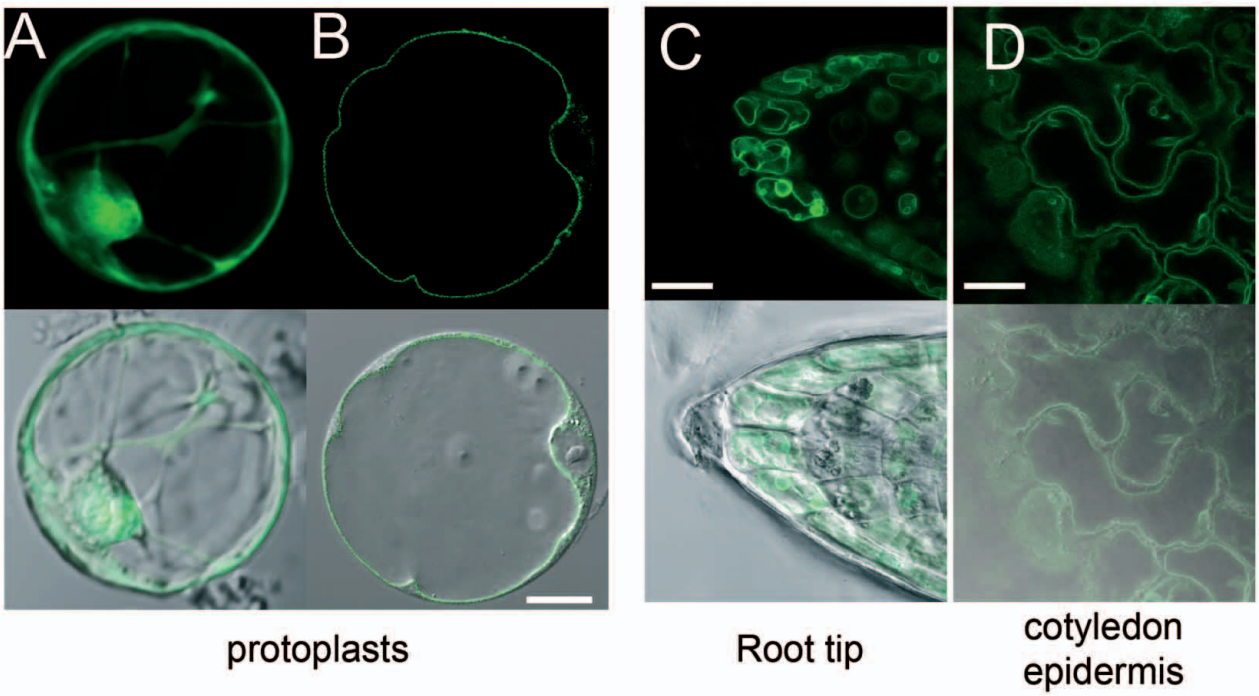

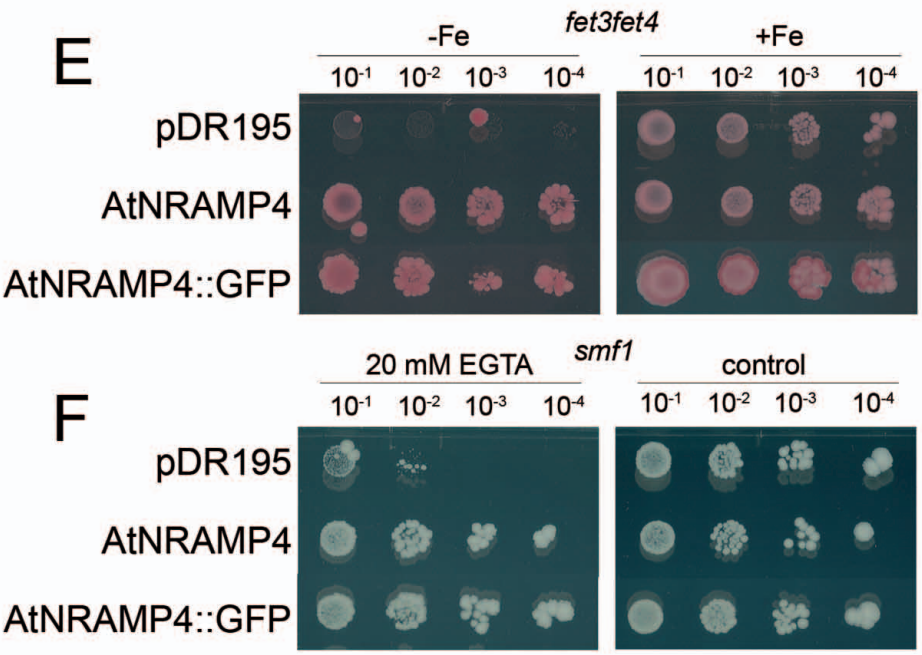

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Figure S3: A functional AtNRAMP4-GFP fusion protein is targeted to the vacuolar membrane

(A-D) AtNRAMP4-GFP is targeted to the vacuolar membrane. Micrographs of protoplasts transiently expressing either free GFP (A) or AtNRAMP4-GFP fusion protein (B). Micrographs of root tip (C) and cotyledon epidermis (D) of 3 day old Arabidopsis seedlings stably transformed with AtNRAMP4-GFP under the control of 35S constitutive promoter. The overlay of fluorescence with the transmission image is shown underneath. Scale bar: 16 μm. DNA constructs, transformations and confocal microscope observations were performed as described in Thomine et al. (2003). (E-F) Expression of AtNRAMP4-GFP rescues the growth defects of *smf1* and *fet3fet4* yeast mutants. Yeast mutants were transformed according to Thomine et al. (2000) with empty pDR195 vector or pDR195 containing AtNRAMP4 or AtNRAMP4-GFP cDNA as indicated. (E) Drop test of transformed *fet3fet4* yeast strains grown from the OD indicated on the top on SD-uracil buffered to pH 5.5 with 50 mM MES containing 0.1 mM BPS (Batho-Phenanthroline di-Sulfonic acid, Sigma, St Louis) supplemented either with 30 μM $FeCl_3$ (-Fe) or 200 μM $FeCl_3$ (+Fe). (F) Drop test of transformed *smf1* yeast strains grown from the OD indicated on the top on SD-uracil buffered to pH 6 with 50 mM MES supplemented with 20 mM EGTA (20 mM EGTA) or not (control). The growth defect of *smf1* on EGTA could also be rescued by adding 0.1 mM $MnCl_2$ to the medium.