Supplementary Figures

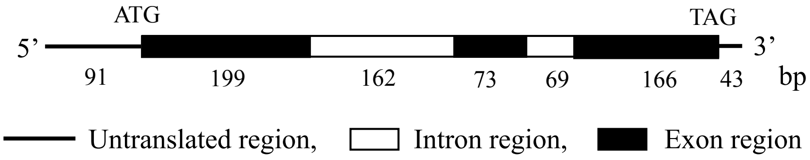
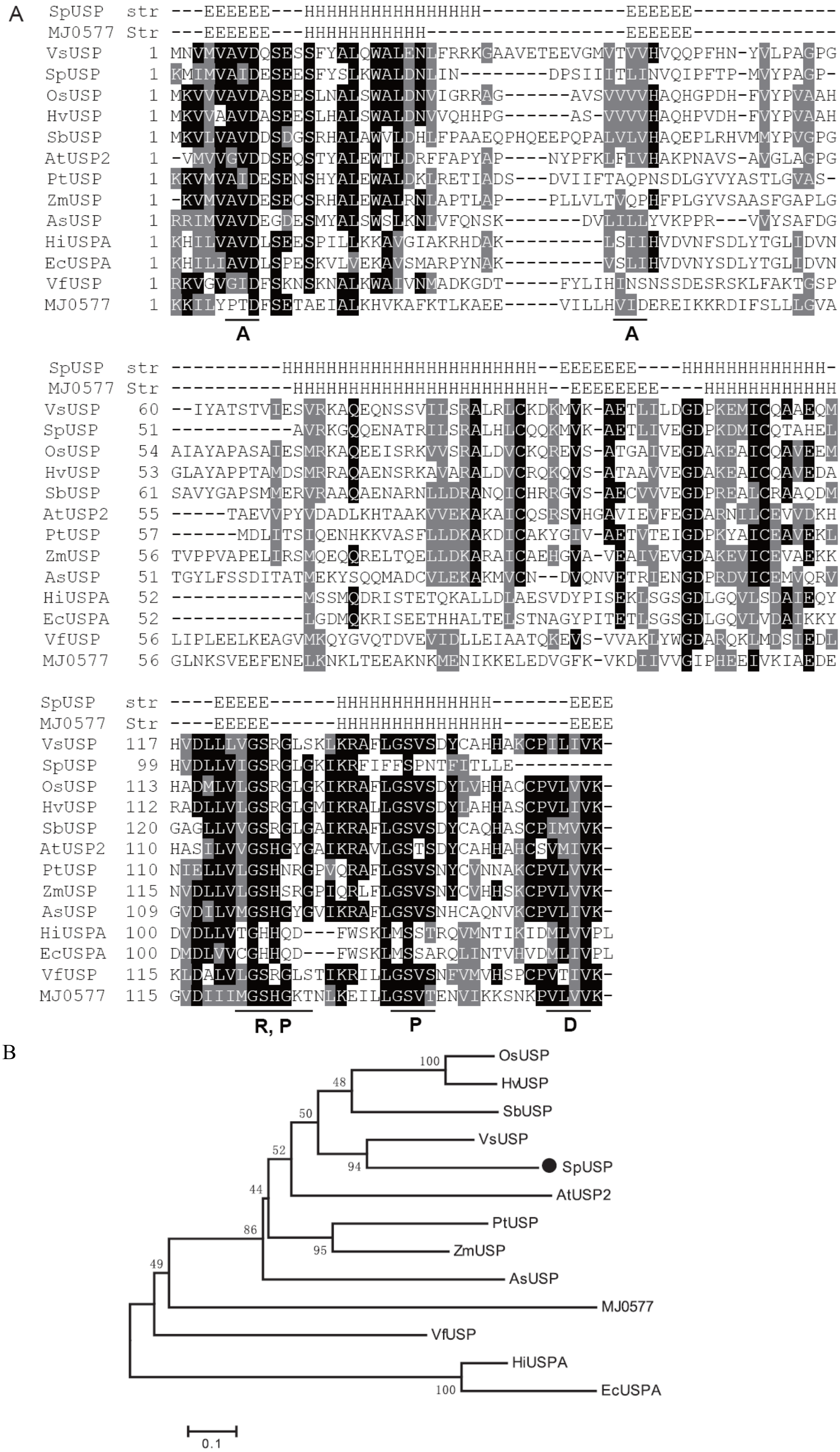


Fig. S1. *S*tructure of the *SpUSP* gene



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A
SpUSP   str ---EEEEEE---HHHHHHHHHHHHHHHH-------------EEEEEE-------------
MJ0577  Str ---EEEEEE---HHHHHHHHHHH------------------EEEEEE-------------
VsUSP     1 MNVMVAVDQSESSFYALQWALENLFRRKGAAVETEEVGMVTVVHVQQPFHN-YVLPAGPG
SpUSP     1 KMIMVAIDESEESFYSLKWALDNLIN--------DPSIIITLINVQIPFTP-MVYPAGP-
OsUSP     1 MKVVVAVDASEESLNALSWALDNVIGRRAG------AVSVVVVHAQHGPDH-FVYPVAAH
HvUSP     1 MKVVAAVDASEESLHALSWALDNVVQHHPG------AS-VVVVHAQHPVDH-FVYPVAAH
SbUSP     1 MKVLVAVDDSDGSRHALAWVLDHLFPAAEQPHQEEPQPALVLVHAQEPLRHVMMYPVGPG
AtUSP2    1 -VMVVGVDDSEQSTYALEWTLDRFFAPYAP----NYPFKLFIVHAKPNAVS-AVGLAGPG
PtUSP     1 KKVMVAIDESENSHYALEWALDKLRETIADS---DVIIFTAQPNSDLGYVYASTLGVAS-
ZmUSP     1 -KVMVAVDESECSRHALEWALRNLAPTLAP----PLLVLTVQPHFPLGYVSAASFGAPLG
AsUSP     1 RRIMVAVDEGDESMYALSWSLKNLVFQNSK-------DVLILLYVKPPR---VVYSAFDG
HiUSPA    1 KHILVAVDLSEESPILLKKAVGIAKRHDAK---------LSIIHVDVNFSDLYTGLIDVN
EcUSPA    1 KHILIAVDLSPESKVLVEKAVSMARPYNAK---------VSLIHVDVNYSDLYTGLIDVN
VfUSP     1 RKVGVGIDFSKNSKNALKWAIVNMADKGDT-----FYLIHINSNSSDESRSKLFAKTGSP
MJ0577    1 KKILYPTDFSETAEIALKHVKAFKTLKAEE-----VILLHVIDEREIKKRDIFSLLLGVA

SpUSP   str ----------HHHHHHHHHHHHHHHHHHHHHHH--EEEEEEE----HHHHHHHHHHHHH-
MJ0577  Str -----------HHHHHHHHHHHHHHHHHHHHHHH--EEEEEEEE----HHHHHHHHHHHH
VsUSP    60 --IYATSTVIESVRKAQEQNSSVILSRALRLCKDKMVK-AETLILDGDPKEMICQAAEQM
SpUSP    51 -----------AVRKGQQENATRILSRALHLCQQKMVK-AETLIVEGDPKDMICQTAHEL
OsUSP    54 AIAYAPASAIESMRKAQEEISRKVVSRALDVCKQREVS-ATGAIVEGDAKEAICQAVEEM
HvUSP    53 GLAYAPPTAMDSMRRAQAENSRKAVARALDVCRQKQVS-ATAAVVEGDAKEAICQAVEDA
SbUSP    61 SAVYGAPSMMERVRAAQAENARNLLDRANQICHRRGVS-AECVVVEGDPREALCRAAQDM
AtUSP2   55 -----TAEVVPYVDADLKHTAAKVVEKAKAICQSRSVHGAVIEVFEGDARNILCEVVDKH
PtUSP    57 ------MDLITSIQENHKKVASFLLDKAKDICAKYGIV-AETVTEIGDPKYAICEAVEKL
ZmUSP    56 TVPPVAPELIRSMQEQQRELTQELLDKARAICAEHGVA-VEAIVEVGDAKEVICEVAEKK
AsUSP    51 TGYLFSSDITATMEKYSQQMADCVLEKAKMVCN--DVQNVETRIENGDPRDVICEMVQRV
HiUSPA   52 -------------MSSMQDRISTETQKALLDLAESVDYPISEKLSGSGDLGQVLSDAIEQY
EcUSPA   52 -------------LGDMQKRISEETHHALTELSTNAGYPITETLSGSGDLGQVLVDAIKKY
VfUSP    56 LIPLEELKEAGVMKQYGVQTDVEVIDLLEIAATQKEVS-VVAKLYWGDARQKLMDSIEDL
MJ0577   56 GLNKSVEEFENELKNKLTEEAKNKMENIKKELEDVGFK-VKDIIVVGIPHEEIVKIAEDE

SpUSP   str ----EEEEE------HHHHHHHHHHHHHH-------EEEE
MJ0577  Str ----EEEEE------HHHHHHHHHHHHHHH------EEEE
VsUSP   117 HVDLLLVGSRGLSKLKRAFLGSVSDYCAHHAKCPILIVK-
SpUSP    99 HVDLLVIGSRGLGKIKRFIFFSPNTFITLLE---------
OsUSP   113 HADMLVLGSRGLGKIKRAFLGSVSDYLVHHACCPVLVVK-
HvUSP   112 RADLLVLGSRGLGMIKRALLGSVSDYLAHHASCPVLIVK-
SbUSP   120 GAGLLVVGSRGLGAIKRAFLGSVSDYCAQHASCPIMVVK-
AtUSP2  110 HASILVVGSHGYGAIKRAVLGSTSDYCAHHAHCSVMIVK-
PtUSP   110 NIELLVLGSHNRGPVQRAFLGSVSNYCVNNAKCPVLVVK-
ZmUSP   115 NVDLLVLGSHSRGPIQRLFLGSVSNYCVHHSKCPVLVVK-
AsUSP   109 GVDILVMGSHGYGVIKRAFLGSVSNHCAQNVKCPVLIVK-
HiUSPA  100 DVDLLVTGHHQD---FWSKLMSSTRQVMNTIKIDMLVVPL
EcUSPA  100 DMDLVVCGHHQD---FWSKLMSSARQLINTVHVDMLIVPL
VfUSP   115 KLDALVLGSRGLSTIKRILLGSVSNFVMVHSPCPVTIVK-
MJ0577  115 GVDIIIMGSHGKTNLKEILLGSVTENVIKKSNKPVLVVK-
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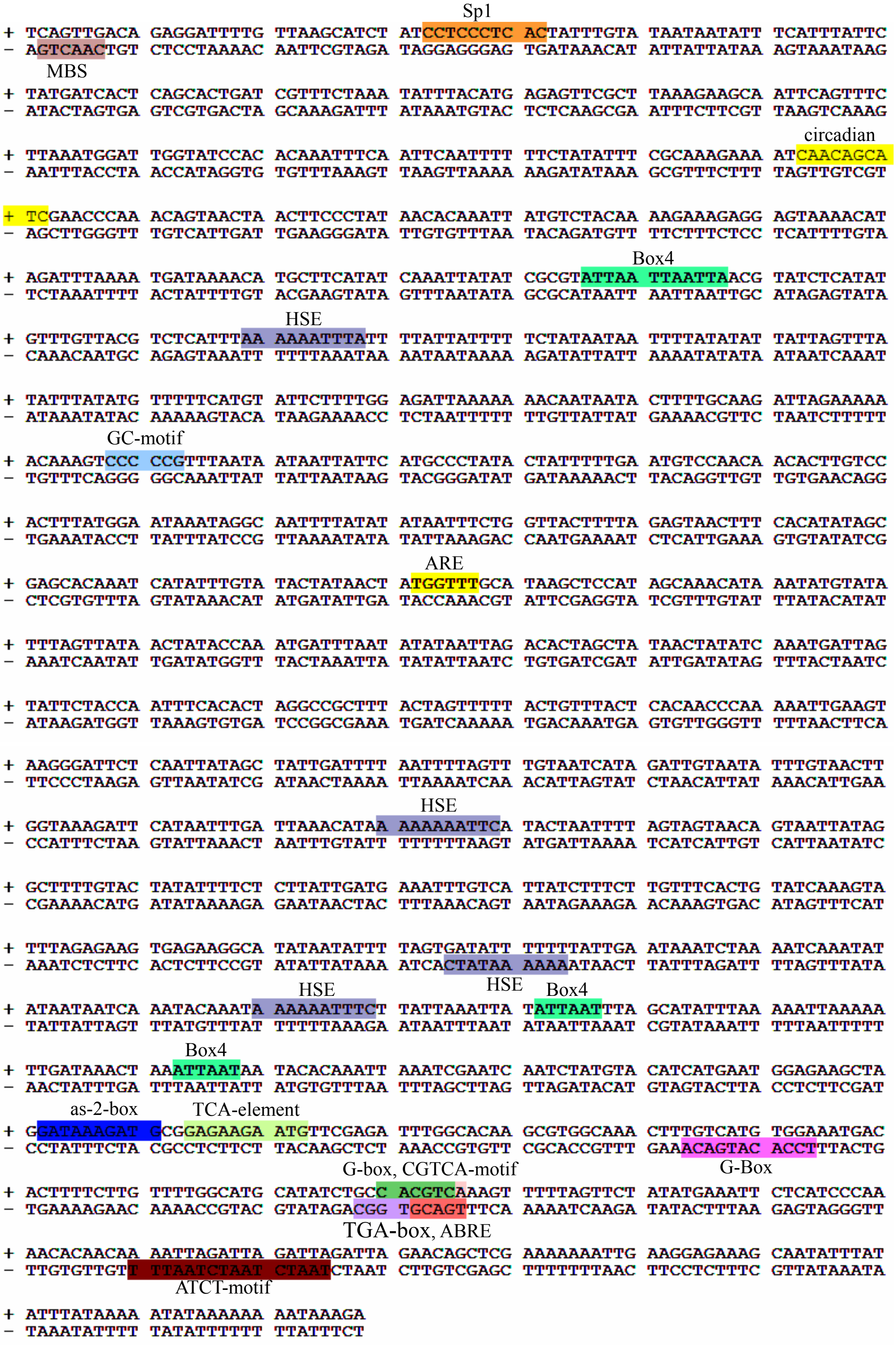
Fig. S2. Multiple sequence alignment and phylogenetic tree analysis of USPs and USP-like proteins from different species. (A) Multiple sequence alignment of USP proteins from tomato (*SpUSP*, SGN-U214690), *Populus trichocarpa* (PtUSP, XP_002312405), grape (VsUSP, XP_002266746), Arabidopsis (AtUSP2, NP_566108), maize (ZmUSP, ACG26386), rice (OsUSP, AAR87267), barley (HvUSP, ADB54816), sorghum (SbUSP, XP_002467191), *Astragalus sinicus* (AsUSP, ABB13620), *Vicia faba* (VfUSP, CAC18556), *Escherichia coli* (EcUspA, U00039), *Haemophilus infuenzae* (HiUspA, YP_248522), and *Methanococcus jannaschii* (MJ0577, NP_247556). Multiple sequence alignments are done using ClustalW, and then treated with BoxShade (http://www.ch.embnet.org/software/BOX_form.html). Amino acids with identities over 75% are shaded in black, whereas those with identities between 50% and 75% are shaded in gray. The black bars below the sequences indicate either residues facing adenine of ATP (A) phosphate of ATP (P) or ribose of ATP (R) or located in the dimerization domain (D) as derived from the crystal structure of *M. jannaschii* MJ0577. The secondary structure elements of *SpUSP* predicted by the Jpred program and the secondary structure of the MJ0577 protein based on its crystal structure are shown above the alignment [E, extended conformation (β-strand); H, α-helix]. Gaps in the alignment and secondary structure are indicated by dashes. The amino acids are numbered on the right. (B) Phylogenetic tree analysis of USP members from different species. The accession numbers are the same as above.



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+ TATGATCACT CAGCACTGAT CGTTTCTAAA TATTTACATG AGAGTTCGCT TAAAGAAGCA ATTCAGTTTC
− ATACTAGTGA GTCGTGACTA GCAAAGATTT ATAAATGTAC TCTCAAGCGA ATTTCTTCGT TAAGTCAAAG

+ TTAAATGGAT TGGTATCCAC ACAAATTTCA ATTCAATTTT TTCTATATTT CGCAAAGAAA ATCAACAGCA
− AATTTACCTA ACCATAGGTG TGTTTAAAGT TAAGTTAAAA AAGATATAAA GCGTTTCTTT TAGTTGTCGT

+ TCGAACCCAA ACAGTAACTA ACTTCCCTAT AACACAAATT ATGTCTACAA AAGAAAGAGG AGTAAAACAT
− AGCTTGGGTT TGTCATTGAT TGAAGGGATA TTGTGTTTAA TACAGATGTT TTCTTTCTCC TCATTTTGTA

+ AGATTTAAAA TGATAAAACA TGCTTCATAT CAAATTATAT CGCGTATTAA TTAATTAACG TATCTCATAT
− TCTAAATTTT ACTATTTTGT ACGAAGTATA GTTTAATATA GCGCATAATT AATTAATTGC ATAGAGTATA

+ GTTTGTTACG TCTCATTTAA AAAATTTATT TTATTATTTT TCTATAATAA TTTTATATAT TATTAGTTTA
− CAAACAATGC AGAGTAAATT TTTTAAATAA AATAATAAAA AGATATTATT AAAATATATA ATAATCAAAT

+ TATTTATATG TTTTTCATGT ATTCTTTTGG AGATTAAAAA AACAATAATA CTTTTGCAAG ATTAGAAAAA
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+ ACAAAGTCCC CCGTTTAATA ATAATTATTC ATGCCCTATA CTATTTTTGA ATGTCCAACA ACACTTGTCC
− TGTTTCAGGG GGCAAATTAT TATTAATAAG TACGGGATAT GATAAAAACT TACAGGTTGT TGTGAACAGG

+ ACTTTATGGA ATAAATAGGC AATTTTATAT ATAATTTCTG GTTACTTTTA GAGTAACTTT CACATATAGC
− TGAAATACCT TATTTATCCG TTAAAATATA TATTAAAGAC CAATGAAAAT CTCATTGAAA GTGTATATCG

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+ AAGGGATTCT CAATTATAGC TATTGATTTT AATTTTAGTT TGTAATCATA GATTGTAATA TTTGTAACTT
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+ GCTTTTGTAC TATATTTTCT CTTATTGATG AAATTTGTCA TTATCTTTCT TGTTTCACTG TATCAAAGTA
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+ TTGATAAACT AAATTAATAA TACACAAATT AAATCGAATC AATCTATGTA CATCATGAAT GGAGAAGCTA
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+ GGATAAAGAT GCGGAGAAGA ATGTTCGAGA TTTGGCACAA GCGTGGCAAA CTTTGTCATG TGGAAATGAC
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+ ACTTTTCTTG TTTTGGCATG CATATCTGCC ACGTCAAAGT TTTTAGTTCT ATATGAAATT CTCATCCCAA
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+ AACACAACAA AATTAGATTA GATTAGATTA GAACAGCTCG AAAAAAATTG AAGGAGAAAG CAATATTTAT
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+ ATTTATAAAA ATATAAAAAA AATAAAGA
− TAAATATTTT TATATTTTTT TTATTTCT
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Fig. S3. *Cis*-acting element analysis of *SpUSP* promoter. ABRE (ACGTGGC), *cis*-acting element involved in abscisic acid responsiveness. ATCT-motif (AATCTAATCT), part of a conserved DNA module involved in light responsiveness.

Box 4 (ATTAAT), part of a conserved DNA module involved in light responsiveness. The CGTCA-motif (CGTCA), *cis*-acting regulatory element involved in MeJA-responsiveness. G-box (TCCACATGGCA), *cis*-acting regulatory element involved in light responsiveness. G-box (TGACGTGG/CACGTC), *cis*-acting regulatory element involved in light responsiveness. HSE (AAAAAATTTC), *cis*-acting element involved in heat stress responsiveness. Sp1 [CC(G/A)CCC], light-responsive element. TCA-element (GAGAAGAATA), *cis*-acting element involved in salicylic acid responsiveness. TGA-box (TGACGTGGC), part of an auxin-responsive element. TGACG-motif (TGACG), *cis*-acting regulatory element involved in MeJA-responsiveness. Circadian (CAANNNNATC), *cis*-acting regulatory element involved in circadian control. GARE-motif (TCTGTTG), gibberellin-responsive element. MBS (CAACTG), MYB binding site involved in drought inducibility. ARE, cis-acting regulatory element essential for the anaerobic induction. GC-motif, enhancer-like element involved in anoxic specific inducibility.

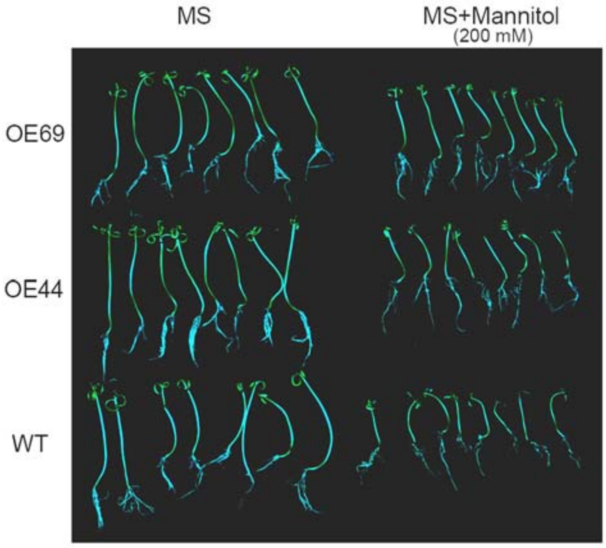

Fig. S4. *SpUSP* Overexpression improves the growth performance of seedlings under osmotic stress. Growth performance of two *SpUSP*-overexpressing lines (OE44 and OE69) in half-strength MS media (left) or supplemented with 200 mM mannitol (right), with wild-type ZS6 as a control.

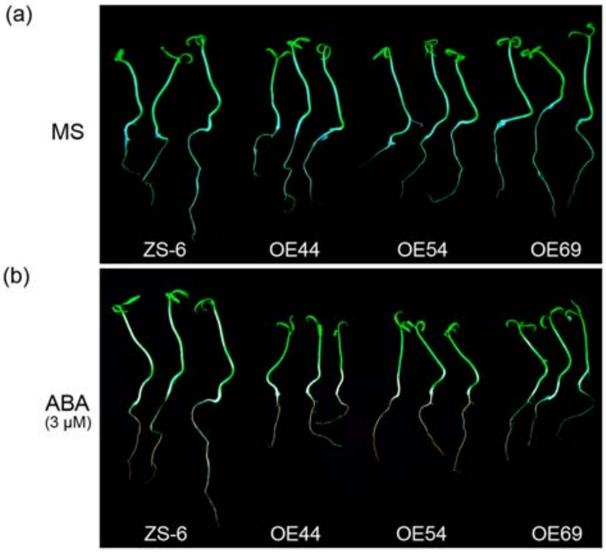

Fig. S5. *SpUSP* Overexpression affects the growth performance of seedlings under ABA treatment. Growth performance of three *SpUSP*-overexpressing (OE44, OE54, and OE69) lines in half-strength MS media (a) or supplemented with 3 μM ABA (b) with wild-type (ZS6) as a control.

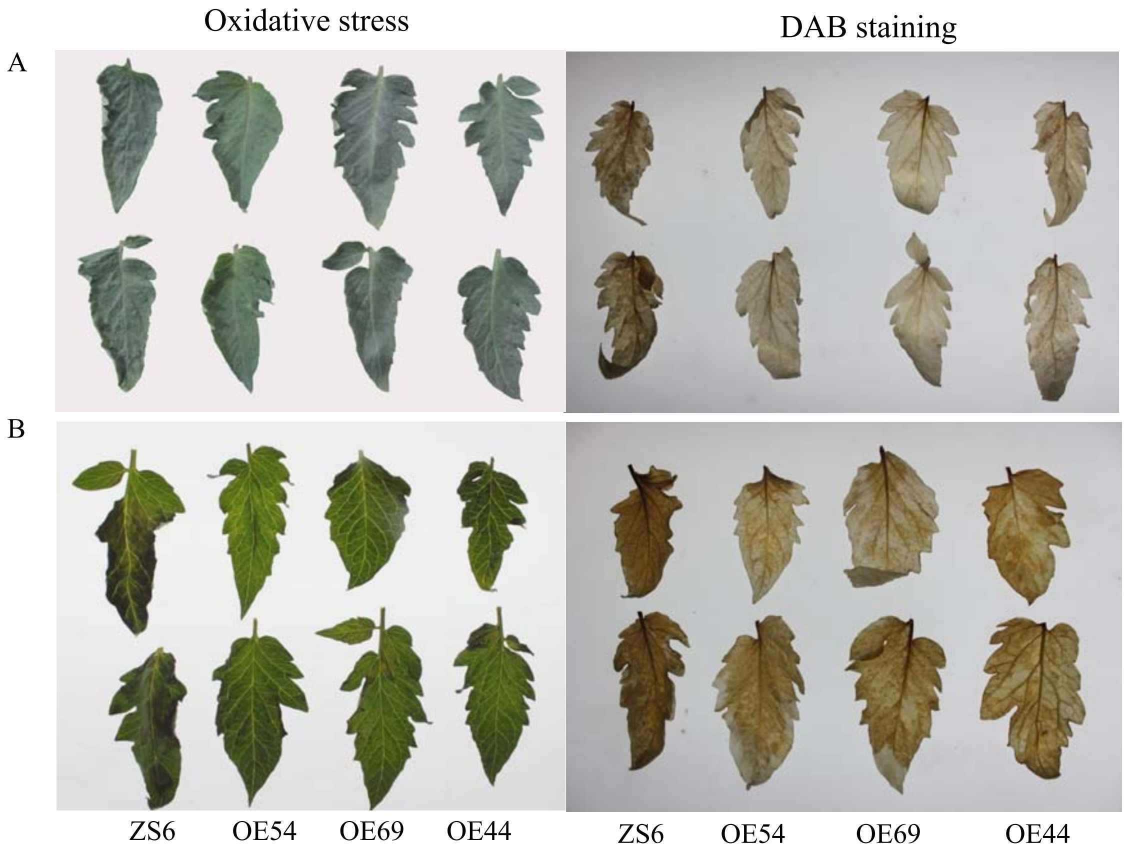

Fig. S6. Oxidative stress assay on the leaves of transgenic and wild-type plants. (A) The leaves of *SpUSP*-overexpressing and wild type (ZS6) plants were detached and exposed to drought stress for 5 h (left), and the phenotype of DAB staining (right). (B) The leaves of the transgenic (OE54, OE44, and OE69) and wild-type (ZS6) plants (left) were sprayed with 100 μM paraquat for 24 h, and the phenotype of DAB staining (right).