Supplementary figures

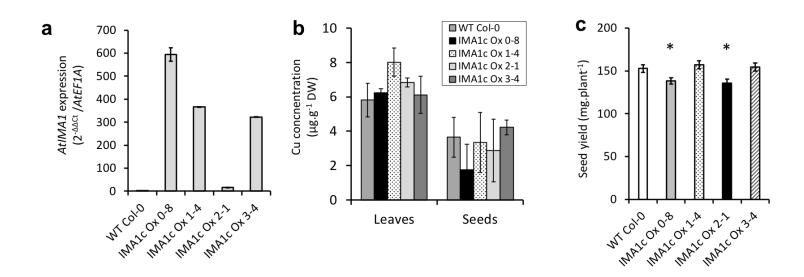


Figure S1. Characterization of IMA1 Ox (*35Spro::AtIMA1_{cDNA}*) lines. (a) Expression level of *AtIMA1*. (b) Cu quantification. (c) seed yield.

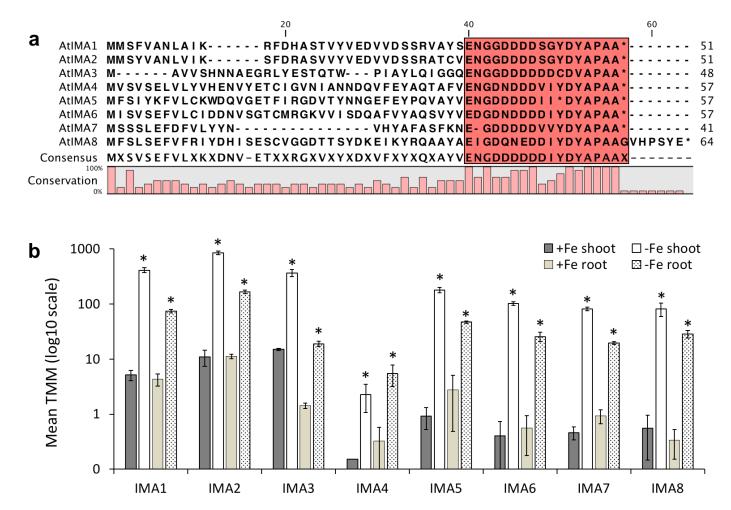


Figure S2: Identification of Arabidopsis IMA genes and their Fe-deficiency regulation. (a) Alignment of putative IMA proteins and (b) Expression level of the 8 Arabidopsis IMA genes in Fe-sufficient and Fe-deficient leaves and roots. TMM values were calculated using reanalyzed data from (11) and (35), stars indicate statistical differences to plants grown on control media (Z-Test).

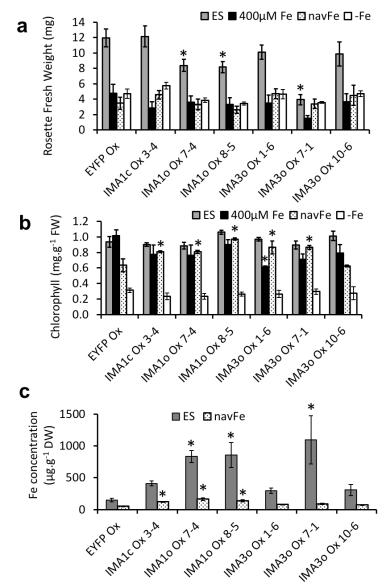
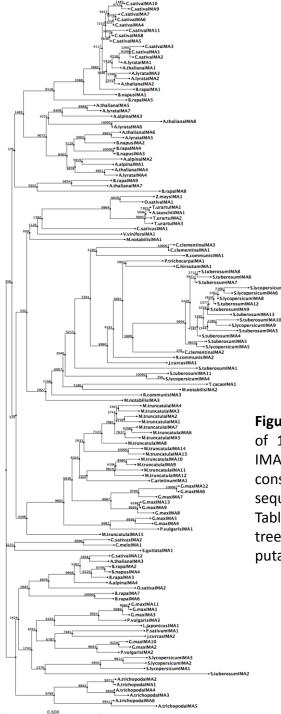


Figure S3. Characterization of IMA1 Ox and IMA3 Ox lines. (a) Biomass production (n = 3 sets of 25 rosettes) and (b) chlorophyll concentration of 13-day-old IMA1 Ox and IMA3 Ox lines grown under various Fe regimes (n = 6 sets of 5 rosettes). (c) Leaf Fe concentration of plants grown on control or navFe media (n = 6 sets of 15 rosettes). ES, Estelle and Somerville media containing 40 μ M FeEDTA; navFe, ES media containing 10 μ M FeCl₃, pH 7; -Fe, ES media without added Fe supplemented with 100 μ M FerroZine (FRZ); IMA1c Ox: 35Spro::AtIMA1_{cDNA}; IMA1o Ox: 35Spro::AtIMA1_{ORF}; IMA3o Ox: 35Spro::AtIMA3_{ORF}. Results show means ± SE. Stars indicate significant difference to control plants grown under the same conditions (Duncan test, $P \le 0.05$).

		20	40	60		80 I	100
A.tauschiilMA1 A.trichopodalMA1	MAPAS-KVMSHIV-QDGGI MLQRYDAPFVGQKWHQKRI	-ADYAVYAAA	APCDAWCG		GRHRKA	SDGDDDDD	-DYDCAPAA 56 -DYDCAPAA 36
A.trichopodalMA2 A.trichopodalMA3	MLQRYDAPFVGQKWHQKR MLDRHDAHLGCQKWHQKK	LR			GI	GDDDDDD	-DYDFAPAA 36 -DYDCAPAT 39
A.trichopodalMA4 A.trichopodalMA5	MLDRHDAHLCCLKWHQKKI MASEENPP	LR			TI	GDDDDDDD	-DYDCAPAA 39 -DYDCAPAT 26
A.trichopodalMA6 A.lyratalMA1	MASEEN MMSEVANLAIKSLDRASAV	YVEDVVDSSR			VAYG	NGGDDDDD	-DYDCAPAA 23 -GYDYAPAA 50
A.lyratalMA2 A.lyratalMA3	MMSFVANLVIKSFYRASAN MMYFFANLVSKSFDRASAN	YVEDWVDSSR YVEDVVDCSR			ATCLI	NGGDDDDS	-GYDYAPAA 50 -GYDYAPAA 50
A.lyratalMA4 A.lyratalMA5	MISVSEIVLYI-HENVYET	SIGVNIANNDK	/FEY		AQSVYVI	N-GDNDDDD	- IYDYAPAA 56 - IYDYAPAA 56 - IYDYAPAA 56
A.lyratalMA7 A.thalianalMA1	MVSIYKFVLCKCDQVRETC	IRGDVTYNNGE-	FEY		HQVAFII		- I YDYAPAA 56 - GYDYAPAA 50
A.thalianalMA2 A.thalianalMA3	MMSYVANLVIKSFDRASV	YVEDVVDSSR			ATCV		-GYDYAPAA 50 -DCDVAPAA 47
A.thalianalMA4 A.thalianalMA5	MISVSEFVLCI-DDNVSGT MFSIYKFVLCKWDQVGETF	CMRGKVVISDQA	FVY		AQSVYVI	D-GDNDDDD	- I YDYAPAA 56 - I XDYAPAA 56
A.thalianalMA6 A.thalianalMA7	MVSVSELVLYV-HENVYET MSSSLEFDFVLYYNVHYAF	ASFKN	/FEY		AQTAFV	N-GDNDDDV	- I YDYAPAA 56 - VYDYAPAA 40
A.thalianalMA8 A.alpinalMA1	MFSLSEFVFRIYDHISESC MISVTEFVLCI-HENVYDH	VGGDTTSYDKE CNGDGIVNNNGA	KY		RQAAYAI	I - GDQNEDD	- IYDYAPAAGVHPSYE 63 - IYDYAPAA 53
A.alpinalMA2 A.alpinalMA3	MVFVFHYVLCKYDEVCETF	IEGNAIKNCAE-	LEY		SQAGYVI	N-GDDDDDN	- I YDYAPAA 53 - VYDYAPAA 56 - DCDVAPAA 47
B.napusIMA1	MAYMSHNKAESKLIES M-SFAANLVIINFYCASAY	CVEELLDNSL			GSYTI		-GYDYAPAA 49 -IYDYAPAA 57
B.napusIMA3 B.napusIMA4	MFSVSEFLFCT-YDNVY MAVMSYNKAEGRLYES	GGDITNNDEA	VIQY		AQAVES	N-GDDDDDV	- I YDYAPAA 52 - DSDVAPAA 47
B.rapalMA1 B.rapalMA2	M-YLVAHLVIKSFDGDYAV MAVMSHNKAEGRLYES	SAEDVVDTSR			IQTLGQ	NGGDDDDG	-GYDYAPAA 49 -DSDVAPAA 47
B.rapalMA3 B.rapalMA4	MAVMSHDKAEDRLYES MFSVSEFLFCT-YDNVY	AHTRPIPYN	VQY		SQIVGQI	SGGDDDDD	-DSDVAPAA 48 -IYDYAPAA 52
B.rapalMA5 B.rapalMA6	MSFAANLVIINFYCASAVC MAVMSHNKAEGRLYESTQT	RLVPY			IQTLGQ	SGGDDDDD	-GYDYAPAA 49 SDVAPAA 47
B.rapalMA7 B.rapalMA8	MAVMSHDKAEDRLYESAHT MCYIKPSFIFFKASFQLSF	VSKRMSAIEFDL	VLHCNGHTQFA				SDVAPAA 48 -VYDYAPAA 63 -VYDYAPAA 41
C.sativalMA1 C.sativalMA2	MMTFVANLLSKSLDRASS	YVEDVVDSSR			VAYG		-GYDYAPAA 41 -GYDYAPAA 50 -GYDYAPAA 50
C.sativalMA3 C.sativalMA4	MMTLLSKSLDRASSV M-SFVANLVIKSFDRASTV	VVEDVVDSSR CVEDVVDSFR			VAYG	NGGDDDDS	-GYDYAPAA 46 -GYDYAPAA 49
C.sativalMA5 C.sativalMA6	MMSSVANLVIKSFDYASTV M-SFVANLVIKSFDRASTV	CVEDVVDSSR CVEDVVDSFR			VAYV	NGGDDDDS	-GYDYAPAA 50 -GYDYAPAA 49
C.sativalMA7 C.sativalMA8	M-SFVANLVIKSFDRASTV MMSSVANLVIKSFDVASTV	CVEDVVDSFR			VAYVI	NGGDDDDS	-GYDYAPVA 49 -GYDYAPAA 50
C.sativalMA9 C.sativalMA10	M-SSVADLVIKSFNHASTV M-SSVADLVIKSFNHASTA	CDEDVVDTFR	VFDAWCG VFV FEY FEY FEY FEY FEY VEY VEY VEY VEY VEY VEY VEY V		AAYV		-GYDYAPAA 49 -GYDYAPAA 49 -GYDYAPAA 50
C.sativalMA11 C.sativalMA12	MAVL IVSRNNGEGRLYES	TRTQPIPY		EVS 80	LQNGGQ		-GYDYAPAA 50 -DCDVAPAA 50 -GYDYAPAA 67
C.clementinalMA1 C.clementinalMA2	MAPMSSSLEGITHGNVHH MSLVSKSVMPSSSWTWCK	DDDS I HVYGCPY	YYRNEPF-EGD	DDDDDDDDGG	DLAPAASM	GDGDDDDDG-	-GYDYAPAA 79 -SYDYAPAA 74
C.clementinalMA3 C.meloIMA1	MSSSLEGITHGNVHH MDVSIFEPVASTMIKNII	DDDS I HVYGCPY YKDVKCGRQF ST	YYRNEPF-EGD	GDDDDDDDGG	LTTTIIRR	GDGDDDDDG	-GYDYAPAA 76 -CYDYAPAA 57
C.sativusIMA1 C.sativusIMA2	MAPISRLPCVLGLKNLGGE M-VSTSKSVASMMIKNNVC	GGHGYREGCDC- EDVKCSRSFPI	D		LTKTIIRR	GDSDDDDG	-GYDFAPAA 56 -CYDYAPAA 55
E.guttatalMA1 G.maxiMA1	MVVFICKEEYGVPLSNDWA	ATHEFGHKFCIS	STA-CV- ELEDYNSGGD VYNNETF-ECD- VYNNETF-ECD- D- NN AATTFIGCNVCNWRGDFVS AATTCIECNVCNWRGDFVS TA-CT-ECDDVRGNDS TA-CT-ECDDVRGNDS TA-C-C-ECDDVRGNDS	FGI	LITQLHDR	GDDDDDDDG-	-GTFVAPAA 58 -VIDVAPAA 50 -GIDIAPAA 51
G.maxIMA3	MVVFLCKEEYGVLLGNDWA	ATHEFGHNFCIS		EVP		GDDDDDDDDG	-VIDVAPAA 50 -GYDYAPAA 66
G.maxIMA5 G.maxIMA6	MSFTSKVIALWCKKHGNDE MALTSKAINO-ECKKHACC		PAATACIEGNVCNWHGDFVS	FVP	VALVI	GDDDDDDG	-GYDYAPAA 70 - IYDYAPAA 68
G.maxIMA7 G.maxIMA8	MAFISMAINLIDCMTHACC	NKDNDWYLY-AF	TACTEGDDPNGDVDS	WF	AYM	GDDD-D	-GYDYAPAA 64 -GYDYAPAA 58
G.maxiMA9 G.maxiMA10	MASMSKAMTP-EIKKHACE MVVFVSCTKSGLPLFSKGM	KKDGVSY-HYDF	PTACAEGDDYNGNINY	VF	LS HQ	GDDDDDG	-GYDYAPAA 65 -GIDIAPAA 50
G.maxIMA11 G.maxIMA12	MVVFICKEEYGVPLSNGWA MALTSKAINQ-ECKKHACC	ATHEFGHKFCIS	TACTEGDDHKGNGDS	CFGH	IAYM	GDDDDDDNG	-VIDVAPAA 50 -IYDYAPAA 68
G.maxIMA13 G.hirsutumIMA1	MASMSKAMTP-ETKKHACL MSP-FSKVVASSCKKHV MSSVLLKALASSWCNNONL	DGDYDDNDGF			DYAPAASLE	GDDDDDG	-GYDYAPAA65 -SYDYAPAA69 -DGDCAPAA72
J.curcasIMA2	MVI-VDSKKLGFF	RLVAGEGQAMA-	NCTEGDDHKGNGDS PTACAEGDDYNGNINY DYAPIACM		CFCMSKQN	GDDDDDDDG -	-GADVAPAA 50
M.truncatulalMA1 M.truncatulalMA2	MVFISMVIA-LNCKQHAY- MASISMVIA-LNCKQHAY-	GEGEVDWFGCT- GEGNWFDYT-	SVS-CIE-E-DYHNTDHDS SVS-CIE-E-DYHNGDRDS		YWI	GDDDDDG	-GYDYAPAA 62 -GYDYAPAA 60
M.truncatulalMA3 M.truncatulalMA4	MASIFTVIA-PLCKQNAC- MTFISTVIA-PKCKQYAYM	GEGNGDWFGYT-	SVS-CIV-E-DYRNGDQDS CVS-CIE-E-DYTNGDRNL		YKI	GDDDDDG	-GYDYAPAA 62 -GYDYAPAA 63
M.truncatulalMA5 M.truncatulalMA6	MASISLAII-PKCEQHGY- MASISLAIT-PKCKHHGY-	GEGNGDWISYT- SEGNGDWFGYT-	CVS-CIE-E-NYHNGDRDS SVS-CIK-E-DNRNGDRDS		CKI	GDDDDDG	-GYDYAPAA 62 -GYDYAPTA 62
M.truncatulaIMA/ M.truncatulaIMA8	MASISMAII-PKCKEHGY- MASISMVIA-PKCKQHAFN	GEGDCDWFGYT-	NIS-CIE-E-DYRNGDRDS		YM	GDDDDDG	-GYDYAPAA62 -GYDYAPAA58 -GYDYAPAA59
M.truncatulaiMA10 M.truncatulaiMA11	MASISIAIA-TRSIKHVY- MASISIPTRSIKNAC-	DEDEWFGYAA	SVS - CIE - E - DYHNTDHDS SVS - CIE - E - DYHNTDHDS SVS - CIV - E - DYHNTDDRS CVS - CIE - E - DYHNTDDRS SVS - CIV - E - DYHNTDRS SVS - CIE - E - DYHTDDVS SVS - CIE - E - DYHTDVS SVS - CI	s	YK	GDDDDDG	-GYDYAPAA 61 -DYDYAPAA 58
M.truncatulalMA12 M.truncatulalMA13	MAFISISIA-TRSFQNAC- MTSISMVNISPKC-NHAAY	DEGEWFCYA- GECDGDWFGYAS	SVG-CIEQDNHIGEEDS	AHL	YRI	GDDDDDG	-GYDYAPAA 60 -GYDYAPAA 70
M.truncatulalMA14 M.truncatulalMA15	MASISIVNTAPKC-NHAAY MSSISNVVAPWCKKHGNDF	GECDVDSFGYAS	STVCIKGNYYIRNEDSGS	ADL	DYPPTVCD	GDDDDDG	-GYDYAPAA 70 -GYDYAPAA 50
M.notabilisIMA1 M.notabilisIMA2	MPH I T FMNMVTARNGKDGE MFPAVDKLIYSESLSKKRE	NGDHCYDHYFQY DGGNHEDGNDG I	SRRYAPTQVMENI	YGASDRDY	NLSAPGI	GDGDDDDDDDDS	-GYDYAPAA 60 -SYDYAPAA 79
M.notabilisIMA3 O.sativalMA1	MAPVS-EA-SPLVHQDGGI MALAKSECEBLAWAL	IASFAVYAGA	STI – CIKGNYYFRNEDSGS STV – CIKGNYYIRNEDSGS Y SFRYAPTQVMENI SFRYAPTQVMENI SFEYNL PC – – CS		ARGRMAN		-GYDYAPAA62 -DYDCAPAA54 -DVDVAPAA43
O.Sativalivitez	MATAKJECEREAWALLEES	ALL			vunnn	R-CDDDDDD	-DVDVAFAA 43
P.vulgarisIMA2 P.vulgarisIMA3	MLLFISCTKSGLPLFIKGM MMFFVCKE-YGLVLSNDWF	ATHDF-HNF	HAPPTFIEESVFNGHGDSVS H- OYAPAACM- GYNEFSMEAPLIEDD- (-FAPPPCI- YYEY-		FPLHH	GDDDDDDDG-	-GIDVAPAA 49 -VIDVAPAA 44
P.trichocarpalMA1 R.communisIMA1	MSPLSKTIIAFCTIHR MVHMAFLTARSSGKCSST	HADGDDDGEYGY	GYNEFSMEAPLIEDD	EGDG-DDDDSD1	DYAPAAPM	GDDDDDG	-DYDYAPAA 71 -DYDYAPAA 84
R.communisIMA2 R.communisIMA3	MSLFAISKAITISCON MSPLSKVIASWCNKPVVE	EMGNDDDRV	/YEY	EGDG-DDDDGDY	APATST	GDDDDDDDG	YDCAPAA 72 -DKDCAPAA 55
5.lycopersicumIMA1 5.lycopersicumIMA2 5.lycopersicumIMA2	MVIVRGNTPFLPG-RIE- MVIVRGNTSPEHPV-FIE	ARPVIN	SHVYESRSM		-FGLNREF	ADDDDDDDDD	-GKKVAPAA 45 -GKKVAPAA 50 -GAKVAPAA 51
S.lycopersicumIMA4 S.lycopersicumIMA5	MSQISTILMNSICNF	CADGD	HVYESRSM	DYAPAASLEGNDDDCD	DYAPAAS	DDGDDDDDDDD	ADYDYAPAA 66 -DYDYAPAA 78
S.lycopersicumIMA6 S.lycopersicumIMA7	MFG-IFKIIGFEKIRRS MSG-ILKIIGFEKIRRS	CLDGDDDGDY	YDYTSAASLE GDDDDGDY YDYAPAACLKRNGDD - DGDY YDYAPAACLERDGDD - DGDY	DYAPAAF LEGDDDDRDY DYAPAAS LEGDDDDRDY	DCAPAATIE	GDDDG	-DYDYAPAA 83 -DYDYAPSGCMK 86
5.lycopersicumiMA8	MSG-TERTIGEERIKRS	CLDGDDDGD1	DFAPAACLERDGDD-DGDY	DTAPAASLEGDDDDRD1	DTVPAASLI		-DTDTAPAA 83
S.tuberosumIMA1 S.tuberosumIMA2	MAGKSGRKVVRGVSKSSKA MVVIMNANNKKVSLGCPDH	FMATEAKLGST	I PGASACLEGGGDDDDDY I FGKSKKYKSCSY I CI DKEC I CI DKEC I CI DKEC	GSEDDDDDEH	DYAPATYL	ADDDDDDG	-NYDYAPAALT 81 -ASKIAPAA 55
S.tuberosumIMA3 S.tuberosumIMA4	MSG-TFKIIGFQK-RRS MST-IFKIIGFQK-RRS MST-IFKIIGFQK-RRS	CSDGDDDSDDG	DYAPAACLEGDGDDNDAYY	DYAPAASLEGDDDDGDY	DYAPAASLE		-DYDYAPAA83 -DYDYAPAA86 -NYDYAPAA78
							-NYDYAPAA 78 -DYDYAPAA 82 -DYDYAPAA 82
S tuberosumIMA8	MSS-IEKIICEON-RRS	YSDCDDDCD>	TOYAPTAYLEGDDDDGDY DYAPAACLERDGDD-DGDY DYAPAACLEGGGDRDDGDY	DYAPVASINGDDDDCD	DYAPATSI	CDDDDC	DYDYAPAA 82
S tuberosumIMA11	MSO I STILMNS I CNI TEED	YHVERGNHDIGS	HVYESTSM	MDRHVIGO	LYVSATWEE	DDGDDDDDDDDDDDD	ADYDYAPAA 78
S.tuberosumIMA12 S.tuberosumIMA13	MSE-IFTIIGFEKIRRS MSSGIFKILGFEK-RRI	CLDGDDDGDY	VYAPASCLERDGDD-DGDY	DYAPAASLEGDDDDRDY	DYVPAASLE	GDDDG	-DYDYAPAA 83
T.cacaolMA1	MSSSKCIMHDEDNIKKIGS MAPAS-KVMSHVV-ODCCI	SSKNIMNDDVHD	DKGRRDGYVSNSKSLVQ	GGNSY	THVPSASVE	GDGDDDDDD	-DYDFAPAA 79
T.urartulMA2 T.urartulMA3 V.viniferalMA1	MAPAS-KIMSHIVVQDGGI MAPAS-KAMSHIV-QDGGI MSSISMAIDCOCMMUDCH	-ATYAVYAA	PCDAWCG		GRHRKA		-DYDCAPAA 57 -DYDCAPAA 55 -GYDFAPAA 65
Z.maysIMA1	MAPVSSEAASYLVLIKGGS	IAASSRAVY	PWDG-CS		ARGRMT	TDSDDDDD	-DYDCAPAA 57
Conservation					ATV		
25							

b



mIMA:

Figure S4. (a) Alignment and consensus plot of 131 amino acid sequences of putative IMAs found by BLAST search of the conserved motif in various EST and genome sequence databases listed in Supplementary Table 2. (b) Phylogenetic neighbour-joining tree inferred from the alignment of the putative IMAs (10000 bootstrap).

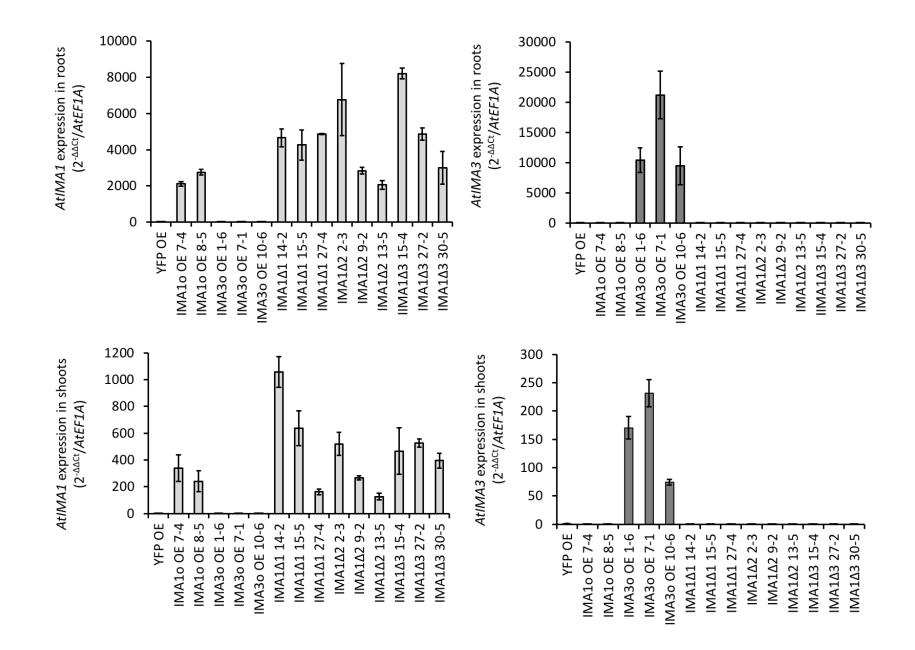


Figure S5. Expression level of *AtIMA1* and *AtIMA3* in roots and leaves of transgenic plants expressing the ORFs of either *AtIMA1*, *AtIMA3*, and mutant versions of *AtIMA1* harboring deletions under the control of the *CaMV 35S* promoter.



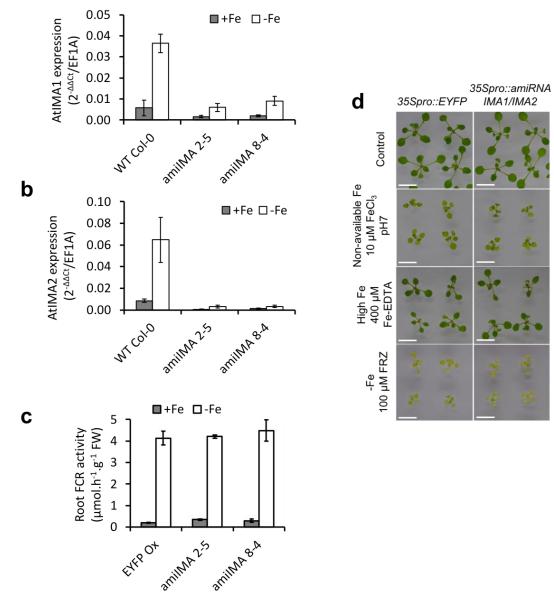


Figure S6: Silencing of AtIMA1 and AtIMA2 using expressing an artificial microRNA targeting both genes. (a) Expression of AtIMA1 and AtIMA2 in Fesufficient and Fe-deficient seedlings, (b) root FCR activity and (c) phenotype of the seedlings under various Fe supply.

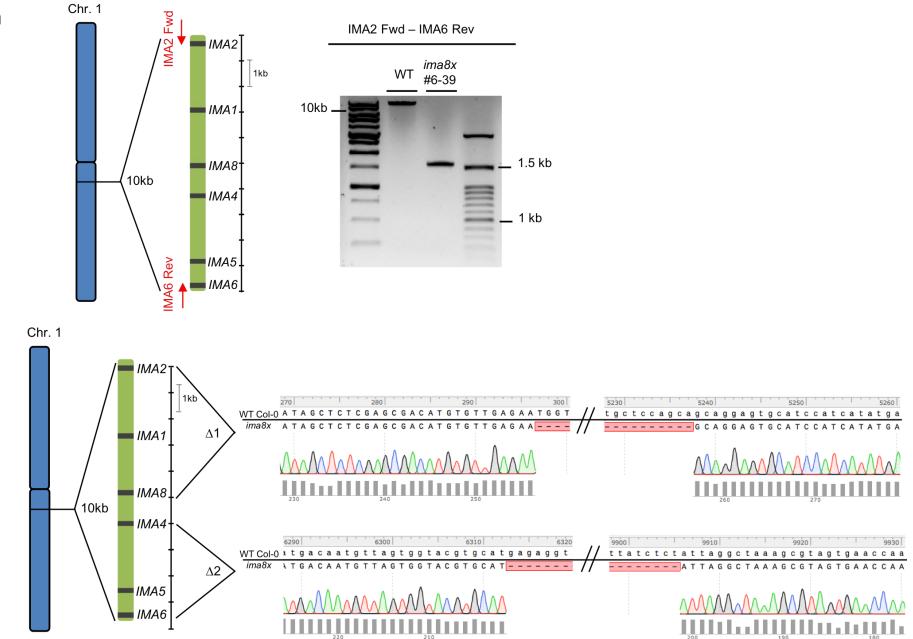


Figure S7. Genotyping of the mutations on chromosome 1 in the *ima8x* mutant line #6-39. (a) PCR of the 10kb region harboring six *IMA* genes. (b) Sequencing of the deletions and alignment with genome sequence of Col-0.

b

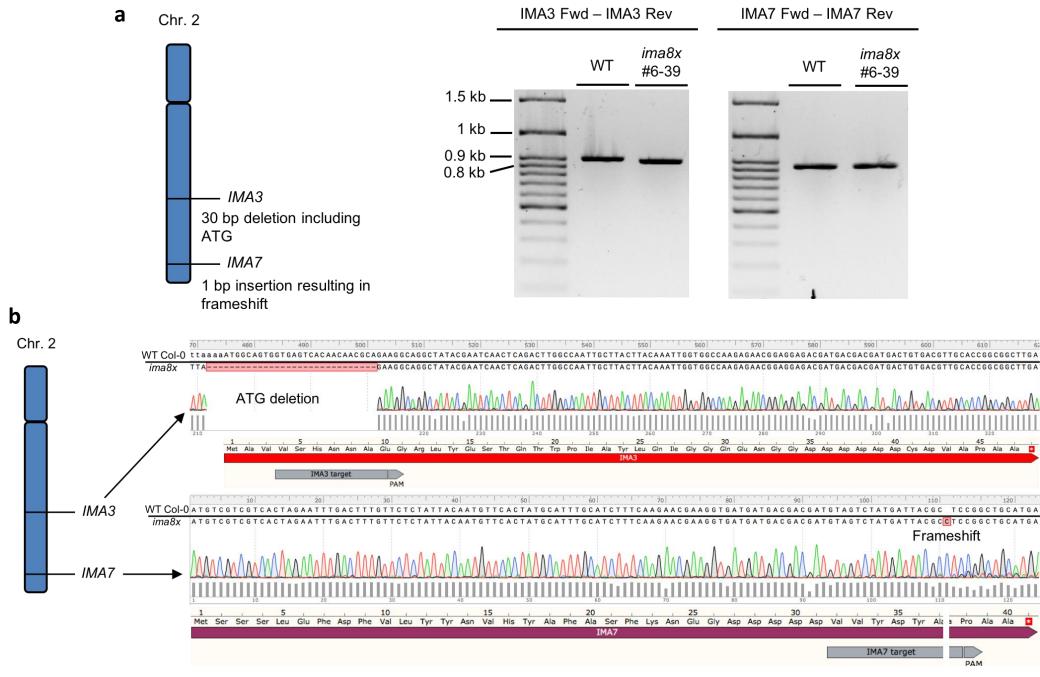


Figure S8. Genotyping of the mutations on chromosome 2 in the *ima8x* mutant line #6-39. (a) PCR amplification of regions harboring either *IMA3 or IMA7* genes. (b) Sequencing of the mutations and alignment with genome sequence of Col-0.

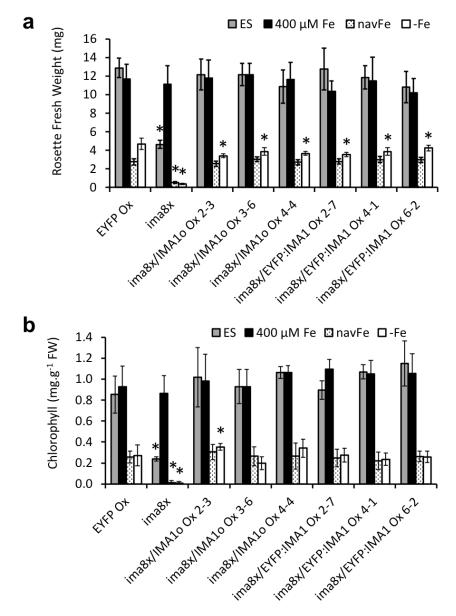


Figure S9. Silencing of eight *IMA* genes by CRISPR-Cas9 gene editing and complementation with *IMA1 and EYFP:IMA1*. (a) Biomass production (n = 3 sets of 25 rosettes). (b) chlorophyll concentration (n = 6 sets of 3 to 8 rosettes). ES, Estelle and Somerville media containing 40 μ M FeEDTA; Non-available Fe, ES media containing 10 μ M FeCl₃, pH 7; -Fe, ES media without added Fe supplemented with 100 μ M FerroZine (FRZ).

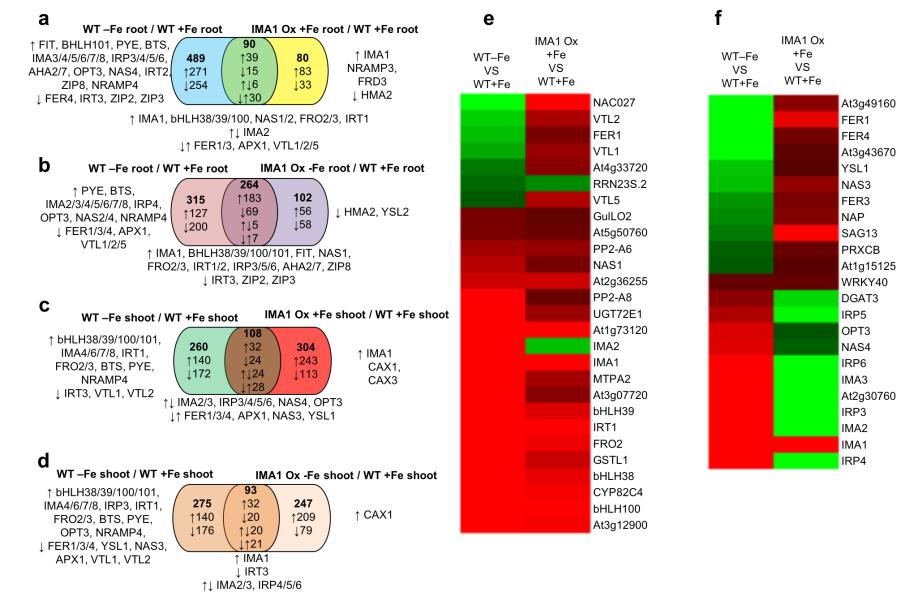


Figure S10. Comparative transcriptome analysis of IMA1 Ox lines grown on Fe-replete (+Fe) and Fe-free (-Fe) media. (a-d) Venn diagrams showing DEGs in wild-type and IMA1 Ox plants. Upward arrows indicate upregulated genes; downward arrows denote downregulated genes. Gene symbols preceded by oppositely oriented arrows indicate genes that are regulated differently in the two genotypes. DEGs denoted in blue were upregulated in Fe-deficient wild-type and down-regulated in IMA1 Ox and DEGs shown in purple were downregulated in wild-type and upregulated in IMA1 Ox. (e, f) Expression changes of the overlapping most differentially expressed genes between IMA1 Ox and control plants (log2 FC > 1) in roots (e) and leaves (f). Data for the wild type are taken from¹¹ and³⁵. All DEGs are listed in Supplementary Dataset 1.

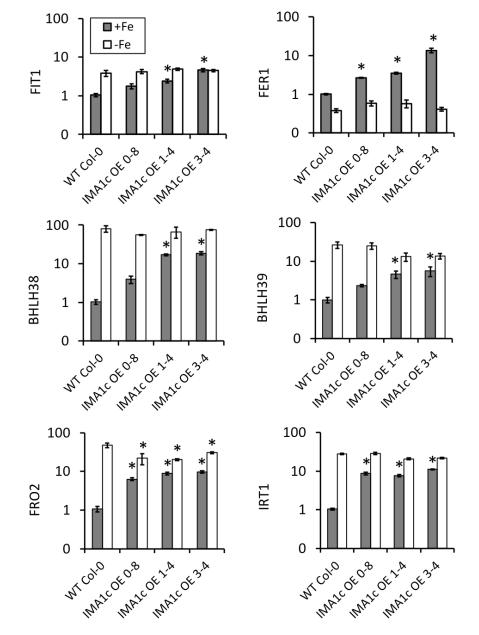


Figure S11. Analysis of *FIT, FER1, BHLH38, BHLH39, FRO2* and *IRT1* gene expression in roots of Fe-replete and Fe-deficient IMA1 Ox (35Spro::AtIMA1cDNA) seedlings. Data are expressed relative to Fe-replete wild-type plants as $2^{-\Delta\Delta Ct}$ using EF1 α as a reference gene.

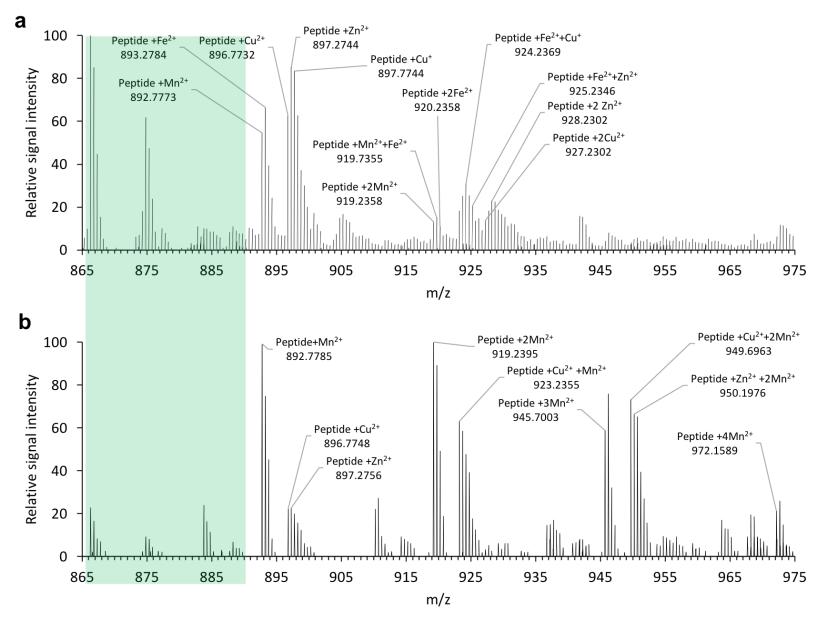


Figure S12. ESI-MS spectra of the IMA1 C-term synthetic peptide in a solution consisting of 100 μ M Fe, 100 μ M Zn, 100 μ M Cu, and 100 μ M Mn buffered at pH5 with 10 mM ammonium acetate in the presence (upper chart) or absence (lower chart) of ascorbic acid. *m*/*z* ratios of peaks corresponding to the ligand-free peptide are indicated by green colour. Peaks corresponding to peptide complexes with ⁵⁶Fe, ⁶³Cu, ⁶⁴Zn, and ⁵⁵Mn have been annotated.

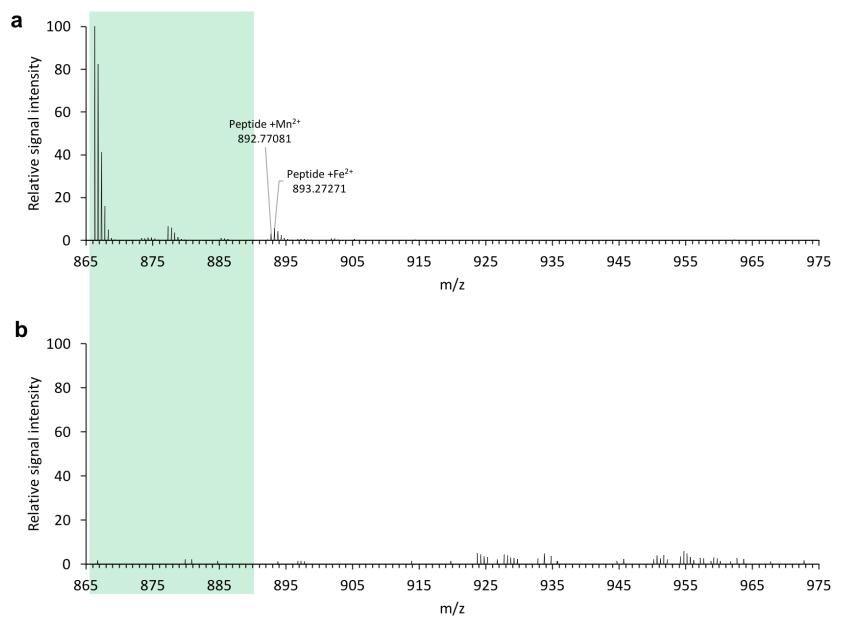
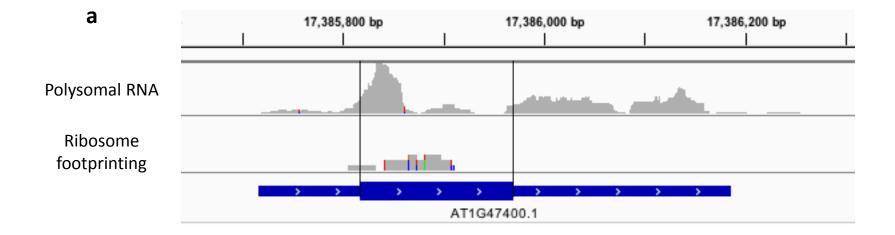


Figure S13. ESI-MS spectra of the IMA1 C-term synthetic peptide. (a) Spectrum obtained after liquid chromatography using acetonitrile with 0.1% formic acid and (b) after addition of 500 μ M Fe, 500 μ M Zn, 500 μ M Cu, and 500 μ M Mn buffered at pH5 with 10 mM ammonium acetate in the presence of ascorbic acid. *m*/*z* ratios of peaks corresponding to the ligand-free peptide are indicated by green colour.



b

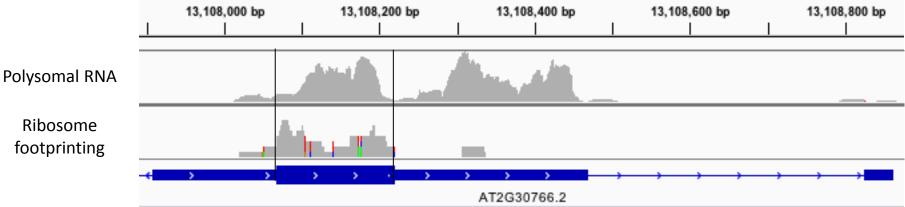


Figure S14. Translation of IMA genes. aligned reads of AtIMA1 (a) and AtIMA3 (b) derived from RNA sequencing of gradient-purified polysomal RNA and ribosome-protected RNA. Data are a courtesy of Dr. Bailey-Serres taken from⁵⁰. AtIMA1 and AtIMA3 mRNAs are associated to polysomes. Reads matching the ORFs are enriched in the ribosome-protected RNA compared to polysomal RNA.