Supplementary Figures and Figure legends

M2 macrophage-secreted Slit3 intensifies sympathetic nerve function in adipose tissue and enhances thermogenesis: A long-term cold adaption way

Yi-Na Wang[1], Yan Tang[1], Zhi-Hui He[1], Hong Ma[2], Yang Liu[1], Qi-Qi Yang[1], Dong-Ning Pan[1], Shu-Wen Qian[1,*], and Qi-Qun Tang[1,*]

[1]Key Laboratory of Metabolism and Molecular Medicine, the Ministry of Education; Department of Biochemistry and Molecular Biology of School of Basic Medical Sciences and Department of Endocrinology and Metabolism of Zhongshan Hospital, Fudan University, Shanghai 200032, China.

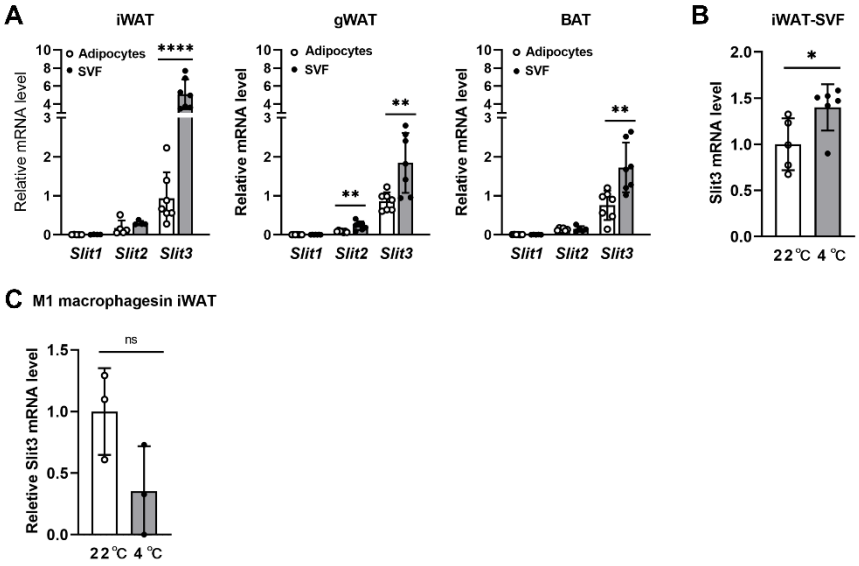
[2]Key Laboratory of Metabolism and Molecular Medicine, the Ministry of Education; Institutes of Biomedical Sciences, Fudan University, Shanghai 200032, China.

*Correspondence to:

Shu-Wen Qian, E-mail: shuwenqian2013@163.com

Qi-Qun Tang, E-mail: qqtang@shmu.edu.cn

Supplementary Figure 1



Supplementary Figure 1: *Slit3* is a cold induced gene in M2 macrophages from iWAT

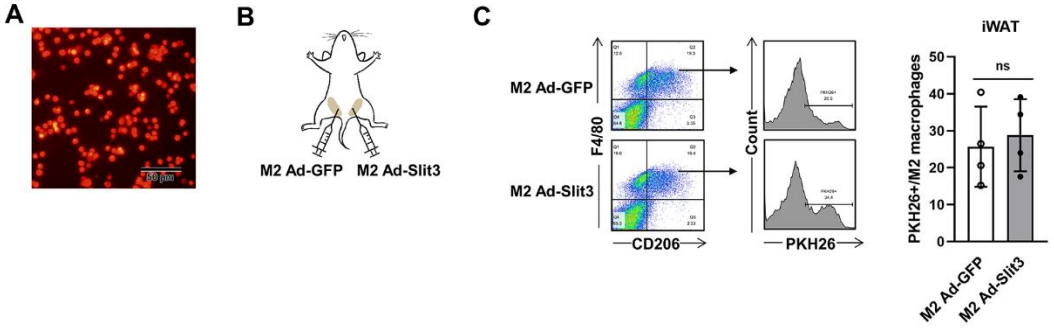
A. Gene expression of *Slit1*, *Slit2* and *Slit3* in SVF isolated from iWAT, gWAT and BAT of WT mice (n =5-7 per group).

B. Gene expression of *Slit3* in SVF isolated from iWAT of WT mice housed at 22°C or exposed to 4°C for 3d (n=5/6).

C. Gene expression of *Slit3* in M1 macrophages in iWAT from mice housed at 22°C or exposed to 4°C for 3d, total SVF pooled from 30 mice in each group were subjected to FACS and M2 macrophages were collected (n=3 per group).

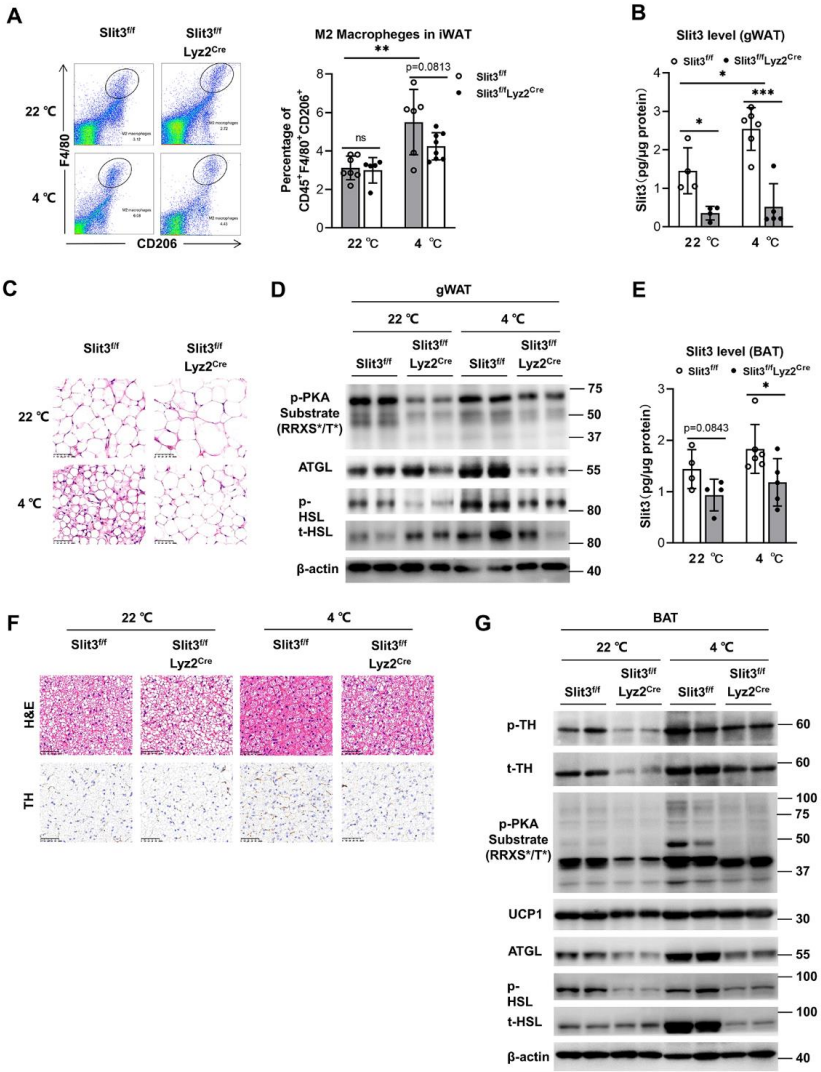
Data are presented as mean ± SEM. Student's t test was used for comparisons. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supplementary Figure 2



Supplementary Figure 2: Implanted M2 macrophages can survive in iWAT
A. PKH26-labeled M2 macrophages.
B. M2 macrophages cultured in vitro were injecteded into the iWAT by contralateral s.c. injection.
C. Flow cytometry analysis for PKH26-labeled M2 macrophages in iWAT 7d after injection.
Data are presented as mean ± SEM. Student's t test was used for comparisons.

Supplementary Figure 3



Supplementary Figure 3: Myeloid deletion of Slit3 exhibit larger adipocytes and impaires thermogenesis

A. Flow cytometry analysis for M2 macrophages (CD45+F4/80+CD206+) in iWAT from Slit3$^{f/f}$ and Slit3$^{f/f}$Lyz2^{Cre} mice housed at 22°C or exposed to 4°C for 24h（n=5-8 per genotype）.

B. Slit3 levels in gWAT of Slit3$^{f/f}$ and Slit3$^{f/f}$Lyz2^{Cre} mice that were housed at 22°C or exposed to 4°C for 24h was determined by ELISA analyze (n=4-6 per genotype). Results were normalized to the total protein levels.

C. H&E staining in gWAT isolated from Slit3$^{f/f}$ and Slit3$^{f/f}$Lyz2^{Cre} mice. Scale bar: 100μm.

D. Western blot analysis of p-(Ser/Thr)-PKA substrate, ATGL, phospho- and total HSL in gWAT isolated from Slit3$^{f/f}$ and Slit3$^{f/f}$Lyz2^{Cre} of mice.

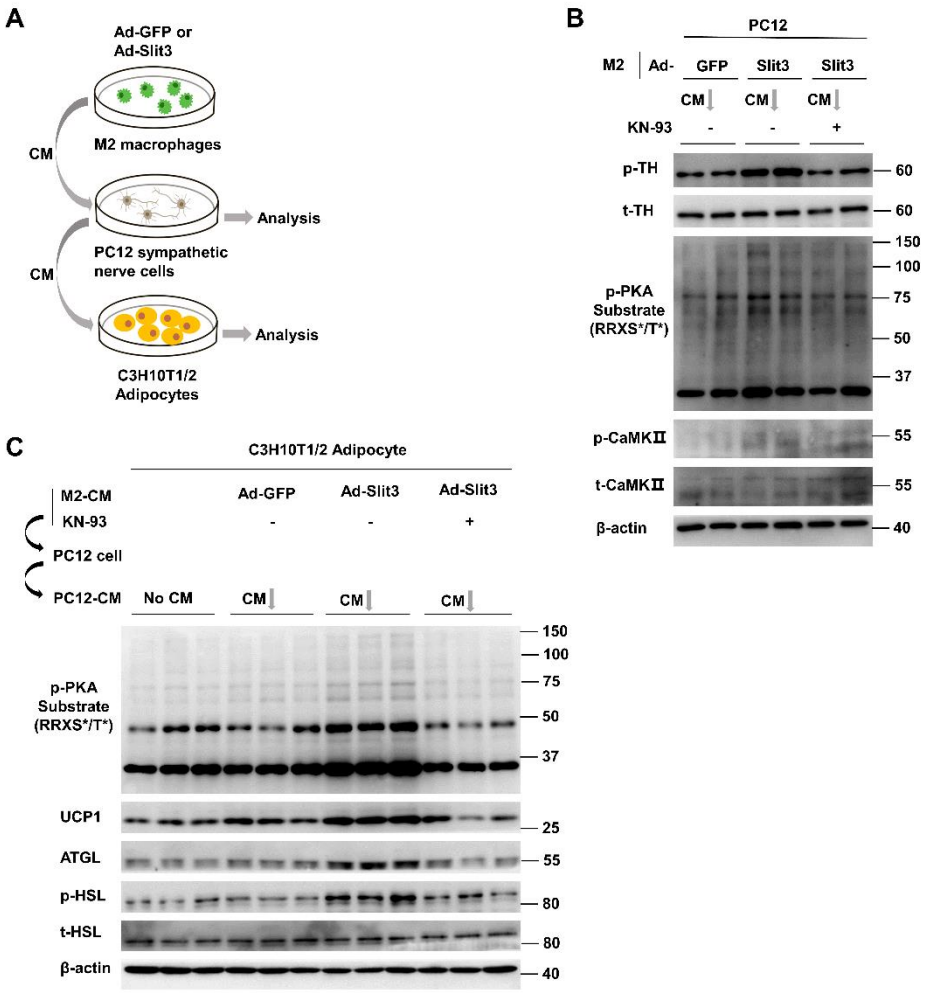
E. Slit3 levels in BAT of Slit3$^{f/f}$ and Slit3$^{f/f}$Lyz2^{Cre} mice that were housed at 22°C or exposed to 4°C for 24h was determined by ELISA analyze(n=4-6 per genotype). Results were normalized to the total protein levels.

F. H&E staining, IHC staining with anti-TH antibody in BAT isolated from Slit3$^{f/f}$ and Slit3$^{f/f}$Lyz2^{Cre} mice. Scale bar: 100μm.

G. Western blot analysis of phospho- and total TH, p-(Ser/Thr)-PKA substrate, UCP1, ATGL, phospho- and total HSL in BATT isolated from Slit3$^{f/f}$ and Slit3$^{f/f}$Lyz2^{Cre} of mice.

Data are presented as mean ± SEM. Student's t test was used for comparisons. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supplementary Figure 4



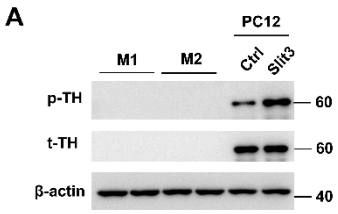
Supplementary Figure 4: M2 macrophages-secreted Slit3 stimulates the phosphorylation of TH

A. Experimental approach to evalute the effect of Slit3 overexpressed M2 macrophages on PC12 sympathetic nerve cells, then the effect of PC12 cells on C3H10T1/2 adipocytes was evaluated. C3H10T1/2 adipocytes.

B. Western blot analysis for phospho- and total TH, p-PKA substrate (RRXS*/T*) and phospho- and total CaMKII in PC12 cells upon 24h-treatment with conditioned medium from M2 macrophages.

C. Western blot analysis for p-PKA substrate (RRXS*/T*), UCP1, ATGL, phospho- and total HSL in C3H10T1/2 adipocytes upon 24h-treatment with conditioned medium from PC12 cells in panel A.

Supplementary Figure 5



Supplementary Figure 5: H is not expressed in macrophages
A. Western blot analysis for phospho- and total TH in M1, M2 macrophages and PC12.