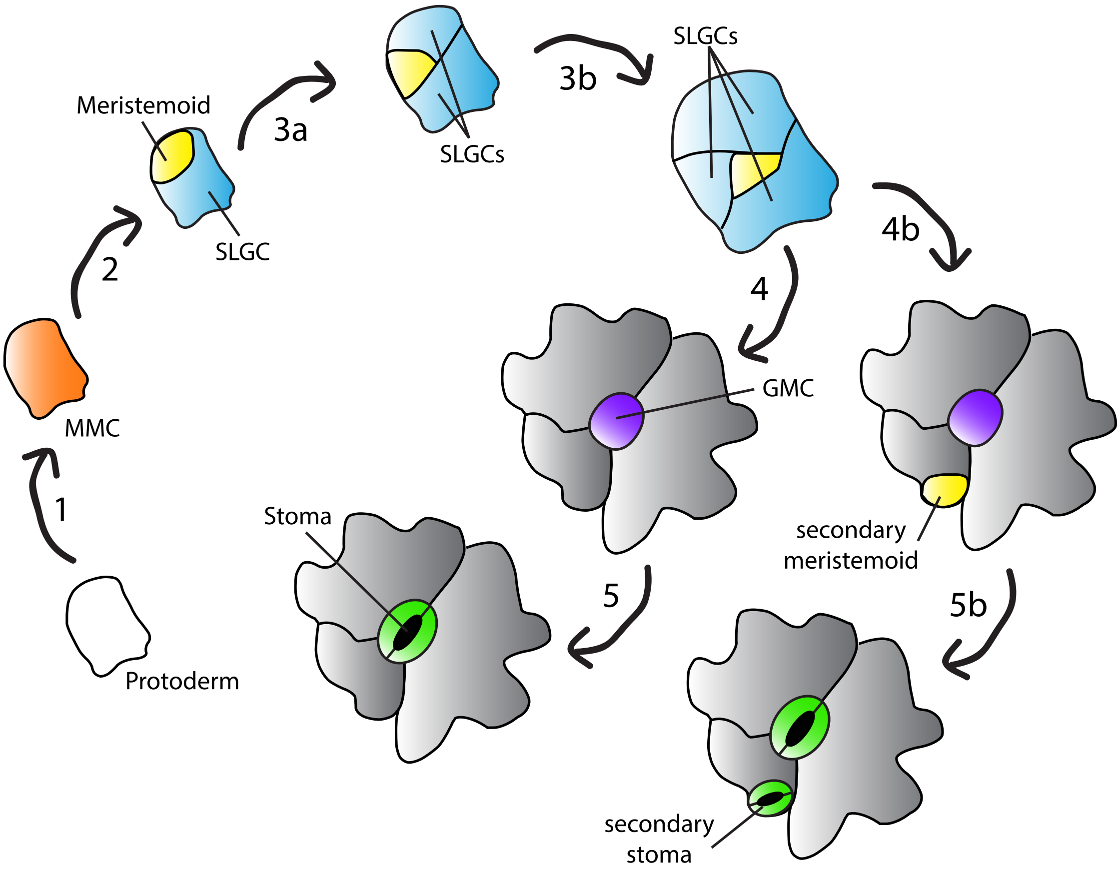
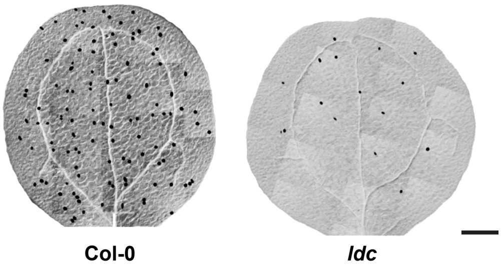


Supplemental Figure 1. Stomatal development in *Arabidopsis*.

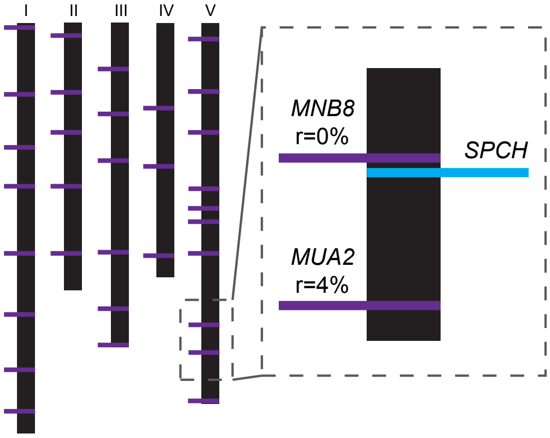
Developmental steps that lead to stomata formation. A protodermal cell acquires MMC fate (orange). The MMC executes the first asymmetric division that generates a meristemoid (yellow) and an SLGC (blue). The meristemoid can undergo additional asymmetric divisions, amplifying the number of SLGCs, and ultimately it differentiates into a GMC (purple), of which the symmetric division produces the guard cell (GC) pair that constitutes the stoma (green). Secondary or satellite stomata can be formed when a SLGC asymmetrically divides away from the primary stoma.



Supplemental Figure 2. Adaxial cotyledon phenotype exhibited by the *ldc* mutant.

DIC images showing complete 15-day-old adaxial cotyledons of Col-0 and *ldc*. Bar = 0.5 mm.

A



B

SPCH (At5g53210) aminoacid sequence

MQEIIPDFLEECEFVDTSLAGDDLFAILESLEGAGEISPTAAST

PKDGTTSSKELVKDQDYENSSPKRKKQRLETRKEEDEEEED

R→W (*spch-5*)
GDGEAEEDNKQDGQQKMSHVTVERNRRKQMNEHLTVLRSL
***   *   ***

bHLH
MPCFYVKRGDQASIIGGVVEYISELQQVLQSLEAKKQRKTYA

EVLSPRVVPSPRPSPPVLSPRKPPLSPRINHHQIHHHLLLPPIS

MPKTD
PRTPQPTSPYRAIPPQLPLIPQPPLRSYSSLASCSSLGDPPPY

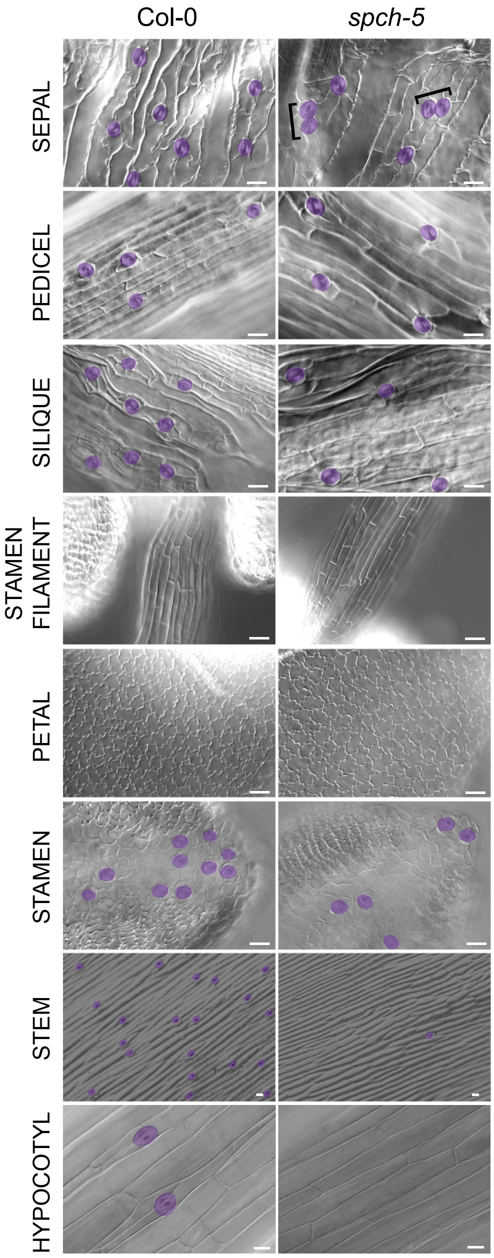
V→M (*spch-2*)
SPASSSSSPSVSSNHESSVINELVANSKSALADVEVKFSGAN

SMF
VLLKTVSHKIPGQVMKIIAALEDLALEILQVNINTVDETMLNSFT

Q→stop (*spch-1*)
IKIGIECQLSAEELAQQIQQTFC

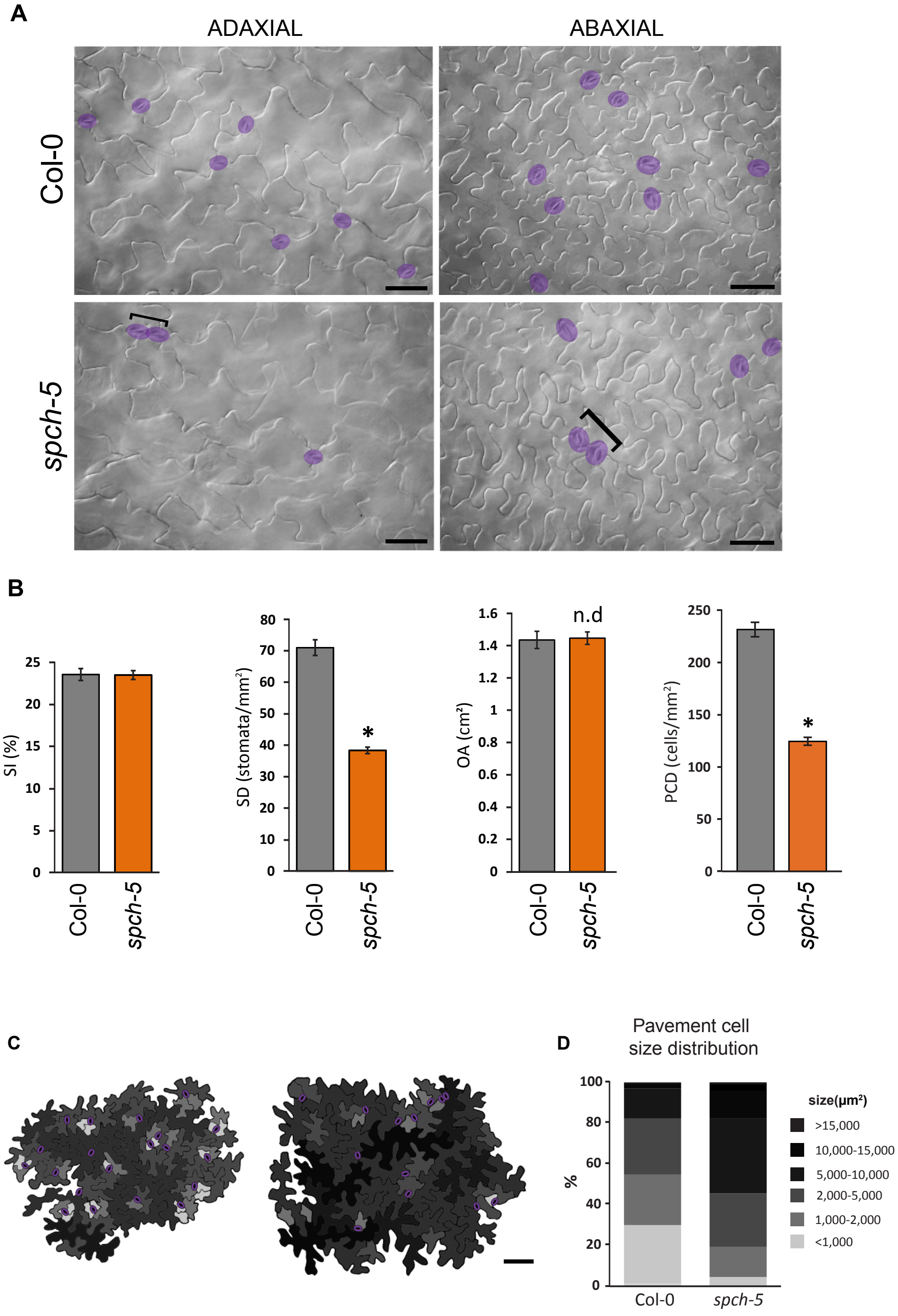
Supplemental Figure 3. Genetic mapping of the *ldc* mutation and SPCH protein sequence indicating known domains and amino acid changes of the point mutants.

(**A**) Genetic map of *Arabidopsis* presenting the 32 microsatellite markers (purple lines) used for the genetic mapping of the *ldc* mutation. Each black rectangle represents a chromosome (I to V). Inset shows the genome region in which the *ldc* mutation had reduced recombination rates (r) and the physical position of the SPCH locus (blue line). **(B)** SPCH amino acid sequence with colored domains (blue for bHLH, yellow for MAP KINASE TARGET DOMAIN [MPKTD], and green for the plant specific C-terminal domain (SPCH, MUTE and FAMA) [SMF]) and point amino acid changes for the different mutants (red).



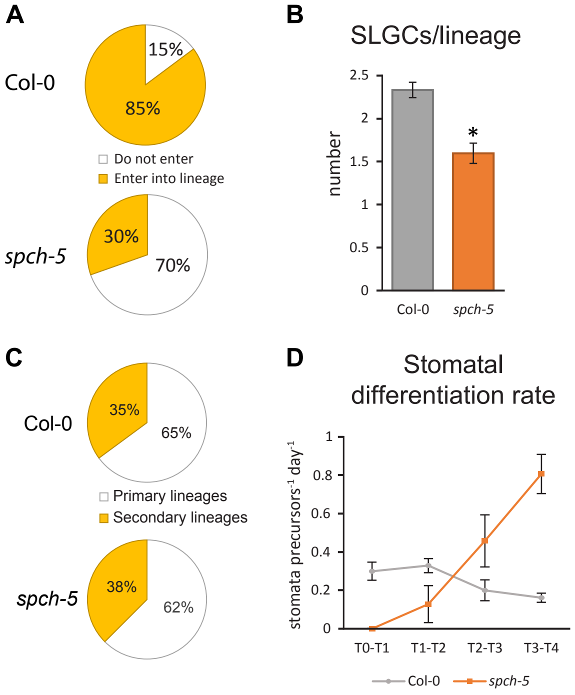
Supplemental Figure 4. Epidermal DIC images of several mature organs in Col-0 and *spch-5*.

Stomata are false-colored in purple for better identification. Brackets mark stomatal clusters. Bars = 20 µm.



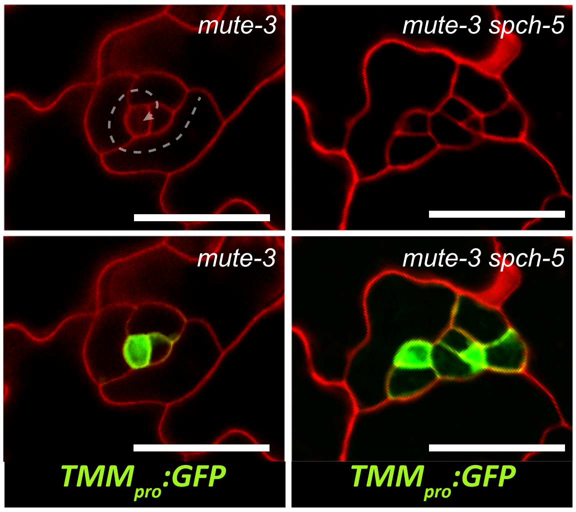
Supplemental Figure 5. *spch-5* third-leaf epidermal phenotype.

(A) DIC images of the adaxial and abaxial epidermis of Col-0 and *spch-5* in third leaves of 28-day-old plants. Stomata are highlighted in purple and clusters marked by brackets. Bars = 50 µm. **(B)** Graphs representing stomatal index, stomatal density, organ area and pavement cell density in abaxial epidermis of 28-day-old third leaves. Grey and orange denote Col-0 and *spch-5*, respectively. Asterisks indicate $P < 0.05$ (Student’s *t* test; n = 10) and n.d $P > 0.05$. Error bars represent SE. **(C)** Representative drawings of Col-0 and *spch-5* with color code as in **(D)**. Stomata are indicated in purple. **(D)** Size distribution of pavement cells in Col-0 and *spch-5*. Frequency of large pavement cells is much higher in the mutant than in the wild type. Bar = 100 µm.



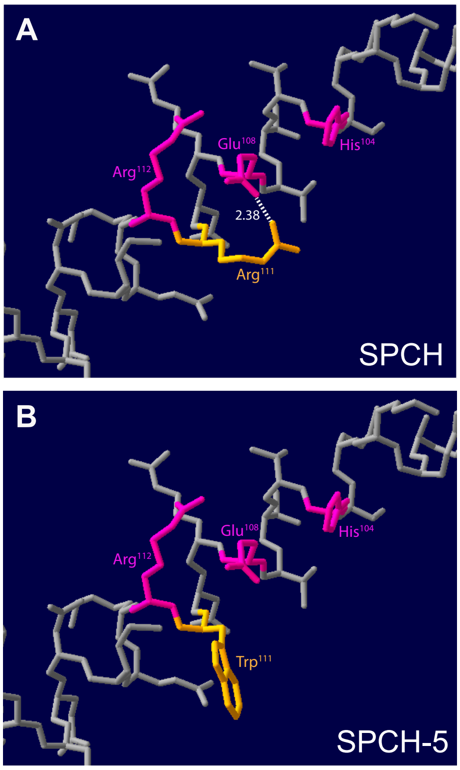
Supplemental Figure 6. Additional data derived from cell tracking of leaf primordia shown in Figure 2.

Graphs elaborated from leaves (n = 5) per genotype and with 50 cells at initial field (T0). **(A)** Percentage of entry divisions. **(B)** Number of SLGCs per lineage. **(C)** Percentage of primary and secondary lineages. **(D)** Stomatal differentiation rate. Asterisk in **(B)** denotes $P < 0.05$ (Student's *t* test). Error bars represent SE.



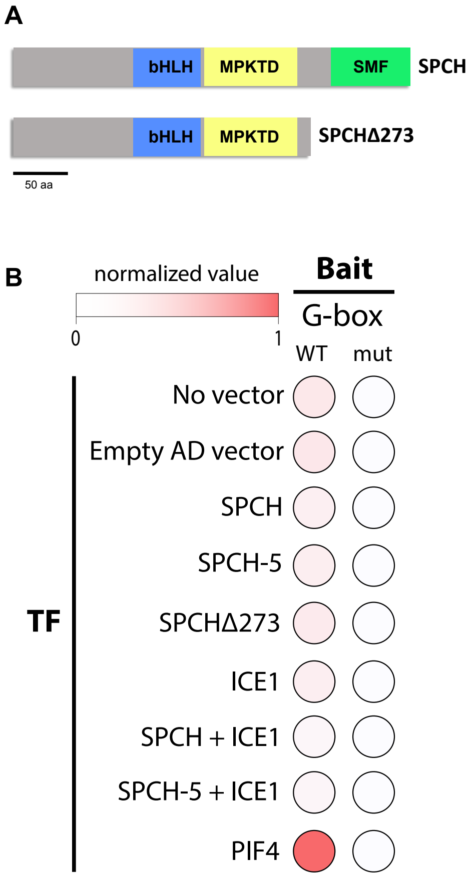
Supplemental Figure 7. Orientation of cell division planes in *spch-5* and *spch-5 mute-3* stomatal lineages.

Confocal images of 13-day-old adaxial cotyledon epidermis, showing cell contours with PI (red) and young lineage cells with GFP (green) expressed from a *TMM*$_{pro}$*:GFP* reporter construct. *mute-3* plants form rosette-like lineages with several small cells in a regular inward spiral arrangement indicated as a dotted white line. Only the most recently formed meristemoid shows a strong *TMM*$_{pro}$*:GFP* expression. *mute-3 spch-5* double mutant lineages also display extra small cells, but arranged in irregular patterns and of which several express *TMM*$_{pro}$*:GFP*. Note the absence of stomata due to the lack of the *MUTE* function. Bars = 50 µm.



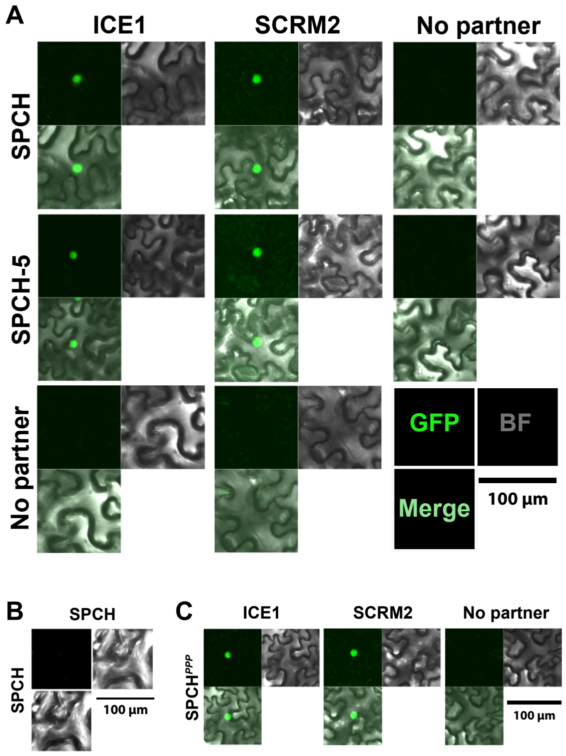
Supplemental Figure 8. Homology-based three-dimensional structural prediction for the SPCH-5 protein.

Modeled protein structures of SPCH versions. **(A)** Wild type SPCH. **(B)** SPCH-5 predicted from *spch-5*. The amino acid triad critical for DNA binding in other bHLH proteins is highlighted (pink). The wild-type Arg^{111} substituted by Trp^{111} in *spch-5* is marked in orange. The hydrogen bond involving this Arg^{111} is indicated as a dashed line with the distance in Ångströms **(A)**, but is lost in the mutant **(B)**.



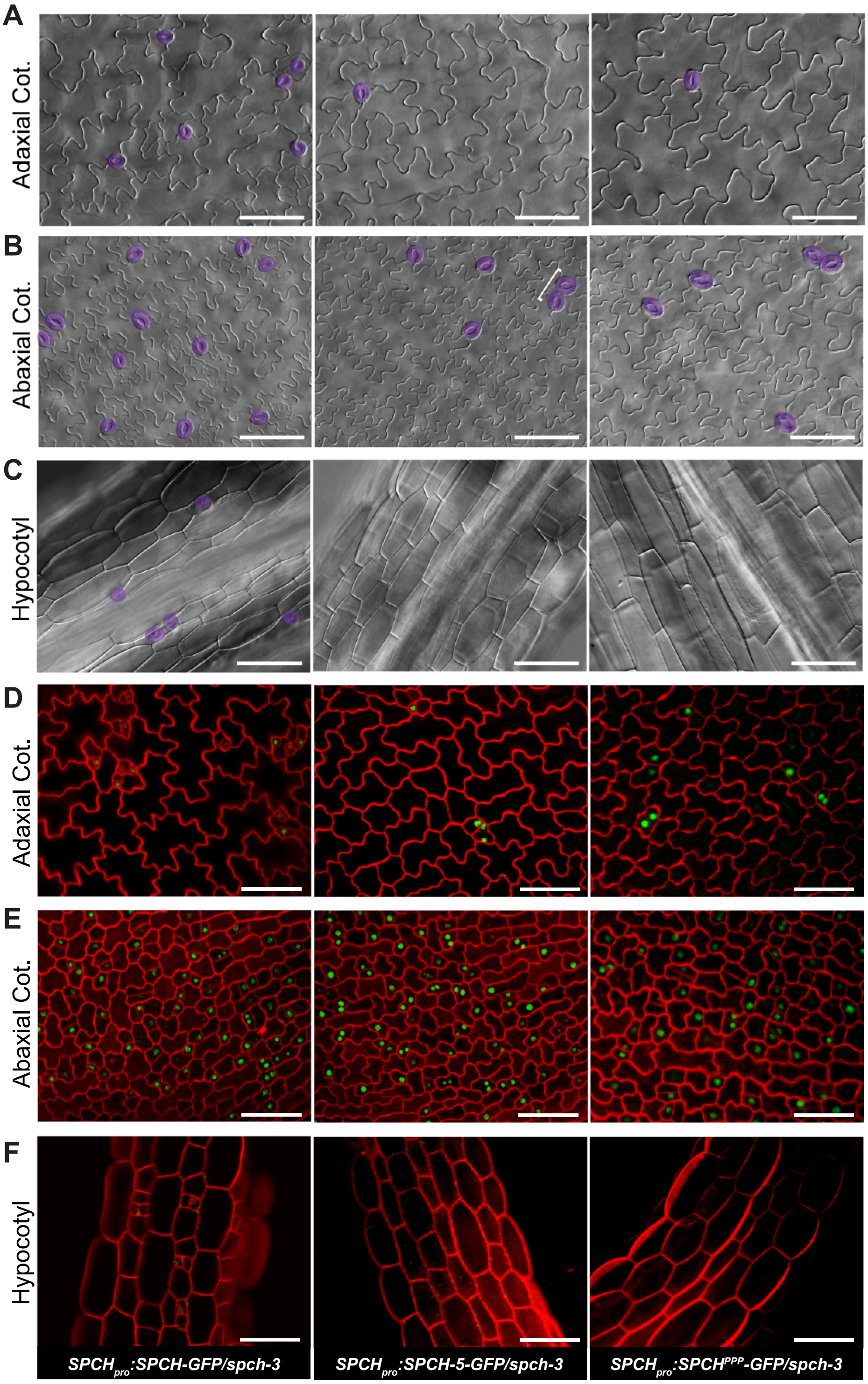
Supplemental Figure 9. DNA-protein interactions involving SPCH-5.

(A) Scheme of the domain structure of SPCH and the truncated SPCHΔ273 version lacking the C-terminal part (the ACT-like or SMF domain). **(B)** Yeast one-hybrid experiment with the tandem-repeated G-box sequence as bait for the wild type (WT) or a mutated version (mut). Colored circles indicate *LacZ* reporter-normalized expression.



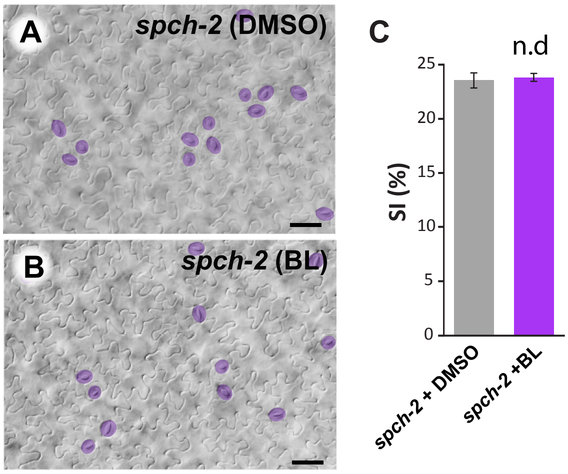
Supplemental Figure 10. Protein--protein interactions involving SPCH-5.

(A) to **(C)** BiFC experiments with transient expression assays in *Nicotiana benthamiana* leaves. Image compositions were made with representative cells showing the recomposed GFP signal diagnostic for physical interactions between proteins.



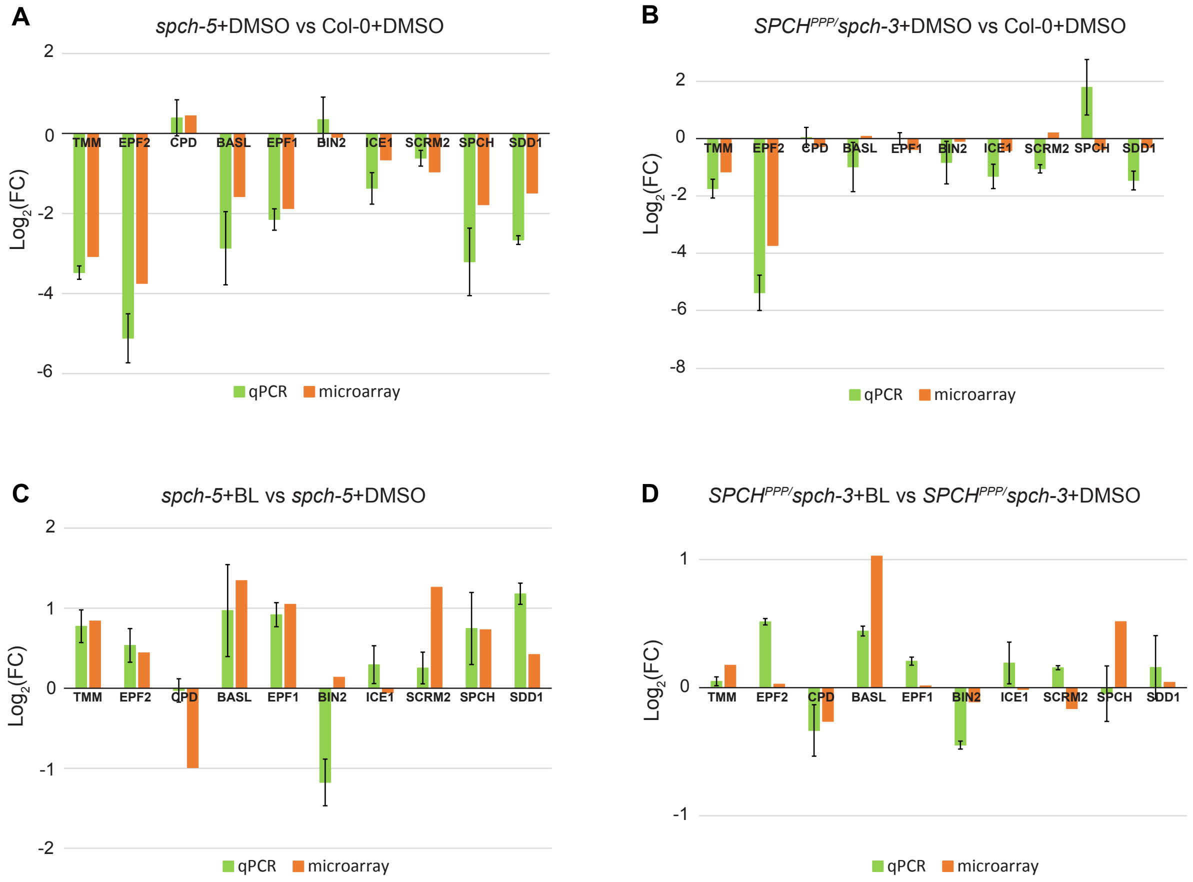
Supplemental Figure 11. Phenotypic and protein expression analyses of *spch-3* lines complemented with different SPCH versions.

(A) to **(C)** DIC images of the epidermis of *spch-3* plants transformed with translational GFP fusions to *SPCH, SPCH-5*, or $SPCH^{PPP}$ under the control of the SPCH promoter. Adaxial **(A)** and abaxial **(B)** 23-day-old cotyledons and 10-days-old hypocotyls **(C)** are shown. Stomata are colored purple. Stomatal clusters are enclosed into brackets. **(D)** to **(F)** Confocal images of the same lines in **(A)** to **(C)**. Red outlines are PI-stained cell contours. Images were taken 3 days after sowing. GFP fluorescence marks the expression of the different protein versions**.** Adaxial **(D)** and abaxial **(E)** cotyledons and hypocotyls **(F)** are shown. Bars = 100 µm.



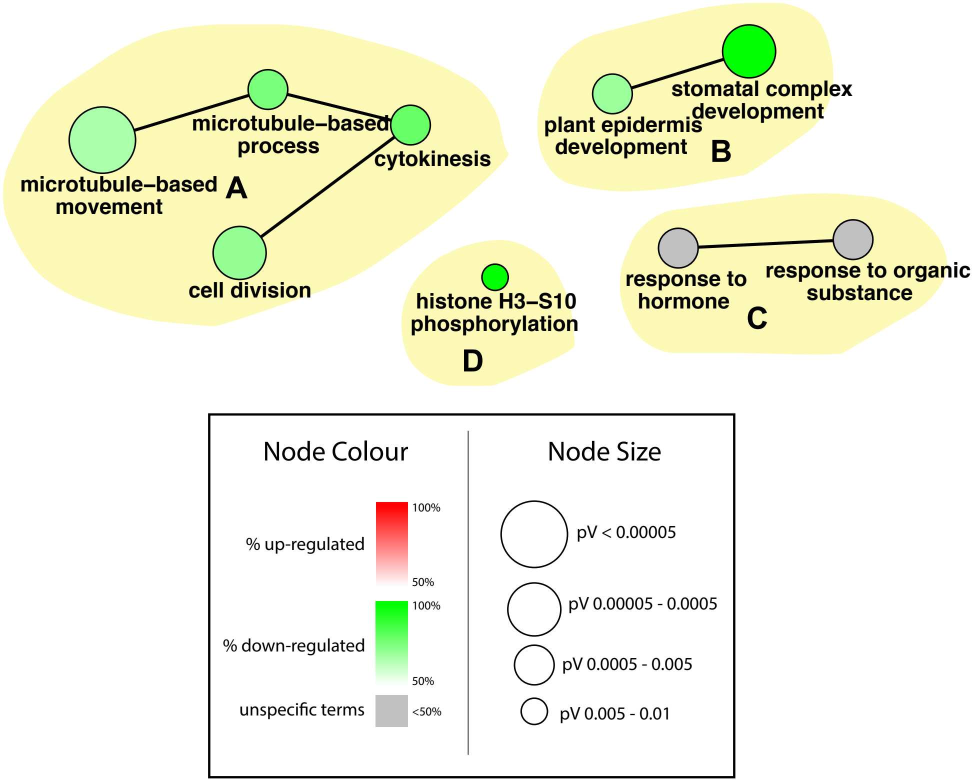
Supplemental Figure 12. BL effect on *spch-2* stomatal development.

(A) and **(B)** DIC images of the abaxial epidermis of *spch-2* in DMSO **(A)** and BL **(B)**. **(C)** Stomatal index quantification of *spch-2* in medium with (purple) and without BL (grey). Error bars represent SE. n.d denotes absence of statistically significant differences between mean values (95% confident Student's *t* test).



Supplemental Figure 13. Quantitative PCR for microarray validation.

Gene expression data in the microarrays (orange) validated by qRT-PCR (green) for 10 genes related to stomata development or BR pathway, selected in pairwise comparisons between genotypes and BL treatments. **(A)** Nontreated *spch-5* vs Col-0. **(B)** Nontreated *SPCH^{PPP}/spch-3* vs Col-0. **(C)** BL-treated vs control *spch-5*. **(D)** BL-treated vs control *SPCH^{PPP}/spch-3*. *UBIQUITIN10* and *ACTIN2* served as reference genes. The Log$_2$-fold change of the qRT-PCR expression data was calculated and represented. The qRT-PCR results were averaged from three (or two in the case of *SPCH^{PPP}/spch-3*) independent experiments, with the error bar representing the SE of the mean.



Supplemental Figure 14. Biological processes overrepresented in the *spch-5* DEGs.

Genes differentially expressed in *spch-5* as compared to Col-0 were analyzed with Gene Ontology (GO) term enrichment by means of ClueGO. Circles represent GO categories and lines depict relationships between nodes. Only the main categories enriched in DEGs in *spch-5* and the involved main nodes are shown. Nodes containing down-regulated genes are in green; nodes containing both up- and down-regulated genes are in grey; no nodes were found with only up-regulated genes. Node size is relative to its *P*-value (inset). **(A)** Cell division and expansion. **(B)** Stomatal and epidermal development. **(C)** Hormonal responses. **(D)** Histone kinases