Supplemental Fig. S1. Expression of the *AtVAMP711 -14* genes in wild type and antisense plants. Total RNA was extracted from wild type and transgenic antisense AtVAMP711 plants, line 2092. Transcription of the *AtVAMP711 -14* genes was assayed by RT-PCR. The amount of RNA was normalized according to expression of *ACTIN-2* gene. Conditions of RT-PCR reaction and primers for the *AtVAMP711 -14* genes (except *AtVAMP712*) were described in Leshem et al (2006). The following primers were used for *AtVAMP712*: Foward- CTT TGT ATG GCT GAT GAA GAC GCC; Reverse- GAA GGT ATT CCC TTG CAT GTT CGC.

Supplemental Fig. S2A. Moisture content of pots during drought treatment. Pots without plants were situated in the same tray with the tested plants. Pots' fresh weight was determined at indicated time points during the drying period and expressed as % of initial FW (fully irrigated pot).

Supplemental Fig. S2B Drought in wild-type and antisense plants grown under long day conditions

Supplemental SFig. 3 Liposome membrane staining with MitoFluor Red 589
Mitofluor Red 589 staining of artificial membranes was done with liposomes, prepared according to Manor *et al* (*Biophysical Journal* 89:563-571, 2005). Briefly, 100mg DMPC (1,2 Disyristoyl-sn-glyero-3-phosphocholine) (SIGMA) or POPC (1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine)(Avanti Polar Lipids Inc., Alabaster, AL) were dissolved in 1ml HFIP (1,1,1,3,3,3-Hexafluoro-2-propanol) (Acros Organics). The incorporation of liposomes was done for 1h at 37^0c, then lyophilized and re-suspended in water at 1:20 ratio for 1h at 37^0C, followed by 4 cycles of freeze-thaw and 2 min. sonication. MitoFlour Red 589 was added to the medium at 0,1 and 10 μM final concentration for 1h at room temp, washed 3 times and transferred to a 48 well plate.

A) Fluorescence was measured at 620nm by Microplate Fluorescence Reader FL600 (Bio-Tek, Blacksburg VI USA).

B) Liposome fluorescence was also visualized at 620 nm by fluorescent microscope (Olympus IX70). Shown are representative POPC liposomes incubated with 1 M MitoFluor Red 589. Same results were observed in DMPC liposomes (data not shown). Pictures were taken with a Coolpix 4500 camera (Nikon, Japan) using identical exposure settings for each set of images

Supplemental SFig 4 - Complete Z stack of guard cells presented in fig. 3B

Supplemental SFig. 5 - Staining of stomata (top row) with epidermal pavement cells (bottom row) with FM 4-64.
FM 4-64 stain of stomata and epidermal pavement cells of wt (left panel) and VAMP AS line 2091 (right panel). Leaf disks were loaded with FM 4-64 (Molecular Probes-Invitrogen) as described by Leshem et al (2006). Images were taken by MRC-1024 confocal microscope (Bio-Rad, USA), fluorescence emission was detected at 695nm.

Supplemental SFig 6 - Nuclear localization of ROS in ABA treated mesophyll cells. Simultaneous staining of nucleus (DAPI – blue) combined with ROS (H_2DCFDA-green) and plastids autofluorescence (red). Staining procedure was performed as described in Fig 3C. Similar results were obtained in the antisense lines (data not shown). Control images are presented in Fig.3B (top panel)