Figure 1

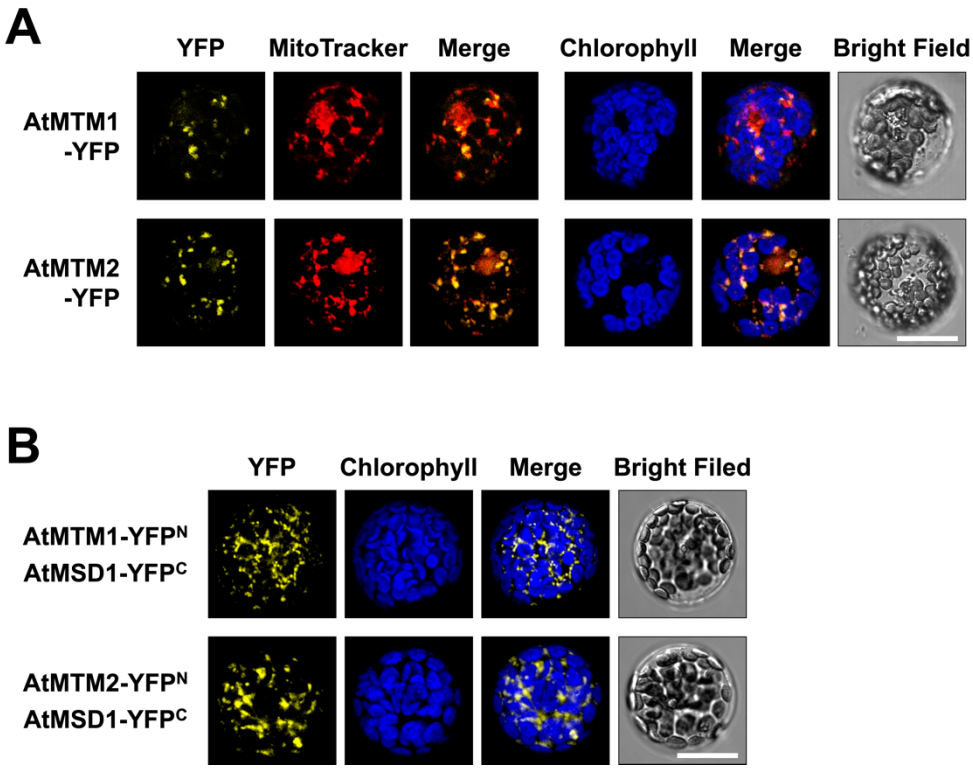

Figure 1. Mitochondrial localization of AtMTM1 and AtMTM2, and their interactions with AtMSD1. (A) Arabidopsis protoplasts were transfected with AtMTM1-YFP and AtMTM2-YFP to observe mitochondrial localization. **(B)** The YFP^N was fused to AtMTM1 and AtMTM2, and the YFP^C was fused to AtMSD1. Constructs were co-transfected in protoplasts for BiFC assay as indicated. The reconstituted YFP signals were observed by confocal microscopy. MitoTracker staining and chlorophyll autofluorescence were used to identify mitochondria and chloroplasts, respectively. Bars = 20 μm.

Figure 2

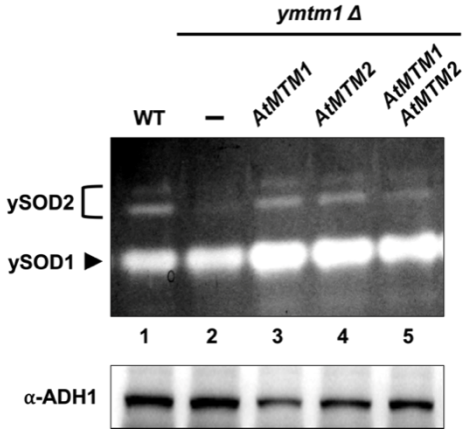

Figure 2. AtMTM1 and AtMTM2 recovered yeast MnSOD activities in *ymtm1Δ* cells. The *ymtm1Δ* cells were transfected with *AtMTM1* and *AtMTM2*, and in-gel SOD activities were analyzed. Yeast CuZnSOD (ySOD1) and MnSOD (ySOD2) activities are indicated by arrowhead and bracket **(top)**, respectively, and immunoblotting used α-alcohol dehydrogenase1 (ADH1) antibody **(bottom)**. ADH1 was an input control.

Figure 3

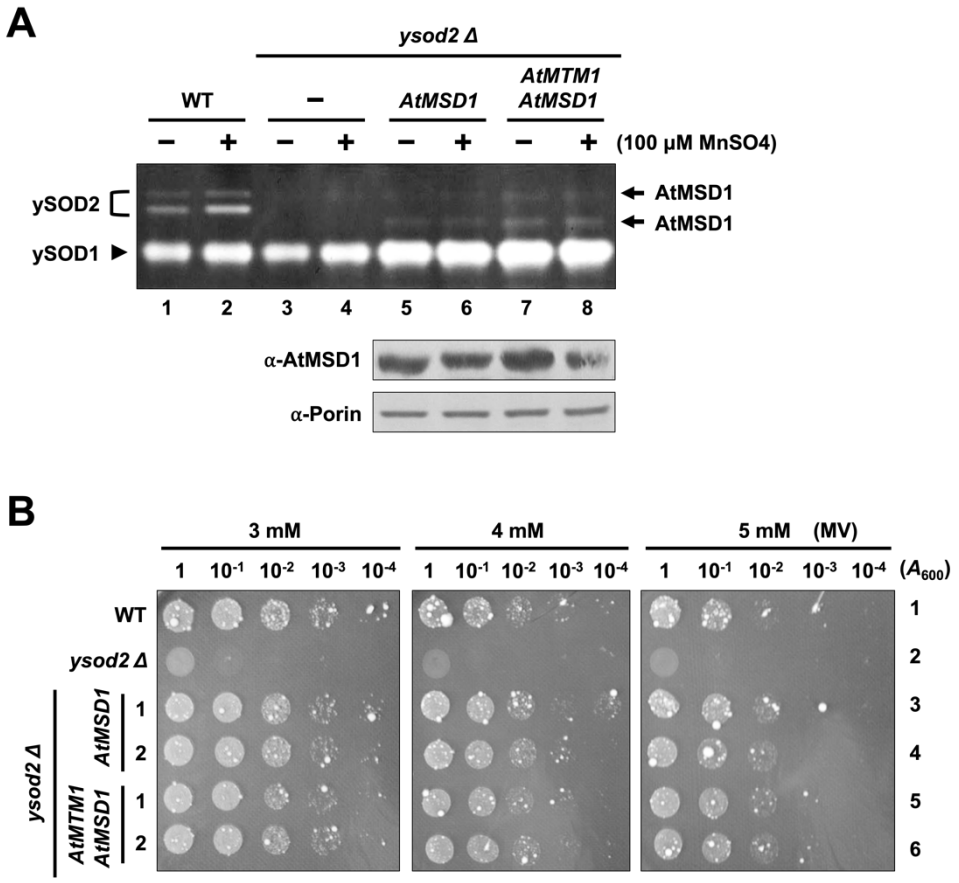

Figure 3. AtMTM1 enhanced AtMSD1 activity and AtMSD1 restored the WT phenotype in *ysod2Δ* cells in response to MV stress. (A) Co-expression of *AtMTM1* enhanced AtMSD1 activity in *ysod2Δ* cells. AtMSD1 activity **(top)** and protein **(bottom)** were analyzed. Yeast without (-) and with (+) 100 μM $MnSO_4$ supplementation are indicated. Immunoblotting used α-AtMSD1 antibody and mitochondrial porin was an input control. **(B)** *AtMSD1* complemented *ysod2Δ* cells to the WT phenotype with MV stress. The amount of 5 μL diluted culture from A_{600} = 1 to 10^{-4} was grown on YPD medium containing 3 to 5 mM MV.

Figure 4

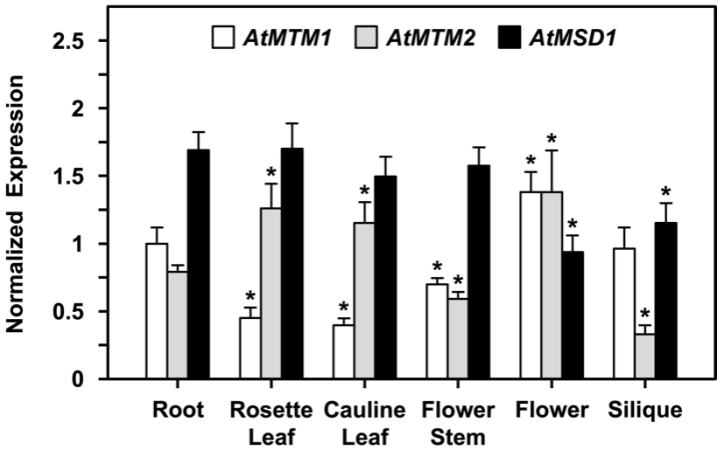

Figure 4. Expressions of *AtMTM1*, *AtMTM2*, and *AtMSD1* in various organs. Two to five-week-old organs were used for qPCR analysis. Expression level was normalized relative to the *AtMTM1* in root. Data are mean ± SE of three biological replicates. *, significant at $P < 0.05$ compared with the root value. *AtPP2A* was an internal control.

Figure 5

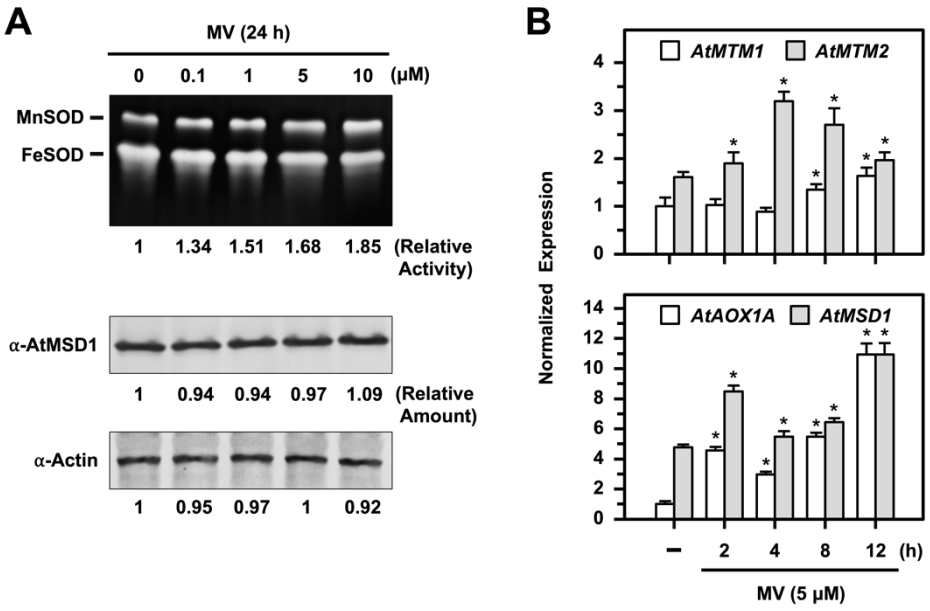

Figure 5. Post-transcriptional regulation of MnSOD and gene expressions of *AtMTM1*, *AtMTM2*, and *AtMSD1* under MV stress. (A) Two-week-old seedlings were treated without or with 0.1 to 10 μM MV for 24 h. An amount of 30 μg protein was used for in-gel SOD activity assay **(top)** and immunoblotting used α-AtMSD1 antibody **(bottom)**. Actin was an input control. AtMSD1 activity and protein were normalized relative to the control without MV treatment. **(B)** Two-week-old seedlings were treated without (-, control) or with 5 μM MV for 2 to 12 h. Expression level was normalized relative to the control of *AtMTM1* or *AtAOX1A*. The oxidation-responsive gene of mitochondrial *AtAOX1A* was used as a reference. Data are mean ± SE of three biological replicates. *, significant at $P < 0.05$ compared with the control. *AtPP2A* was an internal control.

Figure 6

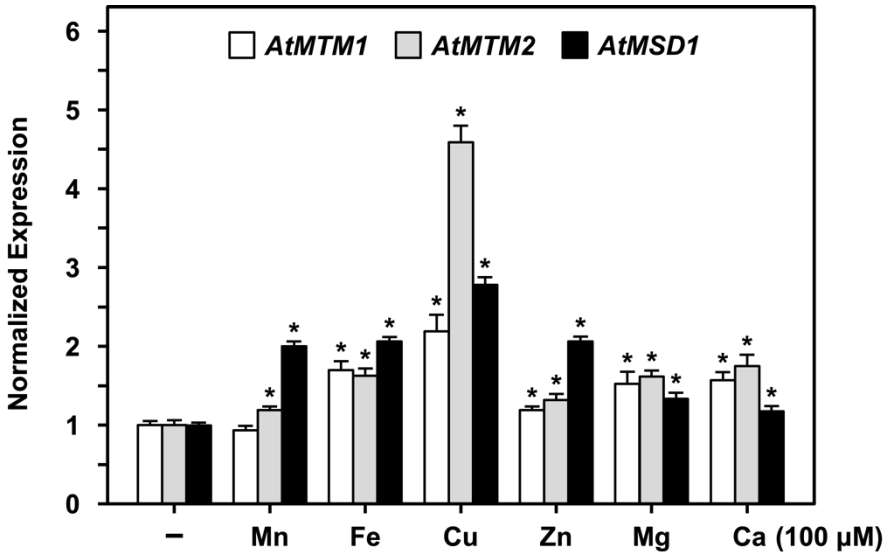

Figure 6. Expressions of *AtMTM1*, *AtMTM2*, and *AtMSD1* in response to different metal treatments. Two-week-old seedlings were treated without (-; control) or with 100 μM metal ions of $MnCl_2$, Fe citrate, $CuSO_4$, $ZnSO_4$, $MgCl_2$, and $CaCl_2$ for 24 h. Expression level was normalized relative to the control. Data are mean ± SE of three biological replicates. *, significant at $P < 0.05$ compared with the control. *AtPP2A* was an internal control.

Figure 7

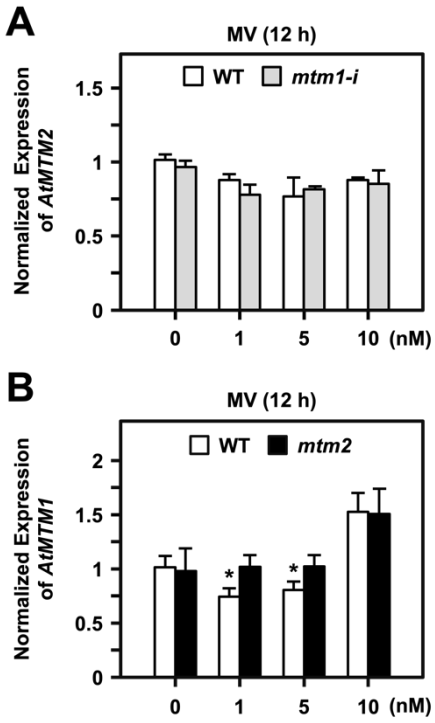

Figure 7. Expressions of *AtMTM2* in *mtm1-i* and *AtMTM1* in *mtm2* mutants in adapting to a shock of MV stress. Two-week-old seedlings were treated without (control) or with 1 to 10 nM MV for 12 h. **(A** and **B)** *AtMTM2* expression in *mtm1-i* mutant and *AtMTM1* expression in *mtm2* mutant were analyzed by qPCR, respectively. Expression level was normalized relative to the control of WT. Data are mean ± SE of three biological replicates. *, significant at $P < 0.05$ compared with the WT. *AtPP2A* was an internal control.

Figure 8

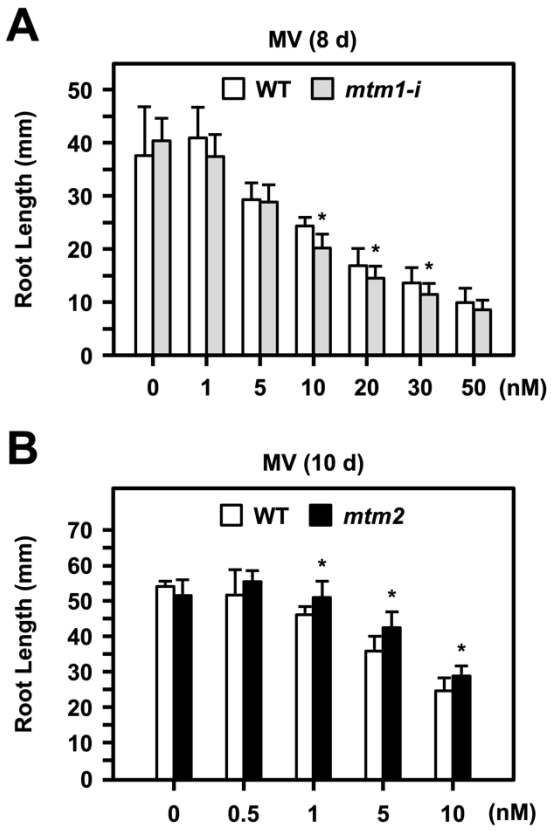

Figure 8. Root lengths of *mtm1-i* and *mtm2* mutants in adaption to long-term MV stress. **(A** and **B)** Root lengths of *mtm1-i* seedlings grown on 1 to 50 nM MV for 8 d and *mtm2* on 0.5 to 10 nM MV for 10 d were measured, respectively. Data are mean ± SE of three biological replicates. *, significant at $P < 0.05$ compared with the WT.

Figure 9

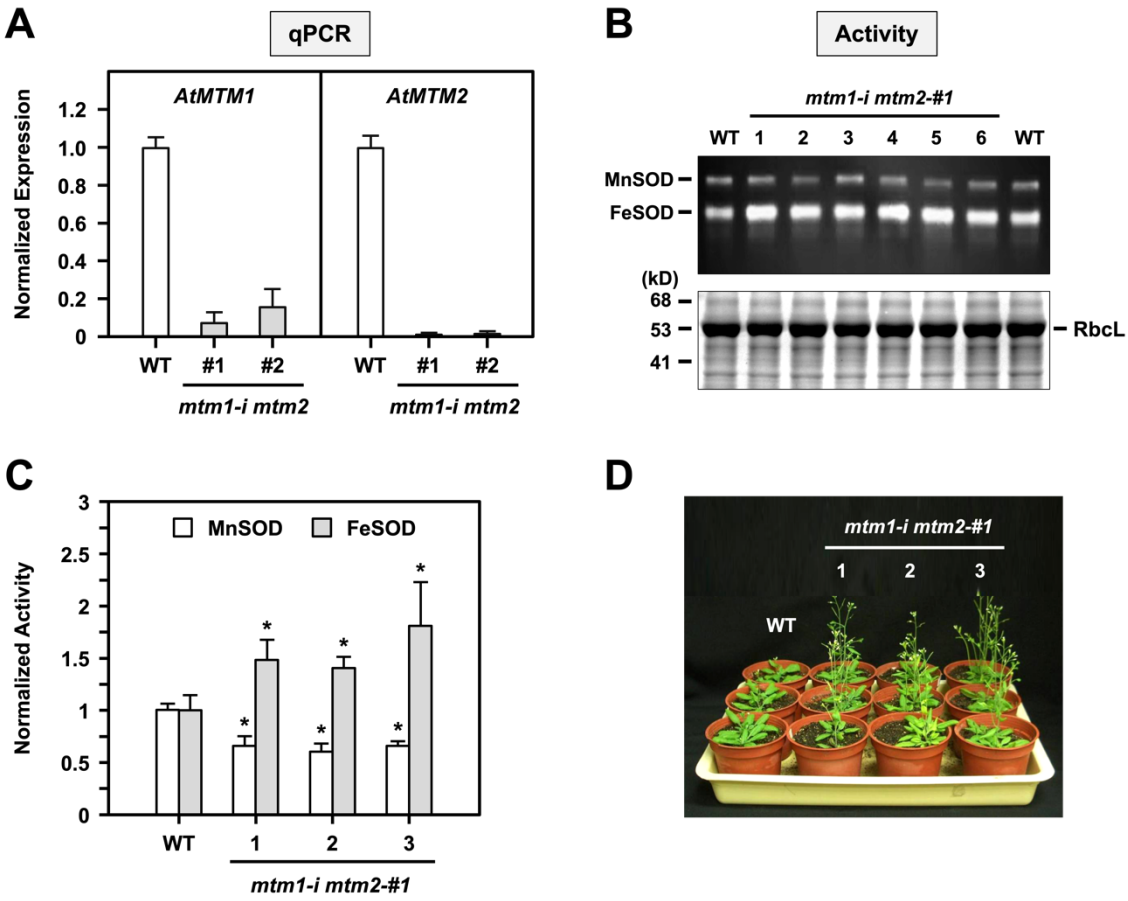

Figure 9. Characterization of *mtm1-i mtm2*-double mutant. (A) Expression level was normalized relative to the WT. *AtPP2A* was an internal control. **(B)** Mn and FeSOD activities in six siblings of *mtm1-i mtm2-#1* line **(top)**. Coomassie blue staining gel showed the ribulose bisphosphate carboxylase large subunit (RbcL) that was used as an input control **(bottom)**. **(C)** Mn and FeSOD activity of three independent plants was normalized relative to the WT, respectively. Data are mean ± SE of three biological replicates. *, significant at $P < 0.05$ compared with the WT. **(D)** Early-flowering phenotype of one-month-old *mtm1-i mtm2-#1* plants.

Figure 10

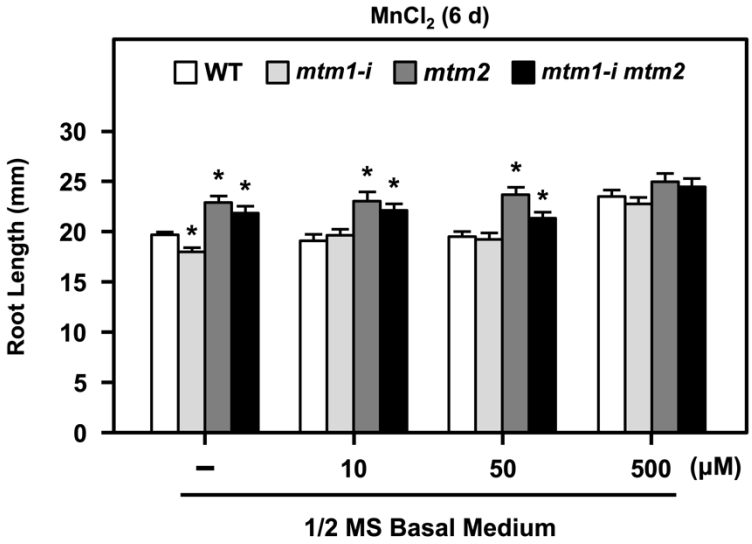

Figure 10. Root lengths of *mtm1-i*, *mtm2*, and *mtm1-i mtm2*-double mutants under Mn supplementation. WT and three mutants grown on 1/2 MS basal medium supplemented without (-) or with additional 10, 50, and 500 μM $MnCl_2$ for 6 d and root lengths were measured. Data are mean ± SE of three biological repeats. *, significant at $P < 0.05$ compared with the WT.

Figure 11

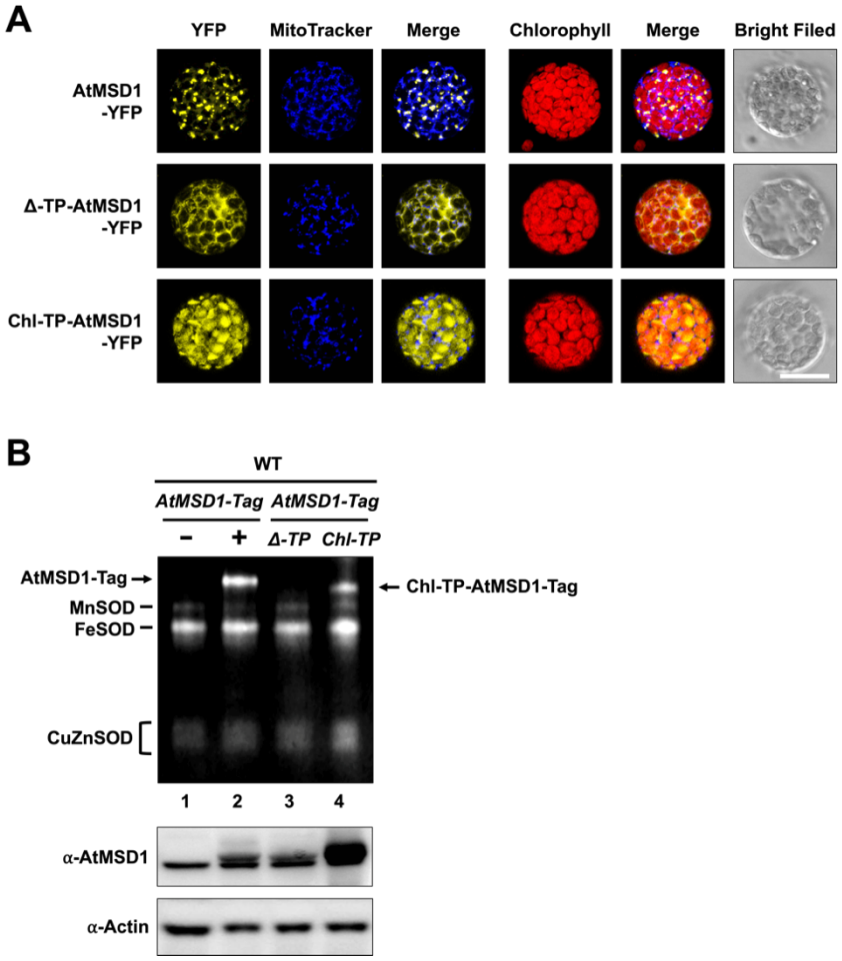

Figure 11. Subcellular localization and SOD activity of modified AtMSD1. (A) Localizations of mitochondrial AtMSD1, cytosol-destined Δ-TP-AtMSD1, and chloroplast-destined Chl-TP-AtMSD1 were analyzed by confocal microscopy. YFP was fused to the C-terminal end of each tester. Bar = 20 μm. **(B)** Transient expressions of *AtMSD1-Tag*, *Δ-TP-AtMSD1-Tag*, and *Chl-TP-AtMSD1-Tag* in WT protoplasts. Transfections without (-) or with (+) 30 μg plasmid DNA of *AtMSD1-Tag* in 10^6 protoplasts are indicated. In-gel SOD activity assay **(top)** and immunoblotting with α-AtMSD1 and α-Actin antibodies **(bottom)** were conducted. Actin was an input control.

Figure 12

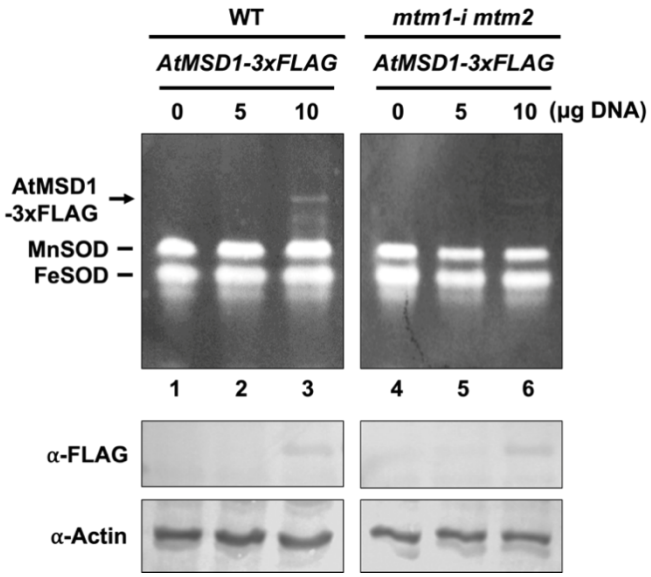

Figure 12. Exogenous expression of *AtMSD1-3xFLAG* in WT and *mtm1-i mtm2* mutant protoplasts. Transfections with 5 and 10 μg plasmid DNA in 10^6 protoplasts are indicated. In-gel SOD activity assay **(top)** and immunoblotting with α-FLAG and α-Actin antibodies **(bottom)** were conducted. Actin was an input control.

Figure 13

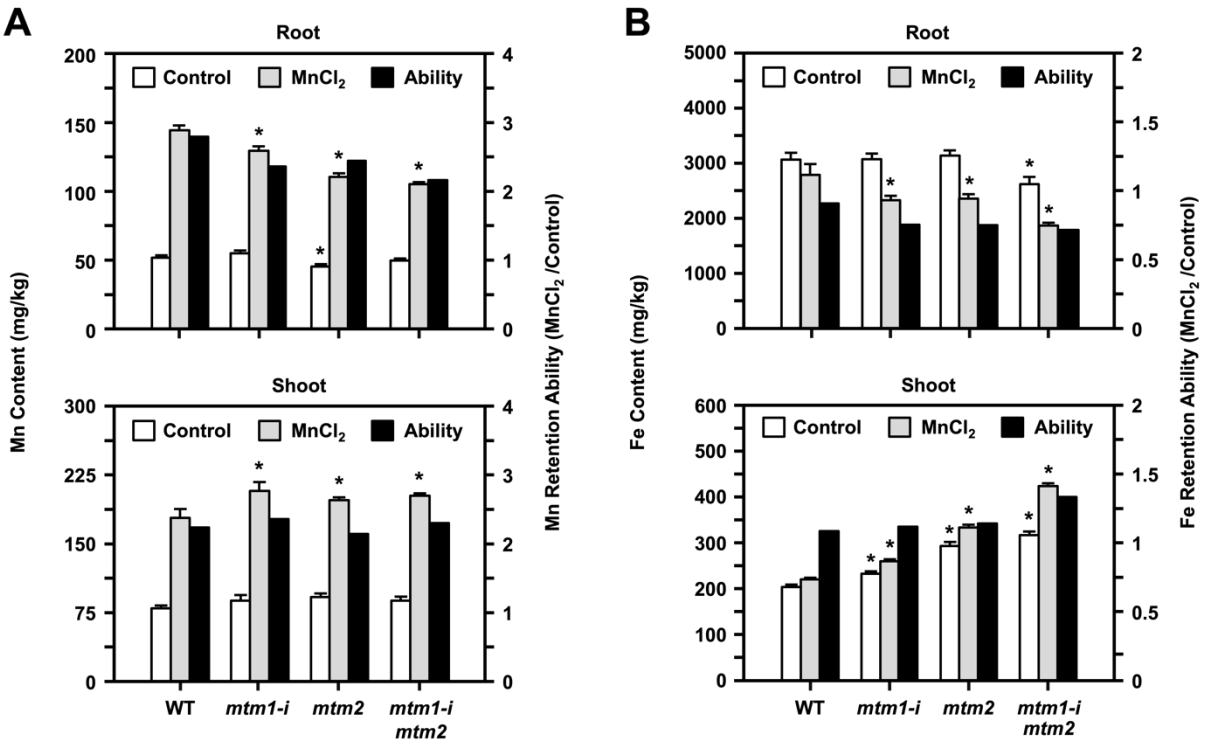

Figure 13. Mn and Fe contents in *mtm1-i*, *mtm2*, and *mtm1-i mtm2* mutants under $MnCl_2$ treatment. Two-week-old seedlings were incubated without (control) or with 100 μM $MnCl_2$ for 24 h. **(A** and **B)** Mn and Fe contents in root and shoot were measured by ICP-OES, respectively. The index of metal retention ability represents the ratio of ion content in the Mn treatment to the control. Data are mean ± SE of three biological repeats. *, significant at $P < 0.05$ compared with the WT.