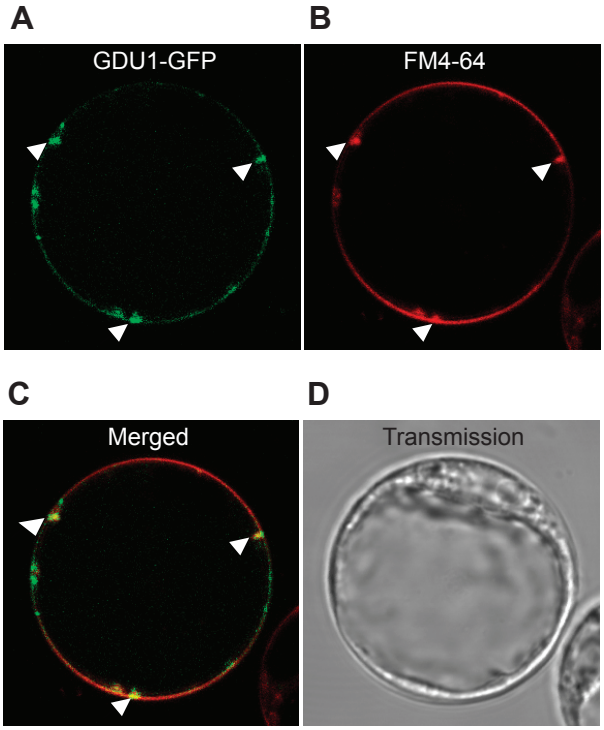

Supplementary figure 2. GDU1 protein is expressed at the plasma membrane in *Arabidopsis* protoplasts. Confocal images of a protoplast expressing GDU1 fused to the GFP. GFP fluorescence is shown in green (A), the fluorescence of the membrane dye FM4-64 is shown in red (B). Arrowheads on the single color and the merged images indicate the perfectly matching bright spots (C).
(D) Transmission image.

Methods:
mGFP4 DNA fragment (Haseloff, PNAS 94 p2122-2127, 1997) was amplified by PCR using the primers TTCTCGAGATGAGTAAAGGAGAAGAAC and TTGGTACCGAGCTCTTATTTGTATAGTTC, and cloned into pBluescript using XhoI and KpnI sites. GDU1 coding sequence was amplified by PCR with the primers TTGGATCCAACAAAAAGAGATTACACAC and TTCTCGAGGTGACTTGTAGTAGTTGTCTCGC, and cloned upstream from the GFP fragment using BamHI and XhoI sites. The resulting DNA fragment was excised and inserted into pAG2370, between the CsVMV promoter and the Nos termination sequence using BamHI and KpnI sites. The whole construct (CsVMV-GDU1-GFP-Nos) was excised by HindIII and PmeI and transferred back into pBluescript using HindIII and EcoRV sites. The resulting plasmid was transferred to *Arabidopsis* cell protoplasts as described (Forreiter, Plant Cell 9 p2171, 1997). Transient expression of the fusion protein GDU1-GFP was observed by confocal microscopy (Leica DMRE microscope equipped with a confocal head TCS SP; Leica, Wetzlar, Germany) the day following the transformation. Membranes were stained for 1 h with 2 µM FM4-64 (Molecular Probes, Leiden, The Netherlands).