OMTN, Volume *12*

Supplemental Information

VSV-G-Enveloped Vesicles for Traceless Delivery of CRISPR-Cas9

Claudia Montagna, Gianluca Petris, Antonio Casini, Giulia Maule, Gian Marco Franceschini, Ilaria Zanella, Luciano Conti, Francesca Arnoldi, Oscar R. Burrone, Lorena Zentilin, Serena Zacchigna, Mauro Giacca, and Anna Cereseto

SUPPLEMENTARY MATERIALS

FIGURES

Figure S1

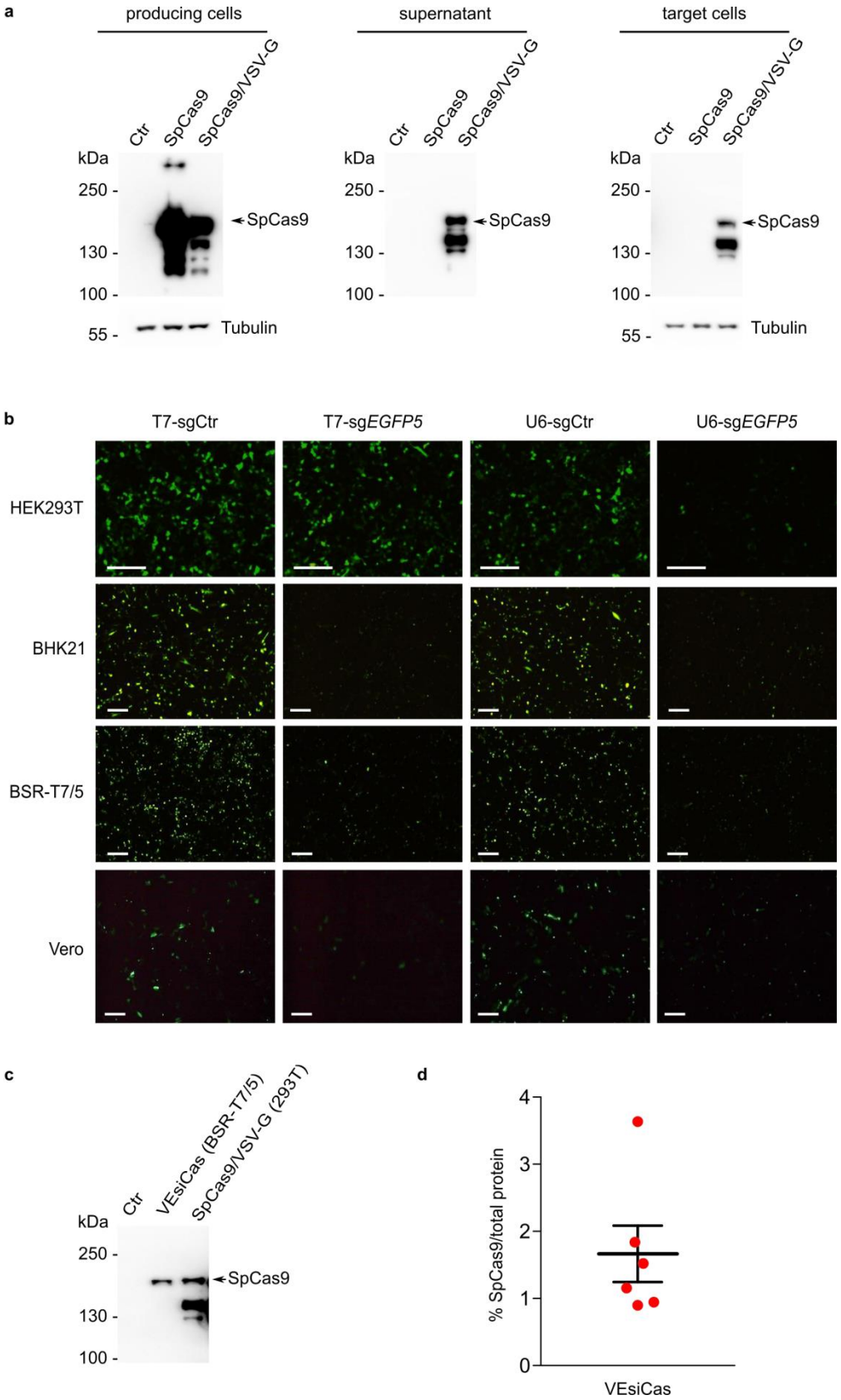

Figure S1. Development of VSV-G Enveloped vesicles for SpCas9-sgRNA delivery. (a) SpCas9/VSV-G vesicles production and delivery in HEK293T cells. Western blot analysis of SpCas9 expression in cell extracts of producing cells (left panel), in the supernatant of producing cells (middle panel) and in target cells 6 hours post transduction (right panel). Ctr corresponds to cells transfected with an empty control plasmid, SpCas9 corresponds to cells over-expressing SpCas9 and sgRNA (sg*EGFP5*), SpCas9/VSV-G corresponds to cells over-expressing SpCas9, sg*EGFP5* and VSV-G. Western blot is representative of n=2 independent experiments. (b) *EGFP* disruption assay in different cell lines using a U6 or T7 promoter sgRNA expression systems. Fluorescence microscopy images obtained from HEK293T, BHK21, BSR-T7/5 (a BHK21 clone stably expressing the T7 RNA polymerase) and Vero cell lines transfected with EGFP and SpCas9 expression plasmids together with plasmids expressing either *EGFP*-targeting (sg*EGFP5*) or non-targeting (sgCtr) sgRNAs from a U6 or a T7 promoter, as indicated. All cells but BSR-T7/5 were also co-transfected with a plasmid expressing the T7 RNA polymerase. *EGFP* knock-out was detected with variable intensity in all cell lines expressing sgRNAs (sg*EGFP5*) driven by the U6 promoter (right panels). Conversely, the sgRNA driven by the T7 RNA Polymerase system was able to induce *EGFP* knock-out only in permissive cells (BHK-21, BSR-T7/5 and Vero cells) but not in HEK293T cells. Scale bar: 100 µm. Data are representative of n=2 independent experiments. (c) Western blot analysis of SpCas9 detected in the supernatant of BSR-T7/5 (VEsiCas) or HEK293T producing cells (SpCas9/VSV-G). The gel was loaded with similar amounts of SpCas9 protein. Western blots were developed with anti-SpCas9 or anti-tubulin antibodies. Western blot is representative of n=2 independent experiments. (d) Efficiency of SpCas9 incorporation into VEsiCas. The dot plot shows the percentage of SpCas9 protein over the total amount of protein content in VEsiCas (mean ± s.e.m. of n=6 independent experiments).

Figure S2

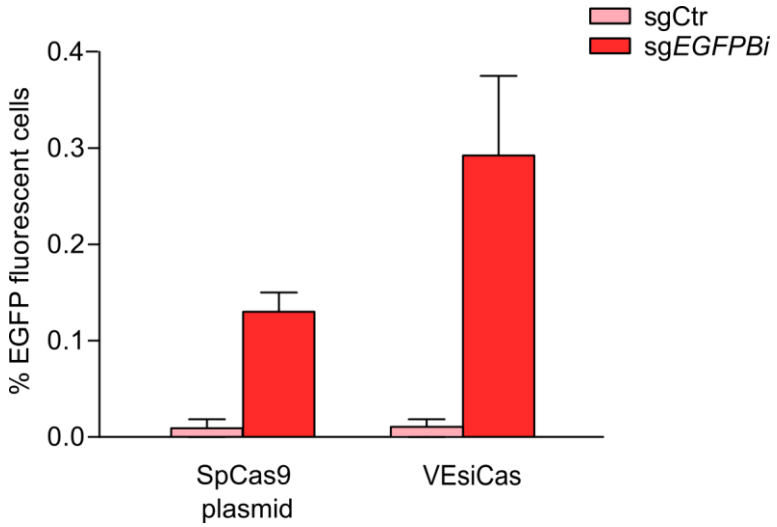

Figure S2. Gene substitution of EGFP-Y66S gene mediated by VEsiCas through homology-directed repair (HDR). Percentage of fluorescent 293-iY66S cells obtained after transfection with a donor DNA plasmid (carrying a non-fluorescent fragment of wt-EGFP) and SpCas9-sgRNA delivery through either plasmid co-transfection or VEsiCas transduction. HDR efficiencies using targeting (*EGFPBi*) or non-targeting (Ctr) sgRNAs are indicated. Data presented as mean ± s.e.m. for n=2 independent experiments.

Figure S3

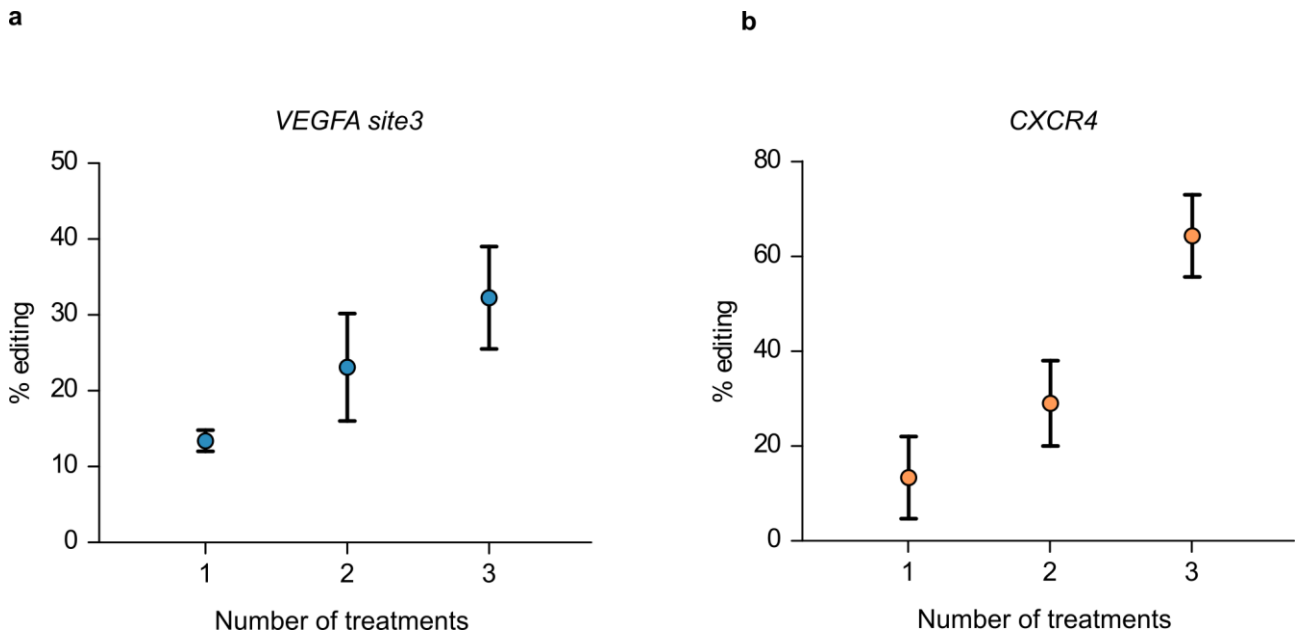

Figure S3. Editing activity by VEsiCas after multiple treatments. (a) *VEGFA site3* and (b) *CXCR4* loci edited by VEsiCas delivering SpCas9/sgRNA complexes in HEK293 cells after the indicated number of treatments performed at 48 hours distance. The percentage of indels was analyzed by TIDE. Data presented as mean ± s.e.m. for n=3 independent experiments.

Figure S4

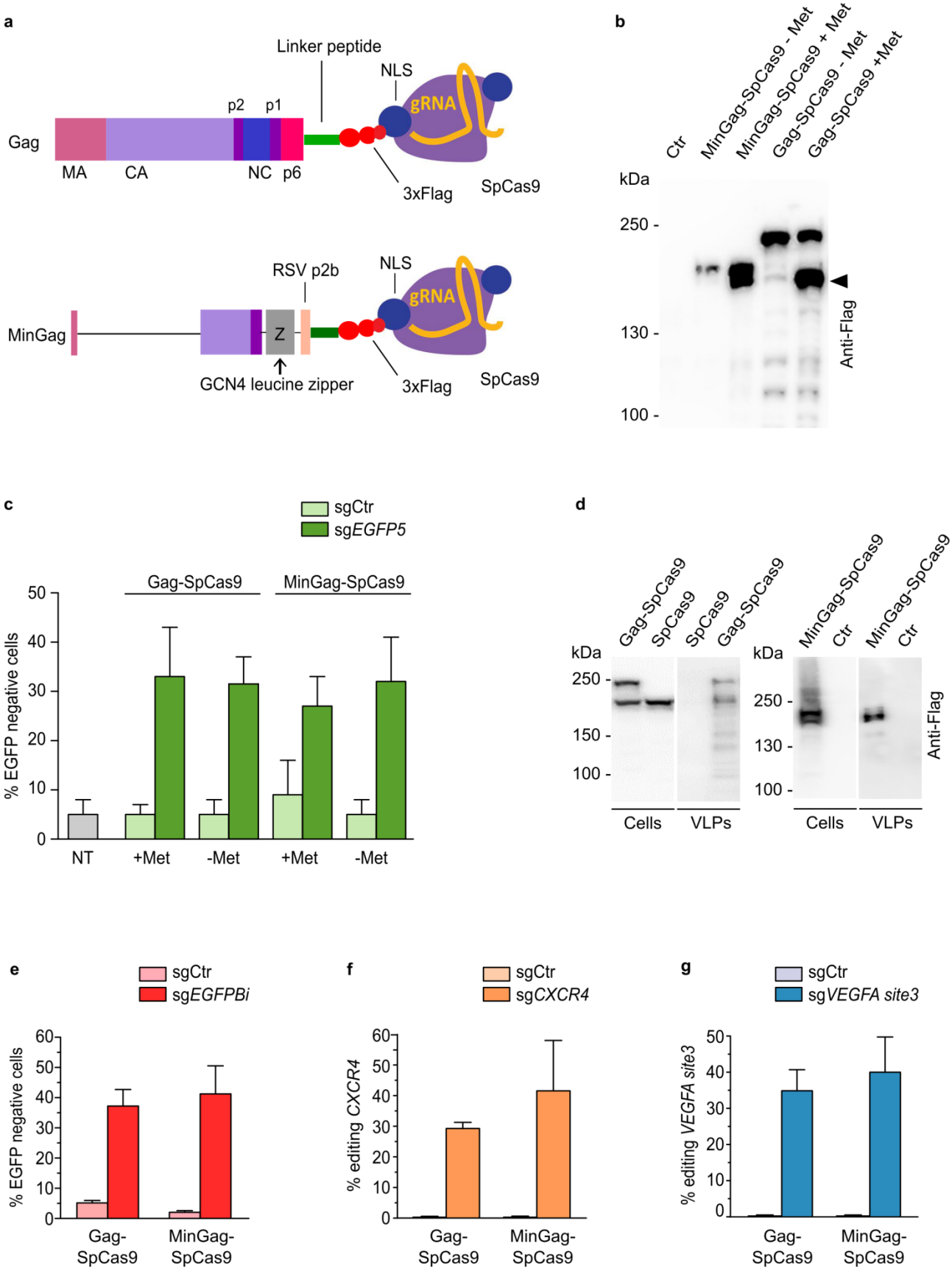
a
Linker peptide
NLS
p2
p1
gRNA
Gag
MA
CA
NC
p6
3xFlag
SpCas9
RSV p2b
NLS
gRNA
MinGag
Z
GCN4 leucine zipper
3xFlag
SpCas9
b
Ctr
MinGag-SpCas9 - Met
MinGag-SpCas9 + Met
Gag-SpCas9 - Met
Gag-SpCas9 +Met
kDa
250 -
130 -
100 -
Anti-Flag
c
sgCtr
sgEGFP5
Gag-SpCas9
MinGag-SpCas9
% EGFP negative cells
50
40
30
20
10
0
NT
+Met
-Met
+Met
-Met
d
Gag-SpCas9
SpCas9
SpCas9
Gag-SpCas9
MinGag-SpCas9
Ctr
MinGag-SpCas9
Ctr
kDa
250 -
150 -
100 -
kDa
250 -
130 -
100 -
Anti-Flag
Cells
VLPs
Cells
VLPs
e
sgCtr
sgEGFPBi
% EGFP negative cells
60
50
40
30
20
10
0
Gag-SpCas9
MinGag-SpCas9
f
sgCtr
sgCXCR4
% editing CXCR4
60
50
40
30
20
10
0
Gag-SpCas9
MinGag-SpCas9
g
sgCtr
sgVEGFA site3
% editing VEGFA site3
50
40
30
20
10
0
Gag-SpCas9
MinGag-SpCas9

Figure S4. Lentiviral-based viral-like particles (lenti-VLPs) for SpCas9 delivery. (a) Scheme of the Gag-SpCas9 and MinimalGag-SpCas9 (MinGag-SpCas9) chimeras. The domains of Gag, Matrix (MA), Capsid (CA), Nucleocapsid (NC) and peptides p1, p2 and p6, are indicated. A linker peptide separates Gag from SpCas9. The position of the nuclear localization signals (NLS) and the 3xFLAG-tag are indicated. MinGag-SpCas9 fusion includes the N-terminal myristoylation signal of MA, the C-terminal part of CA and the p2 peptide. The NC was substituted with the GCN4 leucine zipper domain (Z) to maintain particle assembly. The RSV p2b peptide substitutes p6 for particle formation. (b) Western blot analysis of Gag-SpCas9 and MinGag-SpCas9. Cells were transfected with plasmid encoding Gag-SpCas9 and MinGag-SpCas9 either containing (+Met) or not (-Met) a methionine between the FLAG and the linker peptide. The arrowhead indicates free SpCas9 probably generated by translation starting from the internal Met. Ctr corresponds to cells transfected with an empty control plasmid. Western blot is representative of n=2 independent experiments. (c) Activity of Gag-SpCas9 and MinGag-SpCas9 chimeras in *EGFP* disruption assay. HEK293-EGFP cells were transfected with plasmids expressing Gag-SpCas9 or MinGag-SpCas9 with or without the methionine between the FLAG and the linker peptide. Cells were also co-transfected with sg*EGFP5* or sgCtr. NT = not treated. Data presented as mean ± s.e.m. for n=2 independent experiments. (d) Western blot analysis of producing cells (Cells) and derived supernatants (VLPs) after overexpression of SpCas9, Gag-SpCas9 or MinGag-SpCas9 as indicated. Ctr corresponds to cells transfected with an empty control plasmid. Western blot is representative of n=2 independent experiments. (e-g) Genome editing with lenti-VLPs. Editing activity induced by VSV-G-decorated Gag-SpCas9 or MinGag-SpCas9 lenti-VLPs towards (e) the EGFP (percentage of EGFP negative cells), (f) the *CXCR4* or (g) the *VEGFA site3* loci in HEK293T cells. The percentage of indels was analyzed by TIDE. Data presented as mean ± s.e.m. for n=2 independent experiments.

Figure S5

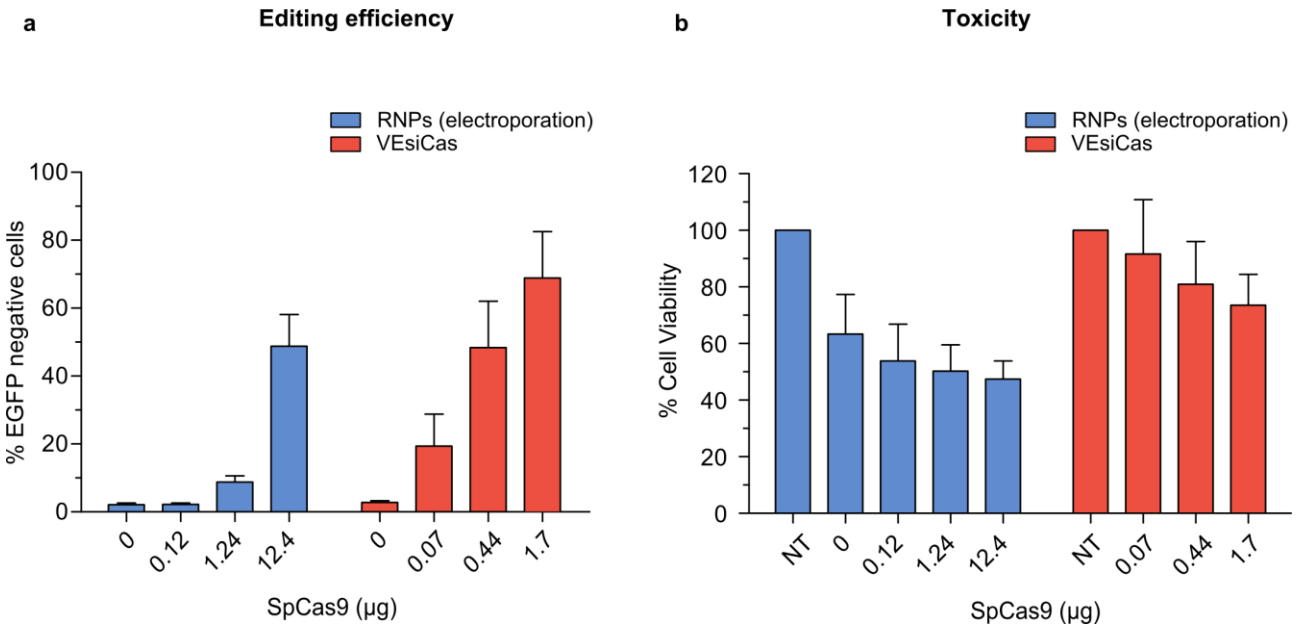

Figure S5. Comparative analysis of editing efficiency and cell toxicity obtained with VEsiCas and SpCas9-sgRNA RNPs electroporation. (a) *EGFP* disruption assay in HEK293-EGFP cells treated with scalar amounts of SpCas9 delivered through VEsiCas or RNPs electroporation. Both RNPs and VEsiCas were loaded with the same sgRNA (sg*EGFPBi*) transcribed by T7 RNA Polymerase either *in vitro* or in BSR-T7/5 cells respectively. (b) Toxicity of VEsiCas compared to RNPs delivered by electroporation. The bar graph shows the percentage of cell viability normalized to untreated cells (NT) 48 hours following delivery different amount of SpCas9 by electroporation or VEsiCas. Data reported as mean ± s.e.m. of n=3 independent experiments.

Figure S6

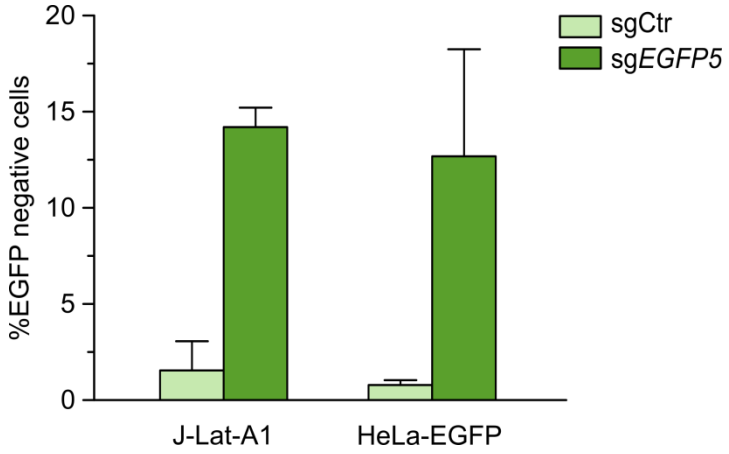

Figure S6. VEsiCas mediated *EGFP* knock-out in J-Lat-A1 and HeLa cells using VEsiCas.

J-Lat-A1 and HeLa stably expressing EGFP were treated with VEsiCas carrying *EGFP* targeting (sg*EGFP5*) or control (sgCtr) sgRNAs. The graph reports the percentages of non-fluorescent cells seven days following treatment (mean ± s.e.m. of n=2 independent experiments).

SEQUENCES

pVAX-T7-sgRNA sequence

GGTACCTAATACGACTCACTATA**GGAGACGGATTGACGTCTCTGTTTAAGAGCTATGCTGGAAACA**
GCATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCT
TGGGTCGGCATGGCATCTCCACCTCCTCGCGGTCCGACCTGGGCATCCGAAGGAGGACGTCGTCCACTCG
GATGGCTAAGGGAGAGCTCGGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCAC
CGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAG
GAACTATATCCGGATCGAGATCC

T7 promoter, protospacer, sgRNA-optimised scaffold, HDV ribozyme, T7 terminator.

pVAX-T7-sgRNA sequence. BsmBI restriction sites to clone protospacer oligonucleotides are underlined, sgRNA sequence is in bold.

pX-Gag-SpCas9 sequence

ATGGGTGCGAGAGCGTCGGTATTAAGCGGGGGAGAATTAGATCGATGGGAAAAAATTCGGTTAAGGCCAG
GGGGAAAGAAGAAGTACAAGCTAAAGCACATCGTATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAA
TCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAATACTGGGACAGCTACAACCATCCCTTCAGACAG
GATCAGAGGAGCTTCGATCACTATACAACACAGTAGCAACCCTCTATTGTGTGCACCAGCGGATCGAGATC
AAGGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAGTCCAAGAAGAAGGCCCAGCAGG
CAGCAGCTGACACAGGACACAGCAATCAGGTCAGCCAAAATTACCCTATAGTGCAGAACATCCAGGGGCAA
ATGGTACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGTAGAAGAGAAGGCTTTCAG
CCCAGAAGTGATACCCATGTTTTCAGCATTATCAGAAGGAGCCACCCCACAGGACCTGAACACGATGTTGA
ACACCGTGGGGGGACATCAAGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAGGAAGCTGCAGAATG
GGATAGAGTGCATCCAGTGCATGCAGGGCCTATTGCACCAGGCCAGATGAGAGAACCAAGGGGAAGTGAC
ATAGCAGGAACTACTAGTACCCTTCAGGAACAAATAGGATGGATGACAAATAATCCACCTATCCCAGTAGG
AGAGATCTACAGGAGGTGGATAATCCTGGGATTGAACAAGATCGTGAGGATGTATAGCCCTACCAGCATTC
TGGACATAAGACAAGGACCAAAAGAACCCTTTAGAGACTATGTAGACCGGTTCTATAAAACTCTAAGAGCT
GAGCAAGCTTCACAGGAGGTAAAAAATTGGATGACAGAAACCTTGTTGGTCCAAAATGCGAACCCAGATTG
TAAGACCATCCTGAAGGCTCTCGGCCCAGCGGCTACACTAGAAGAAATGATGACAGCATGTCAGGGAGTAG
GAGGACCCGGCCATAAGGCAAGAGTTTTGGCTGAAGCAATGAGCCAAGTAACAAATTCAGCTACCATAATG
ATGCAGAGAGGCAATTTTAGGAACCAAAGAAAGATTGTTAAGTGTTTCAATTGTGGCAAAGAAGGGCACAC
AGCCAGAAATTGCAGGGCCCCTAGGAAAAAGGGCTGTTGGAAATGTGGAAAGGAAGGACACCAAATGAAA
GATTGTACTGAGAGACAGGCTAATTTTTTTAGGGAAGATCTGGCCTTCCTACGAGGGAAGGCCAGGGAATTT
TCTTCAGAGCAGACCAGAGCCAACAGCCCCACCAGAAGAGAGCTTCAGGTCTGGGGTAGAGACAACAACTC
CCCCTCAGAAGCAGGAGCCGATAGACAAGGAACTGTATCCTTTAACTTCCCTCAGATCACTCTTTGGCAGC
GACCCCTCGTCACAAGGCGCGCAAGGAAGCCAGAACTATCCAATCGTCCAGACCGGTGCCACC**ATG**GACTA
TAAGGACCACGACGGAGACTACAAGGATCATGATATTGATTACAAAGACGATGACGATAAGATGGCCCCAA
AGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCCGACAAGAAGTACAGCATCGGCCTGGACAT
CGGCACCAACTCTGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGGTG
CTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCCCTGCTGTTCGACAGCGGCGAAA
CAGCCGAGGCCACCCGGCTGAAGAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGCTA
TCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAAGAGTCCT
TCCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTA
CCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCCGACCTGC
GGCTGATCTATCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAAC
CCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAA
CCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCAGACGGCTG
GAAAATCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTCGGAAACCTGATTGCCCTGAGCCT
GGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAGCTGAGCAAGGACA
CCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTTCTGGCCGCC
AAGAACCTGTCCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCCCCT
GAGCGCCTCTATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAAGCTCTCGTGCGGC
AGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATTGAC
GGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAGG
AACTGCTCGTGAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCC
CCACCAGATCCACCTGGGAGAGCTGCACGCCATTCTGCGGCGGCAGGAAGATTTTTACCCATTCCTGAAGG
ACAACCGGGAAAAGATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCTCTGGCCAGGGGA
AACAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAAACCATCACCCCCTGGAACTTCGAGGAAGTGGT
GGACAAGGGCGCTTCCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCCAACGAGA
AGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAACGAGCTGACCAAAGTGAAATAC
GTGACCGAGGGAATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGT
TCAAGACCAACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGAC
TCCGTGGAAATCTCCGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCACATACCACGATCTGCTGAAAAT
TATCAAGGACAAGGACTTCCTGGACAATGAGGAAAACGAGGACATTCTGGAAGATATCGTGCTGACCCTGA
CACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTATGCCCACCTGTTCGACGACAAAGTG

ATGAAGCAGCTGAAGCGGCGGAGATACACCGGCTGGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCC
GGGACAAGCAGTCCGGCAAGACAATCCTGGATTTCCTGAAGTCCGACGGCTTCGCCAACAGAAACTTCATG
CAGCTGATCCACGACGACAGCCTGACCTTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCG
ATAGCCTGCACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTGCAGACAGTG
AAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAAATGGCCA
GAGAGAACCAGACCACCCAGAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCAT
CAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAGCTGT
ACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCAGGAACTGGACATCAACCGGCTGTCCGA
CTACGATGTGGACCATATCGTGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCA
GAAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAGAACTA
CTGGCGGCAGCTGCTGAACGCCAAGCTGATTACCCAGAGAAAGTTCGACAATCTGACCAAGGCCGAGAGA
GGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAAACCCGGCAGATCACAA
AGCACGTGGCACAGATCCTGGACTCCCGGATGAACACTAAGTACGACGAGAATGACAAGCTGATCCGGGAA
GTGAAAGTGATCACCCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTTTACAAAGTGCG
CGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTCGTGGGAACCGCCCTGATCAAAA
AGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGC
CAAGAGCGAGCAGGAAATCGGCAAGGCTACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTTTTCA
AGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAGCGGCCTCTGATCGAGACAAACGGCGAAACCGG
GGAGATCGTGTGGGATAAGGGCCGGGATTTTGCCACCGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAAT
ATCGTGAAAAAGACCGAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCCAAGAGGAACAGCG
ATAAGCTGATCGCCAGAAAGAAGGACTGGGACCCTAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGC
CTATTCTGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTGAAGAGTGTGAAAGAGCTG
CTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCGACTTTCTGGAAGCCAAGGGCTA
CAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGCCTAAGTACTCCCTGTTCGAGCTGGAAAACGGCCGGA
AGAGAATGCTGGCCTCTGCCGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCCAAATATGTGAAC
TTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGCTCCCCCGAGGATAATGAGCAGAAACAGCTGTT
TGTGGAACAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCC
TGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGGGATAAGCCCATCAGAGAGCAG
GCCGAGAATATCATCCACCTGTTTACCCTGACCAATCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACAC
CACCATCGACCGGAAGAGGTACACCAGCACCAAAGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCA
CCGGCCTGTACGAGACACGGATCGACCTGTCTCAGCTGGGAGGCGACAAAAGGCCGGCGGCCACGAAAAA
GGCCGGCCAGGCAAAAAAGAAAAAGTAA

Gag, linker, 3X-FLAG, SV40 NLS, SpCas9, nucleoplasmin NLS

pX-Gag-SpCas9 sequence. Coding sequence of Gag-SpCas9. The internal linker methionine, which mutated to alanine, was identified as responsible of free SpCas9 production is labelled in bold and underlined.

pCDNA3 MinimalGag-SpCas9 sequence

ATGGGTGCGAGAGCGTCAGTATCTAGTCCTACCAGCATTCTGGACATAAGACAAGGACCAAAAG
AACCCTTTAGAGACTATGTAGACCGGTTCTATAAAACTCTAAGAGCCGAGCAAGCTTCACAGGA
GGTAAAAAATTGGATGACAGAAACCTTGTTGGTCCAAAATGCGAACCCAGATTGTAAGACTATT
TTAAAAGCATTGGGACCAGCGGCTACACTAGAAGAAATGATGACAGCATGTCAGGGAGTAGGA
GGACCCGGCCATAAGGCAAGAGTTTTGGCTGAAGCAATGAGCCAAGTAACAAATTCAGCTACCA
TAATGCTGCAGCGTATGAAGCAGCTCGAGGATAAAGTGGAAGAACTCTTAAGCAAAAACTACCA
CCTGGAAAACGAAGTGGCACGTCTGAAAAAGCTTGTGGGTGAGACAGCATCCGCTCCTCCTCCC
CCGTACGTAGGCTCTGGCGGATCCGGCGGGTCCGGTGCCACCATGGACTATAAGGACCACGACGGAGAC
TACAAGGATCATGATATTGATTACAAAGACGATGACGATAAGATGGCCCCAAAGAAGAAGCGGAAGGTCGG
TATCCACGGAGTCCCAGCAGCCGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGGCT
GGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGGTGCTGGGCAACACCGACCGGCA
CAGCATCAAGAAGAACCTGATCGGAGCCCTGCTGTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTG
AAGAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGCTATCTGCAAGAGATCTTCAGCAA
CGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAAGAGTCCTTCCTGGTGGAAGAGGATAAG
AAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCAT
CTACCACCTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCCGACCTGCGGCTGATCTATCTGGCCCTGG
CCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGAC
AAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAACCCCATCAACGCCAGCGGCGT
GGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCAGACGGCTGGAAAATCTGATCGCCCAGCTG
CCCGGCGAGAAGAAGAATGGCCTGTTCGGAAACCTGATTGCCCTGAGCCTGGGCCTGACCCCCAACTTCAA
GAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGAC
AACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCAT
CCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCTCTATGATCAAGA
GATACGACGAGCACCACCAGGACCTGACCCTGCTGAAAGCTCTCGTGCGGCAGCAGCTGCCTGAGAAGTAC
AAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATTGACGGCGGAGCCAGCCAGGAAG
AGTTCTACAAGTTCATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAGGAACTGCTCGTGAAGCTGAAC
AGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGAG
AGCTGCACGCCATTCTGCGGCGGCAGGAAGATTTTTACCCATTCCTGAAGGACAACCGGGAAAAGATCGAG
AAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCTCTGGCCAGGGGAAACAGCAGATTCGCCTGGAT
GACCAGAAAGAGCGAGGAAACCATCACCCCCTGGAACTTCGAGGAAGTGGTGGACAAGGGCGCTTCCGCC
CAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAG
CCTGCTGTACGAGTACTTCACCGTGTATAACGAGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAA
AGCCCGCCTTCCTGAGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAAGT
GACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGACTCCGTGGAAATCTCCGGCG
TGGAAGATCGGTTCAACGCCTCCCTGGGCACATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTC
CTGGACAATGAGGAAAACGAGGACATTCTGGAAGATATCGTGCTGACCCTGACACTGTTTGAGGACAGAGA
GATGATCGAGGAACGGCTGAAAACCTATGCCCACCTGTTCGACGACAAAGTGATGAAGCAGCTGAAGCGG
CGGAGATACACCGGCTGGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCA
AGACAATCCTGGATTTCCTGAAGTCCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGAC
AGCCTGACCTTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTGCACGAGCACAT
TGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCTC
GTGAAAGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCC
AGAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCA
GATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGA
ATGGGCGGGATATGTACGTGGACCAGGAACTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATAT
CGTGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCGGG
GCAAGAGCGACAACGTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAA
CGCCAAGCTGATTACCCAGAGAAAGTTCGACAATCTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAACTG
GATAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCT
GGACTCCCGGATGAACACTAAGTACGACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCCTGA
AGTCCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTTTACAAAGTGCGCGAGATCAACAACTACCAC

CACGCCCACGACGCCTACCTGAACGCCGTCGTGGGAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAG
CGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAAATC
GGCAAGGCTACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGATTACCCTGGC
CAACGGCGAGATCCGGAAGCGGCCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAG
GGCCGGGATTTTGCCACCGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAAAAGACCGAGG
TGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCCAAGAGGAACAGCGATAAGCTGATCGCCAGAAAG
AAGGACTGGGACCCTAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGTGG
CCAAAGTGGAAAAGGGCAAGTCCAAGAAACTGAAGAGTGTGAAAGAGCTGCTGGGGATCACCATCATGGA
AAGAAGCAGCTTCGAGAAGAATCCCATCGACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACC
TGATCATCAAGCTGCCTAAGTACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGCC
GGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCCAAATATGTGAACTTCCTGTACCTGGCCAGCCA
CTATGAGAAGCTGAAGGGCTCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCACT
ACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCCTGGCCGACGCTAATCTGGAC
AAAGTGCTGTCCGCCTACAACAAGCACCGGGATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCACCT
GTTTACCCTGACCAATCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGAGGT
ACACCAGCACCAAAGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACACGG
ATCGACCTGTCTCAGCTGGGAGGCGACAAAAGGCCGGCGGCCACGAAAAAGGCCGGCCAGGCAAAAAAGA
AAAAGTAA

Gag-derived domains, Gcn4, RSV-p2b, linker, 3X-FLAG, SV40 NLS, SpCas9, nucleoplasmin NLS.

pCDNA3 MinimalGag-SpCas9 sequence. Minimal-Gag coding sequence is labelled in bold. The internal linker methionine, which mutated to alanine, was identified as responsible of free SpCas9 production is underlined.

Table S1. Sequences of oligonucleotides used to construct pVAX-T7-sgRNA expression plasmids and sequences of relative target sites

sgRNA	**oligo 1 (*)**	**oligo2 (*)**	**target site (**)**
EGFP5	tataGGAAGTTCGAGGGCGACACCC	aaacGGGTGTCGCCCTCGAACTTCCCC	ggtGAAGTTCGAGGGCGACACCC**TG** **G**tga
EGFPBi	tataGGGCACGGGCAGCTTGCCGG	aaacCCGGCAAGCTGCCCGTGCCCCC	ccaCCGGCAAGCTGCCCGTGCCC**TG** **G**ccc
EGFP3gW	tataGGGCTCGTGACCACCCTGACCTA	aaacTAGGTCAGGGTGGTCACGAGCCCCC	accCTCGTGACCACCCTGACCTA**CGG** cgt
GFPI2	tataGGTGGGCACCGGCTTCCCCG	aaacCGGGGAAGCCGGTGCCCACCCC	ggtGGTGGGCACCGGCTTCCCCG**AG** **G**aca
VEGFA site3	tataGGTGAGTGAGTGTGTGCGTG	aaacCACGCACACACTCACTCACC	gtgGGTGAGTGAGTGTGTGCGTG**TG** **G**ggt
VEGFA OT1	--	--	gtg**A**GTGAGTGAGTGTGTG**T**GTG**GG** **G**ggg
VEGFA OT3	--	--	atg**T**GTG**G**GTGAGTGTGTGCGTG**AG** **G**aca
CXCR4	tataGGAAGCGTGATGACAAAGAGG	aaacCCTCTTTGTCATCACGCTTCCCC	aagGGAAGCGTGATGACAAAG**AGG**agg

(*) Lowercase indicates sticky ends used for cloning into pVAX-T7-sgRNA plasmid.

(**) Mismatches are highlighted in red, PAM is in bold. Context sequence around target site are in lowercase.

Table S2. Sequences of the oligonucleotides used for PCR amplifications.

oligo	**sequence**
T7 fw - HindIII	ACTAAGCTTGTCGACCATGAACACGATTAACATCG
T7 rev - XbaI	TAATCTAGATTACGCGAACGCGAAGTC
T7 promoter fw	GAAATTAATACGACTCACTATAGG
gRNA end rev	AAGCACCGACTCGGTGCCA
EGFP fw	ACCATGGTGAGCAAGGGCGAGGA
EGFP rev	AGCTCGTCCATGCCGAGAGTGATC
VEGFA site3 ON fw	GCATACGTGGGCTCCAACAGGT
VEGFA site3 ON rev	CCGCAATGAAGGGGAAGCTCGA
VEGFA site3 OT1 fw	CAGGCGCCTTGGGCTCCGTCA
VEGFA site3 OT1 rev	CCCCAGGATCCGCGGGTCAC
VEGFA site3 OT3 fw	AGTCAGCCCTCTGTATCCCTGGA
VEGFA site3 OT3 rev	GAGATATCTGCACCCTCATGTTCAC
CXCR4 fw	AGAGGAGTTAGCCAAGATGTGACTTTGAAACC
CXCR4 rev	GGACAGGATGACAATACCAGGCAGGATAAGGCC