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Supplemental Information

Chemically Modified Cpf1-CRISPR RNAs Mediate Efficient Genome Editing in Mammalian Cells

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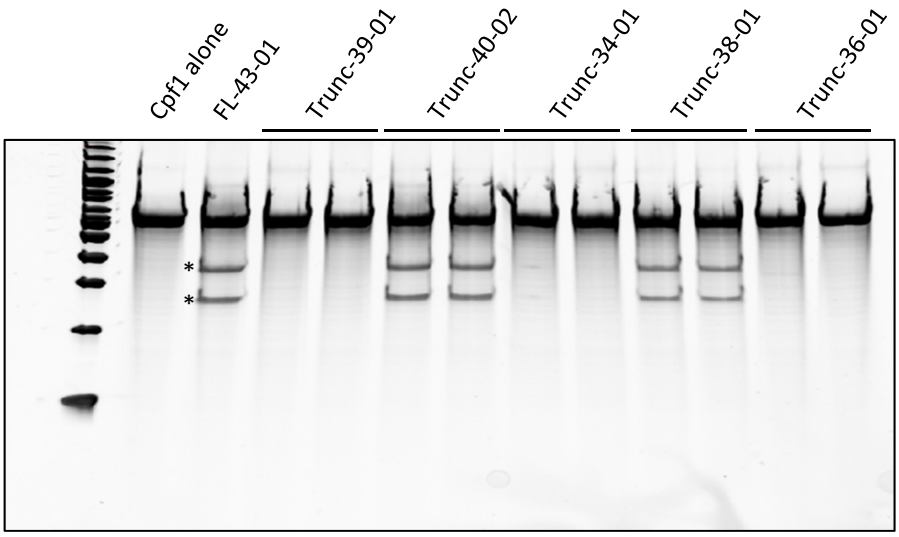
Supplementary Figure S1

A

Cpf1 Direct Repeat	DNMT1 (genomic target)	gBlock	Direct Repeat Length	Target Length	Total RNA Length	Fold Change
taatttctactcttgtagat	CTGATGGTCCATGTCTGTTACTC	FL-43-01	20	23	43	1
ttctactcttgtagat	CTGATGGTCCATGTCTGTTACTC	Trunc-39-01	16	23	39	nd
taatttctactcttgtagat	CTGATGGTCCATGTCTGTTA	Trunc-40-02	20	20	40	1
ttctactcttgtagat	CTGATGGTCCATGTCTGT	Trunc-34-01	16	18	34	nd
taatttctactcttgtagat	CTGATGGTCCATGTCTGT	Trunc-38-01	20	18	38	0.7
ttctactcttgtagat	CTGATGGTCCATGTCTGTTA	Trunc-36-02	16	20	36	nd

B



*Expected bands

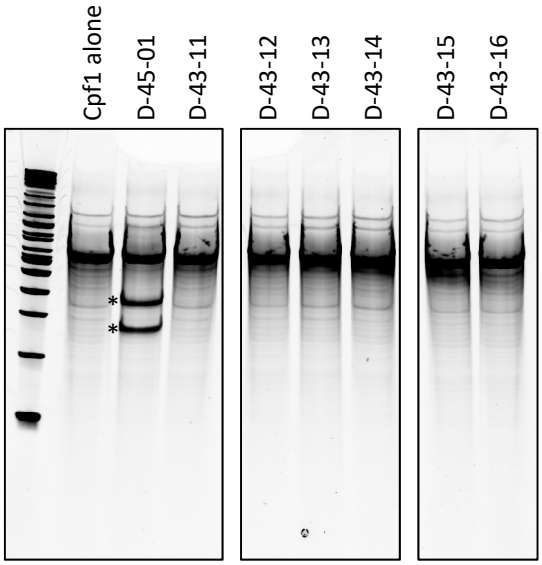
Supplementary Figure S1. crRNAs with a 16 nucleotide direct repeat are not active. (A) Complete sequence and activity of truncated crRNAs targeting DNMT1. No data (nd) was observed for Trunc-39-01, Trunc-34-01 and Trunc-36-01. (B) Surveyor nuclease assay gel with expected bands indicated by an asterisk (*). Duplicate lanes represent biological replicates.

Supplementary Figure S2

A

Name	crRNA sequence (5' to 3')	% Gene Disruption (Absolute)
D-45-01	CTUAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUTACTC	21
D-43-11	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCTGTTACTC	nd
D-43-12	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCATGTCTGTTACTC	nd
D-43-13	UAAUUUCUACUCUUGUAGAUCUGAUGGTCCATGTCTGTTACTC	nd
D-43-14	UAAUUUCUACUCUUGUAGAUCUGAUGGTCCATGTCTGTTACTC	nd
D-43-15	UAAUUUCUACUCUUGUAGAUCTGATGGTCCATGTCTGTTACTC	nd
D-43-16	TAATTTCTACTCTTGTAGATCTGATGGTCCATGTCTGTTACTC	nd
	← Direct Repeat → ← DNA Specificity →	DNA

B



*Expected bands

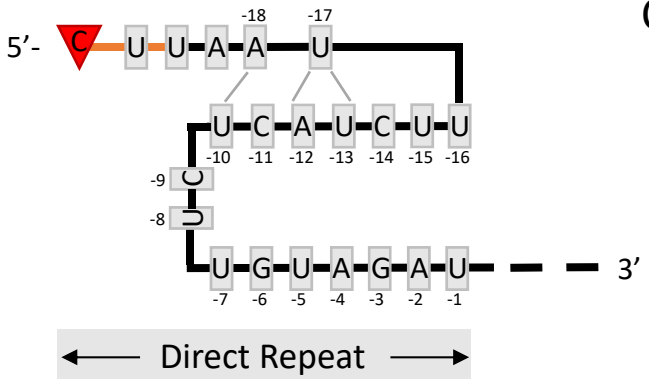
Supplementary Figure S2. scrRNAs with increasing DNA substitutions are not active. (A) Complete sequence and activity of DNA substituted scrRNAs targeting DNMT1. No data (nd) was observed for scrRNAs with eight or more DNA substitutions in the DNA specificity region. (B) Representative surveyor nuclease assay gel with expected bands indicated by an asterisk (*).

Supplementary Figure S3

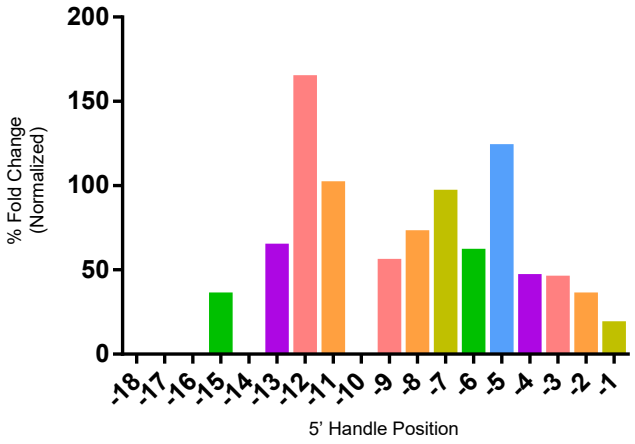
A

Name	crRNA sequence (5’ to 3’)	Fold Change (Normalized)
MPF-40-04	$C_sU_sUAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCU_sG_sU$	0.4
MPF-40-05	$C_sU_sUAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCU_sG_sU$	0.3
MPF40-06	$C_sU_sUAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCU_sG_sU$	0.4
MPF-40-07	$C_sU_sUAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCU_sG_sU$	0.3
MPF-40-08	$C_sU_sUAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCU_sG_sU$	0.3
MPF-40-09	$C_sU_sUAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCU_sG_sU$	nd
	← Direct Repeat → ← DNA Specificity →	2’-OMe, PS, 2’-F, cET

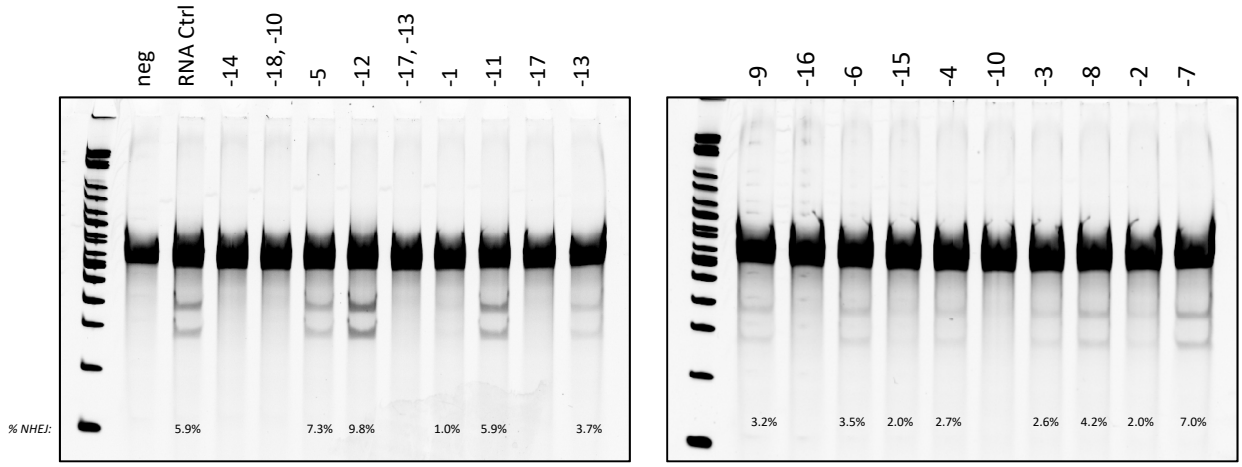
B



C



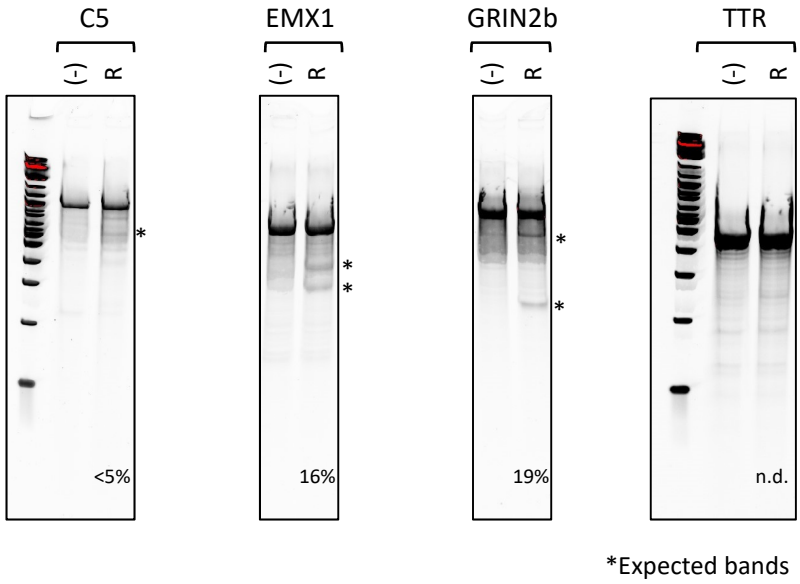
D



Supplementary Figure S3. 2’-F and cET substitution walk in direct repeat. Complete sequence and activity of 40-mer scrRNAs targeting DNMT1 containing single 2’-F and cET substitutions in the direct repeat. No data (nd) was observed for MPF-40-09.

Supplementary Figure S4

Target	crRNA sequence (5’ to 3’)
C5	CUUAAUUUCUACUCUUGUAGAUUACUCCAGACCAGUCAGG
EMX1	CUUAAUUUCUACUCUUGUAGAUUGGUUGCCCACCCUAGUC
GRIN2B	CUUAAUUUCUACUCUUGUAGAUGUGCUCAAUGAAAGGAGA
TTR	CUUAAUUUCUACUCUUGUAGAUUGUCUGAGGCUGGCCCUA
LDLR	CUUAAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCAGG



C5: Complement C5
EMX1: Empty Spiracles Homeobox 1
GRIN2b: Glutamate Ionotropic Receptor NMDA Type Subunit 2B
LDLR: Low Density Lipoprotein Receptor
TTR: Transthyretin

Supplementary Figure S4. Variable activity of crRNAs targeting additional genes. (Top) crRNA target sequences to indicated genes (C5, EMX1, GRIN2b, TTR, and LDLR). (Middle) Representative surveyor nuclease assay gel with % gene disruption indicated at the bottom where n.d.=no data and expected bands marked with an asterisk (*).

Supplementary Figure S5

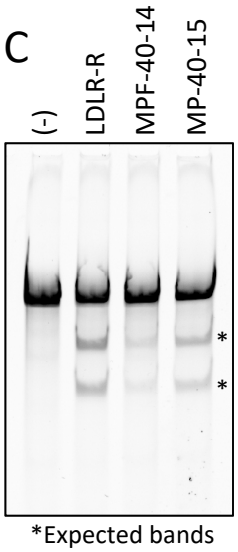
A

Name	crRNA sequence (5' to 3')	% Gene Disruption (Absolute)
U-40-02	CUUAAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCAGG	26
LDLR-**RDP**	C_sU_sUAAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCA$_s$G$_s$G	4
LDLR-**RP**	C_sU_sUAAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCA$_s$G$_s$G	18
LDLR-**RFP**	C_sU_sUAAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCA$_s$G$_s$G	18
	← Direct Repeat → ← DNA Specificity →	2'-OMe, PS, 2'-F, DNA

B

Name	crRNA sequence (5' to 3')	Fold Change (Normalized)
U-40-02	CUUAAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCAGG	1.0
MPD-40-01	C_sU_sUAAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCA$_s$G$_s$G	n.d.
D-43-09	UAAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCAGGTCGTG	1.2
D-43-10	UAAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCAGGTCGTG	1.0
MPD-43-01	U_sA_sAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCAGGUCG$_s$U$_s$G	1.2
MPD-43-02	U_sA_sAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCAGGTCG$_s$U$_s$G	1.1
D-45-02	CTUAAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCAGGTCGTG	1.2
D-45-03	CTUAAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCAGGTCGTG	1.0
MPD-45-01	C_sU_sUAAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCAGGTCG$_s$U$_s$G	1.1
MPD-45-02	C_sU_sUAAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCAGGTCG$_s$U$_s$G	1.2
	← Direct Repeat → ← DNA Specificity →	2'-OMe, PS, DNA

C



*Expected bands

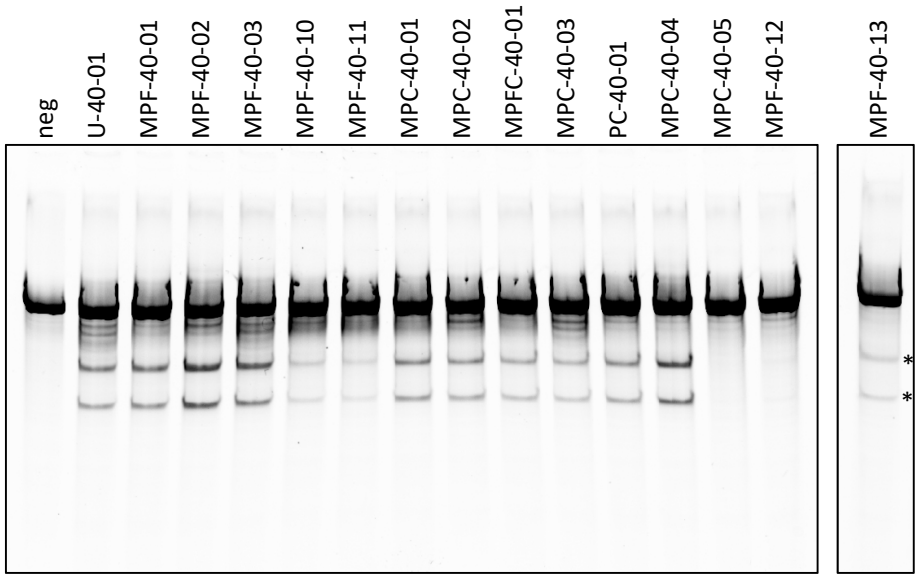
D

Name	crRNA sequence (5' to 3')	Fold Change (Normalized)
U-40-02	CUUAAUUUCUACUCUUGUAGAUCAGCUAGGACACAGCAGG	1.0
MPF-40-14	C_sU_sUAAUUUCUACUCUUGUAGAUCAGCUAG$_s$GA$_s$CA$_s$CA$_s$GC$_s$A$_s$G$_s$G	0.1
MPF-40-15	C_sU_sUAAUUUCUACUCUUGUAGAUCAGCUAG$_s$GA$_s$CA$_s$CA$_s$GC$_s$A$_s$G$_s$G	0.6
	← Direct Repeat → ← DNA Specificity →	
	2'-OMe, PS, 2'-F, cET	

Supplementary Figure S5. Gene disruption activity of scrRNAs targeting LDLR. (A) Absolute gene disruption activity of LDLR scrRNAs with nucleotide substitutions at positions 1, 8 and 9 in the DNA specificity region. (B) Single experiment demonstrating LDLR scrRNAs with DNA substitutions and truncations/extensions of both the DNA specificity region and direct repeat. (C) Surveyor nuclease assay gel with expected bands indicated by an asterisk (*). (D) Complete sequence and activity of 40-mer scrRNAs targeting LDLR.

Supplementary Figure S6

A



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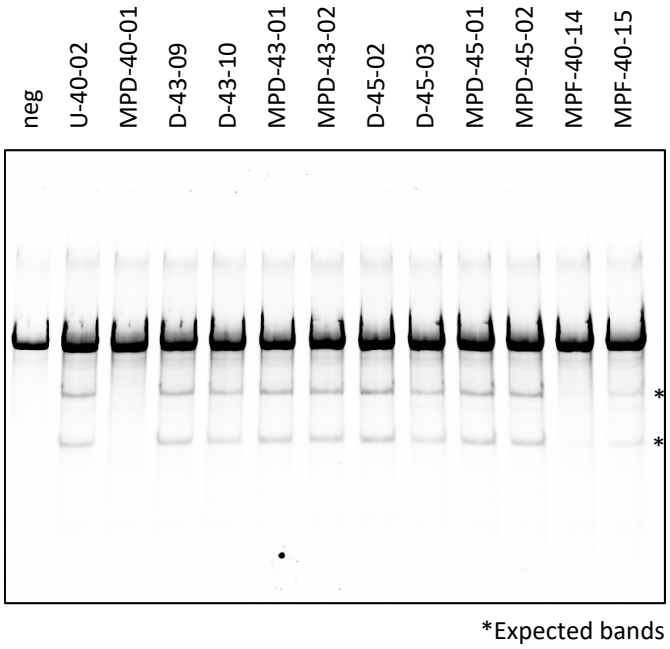
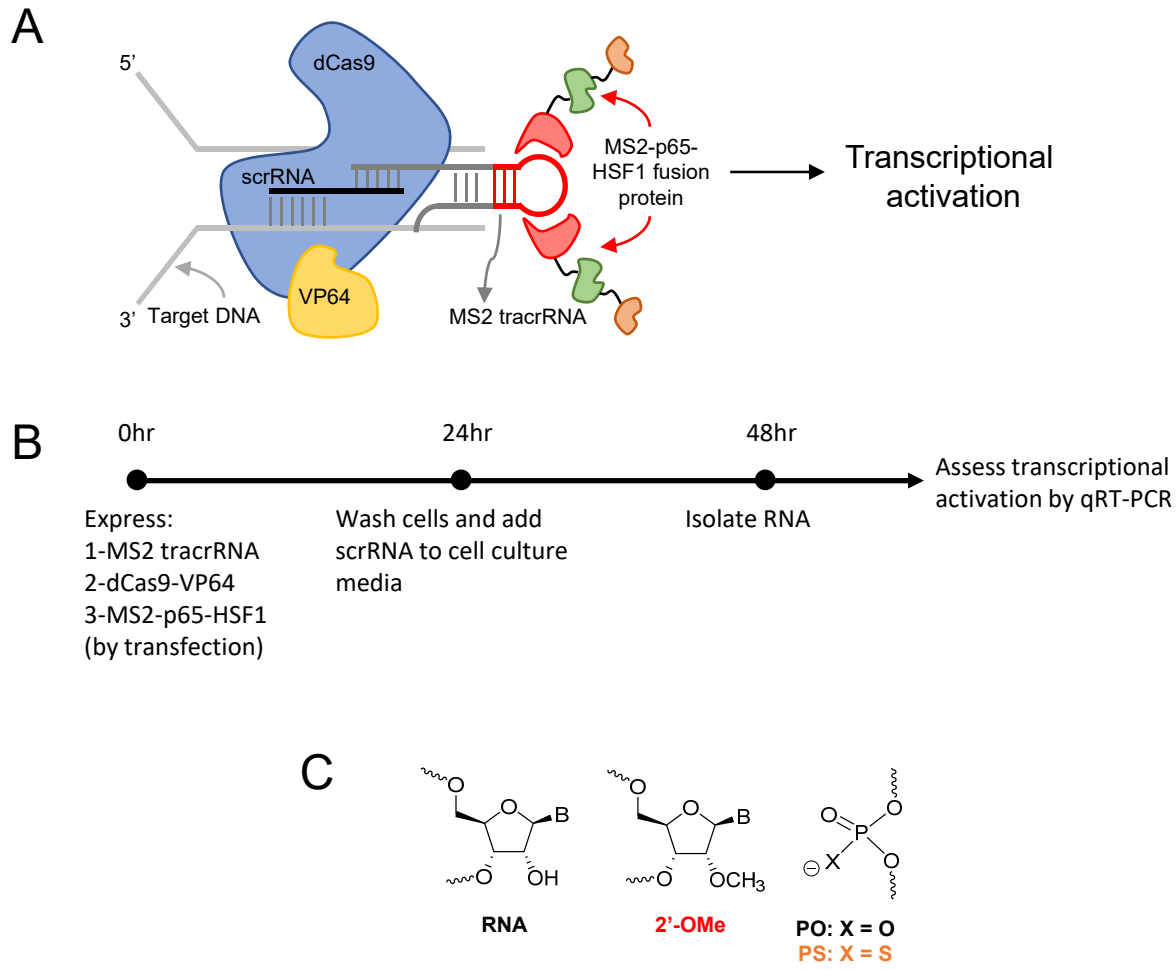


Supplementary Figure S6. Gene disruption activity of scrRNAs targeting DNMT1 and LDLR. (A) Surveyor nuclease assay gel with expected bands indicated by an asterisk (*) for DNMT1 scrRNAs (indicated at the top of the gel) and sequence/chemical modifications and activity in Figure 4A. (B) Same as in (A) except scrRNAs targeting LDLR with sequence/chemical modifications and activity of LDLR scrRNAs indicated in Supplementary Figure S5.

Supplementary Figure S7



scrRNA: 5'-GsAsCsAsAsGsGsUsUsCsAsUsAsUsUsUsGsUsAsUsGsUsUsUsUsAsGsAsGsCsUsAsUsGsCsUsGsUsUsUsUsG-3'

Supplementary Figure S7. A dual RNA system for gene induction using chemically modified synthetic CRISPR RNA. (A) Schematic illustration of DNA target recognition by CRISPR-Cas transcriptional activation complex. The fusion protein of nuclease dead Cas9 (dCas9, blue) and VP64 (yellow) assembles on target DNA (light gray line), specified by synthetic CRISPR RNA (scrRNA, black line). The MS2-tracrRNA (dark gray, red) complexes with scrRNA and recruits the MS2-p65-HSF1 fusion protein (red, green, orange) to induce transcriptional activation. (B) Schematic outlines experimental design for assessing TTR specific transcriptional activation. (C) Structure and sequence of modified nucleotides incorporated into TTR specific scrRNA where red indicates 2'-OMe and orange is phosphorothioate substitution.

Supplementary Figure S8

gBlock gene fragment sequences

Name	Sequence
FL-43-01	AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTG TAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCA TATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG**taatttctactcttgtagat**CTGATGGTCCAT GTCTGTTACTC
Trunc-42-01	AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTG TAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCA TATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG**aatttctactcttgtagat**CTGATGGTCCAT GTCTGTTACTC
Trunc-41-01	AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTG TAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCA TATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG**atttctactcttgtagat**CTGATGGTCCATG TCTGTTACTC
Trunc-40-01	AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTG TAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCA TATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG**tttctactcttgtagat**CTGATGGTCCATGT CTGTTACTC
Trunc-38-01	AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTG TAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCA TATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG**taatttctactcttgtagat**CTGATGGTCCAT GTCTGT
Trunc-37-01	AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTG TAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCA TATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG**aatttctactcttgtagat**CTGATGGTCCAT GTCTGT
Trunc-36-01	AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTG TAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCA TATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG**atttctactcttgtagat**CTGATGGTCCATG TCTGT
Trunc-35-01	AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTG TAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCA TATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG**tttctactcttgtagat**CTGATGGTCCATGT CTGT
Trunc-39-01	AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACT GTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATC ATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG**ttctactcttgtagat**CTGATGGTCCATGT CTGTTACTC
Trunc-40-02	AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACT GTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATC ATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG**taatttctactcttgtagat**CTGATGGTCCA TGTCTGTTA
Trunc-34-01	AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACT GTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATC ATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG**ttctactcttgtagat**CTGATGGTCCATGT CTGT
Trunc-38-01	AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACT GTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATC ATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG**taatttctactcttgtagat**CTGATGGTCCA TGTCTGT
Trunc-36-02	AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACT GTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATC ATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG**ttctactcttgtagat**CTGATGGTCCATGT CTGTTA

Bold: Direct Repeat
Underlined: Primer binding sequence

Supplementary Figure S8. gBlock gene fragment sequences. Complete sequence of double stranded DNA fragments that were cloned into PCR4 blunt topo. Underlined sequences represent forward and reverse primer binding sites and bold sequence represents direct repeat.

Supplementary Figure S9

Target	Forward primer (5' to 3')	Reverse primer (5' to 3')	Ta	Expected Products
C5	catggggtaacccagcaaac	ggaaataagtgatggggcagg	67	729, 258, 471
EMX1	ccatccccttctgtgaatgt	ggagattggagacacggaga	65	639, 363, 276
GRIN2B	gcatactcgcatggctacct	ctccctgcagccccttttta	68	760, 555, 205
LDLR	ggagacccaaatacaacaaatc	ctagactccgtctcaaagaag	62	653, 403, 250
TTR	cagaatcagcaggtttgcag	caaacctaatgcaccaaagc	63	418, 246,172

Supplementary Figure S9. Primer sequences for additional target genes. Forward and reverse primer sequences used for PCR amplification of target gene following transfection with scrRNAs as in Supplementary Figure S5. Actual expected product sizes are indicated (top, right) and marked with an asterisk (*) in Supplementary Figure S5.