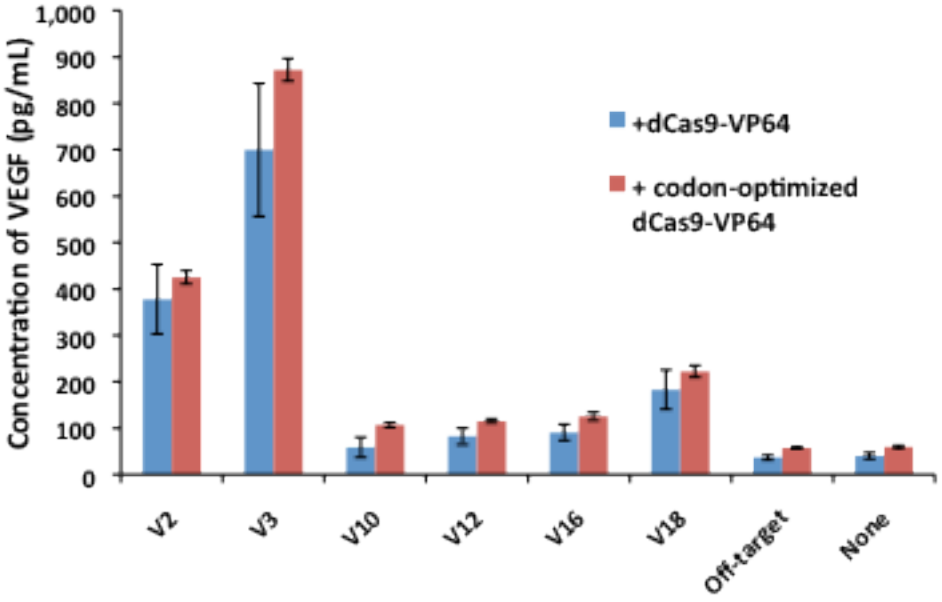
CRISPR RNA-guided activation of endogenous human genes

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

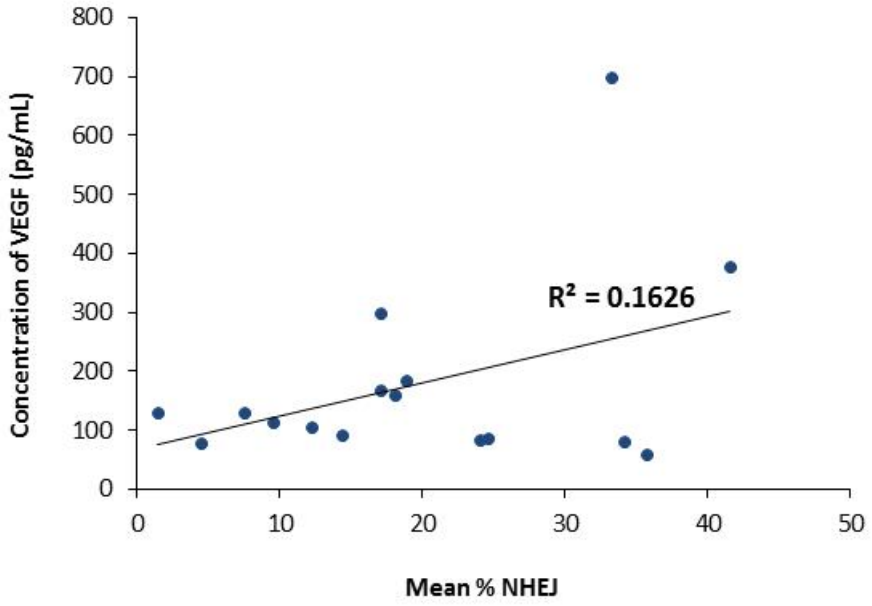
Supplementary Figure 1 Comparison of VEGF activation induced by dCas9-VP64 and a codon optimized dCas9-VP64

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

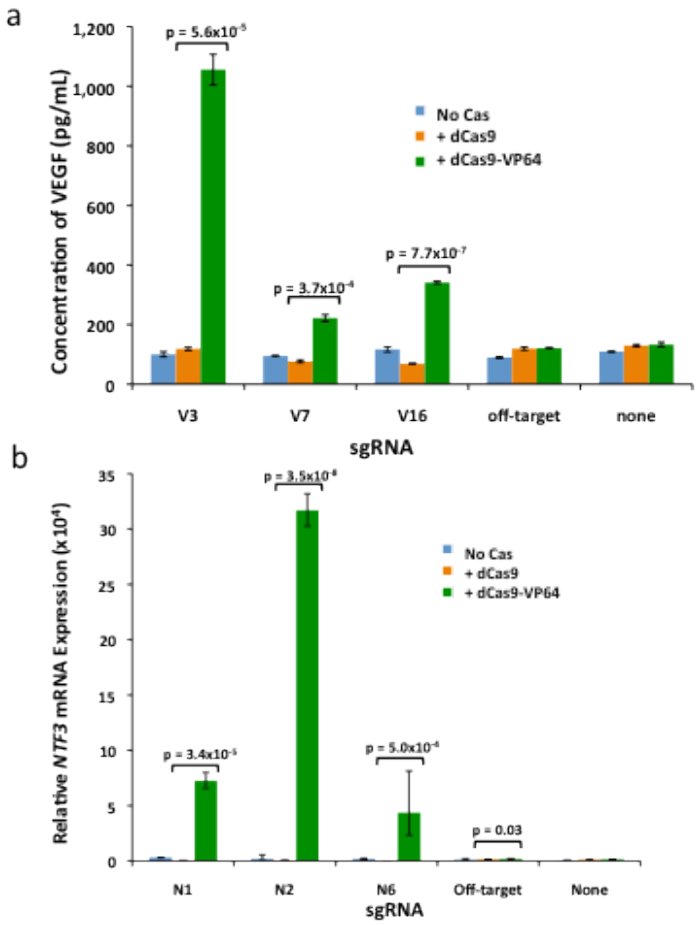
Graph depicts VEGFA protein concentration expressed from 293 cells transfected with 16 sgRNAs as a function of the frequency of NHEJ-induced indel mutations when the same sgRNAs were co-expressed in 293 cells with wild-type Cas9.



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

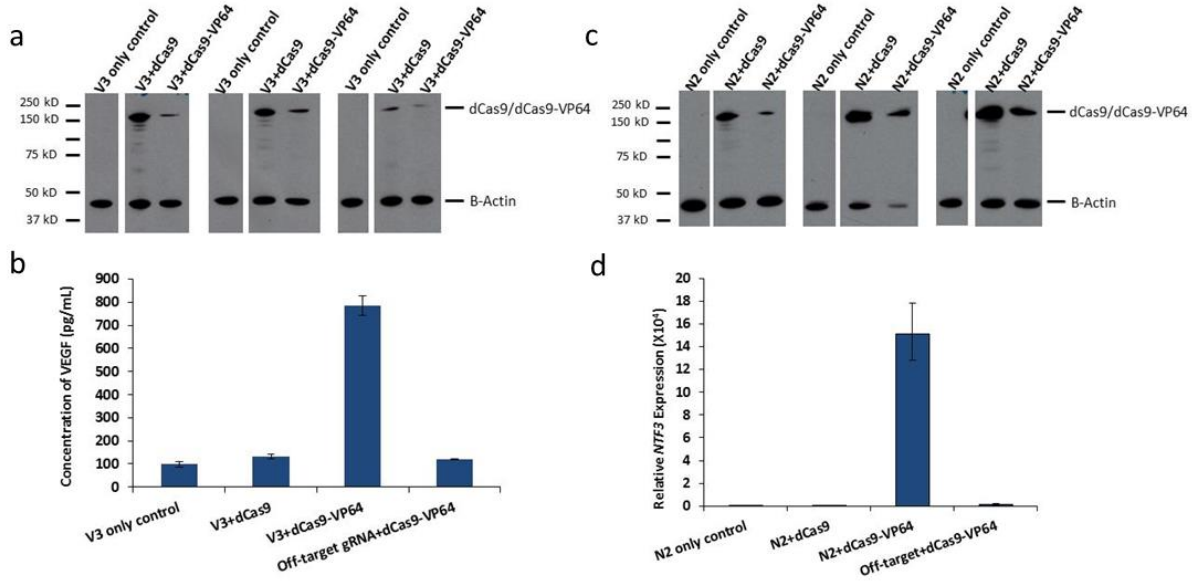
To test whether gene activation by dCas9-VP64 and an associated sgRNA depends on the presence of the VP64 domain, we compared the activities of a subset of sgRNAs each alone, paired with dCas9-VP64, or paired with dCas9 protein lacking VP64. These experiments show that for all sgRNAs tested, activation 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 (deltaCt x 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.

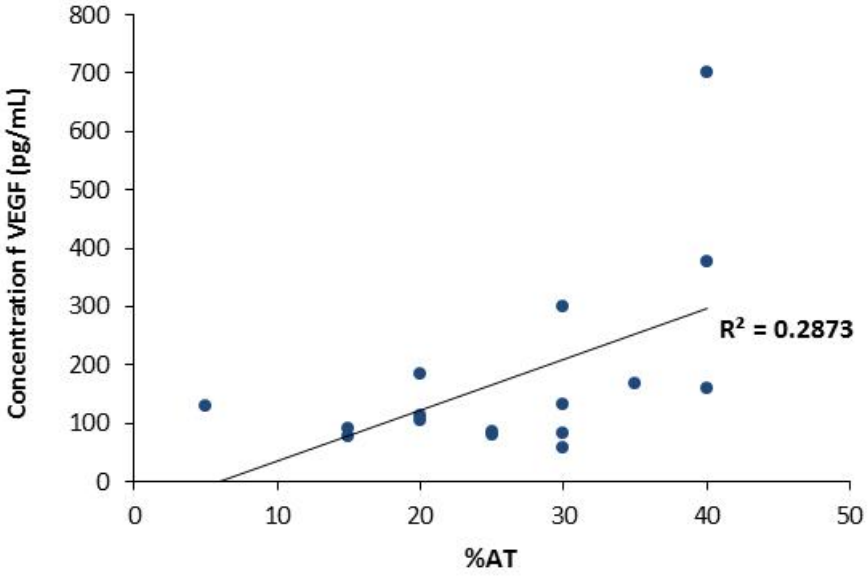


Supplementary Figure 4 dCas9 and dCas9-VP64 are stably expressed in human 293 cells

(**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-VP64as a function of the %AT content of sgRNA target sites.



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

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICY
LQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIK
FRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALS
LGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDE
HHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG
SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQ
SFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFK
KIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKR
RRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAI
KKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLY
LYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNA
KLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQ
FYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITL
ANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY
GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNK
HRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGSPKKKRKVSS
DYKDHDGDYKDHDIDYKDDDDKAAGGGGSGRADALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLGSDALD
DFDLDML.

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 plasmids[1]. 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 (%) ± SEM
V1	18.05 ± 0.47
V2	41.48 ±0.62
V3	33.22 ± 1.05
V4	16.97 ± 0.06
V5	7.46 ± 0.50
V6	16.99 ± 0.51
V7	1.42 ± 0.11
V8	34.07 ± 0.90
V9	24.53 ± 1.40
V10	35.65 ± 1.35
V11	4.45 ± 0.22
V12	23.95 ± 0.41
V13	9.45 ± 0.74
V14	12.17 ± 0.36
V15	14.28 ± 0.54
V16	18.82 ± 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