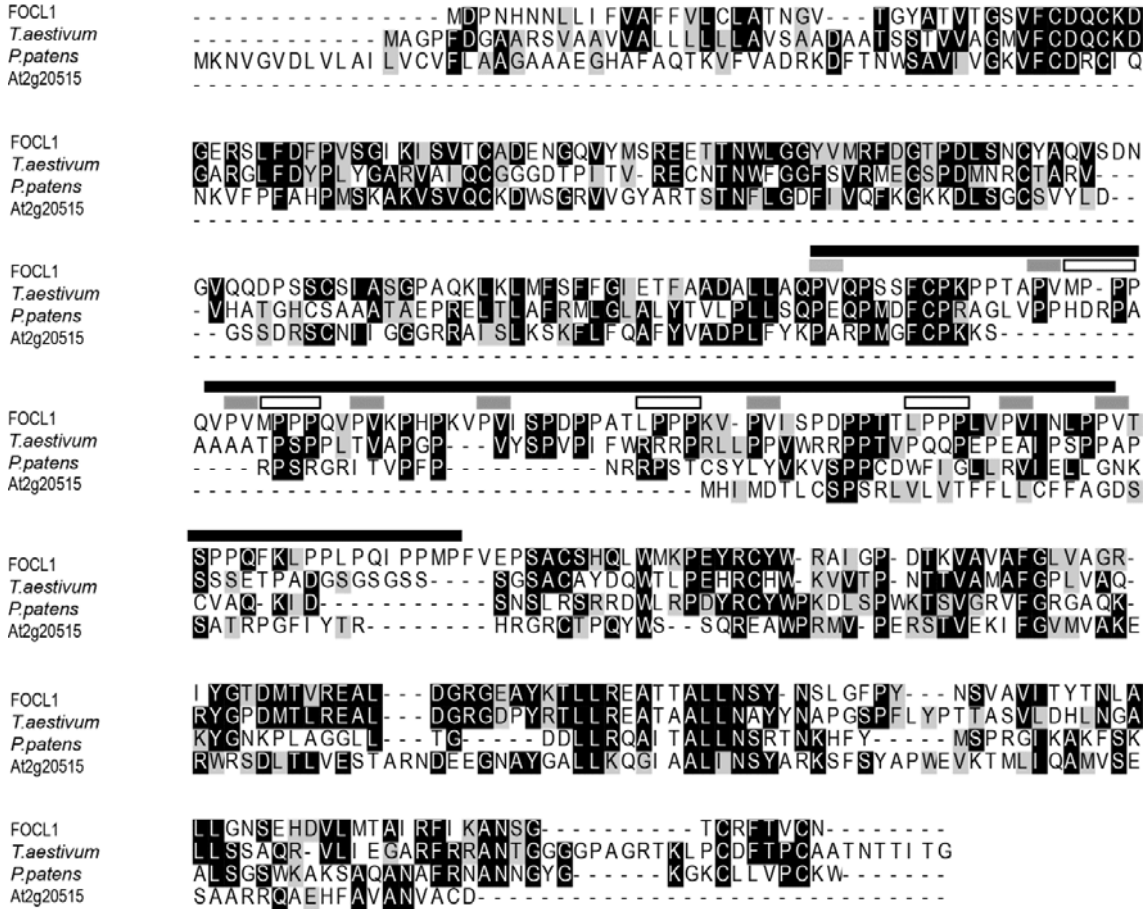


1 **Supplementary Figures**



2  
3 **Figure S1** Alignment of FOCL1 amino acid sequence with orthologues from wheat, and  
4 *Physcomitrella patens* and with closest *Arabidopsis* homologue, At2g20515. Black shading  
5 indicates identity, grey shading indicates similarity. Deduced protein sequences were aligned  
6 with Multalin (<http://multalin.toulouse.inra.fr/multalin>) and displayed using Boxshade  
7 ([http://www.ch.embnet.org/software/BOX\\_form.html](http://www.ch.embnet.org/software/BOX_form.html)). Potential proline hydroxylation sites PV  
8 and PPP are labelled grey and white respectively. Proline rich region is indicated in blue.  
9

10

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FOCL1 MDPNHNLLI FVAF FVLCLATNGVTGYATVITGSVFC DQC- - KDGERSLFD FVPVSGI KI SV
AGP31  - - - - - MGFI GKSVLVSLVAL WCFTSSSVFTEEVNHKTQTPSLAPAPAPYHHGHH

FOCL1 TCADENGQVYMSREETT NWLGGYVMRFDGTPDL SNCYAQVSDNGVQQDPSSCSI ASGPAQ
AGP31  HPHPPHHHPHPHPHPHPAKSPVKPPVKA PVSPPAKPPVKPPVYPPTKAPVKPPTKPPV

FOCL1 K L K L M F S F F G I E T F A A D A L L A Q P V Q P S S F C P K P P T A P V M P P P Q V P V M P P Q V P V K P H P K V
AGP31  K P P V S P P V K P P V K P P V P P T K A P V K P P T K P P V K P P V Y P P T K A P V K P P T K P P V K P P V S P

FOCL1 P V I S P D P P A T L P P P K V P V I S P D P P T T L P P P L V P V I N L P P V T S P P Q F K L P P L P Q I P P M P F V
AGP31  P A K P P V K P P V Y P P T K A P V K P P V S P P T K P P V T P P V Y P P K F N R S L V A V R G T V Y C K S C K Y

FOCL1 E P S A C S H Q L W M K P E Y R C Y W R A I G P D T K V A V A F G L V A G R I Y G T D M T V R E A L D G R G E A Y K T L
AGP31  A A F N T L L G A K P I E G A T V K L V C K S K N I T A E T T T D K N G Y F L L L A P K T V T N F G F R G C R V Y

FOCL1 L R E A T T A L L N S Y N S L G F P Y N S V A V I T Y T N L A L L G N S E H D V L M T A I R F I K A N S G T C R F T V C
AGP31  L V K S K D Y K C S K V S K L F G G D V G A E L K P E K K L G K S T V V V N K L V Y G L F N V G P F A F N P S

FOCL1 N - -
AGP31  C P K
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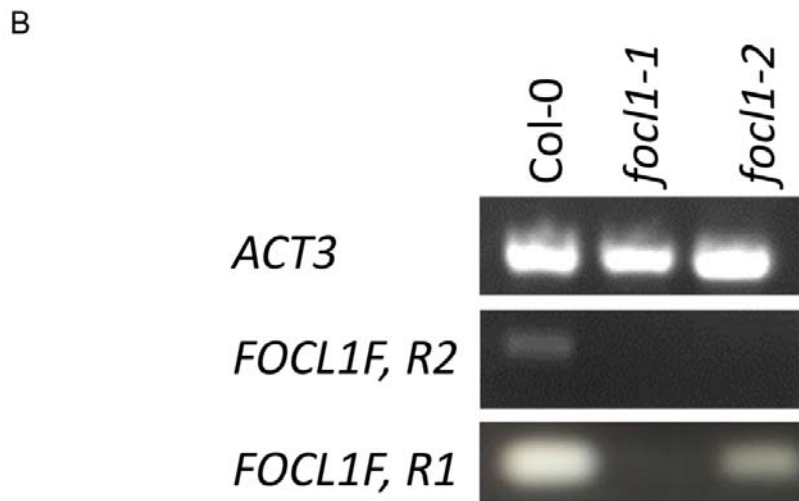
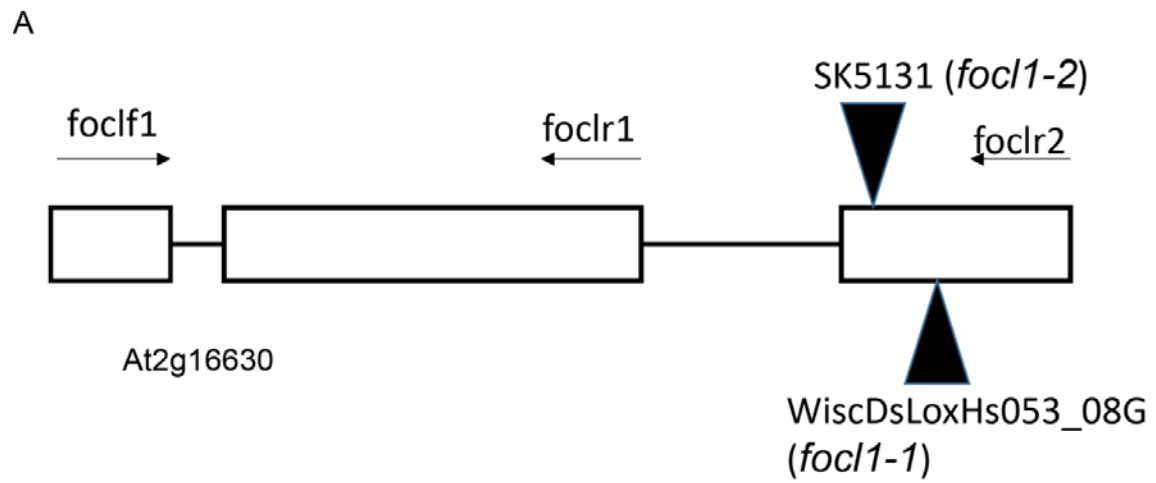
12

13 **Figure S2**

14 Alignment of deduced amino acid sequences of FOCL1 and AGP31. Conserved residues  
15 are shaded. Black shading indicates identity, Grey shading indicates similarity. Background  
16 shading indicates proline-rich region. Repeated sequence indicated above sequence.

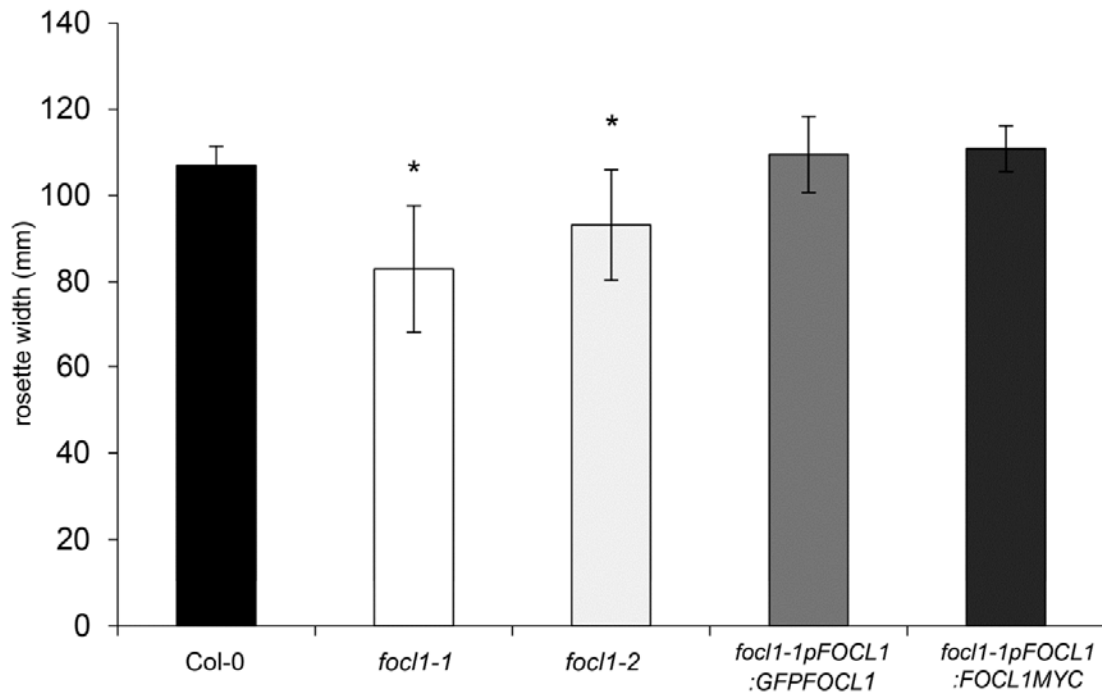
17 Sequences were aligned as for Figure S1

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**Figure S3. Insertion positions and expression of *focl1-1* and *focl1-2***  
*focl1-1* and *focl1-2* are null alleles. cDNA was synthesised from two week old MS plate grown seedlings RNA and amplified for 30 cycles with primers indicated.

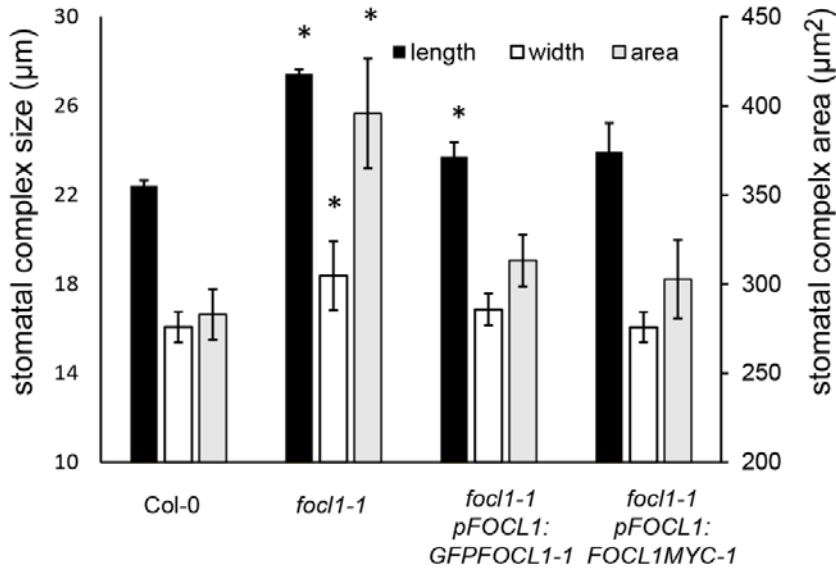


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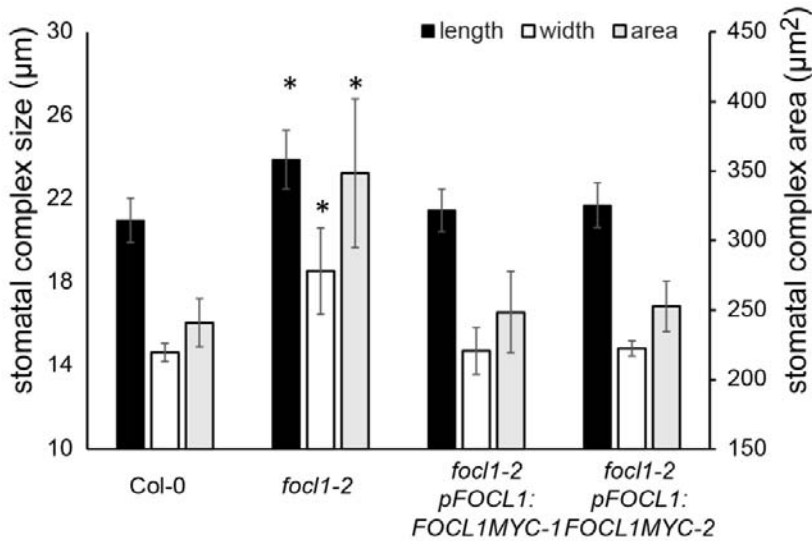
**Figure S4 Rosette widths of *focl1-1* and *focl1-2***

Rosette width at bolting of indicated genotypes (n=4-7 plants) \*= significantly different from Col-0 p<0.05

A



B



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**Figure S5 Complementation of *focl1* restores stomatal complex size to wild type**

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**A Complementation of *focl1-1* restores abaxial stomatal complex size to wild type.** T1

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lines of *focl1-1* transformed with a 3kb genomic construct with either an N-terminal GFP or

34

C-terminal MYC tag reduced stomatal size to wild-type (n=4 plants, 10-20 guard cell pairs

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from 1 leaf of each) \*= significantly different from Col-0 p<0.05

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**B. Complementation of *focl1-2* restores abaxial stomatal complex size to wild type.**

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Two independent T1 lines transformed with a 3kb genomic construct consisting 2kb of

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upstream sequences and in-frame C-terminal MYC tag restores guard cell pair sizes to wild-

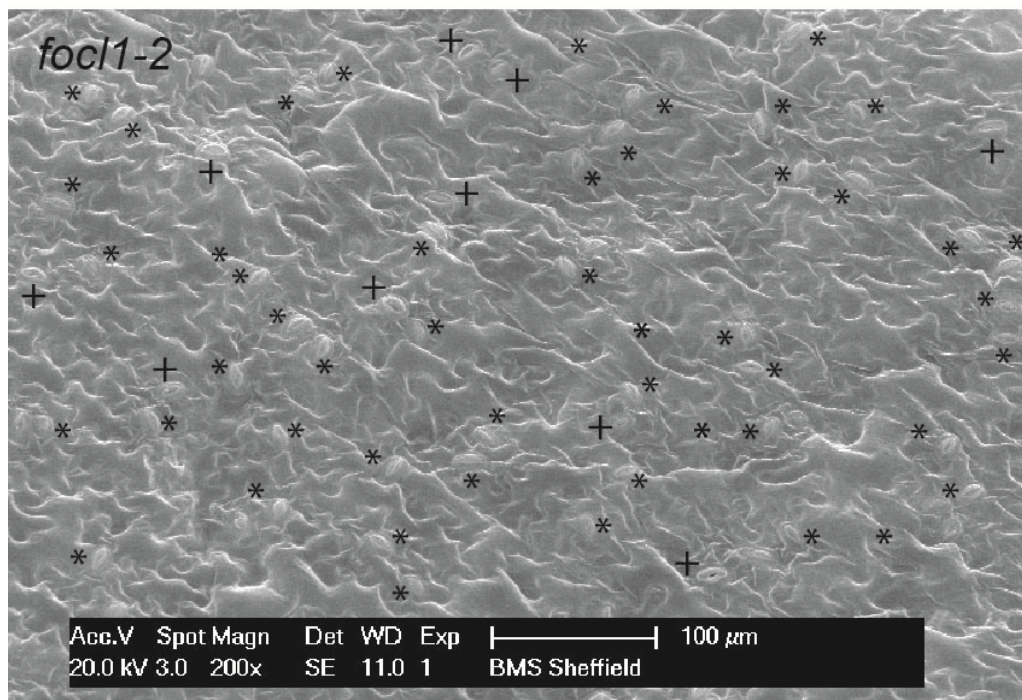
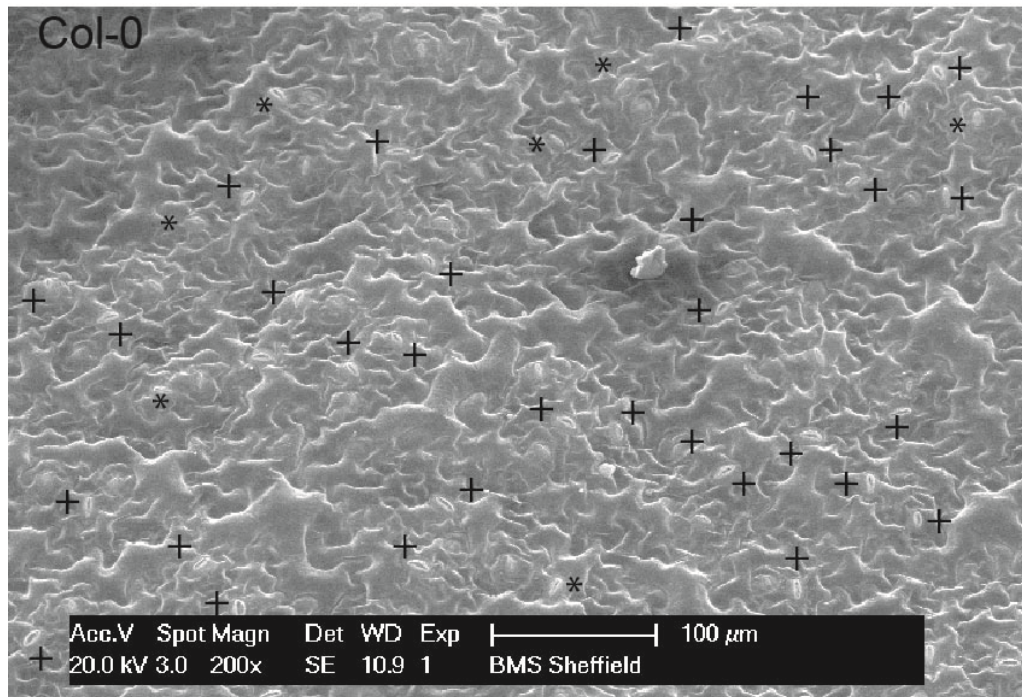
39

type (n=4 plants, 10-20 guard cell pairs from 1 leaf of each) \*= significantly different from

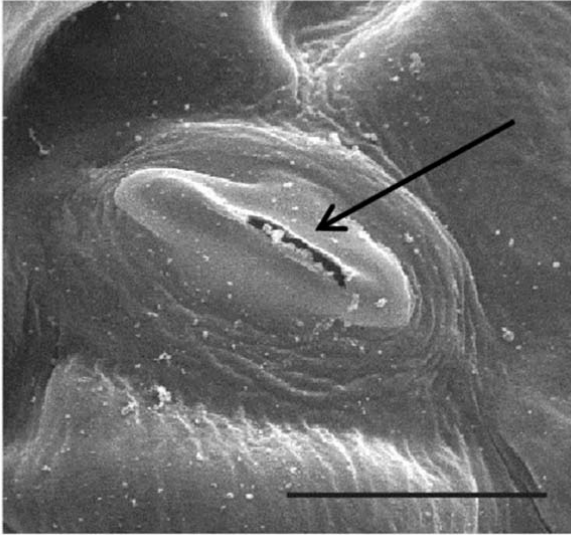
40

Col-0 p<0.05

41



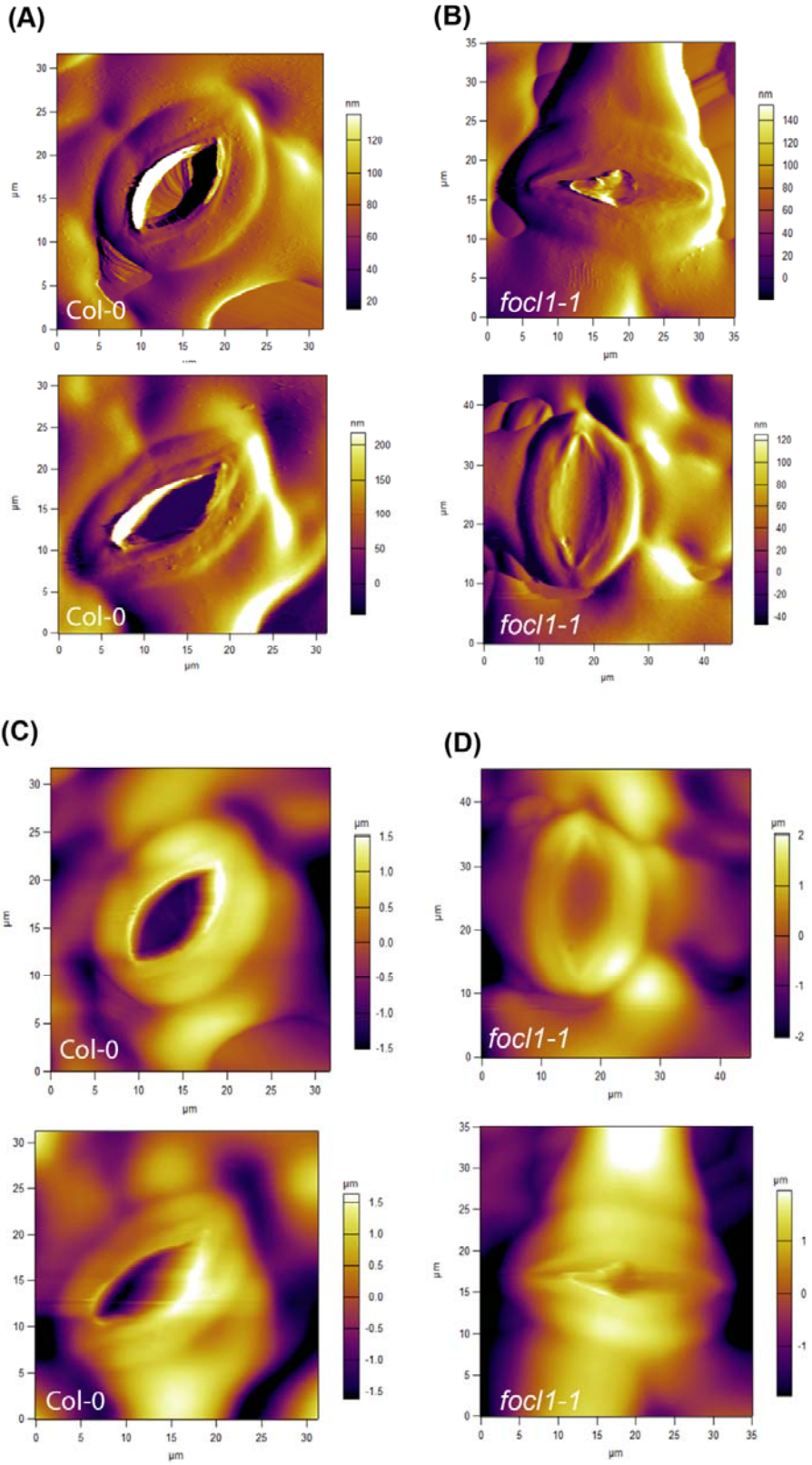
42  
 43 **Figure S6 Wide view of abaxial epidermis of mature leaves of Col-0 and *focl1-2*.**  
 44 Stomata that are judged to have a partial or completely open OCL in a low magnification  
 45 image are indicated with +, closed pores with\*.



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47 **Figure S7** SEM of *focl1-1* stomate showing partial opening (indicated with arrow). Bar

48 =10 $\mu$ m.



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**Figure S8**

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**A & B** .Deflection images for two stomata from *Col* and *focl1-1*. **C & D** Height data for two

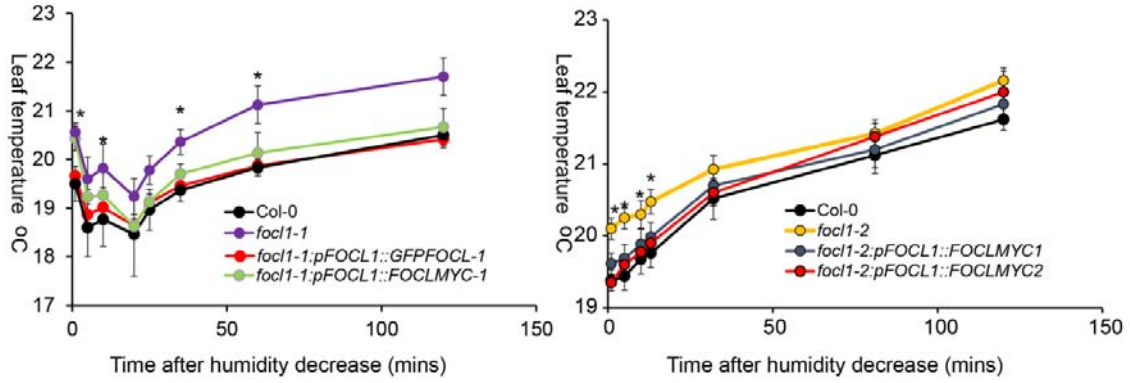
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stomates from *Col-0*



53 **Figure S9** Complementation of *focl1-1* and *focl1-2* with either and N –terminally located GFP  
 54 tag or C –terminally located MYC tag restores leaf temperature to wild type.  
 55 Plants were transferred from high humidity and temperature measured by infra-red  
 56 thermography (n=3-4 plants, 1 measurement from 3 leaves of each) \*= significantly different  
 57 from Col-0 p<0.05) \*

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