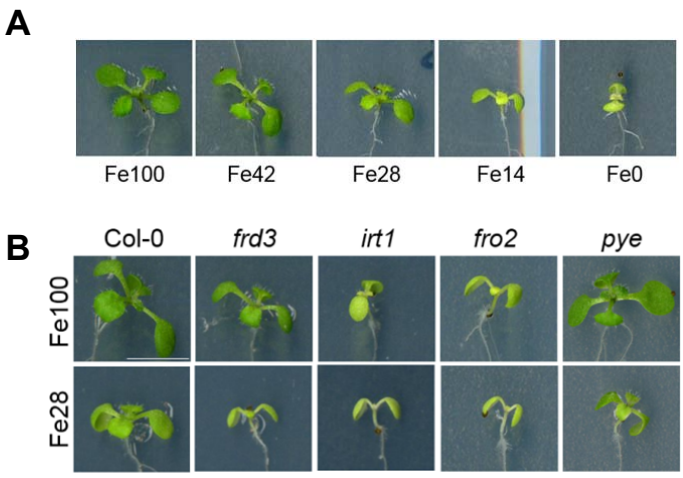
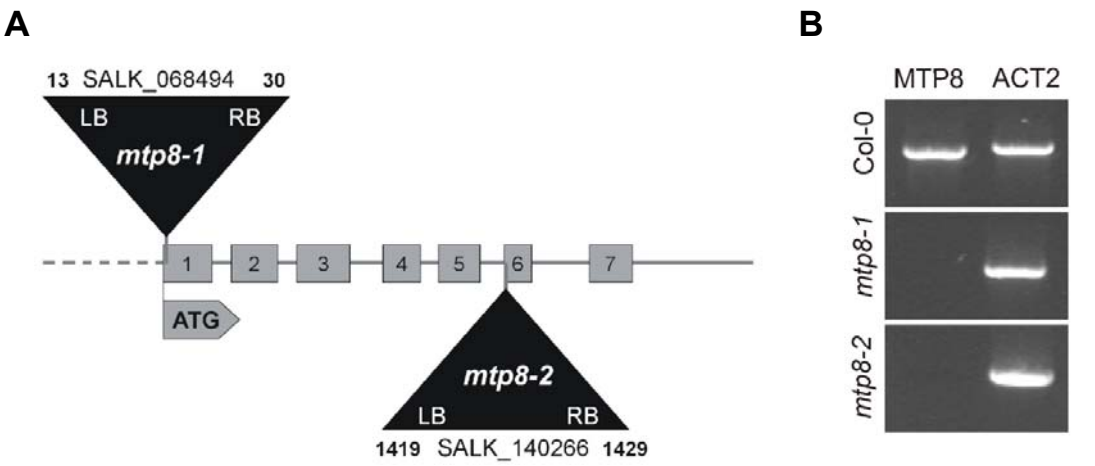
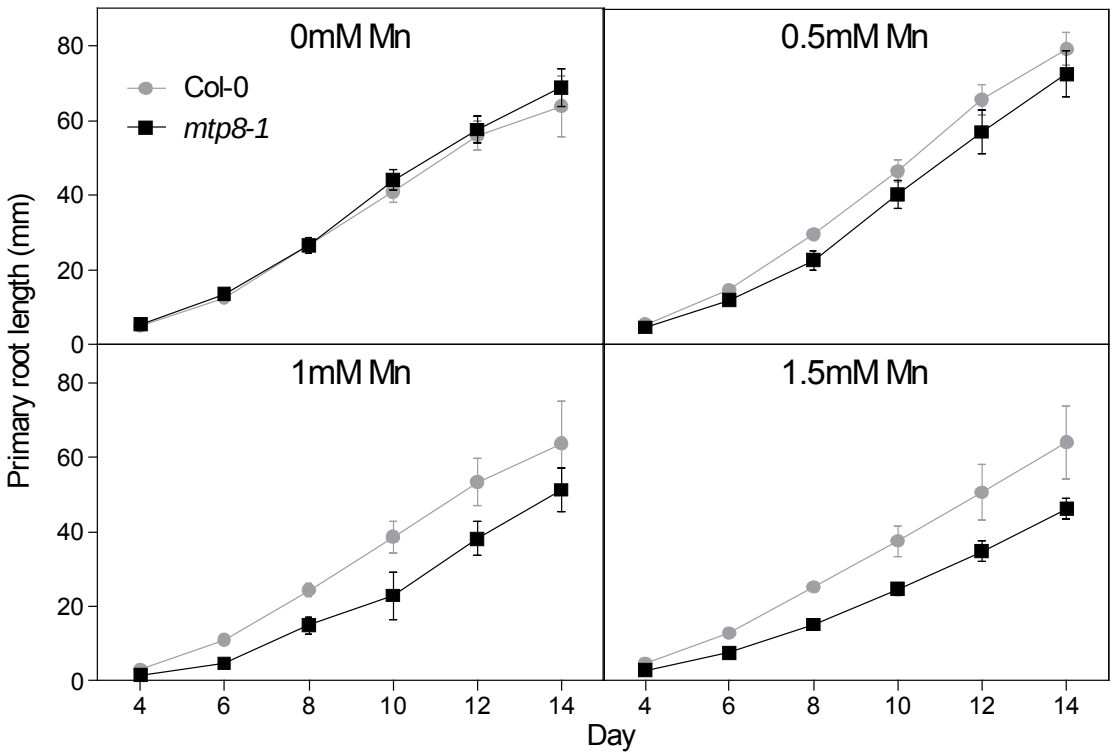


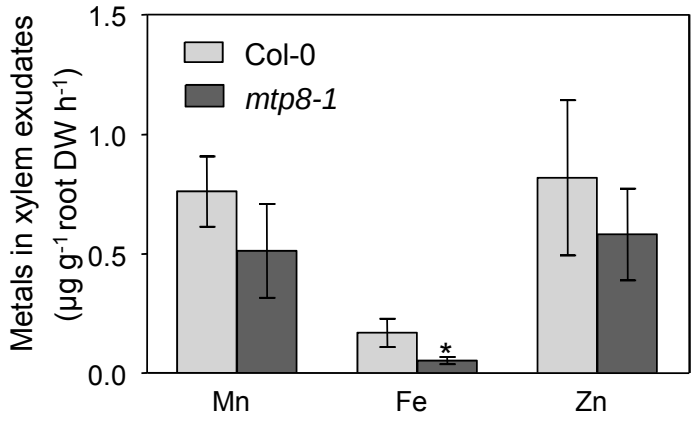
Supplemental Figure S1. Effect of Fe concentration on growth of mutants on high pH medium. A, Col-0 wild type seedlings were cultivated for 12 days on medium containing 100 µM, 42 µM, 28 µM, 14 µM, or no Fe(III)-EDTA. All media were buffered to pH 6.7 with 10 mM MES. B, Growth of Col-0 wild type and *frd3*, *irt1*, *fro2*, and *pye* mutants under Fe-sufficient (Fe100/Mn40) and Fe-deficient (Fe28/Mn40) conditions as employed in the screening. Seedlings were cultivated for 12 days.



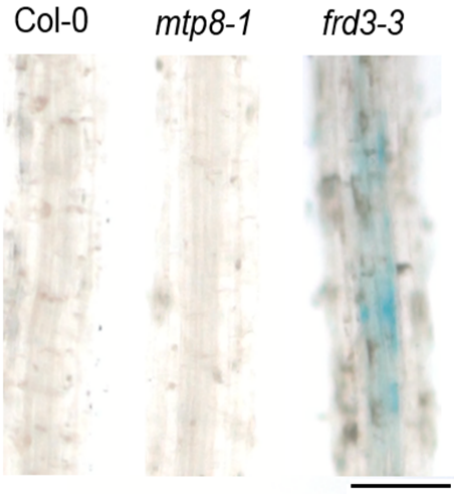
Supplemental Figure S2. Genotypical analysis of *MTP8* T-DNA mutant lines. A, Model of the *AtMTP8* genomic region and the T-DNA insertion sites in the mutant lines *mtp8-1* and *mtp8-2*. Coding regions are presented as grey boxes; introns are shown by a line. The triangle indicates the site of the T-DNA insertions. Numbers indicate the last remaining nucleotide before and the first nucleotide after the T-DNA, counting from the ATG. LB, left T-DNA border; RB, right T-DNA border. B, RT-PCR analysis on RNA isolated from seedlings of wild type and mutant lines showing the absence of the full-length *MTP8* transcript in the mutant lines. *ACT2* served as control.



Supplemental Figure S3. Elongation of *mtp8-1* primary roots is hypersensitive to high Mn concentrations. Primary root lengths of Col-0 (circles) and *mtp8-1* (squares) seedlings on ½ MS medium supplemented with increasing amounts of Mn. Data represent the means ±SD of 16 seedlings per line.



Supplemental Figure S4. Fe translocation in xylem stream is decreased in the *mtp8-1* mutant. Six-week-old Col-0 (light grey) and *mtp8-1* (dark grey) plants growing on liquid Fe28/Mn40 medium were decapitated. Xylem exudate was collected for 2 h and analysed by ICP-MS. Data are from three plants per line. An asterisk indicates that the mean of the mutant line is significantly different from the mean of Col-0 according to Student's t-test ($P<0.05$). Error bars represent ±SD.



Supplemental Figure S5. Roots of the *mtp8-1* mutant do not accumulate iron. Col-0 and *mtp8-1* seedlings were cultivated for 12 days on Fe28/Mn40 agar plates. Roots of the seedlings were excised, incubated in Perls' solution for one hour, washed in distilled water, and observed under the microscope. The *frd3-3* mutant line cultivated on Fe100/Mn40 was used as a positive control for the staining. Bar: 100 µm.

Supplemental Figure S6. Rhizosphere acidification under Fe deficiency is enhanced in *mtp8-1* in the presence of Mn. Plants were precultured on the indicated Fe/Mn regimes for 72 h, transferred to agar plates containing bromocresol purple, and incubated for 24 h. Yellow color around the roots indicates acidification.

Supplemental Table S1. T-DNA mutants screened for sensitivity to low Fe availability.

	Line Code	Gene Code			Line Code	Gene Code			Line Code	Gene Code
1	SALK_144164C	At1g09790		40	SALK_080688C	At4g38950		80	SALK_103699C	At1g11840
2	SALK_147451C	At3g46900		41	SALK_011913C	At4g29220		81	SALK_058904C	At3g47650
3	SALK_109396C	At5g47910		42	SALK_107939C	At3g51200		82	SALK_040185C	At1g23020
4	SALK_132418C	At3g13610		43	SALK_042794C	At3g61930		83	SALK_017016C SALK_135507C	At1g56430
5	SALK_121565C	At5g13740		44	SALK_048526C	At1g05700		84	SALK_096228C	At3g47420
6	SALK_082635C	At2g30660		45	SALK_085412C	At4g21910		85	SALK_096539C	At3g47630
7	SALK_118446C	At3g48450		46	SALK_136932C	At1g60610		86	SALK_043140C	At5g45410
8	SALK_080218C	AT1G19000		47	SALK_008958C	At4g02330		87	SALK_151290C	At1g03080
9	SALK_088311C	At2g47000		48	SALK_015054C	At1g74770		88	SALK_051706C SALK_053278C	At5g03140
10	SALK_057638C	At3g53280		49	SALK_146998C	At5g53450		89	SALK_204565C	At3g55430
11	SALK_043881C	At4g14680		50	SALK_018370C	At5g55620		90	SALK_149052C SAIL_74_B07	At1g74780
12	SALK_009007C	At1g05300		51	SALK_035416	At1g57560		91	SALK_110010C SALK_113040C	At3g57070
13	SALK_036012C	At3g18290		52	SALK_048470C	At1g18910		92	SALK_131929C	At5g03210
14	SALK_003255C	At4g26890		53	SALK_040332C	At5g38820		93	SALK_030632 C SAIL_1255_G10	At1g74790
15	SALK_074896C	At5g37260		54	SALK_094426	At1g09560		94	SALK_029828C SALK_200963C	At1g23030
16	SALK_140266C	At3g58060		55	SALK_078702	At3g50740		95	SALK_006284C	At3g48140
17	SALK_133841C	At2g35930		56	SALK_128043C	At2g45400		96	SALK_204374C SALK_003991C	At3g55440
18	SALK_133954C	At1g49820		57	SALK_080627C	At1g68650		97	SALK_100762C	At2g30090
19	SALK_057798C	At4g25640		58	SALK_023292C	At5g67370		98	SALK_093190C	At5g14960
20	SALK_147453C	At4g25640		59	SALK_081645C	At4g10510		99	SALK_071809 C SALK_090862C	At3g47660
21	SALK_025900	At1g11670		61	SALK_132418C	At3g13610		100	SALK_063364C SALK_107945C	At3g47430
22	SALK_019139C	At2g36885		62	SALK_019569C	At1g17260		101	SALK_005625C	At2g14850
23	SALK_023076C	At1g33090		63	SALK_095937C	At3g06890		102	SALK_141935C SALK_054389C	At5g03230
24	SALK_066923C	At2g20030		64	SALK_016715C	At4g31950		103	SALK_052443C SALK_141821C	At5g14950
25	SALK_035704C	At3g53480		65	SALK_130376C	At3g21690		104	SAIL_703_C10 SAIL_1214_C01	At2g30100
26	SALK_071767C	At5g05250		66	SALK_140776C	At2g19410		105	SALK_052766C SALK_073451C	At1g72460
27	SALK_107837C	At1g77280		67	SALK_076701C	At1g75200		106	SALK_136462 SAIL_1156_C06	At1g72450
28	SALK_045057C	At3g58810		68	SALK_118350C	At2g18960		107	SAIL_267_C12	At1g68580
29	SALK_110829C	At1g01570		69	SALK_120680C	AT4G22880		108	SALK_045661C SALK_111412C	At2g46060
30	SALK_063184	AT1G49960		70	SALK_105742C	AT3G60330		109	SAIL_813_E05	At3g13710
31	SALK_034026	At5g36890		71	SALK_131820 C	At3g48450		110	SAIL_1254_A07	At2g24550
32	SALK_140776C	At2g19410		72	SALK_054106C	At4g14680		111	SALK_025606C	At1g72440
33	SALK_073511C	At4g30120		73	SALK_121588 C	AT4G22880		112	SALK_130654C	At2g15440
34	SALK_020101C	At5g04730		74	GABI_797D03	At3g59030		113	SALK_205573C SALK_205342C	At4g23010
35	SALK_138196	At3g11750		75	SALK_106967C	AT1G49960		114	SALK_205869C	At4g00590
36	SALK_012767	At3g07720		76	SALK_127844C	AT2G24520		115	SAIL_768_F02	At3g02140
37	SALK_099407C	At4g10510		77	SALK_048598C	At1g17260		116	SALK_137966C	At1g03090
38	SALK_072019C	At3g61410		78	SALK_052070C	At5g53450		117	SALK_073394C SALK_200761C	At3g15210
39	SALK_123168C	At1g34760		79	SALK_063470C	At2g35930		118	SALK_120395C	At2g24545
								119	SALK_094145 C ,SA	At5g45307

Supplemental Table S2. Primers used in this study.

Name	Purpose	Sequence
MTP8_494_F	T-DNA screen *mtp08-1* forward	ACGCGCTGATGTGAGCATT
MTP8_494_R	T-DNA screen *mtp08-1* reverse	CCCAATTTGAGATTTGCATGG
MTP8_226_F	T-DNA screen *mtp08-2* forward	CGCAACCGCTATTAAACTCGT
MTP8_226_R	T-DNA screen *mtp08-2* reverse	GATTCACCAATCGCATGAGC
SALK_LBa1	T-DNA screen	TGGTTCACGTAGTGGGCCATCG
SALK_RBb	T-DNA screen	CAGTCATAGCCGAATAGCCTCTCC
MTP8_F-NotI	cloning in pFL61 forward	AAAAAAGCGGCCGCATGGAAGTCAATTATTGTCCGGA
MTP8_R-NotI	cloning in pFL61 reverse	AAAAAAGCGGCCGCTCATAAATCGTTGGGGATTGTAGA
MTP8_F-XmaI	cloning in pART7-EYFP forward	AAAAAACCCGGGATGGAAGTCAATTATTGTCCGGA
MTP8_R-XmaI (nostop)	cloning in pART7-EYFP (gene N-terminal of EYFP) reverse	AAAAAACCCGGGTGCTAAATCGTTGGGGATTGTAGA
MTP8_R-XmaI	cloning in pART7-EYFP (gene C-terminal of EYFP) reverse	AAAAAACCCGGGTCATAAATCGTTGGGGATTGTAGA
MTP8_gDNA_F-XmaI	cloning in pBI101 forward	AAAAAACCCGGGGCATTCCTCCTGTAAACGGAAGC
MTP8_gDNA_R-XmaI	cloning in pBI101 reverse	AAAAAACCCGGGTTCTGGAAAATTATTAAACAAATCATCG
MTP8_rt_F	qRT-PCR forward	TTGTCGAGGTGGATATAGAACTGCC
MTP8_rt_R	qRT-PCR reverse	GGAATGTTCAGGCTTGTGATGACA
At4g05320_rt_F	qRT-PCR forward	CACACTCCACTTGGTCTTGCGT
At4g05320_rt_R	qRT-PCR reverse	TGGTCTTTCCGGTGAGAGTCTTCA
At3g18780_rt_F	qRT-PCR forward	TCCCTCAGCACATTCCAGCAGAT
At3g18780_rt_R	qRT-PCR reverse	AACGATTCCTGGACCTGCCTCATC
At5g60390_rt_F	qRT-PCR forward	TGAGCACGCTCTTCTTGCTTTCA
At5g60390_rt_R	qRT-PCR reverse	GGTGGTGGCATCCATCTTGTTACA
bhlh38_F	qRT-PCR forward	AATCAATACGAAAGCTATTACGGT
bhlh38_R	qRT-PCR reverse	TAAGCTCTTTGAAACCGTTTC
bhlh101_ F	qRT-PCR forward	CTTTCTGATCAAAAGAGGAAGCTGAG
bhlh101 _R	qRT-PCR reverse	GAAACAGATGTCCATTTCGACGT
PYE_F	qRT-PCR forward	CAGGACTTCCCATTTTCCAA
PYE_R	qRT-PCR reverse	CTTGTGTCTGGGGATCAGGT
BTS_F	qRT-PCR forward	GCTCTGGCACAAGTCAATCA

BTS_R	qRT-PCR reverse	CGTTCATCAAATGCCGATAA
at3g12900_F	qRT-PCR forward	GCGGAGCATAGGGTTCGAA
at3g12900_R	qRT-PCR reverse	GGGATTTGGTGCCGTGAA
MYB72_F4	qRT-PCR forward	TCATGATCTGCTTTTGTGCTTTG
MYB72_R4	qRT-PCR reverse	ACGAGATCAAAAACGTGTGGAAC
IRT1_F	qRT-PCR forward	CGGTTGGACTTCTAAATGC
IRT1_R	qRT-PCR reverse	CGATAATCGACATTCCACCG
FIT_F	qRT-PCR forward	GGAGAAGGTGTTGCTCCATCTC
FIT_R	qRT-PCR reverse	GTCTCGAATTTGAACGGATTGG
AtNAS4_F	qRT-PCR forward	ATCGGTTTATCACCCTACCG
AtNAS4_R	qRT-PCR reverse	TCACGTGGATCTTGGAACAG
UBQ2F	qRT-PCR forward	CCAAGATCCAGGACAAAGAAGGA
UBQ2R	qRT-PCR reverse	TGGAGACGAGCATAACACTTG

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