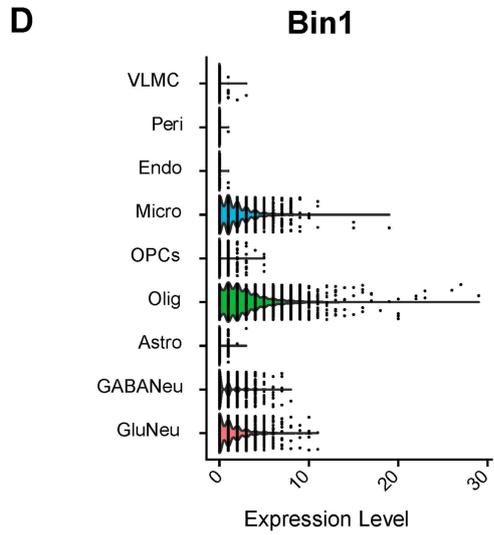
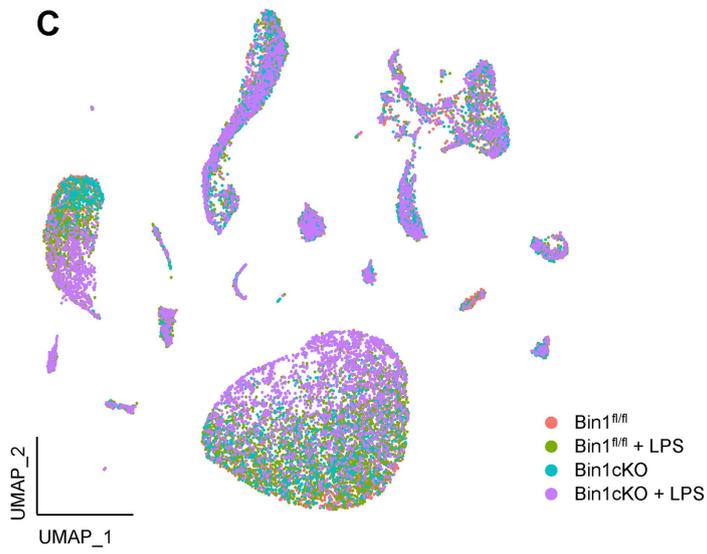
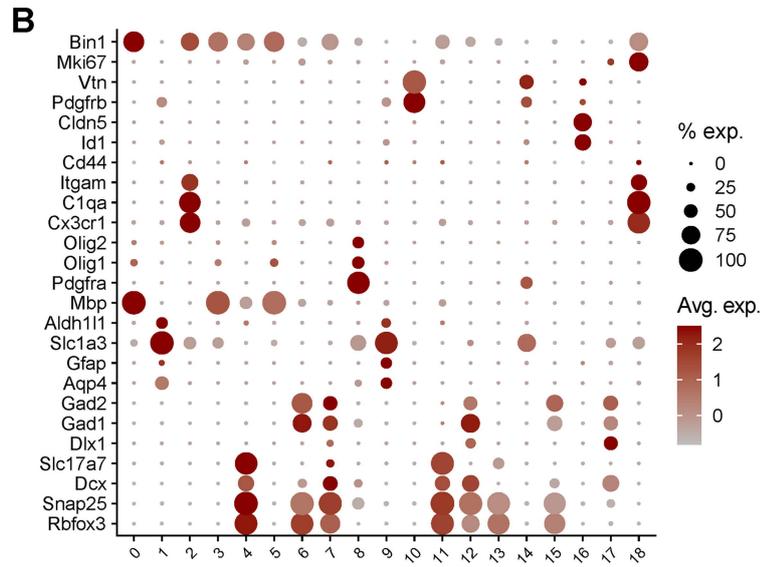
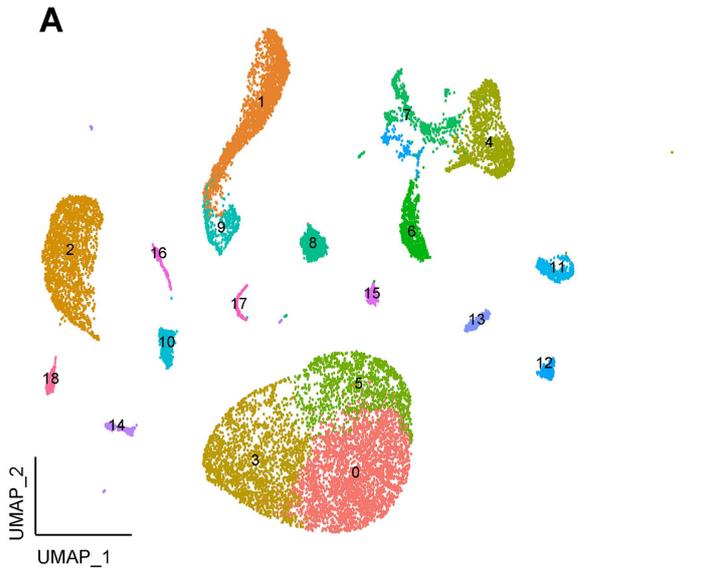


Supplementary Information

Microglial BIN1 deficiency elicits enhanced microglial inflammatory responses that mimic early AD pathology

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Suppl. Figure 1



Suppl. Figure 1: Additional information regarding scRNA-seq data analysis

(A) Uniform manifold approximation and projection (UMAP) plot of snRNA-seq data showing the unsupervised clustering of 18,678 cells from the four different experimental conditions into various cell types.

(B) Dot plot summarizing the expression of cell type-specific marker genes across the identified cell clusters. The size of each dot corresponds to the percentage of cells expressing the marker gene, and the color intensity indicates the average expression level.

(C) UMAP plot of snRNA-Seq data showing the clustering of 18,678 cells into various cell types, indicating their origin from the four different experimental conditions.

(D) Violin plot showing the expression of Bin1 across different cell types.

Suppl. Figure 2: Non cell autonomous effect of microglial Bin1 deletion on astrocytic gene expression in the presence or absence of LPS

(A) Volcano plots presenting differentially expressed genes (DEGs) ($\text{abs}(\text{avg_log2FC}) > 0.25$ & $p \text{ adj value} < 0.05$) in astrocytes originating from the comparisons Bin1^{fl/fl} + LPS vs Bin1^{fl/fl} (top left panel), Bin1cKO + LPS vs Bin1cKO (top right panel), Bin1cKO vs Bin1^{fl/fl} (bottom left panel) and Bin1cKO + LPS vs Bin1^{fl/fl} + LPS (bottom right panel). The upregulated DEGs are presented in red, and the downregulated DEGs are presented in blue.

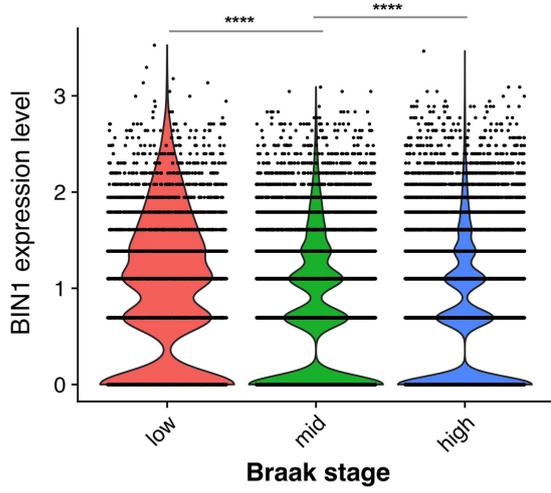
(B) Dot plot presenting the levels of genes related to the inflammatory response (*Ifi27*, *H2-K1*) and astrocytic reactivity (*Lgals3bp*, *C4b*, *Celsr2*, *Gfap*, *Phyh1*, *Nat8f1*, *Fth1* and *Ednrb*) found to be highly expressed in astrocytes from Bin1cKO + LPS cortices.

(C) Dot plot presenting the levels of genes related to Alzheimer's disease pathology (*ApoE*, *Clu*, *Cst3*, *Itih2b*, *Zbtb16* and *Pde10a*), lipid metabolism (*Scd2*, *Acs13* and *Ttyh1*) and neuroprotective astrocytic activities (*Ntrk2*, *Mt1*) or unknown yet role in astrocytes (*Zswim6*) found to be highly expressed in astrocytes from Bin1cKO cortices in the absence of neuroinflammation. The size of each dot corresponds to the percentage of cells expressing the marker gene, and the color intensity indicates the average expression level.

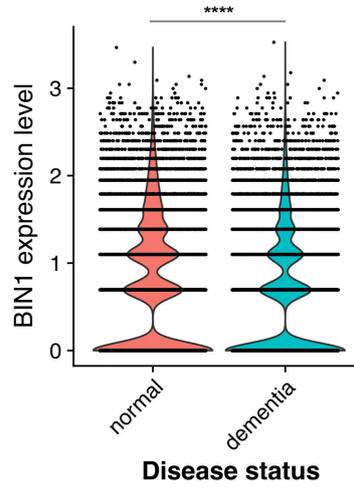
(D) Presentation of enriched Gene Ontology (GO) terms associated with various biological processes (BP) and CCs for the DEGs ($\text{abs}(\text{avg_log2FC}) > 0.25$ & $p \text{ adj value} < 0.05$) identified in astrocytes from the following comparisons: Bin1cKO+LPS vs Bin1cKO (red bars), Bin1fl/fl+LPS vs Bin1fl/fl (purple bars), Bin1cKO+LPS vs Bin1fl/fl+LPS (green bars) and Bin1cKO vs Bin1fl/fl (blue bars).

Suppl. Figure 3

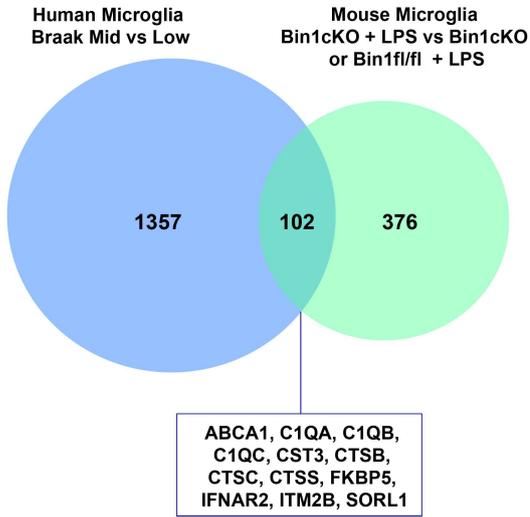
A



B



C



Suppl. Figure 3: Comparison of gene expression alterations between microglia in Bin1cKO + LPS mouse model and those in the brains of AD patients

(A) BIN1 expression levels in human microglia across different Braak stages (****p<0.0001, Wilcoxon rank sum test).

(B) BIN1 expression levels in human microglia across different disease statuses (****p<0.0001, Wilcoxon rank sum test).

(C) Venn diagram showing the overlap of DEGs (avg_log2FC>0.25 and p adj value<0.05) in microglia in our animal model (Bin1cKO + LPS vs Bin1cKO or Bin1^{fl/fl} + LPS) and DEGs in the brains of AD patients at middle vs low Braak stages. Among the commonly affected genes, we highlight *Abca1*, *Ctsb*, *Ctsc*, *Ctss*, *Cst3*, *Fkbp5*, *Itm2b* and *Sorl1* (involved in lipid metabolism and amyloid processing); *C1qa*, *C1qb*, and *C1qc* (part of the complement system); and *Ifnar2* (related to immune regulation and the interferon response).

Suppl. File 1: DEGs ($\log_2FC > 0.25$ and $p \text{ adj value} < 0.05$) found in all major brain cell types across all the different experimental comparisons

Suppl. File 2: DEGs ($\log_2FC > 0.25$ and $p \text{ value} < 0.05$) found in human microglia between individuals clinically diagnosed with dementia or not, or between individuals clinically diagnosed with AD at different Braak stages (high vs mid and mid vs low)

Suppl. File 3: DEGs ($\log_2FC > 0.25$ and $p \text{ adj value} < 0.05$) that constitute the intersect between DEGs in human microglia during the early stages of AD progression (mid vs low Braak stages) and DEGs found in our animal model after Bin1cKO and LPS administration (Bin1cKO + LPS vs Bin1cKO or Bin1fl/fl + LPS)

Suppl. File 4: DEGs ($\log_2FC > 0.25$ and $p \text{ value} < 0.01$) for each microglia cluster compared with all other clusters

Suppl. File 5: Gene Signatures for microglial cells, previously identified under well-defined conditions, extracted from CellID