Supporting Information

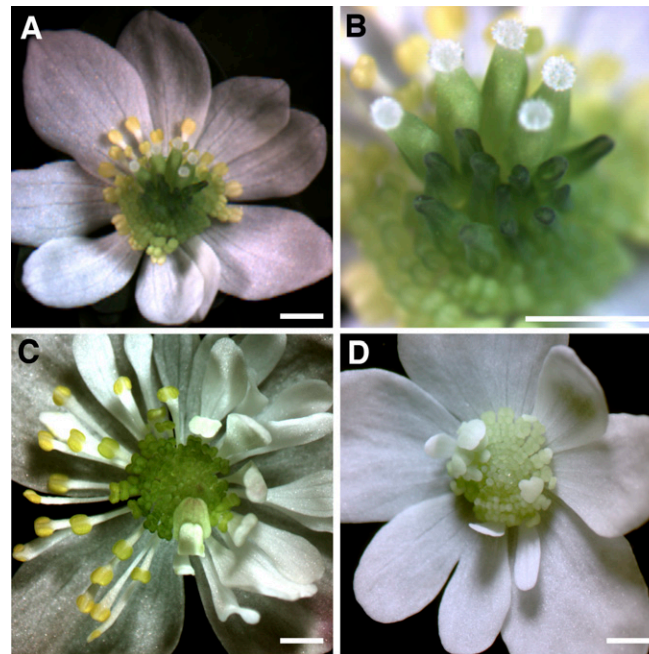## Galimba et al. 10.1073/pnas.1203686109

Fig. S1. Incomplete silencing effects on TRV2-*ThtAG1*–treated flowers of *Thalictrum thalictroides*. (*A*) Weak carpel phenotype consisting of open carpels without stigmas in lower half of flower center. (*B*) Detail of flower center in *A*. (*C*) Partial homeotic conversion of stamens in right half of the flower; (*D*) Homeotic organs among stamens. (Scale bars: 1 mm.)
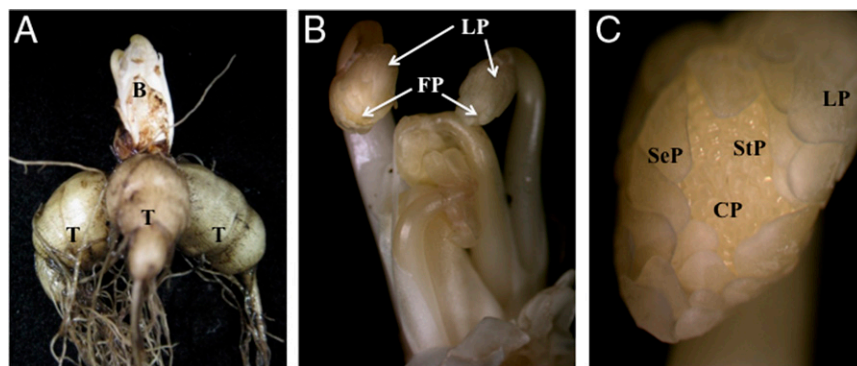

Fig. S2. Tubers with floral primordia. (*A*) Large cluster of radially arranged tubers (T) supporting one bud (B) on the proximal end, containing primordia protected by enclosing bracts. (*B*) Floral (FP) and leaf (LP) primordia in various stages of development with bracts removed. (*C*) Floral primordium showing differentiated carpel (CP), stamen (StP), and sepal (SeP) primordia, with leaf primordia (LP) enveloping the flower.
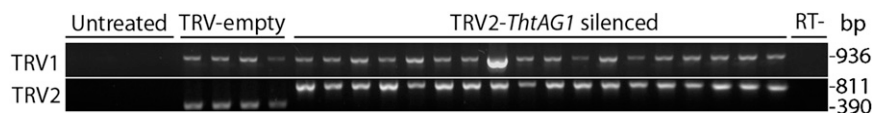

Fig. S3. Detection of TRV transcripts in virus-induced gene silencing (VIGS)-treated flowers showing homeotic phenotypes. RT-PCR with TRV1- and TRV2-specific primers on untreated (*n* = 4), mock-treated (*n* = 4), and VIGS-treated silenced plants (*n* = 18). The smaller band in TRV2-empty plants corresponds to the distance between primers spanning the MCS region in the absence of a target-gene insert; the difference between this band and the bigger band in treated plants corresponds to the length of the target-gene insert (421 bp). A reverse-transcription control confirms the lack of genomic DNA contamination. Size of PCR products, in base pairs (bp), is indicated on the right side.
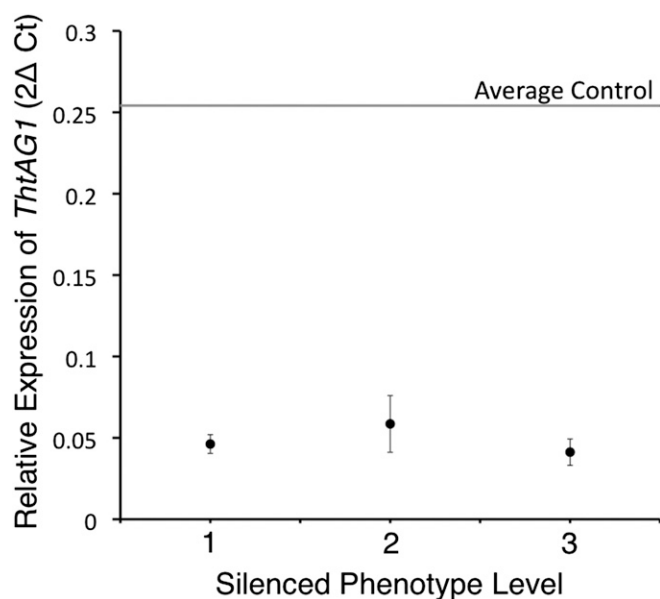
Fig. S4. Relative levels of *ThtAG1* transcript by quantitative PCR in VIGS flowers with increasing intensity of homeotic phenotypes. TRV2-*ThtAG1*–treated flowers in Fig. 3 have been divided according to the intensity of their homeotic phenotype. Mean and SE of phenotype level 1 ($n = 9$), level 2 ($n = 20$), and level 3 ($n = 6$) are shown, normalized with the housekeeping genes *ThtACTIN* and *ThtEEF1*. Average control line represents the mean expression of untreated and empty TRV2 plants (in Fig. 3). The different VIGS phenotypes express comparable amounts of *ThtAG1*. Differences were not significant in a one-way ANOVA; $P = 0.78$, $F = 0.25$. See main text for description of phenotype categories.

BD-ThtSEP3/ AD-Δ

Fig. S5. Interactions among *Thalictrum* MADS-domain proteins as determined with the yeast two-hybrid system. Colony growth on selective -Leu/-Trp/-His + 3 mM 3-AT medium is shown. As in Fig. 5, yeast cells were spotted in 10-fold serial dilution (from left to right) for each interaction tested. Constructs that expressed proteins as fusions with the GAL4 activation domain (AD) are shown horizontally; constructs that express GAL4 binding-domain (BD) fusions are shown vertically. Δ, negative controls in which empty vectors that did not contain a MADS-box gene cDNA insert were used.

Table S1. Primers for amplification and sequencing of *ThtAG1* genomic locus from *T. thalictroides* wild type and 'Double White'

Primer name	Primer sequence
ThtAG1int-cloneF4	5′-GCAAACGAGAGAGCCCCAGAACAT-3′
ThtAG1int-cloneR4	5′-TATAAGATAGGGAGAGATTAAGACACGGCAC-3′
ThtAG1int-cloneF5	5′-CAGCAAGAAGCGTCAAAACTG-3′
ThtAG1int-cloneR5	5′- GCATATACTCTATCTCAGCAAACAGC-3′
ThtAG1int-cloneF6	5′-AACTCTGGATCTGTTTCTGAAGCTAATG-3′
ThtAG1int-cloneF7	5′-GTCTTCTCTACGCGTGGTCG-3′
ThtAG1int-cloneR7	5′-CATTAGCTTCAGAAACAGATCCAGAGTT-3′
ThtAG1int-cloneF8	5′-ATTTCTCTCTCTCTCTTGCTTTC-3′
ThtAG1int-cloneR8	5′-GCCATCTCTTGGTCTATTACTCC-3′
ThtAG1int-cloneF9	5′-ATGCTTCACATAGAACCTCG-3′
ThtAG1int-cloneR9	5′-TTGGTTGCTTCCTGATAGTC-3′
ThtAG1int-cloneF10	5′-CCAAAGGGTAAGAATCAAGACC-3′
ThtAG1int-cloneR10	5′-GCACATTATGCTCTTGGG-3′

Table S2. Primers for detection of expressed alleles of *ThtAG1* in individual plants of *Thalictrum thalictroides* wild type, 'Double White,' and 'Snowball'

Primer sequence	Description
5′-GCTAATGTGCAGTTTTACCAGC-3′	AG1_allele_F1: binding in the Δ3 region (FYQ), designed to amplify only when the FYQ sequence is present
5′-AAAGGGTGCAGAAGACATGAC-3′	AG1_allele_R1_all: binding downstream of both deletions, designed to amplify all alleles
5′-ATTCGGCTTCAAAGAAAAATG-3′	AG1_allele-F2_all: binding upstream of both deletions, designed to amplify all alleles
5′-GCTCCCTGATATTGAGATTGC-3′	AG1_allele_R2: binding in Δ13 region. Designed to amplify only when Δ13 deletion is absent
5′-AGACTCCCCCAACTCTGGAT-3′	AG1 allele test forward:: binding upstream of both deletions
5′-TCTTTTTGGCTCGGATTTTG-3′	AG1 allele test reverse: binding downstream of both deletions

Table S3. Primers for cloning of *T. thalictroides* MADS-box gene cDNAs into yeast two-hybrid vectors

Primer sequence	Description
5′-TAGCGC*GAAT TC*ATGGGAAG GGGAAAGATT GAAA-3′	Binding at the beginning of the MADS box of *ThtAG1*; used for cloning of *ThtAG1; ThtAG1*$_{\Delta3}$*; ThtAG1*$_{\Delta8}$*; ThtAG1*$_{\Delta3\Delta13}$.
5′-AATCGC*GGAT CC*CAAAAGCT TCACAGAAT ATCACC-3′	Binding at the end of the coding sequence and in the 3′ UTR of *ThtAG1*; used for cloning of *ThtAG1; ThtAG1*$_{\Delta3}$*; ThtAG1*$_{\Delta8}$*; ThtAG1*$_{\Delta3\Delta13}$.
5′-AGTGTAGAAT TCAG*CCATGG* GAAGGGGAAA GATTGAA-3′	Binding at the beginning of the MADS box of *ThtAG1*; used for cloning of *ThtAG1; ThtAG1*$_{\Delta3\Delta13}$.
5′-ACTGCA*GTCG AC*CACAGAAT ATCACCCAAG TTG-3′	Binding at the end of the coding sequence and in the 3′ UTR of *ThtAG1*; used for cloning of *ThtAG1; ThtAG1*$_{\Delta3\Delta13}$.
5′-CCTAGT*GAAT TC*ATGGGAAG AGGAAGAGTT GAGT-3′	Binding at the beginning of the MADS box of *ThtSEP3*; used for cloning of *ThtSEP3* and *ThtSEP3ΔC*.
5′-AGTCGAGAAT TCAG*CCATGG* GAAGAGGAAG AGTTGAG-3′	Binding at the beginning of the MADS box of *ThtSEP3*; used for cloning of *ThtSEP3*.
5′-TAGCTT*GAGC TC*TTAAGGTA CGAGAGACAC CACT-3′	Binding at the 3′ UTR of *ThtSEP3*; used for cloning of *ThtSEP3*.
5′-CTATCA*GTCG AC*CTTGCGGA TCAGCTTCAG C-3′	Binding at the 3′ UTR of *ThtSEP3*; used for cloning of *ThtSEP3*.
5′-AAGCTT*GGAT CC*GTCGACTT AATTTGGTTG GCTACCTTCC TC-3′	Used for cloning of *ThtSEP3ΔC*

Restriction enzyme recognition sites used for cloning are in italics.