Figure S1 Diagram of the *CUL1* RNAi construct. LAT52-P, pollen specific promoter; UTR (5'+3'), combined UTR regions of *CUL1* gene; Nos-T, nos terminator; pCAMBIA2301-Nos-Prom-NPTII, modified binary vector pCAMBIA2301.
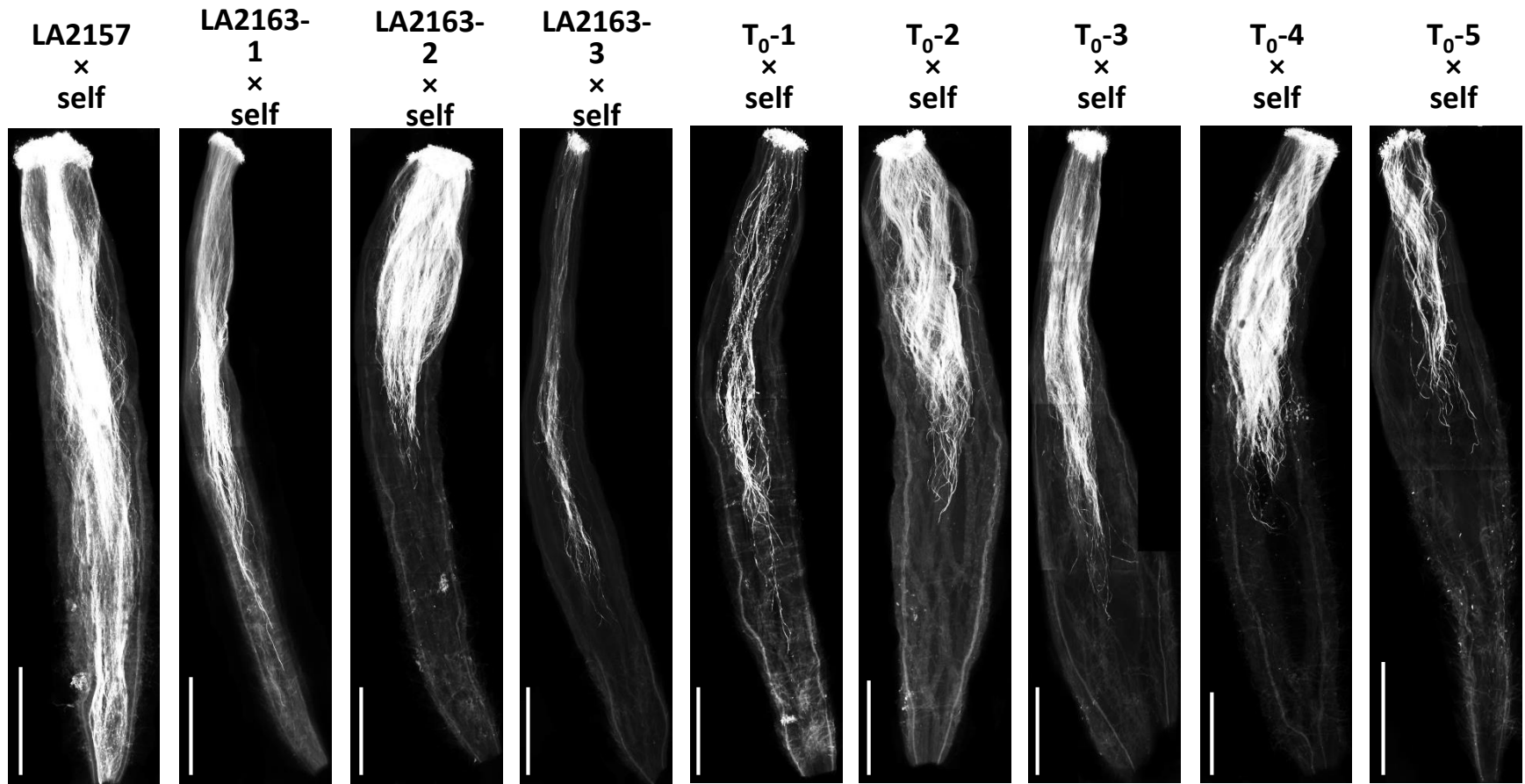
Figure S2 Images of pollen tube growth in pistils after self pollinations. Left to right: LA2157, several independent non-transgenic LA2163 plants, and five independent T_0 *CUL1* RNAi transformants. Styles were fixed 24 hours after pollination and pollen tubes were stained with aniline blue and visualized under UV light. Scale bar is 1 mm.h
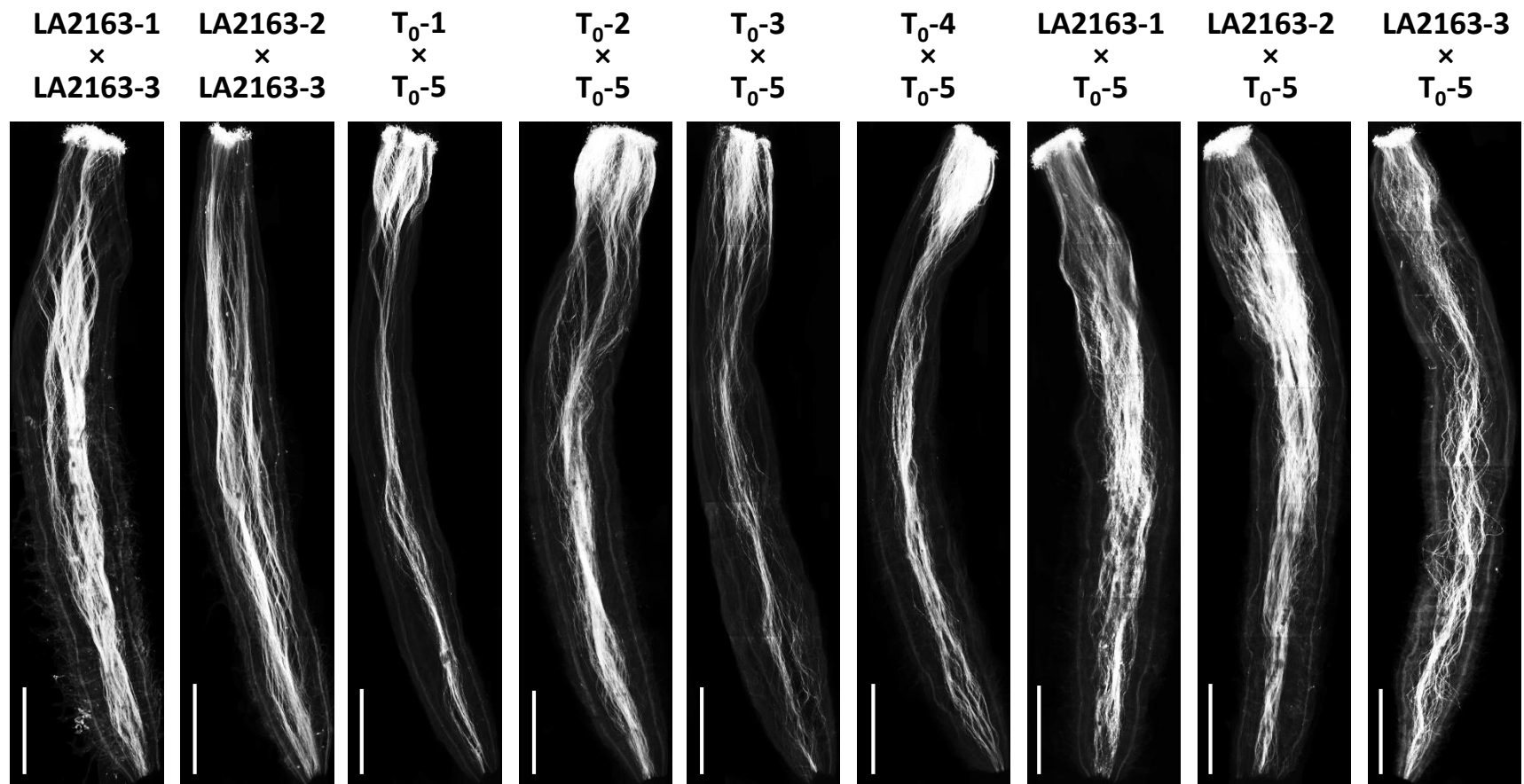
Figure S3 Images of pollen tube growth in pistils after cross pollinations. Left to right: crosses between independent non-transgenic LA2163 plants, crosses between independent T_0 *CUL1* RNAi transformants, and crosses of T_0 plants onto non-transgenic LA2163 pistils. Styles were fixed 24 hours after pollination and pollen tubes were stained with aniline blue and visualized under UV light. Scale bar is 1 mm.

MATERIALS AND METHODS

Plant materials

Two accessions, LA2157 and LA2163, of the wild tomato species *Solanum arcanum* were used in this study. LA2157 is self-compatible, while LA2163 is self-incompatible, but they are otherwise similar in most respects (Rick 1986). They were collected from nearby sites, separated by ~10 Km, in Cajamarca Department, Peru. Seed of these accessions was obtained from the C.M. Rick Tomato Genetics Resource Center (http://tgrc.ucdavis.edu) where they are maintained by cross pollinating all plants in each generation to maximize heterogeneity. The SI accession LA2163 was transformed with a *CUL1* RNAi construct via Agrobacterium transformation using cotyledonary explants. Selected primary transformants (T_0) were used as pollen donors in crosses onto pistils of non-transgenic LA2163 and LA2157. Plants were drawn at random from the corresponding accessions for the transformations and for the control non-transgenic plants. In the case of LA2163, each primary transformant (T_0-1, -2, etc) or non-transgenic control plant (LA2163-1, -2, -3) was expected to carry different *S*-genotypes (e.g. S_1S_2, S_3S_4, etc), and were cross-compatible in test sib pollinations, as predicted (Figure S3). In the case of LA2157, all plants were SC (Figure 2S) and were therefore expected to carry the same *S*-genotype (i.e. S_cS_c).

Pollen tube growth in styles was visualized by the aniline blue staining technique as described previously (Li et al. 2010).

CUL1 RNAi construct and plant transformation

Both conventional digestion-ligation and overlap extension PCR (Ho et al. 1989) were used to synthesize the *CUL1* RNAi construct. For the latter method, the first reaction amplified separate target DNA fragments and a second reaction combined the target fragments in a mixed reaction. Each intermediate primer used for overlap extension PCR had a 5' overhang sequence of ~20 base pairs (bp) that overlapped with the adjacent DNA fragment (Table S1).

To avoid cross-silencing of *Cullin1* genes, 146 and 279 bp 5' and 3' untranslated regions (UTRs) of *CUL1* were combined into a single 425 bp (UTR^{5+3}) sequence and used as the RNAi trigger (Figure S1). The 5' and 3' UTR of *CUL1* from LA2163 were amplified using primers 5UTR-1F/R and 3UTR-1F/R (Table S1) designed from the *SpCul1* sequence (Genbank no. HQ610201). UTR^{5+3} was synthesized by overlap extension PCR using primers 5UTR-1F and 3UTR-1R. The *LAT52* pollen specific promoter was amplified from our previous construct LAT52-SpCUL1 (Li and Chetelat 2010) with primers LAT52-2F/R. The combined LAT52-UTR^{5+3} was obtained by another round of overlap extension PCR using primers LAT52-2F and 3UTR-3R and cloned into pCAMBIA2301. The antisense UTR^{5+3} sequence was amplified with primers 3UTR-2R and 5UTR-2F and ligated to the vector in reverse orientation. The PDK intron was released from pHANNIBAL (Varsha Wesley et al. 2001) and inserted between

the sense and antisense UTR[5+3] copies. To reduce the frequency of non-transgenic plants recovered from kanamycin selection, the strong plant selection cassette 35S-NPTII in pCAMBIA2301 was replaced with the weak selection cassette Nos-NPTII from pBI121 by PCR with primers Nos-Prom-F and NPTII-R (Table S1). The pCAM2301-Nos-NPT-Cul1-RNAi construct was electroporated into *Agrobactericum tumefaciens* LBA4404 using the Gene Pulser II (Bio-Rad) system and introduced into SI *S. arcanum* LA2163 at the Ralph M. Parsons Plant Transformation Facility at the University of California, Davis.

CUL1 gene expression and RT-PCR

CUL1 expression was assayed by RT-PCR. Total RNA was isolated from pollen with TRIzol (Invitrogen). Total RNA (2 mg) was used for cDNA synthesis with SuperScript III (Invitrogen) reverse transcriptase. RT-PCR was performed using the gene specific primer pair Cul-1F/R (Table S1). The PCR conditions for RT-PCR of *CUL1* was 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 sec, 55 °C for 30 sec, and 72°C for 1 min, with a final extension of 5 min at 72 °C. The constitutively expressed *Actin* gene was used as a loading control, and was amplified with the same PCR conditions as *CUL1* except that the cycle number was 25. PCR products were separated in 2% agarose gels.

Transgene segregation tests

To test *CUL1* function in SI, five independent T_0 *CUL1* RNAi transformants were used as pollen parents in crosses onto pistils of SC *S. arcanum* LA2157 and SI *S. arcanum* LA2163 (Table S2). For each T_0 plant, five T_1 progeny arrays -- two from the crosses with LA2157 and three from crosses to three independent non-transgenic LA2163 plants (sibs) -- were genotyped for the presence of the transgene. Data from the two LA2157 progeny arrays of each T_0 plant were pooled since the chi-square tests for heterogeneity were not significant.

A mini-scale DNA extraction method was used to isolate DNA from the T_1 populations as described (Li et al. 2010). The primers 2301-2 and LAT52M-R (Table S1), which spanned the left side of the cloning site where the construct was inserted into the vector, were used to detect the transgene. The presence or absence of the 643 bp amplification product was used to classify the plants as transgenic or non-transgenic, respectively.

Supporting References

Ho, S. N., H. D. Hunt, R. M. Horton, J. K. Pullen, and L. R. Pease (1989) Site-directed mutagenesis by overlap extension using the polymerase chain reaction. Gene 77:51-59.

Varsha Wesley, S. et al. (2001) Construct design for efficient, effective and high-throughput gene silencing in plants. Plant Journal 27: 581-590.

Table S1 Primers used to build the RNAi construct and genotype the transgenic lines. Restriction sites are underlined, and sequences that overlap with adjacent primers for overlap extension PCR are underlined with a wavy line.

Primer	Sequence (5'-3')	Restriction Enzyme
5UTR-1F	CACCAAAAAAAAAAATTCCAATTTAACCAAAATGCAACCGAATTCGACG	
5UTR-1R	CGAGGTATGAGATCAGCACCCTCGGAAAATTCTTGTTAACACGAT	
3UTR-1F	TGTTAACAAGAATTTTCCGAGGGTGCTGATCTCATACCTCGG	
3UTR-1R	GTAAATGTTGGCATTACTGCAACCA	
LAT52-2F	TCTATGGAGCTCGGATAAGGGTAGCTCT	*Sac* I
LAT52-2R	TCGAATTCGGTTGCATTTTGGTTAAATTGGAATTTTTTTTTTTGGTG	
3UTR-3R	GTACGGGGTACCGTAAATGTTGGCATTACTGCAACCA	*Kpn* I
3UTR-2R	TTCGCGGATCCGTAAATGTTGGCATTACTGCAACCA	*Bam*H I
5UTR-2F	GTCTATGGGTCACCACCAAAATGCAACCGAATTCGACG	*Bst*E II
Nos-Prom-F	TCTATGCCAACATGGTGGGATCATGAGCGGAGAATTAAGGGA	*Bst*X I
NPTII-R	TTCGCCTCGAGTCAGAAGAACTCGTCAAGAAGGCGA	*Xho* I
Cul-1F	ATCAGGAACGTGAGGGTGAG	
Cul-1R	CAGCAAAACAGCCTTTCACA	
2301-2	CCAGGCTTTACACTTTATGCTTC	
LAT52M-R	TGCTCCTTCTCTTTGTGTGTGT	

Table S2 Segregation through the pollen of the *CUL1* RNAi T-DNA. Five independent transformants (T_0-1 to -5) were crossed as pollen donors onto SC and SI accessions of *S. arcanum* (LA2157 and LA2163, respectively). T_1 progeny arrays from three independent LA2163 plants (LA2163-1, -2, -3 or -4) were analyzed. Chi-square goodness-of-fit statistics (df = 1, with Yates correction factor) were used to test for deviations from the expected 1:1 (single T-DNA insertion) or 3:1 (two insertions) ratios.

Cross (♀ × ♂)	No. Plants + : - TDNA	Total	% Transgenic Progeny	Ratio Tested	χ^2	P value
LA2157 × T_0-1	71 : 25	96	74.0	3:1	0.01	0.92
LA2163-1 × T_0-1	6 : 42	48	12.5	3:1	96.7	<0.0001
LA2163-2 × T_0-1	7 : 41	48	14.3	3:1	90.3	<0.0001
LA2163-3 × T_0-1	2 : 46	48	4.2	3:1	124.8	<0.0001
LA2157 × T_0-2	42 : 54	96	43.8	1:1	1.26	0.26
LA2163-1 × T_0-2	17 : 79	96	17.7	1:1	38.8	<0.0001
LA2163-2 × T_0-2	12 : 84	96	12.5	1:1	52.5	<0.0001
LA2163-3 × T_0-2	0 : 96	96	0	1:1	94.0	<0.0001
LA2157 × T_0-3	64 : 80	144	44.4	1:1	1.56	0.2117
LA2163-1 × T_0-3	2 : 46	48	4.2	1:1	38.5	<0.0001
LA2163-2 × T_0-3	4 : 44	48	8.3	1:1	31.7	<0.0001
LA2163-3 × T_0-3	0 : 48	48	0	1:1	46.0	<0.0001
LA2157 × T_0-4	50 : 46	96	52.1	1:1	0.1	0.7518
LA2163-1 × T_0-4	6 : 42	48	12.5	1:1	25.5	<0.0001
LA2163-2 × T_0-4	2 : 46	48	4.2	1:1	38.5	<0.0001
LA2163-3 × T_0-4	0 : 48	48	0	1:1	46.0	<0.0001
LA2157 × T_0-5	62 : 82	144	43.1	1:1	2.5	0.1138
LA2163-2 × T_0-5	5 : 43	48	10.4	1:1	28.5	<0.0001
LA2163-3 × T_0-5	0 : 48	48	0	1:1	46.0	<0.0001
LA2163-4 × T_0-5	2 : 46	48	4.2	1:1	38.5	<0.0001

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