

Supplementary Information

Wheat Germination Is Dependent on Plant Target of Rapamycin Signaling

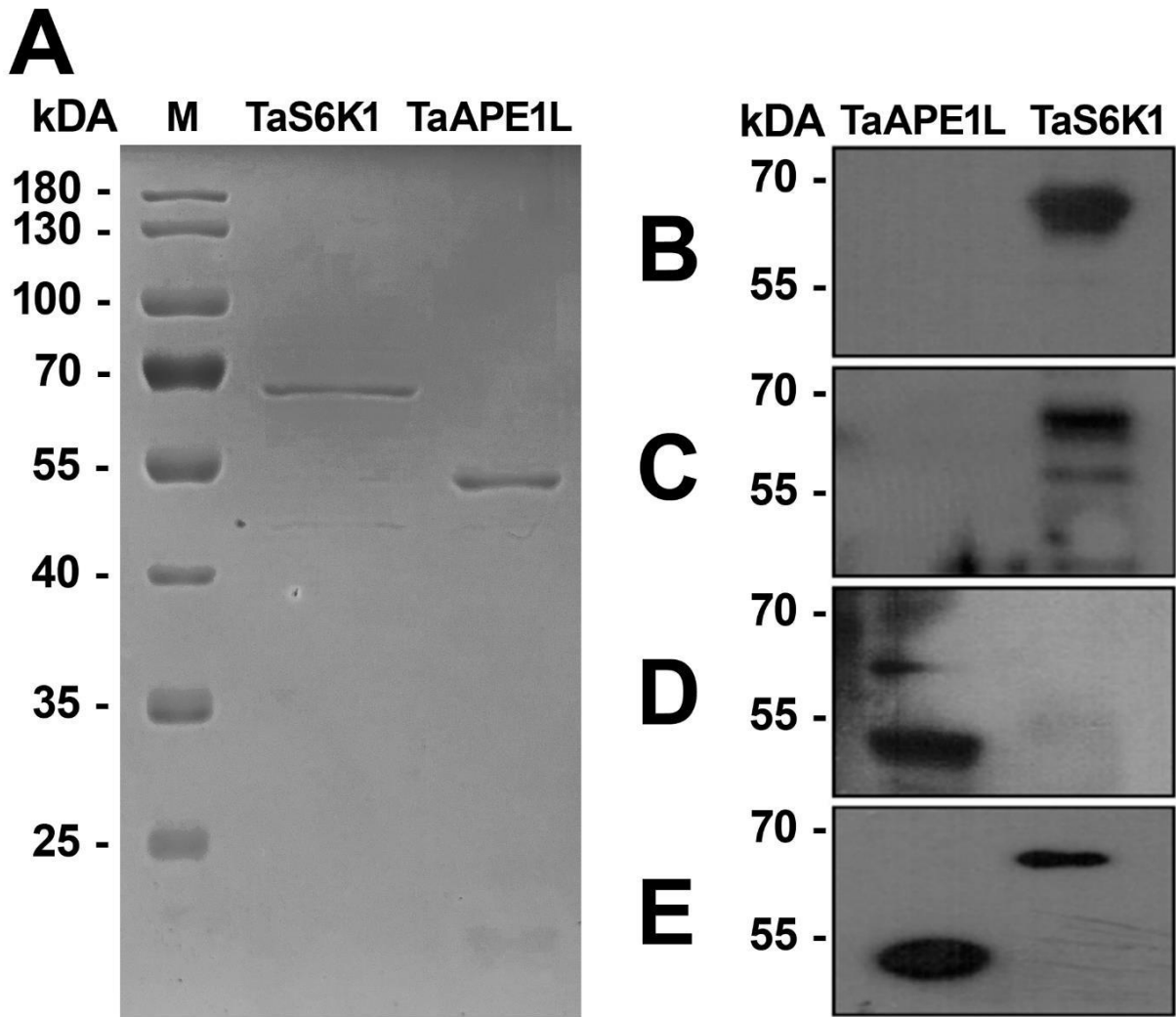
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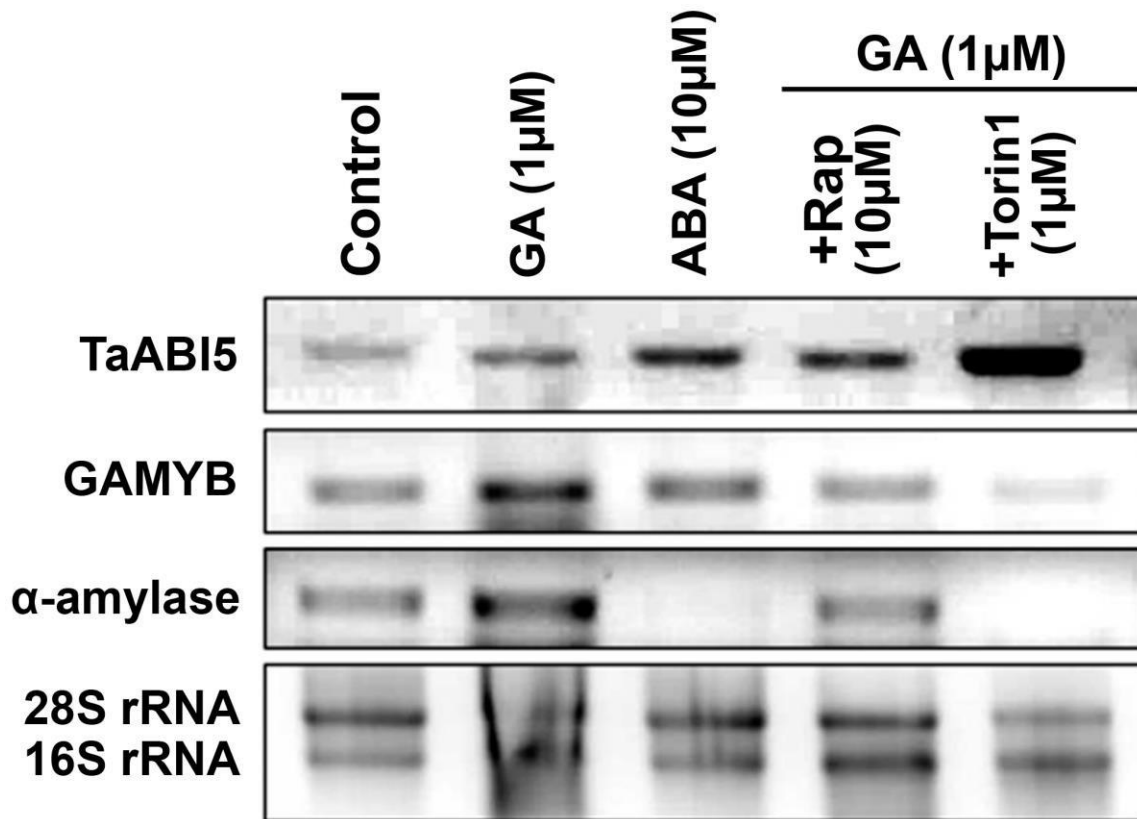
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Supplementary Figure S1. Detection of the purified TaS6K1 protein by the anti-TaS6K1 antibody. (A) SDS-PAGE analysis of the purified recombinant TaS6K1 and TaApe1L proteins. Lane 1, protein size markers; lane 2, TaS6K1, 0.5 µg; lane 3, TaApe1L, 1 µg; 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.



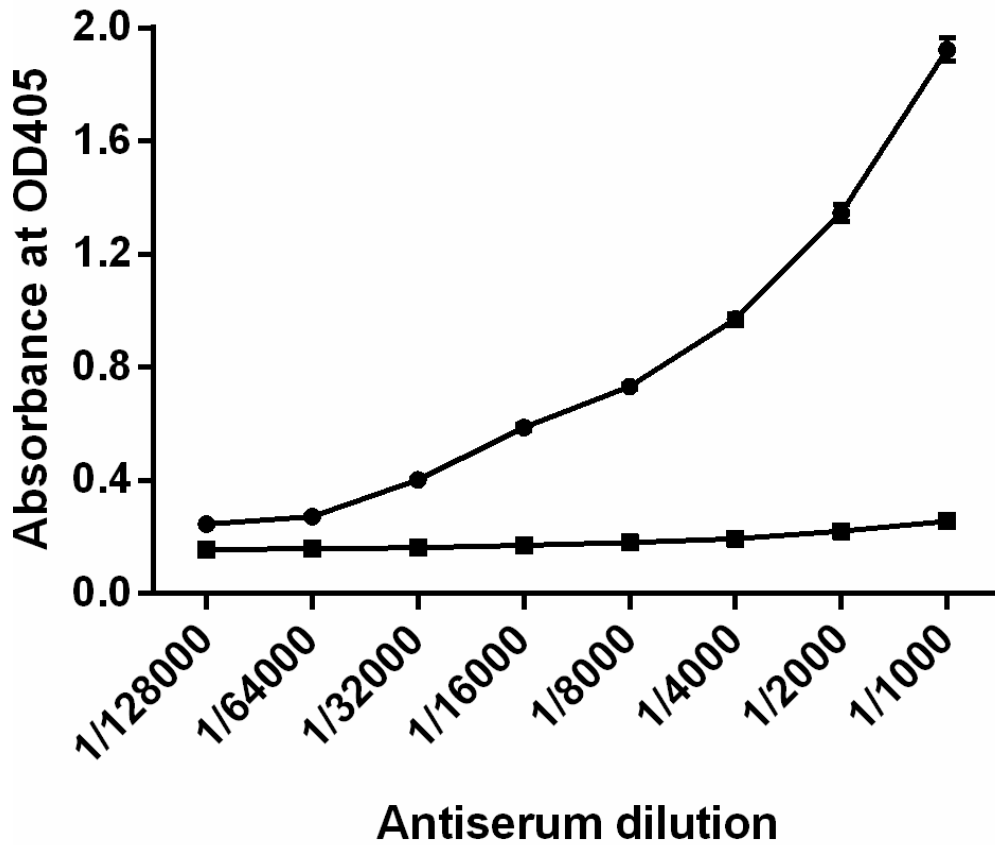
Supplementary Figure S2. The effect of rapamycin and ATP-competitive TOR inhibitors on isolated wheat embryos. The embryos were incubated on the 0.5× MS medium supplemented with ABA alone and rapamycin, torin1, or pp242 as indicated for 4-day incubation.



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.

p70S6K	-----MAGVFDI-----DL	9
AtS6K1	MVSS-----QRPVFNKIQQKQYLSISPSNSVLKDDVELEFSDVFGPLPE-----	44
OsS6K1	MVSSEISSVTTTTHAQGPKLFRGKILLPMGPPDVVPSNEVEFDFSDVFGPTAVQTPTDLSI	60
TaS6K1	MVSSEIIPSVTTTHTQRPKLFTGMILLPKGPPDVVLPENVEFDFNDVFGATAVQTPTEVSI	60
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p70S6K	DQPEDA----GSEDELEEGGQLNESMDHGGVGPYELG-----MEHC	46
AtS6K1	-----EANDIAYDEPAVVYSRSHSLVGPCSLDSHSLKLTCLTLETEDSIDLVEC	94
OsS6K1	LTPDSPAPLTESSEGIYNDPLVIVKRSHSLVGPSSLSVQSLPLSKLTLHESDSALDLEC	120
TaS6K1	LTPGSPAPLAESNEEVYNDPVVITKRSHSLVGPSTSLVSQSLPLSKLTLHESESSLDLEC	120
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p70S6K	EK-----FEISETSVNRGPEKIRPECFELLRVLGKGGYGKVFQVRKV	88
AtS6K1	LEGESLKENDDFSGNDDSDNEKALEGDLVKVSGVVGIDDFEVMKVVGKGAFGKVYQVRKK	154
OsS6K1	TK-EKKSNDQEALSDEEL-----DDTKNENGVVGLDDFEVLKLVGQGAFGKVYQVRKK	171
TaS6K1	LSKEKKSQGQSLSDLEEL-----NDTTKENEAVGLDDFELLKLVGQGAFGKVYQVRKK	172
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p70S6K	TGANTGKIFAMKVLKKAMIVRNAKDTAHTKAERNILEEVKHPFIVDLIYAFQTGGKLYLI	148
AtS6K1	---ETSEIYAMKVMRKDHIMEKN-HAEYMKAERDILTKIDHPFIVQLKYSFQTKYRLYLV	210
OsS6K1	---GTSEIYAMKVMRKDKILEKN-HAEYMKAERDILTKVDHPFVQLRYSFQTKYRLYLV	227
TaS6K1	---CTSDIYAMKVMRKDKILEKN-HAEYMKAERDILTKVDHPFVQLRYSFQTKYRLYLV	228
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p70S6K	LEYLSGGELFMQLEREGIFMEDTACFYLAEISMAILGHLHQKGIYRDLKPENIMLNHQGH	208
AtS6K1	LDFINGGHLFFQLYHQGLFREDLARVYTAEIVSAVSHLHEKGIHRDLKPENILMDTDGH	270
OsS6K1	LDFINGGHLFFQLYQQGLFREELARIYTAEIVSAVAHLHANGIMHRDLKPENILLDADGH	287
TaS6K1	LDFVNGGHLFFQLYQQGLFREELARIYTAEIVSAVAHLHANGIMHRDLKPENILLDAHGH	288
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p70S6K	VKLTDFGLCKESIHDGTVTHTFCGTIEYMAPEILMRSGHNRAVDWWSLALMYDMLTGAP	268
AtS6K1	VMLTDFGLAKEF-EENTRSNSMCGTTEYMAPEIVRGKGDKAADWWSVGIILLYEMLTGKP	329
OsS6K1	AMLTDFGLAKEF-DENTRSNSMCGTVEYMAPEIVQGRGHDKAADWWSVGIILFEMLTGKP	346
TaS6K1	AMLTDFGLAKEF-DENTRSNSMCGTVEYMAPEIVQGRGHDKAADWWSVGIILFEMLTGKP	347
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p70S6K	PFTGENRKKTIKILKCKLNLPPYLTQEARDLLKLLKRNAAASRLGAGPGDAGEVQAHPE	328
AtS6K1	PFLGS-KGKIQQKIVKDKIKLPQFLSNEAHAILKGLLQKEPERRLGSGLSGAEEIKQHKW	388
OsS6K1	PFVGGNRDKVQQKIVKEKIKLPAYLSSEVHSLKGLLHKEAGRRLGCGPGGSNEIKNHKW	406
TaS6K1	PFVGGNRDKIQKIVKEKMKLPTYLSSEVHSLKGLLHKEAGRRLGSGPGGSDEIKNHKW	407
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p70S6K	FRHINWEELLARKVEPPFKPLLQSEEDVSQFDSKFTRQTPVDSRDDSTLSESANQVFLGF	388
AtS6K1	FKGINWKKLEAREVMPSFKPEVSGRQCIANFDKWCWTDMSVLDSPASSPSSDPKANPFTNF	448
OsS6K1	FKSVNWKKLDQRQIQPSFRPNVAGKTCIANFDECWTSMPVLDSPVAVPAA--DSNFVGF	464
TaS6K1	FKAVNWKKLEARQITPSFCPNVAGQTCIANFDECWTSMPVLDSPVAVPAA--DSNFVGF	465
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p70S6K	TYVAPSVLESVKEKFSFEPKIRSPRRFIGSPRTPVSPVKFSPGDFWGRGASASTANPQTP	448
AtS6K1	TYVRPPPSFLHQSTTTL*-----	465
OsS6K1	SYVRPAPFLQRPSPLG*-----	480
TaS6K1	SYVRPEPFLQKPSPLG*-----	481
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p70S6K	VEYPMETSGIEQMDVTMSGEASAPLPIRQPNSGPKYKQAFPMISKRPEHLRMNL*	502
AtS6K1	-----	465
OsS6K1	-----	480
TaS6K1	-----	481

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 \pm standard deviation.