Supplementary Information

Wheat Germination Is Dependent on Plant Target of Rapamycin Signaling

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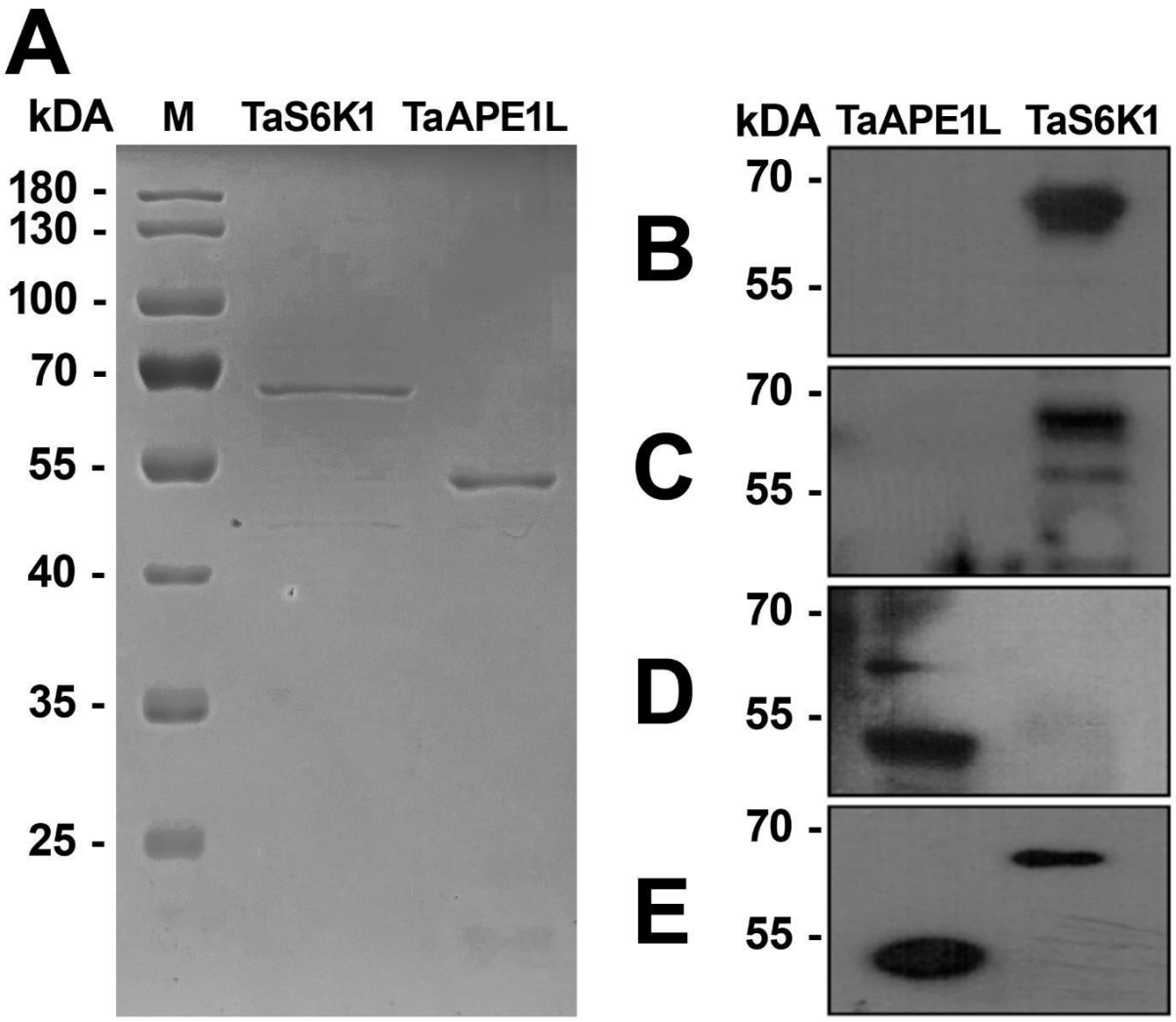
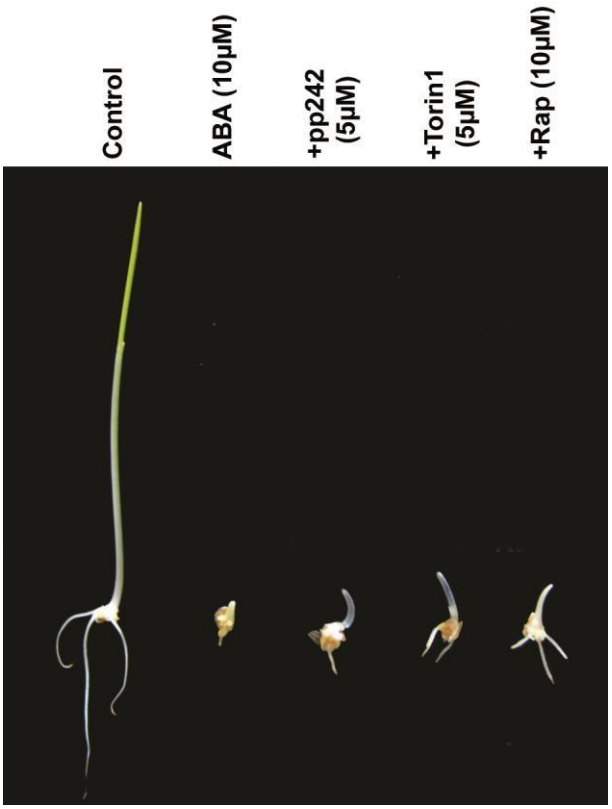
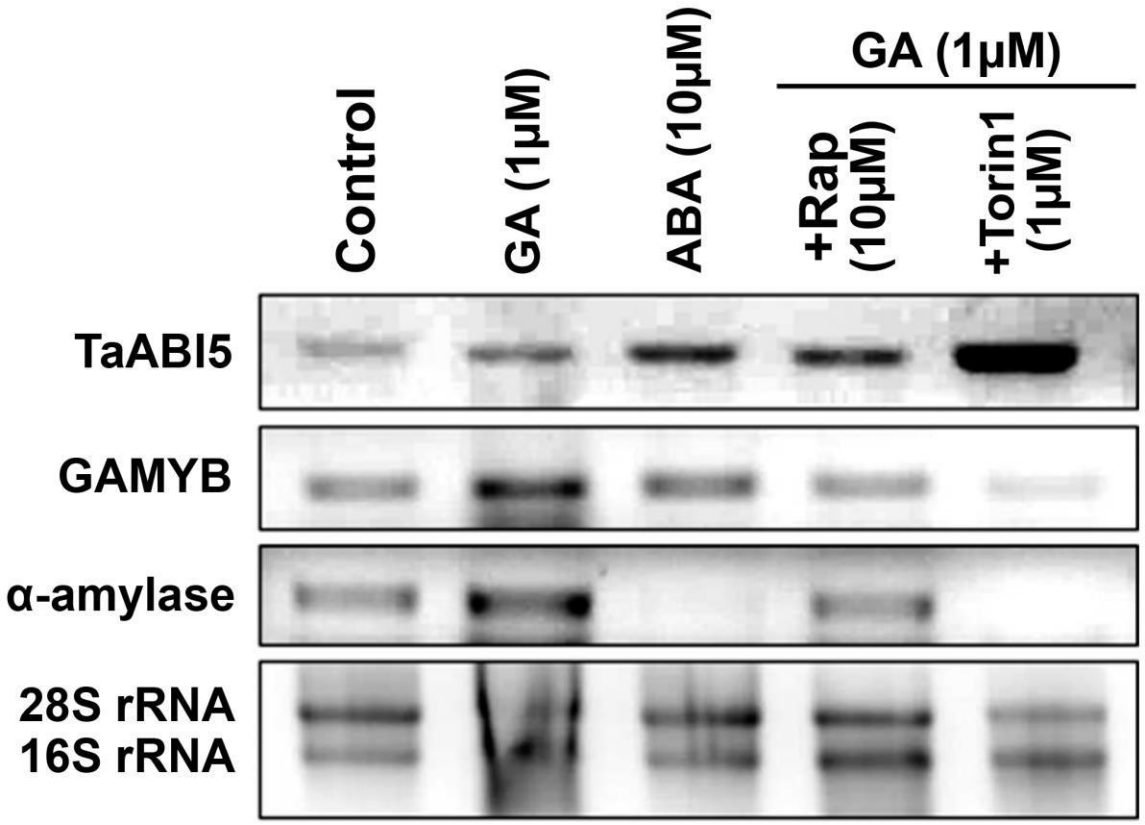


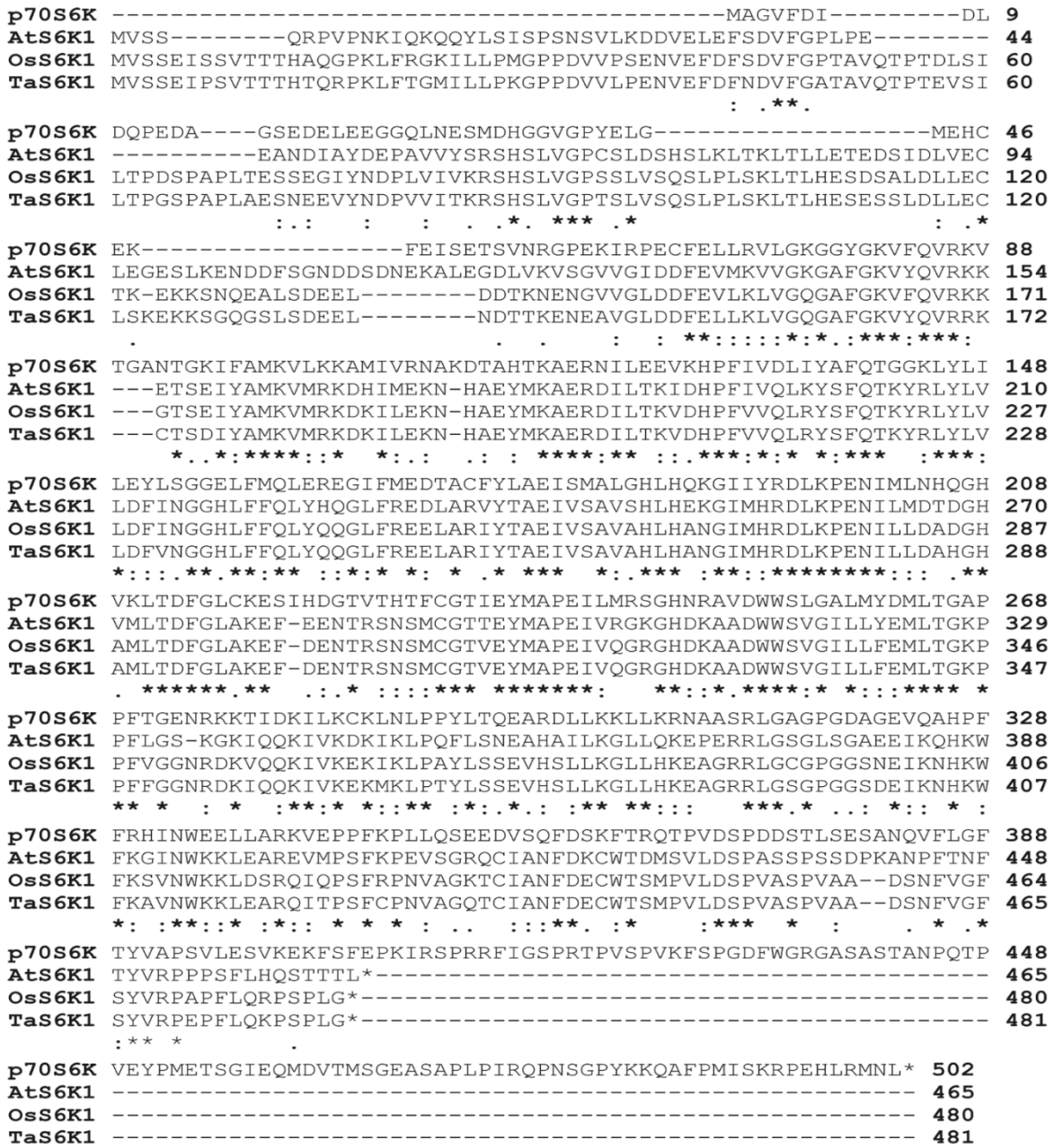
Supplementary Figure S1. Detection of the purified TaS6K1 protein by the anti-TaS6K1 antibody. (A) SDS-PAGE analysis of the purified recombinant TaS6K1 and TaApe1L proteins. Lane 1, protein size markers; lane 2, TaS6K1, 0.5 µg; lane 3, TaApe1L, 1 µg; Western blot analysis with anti-TaS6K1 (B), anti-RPS6KB1 (Ab-389) (C), anti-TaApe1L (D), and anti-His antibodies (E). In all four western blots, 1 µg of purified 6xHis-APE1L was loaded in the first lane and 0.5 µg of purified 6xHis-TaS6K1 in the second lane.



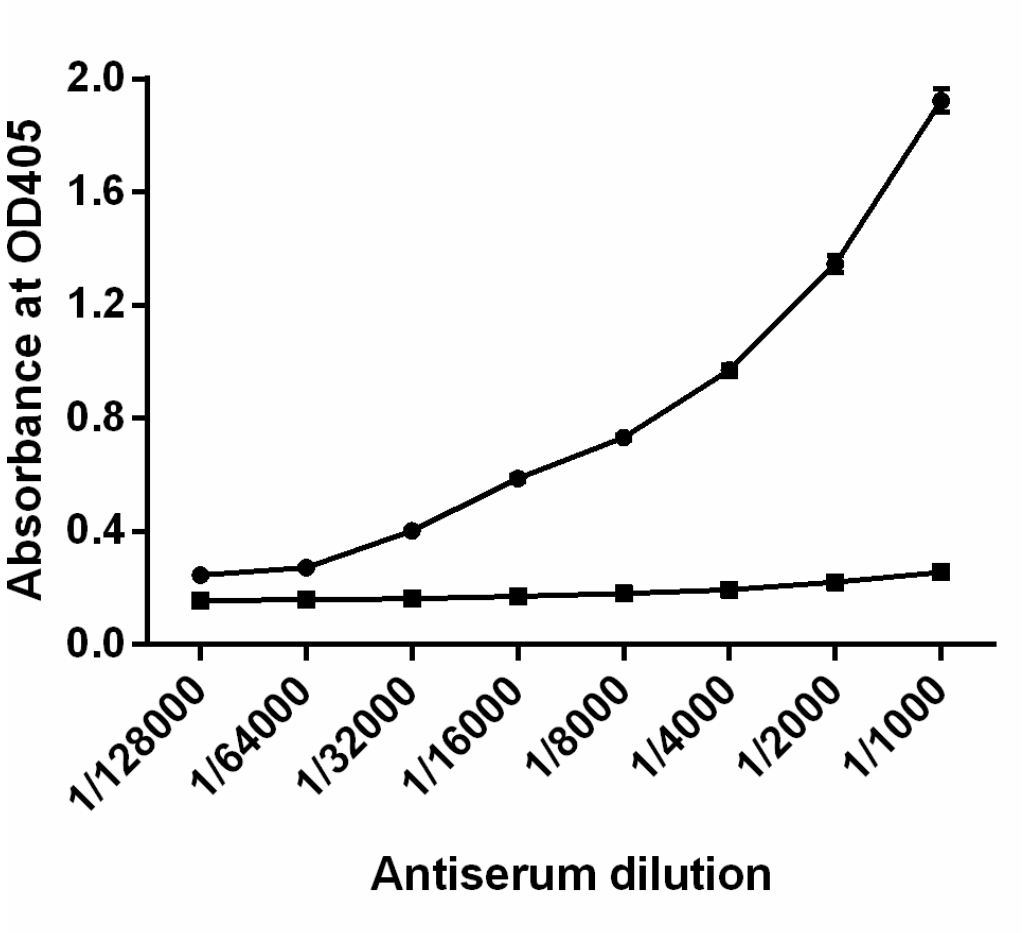
Supplementary Figure S2. The effect of rapamycin and ATP-competitive TOR inhibitors on isolated wheat embryos. The embryos were incubated on the 0.5× MS medium supplemented with ABA alone and rapamycin, torin1, or pp242 as indicated for 4-day incubation.



Supplementary Figure S3. The influence of TOR kinase inhibitors on mRNA levels of the indicated genes GAMYB, α-amylase, and ABI5 in wheat embryos. The wheat embryos dissected from 1-DAG seeds were incubated for 24 h in the presence 10 mM $CaCl_2$ (control) with or without 10 μM ABA, or 1 μM GA, or 1 μM GA with rapamycin or torin1 as indicated. TaABI5, GAMYB, and α-amylase cDNAs were amplified by RT-PCR with gene-specific primers. Ribosomal RNA was included as an internal control of sample loading. Rap: rapamycin.



Supplementary Figure S4. Protein sequence alignment of putative *T. aestivum* S6K1, *A. thaliana* S6K1, *Oryza sativa* S6K1, and human S6K1. The deduced amino acid sequences were aligned in the ClustalX 2.1 software. Asterisks (*), colons (:), and periods (.) indicate identical, conserved, and semiconserved aligned residues, respectively.



Supplementary Figure S5. The titer of antiserum according to the ELISA. The purified antibody was subjected to serial dilution (from 1000- to 128,000-fold) and reacted with the purified rTaS6K1 protein. Preimmunization rabbit serum served as a negative control. The antibody titer is defined as the highest dilution of serum at which the A_{405} ratio (A_{405} of postimmunization serum/A_{405} of preimmunization serum) is greater than 2:1. Data are presented as the mean ± standard deviation.