

Supplemental Figure S1. Localization of non-pollen EXO70 isoforms in growing tobacco pollen tubes. Selected YFP-tagged tobacco EXO70 isoforms were transiently expressed in tobacco pollen tubes and their subcellular localization was examined by spinning disk confocal microscopy. Growing pollen tubes with low expression levels of the transgene are shown. The images shown are representative for ≥ 20 transformed pollen tubes observed in at least two independent experiments. Bar, 10 µm.

NtEXO70A1a	1 M <mark>GMPRNTGG</mark> GGIAVV <mark>GIDMLSER</mark> AAKM <mark>R</mark> EAVH <mark>KSQSITDN</mark> MVSILGSFDHRLSALETAMR60)
NtEXO70A1b	1 M <mark>GIPRNTGG</mark> GVDMLSERAKMMRDAVEKSQSITDNVVNILGSFDHRLSALDTAMR54	
NtEXO70A1a	61 PTQIRTHAIRKAHVNIDKTLKAADVILSQFDLSRQAEAKILKGPHEDLESYLEAIEQLRN 12	20
NtEXO70A1b	55 PTQIRTHAIRKAHGNIDKTLKAAEVILSQFDISRQAEVKILKGPHEDLESYLQAIEQLRN 11	4
NtEXO70A1a	121 NIRFFN <mark>NNKSFKSSDGVLN</mark> NA <mark>NS</mark> LLA <mark>KAISKLEEEFKQ</mark> LLS <mark>SYSKP</mark> VEPERLFECLPNSM 18	30
NtEXO70A1b	115 NIRFFS <mark>NNKSFKSSDGVLN</mark> HA <mark>NS</mark> LLA <mark>KAISK</mark> LEEEFKQLLL <mark>SYSKP</mark> VEPDRLFECLPNSM 17	74
NtEXO70A1a	181 <mark>RPSSGSPG</mark> D <mark>S</mark> NG <mark>KN</mark> HL <mark>S</mark> SSHAEHNNGTDNTVYTPPTLIPPRILPLLHDLVQQMVQAGH 23	38
NtEXO70A1b	175 <mark>RPSSGSPG</mark> HQESS <mark>GKN</mark> RLSNSIAEQ - NGAENAVFTPPTLIPPRILPLLHDLAQQMVQAGH 23	33
NtEXO70A1a	239 QQQLV <mark>KIYRDTRS</mark> PVLEESLRKLGVEKLSKDDVQKMQWEVLEFKIGNWIHFMRIAVKLLF 29)8
NtEXO70A1b	234 QQQFVKIYRDTRSPVLEESLRLLGVEKLSKDDVQKMQWEVLEAKIGNWIHFMRIAVKLLF 29)3
NtEXO70A1a	299 AAER <mark>KVCDQ</mark> MFEGFEHL <mark>KDQ</mark> CFAEV <mark>TT</mark> G <mark>S</mark> VAVLL <mark>S</mark> FGDAIA <mark>KSKRSPEK</mark> LFVLLDMYEIM 35	58
NtEXO70A1b	294 SGEHKVCDQIFEGFDSLKDQCFAEVTTTSVAMLLSFGDAIAKSKRSPEKLFVLLDMYEIM 35	53
NtEXO70A1a	359 <mark>RELHSEIESLFRGKSCNEIRESAFGLSKR</mark> LAQTAQETFRDFEEAVEKDATKTAVSDGTVH 41	8
NtEXO70A1b	354 RELHSEIESLFIGKACNEIRESAFGLTKRLAQTAQETFSDFEEAVEKDATKTAVSDGTVH 41	3
NtEXO70A1a	419 PL <mark>TSYVINYVK</mark> FLFDYQSTLKQLFQEFEN-GDSNSQLASVTMRIMQALQTNLDGKSKQYK 47	'7
NtEXO70A1b	414 PLTSYVINYVKFLFDYQSTLKQLFQEFENGGDSNSQLAAVTMRIMQALQTNLDGKSKQYK 47	'3
NtEXO70A1a	478 DPALTNLFLMNNIHYMVRSVRRSEAKDLLGDDWVQRHRRVVQQHANQYKRIAWAKILQCL 53	37
NtEXO70A1b	474 DPALTHLFLMNNIHYMVRSVRRSEAKDLLGDDWVQRHRRVVQQHANQYKRIAWAKILQCL 53	33
NtEXO70A1a	538 <mark>SIQGLTSSGGSNS</mark> MGVDGQNSSGVSRALVKERLKTFNIQFEELHQRQSQWTVPDTELRES 59)7
NtEXO70A1b	534 <mark>SIQGLTSSGGSNS</mark> GSVDGQNSSGVSRSIVKDRFKTFNVQFEELHQRQTQWTVPDTELRES 59)3
NtEXO70A1a	598 L <mark>R</mark> LAVAEVLL <mark>PAYRS</mark> FI <mark>KRFGPMVENGKNPOKYIRYS</mark> AEDLE <mark>R</mark> MLGEFFEG <mark>KTLNEPKR</mark> 65	56
NtEXO70A1b	594 L <mark>R</mark> LAVAEVLLPAYRSFIKRFGPLVESGKNSQKYIRYSAEDLDRMLGEFFEGKTLNEPKR 65	52

Supplemental Figure S2. Comparison of the NtEXO70A1a and NtEXO70A1b amino acid sequences. Amino acid composition of the proteins is highly conserved (90% of amino acids are identical and 94% are similar) with several amino acid substitutions dispersed throughout the protein sequences. The alignment was generated using the MAFFT algorithm in G-INS-i mode. Identical amino acid residues are colored according to their physico-chemical properties.

YFP:NtEXO70G1a





Supplemental Figure S3. Localization of YFP:NtEXO70G1a in growing tobacco pollen tubes with different expression levels. Top: cytoplasmic localization typical for normally growing pollen tubes. Bottom: localization to cytoplasm, subapical membrane and fibrillar structures typical for pollen tubes with higher expression of the construct and slightly slower growth. Bar, 10 µm.

low expression

B

high expression





Intensity (a. u.)

Supplemental Figure S4. Localization of YFP:NtEXO70B1 and NtEXO70B1:YFP in growing tobacco pollen tubes with different expression levels and colocalization of NtEXO70B1 with NtSEC3a. (A) N-terminally and C-terminally tagged NtEXO70B1 were transiently expressed in tobacco pollen tubes and their subcellular localization was spinning disk confocal microscopy. examined by Left: subapical membrane localization typical for normally growing pollen tubes with low expression levels of the transgene is shown. Right: loss of membrane signal and localization to cytoplasmic particles (most probably inclusion bodies) typical for pollen tubes with higher expression of YFP:NtEXO70B1 or NtEXO70B1:YFP constructs. The images shown are representative for ≥ 30 transformed pollen tubes observed in at three experiments for the YFP:NtEXO70B1 and ≥ 20 transformed pollen tubes observed in two NtEXO70B1:YFP. experiments for the **(B)** NtSEC3a:YFP and mRFP:NtEXO70B1 were transiently coexpressed in tobacco pollen tubes and their subcellular localization was examined by spinning disk confocal microscopy with a representative result shown. Images of individual channels are represented using a color intensity code in order to display local enrichment of the YFP/mRFP signal. In the overlay, YFP is represented by green, mRFP by magenta and white indicates the overlapping signal. Membrane localization of both constructs largely overlaps with membrane localization of NtSEC3a:YFP reaching more to the tip. Red and blue arrowheads mark onset and end of the particular membrane signal. Green arrowheads mark small area near the tip where the NtSEC3a:YFP binds membrane, whereas mRFP:EXO70B1 does not. Bar, 10 µm.

high



Supplemental Figure S5. Mutually exclusive localization of NtEXO70A1a and AtDRP1C in growing tobacco pollen tube. YFP:NtEXO70A1a and AtDRP1C:mRFP were transiently coexpressed in tobacco pollen tubes and their subcellular localization was examined by spinning disk confocal microscopy with representative result shown. Images of individual channels are represented using a color intensity code in order to display local enrichment of the YFP/mRFP signal. In the overlay, YFP is represented by green, mRFP magenta and white indicates bv the overlapping signal. Red and blue arrowheads mark onset and end of the particular membrane signal. Bar, 10 µm.



Supplemental Figure S6. Fluorescence signal intensity distribution in pollen tubes overexpressing major pollen N-terminally YFP-tagged EXO70 isoforms or YFP:GUS control. The quantitative results presented correspond to the dataset used in Figure 10. Data-points represent highest fluorescence levels for individual pollen tubes and red line indicates median value. At least 33 pollen tubes expressing each construct were observed in three independent experiments. GPT, growing pollen tube.



Supplemental Figure S7. Overexpression of the PIP5 kinase 5 results in increased NtSEC3a recruitment to the plasma membrane in pollen tubes but does not recruit NtEXO70C2 to the plasma membrane. NtSEC3a:YFP and YFP:NtEXO70C2 were transiently expressed in tobacco pollen tubes solely (left panels) or coexpressed with high amount of PIP5K5:CFP (5 µg of DNA, right panels) and subcellular localization of the exocvst subunits was examined by spinning disk confocal microscopy. The images shown are representative for \geq 10 transformed pollen tubes observed in at least two independent experiments. Pollen tubes with normal intensity of YFP signal (reflecting level of the tagged exocyst subunits expression) and with high intensity of the CFP signal (reflecting level of the PIP5K5 overexpression) were selected. Morphological deviations of the pollen tubes depicted in right panels are thus result of the PIP5K5:CFP overexpression. Dashed lines mark the site of intensity profiles, which are plotted below the representative micrographs of the pollen tubes. NtSEC3a:YFP shows enrichment at membrane, which is further increased by coexpression of the PIP5K5:CFP, while the intensity profile of neither solely expressed YFP:NtEXO70C2 nor of the YFP:NtEXO70C2 coexpressed with the PIP5K5:CFP does not show any enrichment of the YFP:NtEXO70C2 profile at the plasma membrane. Bar, 10 µm.

EXO70 paralog	Forward primer	Reverse primer	
NtEXO70A1a	AACATCATCTGGGGGCAGTAACTCTATG	CGAGGGTAAGACGTTGAATGAGCCAAAA	
NtEXO70A1b	AACATCATCTGGAGGCAGTAACTCTGGTA	TGAGGGTAAGACGTTAAATGAGCCCAAAC	
NtEXO70A2	AGACGCCGATGATGGTAATTCCAAGGA	AGTTTTTTGAAGGAAAAACCTGGAAAGGACCTTAA	
NtEXO70B1	CTAGTGCTGTTGATTATAGAGGAGAAGTGG	GATAATAATGCTATGTCGCCTACTGGAGCA	
NtEXO70C1a	GCAACATGTAAAATTGGAGGAATCCAATTCC	TGGTAATTCTGCATCAATGGCCAGGAG	
NtEXO70C1b	TGATCACGTTGAGAGCGAAAGCCCAC	CGGCAATCCTACATCTATGGCACGG	
NtEXO70C2	GAGAACATCAAATGATAGAAAGAGCTGATTCAACC	GAAATGCTACGCCATGGGGGGGGG	
NtEXO70D1a	GTGATCCTAACACCCCTGATATGGATTTTG	TATCAAGTTTCACAACACATAAGAAGAAGATCTCAG	
NtEXO70D1b	CGAGTGATCCTAATACACCTGATATGGATTTTA	GTACGCTGTATCACAGCACTTGCG	
NtEXO70E1a	GAAAACTCTTATCGATTATAGCAAAACGCTTGATGA	CTCGAGAACTCTTCTCAAGGAGAGACTA	
NtEXO70E1b	TCTGAAGGACAACGAGCAAGAAGATTTGGT	CTCCATCCTTTCGTTACTTAGGGACGA	
NtEXO70E2	TGGCCAGGCTCGACGTTTCTTCTG	GTCACTGAATCATCAATGGAGGAGGT	
NtEXO70F	AGAGAAGGTAGAGAGTGAATGTGAACCTGA	GTTTTGCACCATATGAAAAGAAAAGGCACGTAG	
NtEXO70G1a	TAAGCAGGGAAGGTCTTATTCTGTTTTCTGGT	TTCAAAGTCCGACATCCTAGTGGAAAATTCAG	
NtEXO70G2	GCTTCTTCTAAATGAGATGCGAAAAATAATAATGGTAATTGAG	TGAGCGAGGAAGGTCTAGTGTTGTTCC	
NtEXO70H1-2	CATCAGCCAACGAGACGCCAACTT	GGGGAAAAGAATTTGGAGGTCTTAGTAAGAT	
NtEXO70H3-4a	GGTGAAAATTGGATATCAAAACAGGAGGGAA	GAGCAAGCTCACCGGAAGCAATCT	
NtEXO70H3-4b	CCGATTCAAACGAATCTCCGGCAC	AACTGATTATTGAATCAGAAAGTTCTTCATCATTTGAG	
NtEXO70H5-8a	TTAACTCGATACGTCATGAACTACCTTGTTTATCTC	GTATGGGATGGAGTGGGACATCTTCG	
NtEXO70H5-8b	AATCGTCGATTGGCCGCTGTCGAT	TTCAGAGCATGGGACGACGTCGTA	
NtEXO70H5-8c	AGTCCAATCGCCGACGATGAGAACTCT	GGTTTTTCAGAAAATAGCAGTACGTCGCACG	

CGACAAATGAAAGATCCTTCGGATGGTAGTAA

GGATGCCTATAGCGCGACAATGGTC

NtEXO70I

Supplemental Table 1. Primers used for RT-PCR expression analysis of EXO70 family.

Supplemental Table 2. Primers used for molecular cloning.

Insert	Forward primer	Reverse primer	Restriction sites
NtEXO70A1a	TAGCCGGCATGGGTATGCCAAGAAACAC	TAGGGCCCTCATCGTTTTGGCTCATTC	NgoMIV, Apal
NtEXO70A1b	ATGCCGGCATGGGTATACCAAGAAACACTGG	ATGGGCCCTCACCGTTTGGGCTCAT	NgoMIV, Apal
NtEXO70A2	TAGTCGACAAATGGCGGCTTACACAGTG	TAGGGCCCTTAAGGTCCTTTCCAGGTTTTT	Sall, Apal
NtEXO70B1	TAGCCGGCATGGCTGAAAATGGCG	TAGGGCCCTCACTTCCTGCCACCAC	NgoMIV, Apal
NtEXO70C1a	TAGCCGGCATGGTGAAGAATCACTTAGATAAAAATG	TAGGGCCCCTATGTCTTCCTCCTGGCC	NgoMIV, Apal
NtEXO70C1b	TAGCCGGCATGGAGAAGAATAATGACAGTGG	TAGGGCCCTTACGTCTTTCTCCGTGCC	NgoMIV, Apal
NtEXO70C2	TAGCCGGCATGGATGCAGAGAAAGTAACACA	TAGGGCCCTTACAATTTCTTCCTCCCCC	NgoMIV, Apal
NtEXO70D1a	TAGTCGACAAATGGAACCACCGGAGA	TAGGGCCCTCACTGAGATCTTCTTCTTATGTGT	Sall, Apal
NtEXO70D1b	TAGTCGACAAATGGAATCACCAGAAAAAAACG	TAGGGCCCTCACTGAGATCTTCTCCGCA	Sall, Apal
NtEXO70E1a	TAGCCGGCATGGGAGACTGTGAAACTCTAGTC	TAGGGCCCTCACTTCCTGTGGGAACC	NgoMIV, Apal
NtEXO70E1b	TAGCCGGCATGTTGTGTGGGTTTAACTATATGG	TAGGGCCCTCACTTCCTGTGAGAACCAT	NgoMIV, Apal
NtEXO70E2	TAGCCGGCATGGGTGATGATAAATCAGTG	TAGGGCCCTCACCTCCTCCATTGATG	NgoMIV, Apal
NtEXO70F	TAGCCGGCATGGCGGCGACTATAGAAG	TAGGGCCCCTACGTGCCTTTTCTTTCATATG	NgoMIV, Apal
NtEXO70G1a	ATGCCGGCATGACGCAGATGGAGAAAG	ATGGGCCCTTATTTAACAGTTGGAGAAGTCTGAT	NgoMIV, Apal
NtEXO70G2	TAGCCGGCATGCATGGGATTGAGAAG	TAGGGCCCTCATGCTGCTGCAGG	NgoMIV, Apal
NtEXO70H1-2	TAGCCGGCATGGCAATTCTTGACATCTCTAAG	TAGGGCCCTTAAGAAAGACATTGCCAAACAC	NgoMIV, Apal
NtEXO70H3-4b	TAGTCGACAAATGCCGAAAAAAGACCTGA	TAGGGCCCTTATTCATCGCAACCGTGA	Sall, Apal
NtEXO70H5-8a	TAGCCGGCATGAGGACCCCCTTTTTC	TAGGGCCCTTATCGGCTTCGACTTGA	NgoMIV, Apal
NtEXO70H5-8b	TAGCCGGCATGAGGACTGGTTTTTTCTCTT	TAGGGCCCTCAACGGCCATGTGAC	NgoMIV, Apal
NtEXO70H5-8c	TAGCCGGCATGACTGGGTTTTTCTCTTCAT	TAGGGCCCTCAACGTAATGGAGATGAGAA	NgoMIV, Apal
AtDRP1C	ATAGCCGGCGGAACAATGGCGACGATGAAAAG	TATGGGCCCCTTCCAAGCCACTGCAT	NgoMIV, Apal