A pathogenesis related protein GmPR10-08 promotes a molecular interaction between the GmSHMT08 and GmSNAP18 in resistance to *Heterodera glycines*

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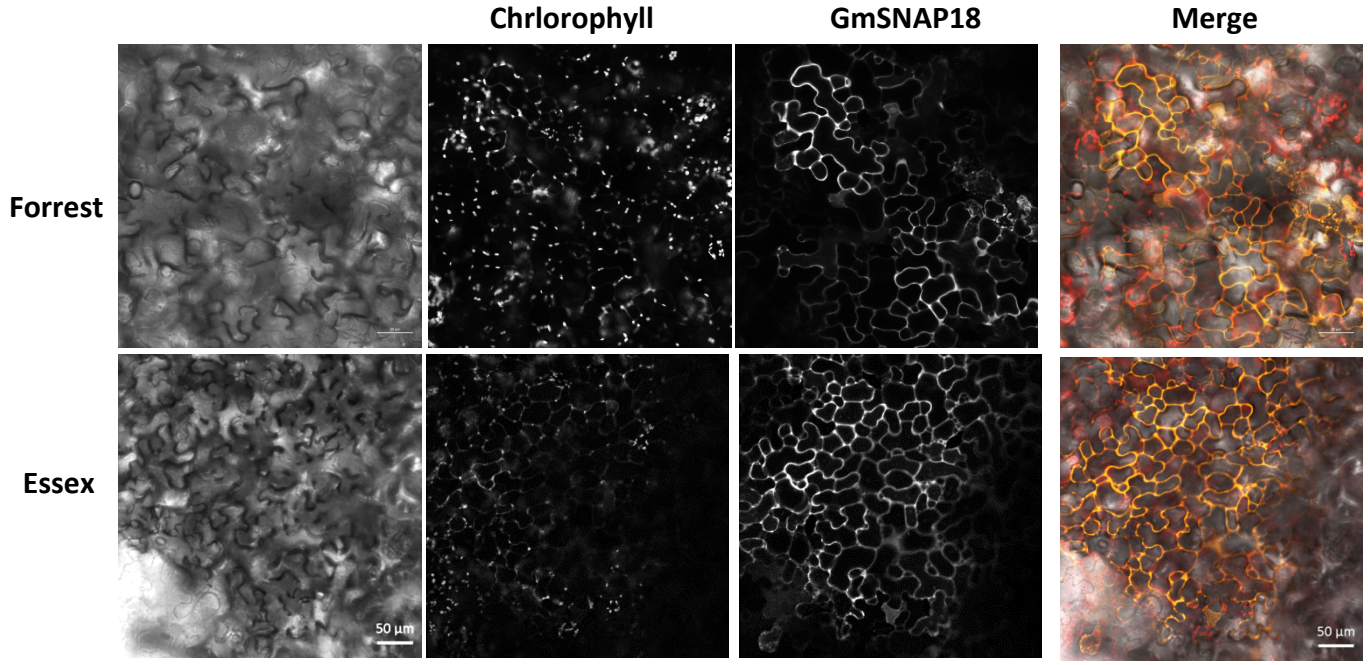
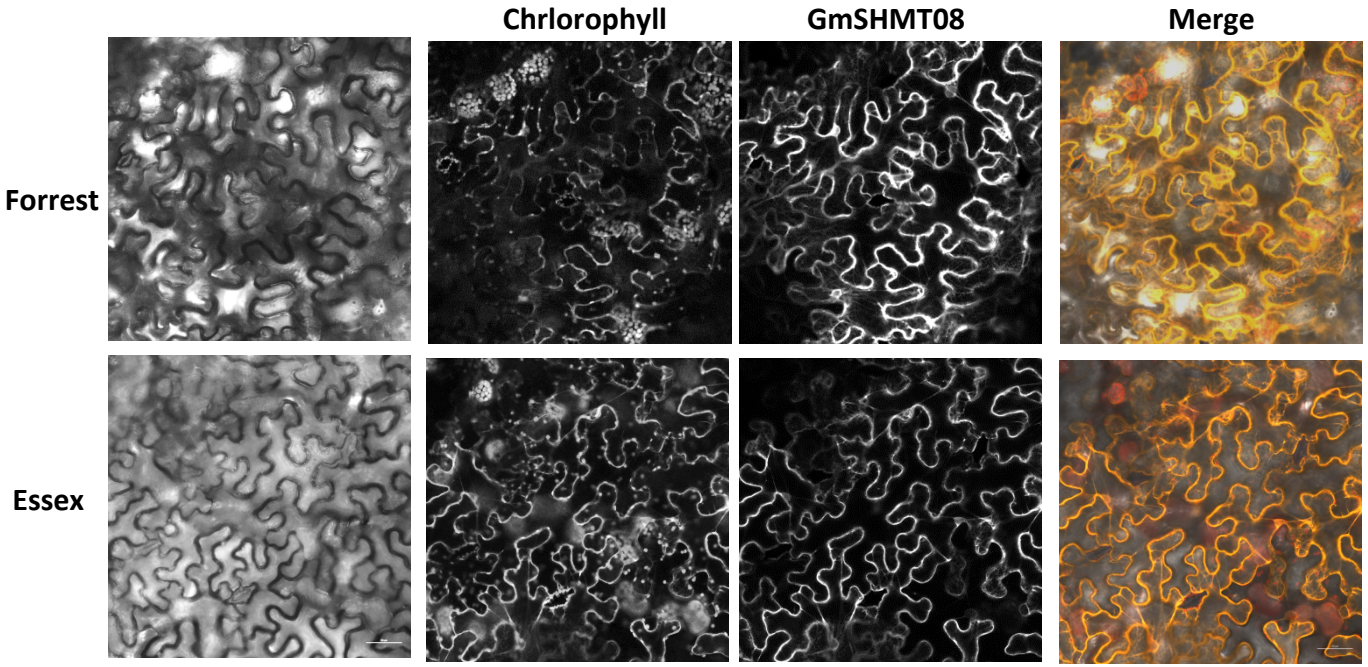
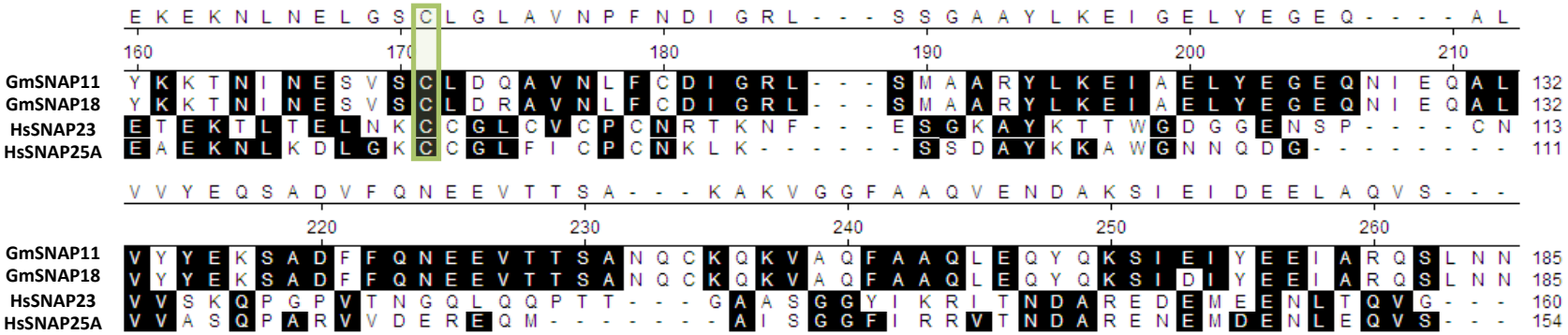
Supplemental Table 1. Genotypes of the soybean lines used for expression analysis and SCN screening. Soybean PIs, elites, cultivars, and ExF RILs carrying different *GmSHMT08* and *GmSNAP18* haplotype combinations are shown below.

PIs, elites, cultivars	Country of Origin	MG	Rhg4 (GmSHMT08)	Rhg1 (GmSNAP18)
ExF 05	U.S.	V	GmSHMT08-	GmSNAP18-
ExF 07	U.S.	V	GmSHMT08+	GmSNAP18+
ExF 24	U.S.	V	GmSHMT08-	GmSNAP18+
ExF 68	U.S.	V	GmSHMT08+	GmSNAP18-
Forrest (Peking-type)	U.S.	V	GmSHMT08+	GmSNAP18+
PI548667 (Essex)	U.S.	V	GmSHMT08-	GmSNAP18-
PI464920B	China	III	GmSHMT08-	GmSNAP18-
PI087617	North Korea	III	GmSHMT08-	GmSNAP18-
PI407184 (Soja)	South Korea	IV	GmSHMT08-	GmSNAP18-
PI438471 (Fiskeby III)	Sweden	0	GmSHMT08-	GmSNAP18-
PI567690	China	III	GmSHMT08-	GmSNAP18-
PI552538 (Dunbar)	U.S.	III	GmSHMT08-	GmSNAP18-
PI518751	Former Serbia	II	GmSHMT08-	GmSNAP18-
PI200508	Japan	I	GmSHMT08-	GmSNAP18-
V71-370	U.S.	VI	GmSHMT08-	GmSNAP18-
PI593258 (Macon)	N/A	III	GmSHMT08-	GmSNAP18-
PI471938	Nepal	V	GmSHMT08-	GmSNAP18-
IA3023	U.S.	III	GmSHMT08-	GmSNAP18-
S07-5049	U.S.	IV	GmSHMT08-	GmSNAP18-
PI404198B	China	IV	GmSHMT08+	GmSNAP18+
PI468915	China	II	GmSHMT08+	GmSNAP18+
PI090763	China	IV	GmSHMT08+	GmSNAP18+
PI658519 (LD00-2817)	N/A	VII	GmSHMT08+	GmSNAP18+
PI548402	China	IV	GmSHMT08+	GmSNAP18+
PI437690	China	III	GmSHMT08+	GmSNAP18+
PI437725	China	IV	GmSHMT08+	GmSNAP18+
PI404166	China	III	GmSHMT08+	GmSNAP18+
PI089772	China	IV	GmSHMT08+	GmSNAP18+
PI437679	China	IV	GmSHMT08+	GmSNAP18+
PI548402 (Peking)	China	IV	GmSHMT08+	GmSNAP18+
PI437654	China	III	GmSHMT08+	GmSNAP18+
PI507354	Japan	I	GmSHMT08+	GmSNAP18+
PI567516C	China	IV	GmSHMT08-	GmSNAP18+
PI567336B	China	IV	GmSHMT08-	GmSNAP18+
PI567387	China	IV	GmSHMT08-	GmSNAP18+
PI424608A	South Korea	IV	GmSHMT08-	GmSNAP18+
PI424298	South Korea	IV	GmSHMT08-	GmSNAP18+
S10-11227	U.S.	N/A	GmSHMT08-	GmSNAP18+
PI407788A	South Korea	IV	GmSHMT08-	GmSNAP18+
PI567230	China	V	GmSHMT08-	GmSNAP18+
PI567305	China	IV	GmSHMT08-	GmSNAP18+
PI603497	China	III	GmSHMT08-	GmSNAP18+

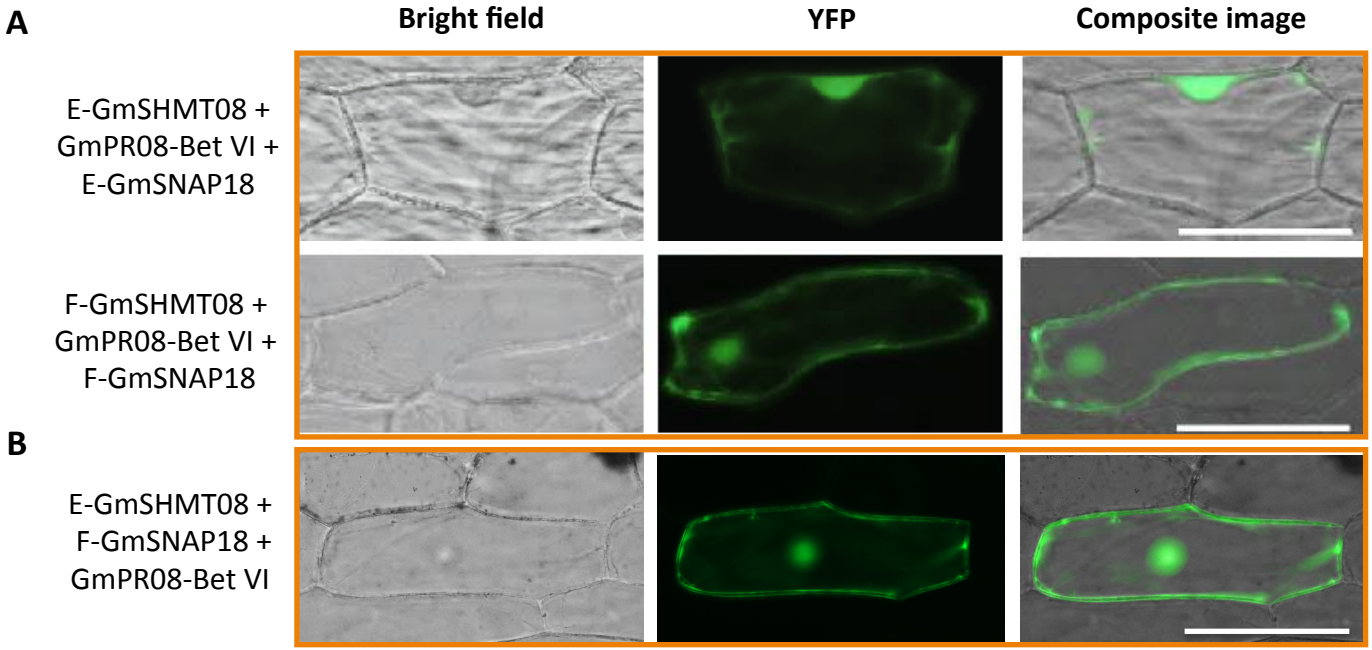




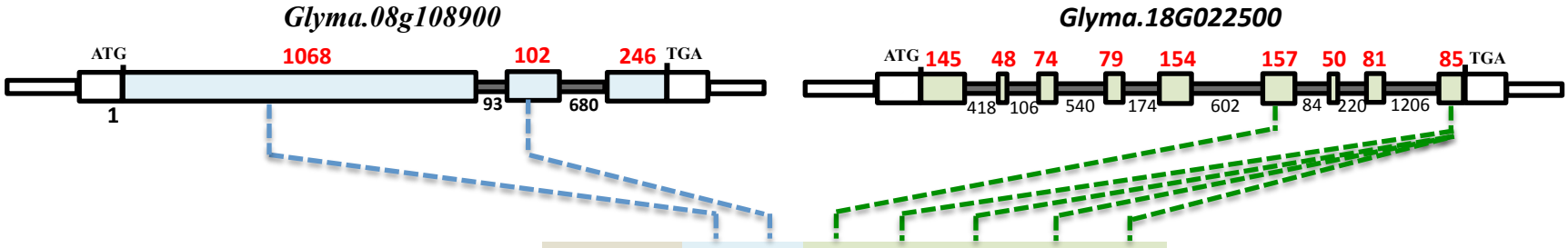
Supplemental Figure 1. Subcellular localization of the GmSNAP18 and GmSHMT08 proteins. Maximum intensity projection of confocal fluorescence and brightfield images of *N. benthamiana* leaves transiently expressing GmSNAP18 or GmSHMT08 - RFP fusion proteins from both Essex and Forrest. In the merge section, chlorophyll fluorescence appears in red, GmSNAP18 or GmSHMT08 appear in yellow. Scale bar corresponds to 50µM.



Supplemental Figure 2. Comparative analysis of the conserved Cysteine Residues in GmSNAP18, GmSNAP11, HsSNAP25 and HsSNAP23. The alignment analysis shows that the "C" residue is conserved in all of the compared predicted proteins (Green box). The conserved cysteine residues in HsSNAP25A, HsSNAP23, and other SNAP25 proteins from other organisms including goldfish, Torpedo, and *Drosophila* (Risinger et al., 1993) contribute to stable membrane association (Hess et al., 1992). This data points to the possibility of the GmSNAP18 to bind the plasma membrane.

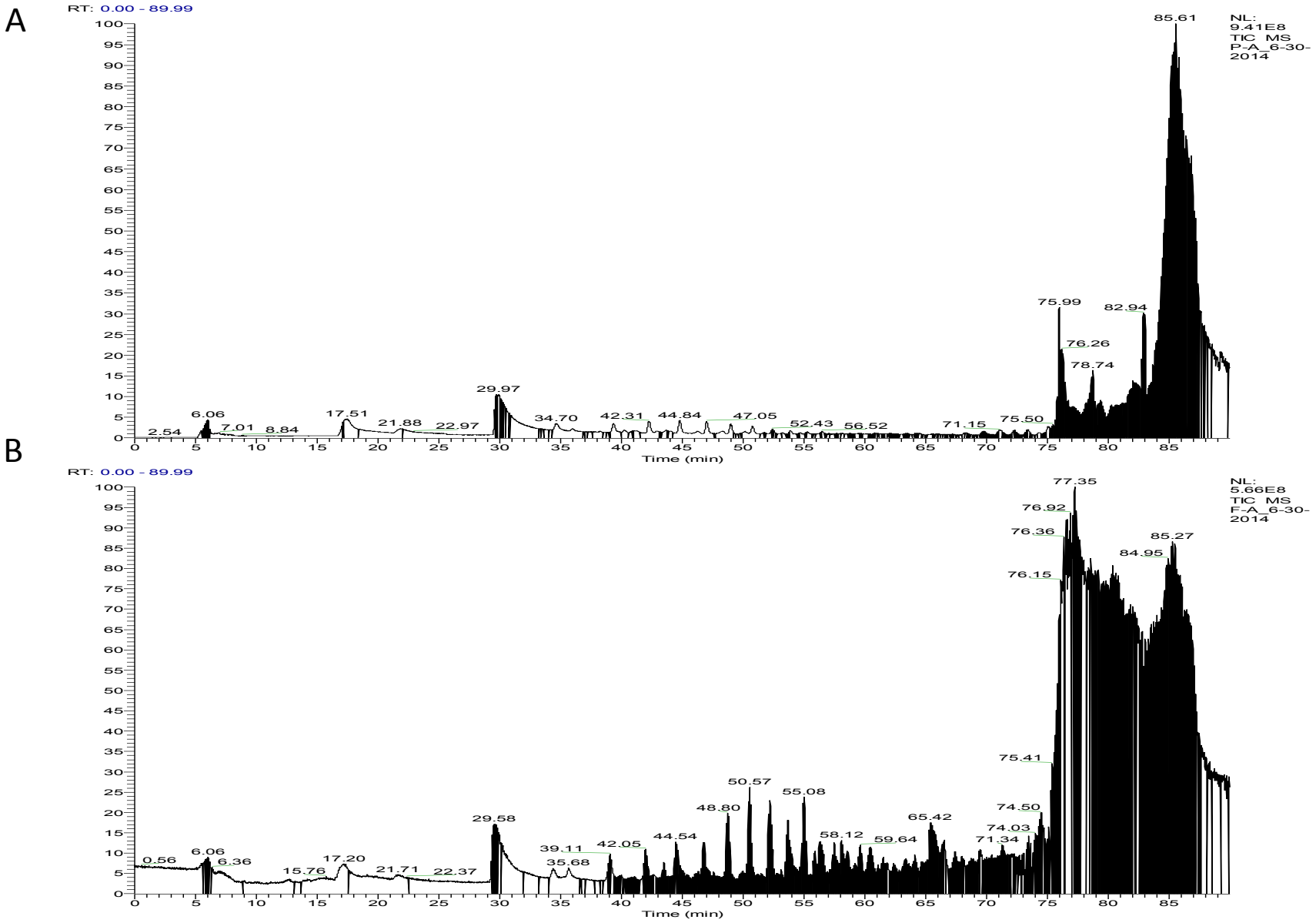


Supplemental Figure 3. BiFC analysis between GmSNAP18, GmSHMT08, and GmPR08-Bet VI from Forrest and Essex. (A) The coding sequence of Forrest and Essex *GmSHMT08* wild-type were cloned into *pSAT4-nEYFP-C1* (E81) to generate *nEYFP-GmSHMT08* fusions. Likewise, *GmSNAP18* from Forrest and Essex wild-type and *GmPR08-Bet VI* coding sequences were cloned into *pG2RNAi2* and *pSAT4-cEYFP-C1-B* (E812) to generate *pG2RNAi2-GmSNAP18* and *cEYFP-GmPR08-Bet VI* fusions. **(B)** *nEYFP-E-GmSHMT08, cEYFP-F-GmSNAP18,* and *pG2RNAi2-GmPR08-Bet VI.* Various combinations of cEYFP and nEYFP fusions including controls (Figure S10) were co-expressed in onion epidermal cells by particle bombardment. Bar = 200 μM.



PI, Elite, Cultivar	SCN races		GmSHMT08 (AA)		SNAP (AA)					
	HG0	HG2.7	200	459	208	286	287	Insert	288	
PI404198B	1	12	R	H	E	Y	E	V	I	GmSHMT08⁺/GmSNAP18⁺
PI468915	1	5	R	H	E	Y	E	V	I	
PI090763	2	2	R	Y	E	Y	E	V	I	
PI658519	1	2	R	Y	E	Y	E	V	I	
PI548402	2	4	R	Y	E	Y	E	V	I	
PI437690	2	4	R	Y	E	Y	E	V	I	
PI437725	2	3	R	Y	E	Y	E	V	I	
PI404166	1	1	R	Y	E	Y	E	V	I	
PI089772	1	1	R	Y	E	Y	E	V	I	
PI437679	1	2	R	Y	E	Y	E	V	I	
PI548402	1	12	R	Y	E	Y	E	V	I	
PI437654	1	2	R	Y	E	Y	E	V	I	
PI507354	2	6	R	Y	E	Y	E	V	I	
Forrest	3	5	R	Y	E	Y	E	V	I	
PI567516C	15	2	P	N	E	Y	E	V	I	GmSHMT08⁻/GmSNAP18⁺
PI567336B	34	1	P	N	E	Y	E	V	I	
PI567387	59	4	P	N	E	Y	E	V	I	
PI424608A	54	5	P	N	E	Y	E	V	I	
PI424298	68	11	P	N	E	Y	E	V	I	
S10-11227	16	3	P	N	E	Y	E	V	I	
PI407788A	64	5	P	N	E	Y	E	V	I	
PI567230	44	**	P	N	E	Y	E	V	I	
PI567305	22	1	P	N	E	Y	E	V	I	
PI603497	60	5	P	N	E	Y	E	V	I	
PI464920B	46	65	P	N	D	D	D	-	L	GmSHMT08⁻/GmSNAP18⁻
PI087617	66	103	P	N	D	D	D	-	L	
PI407184	60	70	P	N	D	D	D	-	L	
PI438471	80	122	P	N	D	D	D	-	L	
PI567690	76	86	P	N	D	D	D	-	L	
PI552538	69	86	P	N	D	D	D	-	L	
PI518751	52	84	P	N	D	D	D	-	L	
PI200508	90	100	P	N	D	D	D	-	L	
V71-370	62	89	P	N	D	D	D	-	L	
PI593258	54	73	P	N	D	D	D	-	L	
PI471938	66	84	P	N	D	D	D	-	L	
IA3023	58	70	P	N	D	D	D	-	L	
S07-5049	62	82	P	N	D	D	D	-	L	
Essex	74	68	P	N	D	D	D	-	L	
W82	78	75	P	N	D	D	D	-	L	

Supplemental Figure 4. Haplotype analysis of the soybean PI, Elite, and cultivars used for SCN screening. Soybean lines carrying the different combinations of *GmSHMT08*$^{+/-}$ and *GmSNAP18*$^{+/-}$ are shown (Patil et al., 2019). Lines carrying the *GmSHMT08*$^{-}$/*GmSNAP18*$^{+}$ lost their SCN resistance to HG0, but maintained their resistance to HG2.7. Female Index in blue represents soybeans resistant to SCN (FI < 10), in green lines with moderate resistance (10 < FI < 30), in orange lines with moderate susceptibility (31 < FI < 60), and in white lines susceptible to SCN (61 < FI).

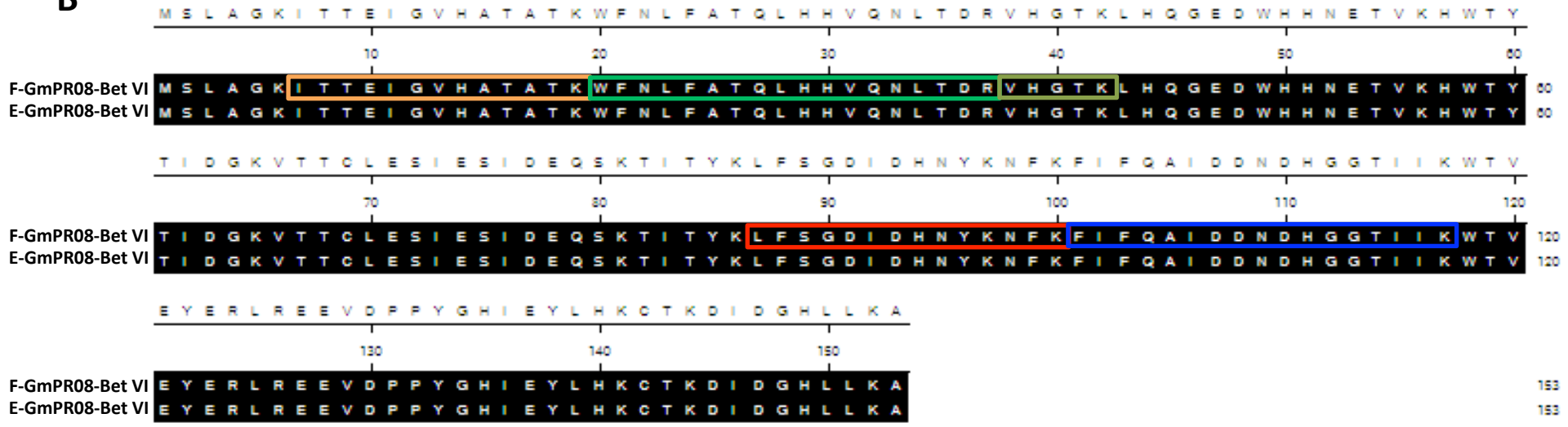


Supplemental Figure 5. Identification of the GmPR08-Bet VI by mass spectrometry. (A) and **(B)** represent LC-MS protein identification of the eluted fraction obtained from the Co-immunoprecipitation using immobilized anti-SHMT08 polyclonal antibodies. Total ion chromatograms showing signal intensity and separation. **(A)** Non infected soybean root samples from Forrest **(B)** SCN infected root samples from Forrest. Non infected soybean roots present a very weak signal in the "heart" of the gradient (35-70min). The SCN-infected soybean sample contains more proteins than the control.

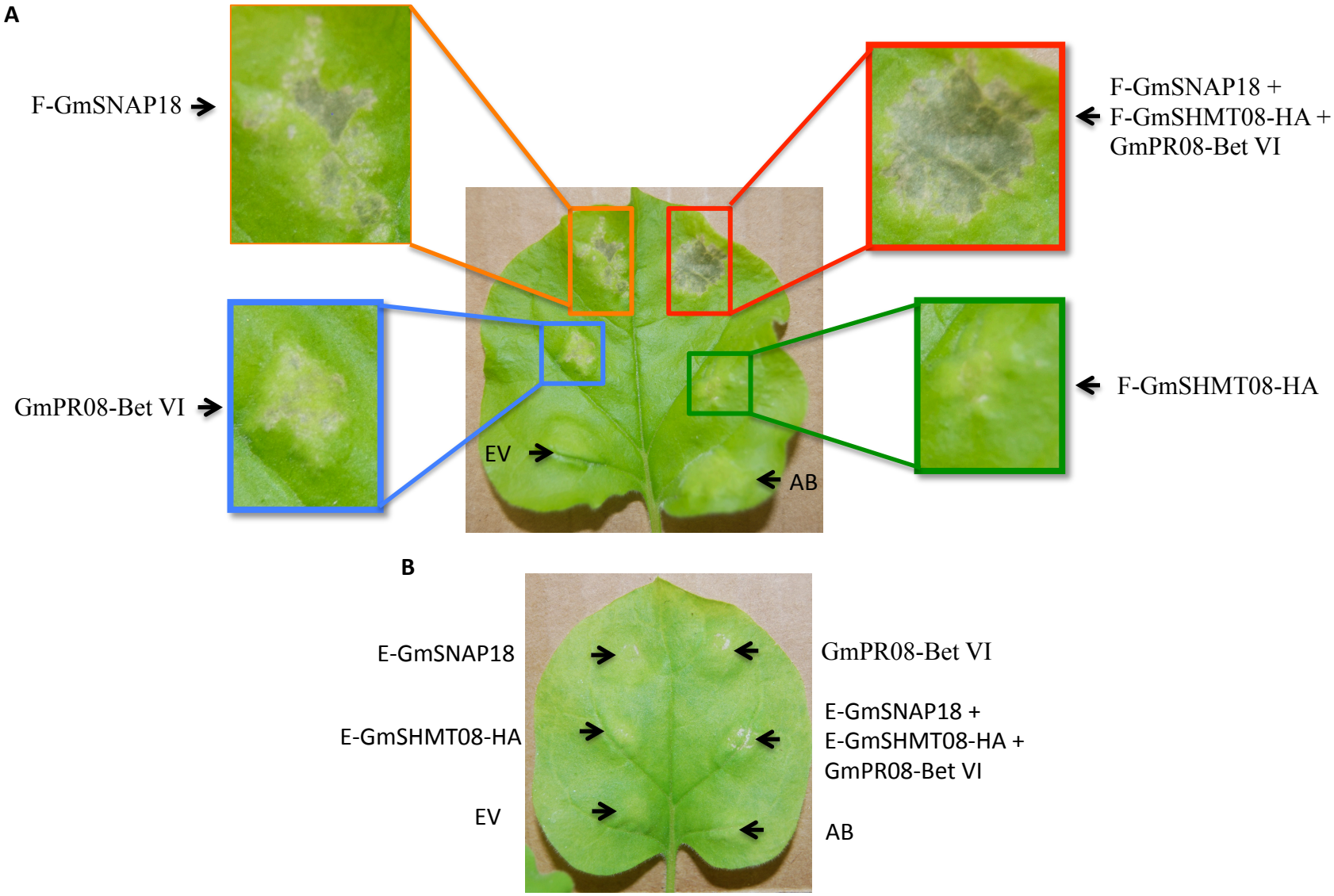
A

Gene Annotation	Protein name in Glycine max	Protein accession	Database sources	Protein (Da)	Probability	Peptide sequence
Glyma.08G230500 (GmPR08-Bet VI)	Pathogenesis-Related Protein Bet VI family	gi|255627117	NCBI_Gmax.fasta	17.762,10	100,00%	FIFQAIDDNDHGGTIIK
	Pathogenesis-Related Protein Bet VI family	gi|255627117	NCBI_Gmax.fasta	17.762,10	100,00%	ITTEIGVHATATK
	Pathogenesis-Related Protein Bet VI family	gi|255627117	NCBI_Gmax.fasta	17.762,10	100,00%	LFSGDIDHNYKNFK
	Pathogenesis-Related Protein Bet VI family	gi|255627117	NCBI_Gmax.fasta	17.762,10	100,00%	WFNLFATQLHHVQNLTDR
	Pathogenesis-Related Protein Bet VI family	gi|255627117	NCBI_Gmax.fasta	17.762,10	100,00%	WFNLFATQLHHVQNLTDRVHGTK

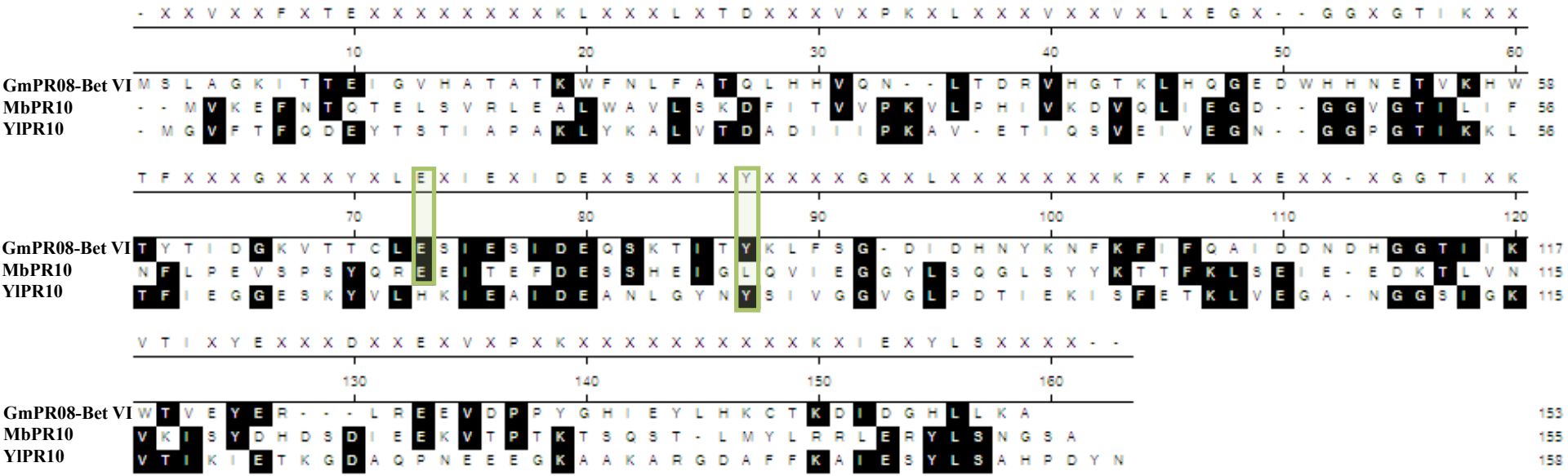
B



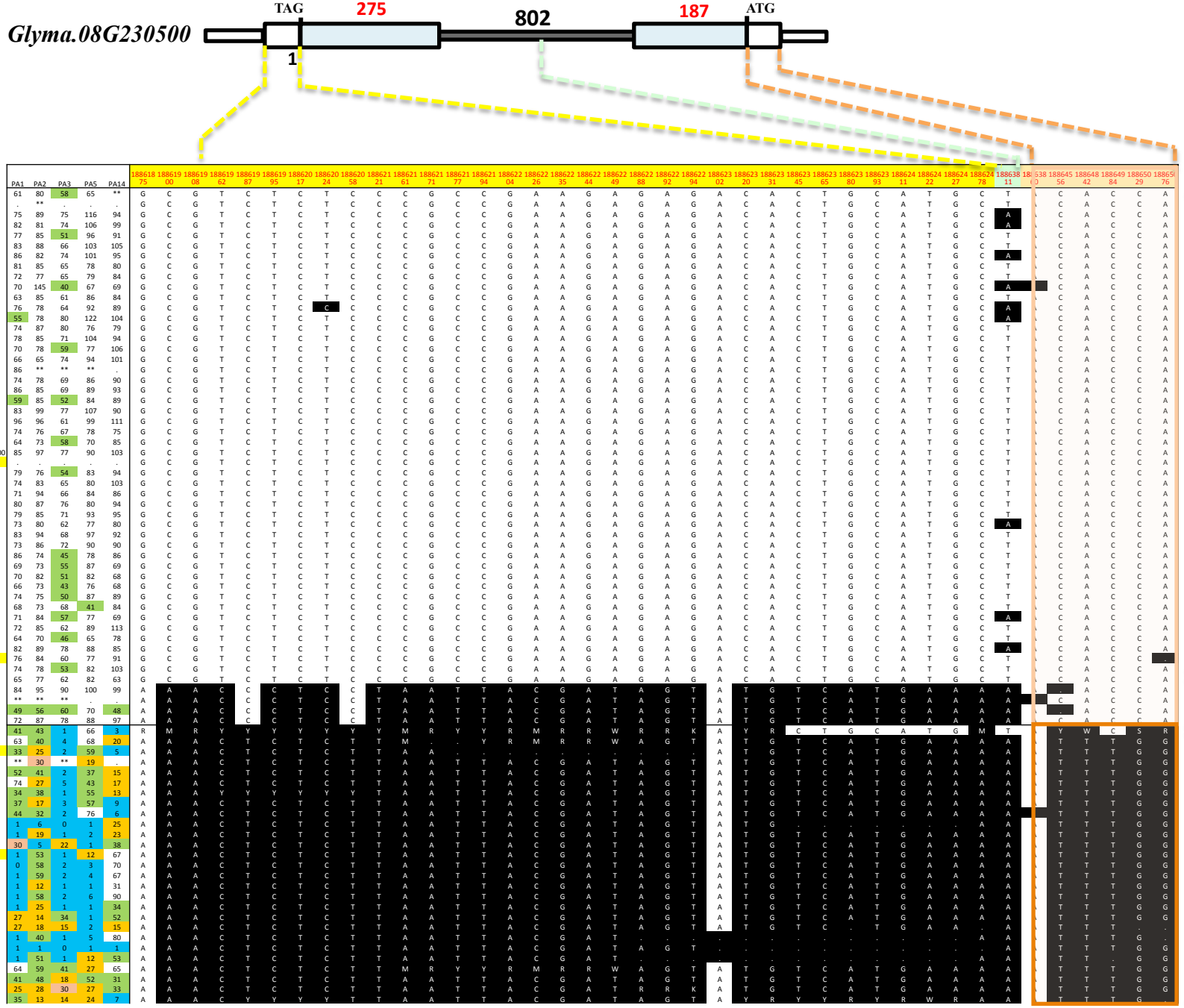
Supplemental Figure 6. Immunoprecipitation of the GmPR08-Bet VI protein by GmSHMT08. LC-MS protein identification of the eluted fraction obtained by immunoprecipitation using immobilized anti-SHMT08 polyclonal antibodies. **(A)** Fragmented peptides identified by LC-MS in SCN infected root samples from Forrest **(B)** Alignment of the GmPR08-Bet VI protein sequence showing the five identified fragmented peptides by LC-MS. E, Essex; F, Forrest.



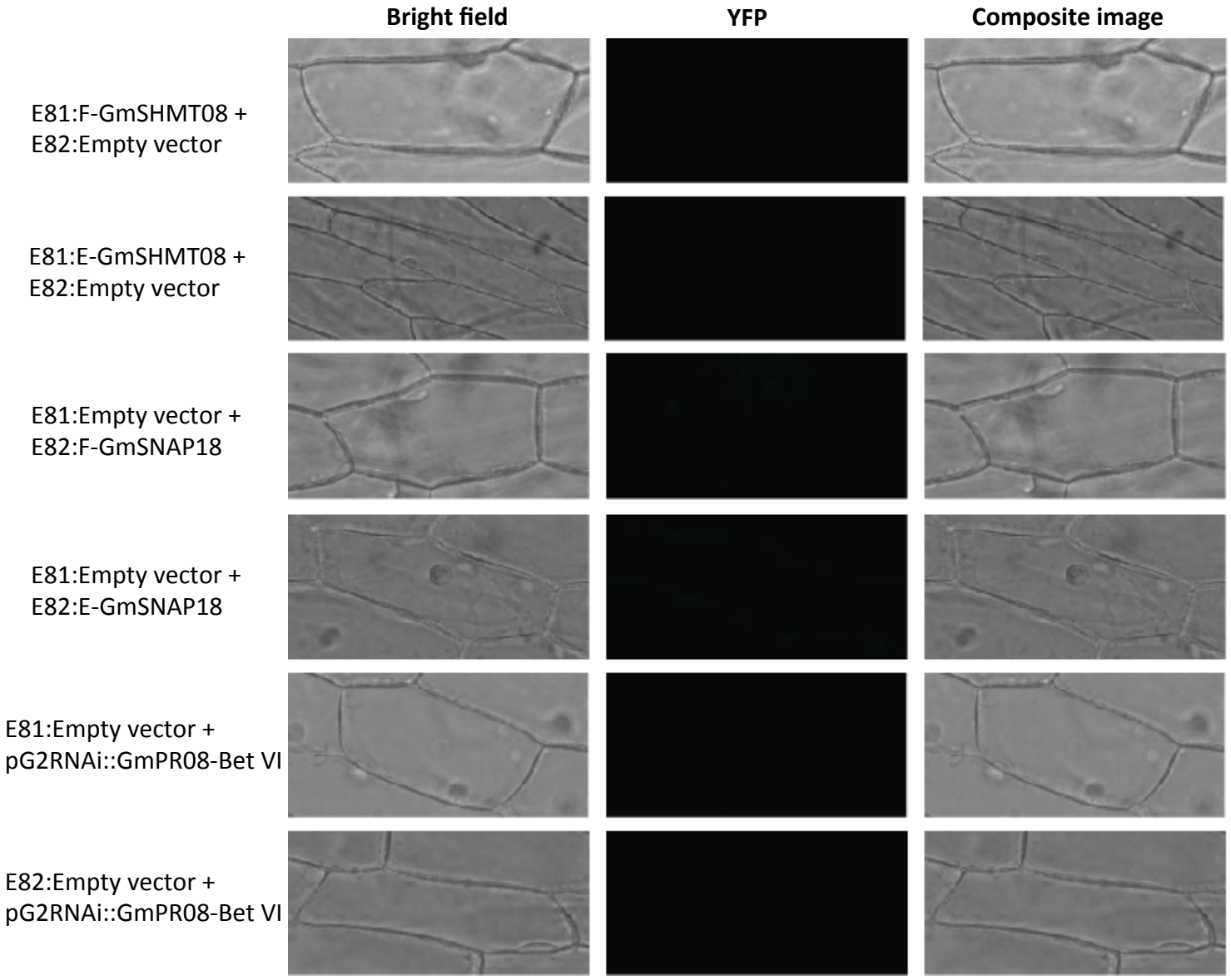
Supplemental Figure 7. Cell-death and necrosis symptoms intensified in *N. benthamiana* when the three *GmSNAP18*, *GmSHMT08*, and *GmPR08-Bet VI* genes were co-agroinfiltrated. The *Agrobacterium* mixture containing the constructs p35S-pGWB-GmSNAP18, p35S-pGWB-GmSHMT08-HA, and/or p35S-pGWB-PR08-Bet VI were mixed with the *P19* (suppression of gene silencing), and then incubated for 4 hours at 28ºC before infiltration. **(A)** and **(B)** *N. benthamiana* leaves after 5 days were co-agroinfiltrated to express either the indicated GmSNAP18, GmSHMT08, and/or GmPR08-Bet VI from Essex or Forrest. **(A)** Cell-death and necrosis symptoms caused when the three proteins were expressed together were also intensified when compared to single infiltrations. **(B)** Cell-death symptoms were very limited in Essex. E, Essex; F, Forrest; EV, Empty pGWB vector; AB, Agro-infiltration Buffer.



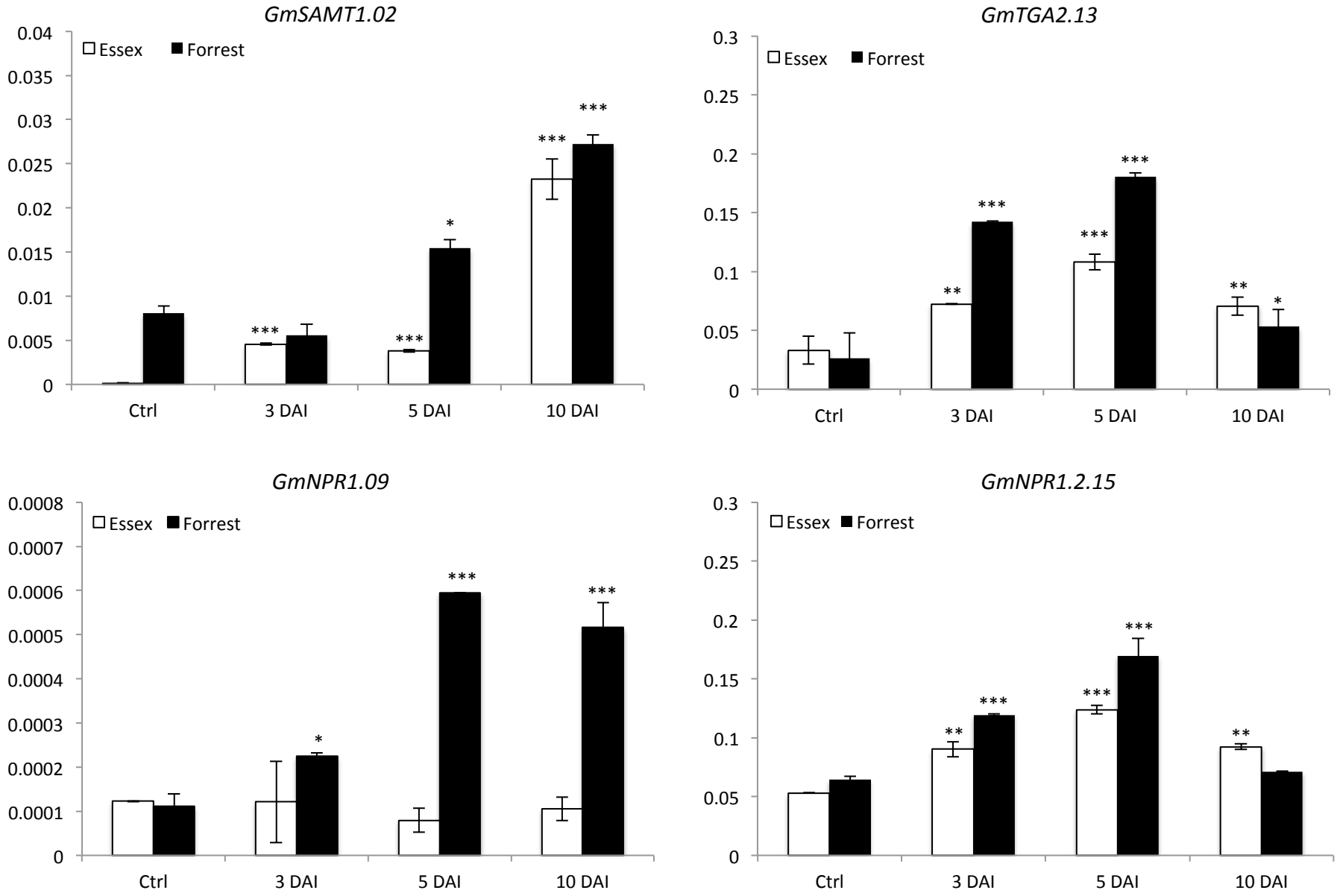
Supplemental Figure 8. Comparative analysis of the conserved Cytokinin (zeatin) binding sites residues at the GmPR08-Bet VI from *Glycine max*, MbPR10 (2FLH) from Mung bean, and a YlPR10 (2QIM) from Yellow lupine. The alignment analysis shows that the two zeatin binding sites; glutamic acid E71 and tyrosine Y85 residues are also conserved in GmPR08-Bet VI (Green box), suggesting that GmPR08-Bet VI may bind cytokinins similarly to the MbGmPR10 and YlGmPR10.



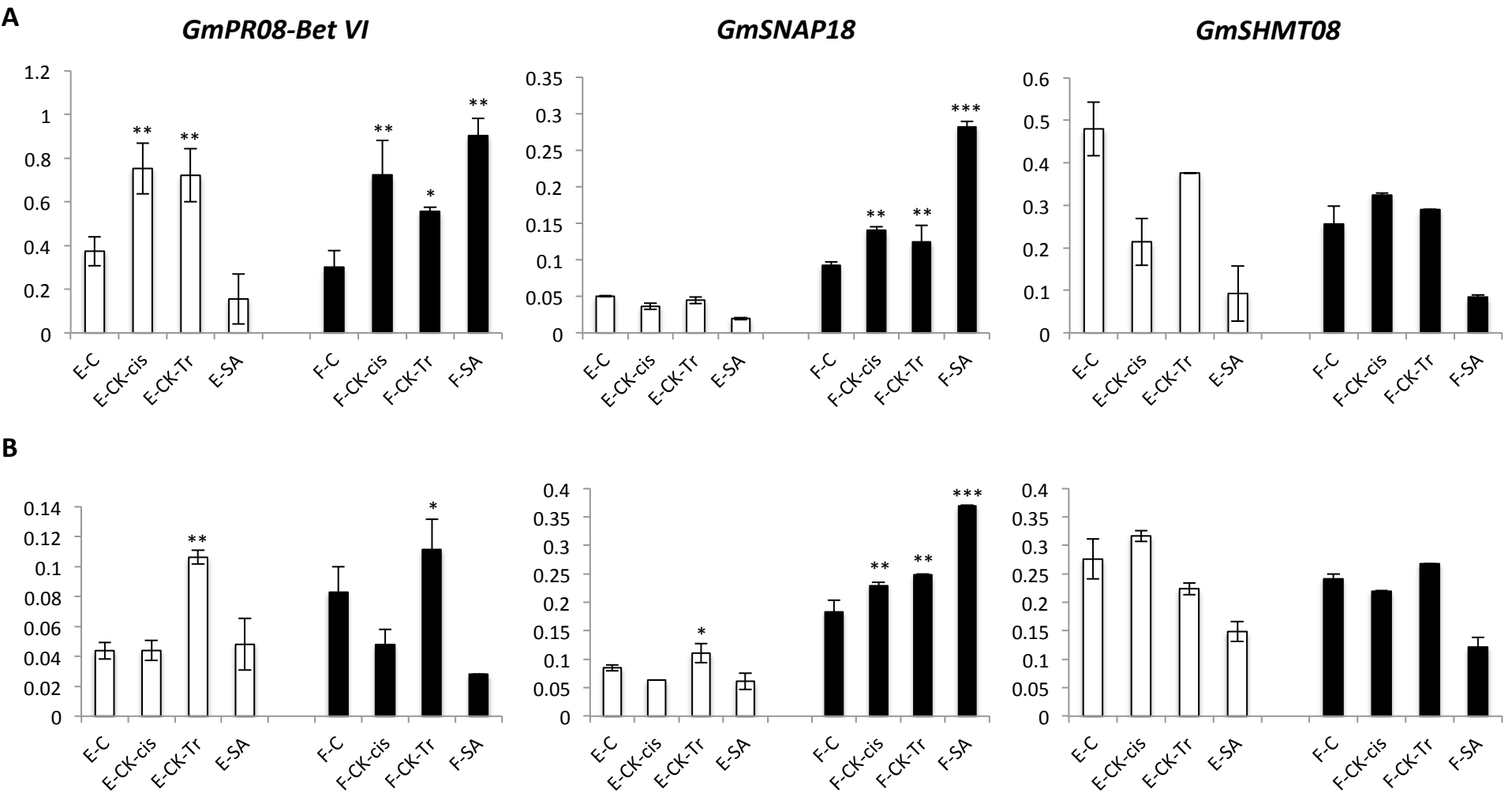
Supplemental Figure 9. *GmPR08-Bet VI* haplotype clustering and correlation with SCN female index in sequenced soybean lines. The lines included non-domesticated, semidomesticated, and elite domesticated introductions belonging to the USDA soybean collection (Patil et al., 2019). The schematic graph shows the position of the SNP/indel for *GmPR08-Bet VI* gene. SNP with a black background at *GmPR08-Bet VI* clustered with soybean lines carrying resistance to different SCN Hg-types. SNPs at the promoter region (orange), intron (Green), and 3′ UTR (yellow) are shown.



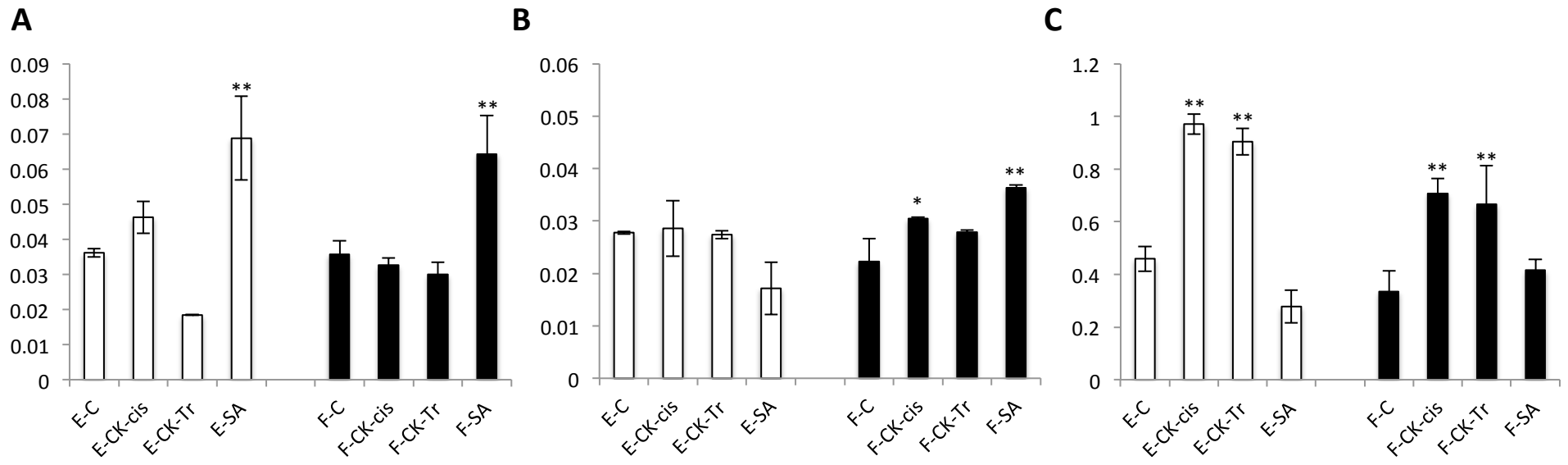
Supplemental Figure 10. Negative controls of the BiFC analysis. Each of the cloned GmSNAP18 and GmSHMT08 in the *pSAT4-nEYFP-C1* (E81) were tested in the presence of the *pSAT4-cEYFP-C1-B* (E82) and/or *pG2RNAi2* empty vectors used on the BiFC analysis in Figures 7 and S3. Various combinations of cEYFP and nEYFP fusions including controls were co-expressed in onion epidermal cells by particle bombardment under the same conditions and experiments.



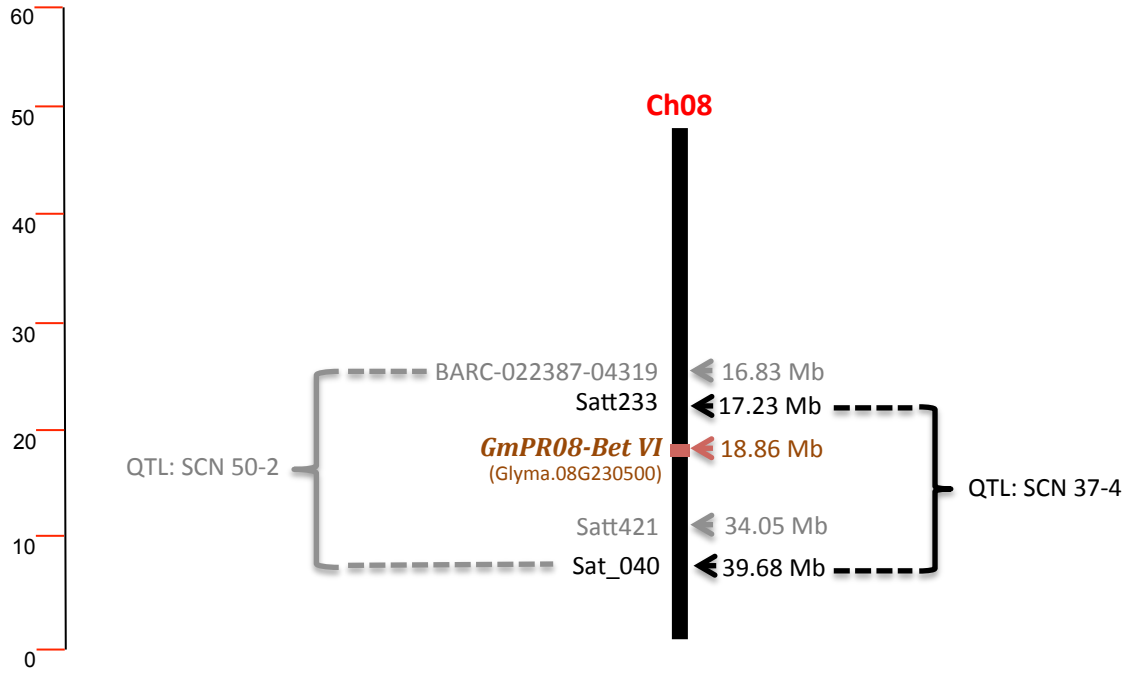
Supplemental Figure 11. Expression analysis of components of the SA signaling pathway reveals that all tested genes are co-regulated in root cells undergoing nematode infection. Transcripts of genes encoding key components of the salicylic acid signaling pathway including the S-adenosyl-L-methionine-dependent salicylic acid methyltransferase (*GmSAMT1.02*), the transcription factor (*GmTGA2.13*), and the two non-inducible pathogenesis-related 1 (*GmNPR1.09* and *GmNPR1.2.15*) were induced and more abundant under SCN infection in the resistant line Forrest than in the susceptible line Essex. The experiment was repeated three times and similar results were obtained. Five plants per line were used for each experiment. Asterisks indicate significant differences between the tested lines in the presence and absence (C) of SCN infection as determined by ANOVA (**** $P < .0001$, ** $P < .01$, * $P < .05$).



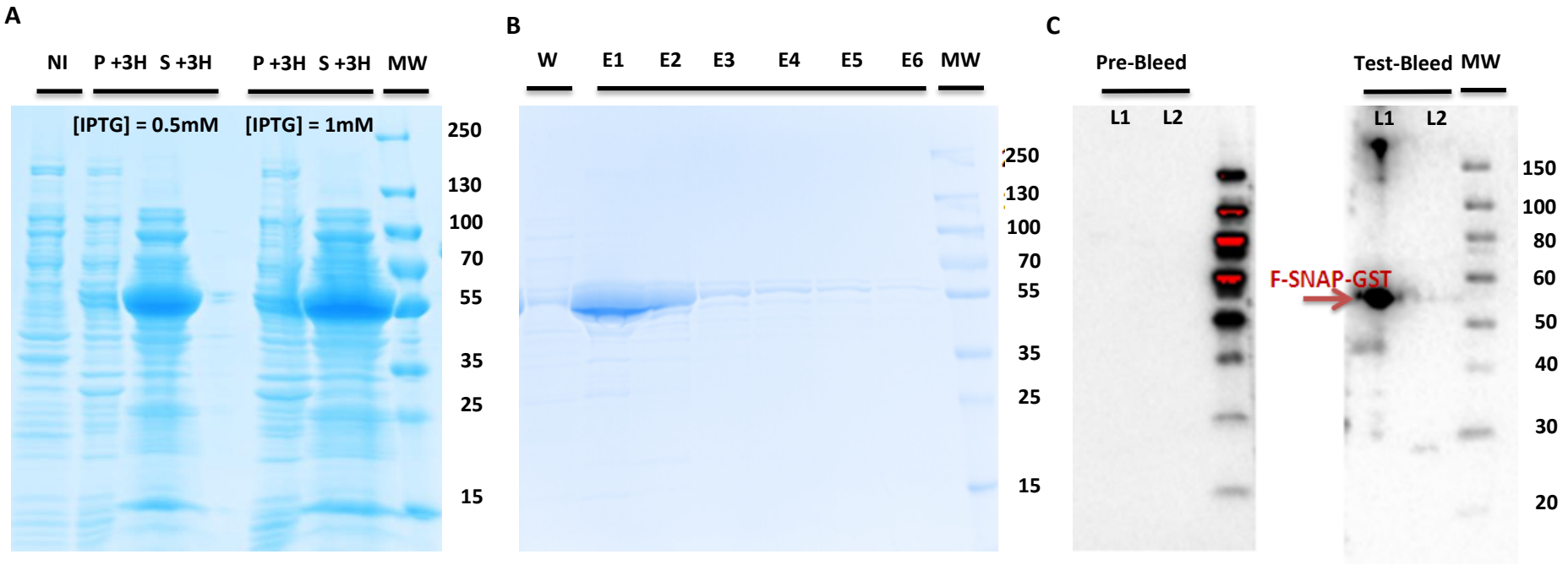
Supplementary Figure 12. Expression analysis of the *GmPR08-Bet VI*, *GmSNAP18*, and *GmSHMT08* under exogenous SA and CKs treatments. Treatments were carried out after **(A)** 12h and after **(B)** 24h using both phytohormones in Forrest and Essex soybean lines. The experiments were repeated three times and similar results were obtained. Five plants per line were used for each experiment. Asterisks indicate significant differences between the tested lines in the presence and absence (C) of SCN infection as determined by ANOVA (**** $P < .0001$, ** $P < .01$, * $P < .05$). CK-cis corresponds to zeatin-cis, and CK-Tr corresponds to zeatin-trans.



Supplementary Figure 13. Expression analysis of the (A) *GmNPR1.2-09*, (B) *GmTGA2-13*, and (C) *GmARR03* genes under exogenous SA and CKs treatments. Hormone treatments have been carried out after 12h in both Forrest and Essex soybean lines. The experiments were repeated three times and similar results were obtained. Five plants per line were used for each experiment. Asterisks indicate significant differences between the tested lines in the presence and absence (C) of SCN infection as determined by ANOVA (** $P < .01$, * $P < .05$). CK-cis corresponds to zeatin-cis, and CK-Tr corresponds to zeatin-trans.



Supplementary Figure 14. Physical positions corresponding to *GmPR08-Bet VI* and the two identified SCN QTLs at chromosome 08 are shown. Glyma.08G230500 (Chr08: 18,862,371 - 18,864,962); QTL: SCN 37-4 (Gm08:17232368 - Gm08:39682787) (Vuong et al. 2010); and QTL: SCN 50-2 (Gm08:16839299 – Gm08:34051964) (Swaminathan et al., 2018).

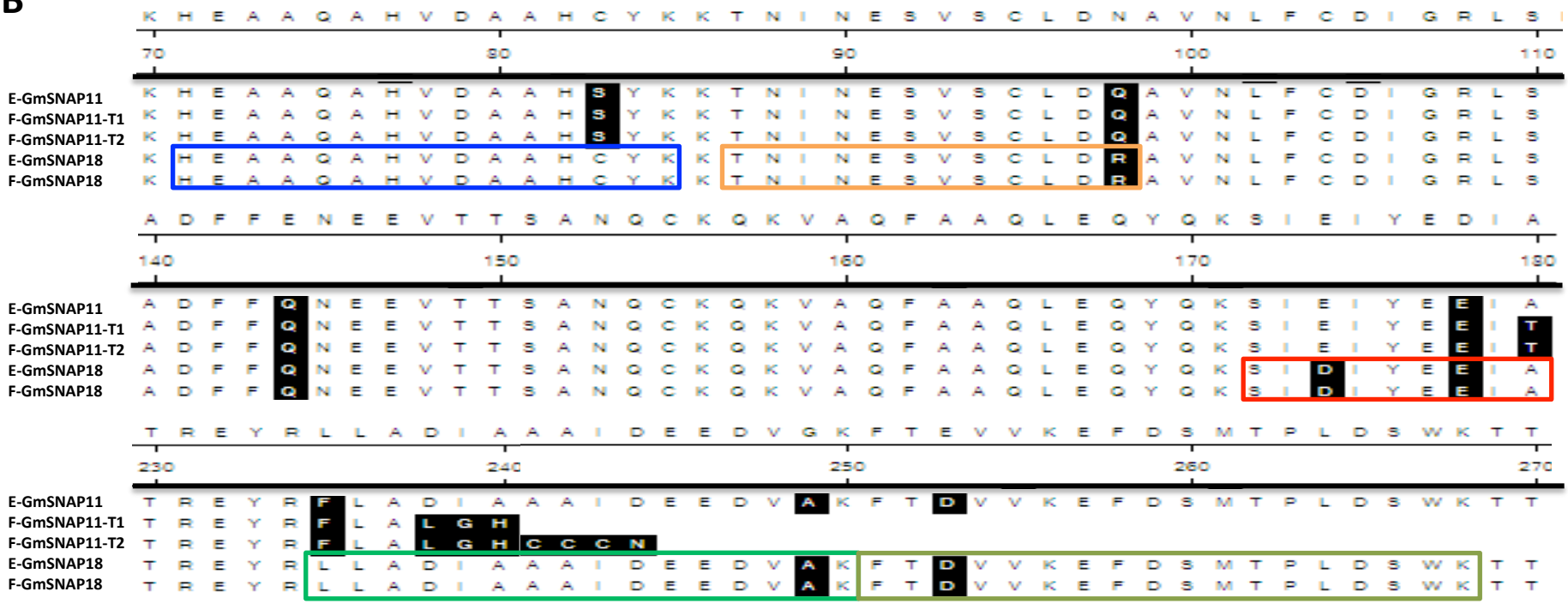


Supplemental Figure 15. ***In vivo*** **assays of GmSNAP18 recombinant protein in** ***E. coli,*** **Antibody Anti-GmSNAP18 production in Rabbit and confirming the specificity of custom-generated anti-GmSNAP18 antibodies. (A)** Recombinant *pGEX-5x-1::GmSNAP18-GST* protein expressed in *E. Coli* strain BL21. Left represent induction with [IPTG] = 0.5 mM and right [IPTG] = 1mM. Dilution 1/100 LB (50 mL) Amp or Cam-Amp, grow until OD600nm = 0.5. Sample at t = 0 (non induced) and 3h induction (Pellet and Supernatant) were revealed in SDS PAGE. **(B)** GST-tagged proteins purification with Glutathione resin (GE). Up to six eluted fractions were performed. Proteins from elution 6 were injected into Rabbits. **(C)** Western-Blot using Rabbit Pre-Bleed (Serum before injecting the purified GmSNAP18 (-1h)) and Test-Bleed (Serum 45 days after injection). Lane 1 = 50 ng purified GST-AB, Lane 2 = 50 ng control purified GST. (NI) Non-induced, (P) Pellet, (S) Supernatant, (MW) Molecular weight in KDa, (W) Wash, (E) Elution, (L) Lane.

A

Gene Annotation	Protein name in Glycine max	Protein accession	Database sources	Protein (Da)	Protein identification probability	Peptide sequence
Glyma.18G022500 (GmSNAP18)	Alpha-Soluble NSF Attachement Protein	gi|255636662	NCBI_Gmax.fasta	32782.2	100,00%	HEAAQAHVDAAHCYK
	Alpha-Soluble NSF Attachement Protein	gi|255636662	NCBI_Gmax.fasta	32782.2	100,00%	TNINESVSCLDR
	Alpha-Soluble NSF Attachement Protein	gi|255636662	NCBI_Gmax.fasta	32782.2	100,00%	SIDIYEEIAR
	Alpha-Soluble NSF Attachement Protein	gi|255636662	NCBI_Gmax.fasta	32782.2	100,00%	LLADIAAAIDEEDVAK
	Alpha-Soluble NSF Attachement Protein	gi|255636662	NCBI_Gmax.fasta	32782.2	100,00%	LLADIAAAIDEEDVAKFTDVVKEFDSMTPLDSWK

B



Supplemental Figure 16. *In vivo* assays and confirming the specificity of custom-generated anti-GmSNAP18 antibodies in Soybean. Only GmSNAP18 protein was immunoprecipitated when using the custom-generated anti-GmSNAP18 antibodies. LC-MS protein identification of the eluted fraction obtained by immunoprecipitation using immobilized anti-SNAP18 antibodies. **(A)** Fragmented peptides identified by LC-MS in root samples from Forrest. **(B)** Alignment of the GmSNAP18 and GmSNAP11 protein sequences showing the five identified fragmented peptides by LC-MS. Three independent experiments were performed showing the specificity of the anti-GmSNAP18 antibodies to bind the GmSNAP18 member only in soybeans. None of the obtained peptides corresponded to the GmSNAP11 member including all polymorphic regions.

Supplemental Table 2. The primers used for genotyping, sequencing, subcloning, qRT-PCR, and *in situ* analysis.

Gene	Gene model	Primers	Primer Sequences	Purpose
GmSHMT08	***Glyma.08g108900***	GmSHMT08-RT-Fw	TAACTTCGCCGTGTTCCCTT	qRT-PCR
		GmSHMT08-RT-Rv	TGTTTCGCGTAGGCCTTAAA	
		GmSHMT08-Fw	ACAACACTCTCTCTTCTCGC	Genotyping
		GmSHMT08-Rv	CAGATTATGAGTTTTGGCCTG	
		GmSHMT08-Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGATCCAGTAAGCGTGTGGGGTA	p35S-SHMT08 construct for Agroinfiltration
		GmSHMT08-Rv	GGGGACCACTTTGTACAAGAAAGCTGGGTCATCCTTGTACTTCATTTCAGATACC	
GmSNAP18	***Glyma.18G022500***	GmSNAP18-RT-Fw	ACAAGGCTGGAGCGACATAC	qRT-PCR
		GmSNAP18-RT-Rv	AGCAATGTGCAGCATCGACA	
		GmSNAP18-Fw	CACTGTGTAAAGTTAATTTTTTTGCTTAC	Genotyping
		GmSNAP18-Rv	CCAATTCAATTAAAACCAAAGCAGG	
		GmSNAP18-Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTCAATGGCCGATCAGTTATCGAAGGGAG	p35S-SNAP18::HA construct for Agroinfiltration
		GmSNAP18-Rv	GGGGACCACTTTGTACAAGAAAGCTGGGTCAGTAATAACCTCATACTCCTCAAGT	
		XmaI-SNAP-Fw	AAACCCGGGAATGGCCGATCAGTTATCGAAGG	pGEX-5x-1-GST::SNAP18 for Antibodies
		XhoI-SNAP- Rv	AAAACTCGAGTCAAGTAATAACCTCATACTCC	
		Probe SNAP-Fw	TGACATTTATGAAGAGATAGC	Probe for *In-situ*
		Probe SNAP-Rv	TTATAGCAACAACCTCCT	
GmPR08-Bet VI	**Glyma.08G230500**	GmPR08-Bet VI-RT-Fw	AGTTCATCTTTCAAGCCATTGATGATA	qRT-PCR
		GmPR08-Bet VI-RT-Rv	CCTCACGAAGCCTCTCGTATT	
GmNPR1.09	**Glyma.09G064700**	GmNPR1.09-RT-Fw	GGACCTGTACATGCCCGAAA	qRT-PCR
		GmNPR1.09-RT-Rv	CCAGTTCCACATCGTCCGAA	
GmNPR1.2.15	**Glyma.15G127200**	GmNPR1.2.15-RT-Fw	GGAATCCATTGGCTGGGGAT	qRT-PCR
		GmNPR1.2.15-RT-Rv	TGCCACTCTGTTCTCAAGGT	
GmSAMT1.02	**Glyma.02G054200**	GmSAMT1.02-RT-Fw	GCTGTGGCAGAACCTATGCT	qRT-PCR
		GmSAMT1.02-RT-Rv	GCCAAGATTTGCTGGTAGCG	
GmTGA2.13	**Glyma.13G193700**	GmTGA2.13-RT-Fw	TCGCCAGCAAACTCTTCAAC	qRT-PCR
		GmTGA2.13-RT-Rv	GTATAGCAAGGAGTGCGCGA	
GmPRXD16	**Glyma.16G164400**	GmPRXD16-RT-Fw	AGGAGGCCCTGATTTTGACG	qRT-PCR
		GmPRXD16-RT-Rv	AGAATGGTGCCGGTAGGTTG	
GmPKR19	**Glyma.19G193100**	GmPKR19-RT-Fw	CCCCGAGCACCCAAATGTTA	qRT-PCR
		GmPKR19-RT-Rv	GTTTAATCTCGGCAGCCCCT	
GmUbiqutin	**Glyma.20G141600**	GmUBI20-RT-Fw	GTGTAATGTTGGATGTGTTCCC	qRT-PCR
		GmUBI20-RT-Rv	ACACAATTGAGTTCAACACAAACCG	