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Supplemental information

Multi-transcriptomics reveals brain cellular responses to peripheral infection in Alzheimer's disease model mice

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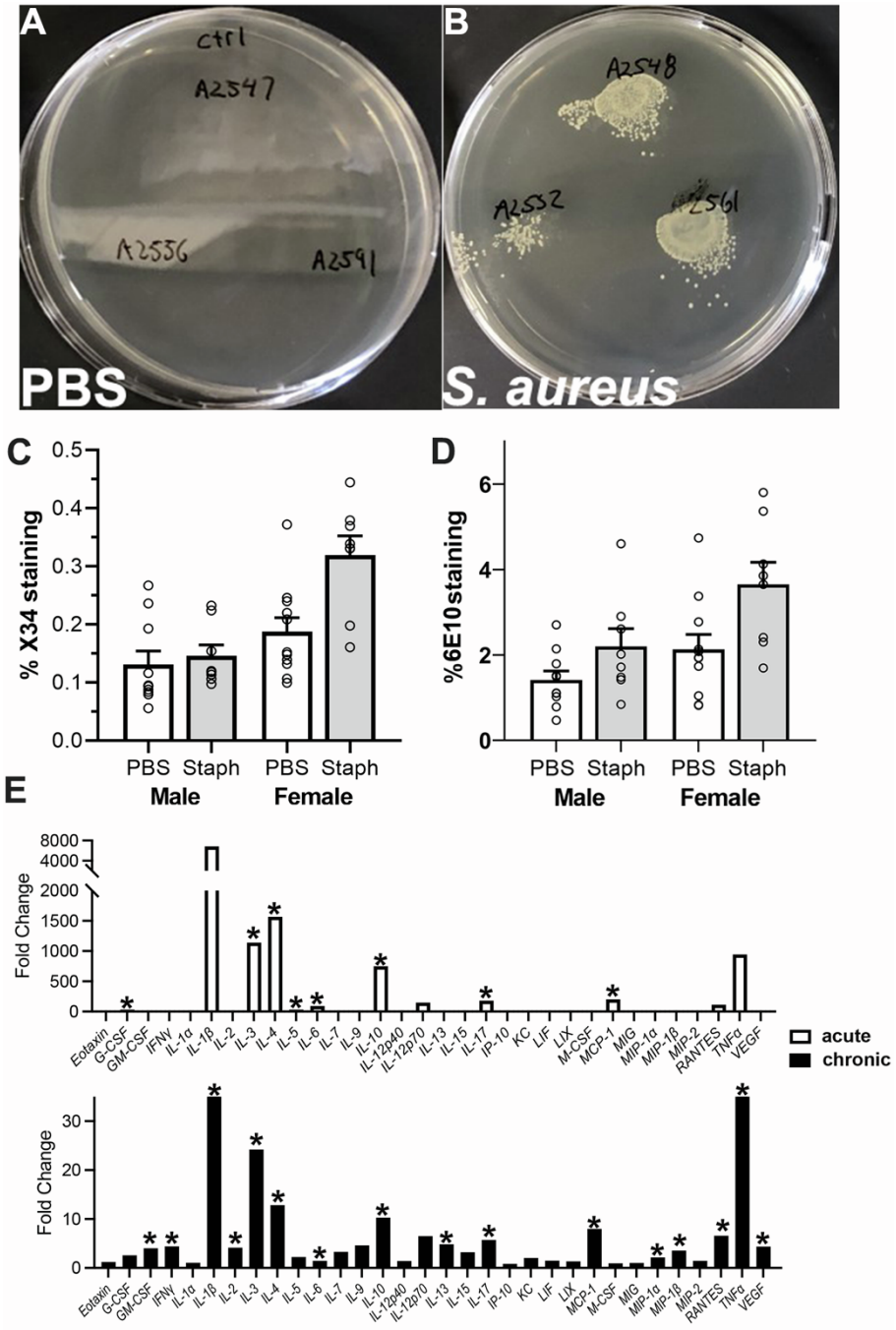

Figure S1. Nasal inoculation of APP/PS mice with Staph. (Supplemental to main Figure 1). Nasal lavage samples grown overnight on Trypticase Soy Agar plates showed no bacterial growth in animals treated with PBS **(A)** and the presence of bacterial colonies from animals treated with *S. aureus* **(B)**. **(C)** Total % X34 staining. Two-way ANOVA of the X34 data shows a significant effect of sex ($F(1, 33) = 20.32$, $p<0.001$) and treatment ($F(1, 33) = 5.195$, $p=0.0292$) and a significant interaction between the two variables ($F(1, 33) = 5.195$, $p=0.0292$). **(D)** Total % of 6E10 staining. Two-way ANOVA of the 6E10 data shows a significant effect of sex ($F(1, 33) = 8.302$, $p=0.0069$) and treatment ($F(1, 33) = 9.434$, $p=0.0042$). There was no significant interaction between the two variables ($F(1, 33) = 0.9672$, $p=0.3325$). **(E-F)** Bar plots representing the plasma cytokine fold change in terms of Staph versus PBS with acute (**E,** n=4 for both group) or chronic (**F**) treatment (n=12 for both group). Statistical analysis was performed with unpaired t test. * $p<0.05$.

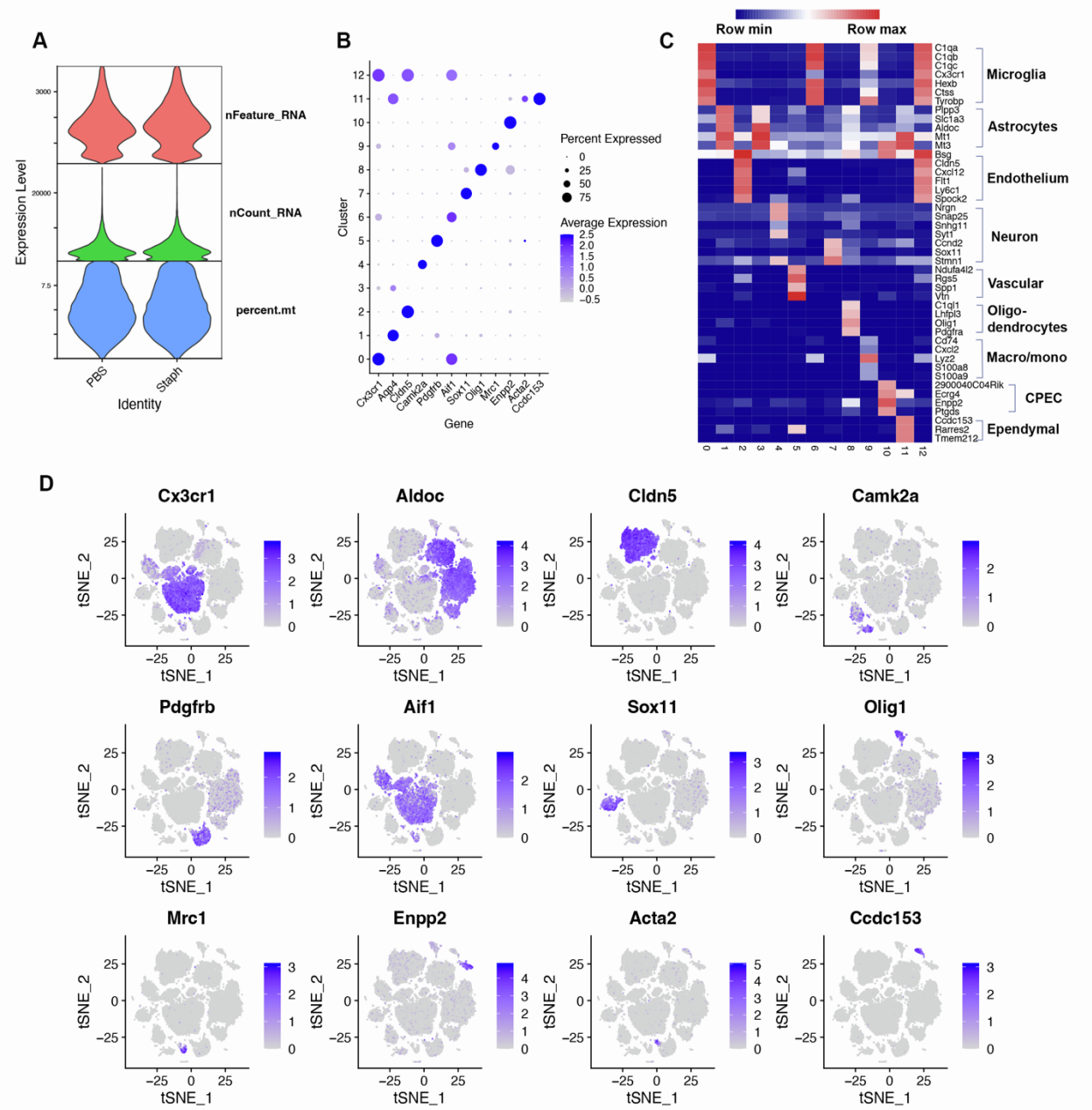

Figure S2. scRNA-seq distinguishes major brain-cell types in APP/PS1 brains (supplemental to main Figure 3). **(A)** Stacked violin plots showing the distribution of number of genes (nFeature_RNA, top), the number of reads (nCount_RNA, middle), and percentage of mitochondrial genes (percent.mt, bottom) in the two groups (left-PBS, right-Staph) of the single cell dataset after filtering. **(B)** Dot plots showing the average expression and percentage of cells expressing the markers genes in each cluster. **(C)** Heatmap showing top differentially expressed genes for each cluster with the cell types marked on side. **(D)** t-SNE plots showing expressions of each cell type markers.

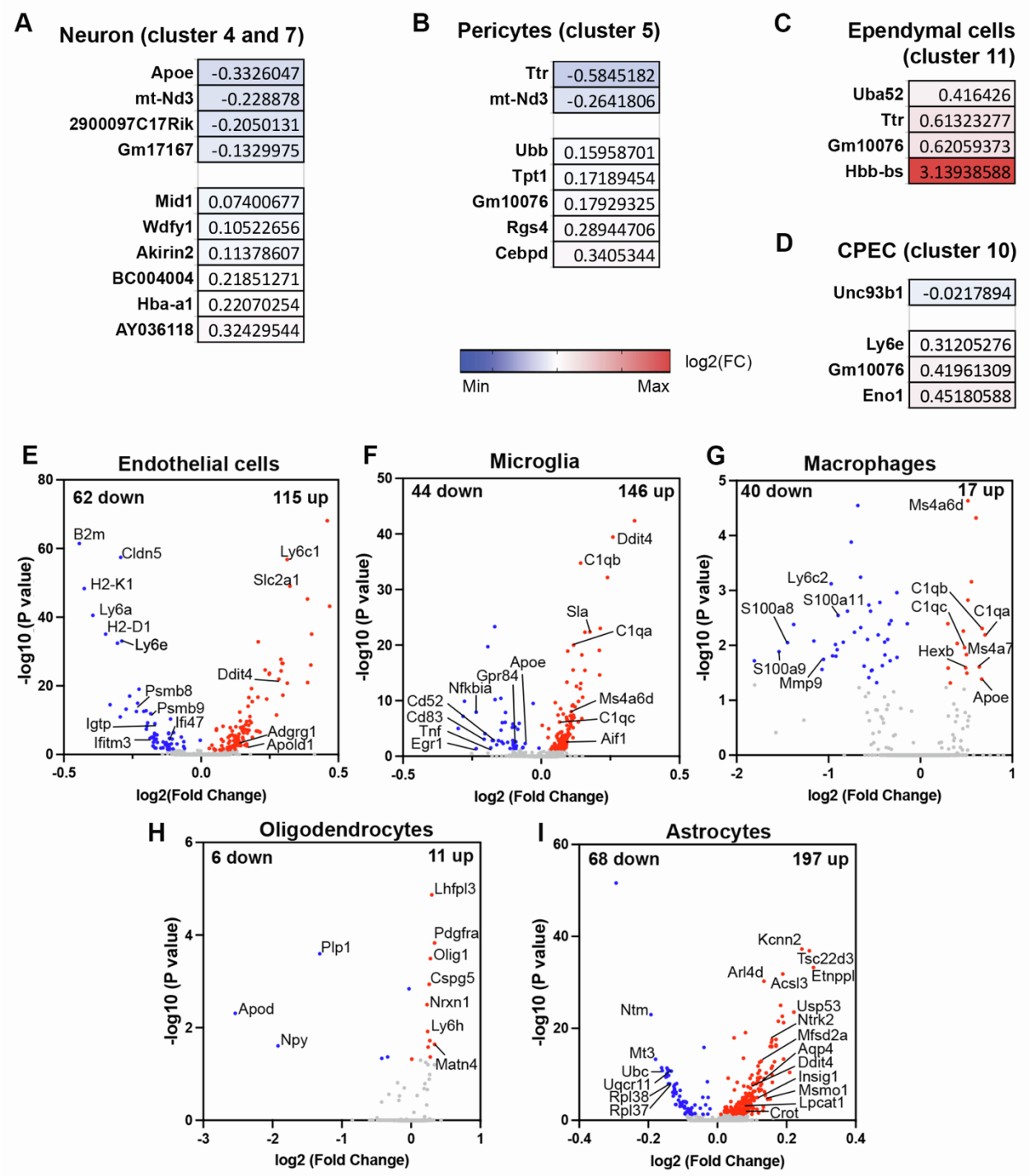

Figure S3. scRNA-seq reveals brain transcriptional changes following Staph treatment. (Supplemental to main Figure 4). Heat maps showing fold changes of DEGs in neuron (**A**, n=3555 cells), pericytes (**B**, n=1853 cells), ependymal cells (**C**, n=346 cells), and choroid plexus epithelial cells (CPEC) (**D**, n=476 cells) in response to Staph treatment. Genes shown had adjusted p-value <0.05, ordered by log2(FC), and results are displayed for comparisons of Staph versus PBS treatment. Volcano plots showing significant differentially expressed genes (DEGs) (adjusted P<0.05) in each cell type of Staph versus PBS treated: endothelial cells (**E**), microglia (**F**), macrophages (**G**), astrocytes (**H**), and oligodendrocytes (**I**).

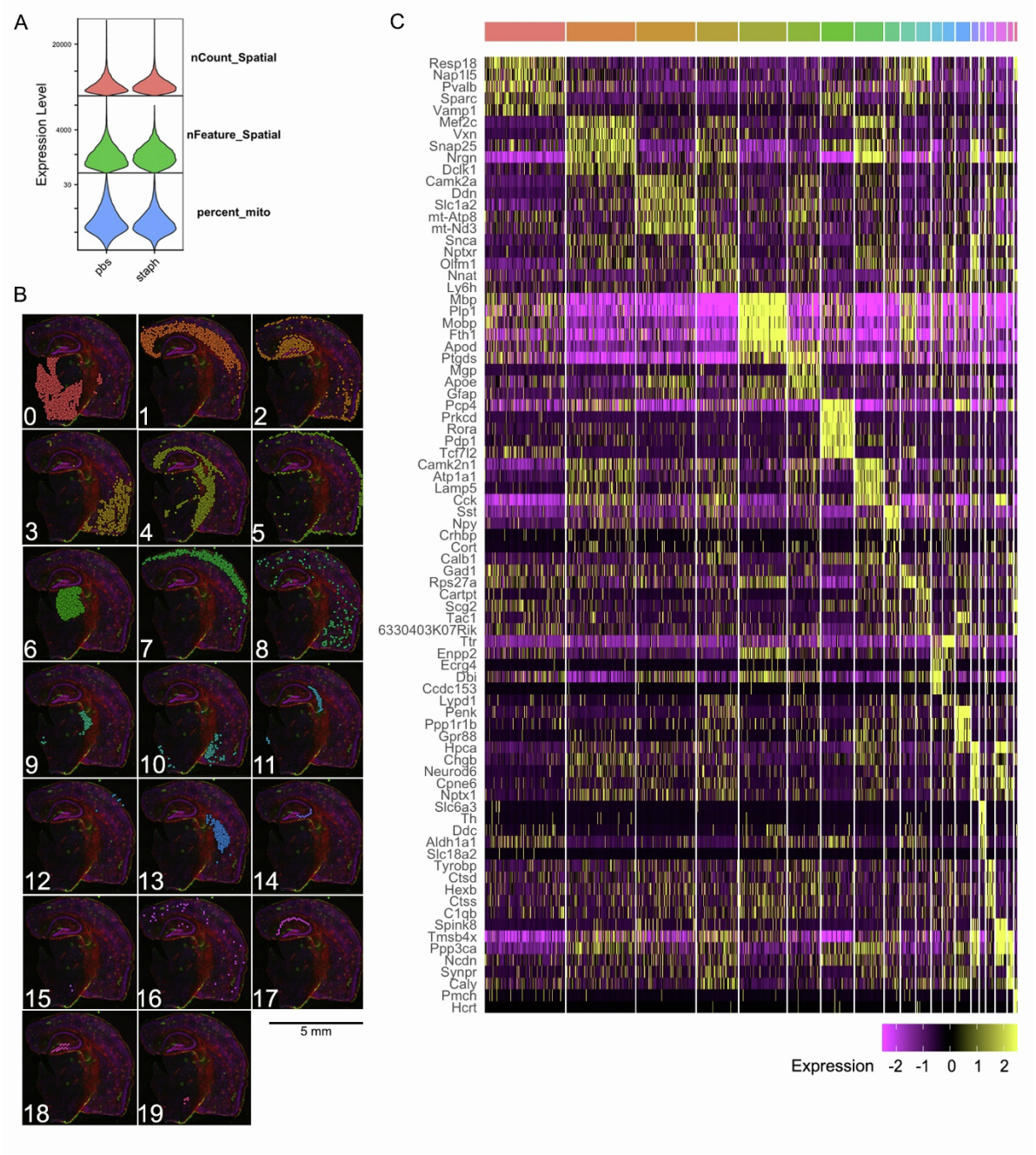

Figure S4. Spatial transcriptomics dataset of APP/PS1 brains. (Supplemental to main Figure 5). **(A)** Spot quality control metrics after filtering of low-quality spots, showing UMI counts per spot (nCoun_Spatial), genes detected per spot (nFeature_Spatial) and percentage of counts from mitochondrial genes (percent_mito), split by PBS and Staph groups. The analysis is raw data detected an average of 2655.8 spots under tissue (55 µm in diameter) for each section, and each spot had 63125.8 mean reads. After preprocessing and filtering of low-quality spots, gene expression analysis was performed with 19,255 genes on 20,800 spots containing 4,215 ± 554 UMI counts and 1,786 ± 574 genes detected per spot. **(B)** Representative spatial plot of each of the 20 clusters identified (0-19) showing tissue location. **(C)** Gene expression of top 3 marker genes of each cluster identity, for all identities (indicated by different colors at the top bar), showing up (yellow) and downregulated (magenta) genes.

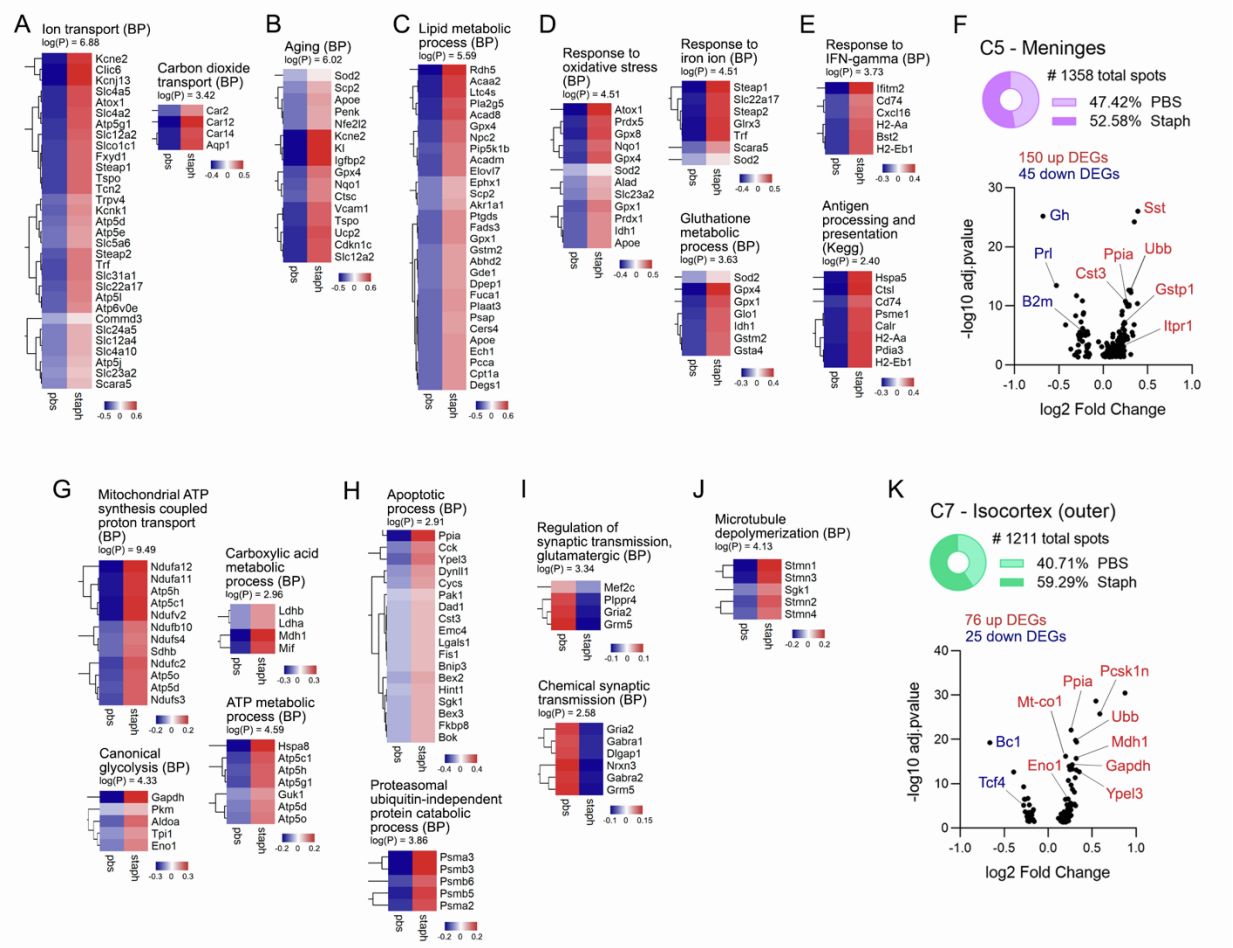

Figure S5. Staph vs. PBS differential expression in Spatial clusters of APP/PS1 brains. (Supplemental to main Figure 6). **(A-E)** Staph vs PBS differential expression in C11 (ventricular surfaces) associated to top GO terms affected. **(F)** On the top, the number of spots in meninges (cluster 5) and distribution (%) of spots between groups. On the bottom, Staph vs PBS differential expression in cluster 5 as a volcano plot showing -log10 adj. p-value and log2 fold change for cluster-specific DEGs (adj. p-value < 0.05), with interest genes labeled in blue (down DEG) or red (up DEG). **(G-J)** Staph vs PBS differential expression in C1 (isocortex, inner layers) associated to top GO terms affected. Heatmaps show SCtransform corrected and gene-level scaled expression values are depicted, with scales indicated. **(K)** On the top, the number of spots in cluster 7 (outer layers of isocortex) and distribution (%) of spots between groups. On the bottom, Staph vs. PBS differential expression in cluster 7 as a volcano plot. Heatmaps show SCtransform corrected and gene-level scaled expression values are depicted, with scales indicated. Volcano plots show -log10 adj. p-value and log2 fold change for cluster-specific DEGs (adj. p-value < 0.05), with interest genes labeled in blue (down DEG) or red (up DEG).

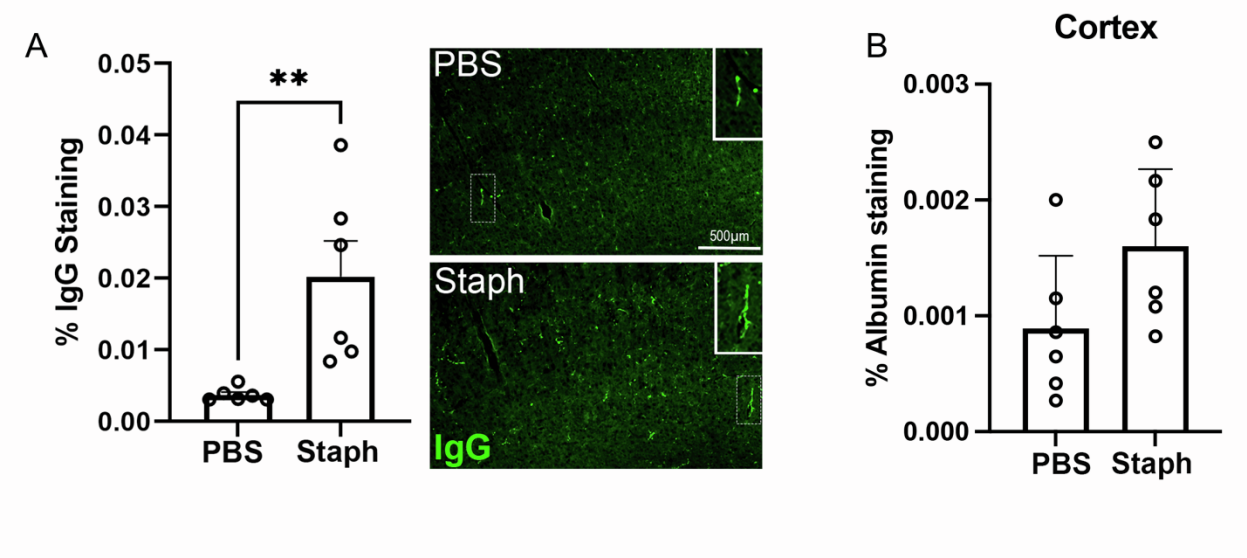

Figure S6. IgG and albumin staining confirmed brain barrier dysregulation. (Supplemental to main Figure 6) **(A)** Left: Percentage of IgG staining (n=6 for both groups) in cortex, hippocampus, and subcortical region in PBS- and Staph-exposed group. ** $p<0.01$; Right: representative images. Green: IgG. **(B)** Percentage of albumin staining (n=6 for both groups) in brain cortex in PBS- and Staph-exposed group.

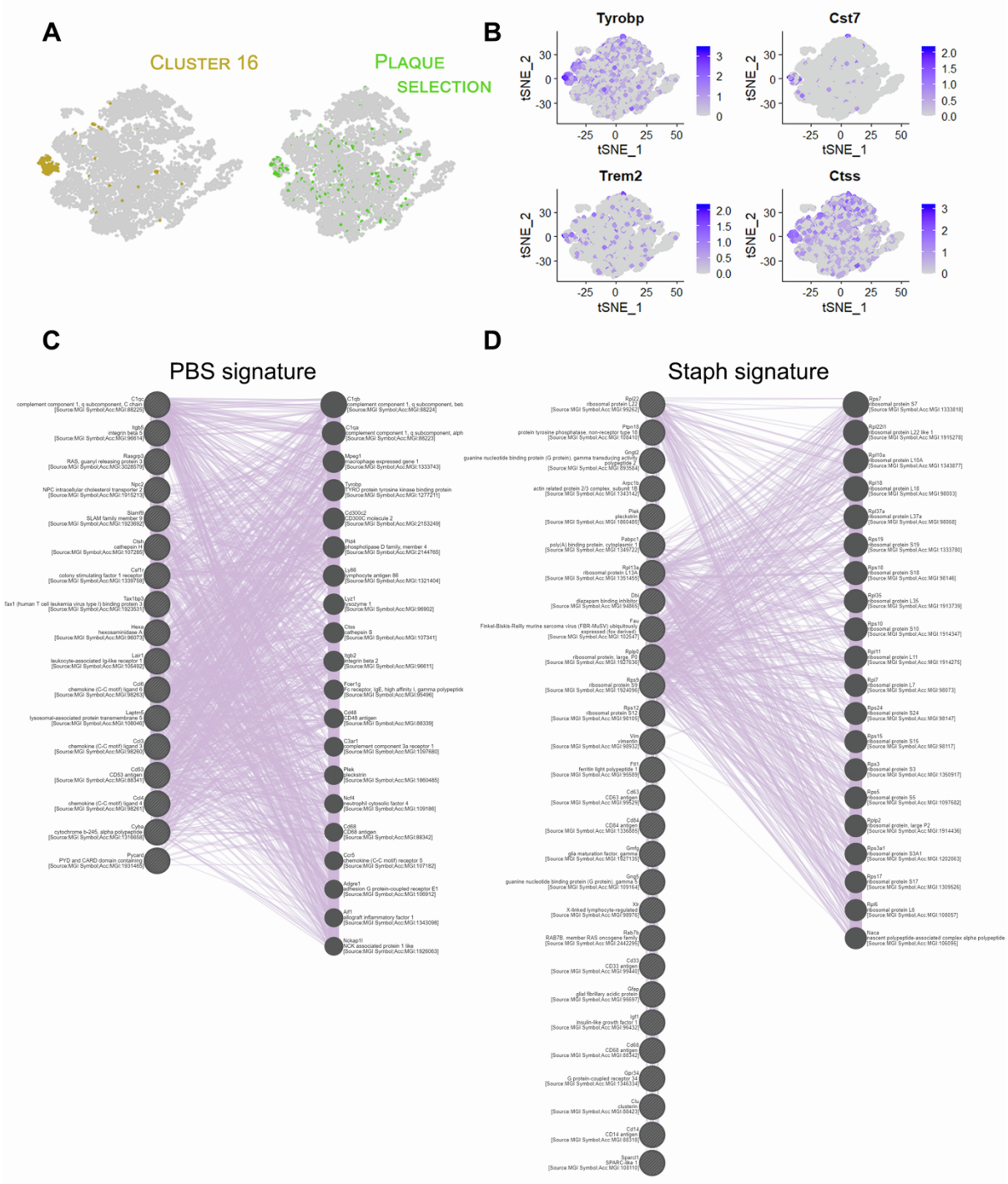

Figure S7. Plaque-associated gene expression in Staph and PBS APP/PS1 mouse brains. (Supplemental to main Figure 7). Subset of plaque-affected areas in APP/PS1 brains spatial dataset, excluding clusters 0, 6, 9, 10, 11, 13, 15, and 9. **(A)** t-SNE of plaque-affected clusters with cluster 16 (Cortex Tyrobp+) highlighted (dark yellow) and with spots manually identified as plaques highlighted (light green). **(B)** t-SNE showing the gene expression levels of Tyrobp, Cst7, Trem2 and Ctss DAM genes, upregulated in both cluster 16 and plaque selection. SCtransform corrected and gene-level scaled expression values are depicted, with scales indicated from grey (low) to blue (high). (**C-D**) Cytoscape analysis of the DEGs in PBS- **(C)** and Staph-exposed **(D)** groups in acute infection experiment.