A mutation-independent approach via transcriptional upregulation of a disease modifier gene rescues muscular dystrophy *in vivo*

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Introductory paragraph

Identification of genetic modifiers has provided critically important insights into the pathogenesis and heterogeneity of disease phenotypes in individuals affected by neuromuscular disorders (NMDs). Targeting modifier genes to improve disease phenotypes could be especially beneficial in cases where the causative genes are large, structurally complex and the mutations are heterogeneous. Here, we report a mutation-independent strategy to upregulate expression of a compensatory disease-modifying gene in Congenital Muscular Dystrophy type 1A (MDC1A) using a CRISPR/dCas9-based transcriptional activation system.

MDC1A is caused by nonfunctional Laminin $\alpha 2$, which compromises muscle fibers stability and axon myelination in peripheral nerves ¹. Transgenic overexpression of *Lama1*, encoding a structurally similar protein Laminin $\alpha 1$, ameliorates muscle wasting and paralysis in the MDC1A mouse models, demonstrating its important role as a disease modifier ². Yet, upregulation of *Lama1* as a postnatal gene therapy is hampered by its large size, which exceeds the current genome packaging capacity of clinically relevant delivery vehicles such as adeno-associated viral vectors (AAVs).

In this study, we use the CRISPR/dCas9-based transcriptional activation system to upregulate *Lama1*. This system is comprised of catalytically inactive *S. aureus* Cas9 (dCas9) fused to VP64 transactivation domains and sgRNAs targeting the *Lama1* promoter. We first demonstrate robust upregulation of *Lama1* in myoblasts, and following AAV9-mediated intramuscular delivery, in skeletal muscles of *dy^{2j}/dy^{2j}* mouse model of MDC1A.

We therefore assessed whether systemic upregulation of *Lama1* would yield therapeutic benefits in *dy^{2j}/dy^{2j}* mice. When the intervention was started early in pre-symptomatic *dy^{2j}/dy^{2j}* mice, *Lama1* upregulation prevented muscle fibrosis and hindlimb paralysis. An important question for future therapeutic approaches for a variety of disorders relates to the therapeutic window and phenotypic reversibility. This is particularly true for muscular dystrophies as it has long been hypothesized that fibrotic changes in skeletal muscle represent an irreversible disease state that would impair any therapeutic intervention at advanced stages of the disease. Here, we demonstrate that dystrophic features and disease progression were significantly improved and reversed when the treatment was initiated in symptomatic 3-week old *dy^{2j}/dy^{2j}* mice with already-apparent hind limb paralysis and significant muscle fibrosis.

Collectively, our data demonstrate the feasibility and therapeutic benefit of CRISPR/dCas9-mediated modulation of a disease modifier gene, which opens up an entirely new and mutation-independent treatment approach for all MDC1A patients. Moreover, this treatment strategy provides evidence that muscle fibrosis can be reversible to some degree, thus extending the therapeutic window for this disorder. Our data provide a proof-of-concept strategy that can be applied to a variety of disease modifier genes and a powerful therapeutic approach for various inherited and acquired diseases.

67 Congenital muscular dystrophy 1A (MDC1A) is an autosomal recessive neuromuscular
68 disease associated with a high degree of morbidity and mortality in early childhood ¹. It is
69 caused by mutations in the *LAMA2* gene encoding Laminin α 2 chain (LAMA2), which
70 interacts with the β 1 and γ 1 chains to form the heterotrimer Laminin-211, an extracellular
71 matrix protein (reviewed in ³). Laminin-211 interacts with α -dystroglycan and α 7 β 1
72 integrin in skeletal muscle and Schwann cells, providing the necessary roles such as
73 survival and stability of myotubes, as well as proper neurite growth, axon myelination
74 and migration of Schwann cells. Lack of Laminin-211 in MDC1A causes degeneration of
75 skeletal muscle and impaired Schwann cell differentiation, resulting in a cascade of
76 secondary events including apoptosis/necrosis of muscle fibers, inflammation, and
77 fibrosis, which ultimately precipitate the disease. Despite significant advances in our
78 understanding of MDC1A pathophysiology, currently, there is no cure.
79 Due to the genetic nature of the disease, gene therapy is a promising treatment option
80 for MDC1A. The large size of *LAMA2* transcript, however, presents a challenge with
81 respect to gene delivery. We have recently overcome this challenge by using
82 CRISPR/Cas9 technology to correct a mutation in *Lama2* gene *in vivo* ⁴. We focused on
83 *dy^{2j}/dy^{2j}* mouse model, which harbors a splice site mutation in *Lama2* causing
84 spontaneous exon skipping and truncation of N-terminal domain of the protein ⁵. We
85 developed an exon inclusion strategy to correct the splice mutation, leading to
86 restoration of full-length *Lama2*. This study established the first direct correction of the
87 primary genetic defect underlying MDC1A in an *in vivo* model.
88 To date, there are over 350 reported pathogenic nonsense-, missense-, splice site- and
89 deletion mutations in the *LAMA2* gene. Given the variety of MDC1A-causing genomic
90 alterations, CRISPR/Cas9-mediated gene editing strategies would require design and
91 thorough analysis of multiple sgRNAs specific for each individual mutation. Moreover,
92 safety concerns regarding CRISPR/Cas9's potential mutagenic nature and the presence
93 of off-target effects after gene editing remain, which together may prove to be
94 challenging from a safety and regulatory point-of-view. In contrast, the attenuation of
95 disease pathogenicity by targeted modulation of disease modifier gene expression would
96 be a potentially safer alternative and beneficial to all individuals with MDC1A.
97 One of the strongest reported disease modifiers for MDC1A is Laminin- α 1 protein, which
98 is structurally similar to Laminin- α 2 (**Fig. 1a**). However, Laminin- α 1 is not expressed in
99 skeletal muscles or Schwann cells. A series of studies previously demonstrated that
100 transgenic expression of *Lama1*, encoding Laminin- α 1, rescued both myopathy and

peripheral neuropathy in dy^{2j}/dy^{2j} ⁶ and dy^{3K}/dy^{3K} ^{2,7-10}; the latter also showed increased lifespan. Although these studies firmly established compensatory function of Laminin- α 1 in MDC1A, utilization of this modifier as a postnatal gene therapy is hampered by the size of *Lama1* cDNA, which exceeds the 4.7 kb packaging capacity of AAV vectors. Advances in CRISPR/Cas9 technologies have provided opportunities for regulating gene expression and creating epigenetic alterations without introducing DNA double-strand breaks (DSBs); commonly termed CRISPR transcriptional activation/inhibition system. The strategy utilizes nuclease-deficient Cas9 (dCas9), which is unable to cleave DNA due to mutations within the nuclease domains, but still retains the ability to specifically bind to DNA when guided by a single guide RNA (sgRNA) ^{11,12}. Using the originally described *S. pyogenes* (*Sp*) dCas9 fused to multiple copies of the VP16 transcriptional activator, our group and others have demonstrated utilization of the CRISPR/dCas9 system to upregulate expression of modifier genes *in vitro* ¹¹⁻¹⁴. A major challenge for *in vivo* applications lies in the large size of *SpdCas9* and its transcriptional activator fusion derivatives that exceed AAV genome packaging capacity. To accommodate this limitation, we sought to adapt the transcriptional upregulation system and utilize a significantly smaller Cas9 protein derived from *S. aureus* (*Sa*)¹⁵ to upregulate MDC1A modifier *Lama1*. We hypothesized that CRISPR/dCas9-mediated transcriptional upregulation of *Lama1* would be sufficient to compensate for the lack of *Lama2* and ameliorate disease phenotypes in dy^{2j}/dy^{2j} mice.

First, we mutagenized *SaCas9* endonuclease catalytic residues (D10A, N580A) to create *SadCas9* and then fused it to transcriptional activators VP64 (four copies of VP16) on both N- and C-termini (**Fig. S1**). We tested the ability of the system, denoted as *SadCas9*-2xVP64, to upregulate the expression of minCMV-driven *tdTomato* gene in 293T cells ¹⁶. In the absence of the *SadCas9*-2xVP64, the expression of *tdTomato* was undetectable due to the low baseline activity of minCMV promoter (**Fig. S1a, b**). In the presence of *SadCas9*-2xVP64 in combination with an sgRNA targeting the minCMV locus, we observed high *tdTomato* fluorescence signal (**Fig. S1c, d**), indicating the general applicability of this system to modulate expression of a gene of interest.

Subsequently, we tailored the system to upregulate *Lama1* expression and designed five sgRNAs, denoted as g1 to g5, within the 500 nucleotides region immediately upstream of the *Lama1* transcription start site (**Figs. 1b, c**). When co-expressed with *SadCas9*-VP64 in C2C12 murine myoblasts, 3 out of 5 sgRNAs, namely g1, g2, and g5 consistently induced significant increase of *Lama1* transcript expression (**Figs. 1d, e**).

Although all sgRNAs were designed to target a chromatin-accessible region derived from DNase1 hypersensitivity- and assay for transposase-accessible chromatin (ATAC-Seq) sites (**Fig. 1b**), g3 and g4 failed to increase expression of *Lama1* above the basal level. This corroborates previously reported findings that chromatin accessibility is not a reliable predictor of successful gene activation^{12,17,18}. Nonetheless, the sgRNA closest to the transcription start site, g1, and the combination of the three most optimum sgRNAs (g1, g2, g5) resulted in a robust increase in Lama1 protein expression in *dy^{2j}/dy^{2j}*-derived myoblasts (**Figs. 1f, 1g**), warranting further investigation *in vivo*.

We then treated 3-week-old *dy^{2j}/dy^{2j}* mice with an AAV9 carrying FLAG-tagged SadCas9-2xVP64 in the absence of sgRNA (no guide) or with g1 (single guide) or a combination of g1, g2 and g5 (three guides) (**Fig. 2a**). Due to the packaging capacity of the AAV, the dCas9-2xVP64 and the three guides were split into two vectors (**Fig. 2a**). Each mouse received a single intramuscular injection of 7.5×10^{11} viral genomes of AAV9, which was doubled to 1.5×10^{12} viral genomes total for the three guide cohort, in the right *tibialis anterior* (TA) and was sacrificed 4 weeks post injection. FLAG expression was detected by western blot in all SadCas9-2xVP64-injected right TA muscles, however, only those injected with guide-containing constructs showed Lama1 upregulation (**Fig. 2b**). Similarly, immunofluorescence staining revealed sarcolemmal expression of Lama1 (**Fig. 2c**, upper), indicating proper protein localization. Furthermore, H&E staining exhibited considerably improved muscle architecture (**Fig. 2c**, lower) in the single- and three guide treatment groups, as compared to the no guide controls.

Next, we sought to investigate whether upregulation of *Lama1* could be achieved systemically *in vivo* and, if administered into pre-symptomatic neonatal *dy^{2j}/dy^{2j}* mice, would prevent the manifestation of dystrophic pathophysiology and paralysis. AAV9 particles carrying either no guide or a combination of three guides were injected into the temporal vein of 2-day-old (P2) *dy^{2j}/dy^{2j}* mice (**Fig. 3a**). Seven weeks post injection, the animals that received the three guides treatment showed an absence of classical hindlimb contracture, unlike the control group (**Fig. 3b; Supplementary videos 1 and 2**). Western blot, immunofluorescence and H&E staining of TA and gastrocnemius muscles demonstrated robust expression of Lama1 on the sarcolemmal membrane (**Fig. 3c, 3d**), leading to ~50% reduction of fibrosis (**Figs. 3e, 3f, S2**).

We subsequently examined the ability of *Lama1* upregulation to reverse established muscular and peripheral nerve dysfunctions by initiating the treatment at 3 weeks of age,

when paralysis was already apparent^{19,20} (**Fig. 4a**). We first tested three different doses, ranging from 7.5×10^{10} to 3×10^{11} viral genome/gram of mouse per AAV9, which was doubled for the three guide cohorts due to the utilization of two vectors, and found that the highest dose resulted in homogeneous Lama1-positive fibers (**Fig. S3a**), significant improvement of muscle function and mobility (**Figs. S3b, S3c**). Longitudinal measurement of animal mobility and stand-up activity in a non-invasive open field activity assay showed significant difference between no guide and three guides-treated mice starting at 5 and 6 weeks old, respectively, which was sustained over time (**Figs. 4b, 4c**). Furthermore, specific tetanic force, which measures the aggregate torque produced by the dorsi flexor muscles, was also improved in the three guides-treated mice (**Fig. 4d**). In line with this finding, we observed a significant increase in nerve conduction velocity, which indicates restoration of the myelination defect and contributes to neuromuscular functionality (**Fig. 4e**). The absence of paralysis in the hind limbs and markedly improved movement of the mice were evident at the end of the treatment regimen (**Supplementary videos 3, 4**).

Molecular analysis of the treated mice revealed strong Lama1 expression by immunostaining and western blot (**Figs. 4f, 4g, S4**), which was accompanied by normalization of $\alpha 4$ chain of the laminin subunit (**Figs. S5, S6**)⁶, significant improvement in muscle histopathology (**Fig. 4h**) and approximately 50% reduction in the fibrotic area (**Fig. 4i**) compared to the no guide control group. There was a trend towards larger fibers in the treated mice, although it did not reach statistical significance due to the large variation between animals (**Fig. 4j**). In addition, upregulation of Lama1 was also observed in the endoneurium of sciatic nerves and resulted in restoration of myelination defect (**Figs. 4k, S7**), supporting the improvement of nerve conduction velocity and lack of paralysis in the mice (**Fig. 4e, Supplementary videos 3, 4**). Quantification of the AAV genome copy number revealed accumulation of most of the viral genome in the liver, which is expected from intravenous delivery. Nevertheless, approximately 1.11 ± 0.4 and 34.1 ± 4.3 copies/diploid genome of the viral genome were detected in the sciatic nerves and skeletal muscles, respectively (**Fig. S8**). Remarkably, even relatively low transduction efficiency in sciatic nerves was sufficient for functionally significant upregulation of Lama1 expression.

Finally, we investigated the genome wide effects of CRISPR/dCas9-mediated Lama1 upregulation by performing RNA-sequencing on quadriceps muscles isolated from mice treated with AAV9 carrying no guide and three guides (**Figs. S9, S10, Supplemental**

Tables 1-3). Age-matched wildtype and dy^{2j}/dy^{2j} mice served as controls. We observed a 3.6- $\log_2$ fold upregulation of *Lama1* (defined by a false discovery rate, FDR <0.05) when comparing the mice treated with AAV9 carrying no guide and three guides (**Fig. S9a**). The transcriptional change was even higher at 9- $\log_2$ fold when comparing untreated dy^{2j}/dy^{2j} with three guide-treated dy^{2j}/dy^{2j} (**Fig. S9b, S9c**). Hierarchical clustering between groups revealed clustering between wildtype and three guides, whereas the untreated cohort was clustered together with no guide-treated mice (**Figs. S9d, S10**). We also computationally predicted 704 potential off-target binding sites for the three sgRNAs targeting *Lama1* promoter in the mouse genome, selected based on the presence of a 6bp PAM-proximal seed sequence and fewer than ten total mismatches to the cognate target sequence (**Supplemental Tables 4-5**). None of the top 100 genes contained a predicted off-target site within 60 kilobases of the gene body, with the average distance from off-target site to the gene body being 2.2 megabases. Taken together, our results provide strong evidence of the robustness and durability of CRISPR/dCas9-mediated *Lama1* upregulation for the treatment of MDC1A. Additionally, we show the therapeutic promise of the strategy for reversal of dystrophic feature in skeletal muscle and peripheral neuropathy in dy^{2j}/dy^{2j} mouse model of MDC1A, which ultimately halts progression of the disease, without any confounding off-target effects.

One challenge in developing a therapy for MDC1A is that the heterogeneity of mutations often leads to variable disease severity and progression. Therefore, there is an urgent need to develop a universal, mutation-independent strategy that provides a treatment approach for all patients with MDC1A. Our study establishes a framework in which CRISPR/dCas9 transcriptional upregulation of a disease modifier gene, such as *Lama1*, ameliorates disease symptoms *in vivo* and has the potential to be applied to all MDC1A patients, irrespective of their mutations.

Advances have been made towards elucidating MDC1A pathogenesis due to the availability of several mouse models with absent or reduced *Lama2* expression. Yet, an important question in the development of therapeutics and clinical trials in MDC1A is the reversibility of symptoms caused by muscle fibrosis and nerve abnormalities. This issue has been challenging to address in patients²¹, however, the ability to modify gene expression in postnatal animals allowed us to address this question and begin to investigate the therapeutic window of intervention.

We have previously demonstrated that an early intervention using CRISPR/Cas9-mediated correction of a splicing defect resulted in robust *Lama2* restoration and prevention of disease manifestation⁴. Here we showed that upregulation of *Lama1*, when initiated at pre-disease-onset, leads to similar prevention. Importantly, when the therapeutic intervention was initiated at older age, significant rescue of the phenotypes was attainable, indicating that post-symptomatic treatment provides a significant benefit in the *dy^{2j}/dy^{2j}* mouse model.

In addition to *Lama1* upregulation described in this study, a number of disease modifying strategies are currently being explored in MDC1A animal models, including treatment with miniaturized agrin²²⁻²⁴ and laminin- α 1 LN-domain nidogen-1 (α LNNd)²⁵⁻²⁷. While the efficacy of α LNNd has only been explored in transgenic mice, AAV-mediated delivery of mini agrin has been shown to normalize most histopathological parameters in skeletal muscle, and improve myelination and regeneration of Schwann cells of the peripheral nerves^{22,24}. Despite the observed phenotypic improvement, the mini agrin-treated mice still have a lower survival rate compared to wild-type animals expressing full-length agrin, suggesting the potential shortcoming of the shortened version of agrin, which may be overcome by its full-length form in native glycosylation state²⁵. It is important to note that many preclinical studies in MDC1A were carried out in the more severe *dy^W/dy^W* mouse model. Therefore, as a follow up on our study, it will be important to evaluate our approach in the *dy^W/dy^W* mice to assess critical parameter such as survival, which is not

possible in the dy^{2j}/dy^{2j} mouse model due to its near-normal life span. Overall, our approach in combining the CRISPR/dCas9-mediated transcriptional upregulation and AAV9 as a delivery vehicle can be translated to many other disease modifiers, such as agrin, as well as conditions where modulation of disease modifiers is required within both skeletal muscles and peripheral nerves.

In fact, neuromuscular disorders have provided excellent examples to demonstrate the role of disease modifiers (recently reviewed in ²⁸). Beyond MDC1A, several studies have demonstrated that upregulation of *Lama1* stabilizes the sarcolemmal membrane in dystrophin-deficient mouse models ²⁹. The most advanced approach is via delivery of Laminin-111 protein, although the efficacy remains low and the need to produce a large amount of bioactive protein is challenging ²⁹. In addition, the utilization of CRISPR/dCas9 system to upregulate *Lama1* in *mdx* mice has been achieved locally via electroporation, which is not easily translatable into clinical settings ³⁰. Our strategy of employing AAV-mediated *S. aureus* dCas9 to upregulate *Lama1* *in vivo* may be tested further as a potential therapy in the context of Duchenne muscular dystrophy.

In addition, since the CRISPR/dCas9-mediated transcriptional modulation acts directly on the endogenous locus, it may be used in conjunction with a mutation-correction approach where the level of restoration of the defective gene is suboptimal, therefore necessitating further amplification to reach therapeutic efficacy ^{4,31-36}.

A very recent study by Liao *et al* described utilization of the CRISPR/Cas9 system to recruit MCP:P65:HSF1 transcriptional activation complex to induce expression of target genes in skeletal muscle, kidney and liver tissues ¹⁸. This resulted in phenotypic augmentation such as enhanced muscle mass and substantial improvement in disease pathophysiology, thereby highlighting the feasibility of using CRISPR/dCas9-mediated transcriptional activation as a possible therapeutic modality. However, their study relied almost exclusively on a Cas9-expressing transgenic mouse model or local intramuscular treatments, and therefore it is difficult to extrapolate the efficacy of this strategy to disease-relevant models. In contrast, we successfully demonstrated robust upregulation of *Lama1* after systemic delivery of therapeutic components in a relevant mouse model of disease that does not constitutively express Cas9.

Finally, the modular nature of the CRISPR/dCas9 system can be utilized to not only to upregulate, but also to downregulate target gene expression. The latter can be achieved by coupling dCas9 with transcriptional repressor such as Kruppel-associated box (KRAB) ^{17,37}. A very recent study described that following sarcolemmal injury, the muscle

290 membrane resealing process is greatly improved upon the deletion of Osteopontin,
 291 which acts in a concerted fashion with protective modifiers such as Latent TGF- β binding
 292 protein (LTBP4) and Annexins 1 and 6^{38,39}. The combinatorial effects of such modifiers,
 293 whether they are additive, synergistic or even opposing in action, represent a new
 294 paradigm for lessening disease phenotypes. A foreseeable application of
 295 CRISPR/dCas9-mediated modulation is in the upregulation of protective disease
 296 modifier genes, such as Lama1 or LTBP4, with concurrent downregulation of detrimental
 297 genes, such as Osteopontin, providing a combinatorial therapeutic approach.
 298 In summary, our study establishes a framework to utilize CRISPR/dCas9 to modulate
 299 gene expression of disease modifiers that should be considered as a mutation-
 300 independent therapeutic strategy not only to MDC1A, but also to various other inherited
 301 and acquired diseases.

Methods

Engineering of activation constructs

A fragment containing a catalytically inactive *SaCas9* coupled to two flanking VP64 transactivator domains was synthesized by BioBasic Canada and cloned into pX601 (Addgene 61591) using *AgeI* and *EcoRI* directional cloning to generate 3XFLAG-VP64-SadCas9 (D10A/N580A)-NLS-VP64 plasmid (**Figs. 2a, S11, Supplemental Table S6**). Each sgRNA (**Supplemental Table S6**) was subsequently introduced using *BsaI* directional cloning. To generate the three guides only construct (**Fig. 2a**), a fragment containing three repetitive regions of U6 promoter and *S. aureus* guide scaffold was assembled, with short linkers in between each region (BioBasic Canada). The fragment was cloned into *KpnI* and *NotI* sites of a pX601-derivative plasmid.

Cell Culture

Primary myoblasts were isolated from the Extensor Digitorum Longus (EDL) Muscle of *dy^{2J}/dy^{2J}* mice, as previously described⁴⁰ and maintained in DMEM supplemented with 1% chicken embryo extract (GeminiBioscience), 10% horse serum, 1% penicillin/streptomycin and 1% L-glutamine (all from Gibco, unless indicated otherwise). HEK293 and C2C12 cells were maintained in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin and 1% L-glutamine (all from Gibco). All cells were maintained at 37°C with 5% CO₂.

Transfection of HEK293T cells was performed as previously described². C2C12 and *dy^{2J}/dy^{2J}* cells were transfected in 12-well plates using the Neon Transfection System (Invitrogen). 400,000 cells were electroporated with 1.5 µg of DNA utilizing optimization program 16 (pulse voltage: 1400V, pulse width: 20ms, pulse number: 2). Cells were grown for 72 hours, after which RNA or protein was subsequently collected for protein analysis and guide screening.

Animals, virus production and injections

dy^{2J}/dy^{2J} mice were purchased from the Jackson Laboratory and maintained in the Toronto Center for Phenogenomics. Both male and female were used in the analyses. All animal experiments were performed according to Animal Use Protocol number 20-0305. SadCas9-2xVP64, single guide and three guides plasmids (**Fig. 2a, Supplemental Table S6**) were packaged into AAV9 vectors by Vigene Biosciences as

previously described³. For intramuscular and temporal vein injection into neonatal pups, the dose of 7.5×10^{11} viral genomes each was used. Due to the limitation in packaging capacity, two AAVs were needed for the three guides cohort (**Fig. 2a**), therefore the total virus injected was 1.5×10^{12} viral genomes per animal. Injection volume was brought to 50 μ l with 1XPBS (Gibco).

For the tail vein injection in young, 3 week old mice, three different doses were initially tested: 7.5×10^{10} , 1.5×10^{11} and 3×10^{11} viral genomes per gram of mouse (**Fig. S3**).

Similar to the intramuscular and temporal vein injections, two AAVs were needed for the three guides cohort, therefore the total dose used in experiments described in **Figs. 4, S4-S10** was 6×10^{11} viral genomes per gram of mouse. Injection volume was brought to 100 μ l with 1XPBS (Gibco).

RNA isolation, guide screening and RT-PCR

RNA was isolated from cultured cells and mouse tissue sections, and cDNA synthesis was performed as previously described³. PCR amplification was utilized to assess the efficiency of each guide in upregulating *Lama1* expression using a primer in *Lama1* exon 55 (RDC 1919) and a second primer spanning the junction of exons 55 and 56 (RDC 1920). Sequences are listed in **Supplemental Table S6**.

qPCR utilizing Fast SYBR green Master Mix (Qiagen) on a Step One Plus Real Time PCR (Applied Biosystems) was performed. *Lama1* expression was analyzed using a primer in *Lama1* exon 55 (RDC 1919) and one spanning the junction of exons 55 and 56 (RDC 1920). Primers against endogenous Gapdh (RDC 345 and 346) were used as an internal control. $\Delta\Delta C_t$ was analyzed to assess fold changes between treated and untreated samples.

Protein Isolation and western blot

Protein was isolated from *dy^{2j}/dy^{2j}* myoblasts and C2C12 cells by adding 150 μ l of a 1:1 part solution of RIPA homogenizing buffer (50-mM Tris HCl pH 7.4, 150-nM NaCl, 1-mM EDTA) and RIPA double-detergent buffer (2% deoxycholate, 2% NP40, 2% Triton X-100 in RIPA homogenizing buffer) supplemented with protease-inhibitor cocktail (Roche). Cells were subsequently scraped from the bottom of each well, collected and incubated on ice for 30 min. Cells then centrifuged at 12000xg for 15 min at 4°C and the supernatant was collected and stored at -80°C. Protein from mouse tissue sections was collected as previously described³. Whole protein concentration was measured using

Pierce BCA protein assay kit according to the manufacturer's protocol (Thermo Fisher Scientific). Western blot was performed as previously described³. Primary antibodies used were rabbit Anti-LN α 1 E3 (a gift from Dr. Peter Yurchenco, 0.6 μ g/ml), mouse monoclonal M2 anti-Flag (Sigma Aldrich F1804, 1:1000) and rabbit polyclonal anti-GAPDH (Santa Cruz sc-25778, 1:5000).

Immunofluorescence and H&E staining

Muscles and nerves were sectioned at 8 μ m thickness and processed for immunofluorescence analyses according to standard procedures. Antibodies used for immunofluorescence staining were rat monoclonal against Laminin α 1 (mAb200, Durbeej Lab, 1:20), α 2 (4H8-2, Sigma, 1:500), γ 1 chain (clone A5, Thermo Fisher Scientific), rabbit polyclonal against Laminin α 4 chain (kindly provided by Dr. Sasaki), mouse monoclonal against NF-H (Biolegend SMI 31, 1:1000), goat polyclonal anti-rat Alexa Fluor 555 (Thermo Fisher Scientific, 1:250) and goat polyclonal anti-mouse Alexa Fluor 488 (Thermo Fisher Scientific, 1:250). H&E staining was performed as previously described⁴. Both immunofluorescence and H&E slides were scanned with the 3Dhistech Panoramic 250 Flash II digital scanner and analyzed with CaseViewer software, with the exception of **Figs. S5, S6**, which were analyzed with a Zeiss Axioplan fluorescence microscope (Carl Zeiss) and images were captured using an ORCA 1394 ER digital camera (Hamamatsu Photonics) and Open Lab software version 4 (Improvision).

Toluidine blue staining and electron microscopy

Freshly isolated mouse sciatic nerves were halved and fixed in a solution of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer until further use. For embedding, the specimens were rinsed with the 0.1M sodium cacodylate buffer, post-fixed in 1% osmium tetroxide in the washing buffer, dehydrated in a graded ethanol series followed by propylene oxide, and embedded in Quetol-Spurr resin overnight at 65°C. Sections of 90nm thickness were cut on a Leica EM UC7 ultramicrotome, stained with uranyl acetate and lead citrate, and imaged on a FEI Tecnai 20 electron microscope at 4400-, 10,000-, and 44,000X magnifications. The same 90nm sections were stained with toluidine blue and imaged on a Leica DM-2000. All reagents were purchased from Electron Microscopy Sciences. Quantification of myelin thickness was measured using ImageJ from at least 14 random axons per field (at least 5 fields per animal)⁴¹.

Open field and *in vivo* muscle force assays

Open field activity test and assessment of *in vivo* muscle force were performed on tail vein injected cohorts at the Lunenfeld-Tanenbaum Research Institute's Centre for Modeling Human Disease Mouse Phenotyping Facility. For the open field test, mice were placed in the frontal center of a transparent Plexiglas open field (41.25 cm × 41.25 cm × 31.25 cm) illuminated by 200 lx. A trained operator, who is unaware of the nature of the projects and treatments, performed experiments. The VersaMax Animal Activity Monitoring System recorded vertical activities and total distance travelled for 20 minutes per animal.

In vivo muscle contraction test was performed using 1300A: 3-in-1 Whole Animal System and analyzed using Dynamic muscle control/analysis (DMC/DMA) High throughput software suite (Aurora Scientific). The mice were anaesthetised with intraperitoneal injection of ketamine/xylazine cocktail at 100 mg/kg and 10 mg/kg of body weight, respectively. Contractile output was measured via percutaneous electrodes that stimulate specific nerves innervating the plantar flexors. Specific tetanic force (200 ms of 0.5-ms pulses at 125 Hz) was recorded and corrected to body weight.

Nerve conduction velocity

Mice were anesthetized with urethane (1.2 mg/g i.p.) and underwent a tracheotomy to maintain their airway but were not artificially ventilated. Core body temperature was maintained at 34-36.5°C using a feedback-controlled heating pad (TR-200; Fine Science Tools). The sciatic nerve was exposed at two locations via incisions (~15 mm) above the left knee (site #1) and along the sacral region of the vertebral column (site #2). After separating the nerve from adjacent tissue, a bipolar hook electrode was applied to the nerve at each site. Each electrode comprised two chlorided silver wires (0.01" diameter, A-M systems) placed ~1 mm apart and bent at the tip to form hooks, and attached to a stimulator (Model DS3, Digitimer Ltd). Stimulating electrodes were insulated from underlying tissue using a small piece of plastic paraffin film. Stimulus duration was fixed at 20 µs and current intensity was varied. Stimulus timing was controlled by computer using a Power1401 computer interface and Signal v5 software (Cambridge Electronic Design, CED). The compound muscle action potential (CMAP) was recorded using needle electrodes (~30 G, BD *PrecisionGlide*™), one inserted into the gastrocnemius muscle and the other (reference) electrode inserted into the Achilles tendon. The CMAP signal was amplified, low-pass filtered at 10 Hz and high-pass filtered at 1 kHz (DAM 80,

World Precision Instruments), and digitized at 40 kHz using the Power1401 and Signal v5 software (CED). To calculate conduction velocity (CV) from CMAP responses, the sciatic nerve was stimulated at just-maximal intensity (beyond which there is no change in CMAP amplitude or latency) three times at site #1 and again three times at site #2. Nerve conduction velocity was calculated as the difference between average CMAP latencies for each stimulation site divided by the length of nerve (7.5-10 mm) separating the sites.

Vector genome quantification

Evaluation of AAV genome distribution was performed as previously published⁴² with a few modifications. Genomic DNA was extracted from tibialis anterior, sciatic nerve, and liver of treated mice using Qiagen Blood and Tissue Kit. 90 ng of DNA was amplified using primers located in between the two inverted tandem repeats (ITRs) (RDC 1687, RDC 1679, **Supplemental Table S7**) using Fast SYBR green master mix (Qiagen) on a Step One Plus Real Time PCR (Applied Biosystems). The Ct value of each reaction was converted to viral genome copy number by interpolating against the copy number of standard curve of a known plasmid containing the sgRNA cassette (RDC 362). The amount of DNA input (90 ng per tissue) was used as a conversion factor to diploid genome (7 picogram DNA = 1 diploid genome).

RNA-Sequencing

Total RNA was isolated from quadriceps muscles using RNeasy kit (Qiagen) and quantified using Qubit RNA HS assay (Thermo Fisher Scientific). RNA-Sequencing was performed by the Centre for Applied Genetics in Toronto using the Illumina HiSeq 2500 system, producing 120bp paired-end reads. Raw transcript reads were aligned to the GRCm38 mouse genome (mm10) using HISAT2⁴³. HTSeq was used to determine the absolute number of read counts for each gene⁴⁴. Only genes with at least 1 read per million in at least three replicates were kept for downstream analysis. Normalization and differential expression analysis was completed using the R packages limma, v3.36.3 and edgeR, v.3.22.3⁴⁵. Differentially expressed genes were defined as genes with a more than twofold change and adjusted $P < 0.05$. Off-target analysis was conducted using a list of 704 computationally predicted using Cas-OFFinder⁴⁶. Using the Bedtools suite “closest” function, each of the top 100 differentially expressed genes was matched to the

nearest off-target loci to determine the shortest distance from off-target loci to differentially expressed gene body.

Statistical Analysis

GraphPad Prism (GraphPad software) was utilized to preform all statistical analyses. Two-tailed Student' t-tests to evaluate statistical significance between two groups was preformed. Significance was considered to be $P < 0.05$.

Data availability

The authors declare that the main data supporting the findings of this study are available within the article and its Supplementary Information files. Extra data are available from the corresponding authors upon request.

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Author contributions

D.U.K., E.A.I., R.D.C. conceived the study, D.U.K., P.S.B., S.E., D.A.B., K.I.G., K.L., E.H., R.K., K.M.P., R.M., performed the experiments, D.A.B., K.I.G., M.D., S.A.P. provided critical reagents, D.U.K., P.S.B, S.E., D.A.B, K.I.G, S.A.P., E.A.I, R.D.C. analyzed data, D.U.K, E.A.I., R.D.C. supervised the study, D.U.K. wrote the manuscript with inputs from the other authors. All authors provided feedback and agreed on the final manuscript.

Figure legends

Figure 1

Fig. 1. SadCas9-2xVP64-mediated upregulation of *Lama1* in vitro. (a) Lama1 and Lama2 protein alignment. Total amino acid (aa) and percentage similarity between each domain are indicated. (b) Analyses of Lama1 proximal promoter. Five sgRNAs (g1-g5) were designed in the proximal promoter region of Lama1 immediately upstream of the transcription start site (TSS), as indicated by CAGE tags (red). Chromatin accessibility in skeletal muscle tissue and cells (retrieved using Digital DNase footprinting and ATAC-Seq) are shown in blue. Data were plotted according to positions from the UCSC Genome Browser. (c) Positions of the five sgRNAs relative to *Lama1* TSS. Arrowheads indicate the direction of each sgRNA. Sequences of each sgRNA (red) are immediately downstream (5') of Sa PAM sequences (NNGRRT) (in blue). ATG indicates translation start site. (d-e) C2C12 myoblasts were transfected with a plasmid containing SadCas9-2xVP64 and the corresponding sgRNA(s) targeting *Lama1*, and 72 hours post-transfection, analyzed by (d) RT-PCR and (e) qRT-PCR. Single and combination of optimal sgRNAs were transfected into *dy^{2j}/dy^{2j}* myoblasts and Lama1 protein expression was assessed by western blot (f). FLAG expression serves as transfection control and used to (g) normalize Lama1 upregulation by densitometry analysis. Data are presented

as mean $\pm$ standard deviation from at least three independent experiments. Statistical analysis was performed using Student's *t*-test. $**P<0.01$.

Figure 2

Fig. 2. Upregulation of Lama1 in *tibialis anterior* (TA) muscles of *dy^{2j}/dy^{2j}* mice following intramuscular administration. (a) Right TA muscles of 3-week old *dy^{2j}/dy^{2j}* mice were injected with AAV9-carrying no guide (n=4; 7.5x10¹¹ viral genomes), single guide (n=4; 7.5x10¹¹ viral genomes) or three guides (n=4; split into two vectors, thus total dose was 2x7.5x10¹¹ viral genomes). ITR: Inverted Terminal Repeats. CMV and U6 promoters are depicted in arrows. Left TA muscles serve as control. (b) Western blot analysis on Lama1, FLAG-tagged SadCas9, and Gapdh expression. (c) Immunofluorescence (upper) and H&E (lower) stainings on cross-sections of right TA muscles from each treatment group. Scale bar: 50 μ m. Representative images from one animal per treatment group are shown.

Figure 3

Fig. 3. Early intervention to upregulate Lama1 prevents disease progression in *dy^{2j}/dy^{2j}* mice. (a) Two-day-old neonatal *dy^{2j}/dy^{2j}* mice were injected with AAV9 carrying no guide (n=3; 7.5x10¹¹ viral genomes) or three guides (n=4; split into two vectors, thus total dose was 2x7.5x10¹¹ viral genomes) via temporal vein and sacrificed 7 weeks later. (b) Photographs of *dy^{2j}/dy^{2j}* from both treatment groups prior to sacrificing. Note the

difference in hindlimb contractures. Lama1 expression was analyzed by (c) western blot and (d) immunofluorescence staining, and general histopathology was evaluated by (e) H&E staining. The muscle groups analyzed are indicated on each panel, except on (c), which shows tibialis anterior (TA) muscles. Scale bars: 100 μ m (d), 200 μ m (e). (f) The percentage of fibrosis from gastrocnemius (upper) and TA (lower) muscles are calculated and presented as mean $\pm$ standard deviation. Statistical analysis was performed using Student's *t*-test. **P*<0.05.

Figure 4

Fig. 4. Upregulation of Lama1 in older dy^{2j}/dy^{2j} mice halts disease progression.

(a) Three-week old dy^{2j}/dy^{2j} mice were injected with AAV9 carrying no guide (denoted as -; n=9; 3×10^{11} viral genomes/gram) or three guides (denoted as +; n=6; split into two vectors, thus total dose was $2 \times 3 \times 10^{11}$ viral genomes/gram) via tail vein. Grey box represents period before treatment. (b, c) Open field activity assay was performed weekly, before (grey-shaded) and after treatment, and the mice were tested for (d) muscle contractile and (e) nerve conduction velocity at the end of the treatment regimen, prior to molecular analyses of the tissues. (f) Immunofluorescence staining and (g) western blot to analyze Lama1 expression and (h) H&E staining to quantify (i) fibrosis and (j) fiber size were performed on gastrocnemius muscles. (k) Sciatic nerves were stained for Lama1 (red) and Neurofilament H (green) (left). Nuclei were counterstained by DAPI (blue). Higher magnification images from the dotted area are shown. Representative toluidine blue staining (middle) and electron micrograph (right) are shown. Asterisks indicate region of amyelinated axon fibers. Scale bars: 100 μ m (f), 50 μ m (h), and 200 μ m (k). Data are presented as mean $\pm$ standard deviation. Statistical analysis was performed using Student's *t*-test. **P*<0.05. ***P*<0.01.

References

1. Dowling, J. J., H. D. G., Cohn, R. D., Campbell, C. Treating pediatric neuromuscular disorders: The future is now. *American journal of medical genetics. Part A* 2017.
2. Gawlik, K., Miyagoe-Suzuki, Y., Ekblom, P., Takeda, S., Durbeej, M. Laminin alpha1 chain reduces muscular dystrophy in laminin alpha2 chain deficient mice. *Human molecular genetics* 13:16. 2004.
3. Yurchenco, P. D., McKee, K. K., Reinhard, J. R., Ruegg, M. A. Laminin-deficient muscular dystrophy: Molecular pathogenesis and structural repair strategies. *Matrix biology : journal of the International Society for Matrix Biology* 2017.
4. Kemaladewi, D. U., Maino, E., Hyatt, E., Hou, H., Ding, M., Place, K. M., Zhu, X., Bassi, P., Baghestani, Z., Deshwar, A. G., Merico, D., Xiong, H. Y., Frey, B. J., Wilson, M. D., Ivakine, E. A., Cohn, R. D. Correction of a splicing defect in a mouse model of congenital muscular dystrophy type 1A using a homology-directed-repair-independent mechanism. *Nat Medicine*. 23:8. 2017.
5. Sunada, Y., Bernier, S. M., Utani, A., Yamada, Y., Campbell, K. P. Identification of a novel mutant transcript of laminin alpha 2 chain gene responsible for muscular dystrophy and dysmyelination in dy2J mice. *Human molecular genetics* 4:6. 1995.
6. Gawlik, K. I., Harandi, V. M., Cheong, R. Y., Petersen, A., Durbeej, M. Laminin alpha1 reduces muscular dystrophy in dy(2J) mice. *Matrix biology : journal of the International Society for Matrix Biology* 2018.
7. Gawlik, K. I., Li, J. Y., Petersen, A., Durbeej, M. Laminin alpha1 chain improves laminin alpha2 chain deficient peripheral neuropathy. *Human molecular genetics* 15:18. 2006.

8. Gawlik, K. I., Mayer, U., Blomberg, K., Sonnenberg, A., Ekblom, P., Durbeej, M. Laminin alpha1 chain mediated reduction of laminin alpha2 chain deficient muscular dystrophy involves integrin alpha7beta1 and dystroglycan. *FEBS letters* 580:7. 2006.
9. Gawlik, K. I., Akerlund, M., Carmignac, V., Elamaa, H., Durbeej, M. Distinct roles for laminin globular domains in laminin alpha1 chain mediated rescue of murine laminin alpha2 chain deficiency. *PloS one* 5:7. 2010.
10. Gawlik, K. I., Durbeej, M. Transgenic overexpression of laminin alpha1 chain in laminin alpha2 chain-deficient mice rescues the disease throughout the lifespan. *Muscle & nerve* 42:1. 2010.
11. Maeder, M. L., Linder, S. J., Cascio, V. M., Fu, Y., Ho, Q. H., Joung, J. K. CRISPR RNA-guided activation of endogenous human genes. *Nature methods* 10:10. 2013.
12. Perez-Pinera, P., Kocak, D. D., Vockley, C. M., Adler, A. F., Kabadi, A. M., Polstein, L. R., Thakore, P. I., Glass, K. A., Ousterout, D. G., Leong, K. W., Guilak, F., Crawford, G. E., Reddy, T. E., Gersbach, C. A. RNA-guided gene activation by CRISPR-Cas9-based transcription factors. *Nature methods* 10:10. 2013.
13. Gonzalez, S., Fernando, R. N., Perrin-Tricaud, C., Tricaud, N. In vivo introduction of transgenes into mouse sciatic nerve cells in situ using viral vectors. *Nature protocols* 9:5. 2014.
14. Wojtal, D., Kemaladewi, D. U., Malam, Z., Abdullah, S., Wong, T. W., Hyatt, E., Baghestani, Z., Pereira, S., Stavropoulos, J., Mouly, V., Mamchaoui, K., Muntoni, F., Voit, T., Gonorazky, H. D., Dowling, J. J., Wilson, M. D., Mendoza-Londono, R., Ivakine, E. A., Cohn, R. D. Spell Checking Nature: Versatility of CRISPR/Cas9 for Developing Treatments for Inherited Disorders. *Am J Hum Genet* 98:1. 2016.
15. Ran, F. A., Cong, L., Yan, W. X., Scott, D. A., Gootenberg, J. S., Kriz, A. J., Zetsche, B., Shalem, O., Wu, X., Makarova, K. S., Koonin, E. V., Sharp, P. A., Zhang, F. In vivo genome editing using Staphylococcus aureus Cas9. *Nature* 520:7546. 2015.
16. Esvelt, K. M., Mali, P., Braff, J. L., Moosburner, M., Yaung, S. J., Church, G. M. Orthogonal Cas9 proteins for RNA-guided gene regulation and editing. *Nature methods* 10:11. 2013.
17. Qi, L. S., Larson, M. H., Gilbert, L. A., Doudna, J. A., Weissman, J. S., Arkin, A. P., Lim, W. A. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell* 152:5. 2013.
18. Liao, H. K., Hatanaka, F., Araoka, T., Reddy, P., Wu, M. Z., Sui, Y., Yamauchi, T., Sakurai, M., O'Keefe, D. D., Nunez-Delgado, E., Guillen, P., Campistol, J. M., Wu, C. J., Lu, L. F., Esteban, C. R., Izpisua Belmonte, J. C. In Vivo Target Gene Activation via CRISPR/Cas9-Mediated Trans-epigenetic Modulation. *Cell* 171:7. 2017.
19. Pasteuning-Vuhman, S., Putker, K., Tanganyika-de Winter, C. L., Boertje-van der Meulen, J. W., van Vliet, L., Overzier, M., Plomp, J. J., Aartsma-Rus, A., van Putten, M. Natural disease history of the dy2J mouse model of laminin alpha2 (merosin)-deficient congenital muscular dystrophy. *PloS one* 13:5. 2018.
20. Kemaladewi, D. U., Benjamin, J., Hyatt, E., Ivakine, E. A., Cohn, R. D. Increased polyamine as protective disease modifier in Congenital Muscular Dystrophy. *Human molecular genetics* 2018.
21. Bonnemann, C. G., Wang, C. H., Quijano-Roy, S., Deconinck, N., Bertini, E., Ferreiro, A., Muntoni, F., Sewry, C., Beroud, C., Mathews, K. D., Moore, S. A., Bellini, J., Rutkowski, A., North, K. N., Members of International Standard of Care Committee for Congenital Muscular, D. Diagnostic approach to the congenital muscular dystrophies. *Neuromuscular disorders : NMD* 24:4. 2014.
22. Qiao, C., Li, J., Zhu, T., Draviam, R., Watkins, S., Ye, X., Chen, C., Li, J., Xiao, X. Amelioration of laminin-alpha2-deficient congenital muscular dystrophy by somatic

- gene transfer of miniagrin. *Proceedings of the National Academy of Sciences of the United States of America* 102:34. 2005.
23. Bentzinger, C. F., Barzaghi, P., Lin, S., Ruegg, M. A. Overexpression of mini-agrin in skeletal muscle increases muscle integrity and regenerative capacity in laminin-alpha2-deficient mice. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 19:8. 2005.
24. Qiao, C., Dai, Y., Nikolova, V. D., Jin, Q., Li, J., Xiao, B., Li, J., Moy, S. S., Xiao, X. Amelioration of Muscle and Nerve Pathology in LAMA2 Muscular Dystrophy by AAV9-Mini-Agrin. *Molecular Therapy* 9:2018.
25. Meinen, S., Barzaghi, P., Lin, S., Lochmuller, H., Ruegg, M. A. Linker molecules between laminins and dystroglycan ameliorate laminin-alpha2-deficient muscular dystrophy at all disease stages. *The Journal of cell biology* 176:7. 2007.
26. Reinhard, J. R., Lin, S., McKee, K. K., Meinen, S., Crosson, S. C., Sury, M., Hobbs, S., Maier, G., Yurchenco, P. D., Ruegg, M. A. Linker proteins restore basement membrane and correct LAMA2-related muscular dystrophy in mice. *Science translational medicine* 9:396. 2017.
27. McKee, K. K., Crosson, S. C., Meinen, S., Reinhard, J. R., Ruegg, M. A., Yurchenco, P. D. Chimeric protein repair of laminin polymerization ameliorates muscular dystrophy phenotype. *The Journal of clinical investigation* 127:3. 2017.
28. Hightower, R. M., Alexander, M. S. Genetic modifiers of Duchenne and facioscapulohumeral muscular dystrophies. *Muscle & nerve* 57:1. 2018.
29. Rooney, J. E., Guppur, P. B., Burkin, D. J. Laminin-111 protein therapy prevents muscle disease in the mdx mouse model for Duchenne muscular dystrophy. *Proceedings of the National Academy of Sciences of the United States of America* 106:19. 2009.
30. Perrin, A., Rousseau, J., Tremblay, J. P. Increased Expression of Laminin Subunit Alpha 1 Chain by dCas9-VP160. *Molecular therapy. Nucleic acids* 6:2017.
31. Yuan, J., Ma, Y., Huang, T., Chen, Y., Peng, Y., Li, B., Li, J., Zhang, Y., Song, B., Sun, X., Ding, Q., Song, Y., Chang, X. Genetic Modulation of RNA Splicing with a CRISPR-Guided Cytidine Deaminase. *Molecular cell* 72:2. 2018.
32. Villiger, L., Grisch-Chan, H. M., Lindsay, H., Ringnalda, F., Pogliano, C. B., Allegri, G., Fingerhut, R., Haberle, J., Matos, J., Robinson, M. D., Thony, B., Schwank, G. Treatment of a metabolic liver disease by in vivo genome base editing in adult mice. *Nature medicine* 24:10. 2018.
33. Bengtsson, N. E., Hall, J. K., Odom, G. L., Phelps, M. P., Andrus, C. R., Hawkins, R. D., Hauschka, S. D., Chamberlain, J. R., Chamberlain, J. S. Muscle-specific CRISPR/Cas9 dystrophin gene editing ameliorates pathophysiology in a mouse model for Duchenne muscular dystrophy. *Nature communications* 8:2017.
34. Nelson, C. E., Hakim, C. H., Ousterout, D. G., Thakore, P. I., Moreb, E. A., Rivera, R. M., Madhavan, S., Pan, X., Ran, F. A., Yan, W. X., Asokan, A., Zhang, F., Duan, D., Gersbach, C. A. In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. *Science* 2015.
35. Tabebordbar, M., Zhu, K., Cheng, J. K., Chew, W. L., Widrick, J. J., Yan, W. X., Maesner, C., Wu, E. Y., Xiao, R., Ran, F. A., Cong, L., Zhang, F., Vandenberghe, L. H., Church, G. M., Wagers, A. J. In vivo gene editing in dystrophic mouse muscle and muscle stem cells. *Science* 2015.
36. Long, C., Amoasii, L., Mireault, A. A., McAnally, J. R., Li, H., Sanchez-Ortiz, E., Bhattacharyya, S., Shelton, J. M., Bassel-Duby, R., Olson, E. N. Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. *Science* 2015.

37. Thakore, P. I., D'Ippolito, A. M., Song, L., Safi, A., Shivakumar, N. K., Kabadi, A. M., Reddy, T. E., Crawford, G. E., Gersbach, C. A. Highly specific epigenome editing by CRISPR-Cas9 repressors for silencing of distal regulatory elements. *Nature methods* 12:12. 2015.
38. Lamar, K. M., Bogdanovich, S., Gardner, B. B., Gao, Q. Q., Miller, T., Earley, J. U., Hadhazy, M., Vo, A. H., Wren, L., Molkentin, J. D., McNally, E. M. Overexpression of Latent TGFbeta Binding Protein 4 in Muscle Ameliorates Muscular Dystrophy through Myostatin and TGFbeta. *PLoS genetics* 12:5. 2016.
39. Quattrocelli, M., Capote, J., Ohiri, J. C., Warner, J. L., Vo, A. H., Earley, J. U., Hadhazy, M., Demonbreun, A. R., Spencer, M. J., McNally, E. M. Genetic modifiers of muscular dystrophy act on sarcolemmal resealing and recovery from injury. *PLoS genetics* 13:10. 2017.
40. Kemaladewi, D. U., de Gorter, D. J., Aartsma-Rus, A., van Ommen, G. J., ten Dijke, P., t Hoen, P. A., Hoogaars, W. M. Cell-type specific regulation of myostatin signaling. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 26:4. 2012.
41. Zhang, Y., Argaw, A. T., Gurfein, B. T., Zameer, A., Snyder, B. J., Ge, C., Lu, Q. R., Rowitch, D. H., Raine, C. S., Brosnan, C. F., John, G. R. Notch1 signaling plays a role in regulating precursor differentiation during CNS remyelination. *Proceedings of the National Academy of Sciences of the United States of America* 106:45. 2009.
42. Hakim, C. H., Wasala, N. B., Pan, X., Kodippili, K., Yue, Y., Zhang, K., Yao, G., Haffner, B., Duan, S. X., Ramos, J., Schneider, J. S., Yang, N. N., Chamberlain, J. S., Duan, D. A Five-Repeat Micro-Dystrophin Gene Ameliorated Dystrophic Phenotype in the Severe DBA/2J-mdx Model of Duchenne Muscular Dystrophy. *Molecular therapy. Methods & clinical development* 6:2017.
43. Kim, D., Langmead, B., Salzberg, S. L. HISAT: a fast spliced aligner with low memory requirements. *Nature methods* 12:4. 2015.
44. Anders, S., Pyl, P. T., Huber, W. HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics* 31:2. 2015.
45. Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., Smyth, G. K. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic acids research* 43:7. 2015.
46. Bae, S., Park, J., Kim, J. S. Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. *Bioinformatics* 30:10. 2014.

Extended data/supplementary figures

Figure S1

Fig. S1. SadCas9-2xVP64 enhances expression of minCMV-driven *tdTomato* in *vitro*. HEK293T was transfected with (a,b) a plasmid containing minCMV-driven *tdTomato* gene only, or (c,d) in combination with a plasmid containing SadCas9-2xVP64 and a sgRNA targeting the minCMV promoter. (b,d) Cells were imaged for *tdTomato* expression by fluorescent microscopy. Scale bar: 50 μ m.

Figure S2

Fig. S2. Expression of Lama1 and muscle architecture following early intervention in neonatal dy^{2j}/dy^{2j} mice. Lower magnification of immunofluorescence and H&E staining images of Fig. 3 in the main manuscript.

Figure S3

Fig. S3. Upregulation of Lama1 corresponds to improvement of muscle functions.

Three-weeks old dy^{2j}/dy^{2j} mice were injected systemically via tail vein with different doses of AAV9: 7.5×10^{10} , 1.5×10^{11} , or 3×10^{11} viral genome (vg)/gram of mouse. Two AAVs were necessary for the three guide cohort, therefore the total doses of virus injected was 1.5×10^{11} , 3×10^{11} , and 6×10^{11} viral genomes/gram of mouse. TA muscles isolated four weeks later were stained for Lama1 expression (**a**). Asterisks indicate Lama1-positive fibers in the low dose cohort. Scale bar: 100 μ m. *In vivo* contractile force assay (**b**) and open field test (**c**) performed in the end of the treatment regimen. Data are presented as average $\pm$ standard deviation. Statistical analysis was performed using one-way ANOVA. NS: not significant. $**P < 0.01$.

Figure S4

Fig. S4. Representative images of Lama1-positive muscle sections

Mice were injected systemically with three guides at the age of three weeks old via tail vein, and sacrificed at the age of 11-12 weeks old. Muscles were stained for Lama1 expression (red). Scale bar: 500 μ m.

Figure S5

Fig. S5. Expression of Laminin (LM) subunits in tibialis anterior muscles.

Expression of LM subunits in tibialis anterior from wild-type, dy^{2j}/dy^{2j} (intravenously injected with AAV carrying no guide or three guides) and $dy^{2j}/LM\alpha1$ (with transgenic overexpression of $LM\alpha1$) mice (n=3 for each group). Expression of $LM\alpha1$ chain in tibialis anterior is comparable between dy^{2j}/dy^{2j} AAV-three guide-treated mice and transgenic mice. No major differences in expression of $LM\alpha2$ and $LM\gamma1$ were detected between the groups. $LM\alpha4$ chain is upregulated in muscle from dy^{2j}/dy^{2j} AAV-no guide-treated mice and its expression is partially normalized in muscle from dy^{2j}/dy^{2j} AAV-three guide treated mice. Scale bar: 50μm.

Figure S6

Fig. S6. Expression of Laminin (LM) subunits in gastrocnemius muscles.

Expression of LM subunits in tibialis anterior from wild-type, dy^{2j}/dy^{2j} (intravenously injected with AAV carrying no guide or three guides) and $dy^{2j}/LM\alpha1$ (with transgenic overexpression of LM $\alpha1$) mice (n=3 for each group). Expression of LM $\alpha1$ chain in tibialis anterior is comparable between dy^{2j}/dy^{2j} AAV-three guide-treated mice and transgenic mice. No major differences in expression of LM $\alpha2$ and LM $\gamma1$ were detected between the groups. LM $\alpha4$ chain is upregulated in muscle from dy^{2j}/dy^{2j} AAV-no guide-treated mice and its expression is partially normalized in muscle from dy^{2j}/dy^{2j} AAV-three guide treated mice. Scale bar: 50 μ m.

Fig. S7. Myelination of sciatic nerves.

Electron microscopy images of sciatic nerves isolated from dy^{2j}/dy^{2j} mice injected with AAV carrying no guide or three guides (n=3 for each group). Myelin thickness was quantified using ImageJ and presented as average $\pm$ standard deviation. Statistical analysis was performed using Student's t -test. * P <0.05. Scale bar: 5 μ m.

Figure S8

Fig. S8. Quantitative evaluation of AAV genome distribution.

Genomic DNA isolated from tibialis anterior muscle, sciatic nerve and liver of dy2j mice injected with AAV carrying three guides (n=8) was amplified for the presence of viral genome by qPCR. Data are presented as average $\pm$ SEM.

Figure S9

Fig. S9. Genome-wide analysis of gene expression of SadCas9-VP64-treated dy^{2j}/dy^{2j} mice.

Differential expression analysis derived from RNA-Sequencing results from the quadriceps of treated and untreated mice (**a-c**), comparing SadCas9-2xVP64 alone and SadCas9-2xVP64 with three guides targeting *Lama1* (**a**), untreated to SadCas9-2xVP64 alone (**b**), and untreated to SadCas9-2xVP64 with three guides targeting *Lama1* (**c**). Gene significantly differentially expressed, (FDR < 0.05), are coloured. Red data points indicate a log-fold change greater than one, while blue data points indicate a log-fold change less than one. Hierarchical clustering was performed on the normalized counts-per-million expression data (**d**).

Figure S10

Fig. S10. Top 100 differentially expressed genes.

Heatmap illustrating normalized log-CPM values for the top 100 genes differentially expressed in SadCas9-2xVP64 with three guide treated quadriceps versus untreated quadriceps. Red indicates higher expression while blue indicates lower expression.

Start 3X FLAG
M - DYKDHDGDYKDHDIDYKDDDDK - HGPR -
VP64
DALDDFDLMDLGSALDDFDLMDLGSALDDFDLMDLGSALDDFDLMDL -
NLS
VN - PKKKRKV - GIHGVPAA -
D10A
KRNILGLA*IGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRL
KRRRRRHRIQRVKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAK
RRGVHNVNEVEEDTGNELSTKEQISRSKALEEKYVAELQLERLKKDGEVRGSINRF
KTSYVKEAKQLLKVKAYHQLDQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKE
WYEMLMGHCTYFPEELRSVKYAYNADLYNALNDLNNLVITRDENEKLEYEKFQIEN
VFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIENAEL
LDQIAKILTIYQSSEDIQEELTNLNSLTQEEIEQISNLKGYTGTHNLSLKAINLILDELWH
TNDNQIAIFNRLKLVPPKVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIIKKYGLP
NDIIELAREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGKENAKYLIEKIKLHDMQE
GKCLYSLEAIPLEDLLNPNFYEVVDHIIIPRSVSFDNSFNKVLVKQEEA*SKKGNRTPF
QYLSSSDSKISYETFFKKHILNLAQKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDT
RYATRGLMNNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKGYKHAEDA
LIANADFIFKEWKKLDKAKKVMENQMFEKQAESMPEIETEQEYKEIFITPHQIKHIKD
FKDYKYSHRVDKKPNRELINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKLKKLINKS
PEKLLMYHHPQTYQKLKLIMEQYGDEKNPLYKYEETGNYLTKYSKKNNGPVIKKIK
YYGNKLNALDITDDYPNSRNKVVKLSLKPYRFDVYLDNGVYKFVTVKNLDVIKKENY
YEVNSKCYEEAKKKKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLLNRIEVMIDIT
YREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG -
NLS
KRPAATKKAGQAKKKK - GS -
VP64
DALDDFDLMDLGSALDDFDLMDLGSALDDFDLMDLGSALDDFDLMDL

Supplemental video legends

Video S1. Phenotype of control dy^{2j}/dy^{2j} mouse following early intervention.

The dy^{2j}/dy^{2j} mouse was injected with AAV9 containing *SadCas9*-2xVP64 (no guide) at P2 (pre-symptomatic stage) via temporal vein and video was taken at the age of 7-week old. Hind limb paralysis, contracture and kyphosis resulting from lack of functional *Lama2* and compensatory *Lama1* are apparent.

Video S2. Phenotype of treated dy^{2j}/dy^{2j} mouse following early intervention.

The dy^{2j}/dy^{2j} mouse was injected with AAV9 containing *SadCas9*-2xVP64 and sgRNAs targeting *Lama1* proximal promoter (three guides) at P2 (pre-symptomatic stage) via temporal vein and video was taken at the age of 7-week old. Upregulation of compensatory *Lama1* expression ameliorates the hind limb paralysis, contracture and kyphosis.

Video S3. Phenotype of control dy^{2j}/dy^{2j} mouse following intervention at symptomatic stage.

The dy^{2j}/dy^{2j} mouse was injected with AAV9 containing *SadCas9*-2xVP64 (no guide) at 3-week old (pre-symptomatic stage) via tail vein. Video was taken at the age of 7-week old. Hind limb paralysis, contracture and kyphosis resulting from lack of functional *Lama2* and compensatory *Lama1* are apparent.

Video S4. Phenotype of treated dy^{2j}/dy^{2j} mouse following intervention at symptomatic stage.

The dy^{2j}/dy^{2j} mouse was injected with AAV9 containing *SadCas9*-2xVP64 and sgRNAs targeting *Lama1* proximal promoter (three guides) at 3-week old (pre-symptomatic stage) via tail vein. Video was taken at the age of 7-week old. Dystrophic features and disease progression were significantly improved and partially reversed following upregulation of *Lama1*.

Supplemental tables

Supplemental table 1. Top 100 genes differentially expressed in “SadCas9-2xVP64 + three guides” compared to “untreated”

Supplemental table 2. Top 100 genes differentially expressed in “SadCas9-2xVP64 + three guides” compared to “SadCas9-2xVP64 + no guide”

Supplemental table 3. Top 100 genes differentially expressed in “SadCas9-2xVP64 + no guide” compared to “untreated”

Supplemental table 4. Analysis showing distance from off-target site to differentially expressed gene body

Supplemental table 5. List of computationally predicted off-target sites

Supplemental table 6. Primers, plasmids and sgRNAs used in this study

Supplemental Table 1. Top 100 genes differentially expressed in "SadCas9-2xVP64 + three guides" compared to "untreated"

SYMBOL	TXCHROM	logFC	AveExpr	t	P.Value	adj.P.Val
Ces1c	chr8	8.06720501	-4.0858727	25.0852651	7.60E-11	1.10E-06
1810053823	chr16	10.5369617	-2.0993569	17.6475033	2.81E-09	6.76E-06
Fabp1	chr6	7.92790193	-3.3988185	18.7401814	1.44E-09	6.76E-06
1700003G13	chr9	7.85466761	-3.8132313	17.9773962	2.19E-09	6.76E-06
Cyp2c69	chr19	6.79884139	-4.4022125	17.5155103	2.77E-09	6.76E-06
Slc7a4	chr16	5.42020681	0.54904376	17.737618	2.38E-09	6.76E-06
Ttr	chr18	8.97781797	-3.6645933	17.0207698	3.88E-09	7.51E-06
Apoa2	chr1	5.40091384	-0.6005275	16.7716387	4.16E-09	7.51E-06
Lama1	chr17	8.98321051	0.34708087	15.7290019	8.52E-09	1.37E-05
ApoB	chr12	7.80234986	-3.4299405	15.0813327	1.26E-08	1.82E-05
Tat	chr8	5.3144494	-3.9814489	14.66332	1.58E-08	2.07E-05
Serpina1c	chr12	8.1709174	-3.2361681	14.3754788	2.04E-08	2.27E-05
Fam25c	chr14	7.39071119	-0.5378939	14.4492017	1.90E-08	2.27E-05
Serpina3k	chr12	9.29387622	-2.7989697	13.9984407	2.72E-08	2.81E-05
Igf1bp1	chr11	6.69308056	-4.2970164	13.6072358	3.38E-08	3.26E-05
Mup3	chr4	7.59035385	-2.0248159	13.5095555	3.70E-08	3.35E-05
Rdh7	chr10	6.12757345	-4.306269	12.9783192	5.31E-08	4.07E-05
Gys2	chr6	4.5777154	-1.5679355	13.0234776	5.00E-08	4.07E-05
Wnt4	chr4	4.20831956	5.03311767	12.9293649	5.35E-08	4.07E-05
Gc	chr5	8.24180382	-3.5843802	12.9381073	5.75E-08	4.07E-05
Klrg2	chr6	4.59908759	-1.1223193	12.8010591	5.92E-08	4.07E-05
Srrm4os	chr5	4.04588484	3.61138985	12.4826437	7.52E-08	4.94E-05
Mat1a	chr14	7.36113899	-3.7343781	12.3707009	8.71E-08	5.47E-05
Alb	chr5	9.586285	0.41592462	12.2174291	1.04E-07	6.01E-05
Serpina1a	chr12	6.95305368	-3.6425688	12.1347225	1.04E-07	6.01E-05
Azgp1	chr5	6.86136461	-4.0619138	11.9945852	1.16E-07	6.46E-05
Fgb	chr3	7.48821994	-3.1735567	11.6312338	1.59E-07	8.50E-05
Cidea	chr18	5.07510399	2.21493926	11.4830743	1.71E-07	8.82E-05
9030619P08	chr15	3.92341807	-0.2600478	11.3463138	1.89E-07	9.42E-05
Fgg	chr3	7.33395248	-3.4152783	11.1329778	2.41E-07	0.00011613
Mmd2	chr5	6.67105341	-3.3145461	11.0364562	2.58E-07	0.00012028
Cyp3a11	chr5	8.17360921	-3.6630995	10.8836274	3.06E-07	0.00013811
Slc27a2	chr2	6.6827	-3.1819203	10.7687412	3.26E-07	0.00014295
Ambp	chr4	6.13758126	-3.4506026	10.6633966	3.54E-07	0.00015057
Fga	chr3	7.62720297	-3.9076478	10.5588289	4.03E-07	0.00016636
Samd11	chr4	4.11178696	-0.4979967	10.4245456	4.25E-07	0.00017075
Fabp4	chr3	2.15462805	9.01447477	10.3115148	4.68E-07	0.00018279
Ighg1	chr12	8.46471745	-3.4389537	10.3557091	4.95E-07	0.00018754
Lctf	chr9	4.2237416	-0.3808795	10.2088981	5.19E-07	0.00018754
Pvalb	chr15	2.52928689	12.8626777	10.2161746	5.11E-07	0.00018754
Apoa5	chr9	6.60004476	-4.1263676	10.219239	5.36E-07	0.00018765
Vps13c	chr9	-2.1668504	3.212495	-10.146085	5.45E-07	0.00018765
Gm4841	chr18	3.86778458	5.35415478	9.78492273	7.72E-07	0.00025932
Prkar2b	chr12	3.97928004	3.13121334	9.7483776	8.00E-07	0.00026274
Pdpr	chr8	-2.203443	3.2904627	-9.4808172	1.03E-06	0.00033107
Hhip1	chr12	-2.7573871	2.31203806	-9.4137394	1.10E-06	0.00034623
Epn3	chr11	4.58955952	-1.5891827	9.3486758	1.19E-06	0.00036635
Aldh1a7	chr19	3.50528717	1.27190747	9.26652168	1.28E-06	0.00038529
Apoa1	chr9	8.39321713	-2.4434213	9.24069066	1.44E-06	0.00042593
Aspg	chr12	4.61360624	-0.6564861	9.09955804	1.53E-06	0.00044273
Adgrd1	chr5	-2.427791	3.44722048	-8.9858424	1.70E-06	0.00048062
Kng1	chr16	5.95942358	-3.7065193	8.99480646	1.75E-06	0.00048561
Ucp1	chr8	11.3963873	-1.5055582	9.07259957	1.85E-06	0.000504
Cps1	chr1	5.87067733	-3.3848414	8.82056311	2.09E-06	0.00055915
Sfrp4	chr13	-3.0424407	4.08490344	-8.7250934	2.23E-06	0.00058511
Jmjd1c	chr10	-3.2313535	1.84643433	-8.5191608	2.77E-06	0.00071562
Orm2	chr4	6.27368915	-2.5305119	8.54126641	2.83E-06	0.00071836
Atp6v1g1	chr4	1.90407218	8.11437097	8.46629447	2.93E-06	0.00072976
NA	NA	3.54327015	-0.8576183	8.37633903	3.24E-06	0.00079375
Elovl6	chr3	3.24419618	3.37792107	8.22580012	3.81E-06	0.0009186
Plg	chr17	4.98742747	-2.84041	8.16368792	4.16E-06	0.00098659
Pzp	chr6	6.39214719	-3.2228911	8.11448596	4.53E-06	0.00105615
Thrsp	chr7	3.26554164	6.03631914	7.85593444	5.77E-06	0.00131835
Gfpt2	chr11	-2.0366124	3.28844052	-7.8437333	5.84E-06	0.00131835
Bhmt	chr13	6.2546489	-3.2262881	7.78687551	6.55E-06	0.00145525
Apoc3	chr9	6.95422106	-2.1511746	7.78192704	6.70E-06	0.00146687
Proca1	chr11	2.63255929	1.56171029	7.68968505	6.97E-06	0.00150381
Hpd	chr5	6.45914605	-3.386459	7.69200292	7.34E-06	0.00156002
Pcsk6	chr7	-1.8922339	4.14311915	-7.6077764	7.67E-06	0.00160525
Dnajb13	chr7	5.86226504	-1.8392095	7.50871687	8.97E-06	0.0018516
NA	NA	-2.8570014	1.63865163	-7.4503139	9.23E-06	0.00187895
Myo5b	chr18	2.30425852	1.18853647	7.43398437	9.40E-06	0.00188746
Fam213b	chr4	2.52777314	7.1852214	7.33239481	1.06E-05	0.00210163
Aldob	chr4	6.06208644	-2.8991635	7.31447554	1.14E-05	0.0021888
Uox	chr3	5.46798424	-3.8910942	7.31419174	1.12E-05	0.0021888
NA	NA	-3.0351581	12.8448432	-7.2578878	1.16E-05	0.00221053
Mfrn	chr10	1.89226589	5.31305923	7.19570826	1.25E-05	0.00234855
Me1	chr9	1.9768026	6.91692293	7.13418269	1.35E-05	0.00243628
Agbl1	chr7	-2.0885962	1.3608529	-7.1465611	1.33E-05	0.00243628
NA	NA	-2.9421529	5.7414647	-7.1386369	1.34E-05	0.00243628
NA	NA	-2.8056988	10.2088761	-7.0774684	1.45E-05	0.00258145
Trpm1	chr7	2.96315703	0.3806044	7.01294075	1.57E-05	0.00276221
Cst6	chr19	2.23495652	4.66958372	7.00007046	1.59E-05	0.00276912
Igtp	chr11	1.86743947	4.54464325	6.89217296	1.82E-05	0.00312869
NA	NA	4.69385795	0.68222882	6.84250559	1.97E-05	0.00331558
Med12	chrX	-2.8614945	3.45721138	-6.8346408	1.96E-05	0.00331558
Serpina1d	chr12	6.32897767	-3.6602287	6.8549536	2.01E-05	0.00334534
NA	NA	4.3761592	4.3735629	6.78718488	2.11E-05	0.00342487
Kcnk3	chr5	3.81800932	1.86995833	6.77998928	2.11E-05	0.00342487
Car5b	chrX	2.17094632	2.52751196	6.69419221	2.34E-05	0.00374934
Aldh1a2	chr9	-3.9155062	1.81350194	-6.6857214	2.38E-05	0.0037799
Grem2	chr1	2.40181819	2.79352205	6.59957294	2.64E-05	0.00414096
Ces1d	chr8	4.10023518	3.35125626	6.59264986	2.69E-05	0.00417708
Ston1	chr17	-2.2562933	0.96729497	-6.573946	2.72E-05	0.00418839
Hpx	chr7	4.63301432	-1.3730015	6.56670755	2.80E-05	0.0042629
ApoH	chr11	2.91855783	-0.5038737	6.52074789	2.92E-05	0.00432487
Ces5a	chr8	2.60133348	0.55171618	6.51934336	2.93E-05	0.00432487
Dpp6	chr5	-2.9196681	0.2619482	-6.5178887	2.93E-05	0.00432487
NA	NA	-3.2449209	-0.3411066	-6.4875991	3.06E-05	0.00445983
Hist1h4i	chr13	1.82127787	3.91078129	6.41227049	3.36E-05	0.00485751

Supplemental Table 2. Top 100 genes differentially expressed in
"SadCas9-2xVP64 + three guides" compared to "SadCas9-2xVP64 no guide"

SYMBOL	TXCHROM	logFC	AveExpr	t	P.Value	adj.P.Val
Ces1c	chr8	7.68192528	-4.0858727	23.7177026	1.33E-10	1.92E-06
Tat	chr8	6.51413216	-3.9814489	18.7406265	1.40E-09	1.01E-05
Slc7a4	chr16	5.42395906	0.54904376	16.666776	4.43E-09	1.30E-05
Cyp2c69	chr19	6.41356165	-4.4022125	16.3517656	5.46E-09	1.30E-05
Fabp1	chr6	7.01430136	-3.3988185	16.2688646	5.82E-09	1.30E-05
1810053823	chr16	8.36567269	-2.0993569	16.2583139	6.04E-09	1.30E-05
Ttr	chr18	8.59253823	-3.6645933	16.1987709	6.30E-09	1.30E-05
Apoa2	chr1	4.31309488	-0.6005275	13.99838	2.46E-08	4.44E-05
1700003G13	chr9	6.16709101	-3.8132313	13.5514729	3.48E-08	5.59E-05
Rdh7	chr10	6.27061455	-4.306269	13.3403682	4.07E-08	5.88E-05
Apob	chr12	6.36042846	-3.4299405	11.8844821	1.26E-07	0.00015264
Alb	chr5	6.60124813	0.41592462	11.8383922	1.31E-07	0.00015264
Gc	chr5	7.61086889	-3.5843802	11.8108876	1.37E-07	0.00015264
Mup3	chr4	5.93703811	-2.0248159	11.5983687	1.58E-07	0.00016265
Igf1bp1	chr11	5.77947999	-4.2970164	11.4236063	1.82E-07	0.00017145
Serpina1a	chr12	6.56777394	-3.6425688	11.3493672	1.97E-07	0.00017145
Fam25c	chr14	4.42384018	-0.5378939	11.2759968	2.02E-07	0.00017145
Klrg2	chr6	4.01555522	-1.1223193	11.016482	2.51E-07	0.000195
Serpina1c	chr12	6.4833408	-3.2361681	10.992408	2.67E-07	0.000195
Fgg	chr3	7.23133838	-3.4152783	10.9526164	2.81E-07	0.000195
Fgb	chr3	7.10294021	-3.1735567	10.9405546	2.83E-07	0.000195
Klf1a	chr1	-3.2035885	1.72216362	-10.578571	3.68E-07	0.0002416
Lama1	chr17	3.60580481	0.34708087	10.3369068	4.59E-07	0.00028082
Serpina3k	chr12	6.46825197	-2.7989697	10.3674744	4.66E-07	0.00028082
Knq1	chr16	6.59377507	-3.7065193	10.145317	5.74E-07	0.00033198
Fga	chr3	7.24192323	-3.9076478	9.94497979	7.04E-07	0.00039142
Cidea	chr18	4.23425211	2.21493926	9.56079601	9.62E-07	0.00050767
Mat1a	chr14	5.91921759	-3.7343781	9.56655238	9.84E-07	0.00050767
Cyp3a11	chr5	7.26000864	-3.6630995	9.49753456	1.09E-06	0.00054116
Cadm4	chr7	-3.0854364	3.43404374	-9.3112336	1.22E-06	0.00058834
Slc27a2	chr2	5.77735647	-3.1819203	9.16142955	1.47E-06	0.00068446
Ucp1	chr8	11.5394284	-1.5055582	9.19742703	1.63E-06	0.00073752
Myo5b	chr18	2.70815964	1.18853647	8.91782918	1.82E-06	0.00079698
Mbp	chr18	-3.1241971	6.32564126	-8.796965	2.07E-06	0.00087745
Uox	chr3	6.38500137	-3.8910942	8.81536963	2.13E-06	0.00087745
NA	NA	7.30546168	-3.4389537	8.74748808	2.33E-06	0.00093426
Prx	chr7	-3.432919	5.10990804	-8.6589476	2.39E-06	0.00093426
Mpz	chr1	-3.1391767	7.72442679	-8.5709125	2.62E-06	0.00095901
Samd11	chr4	3.4319878	-0.4979967	8.53270285	2.74E-06	0.00095901
Apoa5	chr9	5.68644419	-4.1263676	8.55205544	2.76E-06	0.00095901
Azgp1	chr5	5.17378801	-4.0619138	8.54116051	2.77E-06	0.00095901
Ambp	chr4	5.00265745	-3.4506026	8.53232871	2.79E-06	0.00095901
Aspg	chr12	4.49864426	-0.6564861	8.45164468	3.02E-06	0.00101368
9030619P08	chr15	2.99612235	-0.2600478	8.36658783	3.27E-06	0.00105887
Sbsn	chr7	-2.6633714	0.90905622	-8.3572564	3.30E-06	0.00105887
Plg	chr17	5.52507799	-2.84041	8.36109263	3.39E-06	0.00106392
Cldn19	chr4	-3.2653338	2.7388015	-8.2964238	3.53E-06	0.00108534
Gys2	chr6	2.88423499	-1.5679355	8.17357339	4.04E-06	0.00121478
Ahsg	chr16	5.41354129	-0.9425466	8.11597943	4.43E-06	0.00130544
Wnt4	chr4	2.89310387	5.03311767	8.06192403	4.57E-06	0.00132035
Srrm4os	chr5	2.83746235	3.61138985	8.01069135	4.84E-06	0.00137073
Apoa1	chr9	6.68102512	-2.4434213	7.80253925	6.50E-06	0.0018055
Elovl6	chr3	3.14304008	3.37792107	7.70931392	6.83E-06	0.00186108
G6pc	chr11	5.60315183	-3.3951407	7.70806009	7.06E-06	0.00187982
Cps1	chr1	5.23974239	-3.3848414	7.6779702	7.26E-06	0.00187982
Aldob	chr4	6.32598423	-2.8991635	7.69579537	7.29E-06	0.00187982
Arg1	chr10	4.44362967	-1.6097834	7.59014941	7.93E-06	0.00201138
Epn3	chr11	3.69453485	-1.5891827	7.53325568	8.41E-06	0.00209514
Hba-a1	NA	-2.2700334	6.2456611	-7.4965888	8.73E-06	0.00213896
Ddn	chr15	-3.398565	0.55130958	-7.2073462	1.24E-05	0.00298201
Fa2h	chr8	-3.2130863	1.20608522	-7.032031	1.53E-05	0.00363058
Cadm3	chr1	-2.051734	3.75756436	-6.9210036	1.75E-05	0.00403033
Ppp1r3b	chr8	2.57692007	0.40173032	6.92011989	1.76E-05	0.00403033
Pzp	chr6	5.50500956	-3.2228911	6.84788224	1.99E-05	0.00449761
Nfasc	chr1	-2.6849138	1.86436923	-6.7931454	2.06E-05	0.00458172
Orm2	chr4	4.38077136	-2.5305119	6.70807897	2.33E-05	0.00506111
Sox10	chr15	-3.2987824	1.71005186	-6.6733195	2.41E-05	0.00506111
Hpx	chr7	5.41271902	-1.3730015	6.697118	2.41E-05	0.00506111
Prkar2b	chr12	2.93206413	3.13121334	6.65445212	2.46E-05	0.00506111
Bmp7	chr2	-2.8365493	-0.1703501	-6.65022	2.47E-05	0.00506111
Ces5a	chr8	2.72830868	0.55171618	6.64349268	2.49E-05	0.00506111
Serpina1d	chr12	6.18935314	-3.6602287	6.67534697	2.52E-05	0.00506111
Dnajb13	chr7	4.93453349	-1.8392095	6.49479192	3.09E-05	0.00612482
Serpinc1	chr1	3.93724606	-1.3533652	6.28356394	4.02E-05	0.0078515
Dact2	chr17	-2.5238997	0.9490797	-6.1621573	4.69E-05	0.00902999
NA	NA	2.77363083	-0.8576183	6.1509308	4.76E-05	0.0090517
Hpd	chr5	5.36870724	-3.386459	6.15556143	4.90E-05	0.0091922
Acsn3	chr7	4.79325628	-0.8979803	6.08513137	5.32E-05	0.00985906
Dusp15	chr2	-2.832713	1.31355423	-5.9744195	6.05E-05	0.0110674
Mag	chr7	-2.4835744	1.72185278	-5.8808295	6.88E-05	0.01242024
231008N11	chr8	2.47762951	0.0223978	5.78103716	7.90E-05	0.0140903
Aph1c	chr9	2.02542717	1.4446743	5.69597944	8.90E-05	0.01567068
Alas2	chrX	-2.7381939	2.63862482	-5.6882468	9.00E-05	0.01567068
Aldh1a2	chr9	-3.5045852	1.81350194	-5.619936	9.96E-05	0.01713352
Rasgef1c	chr11	-2.7268056	-0.4788461	-5.5831253	0.00010446	0.01760109
Slc9b2	chr3	2.60867469	0.03741035	5.58003047	0.00010488	0.01760109
Sptbn5	NA	-3.1073231	2.1100215	-5.5741903	0.00010597	0.01760109
Aph1	chr11	2.6612831	-0.5038737	5.5623464	0.00010758	0.01766438
Plip	chr8	-2.6544819	2.15418714	-5.5538324	0.00010889	0.01767908
Mgat3	chr15	-2.522372	1.43835928	-5.538076	0.00011133	0.01787536
Spn	chr4	-2.0266173	3.73200949	-5.4989035	0.0001177	0.01857554
Mup11	chr4	6.5359933	-0.3747465	5.54577617	0.00011827	0.01857554
Hbb-bt	NA	-2.1214318	4.13274184	-5.4147076	0.00013289	0.02043237
Klf19a	chr11	-2.4525954	1.56428869	-5.414812	0.00013292	0.02043237
Hepacam	chr9	-3.1235403	-0.7331738	-5.3921781	0.00013769	0.02094399
9130230L23	chr5	2.99937564	-1.7029617	5.3379434	0.00014889	0.0224111
Apo3	chr9	3.89375921	-2.1511746	5.21346237	0.00018033	0.02686402
Scube1	chr15	-2.61022	0.15037746	-5.1910997	0.00018445	0.02719675
Gal3st1	chr11	-2.7637327	-0.2060749	-5.1771827	0.00018838	0.02749575
Gm4841	chr18	2.44516047	5.35415478	5.13981561	0.00019895	0.02874825

Supplemental Table 3. Top 100 genes differentially expressed in
SadCas9-2xVP64-no guide compared to "untreated"

SYMBOL	TXCHROM	logFC	AveExpr	t	P.Value	adj.P.Val
Hba-a1	NA	3.12223892	6.2456611	12.331034	8.43E-08	0.00121768
Mpz	chr1	3.67608141	7.72442679	10.6882893	3.34E-07	0.00241497
Hbb-bt	NA	3.16484723	4.13274184	9.89369815	6.93E-07	0.00244925
Hba-a2	chr11	3.23794544	4.91567042	9.85619618	7.18E-07	0.00244925
Cadm4	chr7	3.13536782	3.43404374	9.68323067	8.47E-07	0.00244925
Mbp	chr18	3.22961817	6.32564126	9.26281665	1.28E-06	0.00308948
Lama1	chr17	5.3774057	0.34708087	8.31143772	3.57E-06	0.0073592
Hbb-bs	chr7	2.8634898	6.55027296	8.1395833	4.19E-06	0.00756795
Prx	chr7	3.18266127	5.10990804	7.96374203	5.11E-06	0.00765789
Cldn19	chr4	3.06808525	2.7388015	7.89212771	5.53E-06	0.00765789
Tlr13	chrX	-2.5987846	1.26055872	-7.8376704	5.88E-06	0.00765789
1700001O22Rik	chr2	3.76078395	-0.8282985	7.77422963	6.36E-06	0.00765789
Ccl2	chr11	-2.7593297	1.1058906	-7.6753655	7.09E-06	0.00788188
Mx1	chr16	-3.4609353	0.74527914	-7.5588781	8.15E-06	0.00804522
Fa2h	chr8	3.32272314	1.20608522	7.53694641	8.35E-06	0.00804522
CSar1	chr7	-1.8891035	2.10571941	-7.258534	1.16E-05	0.01013989
Klf1a	chr1	2.32436318	1.72216362	7.23538702	1.19E-05	0.01013989
Fam178b	chr1	3.0042875	1.76045364	7.10719561	1.40E-05	0.01120779
Ddn	chr15	3.14993822	0.55130958	6.92964353	1.74E-05	0.01322915
Lctl	chr9	3.26770545	-0.3808795	6.88735709	1.83E-05	0.01325713
Plip	chr8	3.06580561	2.15418714	6.82876119	1.97E-05	0.01357527
Lrrc15	chr16	-2.4883765	0.74755506	-6.7582519	2.15E-05	0.01414289
NA	NA	-1.9501553	3.38202652	-6.6526	2.46E-05	0.01547083
Alas2	chrX	2.9899336	2.63862482	6.51840873	2.93E-05	0.01765268
Xist	chrX	-3.2767212	6.77693285	-6.4385364	3.26E-05	0.01864379
Ncmmap	chr4	3.08130356	2.86901463	6.39750506	3.43E-05	0.01864379
Gjb1	chrX	3.46954089	0.2018106	6.38853084	3.48E-05	0.01864379
Mag	chr7	2.56287891	1.72185278	6.33342043	3.73E-05	0.01922711
SncA	chr6	3.12063457	0.87022355	6.30932249	3.86E-05	0.01922711
Ilrl1	chr1	-2.5405179	0.79307145	-6.2535459	4.15E-05	0.01968275
Pvalb	chr15	1.9371664	12.8626777	6.2397791	4.22E-05	0.01968275
Dusp15	chr2	2.72326203	1.31355423	5.89258602	6.77E-05	0.02962619
Adam12	chr7	-2.0037212	2.41708454	-5.8606882	7.07E-05	0.02962619
Ccl7	chr11	-2.540678	0.66322051	-5.838118	7.30E-05	0.02962619
Cd300lb	chr11	-2.4326089	0.71920982	-5.8322784	7.35E-05	0.02962619
Bmp7	chr2	2.48796642	-0.1703501	5.82971426	7.38E-05	0.02962619
Arg1	chr10	-3.6796601	-1.6097834	-5.8042644	7.70E-05	0.03006634
Sfrp1	chr8	-1.9250563	3.06171827	-5.6945804	8.91E-05	0.03389067
Aoah	chr13	-2.230976	0.59804822	-5.5991203	0.00010202	0.0377995
Msr1	chr8	-2.5214491	1.6301269	-5.5421659	0.00011069	0.03942958
Cmtm5	chr14	2.84161366	-0.3319268	5.5243583	0.00011364	0.03942958
Clec4d	chr6	-2.2426926	-0.1450714	-5.5175924	0.0001146	0.03942958
Mmd2	chr5	4.10127424	-3.3145461	5.35972024	0.00014594	0.04854376
Gal3st1	chr11	2.75795131	-0.2060749	5.34218678	0.00014781	0.04854376
Sbspon	chr1	2.22808049	0.44090614	5.22117943	0.00017632	0.05546403
Adams6	chr13	-2.4536128	1.59657982	-5.2204485	0.00017656	0.05546403
Hhip1	chr12	-1.9118394	2.31203806	-5.2007135	0.00018171	0.05586471
Hepacam	chr9	2.91504751	-0.7331738	5.16779439	0.00019114	0.0574251
F2rl1	chr13	-2.2231625	1.71289475	-5.1068931	0.00020888	0.06109392
Gm42067	NA	1.85312529	2.04012111	5.09877039	0.0002114	0.06109392
Adcy7	chr8	-1.881753	4.6834493	-5.0461447	0.00022871	0.06480185
Klf19a	chr11	2.27988907	1.56428869	5.02136549	0.00023743	0.06597873
Tlr8	chrX	-4.0365645	-1.2570211	-4.9449509	0.00027029	0.07369187
Smox	chr2	1.81483923	5.4987099	4.89994085	0.00028521	0.07513181
C3ar1	chr6	-3.095913	2.52727176	-4.897357	0.00028728	0.07513181
Eps8l1	chr7	-2.4924048	2.20261789	-4.8867435	0.00029117	0.07513181
Adgre1	chr17	-1.5575189	3.45468859	-4.8714084	0.00029787	0.07551336
Sfrp4	chr13	-2.0811203	0.08490344	-4.8359387	0.00031448	0.07742878
Sfrn4	chr11	-2.7773743	-1.1861611	-4.8334332	0.00031615	0.07742878
Lilr4b	chr10	-3.0117057	2.19904976	-4.7560936	0.00035649	0.08523435
NA	NA	2.2188592	1.46909467	4.74477333	0.00036179	0.08523435
Dupd1	chr14	1.83532553	6.3387283	4.73767715	0.00036571	0.08523435
Cdh1	chr8	2.69435584	0.15753197	4.7105409	0.00038186	0.08758428
Ctbp100	chr6	2.68467385	-0.1085207	4.65308887	0.00041746	0.09425372
Chil3	chr3	-4.4041995	-0.8908455	-4.6368929	0.00043783	0.09733231
NA	NA	-2.032801	1.63865163	-4.5516428	0.00048859	0.10697147
Ms4a7	chr19	-2.0914278	2.7993614	-4.4397996	0.0005829	0.12571458
Pmp22	chr11	1.97966029	7.02265612	4.41974373	0.00060171	0.12720169
Sox10	chr15	2.30301682	1.71005186	4.41400578	0.0006074	0.12720169
Tnc	chr4	-2.1869894	6.29769631	-4.323846	0.00070125	0.14475757
Dclre1c	chr2	-1.798468	1.83339495	-4.2666147	0.00076856	0.15522687
Ccl6	chr11	-1.8144934	4.30576424	-4.2626793	0.00077345	0.15522687
A930016O22Rik	chr7	1.77831878	7.34616069	4.2491595	0.00079047	0.15646948
Spire2	chr8	2.46586051	-0.8626346	4.23290116	0.00081207	0.15857376
Pdpr	chr8	-1.5137564	3.29044627	-4.2177999	0.00083148	0.1601984
Ms4a4a	chr19	-3.3012903	2.04305875	-4.2086143	0.0008496	0.16153672
Retnla	chr16	1.70702394	3.83252031	4.18081523	0.00088276	0.16566021
Igsf6	chr7	-2.1161057	1.14725297	-4.1698631	0.00089871	0.16649164
Bmper	chr9	-2.3781522	3.01926601	-4.1196582	0.00097551	0.17808689
Kcnmb4	chr10	2.06573485	-0.0707184	4.1071686	0.00099511	0.17808689
Cenpi	chrX	-3.2736233	-0.9010077	-4.1092747	0.00099827	0.17808689
Tfec	chr6	-2.4975075	-0.9890424	-4.0905242	0.00102332	0.18032955
Ccr1	chr9	-3.5459938	0.74382016	-4.0436169	0.00111601	0.19199831
Serpine1	chr5	-1.6929341	4.20045863	-4.0366477	0.00111654	0.19199831
G530011O06Rik	chrX	-3.0056326	0.28110767	-4.0255271	0.00114176	0.19199831
Ptger2	chr14	-2.2297542	-0.7377989	-4.0227253	0.00114269	0.19199831
Lilrb4a	chr10	-4.0994493	0.16994375	-3.9912293	0.00123047	0.20437115
Rnf180	chr13	-1.9538557	-0.0378799	-3.9618891	0.00126259	0.20522254
Ccl12	chr11	-2.0439673	-0.1216011	-3.9612473	0.001264	0.20522254
Jmjd1c	chr10	-1.8859772	1.84643433	-3.942432	0.00130374	0.20875585
Myh4	chr11	1.82246949	13.9035537	3.93272021	0.00132479	0.20875585
Cd33	chr7	-2.5021255	1.96787566	-3.931379	0.0013291	0.20875585
Fam25c	chr14	2.96687101	-0.5378939	3.90989754	0.00138126	0.21461452
Ccr5	chr9	-3.1584851	1.7062355	-3.9018754	0.0014026	0.21561211
Sostdc1	chr12	2.54871926	-0.5067107	3.88711315	0.00143042	0.21757481
Ccnb1	chr13	-2.1828154	0.91827463	-3.8617101	0.0014905	0.22309683
Nuf2	chr1	-2.3761242	0.99193462	-3.8590906	0.00149761	0.22309683
Fgf16	chrX	-2.4858276	0.08167528	-3.850861	0.00151877	0.22347127
Naip6	chr13	-3.0919726	-0.73325	-3.8332285	0.00157101	0.22347127

Supplemental Table 4. Analysis showing distance from off-target site to differentially expressed gene body

SYMBOL	TXCHROM	logFC	adj.P.Val	Gene start (bp)	Gene end (bp)	Off-Target Sequence	Chromosome	Site (bp)	to Gene (bp)	matches	Strand	Lama1 Guide
Ces1c	chr8	8.06720501	1.10E-06	93099015	93131283	aggggGcGagGgaGcCaGcCAGGCGCTGAGT	chr8	90241651	2857364	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Slc7a4	chr16	5.4202681	6.76E-06	17572018	17583214	tgCaAAGaGatTctGtCCcAGGACGAAAT	chr16	8237459	9345359	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Fabp1	chr6	7.92790193	6.76E-06	71199827	71205023	CagCtGcGagCTGtGcCAGGCTGGAAT	chr6	72989175	1784152	8	-	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
1700003G13Rik	chr9	7.85466761	6.76E-06	45318265	45322079	ataCaAaacGCTcAGtGcCAGGCTAGAT	chr9	43733430	1584835	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Cyp2c69	chr19	6.79884139	6.76E-06	39842660	39886769	gACgGcCaGagCctGgCtGcCAGGCGAGGT	chr19	40318577	413808	9	-	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
1810053B23Rik	chr16	10.5369617	6.76E-06	9342504	93366668	CACtAGaTtCctGtGcCAGGCTGGGT	chr16	93058037	284467	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Apoa2	chr1	5.40091384	7.51E-06	171225054	171226379	tgGcCaCGGCGaGcGgCaGAGGCTAGAT	chr1	171081055	143999	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Ttr	chr18	8.97781797	7.51E-06	20665250	20674324	AtgGcAGcagGcCaGcCAGGCGAAGAT	chr18	24331496	3657172	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Lama1	chr17	8.98321051	1.37E-05	67697265	67822645	N/A	N/A	N/A	N/A	N/A	N/A	N/A
ApoB	chr12	7.80234986	1.82E-05	7977648	8016835	CAGcgcGcgagGcCaGcCAGGCGAGCTGGGT	chr12	8858012	841177	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Tat	chr8	5.3144494	2.07E-05	109990437	109999803	aACcttGagGcCatCtGtCAGGCGCTGAGT	chr8	110878472	878669	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Fam25c	chr14	7.39071119	2.27E-05	34351881	34355433	aACtGACCCAGtGgGctGgGAGGCTGGGAGT	chr14	32562154	1789727	8	+	CACCGACCCCACTGGAGTGAGGCTNNNNN
Serpina1c	chr12	8.17091474	2.27E-05	103894926	103904887	ttCacActtgAAtTGAAGgGAGGCTGGGGT	chr12	105870943	1966056	9	+	CACCGACCCCACTGGAGTGAGGCTNNNNN
Serpina3k	chr12	9.29387622	2.81E-05	104338486	104346144	ttCacActtgAAtTGAAGgGAGGCTGGGGT	chr12	105870943	1524799	9	+	CACCGACCCCACTGGAGTGAGGCTNNNNN
Igf1bp1	chr11	6.69308056	3.26E-05	7197782	7202546	cttttAAAGGgATgAGcCtCAGGCTGGGAT	chr11	7421018	218472	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Mup3	chr4	7.59035385	3.35E-05	62083476	62087342	CAGaGAAGGGCTgaactcCAGGCTAGAGT	chr4	63232225	1144883	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Gys2	chr6	4.5777154	4.07E-05	142422613	142473109	CAGgATtGtGttacCGACcAGGCTGGAAT	chr6	143140787	667678	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Wnt4	chr4	4.20831956	4.07E-05	137277489	137299726	tcCTtggGgGcCaGcCtCAGGCTGGGAT	chr4	137673872	374146	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Rdh7	chr10	6.12757345	4.07E-05	127884028	127888817	ccCGCAACagcGTaCagcCAGGCTGGGAT	chr10	128913811	1024994	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Gc	chr5	8.24180382	4.07E-05	89417522	89457898	CAGgttTGtGcgGgCaGcCtCAGGCTAGGAT	chr5	101591226	12133328	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Klrf2	chr6	4.59908759	4.07E-05	38625693	38637242	CgggctGctGgCGGgCAGGCGAGCGGGGT	chr6	38551799	73894	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Srrm4os	chr5	4.04588484	4.94E-05	116438720	116465487	CcCGAAtCtCtAtgTGAAGgGAGGCTGAGAT	chr5	116290065	148655	8	-	CACCGACCCCACTGGAGTGAGGCTNNNNN
Matta1	chr14	7.36113899	5.47E-05	41105035	41124412	gACcCaCGGGCtAtGtCAGGCGAGCAAGGT	chr14	41192606	68194	7	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Alb	chr5	9.586285	6.01E-05	90460897	90476602	CAGgttTGtGcgGgCaGcCtCAGGCAAGAT	chr5	101591226	11114624	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Serpina1a	chr12	6.95305368	6.01E-05	103853589	103863562	ttCacActtgAAtTGAAGgGAGGCTGGGGT	chr12	105870943	2007381	9	+	CACCGACCCCACTGGAGTGAGGCTNNNNN
Azgp1	chr5	6.86136461	6.46E-05	137981520	137990233	CAGtAAtCtCtAtgTGAAGgCAGGCTGGGAT	chr5	139375397	1385164	8	-	CACCGACCCCACTGGAGTGAGGCTNNNNN
Fgb	chr3	7.48821994	8.50E-05	83040141	83049863	CACcagctGagTcCaCtCtCAGGCGAAGT	chr3	87805463	4755600	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Cidea	chr18	5.07510399	8.82E-05	67343564	67367794	gACtAAtCtCtAtgTGAAGgGAGGCTGGGGT	chr18	69341102	1973308	9	+	CACCGACCCCACTGGAGTGAGGCTNNNNN
9030619P08Rik	chr15	9.92341807	9.42E-05	75427606	75431829	aACtAGaGAGcCaGcGtCtCAGGCGAGAT	chr15	76107757	675928	8	-	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Fgg	chr3	7.33395248	0.00011613	83007724	83015049	CACcagctGagTcCaCtCtCAGGCGAAGT	chr3	87805463	4790414	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Mmd2	chr5	6.67105341	0.00012028	142562358	142608800	cttttGAAGGAGGgGgaaACcAGGCGTGGGT	chr5	142010113	552245	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Cyp3a11	chr5	8.17360921	0.00013811	145854426	145879964	aggCGGGGgagTGAAGCGAGGCTAGAT	chr5	145646342	208084	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Slc27a2	chr2	6.6827	0.00014295	126552407	126588243	cttCtACCCactCaGagGagGAGGCTGGGGT	chr2	126819405	231162	9	+	CACCGACCCCACTGGAGTGAGGCTNNNNN
Ambp	chr4	6.13758126	0.00015057	63143275	63154799	CAGaGAAGGGCTgaactcCAGGCTGGGAT	chr4	63232225	77426	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Fga	chr3	7.62720297	0.00016636	83026076	83033627	CACcagctGagTcCaCtCtCAGGCGAAGT	chr3	87805463	4771836	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Samd11	chr4	4.11178696	0.00017075	156246966	156255657	CACAGcagTgGaaaaaCGAGGCGAGGAGT	chr4	155503906	743060	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Fabp4	chr3	2.15462805	0.00018279	10204088	10208576	cttGcCaAaGcCtGtGcCAGGCTCGAT	chr3	10144068	60020	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Pvalb	chr15	2.52928689	0.00018754	78191114	78206400	cttCtAAtCtAtgTGAAGgGAGGCTGGGAT	chr15	78664606	458206	9	+	CACCGACCCCACTGGAGTGAGGCTNNNNN
Lct1	chr9	4.2237416	0.00018754	64117147	64138118	aACtGtGagGcCatCtGtCAGGCGTGGAT	chr9	64028355	88792	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
NA	NA	8.46471745	0.00018754	113325240	113330523	CgCtAGtAGtGacTGAAGcACCGAGCTAAT	chr12	118303251	4972728	8	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Apoa5	chr9	6.60004476	0.00018765	46268633	46271919	aACaAGGCGCTGgGaaCAGGCGCAGGCT	chr9	46913410	641491	6	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Vps13c	chr9	2.1668504	0.00018765	67840396	67995638	aACCGtGAGGcCacCtGtCAGGCGTGGGT	chr9	69433414	1437776	8	-	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Kom4841	chr18	3.86778458	0.00025932	60268301	60273267	AtcCGtAaagCGTGCtCtCAGGCTAGAT	chr18	61821232	1547965	7	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Pkr2b	chr12	3.97928004	0.00026274	31958476	32061296	acCtttGgGgagGgCaAGGCGAGGCGGGAT	chr12	30913970	1044506	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Pdpr	chr8	-2.203443	0.00033107	111094630	111137074	aACtCtGtGcCacCtGtCtCAGGCGTGAAT	chr8	110878472	216158	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Hhsp11	chr12	-2.7573871	0.00034623	108306270	108330869	tgaCtGGtGtGtCaGcCAGGCGAGCTAGAT	chr12	106527437	1778833	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Epn3	chr11	4.58955952	0.00036635	94489599	94499974	CACaCaCaCaCaGtAGaGAGGCTTGGGT	chr11	95563937	1063963	8	-	CACCGACCCCACTGGAGTGAGGCTNNNNN
Aldh1a7	chr19	3.50528717	0.00038529	20692953	20727562	attCtGAAGGGGcCtGtGtCAGGCGAAGGT	chr19	17118070	3574883	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Apoa1	chr9	8.39321713	0.00042593	46228580	46230466	aACaAGGCGCTGgGaaCAGGCGCAGGCT	chr9	46913410	682944	6	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Aspg	chr12	4.61360624	0.0004273	112106679	112127559	cttCtGGtGtGtCaGcCAGGCGAGCTAGAT	chr12	106527437	5579242	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Adgrd1	chr5	-2.427791	0.00048062	129096750	129204599	CAGtCaAGtGcCaGtCtCAGGCTGGGGT	chr5	128632023	464727	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Kng1	chr16	5.95942358	0.00048561	23057865	23082075	gACtAGtGtGtGgAGtGtCAGGCGAGGAT	chr16	29714613	6632535	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Ucp1	chr8	11.3963873	0.000504	129330352	12938455	gggtGAGCCACcTtCaAGTGGAGGCTAAGAT	chr8	80336949	2953403	8	-	CACCGACCCCACTGGAGTGAGGCTNNNNN
Cps1	chr1	5.8706773	0.00055915	67123026	67231259	CAtgTGgGgGgGtCtGtCAGGCGTGGGT	chr1	74579710	7348451	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Sfrp4	chr13	-3.0424407	0.00058511	19623104	19632994	gACaaAAGtGtCtGtggCAGGCTAGAT	chr13	20914228	1281234	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Jmjd1c	chr10	-3.2313353	0.00071562	67096125	67256322	tggaacGtGtGtCtGtGcCAGGCGAGGCT	chr10	70037110	2780784	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Orm2	chr4	6.27368915	0.00071836	63362449	63365878	CAGaGAAGGGCTgaactcCAGGCTAGAT	chr4	63232225	130224	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
AtPdv1g1	chr4	1.90407218	0.00072976	63544772	63550750	CAGaGAAGGGCTgaactcCAGGCTAGAT	chr4	63232225	312547	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
NA	NA	3.54327015	0.00079375	21753529	21800773	CAtGtGtGGGgAGcGtgaTGGCAGCGAAT	chr3	19951663	1801866	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Elovl6	chr17	3.24419618	0.00091886	129532355	129638493	cttCtGtGtGtGcGgGgGgCAGGCTGGAT	chr17	130060315	421820	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Plg	chr3	4.98742747	0.00098659	12378609	12414384	cttCCagAGaGaaTgGgAaCAGGCGAAGAT	chr17	11877092	501517	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Gp2	chr6	6.39214719	0.00101355	128483567	128526720	gACaaaGGGtGtGtGtGgGgCAGGCTGGGT	chr6	122781164	5702403	8	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
FtFt12	chr11	-2.0366214	0.00113835	49794178	49838613	gAtCtGtGtTtGtGtGtGtCAGGCTAGGT	chr11	48443528	1350650	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Thrsp	chr7	3.26554164	0.00113835	97412938	97417730	CtCtAGtGgGgCaGgAGGCGAGGCTGGGT	chr7	98123842	706112	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Bhmt	chr13	6.2546489	0.00145525	93616675	93637961	ccCtCAcAGtGgaGtGgaCAGGCGAGAGT	chr13	93929752	291791	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
ApoC3	chr9	6.95422106	0.00146687	46232933	46235636	aACaAGGCGCTGgGaaCAGGCGCAGGCT	chr9	46913410	677774	6	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Proca1	chr11	2.63255929	0.00150381	78193392	78205763	tgtaaaAAGGcCaTgGtCAGGCGAGGCT	chr11	80531260	2325497	8	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Hpd	chr5	6.45914609	0.00156002	123171807	123182727	tcCtCAGGtGtCtGtGtCAGGCGATGAT	chr5	120699033	2472774	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Pcsk6	chr7	-1.8923239	0.00160525	58661734	6050386	tAggGgAatGcCaGcCtCAGGCGAGGAT	chr7	18273824	2073438	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Dnajb13	chr7	5.86226504	0.0018516	100501716	100514960	aAGaGAGaGcCCTGtggCAGGCGTGAT	chr7	101044600	529640	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
NA	NA	-2.8570014	0.00187895	59672814	59676700	CAGgacGcagGcCaGgCAGGCGAGCTAGAT	chr9	60247801	571101	9	+	CACCGCGGGGCGCGCAGGCGAGGCGNNNNN
Myo5b	chr18	2.30425852	0.00188746	74440936	74771493	atCtGAcAGGtGtGtGtCtCAGGCGGGGT	chr18	75554852	783359	9	+	CACCGAAGGGCTGCGCAGCAGGCGNNNNN
Fam213b	chr4	2.52777314	0.00210163	154895504	154899135	AGgacAGGcagGcCaGgCAGGCGAGGAG						

Supplemental Table 5. List of computationally predicted off-target sites

Number	3'aa1 Guide	Chromosome	Off-Target Locus	Off-Target Sequence	Strand	Mismatches
1	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr1	16312174	CcAagCTcagTgAGTGGAGGGTGGGAAT	+	9
2	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	21512452	CACaGAGaGacATTCmCCAGGCTCGAT	-	9
3	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	38624395	hCtCAAGGCTCGgTgACAGGATAGGAT	-	9
4	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	52143894	hCtCAAGGCTCGgTgACAGGATAGGAT	-	9
5	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	55058127	CtCCAGAGmCaaGAGCAGGACATGAT	+	9
6	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	57261402	gggCAAGGCTTCGCTCCAGGACAGAT	-	8
7	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr1	57934949	CAGtGatGatGatGtCaaCAGCAGGACAGGAT	-	8
8	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr1	57934949	CAGtGatGatGatGtCaaCAGCAGGACAGGAT	-	8
9	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr1	57934626	CAGtGatGatGatGtCaaCAGCAGGACAGGAT	-	8
10	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr1	57979710	CAGtGatGatGatGtCaaCAGCAGGACAGGAT	+	9
11	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	82782223	hCtCAAGGCTCGgTgACAGGATAGGAT	+	9
12	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr1	87961823	hCtCAAGGCTCGgTgACAGGATAGGAT	+	9
13	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	90031346	hCtCAAGGCTCGgTgACAGGATAGGAT	-	9
14	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr1	91995313	hCtCAAGGCTCGgTgACAGGATAGGAT	+	9
15	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	92255646	CtGtGAGAGCTTCmCCAGGATAGGAT	+	9
16	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr1	109684154	gggTGAAGGCTCGgTgACAGGATAGGAT	+	9
17	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr1	12648848	CAGtGatGatGatGtCaaCAGCAGGACAGGAT	-	9
18	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	134277663	CtGtGAGAGCTTCmCCAGGATAGGAT	-	9
19	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	135011972	hCtCAAGGCTCGgTgACAGGATAGGAT	-	9
20	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr1	141154277	CAGtGAGAGCTTCmCCAGGATAGGAT	-	8
21	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	150717933	hCtCAAGGCTCGgTgACAGGATAGGAT	+	9
22	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr1	157191498	hCtCAAGGCTCGgTgACAGGATAGGAT	+	9
23	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	159690338	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
24	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	161901332	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
25	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr1	16320275	hCtCAAGGCTCGgTgACAGGATAGGAT	+	9
26	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr1	171065806	gggCtCCAGGCTCGgTgACAGGATAGGAT	+	9
27	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr1	171073441	gggCtCCAGGCTCGgTgACAGGATAGGAT	+	9
28	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr1	171081075	gggCtCCAGGCTCGgTgACAGGATAGGAT	+	9
29	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr1	17969166	gggTGAAGGCTCGgTgACAGGATAGGAT	+	9
30	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr1	179800672	hCtCAAGGCTCGgTgACAGGATAGGAT	+	8
31	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	180373924	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
32	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	180487362	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
33	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	181119150	hCtCAAGGCTCGgTgACAGGATAGGAT	+	9
34	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	182612878	hCtCAAGGCTCGgTgACAGGATAGGAT	-	9
35	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	182861831	hCtCAAGGCTCGgTgACAGGATAGGAT	-	9
36	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	185426468	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
37	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr1	185917332	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
38	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	6479941	CtCAAGGCTCGgTgACAGGATAGGAT	+	9
39	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr2	174417051	CtGtGAGAGCTTCmCCAGGATAGGAT	+	9
40	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr2	25425905	CtCtCAAGGCTTCmCCAGGATAGGAT	+	9
41	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	25995977	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
42	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	28820200	hCtCAAGGCTCGgTgACAGGATAGGAT	-	9
43	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	31672867	CAGtGAGAGCTTCmCCAGGATAGGAT	-	8
44	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	32697101	hCtCAAGGCTTCmCCAGGATAGGAT	-	8
45	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	33173871	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
46	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr2	33490722	CtCAAGGCTCGgTgACAGGATAGGAT	+	9
47	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	33501232	CACCGAAGGCTCGgTgACAGGATAGGAT	+	8
48	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	33951091	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
49	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	34571771	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
50	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	47931225	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
51	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	49708239	CtCAAGGCTCGgTgACAGGATAGGAT	+	9
52	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	57243174	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
53	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	70241763	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
54	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr2	73434966	gggTGAAGGCTCGgTgACAGGATAGGAT	+	9
55	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr2	82053716	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
56	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	84994867	CtCAAGGCTTCmCCAGGATAGGAT	-	9
57	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr2	86791796	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
58	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	9364802	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
59	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr2	93611661	gggTGAAGGCTCGgTgACAGGATAGGAT	+	9
60	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	93645694	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
61	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	10508767	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
62	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	105117350	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
63	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr2	10860572	CtCAAGGCTCGgTgACAGGATAGGAT	+	9
64	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	117143318	hCtCAAGGCTTCmCCAGGATAGGAT	-	8
65	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	119080135	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
66	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr2	126819405	CtCAAGGCTCGgTgACAGGATAGGAT	+	9
67	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr2	127089292	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
68	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	130267469	CtGtGAGAGCTTCmCCAGGATAGGAT	-	9
69	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	130540758	CtCAAGGCTTCmCCAGGATAGGAT	+	9
70	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr2	131044485	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
71	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr2	160713046	gggTGAAGGCTCGgTgACAGGATAGGAT	+	9
72	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	160802250	CAGtGAGAGCTTCmCCAGGATAGGAT	+	7
73	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	162278882	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
74	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	16474692	gggTGAAGGCTCGgTgACAGGATAGGAT	+	9
75	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr2	164089776	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
76	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr2	164618056	CAGtGAGAGCTTCmCCAGGATAGGAT	-	7
77	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	164852526	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
78	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	168041391	CtCAAGGCTTCmCCAGGATAGGAT	+	9
79	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	170015101	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
80	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	179914167	CtCAAGGCTTCmCCAGGATAGGAT	-	7
81	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	180212667	CtCAAGGCTTCmCCAGGATAGGAT	-	9
82	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr2	18144088	CtCAAGGCTTCmCCAGGATAGGAT	+	9
83	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	11081365	CtCAAGGCTTCmCCAGGATAGGAT	+	9
84	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	18621066	CtCAAGGCTTCmCCAGGATAGGAT	+	7
85	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr3	19951163	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
86	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr3	21671508	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
87	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr3	34729023	hCtCAAGGCTTCmCCAGGATAGGAT	+	8
88	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr3	38817310	CtGtGAGAGCTTCmCCAGGATAGGAT	-	8
89	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr3	48228320	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
90	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	5179584	CtCAAGGCTTCmCCAGGATAGGAT	+	9
91	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	52184374	CtCAAGGCTTCmCCAGGATAGGAT	+	9
92	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	55076356	CtCAAGGCTTCmCCAGGATAGGAT	+	9
93	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr3	57982882	CAGtGAGAGCTTCmCCAGGATAGGAT	+	8
94	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	60211721	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
95	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	69549643	CtCAAGGCTTCmCCAGGATAGGAT	+	9
96	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	87805461	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
97	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	89390004	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
98	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr3	89911371	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
99	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	98329680	CACCGAAGGCTTCmCCAGGATAGGAT	+	8
100	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	108428269	CtCAAGGCTTCmCCAGGATAGGAT	-	9
101	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr3	115947255	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
102	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	11688897	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
103	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	11753763	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
104	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	119127176	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
105	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr3	127683845	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
106	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr3	130680135	CtCAAGGCTTCmCCAGGATAGGAT	+	9
107	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	130315796	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
108	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr3	131116757	gggTGAAGGCTCGgTgACAGGATAGGAT	-	9
109	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	131471628	CtCAAGGCTTCmCCAGGATAGGAT	-	8
110	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	147117145	CtCAAGGCTTCmCCAGGATAGGAT	-	9
111	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr3	153279735	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
112	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr3	153488946	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
113	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr3	154251972	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
114	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr4	8579745	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
115	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr4	18021813	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
116	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr4	24631445	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
117	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr4	31636941	CtCAAGGCTTCmCCAGGATAGGAT	+	9
118	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr4	37414488	CtCAAGGCTTCmCCAGGATAGGAT	-	9
119	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr4	37947240	CtCAAGGCTTCmCCAGGATAGGAT	-	7
120	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr4	37063381	CtCAAGGCTTCmCCAGGATAGGAT	-	9
121	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr4	38565722	gggTGAAGGCTCGgTgACAGGATAGGAT	-	9
122	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr4	41611043	CAGtGAGAGCTTCmCCAGGATAGGAT	+	8
123	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr4	41611043	CAGtGAGAGCTTCmCCAGGATAGGAT	+	8
124	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr4	43631260	CAGtGAGAGCTTCmCCAGGATAGGAT	+	8
125	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr4	46610409	CtCAAGGCTTCmCCAGGATAGGAT	-	9
126	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr4	48027877	hCtCAAGGCTTCmCCAGGATAGGAT	-	9
127	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr4	49151863	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
128	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr4	53545042	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
129	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr4	56611614	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
130	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr4	57847249	CAGtGAGAGCTTCmCCAGGATAGGAT	-	8
131	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr4	62212225	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
132	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr4	6387793	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
133	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr4	103500698	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
134	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr4	107496668	CtCAAGGCTTCmCCAGGATAGGAT	-	9
135	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr4	120015417	CAGtGAGAGCTTCmCCAGGATAGGAT	+	9
136	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr4	109802772	CACCGAAGGCTTCmCCAGGATAGGAT	-	9
137	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr4	114586084	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
138	CACCGGCGGGCGCGGACAGCAGGNNNNNN	chr4	114597689	CAGtGAGAGCTTCmCCAGGATAGGAT	-	9
139	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr4	118003013	gggTGAAGGCTCGgTgACAGGATAGGAT	+	9
140	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr4	119990866	hCtCAAGGCTTCmCCAGGATAGGAT	+	9
141	CACGCCCCCACTGAGTGGAGGTNNNNNN	chr4	12074899	hCtCAAGGCTTCmCCAGGATAGGAT	-	9
142	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr4	123704443	CAGtGAGAGCTTCm		

298	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	31150434	hCCGAGGCaGgIggACCGAGGAGGAT	+	9
299	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	38878074	CAhCGGgGgCTgTgggCAGGCTGGAT	+	8
300	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	38883608	ChCAGGgGgGCThCAGGAGGCTGGAT	+	9
301	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	37092813	CACCGAtmCmupgCACCGAGCTGGGT	-	9
302	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr7	38123045	CAgCattGaaGgGCGCAGGCTGGGGT	-	9
303	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	44972382	hTgGAGGCGCTGgaaACAGGAGGAT	-	7
304	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr7	61987142	hTgGAGGCGCTGgaaACAGGCTGGGT	-	9
305	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	68123824	hAggGAGGCTgGgCACCAGGAGGAT	+	9
306	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr7	79214171	CACCArGgGCTGgaaAGCAGGCTAGGGT	+	9
307	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	79451983	hChCAGGAGGCTGgCACCAGGCTAGGT	+	8
308	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr7	79727951	hTgGAGGCGCTGgaaACAGGCTGGGT	+	9
309	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr7	79801854	CAGgCAGGCGAGGCGCCAGGCTGGGT	-	8
310	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr7	80062652	CgCGCaaGAGCTGcAGGCTAGGAGGAT	-	9
311	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	80994047	hCCGCTGAGGCGCTgCAGGAGGAGAT	+	9
312	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	81468367	hACAGGAGGCTGCThCAGGCTGGGT	+	9
313	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	81578428	CAGgTtGCTTCTGCTCAGGCTGGAGT	-	9
314	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	81778798	CAaGAGGCTGgGgAGCAGGCTGGAGT	+	8
315	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr7	82771251	hggCaaGAGGAGGCGCAGGCGGGGAT	-	9
316	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr7	82771352	hggCaaGAGGAGGCGCAGGCGGGGAT	-	9
317	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr7	82771740	hggCaaGAGGAGGCGCAGGCGGGGAT	-	9
318	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr7	82771840	hggCaaGAGGAGGCGCAGGCGGGGAT	-	9
319	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	87666635	hACCGAGacCTTgGcCACAGGCTGGAGT	-	8
320	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr7	98123842	hACAGGAGGCTGgaaACAGGCTGGGT	+	9
321	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	98410864	hACAGAGGCTGgaaACAGGCTAGAGT	-	9
322	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	10104600	hAgpAGAGGCTCTGgGcCAGGCTGGAT	-	9
323	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	101349375	CACGhGgAGGCTgGgCAGGCGGGGT	-	9
324	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	111678449	CACAGAGGAGGCTGgaaACAGGCTAGAGT	+	8
325	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr7	111695318	hAgpAGGAGGCTGgaaACAGGCTAGAGT	-	9
326	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	112773259	CtGgAGAGGCTCAhCTCAGGCGCAGAT	-	8
327	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr7	113018110	hAgpAGGAGGCTGgaaACAGGCTAGAGT	+	9
328	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr7	114841001	hTaaGAGGAGGCTGgaaACAGGCTAGAGT	+	8
329	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	116158895	hTtGAGGAGGCTTtGaaACAGGCTAGAGT	+	9
330	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	117001778	CAhAGAGGAGGCTGgaaACAGGCTAGAGT	+	7
331	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	117002279	CAhAGAGGAGGCTGgaaACAGGCTAGAGT	+	7
332	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	127412134	hTgAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
333	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	134110658	hTgAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
334	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	135510409	hTgAGAGGAGGCTGgaaACAGGCTAGAGT	+	8
335	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	138607181	hTtGAGAGGCTGgaaACAGGCTAGAGT	+	9
336	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	140210641	hAggGAGGAGGCTGgaaACAGGCTAGAGT	-	9
337	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	143110786	hAggGAGGAGGCTGgaaACAGGCTAGAGT	-	9
338	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr7	142624194	CAhAGAGGAGGCTGgaaACAGGCTAGAGT	+	7
339	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	142960249	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
340	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr7	144513026	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
341	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr7	146295997	CAhAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
342	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr8	3624632	CtGgAGAGGCTGgaaACAGGCTAGAGT	+	9
343	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr8	12853787	CAGgAGGCTGgaaACAGGCTAGAGT	-	8
344	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr8	14926525	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
345	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr8	25201736	hTgAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
346	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr8	26974740	hTgAGAGGAGGCTGgaaACAGGCTAGAGT	-	9
347	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr8	31922609	CAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
348	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr8	44554008	CAGgAGGAGGCTGgaaACAGGCTAGAGT	+	9
349	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr8	46514404	CAhAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
350	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr8	46654050	CtGgAGAGGCTGgaaACAGGCTAGAGT	+	8
351	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr8	47635899	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	9
352	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr8	48818823	CAhAGAGGAGGCTGgaaACAGGCTAGAGT	-	7
353	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr8	70131798	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
354	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr8	71352363	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
355	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr8	78585857	CAGgAGGAGGCTGgaaACAGGCTAGAGT	+	9
356	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr8	80336949	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	8
357	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr8	90241651	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
358	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr8	101340162	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	8
359	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr8	104182220	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
360	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr8	104985257	CAhAGAGGAGGCTGgaaACAGGCTAGAGT	-	9
361	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr8	105492213	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
362	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr8	108867846	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
363	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr8	110878472	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	9
364	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr8	114816077	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	9
365	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr8	119478902	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	7
366	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr8	120681114	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	9
367	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr8	120860571	CtGgAGAGGCTGgaaACAGGCTAGAGT	-	8
368	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr8	120974938	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
369	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr8	123041378	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	9
370	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr8	125101189	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
371	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr8	125556642	CAGgAGGAGGCTGgaaACAGGCTAGAGT	+	9
372	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr8	126158536	CtGgAGGAGGCTGgaaACAGGCTAGAGT	-	8
373	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	621347	CAhAGAGGAGGCTGgaaACAGGCTAGAGT	+	8
374	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	14751375	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
375	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	21916518	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
376	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr9	22034827	CACCGAGGAGGCTGgaaACAGGCTAGAGT	-	9
377	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	22617858	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
378	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	23021171	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
379	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr9	23123824	CAGgAGGAGGCTGgaaACAGGCTAGAGT	+	8
380	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr9	26613462	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	9
381	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr9	27811492	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	8
382	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr9	40241451	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
383	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	42855882	CAhAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
384	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	43731430	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
385	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	46913410	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	6
386	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr9	49517223	CAGgAGGAGGCTGgaaACAGGCTAGAGT	+	9
387	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	52793660	CAGgAGGAGGCTGgaaACAGGCTAGAGT	+	7
388	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr9	53321364	CtGgAGGAGGCTGgaaACAGGCTAGAGT	+	8
389	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	57503474	CACCGAGGAGGCTGgaaACAGGCTAGAGT	+	7
390	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr9	60247801	CAGgAGGAGGCTGgaaACAGGCTAGAGT	-	9
391	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr9	61384373	CtGgAGGAGGCTGgaaACAGGCTAGAGT	-	9
392	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	64028355	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	9
393	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr9	64321431	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	7
394	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	64744207	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
395	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr9	65128873	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
396	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	69431414	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	8
397	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr9	77945476	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	8
398	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr9	86448571	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
399	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	87740653	CACCGAGGAGGCTGgaaACAGGCTAGAGT	-	9
400	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	90022393	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	9
401	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr9	96933884	CAhAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
402	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	96977120	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	8
403	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr9	98334796	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
404	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	106552854	CACCGAGGAGGCTGgaaACAGGCTAGAGT	+	7
405	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	106652201	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
406	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr9	107778877	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	9
407	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr9	109134664	CAGgAGGAGGCTGgaaACAGGCTAGAGT	+	9
408	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr9	111481582	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
409	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr9	115916828	CAGgAGGAGGCTGgaaACAGGCTAGAGT	-	8
410	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr9	116019882	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
411	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr9	119728955	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	7
412	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr10	3754863	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	8
413	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr10	4744761	CAhAGAGGAGGCTGgaaACAGGCTAGAGT	-	9
414	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	5147787	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	8
415	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr10	10301849	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
416	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	12721008	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	8
417	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	21806329	CAhAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
418	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	26217098	CAGgAGGAGGCTGgaaACAGGCTAGAGT	+	7
419	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	29534725	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
420	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	31382172	CAGgAGGAGGCTGgaaACAGGCTAGAGT	-	8
421	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	39018711	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
422	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	39514818	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	8
423	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	62424916	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
424	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	62807098	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	8
425	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr10	70037110	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
426	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr10	70813707	CAGgAGGAGGCTGgaaACAGGCTAGAGT	-	9
427	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr10	71101122	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	7
428	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr10	78196582	CAGgAGGAGGCTGgaaACAGGCTAGAGT	-	7
429	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	78623512	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	8
430	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	78653456	CACCGAGGAGGCTGgaaACAGGCTAGAGT	+	5
431	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	79923552	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
432	CACCGGCGGGCGCGCCAGCAGGNNNNNN	chr10	83675226	CAGgAGGAGGCTGgaaACAGGCTAGAGT	+	9
433	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	11706251	CAGgAGGAGGCTGgaaACAGGCTAGAGT	+	8
434	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	12094625	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	-	8
435	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	120738425	hTtGAGAGGAGGCTGgaaACAGGCTAGAGT	+	9
436	CACCGAAGGCGCTGGCAGCAGGNNNNNN	chr10	128913813	CACCGAGGAGGCTGgaaACAGGCTAGAGT	-	9
437	CACCGACCCCAACTGAGTGGAG					

448	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	5886272	AtaGAAGGgTGtSggGCTAGCGGGGGAT	-	9
449	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	5897089	BAtTAAGGgTGtTGCACAGCGCTGGAT	-	9
450	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	6066293	BAtTAAGGgTGtTGCACAGCGCTGGAT	-	9
451	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	6061500	BAtTAAGGgTGtTGCACAGCGCTGGAT	-	9
452	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	60624712	BAtTAAGGgTGtTGCACAGCGCTGGAT	-	9
453	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	60647231	BAtTAAGGgTGtTGCACAGCGCTGGAT	-	9
454	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	60663843	BAtTAAGGgTGtTGCACAGCGCTGGAT	-	9
455	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	61537636	CACAGAGGCTTGAGGCTAGGCTGAAT	+	7
456	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr11	63110471	CACGtChAACAGGgGGAGGgGTGGAT	+	9
457	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr11	63914474	ggCTGAGGCGgAGgCTTGACAGCTAGGAT	+	9
458	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	67961116	ChAtTAAGGgTGtTGCACAGCGCTGGAT	+	9
459	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	69651321	CcAgGAAGGgTGtTGCACAGCGCTGAAT	+	9
460	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	72116055	gCTGgAGgGCTGGGCTAGGCGAGGAT	+	9
461	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	80511260	ggTAAGGgTGtTGCACAGCGCTGGAT	+	8
462	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr11	81781637	CAGgAGGgTGtTGCACAGCGCTGAAT	+	9
463	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	83327345	CAGggGAGGgTGtTGCAGGCTGGAT	-	8
464	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr11	85710844	CAGtGGGgAGgGGGCGCAGGCTGAGT	-	9
465	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	85753263	ggCAgAGGgTGtTGCACAGCGCAAGAT	+	9
466	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	85811247	ChAtTAAGGgTGtTGCACAGCGAGAT	+	9
467	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	88504549	ggCTGAGGCGgCTTGCTAGGCTGAAT	-	8
468	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr11	91269261	BtAaCACTgACtTAAGgAGGgGCTCTGAT	+	9
469	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr11	95563937	CACaCACTgACtTAAGgAGGgGCTCTGAT	-	8
470	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	95941086	CAGCTGGGgAGGgCAGCAGGATAGAT	+	9
471	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr11	9819292	CTCCACAGGgTGtTGCAGGCTAGGAT	+	9
472	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	98185411	hTCCAAAGGCTGAGGCTAGGCTGGAT	-	9
473	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr11	98498861	CAGGgAGGgTGtTGCAGGCTGGAT	+	9
474	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	98962560	BtAaCACTgACtTAAGgAGGgGCTCTGAT	+	9
475	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr11	99006518	CAGGgAGGgTGtTGCAGGCTGGAT	+	9
476	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr11	102217537	CACGtGcAGGgTGtTGCAGGCTAGGAT	+	7
477	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr11	102769375	BtAaCACTgACtTAAGgAGGgGCTCTGAT	-	9
478	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	103682781	BtAaCACTgACtTAAGgAGGgGCTCTGAT	+	9
479	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr11	103682784	BtAaCACTgACtTAAGgAGGgGCTCTGAT	+	9
480	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	106361263	hACGAAGGgTGtTGCAGGCTGGAT	-	9
481	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	107518125	CtAaAGGgTGtTGCAGGCTAGGAT	+	9
482	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	107975130	BtAaCACTgACtTAAGgAGGgGCTCTGAT	-	9
483	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	112270871	hACGAAGGgTGtTGCAGGCTGGAT	+	9
484	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	113070859	BtAaCACTgACtTAAGgAGGgGCTCTGAT	+	8
485	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	114783454	CTCAtGtGCTTGAAGCAGGCGAGAT	+	9
486	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	11846890	TCGCGAGGgTGtTGCAGGCTGGAT	+	8
487	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr11	11901932	CTCCACAGGCTTGAAGCAGGCTAGAT	+	9
488	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr11	120476342	CACAgAGGgTGtTGCAGGCGAGGAT	-	9
489	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr12	8858012	CAGgGtGAGgTGtTGCAGGCGAGGAT	+	9
490	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr12	16463830	ggCACTAGGgTGtTGCAGGCTGGAT	+	8
491	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr12	17695510	ChAtTAAGGgTGtTGCACAGGCTGGAT	+	9
492	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr12	21518525	CAGtGAGGgTGtTGCAGGCTGGAT	-	8
493	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr12	24731791	CACGtGGGgTGtTGCAGGCGAGAT	-	9
494	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr12	25120352	hTCCAGGgTGtTGCAGGCTGGAT	-	9
495	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr12	30913975	CTChAGGgTGtTGCAGGCGAGAT	+	9
496	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr12	3936480	ggAaGAGCTTGAAGCAGGCTAGAT	+	9
497	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr12	37539562	hTCAAGGCTTGAAGCAGGCTGGAT	+	9
498	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr12	42511226	CACGAAGGgTGtTGCAGGCGAGGAT	-	9
499	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr12	11546130	ChAtTAAGGgTGtTGCAGGCGAGGAT	+	9
500	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr12	55776121	hACGAAGGCTTGAAGCAGGCTGGAT	-	9
501	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr12	66280707	CAGgGAAGgTGtTGCAGGCGAGGAT	+	9
502	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr12	71431937	AggTAAGGgTGtTGCAGGCGAGGAT	+	9
503	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr12	7904969	ChAtTAAGGgTGtTGCAGGCGAGGAT	+	9
504	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr12	79061547	BtAaCACTgACtTAAGgAGGgGCTCTGAT	+	9
505	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr12	82361332	CACGtGGGgTGtTGCAGGCGAGAT	+	8
506	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr12	82551317	CACGAAGGgTGtTGCAGGCGAGGAT	-	9
507	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr12	86398817	hAgAGGgTGtTGCAGGCGAGGAT	+	9
508	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr12	86621523	hAgGAAGGgTGtTGCAGGCGAGGAT	+	9
509	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr12	100325518	BtAaCACTgACtTAAGgAGGgGCTCTGAT	-	9
510	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr12	105870943	hTCACTAGGgTGtTGCAGGCGAGGAT	+	9
511	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr12	106577437	BtAaCACTgACtTAAGgAGGgGCTCTGAT	-	9
512	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr12	118301253	CAGtAGGgTGtTGCAGGCTAGAT	+	9
513	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr13	12179661	BtAaCACTgACtTAAGgAGGgGCTCTGAT	-	7
514	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	13964861	CACgAggGCTTGCAGGCGCGGAT	-	9
515	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	20914278	hACGAAGGCTTGAAGCAGGCTGGAT	-	9
516	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	24917802	CTChAGGgTGtTGCAGGCGAGAT	+	9
517	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr13	34206479	CAGaCTGGAGGgTGtTGCAGGCTGGAT	-	9
518	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	37149519	hAgGAAGGgTGtTGCAGGCGAGGAT	-	9
519	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	42978966	AggTAAGGgTGtTGCAGGCGAGGAT	-	9
520	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	43421534	hAgAGGgTGtTGCAGGCGAGGAT	+	9
521	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr13	44055834	CTCCAGGgTGtTGCAGGCGAGGAT	-	9
522	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	44229402	hAgGAAGGgTGtTGCAGGCTGGAT	+	8
523	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	48697713	CAGtAGGgTGtTGCAGGCGAGGAT	-	8
524	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	51863895	CTChAGGgTGtTGCAGGCGAGGAT	+	9
525	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	52045981	CAGtACAGGgTGtTGCAGGCGAGGAT	+	9
526	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	52831465	CAtTAAGGgTGtTGCAGGCGTGGAT	+	8
527	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr13	53100751	hAgAGGgTGtTGCAGGCGAGGAT	-	9
528	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr13	54401068	CAGgAGGgTGtTGCAGGCGAGGAT	+	9
529	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr13	55391654	hACGAAGGgTGtTGCAGGCGAGGAT	+	9
530	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	58899317	CACaCACTgACtTAAGgAGGgGCTCTGAT	+	9
531	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	70126602	hTCAAGGgTGtTGCAGGCGAGGAT	-	9
532	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr13	71070081	hAgAGGgTGtTGCAGGCGAGGAT	+	9
533	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	72366003	CACGtGGGgTGtTGCAGGCGAGGAT	-	9
534	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr13	73239445	ggCACTAGGgTGtTGCAGGCTAGAT	-	8
535	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	73820815	CACAGGgTGtTGCAGGCGAGGAT	-	8
536	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	79327572	CTCCAGGgTGtTGCAGGCGAGGAT	+	9
537	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	98475919	CcAGAGGgTGtTGCAGGCGAGGAT	+	9
538	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr13	98754007	CAGtCACTGGgGAGGAGGCGGAGAT	+	9
539	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr13	100689073	ggAGGgTGtTGCAGGCGAGGAT	-	9
540	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	10794961	hAgGAAGGgTGtTGCAGGCGAGGAT	+	9
541	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr13	102681498	CTCCAGGgTGtTGCAGGCGTGGAT	-	8
542	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr13	105444228	CACGtCACTGGgGAGGAGGCTAGAT	+	7
543	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr13	111052405	CAGgAGGgTGtTGCAGGCGAGGAT	-	8
544	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr13	11273181	hAtTAAGGgTGtTGCAGGCGAGGAT	+	9
545	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr13	114136231	CACGAAGGgTGtTGCAGGCTAGAT	-	7
546	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr14	11910722	CAGgAGGgTGtTGCAGGCGAGGAT	+	9
547	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	12509076	CACGAAGGgTGtTGCAGGCGAGGAT	+	8
548	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr14	14986967	CTChAGGgTGtTGCAGGCGAGGAT	+	9
549	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	23975031	CAGGAAGGgTGtTGCAGGCGAGGAT	+	9
550	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	25304711	CTCCAGGgTGtTGCAGGCGAGGAT	-	8
551	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr14	25777328	CTCACTAGGgTGtTGCAGGCGAGGAT	-	9
552	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	26531051	CAGgAGGgTGtTGCAGGCGAGGAT	+	7
553	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr14	28401010	CAGgAGGgTGtTGCAGGCGAGGAT	+	9
554	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr14	30801201	CAGgAGGgTGtTGCAGGCGAGGAT	-	9
555	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	30612628	CAGgAGGgTGtTGCAGGCGAGGAT	-	9
556	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr14	32941154	hTCAAGGgTGtTGCAGGCGAGGAT	+	8
557	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr14	41192608	CACGtGGGgTGtTGCAGGCGAGGAT	+	9
558	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr14	53622914	CAGgAGGgTGtTGCAGGCGAGGAT	+	9
559	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	57798611	CTCCAGGgTGtTGCAGGCGAGGAT	-	8
560	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	62801514	hAgGAAGGgTGtTGCAGGCGAGGAT	+	7
561	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	63491007	CTCCAGGgTGtTGCAGGCGAGGAT	+	9
562	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	63703799	hAgGAAGGgTGtTGCAGGCGAGGAT	-	8
563	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	66151363	CAtTAAGGgTGtTGCAGGCTGGAT	+	9
564	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr14	70861731	CAGgAGGgTGtTGCAGGCGAGGAT	+	9
565	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr14	70895904	CACGtGGGgTGtTGCAGGCGAGGAT	+	8
566	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	72181539	CACCAAGGgTGtTGCAGGCTAGAT	-	9
567	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	74495784	CAGGAAGGgTGtTGCAGGCGAGGAT	-	9
568	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	75613804	hAgGAAGGgTGtTGCAGGCGAGGAT	-	9
569	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	76617125	CTChAGGgTGtTGCAGGCGAGGAT	+	9
570	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr14	78674124	CAGgAGGgTGtTGCAGGCGAGGAT	+	9
571	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	80562162	CTChAGGgTGtTGCAGGCTGGAT	-	9
572	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	83416366	CTChAGGgTGtTGCAGGCTGGAT	+	9
573	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	84125726	CTChAGGgTGtTGCAGGCGAGGAT	+	9
574	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr14	86951751	ggCTCACTGGgAGGAGGAGGCGAGGAT	+	9
575	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	88169881	CTChAGGgTGtTGCAGGCTGGAT	+	9
576	CACCGACCCCAACTGAGTGGAGGTTNNNNN	chr14	101214710	CTChAGGgTGtTGCAGGCTGGAT	+	9
577	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr14	10514141	CTChAGGgTGtTGCAGGCTGGAT	+	9
578	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	118049261	hAgGAAGGgTGtTGCAGGCGAGGAT	-	9
579	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr14	121666531	CTChAGGgTGtTGCAGGCGAGGAT	+	8
580	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr15	45359774	hACGAGGgTGtTGCAGGCGAGGAT	+	8
581	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr15	45939662	hAgGAAGGgTGtTGCAGGCGAGGAT	+	9
582	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr15	12809175	CTChAGGgTGtTGCAGGCGAGGAT	-	9
583	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr15	35255002	CACAGGgTGtTGCAGGCGAGGAT	+	9
584	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr15	37068666	CACAGGgTGtTGCAGGCGAGGAT	+	9
585	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr15	47999921	CTChAGGgTGtTGCAGGCGAGGAT	+	9
586	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr15	95450985	CACGtGGGgTGtTGCAGGCGAGGAT	-	9
587	CACCGAAGGCGCTGGCACCAGCGNNNNNN	chr15	63954610	CAGtGAAGGgTGtTGCAGGCTAGAT	-	9
588	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr15	65109882	CAtTAAGGgTGtTGCAGGCGAGGAT	+	9
589	CACCGGCGGGCGCGGCGCAGCGNNNNNN	chr15	78017171	hAgGAAGGgTGtTGCAGGCGAGGAT	+	9
590	CACCG					

Supplemental Table 6. Primers, plasmids and sgRNAs used in this study

	Name	Sequences (5' to 3')	ID Number
Primers	U6_gRNA_PCR_F	GAG GGC CTA TTT CCC ATG ATT CCT	RDC 255
	dTomato_guide_B	AAA CCA CTG TGG GGT GGA GGG GAC	RDC 1194
	mGAPDH F	TGTTTGTGATGGGTGTGAACC	RDC 345
	mGAPDH R	ACTGTGGTCATGAGCCCTTC	RDC 346
	Lama1 Primer FW	GGAAGGTTACAAAGTTCGATTGG	RDC 977
	Lama1 Primer RV	ACGTGAAATAAGACCTTGCCATC	RDC 978
	Lama1 qPCR 1 KL F	TTTCTTTGAAGGAAGCGGATATG	RDC 1918
	Lama1 qPCR 2 KL F	TGGGTCCATTAACAGAAGGAAAG	RDC 1919
	Lama1 qPCR 2 KL R	ATGGGTGAAGTCCAAAAGTTGAG	RDC 1920
	Vector genome F	ATGGTGAGCAAGGGCGAGG	RDC 1687
	Vector genome R	AGCTTGCCGTAGGTGGCATC	RDC 1679

	Name	ID number
Plasmids	minCMV-tdTomato	RDC 262
	px601 SadCas9-VP64	RDC 153
	3-guide cassettes	RDC 367

	Name	Sequences (5' to 3')	PAM
sgRNAs	<i>tdTomato</i> sgRNA Top	CACCGTCCCCTCCACCCACAGTG	AAGAAT
	<i>tdTomato</i> sgRNA Bottom	AAACCACTGTGGGGTGGAGGGGAC	
	<i>Lama1</i> sgRNA1 Top	CACCGAAGGGCCTGCGCACCAGGC	CAGGGT
	<i>Lama1</i> sgRNA1 Bottom	AAACGCCTGGTGCAGGCCCTTC	
	<i>Lama1</i> sgRNA2 Top	CACCGCGGGGCGCGGCCAGGCAGGC	GGGGGT
	<i>Lama1</i> sgRNA2 Bottom	AAACGCCTGCCTGGCCGCGCCCCGC	
	<i>Lama1</i> sgRNA3 Top	CACCGTCCAGGGGGAGCCCCGCCGTG	CGGAGT
	<i>Lama1</i> sgRNA3 Bottom	AAACCACGGCGGGGCTCCCCCTGGAC	
	<i>Lama1</i> sgRNA4 Top	CACCGGTGGTGGCGGGGTGCCTCTC	CTGGGT
	<i>Lama1</i> sgRNA4 Bottom	AAACGAGAGGCACCCCGCCACCACC	
	<i>Lama1</i> sgRNA5 Top	CACCGACCCCACTGGAGTGGAGGGT	CAGAGT
	<i>Lama1</i> sgRNA5 Bottom	AAACACCCTCCACTCCAGTTGGGGTC	