

Supplementary data

Characterization of the Histidine-rich Loop of *Arabidopsis* Vacuolar Membrane Zinc Transporter AtMTP1 as A Sensor of Zinc Level in Cytosol

Natsuki Tanaka, Takashi Fujiwara, Rie Tomioka, Ute Krämer, Miki Kawachi, and Masayoshi Maeshima

Legends for supplemental figures

Fig. S1. Membrane topology of unmodified AtMTP1 (upper panel) and His-half-deleted AtMTP1 (His-half AtMTP1, lower panel). A schematic membrane topology of AtMTP1 was constructed by the homology modeling of *E. coli* YiiP (Lu et al. 2007, Kawachi et al. 2012). The His-half mutant lacks the first half (His-182 to His-216) of the whole His-loop from His-182 to Asn-251.

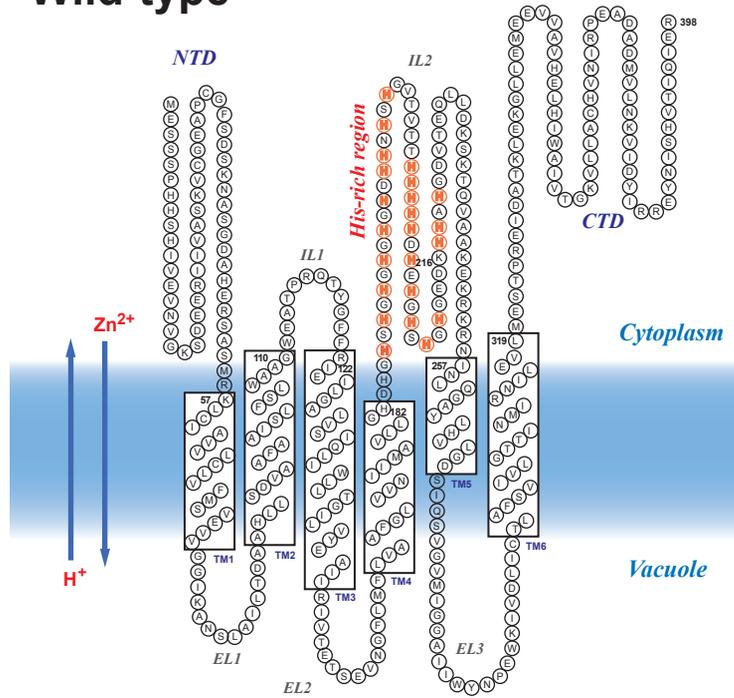
Fig. S2. The growth suppression of *mtp1-1* under Zn-excess conditions was recovered by expressing the AtMTP1 (35S-MTP1) and the His-half AtMTP1 (35S-His-half). *Ws*, *mtp1-1*, 35S-MTP1, and 35S-His-half lines were grown in the modified Hoagland medium containing Zn at concentrations of 0, 100, 200, or 250 μ M. After 10 d, root length was measured. Values are expressed as mean \pm SD; $n = 21$. Significant differences from *mtp1-1* at each Zn concentration are indicated by asterisks ($*P < 0.00002$).

Fig. S3. Expression levels of AtMTP1 and AtMTP3 in *Ws* and mutant lines. Total RNA fractions were prepared from 22-d-old whole plants of *Ws*, *mtp1-1*, 35S-MTP1, and 35S-His-half lines and then subjected to real-time RT-PCR analyses of mRNA levels of *AtMTP1* and *AtMTP3*. Three replicates with 72 plants were averaged and the SD is shown. Seedlings were grown in modified Hoagland medium with (5 μ M; A and B) or without (0 μ M; A) Zn. Values are expressed as mean \pm SD; $n = 3$. Significant differences from *Ws* are indicated by asterisks ($*P < 0.006$). The mRNA levels of MTP3 showed no significant difference.

Fig. S4. 35S-His-half lines grew poorly with TPEN. Seedlings of *Ws*, *mtp1-1*, 35S-MTP1, and 35S-His-half lines were grown in modified Hoagland medium without Zn and supplemented with 1 μ M TPEN, a Zn chelator. Typical 7-week-old plants were photographed (A) and measured for shoot fresh weight (B). For each line, four plants were independently analyzed. Values are expressed as mean \pm SD. No significant difference from *Ws* was detected.

Fig. S1 (Tanaka et al.)

Wild-type



His-half

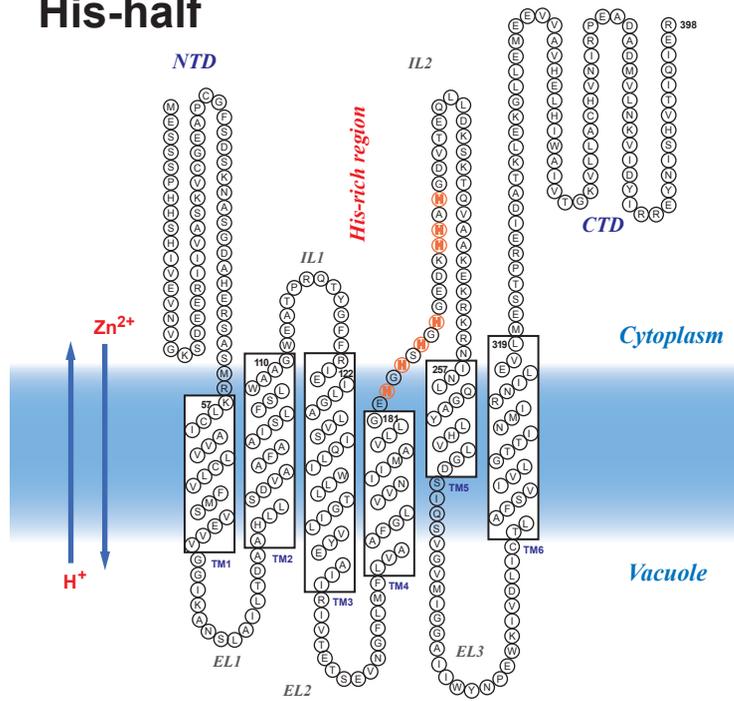


Fig. S2 (Tanaka et al.)

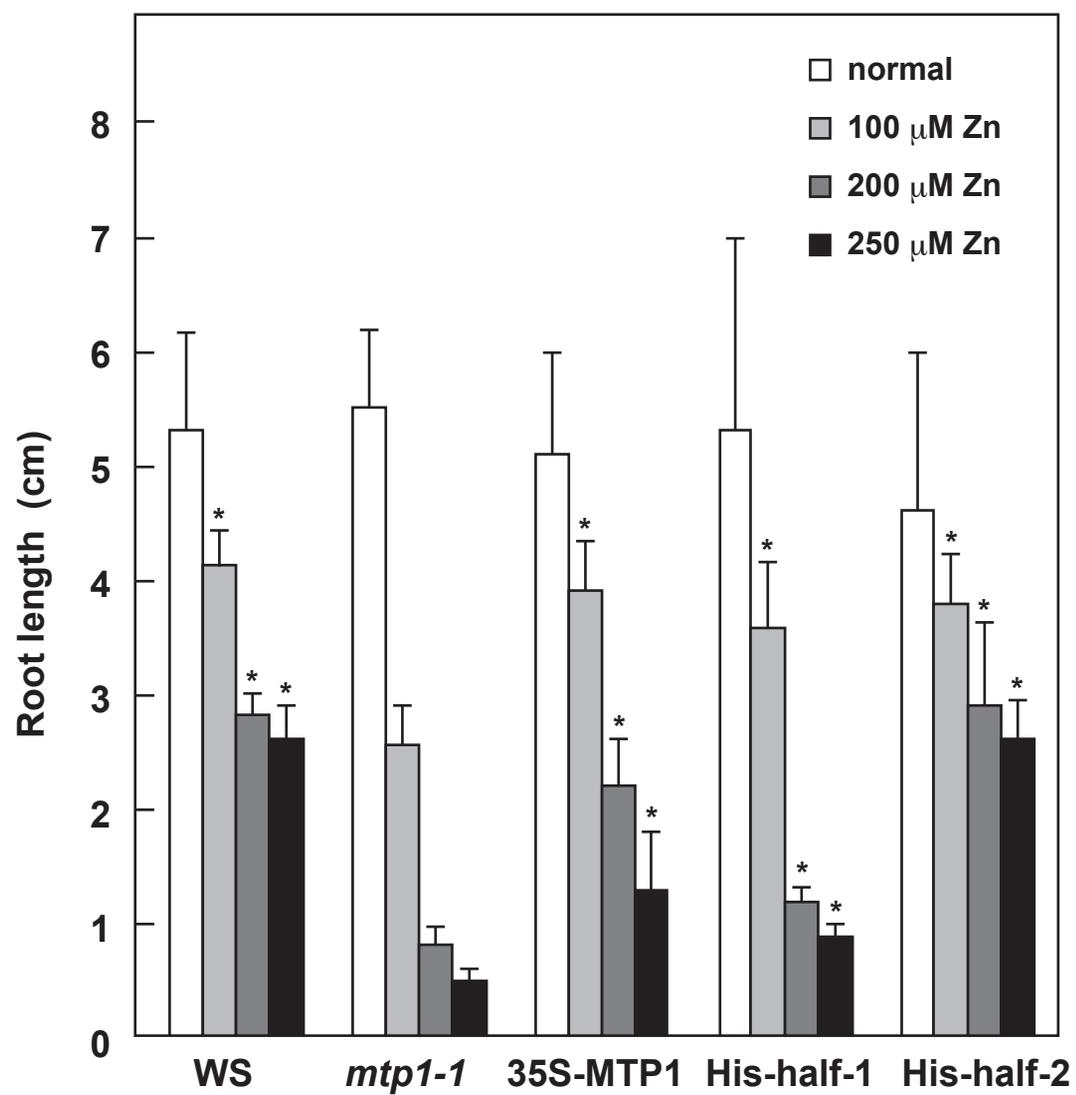
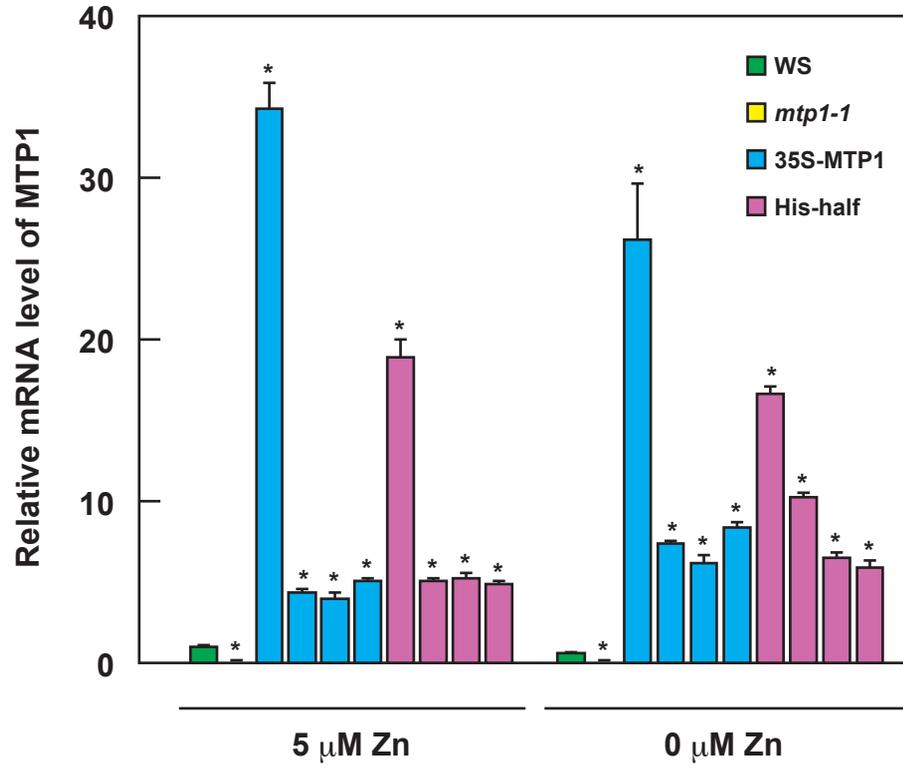


Fig. S3 (Tanaka et al.)

A



B

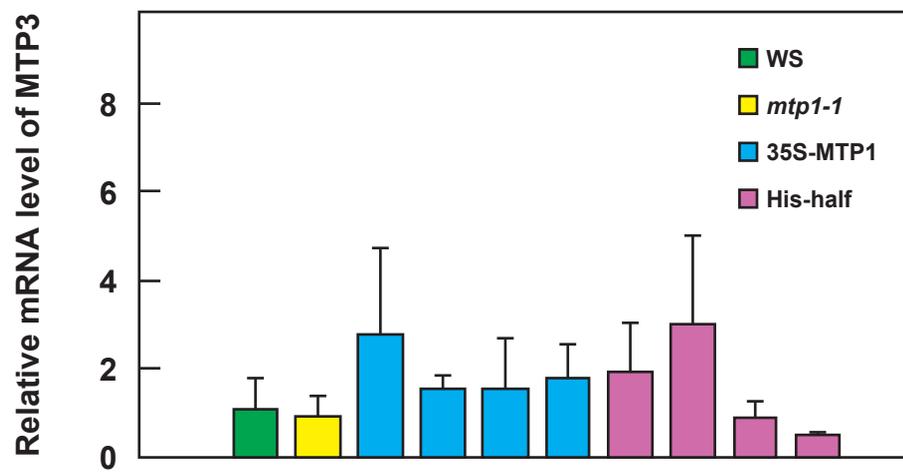
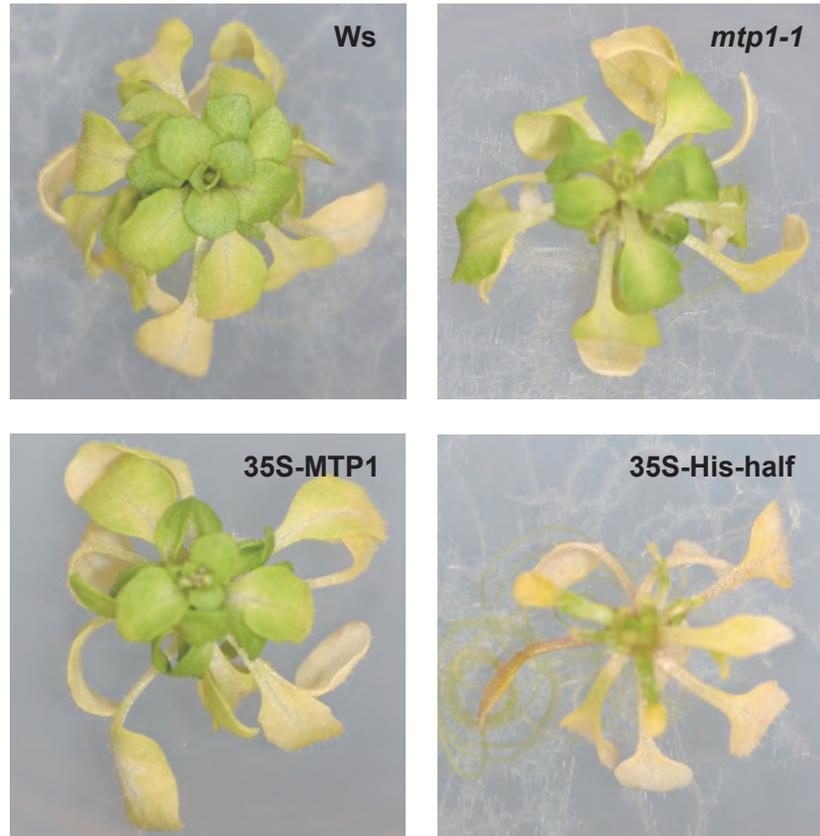


Fig. S4 (Tanaka et al.)

A



B

