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Supplemental Information

In Vivo Knockout of the *Vegfa* Gene by Lentiviral Delivery of CRISPR/Cas9 in Mouse Retinal Pigment Epithelium Cells

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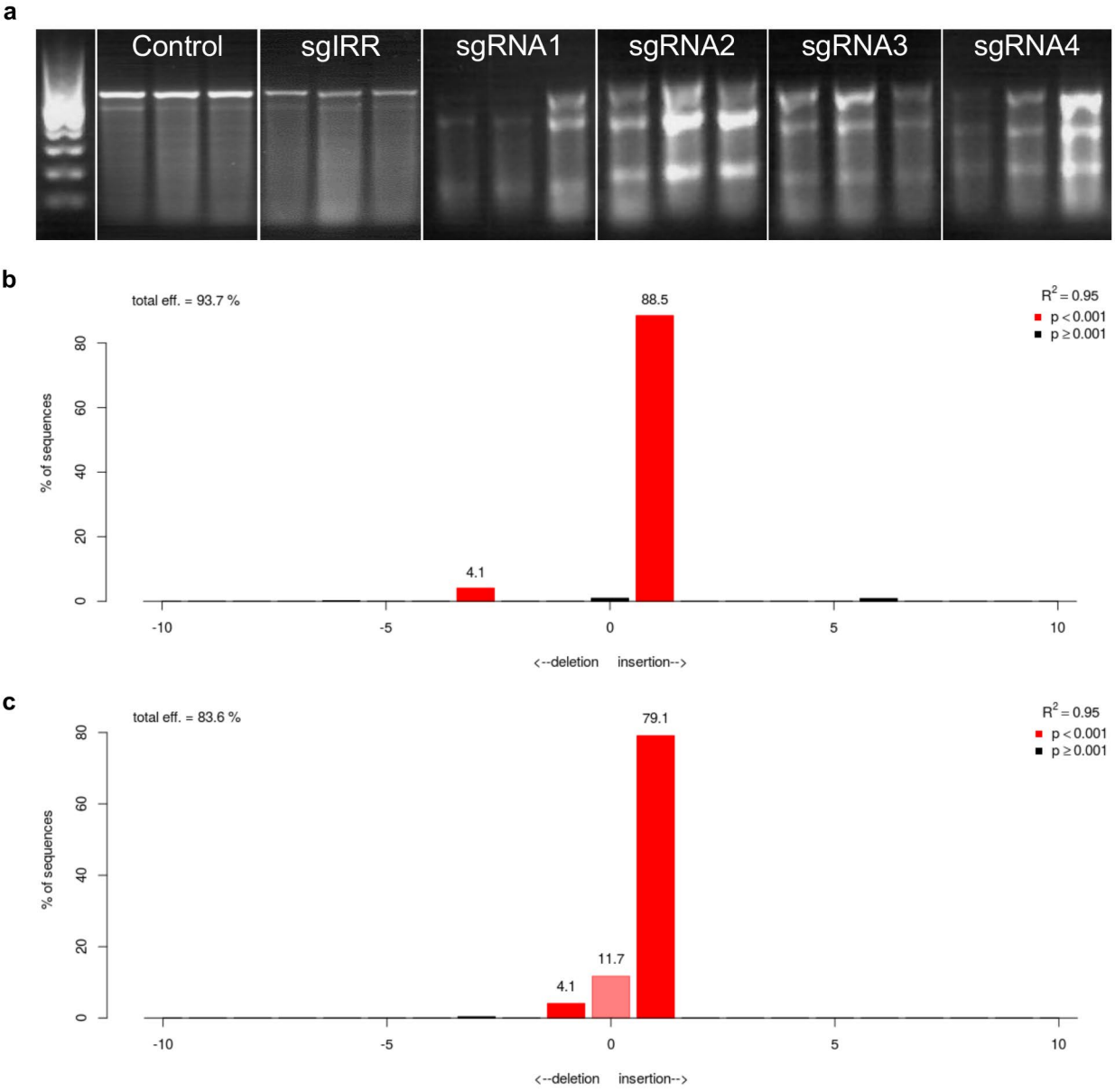
a
Control
sgIRR
sgRNA1
sgRNA2
sgRNA3
sgRNA4
b
total eff. = 93.7 %
88.5
4.1
R2 = 0.95
p < 0.001
p ≥ 0.001
% of sequences
0
20
40
60
80
-10
-5
0
5
10
<--deletion insertion-->
c
total eff. = 83.6 %
79.1
11.7
4.1
R2 = 0.95
p < 0.001
p ≥ 0.001
% of sequences
0
20
40
60
80
-10
-5
0
5
10
<--deletion insertion-->

Supplementary Figure 1 Analysis of genomic knockout. (a) Surveyor Nuclease Assay gel electrophoresis performed on gDNA from LV/Cas9-sgRNA(1-4)- and LV/Cas9-sgRNA-Irr- transduced HEK293-VEGFA cells. Non-transduced cells were used as control. Results from the quantification of the resulting DNA fragments are presented in Figure 1e (black columns). **(b)** Representative TIDE analysis of gDNA from LV/Cas9-sgRNA3 transduced HEK293-VEGFA cells. The results from the TIDE analysis of all the investigated LV/Cas9-sgRNAs are shown in Figure 1e (white columns). **(c)** TIDE analysis of FACS-sorted eGFP-positive LV/Cas9-sgRNA3 transduced RPE cells corresponding to the TIDE knockout score presented Figure 4e.

Supplementary Table 1 Designed single guide RNAs. Calculated on-target score assessed by Broad Institute and off-target score estimated by CRISPR Design (0-100 score). sgRNA1 and sgRNA4 were chosen based on high off-target and above-average on-target scores. sgRNA2 and sgRNA3 were selected based on highest possible on-target score. Primary selection criteria marked in bold. Sequences are shown in 5´-3´orientation.

Name	Sequence	Broad Institute	CRISPR Design
sgRNA1	TCGGACGGCAGTAGCTTCGC	53,6	**96**
sgRNA2	CCGTCCGATTGAGACCCTGG	**71,5**	89
sgRNA3	CTCCTGGAAGATGTCCACCA	**75,3**	55
sgRNA4	AAGATGTACTCTATCTCGTC	69,5	**95**
sgRNA-Irr	ACGGAGGCTAAGCGTCGCAA	-	-

Supplementary Table 2 Primers used for PCR amplification. For *in vitro* investigation of indel formation in HEK293-VEGFA cells, mVEGFA-forward and mVEGFA-reverse primers (No 1 and 2) were used to amplify the cDNA construct of the murine *Vegfa* gene. Following purification of genomic DNA from FACS sorted RPE cells, mExon3-forward and mExon3-reverse primers (No 3 and 4) were used to amplify exon 3 of the murine *Vegfa* gene for TIDE and TOPO cloning analyzes. Sequences are shown in 5´-3´orientation.

No	Name	Sequence
1	mVEGFA-forward	ATGAACTTTCTGCTCTCTTG
2	mVEGFA-reverse	CCTTGGCTTGTCACATCT
3	mExon3-forward	AAAGGTCACGAAAGCAGATGGTCAA
4	mExon3-reverse	GTGTATATACATAGCTGTCCCCGGT