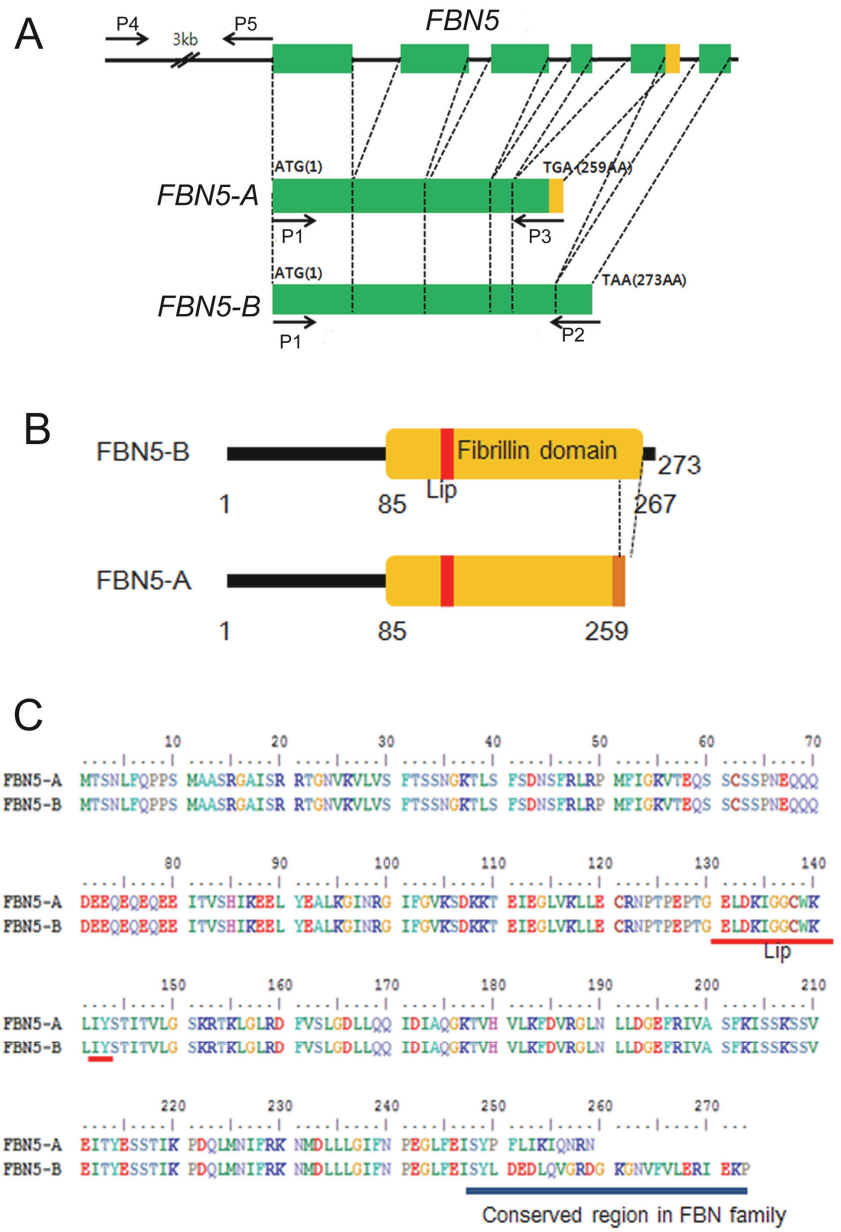
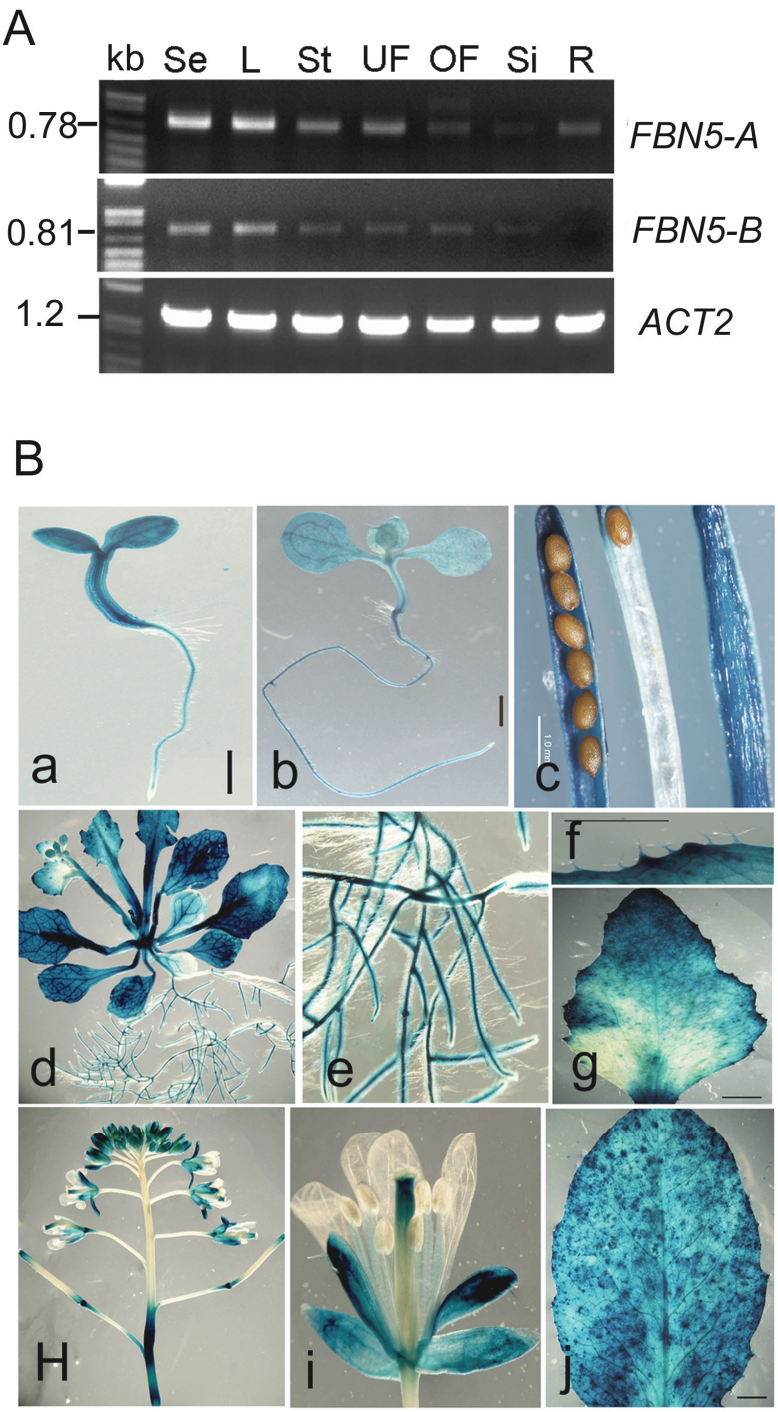


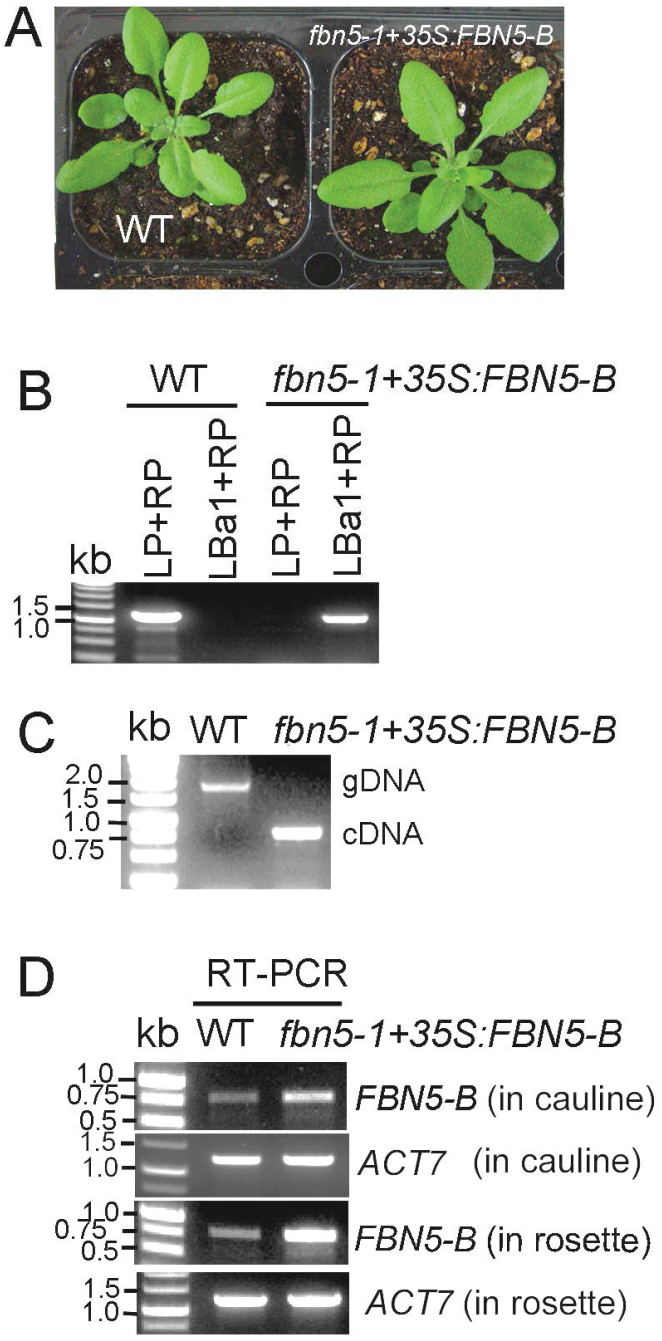
Supplemental Figure 1. Gene Structure of *FBN5*. (A) *FBN5* genomic structure and two alternatively spliced transcripts, *FBN5-A* and *FBN5-B.* (B) Two alternative-splicing products, FBN5-A and FBN5-B. A fibrillin domain was predicted at amino acids 8–267 by the Pfam database (http://pfam.xfam.org/). The numbers indicate the amino acid positions. Possible lipocalin motif 1 (Lip) sequences are shown red. (C) Sequence alignment of FBN5-A and FBN5-B. The N-terminal 249 residues are identical. FBN5-A is 14 amino acids shorter than FBN-5 and contains 10 unique residues at its carboxyl terminus. Predicted Lip motif 1 and the conserved domain near the fibrillin C-terminal residues are shown, according to Singh and McNellis (2011).

REFERENCE

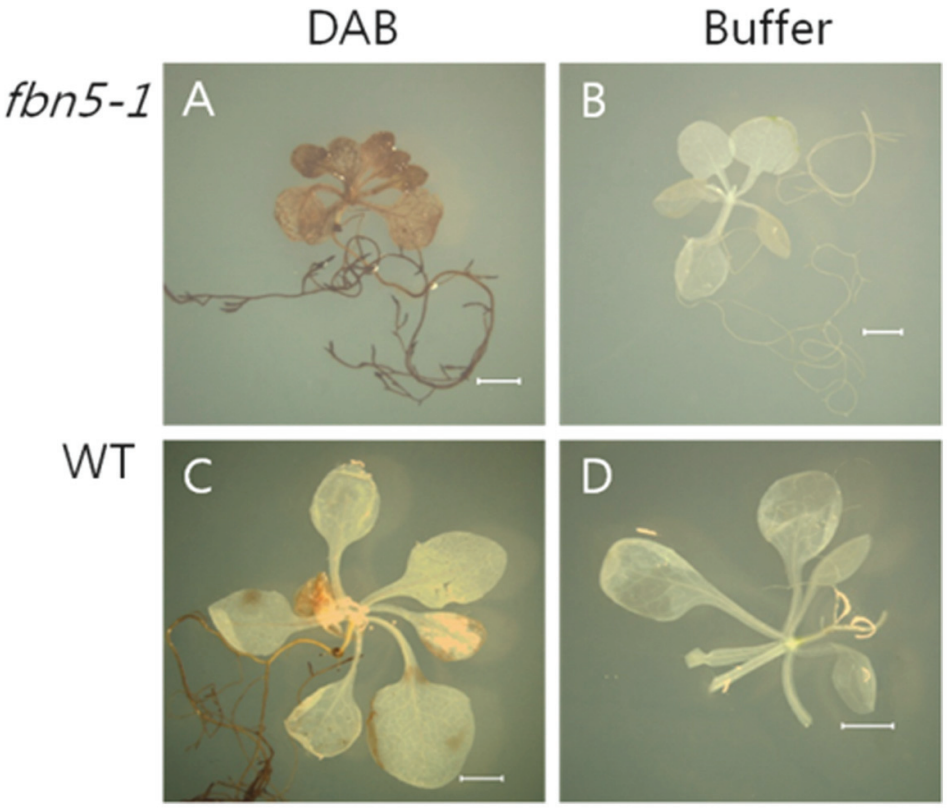
Singh, D.K. and McNellis, T.W. (2011). Fibrillin protein function: the tip of the iceberg? Trends Plant Sci 16: 432-441.



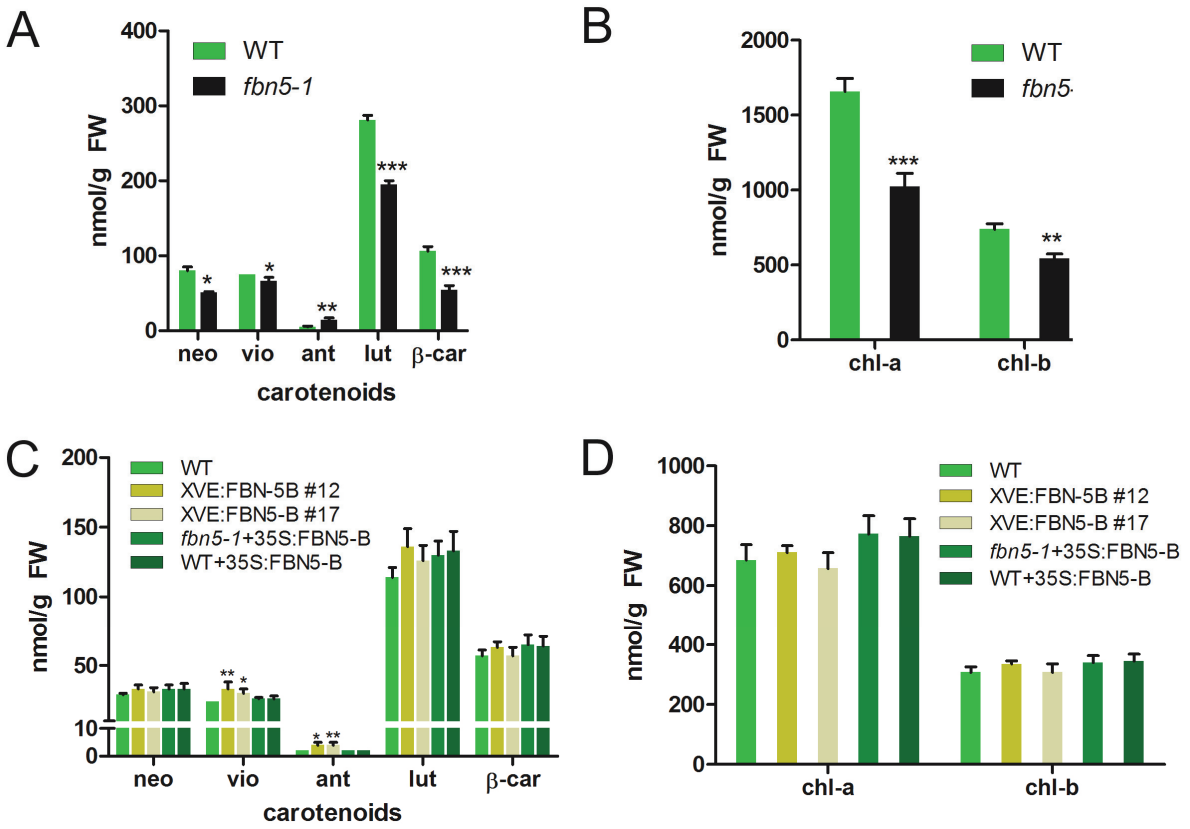
Supplemental Figure 2. Expression Patterns of *FBN5*. (A) RT–PCR analysis of *FBN5* transcripts in various tissues. Se, seedling; L, leaf; St, stem; UF, unopened flower; OF, open flower; Si, silique; and R, root. (B) *FBN5* promoter–GUS expression during the development of transgenic *Arabidopsis*. a, Two-day-old seedling; b, 6-day-old seedling; c, silique of 30-day-old plant; d, rosette of 18-day-old plant; e, root of 18-day-old plant; f, trichome; g, cauline leaf; h, flower cluster; i, flower after opening; and j, rosette leaf.



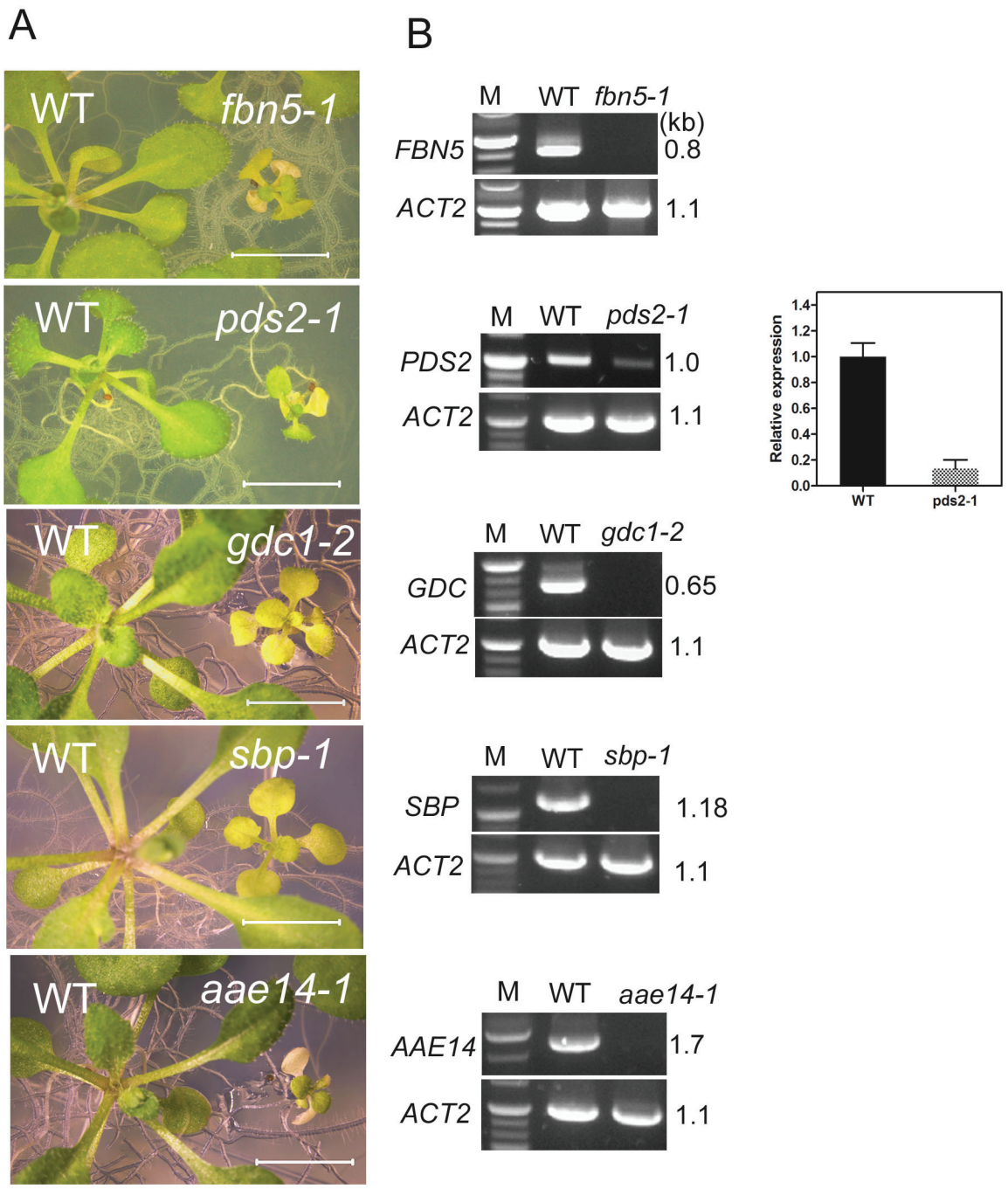
Supplemental Figure 3. Genetic Complementation of Seedling-Lethal Phenotype of the *fbn5-1* Plant with *FBN5-B* cDNA. (A) WT and *fbn5-1+35S-FBN5-B Arabidopsis* plants show similar phenotypes when grown in soil. (B) Genotyping WT and *fbn5-1+35S-FBN5-B* plants with PCR. A 1.2-kb PCR product was amplified from the WT plants but not the *fbn5-1+35S-FBN5-B* plants when the LP + RP primers were used. A 1.0-kb PCR product was detected in the *fbn5-1+35S:FBN5-B* plants but not in the WT when the LBa1 + RP primers were used. (C) 1.7-kb (1,669 bp) and 822-bp PCR products were amplified with P1 + P2 primers from the WT and *fbn5*-1+*35S:FBN5-B* plants, respectively. (D) Expression levels of full-length *FBN5-B* transcripts using the P1 + P2 primers and cDNA from cauline and rosette leaves of WT and *fbn5-1+35S:FBN5-B* plants. *ACT7*, internal control.



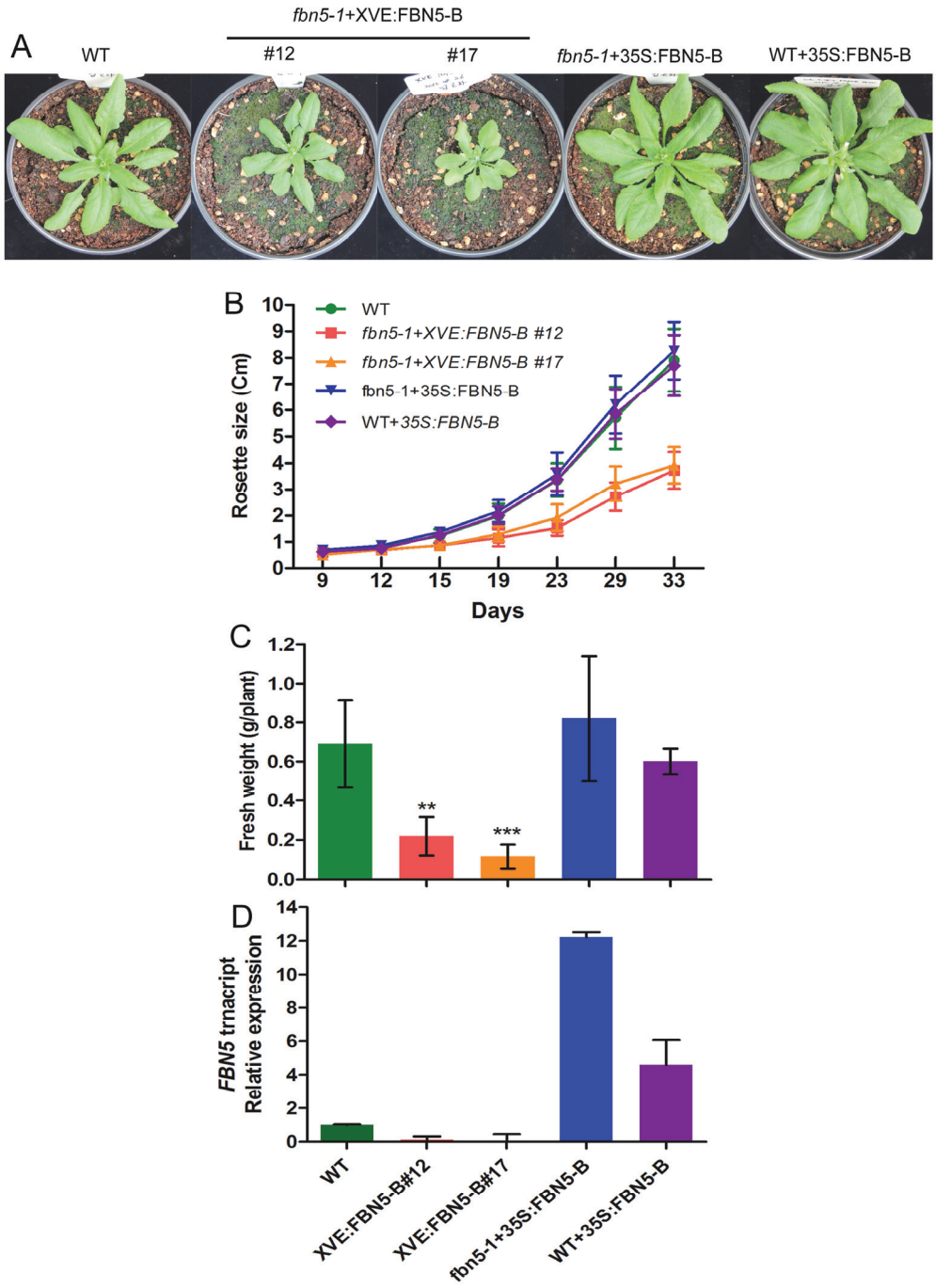
Supplemental Figure 4. H_2O_2 Detection in *fbn5-1* and WT Plants by Staining with DAB (3,3′-diaminobenzidine). The plants were grown on MS medium supplemented with 1% sucrose for 3 weeks, and infiltrated with DAB solution (A, C) or with 10 mM NaH_2PO_4 buffer (B, D) as a control. Bars = 2 mm.



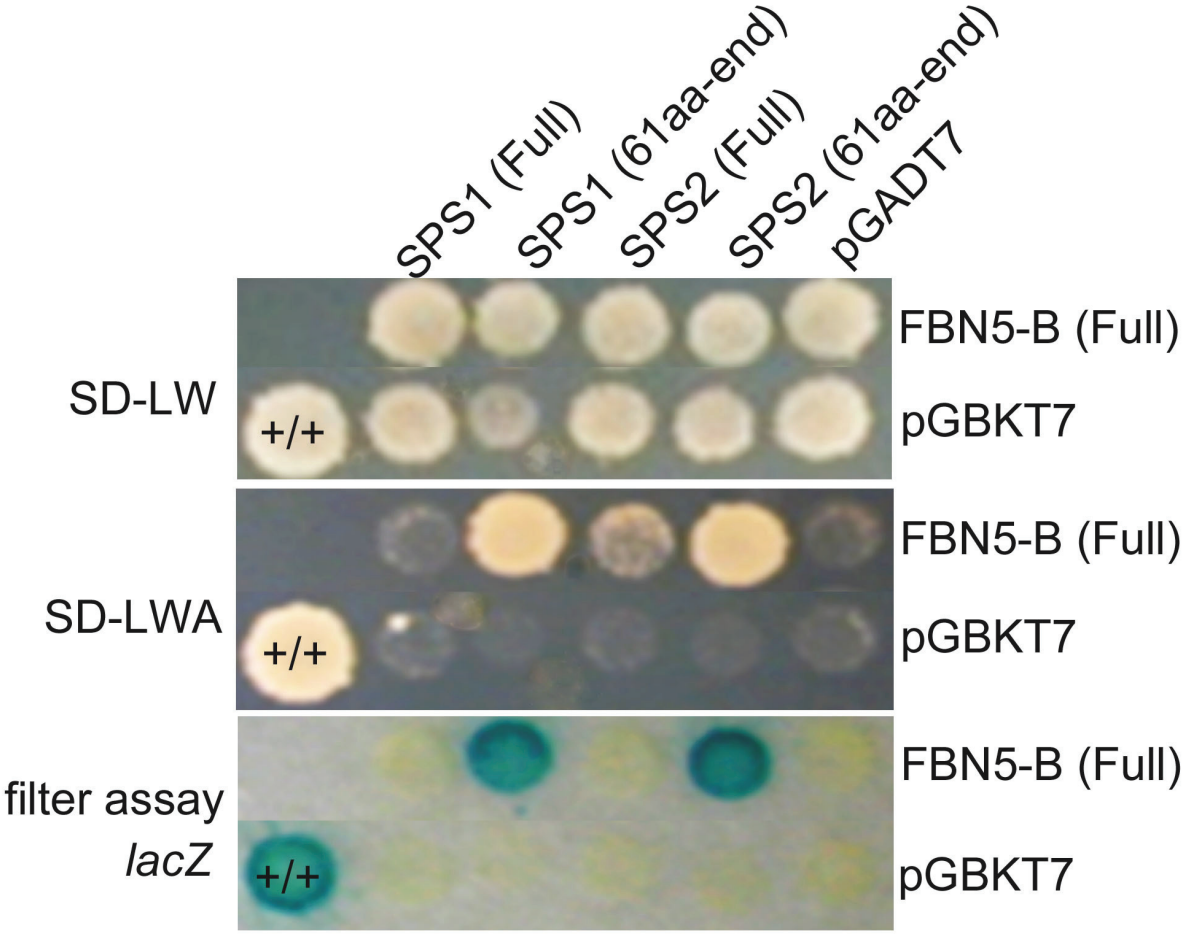
Supplemental Figure 5. Quantification of Carotenoids and Chlorophylls in *Arabidopsis* Leaves with Reversed-phase HPLC. Total lipids were extracted from the leaves of 3-week-old WT and *fbn5-1* homozygous mutant plants grown on MS medium (+1% sucrose) (A, B) and from 6-week-old WT and transgenic plants grown in soil (C, D), and quantified with HPLC. The quantities of individual carotenoids and chlorophylls were determined relative to standards. neo, neoxanthin; vio, violaxanthin; ant, antheraxanthin; lut, lutin; β-car, β-carotene; chl-a, chlorophyll *a*; chl-b, chlorophyll *b*. Asterisks represent the significance of individual components relative to WT, calculated with Student's *t* test; **P* < 0.05, ** *P* < 0.01, and ****P* < 0.001. Data are means ± SD (n = 3–4).



Supplemental Figure 6. Phenotypes of Pale-Green Mutants and WT Plants on 1%-Sucrose-Supplemented MS Medium. (A) Representative photographs of 3-week-old plants (A) and RT–PCR analysis of transcript levels in WT and pale-green mutants (B). All five pale-green mutants were null, except *pds2-1*, which showed a 90% reduction in *PDS2* expression compared with the WT. Numbers are transcript sizes. *fbn5-1*, allele SALK_064597 of At5g09820; *pds2-1*, allele SALK_024357 of At3g11945; *gdc1-2*, allele SALK_151530C of At1g50900; *sbp-1*, allele SALK_090549C of At3g55800; *aae14-1*, allele SALK_060226 of At1g30520. *ACT2*, internal control. Bars = 0.5 cm.



Supplemental Figure 7. Growth and Fresh Weight of *FBN5* Transgenic Plants. (A) Representative photographs of 5-week-old plants. (B) Growth rate was assessed by measuring the rosette sizes of the plants at each time point after seeds were sown (n = 8). (C) Fresh weight of 33-day-old plants (n = 4). Data are means ± SD (n = 3–4). Asterisks represent significance levels of each transgenic line relative to the WT plants, on Student's *t* test: $^{**}P < 0.01$ and $^{***}P < 0.001$ (D) Determination of *FBN5* gene expression in leaves with real-time RT–PCR. The expression levels in *FBN5* of *fbn5* knockdown, complemented, and overexpressed transgenic plants was normalized to that of *ACTIN2* and is shown relative to its expression in WT plants. Data are the means ± SE of three independently prepared pools of plants.



Supplemental Figure 8. Interaction of Preprotein and Mature SPSs with Full-length FBN5-B. A yeast two-hybrid analysis was performed using FBN5-B as the bait and either full-length (preprotein) or mature SPS1 or SPS2 as the prey. Each transformant (PBN204) was dropped onto selective medium lacking Leu and Trp (SD-LW) or selective medium also lacking Ade (SD-LWA). β-Galactosidase activity was tested in each colony on SD-LWA medium. The positive control was yeast transformed with the PTB bait plasmid and PTB prey plasmid (+/+). PTB is a homodimeric protein. The negative control was a cell transformed with the parental bait vector (pGBKT7) and prey vector (pGADT7).

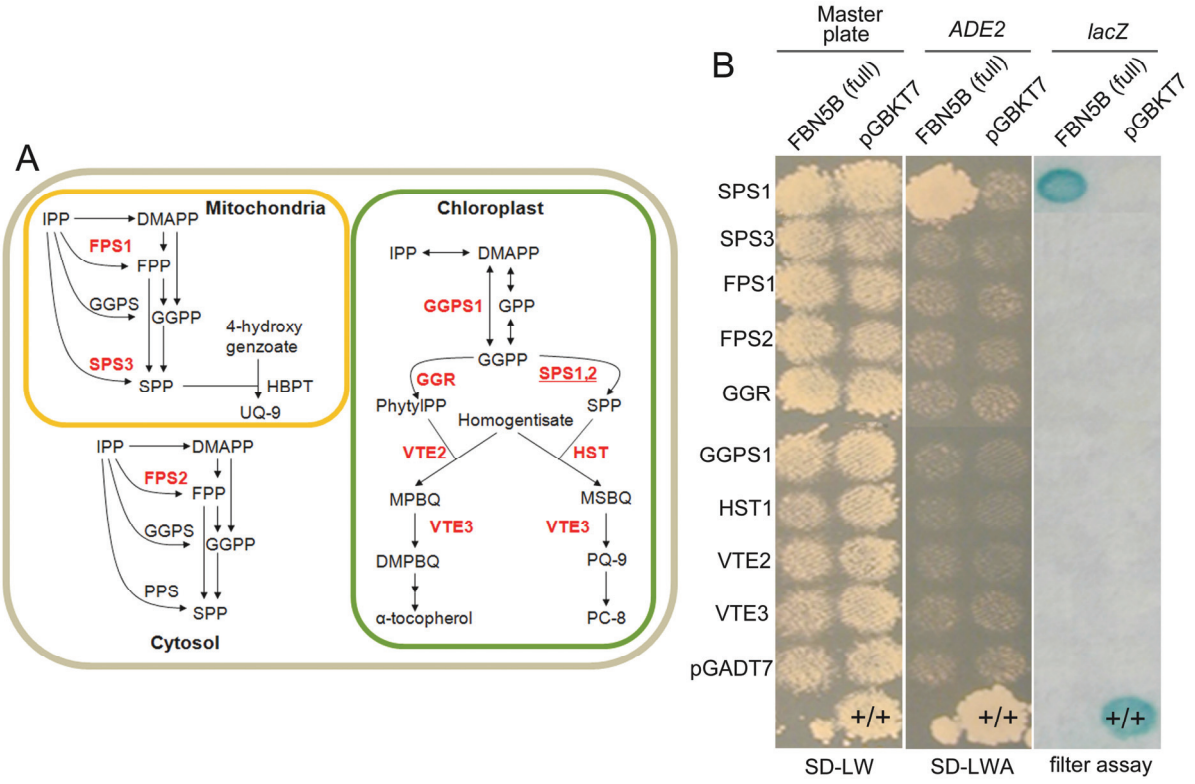
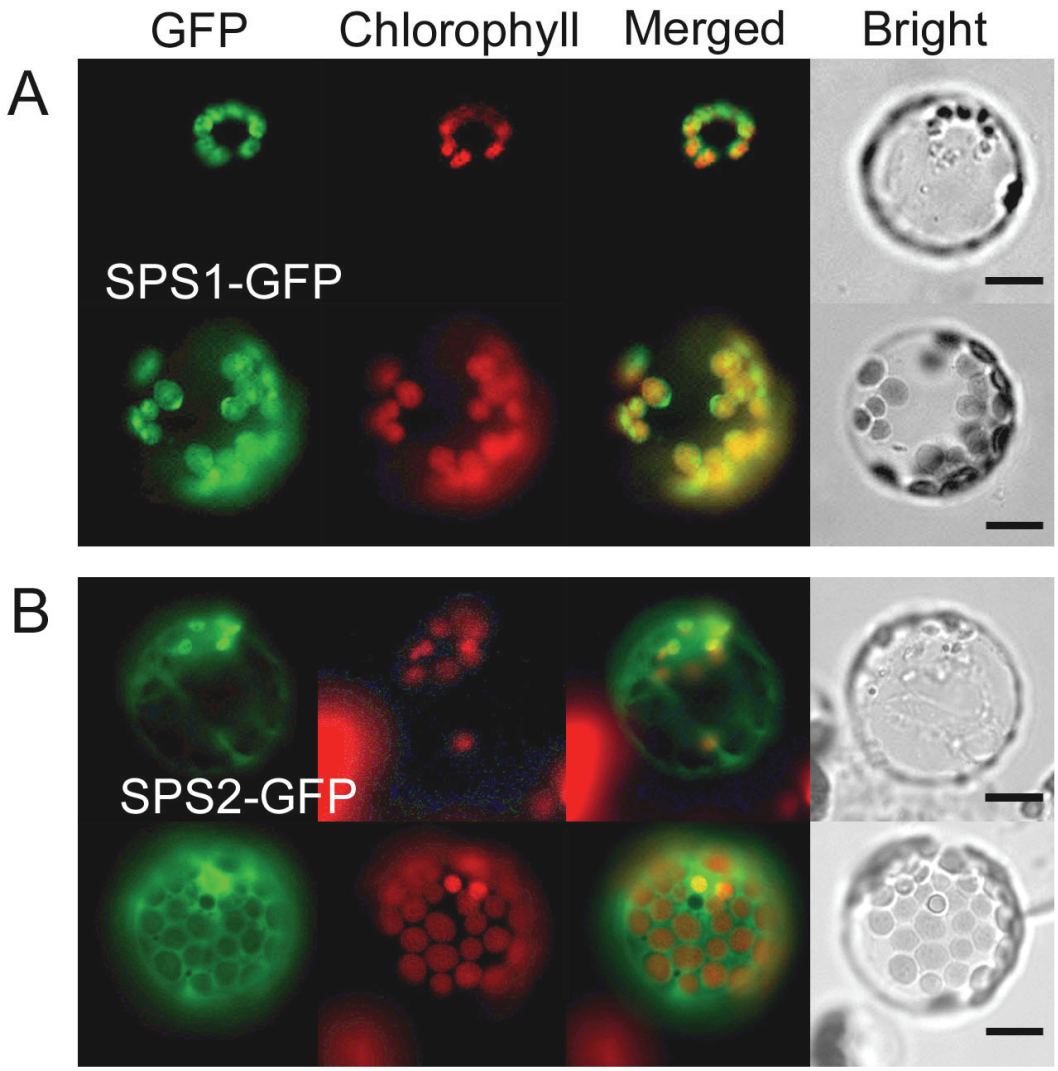


Figure 9. Enzymes Tested for Interaction with FBN5-B in a Yeast Two-hybrid Analysis (Y2H). (A) Generalized overview of the tocochromanol and benzoprenylquinone biosynthetic pathways in *Arabidopsis*. Enzymes that are candidate interacting partners of FBN5-B, used for the Y2H experiments, are shown in red font. (B) Y2H experiments with full-length FBN5-B and candidate enzymes. Only SPS1 is in the mature form; the other enzymes are in the preprotein forms. The interactions between full-length FBN5-B and enzymes fused to the GAL4 DNA-activation domain were examined in each transformant by testing their growth on Leu$^-$, Trip$^-$, and Ade$^-$ SD-LWA medium. β-Galactosidase activity was tested in each colony on SD-LWA medium. Compartmentalization of *Arabidopsis* enzymes is based on the pathway constructed by Vranová et al., (2012) slightly modified based on recent findings. IPP, isopentenyl diphosphate (C5); DMAPP, dimethylallyl diphosphate (C5); GPP, geranyl diphosphate (C10); GGPS, geranylgeranyl diphosphate (GGPP, C20) synthase; FPS, farnesyl diphosphate (FPP, C15) synthase; SPS, solanesyl diphosphate (SPP, C45) synthase; PPS, polyprenyl diphosphate (PPP, C50) synthase; GGR, geranylgeranyl reductase; HST, homogentisate solanesyltransferase; VTE2, homogentisate phytyltransferase; VTE3, MPBQ/MSBQ methyltransferase; MPBQ, 2-methyl-6-phytyl-1,4-benzoquinone; MSBQ, 2-methyl-6-solanesyl-1,4-benzoquinone; PQ-9, plastoquinone-9; PC-8, plastochromanol-8; HBPT, 4-hydroxylgenzoate polytransferase.

REFERENCE

Vranová, E., Coman, D., and Gruissem, W. (2012). Structure and dynamics of the isoprenoid pathway network. Mol. Plant **5**: 318-333.



Supplemental Figure 10. Subcellular Localization of SPS1 (A) and SP2 (B). Representative confocal micrographs of the green fluorescence from sGFP (GFP), red fluorescence from chlorophyll, and merged signals are shown in the three left panels. Transmission micrographs (Bright) for *Arabidopsis* protoplasts expressing SPS1–sGFP (**A**) and SPS2–sGFP (**B**) are shown. Bars = 20 μm.

Supplemental Tables

Supplemental Table 1. Quantification of Tocochromanols and Prenylquinones in Leaves of Each Pale-Green Mutant

	Concentration, nmol/g (fresh weight)						
Line	δ-toc	γ-toc	α-toc	PQ-9	PC-8	Total Toc	PQ-9+PC-8
WT	1.0±0.1	1.1±0	32.9±7.0	245.5±6.1	11.8±1.0	34.9±6.9	257.3±7.0
fbn5-1	4.1±1.1	9.7±2.5	30.5±3.8	8.5±0.5	n.d.	44.3±5.0	8.5±0.5
pds2-1	3.5±0.3	18.6±2.0	20.4±1.2	17.4±0.9	n.d.	42.5±3.3	17.4±1.5
gdc1-2	1.8±0.2	4.4±0.3	47.1±3.3	135.0±11	1.5±0.3	53.3±3.7	136.9±11.7
sbp-1	1.1±0.3	2.3±0.8	33.1±1.1	31.3±0.9	0.9±0.1	36.5±0.9	32.3±0.9
aae14-1	1.5±0.2	20.7±9.1	13.2±4.7	39.5±12	0.8±0.1	35.4±13	40.3±12

Arabidopsis plants were grown on 0.5× MS medium with 1% sucrose for 18–21 days. For each measurement, the leaves of several plants, corresponding to approximately 15–20 mg, were combined. Pigments were analyzed with reversed-phase HPLC. Data are the mean values of three measurements ± SD. n.d., not detected

Supplemental Table 2. Quantification of Carotenoids and Chlorophylls in Leaves of Each Pale-Green Mutant

Concentration, nmol/g (fresh weight)									
Line	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	β-carotene	Chlorophyll *b*	Chlorophyll *a*	Total Carotenoids	Total Chlorophylls
WT	80±5	75±0	5±1	281±6	106±6	739±37	1658±88	547±13	2397±125
fbn5-1	51±1	66±5	14±3	195±5	54±6	544±30	1023±60	381±10	1567±120
pds2-1	65±6	68±4	14±2	243±20	66±13	635±37	1193±166	455±42	1827±234
gdc1-2	10±1	27±4	5±1	63±10	40±6	56±6	284±25	144±22	340±31
sbp-1	35±3	34±11	16±8	123±15	28±4	312±18	553±45	234±24	865±63
aae14-1	9±1	13±2	5±1	51±5	7±2	58±12	124±28	85±10	182±40

Arabidopsis plants were grown on 0.5× MS medium with 1% sucrose for 18–21 days. For each measurement, leaves of several plants, corresponding to approximately 15–20 mg, were combined. Pigments were analyzed with reversed-phase HPLC. Data are the means ± SD of three measurements

Supplemental Table 3. Oligonucleotides Used in this Study

Primer name	Use of primers	Primer
Yeast two hybrid study		
FBN5-B-Y2HF	Y2H bait, *Eco*RI	GCGAATTCACGAGTAACCTTTTCCAGCC
FBN5-B-Y2HR	Y2H bait, *Bam*HI	GCGGATCCTTAAGGTTTCTCTATTCTTTCC
FBN5-A-Y2HF	FBN5A bait, *Eco*RI	AGCGAATTCATGACGAGTAACCTTTTCCAG
FBN5-A-Y2HR	FBN5A bait, *Bam*HI	AGCGGATCCCTAGTTACGATTTTGGATTTTAAT
SPS1-Y2HF	Preprotein SPS1 prey, *Eco*RI	AGCGAATTCATGATGACGTCATGTCGGAAT
SPS1-Y2HR	Preprotein SPS1 prey, *Bam*HI	CGGGATCCCTAATCAATTCTTTCGAGGTTATA
SPS2-Y2HF	Preprotein SPS2 prey, *Eco*RI	AGCGAATTCATGATGACGTCATGTCGGAAT
SPS2-Y2HR	Preprotein SPS2 prey, *Bam*HI	ACGGATCCCCTCTGCCAAATACCCTTTG
SPS3-Y2HF	Preprotein SPS3 prey, *Eco*RI	TCGAATTCATGTTATTCACGAGGAGTGTTGCT
SPS3-Y2HR	Preprotein SPS3 prey, *Bam*HI	ACGGATCCTCACTTGTTTCTGGTGATGACTC
FPS1-Y2HF	Preprotein FPS1 prey, *Nde*I	GTCATATGATGAGTGTGAGTTGTTGTTGTAGG
FPS1-Y2HR	Preprotein FPS1 prey, *Bam*HI	ACGGATCCCTACTTCTGCCTCTTGTAGATCTTAG
FPS2-Y2HF	Preprotein FPS2 prey, *Nde*I	GTCATATGATGGCGGATCTGAAATCA
FPS2-Y2HR	Preprotein FPS2 prey, *Bam*HI	ACGGATCCCTACTTCTGCCTCTTGTAGATCTTA
VTE2-Y2HF	Preprotein VTE2 prey, *Eco*RI	TCGAATTCATGGAGTCTCTGCTCTCTAGTTCTT
VTE2-Y2HR	Preprotein VTE2 prey, *Bam*HI	ACGGATCCCAAAAATCCATACGTTCTGCAA
VTE3-Y2HF	Preprotein VTE3 prey, *Eco*RI	TCGAATTCAACTTGGTGGATCTGTCATCG
VTE3-Y2HR	Preprotein VTE3 prey, *Bam*HI	ACGGATCCTCAGATGGGTTGGTCTTTGG
HST-Y2HF	Preprotein HST prey, *Nde*I	GTCATATGATGGAGCTCTCGATCTCACA
HST-Y2HR	Preprotein HST prey, *EcoRI*	TCGAATTCCTAGAGGAAGGGGAATAACAGA
GGR-Y2HF	Preprotein GGR prey, *Nde*I	GTCATATGATGGCGACGACGGTTACA
GGR-Y2HR	Preprotein GGR prey, *Bam*HI	ACGGATCCTTAAACACTAAGCTTCTCAATCTCTCT
PTB-Y2HF	Positive control, *EcoRI*	TCGAATTCATGGACGGCATCGTCCCAG
PTB-Y2HR	Positive control, *BamHI*	ACGGATCCCTAGATGGTGGACTTGGAG
SPS1-Y2HMF	Mature SPS1 prey, *Nde*I	GCCCATATGGCTGTTCCGGCTAAATCCAA
SPS2-Y2HMF	Mature SPS2 prey, *Nde*I	GCCCATATGGCTGTTCCGGCTAAATCCAA
SPS3-Y2HMF	Mature SPS3 prey, *EcoRI*	GC GAA TTC GCA ATC ATT CCC GAT CAG GG
HST-Y2HMF	Mature HST prey, *NdeI*	GGCCAT ATG GCA TGT TCT CAG GTT GGT GC
VTE2-Y2HMF	Mature VTE2 prey, *EcoRI*	GCGAA TTC TGT GAT TCG AGT AAA GTT GTC G
Yeast two hybrid analysis for FBN5-B domain interacting with SPS1 or SPS2		
1-61aa-F	Forward, *Eco*RI	AGCGAATTCATGACGAGTAACCTTTTCCAG
1-61aa-R	Reverse, *Bam*HI	AGCGGATCCTTAAGAACTCTGTTCTGTGAC
1-130aa-F	Forward, *Eco*RI	AGCGAATTCATGACGAGTAACCTTTTCCAG
1-130aa-R	Reverse, *Bam*HI	AGCGGATCCTTATCCAGTCGGCTCCGG
31-273aa-F	Forward, *Eco*RI	AGCGAATTCTTCACCAGTTCTAATGGCAAAA
31-273aa-R	Reverse, *Bam*HI	AGCGGATCCTTAAGGTTTCTCTATTCTTTCC
41-273aa-F	Forward, *Eco*RI	AGCGAATTCTTTAGTGACAACTCATTTAGGC
41-273aa-R	Reverse, *Bam*HI	AGCGGATCCTTAAGGTTTCTCTATTCTTTCC
51-273aa-F	Forward, *Eco*RI	AGCGAATTCATGTTCATCGGGAAAGTCACAG
51-273aa-R	Reverse, *Bam*HI	AGCGGATCCTTAAGGTTTCTCTATTCTTTCC
62-273aa-F	Forward, *Eco*RI	ACGAATTCTGTTCCTCCCCAAATGAACA
62-273aa-R	Reverse, *Bam*HI	AGCGGATCCTTAAGGTTTCTCTATTCTTTCC
62-130aa-F	Forward, *Eco*RI	GACGAATTCTGTTCCTCCCCAAATGAACAA
62-130aa-R	Reverse, *Bam*HI	ACGGGATCCTTATCCAGTCGGCTCCGG
131-273aa-F	Forward, *Eco*RI	AGCGAATTCGAGTTAGACAAGATCGGAGG
131-273aa-R	Reverse, *Bam*HI	AGCGGATCCTTAAGGTTTCTCTATTCTTTCC
Subcellular localization		

FBN5A-sGFP-F	*Bam*HI	AGCGGATCCATGACGAGTAACCTTTTCCAG
FBN5A-sGFP-R	*Bam*HI	AGCGGATCCCTAGTTACGATTTTGGATTTTAAT
FBN5B-sGFP-F	*Bam*HI	AGCGGATCCATGACGAGTAACCTTTTCCAG
FBN5B-sGFP-R	*Bam*HI	AGCGGATCCAGGTTTCTCTATTCTTTCCAAA
SPS1-sGFP-F	*Bam*HI	ACGGATCCATGATGACGTCATGTCGGAAT
SPS1-sGFP-R	*Bam*HI	ACGGATCCATCAATTCTTTCGAGGTTATACAACA
SPS2-sGFP-F	*Bam*HI	AGCGGATCCATGATGATGTCATGTCGGAATA
SPS2-sGFP-R	*Bam*HI	ACGGATCCATCAATCCTTTCAAGATTAAACATTA
Co-immunoprecipitation		
SPS1-HA tag-F	*Xba*I	ACGTCTAGAATGATGACGTCATGTCGGAAT
SPS1-HA tag-R	*Bam*HI	AGCGGATCCATCAATTCTTTCGAGGTTATACAACA
SPS2-HA tag-F	*Xba*I	ACGTCTAGAATGATGATGTCATGTCGGAATA
SPS2-HA tag-R	*Bam*HI	AGCGGATCCATCAATCCTTTCAAGATTAAACATTA
BiFC analysis		
FBN5-A-BiFC-F	pENTR/D-TOPO	CACCATGACGAGTAACCTTTTCCAG
FBN5-B-BiFC-F	pENTR/D-TOPO	CACCATGACGAGTAACCTTTTCCAG
FBN5-B-BiFC-R	pENTR/D-TOPO	AGGTTTCTCTATTCTTTCCAAA
SPS1-BiFC-F	pENTR/D-TOPO	CACCATGATGACGTCATGTCGGAAT
SPS1-BiFC-R	pENTR/D-TOPO	ATCAATTCTTTCGAGGTTATACAACA
SPS2-BiFC-F	pENTR/D-TOPO	CACCATGATGATGTCATGTCGGAATA
SPS2-BiFC-R	pENTR/D-TOPO	ATCAATCCTTTCAAGATTAAACAT
RT-PCR		
P1	*FBN5-B*	ATGACGAGTAACCTTTTCCAG
P2	*FBN5-B*	TTAAGGTTTCTCTATTCTTTCC
P3	*FBN5-A*	CTAGTTACGATTTTGGATTTTAAT
Actin2-F	*ACTIN2*	GGTGTCATGGTTGGGATGAA
Actin2-R	*ACTIN2*	GATTCCTGGACCTGCCTGAT
Actin7-F	*ACTIN7*	TCCATTTCTCTATCTTTCTCTCTCGCTG
Actin7-R	*ACTIN7*	CAAACTCACCACCACGAACCAGATAA
PDS2-1-RT-F	*PDS2*	CTCGATCTCACAATCACCGC
PDS2-1-RT-R	*PDS2*	AGCCTGAAGCCAAGATCACA
AAE14-RT-F	*AAE14*	GGCTAATCACTCTCGGCCTC
AAE14-RT-R	*AAE14*	AGAGAGAGCTAGTCATGATTTGGAA
GDC1-RT-F	*GDC1*	ATGGCTTCTTCTTCAATCTCA
GDC1-RT-R	*GDC1*	CGCCAAACACCATAGAACA
SBP-RT-F	*SBP*	ATGGAGACCAGCATCGC
SBP-RT-R	*SBP*	CTAAGCGGTAACTCCAATG
Genotyping		
SALK_064597-LP	*fbn5-1*	GAGACGAAATCTCGAAGACCC
SALK_064597-RP	*fbn5-1*	AGAGGCATCGTATGGTGAATG
SALK_024357-LP	*pds2-1*	TTATGAGCTGCCCAGTTTCAG
SALK_024357-RP	*pds2-1*	AGAGAACCGAAACCAAAGCTC
SALK_024357-LP	*aae14-1*	TCGTCACAGTCTACGGAAACC
SALK_024357-RP	*aae14-1*	TGTGCAAATACACCTGCAGAG
SALK_151530C-LP	*gdc1-2*	ATGCAGACGAAAACGGATATG
SALK_151530C-RP	*gdc1-2*	CTCATTCTCCTGTGCACCTTC
SALK_090549C-LP	*sbp-1*	GGAATCTTATGACATTCCAAAAATG
SALK_090549C-RP	*sbp-1*	TCAATCCCTTGTCAGGAGTTG
T-DNA-1	LBb1.3	ATTTTGCCGATTTCGGAAC
T-DNA-2	Lba1	TGG TTC ACG TAG TGG GCC ATC G
Real-time PCR		
FBN5B-F	*FBN5-B*	TAGGCTCAGACCCATGTTCA
FBN5B-R	*FBN5-B*	CTAACTCTCCAGTCGGCTCC
PDS2-F	*PDS2*	CCCAGTTGCAGCATTTCTTA
PDS2-R	*PDS2*	CAGGTGCACTCCACTGAAAT
SPS1-F	*SPS1*	CACTGCAAGCTTGATACACG
SPS1-R	*SPS1*	GAACTGTTTCCTTTCCTGCAA
SPS2-F	*SPS2*	TCTTGTTTGGTTTCAAGCGA
SPS2-R	*SPS2*	CGGGTTCAAGACACATCATC
Actin-2-QR-F	*ACTIN2*	AGCACATTCCAGCAGGTAAA
Actin-2-QR-R	*ACTIN2*	TCTGTGAACGATTCCTGGAC

GUS expression		
P4	*FBN5* promoter	CACCAACGTCACCGAAGGCTGTGATTGT
P5	*FBN5* promoter	TAGCAGTTTATACACCAGAATCTCTAA