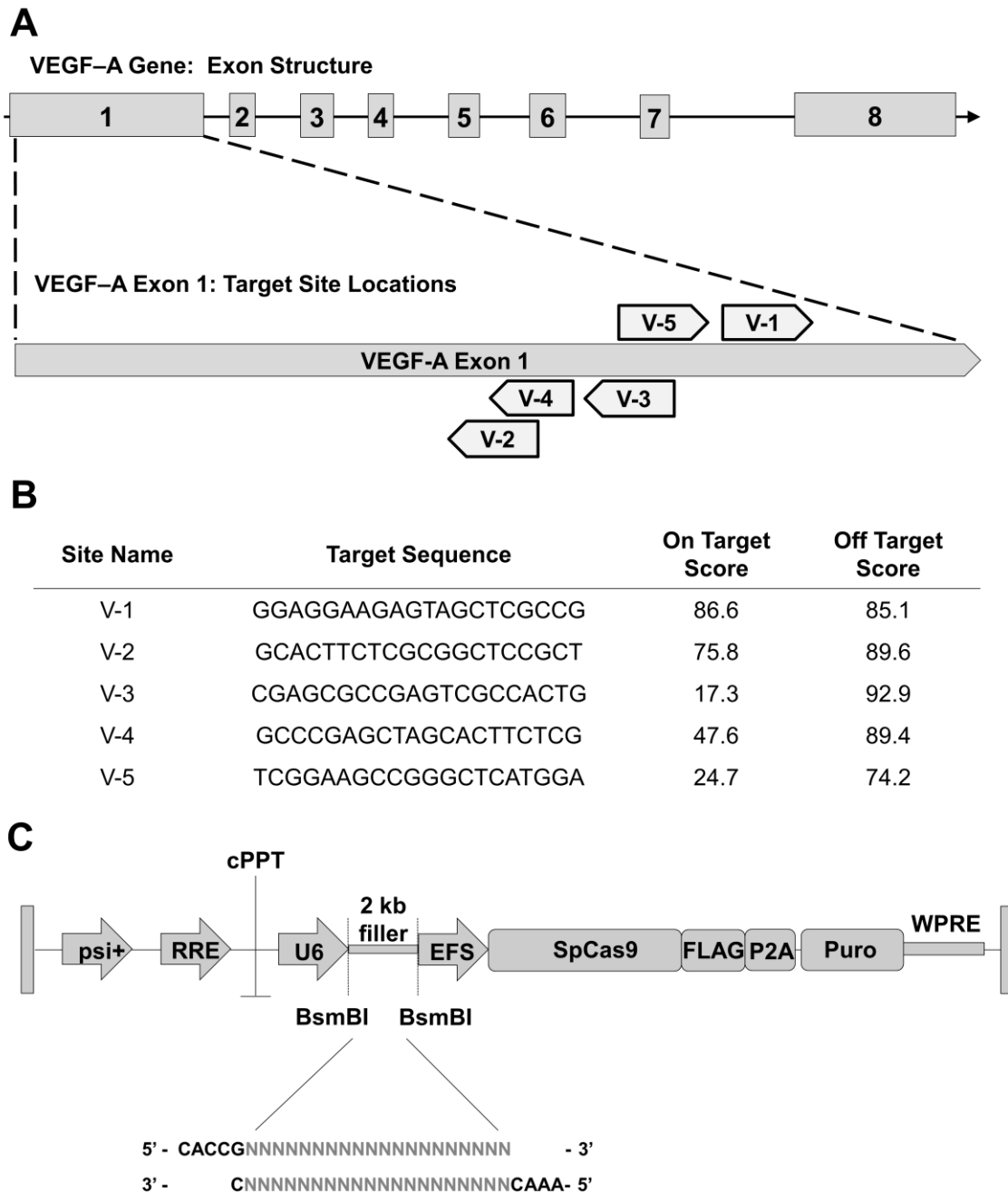


Supplemental Table 1. Primers for T7E1 Assay and DNA Sequencing

Primer Name	Primer Sequence (5' → 3')	Targets	Assay
VEGFA-T7E1-F-a	CGACGGCTTGGGGAGATTGCTC	3, 4	T7E1*
VEGFA-T7E1-R-a	CCCGCAGCAATCCACCCCAAAA		
VEGFA-T7E1-F-b	AGAGACGGGGTCAGAGAGAGCG	1, 5	T7E1
VEGFA-T7E1-R-b	TGAGCCTCTCTCCGGGTACCCT		
VEGFA-T7E1-F-c	AGCTCCAGAGAGAAGTCGAGGA	2	T7E1
VEGFA-T7E1-R-c	TGCTCAGACTTCCAGTTCGTCC		
VEGFA-Dseq-F-a	AGAGACGGGGTCAGAGAGAGCG	1	Deep Sequencing
VEGFA-Dseq-R-a	TGAGCCTCTCTCCGGGTACCCT		
VEGFA-Dseq-F-b	CTGTTCTCGCTTCGGAGGAGC	2, 4	Deep Sequencing
VEGFA-Dseq-R-b	CTCGGCGAGCTACTCTTCCTC		
VEGFA-Dseq-F-c	AGAGAAGGAAGAGGAGAGGGGG	3, 5	Deep Sequencing
VEGFA-Dseq-R-c	CAATGCACCCAAGACAGCAGAA		
VEGFA-Sseq-1	TGACGGACAGACAGACAGACAC	1	Sanger Sequencing
VEGFA-Sseq-2	AAAGTTCATGGTTTTCGGAGGCC	2	Sanger Sequencing
VEGFA-Sseq-3	GCACCCAAGACAGCAGAAAGTT	3	Sanger Sequencing
VEGFA-Sseq-4	CTTACCTTGGCATGGTGGAGGT	4	Sanger Sequencing
VEGFA-Sseq-5	AGGGGCAAAGTGAGTGACCTG	5	Sanger Sequencing

*T7 endonuclease I assay

Supplemental Fig. 1



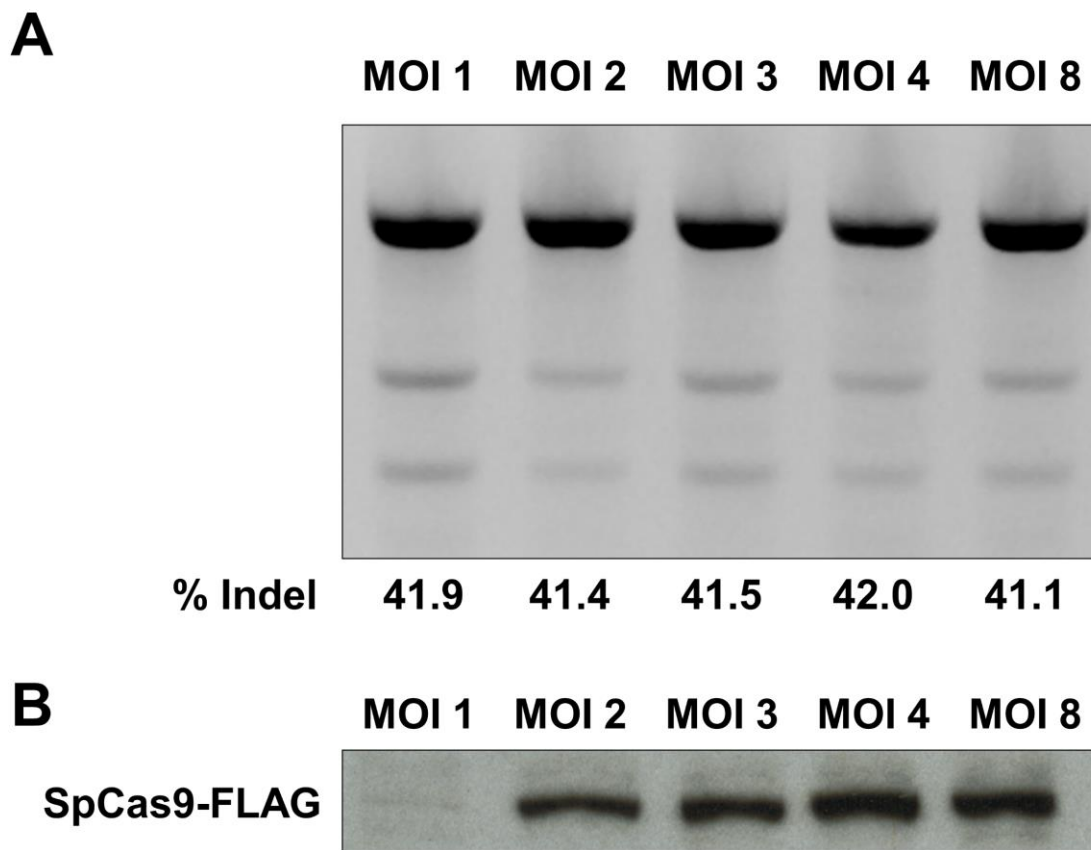
Supplemental Figure 1. Target CRISPR sequences for VEGF-A gene and lentiviral vector design.

(A) Schematic diagram of the VEGF-A gene structure and target CRISPR sequences

located in exon 1. (B) Target CRISPR sequences with on-target and off-target scores to

predict Cas9 activity and probability of off-target binding, respectively. (C) Schematic of LentiCRISPRv2 lentiviral vector, which includes *Strep pyogenes* Cas9 endonuclease (SpCas9) and a guide RNA (gRNA) cassette with BsmBI restriction sites for insertion of target CRISPR sequences. Abbreviations: psi+, Psi packaging signal; RRE, Rev response element; cPPT, central polypurine tract; U6, U6 promoter; EFS, elongation factor 1 α short promoter; FLAG, Flag octapeptide tag; P2A, 2A self-cleaving peptide; Puro, puromycin selection marker; WPRE, woodchuck post-transcriptional regulatory element.

Supplemental Fig. 2



Supplemental Figure 2. Indel formation at different viral concentrations.

(A) T7E1 mismatch detection assay showing similar frequencies of indel formation in VEGF-A at increasing viral concentrations, expressed as multiplicity of infection (MOI). (B) Western blot demonstrating greater SpCas9-FLAG expression at increasing viral concentrations, using rabbit anti-FLAG primary antibody (1:500, F7425, Sigma-Aldrich, St. Louis, MO), and rabbit HRP-linked IgG secondary antibody (1:5000, NA934, GE Life Sciences, Pittsburgh, PA), followed by development with a chemiluminescent reagent (Amersham ECL, GE Life Sciences, Pittsburgh, PA). Greater viral concentrations resulted in toxicity and cell death.

Supplemental Fig. 3

V-2	PAM	▼		V-3	PAM	▼	
Wild Type	CCGAGCCGAGCGGAGCCGCG			Wild Type	CCGCAGTGGCGACTCGGCGC		
	CCGAGCCGAGCGGAGCCGCG				CCGCAGTTGGCGACTCGGCGC		
	CCGAGCCG-----CG				CCGCAG-GGCGACTCGGCGC		
	CCGAGCCGAGCG-AGCCGCG				C-----GACTCGGCGC		
	CCGAGCCGAG-----CCGCG				CCGCAG---CGACTCGGCGC		
	-----GGAAGCCGCG				CCGCAG--GCGACTCGGCGC		
	CCGAGCCGAG-----				CCGCAGGTGGCGACTCGGCGC		
	CCGAGCCGAGCCGAG-CGCG				CCGCAGTGTGGCGACTCGGC		
	CCGAGCCGA-----GCG				CCGCAG-CTGGCGACTCGGC		
	CCGAGCCGAGCTTCCCGCG				CCGCAGA-TGGCGACTCGGC		
	-----GGGAGCCGCG				CCGC--TGGCGACTCGGCGC		
	C-----CG				CCGCAGA-----G		
	CCGAG-----				CCGC-----ACTCGGC		
	-----CGCGGGGAGCCGCG				CCGCAG-----CTCGGC		
	CCGAGCCGAG-----CGCG				CCGCA-TGGCGACTCGGCGC		
	CCGAGCCGAGCTTCCC----						
V-4	PAM	▼		V-5		▼	PAM
Wild Type	CCGCGAGAAGTGCTAGCTCG			Wild Type	GAAGCCGGGCTCATGGACGG		
	CCGCGAAGAAGTGCTAGCTC				GAAGCCGGGCTCATTTGGACG		
	CCGCGACGAAGTGCTAGCTC				GAAGCCGGGCTCATATGGAC		
	CCGC--GAAGTGCTAGCTCG				GAAGCC-----GGACG		
	CCGCGATGAAGTGCTAGCTC				-----CTCGGAAGCCG		
	CGCGGGGAAGTGCTAGCTC				GAAGCCGGG-----ACGG		
	CCGCG-GAAGTGCTAGCTCG				GAAGCCGGGCTCATTTGGAC		
	CCGCGAGGAAGTGCTAGCTC				-----CTCGGAGGACG		
	GGGGGAAGCCGAGCTAGCTC						

Supplemental Figure 3. Deep sequencing reveals Cas9-mediated VEGF-A gene disruption.

Representative deep sequencing results for V-2, V-3, V-4, and V-5 confirming indel formation at the predicted cut site (red arrowhead), including insertions (bases in red) and deletions (dashes).