

Endogenous Gdf15 activation limits adiposity and increases fecal energy excretion in mice

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Abstract

Growth differentiation factor 15 (GDF15) is an emerging target for obesity, but the effects of sustained physiological increases in endogenous GDF15 remain unclear. Here we use muscle-targeted CRISPR activation to elevate endogenous Gdf15 expression in mice. High-physiological circulating GDF15 prevents diet-induced obesity by limiting fat accumulation while preserving lean mass. This protection requires the endogenous Gdf15 locus and GFRAL signaling, without a detectable increase in energy expenditure. Alongside modest, inconsistent reductions in food intake, Gdf15 activation increases fecal energy excretion, accompanied by gut microbiota remodeling and altered ileal transcriptional programs. In mice with established obesity, Gdf15 activation reduces adiposity and increases fecal energy excretion without a detectable reduction in food intake. These findings identify fecal energy excretion as a GDF15-regulated component of energy balance and support a role for endogenous GDF15 beyond appetite regulation.

Introduction

Recent breakthroughs with GLP-1 (glucagon-like peptide 1) receptor agonist-based anti-obesity medications have shown weight reductions exceeding 15% in humans, and next-generation drugs appear even more effective¹. While this shows that significant progress has been made in developing molecules with effective weight loss properties, 10–30% of patients fail to respond (i.e. less than 5% weight loss) to the medication². Another significant future obstacle will be the maintenance of weight loss which likely poses distinct biomedical challenges. A multifaceted, tailored approach will likely be required for sustained weight loss. Thus, while GLP-1 receptor agonists represent a major advance, additional targets influencing energy balance may further optimize obesity treatment and, importantly, help prevent weight regain.

GDF15 (growth differentiation factor 15), a divergent member of the TGF- β superfamily which was first identified as macrophage inhibitory cytokine³, has emerged as a potential candidate for an anti-obesity treatment. In 2017, GFRAL (GDNF family receptor alpha-like) was identified as the hindbrain-restricted receptor through which GDF15 suppresses food intake by activation of the GFRAL/RET pathway^{4–6}. In obese rodents, treatment with recombinant human GDF15 reduces food intake and results in weight loss, primarily by reducing fat mass while fat-free mass remains stable⁷. First clinical testing with long-acting GDF15 analog showed modest effects on weight loss, but notable reductions in food intake and appetite in humans⁸.

Endogenous GDF15 is produced by many cell types and is induced by cellular, tissue, or metabolic stress⁹. Circulating GDF15 increases during chronic pathological conditions such as cancer, obesity, and autoimmune disease, but also during physiological challenges, including pregnancy¹⁰, aging¹¹, and exercise^{9,12,13}. Most studies linking GDF15 to decreased food intake and weight loss have relied on pharmacological doses^{13–16} or transgenic mouse models^{17–20} that generate circulating concentrations

of GDF15 far exceeding those observed under physiological conditions⁹. For example, in a transgenic mouse line overexpressing human GDF15²¹ circulating concentrations are 50 ng/mL, and thus approximately 1000-500-fold higher than levels observed in corresponding wildtype mice²⁰. Similarly, recombinant human GDF15 administered at anorectic doses results in peak plasma levels of 80–90 ng/mL^{13,22}, which are magnitudes higher than those induced by, for example, exercise, treatment with metformin, or intake of dietary fatty acids^{12,13,23–26}. These observations indicate that although pharmacological and transgenic models have been instrumental in demonstrating GDF15's anorectic effects, the supraphysiological concentrations achieved in these settings mimic or surpass pathophysiological levels of GDF15 and thus limit our ability to infer the metabolic consequences of physiological increases in endogenous GDF15.

In addition to pharmacological interventions or transgenic models, there are GDF15 secretagogues that elevate endogenous GDF15 within a physiological to high-physiological range. These secretagogues have yielded inconsistent conclusions regarding necessity of endogenous GDF15 for weight regulation. For example, metformin increases circulating GDF15 in obese mice 5–10-fold, an elevation reported to be sufficient to drive GDF15–GFRAL–dependent weight loss²³. However, this conclusion is not universal with another study reporting that metformin-induced weight loss persists in mice lacking either GDF15 or GFRAL²⁴. Similarly, while cisplatin-induced weight loss has been partially attributed to increased circulating GDF15^{27,28}, endotoxin-induced weight loss occurs independently of the GDF15–GFRAL axis, despite endotoxin elevating plasma GDF15 levels 20–50-fold^{29–31}. A major challenge in interpreting these findings is that these secretagogues do not elevate GDF15 in isolation but instead trigger pleiotropic responses. These concurrent responses may, therefore, override, blunt, or potentiate GDF15 action, obscuring its specific contribution to energy balance. As a result, the impact of physiological increases in GDF15 acting as a singular signal remains poorly defined, and studies directly addressing this gap are lacking.

We therefore set out to directly define the energy balance consequences of an isolated, physiological elevation of GDF15. Using a CRISPR/Cas9-based transcriptional activation (CRISPRa) strategy, we selectively targeted endogenous *Gdf15* expression *in vivo* without altering the genomic sequence. This approach enabled a controlled increase in circulating GDF15, allowing us to determine whether such a physiological elevation is sufficient to modulate energy balance, metabolism and body weight.

Results

CRISPRa induces GDF15 secretion in muscle cells

To establish upregulation of endogenous *Gdf15* expression in muscle cells, we used C2C12 myoblasts and a CRISPRa system with multiple activators (see Methods) (Fig. 1A). We designed multiple guide RNAs (gRNAs) targeting promoter regions upstream of the two annotated *Gdf15* transcription start sites (TSSs), which control expression of the short (NM_011819.4) and long (NM_001330687.1), respectively,

Gdf15 transcript isoform (Fig. 1B). Experimental testing of all gRNAs revealed that gRNAs 4–8 (Fig. 1C), targeting the short isoform promoter, and gRNAs 14 and 15 (Fig. 1D), targeting the long isoform promoter, led to a marked upregulation of *Gdf15* transcript levels. Notably, increased GDF15 protein secretion was observed only with gRNA 4–8, but not with gRNA 14 or 15, suggesting that enhanced GDF15 secretion may specifically require activation of the short isoform promoter (Fig. 1E).

To determine whether distal regulatory elements were required for CRISPRa-mediated activation, we tested promoter fragments of different lengths using luciferase reporter assays. We used a dual-luciferase reporter construct (pAAVS1-*Gdf15*ShortPromoter-Gaussia SP-NanoLuc:eEF1-FLuc) in which the *Gdf15* promoter fragment drives secretion of NanoLuc, while Firefly luciferase under the constitutive EF1 promoter serves as an internal normalization control. Activation with gRNAs 4–8 strongly increased reporter expression regardless of whether a short (0.25 kb) or long (~ 3.6 kb) promoter fragment was used (Fig. 1F,G), suggesting that CRISPRa of *Gdf15* primarily depends on elements close to the core promoter region.

Next, we tested combinations of gRNAs targeting the short isoform promoter, to determine whether such multiplexing would further enhance GDF15 secretion. In general, combining multiple gRNAs augmented GDF15 secretion. Among the tested combinations, the gRNA pair 5 + 7 resulted in one of the highest levels of secreted GDF15 protein, suggesting a synergistic effect (Fig. 1H), and was therefore selected for subsequent *in vitro* and *in vivo* experiments.

Adeno-associated viruses (AAV) are frequently used for *in vivo* gene delivery because they combine low immunogenicity and nonpathogenicity with efficient transduction of cells and long-term episomal expression³². A challenge for applying CRISPRa *in vivo* is that its components exceed the ~ 4.7 kb packaging capacity of AAVs. To address this limitation, we employed an intein-split CRISPRa system (Fig. 1I) (see Methods), which enables strong activation of target genes while fitting within the packaging constraints³³. Using this split system in C2C12 cells, targeting *Gdf15* with gRNAs 5 + 7 increased GDF15 secretion to a similar extent as the conventional full CRISPRa system (Fig. 1J).

Together, these data highlight that CRISPRa targeting the promoter of the short *Gdf15* isoform is sufficient to enhance GDF15 secretion from muscle cells. Moreover, these findings demonstrate the feasibility of an AAV-compatible split CRISPRa system for subsequent *in vivo* activation of *Gdf15*.

Muscle-targeted *Gdf15* activation prevents obesity

We next sought to deliver the split CRISPRa system targeting *Gdf15* (CRISPRa-*Gdf15*) to skeletal muscle *in vivo*, as skeletal muscle is a large, easily accessible, metabolically resilient post-mitotic tissue with the proven capacity to secrete GDF15 into the circulation¹². We used a muscle-tropic AAV vector, termed MyoAAV2a³⁴, with the split CRISPRa system distributed across three MyoAAV2a vectors: two encoding the N- and C-terminal halves of dCas9–VP64, respectively, and one encoding *Gdf15*-targeting gRNAs together with additional transcriptional activation components (see Methods). We first assessed dose responsiveness following intravenous delivery and found that increasing titers of MyoAAV2a carrying

CRISPRa-*Gdf15* produced a dose-dependent trend toward higher *Gdf15* expression in skeletal muscle and resulted in an approximately fivefold increase in circulating GDF15 at the highest titer (Supplementary Fig. 1A,B).

We next examined the impact of physiological elevation of GDF15 on energy balance and body weight. Chow-fed female mice received intravenous MyoAAV2a encoding CRISPRa-*Gdf15*, while control groups received vehicle, MyoAAV2a encoding GFP, or MyoAAV2a encoding CRISPRa components but lacking gRNAs. One week later, mice were switched to a high-fat, high-sucrose diet (HFHSD) (Fig. 2A) to induce diet-induced obesity³⁵. Systemic delivery of CRISPRa-*Gdf15* increased *Gdf15* expression in skeletal and cardiac muscle, but not in liver or adipose tissues (Supplementary Fig. 2A–H), and stably increased circulating GDF15 by seven- to eightfold (Fig. 2B). This sustained elevation of GDF15 conferred resistance to diet-induced obesity: whereas control mice gained approximately 10 g of body weight, predominantly as fat mass, CRISPRa-*Gdf15* mice remained weight stable throughout the study, with no significant changes in fat mass and fat-free mass (Fig. 2C,D). Food intake was reduced (Fig. 2E), whereas energy expenditure remained unchanged (Fig. 2F). Glucose tolerance was improved in the CRISPRa-*Gdf15* animals (Fig. 2G).

Comparable results were also obtained following intramuscular delivery of MyoAAV2a encoding CRISPRa-*Gdf15* simultaneously into multiple muscles (bilateral gastrocnemius, tibialis anterior, quadriceps, and triceps) of male mice, with corresponding control groups receiving vehicle, MyoAAV2a-GFP, or MyoAAV2a encoding CRISPRa without gRNAs (Supplementary Fig. 3A). Multi-muscle delivery resulted in a stable five- to sixfold increase in circulating GDF15 that persisted for at least 13 weeks (Supplementary Fig. 3B). Higher GDF15 levels again prevented diet-induced obesity, by preventing adiposity while preserving lean mass (Supplementary Fig. 3C,D). CRISPRa-*Gdf15* mice exhibited a moderate reduction in food intake (Supplementary Fig. 3E), with no changes in energy expenditure (Supplementary Fig. 3F), and improved glucose tolerance (Supplementary Fig. 3G).

Together, these findings indicate that *in vivo* delivery of CRISPRa-*Gdf15* via MyoAAV2a enables sustained, high-physiological elevation of circulating GDF15 that is sufficient to prevent diet-induced obesity by preventing fat mass accumulation without negatively impacting fat-free mass.

GDF15–GFRAL dependence and voluntary running

To determine whether the metabolic effects of CRISPRa-*Gdf15* are mediated specifically through the GDF15–GFRAL signaling axis, we tested the requirement for both the GDF15 receptor, GFRAL, and the endogenous *Gdf15* locus using two complementary loss-of-function mouse models.

First, we assessed whether the protective effects of CRISPRa-*Gdf15* require GFRAL signaling (Fig. 3A). CRISPRa-*Gdf15* increased circulating GDF15 in both *Gfral* WT and *Gfral* KO mice to comparable levels (600–700 pg/mL) (Fig. 3B), but only *Gfral* WT mice were protected from diet-induced weight gain. In contrast, *Gfral* KO mice gained body weight and fat mass at rates indistinguishable from CRISPRa-Scr

controls (Fig. 3C–E). Fat-free mass remained unchanged across all groups, indicating preservation of lean mass (Fig. 3D,E). Consistent with the known role of GFRAL in mediating anorectic signaling, reduced food intake due to higher GDF15 levels was only observed in *Gfral* WT mice (Fig. 3F). Elevated GDF15 levels did not affect glucose tolerance in either WT or *Gfral* KO animals (Fig. 3G).

Second, we assessed whether the protective effects specifically depend on activation of the endogenous *Gdf15* locus. To address this, we performed studies in female *Gdf15* WT (*Gdf15*^{+/+}) and *Gdf15* KO (*Gdf15*^{-/-}) mice (Fig. 4A). In *Gdf15* WT mice, CRISPRa-*Gdf15* increased circulating GDF15 approximately fivefold (Fig. 4B). In contrast, circulating GDF15 was undetectable in *Gdf15* KO mice regardless of treatment, confirming the absence of endogenous GDF15 (Fig. 4B). CRISPRa-*Gdf15* protected *Gdf15* WT mice from diet-induced weight gain, whereas *Gdf15* KO mice accumulated body weight and fat mass similarly to control animals (Fig. 4C–E). Fat-free mass was unaffected in all groups, indicating preserved lean mass (Fig. 4D,E). Notably, food intake was not reduced statistically with high GDF15 in WT mice (Fig. 4F). High GDF15 levels were associated with improved glucose tolerance in WT, but not in *Gdf15* KO animals (Fig. 4G).

We have previously shown that high-dose pharmacological administration of recombinant GDF15 suppresses the motivation to exercise via the GDF15-GFRAL axis. We therefore herein investigated whether the physiological elevation of endogenous GDF15 elicits similar effects. Running behavior was assessed 6–7 weeks after intravenous delivery of CRISPRa-*Gdf15* or CRISPRa-Scr in *Gdf15* WT and *Gdf15* KO mice. We found that all mice exhibited a robust diurnal running pattern (Fig. 4H) and that CRISPRa-*Gdf15* did not reduce running distance in *Gdf15* WT or *Gdf15* KO mice (Fig. 4I). Interestingly, in *Gdf15* KO mice, running distance increased in mice treated with CRISPRa-*Gdf15* (Fig. 4I).

Collectively, these studies establish that a physiological increase in circulating GDF15 confers resistance to diet-induced obesity through the GDF15-GFRAL axis without suppressing voluntary exercise behavior that has been reported with pharmacological administration of recombinant GDF15.

Fecal energy loss and intestinal profiling

Because energy expenditure was unchanged and reductions in food intake were moderate across several experiments, we assessed fecal energy excretion, as it is a component of energy balance³⁶. We found that *Gdf15* WT mice treated with CRISPRa-*Gdf15* produced more fecal mass, and their feces had a higher energy density compared to CRISPRa-Scr animals (Fig. 5A,B). This translated into ~ 50% greater total energy excretion, corresponding to an increase of ~ 1,000 J per day, an effect that was even more pronounced when normalized to body weight (Fig. 5C,D). In *Gdf15* KO mice, these effects were absent, indicating that the increased fecal energy excretion depends on the presence of the *Gdf15* locus and elevated GDF15 levels. Similarly, increased energy excretion linked to higher circulating GDF15 required intact GFRAL signaling (Supplementary Fig. 4A–D).

The observed increase in fecal energy loss prompted us to examine two potential intestinal determinants of energy extraction: the gut microbiome and host intestinal transcriptional programs. We profiled the cecal microbiome and performed RNA-seq of the distal small intestine to identify pathways that could link elevated GDF15 to reduced nutrient assimilation and, conversely, increased fecal energy excretion.

Using 16S rRNA gene amplicon sequencing, we found that elevated GDF15 levels were linked to higher cecal microbiome richness in *Gdf15* WT mice relative to all other groups (Fig. 5E). Consistently, microbiome composition of *Gdf15* WT mice with elevated GDF15 clustered separately from all other groups, reflecting a pronounced shift in the microbial community (Fig. 5F). At phylum level, this was characterized by reduced abundance of Bacteroidota in *Gdf15* WT mice and higher relative abundance of Bacillota (Fig. 5G,H). The lower Bacteroidota abundance was driven largely by a depletion of the genus *Parabacteroides* with a notable concomitant reduction in *Alistipes* (Fig. 5I,J). By contrast, the greater prevalence of Bacillota in *Gdf15* WT mice was mainly attributable to *Faecalibaculum*, a health-associated, SCFA-producing member of this phylum that is often enriched under low-fat diets³⁷ and was also elevated in the presence of higher GDF15 levels (Supplementary Fig. 4E).

To identify host intestinal transcriptional programs that might be linked to reduced nutrient assimilation, we performed RNA-seq of jejunal and ileal tissue from *Gdf15* WT and *Gdf15* KO mice. In the jejunum, higher GDF15 levels significantly altered 25 genes in *Gdf15* WT animals ($p < 0.001$, DESeq2), whereas 9 differentially expressed genes were detected in *Gdf15* KO mice (Supplementary Fig. 4F,G). In the ileum, elevated GDF15 was associated with a broader transcriptional response, with 122 genes differentially expressed in *Gdf15* WT animals; while only 2 were altered by CRISPRa-*Gdf15* in *Gdf15* KO mice (Fig. 5K,L). These data indicate a spatially- and largely genotype-dependent effect of GDF15 on small intestinal tissue. The GDF15-induced changes in the ileum of *Gdf15* WT animals comprised increased expression of antimicrobial and innate immune-linked transcripts, including *Saa1*, *Reg3g*, *Reg3b*, *Defa34*, *Pla2g2a*, *Pla2g5*, *Il18*, *Il4ra*, *Tifa*, *Alpk1*, *Duox2*, *Oas1a*, *Ctss*, and *Lgmn*, along with epithelial/remodeling and trafficking genes (*Ptk6*, *Plet1*, *Rab8b*, *Ehd4*). In contrast, multiple transporters and metabolic genes, including *Slc23a2*, *Slc20a1*, *Slc22a3*, *Pgam1*, *Aldh1b1*, *Plin2*, *Ucp2*, *Far2*, and *G0s2*, were decreased with higher GDF15. GO Biological Process enrichment of the regulated gene set (Fig. 5M) in the ileum highlighted two functional themes. First, multiple terms converged on endocrine/metabolic remodeling, driven by enrichment of hormone metabolic process and the more specific C21-steroid hormone metabolic process, alongside broader organic acid metabolic process. Second, the gene set showed strong host-microbe/immune-related signatures, including defense responses to microbial stimuli from small intestinal lumen.

Together, these data identify a GDF15–GFRAL–dependent increase in fecal energy loss that coincides with remodeling of the gut microbiome and ileal epithelial function. These changes are consistent with reduced dietary energy extraction and may therefore contribute to the protection from diet-induced obesity observed with CRISPRa-*Gdf15* treatment and higher endogenous GDF15.

Gdf15 activation in mice with established obesity

Having observed that CRISPRa-*Gdf15* protects against the development of diet-induced obesity, we next asked whether this approach could also exert therapeutic effects once obesity was already established. To address this, female mice were maintained on HFHSD until they had developed obesity and subsequently received intravenous MyoAAV2a encoding CRISPRa-*Gdf15* or CRISPRa-Scr (Supplementary Fig. 5A). Fourteen weeks after administration, CRISPRa-*Gdf15* mice had approximately 15% lower body weight than CRISPRa-Scr controls (Supplementary Fig. 5A,B). Cumulative food intake was indistinguishable between groups throughout the intervention (Supplementary Fig. 5C), indicating that the lower body weight was not explained by reduced energy intake. Body composition analysis revealed that the difference in body weight was largely driven by lower fat mass in CRISPRa-*Gdf15* mice, whereas fat-free mass remained unchanged (Supplementary Fig. 5D,E). Glucose tolerance was moderately improved in CRISPRa-*Gdf15* mice (Supplementary Fig. 5F).

Given the absence of differences in food intake, and our previous observation that elevated GDF15 increases fecal energy loss during prevention of diet-induced obesity, we assessed fecal energy excretion. CRISPRa-*Gdf15* mice showed trends toward greater fecal output and higher fecal energy density compared with CRISPRa-Scr controls (Supplementary Fig. 5G,H). Together, these changes resulted in significantly increased total fecal energy excretion in CRISPRa-*Gdf15* mice (Supplementary Fig. 5I), an effect that was even more pronounced when normalized to body weight (Supplementary Fig. 5J).

Together, these findings indicate that CRISPRa-mediated elevation of endogenous GDF15 can reduce adiposity even after diet-induced obesity is established, while preserving lean mass. In this setting, the lower body weight occurs in the absence of reduced food intake and is accompanied by increased fecal energy loss.

Discussion

We show that a high-physiological elevation in endogenous circulating GDF15 is sufficient to prevent diet-induced obesity. This protection is linked to a selective blockade of fat mass accumulation while preserving lean mass. We report for the first time a link between the GDF15-GFRAL axis and intestinal energy handling, gut microbial community architecture, and transcriptional reprogramming of the ileal epithelium. Finally, we found that targeting of endogenous *Gdf15* was not only protective against the development of obesity but also reduced adiposity and increased fecal energy excretion when initiated after diet-induced obesity was already established. Our work identifies a potential new facet of GDF15 biology that impacts energy assimilation with consequences for energy balance.

A relatively modest induction of GDF15 was sufficient to prevent body weight gain during high-fat, high-sucrose feeding. Across our different studies, circulating GDF15 increased from 100 pg/mL in control animals to 500 to 800 pg/mL in treated groups. Concentrations of 500 pg/mL were already sufficient to

confer protection against obesity. Many chemicals/drugs^{23,24,27,28,30,38}, and interventions, including exercise^{12,13} and certain nutritional approaches^{25,26}, have been reported to elevate circulating GDF15 in mice. These increases at times coincide with weight loss that is mediated through GDF15 and GFRAL signaling, whereas in others, weight loss does not require the GDF15-GFRAL axis. These discrepancies are not related to the magnitude of GDF15 induction. For example, endotoxin administration increases plasma GDF15 by 20 to 50-fold, yet endotoxin-induced weight loss occurs largely independent of GDF15 and GFRAL signaling^{29–31}.

Interventions such as exercise, certain medications, or dietary challenges that raise endogenous GDF15 do not act in isolation, but instead trigger a broader constellation of circulating signals, with distinct, intervention-specific signatures. These concomitant changes could modulate GDF15 action at multiple levels, by potentiating, blunting, or functionally overriding its effects on feeding and body weight. For example, we have shown that dietary medium chain fatty acids acutely increase circulating GDF15, but this occurs alongside pronounced remodeling of the liver transcriptome, including altered expression of multiple secreted factors such as *Fgf21*, with a corresponding rise in circulating FGF21^{25,39}. This highlights the difficulty of inferring whether a physiological increase in GDF15 is by itself sufficient to alter energy balance in response to these interventions. We therefore used targeted CRISPRa to assess the consequences of inducing endogenous *Gdf15*.

Our data also raise the question of why pharmacological administration of recombinant human GDF15 in mice requires doses that produce circulating peak concentrations far higher than those we observed in order to suppress food intake. One possibility is species-specific sensitivity, with mice potentially responding more strongly to mouse GDF15 than to the human version. Notably, mature human and mouse GDF15 share only 66% homology³. Another explanation may lie in exposure dynamics. The elevation in GDF15 achieved here is tonic, in contrast to the transient spike produced by pharmacological delivery. Thus, even at much lower absolute concentrations, sustained elevation over time may be sufficient to fully engage GFRAL signaling. However, if this tonic exposure is key for the effects on food intake and body weight, it is notable that they occurred without a reduction in exercise motivation, as has been reported following pharmacological administration of recombinant human GDF15¹³. We also found no effect of elevated GDF15 on energy expenditure, assessed across multiple studies both after one week and after several weeks of increased GDF15 levels, indicating that increased energy turnover does not explain the observed protection against diet-induced obesity. This contrasts with recent findings showing that longer-term daily treatment with recombinant GDF15 increased energy expenditure and thereby promoted weight loss, albeit in mice that were housed at thermoneutrality¹⁴. Nevertheless, an effect of GDF15 on energy expenditure has not been observed consistently across studies⁹. Whether these discrepancies reflect differences in the magnitude or duration of GDF15 exposure remains to be determined.

Another key distinction of our approach is that CRISPRa induces *endogenous* GDF15, which may include the generation of GDF15-associated fragments produced during GDF15 maturation. GDF15 is

synthesized as pre-pro-GDF15 and processed to pro-GDF15, which exists either as a monomer or as a disulfide-linked homodimer. Proteolytic cleavage then releases the mature, secreted GDF15 homodimer⁹. Yet, fragment products of pro-GDF15 have been suggested to have unique biological functions^{40,41}, and a recent publication identified a novel 12-amino-acid fragment generated during GDF15 maturation that reduces food intake and body weight in obese mice, suggesting cooperative regulation of body weight with GDF15⁴². Thus, unlike pharmacological treatment with recombinant GDF15, *endogenous* GDF15 should be viewed as a qualitatively distinct biological factor rather than a reduced-dose analog of pharmacological GDF15.

One of the most intriguing observations was the increase in fecal energy excretion associated with elevated GDF15. The increase in fecal energy excretion was particularly notable because it was also observed when food intake was unchanged, both in the Gdf15 WT/KO study and in mice with established diet-induced obesity. Moreover, lower food intake or body weight would, if anything, be expected to reduce absolute fecal energy excretion, suggesting that this phenotype reflects a direct consequence of elevated GDF15 rather than a secondary effect of altered energy intake or body size. This finding could contribute to protection against diet-induced obesity by reducing the amount of dietary energy assimilated each day. Integrating cecal microbiome profiling with ileal transcriptomics, we identified GDF15-dependent changes that may help explain reduced dietary energy extraction and point to a potentially new dimension of GDF15 biology.

Elevated GDF15 levels were linked to a gut microbial signature of increased community diversity, typically associated with improved intestinal homeostasis and enhanced metabolic health. Higher GDF15 levels were also linked to differences in community composition, including lower Bacteroidota abundance, driven largely by depletion of Parabacteroides, alongside a concomitant reduction in Alistipes. Given human data linking a Bacteroidota-dominated enterotype to lower stool energy density and more efficient dietary energy extraction⁴³, in particular from saccharolytic and proteolytic metabolism⁴⁴, the reduction in Bacteroidota observed here is directionally consistent with the increased fecal energy excretion we found. In parallel, the ileal molecular signature pointed to a coordinated shift away from a predominantly absorptive, metabolically mature enterocyte program and toward an epithelial defense and remodeling state, one typically associated with stress responses and well positioned to influence both nutrient harvest and microbial ecology. Upregulated transcripts were enriched for host–microbe interface pathways, including Paneth cell–associated antimicrobials (α -defensins and Reg3 family members), phospholipases, DUOX2/DUOXA2-mediated reactive oxygen species defense, and pIgR-dependent luminal IgA transport. Some of these molecules possess bactericidal activity toward Gram-negative bacteria⁴⁵ that shape mucosal colonization and impose selective pressure on luminal and mucus-associated bacteria⁴⁶. These changes co-occurred with induction of innate inflammatory mediators (including *Il18* and *Casp4*) and additional cellular stress and regenerative regulators. Conversely, downregulated genes broadly mapped to mature enterocyte metabolic functions, including lipid droplet biology and fatty-acid handling (for example, *Plin2* and *G0s2*), oxidation and redox enzymes, xenobiotic and steroid metabolism (AKR, HSD, and UGT families).

Together, this suggests reduced epithelial nutrient-processing capacity and/or a state of increased exposure of the ileal epithelium to microbial antigens, both affecting composition of the downstream cecal microbiome. These data raise the interesting hypothesis that this transcriptional reprogramming may contribute to the physiological phenotype: reduced small intestinal energy assimilation could increase luminal caloric substrate delivery to colonic bacteria, thereby restructuring community composition and promoting higher alpha diversity. Our loss-of-function experiments indicate that the effects on fecal energy excretion, microbial ecology, and ileal reprogramming depend on GDF15. Whether activation of a central neural circuit by GDF15 drives this coordinated intestinal remodeling program, or whether alternative mechanisms are involved, remains to be resolved.

Several limitations should be considered. First, the work was performed in mice, and whether sustained physiological elevation of endogenous GDF15 similarly alters intestinal energy handling in humans remains unknown. Second, although increased fecal energy excretion was consistently associated with elevated GDF15, our measurements do not resolve the underlying source of this additional energy loss. In particular, whether it reflects reduced nutrient absorption, altered gastrointestinal transit or secretion, changes in microbial metabolism, or other processes remains to be determined. Third, the accompanying changes in the gut microbiome and ileal transcriptional programs are associative, and their causal contribution to increased fecal energy excretion remains unresolved. Finally, sex-specific effects were not systematically examined across experiments, limiting conclusions regarding potential differences between male and female mice.

To conclude, CRISPRa-mediated activation of endogenous *Gdf15* produces high-physiological GDF15 levels that limit adiposity, an effect associated with increased fecal energy excretion and pointing to altered intestinal energy handling as a novel component of GDF15 action.

Methods

Reagents and resources (Table 1)

Materials availability

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Maximilian Kleinert (maximilian.kleinert@dife.de).

All unique materials generated in this study are available from the lead contact without restriction.

Cell culture

C2C12 myoblasts were seeded at a density of 150,000 cells per well in 12-well plates. The next day, cells were transfected with CRISPRa plasmids using Lipofectamine 3000 (#L3000001, Thermo Fisher Scientific) according to the manufacturer's instructions. For each well, 400 ng of Lenti MS2-P65-HSF1_Hygro and SP-dCas9-VPR plasmids, together with 20 ng of Lenti sgRNA(MS2)_zeo, were used. After 24 h, the medium was changed to fresh cell culture medium. The cells were harvested 48 h after

the transfection by adding 350 μ L Qiazol per well and cell pellets were frozen on dry ice and stored at -80°C until further analysis.

For luciferase reporter experiments, C2C12 cells (1.5×10^3 per well) were seeded in Thermo Nunc F-bottom 96-well plates. After 24 h, cells were transfected using Lipofectamine 3000 and 40 ng of Lenti MS2-P65-HSF1_Hygro and SP-dCas9-VPR plasmids, together with 2 ng of Lenti sgRNA(MS2)_zeo, and 1 ng of the corresponding luciferase reporter plasmid.

Animals and ethical approval

Mice were housed in individually ventilated cages (IVC) at a temperature of $22 \pm 1^{\circ}\text{C}$ with a 12-hour light/12-hour dark cycle and ad libitum access to food and drinking water. Procedures were reviewed and approved by the Landesamt for Verbraucherschutz und Gesundheit (LAVG) Brandenburg, Germany under the registration AZ2347-4-2021; or conducted at the Department of Nutrition, Exercise and Sports, University of Copenhagen, with approval from the Danish Animal Experiments Inspectorate (license no. 2020-15-0201-00628). At the University of Copenhagen, mice were housed in open cages at $22 \pm 1^{\circ}\text{C}$ with a 12-hour light/12-hour dark cycle and ad libitum access to food and drinking water.

CRISPRa and gRNA design

Guide RNAs were designed using CRISPOR⁴⁸, selecting target sequences located within ~ 250 bp upstream of the two *Gdf15* TSSs annotated in MGI. Target-specific gRNA sequences were cloned into the lenti sgRNA(MS2)_zeo backbone plasmid (Addgene #61427) using Golden Gate cloning. For every TSS, 7 to 8 distinct sgRNAs were chosen. The corresponding sequences are provided below (Table 2). gRNA gene activation efficiencies were evaluated *in vitro* using the SP-dCas9-VPR plasmid (Addgene #63798), encoding the tripartite activator VP64-p65-Rta (VPR) fused to the C-terminus of dCas9⁴⁹, and the lenti MS2-P65-HSF1_Hygro plasmid (Addgene #61426), encoding the MS2-p65-HSF1 fusion protein, which binds to hairpin aptamers appended to the gRNA. The luciferase reporter plasmid was adapted from *pAAVS1-CMVdel_SV40_minP_mNeonGreen bpA-miniU6-attP-Inv(minipA-HSV-TKArtfEf1a)-attP-Ef1a-PuroR-P2A-mRuby-TypA* (gift from the Institute of Developmental Genetics, Helmholtz Munich) by Gibson assembly-mediated replacement of the mNeonGreen cassette with Gaussia Signal Peptide-NanoLuc (NLuc) as the primary reporter and mRuby with Firefly luciferase as the background reporter. To generate the *Gdf15* promoter-activated Luciferase assay plasmid, the 3.64 kb (LP) or 0.25 kb (SP) region upstream of the *Gdf15* TSS81640 (MGI:6009407) was cloned into the plasmid via Gibson Assembly.

Intein-split dCas9-SAM activator system

The AAV constructs were designed as split-Cas system with N-dCas9 (SpCas92–573) fused to DnaE-N-Intein and C-dCas9-(SpCas9574–1368) fused to VP64 and DnaE-C-Intein, as described in³³ and synthesized by VectorBuilder. The third AAV plasmid, containing MS2-P65-HSF1:hU6-gRNA5-hH1-gRNA7, was cloned following the STAgR (String Assembly gRNA Cloning) approach⁵⁰. The plasmid pAAV-MS2-P65-HSF1:hU6-gRNAscramble was generated from pAAV-MS2-P65-HSF1:hU6-gRNA5-hH1-gRNA7 by

replacing the hU6-gRNA5-hH1-gRNA7 cassette with a hU6-gRNA scramble sequence using Gibson assembly.

All AAVs were produced by the viral core facility (VCF) of the Charité – Universitätsmedizin Berlin (vcf.charite.de) in HEK293T cells, using standard protocols followed by iodixanol gradient ultracentrifugation and subsequent iodixanol-PBS exchange using Millipore Amicon Ultra Centrifugal filtration. For the Rep/Cap helper plasmid a muscle specific capsid was used, MYOAAV2a, an AAV9 capsid derivative (kind gift of Mohammadsharif Tabebordbar³⁴)

Luciferase reporter assay

The luciferase assay was performed 24 h after medium change using the Nano-Glo Dual-Luciferase Reporter Assay System (Promega) and quantified with a Varioskan Lux microplate reader (Thermo Fisher Scientific) according to the manufacturer's instructions. Firefly luciferase activity was measured first, followed by NanoLuc activity in the same wells, as the Firefly substrate also provides the lysis buffer, while the NanoLuc substrate quenches the Firefly signal.

Viral vectors (Table 3)

Study 1a: intravenous dose response

Supplementary Fig. 1

Study design

Chow-fed 7-10-week-old male C57BL/6J were injected intravenously with vehicle or MyoAAV2a encoding for different component of the CRISPRa system. Vectors were administered at three titers to explore the relationship between dose of vector and expression of *Gdf15*. Three weeks later mice were euthanized and plasma and organs were collected.

Viral vector administration

Mice were anesthetized with 3% isoflurane in O₂ and injected intravenously with vehicle (PBS) or MyoAAV2a-C-dCas9-VP64 + MyoAAV2a-N-dCas9 (MyoAAV-dCas9-VP64) at a titer of 5×10^{11} vg as controls. Treatment groups received MyoAAV2a-C-dCas9-VP64, MyoAAV2a-N-dCas9, and MyoAAV2a-SAM-gRNA5+7 (MyoAAV-CRISPRa-*Gdf15*) at three different titers: low (1×10^{11} vg), medium (2.5×10^{11} vg), or high (5×10^{11} vg). Vehicle and vectors were injected at a volume of 50 - 100 µl.

Study 1b: intravenous delivery in wildtype mice

Fig. 2 and Supplementary Fig. 2

Study design

Chow-fed 11-week-old C57BL/6J female mice were injected intravenously 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 high fat high sucrose diet (HFHSD; Research Diets #D12331) containing 58 kcal% fat. Mice were maintained on this diet for 12 weeks, and body weight and food intake were monitored weekly. Every 2 weeks, body composition was assessed by NMR (Echo Medical Systems, EchoMRI 700), and blood was collected from the tail vein to measure circulating GDF15 by ELISA. After 6 weeks on HFHSD, energy expenditure was determined by indirect calorimetry (Phenomaster, TSE Systems, Germany). After 12 weeks on HFHSD, a glucose tolerance test (GTT) was performed, and mice were euthanized the following day and plasma and organs were collected.

Viral vector administration

Mice were anesthetized with 3% isoflurane and injected intravenously with vehicle (PBS), MyoAAV2a-eGFP (MyoAAV-GFP), or MyoAAV2a-C-dCas9-VP64 + MyoAAV2a-N-dCas9 (MyoAAV-dCas9-VP64) at a titer of 5×10^{11} vg as controls. The treatment group received MyoAAV2a-C-dCas9-VP64 + MyoAAV2a-N-dCas9 + MyoAAV2a-SAM-gRNA5+7 (MyoAAV-CRISPRa-*Gdf15*) at a titer of 5×10^{11} vg. Vehicle and vectors were injected at a volume of 50 – 100 μ l.

Indirect calorimetry

In week 6 on HFHSD, mice were transferred to an indirect calorimetry system (PhenoMaster metabolic cage system, TSE Systems, Bad Homburg, Germany) and acclimatized to the new environment for 3 days before continuous assessment of whole body energy metabolism. In week 7, the system recorded oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory exchange ratio (RER), and energy expenditure (EE), along with habitual locomotor activity quantified by infrared beam breaks in the x, y, and z axes. Mice had ad libitum access to HFHSD and water throughout the study period. Afterwards, mice were returned to their home cages.

Glucose tolerance test

Mice were fasted for 5 hours from the onset of the light phase. At the end of the fasting period, a blood sample was collected from the tail vein to measure basal blood glucose. Mice were then injected intraperitoneally with glucose at a dose of 2 g/kg body weight, and blood glucose was measured using handheld glucometers at 20, 40, 60, 90, and 120 minutes after injection.

Study 2: intramuscular delivery in wildtype mice

Supplementary Fig. 3

Study design

Chow-fed 12-week-old male C57BL/6J mice were intramuscularly injected 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. Mice were kept on this diet for 16 weeks and body weight and food intake were monitored on a weekly basis. Every two weeks, body composition was determined by NMR (EchoMRITM-Analyzer (Echo Medical Systems)), and blood was taken from the tail to measure circulating GDF15 by ELISA. After 13 weeks on HFHSD, mice underwent a GTT. Energy expenditure was determined by indirect calorimetry (Phenomaster, TSE Systems, Germany) in week 14; and mice were euthanized in week 16 and plasma and organs were collected.

Viral vector administration

Mice were anesthetized with 2-4% isoflurane. The injection sites (both tibialis anterior, both triceps, both quadriceps, and both gastrocnemius muscles) were disinfected and injected with vehicle (PBS), MyoAAV2a-eGFP (MyoAAV-GFP), or MyoAAV2a-C-dCas9-VP64 + MyoAAV2a-N-dCas9 (MyoAAV-dCas9-VP64) at a titer of 1×10^{10} vg per muscle injected as controls. The treatment group received MyoAAV2a-C-dCas9-VP64 + MyoAAV2a-N-dCas9 + MyoAAV2a-SAM-gRNA5+7 (MyoAAV-CRISPRa-*Gdf15*) at a titer of 1×10^{10} vg per muscle injected. Vehicle and vectors were injected at a volume of 30 μ l per muscle. For analgesia, mice were treated with 0.1 mg/mL Tramadol in drinking water for two days before and three days after injections. Furthermore, mice received a subcutaneous injection of 5 mg/kg Carprofen right before intramuscular injections.

Glucose tolerance test

Mice were fasted for six hours from the onset of the light phase. At the end of the fasting period, an initial blood sample was collected from the tail to measure basal blood glucose. Then mice were injected intraperitoneally (i.p.) with a dose of 2 g of glucose per kg of body weight, and blood glucose was measured using handheld glucometers 15 min, 30 min, 60 min and 120 min after injection.

Indirect calorimetry

Energy expenditure was determined using the TSE Phenomaster System. Mice were single-housed in TSE cages for three days for acclimatization to the new environment, as well as feeding and drinking systems. Afterwards daily energy expenditure was measured inside the TSE Phenomaster at $23 \pm 1^\circ\text{C}$ for three days. Afterwards mice were returned to their home cages.

Study 3: intravenous delivery in *Gfral* knockout mice

Fig. 3 and Supplementary Fig. 4

Study design

Chow-fed 12-17-week-old female *Gfral* wildtype (*Gfral*^{+/+}, WT) and knockout (*Gfral*^{-/-}, KO) mice⁵¹ were injected intravenously with MyoAAV2a encoding for different component of the CRISPRa system with either a scramble gRNA or one targeting *Gdf15*. One week later, all mice were switched to a high fat high

sucrose diet (HFHSD; Research Diets #D12331) containing 59 kcal% fat. Mice were maintained on this diet for 11 weeks, and body weight and food intake were monitored weekly. Every 2 weeks, body composition was assessed by NMR (Echo Medical Systems, EchoMRI 700), and blood was collected from the tail vein to measure circulating GDF15 by ELISA. After 10 weeks on HFHSD, a GTT was performed, feces were collected, and mice were euthanized the following week and plasma and organs were collected.

Viral vector administration

Mice were anesthetized with 3% isoflurane and injected intravenously with MyoAAV2a-C-dCas9-VP64 + MyoAAV2a-N-dCas9 + MyoAAV2a-SAM-gRNAscr (MyoAAV-CRISPRa-Scr) as control and MyoAAV2a-C-dCas9-VP64 + MyoAAV2a-N-dCas9 + MyoAAV2a-SAM-gRNA5+7 (MyoAAV-CRISPRa-*Gdf15*) as treatment. Vectors were injected at a titer of 5×10^{11} vg and in a volume of 80 - 100 μ l.

Glucose tolerance test

Mice were fasted for 5 hours from the onset of the light phase. At the end of the fasting period, blood was collected from the tail vein to measure basal blood glucose. Mice were then injected intraperitoneally with glucose at a dose of 2 g/kg body weight, and blood glucose was measured using handheld glucometers at 20, 40, 60, 90, and 120 minutes after injection.

Feces collection

Feces produced over 7 days during week 11 after the start of HFHSD were collected, freeze dried, and stored frozen in sealed tubes until further processing.

Study 4: intravenous delivery in *Gdf15* knockout mice

Fig. 4

Study design

Chow-fed 12-14-week-old female *Gdf15* wildtype (*Gdf15*^{+/+}, WT) and knockout (*Gdf15*^{-/-}, KO) mice⁵² were injected intravenously with MyoAAV2a encoding for different component of the CRISPRa system with either a scramble gRNA or one targeting *Gdf15*. One week later, all mice were switched to a HFHSD (EF D12331, ssniff Spezialdiäten GmbH, Germany) containing 58 kcal% fat. Mice were maintained on this diet for 15 weeks, and body weight and food intake were monitored weekly. Every two weeks, body composition was assessed by NMR (EchoMRITM-Analyzer (Echo Medical Systems)). Blood was collected from the tail vein in week 1, 3, 5, 9 and 15 to measure circulating GDF15 by ELISA. After 6 weeks all mice were given access to a running wheel for seven days. Feces produced over seven days were collected in week 6. After 14 weeks a GTT was performed and mice were euthanized the following week and plasma and organs were collected.

Viral vector administration

Mice were injected intravenously with MyoAAV2a-C-dCas9-VP64 + MyoAAV2a-N-dCas9 + MyoAAV2a-SAM-gRNA_{scr} (MyoAAV-CRISPRa-Scr) as control and MyoAAV2a-C-dCas9-VP64 + MyoAAV2a-N-dCas9 + MyoAAV2a-SAM-gRNA₅₊₇ (MyoAAV-CRISPRa-*Gdf15*) as treatment. Vectors were injected at a titer of 5×10^{11} vg and in a volume of 100 μ l.

Voluntary wheel running

Three days before the start of the running wheel experiment, mice were acclimatized to open cages in the experimental room by removing the lid of the IVC cages. After acclimatization, mice were single-housed in the running wheel cages (TSE Systems GmbH, Germany) for 7 days. The first four days were considered as acclimatization to the running wheels, and data from the last three days are presented. At the end of the experiment, mice were placed back in their IVC cages.

Feces collection

Feces produced over 7 days during week 6 after start of HFHSD were collected, freeze-dried and stored in desiccator until further processing.

Glucose tolerance test

In week 14, mice were fasted for six hours from the onset of the light phase. After fasting, a blood sample was taken from the tail tip to measure basal blood glucose levels using a Contour Next handheld glucometer (Bayer). Then, mice received an intraperitoneal injection of 2 g of glucose per kg of body weight. Blood glucose levels were measured 15, 30, 60, and 120 minutes after the injection.

Study 5: intravenous delivery in obese mice

Supplementary Fig. 5

Study design

Eight-week-old female C57BL/6J mice were switched from chow diet to a high-fat high-sucrose diet (HFHSD; Research Diets #D12331) containing 58 kcal% fat to induce obesity. After 28 weeks on HFHSD, at 36 weeks of age, mice were injected intravenously with MyoAAV2a encoding the CRISPRa system with either a scrambled gRNA or gRNAs targeting *Gdf15*. Mice were maintained on HFHSD for an additional 14 weeks after viral vector administration. Body weight and food intake were monitored weekly, and body composition was assessed by NMR (Echo Medical Systems, EchoMRI 700). In week 14 after viral vector administration, a glucose tolerance test (GTT) was performed and feces were collected over 7 days to determine fecal output, energy density, and total fecal energy excretion. At the end of the study, mice were euthanized and plasma and organs were collected.

Viral vector administration

Mice were anesthetized with 3% isoflurane and injected intravenously with MyoAAV2a-C-dCas9-VP64 + MyoAAV2a-N-dCas9 + MyoAAV2a-SAM-gRNAscr (MyoAAV-CRISPRa-Scr) as control or MyoAAV2a-C-dCas9-VP64 + MyoAAV2a-N-dCas9 + MyoAAV2a-SAM-gRNA5+7 (MyoAAV-CRISPRa-Gdf15) as treatment. Vectors were injected at a titer of 5×10^{11} vg and in a volume of approximately 100 μ l.

Glucose tolerance test

In week 14 after viral vector administration, mice were fasted for 5 hours from the onset of the light phase. At the end of the fasting period, blood was collected from the tail vein to measure basal blood glucose. Mice were then injected intraperitoneally with glucose at a dose of 2 g/kg body weight, and blood glucose was measured using handheld glucometers at 20, 40, 60, 90, and 120 minutes after injection.

Feces collection

Feces produced over 7 days during week 14 after viral vector administration were collected, freeze dried, and stored frozen in sealed tubes until further processing. Fecal energy density was determined by bomb calorimetry, and total fecal energy excretion was calculated from fecal dry mass and energy density and expressed both as absolute energy excretion and normalized to body weight.

Tissue and blood analyses

Measurement of plasma GDF15. Plasma levels of GDF15 (Mouse/Rat GDF-15 Quantikine ELISA Kit, MGD150, R&D Systems, Inc.) were determined according to the manufacturer's instructions. Plasma samples were diluted 1:5 with sample buffer provided in the ELISA for all analyses.

Fecal energy excretion

For assessment of excreted energy via feces⁵³, freeze-dried feces were weighed, homogenized and pressed into a pellet of approximately 1 g and total energy content was determined by bomb calorimetric combustion (catalog no. IKA C5003; IKA Werke).

RNA isolation and quantitative PCR

RNA from tissues was isolated by Trizol-Chloroform (peqGOLD TriFast™, 30-2010P, VWR) extraction in homogenized tissues and precipitation with Isopropanol. Genomic DNA was removed (DNase I, EN0521, Fisher Scientific; RiboLock RNase Inhibitor, E00382, Fisher Scientific) and 1 μ g RNA was transcribed into cDNA using LunaScript RT Super Mix (E3010L, New England Biolabs). SYBR green qPCR was performed using Precision plus qPCR Mastermix containing SYBR green (Luna Universal qPCR Master Mix, M3003E, New England Biolabs). Primers used were *Gdf15*: F: CCGAGAGGACTCGAACTCAG; R: ACCCAATCTCACCTCTGGA, *Hprt*: F: TCAGTCAACGGGGGACATAAA; R: GGGGCTGTACTGCTTAACCAG, *Tbp*: F: AGAACAATCCAGACTAGCAGCA; R: GGGAACCTTCACATCACAGCTC. qPCR was performed in 384-well plates on a VIA7 Real Time PCR System (4453534, Thermo Fisher Scientific) using 2 min

preincubation at 95°C followed by 45 cycles of 60 s at 60°C and melting curves were performed by stepwise increasing the temperature from 60°C to 95°C. Quantification of mRNA expression was performed according to the delta-delta Ct method.

RNA sequencing and analysis

The RNA sequencing was carried out by Novogene via DNBseq, generating ~ 65 million raw reads per sample (read length 150bp, paired-end reads). After adapter trimming and quality filtering, ~63 million cleaned reads per sample have been aligned to the mouse reference mm39 using HISAT2 v2.2.1. Quantification has been performed via Counts v2.0.6. Gene expression values have been delivered by Novogene. Differential gene expression was calculated using DESeq2 (v1.50.2). GO Biological Process (BP4) enrichment of 122 differentially expressed genes was performed using the DAVID online tool^{54,55} (<https://davidbioinformatics.nih.gov/>) with EASA at 0.05 and minimum gene count cutoff of 5.

16S rRNA gene amplicon sequencing analysis

Cecal content was subjected to 16S rRNA gene amplicon sequencing. DNA was extracted from 600 µL of the sample–stabilizer mixture after bead-beating with 0.1-mm glass beads (FastPrep-24, MP Biomedicals) and purified using the Maxwell® RSC Fecal Microbiome DNA Kit (Promega, USA) on the Maxwell® instrument according to the manufacturer's instructions.

The V3–V4 region of the bacterial 16S rRNA gene was amplified in a two-step PCR to minimize amplification bias. In the first PCR, primers 341F (5'-CCTACGGNGGCWGCAG-3') and 785R (5'-GACTACHVGGGTATCTAATCC-3'), each containing adapter sequences, were used. In the second PCR, sample-specific barcodes and Illumina sequencing adapters (P5/P7) were attached. PCR products were cleaned using magnetic beads (Beckman Coulter), quantified fluorometrically, normalized to 2 nM, and pooled. The multiplexed samples were sequenced on an Illumina MiSeq platform in paired-end mode (2 × 250 bp, v3 chemistry). Quality control included sequencing a mock community (ZymoBIOMICS Microbial Community Standard, D6300) as a positive control, as well as negative extraction and PCR controls.

Raw sequencing data were processed using the IMNGS pipeline⁵⁶, based on the UPARSE approach⁵⁷. Reads were demultiplexed, quality trimmed at the first base with a Phred score < 3, and merged. Reads shorter than 300 bp or longer than 600 bp, or with an expected error rate > 3, were excluded. To minimize GC bias, 5 nucleotides were trimmed from both ends of remaining reads. Chimeric sequences were removed using UCHIME⁵⁸. OTUs were clustered at 97% similarity, and those occurring at a relative abundance < 0.25% in all samples were discarded to avoid spurious taxa⁵⁹. Taxonomy was assigned using the RDP classifier (v2.11) with confirmation against the SILVA database at 80% confidence⁶⁰. Statistical analyses were performed in R v 4.x using the Rhea pipeline⁶¹. Absolute read counts were normalized by Total Sum Count to the minimum sequencing depth across samples. Sequencing depth was evaluated via rarefaction curves. α -diversity was calculated using species richness (e.g., OTU

assigned to species) and the Shannon effective number based on generalized UniFrac distances⁶². Statistical significance was set at $p \leq 0.05$, with multiple testing correction by the Benjamini–Hochberg method. Taxa with $\geq 30\%$ prevalence in at least one group were included in downstream analyses.

Statistical analysis

Data (excl. RNA-seq and microbiome data) were analyzed with GraphPad Prism (GraphPad Software, San Diego, CA, version 10.2.3). Values are presented as means $\pm$ standard error of the mean. $p < 0.05$ was considered statistically significant. The type of statistical testing performed is described in the figure legends. Differences in energy expenditure were calculated using ANCOVA with body weight as covariate using MMPC Energy Expenditure Analysis (<https://www.mmpc.org/shared/regression.aspx>).

Declarations

Competing interests

The authors declare no competing interests.

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Data availability

The RNA-seq and 16S rRNA gene amplicon sequencing datasets will be deposited in public repositories, and accession numbers will be provided upon publication.

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Tables

Table 1. Reagents and resources

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
MyoAAV2a-C-dCas9-VP64	Viral Core Facility, Charité / VectorBuilder	N/A
MyoAAV2a-N-dCas9	Viral Core Facility, Charité / VectorBuilder	N/A
MyoAAV2a-SAM-gRNA5+7	Viral Core Facility, Charité / this paper	N/A
MyoAAV2a-eGFP	Viral Core Facility, Charité	N/A
MyoAAV2a-SAM-gRNAscr	Viral Core Facility, Charité / this paper	N/A
Critical commercial assays		
Mouse/Rat GDF-15 Quantikine ELISA Kit	R&D Systems	MGD150
Nano-Glo Dual-Luciferase Reporter Assay System	Promega	N/A
LunaScript RT Super Mix	New England Biolabs	E3010L
Luna Universal qPCR Master Mix	New England Biolabs	M3003E
Experimental models: Cell lines		
C2C12 mouse myoblasts	ATCC	CRL-1772
HEK293T cells used for AAV production	ATCC	CRL-3216
Experimental models: Organisms/strains		
Mouse: C57BL/6J	Charles River Laboratories	
Mouse: Gfral WT and KO	In house	Reference 52
Mouse: Gdf15 WT and KO	In house	Reference 53
Recombinant DNA		
lenti sgRNA(MS2)_zeo	Addgene	61427
SP-dCas9-VPR	Addgene	63798
lenti MS2-P65-HSF1_Hygro	Addgene	61426
Split dCas9-SAM AAV constructs	VectorBuilder / this paper	
Deposited data		

REAGENT or RESOURCE	SOURCE	IDENTIFIER
RNA-seq dataset	This paper	Accession to be provided upon publication
16S rRNA gene amplicon dataset	This paper	Accession to be provided upon publication
Software and algorithms		
CRISPOR		Reference 49
GraphPad Prism	GraphPad Software	Version 10.2.3
DESeq2	Bioconductor	TBD
DAVID		References 55 and 56
IMNGS / UPARSE workflow		References 57-61
Rhea		Reference 62
Other		
High-fat, high-sucrose diet D12331	Research Diets	D12331
High-fat, high-sucrose diet EF D12331	ssniff Spezialdiäten GmbH	EF D12331

Table 2. gRNA sequences used in this study

Name	Sequence (5' - 3')
Gdf15 gRNA1	CTTA ACTCAAAGACACACTC
Gdf15 gRNA2	GGGTATAAAGGCAGCTGCAG
Gdf15 gRNA3	CGGAGCGGACAGAGTCCAGT
Gdf15 gRNA4	CCCCGTGTGCGATGCCAACT
Gdf15 gRNA5	GGTGACCAGGCTAATTCGGG
Gdf15 gRNA6	GGGAATCCCAGGGCTCCGCA
Gdf15 gRNA7	GTGGGAGGGAGGGAAACCGC
Gdf15 gRNA8	CGTCCCCACAGAACATGTGA
Gdf15 gRNA9	ATACTGGGAGGGACCCCAGC
Gdf15 gRNA10	GGAGTGACCCACAGGTCAG
Gdf15 gRNA11	CTTGTCTTCACAGGGGGACA
Gdf15 gRNA12	TCTTGGACAAAGGAGATGGC
Gdf15 gRNA13	GGATCTGAGGAGGAGTGGTC
Gdf15 gRNA14	TAGTTCTGGGCAGTGCATCA
Gdf15 gRNA15	AGAGTCACCCCCGTAGGCAA
Gdf15 gRNA16	GCCCACAGCTGCGATCCCAA
Scramble gRNA	GGAGACGGGATACCGTCTCT

Table 3. Viral vectors used in the in vivo studies

Serotype	Vector name	Short name
MyoAAV2a	pAAV-C-dCas9aa574–1368-VP64-C-intein	MyoAAV2a-C-dCas9-VP64
MyoAAV2a	pAAV-N-dCas9aa1–573-N-intein	MyoAAV2a-N-dCas9
MyoAAV2a	pAAV-MS2-P65-HSF1:hU6-gRNA5-hH1-gRNA7	MyoAAV2a-SAM-gRNA5+7
MyoAAV2a	pAAV-CAG:eGFP	MyoAAV2a-eGFP
MyoAAV2a	pAAV-MS2-P65-HSF1:hU6-gRNAscramble	MyoAAV2a-SAM-gRNAscr

Figures

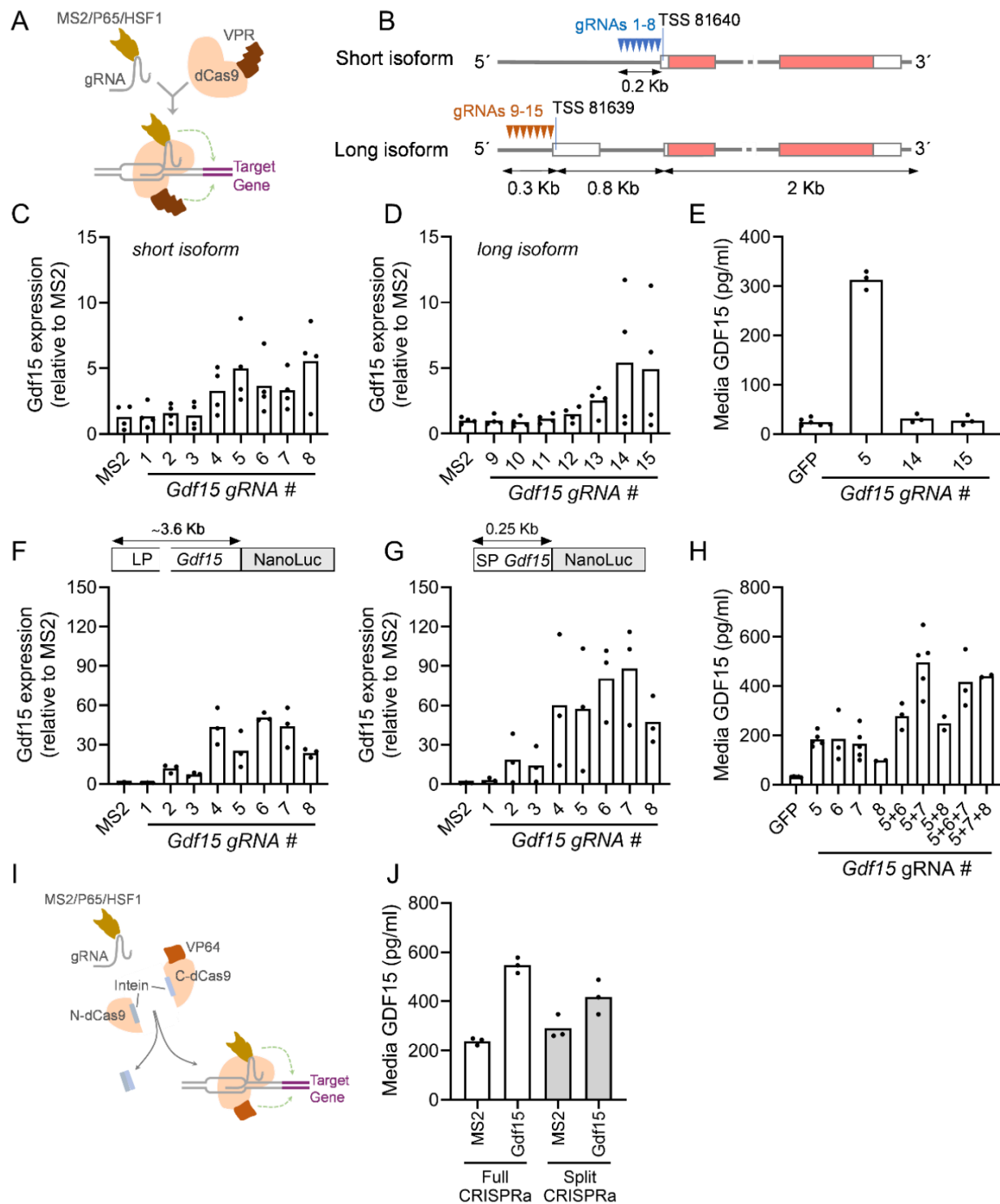


Figure 1

CRISPRa of *Gdf15* triggers GDF15 release from muscle cell *in vitro*. (A) Schematic of CRISPR activation (CRISPRa) systems comprising a nuclease-deficient Cas9 (dCas9) fused with VPR; and guide RNA (gRNA) tethered to MS2-P65-HSF1. (B) Two sets of gRNAs targeting either the promoter region of the short (gRNAs 1-8) or long (gRNAs 9-15) *Gdf15* isoform. (C,D) *Gdf15* mRNA abundance and (E) media GDF15 protein levels from C2C12 myoblasts 48 h after transfection with plasmids encoding the CRISPRa

system with indicated gRNAs. (F-G) CRISPRa-mediated *Gdf15* promoter activation determined by use of luciferase reporter constructs with short (SP) and long (LP) promoter fragments. (H) Media GDF15 levels from C2C12 myoblasts 48 h post-transfection with plasmids encoding CRISPRa system with indicated combinations of gRNAs. (I) Schematic of intein-split CRISPRa system with dCas9-VP64 split into an N- and C-terminal portion. (J) Media GDF15 levels from C2C12 myoblast cells 48 h post transfection with plasmid containing full (A) or split (I) CRISPRa system with gRNAs 5+7. (C-H,J) Data are presented as mean values plus individual data points. Data are shown as mean with each dot representing a biological replicate.

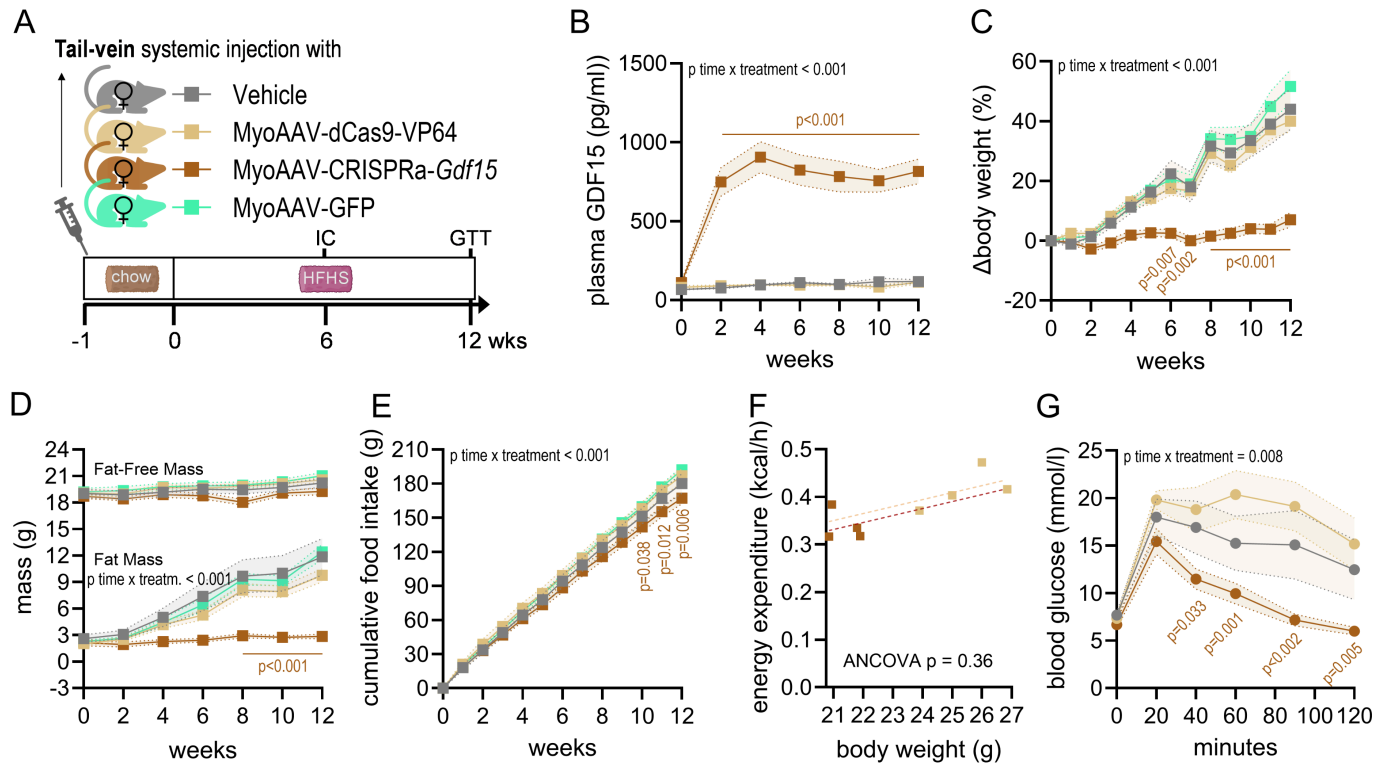


Figure 2

Muscle-targeted CRISPRa of *Gdf15* elevates circulating GDF15 and protects against diet-induced obesity. (A) Schematic of study design (with sample size per group shown except for E and F) with chow-fed female mice receiving an intravenous injection in the tail vein with vehicle (PBS), MyoAAV2a encoding for split dCas9-VP64, MyoAAV2a encoding for split CRISPRa system targeting *Gdf15* (with gRNA 5+7), or MyoAAVs encoding for GFP. One week after the injection, mice were switched to a high-fat, high-sucrose (HFHS) diet. 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 ($n = 4$, because mice were double-housed), were measured. (F) Energy expenditure was determined by indirect calorimetry ($n = 4$, because mice were double-housed) in week 7. (H) Glucose tolerance was tested in week 12. (B-E, H) data are presented as mean values $\pm$ SEM. Statistic: (B-E, H) repeated measures two-way (time $\times$

treatment) ANOVA with Šidák post hoc testing. Data in (F) were analyzed using ANCOVA with body weight as co-variate.

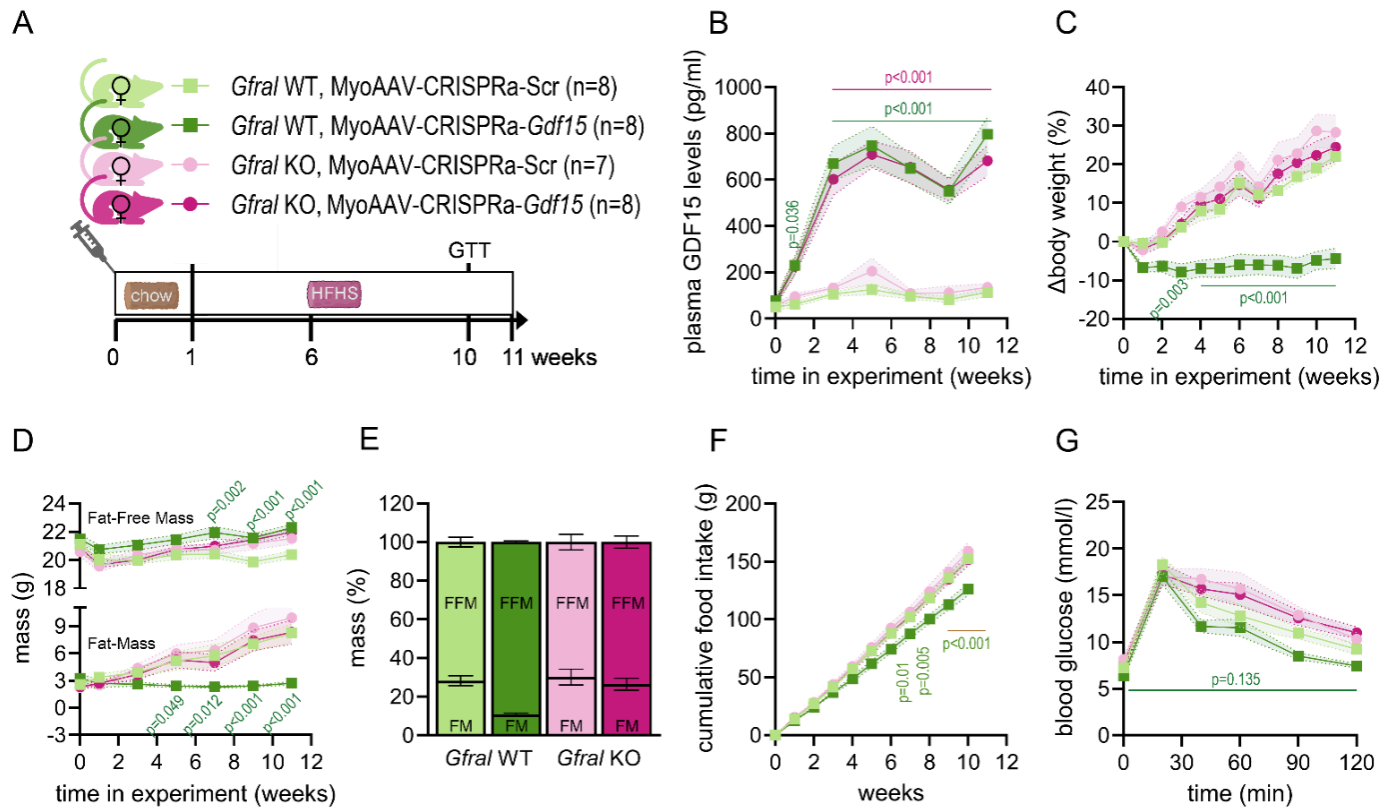


Figure 3

CRISPRa-*Gdf15* confers resistance to diet-induced obesity via the GDF15-GFRAL-axis. (A) Schematic of study design (with sample size per group except for F) with chow-fed female *Gfral* WT or *Gfral* KO mice received an injection in the tail vein with MyoAAV2a encoding for the split CRISPRa system targeting *Gdf15* (with gRNA 5+7) or a gRNA with a scrambled (Scr) targeting sequence. One week after the injection, mice were switched to a high-fat, high-sucrose (HFHS) diet. Over the course of the experiment, (B) plasma GDF15 levels, (C) body weight change, (D, E) fat-free mass and fat mass, and (F) cumulative food intake (n = 4 because mice were double housed when possible), were measured. (G) Glucose tolerance was determined in week 11. (B-G) data are presented as mean values +/- SEM. Statistic: (B-D, F, G) repeated measures two-way (time x genotype+treatment) ANOVA with Šidák post hoc testing.

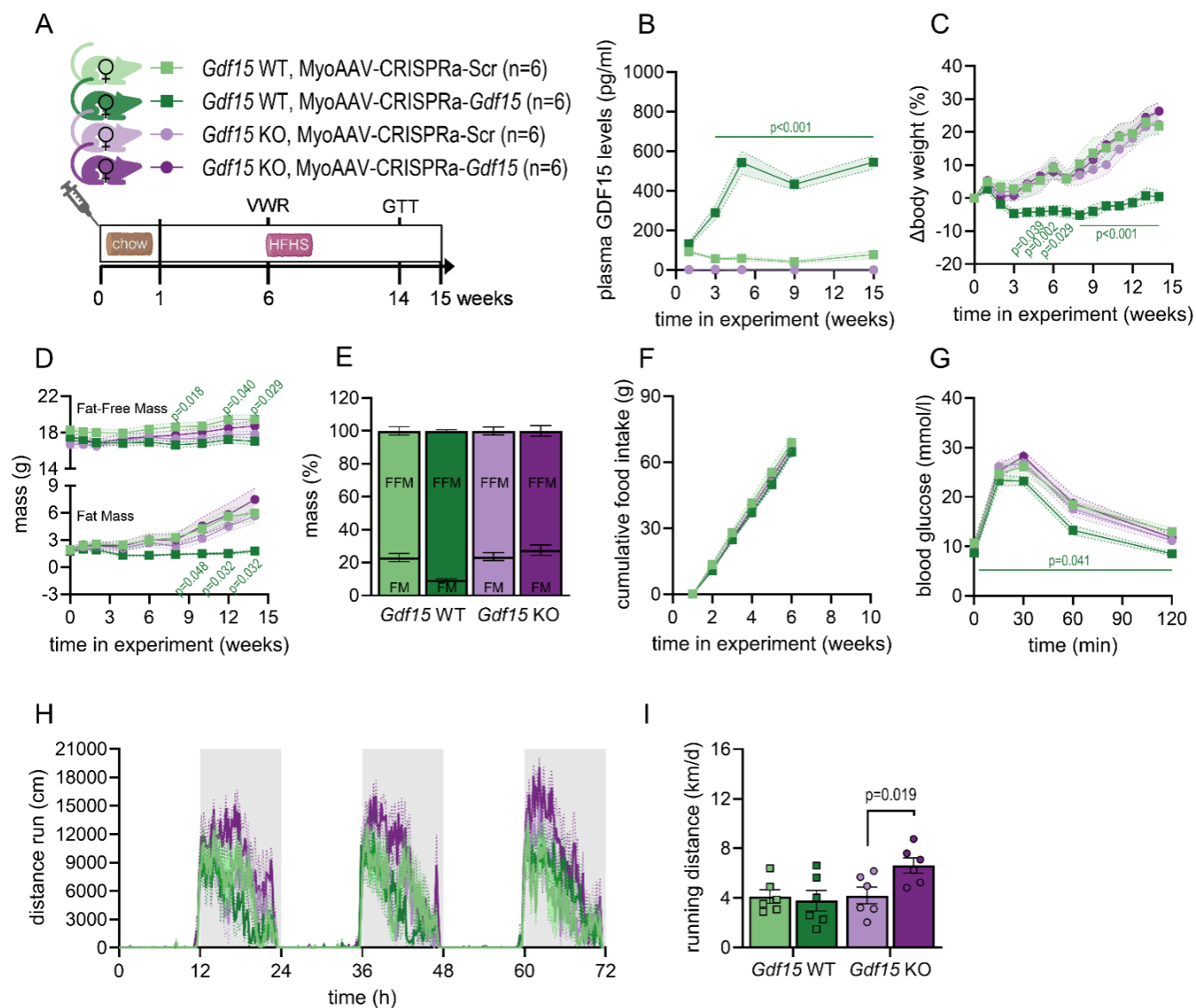


Figure 4

CRISPRa-*Gdf15* confers resistance to diet-induced obesity via the *Gdf15* locus axis and in the absence of suppression of exercise motivation. (A) Schematic of study design (with sample size per group shown except for F) with chow-fed female *Gdf15* WT or *Gdf15* KO mice received an injection in the tail vein with MyoAAV2a encoding for the split CRISPRa system targeting *Gdf15* (with gRNA 5+7) or a gRNA with a scrambled (Scr) targeting sequence. One week after the injection, mice were switched to a high-fat, high-sucrose (HFHS) diet. Over the course of the experiment, (B) plasma GDF15 levels, (C) body weight change, (D, E) fat-free mass and fat mass, and (F) cumulative food intake ($n = 3$ because mice were double housed), were measured. (G) Glucose tolerance was determined in week 14. (H, I) During week 6 all mice were given access to a running wheel and the distance run during the light and dark phase were recorded. (B-I) data are presented as mean values $\pm$ SEM. Statistic: (B-D, F, G) repeated measures two-way (time $\times$ genotype+treatment) ANOVA with Šidák post hoc testing. (H, I) Two-way (genotype $\times$ treatment) ANOVA with Šidák post hoc testing.

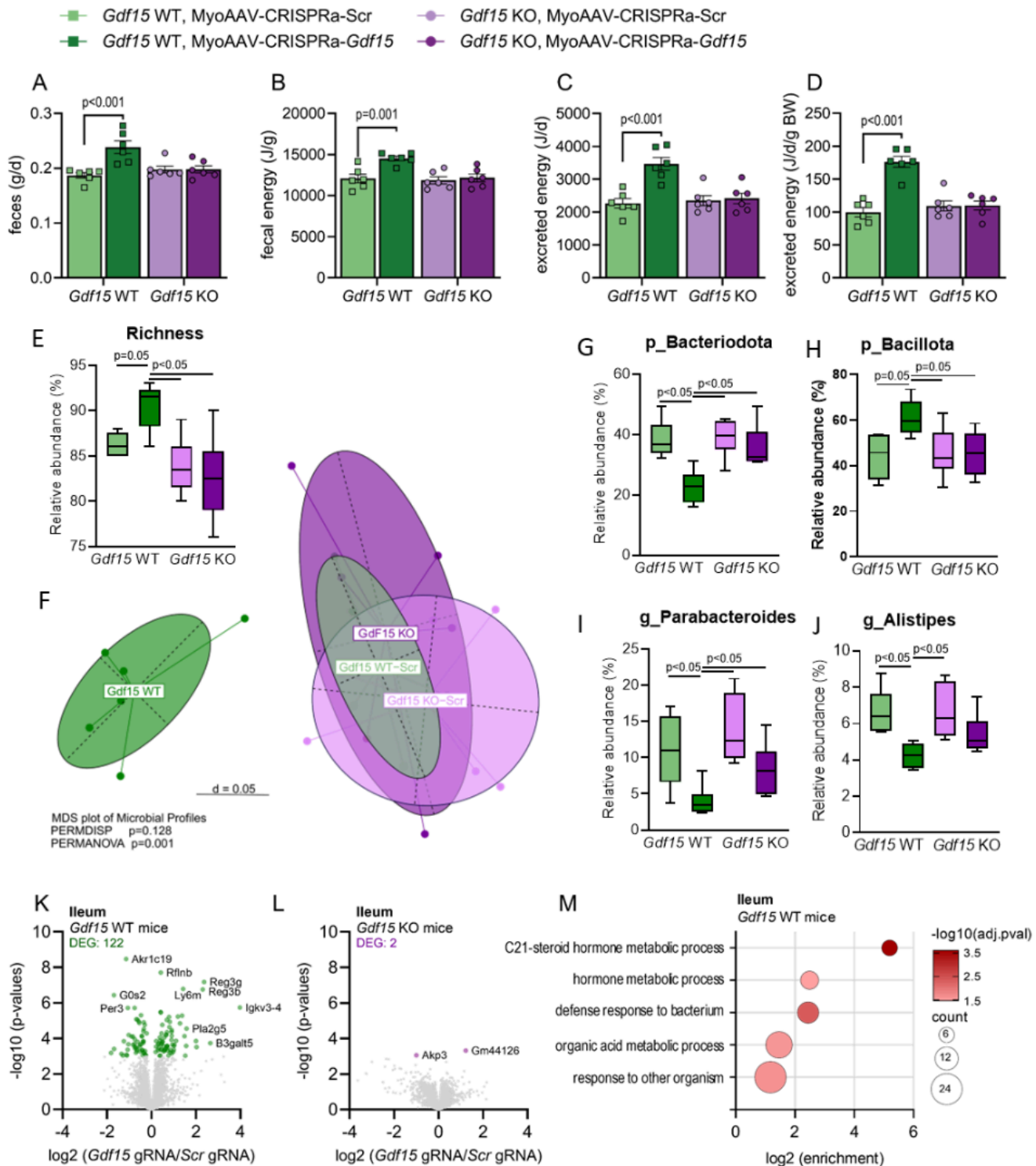


Figure 5

Elevated GDF15 is linked to fecal energy loss, altered microbiome composition, and ileal transcriptional reprogramming. In the study shown in Figure 4, feces were collected in week 6 to determine (A) total feces mass, (B) fecal energy density and (C,D) overall energy excreted. Cecal content and small intestine segments were collected at the end of the study. The cecal microbiome composition was profiled and (E) richness as marker of alpha-diversity, (F) beta-diversity, and (G-J) differential abundances at phylum

and genus level were determined. (K,L) Show differentially expressed genes (DEG) in the ileum of (K) *Gdf15* WT and (L) *Gdf15* KO mice, with (M) displaying the top 5 GO Biological Process BP4 enriched in *Gdf15* WT mice. (A-D) data are presented as mean values $\pm$ SEM with individual dots represent distinct animals. (E, G-J) data are presented as Tukey box plots with median, IQR, and whiskers. (F) Beta-diversity shown in multidimensional scaling (MDS) plot. Statistic: (A-D) Two-way (genotype x treatment) ANOVA with with Šidák post hoc testing. (E,G-J) Wilcoxon Rank Sum Test with multiple testing correction by the Benjamini–Hochberg method for adjusted p-values. (F) Permutational multivariate analysis of variance (PERMANOVA) (K,L) DESeq2 analyses with p-value <0.001 considered as a DEG. For E to J the 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 K and L 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).

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