SUPPLEMENTARY INFORMATION

Evolution of a split RNA polymerase as a versatile biosensor platform

Jinyue Pu, Julia Zinkus-Boltz, Bryan C. Dickinson

Department of Chemistry, The University of Chicago, Chicago, Illinois, USA

Email: Dickinson@UChicago.edu

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SUPPLEMENTARY RESULTS

Vector name	Antibiotic resistance	Origin	Purpose	Map
p2-22	carb/amp	SC101	P_{T7} luciferase reporter plasmid	a
pJin129	spec	P15a	Split N-terminal T7 RNAP expression plasmid	b
p3-7	spec	P15a	Split N-terminal T7 RNAP-linker-ZA expression plasmid	b
p2-55	chlr	CloDF13	Split C-terminal T7 RNAP expression plasmid	c
p2-39	chlr	CloDF13	ZB-linker-split C-terminal T7 RNAP expression plasmid	c
p2-57	chlr	CloDF13	ZBneg-linker-split C-terminal T7 RNAP expression plasmid	c
p5-71	spec	P15a	N-29-1 split N-terminal T7 RNAP variant-linker-ZA expression plasmid	b
p5-72	spec	P15a	N-29-8 split N-terminal T7 RNAP variant-linker-ZA expression plasmid	b
p5-74	spec	P15a	N-29-1 split N-terminal T7 RNAP variant-linker-ZA (L13I, L20I) expression plasmid	b
p5-75	spec	P15a	N-29-8 split N-terminal T7 RNAP variant-linker-ZA (L13I, L20I) expression plasmid	b
p5-79	spec	P15a	N-29-1 split N-terminal T7 RNAP variant-GGSGSGSS-FRB expression plasmid	b
p6-27	spec	P15a	N-29-1 split N-terminal T7 RNAP variant-GGSGSGSS-iLID expression plasmid	b
p6-29	chlr	CloDF13	SspB Nano-linker-Split C-terminal T7 RNAP expression plasmid	c
p5-39	chlr	CloDF13	FKBP-TSGGSG-Split C-terminal T7 RNAP expression plasmid	c
p5-40	chlr	CloDF13	FKBP-GG-Split C-terminal T7 RNAP expression plasmid	c
p7-68	chlr	CloDF13	FKBP-TSGGSGTSGGSG-Split C-terminal T7 RNAP expression plasmid	c
p5-70	spec	P15a	N-29-1 split N-terminal T7 RNAP variant-GG-FRB expression plasmid	b
p7-69	spec	P15a	N-29-1 split N-terminal T7 RNAP variant-GGSGSSGGSGSGSS-FRB expression plasmid	b
pJin200	spec	P15a	N-29-1 split N-terminal T7 RNAP variant-linker-ZA(L13I, L20I)-linker-FRB expression plasmid	b
p3-13	chlr	CloDF13	ZB-linker-split C-terminal CGG RNAP expression plasmid	c
p4-32	chlr	CloDF13	ZBneg-linker-split C-terminal CGG RNAP expression plasmid	c
p2-64	carb/amp	SC101	P_{CGG} luciferase reporter plasmid	a
pJin216	carb/amp	SC101	P_{T7} luciferase, P_{CGG} DsRed-Express2, reporter plasmid	d
pJin207	chlr	CloDF13	ZB-C-terminal CGG/FKBP-C-terminal T7 expression plasmid	e
pJin208	chlr	CloDF13	ZBneg-C-terminal CGG/FKBP-C-terminal T7 expression plasmid	e
pJin141	kan	pBR322	rapa-T7-F30-2xdBroccoli	f
p6-8	kan	pBR322	rapa-T7- mRNA(GFP)	f
pJin140	kan	pBR322	rapa-T7-shRNA(GFP)	f
p1-53	carb/amp	CloE1	eGFP expression vector	g
p3-62	kan	pUC	mRFP expression vector	h
p8-61	chlr	CloDF13	FKBP-linker-split C-terminal mEGFP (158+) expression plasmid	c
p8-62	spec	P15a	split N-terminal mEGFP (1-157)-linker-FRB expression plasmid	b
pJin210	chlr	CloDF13	FKBP-linker-split C-terminal scEGFP (158+) expression plasmid	c
pJin211	spec	P15a	split N-terminal scGFP (1-157)-linker-FRB expression plasmid	b

Supplementary Table 1: List of all non-PACE constructs used in this work. Vector maps for each construct type shown in **Supplementary Fig. 1**.

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evolutionary date	positive AP					negative AP				
	Vector name	Map	Origin	RBS1	RBS2	Vector name	Map	Origin	RBS3	RBS4
day1	pJin69	g	SC101	SD8	sd8					
day2	pJin69	g	SC101	SD8	sd8					
day3	pJin69	g	SC101	SD8	sd8					
day4	pJin177	h	P15A	SD8	sd8	pJin173	i	pBR322	SD4	sd2
	pJin177	h	P15A	SD8	sd8	pJin173	i	pBR322	SD4	sd2
day5	pJin177	h	P15A	SD8	sd8	pJin172	i	pBR322	sd5	sd6
day6	pJin177	h	P15A	SD8	sd8	pJin172	i	pBR322	sd5	sd6
day7	pJin177	h	P15A	SD8	sd8	pJin172	i	pBR322	sd5	sd6
day8	pJin177	h	P15A	SD8	sd8	pJin104	i	pBR322	SD8	sd8
day9	pJin177	h	P15A	SD8	sd8	pJin104	i	pBR322	SD8	sd8
	pJin177	h	P15A	SD8	sd8	pJin104	i	pBR322	SD8	sd8
day10	pJin182	h	P15A	sd5	sd5	pJin172	i	pBR322	sd5	sd6
day11	pJin182	h	P15A	sd5	sd5	pJin172	i	pBR322	sd5	sd6
day12	pJin182	h	P15A	sd5	sd5	pJin172	i	pBR322	sd5	sd6
day13	pJin182	h	P15A	sd5	sd5	pJin172	i	pBR322	sd5	sd6
day14	pJin185	h	P15A	SD4	sd8	pJin194	i	pBR322	SD8	SD4
	pJin185	h	P15A	SD4	sd8	pJin194	i	pBR322	SD8	SD4
day15	pJin196	h	P15A	SD4	sd5	pJin194	i	pBR322	SD8	SD4
day16	pJin196	h	P15A	SD4	sd5	pJin194	i	pBR322	SD8	SD4
day17	pJin196	h	P15A	SD4	sd5	pJin194	i	pBR322	SD8	SD4
day18	pJin196	h	P15A	SD4	sd5	pJin194	i	pBR322	SD8	SD4
day19	pJin196	h	P15A	SD4	sd5	pJin194	i	pBR322	SD8	SD4
day20	pJin70	g	SC101	SD8	sd8	pJin104	i	pBR322	SD8	sd8
day21	pJin70	g	SC101	SD8	sd8	pJin104	i	pBR322	SD8	sd8
day22	pJin178	g	SC101	SD8	sd6	pJin172	i	pBR322	sd5	sd6
day23	pJin178	g	SC101	SD8	sd6	pJin172	i	pBR322	sd5	sd6
day24	pJin178	g	SC101	SD8	sd6	pJin172	i	pBR322	sd5	sd6
day25	pJin178	g	SC101	SD8	sd6	pJin172	i	pBR322	sd5	sd6
day26	pJin178	g	SC101	SD8	sd6	pJin172	i	pBR322	sd5	sd6
	pJin178	g	SC101	SD8	sd6	pJin172	i	pBR322	sd5	sd6
day27	pJin178	g	SC101	SD8	sd6	pJin171	i	pBR322	SD4	sd8
	pJin178	g	SC101	SD8	sd6	pJin172	i	pBR322	sd5	sd6
day28	pJin178	g	SC101	SD8	sd6	pJin171	i	pBR322	SD4	sd8
day29	pJin178	g	SC101	SD8	sd6	pJin171	i	pBR322	SD4	sd8

Supplementary Table 2: Full evolutionary protocol for PACE experiment to evolve proximity-dependent split RNAP. Vector names and details are provided for each day of PACE. Vector maps for the posAP and negAP vectors are shown in **Supplementary Figure 1 i, j, and k**. Two sets of posAP/negAP vectors listed on the same day indicates that a mixed selection pressure was utilized, in which two types of host cells, each containing one set of the posAP/negAP plasmids were added to a lagoon simultaneously. The relative RBS strengths were obtained from previous studies[50]. Note: pJin69 utilized T7 C-term RNAP and P_{T7}, unlike the rest of the positive APs, which used CGG C-term RNAP and P_{CGG}.

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day 3																					
N-3-1																					
N-3-2																					
N-3-3																					
N-3-4									D87N							S128N					
N-3-5		D26Y											I109N								
N-3-6																					
N-3-7																					
N-3-8																					
day 7																					
N-7-1			L32S			E63K					K98R	Q107K									
N-7-2			L32S			E63K					K98R	Q107K									
N-7-3			L32S								K98R	Q107K	I109T								
N-7-4						E63K					K98R	Q107K									
N-7-5						E63K					K98R	Q107K									
N-7-6			L32S							E91G	K98R	Q107K									
N-7-7			L32S			E63K					K98R	Q107K									
N-7-8	E25D		L32S							E91G	K98R	Q107K									
day 8																					
N-8-1			L32S							E91G	K98R	Q107K									
N-8-2			L32S			E63K					K98R	Q107K									
N-8-3						E63K					K98R	Q107K									
N-8-4			L32S								K98R	Q107K	I109T							G152D	
N-8-5			L32S			E63K					K98R	Q107K									
N-8-6						E63K					K98R	Q107K									
N-8-7			L32S	E35D						E91G	K98R	Q107K									
N-8-8			L32S			E63K					K98R	Q107K									
day 13																					
N-13-1			L32S	E35G						E91G	K98R	Q107K						A144T			
N-13-2						E63K					K98R	Q107K									
N-13-3		D26G				E63K					K98R	Q107K						A144T			A159S
N-13-4			L32S	E35G						E91G	K98R	Q107K									
N-13-5			L32S	E35G						E91G	K98R	Q107K									
N-13-6			L32S	E35G						E91G	K98R	Q107K									
day 21																					
N-21-1			L32S	E35G						E91G	K98R	Q107K									
N-21-2			L32S	E35G						E91G	K98R	Q107K							A149T		
N-21-3			L32S	E35G						E91G	K98R	Q107K									
N-21-4			L32S	E35G	A49S					E91G	K98R	Q107K							A149T		
N-21-5			L32S	E35G						E91G	K98R			A124S							
N-21-6			L32S	E35G						E91G	K98R	Q107K									
N-21-7			L32S	E35G			A83T			E91G	K98R	Q107K									
N-21-8			L32S	E35G			A83T			E91G	K98R	Q107K									
N-21-9			L32S	E35G			A83T			E91G	K98R	Q107K									
N-21-10			L32S	E35G		E63K			D87E		K98R	Q107K	T122S					A144T			
day27																					
N-27-2			L32S	E35G						E91G	K98R	Q107K		A124S							
N-27-3			L32S	E35G						E91G	K98R	Q107K		A124S							
N-27-4			L32S	E35G						E91G	K98R	Q107K		A124S							
N-27-5			L32S	E35G		E63K					K98R	Q107K	T122S					A144T			
N-27-6			L32S	E35G		E63K					K98R	Q107K	T122S					A144T			
N-27-7			L32S	E35G		E63K						Q107K	T122S					A144T			
N-27-8			L32S	E35G		E63K		N86S				Q107K	T122S					A144T			
day29																					
N-29-1			L32S	E35G							K98R	Q107K	T122S					A144T			
N-29-2			L32S	E35G		E63K					K98R	Q107K	T122S		T127A		A136D	A144T			
N-29-3			L32S	E35G							K98R	Q107K	T122S					A144T			
N-29-4			L32S	E35G		E63K					K98R	Q107K	T122S					A144T			
N-29-5			L32S	E35G		E63K					K98R	Q107K	T122S					A144T			
N-29-7			L32S	E35G		E63K					K98R	Q107K	T122S					A144T			
N-29-8			L32S	E35G		E63K					K98R	Q107K	T122S					A144T			

Supplementary Table 3: Mutational analysis of evolving split RNAP. Single phage sequenced during the course of the PACE experiment and coding mutations are shown for a set of variants assayed at each time point. The final variants selected for further assay (N-29-1 and N-29-8) are highlighted yellow.

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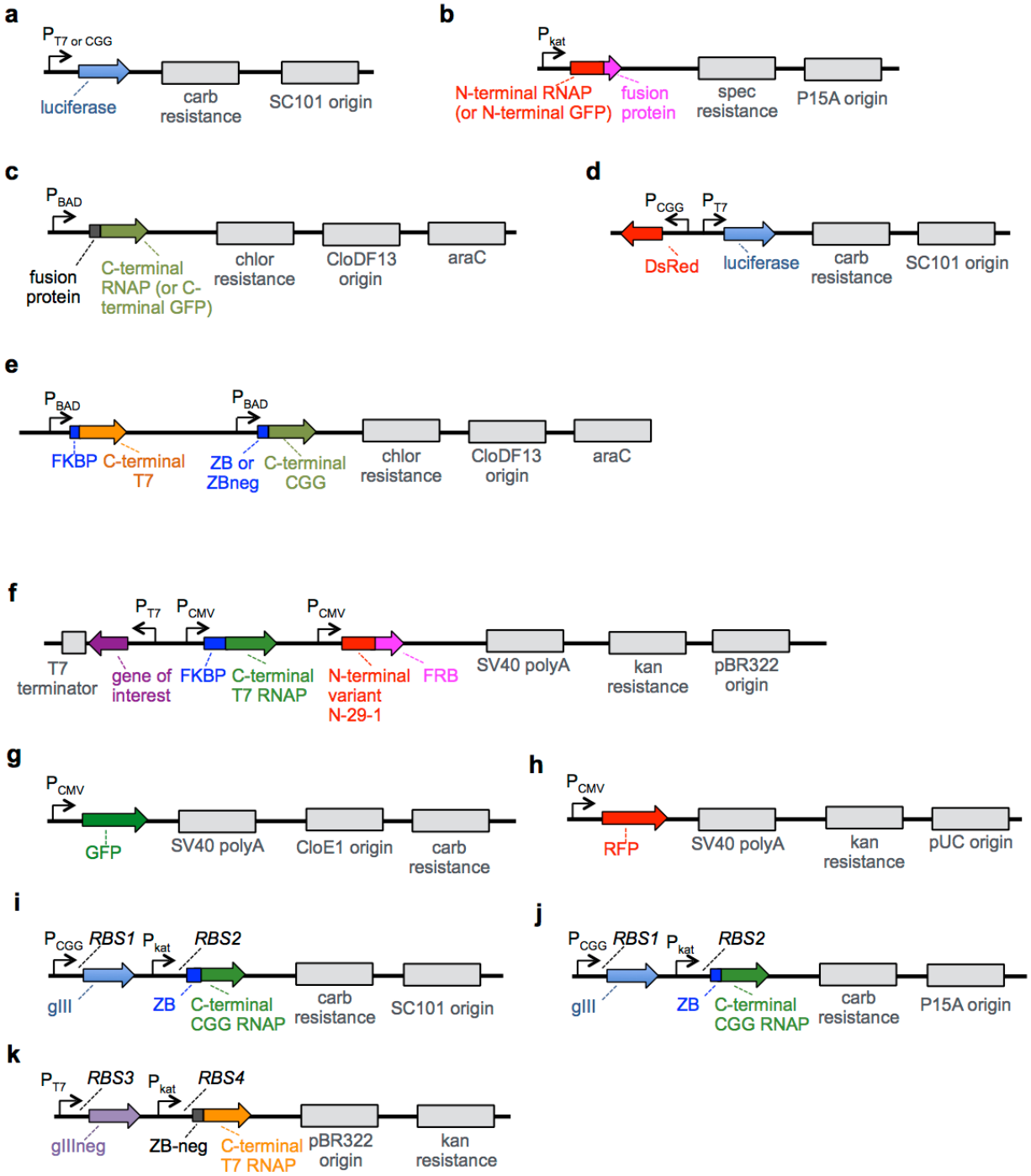

Supplementary Figure 1: Vector maps for all constructs used in this work. Vectors corresponding to the vectors utilized in this work outlined in **Supplementary Tables. 1 and 2**.

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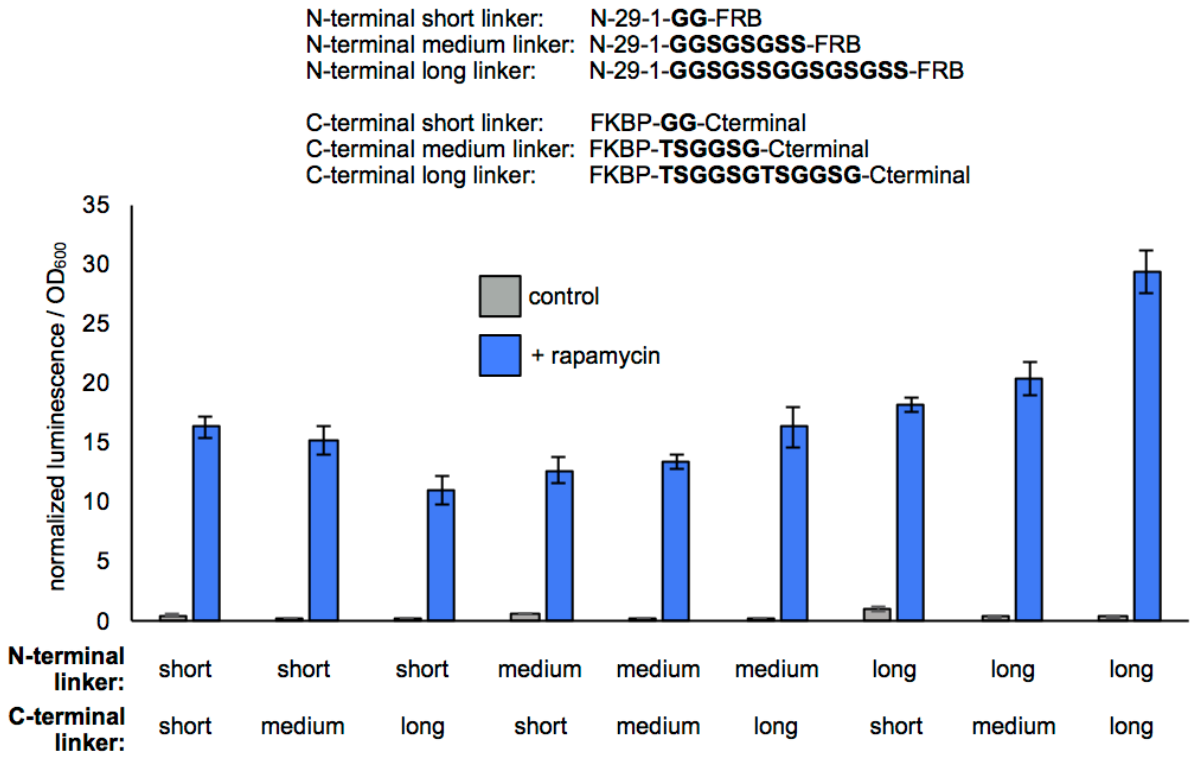

Supplementary Figure 2: Linker effects of AR system. Transcription response of the rapamycin-inducible RNAP system in *E. coli.* Cells transformed with expression vectors for the two halves of the small molecule-inducible RNAP and a reporter vector, then induced with either DMSO or 20 μM rapamycin for 3 h prior to transcription analysis (error bars std. error, n = 5). The linkers tethering the binding domains to each half of the split RNAP were varied as indicated in the figure.

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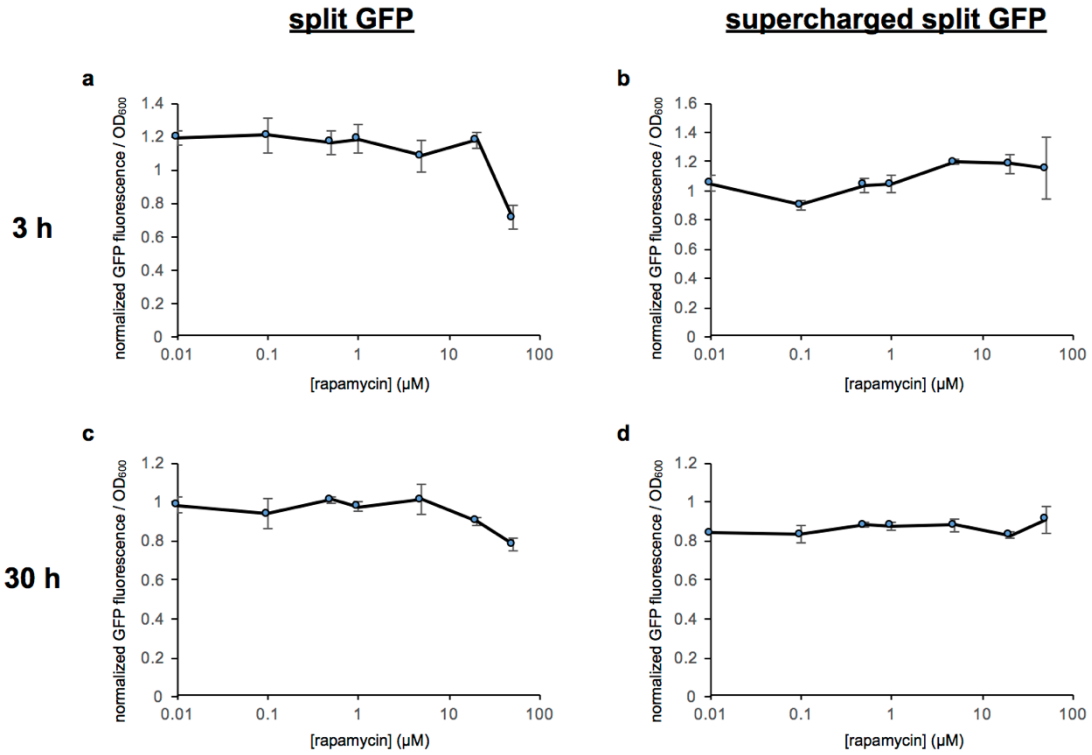

Supplementary Figure 3: Comparison of ARs with split GFP under identical experimental conditions. *E. coli* cells were transformed with expression vectors for the two halves of a split GFP system, either split GFP (a and c) or supercharged split GFP (b and d), fused to FRB and FKBP, in the same vector systems and conditions used for the AR fusions assayed in Fig. 3d. The cells were then induced with varying concentrations of rapamycin for either 3 h (a and b) or 30 h (c and d) prior to analysis of GFP fluorescence (error bars std. error, n = 5). No increase in signal is observed. This is possibly due to either the low concentrations of sensor available under these conditions and the corresponding enhanced sensitivity of the AR system, the signal-to-noise of GFP, the folding kinetics, or other properties of the split GFP system.

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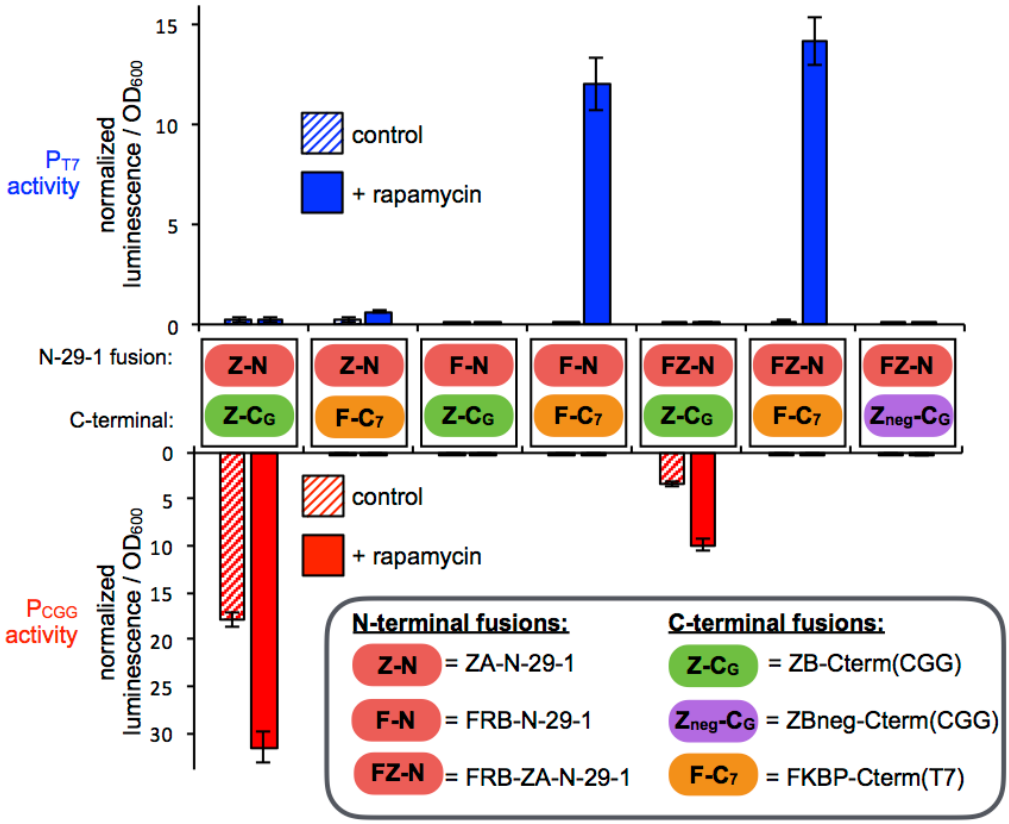

Supplementary Figure 4: Validation of trimolecular interaction system. Transcriptional response and selectivity in *E. coli* of bimolecular interactions of the synthetic parts shown in **Fig. 4a**. Cells transformed with expression vectors for N-29-1 fused to ZA ("Z-N", p5-74), N-29-1 fused to FRB ("F-N", p5-79), or N-29-1 fused to FRB and ZA ("FZ-N", pJin200); either Z-C_G (p3-13), Z_{neg}-C_G (p4-32), or F-C_7 (p5-39); and a reporter vector with either T7-(p2-22) or CGG-promoter (p2-64) driven luciferase. The cells were treated with either DMSO or 20 μM rapamycin for 2 h prior to transcriptional analysis (error bars std. dev., n = 4). As predicted, the FZ-N-F-C_7 interaction is rapamycin-inducible and selectively drives transcription from the T7 promoter, while the FZ-N-Z-C_G interaction is constitutive and selective for the CGG promoter.

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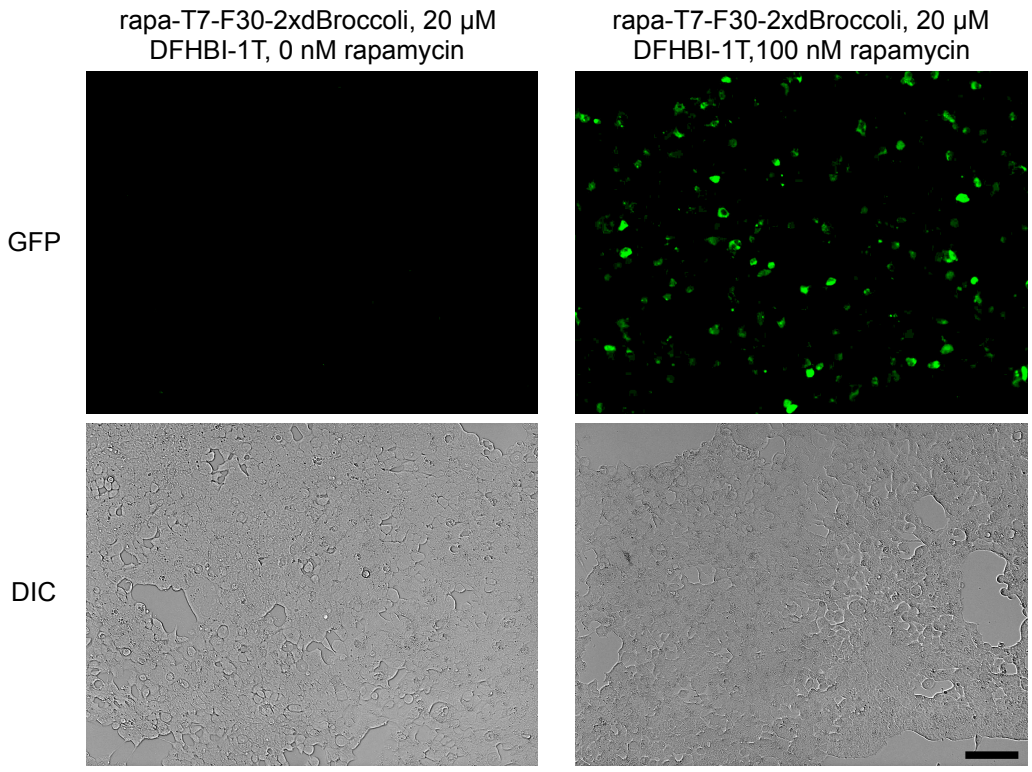

Supplementary Figure 5: Fluorescence imaging of rapa-T7-F30-2xdBroccoli vector in HEK293T cells. (Expanded data from **Fig. 5b**) HEK293T cells transfected with the rapa-T7-F30-2xdBroccoli vector induced with 0 or 100 nM rapamycin for 30 min in the presence of DFHBI-1T then analyzed by fluorescence microscopy. DIC and fluorescence images shown for each set of conditions and 100 μm scale bar shown.

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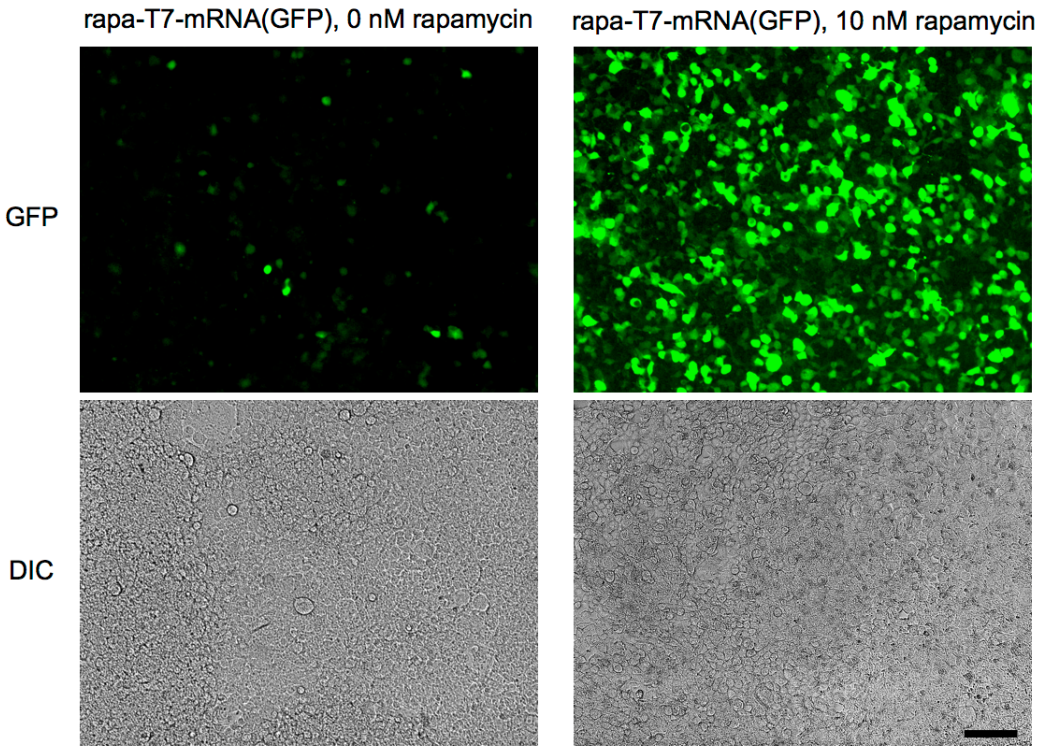

Supplementary Figure 6: Fluorescence imaging of rapa-T7-mRNA(GFP) vector in HEK293T cells. (Expanded data from **Fig. 5c**) HEK293T cells transfected with the rapa-T7-mRNA(GFP) vector induced with 0 or 10 nM rapamycin for 24 h and then analyzed by fluorescence microscopy. DIC and fluorescence images shown for each set of conditions and 100 μm scale bar shown.

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