

Figure S1. Chow-fed 7–10-week-old male C57BL/6J mice were injected intravenously with vehicle or MyoAAV-dCas9-VP64 control vectors (MyoAAV2a-C-dCas9-VP64 plus MyoAAV2a-N-dCas9; 5×10^{11} vg). Treatment groups received MyoAAV-CRISPRa-Gdf15 (MyoAAV2a-C-dCas9-VP64, MyoAAV2a-N-dCas9, and MyoAAV2a-SAM-gRNA5+7) at low (1×10^{11} vg), medium (2.5×10^{11} vg), or high (5×10^{11} vg) doses. Three weeks later mice were euthanized and (a) Gdf15 mRNA were determined in gastrocnemius muscle and (b) plasma GDF15 was measured by ELISA. Data are means \pm SEM with each dot representing a distinct animal.

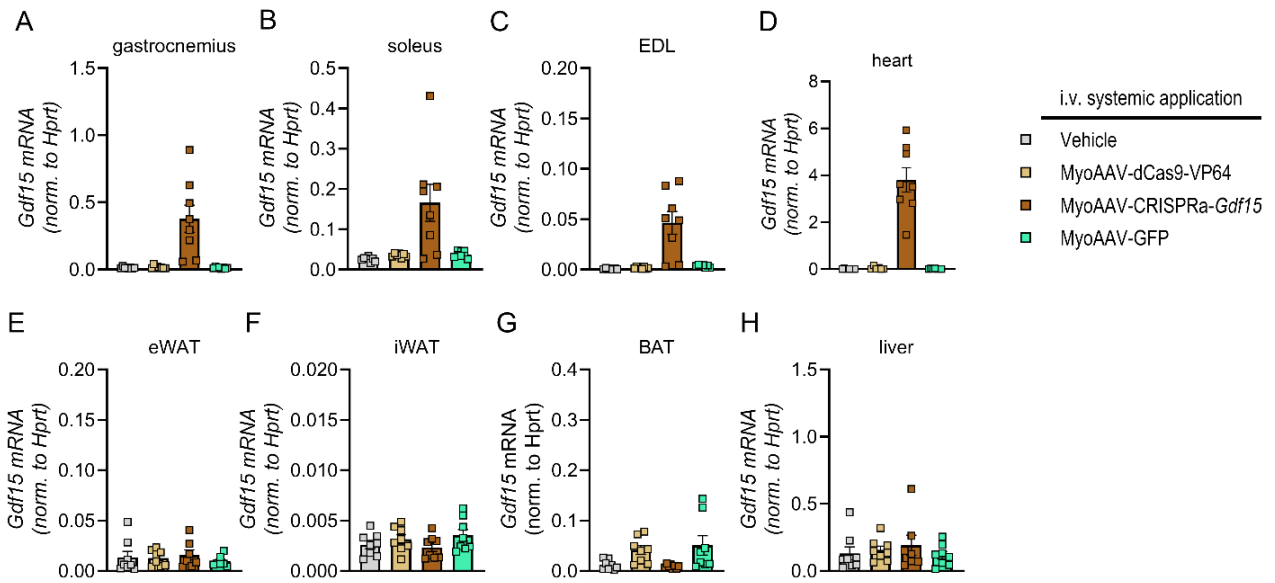


Figure S2. Effect of intravenous delivery of CRISPRa-*Gdf15* on *Gdf15* expression in different tissues in wildtype mice. *Gdf15* mRNA abundance was determined in the indicated (a-h) tissues from the study described in Figure 2. Data are means \pm SEM with each dot representing a distinct animal.

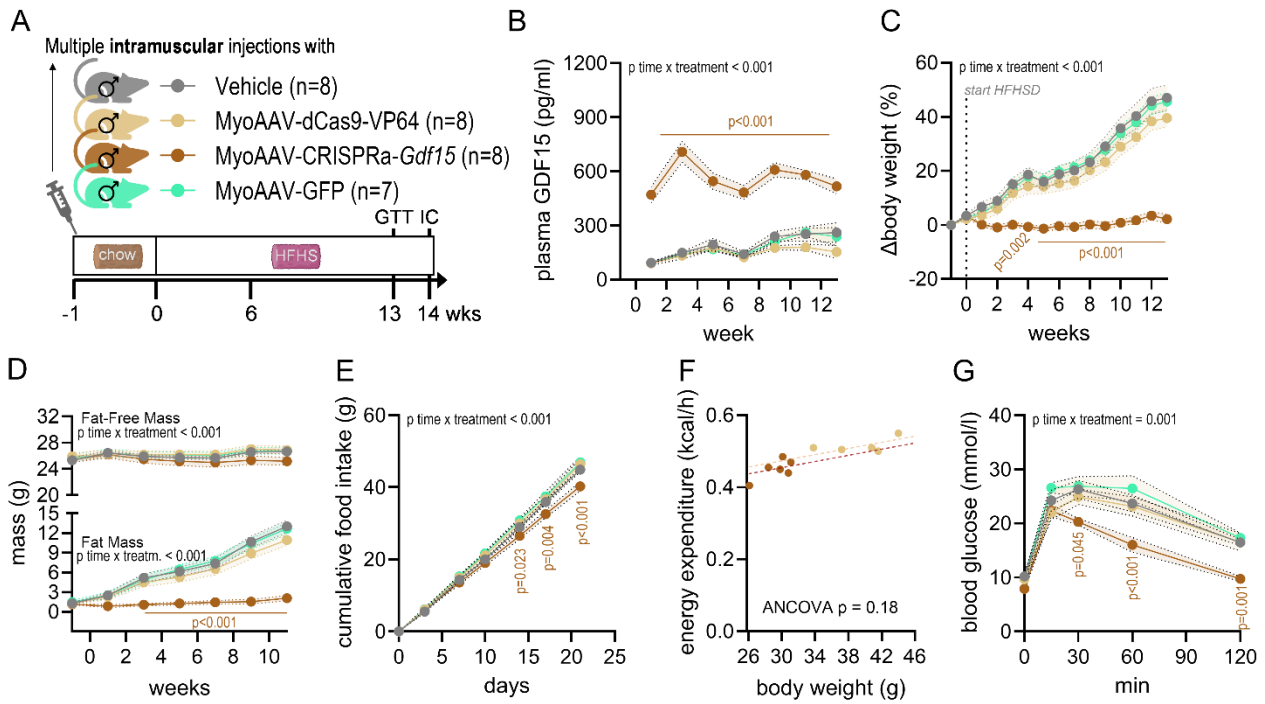


Figure S3. Metabolic phenotyping after intramuscular delivery of CRISPRa-*Gdf15* in wildtype mice. (A) Schematic of study design (with samples size per group shown except for E and F) with chow-fed male mice receiving intramuscularly injections with vehicle or MyoAAV2a encoding for eGFP or MyoAAV2a encoding for different component of the CRISPRa system. One week later all mice were switched to a HFHSD (EF D12331, ssniff Spezialdiäten GmbH, Germany) containing 58 kcal% fat. Over the course of the experiment, (B) plasma GDF15 levels, (C) body weight change, (D) fat-free mass and fat mass, and (E) cumulative food intake ($n = 3-4$ with mice double-housed), were measured. (F) Energy expenditure was determined ($n = 5-6$) by indirect calorimetry in week 14. (H) Glucose tolerance was tested in week 13. (B-E, H) data are presented as mean values \pm SEM with individual dots representing distinct animals. Statistic: (B-E, H) repeated measures two-way (time x treatment) ANOVA with Šidák post hoc testing. Data in (F) were analyzed using ANCOVA with body weight as co-variate.

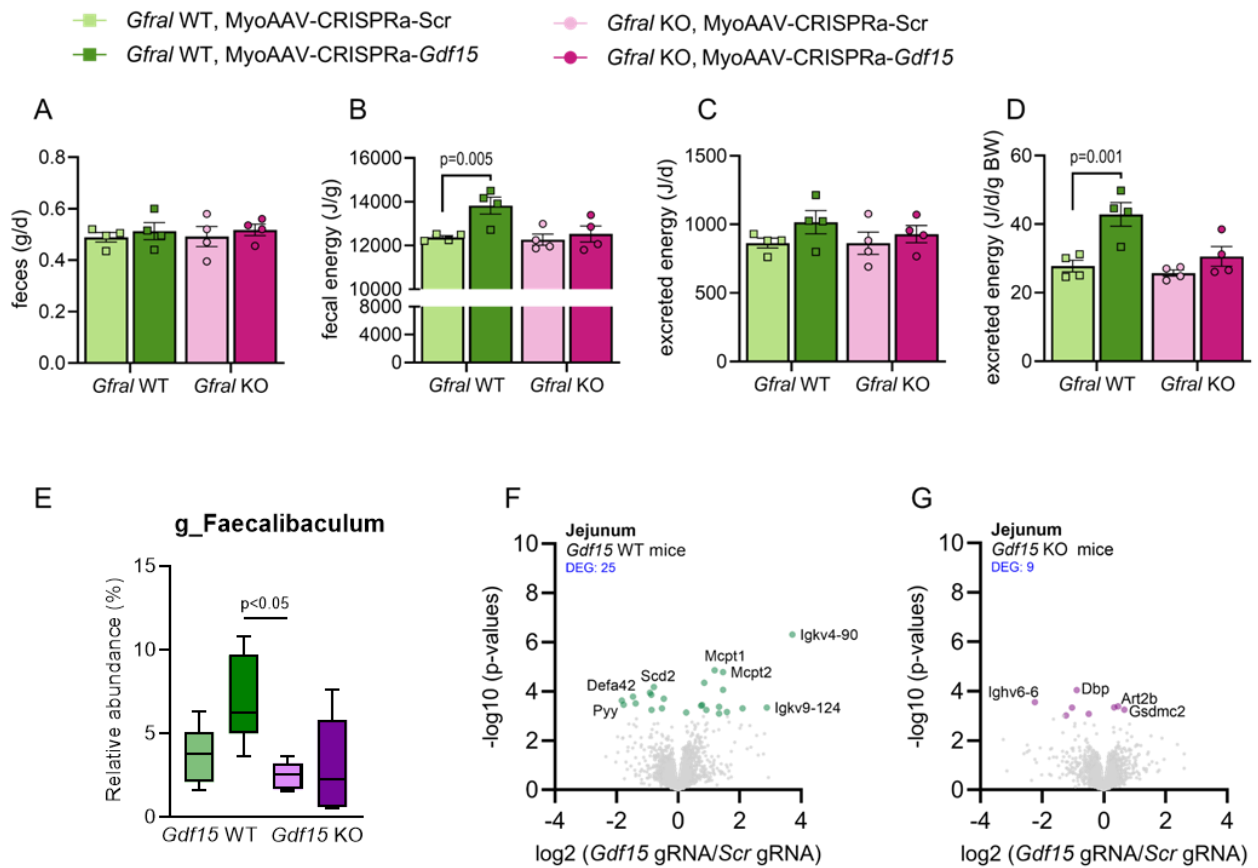


Figure S4. Effect of intravenous delivery of CRISPRa-*Gdf15* on energy excretion in *Gfral* WT and *Gdf15* KO mice (A-D): In the study shown in Figure 3, feces were collected in week 11 to determine (A) total feces mass, (B) fecal energy density and (C,D) overall energy excreted. (E) differential abundance of microbial genera in cecum as described in Figure 5 and on (F,G) gene expression (RNAseq) in jejunum from *Gdf15* WT and *Gdf15* KO mice as described in Figure 5. (A-D) data are presented as mean values \pm SEM with individual dots representing the average of two animals (mice were double housed). (E) data are presented as Tukey box plots with median, IQR, and whiskers. Statistic: (A-D) Two-way (genotype \times treatment) ANOVA with Šidák post hoc testing. (E) Wilcoxon Rank Sum Test with multiple testing correction by the Benjamini–Hochberg method for adjusted p-values. For E sample sizes are: *Gdf15* WT, MyoAAV-CRISPRa-Scr (n = 5); *Gdf15* WT, MyoAAV-CRISPRa-*Gdf15* (n = 6); *Gdf15* KO, MyoAAV-CRISPRa-Scr (n = 6); *Gdf15* KO, MyoAAV-CRISPRa-*Gdf15* (n = 6). For F and G samples sizes are: *Gdf15* WT, MyoAAV-CRISPRa-Scr (n = 5); *Gdf15* WT, MyoAAV-CRISPRa-*Gdf15* (n = 5); *Gdf15* KO, MyoAAV-CRISPRa-Scr (n = 6); *Gdf15* KO, MyoAAV-CRISPRa-*Gdf15* (n = 6).