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Supplementary Materials for

SBP2 deficiency in adipose tissue macrophages drives insulin resistance in obesity

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This PDF file includes:

Supplementary Materials

Supplemental Experimental Details

The in vivo delivery system

β-1,3-d-glucan were prepared from baker's yeast. A suspension of glucan particles encapsulated tRNA/PEI cores was prepared. For knockdown experiment, 2 μg/10 g b.w. siRNA were mixed with 50 nmol Endo-Porter (EP; Gene Tools) in 30 mM sodium acetate (pH 4.8) in a final volume of 20 μL for 15 min at room temperature. 1 mg ($\sim 10^9$) of glucan shells were added to the plasmid/EP mixture, followed by 1 h incubation with vortexing in PBS for injection. For re-expression experiment, 2 μg/10 g b.w. customized TALE-TF plasmids (Genecopoeia, USA) were mixed with glucan shells encapsulated tRNA/PEI ($\sim 10^9$) and mixed 1 h before injection. To examine the in vivo distribution of siRNA-loaded GeRPs, the siRNA/EP mixture was added to 1 mg of 5-(4,6-dichlorotriazinyl) aminofluorescein (Invitrogen; 1 mg/mL in ethanol)-labeled glucan shells to prepare fluorescent-labeled GeRPs for injection.

FACS and quantitative real-time PCR

Equal weights of visceral fat pads were digested in digestion buffer (2 mg/mL collagenase in Hanks' balanced salt solution (HBSS) containing 2% FBS) at 37 °C for 45 min, followed by centrifugation at 800 g for 10 min. The pelleted cells were collected and labeled with live/dead dye (Lifetechnologies, USA), CD45, CD11b, CD64, F4/80 antibody (eBioscience, USA) and loaded in a BD Aria I for sorting. Both SVFs, CD11b+F4/80+ and CD11b-F4/80- populations were collected for further analyses. Total RNA was extracted using an mRNeasy kit (Qiagen, Germany). First strand synthesis was performed (Takara, Japan), and qPCR assays were performed using SYBR Green reagent (Takara, Japan) and an LC480 system (Roche, USA). The primers used were shown in Table S2.

In vivo examination of ATMs proliferation and infiltration

For studying the local proliferation of ATMs in adipose tissue, Edu was injected intraperitoneally into mice at the dose of 10μg/g B.W. 5 h in before sacrifice. Adipose tissue was removed and digested to isolate the cells. The Click-iT Edu assay was then performed according to the manufacturer's instruction (Lifetechnologies, USA). For the study of the infiltration of macrophages into the adipose tissue, 200μl of 2.5μM PKH26PCL dye (Sigma-Aldrich, USA) was intraperitoneally injected to the mice 24 h before sacrifice. Adipose tissue was removed and digested to isolate the cells. CD11b+F4/80+ cells were gated to analyze the proliferation and infiltration of macrophage *in vivo*.

Histology

Tissues were fixed in a 4% paraformaldehyde solution and then subjected to gradual dehydration. Samples were then embedded in paraffin and stained with hematoxylin and eosin (H&E). Images were captured using a BX43 Microscope and Imaging System (Olympus, Japan).

Co-immunoprecipitation and Immunoblotting

Total protein was extracted using NP-40 buffer, and samples were incubated with SBP2 or caspase-1 antibody (Abcam, UK)-conjugated magnetic beads (Millipore, USA) for 1 h. Samples were then washed three times with PBS containing 0.1% Tween 20 and eluted using radioimmunoprecipitation assay (RIPA) buffer mixed with sodium dodecyl sulfate (SDS) loading buffer (Thermo Fisher, USA). Proteins were separated on SDS-PAGE gels and transferred onto polyvinylidene difluoride (PVDF) membranes (Roche, USA). The expression of particular proteins was determined using an ECL select substrate (GE Healthcare, Germany) after incubation with the appropriate primary and secondary antibodies. Images were captured using an XRS+ chemiluminescence system (Biorad, USA).

Bone marrow-derived macrophage (BMDM) differentiation, polarization and inflammasome activation

BMDMs were isolated from the femurs of C57BL/J mice using Ficoll methods. The isolated monocytes were stimulated with 10 ng/mL M-CSF (eBioscience, USA). To modify the expression of the SBP2 gene, siRNA/plasmids were transfected into differentiated BMDMs using the Lipofectamine/FuGene transfection reagent on the second day. Transfection was performed every 2 days. 10 ng/mL LPS and 20 ng/mL IFNγ were added for 16 h to stimulate M1-like polarization, while 20 ng/mL IL4 and 20 ng/mL IL13 treatment were added for 16 h to stimulate M2-like polarization. To induce metabolic activation of BMDMs, 10mM glucose, 10μM insulin or 500μM BSA-conjugated palmitate was added to treat BMDMs for 24 h. To induce the activation of the inflammasome, differentiated BMDMs were primed with 100 ng/mL LPS for 16 h, followed by 4 h of treatment with 5 mM ATP, 150 μg/mL MSU or 100 μM nigericin.

Supplemental Figures

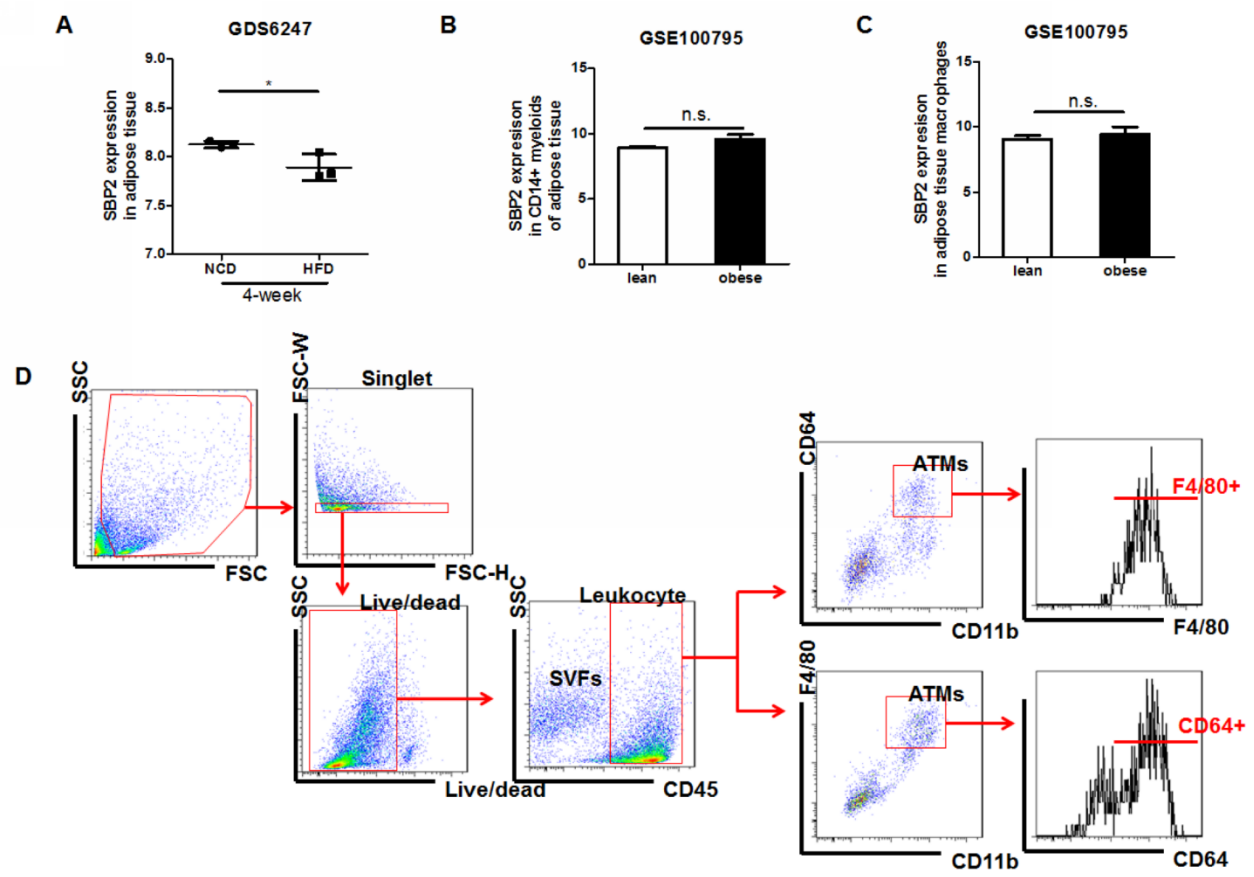

Fig. S1. Determination of SBP2 expression in SBP2 tissue. Previous transcriptomic data were collected from a Gene Expression Omnibus (GEO) datasets. Expression of SBP2 was retrieved and analyzed. A) Data from GDS6247 found 4-week HFD treatment significantly suppressed SBP2 expression in adipose tissue of mice; data from dataset GSE100795 showed no significant difference of SBP2 expression in B) macrophages or C) CD14+ myeloid cells from adipose tissue of non-obese or obese female patients; D) Fluorescence-activated cell sorting (FACS) gating strategy. Cells were separated from debris by a forward scatter (FSC) v.s. side scatter (SSC); then singlet cells were identified by plotting FSC-area (FSC-A) against FSC-width (FSC-W); Live cells were identified from singlet cells by gating on Live/dead dye-negative population; SVFs (CD45-) and leukocytes (CD45+) were differentiated by CD45+ staining on live singlet cells; The fraction of CD11b+F480+ or CD11b+CD64+ within live leukocyte was considered ATMs. The CD11b+F480+ and CD11b+CD64+ were mostly overlapped.

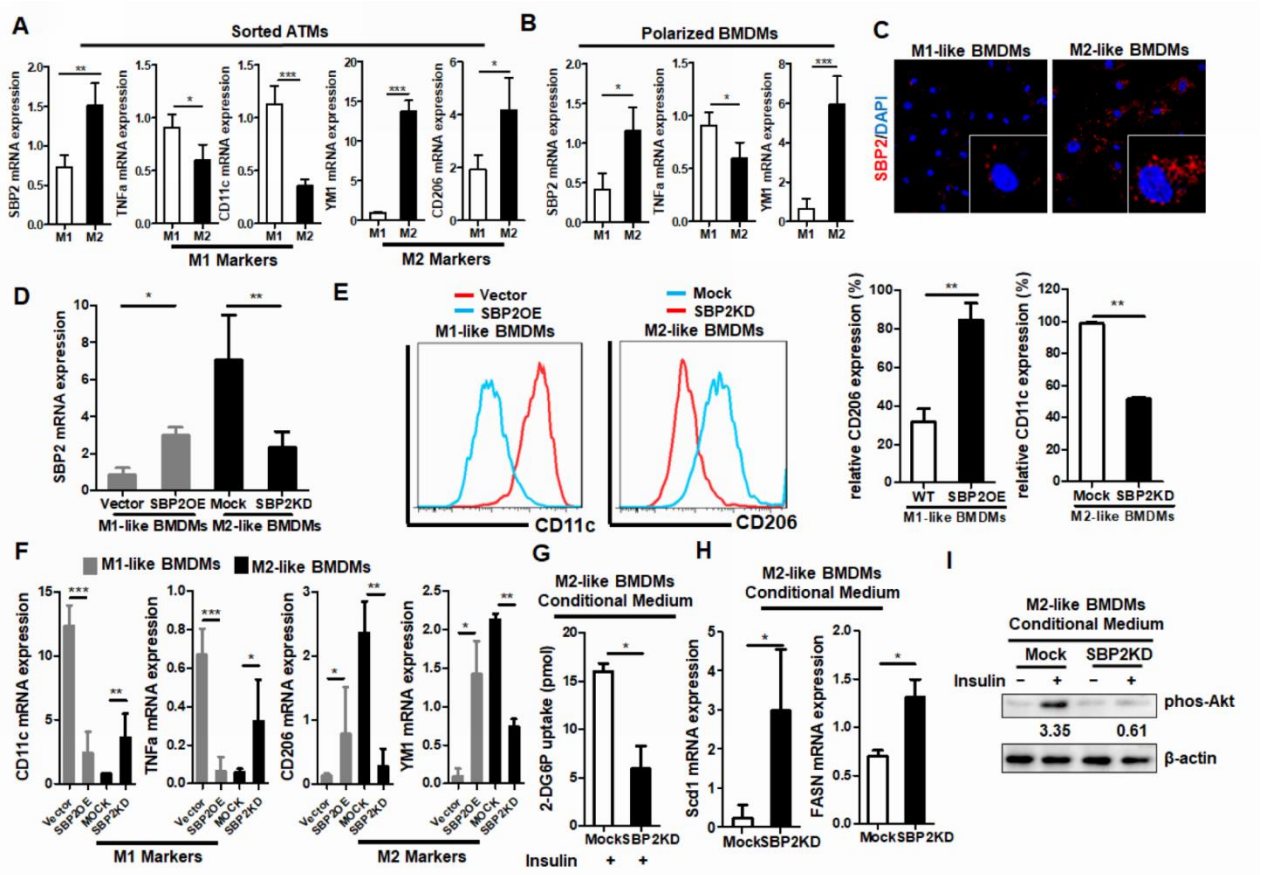

Fig. S2. SBP2 expression controls the phenotype polarization of ATMs. F4/80+CD11c ATMs and F4/80+CD206+ ATMs were sorted from adipose tissue of HFD mice. A) Expression of SBP2 was determined by qRT-PCR. SBP2 expression in F4/80+CD206+ ATMs was significantly higher than that in F4/80+CD11c+ ATMs. F4/80+CD206+ ATMs expressed higher M2-like markers while F4/80+CD11c+ ATMs expressed higher M1-like markers. BMDMs were isolated, differentiated and polarized. B) The mRNA and C) protein expression of SBP2 was significantly higher in M2-like BMDMs than that in M1-like population. D) M1-like BMDMs were transfected with expression plasmid encoding SBP2 while M2-like BMDMs were transfected with siRNA against SBP2. E) & F) Overexpression of SBP2 in M1-like BMDMs induced M2-like cell surface marker CD206 and M2-like gene expression. Knockdown of SBP2 in M2-like BMDMs induced M1-like cell surface marker CD11c and M1-like gene expression. 3T3-L1 pre-adipocytes were treated with conditional medium (CM) from M2-like BMDMs with or without SBP2 knockdown for 48 h. G) 2-DG6P uptake in the presence of insulin was significantly lower in adipocytes treated with CM from SBP2-knockdown M2-like BMDMs. H) Expression of adipogenesis markers Scd1 and FASN were induced in adipocytes treated with CM from SBP2-knockdown M2-like BMDMs, while I) response of Akt signaling to insulin was blunted. *p<0.05, **p<0.01, ***p<0.001

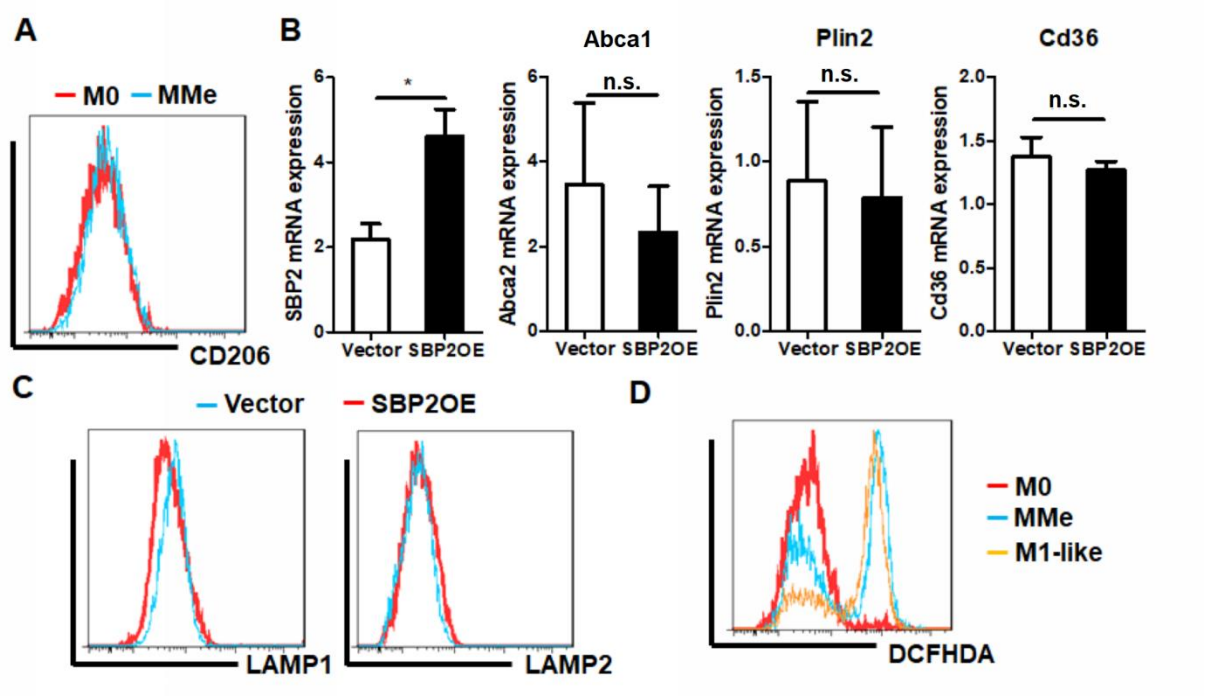

Fig. S3. SBP2 overexpression had no significant effect on the lysosome exocytosis of MMe. A) Palmitate-activated MMe showed no significant changes in CD206 than unstimulated BMDMs. Overexpression of SBP2 had minimal effect on B) the expression of genes related lipid metabolism as well as C) cell surface presentation of LAMP1 and LAMP2, suggesting that SBP2 overexpression did not alter the lysosome exocytosis property of MMe. D) Both MMe and M1-like macrophages expressed a higher level of intracellular ROS than unstimulated BMDMs.

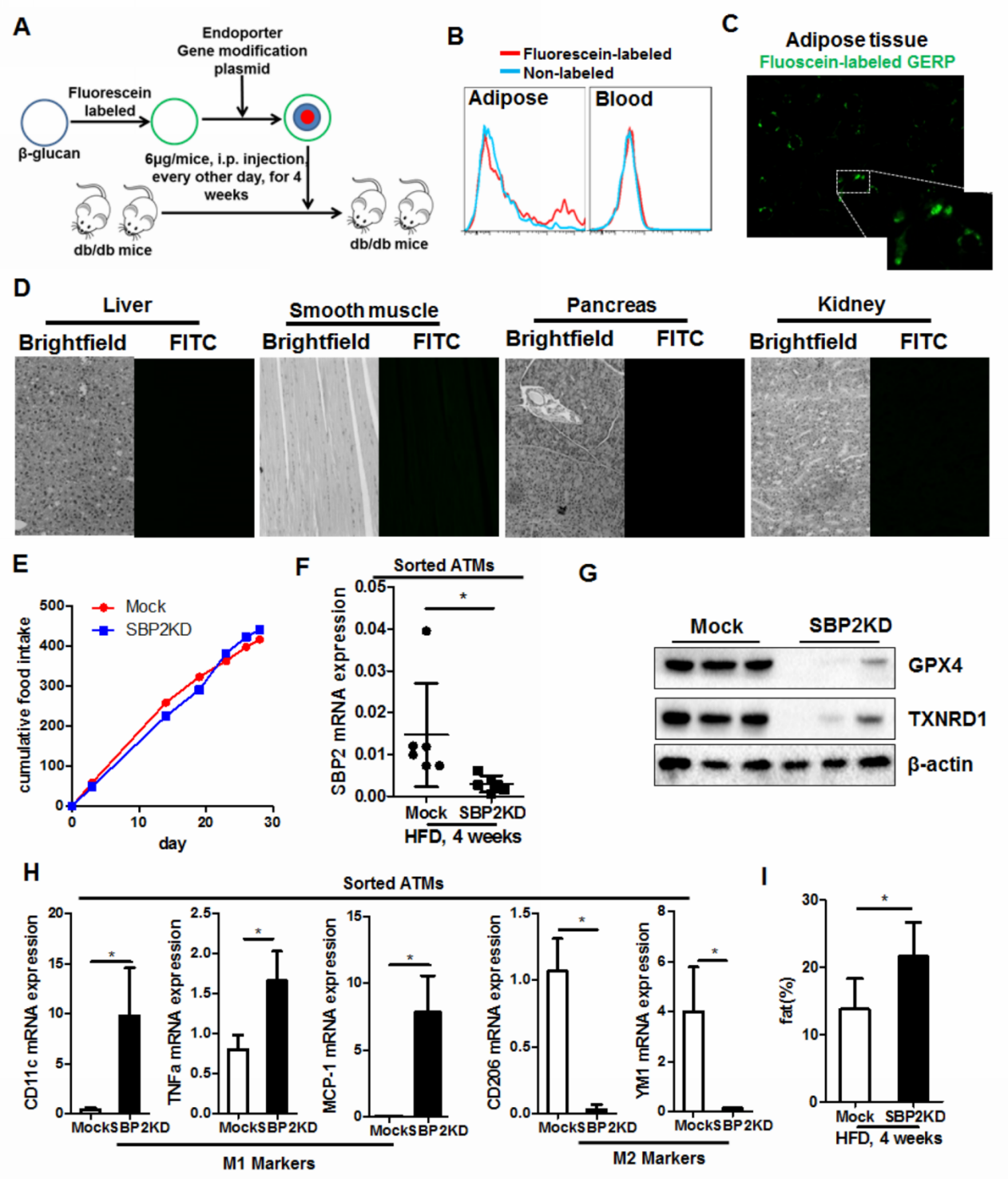

Fig. S4. Glucan-mediated macrophage-targeted delivery specifically suppressed SBP2 in ATMs. A) flowchart of the macrophage-specific knockdown of SBP2 in DIO mice; B) and C) showed the GeRP system delivered siRNA specifically targeted SBP2 in ATMs without affecting circulating mononuclear cells. D) showed that GeRP system did not deliver siRNA into other major organs including liver, pancreas, smooth muscles and kidney. E) Administration of SBP2 siRNA-carrying GeRPs had no significant effect on the food intake of mice, and F) quantitative real-time PCR analysis suggested GeRPs suppressed SBP2 expression in ATMs; Suppression of SBP2 in ATMs G) reduced expression of GPX4 and TXNRD1 in adipose tissue, H) induced M1-like polarization of M2-like ATMs, and I) increased percentage of fat tissue in the body composition . *p<0.05

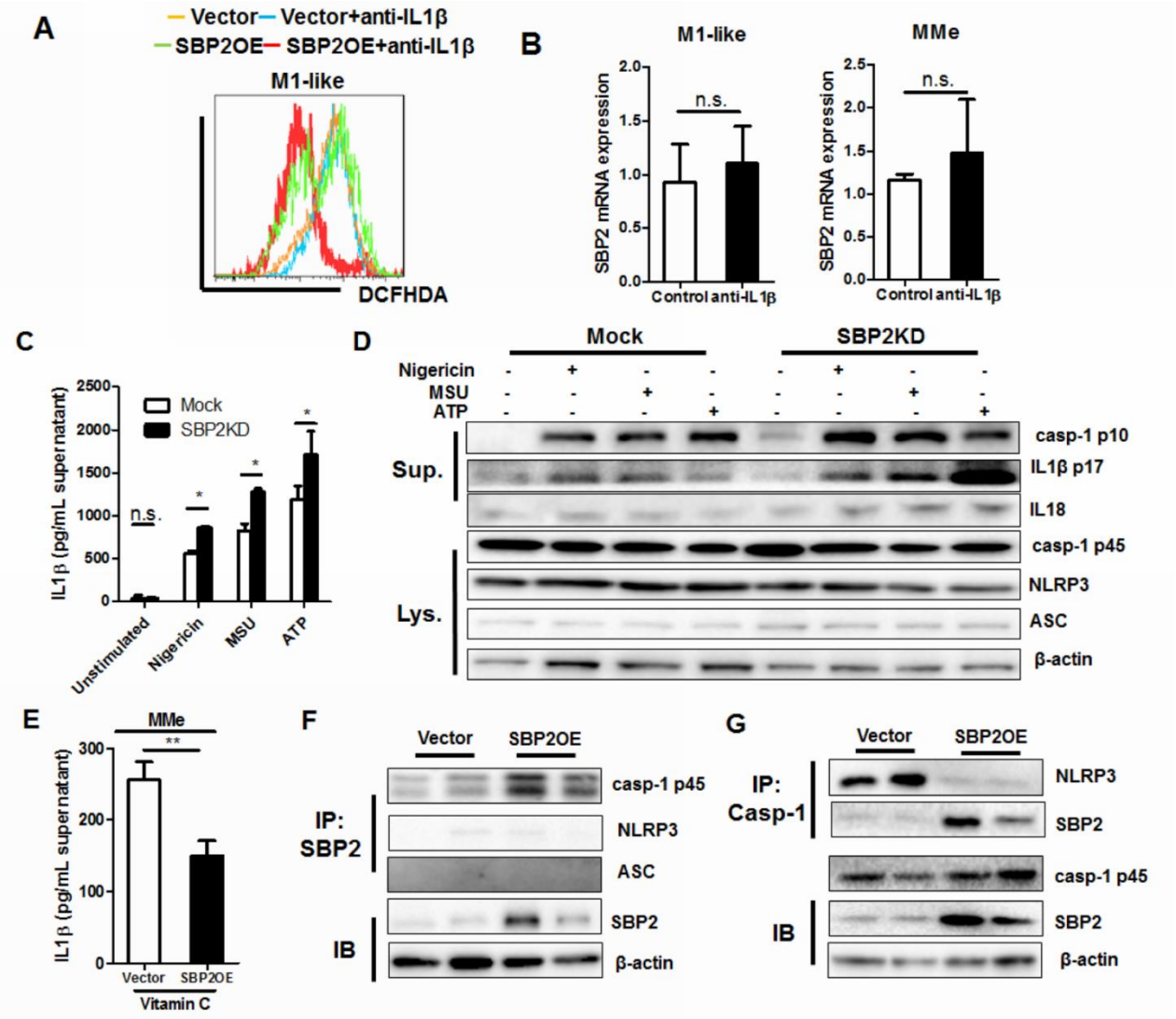

Fig. S5. SBP2 suppressed activation of caspase-1 by the inflammasome. A) In M1-like macrophages, overexpression of SBP2 significantly suppressed ROS production, while the presence of IL1β neutralizing antibody further reduced the ROS level, however, B) presence of IL1β neutralizing antibody had minimal effect in increasing the expression of SBP2 in both MMe and M1-like macrophages. C) SBP2 knockdown further increased the production of IL1β in BMDMs treated with secreted inflammasome inducers ATP, MSU, and nigericin, which was associated with further D) activation of caspase-1 and production of IL1β and IL18 by inflammasome in macrophages without SBP2; E) SBP2 overexpression was able to further suppress IL1β production in MMe in the presence of ROS scavenger vitamin C; F) SBP2 bound to pro-caspase-1 to reduce its activation by the association with inflammasome. G) This could be due to the competitive binding of SBP2 with caspase-1 to block its association with inflammasome.*$p<0.05$; **$p<0.01$*

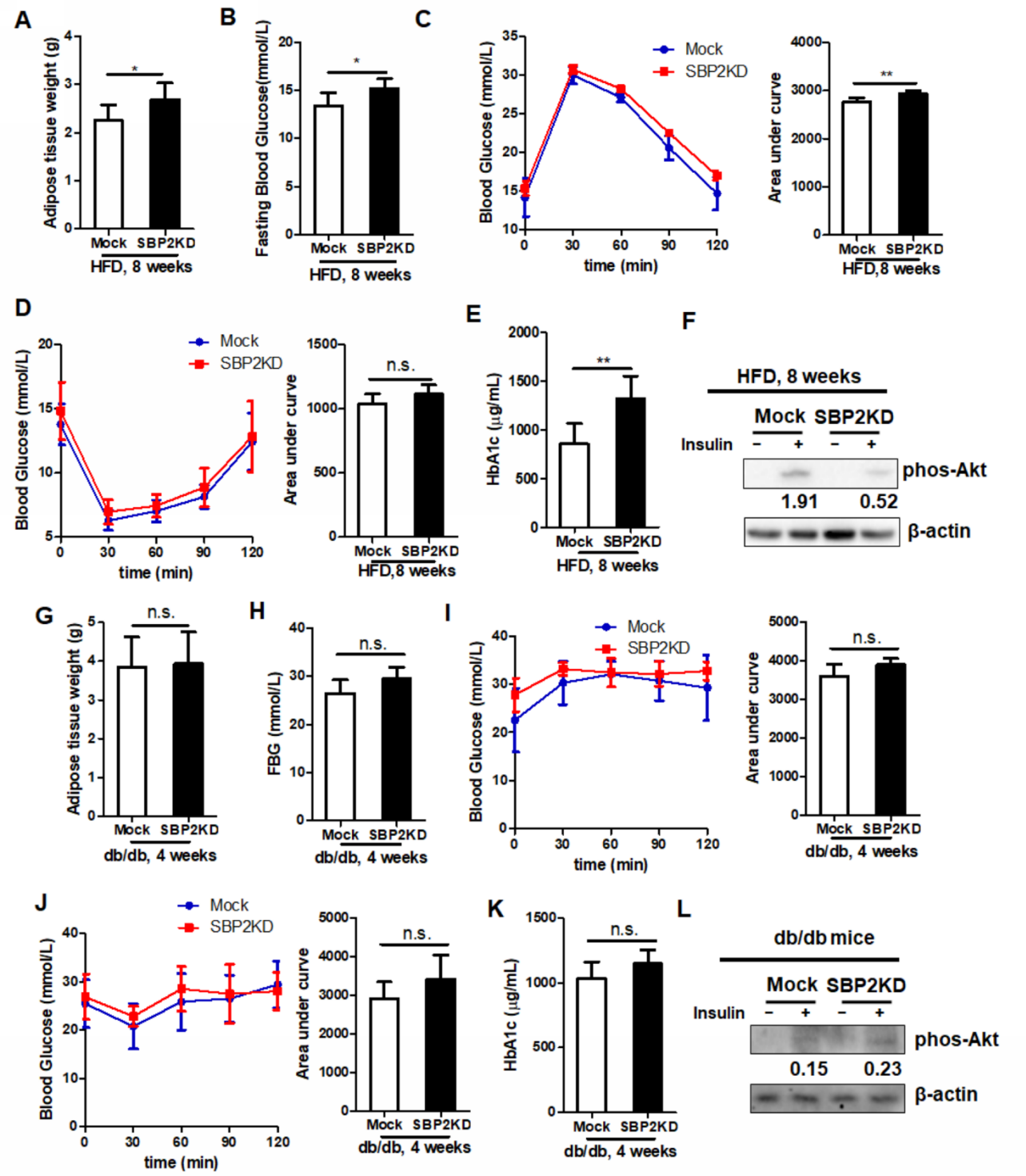

Fig. S6. Eight-week knockdown of SBP2 in ATMs accelerated insulin resistance in DIO mice but not in diabetic db/db mice (*n* = 6). A) Knockdown of SBP2 for 8 weeks accelerated gain of adipose weight in mice fed with HFD. Knockdown of SBP2 for 8 weeks in DIO mice B) elevated fasting blood glucose (FBG), reduced C) glucose tolerance, E) increased serum HbA1c levels, and F) blunted adipose tissue Akt signal activity in response to insulin. However, its effect on D) insulin sensitivity was not significantly pronounced. 4-weeks SBP2 knockdown in ATMs of db/db mice (n=6) did not further exacerbate insulin resistance. Knockdown of SBP2 had minimal effects on G) the adipose weight, H) fasting blood glucose (FBG), I) glucose tolerance, J) insulin sensitivity, K) serum HbA1c levels, and L) adipose tissue Akt signal activity in response to insulin in db/db mice *p<0.05, **p<0.01, ***p<0.001

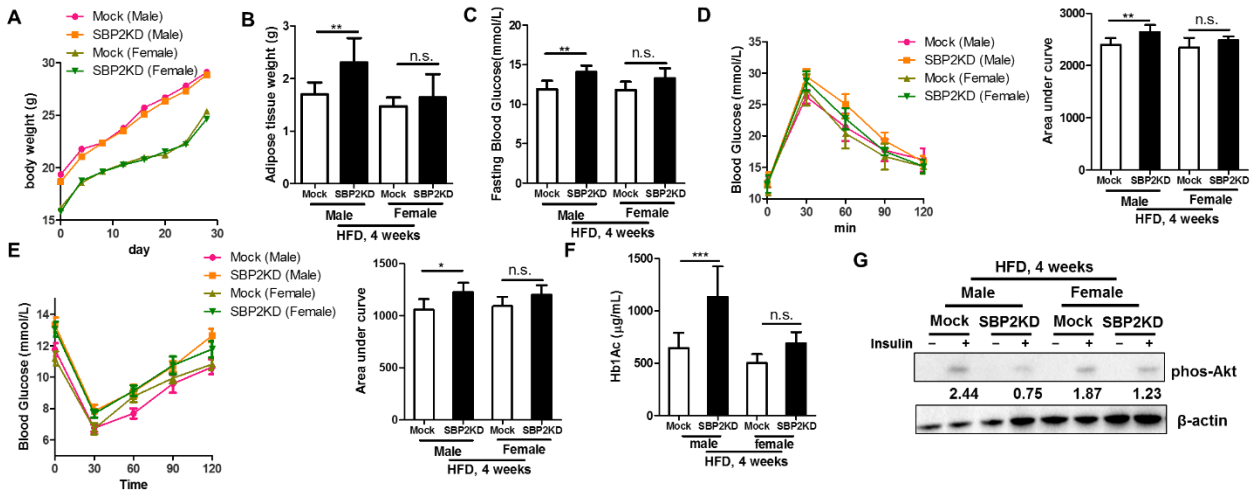

Fig. S7. SBP2 knockdown in ATMs produced a more pronounced effect in male mice than female mice. *In vivo* SBP2 knockdown was performed in both male and female mice for 4 weeks (n=6). A) Knockdown of SBP2 had minimal effects on the body weight of either male or female mice, but B) increased adipose tissue weight gain in male mice. Knockdown of SBP2 C) elevated fasting blood glucose (FBG), reduced D) glucose tolerance and E) insulin sensitivity, F) increased serum HbA1c levels, and G) blunted adipose tissue Akt signal activity in response to insulin in male mice but not female mice. *p<0.05, **p<0.01, ***p<0.001

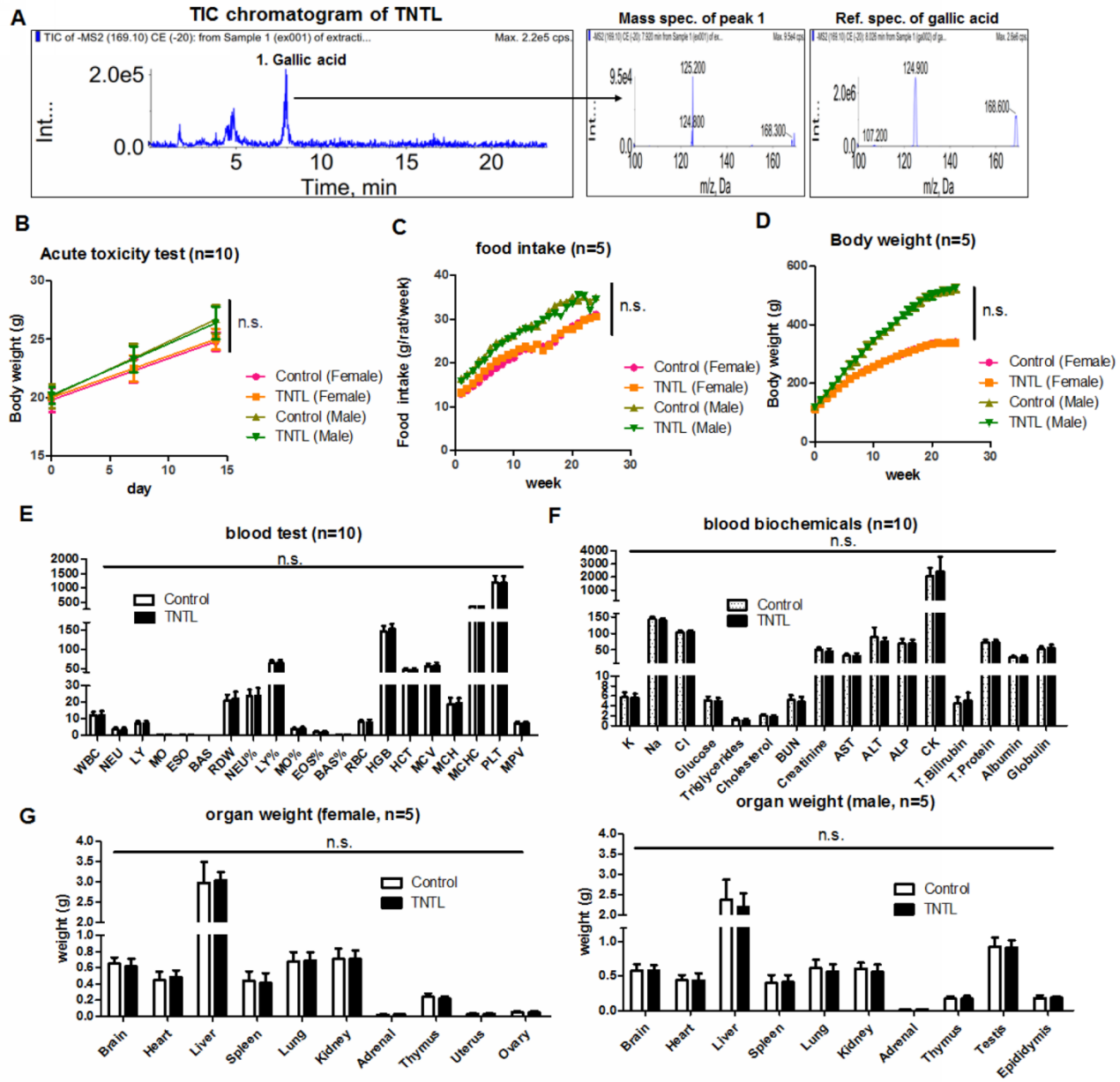

Fig. S8. Chemical and safety analysis of TNTL. TNTL was subjected to LC/MS/MS analysis and eluted with water-acetonitrile (98:2, 0.5 mL/min); A) the results showed that it was composed mainly of gallic acid. B) A single administration of high dose TNTL (36g/kg, p.o.) had no significant impact on the body weight of mice after 7- and 14-day observation. Long-term treatment of TNTL at 3.6 g/kg/day had minimal effect on the C) food intake; D) body weight; E) hematology; F) biochemical blood parameter, and G) organ weight of rats.

Supplemental Tables

Table S1. Basic information of patients observed in the study.

Items	Characteristics (n=20)
Gender, male, n (%)	15(75%)
Age, Mean (SD), yrs	56.35(10.35)
Height, Mean (SD), cm	162.95(9.62)
Weight, Mean (SD), kg	66.75 (11.78)
Fasting blood glucose, Mean (SD), mmol/L	8.96(3.76)
Waist Circumference, Mean (SD), mmol/L	11.457(3.64)
HbA1c, Mean (SD), %	8.14%(1.95)
BMI, Mean (SD), kg/m2	24.98(2.98)
Year since diagnosis, Mean(SD), yrs	4.70(4.45)
Antidiabetic treatment ever used	Metformin, Insulin, Gliclazide, Glimepiride, Acarbose, alone or in combination.

Table S2. Primers to be used for quantitative reverse transcription polymerase chain reaction.

	forward (5' to 3')	reverse (5' to 3')
TNFα	TGCCATCTCTGGGACAGG	TGGGAGTAGACAAGGTACAACC
CD11c	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTC
CD206	CAGGTGTGGGCTCAGGTAGT	TGTGGTGAGCTGAAAGGTGA
Ym1	GGGCATACCTTTATCCTGAG	CCACTGAAGTCATCCATGTC
MCP-1	ATCCCAATGAGTAGGCTGGAGAGC	CAGAGTTGCTTGAGGTGGTTGTG
FASN	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT
SBP2	GAAGCAGAGGGAGATACCTAAGGC	GGCTCACAGCACTTTCTTGGAG
Scd1	TTCTTCTCTCACGTGGGTTG	CGGGCTTGTAGTACCTCCTC
SREBP1c	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
Dgat1	TTCCGCCTCTGGGCATT	AGAATCGGCCCACAATCCA
Cd36	TCCTCTGACATTTGCAGGTCTATC	AAAGGCATTGGCTGGAAGAA
Plin2	GATTGAATTCGCCAGGAAGA	TGGCATGTAGTCTGGAGCTG
Abca1	GGACATGCACAAGGTCCTGA	CAGAAAATCCTGGAGCTTCAAA
β-actin	CTCTGAGGCTCTTTTCCAGCC	TAGAGGTCTTTACGGATGTCAACGT