
**Figure S1. FPN3-FLAG is targeted to the mitochondria in yeast.**

(A) Western blots with fractions containing FPN3-FLAG and the mitochondrial marker, porin. FPN3-FLAG is detected in the fractions enriched with porin. (B) Western blots with total cell lysate (cell) or purified mitochondria expressing FPN3-FLAG. Antibodies against FLAG was used to detect FPN3-FLAG. To detect subcellular markers, Western blots were conducted using antibodies against porin for mitochondria, plasma membrane ATPase 1 (PMA1) for plasma membrane, carboxypeptidase Y (CPY) for vacuole, and dolichol phosphate mannose synthase (DPM1) for ER. As a loading control, Coomassie staining was conducted.

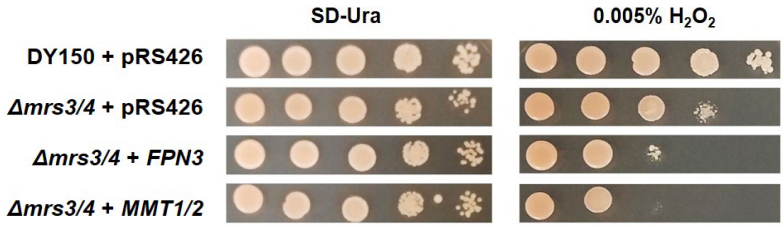


**Figure S2. Heterologous expression of *FPN3* in *Δmrs3/4* yeast.** (A) Spot assays under oxidative stress conditions with *Δmrs3/4* cells expressing *FPN3* or *MMT1/2*. Wild type (DY150) and *Δmrs3/4* cells transformed with an empty vector, *pRS426*, were used as controls.

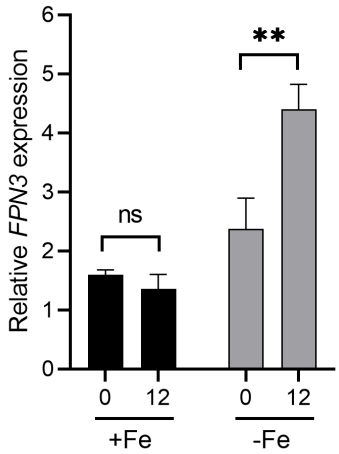


**Figure S3. *FPN3* expression in split root seedlings transferred to plates with only iron sufficient or iron deficient medium.** *FPN3* transcript levels in split roots 0 or 12 hours after transferring to the same conditions, i.e., from iron sufficient to iron sufficient medium, and iron deficient to iron deficient medium. Mean values of triplicates are shown with SD. Statistical difference was compared between 0 and 12 hours after transfer (student's t-test; **: $p<0.01$; ns: not significant).

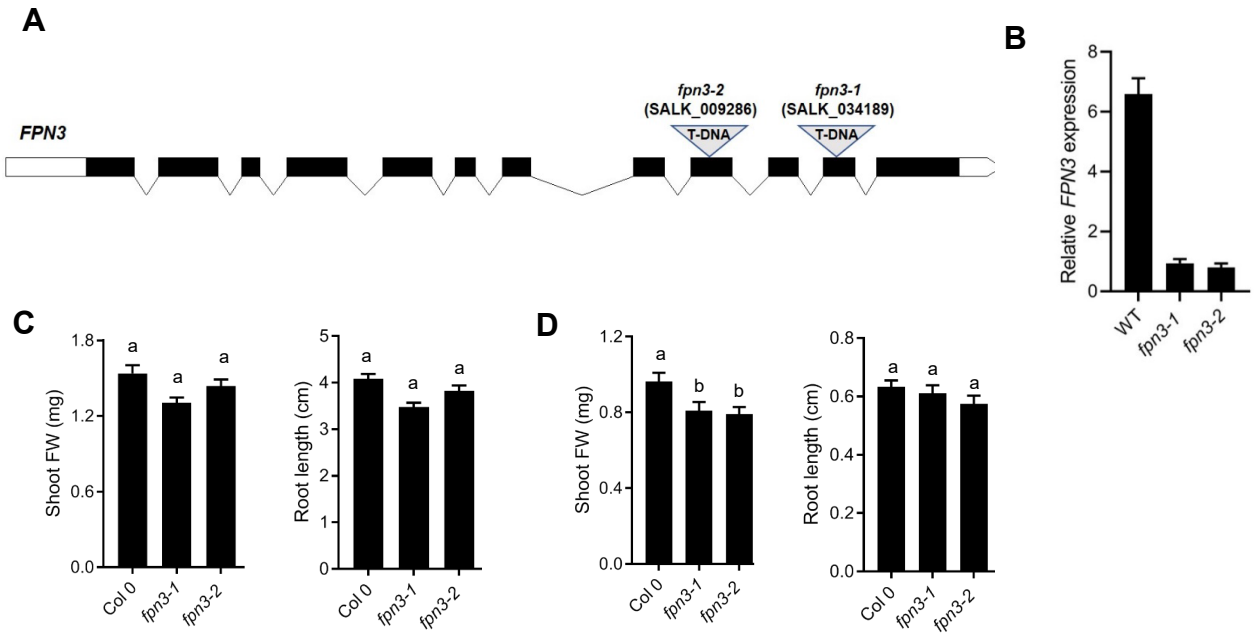


**Figure S4. *FPN3* T-DNA insertion lines, steady-state expression level of *FPN3* in *fpn3,* and quantification of shoot fresh weight and root length from plate grown plants.** (A) Schematic diagram of T-DNA insertions in *fpn3-1* (SALK_034189) and *fpn3-2* (SALK_009286). Black blocks with numbers represent exons and the gray lines are the introns. The light gray boxes before the first exon and after the last exon represent UTRs. (B) Steady state transcript level of *FPN3* was detected by RT-qPCR and normalized with *ACT2*. Normalized FPN3 level in *fpn3-1* was set to 1 and the relative level of normalized *FPN3* in each line was plotted. Mean values of triplicate samples normalized with *ACT2* are shown (n=3; error bars=SE). The following primers were used: *FPN3* forward, 5'- GTGGGTTCTTTGCCAA-CCATGAC-3'; *FPN3* reverse, 5'-TTAGGACGGTCCAGAACTCCAG-3'; *ACT2* forward, 5'-CCAAGCTGTTCTCTCCTTGTACGC-3'; *ACT2* reverse, 5'-TCACCAGAATCCAGC-ACAAT-ACC-3. (C, D) Quantified shoot fresh weights and root lengths of wild type (Col 0), *fpn3* single mutants, germinated and grown on plates with iron-sufficient (C; 100 μM Fe) medium or iron-deficient (D; no added iron with 0.3 mM ferrozine) medium. Mean values of at least 19 individuals are shown with standard error. Statistically significant groups are denoted in lower case letters (One-way ANOVA; Dunnett's test; $p < 0.05$).

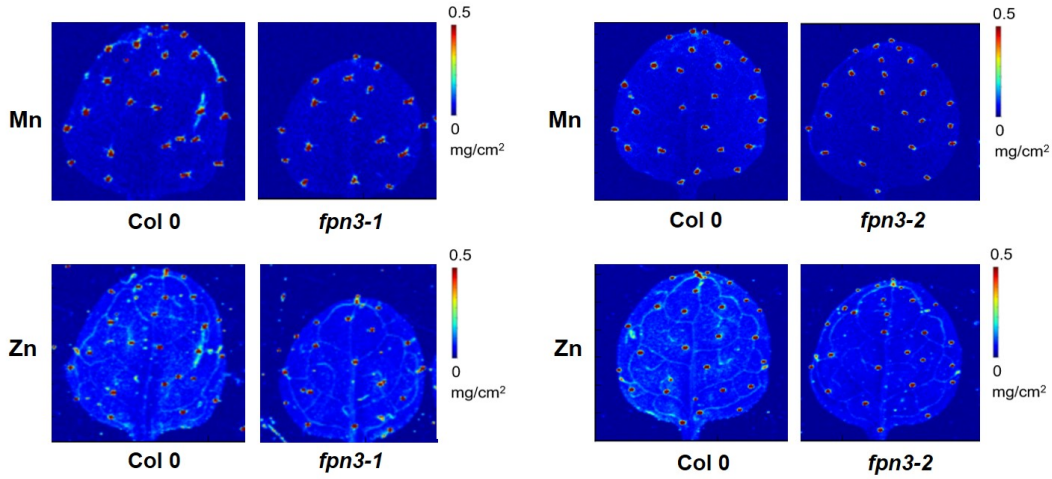


**Figure S5. Elemental map of manganese and zinc in *fpn3-1* and *fpn3-2* leaves.** Syncrhotron x-ray fluorescence microscopy images of the elemental distribution of manganese and zinc in leaves of wild type (Col 0) and *fpn3* single mutants. Distribution of zinc or manganese in Col 0 and *fpn3* was not statistically significant. Lower resolution (40 x 40 µm; 0.2 sec dwell time) images in the first true leaves of 22 day old plants. Representative images of leaves from three individuals are shown.

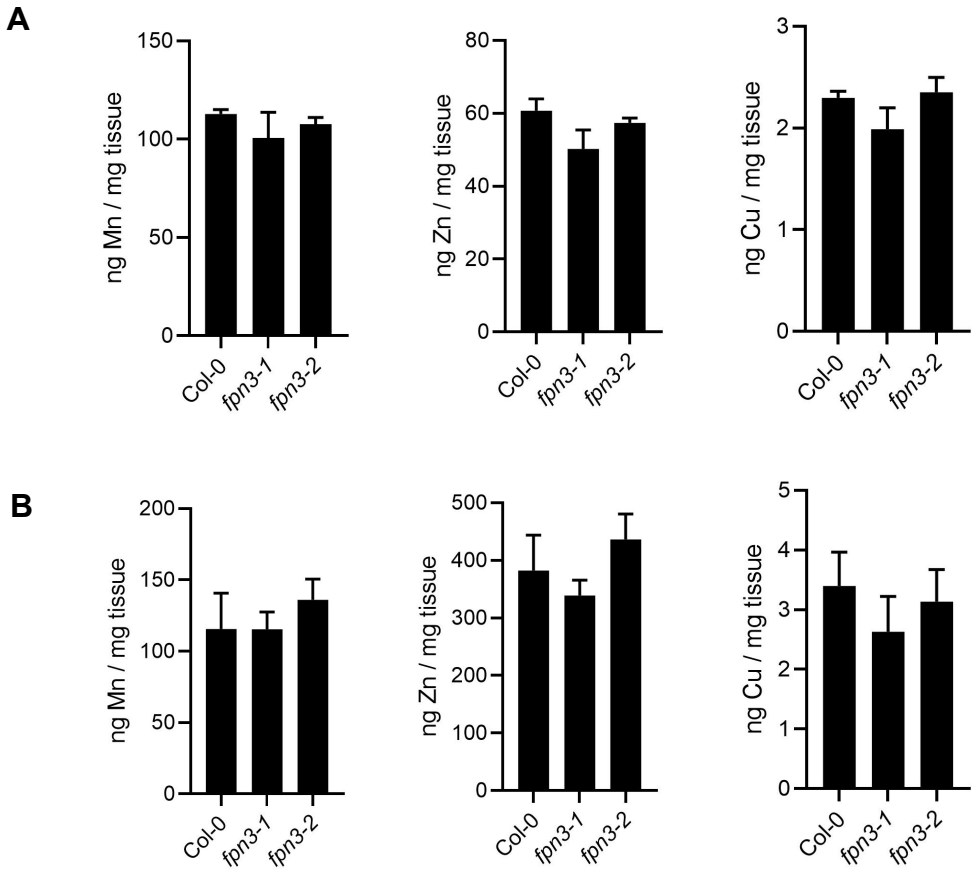


**Figure S6. Manganese, zinc, and copper content in wild type and *fpn3* shoot and root tissue.** ICP-MS was conducted with shoot (A) and root (B) tissue. Mean values of triplicate samples are shown with standard deviation. No significant differences were detected among the three lines (One-way ANOVA).

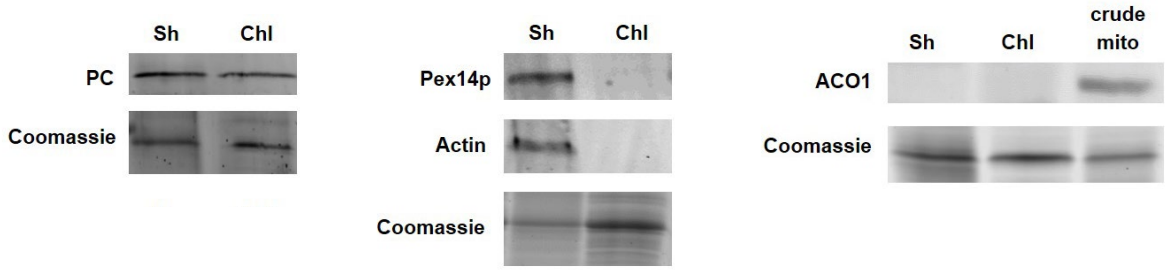


**Figure S7. Western blots with isolated chloroplast samples.**

The purity of chloroplast samples was assessed by Western blots using antibodies against the following subcellular compartment markers: plastocyanin (PC), a chloroplast marker; Pex14p, a peroxisome marker; actin, a cytosolic marker; and aconitase 1 (ACO1), a mitochondrial marker. Shoot protein (Sh) was loaded as a control alongside the chloroplast (Chl) samples. As a loading control, Coomassie staining was conducted with each set of Western blots. Because ACO1 was not detectable in our shoot samples due to its low abundance, a sample with crude mitochondrial prep (crude mito) was included as a positive control for the Western blot with anti-ACO1 antibodies.

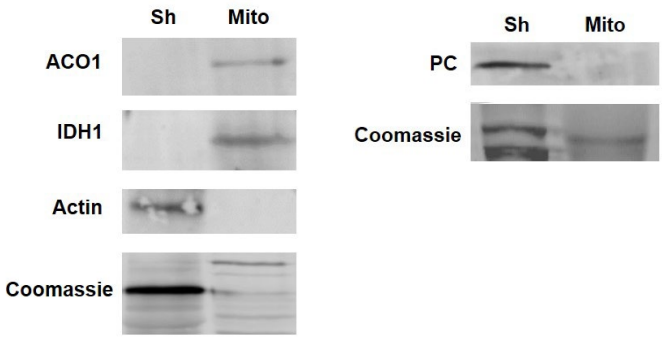


**Figure S8. Western blots with isolated mitochondria samples.**

The purity of mitochondrial samples was assessed by Western blots using antibodies against the following subcellular compartment markers: aconitase 1 (ACO1) and isocitrate dehydrogenase (IDH), mitochondrial markers, plastocyanin (PC), a chloroplast marker; Pex14p, a peroxisome marker; and actin, a cytosolic marker. Shoot protein (Sh) was loaded as a control alongside the chloroplast (Chl) samples. As a loading control, Coomassie staining was conducted with each set of Western blots. Because a small amount of ACO1 may also be found in the cytoplasm, antibodies against an additional mitochondrial marker, IDH, was also used to confirm our mitochondrial sample.

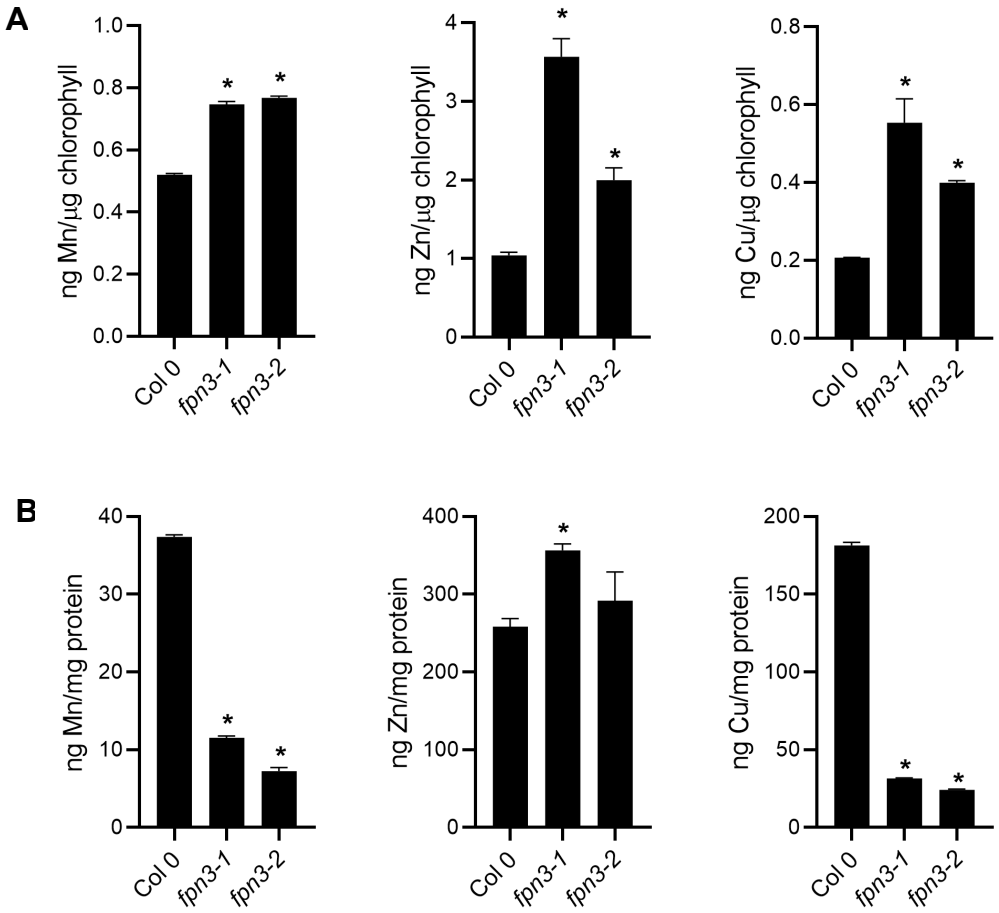


**Figure S9. Manganese, zinc, and copper content in wild type and *fpn3* chloroplasts and mitochondria.** ICP-MS was conducted with chloroplasts (A) and mitochondria (B) isolated from wild type (Col 0) and *fpn3*. (A) Metal content of chloroplast samples normalized with chlorophyll content. (B) Metal content of mitochondrial samples normalized with total protein. Mean values of quadruplicates for chloroplast samples and triplicates for mitochondrial samples are shown with standard error. Significant differences compared to the wild type, Col 0, are denoted (One-way ANOVA; Dunnett's test; *: $p < 0.05$).

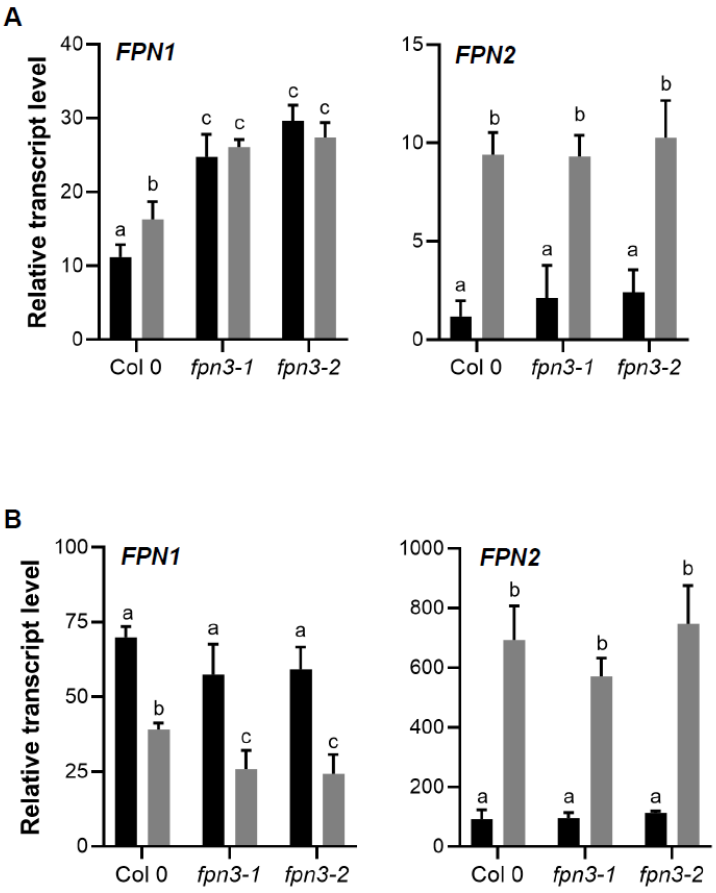


**Figure S10. Expression of *FPN1* and *FPN2* in wild type and *fpn3*.**

Steady-state transcript levels of *FPN1* and *FPN2* were detected by RT-qPCR from shoot (A) and root (B) tissue of wild type (Col 0), *fpn3-1*, and *fpn3-2* grown under iron sufficient and iron deficient conditions. Statistically significant differences are denoted with different letters (One-way ANOVA; Dunnett's test; *: $p < 0.05$; n=3; error bars = SD).