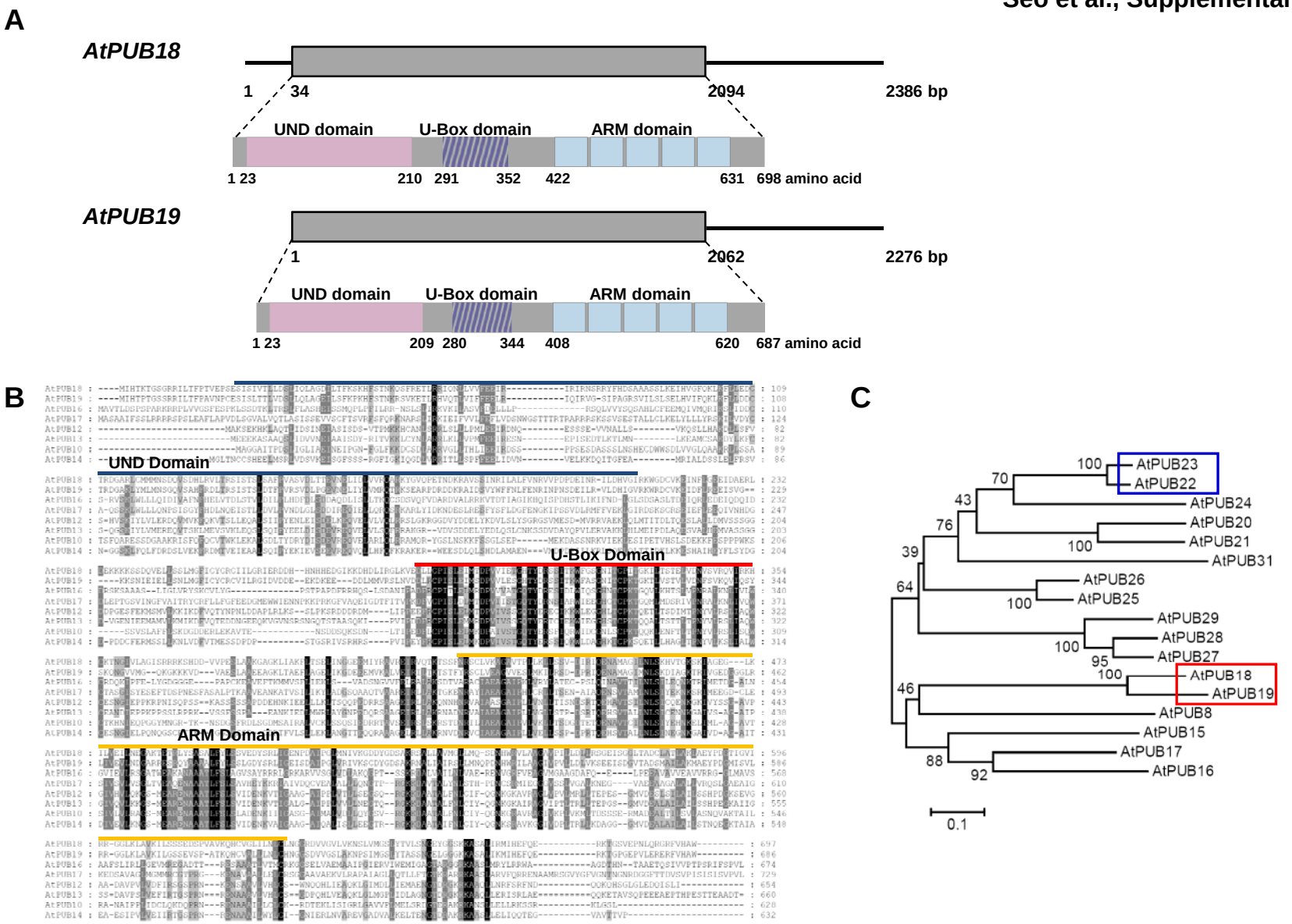
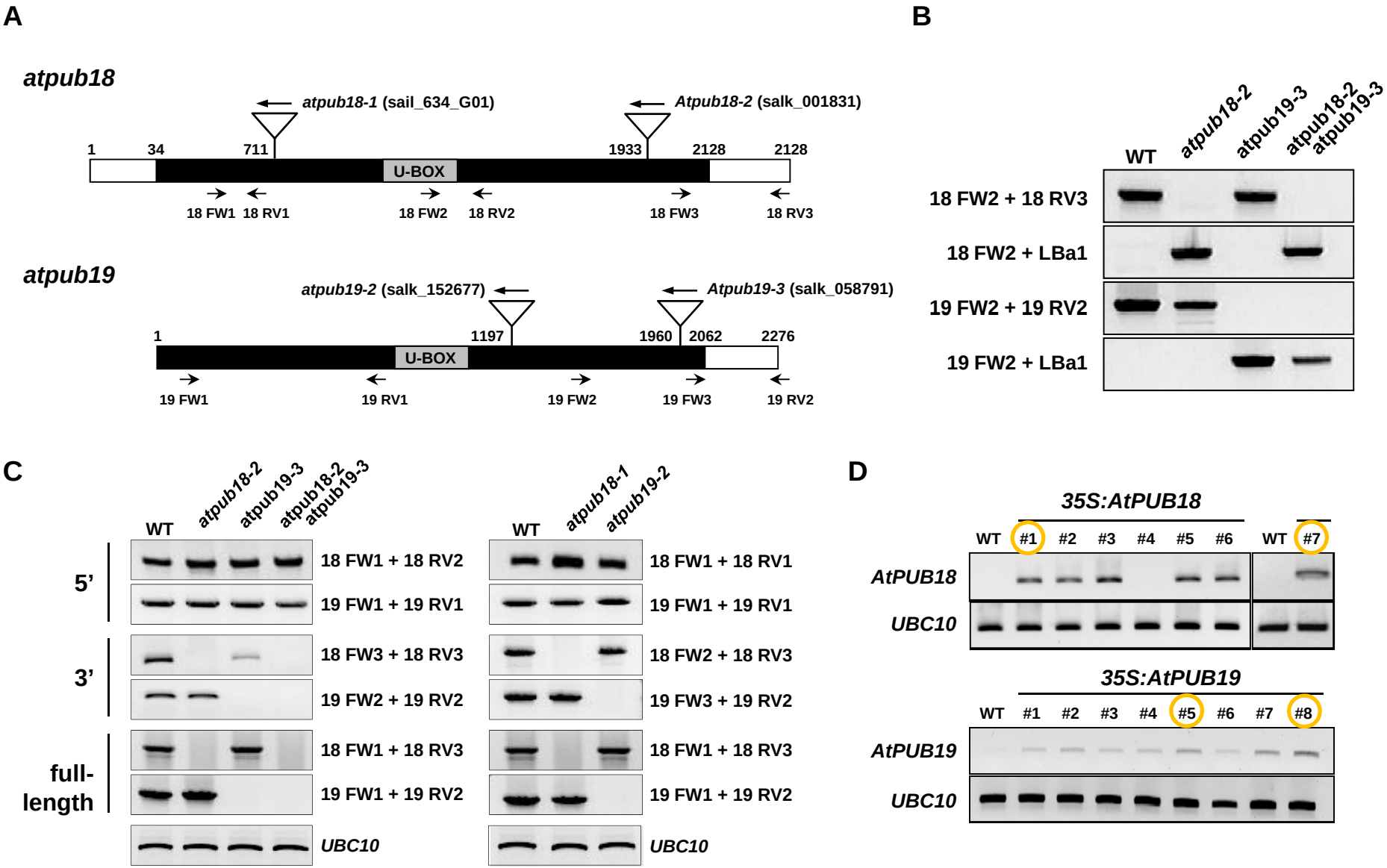
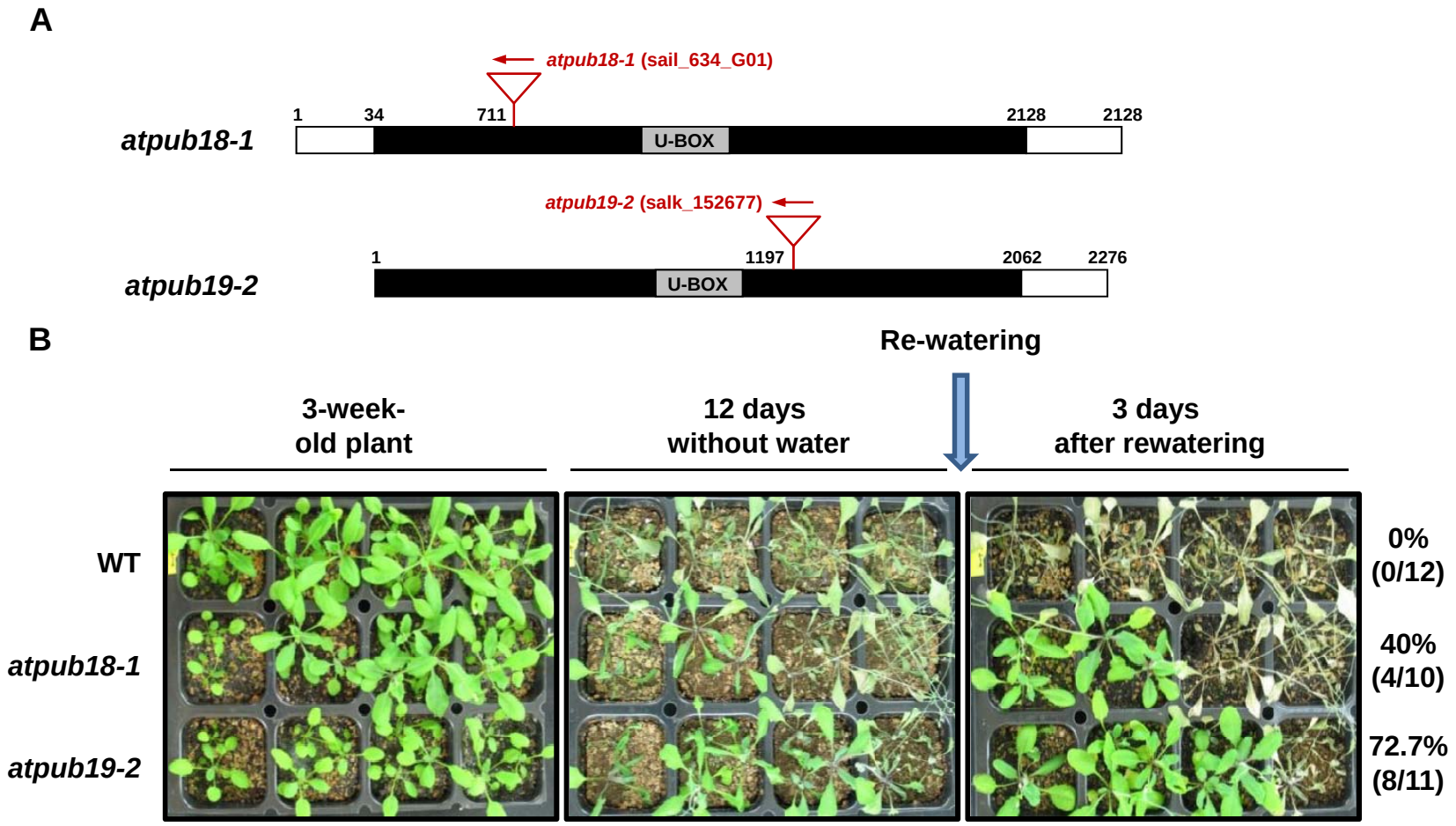


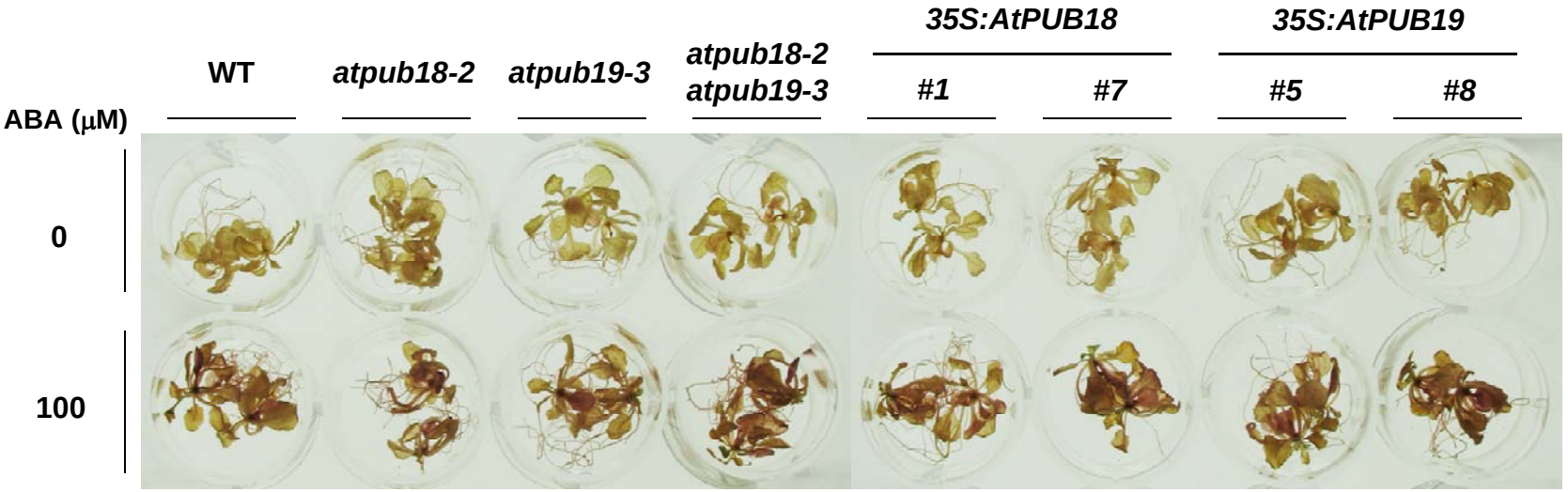
Supplemental Figure S1. Sequence analysis of AtPUB18 and AtPUB19. A, Schematic structures of AtPUB18 and AtPUB19. B, Multiple alignment of amino acid sequences of Arabidopsis U-box E3 Ub ligases. C, Phylogenetic analysis of Arabidopsis U-box E3 Ub ligases.


A
atpub18
atpub18-1 (sail_634_G01)
Atpub18-2 (salk_001831)
1
34
711
1933
2128
2128
U-BOX
18 FW1
18 RV1
18 FW2
18 RV2
18 FW3
18 RV3
atpub19
atpub19-2 (salk_152677)
Atpub19-3 (salk_058791)
1
1197
1960
2062
2276
U-BOX
19 FW1
19 RV1
19 FW2
19 FW3
19 RV2
B
WT
atpub18-2
atpub19-3
atpub18-2 atpub19-3
18 FW2 + 18 RV3
18 FW2 + LBa1
19 FW2 + 19 RV2
19 FW2 + LBa1
C
WT
atpub18-2
atpub19-3
atpub18-2 atpub19-3
WT
atpub18-1
atpub19-2
5'
3'
full-length
18 FW1 + 18 RV2
19 FW1 + 19 RV1
18 FW3 + 18 RV3
19 FW2 + 19 RV2
18 FW1 + 18 RV3
19 FW1 + 19 RV2
UBC10
18 FW1 + 18 RV1
19 FW1 + 19 RV1
18 FW2 + 18 RV3
19 FW3 + 19 RV2
18 FW1 + 18 RV3
19 FW1 + 19 RV2
UBC10
D
35S:AtPUB18
WT
#1
#2
#3
#4
#5
#6
WT
#7
AtPUB18
UBC10
35S:AtPUB19
WT
#1
#2
#3
#4
#5
#6
#7
#8
AtPUB19
UBC10

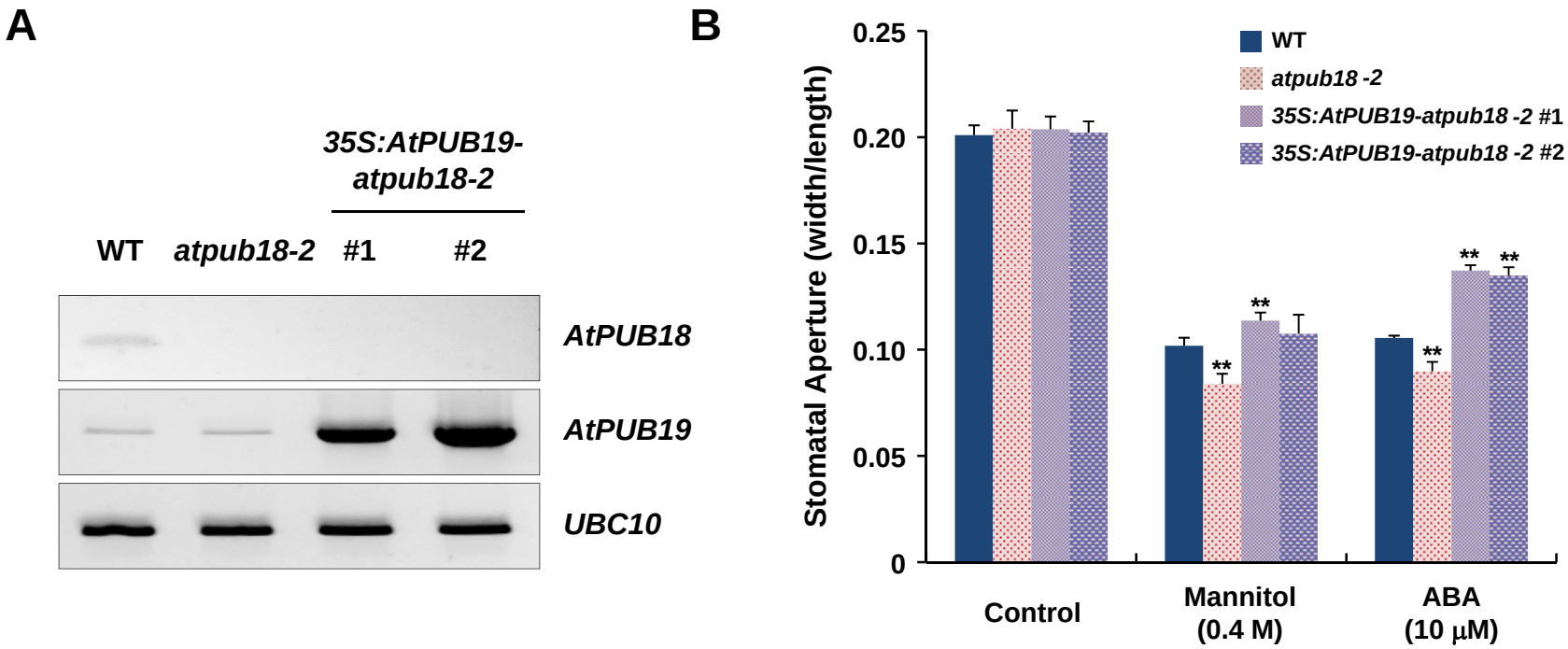
Supplemental Figure S2. Characterization of *atpub18-2*, *atpub19-3*, and *atpub18-2atpub19-3* knock-out mutant and *35S:AtPUB18* and *35S:AtPUB19* over-expressing transgenic plants. A, Schematic representations of *atpub18* and *atpub19* alleles. Inverted triangles and arrows indicate T-DNA insertion sites and gene-specific primers, respectively. DNA sequences of primers are listed in Table S1. B, Genotyping PCR of *atpub18-2*, *atpub19-3*, and *atpub18-2atpub19-3* mutant plants. C, Semi-quantitative RT-PCR analysis to detect the presence of partial or full-length transcripts in *atpub18-1*, *atpub18-2*, *atpub19-2*, and *atpub19-3* mutant alleles and *atpub18-2atpub19-3* double mutant plants. *UBC10* was used as a loading control. D, Semi-quantitative RT-PCR analysis for the selection of *35S:AtPUB18* (lines #1 and #7) and *35S:AtPUB19* (lines #5 and #8) transgenic plants. *UBC10* was used as a loading control.



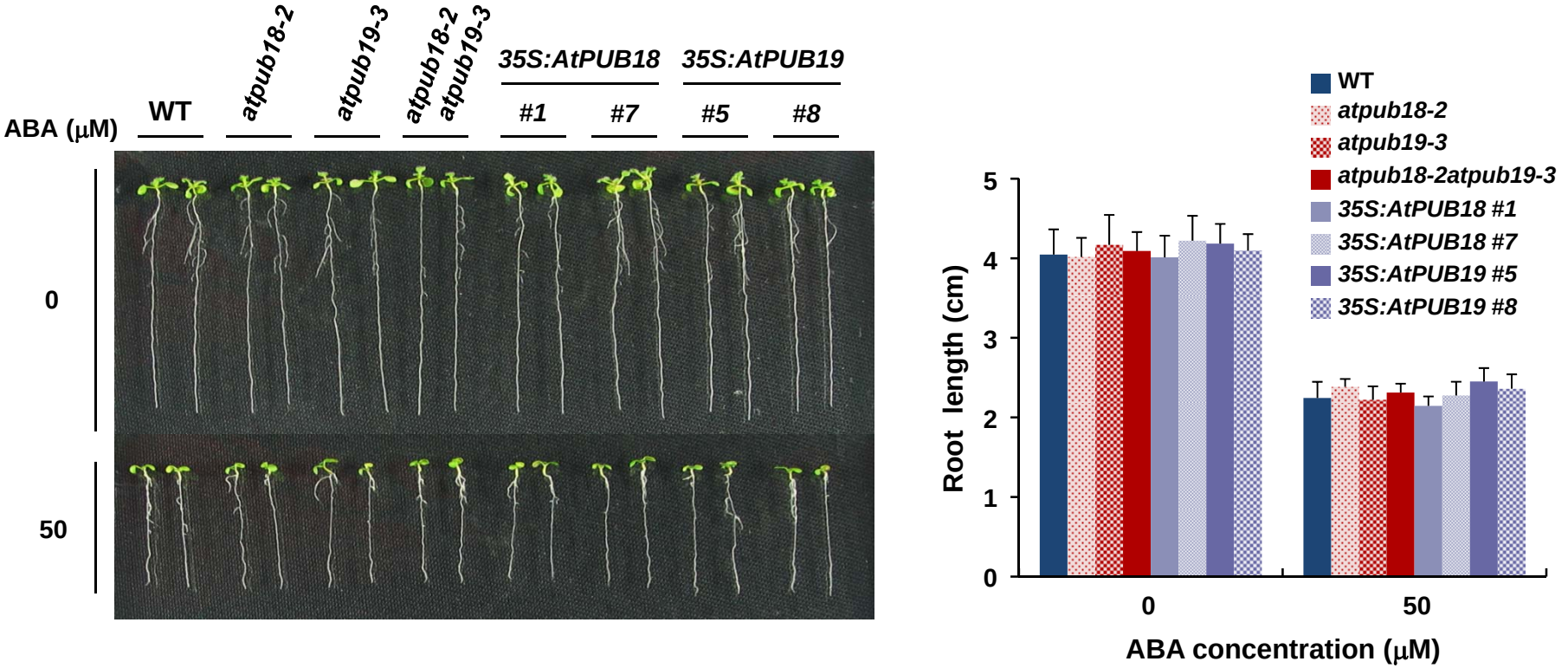
Supplemental Figure S3. Drought-tolerant phenotypes of *atpub18-1* and *atpub19-2* second allele mutant lines. A, Schematic representation of *atpub18-1* and *atpub19-2* alleles. B, Drought phenotypes of wild-type, *atpub18-1*, and *atpub19-2* knock-out mutant plants. Wild-type and mutant plants were grown for 3 weeks under normal growth conditions and exposed to drought stress without water supply for 12 days. Survival rates were recorded 3 days after irrigation.



Supplemental Figure S4. ROS accumulation in wild-type, *atpub18-2*, *atpub19-3*, and *atpub18-2atpub19-3* knock-out mutant, and *35S:AtPUB18* and *35S:AtPUB19* over-expressing transgenic plants in response to ABA. Light-grown, 2-week-old seedlings were treated with or without 100 μM ABA for 2 h and transferred to solution containing 100 ug/ml of 3,3'-diaminobenzidine (DAB). Chlorophylls of DAB-stained seedlings were removed by boiling in 95% (v/v) ethanol. ROS levels were visualized as a dark brown color.



Supplemental Figure S5. Characterization of *35S:AtPUB19-atpub18-2* complementation transgenic plants and their stomatal movements in response to mannitol and ABA treatments. A, Semi-quantitative RT-PCR analysis of wild-type, *atpub18-2*, and *35S:AtPUB19-atpub18-2* transgenic plants. *UBC10* was a loading control. B, Mannitol- and ABA-induced stomatal closure in T_2 *35S:AtPUB19-atpub18-2* transgenic plants. Three replicates were performed for each experiment. Error bars represent ±SD (n = 90, ** $P < 0.005$, Student's *t*-test).



Supplemental Figure S6. ABA-induced root growth inhibition of wild-type, *atpub18-2*, *atpub19-3*, and *atpub18-2atpub19-3* knock-out mutant, and *35S:AtPUB18* and *35S:AtPUB19* over-expressing transgenic plants. Seedlings were grown on vertical MS agar plate for 5 days and transferred to MS agar medium containing 50 μM of ABA. Roots of seedlings were photographed 4 days after transfer and analyzed with Multi Gauge v3.1 software (Fuji Film, Tokyo, Japan).