Histone Deacetylase HD2 interacts with ERF1 and is involved in longan fruit senescence

Jian-fei Kuang, Jian-ye Chen, Ming Luo, Ke-qiang Wu, Wei Sun, Yue-ming Jiang, and Wang-jin Lu

Supplementary Material

Supplementary data

Supplementary Figure S1. Schematic maps of the constructs used in the transcriptional activation analysis in yeast cells.

Supplementary Figure S2. Schematic maps of the constructs used in the subcellular localization analysis.

Supplementary Figure S3. Schematic maps of bait and prey constructs used in the yeast two-hybrid assay.

Supplementary Figure S4. Schematic maps of the constructs for the BiFC assay.

Supplementary Figure S5. Amino acid sequence alignment of the DlHD2 protein with other plant HD2 proteins.

Supplementary Figure S6. Phylogenetic tree of the deduced amino acid sequences of DlHD2 and other plant HD2.

Supplementary Figure S7. Amino acid alignment of the AP2/ ERF domain of DlERFs and other ERF proteins.

Supplementary Figure S8. Phylogenetic analysis of DlERFs with other AP2/ERF proteins.

Supplementary Figure S9. Amino acid sequence alignment of Group III ERFs.

Supplemental Figure S10. Amino acid sequence alignment of Group IV ERFs.

Supplementary Figure S11. Subcellular localization of DlHD2 in *Arabidopsis* mesophyll protoplasts.

Supplementary Figure S12. Relative quantification of *DlHD2* (A), *DlERF1* (B) and *DlERF2* (C) in aril tissues of control and NO-treated longan fruit stored at room temperature.

Supplementary Figure S13. Relative quantification of *DlHD2* (A), *DlERF1* (B) and *DlERF2* (C) in aril tissues of longan fruit stored at low temperature.

Supplementary Figure S14. Relative quantification of *DlHD2* (A), *DlERF1* (B) and *DlERF2* (C) in aril tissues of longan fruit stored for 40 d at 4 °C and then transferred to 25 °C.

Supplementary Figure S15. Western blot analysis of histone H3 acetylation levels in aril tissues of control and NO-treated longan fruit stored at room temperature (25 °C) for 5 days.

Supplementary Figure S16. BiFC visualization of the DlHD2 and DlERF1 interaction in transiently coexpressed *Arabidopsis* mesophyll protoplasts.

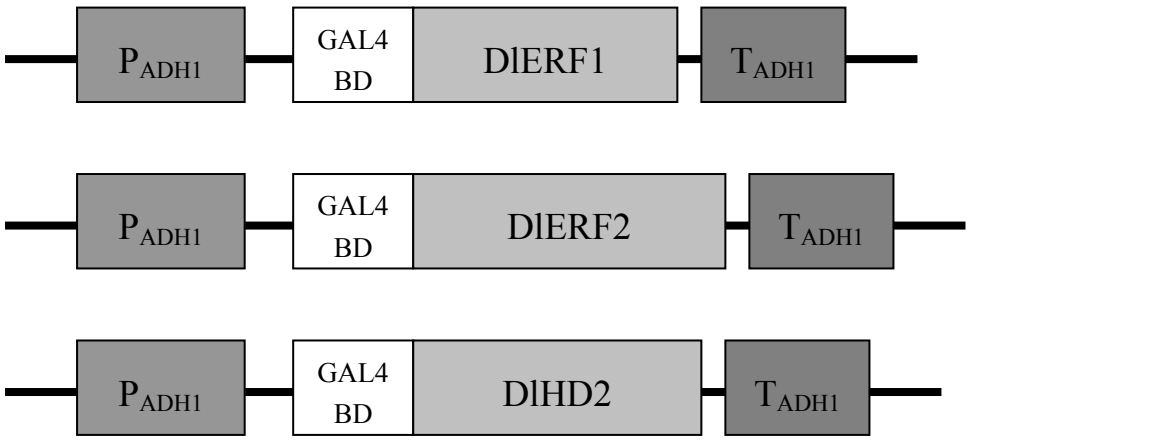
Supplementary Table S1. Primer sequences used for cloning *DlHD2*, *DlERF1* and *DlERF2*.

Supplementary Table S2. Primer sequences used for subcloning into pGBK-T7.

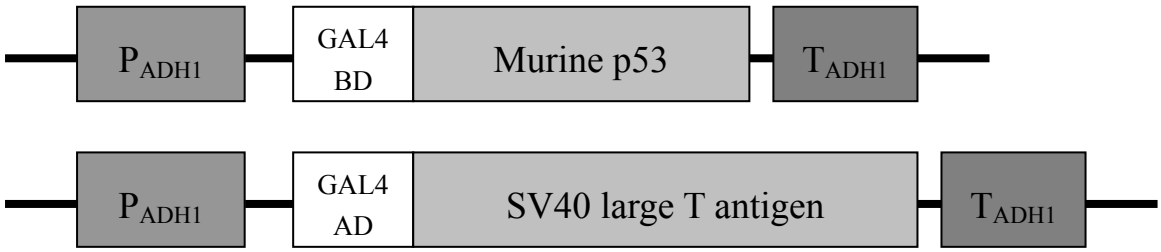
Supplementary Table S3. Primer sequences used for fusing GFP.

Supplementary Table S4. Primer sequences used for synthesis of DIG-labeled probes for Northern blotting.

Supplementary Table S5. Primer sequences used for Yeast Two-Hybrid and BiFC assays.

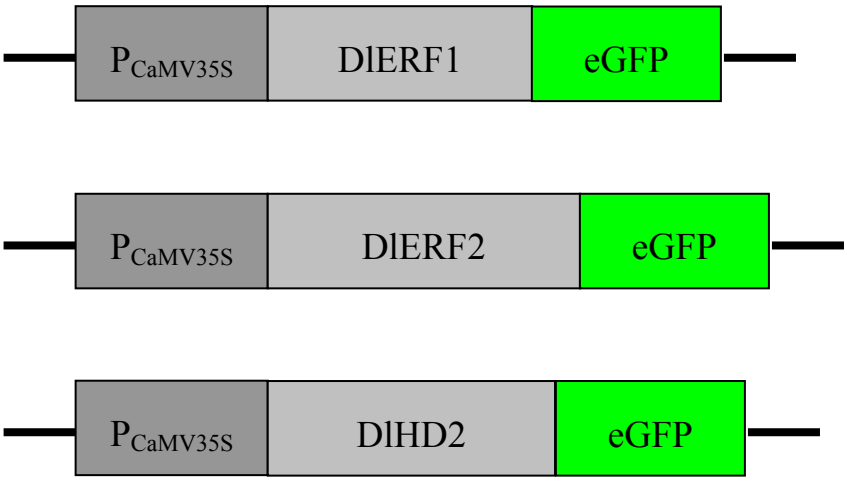


Positive control



Negative control

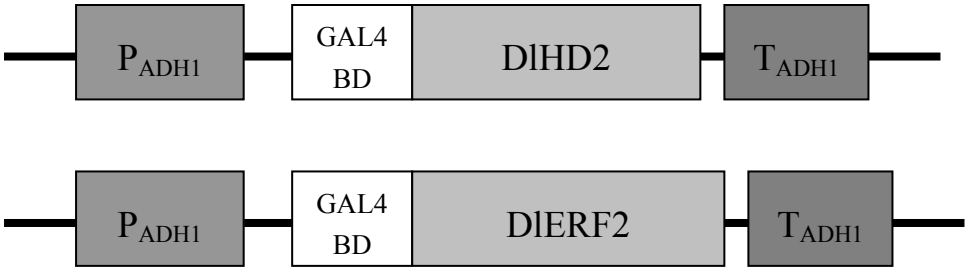
Figure S1. Schematic maps of the constructs used in the transcriptional activation analysis in yeast cells.



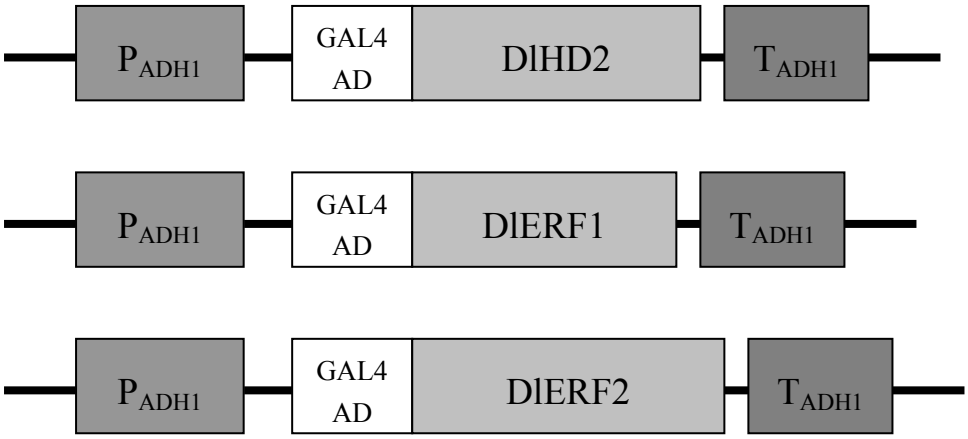
Control

Figure S2. Schematic maps of the constructs used in the subcellular localization analysis.

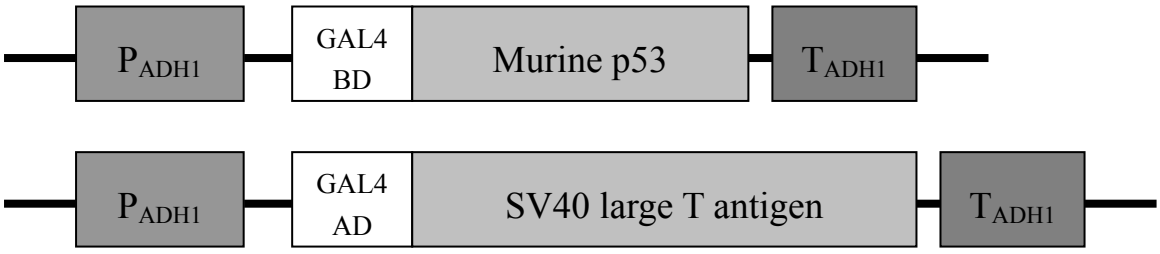
Bait construct



Prey construct



Positive control



Negative control

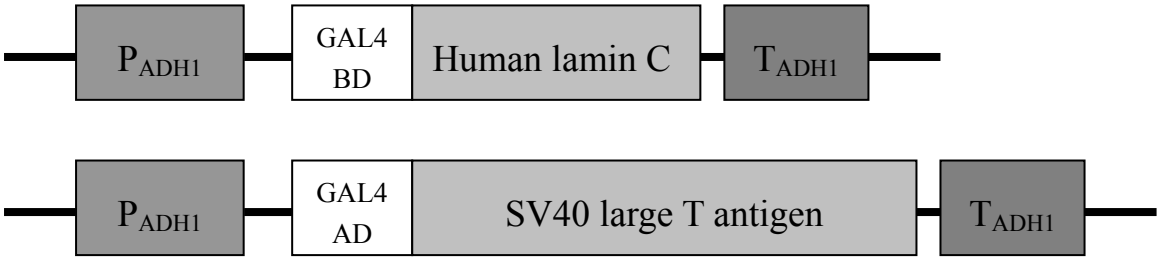
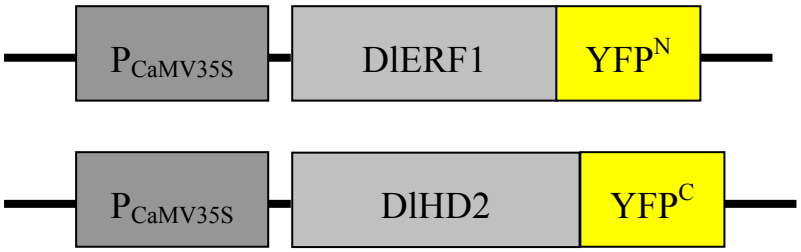
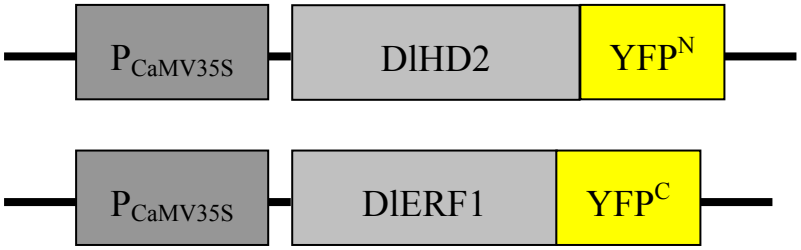


Figure S3. Schematic maps of bait and prey constructs used in the yeast two-hybrid assay.

DlERF1-YN + DlHD2-YC

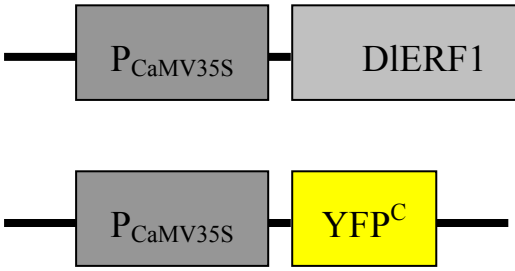


DlHD2-YN + DlERF1-YC



Positive control

Negative control



Emptive vector

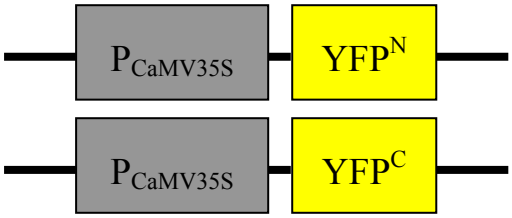


Figure S4. Schematic maps of the constructs for the BiFC assay.

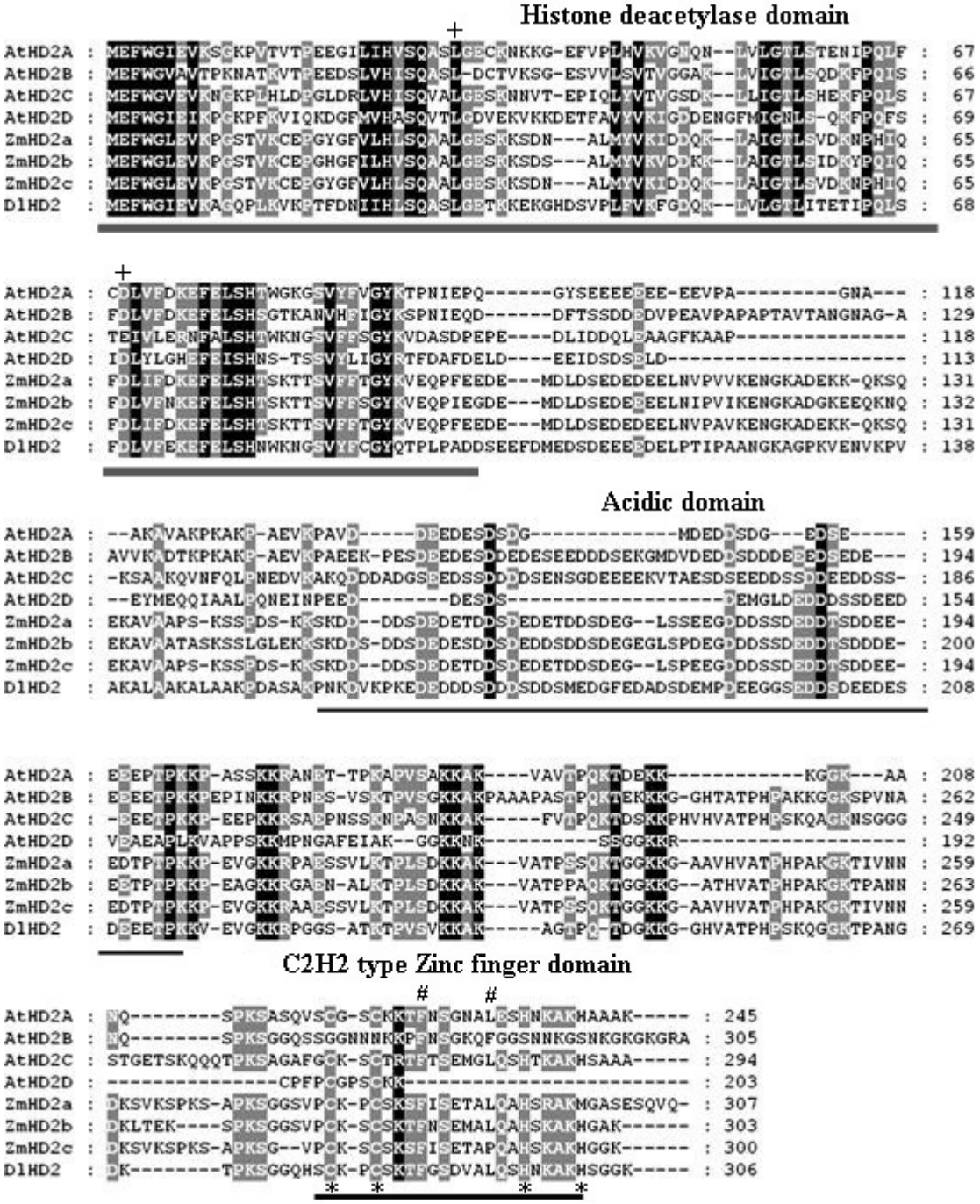


Figure S5. Amino acid sequence alignment of the DlHD2 protein with other plant HD2 proteins. DlHD2 was aligned with *Arabidopsis* AtHD2A (NM_114344), AtHD2B (NM_122171), AtHD2C (NM_120455), and AtHD2D (AF255713); maize ZmHD2a (U82815), ZmHD2b (NM_001112161) and ZmHD2c (AF254073). Identical and similar amino acids were presented by black and gray shading, respectively. Gaps were introduced to optimize alignment. The histone deacetylases domain, acidic domain and a putative C2H2 type zinc finger domain were underlined. Conserved amino acids predicted to be involved in catalytic activity were indicated with '+'. The cysteine and histidine residues, phenylalanine and leucine in the putative zinc finger domain were indicated by '*' and '#', respectively.

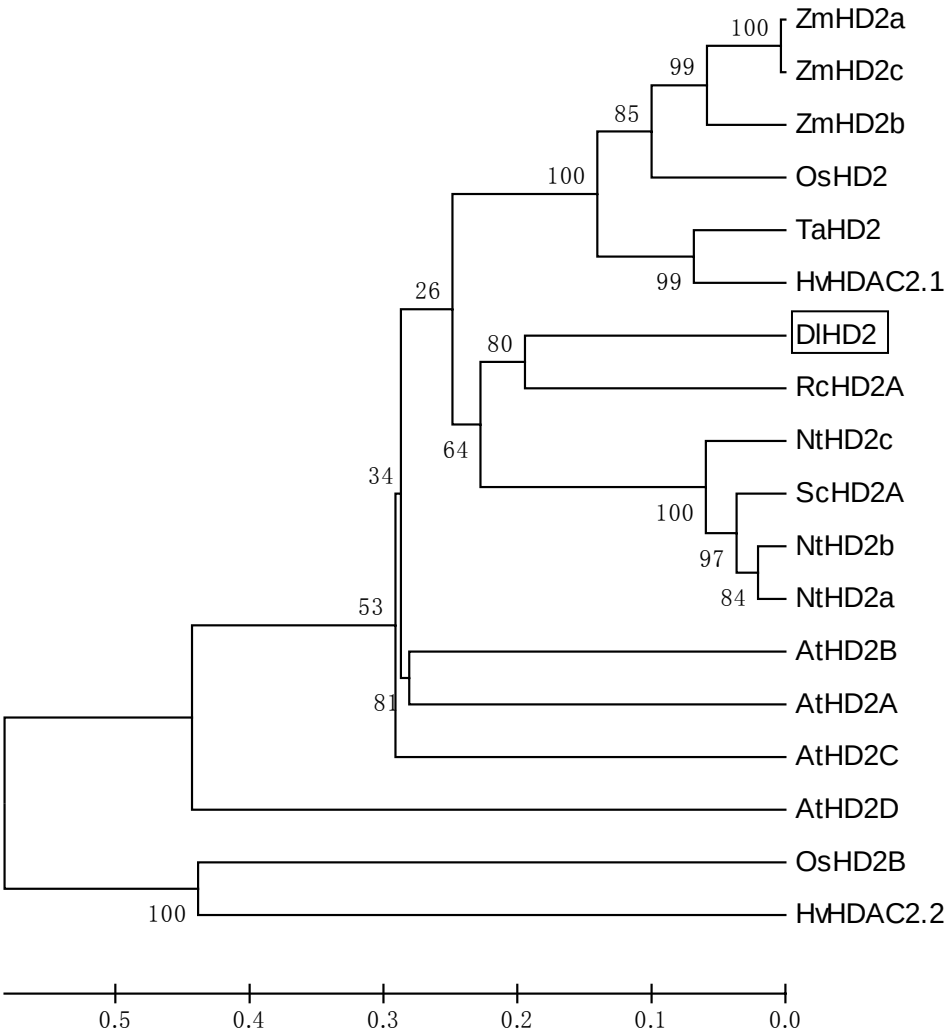


Figure S6. Phylogenetic tree of the deduced amino acid sequences of DlHD2 and other plant HD2. The phylogenetic tree was generated based on an alignment of the full length deduced amino acid sequences of 15 HD2 proteins, including *Arabidopsis* AtHD2A (NM_114344), AtHD2B (NM_122171), AtHD2C (NM_120455), and AtHD2D (AF255713); maize ZmHD2a (U82815), ZmHD2b (NM_001112161) and ZmHD2c (AF254073); rice OsHD2 (AF255711) and OsHD2B (NM_001051686); barley HvHDAC2.1 (EU348775) and HvHDAC2.2 (EU348776), wheat TaHD2 (DQ656602); patato ScHD2A (AY346455); castor bean RcHD2A (XP_002527449). Alignments were made using CLUSTAL X multiple sequence software. The phylogenetic tree was constructed by the Neighbor-Joining method using the MEGA programme with default settings. Numbers at the branchpoints indicated bootstrap values. A scale of distance was shown at the bottom.

Figure S7. Amino acid alignment of the AP2/ ERF domain of DlERFs and other ERF proteins, including *Arabidopsis* AtERF2 (NM_124093), AtERF5 (NM_124094); pepper CaERELP (AAS20427); melon CmERELP (BAD01556); tobacco NtERF1 (Q40476); tomato SlERF1 (Q84XB3), Pti4 (ACF57857) and plum PsERF1a (FJ026009). Black shading identified fully conserved residues, while conservative amino acid substitutions were represented by gray shading. The 14th alanine and 19th aspartic acid residues in the AP2/ERF domain were marked by asterisks.

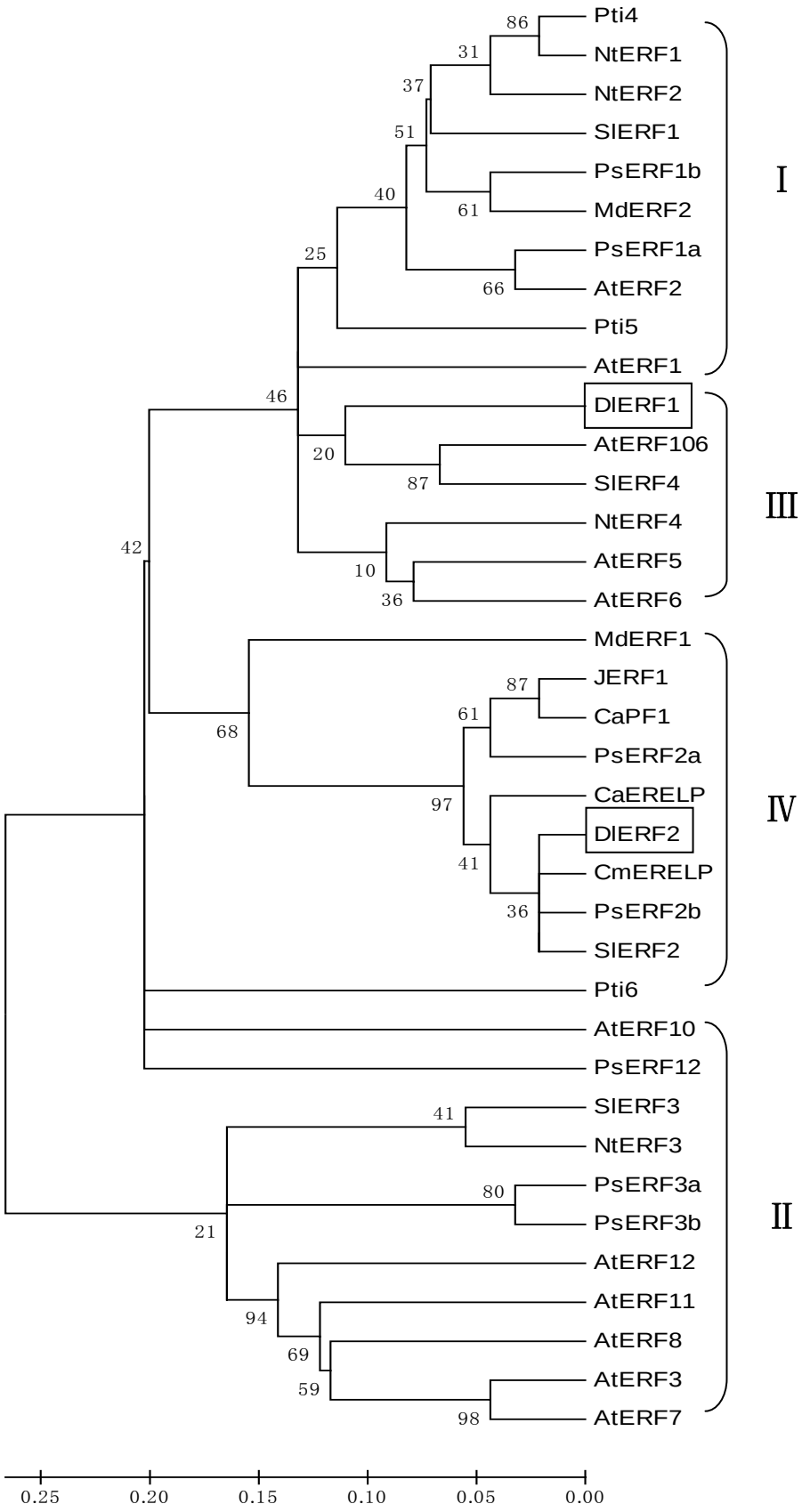


Figure S8. Phylogenetic analysis of DlERFs with other AP2/ERF proteins. The phylogenetic

tree was generated based on an alignment of the full length deduced amino acid sequences of 37 AP2/ERF proteins, including *Arabidopsis* AtERF1 (NM_113225), AtERF2 (NM_124093), AtERF3 (NP_175479), AtERF5 (NM_124094), AtERF6 (NM_117854), AtERF7 (NP_188666), AtERF8 (NP_175725), AtERF10 (NM_100259), AtERF11 (NP_174159), AtERF12 (NP_174158) and AtERF106 (Q9LY05); plum PsERF1a (FJ026009), PsERF1b (FJ026008), PsERF2a (FJ026007), PsERF2b (FJ026006), PsERF3a (FJ026005), PsERF3b (FJ026004) and PsERF12 (FJ026003); tomato SlERF1 (Q84XB3), SlERF2 (AAO34704), SlERF3 (AAO34705), SlERF4 (AAO34706), Pti4 (ACF57857), Pti5 (AAC49740), Pti6 (AAC49741) and JERF1 (AAK95687); tobacco NtERF1 (Q40476), NtERF2 (Q40479), NtERF3 (Q40477) and NtERF4 (Q40478); apple MdERF1 (BAF43419) and MdERF2 (BAF43420); pepper CaPF1 (AAP72289) and CaERELP (AAS20427); melon CmERELP (BAD01556). Alignments were made using CLUSTAL X multiple sequence software. The phylogenetic tree was constructed by the Neighbor-Joining method using the MEGA programme with default settings. Numbers at the branchpoints indicated bootstrap values. A scale of distance was shown at the bottom.

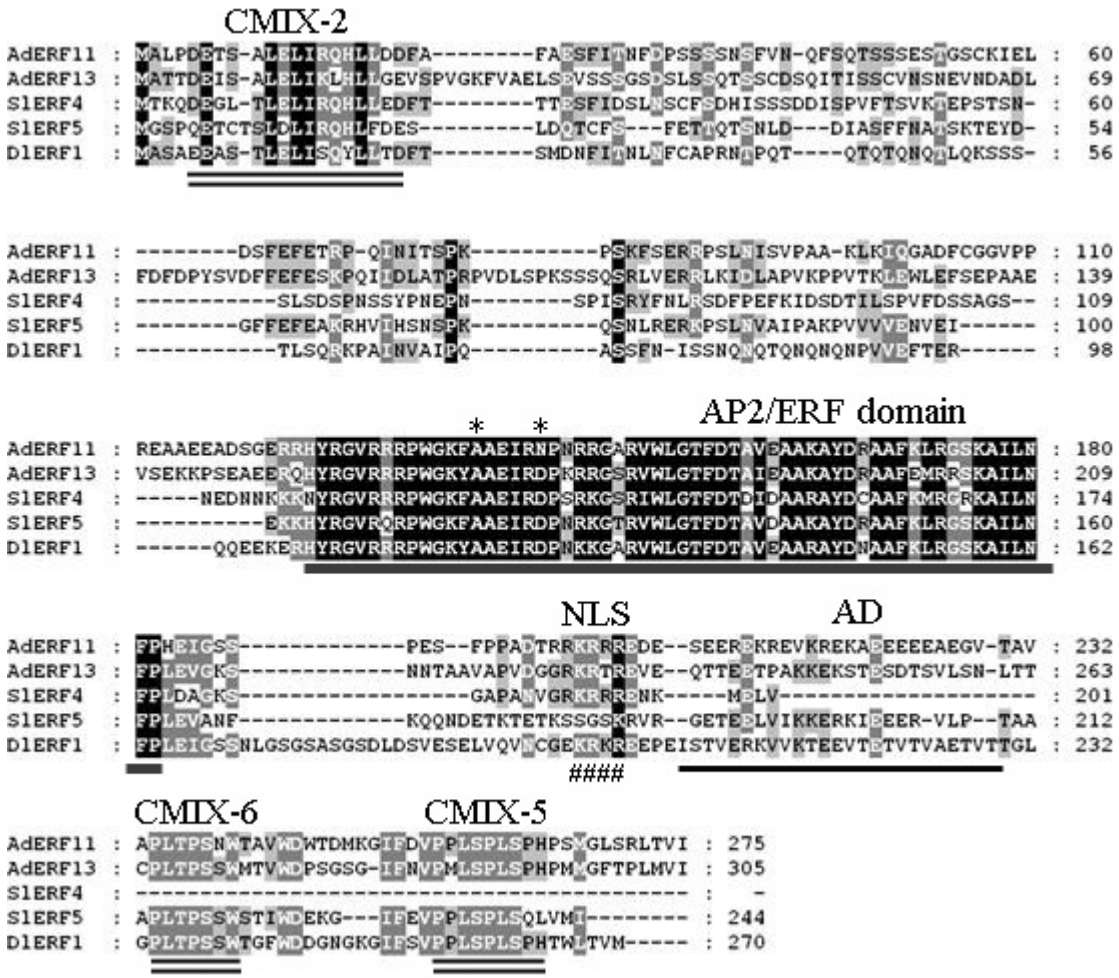


Figure S9. Amino acid sequence alignment of Group III ERFs including DlERF1, AtERF5, AtERF6, AtERF106, NtERF4 and SlERF4. Shaded letters indicated the conserved amino acid residues. AP2/ERF domain and acidic domain (AD) were underlined. The two amino acid residues (Ala and Asp) contributing a functional GCC box-binding activity (Sakuma *et al.*, 2002) and the putative nuclear localization signals (NLS) were indicated by two asterisks and '#', respectively. The CMIX2, CMIX-5 and CMIX-6 motif were double underlined.

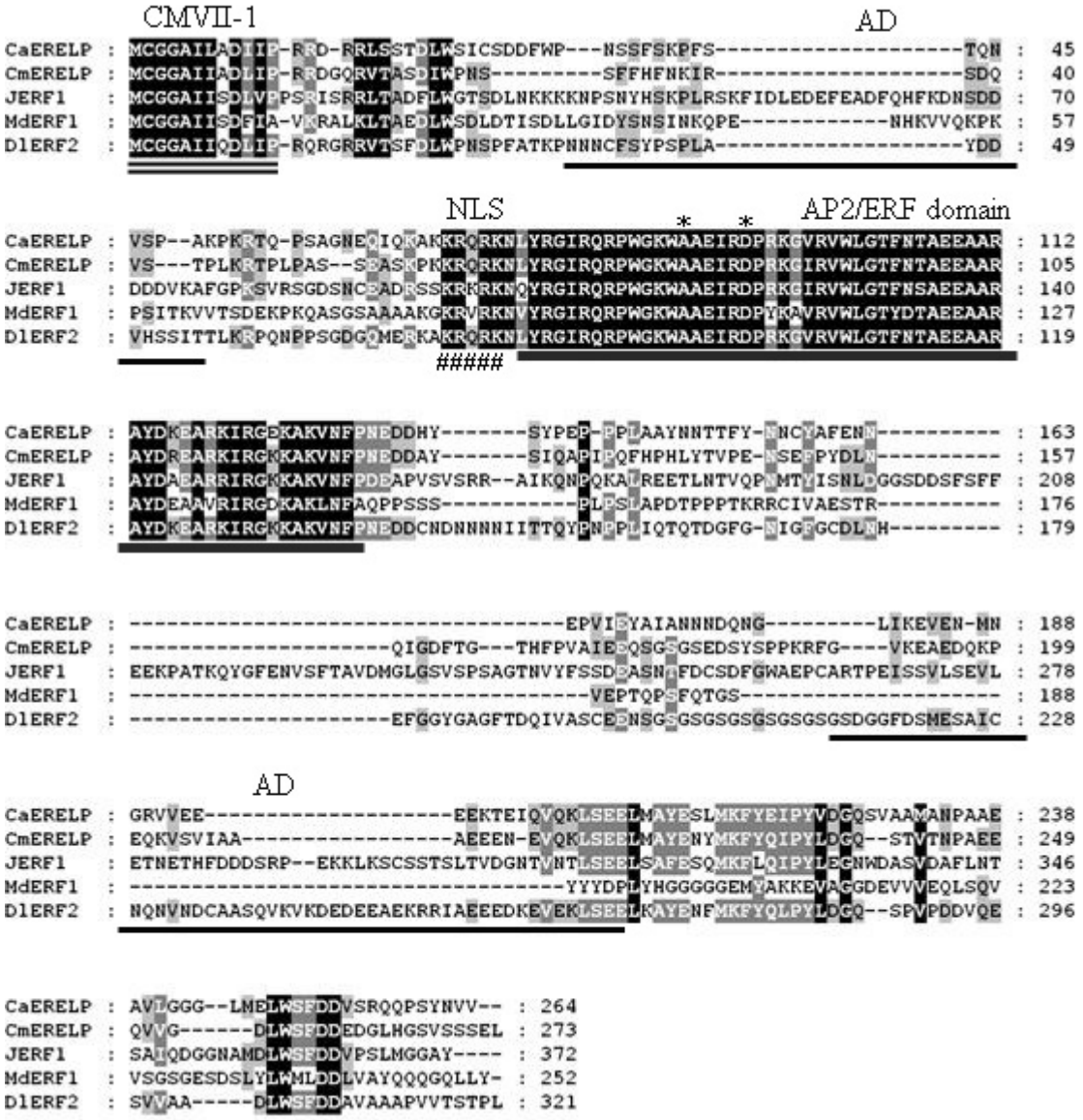


Figure S10. Amino acid sequence alignment of Group IV ERFs including DlERF2, CaPF1, CmERELP, PsERF2a, PsERF2b and SlERF2. Shaded letters indicated the conserved amino acid residues. AP2/ERF domain and acidic domain (AD) were underlined. The two amino acid residues (Ala and Asp) contributing a functional GCC box-binding activity (Sakuma *et al.*, 2002) and the putative nuclear localization signals (NLS) were indicated by two asterisks and '#', respectively. The CMVII-1 motif was double underlined.

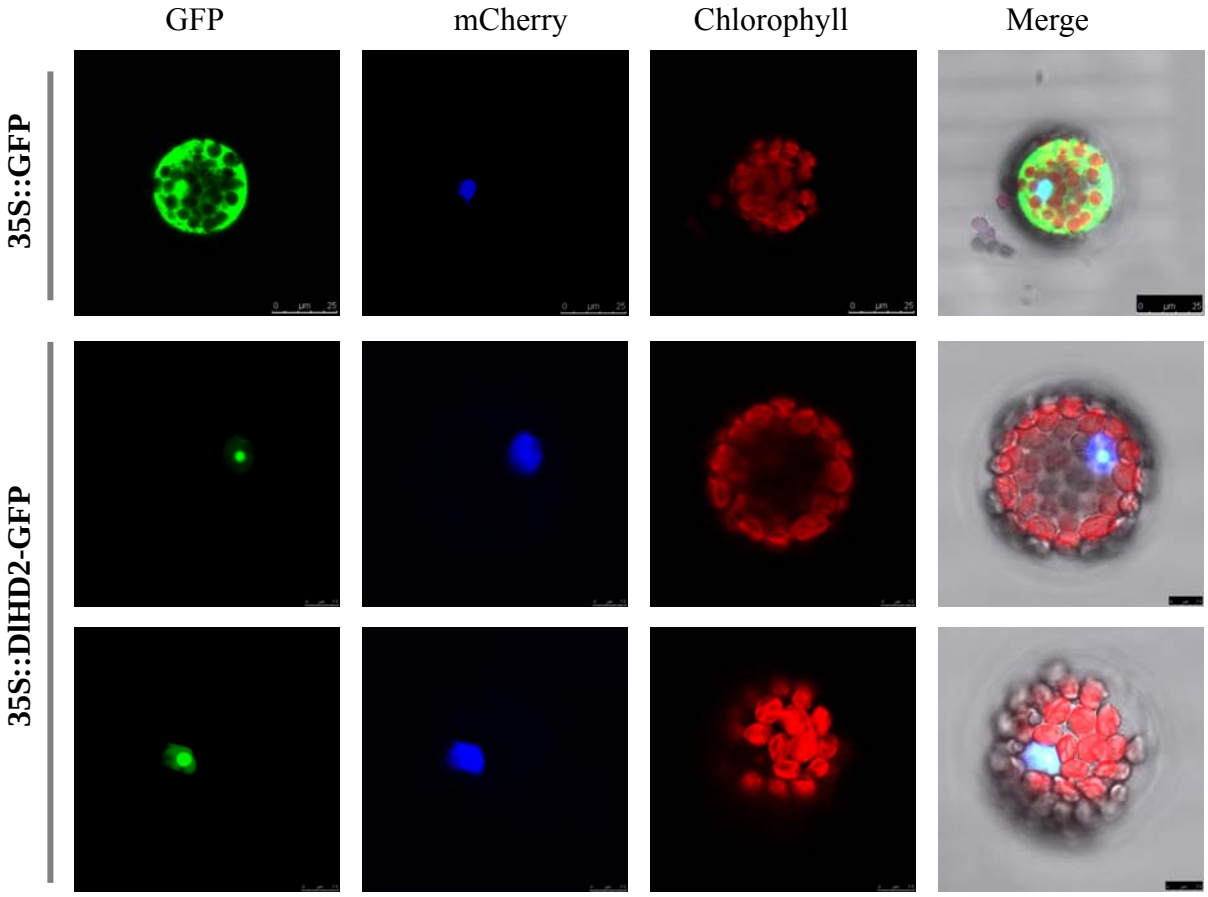


Figure S11. Subcellular localization of DlHD2 in *Arabidopsis* mesophyll protoplasts. The *Arabidopsis* protoplasts were transiently transformed with DlHD2-GFP or GFP vector by a modified polyethylene glycol method. GFP fluorescence was observed with a laser scanning confocal microscope. The mCherry-VirD2NLS was included in each transfection to serve as a control for successful transfection as well as for nuclear localization. The DlHD2-GFP fusion protein was observed in the nucleoli of nucleus. Images were taken in the dark field for green fluorescence, while the outline of the cell and the combination were photographed in a bright field. The length of bar was indicated in the photos.

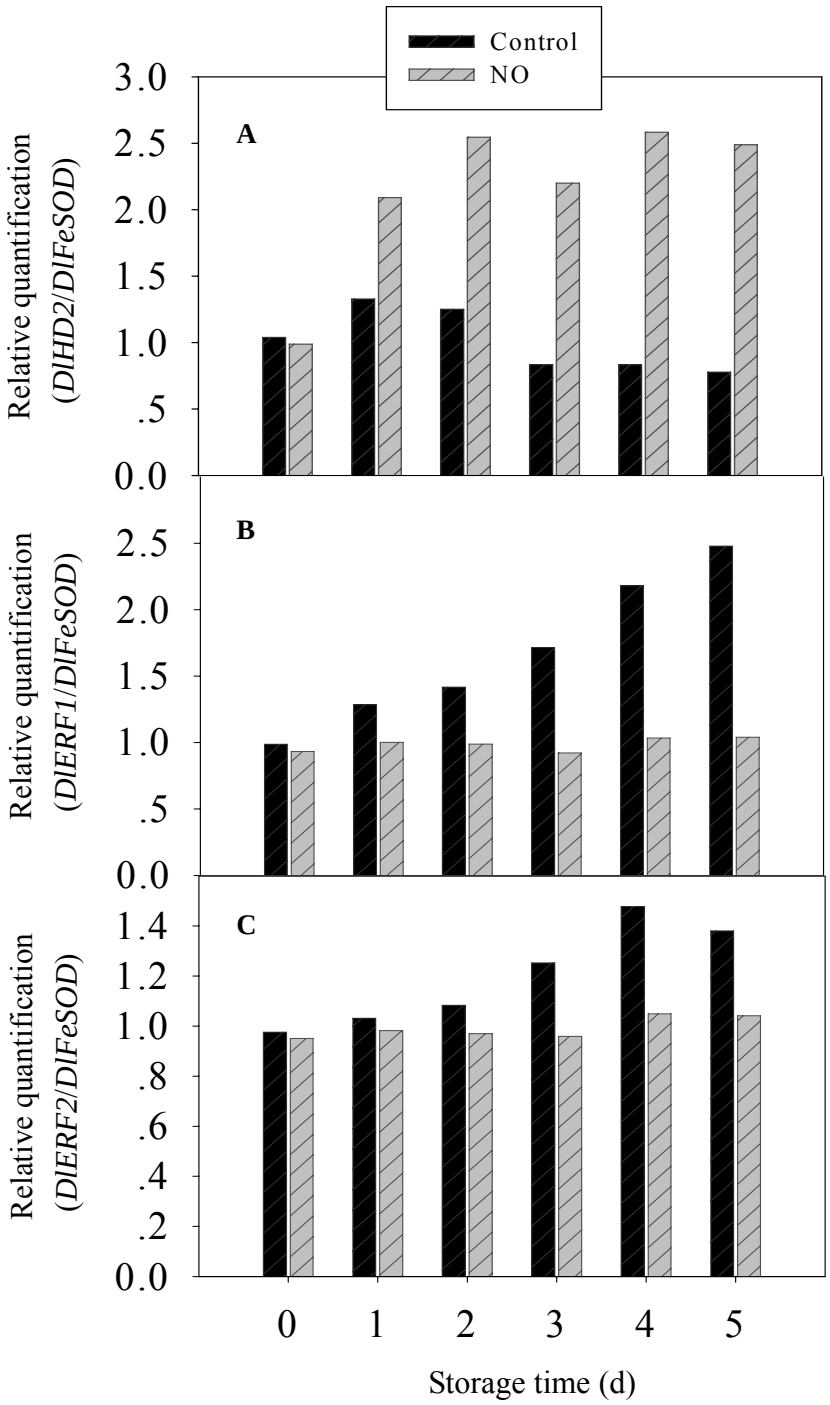


Figure S12. Relative quantification of *DlHD2* (A), *DlERF1* (B) and *DlERF2* (C) in aril tissues of control and NO-treated longan fruit stored at room temperature. The hybridization signals were quantified using Bio-Rad Quantity One software and the relative quantification of each gene was expressed as the ratio of target gene intensity to *FeSOD* intensity.

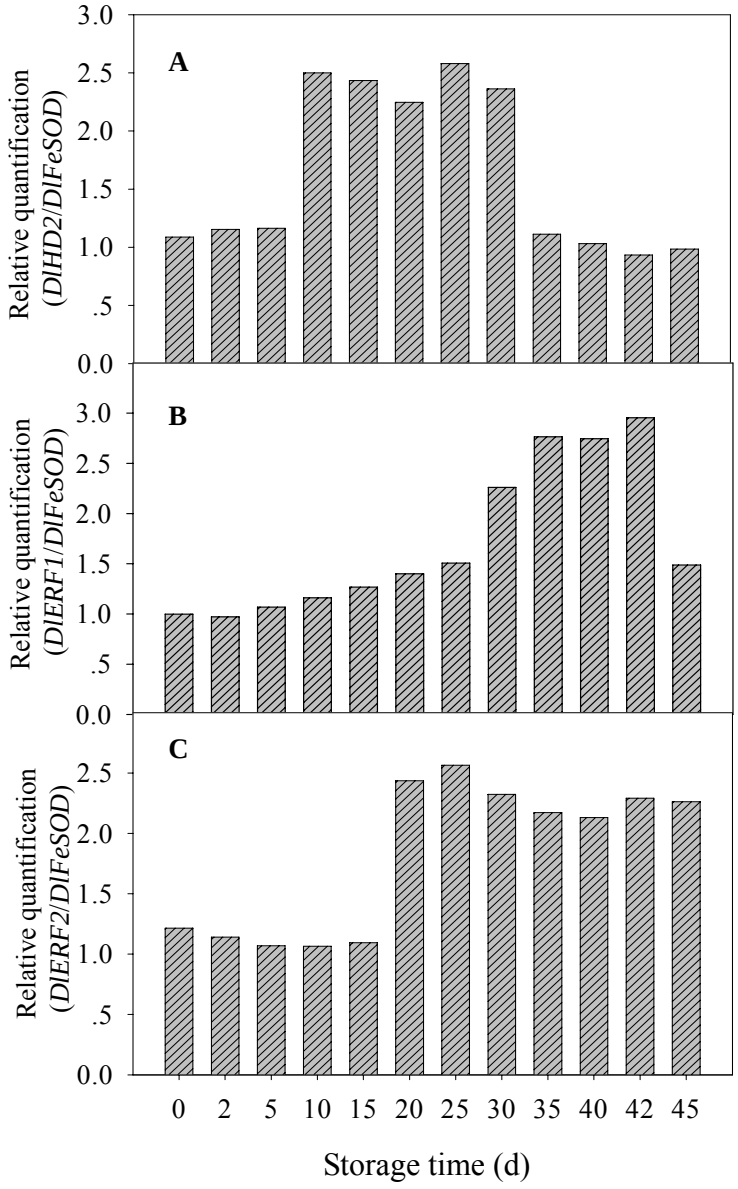


Figure S13. Relative quantification of *DlHD2* (A), *DlERF1* (B) and *DlERF2* (C) in aril tissues of longan fruit stored at low temperature. The hybridization signals were quantified using Bio-Rad Quantity One software and the relative quantification of each gene was expressed as the ratio of target gene intensity to *FeSOD* intensity.

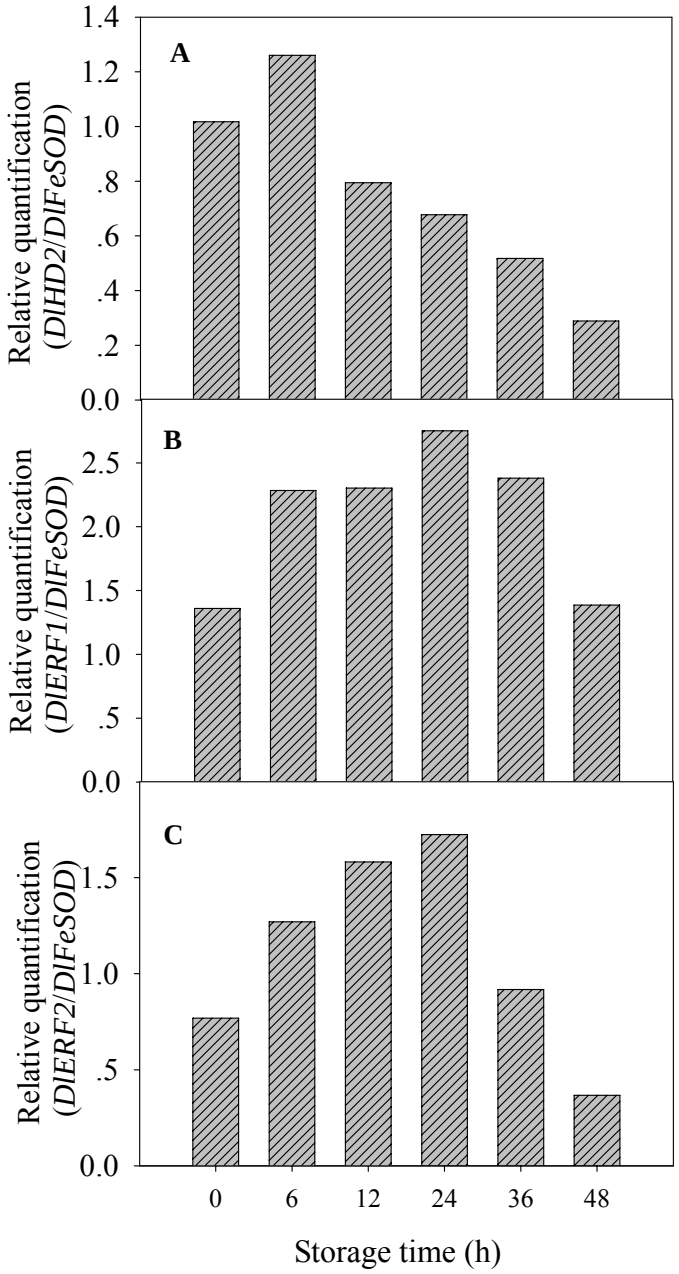


Figure S14. Relative quantification of *DlHD2* (A), *DlERF1* (B) and *DlERF2* (C) in aril tissues of longan fruit stored for 40 d at 4 °C and then transferred to 25 °C. The hybridization signals were quantified using Bio-Rad Quantity One software and the relative quantification of each gene was expressed as the ratio of target gene intensity to *FeSOD* intensity.

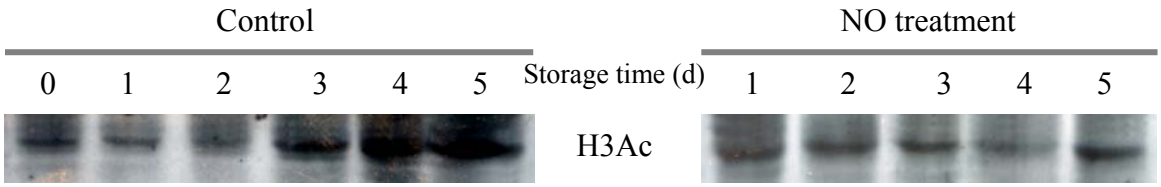


Figure S15. Western blot analysis of histone H3 acetylation levels in aril tissues of control and NO-treated longan fruit stored at room temperature (25 °C) for 5 days. Equal amounts of total protein (20 μg per lane) were subjected to SDS-PAGE and then transferred to nitrocellulose membranes. Thereafter, histone H3 acetylation levels were immunodetected using a polyclonal antibody against acetylated histone H3 (06-599, Millipore) and the secondary antibody goat anti-rabbit IgG-HRP conjugate (Promega). Visualization was performed by using the SuperSignal® West Pico Kit (Pierce) according to the manufacturer's instructions.

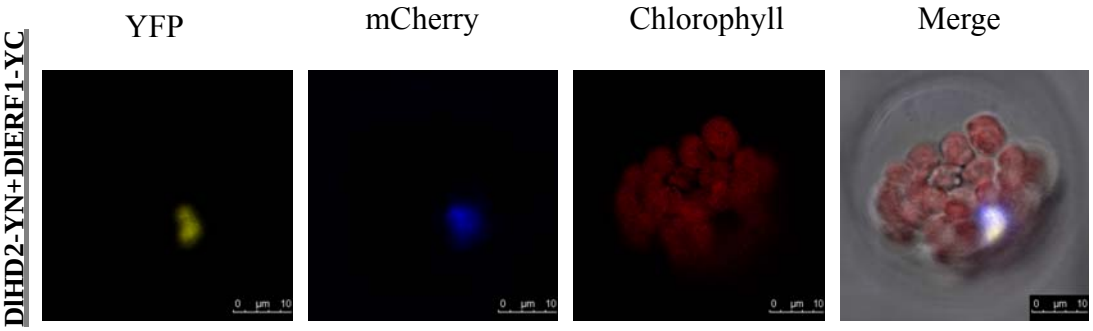


Figure S16. BiFC visualization of the DlHD2 and DlERF1 interaction in transiently coexpressed *Arabidopsis* mesophyll protoplasts. DlHD2 protein was fused with the N-terminal of YFP and DlERF1 protein was fused with C-terminal of YFP. The mCherry-VirD2NLS was to serve as a control for successful transfection as well as for nuclear localization.

Supplementary Table S1. Primer sequences used for cloning *DlHD2*, *DlERF1* and *DlERF2*.

Name	Sequences (5′-3′)
HD2-For	ATGGAGTTCTGGGGTVTNGMRGT
HD2-Rev	AGGATGAGGNGTNGCNACRTG
ERF-For	CCRTGGGGRAAATKKGCGGCK
ERF-Rev	CATAAGCVAVAKBGCRGCTTCYTC
DlHD2-3RACE1	GCTCTTGCTGCTAAGGCTCT
DlHD2-3RACE2	TGATTCTGACGAGATGCCTG
DlERF1-3RACE1	CCATGGGGCAAGTACGCGGCGG
DlERF1-3RACE2	CCGTGACCCGAATAAGAAAGGT
DlERF2-3RACE1	GAGATTCGTGACCCGAGAAA
DlERF2-3RACE2	CACTGCTGAAGAAGCTGCAA
DlHD2-5RACE1	TCTGCTGGTAAGGGGGTCTGGTA
DlHD2-5RACE2	GTTTCAGTGATAAGCGTCCCAAG
DlERF1-5RACE1	CCTTTCCATTTCCGTCATCCCAG
DlERF1-5RACE2	TTCCGTTACCTCCTCCGTCTTCA
DlERF2-5RACE1	AGGCTGGTTGGGAAAGAGAAGGA
DlERF2-5RACE2	AGAGAGGCAGGAAAGGAGGAGGT

Supplementary Table S2. Primer sequences used for subcloning into pGBK-T7.

Name	Sequences (5′-3′)	Restriction Site
DlHD2-BDFor	AcatatgATGGAGTTCTGGGGTATAGAA	*Nde* I
DlHD2-BDRev	AggatccCTATTTGCCACCAGAATGCTTAG	*BamH* I
DlERF1-BDFor	AcatatgATGGCGTCTGCAGAAGAAGCT	*Nde* I
DlERF1-BDRev	AcccgggTTACATGACCGTCAGCCAAGT	*Sma* I
DlERF2-BDFor	AcatatgATGTGTGGCGGTGCTATTATC	*Nde* I
DlERF2-BDRev	AggatccTTACAGAGGCGTCGAGGTCACA	*BamH* I

Supplementary Table S3. Primer sequences used for fusing GFP.

Name	Sequences (5'-3′)	Restriction Site
DlHD2-GFPFor	AgtcgacATGGAGTTCTGGGGTATAGAA	*Sal* I
DlHD2-GFPRev	AgtcgacTTTGCCACCAGAATGCTTAGC	*Sal* I
DlERF1-GFPFor	AgtcgacATGGCGTCTGCAGAAGAAGCT	*Sal* I
DlERF1-GFPRev	AgtcgacCATGACCGTCAGCCAAGTATG	*Sal* I
DlERF2-GFPFor	AgtcgacATGTGTGGCGGTGCTATTATC	*Sal* I
DlERF2-GFPRev	AgtcgacCAGAGGCGTCGAGGTCACAAC	*Sal* I

Supplementary Table S4. Primer sequences used for synthesis of DIG-labeled probes for Northern blotting.

Name	Forward primer (5′-3′)	Reverse primer (5′-3′)	Length
DlHD2	TGATTCTGACGAGATGCCTG	ATCAGACCCGAATGTCTTGC	316 bp
DlERF1	GCTTATGACAACGCTGCCTT	TCCACATCATCCACGACTCT	444 bp
DlERF2	ATTCAATGGAGTCGGCAATC	GCAATTACAGAGGCGTCGAG	309 bp
DlFeSOD	TTTGGGAGCATGCTTATTACCT	GGGAATGTTTCAAAAGTTCTGG	359 bp

Supplementary Table S5. Primer sequences used for Yeast Two-Hybrid and BiFC assays.

Name	Sequences (5′-3′)	Restriction Site
DlHD2-Y2HFor	Acatatg ATGGAGTTCTGGGGTATAGAA	*Nde* I
DlHD2-Y2HRev	Aggatcc CTATTTGCCACCAGAATGCTTAG	*BamH* I
DlERF1-Y2HFor	Acatatg ATGGCGTCTGCAGAAGAAGCT	*Nde* I
DlERF1-Y2HRev	Acccggg TTACATGACCGTCAGCCAAGT	*Sma* I
DlERF2-Y2HFor	Acatatg ATGTGTGGCGGTGCTATTATC	*Nde* I
DlERF2-Y2HRev	Aggatcc TTACAGAGGCGTCGAGGTCACA	*BamH* I
DlHD2-BiFCFor	Atctaga ATGGAGTTCTGGGGTATAGAA	*Xba* I
DlHD2-BiFCRev	Agtcgac TTTGCCACCAGAATGCTTAGC	*Sal* I
DlERF1-BiFCFor	Atctaga ATGGCGTCTGCAGAAGAAGCT	*Xba* I
DlERF1-BiFCRev	Agtcgac CATGACCGTCAGCCAAGTATG	*Sal* I