Supplementary materials

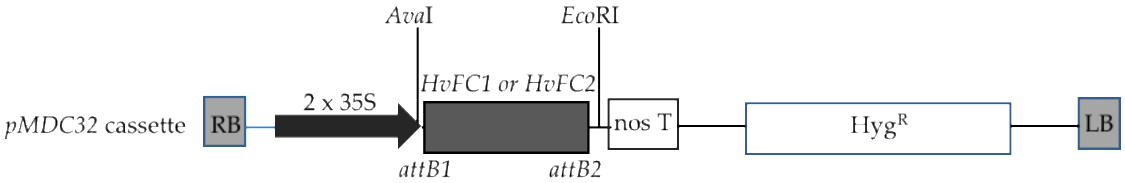
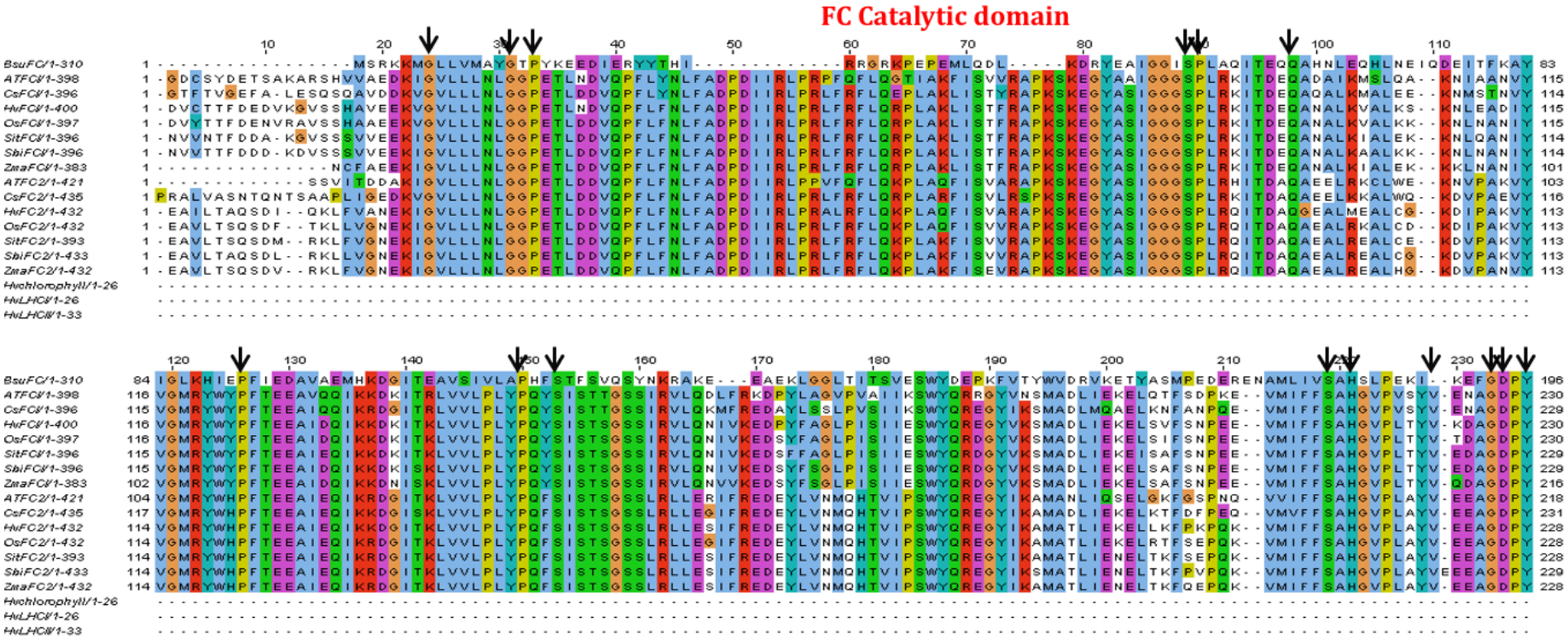


Figure S1. A schematic illustration of the pMDC32 constitutive expression vector used for barley transformation, which harbours a dual 35S promoter, and either *HvFC1* or *HvFC2*.



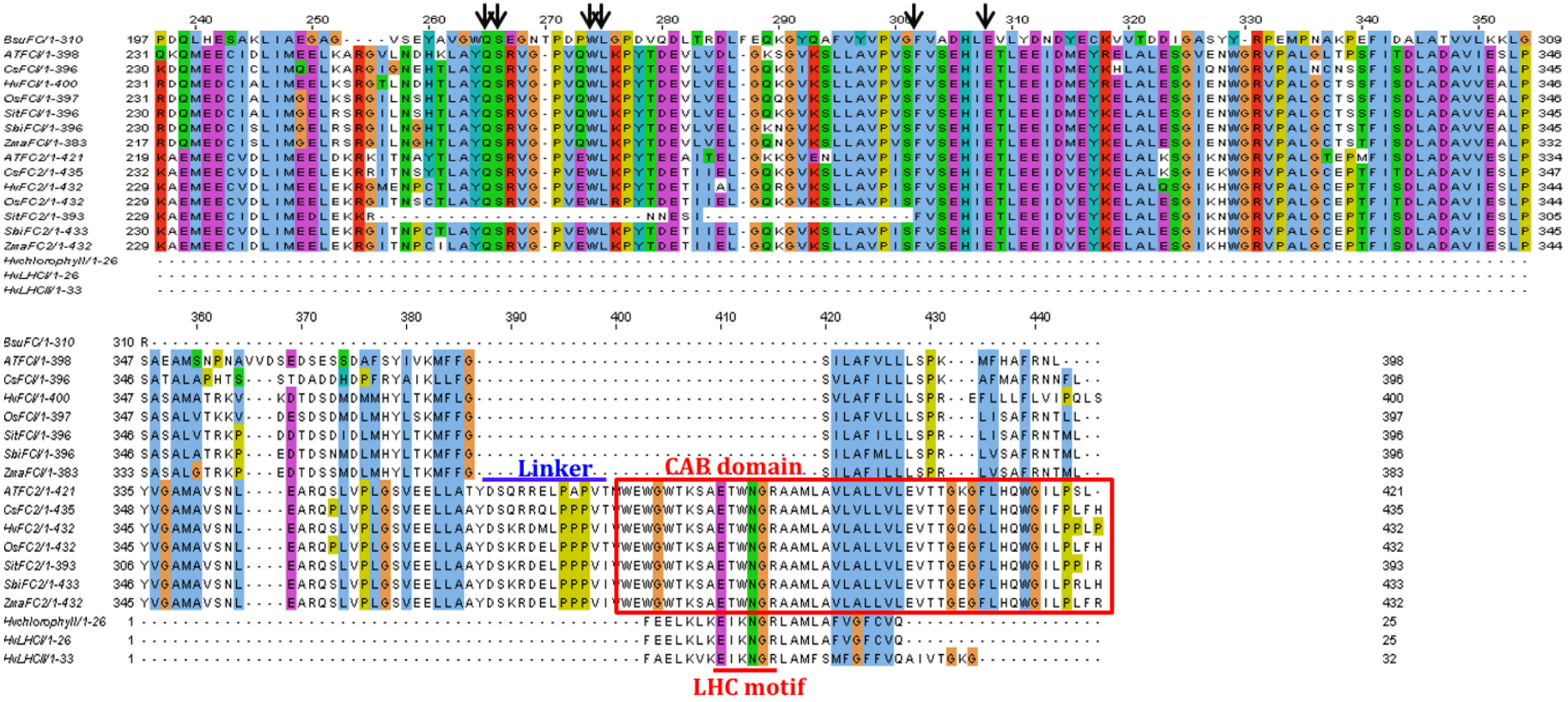


Figure S2. Similarity comparison of primary polypeptide sequences of barley Ferrochelatase 1 (FC1) and barley Ferrochelatase 2 (FC2) to respective FC counterparts of other plant species: Bsu, *Bacillus subtilis*; At-*Arabidopsis* (*Arabidopsis thaliana*); Cs, cucumber (*Cucumis sativa*); Hv, barley (*Hordeum vulgare*); Os, rice (*Oryza sativa*); Sit, foxtail millet (*Setaria italica*); Sbi, Sorghum (*Sorghum bicolor*); Zma, Maize (*Zea maize*) and barley chlorophyll binding proteins (Hvchlorophyll binding protein, HvLHCI, HvLHCII) which contains C-terminal light harvesting complex (LHC) motif. The alignment was generated by using the programs MUSCLE and Jalview. Arrows indicate the conserved residues with deduced functions based on the biochemical studies or from the crystal structure of the *B. subtilis* enzyme (Al-Karadaghi et al., 1997). Red box indicates the truncated N-terminal portion of the chlorophyll a/b binding (CAB) domain which contains LHC motif, the characteristic feature of FC2. Blue line indicates the prolyne-rich linker sequence, which connects CAB domain to the FC catalytic core.

Table S1. Primers used in this study

Promers used for genotyping	Primer orientation	Sequence
Hygromycin	Fwd	CGCTCGTCTGGCTAAGATCG
	Rev	AGGGTGTCACGTTGCAAGAC
Transgene GOI	Fwd	CGAGGCGCGCCAAGCTATCAAA
	Rev	AATTCGAGCTCCACCGCGGT
qRT-PCR primer pairs		
HvFC1	Fwd	CGAGCATATTGAGAGACTGG
	Rev	TCACTGAAGAGTGTTCCGGA
HvFC2	Fwd	GGCCTGCACCGCGTAATTTA
	Rev	GCAGCAGAACGCCAATTTTC
GAPDH	Fwd	GTGAGGCTGGTGCTGATTACG
	Rev	TGGTGCAGCTAGCATTTGAGAC
HSP70	Fwd	CGACCAGGGCAACCGCACCAC
	Rev	ACGGTGTTGATGGGGTTCATG
cyclophilin	Fwd	CCTGTCGTGTCGTCGGTCTAAA
	Rev	ACGCAGATCCAGCAGCCTAAAG
tubulin	Fwd	AGTGTCCTGTCCACCCACTC
	Rev	AGCATGAAGTGGATCCTTGG

Table S2. Phenotypic characterization of transgenic lines ectopically overexpressing *HvFC1* and *HvFC2* relative to WT and null controls

Line	Plant height (cm)	Number of leaves	Tiller number	Shoot dry weight (mg)	Root dry weight (mg)
WT	5.7 bc	4.2 a	4 a	45 abc	14 a
Null	5.4 bc	4.0 a	5 a	50.2 bc	18.6 a
2x35S::FC1-28	5.7 bc	4.0 a	4 a	61.5 c	15.3 a
2x35S::FC1-13	5.3 bc	4.0 a	4 a	38.6 ab	15.1 a
2x35S::FC1-17	4.3 a	3.5 a	5 a	29.4 a	8.7 a
2x35S::FC2-29	5.6 bc	4.3 a	4 a	44 abc	11.3 a
2x35S::FC2-25	6.3 c	4.1 a	4 a	48.8 abc	16.6 a
2x35S::FC2-9	4.9 ab	3.7 a	4 a	53.6 bc	22.2 a

Data are presented as mean ± standard error of five replicates. Means with the same letter within a column are not significantly different at $P<0.05$, one-way ANOVA.