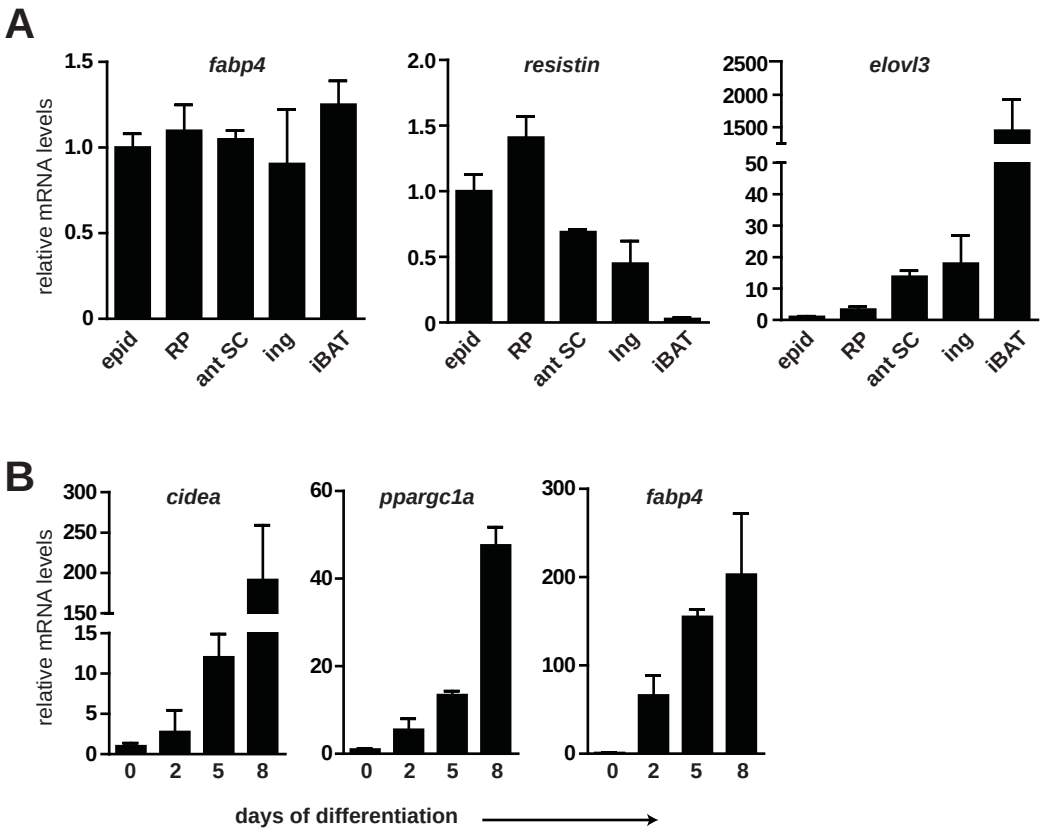

Figure S1. Expression of Prdm16 and a brown fat gene program in subcutaneous adipocytes

(A) Real-time PCR analysis of *fabp4* (general adipocyte marker), *resistin* (WAT-selective gene in mice) and *elovl3* (BAT-selective gene) expression in: WAT (epid [epididymal], RP [retroperitoneal], ant SC [anterior subcutaneous], ing [inguinal subcutaneous]) and iBAT (interscapular BAT) of 8 week old male mice (n=5 mice/group, mean +/- SD). (B) Real-time PCR analysis of the levels of brown fat-selective genes (*cidea*, *ppargc1a*) and the general adipocyte gene, *fabp4* during in vitro adipocyte differentiation of preadipocytes isolated from inguinal WAT (n=3, mean +/- SD).

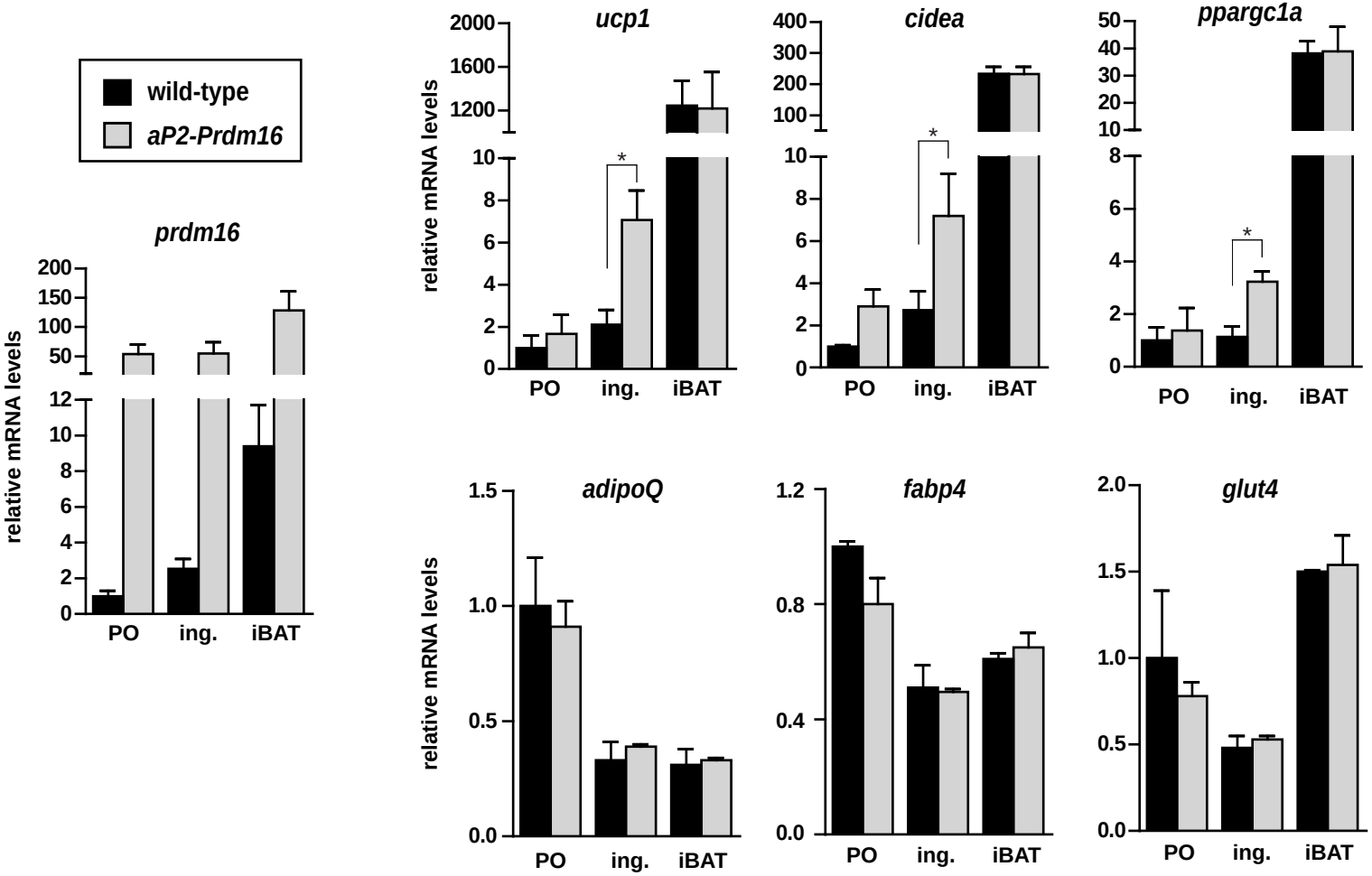

Figure S2. Transgenic expression of Prdm16 induces a brown fat-like gene program in the inguinal WAT of female mice

(A) mRNA levels of: brown fat-selective genes (*prdm16*, *ucp1*, *cidea*, *ppargc1a*); and general adipocyte markers (*adipoQ*, *fabp4*, *glut4*) in the periovarian (PO) WAT, inguinal (ing.) WAT and intercapular BAT (iBAT) of 12-week-old female *aP2-Prdm16* and wild-type animals. (n=4, mean +/- SD). *p<0.05

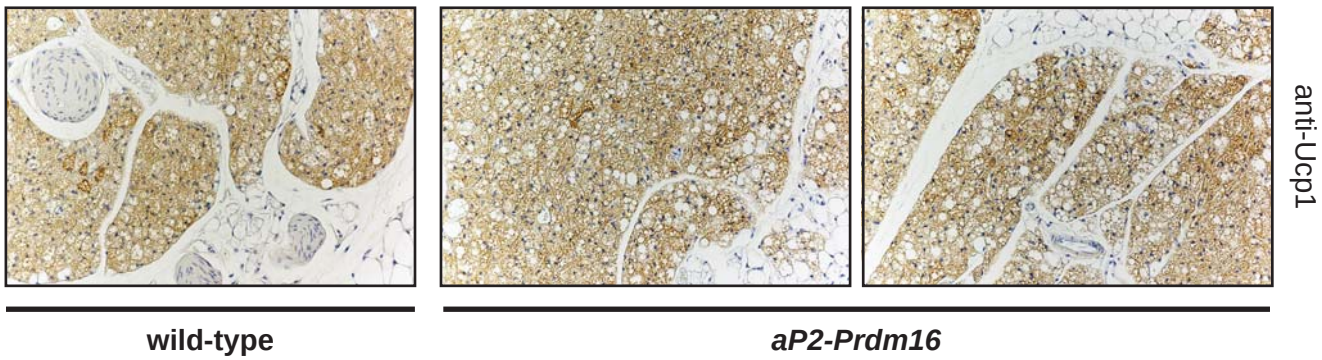

Figure S3. Ectopic Prdm16 expression does not affect the morphology or Ucp1 levels of BAT

Immunohistochemistry for Ucp1 (brown staining) in samples of interscapular BAT from chow fed wild-type and *aP2-Prdm16* animals housed under standard experimental conditions. Hematoxylin was used to counterstain nuclei in blue.

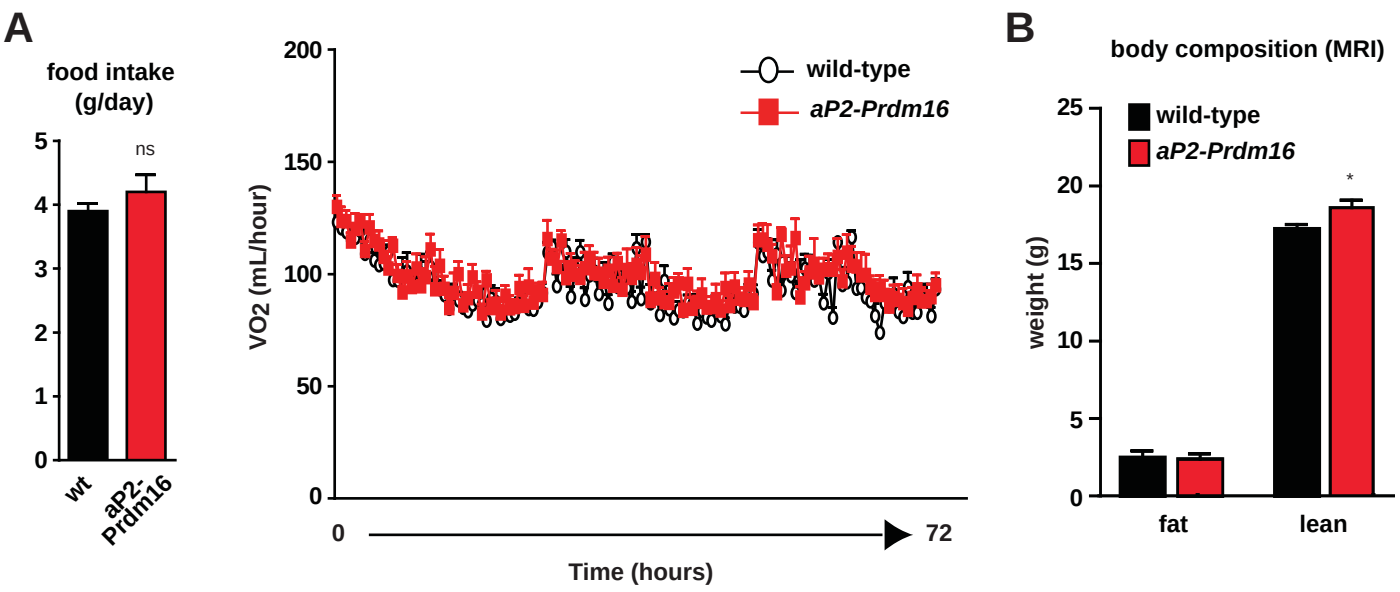

Figure S4. Chow fed *aP2-Prdm16* mice exhibit no alterations in daily energy balance

(A) Metabolic analyses of chow fed wild type (black graphs) and *aP2-Prdm16* (red graphs) mice at 16 weeks of age using a CLAMS (Comprehensive Lab Animal Monitoring System) apparatus. Food intake (left) and energy expenditure (VO_2) (right) was measured over 72 hours. (B) Body composition of wild-type and *aP2-Prdm16* mice (as above) was determined by magnetic resonance imaging (MRI). (n=16 mice/group). *p<0.05

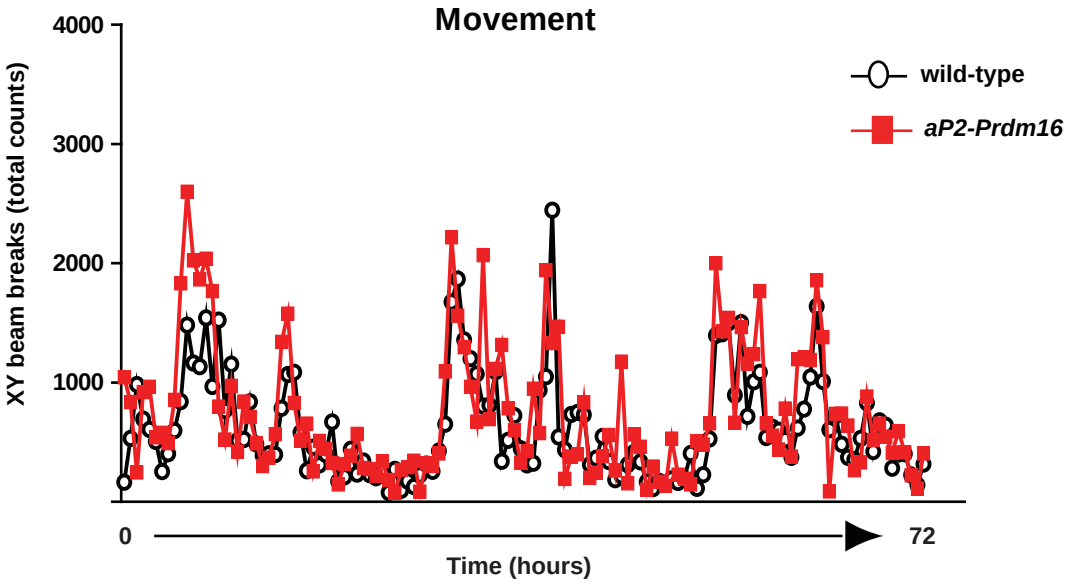

Figure S5. Movement is similar between wild-type and *aP2-Prdm16* animals

Movement of high fat fed wild type (black circles) and *aP2-Prdm16* (red squares) mice was measured by counting laser beam-breaks in a CLAMS (Comprehensive Lab Animal Monitoring System) apparatus. (n=16 mice per study, repeated in 3 separate trials)

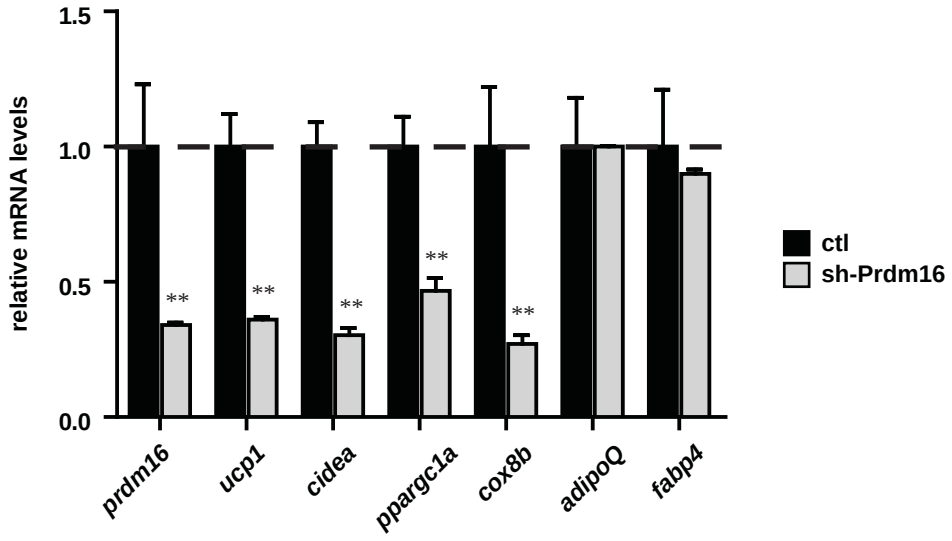

Figure S6. Knock-down of Prdm16 in mature subcutaneous adipocytes reduces the levels of thermogenic genes

Primary stromal-vascular cells from subcutaneous WAT of adult mice were induced to differentiate into adipocytes. At day 7 of differentiation, mature adipocytes were transduced with adenovirus expressing a control (ctl) scrambled short-hairpin (sh) sequence or sh-Prdm16. Relative mRNA levels of: *Prdm16*, brown-fat related genes (*ucp1, cidea, ppargc1a, cox8b*) and general adipocyte differentiation genes(*adipoQ, fabp4*) were measured in ctl and sh-Prdm16 adipocytes 48 hr after transduction. (n= 3,**p<0.01)

Supplemental Table 1. Primers used for real-time PCR analysis

Gene	Forward primer (5' - 3')	Reverse primer (5' - 3')
AdipoQ	GCACTGGCAAGTTCTACTGCAA	GTAGGTGAAGAGAACGGCCTTGT
Elovl3	TCC GCG TTC TCA TGT AGG TCT	GGA CCT GAT GCA ACC CTA TGA
Cidea	TGC TCT TCT GTA TCG CCC AGT	GCC GTG TTA AGG AAT CTG CTG
Cox8b	GAA CCA TGA AGC CAA CGA CT	GCG AAG TTC ACA GTG GTT CC
Fabp4	ACA CCG AGA TTT CCT TCA AAC TG	CCA TCT AGG GTT ATG ATG CTC TTC A
Glut4	GTG ACT GGA ACA CTG GTC CTA	CCA GCC AGT TGC ATT GTA G
Ppargc1a	CCC TGC CAT TGT TAA GAC C	TGC TGC TGT TCC TGT TTT C
Pparγ	GTGCCAGTTTCGATCCGTAGA	GGCCAGCATCGTGTAGATGA
Prdm16	CAG CAC GGT GAA GCC ATT C	GCG TGC ATC CGC TTG TG
Retn	CTG TCC AGT CTA TCC TTG CAC AC	CAG AAG GCA CAG CAG TCT TGA
Tbp	GAA GCT GCG GTA CAA TTC CAG	CCC CTT GTA CCC TTC ACC AAT
Ucp1	ACT GCC ACA CCT CCA GTC ATT	CTT TGC CTC ACT CAG GAT TGG