Supplemental information for:

Post-translational control of genetic circuits using *Potyvirus* proteases

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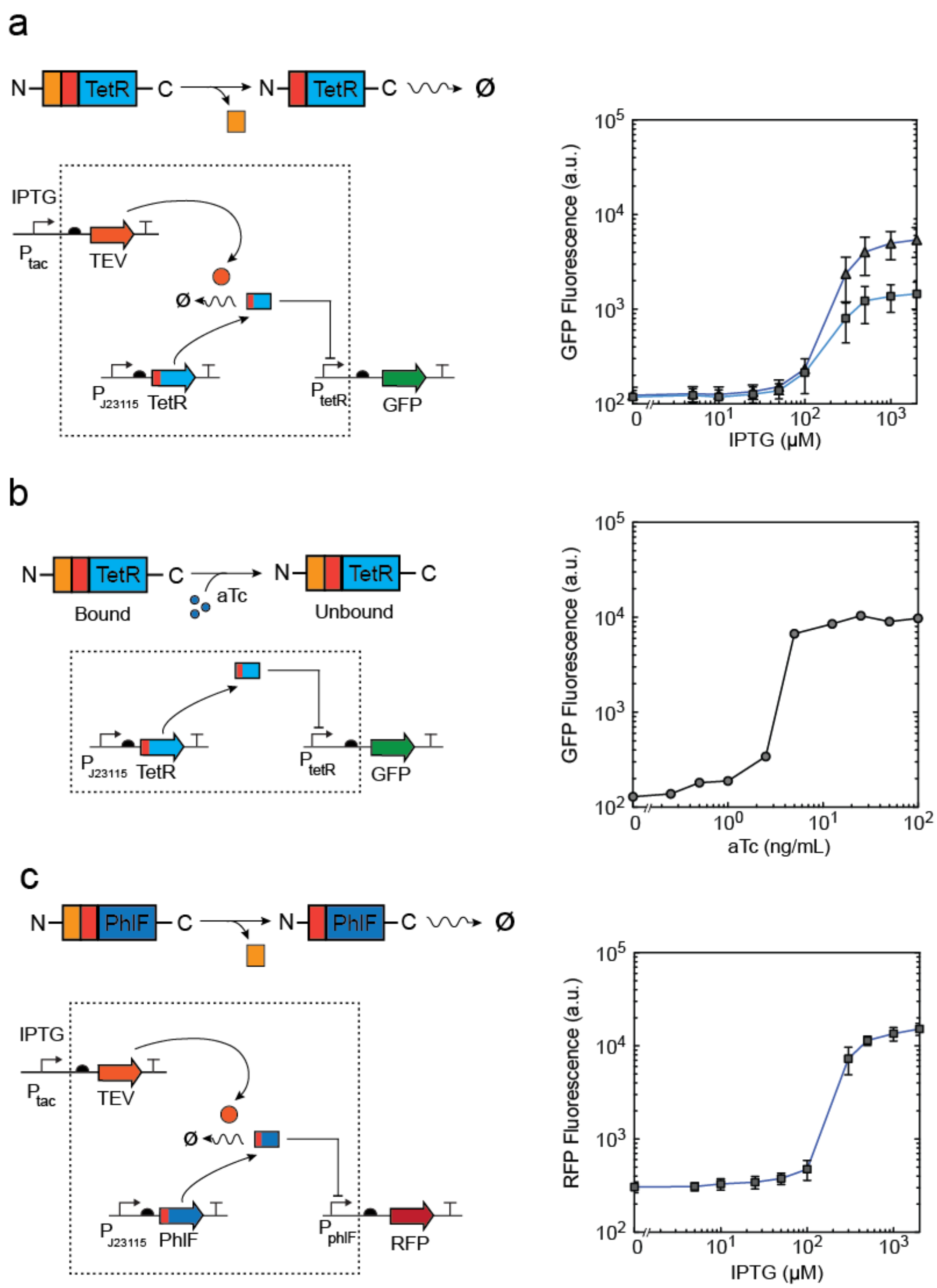
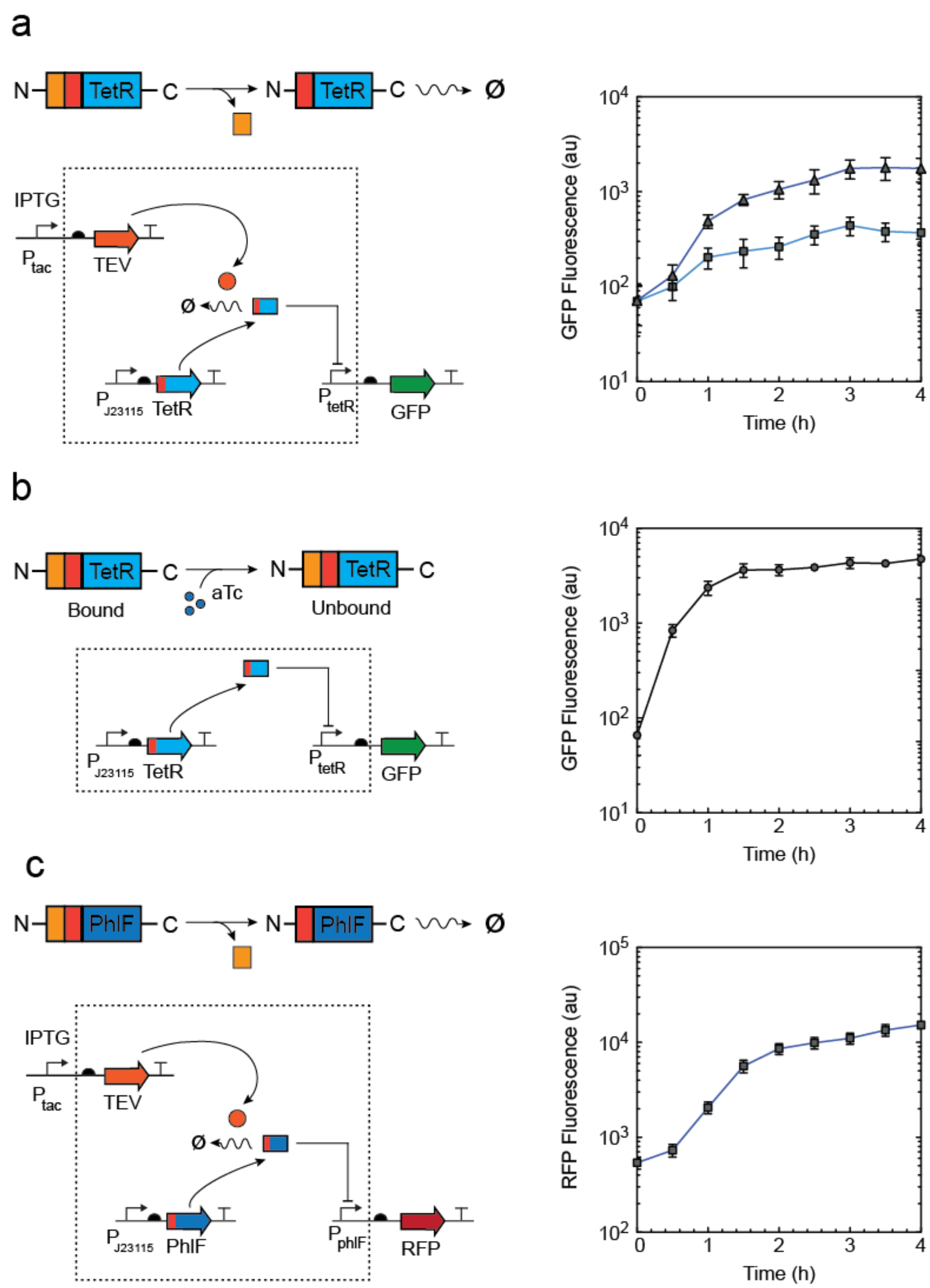
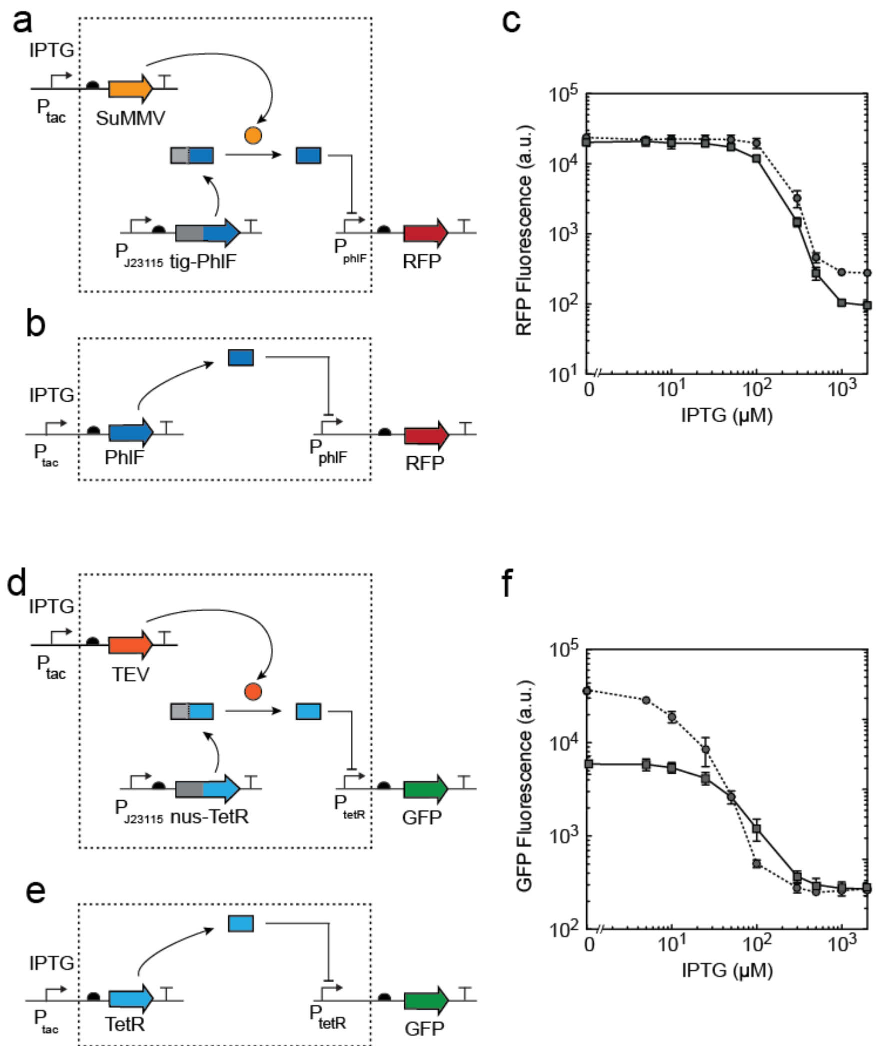


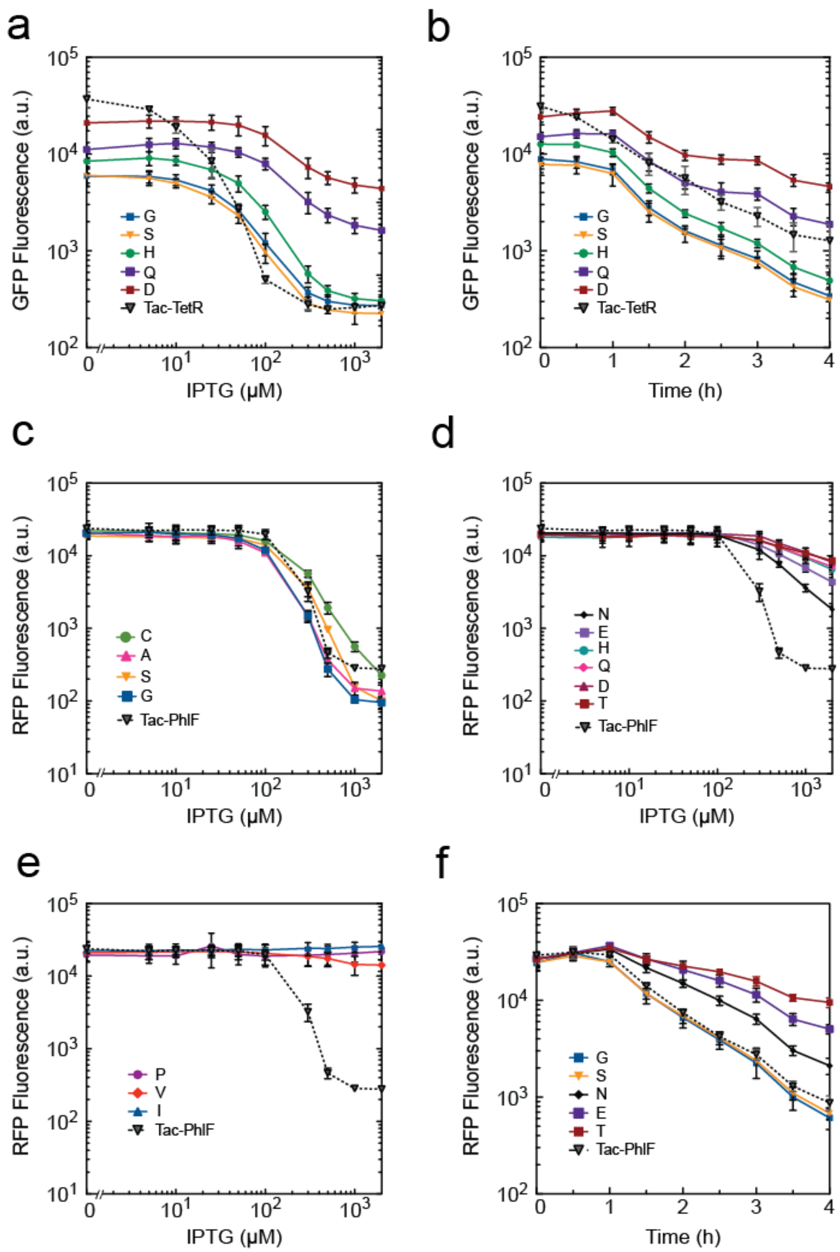
<u>**Supplementary Figure 1:**</u> **Controlled degradation of TetR and PhlF. (A)** The circuit schematic and response function for the TEV-mediated controlled degradation of TetR are shown. Cells containing ptevF-TetR (deep blue line, triangles) or ptevY-TetR (light blue line, squares) and pTac-TEV were grown at different concentrations of IPTG for 6 hours and the resulting GFP fluorescence tracked by flow cytometry. **(B)** The circuit schematic and response function for the activation by anhydrotetracycline (aTc) is shown in cells containing ptevF-TetR without protease. **(B)** The circuit schematic and response function for the TEV-mediated controlled degradation of PhlF is shown. Cells containing ptevF-PhlF and pTac-TEV were grown at different concentrations of IPTG for 6 hours and the resulting RFP fluorescence tracked by flow cytometry. In all panels, error bars represent the standard deviation of three experiments performed in different days.



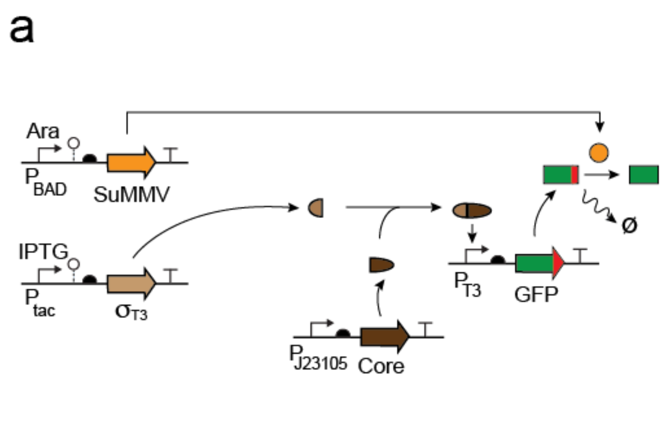
<u>Supplementary Figure 2:</u> **Time course analysis of degron-activated circuits**. **(A)** Cells containing ptevF-TetR (deep blue, triangles) or ptevY-TetR (light blue, squares) in the presence of pTac-TEV were induced with 2 mM IPTG and their fluorescence tracked by flow cytometry every hour. **(B)** Cells containing ptevF-TetR only were treated with 100 ng/mL anhydrotetracycline (aTc) and their fluorescence tracked by flow cytometry every hour. **(C).** Cells containing ptevF-PhlF and pTac-TEV were induced with 2 mM IPTG and their fluorescence tracked by flow cytometry every hour. In all panels, error bars represent the standard deviation of three experiments performed in different days.



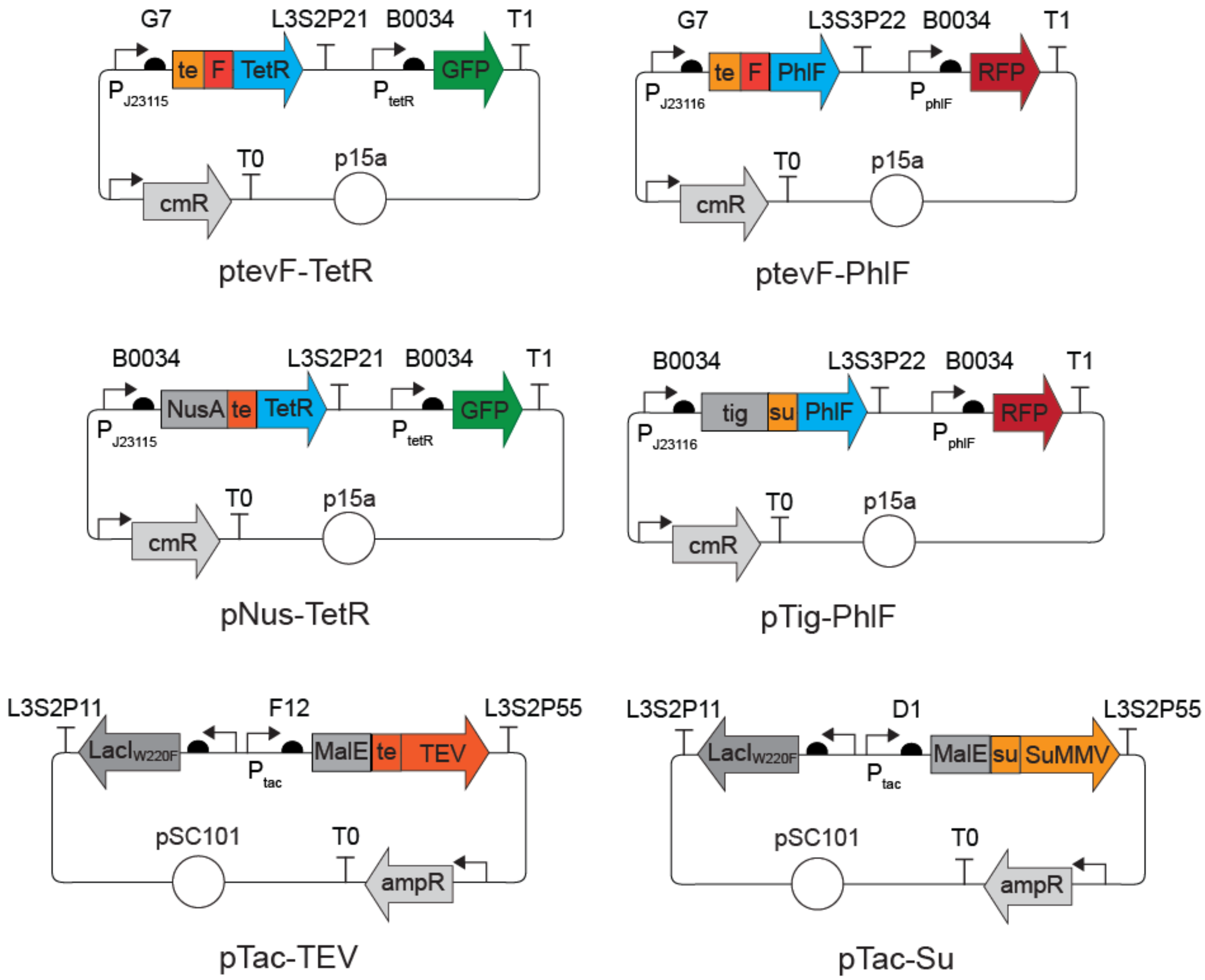
<u>Supplementary Figure 3:</u> **Controlled release of PhlF and TetR compared to P_{tac} induction. (A)** A schematic of controlled release of PhlF mediated by SuMMV is shown. **(B)** A schematic of a P_{tac} inducible PhlF circuit is shown. **(C)** The response function for the SuMMVp-mediated controlled release of PhlF (solid line, squares) as compared to PhlF expressed from a P_{tac} promoter (dashed line, circles) (1). Cells containing pTig-PhlF and pTac-Su or P_{tac}-PhlF were grown at different concentrations of IPTG for 6 hours and the resulting RFP fluorescence tracked by flow cytometry. **(D)** A schematic of controlled release of TetR mediated by TEV is shown. **(E)** A schematic of a P_{tac} inducible TetR circuit is shown. **(F)** The response function for the TEVp-mediated controlled release of TetR (solid line, squares) as compared to TetR expressed from a P_{tac} promoter (dashed line, circles) (1). Cells containing pNus-TetR and pTac-TEV or P_{tac}-TetR were grown at different concentrations of IPTG for 6 hours and the resulting GFP fluorescence tracked by flow cytometry. Error bars represent the standard deviation of three experiments performed in different days.



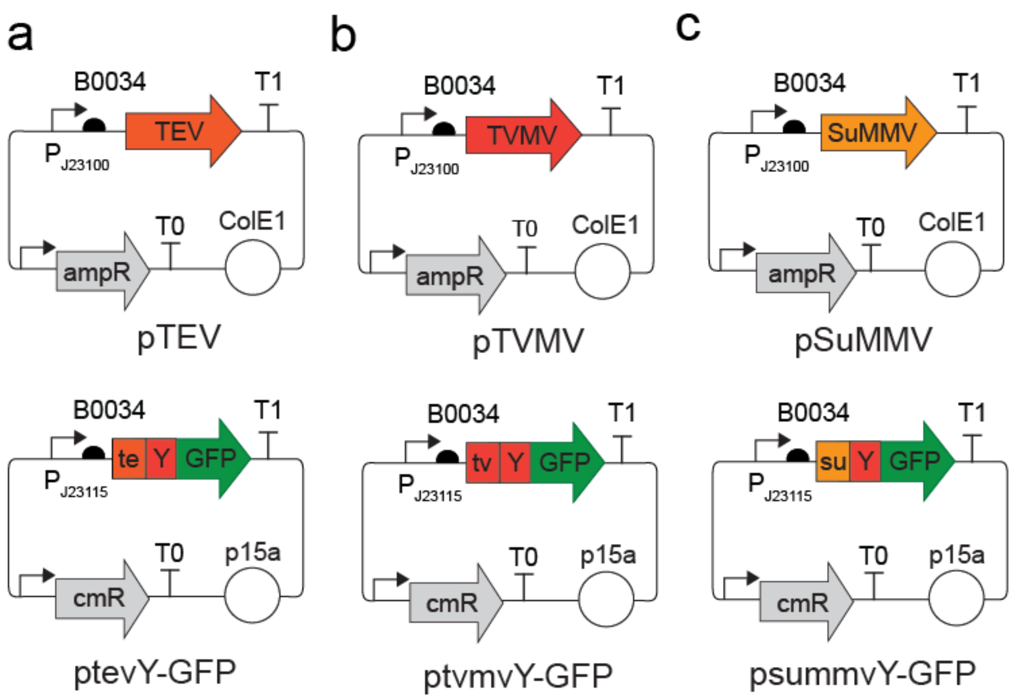
<u>Supplementary Figure 4</u> **Effect of the P1' position on the cleavage efficiency. (A)** IPTG titration of cells containing NusA-tevX-TetR, where X corresponds to a different residue at the tev-P1' position, in the presence of pTac-TEV. The transfer function of pTac-TetR is shown for comparison (dashed black line). **(B)** Time course analysis of the cells shown in (A). **(C), (D), (E)** IPTG titration of cells containing TF-summvX-PhlF, where X corresponds to a different residue at the summv-P1' position, in the presence of pTac-Su. Residues were classified as 'optimal' (C), 'medium' (D) and 'non-cutters' (E). The transfer function of pTac-PhlF is shown for comparison (dashed black line). **(F)** Time course analysis of a subset of P1' variants shown in (C) and (D). Error bars represent the standard deviation of three experiments performed on different days.



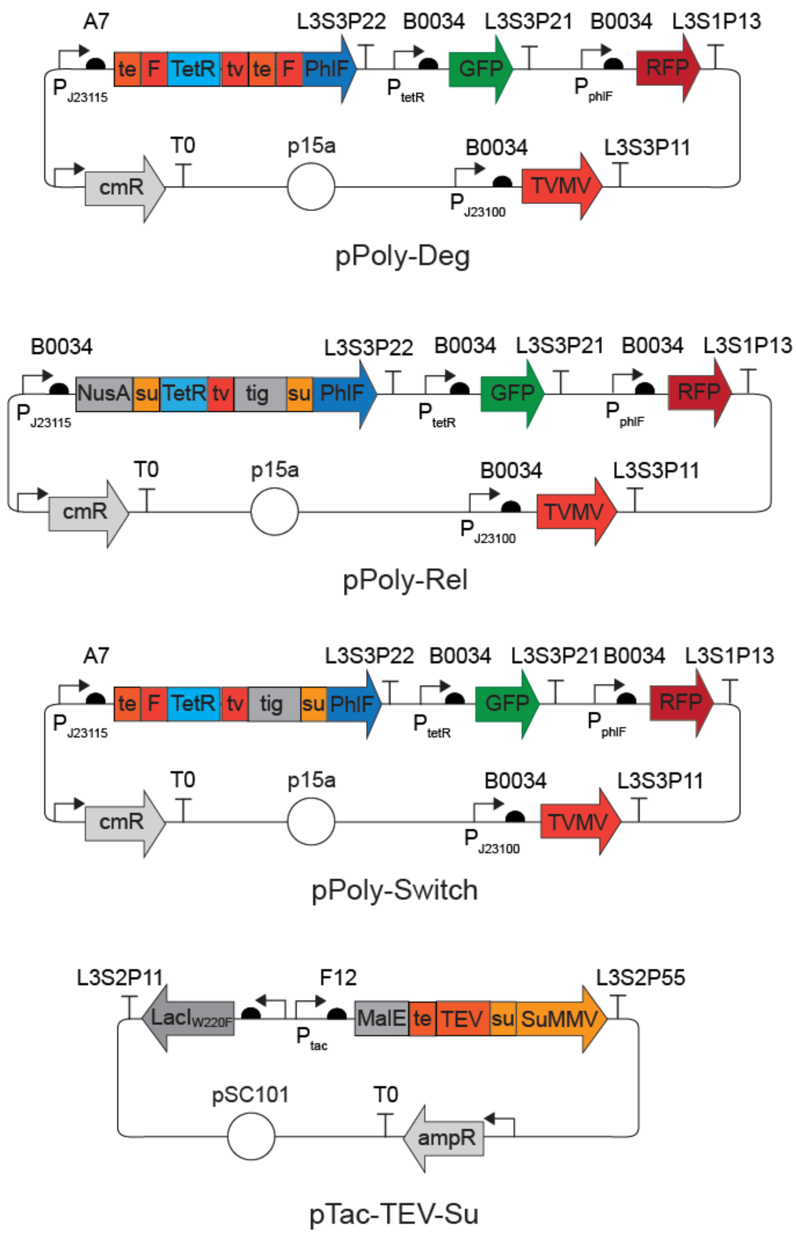
<u>Supplementary Figure 5:</u> Increase of dynamic range by decoupled expression of SuMMV from main circuit. **(A)** A schematic of degradation rescue with expression of the SuMMV protease under the control of P_{BAD} (independent of the main circuit) implemented into the original IPTG-inducible σ_{T3} polymerase system. Red squares, C-terminal degradation tag. o-- symbol, BydvJ ribozyme (SuMMV) and RiboJ ribozyme (T3) (2, 3). **(B)** Response functions of the circuit shown in (A). Cells carrying pCore, pTac-T3, pT3-GFP-LVA and pBAD-Su were grown at different concentrations of IPTG and arabinose for 8 hours and the fluorescence tracked by flow cytometry. Solid grey line, triangles, original split-polymerase system without SuMMV protease and untagged GFP. Dashed grey line, circles, split-polymerase system with degron-tagged GFP output induced with IPTG only. Orange line, the circuit shown in (A) induced with both IPTG and arabinose. Error bars correspond to the standard deviation of three experiments performed on different days.



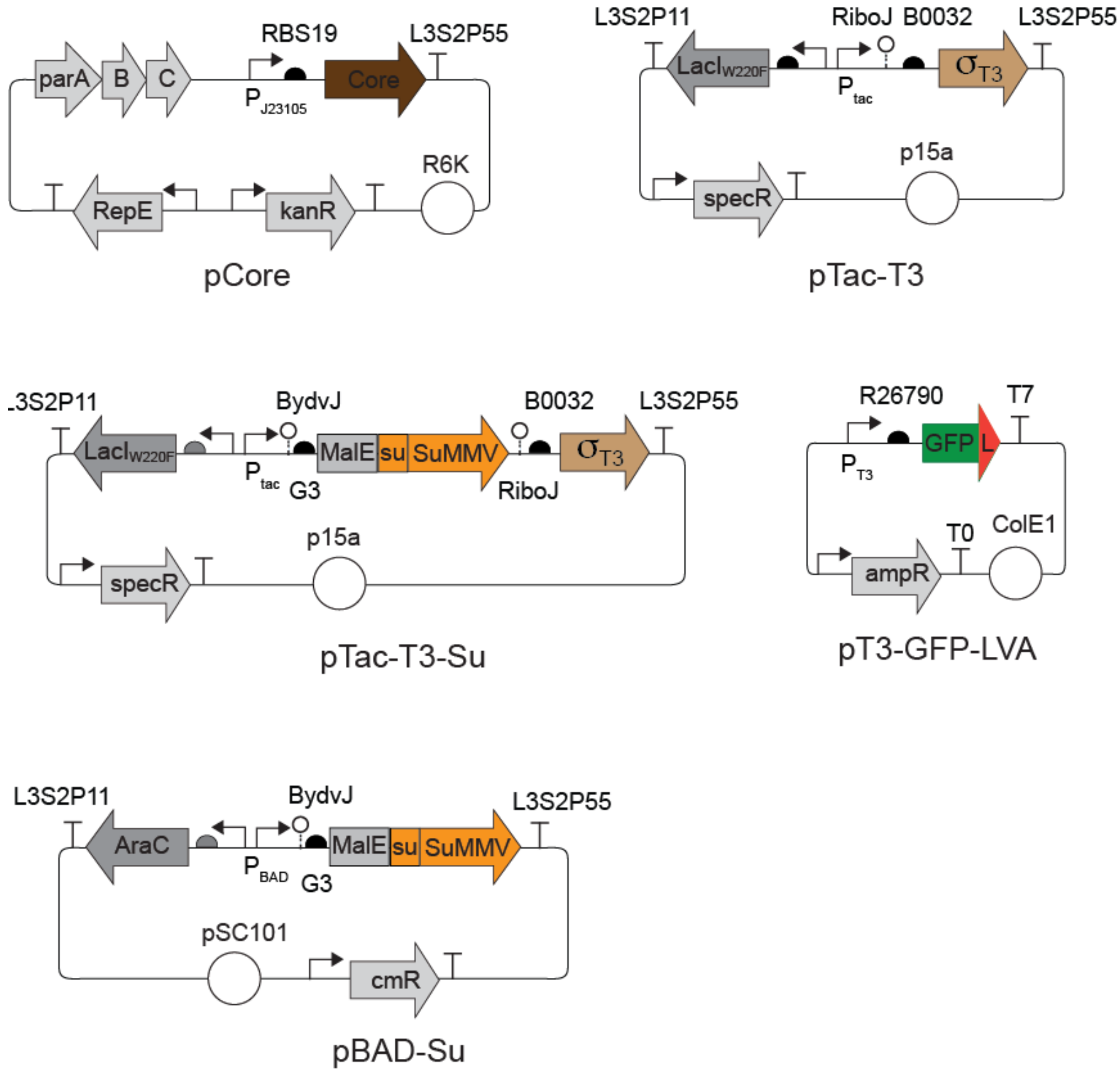
Supplementary Figure 6 **Plasmid maps used in controlled degradation and release experiments. Top row**, the plasmids for controlled degradation are shown. **Middle row**, the plasmids for controlled repressor release are shown. *Nus*, NusA protein (4); *Tig*, trigger factor protein (4); *su*, SuMMVp site. **Bottom row**, the plasmids used to control the expression of TEVp and SuMMVp are shown. $LacI_{W220F}$, a variant of LacI with lower leakage (5). F, degron FLFVQ (6); *te*, TEVp cleavage site; *su*, SuMMVp cleavage site. In all figures, promoters are depicted by arrows; RBS are shown as solid semicircles; terminators are represented by T symbols.



Supplementary Figure 7 **Plasmid maps used in the *Potyvirus* orthogonality experiments. Top row,** the plasmids encoding constitutively expressed, codon-optimized proteases. **Bottom row,** the cognate reporter plasmids for each protease. Y, degron YLFVQ (6); *te*, TEVp cleavage site; *tv*, TVMVp cleavage site; *su*, SuMMVp cleavage site. In all figures, promoters are depicted by arrows; RBS are shown as solid semicircles; terminators are represented by T symbols.



Supplementary Figure 8 **Plasmid maps used in the polyrepressor experiments. Top row**, the plasmid for double degradation of TetR and PhlF; **second row**, the plasmid for double release of TetR and PhlF; **third row,** the plasmid for degradation of TetR and release of PhlF; **bottom row**, the plasmid expressing TEVp and SuMMVp as a polyprotein is shown. *te,* TEVp cleavage site; *tv,* TVMVp cleavage site; *su,* SuMMVp cleavage site; *F*, FLFVQ-degron (6); *Nus*, NusA protein (4); *Tig*, trigger factor protein (4). Promoters are depicted by arrows; RBS are shown as solid semicircles; terminators are represented by T symbols.



<u>Supplementary Figure 9</u> **Plasmid maps used in the degradation rescue experiments. Top row**, the plasmids for the expression of the basic split polymerase components are shown (7). **Middle row**, left, the plasmid containing the controller protease (SuMMVp) and the sigma T3 fragment is shown. *su*, SuMMVp cleavage site**.** Right, reporter plasmid. *L, ssrA* C-terminal tag LVA (8). **Bottom row**, the plasmid containing the protease SuMMV decoupled from the main circuit under the control pf P_{BAD} is shown. Promoters are depicted by arrows; RBS are shown as solid semicircles; terminators are represented by T symbols; the –o symbol shows the *BydvJ* or *RiboJ* ribozymes (2, 3). Genetic parts follow SBOLv format (23).

Table S1. Genetic parts used in this study

Part name	Type	DNA sequence	Ref.
P_{J23100}	Promoter	ttgacggctagctcagtcctaggtacagtgctagc	(10)
P_{J23115}	Promoter	tttatagctagctcagcccttggtacaatgctagc	(10)
P_{J23116}	Promoter	ttgacagctagctcagtcctagggactatgctagc	(10)
P_{J23105}	Promoter	tttacggctagctcagtcctaggtactatgctagc	(10)
P_{tetR}	Promoter	tccctatcagtgatagagattgacatccctatcagtgatagatataatgagc ac	(1)
P_{phlF}	Promoter	tctgattcgttaccaattgacatgatacgaaacgtaccgtatcgttaaggt	(1)
P_{tac}	promoter	tgttgacaattaatcatcggctcgtataatgtgtggaattgtgagcgctcac aatt	(1)
P_{BAD}	Promoter	gaaaccaattgtccatattgcatcagacattgccgtcactgcgtcttttact ggctcttctcgctaaccaaaccggtaaccccgcttattaaaagcattctgta acaaagcgggaccaaagccatgacaaaaacgcgtaacaaaagtgtctataat cacggcagaaaagtccacattgattatttgcacggcgtcacactttgctatg ccatagcatttttatccataagattagcggatcctacctg	(11)
P_{T3}	Promoter	taataaccctcactatagggaga	(7)
B0034	RBS	aaagaggagaaa	(12)
B0032	RBS	tcacacaggaaag	(12)
G7	RBS	tttaaagaggagcaaggtacca	this study
A7	RBS	tttaaagaggagaaagctacca	this study
F12	RBS	ctaaagactagcctttcaatcaggaattcccagg	this study
D1	RBS	ctaaagactaccctttcaatcagggattcccagg	this study
RBS19	RBS	tactagagtcatttatgaaagtactag	(7)
R26790	RBS	tgtcaatttccgcgatagaggaggtaaag	(7)
T1	Terminator	ggcatcaaataaaacgaaaggctcagtcgaaagactgggcctttcgttttat ctgttgtttgtcggtgaacgctctcctgagtaggacaaatccgccgccctag a	(12)
T7	Terminator	tagcataaccccttggggcctctaaacgggtcttgaggggttttttg	(7)
L3S2P21	Terminator	ctcggtaccaaattccagaaaagaggcctcccgaaaggggggccttttttcg ttttggtcc	(13)
L3S3P22	Terminator	ccaattattgaaggccgctaacgcggcctttttttgtttctggtctccc	(13)
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L3S1P13	Terminator	gacgaacaataaggcctccctaacggggggccttttttattgataacaaaa	(13)
L3S3P11	Terminator	ccaattattgaacacccttcggggtgttttttttgtttctggtctccc	(13)
L3S2P11	Terminator	ctcggtaccaaattccagaaaagagacgctttcgagcgtcttttttcgtttt ggtcc	(13)
L3S2P55	Terminator	ctcggtaccaaagacgaacaataagacgctgaaaagcgtcttttttcgtttt ggtcc	(13)
BydvJ	Insulator	ggtgtctcaaggtgcgtaccttgactgatgagtccgaaaggacgaaacacc	(3)
RiboJ	Insulator	ctgtcaccggatgtgctttccggtctgatgagtccgtgaggacgaaacag	(2)
YFLVQ	N-degron	tatctgtttgtgcag	(6)
FLFVQ	N-degron	ttcttattcgtgcaa	(6)
LVA	C-degron	gcagcgaacgacgaaaactatgccctggtagcc	(8)
TEV ENLYFQ	Protease site	gaaaacctgtattttcag	(14)
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araC	gene	atggctgaagcgcaaaatgatcccctgctgccgggatactcgtttaatgccc atctggtggcgggtttaacgccgattgaggccaacggttatctcgattttt tatcgaccgaccgctgggaatgaaaggttatattctcaatctcaccattcgc ggtcagggggtggtgaaaaatcagggacgagaatttgtttgccgaccgggtg atattttgctgttcccgccaggagagattcatcactacggtcgtcatccgga ggctcgcgaatggtatcaccagtgggtttactttcgtccgcgcgcctactgg catgaatggcttaactggccgtcaatatttgccaatacggggttctttcgcc cggatgaagcgcaccagccgcatttcagcgacctgtttgggcaaatcattaa	

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core-sz17	gene	atgaacacgattaacatcgctaagaacgacttctctgacatcgaactggctg ctatcccgttcaacactctggctgaccattacggtgagcgtttagctcgcga acagttggcccttgagcatgagtcttacgagatgggtgaagcacgcttccgc aagatgtttgagcgtcaacttaaagctggtgaggttgcggataacgctgccg ccaagcctctcatcactaccctactccctaagatgattgcacgcatcaacga ctggtttgaggaagtgaaagctaagcgcggcaagcgcccgacagccttccag ttcctgcaagaaatcaagccggaagccgtagcgtacatcaccattaagacca ctctggcttgcctaaccagtgctgacaatacaaccgttcaggctgtagcaag cgcaatcggtcgggccattgaggacgaggctcgcttcggtcgtatccgtgac cttgaagctaagcacttcaagaaaaacgttgaggaacaactcaacaagcgcg tagggcacgtctacaagaaagcatttatgcaagttgtcgaggctgacatgct ctctaagggtctactcggtggcgaggcgtggtcttcgtggcataaggaagac tctattcatgtaggagtacgctgcatcgagatgctcattgagtcaaccggaa tggttagcttacaccgccaaaatgctggcgtagtaggtcaagactctgagac tatcgaactcgcacctgaatacgctgaggctatcgcaacccgtgcaggtgcg ctggctggcatctctccgatgttccaaccttgcgtagttcctcctaagccgt ggactggcattactggtggtggctattgggctaacggtcgtcgtcctctggc gctggtgcgtactcacagtaagaaagcactgatgcgctacgaagacgtttac atgcctgaggtgtacaaagcgattaacattgcgcaaaacaccgcatggaaaa tcaacaagaaagtcctagcggtcgccaacgtaatcaccaagtggaagcattg tccggtcgaggacatccctgcgattgagcgtgaagaactcccgatgaaaccg gaagacatcgacatgaatcctgaggctctcaccgcgtggaaacgtgctgccg ctgctgtgtaccgcaaggacaaggctcgcaagtctcgccgtatcagccttga gttcatgcttgagcaagccaataagtttgctaaccataaggccatctggttc ccttacaacatggactggcgcggtcgtgtttacgctgtgtcaatgttcaacc cgcaaggtaacgatatgaccaaaggactgcttacgctggcgaaaggtaaacc aatcggtaaggaaggttactactggctgaaaatccacggtgcaaactgtgcg ggtgtcgacaaggttccgttccctgagcgcatcaagttcattgaggaaaacc acgagaacatcatggcttgcgctaagtctccactggagaacacttggtgggc tgagcaagattctccgttctgcttccttgcgttctgctttgagtacgctggg gtacagcaccacggcctgagctataactgctcccttccgctggcgtttgacg ggtcttgctctggcatccagcacttctccgcgatgctccgagatgaggtagg tggtcgcgcggttaacttgcttcctagtgaaaccgttcaggacatctacggg attgttgctaagaaagtcaacgagattctacaagcagacgcaatcaatggga ccgataacgaagtagttaccgtgaccgatgagaacggaggttcaggtggtgg atccaacgaaaaagaagaactgaaatccaaaaaagcggaactgcgcaaccgt atcgaacagctgaaacagaaacgtgaacaactgaagcagaaaatcgcgaacc tgcgtaaagaaatcgaagcttacaaataa	(7)
sz18-σ–T3	gene	atgagcatcgcggcgaccctggagaacgatctggcgcgtctggaaaacgaaa acgctcgtctcgaaaaagacatcgcgaacctggaacgtgacctggcgaaact ggagcgtgaagaagcgtacttcggaggttcaggtggtaagaacactggtgaa atctctgagaaagtcaagctgggcactaaggcactggctggtcaatggctgg cttacggtgttactcgcagtgtgactaagcgttcagtcatgacgctggctta cgggtccaaagagttcggcttccgtcaacaagtgctggaagataccattcag ccagctattgattccggcaagggtctgatgttcactcagccgaatcaggctg ctggatacatggctaagctgatttgggaatctgtgagcgtgacggtggtagc tgcggttgaagcaatgaactggcttaagtctgctgctaagctgctggctgct gaggtcaaagataagaagactggagagattcttcgcaagcgttgcgctgtgc attgggtaactcctgatggtttccctgtgtggcaggaatacaagaagcctat tcagaagcgcctggacatgattttcttgggtcaatttcgcttgcaacctacc attaacaccaacaaagatagcgagattgatgcacacaaacaggagtctggta tcgctcctaactttgtacacagccaagacggtagccaccttcgtaagactgt agtgtgggcacacgagaagtacggaatcgaatcttttgcactgattcacgac tccttcggtacgattccggctgacgctgcgaacctgttcaaagcagtgcgcg aaactatggttgacacatatgagtcttgtgatgtactggctgatttctacga ccagttcgctgaccagttgcacgagtctcaattggacaaaatgccagcactt ccggctaaaggtaacttgaacctccgtgacatcttagagtcggacttcgcgt tcgcgtaa	(7)

Table S2. Accession number for plasmids used in this study

Plasmid name	GenBank accession number
ptevF-TetR	KX353594
ptevF-PhlF	KX353595
pNus-TetR	KX353596
pTig-PhlF	KX353597
pTac-TEV	KX353598
pTac-Su	KX353599
pTEV	KX353600
pTVMV	KX353601
pSuMMV	KX353602
ptevY-GFP	KX353603
ptvmvY-GFP	KX353604
psummvY-GFP	KX353605
pPoly-Deg	KX353606
pPoly-Rel	KX353607
pPoly-Switch	KX353608
pTac-TEV-Su	KX353609
pCore	KX353610
pTac-T3	KX353611
pTac-T3-Su	KX353612
pT3-GFP-LVA	KX353613
pBAD-Su	KX353614

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