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1 **Interdependent Iron and Phosphorus Availability Controls Photosynthesis**
2 **Through Retrograde Signaling**

3

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19

20 **Iron deficiency hampers photosynthesis and is associated with chlorosis. We**
21 **recently showed that iron deficiency-induced chlorosis depends on**
22 **phosphorus availability. How plants integrate these cues to control chlorophyll**
23 **accumulation is unknown. Here, we show that iron limitation downregulates**
24 **photosynthesis genes in a phosphorus-dependent manner. Using**
25 **transcriptomics and genome-wide association analysis, we identify two genes,**
26 **a chloroplastic ascorbate transporter (*PHT4;4*) and a nuclear transcription**
27 **factor (*bZIP58*), which prevent the downregulation of photosynthesis genes**
28 **leading to the stay-green phenotype under iron-phosphorus deficiency. Joint**
29 **limitation of these nutrients induces ascorbate accumulation by activating**
30 **expression of an ascorbate biosynthesis gene, *VTC4*, which requires *bZIP58*.**
31 **Exogenous ascorbate prevents iron deficiency-induced chlorosis in *vtc4***
32 **mutants, but not in *bzip58* or *pht4;4*. Our study demonstrates chloroplastic**
33 **ascorbate transport is essential for preventing the downregulation of**
34 **photosynthesis genes under iron-phosphorus combined deficiency. These**
35 **findings uncover a molecular pathway coordinating chloroplast-nucleus**
36 **communication to adapt photosynthesis to nutrient availability.**

37

38 Chloroplasts are sites of photosynthesis, whose function requires numerous proteins
39 encoded in the nuclear genome¹. Although plants tightly orchestrate chloroplast-to-
40 nucleus signaling (retrograde control), it is poorly understood at the mechanistic
41 level. Additionally, the adequate accumulation of nutrients such as iron (Fe) in
42 chloroplasts is required for their optimal performance^{2,3}. Up to 80% of Fe in leaves is
43 located in the chloroplasts^{4,5}, where its ability to donate and accept electrons plays a
44 central role in electron transfer reactions⁶. Fe is found in all electron transfer
45 complexes PSI, PSII, cytochrome b6f complex, and ferredoxins and is required for
46 the biogenesis of cofactors such as hemes and iron-sulfur clusters^{7,8}. Plants grown
47 under Fe-deficient (-Fe) environments show chlorotic symptoms⁹, and compromised
48 photosynthesis^{2,3}. However, chlorotic leaves can also develop under high-
49 phosphorus (P) conditions, despite replete Fe levels¹⁰, challenging the causal
50 connection between Fe concentration and chlorophyll accumulation. Moreover, we
51 recently reported that rice plants grown under a combined Fe and P deficiency (-Fe-
52 P) do not exhibit a chlorotic phenotype¹¹. These observations revealed a gap in our
53 understanding of the interdependent effects of nutrient availability on regulating
54 photosynthesis. Here, we addressed this issue through a combination of global gene
55 expression analyses and genome-wide association studies (GWAS) to find
56 expression quantitative trait loci (eQTLs) and uncovered a regulatory module that
57 controls chlorophyll accumulation in response to Fe and P availability. This module
58 involves an ascorbic acid (AsA) synthesis enzyme named VITAMINC4 (VTC4), a
59 chloroplastic AsA transporter named PHOSPHATE TRANSPORTER 4;4 (PHT4;4),
60 and a putative transcription factor named BASIC LEUCINE-ZIPPER 58 (bZIP58).
61 The functioning of this module sheds light on the importance of chloroplast-nucleus
62 communications under co-occurring nutrient deficiencies in controlling
63 photosynthesis.

64

65 We previously reported that Fe deficiency-induced chlorosis depends on P
66 availability in rice¹¹. To investigate whether the interdependent effects of Fe and P
67 availability on chlorosis are conserved across monocot and eudicot species, we
68 phenotyped *Arabidopsis thaliana* Col-0 (eudicot) and *Lemna gibba* (monocot), along
69 with *Oryza sativa* (monocot), under different regimes of Fe and P availability. Fe
70 deficiency (-Fe) caused chlorosis in all three species, but only in the presence of P (-
71 Fe+P) (Figure 1A-C). Quantification of chlorophyll content confirmed that -Fe

72 significantly reduced the accumulation of chlorophyll in all three species (Figure 1D).
73 However, under -Fe-P conditions, chlorophyll content was comparable to control
74 (+Fe+P) conditions in these species (Figure 1D). Next, we focused on Arabidopsis to
75 gain insights into the physiological and molecular processes underlying the recovery
76 of chlorosis under -Fe-P conditions. First, we asked whether absence of chlorosis
77 under -Fe-P is caused by an increase of Fe levels in shoots. Plants grown in -Fe+P
78 conditions decreased total Fe in shoots by 2-fold compared to +Fe+P conditions
79 (Figure 1E). On the other hand, under +Fe-P conditions, Fe levels increased by 2.2-
80 fold relative to +Fe+P conditions (Figure 1E). Surprisingly, Fe levels in plants grown
81 under -Fe-P were reduced and indistinguishable from the Fe levels in -Fe+P
82 conditions (Figure 1E). Therefore, the lack of chlorosis under -Fe-P is unlikely to be
83 caused by more Fe available in shoots. To further test this hypothesis, we assessed
84 free Fe in leaves. Because FERRITIN1 (*AtFER1*) chelates Fe and its mRNA
85 increases when Fe is in excess, *AtFER1* gene expression can be used as a read-out
86 for intracellular Fe nutritional status¹². We thus quantified the expression of *AtFER1*
87 in shoots under replete or deficient Fe and P in the growth media. Consistent with the
88 total Fe levels, *AtFER1* expression was increased significantly under +Fe-P
89 conditions relative to +Fe+P (Figure 1F). However, *AtFER1* expression decreased
90 under both -Fe+P and -Fe-P conditions relative to +Fe+P (Figure 1F). Interestingly,
91 -Fe-P condition caused a slightly bigger reduction in *AtFER1* expression than -Fe+P
92 did, suggesting that there may be even less free Fe in -Fe-P conditions than in -Fe+P
93 conditions. Taken together, these data show that the onset of chlorosis during -Fe
94 requires sufficient P in the growth media, and that the “stay green” phenotype under
95 the combined -Fe-P deficiency cannot be linked to Fe nutritional status in leaves.

96
97 To understand the cause of chlorophyll reduction in response to -Fe, we first
98 explored the timing of -Fe sensing and photosynthetic function response. Since -Fe
99 affects chlorophyll accumulation and photosystem II (PSII) activity^{13,14}, we monitored
100 their kinetics over 172 hours (h) (Figure 2A-B, Figure S1A-C). Arabidopsis plants
101 were first grown on +Fe+P media for one week, and then transferred to +Fe+P, -
102 Fe+P, or -Fe-P conditions. -Fe+P caused a significant decrease in chlorophyll
103 content observable starting at 52 h after the transfer to -Fe+P (Figure 2A). However,
104 transfer to -Fe-P did not affect chlorophyll content, even at 172 h after the transfer
105 (Figure 2A). To determine how photosynthesis was affected, we measured Fv/Fm,

106 which reflects the quantum yield of photochemistry and is a measure of PSII
107 activity^{13,14}. Plants under -Fe+P decreased Fv/Fm observable starting at 52h,
108 indicative of compromised electron transport through PSII, and which coincides with
109 the decrease of chlorophyll accumulation (Figure 2B). By 172 h, PSII activity was
110 substantially reduced under -Fe+P compared to +Fe+P. However, plants under -Fe-P
111 showed slightly lower but stabilized Fv/Fm compared to those in +Fe+P (Figure 2B).
112 These physiological characterizations showed that chlorophyll accumulation and PSII
113 activity were affected by -Fe, and both responses were P-dependent (Figure 2A-B).

114
115 Based on these findings, we selected three time points at 39, 52, and 76 h after the
116 transfer of plants to +Fe+P, -Fe+P, or -Fe-P to conduct a global gene expression
117 analysis in shoots (Figure 2C, Figure S2A-F). We identified genes whose expression
118 levels were either increased or decreased by -Fe+P relative to +Fe+P by at least 2-
119 fold at a p-value < 0.05 (Figures 2D, Table S1). Even more genes were either
120 upregulated or downregulated in -Fe-P conditions relative to +Fe+P (Figures 2D,
121 Figure S3A-C). A total of 671 and 2434 transcripts were uniquely differentially
122 regulated in response to -Fe+P or -Fe-P, respectively, supporting the existence of
123 different signaling pathways under the two conditions (Figure S 3A-C). To identify
124 functions enriched in genes that were differently regulated by -Fe+P or -Fe-P, we
125 performed Gene Ontology (GO) enrichment analysis. Genes specifically
126 downregulated by -Fe-P but not by -Fe+P at 52 h and 76 h (52 genes; Figure S4A,
127 Table S1) showed an enrichment for ribosomal genes (Figure S4B) while
128 upregulated genes (162 genes, Figure S4C, Table S1) revealed an enrichment for
129 genes involved in cation transport, response to water, and ester hydrolysis (Figure
130 S4D, Table S1). On the other hand, GO analysis of the 32 genes specifically
131 downregulated by -Fe+P but not affected by -Fe-P at 52 h and 76 h (Figure 2E, Table
132 S1) revealed an enrichment of genes related to the chloroplast and photosynthesis-
133 related processes (Figure 2F), while upregulated genes (Figure S4E) were enriched
134 for genes related to cellular respiration, oxidation-reduction process, and energy
135 metabolism (35 genes; Figure S4F). Altogether, the transcriptomics analysis
136 indicated that the control of chloroplast function is an integral component of the
137 nuclear transcriptomic response to -Fe, which is dependent on P availability. We also
138 learned that the photosynthesis-related phenotypes we observed under -Fe+P, but
139 not under -Fe-P, could be due to downregulation of key photosynthesis regulators.

140 To decode the signaling pathways that control expression of the photosynthesis
141 genes in response to -Fe+P, we exploited natural variation in expression of the 32
142 genes that were down-regulated by Fe deficiency in a P-dependent manner in a
143 worldwide collection of *A. thaliana* accessions¹⁵. One way to identify regulatory
144 mechanisms could be to perform expression genome-wide association studies
145 (eGWAS) using the expression level of individual genes across Arabidopsis
146 accessions. Strikingly, we found that expression levels of the 32 genes are
147 predominantly positively associated with each other across 727 Arabidopsis
148 accessions (Figure S5A)¹⁵. We then performed Principal Component Analysis (PCA)
149 to reduce the dimensionality of these expression data. PC1 explained 89.5% of the
150 variation in expression of these genes across Arabidopsis accessions (Figure S5B).
151 The contribution of each accession to PC1 was then used to perform a genome-wide
152 association study (GWAS) (Figure 3A). Our GWAS analysis detected 38 QTLs
153 containing 145 candidate genes, based on a 20-kb window per QTL and using a 5%
154 false discovery rate (FDR) threshold, with the highest peak (Chromosome 2)
155 occurring in an intergenic region (Figure 3A). In this study, we followed up one of the
156 QTLs that contained the inorganic phosphate transporter *PHT4;4* (AT4G00370)
157 (Figure 3A, Figures S6 and S7) given its role in ascorbic acid (AsA) transport into
158 chloroplasts, which was proposed to be important for maintaining the xanthophyll
159 cycle for dissipation of excessive light energy to heat in photosynthesis¹⁶. To
160 determine if *PHT4;4* has any role in -Fe+P dependent chlorosis, we examined
161 mutants with a null *pht4;4* allele. Under -Fe+P, chlorophyll was significantly reduced
162 in *pht4;4* mutants, similar to wild type plants (Figure 3B-C). However, -Fe-P
163 conditions failed to recover chlorosis and chlorophyll reduction in *pht4;4* mutants,
164 unlike wild type plants (Figure 3B-C). Introduction of the wild type *PHT4;4* allele into
165 a *pht4;4* mutant background complemented these phenotypes (Figure 3B-C). The
166 chlorotic phenotype of the *pht4;4* mutant under -Fe-P suggested that transport of AsA
167 into chloroplasts could be important for the “stay green” phenotype.

168
169 To test our hypothesis about the role of AsA in preventing chlorosis under -Fe-P
170 conditions, we first assessed how Fe and P availabilities regulate the expression of
171 *VITAMIN C* (*VTC*) enzymes involved in AsA biosynthesis in plants¹⁷. Our RNA-seq
172 analysis identified that -Fe-P caused a 2 to 3-fold increase in *VTC1*, *VTC2*, and
173 *VTC4* expression, which we confirmed using qRT-PCR (Figure 3D). However, -Fe in

174 the presence of P (-Fe+P) caused a 2-fold decrease in the mRNA abundance of
175 *VTC4* (Figure 3D). *VTC4* is the final enzyme in the AsA biosynthesis pathway¹⁷. This
176 prompted us to test the effect of the absence of *VTC4* on chlorophyll accumulation
177 under -Fe+P and -Fe-P conditions. Under -Fe-P, mutants with a *vtc4* null allele were
178 still chlorotic, similarly to *pht4;4* and in contrast to wild type plants (Figure 3B-C).
179 These data show that AsA contributes to preventing chlorosis in -Fe-P conditions.

180

181 Next, we tested whether the chlorotic phenotype is due to variations in AsA levels. In
182 wild type, AsA levels decreased significantly under -Fe+P at 52 h after the transfer
183 relative to control (+Fe+P), whereas no change was detected under -Fe-P (Figure
184 3E), suggesting that AsA levels were associated with -Fe-mediated chlorosis. To test
185 whether AsA levels were associated with chlorosis in general, we measured AsA
186 levels in AsA synthesis (*vtc4*) mutant plants. Under +Fe+P, *vtc4* plants accumulated
187 35% less AsA than wild type plants, and AsA levels remained unchanged in
188 response to -Fe+P or -Fe-P stress (Figure 3E). However, *vtc4* plants did not show
189 the chlorotic phenotype under +Fe+P, which indicated that the level of AsA
190 contributed to the chlorotic phenotype specifically under -Fe and this contribution was
191 dependent on P availability. In addition, the AsA transporter (*pht4;4*) mutants showed
192 similar AsA levels as the wild type even though *pht4;4* plants were still chlorotic in -
193 Fe-P (Figure 3E). To determine whether AsA accumulation in the cell or its transport
194 to the chloroplast is associated with the development of chlorotic phenotype in -
195 Fe+P, we tested the effect of an exogenous supply of AsA in wild type, *vtc4*, and
196 *pht4;4* plants (Figure 3B-C). Exogenous AsA alleviated the chlorosis caused by -
197 Fe+P in wild type and *vtc4* mutant plants. However, *pht4;4* mutants failed to stay
198 green under -Fe+P+AsA conditions (Figure 3B-C), indicating that the transport of
199 AsA to the chloroplast is required for -P mediated 'stay green' phenotype under Fe
200 deficiency. Our results showed that -P prevents the downregulation of *VTC4* by -Fe
201 and associated changes in AsA accumulation, and that the *PHT4;4*-mediated
202 transport of AsA to chloroplasts is required for the maintenance of chlorophyll content
203 under combined deficiency of Fe and P.

204

205 We next asked whether *PHT4;4*-mediated AsA transport to the chloroplast is
206 important for regulation of the photosynthesis-related genes that were specifically
207 down-regulated by -Fe in a P-dependent manner. First, we tested the effects of

208 *PHT4;4* inactivation on the expression of these photosynthesis related genes using
209 qRT-PCR (Figure 4A). While -Fe+P significantly downregulated the mRNA
210 abundance of these genes in wild type plants (Col-0), -Fe-P prevented this response
211 (Figure 4A). Furthermore, adding AsA to -Fe+P mimicked -Fe-P response in
212 preventing down-regulation of the photosynthesis genes. Under -Fe+P, *pht4;4*
213 mutant plants showed a decrease in the mRNA abundance of these genes
214 comparable to wild type plants (Figure 4A). However, in contrast to the wild type,
215 these genes were still downregulated in *pht4;4* plants under -Fe-P as well as -Fe+P
216 supplemented with AsA (Figure 4A). Taken together, these data indicate that the
217 transport of AsA to chloroplasts via *PHT4;4* is central to preventing the
218 downregulation of photosynthesis-related genes under -Fe-P.

219
220 How does -Fe+P affect the expression of photosynthesis-related genes? To look for
221 potential transcriptional regulators of these genes, we examined the candidate genes
222 from the GWAS analysis. We found that *bZIP58* (AT1G13600) (Figure 3A, Figure S6-
223 S7), a putative transcription factor, underlies one of the strongest QTL peaks (Figure
224 3A). To test whether *bZIP58* responds to Fe and P availability, we performed qRT-
225 PCR of *bZIP58* under various Fe and P availability. *bZIP58* was strongly
226 downregulated by -Fe, and this down-regulation depended on P availability (Figure
227 4B). In addition, *bZIP58* was partially required to induce *VTC4* expression under -Fe-
228 P conditions (Figure 4C). This led us to examine the contribution of *bZIP58* in
229 regulating the -Fe+P specific photosynthesis-related genes under +Fe+P, -Fe+P, -
230 Fe-P conditions (Figure 4A). Mutants with the *bzip58* null allele showed a remarkable
231 constitutive decrease in the expression of these 32 photosynthesis-related genes
232 (Figure 4A). *bZIP58* localizes to the nucleus (Figure 4D), which is consistent with a
233 role as a transcription factor. Taken together, these findings support the idea that
234 *bZIP58* is a key regulator of photosynthesis-related genes, and its absence could
235 alter chlorophyll accumulation regardless of Fe and P availability. Genetic inactivation
236 of *bZIP58* indeed causes a constitutive decrease in chlorophyll content, and the
237 mutant line is chlorotic (Figure 4E-F). The expression of *bZIP58* gene in *bzip58*
238 plants complements the constitutive chlorosis phenotype, and the complemented line
239 responds to Fe and P availability similarly to wild type plants (Figure 4E-F).
240 Furthermore, AsA supplementation could not rescue the chlorotic phenotype of
241 *bzip58* mutants (Figure 4E-F), indicating that *pZIP58* lies downstream of AsA action.

242 These data show that bZIP58 controls the expression of photosynthesis-related
243 genes and is transcriptionally regulated in response to -Fe depending on P
244 availability, likely by mediating the perception of AsA.

245
246 Based on our findings, we propose a model to explain how P availability modulates
247 Fe deficiency-induced chlorosis (Figure 4G). -Fe+P causes a decrease in the
248 expression of AsA biosynthesis gene *VTC4* (Figure 3D). Exogenous AsA supply
249 prevents the development of chlorosis in *vtc4* under -Fe-P, but not in the *pht4;4* that
250 transports AsA to the chloroplast (Figure 3B). We thus propose that AsA deficiency in
251 the chloroplast under Fe limited conditions triggers chlorosis. How does AsA in the
252 chloroplast affect photosynthesis? AsA has an antioxidizing action that detoxifies
253 reactive oxygen species (ROS) through its scavenging properties¹⁸, thus making
254 ROS a potential signaling molecule¹⁹⁻²¹ capable of modulating the expression of
255 photosynthesis-related genes through bZIP58. To test this hypothesis, we measured
256 the relative amount of ROS accumulation in shoots of wild type, *pht4;4* and *bzip58*
257 plants under various Fe and P availability. -Fe+P caused a 2-fold increase in ROS
258 accumulation in shoots of wild type plants, which partially depended on P availability
259 (Figure 4H). *pht4;4* plants displayed comparable ROS accumulation to that of the
260 wild type under +Fe+P and -Fe+P. However, *pht4;4* plants accumulated significantly
261 higher ROS than wild type plants under -Fe-P (Figure 4H). In addition, *bzip58* mutant
262 plants displayed a constitutive increase in ROS accumulation (Figure 4H). To check
263 whether ROS in turn can regulate the expression of *bZIP58*, we quantified *bZIP58*
264 expression in response to foliar application of H₂O₂. ROS treatment caused a 4-fold
265 decrease in *bZIP58* transcript accumulation (Figure 4B). Collectively, our results
266 support the idea that under simultaneous Fe and P deficiency, AsA accumulation in
267 the chloroplast prevents chlorosis by modulating ROS levels that may control the
268 expression of photosynthesis genes via a putative transcription factor bZIP58 (Figure
269 4G). How ROS acts as a retrograde signal to alter nuclear gene expression to control
270 photosynthesis under Fe and P limitation remains to be determined, though we now
271 have several molecular targets with which to explore this field. Modulation of the
272 discovered pathway could have a direct impact on plant growth in the field by
273 improving plant photosynthetic activity while reducing nutrient supply.

274

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367
368 **Figure Legends**

369 **Figure1. Phosphorus deficiency prevents iron deficiency-induced chlorosis in**
370 **evolutionarily distant plant species. A-C)** Duckweed (*Lemna gibba*), rice (*Oryza*
371 *sativa* cv Nipponbare), and *Arabidopsis thaliana* plants grown on media containing
372 iron and phosphorus (+Fe+P), deficient in iron (-Fe+P), or deficient in both elements

373 (-Fe-P). Representative images of *L. gibba* propagated for 28 days (A), 24-day old
374 rice (B) and 14-day old *A. thaliana* (Col-0) (C) are shown. **D**) Chlorophyll
375 accumulation in *L. gibba*, *A. thaliana*, and *O. sativa* grown under +Fe+P, -Fe+P, and -
376 Fe-P conditions. Scale bars, 7 mm (A), 10 mm (B), and 5 mm (C). Data shown are
377 from 3 experiments and 3-10 plants per experiment. Error bars represent 95%
378 confidence interval. FW, fresh weight. **E**) Iron content in shoots of *A. thaliana* plants
379 grown on agar plates containing +Fe+P, -Fe+P, or -Fe-P. Data shown are from 3
380 experiments and 10 plants per experiment. Error bars represent 95% confidence
381 interval. DW, dry weight. **F**) mRNA abundance of *FERRITIN1* (*AtFER1*) relative to
382 *Ubiquitin 10* (*At4g05320*) in *A. thaliana* shoots grown under +Fe+P, -Fe+P, or -Fe-P.
383 Data shown from 3 experiments, with above-ground tissue from 5 plants pooled per
384 experiment for RNA extraction. Error bars represent 95% confidence interval. For D-
385 F, letters above bars represent statistically different means at $P < 0.05$ (one-way
386 ANOVA with a Duncan post-hoc test). Source data are provided as a Source Data
387 file.

388

389 **Figure 2. Kinetics of chlorophyll accumulation, photosystem II activity, and**
390 **transcriptome change in response to iron and phosphorus availability. A-B)**
391 Chlorophyll content and PSII activity (Fv/Fm) in response to iron and/or phosphate
392 deficiency in *A. thaliana*. Seedlings were grown for 7 days in the presence of iron and
393 phosphorus (+Fe+P) and transferred to three different media: +Fe+P, -Fe+P, or -Fe-
394 P for 15h, 28h, 39h, 52h, 76h, 100h, 124h, 148h, 172h. **A**) Chlorophyll data shown
395 are from 3 experiments, each experiment with 8 plants. Error bars represent 95%
396 confidence interval. **B**) Plants were dark-adapted for 30 min before measuring
397 fluorescence kinetics from a leaf. Fv/Fm data shown are from 3 experiments, each
398 experiment with 16 plants. Error bars represent 95% confidence interval. **C**)
399 Experimental design for transcriptomic studies on *A. thaliana* (Col-0) shoots. Plants
400 were grown in media containing iron and phosphorus (+Fe+P) for 7 days, transferred
401 to three different media (+Fe+P, -Fe+P, or -Fe-P) for 39h, 52h, or 76h, and shoots
402 were harvested for RNA extraction and sequencing. **D**) Global expression analysis of
403 genes in response to -Fe+P and -Fe-P relative to +Fe+P. Numbers of genes
404 displaying at least 2-fold change (p -value < 0.05) in their expression are shown for

405 each condition. The 32 genes that were decreased specifically in -Fe+P but not in -
406 Fe-P relative to +Fe+P at 52h and 76h (highlighted in purple) were used to perform
407 genome-wide association studies. **E)** A heatmap showing gene expression patterns
408 of the 32 genes in -Fe+P and -Fe-P relative to control (+Fe+P) at 39h, 52h, 76h after
409 the transfer. LogFC, log₂ fold change. **F)** Gene Ontology analysis for the biological
410 processes (GO-BP) for the 32 genes whose mRNA abundance was specifically
411 decreased by -Fe+P. FDR = false discovery rate. Source data are provided as a
412 Source Data file.

413
414 **Figure 3. PHT4;4 prevents chlorosis under the combined deficiency of iron and**
415 **phosphorus. A)** A Manhattan plot for genome-wide association mapping using
416 principal component 1 that explained 89.5% of expression variation of the 32
417 photosynthesis related genes across 727 *A. thaliana* accessions¹⁵. The five
418 chromosomes are depicted by light and dark blue colors. Dashed lines correspond to
419 FDR 5% threshold (blue) and Bonferroni $\alpha = 0.05$ (red). The light grey rectangle
420 highlights a significant association located in an intergenic region (SNP: 4493712).
421 Two significant associations that were followed up in this study are highlighted in red
422 rectangles. **B)** Representative images of wild-type Col-0, *pht4;4*, *vtc4*, and a line
423 expressing genomic *PHT4;4* in *pht4;4* (*pht4;4-CL*) grown for 7 days in the presence
424 of iron and phosphorus (+Fe+P) and transferred to three different media: +Fe+P, -
425 Fe+P, or -Fe-P for 7 additional days. Scale bars: 7 mm **C)** Total chlorophyll content in
426 Col-0, *pht4;4*, *vtc4*, and *PHT4;4-CL* grown under -Fe+P, +Fe-P, -Fe-P, or -
427 Fe+P+AsA. Data shown are from 10 plants conducted in three independent
428 experiments. **D)** mRNA abundance of *VTC* genes (*VTC1*, *VTC2*, and *VTC4*) relative
429 to *Ubiquitin 10* (*At4g05320*) in shoots of *A. thaliana* Col-0 plants grown in the
430 presence of iron and phosphorus (+Fe+P) for 7 days and transferred to +Fe+P, -
431 Fe+P, -Fe-P or -Fe-P+AsA for 52h. Data shown are from 3 experiments. Error bars
432 represent 95% confidence interval. **E)** Total ascorbic acid (AsA) content in Col-0,
433 *pht4;4*, *vtc4*, and *PHT4;4-CL* plants grown for 7 days in the presence of iron and
434 phosphorus (+Fe+P) and transferred to +Fe+P, -Fe+P, or -Fe-P for 52h. Data shown
435 are from 3 experiments, each with 16 plants. In box plots (C, E) center lines show
436 sample medians; box limits indicate the 25th and 75th percentiles; whiskers extend

437 1.5 times the interquartile range from the 25th and 75th percentiles. For C-E, letters
438 above bars or boxes represent statistically different means at $P < 0.05$ (one-way
439 ANOVA with a Duncan post-hoc test). Data points are plotted as open circles. Source
440 data are provided as a Source Data file.

441

442 **Figure 4. bZIP58 regulates photosynthesis related genes and chlorophyll**
443 **accumulation. A)** mRNA abundance of 32 photosynthesis-related genes relative to
444 *Ubiquitin 10* in shoots of *A. thaliana* Col-0, *pht4;4*, and *bzip58* plants grown for 7 days
445 in the presence of iron and phosphorus (+Fe+P) and transferred to +Fe+P, -Fe+P, -
446 Fe-P, or -Fe-P+AsA for 52h. Data were averaged from three independent
447 experiments. Scale bar represents the relative mRNA abundance. **B)** mRNA
448 abundance of *bZIP58* relative to *Ubiquitin 10* in *A. thaliana* Col-0 shoots of plants
449 grown for 7 days on +Fe+P and transferred to +Fe+P, -Fe+P, -Fe-P, or +Fe+P+H₂O₂
450 for 52h. Data are shown from 3 experiments. Error bars represent 95% confidence
451 interval. **C)** *VTC4* mRNA abundance relative to *Ubiquitin 10* in the shoots of Col-0
452 and *bzip58* mutants grown in the presence of +Fe+P for 7 days and transferred to
453 +Fe+P, -Fe+P, or -Fe-P for 52h. Data shown from 3 experiments. Error bars
454 represent 95% confidence interval. **D)** Confocal microscopy images of
455 p35S::bZIP58::GFP expressing plants (scale bar = 20 μ m) grown for 7 days under
456 +Fe+P. **E)** Representative images of Col-0, *bzip58*, and a line expressing genomic
457 *bZIP58* in *bzip58* mutants (*bzip58-CL*) grown for 7 days in +Fe+P and transferred to
458 +Fe+P, -Fe+P, or -Fe-P for 7 additional days. Scale bars: 7 mm. **F)** Total chlorophyll
459 content in Col-0, *bzip58*, and *bZIP58-CL* plants grown for 7 days in +Fe+P and
460 transferred to -Fe+P, +Fe-P, -Fe-P, or -Fe-P+AsA for 7 days. Data shown are from 4
461 experiments. **G)** A schematic model delineating a signaling pathway that integrates
462 Fe and P availability cues to regulate chlorophyll accumulation and photosynthesis
463 genes. Fe deficiency (-Fe+P) causes a decrease in the expression of *bZIP58* that is
464 central to controlling the transcription of nuclear-encoded photosynthetic genes. P
465 limitation under Fe deficiency (-Fe-P) prevents this downregulation of *bZIP58* and
466 induces *VTC4*. The induction of *VTC4* expression requires bZIP58, whose effect
467 could be direct or indirect, represented here by 'X'. We propose that induction of
468 *VTC4* increases ascorbic acid in the chloroplast mediated by PHT4;4. We

469 hypothesize that the increase of ascorbic acid level prevents ROS accumulation, thus
470 maintaining the expression of bZIP58 and its downstream photosynthesis genes
471 leading to the 'stay green' phenotype. **H**) Accumulation of H₂O₂ (a type of ROS) in
472 shoots of Col-0, *bzip58*, and *bZIP58-CL* plants grown for 7 days in +Fe+P and
473 transferred to +Fe+P, -Fe+P, or -Fe-P for 52h. Data shown from 12 experiments. In
474 box plots (F, H) center lines show sample medians; box limits indicate the 25th and
475 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and
476 75th percentiles. For B, C, F, H, letters above bars or boxes represent statistically
477 different means at $P < 0.05$ (one-way ANOVA with a Duncan post-hoc test). Data
478 points are plotted as open circles. Source data are provided as a Source Data file.

479

480 **Supplemental Figures**

481 **Figure S1. Photosystem II activity in response to Fe and/or P deficiency in *A.***
482 ***thaliana*.** Seedlings were grown for 7 days in the presence of Fe and P (+Fe+P) and
483 transferred to three different media: +Fe+P, -Fe+P, or -Fe-P for 15h, 28h, 39h, 52h,
484 76h, 100h, 124h, 148h, 172h. Plants were dark-adapted for 30 min before measuring
485 the kinetics of fluorescence from a leaf. Data shown are from 3 experiments and 13
486 to 16 plants were measured per experiment. Error bars represent 95% confidence
487 interval. Source data are provided as a Source Data file.

488

489 **Figure S2. Transcriptome kinetics of *A. thaliana* in response to Fe and/or P**
490 **deficiency.** Volcano plots of individual transcript abundance in wild-type plants (Col-
491 0) grown in -Fe+P (A,C,E) or -Fe-P (B,D,F) relative to +Fe+P. Shoot samples were
492 collected from plants grown for 39h (A,B), 52h (C,D), or 76h (E,F). x-axis: fold-
493 changes; y-axis: adjusted p-values based on Benjamini-Hochberg correction; Both
494 axes use log scales. Red: $\log_2\text{FoldChange} > |2|$ and $-\log_{10}P > 6$; Blue:
495 $\log_2\text{FoldChange} < |2|$ and $-\log_{10}P > 6$; Green: $\log_2\text{FoldChange} > |2|$ and $-\log_{10}P < 6$;
496 Grey: $\log_2\text{FoldChange} < |2|$ and $-\log_{10}P < 6$.

497

498 **Figure S3. Common and unique genes regulated by Fe and/or P deficiency. A-**
499 **C)** Venn diagrams show the genes that are commonly and specifically increased or
500 decreased in abundance in shoots of *A. thaliana* wild type (Col-0) plants grown in

501 +Fe+P for 7 days and transferred to -Fe+P or -Fe-P for 39h, 52h, or 76h relative to
502 those transferred to +Fe+P (fold change >2, $p < 0.05$). The Venn diagram was
503 constructed using a web-based tool
504 (<http://bioinformatics.psb.ugent.be/webtools/Venn/>). p = p-values from
505 hypergeometric testing.

506

507 **Figure S4. Gene Ontology enrichment analysis of genes specifically regulated**
508 **by Fe and/or P deficiency. A, C)** Heatmaps representing changes in the expression
509 of genes that were specifically decreased in abundance under -Fe-P but not -Fe+P
510 relative to +Fe+P (**A**) and increased in abundance in -Fe-P but not in -Fe+P relative
511 to +Fe+P (**C**). **E)** A heatmap representing the expression of genes specifically
512 upregulated by -Fe+P but not by -Fe-P relative to +Fe+P. **B, D, F)** Gene Ontology
513 enrichment for biological processes (GO-BP) in the genes that were specifically
514 decreased by -Fe-P relative to +Fe+P (**B**), specifically increased by -Fe-P relative to
515 +Fe+P (**D**), and specifically increased by -Fe+P relative to +Fe+P (**F**) using
516 GENEMANIA²². Number of genes in each functional category and adjusted p-values
517 for the enrichment are shown. FDR = false discovery rate.

518

519 **Figure S5. Correlation in the expression of the 32 photosynthesis genes**
520 **specifically downregulated by -Fe+P across 727 *A. thaliana* accessions. A)** A
521 heatmap of pairwise correlations (Pearson's correlation coefficient) in the 32 genes
522 across 727 Arabidopsis accessions grown under control conditions. The correlations
523 were calculated using normalized read counts. **B)** Principal Component (PC) Analysis
524 was performed using the expression of 32 genes in 727 Arabidopsis accessions. X-
525 and Y-axes show PC 1 and PC 2 that explain 89.5% and 5.9 % of the total variance,
526 respectively.

527

528 **Figure S6. A close-up view of chromosomes 1 and 4 around bZIP58 and**
529 **PHT4;4 respectively.** A Manhattan plot for genome-wide association mapping using
530 PC 1 of the expression profile of the 32 photosynthesis genes across 727
531 accessions. The five *A. thaliana* chromosomes are depicted by light and dark blue
532 colors. Blue and red horizontal dashed lines correspond to FDR 5% threshold and

533 Bonferroni $\alpha = 0.05$, respectively. Light blue rectangles indicate the significant SNPs
534 identified in this study. Below the Manhattan plot shows gene models located within a
535 20-kb genomic region surrounding the two QTLs pursued in this study. Source data
536 are provided as a Source Data file.

537
538 **Figure S7. Effects of Fe and/or P availability on chlorophyll content in *A.***
539 ***thaliana* Col-0 and mutants of candidate genes identified using GWAS.**

540 Chlorophyll content in Col-0 (CS60000), T-DNA insertion mutant lines in AT1G13570
541 (SALK_139877), AT1G13580 (SALK_150849), AT1G13590 (SALK_063177),
542 AT1G13605 (SALK_087271), AT1G13607 (SALK_130208), AT1G13608
543 (SALK_023173), AT1G13609 (SAIL_1243_E04), AT1G13610 (SAIL_897_D11),
544 At4g00355 (N469134), AT4G00360 (SALK_128714), AT4G00380 (SAIL_842_E09),
545 AT4G03585 (SALK_128714), At4g00390 (SAIL_313_F07), and At4g00400
546 (SAIL_633_E10) grown for 7 days in the presence of iron and phosphorus (+Fe+P)
547 and transferred to +Fe+P, -Fe+P, or -Fe-P for an additional week. Data shown from 3
548 experiments. Error bars represent 95% confidence interval. Letters a and b indicate
549 significantly different values at $p < 0.05$ determined by one-way ANOVA and Tukey's
550 honest significant difference (HSD) tests. Source data are provided as a Source Data
551 file.

552
553 **Supplemental tables**

554
555 **Table S1. Differentially expressed genes in response to iron and/or**
556 **phosphorus deficiency in *A. thaliana*.** Gene transcript levels were determined in
557 shoots of Col-0 plants grown in control (+Fe+P) condition for 7 days and then
558 transferred to -Fe+P, -Fe-P, or +Fe+P for 39h, 52h, or 76h.

559
560 **Table S2.** List of primers used in this study.

561
562 **Materials and Methods**

563 *Plants and growth conditions*

564 Seeds of *Arabidopsis thaliana* wild type (ecotype Columbia, Col-0, CS60000) and
565 knock-out mutant lines SALK_139877 (AT1G13570), SALK_150849 (AT1G13580),
566 SALK_063177 (AT1G13590), N571881 (AT1G13600), SALK_087271 (AT1G13605),
567 SALK_130208 (AT1G13607), SALK_023173 (AT1G13608), SAIL_1243_E04
568 (AT1G13609), SAIL_897_D11 (AT1G13610), N469134 (At4g00355), SALK_128714
569 (AT4G00360), N469134 (AT4G00370), SAIL_842_E09 (AT4G00380), N866595
570 (At4g00390), SAIL_633_E10 (At4g00400) and SALK_077222 (AT3G02870) were
571 obtained from the Nottingham Arabidopsis Stock Centre (NASC). Homozygous
572 mutant lines were confirmed by PCR using the primers listed in Table S2. bZIP58
573 complemented lines (bZIP58-CL) were generated by expressing 3896 bp genomic
574 DNA containing *bZIP58* in the *bzip58* mutant background (NASC, N571881).
575 Complementation of *pht4;4* mutant plants (PHT4;4-CL) was obtained by expressing
576 6450 bp genomic DNA containing *PHT4;4* in the *pht4;4* mutant background (NASC,
577 N469134). Arabidopsis plants were grown on control (+Fe+P) plates containing 1.249
578 mM KH_2PO_4 ; 0.25 mM $\text{Ca}(\text{NO}_3)_2$; 0.5 mM KNO_3 ; 1 mM MgSO_4 ; 100 μM
579 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 30 μM H_3BO_3 ; 1 μM ZnCl_2 ; 10 μM MnCl_2 ; 1 μM CuCl_2 ; 0.1 μM
580 $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$; and 50 μM KCl ; 0.05% 2-(N-morpholino)ethanesulfonic acid (MES),
581 without sucrose supplementation, and 0.8% washed agar. The agar was washed 3
582 times with 50 mM EDTA, pH 5.7, with continuous stirring for 16 h, then washed 6
583 times with Milli-Q de-ionized water for 2 hours to reduce mineral contamination²³. P-
584 deficient media contained 12.49 μM KH_2PO_4 (+Fe-P). Fe-free media was obtained by
585 omitting $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ from the growth media (-Fe+P). P- and Fe-deficient media
586 contained 12.49 μM KH_2PO_4 (+Fe-P), and no $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (-Fe-P). Seeds were
587 stratified at 4°C for 3 days and grown on vertical agar plates in a growth chamber
588 with 22 °C, 24 h of light at 100 $\mu\text{mol m}^{-2}\text{s}^{-1}$ fluorescent illumination. *Lemna gibba*
589 (duckweed) plants used during this study were obtained from Duckweeds stock
590 center (stock number 29-DWC131) at Rutgers University (USA). Duckweed plants
591 were grown in 1X Schenk & Hildebrandt (SH) hydroponic medium containing 0.05%
592 2-(N-morpholino)ethanesulfonic acid (MES) and 1% sucrose, and pH adjusted to 5.7.
593 For experiments with duckweeds, P-deficient and Fe-deficient media contained 1%
594 $\text{NH}_4\text{H}_2\text{PO}_4$ and 1% $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, respectively, of 1X SH media. Media were changed
595 every 7 days. The growth condition was 22 °C and 24 h of light at 80 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

596 Rice (*Oryza sativa* cv Nipponbare) plants were grown hydroponically in 0.25X
597 Yoshida media²⁴ under light/dark cycle of 14/10 h, and temperature of 28/25 °C.
598 Single (-P or -Fe) and combined (-P-Fe) nutrient deficiency stresses were applied to
599 10 day-old plants. NaH₂PO₄ (0.33 mM) and Fe-NaEDTA (0.04 mM) present in the
600 complete media were omitted in the P- and/or Fe-deficient media.

601

602 *Iron concentration measurement*

603 Arabidopsis seeds were germinated and grown in the control (+Fe+P) media for 7
604 days, and then transferred to +Fe+P, iron deficient (-Fe+P), phosphate deficient
605 (+Fe-P), or iron and phosphate deficient (-Fe-P) conditions and grown for 7 additional
606 days. Plants were harvested and shoot samples were dried at 70 °C for 3 days. Total
607 iron was extracted by acid digestion in 1N nitric acid using MARSX (CEM) microwave
608 digester. A 1:10 dilution of the digested material was used to quantify total iron with
609 inductively coupled plasma-atomic emission spectrometry (ICP-OES).

610

611 *Analysis of photosystem II activity*

612 Photosystem II (PSII) activity was defined as the maximum quantum yield of the
613 primary quinone acceptor PSII, which was estimated by the ratio of variable
614 fluorescence (Fv) and maximal fluorescence (Fm) of the chlorophyll, Fv/Fm²⁵.
615 Arabidopsis wild type (Col-0) seeds were germinated and grown in control (+Fe+P)
616 for 7 days then transferred to three different media: +Fe+P, iron deficient (-Fe+P),
617 and iron and phosphate deficient (-Fe-P) conditions for 0 h (time of the transfer), 15
618 h, 28 h, 39 h, 52 h, 76 h, 100 h, 124 h, 148 h, and 172 h. Plates containing the
619 seedlings were dark adapted for 30 minutes followed by a very short (160 μs)
620 exposure to a blue measuring beam to determine the minimal fluorescence (F0). The
621 intensity of the detecting and the continuous illumination used was of 156 μE m⁻²s⁻¹.
622 A saturating light flash (2600 μE m⁻²s⁻¹, 250 ms) was applied to measure the
623 maximum fluorescence (Fm). Kinetics were normalized to the maximum fluorescence
624 (Fm). The maximum quantum yield of Photosystem II (Fv/Fm = (Fm – F0)/Fm) was
625 measured for each growth condition²⁵.

626

627 *Chlorophyll content measurement*

628 Seeds of Arabidopsis genotypes were germinated and grown in control (+Fe+P)
629 media for 7 days then transferred to three different media: +Fe+P, iron deficient (-
630 Fe+P), and iron and phosphate deficient (-Fe-P) conditions. Fresh leaves (~30mg)
631 were incubated in 2.5mL of 80% acetone overnight in the dark at 4°C. Total
632 chlorophyll content was measured using a UV-VIS spectrophotometer (Beckman
633 Coulter, DU 530). The absorbance of the supernatant was measured at 645 nm and
634 633 nm. The concentration of total chlorophyll, chlorophyll a, and chlorophyll b were
635 calculated as described previously²⁶.

636

637 *Ascorbic acid content determination*

638 Seeds of Arabidopsis genotypes were germinated and grown in control (+Fe+P)
639 media and then transferred to +Fe+P, -Fe+P, or -Fe-P media for 76h. Ascorbic acid
640 (AsA) content was measured by a colorimetric assay as described previously²⁷.
641 Briefly, shoots were collected and homogenized in ice-cold 6% trichloroacetic acid
642 (TCA) (Sigma Aldrich). In the supernatant, Fe³⁺ (ferric ion) is reduced by AsA to the
643 Fe²⁺ (ferrous ion) that, when coupled with 2,2-dipyridyl, forms a complex with a
644 characteristic absorbance at 525 nm. A standard curve was generated using known
645 concentrations of AsA made in 6% TCA to determine the AsA concentration. Blanks
646 were prepared using only 6% TCA. AsA concentration was expressed as $\mu\text{mol g}^{-1}$
647 fresh weight.

648

649 *Hydrogen peroxide quantification*

650 Seeds of Arabidopsis genotypes were germinated and grown in control (+Fe+P)
651 media and then transferred to +Fe+P, -Fe+P, or -Fe-P media for 76h. Hydrogen
652 peroxide (H₂O₂) (Sigma-Aldrich) was quantified as described previously^{28,29}. Fresh
653 shoot tissues (0.2g) were homogenized with 0.1% (w/v) TCA and were centrifuged at
654 12,000g for 15 min at 4°C. 0.5 ml of supernatant was added to 0.5 ml of 10 mM
655 potassium phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide. The
656 absorbance of the reaction mixture was measured at 390 nm. The amount of H₂O₂
657 was calculated using a standard curve prepared from known concentrations of H₂O₂
658 ranging from 0.1 to 1 mM.

659

660 *RNA-seq experiments*

661 Arabidopsis wild type (Col-0) plants were grown in control (+Fe+P) media for 7 days
662 and transferred to three different media: control (+Fe+P), iron deficiency (-Fe+P), and
663 iron and phosphate deficiency (-Fe-P) conditions. Shoots were collected at 39h, 52h
664 and 76h after the transfer. For RNA-seq experiments, three biological replicates were
665 prepared for each time point (39h, 52h and 76h) and each condition (+Fe+P, -Fe+P
666 and -Fe-P) for a total of 27 samples. Total RNA was extracted from these samples
667 using RNeasy Plant Mini Kit (QIAGEN) using the RLT buffer supplemented with 2-
668 mercaptoethanol, and RNA quality was verified using an Agilent 2100 BioAnalyzer.
669 The mRNAs were subsequently isolated using magnetic KAPA Biosystems oligo-dT
670 beads from KAPA Biosystems (Roche) and then used for library construction using
671 the KAPA Biosystems RNA HyperPrep Kit (Roche). To index the libraries, we used
672 adapters from the KAPA Biosystems Single-Indexed Adapter Set A+B (Roche).
673 Before pooling the libraries, we monitored their quality and concentrations using an
674 Agilent 2100 BioAnalyzer, Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) and
675 the KAPA Library Quantification Kit (Roche). Pooled libraries were then sequenced
676 using the NextSeq 500 System at the Stanford Functional Genomics Facility
677 (Stanford, CA). Raw reads were demultiplexed and aligned to the TAIR10 genome
678 annotation using HISAT2³⁰ on the Galaxy web platform³¹. Finally, mapped read
679 counts were used to perform normalization and a differential expression analysis on
680 R using the DESeq2³² and TxDB.Athaliana.BioMart.plantsmart²⁸ (Bioconductor)
681 packages. In DESeq2, p-values from the Wald test were corrected for multiple
682 hypothesis testing using the Benjamini and Hochberg method. A transcript was
683 considered differentially expressed if the adjusted p-value < 0.05. Volcano plots were
684 generated using the EnhancedVolcano package (version 1.6.0) (Bioconductor) with a
685 default cut-off of $\log_2(\text{Fold Change}) > |2|$ and adjusted p value < $10e^{-6}$. DEGs having
686 a p-value of 0 were converted to 10^{-1} x lowest non-zero p-value.

687

688 *Real-time quantitative reverse-transcription PCR*

689 Seeds of Arabidopsis wild type (Col-0), *bzip58*, and *pht4;4* mutant plants were
690 germinated and grown for 7 days in control (+Fe+P) media, and then transferred to

691 +Fe+P, Fe+P, or -Fe-P. Shoot tissues were collected at 76h after the transfer, and
692 then used for total RNA extraction as described in³³. Each experiment was conducted
693 with 16 plants and 4-6 plants were pooled for RNA extraction, resulting in 3-4
694 biological replicates. Two μ g of the total RNA was used for reverse transcription
695 (Promega) to synthesize cDNA using oligo(dT) primer (Promega). Real-time
696 quantitative reverse-transcription PCR (qRT-PCR) was performed as described in³³
697 using 384-well plates with a LightCycler 480 Real-Time PCR System (Roche
698 diagnostics). The *Ubiquitin 10* mRNA (*UBQ10*: At4g05320) was used as control to
699 calculate the relative mRNA level of each gene. The primers used in this study are
700 listed in Table S2.

701

702 *Genome wide association studies (GWAS)*

703 Gene expression data of the 32 genes that were specifically downregulated by -Fe+P
704 but not by -Fe-P relative to +Fe+P were downloaded from leaf expression data of 727
705 Arabidopsis accessions¹⁵. Normalized RNA-seq read counts of these genes were
706 used to perform Principal Component Analysis, and contributions of the accessions
707 to PC1 that explained 89.5% of the expression variance of the 32 genes were used to
708 run genome-wide association (GWA) analysis. GWA mapping was performed using
709 1001 genomes SNP data³⁴ as implemented in the web application GWAPP³⁵.
710 Bonferroni correction ($\alpha = 0.05$) and false-discovery rate (FDR) at 5%³⁶ were
711 implemented to account for multiple hypothesis tests.

712 *Statistical analysis*

713 Box plots were generated using a web based application "BoxPlotR"³⁷. Statistical
714 analyses of the data were performed using analysis of variance (ANOVA). One-way
715 ANOVA with a Duncan post-hoc test, and two-way analysis of variance (ANOVA) and
716 Tukey's honest significant difference (HSD) test were used to compare mean values.
717 For all the statistical analyses, the difference was considered statistically significant
718 when the test yielded a p-value < 0.05 .

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726

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736

737 **Competing interests**

738 No competing interests declared.

739

740 **Contributions**

741 S.Y.R. and H.R. conceived the project. Experiments were designed by S.Y.R., H.R.,
742 H.N., and Z.S. and mainly carried out by H.N. S.C. performed and analyzed
743 experiments related to photosystem II activity. bZIP58-GFP localization, ascorbic acid
744 quantification and hydrogen peroxide assays were conducted by H.C. and N.B. RNA-
745 seq data were generated and analyzed by Y.D. Gene Ontology analysis was
746 performed K.Z. Z.S. performed the genome-wide association mapping. H.R.
747 performed the qRT-PCR analyses, generated plasmid constructs, the homozygote
748 mutants, and the complemented mutant lines. S.Y.R., H.R. and Z.S. wrote the paper
749 with input from all authors.

750

751 **Data availability**

752 Data supporting the findings of this work are available within the paper and its
753 Supplementary Information and Source Data files. The datasets and plant materials
754 generated and analyzed during the current study are available from the
755 corresponding author H.R. upon request. Transcriptome data were deposited in
756 NCBI's Gene Expression Omnibus (GEO) under a project number GSE163190.

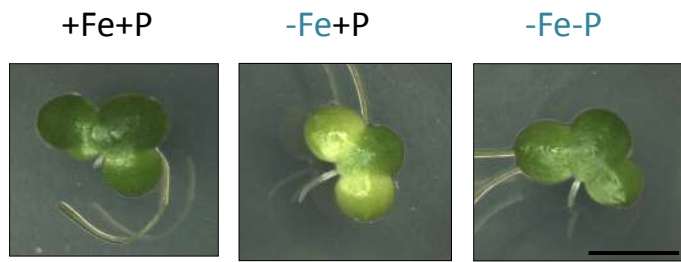
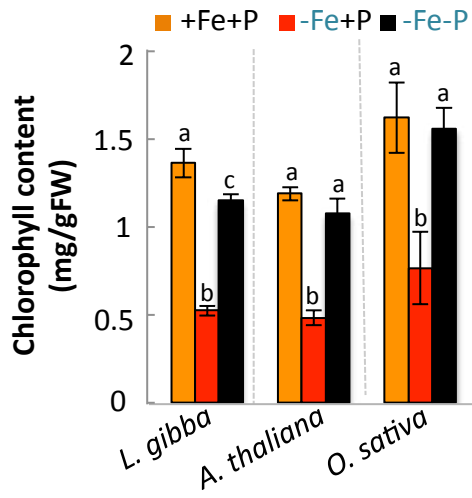
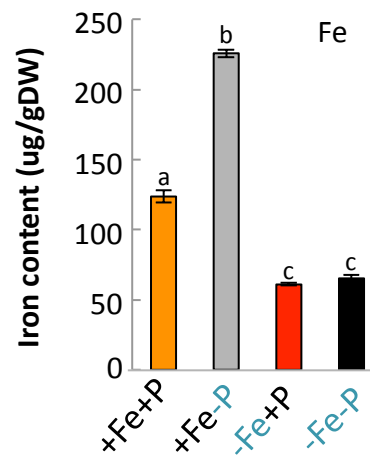
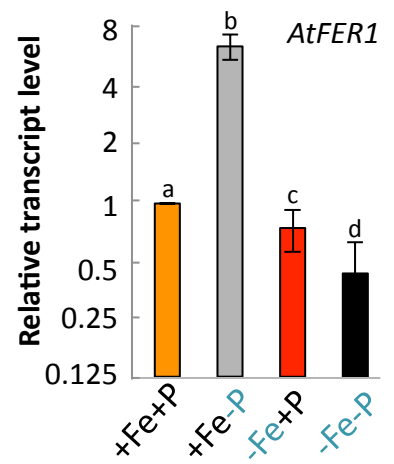
Figure 1**A****B****C****D****E****F**

Figure 2

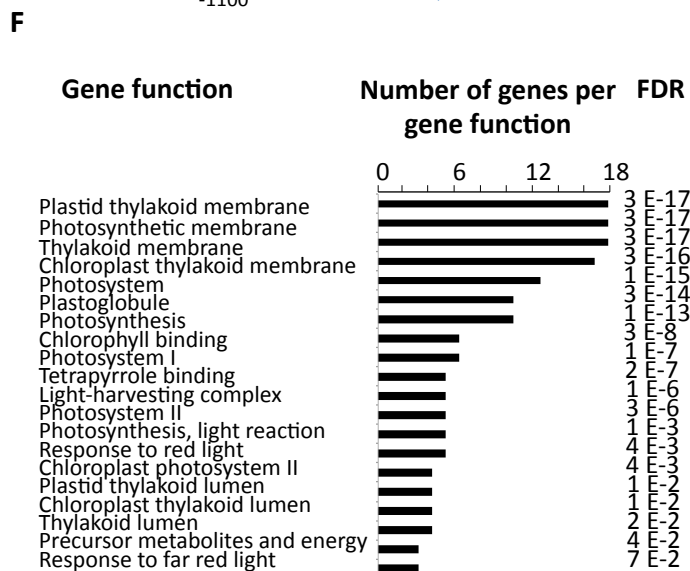
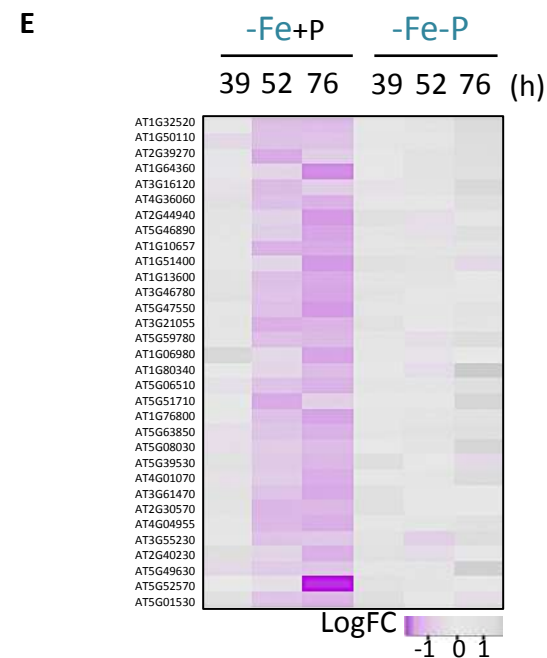
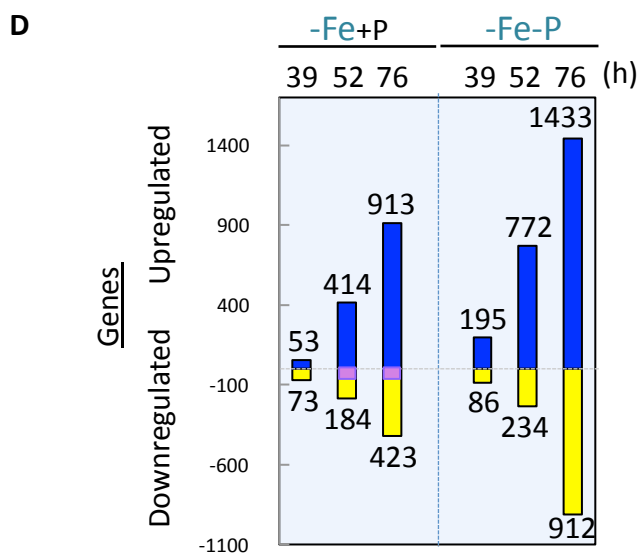
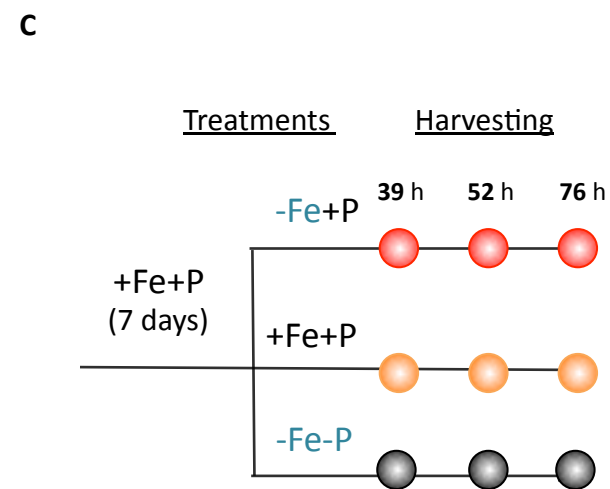
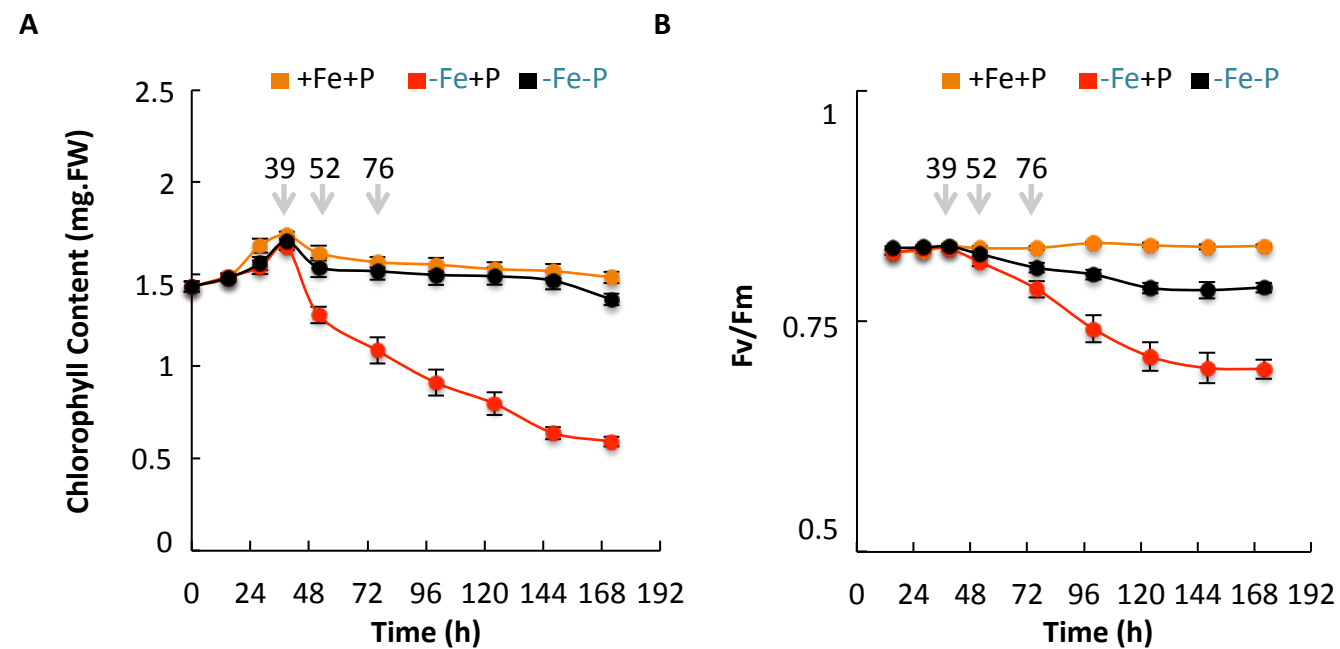


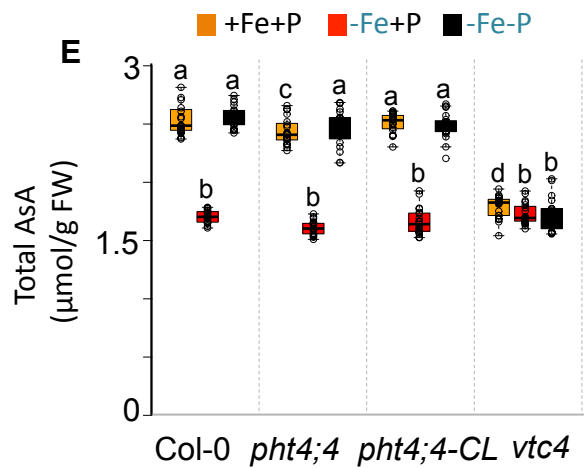
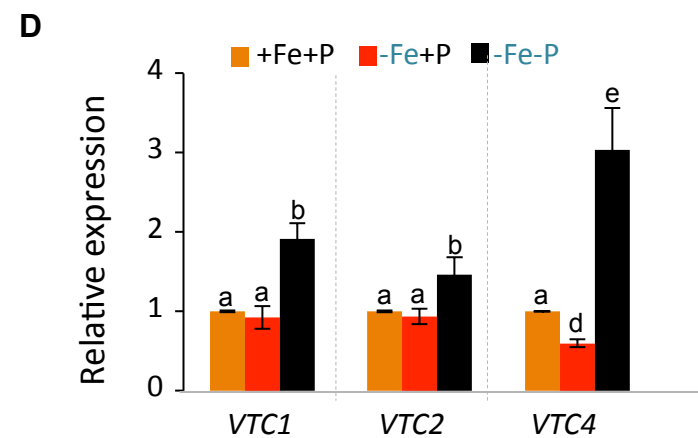
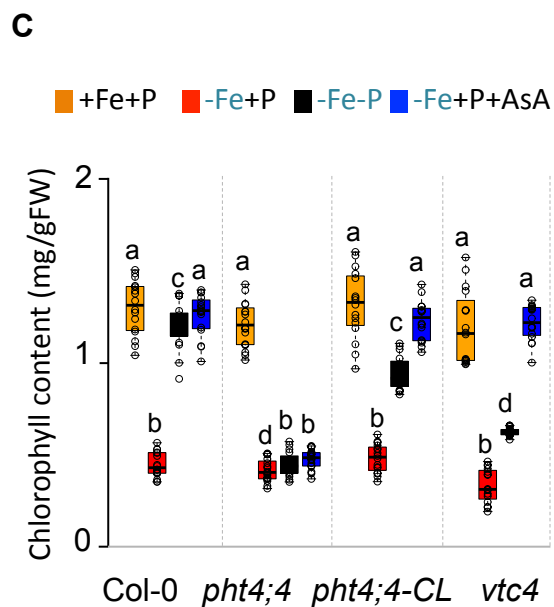
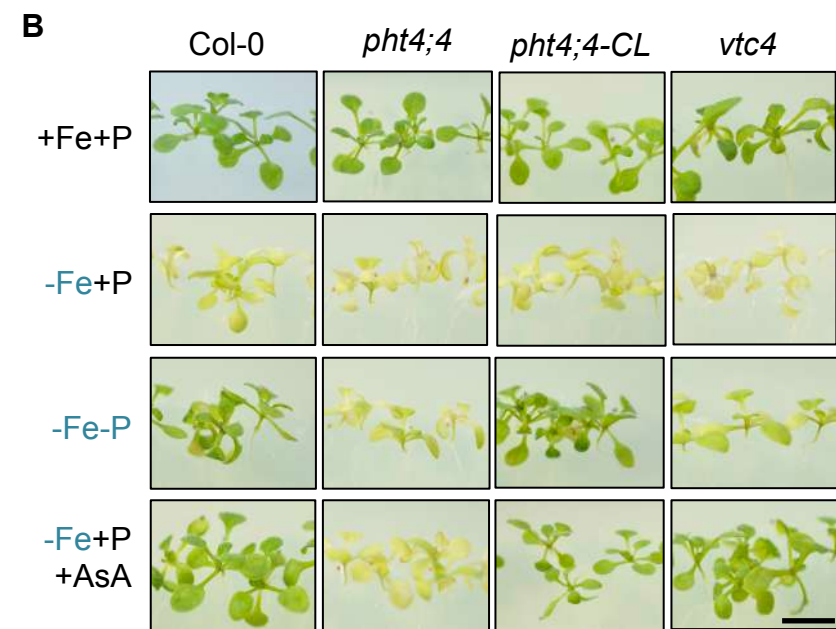
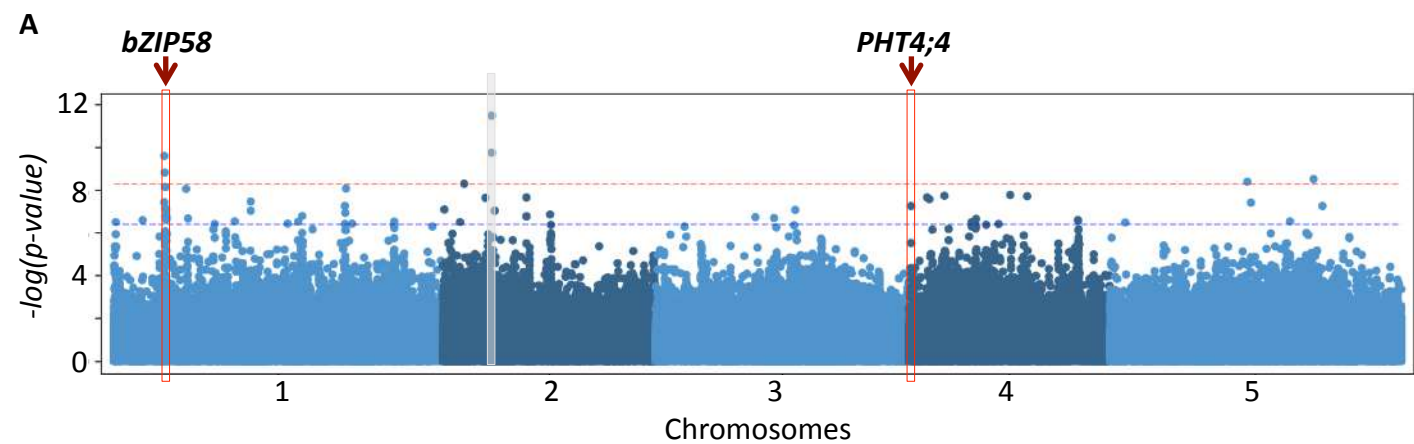
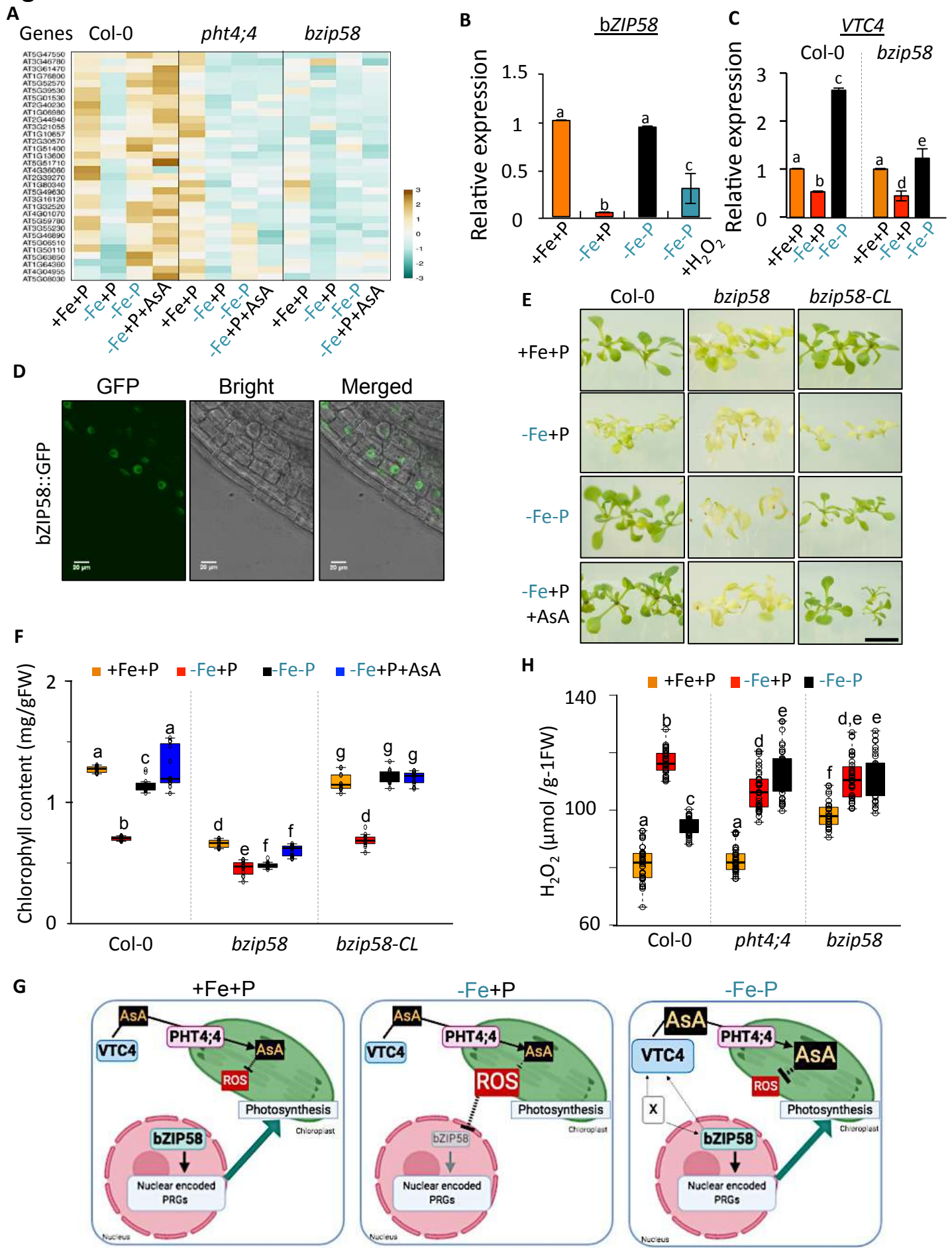
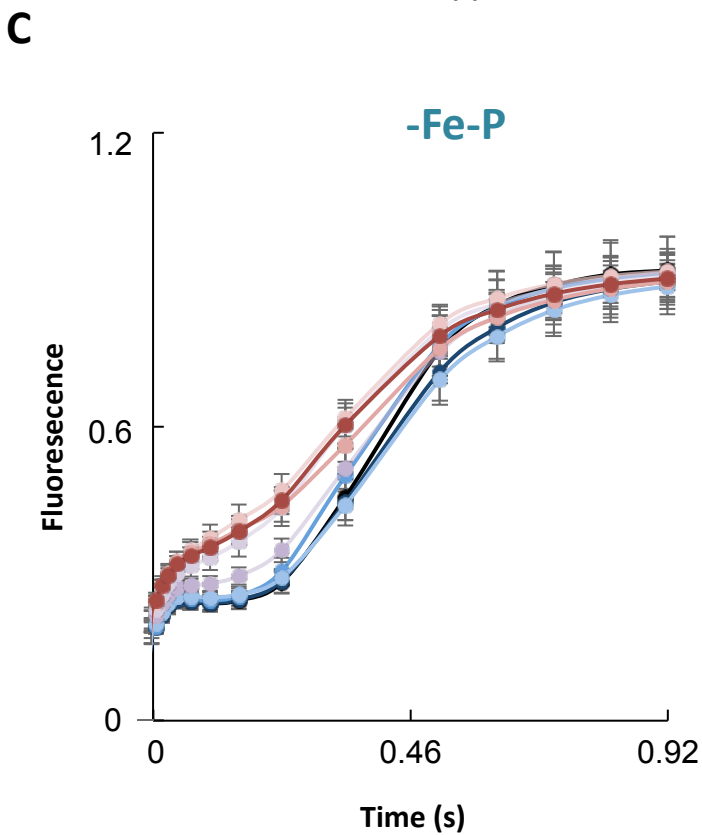
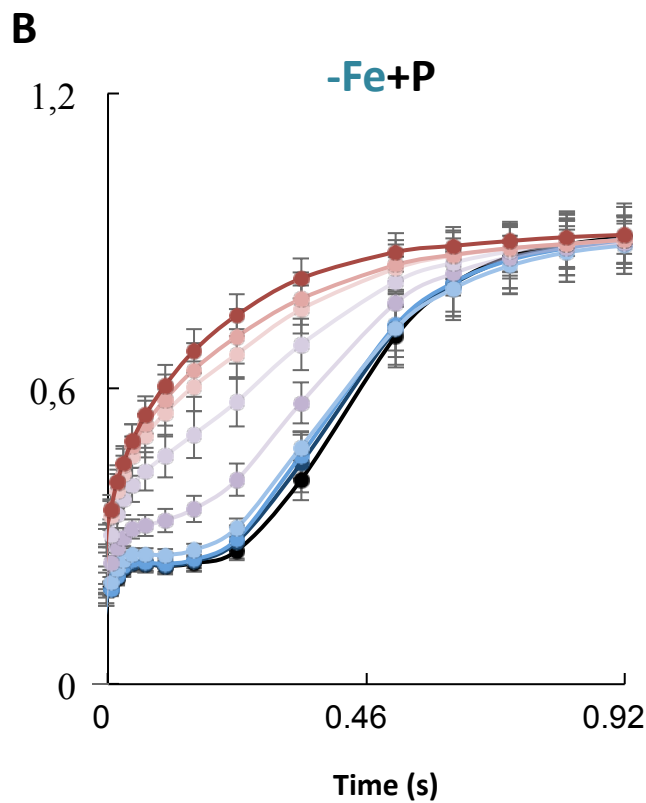
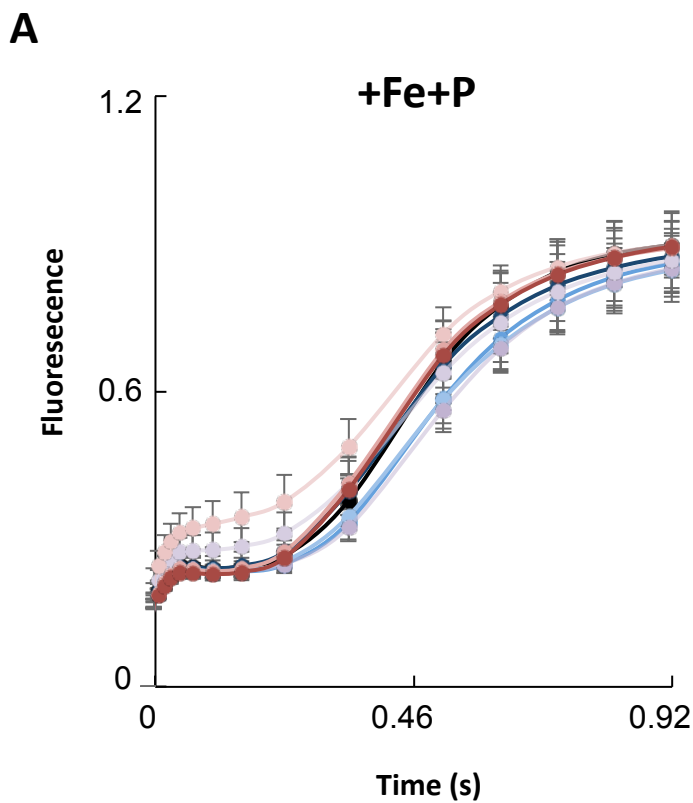
Figure 3

Figure 4

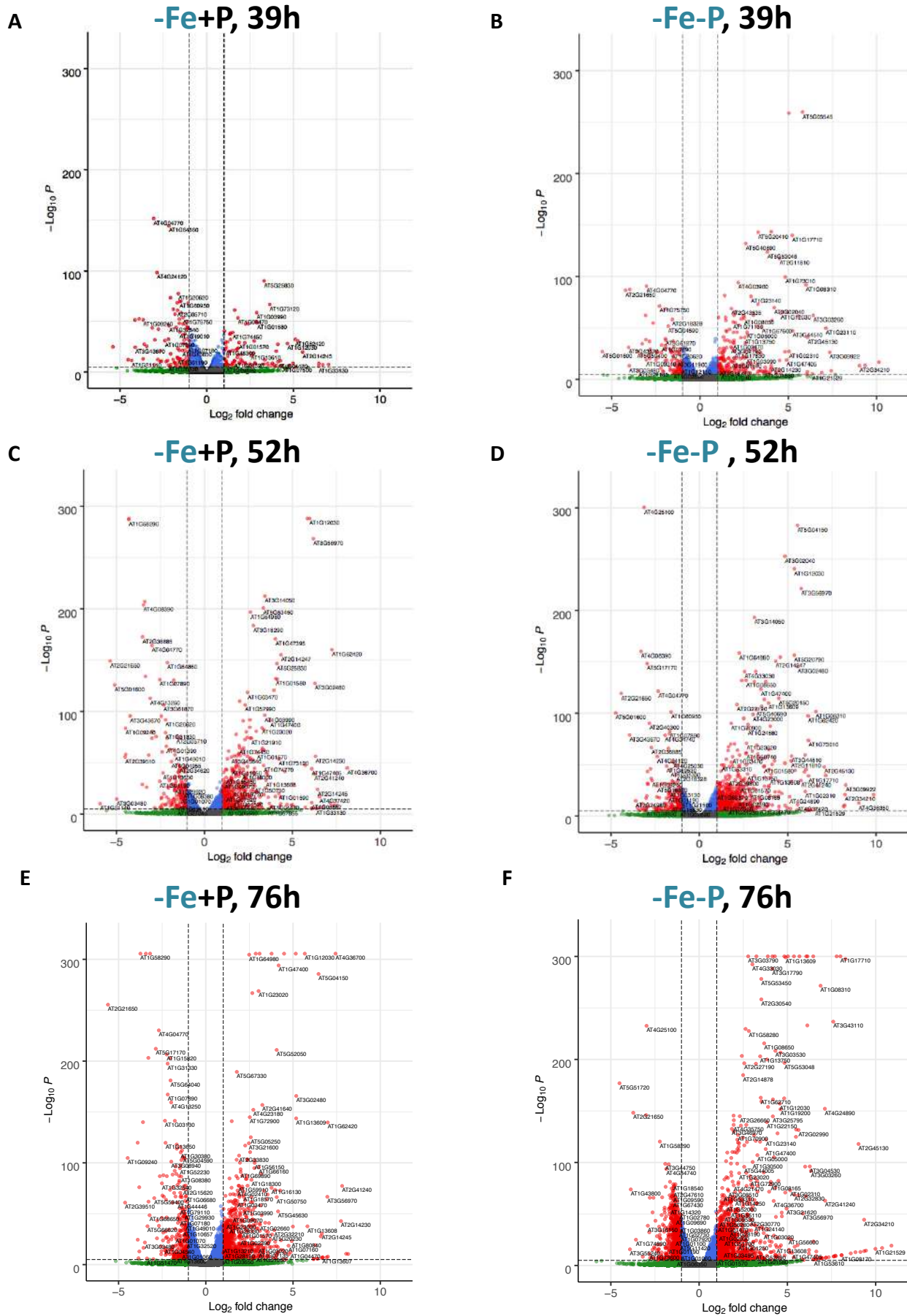


Supplemental Figure 1



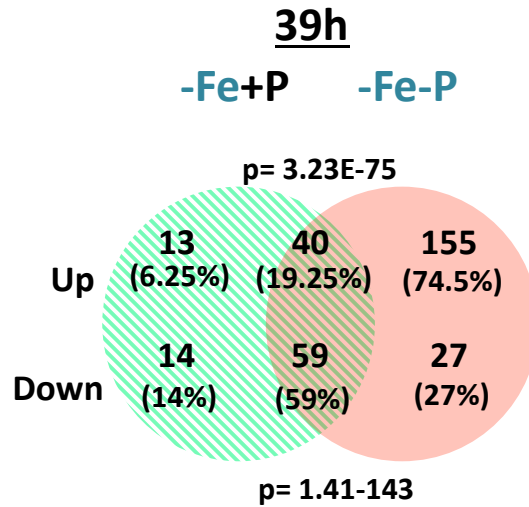
Supplemental Figure 2

● NS ● Log_2 FC ● p-value ● p-value and Log_2 FC

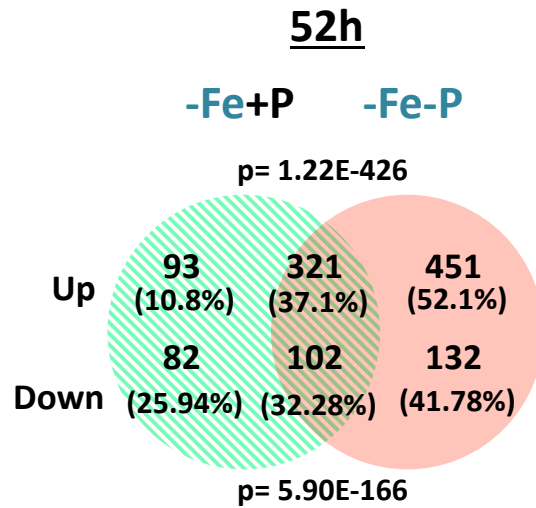


Supplemental Figure 3

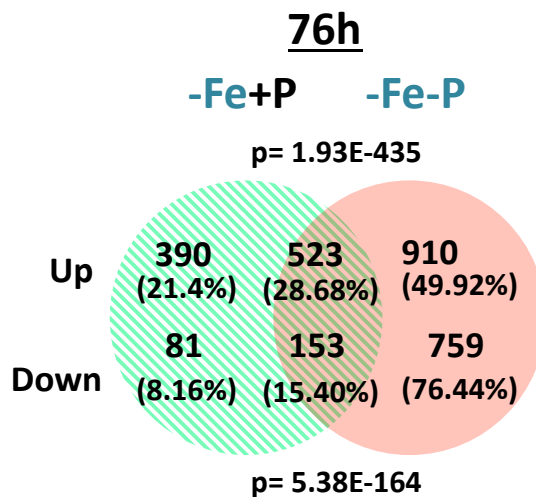
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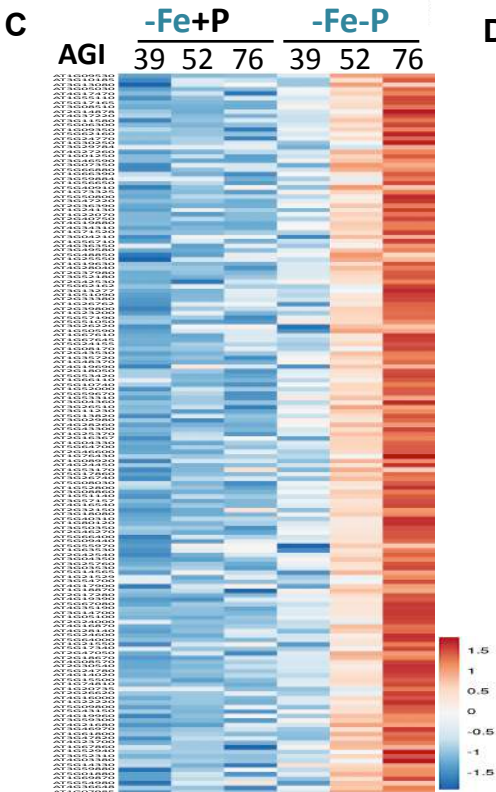
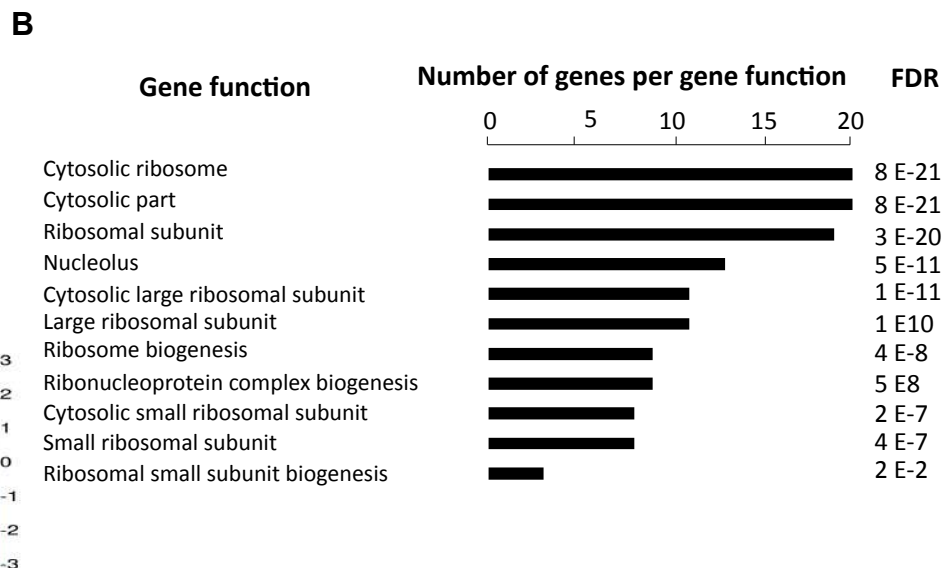
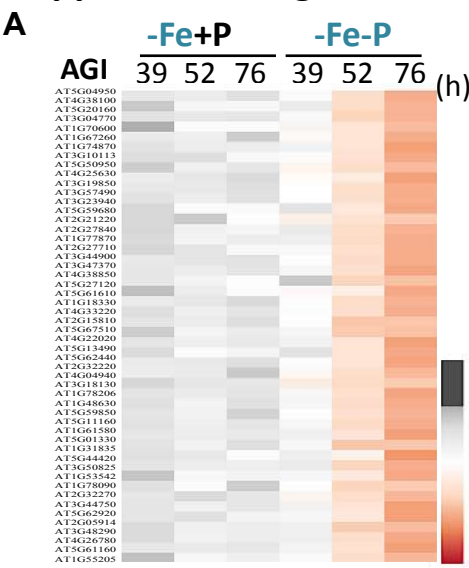
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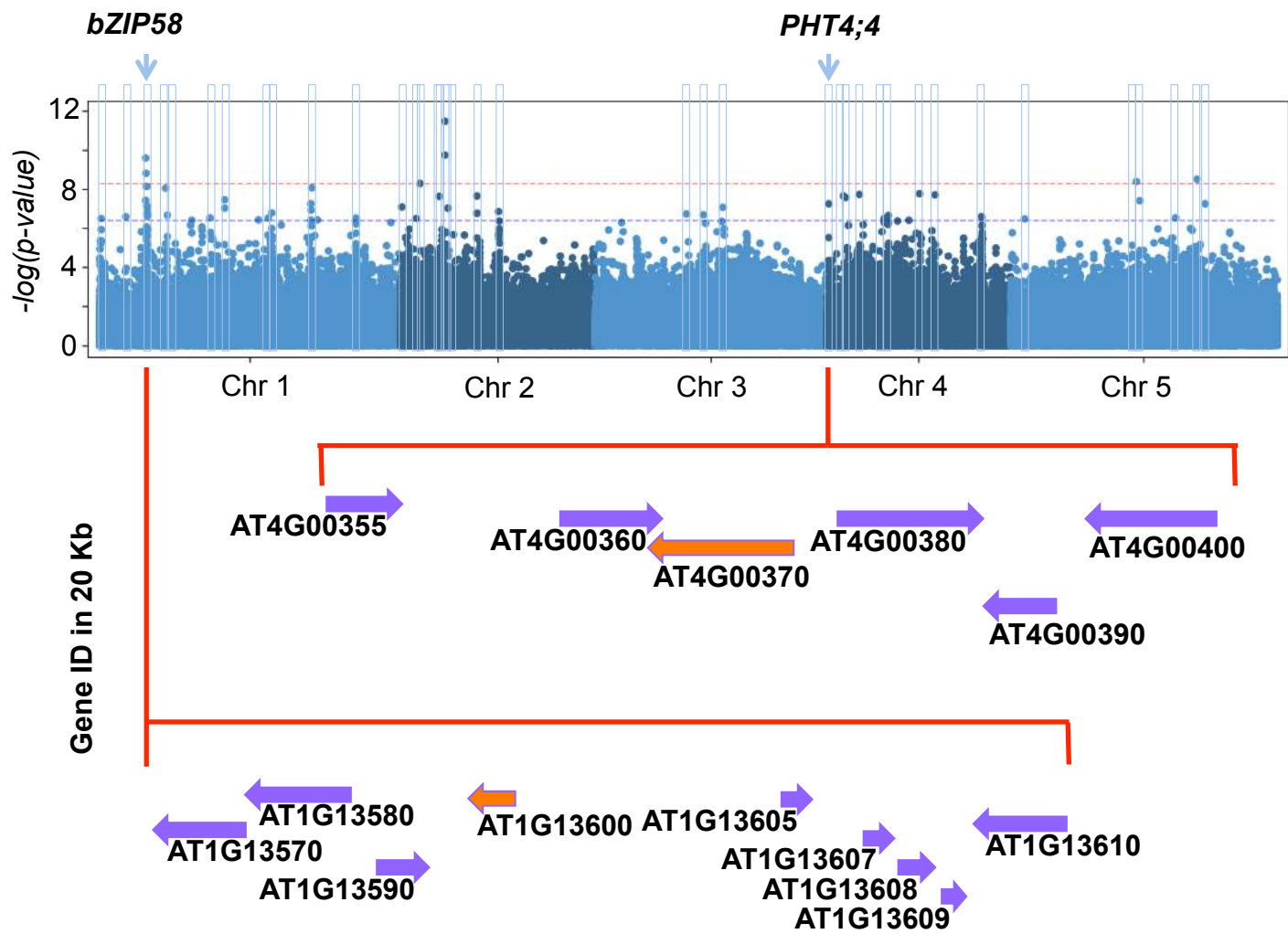
C



Supplemental Figure 4



Supplemental Figure 6



Supplemental Figure 7

