

**Cell Metabolism, Volume 26**

**Supplemental Information**

**Acetyl-CoA Carboxylase 1-Dependent**

**Protein Acetylation Controls Breast**

**Cancer Metastasis and Recurrence**

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## **SUPPLEMENTAL DATA**

## SUPPLEMENTAL FIGURE LEGENDS

**Figure S1. Leptin induces EMT and metabolic changes. Related to Figure 1.** (A) Levels of LepR and (B) leptin in human and murine breast cancer cell lines. (n=3-4). (C) Relative cell numbers representing proliferation of different breast cancer cell lines under leptin treatment. (n=3). (D) Western Blot showing levels of EMT markers in breast cancer cell lines treated with leptin 100ng/ml. (n=3). (E) Colony formation in soft agar of breast cancer cell lines treated with 100ng/ml of leptin, represented as percentage of the control. (n=3) (F) 3D collagen invasion assay in MDA-MB-231 and MCF-7 (G) cells treated with 100ng/ml of leptin. (n=3). (H) mRNA levels of LepR in e0771 and 4T1 cells transfected with scrambled or LepR siRNA. (n=2). (I) Quantification of the western blot shown in Fig 1G (4T1 cells). (J) Invasion of 4T1 and (K) e0771 cells in trans-well assays. (n=2). (L) 4T1 invasion in 3D collagen cultures in the presence or absence of leptin. (n=3) (M) e0771 invasion in 3D collagen cultures in the presence or absence of leptin and (N) representative pictures. (n=3). (O) EMT markers in spheroids of e0771 cells (n=2). (P) Quantification of the immunohistochemistry staining of primary tumors generated by orthotopical injection of e0771 in C57Bl6/J mice from Fig 1D. All data in the figure are shown as the mean  $\pm$  s.e.m. n numbers refer to biological replicates. (F, G, J, K, L) 1-way ANOVA with Tukey's Multiple Comparison Posttest. (C,E,H,P) Student's t-test. (L) 2-way ANOVA with Bonferroni's Multiple Comparison Posttest. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

**Figure S2. ACC regulates EMT and protein acetylation. Related to Figure 2.** (A) Levels of total protein acetylation in 4T1 (low exposition from Fig2A), MCF-7, MDA-MB-231 and T47D breast cancer cells in the presence or absence of 100ng/ml of leptin. (n=3) (B) Western Blot quantification of total protein acetylation in breast cancer cell lines treated with or without 100ng/ml of leptin. (C) Quantification of the western blot shown in Fig 2C. (D) 3D collagen invasion assay of 4T1 cells carrying control or LepR shRNA, transfected with control or ACC1 siRNA. (n=3). All data in the figure are shown as the mean  $\pm$  s.e.m. n numbers refer to biological replicates. (C) 1-way ANOVA with Tukey's Multiple Comparison Posttest. (B) Student's t-test. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

**Figure S3. Smad2 acetylation is downstream of ACC phosphorylation during EMT induction. Related to Figure 3.** (A) Quantification of the western blot shown in Fig 3E. (B) IP of 4T1 cells treated with Tofa and C646 showing levels of Smad2 acetylation (C) Histone H3 acetylation and EMT markers in HEK293T cells transfected with empty vector, RFP-Smad2 wild-type or RFP-Smad2K3R and treated with TGF $\beta$  or Tofa (n=4). (D) Western Blot of pACC, Histone H3 acetylation and EMT markers in 4T1 cells transfected with empty vector, RFP-Smad2 wild-type or RFP-Smad2K3R and treated with Leptin (n=2). Representative images shown.

**Figure S4. TAK1 is the mayor kinase involve in AMPK-ACC phosphorylation during EMT induction. Related to Figure 4.** (A) Densitometric analysis of pACC1 Western blot in Figure 4B, left panel (n=3). (B) Western blot showing levels of pACC and Snail in HEK293T cells transfected with empty vector or CA TAK1 and treated with TGF $\beta$ . (C) Western blot of MCF-7 cells KD for LKB1 or (D) CAMKK2B showing changes in pACC under leptin or TGF $\beta$  treatment (n=3). (E) Western Blot of EMT markers in MCF-7 cells transfected with esiEGFP, esiAMPKa1, esiAMPKa2 or esiAMPKa1/a2 and treated with Leptin or TGF $\beta$ . Representative images shown.

**Figure S5. ACC regulates invasion in vitro and metastasis in vivo. Related to Figure 4.**

(A) Levels of EMT markers in control or ACC1 shRNA-carrying e0771, 4T1, MDA-MB-231 and T47D cell lines. (n=3) (B) Number of lung micrometastases per animal or in total in animals injected with wild-type or ACC1 knockdown e0771 cells into the tail vein. (6 mice per group) (C) *In vitro* ACC1 activity assay of wild-type ACC1, ACC1<sup>S79A</sup> and ACC1<sup>S79E</sup> (n=2). (D) Growth curves of 4T1 cells expressing wild-type ACC1 or ACC1<sup>S79A</sup> (n=2). (E) Representative microphotographs of 4T1 cells transfected with empty vector, ACC1 wild-type or ACC1<sup>S79A</sup>, showing morphological changes after TGFβ treatment. (F) Transwell invasion assay of 4T1 cells expressing ACC1<sup>S79E</sup> in the presence or absence of leptin and TGFβ (n=2). (G) Western blot of 4T1 cells transfected with empty vector or ACC1<sup>S79E</sup> in the presence or absence of TGFβ showing levels of EMT markers (n=2). (H) Western blot of MDA-MB-231 cells transfected with empty vector, ACC1 wild-type or ACC1<sup>S79A</sup> in the presence or absence of TGFβ showing levels of EMT markers. (n=3). (I) Western blot of MDA-MB-231 cells transfected with empty vector, or ACC1<sup>S79A</sup> and incubated for 24h in normoxia or hypoxia, showing levels of EMT markers. (J) Correlation of tumor size and number of lung micrometastases triggered by wild-type ACC1 or ACC1<sup>S79A</sup>-carrying 4T1 cells injected into the mammary fat pad of BalbC mice. Linear regression ACC1<sup>S79E</sup> R<sup>2</sup>= 0,357, ACC1<sup>S79A</sup> R<sup>2</sup>= 0.06743 (7 mice per group). All data in the figure are shown as the mean ± s.e.m. n numbers refer to biological replicates. (B,D,F) 1-way ANOVA with Tukey's Multiple Comparison Posttest. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

**Figure S1**

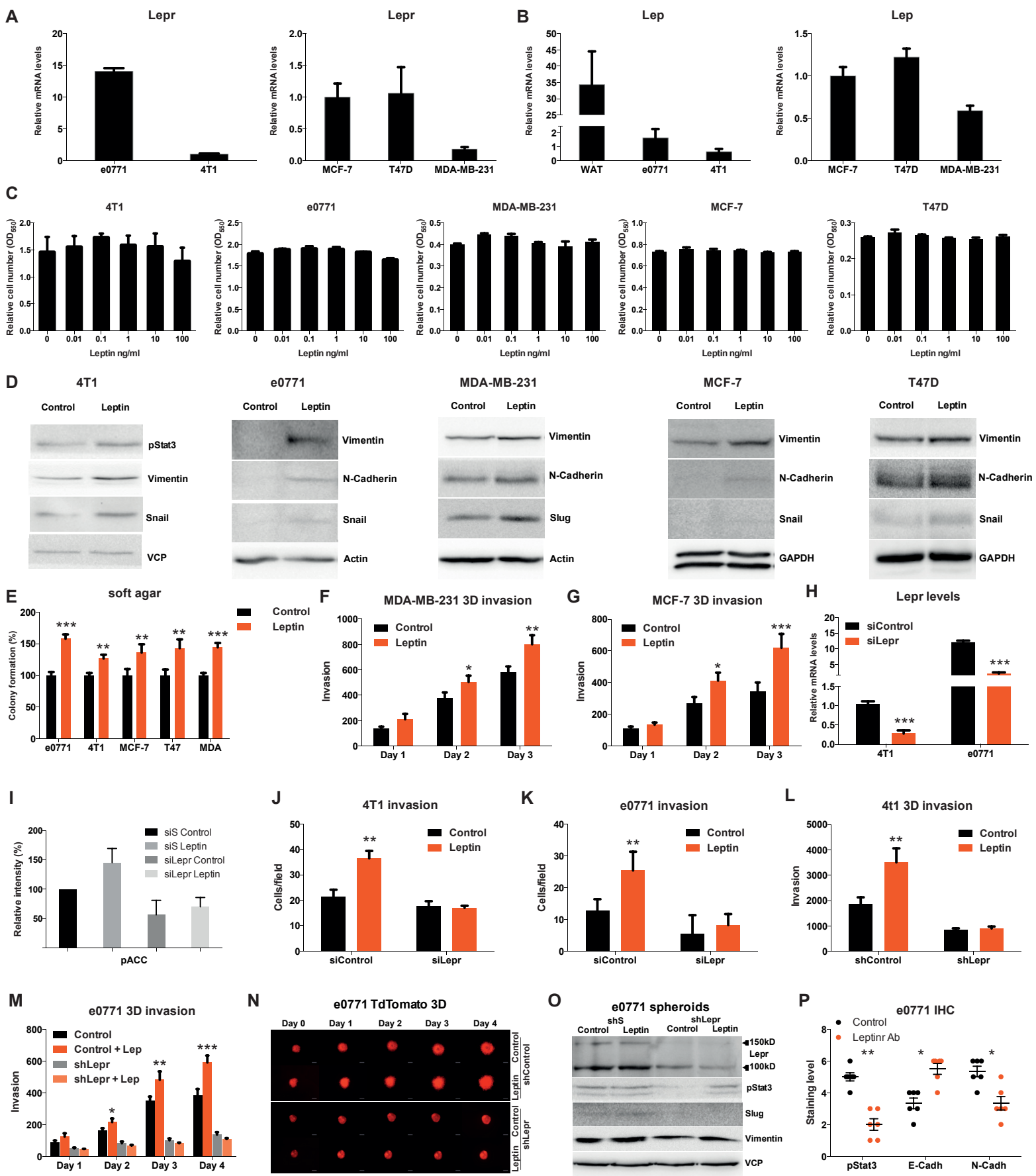
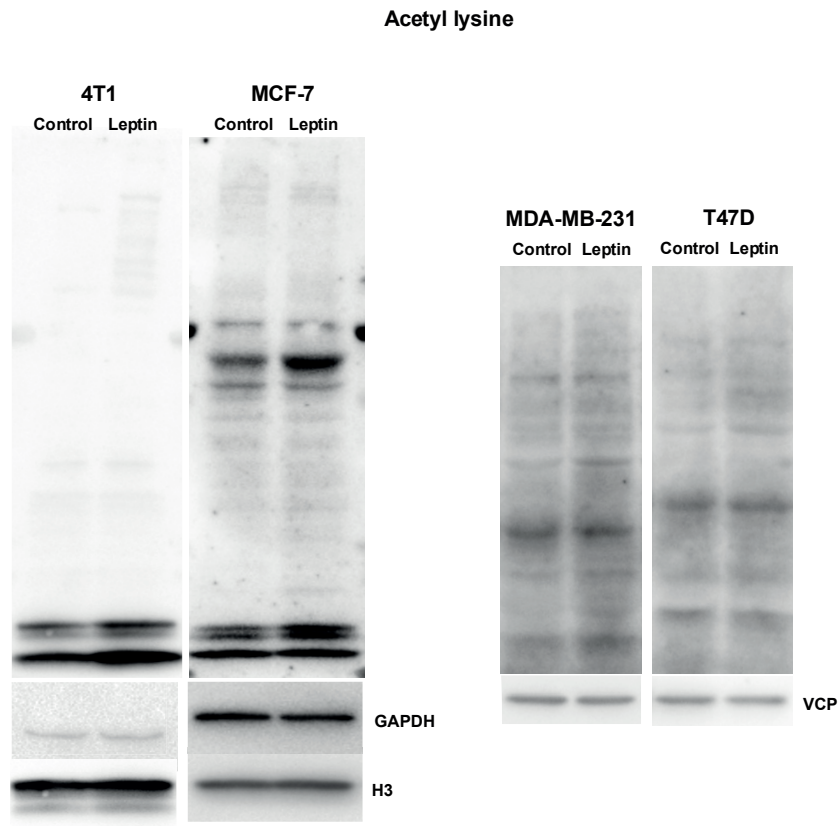
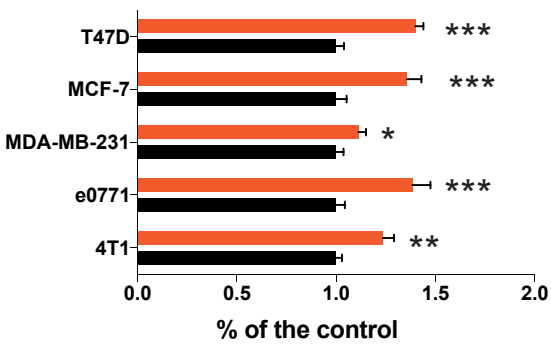


Figure S2

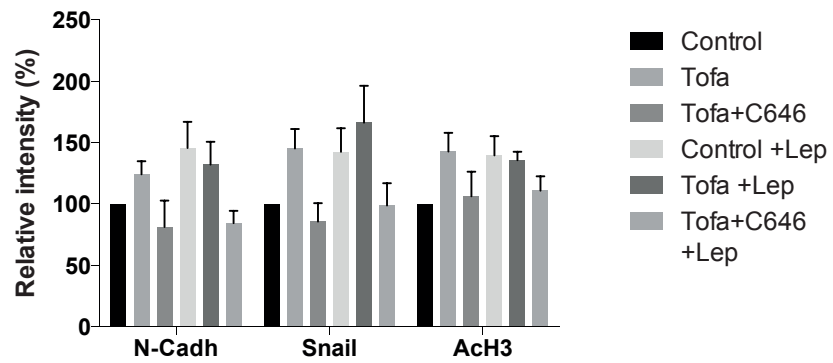
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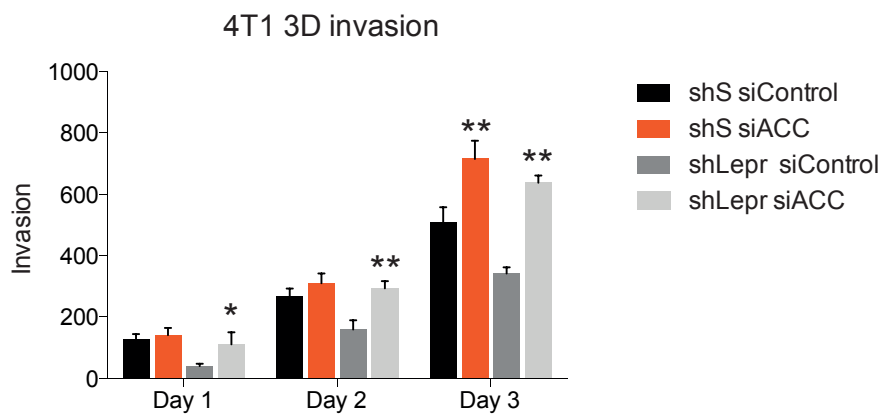
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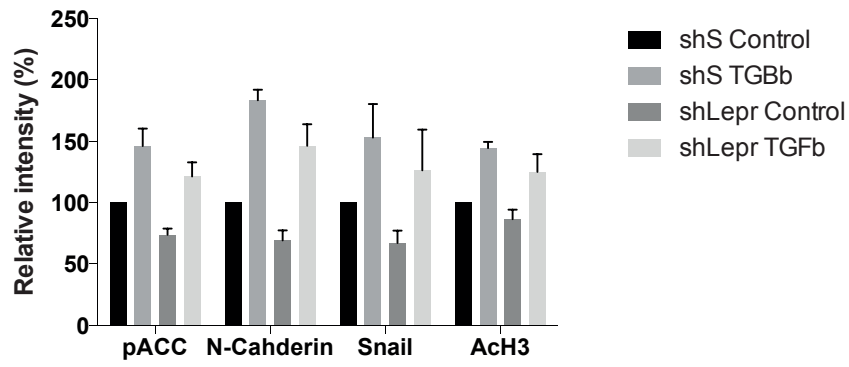


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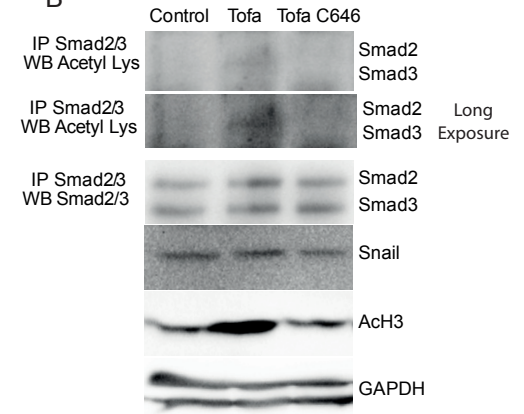


**Figure S3**

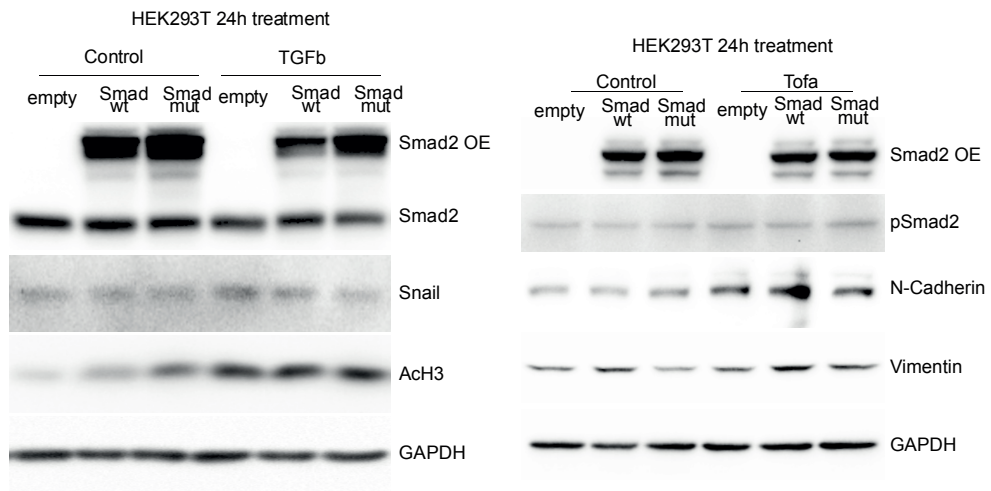
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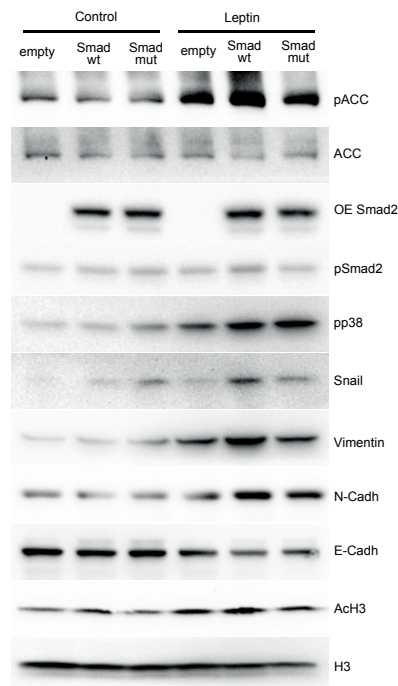
**B**



**C**



**D**





**Figure S5**

