAGGCTCAATCAAGACAGCTGGATGCCG
Caprin1	53872_1_2/2_SE	SE	incl	AGCATTGAGAACAGAATGTTACAGA	TCCAAATATTGTGACTCTCTGGT	AGGGGAAAGGCTCAATCAAGACAGCTGGATGCCG
Caprin1	53872_1_2/2_SE	SE	skip	GCTCTCTAGCATCCGGCAA	AGTTTACCTTTTCTCTCCAGGT	CCGGCCGCCAGACGAGGATGAAG
Cspp1	211660_6_2/2_SE	SE	const	GCGGAAATGAGATCTACAAAC	CCTTGGGCTCTGGAGAGG	CCTTGGGGAAGGGGAGGGTGTCT
Cspp1	211660_6_2/2_SE	SE	incl	GAGGATCTACACCCCTGGG	TCTGCTGTGATCCTATCAA	AGGGGGAGGTGTTGCTCTCTAGAGACGC
Cspp1	211660_6_2/2_SE	SE	skip	GAGGATCTACACCCCTGGG	TGCAAGGACTTACAGATTTCT	AGGGGGAGGTGTTGCTCTCTAGAGACGC
Dmwd	13401_1_2/2_SE	SE	const	ATTGACCCAGCTAGGTG	TTCCTGCACAGGAGGCTC	TGTCCTCCGATCATGAGTGCCTC
Dmwd	13401_1_2/2_SE	SE	incl	CAAGAGGGCCTCATCTGCAC	AACTTGCTGCTCTGCCT	AGGCCTACAGCAGGGAGGCC
Dmwd	13401_1_2/2_SE	SE	skip	CAAGCGGGCATCTCTC	GGGACATCCACATTCACCC	CCAAACAGGAGCAGCTCCCTGGGACT
Dock7	67299_2_2/2_SE	SE	const	TGGGGATGATGCTGCAACT	AAACGGTCTCTCTCTCTCT	AGCTTCCGGCTGCCCCCTACTGTG
Dock7	67299_2_2/2_SE	SE	incl	GAAGTCGAGATCAGTACTGGAG	GGTCAAGTTGCAAGCATCATCC	AGGGGGTCTGGAGGACAGTGG
Dock7	67299_2_2/2_SE	SE	skip	TGATGCGACCAAGAGGCAA	GCTGGTCAAGTGGCAAGCAT	AGGGGGTCTGGAGGACGACAGTGG
Dzip11	72507_1_2/2_SE	SE	const	ATCTCAGCACACCTGGCCAC	GCATGTCCTGTTGGATGT	ACAAGACCTTCTGAACTTCTCCGGGCC
Dzip11	72507_1_2/2_SE	SE	incl	ATCAGGCGATGGCGAGATG	ACTTGACCTTGCGCGAAC	AGCAGGAAAGCCACTGGGGAGGTGCT
Dzip11	72507_1_2/2_SE	SE	skip	GACAAAGCTCTCATGATGCCA	TCTCTAATTCGACATCTGCCA	CCTTCTCCGGGGGACATCCACGAGACA
Epb4.9	13829_3_2/2_SE	SE	const	CAGGTGTTGGGCTACAGGA	GTCAGGTGCTCAATGTC	TCTGGGCTGCATCCCCAAGGACAAGGCCA
Epb4.9	13829_3_2/2_SE	SE	incl	AGCTCTCCTCAGAGCTTCCAG	CTTGTAGCCACACCTGGT	CCCGCTCTCCCTCCAGCATGTG
Epb4.9	13829_3_2/2_SE	SE	skip	CTGAGAAAGGCCAAAGATGGA	ATGTCAGGATGGCTGTGTC	TGTTGGGCTACAAGGATGTGCTGCCATCCCCA
Esy12	52635_5_2/2_SE	SE	const	ATCTCAGATGTCCTCGTC	CATCAGCTCCATGACTGTT	CACCGGTGTTGCAACACTGCTGGTCA
Esy12	52635_5_2/2_SE	SE	incl	GGGCCAGGACCTGGT	AGCAGTTGACTGAGGGAT	TGAGCAGGACATCAGTGTCTGGG
Esy12	52635_5_2/2_SE	SE	skip	GAGGTTGAGGCTCTCCATCT	GCGACGAGACATCTGAGAT	ACGGCCCTCTGTCCTCCAAAGGAGAAAATGCC
Fam172a	68675_2_2/2_SE	SE	const	GTGCAAGTGGCTACTGTCG	CAGATCTGGGCAACT	CCGGGATCTGGAGAGAAAGTCCGAGCAGGGC
Fam172a	68675_2_2/2_SE	SE	incl	CAGAGATCTGGAAGGCCAG	AGACATCGATCTTCAGAACAGT	CCTCTCGCACCTACCTCTCAGATTGAGCT
Fam172a	68675_2_2/2_SE	SE	skip	CAGAGATCTGGAAGGCCAG	CAGCAACATCTGAGCTGTC	CCTCTCGCACCTACCTCTCAGATTGAGCT
Fgd6	13998_1_2/2_SE	SE	const	GGGTTCTCATCTCTCAGC	GGCATACATTCTATGGAGCTG	CTGGCCAGAGAAAGAGTACTGGCTGGAGGCC
Fgd6	13998_1_2/2_SE	SE	incl	AGTATGCAAGAAAAGATCACATT	CCGAGGGGCTAATCTTC	TGCCCCAGAGGAGCTGGCAACT
Fgd6	13998_1_2/2_SE	SE	skip	GGAGGATGTTGGCAGAACG	TCTGAGCTTCTGGGAGA	ACCTGAAAGGCAACTGGCAAGTGGCAG
Gphn	268566_8_2/2_SE	SE	const	TGTTTTCATCTGGGAACTC	ACCTGTTGTAACCCGATCA	AGCTTGTGAGCAGGAACTCAGACTTAATGCCAG
Gphn	268566_8_2/2_SE	SE	incl	AGATGTGTTGTAAGAAACTTGA	AAATGTCATGGGGATGGTGG	AGTACATTGTCATGGGCAAGCTGGCAGGCC
Gphn	268566_8_2/2_SE	SE	skip	TCATGTTGGTGTGAAAGACTTA	TCTTCAGGATTGAGCGGGA	AGTACAAATTCTGGGCAAGCTGGCAGGCC
Htt	15194_13_2/2_SE	SE	E47-E48	ATTCTGACTGTCGGAAGGT	GTCACCCGGTTTCAAAATC	CAACCGTATCCCTGCTGAAGATATGAATGA
Htt	15194_13_2/2_SE	SE	E47-E52	ACCTCTAGATGAGCTGCACCT	CTGAGGATGTTGGCCACCAT	AGTCATGTCACCTGGGCTGGAGATGGTGG
Htt	15194_13_2/2_SE	SE	E52-E53	GAATCCCTGCGATCTGAGT	TCAGGAAACACTGTCGAAA	TGCTGTAAGAACATTTGTTACAGTCTG
Inpp5e	64436_1_2/2_SE	SE	const	GGACTTCAACTCCGCTGA	CAGCACATCCACCTCAGGTT	TGGTGGAGCAGTGGCTGTGAGGCC
Inpp5e	64436_1_2/2_SE	SE	incl	CCCCGAAATGGGCCAGACA	CCCGAACACAGAACGACCTCA	CCGCTAGTGGCAGGGGACTACCCCGT
Inpp5e	64436_1_2/2_SE	SE	skip	skip	CCTCCACACCTGTC	TCTGTTTGGGGACTTCACTTCCGCTGAGTGG
Kdm5a	214899_1_2/2_SE	SE	incl	TCCAATCTGCTGAGCCTT	GGAAATTTCTCTGCTCCCCC	TGAGGTTCTCAGCACTCCAGCTTCCCAGA
Kdm5a	214899_1_2/2_SE	SE	skip	AGAAGAAATGGCTAAAGTGGG	AGAGCTGACATAAGGCTGAC	AGGCCCTGGGATATCTCCAGGAAAGAACGACTGG
Larp4b	217980_3_2/2_SE	SE	const	ACCTCCAGTGTACAGCTCCC	GTGTTGTTGCTGAGCTGTC	AGCAGCAGTGGCTGAGCTGATCACCC
Larp4b	217980_3_2/2_SE	SE	incl	TCAGACAGTATCTCTAGAACGA	TGCACTGAAAGTTAAATTGAGGACT	TGCACTCTAGACAGAGAGGGCTGGCTG
Larp4b	217980_3_2/2_SE	SE	skip	CAGTATCTCTAGAACGGAGAAAA	GGGAAGTGGAGCAGTCCCG	CCCAACACCCAAAAGCCTCATACCGAGCTTC
Lsm14a	67070_2_2/2_SE	SE	const	CAGAGATCTCAATAGTGGTACCT	TCTGGTTTACAGATCTGTGTC	CCAGACAACTGAGCTGGGCTTACACAA
Lsm14a	67070_2_2/2_SE	SE	incl	AGCAATGACACAAAGAGCA	CCTCTTGTGCTTCTGGGCT	CAGGGAGCTGAAGTACACAAAGCTGGCAG
Lsm14a	67070_2_2/2_SE	SE	skip	TCCTTTGGAACAGAACATCA	TTGTGACTTCAGCTGGGC	CCAGAGACATGCACTGGGCTTACACAA
Poldip3	73826_1_2/2_SE	SE	const	GGACTGAGCTGCTTCAAGCT	GGAGCTTCAAGGCCCCCTCTC	CCATGTGCAAGGCCCCCTCTC
Poldip3	73826_1_2/2_SE	SE	incl	GTCATAACCCAGGGCTAAC	CATGTGTTGAAACAGGCTG	TGCTGTCCTCTCCAAAGGAGATGAAGTTGAGGCC
Poldip3	73826_1_2/2_SE	SE	skip	CTGCTGCGTCAACAGCC	TCCAGGTACATAAGTCTGGA	CGCCCATGGGACTGTAACCCAGCTCTGAAGCTC
Polg	18975_1_2/2_SE	SE	const	GACTAGATGACCTCTCTGG	CAGGTAGCTGGGCTACGCT	CCCAAGCGCTTCCAGCCTGCAATGGCG
Polg	18975_1_2/2_SE	SE	incl	GGGAGCTGGGCCCCCTTGG	TAACCGGTTGTTGGGCTT	CCAGCGAGGAGGGAGGAGCTTCAAGGAGCT
Polg	18975_1_2/2_SE	SE	skip	GGAGAGAGATCTGGGCCC	CTCAGCTGCTGTAACCGGGT	CCAGCGAGGAGGGAGGAGCTTCAAGGAGCT
Sirpa	19261_2_2/2_S2E	S2E	const	GGGGATTGACCTGGCTTCTGAA	GGCCCTACTCTCTGTTACCA	TGCACTTGTGACCTCTGTTGCGGTGGGACCC
Sirpa	19261_2_2/2_S2E	S2E	incl	AACCATACCTGGCTGGGAA	TCCAGTTGGGTGGTAGCTATT	TGCACTCTAGGTGATCAAGGGAGCATGCAAAACCT
Sirpa	19261_2_2/2_S2E	S2E	skip	TGTTACTGATAATAGCTACCCACA	AGCAGGACTACAGGAAACAG	TGGAATGTTCTCATGCTGTTGGGGCT
Sltm	66660_7_2/2_SE	SE	const	GTCGAAAGGATTGTCCTGGGA	GTGGCGTTTAAAGAATCTCT	AGAGAGGAGCTGAGAAGGAGCAGCAGCT
Sltm	66660_7_2/2_SE	SE	incl	GAACGCTTGGAAAGGAGAC	TCTCTCTCCGGGAACTCT	CGTATGAAAGGAGCTGAGGAGCTGA
Sltm	66660_7_2/2_SE	SE	skip	GGAAATTCAAGGAGGCCGG	TGGGGCTTAAAGAATCTCT	AGAGAGGAGCTGAGAAGGAGCAGCAGCT
Tcf12	21406_1_2/2_SE	SE	const	GGACTCTGAGAGTGGCTG	AGCTCTTGTGCTGGCTCC	TGAATCCCGAGCAGCGCATGGCCG
Tcf12	21406_1_2/2_SE	SE	incl	GGTATGGATGAGCTGGAGG	AGCTGTTACTCTGGCTTGTG	ACATCTGGGAAACAGTGGTCAACCAAGCCCTCC
Tcf12	21406_1_2/2_SE	SE	skip	GGTATGGCTGGAGGAA	GCTGCTGTTGAAACAGGCTAGATG	CATCTGGGAAACAGTGGTCAACCAAGCCCTCC
Usp16	74112_2_2/2_SE	SE	const	CTTGTGTCAGCGTGTAGC	CCGTGTCCTGGAGCCTCTC	AGTGCAGCATGGGAGACCTGGCCCT
Usp16	74112_2_2/2_SE	SE	incl	GAAACGACCAAGGGGAGAA	CCTTTCTAAGGTGTCGACACA	TGCTTCAGACACGGTGGCCCTAGAGTCTG
Usp16	74112_2_2/2_SE	SE	skip	CTCAGAGTCTGCAAGGGCTG	CGGATCTGGTGTGCTGTAG	TGGCAGAGATTCTCAGGAGCAGCATGGCTGAAGCA

SE = skipped exon

A5SS = alternative 5' splice site

S2E = skipping 2 exons

**Supplementary Table 1 | List of oligonucleotides used to detect alternatively processed RNA species induced in brain by peripheral endotoxemia (see Fig. 7 and Supplementary Data 4).**