

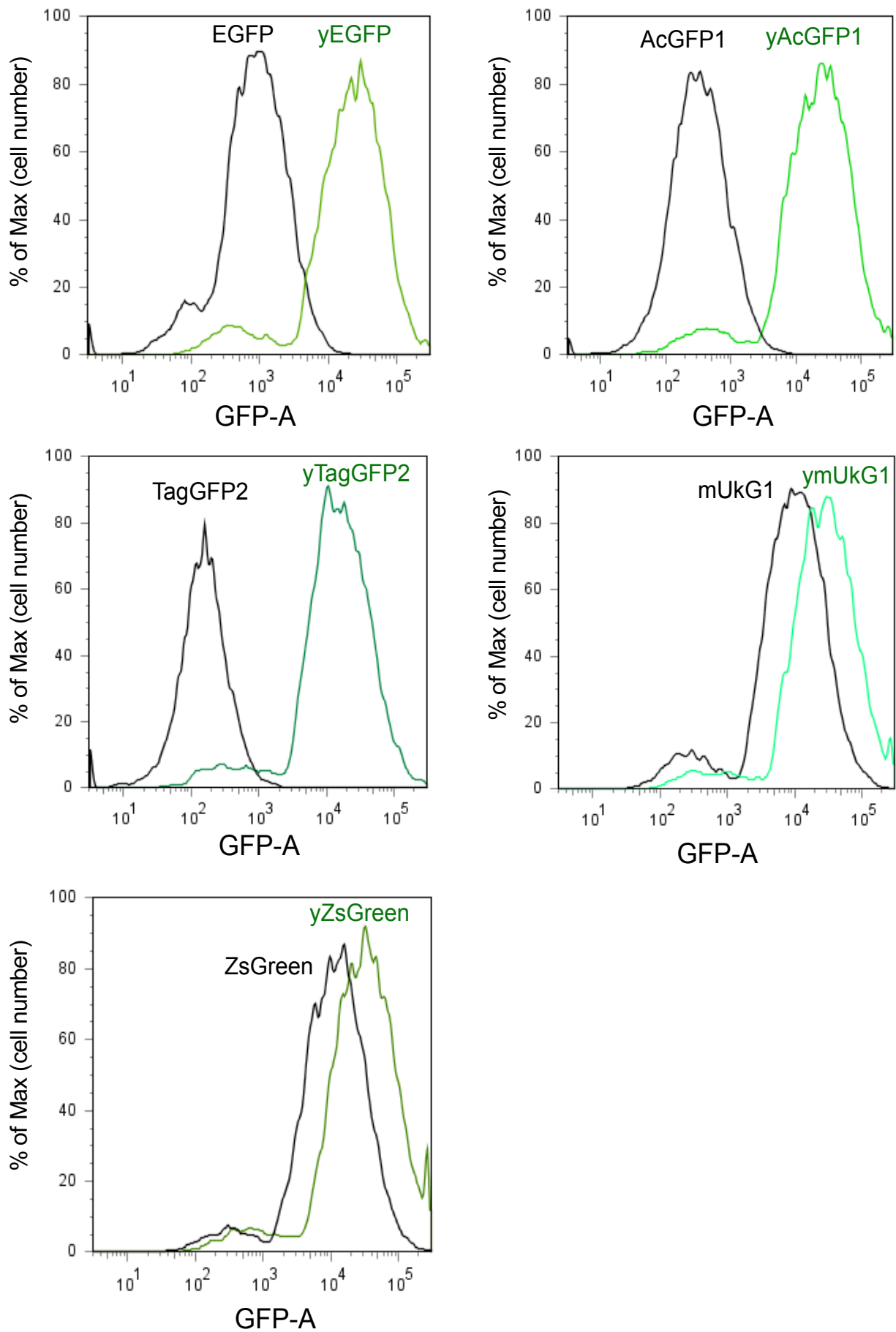
# **Expression of varied GFPs in *Saccharomyces cerevisiae*: codon optimization yields stronger than expected expression and fluorescence intensity**

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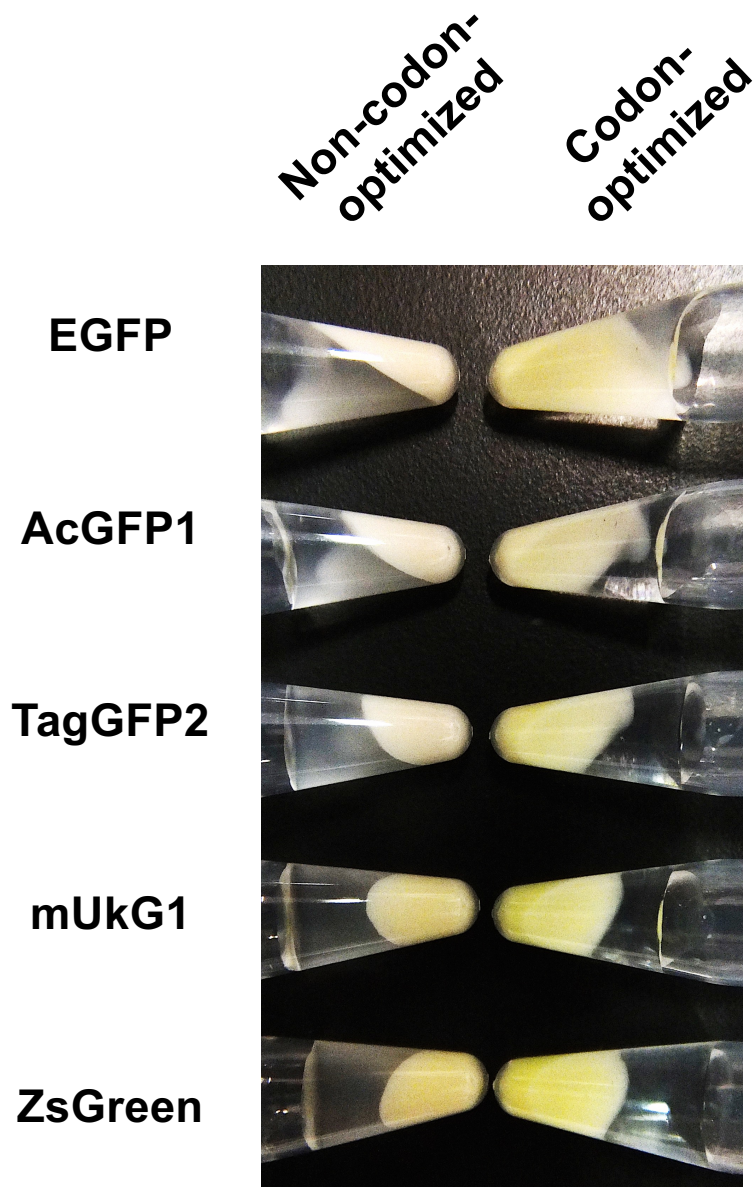
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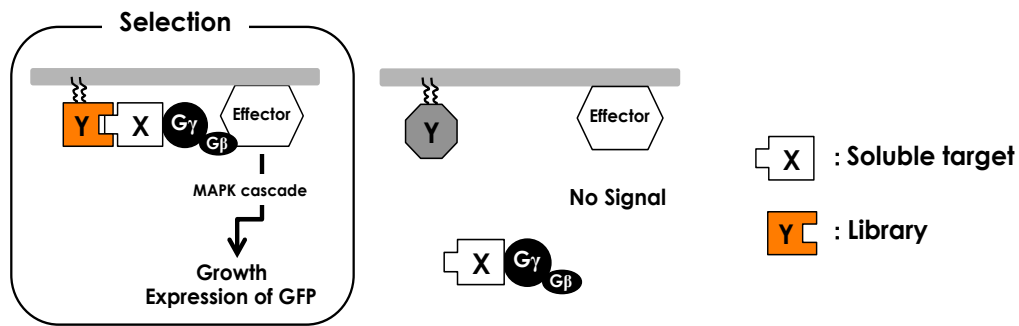


**Figure S1. Histogram plots illustrating codon-optimized and non-codon-optimized GFPs expression in *S. cerevisiae*.** The histogram plots show analytical data measured for 10,000 cells using flow cytometry.

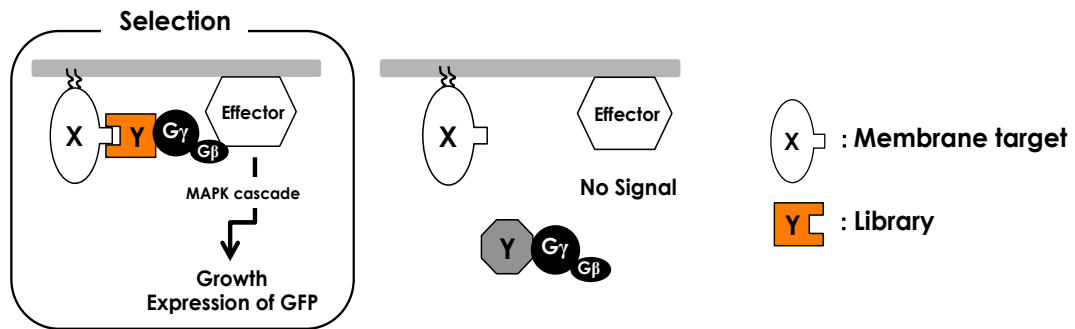


**Figure S2. Visual images of the cells under natural light. Varied codon-optimized and non-codon-optimized GFPs were used in the evaluations.**

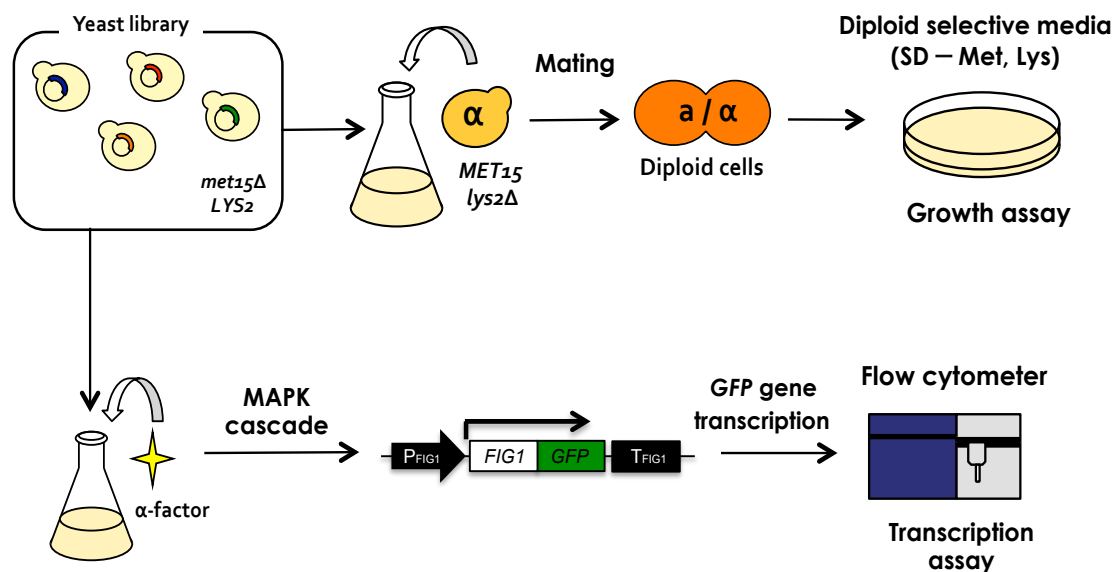
(A) G $\gamma$  recruitment system for soluble cytosolic target protein



(B) G $\gamma$  recruitment system for membrane target protein

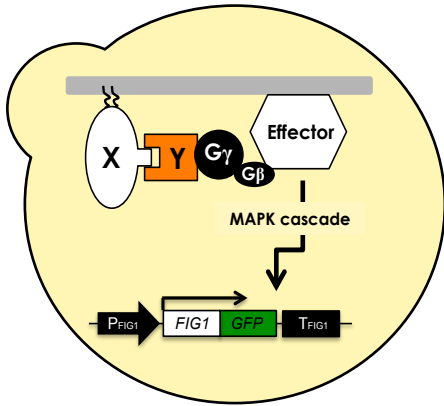


(C)



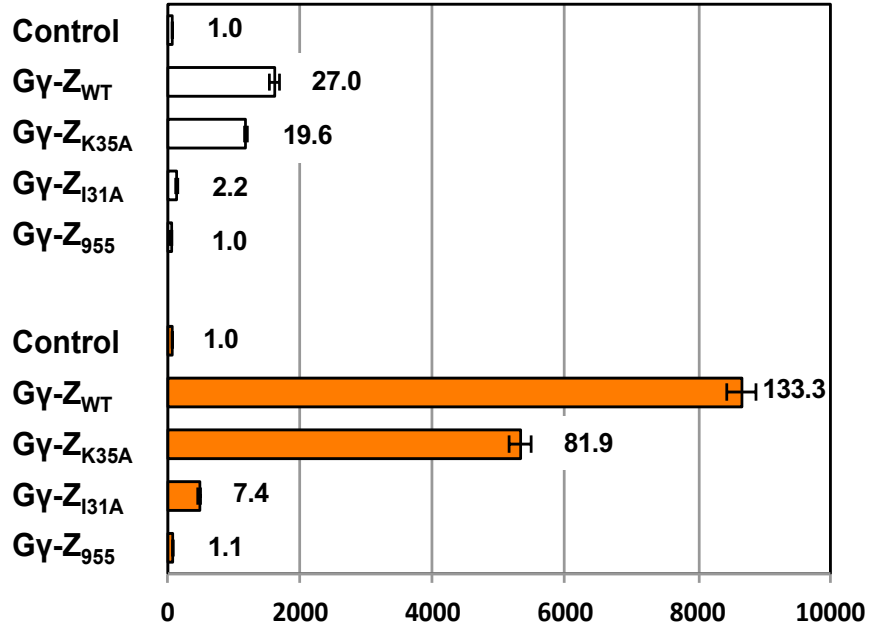
**Figure S3. Schematic diagram of G $\gamma$  recruitment systems used to detect PPIs of cytosolic or membrane target proteins.** (A) Schematic outline of the G $\gamma$  recruitment system for cytosolic target proteins. When target protein 'X', which is fused to G $\gamma$ <sub>cyto</sub>, interacts with candidate protein 'Y<sub>1</sub>', the G $\beta$  and G $\gamma$ <sub>cyto</sub> complex (G $\beta$  $\gamma$ <sub>cyto</sub>) migrates to the inner leaflet of the plasma membrane and restores the signaling function. If protein 'X' cannot interact with protein 'Y', G $\beta$  $\gamma$ <sub>cyto</sub> is released into the cytosol, and signaling is blocked. (B) Schematic outline of the G $\gamma$  recruitment system for membrane protein targets. When membrane target protein 'X' interacts with candidate protein 'Y', which is fused to G $\gamma$ <sub>cyto</sub>, the G $\beta$  $\gamma$ <sub>cyto</sub> complex migrates to the inner leaflet of the plasma membrane and restores the signaling function. If membrane protein 'X' cannot interact with protein 'Y', G $\beta$  $\gamma$ <sub>cyto</sub> is released into the cytosol, and signaling is blocked. (C) Flow diagram of the screening procedure used in the G $\gamma$  recruitment system. Two selection methods are available to screen for new binding proteins. One method uses *GFP* reporter genes. When target candidate proteins are expressed in yeast cells and interact with each other, the proteins induce *GFP* expression, which is detected by flow cytometry.

**G $\gamma$  recruitment system for membrane target protein**



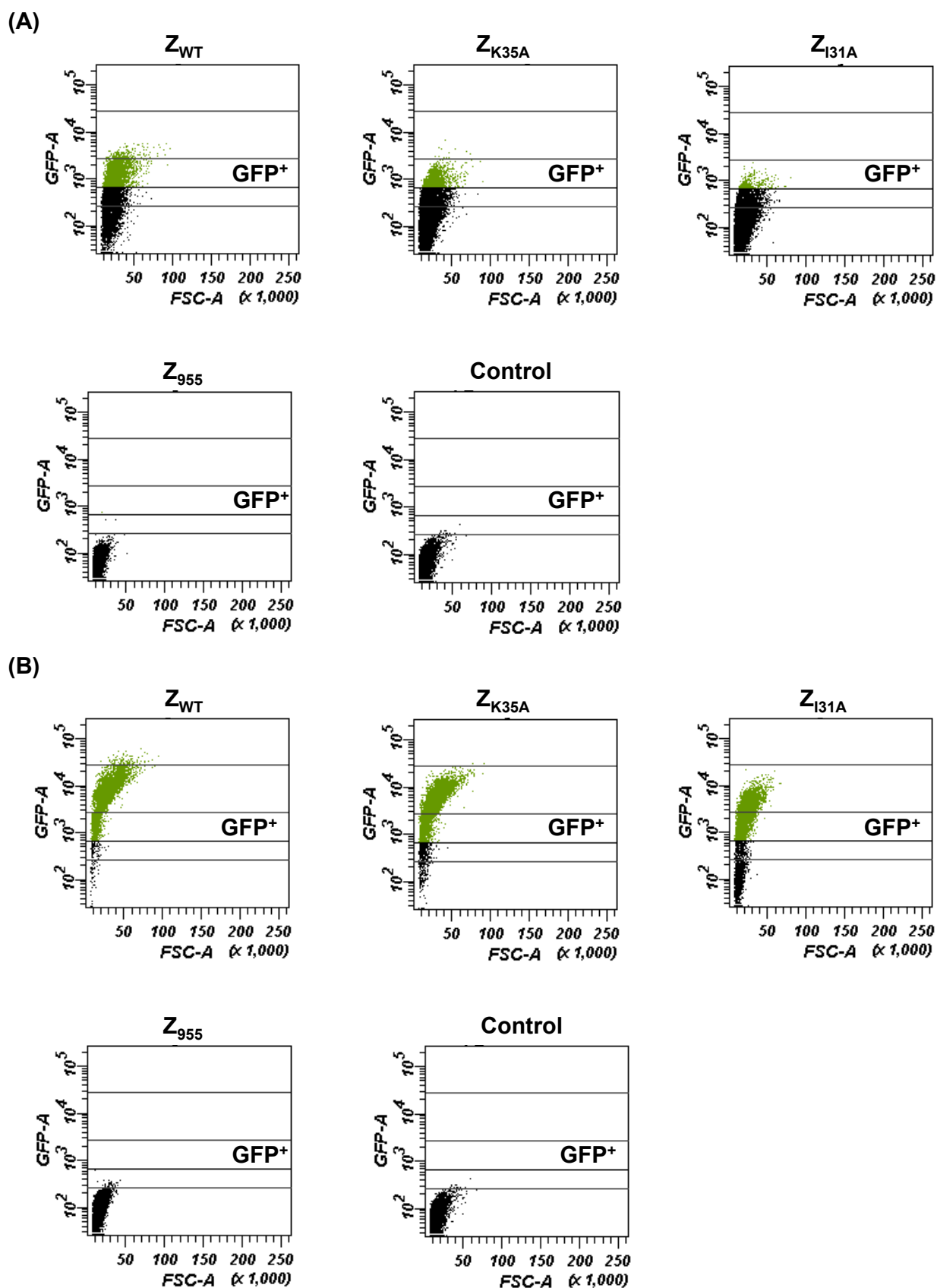
**EGFP**

**ymUkG1**



**Figure S4. Expression of non-codon-optimized EGFP and codon-optimized ymUkG1 as fusion-tagged proteins to detect PPIs using the G $\gamma$  recruitment system.**

Flow cytometry analyses using the G $\gamma$  recruitment system for membrane protein targets. The Fc protein was used as the membrane target protein 'X' and was expressed as a membrane-associated protein with an N-terminal lipid anchor (derived from Gpa1p). Four Z variants (Z<sub>WT</sub>, Z<sub>K35A</sub>, Z<sub>I31A</sub> and Z<sub>955</sub>) were used as the cytosolic candidate 'Y' proteins and were expressed as fusion proteins with G $\gamma$ <sub>cyto</sub> (G $\gamma$ <sub>cyto</sub>-Z variants). 'Control' indicates FN-G0 and UG2-FNG0 yeast strains without the expression of 'G $\gamma$ <sub>cyto</sub>-Y'. The engineered strains were grown in media containing 5  $\mu$ M  $\alpha$ -factor and were used in the analyses of mean fluorescence intensities (MFIs). The MFIs of 10,000 cells were measured by flow cytometry.



**Figure S5. Determination of gate area to dominantly including positive fluorescent cells (GFP<sup>+</sup>).** Data were presented as dot plots (forward scatter, FSC-A vs green fluorescence, GFP). Y-axis is an indication of fluorescence and X-axis is an approximation of relative cell size. Positive cells express membrane-anchored Z<sub>WT</sub>, Z<sub>K35A</sub> and Z<sub>I31A</sub> with G<sub>Ycyto</sub>-Fc. Negative cells express membrane-anchored Z<sub>955</sub> with G<sub>Ycyto</sub>-Fc. 'Control' indicates BFG2118 and UGFG2 yeast strains harboring the pGK413 mock plasmid (without the expression of 'Y'). (A) Flow cytometry analysis of the BFG2118 yeast transformants (EGFP reporter). (B) Flow cytometry analysis of the UGFG2 yeast transformants (ymUkG1 reporter).

**Supplementary Table S1. Nucleic acid sequences of codon-optimized GFPs for the *Saccharomyces cerevisiae***

**yEGFP**

ATGGTCAGTAAGGGTGAAGAATTATTCAGTGGTGTGTTCCAATCTTGGTTGAATTGGATGGTGTGTTAACGGTCAC  
AAGTTTTCTGTTTCTGGTGAAGGTGAAGGTGATGCTACTTATGGTAAATTGACCTTGAAGTTCATCTGTACCACAGGT  
AAATTGCCAGTTCCATGGCCAACCTTTGGTTACTACTTTGACTTATGGTGTCCAATGCTTCTCTAGATACCCAGATCATA  
TGAAGCAACACGACTTTTTCAAATCCGCTATGCCAGAAGGTTACGTTCAGAAAGAACCATCTTCTCAAGGATGAC  
GGTAACTACAAAACCTAGAGCCGAAGTAAAGTTCGAAGGTGATACCTTGGTTAACAGAATCGAATTGAAGGGTATCGAC  
TTCAAAGAAGATGGTAACATCTTGGGTCATAAGTTGGAATACAACCTACAACCTCCACAACGTTTACATTATGGCCGATA  
AGCAAAAAGAACGGTATCAAGGTTAACTTCAAGATCAGACACAACATCGAAGATGGTAGTGTTCATTGGCTGATCACT  
ACCAACAAAACACTCCAATTGGTGTGATGGTCCAGTTTTGTTGCCAGATAACCATTACTTGTCTACCCAATCTGCTTTGT  
CTAAGGACCCAAACGAAAAAAGAGATCACATGGTCTTGTGGAATTCGTTACTGCTGCTGGTATTACTTTGGGTATGG  
ACGAATTATACAAGTAA

**yAcGFP1**

ATGGTTTCTAAGGGTGTGAATTATTCAGTGGTATCGTTCGAATCTTGAATTGAACGGTGTGTTAACGGTCACA  
AGTTTTCTGTTTCTGGTGAAGGTGAAGGTGATGCTACTTATGGTAAATTGACCTTGAAGTTCATCTGTACCACAGGTA  
AATTGCCAGTTCCATGGCCAACCTTTGGTTACTACTTTGTCTTATGGTGTCCAATGCTTCTCTAGATACCCAGATCATAT  
GAAGCAACACGACTTTTTCAAATCCGCTATGCCAGAAGGTTACATCCAAGAAAGAACCATCTTTTTCGAAGATGACG  
GTAACTACAAGTCTAGAGCTGAAGTAAAGTTCGAAGGTGATACCTTGGTTAACAGAATTGAATTGACCGGTACTGACT  
TCAAAGAAGATGGTAACATTTTGGGTAACAAGATGGAATACAACCTACAACGCCATAACGTTTACATCATGACTGATAA  
GGCTAAGAACGGTATCAAGGTTAACTTCAAGATCAGACACAACATCGAAGATGGTTCAGTTCATTGGCTGATCACTA  
CCAACAAAACACTCCAATTGGTGTGATGGTCCAGTTTTGTTGCCAGATAACCATTACTTGTCTACCCAATCTGCTTTGTCT  
AAGGACCCAAACGAAAAAAGAGATCACATGATCTACTTCGGTTTCGTTACTGCTGCTGCTATTACTCATGGTATGGAC  
GAATTATACAAGTAA

**yTagGFP2**

ATGTCTGGTGGTGAAGAATTATTCAGTGGTATAGTTCCAGTCTTGAATTGGATGGTGTGTTTCATGGTCACAAG  
TTTTCTGTTAGAGGTGAAGGTGAAGGTGATGCTGATTATGGTAAATTGGAAATCAAGTTCATCTGCACCACAGGTAAA  
TTGCCAGTTCCATGGCCAACCTTTGGTTACTACTTTGTGTTACGGTATTCAATGCTTCGCTAGATACCCAGAACATATGA  
AGATGAACGACTTCTTCAAATCCGCTATGCCAGAAGGTTACATTCAAGAAAGAACCATTCAATTCCAAGACGACGGTA  
AATACAAAACAGAGGTGAAGTAAAGTTCGAAGGTGATACTTTGGTCAACAGAATCGAATTGAAGGGTAAAGGACTTC

AAAGAAGATGGTAACATCTTGGGTCATAAGTTGGAATACTCATTCAACTCCCACAACGTTTACATCAGACCAGATAAG  
GCTAACAATGGTTTGAAGCTAACTTCAAGACCAGACATAATATTGAAGGTGGTGGTGGTCAATTGGCCGATCATTAT  
CAAATAATGTTCCATTGGGTGACGGTCCAGTTTTGATTCCAATCAATCATTACTTGTCCACCCAACTAAGATCTCCA  
AGGATAGAAATGAAGCCAGAGATCACATGGTCTTGTGGAATCTTTTTCTGCTTGTGTGCATACCCACGGTATGGACG  
AATTATACAGATGA

### **ymUkG1**

ATGGTCAGTGTCAAAAGAAGAAATGAAGATCAAGTTGCACATGGAAGGTAACGTTAATGGTCATGCCTTTGTTATT  
GAAGGTGATGGTAAAGGTAAACCATACGATGGTACTCAAACCTTGAACCTGACTGTCAAAGAAGGTGCTCCATTGCC  
ATTCTCTACGATATTTGACTAACGCCTTCCAATACGGTAATAGAGCTTTACTAAGTACCCAGCCGATATCCCAGATT  
ACTTTAAGCAAACCTTTCCAGAAGGTTACTCCTGGGAAAGAAGTATGTCTTACGAAGATAACGCTATCTGCAACGTCA  
GATCCGAAATTTCTATGGAAGGTGATTGCTTCATCTACAAGATCAGATTCGATGGTAAGAAGCTTTCCACCAAATGGTC  
CAGTCATGCAAAAAAGACTTTGAAGTGGGAACCATCCACCGAAATGATGTATGTTAGAGATGGTTTCTTGATGGGT  
GATGTCAATATGGCTTTGTTGTTGGAAGGTGGTGGTCATCATAGATGTGATTTCAAGACTTCTTACAAGGCCAAGAAG  
GTTGTTCAATTGCCAGATGCTCATAAGATCGATCACAGAATCGAAATCTTGTCCCACGATAGAGATTACTCCAAGGTTA  
AGTTGTACGAAAACGCTGTTGCTAGAAACTCTTTGTTGCCATCTCAAGCTTCTAAGTAA

### **yZsGreen**

ATGGCTCAATCCAAACATGGTTTGACCAAAGAAATGACCATGAAGTACAGAATGGAAGGTTGTGTTGATGGTCACAA  
GTTTCGTTACTGGTGAAGGTATTGGTTACCCATTCAAAGGTAAGCAAGCTATCAACTTGTGTGTTGTTGAAGGTGG  
TCCATTGCCATTTGCTGAAGATATTTGTCTGCTGCTTTCATGTACGGTAACAGAGTTTTTACTGAATACCCACAAGAT  
ATCGCCGACTACTTTAAGAATTCATGTCCAGCTGGTTACACCTGGGATAGATCTTTTTTGTGTTGAAGATGGTGCTGTC  
TGATCTGCAACGCTGATATTACTGTTTCCGTTGAAGAAAAGTGCATGTACCACGAATCTAAGTTCTACGGTGTTAATT  
TTCCAGCTGATGGTCCAGTTATGAAGAAGATGACTGATAATTGGGAACCATCCTGCGAAAAGATTATTCCAGTTCCAA  
AGCAAGGTATCTTGAAGGGTATGTTTCTATGACTTGTATTGAAGGACGGTGGTAGATTGAGATGTCAATTCGATA  
CTGTTTACAAGGCCAAGTCTGTTCCAAGAAAATGCCTGATTGGCATTTCATCCAACATAAGTTGACCAGAGAAGATA  
GATCCGATGCCAAAATCAAAGTGGCATTGACCGAACATGCTATTGCTTCAGGTTCTGCTTTGCCATAA

### **ymWasabi**

ATGGTCAGTAAAGGTGAAGAACTACTATGGGTGTTATCAAGCCAGACATGAAGATCAAGTTGAAGATGGAAGGTAA  
CGTTAACGGTCATGCCTTTGTTATTGAAGGTGAAGGTGAAGGTAAACCATACGATGGTACTAATACCATTAACCTTGA  
AGTCAAAGAAGGTGCTCCATTGCCATTCTCTTACGATATTTGACTACCGCTTCTCATAACGGTAATAGAGCTTTTACT  
AAGTACCCAGATGACATCCCAAACCTACTTCAAGCAATCTTTCCAGAAGGTTACTCTTGGGAAAGAAGTATGACTTTC



GAAGATAAGGGTATCGTCAAGGTTAAGTCCGATATCTCTATGGAAGAAGATTCCTTCATCTACGAAATCCACTTGAAG  
GGTGAAAATTTCCCACCAAATGGTCCAGTCATGCAAAAAGAAACAACCTGGTTGGGATGCTTCTACCGAAAAGAATGTA  
TGTTAGAGATGGTGTCTTAAAAGGTGACGTCAAAATGAAGTTGTTGTTGGAAGGTGGTGGTCATCATAGAGTTGATT  
TCAAGACTATCTACAGAGCTAAGAAGGCTGTTAAGTTGCCAGATTACCATTTGTTGATCACAGAATCGAAATCTTGA  
ACCACGATAAGGATTACAACAAGGTTACCGTTTACGAAATTGCTGTTGCTAGAAACTCTACCGATGGTATGGATGAATT  
ATACAAGTAA

### **ymNeonGreen**

ATGGTCAGTAAGGGTGAAGAAGATAACATGGCTTCTTTGCCAGCTACTCATGAATTGCATATTTTCGGTTCCATCAAC  
GGTGTGATTTGATATGGTTGGTCAAGGTTACTGGTAATCCAAATGATGGTTACGAAGAATTGAACTTGAAGTCTACTA  
AGGGTGACTTGCAATTCTCTCCATGGATTTTGGTTCCACATATTGGTTACGGTTTCCACCAATATTTGCCATATCCAGA  
TGGTATGTCTCCATTTCAAGCTGCTATGGTTGATGGTTCTGGTTACCAAGTTCATAGAACCATGCAATTTGAAGATGGT  
GCTTCTTTGACCGTCAACTACAGATATACTTACGAAGGTTCCCATATCAAAGGTGAAGCTCAAGTTAAGGGTACTGGT  
TTTCCAGCTGATGGTCCAGTTATGACTAATTCTTTGACTGCTGCTGATTGGTGCAGATCTAAAAGACTTACCCAAAC  
GATAAGACCATCATCTCTACTTTCAAGTGGTCTTACTACTGGTAACGGTAAGAGATATAGATCTACTGCTAGAACTA  
CTTACACCTTCGCTAAACCTATGGCTGCTAACTACTTGAAGAATCAACCTATGTACGTTTTTCAGAAAGACCGAATTGAA  
GCACTCCAAAACCTGAATTGAATTTCAAAGAATGGCAAAAAGGCCTTCACCGATGTTATGGGTATGGATGAATTATACAA  
GTAA

**Supplementary Table S2. List of yeast transformants used for expression of various GFPs**

Transformant	Expression of protein	Figure
BY4741 (Control)	–	Fig. 1
BY4741 + pGK416-EGFP	EGFP (mammalian codon-optimized)	Figs. 1 and S2
BY4741 + pGK416-AcGFP1	AcGFP1 (mammalian codon-optimized)	Figs. 1 and S2
BY4741 + pGK416-TagGFP2	TagGFP2 (mammalian codon-optimized)	Figs. 1 and S2
BY4741 + pGK416-mUkG1	mUkG1	Figs. 1 and S2
BY4741 + pGK416-ZsGreen	ZsGreen	Figs. 1 and S2
BY4741 + pGK416-yEGFP	yEGFP (yeast codon-optimized)	Figs. 1 , S1 and S2
BY4741 + pGK416-yAcGFP1	yAcGFP1 (yeast codon-optimized)	Figs. 1 , S1 and S2
BY4741 + pGK416-yTagGFP2	yTagGFP2 (yeast codon-optimized)	Figs. 1 , S1 and S2
BY4741 + pGK416-ymUkG1	ymUkG1 (yeast codon-optimized)	Figs. 1 , S1 and S2
BY4741 + pGK416-yZsGreen	yZsGreen (yeast codon-optimized)	Figs. 1 , S1 and S2
BY4741 + pGK416-ymWasabi	ymWasabi (yeast codon-optimized)	Fig. S1
BY4741 + pGK416-ymNeonGreen	ymNeonGreen (yeast codon-optimized)	Fig. S1
BY4741 + pGK416-EGFP-F	EGFP (mammalian codon-optimized)-FLAG	Fig. 1
BY4741 + pGK416-AcGFP1-F	AcGFP1 (mammalian codon-optimized)-FLAG	Fig. 1
BY4741 + pGK416-TagGFP2-F	TagGFP2 (mammalian codon-optimized)-FLAG	Fig. 1
BY4741 + pGK416-mUkG1-F	mUkG1-FLAG	Fig. 1
BY4741 + pGK416-ZsGreen-F	ZsGreen-FLAG	Fig. 1
BY4741 + pGK416-yEGFP-F	yEGFP (yeast codon-optimized)-FLAG	Fig. 1
BY4741 + pGK416-yAcGFP1-F	yAcGFP1 (yeast codon-optimized)-FLAG	Fig. 1
BY4741 + pGK416-yTagGFP2-F	yTagGFP2 (yeast codon-optimized)-FLAG	Fig. 1
BY4741 + pGK416-ymUkG1-F	ymUkG1 (yeast codon-optimized)-FLAG	Fig. 1
BY4741 + pGK416-yZsGreen-F	yZsGreen (yeast codon-optimized)-FLAG	Fig. 1
BY4741 + pGK416-ymWasabi-F	ymWasabi (yeast codon-optimized)-FLAG	Fig. 1
BY4741 + pGK416-ymNeonGreen-F	ymNeonGreen (yeast codon-optimized)-FLAG	Fig. 1

**Supplementary Table S3. Mean fluorescence intensity (MFI) of yeast transformants used for expression of various GFPs**

GFP	MFI
Control	36
EGFP	1,490
AcGFP1	542
TagGFP2	240
mUkG1	14,194
ZsGreen	15,019
yEGFP	33,551
yAcGFP1	36,177
yTagGFP2	24,250
ymUkG1	47,088
yZsGreen	44,154
ymWasabi	16,987
ymNeonGreen	5,148

**Supplementary Table S4. List of yeast strains used for expression of various Fig1-GFPs as fusion tagged reporters**

Strain	Expression of protein	Figure
BY4741 (Control)	–	Fig. 2
MC-F1	Fig1-EGFP (mammalian codon-optimized)	Fig. 2
BYFAG1	Fig1-AcGFP1 (mammalian codon-optimized)	Fig. 2
BYFTG1	Fig1-TagGFP2 (mammalian codon-optimized)	Fig. 2
BYFUG1	Fig1-mUkG1	Fig. 2
BYFZG1	Fig1-ZsGreen	Fig. 2
BYFEG2	Fig1-yEGFP (yeast codon-optimized)	Fig. 2
BYFAG2	Fig1-yAcGFP1 (yeast codon-optimized)	Fig. 2
BYFTG2	Fig1-yTagGFP2 (yeast codon-optimized)	Fig. 2
BYFUG2	Fig1-ymUkG1 (yeast codon-optimized)	Fig. 2
BYFZG2	Fig1-yZsGreen (yeast codon-optimized)	Fig. 2

**Supplementary Table S5. List of yeast transformants used for the Gy recruitment system for soluble cytosolic target protein**

Transformant	Target	Candidate	Repoter	Figure
	X (cytosol)	Y (membrane)	GFP	
BFG2118 + pGk413 (Control)	Fc	–	EGFP	Figs. 3(A) and S5(A)
BFG2118 + pGK413-ZWTmem	Fc	Z <sub>WT</sub>	EGFP	Figs. 3(A), 4(A), 4(B) and S5(A)
BFG2118 + pGK413-ZK35Amem	Fc	Z <sub>K35A</sub>	EGFP	Figs. 3(A) and S5(A)
BFG2118 + pGK413-ZI31Amem	Fc	Z <sub>I31A</sub>	EGFP	Figs. 3(A), 4(E), 4(F) and S5(A)
BFG2118 + pGK413-Z955mem	Fc	Z <sub>955</sub>	EGFP	Figs. 3(A), 4(A), 4(B), 4(E), 4(F) and S5(A)
UGFG2 + pGk413 (Control)	Fc	–	ymUkG1	Figs. 3(A) and S5(B)
UGFG2 + pGK413-ZWTmem	Fc	Z <sub>WT</sub>	ymUkG1	Figs. 3(A), 4(C), 4(D) and S5(B)
UGFG2 + pGK413-ZK35Amem	Fc	Z <sub>K35A</sub>	ymUkG1	Figs. 3(A) and S5(B)
UGFG2 + pGK413-ZI31Amem	Fc	Z <sub>I31A</sub>	ymUkG1	Figs. 3(A), 4(G), 4(H) and S5(B)
UGFG2 + pGK413-Z955mem	Fc	Z <sub>955</sub>	ymUkG1	Figs. 3(A), 4(C), 4(D), 4(G), 4(H) and S5(B)

**Supplementary Table S6. List of yeast strains used for the Gy recruitment system for membrane target protein**

Strain	Target	Candidate	Reporter GFP	Figure
	X (membrane)	Y (cytosol)		
FC-G0 (Control)	C-terminally membrane-associated Fc	–	EGFP	Fig. 3(B)
FC-GW	C-terminally membrane-associated Fc	Z <sub>WT</sub>	EGFP	Fig. 3(B)
FC-GK	C-terminally membrane-associated Fc	Z <sub>K35A</sub>	EGFP	Fig. 3(B)
FC-GI	C-terminally membrane-associated Fc	Z <sub>I31A</sub>	EGFP	Fig. 3(B)
FC-G9	C-terminally membrane-associated Fc	Z <sub>955</sub>	EGFP	Fig. 3(B)
FN-G0 (Control)	N-terminally membrane-associated Fc	–	EGFP	Fig. S4
FN-GW	N-terminally membrane-associated Fc	Z <sub>WT</sub>	EGFP	Fig. S4
FN-GK	N-terminally membrane-associated Fc	Z <sub>K35A</sub>	EGFP	Fig. S4
FN-GI	N-terminally membrane-associated Fc	Z <sub>I31A</sub>	EGFP	Fig. S4
FN-G9	N-terminally membrane-associated Fc	Z <sub>955</sub>	EGFP	Fig. S4
UG2-FCG0	C-terminally membrane-associated Fc	–	ymUkG1	Fig. 3(B)
UG2-FCGW	C-terminally membrane-associated Fc	Z <sub>WT</sub>	ymUkG1	Fig. 3(B)
UG2-FCGK	C-terminally membrane-associated Fc	Z <sub>K35A</sub>	ymUkG1	Fig. 3(B)
UG2-FCGI	C-terminally membrane-associated Fc	Z <sub>I31A</sub>	ymUkG1	Fig. 3(B)
UG2-FCG9	C-terminally membrane-associated Fc	Z <sub>955</sub>	ymUkG1	Fig. 3(B)
UG2-FNG0	N-terminally membrane-associated Fc	–	ymUkG1	Fig. S4
UG2-FNGW	N-terminally membrane-associated Fc	Z <sub>WT</sub>	ymUkG1	Fig. S4
UG2-FNGK	N-terminally membrane-associated Fc	Z <sub>K35A</sub>	ymUkG1	Fig. S4
UG2-FNGI	N-terminally membrane-associated Fc	Z <sub>I31A</sub>	ymUkG1	Fig. S4
UG2-FNG9	N-terminally membrane-associated Fc	Z <sub>955</sub>	ymUkG1	Fig. S4

**Supplementary Table S7. Summary of the FACS analysis of the Gy recruitment system for cytosolic target proteins.** The percentage of cells belonging to GFP<sup>+</sup> were measured by flow cytometry.

Reporter GFP	Type of Z variant	Percentage of GFP <sup>+</sup>
EGFP	Z <sub>WT</sub>	58.8
EGFP	Z <sub>K35A</sub>	13.9
EGFP	Z <sub>I31A</sub>	2.5
EGFP	Z <sub>955</sub>	0
EGFP	Control	0
ymUkG1	Z <sub>WT</sub>	98.3
ymUkG1	Z <sub>K35A</sub>	95.4
ymUkG1	Z <sub>I31A</sub>	80.3
ymUkG1	Z <sub>955</sub>	0
ymUkG1	Control	0

**Supplementary Table S8. List of primers**

No.	Name	Sequence (5' to 3')
1	Sall-start-AcGFP1-fw	aaaagtcgacatggtgagcaagggc
2	BamHI-end-AcGFP1-rv	tttggatcctcactgtacagctcat
3	Sall-start-TagGFP2-fw	aaaagtcgacatgagcggggcgaggag
4	BamHI-end-TagGFP2-rv	tttggatcctacctgtacagctcgtc
5	Sall-start-mUkG1-fw	aaaagtcgacatggtgagtgattaaa
6	BamHI-end-mUkG1-rv	tttggatcctactcgaagcctgact
7	Sall-start-EGFP(Yeast)-fw	aaaagtcgacatggtcagtaagggtgaa
8	BamHI-end-EGFP(Yeast)-rv	tttggatcctactgtataattcgtc
9	Sall-start-AcGFP1(Yeast)-fw	aaaagtcgacatggttctaaggggtgt
10	BamHI-end-AcGFP1(Yeast)-rv	tttggatcctactgtataattcgtc
11	Sall-start-TagGFP2(Yeast)-fw	aaaagtcgacatgtctggtggaagaa
12	BamHI-end-TagGFP2(Yeast)-rv	tttggatcctcatctgtataattcgtc
13	Sall-start-mUkG1(Yeast)-fw	aaaagtcgacatggtcagtgatcaaaaga
14	BamHI-end-mUkG1(Yeast)-rv	tttggatcctactagaagcttgagatg
15	Sall-start-ZsGreen(Yeast)-fw	aaaagtcgacatggctcaatccaaacat
16	BamHI-end-ZsGreen (Yeast)-rv	tttggatcctatggcaaagcagaacc
17	Sall-start-mWasabi (Yeast)-fw	tagcgtcgacatggtgagcaagggcgagg
18	BamHI-end-mWasabi (Yeast)-rv	tagaccgggttactgtataattcatccatccg
19	Sall-start-mNeonGreen(Yeast)-fw	agcgtcgacatggtcagtaaggggaaga
20	BamHI-end-mNeonGreen (Yeast)-rv	cgggggatcctactgtataattcatc
21	Sall-atg-EGFP-fw	tagcgtcgacatggtgagcaagggcgagga
22	BamHI-end-frag-EGFP-rv	cgggggatcctactgtcatcgtcatcctgtagcctgtacagctcgtccatgccga
23	BamHI-end-frag-AcGFP1-rv	cgggggatcctcactgtcatcgtcatcctgtagcctgtacagctcatccatgccgt
24	BamHI-end-frag-TagGFP2-rv	cgggggatcctactgtcatcgtcatcctgtagcctgtacagctcgtccatgccgt
25	BamHI-end-frag-mUkG1-rv	cgggggatcctactgtcatcgtcatcctgtagcctcgaagcctgactggcagca
26	Sall-atg-ZsGreen-fw	tagcgtcgacatggctcagcaaaagcac
27	BamHI-end-frag-ZsGreen-rv	cgggggatcctcactgtcatcgtcatcctgtagtcgggcaatgcagatccgatgcaa
28	BamHI-end-frag-yEGFP-rv	cgggggatcctactgtcatcgtcatcctgtagcctgtataattcgtccataccca
29	BamHI-end-frag-yAcGFP1-rv	cgggggatcctactgtcatcgtcatcctgtagcctgtataattcgtccataccat



30	BamHI-end-frag-yTagGFP2-rv	cgggggatcctcactgtcatcgatcctttagtctctgtataattcgccataccgt
31	BamHI-end-frag-ymUkG1-rv	cgggggatccttactgtcatcgatcctttagtctcttagaagcttgagatggcaaca
32	BamHI-end-frag-yZsGreen-rv	cgggggatccttactgtcatcgatcctttagtctctggcaaacgagaacctgaagcaa
33	BamHI-end-frag-ymWasabi-rv	cgggggatccttactgtcatcgatcctttagtctctgtataattcatccataccat
34	BamHI-end-frag-ymNeonGreen-rv	cgggggatccttactgtcatcgatcctttagtctctgtataattcatccataccca
35	XhoI-FIG1down-fw	ggggctcgagttttatcctcaataaacat
36	KpnI-FIG1down-rv	ccccgtaccaacagacggtaatgattaga
37	TFIG1hr40-URA3-fw	ttttatcctcaataaacatataagttttgagcggatattttttgtcttttttgattccg
38	XhoI-URA3-rv	gaggataaaactcgaggggtaataactgatataatt
39	SacII-FIG1end50-AcGFP1-fw	aaaaccgcgataggtacaataactactcttcggattcatctacattgcattccaaagtgtgagca agggcgccgagctgtccaccggc
40	AcGFP1-TFIG1hr40-rv	aatatccgctcaaaactatagtgtttattgaggataaaactactgtacagctcatccatgccgtgg gt
41	SacII-FIG1end50-TagGFP2-fw	aaaaccgcgataggtacaataactactcttcggattcatctacattgcattccaaagttagcggg ggcgaggagctgttcgccggc
42	TagGFP2-TFIG1hr40-rv	aatatccgctcaaaactatagtgtttattgaggataaaactactgtacagctctccatgccg
43	SacII-FIG1end50-mUkG1-fw	aaaaccgcgataggtacaataactactcttcggattcatctacattgcattccaaagtgtgagtg tgattaaagagga
44	mUkG1-TFIG1hr40-rv	aatatccgctcaaaactatagtgtttattgaggataaaactactcgaagcctgactggcagc
45	SacII-FIG1end50-ZsGreen-fw	aaaaccgcgataggtacaataactactcttcggattcatctacattgcattccaaagtgtcagtg caaagcacggtct
46	ZsGreen-TFIG1hr40-rv	aatatccgctcaaaactatagtgtttattgaggataaaactcagggaatgcagatccggatgca
47	SacII-FIG1end50-EGFP(Y)-fw	aaaaccgcgataggtacaataactactcttcggattcatctacattgcattccaaagtgtcagta aggggaagaattattcactggt
48	EGFP(Y)-TFIG1hr40-rv	aatatccgctcaaaactatagtgtttattgaggataaaactactgtataattcgccataccc
49	SacII-FIG1end50-AcGFP1(Y)-fw	aaaaccgcgataggtacaataactactcttcggattcatctacattgcattccaaagtgttctaa gggtgctgaatt
50	AcGFP1(Y)-TFIG1hr40-rv	aatatccgctcaaaactatagtgtttattgaggataaaactactgtataattcgccatacca
51	SacII-FIG1end50-TagGFP2(Y)-fw	aaaaccgcgataggtacaataactactcttcggattcatctacattgcattccaaagttctgggg tgaagaattatt
52	TagGFP2(Y)-TFIG1hr40-rv	aatatccgctcaaaactatagtgtttattgaggataaaactactgtataattcgccataccg
53	SacII-FIG1end50-mUkG1(Y)-fw	aaaaccgcgataggtacaataactactcttcggattcatctacattgcattccaaagtgtcagtg

		catcaaagaaga
54	mUkG1(Y)-TFIG1hr40-rv	aatatccgctcaaaactatatgtttattgaggataaaactactagaagctgagatggcaac
55	SacII-FIG1end50-ZsGreen(Y)-fw	aaaaccgcgataggtacaataactactctcggattcatctacattgcattccaaagtgctcaat ccaacatggttt
56	ZsGreen(Y)-TFIG1hr40-rv	aatatccgctcaaaactatatgtttattgaggataaaattatggcaaagcagaacctgaagca
57	FIG1end50-fw	ataggtacaataactactctcggattcat
58	TFIG200-rv	aacagacggtaatgattagatttaggtaa
59	Ste18pro-fw	atattatataatataagggcgt
60	Ste18t-rv	aaattatagaaagcagtagataaaa
61	HIS3pro80-URA3-fw	tatataagtaatgtgatttctcgaagaataactaaaaaatgagcaggcaagataaacgaagg caaagtcaattcatcattttttttattctttt
62	HIS3t end40-rv	ggagccataatgacagcagttggtaggccttctttgt