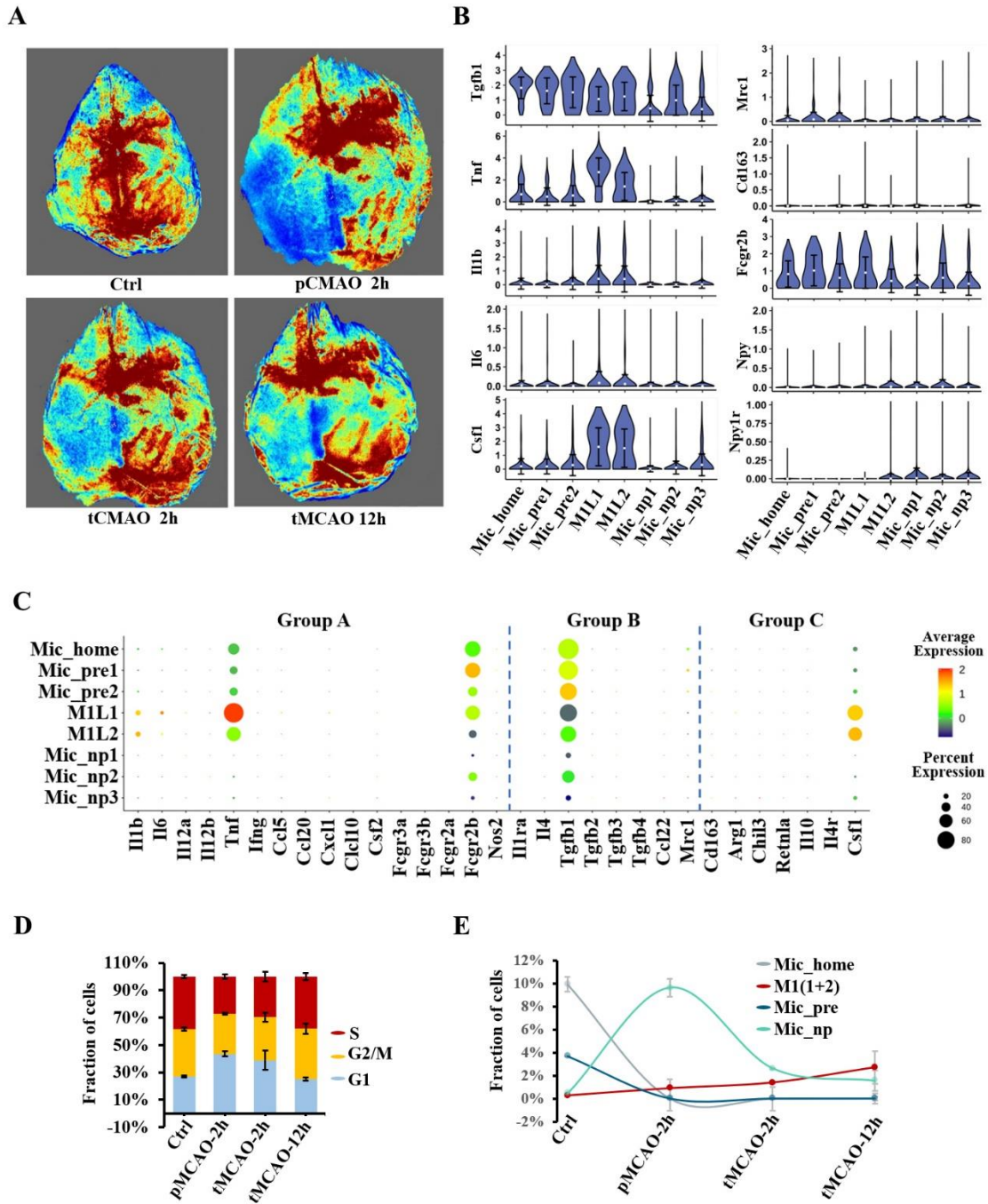


## SUPPLEMENTARY DATA

# **Microglia Exhibit Distinct Heterogeneity Rather than M1/M2 Polarization within the Early Stage of Acute Ischemic Stroke**

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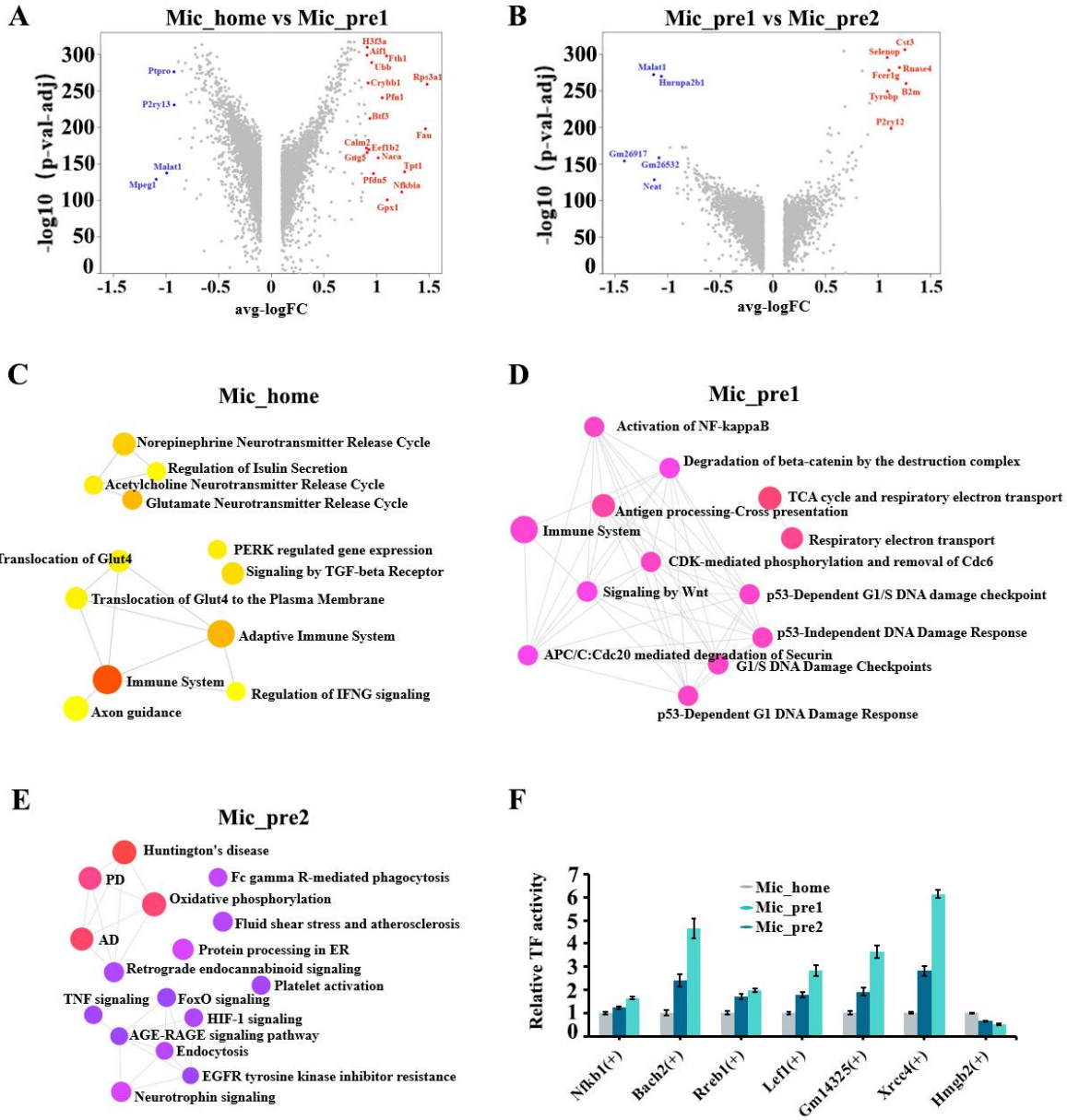
# SUPPLEMENTARY DATA



**Supplementary Figure 1. Supplementary figures for the identification of subpopulation function.** (A) The images of laser speckle flowmetry at the four time points. (B) Violin plot displaying the represented genes related to major microglial function. (C) Dot plot of marker genes for microglial polarization. The genes in groups A, B, and C represent the M1, M2a, and M2b/c phenotypes respectively. (D) The proportion of each cell cycle at four sampling times. (E) Line plot of the temporal change in the fraction of four cell categories.

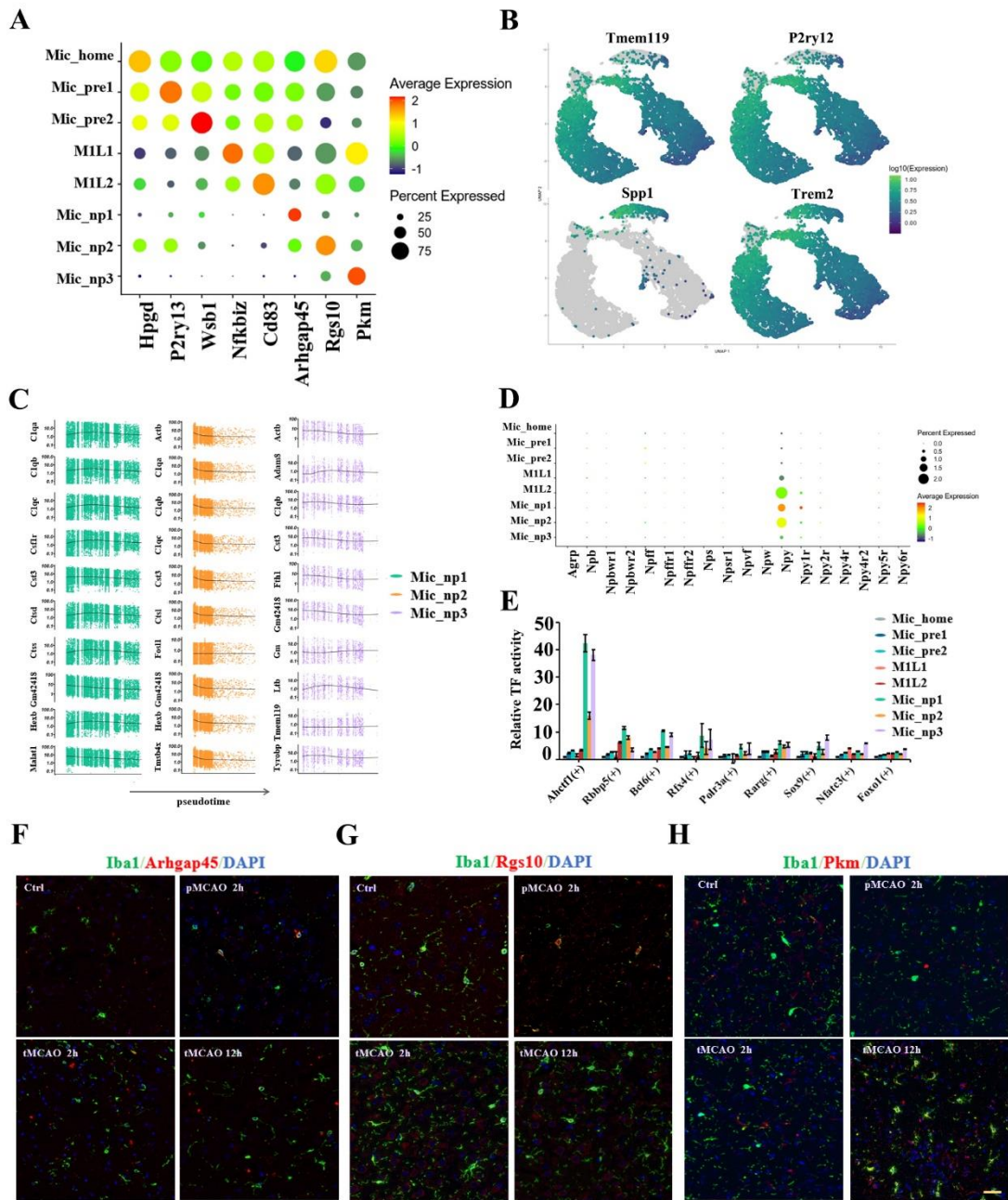


# SUPPLEMENTARY DATA



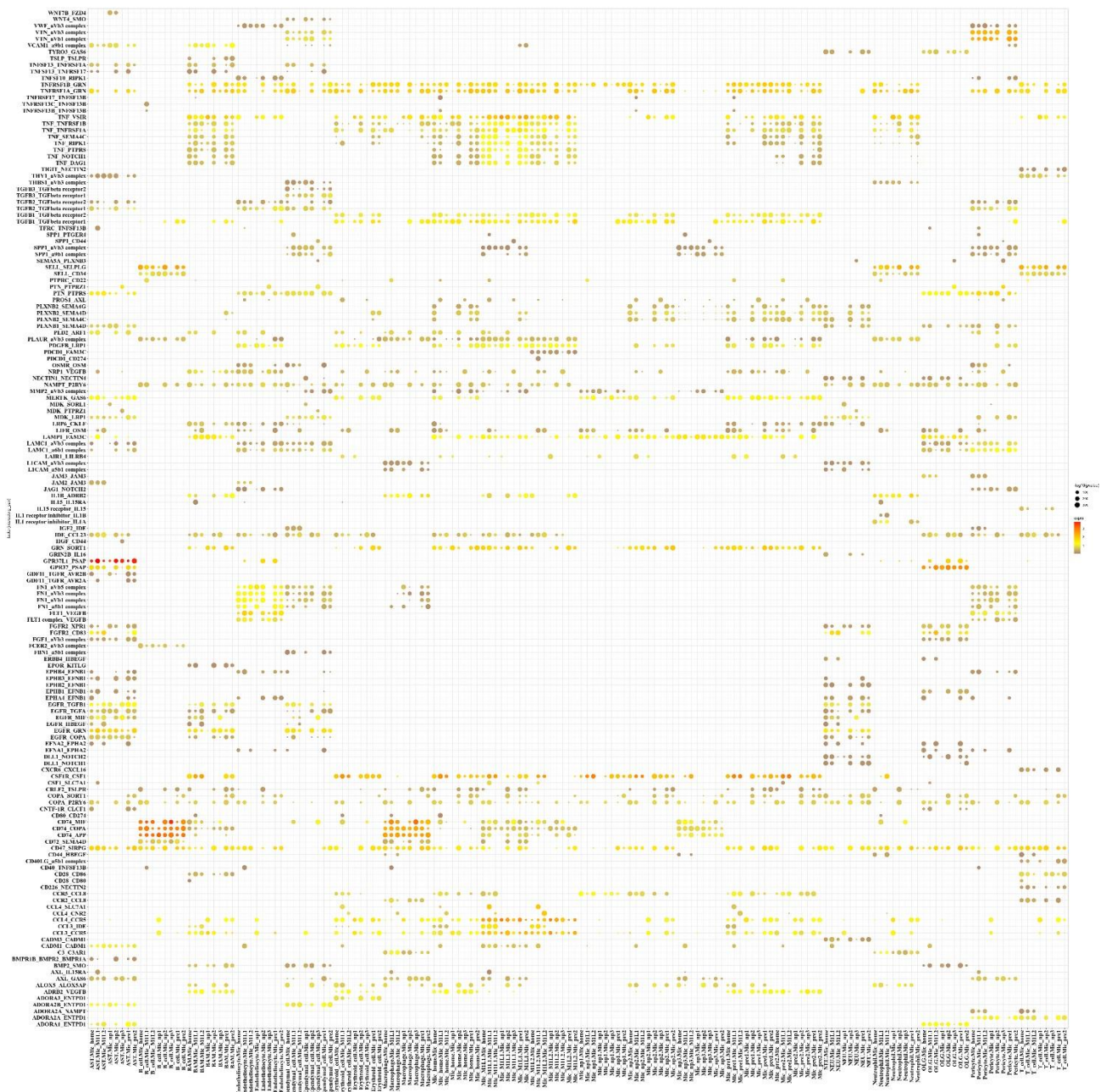
**Supplementary Figure 3. Supplementary figures for function comparison among homeostatic microglial subpopulations.** (A) Volcano plot to compare the differential genes between Mic\_home and Mic\_pre1 clusters. Red dots represent upregulated genes in Mic\_home compared with Mic\_pre1, while blue dots represent the downregulated. (B) Volcano plot to compare the differential genes between Mic\_pre1 and Mic\_pre2 clusters. Red dots represent upregulated genes in Mic\_home compared with Mic\_pre1, while blue dots represent the downregulated. (C-E) Ontology network of the major activated pathways and their interaction in Mic\_home, Mic\_pre1, and Mic\_pre2 subpopulations respectively. (F) Relative activity of the highly expressed transcription factors (TF) in Mic\_pre1 and Mic\_pre2 cells compared with Mic\_home cells. The values of the bars denote the ratio of Mic\_pre1 and Mic\_pre2 cells compared with Mic\_home cells.

# SUPPLEMENTARY DATA



**Supplementary Figure 4. Supplementary figures for the characteristics of neuropeptide-associated microglial subpopulations.** (A) Dot plot of the markers for each subpopulation used for double-immunofluorescent staining. (B) The expression of core microglial marker genes and disease-associated microglia genes in the *Mic\_np3* subpopulation. (C) Expression of the ten genes with the highest Moran's I score alongside the pseudotime trajectory in the *Mic\_np1*, *Mic\_np2*, and *Mic\_np3* subpopulations. (D) Dot plot of the neuropeptide genes. (E) Relative activity of the high expressed transcription factors in *Mic\_np2* and *Mic\_np3* cells compared with *Mic\_np1* cells. (F) Double immunofluorescence staining of *Mic\_np1* cells at each sampling time. Coronal brain sections are all stained with anti-Iba1 (green, representing microglia) and DAPI (blue, representing cell nuclei) antibodies. *Mic\_np1* are marked by *Arhgap45* (red). (N=3) The yellow bar represents 100µm. (G) Double immunofluorescence staining of *Mic\_np2* cells at each sampling time. Coronal brain sections are all stained with anti-Iba1 (green, representing microglia) and DAPI (blue, representing cell nuclei) antibodies. *Mic\_np2* are marked by *Rgs10* (red). (N=3) The yellow bar represents 100µm. (H) Double immunofluorescence staining of *Mic\_np3* cells at each sampling time. Coronal brain sections are all stained with anti-Iba1 (green, representing microglia) and DAPI (blue, representing cell nuclei) antibodies. *Mic\_np3* are marked by *Pkm* (red). (N=3) The yellow bar represents 100µm.

# SUPPLEMENTARY DATA



**Supplementary Figure 5. Dot plot of the major activated ligand-receptor couplings between the subpopulations of microglia and other brain cell populations under control state.**

