SUPPLEMENTAL DATA

Figure S1. PSV identification by autofluorescence.

A: Superimposed lambda scans of Arabidopsis embryos expressing either spRFP-AFVY or α-TIP-YFP. The samples were excited at the indicated wavelengths and their emission monitored within the 405 to 705 nm range. Note that PSV autofluorscent peaks at around 500 nm, which allows for the simultaneous detection of YFP or RFP by sequential scanning.

B: a mature embryo from transgenic Arabidopsis expressing spRFP-AFVY was imaged by sequential scanning at the indicated wavelengths. Note the complete co-localization of PSV autofluorescence and RFP signal, indicating that the protein has reached the PSV.

Figure S2. Three TIP-YFP fusions localise to the tonoplast in tobacco epidermal cells.

Tobacco epidermal cells were co- infiltrated with agrobacteria containing the indicated TIP-YFP (magenta) construct and the plasma membrane marker EGFP-LTI6b (Kurup et al., 2005) (green). Cells were visualised by confocal microscopy. The merged image and the insets clearly show that the TIP-YFP fusions label a membrane underlying the plasma membrane, thus confirming their tonoplast localisation. Scale bar: 10 μm.

Figure S3. The position of the fluorescent protein does not affect TIP targeting to the tonoplast.

Leaves from transgenic Arabidopsis plants expressing the indicated constructs were analyzed by confocal microscopy. GFP was excited at 488 nm and emission detected between 495 and 515 nm. Inset images show clear tonoplast localisation for both GFP-γ and GFP-α constructs. The tonoplast localization of GFP-δ-TIP has been reported previously (Cutler et al., 2000; Tian et al., 2004). Scale bar: 10 μm.

Figure S4. TIP-YFP label large autophagic structures during Arabidopsis seed germination.

Embryos were isolated from transgenic Arabidopsis seeds expressing the indicated constructs after 2 days of germination, and observed by CLSM. The images represent the maximum fluorescence projection of 50 sequential optical z sections. Blue, PSV autofluorescence; Green, TIP-YFP. Scale bar: 10μm

Figure S5. mRNA and protein expression of three Arabidopsis TIP isoforms is developmentally regulated.

A. Expression of α-TIP (At1g73190), γ-TIP (At2g36830) and δ-TIP (At3g16240) was analyzed throughout plant development using the AtGenExpress visualization tool. The result is plotted as normalized mean intensity. Note the sharp drop in expression of both γ-TIP and δ-TIP, which is mirrored by an increase in α-TIP expression

B. Total proteins from seeds of transgenic Arabidopsis expressing the indicated constructs were isolated at the indicated times during germination. Proteins were subjected to immunoblot with anti GFP antiserum. Numbers at left indicate molecular weight markers in kDa.

Figure S6. Expression of native α-TIP-YFP during seed development and maturation.

Intact seeds within siliques (A-C) or the corresponding, isolated embryos (D-F) from transgenic Arabidopsis expressing nat α-TIP-YFP were analyzed by confocal microscopy. YFP is shown in green, chlorophyll autofluorescence is shown in red and PSV autofluorescence is shown in blue. In panels A and B, the transmitted light image is superimposed to the fluorescence signal. Note the absence of nat α-TIP-YFP expression in embryos at the early torped stage. Scale bar: 20 μm

Figure S7. Developmental regulation of the three δ-TIP isoforms of Arabidopsis.

Expression of δ-TIP1 (At3g16240), δ-TIP2 (At4g17340), δ-TIP3 (At5g47450) (Joahanson et al., 2001) was analyzed throughout plant development using the AtGenExpress visualization tool. The result is plotted as normalized mean intensity. Note that all isoforms are not expressed in seeds, and that expression of δ-TIP3 is restricted to roots.

Figure S8. List of all oligonucleotide primers used in this study.