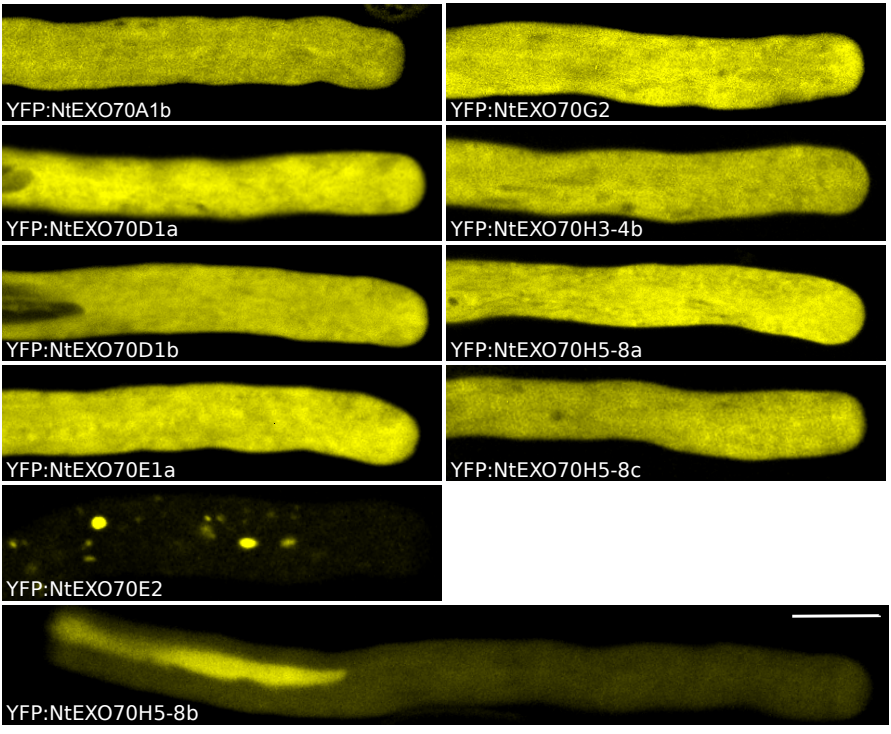
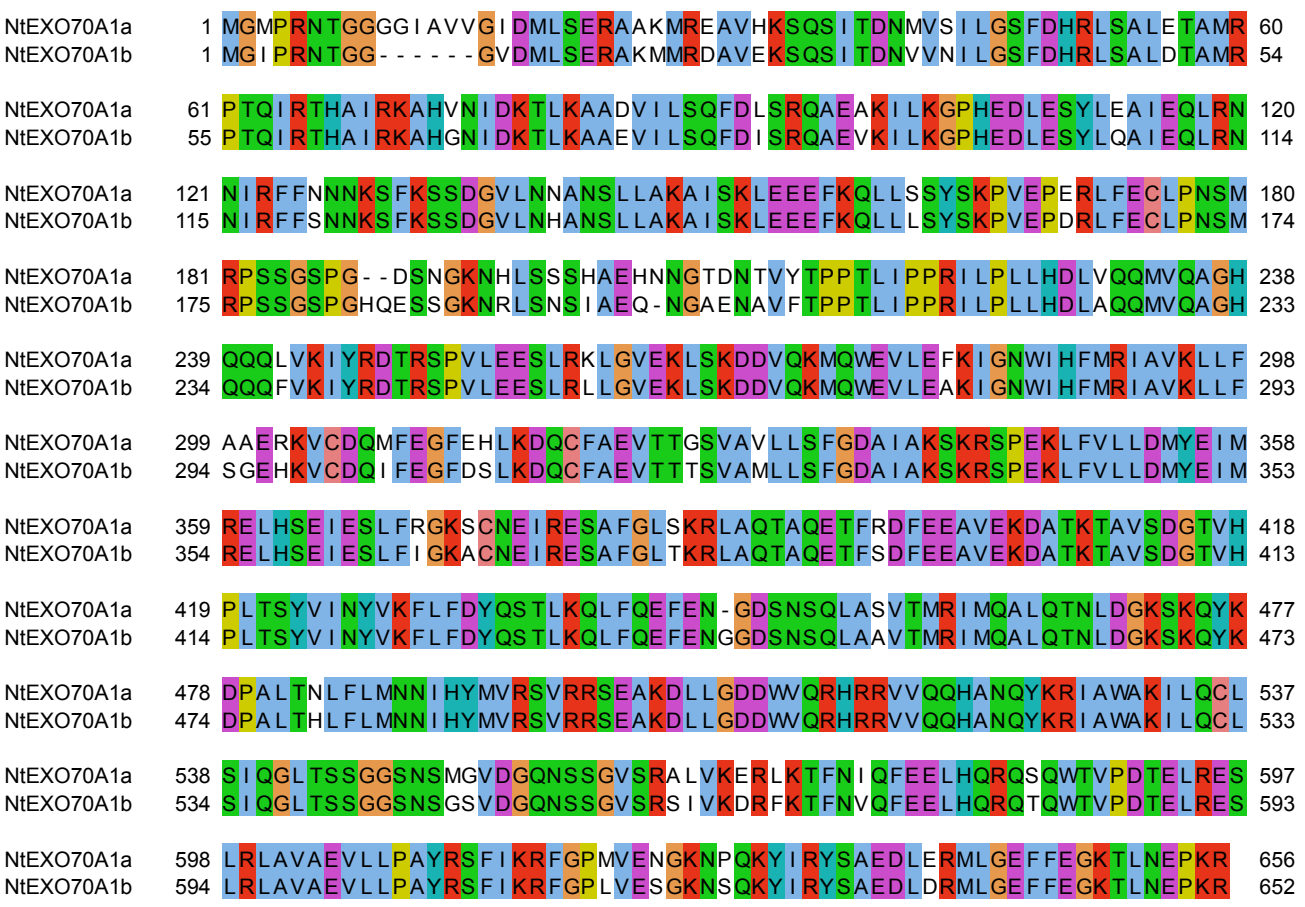
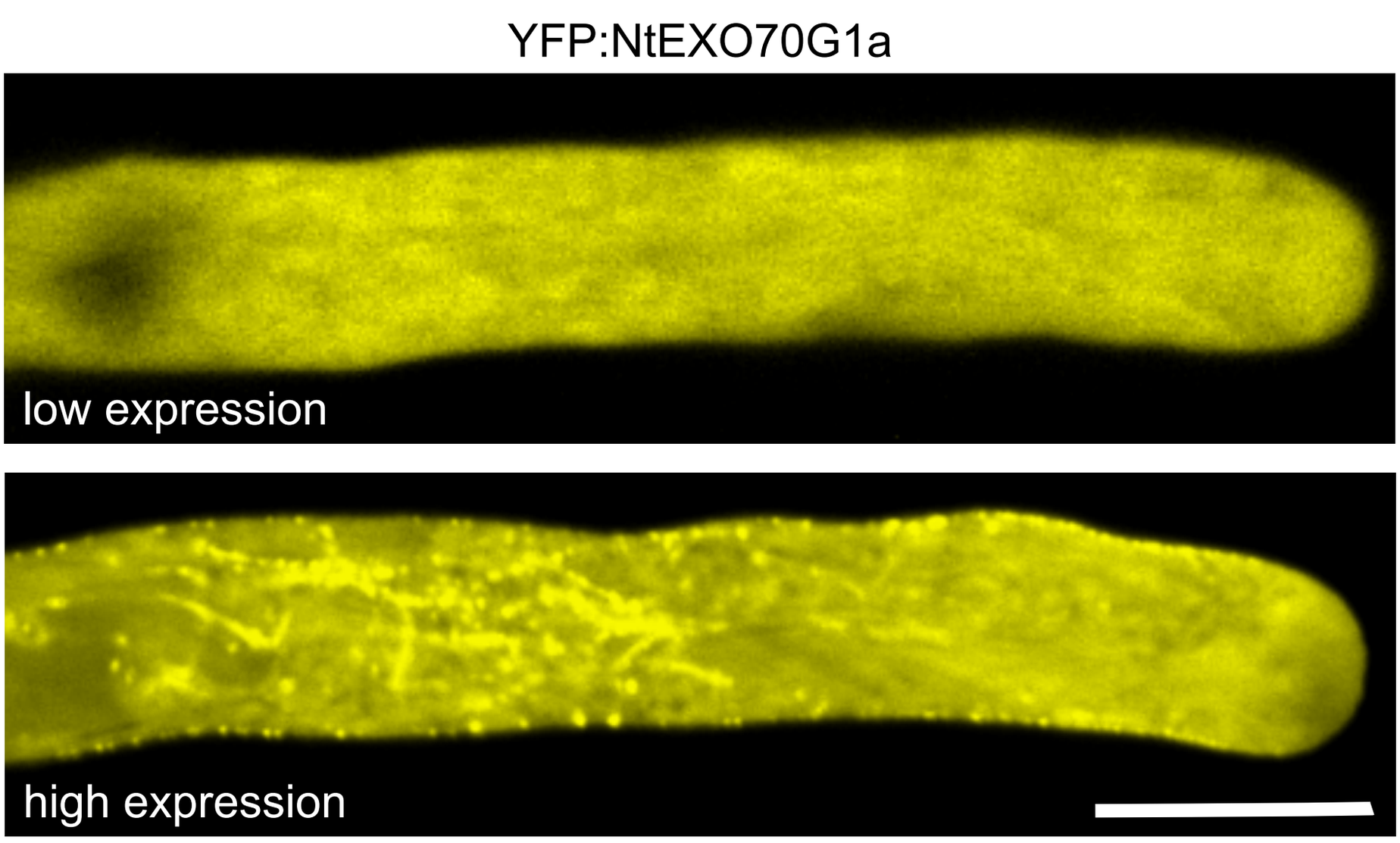


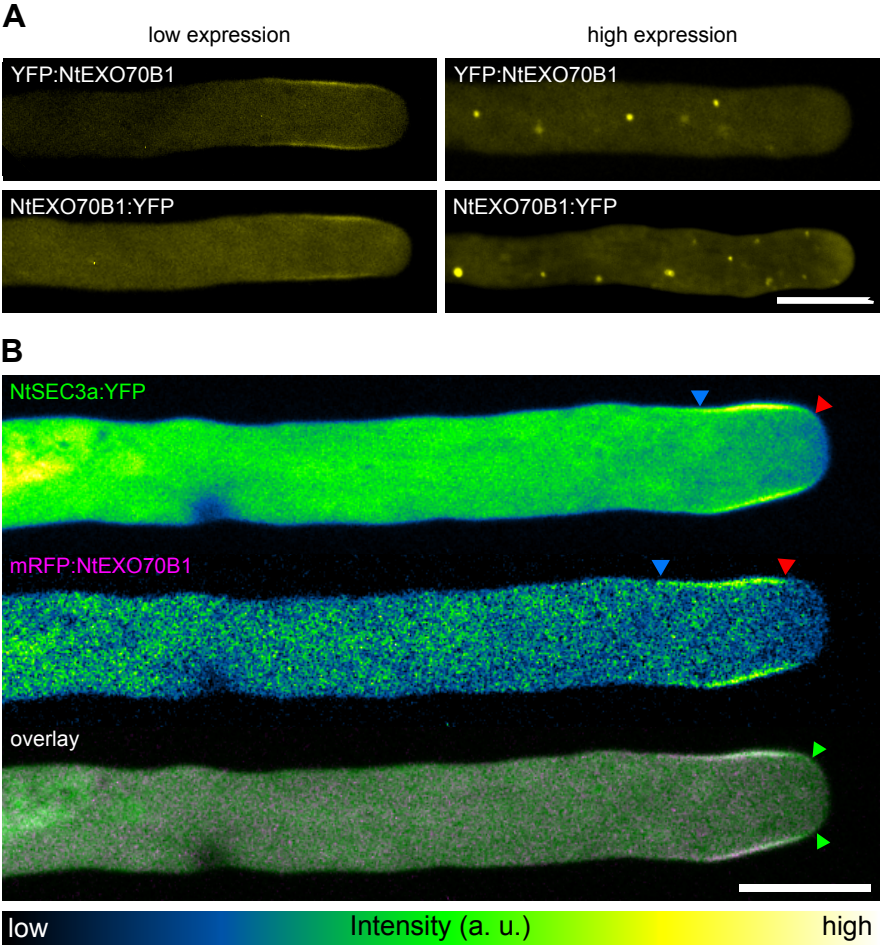
Supplemental Figure S1. Localization of non-pollen EXO70 isoforms in growing tobacco pollen tubes. Selected YFP-tagged tobacco EXO70 isoforms were transiently expressed in tobacco pollen tubes and their subcellular localization was examined by spinning disk confocal microscopy. Growing pollen tubes with 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.



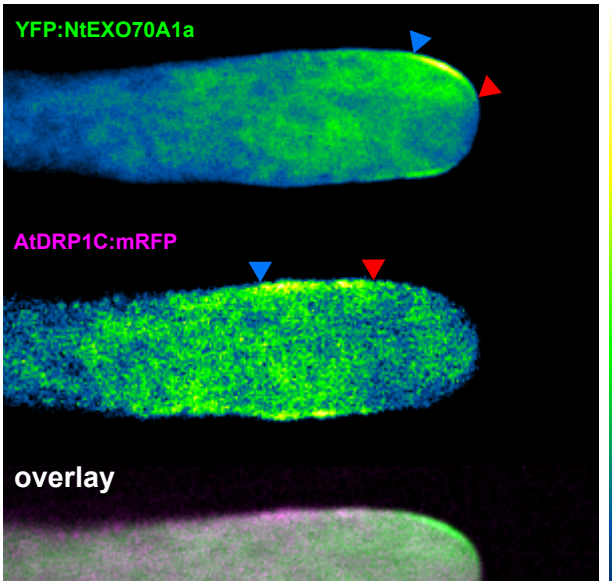
Supplemental Figure S2. Comparison of the NtEXO70A1a and NtEXO70A1b amino acid sequences. Amino acid composition of the proteins is highly conserved (90% of amino acids are identical and 94% are similar) with several amino acid substitutions dispersed throughout the protein sequences. The alignment was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



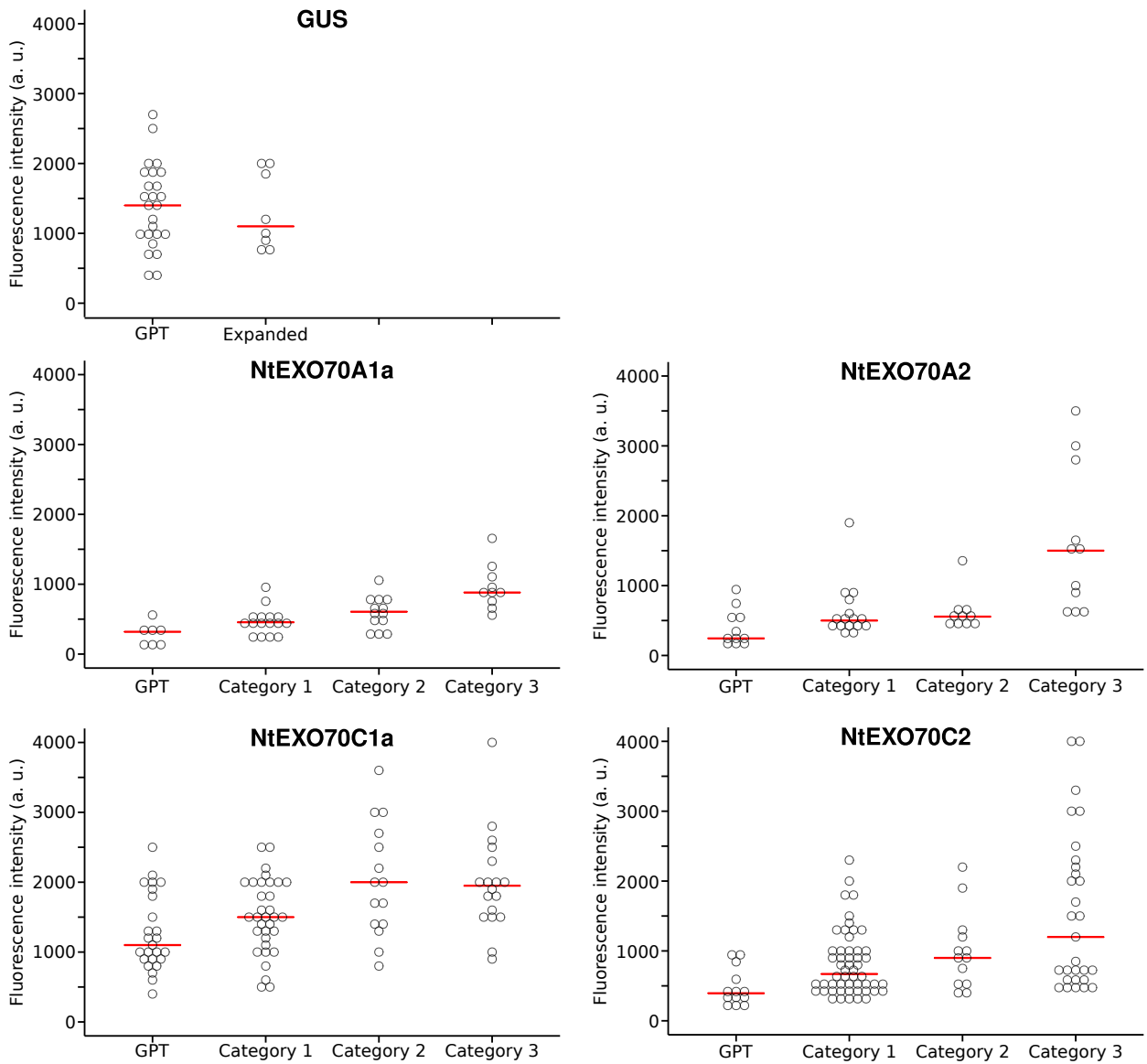
Supplemental Figure S3. Localization of YFP:NtEXO70G1a in growing tobacco pollen tubes with different expression levels. Top: cytoplasmic localization typical for normally growing pollen tubes. Bottom: localization to cytoplasm, subapical membrane and fibrillar structures typical for pollen tubes with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



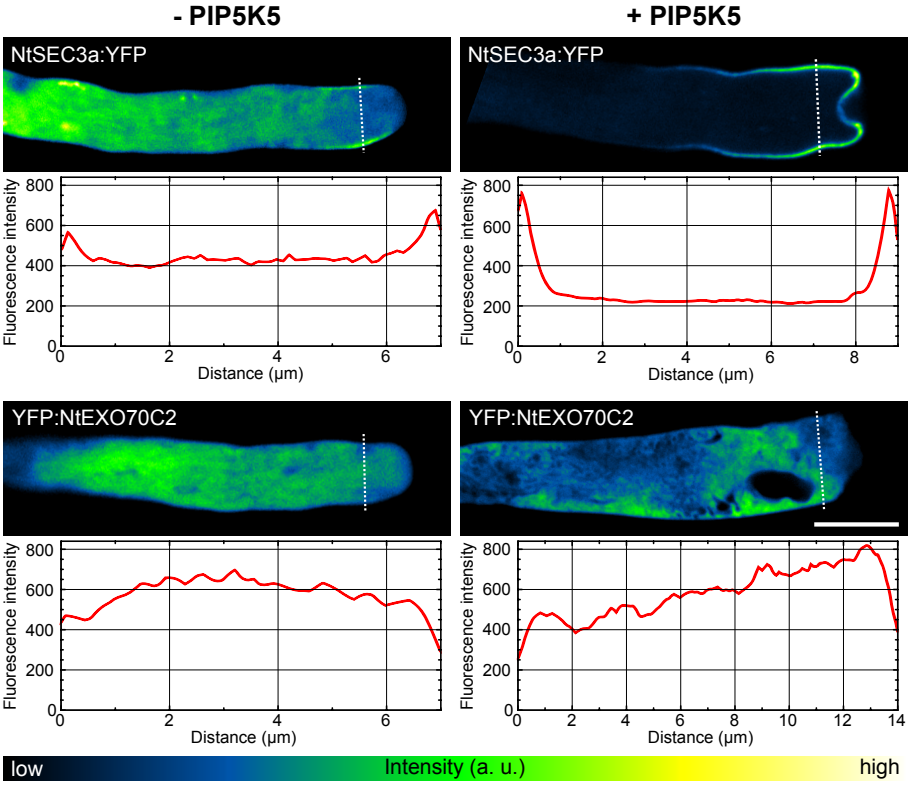
Supplemental Figure S4. Localization of YFP:NtEXO70B1 and NtEXO70B1:YFP in growing tobacco pollen tubes with different expression levels and colocalization of NtEXO70B1 with NtSEC3a. (**A**) N-terminally and C-terminally tagged NtEXO70B1 were transiently expressed in tobacco pollen tubes and their subcellular localization was examined by spinning disk confocal microscopy. 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 experiments for the NtEXO70B1:YFP. (**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.



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 by magenta and white indicates 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 exocyst subunits was examined by spinning disk confocal microscopy. The images shown are representative for ≥ 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.

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

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	GAAATGCTACGCCATGGGGGAGG
NtEXO70D1a	GTGATCCTAACACCCCTGATATGGATTTTG	TATCAAGTTTCACAACACATAAGAAGAAGATCTCAG
NtEXO70D1b	CGAGTGATCCTAATACACCTGATATGGATTTTA	GTACGCTGTATCACAGCACTTGCG
NtEXO70E1a	GAAAACTCTTATCGATTATAGCAAAACGCTTGATGA	CTCGAGAACTCTTCTCAAGGAGAGACTA
NtEXO70E1b	TCTGAAGGACAACGAGCAAGAAGATTTGGT	CTCCATCCTTTCGTTACTTAGGGACGA
NtEXO70E2	TGGCCAGGCTCGACGTTTCTTCTG	GTCACTGAATCATCATCAATGGAGGAGGT
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
NtEXO70I	GGATGCCTATAGCGCGACAATGGTC	CGACAAATGAAAGATCCTTCGGATGGTAGTAA

Supplemental Table 2. Primers used for molecular cloning.

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