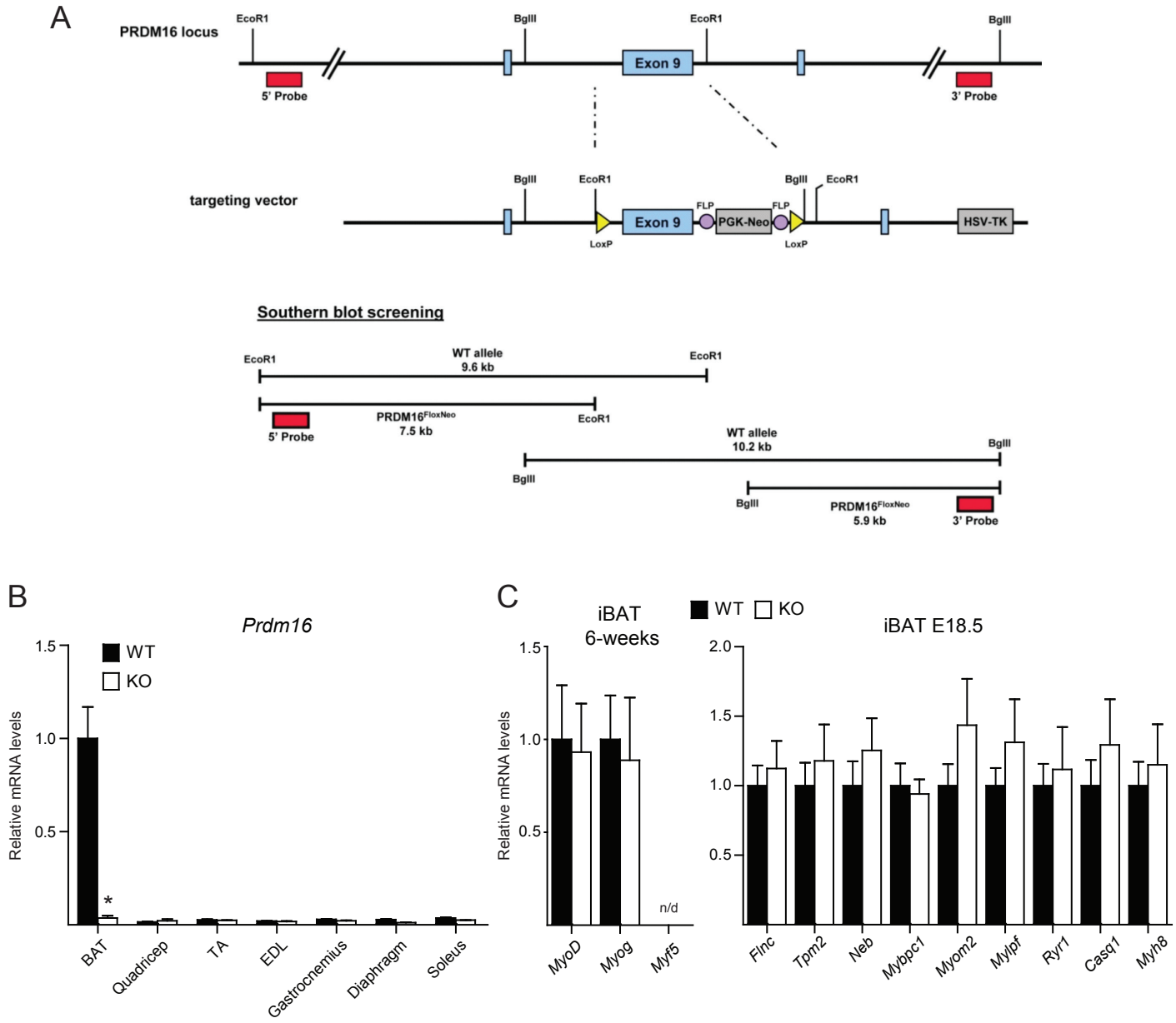

Figure S1. Prdm16 deficiency in BAT does not increase the expression of skeletal muscle genes (related to Figure 1)

(A) Gene targeting strategy for creating *Prdm16*^{flox} mice.
(B) *Prdm16* mRNA levels in interscapular brown adipose tissue (iBAT) and several skeletal muscles (quadricep, tibialis anterior [TA], extensor digitorum longus [EDL], gastrocnemius, diaphragm and soleus) of wildtype (WT) and *Myf5-ΔPrdm16* (KO) mice.
(C) mRNA levels of skeletal muscle-selective genes from the iBAT of 6-week-old and E18.5 WT and KO mice. (B,C: mean ± SEM, n=6-11, *p<0.01).

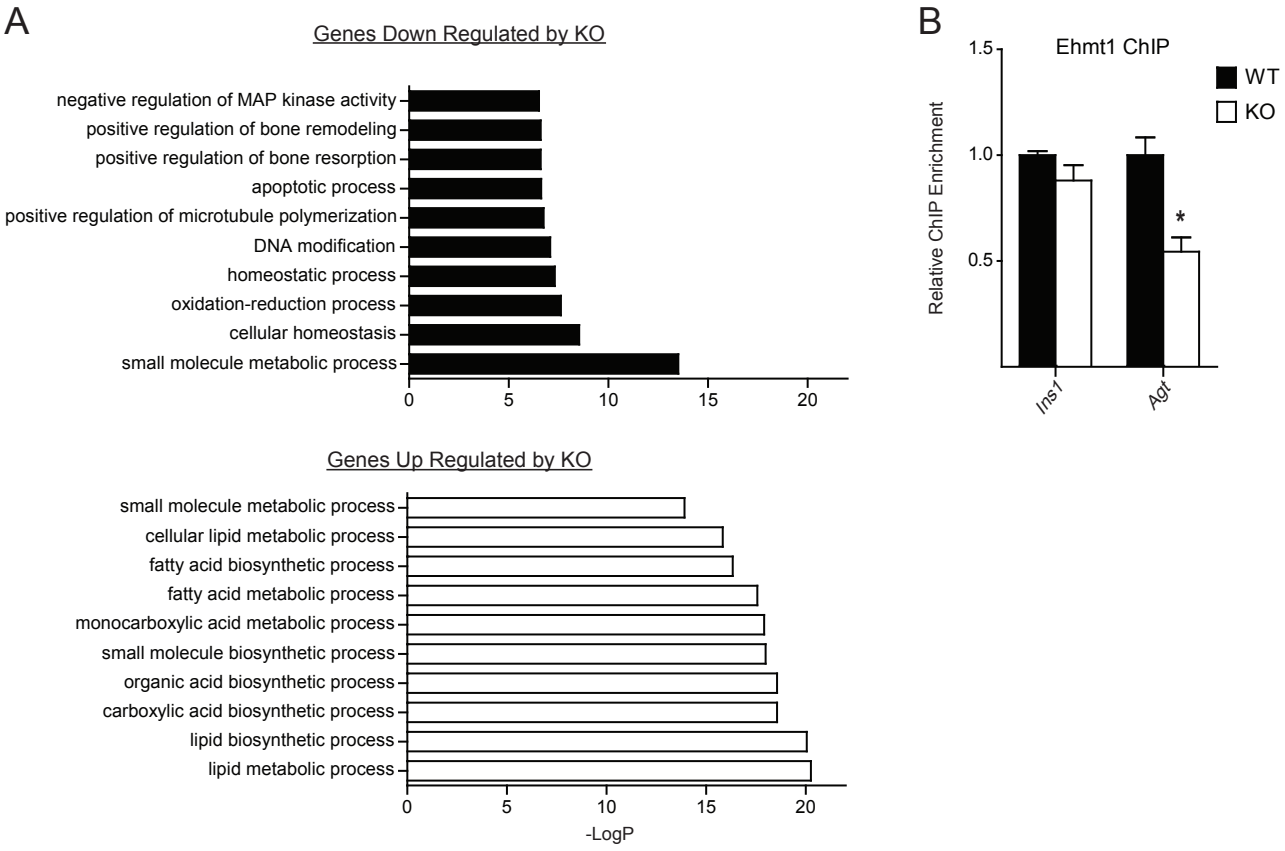

Figure S2. Prdm16 deficient iBAT expresses a white fat-related gene profile (related to Figure 2)

(A) Gene Ontology (GO) analysis of genes that are differentially expressed between wildtype (WT) and Prdm16-deficient interscapular BAT (KO) of 6-week-old mice.
(B) Relative ChIP enrichment for Ehmt1 at the *Ins1* (control) and *Agt* promoter in WT and KO iBAT.
(mean ± stdev, n=3, *p<0.05).

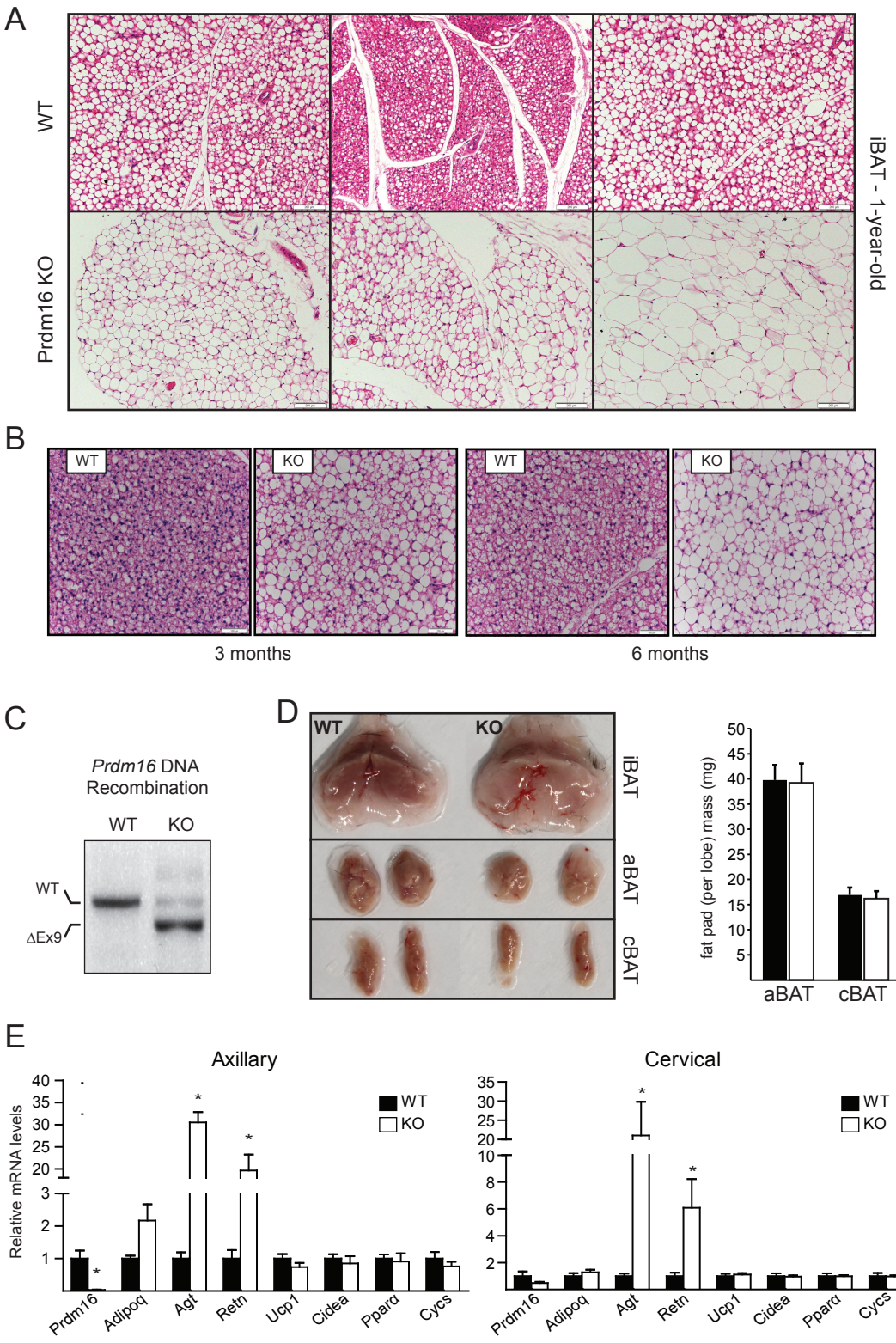

Figure S3. Prdm16-deficiency causes a loss of interscapular brown adipose tissue identity in adult mice (related to Figure 3)

(A) Hematoxylin and eosin (H&E) staining of sections from the interscapular brown adipose tissue (iBAT) of one-year-old WT and *Myf5-ΔPrdm16* (Prdm16 KO) mice.
(B) H&E staining of WT and *Myf5-ΔPrdm16* (KO) iBAT from 3 and 6 month-old-mice.
(C) *Myf5*-Cre driven DNA recombination of the *Prdm16* locus in iBAT from 9-month-old WT and KO mice.
(D) Gross morphology and mass of dissected axillary and cervical BAT depots from WT and KO mice.
(E) mRNA levels of brown and white fat-related genes in the axillary and cervical BAT depots of 6-month-old WT and KO mice (mean ± stdev, n=4, *p<0.05).

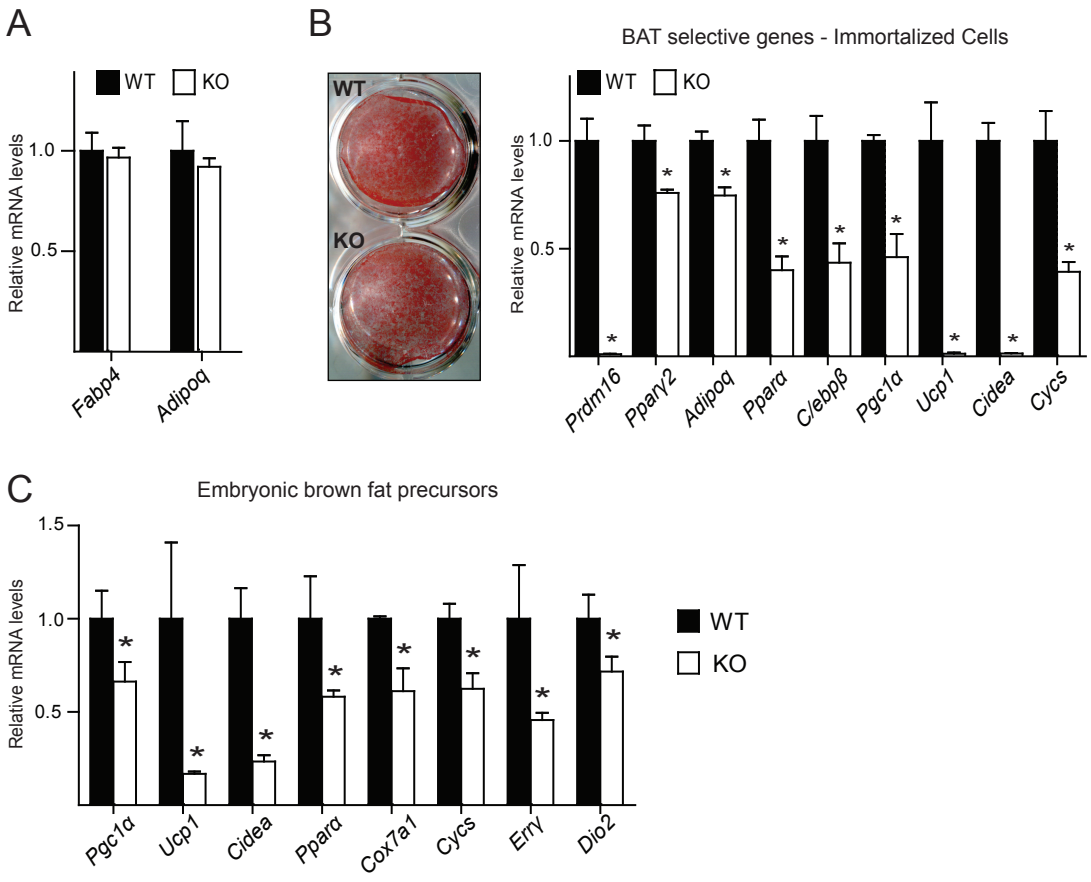

Figure S4. Prdm16 is required for brown fat cell differentiation *ex vivo* (related to Figure 4)

(A) *Fabp4* and *Adipoq* mRNA levels in adipocytes derived from newborn wildtyped (WT) and *Myf5-ΔPrdm16* (KO) brown adipocyte precursors.
(B) Adipocytes from immortalized WT and Prdm16 KO brown precursor cells. Oil-red-O staining (left); mRNA levels of brown fat-selective genes (mean ± stdev, n=3) (right).
(C) Embryonic Pdgfrα+ precursors from the body wall of E14.5 WT and KO embryos were induced to differentiate into adipocytes for 8 days followed by qPCR analysis of brown fat-selective gene levels (mean ± SEM, n=3, *p<0.05).

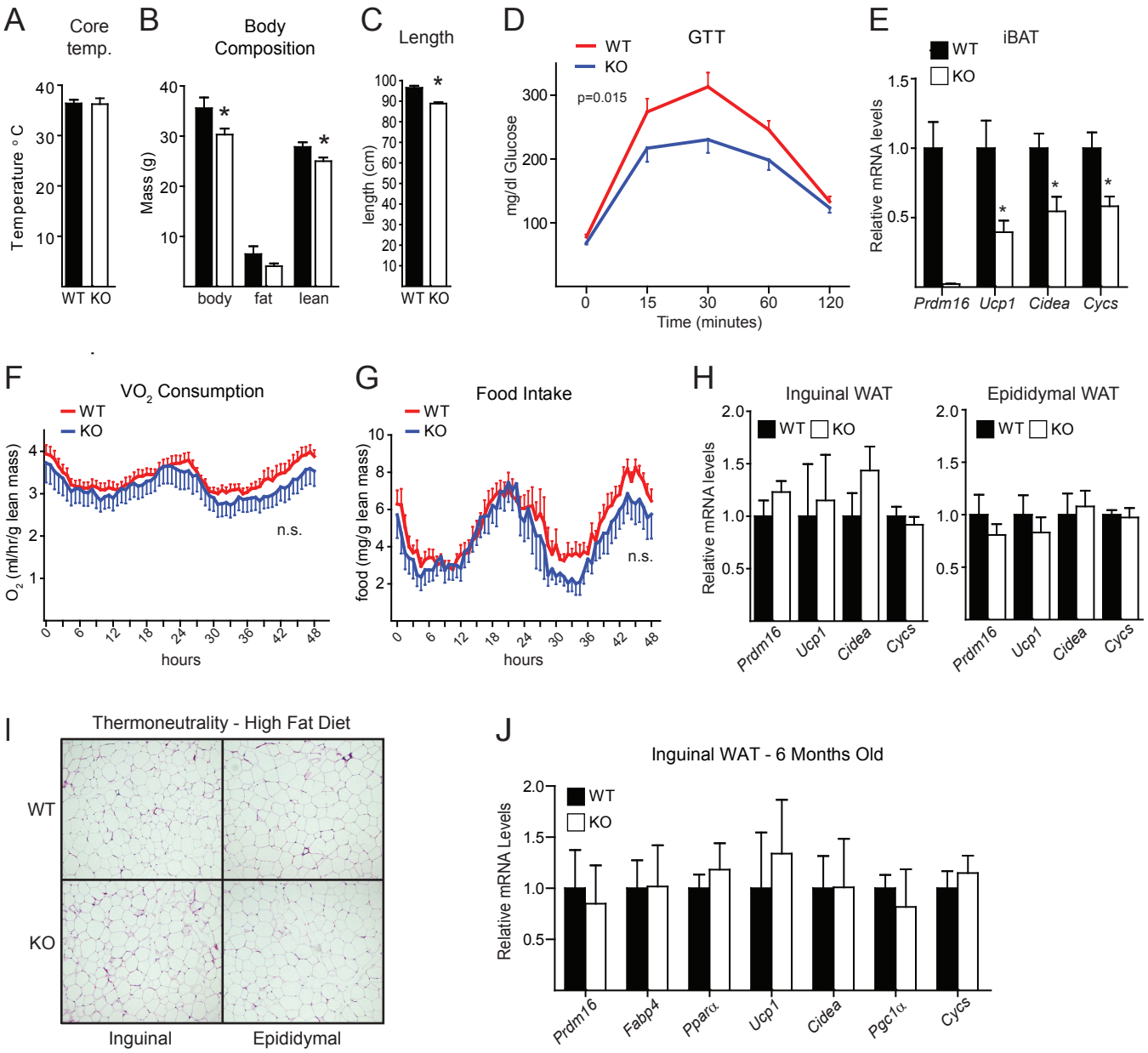

Figure S5. *Myf5-ΔPrdm16* mice are not prone to weight gain and do not display increased browning of white fat (related to Figure 5)

(A) Core body temperatures of 3-month-old WT and *Myf5-ΔPrdm16* (KO) mice, n=4.
(B,C) Body composition (B) and length (C) of 11-month-old WT and KO mice, n=10.
(D) Glucose tolerance test in 11-month-old chow-fed WT and KO mice, n=10.
(E) mRNA levels of brown fat-selective genes in interscapular BAT (iBAT) of 3-month-old WT and KO mice that were maintained at thermoneutrality and fed a high-fat diet, n=8.
(F,G) Oxygen consumption (F) and food intake (G) over 48 hours in individually housed 11-month-old WT and KO mice housed at 22°C. Normalized to lean body mass.
(H) mRNA levels of brown fat-selective genes in inguinal and epididymal white adipose tissue (WAT) from 6-month-old WT and KO mice housed under standard conditions, n=4.
(I,J) Hematoxylin and eosin staining (I) and mRNA expression levels of brown fat-selective genes (J) in inguinal and epididymal WAT from 3-month-old mice maintained at thermoneutrality and fed a high-fat diet for 9 weeks, n=8.
All values are mean ± SEM; *p < 0.05.

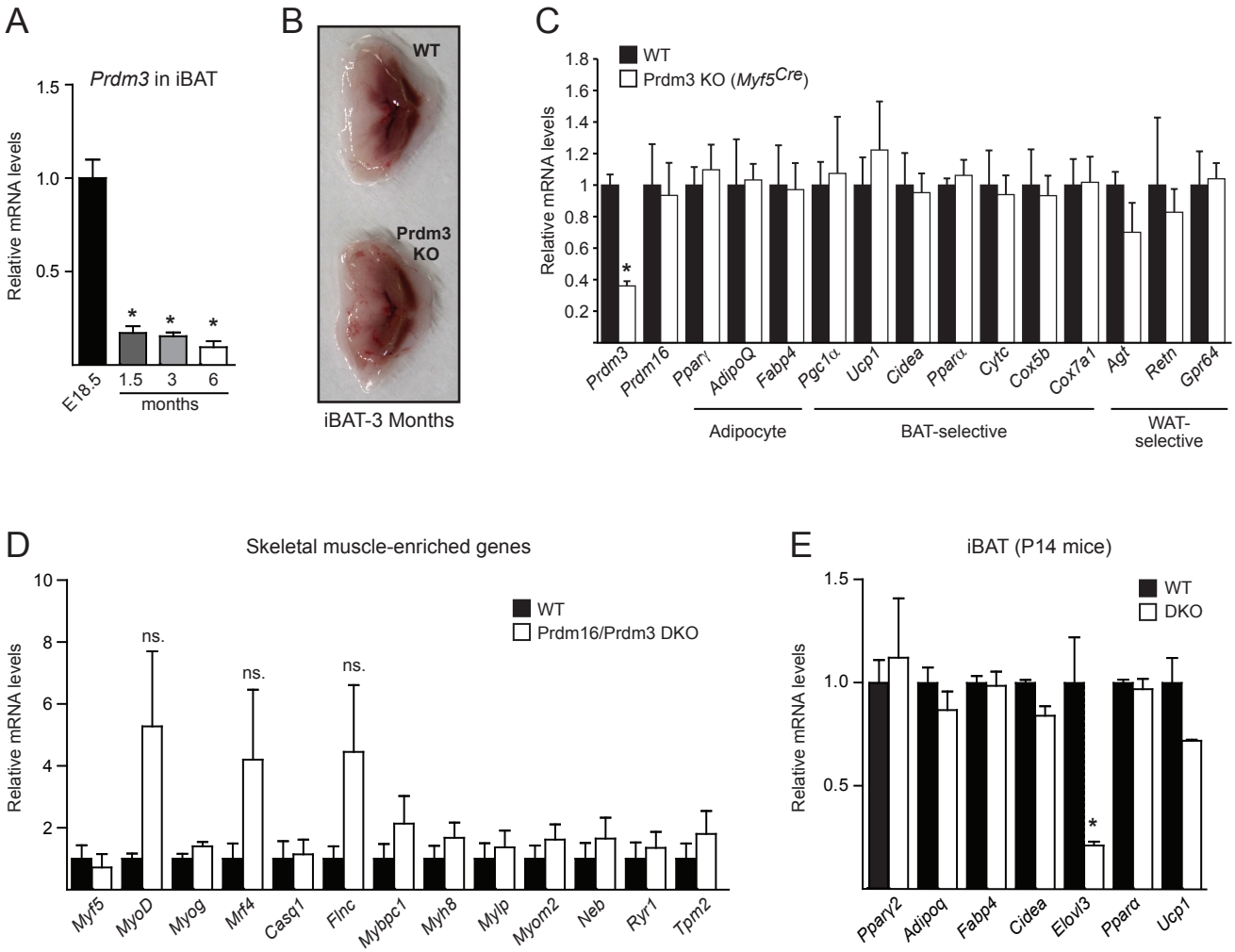

Figure S6. Prdm16 or Prdm3 are required for the postnatal maintenance of brown fat fate (related to Figure 6)

(A) *Prdm3* mRNA levels in interscapular brown adipose tissue (iBAT) of E18.5 embryos (n=5) and 1.5 to 6 month-old mice (as indicated (n=4/group).
(B) iBAT depots from WT and Prdm3 Knockout (KO) (*Myf5*^{Cre}) mice
(C) mRNA levels of *Prdm3*, *Prdm16* and various adipocyte, BAT-selective and WAT-selective markers in the iBAT of 3-month-old WT (n=3) and Prdm3 KO animals (n=4).
(D) mRNA levels of skeletal muscle-selective genes in iBAT of 3-month-old WT (n=7) and *Myf5-ΔPrdm16/Prdm3* (DKO, n=5) mice.
(E) mRNA levels of adipocyte and BAT-selective genes in iBAT of p14 (2-week-old) WT (n=7) and DKO (n=4) mice.
All mRNA expression values are mean ± SEM, *p < 0.05.

Table S1 Primers used for real-time PCR and ChIP-PCR analysis

mRNA	Fwd	Rev
Adipoq	GCACTGGCAAGTTCTACTGCAA	GTAGGTGAAGAGAACGGCCTTGT
Agt	AAGACCCTGCATGATCAGCTC	CTTCCTGCCTCATTCAGCATC
Casq1	ATGAGGTGCTGGCCCTCCTCT	GAGTCCACCAGGCCAAAGCCA
Cidea	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG
Cideb	ATGGTGCTTGAGCAGGGCCAG	ATCGAAGGTGATGCGGGCGAT
Clstn3	AGCCGTGAGGTCATCGAGTGC	CCTCCAGGGTGAGCAGGGACT
Cox5b	GCTGCATCTGTGAAGAGGACAAC	CAGCTTGTAATGGGTTCCACAGT
Cox7a1	CAGCGTCATGGTCAGTCTGT	AGAAAACCGTGTGGCAGAGA
Cycs	GCAAGCATAAGACTGGACCAAA	TTGTTGGCATCTGTGTAAGAGAATC
Cyp2b10	TGCCCCTCTTGGGGAACCTCT	CACAGGCCTTGGTCCCAGGTG
Dgat1	CGGGACAAAGACGGGCGGAC	AGGATCAGCATCACCACACACCA
Dio2	CAGTGTGGTGCACGTCTCCAATC	TGAACCAAAGTTGACCACCAG
Errg	TGGCTGACCGAGAGTTGGTGG	AGCGATCGGTACACAACGCCG
Fabp4	ACACCGAGATTTCCTTCAAACTG	CCATCTAGGGTTATGATGCTCTTCA
Flnc	ATGCCAGAGAGGCCATGCAGC	CGGGTTTGAGCTTGGCCTTGG
Fosl1	GAGACGCGAGCGGAACAA	CTTCCAGCACCAGCTCAAGG
Fosl2	AGCCTCCCGAAGAGGACAG	AGGACATTGGGGTAGGTGAAG
Gpr64	CCACACCAGCCCCATCTGTCC	TCCATCTGGGATACTTGGGCTTCC
Hsph1	ACGGACCTGCCGCTGAACATC	TGCAGGAGCTCAGCACACAGT
Krt19	ACCATCGAGGACTTGCGCGAC	GCTCAGACGCAAGGCGTGTTC
Limk1	GACCTGGGTCGCTCCGAATCC	CCTTGCCCAGCACTTCCCCAT
Mybpc1	CGCAGGGAATTATAGGTGTGAGGTC	CCTGCATCCTCTTGACCTTCTCCA
Myf5	CAGCCCCACCTCCAACTG	GGGACCAGACAGGGCTGTTA
Myh8	CTCCATGAGCCCGGAGTGCTG	CGGCAGCCACTTGTAGGGGTT
Mylpf	GAGAAGGGCAGGAGCGGAAGG	TGGCTGCAAAGGTGTCCCGAA
Myod	CGCCACTCCGGGACATAG	GAAGTCGTCTGCTGTCTCAAAGG
Myogenin	AGCGCAGGCTCAAGAAAGTGAATG	CTGTAGGCGCTCAATGTACTGGAT
Myom2	CGGTCACAGGCTCGGGACAAG	GGGCCCTGCTCATTCGGTCTT
Neb	AGGCAAAGGCTTCTTCCCCCA	GGGCTTGCACCAGGACAGGAG
Nnmt	GGAGCCTTTGACTGGTCCCCA	CCTGCTTGATTGCACGCCTCA
Pck1	TGGCCATGATGAACCCCAGCC	GAGGTGCCAGGAGCAACTCCA
Pgc-1a	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
Ppara	GCGTACGGCAATGGCTTTAT	GAACGGCTTCCTCAGGTTCTT
Pparg1	TGAAAGAAGCGGTGAACCACTG	TGGCATCTCGTGTCAACCATG
Pparg2	TGGCATCTCTGTGTCAACCATG	GCATGGTGCCTTCGCTGA
Prdm3	AACAAAACCTGGAGAGTGAGAG	AATGCCTTGGGACACTGATC
Prdm16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
Retn	CTGTCCAGTCTATCCTTGCACAC	CAGAAGGCACAGCAGTCTTGA
Ryr1	GCACTCATGCCCGCTCCCTAT	GGCCTTGGTCCTCAGTGAGCC
Sgk2	GGTGGTGCTTAGGGGCAGTCC	GAGGTCACAGGCAGCCACTGT
Tbp	GAAGCTGCGGTACAATTCCAG	CCCCTTGTACCCTTCACCAAT
Thbd	CAGGGGCCCAATCCATGTCCC	CGGATCCAGAAGCTCCACGCA
Tpm2	GGGGACAGAGGACGAGGTGGA	GGCGGTTCAGAGAGGCCACAT
Trfr2	GAGCGACCTCCAGGCCATGTT	TGGCGCGAGAGCTTATCGAGG
Trim14	TTGGAAGACGCCGGGGAAAGG	GGCCAGTACTTCCTCTTCATCCAGG
Tubb2a	GTGAGGTCGGGACCATTCGGC	GACAGAGTCCACCAGCTCGGC
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
Ugdh	GCCGATGTGGAAGAGGTGGCA	CGGGCAGATTCAGAGCCTCACA
ChIP		
Ins	GGACCCACAAGTGGAACAAC	GTGCAGCACTGATCCACAAT
Retn -2kb	AGCACAAAGGTGGGGGATGGT	AACCACAGAACAGGAGGCCCAT
Retn -1.5kb	TGGACAGAGGGGTGTCAGGGG	GCATTGCTGGAGACCTGAGGTGA
Retn -1kb	CAGGAGTTCAAGGTCGTTCTTGGC	GCTGTTACACTGGCCTCGATGT
Retn -0.5kb	CTCTTGCTTAGCCCCACCCCC	ACCACACCACCAGACCCTCAC
Retn P.	AGACAACGTCCTGAGAAGACAATC	CCATCCTGCCTTGGATAATAAGTA
Retn 0.5kb	GGAACAGACCCGCCCAGCTAC	GGGGCCTTTTGGAGTGAGGGG
Retn 1.0kb	ATGGGTGCCCCTACACCATGC	GCAGGGGGCGATCTTTGGGT
Retn 1.5kb	CATCTCTGCCTCCCACCTGCC	GCGGGCTGCTGTCCAGTCTAT
Retn 2kb	TGGACCTTGGCAGGACTGAGGT	ATCCTCTGCCCCAGGTGGTGT
Agt P.	CTTGGTCAAGCCTGGATTCTC	CCAACCTAGACAAGCACAGCTATC
Mito DNA		
Ndufv1	CTTCCCCACTGGCCTCAAG	CCAAAACCCAGTGATCCAGC
MT-CO1	TGCTAGCCGCAGGCATTAC	GGGTGCCCAAAGAATCAGAAC