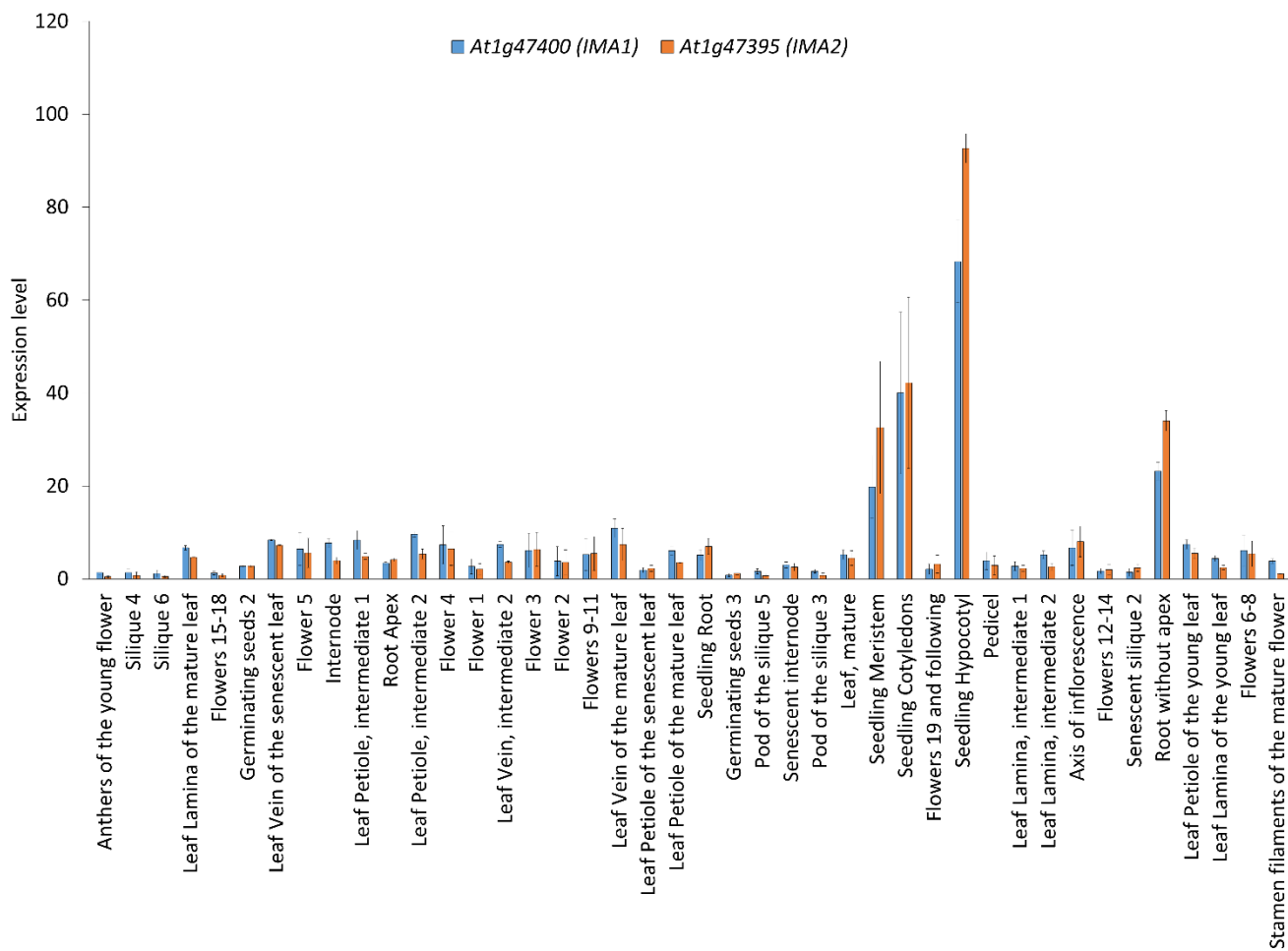
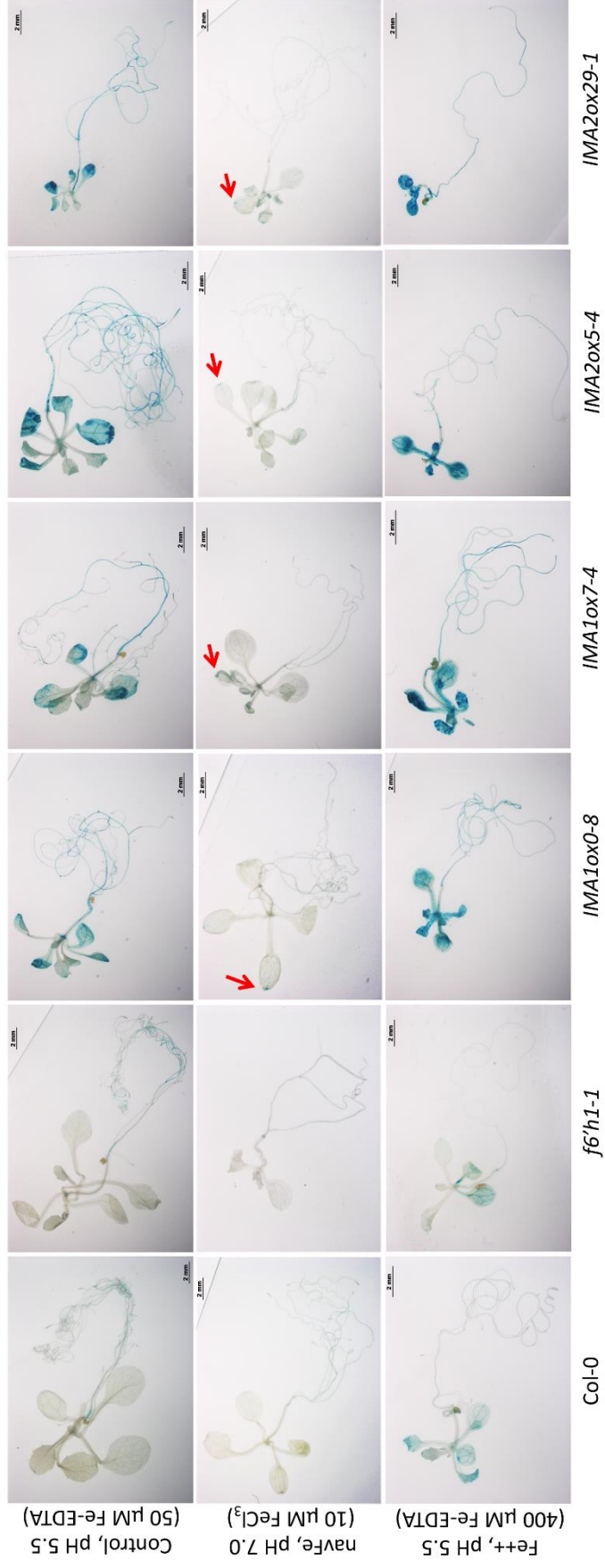


**Supplemental Table S1.** Primers used in this study.

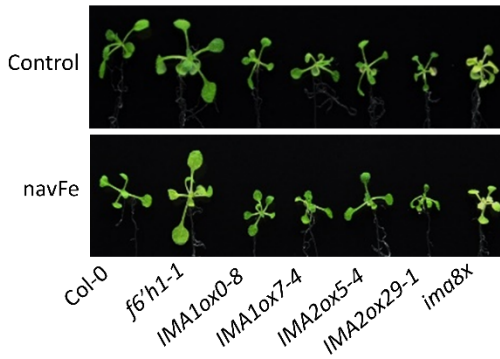
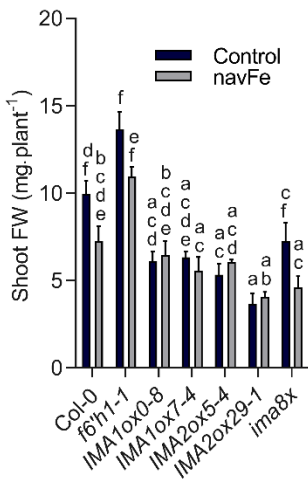
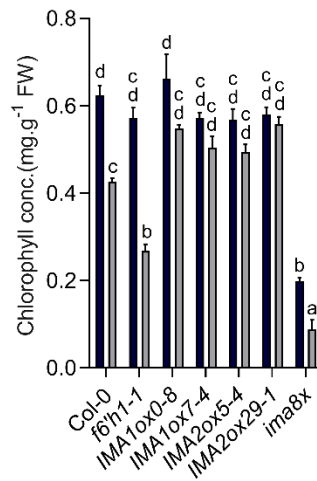
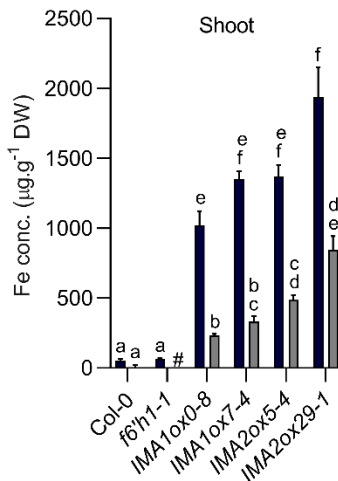
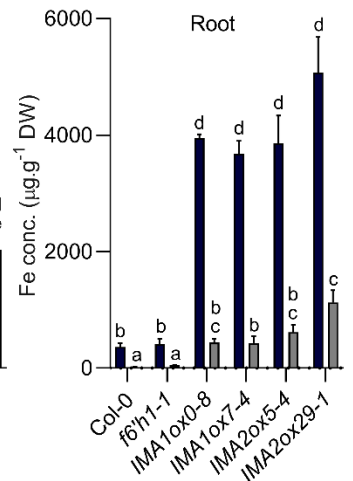
Sr. No.	Primer names	Sequence (5'-3')	References
Primers for RT-qPCR			
1	IMA1 F	GTCTTTTGTGCGAAACTTGG	(Grillet et al., 2018)
2	IMA1 R	CCACCATTCTCACTATATGCCAC	
3	IMA2 F	CGTTGCTAACTTGGTCATC	
4	IMA2 R	AACACATGTCGCTCGAGAG	
5	IMA3 F	GCTGAAGTTGTAGATCGTGC	
6	IMA3 R	AGCTAGTCTCTCAACACTCG	
7	IRT1 F	CGGTTGGACTTCTAAATGC	(Stein & Waters, 2012)
8	IRT1 R	CGATAATCGACATTCCACCG	
9	FRO2 F	CTTGGTCATCTCCGTGAGC	(Wang et al., 2007)
10	FRO2 R	AAGATGTTGGAGATGGACGG	
11	AHA2 F	TGACTGATCTTCGATCCTCTCA	(Stein & Waters, 2012)
12	AHA2 R	GAGAATGTGCATGTGCCAAA	
13	FIT F	GGAGAAGGTGTTGCTCCATC	(Wang et al., 2007)
14	FIT R	TCCGGAGAAGGAGAGCTTAG	
15	bHLH38 F	AAAGGCGGTCGCGAGTTAT	(Kailasam et al., 2018)
16	bHLH38 R	TTGGACCATCACTTCGTTGTCA	
17	bHLH39 F	AAAGGCCGTCGCGAATTATA	(Kailasam et al., 2018)
18	bHLH39 R	TTGGACCATCACTTCGTTGTCA	
19	bHLH100 F	CTTGTCTTCTCCACCAATCA	(Zhang et al., 2015)
20	bHLH100 R	TGCTCTGCAGCTCTGGTATGT	
21	bHLH101 F	ACGCCTGTACTCTCACTTCG	(Zhang et al., 2015)
22	bHLH101 R	CTTGCTCTGCTCTGGTATGATTTTC	
23	EF1 $\alpha$ F	GAGCCCAAGTTTTGAAGA	(Grillet et al., 2018)
24	EF1 $\alpha$ R	CTAACAGCGAAACGTCCCA	
25	F6'H1 F	TGATATCTGCAGGAATGAAACG	(Schippers et al., 2008)
26	F6'H1 R	GGGTAGTAGTTAAGGTTGACTC	
27	S8H F	GGTCATCAACATCGGCGACA	(Tsai et al., 2018)
28	S8H R	ACAACCTCCGGCAAGGGACC	
29	CYP82C2/3/4 F	CCTTACATGGGCCATTTCTC	(Rajniak et al., 2018)
30	CYP82C2/3/4 R	TCCTCGACGTTCCCTGTCTCT	
31	MYB72 F	GGATAAACTATCTGAGACCGGACG	(Zhang et al., 2015)
32	MYB72 R	GAGATGCGTGTTCCACACGTTT	
33	BGLU42 F	ATGGCCTGGGAACTGAAGTC	(Zamioudis et al., 2014)
34	BGLU42 R	ATTTGTCCAACCTCCGATTG	
35	PDR9 F	GCGAAACTCAGAGCTTGTGA	(Sisó-Terraza et al., 2016)
36	PDR9 R	AGTGCGCCGAAGATCAAAGA	
37	COSY F	ACTTCCAAGAGCTGCATCACAAG	(Vanholme et al., 2019)
38	COSY R	TGAATCCGGTCACTCCTGTACC	
Primers for cloning CDS			
39	IMA1 F	ATGATGTCTTTTGTGCGAAACTTGGC	
40	IMA1 R	TCACGCAGCAGGAGCATAATCATAG	
41	IMA2 F	ATGATGTCTTACGTTGCTAACTTGGTC	
42	IMA2 R	TCACGCAGCAGGAGCATAATCATAG	



**Supplemental Figure S1.** Expression patterns of *IMA1* and *IMA2*. Data are extracted from the developmental transcriptome atlas (Klepikova et al., 2016).

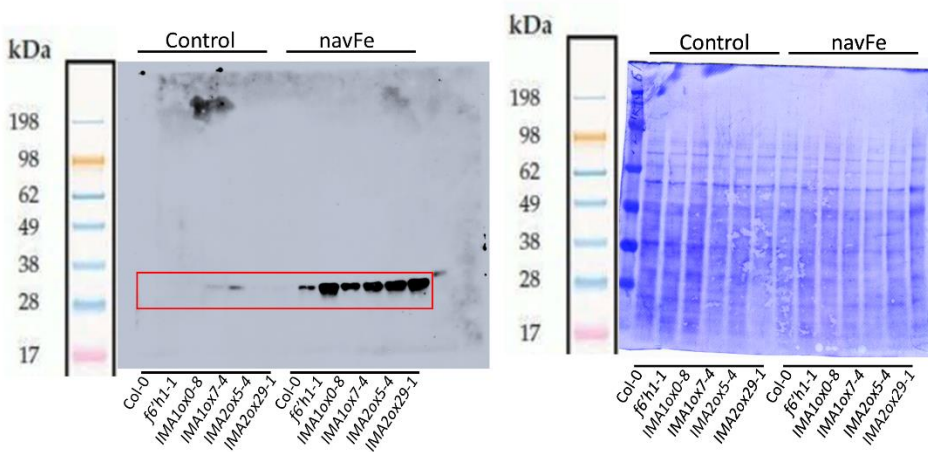


**Supplemental Figure S2.** Perls staining of Col-0, *f6'h1-1*, *IMA1ox* and *IMA2ox* seedlings. Plants were grown for two weeks on ES/control (50  $\mu$ M Fe-EDTA, pH 5.5), navFe (10  $\mu$ M FeCl<sub>3</sub>, pH 7.0), or Fe<sup>++</sup> (400  $\mu$ M Fe-EDTA, pH 5.5) media. navFe, Non-available Fe.

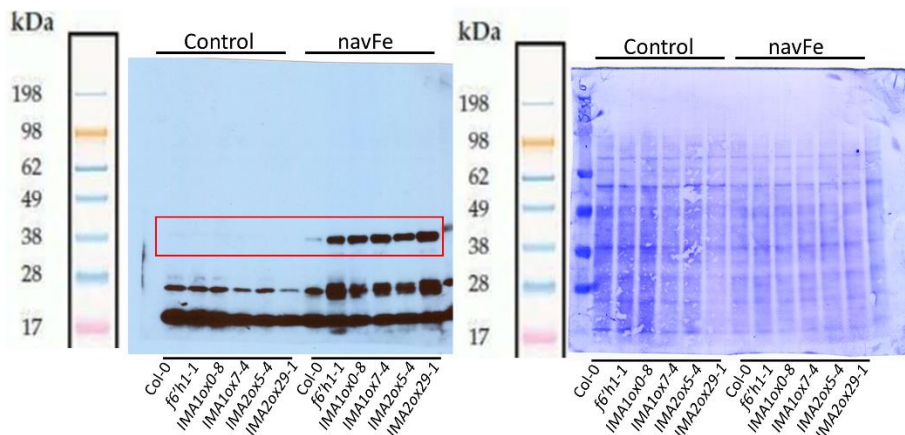
**A****B****C****D****E**

**Supplemental Figure S3.** Phenotypes and Fe concentrations of Col-0, *f6'h1-1*, *IMA1ox*, and *IMA2ox* seedlings. Plants were grown on ES/control media for 7 days and then transferred to either ES/control (50  $\mu$ M Fe-EDTA, pH 5.5) or navFe (10  $\mu$ M FeCl<sub>3</sub>, pH 7.0) media for 5 days. A) Phenotypes of 12-day-old plants. B) Shoot fresh weight (FW). Data represent means  $\pm$  SEM, n = 4. C) Chlorophyll concentration. Data represent means  $\pm$  SEM, n = 4. D) Shoot Fe concentration. Data represent means  $\pm$  SEM, n = 3. E) Root Fe concentration. Data are means  $\pm$  SEM, n = 3. Distinct letters above the bar graph indicate significant differences ( $P < 0.05$ ) in two-way ANOVA followed by Tukey's HSD test. #, Not detected; DW, Dry weight; navFe, Non-available Fe.

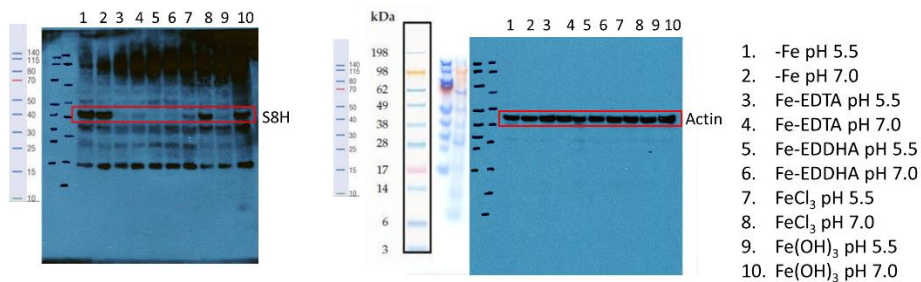
**α** (IRT1 : ~34 kDa )



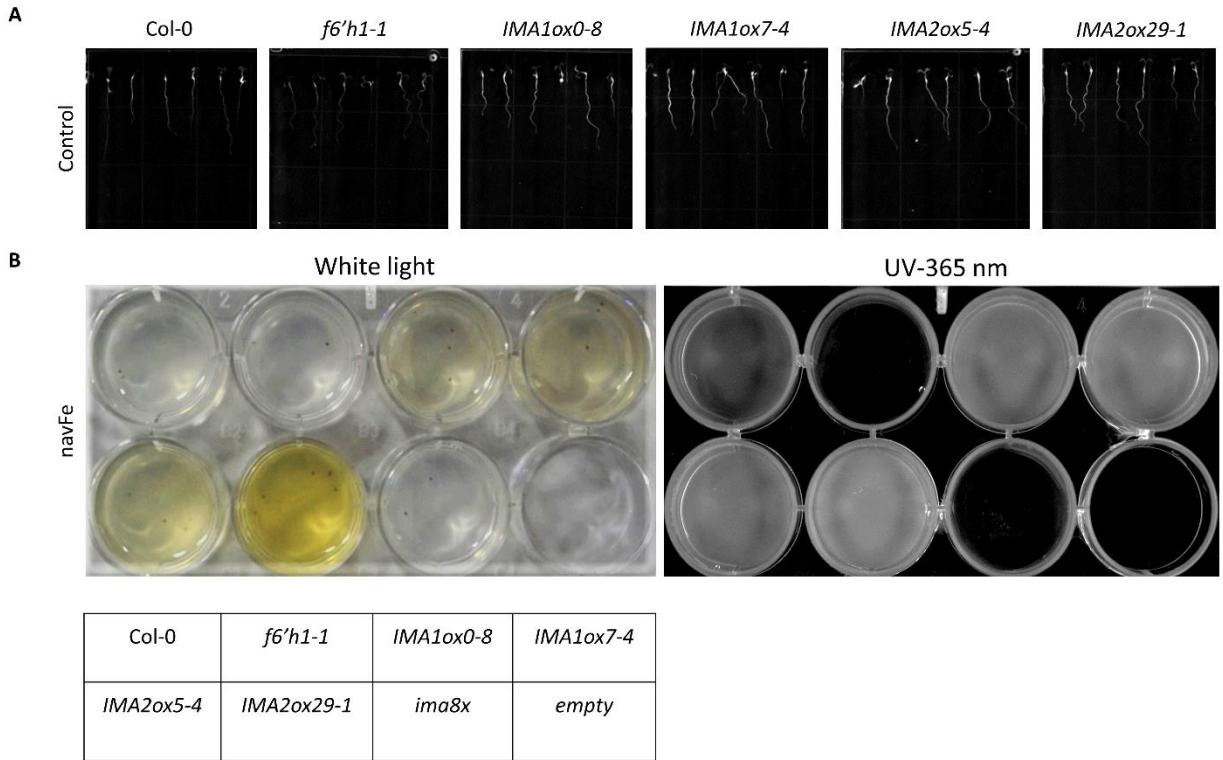
**β** (S8H: ~40 kDa)



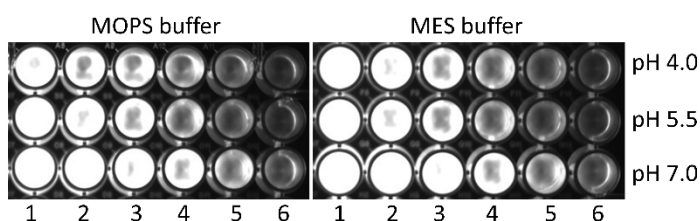
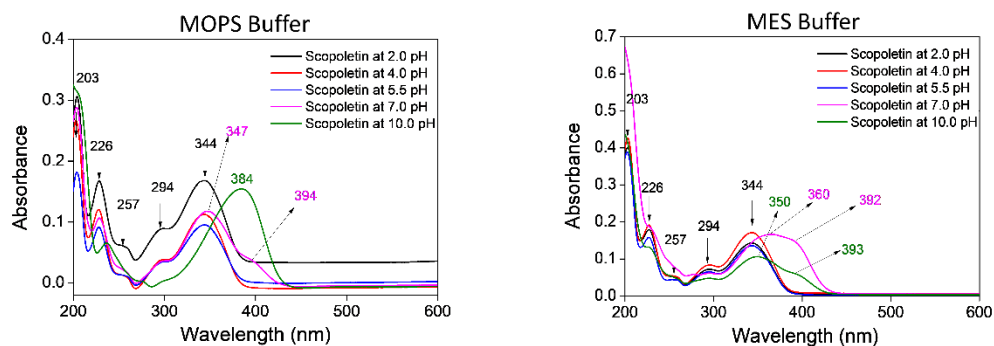
**γ** (S8H : ~40 kDa; Actin (plant): ~45 kDa)



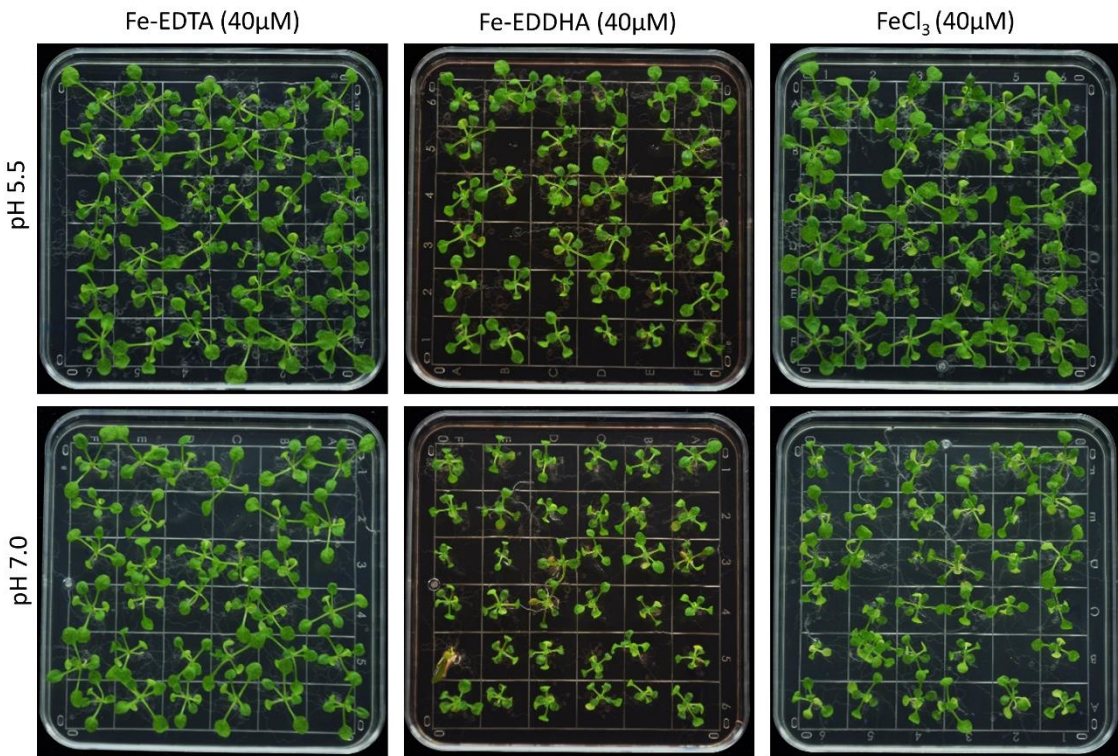
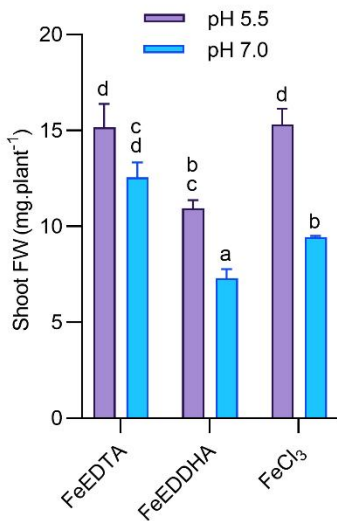
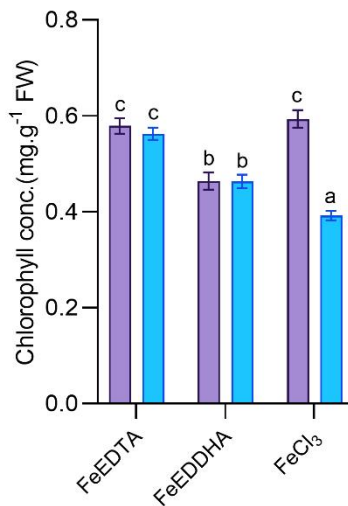
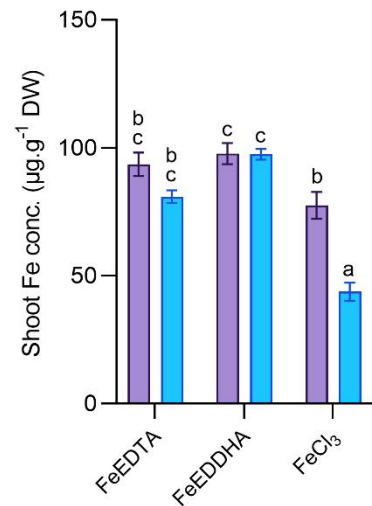
**Supplemental Figure S4.** Full scans of western blots. These figures correspond to the presented data in Fig. 3C(α), 5B(β) and 6B(γ). ES/control (50 μM Fe-EDTA, pH 5.5), navFe (10 μM FeCl<sub>3</sub>, pH 7.0), -Fe (absence of Fe but with 120 μM ferrozine). Fe concentration for all the Fe sources (Fe-EDTA, Fe-EDDHA, FeCl<sub>3</sub>, Fe(OH)<sub>3</sub>) used in Fig. γ is 40 μM. navFe, Non-available Fe.



**Supplemental Figure S5.** Autofluorescence of seedlings and media. A) Visualization of fluorescent compounds in 7-d-old seedlings grown on ES/control (50  $\mu$ M Fe-EDTA, pH 5.5 ) media. B) Imaging of navFe (10  $\mu$ M FeCl<sub>3</sub>, pH 7.0) media color (left) and autofluorescence (right). Seedlings were grown on navFe media for 2 weeks. Subsequently, media were melted and observed under white and UV light at 365 nm. The coumarins secreted by *IMA1ox* and *IMA2ox* plants turned the media color to pale-yellow due to the formation of Fe(III)-catecholic coumarin complexes (Rajniak et al., 2018). The appearance of pale-yellow coloration of the media correlates with the higher fluorescence observed under UV at 365 nm. navFe, Non-available Fe.

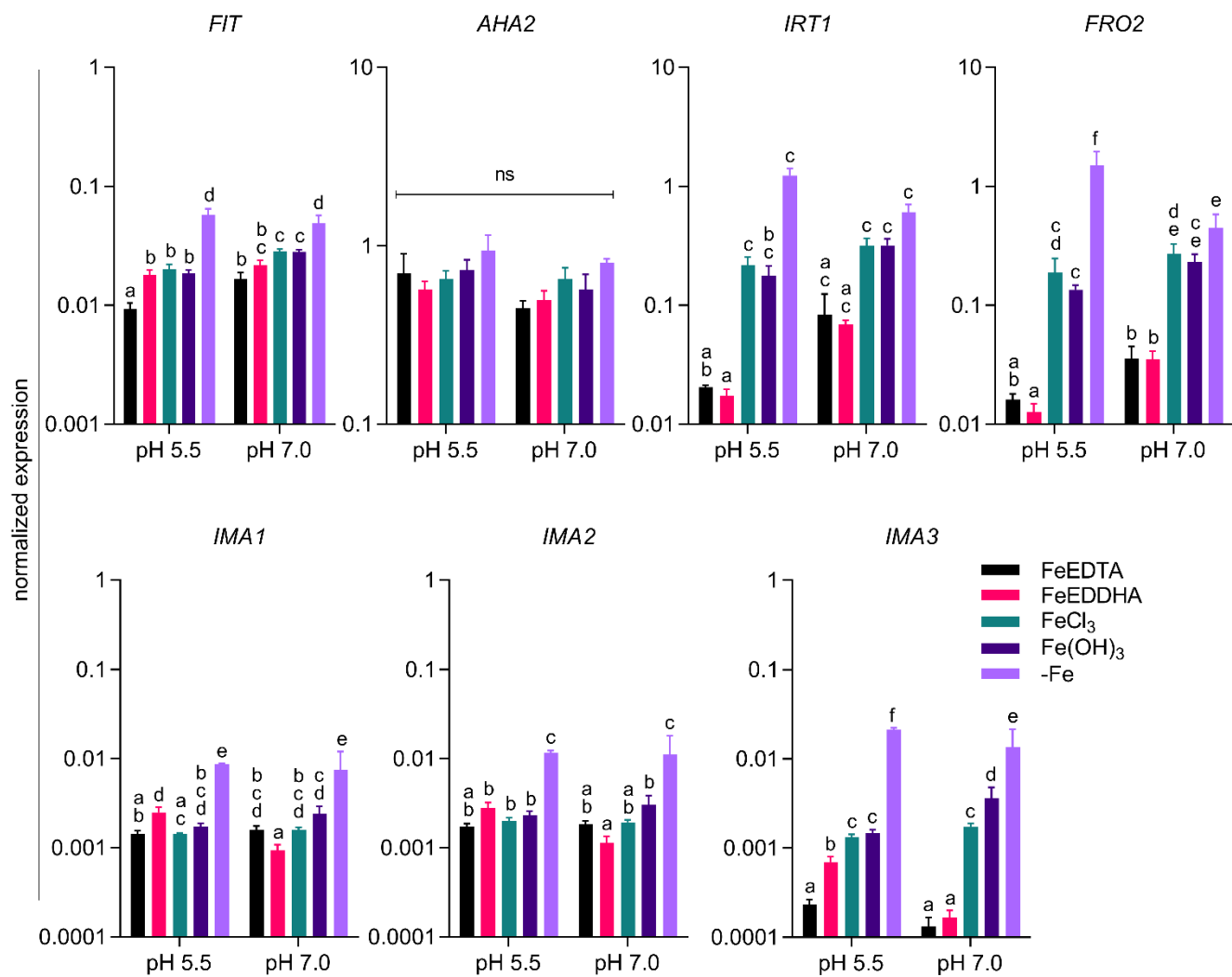
**A****B**

**Supplemental Figure S6.** pH dependence of scooletin fluorescence. A) pH-dependent autofluorescence of scooletin. Scooletin solution was prepared in both MOPS (3-morpholinopropane-1-sulfonic acid) and MES (2-(N-morpholino)ethane sulfonic acid) buffer from a 100 ppm stock solution and serially diluted by 50% at each step from left to right. B) pH-dependent UV absorbance properties of scooletin. UV-Vis absorbance spectra were scanned in water. At pH 7.0 and above, a bathochromic shift in wavelength was observed due to dominance of the anionic form.

**A****B****C****D**

**Supplemental Figure S7.** Phenotype and Fe concentration of wild type seedlings grown on media supplemented with different Fe sources at pH 5.5 and pH 7.0. A) Phenotype of 14-day-old seedlings. B) Shoot fresh weight. Data represents mean  $\pm$  SEM,  $n = 4$ . C) Chlorophyll concentration. Data represents mean  $\pm$  SEM,  $n = 4$ . D) Shoot Fe concentration. Data represents mean  $\pm$  SEM,  $n = 4$ . Distinct letters above the bar graph indicate significant differences ( $P < 0.05$ ) in two-way ANOVA followed by Tukey's HSD test. FW, Fresh weight; DW, Dry weight.





**Supplemental Figure S8. RT-qPCR analysis of key genes involved in Fe homeostasis.** Expression of *FIT*, *AHA2*, *IRT1*, *FRO2*, *IMA1*, *IMA2*, and *IMA3* determined in roots of 14-d-old Col-0 seedlings grown on media supplemented with Fe-EDTA (40  $\mu$ M), Fe-EDDHA (40  $\mu$ M), FeCl<sub>3</sub> (40  $\mu$ M), Fe(OH)<sub>3</sub> (40  $\mu$ M), and in the absence of Fe but with 120  $\mu$ M ferrozine (-Fe), at either pH 5.5 or pH 7.0. Gene expression was calculated using the  $\Delta$ CT method, expression of elongation factor 1 alpha was used as an internal control. Data are means  $\pm$  SEM, n  $\geq$  3. Distinct letters above the bar graph indicate significant differences ( $P < 0.05$ ) in two-way ANOVA followed by Tukey's HSD test.