Electronic supplementary information (ESI)

An intein-cassette integration approach

used for the generation of a split TEV protease activated by conditional protein splicing

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CGACGAGAAGGAGGACGGATCCAGATCTCATCACCATCACCATCACTAA

Figure S1. DNA-sequence of the CPS cassette

The CPS cassette (3207 bp from position 1 of Int^N to position +1 of Int^C) is encoded in the template vector pTS13.

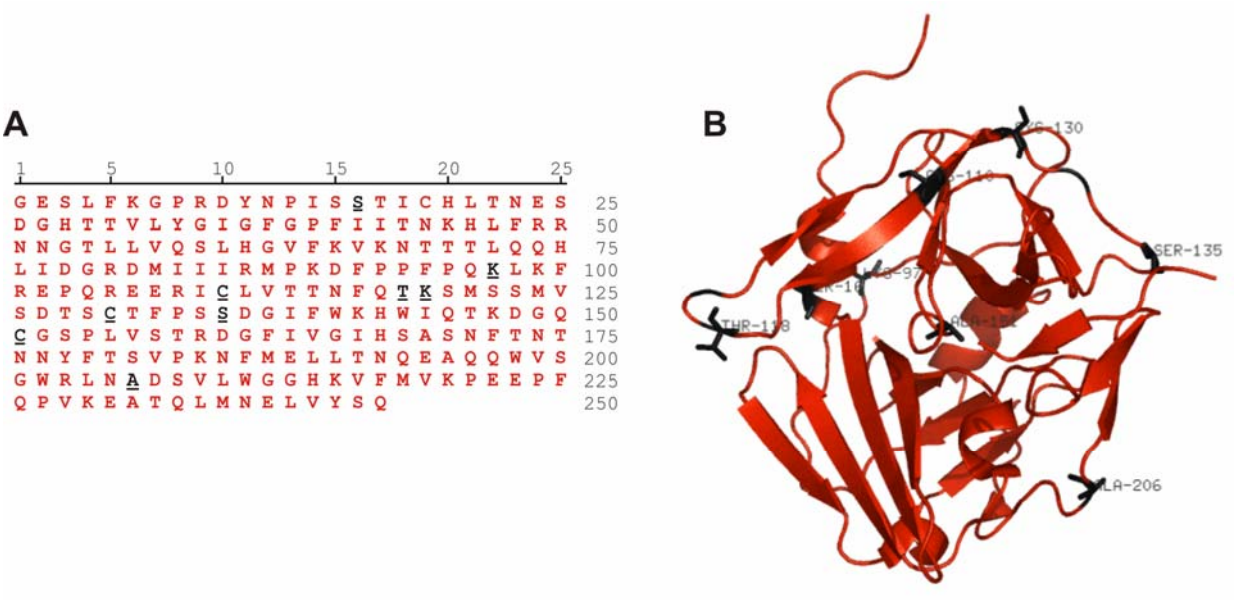

Figure S2. Positions of integration of the CPS cassette into the TEV protease

(A) The primary amino acid sequence of the TEV protease used in these experiments (TEV S219V)[1] is shown in red. Amino acids in black and underlined represent the positions at which the CPS intein cassette was integrated.

(B) Crystal structure of the TEV C151A mutant (PDB ID: 1Q31)[2] with the CPS cassette integration insertion positions highlighted. The figure was generated using PyMOL.

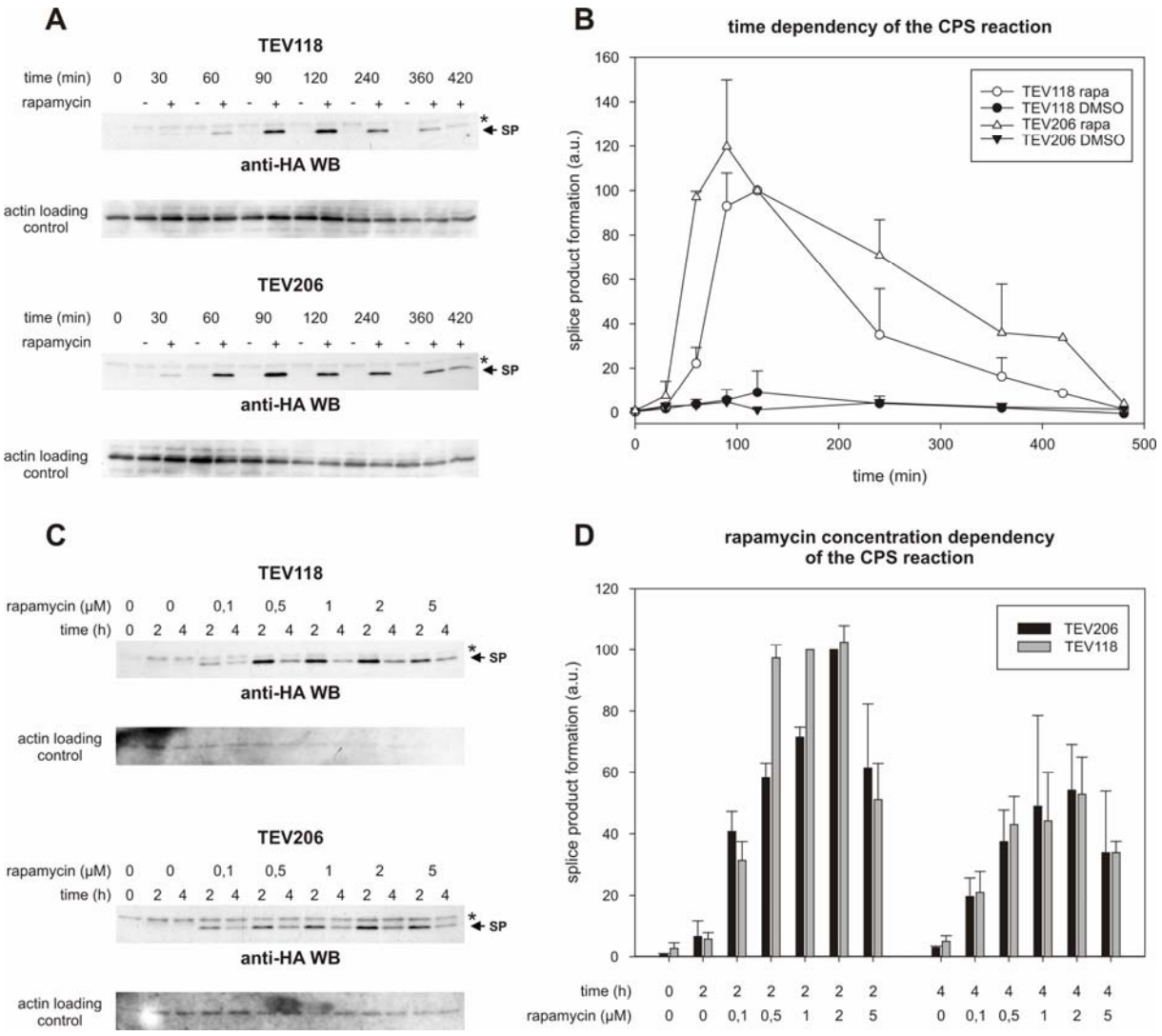

Figure S3. Influence of the incubation time and concentration of the small molecule on the protein *trans*-splicing reaction

(A) Time dependent formation of splice product (SP) in the presence or absence of 2 µM rapamycin.

(B) Normalized time dependent analysis of the individually evaluated splice product formation of split TEV 118 and 206 based upon the densitometric analysis of the splice product band.

(C) Analysis of the splice product (SP) formation at different rapamycin concentrations and for two different time points.

(D) Densitometric analysis of the dose-dependent CPS as shown in c).

Figure S4: Amino acid sequence of the TEV construct and reporter protein

Schematic representation and amino acid sequence of GST-TEV (pTS168) and MBP-TEVsite-EGFP (pTS169).

Experimental Procedures

Small molecules

Rapamycin was purchased from LC Laboratories and was stored in a 1 mM or 100 µM stock solution in DMSO at –20 °C. The rapalog AP23102 was kindly provided by ARIAD Pharmaceuticals and a 1 mM stock solution in ethanol was stored at –20 °C.

Western Blots

For Western Blots from whole cell extracts of yeasts the ECL Kit (GE Healthcare) with a 1:4000 dilution of the secondary antibody was used according to the manufacturer's recommendations. The dilutions of the primary antibodies were as follows: anti-His (Qiagen) 1:2000, anti-GST (Sigma Aldrich) 1:1000, anti-GFP (Covance) 1:4000, anti-HA (Covance) 1:1000, anti-MBP (Fermentas) 1:10000.

All stripped Western Blots were reincubated with the anti-Actin sc-1616 (Santa Cruz Biotechnology) antibody in a 1:500 dilution. The secondary antibody was used in a 1:40000 dilution and subsequently the Western Blot was performed with the ECL Advanced Kit (GE Healthcare) to the manufacturer's recommendations.

General yeast procedures

All *S. cerevisiae* strains were grown at 30°C either in YPD medium or in SD medium lacking the amino acids required for genetic selection of auxotrophic markers.[3] For the SD medium, methionine was used at a concentration of 150 µM to reduce the expression level of the *MET25* promoter.[4] Transformation of yeast was done following a LiAc protocol.[5, 6] Plasmids and genomic DNA were isolated by a glass bead assisted DNA/RNA phenol-chloroform extraction protocol.[3] For the preparation of whole cell protein extracts, always the same amount of cells were centrifuged (1 ml of an OD_{600} = 1.0) and the proteins were

prepared using trichloroacetic acid (TCA) precipitation and resuspension in HU sample buffer containing 8M urea.[7]

Generation of a rapamycin-resistant yeast strain

We generated the *S. cerevisiae* strain TS302 which is based on the W303 strain with two additional mutations in *tor2-1* and *FPR1* (see Table S1). The *tor2-1* mutation was introduced by transformation of W303 with the PCR-product obtained by using the primers oTS25 and oTS26 and genomic DNA of the SBFB4 strain,[8] kindly provided by Pete Belshaw, as a template, followed by selection on YPD-medium plates containing 0.1 µg/ml rapamycin. The *FPR1* gene was deleted by genomic insertion of the *HIS3* marker cassette amplified by PCR from the plasmid pFA6a-HIS3MX6[9] with the primers oTS140 and oTS141.

Genetic insertion of the CPS intein cassette into target genes

For the integration of the CPS cassette into the target genes by homologous recombination a yeast strain (ΔVMA) lacking the *TFP1* gene (Euroscarf accession number: Y03883) was used (see Table S1). The CPS cassette is encoded on plasmid pTS13 and its gene organization and full DNA sequence is given in Figure S1. To insert the cassette at the C-terminus of MBP, the ΔVMA strain was first transformed with the recipient plasmid encoding the target gene (pTS11; 2µ origin). The CPS cassette encoding an additional His_6-tag C-extein sequence (Figure S1) was amplified by PCR using oligonucleotides oTS13 and oTS14 and pTS13 as the template. The ΔVMA-pTS11 strain was then transformed with the purified PCR product and transformants were selected by the *HIS3* marker gene in the cassette.[10] Isolated plasmids were confirmed for the correct integration by DNA-sequencing. Of these, one (pTS15) was used for further experiments.

For the cassette integrations into different positions of the GST-TEV encoding gene, the ΔVMA strain was first transformed with the respective plasmid (pTS55, 2µ origin). The CPS cassette was then

amplified by PCR and inserted as described above using a specific pair of PCR primers for each position (Table S4). The candidate integration plasmids from the selected transformants were isolated by phenol/chloroform extraction. To avoid a potential mixture of plasmids, the isolated plasmids were retransformed into the same strain, the selection was repeated, and plasmids were again isolated from colonies grown in this second round. After confirmation of the correct cassette integration by DNA-sequencing, the final TEV-CPS integration plasmids (pTS89, pTS90, pTS95, pTS99, pTS110, pTS111, pTS114, pTS118, and pTS119) were obtained.

Identification and modification of the active TEV-CPS variants

The rapamycin resistant strain (TS302; see Table S1) was co-transformed with either one of the TEV-CPS integration plasmids or a control plasmid (negative = pTS35, positive = pTS55), respectively, and the reporter plasmid encoding MBP-TEVsite-EGFP (pTS69). Selection was performed according to genetic markers of the plasmids. After inoculation from a plate the strains carrying the different TEV-CPS integration plasmids were grown over night in medium containing either 1 μM rapamycin or 0.1 % (v/v) DMSO. At the following day whole cell extracts were prepared and analysed via Western Blot.

To generate the final TEV118-CPS (pTS165) and TEV206-CPS (pTS170) constructs, the coding region of the active insertion positions 118 (pTS90) and 206 (pTS110) was amplified with the primers oTS186 and oTS187 and subsequently cloned into p424-TDH. In this PCR- amplification step the HA-tag sequence was added to the 3′ end of the C-terminal TEV gene fragment. In the same step, the three terminal amino acids of TEV were deleted to block intramolecular cleavage of the HA-tag. The corresponding C1A splice mutants 118 (pTS184) and 206 (pTS187) were generated via site-directed mutagenesis using the primer pairs oTS182 & oTS183 (template pTS165) and oTS184 & oTS185 (template pTS170). The positive control (pTS165, see Figure S4) coding for GST-TEV-HA was amplified from the plasmid pTS55 using again the primers oTS186 and oTS187. The reporter plasmid coding for MBP-TEVsite-EGFP was derived from pTS69 cloned into the ARS/CEN origin plasmid. In the following growth experiments rapamycin resistant strain (TS302) was cotransfected with two

plasmids, one of which was in all cases reporter plasmid (pTS169, see Figure S4) encoding MBP-TEVsite-EGFP. As the negative control the p424-TDH plasmid was used.

In the growth experiments of the TEV118-CPS and TEV206-CPS constructs over-night cultures grown in SD medium were used to inoculate 10 mL of the same medium to an OD_{600} = 0.3. These cultures were grown until they reached exponential growth phase (OD_{600} ~ 0.5), which took about 3 h. Then each culture was split into 2 flasks and further incubated at the same temperature with either the depicted concentrations of rapamycin or just the same volume of DMSO. At the indicated time points aliquots were removed and whole cell extracts were prepared.

TEV-CPS mediated manipulation of EGFP localization and analysis by fluorescence microscopy

To observe the localization of EGFP in yeast cells the rapamycin resistant strain SBFB4 was cotransformed with a plasmid encoding STE2-TEVsite-EGFP (pTS229) and the plasmids encoding the split TEV118-CPS or TEV206-CPS (pTS165, pTS170). For comparison, the plasmids encoding the respective splice mutants (pTS184, pTS187), the positive (pTS168) and negative (p424-TDH) controls, were cotransformed.

Following inoculation in 10 ml fresh medium to an OD_{600} = 0.15 from an over night culture these cultures were split and grown for eight hours either in the presence of 2 µM rapamycin or 0.2 % DMSO (v/v). Afterwards the cells were used for the preparation of whole cell extracts and also for live cell fluorescence microscopy experiments. For the latter procedure, the cells were pelleted by centrifugation, washed once with PBS, and resuspended in 200 µl of PBS containing 2% glucose to an OD_{600} = 0.5. Half of this solution was added for cell immobilization to MatTek dishes that were coated with concanavalin A according to the following procedure: The glass surface of the MatTek dishes were briefly incubated with 75 µl of 1M NaOH by pipetting the solution three times up and down. The same was subsequently performed for 75 µl of pure ethanol and was followed by two washing steps with H_2O. Then the dishes were incubated for three hours with 100 µl of 1% concanavalin A (Carl Roth) in PBS solution. After removal of the solution the dishes were washed twice with PBS and the yeast samples were placed onto

them. The dishes were mounted on a Leica TCS SP5 confocal microscope with a HCX PL APO 63x oil objective. For excitation at 488 nm an argon laser was used and EGFP fluorescence was detected from 504 to 530 nm. At least 200 cells per experiment were manually analysed for the localization of EGFP for each strain carrying different plasmids and grouped into i) predominant membrane localization, ii) predominant cytosolic localization, and iii) predominant vacuolar localization.

Table S1. *S. cerevisiae* strains used in this study

name	reference	genotype
ΔVMA	Y03883 (EUROSCARF)	*MATα*; *his3Δ1*; *leu2Δ0*; *met15Δ0*; *ura3Δ0*; *tfp1*Δ::*kanMX4*
SBFB4	[8]	*MAT*a; *trp1-901*; *leu2-3,112*; *ura3-52*; *his3-200*; *gal4*Δ; *gal80*Δ; *GAL2-ADE2*; *LYS2*::*GAL1-HIS3*; *met2*:: *GAL7-lacZ; tor2-1*; Δ*fpr1*
TS302	this work; based on the W303 strain[11]	*MATα*, *ura3-1*, *ade2-1*, *trp1-1*, *his3-11*, *leu2-3, tor2-1, fpr1*Δ*::HIS3MX6*
W303	[11]	*MATα, ura3-1*, *ade2-1*, *trp1-1*, *his3-11*, *leu2-3*

Table S2. List of generated plasmids

Plasmid name	Coding region	Vector used for generation	Insert/template DNA	Primer used	Generated via restriction sites / integration / fusion PCR
pTS8	*FRB- C'VMA-His$_6$*	p416-MET25	pEB3[12]	-	restriction sites (*Hin*dIII and *Xho*I)
pTS11	*MBP*	pYES2 (Invitrogen)	pHM45[12]	-	restriction sites (*Hin*dIII and *Xho*I)
pTS13	*N'VMA-FKBP_HIS3_pMET25-FRB-C'VMA-His$_6$*	pBluescript KS- (GenBank Acc. No.: X52329)	pHM41,[12] pFA6a-HIS3MX6,[9] pTS08	oTS007 & oTS008, oTS005 & oTS006, oTS009 & oTS010; fusion PCR = oTS007& oTS010	fusion PCR [13], restriction sites (*Nhe*I and *Xho*I)
pTS15	*MBP-N'VMA-FKBP_HIS3_FRB-C'VMA-His$_6$*	pTS11 (pYES2)	pTS13 (CPS cassette)	oTS13 & oTS14	integration
pTS34	*MBP-TEV(site)-EGFP*	pHM45[12]	pEGFP-N1 (Clontech)	oTS75 & oTS76	restriction sites (*Eco*RI and *Hin*dIII)
pTS35	none (*GAL1* promoter)	p426-GPD	pYES2 (GAL1 promoter)	oTS71 & oTS72	restriction sites (*Sac*I and SpeI)
pTS40	*MBP-TEVsite-EGFP*	p425-TDH	pTS34	oTS77 & oTS76	restriction sites (*Pst*I and SpeI)
pTS55	*His$_6$-GST-TEV(S219V)*	pTS35 (p426-GPD)	pGAT_TEVS219V*	-	restriction sites (*Spe*I and *Xho*I ; *Xba*I instead of *Spe*I in pGAT_TEVS219V*)
pTS68	*MBP-TEVsite-EGFP*	pTS34	pEGFP-N1 (Clontech)	oTS97 & oTS98	restriction sites (*Eco*RI and *Hin*dIII)
pTS69	*MBP-TEVsite-EGFP*	pTS40 (p425-TDH)	pTS68	-	restriction sites (*Nde*I and *Hin*dIII)
pTS89	*His$_6$-GST-N'TEVCys151-N'VMA-FKBP_HIS3_FRB-C'VMA-C'TEVCys151*	pTS55 (p426-GPD)	pTS13 (CPS cassette)	oTS91 & oTS92	integration
pTS90	*His$_6$-GST-N'TEV118-N'VMA-FKBP_HIS3_FRB-C'VMA-C'TEV118*	pTS55 (p426-GPD)	pTS13 (CPS cassette)	oTS95 & oTS96	integration
pTS95	*His$_6$-GST-N'TEV130-N'VMA-FKBP_HIS3_FRB-C'VMA-C'TEV130*	pTS55 (p426-GPD)	pTS13 (CPS cassette)	oTS93 & oTS94	integration
pTS99	*His$_6$-GST-N'TEV97-N'VMA-FKBP_HIS3_FRB-C'VMA-C'TEV97*	pTS55 (p426-GPD)	pTS13 (CPS cassette)	oTS128 & oTS129	integration
pTS110	*His$_6$-GST-N'TEV206-N'VMA-FKBP_HIS3_FRB-C'VMA-C'TEV206*	pTS55 (p426-GPD)	pTS13 (CPS cassette)	oTS124 & oTS125	integration
pTS111	*His$_6$-GST-N'TEV16-N'VMA-FKBP_HIS3_FRB-C'VMA-C'TEV16*	pTS55 (p426-GPD)	pTS13 (CPS cassette)	oTS130 & oTS131	integration

Plasmid name	Coding region	Vector used for generation	Insert/template DNA	Primer used	Generated via restriction sites / integration / fusion PCR
pTS114	*His_6-GST-N'TEV135-N'VMA-FKBP_HIS3_FRB-C'VMA-C'TEV135*	pTS55 (p426-GPD)	pTS13 (CPS cassette)	oTS126 & oTS127	integration
pTS118	*His_6-GST-N'TEV110(Ile)-N'VMA-FKBP_HIS3_FRB-C'VMA-C'TEV110*	pTS55 (p426-GPD)	pTS13 (CPS cassette)	oTS121 & oTS122	integration
pTS119	*His_6-GST-N'TEV110(Ala)-N'VMA-FKBP_HIS3_FRB-C'VMA-C'TEV110*	pTS55 (p426-GPD)	pTS13 (CPS cassette)	oTS123 & oTS122	integration
pTS142	*FRB-C'VMA-C'TEV118-HA*	p426-GPD	pTS90	oTS160 & oTS161	restriction sites (*Bam*HI and *Xho*I)
pTS144	*FRB-C'VMA-C'TEV206-HA*	p426-GPD	pTS110	oTS160 & oTS161	restriction sites (*Bam*HI and *Xho*I)
pTS165	*His_6-GST-N'TEV118-N'VMA-FKBP_HIS3_FRB-C'VMA-C'TEV118-HA*	p424-TDH	pTS90	oTS186 & oTS187	restriction sites (*Spe*I and *Xho*I; *Nhe*I instead of *Spe*I in PCR of pTS90)
pTS168	*His_6-GST-TEV-HA*	p424-TDH	pGAT_TEVS219V*	oTS186 & oTS187	restriction sites (*Spe*I and *Xho*I; *Nhe*I instead of *Spe*I in PCR of pGAT_TEVS219V)
pTS169	*pTDH-MBP-TEVsite-EGFP*	p415-MET	pTS69; pTS69	-	restriction sites (p415-MET:*Sac*I and *Xho*I; pTS69: *SacI* and *NdeI;* pTS69: *NdeI* and *XhoI*)
pTS170	*His_6-GST-N'TEV206-N'VMA-FKBP_HIS3_FRB-C'VMA-C'TEV206-HA*	p424-TDH	pTS110	oTS186 & oTS187	restriction sites (*Spe*I and *Xho*I; *Nhe*I instead of *Spe*I in PCR of pTS110)
pTS184	*His_6-GST-N'TEV118-N'VMA(AFAK)-FKBP_HIS3_FRB-C'VMA-C'TEV118-HA*	p424-TDH	pTS165	oTS182 & oTS183	-
pTS187	*His_6-GST-N'TEV206-N'VMA(AFAK)-FKBP_HIS3_FRB-C'VMA-C'TEV206-HA*	p424-TDH	pTS170	oTS184 & oTS185	-
pTS201	*ssTM-PARPD-TEVsite-EGFP*	p415-MET	ssTM-PARPD-GAL4**,[14] pTS169	oTS197 & oTS204	restriction sites (p415-MET: *Spe*I and *Hin*dIII; PCR ssTM-PARPD-GAL4: *Spe*I and *Eco*RI*;* pTS169: *Eco*RI *and Hin*dIII)
pTS229	*STE2-TEVsite-EGFP*	pTS201 (p415-MET)	STE2 (chromosomal DNA SBFB4)	oTS221 & oTS222	restriction sites (*Spe*I and *Nde*I)

The yeast vectors (p42X and p41X based)[4, 15] were a kind gift of Roland Lill.
*= The TEV protease containing plasmid was a kind gift of Kirill Alexandrov.
**= The ssTM-PARPD-GAL4 plasmid was kindly provided by Christian Haass.

Table S3. List of primers

primer name	description / template	sequence (5' to 3')
oTS005	HIS3 marker for CPS cassette FP	GTGGAGCTTCTAAAACTGGAAACTAGTTATTAACGTACGCTGC AGGTCGAC
oTS006	HIS3 marker for CPS cassette RP	CGAACCCTTGCATCCGAGCTCCAGCTTTTGATCGATGAATTCGA GCTCG
oTS007	VMA-FKBP FP	ATATGCTAGCGAATTCCTTAAGGGGTGCTTTGC
oTS008	VMA-FKBP RP	TTAATAACTAGTTTCCAGTTTTAGAAG
oTS009	pMET25-FRB-C'VMA-His_6 FP	CAAAAGCTGGAGCTCGGATGC
oTS010	pMET25-FRB-C'VMA-His_6 RP	CATGACTCGAGTTAGTGATGGTGATG
oTS013	integration CPS cassette in pTS11 FP	ACAATAACAACAACCTCGGGATCGAGGGAAGGATTTCAGAATT CCTTAAGGGGTGCTTTG
oTS014	integration CPS cassette in pTS11 RP	GAGTTAGTGATGGTGATGGTGATGAGATCTGGATCCGTCCTCCT TCTCGTCGCAATTGTG
oTS071	pGAL1 FP	ATAGAGCTCACGGATTAGAAGCCGCCGAGC
oTS072	pGAL1 RP	ATAACTAGTGTTTTTTCTCCTTGACGTTAAAG
oTS075	TEVsite-EGFP FP	ATAGAATTCGAGAATCTTTATTTTCAGGGCCGGGATCCACCGGT CGCCACC
oTS076	EGFP-HIS_6 RP	ATAAAGCTTTTAGTGATGGTGATGGTGATGCTGCAGCGATCCCT TGTACAGCTCGTCCATGCC
oTS077	MBP FP	ATAACTAGTAGCATATGAAAATCGAAGAAG
oTS091	integration CPS cassette in TEV151 FP	TATTCTGGAAGCATTGGATTCAAACCAAGGATGGGCAGTGCTT TGCCAAGGGTACCAA
oTS092	integration CPS cassette in TEV151 RP	ATGAACCCATCTCTAGTTGATACTAATGGACTGCCACAATTGTG CACGACAACCTGG
oTS093	integration CPS cassette in TEV130 FP	AAACTAAGAGCATGTCTAGCATGGTGTCAGACACTAGTTGCTTT GCCAAGGGTACCAA
oTS094	integration CPS cassette in TEV130 RP	ATGCTTCCAGAATATGCCATCAGATGAAGGGAATGTGCAATTG TGCACGACAACCTGG
oTS095	integration CPS cassette in TEV118 FP	AGGGAAGAGCGCATATGTCTTGTGACAACCAACTTCCAAACTG TCGGGTGCTTTGCCAAGGGTACCAA
oTS096	integration CPS cassette in TEV118 RP	AATGTGCAACTAGTGTCTGACACCATGCTAGACATGCTCTTTTC TCCGCAATTGTGCACGACAACCTGG
oTS097	TEVsite-EGFP FP	ATAGAATTCGCTGATGAGAATCTTTATTTTCAGGGCGGGGCCAC CATGGTGAGCAAGGG
oTS098	EGFP RP	ATAAAGCTTTTACTTGTACAGCTCGTCCA
oTS121	integration CPS cassette in TEV110(Ile) FP	AAAGCTGAAATTTAGAGAGCCACAAAGGGAAGAGCGCATATG CTTTGCCAAGGGTACCAA
oTS122	integration CPS cassette in TEV110 RP	TAGACATGCTCTTAGTTTGGAAGTTGGTTGTCACAAGACAATTG TGCACGACAACCTGG
oTS123	integration CPS cassette in TEV110(Ala) FP	CAAAAGCTGAAATTTAGAGAGCCACAAAGGGAAGAGCGCGCA TGCTTTGCCAAGGGTACCAA
oTS124	integration CPS cassette in TEV206 FP	AAATCAGGAGGCGCAGCAGTGGGTTAGTGGTTGGCGATTAGGT TGCTTTGCCAAGGGTACCAA
oTS125	integration CPS cassette in TEV206 RP	TCACCATGAAAACTTTATGGCTCCCCCACAATACTGAGTCGCAA TTGTGCACGACAACCTGG
oTS126	integration CPS cassette in TEV135 FP	GTCTAGCATGGTGTCAGACACTAGTTGCACATTCCCTTCATGCT TTGCCAAGGGTACCAA
oTS127	integration CPS cassette in TEV135 RP	CATCCTTGGTTTGAATCCAATGCTTCCAGAATATGCCATCGCAA TTGTGCACGACAACCTGG
oTS128	integration CPS cassette in TEV97 FP	AATTATTCGCATGCCTAAGGATTTCCCACCATTTCCTCAATGCT TTGCCAAGGGTACCAA
oTS129	integration CPS cassette in TEV97 RP	GACATATGCGCTCTTCCCTTTGTGGCTCTCTAAATTTCAGGCAA TTGTGCACGACAACCTGG
oTS130	integration CPS cassette in TEV16 FP	AAGCTTGTTTAAGGGACCACGTGATTACAACCCGATATCGTGCT TTGCCAAGGGTACCAA
oTS131	integration CPS cassette in TEV16 RP	TTGTGTGCCCATCAGATTCATTCGTCAAATGACAAATGGTGCAA TTGTGCACGACAACCTGG

primer name	description / template	sequence (5' to 3')
oTS160	FRB FP	ATAGGATCCACCATGGCTTCTAGAATCCTCTGGC
oTS161	C'TEV-HA RP	ATACTCGAGTTATTAGGCGTAATCTGGGACGTCGTATGGGTATC CACCTTGCGAGTACACCAATTCATTC
oTS162	His_6-GST-N'TEV118-N'VMA-FKBP FP	ATAGAATTCACCATGAACACCATTCATCACCATC
oTS163	His_6-GST-N'TEV118-N'VMA-FKBP RP	ATACTCGAGTTATTAATAACTAGTTTCCAGTTTTAG
oTS182	SDM TEV118 FP	CAAACTGTCGGGGCCTTTGCCAAGGGTACC
oTS183	SDM TEV118 RP	GGTACCCTTGGCAAAGGCCCCGACAGTTTG
oTS184	SDM TEV206 FP	GTTGGCGATTAGGTGCCTTTGCCAAGGGTAC
oTS185	SDM TEV206 RP	GTACCCTTGGCAAAGGCACCTAATCGCCAAC
oTS186	His_6-GST FP	ATAGCTAGCTCCATGAACACCATTCATCACC
oTS187	C'TEV-HA RP	ATACTCGAGTTATTAGGCGTAATCTGGGACGTCGTATGGGTATC CACCCACCAATTCATTCATGAGTTGAG
oTS197	ssTM-PARPD FP	ATAACTAGTGCCATGGAGTTACCTGCACC
oTS204	ssTM-PARPD RP	ATAGAATTCATCGCCTTTTCTCTTTCCTTC
oTS221	STE2 FP	ATAACTAGTATGTCTGATGCGGCTCCTTCATTGAG
oTS222	STE2 RP	ATACATATGTAAATTATTATTATCTTCAGTCCAG

FP: forward primer, RP: reverse primer, SDM: site-directed mutagenesis, and iPCR: inverse PCR.

Table S4. Primers and plasmids for the integration of the CPS cassette into *GST-TEV*

TEV protease insertion position (into GST-TEV, pTS55)	Primers used for amplification of the CPS cassette (pTS13)	Resulting integration plasmid
16	oTS130 & oTS131	pTS111
97	oTS128 & oTS129	pTS99
110 (I)	oTS121 & oTS122	pTS118
110 (A)	oTS123 & oTS122	pTS119
118	oTS095 & oTS096	pTS90
130	oTS093 & oTS094	pTS95
135	oTS126 & oTS127	pTS114
151	oTS091 & oTS092	pTS89
206	oTS124 & oTS125	pTS110

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