HOKKAIDO UNIVERSITY

Title	Glucagon-Like Peptide-1 Is Involved in the Thermic Effects of Dietary Proteins in Male Rodents
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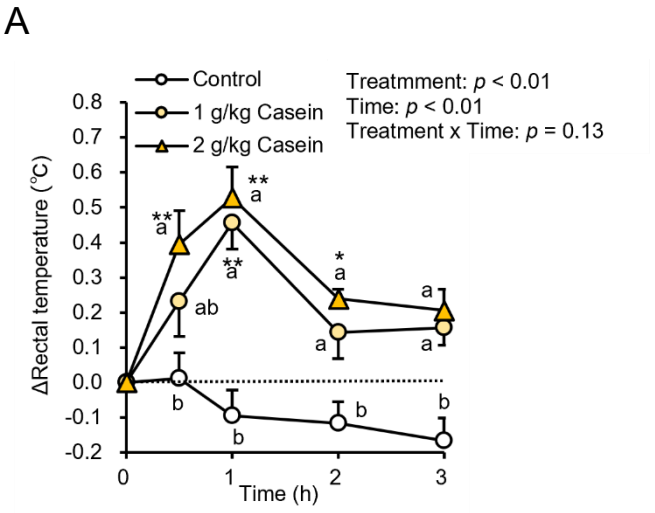
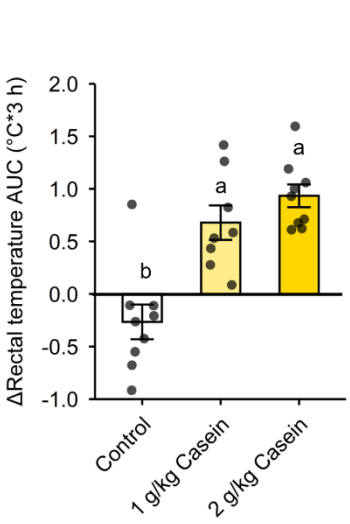
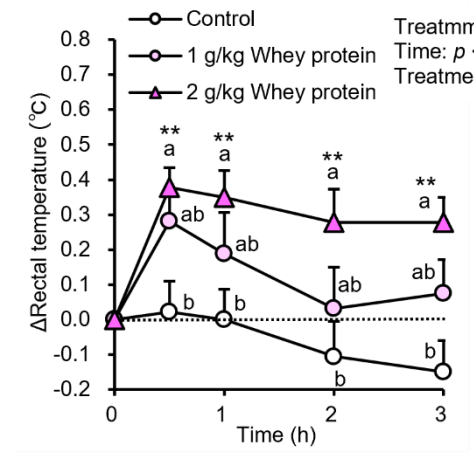
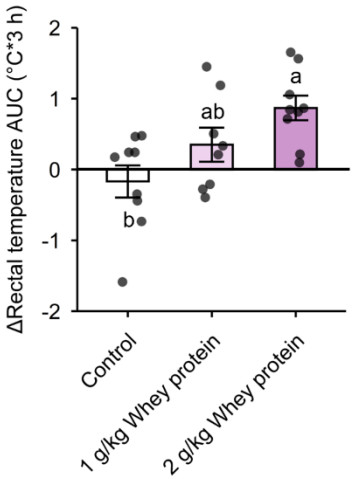
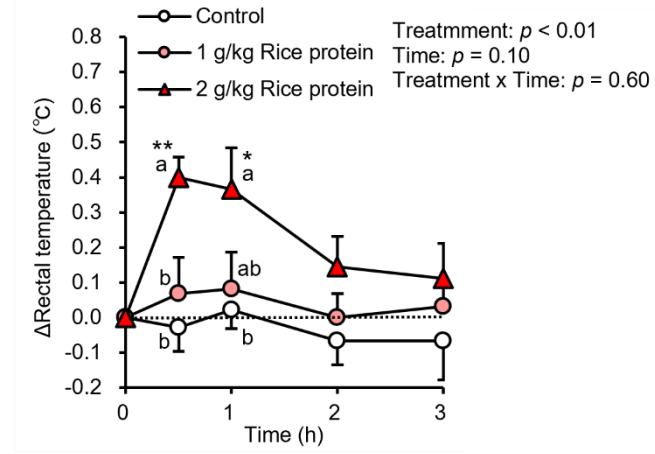
Supplementary Figures

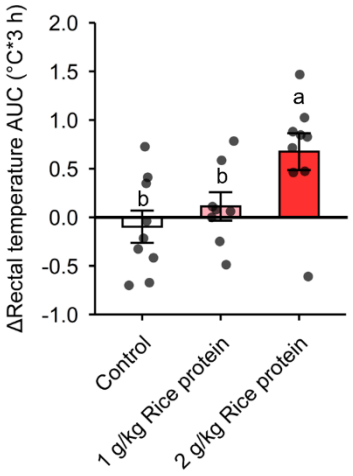


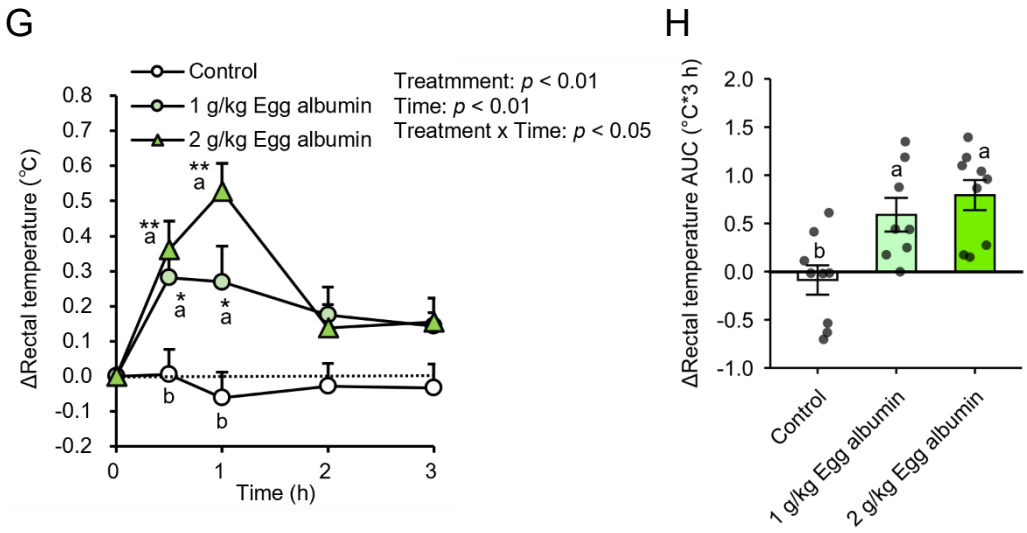




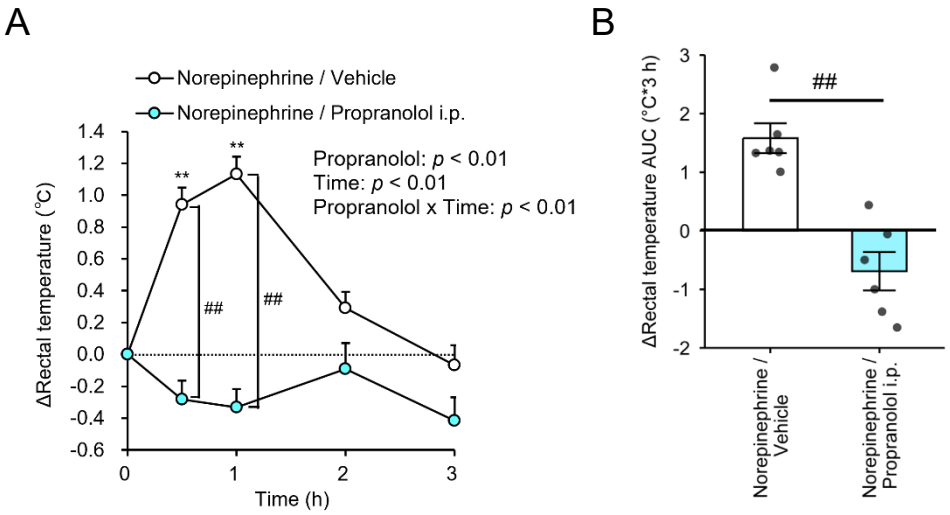




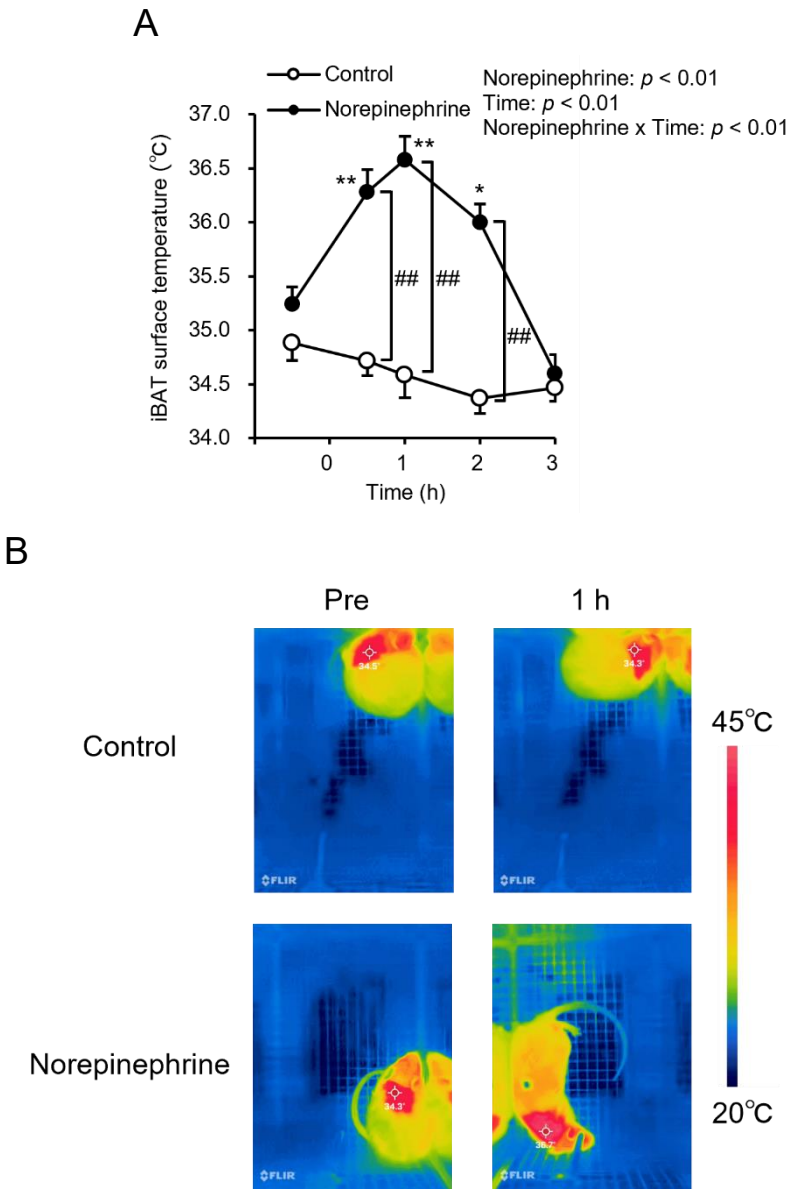




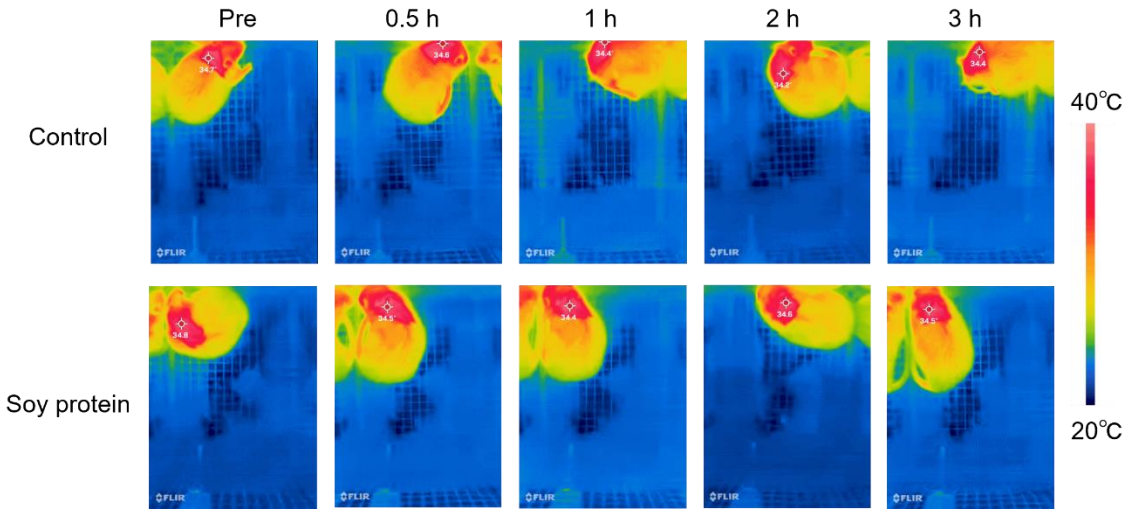
Supplementary Fig. 1. Dose-dependence of various oral proteins on rectal temperature increase. Changes in rectal temperatures after oral administration of different protein doses (1 or 2 g/kg) or water (control, 15 mL/kg) in 4 h-fasted rats and its AUC. (A, B) Casein. (C, D) Whey protein. (E, F) Rice protein. (G, H) Egg albumin. n = 8-9, respectively. Values are expressed as the mean ± SEM. Different letters indicate $p < 0.05$ by Tukey's test. In (A, C, E, G), the p values of the mixed model with unstructured covariance are shown in each panel, and plots with asterisks (*) show significant differences compared with 0 h values within each treatment ($^{*}p < 0.05$ and $^{**}p < 0.01$, Dunnett's test).



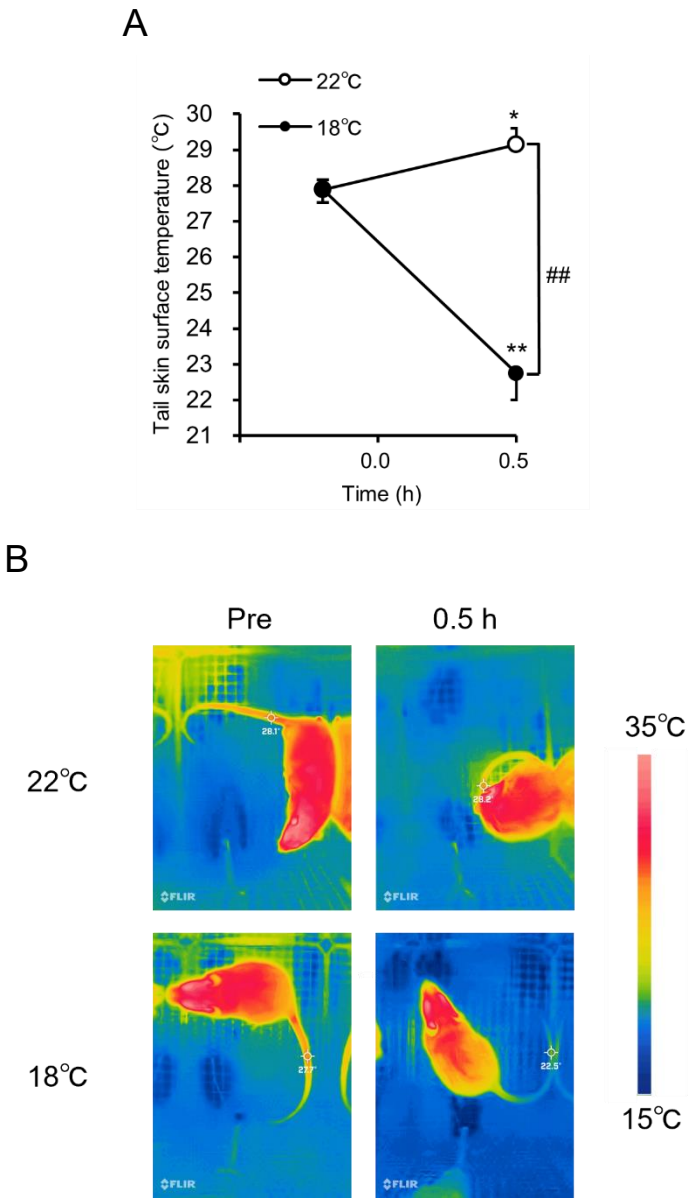
Supplementary Fig. 2. Nonselective β-adrenergic receptor antagonist abolishes norepinephrine-induced increase in rectal temperature. Propranolol (3 mg/kg) or its vehicle (saline) was intraperitoneally injected at a dose of 1 mL/kg immediately after subcutaneous administration (1 mL/kg) of norepinephrine (0.3 mg/kg) in 4 h-fasted rats. (A) Changes in rectal temperatures and (B) its AUC (n = 6). Values are expressed as the mean ± SEM. Plots with asterisks (*) show significant differences compared with baseline values within each treatment ($^{**}p < 0.01$, Dunnett's test), and comparisons between treatments were performed using Student's t-test ($^{\#\#}p < 0.01$). The p values of the mixed model with unstructured covariance are shown in the panel.



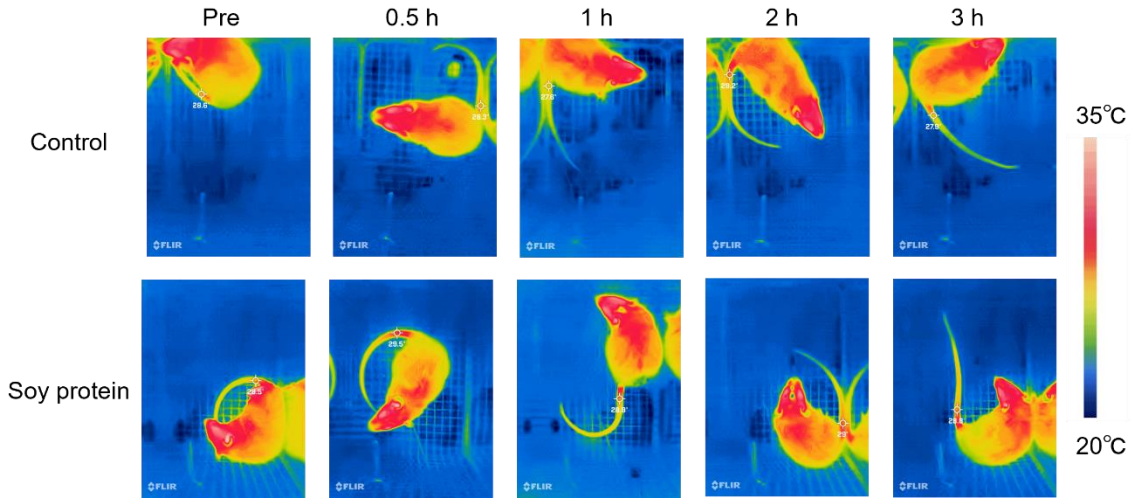
Supplementary Fig. 3. Subcutaneous norepinephrine increases iBAT surface temperature of rats. (A) iBAT surface temperatures before and after subcutaneously injection of saline (control, 1 mL/kg) or 1 mg/kg norepinephrine in 4 h-fasted rats (n = 5-6) and (B) representative infrared pictures from video recordings before and 1 h after the injection (upper row, control; lower row, norepinephrine). Values are expressed as the mean ± SEM. Plots with asterisks (*) show significant differences compared with baseline values within each treatment ($^{*}p < 0.05$ and $^{**}p < 0.01$, Dunnett's test), and comparisons between treatments were performed using Student's t-test ($^{\#\#}p < 0.01$). The p values of the mixed model with unstructured covariance are shown in the panel.



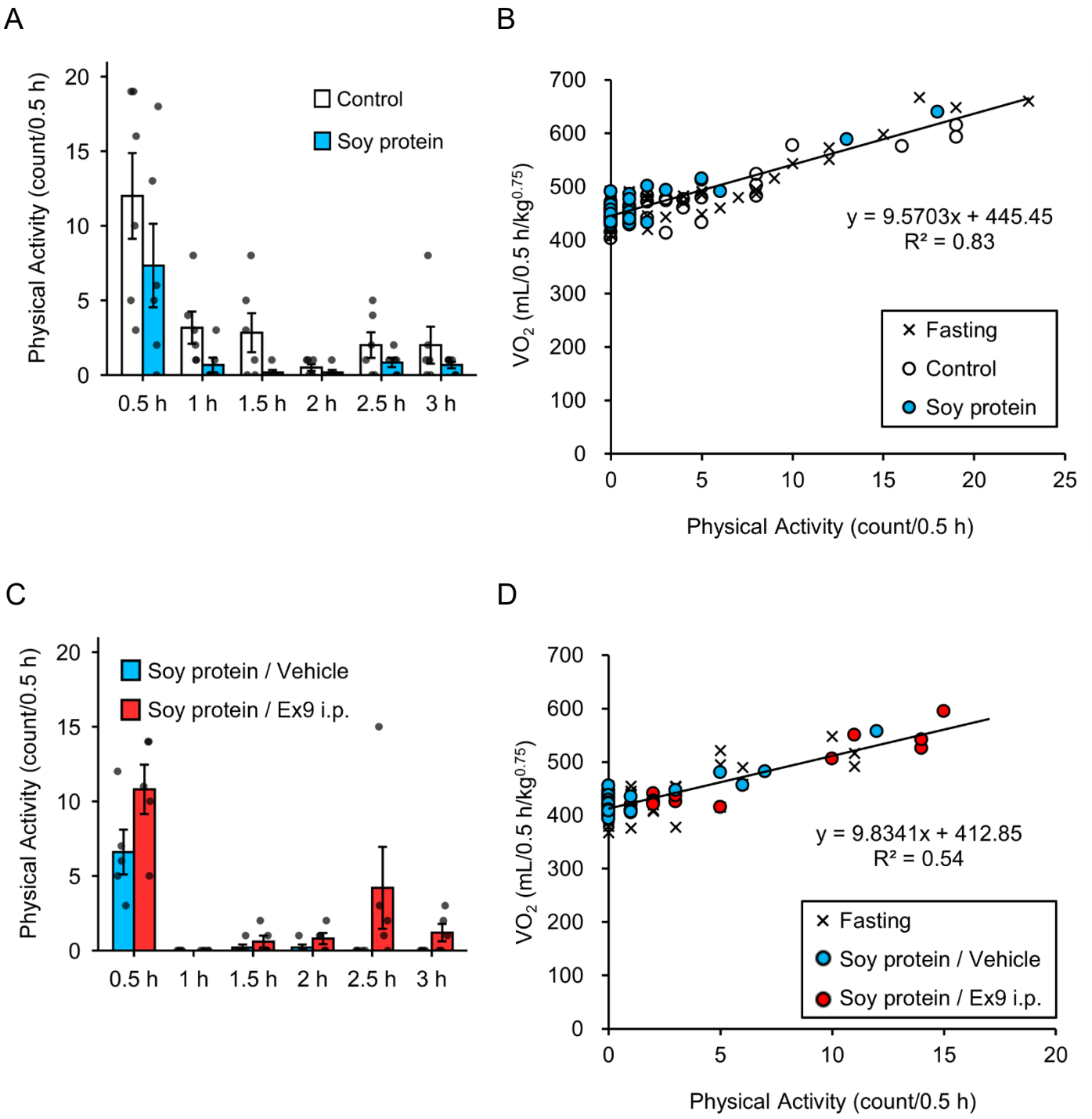
Supplementary Fig. 4. Representative infrared pictures from video recordings, for measurement of iBAT surface temperature, before and after oral administration of water (control, 15 mL/kg) or 2 g/kg soy protein in 4 h-fasted rats (n = 5-6; upper row, control; lower row, soy protein).



Supplementary Fig. 5. Mild cold exposure lowers the tail skin surface temperature of rats. One group of 4 h-fasted rats was moved into a room at 18°C from a room at 22°C, while the other group was placed in a room at 22°C. (A) Tail skin surface temperatures before and 0.5 h after treatments (n = 6) and (B) representative infrared pictures (upper row, 22°C group; lower row, 18°C group). Values are expressed as the mean ± SEM. Plots with asterisks (*) show significant differences compared with baseline values within each treatment ($^{*}p < 0.05$ and $^{**}p < 0.01$, Dunnett's test), and comparisons between treatments were performed using Student's t-test ($^{\#\#}p < 0.01$).

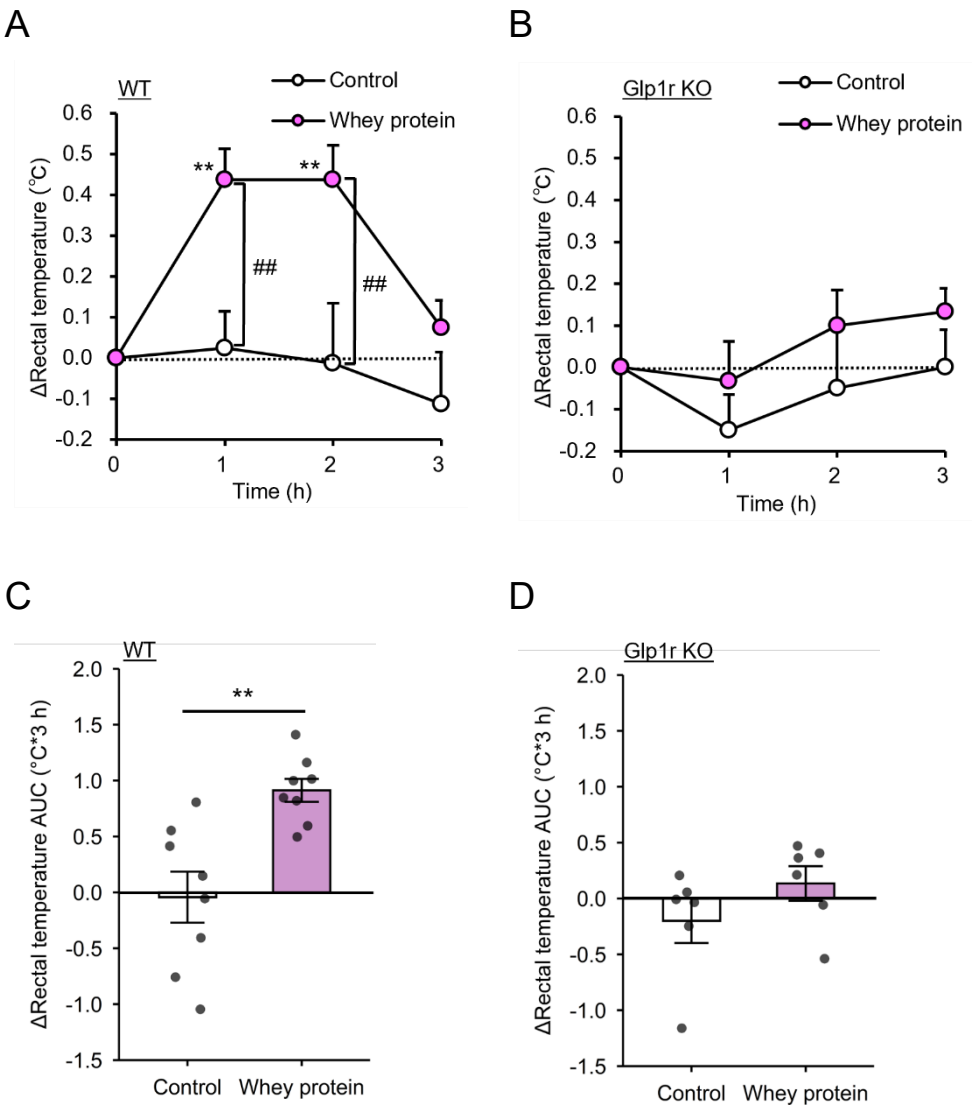


Supplementary Fig. 6. Representative infrared pictures, for measurement of tail skin surface temperature, before and after oral administration of water (control, 15 mL/kg) or 2 g/kg soy protein in 4 h-fasted rats (n = 6; upper row, control; lower row, soy protein).

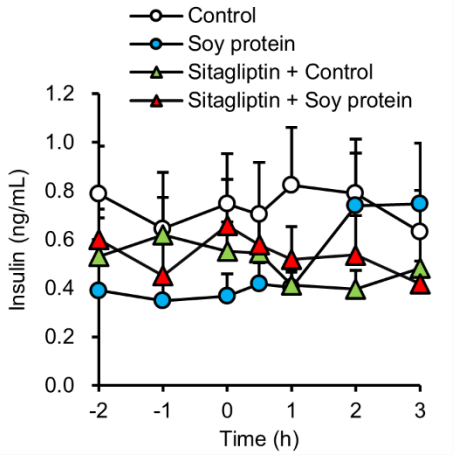


Supplementary Fig. 7. Physical activity and correlation between oxygen consumption (VO_2). (A, B) Soy protein (2 g/kg) or water (control, 15 mL/kg) was orally administered in 4 h-fasted rats. (C, D) Ex9 (30 nmol/kg) or its vehicle (saline) was intraperitoneally injected at a dose of 1 mL/kg immediately after oral administration of soy protein (2 g/kg) in 4 h-fasted rats. (A, C) Physical activity after administration (A, n = 6; C, n = 5). Values are expressed as the mean ±

SEM. Comparisons between treatments were performed by paired *t*-test (crossover design). (B, D) Correlation between physical activity and VO_2 in rats pre- and post-administration. In (B), crosses are the data from fasted (pre-administered) rats, and open and gray circles indicate the data from water-administered or soy protein-administered rats, respectively. In (D), crosses are the data from fasted (pre-administered) rats, and open and gray circles indicate the data from saline-injected or Ex9-injected rats, respectively.



Supplementary Fig. 8. GLP-1 receptor is involved in the whey protein-induced increment of rectal temperature. Changes in rectal temperatures after oral administration of whey protein (2 g/kg) or saline (control, 20 mL/kg) and its AUC in (A, C) WT mice (n = 8) and (B, D) *Glp1r* KO mice (n = 6) fasted 5 h. Values are expressed as the mean ± SEM; those of the control group of WT mice are the same as Fig. 7. In (A, B), plots with asterisks (*) show significant differences compared with 0 h values within each treatment ($^{**}p < 0.01$, Dunnett's test), and comparisons between treatments were performed using Student's t-test ($^{\#\#}p < 0.01$). (C, D) $^{**}p < 0.01$ by Student's t-test.



Supplementary Fig. 9. Oral soy protein and DPP-4 inhibition did not affect insulin levels. Sitagliptin (50 mg/kg) or water (5 mL/kg) was administered orally at 2 h before the oral administration of soy protein (2 g/kg) or water (control, 15 mL/kg) in rats fasted for 2 h. Insulin concentrations in tail vein plasma were measured (n = 6-7). Values are expressed as the mean ± SEM. No significant differences were detected within (vs. 0 h, Dunnett's test) and between treatments (Tukey's test), respectively.