Overexpression of the aspartic protease *ASPG1* gene confers the drought avoidance in *Arabidopsis*. Xuan Yao, Wei Xiong, Tiantian Ye, and Yan Wu

SUPPLEMENTARY DATA

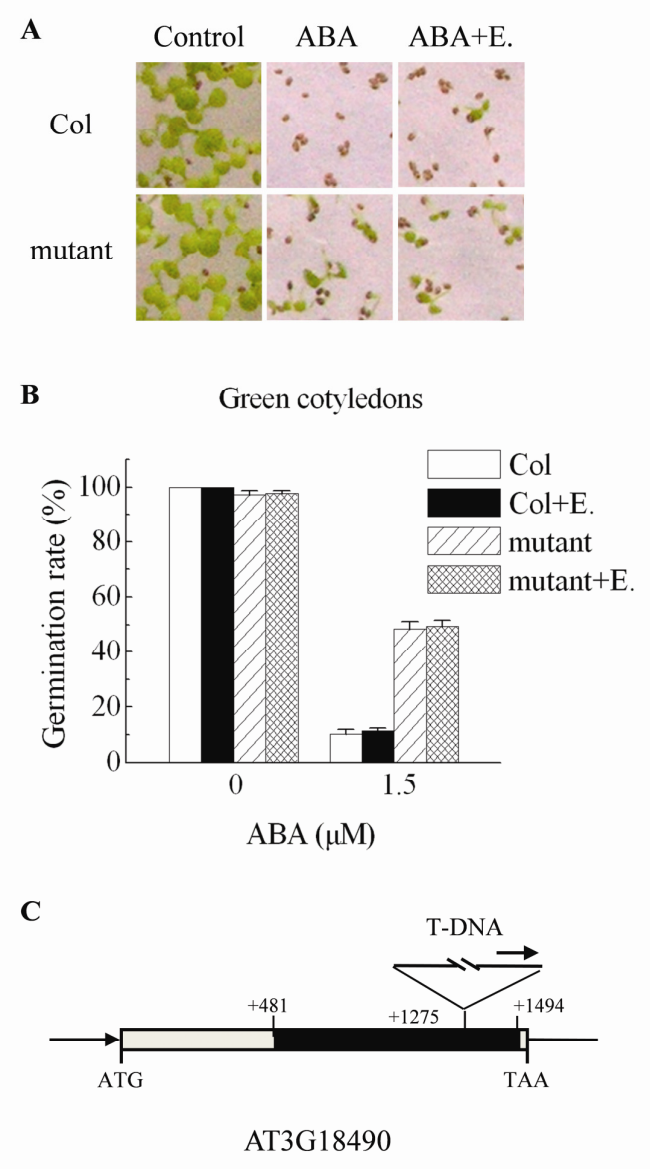

Fig. S1. The original screening with the XVE-tagging T-DNA insertion mutant lines. (A) Seeds of XVE T-DNA-tagging lines were screened on the MS plate containing 1% sucrose, 0.8% (w/v) agar, with or without 10 μM 17-β-estradiol (E.) and 1.5 μM ABA. Photographs were taken to show the germination phenotypes at the day 7 after stratification. (B) Germination rates (%) were analyzed at the day 7 after stratification by scoring the open green cotyledons. Values are means ±SE from three independent experiments (n=100). (C) Schematic drawing (not in scale) to show T-DNA insertion site in gene AT3G18490 revealed in the original mutant screen. Putative aspartic protease domain: +481 bp to +1494 bp (black box). Arrows denote the orientation of gene transcription.

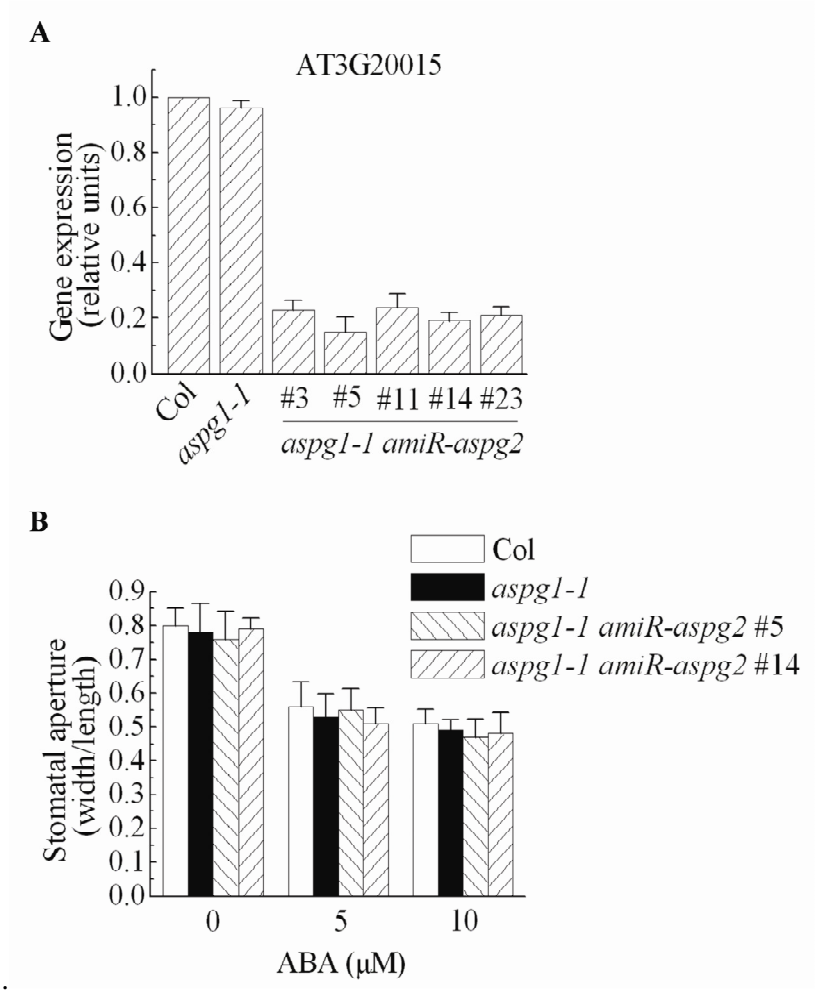

Fig. S2. The response to ABA of the artificial microRNA (amiRNA) lines.
(A) Analyses of gene expression of *ASPG2* (AT3G20015) in Col, *aspg1-1* and *aspg1-1 amiR-aspg2* lines (#3, #5, #11, #14 and #23). The expression levels were analyzed as the relative unit against with the level of *ASPG2* in Col plants, which was taken as "1". Each value is the mean ±SE of three independent experiments. (B) ABA-induced stomatal closure. Values are means ±SE from three independent experiments (n=50). The leaves from 4-week-old plants of Col, *aspg1-1* and *aspg1-1 amiR-aspg2* lines (#5 and #14) were first incubated in the light for 3 hours to induce stomatal opening and then treated with ABA (0, 5, and 10 μM ABA) for 3 hours.

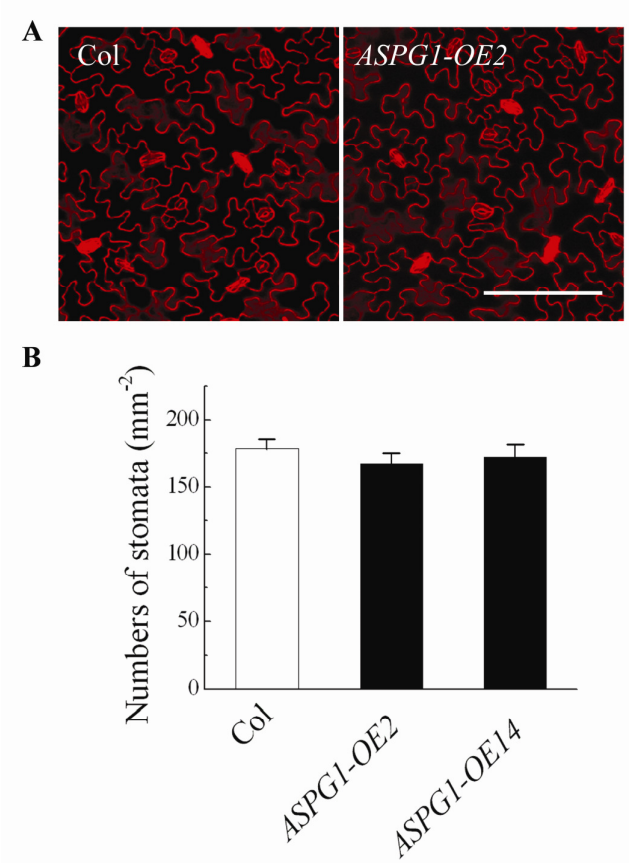

Fig. S3. Overexpression of *ASPG1* has no effect in the development of guard cells. (A) The epidermis of the abaxial surface of rosette leaves from Col and *ASPG1-OE2* plants (Bar = 80 μm). (B) Numbers of stomata per square millimeter in the epidermis of the abaxial surface of rosette leaves of Col, *ASPG1-OE2*, and *ASPG1-OE14* lines were determined. Values are the means ± SE from leaves of three individual plants of Col and *ASPG1-OE* lines. Three independent counts were performed on each leaf.

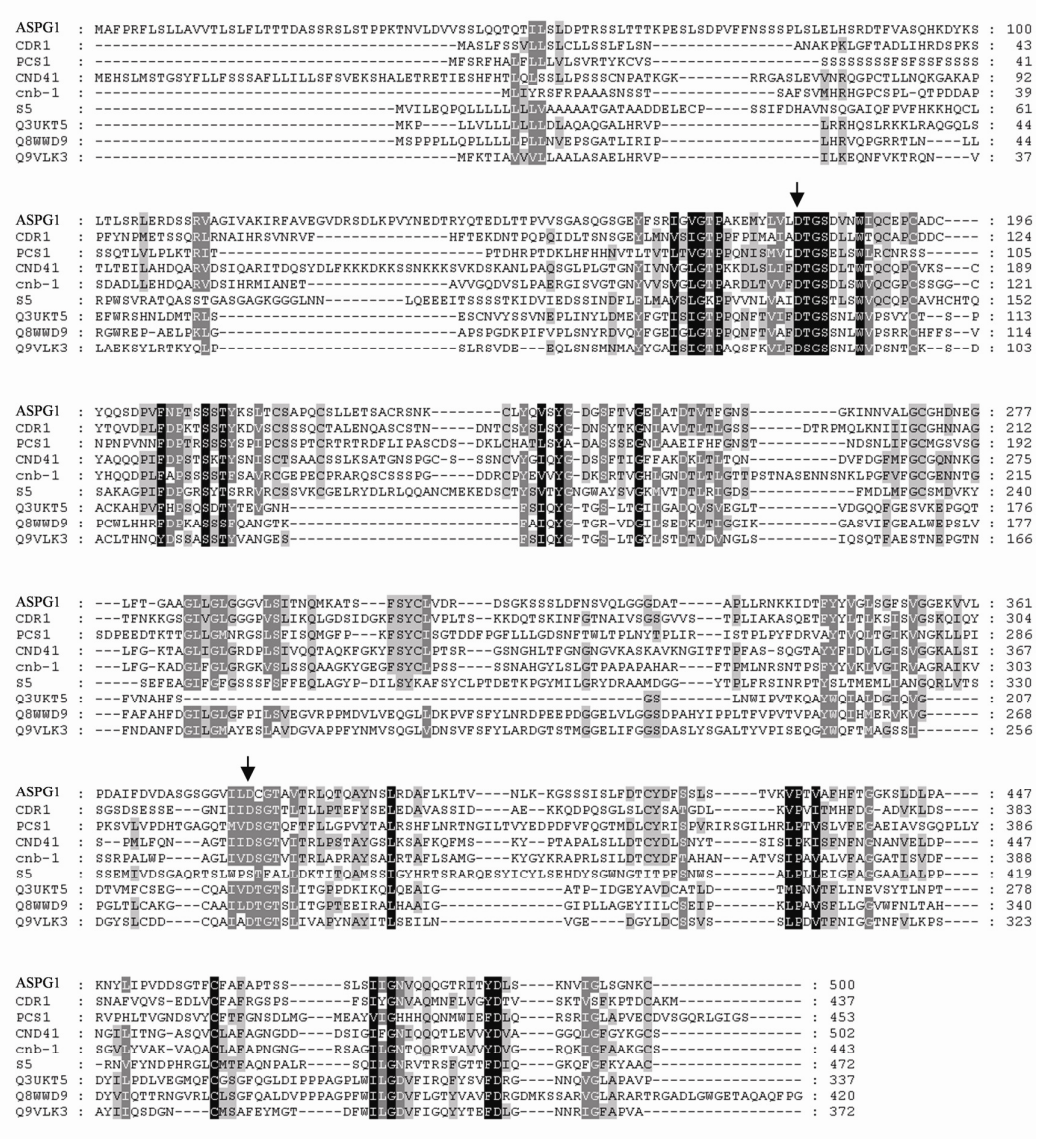

Fig. S4. Two conserved putative aspartic activation sites in ASPG1 protein.
The alignments between predicted ASPG1 protein sequences containing 2 aspartic proteases in a number of organisms by using Clustalx and GeneDoc3.2 tools. CDR1 and PCS1 are from *Arabidopsis thaliana*. CND41 is from *Nicotiana tobacum*. CNB-1 is from *Brachypodium sylvaticum.* S5 is from *Oryza sativa*. Q3UKT5 is from *Mus musculus*. Q9VLK3 is from *Drosophila melanogaster*. Q8WWD9 is from *Homo sapiens*. Arrows indicate the two catalytic aspartic acid residues in ASPG1 protein.

Table S1 Primer sequences were used for plasmids constructions in this study.	
ProASPG1-GUS	F: 5'-TTTCCTGCAGGTTGTGGATGTTAGAGACG-3'
	R: 5'-TCCCCCGGGTATTTCCGGCGAAGGT-3'
p35S-CFP-ASPG1	F: 5'-GGGGTACCATGGCTTTCCCGCGATTT-3'
	R: 5'-TCCCCGCGGTTAGCATTTATTCCCT-3'
pET-30c-ASPG1	F: 5'-CGGGATATCTAATGGCTTTCCCGCG-3'
	R:5'-ATAAGAATGCGGCCGCTTAGCATTTATTCCCTGAC-3'
pET-30c-ASPG1$_{D180N}$	F: 5'-CTTGGTTCTCAACACCGGAAGCGACGTAAATTG-3'
	R: 5'-CGCTTCCGGTGTTGAGAACCAAGTACATCTCTT-3'
pET-30c-ASPG1$_{D379N}$	F: 5'-AGTGATCTTGAATTGTGGAACCGCCGTGACTCG-3'
	R: 5'-CGGTTCCACAATTCAAGATCACTCCTCCGCTTC-3'
pET-30c-ASPG1$_{D180N/D379N}$	See pET-30c-ASPG1$_{D180N}$ and pET-30c-ASPG1$_{D379N}$
p35S-ASPG1	F: 5'-GGGGTACCATGGCTTTCCCGCGATTT-3'
	R: 5'-TCCCCGCGGTTAGCATTTATTCCCT-3'
p35S- ASPG1$_{D180N}$ p35S- ASPG1$_{D379N}$ p35S- ASPG1$_{D180N/D379N}$	See p35S-ASPG1

Table S2 Primer Sequences were used for semi-quantitative and real-time RT-PCR experiments in this study.	
ACTIN2	F: 5'-ATGGCAGACGGTGAGGATATTCA-3'
	R: 5'-GCCTTTGCAATCCACATCTGTTG-3'
ASPG1	F: 5'-ATGGCTTTCCCGCGATTTCTT-3'
	R: 5'-GCATTTATTCCCTGACAATCCGAT-3'
KAT1	F: 5'-TTAGCAGCTGTGTAATTGTTCAC-3'
	R: 5'-ACATGTTCTCACTGATGGATGATG-3'
CBP	F: 5'-CTTCAAACTTAAAGACGGAGGC-3'
	R: 5'-TGCTCTTGCTCGCTGACC-3'
β-ACTIN8	F: 5'-AGTGGTCGTACAACCGGTATTGT-3'
	R: 5'-GAGGATAGCATGTGGAAGTGAGAA-3'
ASPG1	F: 5'-TCGACGTCGTTTCATCTCTC-3'
	R: 5'-GTTTGGTGGTGGTGAGTGAG-3'
DREB2A	F: 5'-AACCTGTCAGCAACAACAGC-3'
	R: 5'-AAACACATCGTCGCCATTTA-3'
DREB2B	F: 5'-AAGCTGCTTCCGCTTATGAT-3'
	R: 5'-AGGGAAGTTAAGACGAGCCA-3'
ABF2	F:5'-TTACAGGCAAGGATCATGGA-3'
	R:5'-CACGGAAACAAACAACCAAG-3'
MYB2	F:5'-ATGGACCGAGGAAGAAGATG-3'
	R:5'-TTGATGATACCAGAGGAACGA-3'
MYC2	F:5'-GGTGAGAACGACCCGTCTAT-3'
	R:5'-CGTTACCCGGTTCGTTAGAT-3'
KIN1	F: 5'-GCAATGTTCTGCTGGACAAG-3'
	R: 5'-TACACTCTTTCCCGCCTGTT-3'
KIN2	F: 5'- CAGAGACCAACAAGAATGCC-3'
	R: 5'-GAAAGAGTACCTCAGCTTTGCC-3'

Table S2 Primer Sequences were used for semi-quantitative and real-time RT-PCR experiments in this study (continued).	
RAB18	F: 5'-AGCTCTAGCTCGGAGGATGA-3'
	R: 5'-CATGATGACCTGGCAACTTC-3'
RD20	F: 5'-TATGGCAGGCTTTCAAACAA-3'
	R: 5'-GAGAATTGGCCCTCTCTTTG-3'
RD22	F: 5'-TTATTGAAGGTAGTGGCGATTG-3'
	R: 5'-ATGGAGAGAGTTGGGAATGG-3'
RD20	F: 5'-TATGGCAGGCTTTCAAACAA-3'
	R: 5'-GAGAATTGGCCCTCTCTTTG-3'
RD26	F:5'-TGTTACAGTTGGATGATTGGG-3'
	R:5'-AGAAGACGATGACGACCCAT-3'
RD29A	F: 5'-AGGAACCACCACTCAACACA-3'
	R: 5'- GCTCATGCTCATTGCTTTGT-3'
RD29B	F: 5'- ACGAGCAAGACCCAGAAGTT-3'
	R: 5'- AGGAACAATCTCCTCCGATG-3'
ABI1	F: 5'- AGATGGTCGGTTTGATCCTC-3'
	R: 5'- AGTTCGCTACCTGAGAACCG-3'
ABI2	F: 5'-TCAAGATCCATTGGCGATAG-3'
	R: 5'-CAAATCGCACACTTCTTCGT-3'
OST1	F: 5'- TGGAATATGCATCTGGAGGA-3'
	R: 5'- AGAACAACCTCGTCTTCGCT-3'
RbohD	F: 5'- AACAACAGGTGGCTGTTTACC-3'
	R: 5'-TGTGATTGAGAAAGGATGCC-3'
RbohF	F: 5'-TTCAGTATCCGTGGGCAATA-3'
	R: 5'-CACTCCTGCGAAAGATCAAA-3'
NCED3	F: 5'-TTGATGCTCCAGATTGCTTC-3'
	R: 5'-GGACCCTATCACGACGACTT-3'
ABA3	F: 5'-AAGTCCATGGATCCACACAA-3'
	R: 5'-TTATCATCTGGCACCGGTTA-3'