Article:
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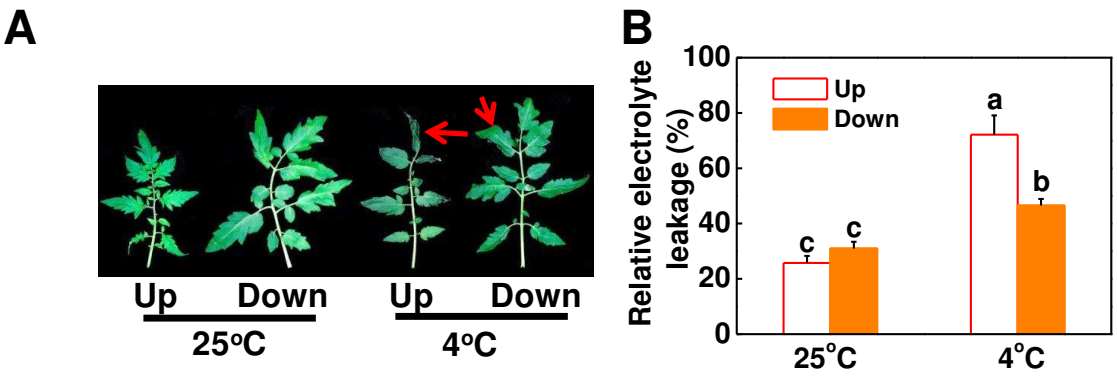
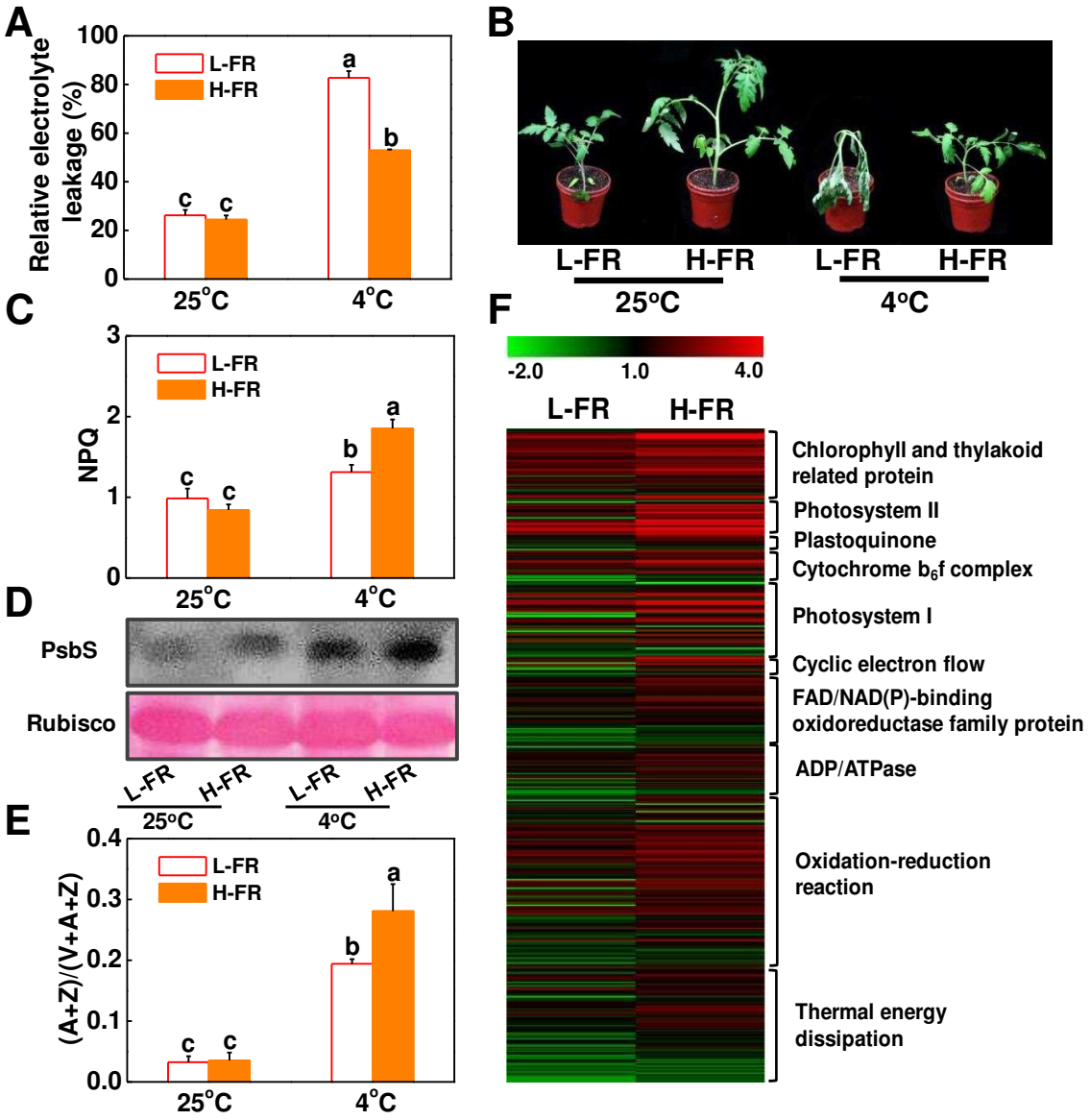
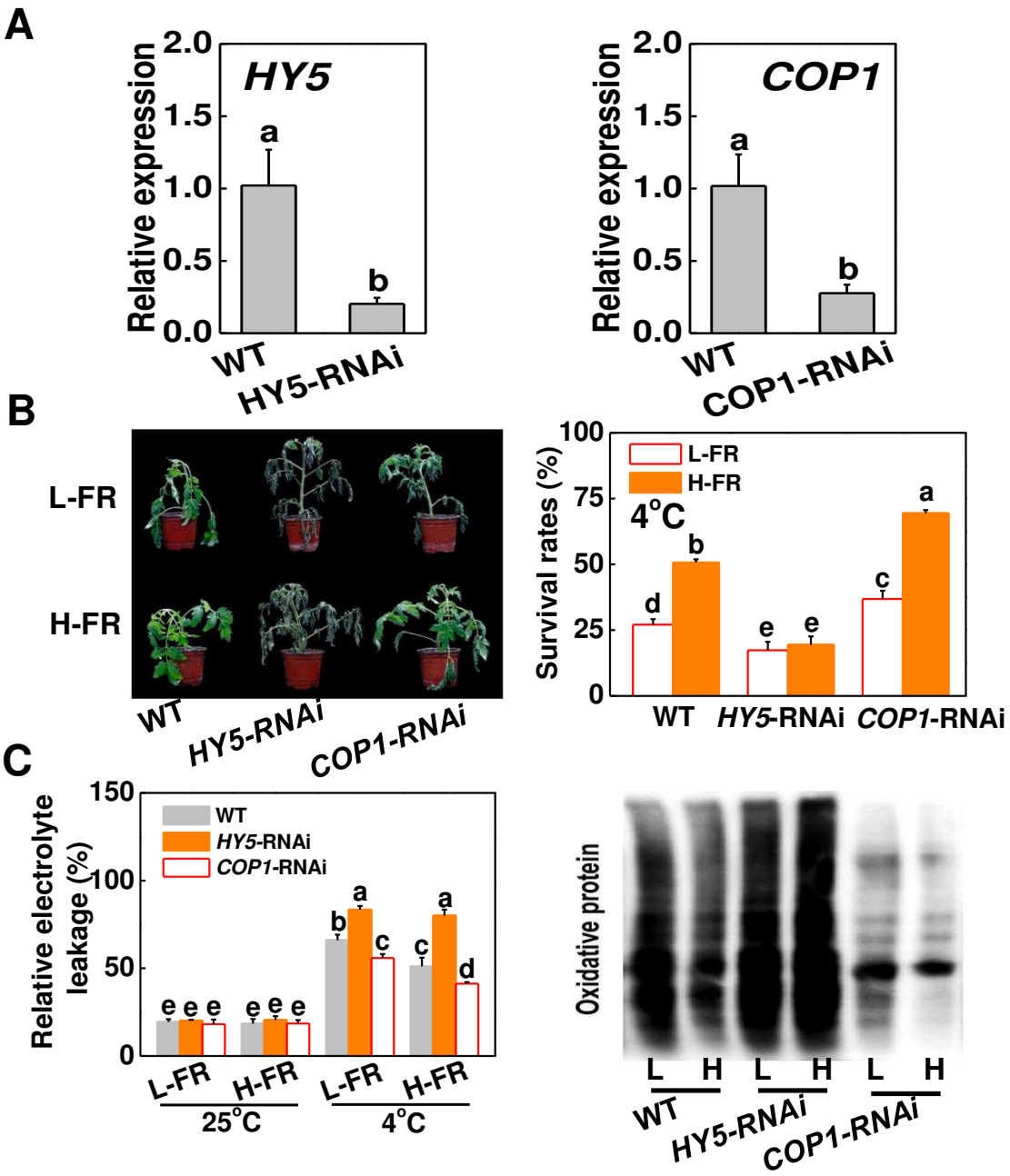


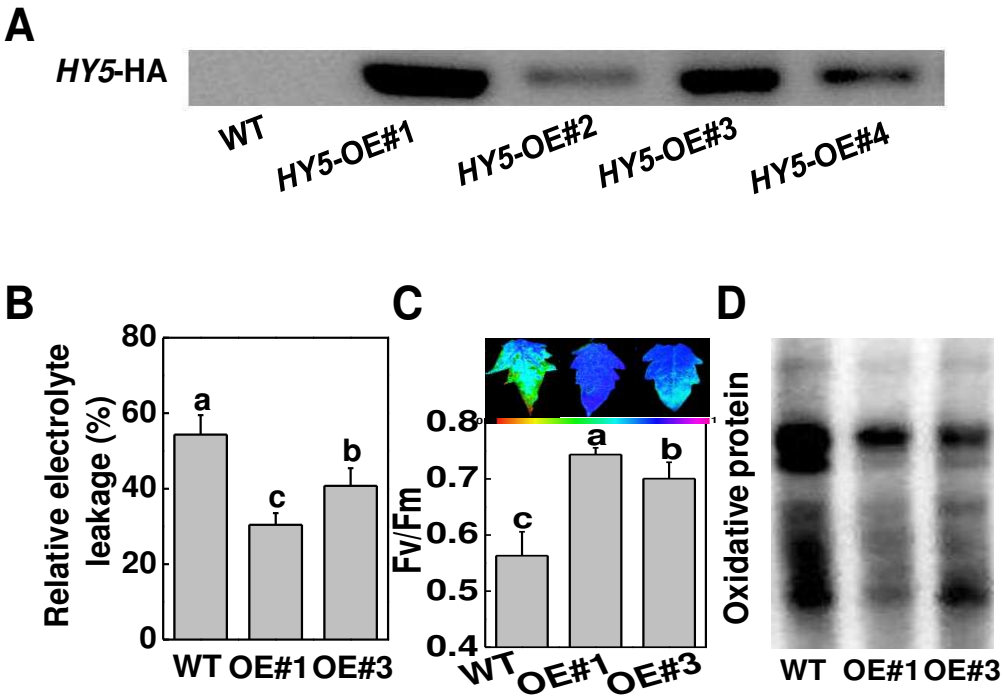
Supplemental Figure S1. Effect of spatial variation on the cold tolerance of tomato. A and B, Phenotypes (A) and the relative electrolyte leakage (B) in leaves at the 9th (Up) and 5th (Down) ranks from the base in plants at 11-leaf stage under white light conditions after exposure to 4 °C for 7 d. Data are presented as the mean of 4 biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.



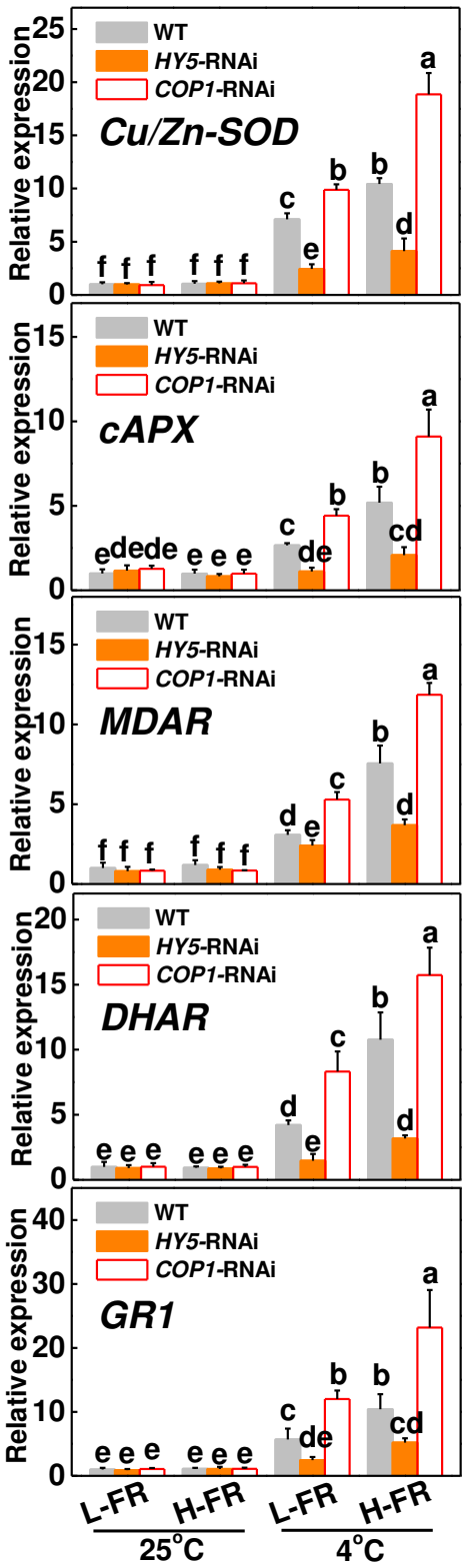
Supplemental Figure S2. Effect of FR intensity on the cold tolerance of tomato. A and B, Phenotypes (A) and the relative electrolyte leakage (B) at 4th leaves of the tomato plants at 6-leaf stage grown in temperature-controlled chambers at 25 °C or 4 °C under L-FR or H-FR light conditions for 7 d. C and D, Changes of NPQ (C) and PsbS protein (D) at 4th leaves of the tomato plants at 6-leaf stage grown at 25 °C or 4 °C under L-FR or H-FR light conditions for 3 d and 1 d, respectively. E, De-epoxidation state of the xanthophyll cycle in the tomato plants after exposure to 25 °C or 4 °C for 3 d under L-FR or H-FR light conditions. F, Transcriptome analysis of differentially expressed genes in the photosystems and photoprotection of tomato plants after exposure to a cold at 4 °C for 6 h under H-FR and L-FR light conditions. The color (from green to red) represents gene expression intensity (based on $\log_{10}$ RPKM values) from low to high. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 µmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 µmol m^{-2} s^{-1} and 400 µmol m^{-2} s^{-1}). Data are presented as the mean of 4 biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.



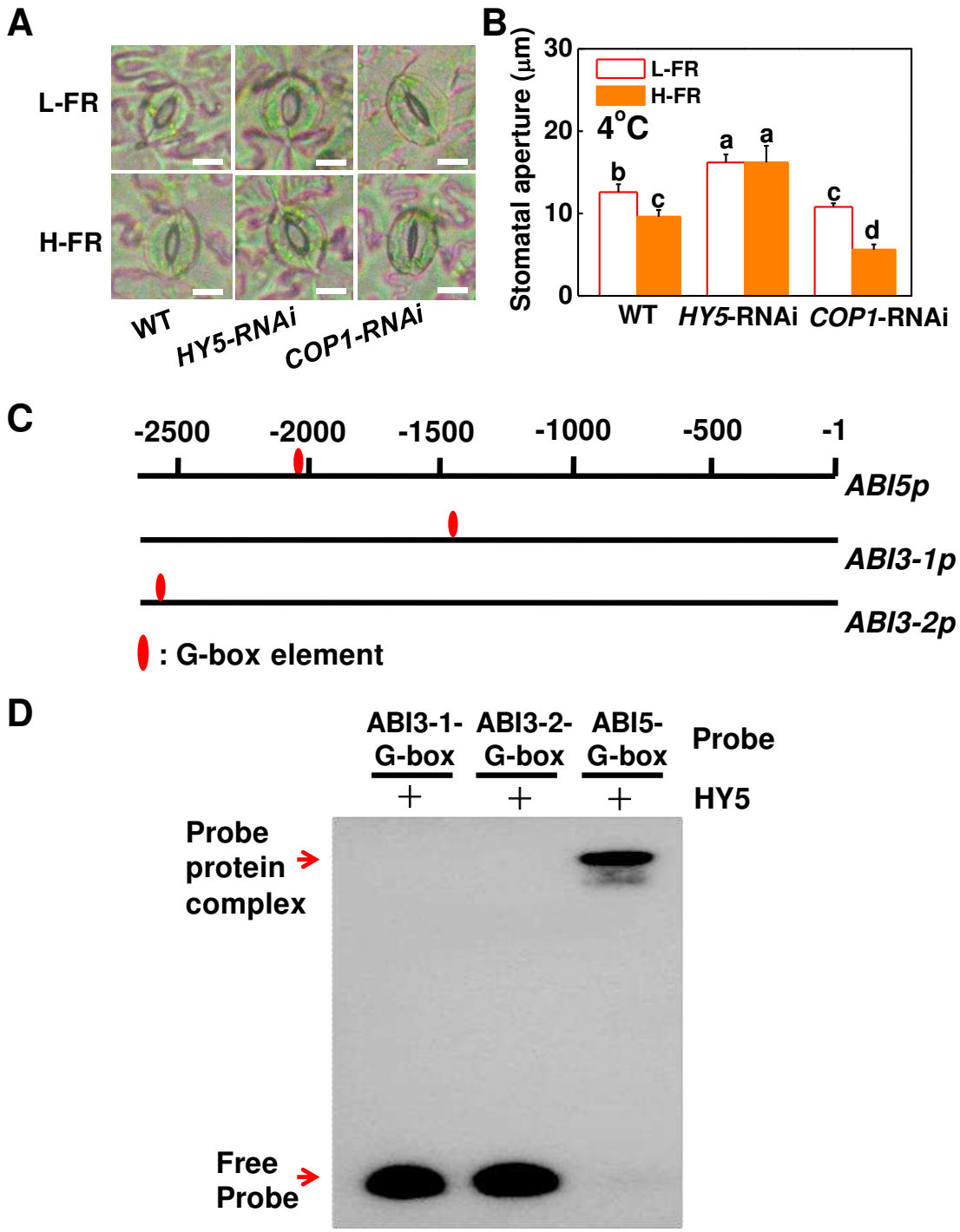
Supplemental Figure S3. Silencing efficiency and cold tolerance of HY5-RNAi and COP1-RNAi tomato plants. A, Relative expression of HY5 and COP1 in the respective HY5-RNAi and COP1-RNAi plants. Samples are from the 4th leaf of RNAi plants at 4-leaf stage. Relative gene expression for HY5 and COP1 genes were calculated using the wild type (WT) plants as 1. B, Phenotypes and survival rates of wild type (WT), HY5-RNAi and COP1-RNAi tomato plants grown in temperature-controlled chambers at 4 °C under L-FR or H-FR light conditions for 7 d. Survival rates were measured by recovery at 25 °C for 6 d after the chilling treatment (4 °C for 7 d) (n = 4), each replicate had 16 plants. C, The relative electrolyte leakage and oxidized protein (proteins with carbonyl groups, a marker for oxidative damage in vivo) in leaves after 7 d and 3 d cold stress, respectively, under L-FR or H-FR light conditions. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 µmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 µmol m^{-2} s^{-1} and 400 µmol m^{-2} s^{-1}). Data are presented as the mean of 4 biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.



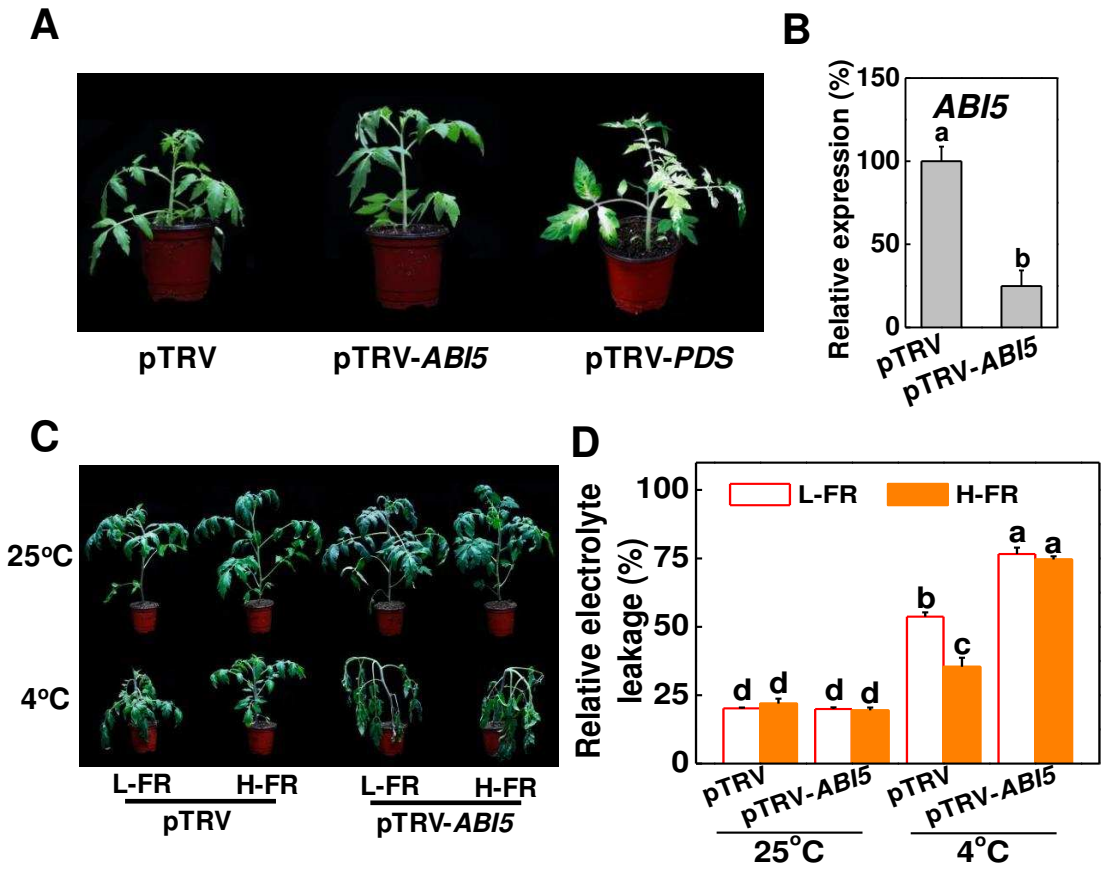
Supplemental Figure S4. Cold tolerance of HY5-overexpressing transgenic tomato lines. A, Western blotting analysis of transgenic plants expressing the 3HA-tagged HY5 transgene. Total proteins were extracted from the leaves and equal amounts of proteins (10 μg) were subjected to SDS-PAGE, and probed with an anti-HA monoclonal antibody. Three independent experiments were performed with similar results. OE, overexpressing; #1, #2, #3 and #4, four independent lines of HY5 overexpressing (HY5-OE) plants. B and C, The relative electrolyte leakage (B) and changes in maximum photochemical efficiency of PSII (Fv/Fm) (C) in wild type (WT) and independent transgene-positive lines (HY5-OE#1 and OE#3) after exposure to 4 °C at H-FR (R/FR ratio, 0.5) light conditions for 7 d. The false color code depicted at the bottom of the image ranges from 0 (black) to 1.0 (purple), represented the level of damage in leaves. D, Western Blot detection of oxidized proteins in leaves after 3 d cold stress under H-FR light conditions. For the H-FR, R/FR ratio at 0.5, plants were kept at R conditions (200 μmol m^{-2} s^{-1}) supplemented with FR (400 μmol m^{-2} s^{-1}). Three independent experiments were performed with similar results. Data are presented as the mean of 4 biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.



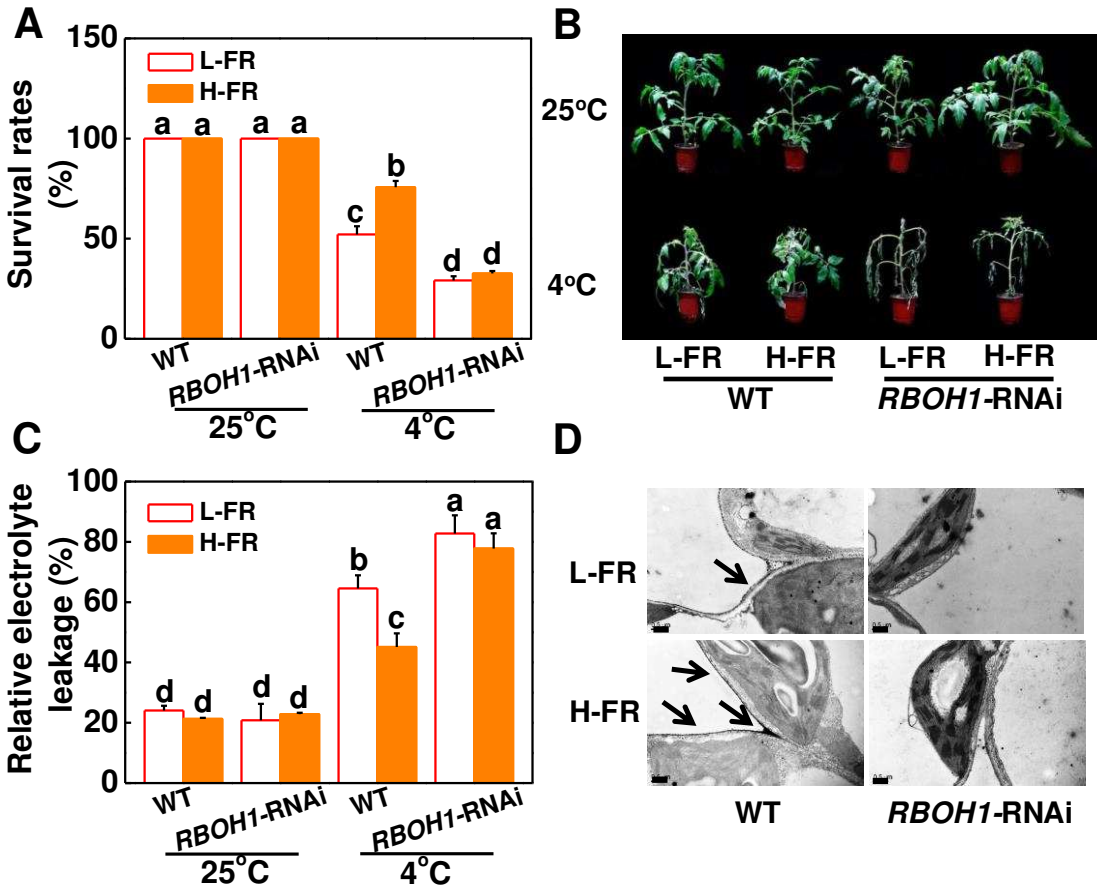
Supplemental Figure S5. Transcripts of genes involved in Foyer-Halliwell-Asada cycle in WT, HY5-RNAi and COP1-RNAi tomato plants. Gene expression of Cu/Zn-SOD, tAPX, MDAR, DHAR and GR1 after tomato plants exposed to 25 °C or 4 °C under L-FR or H-FR light conditions for 3 d. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 μmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 and 400 μmol m^{-2} s^{-1}). Data are presented as the mean of 4 biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.



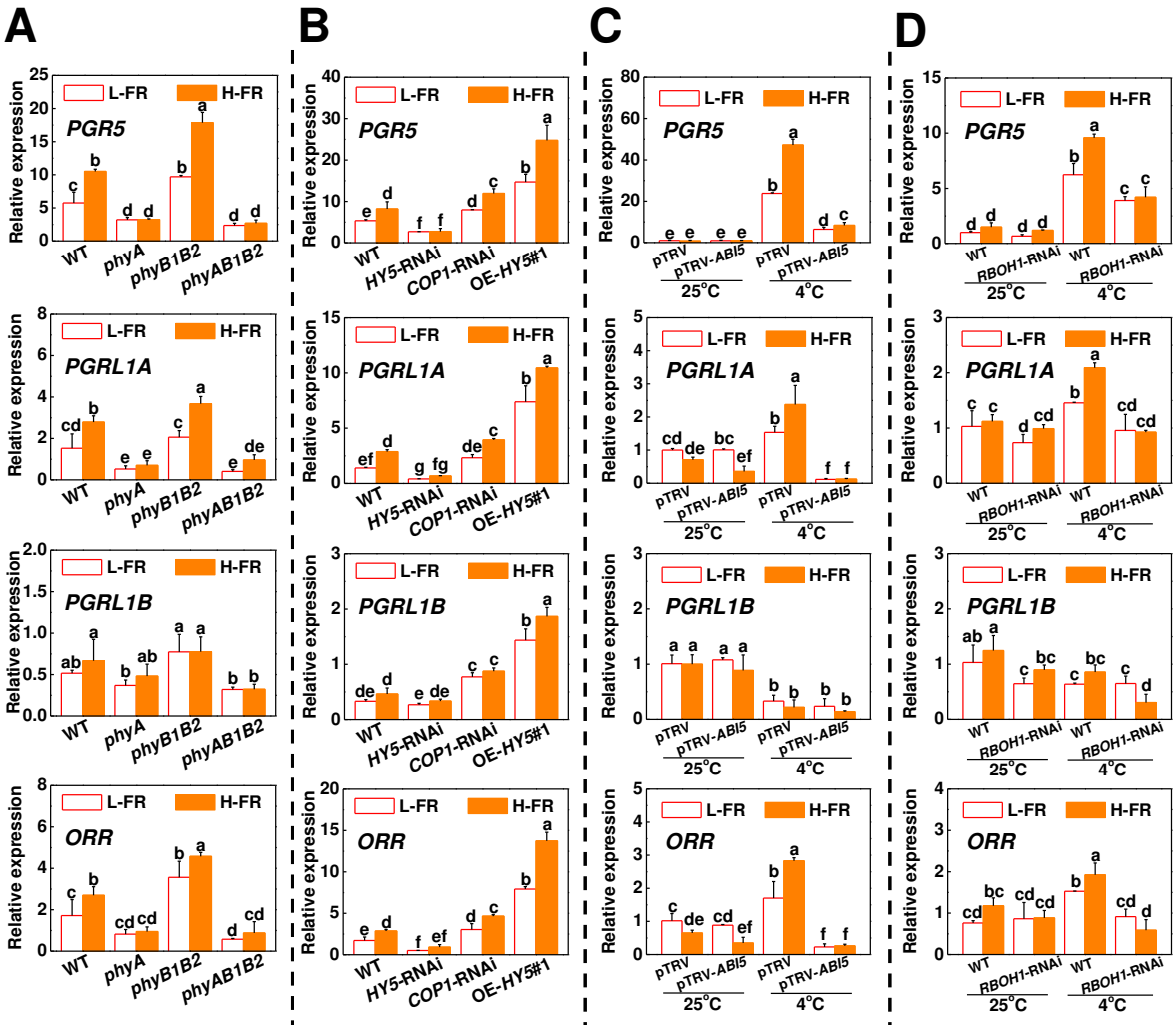
Supplemental Figure S6. HY5 regulated ABA-mediated stomatal movement and directly binds to the G-boxes of the ABI promoters in vitro. A and B, Representative light microscopy image of stomata (A) and stomatal aperture (B) in response to different FR light intensities after exposure to 4 °C for 1 d. B, G-box elements in the promoters of tomato ABI (ABI3-1, ABI3-2 and ABI5) genes. Numbering is from predicted transcriptional start sites. C, HY5 directly binds to the G-boxe of ABI5 promoter in vitro. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 μmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 and 400 μmol m^{-2} s^{-1}). Stomatal aperture are the average means of three biological replicates, and each replicate is the average value of stomata in a field of microscope (with about 25 stomata) under each treatment. Scale bars = 10 μm. Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.



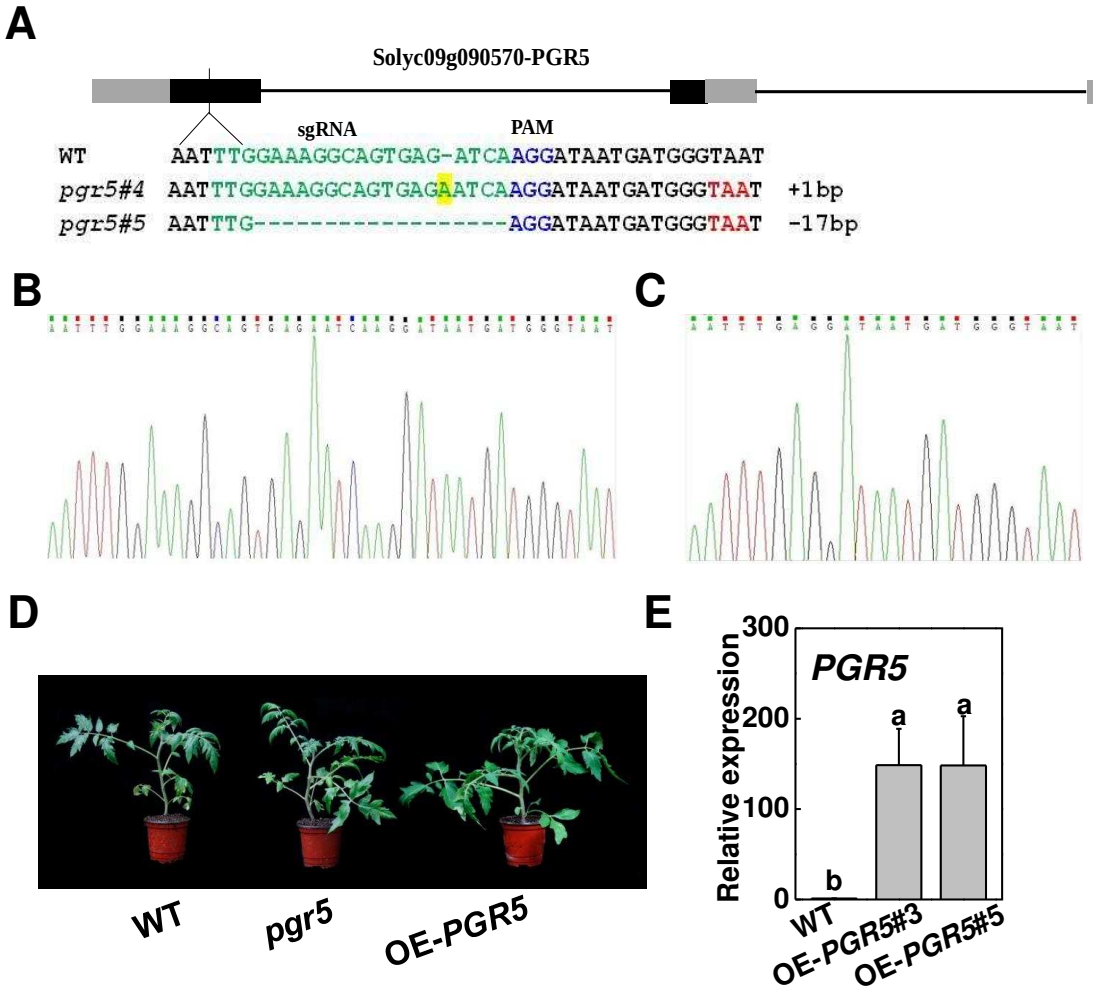
Supplemental Figure S7. Silencing efficiency and cold tolerance of ABA INSENSITIVE 5 (ABI5) silenced (pTRV-ABI5) plants. A, Phenotypes of non-silenced (pTRV) and silenced (pTRV-ABI5) tomato plants. PDS (phytoene desaturase gene, a gene involved in chlorophyll biosynthesis)-silenced (pTRV-PDS) tomato plants was used to monitor the virus-induced gene silencing (VIGS) progression. Photographs were taken 3 weeks after TRV infection. B, Relative expression of ABI5 in the VIGS plants was calculated using the pTRV plants as 100%. Samples are from the 4th leaf of six silenced plants. C and D, Phenotypes (C) and the relative electrolyte leakage (D) of pTRV and pTRV-ABI5 tomato plants grown in temperature-controlled chambers at 25 °C or 4 °C under L-FR or H-FR light conditions for 7 d. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 µmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 and 400 µmol m^{-2} s^{-1}). Data are presented as the mean of 4 biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.



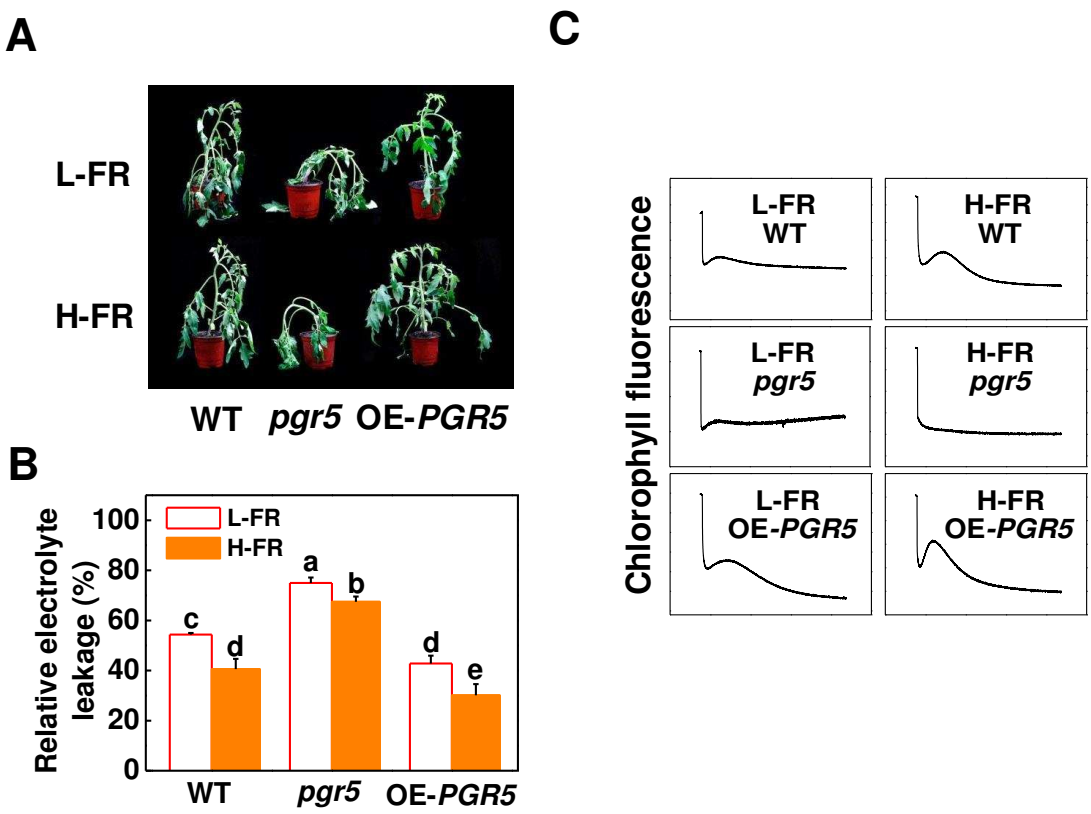
Supplemental Figure S8. Cold tolerance of WT and RBOH1-RNAi transgenic tomato plants. A, Survival rates of WT and RBOH1-RNAi tomato plants exposed to 25 °C or 4 °C under L-FR or H-FR light conditions for 7 d, and recovered at 25 °C for 6 d after the chilling treatment (n = 4), each replicate had 16 plants. B and C, Phenotypes (B) and the relative electrolyte leakage (C) of WT and RBOH1-RNAi tomato plants grown in temperature-controlled chambers at 25 °C or 4 °C under L-FR or H-FR light conditions for 7 d. D, Cytochemical localization of H_2O_2 accumulation in leaf mesophyll cells of WT and RBOH1-RNAi as visualized by $CeCl_3$ staining and TEM. Samples were harvested 1 d after cold treatment. The arrows indicate $CeCl_3$ precipitates. Scale bars = 0.5 μm. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 μmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 and 400 μmol m^{-2} s^{-1}). Data are presented as the mean of 4 biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.



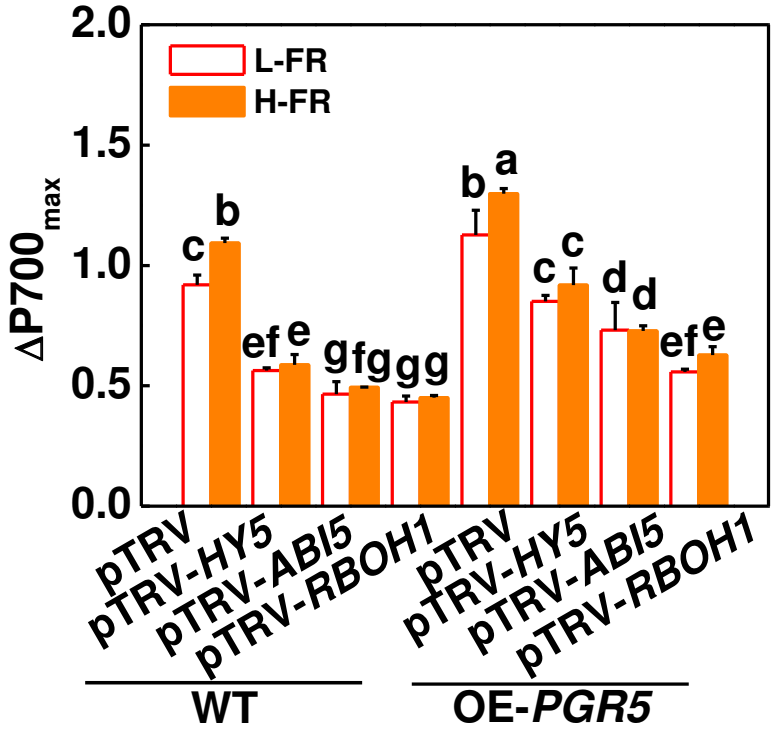
Supplemental Figure S9. Relative expression of CEF related genes in response to cold stress and far red. A and B, Transcription of PGR5, PGRL1A, PGRL1B and ORR genes in the tomato phytochrome mutant plants (A) and HY5-RNAi, COP1-RNAi, OE-HY5 plants (B), which exposed to 4 °C for 6 h under L-FR or H-FR light conditions. C and D, Transcription of PGR5, PGRL1A, PGRL1B and ORR genes in the tomato ABI5-silenced plants (C) and RBOH1-RNAi plants (D), which exposed to 25 °C or 4 °C for 6 h under L-FR or H-FR light conditions. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 µmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 and 400 µmol m^{-2} s^{-1}). Data are presented as the mean of 4 biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.



Supplemental Figure S10. Transgenic tomato of pgr5 mutant and PGR5-overexpressing plants. A, The diagram displays the structure of the PGR5 gene. The PGR5 gene has two exons (black closed boxes) and two introns (black horizontal lines). PAM is indicated by blue, single guide RNA (sgRNA) is indicated by green, one insert mutation of a single nucleotide is indicated by yellow, and stop codon is in red. B and C, Sequencing results in pgr5#4 (B) and pgr5#5 (C) mutants. D and E, Phenotypes in the WT, pgr5#5 mutant and OE-PGR5#3 tomato plants (D) and relative expression of PGR5 gene in the PGR5-overexpressing (OE-PGR5#3 and OE-PGR5#5) transgenic tomato plants (E). Samples are from the 4th leaf of PGR5-overexpressing transgenic tomato plants at 4-leaf stage. Data are presented as the mean of 4 biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.



Supplemental Figure S11. Cold tolerance and cyclic electron flux (CEF) around PSI in pgr5 mutant and OE-PGR5 plants. A and B, Phenotypes (A) and the relative electrolyte leakage (B) of WT, pgr5#5 mutant and OE-PGR5#3 tomato plants grown at 4 °C under L-FR or H-FR light conditions for 7 d. C, Post-illumination chlorophyll fluorescence (CEF around PS I) of WT, pgr5#5 mutant and OE-PGR5#3 tomato plants grown at 4 °C under L-FR or H-FR light conditions for 3 d. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 µmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 and 400 µmol m^{-2} s^{-1}). Data are presented as the mean of 4 biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.



Supplemental Figure S12. Changes of $\Delta P700_{max}$ in WT and OE-PGR5 tomato plants as altered by the silencing of HY5, ABI5 or RBOH1. Tomato plants grown at 4 °C under L-FR or H-FR light conditions for 7 d. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 μmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 and 400 μmol m^{-2} s^{-1}). Data are presented as the mean of 4 biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.

Supplemental Table S3. PCR primer sequences used for vector construction.

Vector		primer
OE-HY5	**Forward**	5′-TTGgcgcgccATGCAAGAGCAAGCGACGAG-3′
	Reverse	5′-ACGCgtcgacCTTCCTCCCTTCCTGTGCAC-3′
pET-32a-HY5	**Forward**	5′-CGCggatccATGCAAGAGCAAGCGACGAG-3′
	Reverse	5′-CGgagctcCTACTTCCTCCCTTCCTGTG-3′
pTRV2-HY5	**Forward**	5′-CCGgaattcCACAGGAAGGGAGGAAGTAG-3′
	Reverse	5′-CGCggatccCTTTTACACCGAGTCTTATC-3′
pTRV2-ABI5	**Forward**	5′-CGCgaattcGGGAAATGTTTCGTTGGA-3′
	Reverse	5′-CGCggatccGAGCTGAATTGCCCTGTT-3′
pTRV2-RBOH1	**Forward**	5′-ATACGCgagctcAAGAATGGGGTTGATATTGT-3′
	Reverse	5′- ATACCGctcgagCTCTGACTTATTCCTTAC-3′
RBOH1-RNAi	**Forward**	5′-GGCCatttaaatggatccCGTTCAGCTCTCATTACC-3′
	Reverse	5′-TTggcgcgcctctagaCCGAAGATAGATGTGTGT-3′
pgr5	**Forward**	5′-gattgTTGGAAAGGCAGTGAGATCA-3′
	Reverse	5′-aaacTGATCTCACTGCCTTTCCAAc-3′
OE-PGR5	**Forward**	5′-TTggcgcgccATGGCAATTACAAGTTCAATTGCA-3′
	Reverse	5′-CGGggtaccAGCAAGAAATCCAAGTTTTTCACC-3′

Supplemental Table S4. List of primer sequences used for qRT-PCR analysis.

Gene	Accession number	Forward primer (5'-3')	Reverse primer (5'-3')
HY5	NM_001247891	GCAAGCGACGAGTTCTAT	ATCTCCGGCACTCTTCTG
COP1	NM_001247118	TGGGACAGTGACAGAATGGG	TTGGGATAGAGTTGACTGGTAG
Cu/Zn-SOD	Solyc11g066390	GGCCAATCTTTGACCCTTTA	AGTCCAGGAGCAAGTCCAGT
cAPX	Solyc06g005160	TCTGAATTGGGATTTGCTGA	CGTCTAACGTAGCTGCCAAA
MDAR	DQ665255	TCCGAACAAACATACCTGGA	CGTGTGTGCAGTTAGCAATG
DHAR	DQ521269	ATGGGCAGAATGTTTGTTCA	TTTCAGGCACACTCCACTTC
GR1	Solyc09g091840	TTGGTGGAACGTGTGTTCTT	TCTCATTCACTTCCCATCCA
ABI5	XM_010327617	AGAGCAGCAACAGAACAACG	GCGTCATTTCACCGAACGTA
RBOH1	Solyc08g081690	GGAGCTCCAGCACAAGATTA	CTTGTTGCAGCACTCATGTC
PGR5	Solyc09g090570	ATCAACTTAGGGGCAAAGCT	CCTTTGCTTCTGATCTGCTCC
PGRL1A	Solyc08g080050	CGATGATTTGACTGGATTCG	CCACAATATGAAGGGCAATG
PGRL1B	Solyc08g007770	CGATTTGACTGGATTCGAGA	AACAATGGCGTTTGTGATTG
ORR	Solyc04g057980	CTCCATTGACGGAGTACACG	ACCCAACTCTTGGTTTCCC
ACTIN2	Solyc11g005330	TGTCCCTATTTACGAGGGTTATGC	CAGTTAAATCACGACCAGCAAGAT

Supplemental Table S5. Primers used for ChIP-qPCR assays.

Gene	Accession number	Forward primer (5'-3')	Reverse primer (5'-3')
ABI5	XM_010327617	ACACGTGGAGAAGTAGGGTG	TGCATGGAGAGATTGACACG