Cell Reports, Volume *26*

Supplemental Information

Parabrachial Interleukin-6 Reduces Body Weight and Food Intake and Increases Thermogenesis to Regulate Energy Metabolism

Devesh Mishra, Jennifer E. Richard, Ivana Maric, Begona Porteiro, Martin Häring, Sander Kooijman, Saliha Musovic, Kim Eerola, Lorena López-Ferreras, Eduard Peris, Katarzyna Grycel, Olesya T. Shevchouk, Peter Micallef, Charlotta S. Olofsson, Ingrid Wernstedt Asterholm, Harvey J. Grill, Ruben Nogueiras, and Karolina P. Skibicka

SI

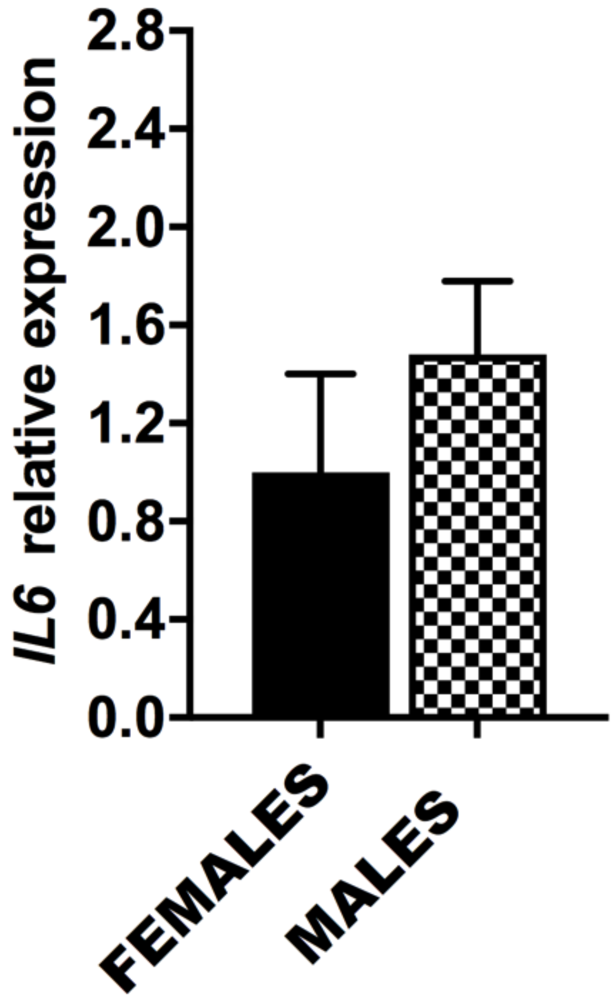


Figure S1: Comparison of IL-6 gene expression levels in chow-fed 13-week-old male and female mice (see figure 1 in the main text) in the parabrachial nucleus. Expression levels were detected by quantitative real-time PCR. Unpaired Student's t-test n=10 males and n=6 females.

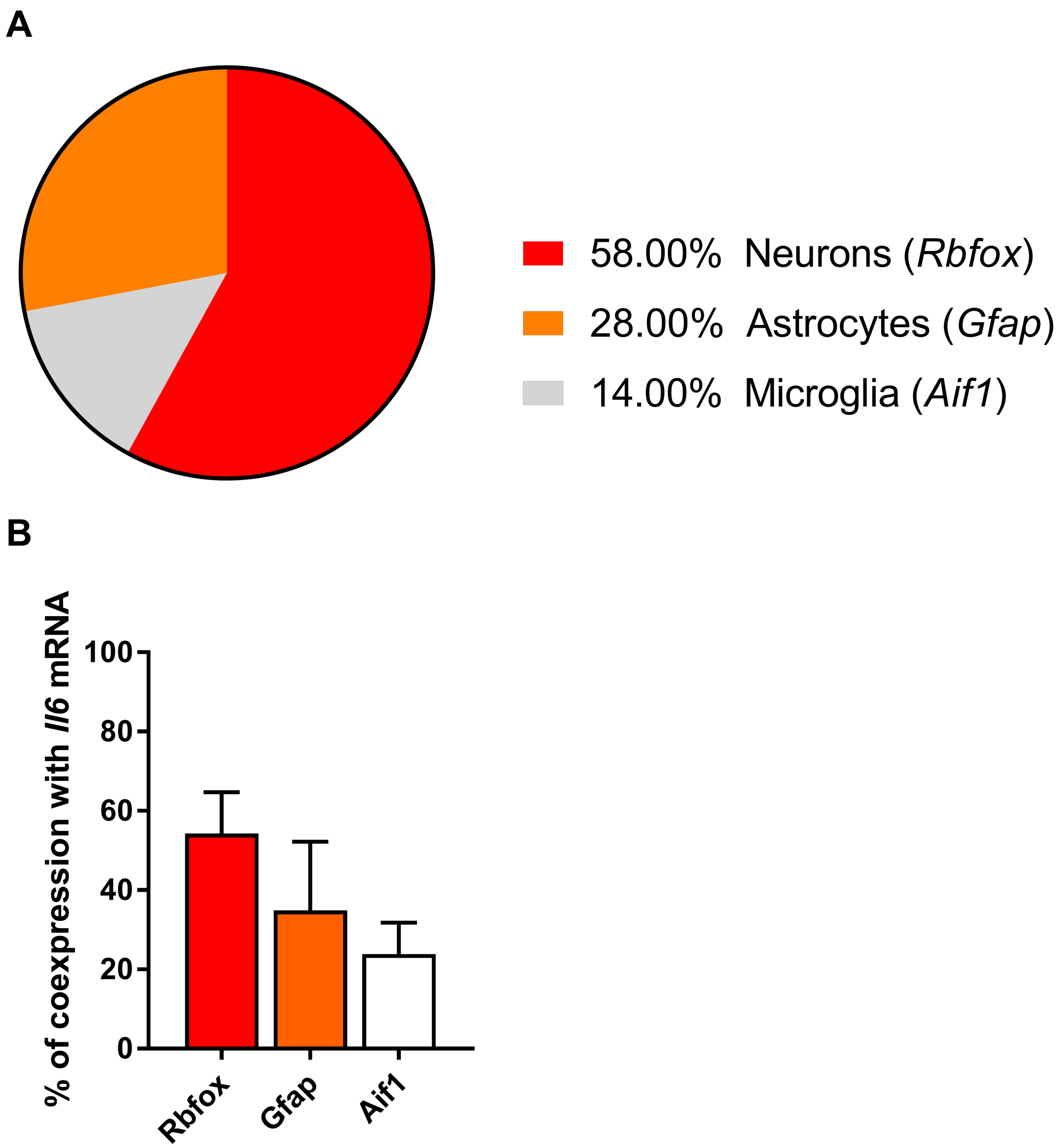


Figure S2: **Results of approximate quantification of co-expression of cellular markers with IL-6 mRNA in the lPBN of chow-fed 10-week-old male rats. Related to Fig 1Q-S in the main text.** (A) Diagram illustrating the proportion (by %) of IL-6 mRNA expressed by neurons, astrocytes, and microglia. (B) Proportion (by %) of each cell type co-expressing IL-6 mRNA at bregma -9.2 mm level. For Rbfox and Gfap experiments, the lPBN from three male rats was used, Aif1 counts are taken from two rats since the third rat lPBN showed levels of Aif1 close to that of negative control. Data represent mean ± SEM.

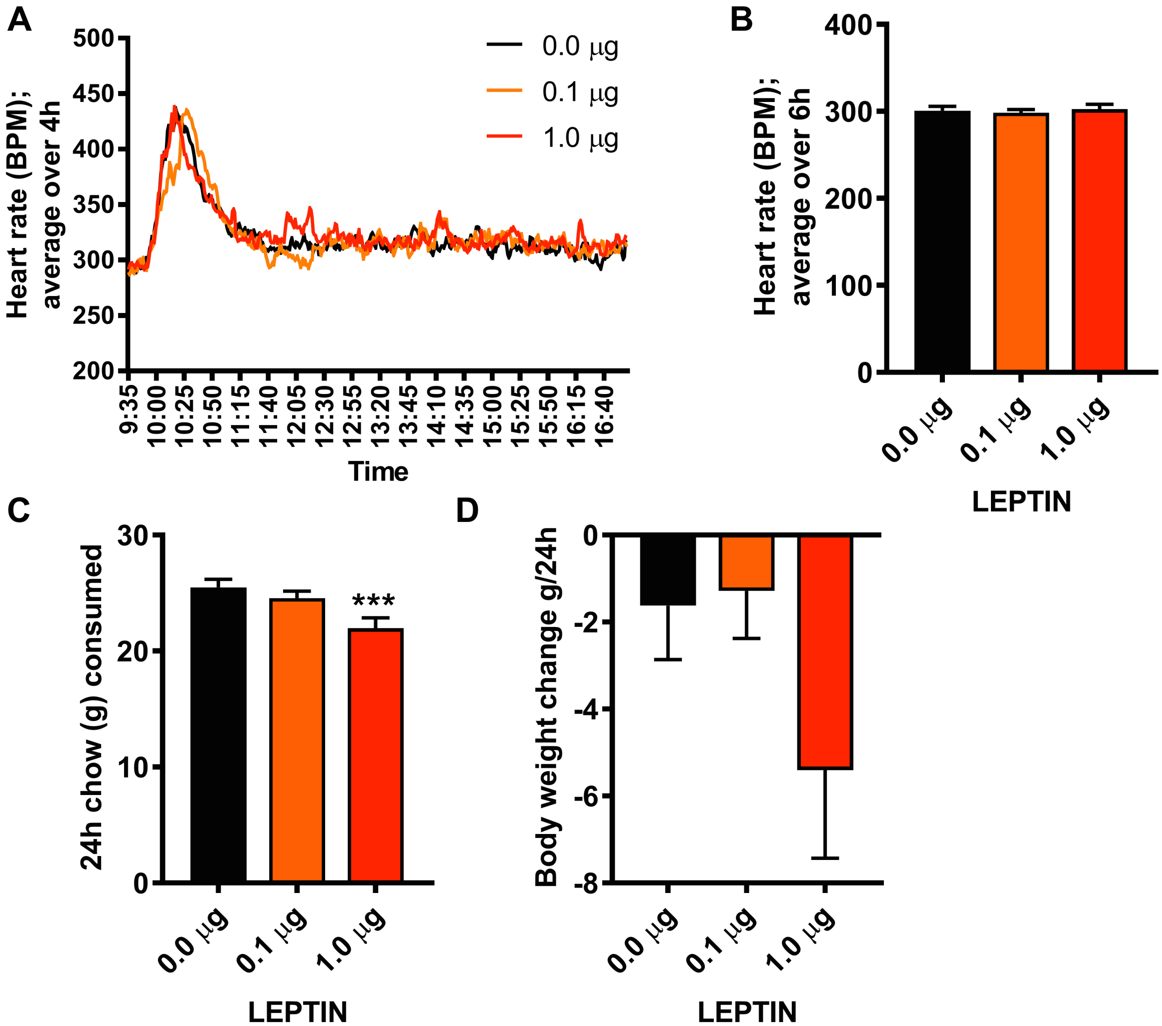


Figure S3: **Measurement of lPBN leptin-administration on cardiovascular activation, feeding and body weight. Related to Fig 3 in the main text.** Heart rate, food intake and body weight gain were monitored after intra-lPBN leptin injections in chow-fed male rats. (A) Line graph derived from continues telemetric hear rate monitoring in male rats. 10.00 injection time. (B) Heart rate averaged over 6h post-injection period. (C) Chow intake 24h after leptin injection (18h after food was offered since food was not available during telemetric recordings). (D) Body weight change in male rats after leptin administration during the light phase. Data represent mean ± SEM. ****P*<0.001. One-way ANOVAs were used followed by Sidak's post-hoc test.

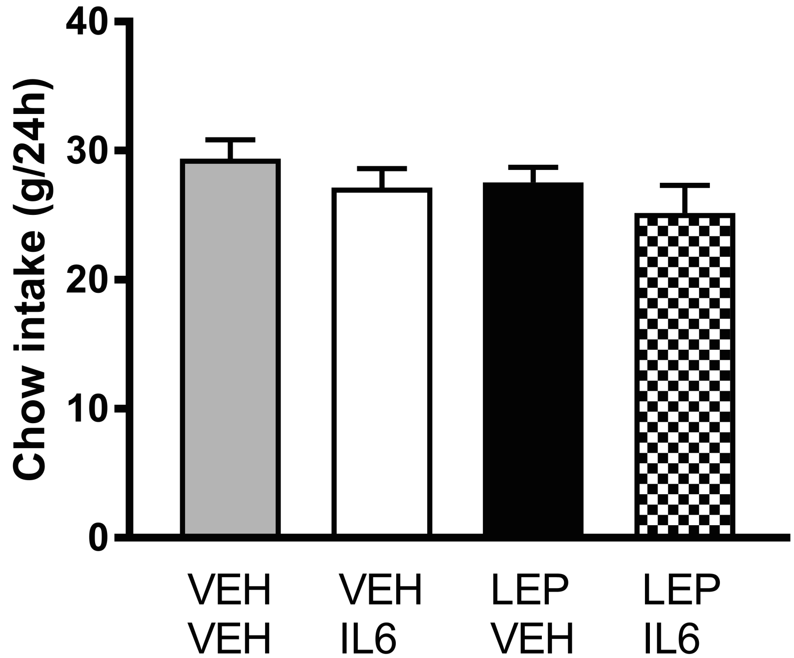
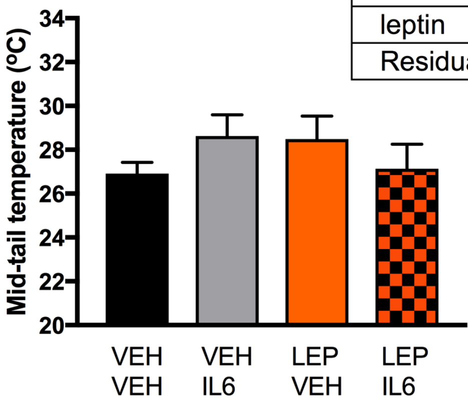


Figure S4. Effect of subthreshold doses of leptin and IL-6 on 24h food intake in male rats. Related to Fig 3. Subthreshold doses of IL-6 and leptin alone, or in combination, were injected into the lPBN of male chow-fed rats and cumulative 24h chow intake was measured (two-way ANOVA, n=12). These rats were 11 weeks old during experimentation.

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	14.15	1	14.15	F (1, 20) = 2.636	P=0.1201
il6	0.0117	1	0.0117	F (1, 20) = 0.00218	P=0.9632
leptin	0.1926	1	0.1926	F (1, 20) = 0.03588	P=0.8517
Residual	107.4	20	5.368		



ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	5.51	1	5.51	F (1, 20) = 2.393	P=0.1375
il6	2.884	1	2.884	F (1, 20) = 1.253	P=0.2763
leptin	5.762	1	5.762	F (1, 20) = 2.503	P=0.1293
Residual	46.05	20	2.302		

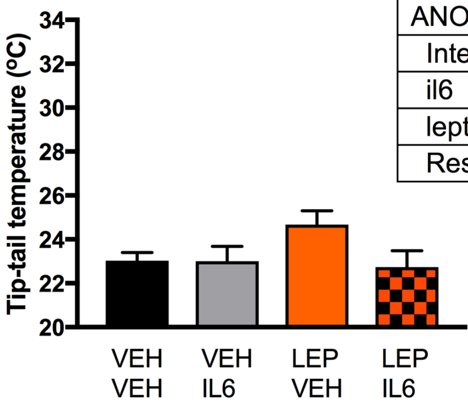


Figure S5: Effect of leptin and IL-6 on tail surface temperature in male rats. Related to Fig 3, new cohort of rats was used for this study. IL-6, or leptin each applied at a dose of 0.1 µg, or the combination of the two substances was delivered into the lPBN of male chow-fed rats. Tail temperature was measured here at mid and tip sections of the rat tail with the FLIR infrared imaging. Data represent mean ± SEM, for detailed statistics see tables embedded in this figure.

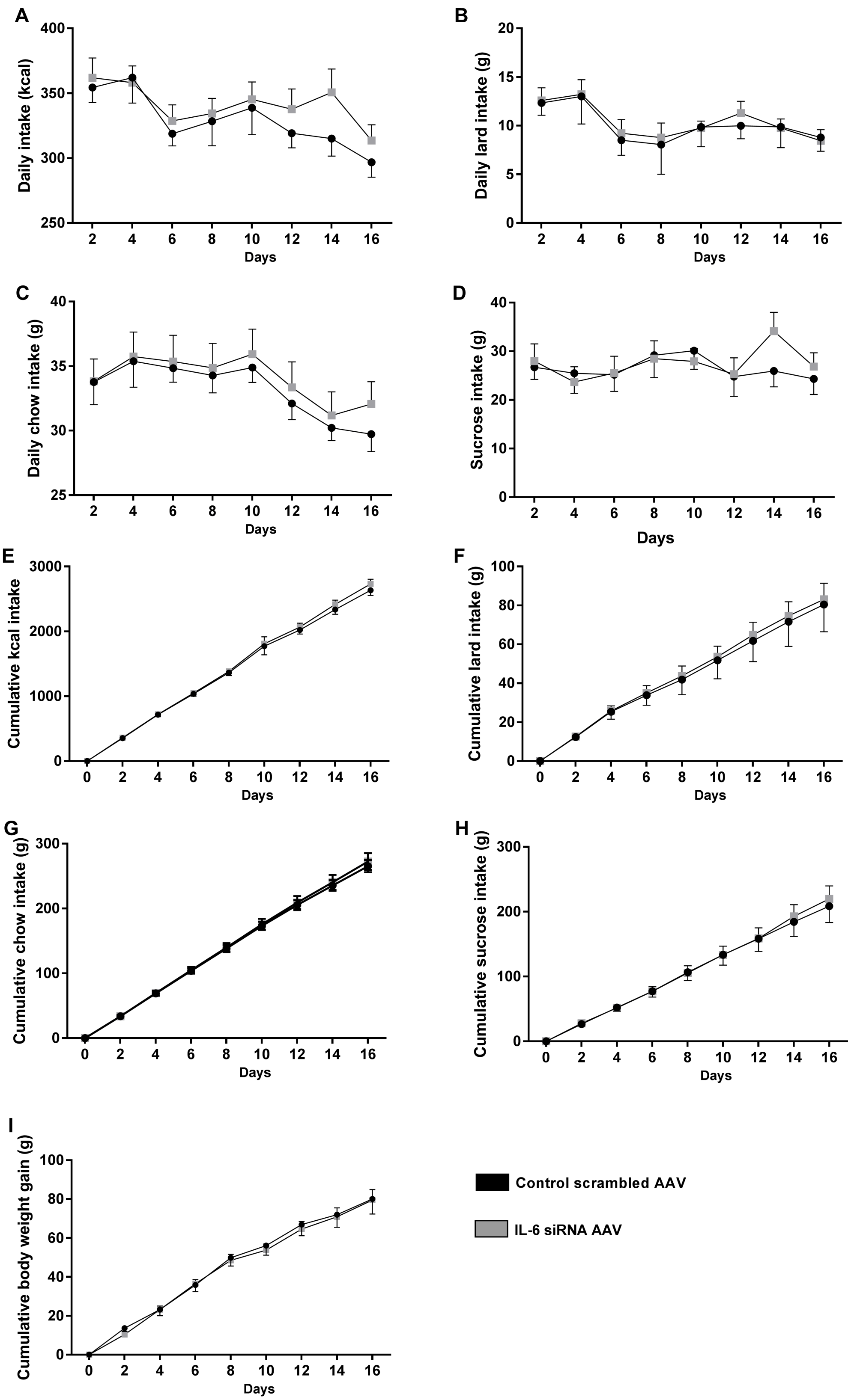

A
Daily intake (kcal)
Days
B
Daily lard intake (g)
Days
C
Daily chow intake (g)
Days
D
Sucrose intake (g)
Days
E
Cumulative kcal intake
Days
F
Cumulative lard intake (g)
Days
G
Cumulative chow intake (g)
Days
H
Cumulative sucrose intake (g)
Days
I
Cumulative body weight gain (g)
Days
Control scrambled AAV
IL-6 siRNA AAV

Figure S6: I**nactive (light) period intake was selectively affected by the lPBN kd in rats fed an obesogenic diet (see Figure 4 I-M), however the overall 24h intake, shown here, was not affected**. AAV-delivered siRNA was utilized to achieve IL-6 knockdown in the lPBN of male rats (as shown in figure 4) offered high-fat/high-sugar choice diet. (A) Daily caloric intake, (B) lard intake, (C) chow intake, (D) and sucrose intake were measured for the first 16 days on the diet. (E-H) Cumulative analysis of intake. (I) Cumulative weight gain. Note that for the high-fat/high-sugar diet feeding experiments, the rats were placed on the diet at least four weeks after AAV-siRNA infusions, thus there was sufficient time for the AAV siRNA to knockdown the gene of interest. Two-weeks of diet exposure are shown here; the rats were followed for a total of four weeks without any changes between the scrambled and IL-6 kd detected. Two-way ANOVA for cumulative comparisons with Sidak's post-hoc test. n=10-12.

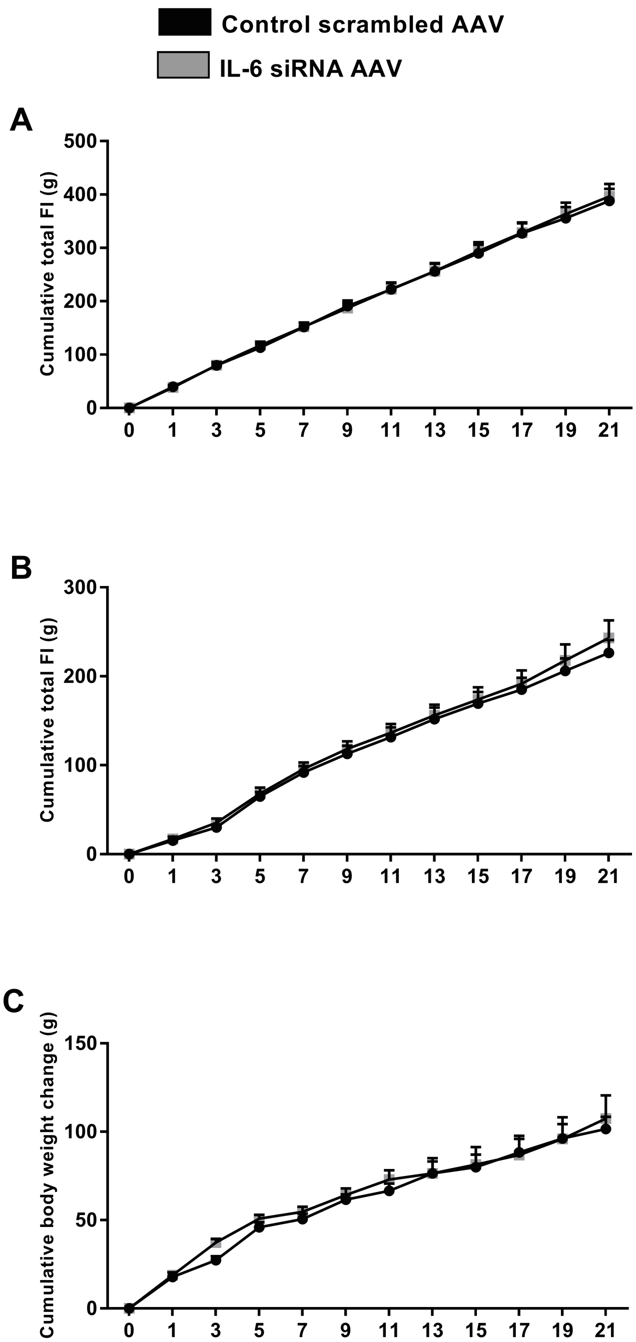


Figure S7: **Unlike the chow-fed IL-6 kd rats, rats fed the high-fat/high-sugar diet, did not gain more weight or did not display chronic daily hyperphagia when maintained on the palatable diet.** Related to fig 4 in the main text. The first diet offered a free choice of lard as solid, and sugar, as a sucrose solution, and chow (see Fig S6). In order to determine whether the lack of weight gain was driven by the specific diet used, a new group of rats, with lPBN IL-6 kd, was offered a different diet, where sugar and lard were mixed (1:1 by weight) and provided in a solid form in addition to water and chow. (A) Intake of chow or (B) the palatable mix, and (C) body weight were measured for 3 weeks. 2-way ANOVA, Sidak's post-hoc test with n=9 in each group. FI, food intake.