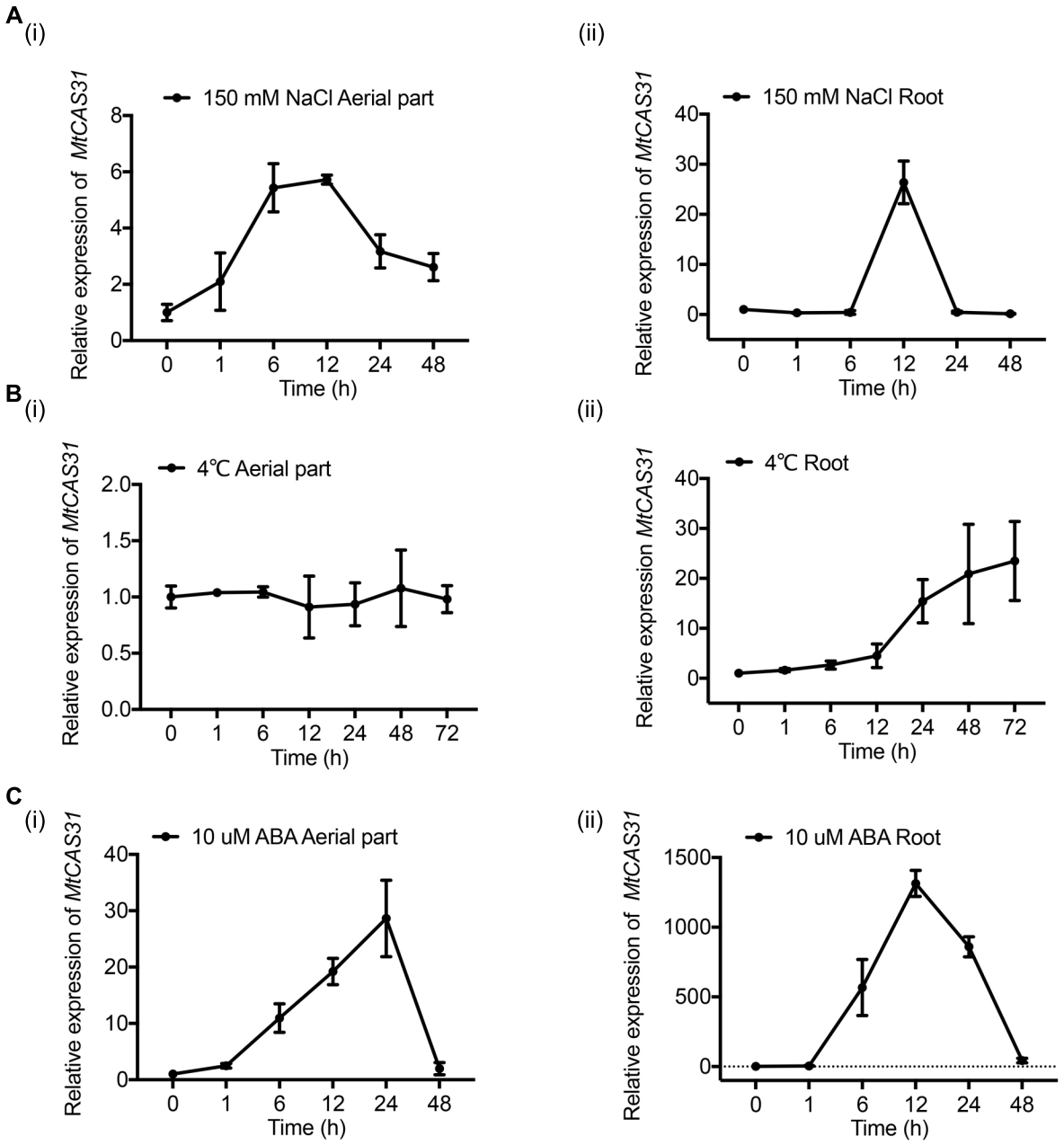


**Figure S1**. Expression patterns of *MtCAS31* in different M. truncatula tissues under abiotic stresses. (**A**) Relative expression of *MtCAS31* in aerial parts (i) and roots (ii) under 150 mM NaCl treatment at the indicated time points. (**B**) Relative expression of *MtCAS31* in aerial parts (i) and roots (ii) under cold (4°C) at the indicated time points. (**C**) Relative expression of *MtCAS31* in aerial parts (i) and roots(ii) under 10 μM ABA treatment at the indicated time points. All the values in (**A**), (**B**) and (**C**) were normalized to *ACTIN* expression. The data represent the mean±SD of 3 technical replicates.

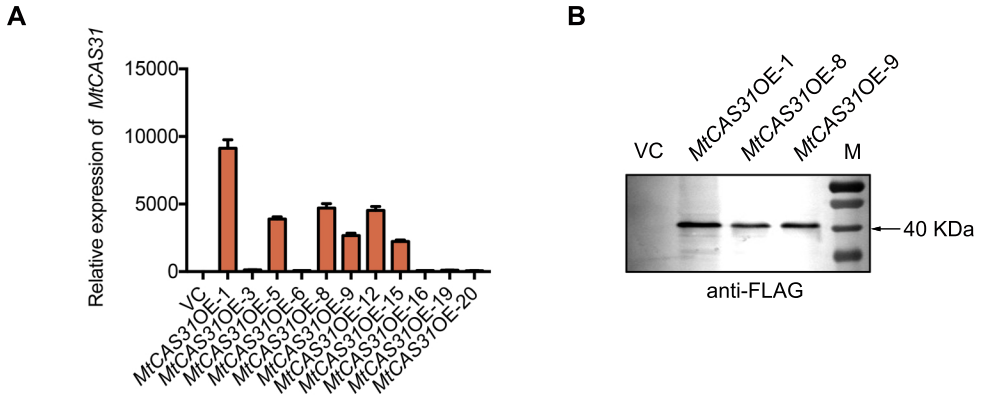


**Figure S2.** Identification of *MtCAS31*OE. (**A**) Relative expression of *MtCAS31* in T1 *MtCAS31*OE transgenic plants by qRT-PCR. The values were normalized against *ACTIN* expression. VC, vector control. (**B**) Immunoblotting blotting analysis of *MtCAS31*OE transgenic plants with anti-FLAG.

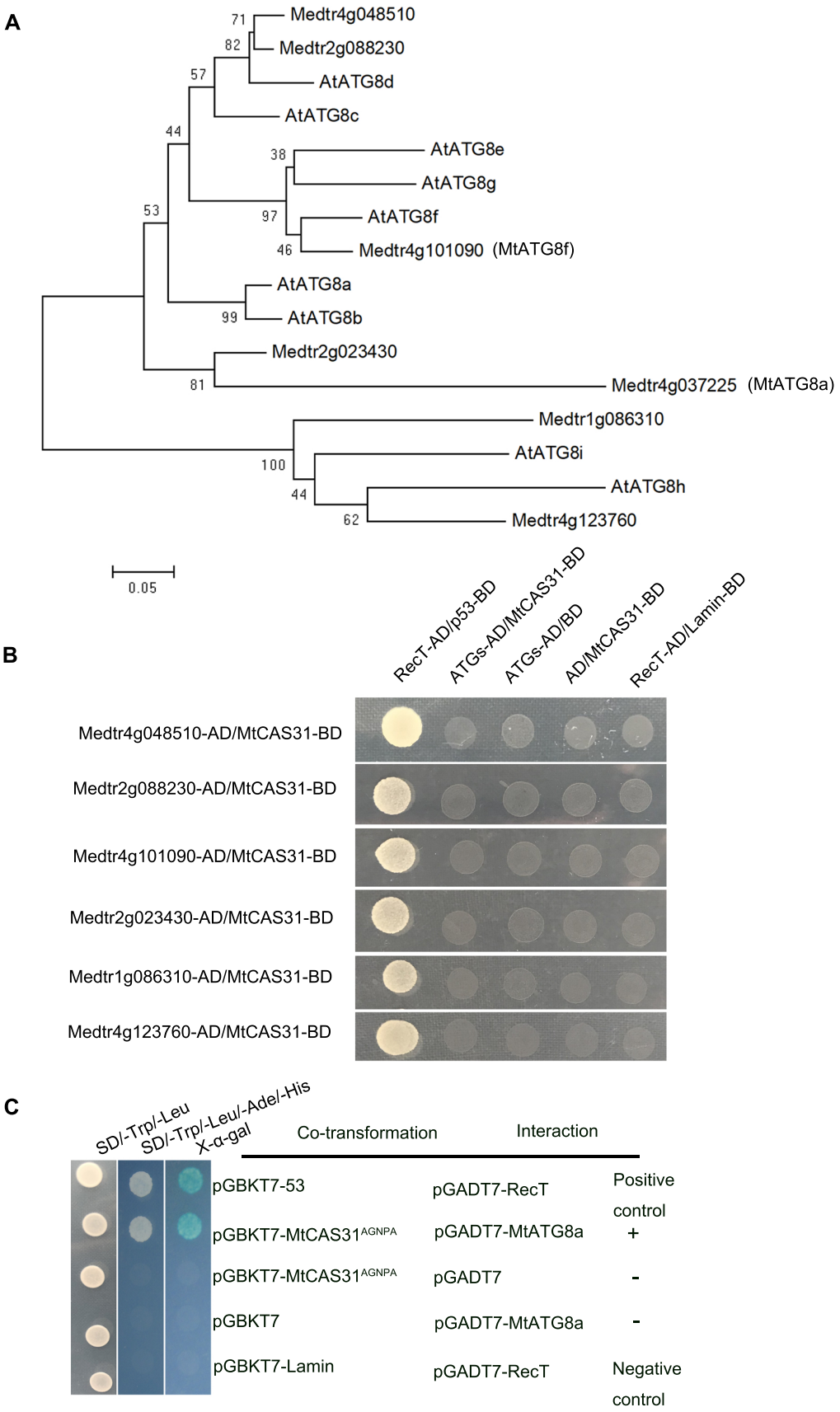


**Figure S3.** MtCAS31 cannot interact with other ATG8 proteins in yeast. (**A**) phylogenetic analysis of ATG8 in M.truncatula and A. thaliana. (**B**) MtCAS31 can't interact with ATG8s (Medtr4g048510, Medtr2g088230, Medtr1g101090, Medtr2g023430,Medtr1g086310, Medtr4g123760). (**C**) The AIM-like motif YGNPI22 of MtCAS31 doesn't affect the interaction with MtATG8a. When the AIM-like motif, YNGPI22, was mutated to ANGPA, MtCAS31 still could interact with MtATG8a.

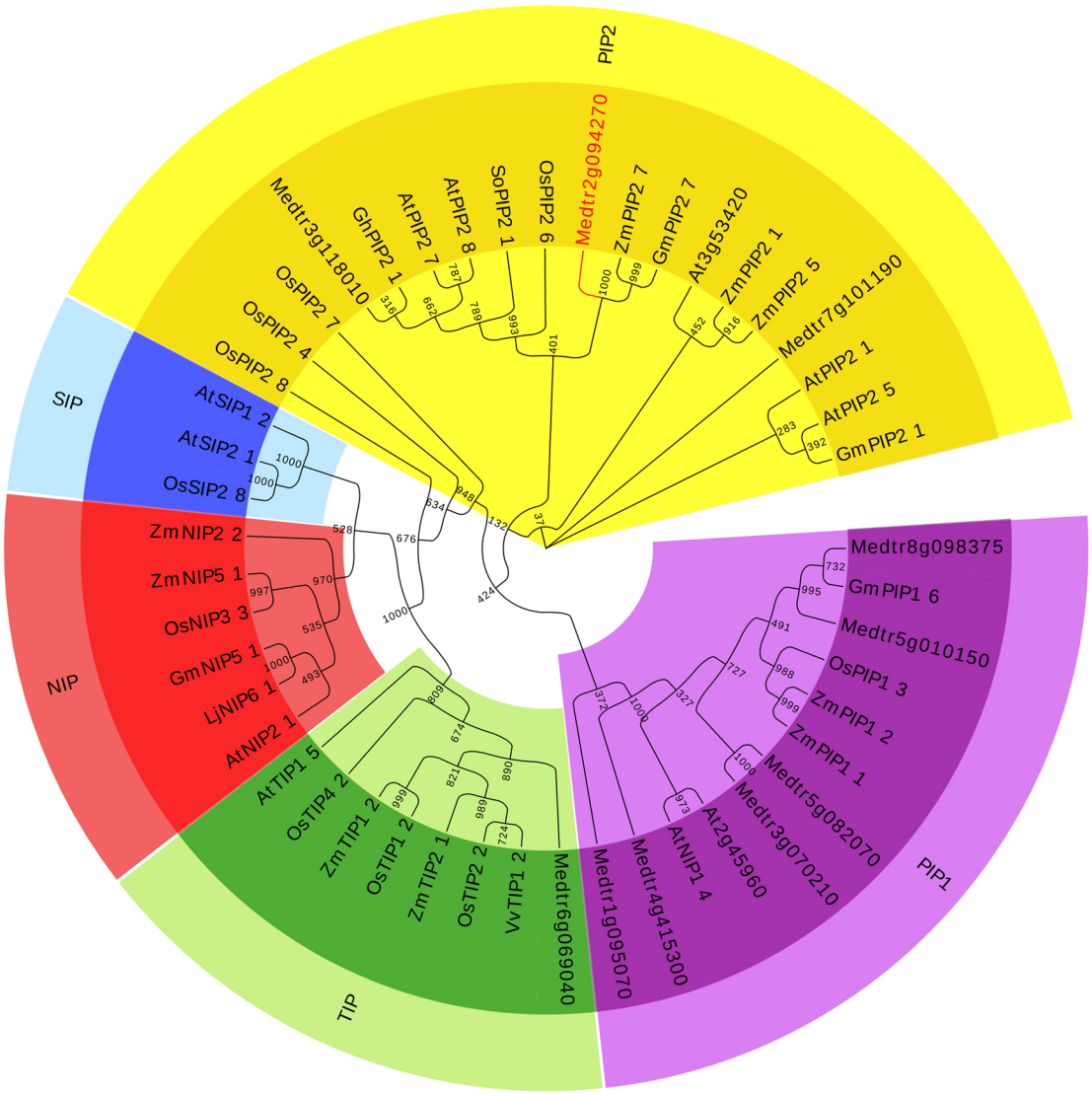


**Figure S4.** Phylogenetic analyses of MtPIP2;7 (Medtr2g094270). The purple background represents the PIP1 subfamily, the yellow background represents the PIP2 subfamily, and the green background represents the TIP2 subfamily. The red background represents the NIP subfamily. The blue background represents the SIP subfamily. MtPIP2;7 (Medtrt2g094270.1) is highlighted by the red branch and red color. The node labels present the statistical support obtained from 1,000 bootstrap repetitions.

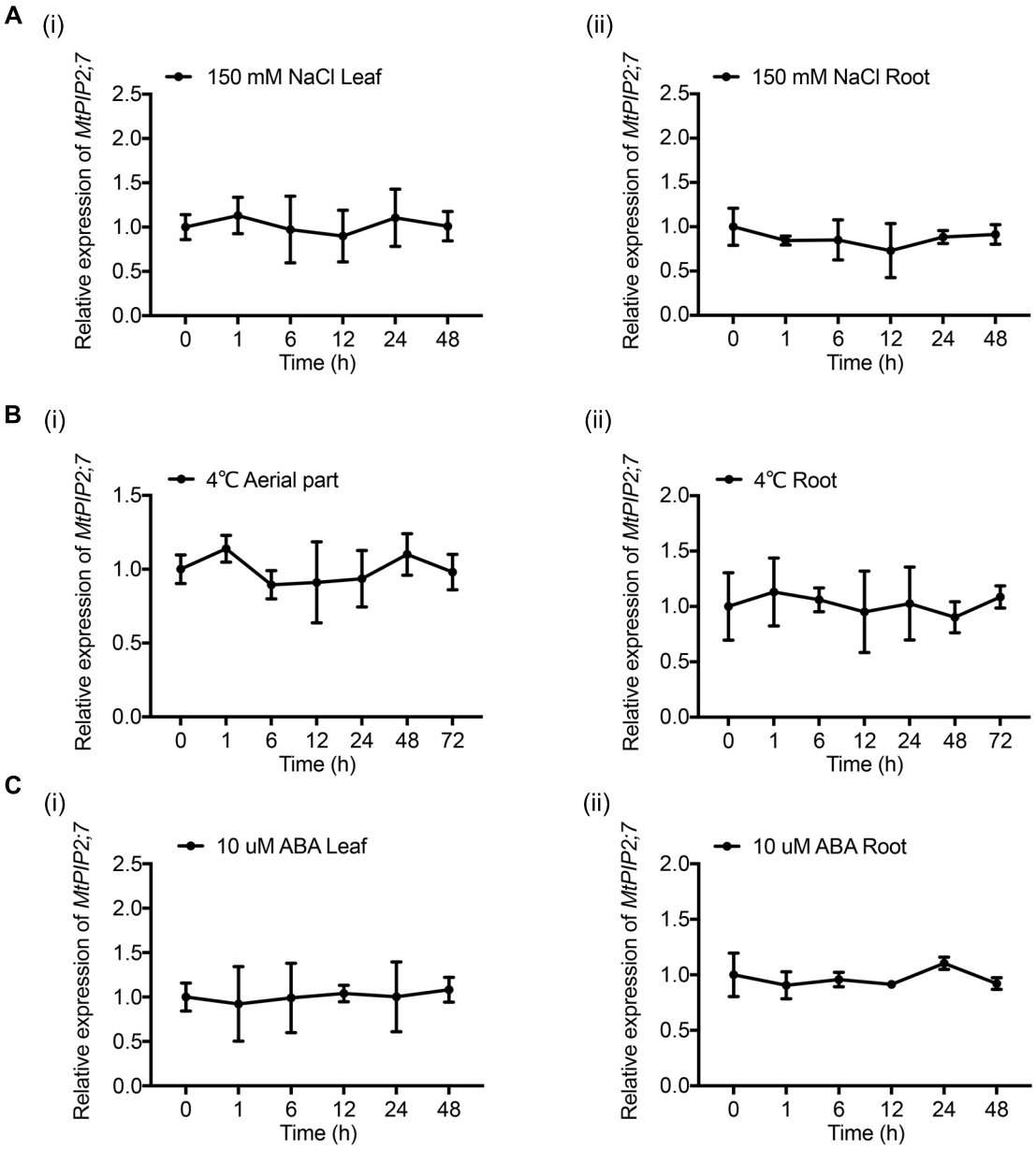


**Figure S5.** The expression patterns of *MtPIP2;7* under abiotic stress. (**A**) The relative expression of *MtPIP2;7* in aerial parts (i) and root (ii) under 150 mM NaCl treatment under indicated time points by qRT-PCR. (**B**) The relative expression of *MtPIP2;7* in aerial parts (i) and root (ii) under 4°C treatment. (**C**) The relative expression of *MtPIP2;7* in aerial parts (i) and root (ii) under 10 μM ABA treatment under indicated time points by qRT-PCR. All data in (**A**), (**B**) and (**C**) represent the mean ± SD of three technical replicates. The values were normalized to *ACTIN* expression.

**A**

```
WT        a GCAAAGATGTTGAGGTTCAAGAACAAGGTGGTGAATTTTCAGCTAAGGATTACCAAGATCCACCACCAGCACCTTTGTTTGATTTAGATGAGTTAACAAAGT  WT
          b GCAAAGATGTTGAGGTTCAAGAACAAGGTGGTGAATTTTCAGCTAAGGATTACCAAGATCCACCACCAGCACCTTTGTTTGATTTAGATGAGTTAACAAAGT

pip2;7-59
          T0 generation
          a GCAAAGATGTTGAGGTTCA----------GCTAAGGATTACCAAGATCCACCACCAGCACCTTTGTTTGATTTAGATGAGTTAACAAAGT  -22
          b GCAAAGATGTTGAGGTTCA----------GCTAAGGATTACCAAGATCCACCACCAGCACCTTTGTTTGATTTAGATGAGTTAACAAAGT  -22
          T1 generation
          a GCAAAGATGTTGAGGTTCA----------GCTAAGGATTACCAAGATCCACCACCAGCACCTTTGTTTGATTTAGATGAGTTAACAAAGT  -22
          b GCAAAGATGTTGAGGTTCA----------GCTAAGGATTACCAAGATCCACCACCAGCACCTTTGTTTGATTTAGATGAGTTAACAAAGT  -22

pip2;7-69
          T0 generation
          a GCAAA----------GT  -95
          b GCAAAGATGTTGAGGTT----------CACCTTTGTTTGATTTAGATGAGTTAACAAAGT  -52
          T1 generation
          a GCAAA----------GT  -95
Line 1    b GCAAAGATGTTGAGGTT----------CACCTTTGTTTGATTTAGATGAGTTAACAAAGT  -52

          a GCAAA----------GT  -95
Line 2    b GCAAA----------GT  -95

          a GCAAAGATGTTGAGGTT----------CACCTTTGTTTGATTTAGATGAGTTAACAAAGT  -52
Line 3    b GCAAAGATGTTGAGGTT----------CACCTTTGTTTGATTTAGATGAGTTAACAAAGT  -52

pip2;7-77
          T0 generation
          a GCAAAGATGTTGAGGTTCA----------CAAAGT  -77
          b G ----------GCTAAGGATTACCAAGATCCACCACCAGCACCTTTGTTTGATTTAGATGAGTTAACAAAGT  -40
          T1 generation
          a GCAAAGATGTTGAGGTTCA----------CAAAGT  -77
Line 1    b G ----------GCTAAGGATTACCAAGATCCACCACCAGCACCTTTGTTTGATTTAGATGAGTTAACAAAGT  -40

          a G ----------GCTAAGGATTACCAAGATCCACCACCAGCACCTTTGTTTGATTTAGATGAGTTAACAAAGT  -40
Line 2    b G ----------GCTAAGGATTACCAAGATCCACCACCAGCACCTTTGTTTGATTTAGATGAGTTAACAAAGT  -40

          a GCAAAGATGTTGAGGTTCA----------CAAAGT  -77
Line 3    b GCAAAGATGTTGAGGTTCA----------CAAAGT  -77
```

**B**

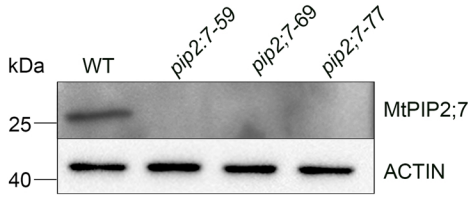


**Figure S6.** Identification of *pip2;7* knockout mutants generated by TALEN technology. (**A**) T0 and T1 generation of 3 different lines of *pip2;7* (*pip2;7-59*, *pip2;7-69*, *pip2;7-77*) generated by TALEN was sequenced. Dotted lines represent missing bases. The digits represent the number of missing bases in each mutant, and a/b represent the homologous chromosome. T0 generation of *pip2;7-59* was homozygous missing 22 bp in the both 2 chromosomes. T1 generation of *pip2;7-59* was used for further study. T0 generation of *pip2;7-69* was heterozygous but the *MtPIP2;7* is deleted in both homologous chromosome with different missing bases (95 bp missing in one chromosome and 52 bp missing in the other chromosome). T1 generation of *pip2;7-69* contains 3 forms. (Line 1, 95 bp missing in one chromosome and 52 bp missing in the other chromosome. Line 2, 95 bp missing in both chromosome. Line 3, 52 bp missing in both chromosome). Line 2 of *pip2;7-69* T1 generation (green, 95 bp missing) was used for further studies. Similar to *pip2;7-69*, T1 generation of *pip2;7-77* with 77 bp missing was selected (blue, 77 bp missing). (**B**) Immunoblotting analysis to identify the protein level of MtPIP2;7 in T1 generation *pip2;7* mutants.

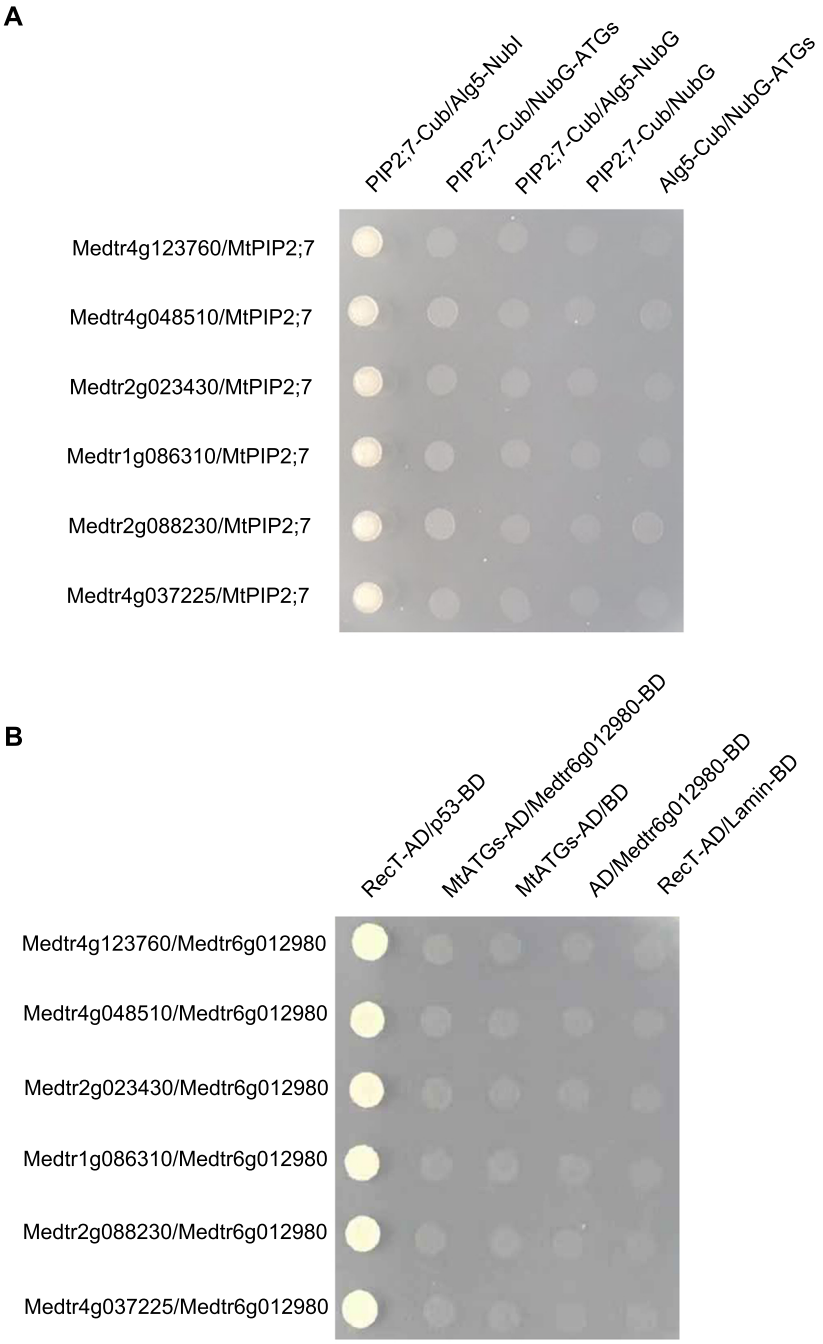


**Figure S7.** MtPIP2;7 and Medtr6g012980 cannot interact with ATG8 proteins in yeast. (**A**) Interaction between MtPIP2;7 and ATG8s (Medtr4g048510, Medtr2g088230, Medtr1g101090, Medtr2g023430, Medtr1g086310, Medtr4g123760). (**B**) Interaction between Medtr6g012980 and ATG8s (Medtr4g048510, Medtr2g088230, Medtr1g101090, Medtr2g023430, Medtr1g086310, Medtr4g123760).