Supplementary Material: Yant L et al. Molecular basis for three-dimensional elaboration of the *Aquilegia* petal spur.

Supplementary Methods

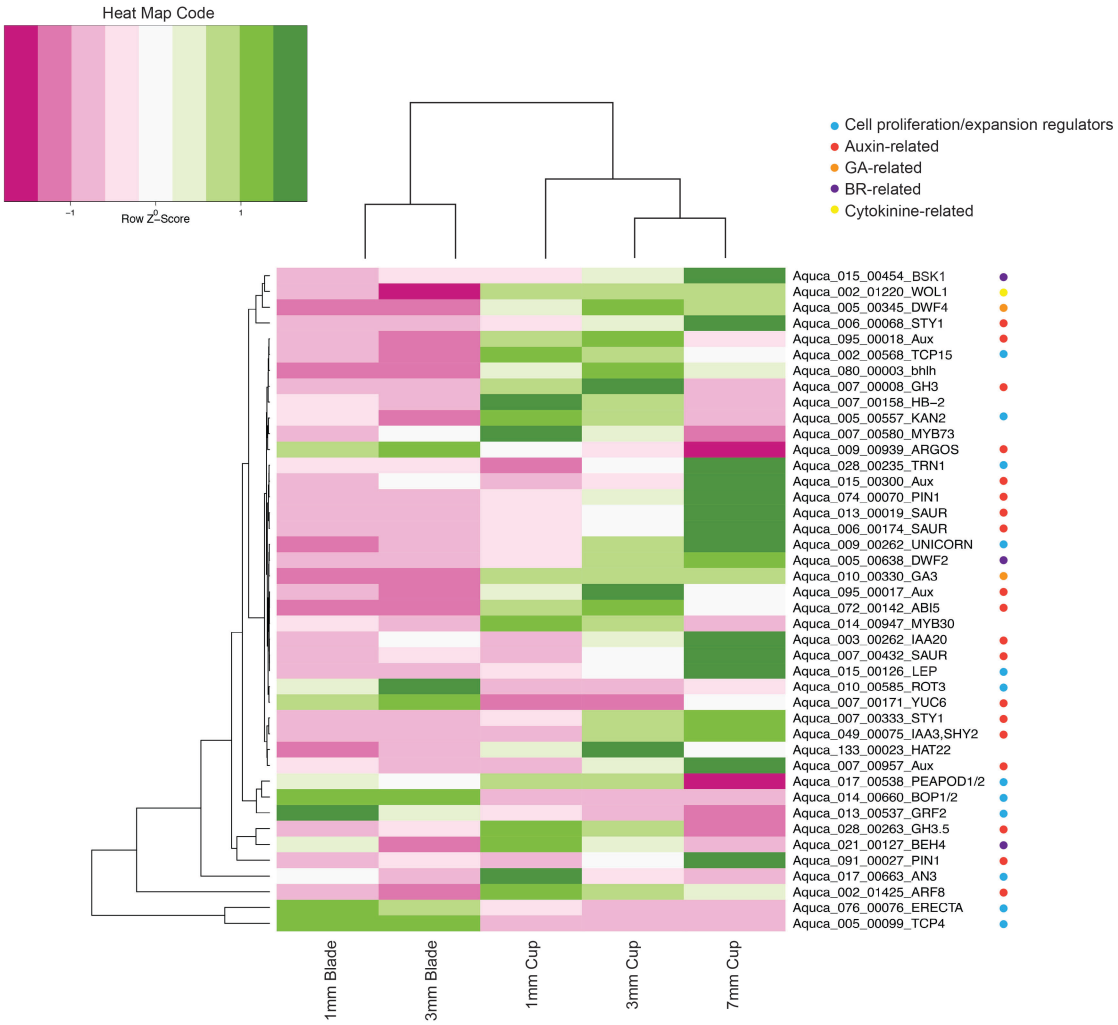
Identification of additional candidate genes. Nine *Aquilegia* homologs of the KNOX homeodomain family were identified: Aquca_002_00078.1, Aquca_002_00080.1, Aquca_003_00549.1 (*AqKXL1*), Aquca_012_00046.1 (*AqSTM1*), Aquca_013_00468.2 (*AqKXL3*), Aquca_016_00110.1 (*AqKXL4*), Aquca_032_00056.1, Aquca_032_00057.1 and Aquca_133_00021.1 (*AqKN*). The pairs of Aquca_002_00078.1/Aquca_002_00080.1 and Aquca_032_00056.1/Aquca_032_00057.1 appear to represent the respective 5' and 3' ends of single loci (termed *AqKXL2* and *AqSTM2*, respectively) that correspond to separate ESTs. In both cases, manual examination of the genome sequence allowed prediction of a contiguous reading frame, which was confirmed using RT-PCR followed by Sanger sequencing. For the TCP genes, we identified thirteen annotated loci as TCP family members but only three of these fall into class I of the TCP-C subfamily based on characteristics outlined in Navaud et al. 2007 (24): Aquca_005_00099 (*AqTCP4*), Aquca_057_00119.1 (*AqTCP5*) and Aquca_002_00878.1 (*AqTCP2*). In order to establish orthology of all these sequences, phylogenetic analyses were performed using RAxML on amino acid alignments of KNOX and TCP family homologs from across the angiosperms (electronic supplementary material, figure S2). The highly supported clades in each tree are generally consistent with canonical angiosperm relationships, with representation from both monocots and dicots. Clearly, both of these families have experienced multiple ancient duplication events that gave rise to various broadly represented lineages. The predicted KNOX and TCP cDNAs are deposited in GenBank under accession numbers KC854334-KC854336 and KP331533-KP331539.

AqSTM1 expression is localized to actively dividing regions of meristems and compound leaves but not petals

Our first step in characterizing the *Aquilegia AqSTM1* locus was to examine its expression in vegetative apices and developing compound leaves. We used *in situe* hybridization of *AqHIS4* to determine the correspondence between *AqSTM1* expression and localized cell divisions. Consistent with what has been found in other dicots, we observed close association between the localization of *AqHIS4* and *AqSTM1* in both the shoot apical meristem and early developing compound leaves, especially the tips of early primordia and leaflets of older leaves (electronic supplementary material, figure S3*a-c* and *d-f*). The one exception to this overlap was the down-regulation of *AqSTM1* in the flanks of some meristem sections (electronic supplementary material, figure S3*f*). This indicates that *AqSTM1* is down-regulated in incipient leaf primordia but then is quickly reactivated once compound leaf development begins.

The next step was to determine whether the association of *AqSTM1* expression with regions of prolonged cell division would also be observed in petals. Hybridization to inflorescences revealed broad expression in young stage 2 floral buds in which no primordia had initiated (electronic supplementary material, figure S4*a*). By stage 3, when sepal primordia had formed, the signal is restricted to the internal region of the meristem with no signal in the young sepals (electronic supplementary material, figure S4*b*).

Similarly, *AqSTM1* progressively disappears from the petal, stamen and staminodium primordia as they are formed, but can still be detected in the apical domain of the floral meristem until it is finally consumed by carpel initiation (electronic supplementary material, figure S4*c*). No expression is observed in developing petals (electronic supplementary material, figure S4*d*-*g*). The expression of *AqKN* is very similar to that observed for *AqSTM1* (electronic supplementary material, figure S5).



Gene Name	Locus	1mmC	1mmB	3mmC	3mmB	7mmC
AqSTM1	Aquca_012_00046.1		Filtered due to low expression			
AqSTM2	Aquca_032_00056.1/57.1		Filtered due to low expression			
AqKN	Aquca_133_00021.1		Filtered due to low expression			
AqKXL1	Aquca_003_00549.1	1	2	1	1	0
AqKXL2	Aquca_002_00078.1/80.1		Filtered due to low expression			
AqTCP4	Aquca_005_00099	305	1063	270	984	179
AqAN3	Aquca_017_00663	693	272	129	54	20
AqARF3	Aquca_036_00025	192	91	237	156	501
AqERECTA	Aquca_076_00076	485	1480	279	1205	184
AqAP3-3	Aquca_007_00336	234	227	281	269	502
AqAG1	Aquca_136_0010.1/09.1		Filtered due to low expression			

Figure S1. Expression of selected genes discussed in the text and coregulated family members. Heat map shows clustered cpm values for each tissue sample after averaging biological triplicates. Each locus is marked with a colored dot to indicate its associated developmental module, as inferred by homology to *Arabidopsis* sequences. The table shows cpm values for all five type I KNOX genes as compared to other DE loci that are

more highly expressed. For reference, we also included an organ identity gene known to control petal identity, *AqAP3-3*, and one that does not, *AqAG1*. Clustering was performed with the heatmap function in R3.1.

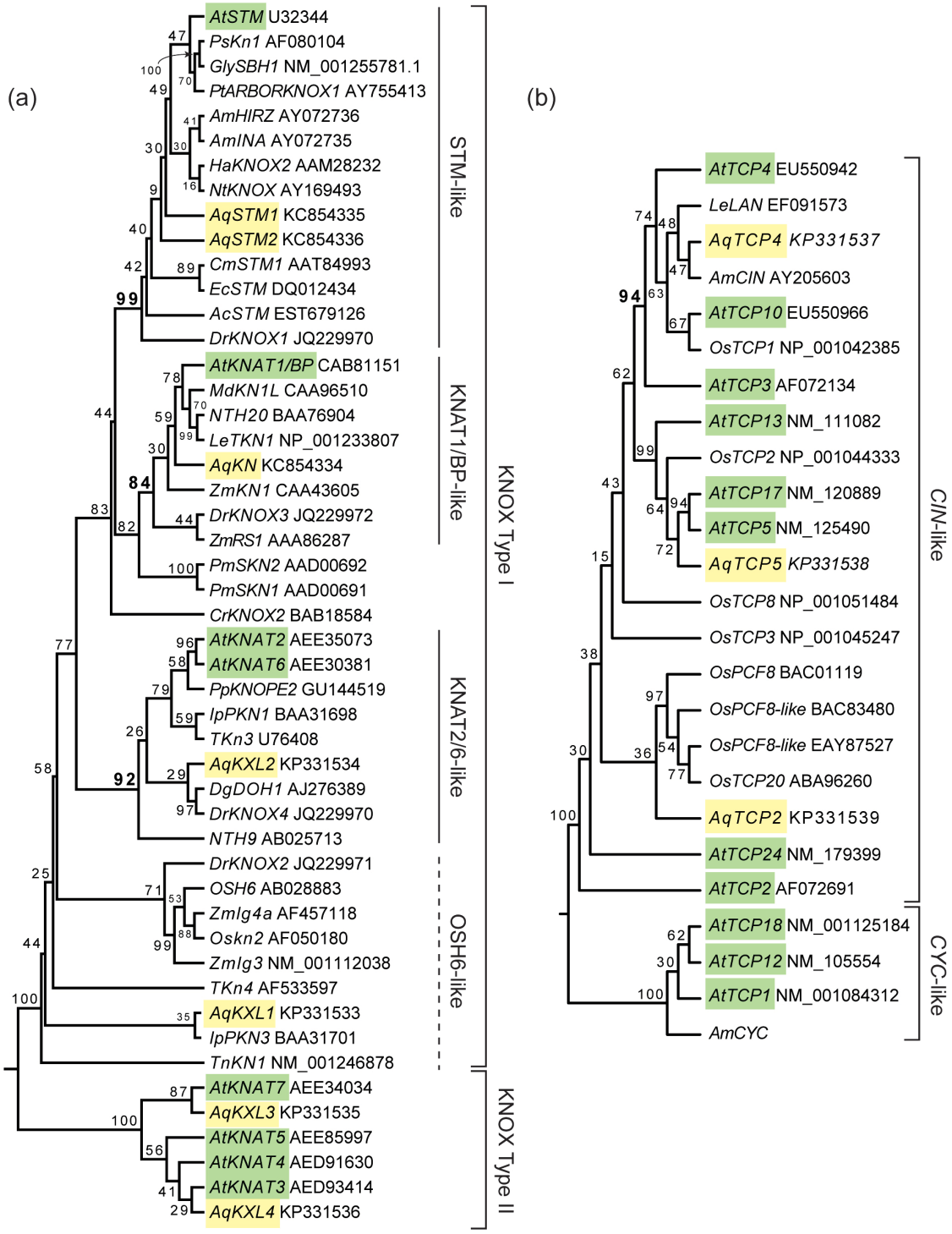


Figure S2. Phylogenetic analysis of *Aquilegia* KNOX and TCP family members *(a)* Maximum likelihood analysis of type I KNOX gene homologs with ML bootstrap values shown at the nodes. Type II KNOX sequences are used as outgroup. Critical support

values for *STM* and *KN* clades are shown in bold. *(b)* Maximum likelihood analysis of *CIN*-like TCP gene homologs with ML bootstrap values shown at the nodes. *CYC*-like TCP sequences are used as outgroup. Critical support value for *TCP4* clade is shown in bold. Key to taxonomic abbreviations: Ac = *Allium cepa*; Am = *Antirrhinum majus*; At = *Arabidopsis thaliana*; Aq = *Aquilegia coerulea* 'Origami'; Cm = *Chelidonium majus*; Cr = *Ceratopteris richardii*; Dg = *Dendrobium* grex Madame Thong-In; Gly = *Glycine max*; Ha = *Helianthus annus*; Ip = *Ipomoea nil*; Le = *Lycopersicon esculentum*; Md = *Malus domestica*; Nt = *Nicotiana tabacum*; Os = *Oryza sativa*; Pm = *Picea mariana*; Ps = *Pisum sativa*; Pt = *Populus tremula*; Zm = *Zea mays*. All loci are followed by their GenBank accession numbers.

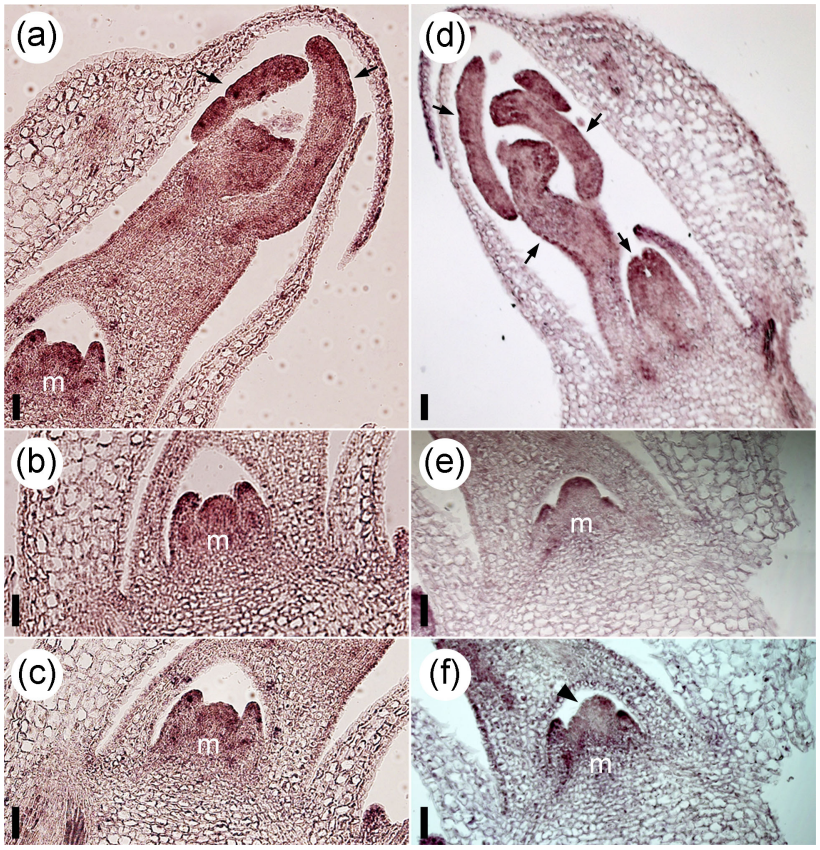


Figure S3. *AqHIS4* and *AqSTM* expression in young leaves and vegetative meristems. (*a-c) AqHIS4* expression as a marker of cell division. Signal is detected in the apical meristem (m) and in developing leaflets (arrows). (*d-f) AqSTM* expression is detected in the distal regions of complex leaves (arrows in *d*) and in the shoot apical meristem (m in *e-f*). *AqSTM* expression appears to be down-regulated in the flanks of the meristem in some cases (arrowhead in *f*), most likely in association with the initiation of new leaf primordia. Size bars = 100 μm.

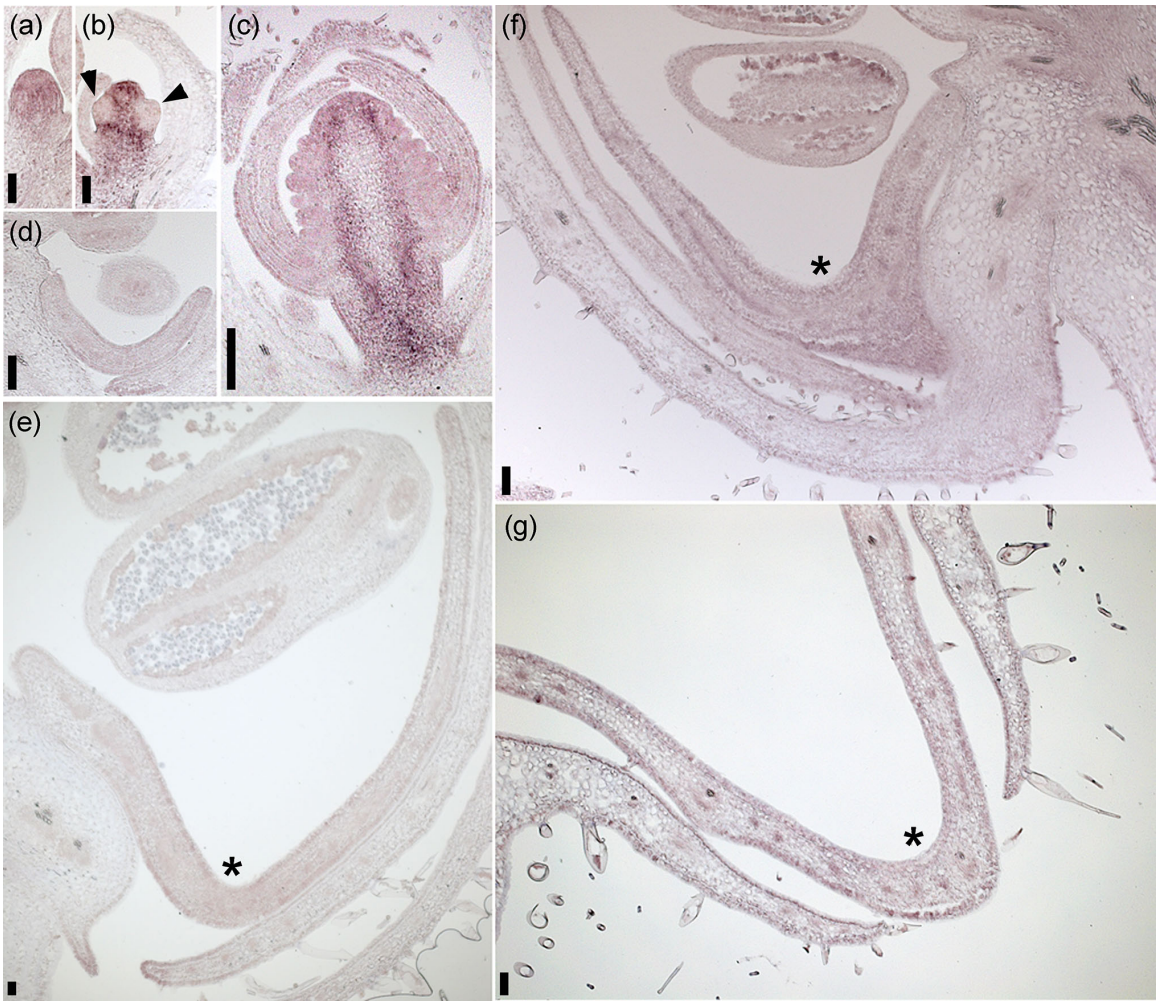


Figure S4. *AqSTM* expression in developing floral meristems and petal primordia. (*a)* Stage 1 floral meristem. *(b)* Stage 3 floral meristem showing down-regulation of *AqSTM* in the incipient sepals (arrowheads). *(c)* Stage 6 floral meristem. *AqSTM* is absent from primordia but still detectable at apex where carpel initiation is yet to occur. *(d)* Petal primordium from a stage 8 meristem. *(e)* Petal primordium from a stage 10 meristem. *(f)* Petal primordium from an early stage 11 meristem. *(g)* Petal primordium from a late

stage 11 meristem. Asterisks in *f-g* indicate the developing nectar spur. Size bars = 50 μm in *a-b*, 100 μm in *c-g*.

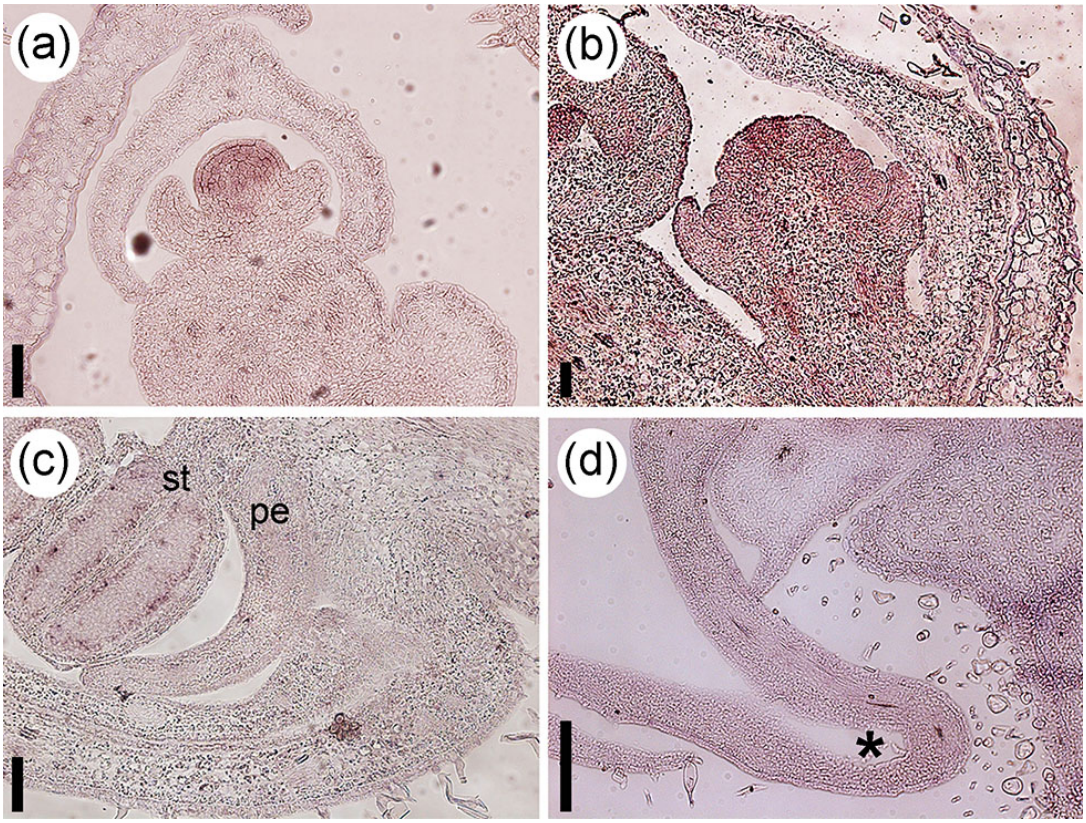


Figure S5. *AqKN* expression in developing floral meristems and petal primordia. *(a)* Stage 3 floral meristem. *(b)* Stage 5 floral meristem. *(c)* Stamen (st) and petal (pe) primordia in stage 8 floral meristem. *(d)* Petal spur (asterisk) in early stage 11 floral meristem. Size bars = 50 µm in *a*-*b*, 100 µm in *c*, 1 mm in *d*.

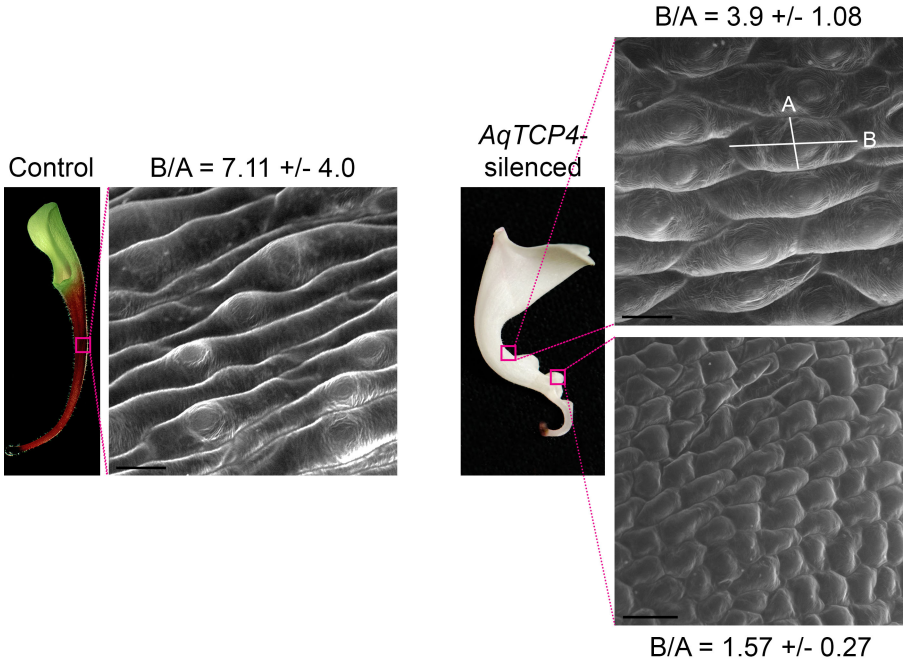


Figure S6. Cell morphology of control and *AqTCP4*-silenced petal spur epidermal cells. In control petals (left), the spur epidermal cells are greatly elongated with a long axis-to-short axis ratio (B/A) of 7.11 +/- 4.0. In *AqTCP4*-silenced petals (right), the undistorted regions of the spur have reduced elongation (top), with B/A = 3.9 +/- 1.08, but the cells in the epidermis of the outgrowths are much smaller and more isotropic (bottom), with B/A = 1.57 +/- 0.27. Cells were measured from 3 control petals and 6 silenced petals as described in Puzey et al. 2011. Size bars = 20 μm in control and top silenced panel, = 50 μm in bottom silenced panel.

Table S1. Primer sequences for *in situ* probe preparation and qRT-PCR

***In situ* Probe Primers**			
Locus	**Primer Sequence**	**Full length**	**Hydrolyzed length**
AqHIS4	F 5' AAGGCGTGGTGGTGTTAAGCGTATCA R 5' GAATTACAAGAAAGTAGTAGATCAGAATCCAAC		
AqTCP4	F 5' TCCCATTGCTGCTGCTACATC R 5' TGAAGACAAATCCTCCTCCACCTC	344 bp	200 bp
AqSTM1	F 5' ATTATCCAAGGCTCTTAGCTTG R 5' CCGGTCAAAAGCATCACCAC	308 bp	200 bp
AqKN	F 5' TCAAGGAGGAGAAAGGATTGG R 5' CTCGTGGATCAATTTCAGGAAC	473 bp	75
AqTCP4 (qRT-PCR)	F 5' CCTGTTTAGGTTGGACTCTATGAGCTCTAC R 5' GCATCAGCCATCTTTGTTGGTTTACT	196 bp	n/a

Table S2. Floral developmental stages in *Aquilegia* and *Arabidopsis thaliana* (Ballerini *et al.*, 2011).

***Arabidopsis* and *Aquilegia* floral developmental stages**		
Stage	***Arabidopsis***	***Aquilegia***
1	Floral buttress arises	Pre-floral meristem arises
2	Flower primordium forms	Two bracteole meristem
3	Sepal primordia arise	Sepals arise (true floral meristem)
4	Sepal overlie flower meristem	Petal primordia arise
5	Petal and stamen primordia arise	Stamen primordia begin arising
6	Sepals enclose bud	Sepals enclose bud, stamen primordia continue appearing
7	Long stamen primordia stalked at base	Carpels initiate, staminodia distinguishable, first whorl of stamens becoming stalked at base
8	Locules appear in long stamens	Petal primordia begin to differentiate, first whorl of stamens begin to differentiate locules, folded carpels remain open
9	Petal primordia stalked at base	Petals continue to differentiate and elongate to same length as first whorl stamens, all stamens differentiating, staminodia still filamentous, carpels elongated to same height as innermost stamens but remain open, staminodia begin to flatten
10	Petals level with short stamens	Spur formation initiates on petals, all organs elongating
11	Stigmatic papillae appear	Spur elongation continues, carpels close, stamens become apiculate
12	Petals level with long stamens	Spurs and all floral organs reach final length
13	Bud opens, petals visible, anthesis	Sepals undergo final expansion and reflex, anthesis

Table S3. Number of Illumina reads passing quality filter in each replicate

sample	reads passing quality filter
1mm blade (Bioreplicate 1)	31,897,244
1mm blade (Bioreplicate 2)	24,424,763
1mm blade (Bioreplicate 3)	18,322,717
1mm cup (Bioreplicate 1)	45,808,529
1mm cup (Bioreplicate 2)	35,248,356
1mm cup (Bioreplicate 3)	52,029,980
3mm blade (Bioreplicate 1)	67,593,830
3mm blade (Bioreplicate 2)	54,564,247
3mm blade (Bioreplicate 3)	27,589,892
3mm cup (Bioreplicate 1)	58,419,420
3mm cup (Bioreplicate 2)	66,893,683
3mm cup (Bioreplicate 3)	23,460,945
7mm cup (Bioreplicate 1)	31,767,957
7mm cup (Bioreplicate 2)	42,649,438
7mm cup (Bioreplicate 3)	54,111,734
total:	634,782,735

Table S4. Number of genes analyzed after filtering

	1mmBC	3mmBC
predicted gene models in genome	24823	24823
genes with any reads in library	24819	24817
remove low abundant genes	16393	16515

Table S5 genes significantly DE in each tissue

	1mm Blade	3mm Cup
1mm Cup	653	859
3mm Blade	-	1802

Table S6 genes DE over time in cup

	3mm Cup	7mm Cup
1mm Cup	859	2482
3mm Cup	-	1067