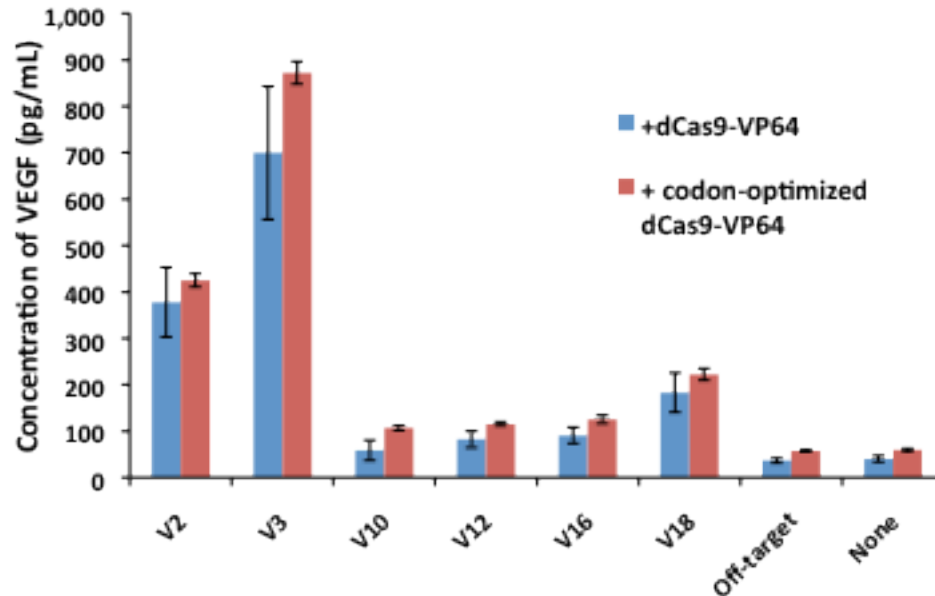


CRISPR RNA-guided activation of endogenous human genes

Morgan L Maeder, Samantha J Linder, Vincent M Cascio, Yanfang Fu, Quan H Ho, J Keith Joung

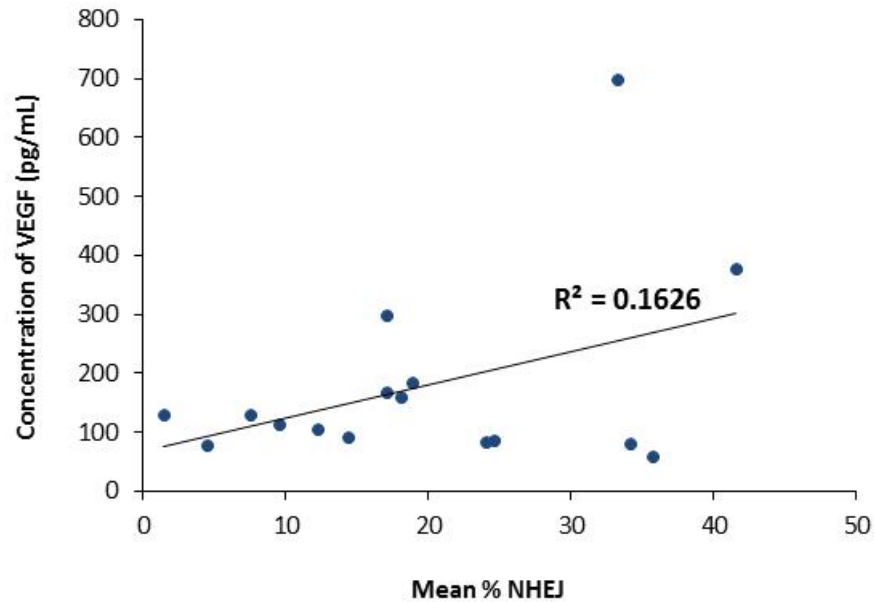
Supplementary Figure 1 Comparison of VEGF activation induced by dCas9-VP64 and a codon optimized dCas9-VP64

Concentration of VEGFA protein expressed from 293 cells transfected with plasmids expressing various sgRNAs and either dCas9-VP64 (blue bars) or a codon optimized version of dCas9-VP64 (red bars). There were no significant differences between the concentrations of VEGFA protein induced by the two different plasmids expressing dCas9-VP64 as determined by a two-sided t-test ($P < 0.05$).



Supplementary Figure 2 Activation of VEGFA by RNA-guided activators does not correlate with NHEJ-mediated indel mutation rates induced by the same sgRNAs co-expressed with active Cas9 nuclease

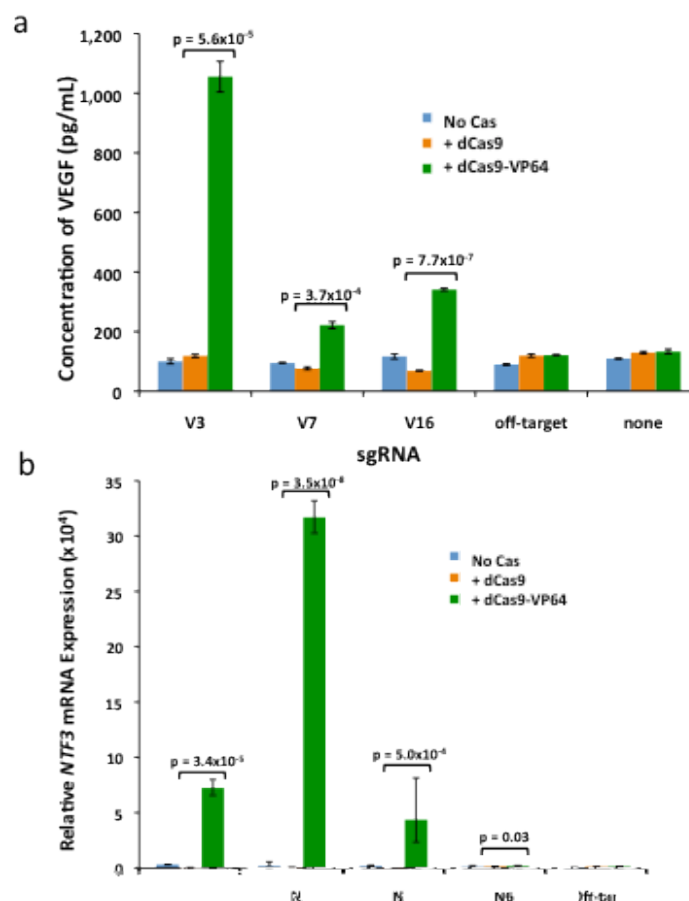
Graph depicts VEGFA protein concentration expressed from 293 cells transfected with 16 sgRNAs as a function of the frequency of NHEJ-induced indel mutations when the same sgRNAs were co-expressed in 293 cells with wild-type Cas9.



Supplementary Figure 3 The VP64 domain in dCas9-VP64 is required for RNA-guided activation of the human *VEGFA* and *NTF3* genes

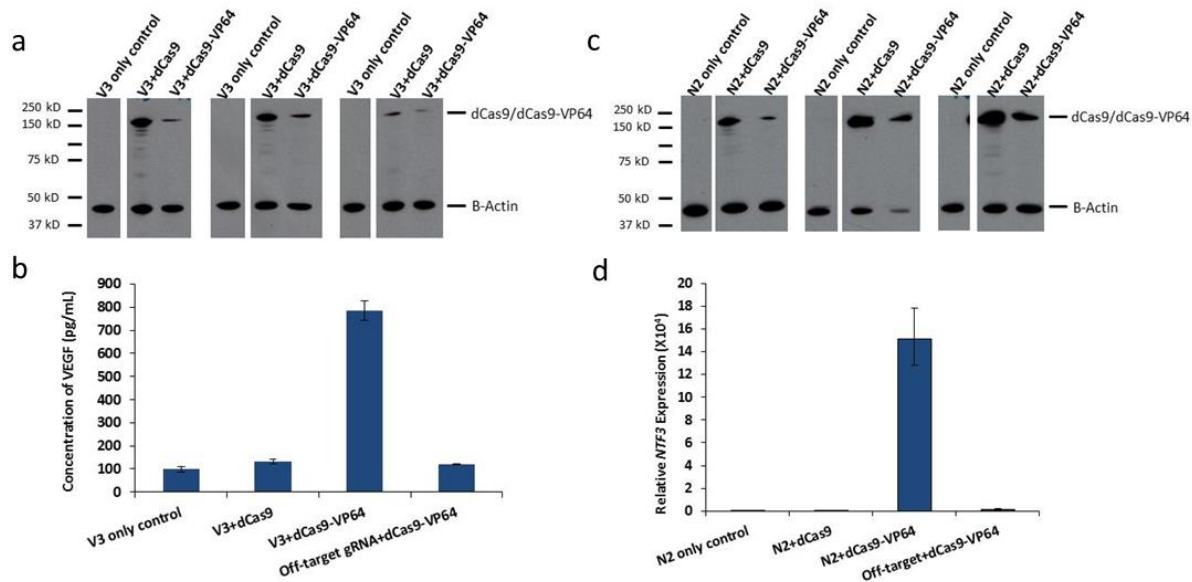
To test whether gene activation by dCas9-VP64 and an associated sgRNA depends on the presence of the VP64 domain, we compared the activities of a subset of sgRNAs each alone, paired with dCas9-VP64, or paired with dCas9 protein lacking VP64. These experiments show that for all sgRNAs tested, activation only occurs in the presence of dCas9-VP64 and not in the presence of dCas9.

(a) Activation of *VEGFA* protein expression by RNA-guided dCas9-VP64 in 293 cells. Concentrations of *VEGFA* protein produced by cells expressing various indicated sgRNAs, each expressed alone (blue bars), with dCas9 (orange bars) or with dCas9-VP64 (green bars). All experiments were performed in triplicate and error bars represent standard errors of the mean. *P* values for the indicated differences were determined by a one-sided t-test. **(b)** Activation of *NTF3* gene expression by RNA-guided dCas9-VP64 in 293 cells. Relative expression of *NTF3* mRNA, detected by quantitative RT-PCR and normalized to a *GAPDH* control ($\Delta\text{Ct} \times 10^4$), is shown for 293 cells co-transfected with various indicated sgRNAs, each expressed alone (blue bars), with dCas9 (orange bars) or with dCas9-VP64 (green bars). All experiments were performed in triplicate with error bars representing standard errors of the mean. *P* values were determined by a paired, one-sided t-test.

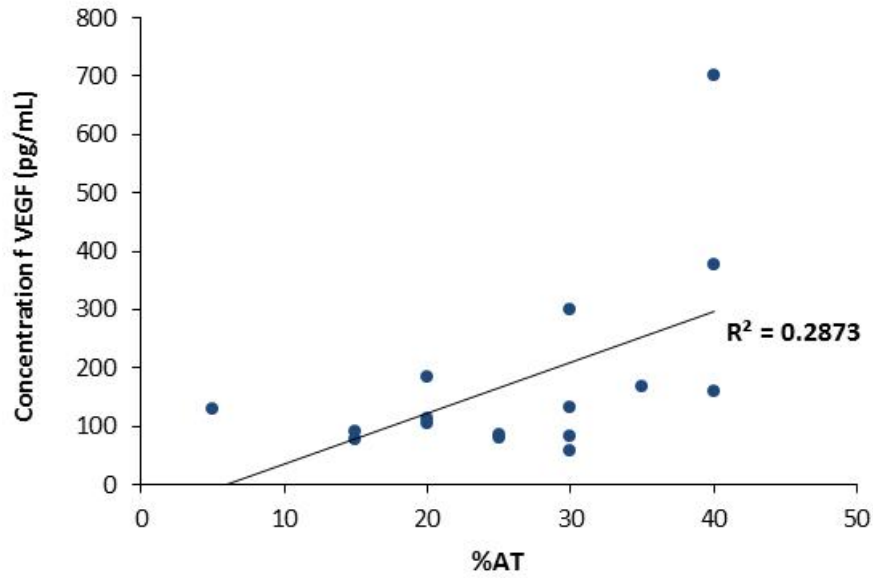


Supplementary Figure 4 dCas9 and dCas9-VP64 are stably expressed in human 293 cells

(a) Western blot analysis performed on 293 cells transfected with plasmid expressing the *VEGF*-targeted V3 sgRNA alone or in combination with dCas9 or dCas9-VP64-expression plasmids. Ten independent transfections were pooled to yield enough cells for Western blotting and each experiment was performed in triplicate. (b) Levels of VEGFA protein expression in the same samples used in (a) as well as an additional control in which an off-target sgRNA and dCas9-VP64 were co-expressed in cells. Error bars represent standard errors of the mean. (c) Western blot analysis performed on 293 cells transfected with plasmid expressing the *NTF3*-targeted N2 sgRNA alone or in combination with dCas9 or dCas9-VP64-expression plasmids. Ten independent transfections were pooled to yield enough cells for Western blotting and each experiment was performed in triplicate. (d) Relative *NTF3* mRNA expression in the same samples used in (b) as well as an additional control in which an off-target sgRNA and dCas9-VP64 were expressed. Error bars represent standard errors of the mean.



Supplementary Figure 5 Activation of VEGFA by RNA-guided activators does not correlate with nucleotide composition of sgRNAs Graph depicts concentration of VEGFA protein in cells transfected with 16 sgRNAs and dCas9-VP64 as a function of the %AT content of sgRNA target sites.



Supplementary Figure 6 Amino acid sequence of the dCas9-VP64 fusion protein

MDKKYSIGLAIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICY
LQEFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYLAHAMIK
FRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALS
LGLTPNFKSNFDLAEDAKLQLSKDQYDDDLNLLAQIGDQYADLFLAAKNLSDAILSDILRVNTEITKAPLSASMIKRYDE
HHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG
SIPHQIHLGELHAILRRQEDFYFPFLKDNREKIEKILTFRIPIYVVGPLARGNSRFAWMTRKSEETITPWNFEVVDKGASAQ
SFIERMTNFDKNLPNEKVLPHKSLLEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFK
KIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTTLFEDREMIEERLKTYAHLFDDKVMKQLKR
RRYTGWGRLSRKLINGIRDQKSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAI
KKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGKELGSQILKEHPVENTQLQNEKLY
LYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNA
KLITQRKFDNLTKAERGGLSELDKAGFIKRLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQ
FYKVRINNYYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVDVRKMIKSESEQEIGKATAKYFFYSNIMNFFKTEITL
ANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSPMPQVNIKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY
GGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSEFKNPIDFLEAKGYKEVKKDLIIKLPKYSLEFENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVL SAYNK
HRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLIHQSTGLYETRIDLSQLGGDGSPKKRKRKVS
DYKDHDGDYKDHDIDYKDDDDKAAGGGGSGRADLDDFDLMLGSDALDDFDLMLGSDALDDFDLMLGSDALD
DFDLML.

Supplementary Table 1 Frequencies of indel mutations induced by *VEGFA*-targeted sgRNAs and Cas9 nuclease in human 293 cells

sgRNA and Cas9 expression vectors were transfected into 293 cells in a 1:3 ratio because previous optimization experiments demonstrated a high level of Cas9-induced DNA cleavage in U2OS cells using this ratio of plasmids¹. All 16 sgRNAs were able to mediate the efficient introduction of Cas9-induced indel mutations at their respective target sites as assessed using a previously described T7E1 genotyping assay.

gRNA	Mean Indel Mutation Frequency (%) \pm SEM
V1	18.05 \pm 0.47
V2	41.48 \pm 0.62
V3	33.22 \pm 1.05
V4	16.97 \pm 0.06
V5	7.46 \pm 0.50
V6	16.99 \pm 0.51
V7	1.42 \pm 0.11
V8	34.07 \pm 0.90
V9	24.53 \pm 1.40
V10	35.65 \pm 1.35
V11	4.45 \pm 0.22
V12	23.95 \pm 0.41
V13	9.45 \pm 0.74
V14	12.17 \pm 0.36
V15	14.28 \pm 0.54
V16	18.82 \pm 1.48

Supplementary Table 2 *VEGFA* and *NTF3* gene target sites and associated oligonucleotides used to construct sgRNA expression plasmids.

See attached Excel file.

Supplementary Reference

1. Fu, Y. *et al.* High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat Biotechnol* (2013). doi:10.1038/nbt.2623