

Supplemental Tables 1-3.

The three tables show the results of pollination assays of stigmas from stage-12 flower buds that were pollinated with SCRb-expressing pollen grains. The numbers represent the average number of pollen tubes observed per pollinated stigma. Each pollination assay used at least 3 stigmas from each plant and was repeated on at least two separate dates. A pollen-tube count of >100 per stigma indicates a compatible response while pollen-tube counts of 0 or <5 per stigma indicate an incompatible response.

Supplemental Table 1. Pollination assays of F1 plants derived by crossing each of three independent high-expressing *AtS1pr:SCRb* transformants (b1, b2, and b3) with an *AtS1pr:cYFP-SRKb* homozygote.

Cross	F1 plants	Number of pollen tubes
<i>AtS1pr:cYFP-SRKb</i> X <i>AtS1pr:SCRb</i> (b1)	1	>100
	2	>100
	3	>100
	4	>100
	5	>100
	6	>100
	7	>100
	8	>100
<i>AtS1pr:cYFP-SRKb</i> X <i>AtS1pr:SCRb</i> (b2)	1	>100
	2	>100
	3	>100
	4	>100
	5	>100
	6	>100
	7	>100
	8	>100
<i>AtS1pr:cYFP-SRKb</i> X <i>AtS1pr:SCRb</i> (b3)	1	>100
	2	>100
	3	>100
	4	>100
	5	>100
	6	>100
	7	>100
	8	>100

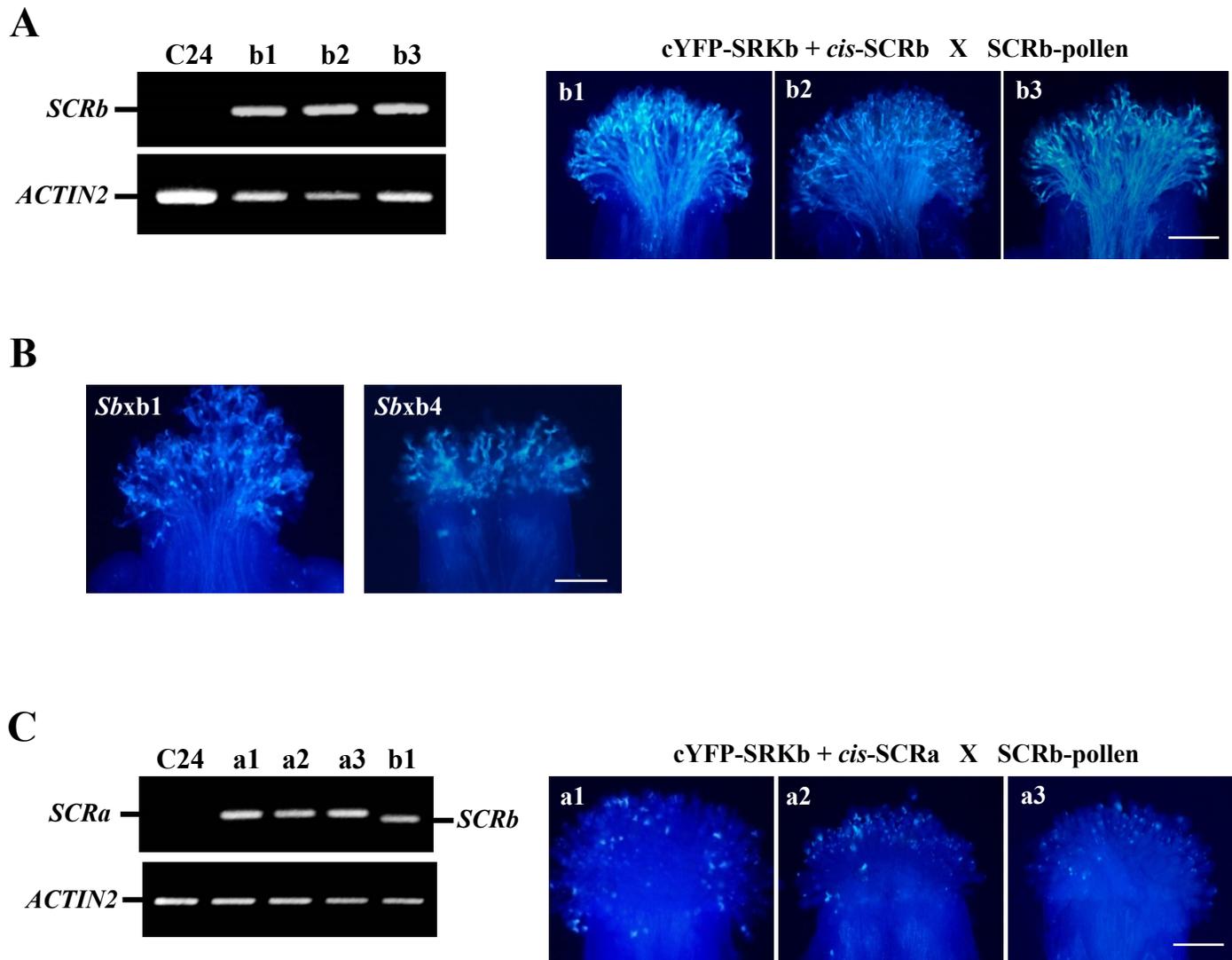
Supplemental Table 2. Pollination assays of *AtS1pr:cYFP-SRKb*-containing F2 plants derived from crossing each of two independent highly-expressing *AtS1pr:SCRb* transformants (b1 and b2) with the *AtS1pr:cYFP-SRKb* homozygote.

Cross	F2 plants containing <i>AtS1pr:cYFP-SRKb</i>	<i>AtS1pr:SCRb</i> present?	Number of pollen tubes
<i>AtS1pr:cYFP-SRKb</i> X <i>AtS1pr:SCRb</i> (b1)	1	no	<5
	2	no	<5
	3	no	<5
	4	yes	>100
	5	yes	>100
	6	no	<5
	7	yes	>100
	8	yes	>100
	9	yes	>100
	10	no	<5
	11	no	<5
	12	yes	>100
	13	yes	>100
	14	yes	>100
	15	no	<5
	16	yes	>100
	17	yes	>100
	18	no	<5
	19	yes	>100
	20	yes	>100
	21	yes	>100
	22	yes	>100
	23	yes	>100
	24	no	<5
	25	no	<5
<i>AtS1pr:cYFP-SRKb</i> X <i>AtS1pr:SCRb</i> (b2)	1	yes	>100
	2	yes	>100
	3	no	<5
	4	no	<5
	5	yes	>100
	6	yes	>100
	7	yes	>100
	8	no	<5
	9	yes	>100
	10	yes	>100
	11	yes	>100
	12	yes	>100
	13	yes	>100
	14	yes	>100
	15	yes	>100

Supplemental Table 3. Pollination assays of F1 plants derived by crossing each of three independent *AtS1pr:SCRa* transformants (a1, a2, and a3) with an *AtS1pr:cYFP-SRKb* homozygote.

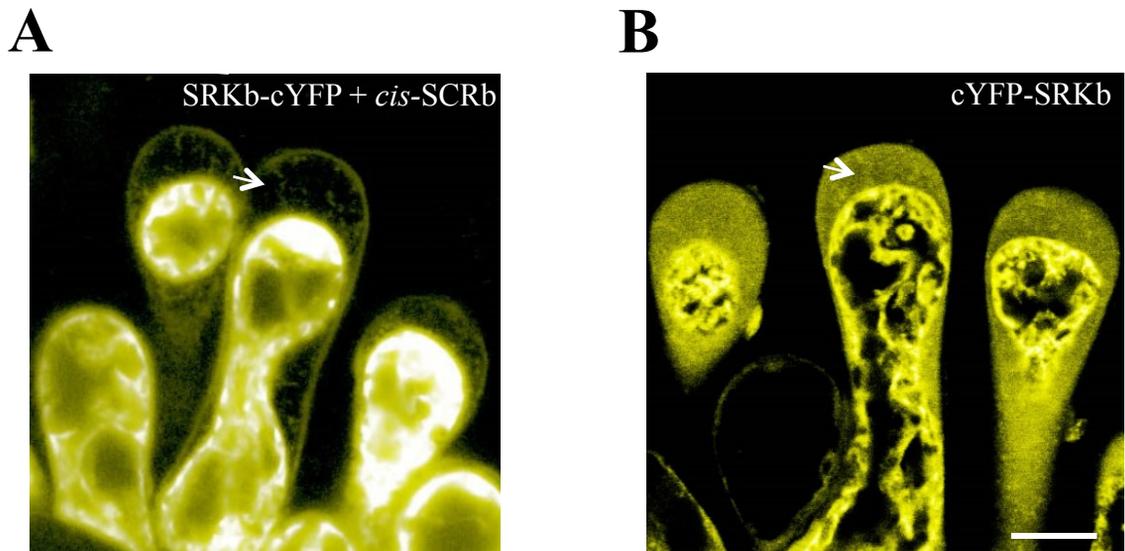
Cross	F1 plants	Number of pollen tubes
<i>AtS1pr:cYFP-SRKb</i> X <i>AtS1pr:SCRa</i> (a1)	1	<5
	2	0
	3	<5
	4	<5
	5	0
	6	<5
<i>AtS1pr:cYFP-SRKb</i> X <i>AtS1pr:SCRa</i> (a2)	1	0
	2	<5
	3	<5
	4	0
	5	0
	6	<5
<i>AtS1pr:cYFP-SRKb</i> X <i>AtS1pr:SCRa</i> (a3)	1	0
	2	0
	3	0
	4	<5
	5	<5
	6	0

Supplemental Figure 1



Supplemental Figure 1. Expression levels of stigma-expressed *SCRb* (*cis-SCRb*) and stigma-expressed *SCRa* (*cis-SCRa*) and their effects on the SRKb-mediated SI response. (A) *cis-SCRb*: The left panel shows RT-PCR of *SCRb* transcripts in the stigmas of an untransformed C24 stigma control sample and the three independent highly-expressing *AtSlpr:SCRb* b1, b2, and b3 transgenic lines. The right panel presents representative images of pollination assays (performed as described in Materials and Methods) in which SCRb-pollen was manually applied to the stigmas of F1 plants derived by crossing each of the *AtSlpr:SCRb* b1, b2, and b3 transgenic lines to an *AtSlpr:cYFP-SRKb* homozygote. Note the profuse pollen tube growth indicative of breakdown of SI. (B) Concentration-dependent effect of stigma-expressed *SCRb* on the growth of SCRb pollen tubes in F1 plants derived by crossing the *AtSlpr:SCRb* b1 and b4 lines to an *SRKb-SCRb* [*Sb*] homozygote. The pollination images are whole-stigma images of the images shown in Figure 1D. (C) *cis-SCRa*: The left panel shows RT-PCR of *SCRa* transcripts in the stigmas of an untransformed C24 stigma control sample, three independent *AtSlpr:SCRa* (a1, a2, and a3) transgenic lines that express high levels of *SCRa*, and in the stigmas of *AtSlpr:SCRb*(b1) (shown for comparison to stigma-expressed *SCRb* transcript levels). The right panel presents representative images of pollination assays in which SCRb-pollen was manually applied to the stigmas of F1 plants derived by crossing each of the *AtSlpr:SCRa* a1, a2, and a3 transgenic lines to an *AtSlpr:cYFP-SRKb* homozygote. The severe inhibition of SCRb-pollen demonstrates that SCRa does not affect SRKb function. Bar in pollination images = 0.1mm.

Supplemental Figure 2



Supplemental Figure 2. Confocal images of cYFP-labeled SRKb proteins in plasmolyzed stigma epidermal cells. To generate these panels, the images depicted in Figures 3D and 4A were manipulated by increasing overall brightness, which allows visualization of Hechtian strands. (A) Image from Figure 3D showing that, in the presence of *cis*-SCRb, cYFP-labeled Hechtian strands are weak but visible, indicating that a very small fraction of the full-length SRKb-cYFP receptor localizes to the plasma membrane (arrow). The uniform weak signal that outlines the boundary of stigma epidermal cells is due to autofluorescence of the cell wall, which is typically barely noticeable (Rea and Nasrallah, 2015) but become evident upon increasing image brightness. (B) Image from Figure 4A showing the distribution of the three SRKb isoforms in *AtSlpr*:cYFP-SRKb stigmas. Note that cYFP-labeled Hechtian strands (arrow) are visible over the strong apoplastic signal emanating from the cYFP-eSRKb isoform. Bar=10 μ m.