SUPPLEMENTAL FIGURES AND TABLES

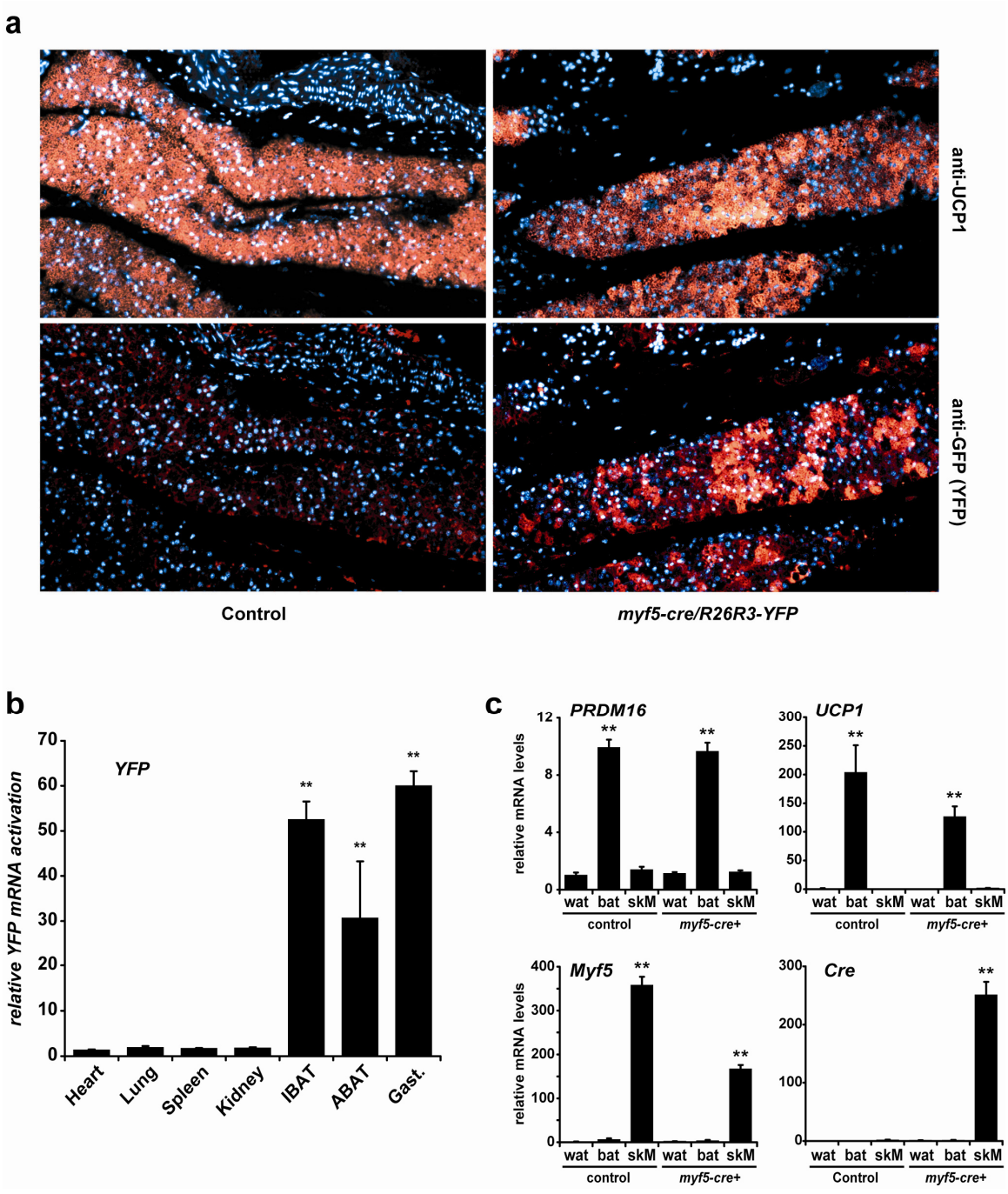

Fig. S1. *myf5*-expressing cells give rise to brown fat depots and skeletal muscle

(a) Perirenal BAT from control (*cre* negative) and *myf5-cre:R26R3-YFP* mice were analyzed by immunohistochemistry to detect YFP (anti-GFP) and UCP1 protein. **(b)** Real-time PCR analysis of *YFP* gene activation in: heart, lung, spleen, kidney, interscapular BAT (IBAT), axial BAT (ABAT) and gastrocnemius muscle (Gast.) from *myf5-cre*:*R26R3-YFP* mice **(c)** Real time PCR analysis of *PRDM16*, *UCP1*, *myf5* and *cre* recombinase expression in WAT, BAT and skeletal muscle (skM) (n=4-6 mice/group; error bars represent ± SEM). **p<0.01.

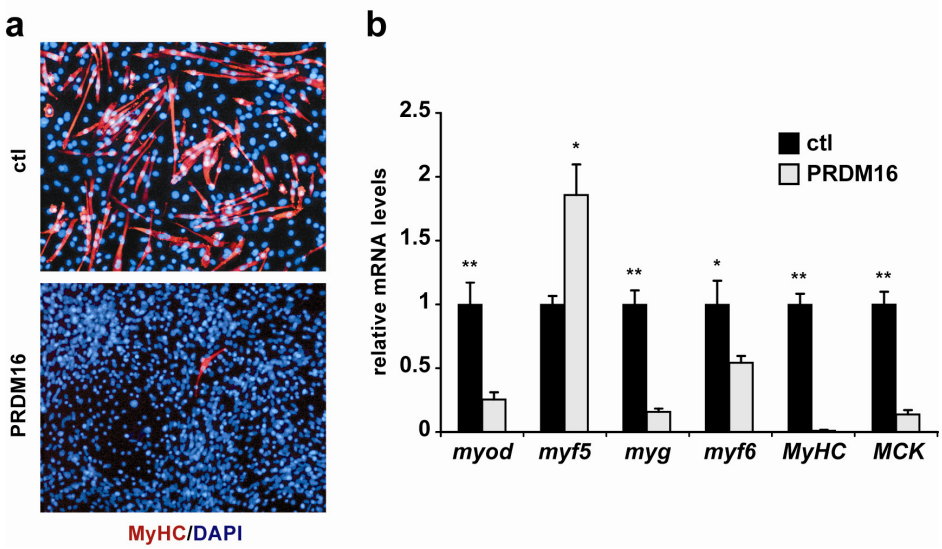

Fig. S2. PRDM16 expression blocks myogenic differentiation

(a) C2C12 myoblast cultures expressing retroviral PRDM16 or vector control (ctl) were induced to undergo muscle differentiation in 2% horse serum and analyzed by immunocytochemistry for expression of skeletal Myosin Heavy Chain (MyHC) protein. **(b)** Real-time PCR analysis of skeletal muscle-specific genes (n=3; error bars represent± SD; *p<0.05 **p<0.01).

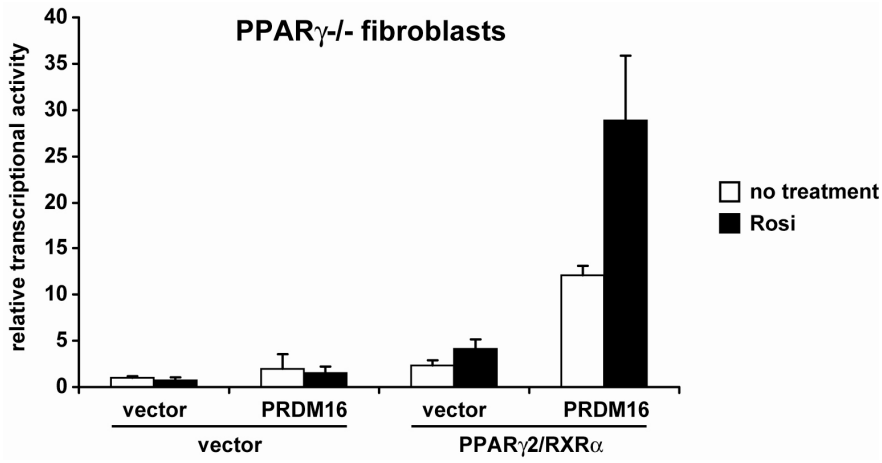

Fig. S3. PRDM16 coactivates exogenous PPARγ in *PPARγ*-deficient cells

Transcriptional activity of a PPAR-driven reporter gene in response to PRDM16 or vector expression in *PPARγ-/-* cells with or without exogenous PPARγ/RXRα (+/- 1 μM rosiglitazone) (n=3; error bars represent ± SD; **p<0.05).

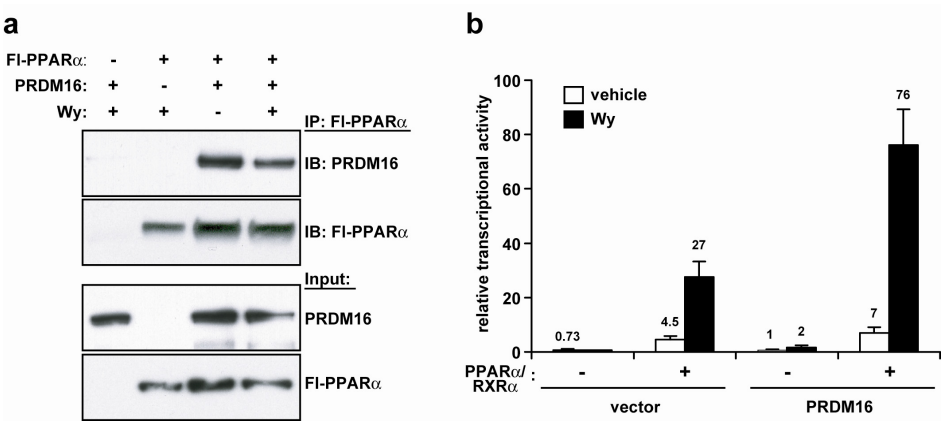

Fig. S4. PRDM16 binds and activates the transcriptional function of PPARα

(a) Flag-PPARα was immunoprecipitated from COS-7 cells expressing exogenous PRDM16 and/or Flag-PPARα followed by western blot analysis to detect PRDM16. The interaction was examined in the presence or absence of 1 μM of a specific PPARα ligand, WY-14643 (Wy). **(b)** Transcriptional activity of a PPAR-driven reporter gene (3x DR1) in response to PPARα/RXRα and PRDM16 or vector expression in COS-7 cells (+/- 1 μM Wy). (n=3; error bars represent ± SD; **p<0.05).

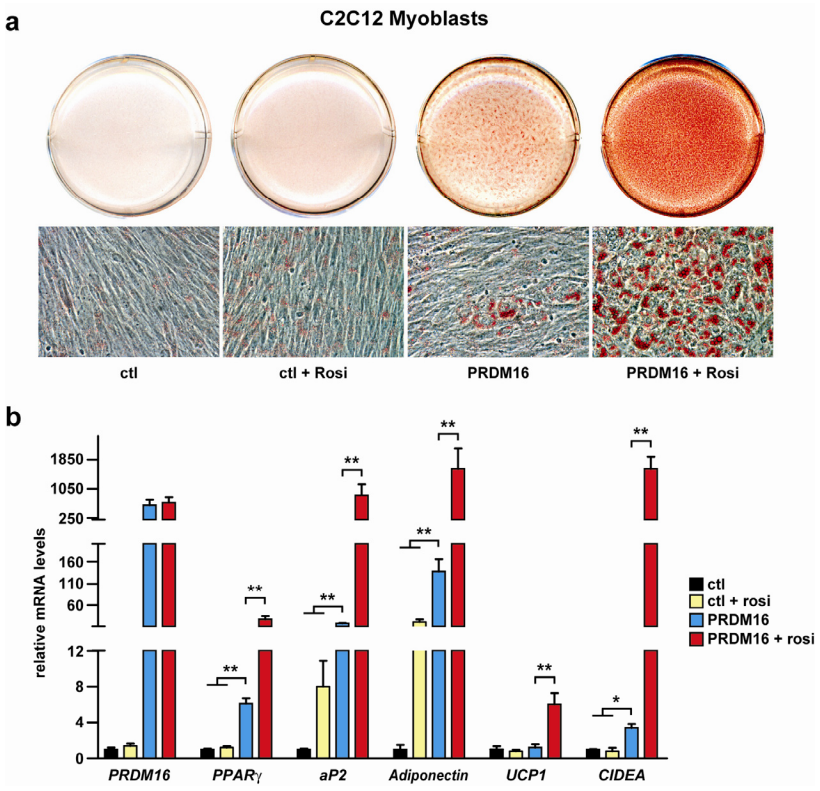

Fig. S5. PPARγ activation is required for the adipogenic action of PRDM16

(a) Oil-Red-O staining of C2C12 myoblast cultures expressing retroviral PRDM16 or vector control (ctl) 6 days after inducing adipocyte differentiation in the presence or absence of 1 μM of the PPARγ-specific agonist, rosiglitazone. **(b)** Real-time PCR analysis of: *PRDM16*; adipocyte markers (*PPARγ, aP2, Adiponectin*); and BAT-selective genes (*CIDEA, UCP1*) (n=3; error bars represent ± SD; **p<0.05).

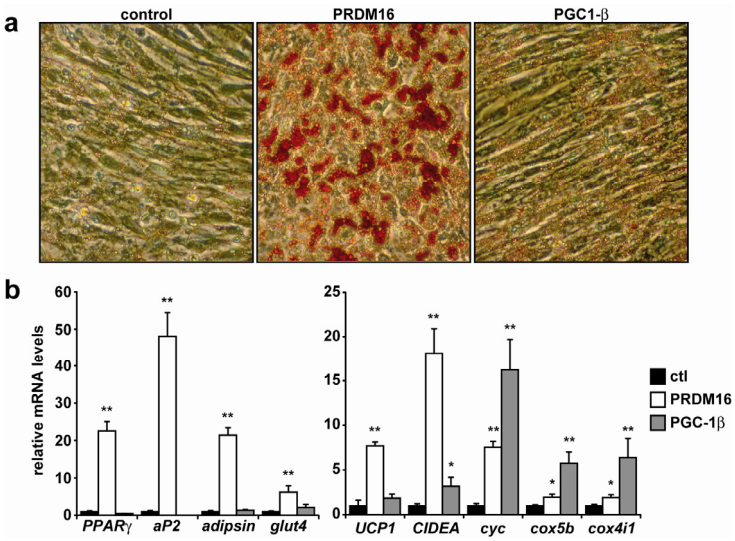

Fig. S6. PGC-1β expression does not stimulate adipogenesis in myoblasts

(a) Oil-Red-O staining of C2C12 myoblast cultures transduced with retroviral PRDM16, PGC-1β or vector control 7 days after inducing adipocyte differentiation. **(b)** Real-time PCR analysis of: adipocyte markers (left), BAT genes (*UCP1*, *CIDEA*); and mitochondrial genes (*cyc*, *cox5b*, *cox4i1*) (n=3, error bars are ± SD; *p<0.05 **p<0.01).

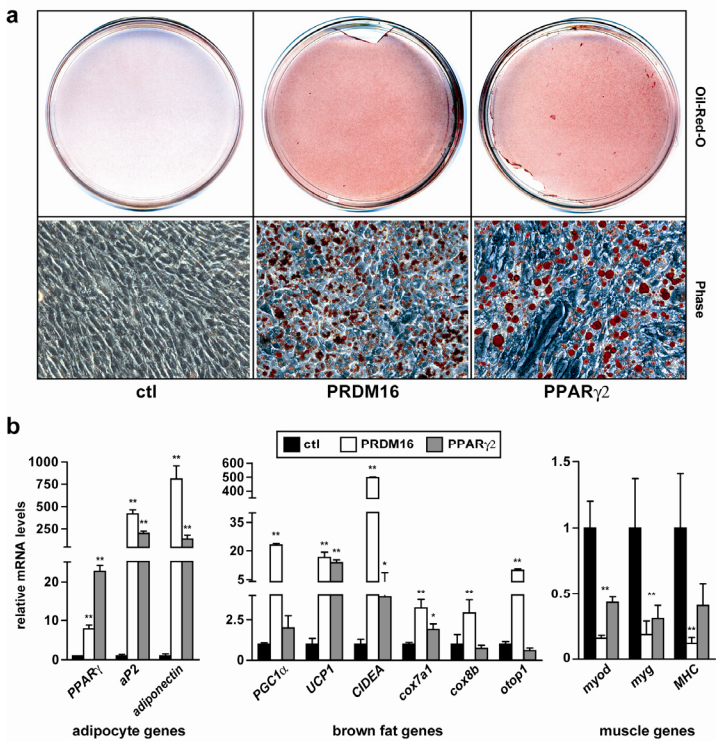

Fig. S7. PPARγ alone drives adipogenesis but does not induce the brown fat cell gene program in C2C12 myoblasts

(a) Oil-Red-O staining of C2C12 myoblast cultures expressing retroviral PRDM16, PPARγ2 or empty vector control (ctl) 7 days after induction of adipocyte differentiation. **(b)** Real-time PCR analysis of: adipocyte markers; BAT-selective genes; and skeletal muscle-specific genes (n=3 per group, error bars represent ± SD; *p<0.05 **p<0.01).

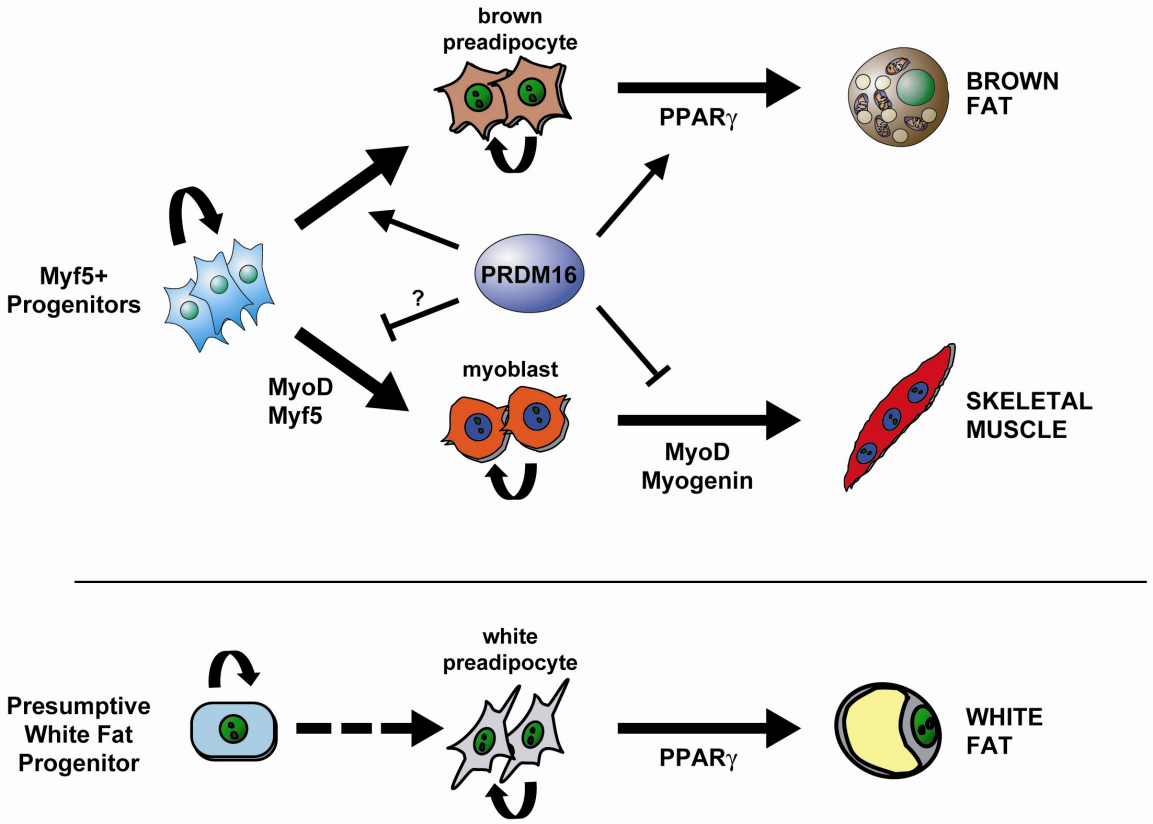

Fig. S8. Model of PRDM16 function in the specification of brown fat fate

Lineage tracing studies reveal that *myf5*-expressing precursors give rise to skeletal muscle and BAT but not WAT or any other tissues examined. PRDM16 expression in myoblasts induces BAT development and represses skeletal muscle differentiation. PRDM16 stimulates brown adipogenesis in myoblasts, at least in part, via binding and coactivating PPARγ. Together, these studies suggest that PRDM16 expression in bipotent muscle/brown fat progenitor cells determines brown fat identity. The mechanisms by which PRDM16 promotes brown fat determination and represses the muscle lineage in this upstream progenitor cell remain to be defined.

Table S1. Proteins identified by MALDI-TOF-MS/MS

MW		Protein Identification
~30 kda		splicing factor, arginine/serine-rich 1 isoform 2 (~22 KDa); NCBI# 26383576; Sequence: I Y V G N L P P D I R
~35 kda		ribosomal protein L6 (~33 kDa); NCBI# 84662736; Sequence: A V P Q L Q G Y L R
~42 kda	A:	β-actin (~42 kDa); NCBI# 74190672
	B:	tropomodulin 3 (~40 kDa); NCBI# 8394460 ; Sequence: F G Y Q F T Q Q G P R
~48 kda	A:	β -actin (~42 kDa); NCBI# 74190672
	B:	C-terminal binding protein 1 (~48 kDa); NCBI# 7304989
	C:	C-terminal binding protein 2 (~46 kDa); NCBI# 2909779 Sequence: I R G E T L G L I G F G R;
	D:	heterogeneous nuclear ribonucleoprotein F (~46 kDa); NCBI# 19527048 Sequence: H S G P N S A D S A N D G F V R
~55 kda	A:	Trim35 (~55kda); NCBI # 32425788; Sequence: I R D E F D K L R
	B:	polymerase I and transcript release factor (~43kda); NCBI # 6679567 Sequence: K S F T P D H V VYAR
	C:	heterogeneous nuclear ribonucleoprotein H2 (~49 kDa); NCBI# 9845253 Sequence: H T G P N S P D T A N D G F V R
	D:	immunoglobulin heavy chain constant region (~36 kDa); NCBI# 14091948 Sequence: A P Q V Y T I P P P K E Q M A K
~65 kda	A:	DEAD box polypeptide 5 (~69 kDa); NCBI# 120538559
	B:	**PPARγ (~57kDa); NCBI # 18255316** Sequence: H I T P L Q E Q S K
~70 kda	A:	HSP 70, protein 5 (~72 kDa); NCBI# 31981722
	B:	HSP 8 (~71kDa); NCBI# 42542422
	C:	HSP 9A (~74 kDa); NCBI# 6754256
	D:	heterogeneous nuclear ribonucleoprotein M (~78 kDa); NCBI# 21313308
~85 kda		gelsolin (~86kDa); NCBI# 28916693
~100 kda	A:	myosin IC isoform b (~118kDa); NCBI# 6678986
	B:	major vault protein (MVP); NCBI # 17433104 Sequence: V P H N A A V Q V Y D Y R;
	C:	gelsolin (~86kDa); NCBI# 28916693
~125 kda		pyruvate carboxylase (~130 kDa); NCBI# 6679237
~150 kda		PRDM16 protein (~130 kDa); NCBI# 37590584
~175 kDa		PRDM16 protein (~130 kDa); NCBI# 37590584
~200 kda		myosin, heavy polypeptide 9, non-muscle (~226 kDa); NCBI# 114326446
~300 kDa	A:	α-spectrin (~270kDa); NCBI # 115496850
	B:	β-spectrin (~270kDa); NCBI # 117938332

Note: All samples contained peptides derived from PRDM16; this information was removed before identifications were made for each protein sample.

Table S2. Primers used for real-time PCR analysis

Gene	Forward primer (5' - 3')	Reverse primer (5' - 3')
adiponectin	GCACTGGCAAGTTCTACTGCAA	GTAGGTGAAGAGAACGGCCTTGT
aP2	ACA CCG AGA TTT CCT TCA AAC TG	CCA TCT AGG GTT ATG ATG CTC TTC A
cidea	TGC TCT TCT GTA TCG CCC AGT	GCC GTG TTA AGG AAT CTG CTG
cox4i1	ACCAAGCGAATGCTGGACAT	GGCGGAGAAGCCCTGAA
cox5b	GCTGCATCTGTGAAGAGGACAAC	CAGCTTGTAATGGGTTCCACAGT
cox7a1	CAG CGT CAT GGT CAG TCT GT	AGA AAA CCG TGT GGC AGA GA
cox8b	GAA CCA TGA AGC CAA CGA CT	GCG AAG TTC ACA GTG GTT CC
Cre	GCG GTC TGG CAG TAA AAA CTA TC	GTG AAA CAG CAT TGC TGT CAC TT
cyc	GCAAGCATAAGACTGGACCAAA	TTGTTGGCATCTGTGTAAGAGAATC
elovl3	TCC GCG TTC TCA TGT AGG TCT	GGA CCT GAT GCA ACC CTA TGA
glut4	GTG ACT GGA ACA CTG GTC CTA	CCA GCC AGT TGC ATT GTA G
MCK	GCA AGC ACC CCA AGT TTG A	ACC TGT GCC GCG CTT CT
MHC (embryonic)	TCC AAA CCG TCT CTG CAC TGT T	AGC GTA CAA AGT GTG GGT GTG T
myod	CGC CAC TCC GGG ACA TAG	GAA GTC GTC TGC TGT CTC AAA GG
myg	AGC GCA GGC TCA AGA AAG TGA ATG	CTG TAG GCG CTC AAT GTA CTG GAT
myf5	CAG CCC CAC CTC CAA CTG	GGG ACC AGA CAG GGC TGT TA
myf6	ATC AGC TAC ATT GAG CGT CTA CA	CCT GGA ATG ATC CGA AAC ACT TG
otopetrin1	ACT AGG ACC CCG TCG AAT CT	ACC ATG CTC TAC GTG CTG TG
PGC-1α	CCC TGC CAT TGT TAA GAC C	TGC TGC TGT TCC TGT TTT C
PGC-1β	TCC TGT AAA AGC CCG GAG TAT	GCT CTG GTA GGG GCA GTG A
PPARγ	GTGCCAGTTTCGATCCGTAGA	GGCCAGCATCGTGTAGATGA
PRDM16	CAG CAC GGT GAA GCC ATT C	GCG TGC ATC CGC TTG TG
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
YFP	CACGACTTCTTCAAGTCCGCCATG	GCGGATCTTGAAGTTCACCTTGAT