

Supplementary Materials for

The obesity-induced adipokine sST2 exacerbates adipose T_{reg} and ILC2 depletion and promotes insulin resistance

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Figs. S1 to S5
Table S1

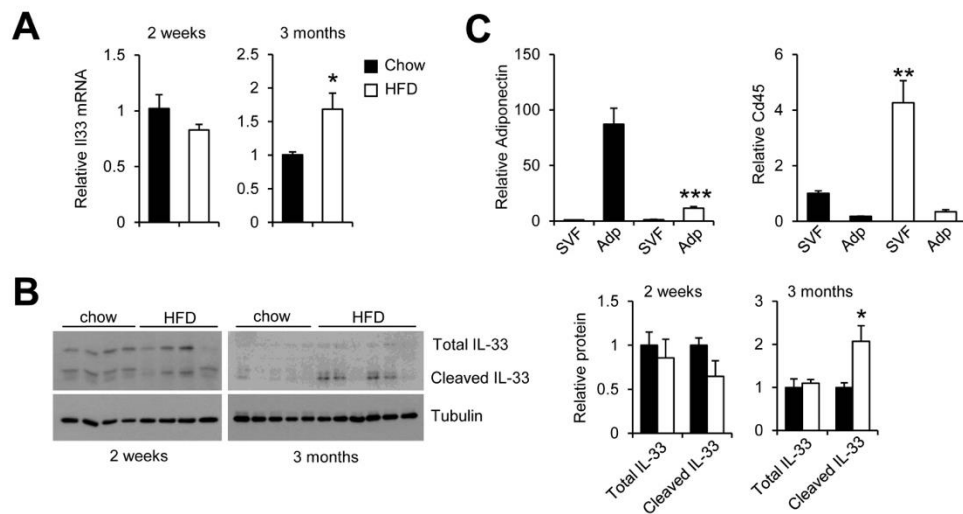


Fig. S1. Regulation of IL-33 expression in mouse eWAT. (A) qPCR analysis of IL33 expression in eWAT from mice fed chow (filled, n=4) or HFD (open, n=4) for 2 weeks (left), and chow (filled, n=5) or HFD (open, n=6) for 3 months (right). (B) Immunoblots of total eWAT lysates from HFD-fed mice. Quantitation of IL-33 protein levels is shown on the right. Data in A-B represent mean \pm SEM. * $p < 0.05$, Chow vs. HFD, two-tailed unpaired Student's t-test. (C) qPCR analysis of gene expression in stromal vascular fraction (SVF) and adipocytes (Adp) isolated from eWAT from mice fed chow (filled, n=3) or HFD (open, n=3). Data represent mean \pm SEM. ** $p < 0.01$, *** $p < 0.001$, SVF vs. Adp, two-way ANOVA.

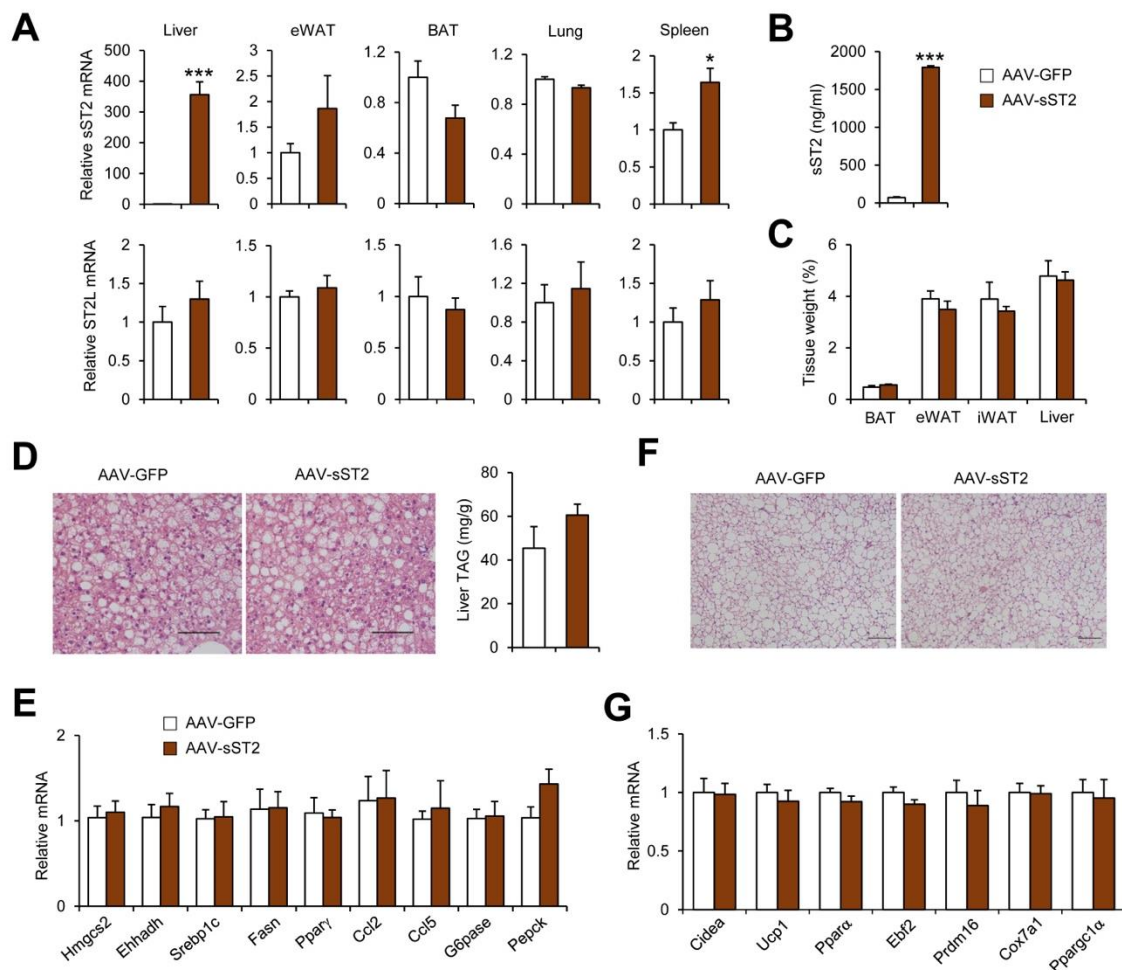


Fig. S2. Effects of sST2 overexpression on metabolic parameters following HFD feeding. (A) qPCR analysis of sST2 and ST2L expression in tissues from mice transduced with AAV-GFP (open, n=5) or AAV-sST2 (filled, n=6) and fed HFD for 10 weeks. (B) Plasma sST2 concentration in transduced mice. (C) Tissue to body weight ratio. (D) H&E staining of liver sections (left) and liver triglyceride (TAG) content (right) in transduced mice. (E) qPCR analysis of hepatic gene expression. (F) H&E staining of BAT sections. (G) qPCR analysis of BAT gene expression. Data in A-D and E, G represent mean \pm SEM. * $p < 0.05$, *** $p < 0.001$, GFP vs. sST2, two-tailed unpaired Student's t-test.

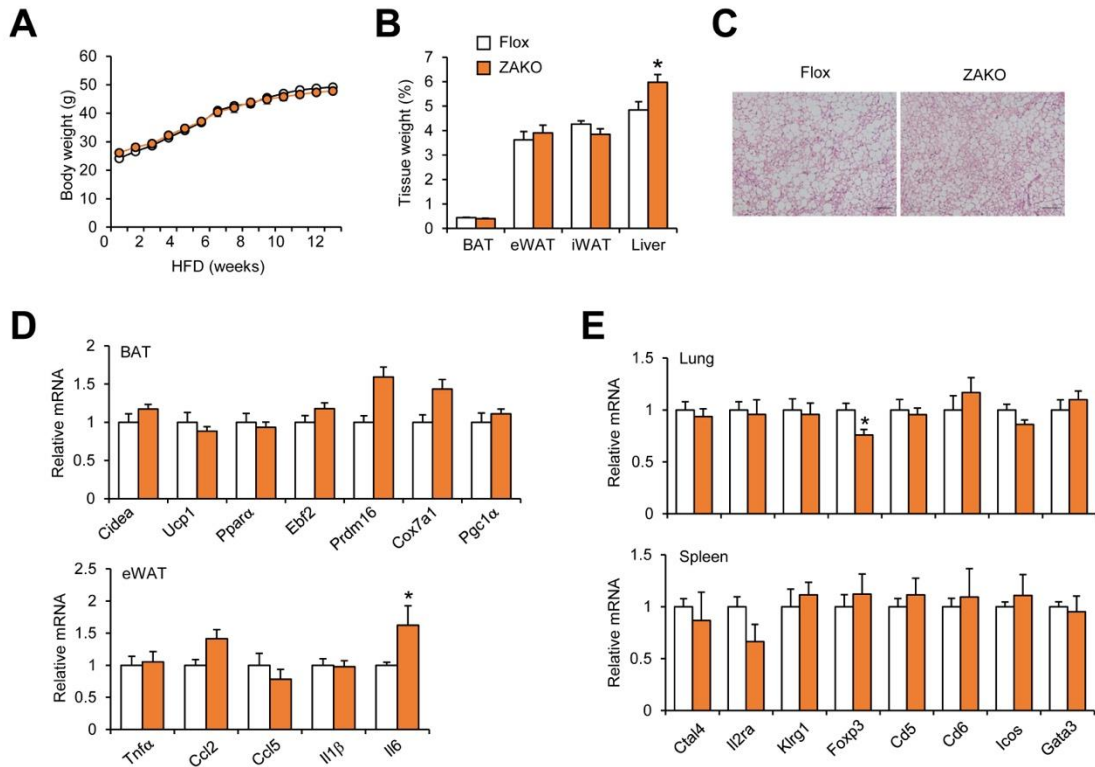


Fig. S3. Effects of *Zbtb7b* inactivation on metabolic parameters following HFD feeding. (A). Body weight of Flox (open, n=7) and ZAKO (filled, n=5) mice fed HFD for 13 weeks. Data represent mean \pm SEM; two-way ANOVA with multiple comparisons. (B). Tissue to body weight ratio in HFD-fed mice. (C). H&E staining of BAT section. (D) qPCR analysis of BAT (top) and eWAT (bottom) gene expression. (E) qPCR analysis of lung (top) and spleen (bottom) gene expression. Data in B and D-E represent mean \pm SEM. * $p < 0.05$, Flox vs. ZAKO, two-tailed unpaired Student's t-test.

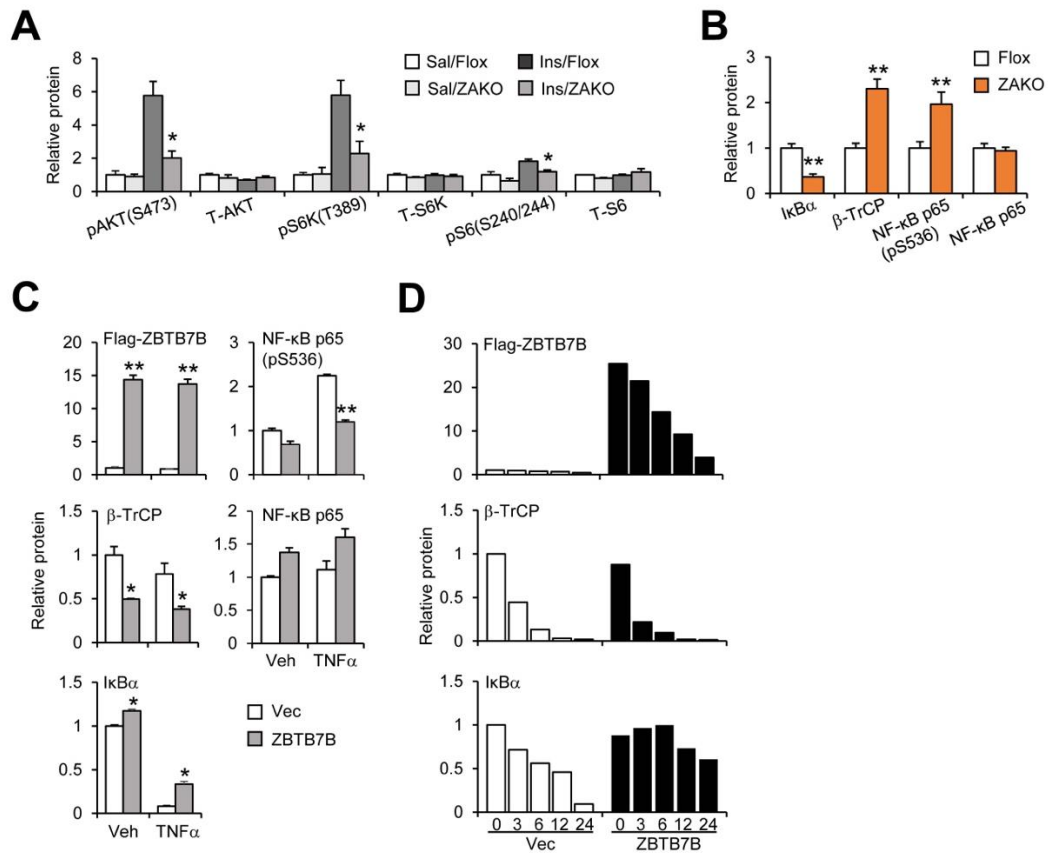


Fig. S4. Quantitation of immunoblots. (A) Quantitation of immunoblots in Fig. 4C. (B) Quantitation of immunoblots in Fig. 6A. (C) Quantitation of immunoblots Fig. 6B. (D) Quantitation of immunoblots Fig. 6D. Data in A-C represent mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, Flox vs. ZAKO (A-B), Vec vs. ZBTB7B (C), two-tailed unpaired Student's t-test.

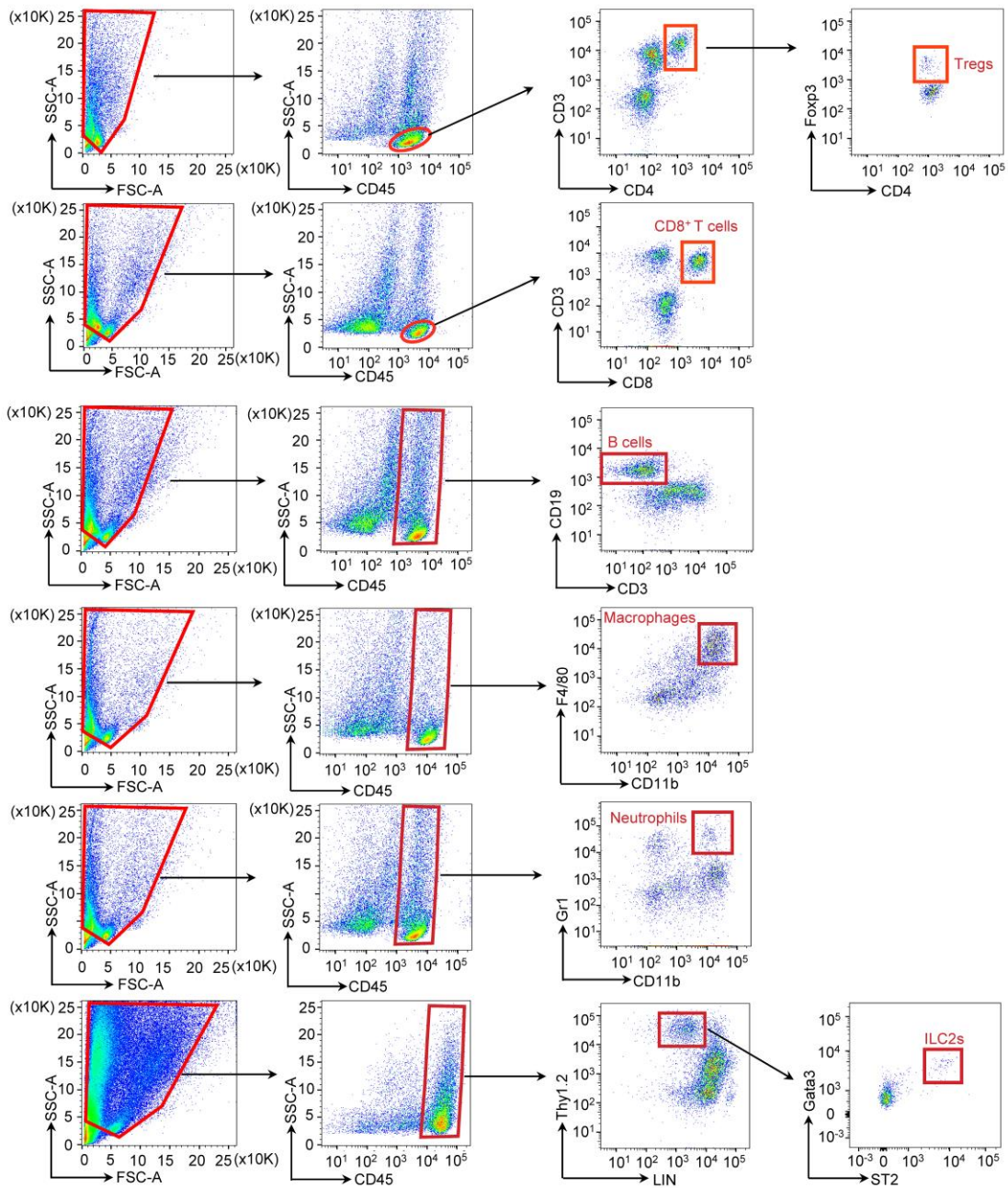


Fig. S5. Flow cytometry gating strategies. Gating strategies for flow cytometry analysis of adipose tissue Tregs, CD8⁺ T cells, B cells, Macrophages, Neutrophils, and ILC2s.

Table S1: List of qPCR primer sequences.

Gene	5' Primer	3' Primer
sST2	AAGGTCGAAATGAAAGTTCCAGC	GCCAATTTATTCAAGCAATGTGTG
ST2L	TGCATTTATGGGAGAGACCTGTTA	TGTGCAGAGCAATCTCCTGC
Il33	GGTCCCGCCTTGCAAATA	CTCTTCATGCTTGGTACCCGAT
Il6	GGGAAATCGTGGAAATGAG	TGAAGGACTCTGGCTTTGTC
Ccl2	AGGTCCCTGTCATGCTTCTG	TCTGGACCCATTCCTTCTTG
Ccl5	TGCCACGTCAAGGAGTATTT	TTCTCTGGGTTGGCACACACT
Clec4d	ACCATCAACACCGAAGCAGAAC	TCCCCCTTTTCCCAGAATACC
Ctla4	TCACCATCCAAGGACTGAGAGC	CGACAAGGATCCAAAGGAGGA
Il2ra	AACCACCACAGACTTCCCACAA	TTCTCCATCTGTGTTGCCAG
Klrg1	TGTATCAACGGATCCTGTGCTG	GGAGATGTGAGCCTTTGTCTGC
Foxp3	CCTTCCCAGAGTTCTTCCACAA	GCGAACATGCGAGTAAACCAAT
Cd5	ATGCCAAGACCCAAACCCA	CCACTGACGCTGCTTTTTTCTG
Cd6	TTCCTGGCGGTTCAACAAC	TCCTTATCCTTCACGCTCACC
Icos	AAACAACCCAGACAGCTCCCA	ACAACGAAAGCTGCACACCCT
Gata3	ACCACCCCATTACCACCTATCC	AGTTCACACACTCCCTGCCTTC
Il5	ACGATGAGGCTTCCTGTCCCTA	CACTTCTCTTTTTGGCGGTCAA
Mmp2	CAACGGTTCGGGAATACAGCAGC	TGGAAGCGGAACGGGAAGTTG
Mmp12	TGGAGCTCACGGAGACTTCAA	CAACAAGGAAGAGGTTTGTGCC
Colla1	AAGAGGCGAGAGAGGTTTCC	AGAACCATCAGCACCTTTGG
Colla2	AGGTCCCTAATGGAGATGCCG	CACAGGGCCTTCTTTACCAG
Acta2	CTGACAGAGGCACCACTGAA	CATCTCCAGAGTCCAGCACA
Lox12	TGCAACAAACACTGGACAGCC	TGGAGATATGCGCTTCAGTGC
Mmp13	TGCTTCTGATGATGACGTTCAAGG	TGGGATGCTTAGGGTTGGGGTC
Tgfb1	ACCATGCCAACTTCTGTCTGGGAC	ACAACCTGCTCCACCTTGGGCTTG
Zbtb7b	CTCACCCATCCCTTGACCTA	CCAGCTCCTCTGGTGATAGC
Hmgcs2	GACATCAACTCCCTGTGCCTG	GATGTCAGTGTTGCCTGAATC
Ehhadh	CAGATGAAGCACTCAAGCTTG	ACCTTGGCAATGGCTTCTGCA

Srebp1c	ATCGGCGCGGAAGCTGTCCG	GGGAAGTCACTGTCTTGGTTG
Fasn	GGTTACACTGTGCTAGGTGTTG	TCCAGGCGCATGAGGCTCAGC
Ppar γ	CCGTAGAAGCCGTGCAAGAG	GGAGGCCAGCATCGTGTAGA
G6pase	ACACCGACTACTACAGCAACAG	CCTCGAAAGATAGCAAGAGTAG
Pepck	CATATGCTGATCCTGGGCATAAC	CAAACCTTCATCCAGGCAATGTC
Cidea	GCAGCCTGCAGGAACTTATCAGC	GATCATGAAATGCGTGTTGTCC
Ucp1	GGCATTTCAGAGGCAAATCAGCT	CAATGAACACTGCCACACCTC
Ppar α	GCAGTGCCCTGAACATCGA	CGCCGAAAGAAGCCCTTAC
Ebf2	GGAACCGGAACGAGACCCCT	TCCCTTGGGTTTCCCGCTGT
Prdm16	CGGAAGAGCGTGAGTACAAATG	TCCGTGAACACCTTGACACAGT
Cox7a1	GTCTCCCAGGCTCTGGTCCG	CTGTACAGGACGTTGTCCATTC
Pgc-1 α	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
TNF α	AGCCCCAGTCTGTATCCTT	CTCCCTTTGCAGAACTCAGG
Il1 β	TGGCAACTGTTCCCTGAACTCAA	AGCAGCCCTTCATCTTTTGG