Supplemental materials attached to the following manuscript:

**Expression and functional analyses of EXO70 genes in Arabidopsis implicate their roles in regulating cell type-specific exocytosis**

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Supplementary Data

The following materials are available in the online version of this article.

**Supplemental Figure S1.** Phylogenetic analysis of EXO70 proteins in different organisms
**Supplemental Figure S2.** Homology matrix tree of 23 EXO70 protein sequences.
**Supplemental Figure S3.** Alignment of the QR motif located near the C-termini of EXO70s from rice and Arabidopsis
**Supplemental Figure S4.** Expression pattern of *EXO70A1.*
**Supplemental Figure S5.** Expression pattern of *EXO70A2.*
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**Supplemental Figure S26.** Expression pattern of *EXO70H8.*
**Supplemental Table S1.** Primers used for the analysis of the expression of *EXO70* genes
**Supplemental Table S2.** Primers used for amplifying *EXO70* promoters for GUS fusion.
**Supplemental Figure S1  Phylogenetic analysis of Exo70 proteins in different organisms**

The tree was constructed using ClustalX, as described in Materials and Methods. Three clades (Exo70.1, Exo70.2, Exo70.3), 9 floral plant-conserved clusters (A to I) and one monocot-specific cluster Fx were marked at the right. Four sets of Exo70 proteins identified from completely sequenced genomes of Arabidopsis, rice, sorghum and poplar were used for this analyses. Within the 9 clusters, dicot- and monocot-specific Exo70 proteins were marked in blue and red boxes, respectively. Species abbreviations: Amoeba, *Dictyostelium discoideum*; At, *Arabidopsis thaliana*; Ce, *Caenorhabditis elegans*; Cr, *Chlamydomonas reinhardtii*; Drosophila, *Drosophila melanogaster*; Hs, *Homo sapiens*; Os, *Oryza sativa*; Pp, *Physcomitrella patens*, Pt, *Populus trichocarpa*; Sb, *Sorghum bicolor*; Sc, *Saccharomyces cerevisiae*; Sm, *Selaginella moellendorffii*.

For the phylogenetic analysis, full-length sequences of Exo70 proteins from completely sequenced genomes of 4 angiosperms (rice, sorghum, Arabidopsis and poplar), an ancient vascular plant *Selaginella moellendorffii* and a moss *Physcomitrella patens* were obtained from GenBank (National Centre for Biotechnology Information, NCBI) by methods described before (Chong et al., 2009). Some monocot-specific Exo70s in the Exo70FX subclade carrying large omissions from their N- or C-termini were not included in this analysis. The alignments were generated with ClustalX (ftp://ftpigbmc.u-strasbg.fr/pub/ClustalX; Jeanmougin et al., 1998) and then manually adjusted. DND output files were analyzed with TreeView 1.6.6 (http://rana.lbl.gov/EisenSoftware.htm; Zhai et al., 2002). Both distance-based (neighbor-joining) and Kimura's two-parameter methods were used (Kimura, 1980), yielding trees with very similar topology. Reliability of the tree was estimated by using 1000 bootstrap replications.
Supplemental Figure 2. Homology Matrix Tree of 23 EX070 Proteins in Arabidopsis
Supplementary Figure S3: Alignment of the C2 Motifs of EXOR70s from Rice and Arabidopsis
Supplemental Figure S4. Expression pattern of **EXO70A1**

A, the AVT expression profile of this member; B, a 3-day-old seedling, showing GUS signal in developing vascular bundles; C, an enlarged view of the hypocotyl of 3-day-old seedling, showing GUS expression in developing xylem; D, leaves and cotyledons of a 7 day-old seedling, showing GUS signal in newly formed vascular bundles; E, Inflorescence, showing signal in carpels; F, GUS expression is associated with protoxylem of young carpel.

Bars=1mm in B, E; Bar=50 μm in C; Bars=0.5mm in D, F.
Supplemental Figure S5. Expression pattern of EXO70A2
A, the AVT expression profile of this member; B, a 3-day-old seedling, no GUS signal was found; C, a 7 day-old seedling, showing very weak GUS expression in vascular bundles, but strong signal in newly formed leaves; D, an enlarged view of selected zone in C, showing GUS signal in the vascular bundle of roots; E, guard cells of young leaf observed in C, showing strong GUS expression in stomata cells of early stage; F, no GUS signal was found in inflorescence. Bar=0.5mm in B; Bars=1mm in C, F; Bars=50 μ m in D, E.
Supplemental Figure S6. Expression pattern of *EXO70A3*

A, the AVT expression profile of *EXO70A3*; B, a 3-day-old seedling, showing strong GUS signal in root tip; C, a 5 day-old seedling with GUS signal in root tip; D, a root tip from 3-day-old seedling, GUS signal located in root cap cells; E, an enlarged view of selected region in C (note that at this stage, only the border cells were stained); F, no GUS expression was observed in inflorescence.

Bars=0.5mm in B,F; Bar=1mm in C; Bars=50 μm in D, E.
Supplemental Figure S7. Expression pattern of *EXO70B1*
A, the AVT expression profile of this member; B, a 3-day-old seedling, no GUS signal was detected; C, a 7 day-old seedling, weak signal was found both in primary and lateral root; D, an enlarged view of selected region in C; E, the weak GUS signal was localized in endodermal layer of root; F, inflorescence and siliques, no GUS expression was found. Bars=0.5mm in B,D; Bars=1mm in C,F; Bar=50 μm in E.
Supplemental Figure S8. Expression pattern of *EXO70B2*

A, the AVT expression profile of this member; B, a 3-day-old seedling, no GUS signal was detected; C, a 7 day-old seedling, no signal was found; D and E, no GUS signal was found in inflorescence and siliques. The GUS expression data are not consistent with AVT and RT-PCR data. The regulatory elements might be missed in the construct. Bar=0.5mm in B; Bars=1mm in C, D; Bar=0.25mm in E
Supplemental Figure S9. Expression pattern of *EXO70C1*

A, the AVT expression profile of this member; B, a 3-day-old seedling, the GUS signal was detected in vascular tissue and guard cells of cotyledons; C, a 7 day-old seedling, strong GUS signal was found in root and leaves; D, an enlarged view of a root in C, showing the GUS signal started from elongation region and became stronger at root hair region, and then turned weak after this region; E, guard cells of the cotyledon, strongest signal was found in guard cells at early developmental stage; F, inflorescence, showing GUS signal in mature anthers; G, stage 13 flower, showing the GUS signal observed in the pollen in anther but not in the released ones on the stigma. Bars=0.5mm in B, F,G; Bar=1mm in C; Bar=50 μm in D,E.
Supplemental Figure S10. Expression pattern of EXO70C2

A, the AVT expression profile of this member; B, a 3-day-old seedling, showing GUS signal in whole root except the root tip; C, a 7 day-old seedling, GUS signal become less abundant in roots except regions with developing root hairs; D, root hair cells of 7 day-old seedlings, GUS signal was only observed in the hair cells undergoing tip growth; E, the GUS expression in mature anthers; F, strong GUS signal was observed in mature pollen and pollen tubes in the style tissue. Bar=0.5mm in B; Bars=1mm in C,E; Bars=50 μm in D, F.
Supplemental Figure S11. Expression pattern of *EXO70D1*

A, the AVT expression profile of this member; B, a 3-day-old seedling, showing the GUS signal in the root tip; C, a 5 day-old seedling, showing the GUS signal in the root tip; D, root tip of 3-day-old seedling, the GUS signal was localized in the meristematic zone, excluding QC, lateral root cap and columella; E, the GUS expression was detected in the lateral root primordium; F, no GUS signal was found in floral organs. Bars=1mm in B,C,F; Bars=50 μm in D, E.
Supplemental Figure S12. Expression pattern of *EXO70D2*

A, the AVT expression profile of this member; B, a 3-day-old seedling, the GUS signal was found in root tip, hypocotyls and cotyledons; C, a 7 day-old seedling, *GUS* expression was observed in hypocotyls, cotyledon and leaf. In addition, a weak signal was localized at root tip; D, the *GUS* expression was detected in lateral root cap in 7 day-old seedlings; E, the GUS signal was associated with trichome cells of leaf, especially in newly formed ones; F, the *GUS* expression was found in node of inflorescence and carpels (inset). Bar=0.5mm in B; Bars=1mm in C,F; Bar=0.1mm in E; Bar=50 μm in D.
Supplemental Figure S13. Expression pattern of *EXO70D3*

A, the AVT expression profile of this member; B, a 3-day-old seedling, showing GUS signal in the root tip, and weak signal in the hypocotyl; C, a 7 day-old seedling, showing GUS signal in leaves; D, a root tip of a 7 day-old seedling, *GUS* expression was located in lateral root cap; E, a lateral root tip of a 7-day old seedling, GUS signal was detected in the lateral root cap; F, a 7-day old seedling, the *GUS* expression was observed in developing trichomes on the leaf surface; G, the GUS signal was not found in inflorescences. Bars=0.5mm in B, F; Bars=1mm in C, G; Bars=50 μm in D, E.
Supplemental Figure S14. Expression pattern of EXO70E1

A, AVT expression profile of this member; B, 3-day-old seedling, GUS signal was found in cotyledon and root hair region in root; C, 7 day-old seedling, showing strong signal in cotyledon, leaf and root excluding root tip; D, an enlarged view of root tip, GUS expression was started from elongation region and restricted to root hair cell in root tip. In the other part of root (near hypocotyl), the GUS was distributed in all cell types; E, the GUS expression of root hair cells showed strongest signal in the cells undergoing hair initiation; F, GUS staining was not found in inflorescence. Bar=0.5mm in B; Bars=1mm in C,F; Bar=50 μm in D; Bar=0.25 μm in E.
Supplemental Figure S15. Expression pattern of EXO70E2

A, AVT expression profile of EXO70E2 (At5g61010) ; B, 3-day-old seedling, the GUS signal was found in hypocotyl and root hair region; C, 7 day-old seedling, the GUS signal was found strongly expressed in root, leaf and hinge of leaves; D, the inflorescence, showing GUS staining in node of inflorescence.
Bars=0.25mm in B, C; Bar=0.5mm in D.
Supplemental Figure S16. Expression pattern of EXO70F1

A, AVT expression profile of EXO70F1; B, a 3-day-old seedling, GUS signal was found in the root close to hypocotyl; C, a 7 day-old seedling, the signal was found in root and hypocotyl; D, an enlarged view of leaves in C, showing GUS signal from stipules and hinge of leaves; E, the GUS expression was detected in siliques; F, the GUS signal was found in base of siliques. Bars=0.5mm in B, F; Bars=1mm in C, E; Bar=100 μm in D.
Supplemental Figure S17. Expression pattern of *EXO70G1*

A, AVT expression profile of *EXO70G1*; B, a 3-day-old seedling, GUS signal was found in root tip; C, a 7 day-old seedling, the signal was found in similar pattern as 3-day-old seedling; D, an enlarge view of root tip in B, showing GUS signal in root tip excluding epidermal cells, lateral root cap and columella; E, strong GUS signal was found in newly formed lateral root tip with similar staining pattern as primary root; F, Inflorescence, showing GUS signal located in young flower buds; G, GUS expression was observed in the tapetum during early pollen development. Bar=0.5mm in B; Bars=1mm in C,F; Bars=50 μm in D, E, G.
Supplemental Figure S18. Expression pattern of *EXO70G2*

A, AVT expression profile of *EXO70G2*; B, a 3-day-old seedling, GUS signal was associated with vascular tissue in root. Some strong staining sites were observed along the root, these sites were presumed the location where the lateral root will be formed; C, Inflorescence, showing GUS signal was in base of siliques and anthers; D, the GUS expression was detected in the endodermal and pericycle cells close to the promidia, as the LR undergoing protrusion; E, the cotyledon, showing GUS signal in the sites where the vascular tissue undergoing differentiation; F, stage 13 flower, showing the signal in pollen, but not in germinated pollen on the stigma. Bar=0.5mm in B; Bars=1mm in C; Bars=50μm in D, E; Bars=100μm in F.
Supplemental Figure S19. Expression pattern of *EXO70H1*

A, AVT expression profile of *EXO70H1*; B, a 3-day-old seedling, obvious GUS signal was found in root except root tip; C, a 7 day-old seedling, the signal was found in similar patterns as 3-day-old seedling, but the expression restricted to root hair region where the root hair undergoing growth; D, an enlarged view of the root tip in B, GUS signal started from elongation zone; E, an enlarged view of root hair region in B, the growing root hair cells was strongly stained; F, Inflorescence, weak GUS signal was located in the small population of cells underneath stigma hair of open flowers (inset). Bar=0.25mm in B; Bars=1mm in C,F; Bars=50 μm in D, E.
Supplemental Figure S20. Expression pattern of EXO70H2
A, AVT expression profile of EXO70H2; B, a 3-day-old seedling, showing GUS signal in the root hair region and above, no signal detected in hypocotyl and cotyledon; C, a 7-day old seedling, showing similar patterns as the 3-day-old one; D, inflorescence, showing GUS signal in the upper part of the filaments. Inset showed the signal in the whole filament during its elongation; E, GUS signal was localized in the joint between stalk and stamen just before the flower opens. Bars=0.5mm in B, D; Bar=1mm in C; Bars=0.1mm in inset of D and E.
**Figure S21. Expression pattern of EXO70H3**

A, AVT expression profile of this member; B, a 3-day-old seedling, no GUS signal was observed; C, a 7-day old seedling, showing no GUS signal; D, inflorescence, showing the GUS signal in mature anthers; E, signal was observed in pollens at flower stage 12; F, GUS expression was found in the pollen tubes growing in the carpel.

Bar=0.5mm in B; Bars=1mm in C and D; Bar=100 μm in E,F.
Supplemental Figure S22. Expression pattern of *EXO70H4*

A, AVT expression profile of this member; B, a 3-day-old seedling, showing the GUS signal in the root hair region and above; C, a 7-day old seedling, showing GUS signal in guard cells of young leaves and the root hair regions of root; D, leaf, showing strong GUS signal in the developing guard cells; E, an enlarged view of the root from the 3-day-old seedling, all the cell layers were stained in the root hair region; F, inflorescence, showing the weak GUS signal in the pistil (inset). Bar=0.5 mm in B; Bars=1 mm in C, F; Bars=50 μm in D and E.
Supplemental Figure S23. Expression pattern of EXO70H5

A, AVT expression profile of this member; B, a 3-day-old seedling, no GUS signal was found; C, a 7-day old seedling, showing no GUS signal; D, inflorescence, showing GUS signal in mature anthers; E, a flower 10 hours after pollination, showing that mature pollen grains and pollen tubes in carpel were stained. Bars=1mm in B, C, D, E.
Supplemental Figure S24. Expression pattern of EXO70H6
A, AVT data was absent for this member; B, a 3-day-old seedling, no GUS signal was observed; C, a 7-day old seedling, showing weak GUS signal in the junction between the root and the hypocotyl; D, an enlarged view of C; E, inflorescence, showing signal in the pollen tubes growing in transmitting tissue of ovary; F, an enlarged view of an ovule one day after pollination, showing GUS signal in the pollen tube and micropylar end.
Bar=0.5 mm in B; Bars=1 mm in C,E; Bar=50 μm in D; Bar=25 μm in F
**Supplemental Figure S25. Expression pattern of EXO70H7**

A, AVT expression profile of this member; B, a 3-day-old seedling, showing the GUS signal in the vascular tissue and root tip and exhibiting weak signal in cotyledons; C, a 7-day old seedling, with similar pattern as the 3-day-old seedling, but strong signal was seen in newly formed leaves; D, an enlarged view of root tip of a 3-day-old seedling, showing patched pattern of GUS expression in the meristem zone; E, the root hair region of 3-day-old seedling, showing the GUS signal restricted to vascular bundle in this region; F, Inflorescence, showing signal in carpals (inset). Bar=0.5mm in B; Bars=1mm in C,F; Bars=50 μm in D, E.
Supplemental Figure S26. Expression pattern of *EXO70H8*

A, AVT expression profile of this member; B, a 3-day-old seedling, showing GUS signal in vascular bundle; C, a 7-day old seedling, showing similar pattern as the 3-day-old seedling, but the staining was restricted to the region close to the root tip; D and E, an enlarged views of the root, showing the GUS signal in the vascular bundle of root hair region. F, Inflorescence, no GUS signal was detected. Bars=0.5mm in B, D, F; Bar=1mm in C; Bar=50 μm in E.
### Supplemental Table S1. Primers used in RT-PCR analyses of expressions of AtEXO70 genes in Arabidopsis

| Gene names | Forward primers | Reverse primers |
|------------|-----------------|-----------------|
| AtEXO70A1  | AAAAGTATTCTCCAGTTTTGGGAAT | ATCGAATTGAGACAAATATTCCA |
| AtEXO70A2  | CTGTAGACATGTTTTTCTTGTAGTGA | CAGTATCTGTCGACTCATCTTCTT |
| AtEXO70A3  | TTAGACAAGACTTCTTCCAGACG | TATAATCAGTTTTCTCTGAAGCTT |
| AtEXO70B1  | TTACAGAAGCTGAGATTGGAAGAG | GAGGTAATTTCATCATAAACGAGT |
| AtEXO70B2  | GAAGAGACACACGACACAAAG | CGATCAAGAAATAGCGGTTT |
| AtEXO70C1  | GATTTCTATTCAAACACTTTTCTTCC | ACATACAAACACTCTCTGCTTCTGAAAC |
| AtEXO70C2  | ATTTTAGAGAATCTGAGACCAAA | GAAATCCCTCTGCAAGTCTT |
| AtEXO70D1  | CTCTCTAGATCTCCTGTTTCTTCC | ACCTGATGAGAATCTCTGTTCTT |
| AtEXO70D2  | ATGCTACAATTCAATCTAGAAGAGA | TTCAAGATTTTTCAACAGAGATTTTC |
| AtEXO70D3  | TTGCACTAGAGGTTTTTCTAGT | TATAGGCTTGGAGTTTCTCTTCAT |
| AtEXO70E1  | CTTTGAAGACTTGAAGGAG | GAAGAGGAGGTTTCTCTGTTCTT |
| AtEXO70E2  | GACTTTTTATAATTCTGATTTTGTTT | AAGTAGTTCTTACATACCTGTAA |
| AtEXO70F1  | GTGTGAGAAGCTGAGATTGAAGAGA | TAATTCATCATAAAGCTATTCAGT |
| AtEXO70G1  | ACAGGTACCATTTCCAGATG | TGTGCAATACGAGTTCTACAA |
| AtEXO70G2  | CTCAACATACTGAGAGAGATTGCT | CTCTCTCTGTTTCTCTTCTT |
| AtEXO70H1  | TTACAAGGATGTTTTTCTTTCTTAC | TTGTGTAAGATCCTCTGTTCTA |
| AtEXO70H2  | GATATGGAAAAGAGTGCAATAAAGAG | GCTGATTCTCAGAGAGATGATA |
| AtEXO70H3  | TTTGAACTCATAATCGTAAAGAC | GATCTAGTCAGAGCTACCTCA |
| AtEXO70H4  | TCAAGGTTGAGAAGAGATTGGT | TTGAAGTCAAGACTCTGCA |
| AtEXO70H5  | TCTGAAATCGACAGCAGAAATC | ACGTCTGATTCTGAATC |
| AtEXO70H6  | CAAGGAGTACGCGTACCTTAC | AGTTTTGGATCGGCGGACGGATC |
| AtEXO70H7  | TAAAGCTCAGCCGTTAAGA | AGCCTCTAGACCCAAAGTAA |
| AtEXO70H8  | CGTCTCTATAACGCGTACGC | TAAAGCAGAAACCCGAGG |
## Supplemental Table S2 Primers used for amplifying AtEXO70 promoters in Arabidopsis for GUS fusion

The AttB1 and AttB2 primers were added to the 5’ ends of gene-specific forward and reverse primers, respectively, for Gateway (Invitrogen) conversions.

| Gene names | Forward primers | Reverse primers | Sizes of PCR products |
|------------|-----------------|-----------------|-----------------------|
| AtEXO70A1  | CATTAACATCATTTCCTC | GGCGAATTCCAAAAC | 1761bp |
| AtEXO70A2  | TCTAAAGGATGGATTTTTTCG | TTTCTTGATTTGGATCG | 1127bp |
| AtEXO70A3  | AAGGTATCTTAGGAATATC | CTGATTATAACAAAAAAAAG | 2246bp |
| AtEXO70B1  | AGAAAAGCGTGCAGACTTCATG | GATTAAGACGATGGTG | 1266bp |
| AtEXO70B2  | CGTCTGTTCTATAATTTCG | CTATTCAATTGGAATCAG | 1236bp |
| AtEXO70C1  | ATGGCAACAATGACTTG | TTTCGCGCGGGAAAAA | 1225bp |
| AtEXO70C2  | ATATATTCTCTAAAGAAAAAG | GGTTCGTTATTATTACTG | 1055bp |
| AtEXO70D1  | TTGTGTTGTATCGTTTGGTG | GTTTCATGGAATAATAG | 2127bp |
| AtEXO70D2  | ATTATTCTCATTTGCAG | ATACGAAATCTGAACACTG | 1948bp |
| AtEXO70D3  | GAAAGAGAAAAAAAAATC | CTAATAAATCAATCAGAC | 1535bp |
| AtEXO70E1  | AATTAACATAATACAGG | CTCCACACAAATCAC | 1496bp |
| AtEXO70E2  | AATGTTCAATTTCTGAAAG | AACCACACACAAAAAG | 1011bp |
| AtEXO70F1  | CATGGAACACTCAAG | CATGGAGAAAATTG | 1599bp |
| AtEXO70G1  | ATACGACGAGGAG | AAATTCATAAAAATAAAC | 2128bp |
| AtEXO70G2  | CAGGTTTCTGGGGCTTTC | TGTTTTGCTGGTTACTC | 1074bp |
| AtEXO70H1  | CATTGTAAGAGAAGAAG | TGAAGAAAGGAAGAAGCTG | 2116bp |
| AtEXO70H2  | GATICTCATAACATTAC | TGAAGAGATTACTAC | 1657bp |
| AtEXO70H3  | TGAAGGAGCGGTGTTT | CTGGATCTTCTAGTCTG | 1004bp |
| AtEXO70H4  | CATGATTGGCTCAATGC | CAAGTCGTCGTG | 1045bp |
| AtEXO70H5  | TTGCTTTGGTTTTAATTTC | TTTGTTTTGTGTGTGTTT | 1436bp |
| AtEXO70H6  | GTTGGTCCTTTTGGTGTTT | GTTTTGTTGGTTGGATG | 1565bp |
| AtEXO70H7  | AAACACTAGTGCTCTAAAAAG | TTTGTTGTTTGCTCTG | 1947bp |
| AtEXO70H8  | TAGATGTTGACGGTGTG | CTTTTTGTTGGTTG | 1633bp |
| AttB1      | GGGGACAAGTTTGTACAAAAAGCAGGCT | |
| AttB2      | GGGGACACTTTTGTTACAGAAAGCAGCTGGT | |