

Overexpression mutants reveal a role for a chloroplast MPD protein in regulation of reactive oxygen species during chilling in Arabidopsis

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Supplementary data:

Table S1: Growth measurements of Col-0, *mpd1-1* and *mpd1-2* at 22°C.

Table S2: The Mpv17_PMP22 family in Arabidopsis.

Table S3: Growth measurements of MPD1-OX3 at 22°C.

Table S4. List of primers used for quantitative PCR analysis.

Fig. S1: Transmembrane domains of MPD1.

Fig. S2: *mpd1-1* is an overexpression allele.

Fig. S3: Level of *MPD1* transcript and phenotype for two MPD1 overexpression lines.

Fig. S4: Chilling phenotype of *MPD1-OX3* lines after recovery.

Table S1. Growth measurements of *mpd1-1* and *mpd1-2* at 22°C

Allele	Rosette diameter (2wks 22°C)	Rosette diameter (5wks 22°C)	Dry weight
Col-0	3.04 ±0.16	10.2 ±0.2	320.3 ±21.3
<i>mpd1-1</i>	3.02 ±0.15	10.2 ±0.2	327.0 ±20.0
<i>mpd1-2</i>	3.07 ±0.16	10.25 ±0.0	328.7 ±20.2

Rosette diameters of plants grown at 22°C measured after two and five weeks of growth. The plants were then cultivated to maturity and the above-soil biomass collected, dried, and weighed. Data are means ± SD for n=30 plants (ten in each of three independent replicates).

Table S2. The Mpv17_PMP22 family in Arabidopsis.

AGI Locus	SUBA targeting¹	Predicted protein length	Identity (%)	Similarity (%)	Similarity Bit value	Chance expectation
At4g03410 (MPD1)	Chloroplast	361	100	100	713	0
At1g52870	Chloroplast	366	81	91	412	8e-144
At5g19750	Chloroplast	288	21	47	57	8e-09
At4g04470 (PMP22)	Peroxisome	190	20	44	97	3e-04
At2g42770	Peroxisome	232	23	44	96	6e-04
At4g14305	Peroxisome	185	25	43	55	9e-09
At2g14860	Mitochondria	252	28	46	80	5e-17
At4g33905	Mitochondrion	261	28	47	82	2e-17
At3g24570 (MPV17)	Mitochondrion	235	23	41	60	3e-10
At5g43140	Mitochondrion	254	23	46	72	3e-14

¹Cellular localization as predicted by SUBA4 (<http://suba.live>). The Arabidopsis Araport11 database was searched with the MPD1 predicted protein sequence through the TAIR BLAST tool (<https://www.Arabidopsis.org/Blast/>) with no other significant homologies detected.

Table S3. Growth measurements of *MPD1-OX3* at 22°C.

Allele	Rosette diameter (2wks 22°C)	Rosette diameter (5wks 22°C)	Dry weight
Col-0	3.04±0.16	10.2±0.2	320.3±21.3
<i>mpd1-1</i>	3.02±0.15	10.2±0.2	327.0±20.0
<i>MPD1-OX3</i>	3.00±0.13	10.3±0.2	325.1±18.0
<i>iso-OX3</i>	3.11±0.12	10.2±0.1	318.5±18.4

Rosette diameters of plants grown at 22°C measured after two and five weeks of growth. The plants were then cultivated to maturity and the above soil biomass collected, dried, and weighed. Data are means ± SD for n=30 plants (ten in each of three independent replicates).

Table S4. List of primer used for RT-qPCR in this study.

Name	Sequence
Zat12-F	AGTTTCATTCGTTCCAAGCC
Zat12-R	TCTCGTTCCTGTGTCTCCTC
DHAR1-F	AACCTCTCTGACAAACCCC
DHAR1-R	TCTTTGCTCTTCAAGAATGTCC
CAT1-F	TTCCCCTTTCTTCACTACAAAC
CAT1-R	AAACCCTTAGCACTGGCTC
SOD1-F	GATGGTGTGACCACTGTGAGTGGAA
SOD1-R	GGGCACCGTGTGTTTTACCATCG
DHAR3-F	GGCGGCGAGTCCTCTTGAAATCT
DHAR3-R	GAAGCCTTTTCAGGTGGGGTAGCA
SOD2-F	CGTCGAAAGCGTTGACAGTTGTTTC
SOD2-R	GAGCTCCGTGTGTCATGTTGTTAGG
tAPX1-F	TCCCGAGGGCATAGTCATTGAAAAC
tAPX1-R	GGGTAATGGCTTATCTGGGCTTCC
Tub2-F	ACTGTCTCCAAGGGTTCCAGGTTT
Tub2-R	ACCGAGAAGGTAAGCATCATGCGA
mpd1-F	GAAGGAAAACCGCTGTTTGAGTTTG
mpd1-R	TGGAAACGCAAGAGCCCTAAAACCTG

MAALCCCPTTIIVSCKVSSRIKTACPLERINPFKNQKRLITERRNLIVKSIIEDREAIDVKND
 NFKAE E E E L S E D K V E D T D R L M S R G I N A A I V L A A G T V A V T K L L T I D H D Y W Q G W T L Y E I L R Y A P E H
 N W F A Y E Q I L K T N P V L A K M A I S G I V Y S L G D W I A Q C Y E C K P L E F D R T R V L R S G L V G F T L H G S L S
 H Y Y Y Q F C E A L F P F Q E W W V V P A K V A F D Q T V W S A I W N S I Y F T V L G L L R F Q S P A D I F S E I K T T F L P
 M L T A G W K L W P L A H L V T Y G V I P V D Q R L L W V D C I E L I W V T I L S T Y S N E K A E A Q A S E E T N S S S H S S
 E V C Q V E L F S K N L S S K I S L E R L N V C S E C S S K L I C A C K Y L T G L G D I S M

Fig. S1. Transmembrane domains of MPD1. Analysis of the MPD1 protein sequence with the HMMTOP 2.0 server (<http://www.enzim.hu/hmmtop/>) reveals five predicted transmembrane domains, underlined in green. The Mpv17_Pmp22 domain (red) and chloroplast targeting signal (blue) are indicated as in Figure 2b.

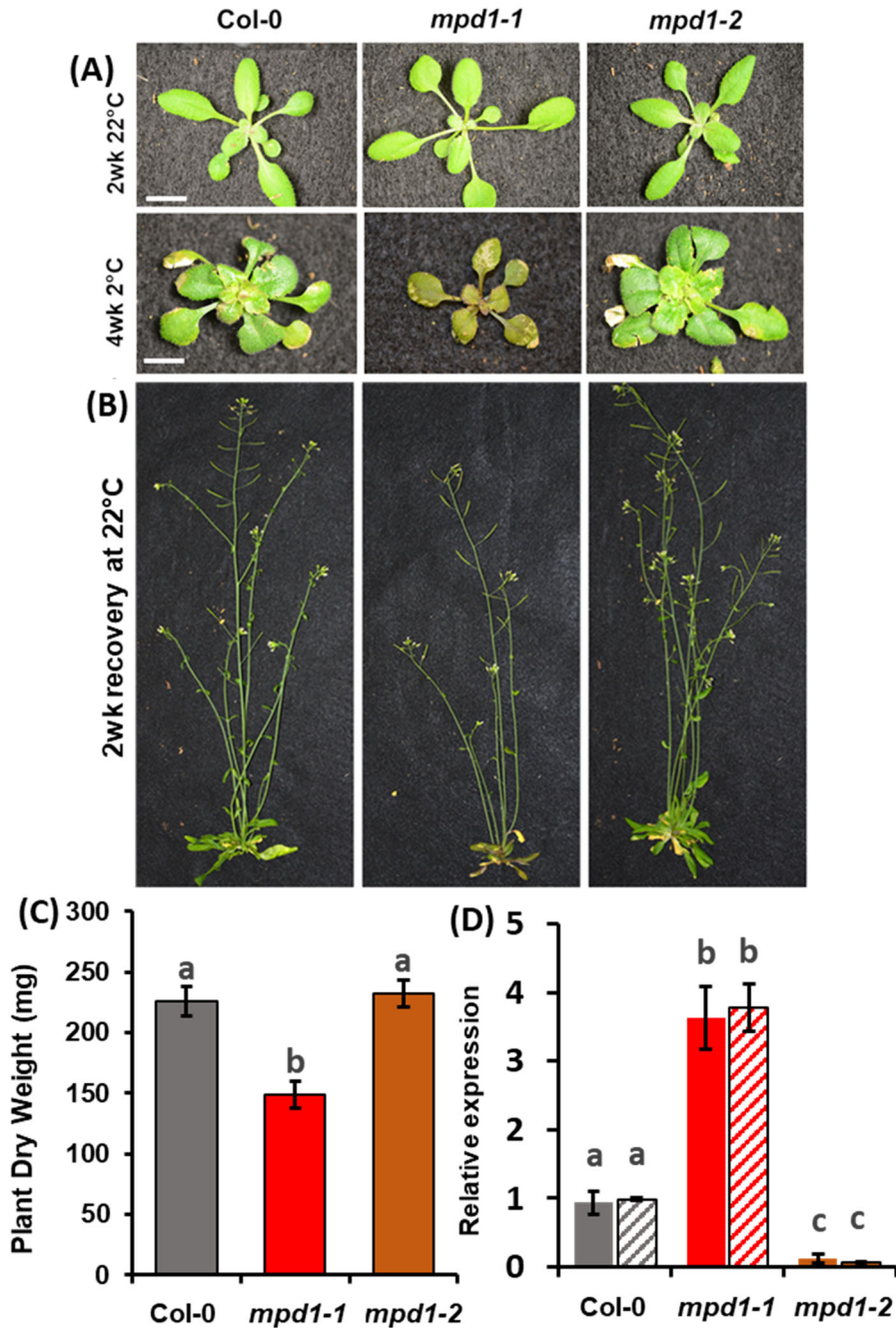


Fig. S2. *mpd1-1* is an overexpression allele. (A) After two-weeks growth at 22°C, *mpd1-1* and *mpd1-2* mutants resemble Col-0. After four weeks at 2°C, *mpd1-1* plants exhibit chilling damage, but *mpd1-2* plants still resemble Col-0. (B) After eight weeks at 2°C and two weeks recovery at 22°C, *mpd1-1* plants remain smaller than Col-0 and *mpd1-2*. (C) Dry weights after eight weeks at 2°C and two weeks recovery at 22°C for Col-0, *mpd1-1* and *mpd1-2*. (D) Expression of *MPD1* relative to *tub2* in plants after two weeks at 22°C (solid) and after a further four weeks at 2°C (hatched). In (A) scale bar is 1cm. In (C) data are means \pm SD for n=30 plants (ten in each of three independent replicates). In (D) data are means \pm SD for n=3 biological replicates of leaves pooled from 10 plants. Statistical analysis by one-way ANOVA and post hoc Tukey test, letters denoting significance ($p < 0.001$).

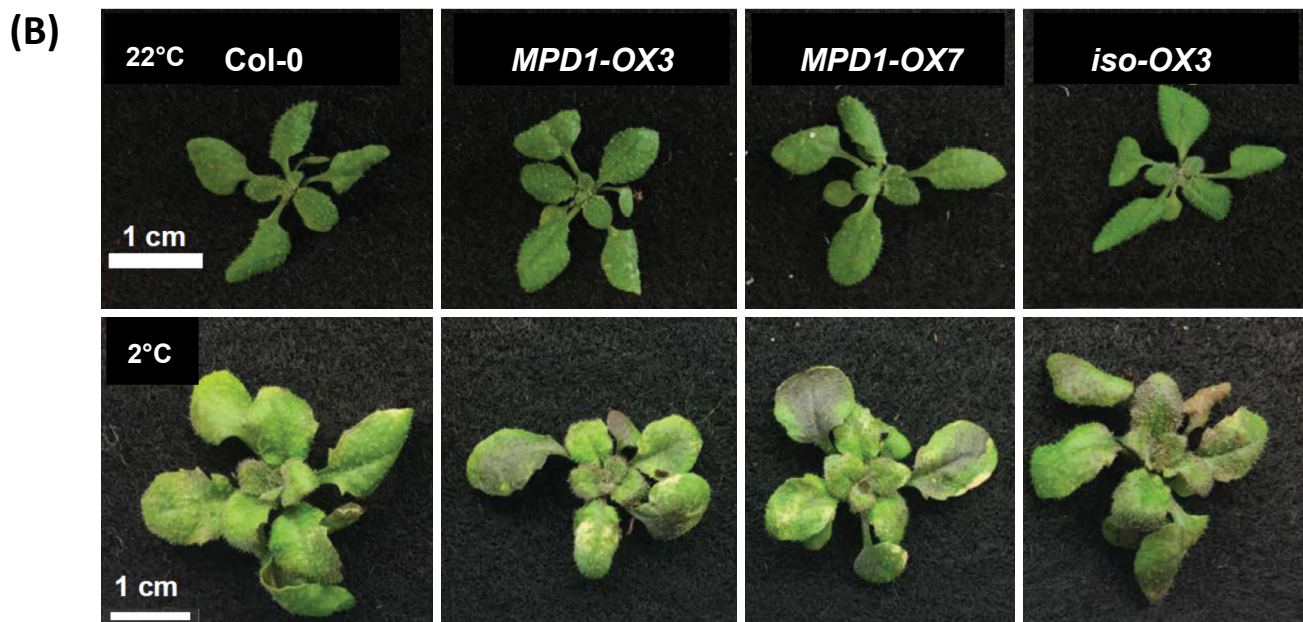
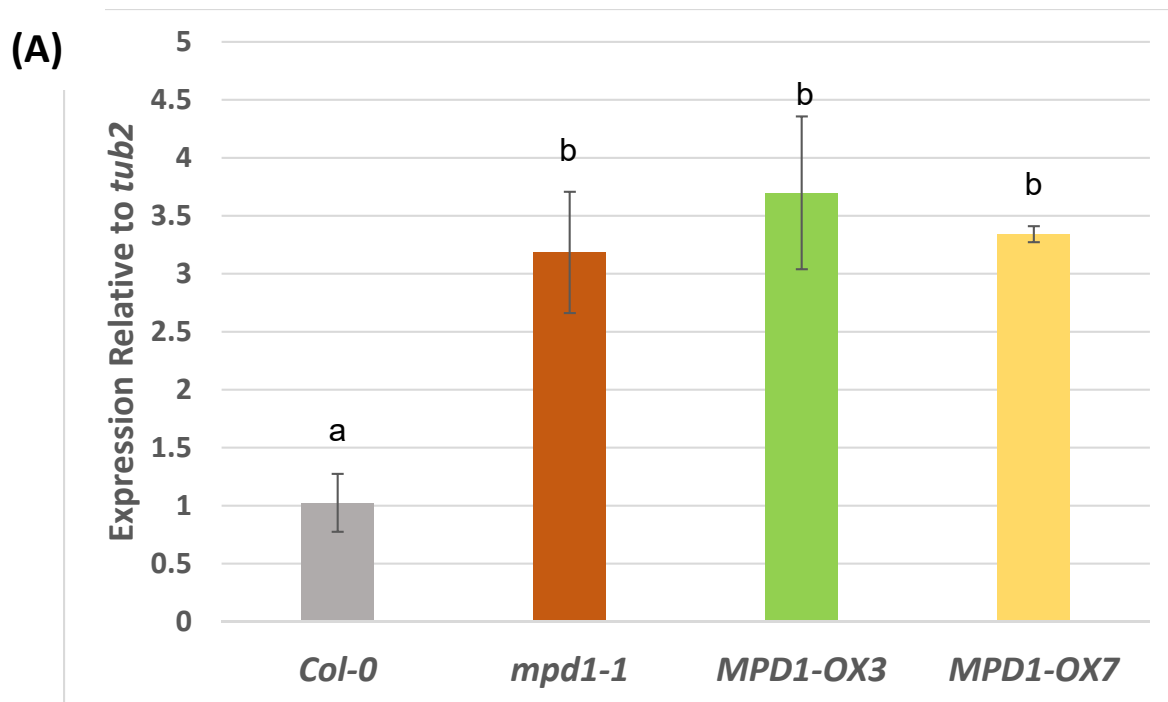


Fig. S3: Level of *MPD1* transcript and phenotypes for two *MPD1*-overexpression lines. (A) Relative expression *MPD1* transcript in leaves of *Col-0*, *mpd1-1*, *MPD1-OX3*, and *MPD1-OX7* plants, normalized to expression of the constitutive *tub2* (*At5g62690*) gene. (B) Phenotypes of *Col-0*, *MPD1-OX3* and *MPD1-OX7* lines after two weeks at 22°C (top) and after four weeks at 2°C (bottom). *iso-OX3* is an untransformed isogenic segregant from the parent of *MPD1-OX3*. (A) Means \pm SE for $n=3$ biological replicates of leaves pooled from 10 plants. Analysis by one-way ANOVA with post hoc Tukey test; letters applied to columns mark significance ($p < 0.001$).

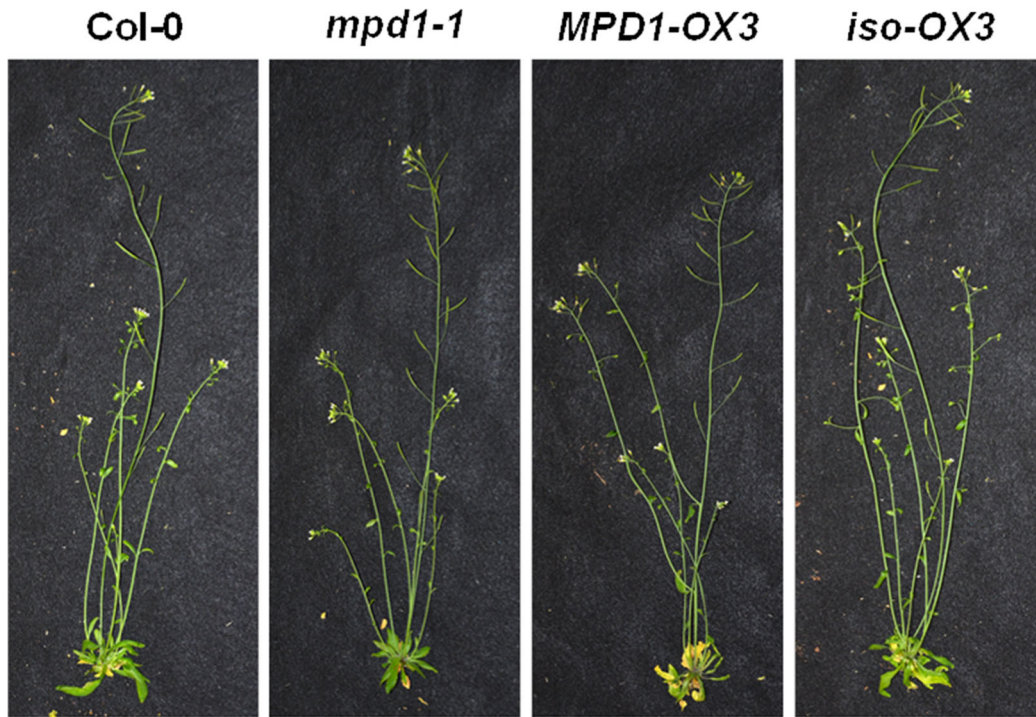


Fig. S4: Chilling recovery phenotype of *MPD1-OX3* line. Representative *mpd1-1*, *MPD1-OX3*, and *iso-OX3* plants compared to Col-0. After initial cultivation at 22°C for two weeks the plants were subjected to growth at 2°C for 8 weeks, then allowed to recover at 22°C for two weeks before they were photographed. The *iso-OX3* line is an isogenic sibling segregant of *MPD1-OX3*.