Fig. S1. Wild-type α-SNAP expression is reduced in $Rhg1_{Low Copy}$ soybeans. (*A*) Independent immunoblot like Fig. 1B and incorporated into NSF densiometric analyses shown in Fig. 1C. Immunoblot of wild-type α-SNAPs and NSF expression in HG-Type soybean roots. $Rhg1_{LC}$ varieties: PI 548402 (Peking), PI 89772, PI 437654, PI 90763; $Rhg1_{HC}$ varieties: PI 88788, PI 209332, PI 548316 (7 copy). PonceauS staining shows total protein loaded per lane. (*B*) Modeling of α-SNAP$_{Ch11}$ to Sec17 crystal structure (yeast α-SNAP, PDB ID 1QQE) suggests early termination of alpha-helix 12 in the intron-retention mutant. Pre-mature translational termination point shown red. (*C*) Immuno-blots for total WT α-SNAP and α-SNAP$_{Rhg1}$LC levels in Forrest ($Rhg1_{LC}$) transgenic roots transformed with the native WT α-SNAP$_{Ch11}$ locus from Wm82 or an EV (empty vector) control. (*D*) Agarose gel showing PCR amplicons of the promoter regions of the α-SNAP$_{Ch11}$-IR allele or the WT α-SNAP$_{Ch11}$ allele from Wm82.

NSF RAN07 alignment to Wild-Type NSF_Ch07 (Wm82)

A

```
Wms82    MASRFGLSSSSSSASSMRVTNTPASDLALTNLAFCSPSDLRNFAVPGHNNLYLAAVADSF
RAN07    MASQFGLSSSSSSASSMRVTYTPANDLALTNLAFCSPSDLRNFAVPGHNNLYLAAVADSF
         ***:************** ***.*************************************


Wms82    VLSLSAHDTIGSGQIALNAVQRRCAKVSSGDSVQVSRFVPPEDFNLALLTLELEF VKKGS
RAN07    VLSLSAHDTIGSGQIALNAVQRRCAKVSSGDSVQVSRFVPPEDFNLALLTLELEFFVKKGS
         ****************************************************** *****


Wms82    KSEQIDAVLLAKQLRKRFMNQVMTVGQKVLFEYHGNNYSFTVSNAAVEGQEKSNSLERGM
RAN07    KSEQIDAVLLAKQLRKRFMNQVMTVGQKVLFEYHGNNYSFTVSNAAVEGQEKSNSLERGI
         ***********************************************************:


Wms82    ISDDTYIVFETSRDSGIKIVNQREGATSNIFKQKEFNLQSLGIGGLSAEFADIFRRAFAS
RAN07    ISDDTYIVFETSRDSGIKIVNQREGATSNIFKQKEFNLQSLGIGGLSAEFADIFRRAFAS
         ************************************************************


Wms82    RVFPPHVTSKLGIKHVKGMLLYGPPGTGKTLMARQIGKILNGKEPKIVNGPEVLSKFVGE
RAN07    RVFPPHVTSKLGIKHVKGMLLYGPPGTGKTLMARQIGKILNGKEPKIVNGPEVLSKFVGE
         ************************************************************


Wms82    TEKNVRDLFADAEQDQRTRGDESDLHVIIFDEIDAICKSRGSTRDGTGVHDSIVNQLLTK
RAN07    TEKNVRDLFADAEQDQRTRGDESDLHVIIFDEIDAICKSRGSTRDGTGVHDSIVNQLLTK
         ************************************************************


Wms82    IDGVESLNNVLLIGMTNRKDMLDEALLRPGRLEVQVEISLPDENGRLQILQIHTNKMKEN
RAN07    IDGVESLNNVLLIGMTNRKDMLDEALLRPGRLEVQVEISLPDENGRLQILQIHTNKMKEN
         ************************************************************


Wms82    SFLAADVNLQELAARTKNYSGAELEGVVKSAVSYALNRQLSLEDLTKPVEEENIKVTMDD
RAN07    SFLAADVNLQELAARTKNYSGAELEGVVKSAVSYALNRQLSLEDLTKPVEEENIKVTMDD
         ************************************************************


Wms82    FLNALHEVTSAFGASTDDLERCRLHGMVECGDRHKHIYQRAMLLVEQVKVSKGSPLVTCL
RAN07    FLNALHEVTSAFGASTDDLERCRLHGMVECGDRHKHIYQRAMLLVEQVKVSKGSPLVTCL
         ************************************************************


Wms82    LEGSRGSGKTALSATVGIDSDFPYVKIVSAESMIGLHESTKCAQIIKVFEDAYKSPLSVI
RAN07    LEGSRGSGKTALSATVGIDSDFPYVKIVSAESMIGLHESTKCAQIIKVFEDAYKSPLSVI
         ************************************************************


Wms82    ILDDIERLLEYVPIGPRFSNLISQTLLVLLKRLPPKGKKLMVIGTTSELDFLESIGFCDT
RAN07    ILDDIERLLEYVPIGPRFSNLISQTLLVLLKRLPPKGKKLMVIGTTSELDFLESIGFCDT
         ************************************************************


Wms82    FSVTYHIPTLNTTDAKKVLEQLNVFTDEDIDSAAEALNDMPIRKLYMLIEMAAQGEHGGS
RAN07    FSVTYHIPTLNTTDAKKVLEQLNVFTDEDIDSAAEALNDMPIRKLYMLIEMAAQGEHGGS
         ************************************************************


Wms82    AEAIFSGKEKISIAHFYDCLQDVVRL
RAN07    AEAIFSGKEKISIAHFYDCLQDVVRL
         **************************
```
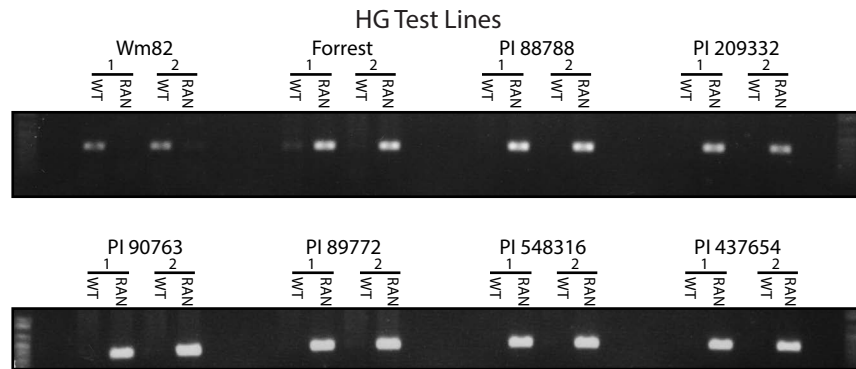
B

HG Test Lines

C

Gene	Young leaf	Flower	One cm pod	Pod shell 10DAF	Pod shell 14DAF	Seed 10DAF	Seed 14DAF	Seed 21DAF	Seed 25DAF	Seed 28DAF	Seed 35DAF	Seed 42DAF	Root	Nodule
Glyma.07G195900	5	8	8	9	9	3	4	6	5	2	4	2	11	7
Glyma.13G180100	6	6	8	6	5	3	4	3	2	1	3	1	12	7

Normalized RNA-seq reads from Wm82 (soybase.org)

Fig. S2. The *NSF*~RAN07~ allele is present within all examined *Rhg1* HG-Type test lines. (*A*) NSF$_{RAN07}$ amino acid alignment with NSF$_{Ch07}$ of soybean reference genome Williams 82 (Wm82). N-domain amino acid polymorphisms unique to NSF$_{RAN07}$ shown red. Corresponding residues of Wm82 encoded NSF$_{Ch07}$ (wild-type) shown boldface. (*B*) Agarose gel showing PCR amplicons generated with *NSF*$_{RAN07}$ (RAN) or *NSF*$_{Ch07}$ WT (WT) allele specific primers on the HG-Type soybeans or soybean Wm82. (*C*) Wm82 normalized RNA-seq reads for both NSF$_{Ch07}$ and NSF$_{Ch13}$ across soybean tissues. RNA-seq data from Severin *et al* (1) and this RNA-seq atlas data is publically available at Soybase.org. DAF: days after fertilization.
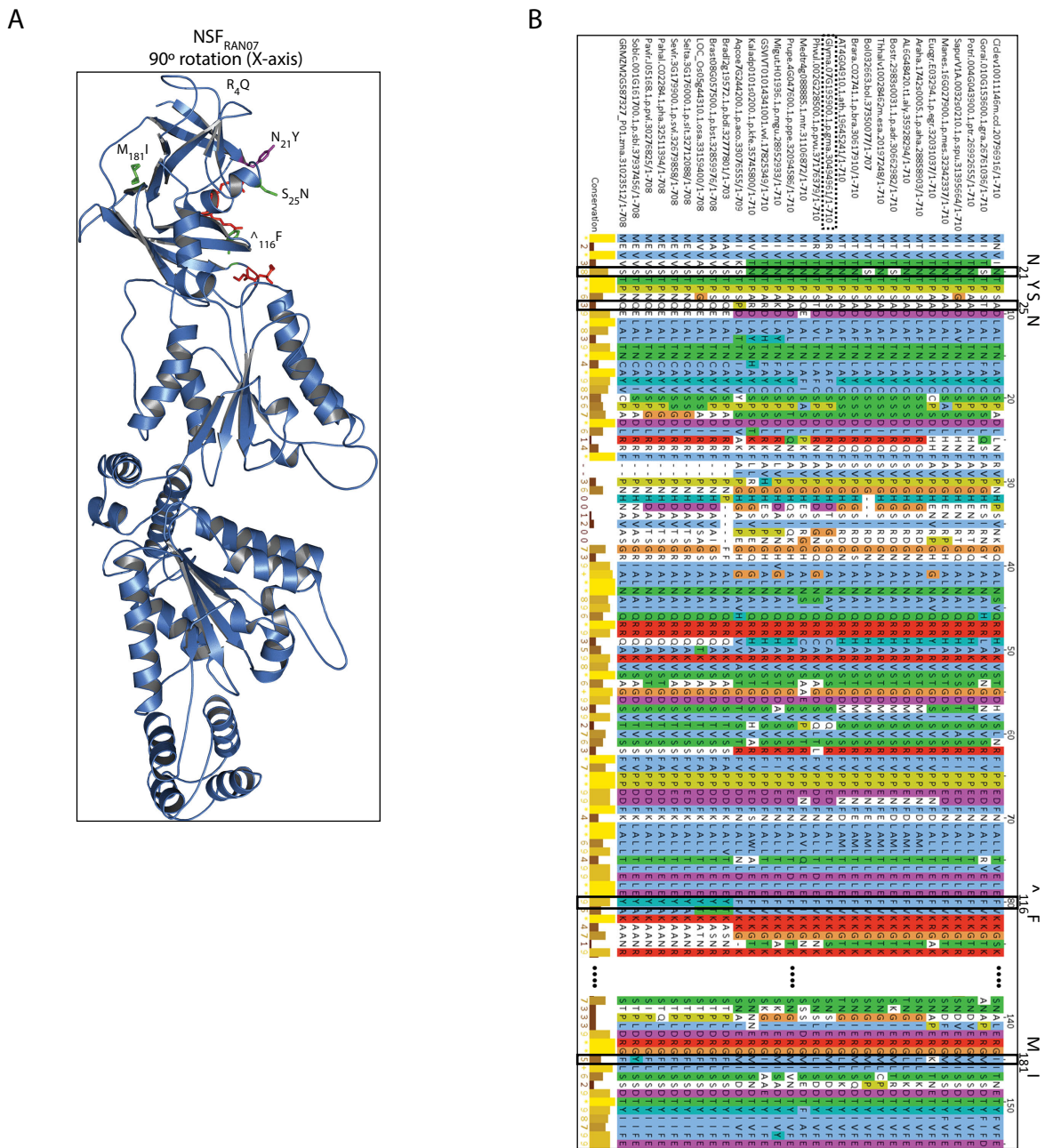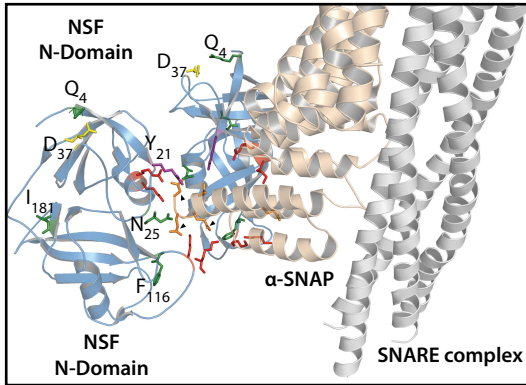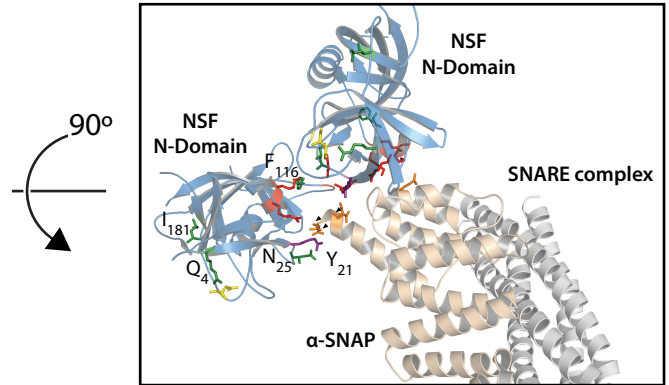
Fig. S3. (*A*) NSF_{RAN07} modeled to NSF_{CHO} cryo-EM structure as in Fig. 2A, but rotated 90° on X-axis relative to Fig. 2*B*. NSF residue patches implicated in α-SNAP binding colored red and labeled I, II or III, respectively. (*B*) Alignment of NSF N-domain using available plant NSF amino acid sequences from Phytozome.org (2). Alignment generated with Jalview starting at a conserved methionine residue corresponding to NSF_{RAN07} methione 17. Residues polymorphic in NSF_{RAN07} are outlined with a box with the corresponding NSF_{RAN07} polymorphism/position labeled above. "⋯" indicates a gap of residues not polymorphic in NSF_{RAN07}.
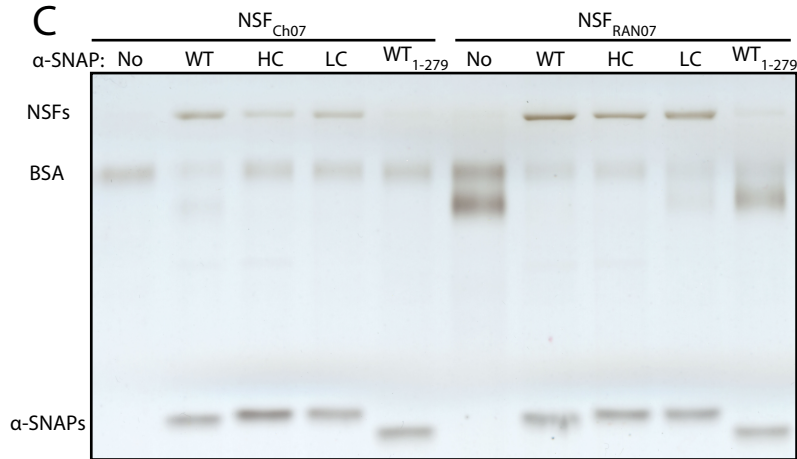
A

NSF
N-Domain

Q₄
D₃₇   Q₄
                       D₃₇
        Y₂₁
I₁₈₁
                N₂₅
                              α-SNAP
        F₁₁₆

NSF
N-Domain   SNARE complex

B

90°

NSF
N-Domain

NSF
N-Domain

F₁₁₆   SNARE complex

I₁₈₁
        N₂₅   Y₂₁
Q₄
        α-SNAP

C

	NSF_Ch07					NSF_RAN07			
α-SNAP:	No	WT	HC	LC	WT₁₋₂₇₉	No	WT	HC	LC	WT₁₋₂₇₉

NSFs

BSA

α-SNAPs

D

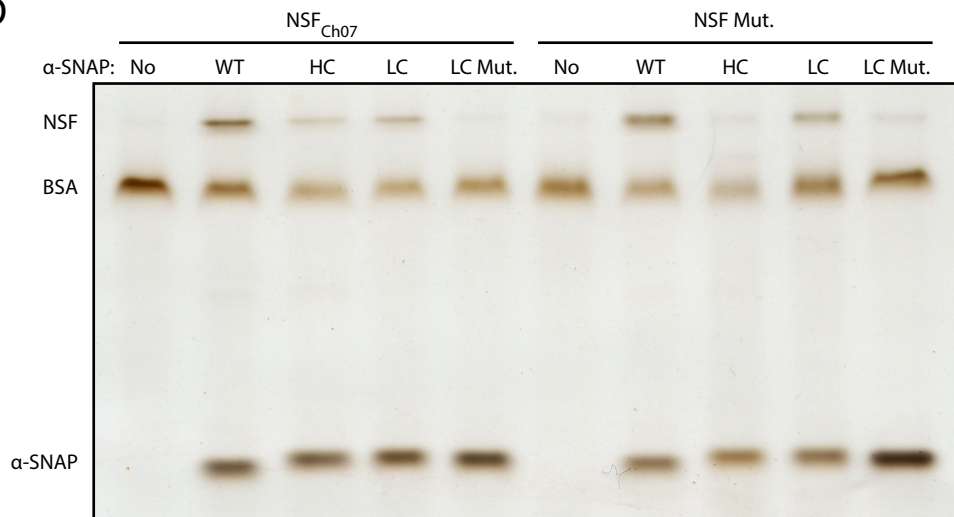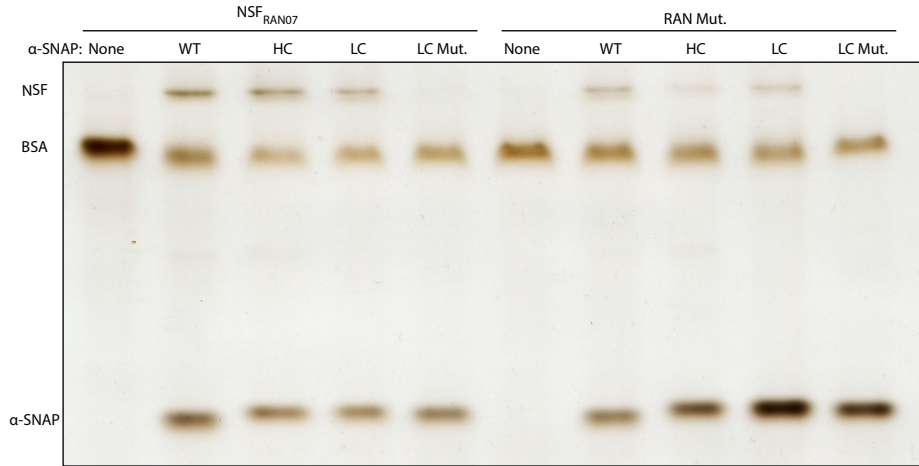|	NSF_Ch07					NSF Mut.				
α-SNAP:	No	WT	HC	LC	LC Mut.	No	WT	HC	LC	LC Mut.

NSF

BSA

α-SNAP

Fig. S4. NSF_{RAN07} polymorphisms are at the α-SNAP binding interface. NSF_{RAN07} and NSF_{Ch07} binding with α-SNAP is dependent on α-SNAP C-terminal polymorphisms, and two NSF_{RAN07} polymorphisms enhance binding by *Rhg1* resistance type α-SNAPs. (*A*) Like Fig. 2*C*, cryo-EM structure of mammalian 20S supercomplex showing SNARE bundle (white), one α-SNAP (yellow) and two NSF N-domains (light blue). Conserved NSF N-domain patches (R_{10}; RK_{67-68}; $KK_{104-105}$) shown red, α-SNAP C-terminal contacts ($D_{217}DEED_{290-293}$) shown orange and α-SNAP residues corresponding to *Rhg1* polymorphisms indicated by black arrows, NSF_{RAN07} polymorphisms (R_4Q, $S_{25}N$, $^\wedge_{116}F$, $M_{181}I$, ^=insertion) colored green, except polymorphisms $N_{21}Y$ colored in purple. NSF_{RAN07} polymorphism R_4Q positions near an acidic residue D_{37} (shown yellow). (*B*) Same as A, but rotated 90° on Y-axis. (*C*) Same as Fig. 2*D*, except recombinant NSF_{Ch07} or NSF_{RAN07} bound *in vitro* by no-α-SNAP (*D,E*). Silver-stained SDS-PAGE showing amount of NSF_{Ch07}, NSF_{RAN07}, or mutants of either ("NSF Mut.", "RAN Mut.") bound to constant amount of *Rhg1* α-SNAPs, including α-SNAP$_{Rhg1}$LC 286$_{AAAA}$289 ("LC Mut."), which has alanine substitutions at the *Rhg1* polymorphisms . NSF Mut. is $NSF_{Ch07}N_{21}A$ $F_{115}A$; RAN Mut. is NSF_{RAN07} $Y_{21}N$ $F_{116}^\wedge$. WT, HC or LC refers to α-SNAP$_{Rhg1}$WT, α-SNAP$_{Rhg1}$HC or α-SNAP$_{Rhg1}$LC, while "None" is a no α-SNAP negative binding control. Entirely independent replicate binding experiments were performed as in C, D and E with similar results.
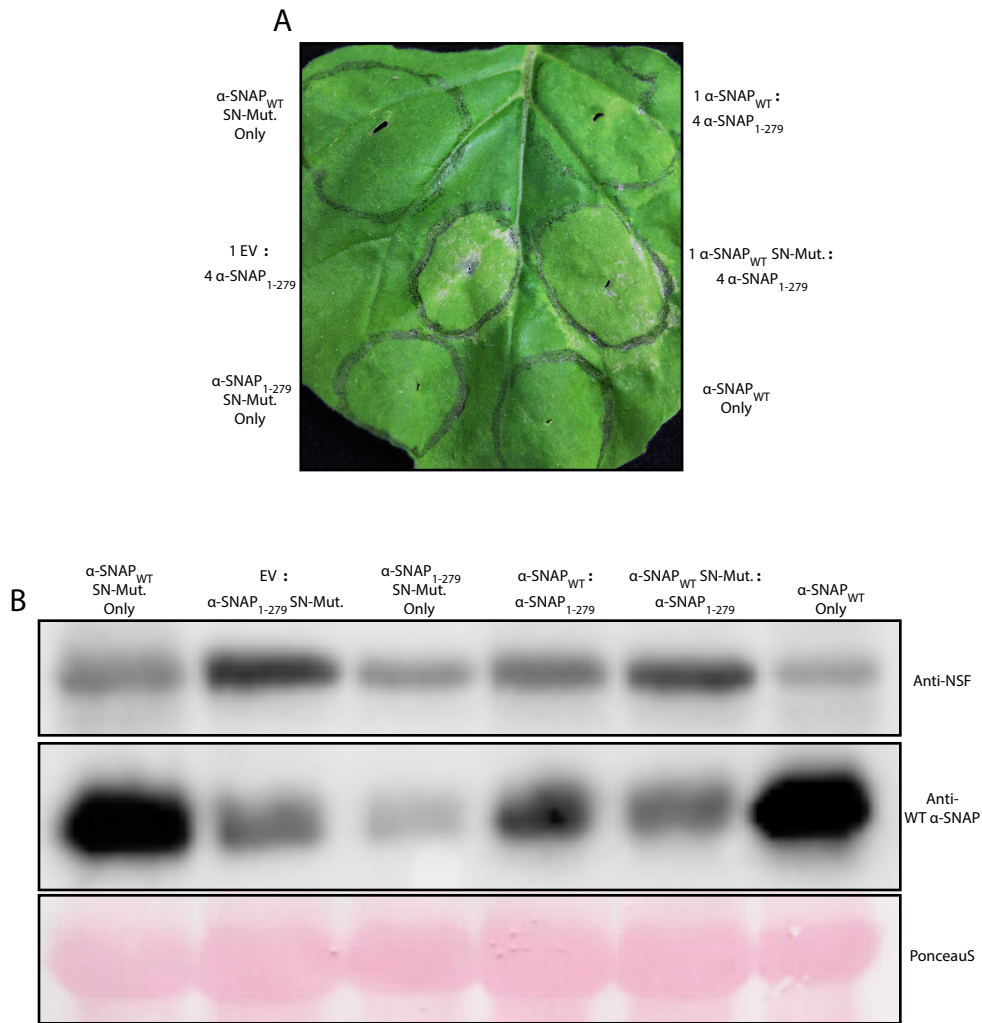
A

α-SNAP$_{WT}$ SN-Mut. Only

1 α-SNAP$_{WT}$: 4 α-SNAP$_{1-279}$

1 EV : 4 α-SNAP$_{1-279}$

1 α-SNAP$_{WT}$ SN-Mut. : 4 α-SNAP$_{1-279}$

α-SNAP$_{1-279}$ SN-Mut. Only

α-SNAP$_{WT}$ Only

B

| α-SNAP$_{WT}$ SN-Mut. Only | EV : α-SNAP$_{1-279}$ SN-Mut. | α-SNAP$_{1-279}$ SN-Mut. Only | α-SNAP$_{WT}$: α-SNAP$_{1-279}$ | α-SNAP$_{WT}$ SN-Mut. : α-SNAP$_{1-279}$ | α-SNAP$_{WT}$ Only | |

Anti-NSF

Anti-WT α-SNAP

PonceauS

Fig. S5. Soybean WT α-SNAPs mutated at residues known in other α-SNAPs to promote SNARE-bundle interactions are deficient in rescuing the *N. benthamiana* cell death induced by toxic α-SNAP types, and α-SNAP$_{1-279}$ (which lacks the final 10 C-terminal residues and induces *N. benthamiana* cell death) becomes unable to cause cell death when mutated at the same SNARE-bundle interaction residues . (*A*) Representative *N. benthamiana* leaf infiltrated with individual or mixed Agrobacterium cultures expressing the indicated α-SNAP constructs. The α-SNAP SNARE-bundle interaction mutant ("SN-Mut.") is K$_{193}$E R$_{230}$E, as described in Zhao *et al* (3). α-SNAP$_{WT}$ is the WT (SCN-susceptible Williams 82) chromosome 18 α-SNAP$_{Rhg1}$WT. (*B*) Immunoblot of total WT α-SNAP or NSF proteins in *N. benthamiana* leaves expressing only the indicated solo proteins, or construct mixes. PonceauS staining indicates similar levels of total protein loading.
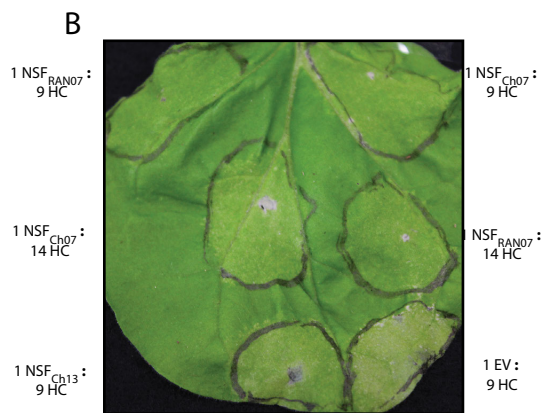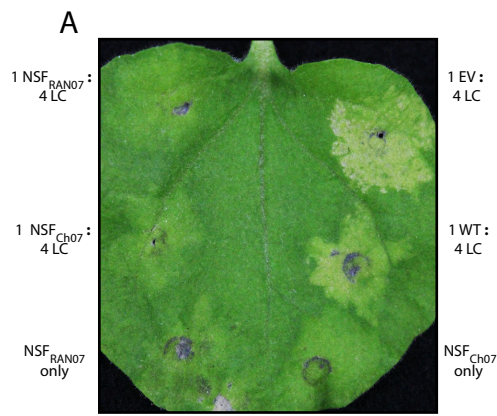
A

1 NSF$_{RAN07}$:
4 LC

1 EV:
4 LC

1 NSF$_{Ch07}$:
4 LC

1 WT:
4 LC

NSF$_{RAN07}$
only

NSF$_{Ch07}$
only

B

1 NSF$_{RAN07}$:
9 HC

1 NSF$_{Ch07}$:
9 HC

1 NSF$_{Ch07}$:
14 HC

NSF$_{RAN07}$:
14 HC

1 NSF$_{Ch13}$:
9 HC

1 EV:
9 HC

Fig. S6. Coexpression of soybean NSFs reduces cell-death symptoms caused by *Rhg1* resistance α-SNAPs; NSF$_{RAN07}$ gives strongest protection. (*A*) *N. benthamiana* leaves ~6 days post agro-infiltration with 1:4 mixed cultures of NSF$_{Ch07}$ or NSF$_{RAN07}$ or α-SNAP$_{Rhg1}$WT or empty vector to α-SNAP$_{Rhg1}$LC (four parts *Agrobacterium* delivering α-SNAP$_{Rhg1}$LC to one part *Agrobacterium* delivering a soybean NSF, or α-SNAP$_{Rhg1}$WT or empty vector control). (*B*) Like Fig. 3*A*, but using α-SNAP$_{Rhg1}$HC instead of α-SNAP$_{Rhg1}$LC in the corresponding mixture cultures of NSF$_{Ch07}$ or NSF$_{RAN07}$ or empty vector.
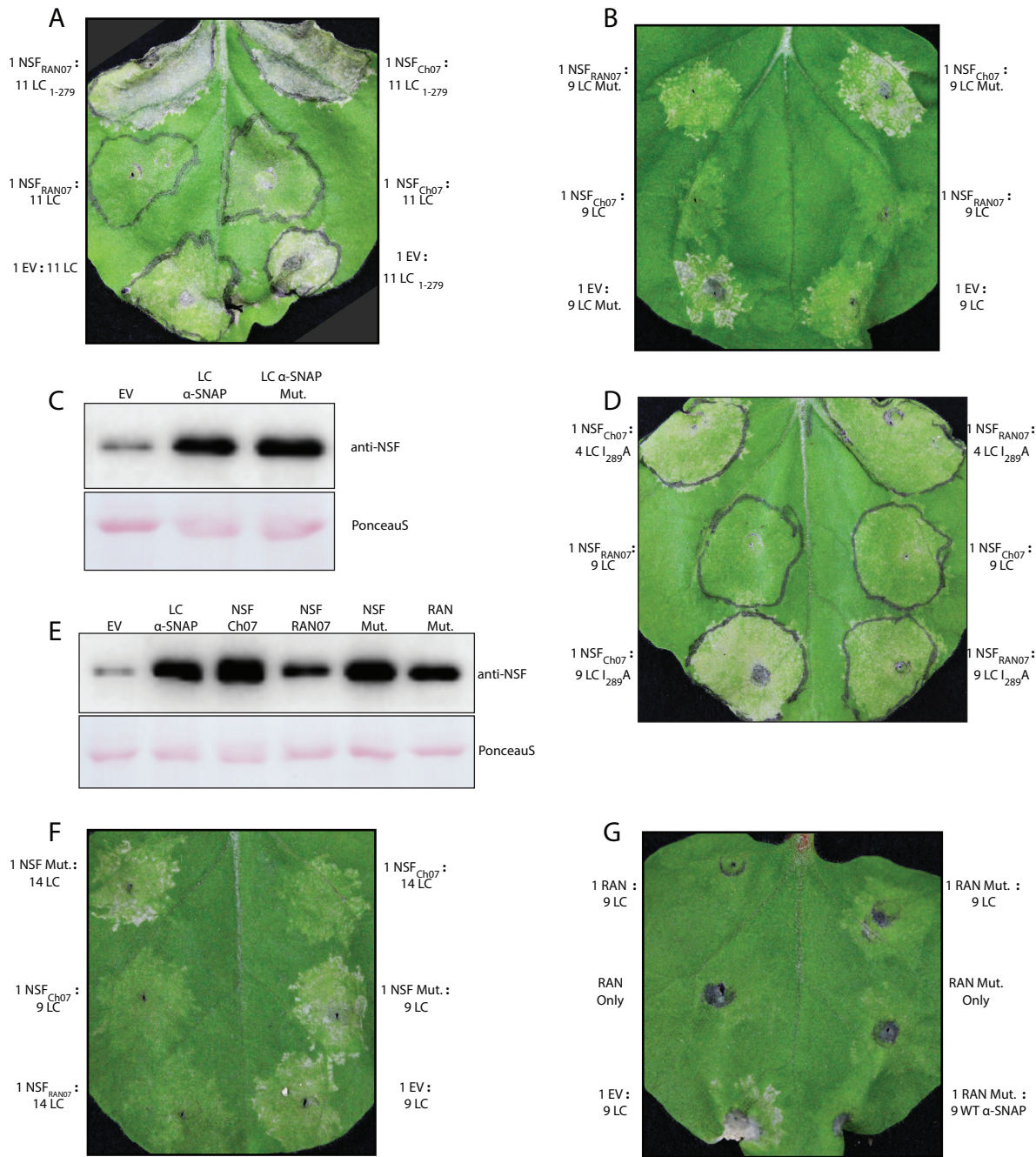
```
Ch07    MASRFGLSSSSSSSASSMRVTNTPASDLALTNLAFCSPSDLRNFAVPGHNNLYLAAVADSF
Nben    MAGRFG-----SGASTMIVTNTPAKDLAYTNCAYCSPADLRNFLVPGSK-LAYGLIADAF
              *.**:* ******.*** ** *:***:***** *** : *   . :**:*

Ch07    VLSLSAHDTIGSGQIALNAVQRRCAKVSSGDSVQVSRFVPPEDFNLALLTLELEFVKKGS
Nben    VLTLAAHDGIPNGHLGLNAIQRRYAKVSTGDTISVNRFVPPDDFNLALLTIDLEFVKKGT
              **:*:*** * .*::.***:*** ****:**::.*.*****:*********:.*******:

Ch07    KSEQIDAVLLAKQLRKRFMNQVMTVGQKVLFEYHGNNYSFTVSNAAVEGQEKSNSLERGM
Nben    RDEQVDAVSLANQVRKKFANQIMSTGQKVTFEYHGNSYIFTVNQATVEGQEKSN-IERGM
              :.**:*** **:*:**:* **:*:.**** ******.* ***.:*:******** :****

Ch07    ISDDTYIVFETSRDSGIKIVNQREGATSNIFKQKEFNLQSLGIGGLSAEFADIFRRAFAS
Nben    ISADTYIIFEAANSSGIKIVNQREAASSSIFRQKEFNLQSLGIGGLSAEFADIFRRAFAS
              ** ****:**::..********** .*:*.**:***********************

Ch07    RVFPPHVTSKLGIKHVKGMLLYGPPGTGKTLMARQIGKILNGKEPKIVNGPEVLSKFVGE
Nben    RVFPPHVTSKLGIKHVKGMLLYGPPGTGKTLMARQIGKMLNGKEPKIVNGPEVLSKFVGE
              **************************************:*********************

Ch07    TEKNVRDLFADAEQDQRTRGDESDLHVIIFDEIDAICKSRGSTRDGTGVHDSIVNQLLTK
Nben    TEKNVRDLFADAEQDQRTKGDQSELHVIIFDEIDAICKSRGSTRDGTGVHDSIVNQLLTK
              ******************.**:*.:*********************************

Ch07    IDGVESLNNVLLIGMTNRKDMLDEALLRPGRLEVQVEISLPDENGRLQILQIHTNKMKEN
Nben    IDGVESLNNVLLIGMTNRKDLLDEALMRPGRLEVQVEISLPDENGRLQILQIHTNQMKEN
              ********************:*****:**********************:****

Ch07    SFLAADVNLQELAARTKNYSGAELEGVVKSAVSYALNRQLSLEDLTKPVEEENIKVTMDD
Nben    SFLSPDVNLQELAARTKNYSGAELEGVVKSAVSFALNRQLSMDDLTKPVDEESIKVTMDD
              ***:.**********************************:*******:::*******:**.*******

Ch07    FLNALHEVTSAFGASTDDLERCRLHGMVECGDRHKHIYQRAMLLVEQVKVSKGSPLVTCL
Nben    FLHALGEVRPAFGASTDDLERCRLNGIVDCGERHQHIYRRTMLLAEQVKVSRGSPLITCL
              **:** ** .*************:*:*:**:**:***:*:*** .******.****:***

Ch07    LEGSRGSGKTALSATVGIDSDFPYVKIVSAESMIGLHESTKCAQIIKVFEDAYKSPLSVI
Nben    LEGPSGSGKTAMAATVGIESDFPYVKIISAETMIGLSESSKCAQIVKVFEDAYKSPLSIV
              ***. ******::*****:*****:***:**** **:*****:*************::

Ch07    ILDDIERLLEYVPIGPRFSNLISQTLLVLLKRLPPKGKKLMVIGTTSELDFLESIGFCDT
Nben    VLDGIERLLEYVAIGPRFSNLISQTLLVLLKRLPPKGKKILVIGTTSEAGFLDSVGLCDA
              :**.******** .***********************::.****** .**:*:*:**:

Ch07    FSVTYHIPTLNTTDAKKVLEQLNVFTDEDIDSAAEALNDMPIRKLYMLIEMAAQGEHGGS
Nben    FSVTYHVPTLKTEDAKKVLQQLNVFSNDDVDSAAEALNDMPIKKLYMVVEMAAQGEHGGT
              ******:***:* ******:*****:::*:*************:****:.:*********:

Ch07    AEAIFSGKEKISIAHFYDCLQDVVRL
Nben    AEAIYSGKEKIQISHFYDCLQDIARY
              ****:******.*:*********:.*
```

Fig. S7. Amino acid sequence of NSF clone from *N. benthamiana* aligned with NSF$_{Ch07}$ from soybean Williams82. NSF N-domain residues conserved in α-SNAP binding are shown red in boldface.

A

1 NSF$_{RAN07}$:
11 LC$_{1-279}$

1 NSF$_{Ch07}$:
11 LC$_{1-279}$

1 NSF$_{RAN07}$:
11 LC

1 NSF$_{Ch07}$:
11 LC

1 EV : 11 LC

1 EV :
11 LC$_{1-279}$

B

1 NSF$_{RAN07}$:
9 LC Mut.

1 NSF$_{Ch07}$:
9 LC Mut.

1 NSF$_{Ch07}$:
9 LC

1 NSF$_{RAN07}$:
9 LC

1 EV :
9 LC Mut.

1 EV :
9 LC

C

EV	LC α-SNAP	LC α-SNAP Mut.	
			anti-NSF
			PonceauS

D

1 NSF$_{Ch07}$:
4 LC I$_{289}$A

1 NSF$_{RAN07}$:
4 LC I$_{289}$A

1 NSF$_{RAN07}$:
9 LC

1 NSF$_{Ch07}$:
9 LC

1 NSF$_{Ch07}$:
9 LC I$_{289}$A

1 NSF$_{RAN07}$:
9 LC I$_{289}$A

E

EV	LC α-SNAP	NSF Ch07	NSF RAN07	NSF Mut.	RAN Mut.	
						anti-NSF
						PonceauS

F

1 NSF Mut. :
14 LC

1 NSF$_{Ch07}$:
14 LC

1 NSF$_{Ch07}$:
9 LC

1 NSF Mut. :
9 LC

1 NSF$_{RAN07}$:
14 LC

1 EV :
9 LC

G

1 RAN :
9 LC

1 RAN Mut. :
9 LC

RAN
Only

RAN Mut.
Only

1 EV :
9 LC

1 RAN Mut. :
9 WT α-SNAP

Fig. S8. Coexpression of soybean NSFs reduces cell-death symptoms caused by *Rhg1* resistance-type α-SNAPs; changes to the polymorphic α-SNAP$_{Rhg1}$LC C-terminus reduce cell death protection, as do site-directed mutations at implicated NSF residues. (*A*) Like Fig. 3A, *N. benthamiana* leaves ~6 days post agro-infiltration but with an 11:1 mixed culture of α-SNAP$_{Rhg1}$LC or α-SNAP$_{Rhg1}$LC$_{1-279}$ (lacks the final C-terminal residues) co-expressed with NSF$_{Ch07}$ or NSF$_{RAN07}$ or empty vector. (*B*) A 9:1 mixed culture of α-SNAP$_{Rhg1}$LC or α-SNAP$_{Rhg1}$LC 286$_{AAAA}$289 ("LC Mut.") co-expressed with NSF$_{Ch07}$ or NSF$_{RAN07}$ or empty vector. (*C*) Immunoblot of total NSF protein expression from *N.benthamiana* leaves expressing empty vector (EV), α-SNAP$_{Rhg1}$LC or α-SNAP$_{Rhg1}$LC 286$_{AAAA}$289 (LC Mut.). PonceauS staining indicates similar loading of total proteins. (*D*) Like A and B, but 4:1 or 9:1 mixed cultures of α-SNAP$_{Rhg1}$LC or α-SNAP$_{Rhg1}$LC-I$_{289}$A co-expressed with NSF$_{Ch07}$ or NSF$_{RAN07}$. (*E*) Immunoblot of total NSF protein expression from *N.benthamiana* leaf tissues expressing empty vector (EV), α-SNAP$_{Rhg1}$LC, or the indicated NSF constructs. PonceauS staining indicates similar loading of total proteins. (*F*) A 9:1 mixed culture of α-SNAP$_{Rhg1}$LC co-expressed with either EV, NSF$_{Ch07}$,or NSF$_{RAN07}$ or NSF$_{Ch07}$ N$_{21}$A F$_{115}$A (NSF Mut.). (*G*) A 9:1 mixed culture of α-SNAP$_{Rhg1}$LC co-expressed with either EV, NSF$_{RAN07}$ or NSF$_{RAN07}$ Y$_{21}$N F$_{116}$^ (RAN Mut.).
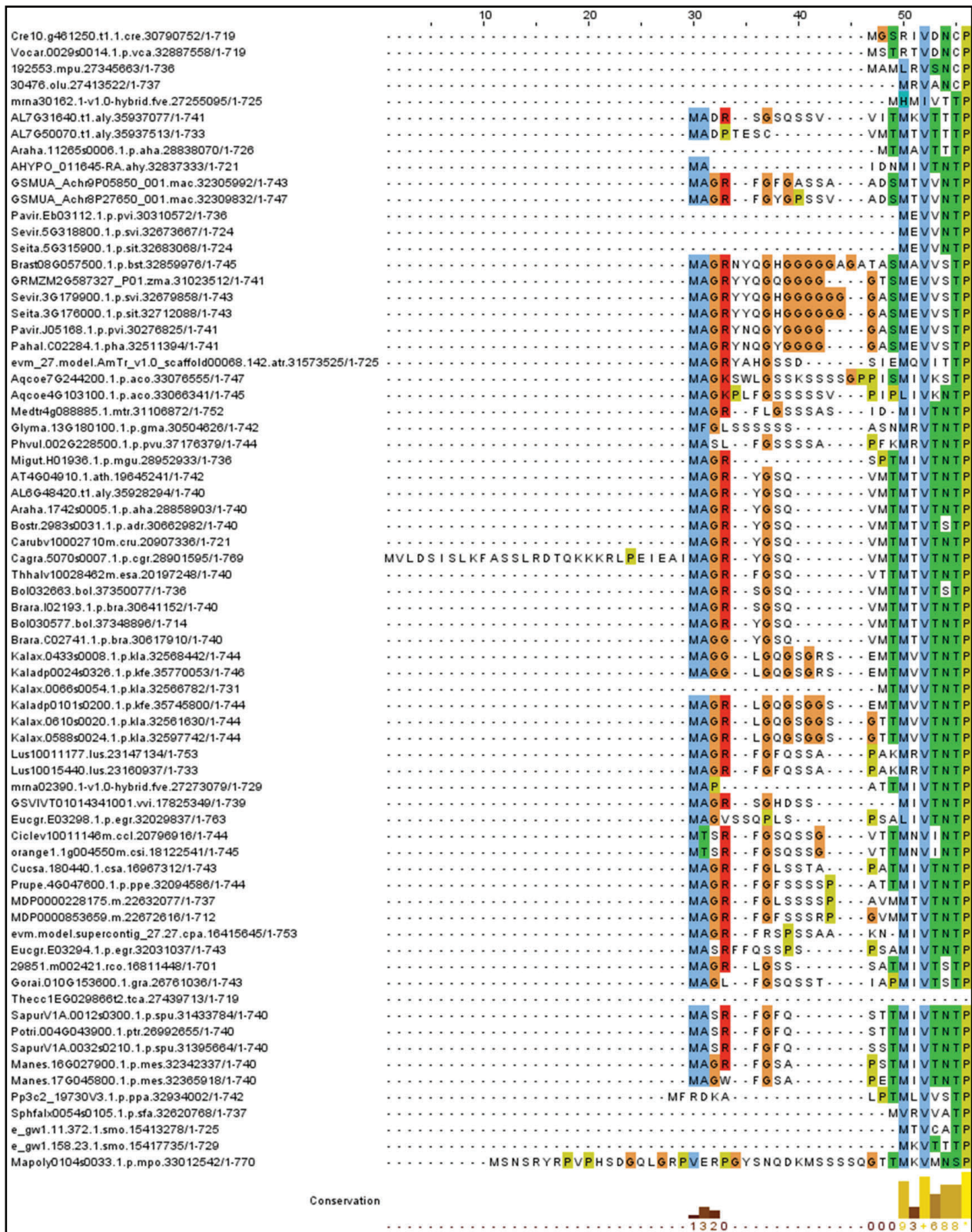
Fig. S9. Alignment of available plant NSF sequences starting at predicted residue 1. General consensus of R_4 is observed across a majority of plant species. Alignment generated with Jalview using all available angiosperm NSF sequences from Phytozome.org (2). Only NSF sequences of residue lengths comparable to known NSF sequences (~700-800 residues) were used for the alignment.
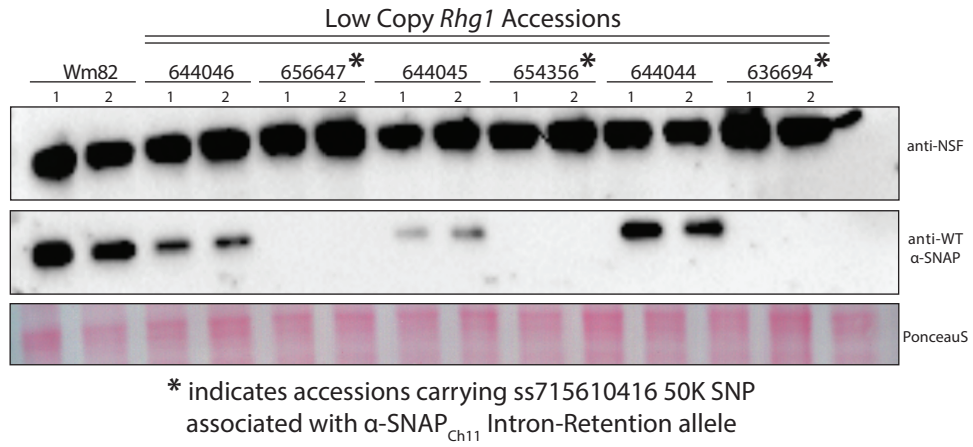
Low Copy *Rhg1* Accessions

Wm82 644046 656647 * 644045 654356 * 644044 636694 *
1 2   1 2   1 2   1 2   1 2   1 2   1 2

anti-NSF

anti-WT
α-SNAP

PonceauS

* indicates accessions carrying ss715610416 50K SNP
associated with α-SNAP$_{Ch11}$ Intron-Retention allele

Fig. S10. Low copy *Rhg1* varieties carrying ss715610416 SNP associated with Ch11 α-SNAP-Intron Retention (IR) allele have lower WT α-SNAP abundance. (*A*) Immunoblots for WT α-SNAP and NSF of Wm 82 or of low copy *Rhg1* accessions PI 644046, PI 656647, PI 644045 PI 654356, PI 636694, which are positive (***)** or negative for the ss715610416 SNP associated with the *α-SNAP$_{Ch11}$-IR* allele. PonceauS indicates relative total protein abundance per each line.

Soybean Accession	Rhg1 Copy #	SCN Resistant	Rhg1 WT α-SNAP	Rhg1 HC α-SNAP	Rhg1 LC α-SNAP	Ch11 WT α-SNAP	Ch11 I.R. α-SNAP	Ch07 NSF WT	Ch07 NSF RAN07
Williams 82	1	—	✓	—	—	✓	—	✓	—
PI 548402	3	✓	—	—	✓	—	✓	—	✓
PI 90763	3	✓	—	—	✓	—	✓	—	✓
PI 437654	3	✓	—	—	✓	—	✓	—	✓
PI 89772	3	✓	—	—	✓	—	✓	—	✓
PI 548316	7	✓	✓	✓	—	—	✓	—	✓
PI 88788	9	✓	✓	✓	—	✓	—	—	✓
PI 209332	10	✓	✓	✓	—	✓	—	—	✓

Table S1. *Rhg1* copy number and relevant α-SNAP and NSF alleles present in Wm82 or in the SCN-resistance pheno-typing "HG-Type" soybeans. *Rhg1* haplotypes color coded: blue (WT, Single Copy *Rhg1*), red (LC, Low Copy *Rhg1*) or orange (HC, High Copy *Rhg1*). A grey checkmark indicates presence of certain trait or allele and a black minus sign denotes absence. WT is Wild-type allele, I.R. is intron-retention allele of Ch11 α-SNAP (*Glyma.11G234500*) and RAN07 is *Rhg1* associated NSF on Ch07 allele of *Glyma.07G195900*.

Line	*Rhg1* Haplotype	NSF$_{Ch07}$	NSF$_{Ch13}$	α-SNAP$_{Ch11}$
Peking	*Rhg1* $_{LC}$	*Rhg1* Assoc. Allele	WT (Wm82-type)	Intron Retention
90763	*Rhg1* $_{LC}$	*Rhg1* Assoc. Allele	V555I	Intron Retention
437654	*Rhg1* $_{LC}$	*Rhg1* Assoc. Allele	WT (Wm82-type)	Intron Retention
209332	*Rhg1* $_{HC}$	*Rhg1* Assoc. Allele	V555I	WT
89772	*Rhg1* $_{LC}$	*Rhg1* Assoc. Allele	V555I	Intron Retention
548316	*Rhg1* $_{HC}$	*Rhg1* Assoc. Allele	V555I	Intron Retention
Prohio	Susceptible	WT (Wm82-type)	V555I	WT
NE3001	Susceptible	WT (Wm82-type)	Y260F	WT
4J105-34	*Rhg1* $_{HC}$	*Rhg1* Assoc. Allele	V555I, L738F	WT
CL0J095-46	*Rhg1* $_{HC}$	*Rhg1* Assoc. Allele	V555I	WT
IA3023	Susceptible	WT (Wm82-type)	V555I	WT
LD00-3309	*Rhg1* $_{HC}$	*Rhg1* Assoc. Allele	WT (Wm82-type)	WT
LD02-4485	*Rhg1* $_{HC}$	*Rhg1* Assoc. Allele	WT (Wm82-type)	WT
LG05-4292	*Rhg1* $_{HC}$	*Rhg1* Assoc. Allele	WT (Wm82-type)	Intron Retention
LD01-5907	*Rhg1* $_{LC}$	*Rhg1* Assoc. Allele	V555I	Intron Retention
LD02-9050	*Rhg1* $_{HC}$	*Rhg1* Assoc. Allele	V555I	WT
Magellan	Susceptible	WT (Wm82-type)	WT (Wm82-type)	WT
Maverick	*Rhg1* $_{HC}$	*Rhg1* Assoc. Allele	V555I	Intron Retention

Table S2. α-SNAP or NSF alleles identified by whole genome sequencing of HG-Type test lines and *Rhg1*-containing NAM parents. All multi-copy *Rhg1* haplotype lines contained a unique *Glyma.07g195900* NSF$_{Ch07}$ allele (*Rhg1* associated NSF on chromosome 07; NSF$_{RAN07}$). An α-SNAP$_{Ch11}$ intron-retention allele was present among some, but not all multi-copy*Rhg1* haplotypes. A *Glyma.13G180100* (NSF$_{Ch13}$) allele was also detected in some but not all *Rhg1* containing HG-Type and NAM lines, but was also found in some SCN-susceptible varieties.

Soybean Line	ss715597431				ss715597413			ss715597410	
	Glyma.07g195900 NSF (RAN07)	Glyma.07g195800 Rubber Elongation Factor	Glyma.07g195700 DNA Mismatch Repair MutS2	Glyma.07g195600 No annotated domains	Glyma.07g195500 TFII H Polypetide 4	Glyma.07g195400 E3 Ubiquitin Ligase	Glyma.07g195300 Asparagine Synthase	Glyma.07g195200 Uncharacterized Conserved Protein	Glyma.07g195100 LRR Containing Protein
Maverick	R_4Q, $N_{21}Y$, $S_{25}N$, $\wedge_{116}F$, $M_{183}I$	K_3N, $F_{137}S$	$H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $Q_{618}K$	WT	WT	WT	WT	WT	WT
CLOJ095-4-6	R_4Q, $N_{21}Y$, $S_{25}N$, $\wedge_{116}F$, $M_{183}I$	K_3N, $L_{42}R$, $F_{137}S$	$T_{21}A$, $K_{23}R$, $G_{109}C$, $H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $Q_{618}K$	WT	WT	WT	WT	WT	WT
LD00-3309	R_4Q, $N_{21}Y$, $S_{25}N$, $\wedge_{116}F$, $M_{183}I$	K_3N, $L_{42}R$, $F_{137}S$	$T_{21}A$, $K_{23}R$, $G_{109}C$, $M_{406}T$, $G_{516}C$, $Q_{618}K$	WT	WT	WT	WT	WT	WT
IA3023	WT	$L_{42}R$, $F_{137}S$	$D_{364}N$, $M_{406}T$, $Y_{576}F$	WT	WT	WT	$E_{49}G$	$D_{60}A$, $S_{64}P$	WT
Magellan	WT	$L_{42}R$, $F_{137}S$	$D_{364}N$, $M_{406}T$	WT	WT	WT	$E_{49}G$	$D_{60}S$, $S_{64}P$	WT
PI 548316	R_4Q, $N_{21}Y$, $S_{25}N$, $\wedge_{116}F$, $M_{183}I$	K_3N, $F_{137}S$	$H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $Q_{618}K$	WT	WT	WT	WT	WT	WT
PI 89772	R_4Q, $N_{21}Y$, $S_{25}N$, $\wedge_{116}F$, $M_{183}I$	K_3N, $F_{137}S$	$T_{21}A$, $K_{23}R$, $G_{109}C$, $H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $Q_{618}K$	WT	WT	WT	WT	WT	WT
PI 209332	R_4Q, $N_{21}Y$, $S_{25}N$, $\wedge_{116}F$, $M_{183}I$	K_3N, $F_{137}S$	$T_{21}A$, $K_{23}R$, $G_{109}C$, $H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $Q_{618}K$	WT	WT	WT	WT	WT	WT
PI 437654	R_4Q, $N_{21}Y$, $S_{25}N$, $\wedge_{116}F$, $M_{183}I$	K_3N, $L_{42}R$, $F_{137}S$	$T_{21}A$, $K_{23}R$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $Q_{618}K$	WT	WT	WT	WT	WT	WT
PI 90763	R_4Q, $N_{21}Y$, $S_{25}N$, $\wedge_{116}F$, $M_{183}I$	K_3N, $F_{137}S$	$T_{21}A$, $K_{23}R$, $G_{109}C$, $H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $Q_{618}K$	WT	WT	WT	WT	WT	WT
PI 548402	R_4Q, $N_{21}Y$, $S_{25}N$, $\wedge_{116}F$, $M_{183}I$	K_3N, $F_{137}S$	$T_{21}A$, $K_{23}R$, $G_{109}C$, $H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $Q_{618}K$	WT	WT	WT	WT	WT	WT

Table S3. Amino acid polymorphisms of genes within the chromosome 07 interval co-segregating with *Rhg1*. Polymorphisms are relative to predicted residues of the Williams82 (SCN-susceptible) reference genome. The predicted amino acid sequence of most candidate loci matches Wm82. Among candidate loci with residue substitutions, only the *NSF RAN07* allele has identical amino acid changes consistent across all *Rhg1*-containing germplasm. SCN-susceptible soybean varieties highlighted in green.

Diverse Parent	RR / RR (Ch07/Ch18)	RR / SS (Ch07/Ch18)	SS / RR (Ch07/Ch18)	SS / SS (Ch07/Ch18)
4J105-3-4	41	41	2	31
CL0J095-4-6	35	45	0	37
LD00-3309	38	45	1	27
LD01-5907	32	32	1	42
LD02-4485	37	50	1	28
LD02-9050	43	31	2	34
Maverick	31	34	0	41
LG05-4292	44	41	1	30
Totals	301	319	8*	270

R refers to allele from *Rhg1* resistant parent.

S refers to allele from SCN-susceptible parent

Genotype order: first allele is chr 7 (RAN07 interval) and second is chr 18 (*Rhg1* interval)

*All 8 re-examined RILs that inherited $Rhg1_{HC}$ or $Rhg1_{LC}$ also inherited the NSF_{RAN07} ^116 F and M_{181}I mutations meaning that all 309 RILs that carried the resistance associated *Rhg1* also carried NSF_{RAN07}

Table S4. NSF_{RAN07} co-segregates with *Rhg1* in all *Rhg1*-containing F2:5 offspring derived from $Rhg1^+$ X *rhg1⁻* parental crosses. Segregating lines and 6K SoySNP genotyping were developed and performed in the soybean NAM (nested association mapping) project of Song *et al.*, 2017 (4).

Supporting Information

Bayless *et al*. "An atypical NSF (N-ethylmaleimide Sensitive Factor) enables the viability of nematode-resistant *Rhg1* soybeans"

SI Materials & Methods

Recombinant Protein Production

Vectors encoding recombinant α-SNAP$_{Rhg1}$HC, α-SNAP$_{Rhg1}$LC, α-SNAP$_{Rhg1}$WT, α-SNAP$_{Rhg1}$WT$_{1-279}$ and the WT alleles of NSF *Glyma.07G195900* (NSF$_{Ch07}$) and *Glyma.13G180100* (NSF$_{Ch13}$) were generated in Bayless *et al.*, 2016. The open reading frames (ORFs) encoding the soybean NSFRAN07 allele of *Glyma.07G195900* or *N.benthamiana* NSF were cloned into the expression vector pRham N-His-SUMO according to manufacturer instructions (Lucigen). Recombinant α-SNAP and NSF proteins were also produced and purified as in Bayless *et al*. 2016. All expression constructs were chemically transformed into the expression strain "E. cloni 10G" (Lucigen), grown to OD$_{600}$ ~0.60-0.70, and induced with 0.2% L-Rhamnose (Sigma) for either 8 hr at 37°C or overnight at 28°C. Soluble, native recombinant His-SUMO-α-SNAPs or His-SUMO-NSF proteins were purified with PerfectPro Ni-NTA resin (5 PRIME), with similar procedures as described in (5) and eluted with imidazole, though no subsequent gel filtration steps were performed. Following the elution of the His-SUMO–fusion proteins, overnight dialysis was performed at 4 °C in 20 mM Tris (pH 8.0), 150 mM NaCl, 10% (vol/vol) glycerol, and 1.5 mM Tris (2-carboxyethyl)-phosphine. The His-SUMO affinity/solubility tags were cleaved from α-SNAP or NSF using 1 or 2 units of SUMO Express protease (Lucigen) and separated by rebinding of the tag with Ni-NTA resin and collecting the recombinant protein from the flow-through. Recombinant protein purity was assessed by Coomassie blue staining and quantified via a spectrophotometer.

In vitro NSF-α-SNAP Binding Assays

In vitro NSF binding assays were performed essentially as described in (5, 6). Briefly, 20 μg of each respective recombinant α-SNAP protein was added to the bottom of a 1.5-mL polypropylene tube and incubated at 25°C for 20 min. Unbound α-SNAP proteins were then washed by adding α-SNAP wash buffer [25 mM Tris, pH 7.4, 50 mM KCl, 1 mM DTT, 0.1 mg/mL bovine serum albumin (BSA)]. After removal of wash buffer, 20 μg of recombinant NSF (1 μg/μL in NSF binding buffer), was then immediately added and incubated on ice for 10 min. The solution was then removed and samples were immediately washed 2X with NBB to remove any unbound NSF. Samples were then boiled in 1X SDS loading buffer and separated on a 10% Bis-Tris SDS-PAGE, and silver-stained using the ProteoSilver Kit (Sigma-Aldrich), according to the manufacturer directions. The percentage of NSF bound by α-SNAP was then calculated using densitometric analysis with ImageJ.

Antibody Production and Validation

Affinity-purified polyclonal rabbit antibodies raised against α-SNAP$_{Rhg1}$HC, α-SNAP$_{Rhg1}$LC and wild-type α-SNAPs were previously generated and validated using recombinant proteins in

Bayless 2016. The epitopes for these custom antibodies are the final six or seven C-terminal α-SNAP residues: "EEDDLT," "EQHEAIT," or "EEYEVIT" for wild-type, high-, or low-copy α-SNAPs, respectively. For NSF, a synthetic peptide, "ETEKNVRDLFADAEQDQRTRGDESD," corresponding to residues 300 to 324 of the *Glyma.07G195900* encoded protein was used. This same epitope is also present in *Glyma.13G180100* encoded NSF$_{Ch13}$ and this NSF antibody was also previously shown to be cross-reactive with the *N.benthamiana*-encoded NSF.

Immunobloting

Tissue preparation and immunoblots were performed essentially as in (5, 7). Soybean roots or *N. benthamiana* leaf tissues were flash-frozen in $N_2(L)$, massed, and homogenized in a PowerLyzer 24 (MO BIO) for three cycles of 15 seconds, with flash-freezing in-between each cycle. Protein extraction buffer [50 mM Tris·HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 0.2% Triton X-100, 10% (vol/vol) glycerol, 1/100 Sigma protease inhibitor cocktail] was then added at a 3:1 volume to mass ratio and samples were centrifuged and stored on ice. In noted experiments, Bradford assays were performed on each sample, and equal OD amounts of total protein were loaded in each sample lane for SDS/PAGE. Immunoblots for either *Rhg1* α-SNAP were incubated overnight at 4 °C in 5% (wt/vol) nonfat dry milk TBS-T (50 mM Tris, 150 mM NaCl, 0.05% Tween 20) at 1:1,000. NSF immunoblots were performed similarly, except incubations were for 1 h at room temperature. Secondary horseradish peroxidase-conjugated goat anti-rabbit IgG was added at 1:10,000 and incubated for 1 h at room temperature on a platform shaker, followed by four washes with TBS-T. Chemiluminescence detection was performed with SuperSignal West Pico or Dura chemiluminescent substrate (Thermo Scientific) and developed using a ChemiDoc MP chemiluminescent imager (Bio-Rad).

Transgenic Soybean Root Generation

Binary expression constructs were transformed into *Agrobacterium rhizogenes* strain, "Arqua1". Transgenic soybean roots were produced from cotyledons of the noted genetic background as described in (8).

Transient Agrobacterium Expression in *Nicotiana benthamiana*. *Agrobacterium tumefaciens* strain GV3101 was used for transient protein expression of all constructs via syringe-infiltration at OD$_{600}$ 0.60 for NSF constructs or OD$_{600}$ 0.80 for α-SNAP constructs into young leaves of ~4-wk-old *N. benthamiana* plants. GV3101 cultures were grown overnight at 28°C in 25 μg/mL kanamycin and rifampicin and induced for ~3.5 h in 10 mM Mes (pH 5.60), 10 mM MgCl$_2$, and 100μM acetosyringone prior to leaf infiltration. *N. benthamiana* plants were grown in a Percival set at 25 °C with a photoperiod of 16 h light at 100 μE·m−2·s−1 and 8 h dark. For α-SNAP complementation assays, GV3101 cultures were well-mixed with one volume of an empty vector control, or of the respective NSF construct immediately before co-infiltration. NSF$_{RAN07}$ or the *N. benthamiana* NSF were PCR amplified from a root cDNA library of *Rhg1*$_{LC}$ variety, "Forrest" or a *N. benthamiana* leaf cDNA library using KAPA HiFi polymerase, respectively. Expression cassettes for NSF$_{N.benthamiana}$, NSF$_{Ch13,}$ NSF$_{Ch07}$ and NSF$_{RAN07}$ ORFs were directly assembled into a pBluescript vector containing the previously described soybean ubiquitin (GmUbi) promoter and NOS terminator using Gibson assembly (8). The NSF expression cassettes were then digested with the restriction enzymes NotI-SalI and ligated with T4 DNA

ligase into the previously described binary vector, pSM101-linker, which was cut with PspOMI-SalI restriction sites. The ORF encoding the α-SNAP$_{Ch11}$ Intron-Retention (IR) allele was amplified with Kapa HiFi from a root cDNA library of $Rhg1_{LC}$ variety "Forrest" while the ORF encoding WT α-SNAP$_{Ch11}$ was previously generated in (5). The ORFs encoding either α-SNAP$_{Ch11}$ and α-SNAP$_{Ch11}$IR were Gibson assembled into a pBluescript vector containing a GmUbi-N-HA tag and NOS terminator, cut with PstI-XbaI and ligated into the binary vector, pSM101, cut with the same restriction pair. An 11.14 kb native genomic region encoding α-SNAP$_{Rhg1}$WT was amplified with Kapa HiFi from a previously described fosmid subclone (Fosmid 19) with AvrII-SbfI restriction ends, and then digested and ligated into the binary vector, pSM101, cut with XbaI-PstI. A 6.85 kb native locus encoding α-SNAP$_{Ch11}$ was amplified from gDNA of Williams82 in two separate fragments (3.25 kb and 3.60 kb fragments) and Gibson assembled into the binary pSM101 vector cut with BamHI-PstI.

Segregating NAM Crosses

Soybean parental crosses and 6K SNP genotyping mapping were developed and performed by (4).

Protein Structure Modeling

NSF$_{RAN07}$, α-SNAP$_{Ch11}$ and α-SNAP$_{Ch11}$IR structural homology models were generated using SWISS-MODEL and the resulting PDB files were analyzed with PyMol (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC). NSF$_{RAN07}$ was modeled to NSF$_{CHO}$ (*Cricetulus griseus*, Chinese hamster ovary) (PDB 3j97.1) cryo-EM structure from Zhao *et al* (Brunger group). NSFs in many plants, including soybean, encode a variable length polyserine/glycine patch, starting at ~residue 6, hence modeling to NSF$_{CHO}$ began at residue 14. Primary amino acid sequence alignment indicated that residues N$_{21}$, RR$_{82-83}$ and KK$_{117-118}$ in the soybean NSF N-domain and residues D$_{208}$, DEED$_{243-246}$ and EEDD$_{284-287}$ in the α-SNAP C-terminus correspond to NSF$_{CHO}$ (R$_{10}$, RK$_{67-68}$, KK$_{104-105}$) and rat α-SNAP (D$_{217}$E$_{249}$EE$_{252-253}$, DEED$_{290-293}$), respectively. 20S supercomplex modeling was also generated using PDB 3j97, with α-SNAPs and SNARE complexes (VAMP-2, Syntaxin-1A, SNAP-25) of *Rattus norvegicus* origin (3). α-SNAP$_{Ch11}$ and α-SNAP$_{Ch11}$IR were modeled to sec17 (yeast α-SNAP) crystal structure 1QQE donated courtesy of Rice *et al* (Brunger group)(9).

Sequence Logo and Alignments

The R$_4$Q NSF amino acid consensus logo was generated using the first 10 NSF amino acids of the model eukaryotic organisms using WebLogo (10). The NSF amino acid sequences of these organisms were retrieved from publicly available sequence data at the National Center for Biotechnology Information (NCBI). Plant NSF sequences were obtained from Phytozome.org and aligned using Jalview (11).

DNA Sequence and SNP Analysis

Whole-genome sequencing data of 12 soybean varieties was obtained from previously published studies (4, 12). Illumina sequencing reads were aligned to the Williams82 reference genome (Wm82.a2.v1) using BWA (version 0.7.12)(13). Reads were initially mapped using the

default settings of the *aln* command with the subsequent pairings performed with the *sampe* command. Alignments were next processed using the program Picard (version 2.9.0) to add read group information (AddOrReplaceReadGroups), mark PCR duplicates (MarkDuplicates, and merge alignments from separate sequencing runs (MergeSamFiles). The processed .bam files were then converted to vcf format using a combination of samtools (version 0.1.19) and bcftools (version 0.1.19). Finally, consensus sequences were generated from these .vcf files using the FastaAlternateReferenceMaker tool within GATK (version 3.7.0)(14).

Oligonucleotide Primers

N. ben NSF Rev	TCAATATCGAGCAATGTCCTGA
N. ben NSF For	ATGGCAGGGAGATTTGGTTCC
N. ben Gmubi For	GATTTATCTGTGATTGTTGACTCGACAGATGGCAGGGAGATTTGGTTCC
N. ben NSF NOS Rev	GAAAGCTGGGTCGAATTCGCCCTTTTCAATATCGAGCAATGTCCTGAAGGC
N. ben pRham For	CACCGCGAACAGATTGGAGGTGCAGGGAGATTTGGTTCC
N. ben pRham Rev	GTGGCGGCCGCTCTATTATCAATATCGAGCAATGTCCTGA
NSF Ch07 UTR For	GCCATTGCTATTGTGGTGCGA
NSF Ch07 UTR Rev	CTATCAGCACAACCAAACAAACACTG
RAN07 Gmubi For	GATTTATCTGTGATTGTTGACTCGACAGATGGCGAGTCAGTTCGGG
RAN07 NOS Rev	AGCTGGGTCGAATTCGCCCTTTTCATAACCTAACAACATCCTGGAGGC
RAN07 pRham For	CTCACCGCGAACAGATTGGAGGTGCGAGTCAGTTCGGGTTATCG
RAN07 pRham Rev	CAGCGGTGGCGGCCGCTCTATTATAACCTAACAACATCCTGGAGGC
RAN07 Detect For	CTACACGCCCGCGAACGAC
NSF07 Detect Rev	CTCACTTGTACGGAATCACCGG
WT NSF Detect For	CAACACGCCCGCGAGCGAC
NSF 13 UTR For	GCCAAGAAACAGAGAAACATAGAGGC
NSF 13 UTR Rev	CTGAACAGTAACAAGCAATGTAGGAATG
NSF 13 NOS Rev	GAAAGCTGGGTCGAATTCGCCCTTTTcATCTAACAACATCCTGGAGGCAATC
NSF 13 Gmubi For	GATTTATCTGTGATTGTTGACTCGACAGATGTTCGGCTTATCGTCTTCGTCTTCC-TC
Ch11 Native 1 For	CTAATCTGGGGACCTGGGTACCCGGGCTCGAACACGTATAAAGGACCTGAGG
Ch11 Native 1 R	GGATCAATATCTCGAAGCTGAATATGGGC
Ch11 Native 2 For	CCCATATTCAGCTTCGAGATATTGATCC
Ch11 Native 2 Rev	GGGCCCACAGATATTAATTAAGACATCTGCAGCATCTCTCTTTATTTCCATG-CACGCG
Ch11 UTR For	CGATCAATCCATCCATCTTCACTTGC
Ch11 UTR Rev	CAAACAATAGGTCCAACCGCCAG
Ch18 Native For	CCTGCAGGGAGCAGTAGGCTTCTTTGGAACTTG
Ch18 Native Rev	GTTTGCCACTTTAGGAACCCTAGG

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