

SUPPLEMENTARY INFORMATION

Modular and Signal-Responsive Transcriptional Regulation Using CRISPRi-Aided Genetic Switches in *Escherichia coli*

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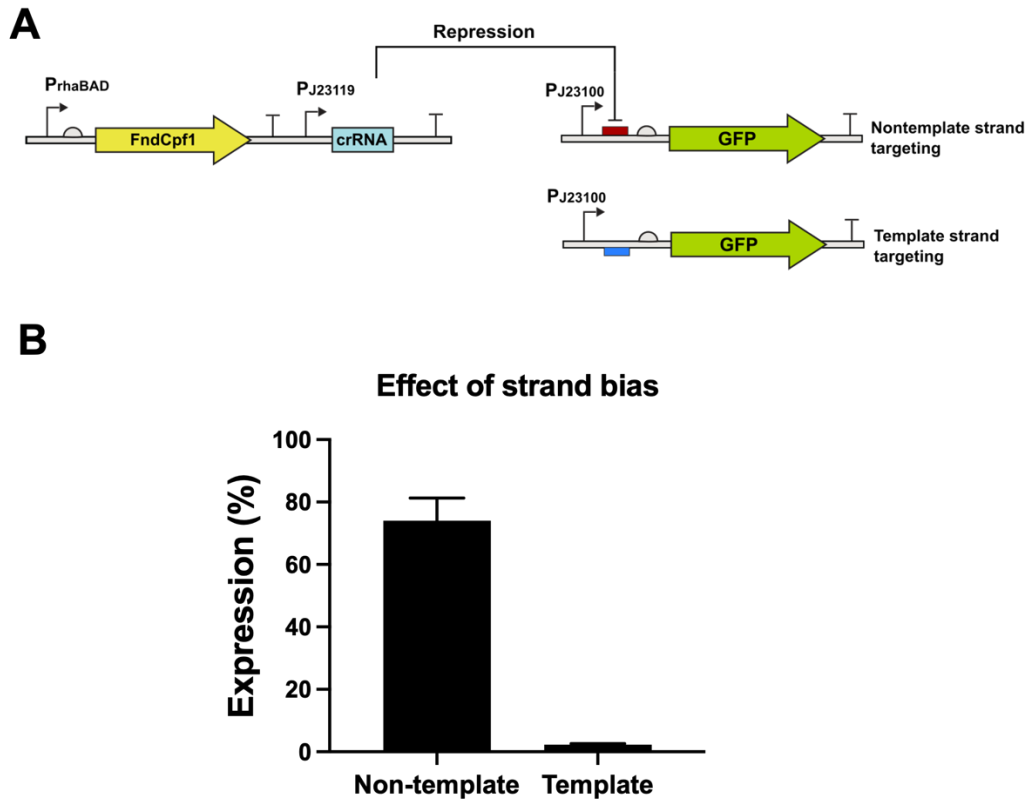


Figure S1. Effect of strand bias on CRISPRi activity of FndCas12a targeting the 5' UTR region. (A) Schematic representation of the FndCas12a expression cassette, crRNA(T1) expression cassette, and *gfp* reporter cassette with binding sites targeting either the non-template or template strands in the 5' UTR region. (B) Fluorescence reporter assay to evaluate strand bias. *E. coli* DH5 α strains harboring CRISPRi and reporter plasmids were cultivated for 8 hours with 1 mM L-rhamnose to induce FndCas12a expression. GFP fluorescence was measured using a fluorescence microplate reader, and "Expression (%)" was calculated relative to *E. coli* DH5 α strains harboring the pSEVA221 control plasmid and corresponding reporter plasmids. Data are presented as the mean $\pm$ standard deviation (SD) from three biological replicates.

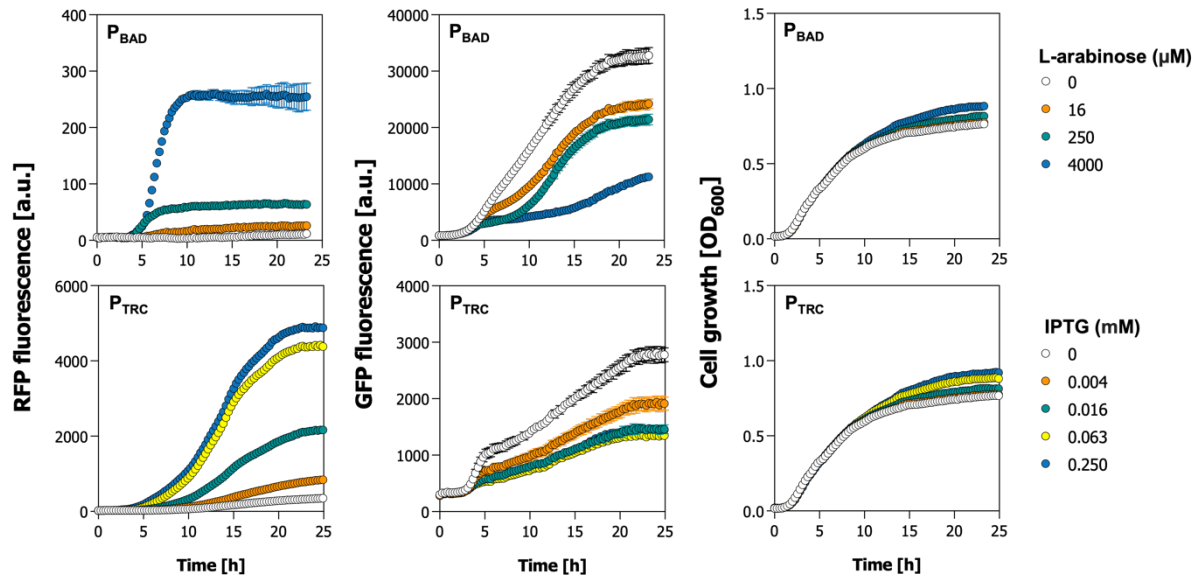


Figure S2. Effect of crRNA levels on the performance of CRISPRi-aided genetic switches targeting an episomal *gfp* gene. *E. coli* DH5α strains harboring each genetic circuit (depicted in Figure 1) were cultivated with 1 mM L-rhamnose for FndCas12a expression and varying concentrations of L-arabinose or IPTG to adjust *rfp*-crRNA(T1) cassette expression levels. The upper panels represent the P_{BAD} -driven genetic switch (L-arabinose-inducible), while the bottom panels represent the P_{TRC} -driven genetic switch (IPTG-inducible). RFP fluorescence, GFP fluorescence, and cell growth (OD_{600}) were monitored using an Infinite 200 PRO microplate reader. Data are presented as the mean $\pm$ standard deviation (SD) from three biological replicates.

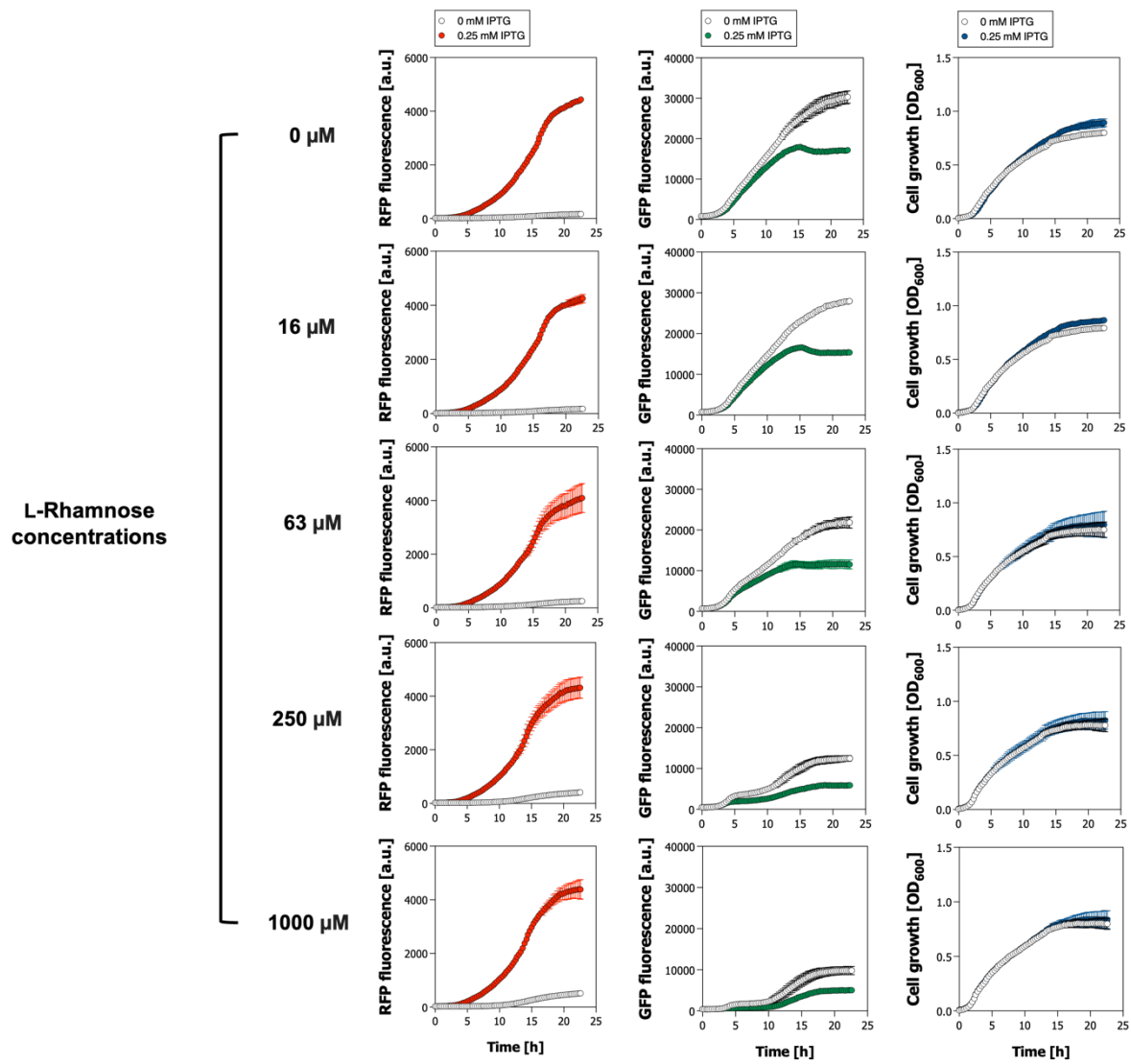


Figure S3. Effect of FndCas12a levels on the performance of IPTG-inducible CRISPRi-aided genetic switches targeting episomal plasmids. *E. coli* DH5 α strains harboring each genetic circuit (depicted in Figure 1B) were cultivated with 0.25 mM IPTG and varying concentrations of L-rhamnose to adjust FndCas12a expression levels. RFP fluorescence (red circles), GFP fluorescence (green circles), and OD₆₀₀ (blue circles) were monitored using a multimode microplate reader. Control values (cultures without IPTG) are represented as open circles. Data are presented as the mean $\pm$ standard deviation (SD) from two biological replicates.

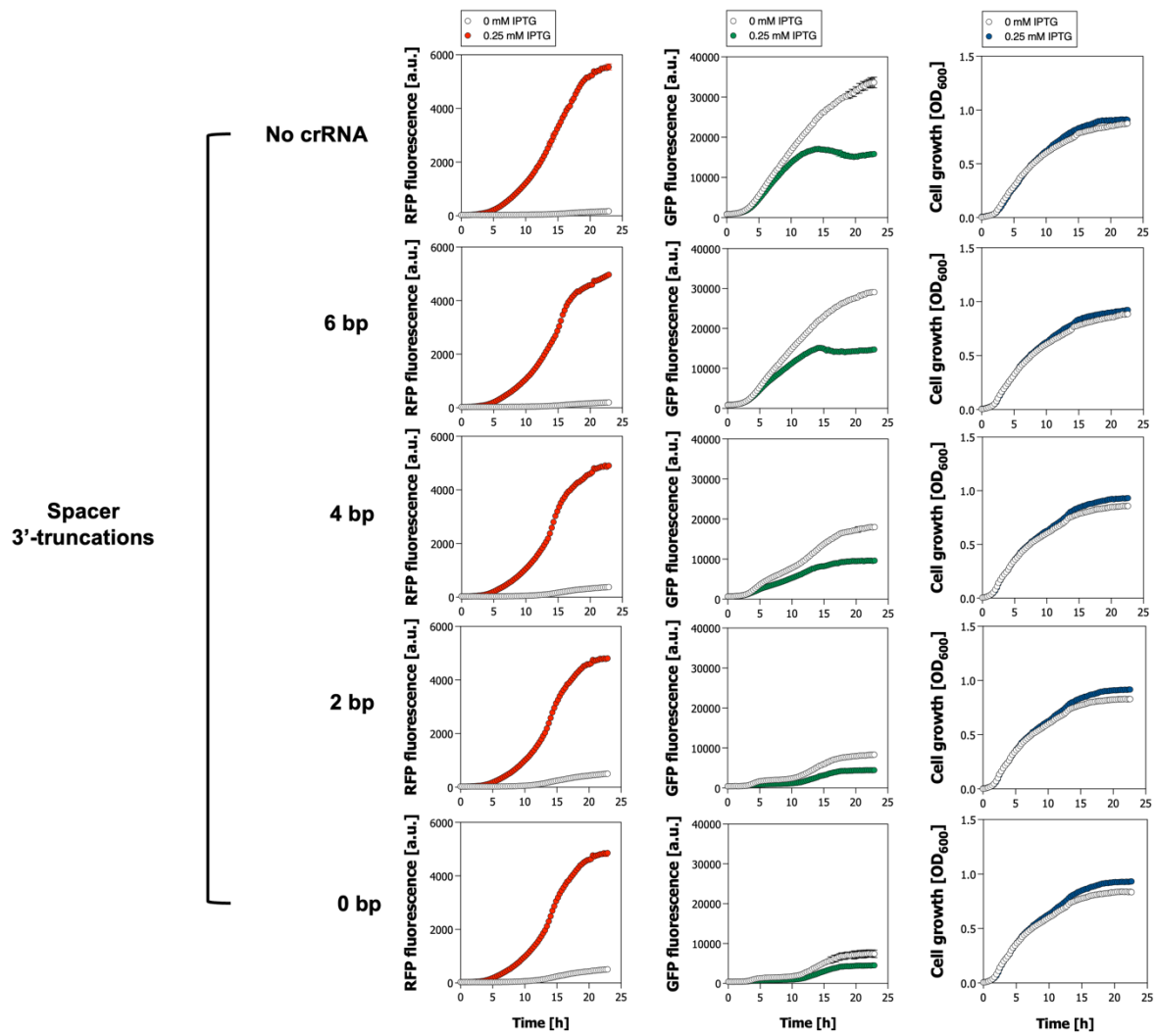


Figure S4. Effect of spacer truncations on the performance of IPTG-inducible CRISPRi-aided genetic switches targeting episomal plasmids. *E. coli* DH5 α strains harboring each genetic circuit (as depicted in Figure 1B, except for spacer length differences) were cultivated with 0.25 mM IPTG and 1 mM L-rhamnose to induce FndCas12a expression. RFP fluorescence (red circles), GFP fluorescence (green circles), and OD₆₀₀ (blue circles) were monitored using a multimode microplate reader. Control values (cultures without IPTG) are represented as open circles. Spacer sequences are as follows: 0 bp (5'-caccagaatcggcacaacg-3'), 2 bp (5'-caccagaatcggcacaac-3'), 4 bp (5'-caccagaatcggcaca-3'), 6 bp (5'-caccagaatcggca-3'), and No crRNA ($P_{TRC-rfp}$). Data are presented as the mean $\pm$ standard deviation (SD) from two biological replicates.

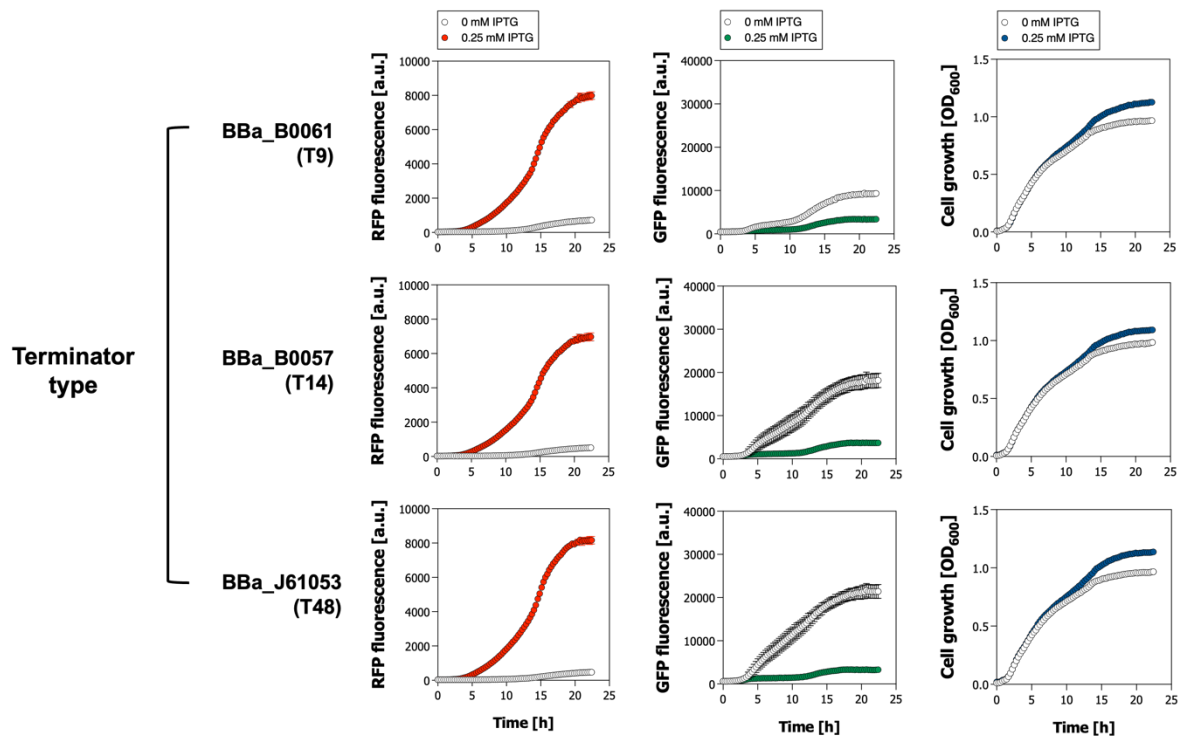


Figure S5. Effect of various terminator filters on the performance of IPTG-inducible CRISPRi-aided genetic switches. *E. coli* DH5 α strains harboring each genetic circuit (as depicted in Figure 2C, equipped with various terminator filters) were cultivated with 0.25 mM IPTG and 1 mM L-rhamnose to induce FndCas12a expression. RFP fluorescence (red circles), GFP fluorescence (green circles), and OD₆₀₀ (blue circles) were monitored using a multimode microplate reader. Control values (cultures without IPTG) are represented as open circles. Data are presented as the mean $\pm$ standard deviation (SD) from three biological replicates.

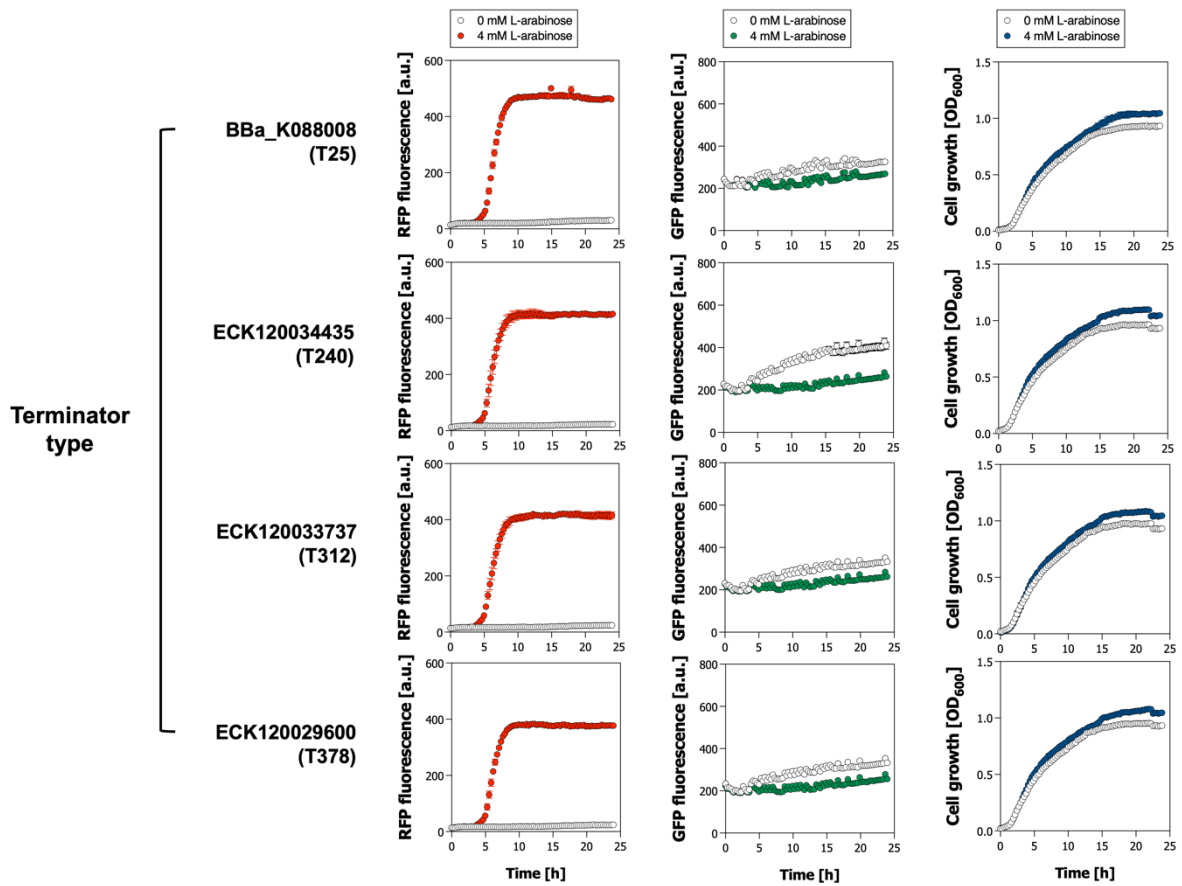


Figure S6. Effect of various terminator filters on L-arabinose-inducible CRISPRi-aided genetic switches targeting a chromosomal reporter gene. *E. coli* DH5α strains harboring genetic circuits (P_{BAD} -*rfp*-T25-crRNA(T1), P_{BAD} -*rfp*-T240-crRNA(T1), P_{BAD} -*rfp*-T312-crRNA(T1), and P_{BAD} -*rfp*-T378-crRNA(T1) in combination with P_{rhaBAD} -FndCas12a were tested. Input signal was provided with 4 mM L-arabinose, and FndCas12a expression was induced with 1 mM L-rhamnose. RFP fluorescence (red circles), GFP fluorescence (green circles), and OD₆₀₀ (blue circles) were monitored using a multimode microplate reader. Control values (cultures without L-arabinose) are represented as open circles. Data are presented as the mean $\pm$ standard deviation (SD) from three biological replicates.

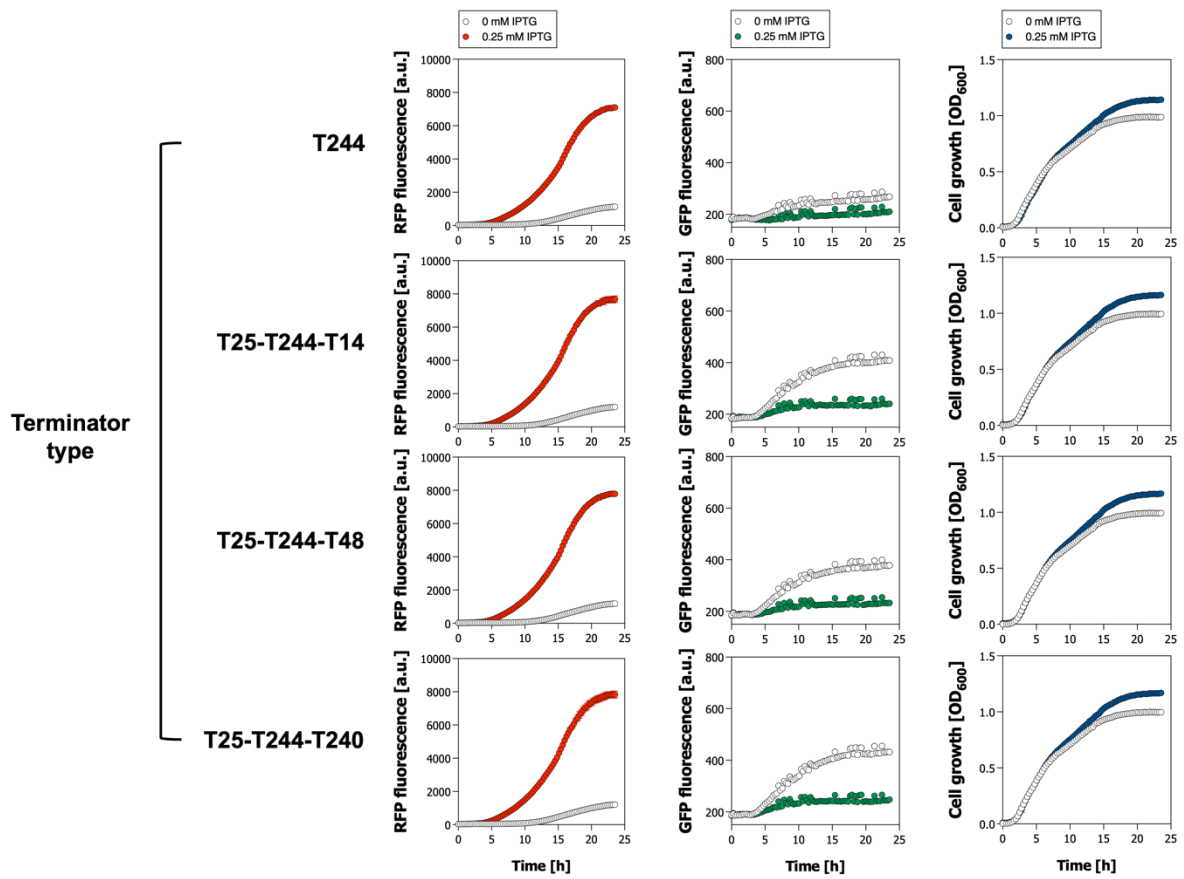


Figure S7. Effect of various terminator filters on IPTG-inducible CRISPRi-aided genetic switches targeting a chromosomal reporter gene. *E. coli* DH5 α strains harboring genetic circuits ($P_{\text{TRC}}\text{-}rfp\text{-T244-crRNA(T1)}$, $P_{\text{TRC}}\text{-}rfp\text{-T25-T244-T14-crRNA(T1)}$, $P_{\text{TRC}}\text{-}rfp\text{-T25-T244-T48-crRNA(T1)}$, and $P_{\text{TRC}}\text{-}rfp\text{-T25-T244-T240-crRNA(T1)}$ in combination with $P_{\text{rhaBAD}}\text{-FndCas12a}$ were tested. Input signal was provided with 0.25 mM IPTG, and FndCas12a expression was induced with 1 mM L-rhamnose. RFP fluorescence (red circles), GFP fluorescence (green circles), and OD₆₀₀ (blue circles) were monitored using a multimode microplate reader. Control values (cultures without IPTG) are represented as open circles. Data are presented as the mean $\pm$ standard deviation (SD) from three biological replicates.

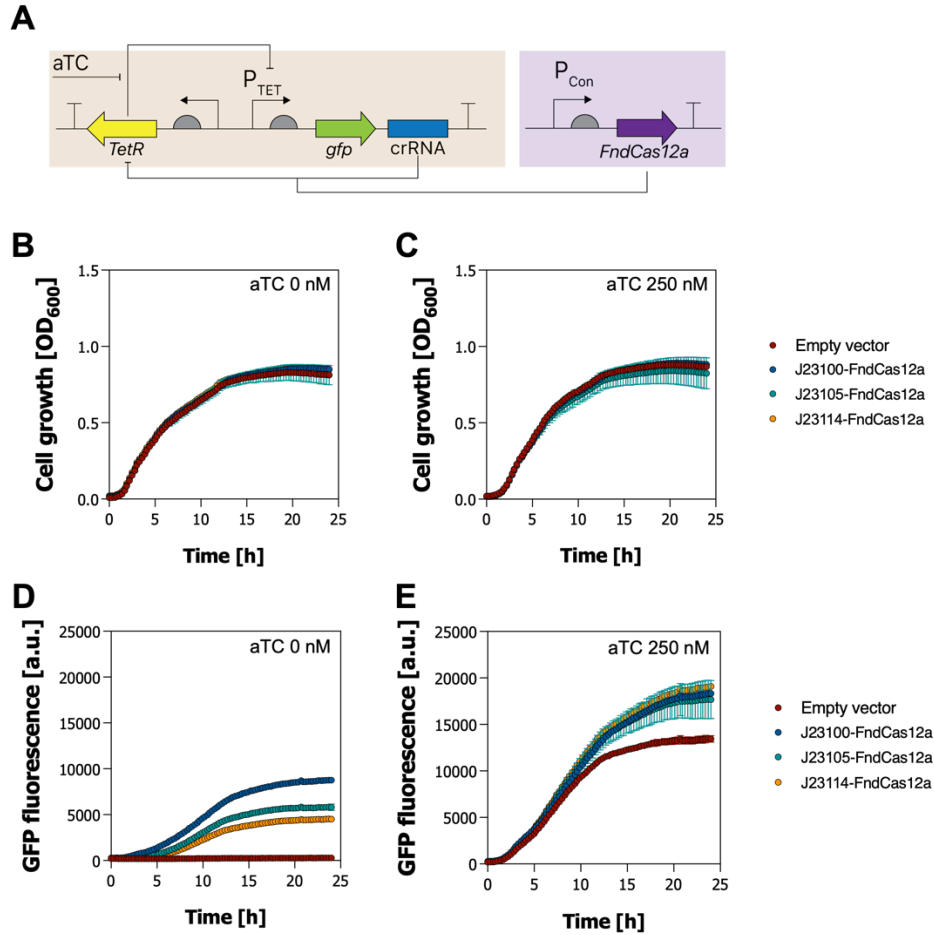


Figure S8. Impact of constitutive FndCas12a expression on cell growth and GFP fluorescence in aTC-inducible CRISPRi-aided genetic switches targeting the TetR repressor. (A) Schematic representation of the aTC-inducible P_{TET} -gfp-crRNA(tetR) circuit. Various constitutive promoters (P_{con}) in the CRISPRi plasmid were used to drive FndCas12a expression to evaluate its effect on cell growth (OD_{600nm}) and GFP fluorescence (a.u.). (B, C) Cell growth (OD_{600nm}) of the P_{TET} -gfp-crRNA(TetR) circuit co-transformed with either P_{con} -FndCas12a (J23100, J23105, or J23114) or the pSEVA221 control plasmid (Empty vector). (D, E) GFP fluorescence (a.u.) of the P_{TET} -gfp-crRNA(TetR) circuit co-transformed with either P_{con} -FndCas12a (J23100, J23105, or J23114) or the pSEVA221 control plasmid (Empty vector). Data are presented as the mean $\pm$ standard deviation (SD) from three biological replicates.

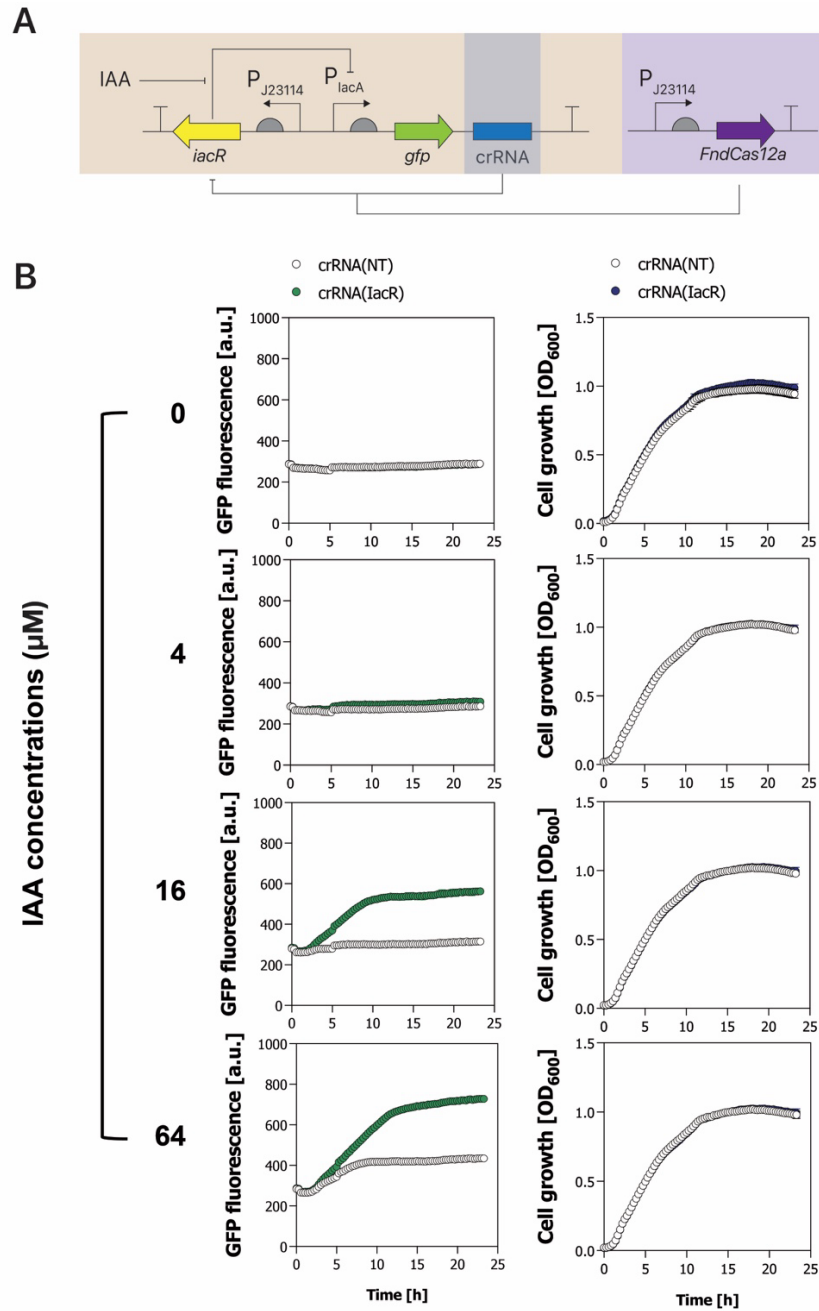


Figure S9. Genetic signal amplification using CRISPRi-aided genetic switches targeting the IAA biosensor system. (A) Schematic representation of an IAA-inducible genetic switch based on the *lacR* transcription factor from *Pseudomonas putida* 1290. A crRNA cassette targeting either a non-target sequence (crRNA(NT)) or the *iacR* gene (crRNA(lacR)) was tested. FndCas12a was constitutively expressed under the control of the P_{J23114} promoter. (B) GFP fluorescence (a.u.) and cell growth (OD_{600}) of the *E. coli* strains harboring the genetic circuits in response to various IAA concentrations (0, 4, 16, and 64 μM) over 24 hours. Open circles represent the crRNA(NT) control, and filled circles represent the crRNA(lacR) condition. GFP fluorescence and OD_{600} were measured using an Infinite 200 PRO microplate reader. Data are presented as the mean $\pm$ standard deviation (SD) from four biological replicates.

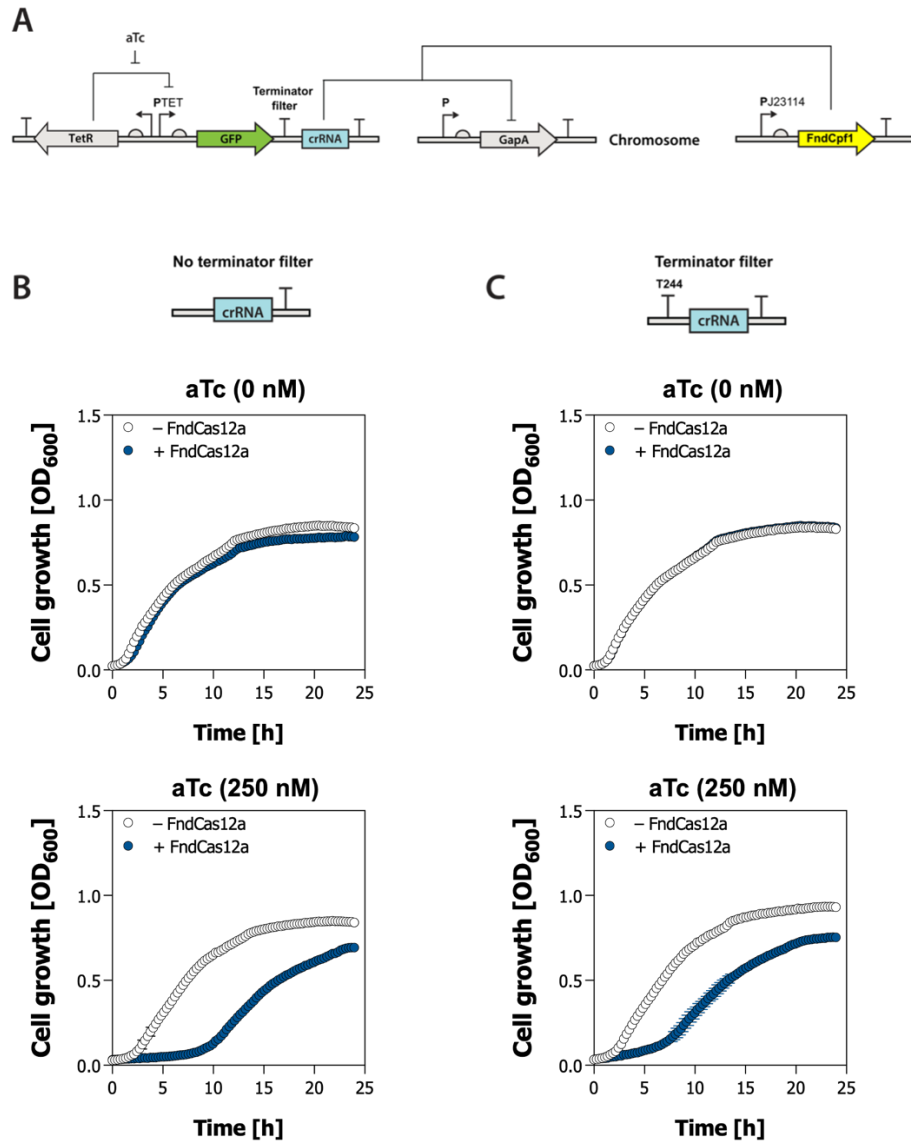


Figure S10. Cell growth defect using aTC-inducible CRISPRi-aided genetic switches targeting the chromosomal essential gene *gapA*. (A) Schematic representation of an aTC-inducible *gfp* gene linked to a crRNA cassette targeting the *gapA* gene (crRNA(GapA)) with a T244 terminator filter. FndCas12a was constitutively expressed from the P_{J23114} promoter. (B, C) Cell growth (OD_{600}) of *E. coli* DH5 α strains harboring P_{TET} -*gfp*-crRNA(GapA) (B) or P_{TET} -*gfp*-T244-crRNA(GapA) (C) co-transformed with either P_{J23114} -FndCas12a (blue circles) or the pSEVA221 control plasmid (open circles). Data are presented as the mean $\pm$ standard deviation (SD) from three biological replicates.

Table S1. Plasmids used in this study

Name	Description	Reference
pSEVA221	3.8 kb, RK2 ori, Km ^R	[1]
pREGFP3	P _{J23100} -gfp in pMW7, Ap ^R	[2]
pREGFP3(NT1)	pREGFP3 containing non-template strand binding site of crRNA(T1) in 5' -UTR of GFP	[3]
pREGFP3(T1)	pREGFP3 containing template strand binding site of crRNA(T1) in 5' -UTR of GFP	[3]
pSECRVi	L-Rhamnose inducible EedCas12a in pSEVA221	[3]
pG-crRNA	P _{J23119} -crRNA in pGEM-B1	[3]
pA-RFP	mCherry expressing plasmid in pACBB	[4]
pT-GFP	GFP expressing plasmid in pTrc99A	[4]
placA-sfGFP	indoleacetic acid inducible GFP in pSEVA131	[5]
pBAD/His	L-Arabinose inducible plasmid, p15A ori, Cm ^R	Thermo Fisher
pY002	Plasmid containing <i>Cas12a</i> gene from <i>Francisella novicida</i>	Addgene (#69975)
pdCas9-bacteria	aTC inducible SpdCas9, p15A ori, Cm ^R	Addgene (#44249)
pY002-FndCas12a	Plasmid containing <i>FndCas12a</i> gene	This study
pFnSECRVi	L-Rhamnose inducible FndCas12a in pSEVA221	This study
pFnSECRiV(T1)	L-Rhamnose inducible FndCas12a and J23119 promoter-crRNA(T1) expression cassette in pSEVA221	This study
pJ23114-FnSECRVi	Constitutive J23114 promoter-FndCas12a in pSEVA221	This study
pG-FncrRNA	P _{J23119} -crRNA from <i>Francisella novicida</i> in pGEM-B1	This study
pBAD-RFP	L-Arabinose inducible mCherry, p15A ori, Cm ^R	This study
pBAD-RFP-FncrRNA(T1)	L-Arabinose inducible mCherry and crRNA(T1) expression for FndCas12a	This study
pBAD-RFP-T25-FncrRNA(T1)	L-Arabinose inducible mCherry and BBa_K088008 terminator-crRNA(T1) expression for FndCas12a	This study
pBAD-RFP-T240-FncrRNA(T1)	L-Arabinose inducible mCherry and ECK120034435 terminator-crRNA(T1) expression for FndCas12a	This study
pBAD-RFP-T244-FncrRNA(T1)	L-Arabinose inducible mCherry and pheA-1 terminator-crRNA(T1) expression for FndCas12a	This study
pBAD-RFP-T312-FncrRNA(T1)	L-Arabinose inducible mCherry and ECK120033737 terminator-crRNA(T1) expression for FndCas12a	This study
pBAD-RFP-T378-FncrRNA(T1)	L-Arabinose inducible mCherry and ECK120029600 terminator-crRNA(T1) expression for FndCas12a	This study
pTRC-RFP	IPTG inducible mCherry, p15A ori, Cm ^R	This study
pTRC-RFP-FncrRNA(T1)	IPTG inducible mCherry and crRNA(T1) expression for FndCas12a	This study

pTRC-RFP-T9-FncrRNA(T1)	IPTG inducible mCherry and BBa_B0061 terminator-crRNA(T1) expression for FndCas12a	This study
pTRC-RFP-T14-FncrRNA(T1)	IPTG inducible mCherry and BBa_B0057 terminator-crRNA(T1) expression for FndCas12a	This study
pTRC-RFP-T25-FncrRNA(T1)	IPTG inducible mCherry and BBa_K088008 terminator-crRNA(T1) expression for FndCas12a	This study
pTRC-RFP-T48-FncrRNA(T1)	IPTG inducible mCherry and BBa_J61053 terminator-crRNA(T1) expression for FndCas12a	This study
pTRC-RFP-T244-FncrRNA(T1)	IPTG inducible mCherry and pheA-1 terminator-crRNA(T1) expression for FndCas12a	This study
pTRC-RFP-T25-T244-FncrRNA(T1)	IPTG inducible mCherry and BBa_K088008, pheA-1 dual terminators-crRNA(T1) expression for FndCas12a	This study
pTRC-RFP-T25-T244-T14-FncrRNA(T1)	IPTG inducible mCherry and BBa_K088008, pheA-1, BBa_B0057 tripple terminators-crRNA(T1) expression for FndCas12a	This study
pTRC-RFP-T25-T244-T48-FncrRNA(T1)	IPTG inducible mCherry and BBa_K088008, pheA-1, BBa_J61053 tripple terminators-crRNA(T1) expression for FndCas12a	This study
pTRC-RFP-T25-T244-T240-FncrRNA(T1)	IPTG inducible mCherry and BBa_K088008, pheA-1, ECK120034435 tripple terminators-crRNA(T1) expression for FndCas12a	This study
pTET-RFP	aTC inducible RFP, p15A ori, Cm ^R	This study
pTET-GFP	aTC inducible sfGFP, p15A ori, Cm ^R	This study
pTET-GFP-FncrRNA(TetR)	aTC inducible sfGFP and crRNA(TetR) expression for FndCas12a	This study
pTET-GFP-T25-FncrRNA(TetR)	aTC inducible sfGFP and BBa_K088008 terminator-crRNA(TetR) expression for FndCas12a	This study
pTET-GFP-T244-FncrRNA(TetR)	aTC inducible sfGFP and pheA-1 terminator-crRNA(TetR) expression for FndCas12a	This study
pTET-GFP-T240-FncrRNA(TetR)	aTC inducible sfGFP and ECK120034435 terminator-crRNA(TetR) expression for FndCas12a	This study
pTET-GFP-T25-T244-FncrRNA(TetR)	aTC inducible sfGFP and BBa_K088008, pheA-1 dual terminators-crRNA(TetR) expression for FndCas12a	This study
pTET-GFP-T25-T240-FncrRNA(TetR)	aTC inducible sfGFP and BBa_K088008, ECK120034435 dual terminators-crRNA(TetR) expression for FndCas12a	This study
pTET-GFP-T25-T244-T240-FncrRNA(TetR)	aTC inducible sfGFP and BBa_K088008, pheA-1, and ECK120034435 tripple terminators-crRNA(TetR) expression for FndCas12a	This study
placA-sfGFP-FncrRNA(lacR)	indoleacetic acid inducible GFP and crRNA(lacR) expression for FndCas12a	This study
pTET-GFP-FncrRNA(GapA)	aTC inducible sfGFP and crRNA(GapA) expression for FndCas12a	This study

pTET-GFP-T244-FncrRNA(GapA)	aTC inducible sfGFP and pheA-1 terminator-crRNA(GapA) expression for FndCas12a	This study
pTET-GapC-T244-FncrRNA(GapA)	aTC inducible GapC of <i>Chlostridium acetobutylicum</i> and pheA-1 terminator-crRNA(GapA) expression for FndCas12a	This study

Table S2. Oligonucleotides used in this study

Primers	Sequences of the oligonucleotides (5'-3')	Remarks
FndCpf1-VF	attaagtatagcgagaggtgaaagacatttagc	pY002-FndCas12a plasmid
FndCpf1-VR	caaaggttagttcttctgccaatctttttaa	
FndCpf1-IF	ttggcagaagaactaacctttgatattgacta	
FndCpf1-IR	ttcacctctcgctatacttaatatatgaacat	
pFnSECRVi-VF	gaataactaactcgagtaaggatctccagg	pFnSECRVi plasmid
pFnSECRVi-VR	aaattgacatatggtgatcctgctgaattt	
pFnSECRVi-IF	ggatcaccatatgtcaatttatcaagaatt	
pFnSECRVi-IR	cttactcgagttagttattcctattctgca	
FncrRNA-F	aatttctactgttagatcaccagaatcggcac	pG-FncrRNA plasmid
FncrRNA-R	actagtattatacctaggac	
pTRC-VF	agggttaaaccatggtgagcaagggcgagga	pTRC-RFP-FncrRNA plasmid
pTRC-VR	catgccgcttgggctgacttcaggtgctac	
pTRC-IF	aagtcagcccaagcggcatgcattacgtt	
pTRC-IR	tgctcaccatgggttaacctcctgtgtgaa	
crRNA-F	Tctagagtcgacctgcaggcatgcagtctaagaactttaataatttctactgtgtagatcaccagaatc	
crRNA-R	Cttctctcatccgcaaaacagccagctcttcagcgttgccgattctggtgatctacaacagtagaaatta	
pBAD-VF	ggaattaaccatggtgagcaagggcgagga	pBAD-RFP-FncrRNA plasmid
pBAD-VR	acattatgcagggctgacttcaggtgctac	
pBAD-IF	aagtcagccctgcataatgtgcctgtcaaa	
pBAD-IR	tgctcaccatgggttaattcctcctgttagc	
pTET-VF	ctacaaactctaactcgagtaaggatctcc	pTET-RFP plasmid
pTET-VR	tgctcaccatagatcctttctcctcttag	
pTET-IF	gaaaggatctatggtgagcaagggcgagga	
pTET-IR	actcgagttagagttttagaaaacgcaaaa	
GFP-VF	cgaaaaataaggtacctctagagtcgacct	pTET-GFP plasmid
GFP-VR	ctttgctcatagatcctttctcctcttag	
GFP-IF	gaaaggatctatgagcaaaggtgaagaact	
GFP-IR	tagaggtagcttatttttcgaactcgcat	

Table S3. Binding sites of crRNA used in this study

Name	Target gene	Binding site with PAM sequence (5' to 3')
crRNA(T1)	5' -UTR of GFP	TTC caccagaatcggcacaacgc
crRNA(TetR)	ORF of TetR	TTA acaacccgtaaactcgccca
crRNA(GapA)	ORF of GapA	TTC cgtgctgctcagaaacgttc
crRNA(lacR)	ORF of lacR	TTC cgtaaagaagacttcccggt

Table S4. Terminator filters used in this study

Name	Average strength[6]	Sequence (5' to 3')
BBa_B0061(T9)	9	aagtcaaaagcctccggtcggaggcttttgacttt
BBa_B0057(T14)	14	cagaaatcatccttagcgaaagctaaggattttttatctg
BBa_K088008(T25)	25	agaaacagcaaacaatccaaaacgccgcgttcagcgg cgtttttctgcttttct
BBa_J61053(T48)	48	gcttgctgaggatcctaaagccccgaatttttataaattcg gggcttttt
ECK120034435(T240)	240	ctcgggtaccaaattccagaaaagagacgctgaaaagcgt cttttttcgttttgggtcc
pheA-1(T244)	244	gacgaacaataaggcctcccaaatcggggggccttttta ttgataacaaaa
ECK120033737(T312)	312	ggaaacacagaaaaaagcccgccacgtgacagtgcggg cttttttttcgaccaaagg
ECK120029600(T378)	378	ttcagccaaaaaacttaagaccgccggtcttgtccacta ccttgacagtaatgcggtggacaggatcggcggttttctttc tcttctcaa

Table S5. ON/OFF ratios for genetic switches with various terminator filters across different aTC concentrations (nM)

Name	0 nM	16 nM	31 nM	63 nM	125 nM	250 nM
No crRNA (Control)	1.0	30	59	111	160	183
crRNA Only	1.0	4.2	4.3	4.3	4.3	4.6
T25-crRNA	1.0	146	164	178	182	188
T244-crRNA	1.0	300	336	355	361	381
T240-crRNA	1.0	276	332	363	378	395
T25-T244-crRNA	1.0	248	328	375	393	402
T25-T240-crRNA	1.0	196	331	394	423	436
T25-T244-T240-crRNA	1.0	51	105	210	338	463

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