Supplemental materials

NSun2 Regulates Aneurysm Formation by Promoting Autotaxin Expression and T Cell Recruitment

Yutong Miao[1#], Yang Zhao[2#], Lulu Han[1], Xiaolong Ma[1], Jiacheng Deng[3], Juan Yang[1], Silin Lü[4], Fangyu Shao[1], Wei Kong[1], Wengong Wang[5], Qingbo Xu[3], Xian Wang[1*], Juan Feng[1*]

[1]Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Peking University, Key Laboratory of Molecular Cardiovascular Science, Ministry of Education, Beijing, P. R. China
[2]Department of Laboratory Medicine, Peking University Third Hospital, Beijing, P. R. China
[3]Cardiovascular Division, BHF Center for Vascular Regeneration, King's College London, London, UK
[4]State Key Laboratory of Bioactive Substances and Function of Natural Medicine, Institute of Materia Medica, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, P. R. China
[5]Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Peking University, Beijing, P. R. China

[#] These authors contributed equally to this article.
*Corresponding authors are Juan Feng and Xian Wang.

Supplemental Figures

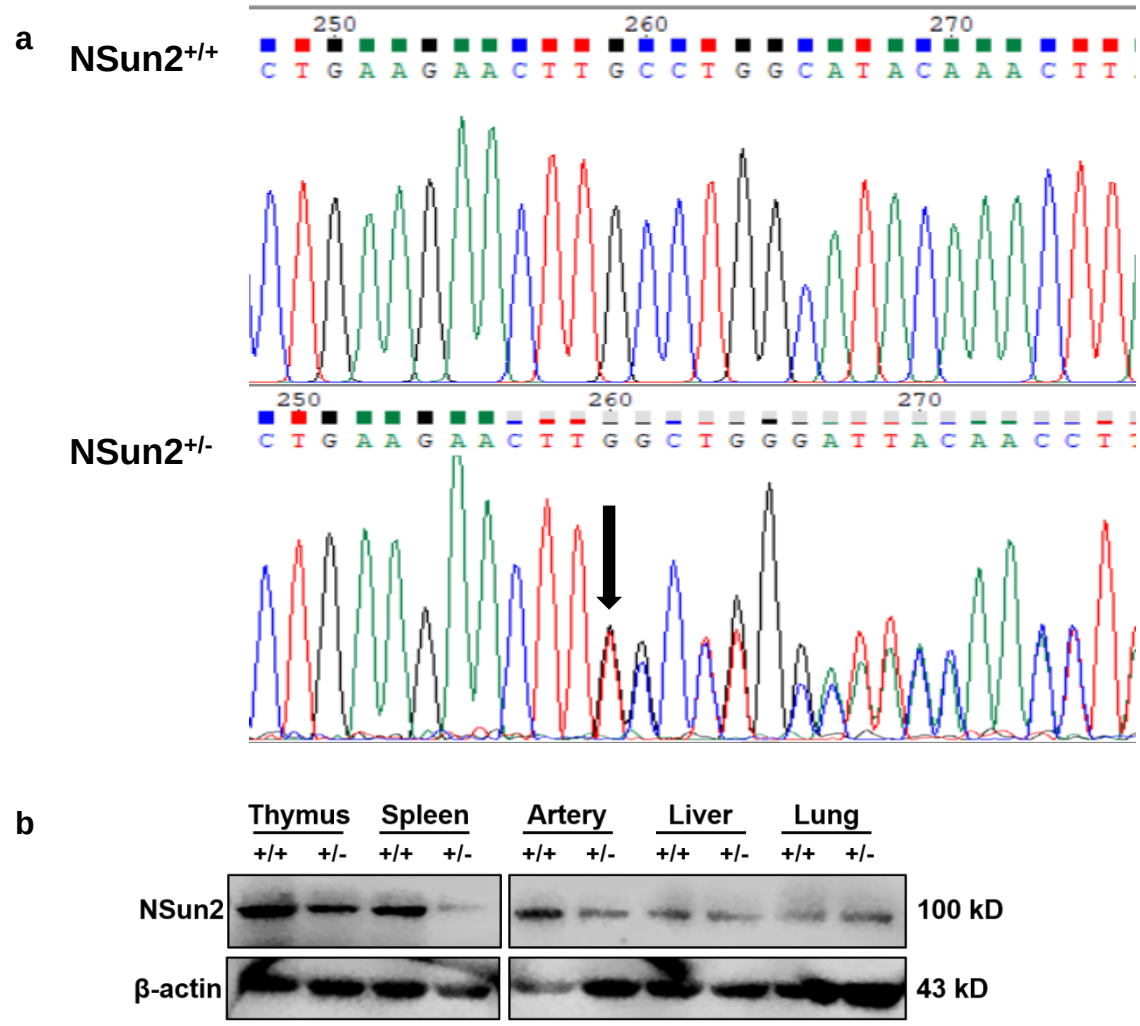

Fig. S1 Validation of NSun2$^{+/-}$ mice. **a** DNA sequence analysis was performed to confirm the deletion of NSun2 in the NSun2$^{+/-}$ mice. The deleted nucleotides are indicated. **b** Representative Western blot analysis of NSun2 expression in various tissues from WT and NSun2$^{+/-}$ mice.

Supplemental Figures

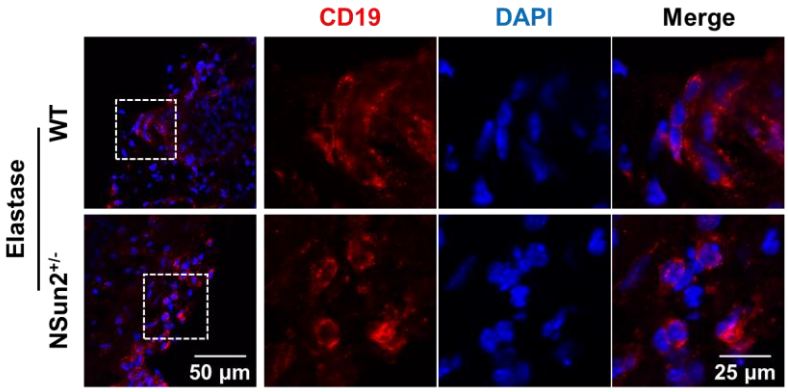

Fig. S2 NSun2 deficiency does not affect the recruitment of B cells to the aortic wall during AAA formation. Infrarenal abdominal aortas of 10- to 11-week-old WT and NSun2$^{+/-}$ mice were treated with saline or elastase to induce abdominal aortic aneurysm. Representative immunofluorescent staining of B cells (CD19) in infrarenal abdominal aortas. Scale bar, 25 μm.

Supplemental Figures

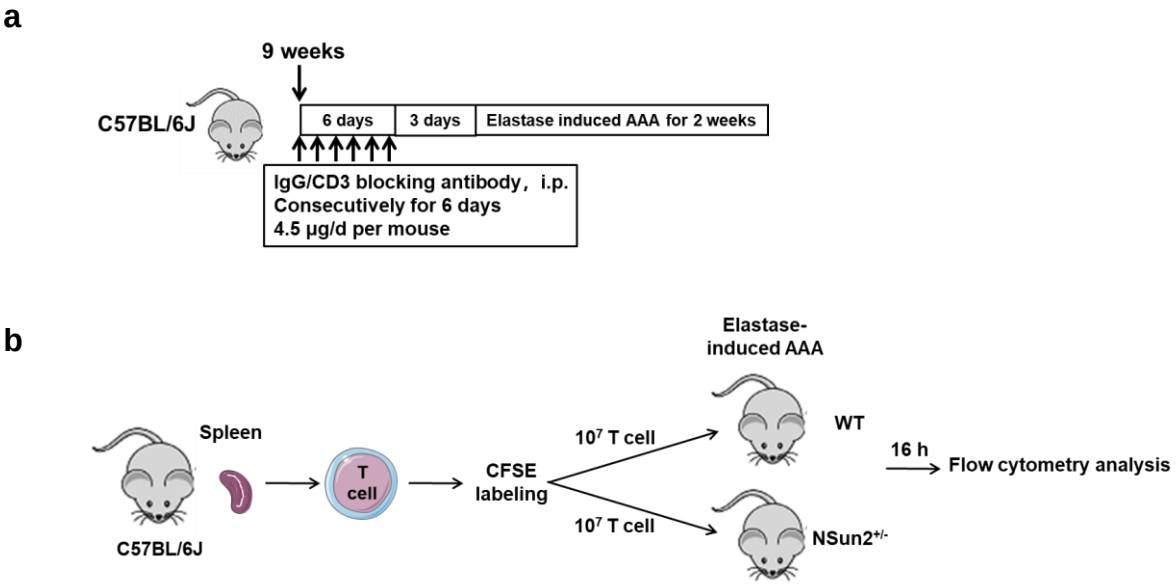

Fig. S3 Schematic protocols in Fig. 2. **a** Recipient C57BL/6J mice were intraperitoneally injected with anti-CD3ε F(ab')2 fragments or IgG isotype for six consecutive days, followed by an AAA-inducing operation. **b** $CD3^+$ T cells from WT mice were labeled with CFSE. A total of 10^7 labeled cells were intravenously injected into one recipient WT or $NSun2^{+/-}$ mouse whose infrarenal abdominal aorta had been treated with elastase for 3 days. Sixteen hours later, the aortas were digested into single cells and the proportions of donor cells in infrarenal abdominal were measured by flow cytometry.

Supplemental Figures

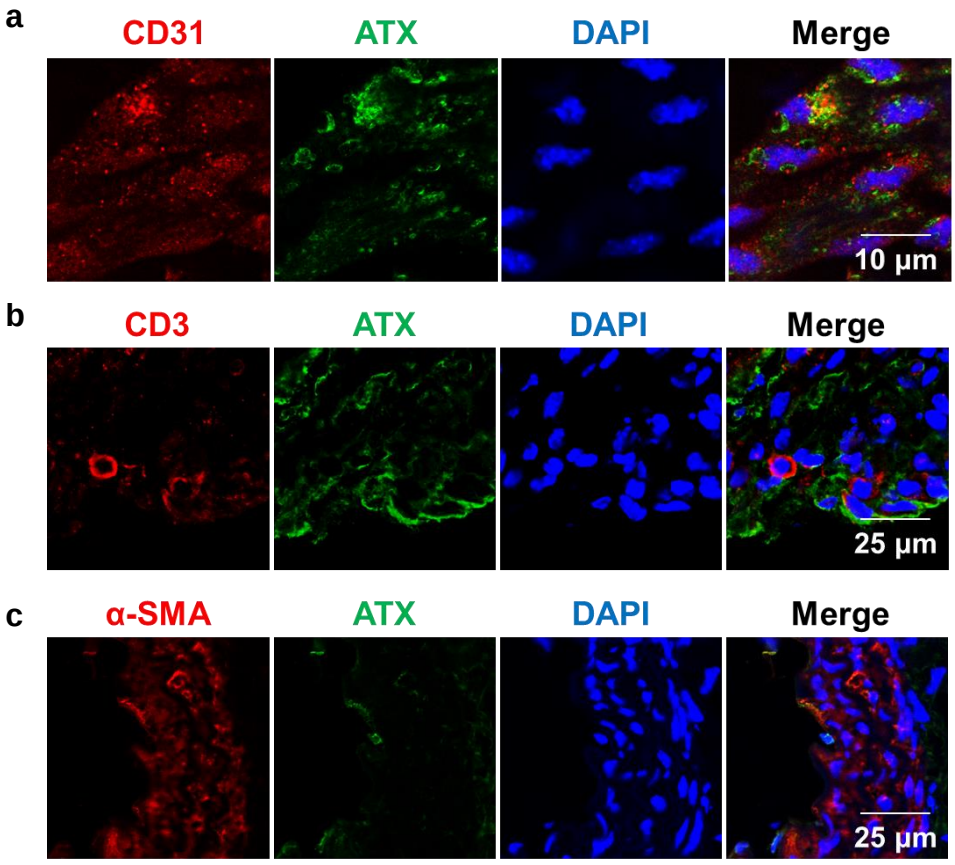

Fig. S4 T cells and smooth muscle cells do not express ATX in elastase-induced AAA. Infrarenal abdominal aortas of 10- to 11-week-old WT mice were treated with elastase to induce abdominal aortic aneurysm. **a** Representative immunofluorescent *enface* staining of ATX expression in the endothelial layer of infrarenal abdominal aortas. Scale bar, 10 μm. **b-c** Representative immunofluorescent staining of ATX expression in T cells (CD3; **b**) and SMC (α-SMA; **c**) in infrarenal abdominal aortas. Scale bar, 25 μm.

Supplemental Figures

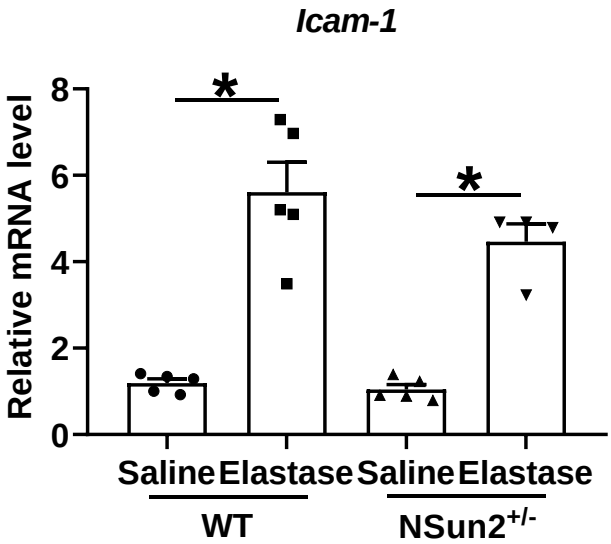

Fig. S5 ICAM-1 expression is upregulated in AAA model. Real-time PCR quantification of mRNA levels of Icam-1 in of infrarenal abdominal aortas (n=4-5). Data shown are mean ± SEM. *P<0.05, by two-way ANOVA followed by Tukey's test for multiple comparisons.

Supplemental Figures

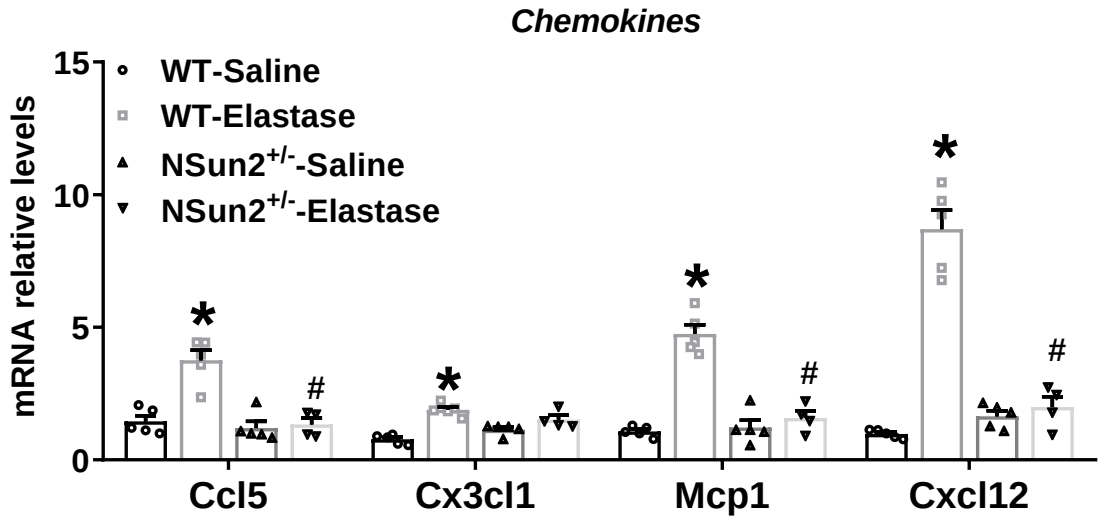

Fig. S6 NSun2 deficiency inhibits the chemokines expression in AAA model. Real-time PCR quantification of mRNA levels of Ccl5, Cx3cl1, Mcp-1 and Cxcl12 in of infrarenal abdominal aortas (n=4-5). Data shown are mean ± SEM. *P<0.05, compared with WT-Saline, #P<0.05, compared with WT-Elastase, by two-way ANOVA followed by Tukey's test for multiple comparisons.

Supplemental Figures

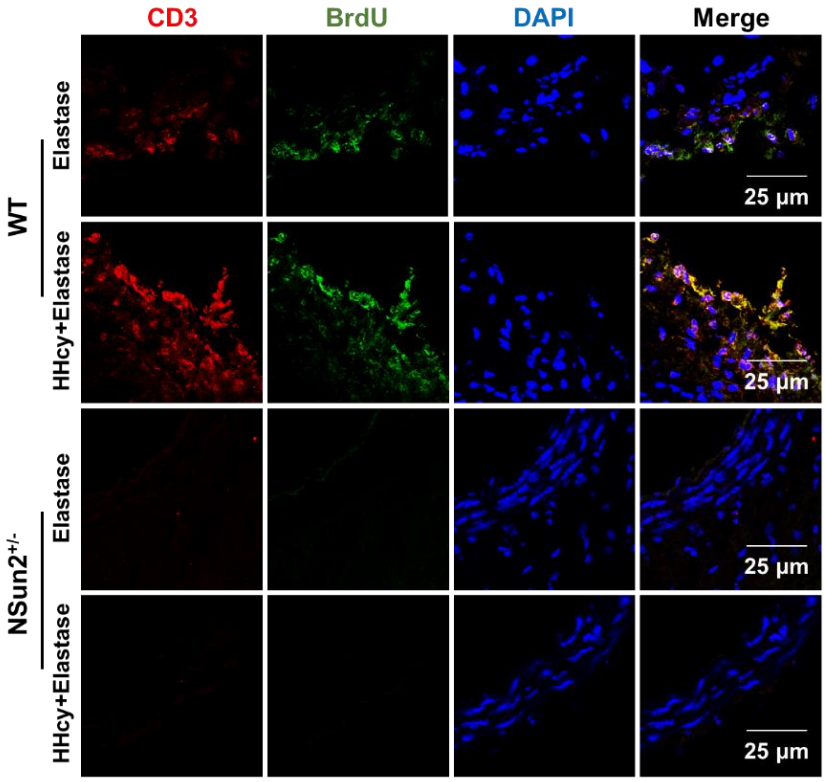

Fig. S7 NSun2 deficiency inhibits HHcy-increased T cell proliferation in AAA lesions. Infrarenal abdominal aortas of 10- to 11-week-old WT and NSun2$^{+/-}$ mice were treated with elastase to induce abdominal aortic aneurysm after the mice were fed a normal chow diet and given drinking water supplemented with or without 1.8 g/L Hcy for 2 weeks. Representative immunofluorescent staining of CD3 and BrdU in infrarenal abdominal aortas. Scale bar, 25 μm.

Supplemental Figures

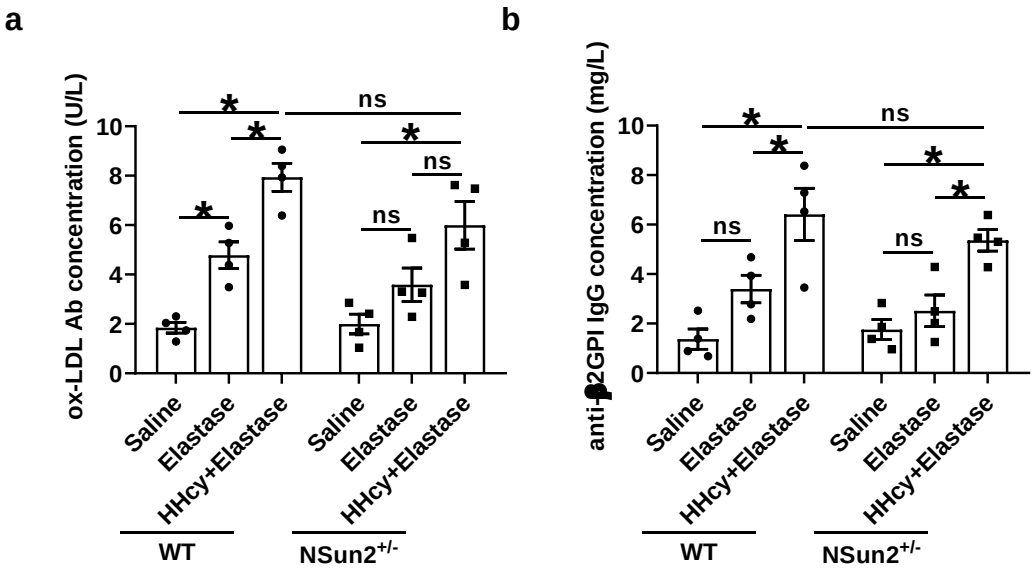

Fig. S8 HHcy increases autoantibody production in AAA model. Infrarenal abdominal aortas of 10- to 11-week-old WT and NSun2$^{+/-}$ mice were treated with elastase to induce abdominal aortic aneurysm after the mice were fed a normal chow diet and given drinking water supplemented with or without 1.8 g/L Hcy for 2 weeks. Plasma ox-LDL IgG (**a**) and anti-β2GPI IgG (**b**) levels were measured via ELISA (n=4). Data shown are mean ± SEM. *P<0.05, by two-way ANOVA followed by Tukey's test for multiple comparisons (**a, b**). ns, no significance.

Supplemental Tables

Table S1. Baseline characteristics of the patients.

	Nonhyperhomocysteinemia	Hyperhomocysteinemia
Sex (Male/Female)	13/11	26/6
Age (years)	45.78 ± 2.18	46.16 ± 2.82
BMI (kg/m^2)	24.10 ± 0.79	25.19 ± 0.57
Systolic BP (mmHg)	125.79 ± 3.69	133.90 ± 3.29
Diastolic BP (mmHg)	75.00 ± 2.91	76.30 ± 1.43
Homocysteine (μmol/L)	9.74 ± 0.57	26.55 ± 1.84 *
Total cholesterol (mmol/L)	4.79 ± 0.17	4.67 ± 0.15
Triglycerides (mmol/L)	1.34 ± 0.19	1.84 ± 0.31
HDL cholesterol (mmol/L)	1.32 ± 0.07	1.19 ± 0.04
LDL cholesterol (mmol/L)	3.05 ± 0.15	2.89 ± 0.13

Data shown are mean ± SEM. *P<0.05, by unpaired Student t test.

Table S2. Primers for RT-PCR.

Primer name	Sequence
mus-β-actin	forward, 5'-GTGACGTTGACATCCGTAAAG-3'; reverse, 5'-GCCGGACTCATCGTACTCC-3'
mus-Enpp2	forward, 5'-TCCTCCCTATCTGAGCTCTTC-3'; reverse, 5'-GGTGATGATGCTGTAGTAGTGG-3'
mus-Itga4	forward, 5'-CCTCAGTGGTCAATCCTGGG-3'; reverse, 5'-CACAAGTCACGATAGAGCCATT-3'
mus-Itgal	forward, 5'-ACCAGGTCTTGCAGATTGAAG-3'; reverse, 5'-TGCTTGTTCGGCAGTGATAGA-3'
mus-Itgb1	forward, 5'-ATGCCAAATCTTGCGGAGAAT-3'; reverse, 5'-TTTGCTGCGATTGGTGACATT-3'
mus-Itgb2	forward, 5'-CAGGAATGCACCAAGTACAAAGT-3'; reverse, 5'-GTCACAGCGCAAGGAGTCA-3'
mus-Itgb7	forward, 5'-AAACGGTGCTGCCCTTTGTAA-3'; reverse, 5'-CTCTCTCTCGAAGGCTTGAGC-3'
mus-Ccl5	forward, 5'-TTTGCCTACCTCTCCCTCG-3'; reverse, 5'-CGACTGCAAGATTGGAGCACT-3'
mus-Ccl2	forward, 5'-TAAAAACCTGGATCGGAACCAAA-3'; reverse, 5'-GCATTAGCTTCAGATTTACGGGT-3'
mus-Cx3cl1	forward, 5'-CTGGCCGCGTTCTTCCATT-3'; reverse, 5'-GCACATGATTTCGCATTTCGT-3'
mus-Cxcl12	forward, 5'-TGCATCAGTGACGGTAAACCA-3'; reverse, 5'-CACAGTTTGGAGTGTTGAGGAT-3'
mus-Icam1	forward, 5'-GTGATGCTCAGGTATCCATCCA-3'; reverse, 5'-CACAGTTCTCAAAGCACAGCG-3'
human-β-actin	forward, 5'-ATCTGGCACCACACCTTC-3'; reverse, 5'-AGCCAGGTCCAGACGCA-3'
human-Enpp2	forward, 5'-ACTTTTGCCGTTGGAGTCAAT-3'; reverse, 5'-GGAGTCTGATAGCACTGTAGGA-3'

Table S3. The lipid profile and leukocyte profile in peripheral blood of WT and NSun2$^{+/-}$ mice.

	WT	NSun2$^{+/-}$
n	16	16
Body weight (g)	23.78 ± 0.47	20.75 ± 0.33 *
Cholesterol (mmol/L)	4.95 ± 0.14	4.81 ± 0.17
Triglycerides (mmol/L)	1.49 ± 0.30	1.49 ± 0.03
Leukocyte (×10^9/L)	6.45 ± 0.64	6.89 ± 0.68
Lymphocyte (×10^9/L)	4.71 ± 0.42	5.58 ± 0.60
Monocyte (×10^9/L)	0.17 ± 0.03	0.25 ± 0.06
Neutrophil (×10^9/L)	0.30 ± 0.07	0.28 ± 0.05

Data shown are mean ± SEM. *P<0.05, by unpaired Student t test.