Expanded View Figures

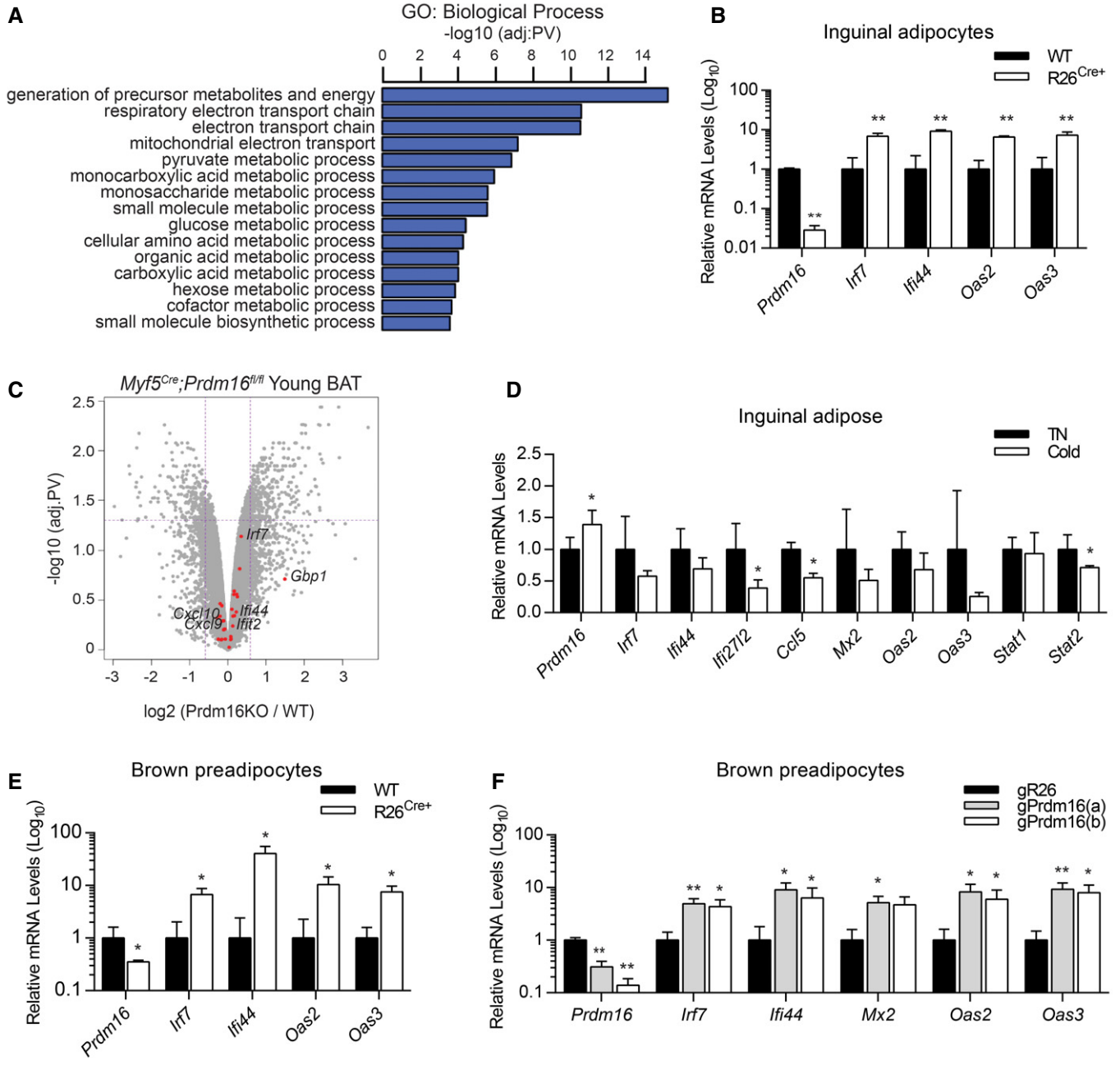

Figure EV1. PRDM16 is required for repression of interferon-stimulated genes.

A Gene ontology (GO) of downregulated genes in *Prdm16* KO cells (green cluster Fig 1B).

B Relative mRNA levels of *Prdm16* and ISGs in *Prdm16* $^{fl/fl}$ (WT) and *R26*^{CreER}; *Prdm16* $^{fl/fl}$ (*R26*$^{Cre+}$) inguinal adipocytes treated with 1 μM 4-hydroxytamoxifen (4OHT).

C Volcano plot comparing gene expression between young *Prdm16* $^{fl/fl}$ (WT) and *Prdm16* KO BAT. Red dots indicate type I ISGs found in the blue cluster of Fig 1B heat map.

D Relative mRNA levels of *Prdm16* and ISGs in inguinal adipose from wild-type mice incubated in TN (n = 5) or cold (n = 5).

E Relative mRNA levels of *Prdm16* and ISGs in WT (n = 7) and *R26*$^{Cre+}$ (n = 13) brown preadipose cells treated with 4OHT.

F Relative mRNA of *Prdm16* and ISGs in brown adipocyte precursor cells transduced with CRISPR lentiviral vectors expressing Cas9 and guide RNA sequences for *Rosa26* (gR26) or *Prdm16* (gPrdm16a, gPrdm16b) (n = 3).

Data information: Data are presented as mean ± standard deviation (B, E, F) and mean ± SEM (D). *$P \leq 0.05$, **$P \leq 0.01$ (Student's t-test).

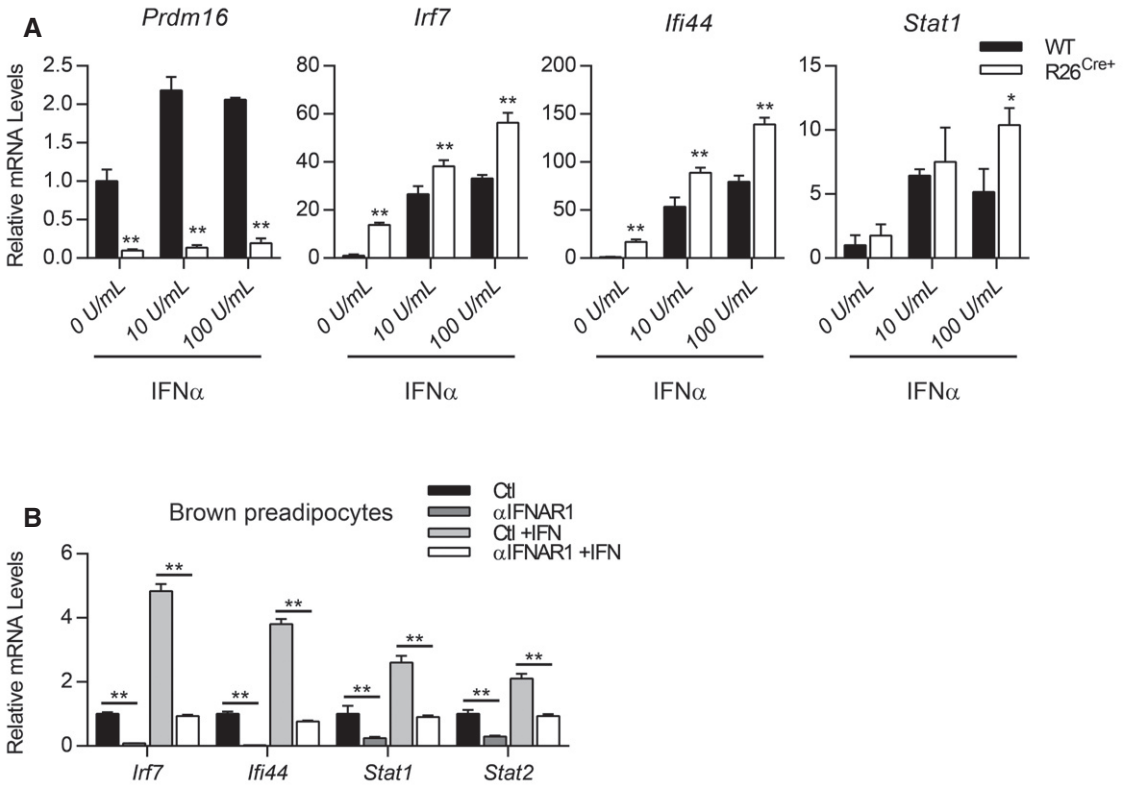

Figure EV2. PRDM16 blocks type I IFN signaling downstream of IFNAR receptor.

A Relative mRNA levels of *Prdm16*, *Irf7*, *Ifi44*, and *Stat1* in WT and $R26^{Cre+}$ inguinal precursors treated with increasing doses of recombinant mouse IFNα.

B Relative mRNA levels of ISGs in brown preadipocytes treated with vehicle, anti-IFNAR (αIFNAR) neutralizing antibody, mouse IFNα, or a combination of αIFNAR and IFNα.

Data information: Data represent ($n = 3$) mean ± standard deviation. $^*P \leq 0.05$, $^{**}P \leq 0.01$ (Student's *t*-test).

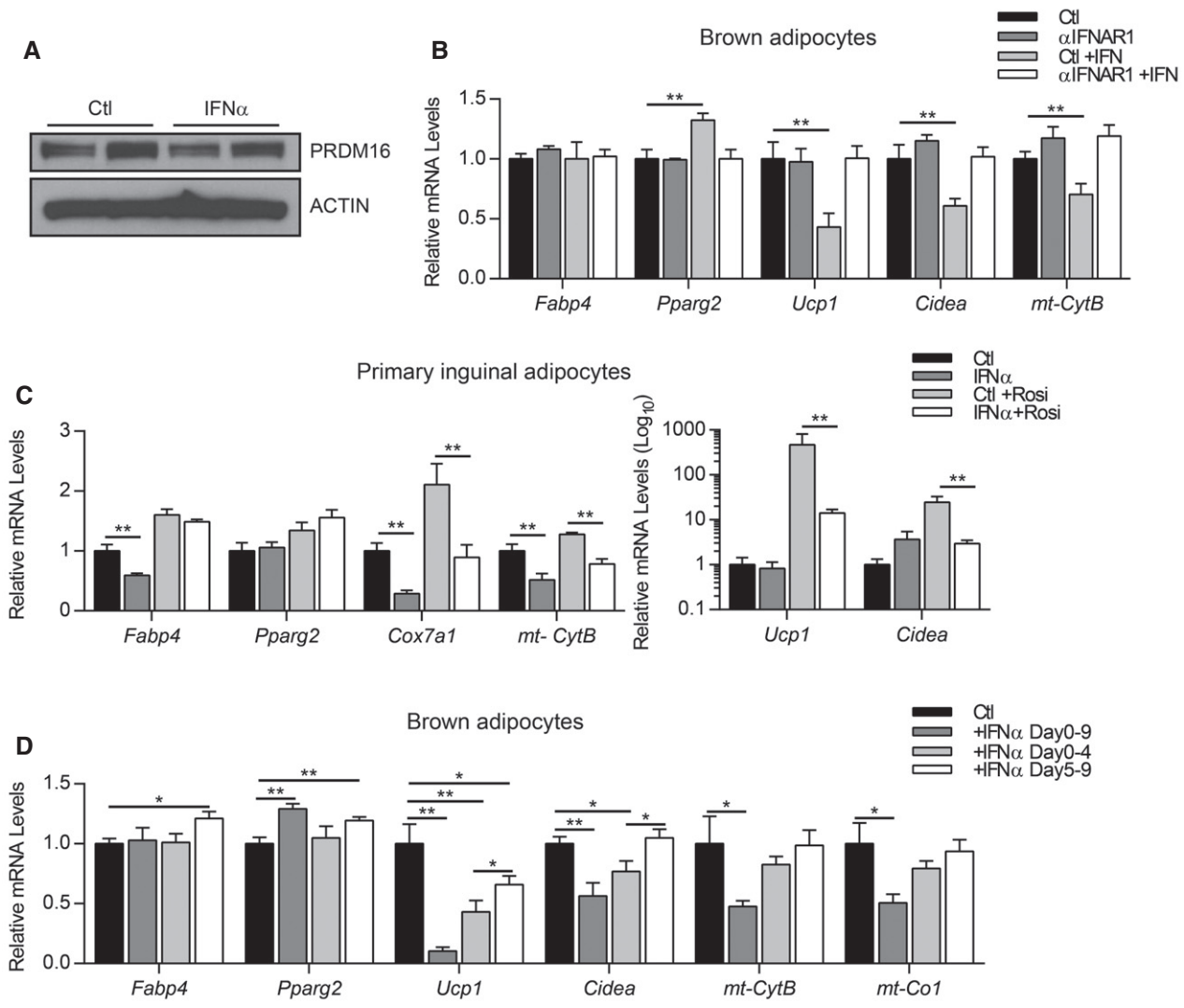

Figure EV3. Type I IFN disrupts mitochondrial structure and function in adipocytes.

A, B Western blot analysis of PRDM16 and actin protein (A) and relative mRNA levels of pan-adipogenic genes (*Fabp4*, *Pparg2*) and brown-selective genes (*Ucp1*, *Cidea*) (B) in brown adipocytes treated with vehicle, anti-IFNAR (αIFNAR) neutralizing antibody, mouse IFNα, or αIFNAR + IFNα.

C Relative mRNA levels of general adipocyte markers (*Fabp4*, *Pparg2*), mitochondrial genes (*Cox7a1*, *mt-Cytb*), and brown fat-selective genes (*Ucp1*, *Cidea*) in primary inguinal adipocytes treated with IFNα or vehicle (Ctl) +/− 1 μM rosiglitazone (Rosi).

D Relative mRNA levels of general adipocyte markers (*Fabp4*, *Pparg2*), brown fat-selective genes (*Ucp1*, *Cidea*), and mitochondrial genes (*mt-Cytb*, *mt-Co1*) in brown adipocytes treated with vehicle (Ctl) or mouse IFNα for varying periods during differentiation.

Data information: Data represent ($n = 3$) mean ± standard deviation. *$P \leq 0.05$, **$P \leq 0.01$ (Student's *t*-test).

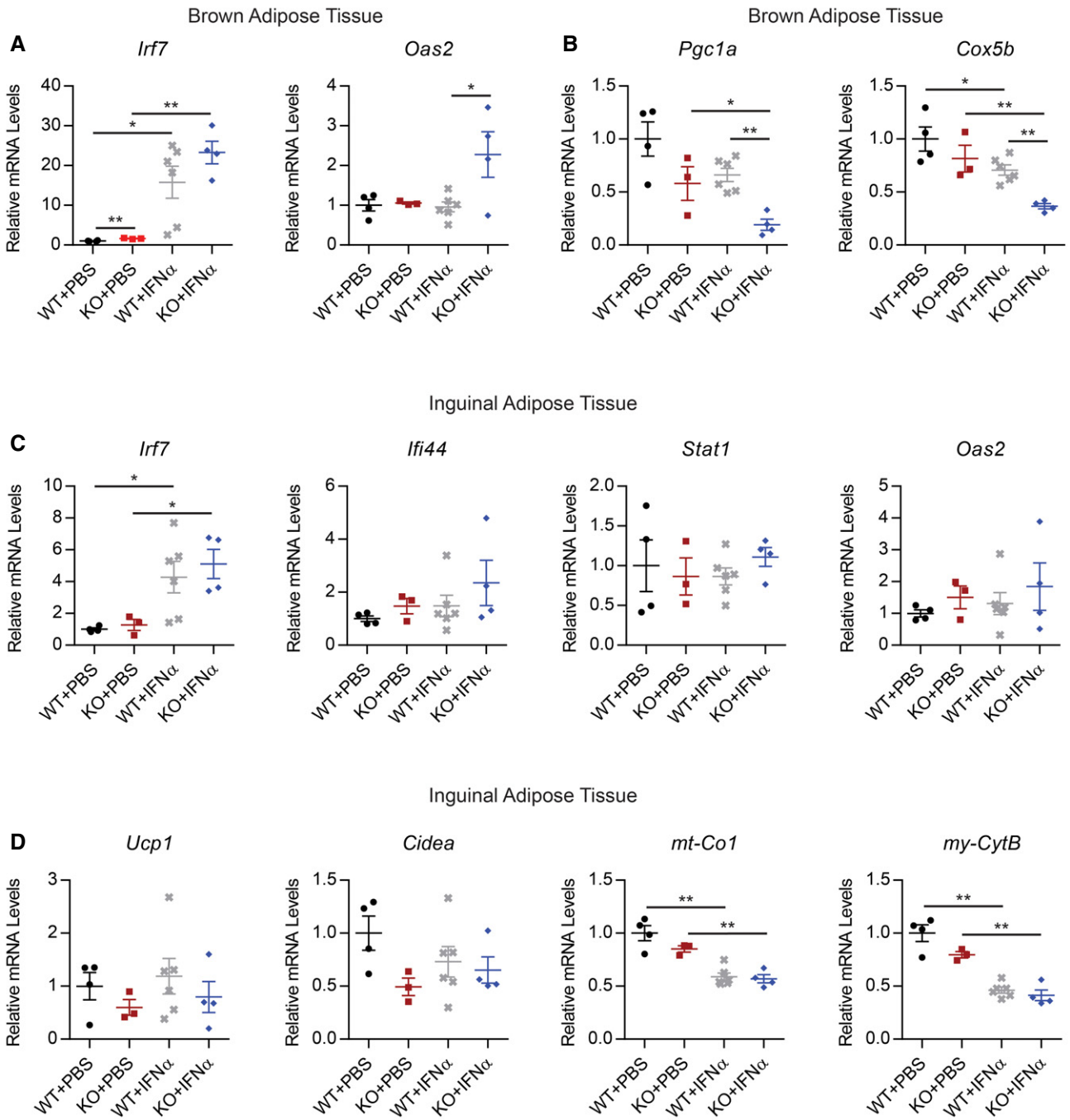

Figure EV4. PRDM16 opposes type I IFN signaling *in vivo*.

A, B Relative mRNA levels of ISGs (A) and mitochondrial genes (B) in brown adipose of *Prdm16* $^{fl/fl}$ (WT) and *Myf5*^{Cre}; *Prdm16* $^{fl/fl}$ (KO) mice treated with IFNα or phosphate-buffered saline (PBS) for 2 weeks.

C, D Relative mRNA levels of ISGs (C), as well as brown fat-selective genes (*Ucp1*, *Cidea*) and mitochondrial genes (*mt-Co1*, *mt-CytB*) (D) in inguinal tissue from the same experimental mice in (A, B).

Data information: Experimental groups: WT+PBS (n = 4), KO+PBS (n = 3), WT+IFN (n = 6), KO+IFN (n = 4). Data are presented as mean ± SEM. *$P \leq 0.05$, **$P \leq 0.01$ (paired two-way ANOVA).

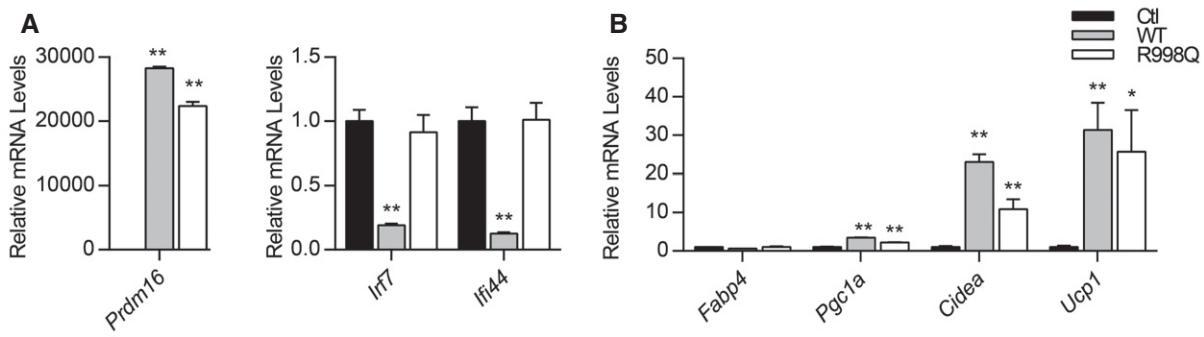

Figure EV5. PRDM16 represses ISGs through direct binding at gene promoters.

A, B Relative mRNA levels of *Prdm16* and ISGs (A) and relative mRNA levels of adipogenic (*Fabp4*) and brown fat-selective genes (*Pgc1a*, *Cidea*, *Ucp1*) in *Prdm16* KO brown adipocytes cells transduced with retroviral vectors expressing wild-type (WT) or DNA-binding mutant (R998Q) PRDM16, or empty vector (Ctl).

Data information: Data represent ($n = 3$) mean ± standard deviation. *$P \leq 0.05$, **$P \leq 0.01$ (Student's *t*-test).

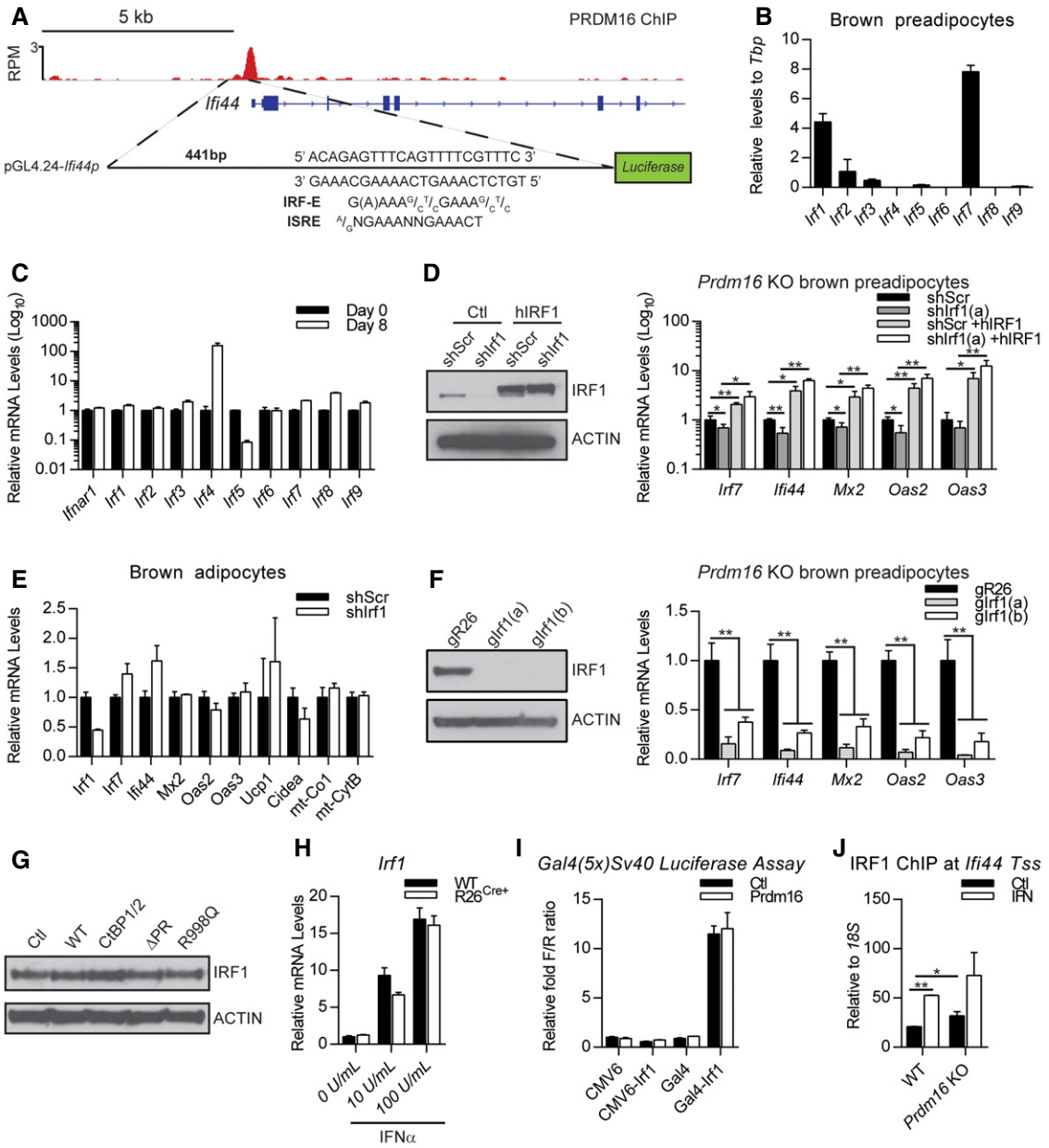

Figure EV6. PRDM16 blocks IRF1 function at IRF-E elements.

A Schematic showing the ChIP-seq track of PRDM16 binding at *Ifi44* promoter and the identified IFN-stimulated response element (ISRE)/IRF-binding element (IRF-E) that was inserted into the luciferase reporter plasmid (pGL4.24-*Ifi44*p).

B Relative mRNA levels of IRF genes in brown preadipose cells.

C Relative mRNA levels of *Ifnar1* and *Irfs* in brown preadipocytes (D0) and mature brown adipocytes (D8).

D Western blot analysis of IRF1 and actin protein levels and relative mRNA levels of ISGs in *Prdm16* KO brown adipocytes cells transduced with lentiviral short-hairpin RNA directed against *Irf1* (shIrf1) or a scrambled control (shScr) and either retroviral expression vectors expressing human IRF1 (hIRF1) or puromycin control (Ctl).

E Relative mRNA levels of *Irf1* and ISGs and of brown fat-selective genes (*Ucp1*, *Cidea*) and mitochondrial genes (*mt-Co1*, *mt-CytB*) in brown adipocytes transduced with lentiviral short-hairpin RNA directed against *Irf1* (shIrf1) or a scrambled control (shScr).

F Western blot analysis of IRF1 and actin protein levels and relative mRNA levels of ISGs in *Prdm16* KO brown adipocytes cells transduced with CRISPR lentiviral vectors expressing Cas9 and guide RNA sequences for *Rosa26* (gR26) or *Irf1* (gIrf1a, gIrf1b).

G Western blot analysis of IRF1 and actin (loading control) protein levels in cells from Fig 5C.

H Relative mRNA levels of *Irf1* in *Prdm16* $^{fl/fl}$ (WT) and *R26*^{CreER}; *Prdm16* $^{fl/fl}$ (*R26*$^{Cre+}$) inguinal adipocytes treated 1 μM 4OHT and increasing doses of IFNα.

I Transcriptional activity of a Gal4 UAS-driven *luciferase* gene in response to expression of GAL4 DNA-binding domain alone (Gal4), IRF1, or GAL4-IRF1+/− PRDM16.

J ChIP-qPCR showing IRF1 binding at *Ifi44* transcriptional start site (Tss) in WT and *Prdm16* KO cells +/− IFNα.

Data information: Data represent (n = 3) mean ± standard deviation (B–F, H, J) and (n = 3) mean ± SEM (I). *$P \leq 0.05$, **$P \leq 0.01$ (Student's t-test).