Supplemental Material

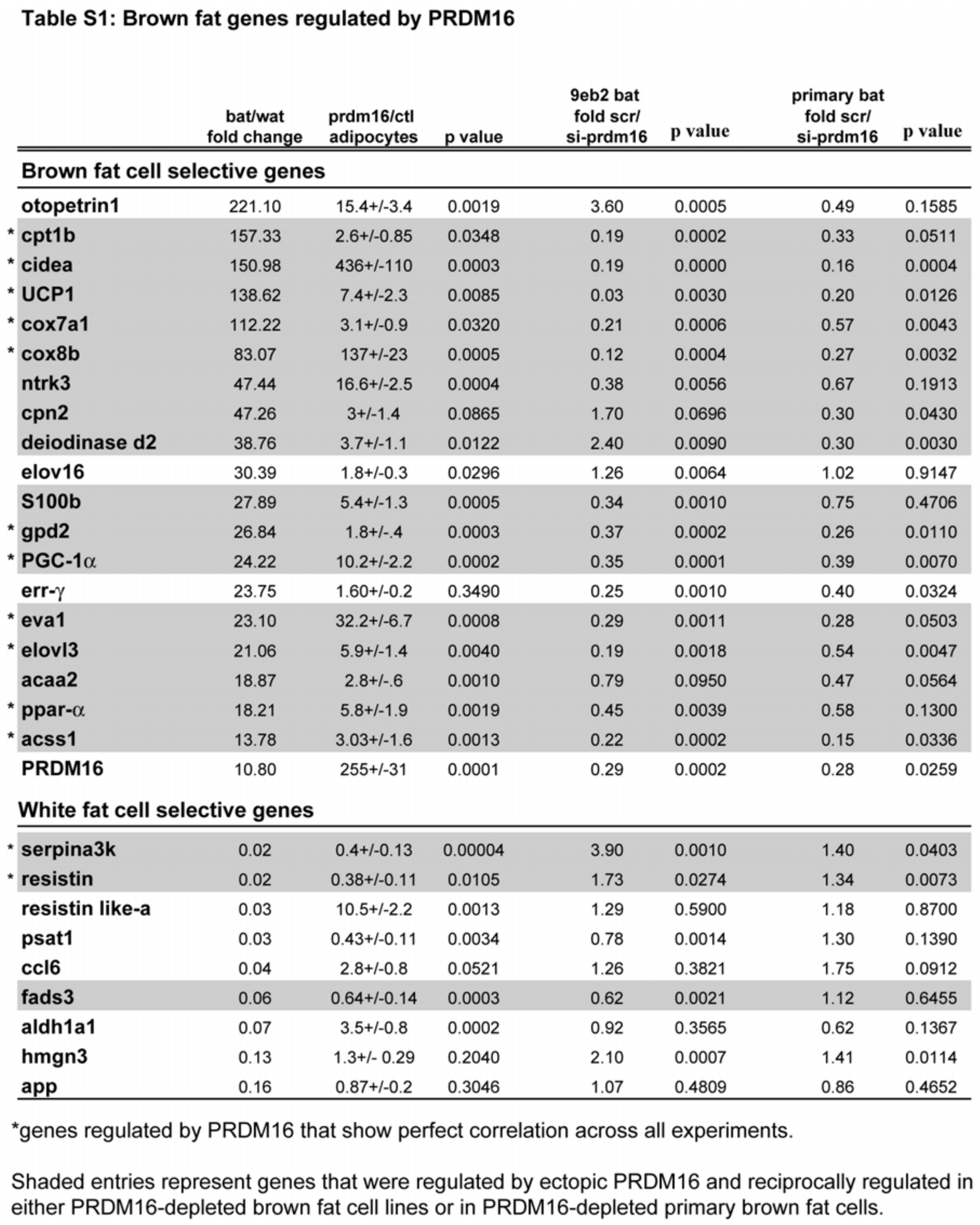

Table S1: Brown fat genes regulated by PRDM16

	bat/wat fold change	prdm16/ctl adipocytes	p value	9eb2 bat fold scr/ si-prdm16	p value	primary bat fold scr/ si-prdm16	p value
Brown fat cell selective genes							
otopetrin1	221.10	15.4+/-3.4	0.0019	3.60	0.0005	0.49	0.1585
* **cpt1b**	157.33	2.6+/-0.85	0.0348	0.19	0.0002	0.33	0.0511
* **cidea**	150.98	436+/-110	0.0003	0.19	0.0000	0.16	0.0004
* **UCP1**	138.62	7.4+/-2.3	0.0085	0.03	0.0030	0.20	0.0126
* **cox7a1**	112.22	3.1+/-0.9	0.0320	0.21	0.0006	0.57	0.0043
* **cox8b**	83.07	137+/-23	0.0005	0.12	0.0004	0.27	0.0032
ntrk3	47.44	16.6+/-2.5	0.0004	0.38	0.0056	0.67	0.1913
cpn2	47.26	3+/-1.4	0.0865	1.70	0.0696	0.30	0.0430
deiodinase d2	38.76	3.7+/-1.1	0.0122	2.40	0.0090	0.30	0.0030
elov16	30.39	1.8+/-0.3	0.0296	1.26	0.0064	1.02	0.9147
S100b	27.89	5.4+/-1.3	0.0005	0.34	0.0010	0.75	0.4706
* **gpd2**	26.84	1.8+/-.4	0.0003	0.37	0.0002	0.26	0.0110
* **PGC-1α**	24.22	10.2+/-2.2	0.0002	0.35	0.0001	0.39	0.0070
err-γ	23.75	1.60+/-0.2	0.3490	0.25	0.0010	0.40	0.0324
* **eva1**	23.10	32.2+/-6.7	0.0008	0.29	0.0011	0.28	0.0503
* **elovl3**	21.06	5.9+/-1.4	0.0040	0.19	0.0018	0.54	0.0047
acaa2	18.87	2.8+/-.6	0.0010	0.79	0.0950	0.47	0.0564
* **ppar-α**	18.21	5.8+/-1.9	0.0019	0.45	0.0039	0.58	0.1300
* **acss1**	13.78	3.03+/-1.6	0.0013	0.22	0.0002	0.15	0.0336
PRDM16	10.80	255+/-31	0.0001	0.29	0.0002	0.28	0.0259
White fat cell selective genes							
* **serpina3k**	0.02	0.4+/-0.13	0.00004	3.90	0.0010	1.40	0.0403
* **resistin**	0.02	0.38+/-0.11	0.0105	1.73	0.0274	1.34	0.0073
resistin like-a	0.03	10.5+/-2.2	0.0013	1.29	0.5900	1.18	0.8700
psat1	0.03	0.43+/-0.11	0.0034	0.78	0.0014	1.30	0.1390
ccl6	0.04	2.8+/-0.8	0.0521	1.26	0.3821	1.75	0.0912
fads3	0.06	0.64+/-0.14	0.0003	0.62	0.0021	1.12	0.6455
aldh1a1	0.07	3.5+/-0.8	0.0002	0.92	0.3565	0.62	0.1367
hmgn3	0.13	1.3+/- 0.29	0.2040	2.10	0.0007	1.41	0.0114
app	0.16	0.87+/-0.2	0.3046	1.07	0.4809	0.86	0.4652

*genes regulated by PRDM16 that show perfect correlation across all experiments.

Shaded entries represent genes that were regulated by ectopic PRDM16 and reciprocally regulated in either PRDM16-depleted brown fat cell lines or in PRDM16-depleted primary brown fat cells.

Table S2. Primers used for real-time PCR analysis

Gene	Forward primer (5’ - 3’)	Reverse primer (5’ - 3’)
acaa2	GCC TCC ACT CAC ATT GGT TT	ATT GAA GAA AGC TGG GCT GA
acss1	TCC TCC AGG GTA GTG GTG TC	ACC AGA TCC TGG TGG TGA AG
adiponectin	GCACTGGCAAGTTCTACTGCAA	GTAGGTGAAGAGAACGGCCTTGT
aldh1a1	TTT GGC CAC ACA CTC CAA TA	GGG CTG ACA AGA TTC ATG GT
ap2	ACA CCG AGA TTT CCT TCA AAC TG	CCA TCT AGG GTT ATG ATG CTC TTC A
ccl6	GTG GCA TAA GAG AAG CAG CAG TC	TAT CCT TGT GGC TGT CCT TGG
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
coxIII	GCAGGATTCTTCTGAGCGTTCT	GTCAGCAGCCTCCTAGATCATGT
cpn2	GCG TTG TCC TTC AGG CTT AG	TCC CCA CAT CTG TTC TCA CA
cpt1b	CGA GGA TTC TCT GGA ACT GC	GGT CGC TTC TTC AAG GTC TG
cyt-c	GCAAGCATAAGACTGGACCAAA	TTGTTGGCATCTGTGTAAGAGAATC
Dio2	CAGTGTGGTGCACGTCTCCAATC	TGAACCAAAGTTGACCACCAG
elovl3	TCC GCG TTC TCA TGT AGG TCT	GGA CCT GAT GCA ACC CTA TGA
elovl6	TGC TGC ATC CAG TTG AAG AC	TGC CAT GTT CAT CAC CTT GT
err-γ	TTC TGC ACA GCT TCC ACA TC	GGA AGA ATT CGT CAC CCT CA
eva1	GTC CCA ACC AGA CCA TCA AC	CTC CAT CTT GCT CTG GAA GC
fads3	GTG ATC CAC ACG AAC CAG TG	TCC CGC TTT TTC TTG TCC TA
glut4	GTG ACT GGA ACA CTG GTC CTA	CCA GCC AGT TGC ATT GTA G
GyK	CCGCGAAGAAAGCAGTTCTG	CAAAAAACGTGTCGAGCTGGTA
hmgn3	AGA CTC CGG TTT TGG TGG AAC	CAC AGA GGG CAA AGA TGG AAC
lhx8	ACA CGA GCT GCT ACA TTA AGG A	CCC AGT CAG TCG AGT GGA TG
ntrk3	TGG CTC ACA CTG ATC TCT GG	GCC AGA GCC TTT ACT GCA TC
otopetrin1	ACT AGG ACC CCG TCG AAT CT	ACC ATG CTC TAC GTG CTG TG
pgc1α	CCC TGC CAT TGT TAA GAC C	TGC TGC TGT TCC TGT TTT C
ppar-α	GCGTACGGCAATGGCTTTAT	GAACGGCTTCCTCAGGTTCTT
ppar-γ	GTGCCAGTTTCGATCCGTAGA	GGCCAGCATCGTGTAGATGA
prdm16	CAG CAC GGT GAA GCC ATT C	GCG TGC ATC CGC TTG TG
prdm16-utr	TGG CCT TCA TCA CCT CTC TGA A	TTT CTG ATC CAC GGC TCC TGT GA
psat1	TAC CGC CTT GTC AAG AAA CC	AGT GGA GCG CCA GAA TAG AA
resistin	CTG TCC AGT CTA TCC TTG CAC AC	CAG AAG GCA CAG CAG TCT TGA
retn-like-α	TCC ACT CTG GAT CTC CCA AGA	TCC CTC CAC TGT AAC GAA GAC TC
s100b	GTC CAG CGT CTC CAT CAC TT	GGT GAC AAG CAC AAG CTG AA
serpina3k	GGC TGA AGG CAA AGT CAG TGT	TGG AAT CTG TCC TGC TGT CCT
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
zic1	CTG TTG TGG GAG ACA CGA TG	CCT CTT CTC AGG GCT CAC AG

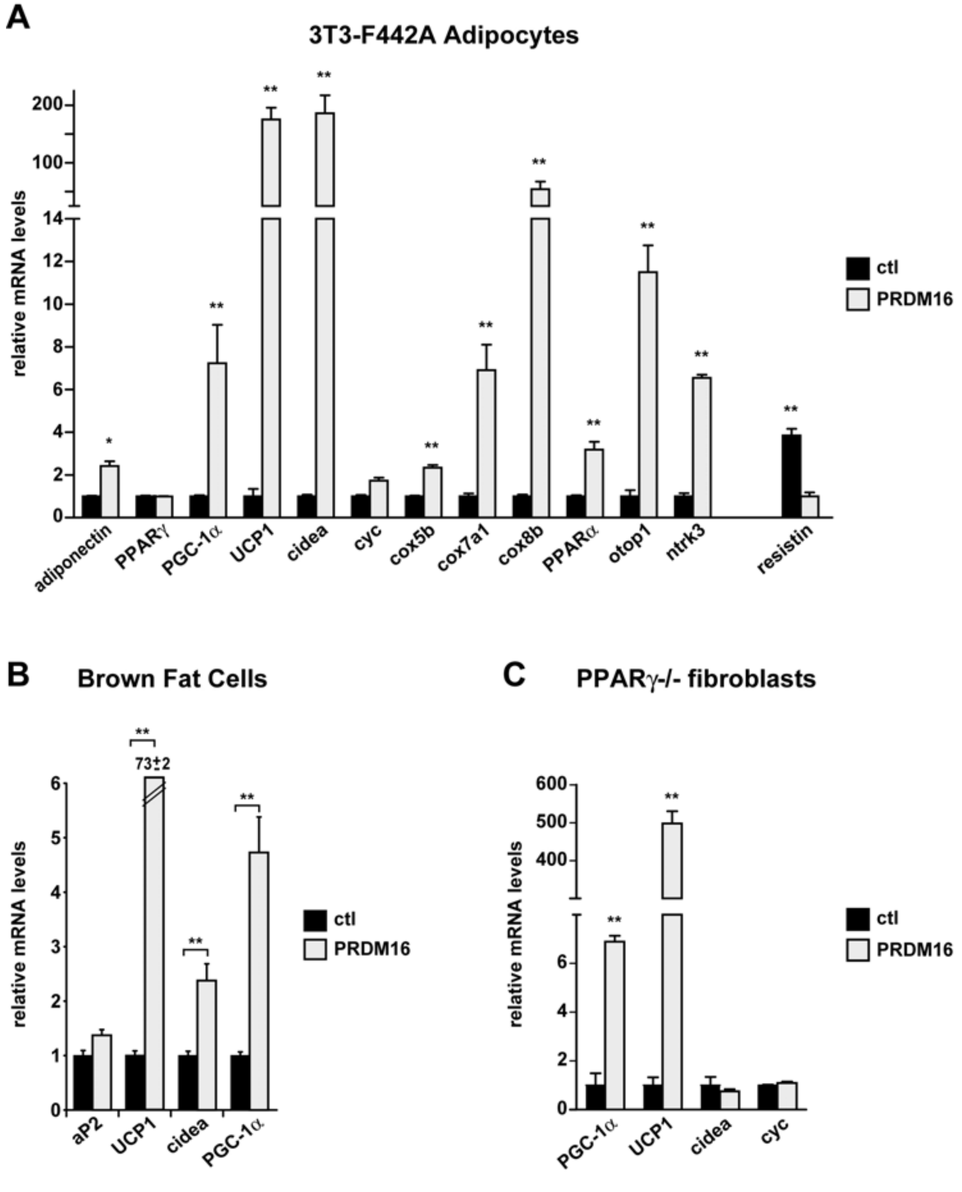

Figure S1. PRDM16 activates BAT genes in other fat cellular systems

(A) Real-time PCR analysis of gene expression (as indicated) in adipocytes (day 6) from 3T3-F442A preadipocytes expressing retroviral- PRDM16 or –vector control (ctl). (B) Real-time PCR analysis of fat-related gene expression (as indicated) in adipocytes (day 4) from brown fat preadipocytes expressing retroviral- PRDM16 or –vector control (ctl). (C) Real-time PCR analysis of gene expression (as indicated) in PPARγ–deficient fibroblasts expressing retroviral- PRDM16 or –vector control (ctl) that had been treated with the adipogenic differentiation protocol. (n=3, mean ± SD). * $p < 0.05$; ** $p < 0.01$

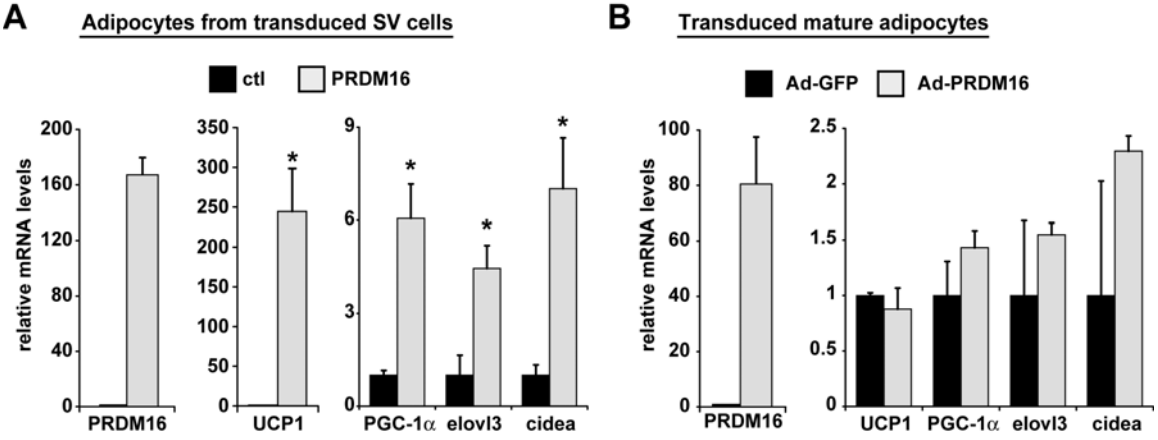

Figure S2. PRDM16 function is required before or during adipocyte differentiation

(A, B) Real-time PCR was used to measure the expression of brown fat cell-selective genes (as indicated) in adipocytes from PRDM16- or ctl- transduced stromal-vascular cells isolated from mouse white fat (A); and in mature fat cells from the same tissue transduced with adenoviral-PRDM16 or -GFP control (B). (n=3, mean ± SD). * $p < 0.05$; ** $p < 0.01$

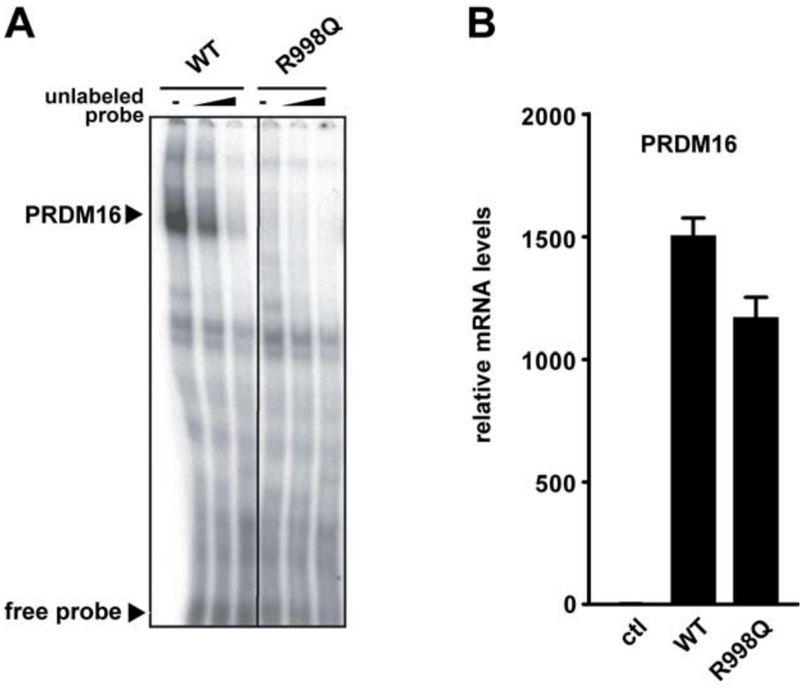

Figure S3. A R998Q mutant allele of PRDM16 does not bind DNA

(A) Electrophoretic mobility shift assays (EMSA) were performed using *in vitro* translated wildtype PRDM16 and R998Q mutant proteins to investigate their ability to bind DNA containing a consensus binding site for PRDM16. (B) Real-time PCR analysis of wildtype and R998Q mutant PRDM16 mRNA levels in adipocytes at day 6 of differentiation (n=3, mean ± SD).

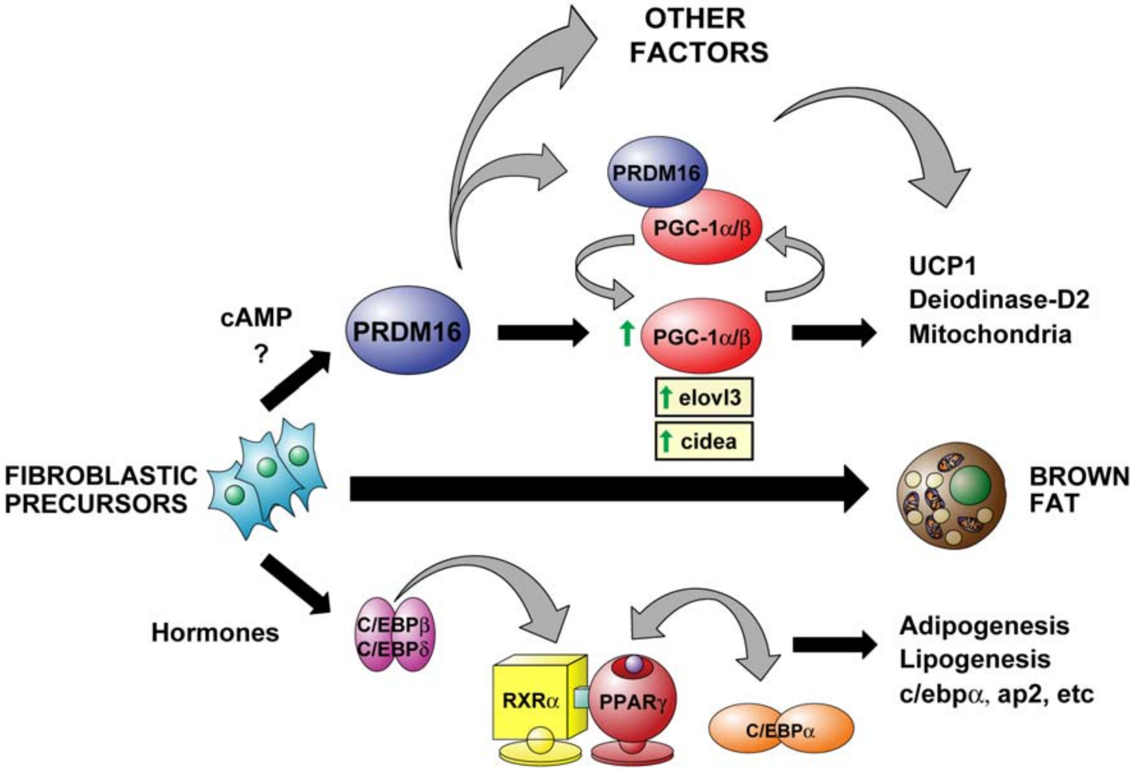

Figure S4. **Model of PRDM16 function in brown fat determination**

PRDM16 expression is induced in fat cell precursors during brown fat adipogenesis. As shown here, PRDM16 activates a broad program of brown fat determination including expression of PGC-1α, elovl3, cidea and PRDM16 itself. Importantly, PRDM16 action renders fat cells responsive to adrenergic signals, such that expression of UCP1 and other thermogenic genes can be further increased by cAMP. The function of PRDM16 is mediated, at least in part, via its physical interaction with PGC-1α and PGC-1β. PRDM16 regulates brown fat character without influencing the general program of adipocyte differentiation (common to BAT and WAT) that is controlled by PPARγ and C/EBP family members.