Supplementary Information

Directed evolution improves the catalytic efficiency of TEV protease

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Supplementary Text 1: Optimization of TEVΔ yeast selection platform

Using C-terminally truncated, low-affinity wild-type TEV (TEVΔ219 [1], or "TEVΔ") as our starting template, we optimized a number of features of the platform. We found that truncation of the STE2-based plasma membrane anchor (Supplementary Figure 1A) [2] and incorporation of a BFP linker both increased Citrine signal, likely because of improved membrane trafficking of the TF construct. We also increased expression of the membrane-anchored TF by optimizing its promoter [3] (Figure 1C), incorporated a terminator [4] (Figure 1C), and tested alternative activation domains (Supplementary Figure 1C). By performing a time course, we determined that 6 hours was sufficient to reach maximal Citrine expression post light-induction of TF release (Supplementary Figure 1D). Omission of either light or CRY abolished the Citrine signal (Figure 1D).

Supplementary Text 2: Optimization of full-length TEV yeast selection platform

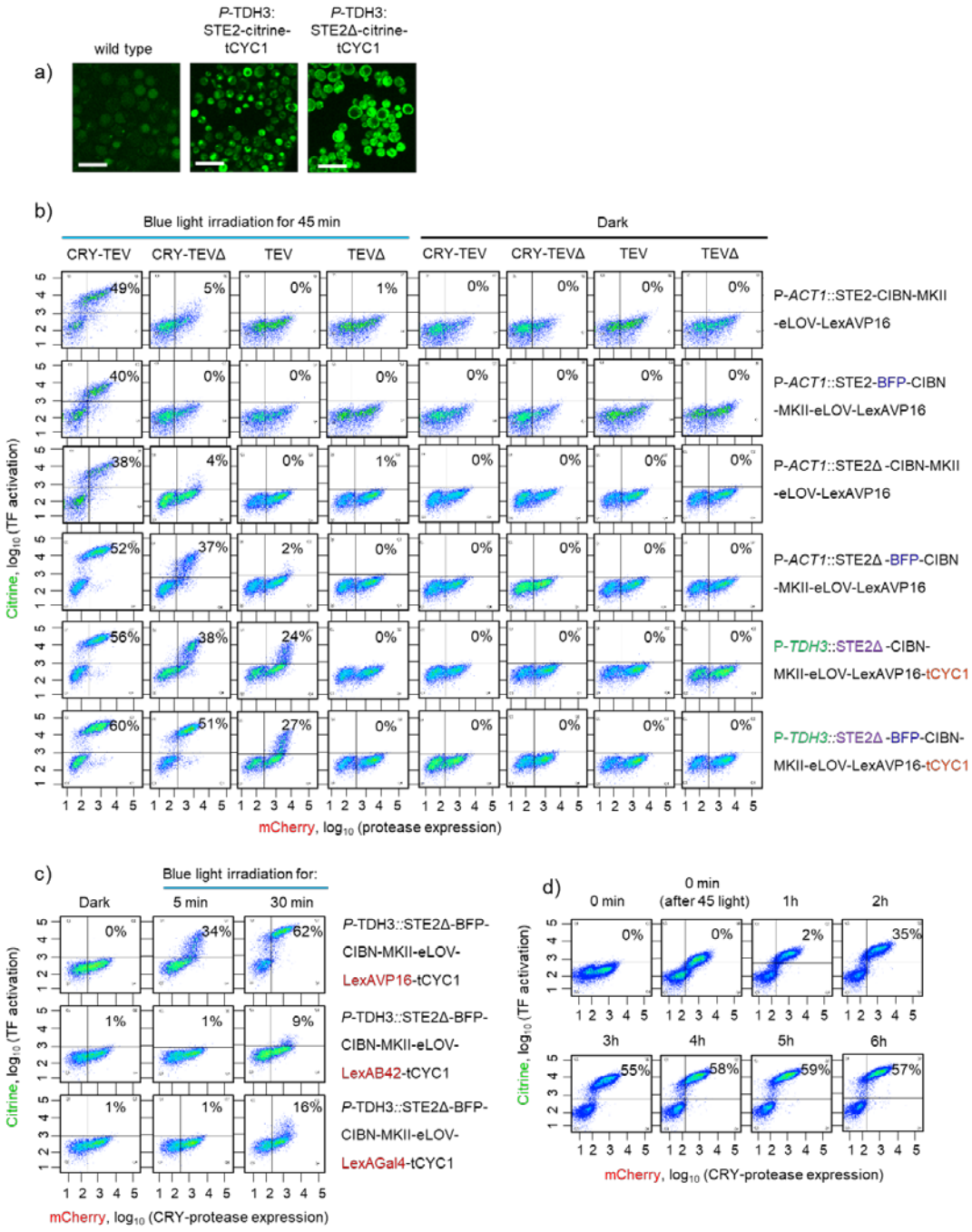
While establishing the yeast platform for evolution of full-length, high-affinity TEV proteases, we initially observed signal saturation due to the high affinity between wild-type full-length TEV and its TEVcs. To reduce the sensitivity, and thereby increase the dynamic range of our platform, we experimented with alternative TFs and promoters (Supplementary Figure 6B). We found that by reducing the number of LexA boxes in the promoter of our reporter gene, we could diminish the Citrine response to wild-type TEV [5]. We then tested the mutations enriched in our previous selection (Figure 3B and Supplementary Figure 6C) in the context of full-length TEV. We found that the S153N (from uTEV1Δ) improved activity and therefore incorporated this mutation in our starting template (full-length uTEV1).

Supplementary Text 3: Directed evolution of full-length TEV

Using the selection scheme in Figure 3A, we induced protease expression with galactose 12 hours prior to blue light irradiation for 45 seconds (round 1), 30 seconds (round 2), or 5 seconds (round 3). The yeast populations after each round were compared in Figure 3C. The selection clearly enriched activity, but there was little difference between light and dark conditions (Figure 3C and Supplementary Figure 7A), suggesting that eLOV does not effectively cage the high-affinity TEVcs from full-length protease under these conditions. Even without the advantage of light-based kinetic selection as in Figure 1A, however, we nevertheless enriched mutations that improved the catalytic efficiency of full-length TEV. Sequencing of round 3 showed that the mutations S135F, I138T and T180A had been enriched (Supplementary Figure 7B). Assays on individual mutants in yeast showed that all three mutations contributed to improved TEV activity (Supplementary Figure 8A), and combining the mutations had an additive effect (Supplementary Figure 8B). The best final clone, uTEV3, combines these three beneficial mutations (I138T/S153N/T180A).

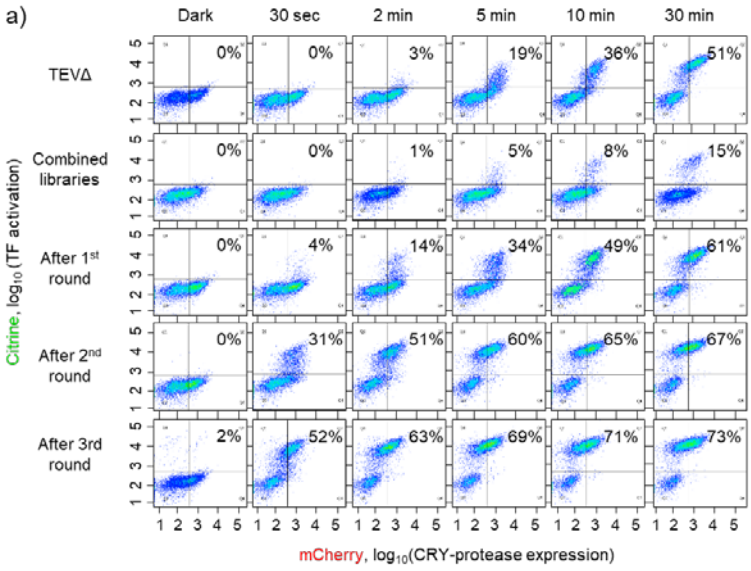
Supplementary Text 4: Comparison to previous evolved TEVs

Previously, Iverson et al. used directed evolution in the yeast secretory pathway to evolve a full-length TEV mutant (G79E/T173A/S219V) with higher activity than wild-type TEV [6]. In another study, a TEV mutant (L56V/S153G/S219V) was designed *in-silico* and shown to exhibit improved solubility and stability [7, 8]. We compared these TEV variants to our own, using our transcriptional assay in the yeast cytosol (Figure 3A). Supplementary Figure 10A shows that uTEV3 is more active than both the previous full-length TEVs. We also performed a comparison in the context of truncated TEVΔ for proximity-dependent cleavage. Supplementary Figure 10B shows that uTEV2Δ was the most active (against the low-affinity TEVcs ENLYFQ/M), while the Iverson mutant (truncated form) and wild-type TEVΔ exhibited comparable activity. In the context of FLARE, the Iverson mutant (truncated form) also performed more poorly than uTEV1Δ (Supplementary Figure 11C). Note that the Iverson mutant was not evolved against the low-affinity TEVcs and likely has lower k_{cat} towards it than do uTEV1Δ and uTEV2Δ.



Supplementary Figure 1. Optimization of membrane-anchored transcription factor for yeast directed evolution. Related to Figure 1C. (**A**) BY4741 yeast constitutively expressing STE2-citrine or STE2Δ(1-300)-citrine; the latter have much improved surface localization. Scale bars, 10 μm (**B**) Left four columns: FACS plots showing yeast cells 6 hours after 45-minute blue light irradiation. Percentages reflect fraction of cells with Citrine signal (cells that release TF to drive Citrine expression) and are given in the table in Figure 1C. Right four columns: Control cells without light exposure. Each condition performed twice, n = 10,000 cells. (**C**) Optimization of the LexA transcriptional activator. Yeast co-expressing the TF and mCherry-CRY-TEVΔ were analyzed by FACS 6 hours after variable amounts of blue light exposure. Percentages reflect the fraction of Citrine-positive cells. (**D**). Optimizing the time for reporter transcription and translation. FACS plots collected at various timepoints after 45-min blue light exposure to induce TF release. Percentages give fraction of cells in Q1 + Q2 quadrants. Each plot representative of two replicates, n = 20,000 cells. We selected 6 hours as our expression time window.

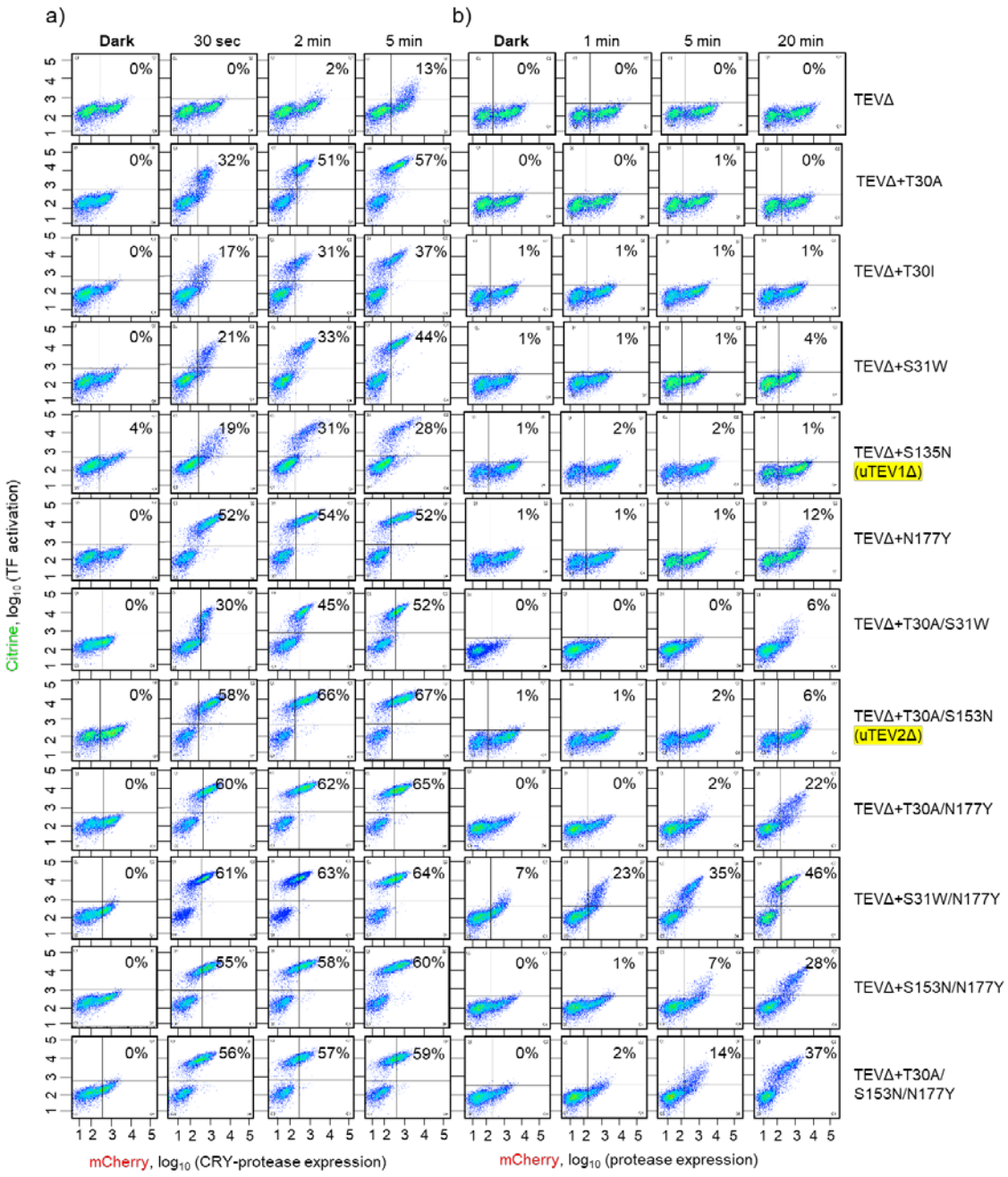
a)



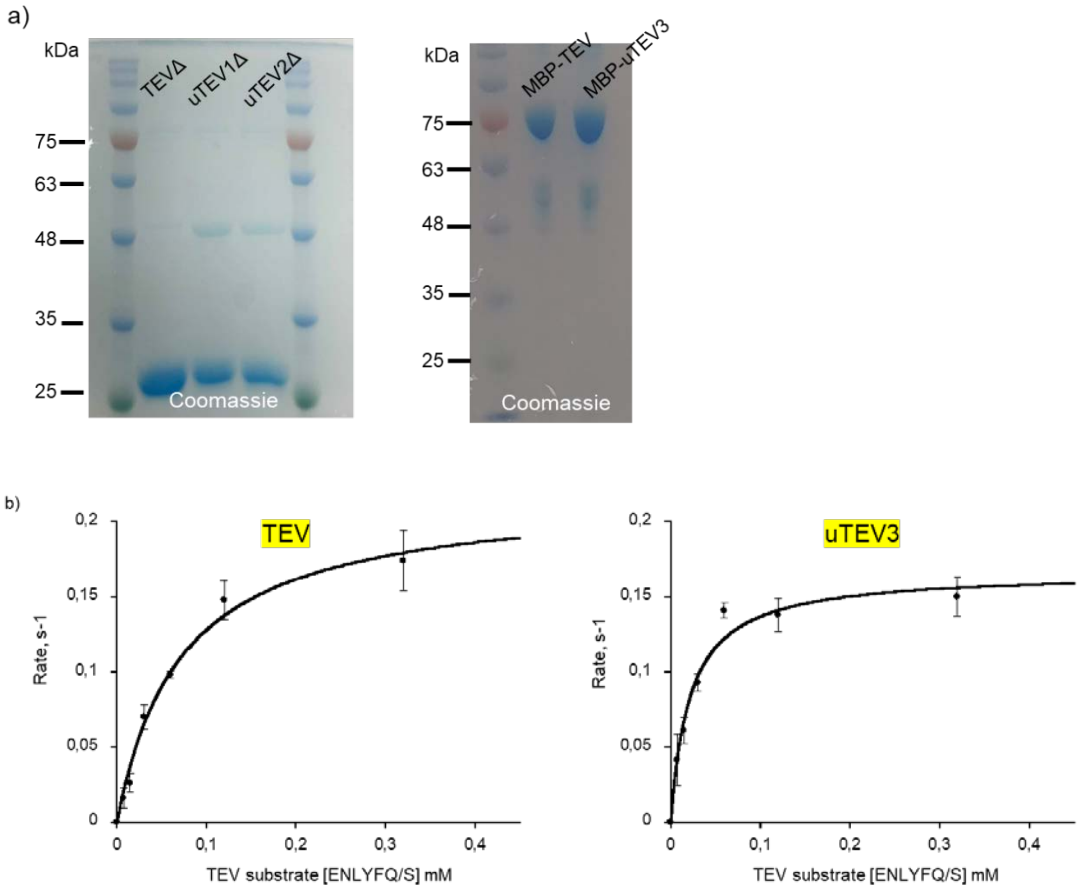
b)

#1						S31W													
#2					T30A													S153N	
#3									L56S										N177Y
#4			K67E	K89E															
#5																			N177Y
#6																			N177Y
#7								T54A											N177Y
#8						S31W													
#9										F64L									N177Y
#10						S31W													
#11		S16I					R50G				D90G								N177Y
#12																		S153N	
#13					T30A													S153N	
#14																			N177Y
#15					T30I											D136V			
#16																			N177Y
#17												I100V							
#18																			
#19																		S153N	
#20															S135F				N177Y
#21																	I138T		
#22						S31W							C101RT114A						
#23	S3F																		N177Y
#24					T30A														

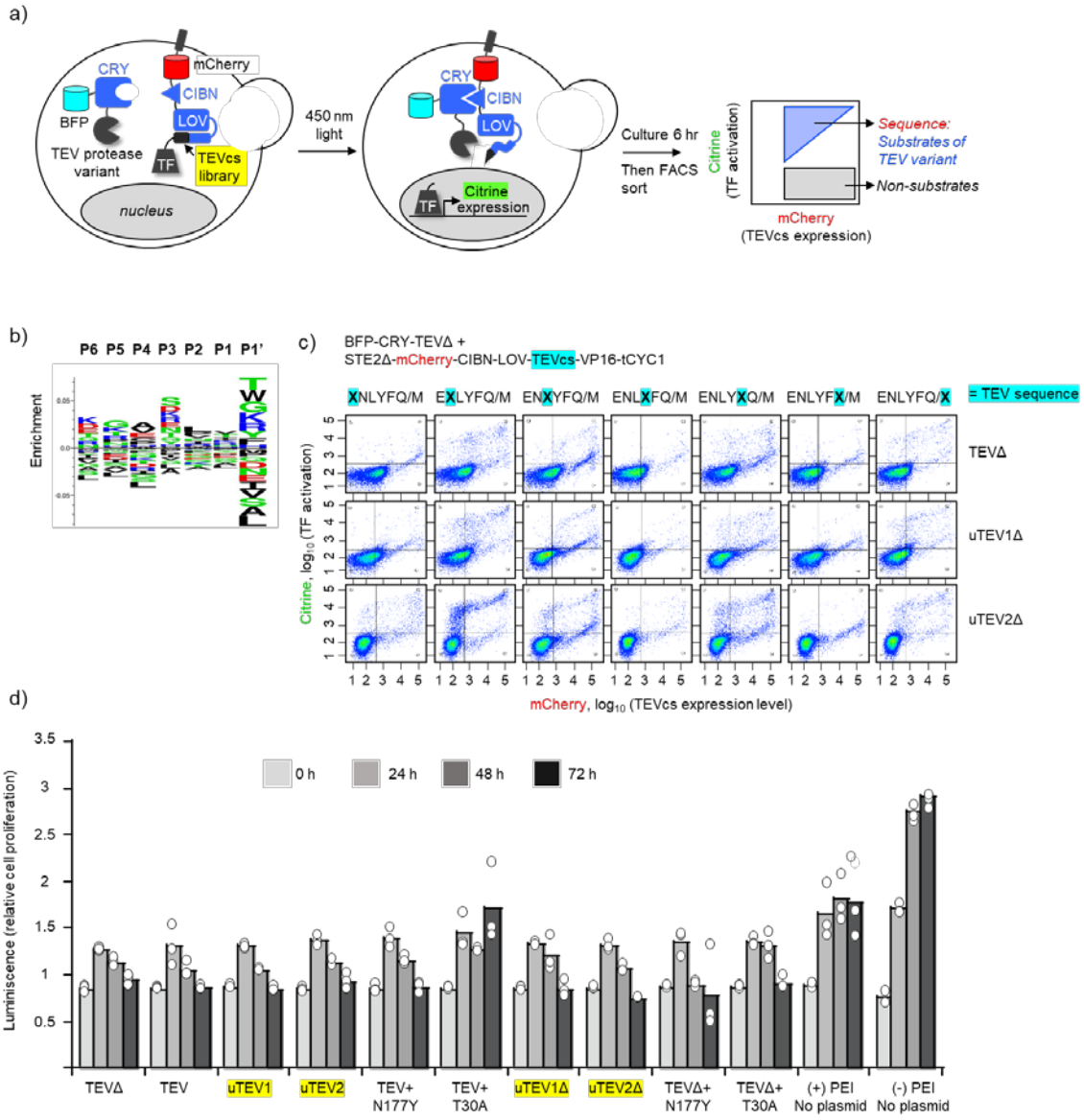
Supplementary Figure 2. Analysis of selected yeast populations and TEV clones. Related to Figure 1F. (**A**) Same as Figure 1F, but more conditions are shown. (**B**) Sequencing after round 3. 24 clones were sequenced and mutations found in each, relative to original TEVΔ, are shown.



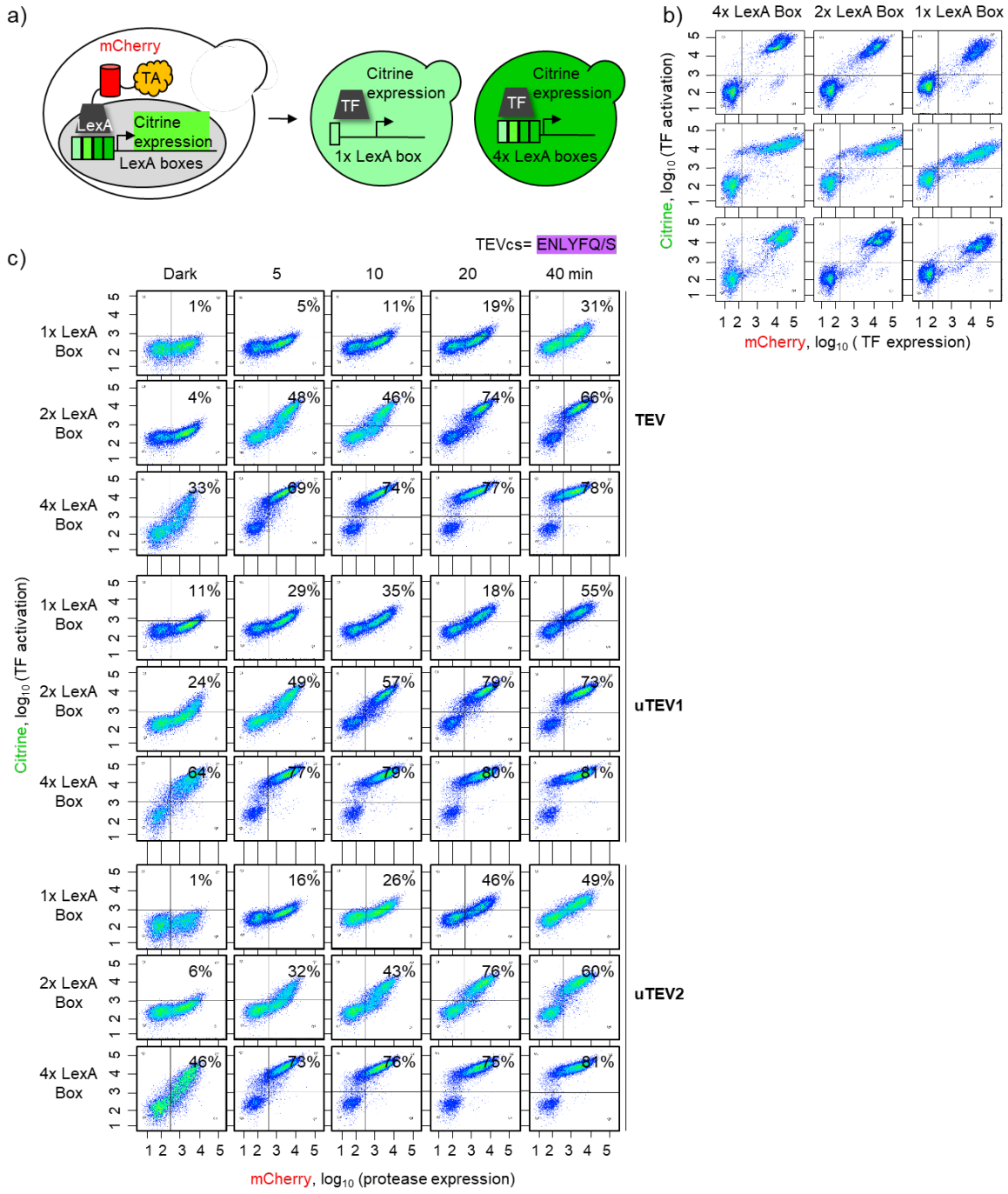
Supplementary Figure 3. Characterization of evolved TEVΔ mutants in yeast. Related to Figure 2A. (**A**) FACS plots were collected 6 hours after blue light exposure for the indicated times (0.5, 2, and 5 min). Percentages of Citrine-positive cells are shown in the graph in Figure 2A. (**B**) Same as (A) but with CRY omitted to test for proximity-dependence of TEVΔ-TEVcs interaction (cells express TEVΔ-mCherry instead of CRY-TEVΔ-mCherry). Each plot represents two replicates, n = 10,000 cells.



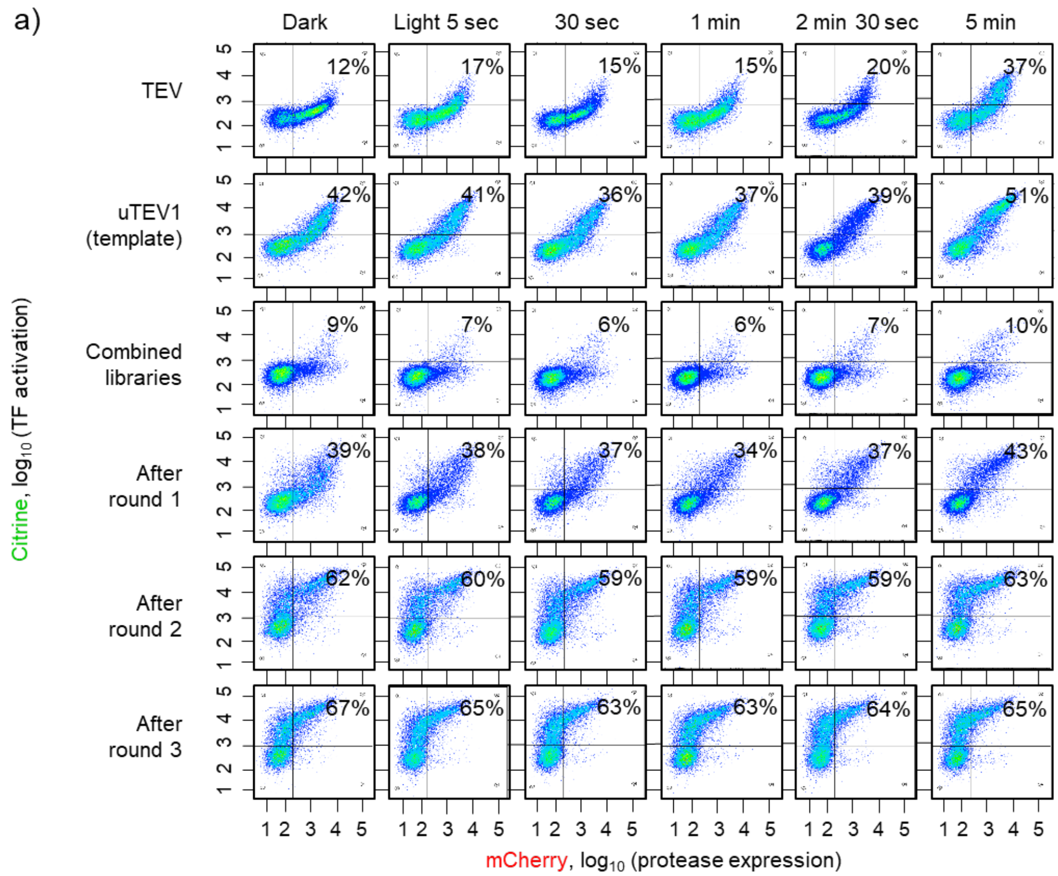
Supplementary Figure 4. **TEV purification and kinetics**. Related to Figures 2C-D and 3E-F. (**A**) SDS-PAGE (9%) of purified TEV proteases. TEVΔ is 25 kD. MBP-TEV (full-length) is 75 kD. (**B**) Michaelis-Menten plots for wild-type full-length TEV and uTEV3 (containing mutations I138T, S135N, and T180A). Reactions were assembled with 100 nM purified protease and variable amounts (0.0075-0.32 mM) of substrate protein MBP-TEVcs(ENLYFGS)-GFP at 30 °C. Initial proteolysis rates were determined for each starting substrate concentration, using the in-gel fluorescence assay shown in Figures 2C and 3E. Data was fit to a Michaelis-Menten enzyme kinetics model with center values representing the mean and error bars representing the standard deviation of three technical replicates.



Supplementary Figure 5. Profiling the sequence specificity of TEVΔ variants in yeast. Related to Figure 2F-G**.** (**A**) Assay for profiling yeast sequence specificity. The protease variant of interest is co-expressed with a library of TEVcs sequences (flanked by LexA-VP16 TF at the C-terminal end, and a plasma membrane anchor, mCherry, CIBN, and LOV at the N-terminal end). Upon exposure of cells to blue 450 nm light, the CRY-CIBN interaction brings the protease proximal to TEVcs, and the LOV domain changes conformation to expose TEVcs. Sequences sensitive to TEV proteolysis will release the TF, which translocates to the nucleus and drives expression of the reporter gene Citrine. (**B**) Sequence profile of the seven TEVcs libraries with randomized nucleotides (pooled together) before sorting. Each of the seven TEVcs libraries is randomized at a single position only. (**C**) Analysis of single randomized positions in the TEV cleavage site, using wild-type TEVΔ, uTEV1Δ, and uTEV2Δ. Sample FACS plots 6 hours after blue light exposure. Each plot represents one replicate, n = 10,000 cells. (**D**) Viability assays in HEK 293T expressing the evolved TEV proteases. This experiment was performed once, with three biological replicates per sample. White dots indicate individual technical replicates.



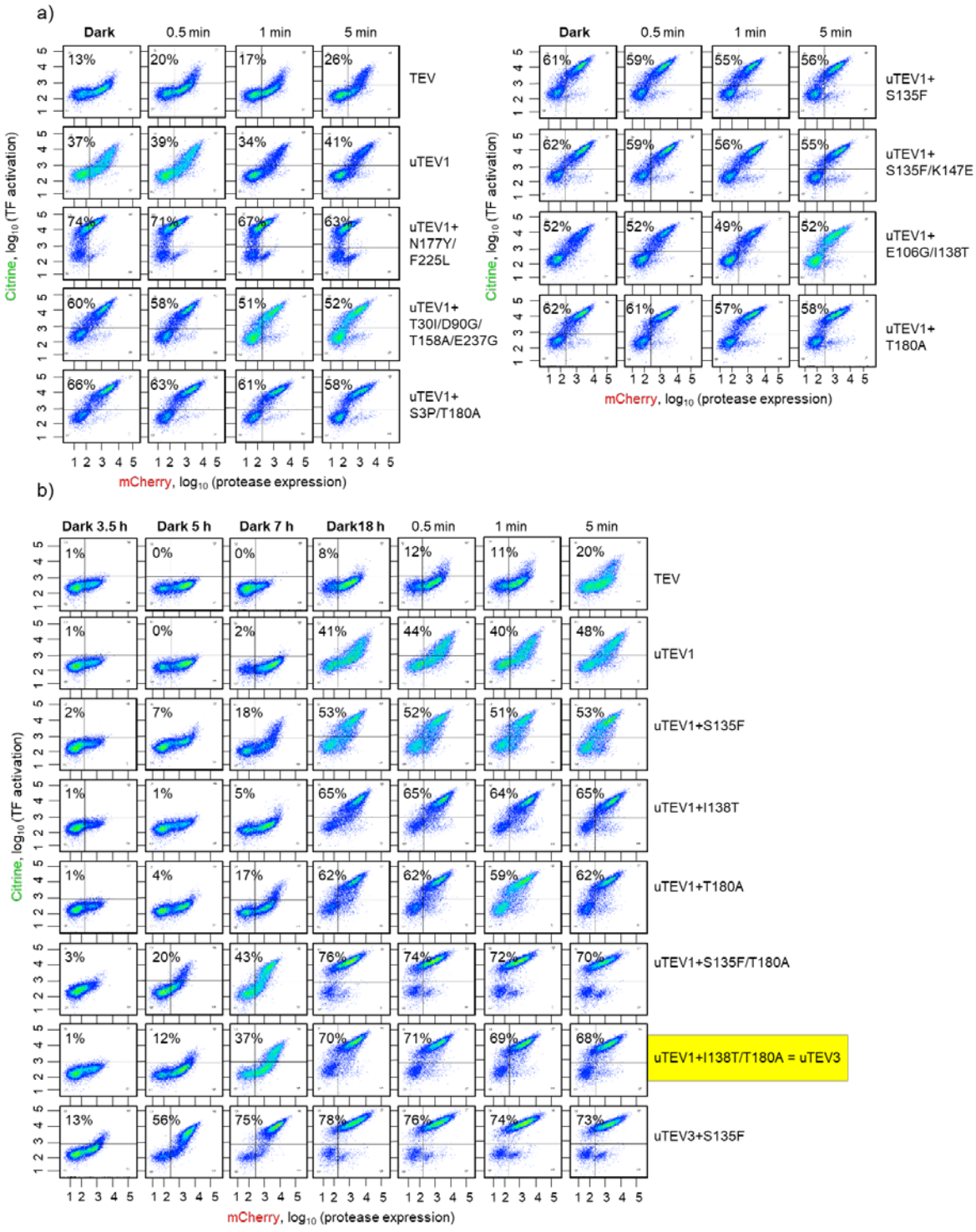
Supplementary Figure 6. Optimizing the yeast platform for evolution of full-length high-affinity proteases. Related to Figure 3. (**A**) To tune the dynamic range of the platform, the LexA DNA-binding domain was fused to one of 3 different transcriptional activators (TAs): VP16, B42, or Gal4. The constructs were expressed in yeast containing different numbers of LexA boxes upstream of the Citrine reporter gene. (**B**) FACS data showing the effect of varying the number of LexA boxes in the promoter with different TAs. FACS data collected 12 hours following galactose induction. Each plot represents two replicates, n = 20,000 cells. (C) Comparison of different full-length TEVs in the setup shown in Figure 3A. The TEVcs was the high-affinity sequence ENLYFQ/S. FACS plots obtained 6 hours after blue light exposure for the indicated times (5, 10, 20 and 40 min). Each plot represents one replicate, n = 20,000 cells. Percentage of Citrine-positive cells in each condition used to generate the graph in Figure 3B.



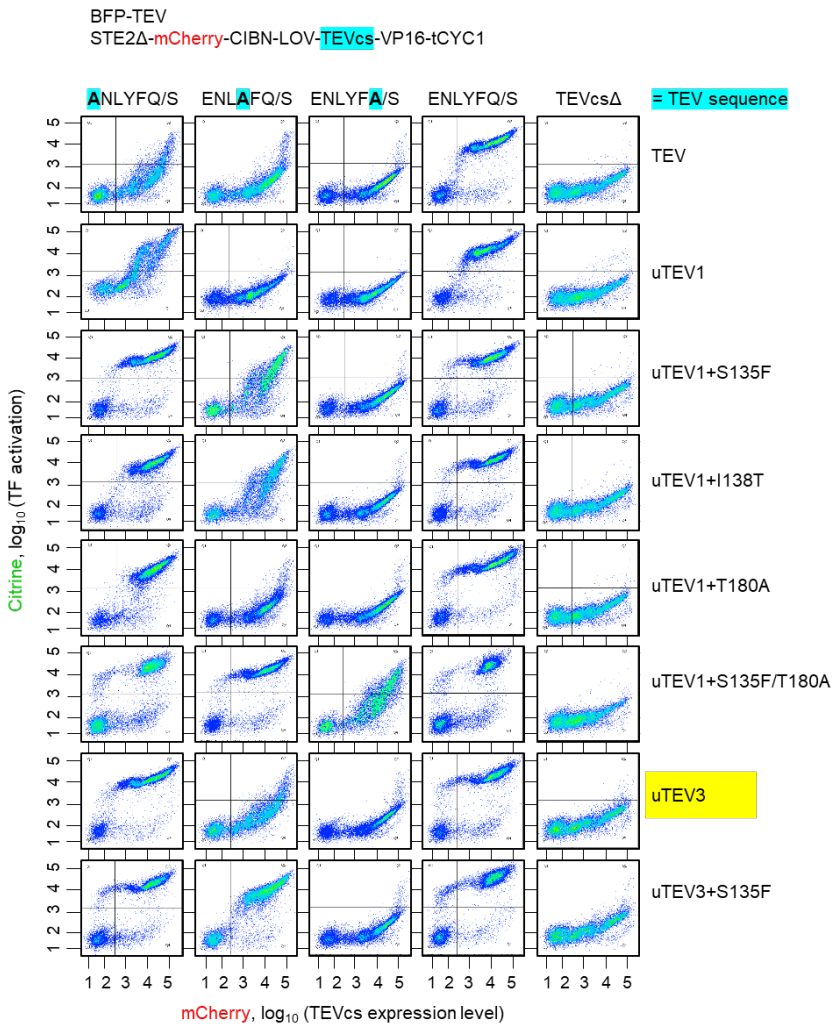
b)

Clone	Mutations
#1	S3P, T180A
#2	N177Y, F225L
#3	S135F
#4	T30I, D90G, T158A, E237G
#5	K119E, T180A, N236S
#6	template
#7	N68D, P93S, S122P, S135F
#8	K67E, S135F, M218T
#9	M87L, T180A
#10	M87T, I138T
#11	F132S, N153D
#12	T180A
#13	K141R
#14	S135F, K147E
#15	template
#16	S122P, T180A
#17	P224S, N236S
#18	N205D
#19	E106G, I138T
#20	T180A
#21	inserted linker (GSASGSGKLSGGG), D90G, I138T, K220R
#22	empty vector
#23	T180A
#24	S135F

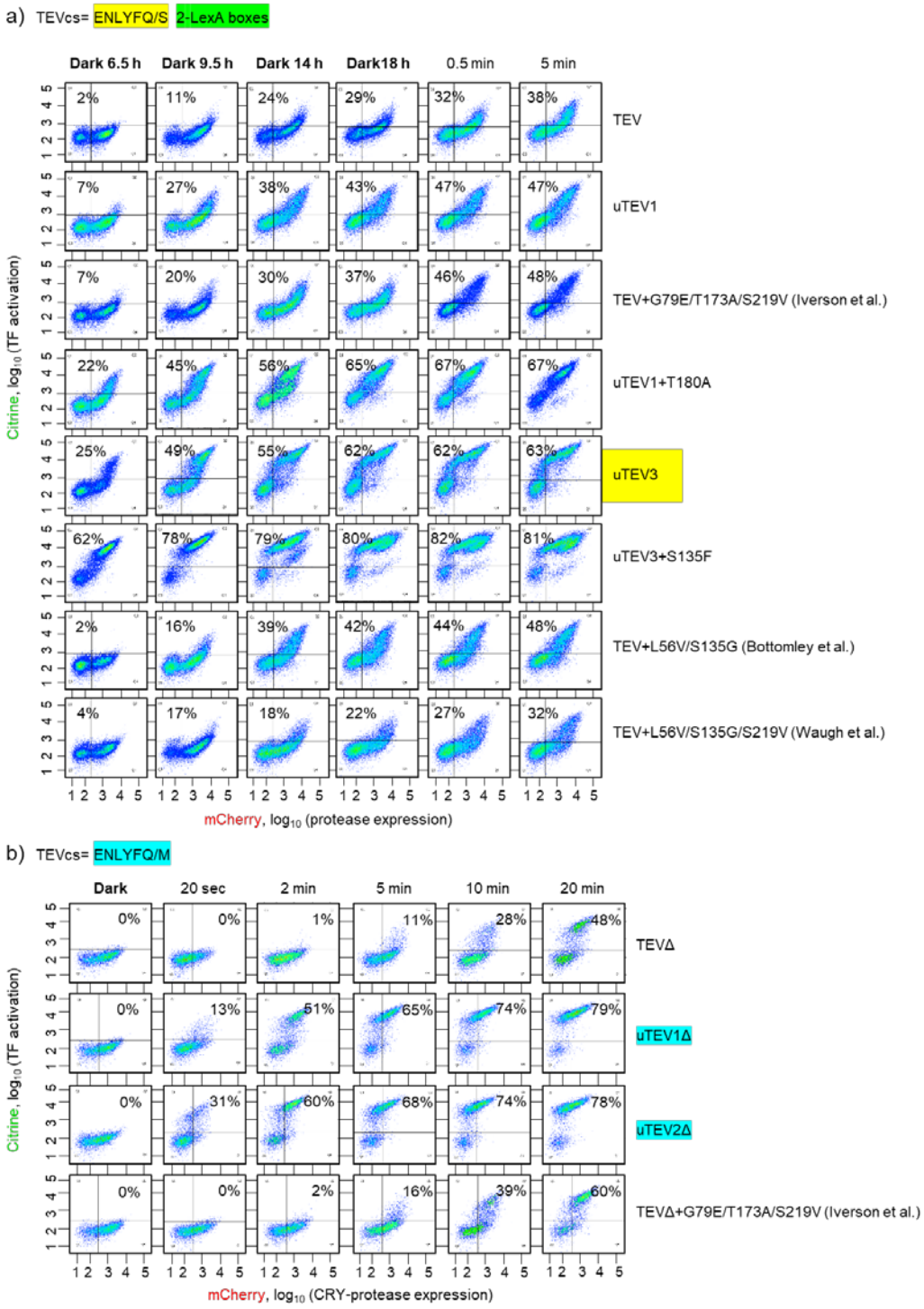
Supplementary Figure 7. Analysis of selected yeast populations and full-length TEV clones. Related to Figure 3. (**A**) Same as Figure 3C but showing more conditions. (**B**) Sequencing after round 3. 24 clones were sequenced and mutations found in each, relative to the original template uTEV1, are shown.



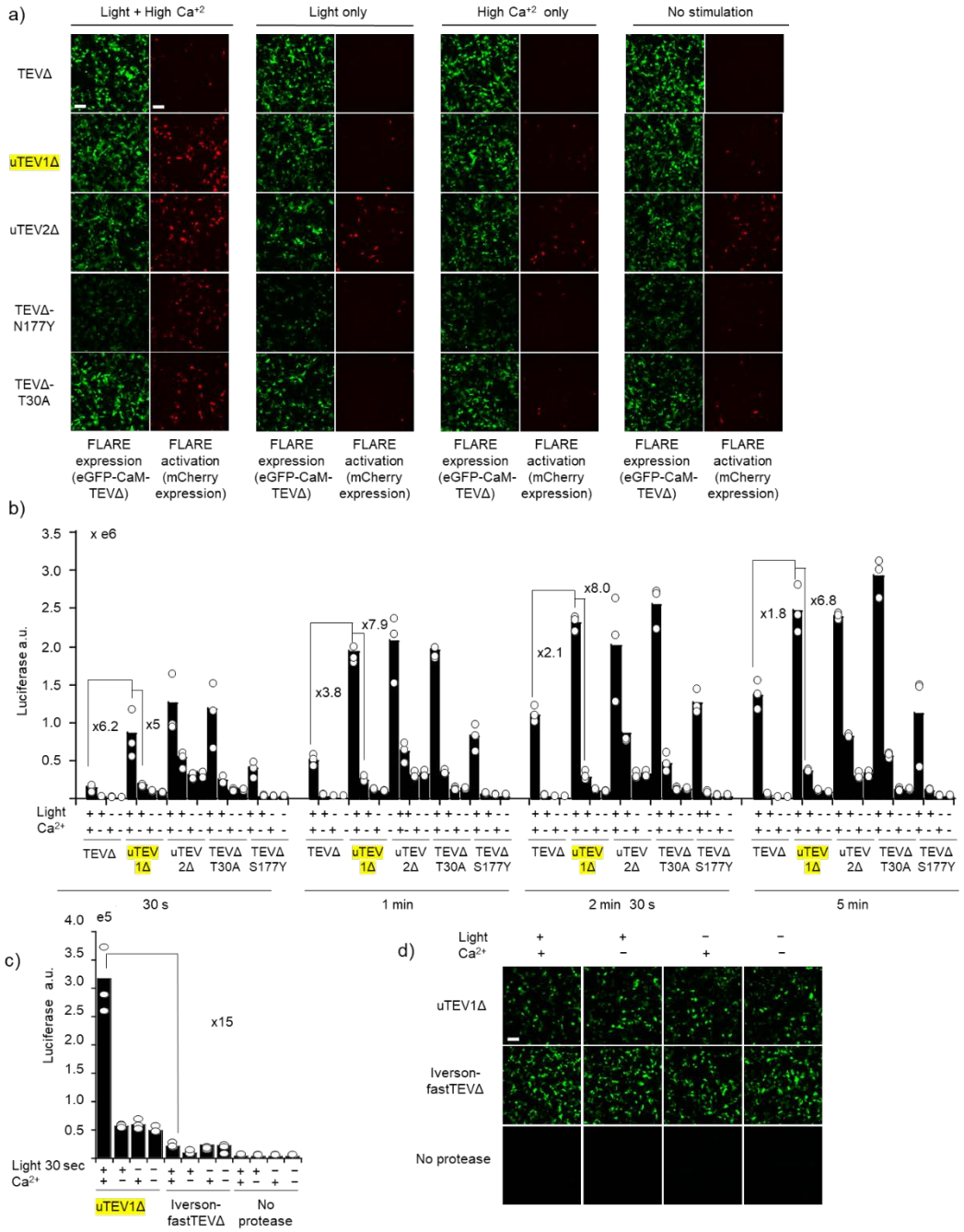
Supplementary Figure 8. Characterization of evolved full-length TEV mutants. Related to Figure 3D. (**A**) Proteases were expressed in a yeast strain with 2 LexA boxes and high-affinity TEVcs (ENLYFQ/S) (configuration shown in Figure. 3A). FACS analysis performed 6 hours after light irradiation. Each condition repeated once. n = 20,000 cells. (**B**) Same as (A) with additional TEV mutants and additional conditions. The first 3 columns show shorter protein induction times in the dark (standard induction time is 12 hours). Right 3 columns show cells 6 hours following blue light irradiation for the indicated times. Each condition performed twice. n = 20,000 cells.



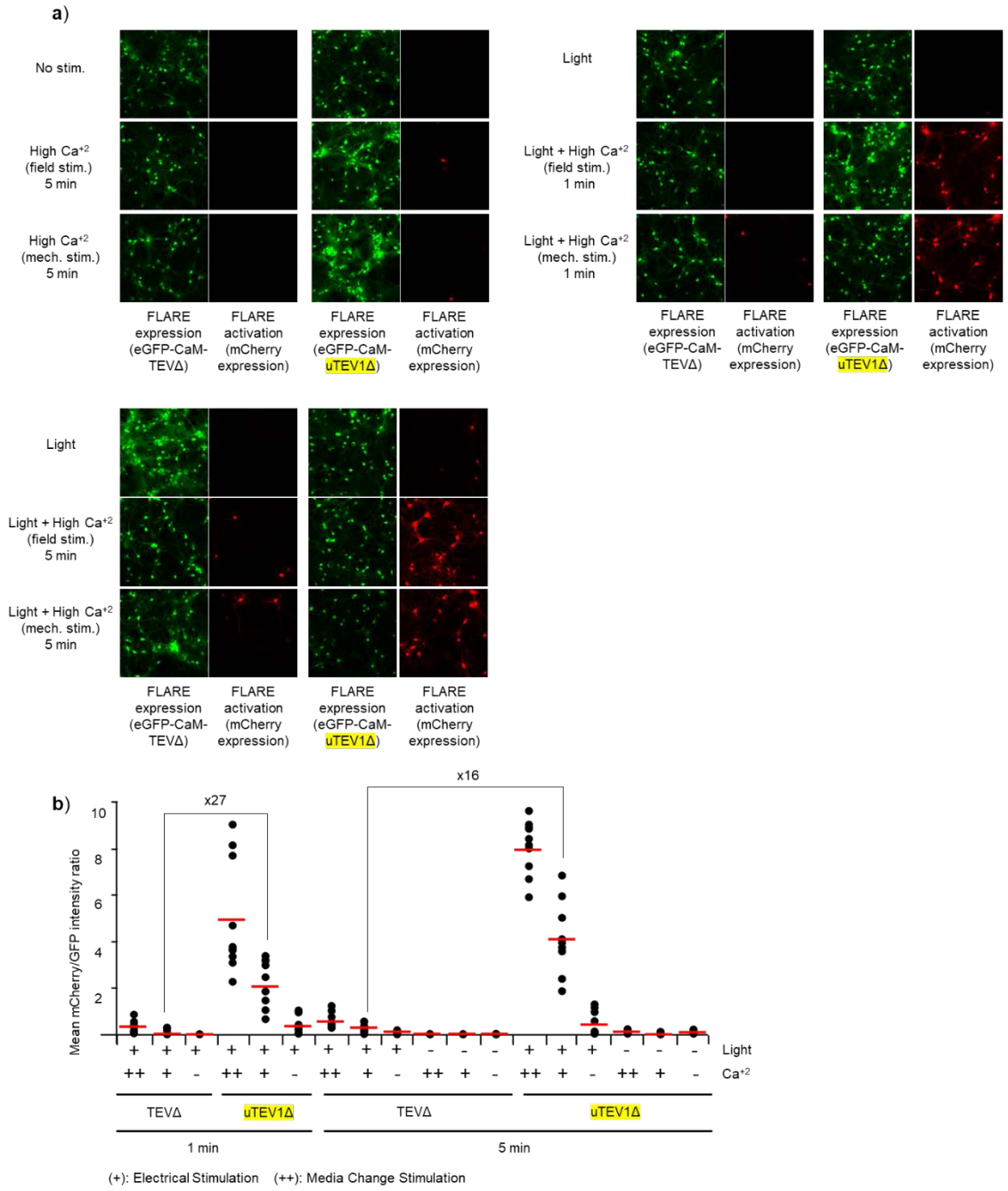
Supplementary Figure 9. Profiling the sequence specificity of full-length TEV mutants in yeast. Same assay as in Figure 2F. FACS analysis performed 12 hours after galactose induction. Each condition performed once, n = 20,000 cells.



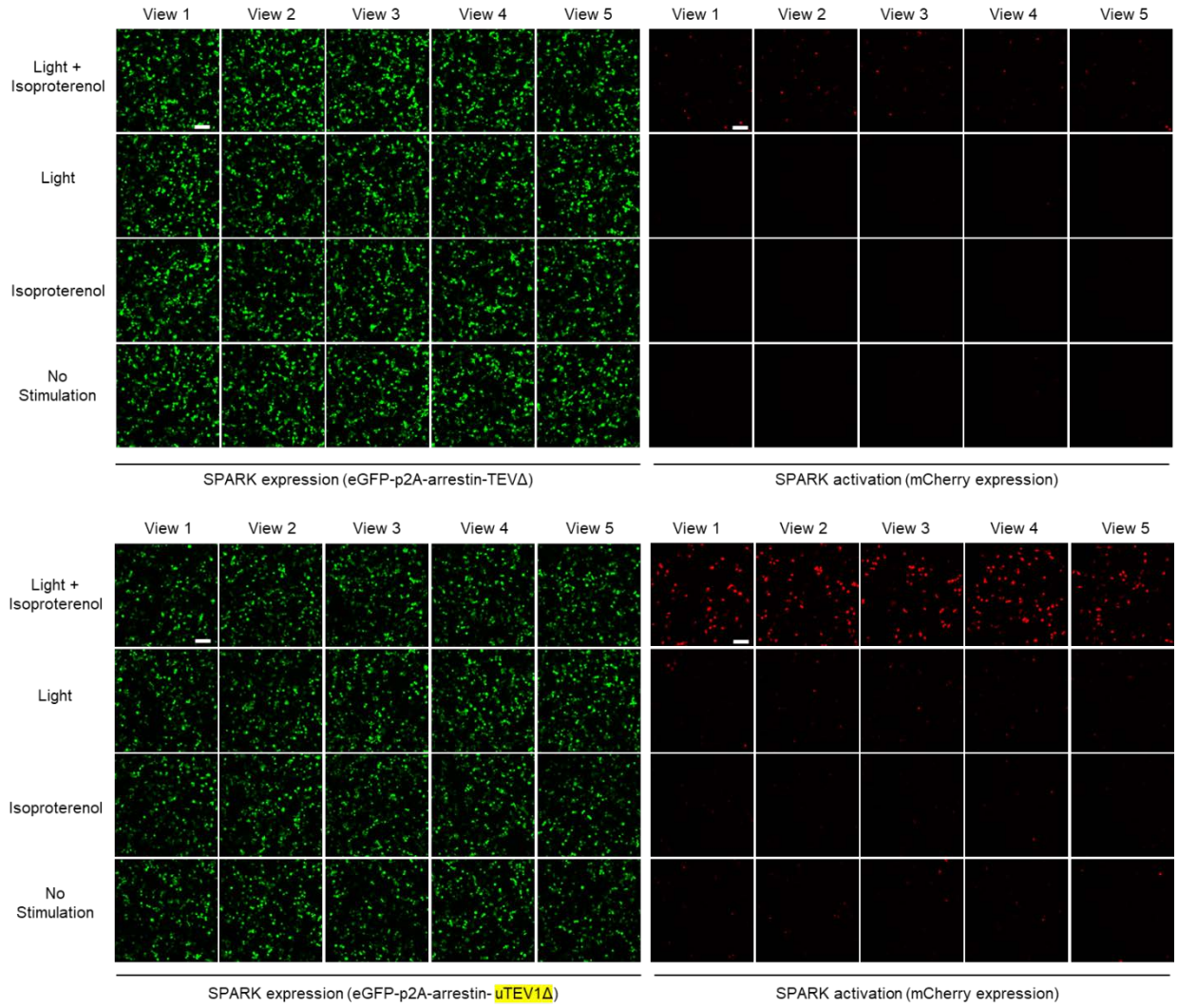
Supplementary Figure 10. Comparison of evolved TEVs with Iverson, Bottomley and Waugh TEV mutants. (**A**) Side-by-side comparison in yeast, with full-length proteases and the high-affinity TEVcs (ENLYFQ/S). First four columns show yeast induced with galactose in the dark for 6.5 to 18 hours before FACS analysis. Last two columns were irradiated with light before FACS analysis 6 hours later. (**B**) Side-by-side comparison of truncated proteases using the low-affinity TEVcs (ENLYFQ/M). FACS analysis was performed 6 hours after blue light exposure for the indicated times. Each condition was repeated once, n = 20,000.



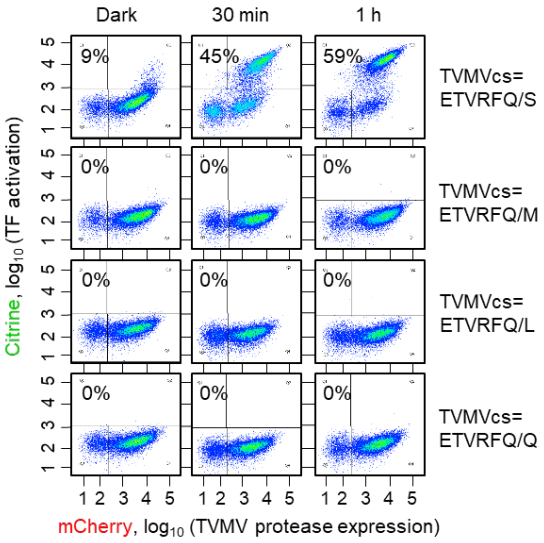
Supplementary Figure 11. **Testing evolved TEV mutants in FLARE**. Related to Figure 4E. (**A**) HEK293T cells were transiently transfected with FLARE constructs (as in Figure 4A) incorporating the indicated TEV protease. Stimulation was performed using 5 mM $CaCl_2$ and ionomycin for 30 seconds in the presence of blue light (467 nm, 60 mW/cm2, 10% duty cycle (0.5s light every 5s). Nine hours later, cells were fixed and imaged. This experiment was performed independently two times with similar results. (**B**) Same experiment as in (A) but with luciferase as the reporter gene instead of mCherry. Stimulation times varied from 30 seconds to 5 minutes. This experiment was performed once with three technical replicates per condition. (**C**) Comparison of uTEV1Δ with the truncated version of Iverson's TEV in the context of FLARE. Cells were stimulated and analyzed as in (B). This experiment was performed once, with three technical replicates per condition. (**D**) Samples from (C) were imaged by confocal microscopy to confirm protease expression. GFP channel shown. Scale bar, 10 µm.



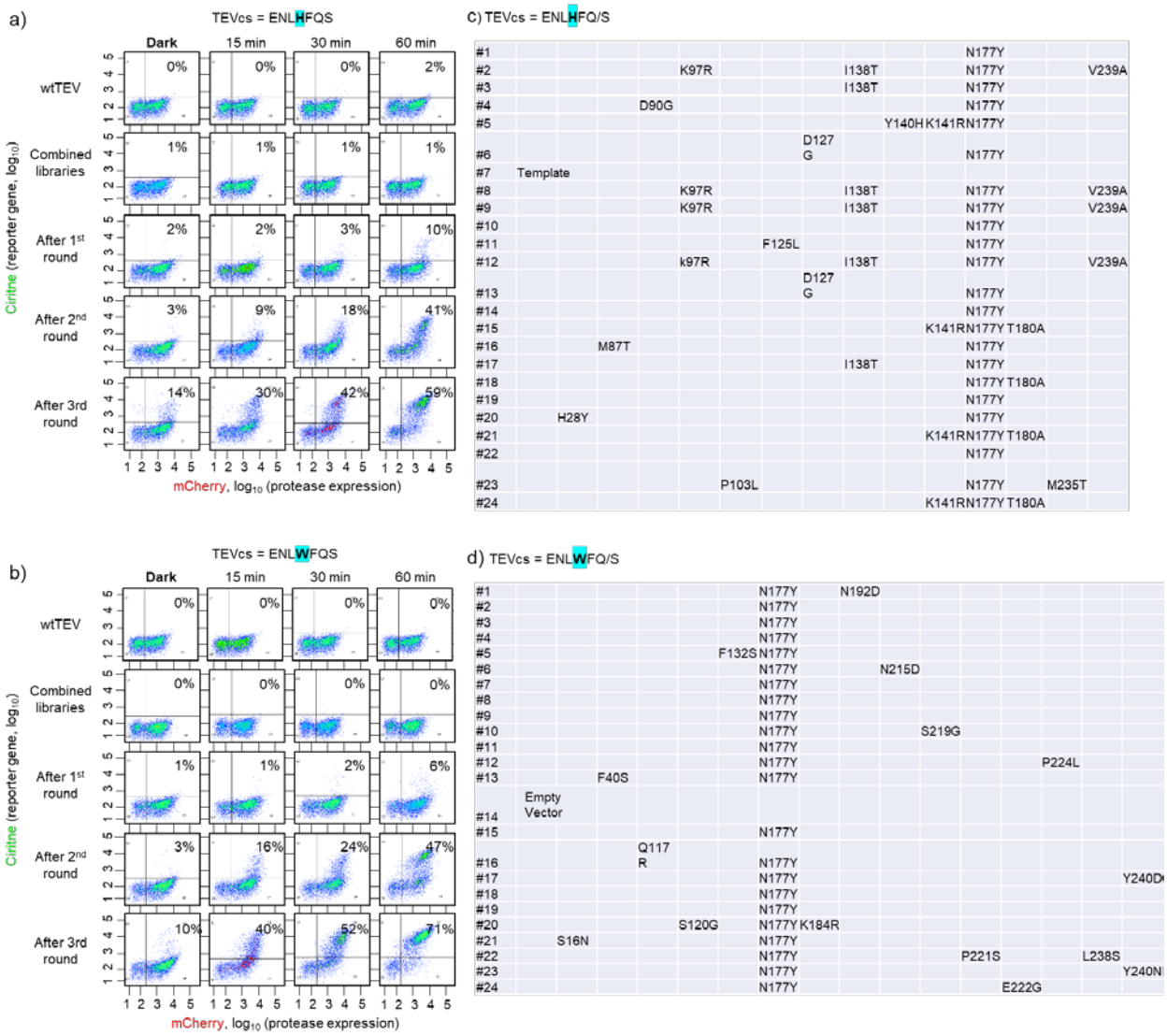
Supplementary Figure 12. **Evaluation of uTEV1Δ in the context of FLARE in neurons**. Related to Figure 4F. (**A**) Rat cortical neurons were transduced at day 12 with FLARE constructs (packaged into AAV1/2 viruses) containing either the original TEVΔ protease or our evolved uTEV1Δ protease. At day 18 in vitro (DIV18), we stimulated the neurons using either field stimulation (3-s trains consisting of 32 1-ms 50 mA pulses at 20 Hz for a total of 1 or 5 min), or via replacement of culture media with media of identical composition (this mechanically stimulates the cultures and also provides a fresh source of glutamate). Light source was 467 nm, 60 mW/cm2, 10% duty cycle (0.5s light every 5s). Imaging was performed 18 hours later. This experiment was replicated three times for each condition. Scale bars, 10 μm. (**B**) Quantitation of data from (A). Signal ratios were calculated from mean mCherry and mean GFP intensities across >50 cells per field of view. 10 fields of view quantified per condition. Red lines indicates the mean of 10 FOVs.



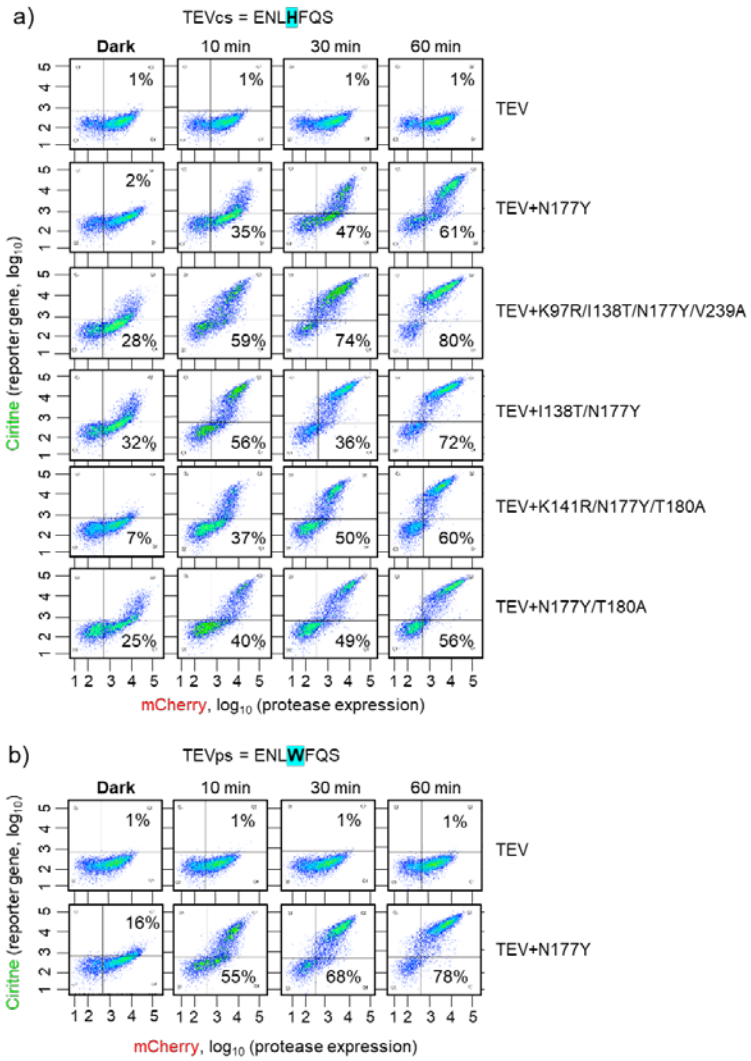
Supplementary Figure 13. **Testing the evolved protease uTEV1Δ in SPARK**. Related to Figure 4G. HEK293T cells were transiently transfected with SPARK constructs (Figure 4B) containing the indicated protease variant. Cells were stimulated with 10 μM isoproterenol for 60 sec in the presence or absence of blue light (467 nm, 60 mW/cm2, 10% duty cycle (0.5s light every 5s)). Nine hours later, cells were imaged. This experiment was replicated two times. Scale bars, 10 μm.



Supplementary Figure 14. Testing a different protease (TVMV) in the yeast platform. Constructs were designed as in Figure 1A, but TEV was replaced with TVMV protease, and TEVcs was replaced with the TVMV substrate sequences shown at right. FACS plots were collected 6 hours after blue light irradiation for the indicated times. Percentages give the fraction of Citrine-positive cells. Each plot representative of two replicates, n = 20,000 cells.



Supplementary Figure 15. Selections to alter the sequence-specificity of TEV. Analysis of full-length TEV libraries after 3 rounds of sorting against mutated TEV cleavage sequences: H at the P3 position of TEVcs in (**A**), and W in the P3 position of TEVcs in (**B**). The configuration of constructs was the same as in Figure 3A. FACS plots were obtained 6 hours after blue light exposure for the indicated times. Percentages quantify Citrine-positive cells. This experiment was performed once, n = 10,000 cells. (**C**) and (**D**) Sequencing after round 3. 24 clones were sequenced from each selection and mutations found in each, relative to the original template wild-type TEV, are shown.



Supplementary Figure 16. Characterization of specificity-altered TEV mutants in yeast. Related to Supplementary Figure 15. The indicated TEV mutants were compared to wild-type full-length TEV, using the altered TEVcs substrates P3 = H (**A**) and P3 = W (**B**). FACS plots obtained 6 hours after blue light exposure for the indicated times. Percentages reflect the fraction of Citrine-positive cells**.** Each plot representative of two replicates, n = 10,000 cells.

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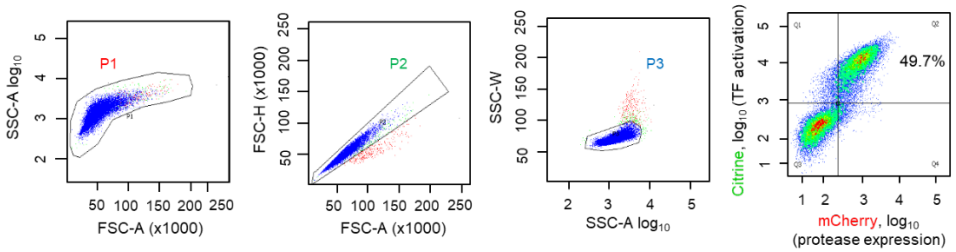
Plasmid table

ID	Plasmid name	Plasmid Vect	Promoter	Terminator	Expression in	Tags	More Details	Ab. R	Used in figures
p1	STE2-V5-CIBN-M13-eLOV-TEVcs(ENLYFQM)-LexAVP16	pRS-derived	ACT1		Yeast / ApaI digestion to integrate	V5	HIS3 selectable marker	Amp	Fig. 1 / S. Fig 1B
p2	STE2-V5-BFP-CIBN-M13-eLOV-TEVcs(ENLYFQM)-LexAVP16	pRS-derived	ACT1		Yeast / ApaI digestion to integrate	V5 / BFP	HIS3 selectable marker	Amp	Fig. 1 / S. Fig 1B
p3	STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs(ENLYFQM)-LexAVP16-tC	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	flag / BFP	LEU2 selectable marker	Amp	Fig. 1 / S. Fig 1B
p4	STE2Δ-CIBN-MKII-eLOV-TEVcs(ENLYFQM)-LexAVP16-tCYC	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	flag / BFP	LEU2 selectable marker	Amp	Fig. 1 / S. Fig 1B
p5	STE2Δ-V5-CIBN-M13-eLOV(ENLYFQM)-TEVcs-LexAVP16	pRS-derived	ACT1		Yeast / AscI digestion to integrate	V5	LEU2 selectable marker	Amp	Fig. 1 / S. Fig 1B
p6	STE2Δ-V5-BFP-CIBN-M13-eLOV(ENLYFQM)-TEVcs-LexAVP1	pRS-derived	ACT1		Yeast / AscI digestion to integrate	V5 / BFP	LEU2 selectable marker	Amp	Fig. 1 / S. Fig 1B
p7	mCherry-CRY-TEV	pRSII415	Gal	tCYC1	Yeast / episomally	mCherry	LEU2 selectable marker	Amp	Fig. 1 / S. Fig 1B
p8	mCherry-CRY-TEVΔ/S219V	pRSII415	Gal	tCYC1	Yeast / episomally	mCherry	LEU2 selectable marker	Amp	Fig. 1 / S. Fig 1B
p9	mCherry-TEV	pRSII415	Gal	tCYC1	Yeast / episomally	mCherry	LEU2 selectable marker	Amp	Fig. 1 / S. Fig 1B
p10	mCherry-TEVΔ-S219V	pRSII415	Gal	tCYC1	Yeast / episomally	mCherry	LEU2 selectable marker	Amp	Fig. 1 / S. Fig 1B
p11	mCherry-CRY-TEV	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 1 / S. Fig 1B
p12	mCherry-CRY-TEVΔ/S219V	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 1 / S. Fig 1B
p13	mCherry-TEV	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 1 / S. Fig 1B/
									Fig. 2 S. Fig. 3
p14	mCherry-TEVΔ/S219V	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 1 / S. Fig 1B/ Fig. 2
									S. Fig. 3 / S. Fig 10
p15	STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs-LexAB42	pRS-derived	ACT1	tCYC1	Yeast / AscI digestion to integrate	V5 / BFP	LEU2 selectable marker	Amp	S. Fig 1C
p16	STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs-LexAGal4	pRS-derived	ACT1	tCYC1	Yeast / AscI digestion to integrate	V5 / BFP	LEU2 selectable marker	Amp	S. Fig 1C
p17	mCherry-CRY-TEVΔ/S219V+S31W	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p18	mCherry-CRY-TEVΔ/S219V+S153N	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3 / S. Fig 10
p19	mCherry-CRY-TEVΔ/S219V+T30A+S153N	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p20	mCherry-CRY-TEVΔ/S219V+T30I	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p21	mCherry-CRY-TEVΔ/S219V+N177Y	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p22	mCherry-CRY-TEVΔ/S219V+T30A	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p23	mCherry-TEVΔ/S219V+S31W	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p24	mCherry-TEVΔ/S219V+S153N	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p25	mCherry-TEVΔ/S219V+T30A+S153N	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p26	mCherry-TEVΔ/S219V+T30I	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p27	mCherry-TEVΔ/S219V+N177Y	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p28	mCherry-TEVΔ/S219V+T30A	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p29	mCherry-CRY-TEVΔ/S219V+T30A+N177Y	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p30	mCherry-CRY-TEVΔ/S219V+S153N+N177Y	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p31	mCherry-CRY-TEVΔ/S219V+S31W+N177Y	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p32	mCherry-CRY-TEVΔ-S219V- T30A- S153N + Asn177Tyr-tCYC1	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p33	mCherry-CRY-TEVΔ-S219V- Thr30Ala + Ser31Trp-tCYC1	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p34	mCherry-TEVΔ/S219V+T30A+N177Y	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p35	mCherry-TEVΔ/S219V+S153N+N177Y	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p36	mCherry-TEVΔ/S219V+S31W+N177Y	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p37	mCherry-TEVΔ/S219V+T30A+S153N+N177Y	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p38	mCherry-TEVΔ/S219V+T30A+S31W	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2 / S. Fig 3
p39	mCherry-CRY-TEVΔ/G79E+T173A+S219V	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	S. Fig 10B
p40	mCherry-TEV/S219V	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 3B / S. Fig. 8A
p41	mCherry-TEV/S153N+S219V	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 3B / S. Fig. 8A
p42	mCherry-TEV/T30A+S153N+S219V	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 3B
p43	mCherry-TEV/G79E+T173A+S219V	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	S. Fig 10A
p44	mtagBFPII-CRY-TEVΔ	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	mtagBFPII	LEU2 selectable marker	Amp	Fig. 2G / S. Fig. 5B, C
p45	mtagBFPII-CRY-TEVΔ/S219V+S153N	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	mtagBFPII	LEU2 selectable marker	Amp	Fig. 2G / S. Fig. 5B, C
p46	mtagBFPII-CRY-TEVΔ/s219V+T30A+S153N	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	mtagBFPII	LEU2 selectable marker	Amp	Fig. 2G / S. Fig. 5B, C
p47	STE2Δ-mCherry-CIBN-PIF6-eLOV-TEVcs-LexAVP16	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2F
p48	STE2Δ-mCherry-CIBN-PIF6-eLOV-TEVcs-Ala P6-LexAVP16	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2F
p49	STE2Δ-mCherry-CIBN-PIF6-eLOV-TEVcs-Ala P3-LexAVP16	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2F
p50	STE2Δ-mCherry-CIBN-PIF6-eLOV-TEVcs-Ala P1-LexAVP16	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 2F
p51	MBP-TEVcs-7xHis-TEVΔ/S219V	pRK793	tac P		E. Coli			Amp	Fig. 2C, D, E
p52	MBP-TEVcs-7xHis-TEVΔ/S219V+S153N	pRK793	tac P		E. Coli			Amp	Fig. 2C, D, E
p53	MBP-TEVcs-7xHis-TEVΔ/S219V+T30A+S153N	pRK793	tac P		E. Coli			Amp	Fig. 2C, D, E
p54	MBP-TEVcs(ENLYFQ/S)-eGFP	pYFJ16	tac P		E. Coli			Amp	Fig. 3E, G
p55	UAS-mCherry	AAV	UAS	WPRE/PolyA	mammalian / HEK			Amp	Fig. 4E, G / S. Fig. 11A, 13
p56	CD4-HA-CIBN-2xMKII-NNES-heLOV-GAL4	AAV	CMV	PolyA	mammalian / HEK	HA		Amp	Fig. 4E, G / S. Fig. 11A, 14
p57	CMV-eGFP-CaM-TEVΔ/S219V	AAV	CMV	PolyA	mammalian / HEK	V5 / eGFP		Amp	Fig. 4D / S. Fig. 11A, B.
p58	CMV-eGFP-CaM-TEVΔ/S219V+S153N	AAV	CMV	PolyA	mammalian / HEK	V5 / eGFP		Amp	Fig. 4D / S. Fig. 11A, B.
p59	CMV-eGFP-CaM-TEVΔ/S219V+T30A+S153N	AAV	CMV	PolyA	mammalian / HEK	V5 / eGFP		Amp	Fig. 4D / S. Fig. 11A, B.
p60	CMV-eGFP-CaM-TEVΔ/S219V+N177Y	AAV	CMV	PolyA	mammalian / HEK	V5 / eGFP		Amp	Fig. 4D / S. Fig. 11A, B.
p61	CMV-eGFP-CaM-TEVΔ/S219V+T30A	AAV	CMV	PolyA	mammalian / HEK	V5 / eGFP		Amp	Fig. 4D / S. Fig. 11A, B.
p62	TRE-mCherry	AAV	TRE	WPRE/PolyA	mammalian / Neuron			Amp	Fig. 4F / S. Fig. 12A
p63	tNeu-CIBN-2xMKII-heLOV-tTA	AAV	Syn	PolyA	mammalian / Neuron	HA/ flag/VP16		Amp	Fig. 4F / S. Fig. 12A
p64	AAV-EGFP-CaM5f-V5-TEVΔ/S219V	AAV	Syn	WPRE/PolyA	mammalian / Neuron	V5/ eGFP		Amp	Fig. 4F / S. Fig. 12A
p65	AAV-eGFP-CaM5f-V5-TEVΔ/S219V+S153N	AAV	Syn	WPRE/PolyA	mammalian / Neuron	V5/ eGFP		Amp	Fig. 4F / S. Fig. 12A
p66	AAV-EGFP-CaM5f-V5-TEVΔ/S219V+T30A+S153N	AAV	Syn	WPRE/PolyA	mammalian / Neuron	V5/ eGFP		Amp	
p67	AAV-EGFP-CaM5f-V5-TEVΔ/S219V+N177Y	AAV	Syn	WPRE/PolyA	mammalian / Neuron	V5/ eGFP		Amp	
p68	AAV-EGFP-CaM5f-V5-TEVΔ/S219V+T30A	AAV	Syn	WPRE/PolyA	mammalian / Neuron	V5/ eGFP		Amp	
p69	B2Ar-heLOV-TEVcs-Gal4-V5	plx208	CMV		mammalian / HEK	HA/ eGFP	Hygromycin gene deleted	Amp	Fig. 4G / S. Fig. 13
p70	EGFP-p2a-HA-arrestin-TEVΔ/S219V	plx208	CMV		mammalian / HEK	HA/ eGFP	Hygromycin gene deleted	Amp	Fig. 4G / S. Fig. 14
p71	EGFP-p2a-HA-arrestin-TEVΔ/S153N	plx208	CMV		mammalian / HEK	HA/ eGFP	Hygromycin gene deleted	Amp	Fig. 4G / S. Fig. 15
p72	pAAV1							Amp	Fig. 4F / S. Fig. 12A
p73	pAAV2							Amp	Fig. 4F / S. Fig. 12A
p74	pDF6							Amp	Fig. 4F / S. Fig. 12A
p75	mCherry-TEV/S153N+T180A+S219V	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 3D / S. Fig. 8A
p76	mCherry-TEV/S135F+S153N+T180A+S219V	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 3D / S. Fig. 8A
p77	mCherry-TEV/I138T+S153N+T180A+S219V	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 3D / S. Fig. 8A
p78	mCherry-TEV/S135F+I138T+S153N+T180A+S219V	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 3D / S. Fig. 8A
p79	mCherry-TEV/I138T+S153N+S219V	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	Fig. 3D / S. Fig. 8A
p80	mtagBFPII-TEV/S153N+T180A+S219V	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	mtagBFPII	HIS3 selectable marker	Amp	S. Fig. 9
p81	mtagBFPII-TEV/S135F+S153N+T180A+S219V	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	mtagBFPII	HIS3 selectable marker	Amp	S. Fig. 9
p82	mtagBFPII-TEV/I138T+S153N+T180A+S219V	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	mtagBFPII	HIS3 selectable marker	Amp	S. Fig. 9
p83	mtagBFPII-TEV/S135F+I138T+S153N+T180A+S219V	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	mtagBFPII	HIS3 selectable marker	Amp	S. Fig. 9
p84	mtagBFPII-TEV/I138T+S153N+S219V	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	mtagBFPII	HIS3 selectable marker	Amp	S. Fig. 9
p85	mtagBFPII-TEV/I153F+S153N+S219V	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	mtagBFPII	HIS3 selectable marker	Amp	S. Fig. 9
p86	STE2Δ-mCherry-CIBN-PIF6-eLOV-TEVcs-Ala P6-LexAVP16	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	S. Fig. 9
p87	STE2Δ-mCherry-CIBN-PIF6-eLOV-TEVcs-Ala P3-LexAVP16	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	S. Fig. 9
p88	STE2Δ-mCherry-CIBN-PIF6-eLOV-TEVcs-Ala P1-LexAVP16	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	S. Fig. 9
p89	STE2Δ-mCherry-CIBN-PIF6-eLOV-TEVcs-LexAVP16	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	S. Fig. 9
p90	mCherry-TEV/L56V+S135G	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	S. Fig. 8B

ID	Plasmid name	Plasmid Vect	Promoter	Terminator	Expression in	Tags	More Details	Ab. R	Used in figures
p91	mCherry-TEV/L56V+S135G+S219V	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	S. Fig. 8B
p92	HIS6-MBP-TEV	pYFJ16	tac P		E. Coli	MBP / His6	S219V	Amp	Fig. 4E, G.
p93	HIS6-MBP-TEV I138T S153N T180A	pYFJ16	tac P		E. Coli	MBP / His6	S219V	Amp	Fig. 4E, G.
p94	mCherry-TVMV-tCYC1	pRSII413	Gal		Yeast / episomally	mCherry	HIS3 selectable marker	Amp	S. Fig. 14
p95	STE2Δ-BFP-ClBN-MKII-eLOV-TVMV(ETVRFQS)-LexAVP16	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	BFP	LEU2 selectable marker	Amp	S. Fig. 14
p96	STE2Δ-BFP-ClBN-MKII-eLOV-TVMV(P1'=M)-LexAVP16	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	BFP	LEU2 selectable marker	Amp	S. Fig. 14
p97	STE2Δ-BFP-ClBN-MKII-eLOV-TVMV(P1'=L)-LexAVP16	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	BFP	LEU2 selectable marker	Amp	S. Fig. 14
p98	STE2Δ-BFP-ClBN-MKII-eLOV-TVMV(P1'=Q)-LexAVP16	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	BFP	LEU2 selectable marker	Amp	S. Fig. 14
p99	LexA-mCherry-VP16	pRSII413	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	S. Fig. 8B
p100	LexA-mCherry-B42	pRSII414	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	S. Fig. 8B
p101	LexA-mCherry-Gal4	pRSII415	Gal	tCYC1	Yeast / episomally	mCherry	HIS3 selectable marker	Amp	S. Fig. 8B
p102	MBP-TEVcs(ENLYFQ/M)-eGFP	pYFJ16	tac P		E. Coli	MBP/eGFP		Amp	Fig. 2C, D, E
p103	STE2-citrine	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	citrine	LEU2 selectable marker	Amp	S. Fig 1A
p104	STE2Δ-citrine	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	citrine	LEU2 selectable marker	Amp	S. Fig 1A
p105	STE2Δ-BFP-ClBN-eLOV-TEVcs(ENLHFQS, P3=H)-LexAVP16	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	BFP / flag	LEU2 selectable marker	Amp	S. Fig 16
p106	STE2Δ-BFP-ClBN-eLOV-TEVcs(ENLWFQS, P3=W)-LexAVP16	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	BFP / flag	LEU2 selectable marker	Amp	S. Fig 16
p107	STE2Δ-BFP-ClBN-eLOV-TEVcs(ENLYFQS)-LexAVP16	pRS-derived	TDH3	tCYC1	Yeast / AscI digestion to integrate	BFP / flag	LEU2 selectable marker	Amp	Fig. 3B , C / S. Fig. 6C,
									7A, B, 8A, B, 10A
p108	mCherry-TEVΔ	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p109	mCherry-TEV	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p110	mCherry-TEV/S31W+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p111	mCherry-TEV/S153N+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p112	mCherry-TEV/T30A+S153N+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p113	mCherry-TEV-T30I+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p114	mCherry-TEV/N177Y+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p115	CMS817-CMV-mCherry-TEV/T30A S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p116	mCherry-TEV/N177Y+T30A+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p117	mCherry-TEV/N177Y+S153N+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p118	mCherry-TEV/N177Y+S31W+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p119	mCherry-TEV/T30A+S153N+N177Y+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p120	mCherry-TEV/T30A+S31W+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p121	mCherry-TEVΔ/S31W+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p122	mCherry-TEVΔ/S153N+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p123	mCherry-TEVΔ/T30A+S153N+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p124	mCherry-TEVΔ/T30I+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p125	mCherry-TEVΔ/N177Y+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5
p126	mCherry-TEVΔ/T30A+S219V	AAV	CMV	PolyA	mammalian / HEK	mCherry		Amp	S. Fig. 5

Yeast table

The original strain used is BY4741. **Genotype**: MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0. **Description**: S288C-derivative laboratory strain		
ID	**Plasmid used**	**Auxotrophic selection marker**
YS-FRP791	Addgene plasmid # 58432 (FRP791)	URA3
YS-FRP792	Addgene plasmid # 58433 (FRP792)	URA3
YS-FRP793	Addgene plasmid # 58434 (FRP793)	URA3
YS1	P3-pTDH3-STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs(ENLYFQ/**M**)-LexAVP16-tCYC	URA3/LEU2
YS2	P105-pTDH3-STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs(ENL**H**FQ/S P3=H)-LexAVP16-tCYC	URA3/LEU2
YS3	P106-pTDH3-STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs(ENL**W**FQ/S P3=W)-LexAVP16-tCYC	URA3/LEU2
YS4	P107-pTDH3-STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs(ENLYFQ/**S**)-LexAVP16-tCYC.ape	URA3/LEU2
YS5	P44-mtagBFPII-CRY-TEVΔ/S219V	URA3/LEU2
YS6	P45-mtagBFPII-CRY-TEVΔ/S153N+S219V	URA3/LEU2
YS7	P46-mtagBFPII-CRY-TEVΔ/T30A+S153N+S219V	URA3/LEU2
YS8	P79-mtagBFPII-TEV/S153N+T180A+S219V	URA3/LEU2
YS9	P80-mtagBFPII-TEV/S135F+S153N+T180A+S219V	URA3/LEU2
YS10	P81-mtagBFPII-TEV/I138T+S153N+T180A+S219V	URA3/LEU2
YS11	P82-mtagBFPII-TEV/S135F+I138T+S153N+T180A+S219V	URA3/LEU2
YS12	P83-mtagBFPII-TEV/I138T+S153N+S219V	URA3/LEU2
YS13	P84-mtagBFPII-TEV/I153F+S153N+S219V	URA3/LEU2
YS14	P94-STE2Δ-BFP-CIBN-MKII-eLOV-TVMVcs(ETVRFQ/S)-LexAVP16	URA3/LEU2
YS15	P95-STE2Δ-BFP-CIBN-MKII-eLOV-TVMVcs(ETVRFQ/**M** P1'=M)-LexAVP16	URA3/LEU2
YS16	P96-STE2Δ-BFP-CIBN-MKII-eLOV-TVMVcs(ETVRFQ/**L** P1'=L)-LexAVP16	URA3/LEU2
YS17	P97-STE2Δ-BFP-CIBN-MKII-eLOV-TVMVcs(ETVRFQ/**Q** P1'=Q)-LexAVP16	URA3/LEU2
ID	**Genetic Background**	**Used in figures**
YS-FRP791	BY4741::insul-(lexA-box)1-PminCYC1-Citrine-TCYC1 (URA3)	Fig. 3
YS-FRP792	BY4741::insul-(lexA-box)2-PminCYC1-Citrine-TCYC1 (URA3)	Fig. 3
YS-FRP793	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3)	Fig. 1,2 ,3.
YS1	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3),pTDH3-STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs(ENLYFQM)-LexAVP16-tCYC (LEU2)	Fig. 1 ,2. / Supp. 1,
YS2	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3),pTDH3-STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs(ENLHFQS)-LexAVP16-tCYC (LEU2)	Supp. Fig. 15/16
YS3	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3),pTDH3-STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs(ENLWFQS)-LexAVP16-tCYC (LEU2)	Supp. Fig. 15/16
YS4	BY4741::insul-(lexA-box)2-PminCYC1-Citrine-TCYC1 (URA3),pTDH3-STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs(ENLYFQS)-LexAVP16-tCYC (LEU2)	Fig. 3. / Supp. 7, 8,
YS5	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3), pTDH3-mtagBFPII-TEVΔ/S219V (LEU2)	Supp. Fig. 5C
YS6	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3), pTDH3-mtagBFPII-TEVΔ/S153N+S219V (LEU2)	Supp. Fig. 5C
YS7	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3), pTDH3-mtagBFPII-TEVΔ/T30A+S153N+S219V (LEU2)	Supp. Fig. 5C
YS8	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3), pTDH3-mtagBFPII-TEVΔ/S153N+T180A+S219V (LEU2)	Supp. Fig. 9
YS9	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3), pTDH3-mtagBFPII-TEVΔ/S135F+S153N+T180A+S219V (LEU2)	Supp. Fig. 9
YS10	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3), pTDH3-mtagBFPII-TEVΔ/I138T+S153N+T180A+S219V (LEU2)	Supp. Fig. 9
YS11	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3), pTDH3-mtagBFPII-TEVΔ/S135F+I138T+S153N+T180A+S219V (LEU2)	Supp. Fig. 9
YS12	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3), pTDH3-mtagBFPII-TEVΔ/I138T+S153N+S219V (LEU2)	Supp. Fig. 9
YS13	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3), pTDH3-mtagBFPII-TEVΔ/I153F+S153N+S219V (LEU2)	Supp. Fig. 9
YS14	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3),pTDH3-STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs(ETVRFQ/S)-LexAVP16-tCYC (LEU2)	Supp. Fig. 14
YS15	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3),pTDH3-STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs(ETVRFQ/M P1'=M)-LexAVP16-tCYC (LEU2)	Supp. Fig. 14
YS16	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3),pTDH3-STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs(ETVRFQ/L P1'=L)-LexAVP16-tCYC (LEU2)	Supp. Fig. 14
YS17	BY4741::insul-(lexA-box)4-PminCYC1-Citrine-TCYC1 (URA3),pTDH3-STE2Δ-BFP-CIBN-MKII-eLOV-TEVcs(ETVRFQ/Q P1'=Q)-LexAVP16-tCYC (LEU2)	Supp. Fig. 14



Tube: Example

Population	#Events	%Parent	%Total
All Events	20,000	####	100.0
P1	19,995	100.0	100.0
P2	19,709	98.6	98.5
P3	19,554	99.2	97.8
Q1	1,319	6.6	6.6
Q2	8,616	43.1	43.1
Q3	9,389	46.9	46.9
Q4	676	3.4	3.4

Sample FACS plots showing the gating parameters. Percentage values reflect the fraction of cells with high Citrine intensity, i.e., cells in the upper FACS quadrants Q1 + Q2.

TEV sequences

TEVΔ-S219V

Ggagaatccttgtttaagggaccacgtgattacaacccgatatcgagcaccatttgtcatttgacgaatgaatctgatgggcacacaacatcgttgtatggtattggattt
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acacctcattgatgggagggacatgataattattcgcatgcctaaggatttcccaccatttcctcaaaagctgaaatttagagagccacaaagggaagagcgcatatg
tcttgtgacaaccaacttccaaactaagagcatgtctagcatggtgtcagacactagttgcacattcccttcatctgatggcatattctggaagcattggattcaaaccaa
ggatgggcagtgtggcagtccattagtatcaactagagatgggttcattgttggtatacactcagcatcgaatttcaccaacacaaacaattatttcacaagcgtgccga
aaaacttcatggaattgttgacaaatcaggaggcgcagcagtgggttagtggttggcgattaaatgctgactcagtattgtgggggggccataaagttttcatg**gtg.**

GESLFKGPRDYNPISSTICHLTNESDGHTTSLYGIGFGPFIITNKHLFRRNNGTLLVQSLHGVFKVKNTTTLQQHLIDGR
DMIIIRMPKDFPPFPQKLKFREPQREERICLVTTNFQTKSMSSMVSDTSCTFPSSDGIFWKHWIQTKDGQCGSPLVST
RDGFIVGIHSASNFTNTNNYFTSVPKNFMELLTNQEAQQWVSGWRLNADSVLWGGHKVFM**V**

TEV1Δ-(S135N/S219V)

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ggatgggcagtgtggc**aat**ccattagtatcaactagagatgggttcattgttggtatacactcagcatcgaatttcaccaacacaaacaattatttcacaagcgtgccga
aaaacttcatggaattgttgacaaatcaggaggcgcagcagtgggttagtggttggcgattaaatgctgactcagtattgtgggggggccataaagttttcatg**gtg.**

GESLFKGPRDYNPISSTICHLTNESDGHTTSLYGIGFGPFIITNKHLFRRNNGTLLVQSLHGVFKVKNTTTLQQHLIDGR
DMIIIRMPKDFPPFPQKLKFREPQREERICLVTTNFQTKSMSSMVSDTSCTFPSSDGIFWKHWIQTKDGQCG**N**PLVST
RDGFIVGIHSASNFTNTNNYFTSVPKNFMELLTNQEAQQWVSGWRLNADSVLWGGHKVFM**V**

TEV2Δ-(T30A/S135N/S219V)

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ggtcccttcatcattacaaacaagcacttgtttagaagaaataatggaacactgttggtccaatcactacatggtgtattcaaggtcaagaacaccacgactttgcaaca
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ggatgggcagtgtggc**aat**ccattagtatcaactagagatgggttcattgttggtatacactcagcatcgaatttcaccaacacaaacaattatttcacaagcgtgccga
aaaacttcatggaattgttgacaaatcaggaggcgcagcagtgggttagtggttggcgattaaatgctgactcagtattgtgggggggccataaagttttcatg**gtg.**

GESLFKGPRDYNPISSTICHLTNESDGHT**A**SLYGIGFGPFIITNKHLFRRNNGTLLVQSLHGVFKVKNTTTLQQHLIDGR
DMIIIRMPKDFPPFPQKLKFREPQREERICLVTTNFQTKSMSSMVSDTSCTFPSSDGIFWKHWIQTKDGQCG**N**PLVST
RDGFIVGIHSASNFTNTNNYFTSVPKNFMELLTNQEAQQWVSGWRLNADSVLWGGHKVFM**V**

TEV-S219V

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RDGFIVGIHSASNFTNTNNYFTSVPKNFMELLTNQEAQQWVSGWRLNADSVLWGGHKVFM**V**KPEEPFQPVKEATQL
MNELVYSQ

uTEV3-(I138T/S153N/T180A/S219V)

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MNELVYSQ