Supplemental Information

Split Nano Luciferase Complementation for Probing Protein-Protein Interactions in Plant Cells

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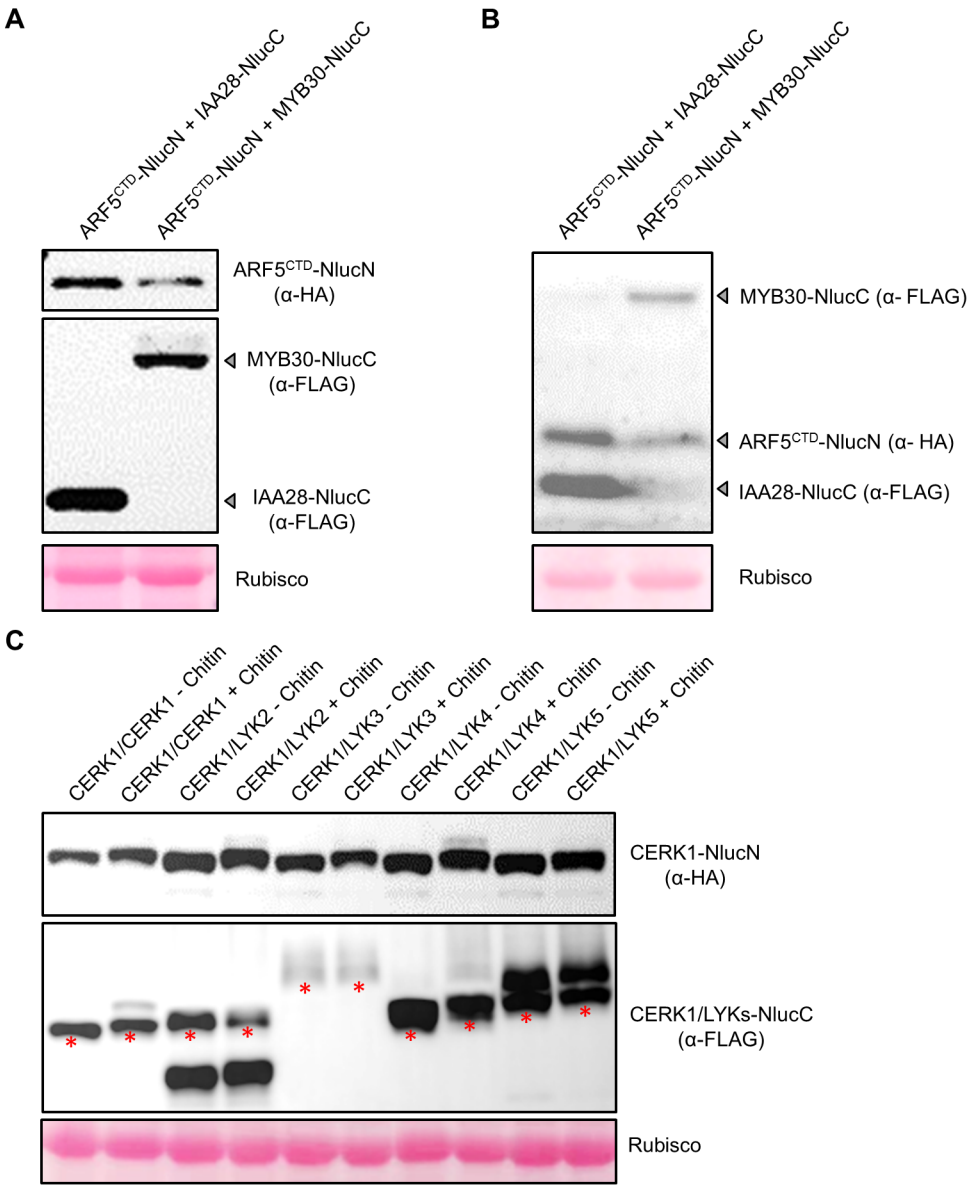

Figure S1. Western blot analysis for the expression of ARF5^{CTD}, IAA28, MYB30 and LysM-RLKs in the Nluc-based SLC assays

(A, B) ARF5^{CTD}-NlucN, IAA28-NlucC, and MYB30-NlucC are expressed in *Arabidopsis* protoplasts **(A)** and *N. benthamiana* leaves **(B)**. **(C)** CERK1/LYK2/3/4/5-NlucC (labeled by asterisks) and CERK1-NlucN are all expressed in *Arabidopsis* protoplasts. Note that LYK3-NlucC has a low abundance presumably due to protein instability.

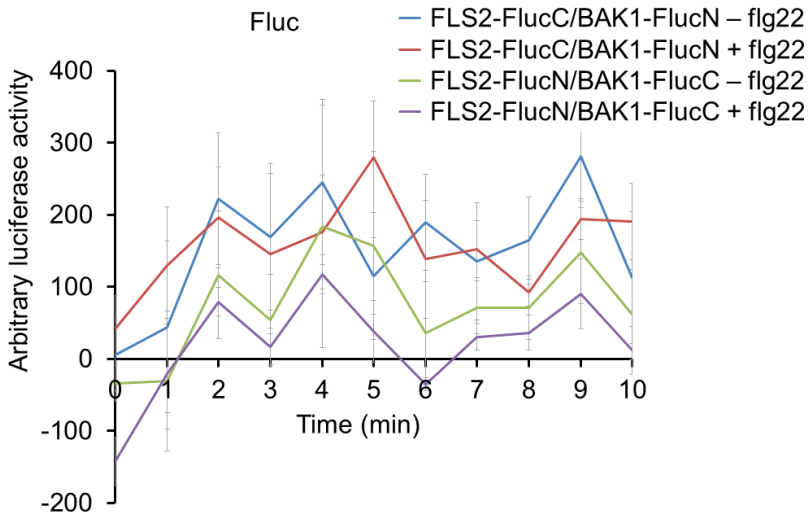

Figure S2. Flg22-induced FLS2-BAK1 interaction cannot be detected by the Fluc-based SLC assay

The *Arabidopsis* protoplasts co-expressing *FLS2-FlucC* and *BAK1-FlucN* or *FLS2-FlucN* and *BAK1-FlucC* for 12 h were divided into two equal aliquots, one treated with 100 nM flg22 and the other treated with water as mock.

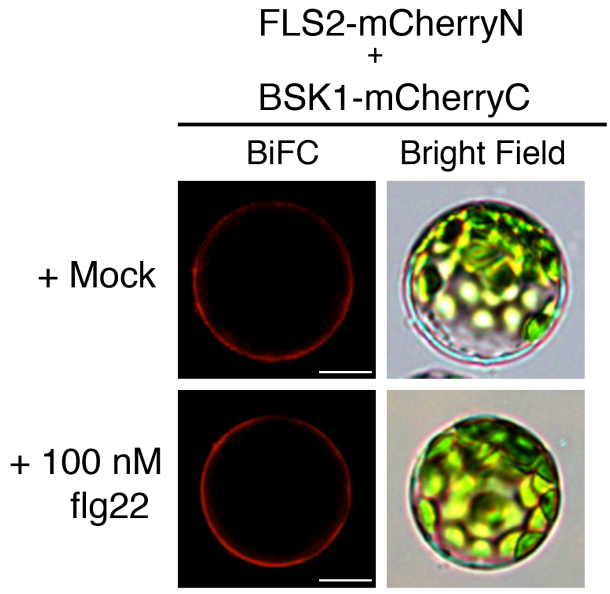

Figure S3. Flg22-induced FLS2-BSK1 dissociation cannot be reflected by the mCherry-based BiFC assay

The *Arabidopsis* protoplasts co-expressing *FLS2-mCherryN* and *BSK1-mCherryC* were divided into two equal aliquots, one treated with 100 nM flg22 and the other treated with mock (water). mCherryN and mCherryC encode aa1-159 and aa160-235 of mCherry, respectively. Scale bar = 10 μm.

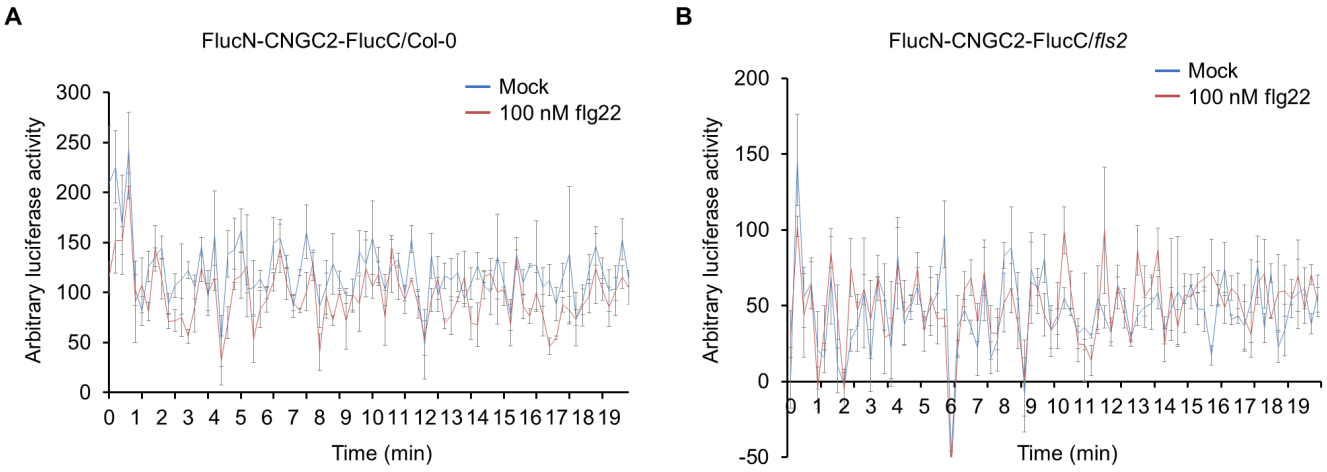

Figure S4. Flg22-induced allosteric change of CNGC2 can be barely detected by split Fluc complementation

The *Arabidopsis* Col-0 **(A)** or *fls2* **(B)** protoplasts expressing *FlucN-CNGC2-FlucC* for 12 h were divided into two equal aliquots, one treated with 100 nM flg22 (red) and the other treated with water as mock (blue). Note that, overall, the red line is slightly lying underneath the blue line in **(A)** but not in **(B)**.

Table S1. Primers used in this study

Primer name	Sequence (5'-3')	Restriction site	Usage
NlucN-F-*Bgl*II	CGAAGATCTATGGTTTTCACCCTTGAGGATTTCGTT	*Bgl*II	For construction of the vector *NlucN-gene-HA* or *NlucN-NlucC-FLAG*
NlucN-gene-R-*Stu*I	CGAAGGCCTGGATCCGCCACCAGAGCCACCGCCACCAGAGCCACCAGAGTTGATGGTAACTCT	*Bam*HI/*Stu*I	
NlucN-Gene-F-*Stu*I	CGAGGATCCAGGCCTGGCGGAAGCGGCGGAGGCGGAAGCGGCGGAATGGTTTTCACCCTTGAGGATTTCGTT	*Bam*HI/*Stu*I	For construction of the vector *Gene-NlucN-HA*
NlucN-R-*Sma*I	TCCCCCGGGAGAGTTGATGGTAACTCTGAA	*Sma*I	
NlucC-F-*Bgl*II	AGATCTATGGTTACCGGATACAGACTTTTCGAGG	*Bgl*II	For construction of the vector *NlucC-gene-FLAG*
NlucC-gene-R-*Stu*I	CGAAGGCCTGGATCCGCCACCAGAGCCACCGCCACCAGAGCCACCAAGGATCTCCTCGAAAAGTCTGTATCC	*Bam*HI/*Stu*I	
NlucC-gene-F-*Stu*I	CGAGGATCCAGGCCTGGCGGAAGC	*Bam*HI/*Stu*I	For construction of the vector *Gene-NlucC-FLAG*
NlucC-R-*Sma*I	TCCCCCGGGAAGGATCTCCTCGAAA	*Sma*I	
IAA28-*Bam*HI-F	CGAGGATCCATGGAAGAAGAAAAGAGATTGGAGC	*Bam*HI	PCR *IAA28* to construct *IAA28-NlucC-FLAG*
IAA28-*Stu*I-R	CGAAGGCCTTTCCTTGCCATGTTTTCTAGGTG	*Stu*I	
ARF5^{CTD}-*Bam*HI-F	CGAGGATCCATGGCGACACCCCGCGTCC	*Bam*HI	PCR *ARF5^{CTD}* to construct *ARF5^{CTD}-NlucN-HA*
ARF5^{CTD}-*Sma*I-R	CGACCCGGGTGAAACAGAAGTCTTAAGATCGTTAATGC	*Sma*I	
MYB30-*Bam*HI-F	CGAGGATCCATGGTGAGGCCTCCTTGTT	*Bam*HI	PCR *MYB30* to construct *MYB30-NlucC-FLAG*
MYB30-*Sma*I-R	CGACCCGGGGAAGAAATTAGTGTTTTCATCCAA	*Sma*I	
FKBP-*Bam*HI -F	CGAGGATCCATGGGAGTGCAGGTGGAAAC	*Bam*HI	PCR *FKBP* to construct *NlucC-FKBP-FLAG*
FKBP-*Stu*I-R	CGAAGGCCTTTCCAGTTTTAGAAGCTCCACATCG	*Stu*I	

FRB-*Bam*HI-F	CGAGGATCCGAACTTATCAGGGTTGCCATACTTTG	*Bam*HI	PCR *FRB* to construct *NlucN-FRB-HA*
FRB-*Stu*I-R	CGAAGGCCTCTGTTTGTCAATCCGTTTGAAAACG	*Stu*I	
FLS2-*Bam*HI-F	CGAGGATCCATGAAGTTACTCTCAAAG	*Bam*HI	PCR *FLS2* to construct *FLS2-NlucC-FLAG and FLS2-NlucN-HA*
FLS2-S*ma*I-R	CGACCCGGGAACTTCTCGATCCTCGTT	*Stu*I	
BAK1-*Bam*HI-F	CGAGGATCCATGGAACGAAGATTAATG	*Bam*HI	PCR *BAK1* to construct *BAK1-NlucN-HA*
BAK1-S*tu*I-R	CGAAGGCCTTCTTGGACCCGAGGGGTA	*Stu*I	
BSK1-*Bam*HI-F	CGAGGATCCATGGGTTGTTGTCAATCCTTGTT	*Bam*HI	PCR *BSK1* to construct *BSK1-NlucC-FLAG*
BSK1-*Sma*I-R	CGACCCGGGAGATCCTCTGCCGCCTCG	*Sma*I	
CERK1-*Bam*HI-F	CGAGGATCCATGAAGCTAAAGATTTCTCTAATCGCTC	*Bam*HI	PCR *CERK1* to construct *CERK1-NlucC-FLAG and CERK1-NlucN-HA*
CERK1-*Sam*I-R	CGACCCGGGCCGGCCGGACATAAGACT	*Sma*I	
CERK1-A138H-F	GCGAGGAATCCTTTTCCGCACACTAACATACCTCTCTCT		Introduce the A138H mutation into *CERK1* to construct $CERK1^{A138H}$-*NlucC-FLAG and* $CERK1^{A138H}$*-NlucN-HA*
CERK1-A138H-R	AGAGAGAGGTATGTTAGTGTGCGGAAAAGGATTCCTCGC		
LYK2-*Bam*HI-F	GGATCCATGGCTGTTTCAGTTAGTAAGCAGTAC	*Bam*HI	PCR *LYK2* to construct *LYK2-NlucC-FLAG*
LYK2-*Stu*I-R	CGAAGGCCTATCTATTATACTACTCTTCTTTACCAAAGGCT	*Stu*I	
LYK3-*Bam*HI-F	CGAGGATCCATGAATCTTACCTTCTACATCTTCTTCTT	*Bam*HI	PCR *LYK3* to construct *LYK3-NlucC-FLAG*
LYK3-*Stu*I-R	CGAAGGCCTTCTTCCTTGGACTAGACCACTAAAGAC	*Stu*I	
LYK4-*Bam*HI-F	CGAGGATCCATGATCTCGTTTTCATTTCATCTCCTCG	*Bam*HI	PCR *LYK4* to construct *LYK4-NlucC-FLAG*
LYK4- *Stu*I-R	CGAAGGCCTGTACGACGATTCTTCCCAGTTCTG	*Stu*I	
LYK5-*Bam*HI-F	GGATCCATGGCTGCGTGTACACTCCA	*Bam*HI	PCR *LYK5* to construct *LYK5-NlucC-FLAG*

LYK5-*Stu*I-R	CGAAGGCCTGTTGCCAAG AGAGCCGGAAC	*Stu*I	
CNGC2-*Bam*HI-F	GGTGGCTCTGGTGGCGGAT CCATGCAATTCCTTATAATC TCGC	*Bam*HI	PCR *CNGC2* to construct *NlucN-CNGC2-NlucC*
CNGC2-*Stu*I-R	CTCCGCCGCTTCCGCCAGG CCTTTCGAGATGATCATGC GGTCG	*Stu*I	
CNGC4-*Bam*HI-F	GGATCCATGGCCACAGAAC AAGAATTC	*Bam*HI	PCR *CNGC4* to construct *NlucN-CNGC4-NlucC*
CNGC4-*Stu*I-R	CCGACGATTTTGATGATTAT AGGCCT	*Stu*I	
pFGC-35S-F	CTCAGAGAGGATATCGGC GCGCCTACTCCAAGAATAT CAAAGATACAG	*Asc*I	To amplify and transfer the expression cassettes from the HBT vector to the binary pFGC-RCS vector
NOS-pFGC-R	TTCCTCGAGGGATCCTCTA GAGATCTAGTAACATAGAT GACACCGCG	*Xba*I	
HBT-35SPPDK-F	GTCACGTAGTAAGCAGCTC TCGG		For sequencing the HBT constructs
HBT-NOS-R	ATCGCAAGACCGGCAACA GGA		
pFGC-RCS-F	AATAAAAACTGACTCGGA		For sequencing the pFGC constructs
pFGC-RCS-R	AGTTGGGTAACGCCAGGG		

SUPPLEMENTAL SEQUENCES

>*NlucN-Gene-HA* expression cassette
[*35SPPDK* promoter is in blue; *NlucN* is in green; the coding sequence for $(GGSGG)_2$ linker is in cyan; the coding sequence for the double-HA tag is in magenta; *NOS* terminator is in red; *Bam*HI (GGATCC) and *Stu*I (AGGCCT) restriction sites for gene cloning are underlined; the start codon and stop codon are in bold]

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>*NlucC-Gene-FLAG* expression cassette
[*35SPPDK* promoter is in blue; *NlucC* is in brown; the coding sequence for $(GGSGG)_2$ linker is in cyan; the coding sequence for the double-FLAG tag is in magenta; *NOS* terminator is in red; *Bam*HI (GGATCC) and *Stu*I (AGGCCT) restriction sites for gene cloning are underlined; the start codon and stop codon are in bold]

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ATCTATGTTACTAGATC

>*Gene-NlucN-HA* expression cassette
[*35SPPDK* promoter is in blue; *NlucN* is in green; the coding sequence for $(GGSGG)_2$ linker is in cyan; the coding sequence for the double-HA tag is in magenta; *NOS* terminator is in red; *Bam*HI (GGATCC) and *Stu*I (AGGCCT) restriction sites for gene cloning are underlined; the stop codon is in bold]
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ACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGATC

>*Gene-NlucC-FLAG* expression cassette
[*35SPPDK* promoter is in blue; *NlucC* is in brown; the coding sequence for $(GGSGG)_2$ linker is in cyan; the coding sequence for the double-FLAG tag is in magenta; *NOS* terminator is in red; *Bam*HI (GGATCC) and *Stu*I (AGGCCT) restriction sites for gene cloning are underlined; the stop codon is in bold]
TACTCCAAGAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCTATTGAGACTTTTCA
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>*NlucN-Gene-NlucC-FLAG*
(*35SPPDK* promoter is in blue; *NlucN* is in green; *NlucC* is in brown, the coding sequences for $(GGSGG)_2$ linkers are in cyan; the coding sequence for the double-FLAG tags is in magenta; *NOS* terminator is in red; *Bam*HI (GGATCC) and *Stu*I (AGGCCT) restriction sites for gene cloning are underlined; the start codon and stop codon are in bold)

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