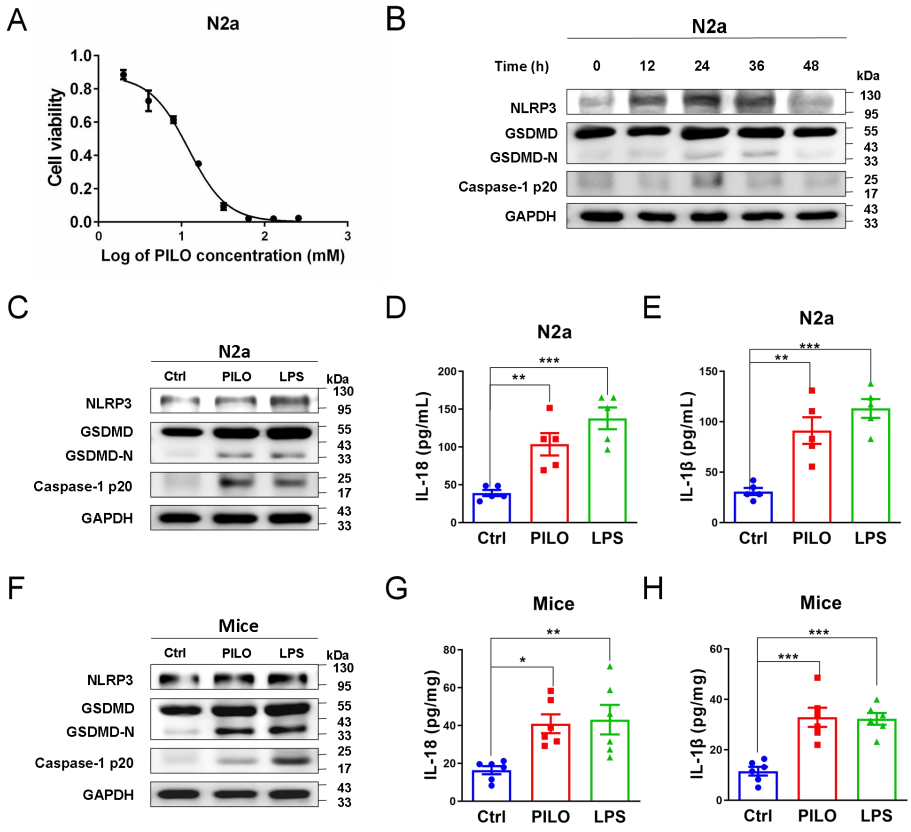
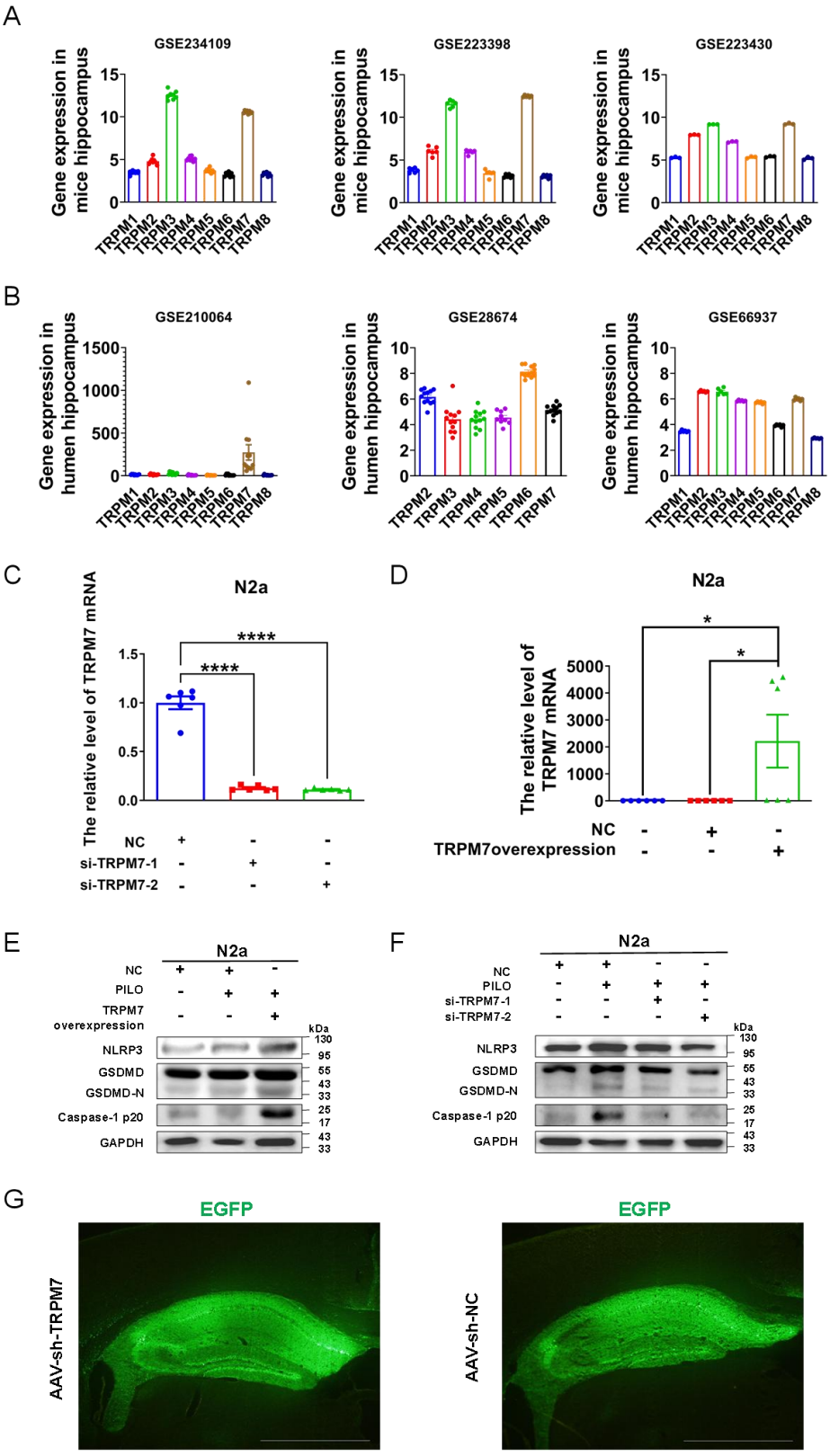
Supplemental Figure 1



Supplemental Figure 1. PILO-treatment induced pyroptosis in N2a cells and mice.

A IC_{50} values of PILO in N2a cells was measured by CCK-8 assays and calculated using GraphPad. **B** The representative protein bands of NLRP3, GSDMD and caspase-1 p20 expression in N2a cells treated with 12 mM PILO for 0 h, 12 h, 24 h, 36 h, or 48 h ($n = 5$ or 6). **C-E** The representative protein bands (n = 6) and ELISA analysis (n = 5) of N2a cells treated with PILO for 24 h or pretreated with 1 μg/mL LPS for 4 h and then treated with 15 μM Nigericin for 1 h. **F-H** The representative protein bands and ELISA analysis in hippocampus of C57BL/6J mice treated with PILO (300 mg/kg) or LPS (20 mg/kg) (n = 6). Full scans of all the blots are in the Supplementary Note. *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

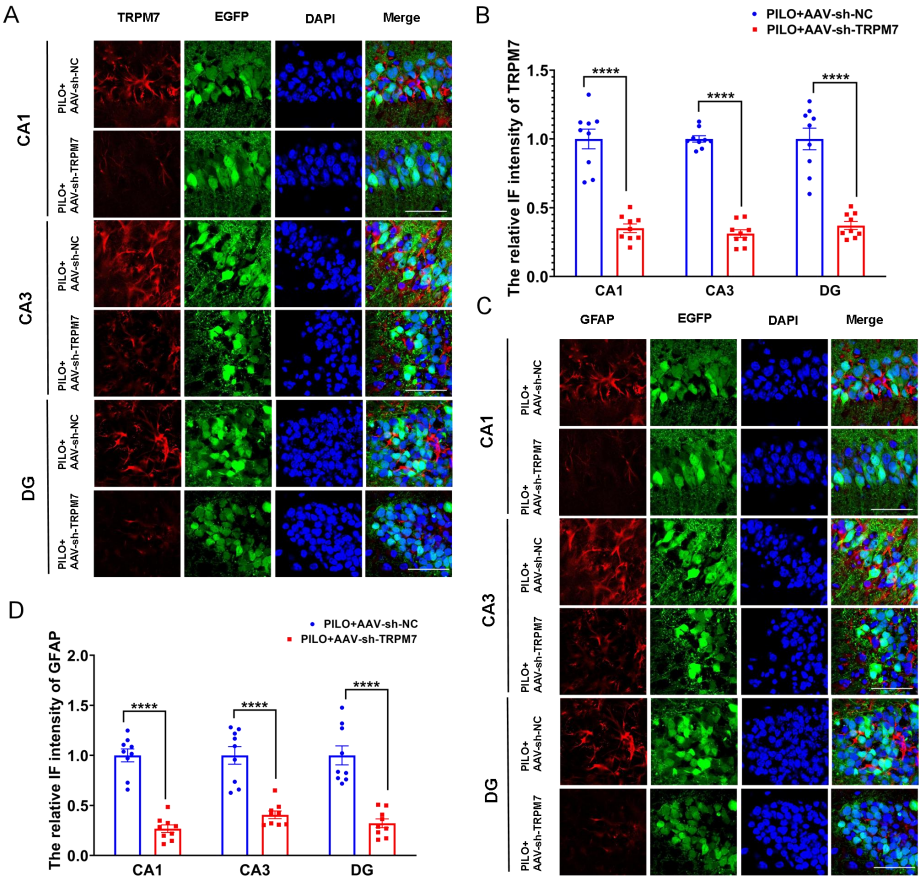
Supplemental Figure 2



Supplemental Figure 2. TRPM7 participated in pyroptosis.

A The gene expression of TRPM subfamily in hippocampi from healthy mice, which was measured by three independent GEO datasets (n = 3, 6 or 8). **B** The gene expression of TRPM subfamily in hippocampi from healthy human, which was measured by three independent GEO datasets (n = 6, 11 or 12). **C, D** The mRNA expression of TRPM7 in PILO-treated N2a cells after TRPM7 knockdown (C) or TRPM7 overexpression (D) (n = 6). **E** The representative protein bands of NLRP3, GSDMD, and caspase-1 p20 in PILO-treated N2a cells after TRPM7 overexpression (n = 5 or 6). **F** The representative protein bands of NLRP3, GSDMD, and caspase-1 p20 in PILO-treated N2a cells after TRPM7 knockdown (n = 5 or 6). **G** Immunofluorescence analysis of EGFP (green) in hippocampus (4x lens) of AAV-sh-TRPM7 and AAV-sh-NC transfected C57BL/6J mice. Scale bar, 500 μm. Full scans of all the blots are in the Supplementary Note. *$P < 0.05$; ** $P < 0.01$.

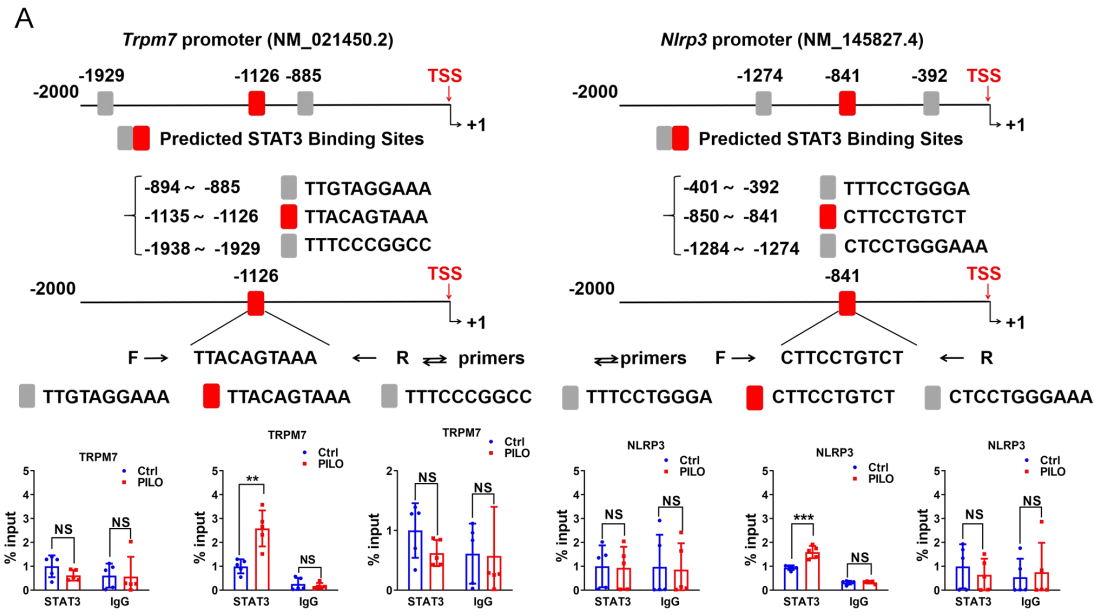
Supplemental Figure 3



Supplemental Figure 3. Silencing TRPM7 downregulated TRPM7 expression and alleviated neuronal damage in PILO-treated SE mice.

A, B Immunofluorescence analysis of TRPM7 (red) expression in hippocampus (60x lens) of PILO-treated C57BL/6J mice after AAV-sh-TRPM7-EGFP (green) or AAV-sh-NC-EGFP (green) transfection, including the CA1, CA3, and DG regions ($n = 9$). Scale bar: 100 μm. DAPI (blue) is used to label nucleus. **C, D** Immunofluorescence analysis of GFAP (red) expression in hippocampus (60x lens) of PILO-treated C57BL/6J mice after AAV-sh-TRPM7-EGFP (green) or AAV-sh-NC-EGFP (green) transfection, including the CA1, CA3, and DG regions ($n = 9$). Scale bar: 100 μm. DAPI (blue) is used to label nucleus. **** $P < 0.0001$.

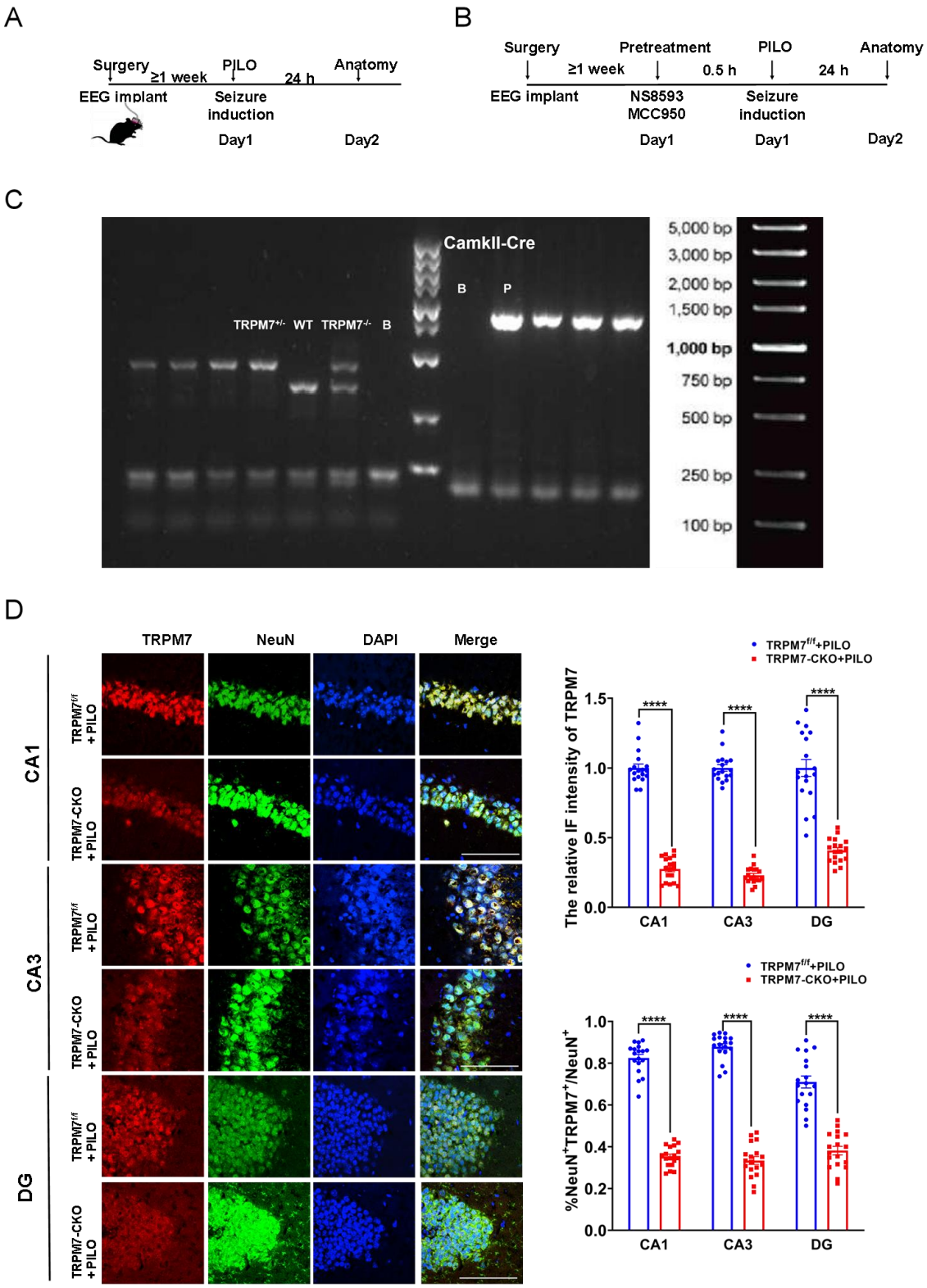
Supplemental Figure 4



Supplemental Figure 4. STAT3 was recruited to the promoter regions of *Trpm7* and *Nlrp3*.

A Top: A sketch applied to show the predicted binding sites of STAT3 in *Trpm7* or *Nlrp3* promoter. Bottom: ChIP assays performed on the promoter regions of *Trpm7* or *Nlrp3* with the STAT3 antibodies in N2a cells with or without PILO (10 μM) treatment ($n = 5$). TSS indicates transcription start site. ** $P < 0.01$; *** $P < 0.001$; NS not significant.

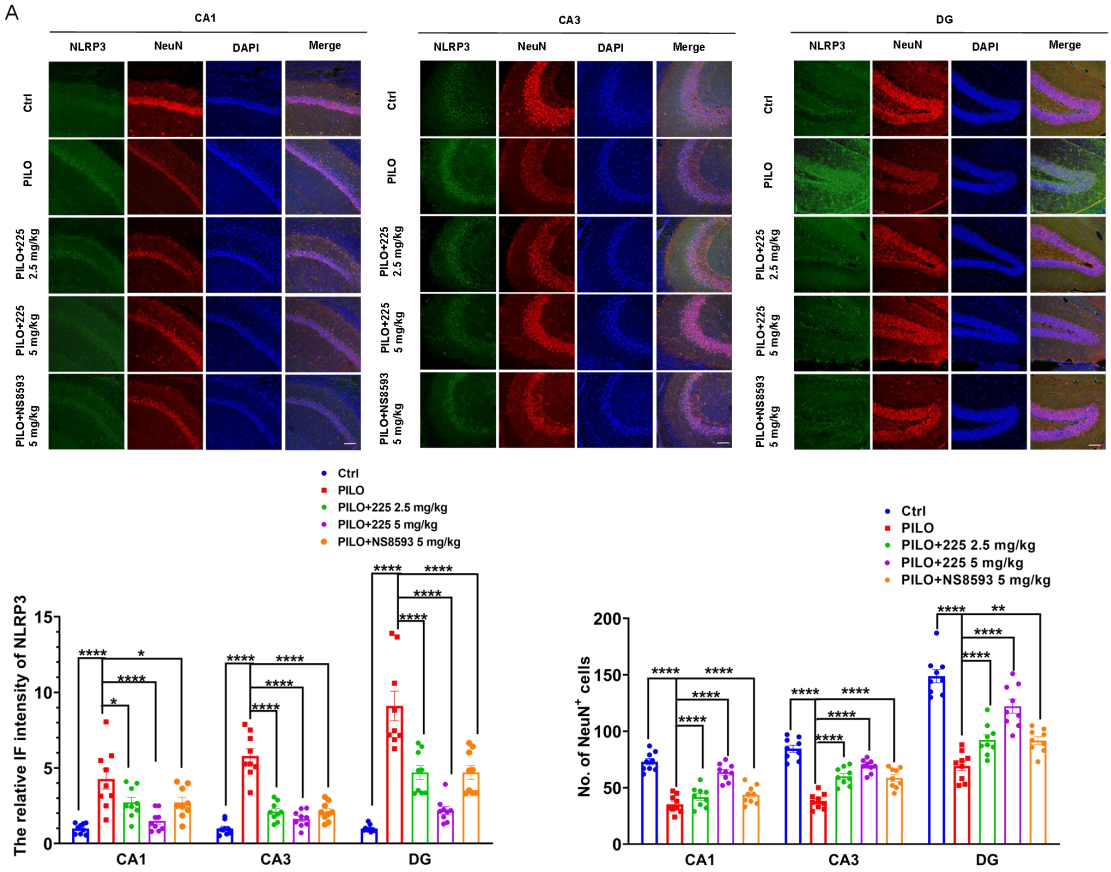
Supplemental Figure 5



Supplemental Figure 5. TRPM7 conditional knockout mice alleviated neuronal damage.

A Schematic diagram showing the injection protocol of PILO-treated C57BL/6J mice after AAV-sh-TRPM7-EGFP (green) or AAV-sh-NC-EGFP (green) transfection. **B** Schematic diagram showing the injection protocol of PILO-treated C57BL/6J mice after injected with NS8593 or MCC950 or both. **C** Mice were genotyped using DNA extracted from tail biopsies and PCR with gene-specific primers. **D** Immunofluorescence analysis of TRPM7 (green) and NeuN (red) expression in hippocampus (100x lens) of TRPM7-CKO mice, including the CA1, CA3 and DG regions (n = 18). Scale bar: 100 μm. DAPI (blue) was used to label nucleus. **** $p <$ 0.0001.

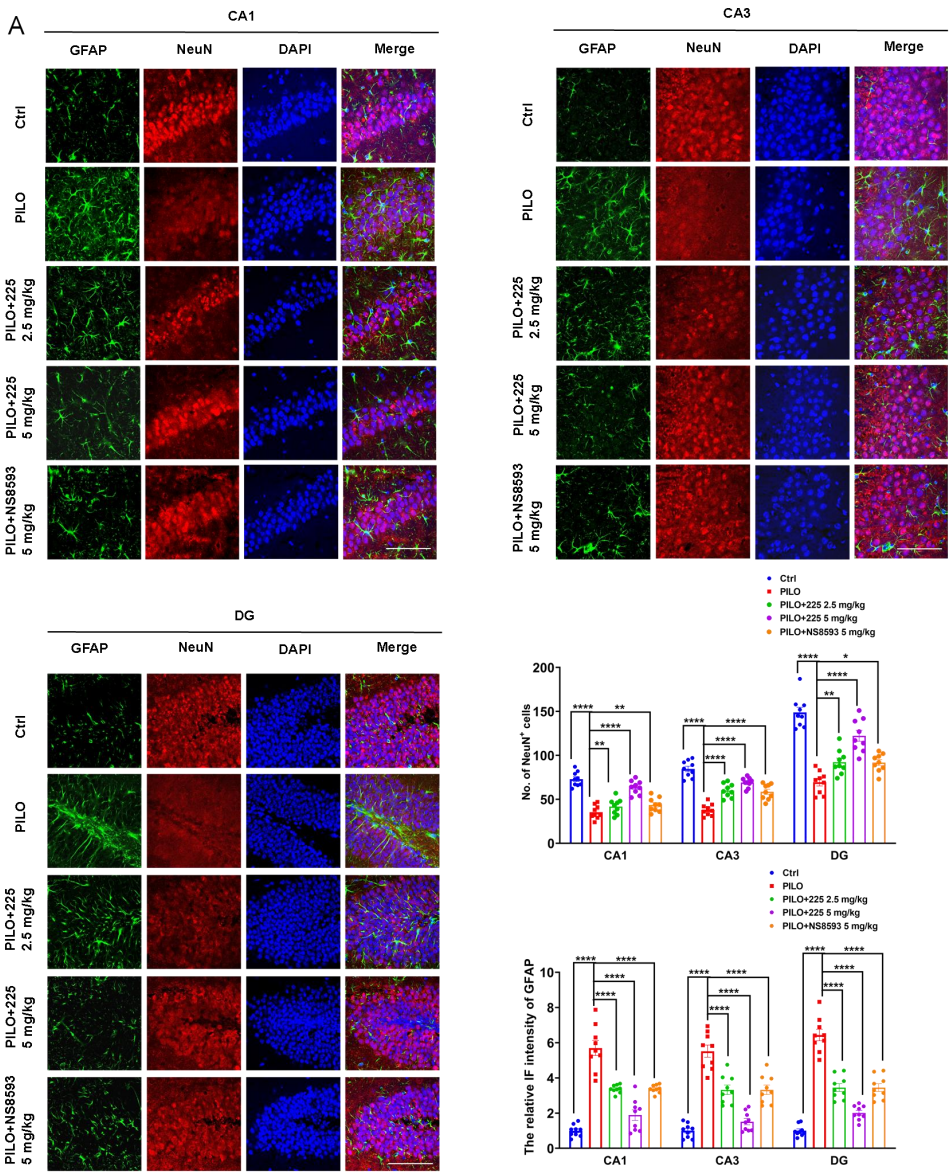
Supplemental Figure 6



Supplemental Figure 6. The TRPM7 inhibitor, SDUY-225, alleviated pyroptosis in PILO-treated SE mice.

A Immunofluorescence analysis of NeuN (red) and NLRP3 (green) expression in hippocampus (60x lens) of PILO-treated C57BL/6J mice after 225 (low-dosage and high-dosage) or NS8593 pretreatment, including the CA1, CA3, and DG regions. ($n = 9$). Scale bars: 100 μm. DAPI (blue) was used to label nucleus. *$P < 0.05$; ** $P < 0.01$; **** $P < 0.0001$.

Supplemental Figure 7



Supplemental Figure 7. The TRPM7 inhibitor, SDUY-225, alleviated neuronal damage in PILO-treated SE mice.

A Immunofluorescence analysis of NeuN (red) and GFAP (green) expression in hippocampus (60x lens) of PILO-treated C57BL/6J mice after 225 (low-dosage and high-dosage) or NS8593 pretreatment, including the CA1, CA3, and DG regions. ($n = 9$). Scale bars: 100 μm. DAPI (blue) was used to label nucleus. Results were shown as mean ± SEM. *$P < 0.05$; ** $P < 0.01$; **** $P < 0.0001$.