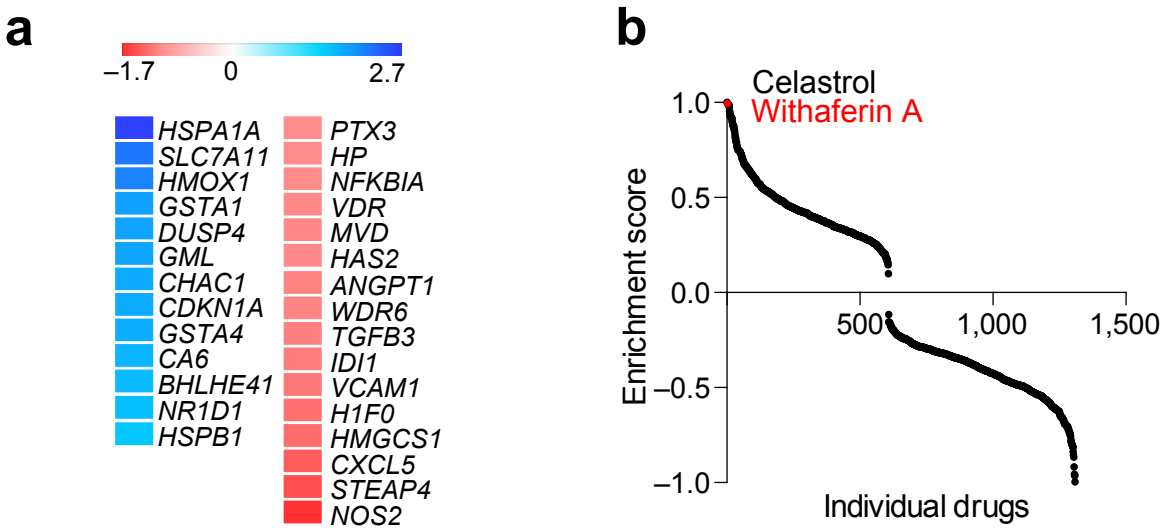
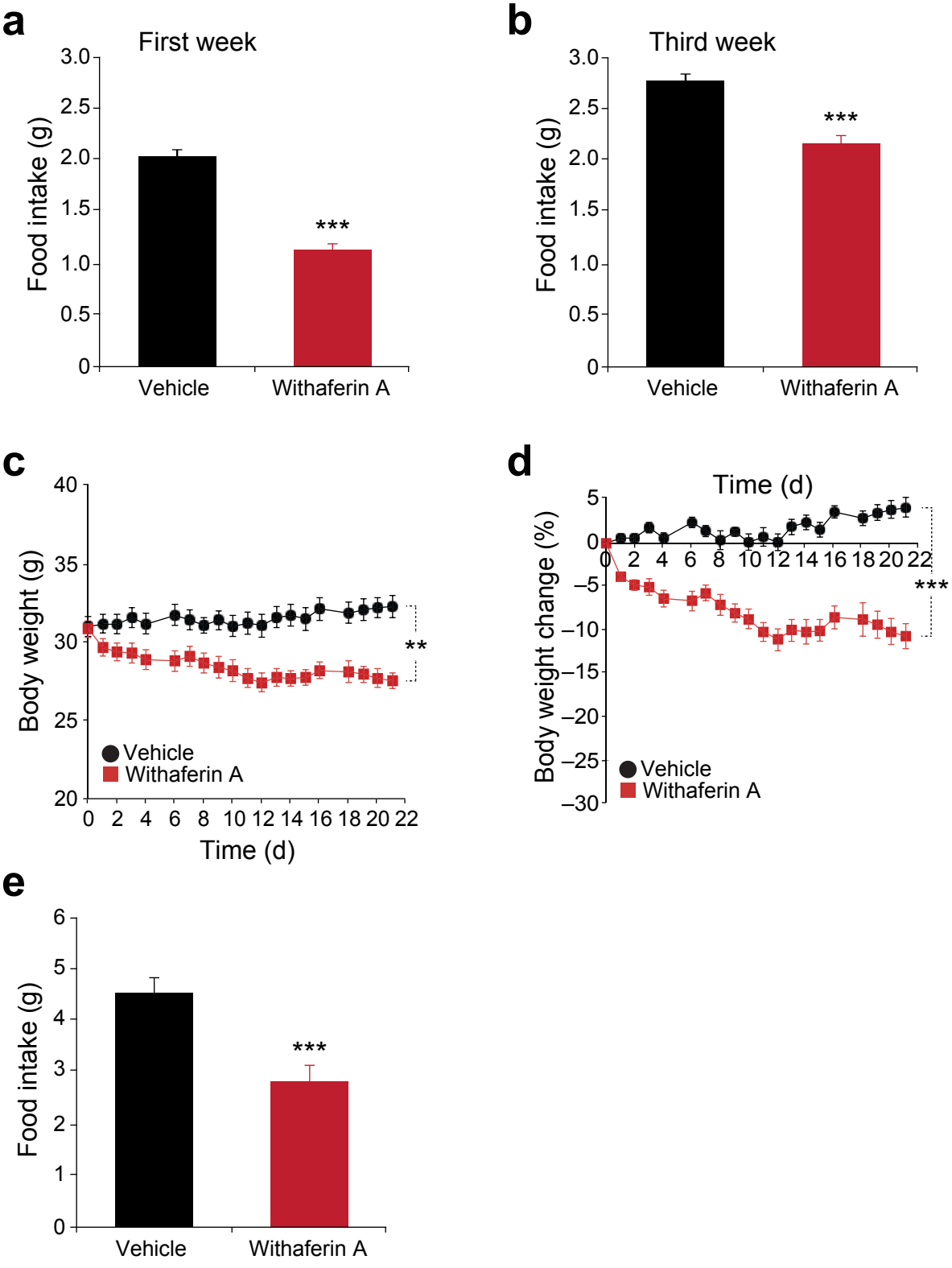
Supplementary Figure 1



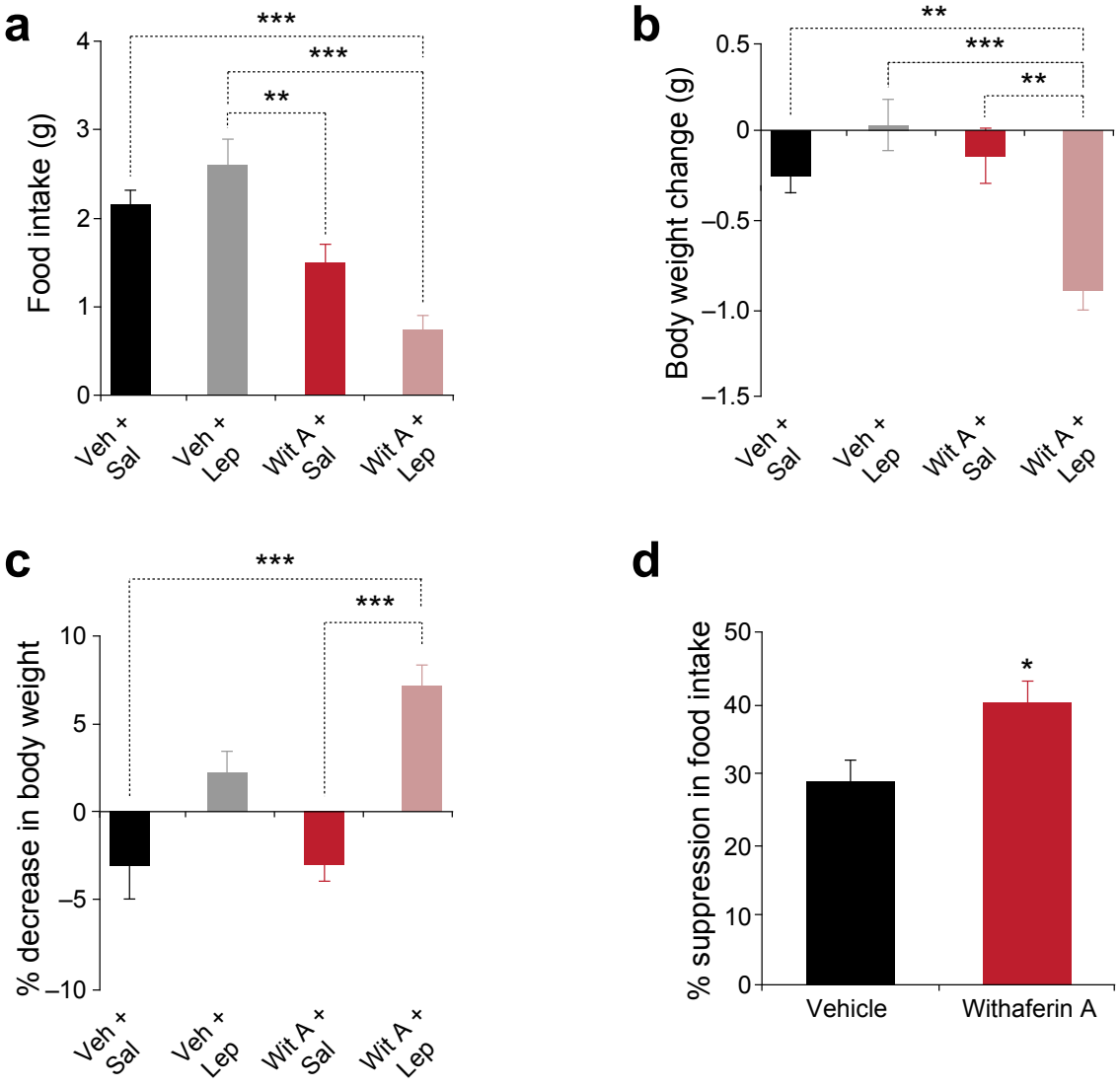
Supplementary Figure 1. Idenfication of withaferin A as an anti-obesity compound. (**a**) Heatmap of corresponding human homologs of the gene expression signature in **Fig. 1b**, which was used as CMAP query. (**b**) Distribution of enrichment scores of the individual compounds from the CMAP result using celastrol's gene expression signature from **a**.

Supplementary Figure 2



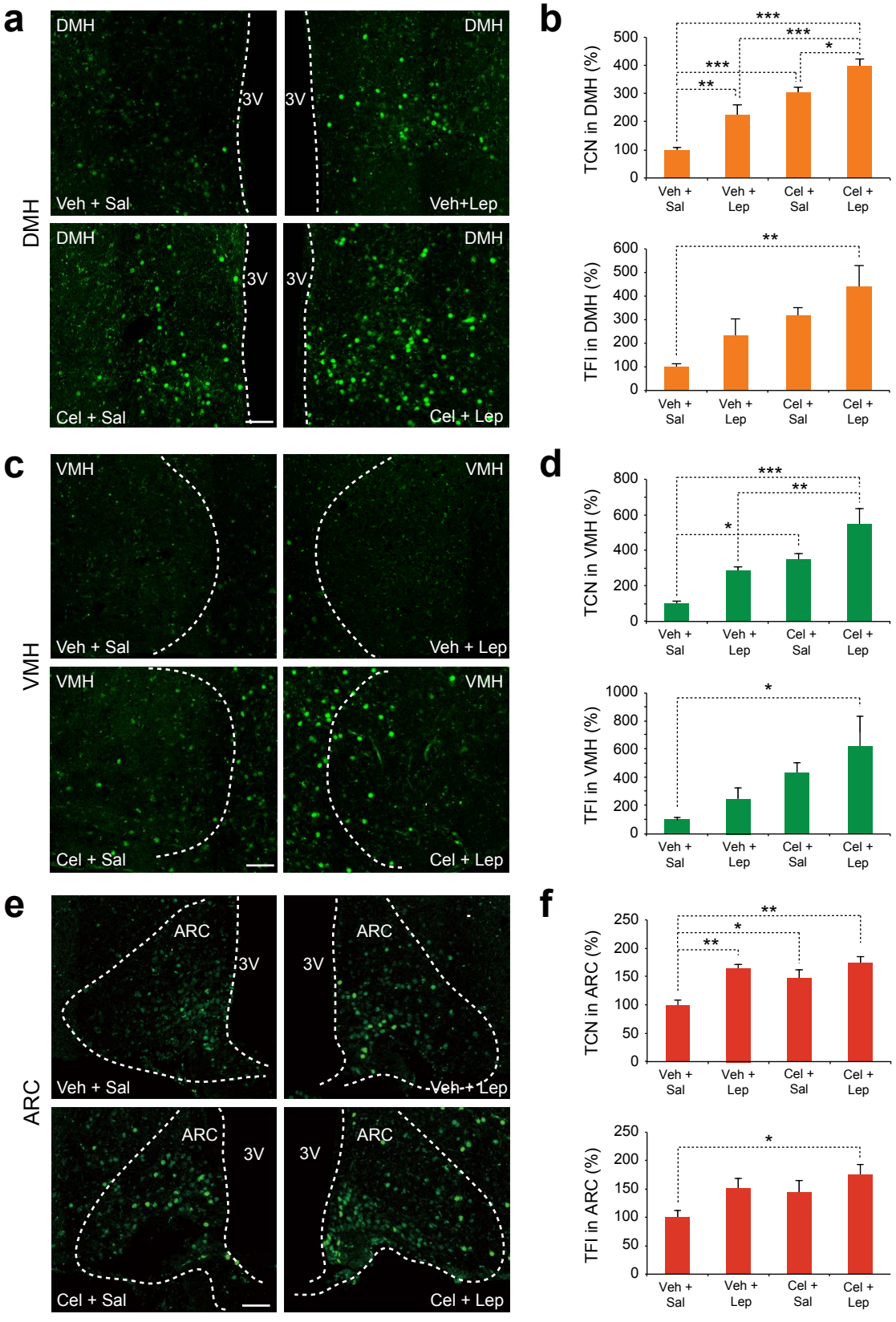
Supplementary Figure 2. Withaferin A reduces body weight and food intake of diet-induced obese (DIO) and heavier mice on chow diet. (**a**,**b**) DIO mice received DMSO or withaferin A (1.25 mg/kg) daily. Daily food intake from the average of 1 week food intake during (**a**) the first week and (**b**) the third week of treatment. Bar graphs represent the average of four independent experiments. (**c**–**e**) Heavier mice on chow diet (with 31 g of average body weight) received DMSO or withaferin A (2 mg/kg) daily. (**c**) Body weight and (**d**) percent change of body weight during 3-week treatment (n = 10, vehicle; n = 9, withaferin A). (**e**) Daily food intake from the average of 3-d food intake during the first week of treatment. Values are averages ± s.e.m. Statistical significance was determined by two-way ANOVA (**c**,**d**) or Student's t test (**a**,**b**,**e**) *** $P < 0.001$.

Supplementary Figure 3



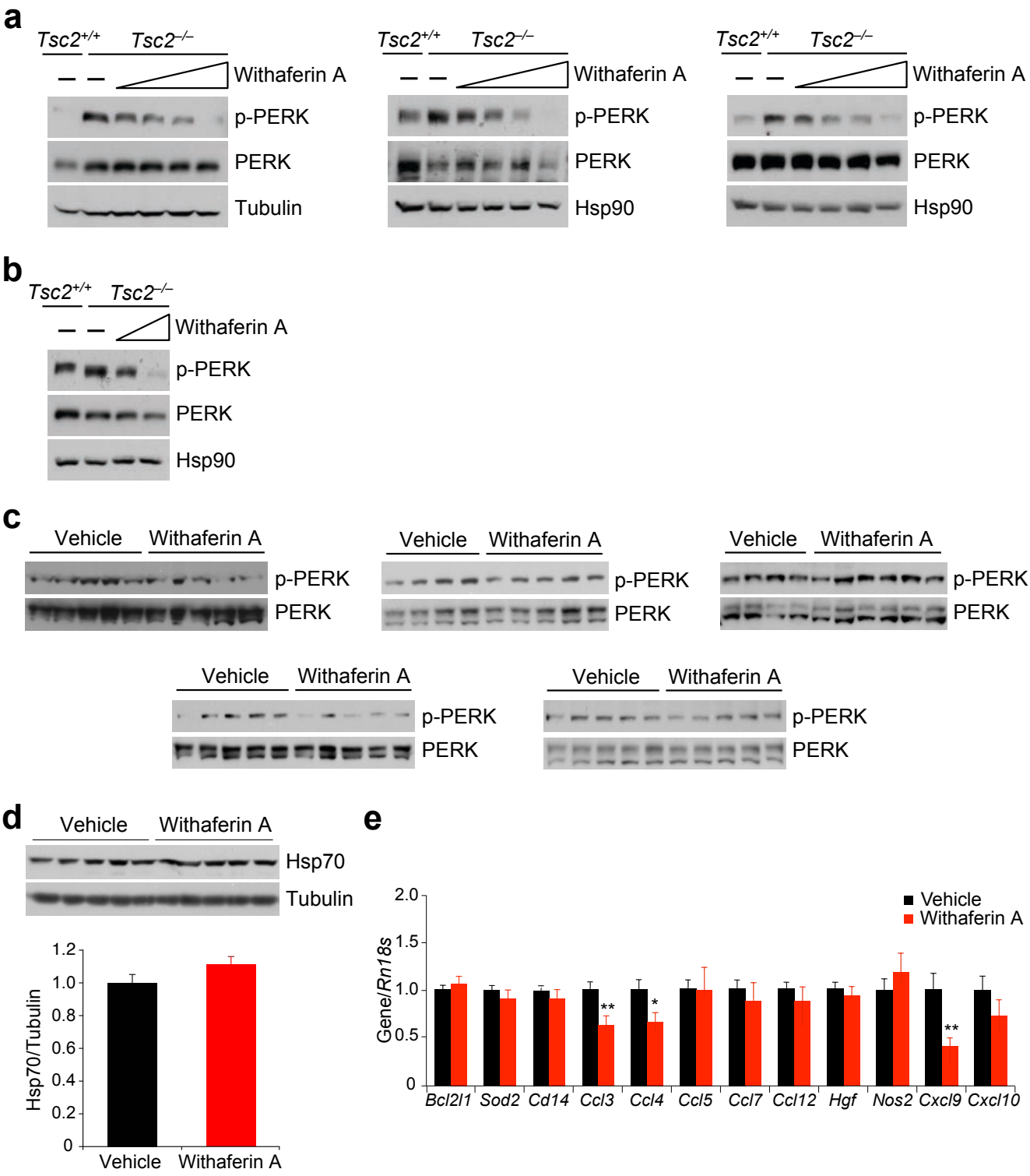
Supplementary Figure 3. Withaferin A potentiates the anorexigenic and weight reducing effect of leptin. (**a**,**b**) DIO mice were treated with vehicle or withaferin A (1.5 mg/kg) for 2 d. After the second treatment, groups were divided into two subgroups and each subgroup received saline or leptin (1 mg/kg). (**a**) Food intake and (**b**) body weight change during 15-h period after saline/leptin injection. Data in **a**,**b** are average of two independent cohorts (n = 10 for Veh + Sal; n = 11 for Veh + Lep, Wit A + Sal and Wit A + Lep). (**c**–**d**) 8-week-old male *ob/ob* mice received vehicle or withaferin A (1 mg/kg) for 5 d. Subsequently, vehicle or withaferin A pre-treated mice were further divided into two subgroups to receive either saline or leptin (0.1 mg/kg) together with vehicle or withaferin A for additional 2 weeks (n = 5 per group). (**c**) Percent weight loss after 2 weeks of leptin treatment. (**d**) Percent of food intake suppression during the second week of leptin treatment. Values are averages ± s.e.m. P values are determined by one-way ANOVA with Bonferroni *post hoc* test (**a**–**c**) or Student's t test (**d**). See Online Methods for the details of statistical analysis. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplementary Figure 4



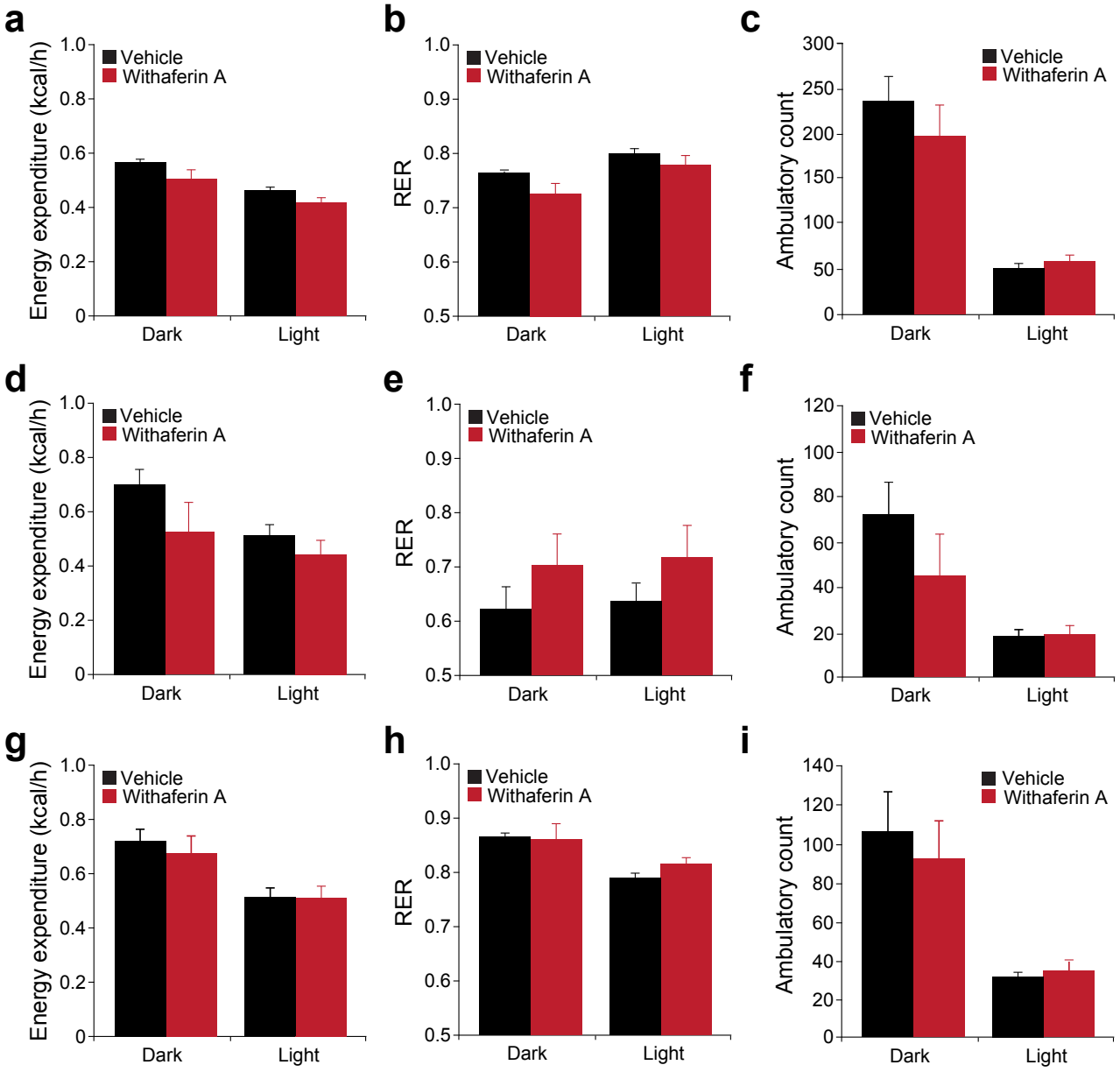
Supplementary Figure 4. Celastrol increases leptin sensitivity in the hypothalamus of DIO mice. (**a**–**f**) DIO mice were administered with vehicle or celastrol for 4 d (100 μg/kg for first 3 d and 200 μg/kg on day 4). Each group of mice subsequently received a single dose of saline or leptin (1 mg/kg). The hypothalamic samples were analyzed by immunohistofluorescence staining using p-STAT3^{Tyr705} specific antibodies. The representative image of p-STAT3^{Tyr705} positive cells in the (**a**) dorsomedial hypothalamus (DMH), (**c**) ventromedial hypothalamus (VMH) and (**e**) arcuate nucleus (ARC) (total numbers of analyzed images are provided in Online Methods). The quantified results of total p-STAT3^{Tyr705} positive cell numbers (TCN, top panel) and fluorescence intensities (TFI, bottom panel) in (**b**) DMH, (**d**) VMH and (**f**) ARC. Bar graphs represent average of two independent experiments (total n = 7 mice per group). Values are averages ± s.e.m. P values are determined by one-way ANOVA with Tukey *post hoc* test (**b**,**d**,**f**). See Online Methods for the details of statistical analysis. * P < 0.05, ** P < 0.01, *** P < 0.001. Scale bars, 100 μm.

Supplementary Figure 5



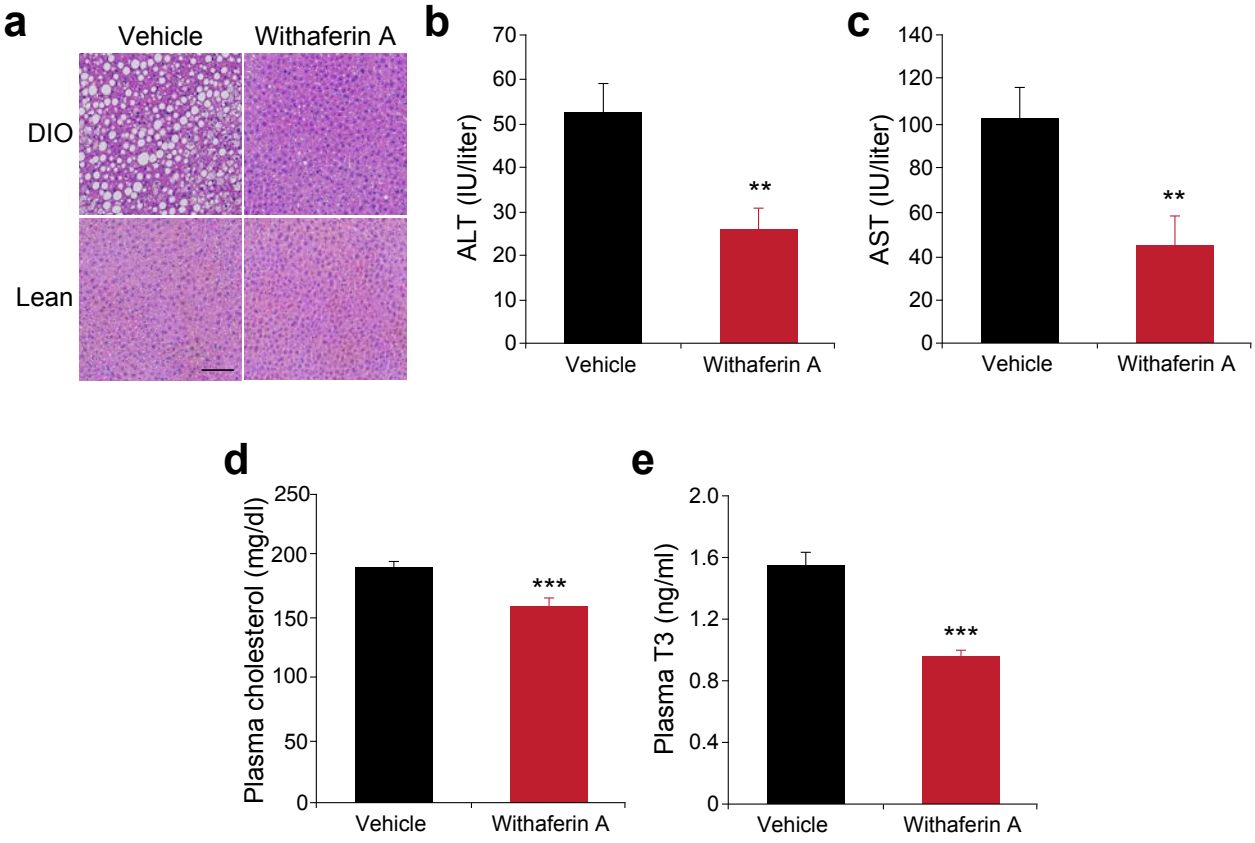
Supplementary Figure 5. Withaferin A administration decreases endoplasmic reticulum stress in MEFs and in the hypothalamus of DIO mice. (**a**) Phospho-PERK^{Thr980} (p-PERK), total PERK, tubulin and Hsp90 immunoblotting from *TSC2*$^{-/-}$ MEFs treated with increasing doses of withaferin A (0.5, 1, 2 and 4 μM) or vehicle for 16 h. As a control, *TSC2*$^{+/+}$ MEFs were treated with vehicle. (**b**) p-PERK, total PERK and Hsp90 immunoblotting from *TSC2*$^{-/-}$ MEFs treated with increasing doses of withaferin A (0.5 and 1 μM) or vehicle for 24 h. (**c**–**e**) DIO mice received vehicle or withaferin A (2 mg/kg) for 3 d (see online method for the detailed procedure). (**c**) p-PERK and total PERK immunoblotting from the hypothalamic lysates. The quantified ratio between phospho- and total PERK from blots in **c** was analyzed together in two-way ANOVA and the result is depicted in **Fig. 5b**. (**d**) Hsp70 and tubulin protein levels in western blot (top) and the quantified ratio of signal intensities of Hsp70 to tubulin (bottom). The experiments in **d** were repeated in five independent cohorts (total n = 25 for vehicle and n = 26 for withaferin A). (**e**) NF-κB target gene expression analyzed by real-time PCR. Bar graphs represent the average of four independent experiments (total n = 21 per group). Values are averages ± s.e.m. Statistical significance was determined by Student's t test (**d**,**e**). * $P < 0.05$, ** $P < 0.01$.

Supplementary Figure 6



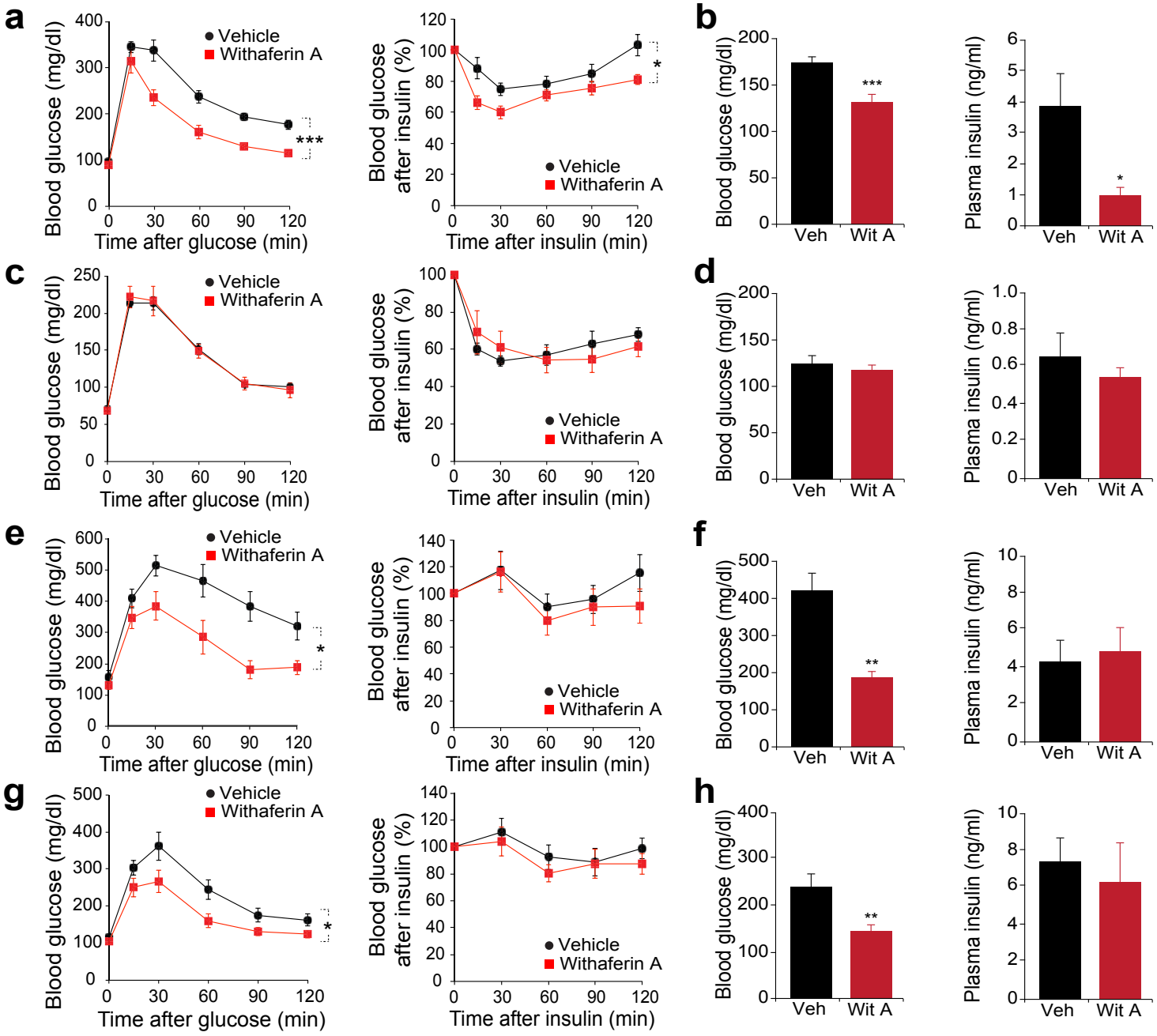
Supplementary Figure 6. There is no difference in metabolic parameters of mice after chronic withaferin A treatment. (**a**–**c**) DIO (total *n* = 15 for vehicle and total *n* = 13 for withaferin A from two independent cohorts), (**d**–**e**) *ob/ob* (*n* = 8 for vehicle, *n* = 5 for withaferin A) and (**g**–**i**) *db/db* mice (*n* = 4 per group) received vehicle or withaferin A (2 mg/kg) for 18 d. Following 18-d treatment, mice were placed into metabolic chambers and treated continuously with either vehicle or withaferin A (2 mg/kg) for additional 3 d (see Online Methods for the detailed procedure). Energy expenditure (kcal/h) of (**a**) DIO, (**d**) *ob/ob* and (**g**) *db/db* mice. Respiratory exchange ratios (RER) (VCO_2/VO_2) of (**b**) DIO, (**e**) *ob/ob* and (**h**) *db/db* mice. Ambulatory physical activity of (**c**) DIO, (**f**) *ob/ob* and (**i**) *db/db* mice. Bar graphs represent 24 h data (single dark and light cycles) collected after 24 h (**a**–**c**), 72 h (**g**–**i**) or 12 h (**j**–**l**) in metabolic chambers. Results in **a**–**c** were the average of two independent cohorts. Values are averages ± s.e.m. *P* values are determined by Student's *t* test.

Supplementary Figure 7



Supplementary Figure 7. Withaferin A reverses HFD-induced metabolic abnormalities. (**a**–**e**) DIO and lean mice received either vehicle or withaferin A (1.25 mg/kg for **a**–**d**, 2 mg/kg for **e**, 3 weeks). (**a**) Representative hematoxylin and eosin (H&E) staining of liver sections of DIO and lean mice at the end of the treatment (two images per mouse; *n* = 9, lean vehicle; *n* = 10, lean withaferin A; *n* = 9, DIO vehicle; *n* = 7, DIO withaferin A). (**b**) Plasma alanine transaminase (ALT) (*n* = 10 per group), (**c**) aspartate transaminase (AST) (*n* = 10 per group) (**d**) total plasma cholesterol (*n* = 38 for vehicle, *n* = 35 for withaferin A) and (**e**) triiodothyronine (T3) concentrations (*n* = 7 per group) of DIO mice after 3 week of treatment. The results in **a**–**c** were repeated with three (**a**) or four (**b**,**c**) independent cohorts. The result in **d** is the average of four independent experiments. Values are averages ± s.e.m. *P* values are determined by Student's *t* test (**b**–**e**). ** $P < 0.01$, *** $P < 0.001$. Scale bar, 100 μm.

Supplementary Figure 8



Supplementary Figure 8. Withaferin A improves glucose homeostasis of obese mice. (**a**–**h**) DIO, lean**,** *db/db* and *ob/ob* mice received vehicle or withaferin A (1.25 mg/kg) for 3 weeks. (**a**,**c**,**e**,**g**, left panels) Glucose tolerance test of (**a**) DIO (n = 9 per group), (**c**) lean (n = 7, vehicle; n = 5, withaferin A), (**e**) *db/db* (n = 10, vehicle; n = 8, withaferin A) and (**g**) *ob/ob* (n = 9 per group) mice after 1 week (**a**) or 2 weeks (**c**,**e**,**g**) of treatment. (**a**,**c**,**e**,**g**, right panels) Insulin tolerance test of (**a**) DIO (n = 10 per group), (**c**) lean (n = 9 per group), (**e**) *db/db* (n = 10, vehicle; n = 8, withaferin A) and (**g**) *ob/ob* (n = 10, vehicle; n = 7, withaferin A) mice after 3-week treatment. (**b**,**d**,**f**,**h**, left panels) 6-h fasting blood glucose concentrations of (**b**) DIO (n = 9 per group), (**d**) lean (n = 9, vehicle; n = 10, withaferin A), (**f**) *db/db* (n = 9, vehicle; n = 7, withaferin A) and (**h**) *ob/ob* (n = 9 per group) mice after 3-week treatment. (**b**,**d**,**f**,**h**, right panels) Plasma insulin concentrations of (**b**) DIO (n = 9, vehicle; n = 6, withaferin A), (**d**) lean (n = 9, vehicle; n = 10, withaferin A), (**f**) *db/db* (n = 10, vehicle; n = 7, withaferin A) and (**h**) *ob/ob* (n = 10, vehicle; n = 7, withaferin A) mice after 3-week treatment. Values are averages ± s.e.m. P values are determined by two-way ANOVA (**a**,**c**,**e**,**g**) or Student's t test (**b**,**d**,**f**,**h**). * P < 0.05, ** P < 0.01, *** P < 0.001.