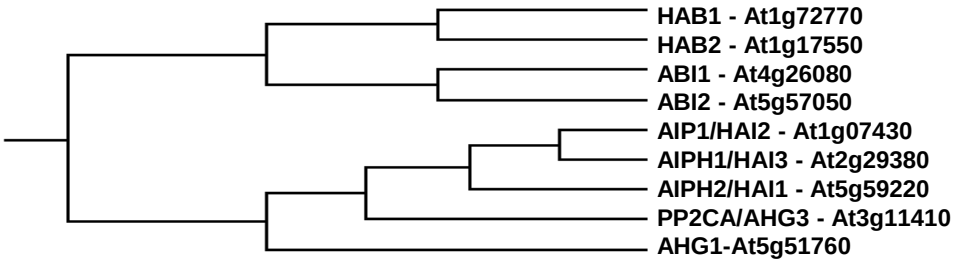
A



B

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PP2CA     :  MAGICCG-----------VVGETEPAAP--VDSTSRASLRRRLDLLPSIKIVADSAVAPPLENCRKRQKRETVVL  :  62
At5g59220 :  MAEICYENETMMIETTATVVKKATTTTRRRERSSSQAARRRRMEIRRFKFVSGEQEPVFVDGDLQRRRRRESTVA  :  75

PP2CA     :  STLPGNLDLDSNVRSENKKARSAVTNS-NSVTEAESFFSDVPKIGTTSVCGRRRDMEDAVSIHPSFLQRNSEN--  : 134
At5g59220 :  AST-------STVFYETAKEVVVLCESLSSTVVALPDPEAYPKYGVASVCGRRREMEDAVAVHPFFSRHQTEYSS  : 143

PP2CA     :  --HHFYGVFDGHGCSHVAEKCRERLHDIVKKEVEVMASDEWTETMVKSFQKMDKEVSQRECNLVVNGATRSMKNS  : 207
At5g59220 :  TGFHYCGVYDGHGCSHVAMKCRERLHELVREEFEADA--DWEKSMARSFTRMDMEVVA----LNADGAAK-----  : 207

PP2CA     :  CRCELQSPQCDAVGSTAVVSVVTPEKIIVSNCGDSRAVLCRNGVAIPLSVDHKPDRPDELIRIQQAGGRVIYWDG  : 282
At5g59220 :  CRCELQRPDCDAVGSTAVVSVLTPEKIIVANCGDSRAVLCRNGKAIALSSDHKPDRPDELDRIQAAGGRVIYWDG  : 282

PP2CA     :  ARVLGVLAMSRAIGDNYLKPYVIPDPEVTVTDRTDEDECLILASDGLWDVVPNETACGVARMCLR----------  : 347
At5g59220 :  PRVLGVLAMSRAIGDNYLKPYVISRPEVTVTDRANGDDFLILASDGLWDVVSNETACSVVRMCLRGKVNGQVSSS  : 357

PP2CA     :  ------GAGAG----DDSDAAHNACSDAALLLTKLALARQSSDNVSVVVVDLRKRRNNQASS  : 399
At5g59220 :  PEREMTGVGAGNVVVGGGDLPDKACEEASLLLTRLALARQSSDNVSVVVVDLRRDT------  : 413
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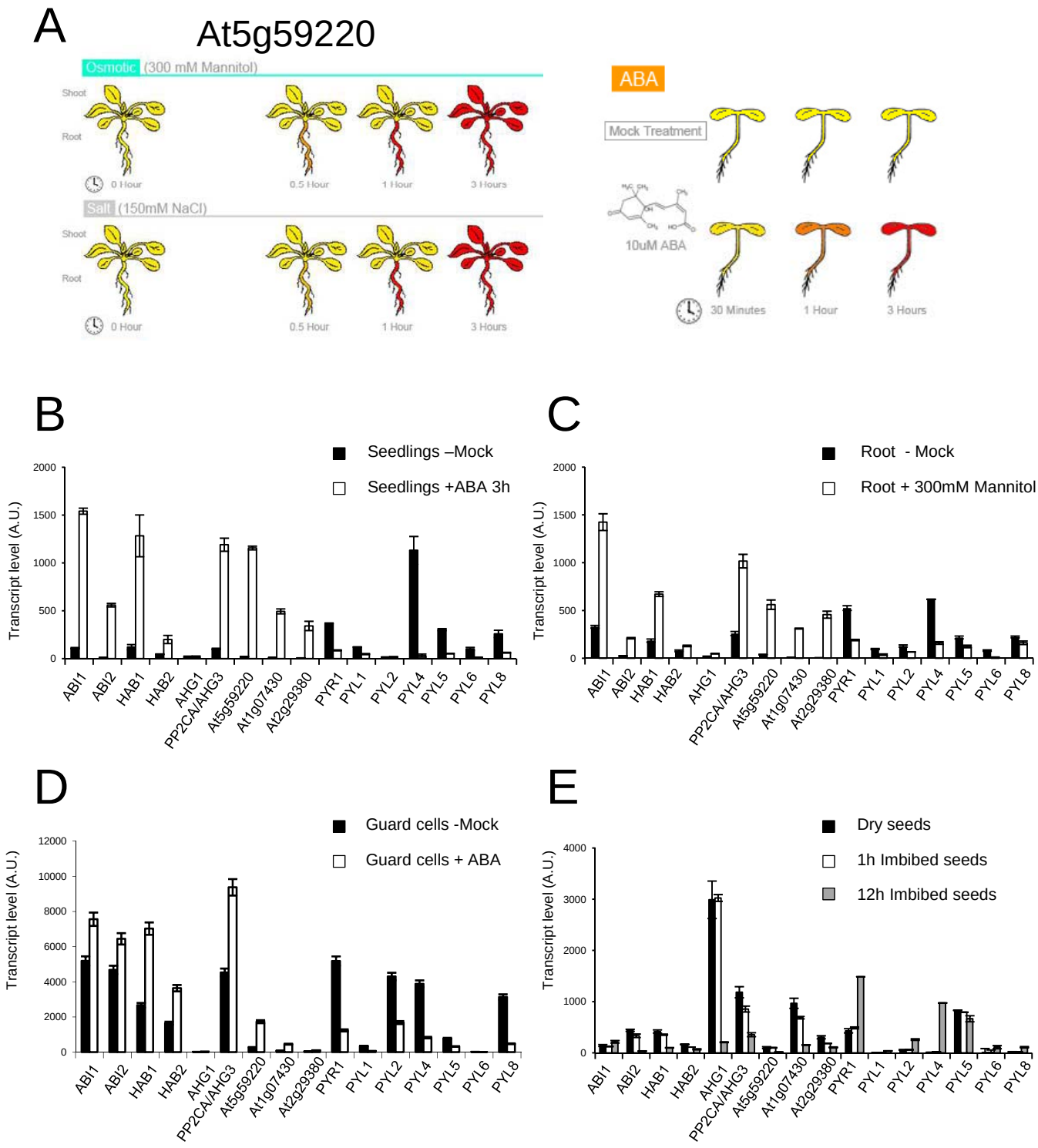
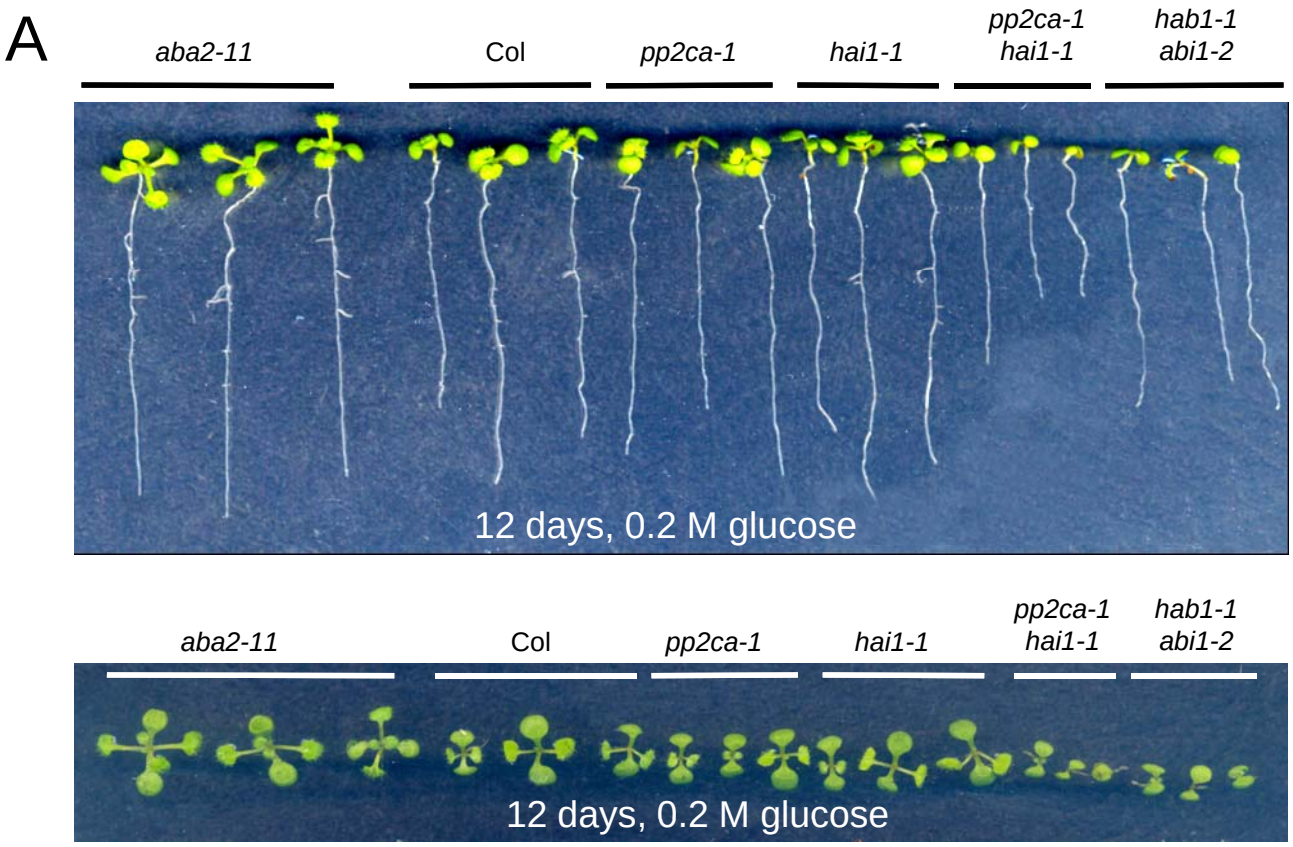
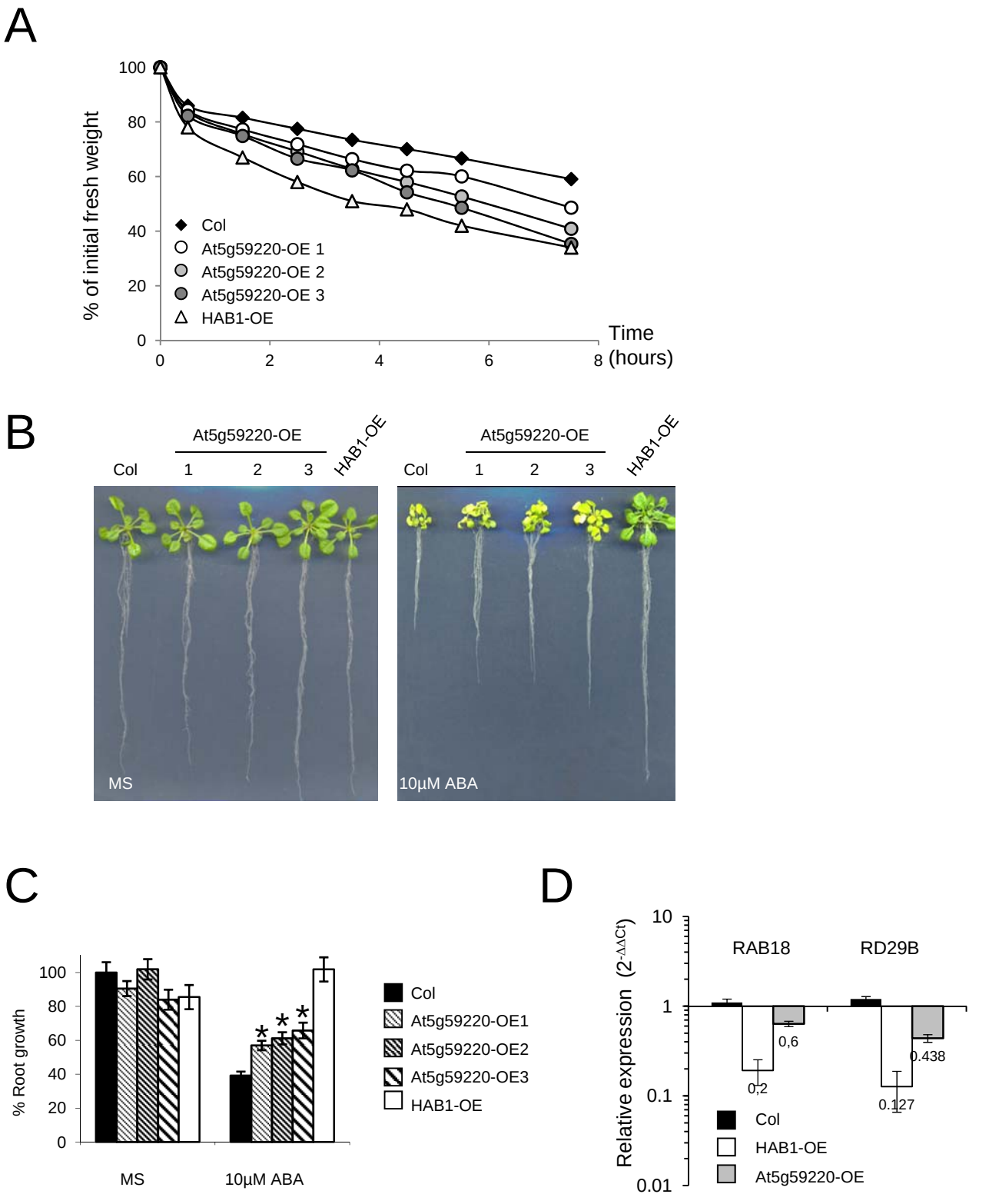
Supplemental Figure S1. Cladogram and nomenclature of Clade A PP2Cs. Position of nuclear localization signals and the conserved Trp residue in PP2CA and At5g59220. A, Cladogram, according to Schweighofer et al., (2004). B, Position of nuclear localization signals and the conserved Trp residue in PP2CA and At5g59220. A discontinuous line indicates the bipartite nuclear localization of At5g59220, whereas continuous lines mark the four basic residues of both PP2CA and At5g59220. An asterisk indicates the position of the conserved Trp residue described in the text.



Supplemental Figure S2. Upregulation of At5g59220 gene expression by osmotic stress (300 mM mannitol or 150 mM NaCl) and ABA. Expression levels of clade A PP2Cs and seven PYR/PYLs in whole 7-day-old seedlings, root, guard cells and seeds. A, Mannitol, NaCl and ABA induce At5g59220 expression. Data were obtained from the Bio-Array Resource for Arabidopsis Functional Genomics (http://bar.utoronto.ca) (Winter et al., 2007). B, Expression levels of clade A PP2Cs in whole 7-day-old seedlings that were either mock- or ABA-treated for 3 h (data produced by the AtGen-Express Consortium; http://web.uni-frankfurt.de/fb15/botanik/mcb/AFGN/atgenex. htm). C, Expression levels in roots that were either mock- or 300 mM mannitol treated (Kilian et al., 2007). D, Expression levels in guard cells that were either mock- or ABA-treated (Yang et al., 2008). E, Expression levels in dry seeds, or 1 and 12h imbibed seeds (Nakabayashi et al., 2005).



Supplemental Figure S3. Glucose-hypersensitive growth inhibition of *pp2ca-1 hai1-1* and *hab1-1 abi1-2* double mutants compared to wt and single parental mutants. A, Seedling growth after 12 days of *aba2-11*, Col wt, *pp2ca-1*, *hai1-1, pp2ca-1 hai1-1* and *hab1-1 abi1-2* double mutants in medium supplemented with 0.2 M glucose. Approximately 200 seeds of each genotype were sowed on MS plates supplemented with 0.2 M glucose. After 12 days, representative seedlings were removed from the medium, rearranged in a new plate and photographed under a Nikon SMZ800 binocular glass. B, Germination and seedling establishment of Col wt, *pp2ca-1*, *hai1-1, pp2ca-1 hai1-1* and *hab1-1 abi1-2* double mutants in medium lacking or supplemented with either ABA, glucose or mannitol. The photograph was taken 5 days after sowing.



Supplemental Figure S4. Analysis of water-loss, ABA-mediated growth inhibition and expression of two ABA-responsive genes in 35S:HAB1 and 35S:At5g59220 lines compared to wt. A, Enhanced water loss of 35S :HAB1 and 35S:At5g59220 lines compared to Col wt. Five leaves at the same developmental stage were detached from 21-d-old plants and fresh weight was determined after submitting them to the drying atmosphere of a flow laminar hood (*n* =4 plants per experiment). B, C ABA-hypersensitive root growth inhibition of 35S :HAB1 and three 35S:At5g59220-OE lines compared to wild-type. B, Photograph of representative seedlings 10 days after the transfer of 4-day-old seedlings from MS medium to plates lacking or supplemented with 10 µM ABA. Root growth was scored after 10 days. Data are averages ±SE from three independent experiments (n=20 seedlings per experiment). Asterisk indicates P < 0.01 (Student's t test) when comparing data for each genotype versus the wild-type under the same assay conditions. D, Relative expression of two ABA-responsive genes in 35S:HAB1 and 35S:At5g59220 plants compared to wt. RT-qPCR analyses were made in triplicate on RNA samples of 2-week-old seedlings that were either mock or 10 µM ABA-treated for 3 h. Numbers indicate the induction level of the genes in each over-expression line with respect to the wt (value 1).