Supporting information

The regulatory protein SnoN antagonizes Activin/Smad2 signaling and thereby promotes adipocyte differentiation and obesity in mice

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Supplementary Figure 1-4
Supplementary Table 1

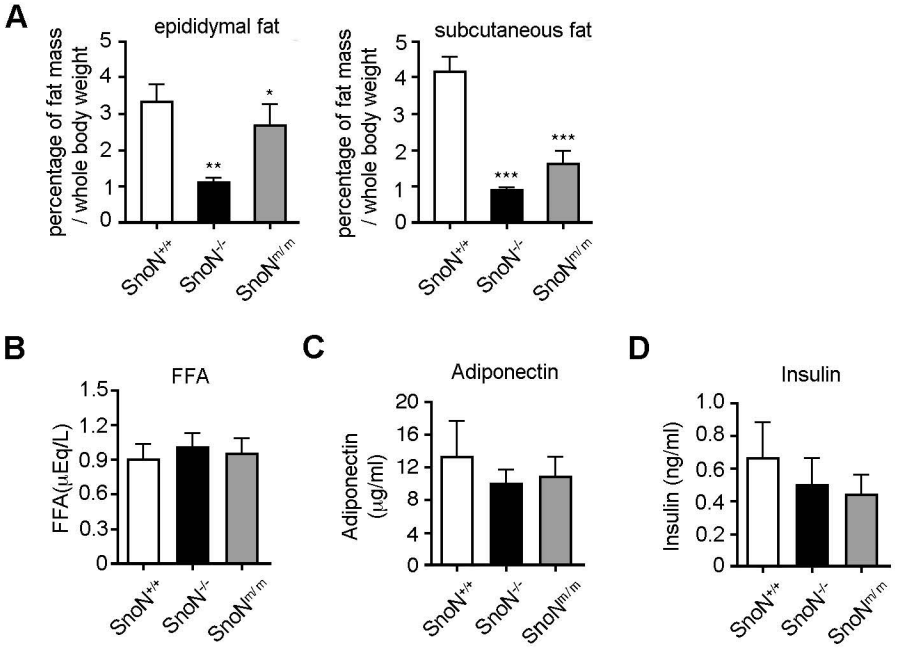

Figure S1. **Mice lacking SnoN are resistant to HFD-induced weight gain.**
(A) Mean weight of the epididymal adipose tissue (**left**) and subcutaneous adipose tissue (**right**) of $SnoN^{+/+}$, $SnoN^{-/-}$ and $SnoN^{m/m}$ male mice after 16 weeks on HFD. Values are expressed as percentage of body weight. Data are represented as means ± SD (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). **(B-D)** No significant difference was observed between HFD-fed WT and SnoN deficiency mice in the levels of plasma FFA (**B**), blood Adiponectin (**C**) and insulin (**D**). The serum/plasma was collected after 6 hr of fasting. Data are represented as means ± SD ($p > 0.05$).

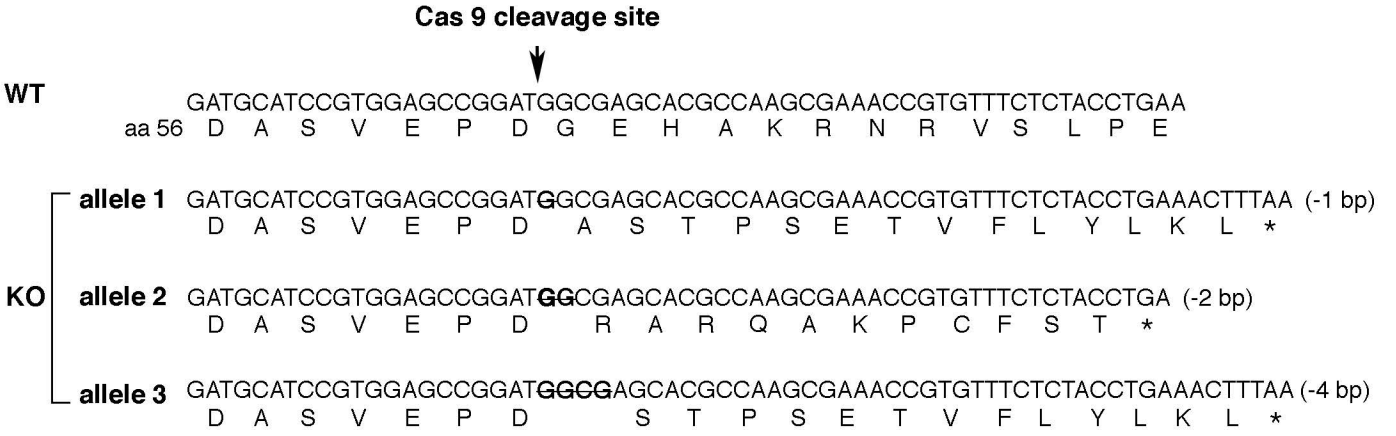

Figure S2. Generation of SnoN$^{-/-}$ CH310T1/2 cells using CRISPR/Cas9. 10 cloned DNA fragments containing regions of *snoN* gene targeted by the sgRNA were PCR-amplified from SnoN$^{-/-}$ cells and subjected to Sanger sequencing. The DNA and amino acid sequences for WT SnoN are shown on the top with the intended Cas9 cleavage site indicated by an arrowhead. Sequences of all three mutant SnoN alleles found in the 10 clones are listed. Deleted nucleotides (1, 2 and 4 nucleotides) are denoted by the strike-through bold letters, and the premature stop codons resulted from the frame-shift mutations are indicated by stars.

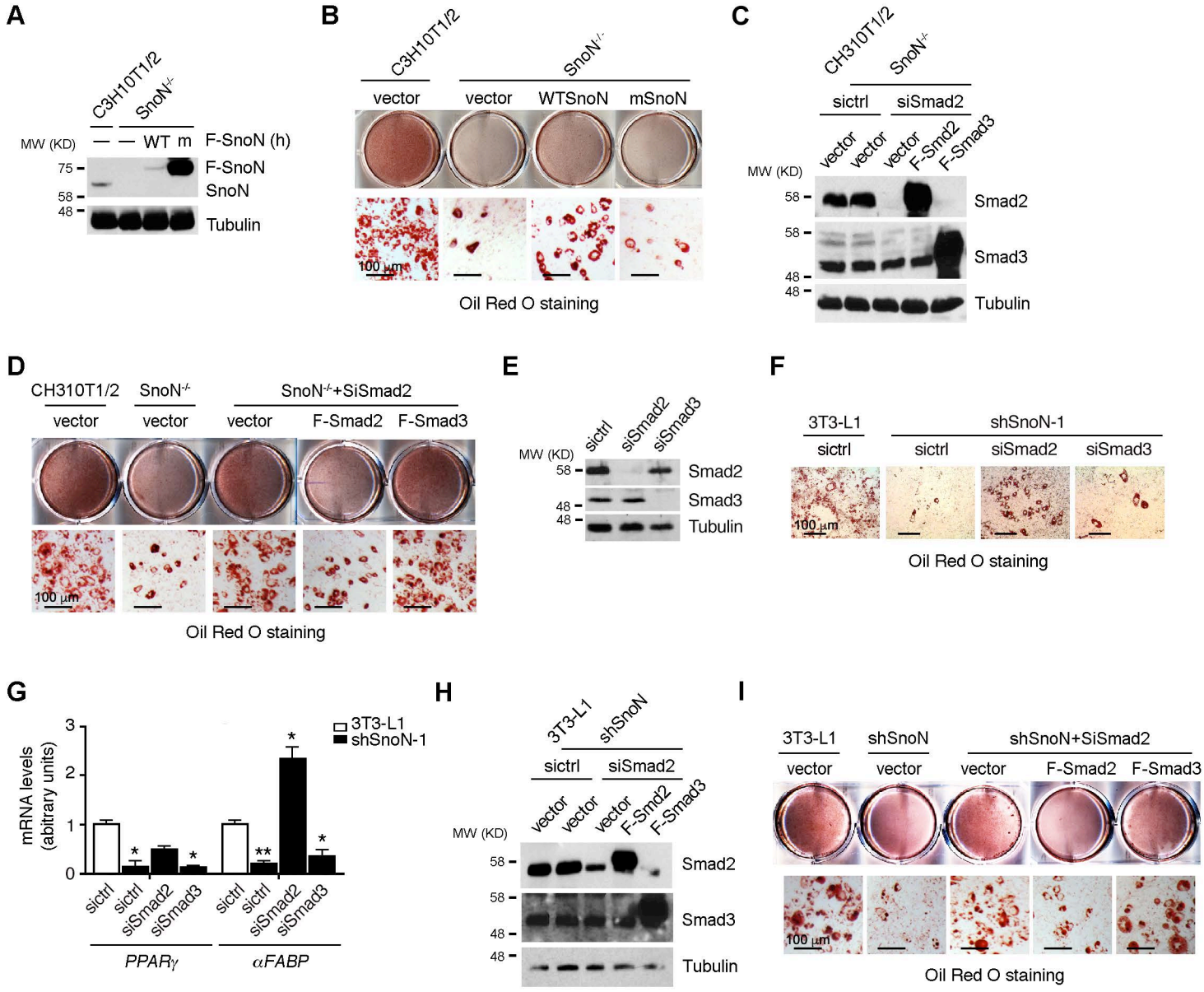

Figure S3. Smad pathway is targeted by SnoN to regulate adipocyte differentiation in C3H10T1/2 and 3T3L1 cells.
(A-B) Re-expression of WT SnoN (WTSnoN), but not Smad binding mutant SnoN (mSnoN) in C3H10T1/2 SnoN$^{-/-}$ cells by retro-viral infection partially restored their ability to differentiate into adipocytes. SnoN protein levels were examined by western blotting with anti-SnoN **(A)**. Tubulin was used as a loading control. Adipocyte differentiation was assessed by Oil Red O staining at day 7 **(B)**. **(C-D)** Re-expression of human Flag-tagged Smad2 (F-Smad2), but not Smad3 (F-Smad3), in C3H10T1/2 SnoN$^{-/-}$+SiSmad2 cells reversed the adipogenic differentiation. Smad2 and Smad3 expression levels in parental, SnoN$^{-/-}$, SnoN$^{-/-}$+siSmad2 and Smad2- or Smad3-rescue cell lines were examined by western blotting **(C)**. Tubulin was used as a loading control. Cell differentiation was assessed by Oil Red O staining at day 7 **(D)**. **(E-I)** Smad2 is targeted by SnoN to regulate adipocyte differentiation in 3T3L1 cells. Control siRNA (sictrl), siSmad2 or siSmad3 were transfected into 3T3L1 shSnoN-1 cells. The knockdown efficiency was examined by western blotting **(E)**. Cell differentiation was assessed by Oil Red O staining **(F)** and the mRNA levels of *PPARγ* and *αFABP* were quantified by qRT-PCR on day 7 **(G)**. Rescue experiments by re-expressing F-Smad2 or F-Smad3 in 3T3-L1 shSnoN+siSmad2 cells were performed **(H-I)**. Levels of Smad2 and 3 were examined by western blotting **(H)**, and cell differentiation assessed by Oil Red O staining **(I)**. Data in **(G)** are represented as means ± SD (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). Scale bars =100 μm.

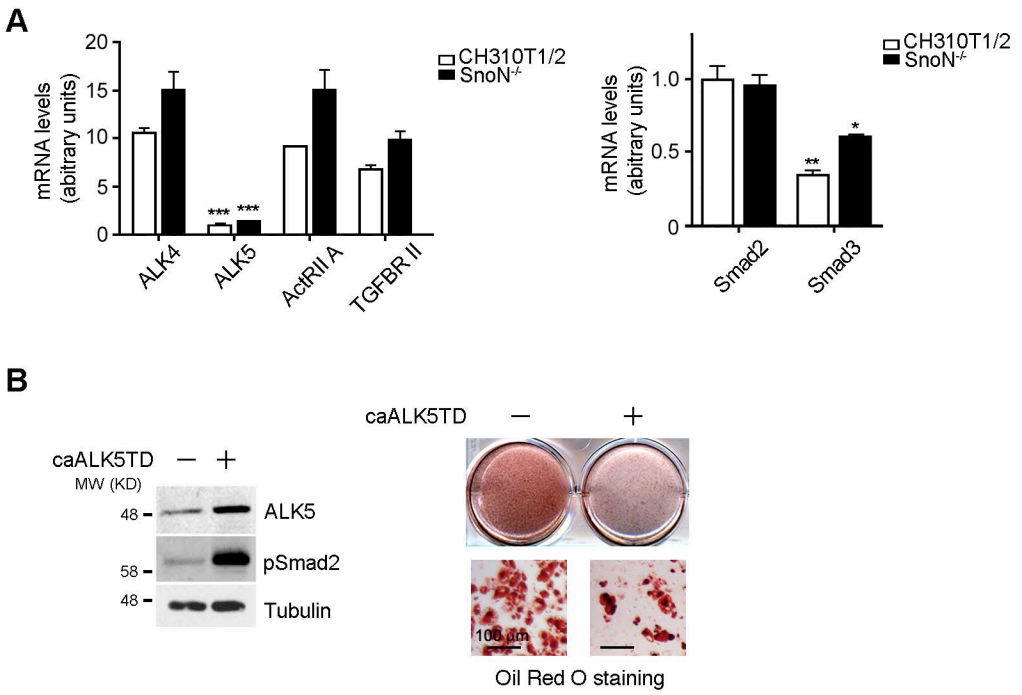

Figure S4. The expression and activity of TGFβ/ALK5 pathway in C3H10T1/2 cells.
(A) CH310T1/2 cells expressed lower levels of *ALK5* and *Smad3*. The mRNA levels of activin A receptors *ALK4* and *ActRIIA*, and TGFβ receptors *ALK5* and *TGFBRII*, as well as *Smad2* and *Smad3* in CH310T1/2 cells were examined by qRT-PCR. **(B)** Overexpression of a constitutively active form of ALK5 (caALK5TD) effectively inhibited adipogenic differentiation of CH310T1/2 cells. Protein levels of ALK5 and pSmad2 were examined by western blotting (left panels). Cell differentiation was assessed by Oil Red O staining at day 7 (right panels). Scale bar: 100 μm.

Table S1: qRT-PCR primer sequences

Gene	Forward	Reverse
PPARγ	TTGATTTCTCCAGCATTTC	TGATCGCACTTTGGTATT
C/EBPβ	GCAAGAGCCGCGACAAG	GGCTCGGGCAGCTGCTT
αFABP	TCACCATCACCTATGGACCCA	TCCAGTTCGCACTCCTCCC
Adipsin	CATGCTCGGCCCTACATGG	CACAGAGTCGTCATCCGTCAC
nanog	CTTTCACCTATTAAGGTGCTTGC	TGGCATCGGTTCATCATGGTAC
Acvr2a	TTGACTTTCCTCCCAAAGAATC	TTCCTTAGCTTAGCAGCTCCA
Tgfbr2	CAACACCAGTGGGTTCCATT	GTGCGCCATTCAAATCCT
ALK4	TGCTTGAGCTTTCTGTGCAT	GAGAAGCAGCAGCACTCAGA
ALK5	AGTCAGTCCGTTGGGTCTTC	GTAAAACCCAGGCTCAACCA
Smad2	ATGTCGTCCATCTTGCCATTC	AACCGTCCTGTTTTCTTTAGCTT
Smad3	CACGCAGAACGTGAACACC	GGCAGTAGATAACGTGAGGGA
CD206	CTCTGTTCAGCTATTGGACGC	CGGAATTTCTGGGATTCAGCTTC
CD11c	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTC
Arg1	CTGGATAGCCTTTCTTCTGCTG	ATGGAAGAGACCTTCAGCTAC
CCL17	CCCATGAAGACCTTCACCTC	CATCCCTGGAACACTCCACT
IL1β	GTGTGGATCCAAAGCAATAC	GTCTGCTCATTCATGACAAG
iNOS	AGGAACCTACCAGCTCACTCTG	TTTCCTGTGCTGTGCTACAGTT
IL10	AGAAAAGAGAGCTCCATCATGC	TTATTGTCTTCCCGGCTGTACT
TNF-α	CTGTAGCCCACGTCGTAGC	TTGAGATCCATGCCGTTG
STAT1	TCACAGTGGTTCGAGCTTCAG	GCAAACGAGACATCATAGGCA
STAT6	CTCTGTGGGGCCTAATTTCCA	CATCTGAACCGACCAGGAACT
TGFβ	CTCCCGTGGCTTCTAGTGC	GCCTTAGTTTGGACAGGATCTG
Activin A	TGAGAGGATTTCTGTTGGCAAG	TGACATCGGGTCTCTTCTTCA
SnoN	AATAAAAAGCTGAACGGCATGGA	GGGTTTTCCCATTGGCATGAAT
18 S rRNA	ACCGCAGCTAGGAATAATGGA	GCCTCAGTTCCGAAAACCA
GAPDH	GGTCCTCAGTGTAGCCCAAG	AATGTGTCCGTCGTGGATCT
β-actin	CCAACCGTGAAAAGATGACC	CCATCACAATGCCTGTGGTA