Supplemental Information

Supplementary Figures

Supplementary Figure 1

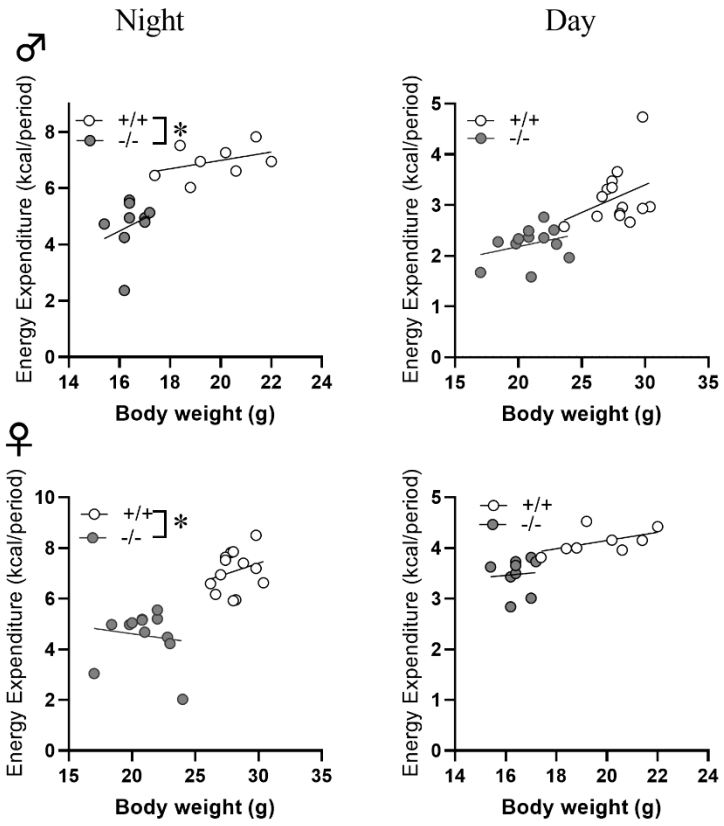

Figure S1: Analysis of covariance of energy expenditure in active and inactive phase, Related to Figure 2

Analysis of covariance (ANCOVA) of energy expenditure measured during day and night period (12h Light: 12h Dark) in $Ptprd^{+/+}$ and $Ptprd^{-/-}$ male and female mice housed in Promethion metabolic chambers for 3 days under ad libitum normal chow feeding condition. For statistical significance, alpha (α) was set at 0.05.

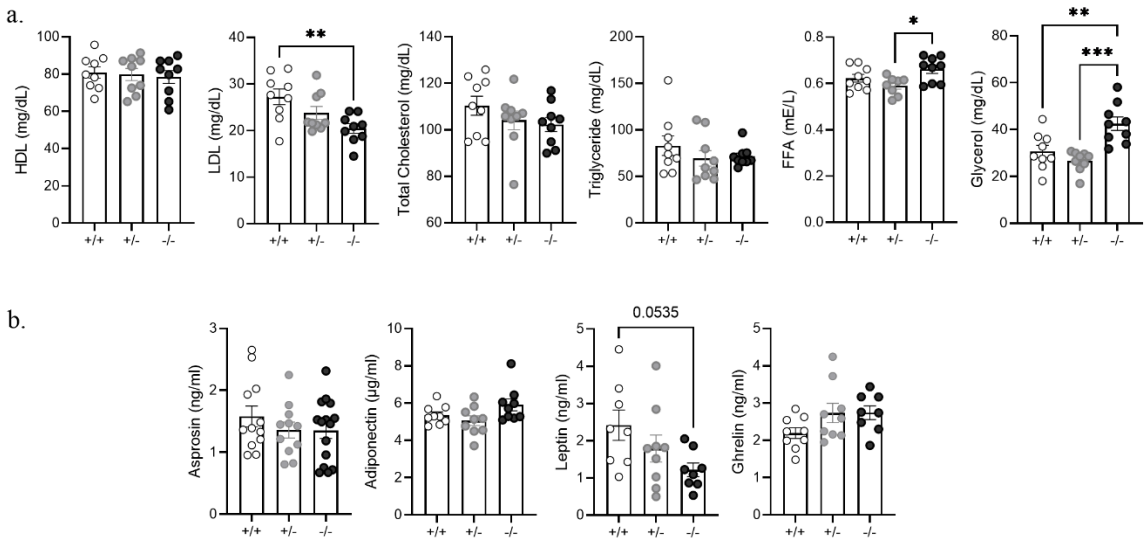

Figure S2: Plasma lipid and metabolic hormone profile associated with *Ptprd* ablation, Related to Figure 2.

(a) Plasma levels of low-density lipoproteins (LDL), high-density lipoprotein (HDL), total cholesterol (TC), triglycerides (TG), free fatty acids (FFA), and glycerol measured in plasma of 14-week-old ad libitum fed $Ptprd^{+/+}$, $Ptprd^{+/-}$, $Ptprd^{-/-}$ male mice (n = 9/group).

(b) Plasma levels of key metabolic hormones, asprosin, adiponectin, ghrelin, and leptin, measured in plasma of 14-week-old ad libitum fed $Ptprd^{+/+}$, $Ptprd^{+/-}$, $Ptprd^{-/-}$ male mice (n = 9/group except plasma asprosin measured in n = 12 $Ptprd^{+/+}$, n = 12 $Ptprd^{+/-}$ and n = 15 $Ptprd^{-/-}$ mice).

Asterisk (*) indicate the range of alpha; *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001; by two-tailed T-test. Data are represented as mean ± SEM.

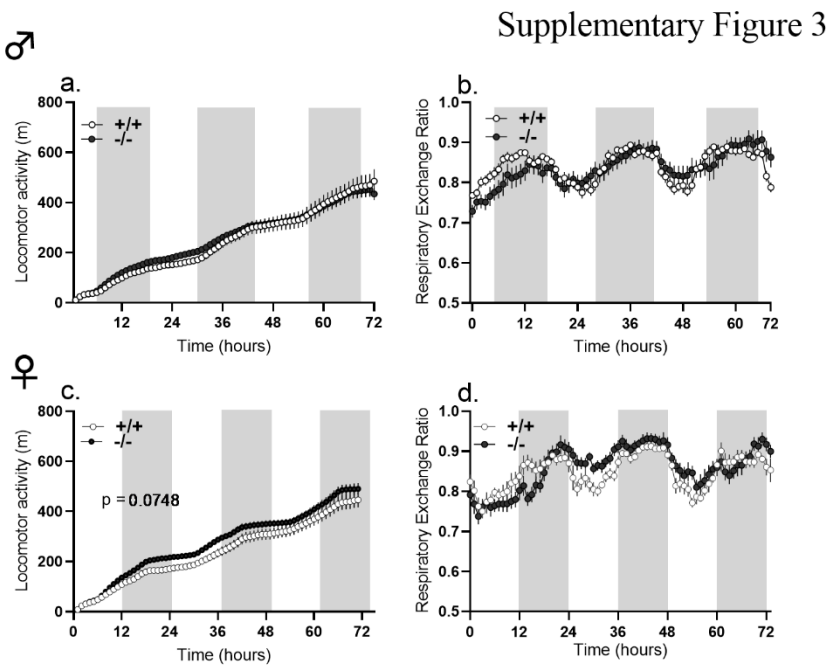

Figure S3: Locomotor activity and respiratory exchange ratio of Ptprd-null mice, Related to Figure 2

Cumulative locomotor activity (a,c) and hourly respiratory exchange ratio (R.E.R.; b,d) measured in 12-week-old male and 10-week-old female *Ptprd*$^{+/+}$ and *Ptprd*$^{-/-}$ mice over 3 days in Promethion metabolic chambers under ad libitum normal chow feeding condition.

For statistical significance, alpha (α) was set at 0.05 with repeated measures Two-way ANOVA. Data are represented as mean ± SEM.

Supplementary Figure 4

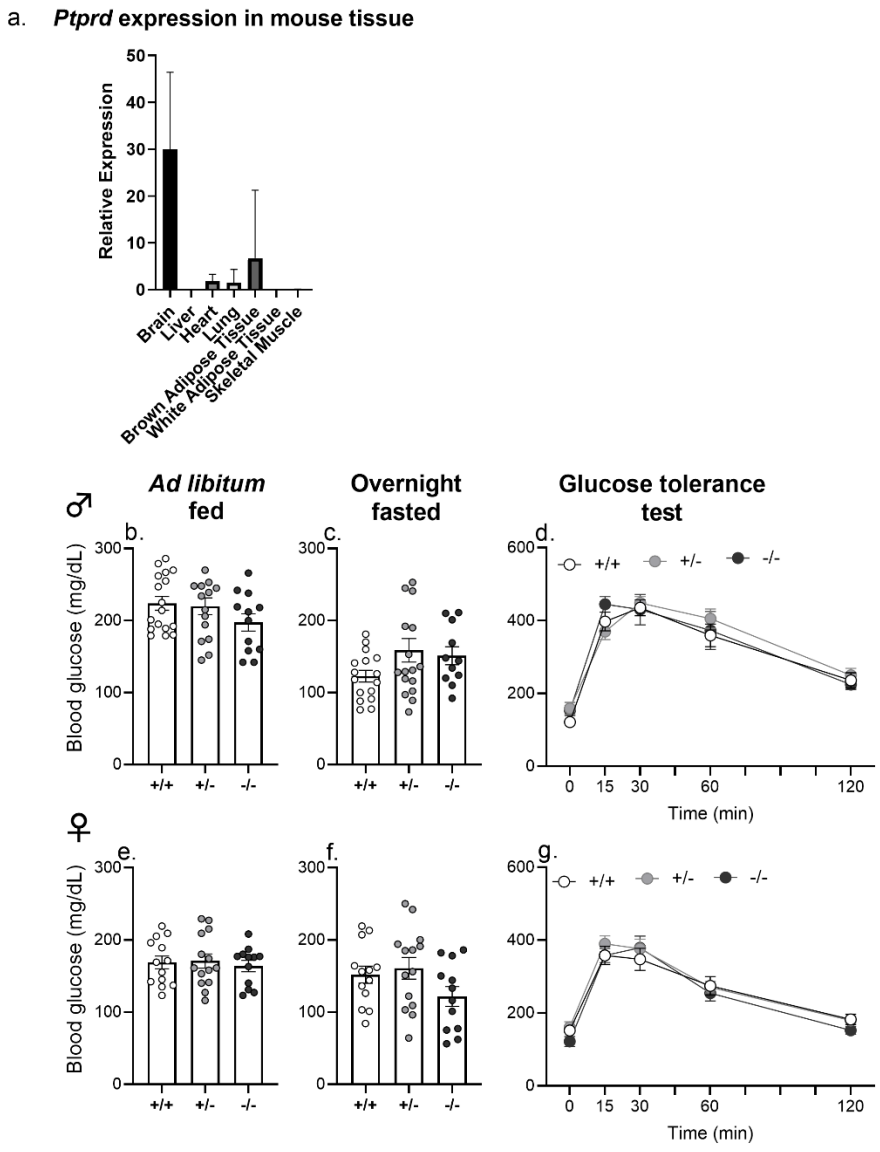

Figure S4: *Ptprd* is dispensable for the glucogenic function of Asprosin, Related to Figure 2

(a) qPCR of *Ptprd* expression in various tissues of wild type male mice (n = 5).

(b-c) Blood glucose levels of *Ad libitum* fed and 18-hour-fasted 12-week-old male mice (n = 17 $Ptprd^{+/+}$, 16 $Ptprd^{+/-}$ and 12 $Ptprd^{-/-}$).

(d) Glucose measured at 0, 15, 30, 60 and 120 min post intraperitoneal (IP) injection of 2mg/kg glucose solution in 18-hour-fasted 12-week-old male mice (n = 15 *Ptprd*$^{+/+}$, 16 *Ptprd*$^{+/-}$ and 10 *Ptprd*$^{-/-}$).

(e,f) Blood glucose levels of *Ad libitum* fed and 24-hour-fasted 10-week-old female mice (n = 13 *Ptprd*$^{+/+}$, 14 *Ptprd*$^{+/-}$ and 12 *Ptprd*$^{-/-}$).

(g) Glucose measured at 0, 15, 30, 60 and 120 min post intraperitoneal injection of 2mg/kg glucose solution in 24-hour-fasted female mice (n = 13*Ptprd*$^{+/+}$, 14 *Ptprd*$^{+/-}$and 12 *Ptprd*$^{-/-}$).

One-way (b,c,e,f) and two-way ANOVA (d,g) tested statistical significance with alpha (α) set at 0.05. Data are represented as mean ± SEM.

Supplementary Figure 5

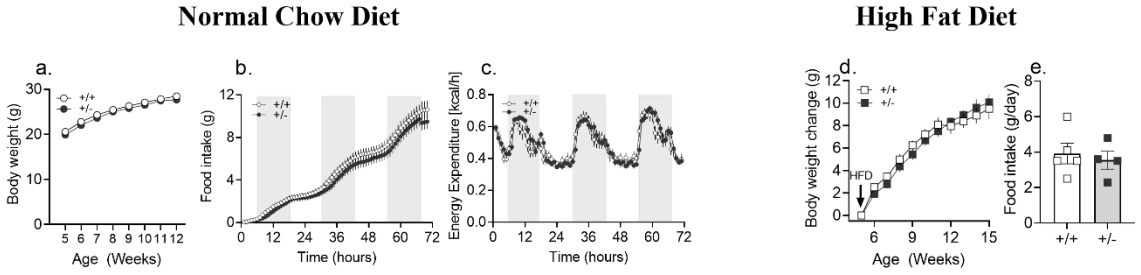

Figure S5: Whole body loss of a single allele is not protective against diet induced obesity in males, Related to Figure 3

(a) Weekly body weight of wildtype (*Ptprd*$^{+/+}$; n = 24) and heterozygous (*Ptprd*$^{+/-}$; n = 25) male mice maintained on *ad libitum* normal chow (NC) diet.

(b,c) Cumulative food intake and hourly energy expenditure of 12-week-old *Ptprd*$^{+/+}$ and *Ptprd*$^{+/-}$ mice on NC over 3 days using the Promethion system (food intake: *Ptprd*$^{+/+}$ n = 14; *Ptprd*$^{+/-}$ n = 15; energy expenditure: *Ptprd*$^{+/+}$ n = 13; *Ptprd*$^{+/-}$ n = 13).

(d,e) Weekly body weight change of *Ptprd*$^{+/+}$ and *Ptprd*$^{+/-}$ males maintained on high fat diet (HFD) from 5 weeks of age, with food intake measured in a randomly selected cohort of 16-week-old mice from each group (Body weight change: *Ptprd*$^{+/+}$ n = 9, *Ptprd*$^{+/-}$ n = 7; food intake: *Ptprd*$^{+/+}$ n = 5; *Ptprd*$^{+/-}$ n = 4).

*P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001; by student T-test (e), 'effect of genotype' determined by repeated measures two-way ANOVA (a-d). Circle and square symbol represent mice maintained on NC diet and HFD, respectively. Data are represented as mean ± SEM.

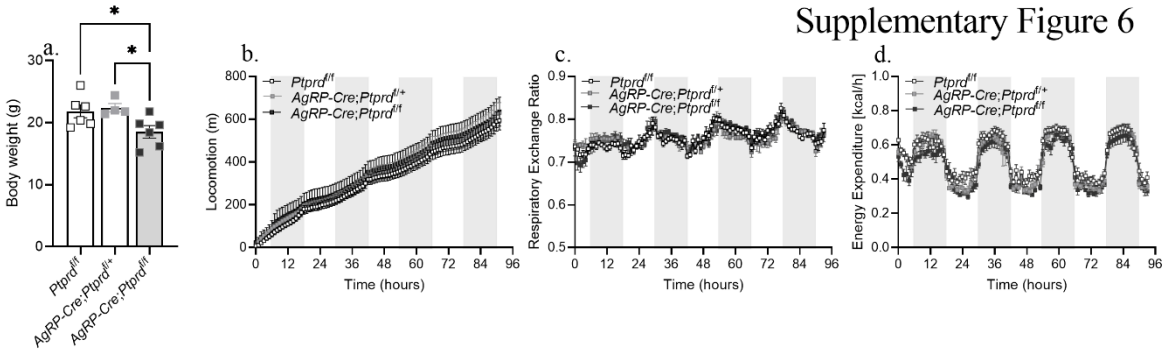

Figure S6: Energy expenditure, locomotor activity and respiratory exchange ratio of AgRP neuron-specific *Ptprd* knock-out female mice on a high fat diet, Related to Figure 3

Body weight (a), cumulative locomotion activity (b), respiratory exchange ratio (c), and hourly energy expenditure of 14-week-old *Ptprd*$^{flox/flox}$ (n = 6), *AgRP-cre*; *Ptprd*$^{flox/+}$ (n = 4) *and AgRP-cre; Ptprd*$^{flox/flox}$ (n = 6) females maintained on high fat diet (HFD) from 5-weeks of age. Change in body weight and food intake presented in Figure 3i,j.

Asterisk (*) indicate the range of alpha; *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001, as determined by one-way ANOVA (a) and two-way ANOVA (b-d). Data are represented as mean ± SEM.

Supplementary Figure 7

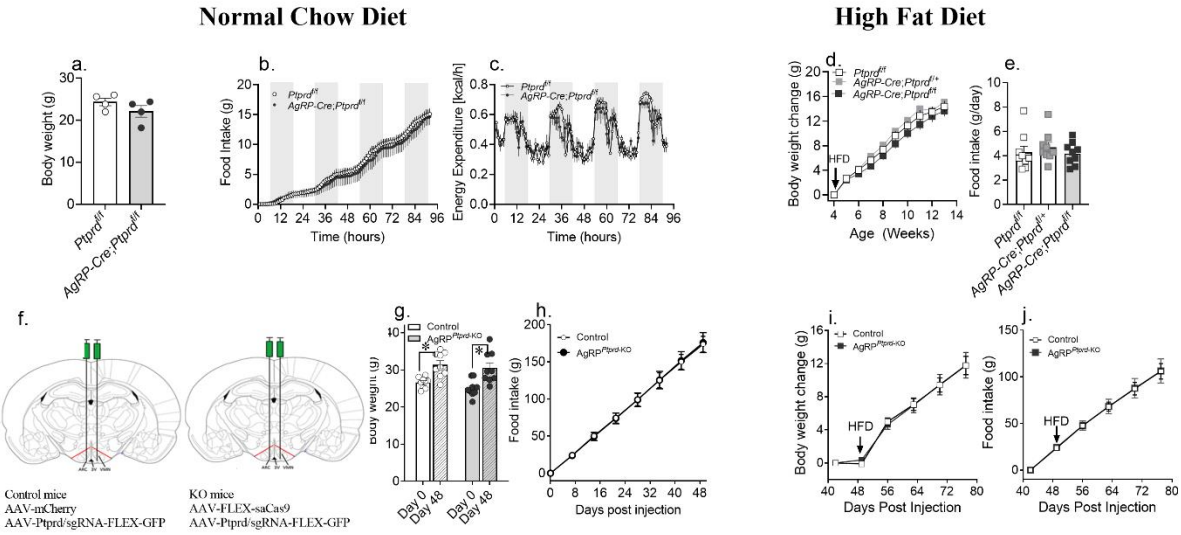

Figure S7: AgRP neuron-specific loss of *Ptprd* is not protective against diet induced obesity in males, Related to Figure 3

(a) Body weight of 8-week-old *Ptprd*$^{flox/flox}$ and *AgRP-cre; Ptprd*$^{flox/flox}$ males maintained on *ad libitum* NC diet (n = 4/group)

(b,c) Cumulative food intake and hourly energy expenditure of 8-week-old *Ptprd*$^{flox/flox}$ and *AgRP-cre; Ptprd*$^{flox/flox}$ male mice maintained on *ad libitum* NC over 4 days using the Promethion system (n = 4/group).

(d,e) Weekly body weight change of *Ptprd*$^{flox/flox}$ (n = 16), *AgRP-cre*; *Ptprd*$^{flox/+}$ (n = 9) *and AgRP-cre; Ptprd*$^{flox/flox}$ (n = 11) males maintained on high fat diet (HFD) from 5 weeks of age, with food intake measured in a randomly selected cohort of 14-week-old mice from each group (n = 9/group).

(f) Schematic showing bilateral stereotactic injection of virus (AAV) containing *Ptprd* sgRNA with AAV expressing mCherry (control) or Cas9 (AgRP$^{Ptprd\text{-KO}}$) in the arcuate (ARC) nucleus of adult AgRP-cre male mice.

(g) Body weight of control and AgRP$^{Ptprd\text{-KO}}$ male mice maintained on NC diet from day 0 to day 48 post stereotactic injection (control: n = 9 and AgRP$^{Ptprd\text{-KO}}$: n = 9).

(h) Cumulative food intake measured every week of control and AgRP$^{Ptprd\text{-KO}}$ male mice maintained on NC diet (control: n = 9 and AgRP$^{Ptprd\text{-KO}}$: n = 9).

(i,j) Body weight change and cumulative food intake of control and AgRP$^{Ptprd\text{-KO}}$ male mice subjected to HFD on day 48 post stereotactic injection of AAVs coding cas9 and *Ptprd* sgRNA (control: n = 9 and AgRP$^{Ptprd\text{-KO}}$: n = 9).

*P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001; by student T-test (e,e,g), 'effect of genotype' determined by repeated measures two-way ANOVA (b-d,h-j). Circle and square symbol represent mice maintained on NC diet and HFD, respectively. Data are represented as mean ± SEM.

Supplementary Figure 8

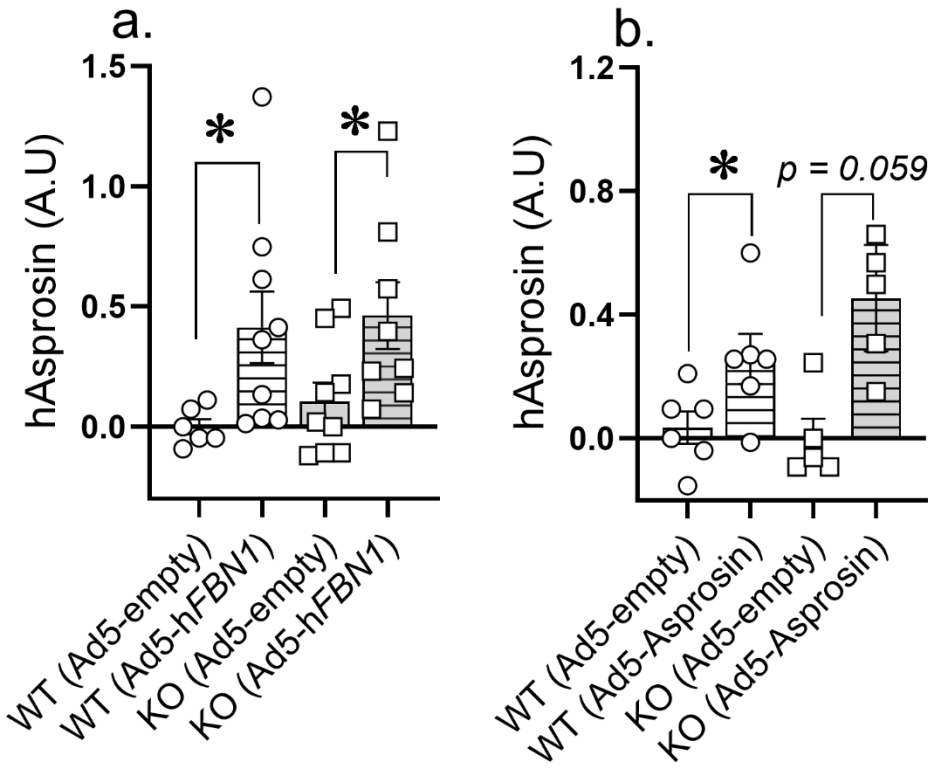

Figure S8: **Adenovirus mediated overexpression of asprosin, Related to Figure 5.**

Human asprosin levels detected in plasma of Ad5-*FBN1*(a) and Ad5-Asprosin (b) injected *Ptprd*$^{+/+}$ and *Ptprd*$^{-/-}$ male mice is plotted relative to the average background signal detected in Ad5-empty injected mice.

Asterisk (*) indicate the range of alpha; *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001, as determined by two-tailed T-test. Data are represented as mean ± SEM.

Supplementary Figure 9

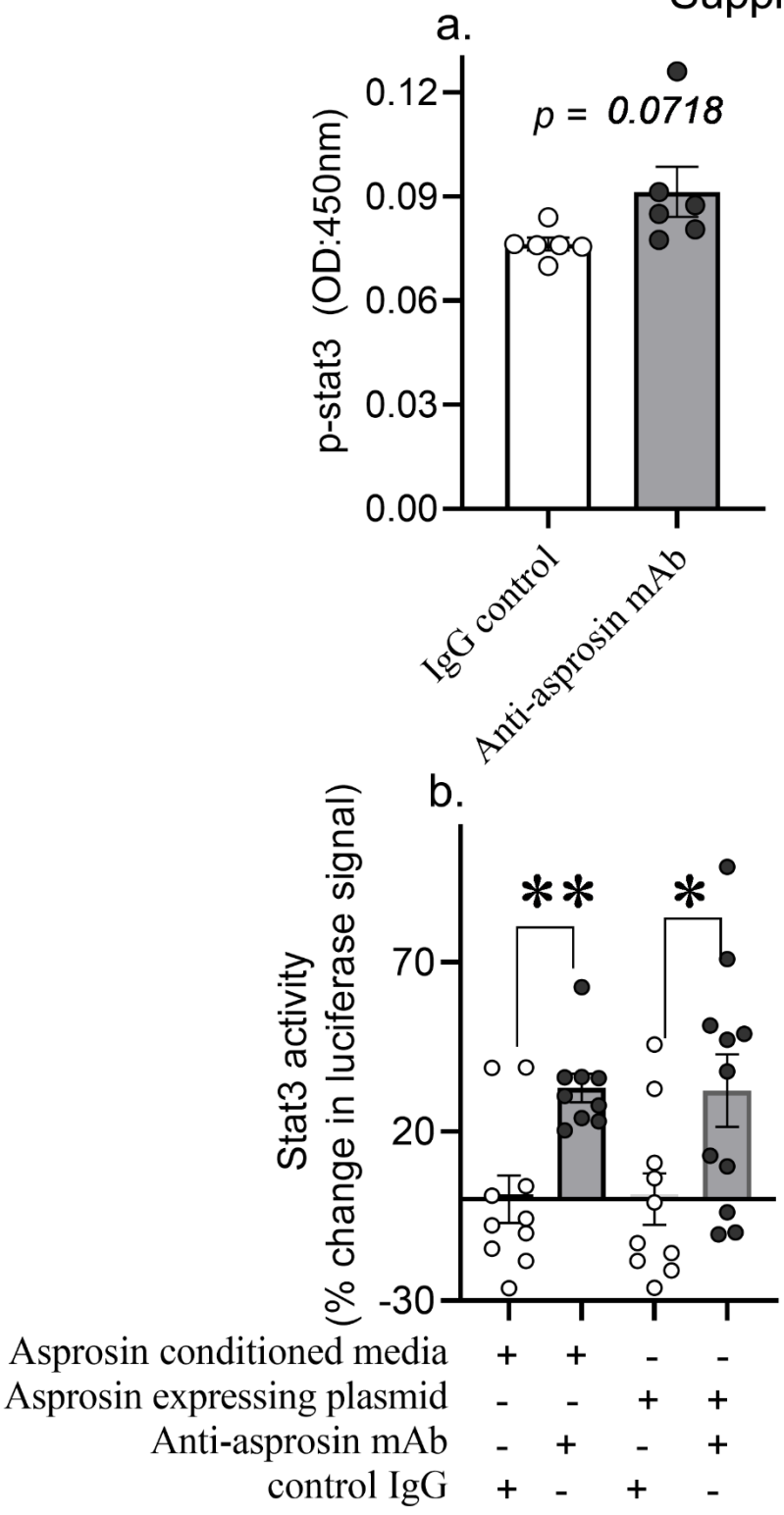

Figure S9: Asprosin neutralization rescues p-Stat3 response in diet-induced obese mice and HEK293T cells, Related to Figure 6

(a) phosphorylated-Stat3 (p-Stat3) levels measured using ELISA in hypothalamic neural lysate diet-induced obese (DIO) male mice, 16 h post intraperitoneal treatment with anti-asprosin monoclonal antibody (mAb) or IgG control (500µg in 500 ml saline/mouse; n = 6/group). Note that mice were without food for the duration of the experiment, demonstrating that the effect on hypothalamic p-stat was independent of the mAb induced changes in food intake and adiposity.

(b) Percent change in Stat3-response element driven luciferase activity measured in pTATA-TK-Luc plasmid transfected HEK293T cells, treated for 18 h with asprosin conditioned media preincubated with anti-asprosin mAb (or IgG control 500 ng/well), or 18 h ectopic treatment of anti-asprosin mAb (IgG control; 500 ng/well) 48 h post co-transfection of Empty or Asprosin expressing mammalian plasmid (n = 10 or 11/group). *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001; by two-tailed t-test. Data are represented as mean ± SEM.

Supplementary Figure 10

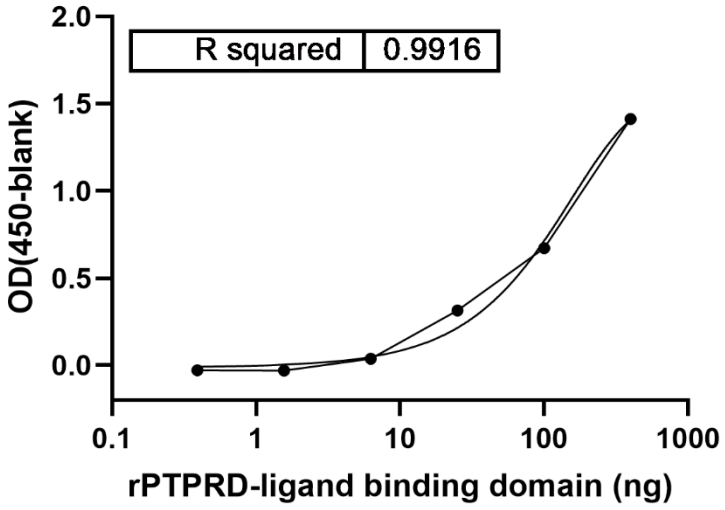

Figure S9 S10: **PTPRD ligand binding domain sandwich ELISA, Related to Figure 7**

A custom-built sandwich ELISA using rabbit anti-PTPRD (100ng/well) as the capture antibody, and mouse anti-his (100ng/well) as the detection antibody. An anti-mouse secondary antibody (1:5000) linked to HRP was used to generate a signal, and recombinant PTPRD was used to generate a standard curve.