SUPPLEMENTARY DATA

Aging-Associated CCL8^{+} Senescent Macrophages Recruit CCR1^{+} Neutrophils to Promote NETs Formation and Impair Meningeal Lymphatic Drainage

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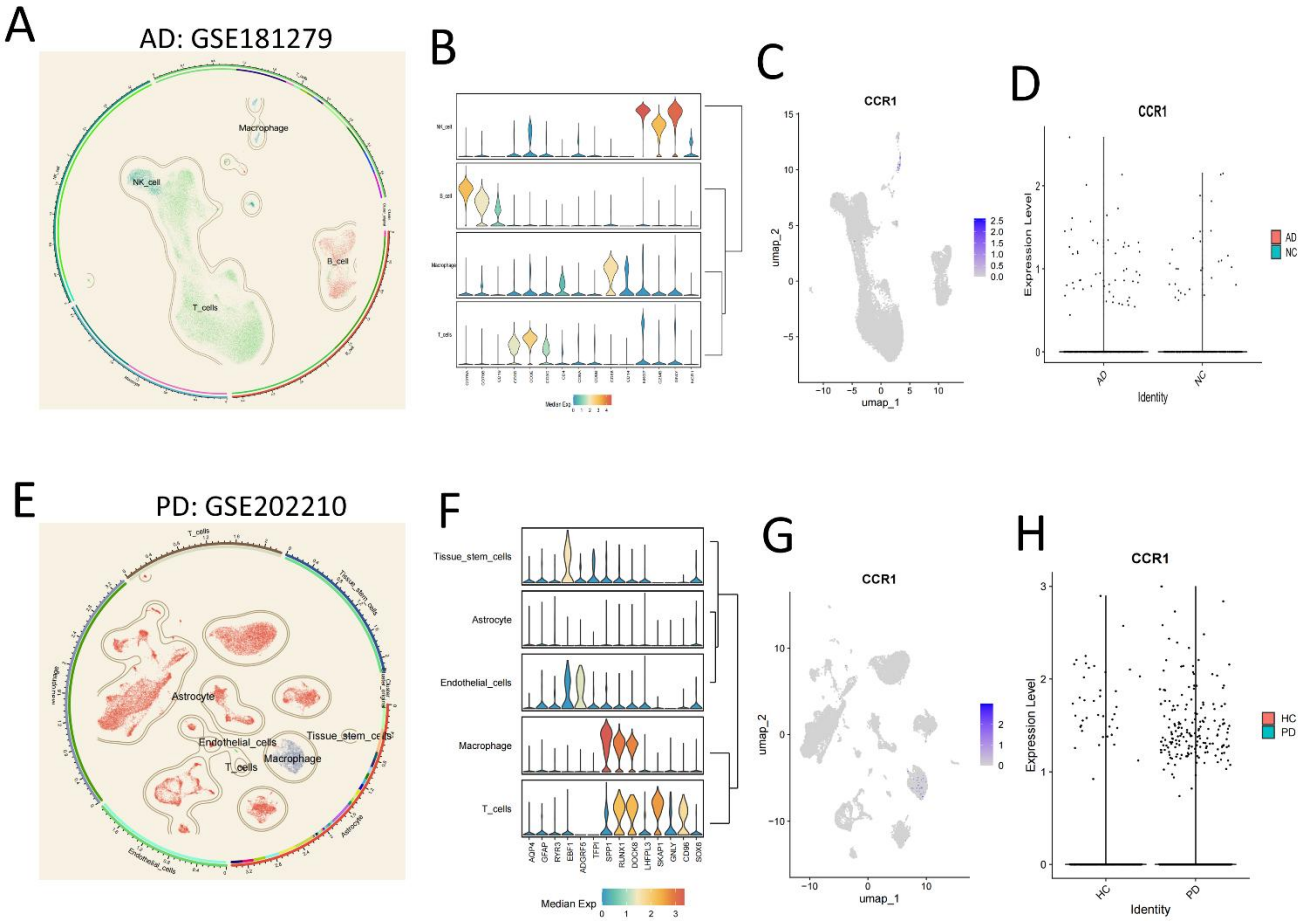
SUPPLEMENTARY DATA

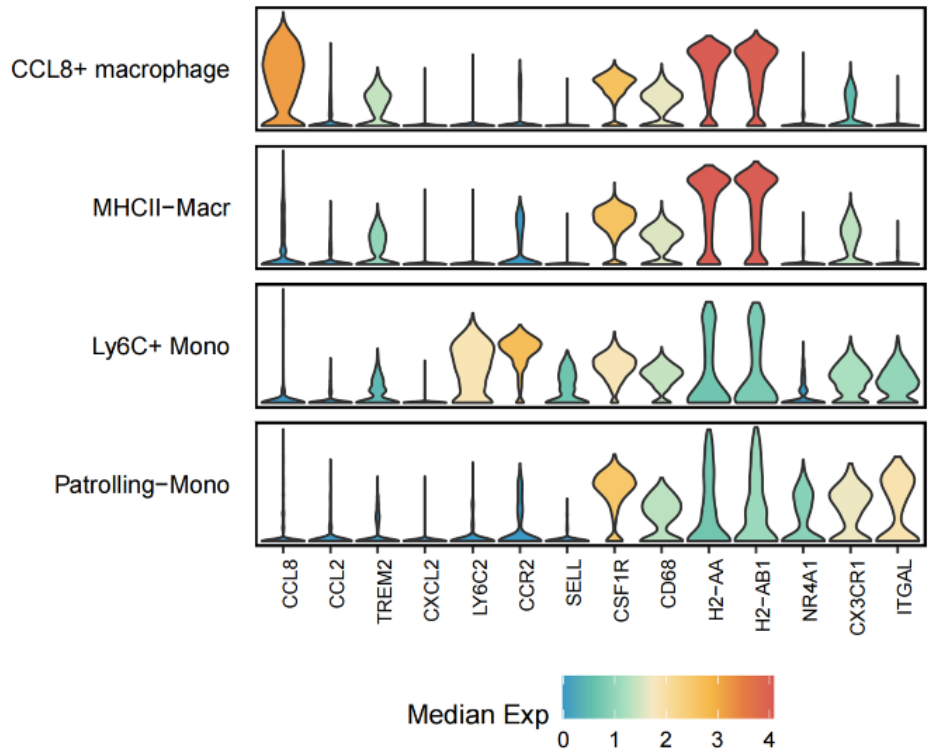
Supplementary Table 1.

Gene	Forward, 5'-3'	Reverse, 5'-3'
IL-1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
IL-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
TNF-α	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
Mmp3	ACATGGAGACTTTGTCCCTTTTG	TTGGTCCATTGGGTATAGTCTTC
Mmp12	CTGCTCCCATGAATGGTGGT	CATGTGACAGTGCTCATCATC
PAI-1	GCGGAGGCACTTTTCCAGAA	AGGGTTGCACTAAACATGTCAG
MCP-1	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
GAPDH	AGGTCGGTGTGAACGGATTTG	GGGGTCGTTGATGGCAACA



Supplementary Figure 1. CCR1 expression in human peripheral blood and brain single-cell RNA-seq datasets from Alzheimer's disease (AD) and Parkinson's disease (PD) patients. (A) UMAP visualization of single-cell RNA-seq data from peripheral blood mononuclear cells (PBMCs) of AD patients and healthy controls (GSE181279). Major immune cell populations—including T cells, NK cells, B cells, and macrophages—are annotated based on canonical marker gene expression. (B) Violin plots displaying the expression levels of lineage-specific marker genes across identified cell clusters in panel (A), confirming cellular identity assignments. Color intensity reflects median expression level per cluster. (C) Feature plot showing spatial distribution of CCR1 expression within the UMAP embedding for AD blood samples. Expression is scaled logarithmically and color-coded by intensity (low to high: white to dark blue). (D) Boxplot with jittered individual data points comparing CCR1 expression levels between AD patients and non-demented controls (NC) in PBMCs. Each dot represents a single cell; horizontal lines indicate medians, boxes denote interquartile ranges (IQR), and whiskers extend to 1.5×IQR. Statistical significance was assessed via two-sided Wilcoxon rank-sum test (E) UMAP representation of single-cell RNA-seq data from brain tissue of PD patients and controls (GSE202210). Annotated cell

types include astrocytes, endothelial cells, T cells, macrophages, and tissue stem cells. (F) Heatmap-style violin plots illustrating expression profiles of key cell-type markers across clusters in panel (E), validating annotation accuracy. Median expression values are color-scaled from low (blue) to high (red). (G) Feature plot depicting CCR1 expression patterns across the brain-derived snRNA-seq UMAP space. Expression intensity is log-transformed and visualized using a continuous color gradient. (H) Comparative boxplot with overlaid scatter points quantifying CCR1 expression differences between PD and HC (healthy control) groups in brain tissue. Data presentation follows same conventions as in panel (D). Group labels correspond to those in the legend (HC = healthy control; PD = Parkinson's disease).



Supplementary Figure 3. Violin plots showing the expression distribution of canonical marker genes across monocyte-macrophage subpopulations.