CasRx-mediated RNA targeting prevents choroidal neovascularization in a mouse model of age-related macular degeneration

Changyang Zhou^{1,2,4}, Xinde Hu^{1,2,4}, Cheng Tang^{1,2,4}, Wenjia Liu^{3,4}, Shaoran Wang^{1,4}, Yingsi Zhou¹, Qimeng Zhao¹, Qiyu Bo³, Linyu Shi¹, Xiaodong Sun^{3,#}, Haibo Zhou^{1,#}, Hui Yang^{1,#}

¹ Institute of Neuroscience, State Key Laboratory of Neuroscience, Key Laboratory of Primate Neurobiology, CAS Center for Excellence in Brain Science and Intelligence Technology, Shanghai Research Center for Brain Science and Brain-Inspired Intelligence, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

²College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

³Department of Ophthalmology, Shanghai General Hospital (Shanghai First People's Hospital), Shanghai Jiao Tong University School of Medicine, Shanghai, China. Shanghai Key Laboratory of Fundus Diseases, Shanghai, China.

⁴These authors contributed equally to this work.

#Correspondence: xdsun@sjtu.edu.cn, hbzhou@ion.ac.cn, huiyang@ion.ac.cn.

Abstract

The smallest Cas13 family protein, CasRx, has a high cleavage activity and targeting specificity, offering attractive opportunity for therapeutic applications. Here we report that delivery of CasRx by adeno-associated virus via intravitreal injection could efficiently knockdown *Vegfa* transcripts and significantly reduce the area of laser-induced choroidal neovascularization in a mouse model of age-related macular degeneration.

Thus, RNA-targeting CRISPR system could be used for *in vivo* gene therapy.

Main

Age-related macular degeneration (AMD), characterized by the development of choroidal neovascularization (CNV), is a leading cause of vision deterioration in adults over age 50¹. An angiogenic growth factor vascular endothelial growth factor A (VEGFA) plays a crucial role in CNV pathogenesis and anti-VEGFA therapy using humanized antibodies has been widely used in treating AMD, with the therapeutic effects maintained by regular injections of antibodies^{2,3}. Two recent studies showed that in a mouse model of AMD that permanent *Vegfa* gene disruption could be induced by SpCas9 or LbCpf1 editing^{4,5}. However, risks associated with permanent DNA modifications, including unwanted off-target and on-target effects, need to be considered^{6,7}.

The Cas13 protein family was recently shown to be a programmable RNA-targeting CRISPR system⁸⁻¹³, which could mediated RNA knockdown with high efficiency and specificity relative to other existing RNA interference approaches^{8,9,12}. Several Cas13 proteins have been identified, among which RfxCas13d (CasRx) has the smallest size and highest RNase activity¹². Here, we examine the potential application of CasRx system for *in vivo* gene therapy, using a laser-induced mouse model of AMD. Our results show that adeno-

associated viral (AAV)-delivered CasRx could knockdown *Vegfa* transcripts efficiently, resulting in the significant reduction of CNV area in this AMD model.

We first identified two CasRx targeting sites that are conserved in the human and mouse *Vegfa* gene. To achieve efficient *Vegfa* mRNA knockdown, two guide RNAs (gRNAs) targeting these two sites respectively were designed (Fig. 1a). We found that transient transfection of vectors expressing CasRx and the gRNA resulted in marked reduction of the *Vegfa* mRNA level in cultured human 293T cells ($12 \pm 3.5\%$, s.e.m.) and mouse N2a cells ($29.5 \pm 8.4\%$, s.e.m.) within two days, as compared to cells transfected with the control vector (Fig. 1b, c). The VEGFA protein levels were also significantly reduced in mouse N2a cells (Fig. 1d). To determine targeting specificity of CasRx, we performed transcriptome-wide RNA-seq analysis. Besides *Vegfa*, the expression levels of many other genes were changed and more than half of top-ranked genes with altered expression were related with *Vegfa* according to previous studies. (Supplementary Fig. 1 and Supplementary Table 1). To investigate the knockdown efficiency of CasRx in the normal mouse retina, we intravitreally injected AAVs encoding CasRx and a dual-gRNA array targeting *Vegfa* (referred as AAV-CasRx-*Vegfa*). Three weeks after injection, choroid-retinal pigment epithelial (RPE) tissue complex was isolated for qPCR analysis (Fig. 1e,f). We observed the expression of AAV-CasRx-*Vegfa* (Fig. 1g) and found that *Vegfa* transcripts in the treated eye were potently suppressed ($65.4 \pm 8.7\%$, s.e.m), as compared to those in the contralateral eye injected with PBS (Fig. 1h).

We next created the AMD mice by inducing CNV in both eyes by laser irradiation (Supplementary Fig. 2a,b, also see Methods). To investigate the potential usefulness of mRNA knockdown approach for treating AMD, we injected AAV-CasRx-*Vegfa* into one eye of the mouse, and PBS in the other eye as control (Fig 1f. Induction of CNV was performed in both eyes three weeks later. After laser burn, we confirmed successful infection of AAV-CasRx-*Vegfa* (Supplementary Fig. 3a). Furthermore, we found that the levels of *Vegfa* mRNA and VEGFA protein were significantly lower in the AAV-injected eye as compared that those in the contralateral PBS-injected eye (mRNA, 22.7 +/- 1.8% s.e.m., $p = 0.002$; protein, 68.2 +/- 8.7%, s.e.m., $p = 0.019$; unpaired t -test) (Fig. 1i,j). Thus, intravitreal injection of the *Vegfa* mRNA-targeting AAV was efficient to knockdown VEGFA expression in the injected eye. The therapeutic effect of this CasRX approach was assessed by quantifying the CNV area 7 days after laser treatment. Our results showed that *Vegfa*-targeting AAV markedly reduced the area of CNV at two different levels of laser irradiation, as compared to the control eyes injected with PBS (Fig. 1k,l and Supplementary Fig. 3b and 4a,b. 180 mW, 66 +/- 7.8%, s.e.m., $n = 6$ mice, $p = 0.004$; 240 mW, 36.5 +/- 6.9%, s.e.m., $n = 4$ mice, $p = 0.002$; unpaired t -test). Reduction of CNV was also confirmed by injecting AAV-CasRx-*Vegfa* into one eye, and AAV-CasRx with no gRNA into the other eye as control (Fig. 1m and Supplementary Fig. 4c). To evaluate the potential toxicity of AAV-CasRx-*Vegfa*-mediated gene knockdown, we performed electroretinography (ERG) recording in mice at one and two months after the subretinal injection. Our results showed that there is no significant change in the

responses in mice injected with AAV-CasRx-*Vegfa* compared to that in mice injected with PBS (Supplementary Fig. 5a,b). In addition, we examined the expression level of opsin in the retina at around 1 month after AAV injection. We found that injection of AAV-CasRx-*Vegfa* did not affect the opsin-positive areas (Supplementary Fig. 5c). Together, these results suggest that AAV-CasRx-mediated *Vegfa* knockdown is a safe way to treat AMD.

In summary, our results demonstrate that AAV-mediated delivery of CasRx can potentially knockdown *Vegfa* mRNA and suppress pathogenic CNV development in a mouse model of AMD, supporting the notion that RNA-targeting CRISPR system could be useful for therapeutic purposes. The small size of CasRx is suitable for packaging with multiple gRNAs in a single AAV vector for *in vivo* delivery. Notably, AAV-delivered CasRx has the potential for sustained corrective effects on protein expression for up to 2 years with a single injection¹⁴. The risks associated with mRNA editing could be lower than that of DNA editing, because of the existence of large number of transcripts, many of which may maintain normal functions. Thus CasRx knockdown approach could complement existing therapeutic strategies such as monoclonal antibodies, antisense oligonucleotides and DNA nuclease editing. Intriguingly, a recent study demonstrated that Cas13 showed potent activity against RNA viruses¹⁵. In the future, it is promising to examine whether CasRx could be used to inhibit the reproduction of recently emerged deadly RNA viruses such as 2019-nCoV, Ebola, MERS and Zika.

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Author contributions: CZ designed and performed experiments. XH, CT, WL and QB performed CNV experiments. SW performed ERG. YZ analyzed the RNA-seq data. LS prepared AAVs. XS, HZ and HY designed experiments and supervised the project. HZ and HY wrote the paper.

Competing Financial Interests: The authors declare no competing financial interests.

References:

- 1 Cheung, L. K. & Eaton, A. Age-related macular degeneration. *Pharmacotherapy* **33**, 838-855, doi:10.1002/phar.1264 (2013).
- 2 Amoaku, W. M. *et al.* Defining response to anti-VEGF therapies in neovascular AMD. *Eye* **29**, 721-731, doi:10.1038/eye.2015.48 (2015).
- 3 Ferrara, N. Vascular endothelial growth factor and age-related macular degeneration: from basic science to therapy. *Nat Med* **16**, 1107-1111,

- doi:10.1038/nm1010-1107 (2010).
- 4 Kim, K. *et al.* Genome surgery using Cas9 ribonucleoproteins for the treatment of age-related macular degeneration. *Genome Res* **27**, 419-426, doi:10.1101/gr.219089.116 (2017).
- 5 Koo, T. *et al.* CRISPR-LbCpf1 prevents choroidal neovascularization in a mouse model of age-related macular degeneration. *Nat Commun* **9**, doi:Artn 1855
10.1038/S41467-018-04175-Y (2018).
- 6 Kosicki, M., Tomberg, K. & Bradley, A. Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements (vol 36, pg 765, 2018). *Nature Biotechnology* **36**, 899-899, doi:Doi 10.1038/Nbt0918-899c (2018).
- 7 Shin, H. Y. *et al.* CRISPR/Cas9 targeting events cause complex deletions and insertions at 17 sites in the mouse genome. *Nat Commun* **8**, doi:Artn 15464
10.1038/Ncomms15464 (2017).
- 8 Abudayyeh, O. O. *et al.* RNA targeting with CRISPR-Cas13. *Nature* **550**, 280-+, doi:10.1038/nature24049 (2017).
- 9 Cox, D. B. T. *et al.* RNA editing with CRISPR-Cas13. *Science* **358**, 1019-1027, doi:10.1126/science.aaq0180 (2017).
- 10 East-Seletsky, A. *et al.* Two distinct RNase activities of CRISPR-C2c2 enable guide-RNA processing and RNA detection. *Nature* **538**, 270-+, doi:10.1038/nature19802 (2016).

- 11 Knott, G. J. & Doudna, J. A. CRISPR-Cas guides the future of genetic engineering. *Science* **361**, 866-869, doi:10.1126/science.aat5011 (2018).
- 12 Konermann, S. *et al.* Transcriptome Engineering with RNA-Targeting Type VI-D CRISPR Effectors. *Cell* **173**, 665-+, doi:10.1016/j.cell.2018.02.033 (2018).
- 13 Shmakov, S. *et al.* Discovery and Functional Characterization of Diverse Class 2 CRISPR-Cas Systems. *Molecular Cell* **60**, 385-397, doi:10.1016/j.molcel.2015.10.008 (2015).
- 14 Mendell, J. R. *et al.* Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy. *New Engl J Med* **377**, 1713-1722, doi:10.1056/Nejmoa1706198 (2017).
- 15 Freije, C. A. *et al.* Programmable Inhibition and Detection of RNA Viruses Using Cas13. *Mol Cell* **76**, 826-837 e811, doi:10.1016/j.molcel.2019.09.013 (2019).

Methods

Ethical compliance. The use and care of animals complied with the guideline of the Biomedical Research Ethics Committee of Institute of Neuroscience, Chinese Academy of Sciences.

Vector and gRNA sequences. The vector information are provided in Supplementary Sequences. gRNA1: 5'-gtgctgtaggaagctcatctctctatgtg-3';

gRNA2: 5'-ggctactcctggaagatgtccaccaggggtct-3'.

Transient transfection, qPCR and RNA-seq. Plasmids transient transfection was performed as previously described¹. 293T and N2a cells were cultured in dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and penicillin/streptomycin, and maintained at 37 °C with 5% CO₂. Cells were seeded in 6-well plates and transfected with 4 µg/well vectors expressing CasRx-GFP and gRNAs-mCherry (CasRx:gRNA-1:gRNA-2 = 2:1:1, see Supplementary sequences) using Lipofectamine 3000 reagent (Thermo Fisher Scientific). Control group was only transfected with 2 µg/well vectors containing CasRx-GFP. GFP⁺mCherry⁺ cells (GFP⁺ cells for control group) were isolated using flow cytometry 3 days after transfection. Total RNA was first purified using Trizol (Ambion) and then transcribed into complementary DNA (HiScript Q RT SuperMix for qPCR, Vazyme, Biotech). qPCR reactions were tracked by SYBR green probe (AceQ qPCR SYBR Green Master Mix, Vazyme, Biotech). For Supplementary Fig. 3, DNA was extracted from the RPE complex and used for qPCR. VEGFA qPCR primers are: Forward, 5'-GGTGGACATCTTCCAGGAGT-3'; Reverse, 5'-TGATCTGCATGGTAGATGTTG-3'.

CasRx qPCR primers are: Forward, 5'-CCCTGGTGTCCGGCTCTAA-3'; Reverse, 5'-GGACTCGCCGAAGTACCTCT-3'. For RNA-seq, around 250000 GFP-positive cells were collected and lysed. Total RNAs were extracted and then converted to cDNA, which was used for RNA-seq. The libraries were sequenced using Illumina Xten platform. Low-quality reads were filtered with SolexaQA (V3.1.7.1) and aligned to mm10 reference genome with Hisat2 (V2.0.4). Read

counts and differentially expressed genes were calculated using htseq-count (v0.11.2) and DESeq2 (1.24.0), respectively. Genes were treated as differentially expressed genes when the fold-change > 2 and FDR < 0.05.

AAV production and intravitreal injection.

AAV-CasRx-*Vegfa* and AAV-CasRx (AAV-PHP.eb capsid²) was packaged by transfection of HEK293T cells using Polyethylenimine (PEI) (50 µg/ml). Viruses were harvested, purified and concentrated 3–7 days after transfection. Mice (C57BL/6) aged 6–8 weeks were anesthetized for intravitreal injection. Intravitreal injection of PBS, AAV-CasRx or AAV-CasRx-*Vegfa* (7.5×10^9 viral genomes in 1 µl) was intravitreally injected using a Hamilton syringe with a 34G needle under an Olympus microscope (Olympus, Tokyo, Japan). Mice with retinal hemorrhage were excluded.

Laser-induced CNV model, CNV staining and ERG

At 2–3 weeks after AAV injection, mice were used for laser burn. CNV models were induced as previously described³. In brief, mice were anesthetized and pupils were dilated with dilating eye drops to enlarge the pupil size. Laser photocoagulation was performed using NOVUS Spectra (LUMENIS). The laser parameters used in this study were: 532 nm wave length, 70 ms exposure time, 240 mW power (otherwise stated) and 50 µm spot size. 4 laser burns (30 laser burns for ELISA) around the optic disc were induced. Mice with vitreous hemorrhage were excluded in the study. 3 days after laser induction, mice were

perfused with saline and RPE complexes were dissociated for ELISA analysis (qPCR was performed 7 days after laser burn). CNV analysis was conducted 7 days later after laser burn. Mice were perfused with PFA and the eyes were then fixed with PFA for 2 hours. The retina was removed from the eyes, and only RPE/choroid/scleral complex was stained with isolectin-B4 (IB4, 10 μ g/ml, I21413, Life Technologies) overnight. RPE complexes were flat-mounted and visualized with microscope (VS120 Olympus). Only eyes with successful AAV-CasRx-*Vegfa* infection were included for quantification. After obtaining CNV images, DNA was extracted from the RPE complex, the copy number of CasRx was evaluated by qPCR. The area of CNV was quantified using ImageJ software by a blind observer. ERG was performed as previously described⁴.

ELISA

RPE complex were collected for ELISA. To perform VEGFA ELISA, 30 laser burns were induced in each eye 3 weeks after AAV injection. Eyes were enucleated 3 days post-induction, the RPE complexes were dissociated from the retinas and lysed with RIPA lysis buffer. VEGFA protein levels were determined using Quantikine ELISA kit (MMV00 , R&D SYSTEMS) according to the standard protocol.

Statistical analysis. All values are shown as mean $\pm$ s.e.m. Statistical significance ($p < 0.05$) is determined by unpaired two-tailed Student's *t* test. Randomization was used in all experiments and no statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported

in previous publications⁵.

- 1 Zhou, H. *et al.* In vivo simultaneous transcriptional activation of multiple genes in the brain using CRISPR-dCas9-activator transgenic mice. *Nat Neurosci* **21**, 440-446, doi:10.1038/s41593-017-0060-6 (2018).
- 2 Chan, K. Y. *et al.* Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems. *Nat Neurosci* **20**, 1172-1179, doi:10.1038/nn.4593 (2017).
- 3 Gong, Y. *et al.* Optimization of an Image-Guided Laser-Induced Choroidal Neovascularization Model in Mice. *PLoS One* **10**, e0132643, doi:10.1371/journal.pone.0132643 (2015).
- 4 Koo, T. *et al.* CRISPR-LbCpf1 prevents choroidal neovascularization in a mouse model of age-related macular degeneration. *Nat Commun* **9**, 1855, doi:10.1038/s41467-018-04175-y (2018).
- 5 Chavez, A. *et al.* Comparison of Cas9 activators in multiple species. *Nat Methods* **13**, 563-567, doi:10.1038/nmeth.3871 (2016).

Figures

Figure 1. AAV-mediated delivery of CasRx reduces the area of CNV in a mouse model of AMD. (a) Schematic illustration of the targeting sites. The CasRx targeting sites are AAV-CasRx in the human and mouse *Vegfa* gene, and all isoforms were targeted. (b, c) Transient transfection of AAV vectors can

potently knock down *Vegfa* in both human 293T ($n = 4$ repeats, $p < 0.0001$, $t = 25.02$) cells and mouse N2a cells ($n = 3$ repeats, $p = 0.0011$, $t = 8.425$). **(d)** VEGFA protein levels ($n = 3$ repeats, $p < 0.01$, $t = 9.675$). **(e)** Schematic showing AAV-CasRx-*Vegfa*. **(f)** Schematic of the experimental procedure. AAV-CasRx-*Vegfa* was intravitreally injected into one eye, and AAV-CasRx was injected into the other eye as a control, 21 days before laser burn. Three weeks after AAV infection, the transcription level of *Vegfa* mRNA was analyzed without laser burn. VEGFA protein levels were quantified by ELISA 3 days after laser burn. CasRx and *Vegfa* mRNA levels as well as the area of CNV were measured 7 days after laser burn. **(g)** CasRx mRNA levels without laser burn, 21 days after AAV injection ($n = 3$ mice). **(h,i)** *Vegfa* mRNA levels before or 7 days after laser burn (Before laser burn: $n = 6$ mice, $p = 0.002$, $t = 4.059$; after laser burn: $n = 3$ mice, $p = 0.002$, $t = 7.583$). **(j)** VEGFA protein levels 3 days after CNV induction ($n = 5$ mice, $p = 0.019$, $t = 2.928$). **(k)** Representative CNV images injected with the PBS or AAV-CasRx-*Vegfa*, 7 days after laser burn. The area of CNV is indicated by the yellow line. Scale bar: 200 μm . **(l,m)** The CNV area. A data point represents a laser burn and in total 4 laser burns were induced in each eye. (PBS + AAV-CasRx-*Vegfa*: $n = 4$ mice, $p = 0.002$, $t = 3.39$; AAV-CasRx + AAV-CasRx-*Vegfa*: $n = 4$ mice, $p = 0.0002$, $t = 4.292$). All values are presented as mean $\pm$ s.e.m.. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, unpaired t-test.

Supplementary Figures.

Supplementary Figure 1. Targeting specificity of CasRx-*Vegfa*. Volcano plot showing the expression levels of all detected genes in RNA-seq libraries of CasRx-*Vegfa* compared to CasRx control. N2a cells, n = 3 independent replicates for both groups. Note that 24 out of 40 top-ranked genes with altered expression were related with *Vegfa* according to previous studies, and these genes are marked in the Figure. For details, see Supplementary Table 1.

Supplementary Figure 2. Induction of CNV with different levels of laser

irradiation. (a) A data point represents a laser burn and in total 4 laser burns were induced in each eye. (b) Increase of *Vegfa* expression 7 days after laser burn (n = 5 retinas per group). All values are presented as mean $\pm$ s.e.m.. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, unpaired t-test.

Supplementary Figure 3. AAV expression and induction of CNV. (a)

Successful infection of AAV-CasRx-*Vegfa* in the RPE complex after laser burn.

(b) Images showing laser-induced CNV (red circle) in the anesthetized mice, 7 days after laser burn. All values are presented as mean $\pm$ s.e.m..

Supplementary Figure 4. Reduction of the area of CNV at two different levels of laser irradiation. (a) Representative images showing CNV was induced in the retina injected with PBS or AAV-CasRx-Vegfa using a laser intensity of 180 mW. The area of CNV is indicated by the yellow line. Scale bar: 200 μ m. (b) The CNV area. A data point represents a laser burn and in total 4 laser burns were induced in each eye. (180 mW, $n = 6$ mice, $p = 0.004$, $t = 3.079$). (c) Representative images showing CNV was induced in the retina injected with AAV-CasRx or AAV-CasRx-Vegfa using a laser intensity of 240 mW. The area of CNV is indicated by the yellow line. Scale bar: 200 μ m. All values are presented as mean $\pm$ s.e.m.. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, unpaired t-test.

Supplementary Figure 5. Injection of AAV-CasRx-Vegfa does not affect the retinal function. (a,b) ERG was performed to evaluate retinal function. Full-field ERG showed that there was no significant change in the scotopic a-wave, scotopic b-wave, or photopic response in mice injected with AAV-CasRx-Vegfa compared to control mice injected with PBS at 1 or 2 months after injection (1 month: n = 16 mice per group; 2 month: n = 15 mice per group). (c), Representative images showing that the size of opsin-positive area was not affected 1 month after injection (n = 3 mice). Scale bar: 50 μm. All values are presented as mean ± s.e.m.. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, unpaired t-test.

Supplementary Tables

Supplementary Table 1. List of all genes from RNA-seq. Note that 24 out of 40 top-ranked genes with altered expression (based on p value, yellow) were reported related with *Vegfa*.

Supplementary Sequences

CasRX-P2A-GFP plasmid:

pCAG-SV40 NLS-CasRx-SV40 NLS-P2A-GFP-WPRE-PolyA

ATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC
CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTT
CCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
ACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGT
AAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCTTA
CTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTTCGAGGTGAG
CCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCAAT
TTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGG
GGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGGC
GGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTC
CGAAAGTTTCTTTTATGGCGAGGCGGCGGGCGGCGGGCGGCCCTATAAAAA
GCGAAGCGCGCGGCGGGCGGGGAGTCGCTGCGACGCTGCCTTCGCCCC
GTGCCCCGCTCCGCGCGCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGA
CCGCGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGG
CTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTCCTTTCTGTGGCTGCGT
GAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCT
CGGGGGGTGCGTGCGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCTC
CGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGT
GCGCTCCGCAGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCGG
CGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTG
TGCCTGGGGGGGTGAGCAGGGGGTGTGGGCGCGTCGGTCGGGCTGCAA
CCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCCGGCTTCGG
GTGCGGGGCTCCGTACGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCG
GGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCTCGGG
CCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCGGAGCGCCGGC
GGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTG
CGAGAGGGGCGCAGGGACTTCTTTGTCCCAAATCTGTGCGGAGCCGAAAT
CTGGGAGGCGCCGCGCACCCCCCTCTAGCGGGCGCGGGGGCGAAGCGGT
GCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGC
CGCGCCGCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGG

GACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTGGCTTCTGGC
GTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCT
TTTTCTACAGCTCCTGGGCAACGTGCTGTTATTGTGCTGTCTCATCATT
TGGCAAAGAATTGGAATTCGCCGCCACCATGCTAAAAAGAAAAGAAAGG
TGGGTTCTGGTATCGAGAAGAAGAAGAGCTTCGCCAAGGGGCATGGGAGTG
AAGAGCACCTGGTGTCCGGCTCTAAGGTGTACATGACCACATTTGCTGAG
GGAAGCGACGCCAGGCTGGAGAAGATCGTGGAGGGCGATAGCATCAGAT
CCGTGAACGAGGGAGAGGCTTTTCAGCGCCGAGATGGCTGACAAGAACGC
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TGGTGGCTAACAACCCACTGTACACCGGACCAGTGCAGCAGGACATGCTG
GGAAGGAGACACTGGAGAAGAGGTACTTCGGCGAGTCCGCCGACG
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CATACGACGAGTTCAAGGATCCAGAGCACCACCGGGCCGCTTTTAACAAC
AACGACAAGCTGATCAACGCCATCAAGGCTCAGTACGACGAGTTCGATAA
CTTTCTGGATAACCCAGGCTGGGCTACTTCGGACAGGCTTTCTTTTCTAA
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GGAGTCTCGGATCAGCCGCACCTGGCTGTACAACCTGGACAAGAACCTGG
ATAACGAGTACATCTCCACACTGAACTACCTGTACGACAGGATCACCAACG
AGCTGACAAACAGCTTCTCCAAGAACTCTGCCGCTAACGTGAACTACATCG
CTGAGACCCTGGGCATCAACCCAGCTGAGTTCGCTGAGCAGTACTTCAGA
TTTTCCATCATGAAGGAGCAGAAGAACCTGGGCTTCAACATCACAAAGCTG
AGAGAAGTGATGCTGGACAGAAAGGATATGTCCGAGATCAGGAAGAACCA
CAAGGTGTTGATTCTATCAGAACCAAGGTGTACACAATGATGGACTTTGT
GATCTACAGGTACTACATCGAGGAGGATGCCAAGGTGGCCGCTGCCAACA
AGAGCCTGCCCCGACAACGAGAAGTCTCTGAGCGAGAAGGATATCTTCGTG
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GATGAGGCCAACAGGATCTGGAGAAAGCTGGAGAACATCATGCACAACAT
CAAGGAGTTCGGGGGAAACAAGACCCGCGAGTACAAGAAGAAGGACGCT
CCAAGGCTGCCTAGGATCCTGCCTGCTGGAAGGGACGTGAGCGCCTTCAG
CAAGCTGATGTACGCCCTGACAATGTTTCTGGACGGAAAGGAGATCAACG
ATCTGCTGACCACACTGATCAACAAGTTCGACAACATCCAGTCTTTTCTGA
AAGTGATGCCTCTGATCGGCGTGAACGCTAAGTTCGTGGAGGAGTACGCC
TTCTTTAAGGACAGCGCCAAGATCGCTGATGAGCTGCGGCTGATCAAGTCC
TTTGCCAGGATGGGAGAGCCAATCGCTGACGCTAGGAGAGCTATGTACATC
GATGCCATCCGGATCCTGGGAACCAACCTGTCTTACGACGAGCTGAAGGC
TCTGGCCGACACCTTCAGCCTGGATGAGAACGGCAACAAGCTGAAGAAG
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GCGGTTTTCACTACCTGATCAGATACGGCGACCCAGCTCACCTGCACGAGA
TCGCTAAGAACGAGGCCGTGGTGAAGTTCGTGCTGGGACGGATCGCCGAT
ATCCAGAAGAAGCAGGGCCAGAACGGAAAGAACCAGATCGACCGCTACT
ACGAGACCTGCATCGGCAAGGATAAGGGAAAGTCCGTGTCTGAGAAGGTG
GACGCTCTGACCAAGATCATCACAGGCATGAACTACGACCAGTTCGATAAG
AAGAGATCTGTGATCGAGGACACCGGAAGGGAGAACGCCGAGAGAGAGA

AGTTTAAGAAGATCATCAGCCTGTACCTGACAGTGATCTACCACATCCTGA
AGAACATCGTGAACATCAACGCTAGATACGTGATCGGCTTCCACTGCGTGG
AGCGCGATGCCAGCTGTACAAGGAGAAGGGATACGACATCAACCTGAAG
AAGCTGGAGGAGAAGGGCTTTAGCTCCGTGACCAAGCTGTGCGCTGGAAT
CGACGAGACAGCCCCGACAAGAGGAAGGATGTGGAGAAGGAGATGGCC
GAGAGAGCTAAGGAGAGCATCGACTCCCTGGAGTCTGCTAACCCTAAGCT
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CATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGT
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TCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTC
CCTTTGGGCCGCTCCCGCATCGATACCGTCGACCTCGACTGTGCCTTCT
AGTTGCCAGCCATCTGTTGTTTGGCCCTCCCGCTGCCTTCTTGACCCTG

GAAGGTGCCACTCCCCTGTCCTTTCCTAATAAAATGAGGAAATTGCATCG
CATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGA
CAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGA

Vegfa-gRNA-1 plasmid:

U6-DR-target ste-1-DR-pCMV-mCherry-WPRE-PolyA

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGT
TAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAGTACAA
AATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAT
ATGTTTTAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTGATT
TCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGCAAGTAACCC
TACCAACTGGTTCGGGGTTTGAACGGTACTCCTGGAAGATGTCCACCAGG
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AGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGG
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 GGCTCTGCGGCCTCTTCCGCGTCTCCGCCTTCGCCCTCAGACGAGTCGGA
 TCTCCCTTTGGCCGCCTCCCCGCCACCGGTTAGGGGGCCCGTTTAAACCCG
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 CTCCCCCGTGCCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTC
 CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATT
 CTGGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGAC
 AATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGC

Vegfa-gRNA-2 plasmid:

U6-DR-target site-2-DR-pCMV-mCherry-WPRE-PolyA
 GAGGGCCTATTTCCCATGATTCTTCATATTTGCATATACGATACAAGGCTGT
 TAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAGTACAA
 AATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTAAAATT
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 GTCTCAAGTAACCCCTACCAACTGGTGGGGTTTGAACCTTTTTTTGTCC
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 CCCC GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAG
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 CCCC GAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTG
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TCAGCTCCTTTCCGGGACTTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGA
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CTCCCCCGTGCTTCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTC
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CTGGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGAC
AATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGC

AAV-CasRx-Vegfa plasmid:

ITR-EFS-SV40 NLS-CasRX-HA-SV40 NLS-SV40 PolyA-U6-DR-target site-1-
DR-target site-2-DR-ITR

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTC
GGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
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 GACCAAAGGTCGCCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAG
 CGAGCGAGCGCGCAGCTGCCTGCAGG

AAV-CasRx plasmid:

ITR-EFS-SV40 NLS-CasRX-HA-SV40 NLS-SV40 PolyA -ITR
 CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTC
 GGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
 GGGAGTGGCCAACCTCCATCACTAGGGGTTCTCGGGCCTCTAGAtaggtcttg
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