

Supplemental Figure 1. Diagram showing differences in first, second, and third order branching in California poppy wild type (left) and *escafl1fl2* plants (right). The terminal flower is yellow, first order flowers are green, second order branches are pink, third order branches are purple.

Supplemental Figure 2. Amino acid alignment of the opium poppy and California poppy FUL-like proteins. FUL-like motif is indicated by the box. Lines below indicate the locations of the primers used to amplify the regions for VIGS constructs from the *PapsFUL-like* genes from opium poppy and the *EscaFUL-like* genes from California poppy. *PapsFL1-PapsFL2* reverse primers are not shown because they bind to the 3'UTR. EcFL Fwd, forward *EscaFL* primer; EcFL Rev, reverse *EscaFL* primer; PapsFL Fwd, forward *PapsFL1- PapsFL2* primers.

Supplemental Figure 3. In situ hybridization controls.

(A) to (C) Controls for opium poppy. (A) *PapsFL1* sense probe. (B) *PapsFL2* sense probe. (C) Expression of *PapsPI* used to test the specificity of the technique in light of the broad expression of *PapsFL1*; results are similar to those obtained by Drea et al. (2007)

(D) to (F) Controls for California poppy (D) *EscaFL* sense probe. (E) and (F) Expression of *EscaAG* used to test the specificity of the technique in light of the broad expression of *EscaFUL-like* genes; results are similar to those of Zahn et al. (2006).

Scale bars: 50 μ m

Supplemental Figure 4. Locus-specific RT-PCR and qRT-PCR using cDNA prepared from organs of VIGS-treated plants.

(A), (C) and (E) RT-PCR analysis of opium poppy plants transformed with (A) TRV2-*PapsFL1*, (C) TRV2-*PapsFL2* and (E) TRV2-*PapsFL1* and TRV2-*PapsFL2* simultaneously, showing preliminary categories of un-silenced (un), mildly (mild), moderately (mod), and strongly (str) silenced plants. Samples were extracted from leaves (l), leaf-like sepals (ls) or fruits (f) and screened for down-regulation of *PapsFL1* and *PapsFL2* as well as presence of the vector (TRV2). Note specific down-regulation of

PapsFL1 in (A), specific down-regulation of *PapsFL2* in (C) and down-regulation of both copies in (E). Samples of wild type leaf (l), sepal (s) and fruit (f) were used for comparison and *ACTIN* was used as a control.

(B), (D) and (F) qRT-PCR of a subset of leaf samples showing the range of silencing (from mildly to strongly down-regulated) of (B) *PapsFL1*, (D) *PapsFL2* and (F) *PapsFL1* and *PapsFL2* simultaneously. Fold change in *PapsFL1* (black bars) and *PapsFL2* (gray bars) expression in samples is shown relative to the wild type leaf gene expression. Error bars show \pm SD among three technical replicates. *GADPH* was used as the endogenous control.

(G) RT-PCR analysis showing preliminary categories of un-silenced (un), mildly (mild), moderately (mod) and strongly (str) silenced tissue samples from California poppy plants transformed with TRV2-*EscaFL1* and TRV2-*EscaFL2*. Samples were collected from the same organs as opium poppy plants. Treated plants were screened for down-regulation of *EscaFL1* and *EscaFL2* and for presence of the vector (TRV2). Note down-regulation of both *EscaFL1* and *EscaFL2*. Similar wild type samples were used for comparison and *UBIQUITIN* was used as a control.

(H) qRT-PCR of a subset of leaf samples showing the range of silencing (from mildly to strongly down-regulated) of *EscaFL1* and *EscaFL2* simultaneously. Fold change in *EscaFL1* (black bars) and *EscaFL2* (gray bars) expression is shown relative to the wild type leaf gene expression. Error bars show \pm SD among three technical replicates. *GADPH* was used as the endogenous control.

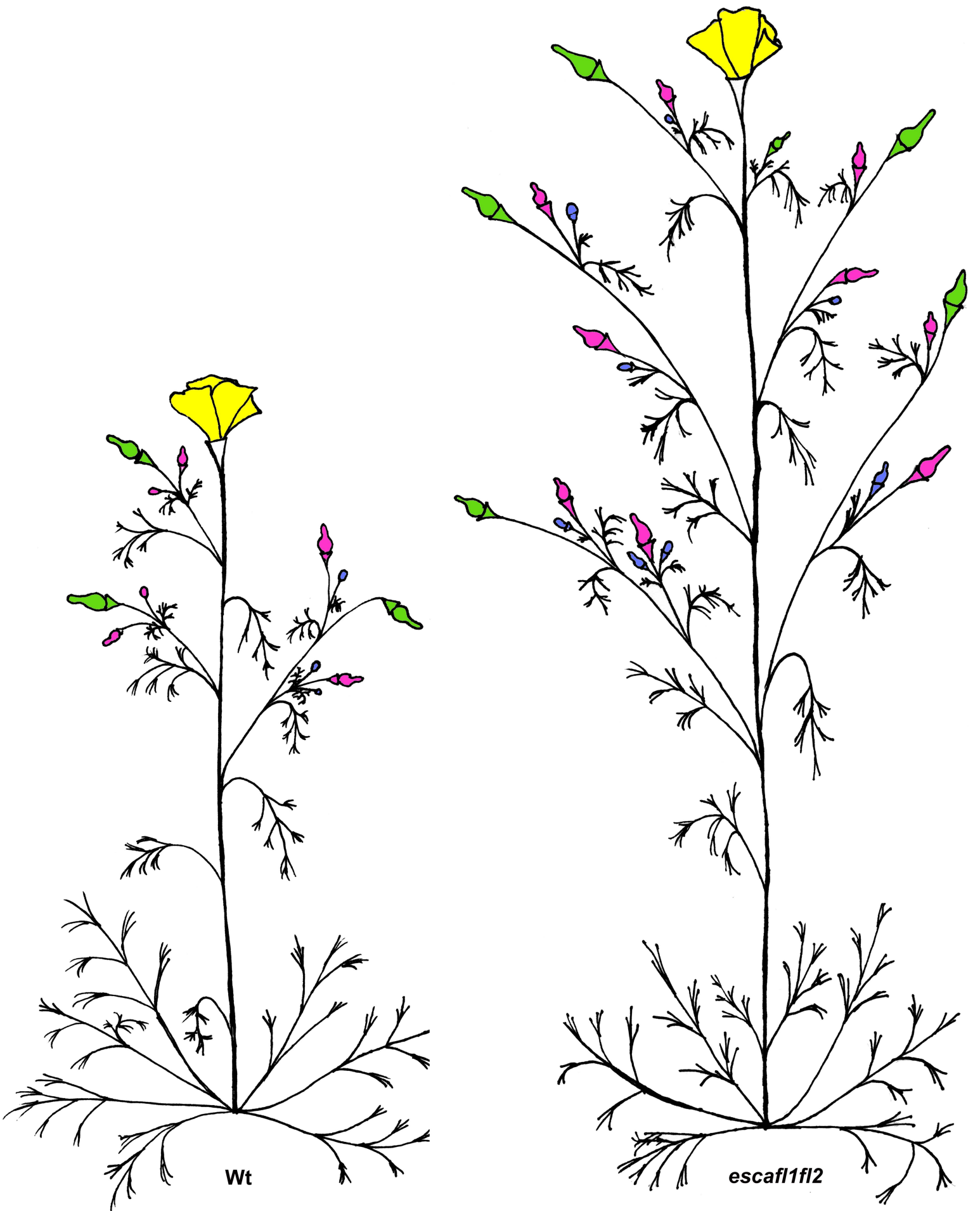
Supplemental Figure 5. Protein interactions between PapsFL1 and PapsFL2 as determined by growth on selective SD medium. Opium poppy proteins were cloned into both activation domain (AD) and binding domain (BK) vectors. Within each column corresponding to an interacting pair, the three columns of colonies represent a dilution series (10^{-5} , 10^{-4} and 10^{-3} colony forming units) of each strain grown on SD medium (-HWL, -AWL and -HAWL). The -HWL medium was supplemented with 2.5 to 30mM 3-amino-1,2,4-triazole.

Supplemental Figure 6. Range of variation of the leaf-like sepal phenotype in *escafl1-fl2* California poppy plants.

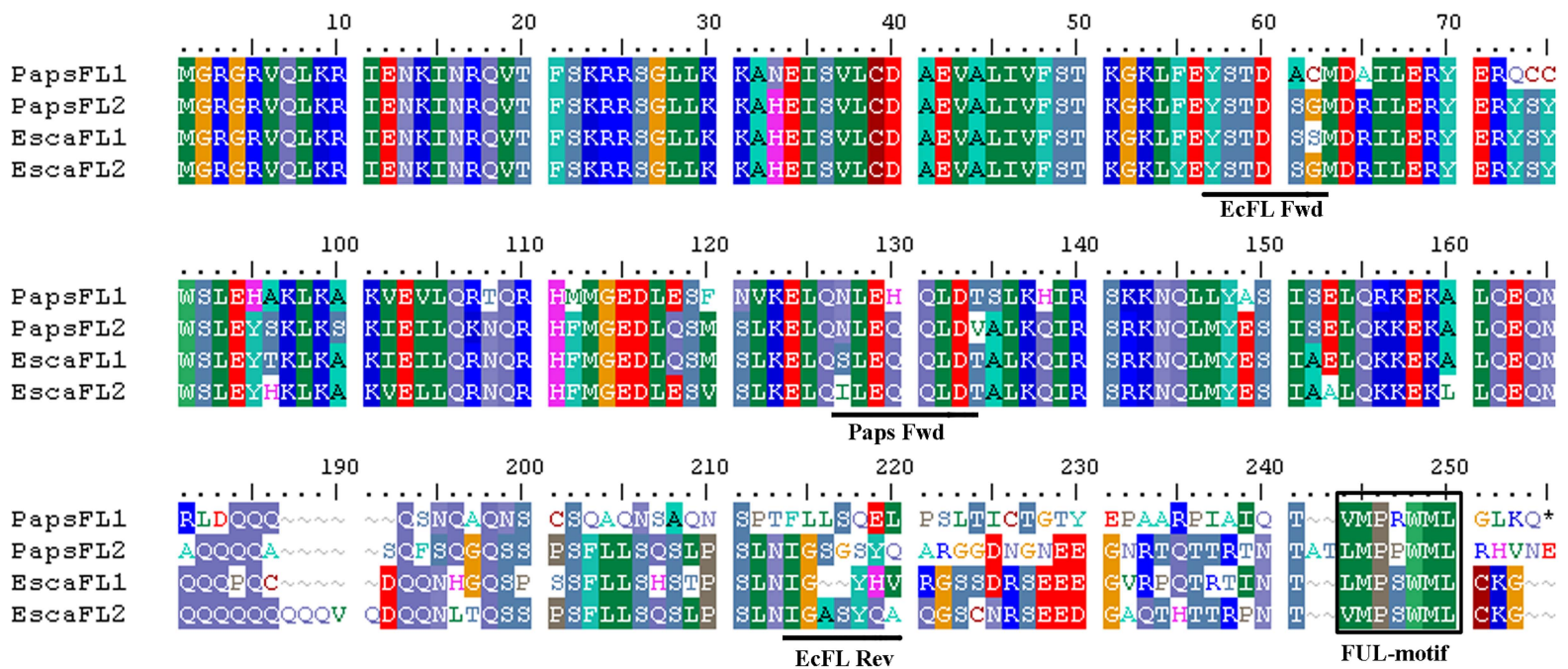
(A) to (C) Slightly abnormal sepals, with leafy edges. In these flowers, floral organs remain trapped inside because sepals are largely fused but not deciduous. Floral cup is visible.

(D) to (I) Strongly abnormal, free (un-fused) sepals with highly dissected leaf-like edges that allow full or partial exposure of the remaining floral organs. Floral cup is present.

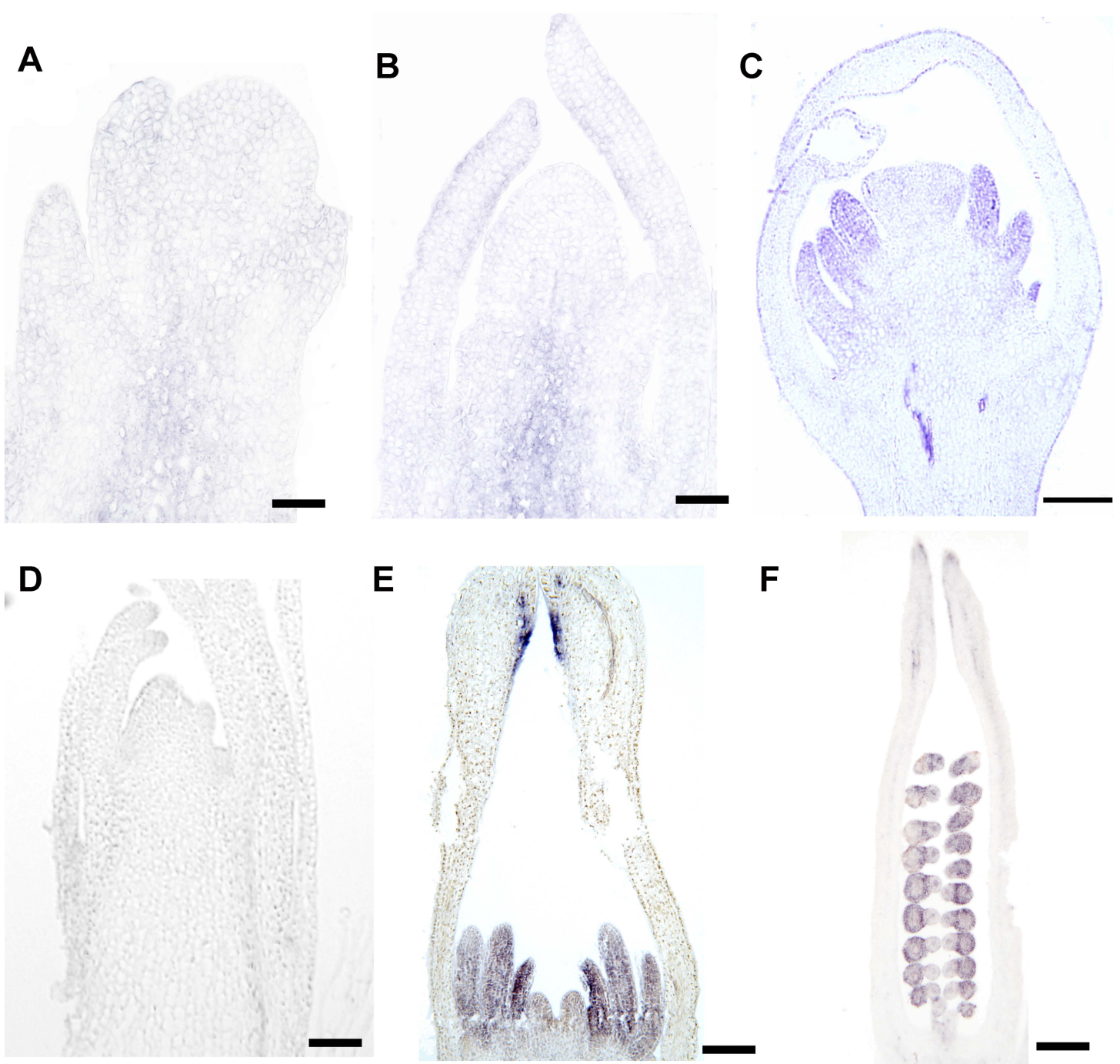
(J) to (L) Complete homeotic transformation of sepals into leaf-like organs, where no remnants of the floral cup are observed. Scale Bars: 0.5 cm



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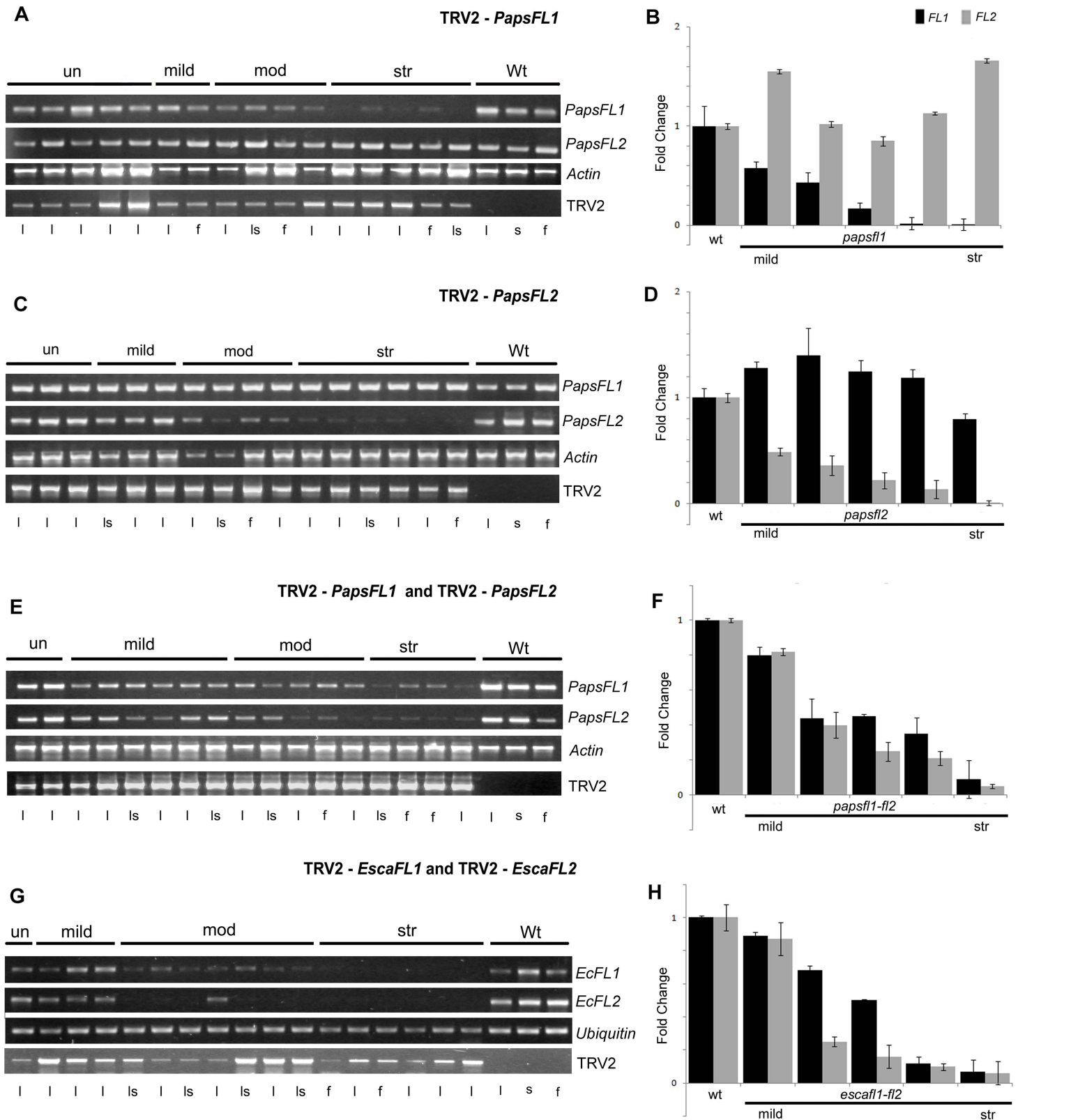
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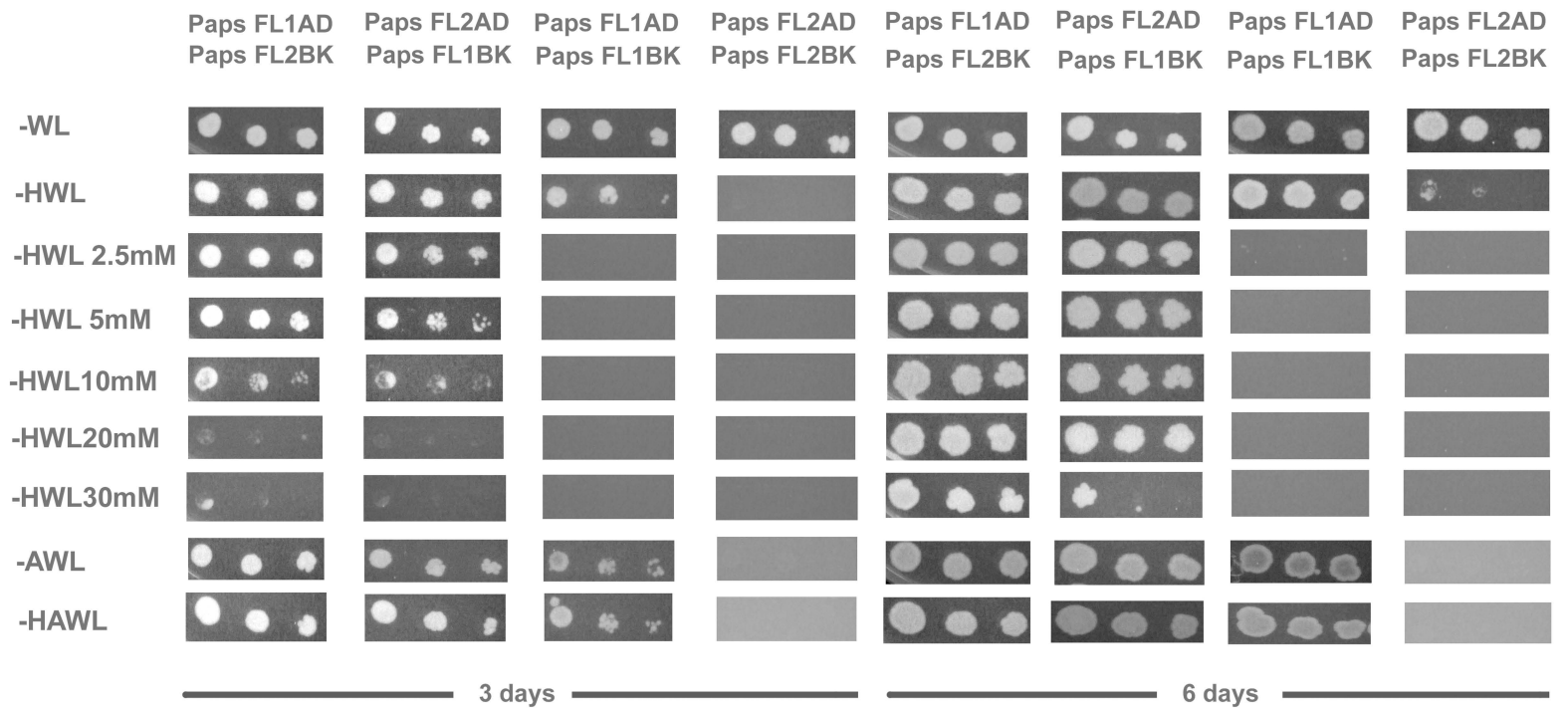
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Supplemental Figure 4. Locus-specific RT-PCR and qRT-PCR using cDNA prepared from organs of VIGS-treated plants. **(A)**, **(C)** and **(E)** RT-PCR analysis of opium poppy plants transformed with (A) TRV2-*PapsFL1*, (C) TRV2-*PapsFL2* and (E) TRV2-*PapsFL1* and TRV2-*PapsFL2* simultaneously, showing preliminary categories of un-silenced (un), mildly (mild), moderately (mod), and strongly (str) silenced plants. Samples were extracted from leaves (l), leaf-like sepals (ls) or fruits (f) and screened for down-regulation of *PapsFL1* and *PapsFL2* as well as presence of the vector (TRV2). Note specific down-regulation of *PapsFL1* in (A), specific down-regulation of *PapsFL2* in (C) and down-regulation of both copies in (E). Samples of wild type leaf (l), sepal (s) and fruit (f) were used for comparison and *ACTIN* was used as a control. **(B)**, **(D)** and **(F)** qRT-PCR of a subset of leaf samples showing the range of silencing (from mildly to strongly down-regulated) of (B) *PapsFL1*, (D) *PapsFL2* and (F) *PapsFL1* and *PapsFL2* simultaneously. Fold change in *PapsFL1* (black bars) and *PapsFL2* (gray bars) expression in samples is shown relative to the wild type leaf gene expression. Error bars show \pm SD among three technical replicates. *GADPH* was used as the endogenous control. **(G)** RT-PCR analysis showing preliminary categories of un-silenced (un), mildly (mild), moderately (mod) and strongly (str) silenced tissue samples from California poppy plants transformed with TRV2-*EscaFL1* and TRV2-*EscaFL2*. Samples were collected from the same organs as opium poppy plants. Treated plants were screened for down-regulation of *EscaFL1* and *EscaFL2* and for presence of the vector (TRV2). Note down-regulation of both *EscaFL1* and *EscaFL2*. Similar wild type samples were used for comparison and *UBIQUITIN* was used as a control. **(H)** qRT-PCR of a subset of leaf samples showing the range of silencing (from mildly to strongly down-regulated) of *EscaFL1* and *EscaFL2* simultaneously. Fold change in *EscaFL1* (black bars) and *EscaFL2* (gray bars) expression is shown relative to the wild type leaf gene expression. Error bars show \pm SD among three technical replicates. *GADPH* was used as the endogenous control.



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Supplemental Table 1. Summary of phenotypes identified using VIGS to silence poppy *FUL-like* genes individually and simultaneously

Construct /(effectiveness ^a)	Phenotypes	N ^o plants
TRV2-PapsFL1/ (27/100)	Branched inflorescences Overgrowth and shape defects in cauline leaves Leaf –like sepals Carpel defects /Premature fruit rupture	21 6 8 4
TRV2-PapsFL2/ (13/80)	Branched inflorescences Overgrowth and shape defects in cauline leaves Leaf –like sepals Carpel defects /Premature fruit rupture	10 5 7 3
TRV2-PapsFL1 and TRV2-PapsFL2/ (15/108)	Delay in reproductive transition Branched inflorescences Overgrowth and shape defects in cauline leaves Leaf –like sepals Mosaic green petals Carpel defects /Premature fruit rupture	12 7 5 5 5 5
TRV2-EscaFL ^b (40/120)	Branched inflorescences Leaf –like sepals Carpel defects /Premature fruit rupture	40 40 4

^aeffectiveness: (Total number of plants down-regulated/ Total number of plants transformed)

^bboth constructs downregulated both gene copies therefore data are combined here.

Supplemental Table 2. Primers used for all the experiments

	Primer name	Primer sequence	
In Situ Hybridization	<i>PapsFL1</i> - F	GCACCAACTTGATACTTCC	
	<i>PapsFL1</i> - R-T7	CTTAATACGACTCACTATAGGGTGATCCCTGATTGCTTTCTT	
	<i>PapsFL2</i> - F	AAGAGCTCCAAAATCTAG	
	<i>PapsFL2</i> - R-T7	CTTAATACGACTCACTATAGGGAGATACAACATTACATGCA	
	<i>PapsPI</i> F	AGTGGAAATTCATGGAAGAGG	
	<i>PapsPI</i> R T7	CTTAATACGACTCACTATAGGGAGTTATAGTAGCAGCTATGATC	
	<i>EcFL1</i> - F	GGAAAACCTTTGAATACTCTACTGATTCCAGCA	
	<i>EcFL1</i> - R-T7	CTTAATACGACTCACTATAGGGTCGCTGCTGCCTCGAACATGGTA	
	<i>EcFL2</i> - F	GCTCTATGAATACTCCACTGATTCTGGTA	
	<i>EcFL2</i> - R-T7	CTTAATACGACTCACTATAGGGTCCTTGCGCCTGATAACTTG	
	<i>EcAG</i> F	GCAACCAAACCTGCGTCAACAAATCG	
	<i>EcAG</i> R T7	CTTAATACGACTCACTATAGGGTGTCTGCTCCTGGTGGGAGTAATG	
Yeast 2 Hybrid	<i>PapsFL1</i> - <i>NdeI</i>	CATATGGGAAGAGGTAGGGTTCAGCTGAAG	
	<i>PapsFL1</i> - <i>EcoRI</i>	GAATTCCTATTGTTTGAGACCAAGCATCCA	
	<i>PapsFL2</i> - <i>NdeI</i>	CATATGGGAAGAGGTAGGGTTCAGCTGAA	
	<i>PapsFL2</i> - <i>EcoRI</i>	GAATTCCTCATTAAACATGGCGAAGCATCCA	
VIGS Insert	<i>EcFL1</i> - <i>KpnI</i> F	GGTACCGGAAAACCTTTGAATACTCTACTGATTCCAGCA	
	<i>EcFL1</i> - <i>SacI</i> R	GAGCTCTCGCTGCTGCCTCGAACATGGTAA	
	<i>EcFL2</i> - <i>KpnI</i> F	GGTACCGCTCTATGAATACTCCACTGATTCTGGTA	
	<i>EcFL2</i> - <i>SacI</i> R	GAGTCCCTTGCGCCTGATAACTGCACCAATG	
	<i>PapsFL1</i> - <i>EcoRI</i> F	GAATTCCTGGAGCACCACACTTGATACTTCCT	
	<i>PapsFL1</i> - <i>XbaI</i> R	TCTAGAAAAGCACTGACCGTGTTCATG	
	<i>PapsFL2</i> - F	AAGAGCTCCAAAATCTAGA	
	<i>PapsFL2</i> - <i>BamHI</i> R	GGATCCGAATAGAGATACAACATTACATGCATG	
Locus specific RT-PCR	TRV2 - pYL156 F	GGTCAAGGTACGTAGTAGAG	
	TRV2 - pYL156R	CGAGAATGTCAATCTCGTAGG	
	<i>ACTIN</i> F	GATGGATCCTCCAATCCAGACACTGTA	
	<i>ACTIN</i> R	GTATTGTGTTGGACTCTGGTGATGGTGT	
	<i>PapsFL1</i> F	CAATAGAAAGATACTTACTTCCATGGT	
	<i>PapsFL1</i> R	AAGCACTGACCGTGTTCATG	
	<i>PapsFL2</i> F	GTAACCGAATAATCTCATATCTATCTC	
	<i>PapsFL2</i> R	GAATAGAGATACAACATTACATGCATG	
	<i>UBIQUITIN</i> F	AACCCTTGAGGTTGAATCATCC	
	<i>UBIQUITIN</i> R	GTCTTCTTTCTGGTAAACGT	
	<i>EcFL1</i> - F	GGAAAACCTTTGAATACTCTACTGATTCCAGCA	
	<i>EcFL1</i> - R	TCGCTGCTGCCTCGAACATGGTAA	
	<i>EcFL2</i> - F	GCTCTATGAATACTCCACTGATTCTGGTA	
	<i>EcFL2</i> -R	TCCTTGCGCCTGATAACTTG	
	VIGS Down-regulation screen	<i>PapsFL1</i> F	GATTTAGAATCCTTCAATGTGA
		<i>PapsFL1</i> R	AAGCACTGACCGTGTTCATG
<i>PapsFL2</i> F		GACTTACAATCCATGAGTCTTAAA	
<i>PapsFL2</i> R		GAATAGAGATACAACATTACATGCATG	
<i>EcFL1</i> - F		TCTCTGTTCTTTGTGATGCTGAAGT	
<i>EcFL1</i> - R		TTGATGGTTCTAGTCTGTGGTCGAA	
<i>EcFL2</i> - F		GAAGTTGCTTTAATTGTCTTCTCTA	
<i>EcFL2</i> -R		AGGCCTCGTCGTATGTGTTGTGC	
qRT-PCR	<i>PapsFL1</i> F	TTATGCCACGGTGGATGCT	
	<i>PapsFL1</i> R	ACAGATCTATATGAAACATCCATCAACA	
	<i>PapsFL2</i> F	GAAGAAGGGAATCGAACTCAGACT	
	<i>PapsFL2</i> R	TGCGACGGATAGACACCCA	
	<i>EcFL1</i> - F	ACAGCAGCAACCTCAGTTGA	
	<i>EcFL1</i> - R	TGCTGCCTCGAACATGGTAA	
	<i>EcFL2</i> - F	ATAACCCAGCAGCAGCAGCAGCA	
	<i>EcFL2</i> -R	GTATGTGTTTGTGCTCCATCTGTGTT	
	<i>GADPH</i> -F	GCTTCCTTCAACATCATTCC	
<i>GADPH</i> -R	AGTTGCCTTCTTCTCAAGTC		