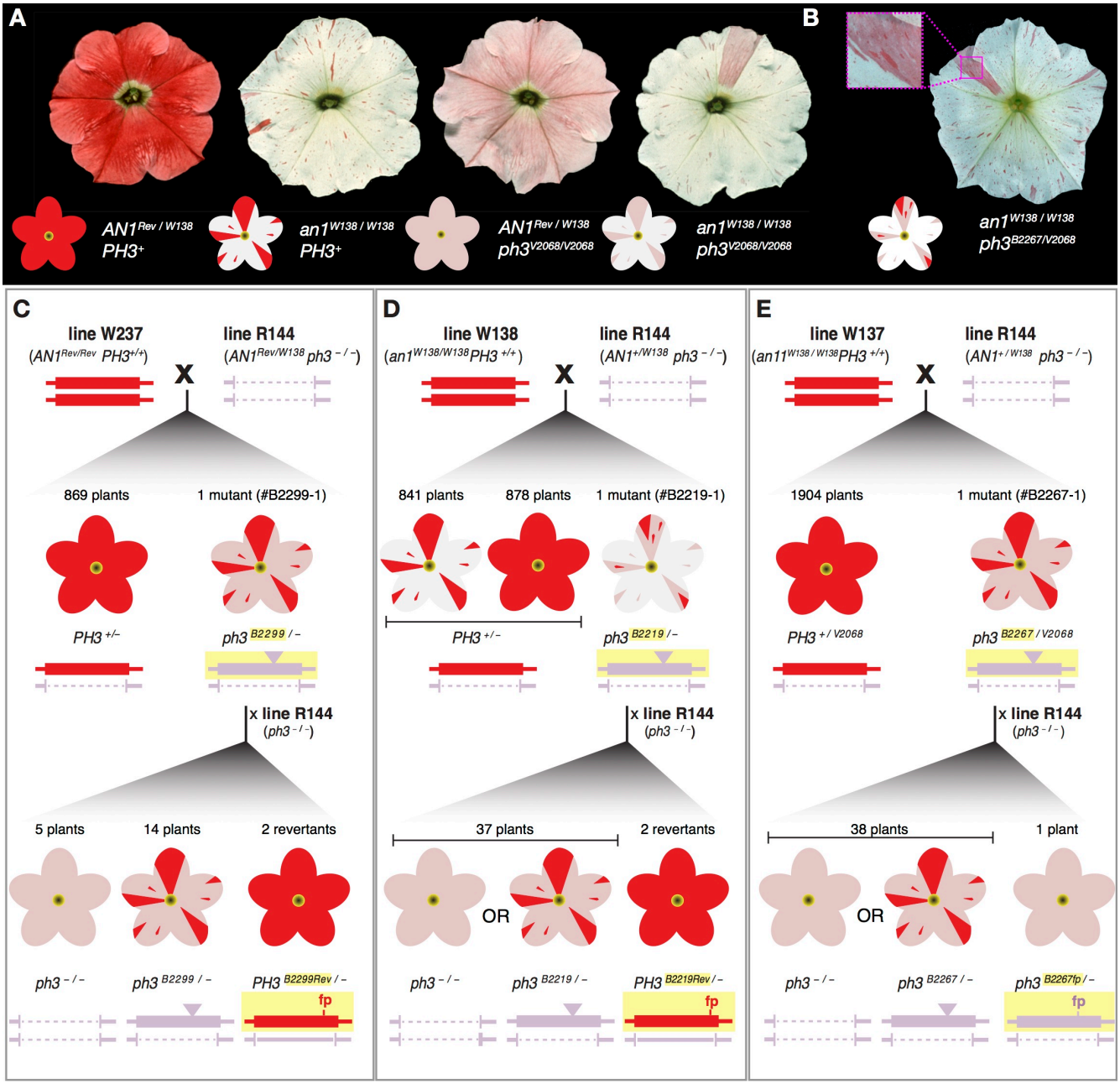
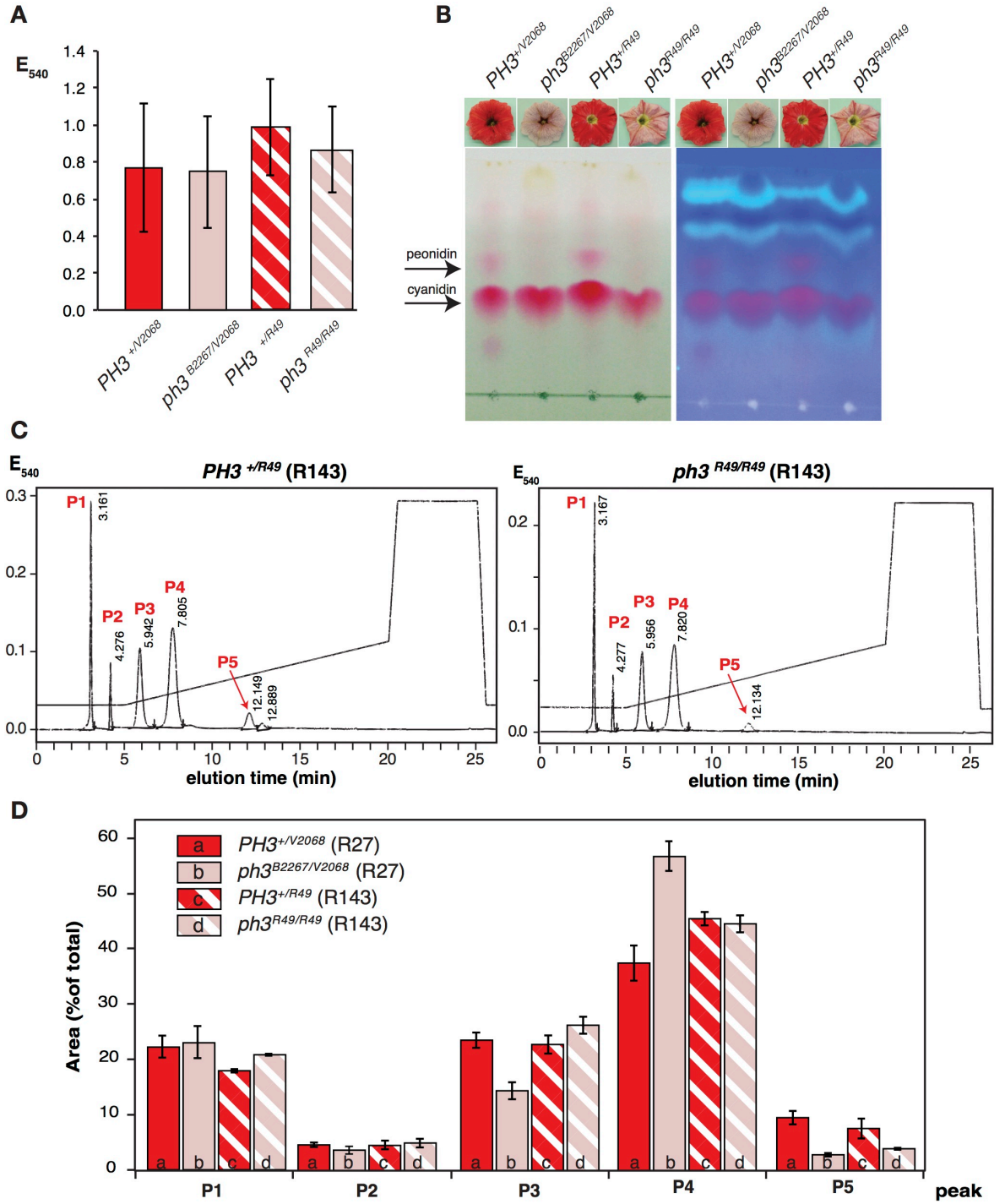


Supplemental Figure 1. Isolation and genetic characterization of *ph3* mutants.

(A) Images and diagrams of flower phenotypes in family V2068 segregating for the mutable $an1^{W138}$ allele (indicated here as $an1^{m}$) and a derived revertant allele ($AN1^{REV}$) as well as a wild type $PH3^{+}$ and the stable recessive $ph3^{V2068}$ allele (indicated here as $ph3^{-}$). Note that colored petal cells ($AN1^{REV}$) acquire either a red or a purplish color depending on the *ph3* genotype.

(**B**) Flower and diagrammatic representation of a transposon tagged mutant (plant B2267-1) with an unstable *an1* ($an1^{W138/W138}$) and *ph3* ($ph3^{B2267/V2068}$) phenotype. The enlarged rectangle shows a detail of a purplish (*ph3*) sector with red $PH3^{REV}$ spots.

(C-E) Pedigrees showing the origin of $ph3^{B2299}$ (C), $ph3^{B2219}$ (D) and $ph3^{B2267}$ (E) and progeny from test crosses. Floral phenotypes are indicated by diagrams as in (A); the structure of the *PH3* locus is shown below. The active *PH3* allele is shown in red, mutant *ph3* alleles with a transposon insertion (triangle) or deletion (dashed line) are shown in purplish. For each generation newly arisen *ph3* or $PH3^{REV}$ alleles are highlighted with yellow background.



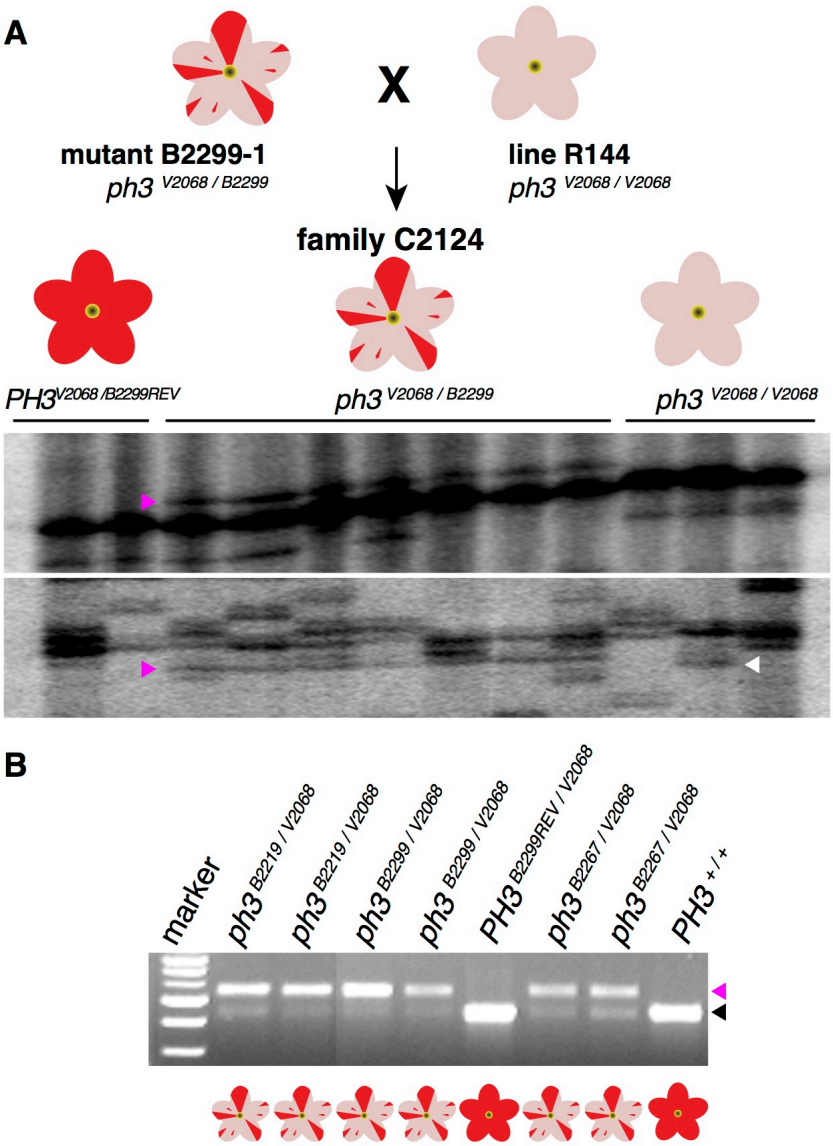
Supplemental Figure 2. Anthocyanin content of *PH3* and *ph3* petals.

(**A**) Total anthocyanin content (E_{540}) of flowers with different genotypes (n=5, mean±SD).

(**B**) TLC analysis of anthocyanidins obtained by acid hydrolysis of anthocyanins from flowers with different genotypes. The TLC plates were photographed under white light (left) and UV light (right)

(**C**) Representative HPLC profiles of anthocyanins in $PH3^{+}$ and *ph3* mutant flowers. P1-P5 indicate numbers of anthocyanin peaks

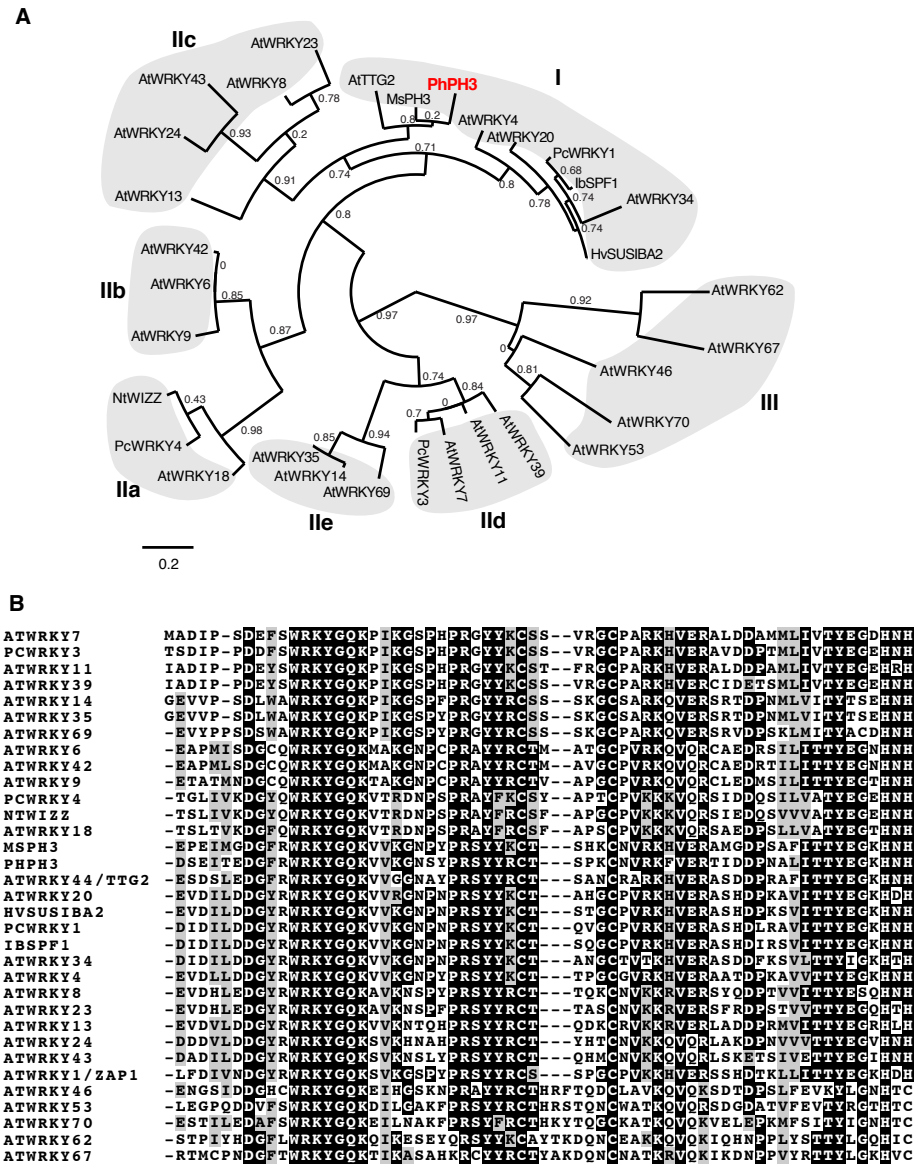
(**D**) Quantification (mean ± SD) of different anthocyanins (expressed as % of total amount anthocyanins) as determined from the area of peaks 1 to 5 (P1-P5) in HPLC profiles from different flowers (n=5).



Supplemental Figure 3. Identification of the *PH3* gene.

(A) Identification of *dTPH1* flanking sequences originating from *PH3* by transposon display. The origin, phenotypes and inferred genotypes of the analyzed plants from family C2124 are indicated by the flower diagrams (*cf*. Supplemental Figure 1C). The gel images are sections from two transposon-display gels showing amplification products obtained with the adaptors *BfaI+T* (top) or *TaqI+G* (bottom). Magenta arrowheads mark two specific *dTPH1* flanking sequences seen in seven unstable $ph3^{V2068/B2299}$ mutants, but not in siblings with a stable recessive ($ph3^{V2068/V2068}$) phenotype or two germinal revertants ($PH3^{V2068/B2299REV}$). The white arrowhead marks an unrelated fragment that is 1 nucleotide shorter.

(**B)** PCR amplification of the genomic region identified in (A) from the three tagged mutants heterozygous for $ph3^{B2219}$, ph^{B2299} or $ph3^{B2267}$ and the stable $ph3^{V2068}$ allele, a derived revertant ($PH3^{B2299REV}$) and the $PH3^{+}$ progenitor line W138. Note that plants with an unstable *ph3* phenotype (purplish flowers with red spots) yield besides amplification productions containing *dTPH1* (magenta arrowhead) a small amount of products with a wild type size (black arrowhead) that result from somatic *dTPH1* excisions. The $ph3^{V2068}$ allele yields no PCR products at all.

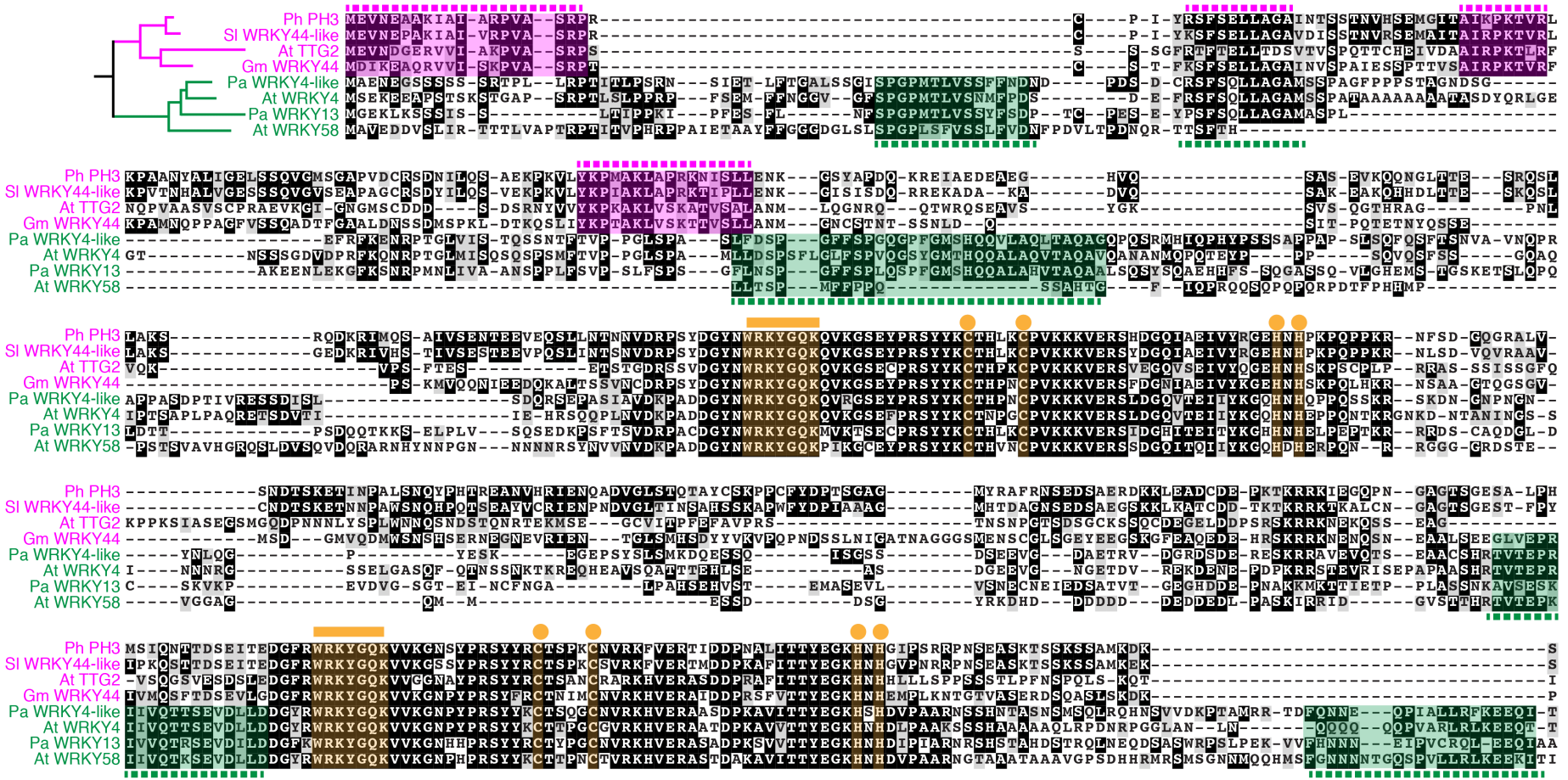


Supplemental Figure 4. PH3 is a type I WRKY protein with similarity to Arabidopsis TTG2.

(A) Phylogenetic tree of a selection of WRKY proteins, representing all mains branches of the family (*cf.* Eulgem et al., 2000). The tree is based on the alignment of sequences spanning the C-terminal WRKY domain and 10 upstream amino acids shown in B. Prefixes denote the species of origin: *Arabidopsis thaliana* (At), parsley *(Petrosellum crispum, Pc)*, *Nicotiana tabaccum* (Nt), barley (*Hordeum vulgare*, Hv) and sweet potato (*Ipomea batatas,* Ib).

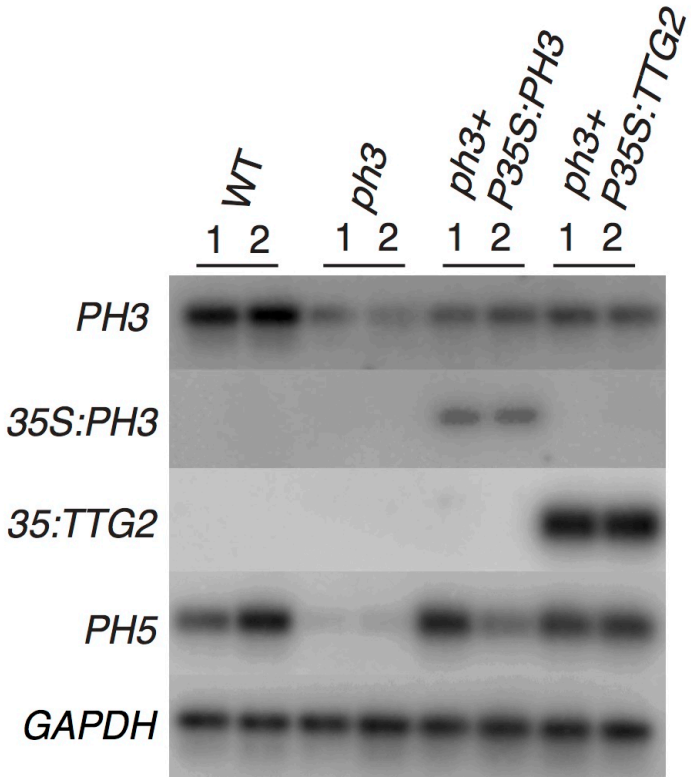
(B) Alignment of the PH3 and TTG2 protein sequence fragments used to generate the phylogenetic tree shown in (A). Black shading denotes sequence identity, grey shading similarity.

Eulgem, T., Rushton, P.J., Robatzek, S., and Somssich, I.E. (2000). The WRKY superfamily of plant transcription factors. Trends Plant Sci **5,** 199-206



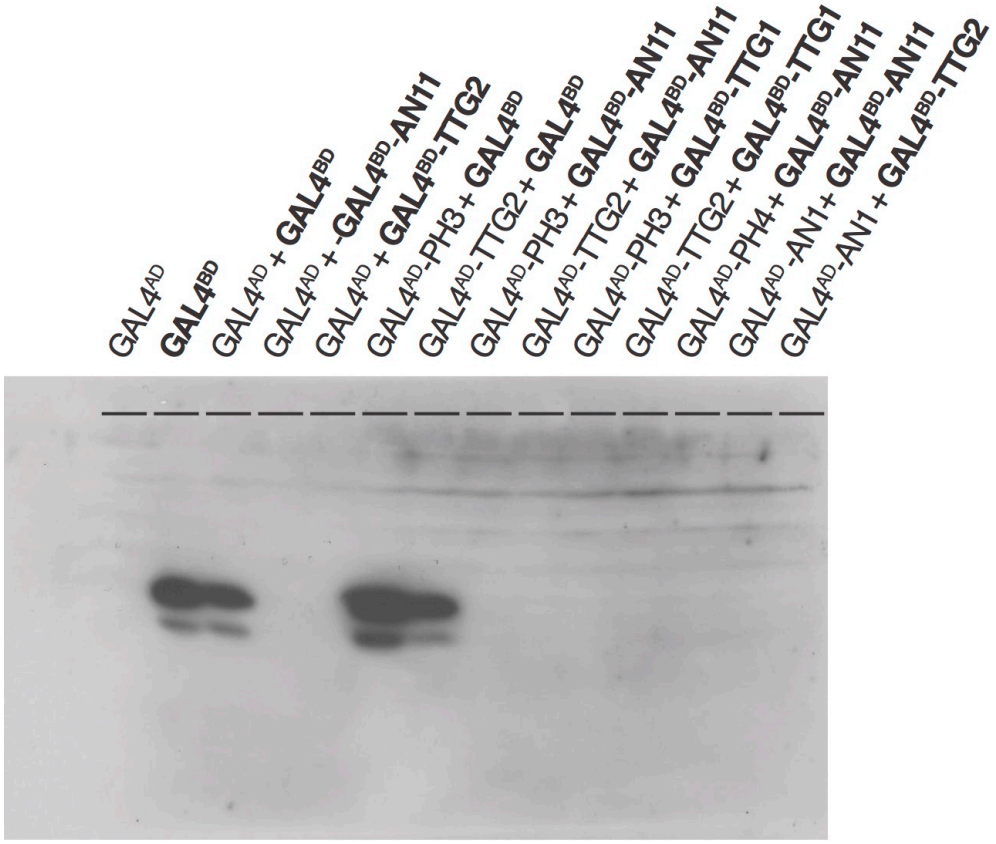
Supplemental Figure 5. Alignment of PH3 with homologs from other species and closely related WRKY proteins.

Alignment of PH3 protein to TTG2 and homologs from soybean (*Glycine max*, Gm) and tomato (*Solanum lycopersicum*, Sl) (names in magenta) and the next most similar proteins (names in green) from *Petunia axillaris* (Pa) and *Arabidopsis* (At). The conserved WRKY motif and C2H2 finger are marked by ochre stripes and dots above the sequence. Dashed lines above and below the sequences denote several regions conserved within the magenta and green group. Black shading denotes sequence identity, grey shading similarity.

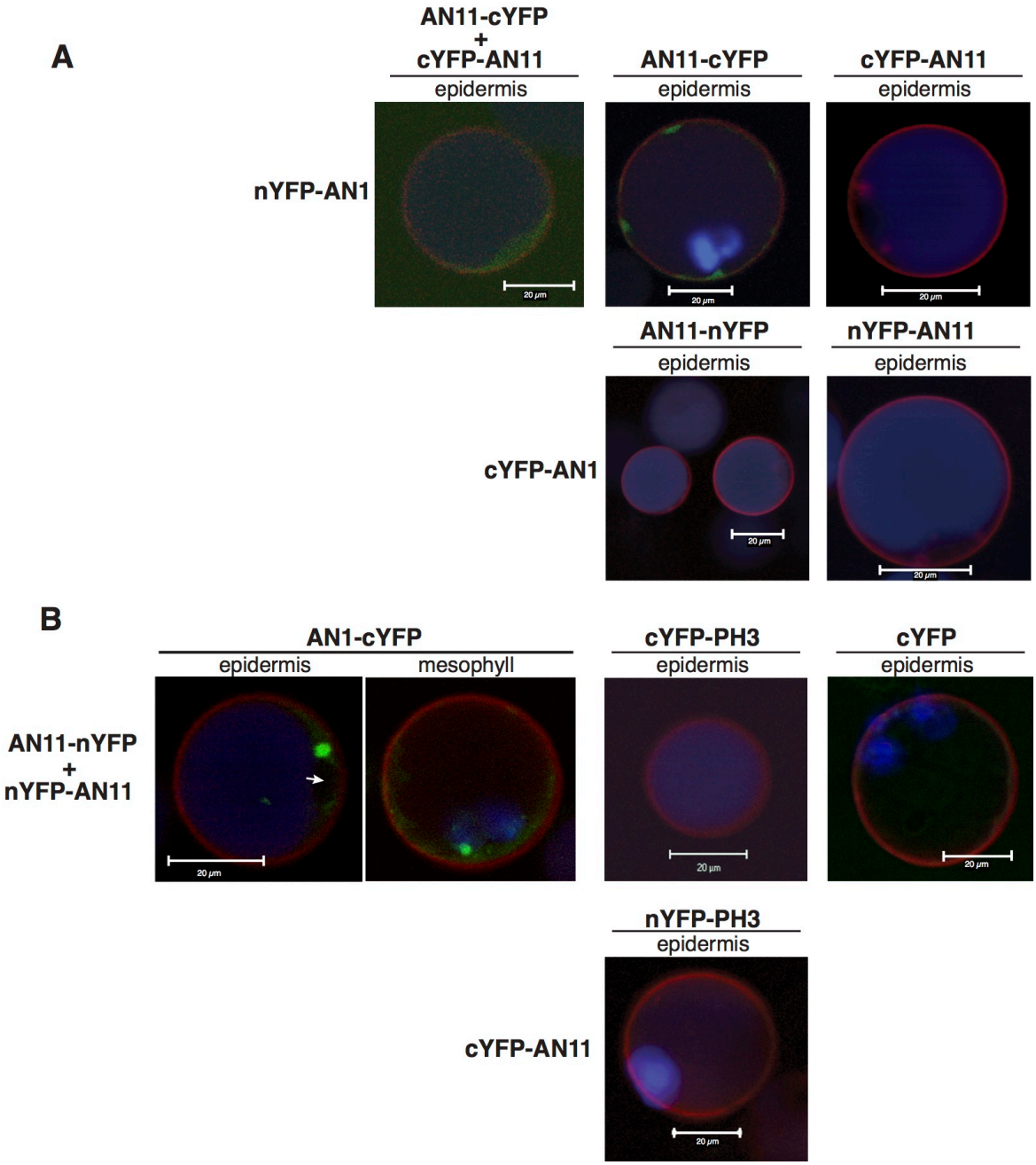


Supplemental Figure 6. Expression *p35S:PH3* and *p35S:TTG2* transgenes rescues *PH5* mRNA expression in *ph3* mutants.

RT-PCR analysis of the expression of the *PH3* endogene, *p35S:PH3*, *p35S:TTG2* and the downstream gene *PH5* in four transgenic lines with red flowers (cf. Figure 5). *GAPDH* expression is shown as an internal control. RNAs were amplified using gene-specific primers and a reduced number of cycles and amplification products were after electrophoresis detected by blot hybridization.



Supplemental Figure 7. Immuno-blot analysis of yeast two hybrid strains**.**
Proteins from yeast strains expressing the indicated GAL4 fusion proteins were detected with anti-Gal4^{BD} serum.



Supplemental Figure 8. Interaction of AN1 and AN11 in petunia petal protoplasts.

(**A**) Protoplast from petal epidermis expressing AN11-cYFP and c-YFP-AN11 in combination with nYFP-AN1.

(**B**) Petal protoplasts from epidermis and mesophyll expressing AN11-nYFP and nYFP-AN11 in combination with AN1-cYFP or cYFP.

Restored YFP fluorescence (BiFC) is shown in green. Fluorescence of the plasma membrane protein RFP-SYP122, which was used as a transformation marker, is shown in red and fluorescence of anthocyanins in dark blue. The white arrows point into the nucleus. Size bars, 20 µm.

Supplemental Table 1: Inbred petunia lines and genotypes used.

		relevant genotype			
line	**background**	**regulatory *AN* genes**	**structural genes**	***PH* genes**	**reference**
R27	R27	*AN1 an4 AN11*	*AN10 hf1 hf2 rt*	*PH2 PH3 PH4*	*a,b,e*
W134	R27	*AN1 an4 an11^{W134}*	*AN10 hf1 hf2 rt*	*PH2 PH3 PH4*	*a*
W137	R27	*AN1 an4 an11^{W137}*	*AN10 hf1 hf2 rt*	*PH2 PH3 PH4*	*a*
W138	R27	*an1^{W138} an4 AN11*	*AN10 hf1 hf2 rt*	*PH2 PH3 PH4*	*b*
W225	R27	*an1^{V2015} an4 AN11*	*AN10 hf1 hf2 rt*	*PH2 PH3 PH4*	*b*
W237	R27	*an1^{W138} an4 AN11*	*an10 hf1 hf2 rt*	*PH2 PH3 PH4*	*c*
R143	[(R49xR3)xM1]xS	*AN1 an4 AN11*	*AN10 hf1 hf2 rt*	*PH2 ph3^{R49} PH4*	*d,f*
R144	R27	*AN1 an4 AN11*	*AN10 hf1 hf2 rt*	*PH2 ph3^{V2068} PH4*	*e*
R149	R27	*AN1 an4 AN11*	*AN10 hf1 hf2 rt*	*PH2 PH3 ph4^{V2153}*	*e*
R160	R27	*AN1 an4 AN11*	*AN10 hf1 hf2 rt*	*ph2 PH3 PH4*	*e*

[1] *a*, de Vetten et al., 1999; *b*, Spelt et al., 2000; *c*, van Houwelingen et al., 1998; *d*, de Vlaming et al., 1983; *e*, Quattrocchio et al., 2006, f, this paper

Supplemental Table 2: *AN1* and *PH3* alleles used.

allele	**background**	**lesion**	**phenotype if homozygous in R27 background**	**reference[1]**
AN1	R27	none, functional allele	red petals, low pH	b
an1^{W138}	R27	highly unstable *dTPH1* insertion allele	white petals with red spots, high pH	b
an1^{V2015}	R27	null allele with footprint derived by excision of *dTPH1* from *an1^{w138}*	white petals, high pH	b
AN11	R27	none, functional allele	red petals, low pH	a
an11^{W137}	R27	unstable *dTPH1* insertion allele	white petals with red spots, high pH	a
an11^{W134}	R27	stable *dTPH1* excision allele with footprint derived from *an1^{W137}*	white petals, high pH	a
PH3	R27	none, functional allele	red petals, low pH	f
ph3^{V2068}	R27	(nearly) complete deletion	purplish petals, high pH,	e,f
ph3^{B2267}	R27	weakly unstable *dTPH1* insertion	purplish petals with red spots, high pH	f
ph3^{B2219}	R27	weakly unstable *dTPH1* insertion	purplish petals with red spots, high pH	f
ph3^{B2299}	R27	weakly unstable *dTPH1* insertion	purplish petals with red spots, high pH	f
ph3^{B2267FP}	R27	stable *dTPH1* excision allele derived from *ph3^{B2267}* with a 7 bp footprint	purplish petals, high pH	f
ph3^{R49}	[(R49xR3)xM1]xS	(nearly) complete deletion	purplish petals, high pH	d,f
PH4	R27	none, functional allele	red petals, low pH	e
ph4^{V2153}	R27	stable *dTPH6* insertion	purplish petals, high pH	e

[1] *a*, de Vetten et al., 1999; *b*, Spelt et al., 2000; *c*, van Houwelingen et al., 1998; *d*, de Vlaming et al.,1983; *e*, Quattrocchio et al., 2006, f, this paper

Supplemental Table 3: Primers used for transposon display.

primer	name	sequence 5'-3'
1115	TaqI-Adapter1	GACGATGAGTCCTGAG
1116	TaqI-Adapter2	CGCTCAGGACTCAT
1118-21	TaqI+N	CGATGAGTCCTGAGCGAA+**N**
701	BfaI-Adapter1	GACGATGAGTCCTGAG
702	BfaI-Adapter2	TACTCAGGACTCAT
1123-26	BfaI+N	ACGATGAGTCCTGAGTAGA+**N**
423	out 11 (*dTPH1*)	CGAAGGGGTGTCAATGCTG
15	out 5 (*dTPH1*)	CCCTTATTAGAATTCYTGGCTCCGC
630	out12 (*dTPH1*)	CAGCATTGACACCCCTTC
631	out 13 (*dTPH1*)	CAGTGTAAATTTTGCGCAAA

Oligonucleotides TaqI-Adapter1 and TaqI-Adapter2 were annealed to generate the double stranded TaqI adapters and BfaI-Adapters and BfaI-Adapter2 to generate the double stranded BfaI adapters. Out11 (nested is out5) and out12 (nested is out13) are *dTPH1*-specific primers used in transposon display.

Supplemental Table 4: Primer used for RACE amplification of full length *PH3* cDNA.

primer	gene	sequence 5'-3'	orientation
1209	*PH3*	TACCACTGACTCTGAAATTACTGAG	forw
1269	*PH3*	GGGCGAGCACTTGTAAGCAAT	forw
1254	*PH3*	CCTGATGTACCTGCTCCATTG	rev
1297	*PH3*	CAGGGCATTCCGAAATTCTGAAG	rev
1296	*PH3*	CATCATAACTAGGGCGATCGAC	rev

Supplemental Table 5: Primer number and sequence used for real time RT-PCR

primer	gene	sequence 5'-3'	orientation
6035	*PH3endo*	GGAAATGGAATTCAATGGGAGC	forward
2378	*PH3endo*	CTGTAAATAGGACATCTAGGCC	reverse
6036	*35S:PH3*	GCTGACAAGCTGACTCTAGC	forward
2378	*35S:PH3*	CTGTAAATAGGACATCTAGGCC	reverse
6036	*35S:TTG2*	GCTGACAAGCTGACTCTAGC	forward
6037	*35S:TTG2*	CGGTAACTGAATCAGTAAGAAGC	reverse
1060	*PH4*	CAATGGCTTTGTGAGTTCTAGGATC	forward
1061	*PH4*	CGCCTCCATCGTCTCCTTGG	reverse
5641	*PH5*	TAGCAATCCTAAATGATGGCACT	forward
5642	*PH5*	CAACTATCAGGTCTTGGAGATGG	reverse
4900	*DFR*	ACCTATGGATTTCGAGTCCAAAGA	forward
4901	*DFR*	CACATGATTCAATGATGCTTAGCAT	reverse
5643	*AN1*	TAGAGCCAATCAGACGGAGGCTAC	forward
5644	*AN1*	CCCTTCCTTGCAACACCTTCAC	reverse
3622	*AN11*	ATGGGATTGATCCCATGTCGA	forward
3559	*AN11*	TACTTTAAGCAATTGCAACTTGTTAGAA	reverse
5922	*Actin11*	TGCACTCCCACATGCTATCCT	forward
5923	*Actin11*	TCAGCCGAAGTGGTGAAAGAG	reverse

Supplemental Table 6: Primers and number of cycles used for RT-PCR analysis.

primer	gene	sequence 5'-3'	orientation	cycles
19	*GAPDH*	CTGGTTATTCCATTACAACTAC	reverse	20x
20	*GAPDH*	GGTCGTTTGGTTGCAAGAGT	forward	
126	*AN1*	TAGGATCCAGCCTTATCTGAGCACT	forward	24 x
123	*AN1*	GGGAATTCTATGGTGTCACCAAG	reverse	
1269	*PH3*	GGGCGAGCACTTGTAAGCAAT	forward	24 x
1254	*PH3*	CCTGATGTACCTGCTCCATTG	reverse	
1317	*PH4*	TTCTCTAGAGATGAGAACCCCATCATCAT	forward	24 x
1233	*PH4*	AAGCTTTCTCTAACTGGGATTATATTGA	reverse	
1812	*PH5*	GGAATCAATGTAAGTGATTGCAGTCCG	forward	22x
1849	*PH5*	CGGAATACCAATAGCTATGCCAAC	reverse	
27	*35S*	AGAAGACGTTCCAACCACGTCT	forward	30x [1]
2230	*PH3*	GATTTTGCTAAGAGACTTTGCCC	reverse	30x [1]
2118	*TTG2*	CTTCTCGTTCTTCCTTCTTTTGCTTC	reverse	30x [1]

[1] primers 27, 2230 and 2118 were used to assay expression of *35S:PH3* and *35S:TTG2*

Supplemental Table 7: Primers used to generate templates for synthesis of RNA probes.

primer	gene	Sequence[1]	orientation
T7 promoter primer	pGEM-Teasy	GTAATACGACTCACTATAGGG	n.a.
SP6 promoter primer	pGEM-Teasy	CTATTTAGGTGACACTATAG	n.a.
3421	*PH3*	ATGGAGGTCAATGAAGCAGCGAAAATAG	forward
1136	*PH4*	ATGAGAACCCCATCATCATC	forward
0097	*DFR*	ACAATGTTCACGCTACTGTTC	forward
0098	*DFR*	GTAGGAACATAGTACTCTGG	reverse

[1] n.a., not applicable

Supplemental Table 8: Primers used to generate *35S:PH3* and *35S:TTG2.*

primer	gene	Sequence[1]	orientation	restriction enzyme used
2254	*PH4*	GCTCTAGAATGGAGGTCAATGAAGCAGCGA	forward	BamHI
1295	*PH3*	TAGGATCCCGTCAATCGACTATGATTTGTC	reverse	XbaI
2224	*TTG2*	GCTCTAGAATGGAGGTGAATGATGGTGAAAGAG	forward	BamHI
1391	*TTG2*	GCGGATCCTCAAATTGTTTGCTTAGAAAGTTG	reverse	XbaI

[1] Magenta letters indicate restriction sites that were introduced to facilitate ligation of PCR fragments in pGreen1H

Supplemental Table 9: Primer number and sequence used for yeast 2-hybrid cloning

primer	gene	Sequence[1]	orientation	restriction enzyme used
3421	*PH3*	GGGAATTCATGGAGGTCAATGAAGCAGCGAA	forward	EcoRI
3422	*PH3*	CCGCTCGAGCTATGATTTGTCTTTCATAGCTGA	reverse	XhoI
3660	*TTG2*	CGGAATTCATGGAGGTGAATGATGGTGAAAG	forward	EcoRI
3661	*TTG2*	CCGCTCGAGTCAAATTGTTTGCTTAGAAAGTTG	reverse	XhoI
0296	*AN11*	TCGAATTCATGGAAAATTCAAGTCAAGAATC	forward	EcoRI
0297	*AN11*	TCCTCGAGCTTATACTTTAAGCAATTGCA	reverse	XhoI
3658	*TTG1*	CGGAATTCATGGATAATTCAGCTCCAGATTCG	forward	EcoRI
3659	*TTG1*	CCGCTCGAGTCAAACTCTAAGGAGCTGCATT	reverse	XhoI
0282	*AN1full*	AAGAATTCATGCAGCTGCAAACCATG	forward	EcoRI
0283	*AN1full*	ATCTCGAGGGACAAAGTGAGAGATC	reverse	XhoI
0451	*AN1N'*	TTTCCTCGAGGCTCTTCTTGTG	reverse	XhoI
0294	*JAF13 N'*	CTCTCGAGCTTGTACTCTCCTTTCAAG	forward	MunI
0325	*JAF13 N'*	GGCAATTGATGGCTATGGGATGCAAAG	reverse	XhoI
1136	*PH4*	GGCAATTGATGAGAACCCCATCATCATC	forward	MunI
1139	*PH4*	GGCTCGAGCTCTAACTGGGATTATATTGATC	reverse	XhoI

[1] Magenta letters indicate restriction sites that were introduced to facilitate ligation of PCR fragments in $GAL4^{AD}$ and $GAL4^{BD}$ yeast vectors

Supplemental Table 10: Primer number and sequence used for GFP and split-YFP constructs

primer	gene	sequence[1]	orientation
6033	*PH3*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGGAGGTCAATGAAGCAG	forward
6034	*PH3*	GGGGACCACTTTGTACAAGAAAGCTGGGTTCTTCTGAGCACGTCAATCGA	reverse
6142	*TTG2*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGAGGTGAATGATGGTG	forward
6143	*TTG2*	GGGGACCACTTTGTACAAGAAAGCTGGGTCGAGTCAAATTGTTTGCTTAG	reverse
3558	*AN11*	CACCATGGAAAATTCAAGTCAAGAATCAC	forward
3559	*AN11*	TACTTTAAGCAATTGCAACTTGTTAGAA	reverse
6140	*TTG1*	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGGATAATTCAGCTCCAG	forward
6141	*TTG1*	GGGGACCACTTTGTACAAGAAAGCTGGGTAAACTCTAAGGAGCTGCATTT	reverse
4274	*AN1*	TACCCATGGTAATGCAGCTGCAAACCATGTTACGG	forward
4275	*AN1*	GATGAATTCAACTCTAGGGATTAACTGGTG	reverse

[1] 5' extensions added to facilitate cloning/recombination using the Gateway cloning system, directional TOPO cloning, or conventional restriction-ligation are indicated in blue, green and magenta respectively