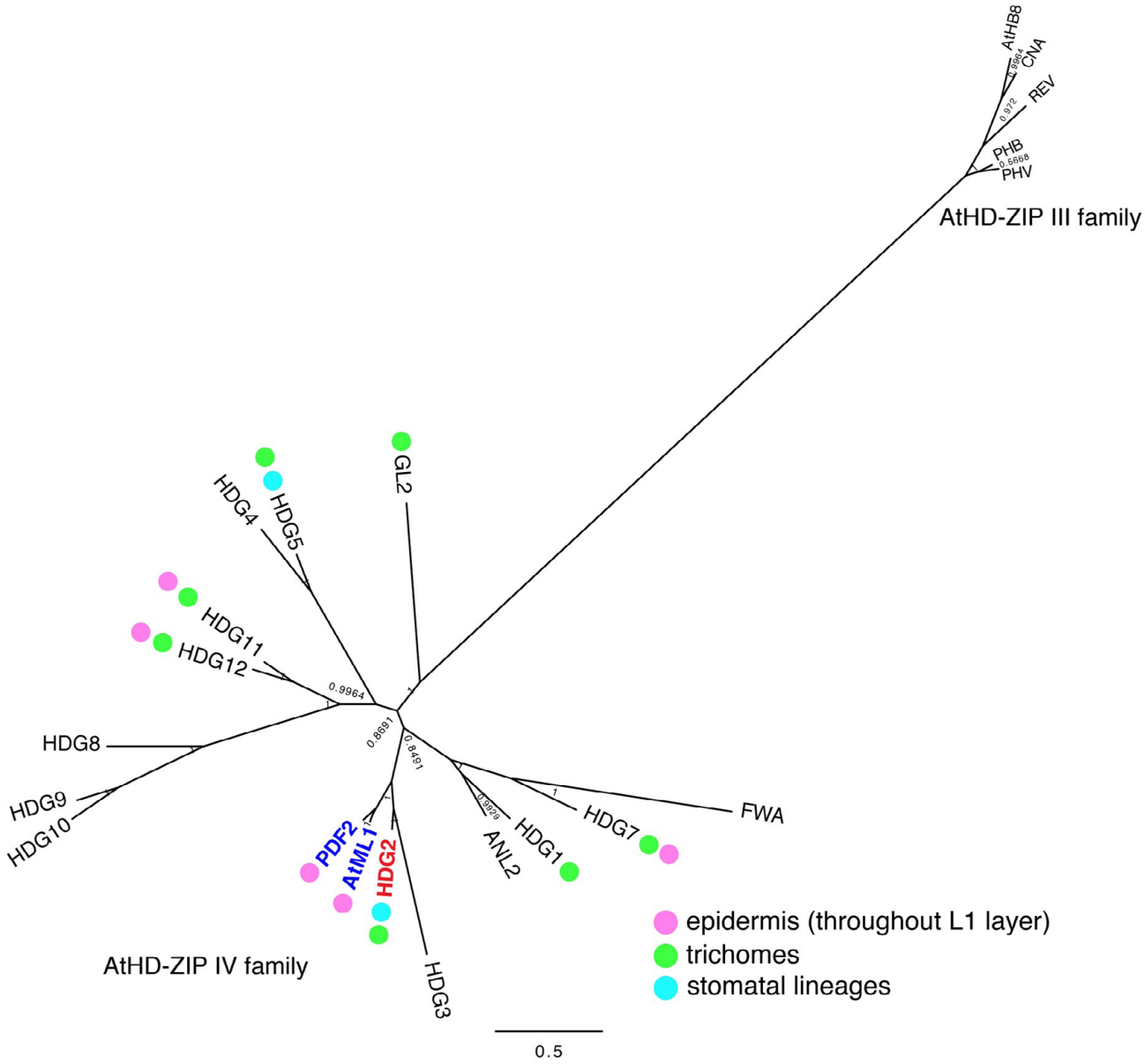


Fig. S1. Molecular phylogenetic analysis of AtHD-ZIP IV family. A phylogenetic tree was constructed using Bayesian analysis with Markov Chain Monte Carlo algorithm for one million generations to obtain a majority-rule consensus tree. Full-length amino acid sequences of 16 AtHD-ZIP IV family and five AtHD-ZIP III (outgroup) were analyzed. Posterior probabilities of key nodes are shown at the respective nodes. The scale bar indicates the number of amino acid changes per branch length. HDG2 is highlighted in red; AtML1 and PDF2 are highlighted in blue to show their close phylogenetic relationship with HDG2. Colored dots indicate known expression/promoter activities within the leaf epidermis with the following cell types: pink, uniformly in the protoderm (L1-layer); green, in trichomes; cyan, in stomatal-lineage cells (Rerie et al. ,1994; Lu et al., 1996; Session et al., 1999; Abe et al. 2003; Nakamura et al., 2006).

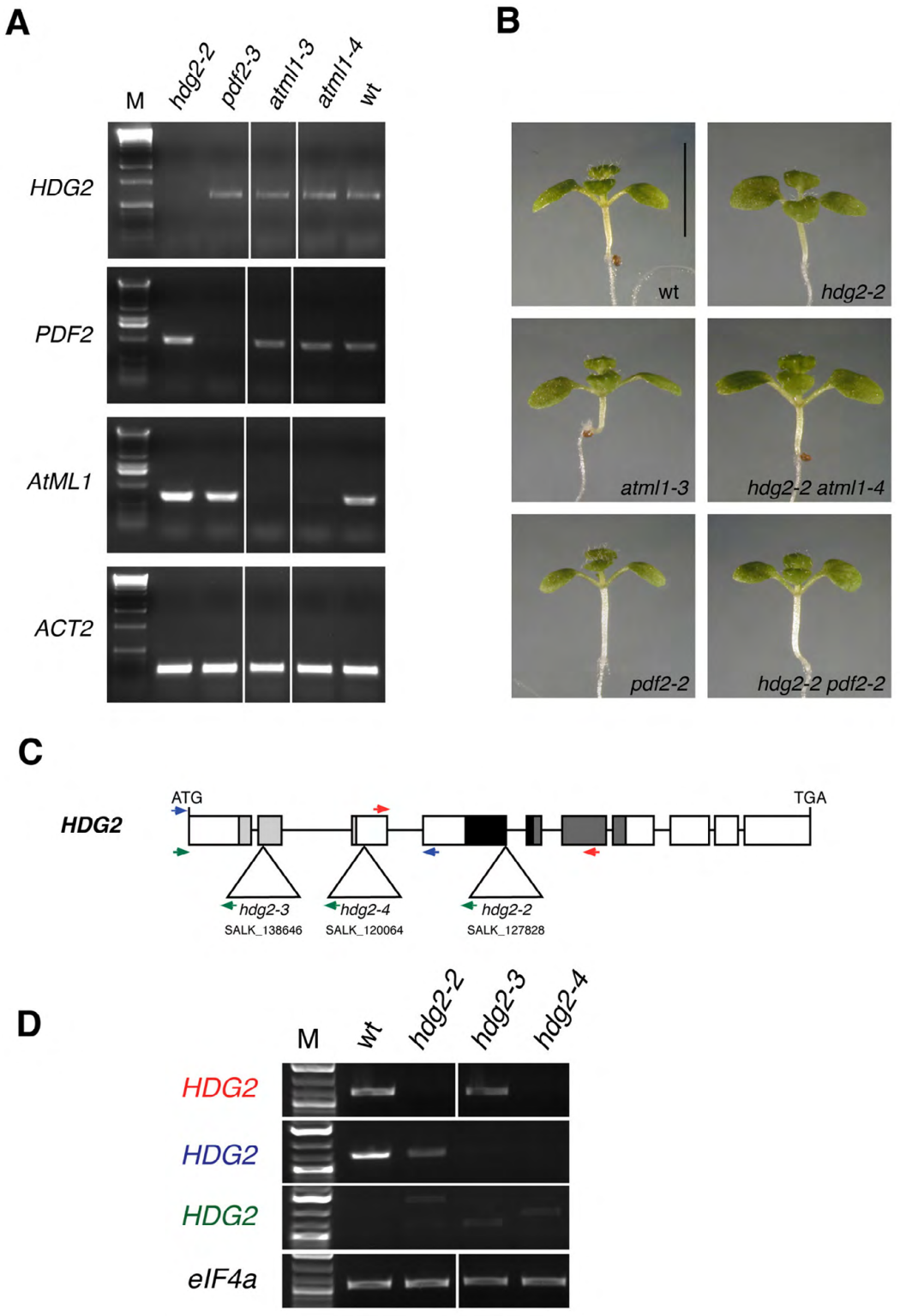


Fig. S2. T-DNA insertion knockout alleles of *HDG2*, *PDF2* and *AtML1* show no obvious growth phenotypes. (**A**) RT-PCR analysis of *HDG2*, *PDF2* and *AtML1* from 10-day-old seedlings of wild-type (wt) and T-DNA insertion alleles, *hdg2-2* (SALK_127828C), *pdf2-2* (SALK_109425C), *atml1-3* (SALK_128172) and *atml1-4* (SALK_033408). Corresponding transcripts are undetectable in these T-DNA insertion alleles. *ACT2* transcripts serve as a control. (**B**) 10-day-old seedlings of the corresponding genotypes. Images were taken under the same magnification. Scale bar: 5 mm. (**C**) Schematic diagram indicating the T-DNA insertion sites for three mutant alleles within the *HDG2* gene. This diagram includes *HDG2* genomic region from translational initiation codon to termination codon. Light-gray boxes, homeodomain; dark-gray boxes, START domain, which also contains a transcriptional activation domain of maize OCL1 (black boxes). Color-coded arrows correspond to locations of primer pairs used for RT-PCR analysis (see D). (**D**) RT-PCR analysis of three *hdg2* T-DNA insertion alleles reveals expressions of aberrant transcripts of partial size, which are not derived from genomic DNA contamination based on their size. Each color code corresponds to the following primer pairs used for analysis: green, primers HDG2_1_XhoI and LBa1; blue, primers HDG2_1_XhoI and HDG2_1750.rc; red, HDG2_1168 and HDG2_2359.rc. See C for locations of primers. For primer sequence, see supplementary materials Table S1.

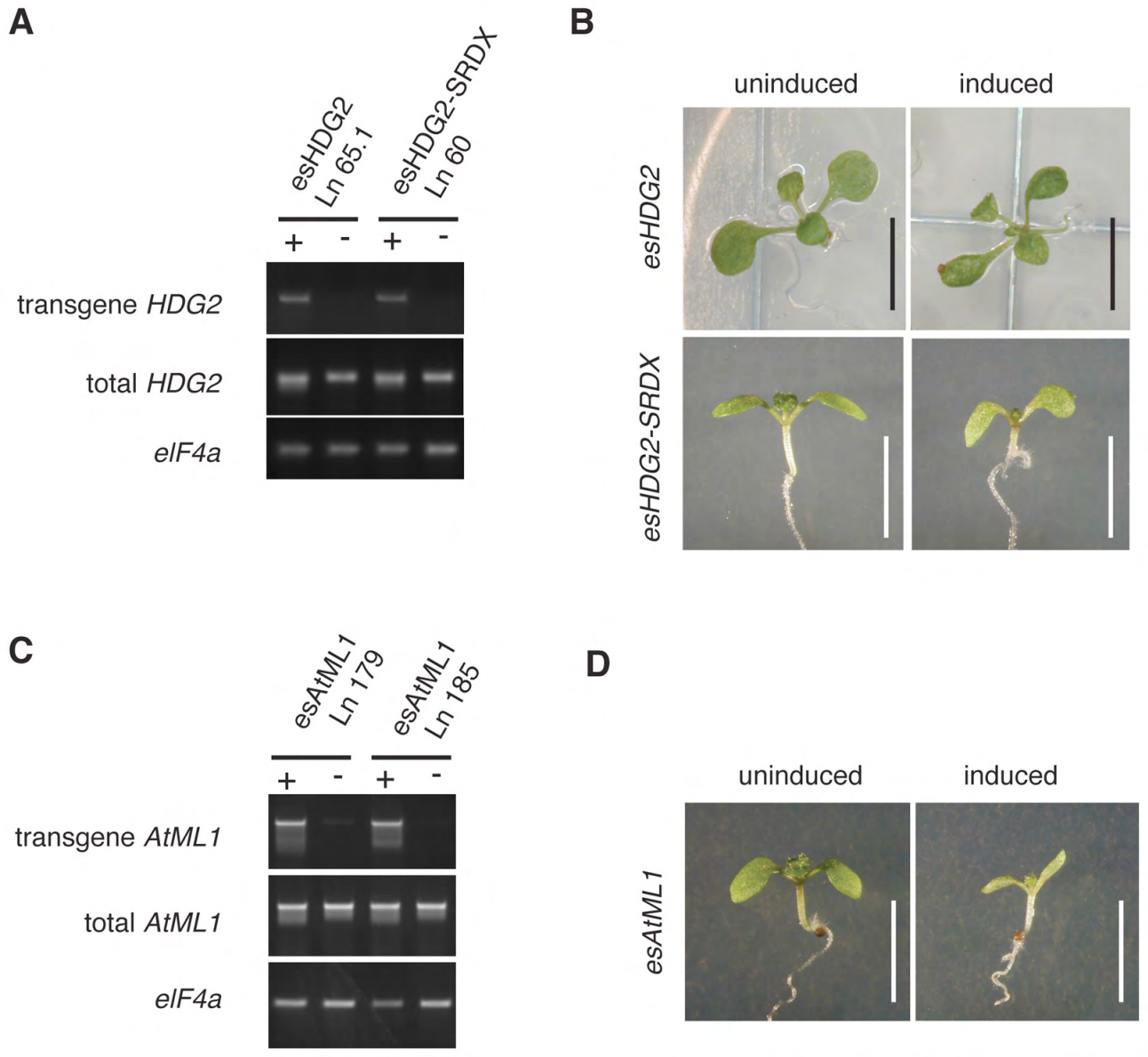


Fig. S3. Inducible ectopic overexpressors of *HDG2*, *HDG2-SRDX* and *AtML1*. (**A**,**C**) RT-PCR analysis of transgenic *Arabidopsis* lines carrying inducible *HDG2*, *HDG2-SRDX* and *AtML1* with (+) or without (–) estradiol induction. *eIF4a* transcripts serve as a control. (**B**,**D**) Seedlings of the corresponding transgenic lines, *HDG2* (top; 2 weeks old) and *HDG2-SRDX* (10 days old) with or without induction. Scale bars: 5 mm.

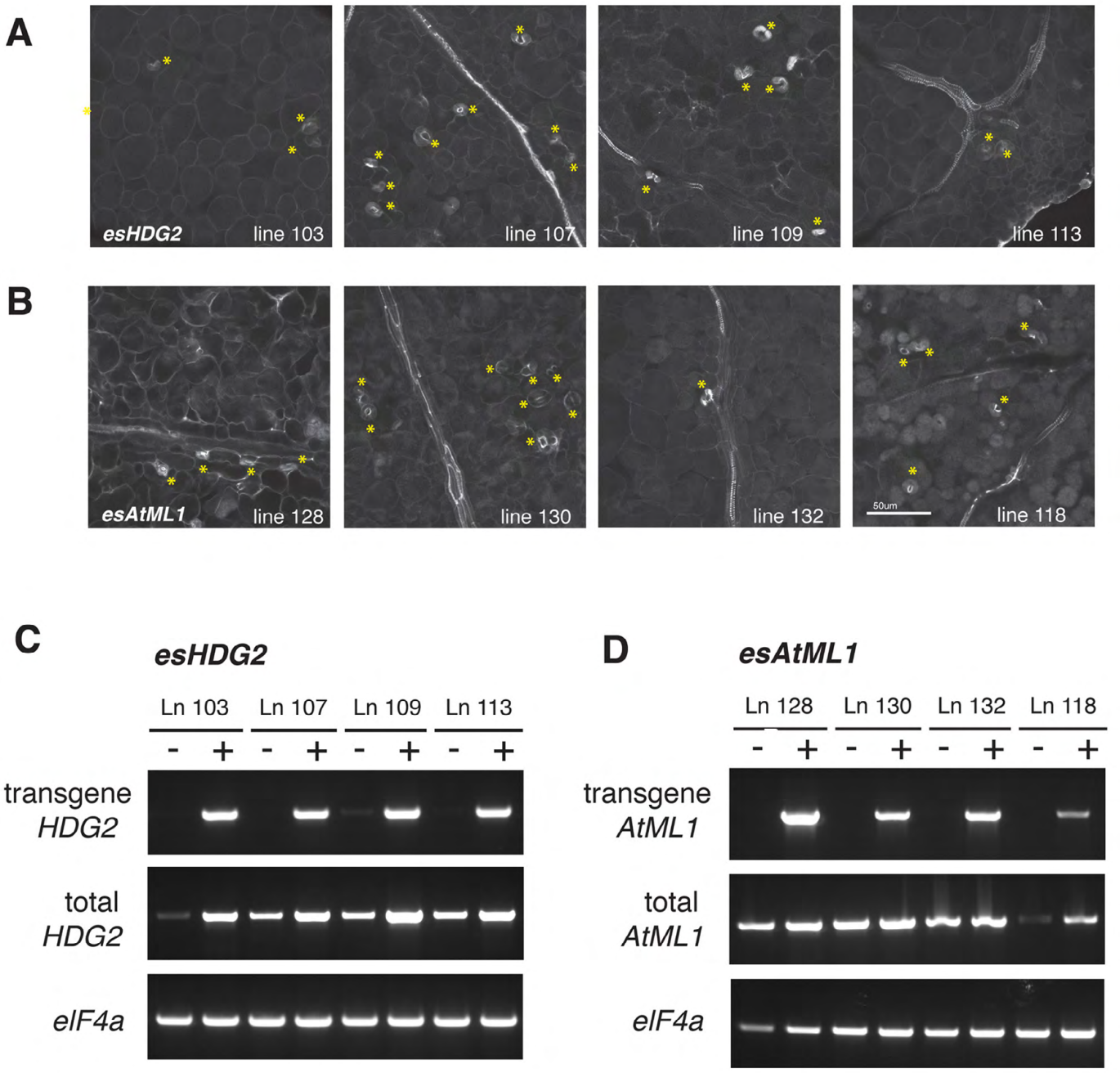


Fig. S4. Induced ectopic overexpression of *HDG2* and *AtML1* in additional four transgenic lines for each construct, each showing ectopic stomatal differentiation in mesophyll layers. (**A**,**B**) mPS-PI stained optical sections of mesophyll layer of 12-day-old cotyledons from four independent transgenic lines harboring inducible *HDG2* transgene (A) and four independent transgenic lines harboring inducible *AtML1* transgene (B). Ectopic differentiation of internal stomata (yellow) is evident. Images were taken under the same magnification. Scale bar: 50 μm. (**C**,**D**) RT-PCR analysis of additional transgenic *Arabidopsis* lines carrying inducible *HDG2* or *AtML1* with (+) or without (–) estradiol induction. *eIF4* transcripts serve as a control. The line number corresponds to the lines examined for phenotypes (A,B).

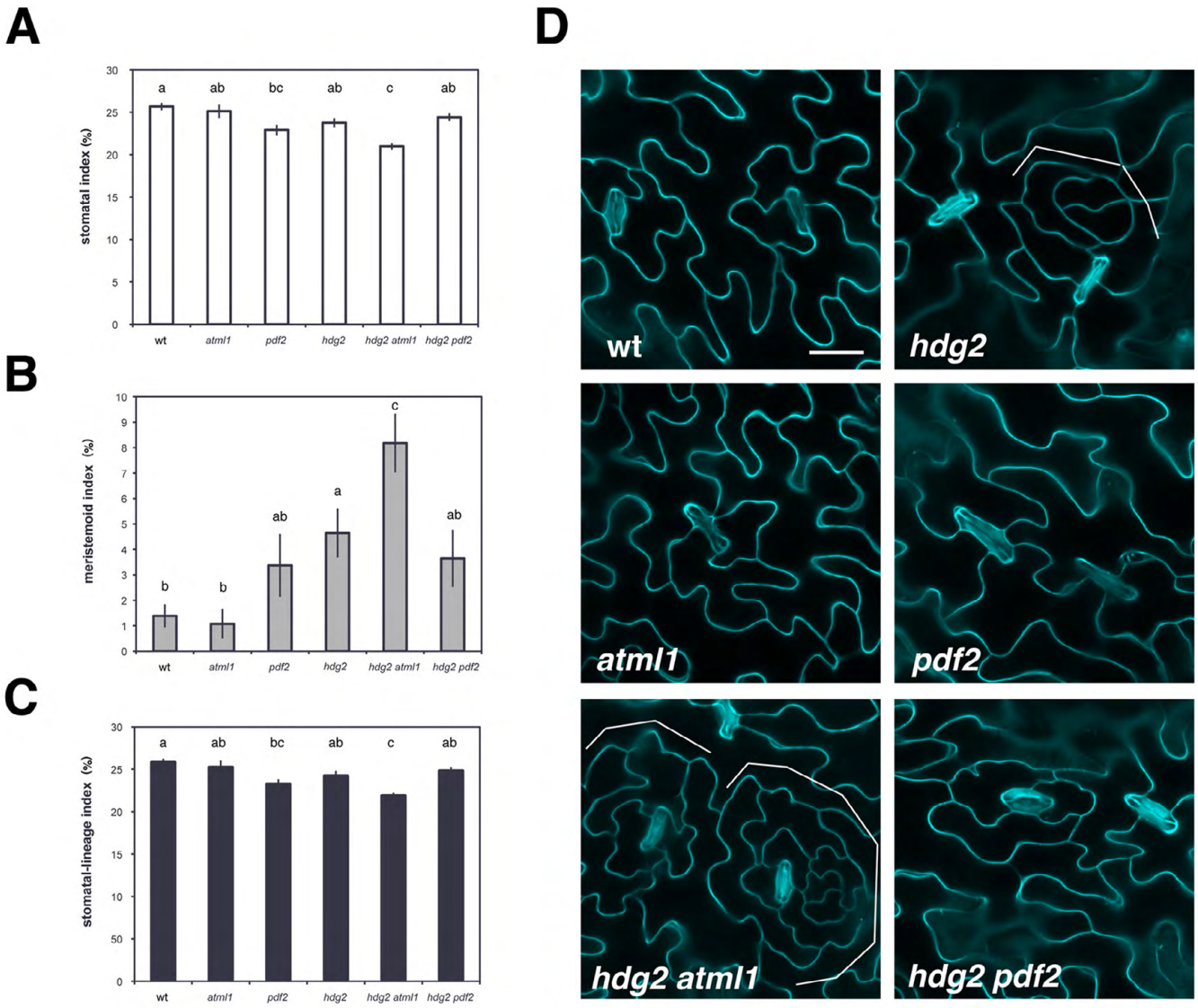


Fig. S5. Stomatal development defects in rosette leaves of *hdg2* mutants and higher-order mutants in closely related *HD-ZIP IV* genes. (**A**) SI of 6-week-old abaxial rosette leaf 4 from wild type, *atml1, pdf2, hdg2, hdg2 atml1* and *hdg2 pdf2*. *hdg2-2* and *atml1-3* alleles were used for the analysis. Bars indicate means (n=16-21); error bars indicate s.e.m. Total numbers of stomata counted: 1875 (wild type), 1224 (*atml1*), 1424 (*pdf2*), 1931 (*hdg2*), 1608 (*hdg2 atml1*) and 1749 (*hdg2 pdf2*). (**B**) MI of the six genotypes described above. Bars indicates means; error bars indicate s.e.m. Total numbers of meristemoids counted: 12 (wild type), 5 (*atml1*), 22 (*pdf2*), 40 (*hdg2*), 58 (*hdg2 atml1*) and 31 (*hdg2 pdf2*). (**C**) SLI of the six genotypes described above. Bars indicate means; error bars indicate s.e.m. Total numbers of cells counted: 7272 (wild type), 4795 (*atml1*), 6229 (*pdf2*), 8010 (*hdg2*), 7585 (*hdg2 atml1*) and 7123 (*hdg2 pdf2*). For B,C, genotypes with non-significant phenotypes were grouped together with a letter (Tukey's HSD test after One-way ANOVA). For C, only one genotype was significantly different from others (Tukey's HSD test; P<0.01). (**D**) Representative confocal images of 6-week-old abaxial rosette leaf 4 epidermis from six different genotypes. Images were taken under the same magnification. Scale bar: 20 μm. *hdg2* mutant rosette leaf epidermis shows characteristic delayed stomatal differentiation, a phenotype exaggerated in *hdg2 atml1* double mutant (brackets).

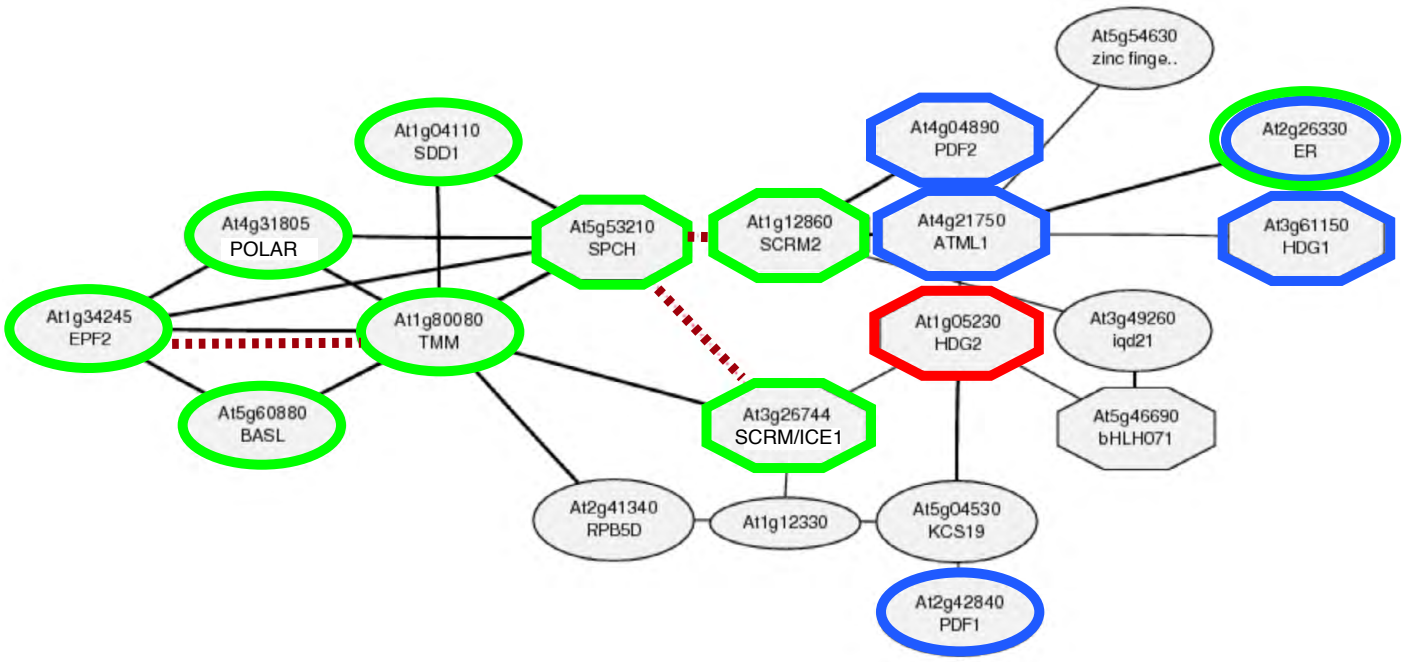


Fig. S6. Co-expression network of genes controlling stomatal development and/or protodermal differentiation. The network diagram was generated using ATTED II network drawer (http://atted.jp/top_draw.shtml#NetworkDrawer). *HDG2* is highlighted in red; known regulators of stomatal development are in green; known genes with protodermal expression are in blue; hexagons represent transcription factors. Thickness of lines (edges) represents mutual rank (MR) of co-expression; the higher the rank, the thicker the line. Dotted red line indicates known protein-protein interactions.

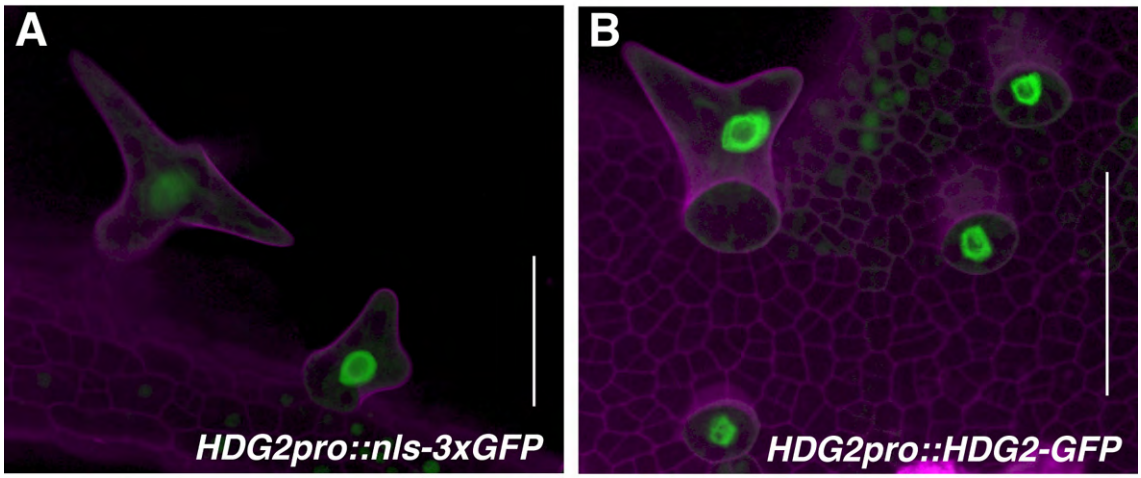


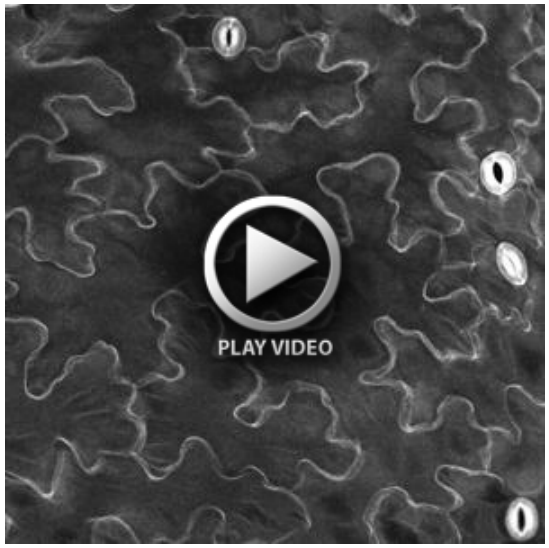
Fig. S7. Expression of transcriptional and translational reporters of *HDG2* in developing trichomes. (**A**,**B**) Confocal images of abaxial rosette leaf epidermis expressing *HDG2pro::nls-3xGFP* (A) and *HDG2pro::HDG-GFP* (B). Each developing trichome has very large nucleus (fluorescing green). Scale bars: 50 μm.

Table S1. List of primer DNA sequences for real-time quantitative RT-PCR and RT-PCR. Download Table S1

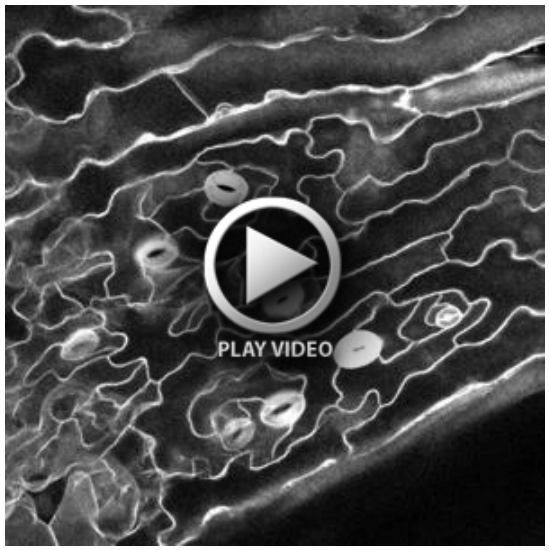
Table S2. List of plasmid constructs generated in this study. Download Table S2

Table S3. DNA sequences used for yeast one-hybrid analysis. Download Table S3

Table S4. Bioinformatic analysis of promoter motifs in stomatal development genes. Overrepresentation analysis of promoter elements in 18 genes involved in stomatal development (*EPF1*, *EPF2*, *ERECTA*, *ERL1*, *ERL2*, *TMM*, *SPCH*, *MUTE*, *FAMA*, *SCRM*, *SCRM2*, *HDG2*, *AtML1*, *POLAR*, *BASL*, *MYB88*, *FLP* and *SDD1*) were performed with the Athena tool (http://www.bioinformatics2.wsu.edu/Athena/). Download Table S4



Movie 1. Complete optical sectioning of a wild-type 10-day-old cotyledon. *Z* stacks were made from mPS-PI stained confocal optical sections in 1 μm intervals.



Movie 2. Complete optical sectioning of a 10-day-old cotyledon from *HDG2* induced ectopic overexpression. *Z* stacks were made from mPS-PI stained confocal optical sections in 1 μm intervals.

Movie 3. Complete optical sectioning of a 10-day-old cotyledon from *AtML1* induced ectopic overexpression. *Z* stacks were made from mPS-PI stained confocal optical sections in 1 μm intervals.