Programmable control of bacterial gene expression with the combined CRISPR and antisense RNA system

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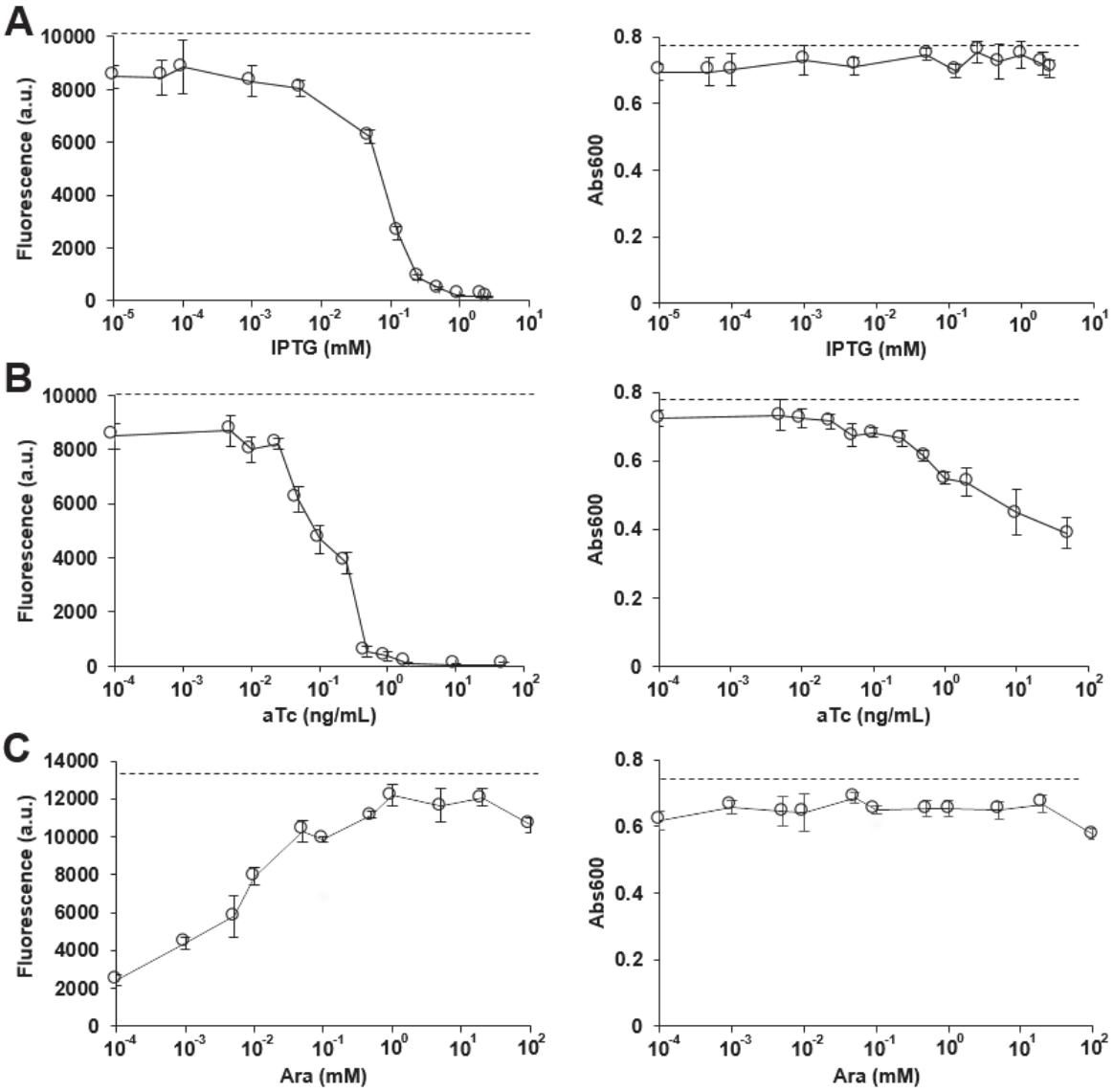
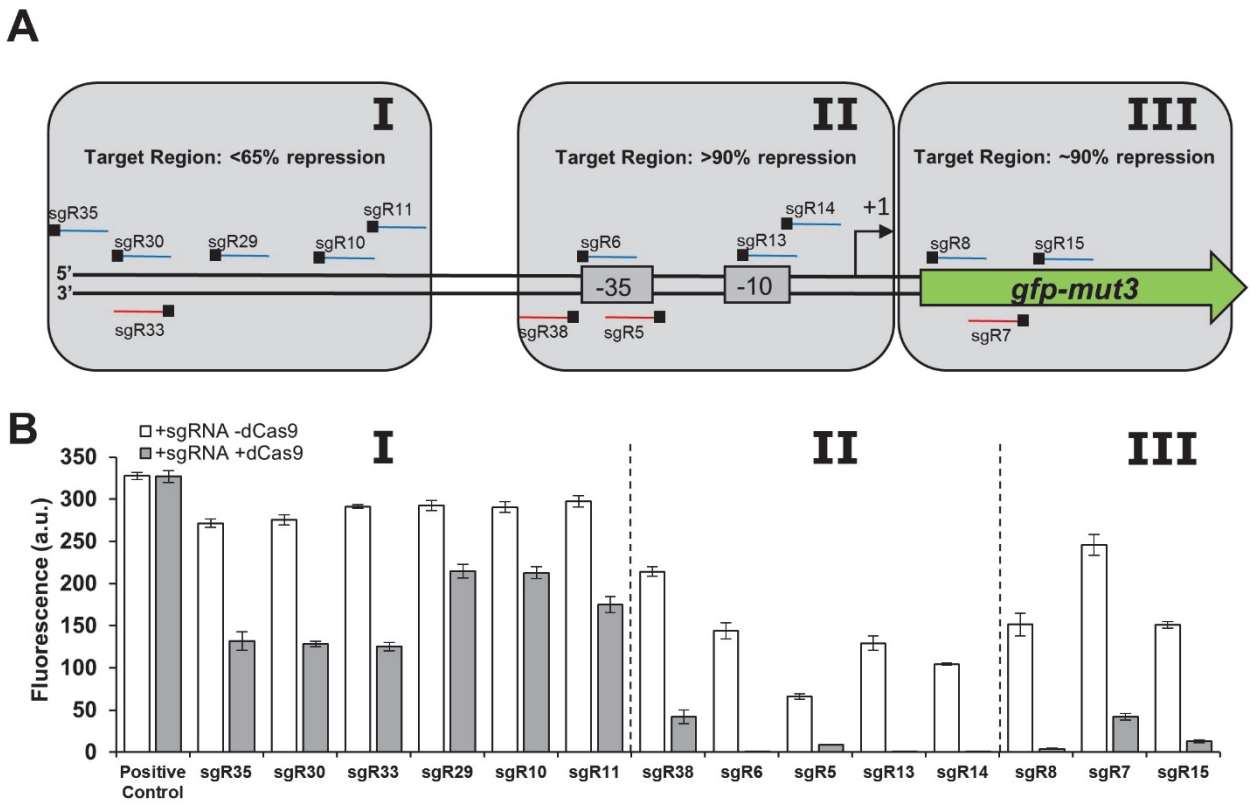
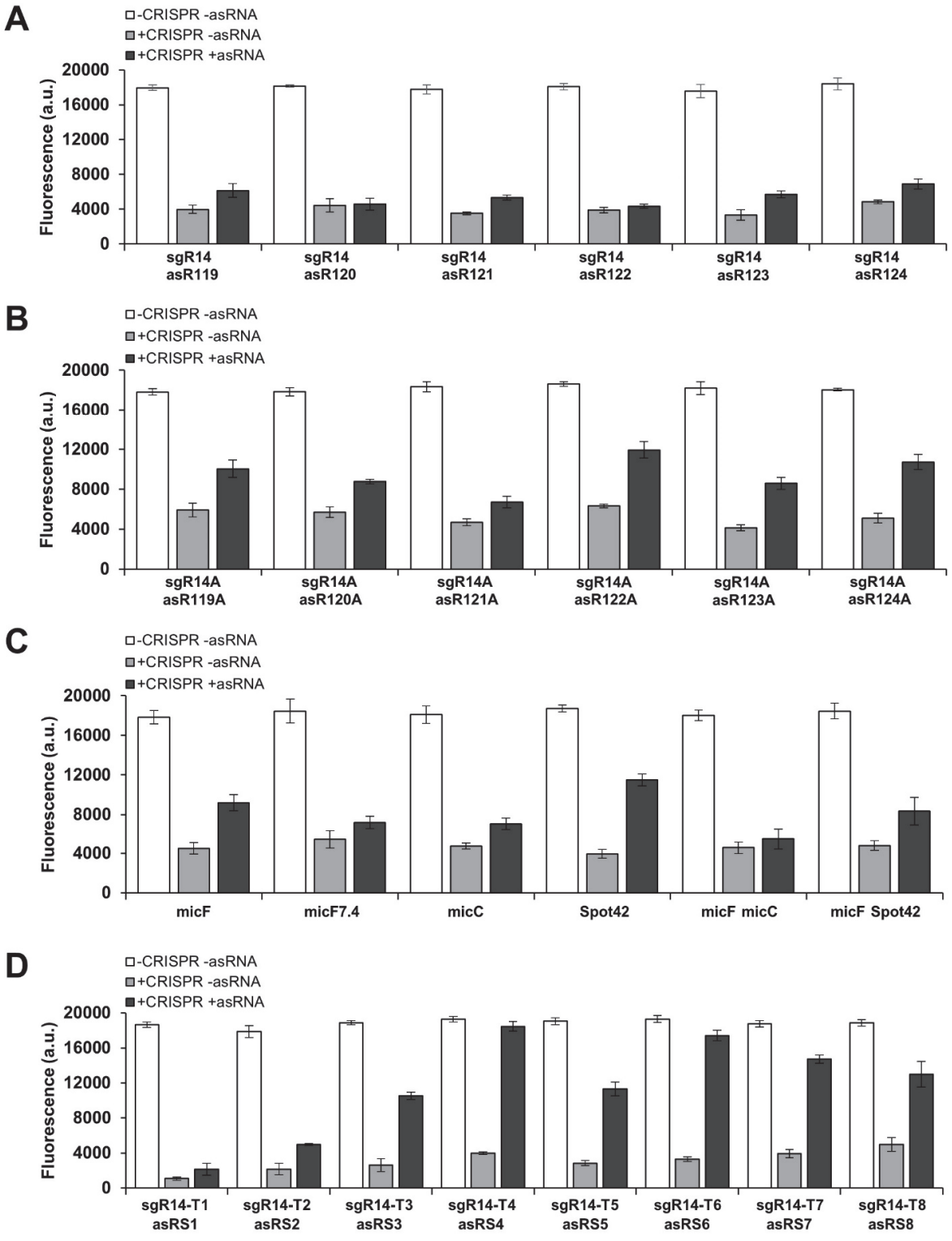


Supplementary Figure S1. The fluorescence and Abs_{600} data without normalization. The fluorescence and Abs_{600} (absorbance at 600 nm) data without normalization are shown with various concentrations of sgRNA (A), dCas9 (B), and asRNA (C). GFP is under the control of the constitutive Bba J23104 promoter. The fluorescence (a.u.) was measured using a microplate reader. See Figure 1 for the genetic circuit and normalized fluorescence data (Figures 1A and 1B for Figures S1A and S1B; Figures 1C and 1D for Figures S1C). The dashed line represents the fluorescence or Abs_{600} of cells grown without any inducer. The error bars represent the standard deviation of the measured values from three biological replicates performed on three different days.

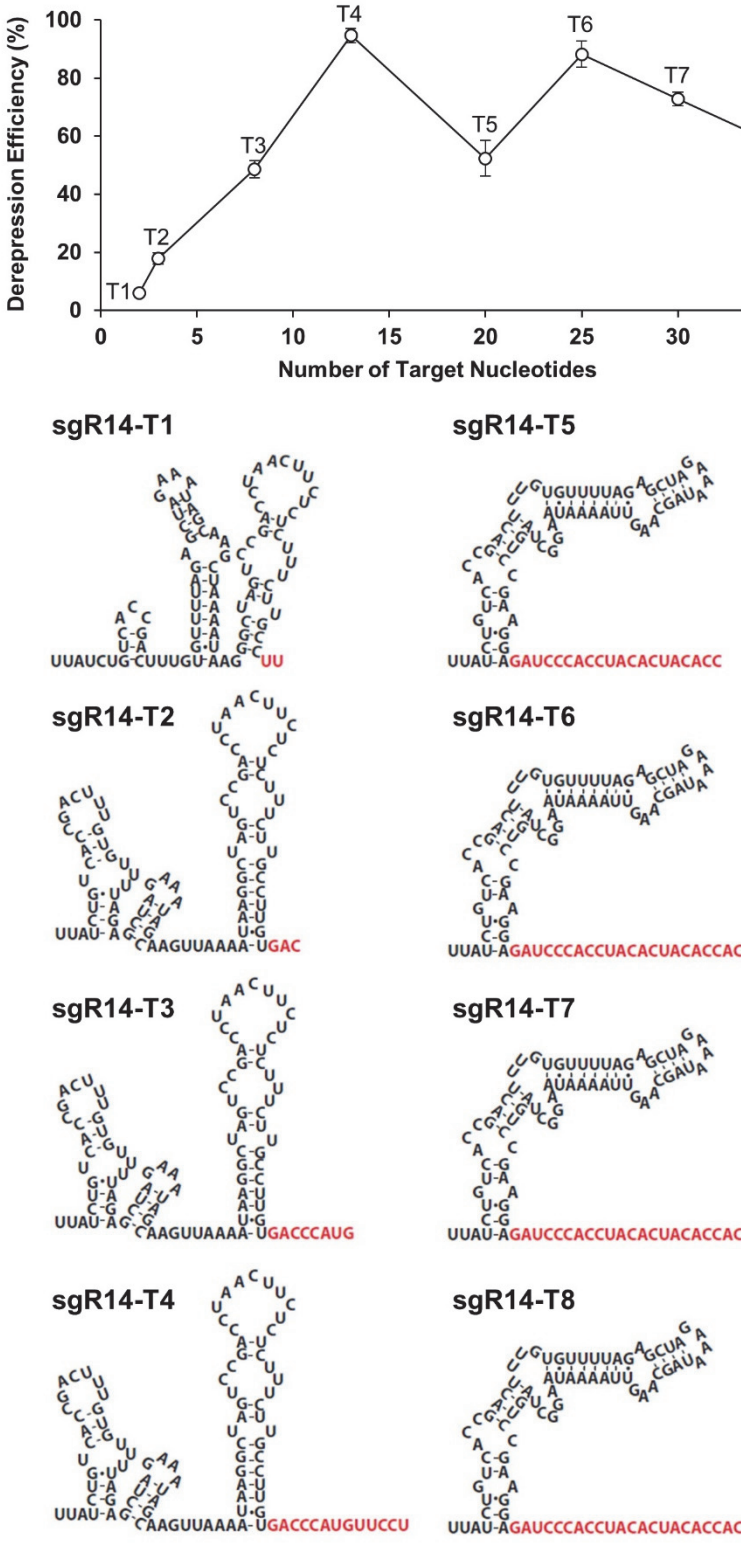


Supplementary Figure S2. Characterization of the CRISPR system. (A) The repression efficiency of the CRISPR system is dependent on the target region of the sgRNA. A variety of sgRNAs were designed to bind to different regions within and surrounding a constitutive promoter (Bba J23104) in order to determine the repression efficiency of each target region. Only the sgRNAs targeting a region between -35 and +1 show repression efficiency greater than 90%. Blue and red lines correspond to sgRNAs that bind to non-template and template DNA strands, respectively. Black squares are PAM sites. For sequence information, see Supplementary Table 2. (B) Samples were grown either in the absence (white bar) or presence (gray bar) of 10 ng/mL aTc to control expression of dCas9. sgRNAs were under the control of the constitutive Bba J23119 promoter. The repression efficiency was calculated by $[1 - (F_{CRISPR}/F_{positive\ control})] \times 100\%$ where $F_{positive\ control}$ is the fluorescence of cells with GFP only (no plasmid containing the CRISPR system). Fluorescence (a.u.) was measured by flow cytometry. The error bars represent the standard deviation of the fluorescence values from three biological replicates performed on three different days.



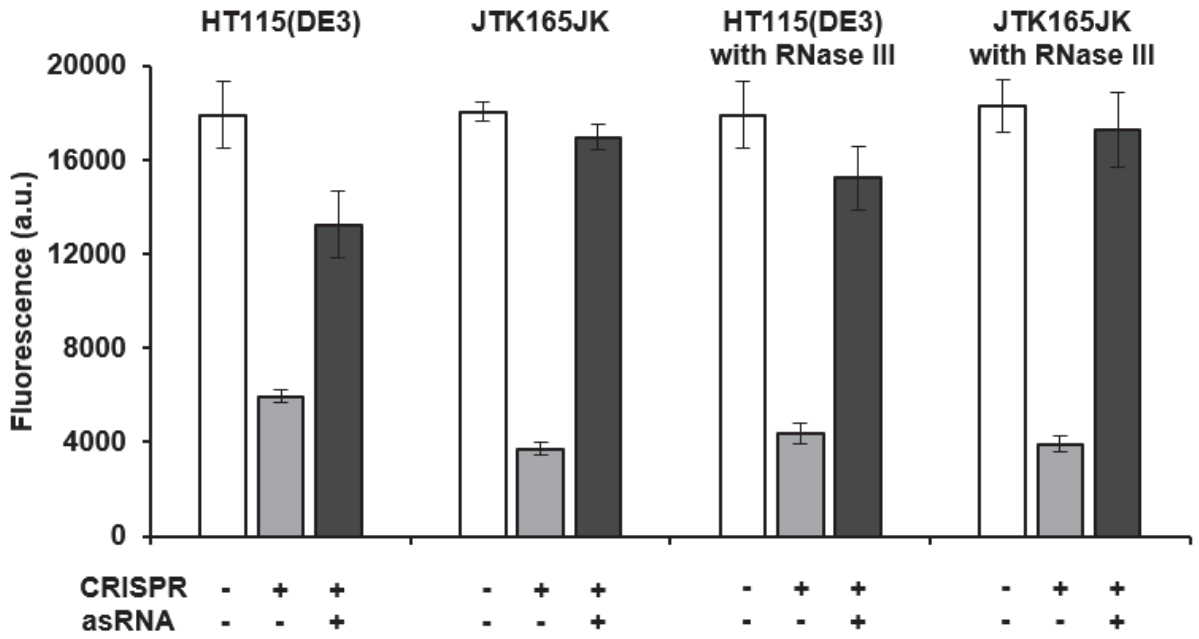
Supplementary Figure S3. Characterization of the combined CRISPR and antisense RNA system. Each category (A-D) corresponds to that of Figure 2, and the experimental conditions are the same as that of Figure 2. (A) sgR14 was selected out of the pool of sgRNAs shown in

Supplementary Figure S2B because it had the highest repression efficiency. asR119-124 are antisense to the DNA binding sequence of sgR14. This initial set of asRNA molecules showed very low derepression efficiency (maximum 15%). (B) Binding affinity between sgR14 and asR119-124 was improved by introducing 10 additional nucleotides (annotated as sgR14A and asR119A-124A). This led to an increased derepression efficiency (up to 43%). (C) Different Hfq binding sites were tested to increase derepression efficiency. Higher derepression efficiency (up to 55%) was achieved by replacing the micF Hfq binding site with the Spot42 Hfq binding site. sgR14A and the target binding region from asR124A were used for this test. micFmicC and micFSpot42 are two Hfq binding sites in tandem. (D) A final increase in derepression efficiency (up to 95%) was observed when the asRNA target region was shifted to T1-8, artificially introduced linkers. asRS1-8 are antisense to T1-8. GFP is under the control of the constitutive Bba J23104 promoter. The fluorescence (a.u.) was measured using a microplate reader. The error bars represent the standard deviation of the fluorescence values from three biological replicates performed on three different days.

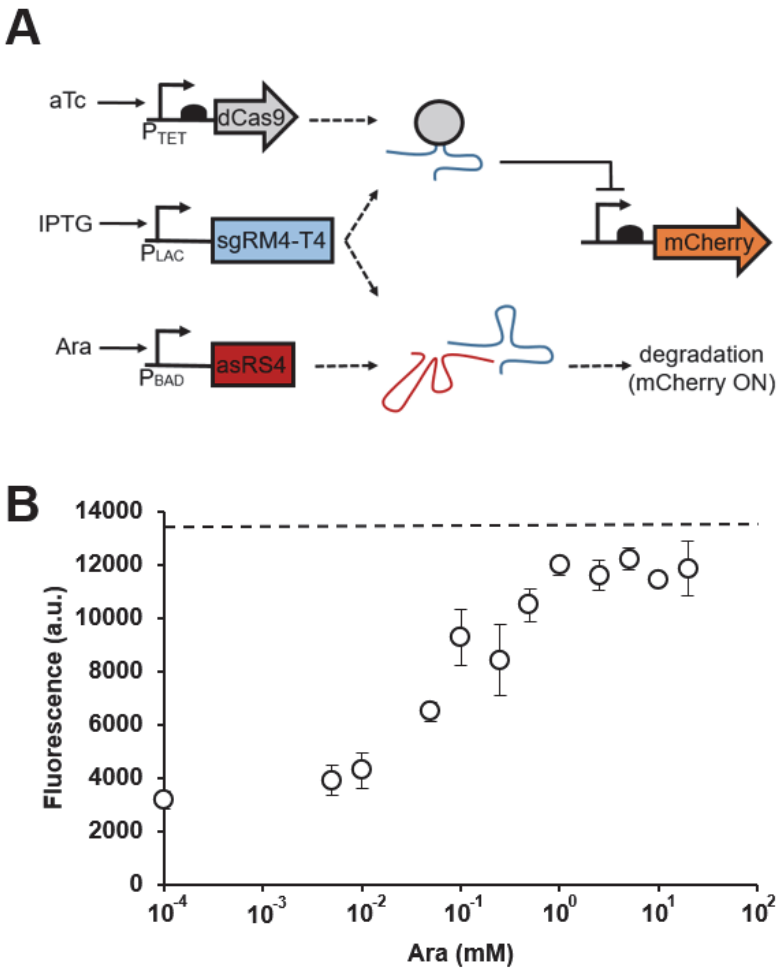


Supplementary Figure S4. A relationship between the number of target nucleotides and derepression efficiency. The length of artificial linkers (T1-T8) available for targeting with

corresponding asRNAs (asRS1-asRS8) affects the derepression efficiency. A linear increase in derepression efficiency was observed as the number of target nucleotides increased (up to T4, which contained 13 nucleotides). A saturation effect (with a change in the predicted secondary structure) was observed beyond 13 nucleotides. The sgRNA structures were predicted by NUPACK at 37℃ (1). The black region (left to right) represents the DNA binding site, dCas9 binding site, and a part of the artificial linker. The red region represents the available nucleotides of the linker for targeting with corresponding asRNAs. GFP is under the control of the constitutive Bba J23104 promoter. Cells were grown in the presence of 0.2 ng/mL aTc, 0.25 mM IPTG, and with or without 5 mM Ara (see Figure 1C for the schematic). The error bars represent the standard deviation of the values from three biological replicates performed on three different days.



Supplementary Figure S5. Fluorescence results obtained from HT115(DE3), JTK165JK, and two rescue strains. Repression and derepression of GFP by sgR14-T6 and asRS6, respectively, in HT115(DE3), JTK165JK, and two rescue strains are shown. Cells were grown in the presence of 0.2 ng/mL aTc and 0.2 mM IPTG (+ for CRISPR); and for asRNA, without (-) or with (+) 5 mM Ara. + and – indicate cells induced and uninduced, respectively. The positive control cells (white bar) were grown without any inducer. See Figure 5 for the normalized transcript abundance of *gfp*, sgRNA, and asRNA in HT115(DE3), JTK165JK, and two rescue strains. For better comparison, the original strains HT115(DE3) and JTK165JK were also transformed using the backbone plasmid that does not contain the RNase III gene (the left two graphs). The fluorescence (a.u.) was measured using a microplate reader. The error bars represent the standard deviation of the fluorescence values from three biological replicates (two technical replicates each; total six replicates) performed on three different days.



Supplementary Figure S6. Derepression of mCherry. (A) A genetic circuit built by combining the CRISPR system with asRNA. Transcribed asRS4 binds to its target sgRM4-T4 and prevents the sgRNA-dCas9 complex from repressing mCherry. The output is "on" when asRS4 is present, and "off" when asRS4 is absent. (B) Response of the combined CRISPR and antisense RNA system (asRS4) shown in (A). mCherry is under the control of the constitutive Bba J23100 promoter. All samples were grown in the presence of 0.2 ng/mL aTc and 0.25 mM IPTG, and with different Ara concentrations (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, and 20 mM). The fluorescence (a.u.) was measured using a microplate reader and reported by calculating $[(F_{experimental}/Abs_{experimental}) - (F_{negative\ control}/Abs_{negative\ control})]$ where the negative control is JTK165JK with no plasmid. The dashed line represents the fluorescence of cells grown without any inducer. The error bars represent the standard deviation of the fluorescence values from three biological replicates performed on three different days.

Supplementary Table 1. Plasmids used in this study.

Name	Parts
pRL01	ColE1 ori; amp-R; Bba J23119-sgR5
pRL02	ColE1 ori; amp-R; Bba J23119-sgR6
pRL03	ColE1 ori; amp-R; Bba J23119-sgR7
pRL04	ColE1 ori; amp-R; Bba J23119-sgR8
pRL05	ColE1 ori; amp-R; Bba J23119-sgR10
pRL06	ColE1 ori; amp-R; Bba J23119-sgR11
pRL07	ColE1 ori; amp-R; Bba J23119-sgR13
pRL08	ColE1 ori; amp-R; Bba J23119-sgR14
pRL09	ColE1 ori; amp-R; Bba J23119-sgR15
pRL10	ColE1 ori; amp-R; Bba J23119-sgR29
pRL11	ColE1 ori; amp-R; Bba J23119-sgR30
pRL12	ColE1 ori; amp-R; Bba J23119-sgR33
pRL13	ColE1 ori; amp-R; Bba J23119-sgR35
pRL14	ColE1 ori; amp-R; Bba J23119-sgR38
pRL15	ColE1 ori; amp-R; P_{LAC}-sgR14
pRL16	ColE1 ori; amp-R; P_{LAC}-sgR14; P_{BAD}-asR119; micF Hfq
pRL17	ColE1 ori; amp-R; P_{LAC}-sgR14; P_{BAD}-asR120; micF Hfq
pRL18	ColE1 ori; amp-R; P_{LAC}-sgR14; P_{BAD}-asR121; micF Hfq
pRL19	ColE1 ori; amp-R; P_{LAC}-sgR14; P_{BAD}-asR122; micF Hfq
pRL20	ColE1 ori; amp-R; P_{LAC}-sgR14; P_{BAD}-asR123; micF Hfq
pRL21	ColE1 ori; amp-R; P_{LAC}-sgR14; P_{BAD}-asR124; micF Hfq
pRL22	ColE1 ori; amp-R; P_{LAC}-sgR14A; P_{BAD}-asR119A; micF Hfq
pRL23	ColE1 ori; amp-R; P_{LAC}-sgR14A; P_{BAD}-asR120A; micF Hfq
pRL24	ColE1 ori; amp-R; P_{LAC}-sgR14A; P_{BAD}-asR121A; micF Hfq
pRL25	ColE1 ori; amp-R; P_{LAC}-sgR14A; P_{BAD}-asR122A; micF Hfq
pRL26	ColE1 ori; amp-R; P_{LAC}-sgR14A; P_{BAD}-asR123A; micF Hfq
pRL27	ColE1 ori; amp-R; P_{LAC}-sgR14A; P_{BAD}-asR124A; micF Hfq
pRL28	ColE1 ori; amp-R; P_{LAC}-sgR14A; P_{BAD}-asR124A; micF7.4 Hfq
pRL29	ColE1 ori; amp-R; P_{LAC}-sgR14A; P_{BAD}-asR124A; micC Hfq
pRL30	ColE1 ori; amp-R; P_{LAC}-sgR14A; P_{BAD}-asR124A; Spot42 Hfq

pRL31	ColE1 ori; amp-R; P_{LAC}-sgR14A; P_{BAD}-asR124A; micFmicC Hfq
pRL32	ColE1 ori; amp-R; P_{LAC}-sgR14A; P_{BAD}-asR124A; micFmicC Hfq
pRL33	ColE1 ori; amp-R; P_{LAC}-sgR14T1; P_{BAD}-asRS1; Spot42 Hfq
pRL34	ColE1 ori; amp-R; P_{LAC}-sgR14T2; P_{BAD}-asRS2; Spot42 Hfq
pRL35	ColE1 ori; amp-R; P_{LAC}-sgR14T3; P_{BAD}-asRS3; Spot42 Hfq
pRL36	ColE1 ori; amp-R; P_{LAC}-sgR14T4; P_{BAD}-asRS4; Spot42 Hfq
pRL37	ColE1 ori; amp-R; P_{LAC}-sgR14T5; P_{BAD}-asRS5; Spot42 Hfq
pRL38	ColE1 ori; amp-R; P_{LAC}-sgR14T6; P_{BAD}-asRS6; Spot42 Hfq
pRL39	ColE1 ori; amp-R; P_{LAC}-sgR14T7; P_{BAD}-asRS7; Spot42 Hfq
pRL40	ColE1 ori; amp-R; P_{LAC}-sgR14T8; P_{BAD}-asRS8; Spot42 Hfq
pRL41	ColE1 ori; amp-R; P_{LAC}-sgR14-T6; P_{LAC}-sgRM4-T4 -P_{BAD}-asRS6; PLUX-asRS4 Spot42 Hfq
pRL42	ColE1 ori; amp-R; P_{LAC}-sgR14-T6; P_{BAD}-asRS4; Spot42 Hfq
pRL43	ColE1 ori; amp-R; P_{LAC}-sgR14-T4; P_{BAD}-asRS6; Spot42 Hfq
pRL44	ColE1 ori; amp-R; P_{LAC}-sgRM4-T4; P_{BAD}-asRS4; Spot42 Hfq
pRL45	p15A ori; cm-R; P_{TET}-dCas9-Bba J23104-*gfpmut3*
pRL46	p15A ori; cm-R; P_{TET}-dCas9
pRL47	R6K ori; kan-R; Bba J23104-*gfpmut3*
pRL48	R6K ori; kan-R; Bba J23104-*gfpmut3*; Bba J23100-*mCherry*
pRL49	pSC101* ori; kan-R; P_{rnc}-*rnc-era-recO-pdxJ-acpS*
pRL50	pSC101* ori; kan-R

Supplementary Table 2. Genetic parts used in this study. ncRNA, noncoding RNA.

Part Name	Type	DNA sequence
sgR5	ncRNA	ttgacagctagctcagtcct
sgR6	ncRNA	ctgagctagctgtcaactcg
sgR7	ncRNA	gaattagatggtgatgttaa
sgR8	ncRNA	catctaattcaacaagaatt
sgR10	ncRNA	ggttaatgtcatgataataa
sgR11	ncRNA	cgtgatacgcctatttttat
sgR13	ncRNA	tgtaggctagcacaatacct

sgR14	ncRNA	ttatctgtcaccgactttgt
sgR15	ncRNA	agtagtgcaaataaatttaa
sgR29	ncRNA	gtttcttagacgtcgatatc
sgR30	ncRNA	cctgataaatgcttcaataa
sgR33	ncRNA	cattattgaagcatttatca
sgR35	ncRNA	agagcattacgctgacttga
sgR38	ncRNA	ataaaaataggcgtatcacg
sgR39	ncRNA	ggtaggatccgctaatctta
sgR14A	ncRNA	cttactcctgttatctgtcaccgactttgt
asR119	ncRNA	aaaacacaaagtcggtgacagataa
asR120	ncRNA	aaaacacaaagtcggtgacagataa
asR121	ncRNA	aaacacaagtcgtgacagataa
asR122	ncRNA	gctctaaaacacaaagtcggtgacagataa
asR123	ncRNA	gctctaaacacaagtcgtgacagataa
asR124	ncRNA	ttctagctctaaaacacaaagtcgtgacgataa
asR119A	ncRNA	aaaacacaaagtcggtgacagataacaggagtaag
asR120A	ncRNA	aaaacacaaagtcggtgacagataacaggagtaag
asR121A	ncRNA	aaacacaagtcgtgacagataacaggagtaag
asR122A	ncRNA	gctctaaaacacaaagtcggtgacagataacaggagtaag
asR123A	ncRNA	gctctaaacacaagtcgtgacagataacaggagtaag
asR124A	ncRNA	ttctagctctaaaacacaaagtcgtgacgataacaggagtaag
asR39	ncRNA	catatgtatatctccttcttaaagatcttttgaattcc
T1	artificial linker	acctaacttctctctttcttgcctt
T2	artificial linker	acctaacttctctctttcttgccttgtgac
T3	artificial linker	acctaacttctctctttcttgccttgtgacccatg
T4	artificial linker	acctaacttctctctttcttgccttgtgacccatgttcct
T5	artificial linker	aaggagatcccacctacactacacc
T6	artificial linker	aaggagatcccacctacactacaccacacc
T7	artificial linker	aaggagatcccacctacactacaccacacccatga
T8	artificial linker	aaggagatcccacctacactacaccacacccatgaccaca
asRS1	ncRNA	ggtattgtgctagcctaca

asRS2	ncRNA	aaggcaagaaagagagaagttaggt
asRS3	ncRNA	gtcacaaggcaagaaagagagaagttaggt
asRS4	ncRNA	catgggtcacaaggcaagaaagagagaagttaggt
asRS5	ncRNA	aggaacatgggtcacaaggcaagaaagagagaagttaggt
asRS6	ncRNA	ggtgtagtgtaggtgggatctcctt
asRS7	ncRNA	ggtgtggtgtagtgtaggtgggatctcctt
asRS8	ncRNA	tcatgggtgtggtgtagtgtaggtgggatctcctt
micF	Hfq binding site (2)	tcatttctgaatgtctgtttacccctatttcaaccggatgcctcgcattcggtttttttt
micF7.4	Hfq binding site (3)	cgtcccgcaaggatgcgggtctgtttacccctatttcaaccggccgcctcgcggccggttttttttt
micC	Hfq binding site (2)	tttctgttgggccattgcattgccactgattttccaacatataaaaagacaagcccgaacagtcgtccgggctttttttctcgag
Spot42	Hfq binding site (3)	atttggctgaatattttagccgccccagtcagtaatgactggggcgtttttta
micF micC	Hfq binding sites in tandem	tcatttctgaatgtctgtttacccctatttcaaccggatgcctcgcattcggtttttttttttctgttggg ccattgcattgccactgattttccaacatataaaaagacaagcccgaacagtcgtccgggct tttttctcgag
micF Spot42	Hfq binding sites in tandem	tcatttctgaatgtctgtttacccctatttcaaccggatgcctcgcattcggtttttttttatttggctg aatattttagccgccccagtcagtaatgactggggcgtttttta
dCas9	dCas9 binding site (4)	gttttagagctagaaatagcaagttaaaataaggctagtccg
Bba_J23104	Promoter (http://parts.igem.org/Part:BBa_J23104)	ttgacagctagctcagtcctaggtattgtgctagc
Bba_J23100	Promoter (http://parts.igem.org/Part:BBa_J23100)	ttgacggctagctcagtcctaggtacagtgctagc
Bba_J23105	Promoter (http://parts.igem.org/Part:BBa_J23105)	tttacggctagctcagtcctaggtactatgctagc
P_{LAC}	Promoter (5)	ataaatgtgagcggataacattgacattgtgagcggataacaagatactgagcac
P_{BAD}	Promoter (5)	agaaaccaattgtccatattgcatcagacattgccgtcactgcgtcttttactggctcttctcgct aaccaaaccggtaaccccgcttattaaaagcattctgtaacaaagcgggaccaaagccatga caaaaacgcgtaacaaaagtgtctataatcacggcagaaaagtccacattgattatttgcacg gcgtcacactttgctatgccatagcatttttatccataagattagcggatcctacctgacgctttt atcgcaactctctactgtttctccatac
P_{TET}	Promoter (4)	taattcctaatttttgttgacactctatcgttgatagagttattttaccactccctatcagtgatagag aaaa
P_{LUX}	Promoter (5)	acctgtaggatcgtacaggtttacgcaagaaaatggtttgttactttcgaataaa
P_{rnc}	Promoter (6)	gaagtttaaggttggcacctccagg
rnc	Gene (6)	atgaaccccatcgtaattaatcggcttcaacggaagctgggctacacttttaatcatcaggaac tgttgcagcaggcattaactcatcgtagtgccagcagtaaacataacgagcgtttagaatttta ggcgactctattctgagctacgttatcgccaatgcgctttatcaccgtttccctcgtgtggatga aggcgatatgagccggatgcgcgccacgctggtccgtggcaatacgctggcggaactggc gcgcgaatttgagttaggcgagtgcttacgtttagggccaggtgaacttaaaagcggtggatt

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pdxJ	Gene (6)	atggctgaattactgttaggcgtcaacattgaccatatcgctacgctgcgcaacgcgcgcggt accgcttacccggatccggtgcaggccgcgtttattgccgagcaggcgggagcggacggc attaccgtgcatttacgtgaagatcgccgtcacattactgaccgcgacgtgcgcatcctgcgt cagacgctggatacccgcatgaatctggagatggcggtgaccgaagagatgctggcgatc gccgttgagacgaagccacatttttgctgcctggtaccggaaaagcgtcaggaagtaacaac cgaaggcggcctggatgtcgcagggcagcgtgacaaaatgcgcgatgcctgcaaacgtct ggcagatgccgggattcaggtttctctgtttattgacgccgatgaagagcagatcaaagctgc ggcagaggttggcgcaccgtttatcgagatccacaccggttgctatgctgatgccaaaactg acgccgaacaggcgcaagagctggcgcgtatcgccaaagccgcgacctttgccgcaagc ctcggtctgaaagttaacgccggacacggtctgacctatcacaacgtgaaagccattgccgc catccctgagatgcatgaactgaatatcggtcatgccattattggtcgtgcagtgatgaccgga ctgaaagatgcggtggcagaaatgaagcgtctgatgctggaagcgcgtggctaa
acpS	Gene (6)	atggcaatattaggtttaggcacggatattgtggagatcgctcgcatcgaagcggtgatcgcc cgatccggtgatcgcctggcacgccgcgtattaagcgataacgaatgggctatctggaaaac gcaccaccagccggtgcgttttctggcgaagcgttttgctgtgaaagaagccgcagcaaaa gcgtttggcaccgggatccgcaatggtctggcgtttaatcaatttgaagtattcaatgatgagct cggcaaaccacggctacggctatggggcgaggcattaaaactggcggaaaagctgggcgt tgcaaatatgcatgtaacgctggcagatgagcggcactatgcttgtgccacggtaattattgaa agttaa
gfp-mut3	Gene (7)	atgagtaaaggagaagaacttttcactggagttgtcccaattcttgttgaattagatggtgatgtt aatgggcacaaattttctgtcagtggagagggtgaaggtgatgcaacatacggaaaacttac ccttaaatttatttgcactactggaaaactacctgttccatggccaacacttgtcactactttgact

		tatggtgttcaatgcttttcaagatacccagatcatatgaaacggcatgactttttcaagagtgcc atgcccgaaggttatgtacaggaaagaactatatttttcaaagatgacgggaactataagaca cgtgctgaagtcaagtttgaaggtgatacacttgttaatagaatcgagttaaaaggtattgatttt aaagaagatggaaacattcttggacacaagttggaatacaactataactcacacaatgtatac atcatggcagacaaacaaaagaatggaatcaaagttaacttcaaaattagacacaacattgaa gatggaagcgttcaactagcagaccattatcaacaaaatactccaattggcgatggccctgtc cttttaccagacaaccattacctgtccacacaatctgccctttcgaaagatcccaacgaaaaga gagaccacatggtccttcttgagtttgtaacagctgctgggattacacatggcatggatgaact atacaaaaggcctgcagcaaacgacgaaaactacgcttaagtagcttaa
mCherry	Gene (8)	atggtgagcaagggcgaggaggataacatggccatcatcaaggagttcatgcgcttcaagg tgcacatggagggctccgtgaacggccacgagttcgagatcgagggcgagggcgagggc cgcccctacgagggcacccagaccgccaagctgaaggtgaccaagggtggcccccctgcc cttcgcctgggacatcctgtcccctcagttcatgtacggctccaaggcctacgtgaagcaccc cgccgacatccccgactacttgaagctgtccttccccgagggcttcaagtgggagcgcgtga tgaacttcgaggacggcggcgtggtgaccgtgacccaggactcctccctgcaggacggcg agttcatctacaaggtgaagctgcgcggcaccaacttccctccgacggccccgtaatgcag aagaagaccatgggctgggaggcctcctccgagcggatgtaccccgaggacggcgccct gaagggcgagatcaagcagaggctgaagctgaaggacggcggccactacgacgctgag gtcaagaccacctacaaggccaagaagcccgtgcagctgcccggcgcctacaacgtcaac atcaagttggacatcacctcccacaacgaggactacaccatcgtggaacagtacgaacgcg ccgagggccgccactccaccggcggcatggacgagctgtacaagtaa

Supplementary Table 3. RT-qPCR primers.

Primer	Sequence	Source
cysG-F	ttgtcggcggtggtgatgtc	(9)
cysG-R	atgcggtgaactgtggaataaacg	(9)
hcaT-F	gctgctcggctttctcatcc	(9)
hcaT-R	ccaaccacgctgaccaacc	(9)
idnT-F	ctgtttagcgaagaggagatgc	(9)
idnT-R	acaaacggcggcgatagc	(9)
gfp-F	ctgtccacacaatctgccct	(10)
gfp-R	gtttgctgcaggccttttgt	(10)
sgRNA-F	ttatctgtcaccgactttgtgttttagagc	this study
sgRNA-R	cggactagccttattttaacttgc	this study
asRNA-F	tccttatttggctgaatattttagcc	this study
asRNA-R	taaaaaacgccccagtcattactgac	this study

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