Supplemental Data. Samuel et al. (2009). Cellular Pathways Regulating Responses to Compatible and Self-Incompatible Pollen in *Brassica* and *Arabidopsis* Stigmas Intersect at Exo70A1, a Putative Component of the Exocyst Complex.

A. Pair-wise amino acid alignment scores of the *Arabidopsis* Exo70 family members with *Brassica napus* Exo70A1

At Exo70	A1	A2	A3	B1	B2	C1	C2	D1	D2	D3	E1	E2	F1	G1	G2	H1	H2	H3	H4	H5	H6	H7	H8
Length (aa)	638	695	547	624	599	653	695	633	622	623	658	639	683	687	660	636	637	637	628	605	615	634	573
% Identity to Bn Exo70A1	94	71	46	32	28	28	28	33	32	30	29	24	33	25	22	22	23	24	22	24	24	23	23

B. Amino Acid Sequence Alignment of *Brassica napus* Exo70A1 to *Arabidopsis* Exo70A1

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Bn Exo70A1 MAVDSRMDLL SERAVLMRES LQKSQTITDN VVSILGSFDS RLSALESAMR PTQIRTHAIR KAHENIDKTL KSAEVILSQF DLLRQAETKV LKGPHEDLES
At Exo70A1 ---------- --------A- ---------- ---------- ------T--- ---------- -------R-- -A-------- ---------- ----------

Bn Exo70A1 YLEAIAQLRK VIRYFSSNKG FKNSDGVLNH ANSLLAKAQS KLEEEFKQLL ASYSKAVEPD RLFDGLPNSL RPSADGEGNG KAHGGHHNDD SETAAYTLPV
At Exo70A1 --D------- I----M---S --S------- ---------- ---------- ---------- ---------- ---S--D-G- -P-------- A--------I

Bn Exo70A1 LIPSRVLPLL HDLAQQMVQA GHQQLLLQIY RETRTFVLEE SLRKLGVEKL SKEDVQRMQW EVLEAKIGNW IHFMRIAVKL LFAGERQVCD QIFRGFDSLS
At Exo70A1 ---------- ---------- ----Q----- -D--S----- --K------- ---------- ---------- ---------- ---------- ----------

Bn Exo70A1 DQCFAEVTVS SVSMLLSFGD AIARSKRSPE KLFVLLDMYE IMRELHSEIE TIFKGKACLE IRNSATGLTK RLAQTAQETF GDFEEAVEKD ATKTAVLDGT
At Exo70A1 ---------- ---------- ---------- ---------- ------T--- ---------- --D------- ---------- ---------- ----------

Bn Exo70A1 VHPLTSYVIN YVKFLFDYQA TLKQLFSEFG NGDDSNSQLA SVTMRIMQAL QNNLEGKSKQ YKDQALTHLF LMNNIHYMVR SVRRSEAKDL LGDDWVQRHR
At Exo70A1 ---------- ---------T ---------- ---------- ---------- ----D----- ---P------ ---------- ---------- ----------

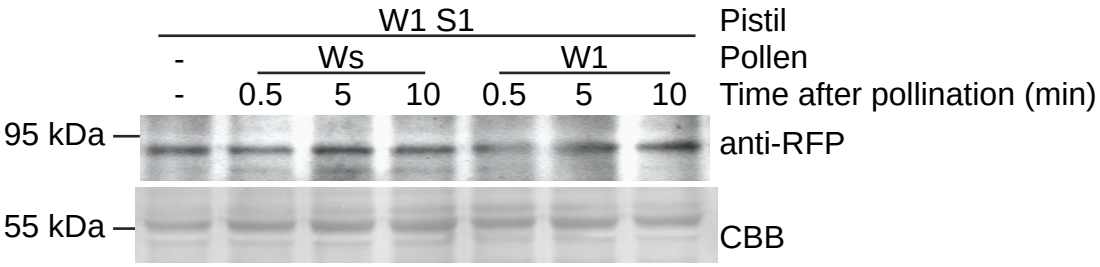
Bn Exo70A1 RVVQQHANLY KRTAWTKILQ TSSAQGLTSS GGGSVEGGNS SGVSRGLLKE RFKMFNMQFD ELHQRQSQWT VPDTELRESL RLAVAEVLLP AYRSFLKRFG
At Exo70A1 -I------Q- --V------- S--------- ----L----- ---------- ---------- ---------- ---------- ---------- ----------

Bn Exo70A1 PLVESGKNSQ RYIKYTAEDL ERLLGELFEG KSMNEPRR
At Exo70A1 --------P- K--------- ---------- --------
```

Supplemental Figure S1. Amino acid alignments of *Brassica napus* Exo70A1 to different members of the *Arabidopsis* Exo70 gene family.

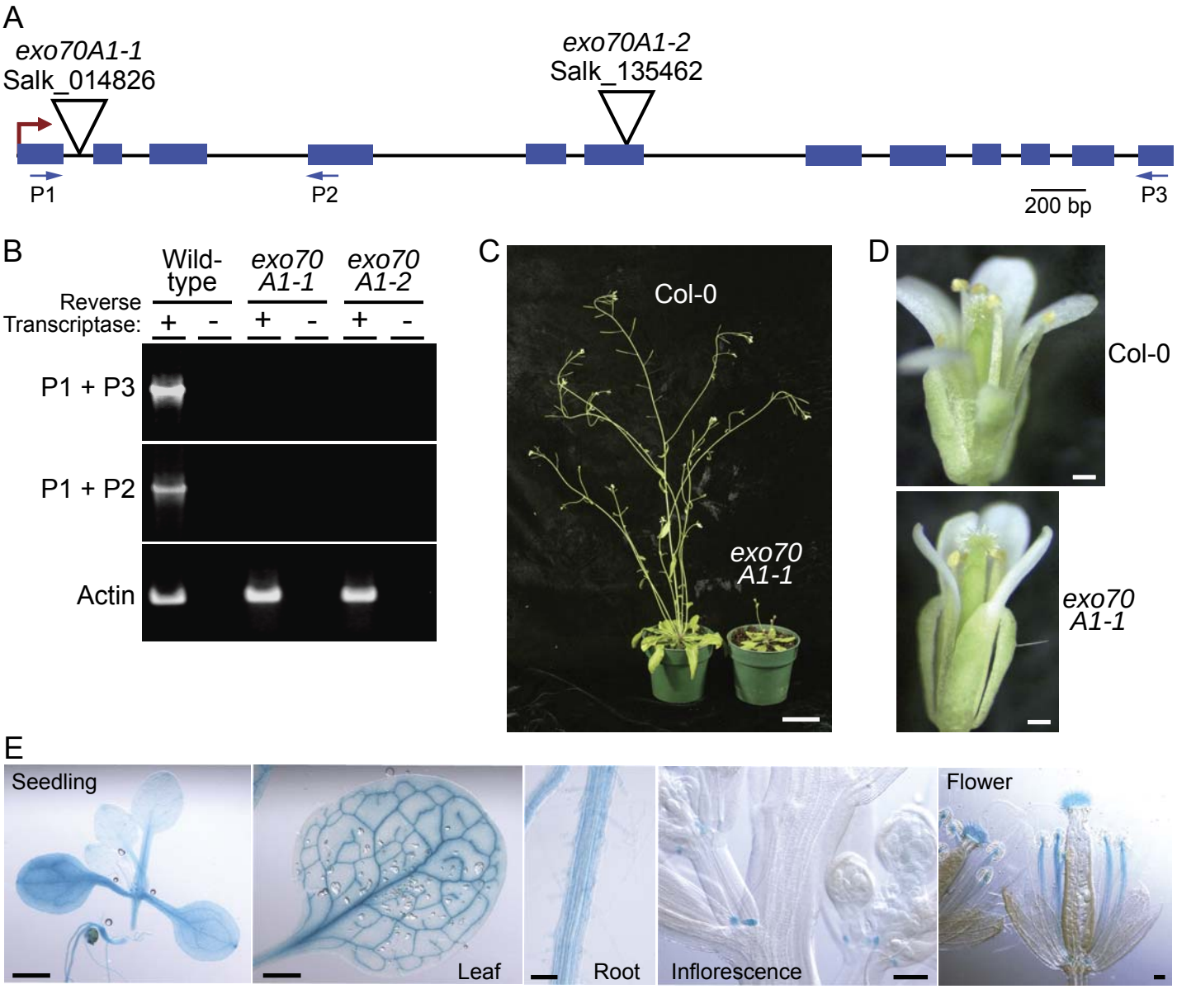
(A) Using BLAST searches, the *Brassica* Exo70A1 amino acid sequence was found to have sequence identity to Exo70s from a number of different organisms. Searches of Arabidopsis genome databases identified *Arabidopsis* Exo70A1 as the closest orthologue to *Brassica* Exo70A1 in the 23-member Exo70 family (Synek et al. Plant J 48, 54-72, 2006).

(B) Alignment of the predicted *Brassica napus* Exo70A1 to *Arabidopsis* Exo70A1 amino acid sequences. A yeast two-hybrid screen with the *Brassica* ARC1 UND domain resulted in the isolation of a cDNA encoding the first 85 amino acids of *Brassica* Exo70A1 (underlined). This sequence was used to identify corresponding *Brassica* EST sequences in the *Brassica* database (http://ukcrop.net/brassica.html). A full-length cDNA sequence was assembled and used to design primers to isolate the full-length *Brassica napus* Exo70A1 cDNA. At least three independent clones were sequenced in both directions to assemble the final sequence.

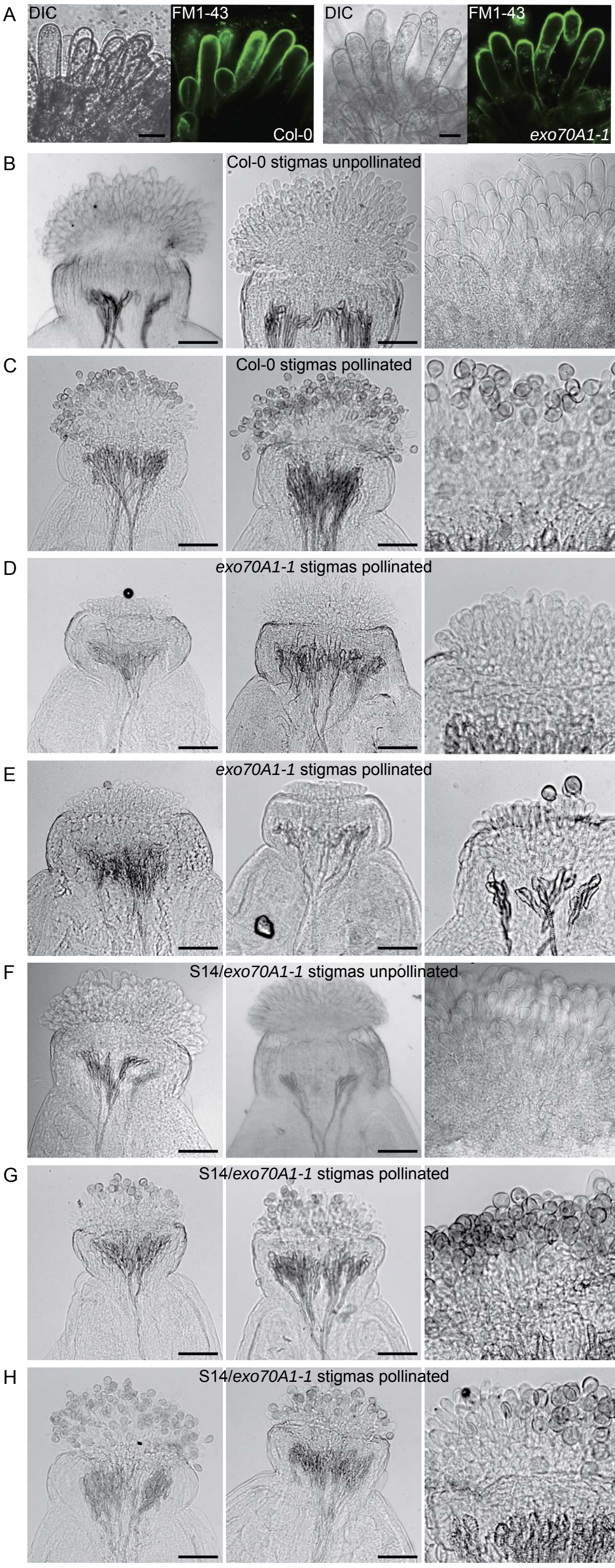


Supplemental Figure S2. RFP:Exo70A1 protein levels following self-incompatible W1 and compatible Westar pollinations.

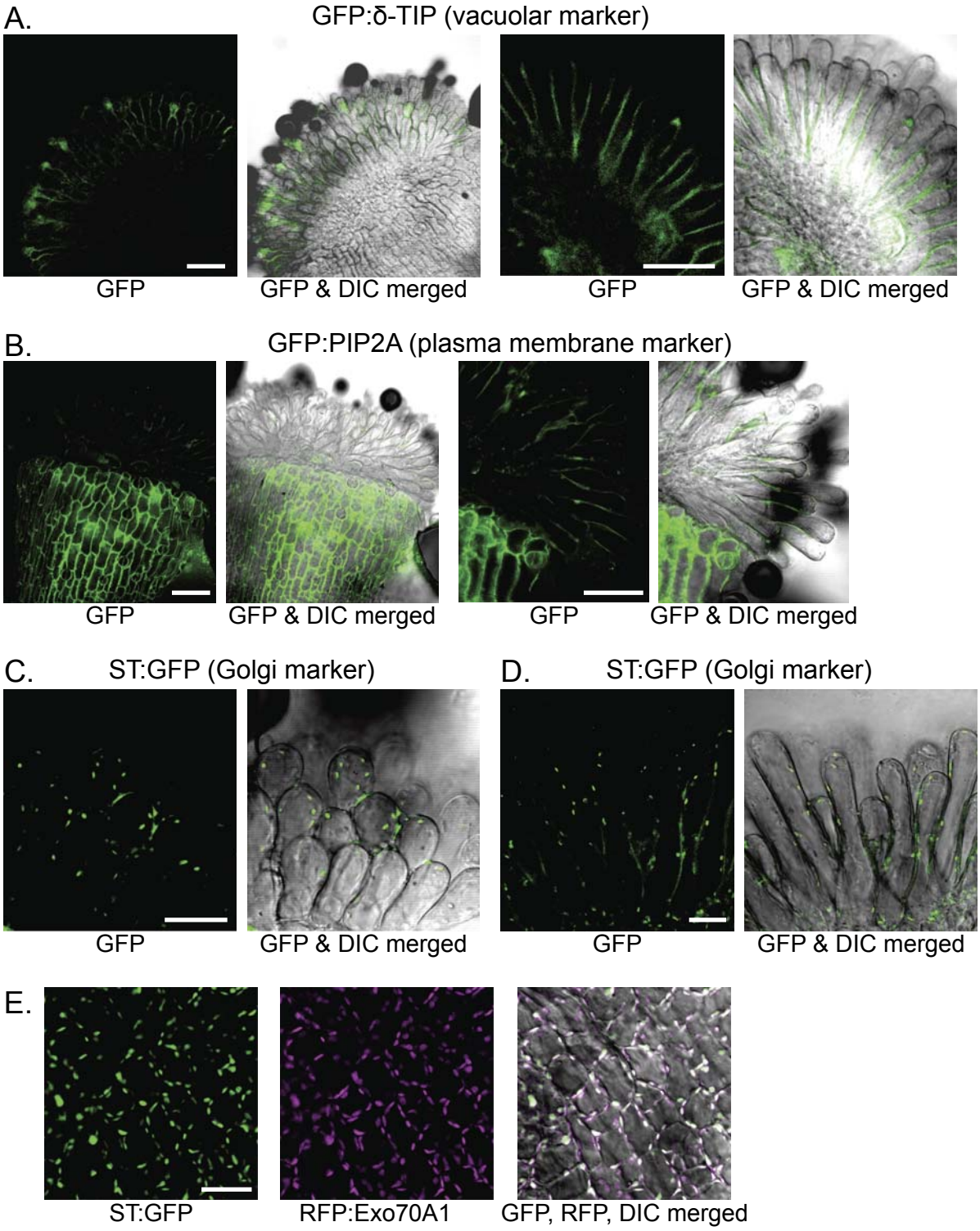
RFP:Exo70A1 (*Brassica* Exo70A1) was detected in protein samples from *Brassica* W1-S1 stigmas using anti-RFP antibodies and immunoblot analysis. The bottom panel is the CBB-stained membrane after immunoblotting.
Protein levels were observed at 0.5, 5, and 10 minutes post-pollination. There may be some decrease in the RFP:Exo70A1 levels observed at 0.5 minutes, but this was not a clearly reproducible difference.



Supplemental Figure S3. *Arabidopsis exo70A1* T-DNA insertional lines.
(A) *Exo70A1* gene structure and T- DNA insertion sites for two SALK lines, *exo70A1-1 & 2* (Alonso et al. Science 301: 653-657, 2003), as previously reported by Synek et al. (Plant J 48:54-72, 2006).
(B) RT-PCR analysis showing the loss of *Exo70A1* expression in the *exo70A1-1 & -2* (35 cycles; 2 biological replicates were performed). Location of the P1, P2 and P3 primer sites are shown in the gene structure.
(C) Col-0 and *exo70A1-1* plants at five weeks of age. As previously reported by Synek et al. (Plant J 48:54-72, 2006), the *exo70A1* mutants have a dwarf phenotype and extended life-span. Scale bar = 3 cm.
(D) Open flowers from Col-0 and *exo70A1-1* plants. The Col-0 flower represents Stage 14 as described by Smyth et al. (Plant Cell 2:755-767, 1990). The *exo70A1* mutant was reported to have immature stigmas at bud opening (Synek et al. Plant J 48:54-72, 2006); hwever, we have observed that this is variable (Supplemental Figure S4D-E). The *exo70A1* mutant also has an extended life span as reported by Synek et al. (2006), and in plants 10-week-old plants and older, we could sometimes observe continued development of the stigma after flower bud opening, to what appeared to be stage 13 flowers as described by Smyth et al. (1990). This is seen in the *exo70A1* mutant flower shown. This flower has been open for a couple of days and appears similar to a freshly-open wild-type stage 13 flower. Scale bars = 200 µm.
(E) *Arabidopsis* Exo70A1$_{pro}$GUS transgenic *Arabidopsis* plants showing GUS activity in the seedling and leaf (scale bars = 2 mm) as well as in the root, stipules in the nodes of inflorescences, and in the anther filaments and stigma of the flower (scale bars = 100 µm).



Supplemental Figure S4. Unpollinated and pollinated stigmas from *Arabidopsis* Col-0, the *exo70A1-1* mutant, and the rescued *exo70A1-1* mutant line expressing *RFP:Exo70A1*.
(A) Confocal sections of Col-0 and *exo70A1-1* elongated stigmatic papillae stained with the membrane impermeable lipophilic styryl dye, FM1-43 (Molecular probes, Invitrogen), to visualize the cuticular layer. Scale bar = 20 µm.
(B-C) DIC images of unpollinated (B) and pollinated (C) Col-0 stigmas.
(D-E) DIC images of pollinated *exo70A1-1* stigmas. Note the variability in the stigmatic papillar elongation and the absence of Col-0 pollen. A very rare example of a pollen grain germinated on an *exo70A1* stigma is shown in the far right panel in (E).
(F) DIC images of unpollinated rescued S14/*exo70A1-1* stigmas.
(G-H) DIC images of pollinated rescued S14/*exo70A1-1* stigmas.
For (B-H), Stage 12 flowers were emasculated, covered, and left overnight. For all pollinations, wild-type Col-0 pollen were applied the next day, and pistils were again left overnight. The following day, all pistils were collected, stained with aniline blue, and visualized under an epifluorescence microscope. Two left panels - 10X objective (scale bars= 100 µm); right panel - 20X objective (scale bars = 50 µm).



Supplemental Figure S5. Transgenic *Arabidopsis* lines expressing different GFP marker proteins in the stigma.

(A) Expression in the stigma of the GFP:δ-TIP vaculolar marker (from Cutler et al. Proc Natl Acad Sci USA 97:3718-3723, 2000). GFP:δ-TIP is visible in the stigmatic papillae and was used to mark the vacuolar membrane in the *RFP:Exo70A1* transgenic *Arabidopsis* plants.

(B) Expression in the stigma of the GFP:PIP2A plasma membrane marker (from Cutler et al. Proc Natl Acad Sci USA 97:3718-3723, 2000). GFP:PIP2A is expressed well in the underlying stigmatic cells, but poorly in the stigmatic papillae and could not be clearly visualized in the *RFP:Exo70A1* transgenic *Arabidopsis* plants.

(C-D) Expression in the stigma of ST:GFP Golgi marker (from Saint-Jore et al. Plant J 29:661-678, 2002). ST:GFP is clearly visible in the stigmatic papillae of both immature (C- stage 12) and mature (D- stage 13) stigmas, and was used to mark the Golgi in the *RFP:Exo70A1* transgenic *Arabidopsis* plants.

(E) RFP:Exo70A1 also co-localizes with ST:GFP in the basal stigmatic cells (stage 13 flower).

Scale bars = 50 μm for A-B; and 20 μm for C-E.

Supplemental Table S1: Primers used in study for cloning and RT-PCR

Primer Name	Sequence
Sma1-EXO70-F	5’ tccccccgggatggccgtcgatagccgaatggatctg 3’
EcoR1-intron-AS	5’cggaattcctatgagctgcaaaaactacttacctccagcagttgctggtgaccagcctgaac 3’
EcoR1-AS	5’cggaattccagcagttgctggtgaccagcctgaac3’
BamH1-For	5’ cgggatccatggccgtcgatagccgaatggatctg3’
Actin3-For	5’ ggctgatggtgaagatattca 3’
Actin3-Rev	5’caagcacaataccagtagtac3’
Rpt2a-F	5’ gctctagaatgggacaaggaccatcggga 3’
Rpt2a-R	5’gctctagattacatgtagaggccttcaggg 3’
BnExo70-fwd	5’cgcccgggatggccgtcgatagccgaa 3’
Bn/AtExo70-rev	5’ cgcgggcccttaccgtcgtggttcattcat 3’
AtExo70-fwd	5’ cgccccggggatggctgttgatacgata 3’
RFP-int-for	5’ gaggtcaagaccacctac 3’