Supporting Information

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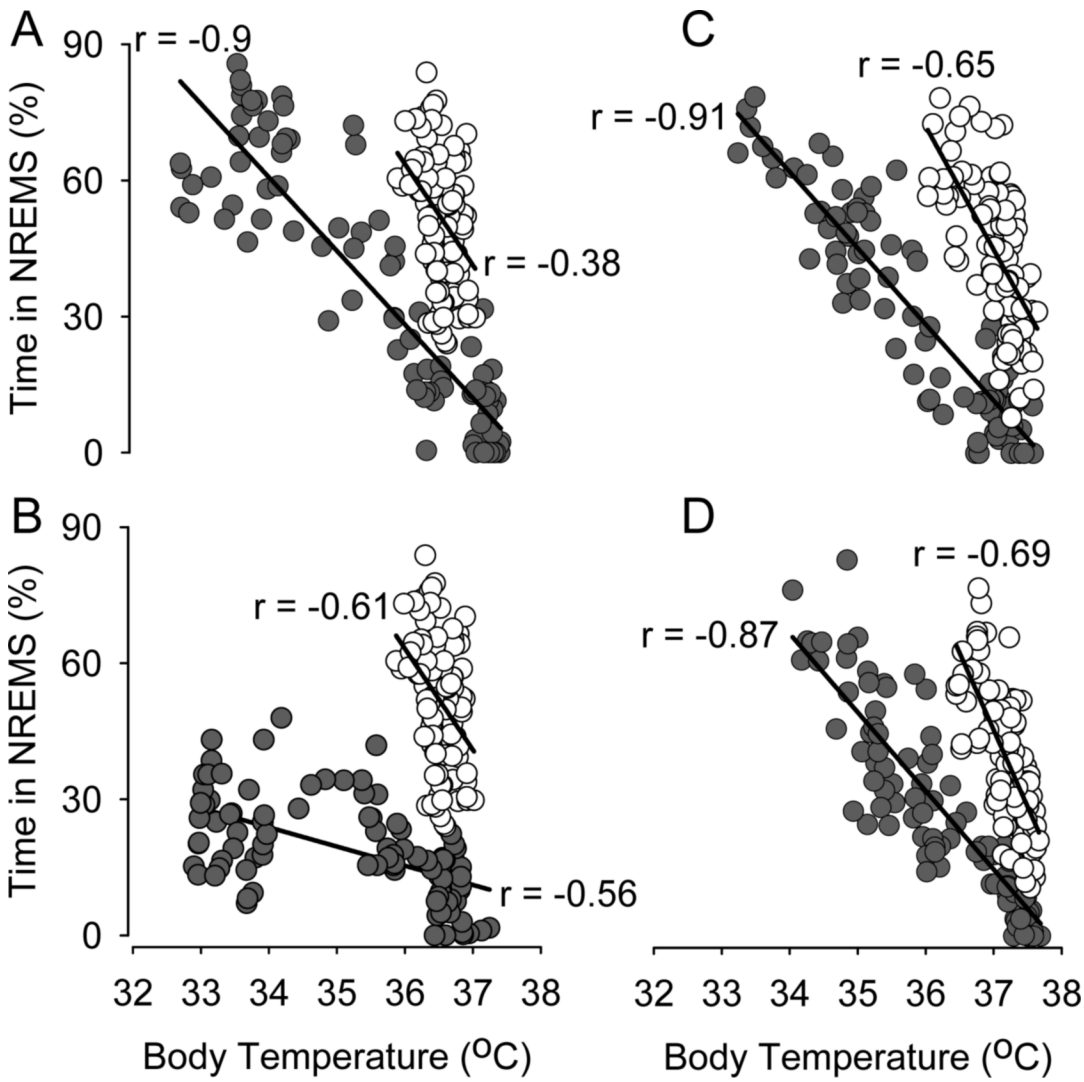

Fig. S1. Correlation between the amount of NREMS and body temperature during hours 1–16 on the baseline (open circles) and fasting days (closed circles) in preproghrelin WT (*A*), preproghrelin KO (*B*), ghrelin receptor WT (*C*), and ghrelin receptor KO (*D*) mice. Individual data points represent 10-min temperature (°C) and NREMS (percentage of recording time) averages. The relatively weak correlation between body temperature and NREMS duration on the baseline day can be explained by the fact that, in addition to NREMS-related decreases in body temperature, there are also sleep-independent, circadian changes in temperature. In response to fasting in a cold environment, the correlation became significantly stronger and the regression line is shifted to the left in preproghrelin WT, ghrelin receptor WT, and KO mice. This reflects the fact that the hypothermic periods were associated with increases in NREMS, but when body temperature was in the 36 °C to 37 °C range the dominant state was wakefulness. In preproghrelin KO mice, the slope of regression line became significantly more horizontal on the fasting day [$F(1, 188) = 112.2$, $P < 0.001$], indicating that the integrative mechanisms linking NREMS duration to body temperature are dependent upon the preproghrelin gene. Multiple regression analyses revealed that the correlation between NREMS time and body temperature on the fasting day in preproghrelin KO mice is significantly different from preproghrelin WT [$F(2, 188) = 148.3$, $P < 0.001$], ghrelin receptor WT [$F(2, 188) = 166.2$, $P < 0.001$], and ghrelin receptor KO mice [$F(2, 188) = 140.5$, $P < 0.001$].

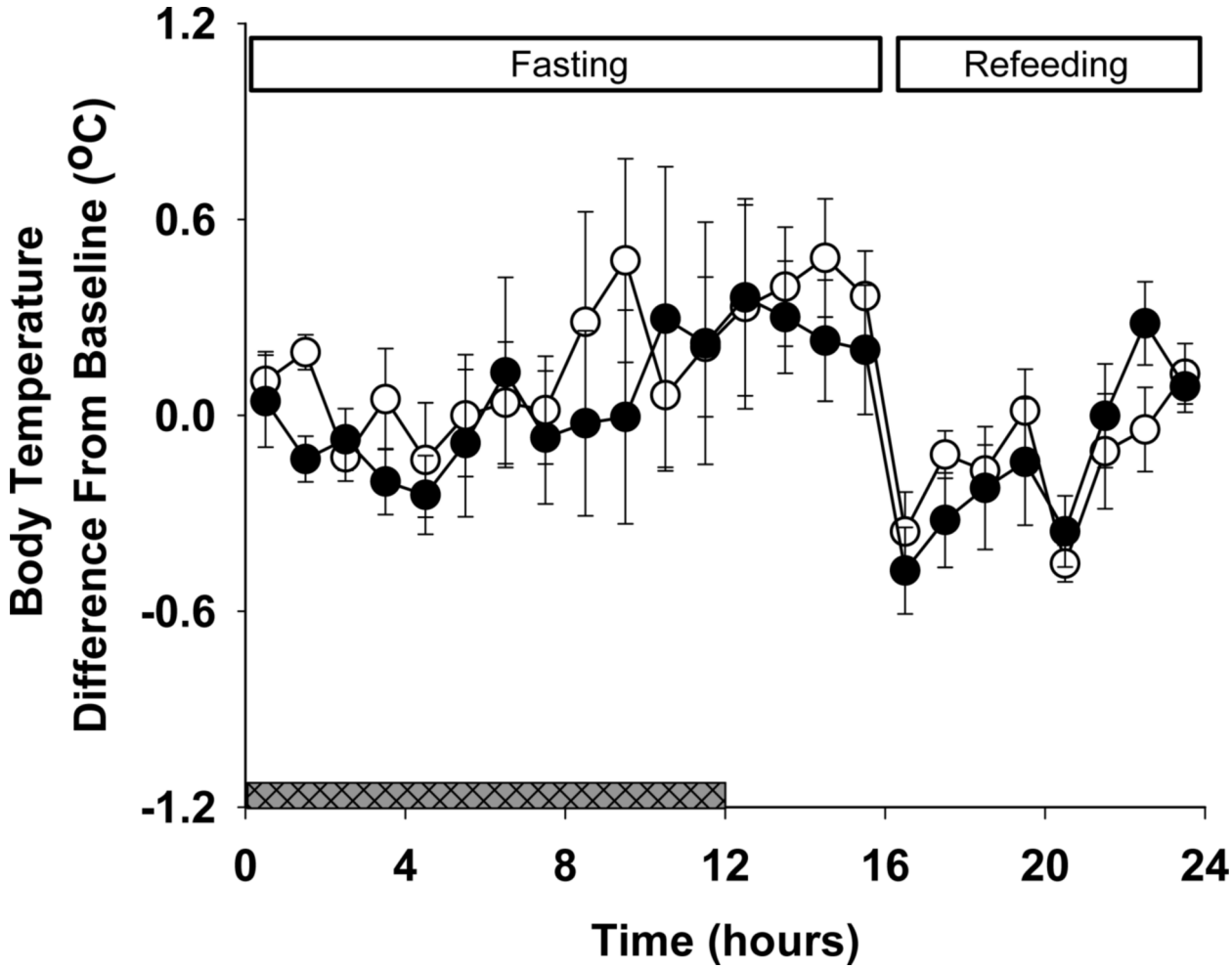

Fig. S2. Body temperature changes in response to fasting for 16 h at 30 °C ± 1 °C ambient temperature in preproghrelin WT (open symbols, $n = 7$) and preproghrelin KO (solid symbols, $n = 8$) mice. There was no apparent difference in the body temperature response of preproghrelin KO and WT mice. Horizontal gray bar: dark phase of the day.

Table S1. Sleep parameters of preproghrelin KO and WT mice on the fasting day

		WT		KO	
Phase	Sleep	Baseline day	Fasting day	Baseline day	Fasting day
Phase 1 (hours 1–6)					
T_b, °C		36.8 ± 0.2	37.2 ± 0.3	37.0 ± 0.2	36.7 ± 0.2
Time in vigilance states, min	NREMS	264.2 ± 25.9	52.3 ± 16.5*	268.3 ± 7.3	58.0 ± 20.1*
	REMS	23.5 ± 4.0	2.9 ± 1.2*	26.5 ± 3.2	5.2 ± 2.1*
Average bout duration, min	NREMS	4.8 ± 0.4	5.2 ± 0.5	5.3 ± 0.3	2.2 ± 0.6*†
	REMS	1.4 ± 0.2	1.6 ± 0.3	1.6 ± 0.1	0.9 ± 0.3
Total no. of bouts	NREMS	34 ± 3.1	5.3 ± 1.5*	33.9 ± 1.9	13.6 ± 5.3*
	REMS	8.4 ± 1.5	1.3 ± 0.6*	9.1 ± 1.2	2.1 ± 0.8*
Phase 2 (hours 7–16)					
T_b, °C		36.4 ± 0.2	34.7 ± 0.4*	36.7 ± 0.2	34.3 ± 0.5*
Time in vigilance states, min	NREMS	560.4 ± 16.7	499.5 ± 36.9*	576.6 ± 19.0	241.1 ± 38.3*†
	REMS	55.4 ± 3.3	25.8 ± 4.3*	69.2 ± 4.2	11.5 ± 5.2*
Average bout duration, min	NREMS	5.8 ± 0.6	11.9 ± 0.7*	6.4 ± 0.4	2.8 ± 0.3*†
	REMS	1.5 ± 0.1	1.7 ± 0.2	1.6 ± 0.1	1.7 ± 0.5
Total no. of bouts	NREMS	65.0 ± 5.8	29.0 ± 2.1*	60.5 ± 3.9	57.6 ± 9.3†
	REMS	20.5 ± 1.6	8.9 ± 1.5*	26.4 ± 2.3	4.1 ± 1.9*
Phase 3 (hours 17–24)					
T_b, °C		36.1 ± 0.2	35.1 ± 0.2	36.3 ± 0.2	28.4 ± 0.6*†
Time in vigilance states, min	NREMS	463.1 ± 15.0	264.9 ± 48.5*	489.4 ± 11.9	18.6 ± 7.4*†
	REMS	49.7 ± 2.4	18.9 ± 4.7*	65.0 ± 4.9†	0.2 ± 0.1*†
Average bout duration, min	NREMS	6.0 ± 0.6	8.8 ± 1.2*	6.7 ± 0.3	2.8 ± 0.5*†
	REMS	1.6 ± 0.1	1.8 ± 0.2	1.8 ± 0.1	N/A
Total no. of bouts	NREMS	51.8 ± 4.4	18.1 ± 2.8*	49.6 ± 2.2	4.6 ± 2.0*†
	REMS	18.6 ± 1.3	6.0 ± 1.4*	22.7 ± 1.4	0.1 ± 0.1*

According to the characteristic changes in body temperature and sleep on the fasting day, 3 response phases were distinguished: phase 1, hours 1–6; phase 2, hours 7–16; and phase 3, hours 17–24. Body temperature, the amount of NREMS and REMS, and the total number and the average duration of NREMS and REMS bouts of preproghrelin KO and WT mice were calculated for these 3 time periods. Data for each measurement were compared by using 3-way ANOVA (genotype factor as independent measure, phase and day factors as repeated measures). T_b, body temperature.

*Significant difference from baseline.

†Significant difference between WT and KO mice.

Table S2. Body temperature and amount of NREMS and REMS of ghrelin receptor KO and WT mice during the 3 phases of the fasting day

		WT		KO	
Phase	Sleep	Baseline day	Fasting day	Baseline day	Fasting day
Phase 1 (hours 1–6)					
T_b, °C		37.3 ± 0.1	37.1 ± 0.1	37.5 ± 0.1	37.5 ± 0.1
Time in vigilance states, min	NREMS	195.1 ± 17.1	64.8 ± 26.3*	176.1 ± 10.3	59.9 ± 17.5*
	REMS	12.1 ± 1.7	2.3 ± 1.3*	11.9 ± 1.6	2.1 ± 0.9*
Phase 2 (hours 7–16)					
T_b, °C		36.8 ± 0.1	35.2 ± 0.4*	37.0 ± 0.1	35.6 ± 0.2*
Time in vigilance states, min	NREMS	516.8 ± 19.0	407.6 ± 23.0*	476.3 ± 11.8	383.8 ± 18.0*
	REMS	57.9 ± 2.8	18.4 ± 5.2*	56.6 ± 5.7	32.6 ± 5.8*
Phase 3 (hours 17–24)					
T_b, °C		36.0 ± 0.1	35.4 ± 0.2*	36.4 ± 0.1	35.4 ± 0.2*
Time in vigilance states, min	NREMS	488.7 ± 5.9	281.4 ± 65.7*	469.7 ± 6.8	376.5 ± 25.3
	REMS	64.6 ± 3.7	26.7 ± 7.6*	68.0 ± 3.4	41.3 ± 6.4*

See Table S1 footnote for details.
*Significant difference from baseline.