**SUPPLEMENTAL MATERIALS**

# EXO70C2 is a key regulatory factor for optimal tip growth of Arabidopsis pollen tubes

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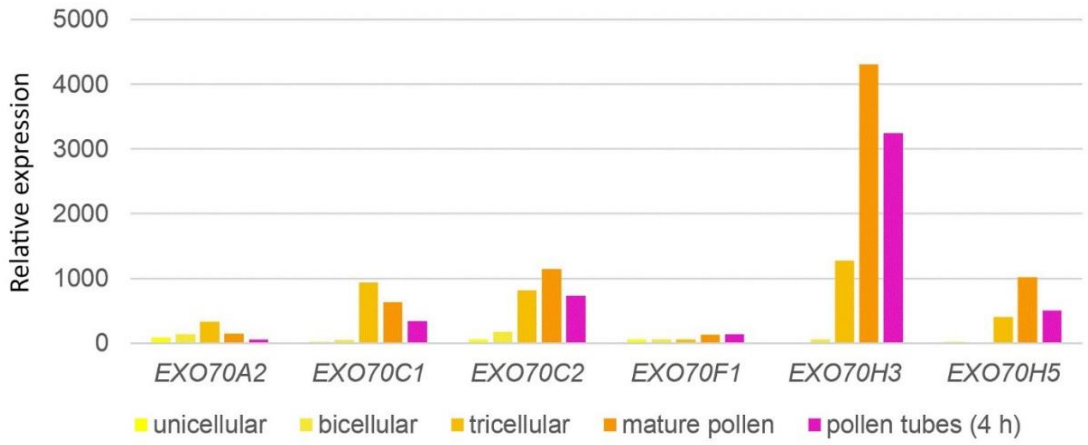
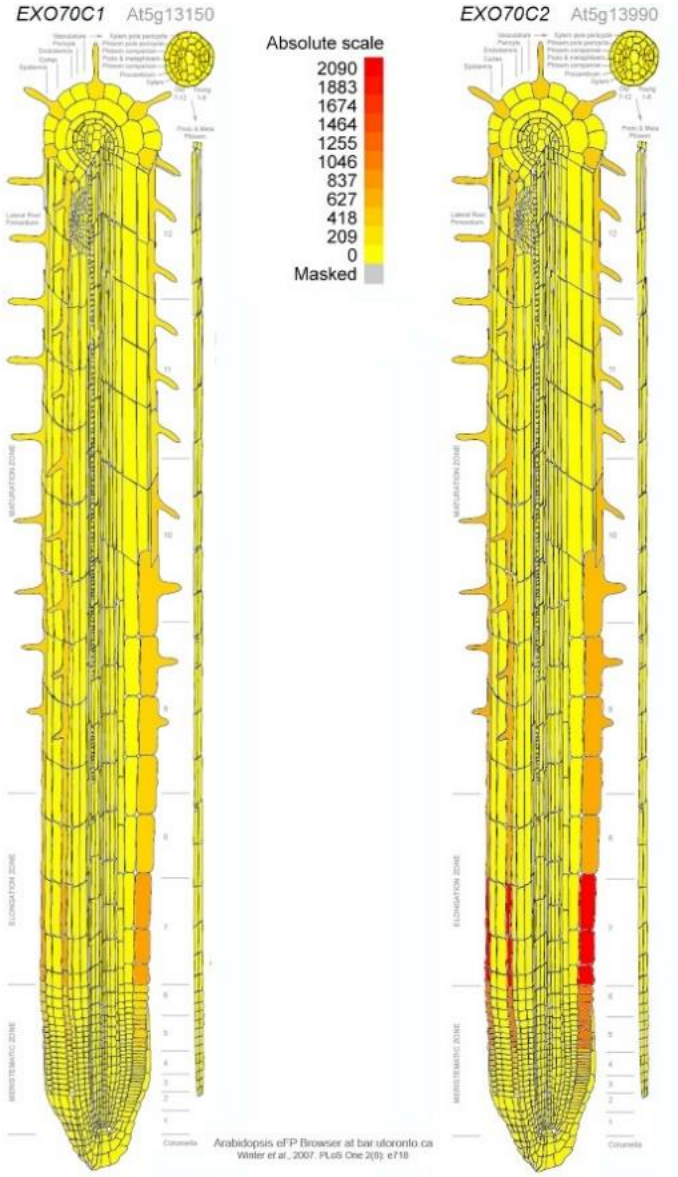


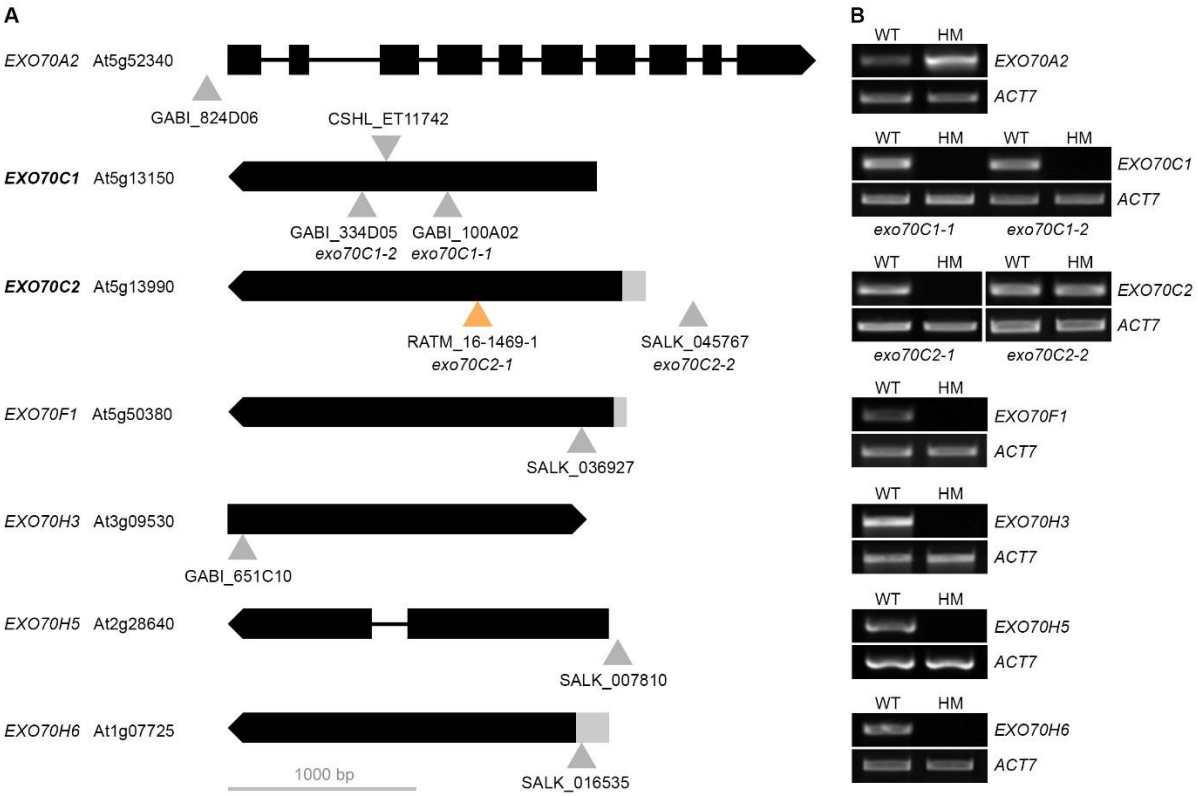

**Supplemental Figure 1.** Expression of candidate pollen *EXO70* genes during pollen development in Arabidopsis.

Expression levels of putative pollen *EXO70* isoforms in unicellular, bicellular, tricellular and mature pollen, and germinated pollen tubes (4 hours) based on microarray data in the Arabidopsis eFP Browser (Winter et al., 2007). *EXO70H6* is undetectable using the ATH1 DNA chip (Affymetrix).



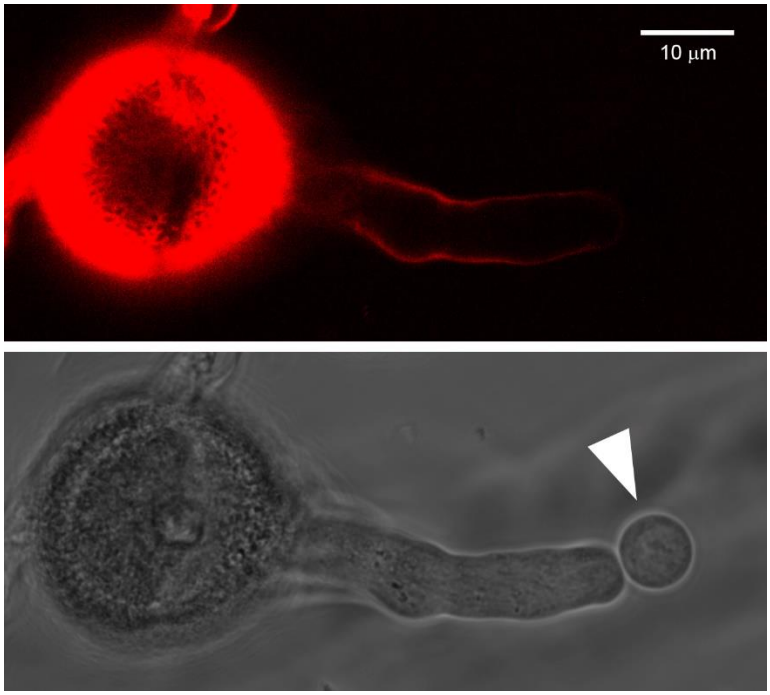

**Supplemental Figure 2.** *EXO70C1* and *EXO70C2* expression in Arabidopsis roots.

*EXO70C1* (left) and *EXO70C2* (right) expression in root cell types based on microarray data as visualized by the Arabidopsis eFP Browser (Winter et al., 2007). Both genes show specific expression in trichoblast cells, and the *EXO70C2* expression obviously exceeds that of *EXO70C1*.



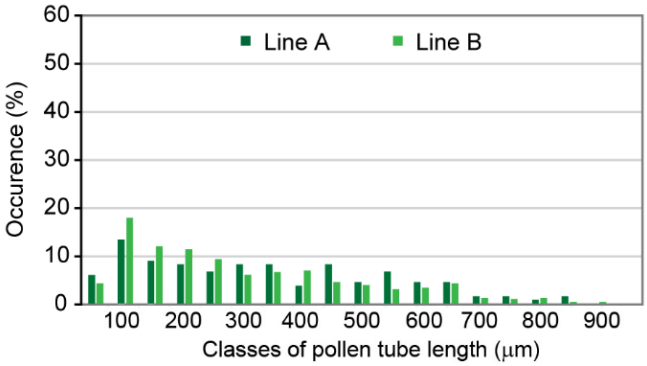

**Supplemental Figure 3.** Positions of insertions in *exo70* mutant lines and semi-quantitative RT-PCR analysis of gene expression of the affected *EXO70* isoforms.

A) Positions of T-DNA and transposon insertions in Arabidopsis mutants analyzed or referred to in this work. Exons are boxed, untranslated exons at the 5'UTR in light gray, introns in lines. In orange, the *exo70C2* insertion resulting in the transmission defect.
B) Expression levels of EXO70 genes in respective mutants as analyzed by semi-quantitative RT-PCR in samples prepared from flowers of wild-type (WT) or homozygous (HM) plants. The *ACT7* gene was used as a quantitative control.



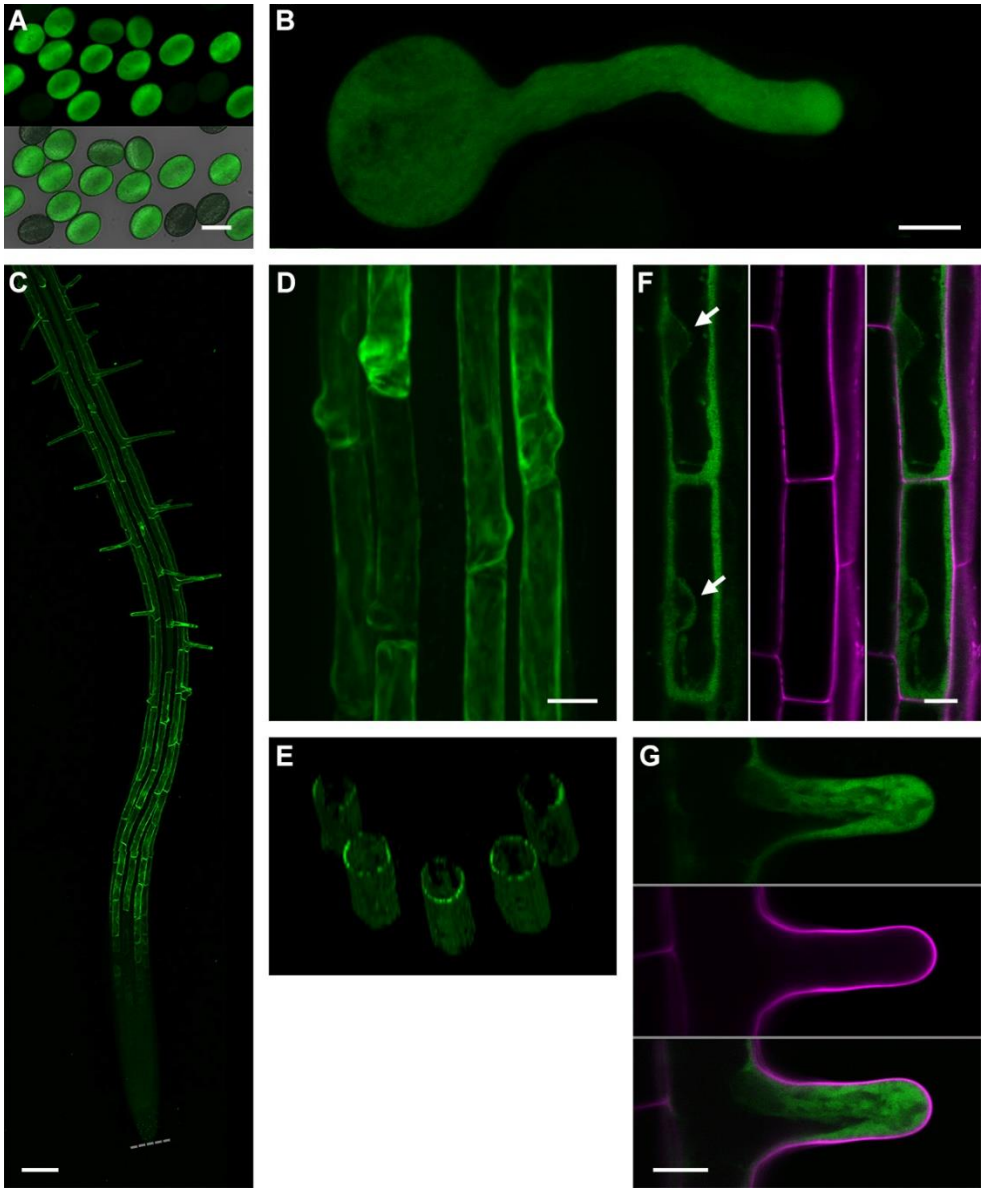

**Supplemental Figure 4.** Propidium-iodide staining of protoplast-like structures emerging from the *exo70C2* pollen tube apex.

The absent propidium iodide signal around the protoplast-like structures suggests a very weak cell wall or limited amount of demethylated pectins is such a cell wall.




**Supplemental Figure 5.** Pollen tube lengths of complemented *exo70C2* lines non-segregating for the *pEXO70C2::EXO70C2:GFP* expression cassette.

Distribution of pollen tube lengths in samples of *in vitro* germinated pollen from homozygous *exo70C2* mutants in which the introduced *pEXO70C2::EXO70C2:GFP* was in a homozygous state resembles the distribution of WT pollen tube length (Figure 2A).




**Supplemental Figure 6.** Localization of *pEXO70C2::*GFP:EXO70C2 is identical to *pEXO70C2::*EXO70C2:GFP.

A) GFP:EXO70C2 in pollen grains of *exo70C2* homozygotes with the GFP expression cassette segregating. Scale bar = 20 μm.
B) GFP:EXO70C2 in the cytoplasm of a pollen tube. Scale bar = 10 μm.
C) GFP:EXO70C2 in a root. Gray dotted line marks the root tip. Scale bars = 100 μm.
D) GFP:EXO70C2 is specifically expressed in trichoblast cells (maximum intensity projection of a confocal Z-stack). Scale bar = 20 μm.
E) Top view at a 3D reconstruction calculated from the Z-stack in (D).
F) GFP:EXO70C2 is localized in the cytoplasm and excluded from the nucleus (marked by arrow) in trichoblasts. Cell walls stained with propidium iodide (in magenta). Scale bar = 10 μm.
G) A detail of the GFP:EXO70C2 cytoplasmic localization in a root hair. Cell walls stained with propidium iodide (in magenta). Scale bar = 10 μm.

**Supplemental Table 1.** List of primers used in this study.

| GENOTYPING | | | |
|---|---|---|---|
| **gene** | **primer** | **sequence** | **purpose** |
| *EXO70A2* | A2-wt2 | GAACAAGCACATAGGTTTGGATTATATTCAAC | genotyping of GABI_824D06 |
| | A2-ins2 | CCAAATCCAGAATCACATTTCAATTTCAACAG | genotyping of GABI_824D06 |
| *EXO70C1* | C1_G100-wt2 | TGAATCAGATTGTTCCACCGCAGGAAT | genotyping of GABI_100A02 |
| | C1_G100-ins3 | TTTCGTCATCTTCTAGATCGTTCCAGAG | genotyping of GABI_100A02 (+ LB3 primer) |
| | C1-G334_wt2 | CGGATTTCGAAATCGGTTATGAGATTG | genotyping of GABI _334D05 |
| | C1-G334_ins2 | GTGATGGGTCTCTGTACAGTCTC | genotyping of GABI _334D06 |
| *EXO70C2* | C2-Rik-WT1 | ATAGCACCGAGGAGAAAGGAG | genotyping of RATM16-1469 |
| | C2-Rik-ins1 | GTCCTTGTATTCGCACGAGTA | genotyping of RATM16-1469 |
| | C2-Rik-WT2 | GATTGAATACCCTGGTTACCCTG | genotyping of RATM16-1469 with GFP:EXO70C2 |
| | C2-Rik-ins3 | TGAGAAAATCAGGAAAAAGTGTTGTTGC | genotyping of RATM16-1469 with GFP:EXO70C2 |
| | C2-S767-LP | CGGCTACACACACACACAAAC | genotyping of SALK_045767 |
| | C2-S767-RP | TCCTTGTCGTTCTTCTCCATG | genotyping of SALK_045767 |
| *EXO70F1* | F1-wt | CCGCTCTGCAATCTCCTTCAAATC | genotyping of SALK_036927 |
| | F1-ins | TTTCGCTGTCTTTGTTCCACTCAAAC | genotyping of SALK_036927 |
| *EXO70H3* | H3_G651-wt3 | CTTGTCTCTTAAGGTGATAGTTTCCG | genotyping of GABI_651C10 |
| | H3_G651-ins1 | GAGACACGTGTACGGAAAAGATGAG | genotyping of GABI_651C10 |
| *EXO70H5* | H5P-wt | GTATCCAACACAACAAGCATACACTACC | genotyping of SALK_007810 |
| | H5P-ins | CGGATTCCGGATCAAGATTCCGTC | genotyping of SALK_007810 |
| *EXO70H6* | H6-SALK-wt | TTCACAGATGGGCTCGAGACTGTT | genotyping of SALK_016535 |
| | H6-SALK-ins | CAACAATAGCATCAGAATTCCCTCCT | genotyping of SALK_016535 |
| Rikken lines | Ds5-2a | TCCGTTCCGTTTTCGTTTTTTAC | genotyping of Rikken lines |
| SALK lines | LBb1.3e | GATTTTGCCGATTTCGGAACCA | genotyping of SALK lines |
| GABI lines | GABI_o8760 | GGGCTACACTGAATTGGTAGCTC | genotyping of GABI lines |

| | | | |
|---|---|---|---|
| | LB3 | TAGCATCTGAATTTCATAACCAATCTCGATACAC | genotyping of GABI_100A02 |

**RT-PCR**

| gene | primer | sequence | purpose |
|---|---|---|---|
| *EXO70A2* | A2-RT-LP | AGCTGCGGTGTTGGAACAGA | RT-PCR |
| | A2 RT-RP | CTC GAC TGA ACC GTG AGA CAC T | RT-PCR |
| *EXO70C1* | C1-RT-5k | CTGCGGTGGAACAATCTGATTCAG | RT-PCR |
| | C1-RT-3k | TCCCTGAATCAAGATGTTGCTTATAC | RT-PCR |
| *EXO70C2* | C2-Rik-ins1 | GTCCTTGTATTCGCACGAGTA | RT-PCR |
| | C2-RT-RP | TTTGAAACAAGATTGTGAGTTTGAGA | RT-PCR |
| *EXO70H3* | H3-RT-LP | ACGGATGTTAACAAGACCATCGAC | RT-PCR |
| | H3_G651-wt3 | CTTGTCTCTTAAGGTGATAGTTTCCG | RT-PCR |
| *EXO70H5* | H5-RT-5k | ATGATGCTACTATTTAAACCGTCTTTA | RT-PCR |
| | H5-RT-3k | CATGCAATTCTTGTGGTTACAGTA | RT-PCR |
| *EXO70H6* | H6-RT-5k | TAAGGTCGGGCCGCAACAGCAA | RT-PCR |
| | H6-RT-3k | GCCGCTGCTACTGAAGTAATCCT | RT-PCR |
| *ACT7* | ACT7_L | CAAACTCACCACCACGAACCA | RT-PCR |
| | ACT7_R | GCCGATGGTGAGGATATTCAGC | RT-PCR |

**amiRNA**

| gene | primer | sequence | purpose |
|---|---|---|---|
| *EXO70C1* | C1A_I_miR-s | GATGTATGACATACAAGACTCTGTCTCTCTTTTGTATTCC | amiRNAxC1 |
| | C1A_II_miR-a | GACAGAGTCTTGTATGTCATACATCAAAGAGAATCAATGA | amiRNAxC1 |
| | C1A_III_miR*s | GACAAAGTCTTGTATCTCATACTTCACAGGTCGTGATATG | amiRNAxC1 |
| | C1A_IV_miR*a | GAAGTATGAGATACAAGACTTTGTCTACATATATATTCCT | amiRNAxC1 |
| *EXO70C2* | C2A_I_miR-s | GATATGCGGAATTCGTGTTCCAATCTCTCTTTTGTATTCC | amiRNAxC2 |
| | C2A_II_miR-a | GATTGGAACACGAATTCCGCATATCAAAGAGAATCAATGA | amiRNAxC2 |
| | C2A_III_miR*s | GATTAGAACACGAATACCGCATTTCACAGGTCGTGATATG | amiRNAxC2 |
| | C2A_IV_miR*a | GAAATGCGGTATTCGTGTTCTAATCTACATATATATTCCT | amiRNAxC2 |
| *pLAT52:: amiRNAxC1/C2* | EXO70C1/2_ Lat52_XmaI | CCCCCGGGAGCTTCGACATACTCGACTC | cloning to pBAR1 |
| | EXO70C1/2_ Lat52_XbaI | TGCTCTAGAGCGATGCCTTAAATAAAGATAA | cloning to pBAR1 |
| *amiRNA* | amiRNA-primerA | CTGCAAGGCGATTAAGTTGGGTAAC | amiRNA cassette detection |
| | amiRNA-primerB | GCGGATAACAATTTCACACAGGAAACAG | amiRNA cassette detection |

**CLONING**

| gene/construct | primer | sequence | purpose |
|---|---|---|---|
| *pEXO70C1:: EXO70C1* | C1-5prom-SalI | ATGTCGACCTTCTTTCTGCTGTCAAAATAAC | cloning to pENTR3C |
| | C1-3end-NS-NotI | AAGCGGCCGCTCTCTTGCGTGCCATAGA | cloning to pENTR3C |
| *pEXO70C2:: EXO70C2* | C2-5prom-BglII | TTAGATCTGCATTGATCCATTATTCCATC | cloning to pENTR3C |
| | C2-3end-NS-NotI | AAGCGGCCGCGATGTTCTTCGCCTGGCGG | cloning to pENTR3C |

| | | | |
|---|---|---|---|
| *EXO70C2* | C2-5k-XbaI | AATCTAGAATGGAGAAGAACGACAAGG | cloning to pBAR1-GFP |
| | C2-3k-XbaI | AATCTAGACTATGTTCTTCGCCTGGC | cloning to pBAR1-GFP |
| *GFP:EXO70C2* | C2+GFP-5end | CGTAAATAATAAGAAACCATGAGTAAAGGAGAAGA | amplification |
| | C2-3end+B2 | AGAAAGCTGGGTCTATGTTCTTCGCCT | amplification |
| *pEXO70C2* | C2prom+B1-5end | AAAAAGCAGGCTGCATTGATCCATTATTCC | amplification |
| | C2prom+GFP-3end | TTCTCCTTTACTCATGGTTTCTTATTATTTACG | amplification |
| *pEXO70C2:: GFP:EXO70C2* | C2prom+B1-5end | AAAAAGCAGGCTGCATTGATCCATTATTCC | cloning to pDONR201 |
| | C2-3end+B2 | AGAAAGCTGGGTCTATGTTCTTCGCCT | cloning to pDONR201 |
| *EXO70C2* | C2-BD_FL_NdeI | CATATGGAGAAGAACGACAAGGACCC | cloning to pGBKT7 and pGADT7 |
| | C2-BD_stop_SmaI | CCCGGGCTATGTTCTTCGCCTGGCGGTGG | cloning to pGBKT7 and pGADT7 |
| *EXO70A2* | A2_Bam_fw | TTGAATTCATGGGGGTGGCTCAAGCAATGGAAG | cloning to pGBKT7 |
| | A2_Sal_rv | TTTGTCGACTTATCTCTTTGGCTCACTCCATGTC | cloning to pGBKT7 |
| *SEC15a* | SEC15a_Bam_fw | TTGGATCCGAATGATGGAGGCCAAACCAA | cloning to pGADT7 |
| | SEC15a_Sal_rv | TTTGTCGACTCAGTTAAATTCCTTGAGTCTC | cloning to pGADT7 |
| *EXO70A1* | EXO70A1-EcoRI_fw | ACTGAATTCGCCATGGCTGTTGATAGC | cloning to pENTR3C |
| | EXO70A1-Not_rv | TTGCGGCCGCCCCCGGCGTGGTTCATT | cloning to pENTR3C |

**The following files are available on-line:**

**Supplemental File 1.** Interactive model of genetic segregation.

**Supplemental Movie 1.** Growth dynamics of *exo70C2* and WT pollen tubes stained with calcofluor white.

Calcofluor white signal (left) inversely correlates with the growth rate (Rainbow LUT: red -the strongest signal, blue the weakest signal, quantification in the graph). Red arrows in the bright field channel (right) highlight pollen tube bursts, which are also highlighted by blue arrowheads in the graph below.

**Supplemental Movie 2.** Growth dynamics of *exo70C2* and WT pollen tubes stained with calcofluor white.

The growth rate of the *exo70C2* pollen tube greatly exceeds the WT rate. At the rate maxima, apical calcofluor signal of *exo70C2* mutant is decreased by 75% with respect to the WT signal (Rainbow LUT: red -the strongest signal, blue the weakest signal, quantification in the graph). Arrow in the bright field channel (right) highlights pollen tube burst followed by a stop of the tube growth.

**Supplemental Movie 3.** Multiple *exo70C2* pollen tube bursts and recovery.

Red arrowheads point to cytoplasmic extrusions and highlight time points of each pollen tube burst followed by recovery.