

TRPM8 in the negative regulation of TNF α expression during cold stress

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Supplementary information includes:

Supplementary Figure S1-S11, Table S1 and S2.

Supplementary information
Figure S1

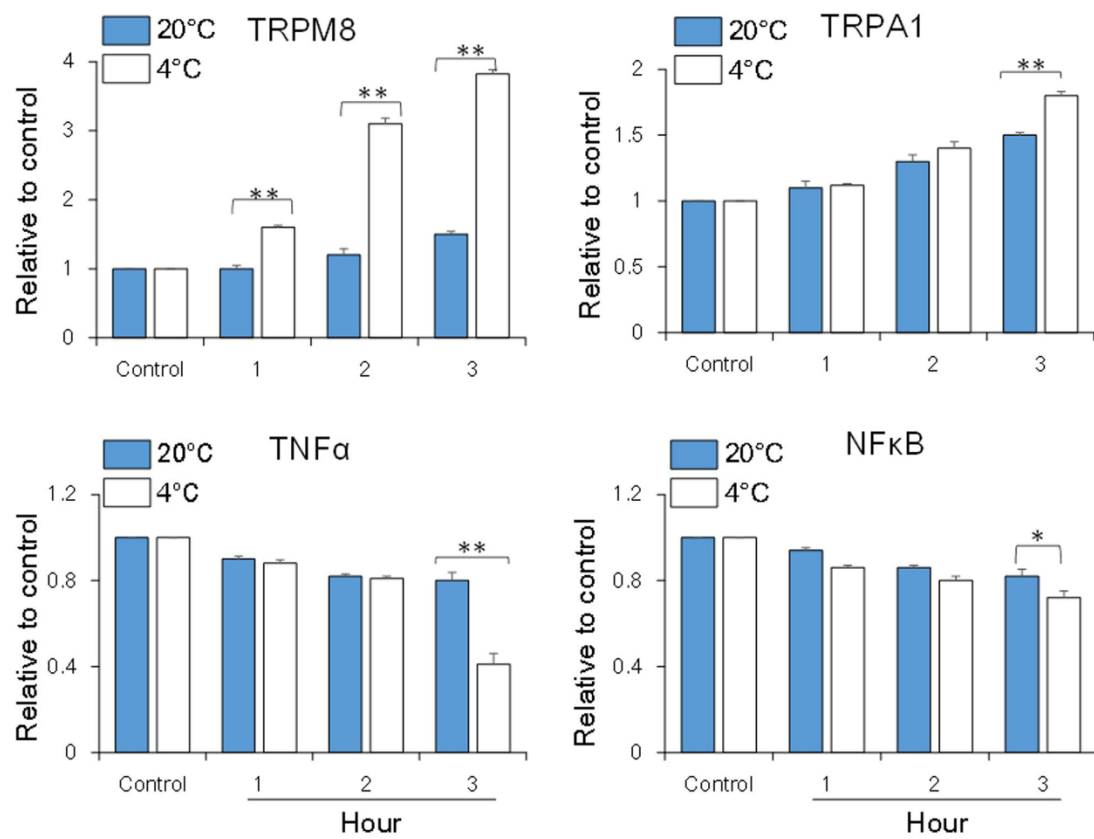


Figure S1. Comparison of the mRNA expressions of TRPM8, TRPA1, TNF α and NF κ B of PC12 cells in between 20 °C and 4 °C conditions. Data are shown as the mean \pm S.D. from three independent experiments. *, $P < 0.05$; **, $P < 0.01$.

Figure S2

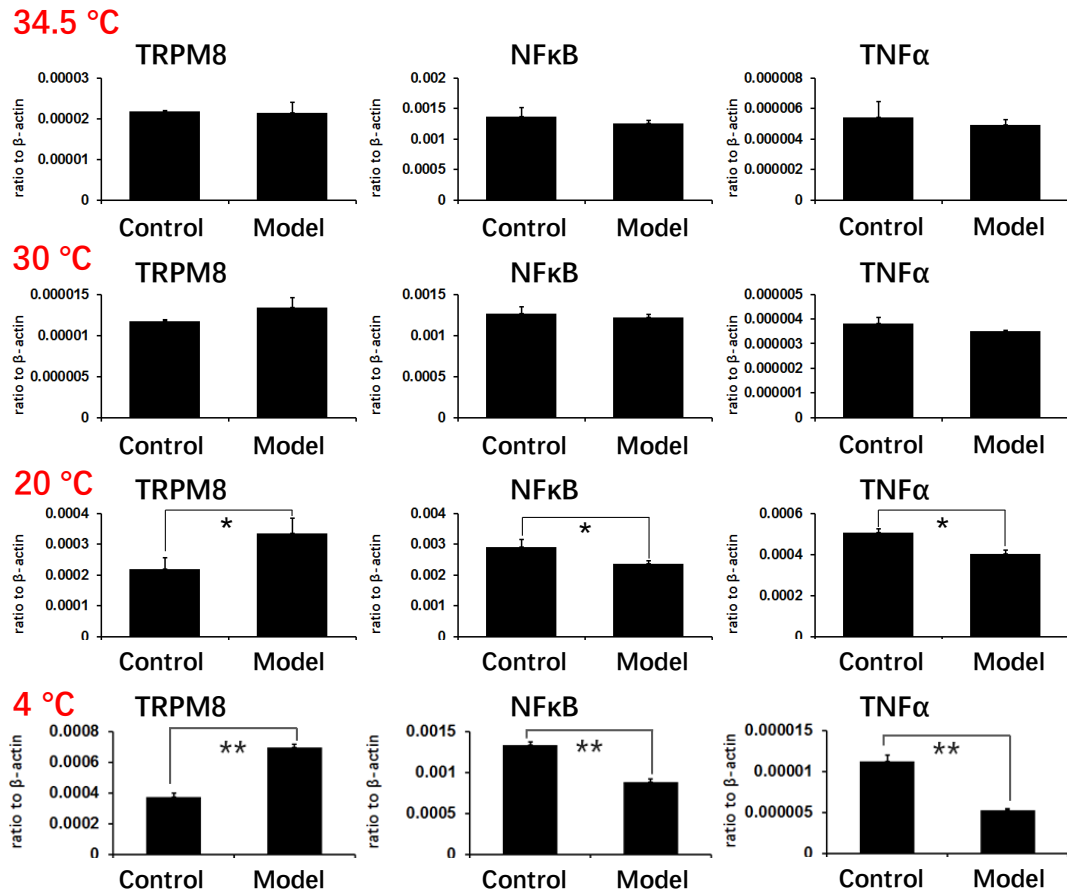
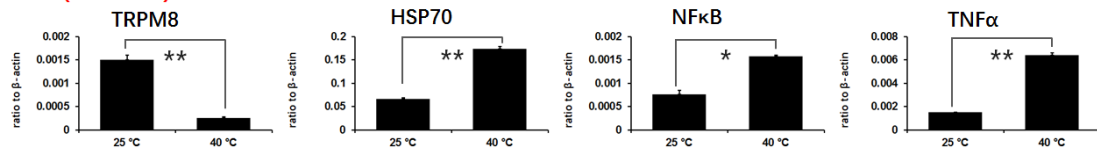


Figure S2. The mRNA expressions of TRPM8, NFκB and TNFα in PC12 cells under different temperature *in vitro*. Control: 37 °C. Model: Cold conditions. Data are shown as the mean ± S.D. from three independent experiments. *, $P < 0.05$; **, $P < 0.01$.

Figure S3

Hot (ICR mice)



Hot (PC12 cell)

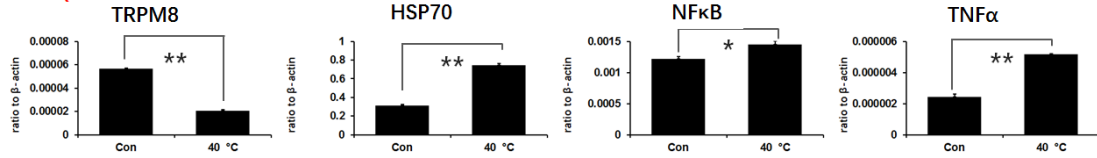


Figure S3. The mRNA expressions of TRPM8, HSP70, NFκB and TNFα in heat stress (40 °C) both *in vivo* and *in vitro*. Data are shown as the mean ± S.D. from five mice in each group *in vivo* and three independent experiments *in vitro*. *, $P < 0.05$; **, $P < 0.01$.

Figure S4

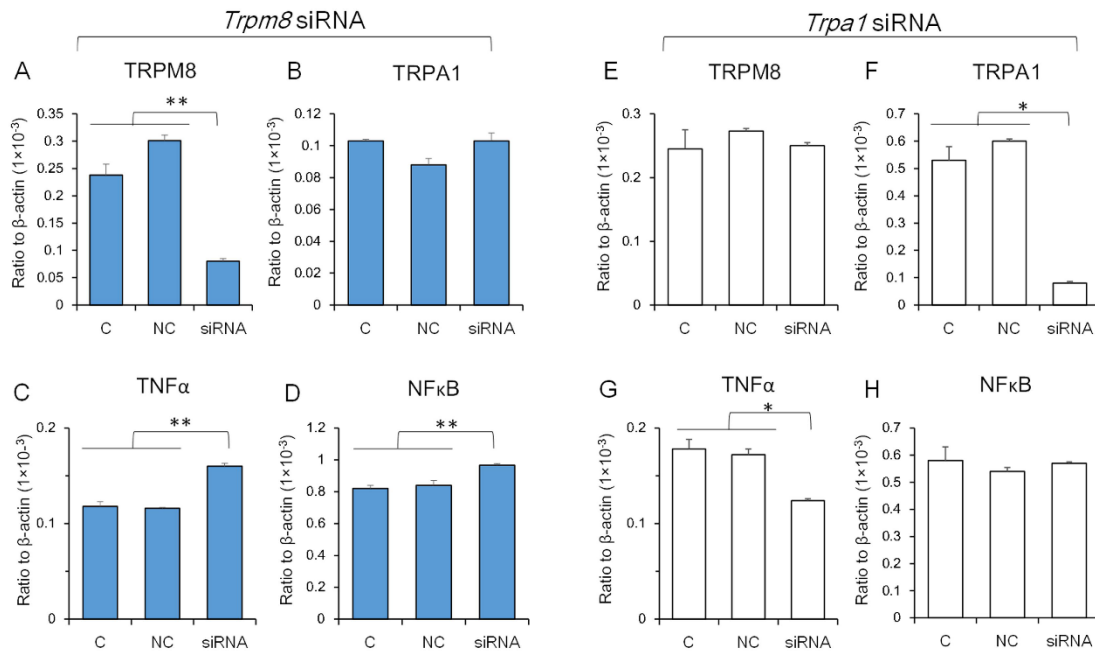


Figure S4. Effect of *Trpm8*siRNA and *Trpa1*siRNA (siRNA) on the mRNA expressions of TRPM8, TRPA1, TNFα and NFκB in PC12 cells in normal conditions (37 °C). (A) - (D): *Trpm8*siRNA. (E) - (H): *Trpa1*siRNA. Data are shown as the mean ± S.D. from three independent experiments. *, $P < 0.05$; **, $P < 0.01$.

Figure S5

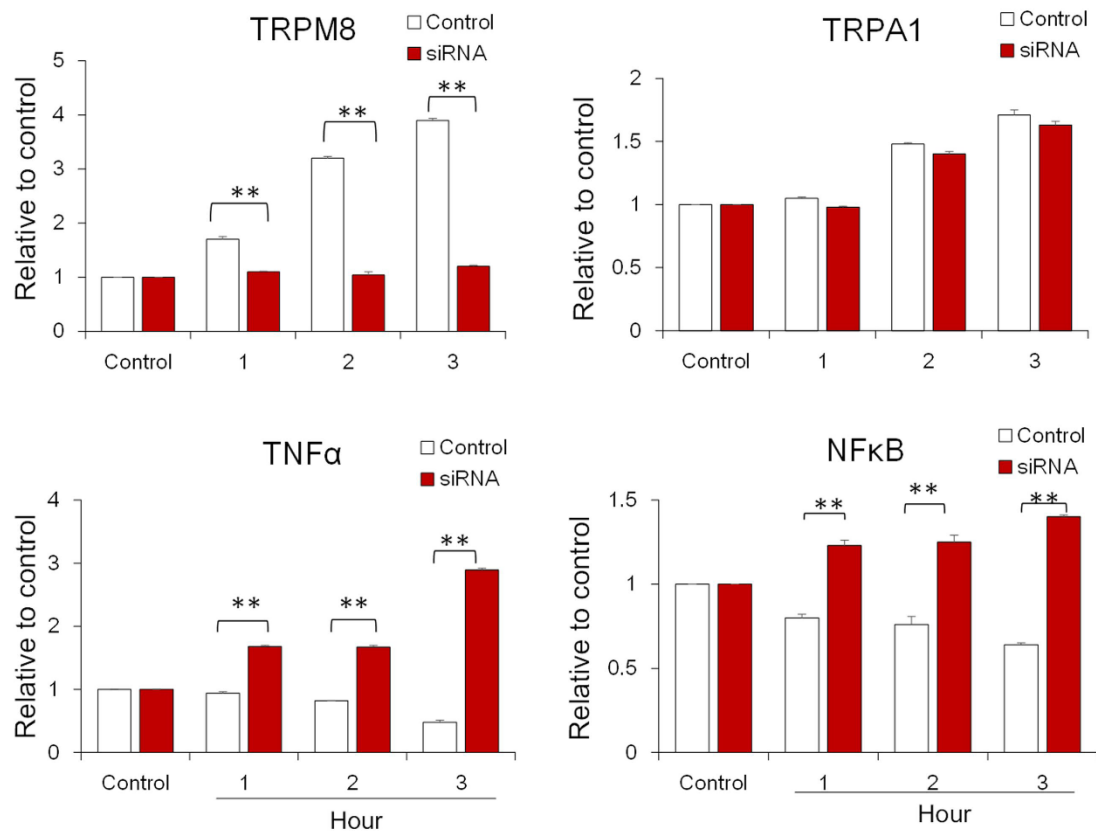


Figure S5. Effect of *Trpm8*siRNA (siRNA) on the mRNA expressions of TRPM8, TRPA1, TNF α and NF κ B in PC12 cells in cold conditions (4 °C). Data are shown as the mean \pm S.D. from three independent experiments. **, $P < 0.01$.

Figure S6

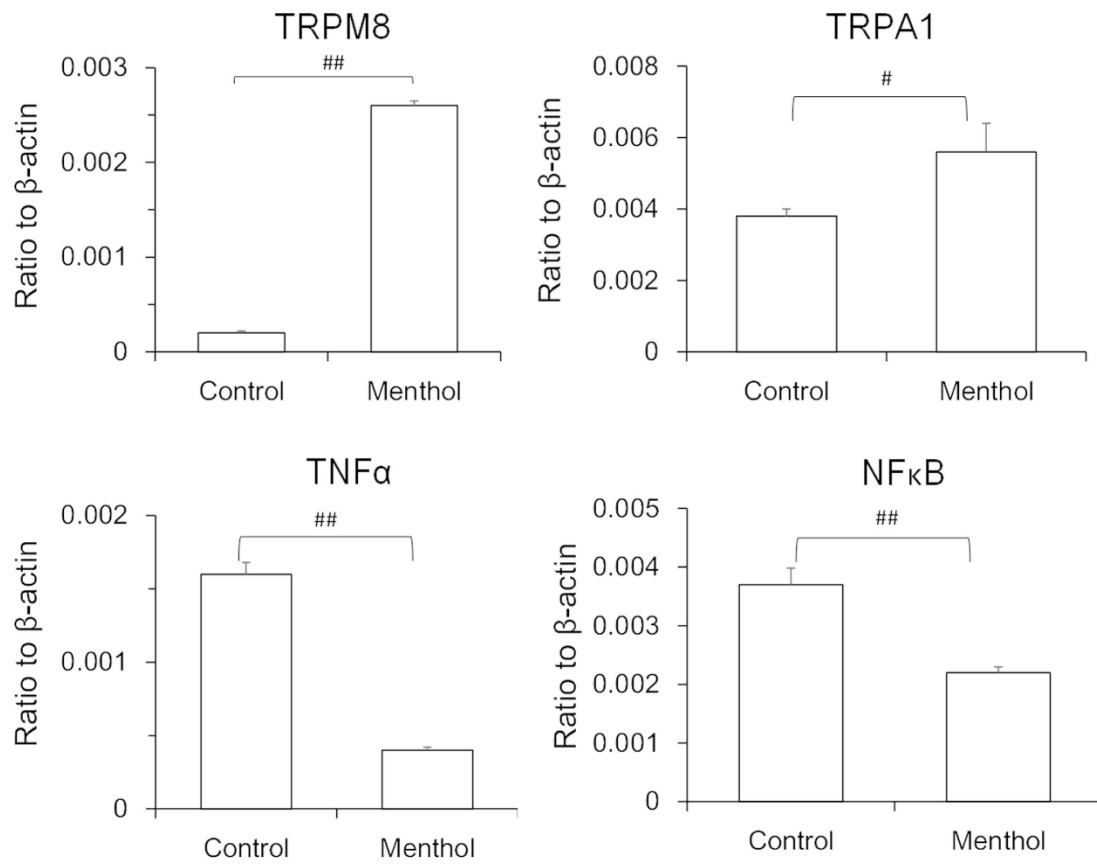
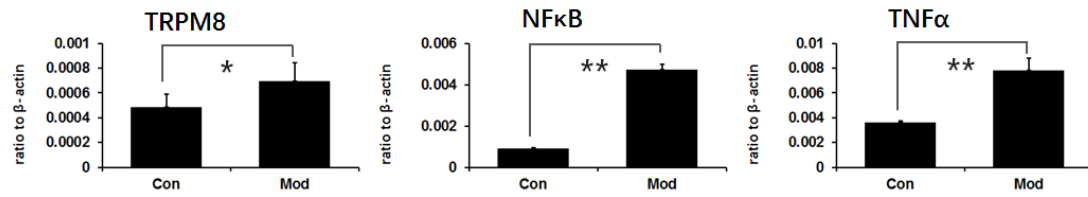


Figure S6. Effect of menthol on the mRNA expressions of TRPM8, TRPA1, TNF α and NF κ B in PC12 cells. Data are shown as the mean \pm S.D. from three independent experiments. #, $P < 0.05$; ##, $P < 0.01$.

Figure S7

CIR (ICR mice)



OGD (PC12 cell)

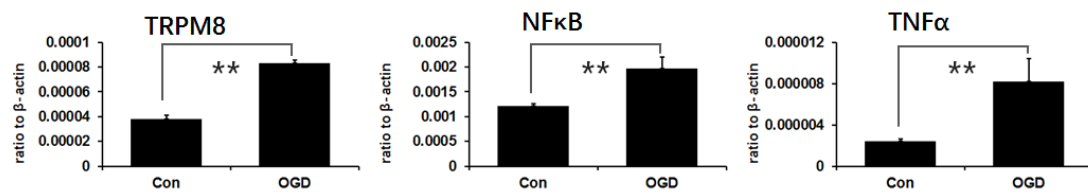
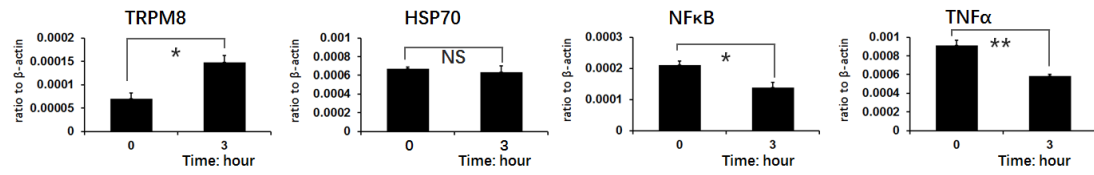


Figure S7. The mRNA expressions of TRPM8, NFκB and TNFα in ischemia and reperfusion stress under normal conditions (*in vivo*: room temperature, *in vitro*: 37 °C). Con represents control, Mod means model. CIR: cerebral ischemia and reperfusion, OGD: oxygen and glucose deprivation. Data are shown as the mean ± S.D. from five mice in each group *in vivo* and three independent experiments *in vitro*. *, $P < 0.05$; **, $P < 0.01$.

Figure S8

Cold (ICR mice)



Cold (PC12)

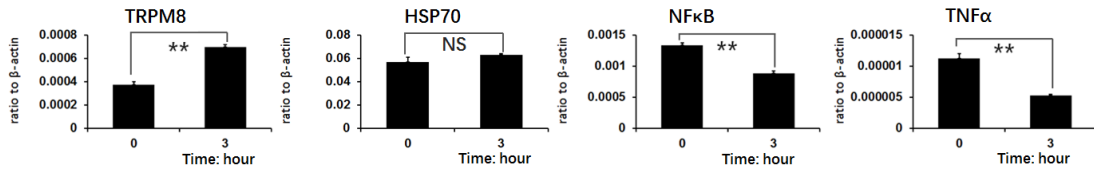


Figure S8. The mRNA expressions of TRPM8, HSP70, NFκB and TNFα under cold stress (4 °C) both *in vivo* and *in vitro*. Data are shown as the mean ± S.D. from five mice in each group *in vivo* and three independent experiments *in vitro*. *, P < 0.05; **, P < 0.01.

Figure S9

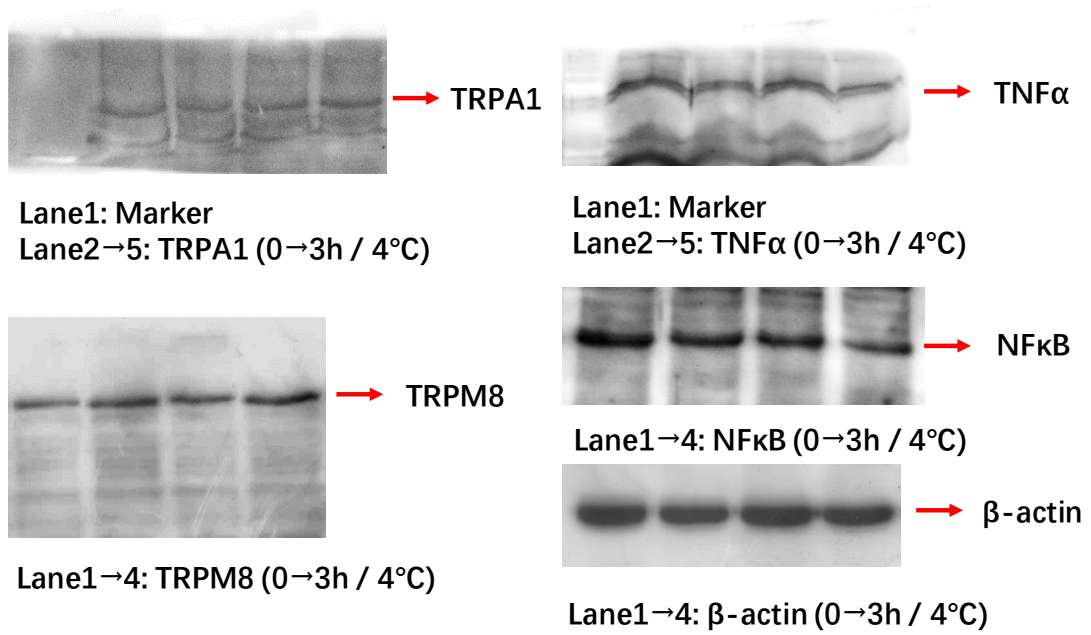


Figure S9. Original blots of cropped western blots of Figure 1. The expression levels of TRPM8, TRPA1, NFκB and TNFα in mouse brains under cold conditions.

Figure S10

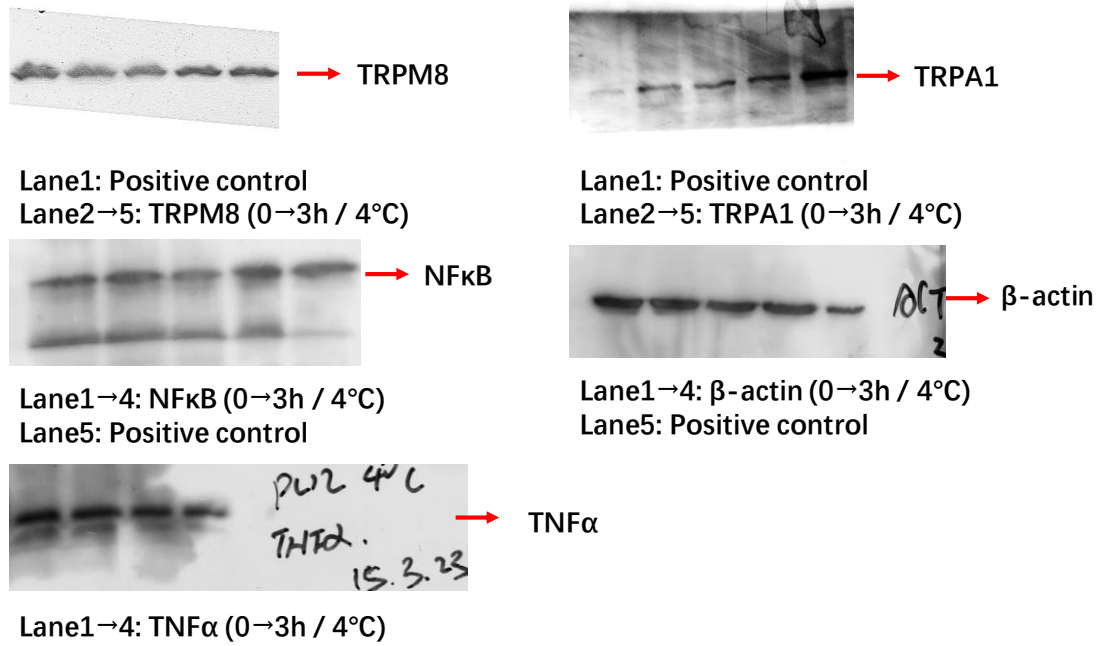


Figure S10. Original blots of cropped western blots of Figure 2. Expression of TRPM8, TRPA1, NFκB and TNFα in PC12 cells under cold conditions.

Figure S11

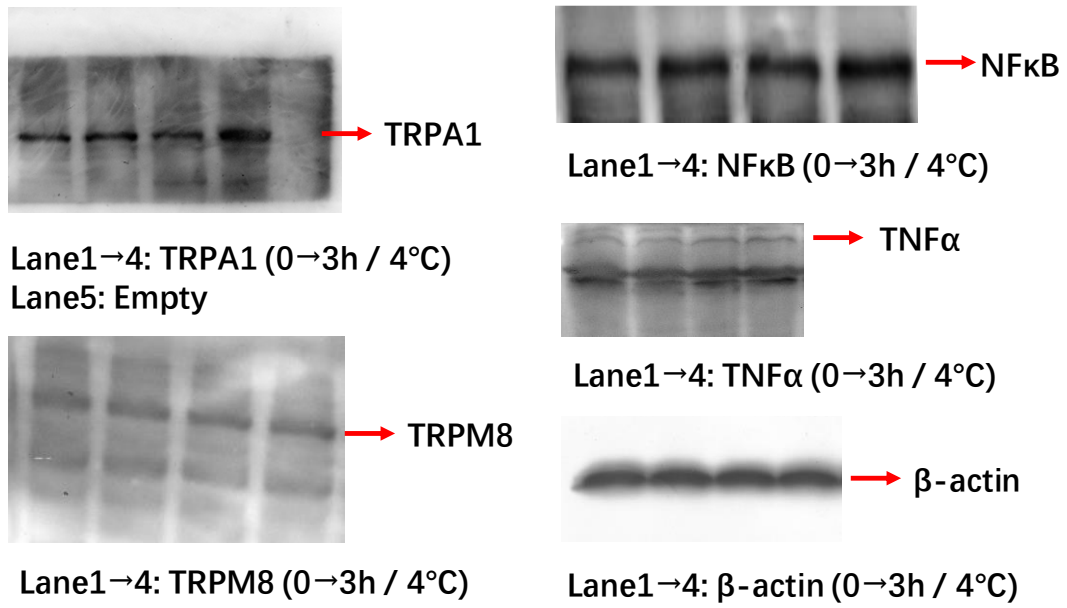
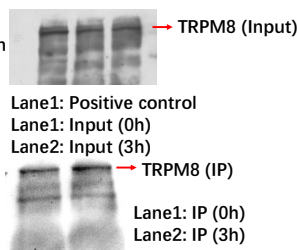
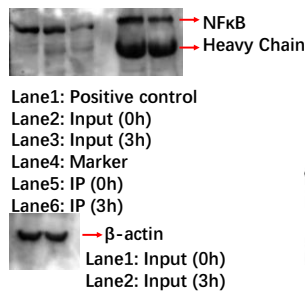


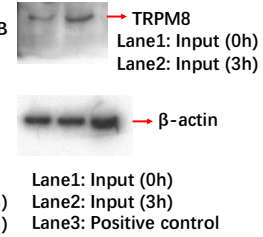
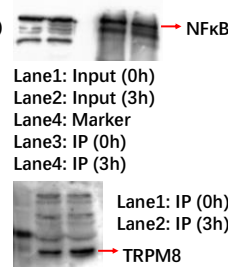
Figure S11. Original blots of cropped western blots of Figure 3. Expression of TRPM8, TRPA1, NFκB and TNFα in PC12 cells with Trpm8 knockdown under cold conditions.

Figure S12

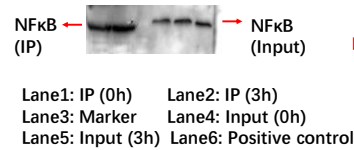
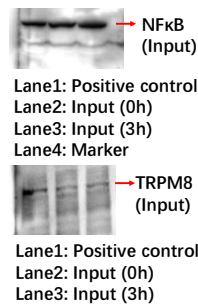
IP: NFκB (WT)



IP: TRPM8 (WT)



IP: NFκB (KD)



IP: TRPM8 (KD)

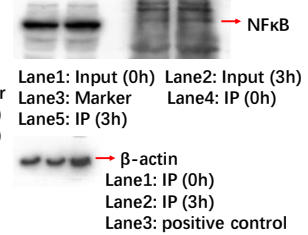
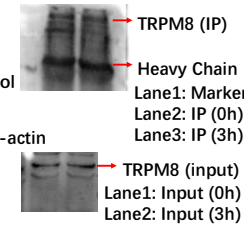


Figure S12. Original blots of cropped western blots of Figure 5. Co-immunoprecipitation (CoIP) of endogenous TRPM8 and NFκB using NFκB antibodies and reverse CoIP performed with TRPM8 antibodies in PC12 (WT) and (KD) respectively.

Figure S13

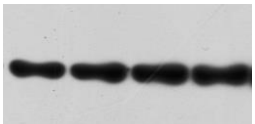
WT

C-NFκB ←  → N-NFκB


Lane1→4: NFκB in Cytoplasm (0→3 h/ 4°C)

Lane5: Marker

Lane6→9: NFκB in Nucleus (0→3 h/ 4°C)

β-actin ←  Lane1→4: β-actin (0→3 h/ 4°C)

KD

C-NFκB ←  Lane1→4: NFκB in Cytoplasm (0→3 h/ 4°C)

N-NFκB ←  Lane1→4: NFκB in Nucleus (0→3 h/ 4°C)

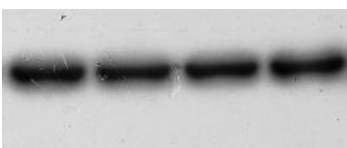
β-actin ←  Lane1→4: β-actin (0→3 h/ 4°C)

Figure S13. Original blots of cropped western blots of Figure 6. Protein expression of NFκB in PC12 cells under cold conditions (4 °C).

Figure S14

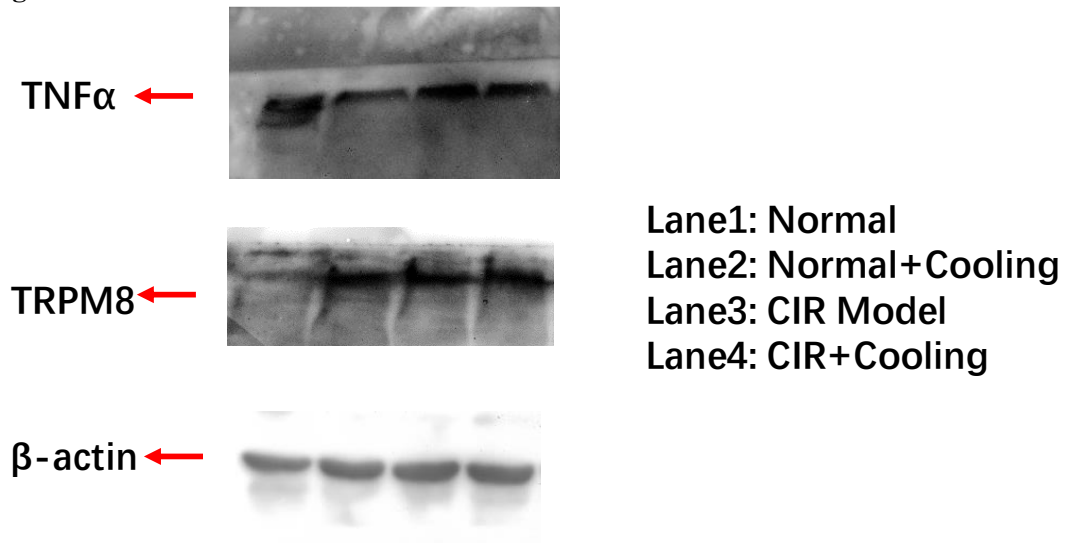


Figure S14. Original blots of cropped western blots of Figure 7. Protein expression of TRPM8 and TNF α in mouse brain with cerebral ischemia-reperfusion (CIR)..

Table S1

Table S1 Primer for mouse

Gene	Sense	Anti-sense
TRPM8	TATGAGACCCGAGCAGTGGA	CAGGCTGAGCGATGAAATGC
TRPA1	GTCCAGGGCGTTGTCTATCG	CGTGATGCAGAGGACAGAGAT
NF κ B	GGAGGCATGTTCCGGTAGTGG	CCCTGCGTTGGATTCGTG
TNF α	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
β -actin	AGGCCACACAAATAGGGTCC	TTGTGGACACTGCCCCATTC

Table S2

Table S2 Primer for PC12 cells (rat)

Gene	Sense	Anti-sense
TRPM8	TCATACCCACCTGCTGCTTG	CCTGGGCAAAACACACGATG
TRPA1	GCAGCATTTTCAGGTGCCAA	CGCTGTCCAGGCACATCTTA
NFκB	CGCCTGAGACCCGAGACAAG	CTGCCTCCTGCTCCACTGAC
TNFα	CTCCAGCTGGAAGACTCCTCCCAG	CCCGACTACGTGCTCCTCACC
β-actin	CCGTAAAGACCTCTATGCCAACA	CGGACTCATCGTACTCCTGCTT