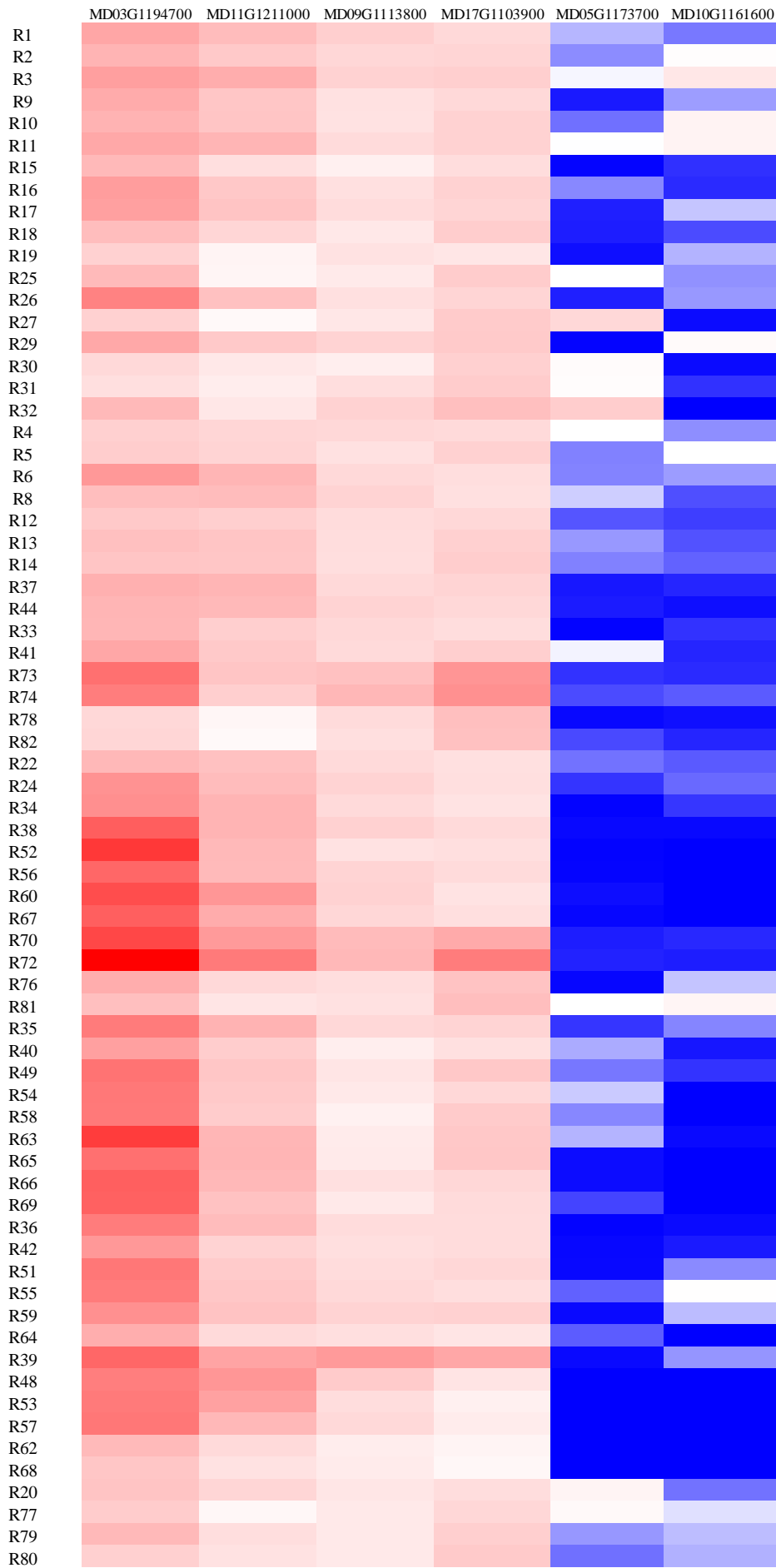


A

FPKM

400

300

200

100

50

40

30

20

10

0

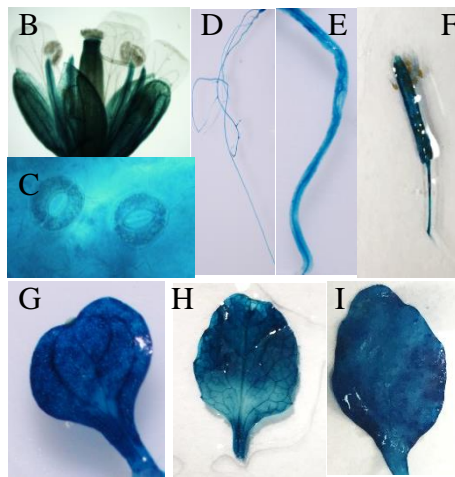


Fig. S1. Expression patterns of apple *SUMO2*s. (A) Expression patterns of apple *SUMO2*s in *Malus fusca*. MD03G1194700 and MD11G1211000, *SUMO2A*; MD17G1103900 and MD09G1113800, *SUMO2B*; MD5G1173700 and MD10G1161600, *SUMO2C*. The detailed information for various tissues was provided in Appendix S1. (B)-(I) Ectopic expression of *MdSUMO2A::GUS* in Arabidopsis flower (B), stoma (C), root (D), stem (E), silique (F), cotyledon (G), rosette leaf (H), and cauline leaf (I). At least six independent transgenic lines were examined.

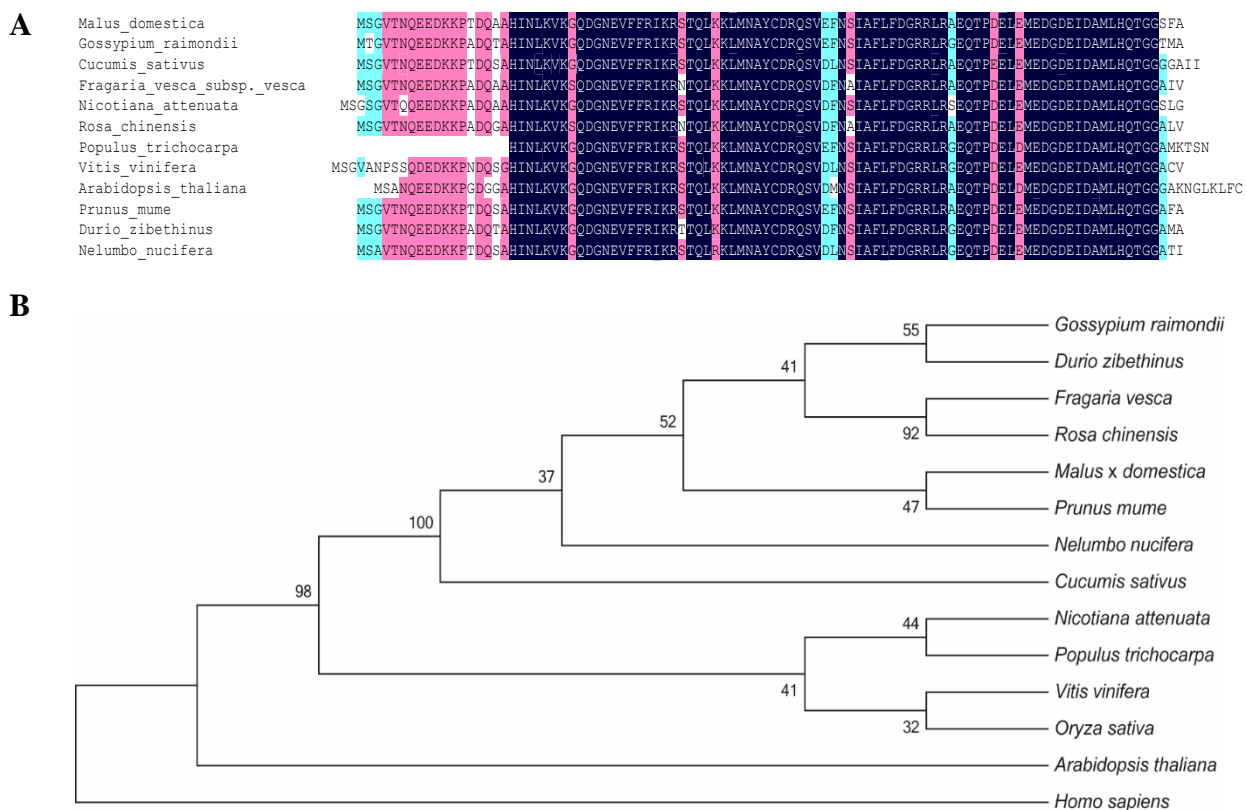


Fig. S2. Protein alignment (A) and phylogenetic analysis (B) of SUMO2A from different species.

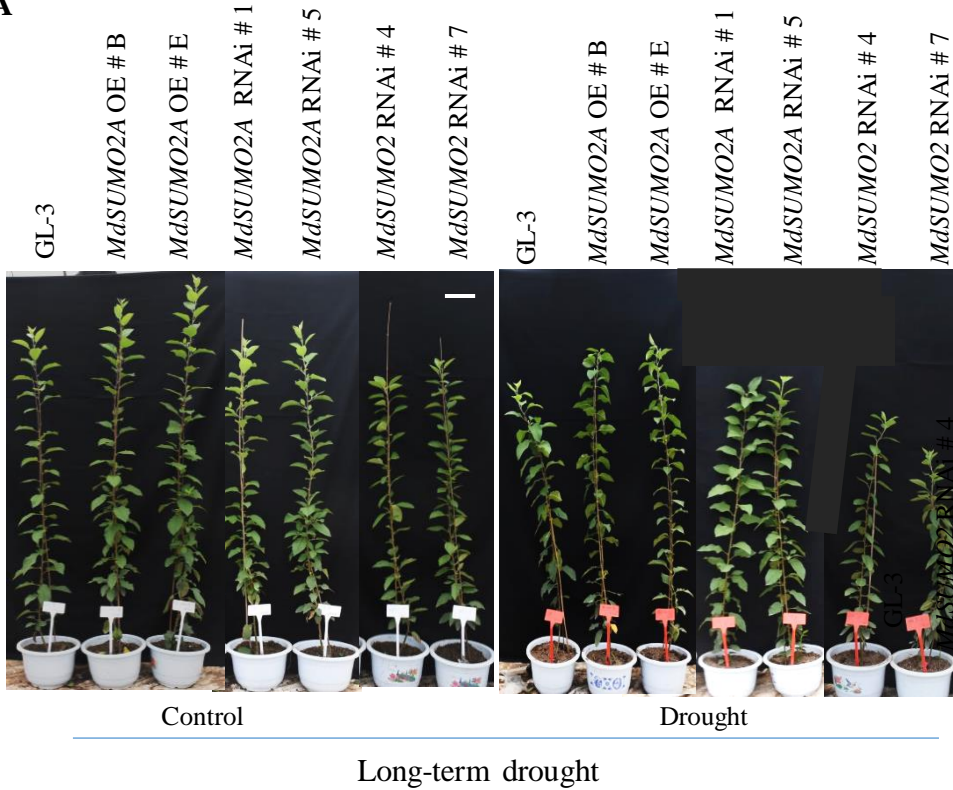
A**B**

Fig. S3. Phenotypes of *MdSUMO2* transgenic plants and GL-3 under prolonged drought stress. (A) Phenotypes of *MdSUMO2* transgenic plants and GL-3 under control and prolonged drought stress for 3 months. Bar = 10 cm. (B) Phenotypes of *MdSUMO2* transgenic plants and GL-3 after prolonged drought for 3 months and subsequent drought stress by withholding water for 10 days.

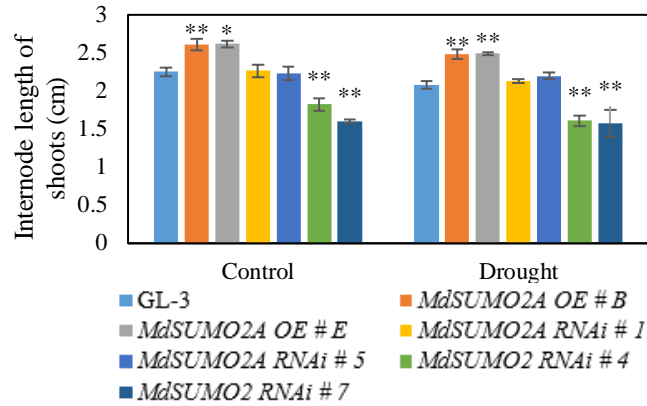


Fig. S4. Internode length of GL-3, *MdSUMO2A* OE plants, *MdSUMO2A* RNAi, and *MdSUMO2* RNAi plants under long-term drought conditions. OE, overexpression. Data are means \pm SE (n = 4). One-way ANOVA was performed and different letters indicate statistically significant differences (*, $P < 0.05$; **, $P < 0.01$).

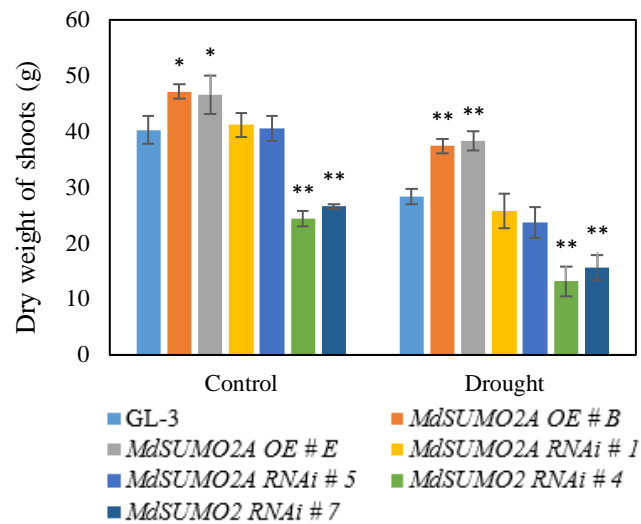


Fig. S5. Dry weight of shoots of *MdSUMO2* transgenic plants and GL-3 under prolonged drought stress. Plants were exposed to drought for up to 3 months. During treatment, 43-48% soil volumetric water content (VWC) was maintained as control and 18-23% of VWC was maintained as drought treatment. Error bars indicate standard error (n = 5). Asterisks indicate significant differences based on one-way ANOVA and Tukey test (*, $P < 0.05$; **, $P < 0.01$). OE, overexpression.

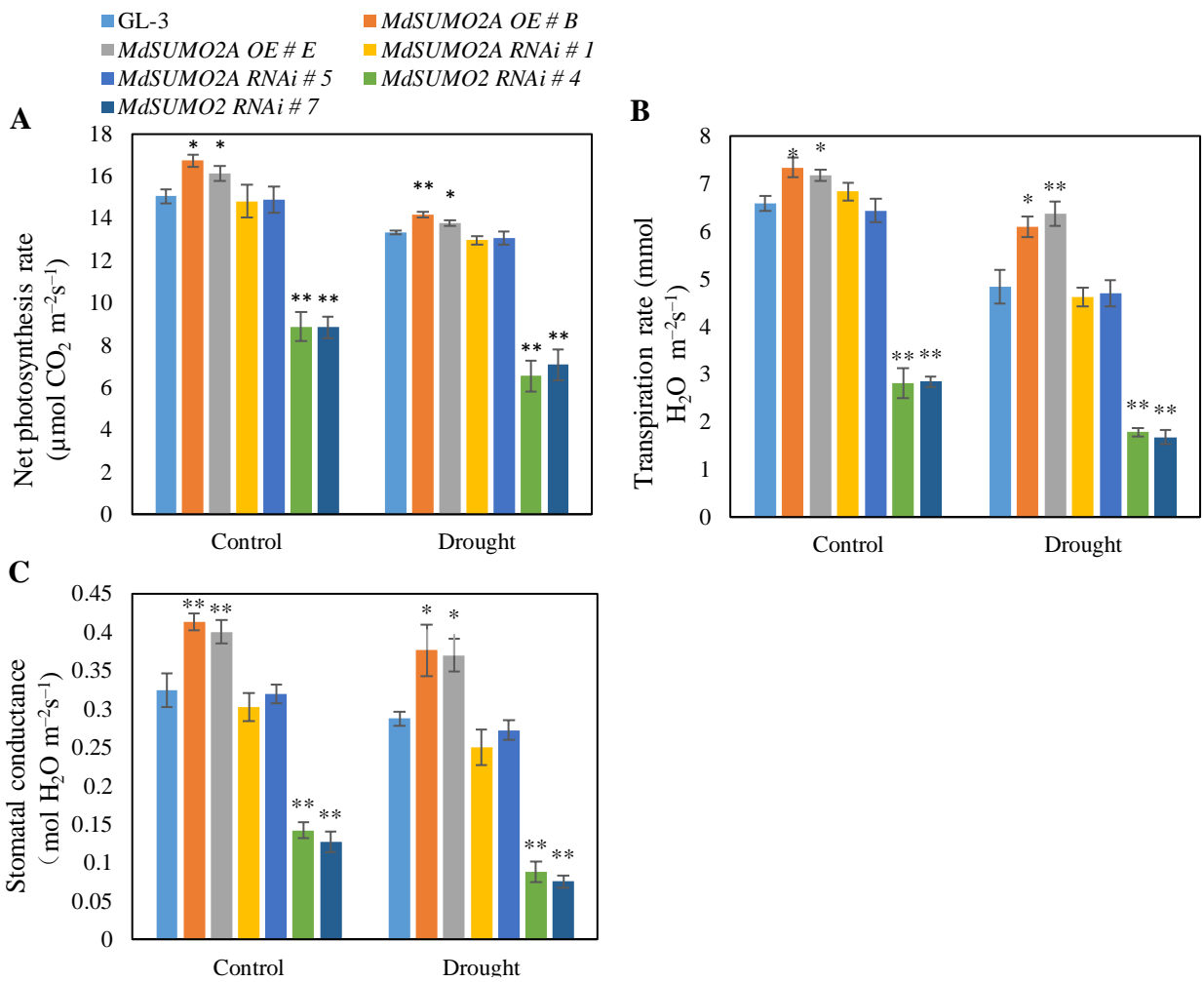


Fig. S6. Photosynthesis related parameters of GL-3, *MdsUMO2A* overexpression plants, *MdsUMO2A* RNAi, and *MdsUMO2* RNAi plants under long-term drought conditions. (A) The rate of net photosynthesis. (B) Rate of transpiration. (C) Stomatal conductance (G_s). Data are means \pm SE ($n = 8$). Asterisks indicate significant differences based on one-way ANOVA and Tukey test (*, $P < 0.05$; **, $P < 0.01$).

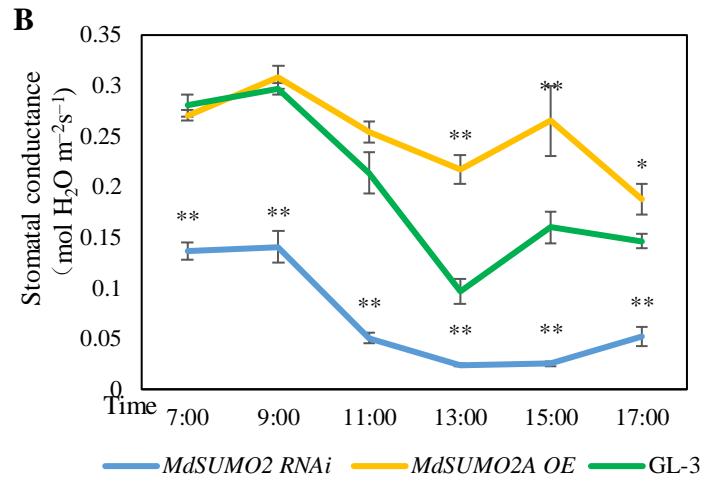
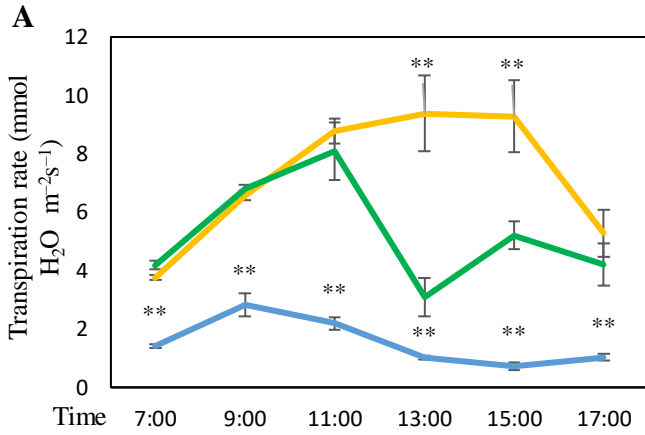


Fig. S7. Transpiration rate (A) and stomatal conductance (B) of *MdSUMO2* transgenic plants and GL-3 under long-term drought stress conditions. Long-term drought-treated plants were used to measure the transpiration rate and stomatal conductance. Data are means \pm SE ($n = 7$). One-way ANOVA (Tukey's test) was performed and statistically significant differences were indicated by * $P < 0.05$ or ** $P < 0.01$.

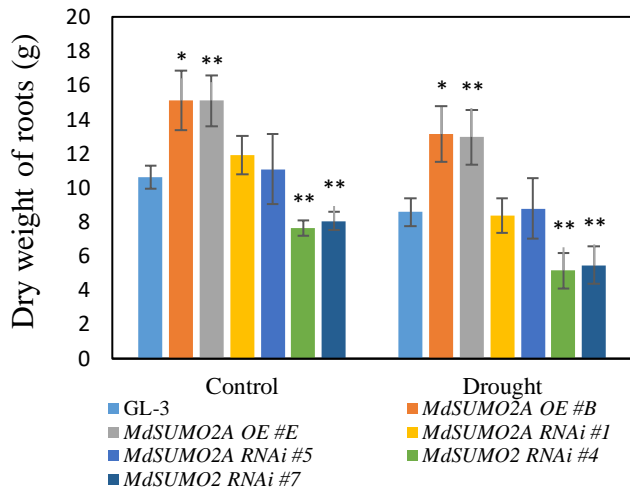


Fig. S8. Dry weight of roots of *MdSUMO2A* transgenic plants and GL-3 under control and long term drought stress condition. Data are means \pm SE (n = 5). Asterisks indicate significant differences based on one-way ANOVA and Tukey test (*, $P < 0.05$; **, $P < 0.01$).

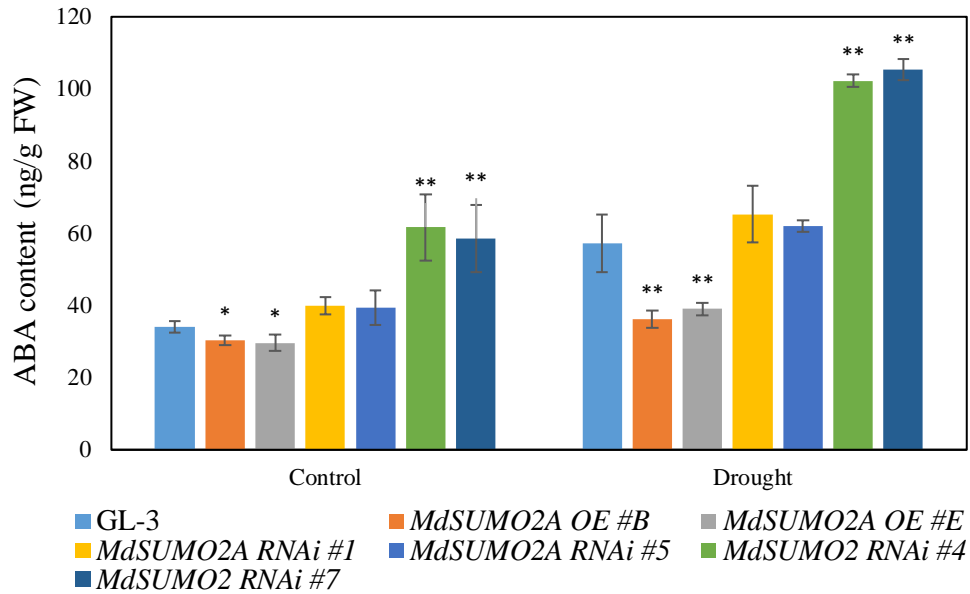


Fig. S9. The ABA content of *MdSUMO2A* transgenic plants and GL-3 under control and long-term drought stress conditions. Data are means \pm SE (n = 6). Asterisks indicate significant differences based on one-way ANOVA and Tukey test (*, $P < 0.05$; **, $P < 0.01$).

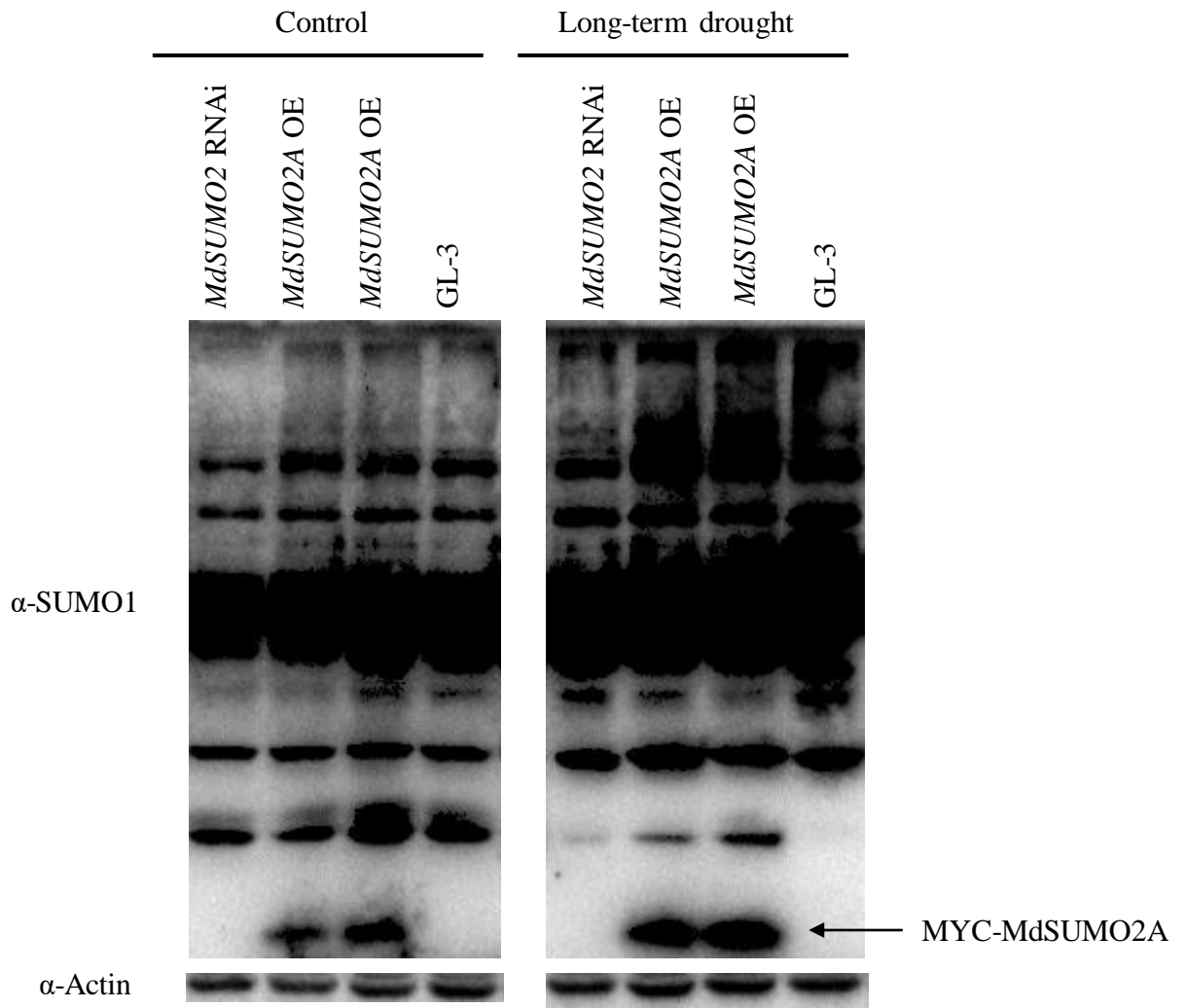


Fig. S10. SUMOylation of *MdSUMO2* transgenic plants and GL-3 under control and long-term drought stress conditions.

A 1 MGAYDQGVNMPSLPLDSSRKRKTRSRRDGNSVADTLEKWKEHNKQLESATEEGKKRKVPA 60
61 KGSKKGCMKGGKGGPENARCNYRGVQRRTWGKVAEIREPDRGSRLWLGTFPTAVDAALAY 120
121 DEAAKAMYGDGARLNLPHAVNNHPSSSQETSSVATPSGSSAAATPGCSTSTSTSSLSLSEVC 180
181 ADENSKLFLDV **K**KEDGEGESRMYPLSGAVPQASGMV **K**QEVNEDFDMDYLRRNNQQQPVG MV 240
241 KPEVNEVFDMDYLRRNNQQQRGVEDHNPNGGDGFVGDYSENFTMGELFDMDEMFDVNELLM 300
301 PPDDISLCNSGPEQVSRPDVGGQPGMETLSSEGSPNLSYQLQYPDAKLLGSLPHMEQAPLD 360
361 FEYSFDFM **K**QEEGSNHTSQNDQGYFNLGPSDLGLGGFTEGTMQSGDVGYNYSMEM. 420

B 1 MAKDIEGAEHGEFATKDYQDPPPTPLFDAEELTKWSFYRAVIAEFVATLLFLYITVLTVIGYKSQ 60
61 SEVDQCGGVGILGIAWAFGGMIFVLVYCTAGISGGHINPAVTFGLFLARKVSLIRAVLYIVAQCL 120
121 GAICGVGLVKAFQKSYVVKYGGGANELADGYNKG TGLGAEIIGTFVLVYTVFSATDPKRNARD 180
181 SHVPVLAPLPIGFAVFIHLATIPITGTGINPARSFGAAVIYNKDKAWDDQWIFWLGPFIGA AIA 240
241 AFYHQYILRAGAI **K**ALGSFRSNA 300

C 1 MLAVFDKSV **K**CPEALQSPQSGSASAL **K**DGFLAQQFASVHPSSVTVNLGASGLIAYS LD 60
61 RQNPLLPRLFAVVDDIFCLFQGHIENVALLKQYGLNKTANE **VIIIVT** EAFRTLDRGYPY 120
121 ADQVVRDIQGFVFLFDSASKATFIATDPDGNVPPFFWGT DSEGHLVLSDDSEVV **K**KGC 180
181 GNSFAPFPKGCFFTS SGG LRSYEHPHNELKAVPRVDSSGEMCGANF **K**VDADT **K**KESSGI 240
241 PRVGSAAWSSNY. 300

Fig. S11. The putative SUMOylation lysine residues in MdDREB2A (A), MdAQP2 (B), and MdALI (C) proteins. The red line indicates AP2 domain in (A). Red box indicates SIM in (C). The SUMOylation lysine residues are highlighted in red.

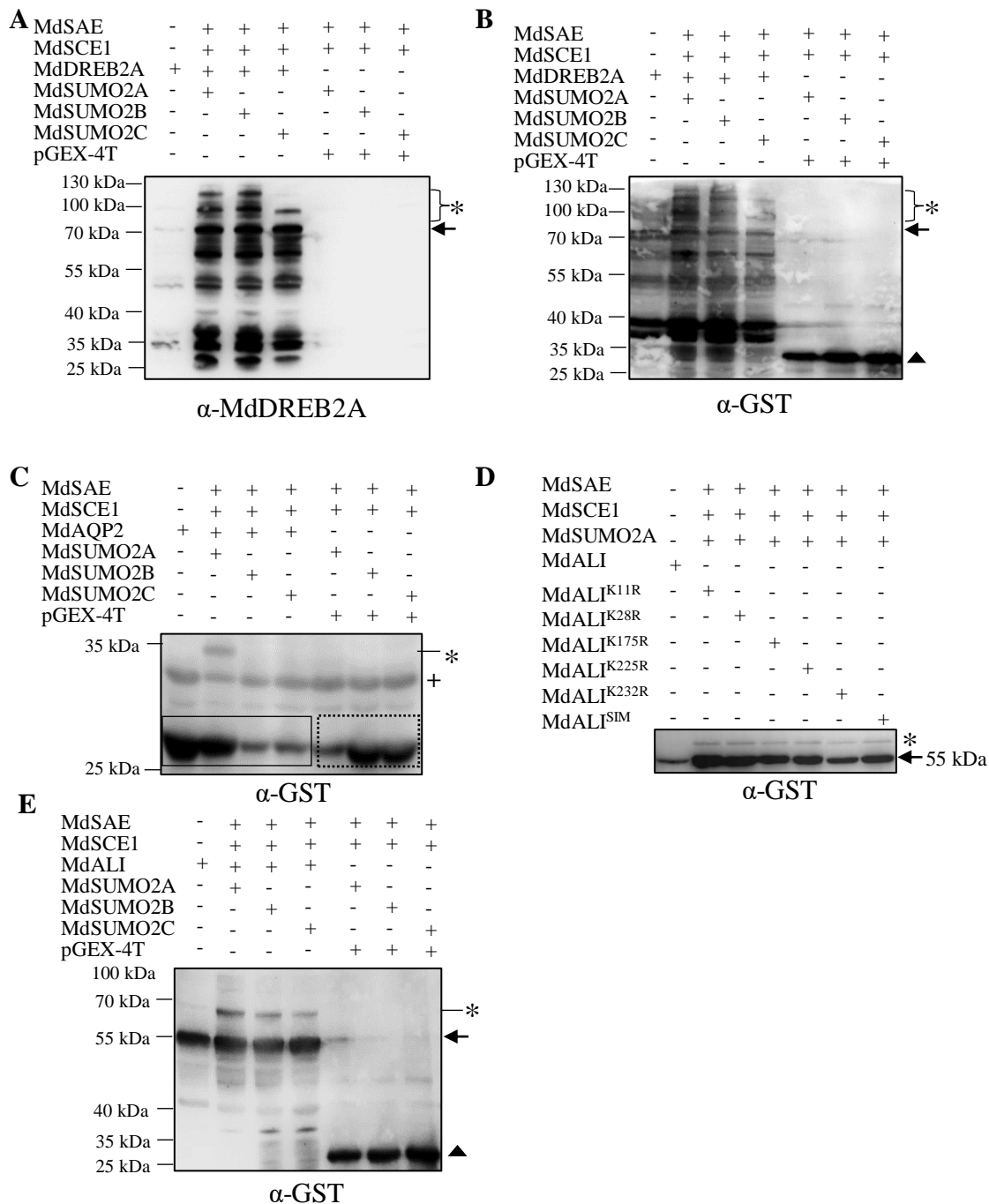


Fig. S12. SUMOylation of MdDREB2A, MdAQP2, and MdALI using *E. Coli* system. (A) SUMOylation of MdDREB2A was detected with anti-MdDREB2A. (B) SUMOylation of MdDREB2A was detected with anti-GST. (C) SUMOylation of MdAQP2 was detected with anti-GST. + indicates non-specific band; solid box indicates the fused GST-MdAQP2 protein; dotted box indicates the GST protein. (D) SUMOylation of mutant MdALI was detected with anti-GST. The SIM (V102 and V105) was mutated alanine (A). Putative SUMOylation sites (K) were mutated to arginine (R). (E) SUMOylation of MdALI was detected with anti-GST. The empty vector carrying GST tag (pGEX-4T) was used as the negative control. * indicates SUMOylated substrates; arrow indicates substrates; triangle indicates the expressed GST protein. OE, overexpression.

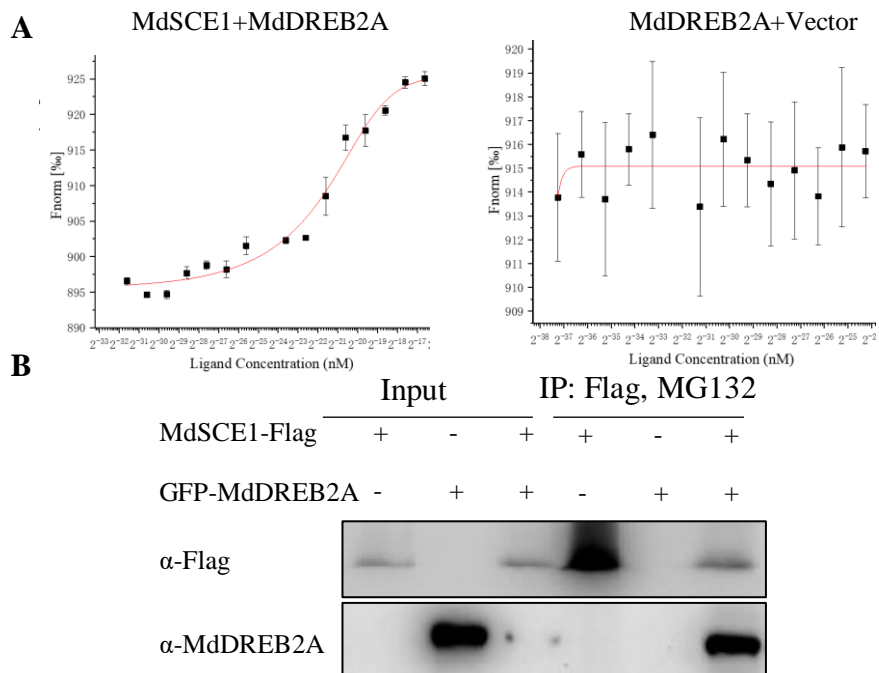
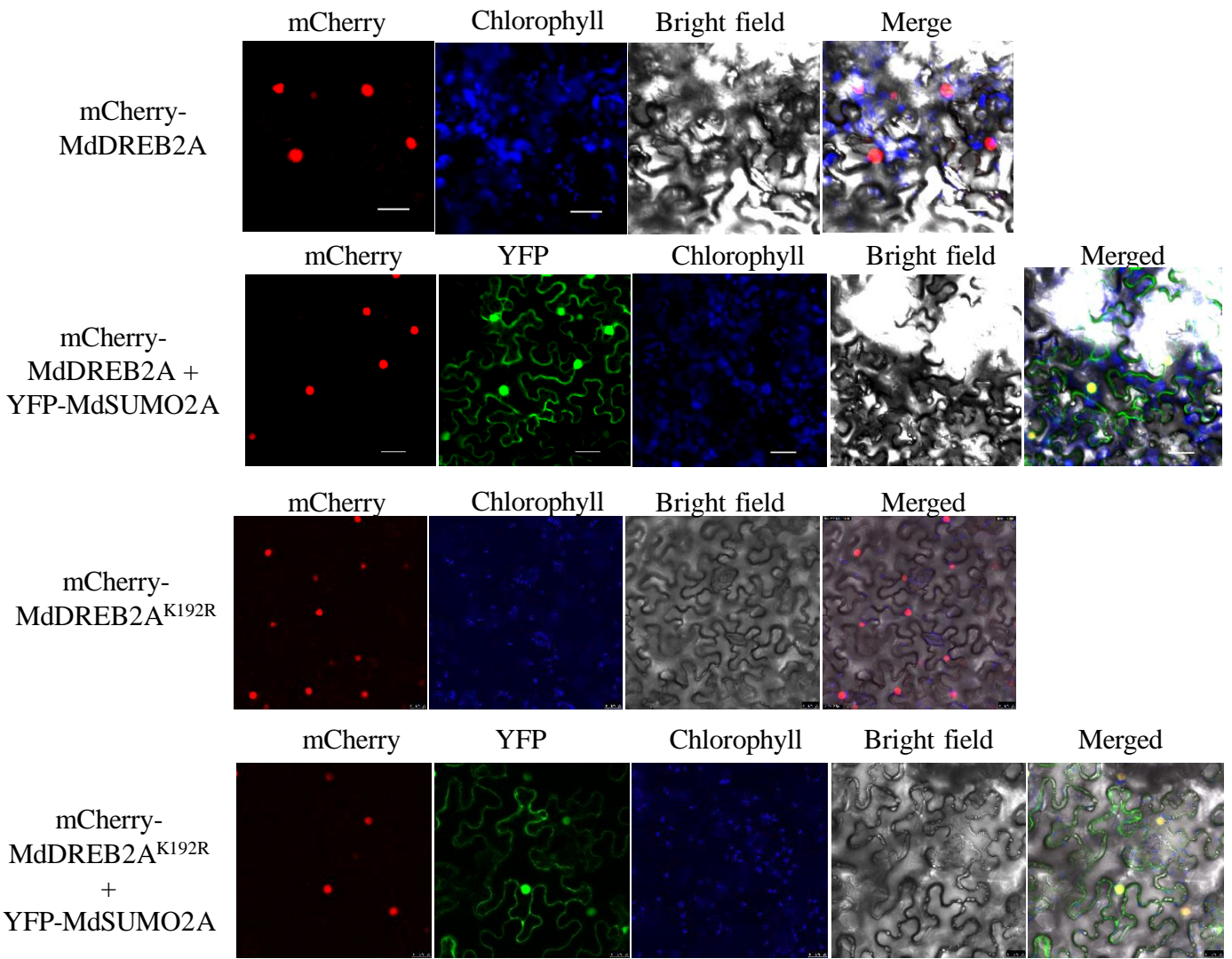


Fig. S13. MdDREB2A interacts with MdSCE1 *in vitro* and *vivo*. (A) The interaction of MdSCE1 and MdDREB2A was examined by MST analysis. (B) CO-IP analysis. MdSCE1-Flag was co-infiltrated with GFP-MdDREB2A into tobacco leaves. Proteins were extracted and immunoprecipitated with an anti-Flag antibody. The immunocomplex was then detected with western blot using anti-Flag or anti-MdDREB2A.



Bars = 40 μ m

Fig. S14. Effects of MdSUMO2A on the subcellular localization of MdDREB2A.

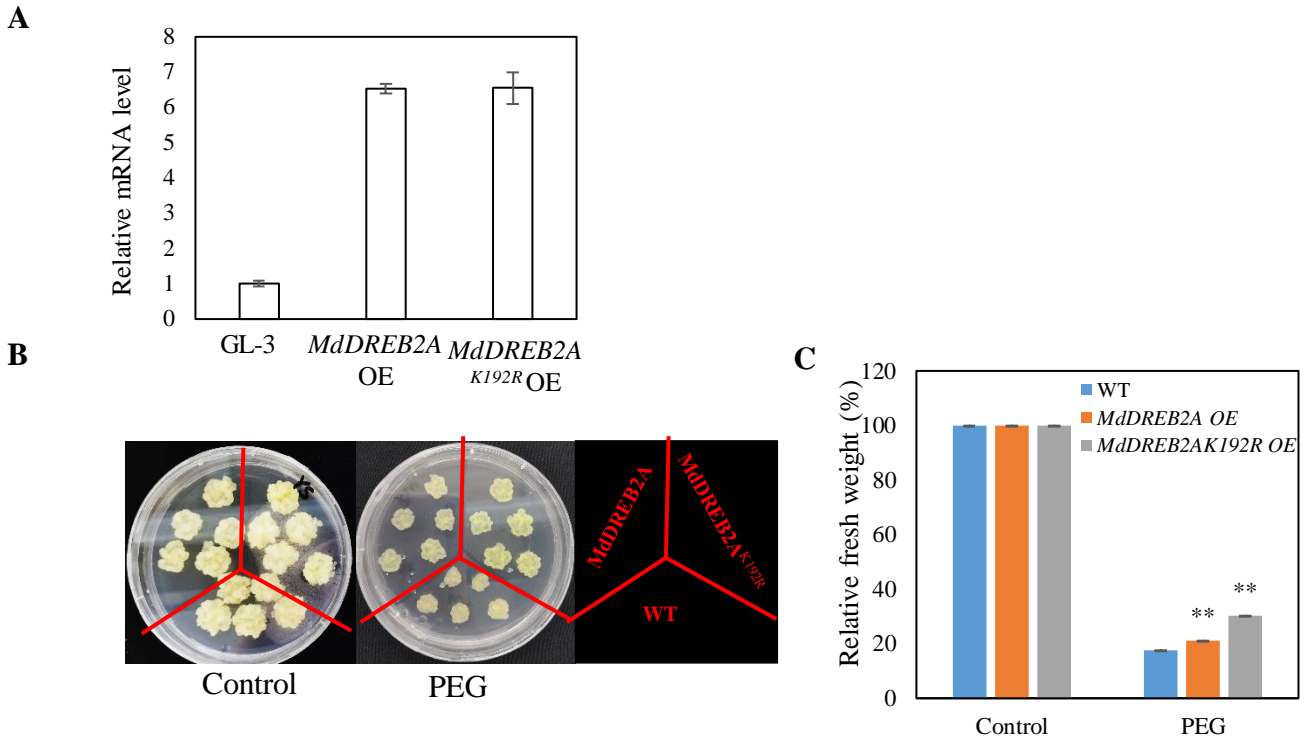


Fig. S15. SUMOylation of MdBREB2A is critical for drought stress tolerance. (A) Expression of *MdBREB2A* in *MdBREB2A* or *MdBREB2A*^{K192R} OE transgenic calli. (B) Phenotypes of 35S::*MdBREB2A* and 35S::*MdBREB2A*^{K192R} calli under PEG treatment for 2 weeks. (C) Relative fresh weight of calli shown in (B). Error bars indicate standard error (n = 3 in A, 10 in C). Asterisks indicate significant differences based on one-way ANOVA and Tukey test (*, $P < 0.05$; **, $P < 0.01$).

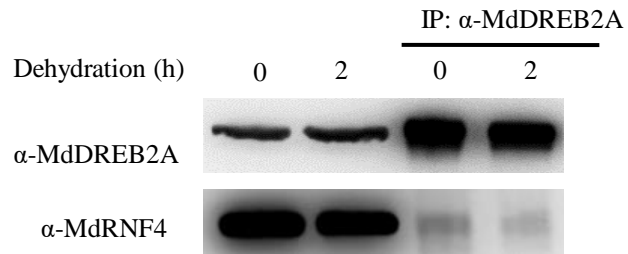


Fig. S16. MdDREB2A interacts with MdRNF4 *in vivo*. Proteins were extracted from GL-3 leaves and immunoprecipitated with an anti-MdDREB2A antibody. The immunocomplex was then detected with western blot using anti-MdRNF4 or anti-MdDREB2A.

A

1 MSTRGARAPHARGRRRKNELNLDLNSAPPGDSREQEGTSTQSVQNNQATLQVQPIPPNTID 62

63 VEAIDDDVISSPTAFAEAKNNSRRNRRT**VVDVE**SERTARVTQNKRRRVGPNQTIINCDFYI 124

125 NLESNSNSTSTKQNVTPPPPPPPPPKEPTFTCPICMAPLVEEMSTKCGHIFCKACIKAAIGAQG 187

188 RCPTCRRKVTMKE**LIRVFL**PTTC. 212

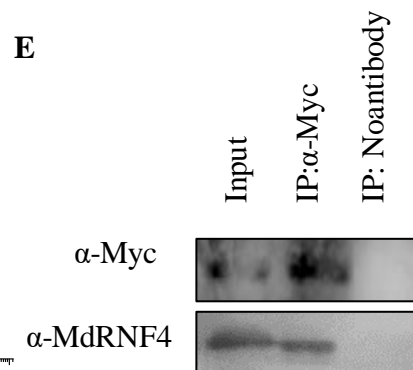
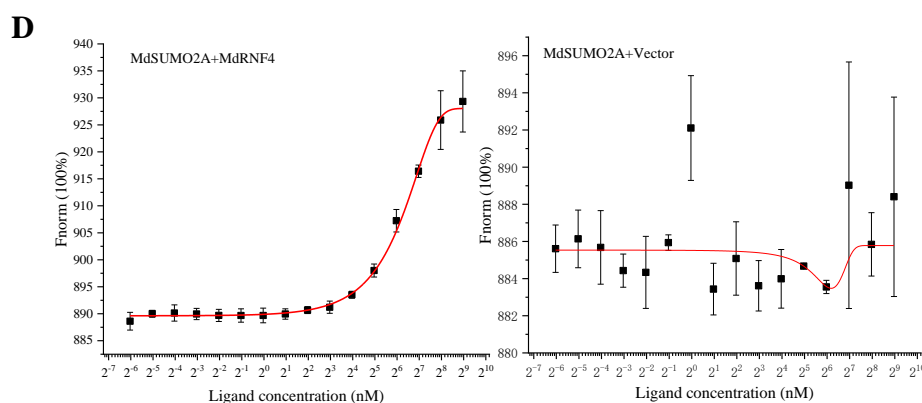
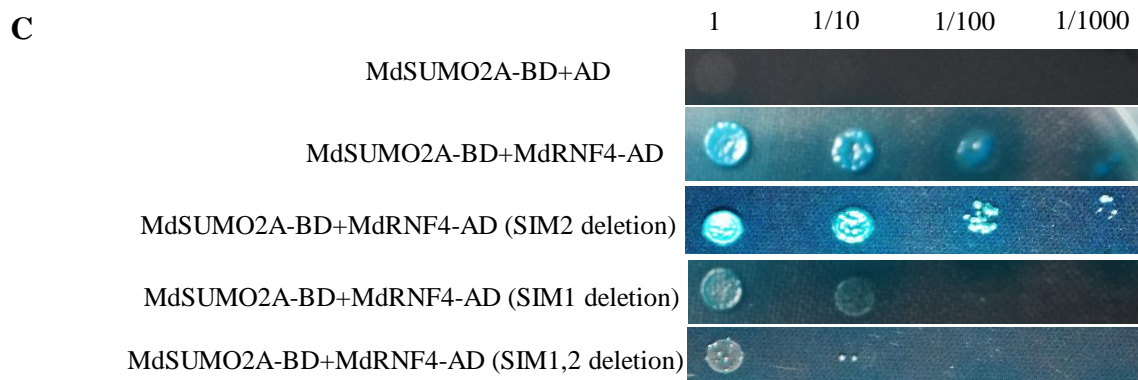
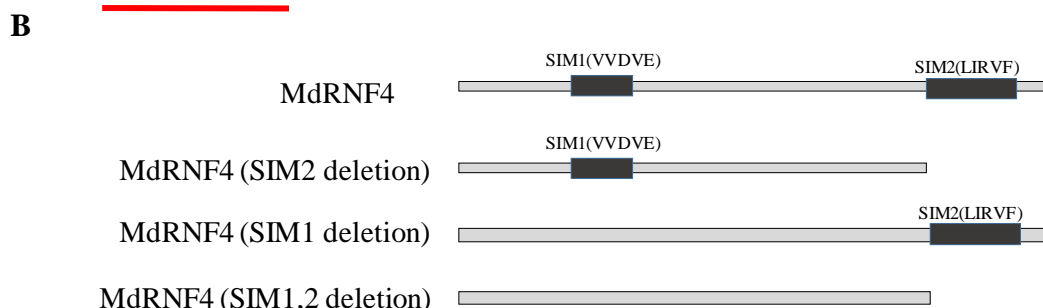


Fig. S17. MdrNF4 interacts with MdrSUMO2A by SIMs. (A) The putative SIMs in MdrNF4 protein. SIMs were indicated by red color and the ring finger domain was underlined in red. (B) The scheme of MdrNF4 SIM deletions. (C) MdrSUMO2A interacted with MdrNF4 by SIMs in yeast two-hybrid analysis. (D) The interaction of MdrNF4 and MdrSUMO2A was verified by MST analysis. (E) Co-IP assay confirmed the interaction of MdrNF4 and MdrSUMO2A. Proteins were extracted from the leaves of *MdSUMO2A* OE (carrying 35S::Myc-*MdSUMO2A*) transgenic plants and immunoblotted with anti-Myc or anti-MdrNF4. No-antibody was used as the negative control.

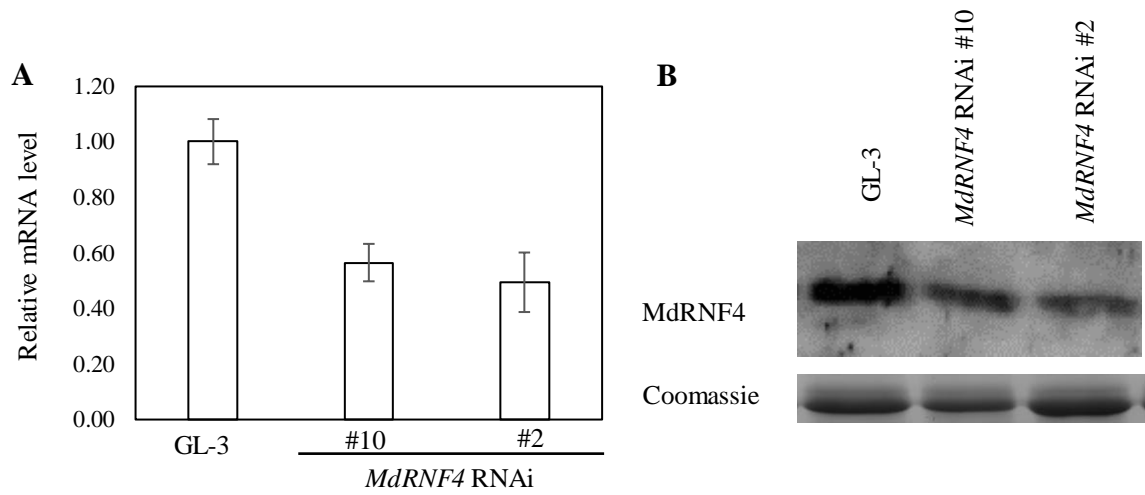


Fig. S18. Expression of *MdRNF4* in *MdRNF4* RNAi transgenic plants.

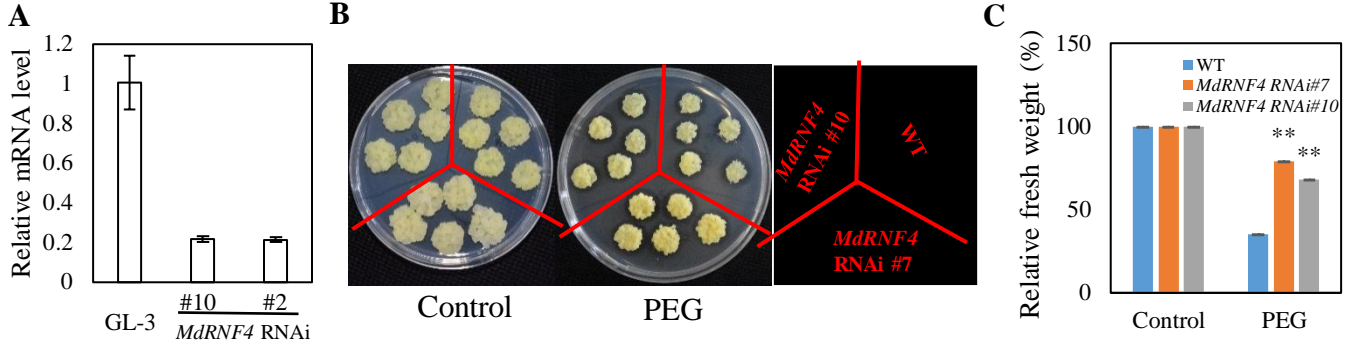


Fig. S19. Tolerance of *MdRNF4* RNAi calli in response to simulated drought stress. (A) Expression of *MdRNF4* in *MdRNF4* RNAi transgenic calli. (B) Tolerance of *MdRNF4* RNAi to PEG treatment. (C) Relative fresh weight of WT and *MdRNF4* RNAi under PEG treatment. Error bars indicate standard error (n = 3 in A, 15 in C). Asterisks indicate significant differences based on one-way ANOVA and Tukey test (*, $P < 0.05$; **, $P < 0.01$).

Malus domesticaMGAYDQGVNMPSLFLDSSRRKRTSRRDG.NSVADTLERKWEHNRKQLESATEE.GKRRKVPKRGSKKGCMMKRGGPEANARCNVRCVQRQWGWKVAEITREPFGSSRLWLCGF	111
Pyrus bretschneideriMGAYDQGVNMPYFLDSSRRKRTSRRDG.NSVADTLERKWEHNRKQLESATEE.GKRRKVPKRGSKKGCMMKRGGPEANARCNVRCVQRQWGWKVAEITREPFGSSRLWLCGF	111
Prunus armeniaca	MQIVEKTINGISLSKLOGMGAYDQGSNEVALTLDSTRKRTSRRDG.TSVAETLERKWEHNRKQLESADNNE.GKTRKVPKRGSKKGCMMKRGGPEANARCNVRCVQRQWGWKVAEITREPFGSSRLWLCGF	128
Prunus mumeMGAYDQGSNEVALTLDSTRKRTSRRDG.TSVAETLERKWEHNRKQLESADNNE.GKTRKVPKRGSKKGCMMKRGGPEANARCNVRCVQRQWGWKVAEITREPFGSSRLWLCGF	111
Fragaria vescaMGAYDQGSNGFSQFLDSSRRKRTSRRDG.GSVEETLSRWEHNRKQLESADNNE.GKTRKVPKRGSKKGCMMKRGGPEANARCNVRCVQRQWGWKVAEITREPFGSSRLWLCGF	110
Rosa chinensisMGAYDQGSNGFSQFLDSSRRKRTSRRDG.ASVVETLSRWEHNRKQLESADNNE.GKTRKVPKRGSKKGCMMKRGGPEANARCNVRCVQRQWGWKVAEITREPFGSSRLWLCGF	111
Zea maysMTLIYSYDDELPCWY..RSMRRKRTSPDSIAETIRWKEHNRKQLESADNNE.GKTRKVPKRGSKKGCMMKRGGPEANARCNVRCVQRQWGWKVAEITREPFGSSRLWLCGF	106
Oryza sativaMAVYDQSGDRNRQTIDTSRRKRSRGGD.TTVAERLKRKWEHNRKQLESADNNE.GKTRKVPKRGSKKGCMMKRGGPEANARCNVRCVQRQWGWKVAEITREPFGSSRLWLCGF	109
Triticum aestivumMTVDRKDAEAAAATAAATPFEIPALQPKRRKRSRDRGPNVSEETIRWKEHNRKQLESADNNE.GKTRKVPKRGSKKGCMMKRGGPEANARCNVRCVQRQWGWKVAEITREPFGSSRLWLCGF	121
Nicotiana attenuataMALLDQASNMMSLFLDYTRKRSRKRKDPKNVAETLAKRWEHNRKQLESADNNE.GKTRKVPKRGSKKGCMMKRGGPEANARCNVRCVQRQWGWKVAEITREPFGSSRLWLCGF	113
ArabidopsisMAVYDQSGDRNRQTIDTSRRKRSRGGD.TTVAERLKRKWEHNRKQLESADNNE.GKTRKVPKRGSKKGCMMKRGGPEANARCNVRCVQRQWGWKVAEITREPFGSSRLWLCGF	109
Malus domestica	TAVDAALAYDEAARAMYGDGARINLPHAVNN...HPS..SSQETSSVATPSGSSAAATPGCSTSTSTSS...LSEVCADENSKFLFDVKKEDGE.GESRMYPISGAVPQASG...MVKQEVNEFDMDY	228
Pyrus bretschneideri	TAVDAALAYDEAARAMYGDGARINLPHGVNN...HPS..SSQETSSVATPSGSSAAATPGCSTSTSTSS...HSEVCGDENSKFLFDVKKEDGE.GESRMYPISGAVPQASG...MVKSEVSELDVDY	228
Prunus armeniaca	TALDAALAYDEAARAMYGPBARINLPHVANY...SPWKESQETSSATTPSGSSAVATPGCGSTSTSS...HSEVCADEDSKGFLDVKTENGE.GESANHDWSKGLNQA...LVKEETREDFEQY	245
Prunus mume	TALDAALAYDEAARAMYGPBARINLPHVASY...SSWKESQETSSATTPSGSSAVATPGCGSTSTSS...HSEVCADEDSKGFLDVKTENGE.GESAIHAWSNALNQT...LVKEETREDFEQY	228
Fragaria vesca	TAVDAALAYDEAARAMYGABARINLPHVANNRNSYVSSWNSSEHETSSVTPSGCSTVAKGCGCESTMNSQSDHSEVCAEES...VVKVTEDEGE.GELTSRAMSNAYTEPASNATQDVVKSEVPMDH	236
Rosa chinensis	TAVDAALAYDEAARAMYGABARINLPHVNNHNSYTSWNSSEHETSSVTPSGCSTVATGCGCESTMNSQSDHSEVCAEES...VVKVTEDEGE.DESTS...HNAFTQPTSNATQDVVKSEVPMYD	234
Zea mays	TAVDAALAYDEAARAMYGPBARINLPHVSSADA...EVTSSTSSQEVCTVETPGGVHVKTEDPDCESKPPSGGVEPMYCLENGAEMKRGVKADKHWLSEFEHNYWSDILKEKQKQGGIVE	198
Oryza sativa	TAQDAASAYDEAARAMYGPBARINLPHVSSADA...EVTSSTSSQEVCTVETPGGVHVKTEDPDCESKPPSGGVEPMYCLENGAEMKRGVKADKHWLSEFEHNYWSDILKEKQKQGGIVE	227
Triticum aestivum	TAQDAASAYDEAARAMYGPBARINLPHVSSADA...EVTSSTSSQEVCTVETPGGVHVKTEDPDCESKPPSGGVEPMYCLENGAEMKRGVKADKHWLSEFEHNYWSDILKEKQKQGGIVE	232
Nicotiana attenuata	TAQDAASAYDEAARAMYGPBARINLPHVSSADA...EVTSSTSSQEVCTVETPGGVHVKTEDPDCESKPPSGGVEPMYCLENGAEMKRGVKADKHWLSEFEHNYWSDILKEKQKQGGIVE	220
Arabidopsis	TAQDAASAYDEAARAMYGPBARINLPHVSSADA...EVTSSTSSQEVCTVETPGGVHVKTEDPDCESKPPSGGVEPMYCLENGAEMKRGVKADKHWLSEFEHNYWSDILKEKQKQGGIVE	227
Malus domestica	LRNMQQPVGMKPEVNEVDFMDYLRNMQQQRVEDHNPNGDGFVGDYSEFTMCELFDMDMFDVNELLMPPDDISLCSNGPEQVSRPD...VQGF...GMETLSS.EGPNLSYQLQYDPAKL	347
Pyrus bretschneideri	LRNMQQPV.VVKPE...RCVEDHNPNGDGFVGDYSEFTMCELFDMDMFDVNELLMPPDDISLCSNGPEQVSRPD...VQGF...GMETLSS.EGPNLSYQLQYDPAKL	330
Prunus armeniaca	FHNDQ..FA..VKPE...AYVGFDFNAGG.PYTGDYLENFTEEEVFDVDELLN...FLDDETVRNPEPEQGLGSD...VQGGCCSGMHPYEC.DRPNLSYQLQYDPAKL	340
Prunus mume	FHNDQ..FA..VKPE...AYVGFDFNAGG.PYTGDYLENFTEEEVFDVDELLN...FLDDETVRNPEPEQGLGSD...VQGGCCSGMHPYEC.DRPNLSYQLQYDPAKL	323
Fragaria vesca	AHTDQ..FG..VQEQ...ALDNI...GPDVNYLQFSLLELLFVDDLVG...PTDDKPIPTLKLQDTSDD...VGRSQFADTAPVES.ATPSSSSYQVQYDGGTT	325
Rosa chinensis	FHTDQ..FG..VQEQ...AHDISTDGFMDYLRQFISIDELFVDDLVG...PMDGKPIAPRLQDLSDD...VGRSQFADTAPVES.ATPSSSSYQVQYDGGTT	327
Zea mays	VIQSA...EET...VLHKEG.DVS...YDFVNEVDVE...MIIVELNADKRFEAHEEYLDGDD...GFSLFAY...GFSLFAY...	256
Oryza sativa	TCQQQ...QQDS...LSVADYGNPN.DVDQSHLD...SSDMFDVDELLR...DLNGDDVDFAGLNQDRYPGNS...VANG...SYR...PESQQ	298
Triticum aestivum	ARSTS...EED...VEFPLE.PISSLPDG...EADGFDIEELLR...LMEADPFEVEPVTTGGSWNCGTNT...LMEADPFEVEPVTTGGSWNCGTNT...GVMGLQEPY	300
Nicotiana attenuata	IVETK...EEP...VAFDYDSVKS.GQDNNLDNLCEFDDEMFVDELLG...VLDSPTLDASAPNQVSGSVFNQDQYVDPSYRQSAAYDNNQFNSLSYQLQYDGGTT	319
Arabidopsis	TCQQQ...QQDS...LSVADYGNPN.DVDQSHLD...SSDMFDVDELLR...DLNGDDVDFAGLNQDRYPGNS...VANG...SYR...PESQQ	298
Malus domestica	LG.SLPHMQAFLDYEDFDPMKQEE.GSNHTSQNDQGYFNLGSDLDLGLGFTGTMQSGDVGYNYSME	414
Pyrus bretschneideri	LG.SLPHMQAFLDYEDFDPMKQEE.GSNHTSQNDQGYFNLGSDLDLGLGFTGTMQSGDVGYNYSME	397
Prunus armeniaca	LG.SLNHMQQFSGGEMNFDFLKR...GVTGEDDQGYNLGLSDLDLGLGFTGTMQPEDGYSNFSMD	404
Prunus mume	LG.SLNHMQQFSGGEMNFDFLKR...DVTGEDDQGYNLGLSDLDLGLGFTGTMQPEDGYSNFSMD	387
Fragaria vesca	SG.SVQHMDQFESVADYGFLLNLQL.QDNGVGVNHRVNMDDDFPHFPGGADN...	378
Rosa chinensis	PGGNVGHMDVESVADYGFLLNLQR.QENNNGVGVNHCYFNVDVDFNFTGGCCGNAN...	384
Zea mays	SG...FDPYQS...LNNGTIPFPQLEG.KDNGFFDLSLVDLEN	256
Oryza sativa	SG...FDPYQS...LNNGTIPFPQLEG.KDNGFFDLSLVDLEN	335
Triticum aestivum	LDLGDQQLGMLQADYPPMWSIEDRAMNPAFHDAEMSEFFEG	346
Nicotiana attenuata	LDSQQMQQAIVDDYDFFLKPGRPEDFNFSLDLDFDFDQ	362
Arabidopsis	SG...FDPYQS...LNNGTIPFPQLEG.KDNGFFDLSLVDLEN	335

Fig. S20. Protein alignment of DREB2A from different species. The amino acids in red line indicate AP2 domain, and the blue frame indicates NRD region.