

Fig. S1. Phylogeny, genomic structure and duplication among *PotriNRAMP* genes. (A) Phylogeny and genomic structure of the NRAMP family genes in *P. trichocarpa*. The intron (black) -exon (yellow) structure of the *PotriNRAMPs* is represented beside the phylogenetic tree. Phylogenetic analyses were conducted as described in Materials and methods. *A. thaliana* and *P. trichocarpa* accession numbers as well as model genes used for these analyses are listed in supplementary table S2. (B) Schematic representation of *PotriNRAMP3* duplication on Chromosome 7. (C) Dot-plots performed with Gepard software V1.30 (Krusmiek et al., 2007), between 150 kb of genomic DNA centred on *PotriNRAMP3.1*-*PotriNRAMP3.2* sequence and the same sequence. Sequence homologies are indicated by black dots. Red arrows highlight the specific duplication of *PotriNRAMP3* sequence. (D) Dot-plots between *PotriNRAMP3.1* and *PotriNRAMP3.2* genomic DNAs. Homology is restricted to the coding sequences. Red squares delimit intron and exon.

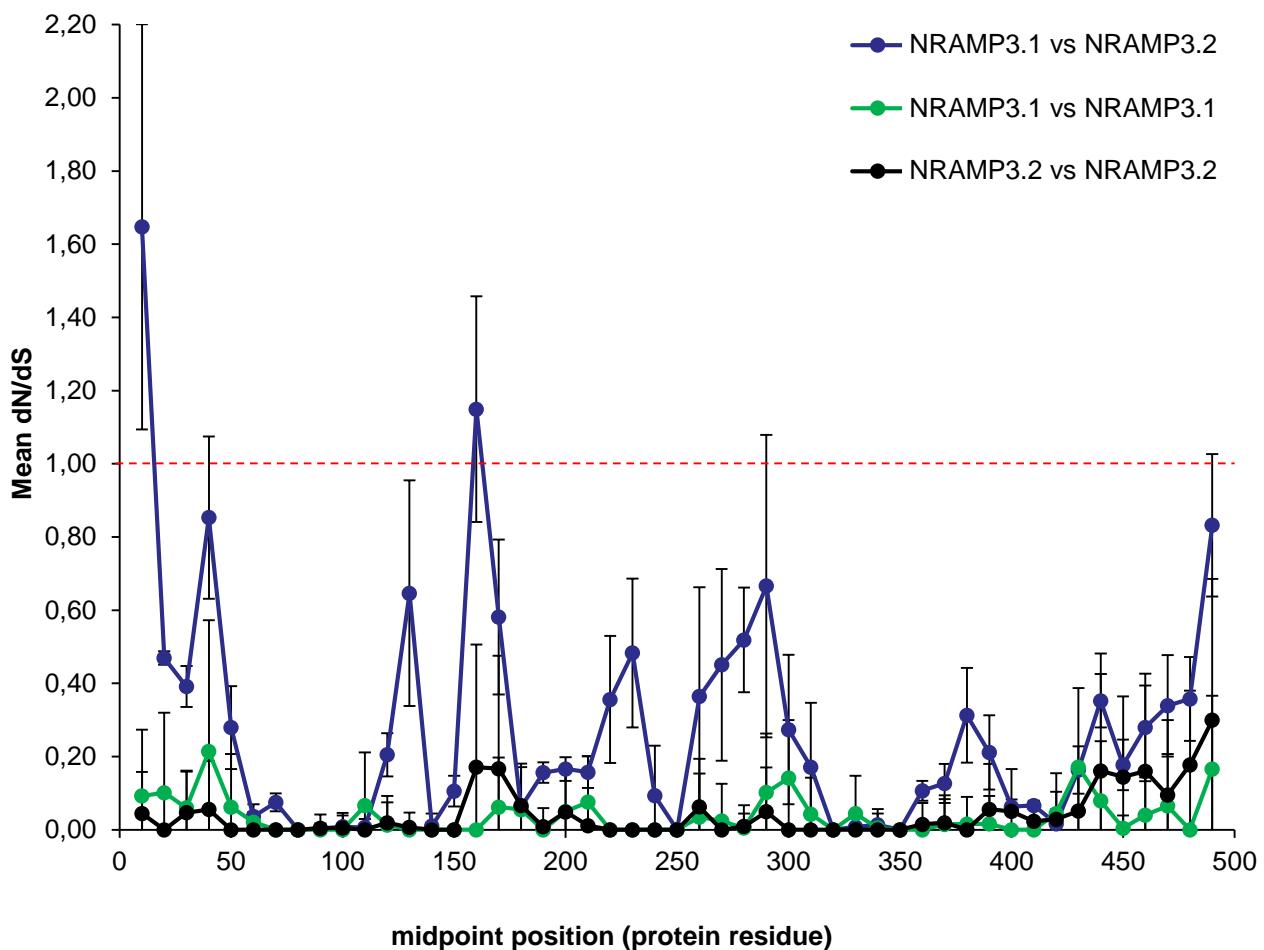


Fig. S2. Non-synonymous (dN) vs synonymous (dS) codon analysis along PotriNRAMP3 coding sequences. Codon alignment were performed with cDNA encoding 13 NRAMP3.1 and 13 NRAMP3.2 proteins from 13 different *Populus* species. dN/dS was calculated for each pair of cDNA (325 comparisons) through the Neij Gobodojori algorithm along the protein sequence using a 20 residue window with shift of 10 residues. Mean dN/dS for each window position was calculated independently for NRAMP3.1 vs NRAMP3.1 comparisons (78), NRAMP3.2 vs NRAMP3.2 comparisons (78) and NRAMP3.1 vs NRAMP3.2 comparisons between species (169). The mean dN/dS \pm SD is shown for each window midpoint position. Analyses were conducted using JCoDA 1.4 (Steinway *et al.*, 2010).

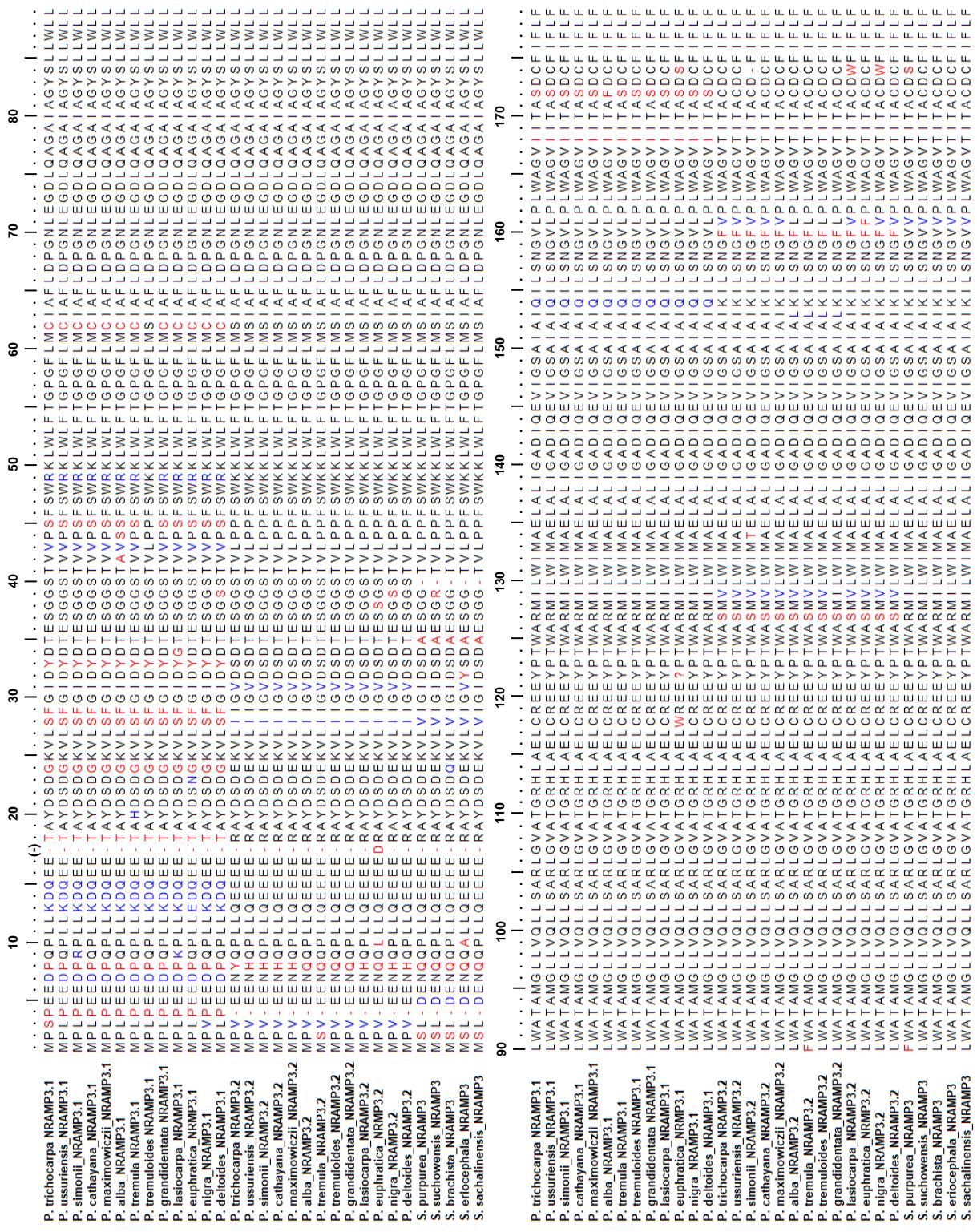


Fig. S3. Alignment of NRAMP3 protein sequences from *Populus* and *Salix* sp. Identical, similar and different residues between sequences are indicated in black, blue and red respectively.

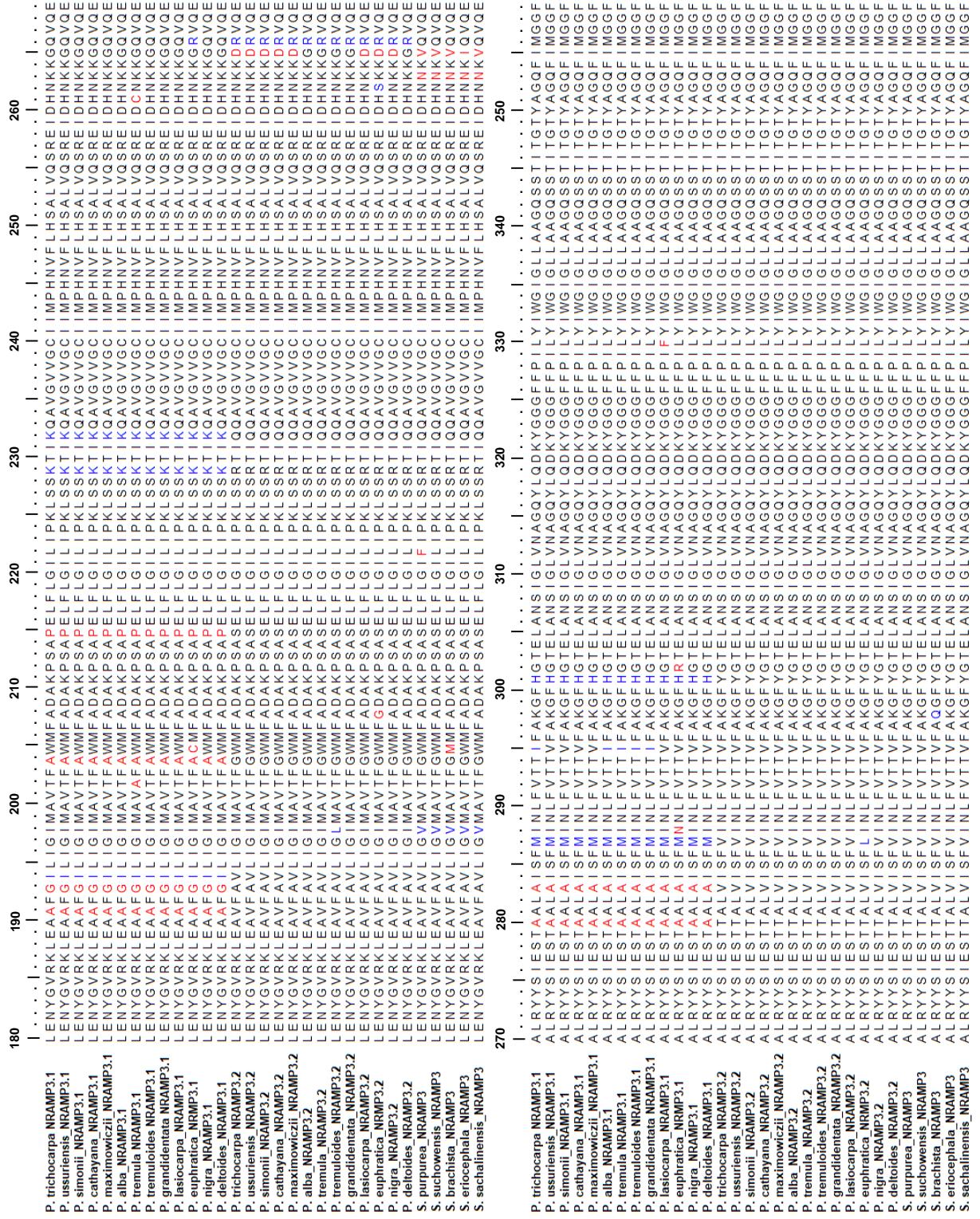


Fig. S3 (continuation). Alignment of NRAMP3 protein sequences from *Populus* and *Salix* sp. Identical, similar and different residues between sequences are indicated in black, blue and red respectively.

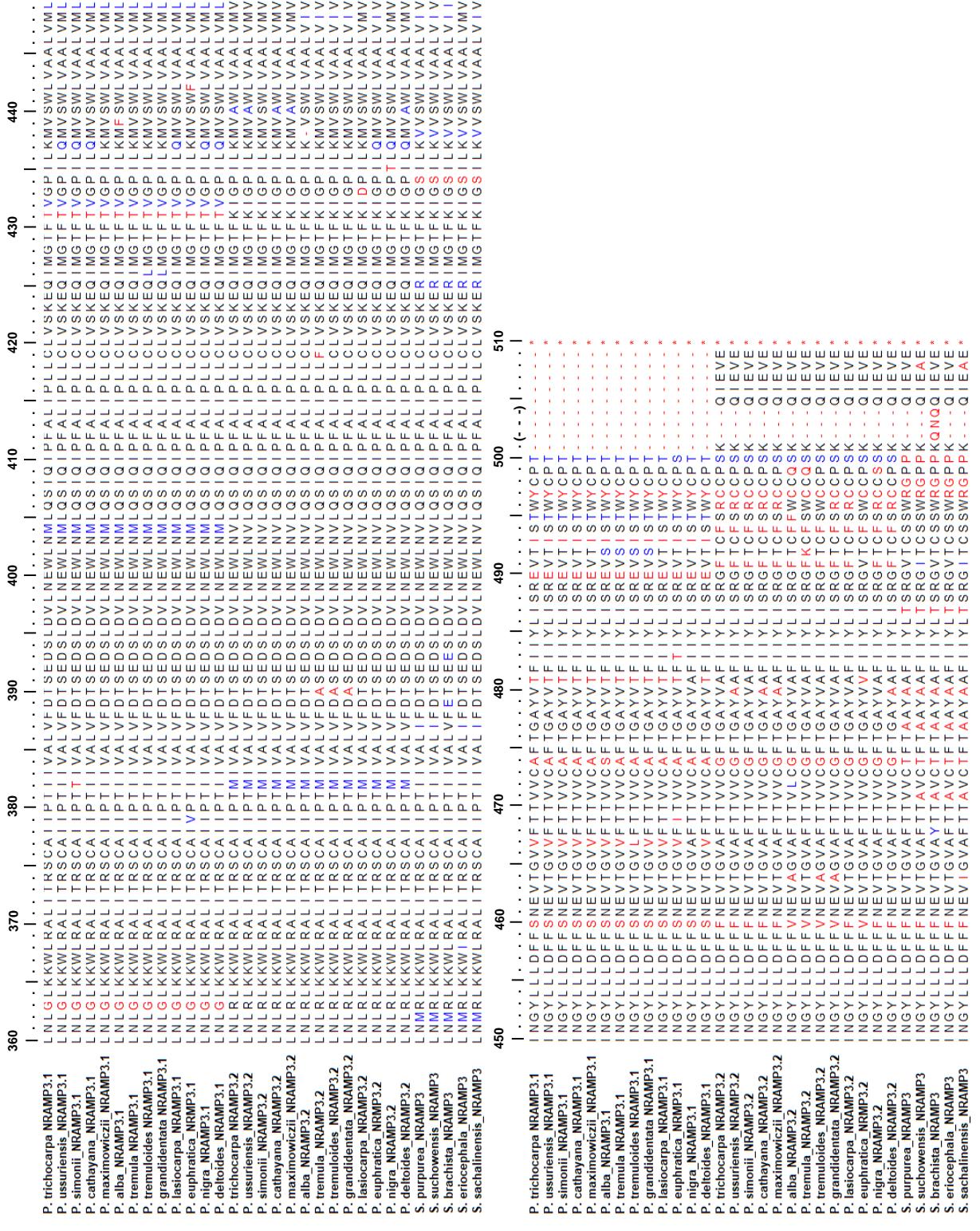


Fig. S3 (continuation). Alignment of NRAMP3 protein sequences from *Populus* and *Salix* sp. Identical, similar and different residues between sequences are indicated in black, blue and red respectively.

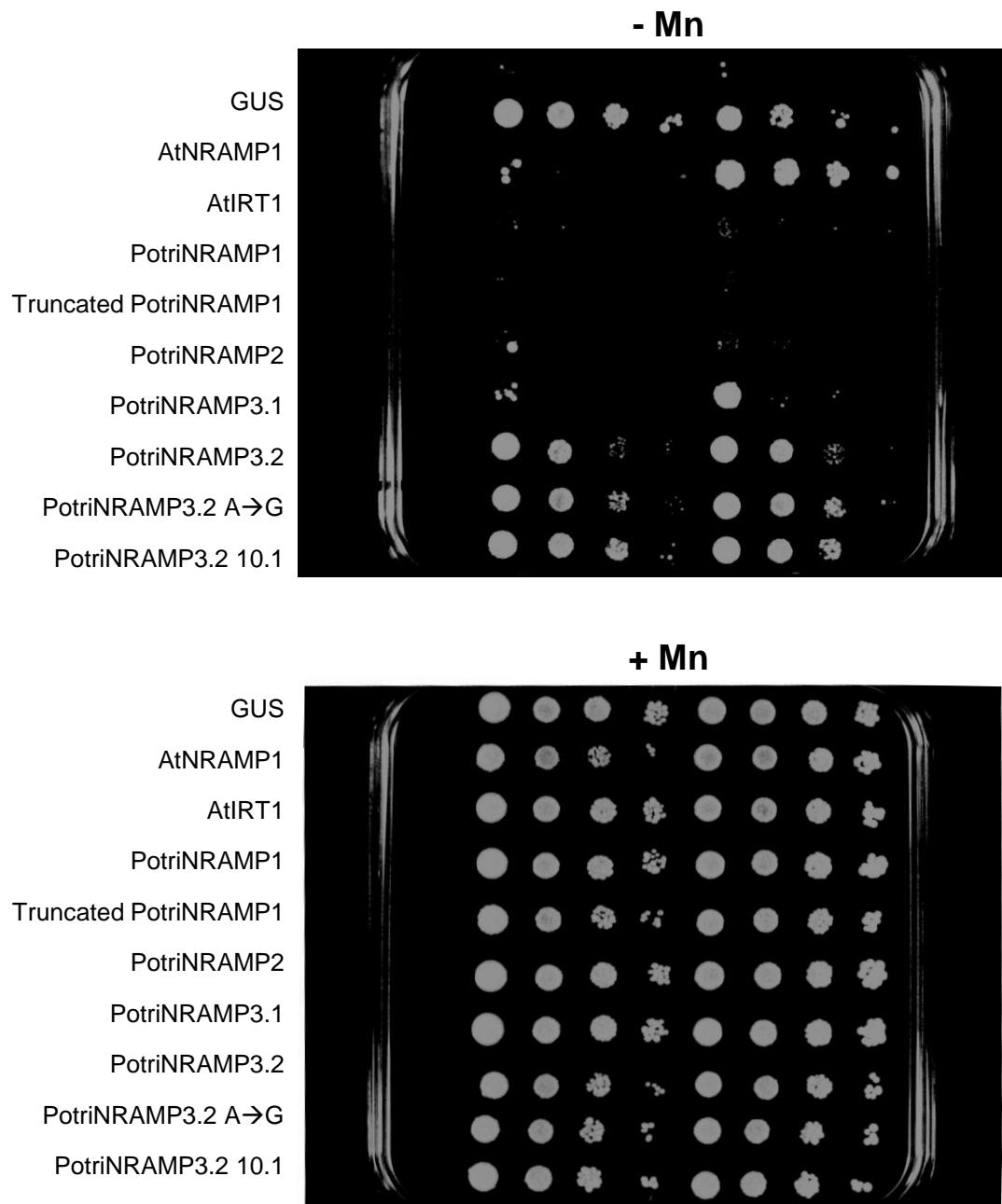


Fig. S4. *smf1* complementation. Yeast cells were transformed with pDR195gtw vector containing the cDNA of GUS, AtNRAMP1, AtIRT1, PotriNRAMP1 (2 variants), PotriNRAMP2, PotriNRAMP3.1 and PotriNRAMP3.2 (3 variants). Transformed *smf1* yeasts were grown overnight in liquid synthetic dextrose -ura. The cultures were diluted to ODs of 1 to 10⁻³ and spotted on synthetic dextrose -ura plates. Transformed *smf1* yeasts were spotted on medium supplemented with 5 mM EGTA and 100 µM MnSO₄ (+ Mn) or with 5 mM EGTA without MnSO₄ (- Mn). The plates were incubated at 30°C for 5 days (*smf1*) before photography. The right and left part of each plate show results obtained with 2 different transformants per construct.

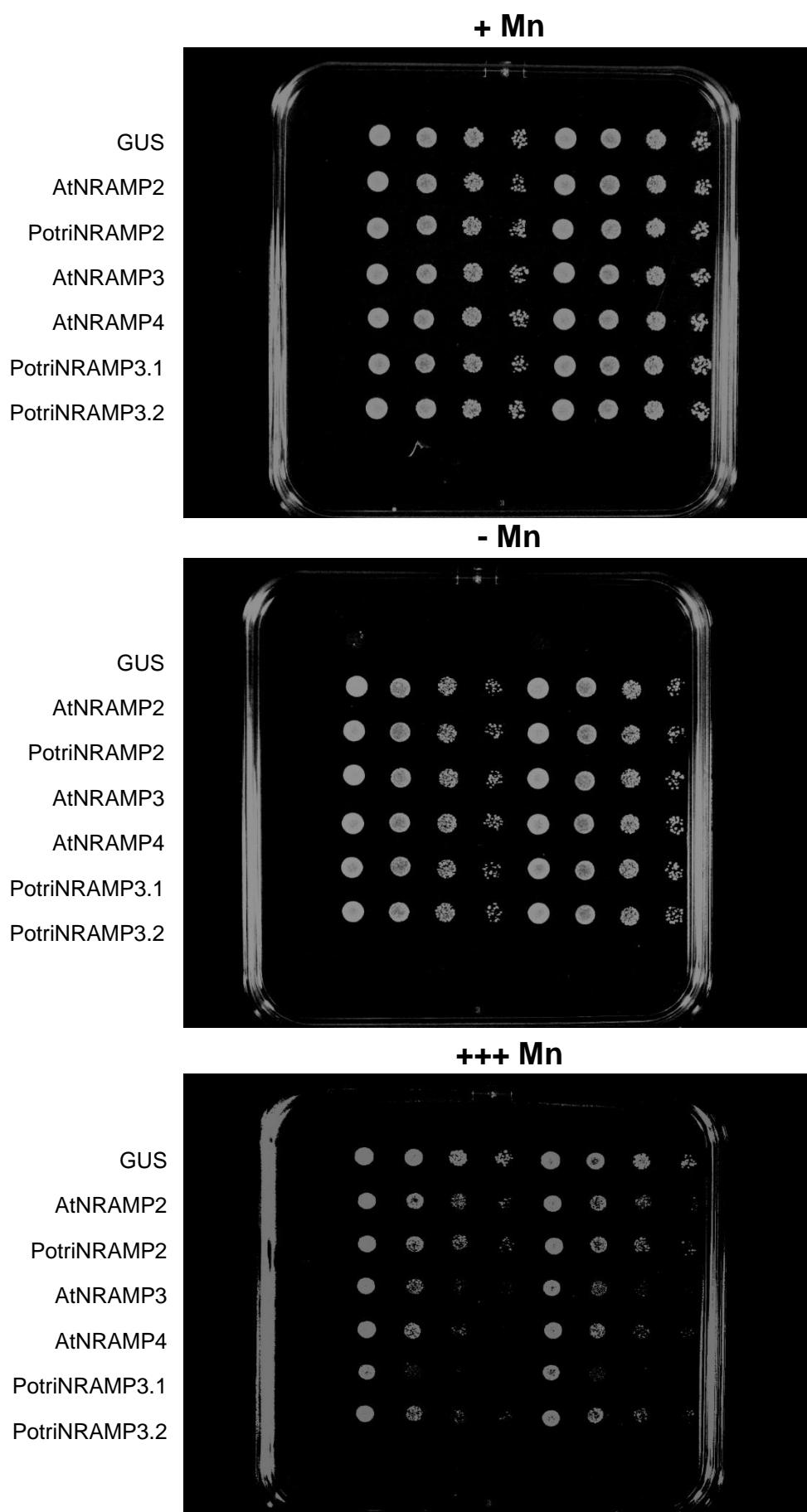


Fig. S5. *smf2* complementation. Yeast cells were transformed with pDR195gtw vector containing the cDNA of *GUS*, *AtNRAMP2*, *PotriNRAMP2*, *AtNRAMP3*, *AtNRAMP4*, *PotriNRAMP3.1* and *PotriNRAMP3.2*. Transformed *smf2* yeasts were grown overnight in liquid synthetic dextrose -ura. The cultures were diluted to ODs of 1 to 10⁻³ and spotted on synthetic dextrose -ura plates. Transformed *smf2* yeasts were spotted on media medium supplemented with 10 mM EGTA and 100 µM MnSO₄ (+ Mn), with 5 mM EGTA without MnSO₄ (- Mn) or with 10 mM MnSO₄ (+++ Mn). The plates were incubated at 30°C for 2 days before photography. The right and left part of each plate show results obtained with 2 different transformants per construct.

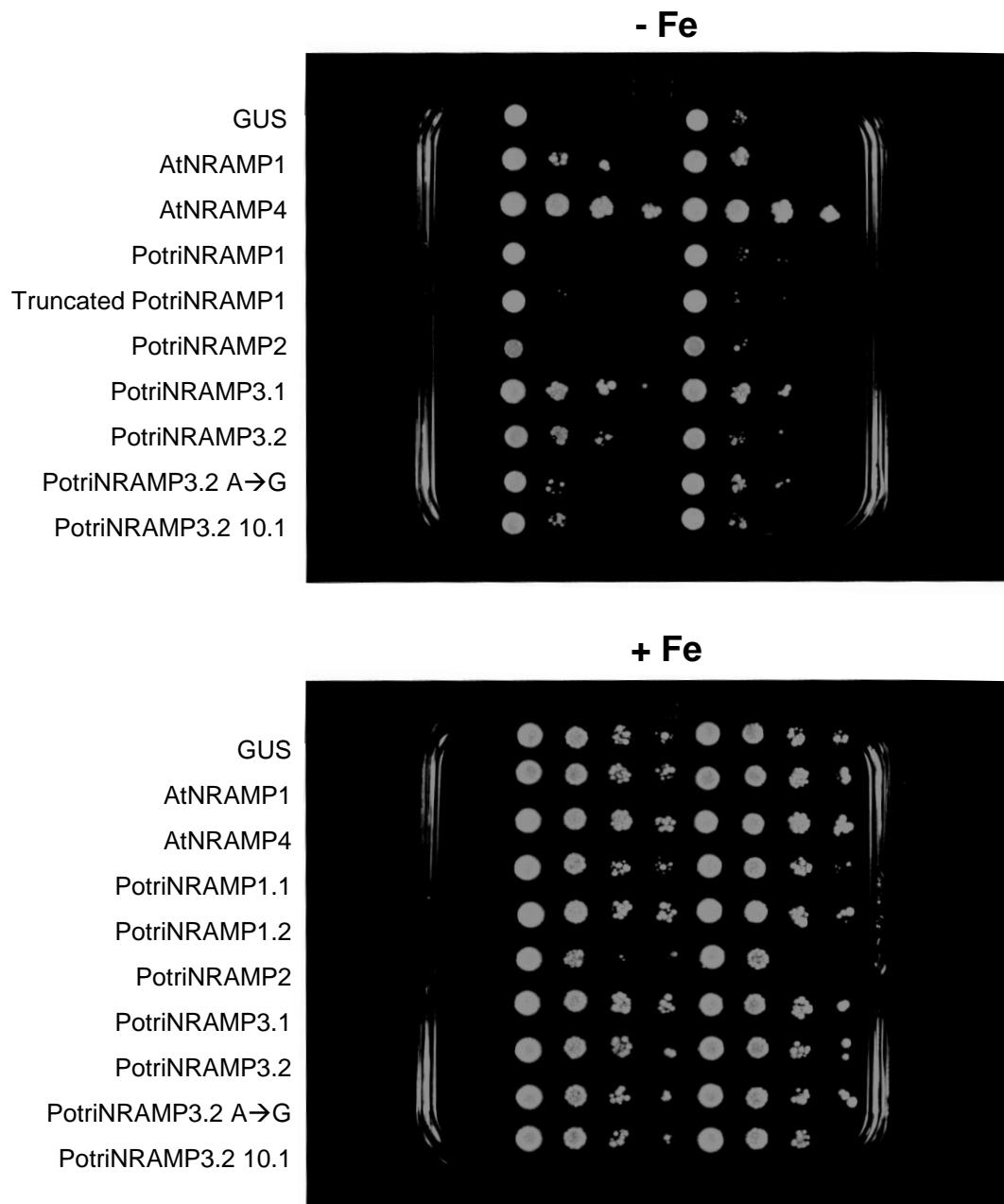


Fig. S6. *fet3fet4* complementation. *fet3fet4* yeast cells were transformed with pDR195gtw vector containing the cDNA of *GUS*, *AtNRAMP1*, *AtNRAMP4*, *PotriNRAMP1* (2 variants), *PotriNRAMP2*, *PotriNRAMP3.1* or *PotriNRAMP3.2* (3 variants). Transformed *fet3fet4* were grown overnight in liquid synthetic dextrose -ura supplemented with 0.2 mM FeCl_3 . Cultures from 2 independent transformed strains for each construct (left-right) were diluted to ODs of 1 to 10^{-3} and spotted on synthetic dextrose -ura plates supplemented with 100 μM of the Fe chelator Bathophenanthroline-Di-Sulfonic acid (BPDS) and 0.2 mM FeCl_3 (+ Fe) or with 100 μM BPDS and 40 μM FeCl_3 (- Fe). The plates were incubated at 30°C for 4 days before photography.

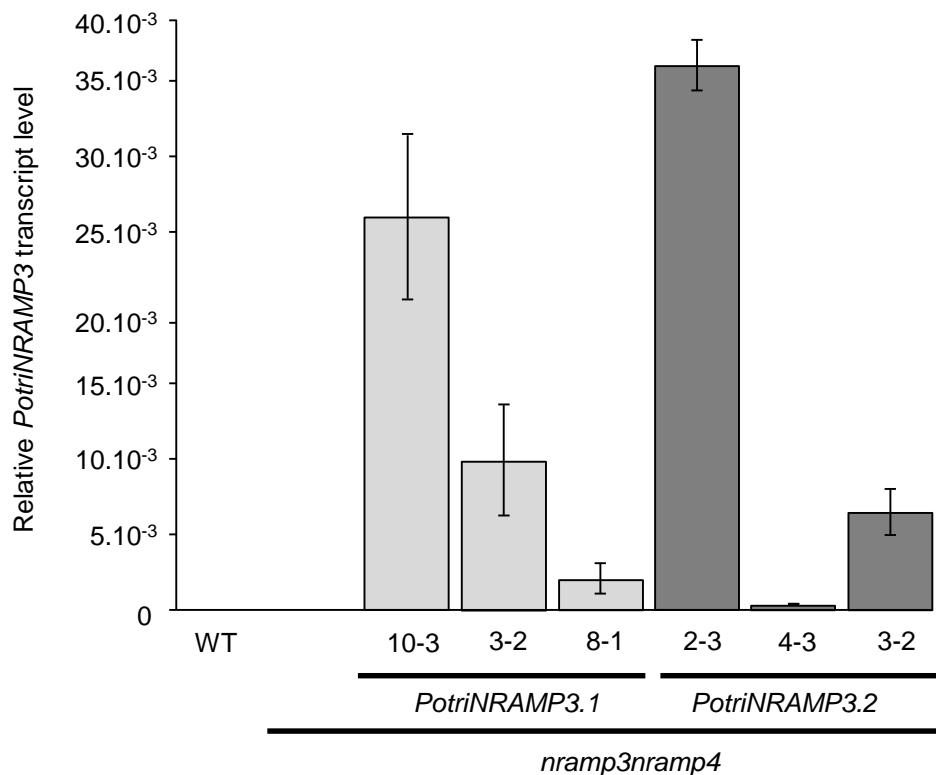


Fig. S7. Transcript levels of *PotriNRAMP3.1* and *PotriNRAMP3.2* in Arabidopsis *nramp3nramp4* transgenic lines. *PotriNRAMP3.1* and *PotriNRAMP3.2* transcript levels were determined by RT-qPCR using specific primers for each isoform and normalization with *AtACTIN* as a reference gene. The graph shows means \pm SD of three replicates on different individuals.

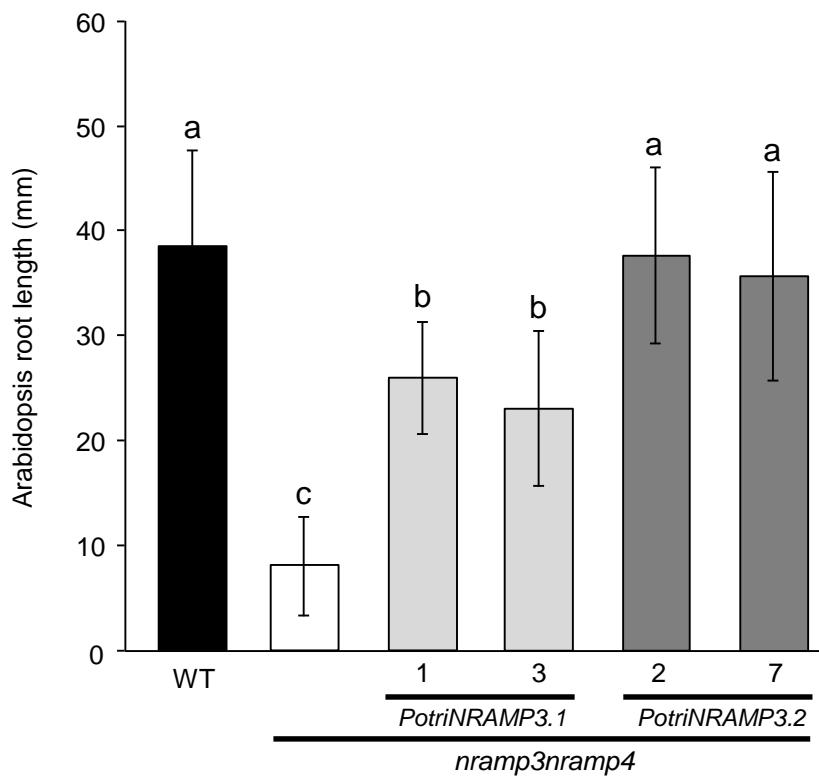


Fig. S8. Root length of Col-0 wild-type, *nramp3nramp4* mutant and representative transformed with *p35S::PotriNRAMP3.1-GFP* or *p35S::PotriNRAMP3.2-GFP*. Roots were measured 12 days after germination on ABIS medium without Fe. The graph shows means \pm SD ($n = 10\text{-}25$ roots). Different letters indicate significant difference between genotypes based on ANOVA analysis ($p < 0.05$).

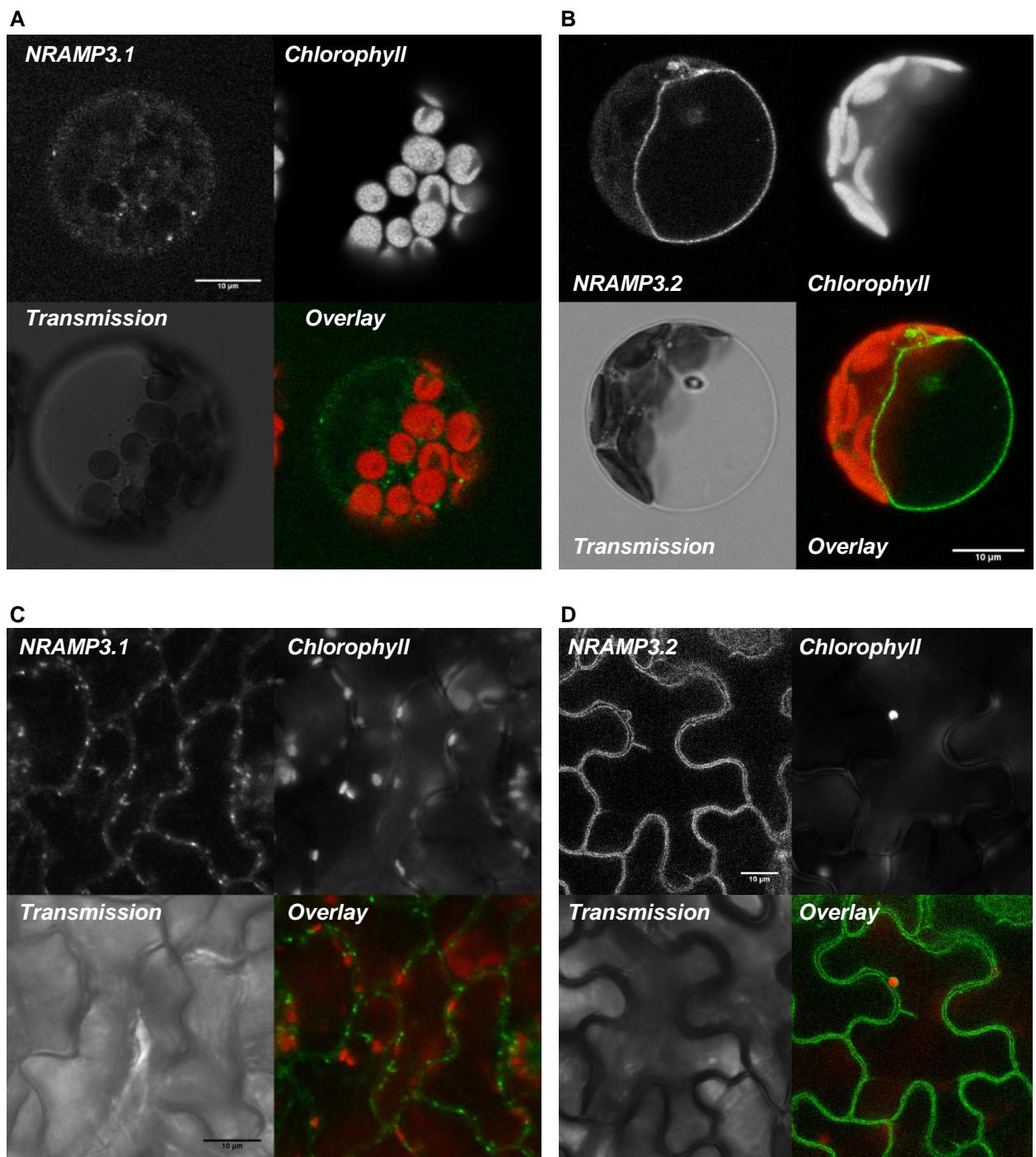


Fig. S9. PotriNRAMP3.1 and PotriNRAMP3.2 subcellular localizations in leaf cells. LSCM images of leaf protoplasts (A, B) or epidermis (C, D) from poplar lines expressing PotriNRAMP3.1-GFP (A, C) or PotriNRAMP3.2 (B, D). The localizations of the two isoforms are similar to those observed in root cells. Scale bars : 10 μ m.

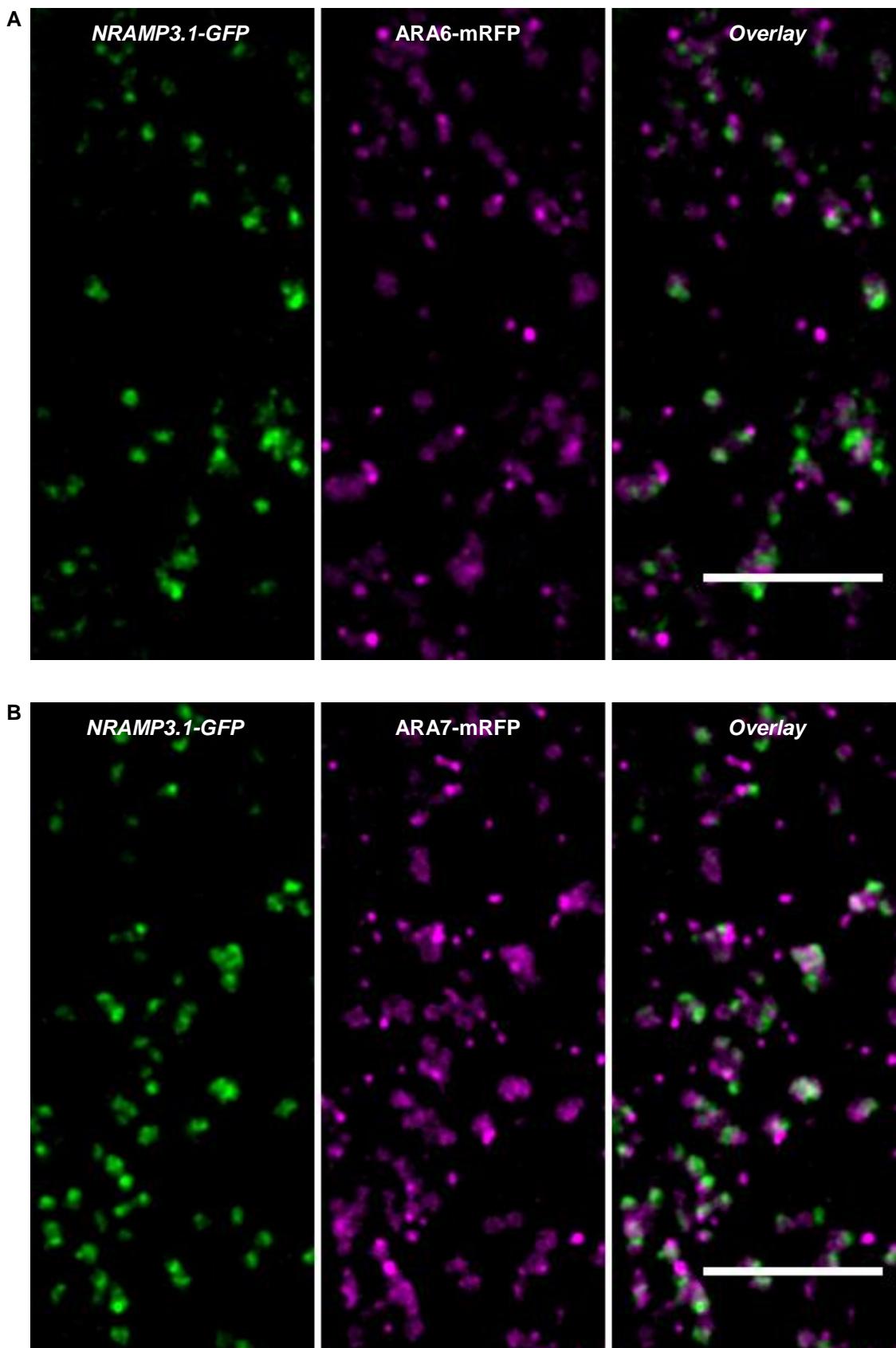


Fig. S10. PotriNRAMP3.1-GFP does not colocalize with late endosomal / MVB markers. PotriNRAMP3.1-GFP fluorescence is often in proximity of ARA6 (A) and ARA7 (B) but has little overlap with these markers of late endosomes / MVBs. On the merged images the overlap of GFP (green) and mRFP (magenta) channels appears white. Spinning disk confocal images were acquired in the cortical planes of root epidermal cells in the early elongation zone of *Arabidopsis* F1 seedlings. Scale bar : 10 μ m.

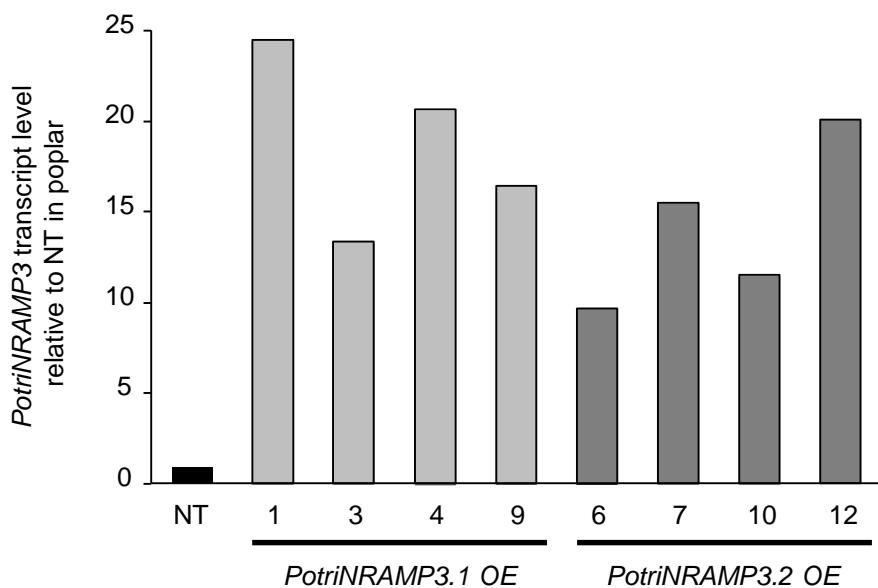


Fig. S11. Transcript level of *PotriNRAMP3.1* and *PotriNRAMP3.2* mRNA in leaves of transgenic poplar lines relative to NT. Relative transcript levels were determined by RT-qPCR using specific primers for each isoform and normalization by three reference genes, *i.e.*, *PtUBQ*, *PtEF1*, and *PtPP2A*.

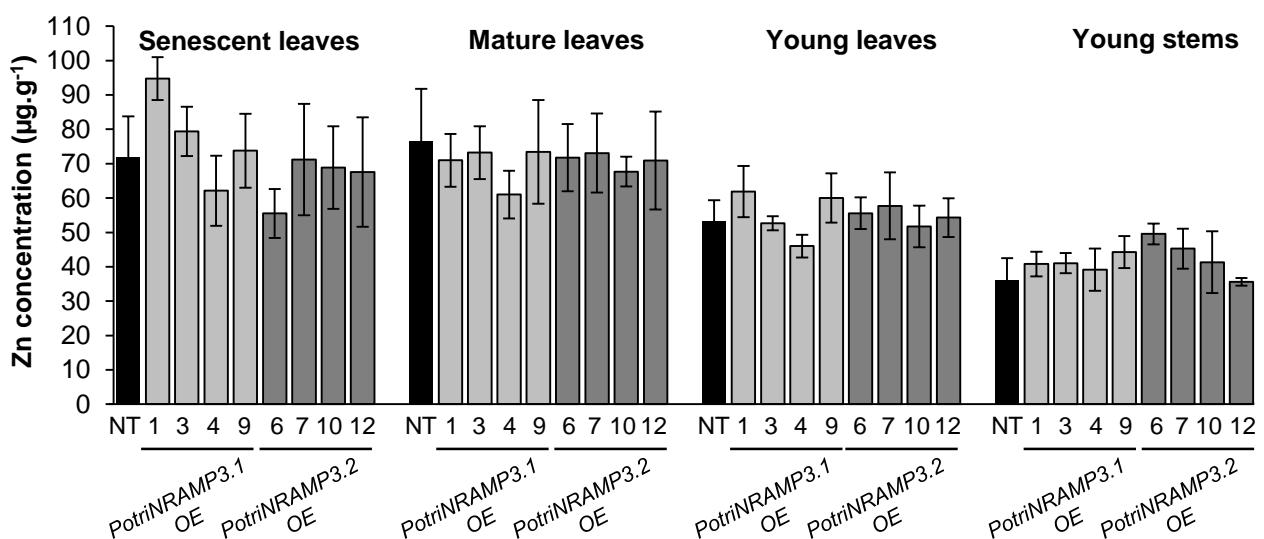
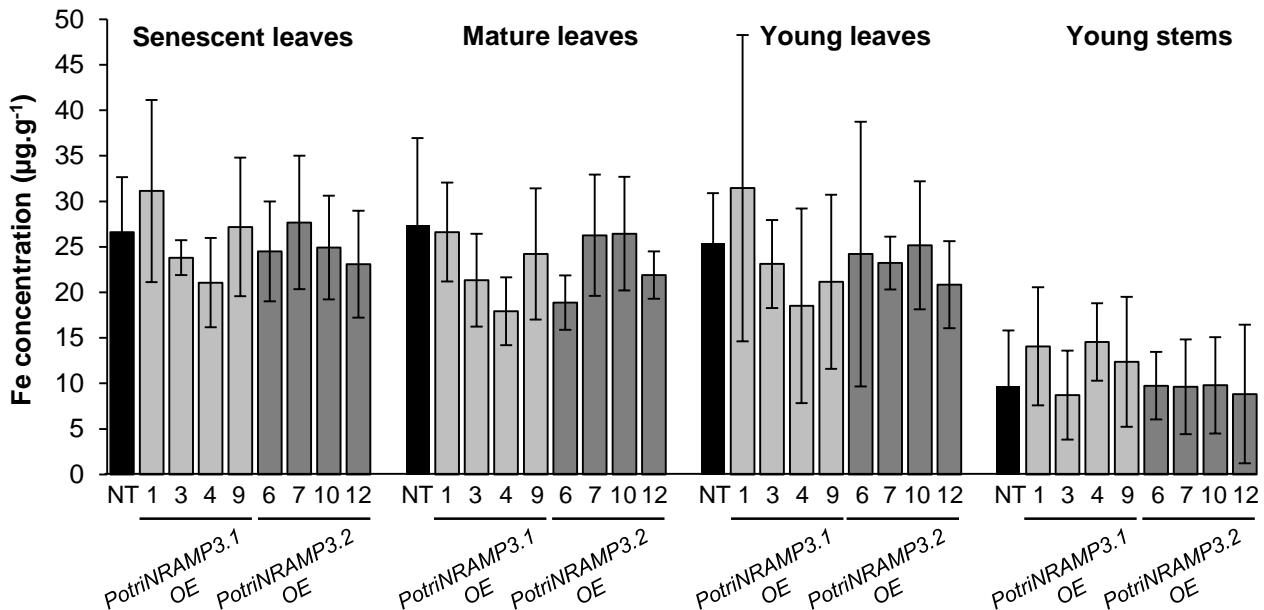


Fig. S12. Metal concentrations in poplar transgenic lines. Ectopic over expression of *PotriNRAMP3.1-GFP* or *PotriNRAMP3.2-GFP* does not perturb Fe or Zn distribution in poplar leaves or stems. Fe and Zn concentrations in senescent, mature, young leaves and young stems of poplars 2 months after transfer from *in vitro* to soil was determined using Atomic Emission Spectroscopy. Mean Fe or Zn concentrations of *PotriNRAMP3.1-GFP* or *PotriNRAMP3.2-GFP* OE lines were compared with NT control. Error bars represent SD ($n = 4-7$). Kruskal Walis multiple comparison tests did not reveal any statistically significant difference among genotypes for any organ.

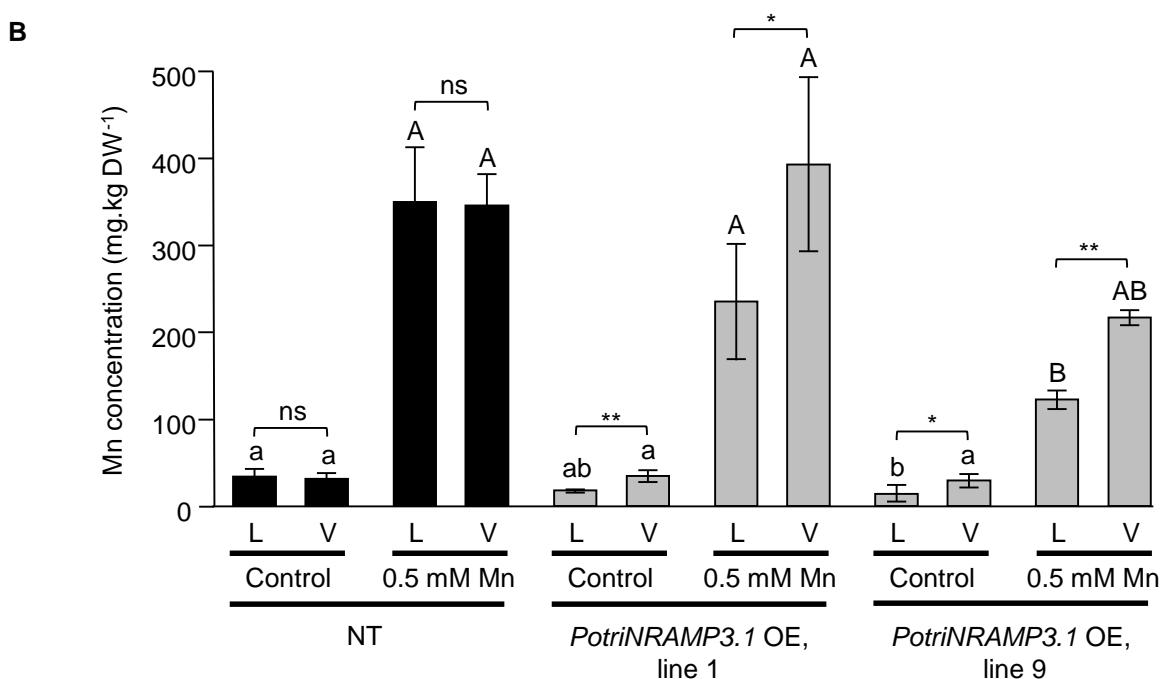
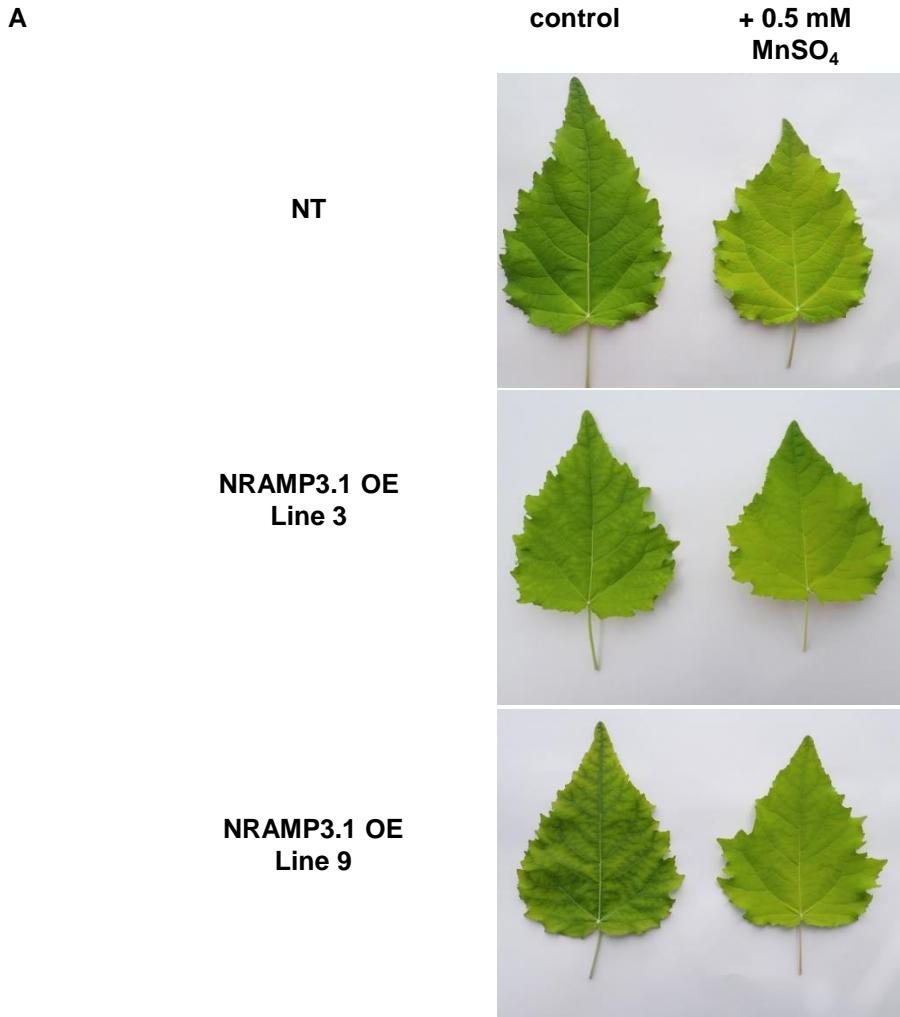


Fig. S14. High Mn supply mitigates the internerval chlorosis in NRAMP3.1 OE lines (A) photographs of mature leaves of the wild type and two lines overexpressing *PotriNRAMP3.1* supplied or not with 0.5 mM $MnSO_4$ through watering. (B) Mn concentration in the lamina (L) and veins (V) of the same leaves. (A) and (B) use leaves number 8-13 starting from the apex. Asterisks denote significant differences between lamina and veins according to a Mann-Whitney test (* $p < 0.05$, ** $p < 0.01$). Different letters denote significant differences among concentrations in control soil (lowercase) and soil supplemented with Mn (capital) according to a Kruskal-Wallis test followed by Dunn's test for multiple comparison ($p < 0.05$).