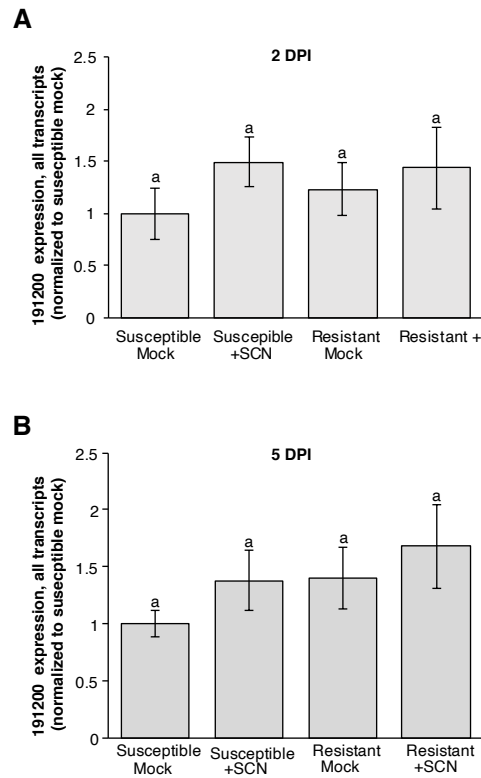
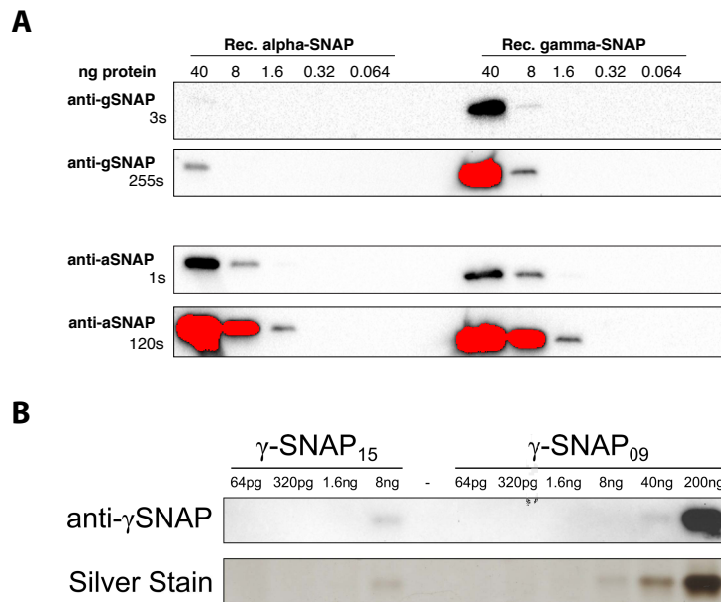


Supplemental Figure S2. An additional splice from results from intron 6 retention.

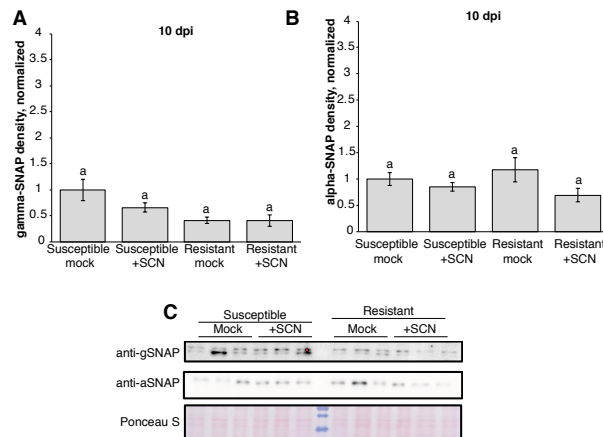
(A) RT-PCR revealed the expression of another alternative splice form, *Glyma.15G191200.4*, which is expressed at a very low level in susceptible (LD10-30080) and resistant (LD10-30110) mock- and SCN-inoculated roots. No RT: cDNA templates made from the same extracted RNA but without reverse transcriptase. (B) A predicted gene model for *Glyma.15G191200.4* with SNPs found in PI 468916. The location of the premature stop codon is indicated with a red asterisk.



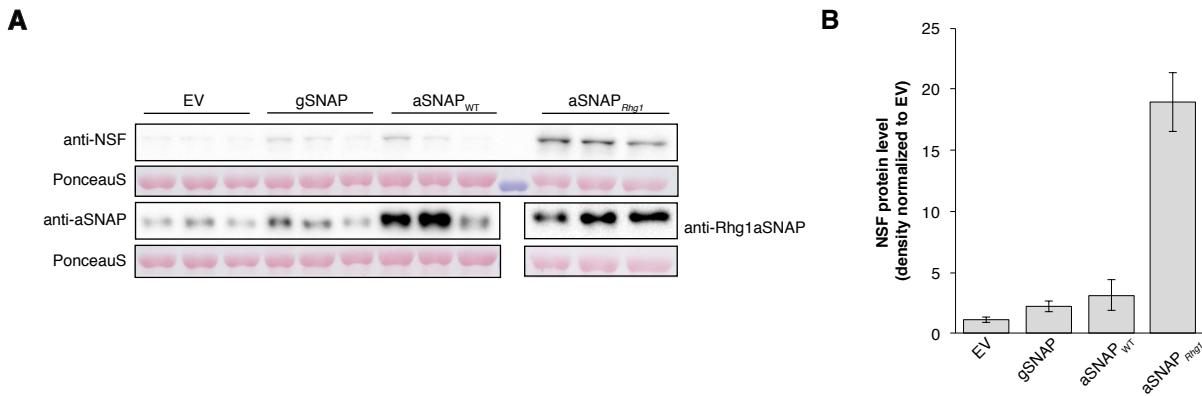
Supplemental Figure S3. No change detected in overall transcription of *Glyma.15G191200* during SCN infection. qPCR results of overall transcription of *Glyma.15G191200* at two (A) and five (B) days post inoculation. qPCR amplification was performed with a primer set that amplifies the first and second exon to detect diverse transcripts from *Glyma.15G191200*, including the identified splice forms. Bars represent mean expression relative to *GmSKP16*, normalized to mean for susceptible mock within the same experiment. Error bars are standard error of the mean. Means for treatments labeled with the same letter are not significantly different ($P > 0.1$, ANOVA Tukey). Data are for four independent experiments, total n for each treatment was: (A) susceptible mock, 14; susceptible +SCN, 13; resistant mock, 11; resistant +SCN, 15. (B) susceptible mock, 14; susceptible +SCN, 16; resistant mock, 13; resistant +SCN, 15.



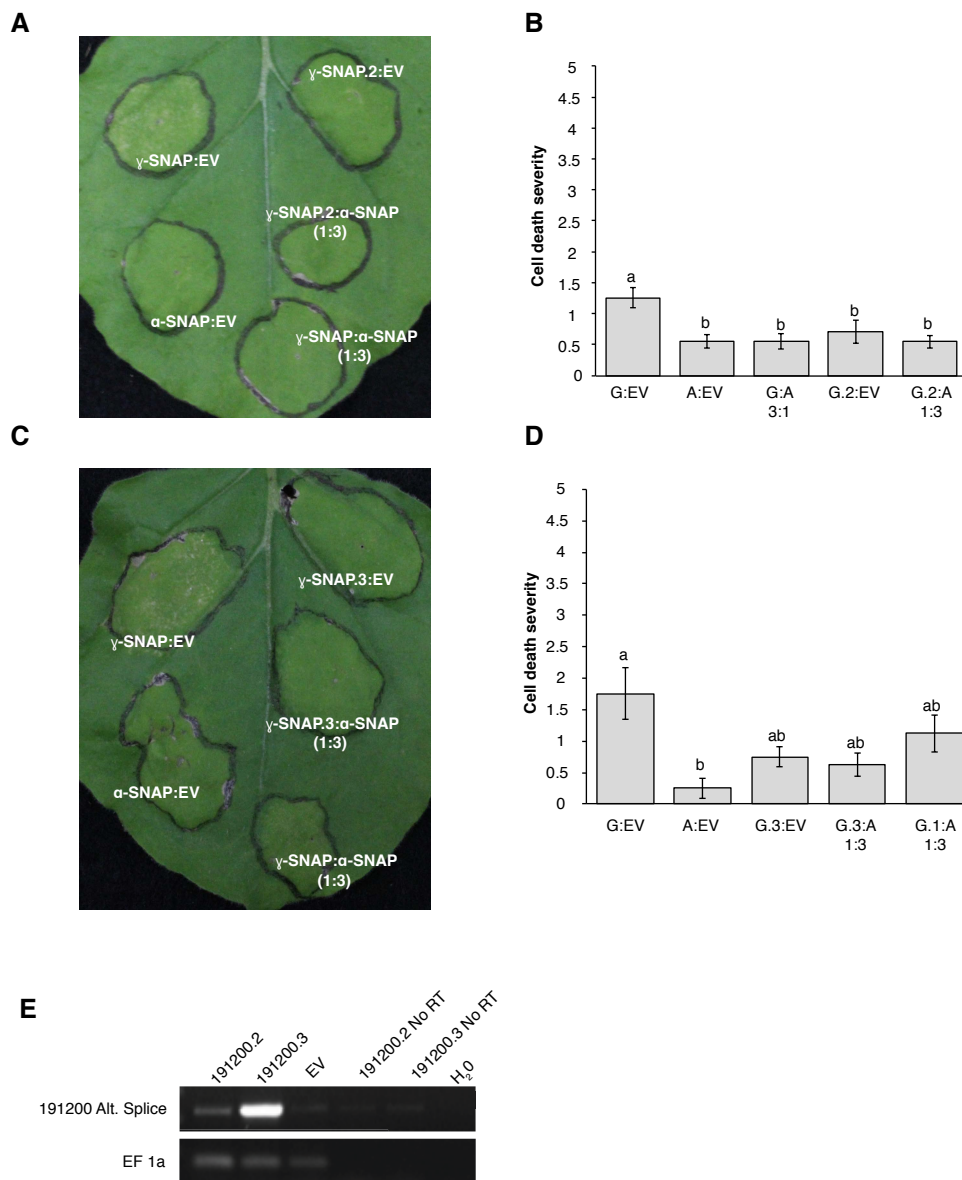
Supplemental Figure S4. Validation of custom γ -SNAP antibody for specificity for γ -SNAP and not α -SNAP. (A) Immunoblots of recombinant (Rec.) γ -SNAP and α -SNAP protein over a five-fold dilution series. Each band was at the expected size for the respective protein. Red indicates overexposed areas. Exposure time on a high-resolution setting indicated. At extremely high levels, α -SNAP can be detected by γ -SNAP antibody, but at a lower affinity than for γ -SNAP. In contrast, the α -SNAP antibody readily detects γ -SNAP with similar affinity. (B) Immunoblots of recombinant protein products of the *cqSCN-006* chromosome 15 locus γ -SNAP and the γ -SNAP encoded on chromosome 9, revealing approximately five-fold greater sensitivity of the polyclonal antibody for the *cqSCN-006* γ -SNAP.



Supplemental Figure S5. γ -SNAP and α -SNAP levels diminish 10 days after inoculation. Densitometry analysis of immunoblot using (A) soybean chromosome 15 γ -SNAP or (B) chromosome 18 α -SNAP antibody in susceptible (LD10-30080) and resistant (LD10-30110) mock and SCN inoculated roots 10 dpi. Densitometry analysis data show mean and standard error of four independent experiments each consisting of two or three samples ($n = 11$); data points normalized for susceptible mock within each experiment, data analysis was performed on raw data. Means for treatments with same letter are not statistically different ($P > 0.05$ ANOVA Tukey test) (C) Representative immunoblots from a single replicate. Images were selected once overexposure of a single band was observed. Ponceau S stains tests equal loading of protein across treatments.



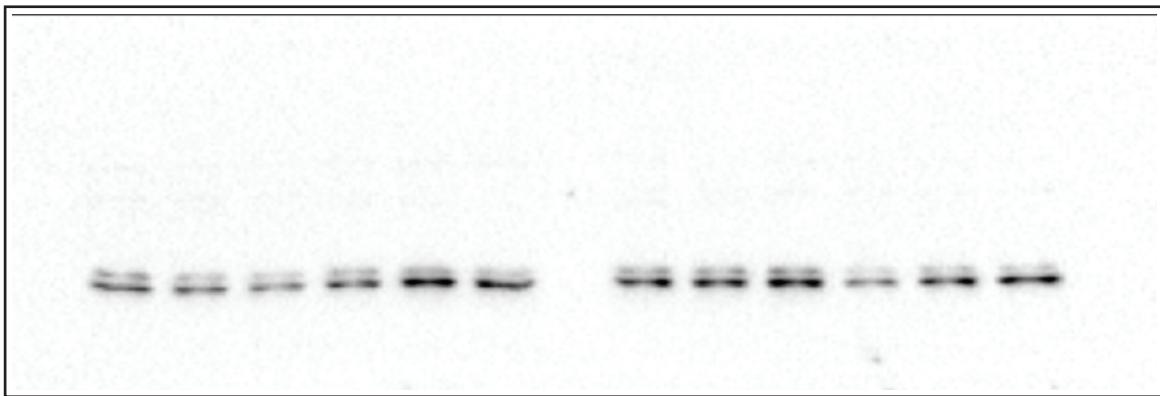
Supplemental Figure S6. Immunoblots demonstrating previously observed increase in NSF upon ectopic expression of a toxic α -SNAP in *N. benthamiana* leaves. (A) immunoblot of protein lysates from *N. benthamiana* leaves expressing γ -SNAP, wild-type α -SNAP, or Peking-type α -SNAP_{Rhg1} or empty vector (EV). Blots were probed with custom antibodies for NSF and wildtype α -SNAP, which also detects γ -SNAP, or Peking-type α -SNAP_{Rhg1} as indicated. Ponceau S tests equal loading of samples. (B) Densitometry analysis of representative blot. $n=3$ for each sample. Data are normalized to EV control.



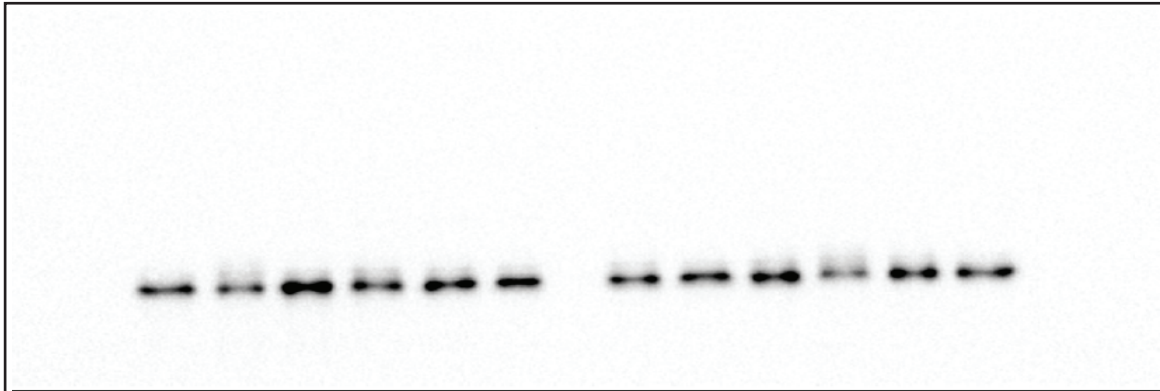
Supplemental Figure S7. γ -SNAP alternative splice forms do not cause cell death when transiently expressed in *N. benthamiana* leaves. *N. benthamiana* leaves expressing γ -SNAP alternative splice forms *Glyma.15G191200.2* (A) or *Glyma.15G191200.3* (C) in various ratios with wildtype soybean α -SNAP and primary transcript *Glyma.15G191200.1* at the indicated ratios. (B, D) Cell death ratings on a 0-5 scales, from multiple independent leaves. Mean treatments with the same letter are not significantly different ($P < 0.05$, pairwise Wilcoxon rank sum test). Data shown are mean and standard error of the mean. $n = 32$ for *Glyma.15G191200.2* and $n = 8$ for *Glyma.15G191200.3*. G = γ -SNAP, A = α -SNAP. (E) RT-PCR of cDNA library from infiltrated areas to detect the indicated alternative transcripts. RT-PCR for EF1a performed as a positive control. RT-PCR using water or mock cDNA templates made from the same extracted RNA but with no reverse transcriptase (no RT) performed as negative controls.

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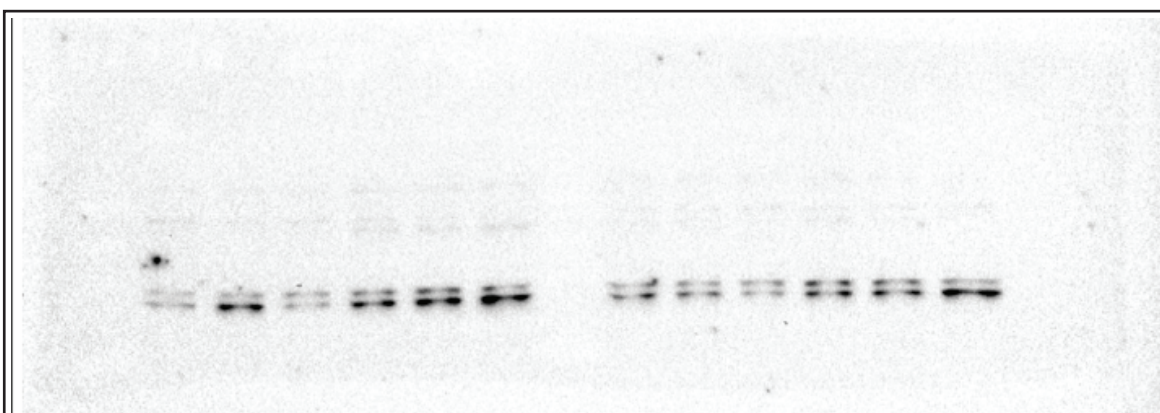
Day Two - anti-gamma-SNAP - full blot



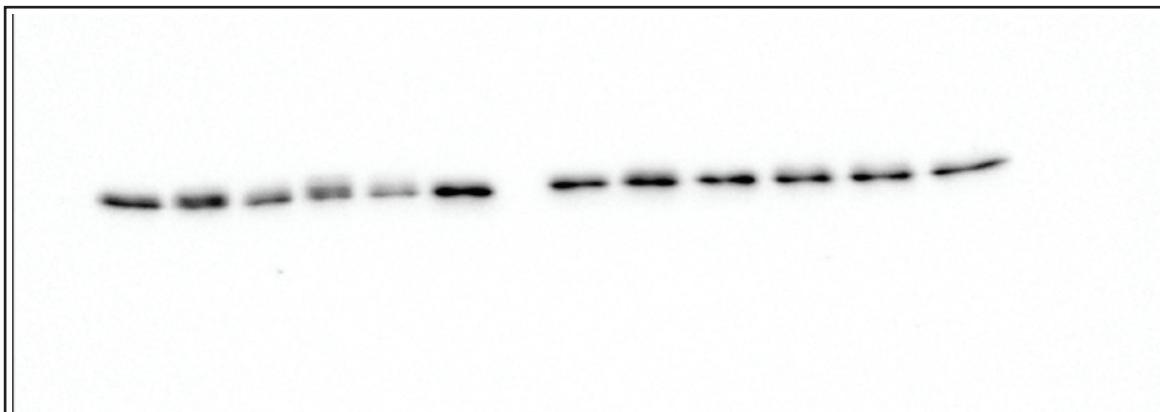
Day Two - anti-alpha-SNAP - full blot



Day Five - anti-gamma-SNAP - full blot

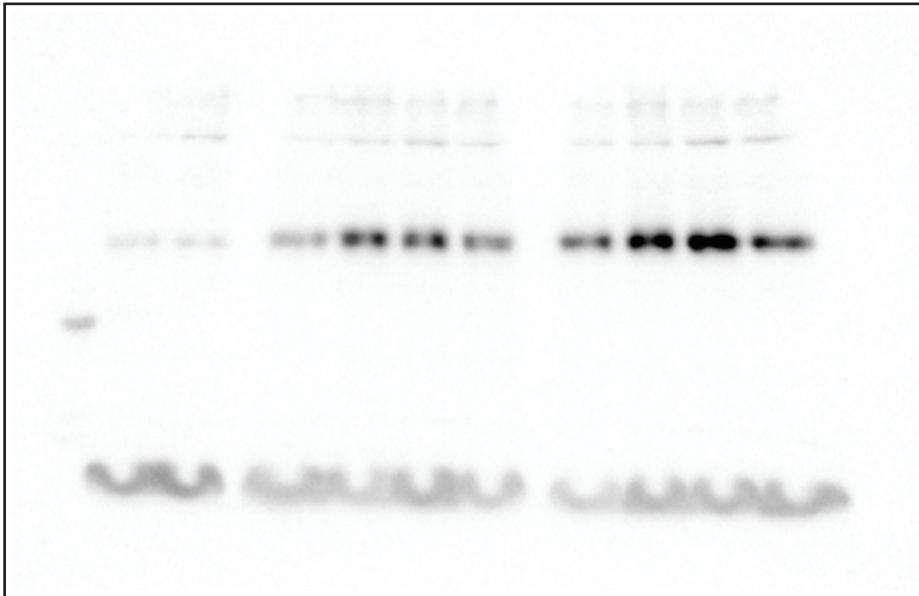


Day Five - anti-alpha-SNAP - full blot

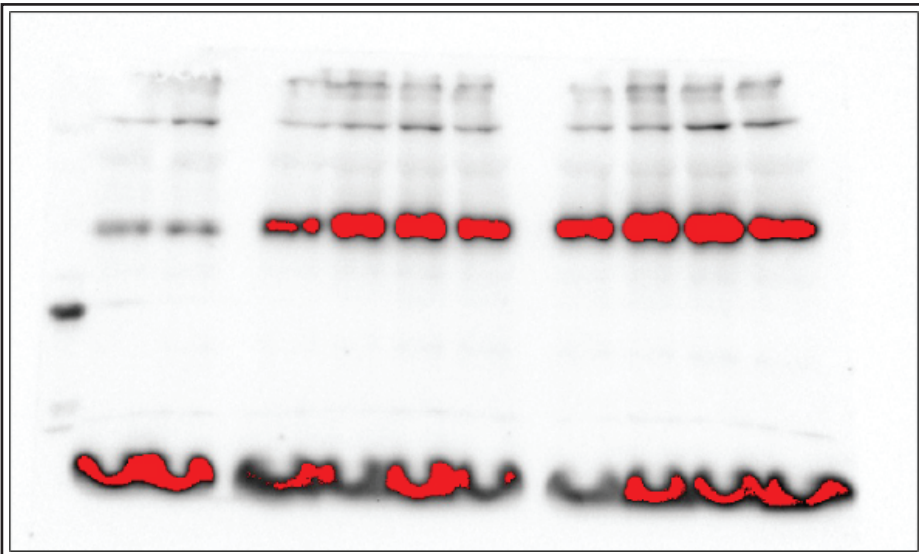


Supplemental Figure S8. Original (uncropped) whole-blot images for Figure 6.

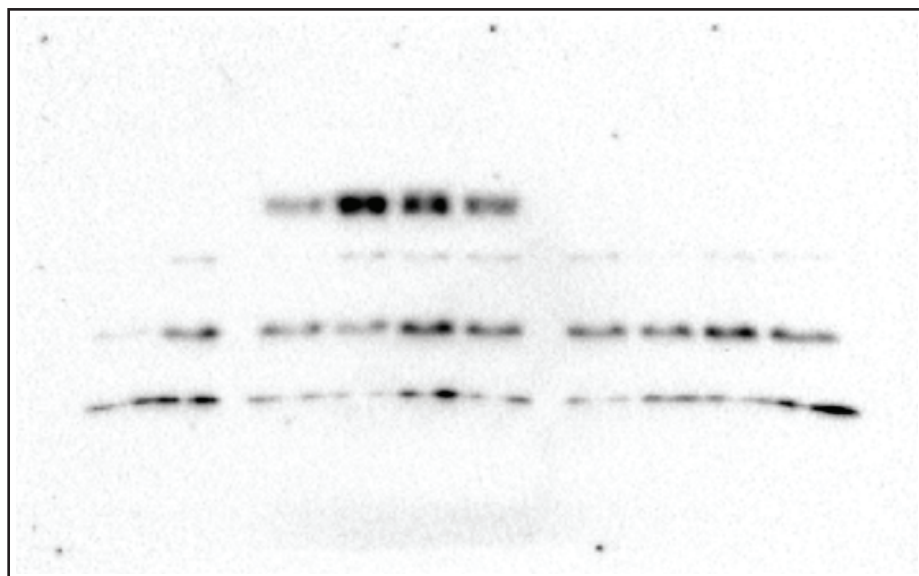
N. benthamiana expression - anti-NSF & anti-alphaSNAPWT (a-SNAP exposure time) - full blot



N. benthamiana expression - anti-NSF & anti-alphaSNAPWT (NSF exposure time) - full blot



N. benthamiana expression - anti-gamma-SNAP - full blot



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Supplemental Figure S9. Original (uncropped) whole-blot images for Figure 7.

Supplemental Table S1. Candidate genes fine-mapped to the *cqSCN-006* interval

Gene name	Annotated Domain	General protein function	<i>A. thaliana</i> homolog	Reported Root Expression (FPKM)¹
<i>Glyma.15G191000</i>	None	None	None	1.297
<i>Glyma.15G191100</i>	None	None	None	0.782
<i>Glyma.15G191200</i>	Gamma Soluble NSF Attachment Protein	Protein movement, vesicle trafficking	AT4G20410.1	25.555
<i>Glyma.15G191300</i>	BED-Finger related	DNA Binding	AT5G17680.1	Not expressed
<i>Glyma.15G191400</i>	BED-Finger related	DNA Binding	AT5G17680.1	Not expressed
<i>Glyma.15G191500</i>	None	Arabinogalactan	AT4G12950.1	1.231

¹ As reported on Phytozome (phytozome.jgi.doe.gov), FPKM – Fragments per kilobase of transcript per million mapped reads (Goodstein et al., 2012)

Modified from (Yu & Diers, 2017).

Supplemental Table S2. DNA sequence analysis for *Glyma.15G191200* across 18 differently soybean lines.

Promoter SNPs		-1941	-1864	-1550	-1513	-1353	-1119	-1064	-1027	-1023	-1005	-910	-897	-851	-838	-775	-766	-656	-653	-541	-498	-420
Line/Location		A	T	G	C	C	CGCA	A	G	A	C	T	C	T	G	A	T	C	G	ATAGGC	G	G
PI 468916		A	T	G	C	C	CGCA	A	G	A	C	T	C	T	G	A	T	C	G	ATAGGC	G	G
LD10-30110		T	T	T	C	C	CGCA	A	G	G	T	T	C	T	G	A	T	C	G	ATAGGC	G	A
LD10-30080		T	G	T	C	C	----	A	A	G	C	T	C	T	G	G	T	C	G	-----	G	G
Williams 82		PI 483463 (G. soja) T A complex																				
PI 483463 (G. soja)		T	G	T	C	C	----	A	A	G	C	T	C	T	G	G	T	C	G	-----	G	G
Maverick		T	G	T	C	C	----	A	A	G	C	T	C	T	G	A	T	C	G	-----	G	G
Magellan		T	G	T	C	C	----	A	A	G	C	T	C	T	G	A	T	C	G	-----	G	G
Peking		T	G	T	C	C	----	A	A	G	C	T	C	T	G	G	T	C	G	-----	G	G
PI 90763		T	G	T	T	T	----	G	G	G	C	A	C	T	G	G	C	C	G	ATAGGC	A	G
PI 437654		T	G	T	T	T	----	G	G	G	C	A	A	T	G	A	C	C	G	-----	A	G
PI 209332		T	G	T	T	T	----	G	G	G	C	A	C	T	G	A	C	C	G	-----	A	G
PI 89772		T	G	T	T	T	----	G	G	G	C	A	C	A	A	G	C	C	G	-----	A	G
Cloud		T	T	T	C	C	----	A	A	G	T	T	C	T	G	A	T	C	G	-----	G	A
IA3023		T	G	T	C	C	----	A	A	G	C	T	C	T	G	G	T	C	G	-----	G	G
LD05-5429		T	G	T	C	C	----	A	A	G	C	T	C	T	G	G	T	C	G	-----	G	G
LD02-9050		T	G	T	C	C	----	A	A	G	C	T	C	T	G	G	T	C	G	-----	G	G
LD02-4495		T	G	T	C	C	----	A	A	G	C	T	C	T	G	G	T	C	G	-----	G	G
LD00-3309		T	G	T	C	C	----	A	A	G	C	T	C	T	G	G	T	C	G	-----	G	G
CL0J095-4-6		T	G	T	C	C	----	A	A	G	C	T	C	T	G	G	T	C	G	-----	G	G
4J105-4-4		T	G	T	C	C	----	A	A	G	C	T	C	T	G	G	T	C	G	-----	G	G

Promoter SNPs		-362	-344	-220	-209	-193	-180	-170	-153	-148	-141	-136	-137	-131	-124	-115	-114	-106	-93	-91	-55	-53
Line/Location		G	G	C	C	G	T	C	A	G	A	C	C	T	C	G	C	G	T	-	A	A
PI 468916		G	G	C	C	G	T	C	A	G	A	C	C	T	C	G	C	G	T	-	A	A
LD10-30110		G	G	C	C	G	C	C	G	G	A	C	C	G	C	G	C	G	T	-	C	A
LD10-30080		G	G	C	T	G	C	C	G	A	A	C	C	T	T	G	T	G	T	-	C	A
Williams 82		G	G	T	C	A	T	G	G	complex	complex	T	C	T	C	G	C	A	C	T	C	A
PI 483463 (G. soja)		G	G	C	T	G	C	C	G	A	A	C	C	T	C	G	T	G	T	-	C	A
Maverick		G	G	C	T	G	C	C	G	A	A	C	C	T	C	G	T	G	T	-	C	A
Magellan		G	G	C	T	G	C	C	G	A	A	C	C	T	C	G	T	G	T	-	C	A
Peking		G	C	C	C	G	C	C	G	G	G	C	T	T	C	A	C	G	T	T	C	C
PI 90763		G	C	C	C	G	C	C	G	G	G	C	T	T	C	A	C	G	T	T	C	C
PI 437654		G	C	C	C	G	C	C	G	G	G	C	T	T	C	A	C	G	T	T	C	C
PI 209332		G	C	C	C	G	C	C	G	G	G	C	T	T	C	A	C	G	T	T	C	C
PI 89772		G	G	C	C	G	C	C	G	G	A	C	C	T	C	G	C	G	T	-	C	A
Cloud		G	G	C	T	G	C	C	G	A	A	C	C	T	C	G	T	G	T	-	C	A
IA3023		G	G	C	T	G	C	C	G	A	A	C	C	T	C	G	T	G	T	-	C	A
LD05-5429		G	G	C	T	G	C	C	G	A	A	C	C	T	C	G	T	G	T	-	C	A
LD02-9050		G	G	C	T	G	C	C	G	A	A	C	C	T	C	G	T	G	T	-	C	A
LD02-4495		G	G	C	T	G	C	C	G	A	A	C	C	T	C	G	T	G	T	-	C	A
LD00-3309		G	G	C	T	G	C	C	G	A	A	C	C	T	C	G	T	G	T	-	C	A
CL0J095-4-6		G	G	C	T	G	C	C	G	A	A	C	C	T	C	G	T	G	T	-	C	A
4J105-4-4		G	G	C	T	G	C	C	G	A	A	C	C	T	C	G	T	G	T	-	C	A

Gene body SNPs		57	304	315	997	1429	1461	1769	1787	2216	2164	3521	3702	3861	4256	4542	4698	4710	4990	5247	5304	5443	6332	6886
Line/Location		C	C	-	G	----	T	T	C	-	T	G	TT	G	T	C	T	G	----	A	A	A	A	C
PI 468916		C	C	-	G	----	T	T	C	-	T	G	TT	G	T	C	T	G	----	A	A	A	A	C
LD10-30110		C	C	-	G	----	T	T	C	-	T	G	TT	G	T	C	T	G	----	A	A	A	A	C
Williams 82		C	C	T	G	----	T	A	C	-	G	G	TT	C	-	C	-	G	----	A	A	A	A	C
PI 483463 (G. soja)		T	C	-	G	TTGTA	C	A	T	CT	G	G	TT	C	-	T	T	A	GGTC	A	T	T	A	T
Maverick		C	C	T	G	----	T	A	C	-	G	G	TT	C	-	C	-	G	----	A	A	A	A	C
Magellan		C	C	T	G	----	T	A	C	-	G	G	TT	C	-	C	-	G	----	A	A	A	A	C
Peking		C	C	T	G	----	T	A	C	-	G	G	TT	C	-	C	-	G	----	A	A	A	A	C
PI 90763		C	-	-	A	----	T	A	C	-	G	A	TT	C	-	C	-	G	----	T	A	A	T	C
PI 437654		C	-	-	A	----	T	A	C	-	G	A	TT	C	-	C	-	G	----	T	A	A	T	C
PI 209332		C	-	-	A	----	T	A	C	-	G	A	TT	C	-	C	-	G	----	T	A	A	T	C
PI 89772		C	-	-	A	----	T	A	C	-	G	A	TT	C	-	C	-	G	----	T	A	A	T	C
Cloud		C	C	T	G	----	T	A	C	-	G	G	TT	C	-	C	-	G	----	A	A	A	A	C
IA3023		C	C	T	G	----	T	A	C	-	G	G	TT	C	-	C	-	G	----	A	A	A	A	C
LD05-5429		C	C	T	G	----	T	A	C	-	G	G	TT	C	-	C	-	G	----	A	A	A	A	C
LD02-9050		C	C	T	G	----	T	A	C	-	G	G	TT	C	-	C	-	G	----	A	A	A	A	C
LD02-4495		C	C	T	G	----	T	A	C	-	G	G	TT	C	-	C	-	G	----	A	A	A	A	C
LD00-3309		C	C	T	G	----	T	A	C	-	G	G	TT	C	-	C	-	G	----	A	A	A	A	C
CL0J095-4-6		C	C	T	G	----	T	A	C	-	G	G	TT	C	-	C	-	G	----	A	A	A	A	C
4J105-4-4		C	C	T	G	----	T	A	C	-	G	G	TT	C	-	C	A	G	----	A	A	A	A	C

Location based on TSS (1bp of UTR is 1, first base pair outside is -1)

Complex = complex variation greater than one nucleotide or simple insertion

Unique to 46916

Other Unique SNPs

No Data

Supplemental Table S3. Oligonucleotides used in this study.

Vector Construction	
MtU6 R	AAGCCTACTGGTTCGCTTGAAG
Scaffold F	GTTTTAGAGCTAGAAATAGCAAGTT
UNS1_MtU6 F	CATTACTCGCATCCATTCTCATGCCTATCTTATATGATCAATGAGG
UNS1_Scaffold R	GAGAATGGATGCGAGTAATGAAAAAAGCACCGACTCGGTG
35SSpeI_Mtu6 F	CGTGCTCCACCATGTTGGGAATGCCTATCTTATATGATCAATGAGG
SpeI_Scaffold R	GTCATGAATTGTAATACGACTCAAAAAAAGCACCGACTCGGTG
191200 gRNA1	GCTATTTCTAGCTCTAAAACATTAGCTGCAACAGAATACCAAGCCTACTGGTTCCG CTTGA
191200 gRNA2	GCTATTTCTAGCTCTAAAACCTTGGCAGAGCAGCAGCGGACAAGCCTACTGGTTC GCTTGA
191200 Gmubi Gibson F	TGTGATTGTTGACTCGACAGATGGCAGCTTCTGATCC
191200 Gmubi Gibson R	GGTCGAATTCGCCCTTTCAAGTGAGGTCATTTTCATCGA
RT-PCR and qRT-PCR	
191200.2/3 RT-PCR F	CGAGACCACAACCTGCATCA
191200.2/3 RT-PCR R	ACAGCTCAATCACATGCAACA
SKP16 qPCR F	GAGCCCAAGACATTGCGAGAG
SKP16 qPCR R	CGGAAGCGGAAGAACTGAACC
Intron 6 retention F	AATGCTACCAATAGCCAGTG
Intron 6 retention R	AACTACCAACATCAGAGTATTCAC
191200.2/3 qPCR For	TCTTTATCGTGCTGCCACTAA
191200.2/3 qPCR Rev	CCATCAAATGTATTGCCTCAATT
191200 ALL qPCR For	TGCTTTCTTCACCTTGGGATGCG
191200 ALL qPCR Rev	TGCCAGTGCTTAGATCCTTTGCG
NbEF-1a For	CTTGCTTTCACTCTTGGTGTC
NBEF-1a Rev	CAATCATATTGTCTCCCTCGA
Glyma.15G191200 Alternative Splice Cloning	
191200 5' UTR For	AGGAGGGTGAGTTCTTC
191200 Splice Intron Rev	GTCTACCCTGTGAAAAATCT
191200 SPLICE Intronic For	GTGTGAATTCTCAAAGTCTTCA
191200 3' UTR Rev	AACAGCGCTCCTCTTAT