SUPPLEMENTARY INFORMATION

Prdm4 induction by the small molecule butein promotes white adipose tissue browning

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SUPPLEMENTARY RESULTS

Supplementary Table 1: *Ucp1* induction by bioactive compounds.

Supplementary figure1. Identification of butein as an *Ucp1* inducer.
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Supplementary figure 18: Effects of Prdm4 knockdown on white and brown adipocyte-selective gene expression in adipose tissues of HFD fed mice.
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Supplementary figure 20: Uncropped versions of immunoblots in the figure 1b.
Supplementary figure 21: Uncropped versions of immunoblots in the supplementary figures 4b, 7c, and 10d.
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Supplementary Notes: Chemical Identity of Butein.
Supplementary data set 1: Lists of butein selective genes

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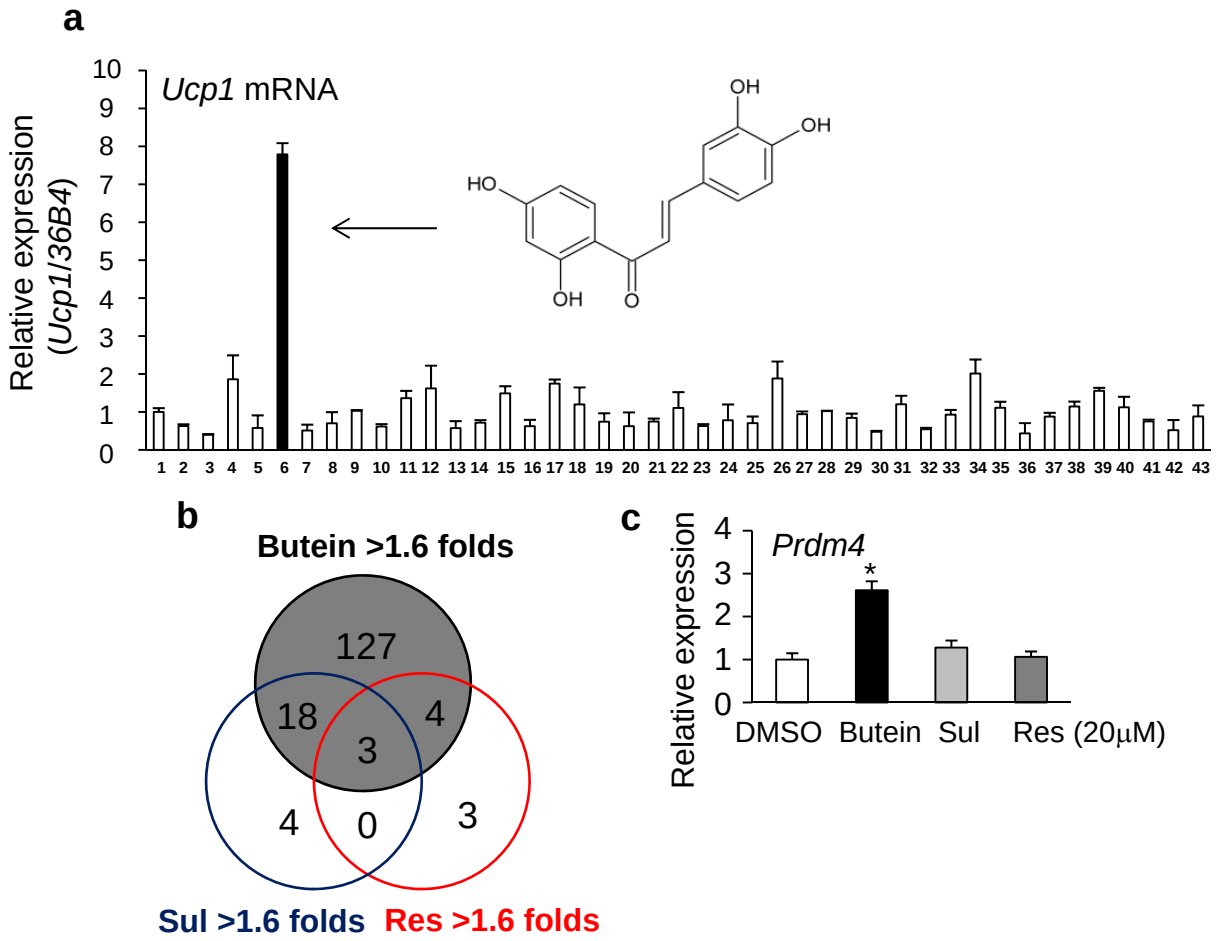
Number	Compound Name	Fold change (UCP-1 expression)
1	DMSO	1
2	BAIBA(3-Aminoisobutyric acid)	0.62992
3	Resveratrol	0.396537
4	r-Oryzanol	1.860922
5	Quercetin	0.576402
6	Butein	7.788245
7	Tannic acid	0.512232
8	Caffeine	0.700894
9	Apigenin	1.030274
10	Cyanidin-3-glucoside	0.614538
11	Peonidin 3-glucoside	1.3633
12	Fisetin	1.620888
13	Ferulic acid	0.573426
14	Coumaric acid	0.716625
15	Conjugate linoleic acid	1.489182
16	Dgat inhibitor	0.623099
17	Sesamol	1.747275
18	Rutin	1.199854
19	Licochalcone A	0.741579
20	Glucosamine	0.62411
21	Sulfuretin	0.747459
22	Glycyrrhizic acid	1.106994
23	γ-aminobutyric acid	0.627506
24	Astaxanthin	0.780454
25	β-Carotene	0.703586
26	Epigallocatechin gallate	1.882942
27	Chitosan	0.938903
28	Dimethylfumarate	1.022605
29	Mono-methyl fumarate	0.84424
30	Bisphenol A	0.477813
31	Daidzein	1.20231
32	Ascorbic acid	0.542047
33	Curcumin	0.928933
34	WP 1066	2.010603
35	Genestein	1.109297
36	Luteolin	0.435046
37	Cordycephine	0.87757
38	Kaempferol	1.142791
39	Thymoquinone	1.556054
40	Myricetin	1.1222
41	β-Estradiol	0.751744
42	*Tert*-butylhydroquinone	0.881284

Supplemental Table 1. *Ucp1* induction by bioactive compounds. C3H10T1/2 adipocytes were treated with bioactive compounds (20 μM) for 6 hours and *Ucp1* expression was measured by real time PCR. Fold increase relative to DMSO treatment was shown. r-Oryzanol is a mixture of steryl and other triterpenyl esters of ferulic acids and Conjugated linoleic acids (CLA) is a family of at least 28 isomers of linoleic acid.



Supplementary Figure 1. Identification of butein as an *Ucp1* inducer. (**a**) C3H10T1/2 adipocytes were treated with various polyphenols isolated from herbal products, including the known anti-adipogenic compounds resveratrol, butein, and sulfuretin. *Ucp1* mRNA expression was measured by real time PCR. Data represent means ± s.d. (n=3). (**b**) C3H10T1/2 adipocytes were treated with butein, resveratrol or sulfuretin for 6 hours and the gene expression profiles were analyzed by microarray. Diagram showing the number of genes (> 1.6 fold) regulated by butein, sulfuretin (Sul), and resveratrol (Res). (**c**) Butein but not resveratrol and sulfuretin induces *Prdm4* mRNA expression. Data represent means ± s.d. (n=3). Statistical significance was determined relative to a control by the Student's *t*-test (* P<0.05).

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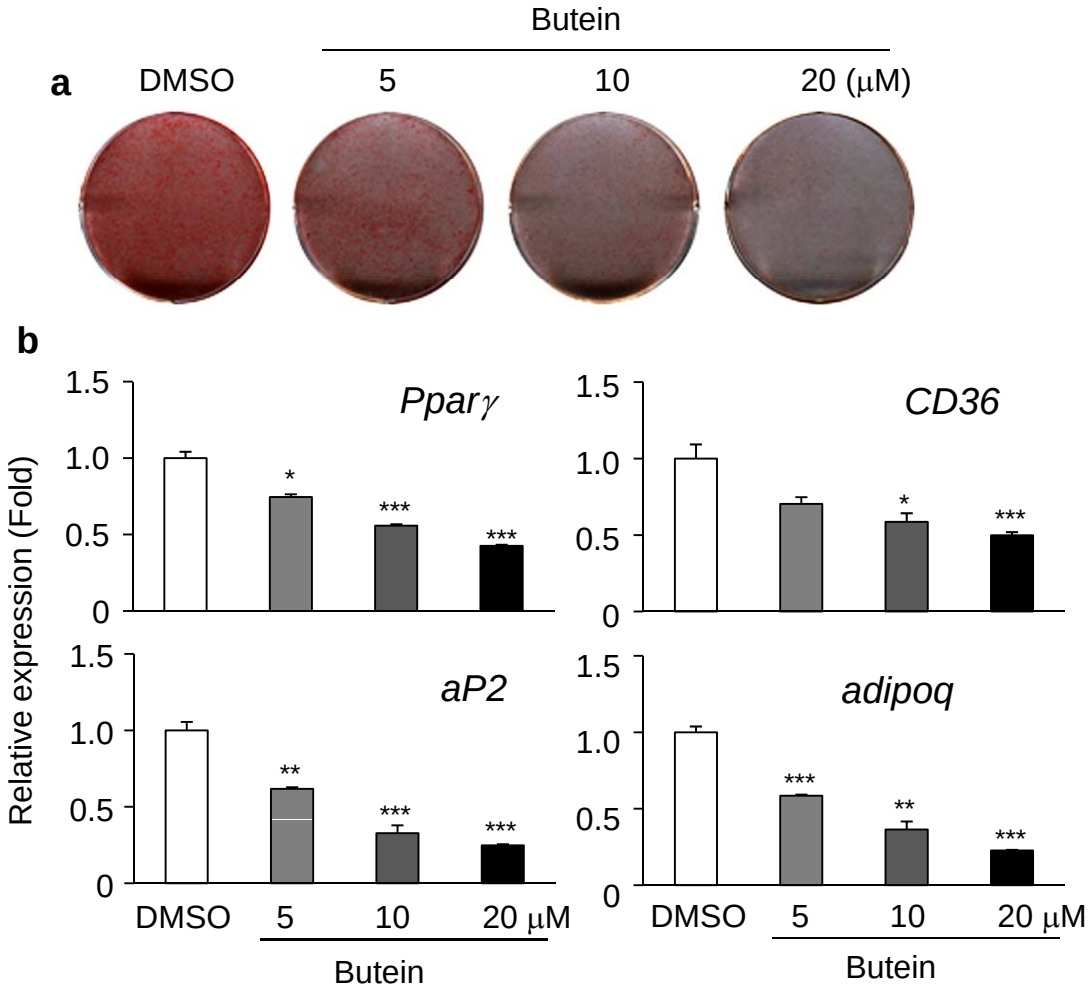
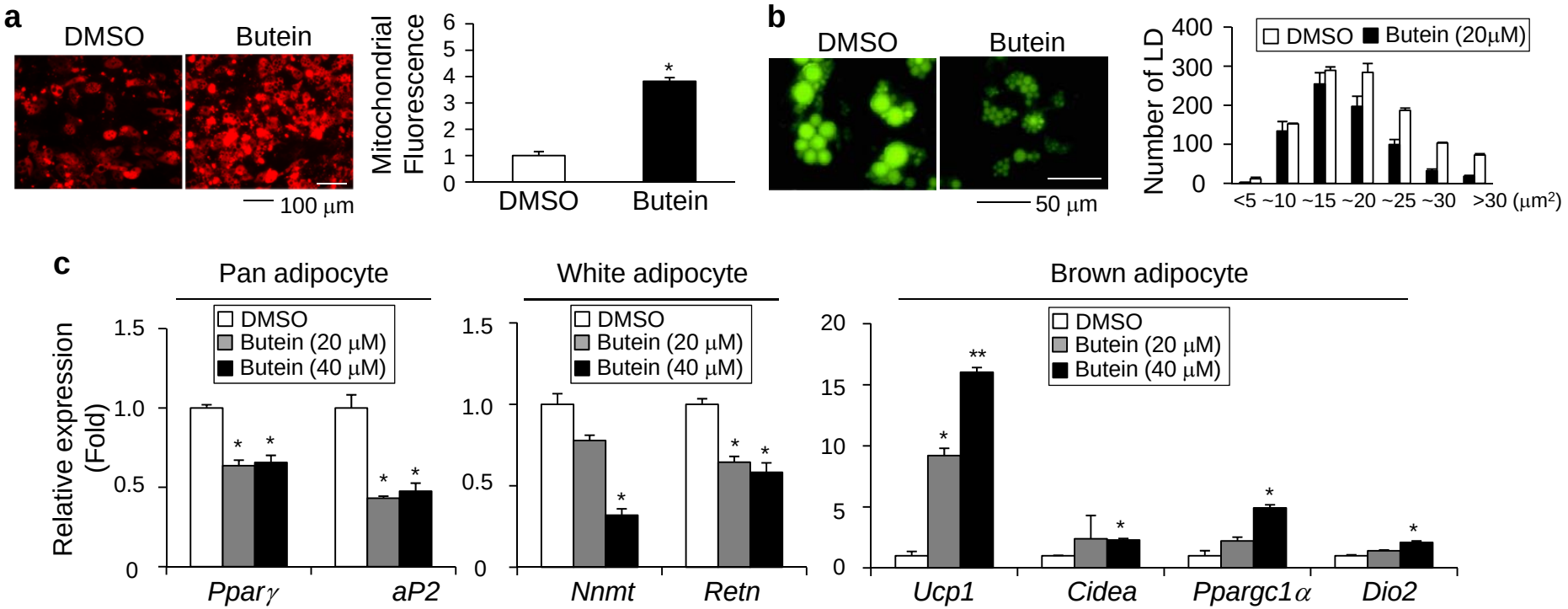
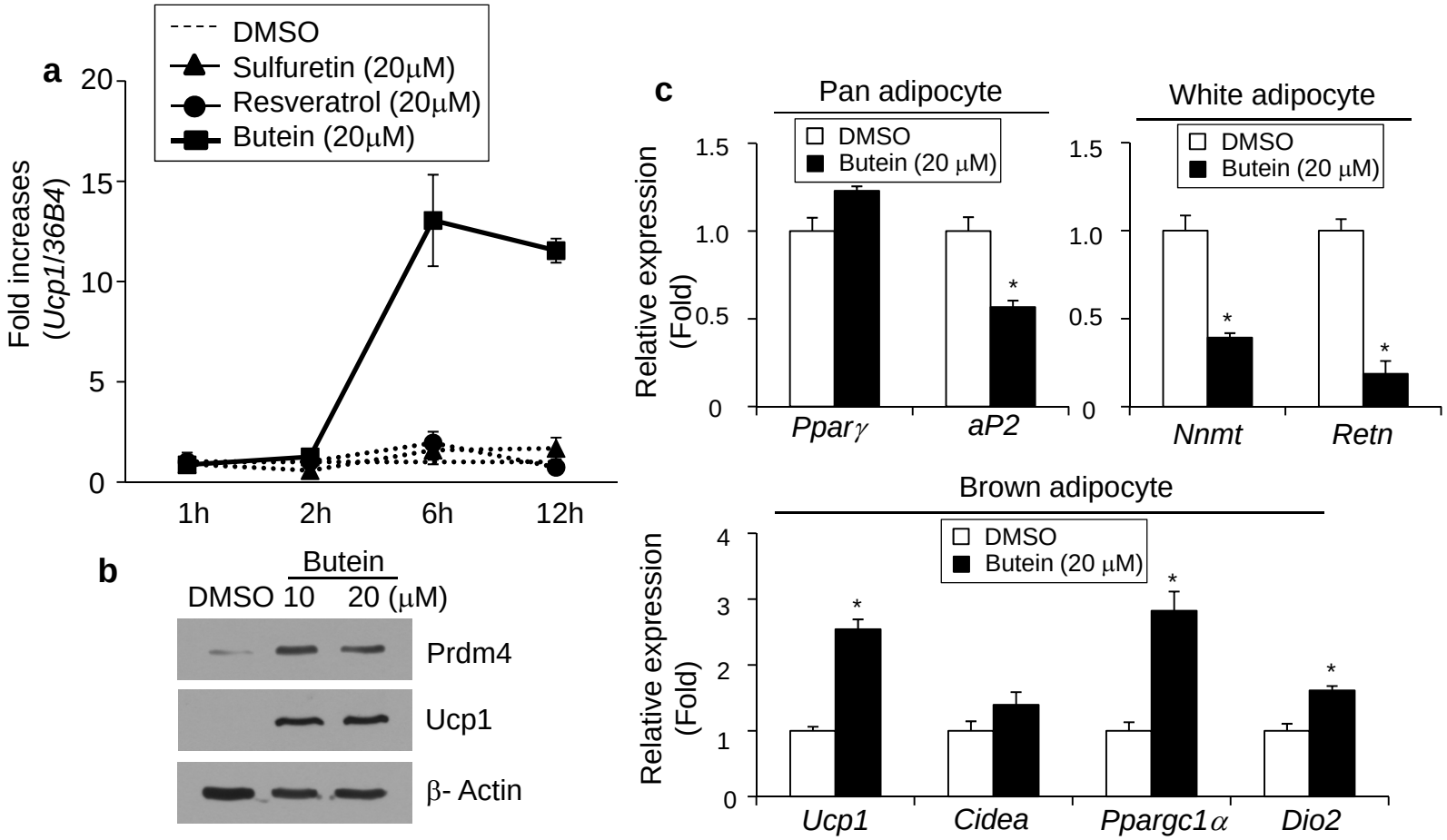


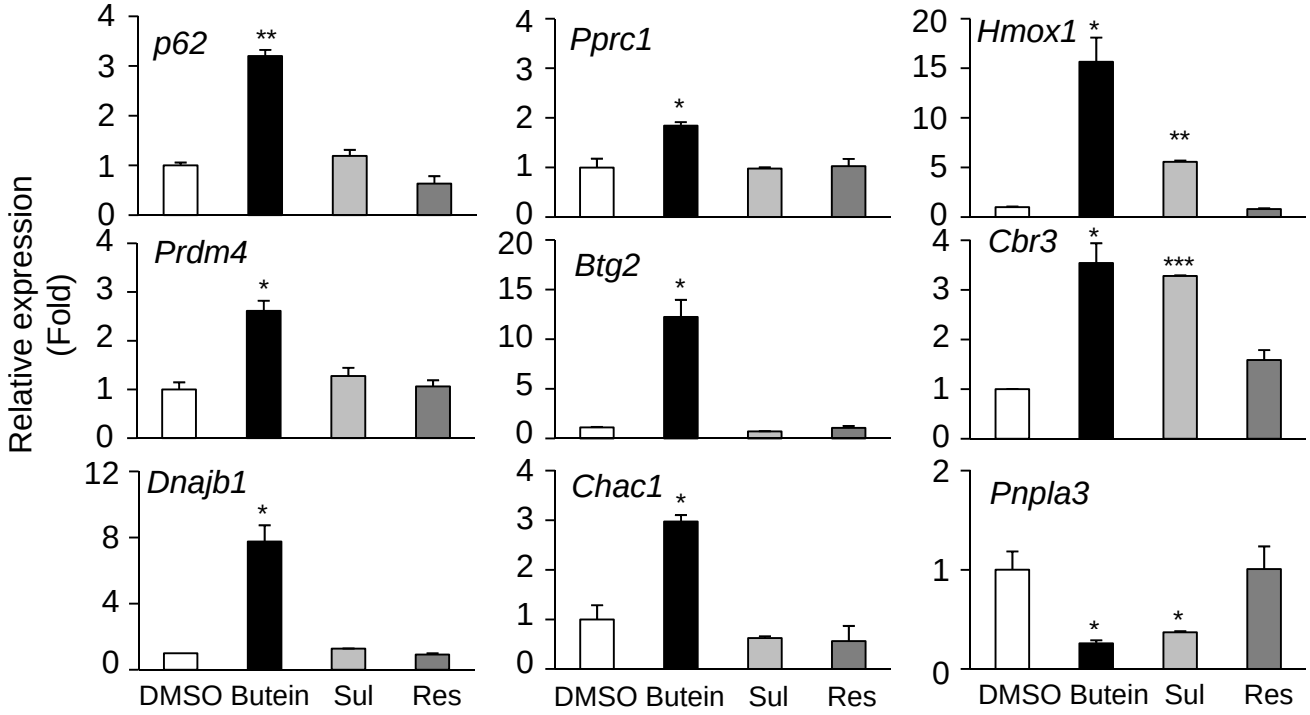
Supplementary Figure 2. Butein inhibits adipogenesis in C3H10T1/2 cells. (**a**) Butein suppresses lipid accumulation in C3H10T1/2 cells. Data are representative of two independent experiments. (**b**) C3H10T1/2 cells were differentiated and treated with butein for 7 days and mRNA expression of *Pparγ*, *aP2*, *CD36*, and *adipoq* (*adiponectin)* were measured by realtime PCR. Data represent means ± s.d. (n=3). Statistical significance was determined relative to a control by Student's *t*-test (* P<0.05; ** P<0.005; *** P<0.0005).



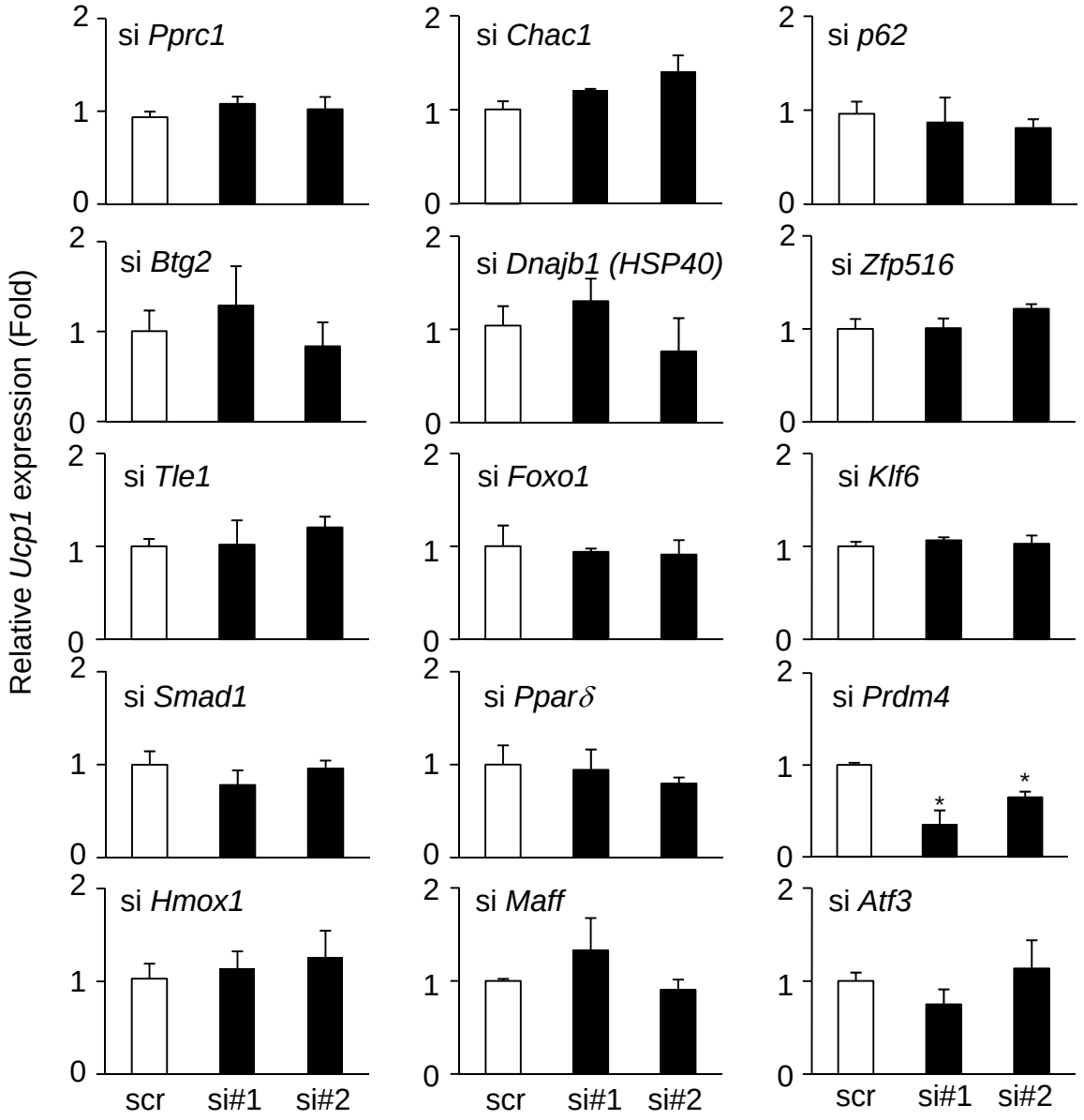
Supplementary Figure 3. Butein induces expression of thermogenic genes in adipocytes. (**a**) Mitochondrial staining by mitochondria specific cytopainter (ab112145, Abcam) in C3H10T1/2 adipocytes (left) and quantified by NIH Image J software (right). Data represent means ± s.d. (n=3). (scale bar = 100 μm) (**b**) C3H10T1/2 adipocytes were treated with butein and stained with bodipy (green). Quantification of changes in lipid droplet (LD) size (right). Data represent means ± s.d. (n=3). (scale bar = 50 μm) (**c**) Primary adipocytes were treated with butein for 24 hours and expression levels of general adipocyte markers (*Pparγ* and *aP2*), white adipocyte-selective markers (*Nnmt* and *Retn*), and thermogenic-selective markers (*Ucp1*, *Cidea*, *Ppargc1α*, and *Dio2*) were measured by realtime PCR. Data represent means ± s.d. (n=3). Statistical significance was determined relative to a control by Student's *t*-test (* P<0.05; ** P<0.005).



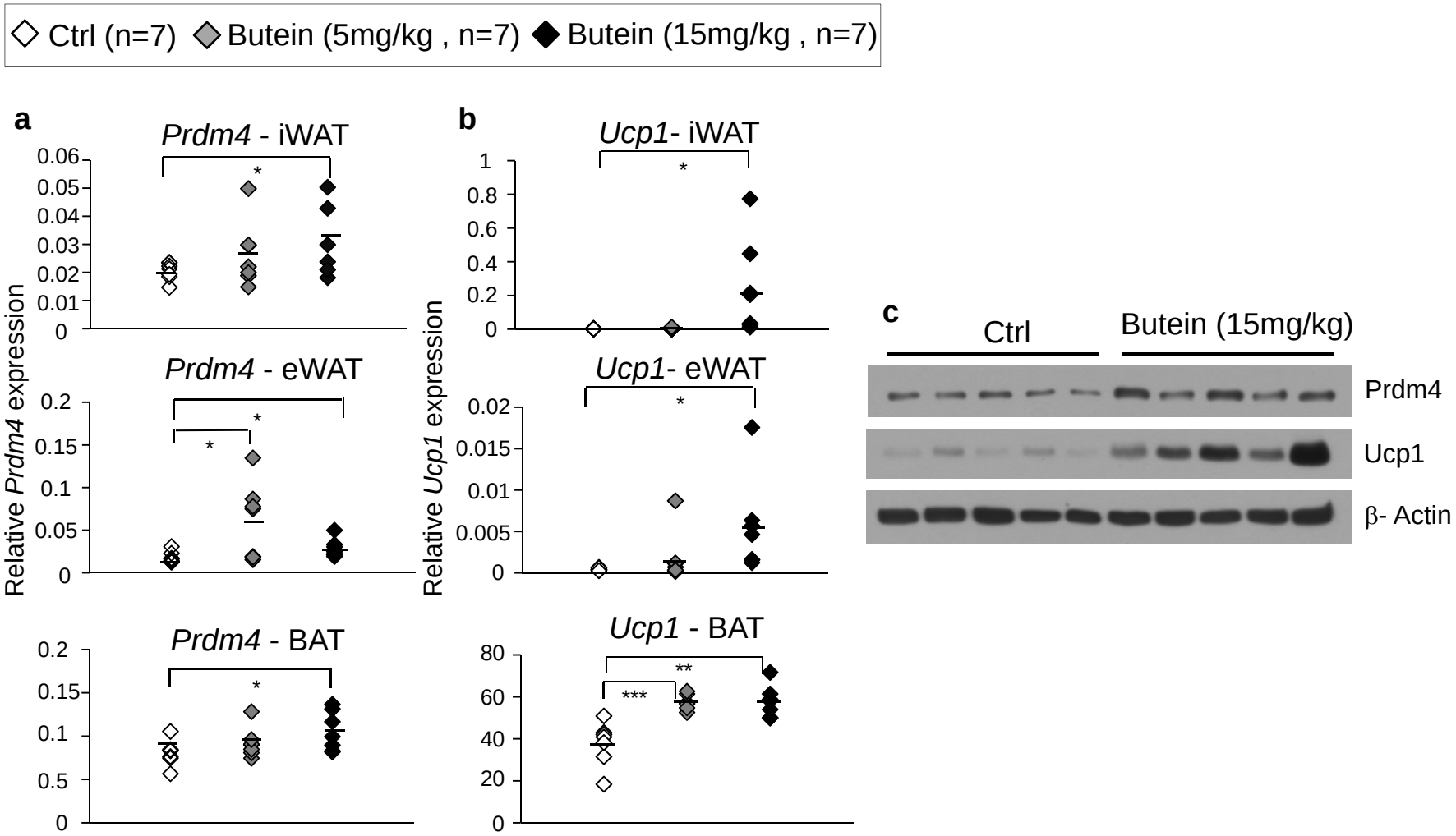
Supplementary Figure 4. Selective induction of *Ucp1* by butein. (**a**) Time course induction of *Ucp1* by sulfuretin, resveratrol, and butein in C3H10T1/2 adipocytes. Data represent means ± s.d. (n=3). (**b**) Butein increases UCP1 and Prdm4 protein expression in T37i cells. T37i cells were treated with DMSO or butein at the indicated concentrations for 24 hours and western blotting was performed. (**c**) Primary brown adipocytes isolated from interscapular brown fats were treated with butein for 24 hours, and the levels of general adipocyte genes (*Pparγ* and *aP2*), white adipocyte-selective genes (*Nnmt* and *Retn*), and thermogenic-selective markers were measured. Data represent means ± s.d. (n=3). Statistical significance was determined relative to a control by Student's *t*-test (* $P<0.05$). Uncropped images of blots are shown in Supplementary Fig. 21.



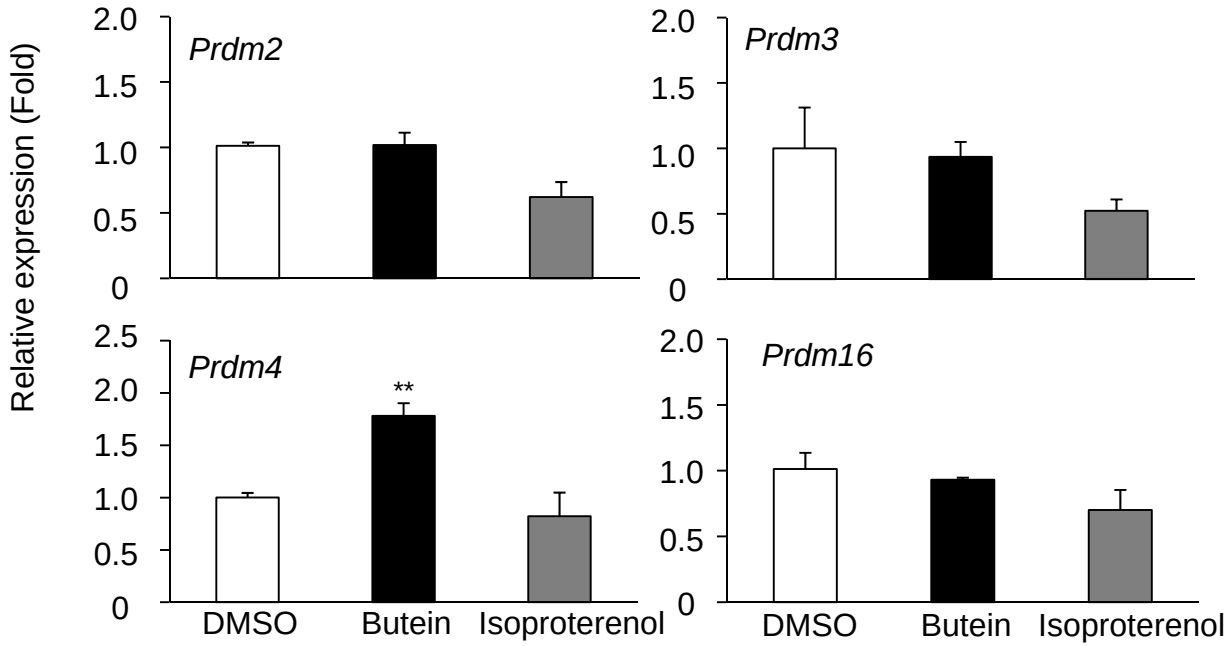
Supplementary Figure 5. Identification of genes selectively regulated by butein. Genes specifically regulated by butein were confirmed by real time PCR. Data represent means ± s.d. (n=3). Statistically significant differences were determined by Student's *t*-test (* P<0.05; ** P<0.005; *** P<0.0005).



Supplementary Figure 6. Effects on *Ucp1* expression by butein-selective genes. Two independent siRNA against butein selective genes and the top three genes most induced by butein (*Hmox-1, Maff,* and *Atf3)* were transfected into C3H10T1/2 adipocytes and *Ucp1* expression was measured by real time PCR. Data represent means ± s.d. (n=3). Statistically significant differences were determined by Student's *t*-test (* P<0.05; ** P<0.005; *** P<0.0005).



Supplementary Figure 7. Induction of *Prdm4* and *Ucp1* by butein *in vivo*. (**a**) Treatment with butein at daily doses of 5 and 15mg/kg for 8 weeks in mice (n=7 per each group) increases the expression of *Prdm4* in inguinal (iWAT) (top), epididymal white adipose tissues (eWAT) (middle), and brown adipose tissues (BAT) (bottom). (**b**) Treatment with butein increases the expression of *Ucp1* in iWAT (top), eWAT (middle), and BAT (bottom). Dots (open and closed) and bars in scatter plots represent expression levels of individual mice and the average, respectively. Statistically significant differences in gene expression between the control and butein-treated mice (n=7 per each group) were determined by Student's *t*-test (* $P < 0.05$; ** $P<0.005$; *** $P<0.0005$). (**c**) Expression of Prdm4 and Ucp1 protein in eWAT of control (n=5) and butein-injected mice (15mg/kg, n=5) was determined by western blotting. Uncropped images of blots are shown in Supplementary Fig. 21.



Supplementary Figure 8. Acute induction of Prdm4 by butein. Butein treatment induces expression of *Prdm4* but not the similar Prdm family members (*Prdm2*, *Prdm3*, and *Prdm16)*. Isoproterenol does not regulate all Prdm family members, including *Prdm4*. Data represent means ± s.d. (n=3). Statistically significant differences were determined by Student's *t*-test (** $P < 0.005$).

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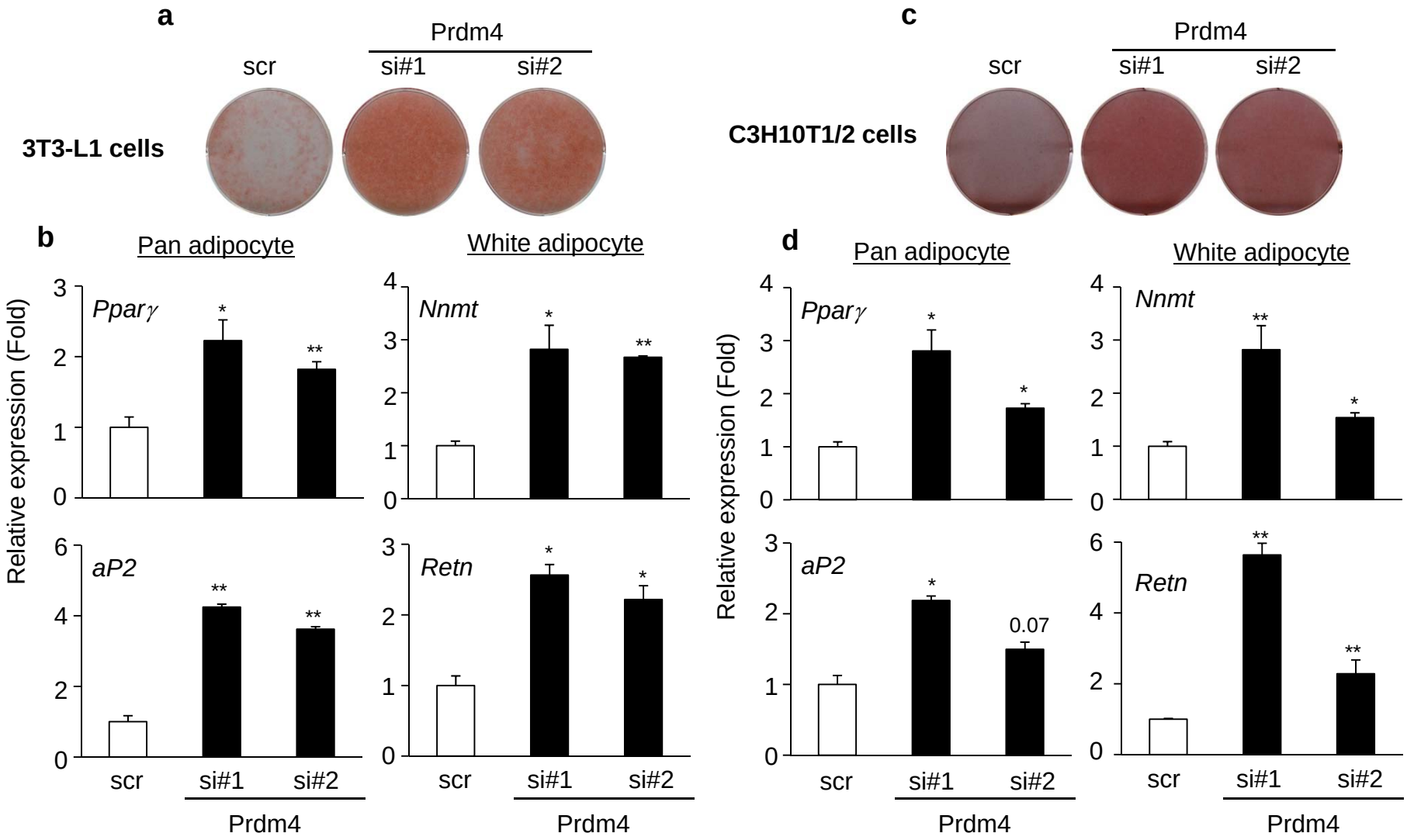
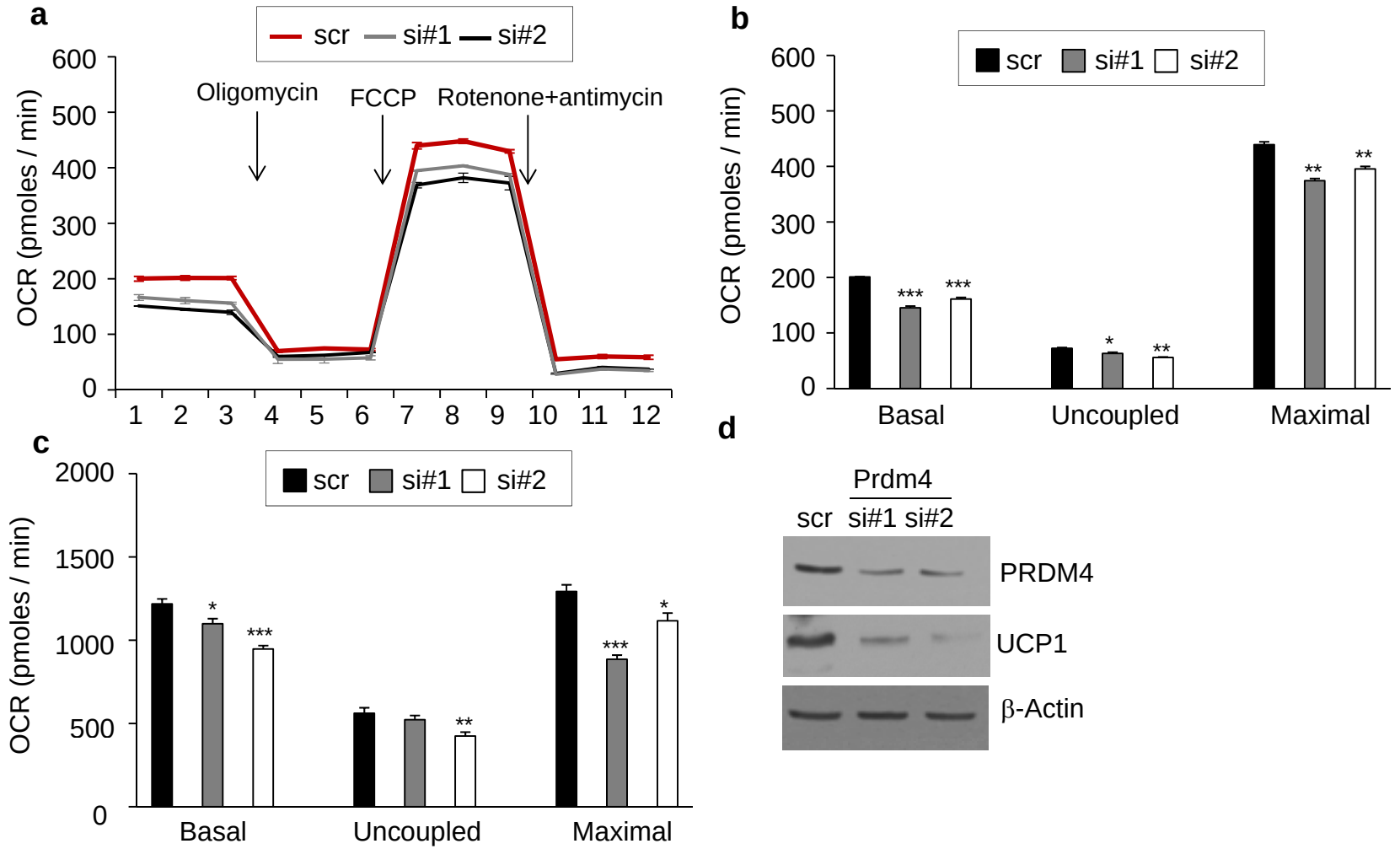
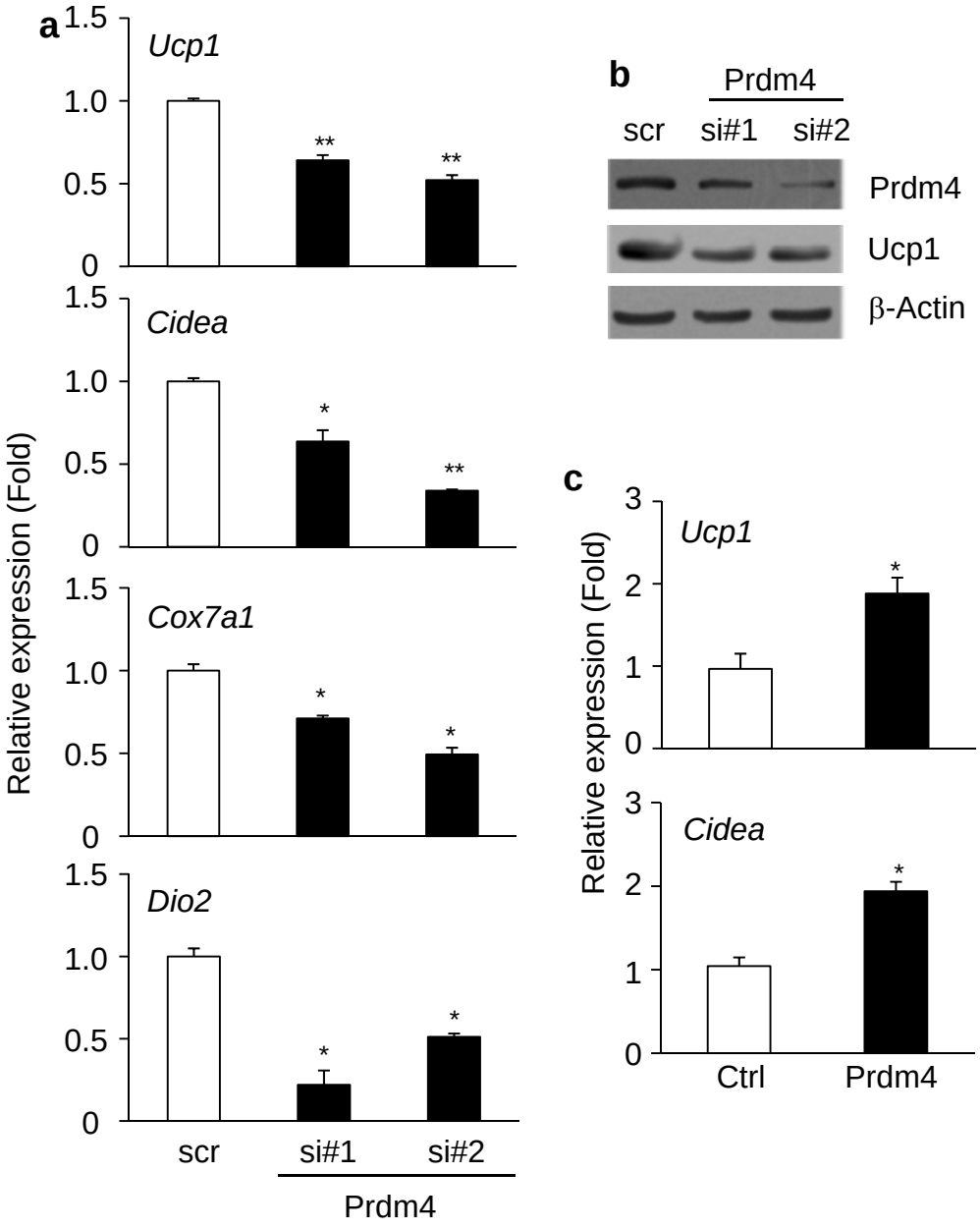


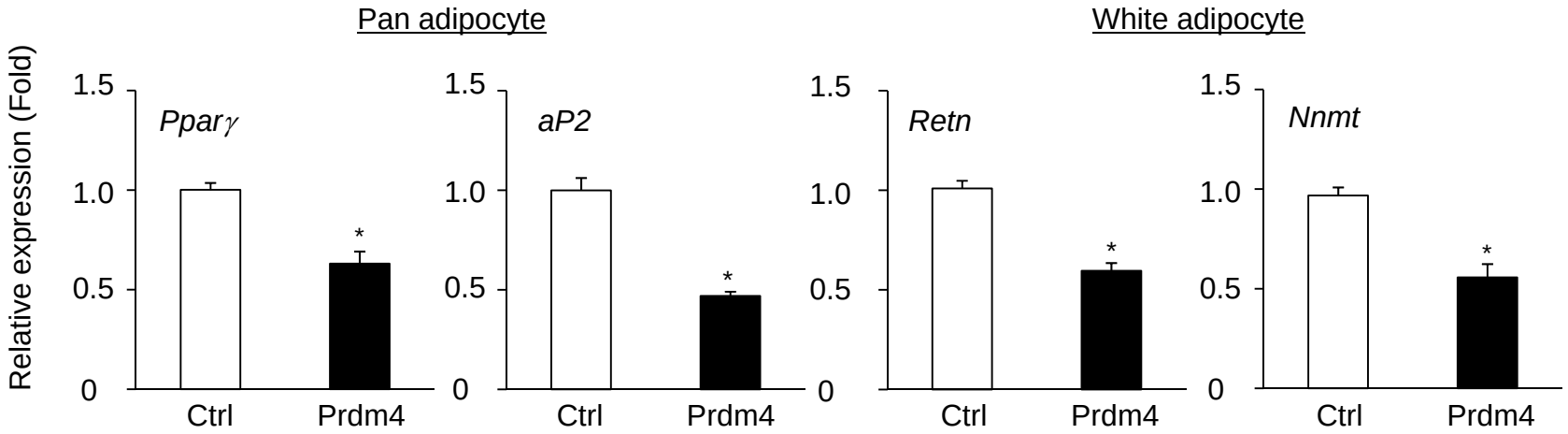
Supplementary Figure 9. Prdm4 silencing increases lipid accumulation and expression of white adipocyte-selective genes in preadipocytes. (**a**) Transient transfection of two independent Prdm4-targeting siRNAs (si#1 and si#2) compared to scrambled control (scr) in 3T3-L1 pre-adipocytes increases lipid accumulation during adipocyte differentiation. Data are representative of two independent experiments. (**b**) Knockdown of Prdm4 by siRNAs increases expression of pan-adipocyte markers (*Pparγ* and *aP2*) and the WAT-selective markers (*Nnmt* and *Retn*). Data represent means ± s.d. (n=3). (**c**) Transient transfection of two independent Prdm4-targeting siRNAs (si#1 and si#2) in C3H10T1/2 cells increases lipid accumulation during adipocyte differentiation. Data are representative of two independent experiments. (**d**) Knockdown of Prdm4 in C3H10T1/2 cells increases the expression of pan- and WAT-selective adipocyte markers. Data represent means ± s.d. (n=3). Statistically significant differences were determined by Student's *t*-test (* P<0.05; ** P<0.005).



Supplementary Figure 10. Prdm4 regulates oxygen consumption rates in C3H10T1/2 cells. (**a-b**) Knockdown of Prdm4 by two independent siRNAs (si#1 and si#2) reduces the oxygen consumption rates (OCR) compared to the scrambled control (scr) siRNA-transfected C3H10T1/2 cells. Data represent means ± s.d. (n=3). (**a**) The OCR was measured in approximately 8 minute intervals. (**b**) Basal respiration, uncoupled respiration (oligomycin), and maximal respiration (FCCP) were determined using XF24 Extracellular Flux Analyzer. (**c**) Knockdown of Prdm4 by two independent siRNAs (si#1 and si#2) reduces the basal, uncoupled, and maximal respiration in differentiated C3H10T1/2 adipocytes. Data were normalized to cell number. Data represent means ± s.d. (n=6). Statistical analysis was determined by Student's *t*-test (* $P < 0.05$; ** $P<0.005$; *** $P<0.0005$). (**d**) Knockdown of Prdm4 by siRNAs in C3H10T1/2 adipocytes decreases Prdm4 and Ucp1 protein expression. Uncropped images of blots are shown in Supplementary Fig. 21.



Supplementary Figure 11. Prdm4 induces Ucp1 and thermogenic genes in T37i cells. (**a. b**) Prdm4 silencing in T37i cells reduces expression of thermogenic genes. (**a**) Knockdown of Prdm4 by two independent siRNAs (si#1 and si#2) reduces the expression levels of *Ucp1*, *Cidea*, *Cox7a1*, and *Dio2* mRNA compared to the scrambled control (scr) siRNA-transfected T37i cells. Data represent means ± s.d. (n=3). (**b**) Two independent Prdm4-specific siRNAs decrease Ucp1 and Prdm4 protein expression. (**c**) Forced expression of Prdm4 induces thermogenic genes in T37i brown adipocytes. Data represent means ± s.d. (n=3). Statistically significant differences were determined by Student's *t*-test (* $P < 0.05$; ** $P<0.005$). Uncropped images of blots are shown in Supplementary Fig. 22.



Supplementary Figure 12. Prdm4 overexpressing cells decrease expression of white adipocyte-selective genes. Forced expression of Prdm4 decreases pan- and white-selective genes in 3T3-L1 adipocytes. Data represent means ± s.d. (n=3). Statistically significant differences were determined by Student's *t*-test (* P<0.05).

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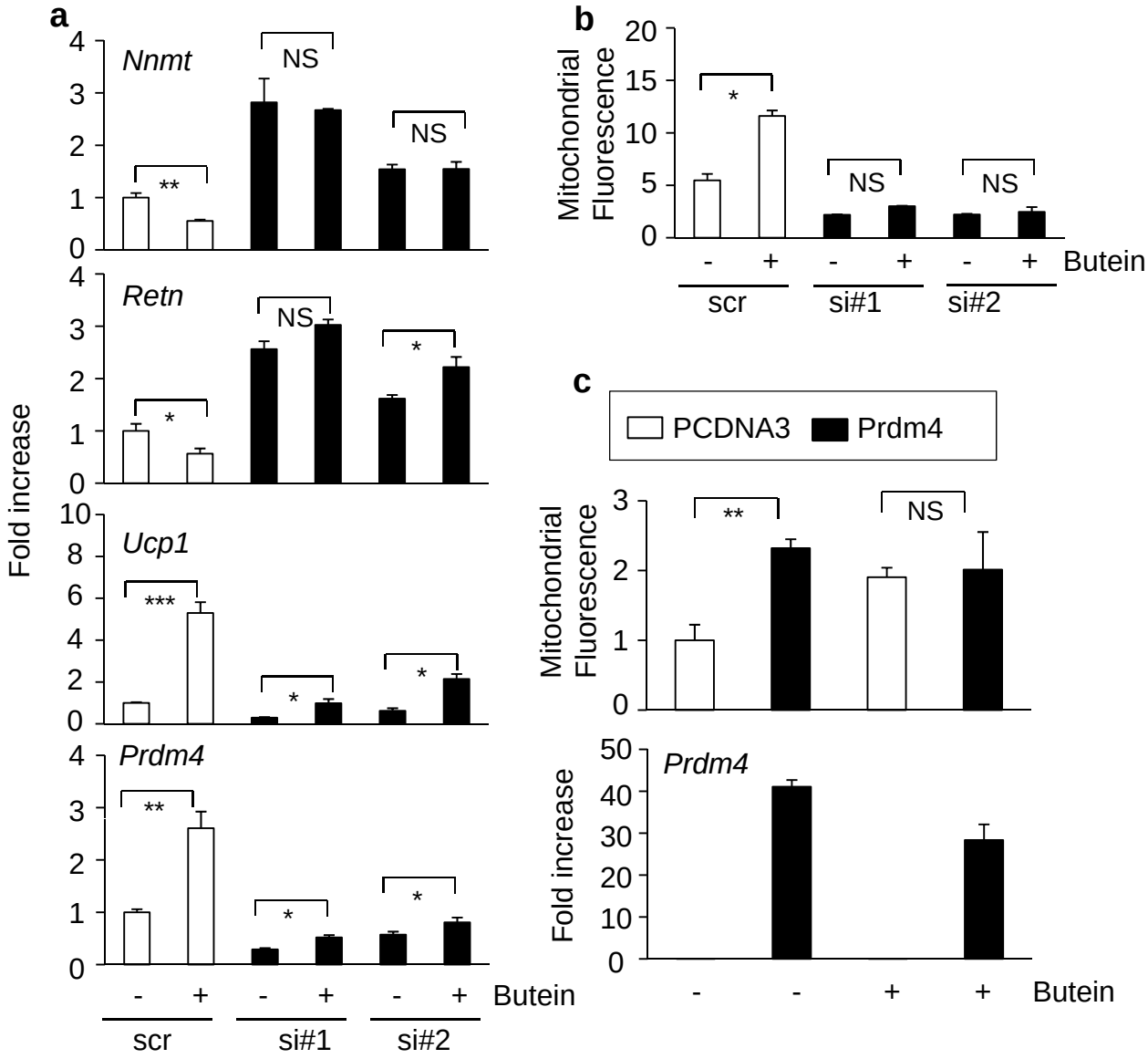
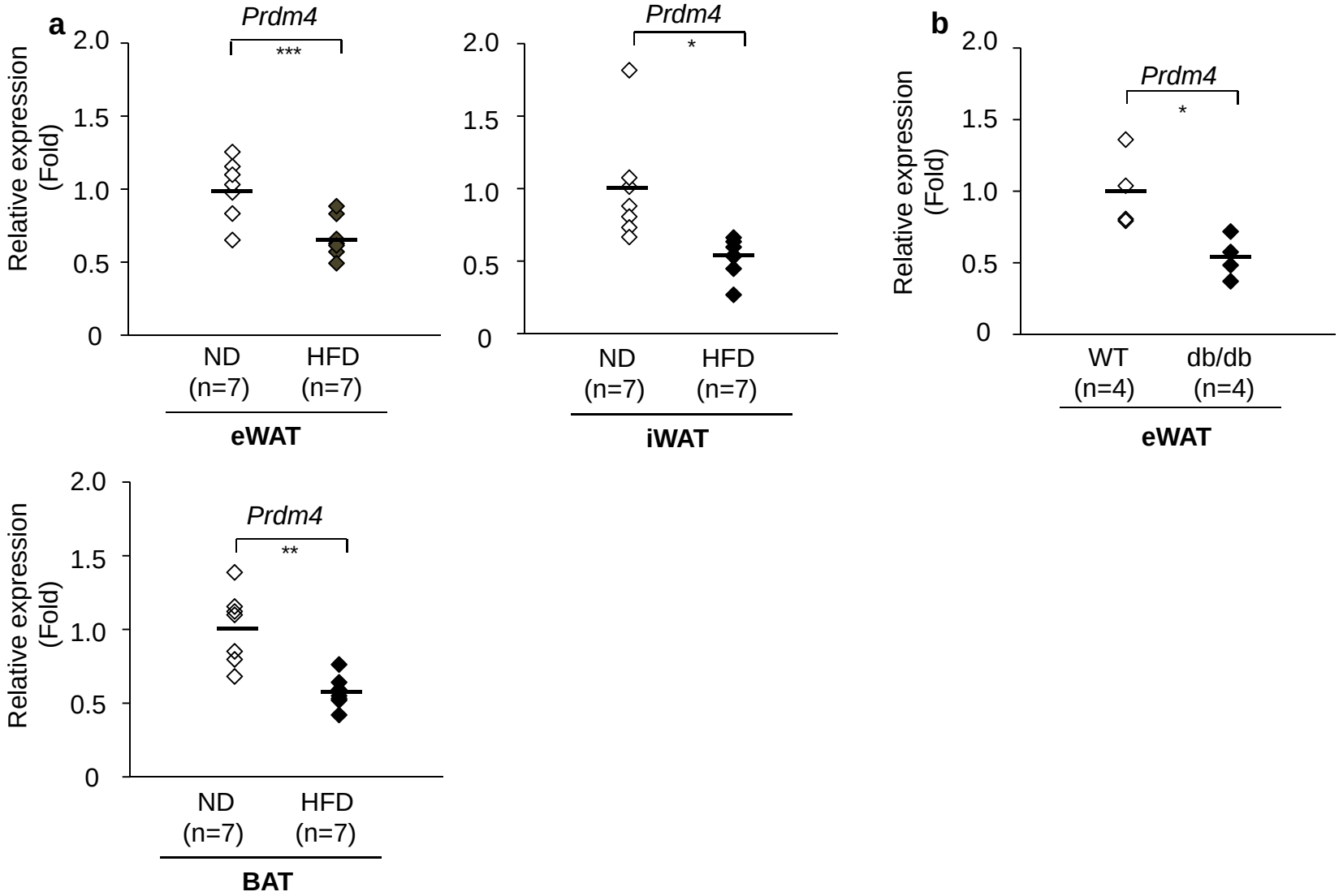
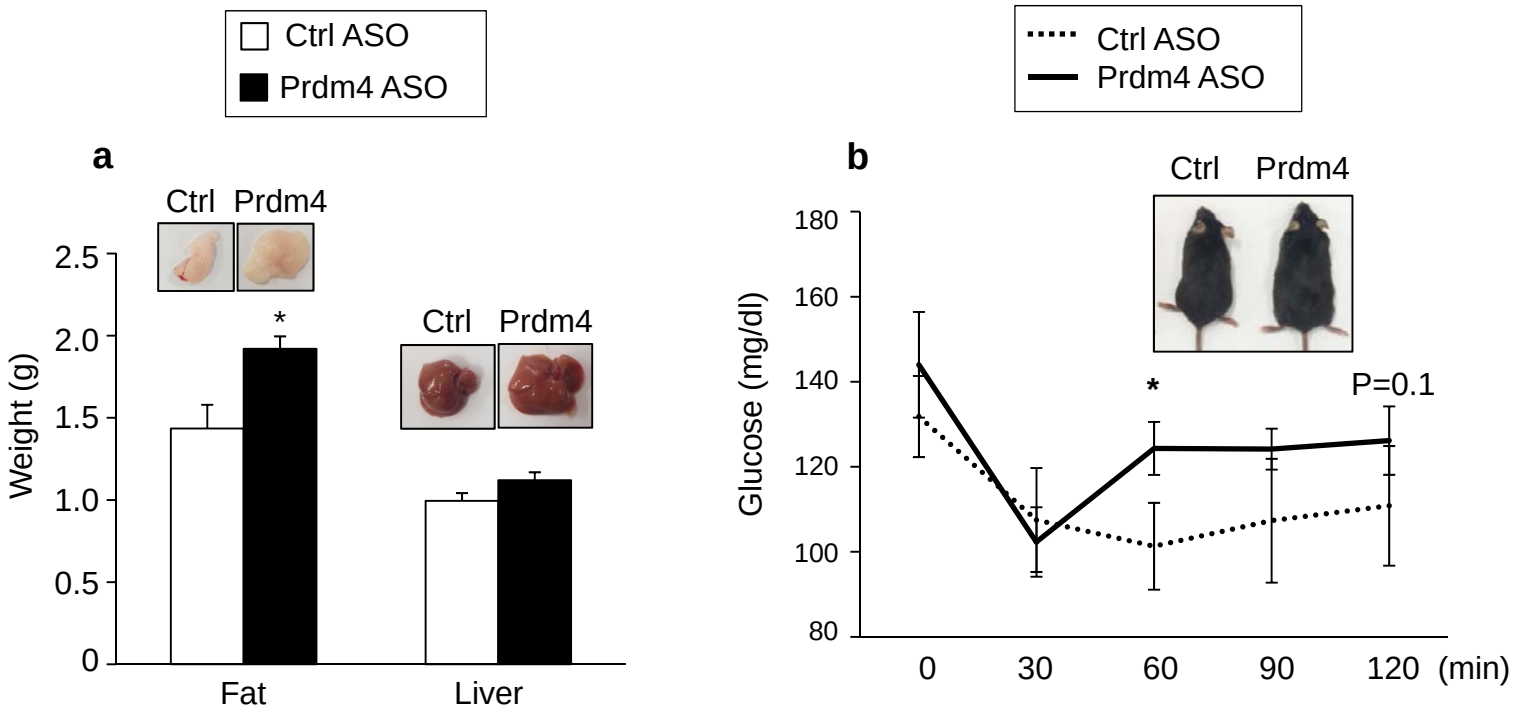


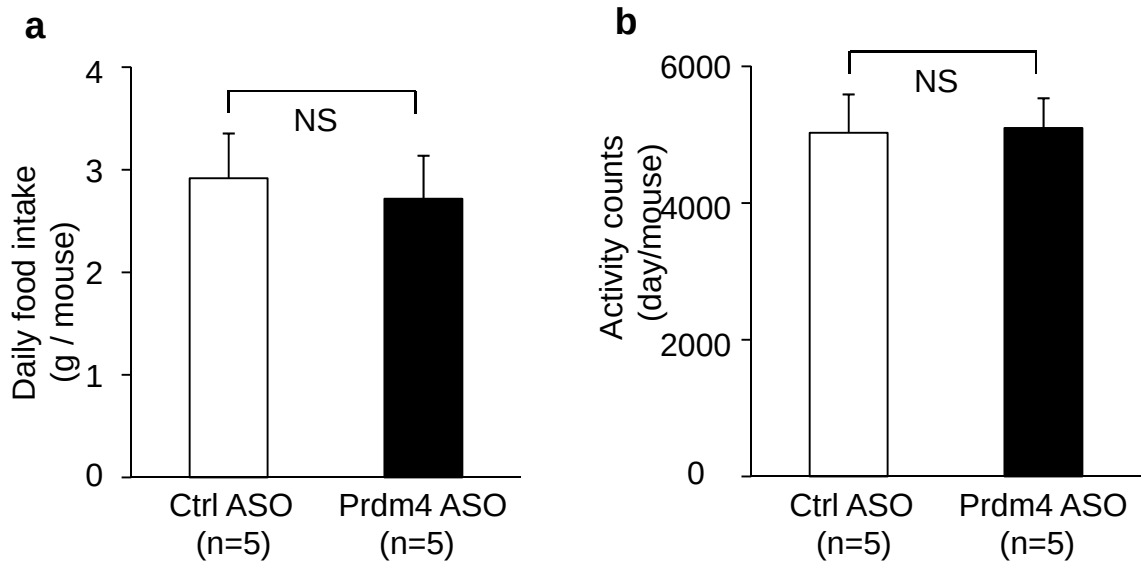
Supplementary Figure 13. Prdm4 is necessary for the effects of butein in thermogenic programming. (**a**) Prdm4 siRNA-transfected cells display blunted effects of butein on expression of *Nnmt*, *Retn*, and *Ucp1* in C3H10T1/2 cells. Data represent means ± s.d. (n=3). (**b**) Increased mitochondrial staining by treatment with butein is compromised in Prdm4 siRNA-transfected C3H10T1/2 adipocytes. Data represent means ± s.d. (n=3). (**c**) Prdm4-overexpressing cells abrogate the effects of butein in increased mitochondrial staining of C3H10T1/2 cells. Data represent means ± s.d. (n=3). Statistically significant differences in the control and butein treatment were determined by Student's *t*-test (* $P < 0.05$; **$P<0.005$; *** $P<0.0005$; NS, not significant).



Supplementary Figure 14. Prdm4 expression is reduced in adipose tissues of obese and diabetic mice. (**a**) *Prdm4* expression in lean and obese fat tissues. *Prdm4* expression is significantly lower in epididymal white adipose tissues (eWAT), inguinal white adipose tissues (iWAT), and brown adipose tissues (BAT) of the HFD-fed obese mice compared to the lean control mice (n=7 per group). Dots (open and closed) and bars in the scatter plots represent individual mice and the average, respectively. (**b**) The expression levels of *Prdm4* in epididymal fats of diabetic mice (*db/db*) are compared to non-diabetic control mice (n=4 per group). Dots (open and closed) and bars in the scatter plots represent individual mice and the average, respectively. Statistically significant differences in the control and butein treatment were determined by Student's *t*-test (* $P < 0.05$; ** $P<0.005$; *** $P<0.0005$).



Supplementary Figure 15. Effects of Prdm4 knockdown on tissue weight gains and insulin sensitivity in HFD fed mice. (**a-b**) C57BL/6 mice on a HFD were treated with Prdm4 ASO or a control ASO twice per week (25mg per kg per dose) for 6 weeks. (**a**) Liver and fat weights of C57BL/6 mice treated with control ASO or Prdm4 ASO. Data represent means ± s.d. (n=5). (**b**) Insulin tolerance test in control and Prdm4 ASO-injected groups. Data represent means ± s.d. (n=5). Statistically significant differences in the control ASO and Prdm4 ASO-injected groups were determined by Student's *t*-test (* $P < 0.05$).



Supplementary Figure 16. Prdm4 knockdown in HFD fed mice does not alter food intake and locomotor activity. C57BL/6 mice on a HFD were treated with Prdm4 ASO or a control ASO twice per week (25mg per kg per dose) for 6 weeks. (**a**) Food intake of HFD-fed control ASO and Prdm4 ASO-treated mice. Data represent means ± s.d. (n=5). (**b**) Physical activity of HFD-fed control ASO and Prdm4 ASO-treated mice. Data represent means ± s.d. (n=5). Statistically significant differences in the control ASO and Prdm4 ASO-injected groups were determined by Student's *t*-test (NS, not significant).

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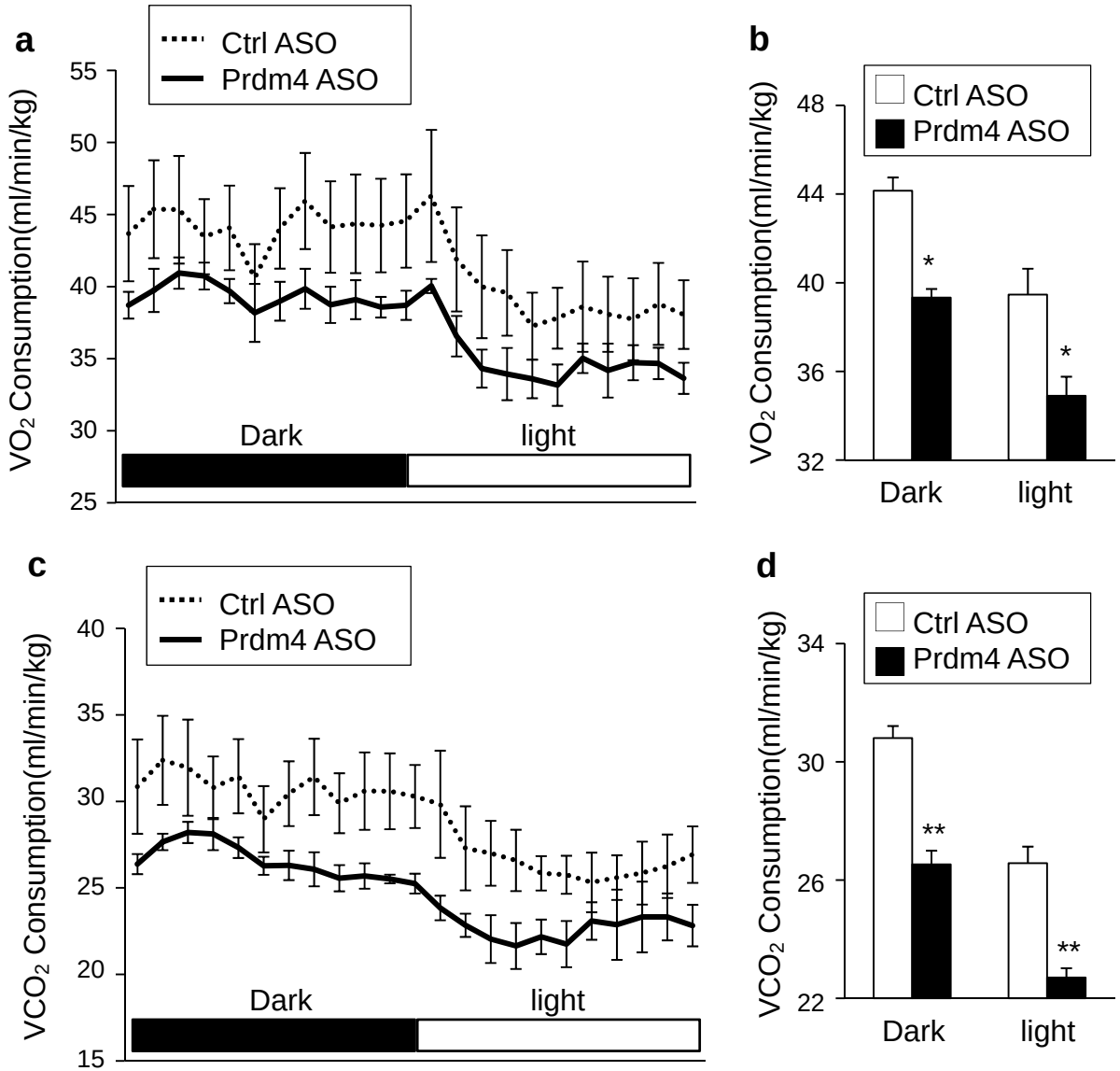
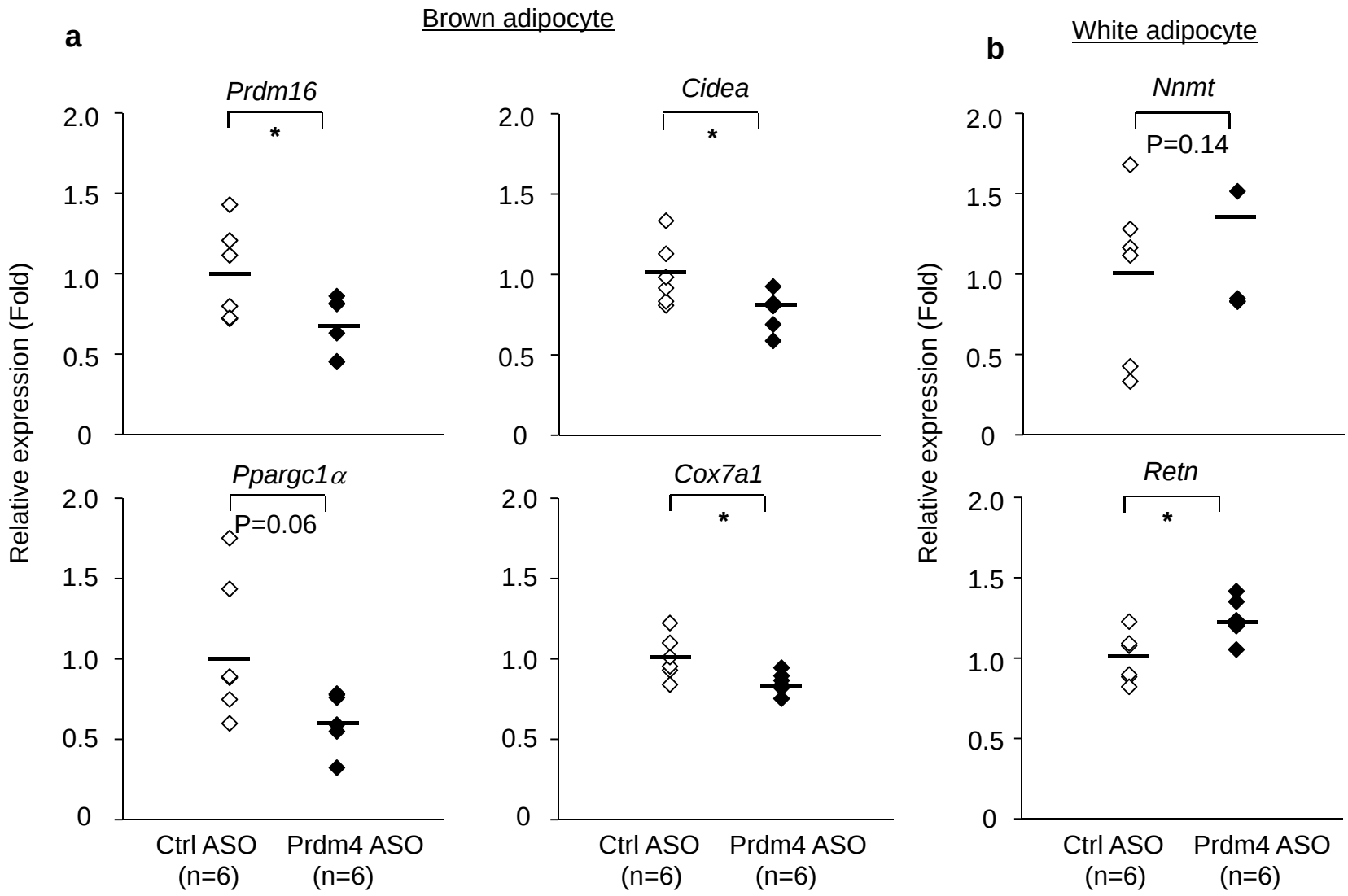
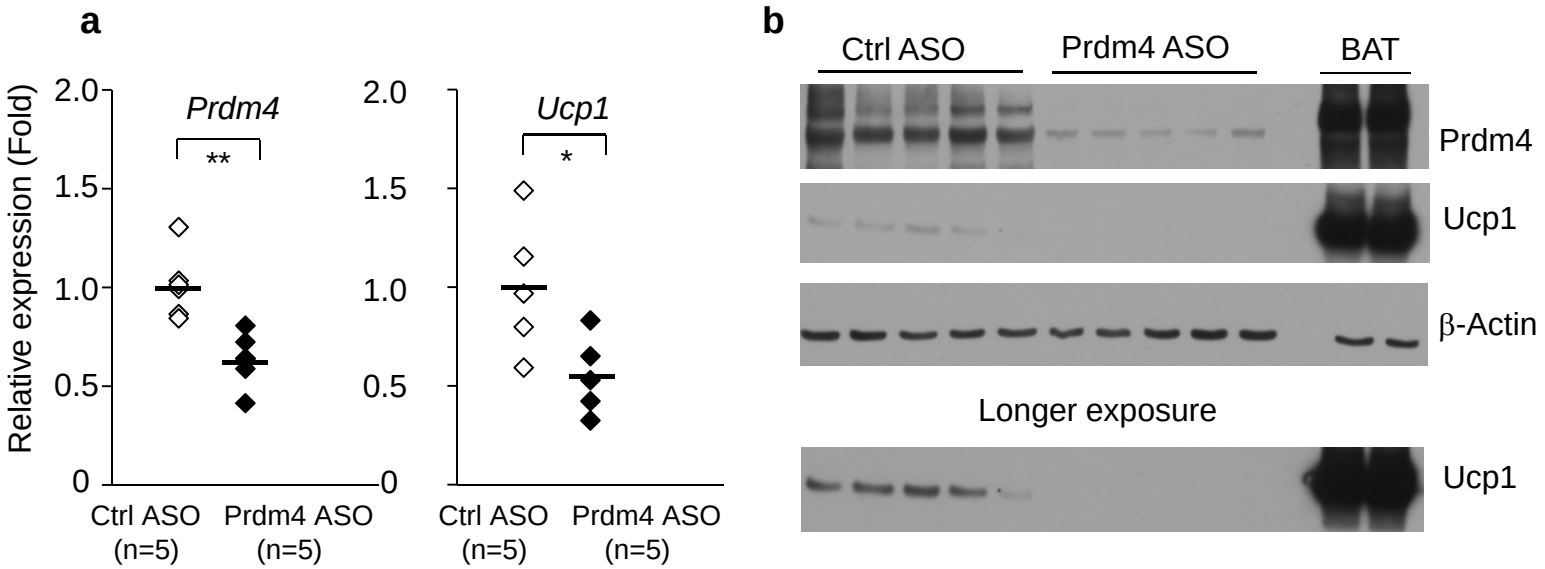


Supplementary Figure 17. Prdm4 knockdown in HFD fed mice decreases energy expenditure. (**a-d**) Mice on a high fat diet were treated with Prdm4 ASO or control ASO for 2.5 weeks and energy expenditure was measured. Data represent means ± s.d. (n=5). (**a**) O_2 consumption was recorded during a 24 hour period. (**b**) averages of O_2 consumption. (**c**) CO_2 production was recorded during a 24 hour period. (**d**) averages of CO_2 production. Statistically significant differences in the control and Prdm4 ASO-treated mice were determined by Student's *t*-test (* $P < 0.05$; **$P<0.005$).



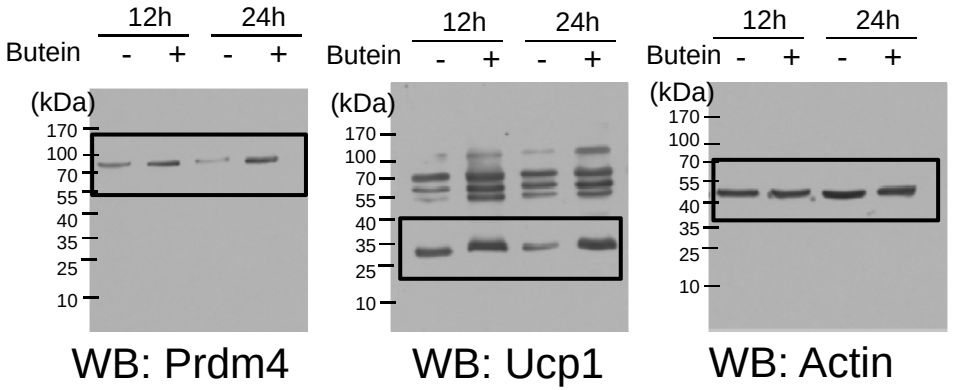
Supplementary Figure 18. Effects of Prdm4 knockdown on white and brown adipocyte selective gene expression in adipose tissues of HFD fed mice. (**a-b**) C57BL/6 mice on a HFD were treated with Prdm4 ASO or a control ASO twice per week (25mg per kg per dose) for 6 weeks. Expression of brown (**a**) and white adipocyte-selective genes (n=6 per group) (**b**) in epididymal adipose tissues of control and Prdm4 ASO-treated mice (n=6 per group). Dots (open and closed) and bars in the scatter plots represent individual mice and the average, respectively. Statistically significant differences in the control ASO and Prdm4 ASO-injected groups were determined by Student's *t*-test (* $P < 0.05$).



Supplementary Figure 19. Effects of Prdm4 knockdown on Ucp1 expression in adipose tissues of HFD fed mice. (**a-b**) C57BL/6 mice on a HFD were treated with Prdm4 ASO or a control ASO twice per week (25mg per kg per dose) for 6 weeks. (**a**) Expression of *Prdm4* and *Ucp1* in epididymal adipose tissues (eWAT) of control and Prdm4 ASO-injected mice (n=5 per group). Dots (open and closed) and bars in the scatter plots represent expression levels of individual mice and the average, respectively. (**b**) Expression of Prdm4 and Ucp1 protein in eWAT of control ASO and Prdm4 ASO-injected mice (n=5 per group) was determined by western blotting. Brown adipose tissues (BAT) were used as controls. Uncropped images of blots are shown in Supplementary Fig. 22.

Original blots

Fig. 1b



Supplementary Figure 20. Uncropped versions of immunoblots in the Fig 1b.

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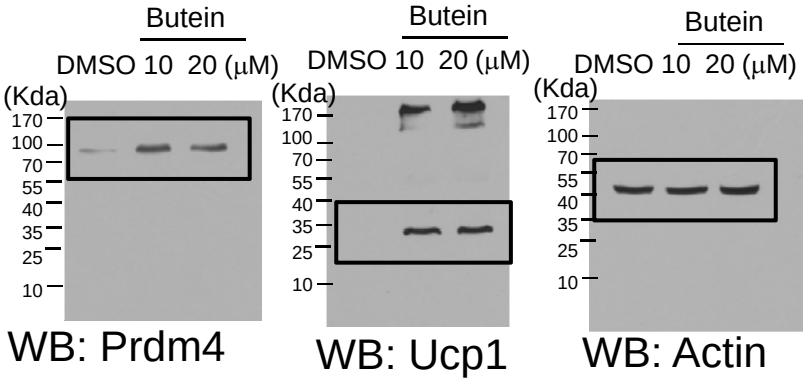
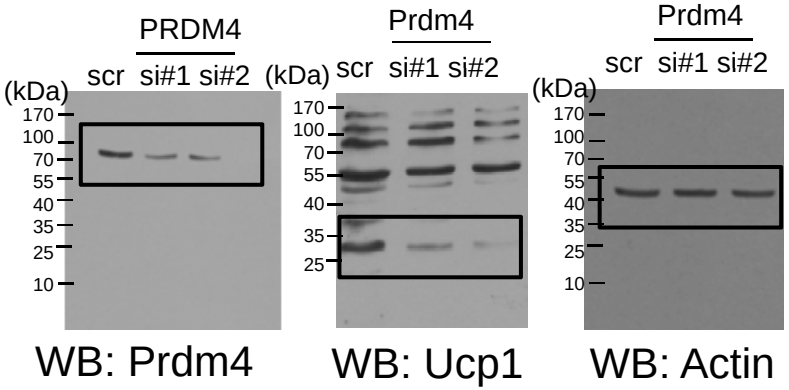
Original blots

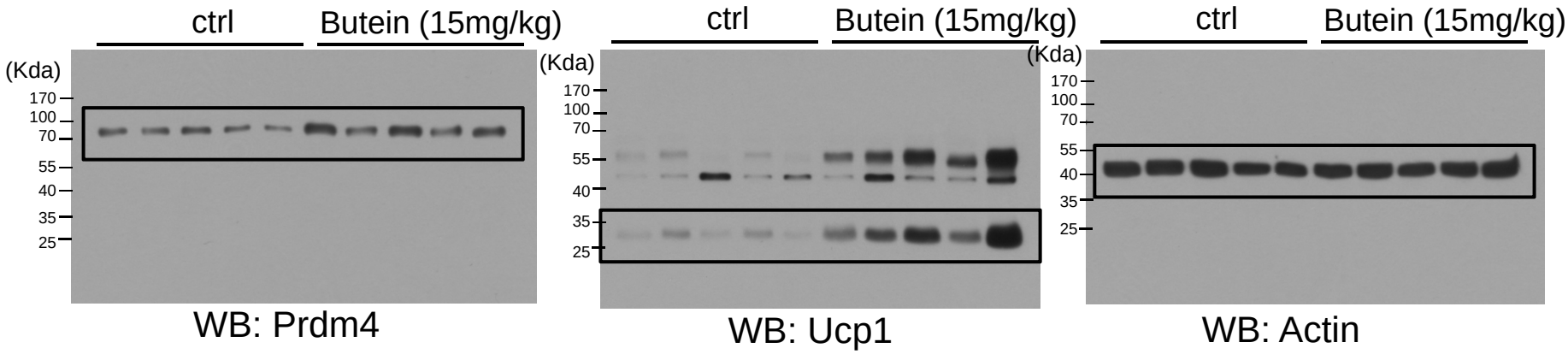
Supplementary Fig.4b



Supplementary Fig 10.d



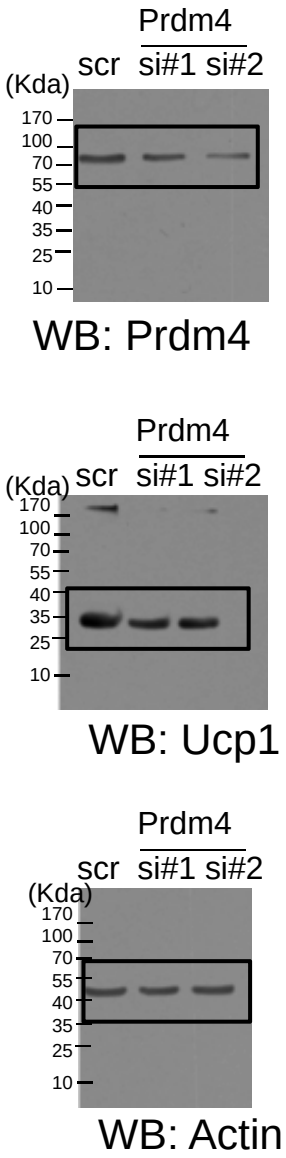
Supplementary Fig.7c



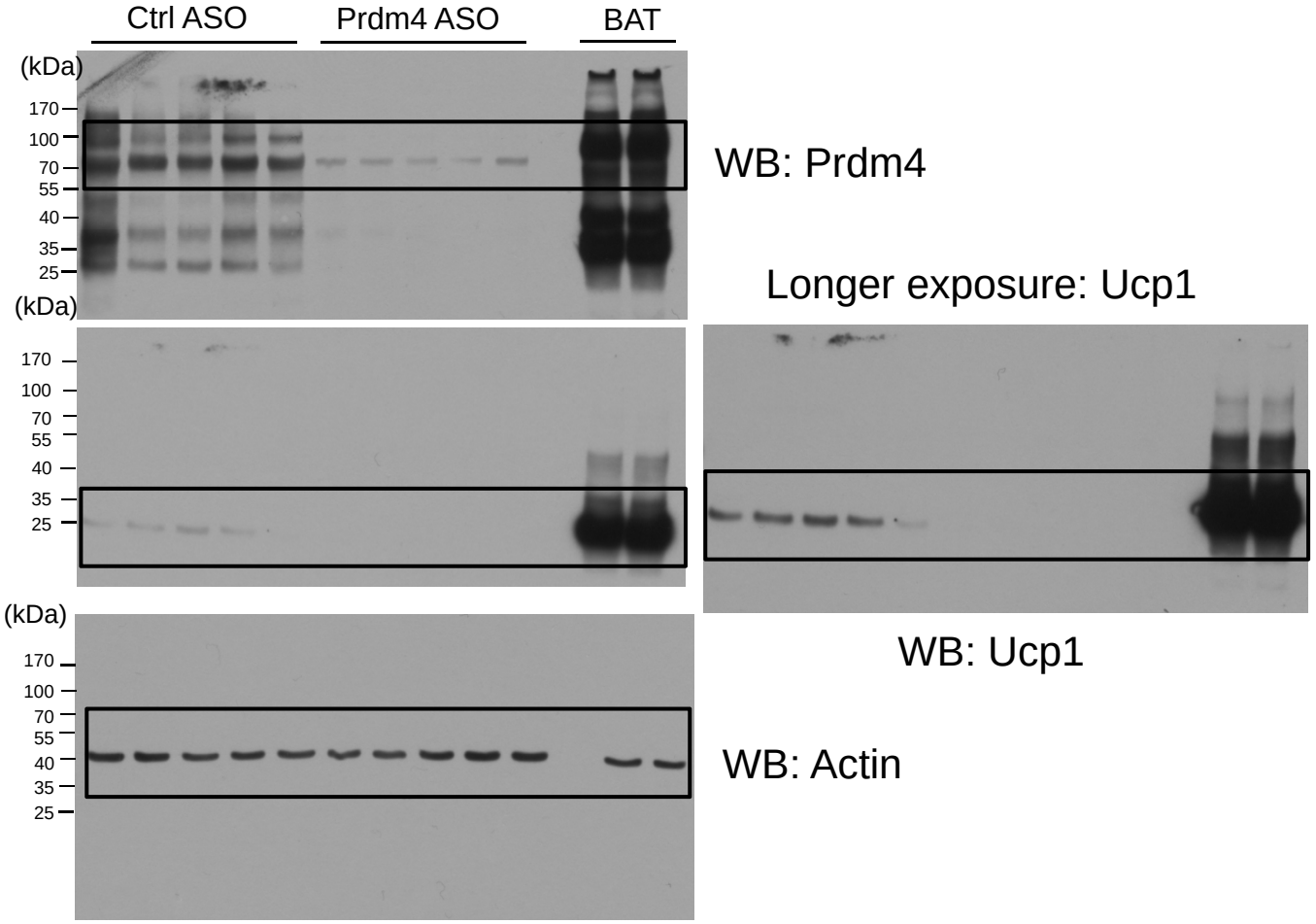
Supplementary Figure 21 Uncropped versions of immunoblots in the supplementary Figure 4b, 7c, and 10d

Original blots

Supplementary Fig.11b



Supplementary Fig 19. b



Supplementary Figure 22. Uncropped versions of immunoblots in the supplementary figure 11b and 18b.