

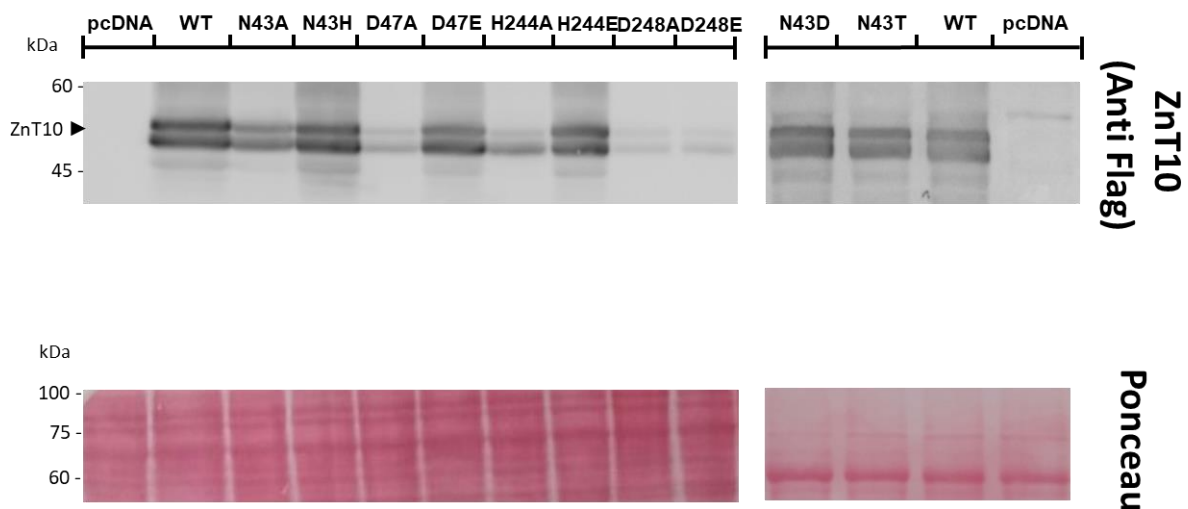
SUPPLEMENTARY

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YiIP      *SILAALVDSLVDIGASLTNL...RADMLHYQSDVMMNGAIL
ZnT1     AMLSDFHMLSDVLALVVAL...RGVFLHVLGDALGSVIVV
ZnT5     GLISDGFHMLFDCSALVMGL...RGVFLHVLADTLGSIGVI
ZnT3     AIMTDAHLLADVGSMMGSL...RAAFVHVLGDLQSFQVL
ZnT4     AIMTDALHMLTDLIAIILTL...RAAFVHALGDLVQSVGVL
ZnT10    ALLSDSFNMLSDLISLCVGL...RGVLLHVMGDALGSVVVV
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* - Leads to Cd²⁺ efflux (changes the transport affinity)

Supp Fig.1| MSA analysis of hZnT10 vs. other hZnT family members. Multiple Sequence Alignment of hZnT10 against different hZnT family members and the bacterial homologues YiiP (*Escherichia coli*), suggested tetrahedral site amino acids are highlighted in yellow as indicated. Note that both YiiP and ZnT10 show dissimilarity in the first residue of the tetrahedral site.



Supp Fig.2| expression of ZnT10 mutants. Western Blot analysis of ZnT10 mutants described in this study expressed in HEK-T cells.



Supp Fig.3| ZnT10 other tetrahedral residues are essential for Mn^{2+} transport. a, Representative traces of Mn^{2+} influx in ZnT10 WT (red), pcDNA (black), ZnT10 H244D (gray) and ZnT10 D47E (yellow) transfected cells. Mn^{2+} influx ($5 \mu M$) was determined as described in Fig. 1c. The horizontal bar indicates when Ringer's solution was replaced with Ringer's solution containing Mn^{2+} ($5 \mu M$). **b,** Mean rates of cytoplasmic Mn^{2+} uptake for ZnT10 WT (n=6), pcDNA (n=7), ZnT10 H244D (n=3) and ZnT10 D47E (n=3) taken from Supp Fig.5a (**p<0.01,***p<0.001 ****p<0.0001).

Supp Table.1: Number of PDB files and metals used for binding preferences analysis of Zn^{2+} , Mn^{2+} and Ca^{2+} .

	Zn^{2+}	Mn^{2+}	Ca^{2+}
# initial PDB files	2529	789	2351
# PDB files with relevant metals	2093	369	1831
# used metals	3938	629	3845

Supp Table.2: Amino acid preferences of Zn²⁺, Mn²⁺ and Ca²⁺.

Residue	Zn ²⁺	Mn ²⁺	Ca ²⁺
Ala	0.2%	0.7%	2.2%
Arg	0.1% (0.0%)	0.2% (0.0%)	0.9% (0.0%)
Asn	0.3% (0.2%)	2.2% (1.9%)	8.1% (6.0%)
Asp	15.2% (15.1%)	38.7% (38.4%)	39.1% (35.8%)
Cys	35.8% (35.7%)	1.1% (1.1%)	0.3% (0.0%)
Gln	0.3% (0.2%)	1.4% (1.2%)	2.3% (1.3%)
Glu	16.6% (16.4%)	25.8% (25.2%)	23.6% (21.8%)
Gly	0.3%	0.7%	4.3%
His	29.4% (29.1%)	24.0% (23.7%)	1.2% (0.5%)
Ile	0.0%	0.4%	1.6%
Leu	0.1%	0.2%	1.7%
Lys	0.4% (0.4%)	0.4% (0.1%)	2.0% (0.1%)
Met	0.2% (0.0%)	1.0% (0.0%)	0.4% (0.0%)
Phe	0.0%	0.3%	1.0%
Pro	0.0%	0.0%	0.7%
Ser	0.5% (0.2%)	1.2% (0.9%)	3.5% (1.6%)
Thr	0.2% (0.1%)	0.7% (0.3%)	3.8% (1.3%)
Trp	0.1% (0.0%)	0.0% (0.0%)	0.2% (0.0%)
Tyr	0.2% (0.1%)	0.1% (0.0%)	1.3% (0.1%)
Val	0.1%	0.9%	1.7%

() refers to the percentage of the polar atoms of the side-chain that are bound to the metal (oxygen, sulfur and/or nitrogen)

Supp Table.3: Coordination number preferences of Zn²⁺, Mn²⁺ and Ca²⁺.

Coordination #	Zn ²⁺	Mn ²⁺	Ca ²⁺
1	0.0%	0.0%	0.0%
2	7.7%	7.6%	2.7%
3	13.2%	8.3%	5.4%
4	57.3%	12.4%	7.4%
5	13.9%	28.1%	11.4%
6	7.0%	38.8%	23.7%
7	0.5%	3.5%	38.8%
8	0.3%	0.6%	9.5%
9	0.1%	0.2%	0.6%
10	0.0%	0.3%	0.3%
11	0.0%	0.0%	0.1%
12	0.0%	0.0%	0.1%

Supp Table.4: A-site composition of all experimentally-determined manganese-sensitive CDF proteins.

Protein	Organism	N43*	D47*	H244*	D248*
sMTP8.1 [§]	<i>Oryza sativa</i>	H	D	H	D
AtMTP8 [§]	<i>Arabidopsis thaliana</i>	D	D	D	D
hvMTP8.1 [§]	<i>Hordeum vulgare</i>	D	D	D	D
hvMTP8.2 [§]	<i>Hordeum vulgare</i>	D	D	D	D
shMTP1 [§]	<i>Stylosanthes hamata</i>	D	D	D	D
csMTP8 [§]	<i>Cucumis sativus</i>	D	D	D	D
AtMTP11 [§]	<i>Arabidopsis thaliana</i>	D	D	D	D
bmMTP11 [§]	<i>Beta vulgaris</i> subsp. <i>maritima</i>	D	D	D	D
bmMTP10 [§]	<i>Beta vulgaris</i> subsp. <i>maritima</i>	D	D	D	D
csMTP9 [§]	<i>Cucumis sativus</i>	D	D	D	D
EmfA [§]	<i>Rhizobium etli</i> CFN 42	E	N	H	D
SMc02724 [§]	<i>Sinorhizobium meliloti</i> 1021	E	N	H	D
spMntE [§]	<i>Streptococcus pneumoniae</i> D39	N	D	D	D
Spy0980 [§]	<i>Streptococcus pyogenes</i> MGAS5005	N	D	D	D

Table data is based on MSA between all proteins and ZnT-10 sequences, by Clustal Omega(7, 8).

* Refers to the residue in the same position in ZnT-10

§ Bacterial protein

§ Plant protein

Relevant references that show that these proteins transport Mn²⁺: 21-32.

Supp Table.5: K_m and V_{max} values of WT and N43T with respect to Mn^{2+} . Data is extrapolated from **Fig.5 h** and **Fig.1 f**.

	WT	N43T
Vmax (a.u)	4.93±0.47	14.25±2.29
Km (μ M)	1.23±0.06	4.61±1.81