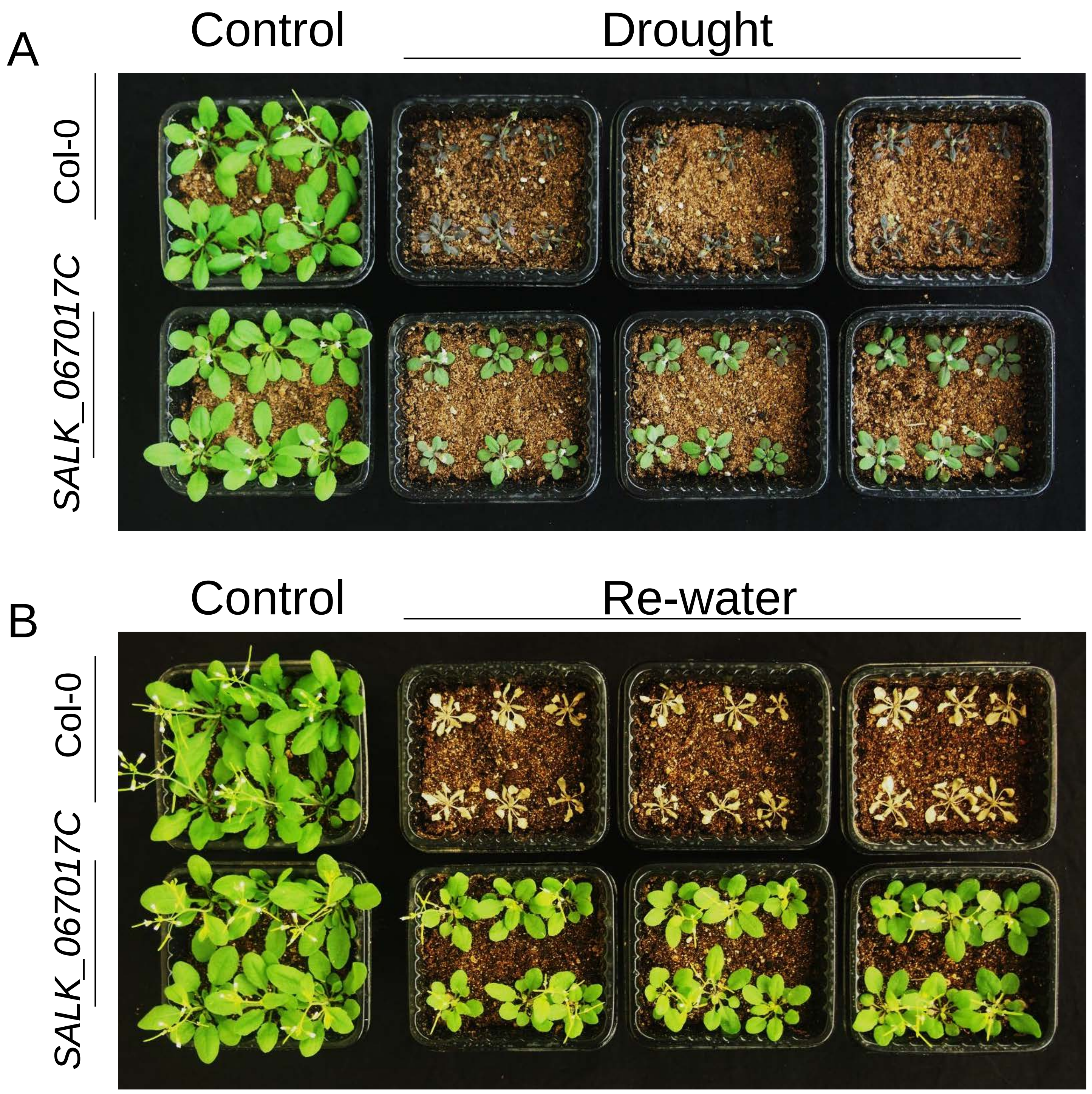
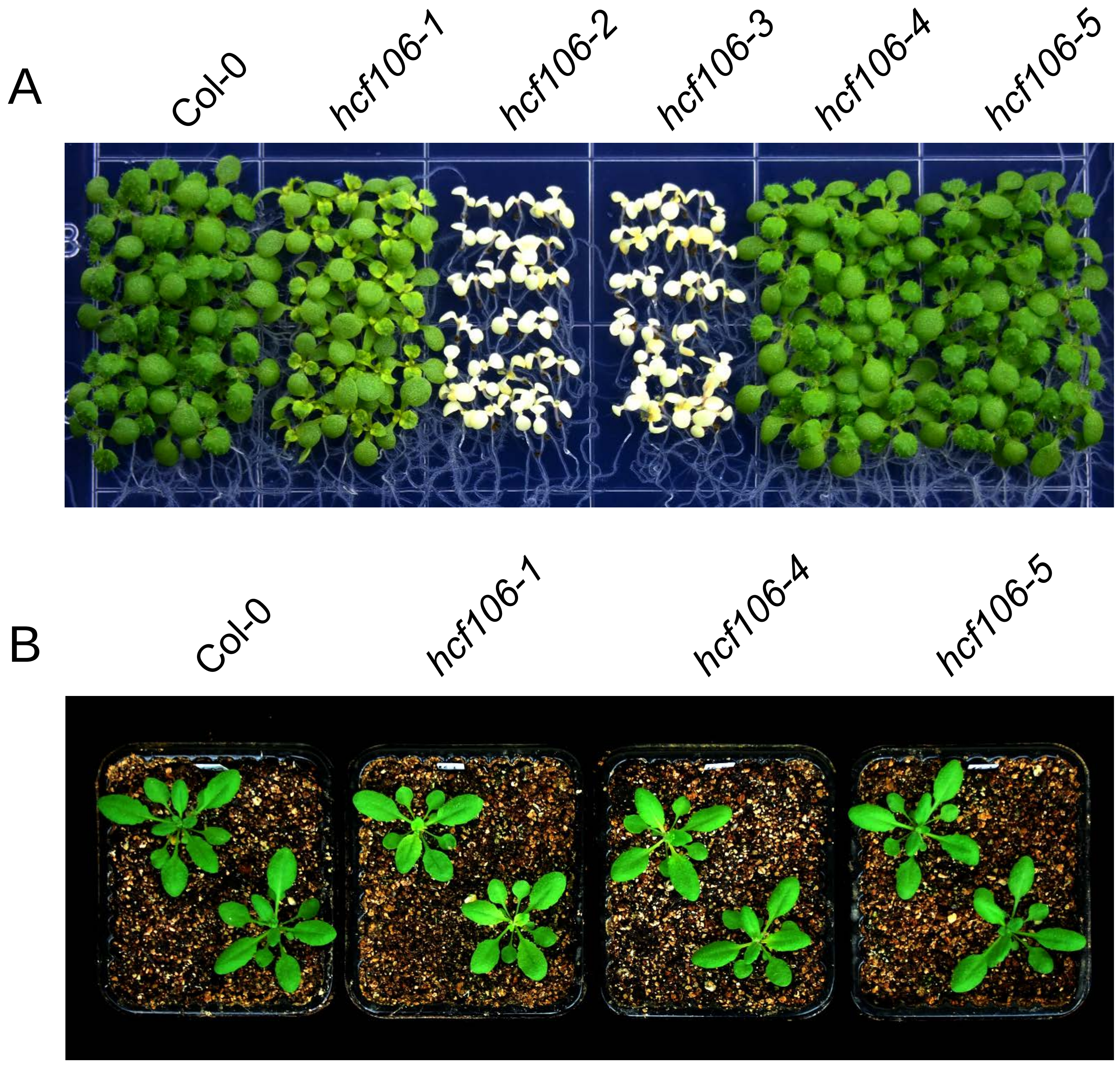
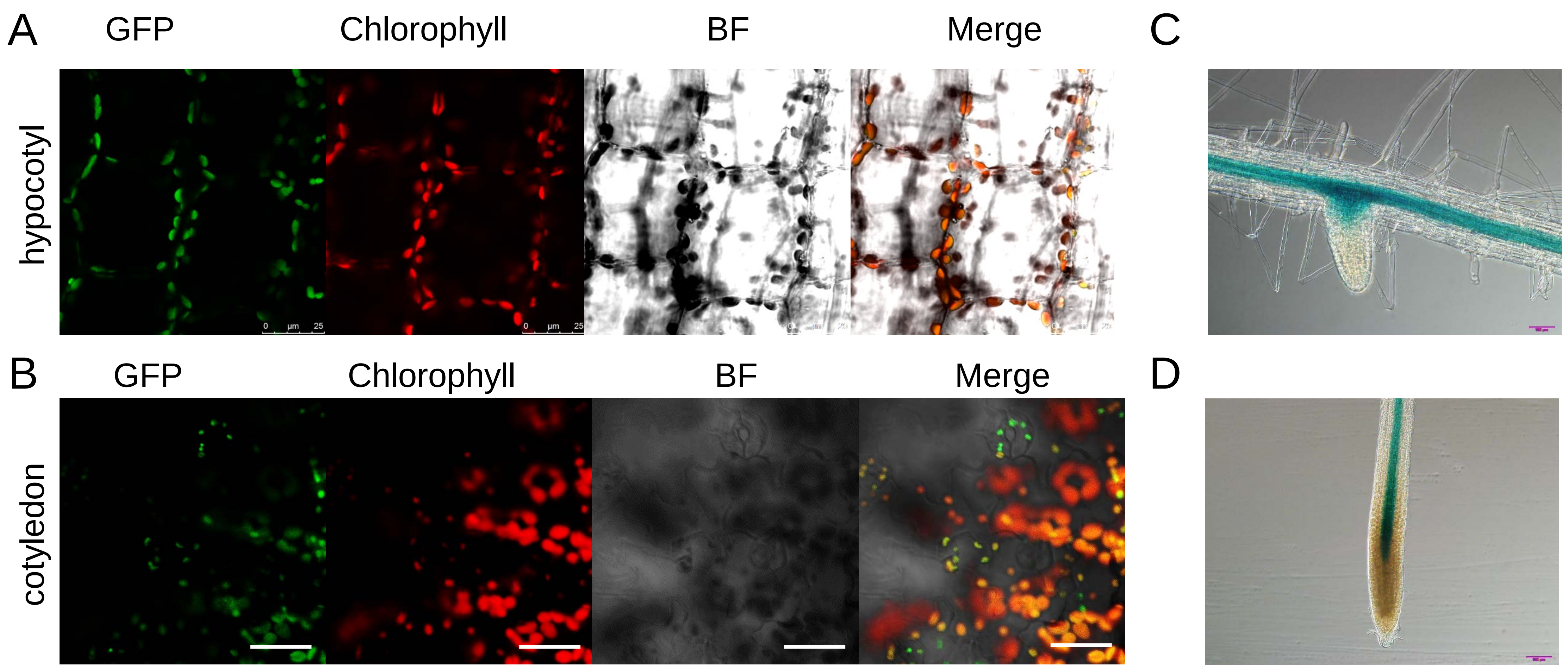


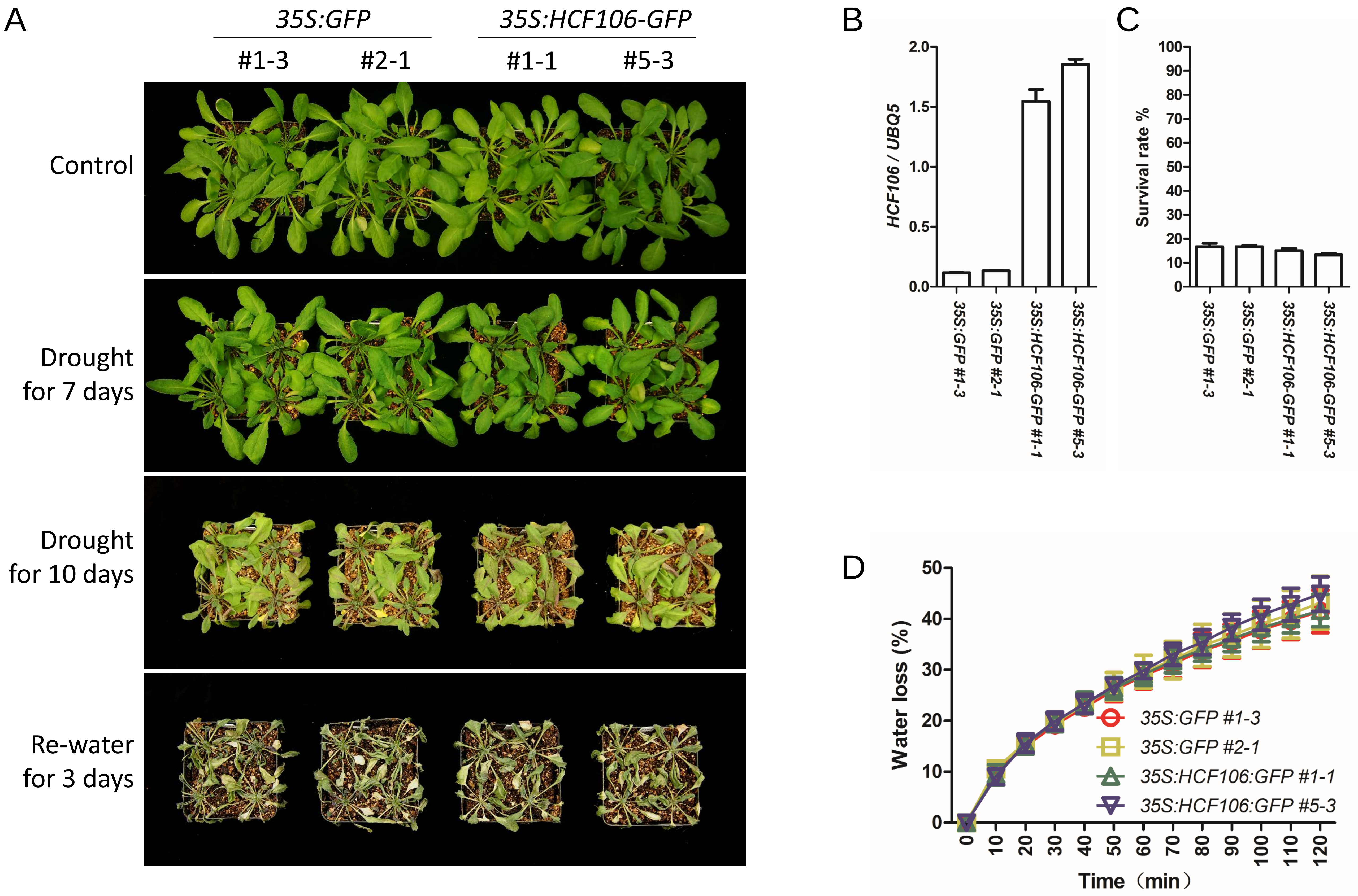
Supplemental Figure 1. Drought resistance assay of SALK_067017C
(A) Col-0 wild type and SALK_067017C plants were grown under normal conditions with 16-h-light / 8-h-dark for 21 days and then drought stressed for 7 days. (B) Re-watered for 3 days. Control, well-watered plants.



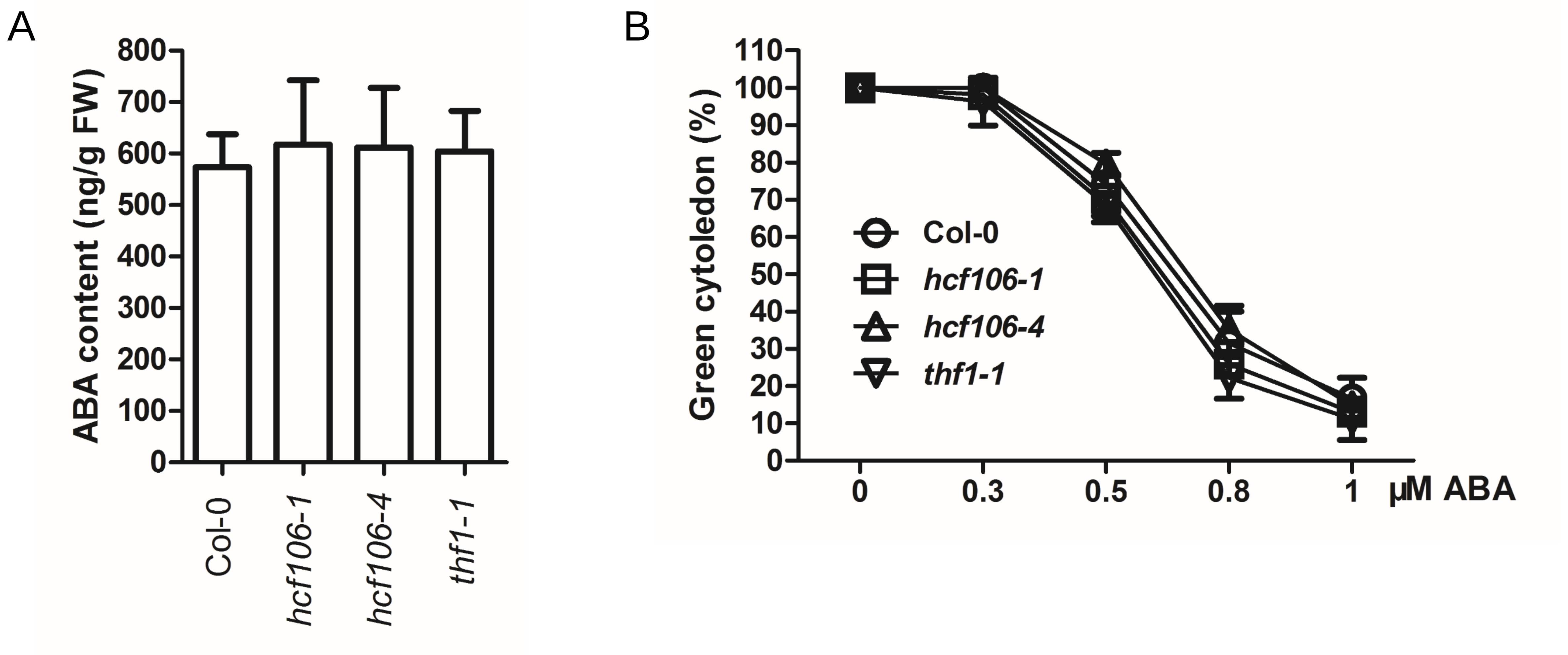
Supplemental Figure 2. Developmental phenotype of the *hcf106* mutants
(A) Phenotypes of 10-day-old Col-0, *hcf106-1*, *hcf106-2*, *hcf106-3*, *hcf106-4*, and *hcf106-5* seedlings under normal growth conditions. (B) Phenotypes of 21-day-old Col-0, *hcf106-1*, *hcf106-4*, and *hcf106-5* plants grown in soil under normal growth conditions.



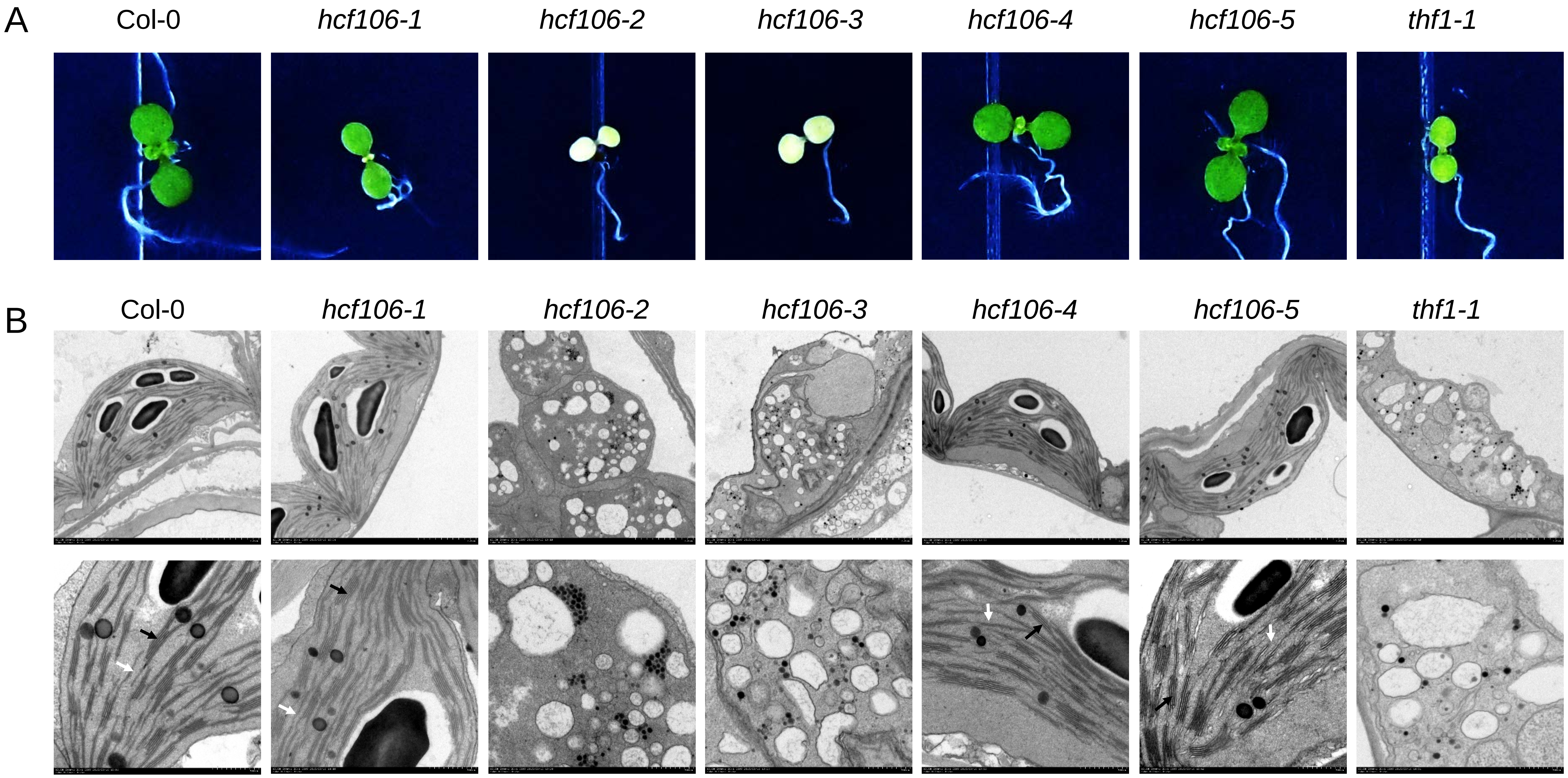
Supplemental Figure 3. Subcellular localization of HCF106-GFP and root expression pattern of *HCF106*
(A) and (B) Subcellular localization of HCF106-GFP. Transgenic Arabidopsis seedlings expressing genomic *HCF106-GFP* driven by its native promoter. GFP, green fluorescence protein. Chlorophyll, auto-fluorescence of chlorophyll. BF, bright field. Scale bars, 20 µm. (C) and (D) GUS activity in the roots of 10-day-old seedlings containing *HCF106pro-GUS*. Scale bars, 100 µm.



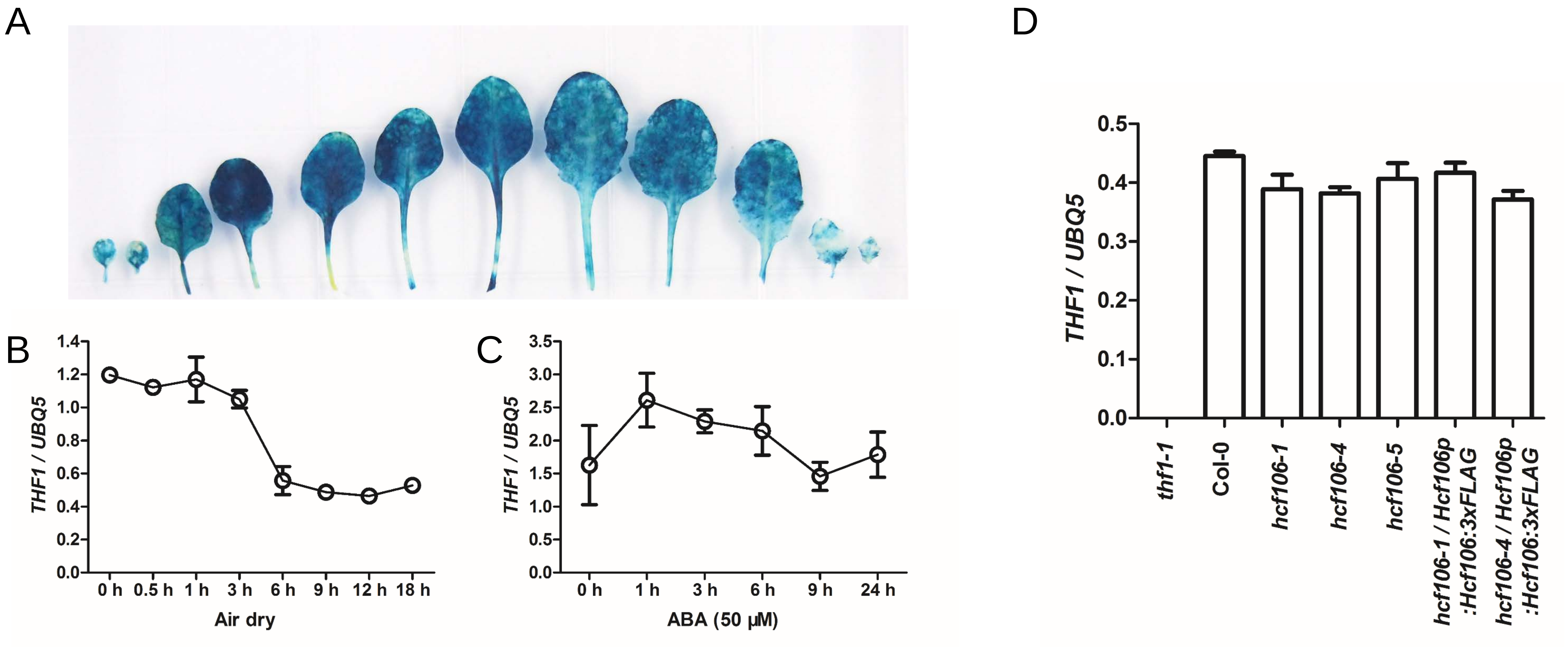
Supplemental Figure 4. Drought assay of the over-expression lines of *HCF106*
(A) Transgenic lines of *35S:GFP* and *35S:HCF106:GFP* grown under normal growth conditions for 21 days were treated with drought for 7 days or 10 days and recovered for 3 days. Control, well-watered plants. (B) *HCF106* transcript levels in the *35S:GFP* and *35S:HCF106:GFP* transgenic lines. Values are means ±SD (n = 3). (C) Survival rate of the *HCF106* overexpression plants after drought and re-watering (for 3 days) treatment (n = 4 biological replicates, 20 plants of each replicate). Data represent means ±SD (n = 3). (D) Water loss in the transgenic plant leaves (n=4, each containing 3 fully expanded leaves from 30-day-old plants). Data represent means ± SD (n = 3). #1-3 and #2-1, two independent transgenic lines of *35S:GFP* ; #1-1 and #5-3, two independent transgenic lines of *35S:HCF106:GFP.*



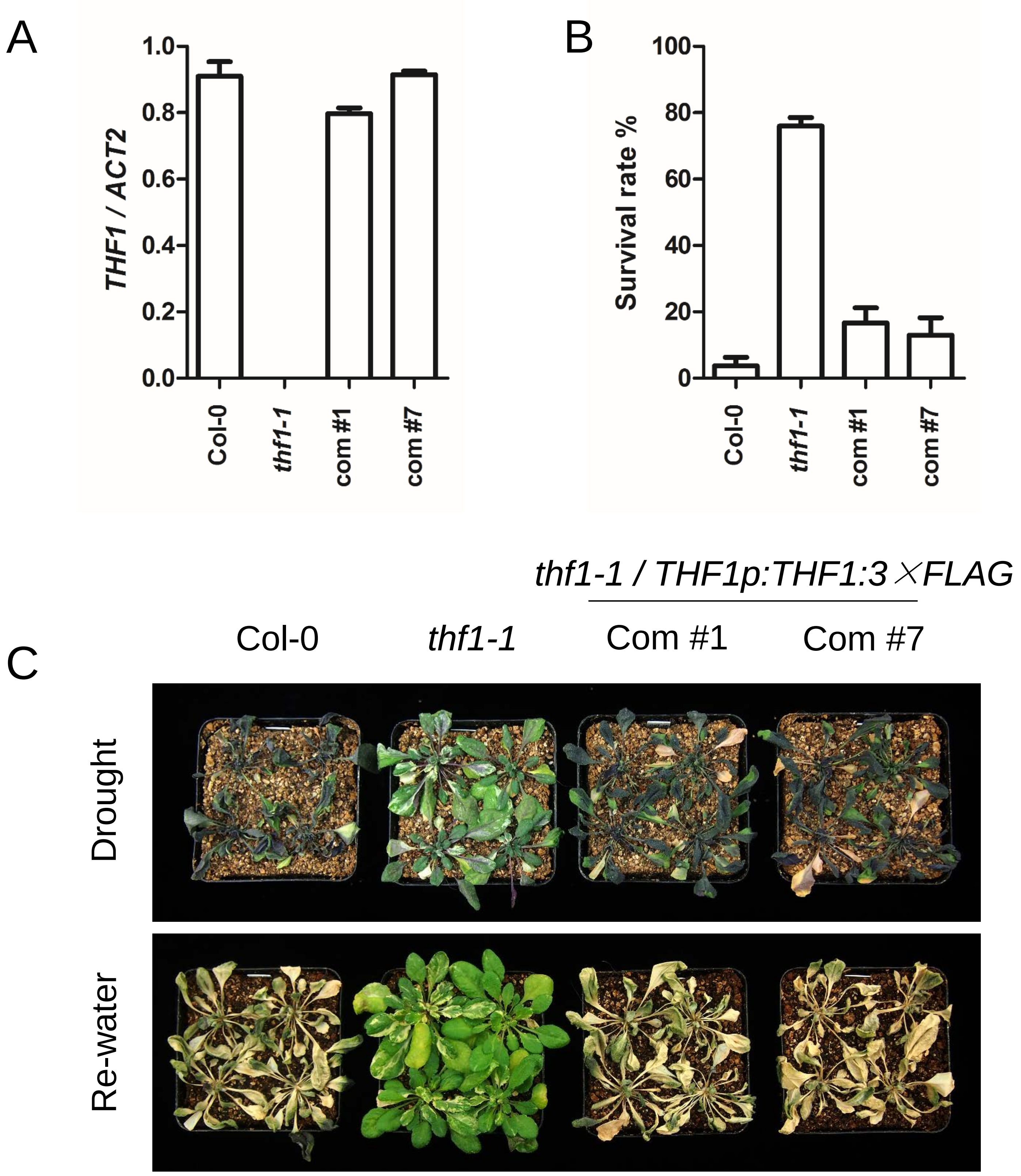
Supplemental Figure 5. ABA contents and germination assay of *hcf106* and *thf1* mutants
(A) ABA contents in the leaves of 4-week-old Col-0, *hcf106-1*, *hcf106-4*, and *thf1-1* plants after drought stress for 7 days. (B) Seed germination rate of Col-0, *hcf106-1*, *hcf106-4*, and *thf1-1* at the seventh day in 1/2 MS with different concentrations of exogenous ABA. Values are means ±SD (n = 3).



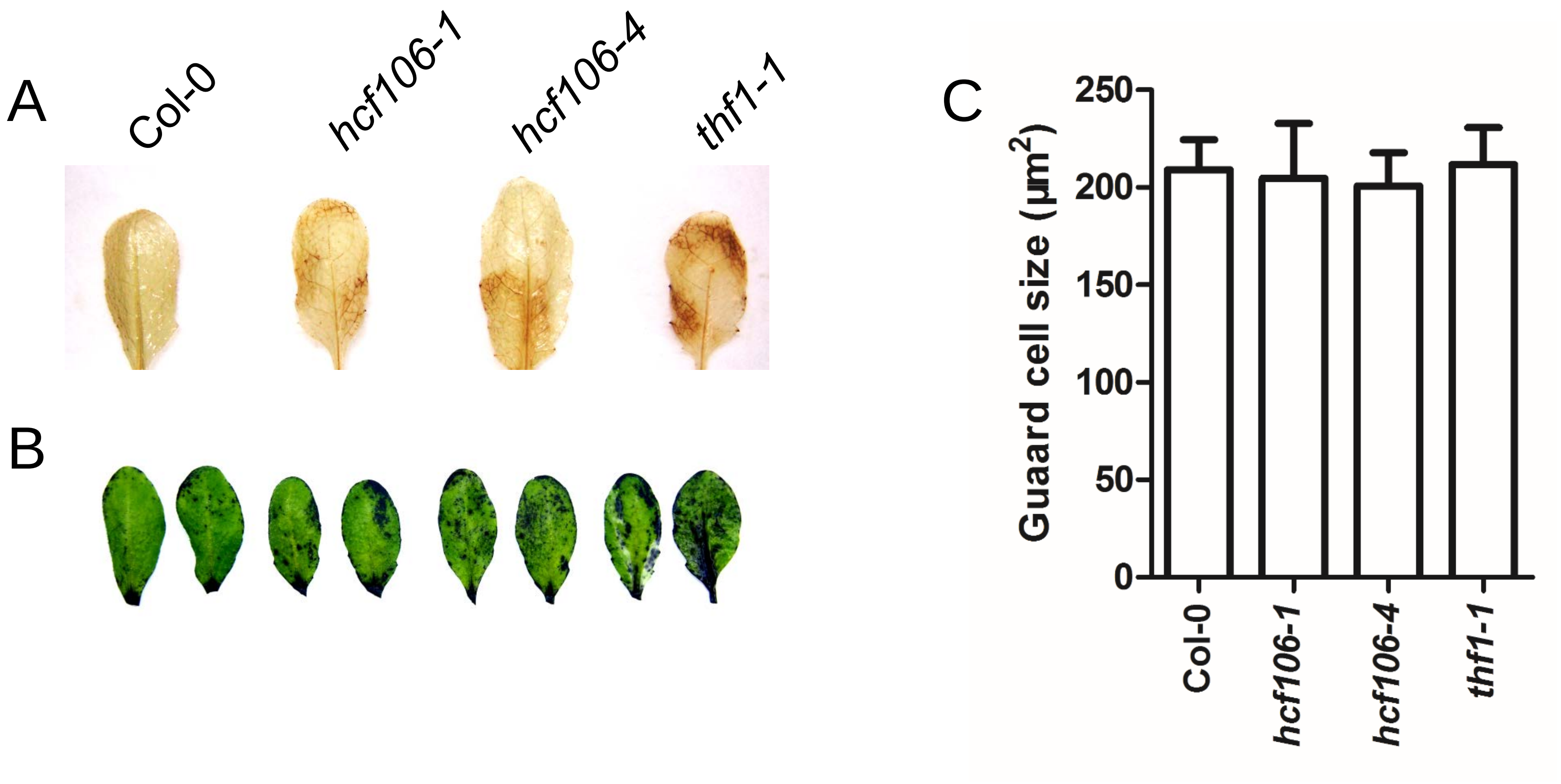
Supplemental Figure 6. Ultrastructure of chloroplasts in the *hcf106* and *thf1* mutants
(A) Phenotypes of 6-day-old Col-0, *hcf106-1*, *hcf106-2*, *hcf106-3*, *hcf106-4*, *hcf106-5* and *thf1-1* seedlings under normal growth conditions. (B) Ultrastructure of chloroplasts from cotyledons of 6-day-old Col-0, *hcf106-1*, *hcf106-2*, *hcf106-3*, *hcf106-4*, *hcf106-5* and *thf1-1*. A representative chloroplast is shown on the upper panel, and the magnified structure is shown on the lower panel. Black arrows, grana thylakoid; White arrow, stromal thylakoid.



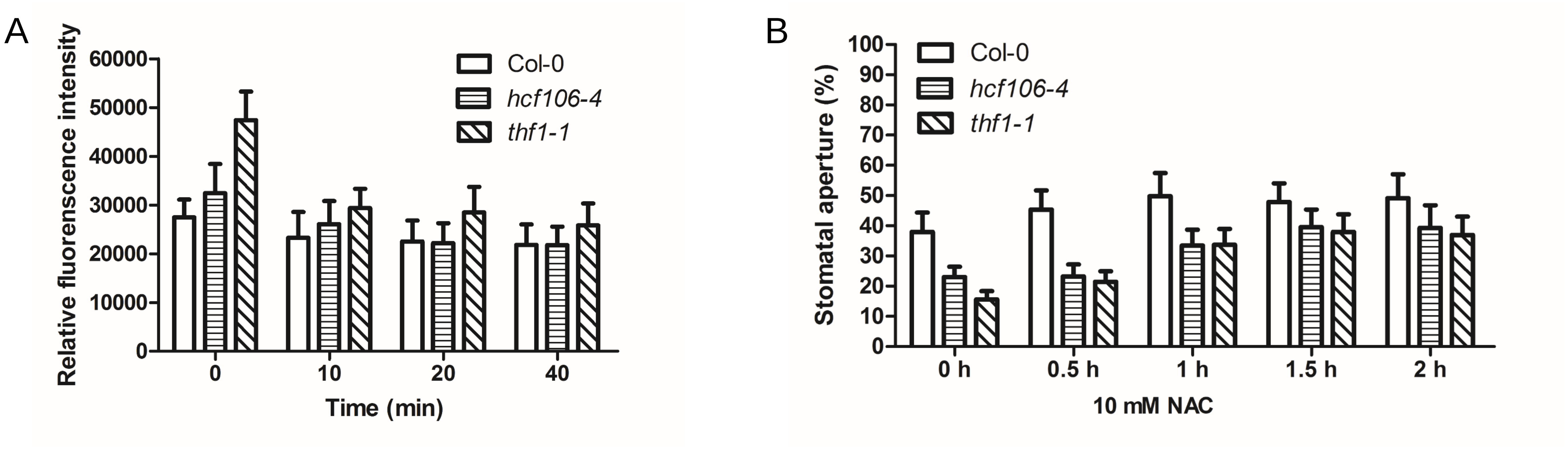
Supplemental Figure 7. Expression pattern of *THF1*
(A) GUS activity in rosette leaves of 21-day-old Arabidopsis containing the fusion *THF1pro-GUS*. (B) and (C) *HCF106* expression levels in 21-day-old plant leaves treated with air drying (B), or 50 μM ABA (C). (D) Relative expression levels of *THF1* in leaves of *thf1-1*, Col-0, *hcf106-1*, *hcf106-4*, *hcf106-5*, *hcf106-1* complementation line, and *hcf106-4* complementation line. Values are means ±SD (n = 3).



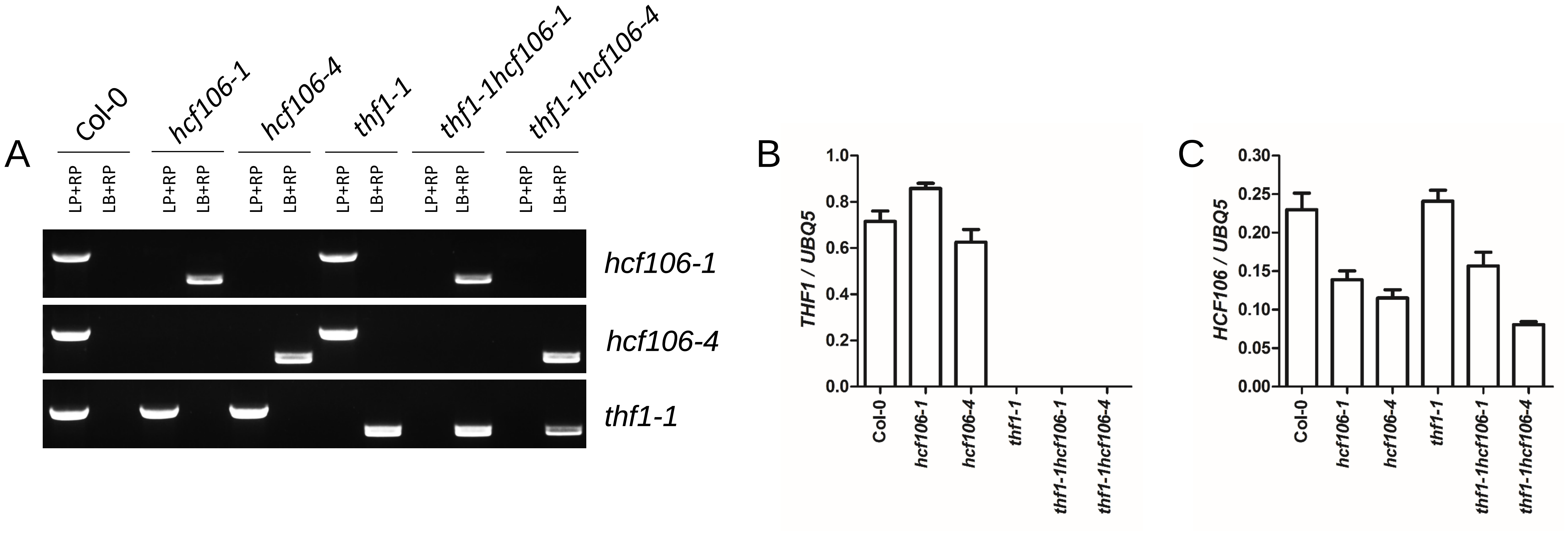
Supplemental Figure 8. Drought assay of *thf1-1* complementation lines
(A) *THF1* expression levels in Col-0, *thf1-1*, and the complementation lines. Values are means ±SD (n=3) (B) Survival rate of the complementation plants after drought and re-watering (for 5 days) treatment (n = 4 biological replicates, 16 plants of each replicate). Data represent means ±SD (n = 3). (C) Col-0, *thf1-1*, Com #1 and Com #7 of the complementation lines grown under normal growth conditions for 21 days and then drought for 12 days, and recovered for 5 days.



Supplemental Figure 9. Determination of ROS and guard cell size in *hcf106* and *thf1* mutants
(A) DAB staining and (B) NBT staining of the mature leaves of Col-0, *hcf106-1, hcf106-4*, and *thf1-1* from 21-day-old plants following drought stress for 7 days. (C) Measurement of guard cell size of Col-0, *hcf106-1*, *hcf106-4*, and *thf1-1* (n = 5 leaves, 5 stomata per leaf of 28-day-old plants). Data represent means ± SD.



Supplemental Figure 10. ROS affects the stomatal aperture in *hcf106 and thf1* mutants
(A) H_2O_2 level in guard cells obtained from CM-H2DCFDA fluorescence imaging in 21-day-old Col-0, *hcf106-4*, *thf1-1* after drought for 7 days. Values are displayed as the mean pixel intensities after treatment with 1 mM NAC for 0, 10, 20, and 40 min (n > 30; mean ±SD). (B) Responses of the stomatal aperture to 10 mM NAC at the indicated time in leaves of 21-day-old Col-0, *hcf106-4*, and *thf1-1* after drought for 7 days. Values are means ± SD (n ≥ 50).



Supplemental Figure 11. Identification of the double mutant *thf1hcf106*.
(A) Genotyping of *thf1-1hcf106-1*, *thf1-1hcf106-4*. LP, left primer. RP, right primer. LB, primer of T-DNA left border. (B) and (C) Expression levels of *THF1* and *HCF106* in Col-0, *hcf106-1*, *hcf106-4*, *thf1-1, thf1-1hcf106-1*, and *thf1-1hcf106-4*. Values are means ± SD (n=3).

Supplemental Table S1. Proteins interacting with HCF106 as identified by mass spectrometry in chloroplast

Protein Name	Accession Number	Description	Number of peptides identified
HCF106	AT5G52440	Bacterial sec-independent translocation protein mttA/Hcf106	55
THF1, PSB29	AT2G20890	photosystem II reaction center PSB29 protein	6
SOQ1	AT1G56500	haloacid dehalogenase-like hydrolase family protein	39
PSAD-2	AT1G03130	photosystem I subunit D-2	32
PSAD-1	AT4G02770	photosystem I subunit D-1	32
LOX2, ATLOX2	AT3G45140	lipoxygenase 2	27
PSBQ, PSBQA, PSBQ-1	AT4G21280	photosystem II subunit QA	26
AOS, CYP74A, DDE2	AT5G42650	allene oxide synthase	19
ATPF	ATCG00130	ATPase, F0 complex, subunit B/B~, bacterial/chloroplast	15
VAR1, FTSH5	AT5G42270	FtsH extracellular protease family	15
LHCB4.2	AT3G08940	light harvesting complex photosystem II	14
	AT5G08670	ATP synthase alpha/beta family protein	12
FTSH1	AT1G50250	FTSH protease 1	12
LHB1B2, LHCB1.5	AT2G34420	photosystem II light harvesting complex gene B1B2	12
LHCA3	AT1G61520	photosystem I light harvesting complex gene 3	11
MFP1	AT3G16000	MAR binding filament-like protein 1	11
TAPX	AT1G77490	thylakoidal ascorbate peroxidase	11
GOX1	AT3G14420	GLYCOLATE OXIDASE 1, Aldolase-type TIM barrel family protein	11
APG2, UNE3, PGA2, TATC	AT2G01110	Sec-independent periplasmic protein translocase	10
ATPC1	AT4G04640	ATPase, F1 complex, gamma subunit protein	10
AAC1	AT3G08580	ADP/ATP carrier 1	10

TLP18.3	AT1G54780	thylakoid lumen 18.3 kDa protein	10
PDE334	AT4G32260	ATPase, F0 complex, subunit B/B~, bacterial/chloroplast	9
ATTIC62, TIC62	AT3G18890	NAD(P)-binding Rossmann-fold superfamily protein	9
PSBP-1, PSII-P, OE23	AT1G06680	photosystem II subunit P-1	9
GOX2	AT3G14415	GLYCOLATE OXIDASE 2, Aldolase-type TIM barrel family protein	8
ATPD	AT4G09650	ATP synthase delta-subunit gene	8
FIB4	AT3G23400	Plastid-lipid associated protein PAP / fibrillin family protein	8
	AT1G26090	P-loop containing nucleoside triphosphate hydrolases superfamily protein	8
TROL	AT4G01050	thylakoid rhodanese-like	8
PSAG	AT1G55670	photosystem I subunit G	8
	AT1G18170	FKBP-like peptidyl-prolyl cis-trans isomerase family protein	7
	AT1G74470	Pyridine nucleotide-disulphide oxidoreductase family protein	6
	AT1G51110	Plastid-lipid associated protein PAP / fibrillin family protein	6
MPPBETA	AT3G02090	Insulinase (Peptidase family M16) protein	6
RCA	AT2G39730	rubisco activase	6
	AT3G61870	unknown protein	5
	AT5G03880	Thioredoxin family protein	5
	AT2G21960	unknown protein	5
PETB	ATCG00720	photosynthetic electron transfer B	5
CCB1	AT3G26710	cofactor assembly of complex C	5
PSBH	ATCG00710	photosystem II reaction center protein H	5
ATPE	ATCG00470	ATP synthase epsilon chain	5
ATLFNR1, FNR1	AT5G66190	ferredoxin-NADP(+)-oxidoreductase 1	5
LPA1	AT1G02910	tetratricopeptide repeat (TPR)-containing protein	5
FTSH8	AT1G06430	FTSH protease 8	4

	AT3G26080	plastid-lipid associated protein PAP / fibrillin family protein	4
AAC3, ATAAC3	AT4G28390	ADP/ATP carrier 3	4
	AT4G02530	chloroplast thylakoid lumen protein	4
PGR5-LIKE A	AT4G22890	PGR5-LIKE A	4
	AT2G35490	Plastid-lipid associated protein PAP / fibrillin family protein	4
LHCA4, CAB4	AT3G47470	light-harvesting chlorophyll-protein complex I subunit A4	4
CRD1, CHL27, ACSF	AT3G56940	dicarboxylate diiron protein, putative (Crd1)	4
NDF2, NDH45	AT1G64770	NDH-dependent cyclic electron flow 1	4
ENH1	AT5G17170	rubredoxin family protein	4
ATG4, G4, CHLG	AT3G51820	UbiA prenyltransferase family protein	4
APX4, TL29	AT4G09010	ascorbate peroxidase 4	4
	AT2G34460	NAD(P)-binding Rossmann-fold superfamily protein	4
PSAA	ATCG00350	Photosystem I, PsaA/PsaB protein	4
PTAC8, TMP14, PSAP	AT2G46820	photosystem I P subunit	4
PSAH2, PSAH-2, PSI-H	AT1G52230	photosystem I subunit H2	4
	AT2G47710	Adenine nucleotide alpha hydrolases-like superfamily protein	4
PSBG	ATCG00430	photosystem II reaction center protein G	4
	AT2G32640	Lycopene beta/epsilon cyclase protein	4
PSAC	ATCG01060	iron-sulfur cluster binding;electron carriers;4 iron, 4 sulfur cluster binding	4
FLU	AT3G14110	Tetratricopeptide repeat (TPR)-like superfamily protein	4
WLIM1	AT1G10200	GATA type zinc finger transcription factor family protein	4
FBA1	AT2G21330	fructose-bisphosphate aldolase 1	4
CRR31, NDHS	AT4G23890	CHLORORESPIRATORY REDUCTION 31, NADH dehydrogenase-like complex	4

Supplemental Table S2. Primers used in this study

Name	Sequence (5'>3')	Purpose
SALK_067017C LP	GACCTCCTCTCAAGTTTTGAG	*hcf106-1* genotyping
SALK_067017C RP	AACCTGGGTTTGAAGATTTCG	*hcf106-1* genotyping
SALK_044421C LP	TCAAATGGCTCCGTATAGACG	*hcf106-2* genotyping
SALK_044421C RP	ATCCCTATGATTCGACGTTTG	*hcf106-2* genotyping
SALK_020680 LP	GTCTCTGTTTGGTGTTGGAGC	*hcf106-3* genotyping
SALK_020680 RP	AGCGGGAGACAAAGCTTTTAG	*hcf106-3* genotyping
SAIL_760_H06 LP	GCCAAAAGGTCATCTATCTCTAGC	*hcf106-4* genotyping
SAIL_760_H06 RP	CCATGTTGACATCAGTCAAAAC	*hcf106-4* genotyping
SAIL_831_E01 LP	GACCTCCTCTCAAGTTTTGAGC	*hcf106-5* genotyping
SAIL_831_E01 RP	AACCTGGGTTTGAAGATTTCG	*hcf106-5* genotyping
SALK_094925 LP	GGATCAGTAGCACTTGCAAGC	*thf1-1* genotyping
SALK_094925 RP	TAACGTGGACAGACAAGGGAC	*thf1-1* genotyping
HCF106_qFP	CGATTAGAGAGCTACAGGATG	qPCR
HCF106_qRP	GTGATTGTGAATCATTGGGATC	qPCR
THF1_qFP	TGAGGATCCTAAGCAATACCG	qPCR
THF1_qRP	CCGATCCACGCTTTTCTTGTT	qPCR
UBQ5_qFP	AGAAGATCAAGCACAAGCAT	qPCR
UBQ5_qRP	CAGATCAAGCTTCAACTCCT	qPCR
ACT2_qFP	TTGTGCTGGATTCTGGTGATG	qPCR
ACT2_qRP	CGCTCTGCTGTTGTGGTG	qPCR
ACT7_qFP	CATTCAATGTCCCTGCCATGT	qPCR
ACT7_qRP	GGTTGTACGACCACTGGCATAG	qPCR
HCF106pg _*EcoR* I_FP	G GAATTC CGGTCGTTATCTAGTCCAGCT	*HCF106p:HCF106:3×FLAG*
HCF106pg _*Sal* I_RP	TTT GTCGAC ATCTTGCCTTGGAGGAGATGC	*HCF106p:HCF106:3×FLAG*
THF1pg_*Kpn* I_FP	GG GGTACC AACAACAACTACATGCTCTGTCT	*THF1p:THF1:3×FLAG*
THF1pg_*Pst* I_RP	TTT CTGCAG AGATTTCCGTTCAACCAAGAAAG	*THF1p:THF1:3×FLAG*
THF1pro_attB1_FP	GGGGACAAGTTTGTACAAAAAAGCAGGCT GC GATGCTTCCTCTACGTCTCCA	*THF1p:GUS*

THF1pro_attB2_RP	GGGGACCACTTTGTACAAGAAAGCTGGGT C CGCTAAATTGAAGTGAGAAAGAG	*THF1p:GUS*
HCF106pro_attB1_FP	GGGGACAAGTTTGTACAAAAAAGCAGGCT GC GATGGTTCATGGTTCCTTTCCT	*HCF106p:GUS*
HCF106pro_attB2_RP	GGGGACCACTTTGTACAAGAAAGCTGGGT C GGGCAGCTTCGAAATTGTAATAT	*HCF106p:GUS*
HCF106 CDS_attB1_FP	GGGGACAAGTTTGTACAAAAAAGCAGGCT GC ATGGCCATGGCGTTACAGATTA	*35S:HCF106:GFP*
HCF106 CDS_attB2_RP	GGGGACCACTTTGTACAAGAAAGCTGGGT C ATCTTGCCTTGGAGGAGATGC	*35S:HCF106:GFP*
HCF106pg_attB1_FP	GGGGACAAGTTTGTACAAAAAAGCAGGCT GC CGGTCGTTATCTAGTCCAGCT	*HCF106p:HCF106:GFP*
HCF106pg_attB2_RP	GGGGACCACTTTGTACAAGAAAGCTGGGT CC ATCTTGCCTTGGAGGAGATGC	*HCF106p:HCF106:GFP*
THF1 CDS_*Sal* I_FP	TTT GTCGAC ATGGCTGCAACTGCAATCTCT	THF1-*N*YFP
THF1 CDS_*Kpn* I_RP	GG GGTACC G AGATTTCCGTTCAACCAAGAAAG	THF1-*N*YFP
HCF106 CDS_*Sal* I_FP	TTT GTCGAC ATGGCCATGGCGTTACAGATTA	HCF106-*C*YFP
HCF106 CDS_*Kpn* I_RP	GG GGTACC G ATCTTGCCTTGGAGGAGATGC	HCF106-*C*YFP
THF1 CDS_*Nde* I_FP	TTT CATATG ATGGCTGCAACTGCAATCTCTT	MBP-THF1
THF1 CDS_*Sal I*_RP	TTT GTCGAC CTAAGATTTCCGTTCAACCAAGA	MBP-THF1
HCF106 CDS_*EcoR I*_FP	G GAATTC ATGGCCATGGCGTTACAGATTA	His-HCF106
HCF106 CDS_*Sal I*_RP	TTT GTCGAC G ATCTTGCCTTGGAGGAGATGC	His-HCF106
THF1 CDS_*Kpn I*_FP	CGG GGTACC ATGGCTGCAACTGCAATCTCTT	THF1-NLuc
THF1 CDS_*Sal I*_RP	TTT GTCGAC AGATTTCCGTTCAACCAAGAAAG	THF1-NLuc
HCF106 CDS_*Kpn I*_FP	CGG GGTACC ATGGCCATGGCGTTACAGATTA	CLuc-HCF106
HCF106 CDS_*Pst I*_RP	TTT CTGCAG TCAATCTTGCCTTGGAGGAGAT	CLuc-HCF106