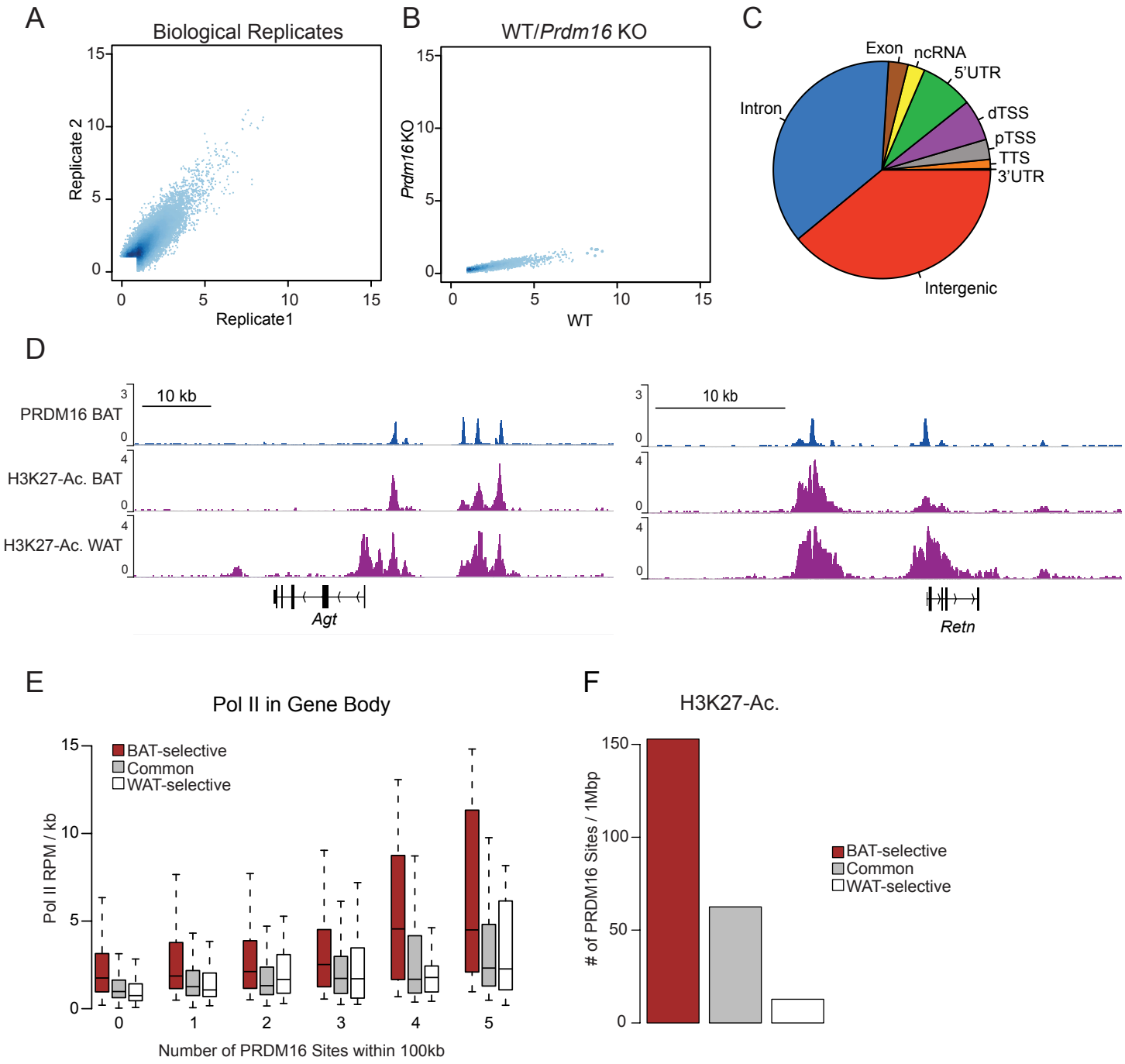


Figure S1. PRDM16 is preferentially bound to BAT-selective genes in BAT

(A) Scatter plot comparing ChIP-seq binding profiles of PRDM16 from independent biological replicates of BAT
(B) Scatter plot showing PRDM16 ChIP-seq signal in wildtype (WT) and *Prdm16* KO BAT using pooled replicates.
(C) Pie chart showing the relative enrichment of PRDM16 in different genomic regions
(D) ChIP-seq stack-height profiles in reads per million (RPM) for PRDM16 in BAT and H3K27-Ac. in BAT and WAT at the *Agt* and *Retn* loci.
(E) Box plot comparing Pol II (RPM/kb) levels within gene bodies of BAT-selective, common and WAT-selective genes with varying number of proximal PRDM16 binding sites
(F) Average number of PRDM16 binding sites (per 1Mbp) at BAT-selective, common and WAT-selective regions of H3K27-Ac. enrichment

A

PRDM16 - activated genes

De Novo Motif	TF	P-Value	Hit %
	EBF	10^{-118}	55.6
	C/EBP	10^{-57}	30.4
	NF1-half	10^{-41}	49.3
	DR1-half	10^{-31}	21.7

PRDM16 - repressed genes

De Novo Motif	TF	P-Value	Hit %
	C/EBP	10^{-97}	43.5
	EBF	10^{-71}	47.5
	NF1-half	10^{-44}	61.3
	DR1	10^{-17}	4.2

B

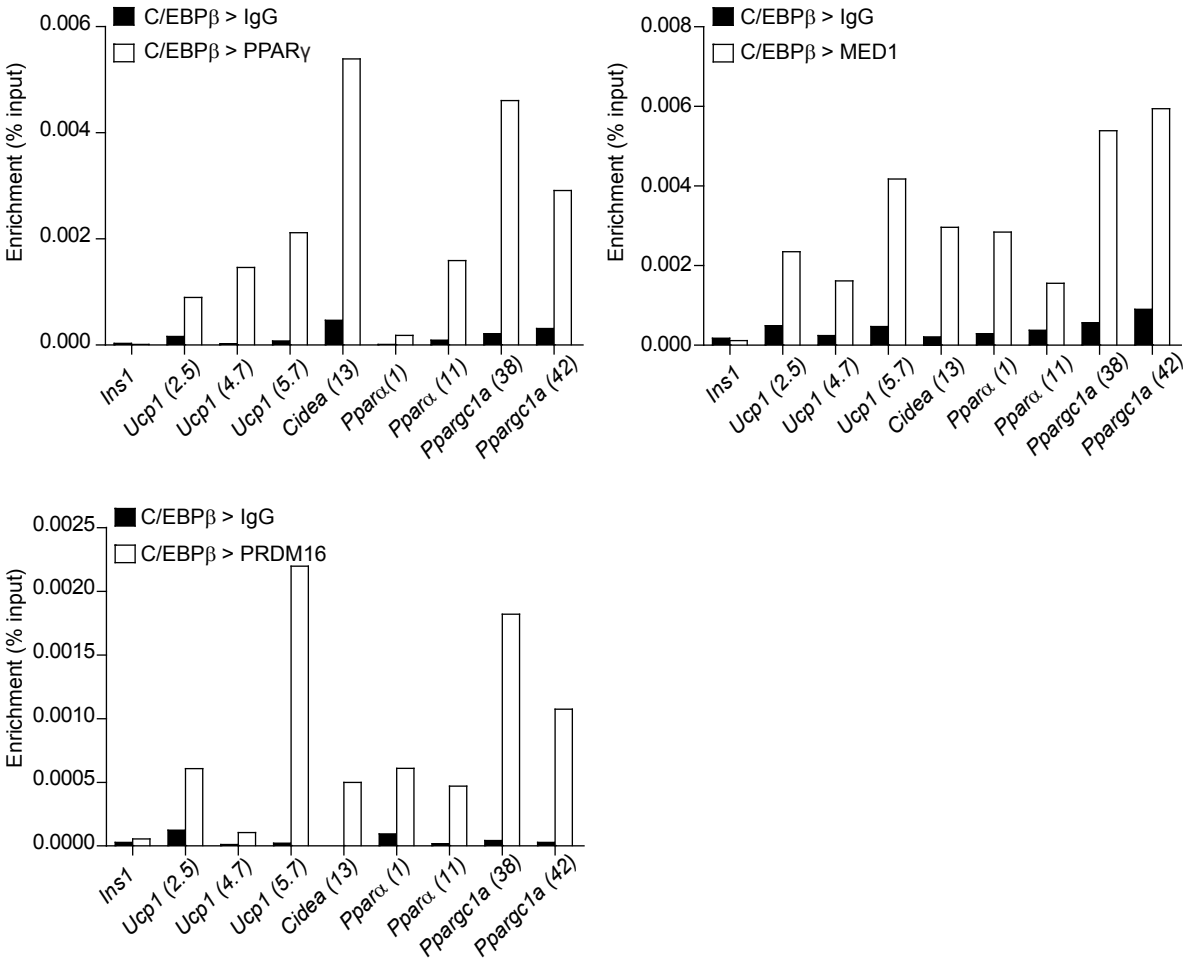


Figure S2. C/EBPβ, PPARγ, PRDM16 and MED1 are co-localized at BAT-selective loci

(A) *De novo* motif analysis at regions of PRDM16 enrichment within 100kb of genes positively or negatively regualted by PRDM16
(B) Sequential ChIP analysis. Chromatin was first immunoprecipitated using an anti-C/EBPβ antibody. Sequential immunoprecipitation was performed comparing IgG to PPARγ, MED1 and PRDM16. Experiments were performed a minimum of 3 times and representative data is shown.

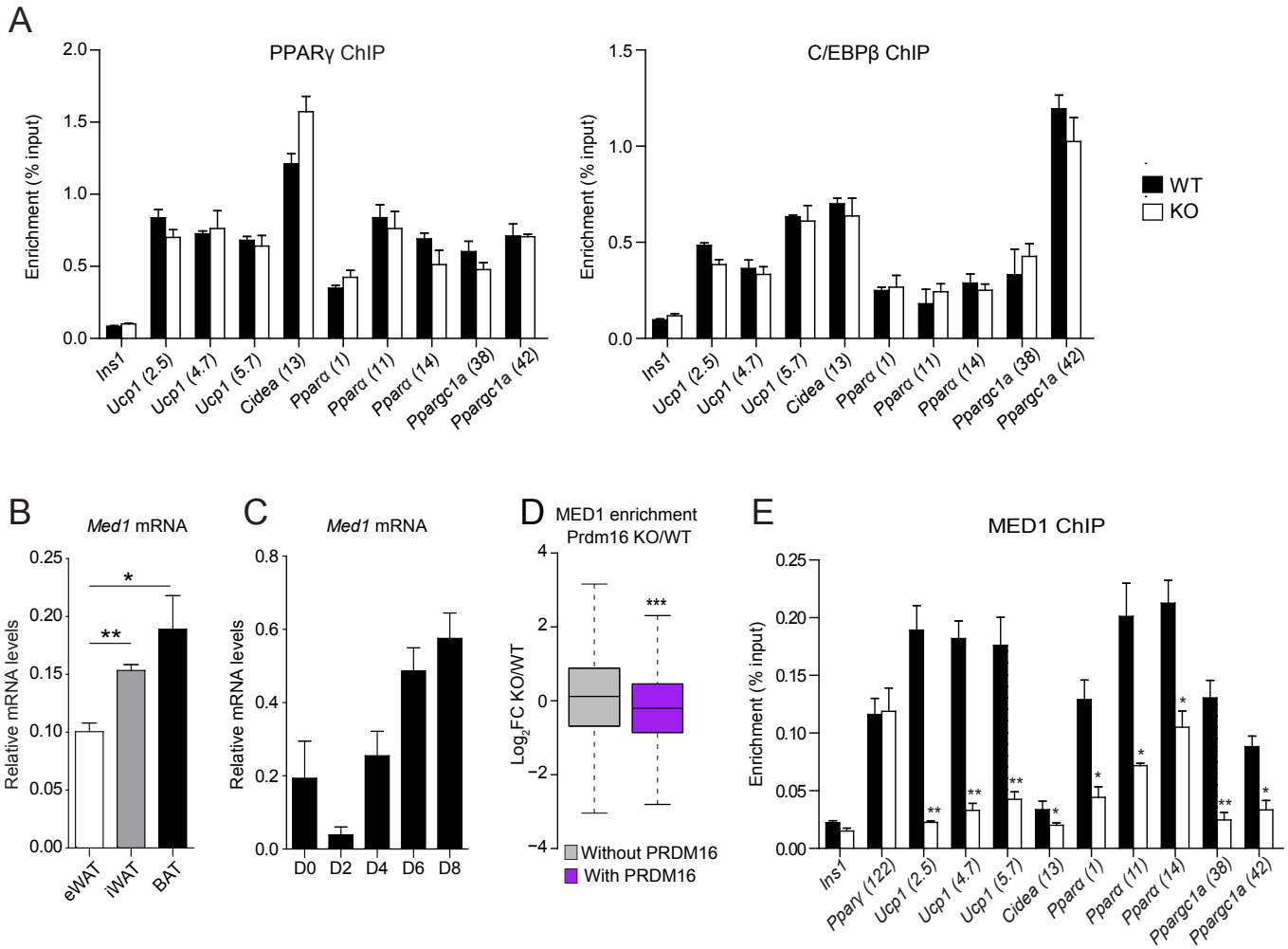


Figure S3. Loss of PRDM16 does not affect PPARγ or C/EBPβ binding levels at BAT-selective genes

(A) ChIP-qPCR analysis of PPARy and C/EBPβ binding at BAT-selective genes in WT and *Prdm16* KO BAT(mean ± SEM; n = 3; *p < 0.05)
(B) Relative Med1 mRNA levels in epididymal WAT, inguuinal WAT and BAT (mean ± SEM; n = 3; *p<0.05; **p<0.01)
(C) Relative Med1 mRNA levels in differentiating brown adipocytes (mean ± SEM; n = 3)
(D) Box plot comparing MED1 occupancy changes upon *Prdm16* KO in BAT at sites that possess or lack PRDM16 binding (***p < 10^{-10})
(E) ChIP-qPCR analysis of MED1 levels at BAT-sel. genes in WT and *Prdm16* KO BAT (mean ± SEM; n = 3; *p < 0.05, **p<0.01

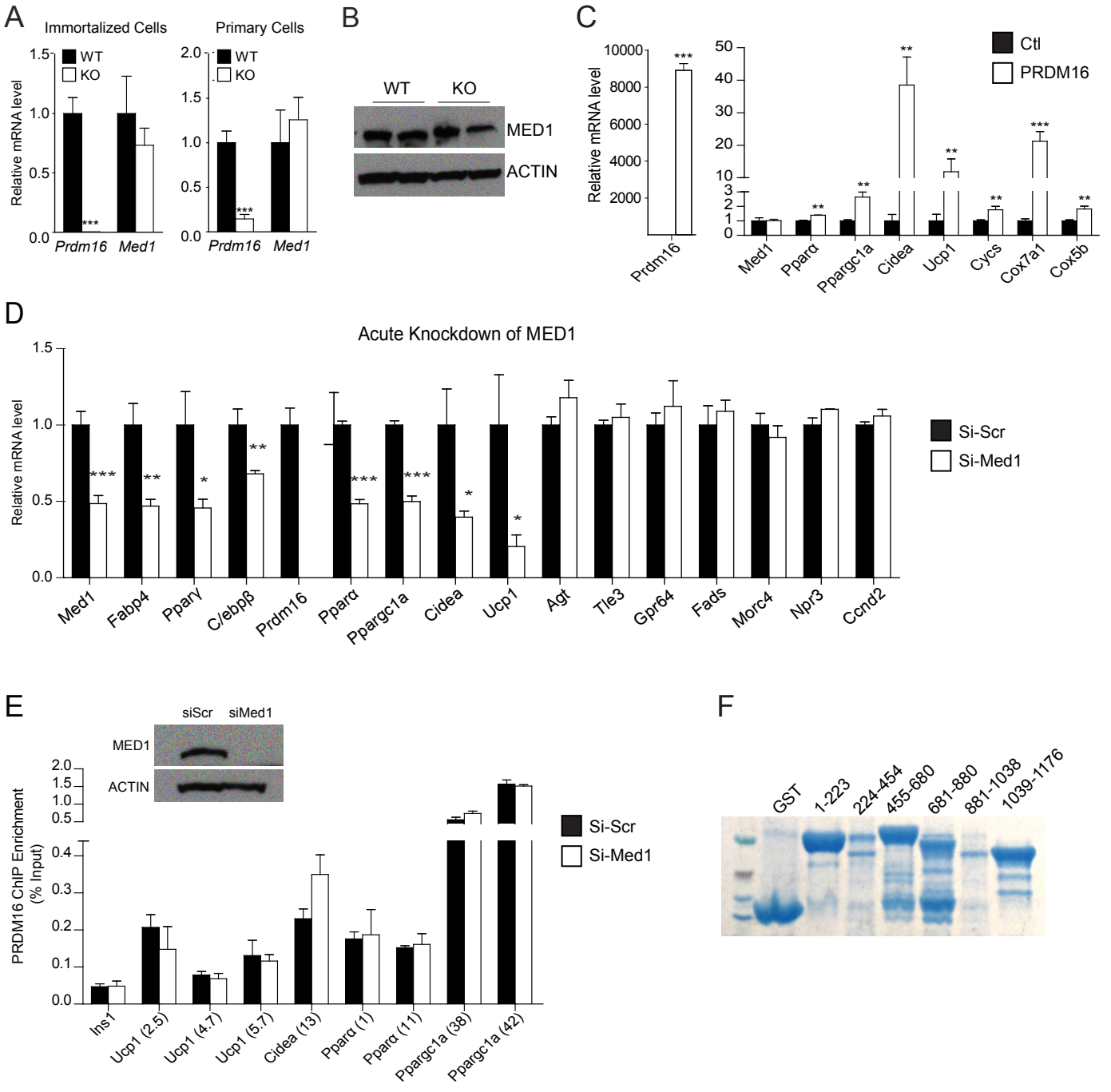
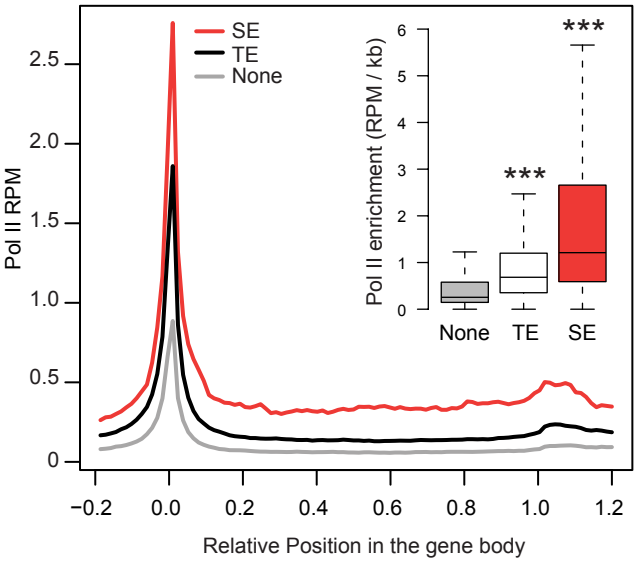


Figure S4. PRDM3 binds and recruits MED1 to BAT-selective loci

(A) Relative mRNA expression levels of *Prdm16* and *Med1* in immortalized brown adipocytes, primary brown adipocytes, and *Prdm16*-deficient brown adipocytes that have been transduced with *Prdm16* (mean ± stdev; n = 3; ***p<0.001)
(B)MED1 protein levels from WT and Prdm16 KO adipocytes
(C) Relative mRNA levels of BAT-sel. genes in PRDM16-deficient brown adipocytes that were transduced with control or PRDM16 -expressing virus. (mean ± Stdev; n = 3; *p < 0.05, **p<0.01, ***p<0.001)
(D) Relative mRNA levels of BAT-selective, common and WAT-selective genes from differentiated primary brown adipocytes that were transfected with siScr or siMed1. (mean ± Stdev; n = 3; *p < 0.05, **p<0.01, ***p<0.001)
(E) ChIP-qPCR analysis of PRDM16 enrichment after acute MED1 deletion at BAT-sel. genes (mean ± Stdev; n = 3).
Inset - Western blot analysis of MED1 levels after acute MED1 deletion
(F) Commassie blue staining of GST and GST-PRDM16 fusion proteins

A



B

GO analysis for genes near SE

Rank	Term	P-Value
1	brown fat cell differentiation	1.92E-13
2	fat cell differentiation	2.22E-10
3	oxidation reduction	4.86E-09
4	fatty acid metabolic process	7.50E-09
5	generation of precursor metabolites and energy	4.84E-08
6	energy derivation by oxidation of organic compounds	5.25E-08
7	organic ether metabolic process	6.73E-07
8	glycerol ether metabolic process	2.94E-06
9	glycerolipid metabolic process	2.96E-06
10	coenzyme metabolic process	2.97E-06

C

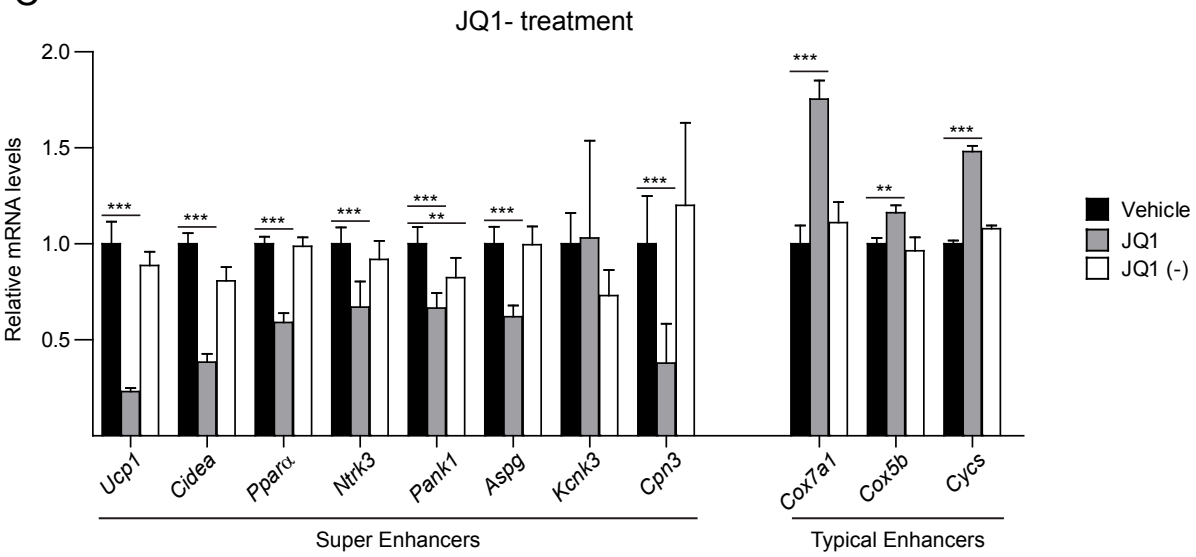


Figure S5. PRDM16 regulates Super Enhancers in BAT

(A) Average Pol II profile (RPM) around gene bodies of genes associated with super enhancers (red), typical enhancers (black), or genes without proximal MED1 occupancy (grey). Insert – box plot representation of Pol II signal (RPM/kb) of same data (***$p < 10^{-50}$)
(B) DAVID gene ontology (GO) analysis for super enhancer-associated genes in WT BAT
(C) Relative mRNA levels of genes marked by SE or TE in differentiated primary brown adipocytes that were treated with either vehicle, JQ1 or an inert JQ1 analog (JQ1(-)) (mean ± Stdev; n = 6; *p < 0.05, **p<0.01, ***p<0.001)

Table S1 **Primers used for real-time qPCR and ChIP-qPCR analysis**

mRNA	Fwd	Rev
Agt	AAGACCCTGCATGATCAGCTC	CTTCCTGCCTCATTCAGCATC
Aspg	ATTGCCTTCAGG GGCTGTGAC	CTGGCCCAGCACATAGGACAGT
C/ebpβ	ACGACTTCCTCTCCGACCTCT	CGAGGCTCACGTAACCGTAGT
Ccnd2	AGCTGTCCCTGATCCGCAAGC	AAGCCCACAGATGGCTGCTCC
Cidea	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG
Cox5b	GCTGCATCTGTGAAGAGGACAAC	CAGCTTGTAATGGGTTCCACAGT
Cox7a1	CAGCGTCATGGTCAGTCTGT	AGAAAACCGTGTGGCAGAGA
Cpn3	GCCGACATCCCTCCGGACATC	TCCAGGTGACGGACCTGAGTGT
Cycs	GCAAGCATAAGACTGGACCAAA	TTGTTGGCATCTGTGTAAGAGAATC
Fabp4	ACACCGAGATTTCCTTCAAACTG	CCATCTAGGGTTATGATGCTCTTCA
Fads	TGCGCAAGTTCCTGAAACCCCT	CATGTCTTCAGCTGCCTGGCG
Gpr64	CCACACCAGCCCCATCTGTCC	TCCATCTGGGATACTTGGGCTTCC
Kcnk3	GCTTCGCCG GCTCCTTCTACTT	CTAGTGTGAGCGGGATGCCCAG
Med1	TGCTTGGAAAATTCCTCAAAA	GATGTCAAAGTGGCTCACCA
Morc4	GTATCCAGGCGCAGGCGGTTA	AGATGGCTTCTAGGCTGGGCA
Npr3	GTGCGCTACATCCAAGGCAGC	TCCACTGGTCATGCCGTGTCTG
Ntrk3	ATGCGAGCCCTACACCTCCTA	GACTGCTATGGACACCCCAAA
Pank1	CGCTGTTCGCCCAGCATGATTC	CAGCTTAACCAGGGTTCCACCGA
Ppargc1a	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
Pparα	GCGTACGGCAATGGCTTTAT	GAACGGCTTCCTCAGGTTCTT
Pparγ2	TGGCATCTCTGTGTCAACCATG	GCATGGTGCCTTCGCTGA
Prdm16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
Tbp	GAAGCTGCGGTACAATTCCAG	CCCCTTGTACCCTTCACCAAT
Tle3	CTCACCCACACCCCGCATCAA	AAACCGCAATGTTCCCGTCGC
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG

ChIP	Fwd	Rev
18s	AGTCCCTGCCCTTTGTACACA	CGATCCGAGGGCCTCACT
Cidea (13)	GGCCACTTGAGGAGCCAACCA	TGGGCACTGGCCTTGTAGCTG
Fabp4	GACAAAGGCAGAAATGCACA	AATGTCAGGCATCTGGGAAC
Ins1	GGACCCACAAGTGGAACAAC	GTGCAGCACTGATCCACAAT
Ppargc1a (38)	TCCGAGTTTCCCTGCTGTGGC	AGGGACTTGCAGCTGTGGTGG
Ppargc1a (42)	GAGGTGGCACCAGGACACCAG	CCCAAGCTCGAGACTCCGCTC
Pparα (1)	GGGGCATGTGCATTCCGTGAC	CACTGGGGCTCTGCCAACTGA
Pparα (11)	AAGAGCATGGGACAGTGGCCG	TGGCCAGCTGAAGGTCACCAC
Pparα (14)	CCTGCCCCATAGGCAGTATGGTC	ACAGGGGCAGAAGCCAAGCTG
Pparγ (122)	AGCTTTGCTGGCTAGAGGTG	TTTCGCAGAACTGAGGTTGA
Ucp1 (2.5)	CAAATGGTGACCGGGTGCCCT	GGGTGACTGACCCTCTGTGACG
Ucp1 (4.7)	CCCCACTGCCTGTCACGTTCA	GAAGCTGCCGAATGGTGCGTC
Ucp1 (5.7)	ACCACACCATTTGGAGCCTGAC	TGAGTTTGCAGGGAGGATGGGC