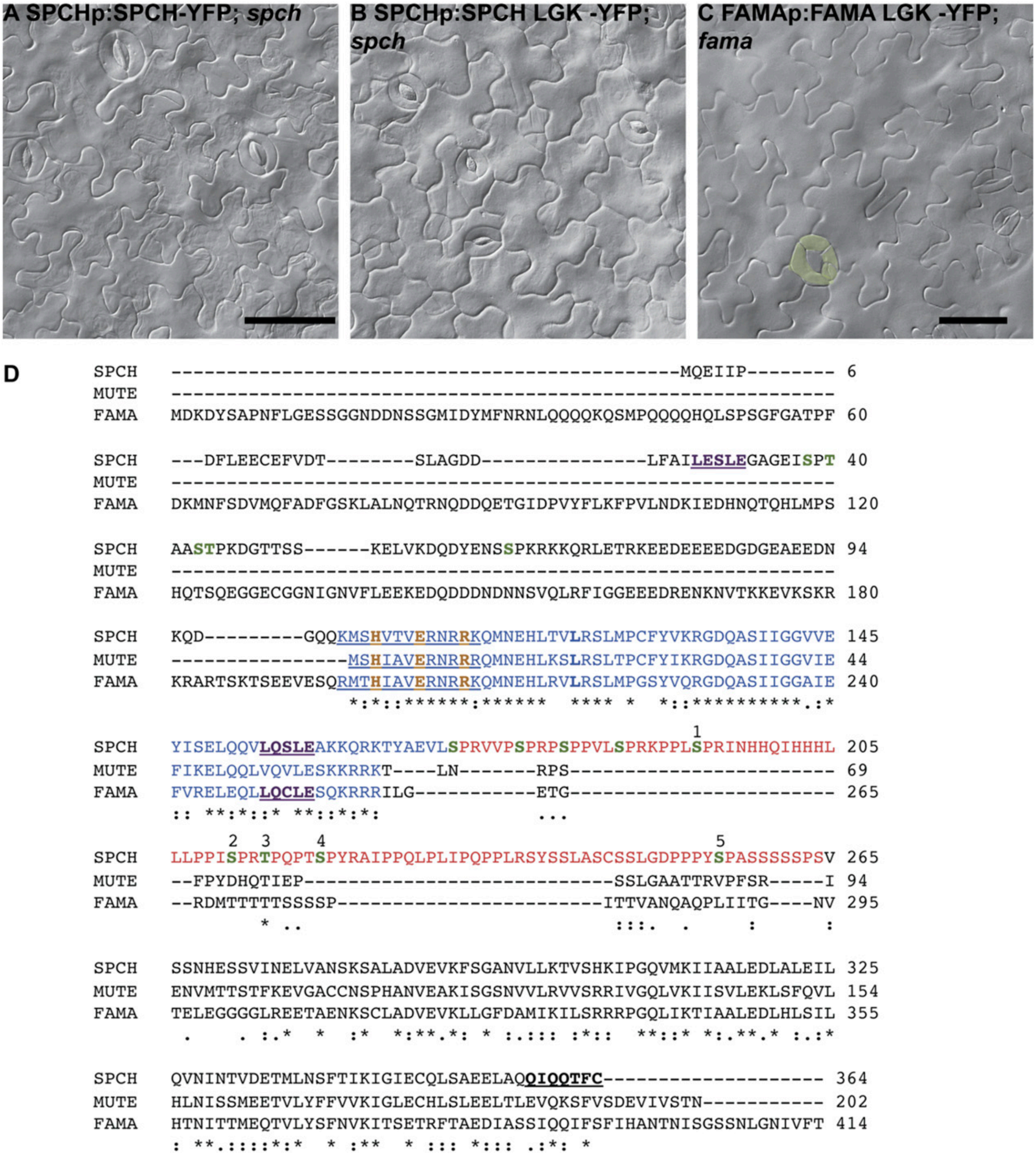
Supporting Information

Davies and Bergmann 10.1073/pnas.1411766111



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SPCH     ----------------------------------------------MQEIIP-------- 6
MUTE     ------------------------------------------------------------
FAMA     MDKDYSAPNFLGESSGGNDDNSSGMIDYMFNRNLQQQQKQSMPQQQQHQLSPSGFGATPF 60

SPCH     ---DFLEECEFVDT--------SLAGDD---------------LFAILESLEGAGEISPT 40
MUTE     ------------------------------------------------------------
FAMA     DKMNFSDVMQFADFGSKLALNQTRNQDDQETGIDPVYFLKFPVLNDKIEDHNQTQHLMPS 120

SPCH     AASTPKDGTTSS------KELVKDQDYENSSPKRKKQRLETRKEEDEEEEDGDGEAEEDN 94
MUTE     ------------------------------------------------------------
FAMA     HQTSQEGGECGGNIGNVFLEEKEDQDDDNDNNSVQLRFIGGEEEDRENKNVTKKEVKSKR 180

SPCH     KQD---------GQQKMSHVTVERNRRKQMNEHLTVLRSLMPCFYVKRGDQASIIGGVVE 145
MUTE     ----------------MSHIAVERNRRRQMNEHLKSLRSLTPCFYIKRGDQASIIGGVIE 44
FAMA     KRARTSKTSEEVESQRMTHIAVERNRRKQMNEHLRVLRSLMPGSYVQRGDQASIIGGAIE 240
                         *:*::******:******  **** *  *::**********.:*

                                                        1
SPCH     YISELQQVLQSLEAKKQRKTYAEVLSPRVVPSPRPSPPVLSPRKPPLSPRINHHQIHHHL 205
MUTE     FIKELQQLVQVLESKKRRKT----LN-------RPS------------------------ 69
FAMA     FVRELEQLLQCLESQKRRRILG-----------ETG------------------------ 265
         :: **:*::* **::*:*:              ...

              2  3    4                                   5
SPCH     LLPPISPRTPQPTSPYRAIPPQLPLIPQPPLRSYSSLASCSSLGDPPPYSPASSSSSPSV 265
MUTE     --FPYDHQTIEP----------------------------SSLGAATTRVPFSR-----I 94
FAMA     --RDMTTTTTSSSSP------------------------ITTVANQAQPLIITG----NV 295
                 * ..                            :::.  .     :      :

SPCH     SSNHESSVINELVANSKSALADVEVKFSGANVLLKTVSHKIPGQVMKIIAALEDLALEIL 325
MUTE     ENVMTTSTFKEVGACCNSPHANVEAKISGSNVVLRVVSRRIVGQLVKIISVLEKLSFQVL 154
FAMA     TELEGGGGLREETAENKSCLADVEVKLLGFDAMIKILSRRRPGQLIKTIAALEDLHLSIL 355
          .    . :.*  *  :*  *:**.*: * :.::: :*::  **::* *:.**.* :.:*

SPCH     QVNINTVDETMLNSFTIKIGIECQLSAEELAQQIQQTFC-------------------- 364
MUTE     HLNISSMEETVLYFFVVKIGLECHLSLEELTLEVQKSFVSDEVIVSTN----------- 202
FAMA     HTNITTMEQTVLYSFNVKITSETRFTAEDIASSIQQIFSFIHANTNISGSSNLGNIVFT 414
         : **.::::*:*  * :**  * ::: *::: .:*: *
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Fig. S1. SPCH does not require its potential RBR interaction motifs. (*A*–*C*) Differential interference contrast images of 6-d postgermination abaxial cotyledons of SPCHp:SPCH YFP *spch* (*A*), SPCHp:SPCH$^{\mathrm{LGK}}$ YFP *spch* (*B*), and FAMAp:FAMA$^{\mathrm{LGK}}$ YFP *fama* (*C*). (Scale bars: 50 μm.) *A* and *B* are at the same magnification. The FAMA$^{\mathrm{LGK}}$ YFP phenotype of a stoma in stoma is highlighted in green. (*D*) ClustalW2 alignment of *Arabidopsis* SPCH, MUTE, and FAMA full-protein sequences. The basic region of the bHLH domain (blue) is underlined, putative DNA-contacting residues are in orange, and the SPCH MPKTD is in pink. Potential RBR interaction motifs are underlined in purple. Asterisks indicate identical residues; colons, conserved substitutions; dots, semiconserved substitutions. Putative phosphorylation sites (1, 2) are in green, with numbers above corresponding to the notation in ref. 1. Bold underlined residues at the C terminus of SPCH are deleted in the spch-1 allele.

1. Lampard GR, Macalister CA, Bergmann DC (2008) *Arabidopsis* stomatal initiation is controlled by MAPK-mediated regulation of the bHLH SPEECHLESS. *Science* 322(5904):1113–1116.
2. Gudesblat GE, et al. (2012) SPEECHLESS integrates brassinosteroid and stomata signalling pathways. *Nat Cell Biol* 14(5):548–554.

Table S1. Transgenic constructs examined for stomatal production in bHLH mutants

			T2 individuals		
Construct (T1) (all have a C-terminal YFP tag)	Line	Rescues	Stomata-producing	No stomata	Notes
pMDC7 SPCH 1–5A in mute/+; no estradiol	A	No	15	37	
pMDC7 SPCH 1–5A in mute/+; 10 μM estradiol	A	Yes	117	15	
MUTEp:SPCH 1–5A mute/+	A	Yes	74	4	
MUTEp:SPCH 1–5A mute/+	B	Yes	64	0	
MUTEp:SPCH 1–5A mute $^{-/-}$	C	Yes, T1 homo	NA	NA	
MUTEp:SPCH 1–5A mute $^{-/-}$	D	Yes, T1 homo	NA	NA	
MUTEp:SPCH 1–4A mute$^{-/-}$	A	Yes, T1 homo	NA	NA	
MUTEp:SPCH 1–4A mute$^{-/-}$	B	Yes, T1 homo	NA	NA	
MUTEp:SPCH 5A mute$^{-/+}$	A	Yes	33	0	1
MUTEp:SPCH 5A mute$^{-/+}$	B	Yes	60	0	1
MUTEp:SPCH 5A mute$^{-/+}$	C	Yes	27	2	1
MUTEp:SPCH 5A mute$^{-/-}$	D	Yes, T1 homo	NA	NA	2
MUTEp:SPCH (WT SPCH) mute/+	A	Ambiguous	66	15	3
MUTEp:SPCH (WT SPCH) mute/+	B	Ambiguous	70	2	4
MUTEp:SPCH Δ93 in mute/+	A	Yes	62	0	
MUTEp:SPCH Δ93 in mute$^{-/-}$	B	Yes, T1 homo	NA	NA	
MUTEp:SPCH ΔN and Δ93 in mute/+	A	Yes	50	2	
MUTEp:SPCH ΔN and Δ93 in mute$^{-/-}$	B	Yes, T1 homo	NA	NA	
SPCHp: 2–4A PGG in spch/+	A	Yes	61	2	
SPCHp: 2–4A PGG in spch/+	B	Yes	54	9	
SPCHp: 2–4A PGG in spch/+	C	Yes	54	8	
SPCHp: 2–4A PGG in spch/+	D	Yes	75	3	
SPCHp: 2–4A PGG in spch/+	E	Yes	64	1	
SPCHp: 2–4A PGG in spch/+	F	Yes	76	0	
SPCHp:SPCHPGG in spch-3/+	A	Yes	71	8	
SPCHp:SPCHPGG in spch-3/+	B	No	37	11	
SPCHp:SPCHPGG in spch-3/+	C	Yes	70	5	
SPCHp:SPCHPGG in spch-3/+	D	Yes	65	11	
SPCHp:SPCHPGG in spch-3/+	E	Yes	75	2	
SPCHp:SPCHPGG in spch-3/+	F	No	64	14	
MUTEp: FAMA PGG in mute/+	A	Yes	84	5	
MUTEp:MUTE PGG in mute/+	A	Yes	70	0	
MUTEp:MUTE PGG in mute/+	B	Yes	79	0	
MUTEp:SPCH 1–4A PGG YFP in mute/+	A	Ambiguous	113	18	5
MUTEp:MUTE in mute/+	A	Yes	46	3	
FAMAp:SPCH in fama/+	A	No	52	13	
FAMAp:SPCH in fama/+	B	No	40	10	
FAMAp:SPCH in fama/+	C	No	31	12	
FAMAp:SPCH 2–4A in fama/+	A	No	43	14	
FAMAp:SPCH 2–4A in fama/+	B	No	50	12	
FAMAp:SPCH 2–4A in fama/+	C	No	28	15	
FAMAp:SPCH 2–4A in fama/+	D	No	40	12	
FAMAp:SPCH 1–4A in fama/+	A	No	40	19	
FAMAp:SPCH 1–4A in fama/+	B	No	39	7	
FAMAp:SPCH 1–4A in fama/+	C	No	41	10	
FAMAp:SPCH 1–4A in fama/+	D	No	40	11	
FAMAp:SPCH 1–5A in fama/+	A	No	36	9	
FAMAp:FAMA PGG in fama/+	A	No	65	13	
SPCHp::SPCH-LGK in spch/+	A	Yes	109	8	
SPCHp::SPCH-LGK in spch/+	B	Yes	107	10	
SPCHp::SPCH-LGK in spch/+	C	Yes	109	10	
SPCHp:SPCHΔC7 in spch-3/+	A	No	27	8	
SPCHp:SPCHΔC7 in spch-3/+	B	No	27	10	
SPCHp:SPCHΔC7 in spch-3/+	C	No	30	12	
SPCHp:SPCHΔC7 in spch-3/+	D	No	28	10	
SPCHp:SPCHΔC7 in spch-3/+	E	No	28	10	
SPCHp:SPCH 2–4A ΔC7 in spch-3/+	A	No	28	11	
SPCHp:SPCH 2–4A ΔC7 in spch-3/+	B	No	29	9	
SPCHp:SPCH 2–4A ΔC7 in spch-3/+	C	No	22	10	
SPCHp:SPCH 2–4A ΔC7 in spch-3/+	D	No	42	13	
SPCHp:SPCH 2–4A ΔC7 in spch-3/+	E	No	28	10	
SPCHp:SPCH 2–4A ΔC7 in spch-3/+	F	No	27	12	

Notes: (1) Many T2 individuals have >50% arrested lineages. (2) In the T1 plant, >50% lineages were arrested. (3) Three or more individuals had very few stomata (one to four per cotyledon). (4) Seven or more individuals had very few stomata (one to four per cotyledon). (5) Seven individuals had no stomata and resembled mute; 11 individuals had no stomata but additional cell divisions; 11 individuals had additional cell divisions and a few stomata per cotyledon; and 102 individuals produced stomata throughout the epidermis.

Table S2. Primers used for cloning

Forward (written 5′-3′)	Reverse	Template	Construct
CACC ATG CAG GAG ATA ATA CCG GAT TTT CT	GGG CCC TTA CTT GTA CAG CTC	pHGY SPCHp:SPCH variants (1)	pENTR SPCH 1–4A YFP pENTR SPCH 1–5A YFP
CAA AAG ATG TCT CCT GTA ACC GTG GGA CGT AAC CGG GGA AAG CAA	TTG CTT TCC CCG GTT ACG TCC CAC GGT TAC AGG AGA CAT CTT TTG	pENTR SPCH, pENTR SPCH 2–4A (1) and pENTR SPCH 1–4A YFP generated above	pENTR SPCH PGG pENTR SPCH 2–4A PGG pENTR SPCH 1–4A PGG YFP
CACC ATG AAGATGTCTCATG TAACCGTGG	GCA GAA TGT TTG CTG AAT TTG TTG	pENTR SPCH delta 93 (1)	pENTR SPCH delta N delta 93
CTC TAC CAA TGG AGG ATC CAA GGG TGG GCG	CGC CCA CCC TTG GAT CCT CCA TTG GTA GAG	pENTR MUTE with stop (2)	pENTR MUTE (mutation of stop codon stop to glycine)
CACC ATG TCT CCC ATC GCT GTT GGA AGG AAT CGA GGA AGG CAA ATG	CAT TTG CCT TCC TCG ATT CCT TCC AAC AGC GAT GGG AGA CAT GGTG	pENTR MUTE (no stop) generated above	pENTR MUTE PGG (no stop)
G ATG ACT CCT ATC GCG GTC GGA AGA AAC CGT GGG AAG CAA AT	ATT TGC TTC CCA CGG TTT CTT CCG ACC GCG ATA GGA GTC ATC	pENTR FAMA (3)	pENTR FAMA PGG
GAT CTA TTT GCC ATC TTA GAG GGT CTT AAA GGT GCC GGA GAG ATA	TAT CTC TCC GGC ACC TTT AAG ACC CTC TAA GAT GGC AAA TAG ATC	pENTR SPCH	pENTR SPCH LGK product 1
TTA CAA CAA GTT CTC CAA GGT TTG AAA GCC AAG AAA CAA CGT AAA A	TTT TAC GTT GTT TCT TGG CTT TCA AAC CTT GGA GAA CTT GTT GTA A	pENTR SPCH LGK product 1	pENTR SPCH LGK final
GGGG ACA ACT TTG TAT AGA AAA GTT G AAG CTT ATC AAA GAA AAA AC	GGGG AC TGC TTT TTT GTA CAA ACT TG TAA CCG GTG GAT TCA GGG	pAR169 ATML1p::mCitrine-RCI2A (4)	pDonr P4 p1R ATML1pro (recombination reaction)
CACC TCCGGAGCAGCTGCGGCTG	ATG AAA TGA TAG CGT AAG GTA TAA GG	pAR169 ATML1p::mCitrine-RCI2A (4)	pENTR RCI2A
AT GCG GCC GC ATG GTG AGC AAG GGC GAG GAG	AT GCG GCC GC CTT GTA CAG CTC GTC CAT GCC	pcr2.1 TOPO mCherry	pENTR mCherry RCI2A (ligated mCherry into above construct using NOT1 restriction site)

1. Lampard GR, Macalister CA, Bergmann DC (2008) *Arabidopsis* stomatal initiation is controlled by MAPK-mediated regulation of the bHLH SPEECHLESS. *Science* 322(5904):1113–1116.
2. MacAlister CA, Ohashi-Ito K, Bergmann DC (2007) Transcription factor control of asymmetric cell divisions that establish the stomatal lineage. *Nature* 445(7127):537–540.
3. Ohashi-Ito K, Bergmann DC (2006) *Arabidopsis* FAMA controls the final proliferation/differentiation switch during stomatal development. *Plant Cell* 18(10):2493–2505.
4. Roeder AH, et al. (2010) Variability in the control of cell division underlies sepal epidermal patterning in *Arabidopsis thaliana. PLoS Biol* 8(5):e1000367.