

**Supplementary information, Figure S5** TTS targets several soluble proteins but not transmembrane proteins to the tonoplast.

(A) CBL5, CIPK14, eIF4A (eukaryotic translation initiation factor 4A) and SND2 (secondary wall-associated NAC domain protein2) were fused in-frame to YFP and then transiently expressed in *Arabidopsis* mesophyll protoplasts to give rise to CBL5-YFP (throughout the cell), CIPK14-YFP (throughout the cell), eIF4A-YFP (in cytosol) and SND2-YFP (in nucleus), respectively (left panels). N-terminal TTS-tagged fusions were also constructed and expressed in protoplasts, resulting in TTS-CBL5-YFP, TTS-CIPK14-YFP, TTS-eIF4A-YFP and TTS-SND2-YFP (right panels). Scale bar = 5  $\mu$ m. (B) SCAMP1 (secretory carrier membrane protein1), GONST1 (Golgi nucleotide sugar transporter1), PIP2A (plasma membrane intrinsic protein2A) were fused to YFP and then transiently expressed in *Arabidopsis* mesophyll protoplasts to give rise to SCAMP1-YFP (in late endosomes), GONST1-YFP (in Golgi apparatus), PIP2A (in endoplasmic reticulum), respectively (left panels). Adding TTS to the N-terminus of these transmembrane proteins does not significantly change their subcellular localizations (right panels). Scale bar = 5  $\mu$ m.