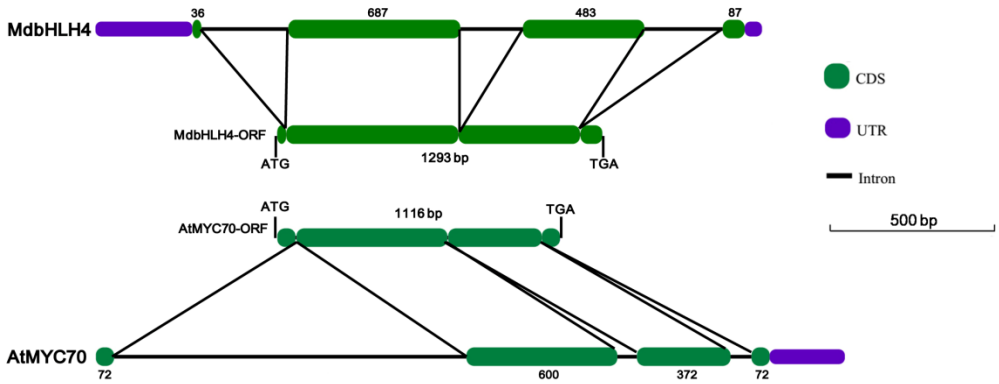
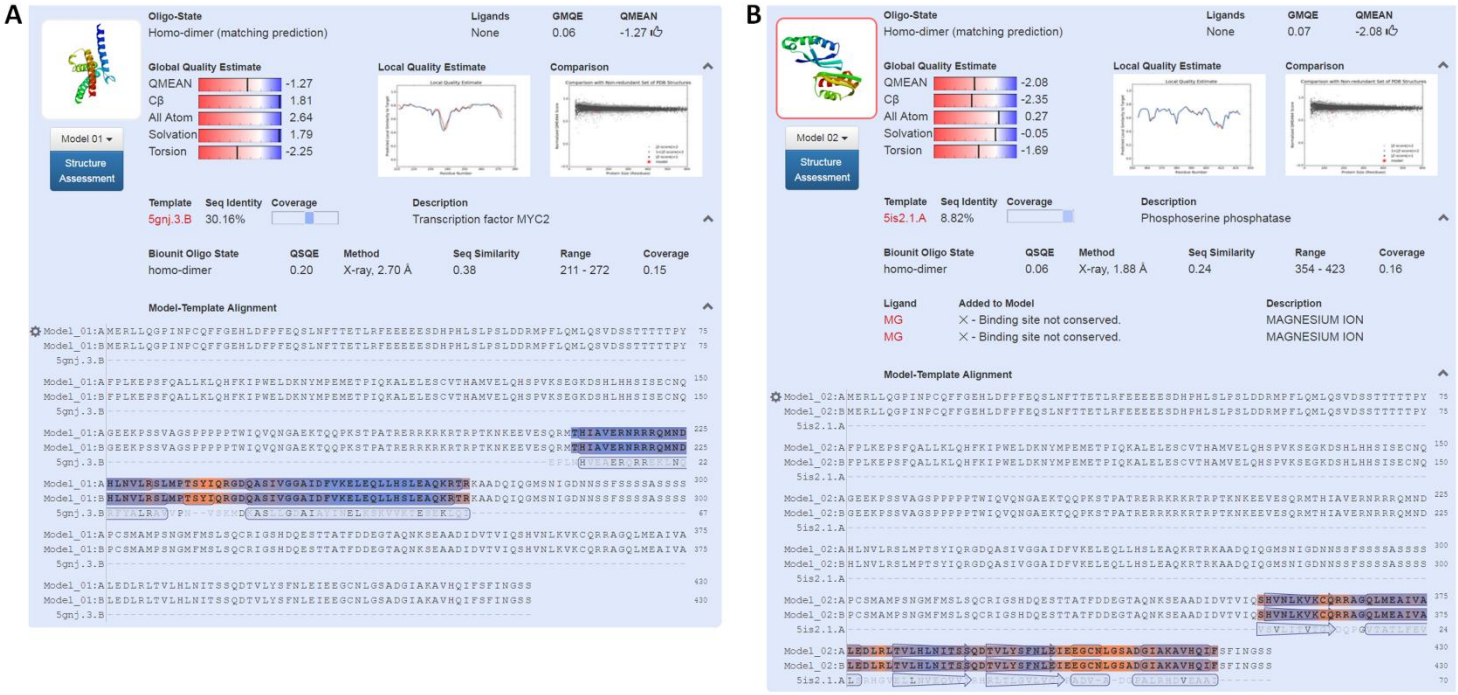
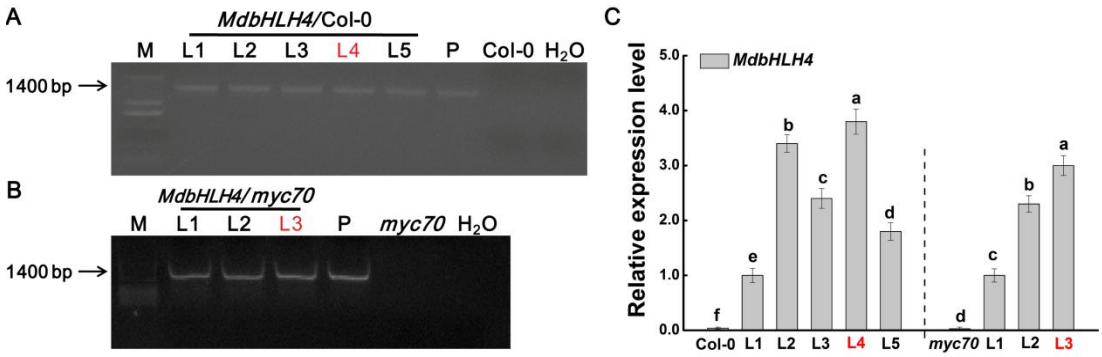


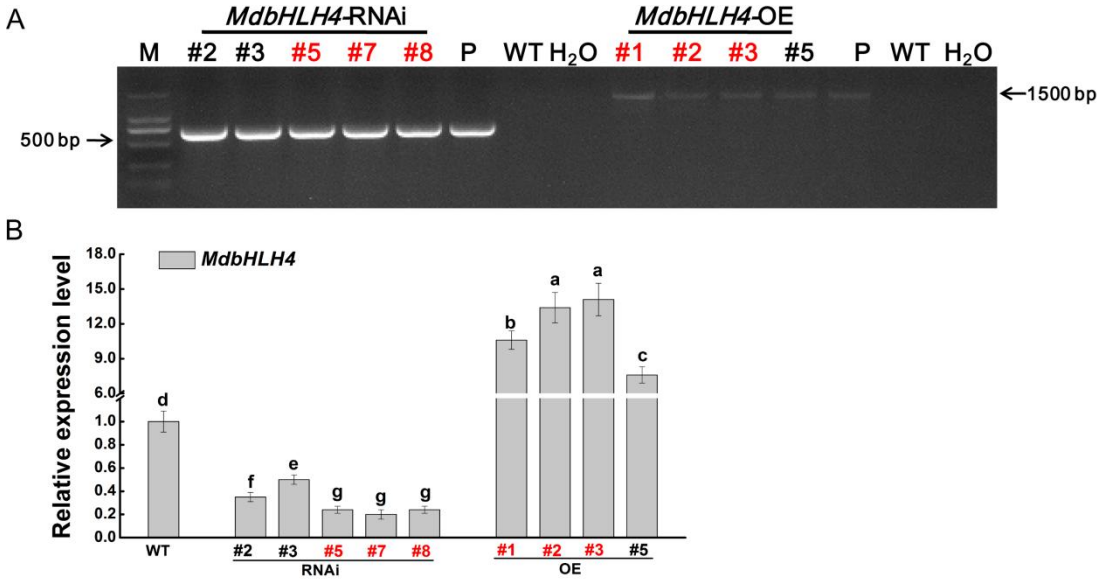
Supplemental Figure S1. Schematic diagram of the gene structures of *MdbHLH4* and *AtMYC70*. Numbers indicate the sizes of different exons.



Supplemental Figure S2. Details of the 3D structure prediction results of MdbHLH4 protein. **A,** Protein structure prediction of the bHLH domain of MdbHLH4 based on the template 5gnj.3.B. **B,** Protein structure prediction of the C-terminal domain of MdbHLH4 based on the template 5is2.1.A.



Supplemental Figure S3. Identification of *MdbHLH4* transgenic *Arabidopsis* seedlings. **A-B,** Genomic PCR identification of *MdbHLH4* transformation in Col-0 (A) and *myc70* mutant (B) backgrounds. M, DNA marker; H_2O, negative control; P, positive control (overexpression vector plasmid). **C,** *MdbHLH4* transcripts in different transgenic lines. Error bars indicate the SD of three biological replicates. Different letters represent significant differences through one-way ANOVA and Duncan's tests ($p < 0.05$).



Supplemental Figure S4. Identification of *MdbHLH4* transgenic apple plants. **A,** Genomic PCR identification of *MdbHLH4* transgenic apple plants. WT: 'GL-3' plants; P, positive control (plasmid DNA of *MdbHLH4-GFP* and *MdbHLH4-RNAi* vectors). Red numbers indicate the transgenic lines used for cold treatment in this study. **B,** *MdbHLH4* transcripts in WT and transgenic lines. Error bars indicate the SD of three biological replicates. Different letters represent significant differences through one-way ANOVA and Duncan's tests ($p < 0.05$).

A

MdCBF1-P1 : 5'-AGCCGTACAATTGTAAGACAGCTGTCGCACT-3'

MdCBF1-P2 : 5'-AAACCACACACTTGGCACACAC-3'

MdCBF1-P2-mut : 5'- AAACCACAAAAAAAGCACACAC -3'

MdCBF1-P3 : 5'-ACAGCAAGTGAGCAACAAATCGGCGAGGAATATGTCTCCC
CAGAGTGCTGCAGCAGGTGCGAAAGTACATCTGTTGG-3'

MdCBF1-P4 : 5'-CTTGCTACAAATGTAAGGTTTGTTTACAAGTGTTTTTA-3'

MdCBF1-P5 : 5'-ATTTGTTCACTTGGTGTCACTC-3'

MdCBF1-P5-mut : 5'- ATTTGTTAAAAAAGTGTCACTC -3'

MdCBF3-P1 : 5'-ATTGTCCACACTTGCGACTTTTCC -3'

MdCBF3-P2 : 5'-GTTCGCGTTTTTCATTTGCCCAGTCGCC-3'

MdCBF3-P2-mut : 5'- GTTCGCGTTTTTAAAAAACCCAGTCGCC -3'

MdCBF3-P3 : 5'-AAATCCTACGACAGCTGTAGGGTTTATTGG-3'

MdCBF3-P4 : 5'-GAATCCACACACTTGGCACACACAAAC-3'

MdCBF3-P4-mut : 5'-GAATCCACAAAAAAAGCACACACAAAC-3'

MdCBF3-P5 : 5'-ATATATTCATGTGGATTGGGTGATGAG-3'

MdCBF3-P5-mut : 5'-ATATATTAAAAAAGATTGGGTGATGAG-3'

B

MdCAX3L-2-P1 : 5'-CTTACTCTACTTCATCTGACAAGATACTATCTAGC-3'

MdCAX3L-2-P1-mut : 5'-CTTACTCTACTTAAAAAAACAAGATACTATCTAGC-3'

MdCAX3L-2-P2 : 5'-CTATCAACAATCTCAATTGCAAAGAAAAATG-3'

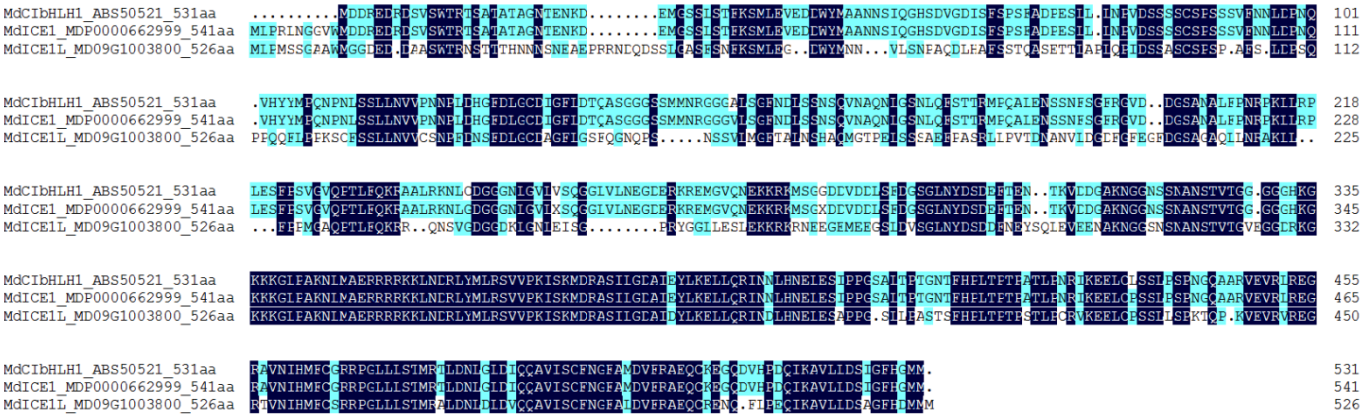
MdCAX3L-2-P3 : 5'-GGTGAGCATTACCACCACTTGAGGGGGTGGATTCGG-3'

MdCAX3L-2-P3-mut : 5'-GGTGAGCATTACCACAAAAAAAGGGGGGTGGATTCGG-3'

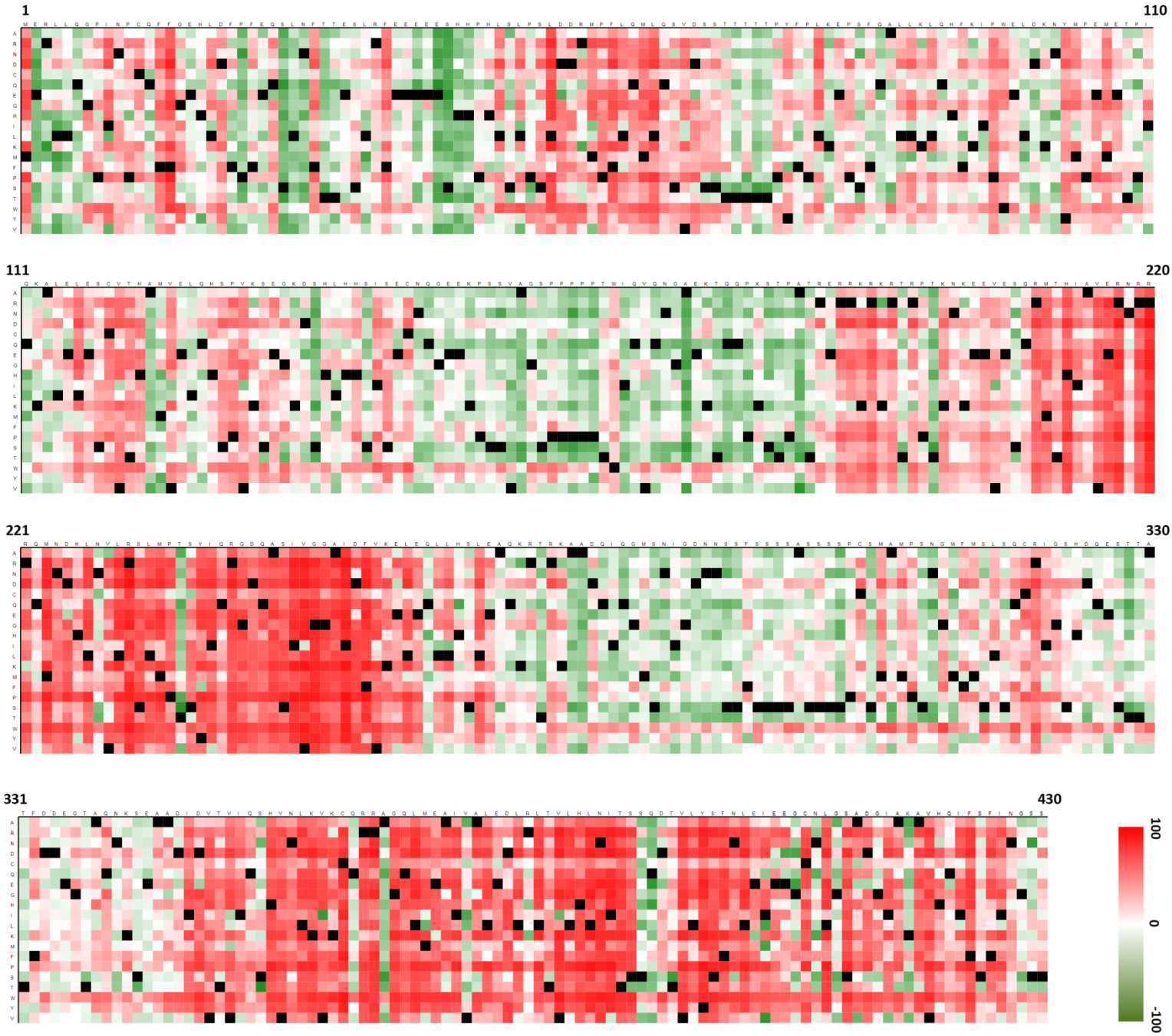
MdCAX3L-2-P4: 5'-CGTTCAGAACTCAGTTGACTGATTCTATAGATAAGG-3'

MdCAX3L-2-P5: 5'-CTAAAACGAGCCATCTGGCCAGCCCTCGG-3'

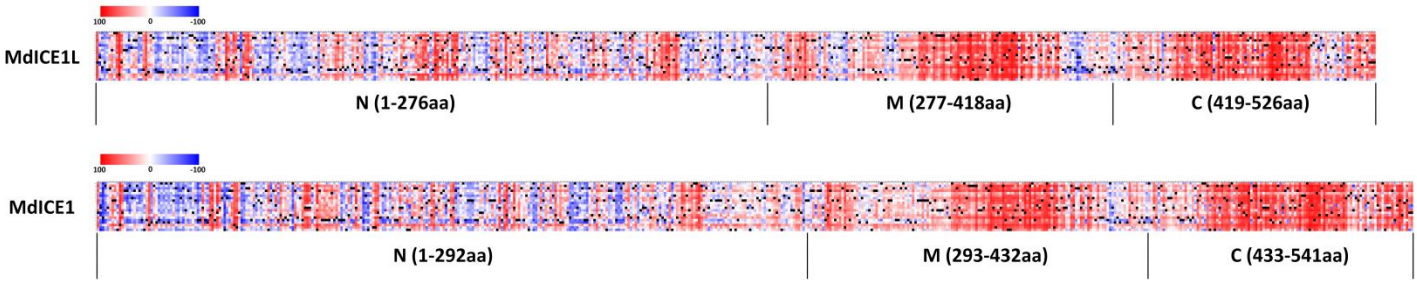
Supplemental Figure S5. Sequences of probes used in EMSAs. P1 to P5 indicate the putative binding sites in *MdCBF1/3* (A) and *MdCAX3L-2* (B) promoters. Red letters indicate the E-box elements and the corresponding mutated sequences in the probes.



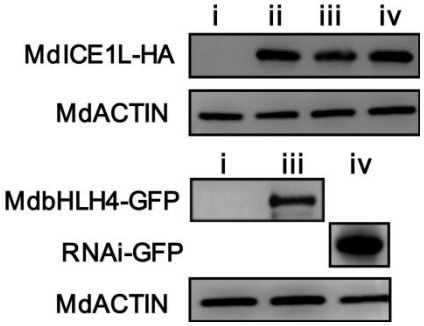
Supplemental Figure S6. Sequence alignment of MdCIbHLH1, MdICE1, and MdICE1L.



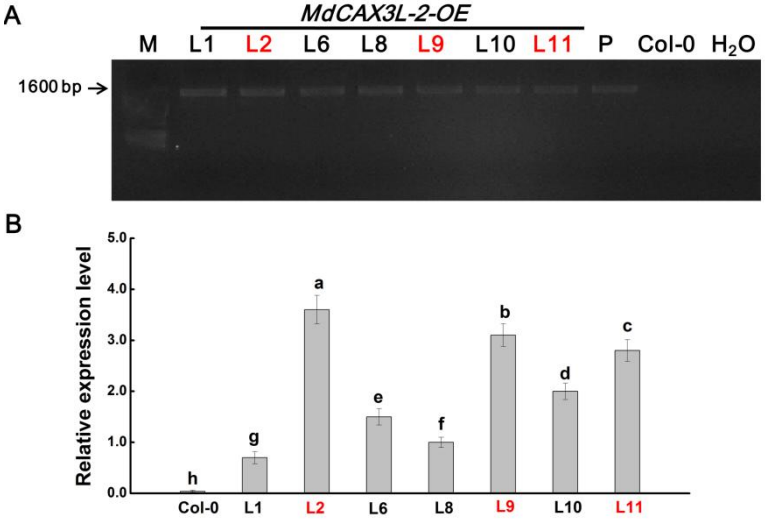
Supplemental Figure S7. Prediction of the effects of point mutations on the MdbHLH4 protein. The heat map shows each independent substitution (for the 20 amino acids) for each position of the MdbHLH4 protein. Dark red indicates a high score (score > 50, strong signal for effect), white indicates weak signals (−50 < score < 50), and green indicates a low score (score < −50, strong signal for neutral/no effect). Black marks correspond to wild-type residues.



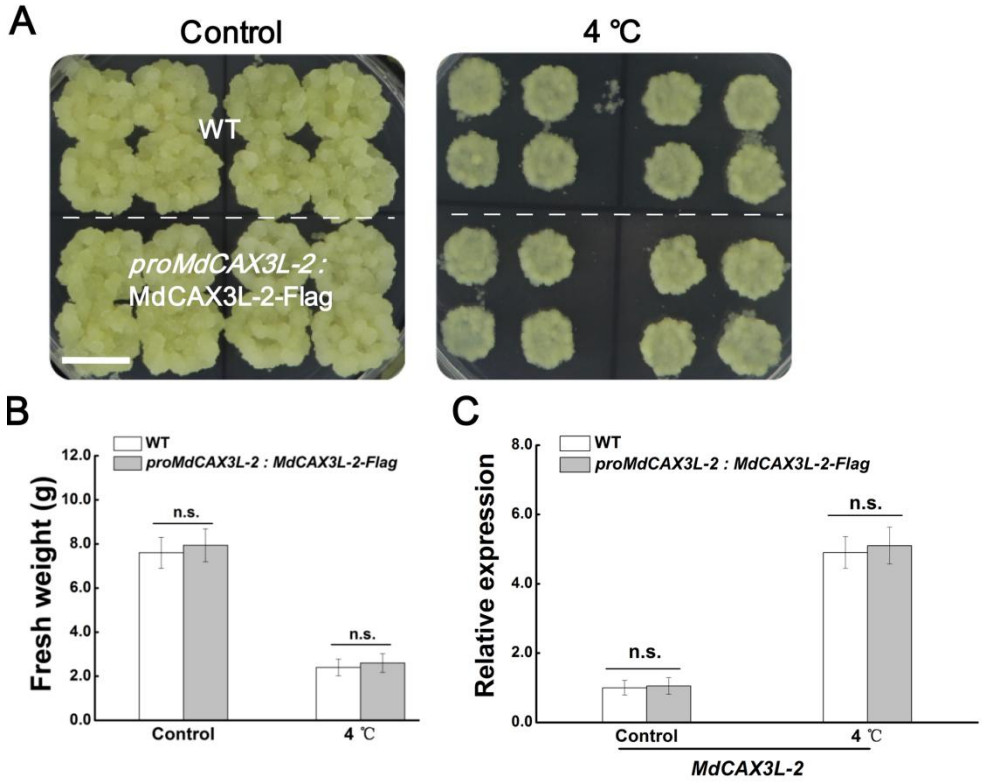
Supplemental Figure S8. Prediction of the effects of point mutations and functional region division of MdICE1L and MdICE1 proteins.



Supplemental Figure S9. Identification of MdICE1L-HA and MdbHLH4-GFP protein expression in transgenic calli. The protein levels of MdICE1L-HA and MdbHLH4-GFP were detected with anti-HA and anti-GFP antibodies, respectively. The meanings of Roman numerals are provided in Figure 5E.



Supplemental Figure S10. Identification of *MdCAX3L-2* transgenic *Arabidopsis* seedlings. **A,** Genomic PCR identification of *MdCAX3L-2* transgenic seedlings. P, positive control (plasmid DNA of the *MdCAX3L-2-Flag* vector). **B,** Relative expression analysis of *MdCAX3L-2* in Col and *MdCAX3L-2* transgenic lines. Error bars indicate the SD of three biological replicates. Different letters represent significant differences according to one-way ANOVA and Duncan's test ($p < 0.05$).



Supplemental Figure S11. Comparison of the growth phenotype (A), fresh weight (B), and *MdCAX3L-2* expression (C) between WT and *proMdCAX3L-2*::*MdCAX3L-2* transgenic apple calli. Scale bars, 1 cm. Error bars indicate the SD of three biological replicates. n.s. means no significant difference relative to the WT according to Student's *t*-test ($p < 0.05$).

Supplemental TableS1. The primers used in this study.

Annotation	Primer name	Sequence(5'-3')
Y2H analysis	MdbHLH4-BT9-F/ MdbHLH4-N-BT9-F/ MdbHLH4-N+M-BT9-F	CGGAATTCCCGGGGATCCTGGAGAGGCTTCTTCA
	MdbHLH4-N55-BT9-F	CGGAATTCCCGGGGATCCTGCCATTTCTCCAGAT
	MdbHLH4-BT9-R/ MdbHLH4-C-BT9-R/MdbHLH4-M+C-BT9-R	GCTTGGCTGCAGGTCGACTCAACTGCTACCATTG
	MdbHLH4-N-BT9-R	GCTTGGCTGCAGGTCGACTCAAGCAACTGAGCTGGGT
	MdbHLH4-M-BT9-F/MdbHLH4-M+C-BT9-F	CGGAATTCCCGGGGATCCGGTCTCCACCACCACC
	MdbHLH4-M-BT9-R/MdbHLH4-N+M-BT9-R	GCTTGGCTGCAG GTCGACTCAGTTGTTGTCACCAATA
	MdbHLH4-C-BT9-F	CGGAATTCCCGGGGATCCGCTCTTTCAGCTCCTC
	MdICE1L-N163-BT9-F	CGGAATTCCCGGGGATCCTGGGTTTCACGGCCCT
	MdICE1L-N163-BT9-R	GCTTGGCTGCAGGTCGACTTACATCATCATATCA
	MdICE2-N158-BT9-F	CGGAATTCCCGGGGATCCTGAACAGGGGAGGTGG
	MdICE2-N158-BT9-R	GCTTGGCTGCAGGTCGACCTACATCATGCCATGG
	MdbHLH4-AD424-F/MdbHLH4-N-AD424-F/MdbHLH4-N+M-AD424-F	TCGAATTCCCGGGGATCCTGGAGAGGCTTCTTCA
	MdbHLH4-AD424-R/MdbHLH4-C-AD424-R/MdbHLH4-M+C-AD424-R	AGATCTCTGCAGGTCGACTCAACTGCTACCATTG
	MdbHLH4-N-AD424-R	AGATCTCTGCAGGTCGACTCAAGCAACTGAGCTGGGT
	MdbHLH4-M-AD424-F/ MdbHLH4-M+C-AD424-F	TCGAATTCCCGGGGATCCGGTCTCCACCACCACC
	MdbHLH4-M-AD424-R/MdbHLH4-N+M-AD424-R	AGATCTCTGCAGGTCGACTCAGTTGTTGTCACCAATA
	MdICE1L-AD424-F	TCGAATTCCCGGGGATCCTGCTTCCGATGTCGAG
	MdICE1L-AD424-R	AGATCTCTGCAG GTCGACTTACATCATCATATCA
	MdICE2-AD424-F	TC GAA TTC CCG G GGATCCTGCTGCCAAGGCTGAA
	MdICE2-AD424-R	AGATCTCTGCAG GTCGACCTACATCATGCCATGG
	MdbHLH4-N55-BKT7-F	GCCATGGAGGCCGAA TTCATGCCATTTCTCCAGAT
	MdbHLH4-N55-BKT7-R	CTGCAGGTCGACGGATCCTCAACTGCTACCATTG
	MdICE1L-N163-BKT7-F	GCCATGGAGGCCGAA TTCATGGGTTTCACGGCCCT
	MdICE1L-N163-BKT7-R	CTGCAGGTCGACGGATCCTTACATCATCATATCA
	AtICE1-ADT7-F	ATGGAGGCCAGTGAATTCATGGGTCTTGACGGAA
	AtICE1-ADT7-R	CTCGAGCTCGATGGATCCTCAGATCATACCAGCA
	AtICE2-ADT7-F	ATGGAGGCCAGTGAATTCATGAACAGCGACGGTG
	AtICE2-ADT7-R	CTCGAGCTCGATGGATCCTCAAACCAAACCAGCG
	MdMPK3a-ADT7-F	ATGGAGGCCAGTGAATTCATGGCCGACCTCACTCCC
	MdMPK3a-ADT7-R	CTCGAGCTCGATGGATCCTTAAGCATACTCTGGATT
	MdMPK3b-ADT7-F	ATGGAGGCCAGTGAATTCATGGCAGACCTCGTTCCC
	MdMPK3b-ADT7-R	CTCGAGCTCGATGGATCCTCAAGCATACTCTGGATT
	MdMPK6a-ADT7-F	ATGGAGGCCAGTGAATTCATGGAGGGAGGAGGGC
	MdMPK6a-ADT7-R	CTCGAGCTCGATGGATCCTCACTGTAGCTGATAC
	MdMPK6b-ADT7-F	ATGGAGGCCAGTGAATTCATGGAGGGAGGAGGGC
	MdMPK6b-ADT7-R	CTCGAGCTCGATGGATCCTCACTGTCGCTGGTAC
Y1H analysis	MdCBF1-pro-pHIS2-F	TCACTATAGGGCGAATTCTCTTCCATGCTCGGTC
	MdCBF1-pro-pHIS2-R	TCGCGAACGCGTGAGCTCTAATTTCTGGGACCAG
	MdCBF3-pro-pHIS2-F	TCACTATAGGGCGAATTCCTTCCAAAAATACTAA
	MdCBF3-pro-pHIS2-R	TCGCGAACGCGTGAGCTCTTCTAGGTACGAGAGT
	MdCAX3L-2-pro-pHIS2-F	TCACTATAGGGCGAATTCGGCCCTGTATTGTTCA
	MdCAX3L-2-pro-pHIS2-R	TCGCGAACGCGTGAGCTCGATCGATGGTGATGCA
	MdbHLH4-pGADT7-F	ATGGAGGCCAGTGAATTCATGGAGAGGCTTCTTCA
	MdbHLH4-pGADT7-R	CTCGAGCTCGATGGATCCTCAACTGCTACCATTG
Dual-luciferase analysis	MdCBF1-pro-0800-F	CTTGATATCGAATTCCTGCAGGTAGATGCTACAATTG
	MdCBF1-pro-0800-R	CGCTCTAGAACTAGTGGATCCTAATTTCTGGGACCAG
	MdCBF3-pro-0800-F	CTTGATATCGAATTCCTGCAGTACGACTTTCAAGTTT
	MdCBF3-pro-0800-R	CGCTCTAGAACTAGTGGATCCTTCTAGGTACGAGAGT
	MdCBF1-pro2-0800-F	CTTGATATCGAATTCCTGCAGCGAGACCGACCTTTGACCC
	MdCBF1-pro2-0800-R	CGCTCTAGAACTAGTGGATCCTTTTCGGAAGTTGCGGG
	MdCBF1-pro5-0800-F	CTTGATATCGAATTCCTGCAGTCCATGCTCGGTCATAC
	MdCBF1-pro5-0800-R	CGCTCTAGAACTAGTGGATCCTAAAATATGTTTGATGTT
	MdCBF3-pro2-0800-F	CTTGATATCGAATTCCTGCAGGGCACCCAGTCCTGCAATT
	MdCBF3-pro2-0800-R	CGCTCTAGAACTAGTGGATCCATTGTTTGTTCTAGTTAG

	MdCBF3-pro4-0800-F	CTTGATATCGAATTCCTGCAGGAAAGCAACAAGATTCATT
	MdCBF3-pro4-0800-R	CGCTCTAGAACTAGTGGATCCAGTTGCGGGCGTGGATTG
	MdCBF3-pro5-0800-F	CTTGATATCGAATTCCTGCAGGAGTCAAAGTATAAATAC
	MdCBF3-pro5-0800-R	CGCTCTAGAACTAGTGGATCCGTCAAAGGTCGGCCTCAC
	MdCAX3L-2-pro-0800-F	CTTGATATCGAATTCCTGCAGCCCTGTATTGTTCATTA
	MdCAX3L-2-pro-0800-R	CGCTCTAGAACTAGTGGATCCGATCGATGGTGATGCAC
	MdbHLH4-62SK-F	GGTGGCGGCCGCTCTAGAATGGAGAGGCTTCTTC
	MdbHLH4-62SK-R	CTGCAGCCCGGGGGATCCTCAACTGCTACCATTG
	MdICE1L-62SK-F	GGTGGCGGCCGCTCTAGAATGCTTCCGATGTCGAG
	MdICE1L-62SK-R	CTGCAGCCCGGGGGATCCCTACATCATGCCATGG
Split-LUC analysis	MdbHLH4-cLUC-F	AAAGCAGGCTTCGGATCCATGGAGAGGCTTCTTCAA
	MdbHLH4-cLUC-R	GTTGTTGATTCAGAATTCTCAACTGCTACCATTGAT
	MdMPK6a-nLUC-F	ATACATATGCCCGTCGACATGGAGGGAGGAGGGCCC
	MdMPK6a-nLUC-R	GAAAGCTGGGTTGGTACCCTGTAGCTGATACTCGGG
	MdMPK6b-nLUC-F	ATACATATGCCCGTCGACATGGAGGGAGGAGGGCGA
	MdMPK6b-nLUC-R	GAAAGCTGGGTTGGTACCCTGTCGCTGGTACTCGGG
Subcellular localization in yeast	MdCAX3L-2-pDR196GFP-F	CCCGGGCTGCAGGAATTCATGGCTTCAAACCAAGA
	MdCAX3L-2-pDR196GFP-R	CTTGCTCACCATGTCGACAGCTCCAAGAACTGCTCC
Protein purification	MdbHLH4-pGEX4T-1-F	CGTGGATCCCCGGAATTCATGGAGAGGCTTCTTCA
	MdbHLH4-pGEX4T-1-R	ACGATGCGGCCGCTCGAGTCAACTGCTACCATTG
	MdICE1L-pET28a-F	AAATGGGTCGCGGATCCATGCTTCCGATGTCGAG
	MdICE1L-pET28a-R	GGCCGCAAGCTTGTCGACCATCATCATATCATGAA
Genetic transformation	MdICE1L-pCambia-3HA-F	GAATTCGAGCTCGGTACCGCTTCCGATGTCGAG
	MdICE1L-pCambia-3HA-R	GGTCGACTCTAGAGGATCCCTACATCATGCCATGG
	MdbHLH4-pCambia-4MYC-3FLAG-F	GCCCATAGGCCTGAATTCGGAGAGGCTTCTTCAAGG
	MdbHLH4-pCambia-4MYC-3FLAG-R	CTGCAGGTCGACTCTAGACAACTGCTACCATTGAT
	MdCAX3L-2-pCambia-4MYC-3FLAG-F	GCCCATAGGCCTGAATTCGGCTTCAAACCAAGA
	MdCAX3L-2-pCambia-4MYC-3FLAG-R	CTGCAGGTCGACTCTAGATAAGCTCCAAGAACTGC
	MdCAX3L-2pro-pCambia-4MYC-MdCAX3L-2-3FLAG-F	AGAAGACCAAAGGGCAATAACTTAAACAACATTTTAG
	MdCAX3L-2pro-pCambia-4MYC-MdCAX3L-2-3FLAG-R	TGGTTTGAAGCCGAATTCGATCGATGGTGATGCACAC
	MdbHLH4-2300GFP-F	CTCGGTACCCGGGGATCCATGGAGAGGCTTCTTCA
	MdbHLH4-2300GFP-R	GCTCACCATGGTGTCGACACTGCTACCATTGATG
	MdCAX3L-2-2300GFP-F	CTCGGTACCCGGGGATCCATGGCTTCAAACCAAGA
	MdCAX3L-2-2300GFP-R	GCTCACCATGGTGTCGACAGCTCCAAGAACTGCTC
	MdCAX3L-2-RNAi-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGTGTGCATCACCATCG
	MdCAX3L-2-RNAi-R	GGGGACCACTTTGTACAAGAAAGCTGGGTACTGTCTGAGAAGGGC
	MdbHLH4-RNAi-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAGGAGGAAGAAGAAT
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RT-qPCR	MdbHLH4-qF	GTCTCCACCACCACCACCTACC
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	AtCOR47-qR	TCCACCACACTCTCCGACACTG
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	AtCOR15A-qR	CGTCACCTTTAGCGGCGTAGATC
EMSA probes	MdCBF1-P1-F	AGCCGTACAATTGTAAGACAGCTGTCGCACT
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	MdCBF3-P2-mut-F	GTTCGCGTTTTTAAAAAACCCAGTCGCC
	MdCBF3-P2-mut-R	GGCGACTGGGTTTTTTAAAAACGCGAAC
	MdCBF3-P4-mut-F	GAATCCACAAAAAAAGCACACACAAAC
	MdCBF3-P4-mut-R	GTTTGTGTGTGCTTTTTTTGTGGATTC
	MdCBF3-P5-mut-F	ATATATTAAAAAAGATTGGGTGATGAG
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	MdCAX3L-2-P2-R	CATTTTTCTTTGCAATTGAGATTGTTGATAG
	MdCAX3L-2-P3-F	GGTGAGCATTACCACCACTTGAGGGGGTGGATTCGG
	MdCAX3L-2-P3-R	CCGAATCCACCCCCTCAAGTGGTGGTAATGCTCACC
	MdCAX3L-2-P4-F	CGTTCAGAACTCAGTTGACTGATTCTATAGATAAGG
	MdCAX3L-2-P4-R	CCTTATCTATAGAATCAGTCAACTGAGTTCTGAACG
	MdCAX3L-2-P5-F	CTAAAACGAGCCATCTGGCCAGCCCTCGG
	MdCAX3L-2-P5-R	CCGAGGGCTGGCCAGATGGCTCGTTTTAG
	MdCAX3L-2-P1-mut-F	CTTACTCTACTTAAAAAAACAAGATACTATCTAGC
	MdCAX3L-2-P1-mut-R	GCTAGATAGTATCTTGTTTTTTTTAAGTAGAGTAAG
	MdCAX3L-2-P3-mut-F	GGTGAGCATTACCACAAAAAAAGGGGGTGGATTCGG
	MdCAX3L-2-P3-mut-R	CCGAATCCACCCCCTTTTTTTGTGGTAATGCTCACC
Chip-qPCR analysis	MdCBF1-pro2-qF	GCAAGCAAAATACAGATTGT
	MdCBF1-pro2-qR	TCCTTTTTCGGAAGTTGCGG
	MdCBF3-pro4-qF	GAGGCCGACCTTTGACCTTG
	MdCBF3-pro4-qR	ATTTTATCCCTCCCTCCC
Mutant identification	LBb1.3	ATTTTGCCGATTTCGGAAC
	myc70-LP	AAACTGCCACAGCCAATAATG
	myc70-RP	TGGGTTGAATGGAAGTTCTTG
Universal primer	pCAM-R	GACCGGCAACAGGATTCAATC
	2300GFP-R	CAGGGTCAGCTTGCCGTAG

	pK7-R1	CCGTAAGAAGAGGCAAGAGTATGA

The red letters refer to the nucleotide sequences in the vectors.