Supplementary Tables

Supplementary Table 1. Amount of transfected plasmids in each well of 96-well plate used for realization of individual logic functions using the firefly luciferase activity readout. Reporter *Renilla luciferase* (10 ng) was used for normalization of transfection.

Input plasmids	ng	Reporter plasmid	ng	Inducer molecule
Protease orthogonality. Figure 1, Supplementary Figure 1d				
TEVp	70	CycLuc_TEVs	100	/
PPV	70			
SbMVp	70			
SuMMVp	70			
TEVp	70	CycLuc_PPVs	100	/
PPV	70			
SbMVp	70			
SuMMVp	70			
TEVp	70	CycLuc_SbMVs	100	/
PPV	70			
SbMVp	70			
SuMMVp	70			
TEVp	70	CycLuc_SuMMVs	100	/
PPV	70			
SbMVp	70			
SuMMVp	70			
Split protease titration. Figure 1				
FRB-nTEVp FKBP-cTEVp	0; 0	CycLuc_TEVs	100	Rapamycin
	10; 10			Rapamycin
	20; 20			Rapamycin
	40; 40			Rapamycin
	70, 70			Rapamycin
	70, 70			/
TEVp	70			/
FRB-nPPVp FKBP-cPPVp	0; 0	CycLuc_PPVs	100	Rapamycin
	10; 10			Rapamycin
	20; 20			Rapamycin
	40; 40			Rapamycin
	70, 70			Rapamycin
	70, 70			/
PPVp	70			/
FRB-nSbMVp FKBP-cSbMVp	0; 0	CycLuc_SbMVs	100	Rapamycin
	10; 10			Rapamycin
	20; 20			Rapamycin
	40; 40			Rapamycin
	70, 70			Rapamycin
	70, 70			/
SbMVp	70			/
FRB-nSuMMVp FKBP-cSuMMVp	0; 0	CycLuc_SuMMVs	100	Rapamycin
	10; 10			Rapamycin
	20; 20			Rapamycin
	40; 40			Rapamycin
	70, 70			Rapamycin
	70, 70			/
SuMMVp	70			/
Optimization of split protease reconstitution. Figure 2b, Supplementary Figure 2				
ABI-cTEVp PYL1-nTEVp	5 10 20	nLuc_AP4_TEVs_P3mS P3_cLuc	10 10	ABA
FRB-nTEVp FKBP-cTEVp	50 80 110	nLuc_AP4_TEVs_P3mS P3_cLuc	10 10	Rapamycin
Protease-inactivated module (logical negation). Figure 2d				
nLuc_TEVs_AP4 P3_PPVp_cLuc	10 5	PPVp	0; 10; 25; 50	
P3_PPVs_cLuc nLuc_TEVs_AP4	10 5	TEVp	0; 10; 25; 50	

Input plasmids	ng	Reporter plasmid	ng	Inducer molecule
Inverter. Supplementary Figure 12a				
FRB-nPPVp FKBP-cPPVp P4_PPVs_cTEVp P3_PPVs_nTEVp	60 60 40 40	nLuc_TEVs_AP10 10 P9_TEVs_cLuc 10	10 10	/
FRB-nPPVp FKBP-cPPVp P4_PPVs_cTEVp P3_PPVs_nTEVp	60 60 40 40	nLuc_TEVs_AP10 10 P9_TEVs_cLuc 10	10 10	Rapamycin
P4_PPVs_cTEVp P3_PPVs_nTEVp	40 40	nLuc_TEVs_AP10 10 P9_TEVs_cLuc 10	10 10	/
PPVp P4_PPVs_cTEVp P3_PPVs_nTEVp	90 40 40	nLuc_TEVs_AP10 10 P9_TEVs_cLuc 10	10 10	/
Double Inverter. Figure 4b, Supplementary Figure 12b				
P9_SbMVs_nPPVp P10_SbMVs_cPPVp P4_PPVs_cTEVp P3_PPVs_nTEVp	20 20 10 10	cycLuc_TEVs	70	Rapamycin
FRB-nSbMVp FKBP-cSbMVp P9_SbMVs_nPPVp P10_SbMVs_cPPVp P4_PPVs_cTEVp P3_PPVs_nTEVp	55 55 20 20 10 10	cycLuc_TEVs	70	/
FRB-nSbMVp FKBP-cSbMVp P9_SbMVs_nPPVp P10_SbMVs_cPPVp P4_PPVs_cTEVp P3_PPVs_nTEVp	55 55 20 20 10 10	cycLuc_TEVs	70	Rapamycin
SbMVp P9_SbMVs_nPPVp P10_SbMVs_cPPVp P4_PPVs_cTEVp P3_PPVs_nTEVp	55 20 20 10 10	cycLuc_TEVs	70	
Two-layer protease cascade. Figure 4a, Supplementary Figure 11				
SbMVp AP4_SbMVs_P3_nTEVp P4_PPVs_cTEVp	90 20 5	cycLuc_TEVs	70	
/ AP4_SbMVs_P3_nTEVp P4_PPVs_cTEVp	 20 5	cycLuc_TEVs	70	
SbMVp cTEVp*_AP4_SbMVs_P3_nTEVp P4_PPVs_cTEVp	90 20 5	cycLuc_TEVs	70	
/ cTEVp*_AP4_SbMVs_P3_nTEVp P4_PPVs_cTEVp	90 20 5	cycLuc_TEVs	70	
TEVp variants. Supplementary Figure 1b				
/ TEVp TEVpE TEVpH	0 100 100 100	Fluc_TEVs	25	
/ TEVp TEVpE TEVpH	0 100 100 100	Fluc_TEVsE	25	
/ TEVp TEVpE TEVpH	0 100 100 100	Fluc_TEVsH	25	

Supplementary Table 2. Amount of transfected plasmids in each well of 96-well plate used for realization of individual logic functions using the firefly luciferase activity readout. Reporter *Renilla luciferase* (10 ng) was used for normalization of transfection.

SPOC-based logic functions. Figure 3. Supplementary Figure 9					
A	**B**	**Input plasmids**	**(ng)**	**Logic function plasmids**	**(ng)**
A					
0	0	/	/		
1	0	pCMV_TEVp	90	nLuc_AP4_TEVs_P3mS	10
0	1	pCMV_PPVp	90	P3_cLuc	10
1	1	pCMV_TEVp, pCMV_PPVp	90; 90		
B					
0	0	/	/		
1	0	pCMV_TEVp	90	nLuc_AP4_PPVs_P3mS	10
0	1	pCMV_PPVp	90	P3_cLuc	10
1	1	pCMV_TEVp, pCMV_PPVp	90; 90		
NOT A					
0	0	/	/		
1	0	pCMV_TEVp	90	nLuc_TEVs_AP4	10
0	1	pCMV_PPVp	20	P3_cLuc	10
1	1	pCMV_TEVp, pCMV_PPVp	90; 20		
NOT B					
0	0	/	/		
1	0	pCMV_TEVp	90	nLuc_AP4	10
0	1	pCMV_PPVp	90	P3_PPV_cLuc	10
1	1	pCMV_TEVp, pCMV_PPVp	90; 90		
AND					
0	0	/	/		
1	0	pCMV_TEVp	90	nLuc_AP4_TEVs_P3mS	10
0	1	pCMV_PPVp	90	AP4_PPV_P3_cLuc	10
1	1	pCMV_TEVp, pCMV_PPVp	90; 90		
OR					
0	0	/	/		
1	0	pCMV_TEVp		nLuc_AP4_TEVs_PPVs_P3mS	10
0	1	pCMV_PPVp		P3_cLuc	10
1	1	pCMV_TEVp, pCMV_PPVp			
NOR					
0	0	/	/		
1	0	pCMV_TEVp	90	nLuc_TEVs_AP4	10
0	1	pCMV_PPVp	90	P3_PPV_cLuc	10
1	1	pCMV_TEVp, pCMV_PPVp	90; 90		
A nimply B					
0	0	/			
1	0	pCMV_TEVp	90	nLuc_AP4_TEVs_P3mS	10
0	1	pCMV_PPVp	20	P3_PPV_cLuc	10
1	1	pCMV_TEVp, pCMV_PPVp	90; 20		
B nimply A					
0	0	/	/		
1	0	pCMV_TEVp	90	AP4mS_PPV_P3_cLuc	10
0	1	pCMV_PPVp	20	nLuc_TEVs_AP4	10
1	1	pCMV_TEVp, pCMV_PPVp	90; 20		
NAND					
0	0	/	/	nLuc_AP10	10
1	0	pCMV_TEVp	90	P9_PPVs_cLuc	10
0	1	pCMV_PPVp	90	AP4mS_PPVs_P3_TEVs_cLuc	20
1	1	pCMV_TEVp, pCMV_PPVp	90; 90	nLuc-TEVs-AP4	20

SPOC-based logic functions Figure 3. Supplementary Figure 9					
A	**B**	**Input plasmids**	**(ng)**	**Logic function plasmids**	**(ng)**
XOR (A nimply B + orthogonal B nimply A)					
0	0	/	/	nLuc_AP4_TEVs_P3mS	10
1	0	pCMV_TEVp	90	P3_PPV_cLuc	10
0	1	pCMV_PPVp	25	nLuc_AP10_PPV_P9mS	10
1	1	pCMV_TEVp, pCMV_PPVp	90; 25	P9_TEVs_cLuc	10
XNOR (AND + orthogonal NOR)					
0	0	/		nLuc_AP4_TEVs_P3mS	10
1	0	pCMV_TEVp	90	AP4_PPV_P3_cLUc	10
0	1	pCMV_PPVp	90	nLuc_PPV_AP10	13
1	1	pCMV_TEVp, pCMV_PPVp	90; 90	P9_TEVs_cLuc	13
A imply B (AND + orthogonal NOT A)					
0	0	/	/	nLuc_AP4_TEVs_P3mS	10
1	0	pCMV_TEVp	90	AP4_PPV_P3_cLuc	10
0	1	pCMV_PPVp	20	nLuc_AP10	10
1	1	pCMV_TEVp, pCMV_PPVp	90; 20	P9_TEVs_cLuc	10
B imply A (A + orthogonal NOR)					
0	0	/		nLuc_AP4_TEVs_P3mS	10
1	0	pCMV_TEVp	90	P3_cLuc	10
0	1	pCMV_PPVp	90	nLuc_PPV_AP10	20
1	1	pCMV_TEVp, pCMV_PPVp	90; 90	P9_TEVs_cLuc	20

Supplementary Table 3. Amount of transfected plasmids in each well of 96-well plate used for realization of individual logic functions using the firefly luciferase activity readout. Reporter *Renilla luciferase* (10 ng) was used for normalization of transfection.

Input plasmids	ng	Reporter plasmid	ng
Orthogonality of split proteases, Supplementary Figure 1f,g			
FRB_nTEVp	60	cycLuc_TEVs	90
FKBP_cTEVp	60		
FRB_nPPVp	60		
FKBP_cPPVp	60		
FRB_nTEVp	60	cycLuc_PPVs	90
FKBP_cTEVp	60		
FRB_nPPVp	60		
FKBP_cPPVp	60		
AND logic function protease titration, Supplementary Figure 10			
pCMV_TEVp	0;10;25;50	nLuc_AP4_TEVs_P3mS	10
pCMV_PPVp	0; 10; 25; 50	AP4mS_PPVs_P3_cLu	10
A logic function titration of autoinhibitor and displacer. Supplementary Figure 5b,c			
pCMV_TEVp	50	nLuc_AP4_TEVs_P3mS	0; 2,5; 5; 10
		P3_cLuc	0; 2,5; 5; 10
pCMV_TEVp	0	nLuc_AP4_TEVs_P3mS	0; 2,5; 5; 10
		P3_cLuc	0; 2,5; 5; 10
A logic function CC variations. Supplementary Figure 6c			
pCMV_TEVp	0; 50	nLuc_AP4_TEVs_P3mS	10
		P3_cLuc	0; 2,5; 5; 10; 15; 20
pCMV_TEVp	0; 50	nLuc_AP4_TEVs_P3mS^{2}A$_{2}$	10
		P3_cLuc	0; 2,5; 5; 10; 15; 20
pCMV_TEVp	0; 50	nLuc_AP4_TEVs_P3mS^{1}A^{2}A	10
		P3_cLuc	0; 2,5; 5; 10; 15; 20
pCMV_TEVp	0; 50	nLuc_AP4_TEVs_P3mS^{1}A$_{2}$^{2}A$_{2}$	10
		P3_cLuc	0; 2,5; 5; 10; 15; 20
Coiled-coil orthogonality. Supplementary Figure 3c			
nLuc_AP4;	25; 50; 100	P3_cLuc	100
nLuc_AP10;	100	P3_cLuc	100
nLuc_AP10;	25; 50; 100	P9_cLuc	100
nLuc_AP4;	100	P9_cLuc	100
Coiled-coil orthogonality. Supplementary Figure 3d			
P3cLuc	0; 25; 50; 100	nLucAP4	10; 25; 50
Protease-inactivated module (logical negation). Supplementary Figure 7b			
nLuc_TEVs_AP4	0; 10; 25; 50	PPVp	0; 10; 25; 50
P3_PPVs_cLuc	5		

Supplementary Table 4. Amount of transfected plasmids in each well of 96-well plate used for realization of individual logic functions using the firefly luciferase activity readout. Reporter *Renilla luciferase* (10 ng) was used for normalization of transfection.

		Input	ng	Logic function plasmids	ng	Reporter	ng
Logic function with HIVp. Figure 4c							
		NIMPLY					
		/	/				
		pCMV_HIVp	80	cTEV*_AP4_HIVs_P3_nTEV	10	cycLuc_TEVs	60
		pCMV_PPVp	90	P4mS_cTEV	5		
		pCMV_HIVp, pCMV_PPVp	80; 90				
Logic function AND ; Supplementary Fig. 13							
		N2A					
0	0	/	/				
1	0	pCMV_TEVp	90	nLuc_AP4_TEVs_P3mS	10		
0	1	pCMV_PPVp	90	AP4_PPV_P3_cLuc	10		
1	1	pCMV_TEVp, pCMV_PPVp	90; 90				
		HeLa					
0	0	/	/				
1	0	pCMV_TEVp	90	nLuc_AP4_TEVs_P3mS	10		
0	1	pCMV_PPVp	90	AP4_PPV_P3_cLuc	10		
1	1	pCMV_TEVp, pCMV_PPVp	90; 90				
		NIH					
0	0	/	/				
1	0	pCMV_TEVp	90	nLuc_AP4_TEVs_P3mS	10		
0	1	pCMV_PPVp	90	AP4_PPV_P3_cLuc	10		
1	1	pCMV_TEVp, pCMV_PPVp	90; 90				
		CHO					
0	0	/	/				
1	0	pCMV_TEVp	90	nLuc_AP4_TEVs_P3mS	10		
0	1	pCMV_PPVp	90	AP4_PPV_P3_cLuc	10		
1	1	pCMV_TEVp, pCMV_PPVp	90; 90				

Supplementary Table 5. Amount of transfected plasmids in each well of 96-well plate used for realization of individual logic functions using the firefly luciferase activity readout. Reporter *Renilla luciferase* (10 ng) was used for normalization of transfection.

Inducible SPOC-based logic functions; Figure 5e-h; Supplementary Figure 14						
A	**B**	**Inducer**	**Input plasmids**	**ng**	**Logic function plasmids**	**ng**
		B				
0	0	/				
1	0	ABA	ABI_cTEVp; PYL1_nTEVp	90; 90	nLuc_AP4_PPVs_P3mS	10
0	1	Rapamycin	FRB_nPPVp; FKBP_cPPVp	10; 10	P3_cLuc	10
1	1	ABA, Rapamycin				
		AND				
0	0	/				
1	0	ABA	ABI_cTEVp; PYL1_nTEVp	90; 90	nLuc_AP4_TEVs_P3mS	10
0	1	Rapamycin	FRB_nPPVp; FKBP_cPPVp	10; 10	AP4_PPV_P3_cLuc	10
1	1	ABA, Rapamycin				
		OR				
0	0	/				
1	0	Rapamycin	FRB_nTEVp; FKBP_cTEVp	70; 70	nLuc_AP4_TEVs_PPVs_P3mS	10
0	1	ABA	ABI_cPPVp; PYL1_nPPVp	30; 30	P3_cLuc	10
1	1	Rapamycin; ABA				

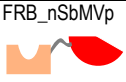
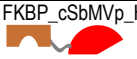
Supplementary Table 6. Amount of transfected plasmids in each well of 96-well plate used for realization of individual logic functions using the firefly luciferase activity readout. Reporter *Renilla luciferase* (10 ng) was used for normalization of transfection.

Input plasmids	ng	Reporter plasmid	ng	Inducer molecule
***In situ* kinetics of split protease reconstitution, Figure 5b,c**				
A				
ABI-cTEVp	10	nLuc_AP4_TEVs_P3mS	10	ABA
PYL1-nTEVp	10	P3_cLuc	10	
B				
FRB-nPPVp	90	nLuc_AP4	10	Rapamycin
FKBP-cPPVp	90	AP4mS_PPVs_P3_cLuc	10	
***In situ* translational activation, Figure 5b,c**				
dCAS9_ABI	25	pmin_fLuc	20	ABA
PYL_VPR	25	sgRNA	25	

Supplementary Table 7. Amount of transfected plasmids in each well of a 6-well plate used for the western blot (**Supplementary Figure 15**).

Input plasmids	ng	Inducer molecule
Imunoblotting split luciferase reporters		
nLUC_AP4_TEVs_P3mS	1000	0
		ABA
		Rapamycin
		ABA+Rapamycin
AP4ms_PPVs_P3_cLuc	1000	0
		ABA
		Rapamycin
		ABA+Rapamycin
pCDNA3	1000	0
		ABA
		Rapamycin
		ABA+Rapamycin
Imunoblotting split protease		
pcDNA3	1000	0
		Rapamycin
PYL1_nTEVp	1000	0
		ABA
ABI_cTEVp	1000	0
		ABA
PYL1_nPPVp	1000	0
		ABA
ABI1_cPPVp	1000	0
		ABA
pcDNA3	1000	0
		ABA

Supplementary Table 8. Amino acid sequences of constructs used in this study

Potyviral proteases	
TEVp 	M EQKLISEEDL GESLFKGPRDYNPISSTICHLTNESDGHTTSLYGIGFGPFIITNKHLFRRNNGTLLVQSLHGVFKVKNTTTLQQHLIDGRDMII IRMPKDFPPFPQKLKFREPQREERICLVTTNFQTKSMSSMVSDTSCTFPSSDGIFWKHWIQTKDGQCGSPLVSTRDGFIVGIHSASNFTNTNNYFTS VPKNFMELLTNQEAQQWVSGWRLNADSVLWGGHKVFMSKPEEPFQPVKEATQLMSELVYSQYPYDVPDYA Dark blue: Myc tag; Black: TEVp
PPVp 	MSKSLFRGLRDYNPIASSICQLNNSSGARQSEMFGLGFGGLIVTNQHLFKRNDGELTIRSHHGEFVVKDTKTLKLLPCKGRDIVIIRLPKDFPPFPK RLQFRTPTTEDRVCLIGSNFQTKSISSTMSETSATYPVDNSHFWKHWISTKDGHCGLPIVSTRDGSILGLHSLANSTNTQNFYAAFPDNFETTYLSN QDNDNWIKQWRYNPDEVCWGSLQLKRDIPQSPFTICKLLTDLDGEFVYTQ Black: PPVp
SbMVp 	MSKSVYKGLRDYSGISTLICQLTNSSDGHKETMFGVGYGSFIITNGHLFRRNNGMLTVKTWHGEFVIHNTTQLKIHFIQGRDVILIRMPKDFPPFGK RNLFRQPKREERVCMVGTNFQEKSLRATVSESSMILPEGKGSFWIHWITTQDGFCGLPLVSVNDGHIVGIHGLTSNDSEKNFFVPLTDGFEKEYLEN ADNLSWDKHWFWEPSKIAWGSLNLVEEQPKEEFKISKLVSDLFGNTVTVQ YPYDVPDYA Dark blue: HA tag; Black: SbMVp
SuMMVp 	MGVSLSRGVRDYNAISSMVCRVTNDSGSSSTTMYGIGYGCYIITNKHLFRENNGRLLITSHHGEYICKNSASLKLSLVPGRDMLLIRLPKDCPPFPS KLKFREPTSEEKAVLVVTNFQEKHLSSMVSESSCVVQREDSPIWRHWISTKDGHCGAPIVSIRDGYIIGSHCGENPMTSNFFTSIPKDFQNLLNGKE ANEWVSGWKYNIDAVCWGGLSVVNDAPSEPFITAKVVSALDTEGIKVQ YPYDVPDYA Dark blue: HA tag; Black: SuMMVp
Inducible split proteases	
FRB_nTEVp 	M EQKLISEEDL ILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLY YHVFRRISK GSGS GESLFKGPRDYNPISSTICHLTNESDGHTTSLYGIGFGPFIITNKHLFRRNNGTLLVQSLHGVFKVKNTTTLQQHLIDGRDM IIIRMPKDFPPFPQKLKFREPQREERICLVTTNFQT Dark blue: Myc tag; Black: FRB; Magenta: nTEVp
FKBP_cTEVp 	M GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHA TLVFDVELLKLE GSG KSMSSMVSDTSCTFPSSDGIFWKHWIQTKDGQCGSPLVSTRDGFIVGIHSASNFTNTNNYFTSVPKNFMELLTNQEAQQW VSGWRLNADSVLWGGHKVFMSKPEEPFQPVKEATQLMSELVYSQ Black: FKBP; Magenta: cTEVp;
PYL1_nTEVp 	M DTYRYI GGGAPTQDEFTQLSQSIAEFHTYQLGNGRCSSLLAQRIHAPPETVWSVVRRFDRPQIYKHFIKSCNVSEDFEMRVGCTRDVNVISGL PANTSRERLDLLDDDRRVTGFSITGGEHRLRNYKSVTTVHRFEKEEEEERIWTVVLESYVVDVPEGNSEEDTRLFADTVIRLNLQKLASITEAMN G SGSS GESLFKGPRDYNPISSTICHLTNESDGHTTSLYGIGFGPFIITNKHLFRRNNGTLLVQSLHGVFKVKNTTTLQQHLIDGRDMIIIRMPKDFP PFPQKLKFREPQREERICLVTTNFQT Dark blue: AU1 tag Black: PYL1; Magenta: nTEVp
ABI_cTEVp 	M EQKLISEEDL TRVPLYGFTSICGRRPEMEAAVSTIPRFLQSSSGSMLDGRFDPQSAAHFFGVYDGHGGSQVANYCRERMHLALAEEIAKEKPML CDGDTWLEKWKKALFNSFLRVDSEIESVAPETVGSTSVVAVVFPSHIFVANCGDSRAVLCRGKTALPLSVDHKPDREDEAARIEAAGGKVIQWNGAR VFGVLAMSRSIGDRYLKPSIIPDPEVTAVKRVKEDDCLILASDGVWDVMTDEEACEMARKRILLWHKKNAVAGDASLLADERRKEGKDPAAMSAAEY LSKLAIQRGSKDNISVVVVDLK GSGS KSMSSMVSDTSCTFPSSDGIFWKHWIQTKDGQCGSPLVSTRDGFIVGIHSASNFTNTNNYFTSVPKNFM ELLTNQEAQQWVSGWRLNADSVLWGGHKVFMSKPEEPFQPVKEATQLMSELVYSQ Dark blue: Myc tag; Black: ABI; Magenta: cTEVp
FRB_nPPVp 	M EQKLISEEDL ILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLY YHVFRRISK GSGS SKSLFRGLRDYNPIASSICQLNNSSGARQSEMFGLGFGGLIVTNQHLFKRNDGELTIRSHHGEFVVKDTKTLKLLPCKGRDI VIIRLPKDFPPFPKRLQFRTPTTEDRVCLIGSNFQT Dark blue: Myc tag; Black: FRB; Cyan: nPPVp
FKBP_cPPVp 	M GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHA TLVFDVELLKLE GSG SKSISSTMSETSATYPVDNSHFWKHWISTKDGHCGLPIVSTRDGSILGLHSLANSTNTQNFYAAFPDNFETTYLSNQDND NWIKQWRYNPDEVCWGSLQLKRDIPQSPFTICKLLTDLDGEFVYTQ DTYRYI Black: FKBP; Cyan: cPPVp Dark blue: AU1 tag
PYL1_nPPVp 	M DTYRYI GGGAPTQDEFTQLSQSIAEFHTYQLGNGRCSSLLAQRIHAPPETVWSVVRRFDRPQIYKHFIKSCNVSEDFEMRVGCTRDVNVISGLP ANTSRERLDLLDDDRRVTGFSITGGEHRLRNYKSVTTVHRFEKEEEEERIWTVVLESYVVDVPEGNSEEDTRLFADTVIRLNLQKLASITEAMN GS GSS KSLFRGLRDYNPIASSICQLNNSSGARQSEMFGLGFGGLIVTNQHLFKRNDGELTIRSHHGEFVVKDTKTLKLLPCKGRDIVIIRLPKDFPPF PKRLQFRTPTTEDRVCLIGSNFQT Dark blue: AU1 tag Black: PYL1; Cyan: nPPVp
ABI_cPPVp 	M EQKLISEEDL TRVPLYGFTSICGRRPEMEAAVSTIPRFLQSSSGSMLDGRFDPQSAAHFFGVYDGHGGSQVANYCRERMHLALAEEIAKEKPML CDGDTWLEKWKKALFNSFLRVDSEIESVAPETVGSTSVVAVVFPSHIFVANCGDSRAVLCRGKTALPLSVDHKPDREDEAARIEAAGGKVIQWNGAR VFGVLAMSRSIGDRYLKPSIIPDPEVTAVKRVKEDDCLILASDGVWDVMTDEEACEMARKRILLWHKKNAVAGDASLLADERRKEGKDPAAMSAAEY LSKLAIQRGSKDNISVVVVDLK GSGS SKSISSTMSETSATYPVDNSHFWKHWISTKDGHCGLPIVSTRDGSILGLHSLANSTNTQNFYAAFPDNFETTYLSNQDNDNWIKQWRYNPDEVCWGS LQLKRDIPQSPFTICKLLTDLDGEFVYTQ Dark blue: Myc tag; Black: ABI; Cyan: cPPVp
FRB_nSbMVp 	M EQKLISEEDL ILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLY YHVFRRISK GSGS SKSVYKGLRDYSGISTLICQLTNSSDGHKETMFGVGYGSFIITNGHLFRRNNGMLTVKTWHGEFVIHNTTQLKIHFIQGRD VILIRMPKDFPPFGKRNLFRQPKREERVCMVGTNFQE Dark blue: Myc tag; Black: FRB; Red: nSbMVc
FKBP_cSbMVp_HA 	M GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHA

TLVFDVELLKLE GSGS KSLRATVSESSMILPEGKGSFWIHWITTQDGFCGLPLVSVNDGHIVGIHGLTSNDSEKNFFVPLTDGFEKEYLENADNL
SWDKHWFWEPSKIAWGSLNLVEEQPKEEFKISKLVSDLFGNTVTVQ YPYDVPDYA
Black: FKBP; Red: cSbMVp; Dark blue: HA tag

PYL1_nSbMVp

M GGGAPTQDEFTQLSQSIAEFHTYQLGNGRCSSLLAQRIHAPPETVWSVVRRFDRPQIYKHFIKSCNVSEDFEMRVGCTRDVNVISGLPANTSRER
LDLLDDDRRVTGFSITGGEHRLRNYKSVTTVHRFEKEEEEERIWTVVLESYVVDVPEGNSEEDTRLFADTVIRLNLQKLASITEAMN GSGSS SKS
VYKGLRDYSGISTLICQLTNSSDGHKETMFGVGYGSFIITNGHLFRRNNGMLTVKTWHGEFVIHNTTQLKIHFIQGRDVILIRMPKDFPPFGKRNLF
RQPKREERVCMVGTNFQE
Black: PYL1; Red: nSbMVp

ABI_cSbMVp

M EQKLISEEDL TRVPLYGFTSICGRRPEMEAAVSTIPRFLQSSSGSMLDGRFDPQSAAHFFGVYDGHGGSQVANYCRERMHLALAEEIAKEKPML
CDGDTWLEKWKKALFNSFLRVDSEIESVAPETVGSTSVVAVVFPSHIFVANCGDSRAVLCRGKTALPLSVDHKPDREDEAARIEAAGGKVIQWNGAR
VFGVLAMSRSIGDRYLKPSIIPDPEVTAVKRVKEDDCLILASDGVWDVMTDEEACEMARKRILLWHKKNAVAGDASLLADERRKEGKDPAAMSAAEY
LSKLAIQRGSKDNISVVVVDLK GSGS KSLRATVSESSMILPEGKGSFWIHWITTQDGFCGLPLVSVNDGHIVGIHGLTSNDSEKNFFVPLTDGFE
KEYLENADNLSWDKHWFWEPSKIAWGSLNLVEEQPKEEFKISKLVSDLFGNTVTVQ
Dark blue: Myc tag; Black: ABI; Red: cSbMVp

FRB_nSuMMVp

M EQKLISEEDL ILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLY
YHVFRRISK GSGS GVSLSRGVRDYNAISSMVCRVTNDSGSSSTTMYGIGYGCYIITNKHLFRENNGRLLITSHHGEYICKNSASLKLSLVPGRDM
LLIRLPKDCPPFPSKLKFREPTSEEKAVLVVTNFQE
Dark blue: Myc tag; Black: FRB; Purple: nSuMMVp

FKBP_cSuMMVp

M GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHA
TLVFDVELLKLE GSGS KHLSSMVSESSCVVQREDSPIWRHWISTKDGHCGAPIVSIRDGYIIGSHCGENPMTSNFFTSIPKDFQNLLNGKEANEW
VSGWKYNIDAVCWGGLSVVNDAPSEPFITAKVVSALDTEGIKVQ YPYDVPDYA
Black: FKBP; Purple: cSuMMVp; Dark blue: HA tag

CC fused split proteases

P3_PPVs_nTEVp

M SPEDEIQQLEEEIAQLEQKNAALKEKNQALKY GGGSGGG **NVVVHQA** EQKLISEEDL GESLFKGPRDYNPISSTICHLTNESDGHTTSLYGI
GFGPFIITNKHLFRRNNGTLLVQSLHGVFKVKNTTTLQQHLIDGRDMIIIRMPKDFPPFPQKLKFREPQREERICLVTTNFQT

Green: P3; **Cyan: PPVs cleavage site;** Dark blue: Myc tag; Magenta: nTEVp

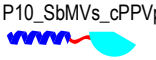
P4_PPVs_cTEVp

M SPEDKIAQLKQKIQALKQENQQLEEENAALEY GGGSGGG **NVVVHQA** KSMSSMVSDTSCTFPSSDGIFWKHWIQTKDGQCGSPLVSTRDGFIV
GIHSASNFTNTNNYFTSVPKNFMELLTNQEAQQWVSGWRLNADSVLWGGHKVFMSKPEEPFQPVKEATQLMSELVYSQ YPYDVPDYA
Green: P4; **Cyan: PPVs cleavage site;** Magenta: cTEVp; Dark blue: HA tag

P9_SbMVs_nPPVp

M SPEDENQALEQKNAQLKQEIAALEQEIAQLEYG GGSGGG **ESVSLQS** GGSGGS SKSLFRGLRDYNPIASSICQLNNSSGARQSEMFGLGFGG
LIVTNQHLFKRNDGELTIRSHHGEFVVKDTKTLKLLPCKGRDIVIIRLPKDFPPFPKRLQFRTPTTEDRVCLIGSNFQT
Blue: P9; **Red: SbMVs cleavage site;** Cyan: nPPVp

P10_SbMVs_cPPVp



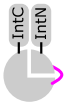
M SPEDKNAQLKEENAALEEKIQQLKEKIQALKYG GGSGGG **ESVSLQS** GGSGG SKSISSTMSETSATYPVDNSHFWKHWISTKDGHCGLPIVS
TRDGSILGLHSLANSTNTQNFYAAFPDNFETTYLSNQDNDNWIKQWRYNPDEVCWGSLQLKRDIPQSPFTICKLLTDLDGEFVYTQ
Blue: P10; **Red: SbMVs cleavage site;** Cyan: cPPVp

cTEVp*-AP4-SbMVs-P3-nTEVp

M KSMSSMVSDTSCTFPSSDGIFWKHWIQTKDGQAGSPLVSTRDGFIVGIHSASNFTNTNNYFTSVPKNFMELLTNQEAQQWVSGWRLNADSVLWGG
HKVFMSKPEEPFQPVKEATQLMSELVYSQYPYDVPDYA GGSGGGSGGS SPEDELAANEEELQQNEQKLAQIKQKLQAIKYG GGSGGG **ESVSLQ**
S GSSGSG SPEDEIQQLEEEIAQLEQKNAALKEKNQALKYG EQKLISEEDL GESLFKGPRDYNPISSTICHLTNESDGHTTSLYGIGFGPFIIT
NKHLFRRNNGTLLVQSLHGVFKVKNTTTLQQHLIDGRDMIIIRMPKDFPPFPQKLKFREPQREERICLVTTNFQT
Magenta: cTEVp*; Green: AP4; **Red: SbMVs cleavage site;** Green: P3; Dark blue: Myc tag; Magenta: nTEVp

Cyclic luciferase with protease cleavage site

CycLuc_TEVs



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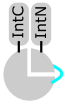
RSLQDYKIQSALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITPEGDDKPGAVGKVVPFFEA
KVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAG
VAGLPDDDAGELPAAVVVLEHGKTMTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILIKAKK GS **ENLYFQS** GG AKNIKK
GPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPAN
DIYNERELLNSMGISQPTVVFVSKKGLQKILNVQKKLPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNEYDFVPESFDRDKTIALIMNSSGSTG
LPKGVALPHRTACVRFSHARDPIFGNQIIP AEYCLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQWHDRGEQEVFEYCLEDG
SLIRATKDHKFMTVDGQMLPIDEIFERELDLMRVDNLPN GGKIAVNSACKNWFSSLSHFVIHLNSHGFPPEVEEQAAGTLPMSCAQESGMDRHPAA
CASARINV
Dark blue: His tag; Green: IntN; Black: CLuc; **Magenta: TEVs cleavage site;** Black: NLuc; Green: Cint

CycLuc_PPVs



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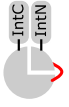
KVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAG
VAGLPDDDAGELPAAVVVLEHGKTMTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILIKAKK GS **NVVVHQA** GSG AKNIK
KGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPA
NDIYNERELLNSMGISQPTVVFVSKKGLQKILNVQKKLPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNEYDFVPESFDRDKTIALIMNSSGST
GLPKGVALPHRTACVRFSHARDPIFGNQIIP AEYCLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQWHDRGEQEVFEYCLED
GSLIRATKDHKFMTVDGQMLPIDEIFERELDLMRVDNLPN GGKIAVNSACKNWFSSLSHFVIHLNSHGFPPEVEEQAAGTLPMSCAQESGMDRHPA
ACASARINV
Dark blue: His tag; Green: IntN; Black: CLuc; **Cyan: PPVs cleavage site;** Black: NLuc; Green: Cint

CycLuc_SbMVs



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KVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAG
VAGLPDDDAGELPAAVVVLEHGKTMTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILIKAKK GS **ESVSLQS** GSG AKNIK
KGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPA
NDIYNERELLNSMGISQPTVVFVSKKGLQKILNVQKKLPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNEYDFVPESFDRDKTIALIMNSSGST

GLPKGVALPHRTACVRFSHARDPIFGNQIIP AEYCLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQWHDRGEQEVFEYCLED GSLIRATKDHKFMTVDGQMLPIDEIFERELDLMRVDNLPN GGKIAVNSACKNWFSSLSHFVIHLNSHGFPPEVEEQAAGTLPMSCAQESGMDRHPA ACASARINV

Dark blue: His tag; Green: IntN; Black: CLuc; **Red: SbMVs cleavage site**; Black: NLuc; Green: Cint

CycLuc_SuMMVs

M HHHHHH M IKIATRKYLGKQNVYDIGVERDHNFALKNGFIASNCFN DTYRYIDTAILSVVPFHHGFGMFTTLGYLICGFRVVLMYRFEEELFL RSLQDYKIQSALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITPEGDDKPGAVGKVVPFFEA KVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAG VAGLPDDDAGELPAAVVVLEHGKTMTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILIKAKK GS **EEIHLQS** GSG AKNIK KGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPA NDIYNERELLNSMGISQPTVVFVSKKGLQKILNVQKKLPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNEYDFVPESFDRDKTIALIMNSSGST GLPKGVALPHRTACVRFSHARDPIFGNQIIP AEYCLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQWHDRGEQEVFEYCLED GSLIRATKDHKFMTVDGQMLPIDEIFERELDLMRVDNLPN GGKIAVNSACKNWFSSLSHFVIHLNSHGFPPEVEEQAAGTLPMSCAQESGMDRHPA ACASARINV

Dark blue: His-tag; Green: IntN; Black: CLuc; **Purple: SuMMVs cleavage site;** Black: NLuc; Green: Cint

SPOC logic building modules

P3_cLuc

M SPEDEIQQLEEEIAQLEQKNAALKEKNQALKY GGGSGGGSGG STMTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILIK AKKGGKIAVNSGSG YPYDVPDYA

Green: P3; Black: cLuc; Dark blue: HA-tag

P3_PPVs_cLuc

M SPEDEIQQLEEEIAQLEQKNAALKEKNQALKY GGGSGGS **NVVVHQA** GGSGG STMTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKL DARKIREILIKAKKGGKIAVNSGSG YPYDVPDYA

Green: P3; **Cyan: PPVs cleavage site;** Black: cLuc; Dark blue: HA-tag

nLuc_AP4

M EQKLISEEDL GSG EDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVC SENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMGISQPTVVFVSKKGLQKILNVQKKLPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNE YDFVPESFDRDKTIALIMNSSGSTGLPKGVALPHRTACVRFSHARDPIFGNQIIPDTAILSVVPFHHGFGMFTTLGYLICGFRVVLMYRFEEELFLR SLQDYKIQSALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITPEGDDKPGAVGKVVPFFEAK VVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAGV AGLPDDDAGELPAAVVVLEHGK GGSGGGSGGS SPEDELAANEEELQQNEQKLAQIKQKLQAIKYG

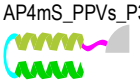
Dark blue: Myc-tag; Black: nLuc; Green: AP4

nLuc_TEVs_AP4

M EQKLISEEDL EDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVCSENS LQFFMPVLGALFIGVAVAPANDIYNERELLNSMGISQPTVVFVSKKGLQKILNVQKKLPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNEYDFV PESFDRDKTIALIMNSSGSTGLPKGVALPHRTACVRFSHARDPIFGNQIIPDTAILSVVPFHHGFGMFTTLGYLICGFRVVLMYRFEEELFLRSLQD YKIQSALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITPEGDDKPGAVGKVVPFFEAKVVDL DTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAGVAGLP DDDAGELPAAVVVLEHGK GGSGGS **ENLYFQS** GGSGGS SPEDELAANEEELQQNEQKLAQIKQKLQAIKYG

Dark blue: Myc-tag; Black: nLuc; **Magenta: TEVs cleavage site;** Green: AP4

AP4mS_PPVs_P3_TEVs_cLuc



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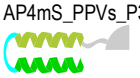
Green: AP4mS; **Cyan: PPVs cleavage site;** Green: P3; **Magenta: TEVs cleavage site;** Black: cLuc; Dark blue: HA tag

AP4mS_PPVs_P3_cLuc



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Green: AP4mS; **Cyan: PPVs cleavage site;** Green: P3; Black: cLuc; Dark blue: HA tag

nLuc_AP4_TEVs_P3mS

M EQKLISEEDL GSG EDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVC SENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMGISQPTVVFVSKKGLQKILNVQKKLPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNE YDFVPESFDRDKTIALIMNSSGSTGLPKGVALPHRTACVRFSHARDPIFGNQIIPDTAILSVVPFHHGFGMFTTLGYLICGFRVVLMYRFEEELFLR SLQDYKIQSALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITPEGDDKPGAVGKVVPFFEAK VVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAGV AGLPDDDAGELPAAVVVLEHGK GGSGGGSGGS SPEDELAANEEELQQNEQKLAQIKQKLQAIKYG GGSGGG **ENLYFQS** GGSGGS SPEDEIQ QLEEEISQLEQKNSQLKEKNQQLKYG

Dark blue: Myc tag; Black: nLuc; Green: AP4; **Magenta: TEVs cleavage site;** Green: P3mS

nLuc_AP4_PPVs_P3mS

M EQKLISEEDL GSG EDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVC SENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMGISQPTVVFVSKKGLQKILNVQKKLPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNE YDFVPESFDRDKTIALIMNSSGSTGLPKGVALPHRTACVRFSHARDPIFGNQIIPDTAILSVVPFHHGFGMFTTLGYLICGFRVVLMYRFEEELFLR SLQDYKIQSALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITPEGDDKPGAVGKVVPFFEAK VVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAGV AGLPDDDAGELPAAVVVLEHGK GGSGGGSGGS SPEDKLAQIKEKLQQIKEELAANEEKLQANKYG GGSGGS **NVVVHQA** GSGGGS SPEDENA QLEQKNAQLKQEISQLEQEISQLEW

Dark blue: Myc tag; Black: nLuc; Green: AP4; **Cyan: PPVs cleavage site;** Green: P3mS

nLuc_AP4_PPVs_TEVs_P3mS

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Myc tag; Black: nLuc; Green: AP4; **Cyan: PPVs cleavage site;** **Magenta: TEVs cleavage site;** Green: P3mS

P9_cLuc

M SPEDENQALEQKNAQLKQEIAALEQEIAQLEYG GGSGGGSGG STMTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILIK AKKGGKIAVNSGSG YPYDVPDYA

Blue: P9; Black: cLuc; Dark blue: HA tag

P9_TEVs_cLuc

M SPEDENQALEQKNAQLKQEIAALEQEIAQLEYG GGSGGG **ENLYFQS** GGSGGS TMTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKL
DARKIREILIKAKKGGKIAVNSSGSG YPYDVPDYA

Blue: P9; **Magenta: TEVs cleavage site;** Black: cLuc; Dark blue: HA tag

nLuc_AP10

M EQKLISEEDL GSGEDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVCS
ENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMGISQPTVVFVSKKGLQKILNVQKKLPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNEY
DFVPESFDRDKTIALIMNSSGSTGLPKGVALPHRTACVRFSHARDPIFGNQIIPDTAILSVVPFHHGFGMFTTLGYLICGFRVVLMYRFEEELFLRS
LQDYKIQSALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITPEGDDKPGAVGKVVPFFEAKV
VDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAGVA
GLPDDDAGELPAAVVVLEHGK GGSGGGSGGS SPEDKLAQIKEKLQQIKEELAANEEKLQANKYG

Dark blue: Myc tag; Black: nLuc; Blue: AP10

nLuc_PPVs_AP10

M EQKLISEEDL GSG EDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVC
SENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMGISQPTVVFVSKKGLQKILNVQKKLPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNE
YDFVPESFDRDKTIALIMNSSGSTGLPKGVALPHRTACVRFSHARDPIFGNQIIPDTAILSVVPFHHGFGMFTTLGYLICGFRVVLMYRFEEELFLR
SLQDYKIQSALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITPEGDDKPGAVGKVVPFFEAK
VVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAGV
AGLPDDDAGELPAAVVVLEHGK GGSGGG **NVVVHQA** GGSGGS SPEDKLAQIKEKLQQIKEELAANEEKLQANKYG

Dark blue: Myc tag; Black: nLuc; **Cyan: PPVs cleavage site;** Blue: AP10

nLuc_AP10_PPVs_P9mS

M EQKLISEEDL GSG EDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVC
SENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMGISQPTVVFVSKKGLQKILNVQKKLPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNE
YDFVPESFDRDKTIALIMNSSGSTGLPKGVALPHRTACVRFSHARDPIFGNQIIPDTAILSVVPFHHGFGMFTTLGYLICGFRVVLMYRFEEELFLR
SLQDYKIQSALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITPEGDDKPGAVGKVVPFFEAK
VVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAGV
AGLPDDDAGELPAAVVVLEHGK GGSGSG **ENLYFQS** GGSGGS SPEDKLAQIKEKLQQIKEELAANEEKLQANKYG GGSGGS **NVVVHQA** GSG
GGS SPEDENAQLEQKNAQLKQEISQLEQEI

Dark blue: Myc tag; Black: nLuc; Blue: AP10; **Magenta: TEVs cleavage site;** **Cyan: PPVs cleavage site;** Blue: P9mS

Transcriptional regulators

PYL1_NLS_VPR

M HHHHHH GGGAPTQDEFTQLSQSIAEFHTYQLGNGRCSSLLAQRIHAPPETVWSVVRRFDRPQIYKHFIKSCNVSEDFEMRVGCTRDVNVISGLP
ANTSRERLDLLDDDRRVTGFSITGGEHRLRNYKSVTTVHRFEKEEEEERIWTVVLESYVVDVPEGNSEEDTRLFADTVIRLNLQKLASITEAMN DP
KKKRKV EASGSGRADALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLINTSGGSGGGSGGSSQYLPDTDDRHRIEEKRK
RTYETFKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFPSGQISQASALAPAPPQVLPQAPAPAPAPAMV
SALAQAPAPVPVLAPGPPQAVAPPAPKPTQAGEGTLSEALLQLQFDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPVAPHTTEPMLMEYP
EAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADMDFSALLGSGSGSRDSREGMFLPKPEAGSAISDVFEGREVCQPKRIRPFHPPGSP
WANRPLPASLAPTPTGPVHEPVGSLTPAPVPQPLDPAPAVTPEASHLLEDPDEETSQAVKALREMADTVIPQKEEAAICGQMDLSHPPPRGHLDELT
TTLESMTEDLNLDSPLTPELNEILDTFLNDECLLHAMHISTGLSIFDTSLF

Dark blue: His tag; Black: PYL1; Red: nuclear localization signal; Orange: transcription activation domain VPR

dCas9_NLS_ABI

M HHHHHH DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNE
MAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIG
DQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPIL
EKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWN
FEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIEC
FDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIR
DKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQ
TTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRG
KSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKL
VSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRK
RPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKK
LKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNE
QKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ
SITGLYETRIDLSQLGGD DPKKKRKV TRVPLYGFTSICGRRPEMEAAVSTIPRFLQSSSGSMLDGRFDPQSAAHFFGVYDGHGGSQVANYCRERM
HLALAEEIAKEKPMLCDGDTWLEKWKKALFNSFLRVDSEIESVAPETVGSTSVVAVVFPSHIFVANCGDSRAVLCRGKTALPLSVDHKPDREDEAAR
IEAAGGKVIQWNGARVFGVLAMSRSIGDRYLKPSIIPDPEVTAVKRVKEDDCLILASDGVWDVMTDEEACEMARKRILLWHKKNAVAGDASLLADER
RKEGKDPAAMSAAEYLSKLAIQRGSKDNISVVVVDLK

Dark blue: His tag; Orange: dCas9; Red: nuclear localization signal; Black: ABI

sgRNA

TCTTCCGTTTCCACATCTTCA GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG
CTTTTTT

Orange: sgRNA; Black: sgRNA scaffold

HIVp logic

cTEVp*-AP4-HIVs-P3-nTEVp

M KSMSSMVSDTSCTFPSSDGIFWKHWIQTKDGQAGSPLVSTRDGFIVGIHSASNFTNTNNYFTSVPKNFMELLTNQEAQQWVSGWRLNADSVLWGG
HKVFMSKPEEPFQPVKEATQLMSELVYSQYPYDVPDYA GGSGGGSGGS SPEDELAANEEELQQNEQKLAQIKQKLQAIKYG GGSGGG **SQVSQN**
YPIVQNLQ GSSGSG SPEDEIQQLEEEIAQLEQKNAALKEKNQALKYG EQKLISEEDL GESLFKGPRDYNPISSTICHLTNESDGHTTSLYGIG
FGPFIITNKHLFRRNNGTLLVQSLHGVFKVKNTTTLQQHLIDGRDMIIIRMPKDFPPFPQKLKFREPQREERICLVTTNFQT

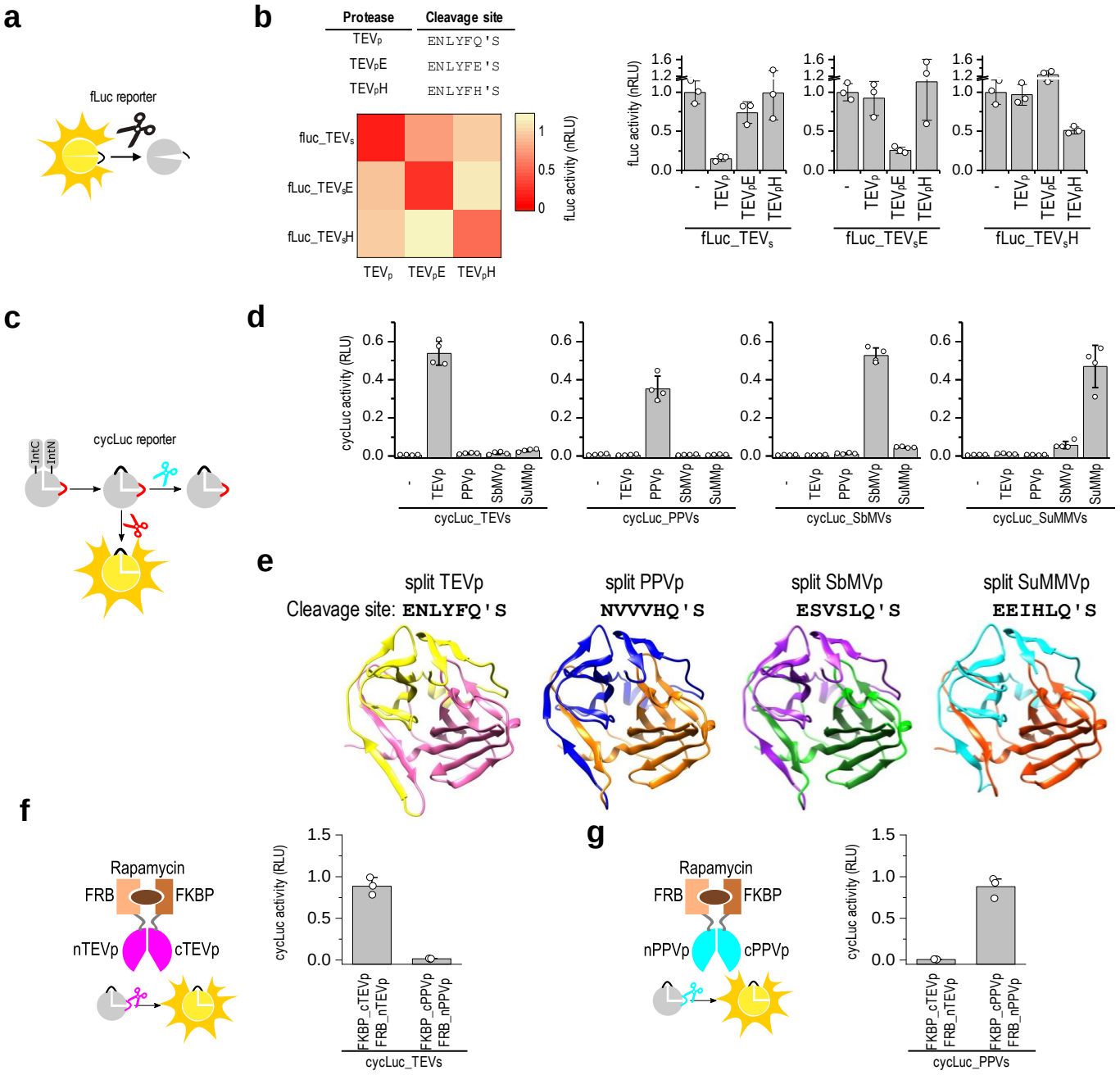
Magenta: cTEVp*; Green: AP4; **Orange: HIVs cleavage site;** Green: P3; Dark blue: Myc tag; Magenta: nTEVp

AP3mS_SbMVs_P4_PPVs_cTEVp

M SPEDKNQQLKEKNQSLKQEIQSLEEEIQQLEYG GGGSGGG ESVSLQS GSGSGG
SPEDKIAQLKQKIQALKQENQQLEEENAALEY GGGSGGG **NVVVHQA** KSMSSMVSDTSCTFPSSDGIFWKHWIQTKDGQCGSPLVSTRDGFIVG
IHSASNFTNTNNYFTSVPKNFMELLTNQEAQQWVSGWRLNADSVLWGGHKVFMSKPEEPFQPVKEATQLMSELVYSQ YPYDVPDYA

Tan: AP3mS; **Red: SbMVp cleavage site;** Green: P4; **Cyan: PPVs cleavage site;** Magenta: cTEVp; Dark blue: HA tag

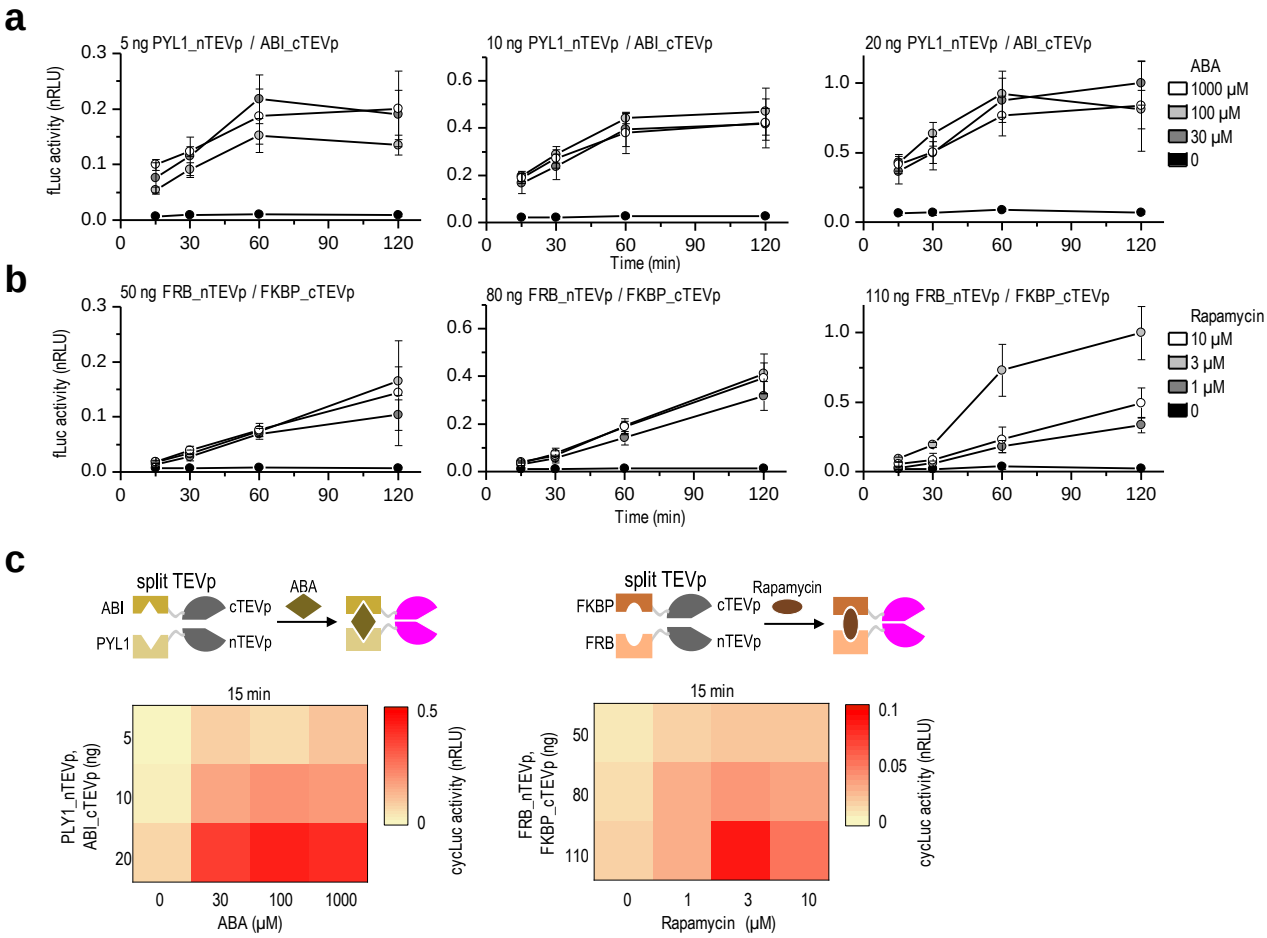
Supplementary Figures



Supplementary Figure 1. Design and orthogonality of split potyviral proteases.

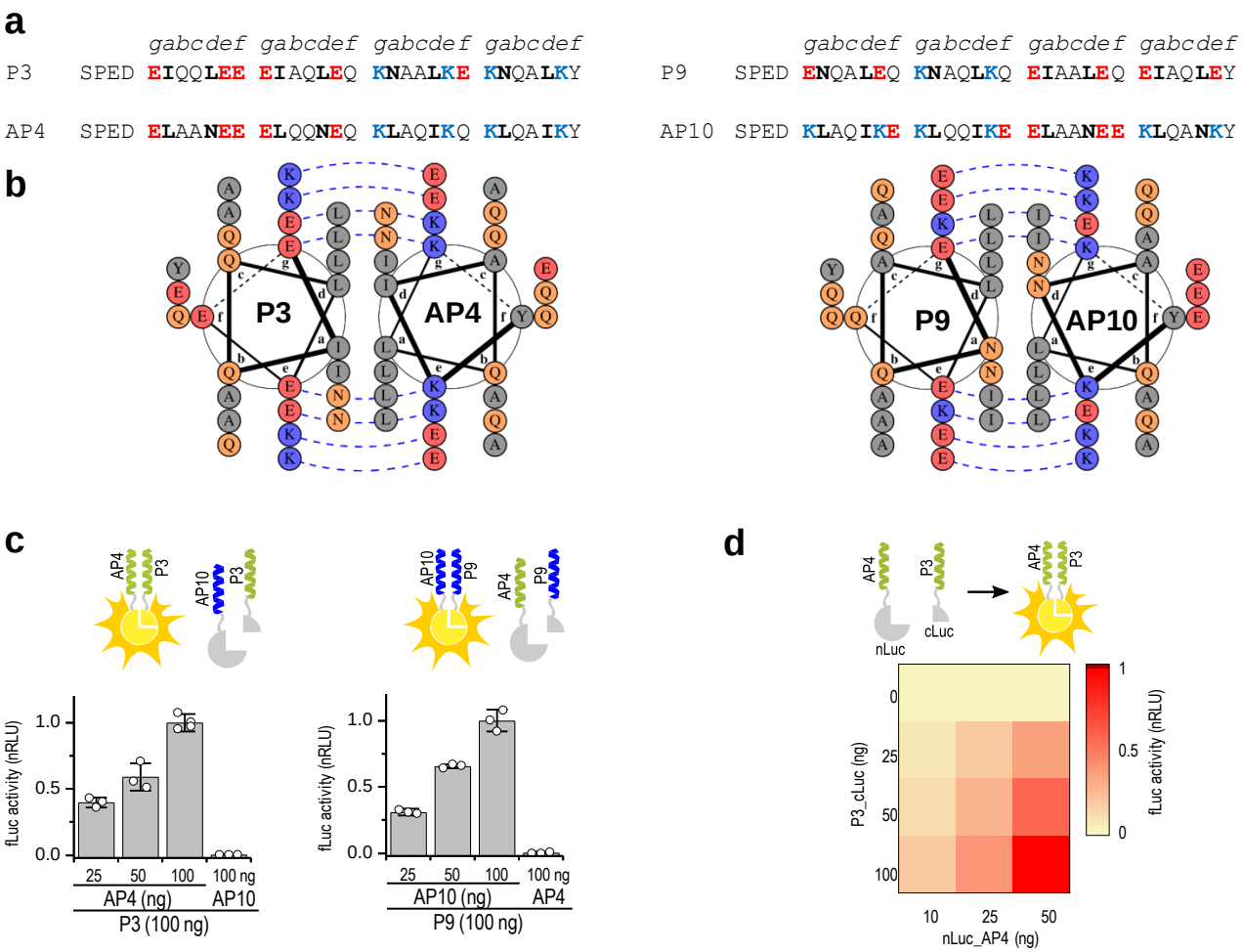
(**a**) Schematic presentation of the cleavable firefly luciferase (fLuc) inverse reporter. The cleavable fLuc reporter is deactivated by proteolysis. (**b**) Recognition sequences for the wild-type tobacco etch virus protease (TEVp) and its variants, as well as orthogonality of TEVp variants with modified cleavage sites. Reduced luciferase activity was detected in the presence of a protease and the cleavable fLuc reporter containing the appropriate protease cleavage site. Heat map showing orthogonality of the TEVp variants. Dark red corresponds to lower luciferase activity as a measure of higher protease activity. (**c**) Schematic presentation of the cyclic firefly luciferase reporter (cycLuc). CycLuc is cyclized by intein excision and

activated by proteolysis. (**d**) Orthogonality of four potyviral protease homologues detected by the cyclic luciferase reporter with matching protease cleavage site. (**e**) Three-dimensional homology models of orthogonal split proteases from the Potyviridae family reconstituted in the active form (using the TEVp crystal structure from PDB 1LVB): nTEVp (residues 1–118 in magenta) and cTEVp (residues 119–242 in yellow), N-plum pox virus protease (nPPVp; residues 1–118 in orange) and cPPVp (residues 119–242 in blue), N-soybean mosaic virus protease (nSbMVp; residues 1–118 in green) and cSbMVp (residues 119–242 in violet), and N-sunflower mild mosaic virus (nSuMMVp; residues 1–118 in orange) and cSuMMVp (residues 119–242 in cyan). (**f**) Orthogonality of split TEVp and PPVp tested on cycLuc reporter with TEVp cleavage site. (**g**) Orthogonality of split TEVp and PPVp tested on cycLuc reporter with PPVp cleavage site. Transfection mixtures are listed in **Supplementary Table 1** and **Supplementary Table 3**. Values are the means of three (**b,f,g**) and four (**d**) cell cultures ± s.d. and are representative of two independent experiments.



Supplementary Figure 2. Chemical regulation of the reconstitution of split potyviral proteases.

(**a**) Kinetics of split TEVp reconstitution based on the abscisic acid (ABA) dimerization system. (**b**) Kinetics of split TEVp reconstitution based on the rapamycin dimerization system. (**c**) Heat map showing ABA (left) and rapamycin (right) dependent reconstitution of split TEVp 15 minutes after induction. Dark red corresponds to higher luciferase activity as a measure of higher protease activity. Transfection mixtures are listed in **Supplementary Table 1.** Values are the means of four cell cultures ± s.d. and are representative of two independent experiments.



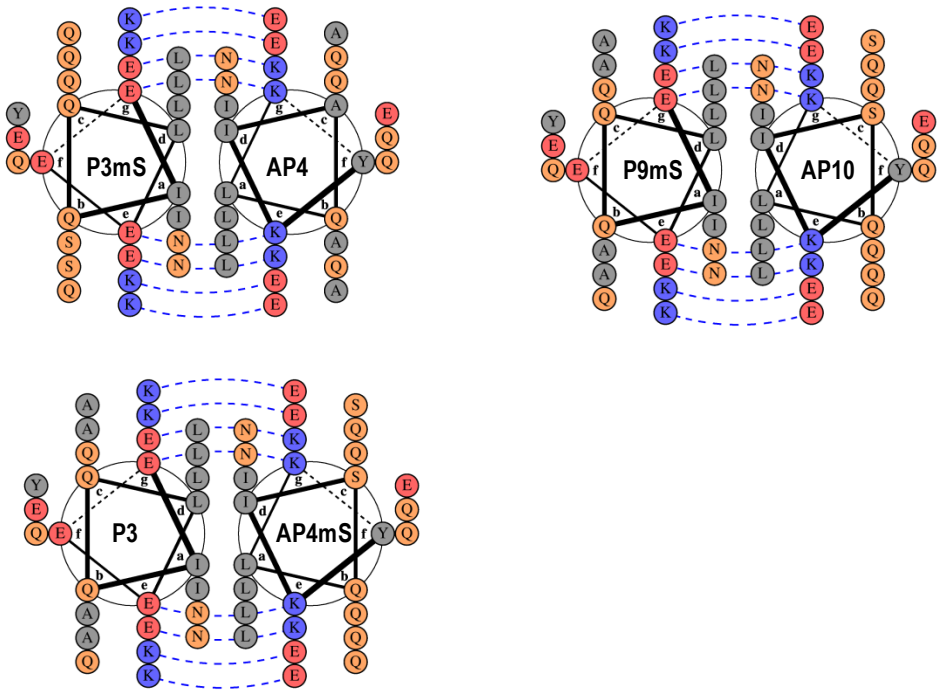
Supplementary Figure 3. Design of antiparallel coiled-coil (CC) pairs.

(**a**) Amino acid sequences of CC peptides in heptad repeats register. Acidic residues and basic residues are represented in red and blue respectively. (**b**) Helical projection representing specific CC interactions in antiparallel orientation (salt bridges between residues *g* and *g'* and *e* and *e'* are shown with dotted lines). Acidic residues and basic residues are represented in red and blue respectively, hydrophobic residues in grey and asparagine and glutamine residues in orange. The helical wheels projections were drawn in DrawCoil 1.0. (**c**) CC orthogonality and split luciferase reconstitution by antiparallel CC dimerization. (**d**) Split luciferase reconstitution with CC (P3/AP4) across 15 input concentration combinations. Transfection plasmid mixtures are listed in **Supplementary Table 3**. Values are the mean of three cell cultures ± s.d. and are representative of two independent experiments.

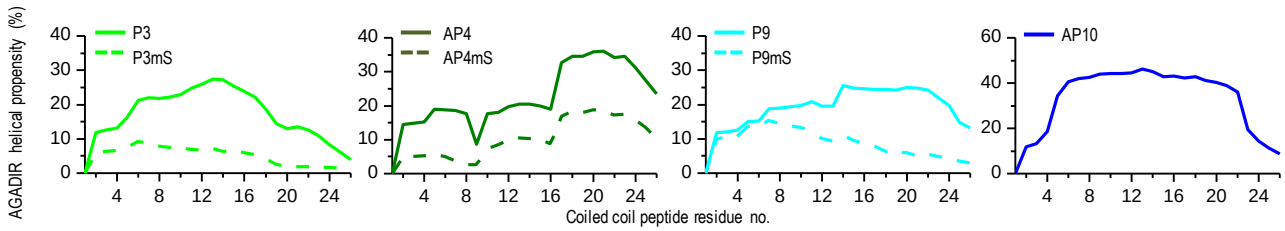
a

```
                    gabcdef gabcdef gabcdef gabcdef
P3      SPED EIQQLEE EIAQLEQ KNAALKE KNQALKY
P3mS    SPED EIQQLEE EISQLEQ KNSQLKE KNQQLKY

AP4     SPED ELAANEE ELQQNEQ KLAQIKQ KLQAIKY
AP4mS   SPED ELQSNEE ELQQNEQ KLQQIKQ KLQSIKY

                    gabcdef gabcdef gabcdef gabcdef
P9      SPED ENQALEQ KNAQLKQ EIAALEQ EIAQLEY
P9mS    SPED ENAQLEQ KNAQLKQ EISQLEQ EISQLEY

AP10    SPED KLAQIKE KLQQIKE ELAANEE KLQANKY
```

b

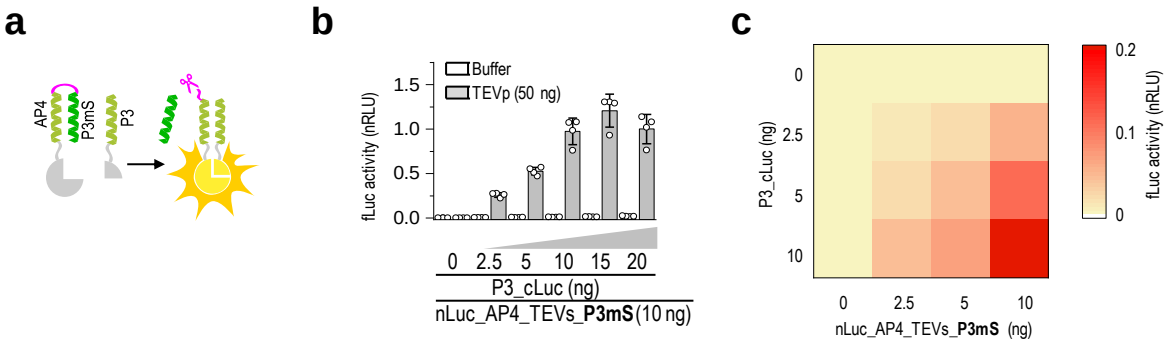


c



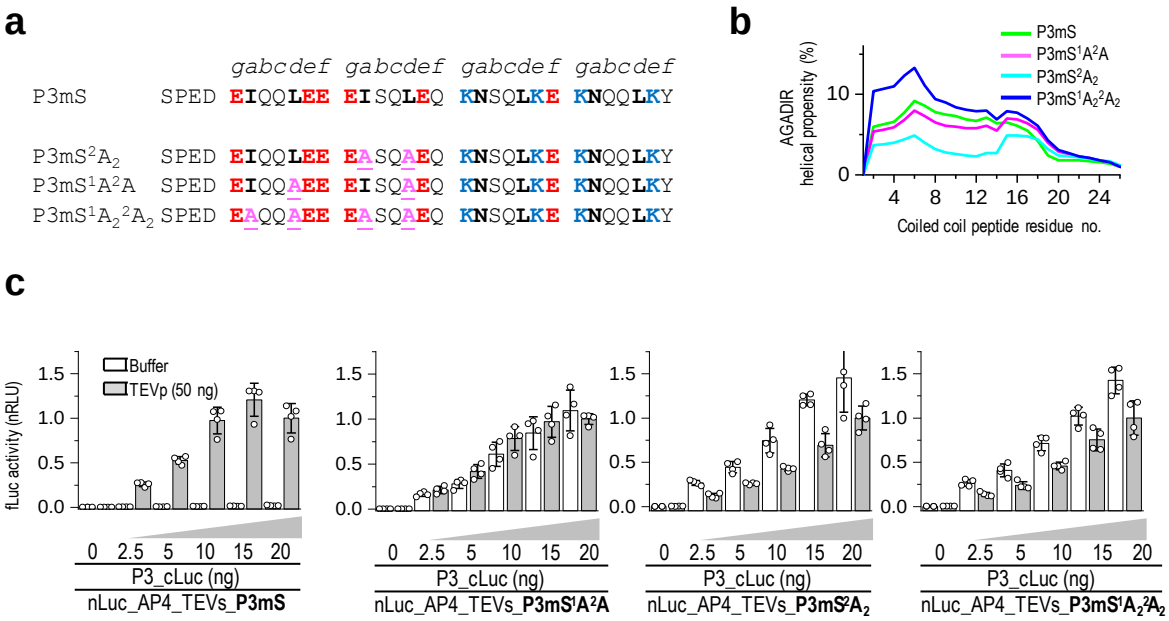
Supplementary Figure 4. Design of autoinhibitory coiled-coil (CC) pairs.

(**a**) Amino acid sequences of CC peptides in heptad repeats register. Acidic residues and basic residues are represented in red and blue respectively. Mutations from the original P3, AP4 and P9 sequences are shown in magenta and underlined. (**b**) Helical projection of destabilized mS CC variants representing specific interactions in antiparallel orientation (salt bridges between residues *g* and *g'* and *e* and *e'* are shown with dotted lines). Acidic residues and basic residues are represented in red and blue respectively, hydrophobic residues in grey and hydrophilic residues (asparagine, glutamine and serine) in orange. The helical wheels projections were drawn in DrawCoil 1.0. (**c**) Per residue helical propensity calculated with AGADIR. Helicity of original peptides (continuous lines) are compared to the helicity of mS version (dotted lines).



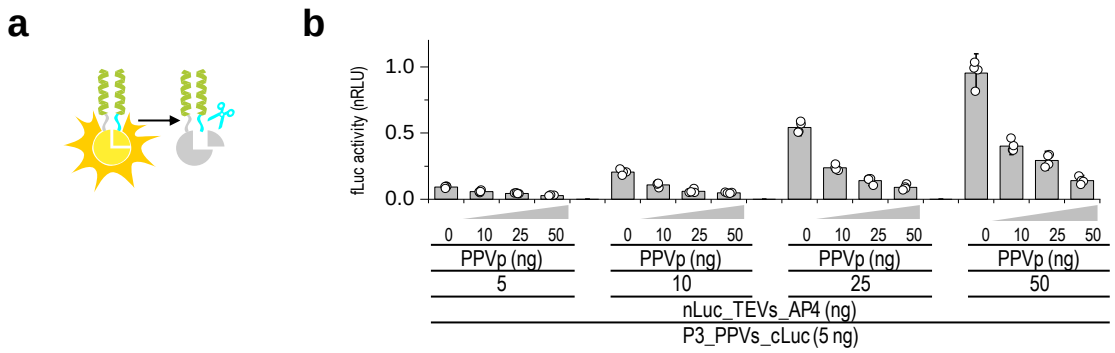
Supplementary Figure 5. Titration of autoinhibitory and displacer peptides.

(**a**) Schematic representation of the SPOC building module used for titration of autoinhibitory and displacer peptides. (**b**) Luciferase reconstitution from the autoinhibited module in presence or absence of TEVp with varying displacer amounts. (**c**) Heat map showing luciferase reconstitution from the autoinhibited module in presence of TEVp across 16 input concentration combinations. After cleavage of the linker by TEVp, the autoinhibitory coil is replaced by a displacer segment with higher affinity to reconstitute the reporter. Transfection plasmid mixtures are listed in **Supplementary Table 3**. Values (**b**,**c**) are the mean of four cell cultures ± s.d. and are representative of two independent experiments.



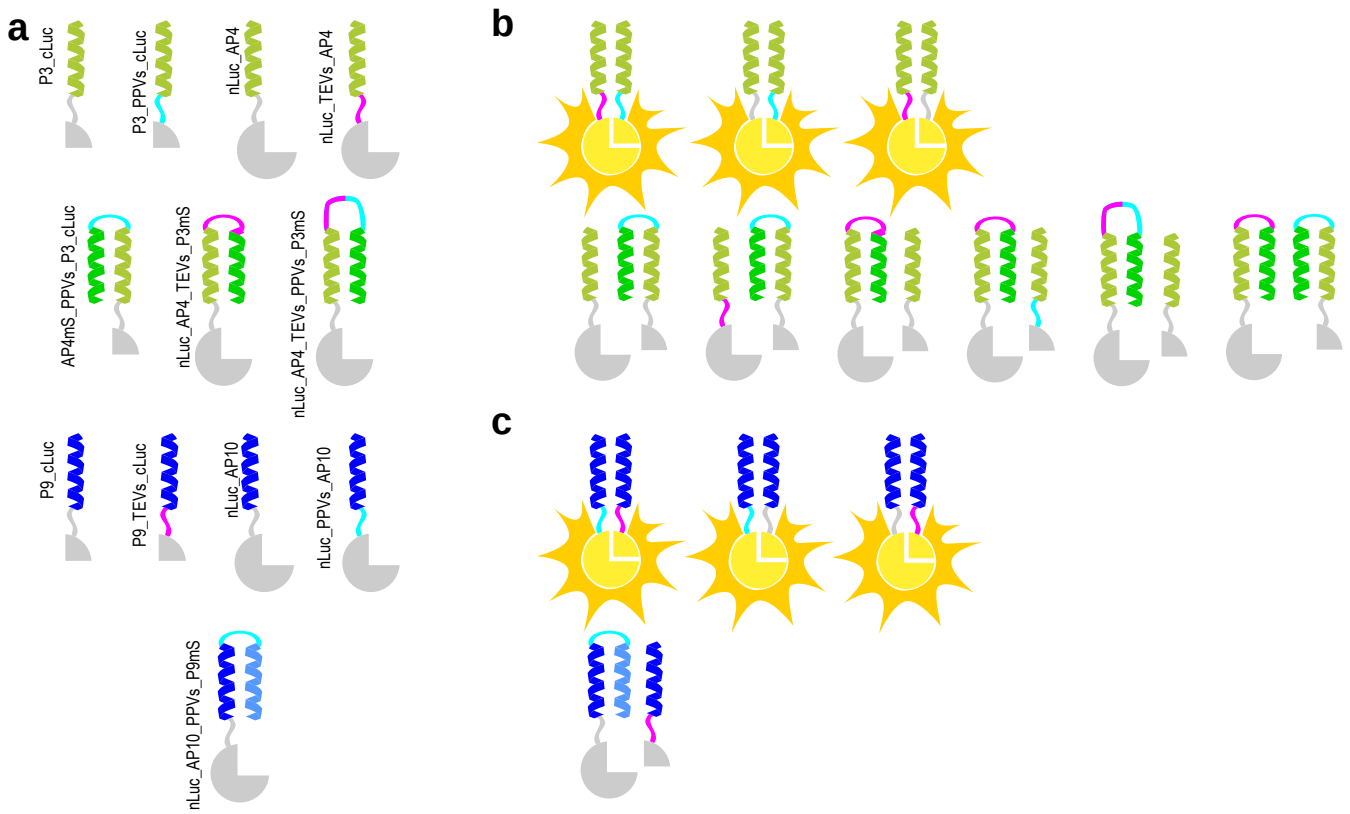
Supplementary Figure 6. Characterization of destabilized coiled-coil (CC) variants.

(**a**) Amino acid sequences of P3mS compared to more destabilized versions reported in heptad repeats register. Acidic residues and basic residues are represented in red and blue respectively, mutations from the P3mS sequence are shown in magenta and underlined. (**b**) Per residue helical propensity for each P3mS version calculated with AGADIR. (**c**) Titration of luciferase reconstitution from four variants of autoinhibited modules by displacement with peptide P3_cLuc. Transfection plasmid mixtures are listed in **Supplementary Table 3**. Values are the mean of four cell cultures ± s.d. and are representative of two independent experiments.



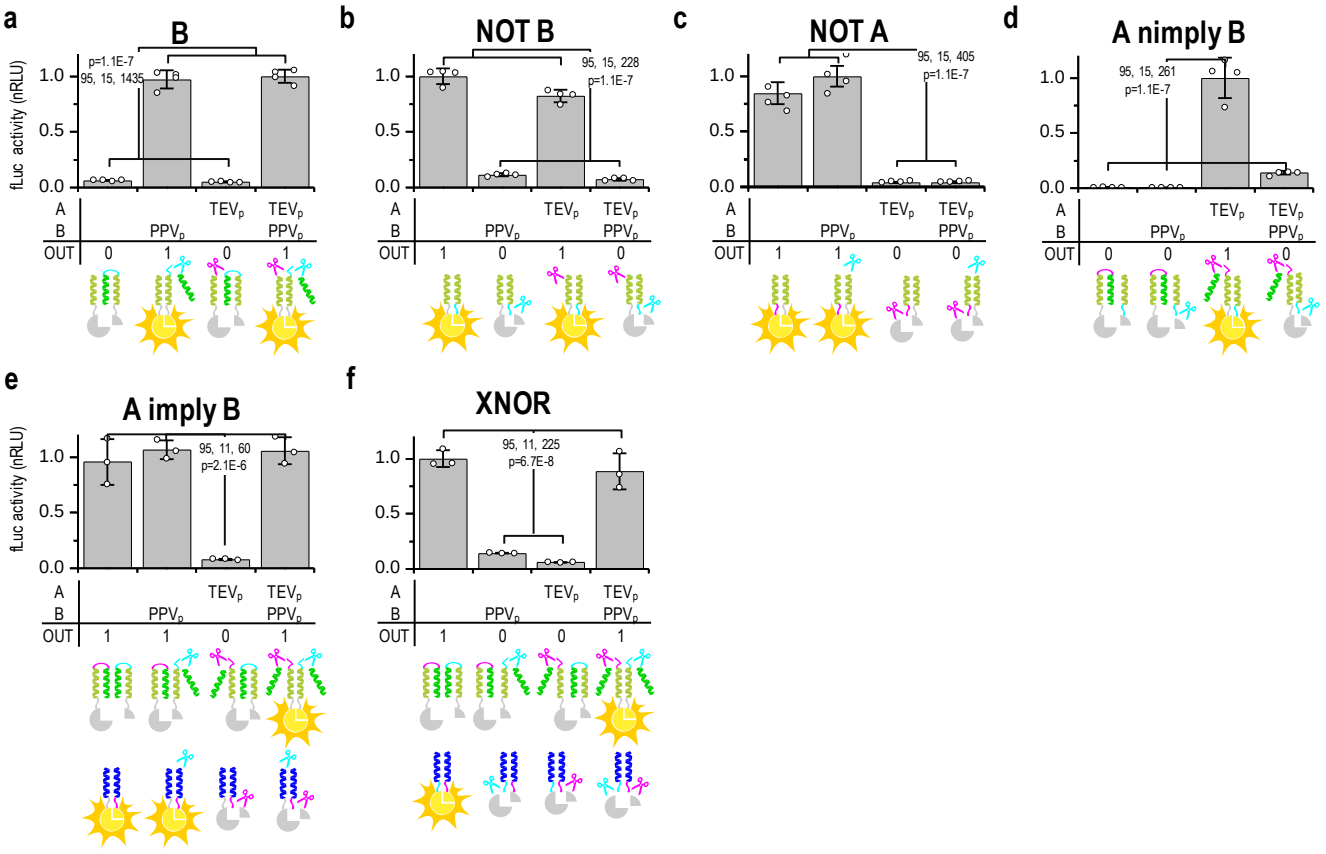
Supplementary Figure 7. Proteolysis inactivatable SPOC building module.

(**a**) Shematic presentation of the inactiivatable SPOC building module (negation logic module). (**b**) Reconstitution of luciferase activity from the negation logic module with varying amounts of complementary fragments and in the presence of varying amounts of PPVp. Transfection plasmid mixtures are listed in **Supplementary Table 3**. Values are the mean of four cell cultures ± s.d. and are representative of two independent experiments.



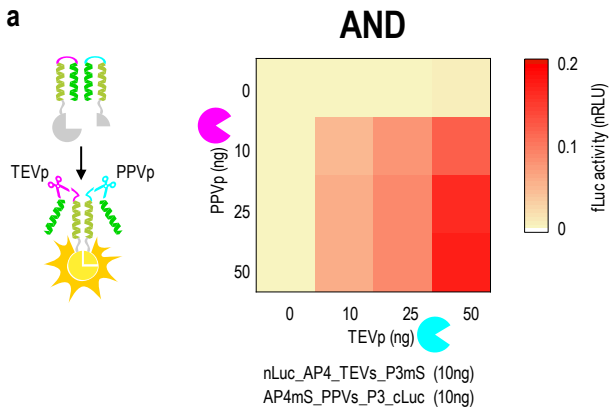
Supplementary Figure 8. Basic modules for implementation of split proteolysis-based signaling and logic circuits.

(**a**) All protease-cleavable orthogonal CC-based (SPOC) interaction modules for luciferase reconstitution used in this study. The cleavage site for plum pox virus protease and tobacco etch virus protease (TEVp) are shown in cyan and magenta respectively, uncleavable linkers and luciferase fragments are shown in grey. Green and blue coils represent the P3/AP4 and P9/AP10 CC pairs, respectively. One or multiple cleavage sites can be located between the CC-forming segments and the reporter fragment or in the linker between the target an autoinhibitory coil. (**b**) Pair arrangements of P3/AP4-based modules used in this study for construction of Boolean logic gates. (**c**) Pair arrangements of P9/AP10-based modules used in this study for construction of Boolean logic gates.

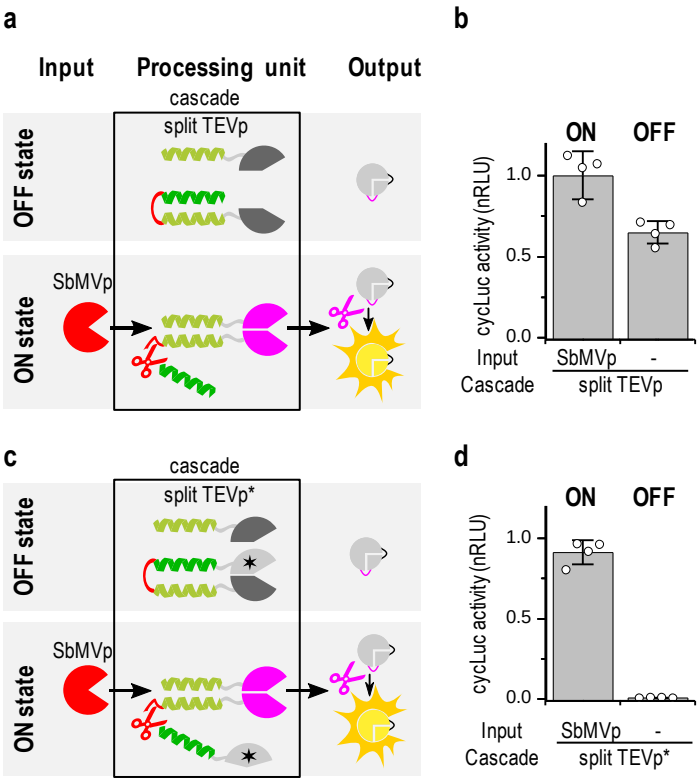


Supplementary Figure 9. Design of proteolytic cleavage responsive binary logic functions

Combinations of modules resulting in behavior as binary functions B, NOT B, NOT A, A nimply B, A imply B and XOR. Input signals are combinations of two orthogonal proteases, and the output is split luciferase activity. Experiments were performed on HEK293T cells. Transfection plasmid mixtures are listed in **Supplementary Table 2**. Values are the mean of three (e, f, g) and four (a,b,c, d) cell cultures ± (s.d.) and are representative of at least two independent experiments, significance tested by 1-way ANOVA with Tukey's comparison (values CI, df, F and p are indicate on graphs).

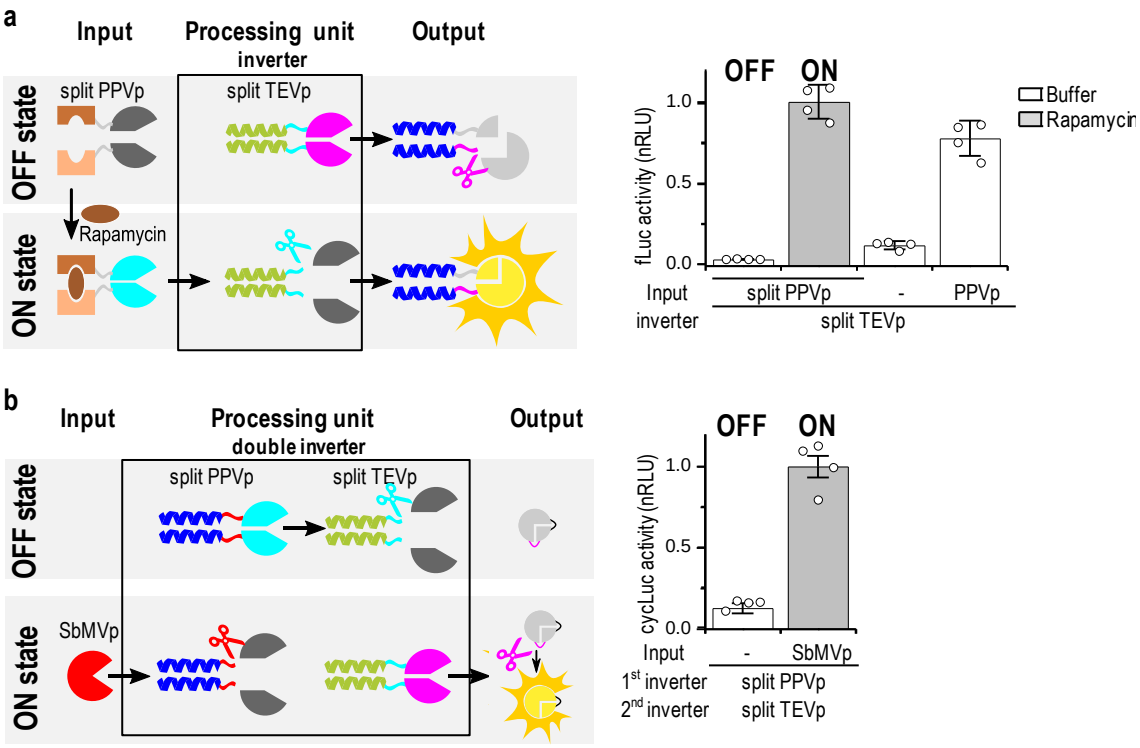


Supplementary Figure 10. Input protease titration.
(**a**) Responses of AND SPOC logic gate across 16 input protease concentration combination. Transfection plasmid mixtures are listed in **Supplementary Table 3**. Values are the mean of four cell cultures and are representative of two independent experiments.



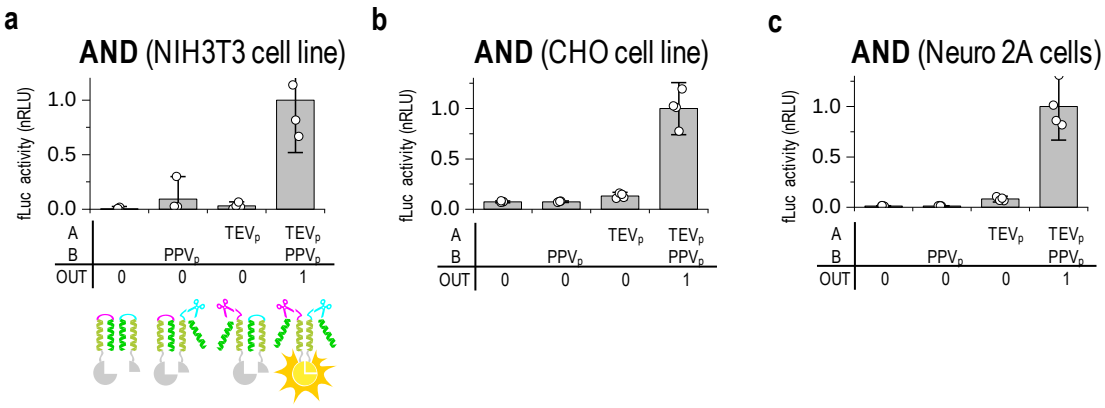
Supplementary Figure 11. Layered protease cascade inverter.

(**a,c**) Shematic presentation of two-layer-protease-cascade function with split TEVp protease inhibited by only an autoinhibitory coil (**a**) or by an autoinhibitory coil with a catalytically inactive split TEVp* fragment **(c)**. (**b,d**) Comparison of two-layer-protease-cascade (**b**) and of the improved design with a catalytically inactive split TEVp* fragment (**d**). Values are the means of four cell cultures ± s.d. and are representative of two independent experiments. Transfection mixtures are listed in **Supplementary Table 1**.



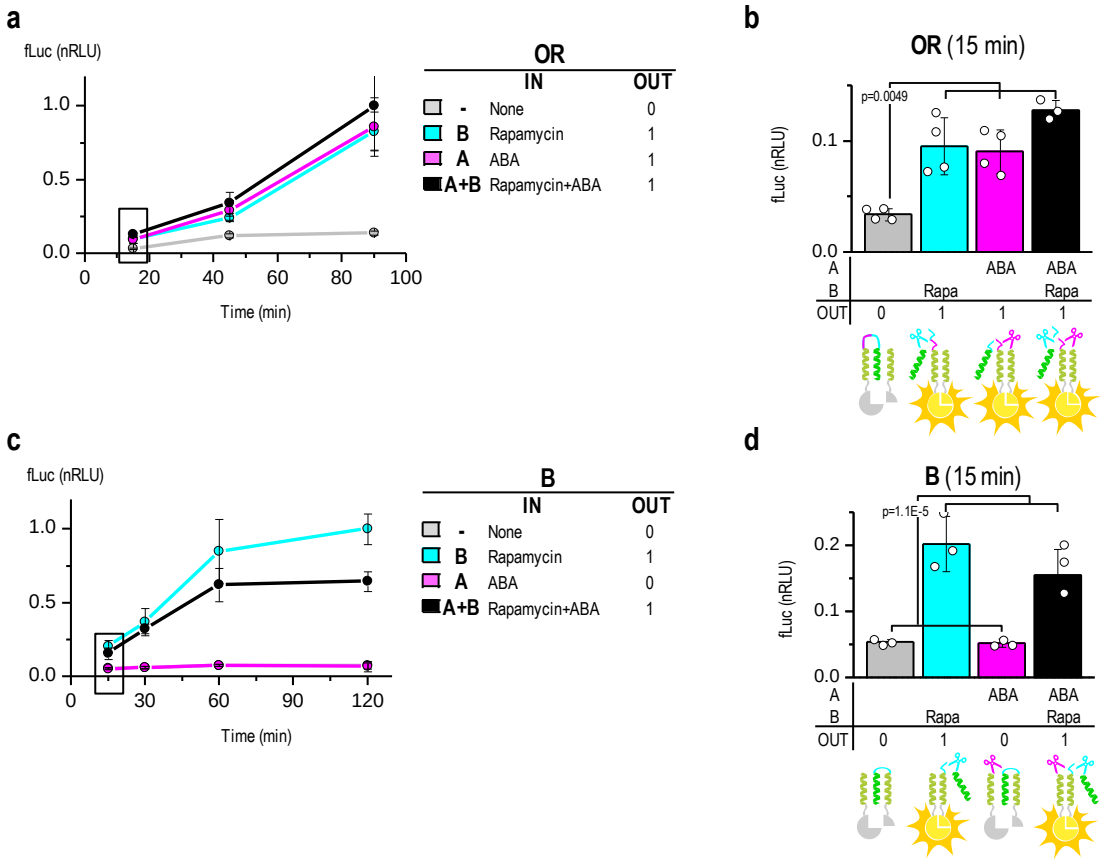
Supplementary Figure 12. Layered protease cascade inverter.

(**a**) The proteolytic inverter consisting of the split plum pox virus protease (PPVp) regulated by rapamycin and the split tobacco etch virus protease (TEVp) fused to a P3/P4 pair of parallel coiled-coils (CCs) with a PPVp cleavage site between the CC segments and TEVp fragments. The output of the proteolytic inverter is measured by a negation function (inverse correspondence to TEVp activity). (**b**) The double proteolytic inverter consist of two inverter layers—a split PPVp fused to a P9/P10 pair of parallel CCs inactivated by the soybean mosaic virus protease (SbMVp) and a split TEVp fused to a P3/P4 CC pair inactivated by PPVp. The output is measured by a cycLuc_TEVs reporter (direct correspondence to TEVp activity). Transfection mixtures are listed in **Supplementary Table 1**. Values are the mean of four cell cultures ± s.d. and are representative of at least two independent experiments.



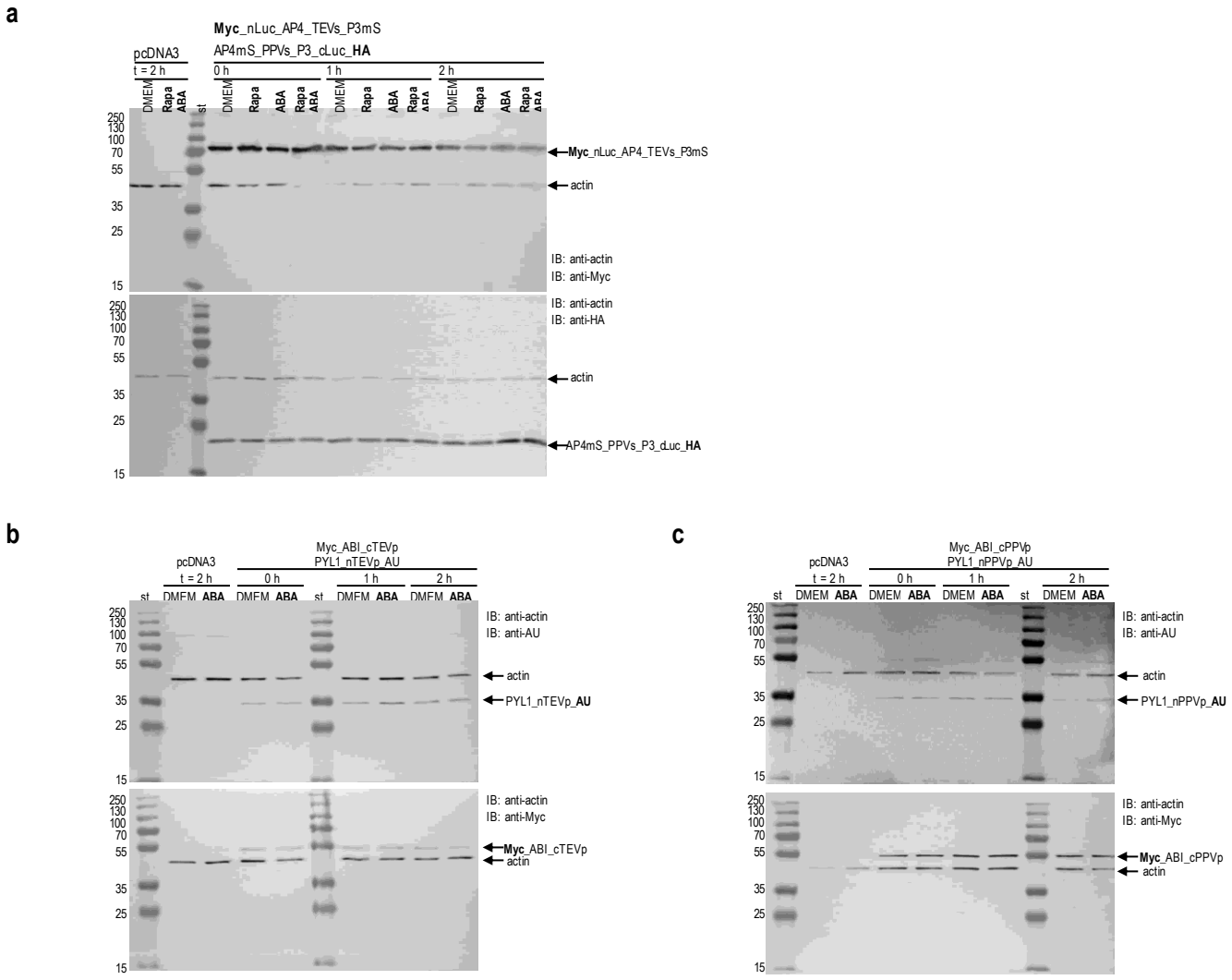
Supplementary Figure 13. SPOC logic gate AND tested on different cell lines.

Behavior of AND logic function in NIH3T3 cell line **(a)**, CHO cell line **(b)** and Neuro2A cells **(c)**. Input signals are combinations of two orthogonal proteases, and the output is split luciferase activity. Transfection plasmid mixtures are listed in **Supplementary Table 4**. Values are the mean of three cell cultures ± (s.d.) and are representative of two independent experiments.



Supplementary Figure 14. Kinetics of the split protease-cleavable orthogonal CC-based logic (SPOC logic) function OR and B.

(**a**) Kinetics of the OR SPOC logic functions at 15–90 min. Only one fragment of split luciferase is proteolytically dependent, but it contains the cleavage sites for both input proteases regulated by rapamycin and abscisic acid (ABA). (**b**) OR function regulated by rapamycin and ABA 15 minutes after induction. (**c**) Kinetics of B SPOC logic function from 15–120 min. Only one fragment of split luciferase depends on plum pox virus (PPV) proteolytic cleavage. (**d**) B function regulated by rapamycin and ABA 15 minutes after induction. Transfection mixtures are listed in **Supplementary Table 5**. Values are the mean of four cell cultures ± standard deviation (s.d.) and are representative of two independent experiments, significance tested by 1-way ANOVA with Tukey's comparison (CI 95%, df=15, F=11 (**b**); CI 95%, df=11, F=66 (**d**))



Supplementary Figure 15. Expression pattern of split proteases and split luciferase reporters.

(a) Plasmids coding for split luciferase reporters were transfected into HEK293T, induced with rapamycin, abscisic acid or both at indicated time points (0, 1 h or 2 h) and their expression was verified by Western blot. An empty plasmid vector (pcDNA3) was transfected as negative control. Bands at approximately 67 kDa corresponds to nLuc_AP4_TEVs_P3mS (Myc tag) and bands at approximately 18 kDa correspond to AP4mS_PPVs_P3_cLuc (HA tag). (**b, c**) Plasmids coding for split proteases in fusion with abscisic acid dimerizing domains (ABI/PYL1) were transfected into HEK293T, with or without addition of inducer. Cells were lysed at indicated time points (0, 1 h or 2 h) and their expression was verified by Western blot. An empty plasmid vector (pcDNA3) was transfected as negative control. Bands at approximately 35 kDa corresponds to Myc_ABI_cTEVp (**b**) or Myc_ABI_cPPVp (**c**) and bands at approximately 48,5 kDa correspond to HA_PYL1_nTEVp (**b**) or HA_PYL1_nPPVp (**c**). Transfection mixtures are listed in **Supplementary Table 7.**