Supporting Information

Pumplin et al. 10.1073/pnas.1110215109

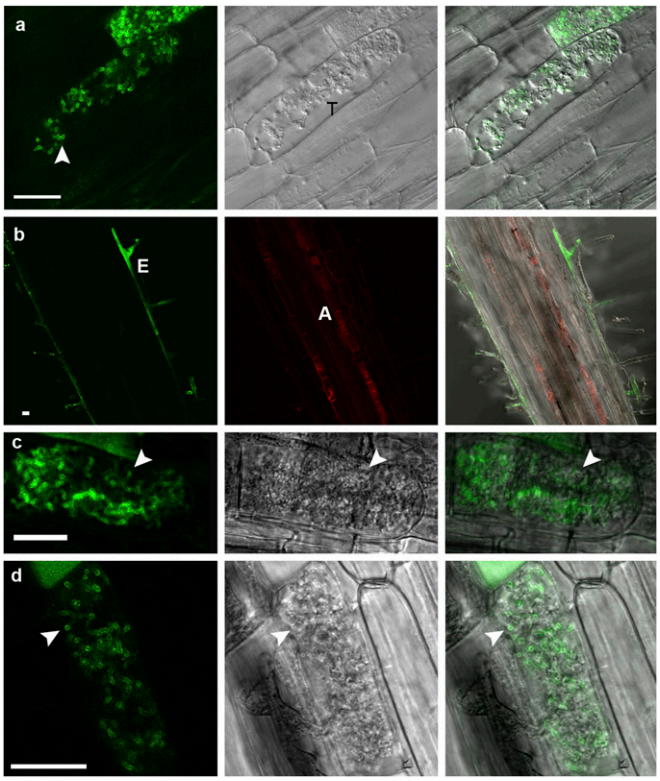

Fig. S1. Localization of MtPT4-GFP and MtPT1-GFP and various putative sorting-motif mutants. *Medicago truncatula* roots colonized by *Glomus versiforme*. (*A*) *pMtPT4*:MtPT4-GFP is located on the periarbuscular membrane around arbuscule branches and is first detected as arbuscules begin to branch. (*B*) *pMtPT1*:MtPT1-GFP is expressed in epidermal and root hair cells (E) and is not detected in cortical cells containing arbuscules (A). These roots also express *pBcp1*:AtPIP2a-mcherry, and colonized regions of the cortex show red fluorescence (A). (*C* and *D*) Mutation of putative sorting motifs present in MtPT4 does not affect localization. Expression of MtPT4 derivatives under the native promoter harboring (*C*) deletion of the soluble carboxy-terminus MtPT4$^{\Delta 506}$-GFP and (*D*) mutation of two tyrosines to alanine from putative YXXΦ endocytosis motifs, MtPT4$^{Y228/239A}$-GFP. (*Left*) GFP fluorescence. (*Center*) Bright-field differential interference contrast (DIC). (*Right*) Overlay. Localization in the periarbuscular membrane around branches is observed for all proteins (arrowheads). (Scale bars: 20 μm.)

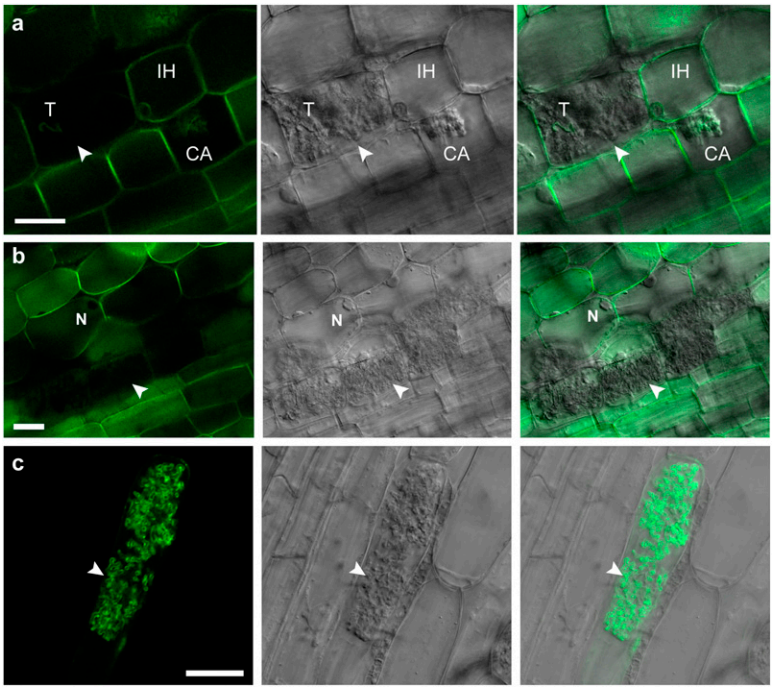

Fig. S2. Localization of MtPT1-GFP is unaffected by 5′ UTR sequence. (*A* and *B*) *M. truncatula* roots colonized by *G. versiforme* and expressing *p35S:MtPT1-GFP* with the MtPT4 5′ UTR sequence display fluorescence in the plasma membrane and in the membrane surrounding the arbuscule trunk (T) but not in the periarbuscular membrane. In some cells additional vacuolar signal arising from degradation of the MtPT1-GFP protein is apparent, particularly in cells in which the 35S promoter is most active. In roots expressing p35S:MtPT1-GFP, the signal intensity is reduced in cells with mature arbuscules. (*C*) *pMtPT4:MtPT1-GFP* with the 35S 5′ UTR sequence is localized in the periarbuscular membrane. (*Left*) GFP fluorescence. (*Center*) Bright-field DIC. (*Right*) Overlay. (Scale bars: 20 μm.) IH, intracellular hypha.

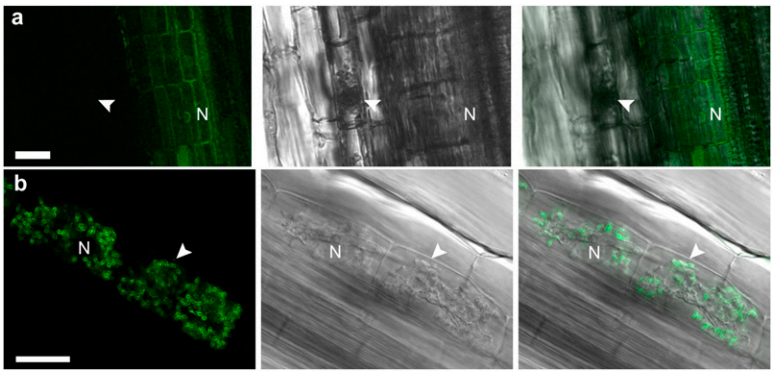

Fig. S3. Localization of MtPT4-GFP is unaffected by 5′ UTR sequence. No difference is observed in the constructs containing MtPT4 5′ UTR in *M. truncatula* roots colonized by *G. versiforme* and expressing (*A*) *p35S:MtPT4-GFP* with the 35S 5′ UTR or (*B*) *pMtPT4:MtPT4-GFP* with the 35S 5′ UTR. (*Left*) GFP fluorescence. (*Center*) Bright-field DIC. (*Right*) Overlay. Arrowheads point to arbuscules. (Scale bars: 20 μm.) N, nucleus.

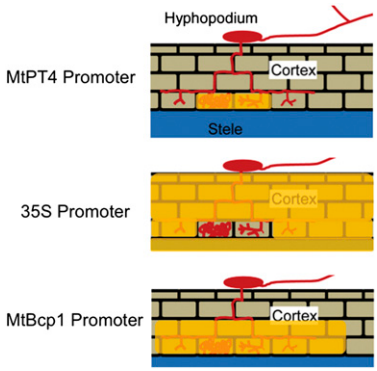

Fig. S4. Spatial expression patterns of the MtPT4, MtBcp1, and 35S promoters. Promoter activity is depicted in yellow, arbuscular mycorrhizal AM fungus in red, and vascular tissue (stele) in blue. The MtPT4 promoter is expressed only in cells with arbuscules, whereas the MtBcp1 promoter is expressed in the cortex in cells with arbuscules and also in noncolonized cells and cells with intracellular hyphae. The 35S promoter is expressed throughout the root, although down-regulation is apparent in cells with arbuscules.

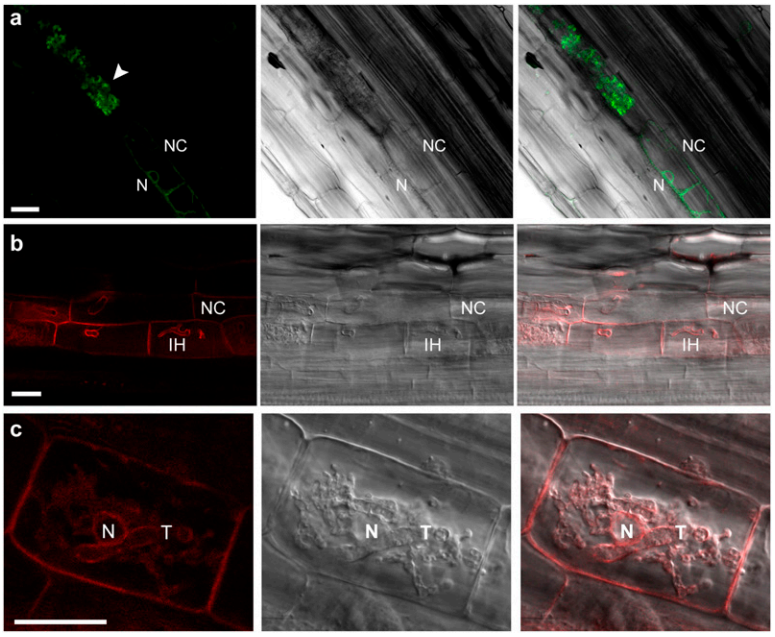

Fig. S5. Localization of MtPT4 and AtPIP2a expressed from the MtBcp1 promoter. (*A*) pMtBcp1:MtPT4-GFP localizes in the periarbuscular membrane in cells with arbuscule branches (arrowhead) but is retained in the endoplasmic reticulum (ER) of noncolonized cells (NC). (*B*) pMtBcp1:AtPIP2a-mCherry localizes in the plasma membrane of noncolonized cells and in cells containing intracellular hyphae (IH). (*C*) pMtBcp1:AtPIP2a-mCherry localizes in the plasma membrane, surrounding arbuscule trunks (T) and the ER, but not in the periarbuscular membrane surrounding arbuscule branches. These localization patterns represent a combination of the localization patterns observed with proteins expressed from the MtPT4 and 35S promoters. (Scale bars: 20 μm.)

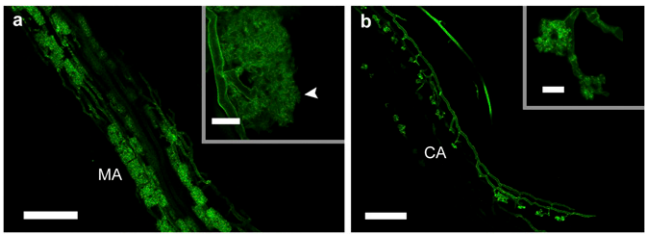

Fig. S6. Phenotype of *mtpt4-3*. Mature arbuscule (MA) morphology in roots of wild-type (*A*) and prematurely collapsed arbuscules (CA) in *mtpt4-3* mutant (*B*) roots, visualized by the chitin stain wheat germ agglutinin (WGA)-Alexa Fluor 488 (green). (Scale bars: 100 μm in *A* and *B*, 10 μm in *Insets*.)

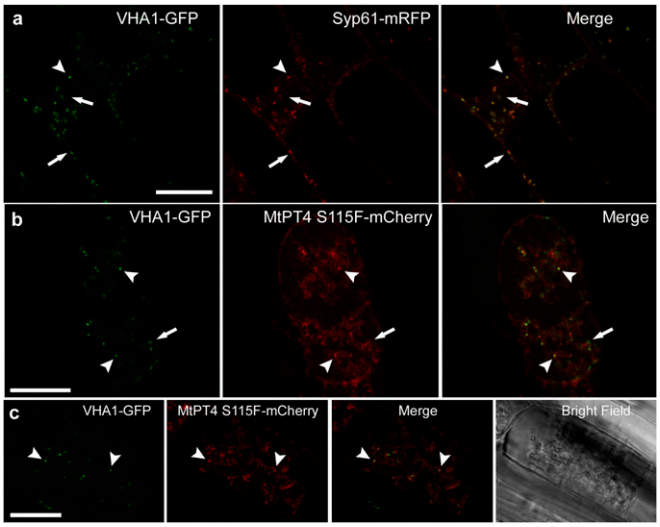

Fig. S7. Colocalization with the TGN marker VHA1. (*A*) Colocalization of VHA1-GFP and Syp61-mRFP reveals strong colocalization (arrowhead), but some labeled compartments do not overlap (arrow). (*B* and *C*) VHA1-GFP coexpressed with pMtPT4:MtPT4^{S115F}-mCherry reveals strong colocalization, but some compartments do not overlap. Because MtPT4^{S115F} resides in Syp61-labeled compartments that mostly overlap with VHA1-labeled compartments, MtPT4^{S115F} is likely retained in the TGN as well as in the ER. Syp61-mRFP is expressed from the 35S promoter, and VHA1-GFP is expressed from the pUbq10 promoter. (Scale bars: 20 μm.)

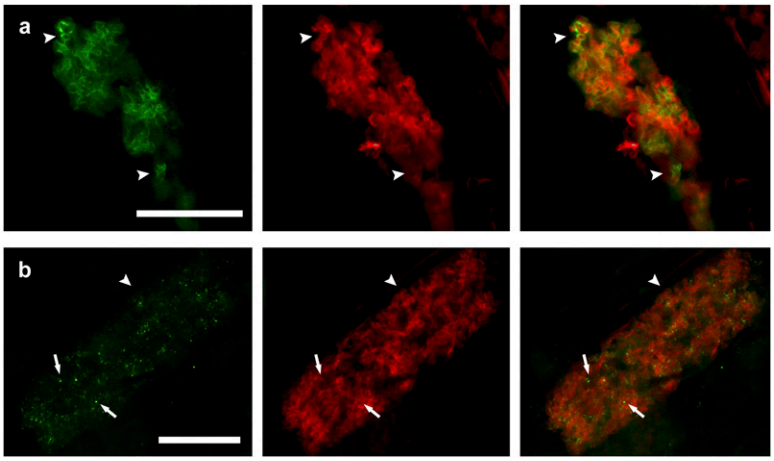

Fig. S8. Immunolocalization of MtPT4 protein in wild-type and *mtpt4-3* roots. MtPT4 protein was detected by native antibody in wild-type (*A*) and *mtpt4-3* mutant (*B*) roots. Wild-type MtPT4 protein localizes around arbuscule branches, whereas mtpt4-3 (MtPT4^{S115F}) is retained in trans-Golgi network (TGN)-like puncta. (*Left*) Detection of MtPT4 antibody by secondary antibody conjugated to Alexa Fluor 488. (*Center*) Visualization of fungal arbuscule by WGA conjugated to Alexa Fluor 594. (*Right*) Overlay. Arrowheads indicate the arbuscule branch; the arrows indicate MtPT4^{S115F}-labeled puncta. (Scale bars: 20 μm.)

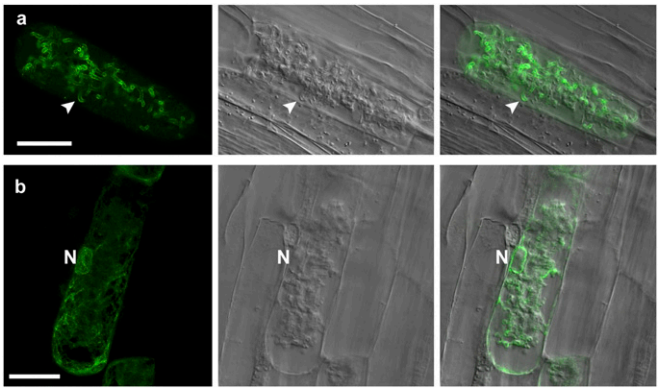

Fig. S9. Mutagenesis of S115 does not support a role for phosphorylation in membrane targeting. (*A*) pMtPT4:MtPT4^{S115A}-GFP is localized in the periarbuscular membrane, indicating that mutation of serine 115 to alanine did not disrupt secretion to the periarbuscular membrane (arrowhead). (*B*) pMtPT4:MtPT4^{S115E}-GFP containing the phosphoserine mimic glutamic acid did not result in periarbuscular membrane but instead caused ER retention, as evidenced by the reticulate pattern and perinuclear signal (N). No TGN localization was observed, and this ER retention likely is caused by a misfolded protein response. (Scale bars: 20 μm.)