Supplementary Information

Supplementary Table 1 All oligonucleotides sequences used in this study
Supplementary Table 2 Primer sequences
Supplementary Figure 1
Supplementary Figure 2
Supplementary Figure 3
Supplementary Figure 4
Supplementary Figure 5
Supplementary Figure 6
Supplementary Data Set 1 Details of GUIDE-Seq analysis of native and 10 DNA crRNAs of three endogenous genes (Excel file)

Supplementary Table 1. All oligonucleotides sequences used in this study

DNA nucleotides are in uppercase (A, T, C, G). RNA nucleotides are described as r plus uppercase (rA, rU, rC, rG)

Name	Sequence (5’ to 3’)	Note
GFP-Native Strand	rGrGrGrCrGrArGrGrArGrCrUrGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	crRNA without any modifications
GFP-2DNA	GGrGrCrGrArGrGrArGrCrUrGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 2 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
GFP-4DNA	GGGCrGrArGrGrArGrCrUrGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 4 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
GFP-6DNA	GGGCGArGrGrArGrCrUrGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 6 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
GFP-8DNA	GGGCGAGGrArGrCrUrGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 8 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
GFP-10DNA	GGGCGAGGAGrCrUrGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 10 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
GFP-12DNA	GGGCGAGGAGCTrGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 12 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
GFP-14DNA	GGGCGAGGAGCTGTrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUr	Substitutes 14 RNA nucleotides with DNA nucleotides at 5’ end of

	UrUrUrG	guide sequence
GFP-16DNA	GGGCGAGGAGCTGTTCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 16 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
GFP-18DNA	GGGCGAGGAGCTGTTCACrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 18 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
GFP-20DNA	GGGCGAGGAGCTGTTCACCGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 20 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
EMX1-Native Strand	rGrArGrUrCrCrGrArGrCrArGrArArGrArArGrArArGrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrG	crRNA without any modifications
EMX1-2DNA	GArGrUrCrCrGrArGrCrArGrArArGrArArGrArArGrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrG	Substitutes 2 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
EMX1-4DNA	GAGTrCrCrGrArGrCrArGrArArGrArArGrArArGrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrG	Substitutes 4 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
EMX1-6DNA	GAGTCCrGrArGrCrArGrArArGrArArGrArArGrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrG	Substitutes 6 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
EMX1-8DNA	GAGTCCGArGrCrArGrArArGrArArGrArArGrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrG	Substitutes 8 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
EMX1-10DNA	GAGTCCGAGCrArGrArArGrArArGrArArGrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrG	Substitutes 10 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
EMX1-12DNA	GAGTCCGAGCAGrArArGrArArGrArArGrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrG	Substitutes 12 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
EMX1-14DNA	GAGTCCGAGCAGAArGrArArGrArArGrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 14 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
EMX1-16DNA	GAGTCCGAGCAGAAGArArGrArArGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 16 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
EMX1-18DNA	GAGTCCGAGCAGAAGAAGrArArGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 18 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence

EMX1-20DNA	GAGTCCGAGCAGAAGAAGAArGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 20 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
tracrRNA	rArArArCrArGrCrArUrArGrCrArArGrUrUrArArArArUrArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArCrUrUrGrArArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCrUrUrUrUrUrUrU	tracrRNA without any modification
GFP-4DNA at 3’ end	rGrGrGrCrGrArGrGrArGrCrUrGrUrUrCACCGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 4 RNA nucleotides with DNA nucleotides at 3’ end of guide sequence
VEGFA-Native Strand	rGrArCrCrCrCrCrUrCrCrArCrCrCrCrGrCrCrUrCrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	crRNA without any modifications
VEGFA-10DNA	GACCCCCTCCrArCrCrCrCrGrCrCrUrCrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 10 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
GFP sgRNA	rGrGrG rCrGrA rGrGrA rGrCrU rGrUrU rCrArC rCrGrG rUrUrU rUrArG rArGrC rUrArG rArArA rUrArG rCrArA rGrUrU rArArA rArUrA rArGrG rCrUrA rGrUrC rCrGrU rUrArU rCrArA rCrUrU rGrArA rArArA rGrUrG rGrCrA rCrCrG rArGrU rCrGrG rUrGrC rUrUrU rUrU	GFP-targeting sgRNA without any modifications
GFP sgRNA-8D	GGG CGA GGrA rGrCrU rGrUrU rCrArC rCrGrG rUrUrU rUrArG rArGrC rUrArG rArArA rUrArG rCrArA rGrUrU rArArA rArUrA rArGrG rCrUrA rGrUrC rCrGrU rUrArU rCrArA rCrUrU rGrArA rArArA rGrUrG rGrCrA rCrCrG rArGrU rCrGrG rUrGrC rUrUrU rUrU	Substitutes 8 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
EMX1 sgRNA	rGrArGrUrCrCrGrArGrCrArGrArArGrArArGrArArG rUrUrU rUrArG rArGrC rUrArG rArArA rUrArG rCrArA rGrUrU rArArA rArUrA rArGrG rCrUrA rGrUrC rCrGrU rUrArU rCrArA rCrUrU rGrArA rArArA rGrUrG rGrCrA rCrCrG rArGrU rCrGrG rUrGrC rUrUrU rUrU	EMX1-targeting sgRNA without any modifications
EMX1 sgRNA-8D	GAGTCCGArGrCrArGrArArGrArArGrArArG rUrUrU rUrArG rArGrC rUrArG rArArA	Substitutes 8 RNA nucleotides with DNA nucleotides at the 5’ end of

	rUrArG rCrArA rGrUrU rArArA rArUrA rArGrG rCrUrA rGrUrC rCrGrU rUrArU rCrArA rCrUrU rGrArA rArArA rGrUrG rGrCrA rCrCrG rArGrU rCrGrG rUrGrC rUrUrU rUrU	guide sequence
VEGFA sgRNA	rGrArCrCrCrCrCrUrCrCrArCrCrCrCrGrCrCrUrCrG rUrUrU rUrArG rArGrC rUrArG rArArA rUrArG rCrArA rGrUrU rArArA rArUrA rArGrG rCrUrA rGrUrC rCrGrU rUrArU rCrArA rCrUrU rGrArA rArArA rGrUrG rGrCrA rCrCrG rArGrU rCrGrG rUrGrC rUrUrU rUrU	VEGFA-targeting sgRNA without modifications
VEGFA sgRNA-8D	GACCCCCTrCrCrArCrCrCrCrGrCrCrUrCrG rUrUrU rUrArG rArGrC rUrArG rArArA rUrArG rCrArA rGrUrU rArArA rArUrA rArGrG rCrUrA rGrUrC rCrGrU rUrArU rCrArA rCrUrU rGrArA rArArA rGrUrG rGrCrA rCrCrG rArGrU rCrGrG rUrGrC rUrUrU rUrU	Substitutes 8 RNA nucleotides with DNA nucleotides at 5' end of guide sequence
RNA mut	GGGCGAGGrUrCrGrArGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 8 RNA nucleotides with DNA nucleotides at 5' end of guide sequence, and mutates 4 RNA nucleotides (position 9-12 from 5' end).
DNA mut-1	GGGCCTCCrArGrCrUrGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 8 RNA nucleotides with DNA nucleotides at 5' end of guide sequence, and mutates 4 DNA nucleotides (position 5-8 from 5' end).
DNA mut-2	CCCGGAGGrArGrCrUrGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 8 RNA nucleotides with DNA nucleotides at 5' end of guide sequence, and mutates 4 DNA nucleotides (position 1-4 from 5' end).
GFP2 Native crRNA	rGrGrGrCrArCrGrGrGrCrArGrCrUrUrGrCrCrGrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	crRNA without any modifications
GFP2 RNA-mut 1	rGrGrGrCrArCrGrGrGrCrArGrCrUrUrGrGrCrGrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	crRNA without any modifications and with 1 nt mismatch mutation at the seed region

GFP2 RNA-mut 2	rGrGrGrCrArCrGrGrCrCrArGrCrUrUrGrCrCrGrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	crRNA without modifications and with 1 nt mismatch mutation at the tail region
GFP2 10DNA	GGGCACGGGCrArGrCrUrUrGrCrCrGrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 10 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
GFP2 10DNA-mut 1	GGGCACGGGCrArGrCrUrUrGrGrCrGrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 10 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence; with 1 nt mismatch mutation at the seed region
GFP2 10DNA-mut 2	GGGCACGGCCrArGrCrUrUrGrCrCrGrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 10 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence; with 1 nt mismatch mutation at the tail region
Mus Pcsk9-2-crRNA	rGrCrU rCrGrC rCrCrU rCrCrC rGrUrC rCrCrA rGrG rG rUrUrU rUrArG rArGrC rUrArU rGrCrU rGrUrU rUrUrG	crRNA without any modifications
Mus Pcsk9-2-10DNA	GCT CGC CCT CrCrC rGrUrC rCrCrA rGrG rG rUrUrU rUrArG rArGrC rUrArU rGrCrU rGrUrU rUrUrG	Substitutes 10 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
293 site 4-crRNA	rGrGrC rArCrU rGrCrG rGrCrU rGrGrA rGrGrU rGrG rG rUrUrU rUrArG rArGrC rUrArU rGrCrU rGrUrU rUrUrG	crRNA without any modifications
293 site 4-10DNA	GGC ACT GCG GrCrU rGrGrA rGrGrU rGrG rG rUrUrU rUrArG rArGrC rUrArU rGrCrU rGrUrU rUrUrG	Substitutes 10 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence
GFP 3nt DNA mismatch	GGGC GTCC rArGrCrUrGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 8 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence; with 3 nt mismatch mutation at the seed region
GFP 3nt RNA mismatch	rGrGrGrC rGrUrCrC rArGrCrUrGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	crRNA without modifications and with 3 nt mismatch mutation at the tail region
GFP 2nt DNA mismatch	GGGC GACCrArGrCrUrGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 8 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence; with 2 nt mismatch mutation at the seed region
GFP 2nt RNA mismatch	rGrGrGrC rGrArCrC rArGrCrUrGrUrUrCrArCrCrGrGrUrUrUrUr	crRNA without modifications and with 2 nt mismatch mutation at the

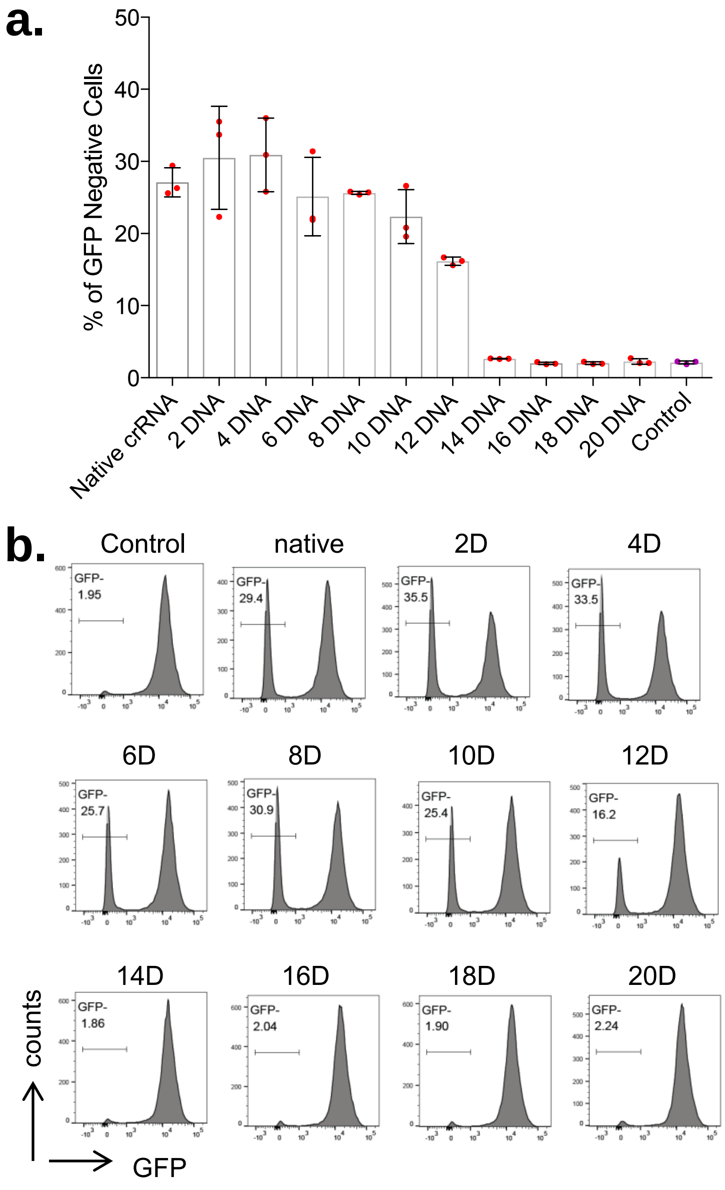
	ArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	tail region
GFP 1nt DNA mismatch	GGGC GAGC rArGrCrUrGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrG	Substitutes 8 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence; with 1 nt mismatch mutation at the seed region
GFP 1nt RNA mismatch	rGrGrGrC rGrArGrC rArGrCrUrGrUrUrCrArCrCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrUrGrUrUrUrUrGrUrG	crRNA without modifications and with 1 nt mismatch mutation at the tail region
GFP-sg10D	GGG CGA GGA GrCrU rGrUrU rCrArC rCrGrG rUrUrU rUrArG rArGrC rUrArG rArArA rUrArG rCrArA rGrUrU rArArA rArUrA rArGrG rCrUrA rGrUrC rCrGrU rUrArU rCrArA rCrUrU rGrArA rArArA rGrUrG rGrCrA rCrCrG rArGrU rCrGrG rUrGrC rUrUrU rUrU	Substitutes 10 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence of sgRNA
EMX-sg10D	GAGTCCGAGCrArGrArArGrArArGrArArG rUrUrU rUrArG rArGrC rUrArG rArArA rUrArG rCrArA rGrUrU rArArA rArUrA rArGrG rCrUrA rGrUrC rCrGrU rUrArU rCrArA rCrUrU rGrArA rArArA rGrUrG rGrCrA rCrCrG rArGrU rCrGrG rUrGrC rUrUrU rUrU	Substitutes 10 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence of sgRNA
VEGFA-sg10D	GACCCCCTCCrArCrCrCrCrGrCrCrUrCrG rUrUrU rUrArG rArGrC rUrArG rArArA rUrArG rCrArA rGrUrU rArArA rArUrA rArGrG rCrUrA rGrUrC rCrGrU rUrArU rCrArA rCrUrU rGrArA rArArA rGrUrG rGrCrA rCrCrG rArGrU rCrGrG rUrGrC rUrUrU rUrU	Substitutes 10 RNA nucleotides with DNA nucleotides at 5’ end of guide sequence of sgRNA
22 DNA-3'	rGrGrGrCrGrArGrGrArGrCrUrGrUrUrCrArCrCrGGTTTTAGAGCTATGCTGTTTTG	Substitutes 22 RNA nucleotides with DNA nucleotides at 3’ end of crRNA
16 DNA-3'	rGrGrGrCrGrArGrGrArGrCrUrGrUrUrCrArCrCrGGrUrUrUrUrArGAGCTATGCTGTTTTG	Substitutes 16 RNA nucleotides with DNA nucleotides at 3’ end of guide sequence of crRNA
8DNA16DNA	GGGCGAGGrArGrCrUrGrUrUrCrArCrCrGGrUrUrUrUrArGAGCTATGCTGTTTTG	Substitutes 8 RNA nucleotides with DNA nucleotides at 5’ end 16 RNA nucleotides with DNA nucleotides at 3’ end of crRNA

Ascpf1	rUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArUrCrUrGrArUrGrGrUrCrCrArUrGrUrCrUrGrUrUrArCrUC	Ascpf1 crRNA without modifications
8DNA-Ascpf1	rUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArUrCrUrGrArUrGrGrUrCrCrArUrGrUrCTGTTACTC	Substitutes 8 RNA nucleotides with DNA nucleotides at 3’ end of guide sequence of Ascpf1 crRNA

Supplementary Table 2. Primer sequences.

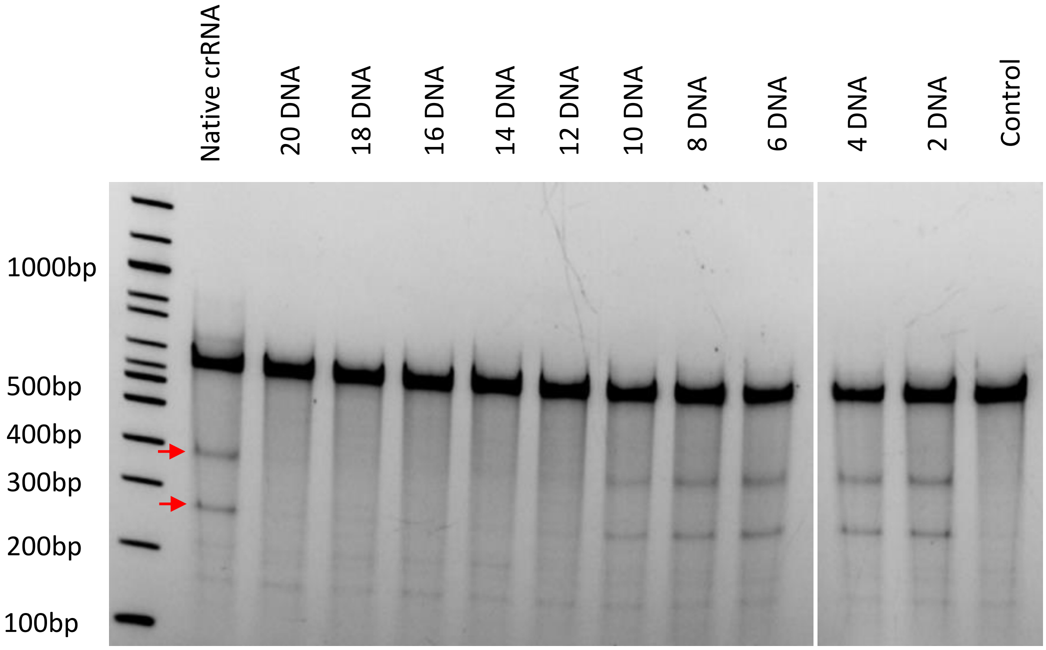
ID	Sequence (5’->3’)	Notes
EF1a-F	TCAAGCCTCAGACAGTGGTTC	GFP PCR primers
GFP-R	TCCTTGAAGTCGATGCCCTT	
VEGFA_For	AGAGAAGTCGAGGAAGAGAGAG	VEGFA PCR Primers
VEGFA _Rev	CAGCAGAAAGTTCATGGTTTCG	
VEGFA_OFF_1_For	CAAGATGTGCACTTGGGCTA	VEGFA Off target site 1
VEGFA _OFF_1_Rev	GCAGCCTATTGTCTCCTGGT	
VEGFA _OFF_2_For	CCAGGTGGTGTCAGCGGAGG	VEGFA Off target site 2
VEGFA _OFF_2_Rev	TGCCTGGCCCTCTCTGAGTCT	
VEGFA _OFF_3_For	TGCTGCAGGTGGTTCCGGAG	VEGFA Off target site 3
VEGFA _OFF_3_Rev	CTGGAACCGCATCCTCCGCA	
Sp-EMX1-F2	CCATCCCCTTCTGTGAATGT	EMX1 PCR Primers
Sp-EMX1-R2	GGAGATTGGAGACACGGAGA	
293-site4F	GAGACCTGCTGAGGGCGGCTTCTCCC	293 site4 PCR primers
293-site4 seq	AGTCAGTCCATGCCTGCAGGGTC	
293-site4R	CCCACTGTAGTCACACAGCACCAGAG	
Pcsk9_For	ATCACCCCAACCCCAAAGCA	Pcsk9 PCR primers
Pcsk9_Rev	CCAGGCGTCCATGTCCTTCC	

Supplementary Figure 1



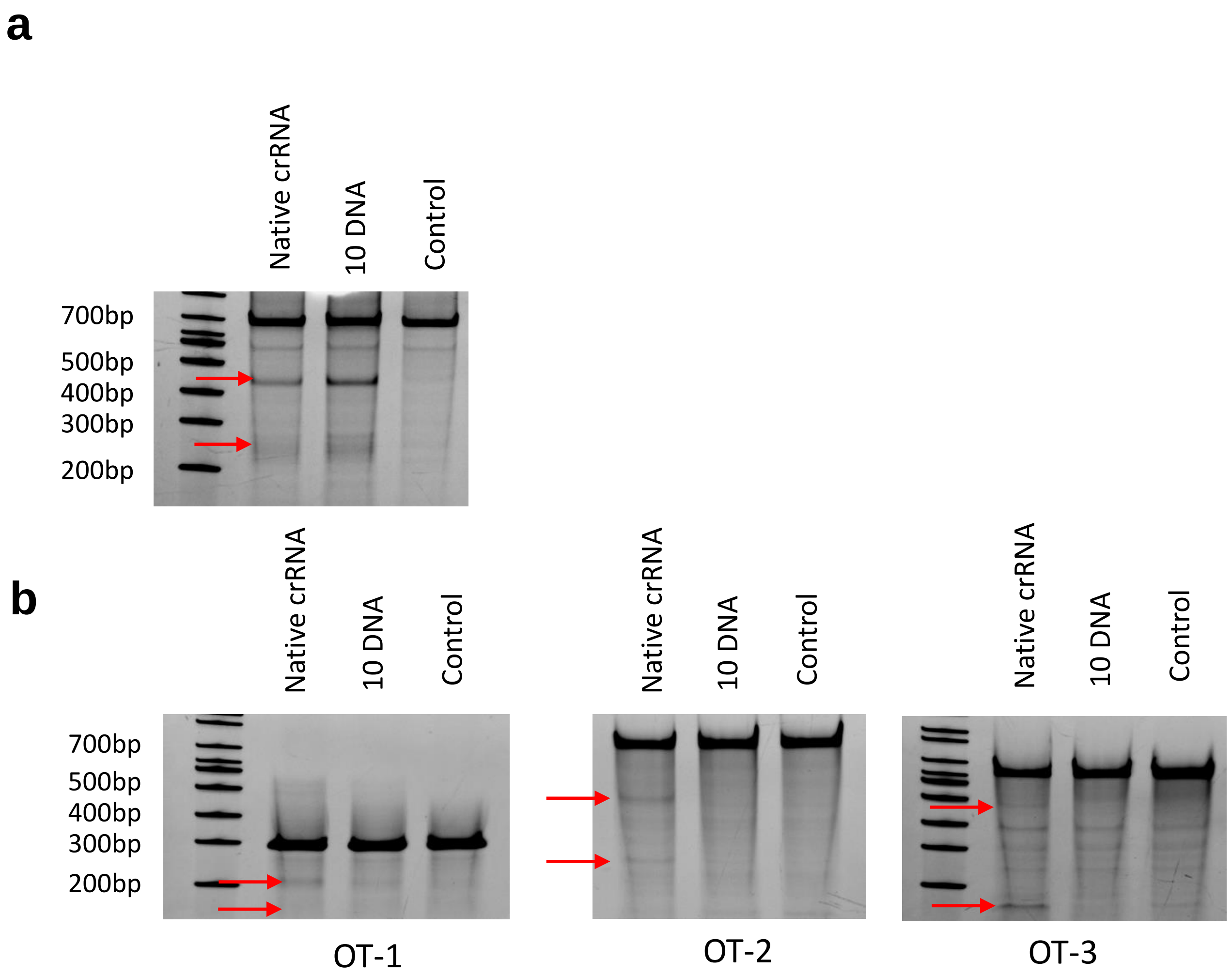
Supplementary Figure 1. Partial DNA replacement at the 5' end of guide sequence of a crRNA induced gene editing in human cells. HEK293T cells stably expressing both EFs promoter-*Sp*Cas9 and EF1a promoter-GFP were transfected with a crRNA targeting GFP and tracrRNA. (a) Percentage of GFP negative cells determined by FACS analysis. n=3 biologically independent samples. Error bars, mean ± s.d.. (b) Representative FACS plots.

Supplementary Figure 2



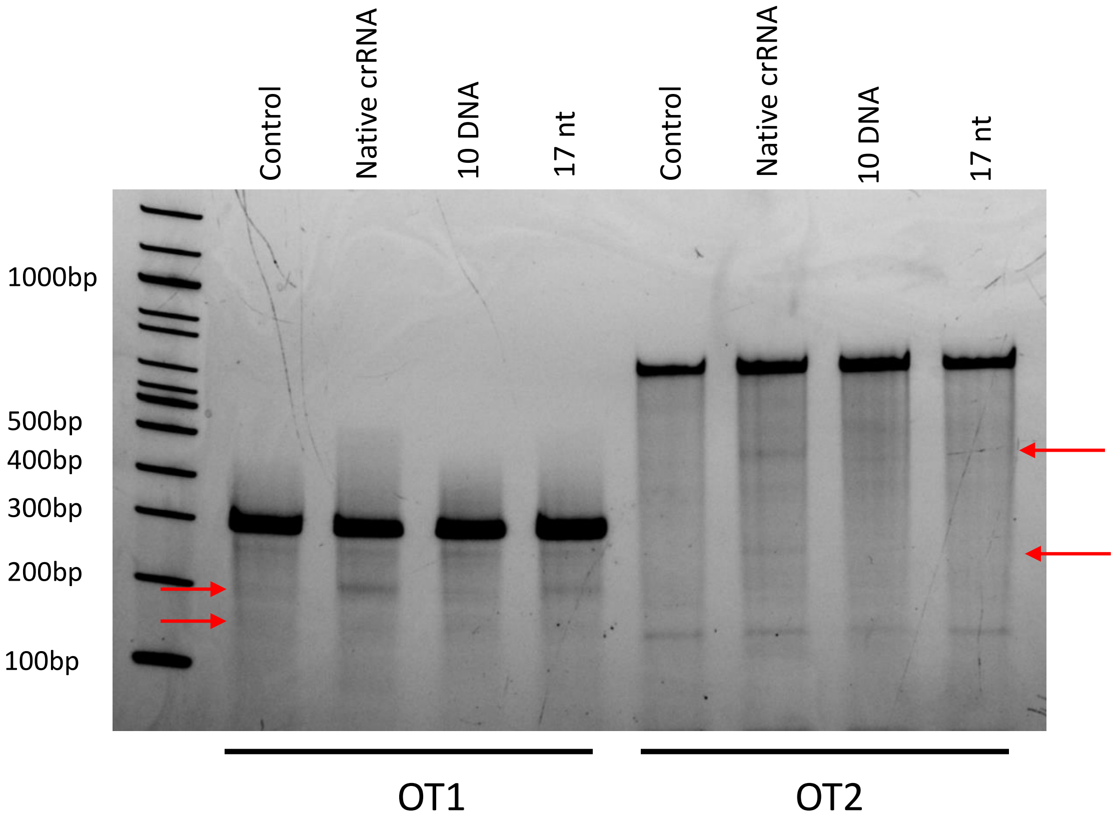
Supplementary Figure 2. Partial DNA replacement at the 5' end of guide sequence of a crRNA targeting EMX1 induced indels in human cells. HEK293T cells stably expressing *Sp*Cas9 were transfected with tracrRNA and an EMX1-targeting crRNA. Surveyor assay were performed to determine indels at EMX1 locus. Red arrowheads indicate surveyor nuclease cleaved fragments of the EMX1 PCR product. These experiments were repeated three times independently with similar results.

Supplementary Figure 3



Supplementary Figure 3. Partial DNA replacement at the 5' end of guide sequence of a crRNA targeting VEGFA efficiently reduced off-target activity in human cells. Surveyor assay were performed to determine indels at a) VEGFA locus and b) 3 top off-target sites of VEGFA guide sequence. Red arrowheads indicate surveyor nuclease cleaved fragments of PCR products of the VEGFA or off-target sites. These experiments were repeated three times independently with similar results.

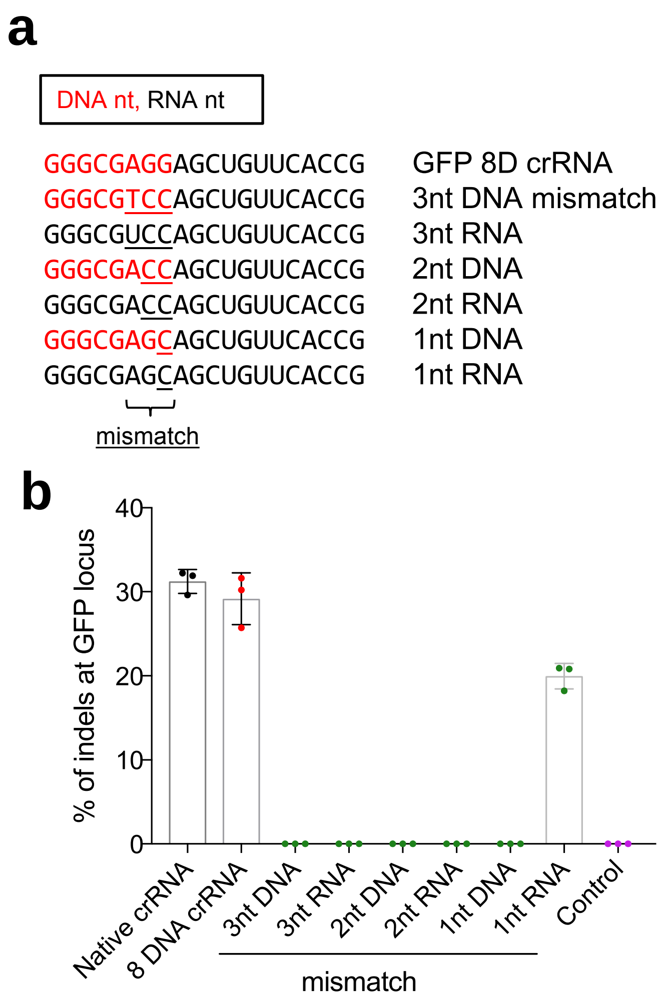
Supplementary Figure 4



Supplementary Figure 4. Partial DNA replacement at the 5' end of guide sequence of a crRNA or truncated crRNA targeting VEGFA efficiently reduced off-target activity in human cells. Surveyor assay was performed to determine indels at 2 top off-target sites of VEGFA guide sequence. Red arrowheads indicate surveyor nuclease cleaved fragments of PCR products of the off-target sites. These experiments were repeated three times independently with similar results.

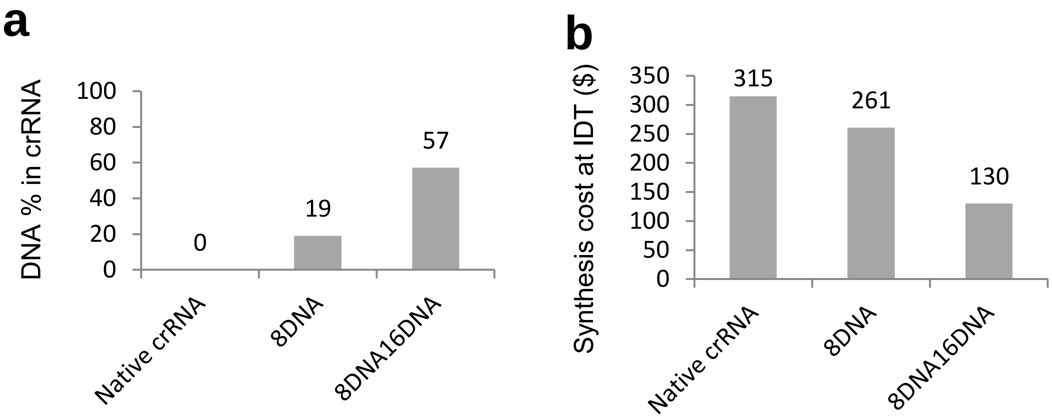
Nature Chemical Biology: doi:10.1038/nchembio.2559

Supplementary Figure 5



Supplementary Figure 5. Native crRNA, but not the 8 DNA crRNA, tolerates single nucleotide mismatch. (a) Illustration of DNA replacement and mismatches at the 20 nt guide region of GFP crRNAs. RNA, DNA, and mismatch are shown in black, red and underlined, respectively. (b) HEK293T-GFP-SpCas9 cells were transfected with tracrRNA and crRNAs in (a). TIDE analysis was performed at day 3 to calculate the % of indel at GFP locus. n=3 biologically independent samples. Error bars, mean ± s.d.

Supplementary Figure 6



Supplementary Figure 6. An optimized DNA-RNA chimeric crRNA enables efficient genome editing in human cells and significantly reduces cost. (a) DNA % in crRNA design in Fig. 4a. **(b)** 8DNA16DNA significantly reduces synthesis cost. Cost shown is for 100nMole custom RNA or DNA-RNA chimeric oligos ordered from IDT.

Supplementary Data Set 1 Details of GUIDE-Seq analysis of native and 10 DNA crRNAs of three endogenous genes (Excel file)

GUIDE-Seq analysis of native and 10 DNA crRNAs targeting mouse *Pcsk9*, and human EMX1 and 293 site 4 was performed using Hepa1-6 cells and HEK293T cells, respectively. The information of the on-target and off-target sites, including location, peak scores, off-target sequence, details of mismatches, etc was showed in the tables. Off target sites for native crRNA and 10D are shown in black or blue, respectively. Each tab in the excel file is one gene locus. The full table shows off-target sites allowing up to 6 mismatches in the guide and 2 in the PAM. Sites with more than 6 mismatches total between the guide and PAM (the threshold defined by the Joung laboratory) are shown in grey. These sites were excluded from our analysis.