CRISPR-mediated activation of a promoter or enhancer rescues obesity caused by haploinsufficiency


INTRODUCTION: Loss-of-function mutations in one gene copy can lead to reduced amounts of protein and, consequently, human disease, a condition termed haploinsufficiency. It is currently estimated that more than 660 genes cause human disease as a result of haploinsufficiency. The delivery of extra copies of the gene by way of gene therapy is a promising therapeutic strategy to increase gene dosage in such conditions. Recombinant adeno-associated virus (rAAV) provides a promising tool for delivery of transgenes in an efficient and safe way for gene therapy. However, it has some limitations, including an optimal DNA packaging constraint of 4700 base pairs and ectopic expression.

RATIONALE: Increasing the expression levels of the normal gene copy by directly targeting the endogenous gene regulatory elements that control it could potentially correct haploinsufficiency. CRISPR-mediated activation (CRISPRa), whereby a nuclease-deficient Cas9 (dCas9) is used to target a transcriptional activator to the gene’s regulatory element (promoter or enhancer), could be used for this purpose. Such an approach could overcome the ectopic expression and DNA packaging limitations of rAAV. Using obesity as a model, we tested in mice whether CRISPR-mediated activation of the existing normal copy of two different genes, Sim1 or Mc4r, where loss-of-function mutations that lead to haploinsufficiency are a major cause of human obesity, can rescue their obesity phenotype.

RESULTS: We first generated a transgenic CRISPRa system using dCas9 fused to a transcriptional activator, VP64, to test whether it can rescue the obesity phenotype in a Sim1 haploinsufficient mouse model. CRISPRa targeting of the Sim1 promoter or its hypothalamus-specific enhancer, which is 270 kilobases away from the gene, in Sim1 haploinsufficient mice increased the expression of the normal copy of Sim1. This up-regulation was sufficient to rescue the obesity phenotype of Sim1 heterozygous mice and led to significantly reduced food intake and body fat content in these mice. We assessed the off-targeting effects of CRISPRa using both RNA sequencing (RNA-seq) and Cas9 chromatin immunoprecipitation sequencing (ChIP-seq) analyses. We found CRISPRa targeting to be highly specific and without any overt changes in the expression of other genes. We also observed that Sim1 up-regulation occurred only in tissues where the regulatory element (promoter or enhancer) that was being targeted was active. Although promoter-CRISPRa–targeted mice up-regulated Sim1 in all the tissues where it is expressed, the enhancer-CRISPRa–targeted mice showed Sim1 up-regulation only in the hypothalamus.

We then delivered CRISPRa packaged into rAAV targeting the Sim1 promoter or its hypothalamus-specific enhancer using either Streptococcus pyogenes or the shorter Staphylococcus aureus CRISPRa system. We show that postnatal injection of CRISPRa-rAAV into the hypothalamus can up-regulate Sim1 expression and rescue the obesity phenotype in Sim1 haploinsufficient mice in a long-lasting manner. To further highlight the therapeutic potential of this approach to rescue other haploinsufficient genes, we targeted Mc4r, where haploinsufficiency leads to severe obesity in mice and humans. CRISPRa-rAAV targeting of the Mc4r promoter rescued the obesity phenotype of Mc4r heterozygous mice.

CONCLUSION: These findings show that the CRISPRa system can rescue a haploinsufficient phenotype in vivo. This CRISPR-mediated activation strategy is different from a conventional gene therapy strategy, as it uses the endogenous regulatory elements to up-regulate the existing functional gene copy. As such, it can overcome the problem of ectopic gene expression. In addition, it could be used for genes that are not amenable to conventional gene therapy because their coding sequences are longer than the rAAV packaging limit. Our results provide a framework to further develop CRISPRa as a potential tool to treat gene dosage–sensitive diseases.

CRISPRa up-regulation of the existing normal gene copy rescues obesity caused by haploinsufficiency. Loss-of-function mutations in one allele lead to reduced amounts of mRNA and protein and can cause human disease, a condition termed haploinsufficiency. By up-regulating the existing normal allele using CRISPR-mediated activation (CRISPRa), whereby a nuclease-deficient Cas9 is fused to a transcriptional activator and targeted to a gene’s regulatory element (promoter or enhancer), the haploinsufficient phenotype could be rescued.
CRISPR-mediated activation of a promoter or enhancer rescues obesity caused by haploinsufficiency

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A wide range of human diseases result from haploinsufficiency, where the function of one of the two gene copies is lost. Here, we targeted the remaining functional copy of a haploinsufficient gene using CRISPR-mediated activation (CRISPRa) in Sim1 and Mc4r heterozygous mouse models to rescue their obesity phenotype. Transgenic-based CRISPRa targeting of the Sim1 promoter or its distant hypothalamic enhancer up-regulated its expression from the endogenous functional allele in a tissue-specific manner, rescuing the obesity phenotype in Sim1 heterozygous mice. To evaluate the therapeutic potential of CRISPRa, we injected CRISPRa-recombinant adeno-associated virus into the hypothalamus, which led to reversal of the obesity phenotype in Sim1 and Mc4r haploinsufficient mice. Our results suggest that endogenous gene up-regulation could be a potential strategy to treat altered gene dosage diseases.

More than 660 genes are currently estimated to cause human disease due to haploinsufficiency (table S1) (1, 2), leading to a wide range of phenotypes that include cancer, neurological diseases, developmental disorders, immunological diseases, metabolic disorders, infertility, kidney disease, limb malformations, and many others (1, 2). Large-scale exome sequencing analyses estimate that there could be over 3000 human genes that are haploinsufficient (3). Gene therapy in which a functional recombinant copy or copies replace the mutant gene holds great promise in addressing diseases caused by haploinsufficiency. Numerous clinical trials are being carried out for gene therapy, most of which use recombinant adeno-associated virus (rAAV) to deliver the transgene (4). rAAV is a preferred gene delivery method because of its ability to provide long-lasting gene expression of the transgene, delivering DNA without integrating into the genome, and with limited pathogenicity (5). However, current rAAV approaches tend to use promoters to drive transgenes that can lead to nondesirable ectopic expression (6, 7). Another crucial limitation is that AAV has an optimal 4.7-kilobase (kb) packaging capacity (8), limiting its gene therapy use for genes longer than 3.5 kb (taking into account additional regulatory sequences needed for its stable expression). Analysis of the 660 haploinsufficiency disease-causing genes and 3230 predicted heterozygous loss-of-function (LoF) genes reveals that 135 (20%) and 730 (23%) of them, respectively, have coding sequences longer than 3.5 kb (fig. S1), rendering them unsuitable for rAAV gene therapy.

CRISPR gene editing can potentially fix haploinsufficient mutations; however, this would require that the editing strategy be customized for each mutation. Moreover, it may not be feasible to correct heterozygous LoF microdeletions. To address these challenges, we devised a strategy that could potentially treat haploinsufficiency by using CRISPR activation (CRISPRa). CRISPRa takes advantage of the RNA-guided targeting ability of CRISPR to direct a nuclease-deficient Cas9 (dCas9) fused with a transcriptional activator or regulatory element(s) of a specific gene, thus increasing its expression (9–15). Here, we tested whether this system can be used to rescue a haploinsufficient phenotype by increasing the transcription of the normal endogenous gene. As a proof-of-concept model, we chose a quantitative trait, obesity caused by haploinsufficiency of either the single-minded 1 (Sim1)–/– gene, the melanocortin 4 receptor (Mc4r) gene.

SIM1 is a transcription factor that is expressed in the developing kidney and central nervous system and is essential for the formation of the supraoptic nuclei (SON) and paraventricular nuclei (PVN) of the hypothalamus (16). SIM1 also plays a role in the maintenance of long-term energy homeostasis by acting downstream of the leptin-melanocortin pathway (17). In humans, haploinsufficiency of SIM1 due to chromosomal aberrations results in hyperphagic obesity (18), and SIM1 coding mutations, many of which are LoF mutations, are thought to be a major cause of severe obesity in humans (19–21). Sim1 homozygous null mice die perinatally, whereas Sim1 heterozygous mice (Sim1+/-) survive, are hyperphagic, and develop early-onset obesity with increased linear growth, hyperinsulinemia, and hyperleptinemia (22). A postnatal conditional knockout of hypothalamic Sim1 leads to a similar phenotype in heterozygous mice (23), delineating an additional role for Sim1 as an important regulator of energy homeostasis in adults. Overexpression of Sim1, by using a human bacterial artificial chromosome in mice, rescues diet-induced obesity and reduced food intake (24), suggesting a potential role for Sim1 in preventing development of an obesity phenotype.

MC4R is a heterotrimeric guanine nucleotide-binding protein (G protein)–coupled receptor that is essential for the long-term regulation of energy homeostasis and other physiological processes. MC4R expression in the PVN of the hypothalamus is both necessary and sufficient for most of its effects on the regulation of body weight (25). Haploinsufficiency in MC4R are the most common cause of monogenic severe obesity, estimated at 2.6 to 5% of cases of early-onset and/or adult class 3 obesity (body mass index >40 kg/m2) (26–28). Mice haploinsufficient for Mc4r become obese with hyperphagia, hyperinsulinemia, and hyperglycemia (29).

We initially tested the ability of a transgenic CRISPRa system to rescue the obesity phenotype in Sim1+/- mice. CRISPRa using a single guide RNA (sgRNA) targeted to either the Sim1 promoter or its ~270-kb distant enhancer up-regulated Sim1 expression and rescued Sim1-mediated obesity in haploinsufficient animals. This transgenic approach also showed that Sim1 up-regulation occurred only in tissues where the promoter or enhancer is active, suggesting that the targeted cis-regulatory elements can determine CRISPRa tissue specificity. We also used transgenic mice to assess the targeting specificity of CRISPRa by using RNA sequencing (RNA-seq) and chromatin immunoprecipitation sequencing (ChiP-seq), which we found to be highly specific and without any apparent off-target effects. To further show that CRISPRa could be used as a potential strategy to treat haploinsufficient phenotypes, we used rAAV-mediated delivery of CRISPRa to the hypothalamus, preventing excessive weight gain in postnatal Sim1+/- mice. To demonstrate that this strategy could be used for other haploinsufficient genes, we also targeted the Mc4r promoter by means of a similar CRISPRa-rAAV approach and reduced weight gain in Mc4r+/- mice. Our results present a potential strategy for treating haploinsufficiency and additional gene dosage–related functional abnormalities.

Results

Up-regulation of Sim1 in vitro by CRISPRa

To increase expression of the wild-type Sim1 gene, we optimized CRISPRa conditions in vitro.
Sim1 has a well-characterized promoter (30) and distant and robust hypothalamic enhancer (~270 kb from the transcription start site) denoted Sim1 candidate enhancer 2 (SCE2 (31)) (Fig. 1A). To target Sin1 using CRISPRa, we designed two sgRNAs for either the Sim1 promoter or SCE2. Using these guides, we tested whether Streptococcus pyogenes dCas9 fused to VP64 (spCas9-VP64), a transcriptional activator that carries four tandem copies of VP16 (a herpes simplex virus type 1 transcription factor) (32), can up-regulate Sim1 in mouse neuroblastoma cells (Neuro-2A). The VP64 activator domain was chosen primarily because of its small size (so that it could later fit in our rAAV plasmid). It is also known to have a moderate activation potential compared to other known activators for a wide variety of genes (33), which could be advantageous in obtaining physiologically relevant Sim1 dosage levels in vivo. Cells were transfected with spCas9-VP64 and the various guide RNAs. After 48 hours, Sim1 mRNA levels were measured by quantitative polymerase chain reaction (qPCR). We identified one sgRNA for either promoter or SCE2 that could up-regulate endogenous Sim1 by 13- and 4-fold respectively (Fig. 1B and fig. S2, A and B). We also carried out ChIP-seq using an antibody against S. pyogenes Cas9 in both CRISPRa-promoter– and CRISPRa-enhancer–transfected cells and found on-target binding for the promoter and enhancer, respectively (fig. S2, C and D). We did not observe any peaks that overlapped with predicted sgRNA off-targets (table S2).

**Up-regulation of Sim1 in vivo by transgenic CRISPRa rescues obesity**

To test the ability of the CRISPRa system to rescue obesity in Sim1−/− mice, we generated knockin mouse lines using TARGATT technology (34). Using this technology, we inserted spCas9-VP64 into the mouse Hipp1 locus (a region that is known to allow robust transgene expression (35)) having three copies of atp (H11P3CAG-dCas9-VP64) and either sgRNA, targeting the Sim1 promoter (R26PrSim1P-gRNA) or SCE2 (R26PrSCE2x3-SgrNA), in the Rosa26 locus that has three atp sites (fig. 1C and fig. S3). We then crossed these mice to Sim1−/− mice that develop severe obesity (22). Mice having all three alleles (Sim1−/− × H11P3CAG-dCas9-VP64 and R26PrSim1P-gRNA or R26PrSCE2x3-SgrNA) were weighed weekly until 10 weeks of age along with wild-type littermates and Sim1−/− and Sim1+/− × H11P3CAG-dCas9-VP64 mice, both of which become severely obese (negative controls). Analysis of at least 10 females and 10 males per condition showed that Sim1−/− mice carrying both spCas9-VP64 and either Sim1 promoter or enhancer sgRNA had a significant reduction in body weight compared to Sim1−/− × H11P3CAG-dCas9-VP64 and Sim1+/− (fig. 1D and E, and fig. S4). Sim1−/− mice carrying spCas9-VP64 and either Sim1 promoter or enhancer sgRNA also showed a reduction in body weight compared to wild-type mice (fig. S4). We also analyzed body fat content and food intake for all genotypes: Sim1−/− × H11P3CAG-dCas9-VP64 × R26PrSim1P-sgRNA (Enh-CRISPRa), Sim1+/− × H11P3CAG-dCas9-VP64 × R26PrSCE2x3-sgRNA (Enh-CRISPRa), Sim1−/−, and wild-type mice. Both Prm-CRISPRa and Enh-CRISPRa mice showed significantly reduced body fat content and food intake compared to Sim1−/− in both females and males (fig. S5). Of note, we observed slight differences in body weight trajectories between female and male mice (i.e., when compared to wild-type mice, Sim1−/− females gained weight more rapidly than males), similar to what was observed in previous Sim1 knockout studies (22, 23). Taken together, these results show that both Prm-CRISPRa and Enh-CRISPRa mice have reduced body weight due to lower food intake, which likely leads to their reduced body fat levels.

**CRISPRa up-regulation of Sim1 is tissue specific**

To test for Sim1 activation levels and tissue specificity in Prm-CRISPRa and Enh-CRISPRa mice, we measured its mRNA expression levels in different tissues. We selected two tissues where Sim1 is expressed, hypothalamus and kidney, and two tissues where it is not expressed, lung and liver, based on previous studies (36, 37) and our analysis of Sim1 expression in different tissues (fig. S6). We first measured spCas9 expression and found that it was expressed in all four tissues, as expected, because we used a ubiquitous cytomegalovirus (CMV) enhancer chicken β-actin (CAG) promoter to drive its expression (Fig. 2A). By contrast, for Sim1, we observed significantly higher mRNA levels in both the hypothalamus and kidney in Prm-CRISPRa mice but only in the hypothalamus of Enh-CRISPRa mice compared to Sim1−/− mice (Fig. 2B). In Sim1+/− mice, we observed half the levels of mRNA expression when compared to wild-type mice, both in the hypothalamus and kidney.

Because we did not observe any significant differences between the obesity phenotype of Prm-CRISPRa and Enh-CRISPRa mice, we speculate that the activation of Sim1 in the hypothalamus is sufficient to rescue the Sim1−/− obesity phenotype. In tissues where Sim1 is not expressed (i.e., liver and lung), we could not detect Sim1 expression in Prm-CRISPRa or Enh-CRISPRa mice despite spCas9 being expressed. These results imply that despite ubiquitous expression, spCas9-VP64 could only up-regulate Sim1 in tissues where its target cis-regulatory elements are active. This suggests that cis-regulatory elements could be used to define the tissue specificity of CRISPRa.

**Sim1 CRISPRa targeting is highly specific**

To check for CRISPRa off-target effects, we undertook two genomic-level approaches: We analyzed the hypothalamic transcriptome (RNA-sequencing) of wild-type, Sim1−/−, Prm-CRISPRa, and Enh-CRISPRa mice. Three males and three females were used for each genotype (total of 24 samples; 6 biological replicates per condition). We also carried out ChIP-seq using an antibody against S. pyogenes Cas9 in the hypothalamus of Prm-CRISPRa, Enh-CRISPRa, and spCas9-VP64 (negative control) mice. A pool of four mice was used for each genotype, and two biological replicates.

In the RNA-seq analyses, we identified 24 differentially expressed genes [at a false discovery rate (FDR) of 0.1] between Sim1−/− and wild-type mice, of which 17 were up-regulated and 7 were down-regulated. For all of the 17 up-regulated and 6 of the 7 down-regulated genes, we observed fold changes that were similar to that of wild-type versus Sim1−/− for Prm-CRISPRa or Enh-CRISPRa when compared to Sim1−/− (Fig. 2, C and D, and table S3). We also observed that genes that were significantly up-regulated or down-regulated in Prm-CRISPRa versus Sim1−/− were also up-regulated or down-regulated in Enh-CRISPRa and vice versa, highlighting that the overall gene expression profile in Prm-CRISPRa and Enh-CRISPRa was similar (Fig. 2, C and D, and table S3). None of the Sim1 neighboring genes within a 500-kb window were differentially expressed in Prm-CRISPRa or Enh-CRISPRa in the RNA-seq analysis. Using qPCR, we also analyzed the mRNA expression levels of Sim1 neighboring genes, activating signal cointegrator 1 complex subunit 3 (Ascc3) and G protein-coupled receptor class C group 6 member A (GprC6a). We did not observe any differences in expression levels for these genes in Prm-CRISPRa and Enh-CRISPRa compared to wild-type mice (fig. S7, A and B). These results suggest that Sim1-CRISPRa changes the transcription profile of Sim1−/− mice to one that is more similar to that of the wild type.

Next, we carried out ChIP-seq analysis to identify off-target spCas9-VP64 binding. We found the most significant on-target enrichment at the Sim1 promoter in Prm-CRISPRa and SCE2 in Enh-CRISPRa mice (fig. S8, A and B, and table S2). In addition, we found 91 and 136 significant peaks (FDR adjusted p value ≤ 10−5) in Prm-CRISPRa and Enh-CRISPRa, respectively (table S2). We then looked for predicted sequence-specific genomic off-targets due to Prm-sgRNA or Enh-sgRNA mismatches, allowing for zero to three nucleotide mismatches, using Cas-OFFinder (38). For the promoter-targeting sgRNA, we found the one expected on-target site, and one off-target site with three nucleotide mismatches (fig. S8B) and table S2). For the enhancer-targeting sgRNA, we found the one expected on-target site and eight off-target sites with three nucleotide mismatches (fig. S8D and table S2). None of the Prm-CRISPRa or Enh-CRISPRa ChIP-seq peaks overlapped a corresponding predicted off-target site (table S2).

Next, we analyzed the RNA-seq datasets for the expression of the neighboring genes (~500 kb upstream and downstream) near the ChIP-seq peaks and sgRNA off-target sites, including the Sim1 target-specific peaks (fig. S8, C and D, and table S3). Of the genes within 500 kb of the off-target ChIP-seq peaks or predicted off-target sites, none showed differential gene expression (Fig. 2, E and F, and fig. S8, E and F). In addition, ChIP for dCas9 followed by qPCR for Ascc3, GprC6a, and the Sim1 promoter or SCE2 showed binding
Fig. 1. CRISPRa Sim1 up-regulation in vitro and obesity rescue in vivo.

(A) Schema of the mouse Sim1 genomic locus, showing the LacZ-driven hypothalamic expression of SCE2 (En) from 56-day-old mice. (B) CRISPRa in Neuro-2a cells targeting the Sim1 promoter (Prm-CRISPRa) or enhancer (Enh-CRISPRa). Results are expressed as mRNA fold increase normalized to Actb using the ΔΔCT method. The data are represented as means ± the lower and upper quartile, and lines represent the minimum and maximum from three independent experiments and three technical replicates. *p < 0.001; ***p < 0.0005 (ANOVA, Tukey test).

(C) Schema showing the mating scheme used to generate Sim1+/- CRISPRa mice. A CAG-dCas9-VP64 cassette was knocked into the Hipp11 (H11P3) locus, and an sgRNA targeting either the Sim1 promoter (U6-Prm-sgRNA) or SCE2 (U6-Enh-sgRNA) was knocked into the Rosa26 locus. (D) Weekly weight measurements of wild-type, Sim1+/−, H11P3CAG-dCas9-VP64 × R26P3Sim1Pr-sgRNA (Prm-CRISPRa), and H11P3CAG-dCas9-VP64 × R26P3SCE2En-sgRNA (Enh-CRISPRa). At least 10 male and female mice were measured per genotype. Mean values ± SD are shown. *p-value statistics are listed in table S5. (E and F) Photos of 26-week-old male mice for each genotype: Sim1+/−, H11P3CAG-dCas9-VP64 × R26P3Sim1Pr-sgRNA (Prm), and wild type (WT) (E) and Sim1+/−, H11P3CAG-dCas9-VP64 × R26P3SCE2En-sgRNA (Enh), and wild type (WT) (F). Genotype, weight, and length of each mouse are depicted below.
Fig. 2. dCas9 and Sim1 mRNA expression levels in CRISPRa transgenic mice. (A) dCas9 mRNA expression in the hypothalamus, kidney, lung, and liver from four Sim1+/− × H1IP3CAG-dCas9-VP64 mice. The data are represented as means ± the lower and upper quartile, and lines represent the minimum and maximum from at least four mice with three technical replicates. (B) Sim1 mRNA expression in the hypothalamus, kidney, lung, and liver for the following genotypes: wild type, Sim1+/−, H1IP3CAG-dCas9-VP64 × R26P3Sim1Pr-sgRNA (Prm-CRISPRa), and H1IP3CAG-dCas9-VP64 × R26P3SCE2En-sgRNA (Enh-CRISPRa). The data are represented as means ± the lower and upper quartile, and lines represent the minimum and maximum from four mice (two females and two males) and three technical replicates. All experiments were determined based on mRNA fold increase compared to wild type and normalized to Actb or Rpl38 using the ΔΔCT method or ΔCT for (A). BDL, below detectable levels. (C and D) A Michaelis-Menten plot showing differentially expressed genes in the hypothalamus between Sim1+/− and wild-type mice on the x axis and Prm-CRISPRa (C) or Enh-CRISPRa (D) versus Sim1+/− mice on the y axis. The larger circles are genes that are differentially expressed with a raw p value ≤ 0.05, and the outlined circles have a FDR ≤ 0.1. (E and F) A Michaelis-Menten plot showing differentially expressed genes in the hypothalamus that are nearby ChIP-seq peaks and predicted off-target sgRNAs between Sim1+/− and wild-type mice on the x axis and Prm-CRISPRa (E) or Enh-CRISPRa (F) versus Sim1+/− mice on the y axis. The outlined circles are genes that show differential expression with a raw p value ≤ 0.05, and the larger circles are genes that overlap nearby off-target sites (both Sim1 promoter and SCE2 were predicted targets even up to three mismatches).
only in the Sim1 promoter for Prm-CRISPRa and SCE2 in Enh-CRISPRa mice (fig. S7, C and D), similar to what we observed in the ChiP-seq data. Our results show that Sim1 Prm-CRISPRa and Enh-CRISPRa are highly specific, without any apparent off-target effects.

**Delivery of Sim1 CRISPRa rAAV to the PVN rescues the weight gain phenotype in Sim1<sup>+/−</sup> mice**

To further investigate the translational potential of this approach to rescue haploinsufficiency in adult mice, we took advantage of rAAV to deliver CRISPRa vectors into the hypothalamus of Sim1<sup>+/−</sup> mice. We generated the following three rAAV vectors: (i) <i>S. pyogenes</i> dCas9-VP64 driven by a CMV promoter (<i>pCMV-spdCas9-VP64</i>); (ii) Sim1 promoter sgRNA along with mCherry (<i>pU6-Sim1Pr-CMV-mCherry</i>); and (iii) SCE2 sgRNA along with mCherry (<i>pU6-SCE2-CMV-mCherry</i>) (Fig. 3A). These vectors were packaged individually into AAV-DJ serotype (<i>39</i>). We first tested if the rAAV CRISPRa vectors could up-regulate Sim1 in vitro using Neuro-2a cells. We observed a four- and fivefold increase in Sim1 mRNA expression when targeting the promoter or enhancer, respectively (Fig. 3B and fig. S9).

Next, we performed stereotactic injections to deliver virus carrying <i>pCMV-spdCas9-VP64</i> and either <i>pU6-Sim1Pr-CMV-mCherry</i> (Prm-CRISPRa-AAV) or <i>pU6-SCE2-CMV-mCherry</i> (Enh-CRISPRa-AAV) into the PVN of the hypothalamus of Sim1<sup>+/−</sup> mice at 4 weeks of age, before the mice start becoming obese. As an injection-based negative control, we also injected Sim1<sup>+/−</sup> mice with <i>pCMV-spdCas9-VP64</i> virus only. We first optimized the stereotactic injection conditions and coordinates (see Methods) and tested for the expression of mCherry from the <i>pU6-Sim1Pr-CMV-mCherry</i> cassette in the PVN by performing immunostaining on the hypothalami of injected mice (Fig. 3C and fig. S10). Next, we carried out stereotactic injections into the PVN of Sim1<sup>+/−</sup> mice at 4 weeks of age using <i>S. pyogenes</i> CRISPRa-AAV. To test whether Sim1 expression levels were increased by delivering CRISPRa-AAV to the hypothalamus of Sim1<sup>+/−</sup> mice, we measured mRNA expression levels for both dCas9 and Sim1 from 11-week-old AAV-injected mice. dCas9 was expressed in the hypothalami of all the <i>pCMV-spdCas9-VP64</i>-AAV-injected mice (Fig. 3D). Sim1 up-regulation was observed in both Prm-CRISPRa-AAV- and Enh-CRISPRa-AAV-injected hypothalami, but not in mice injected with only <i>pCMV-spdCas9-VP64-AAV</i> (Fig. 3E). To observe the extent of Sim1 up-regulation that could be achieved, we injected Prm-CRISPRa-AAV into the hypothalami of wild-type mice using two different titers. We observed up to 1.8-fold up-regulation with the higher viral titer (fig. S11).

Because the length of <i>S. pyogenes</i> dCas9-VP64 exceeds the optimal packaging load for AAV (i.e., 4.7 kb), we generated a <i>Staphylococcus aureus</i>
dCas9–fused VP64 vector, which has an AAV packaging load of 4.3 kb (Fig. 3A; pCMV-sadCas9–VP64). As S. aureus uses a different protosparser adjacent motif site, we designed and cloned sgRNAs for pU6-Sim1Pr-CMV-mCherry and pU6-SCE2-CMV-mCherry. We identified several sgRNAs that can increase Sim1 expression by targeting its promoter or SCE2 in Neuro-2a cells through transient transfection (fig. S12, A and B). We also carried out ChIP-seq using an antibody against S. aureus Cas9 in both Prm-CRISPRa– and Enh-CRISPRa–transfected cells and found on-target binding for the promoter and enhancer, respectively (fig. S12, C and D). We did not observe any peaks that overlapped with predicted sgRNA off-targets (table S2). We then generated an AAV-DJ serotype of the S. aureus CRISPRa vectors, obtaining higher titers for dCas9–VP64 virons than S. pyogenes CRISPRa-AAVs (see Methods).

We infected Neuro-2a cells with the viruses and selected a single sgRNA for the promoter or SCE2 that significantly increased Sim1 expression (Fig. 3B and fig. S12E). Next, we carried out stereotactic injections into the PVN of Sim1+/− mice at 4 weeks of age using S. aureus CRISPRa-AAV. To test whether Sim1 expression levels were increased by delivering S. aureus CRISPRa-AAV to the hypothalamus of Sim1+/− mice, we measured mRNA expression levels from hypothalami of 11-week-old AAV-injected mice. Compared to S. pyogenes dCas9 expression levels (Fig. 3D), we found higher expression levels of S. aureus dCas9 in the hypothalami of all pCMV-sadCas9–VP64–AAV–injected mice (Fig. 3F). We also observed higher Sim1 up-regulation (Fig. 3G) compared to S. pyogenes CRISPRa (Fig. 3E).

Next, we tested whether Sim1 up-regulation by CRISPRa-AAV can lead to a reduction in body weight of Sim1 haploinsufficient mice. CRISPRa-AAV–injected Sim1+/− mice were measured for body weight up to 11 weeks of age (Fig. 4A). We observed a significant weight reduction in the Prm-CRISPRa–AAV– or Enh-CRISPRa–AAV–injected mice compared to the Sim1+/− or pCMV-dCas9–VP64–AAV–injected Sim1+/− mice both for S. pyogenes (Fig. 4B) and S. aureus (Fig. 4C). These results suggest that CRISPRa-AAV can rescue the Sim1 haploinsufficiency obesity phenotype.

Finally, we analyzed whether CRISPRa would have a long-term body weight effect on these mice. Although many of the injected mice were analyzed in the aforementioned gene expression studies, a few were maintained and showed significant weight reduction compared to the Sim1+/− or pCMV-spdCas9–VP64–AAV–injected Sim1+/− mice 9 months after injection (Fig. 4, D and E). Similar results were also observed in S. aureus–injected mice (fig. S13). These results show that CRISPRa-AAV–mediated up-regulation could have a long-lasting effect on phenotype.

Delivery of Mc4r CRISPRa rAAV to the PVN rescues the weight gain phenotype in Mc4r+/− mice

To further investigate whether CRISPRa can rescue an additional haploinsufficient obesity model, we carried out S. aureus CRISPRa targeting of the Mc4r promoter in Mc4r+/− mice, which become obese as a result of heterozygous LoF of Mc4r (29). We first screened five sgRNAs targeting the Mc4r promoter and selected one that led to robust Mc4r up-regulation in Neuro-2a cells using both transient transfection and rAAV infections (fig. S14). We then carried out stereotactic injections into the PVN of 4-week-old Mc4r+/− mice with either pCMV-sadCas9–VP64–AAV as a negative control or pCMV-sadCas9–VP64–AAV and pU6-Mc4rPr-CMV-mCherry (Mc4rPrm-CRISPRa-AAV) (Fig. 5, A and B). We observed an increase in Mc4r expression up to 2.7-fold in Mc4rPrm-CRISPRa-AAV mice (Fig. 5C). Body weight measurements 8 weeks after injection showed a significant weight reduction in the Mc4rPrm-CRISPRa-AAV–injected mice compared to the Mc4r+/− or pCMV-sadCas9–VP64–AAV–injected Mc4r+/− mice (Fig. 5, D and E).

These results further suggest that CRISPRa can be used to rescue other haploinsufficient phenotypes.

Discussion

CRISPR-based gene editing is a promising therapeutic technology for correcting genetic mutations. However, it is a challenging approach for treating haploinsufficiency, limited by low homology-directed repair efficiencies (i.e., editing only a small portion of cells) and the need to customize specific guides and donor sequences for each individual mutation. In addition, it may not be a feasible therapeutic strategy for microdeletions, more than 200 of which are known to cause human disease (40), primarily because of haploinsufficiency. In this study, we used a CRISPR-mediated activation approach to tackle these hurdles and show how a haploinsufficient phenotype could be corrected by increasing the transcriptional output from the existing functional allele with CRISPRa.
Using CRISPRa targeting for either the promoter or enhancer of Sim1, we could rescue the obesity phenotype in a tissue-specific manner in mice that are haploinsufficient for Sim1. Because this approach takes advantage of the existing functional allele, it has several benefits: (i) It overcomes the need to custom-tailor CRISPR gene editing approaches for different haploinsufficiency-causing mutations in the same gene. (ii) This approach could potentially be used to target two or more genes. It could serve as a potential therapeutic strategy for microdeletions-related diseases that are caused by the heterozygous LoF of more than one gene. (iii) CRISPRa-AAV could be used to rescue haploinsufficient phenotypes caused by genes that are longer than its optimal packaging capability. (iv) Tissue specificity is a major concern for gene therapy. CRISPRa-based approaches can take advantage of cis-regulatory elements to guide tissue specificity (Fig. 6A). The availability of large-scale tissue-specific maps of gene regulatory elements could provide ample candidates for this approach. We observed distinct differences in tissue-specific activation of Sim1 based on the targeted cis-regulatory element, which can be attributed to chromatin accessibility of the locus in various tissues. Previous large-scale Cas9 and dCas9 cell culture screens have shown a targeting preference for regions with low nucleosome occupancy (41, 42). Active promoters or enhancers would have lower nucleosome occupancy, thus being more amenable to dCas9 targeting.

CRISPRa uses a nuclease-deficient Cas9 (dCas9) fused to a transcriptional activator and as such does not edit the genome. However, it can lead to transcriptional modulated off-target effects. To test for such effects, we carried out both RNA-seq and ChIP-seq in vitro and in vivo. We did not observe any apparent CRISPRa off-target binding that resulted in significant transcriptional changes. Additional analyses of predicted off-targeting loci due to sgRNA mismatches did not find any transcriptional changes surrounding these loci. The dCas9-VP64 fusion used in our CRISPRa system is known to activate loci that are programmed for transcription, such as promoters or enhancers (14, 42). Taken together, our results suggest that CRISPRa has high specificity.

Our dCas9-VP64 mouse and rAAV vectors can be a useful tool for targeted gene activation in vivo by delivering sgRNA(s) targeted to a specific gene in certain tissues or cell types. This approach could be used to assess gene-gene interactions or for the identification of the target gene(s) of a specific regulatory element in vivo by measuring its expression level following activation. Another potential area of study could be neuronal circuit manipulation. Discrepancies between acute and chronic neuronal circuit manipulations have been observed (43) that could potentially be addressed by rAAV-CRISPRa and transgenic-CRISPRa strategies, respectively.

Haploinsufficiency of SIM1 is associated with severe obesity (19–21) in humans and mice (22). Whether this is caused by the reduction in PVN...
size during development that is observed in Sim1−/− mice (22) or by disturbed energy homeostasis during adulthood was largely unknown. The obesity phenotype observed in the postnatal conditional knockout of hypothalamic Sim1 (23) reinforced the hypothesis that Sim1 does indeed have a role in energy homeostasis later during adulthood. Our results showing phenotypic rescue in adult mice by rAAV CRISPRa further corroborate this role.

Mutations in MC4R are the most commonly found in individuals with class 3 obesity (body mass index >40 kg/m²), with an estimated 2.6 to 2.8% of this population having mutations in this gene are associated with an obesity phenotype (24). Heterozygous LoF mutations in this gene was used in this study, could indeed be rescued in adult mice by rAAV CRISPRa targeting of the Mc4r promoter.

CRISPRa-based gene activation is highly dependent on the nature of the fused activator (33) and sgRNA target (41) and would need to be optimized for a particular gene, along with the delivery method. It is important to note that overexpressing genes beyond their physiologically relevant doses could have undesirable side effects. The use of a shorter dCas9, such as the S. aureus dCas9, which has a shorter length than Cas9. For example, a CRISPR-based approach (CRISPR, Cas9 TGA) was recently shown to ameliorate muscular dystrophy (DMD) (49, 50), survival of motor neuron 2 (SMA2) (48), and utrophin (Utn) (32). Several agonists have been developed to target this receptor (44, 45). Here, we decreased the weight gain in Mekr−/- mice using rAAV-CRISPRa targeting of the Mekr promoter.

CRISPRa-based gene activation is highly dependent on the nature of the fused activator (33) and sgRNA target (41) and would need to be optimized for a particular gene, along with the delivery method. It is important to note that overexpressing genes beyond their physiologically relevant doses could have undesirable side effects. The use of a shorter dCas9, such as the S. aureus dCas9 (46) that was used in this study, could reduce the packaging load and improve dCas9 delivery, with optimal up-regulation levels to activate physiologically relevant results. This approach can also lead to increased expression of the mutant allele, if the targeted promoter or enhancer is not deleted, and may not be useful in cases where this allele is not a complete LoF. In addition, targeting rAAV to specific neuronal populations in primates may require multiple injections or other DNA delivery methods.

We demonstrate that CRISPRa can be used to activate genes in vivo not only by targeting their promoters but also by targeting distal cis-regulatory elements such as enhancers. We were able to rescue a haploinsufficient phenotype in a long-lasting manner (9 months) with CRISPRa rAAV by targeting either the promoter or enhancer of a gene. Previous studies have shown that these elements can be potential therapeutic targets. For example, by targeting a global enhancer with zinc finger nucleases fused to a chromatin opening factor, the LIM domain binding 1 (LDB1) gene, activation of fetal hemoglobin was achieved in vitro, providing a potential therapy for sickle cell disease (47). In another study, reactivation of fetal hemoglobin was achieved by deactivating the enhancer of its repressor B cell CLL/Lymphoma 11A (BCL11A) using CRISPR gene editing (48). Our study provides an approach in preclinical mouse model systems that takes advantage of cis-regulatory elements and can aid in designing potential therapeutic strategies. Numerous phenotypes caused by lower gene dosage could potentially be targeted with CRISPRa (Fig. 6B). In addition, several human diseases could potentially be rescued by the activation of another gene with a similar function (Fig. 6C). These could include, for example, Urothrophin for Duchenne muscular dystrophy (DMD) (49, 50), survival of motor neuron 2 (SM2A) for spinal muscular atrophy (51), or fetal globin for sickle cell disease. For example, a CRISPR-based approach (CRISPR/ Cas9 TGA) was recently shown to ameliorate the dystrophic phenotype upon up-regulation of either the Uroporphin, Klotho, or Fst genes in a mouse model of DMD (50). Further development of gene up-regulation by CRISPRa or other techniques could provide a potential therapy for dosage-related diseases. Materials and methods Plasmids

The pMSCV-LTR-dCas9-VP64-BFP vector, encoding a mammalian codon-optimized Streptococcus pyogenes dCas9 fused to two C-terminal SV40 NLSs and tagBFP along with a VP64 domain and the U6-sgRNA-CMV-mCherry-T2A-Puro plasmids were used for cell line transfections (both kind gifts from J. S. Weissman and S. Qi). sgRNAs (table S4) were cloned using the In-Fusion HD-cloning kit (Clontech) following the manufacturer’s protocol into the Bst XI and Xho I sites. Mouse knockin vectors were cloned by generating dCas9-VP64 and U6-sgRNA-CMV-mCherry expression cassettes from the aforementioned vectors into the TARGATT (CAG + Poly A) plasmid (Applied StemCell). For AAV vectors, pDNA-dCas9-VP64 (Addgene 47807), and U6-sgRNA-CMV-mCherry-WPREPa were cloned replacing the EFta-FAS-hChR2(H134R)-mCherry-WPRE-p4 with that of the U6-sgRNA-CMV-mCherry-WPREPa into the backbone of pAAV-EFta-FAS-hChR2(H134R)-mCherry-WPRE-p4 (Addgene 73090). S. aureus dCas9-VP64 vector was constructed from Addgene Plasmid #68495, AAV_NLS+SimCas9-NLS-VP by removing the RelA(p65) activation domain and Rta Activation domain using XbaI and EcoRI enzymes and introducing a stop codon after the VP64 domain followed by bGHPolyA. We named it pAAV-CMV-sadcas9-VP64-pA. pAAV-U6-sasgRNA-CMV-mCherry-WPREPa was cloned by replacing the CMV-sadcas9-VP64-p4 cassette in pAAV-CMV-sadcas9-VP64-p4 backbone with that of U6-sasgRNA-CMV-mCherry-WPREPa cassette from pLTR-1120-MP177-U6-sasgRNA-mcherry (a kind gift from B. Huang at UCSF). S. aureus sgRNAs (table S4) were cloned using the In-Fusion HD-cloning kit (Clontech) following the manufacturer’s protocol into the Bst XI and Xho I restriction enzyme sites.

AAV production

Particles of rAAV-DJ serotype, which is a chimer of type 2, 8, and 9 that was shown to achieve high expression levels in multiple tissues (39), were produced for all vectors using the Stanford Gene Vector and Virus core. The packaging load for pCMV-spdcas9-VP64 was 5.4 kb and for pU6-Sim1Pr-CMV-mCherry and pU6-SCE2-CMV-mCherry 2.5 kb. Genomic titers were ascertained by WPRE and ITR probes to be 1.40 × 10^9 viral genome (vg)/ml for pCMV-spdcas9-VP64 and around 3.80 × 10^13 vg/ml for pU6-Sim1Pr-CMV-mCherry and 2.20 × 10^13 vg/ml for pU6-SCE2-CMV-mCherry. The packaging load for pCMV-sadcas9-VP64-pA was 4.3 kb and for pU6-sasgRNA-CMV-mCherry was 2.5 kb. Genomic titers for pAAV-CMV- sadcas9-VP64-p4 were 1.60 × 10^12 vg/ml for pU6-sasgRNAASim1Pr-CMV-mCherry 1.58 × 10^13 vg/ml for pU6-sasgRNAASim1Pr-CMV-mCherry 2.0 × 10^13 vg/ml, and for pU6-sasgRNA2M4rprpMV-mCherry 1.0 × 10^13 vg/ml.

Cell culture

Neuroblastoma 2a cells (Neuro-2a; ATCC® CCL-131) were grown following ATCC guidelines. Plasmids were transected into Neuro-2a cells using X-tremeGENE HP DNA transfection reagent (Roche) following the manufacturer’s protocol. AAV particles were infected into Neuro-2a cells at different multiplicity of infection (MOI) ratios (figs. S9 and S12E). Neuro-2a cells were
All RNA-seq data was deposited in NCBI as differential expression between sample groups. RNA was isolated from cells or tissues using RNeasy Mini Kit (Qiagen) following the manufacturer’s protocol. For mice, animals were euthanized and tissues were harvested directly into the RNA lysis buffer of the RNeasy Mini Kit. The hypothalamus was dissected using a mouse brain matrix and slicers (Zivic Instruments). Each hypothalamus was sampled the same way for each animal with 1.0-mm coronal section slice intervals. The coronal brain section of the hypothalamus was sliced with two blades 3 mm apart (fig. S15, A to C). The hypothalamus was dissected out from this coronal section. Care was taken to orient the brain on the matrix and to align the hypothalamus each time corresponding to the same coronal section slots. For qRT-PCR, cDNA was prepared using SuperScript III First-Strand Synthesis System (Invitrogen) using the manufacturer’s protocol along with deoxyriboonuclease I digestion. qPCR was performed with SsoFast EvaGreen Supermix (Bio-Rad) and primers listed in table S4. To further validate our qPCR results for Sim1, we also performed qPCR on wild-type, Sim1+/− and Sim1−/− Prm-CRISPRa-AAV−, Enh-CRISPRa-AAV−, or pCMV-dCas9–VP64–AAV–injected mice with primers that overlap the region that was knocked out (Sim1-5′ primers listed in table S4), obtaining similar results (fig. S13, D to G). The results were expressed as fold-increase mRNA expression of the gene of interest normalized to either Actb or Rpl38 expression by the ΔΔCT method followed by analysis of variance (ANOVA) and Tukey test for statistical analysis.Reported values are the mean ± SEM from three independent experiments performed on different days (N = 3) with technical duplicates that were averaged for each experiment. For RNA-seq, a pool of four mice was used for each genotype and two biological replicates that were averaged for each experiment. For ChIP-seq, we analyzed three independent integrants were used from each line to set up matings. H11P3′-AG-dCas9–VP64− and R26P3′-Sim1Pr-sgRNA mice were fed ad libitum Picolab mouse diet 20, 5058 containing 20% protein, 9% fat, 4% fiber for the whole study. Calories were provided by protein (23.21%), fat (ether extract) (21.55%), and carbohydrates 55.23%. All animal work was approved by the UCSF Institutional Animal Care and Use Committee.

**Transgenic mice body weight measurements**

H11P3′-AG-dCas9–VP64−, R26P3′-Sim1Pr-sgRNA and R26P3′-C2EEn–aSGNA mice were fed with FVB mice for three to five generations to assess germ line transmission. Three independent integrants were used from each line to set up matings. H11P3′-AG-dCas9–VP64− were mated with Sim1+/− and subsequent Sim1−/− × H11P3′-AG-dCas9–VP64− mice were crossed with either R26P3′-Sim1Pr-sgRNA or R26P3′-C2EEn–aSGNA to generate mice having all three unlinked alleles. Mice were maintained at Picodiet.5058 throughout the study, and at least 10 females and 10 males from all genotypes (wild type, Sim1+/−, Sim1−/− × H11P3′-AG-dCas9–VP64− Sim1−/− × H11P3′-AG-dCas9–VP64− × R26P3′-Sim1Pr-sgRNA Sim1−/− × H11P3′-AG-dCas9–VP64− × R26P3′-C2EEn–aSGNA) were measured for their body weights from 4 to 16 weeks of age on a weekly basis.

**Body composition and food intake analyses**

Body composition was measured using either dual energy x-ray absorptiometry (DEXA) or Echo Magnetic Resonance Imaging (EchoMRI; Echo Medical System). For DEXA, mice were anesthetized with isoflurane and measured for bone mineral density and tissue composition (fat mass and lean mass) with the Lunar PIXImus. EchoMRI (Echo Medical System) was used to measure whole-body composition parameters such as total body fat, lean mass, body fluids, and total body water in live mice without the need for anesthesia or sedation. Food intake was measured by using the Columbus Instruments Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments). Mice were housed individually and acclimated on powdered picodiet 5058 for 3 to 4 days, and food intake measurements were done over 4 to 5 days. Three males and three females from each genotype: wild-type littermates, Sim1+/−, Sim1−/− × H11P3′-AG-dCas9–VP64−, R26P3′-Sim1Pr-sgRNA Sim1−/− × H11P3′-AG-dCas9–VP64− × R26P3′-C2EEn–aSGNA were measured.

**Stereotaxic injections**

Four-week-old Sim1+/− or Mc4r−/− males or females, weighing between 20 and 26 g were housed individually in cages for at least 2 days before surgical interventions. Mice were anesthetized with 3% isoflurane for induction and 1% isoflurane for maintenance in a vaporizer chamber. The skull was immobilized in a stereotaxic apparatus (Model 1900, Stereotaxic Alignment Systems, 1micron resolution, David Kopf Instruments). The stereotaxic coordinates for injection into the PVN were 0.80 mm caudal to bregma, 0 mm at the midline, and 5.2 mm below the surface of the skull, similar to the midline location.
AAV along with 2.5 × 10⁶ vg/kg of spdCas9-VP64-the PVN unilaterally over a 10-min period. This 24 hours in 4% PFA, and then equilibrated in hyde (PFA). Brains were removed, postfixed for 10 ml of phosphate-buffered 4% paraformaldehyde (7.5 mg/0.15 ml, intraperitoneally) staining as they were used for RNA analyses. stronger mCherry expression (Fig. 3C and fig. S10). Used to validate PVN injection coordinates 8 weeks because of a missed injection. Immuno- the experiment. All surviving mice were included in surgery; (ii) having profuse bleeding during sur- from the study for the following reasons (table S6): prevent reflux and slowly withdrawn in several steps, over 10 min. Mice were administered two doses of buprenorphine (100 mg/kg) before and 24 hours after surgery. Mice were only excluded from the study for the following reasons (table S6): (i) having shorter bregma length during surgery; (ii) having profuse bleeding during sur- or (iii) did not survive surgery or died during the experiment. All surviving mice were included in the phenotypic analysis, and we did not elim- nate mice because of a missed injection. Immuno- staining for mCherry, as described below, was used to validate PVN injection coordinates 8 weeks after injection in several mice with single midline injections showing one side of the PVN to have stronger mCherry expression (Fig. 3C and fig. S10). The majority of mice did not undergo immuno- staining as they were used for RNA analyses. Mice were maintained on a picodiet 5058 and weighed on a weekly basis. Immunostaining

For immunostaining, mice were anesthetized with pentobarbital (7.5 mg/0.15 ml, intraperitoneally) and transcardially perfused with 10 ml of hepa- rinized saline (10 U/ml, 2 ml/min) followed by 10 ml of phosphate-buffered 4% paraformalde- hyde (PFA). Brains were removed, postfixed for 24 hours in 4% PFA, and then equilibrated in 30% sucrose in PBS for 72 hours. Brains were coronally sectioned (35 μm for immunostaining, 50 μm for stereology) on a sliding microtome (Leica SM 2000R). Immunohistochemistry was performed as previously described (24, 57, 58). Coronal brain sections that had been stored in PBS at 4°C were permeabilized and blocked in 3% normal goat serum–0.3% Triton X-100 for 1 hour and incubated overnight at 4°C overnight using an antibody to mCherry at a dilution of 1:500 (Abcam ab167453). Sections were placed in 4β-diamidino-2-phenylindole (DAPI) (0.2 g/ml; 236276; Roche) for 10 min and then mounted on Plus coated slides and coverslipped using Vectashield (H-1000; Vector Laboratories). Images of sections containing PVN were captured on a Zeiss Axioplan.
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Data and materials availability: All RNA-seq data were deposited in NCBI as Bioproject PRJNA438712. All CHIP-seq data were deposited in NCBI as Bioproject PRJNA438723.

SUPPLEMENTARY MATERIALS

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Figs. S1 to S5
Tables S1 to S6
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CRISPRa corrects haploinsufficient obesity

Loss-of-function mutation in one gene copy, termed haploinsufficiency, can lead to insufficient protein levels and result in human disease. Matharu et al. tested whether a CRISPR-based activation system (CRISPRa) could rescue a haploinsufficient phenotype by increasing the gene expression levels of the existing normal copy (see the Perspective by Montefori and Nobrega). By delivering this system into the mouse hypothalamus using adeno-associated virus, they rescued the obesity phenotype caused by haploinsufficiency of either of two genes known to promote obesity when mutated in mice and humans. These results highlight the translational potential of the CRISPR activation system to treat haploinsufficient disease.

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