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Supplemental Information

Cbx4 Sumoylates Prdm16 to Regulate Adipose Tissue Thermogenesis

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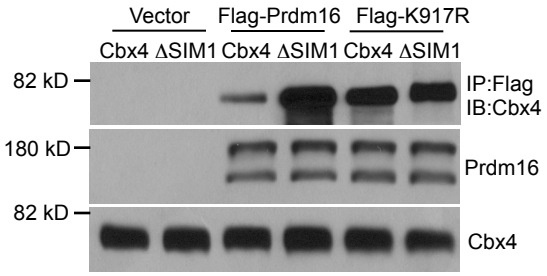

Figure S1, related to Figure 3. Prdm16 K917R mutant interacts with Cbx4. HEK293 cells were co-transfected with indicated plasmids. Immunoprecipitation was performed with anti-Flag beads, and presence of Cbx4 in the immunoprecipitate complex was examined with an anti-Cbx4 antibody.

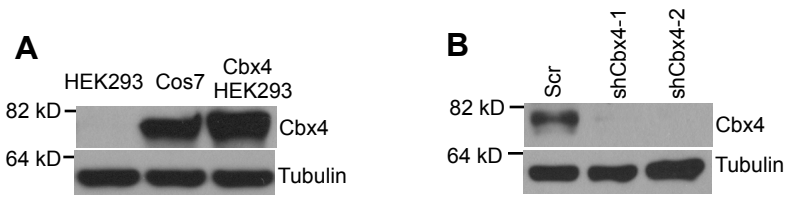

Figure S2, related to Figure 3. (A) Expression of endogenous Cbx4 protein in HEK293 cells and Cos7 cells. (B) Cbx4 protein level in Cos7 cells after lentiviral knockdown.

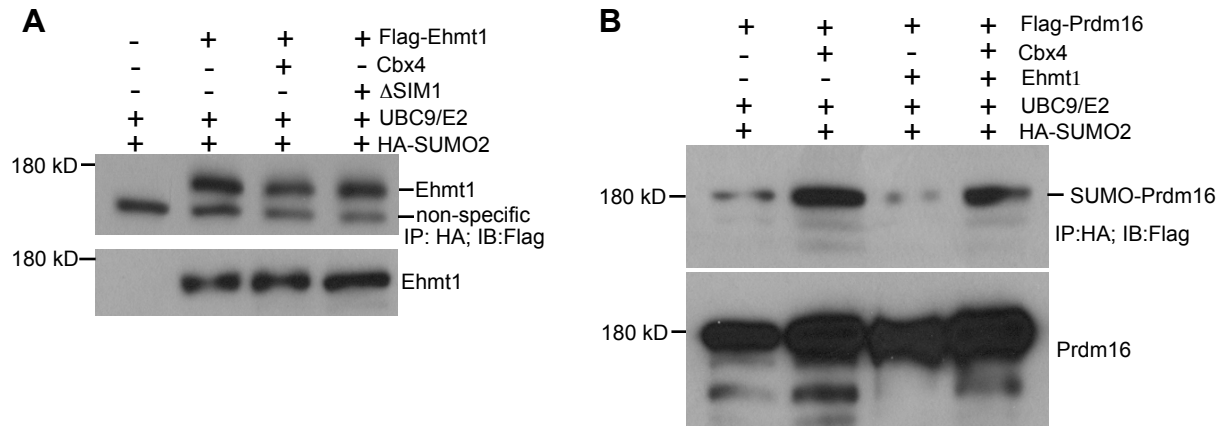

Figure S3. Related to Figure 3. Cbx4 does not sumoylate Ehmt1 (A) and Ehmt1 does not increase Cbx4 sumoylation activity (B). HEK293 cells were transfected with indicated plasmids and treated with MG132 for 16 hr before harvesting for immunoprecipitation.
(A) Sumoylation of Ehmt1. (B) Sumoylation of Prdm16.

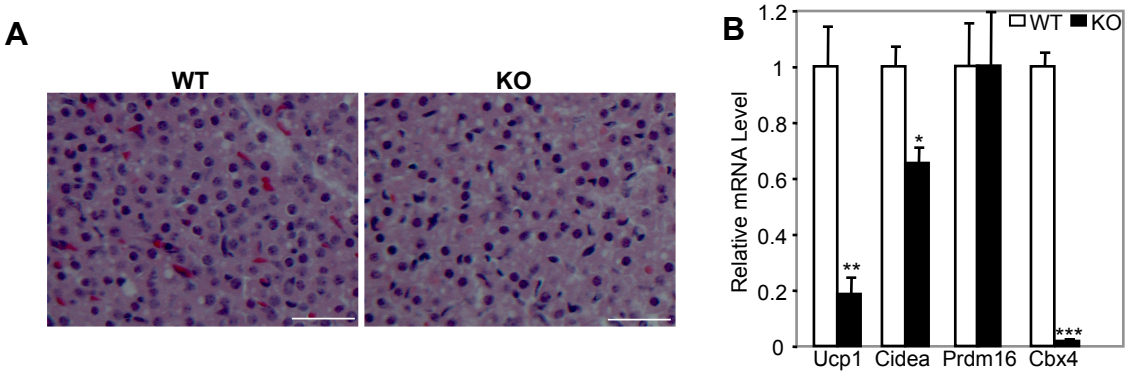

Figure S4, related to Figure 5. (A) H&E staining of BAT isolated from neonatal Cbx4 null mice. Shown are representative images of three mice per group. Scale bar=200 μm.
(B) Gene expression in BAT isolated from 18.5 Day embryos of Cbx4 null. Data represent mean ± SEM. *p<0.05,**p<0.01,***p<0.001.

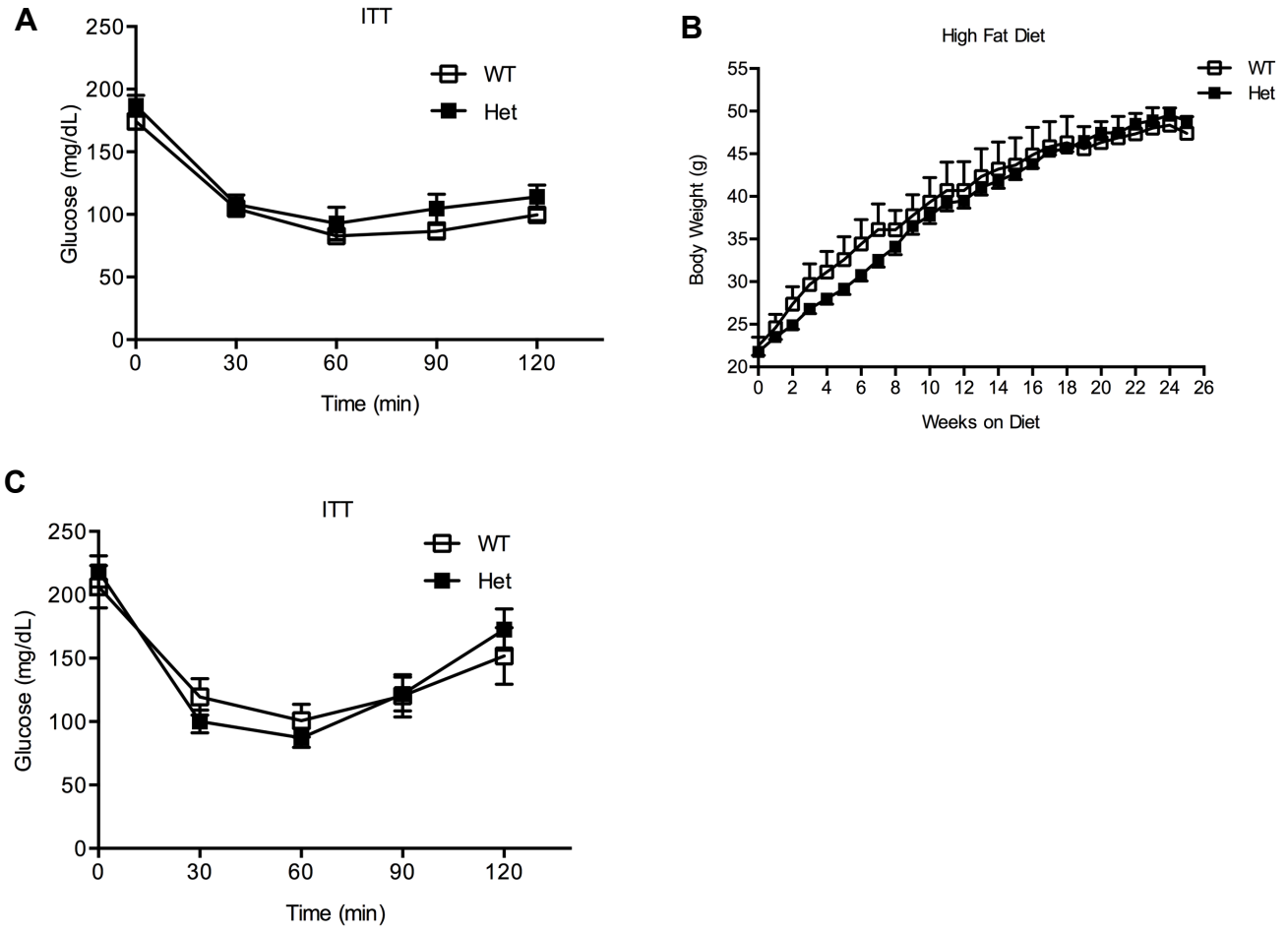

Figure S5, related to Figure 6. (A) Insulin tolerance test of 10-month-old male wild type and Cbx4 heterozygous mice (n=6-8 mice/group). (B) Body weights of male wild type and Cbx4 heterozygous mice fed a high fat diet (n=6 mice/group). (C) Insulin tolerance test of male wild type and Cbx4 heterozygous mice fed a high fat diet for 25 weeks (n=5-7 mice/group). Data represent mean ± SEM.

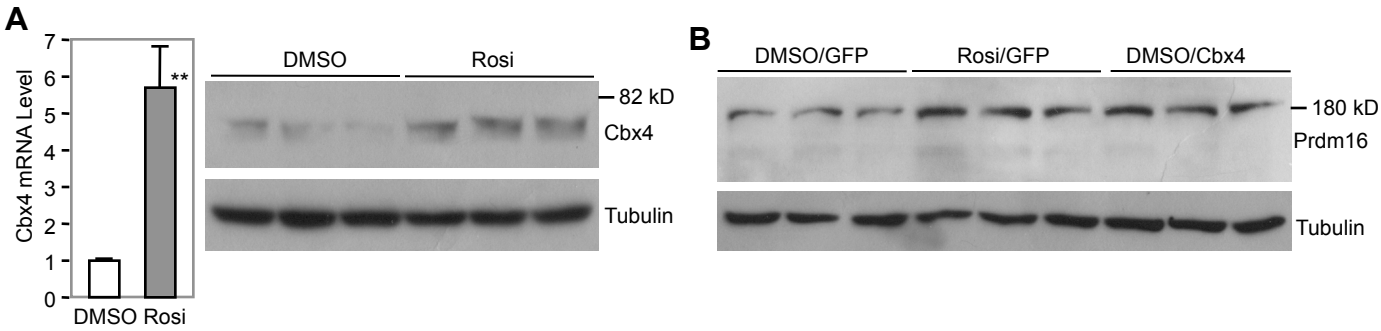

Figure S6, related to Figure 4. (A) Primary iWAT adipocytes were treated with Rosiglitazone (1 μM) during differentiation, and Cbx4 mRNA (n=4) and protein were measured. Data represent mean ± SEM. **p<0.01.

(B) Adipocytes were treated with Rosiglitazone or were infected with Cbx4 adenovirus, and Prdm16 protein was measured.