

## Supplemental Figures and Tables

### **Three CNGC Family Members, CNGC5, CNGC6 and CNGC9, Are Required for Constitutive Growth of Arabidopsis Root Hairs as Ca<sup>2+</sup>-Permeable Channels**

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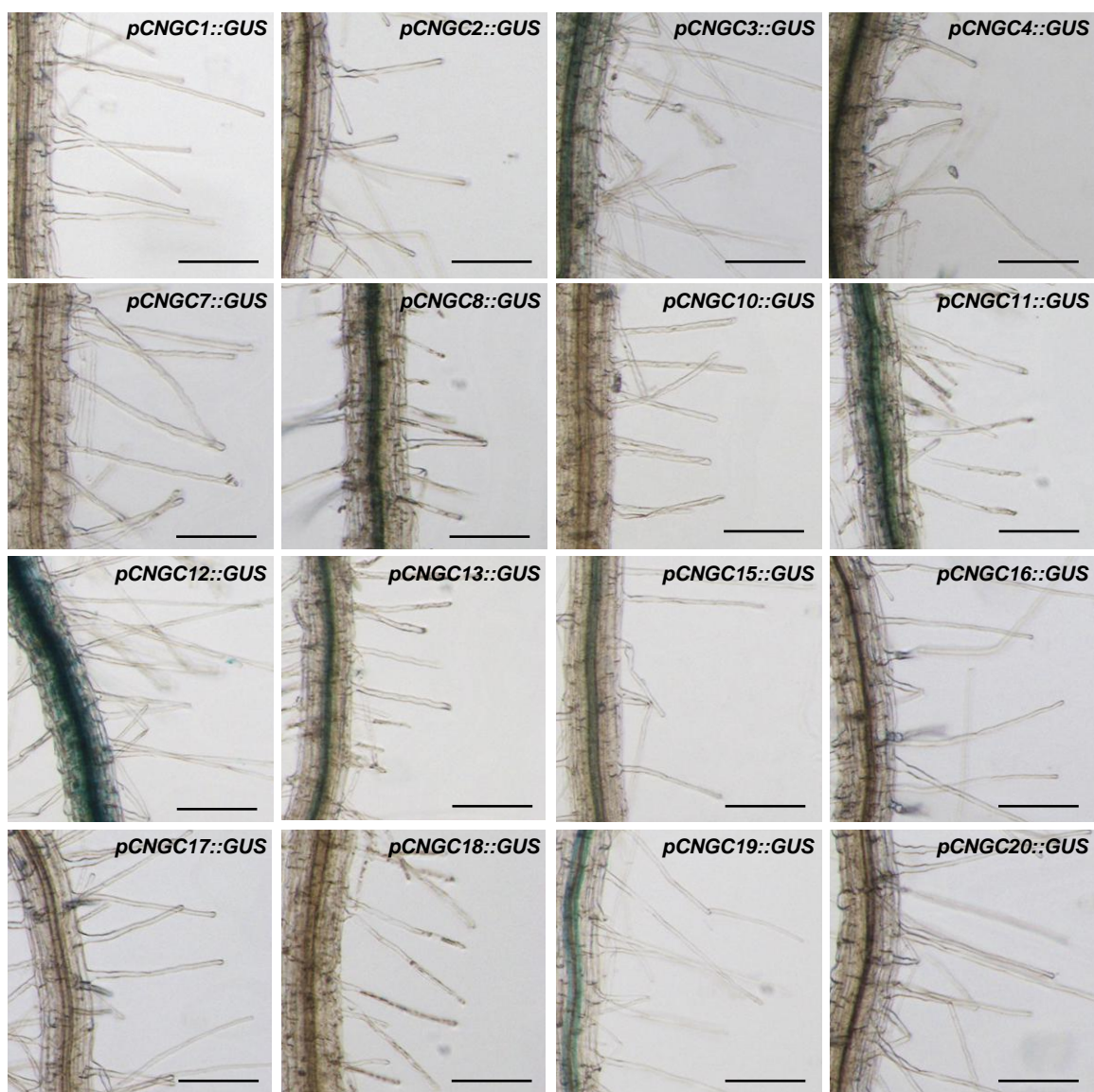
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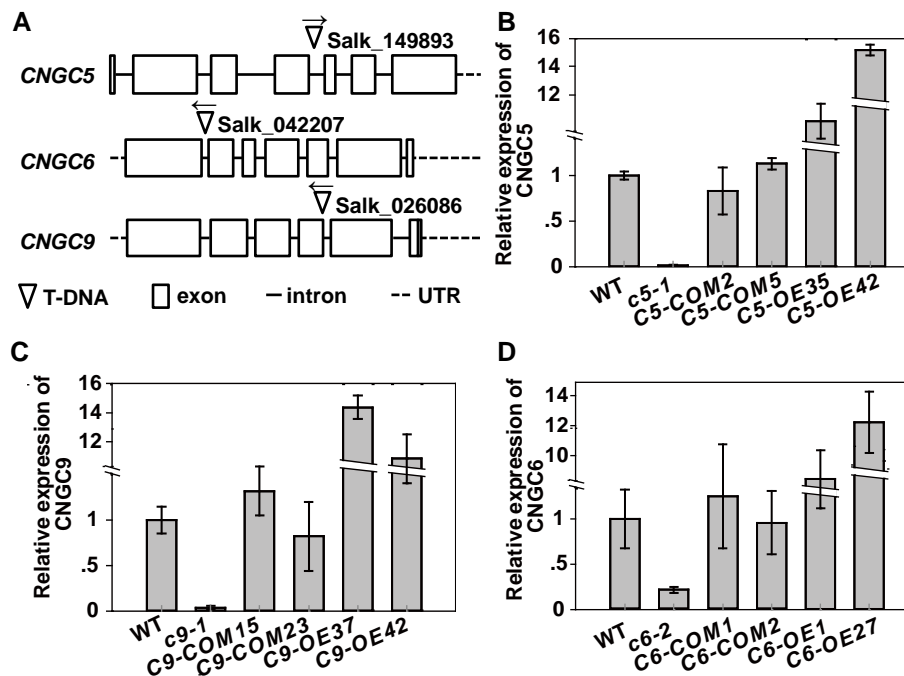
The supplemental file includes 8 Supplemental Figures and 2 Supplemental Tables.

## Supplemental Figure 1



**Supplemental Figure 1.** GUS Staining Analysis for CNGC Members Except CNGC5, 6, 9 and 14 in Arabidopsis RHs. Scale bars equal to 0.1 mm.

## Supplemental Figure 2

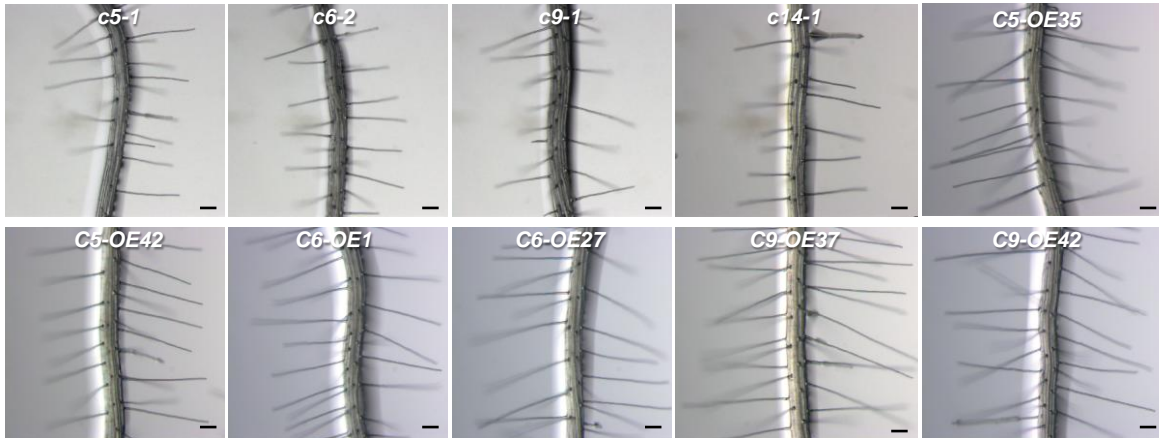


**Supplemental Figure 2.** qRT-PCR Analysis of *Shrh1* Mutant and Transgenic Lines in Arabidopsis. (Supports Figure 1.)

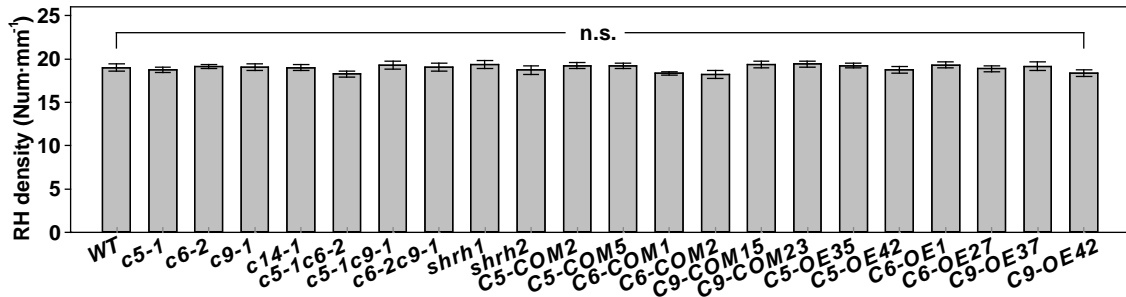
(A) Schematic models showing T-DNA insertion sites in *CNGC5*, *CNGC6* and *CNGC9*. (B) to (D) qRT-PCR analysis showing the relative expression levels of *CNGC5* (B), *CNGC9* (C) and *CNGC6* (D) in Arabidopsis plants. 3 biological replicates were conducted. Error bars depict means  $\pm$  SEM.

### Supplemental Figure 3

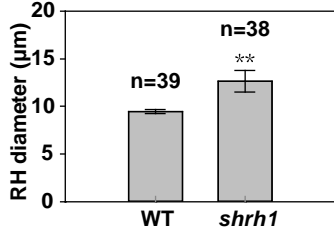
**A**



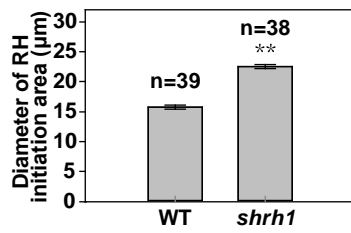
**B**



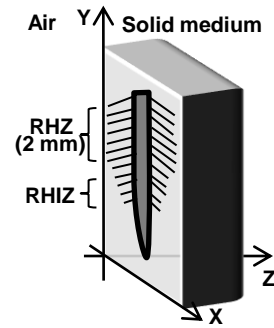
**C**



**D**



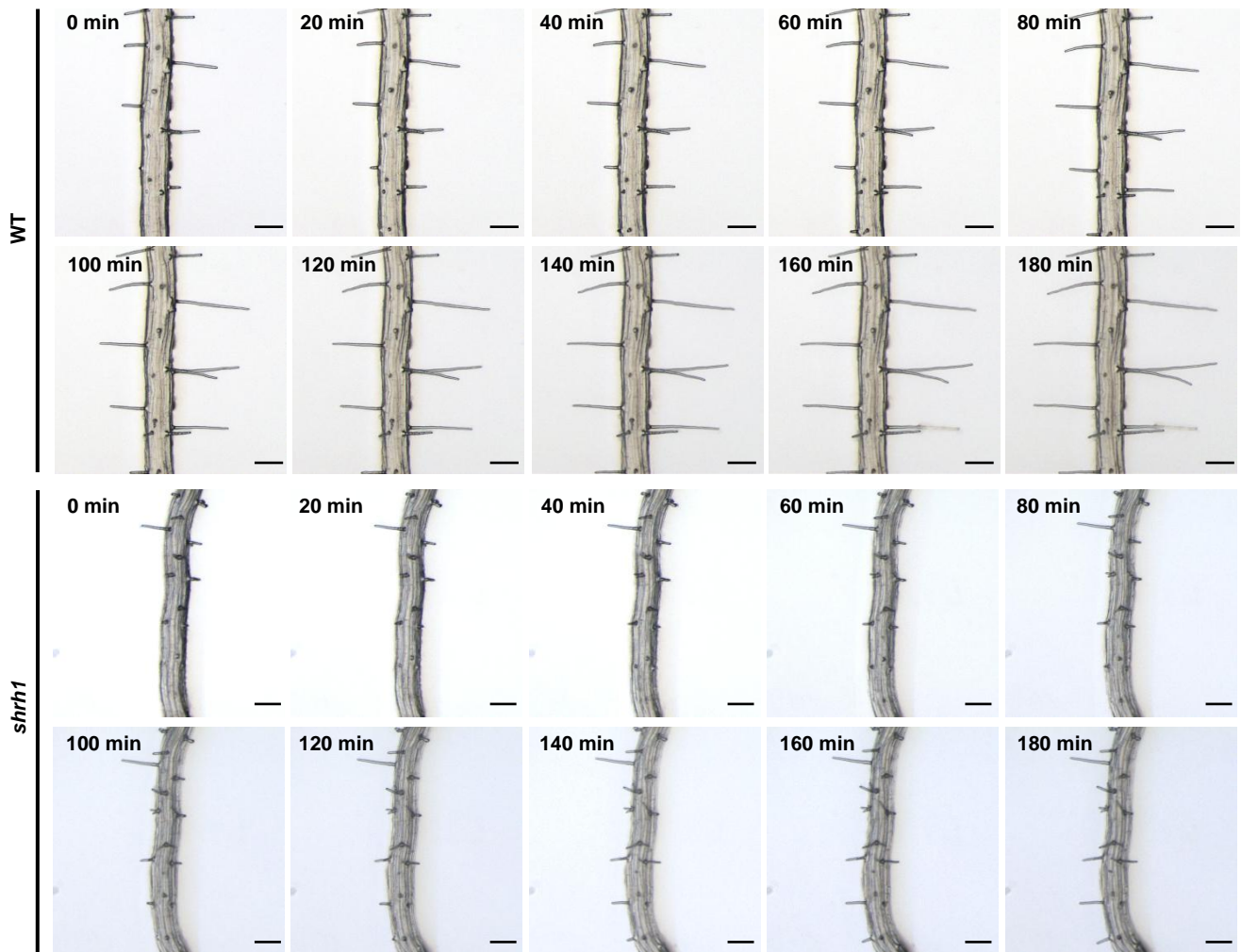
**E**



**Supplemental Figure 3. RH Phenotypic Analysis in Arabidopsis.**

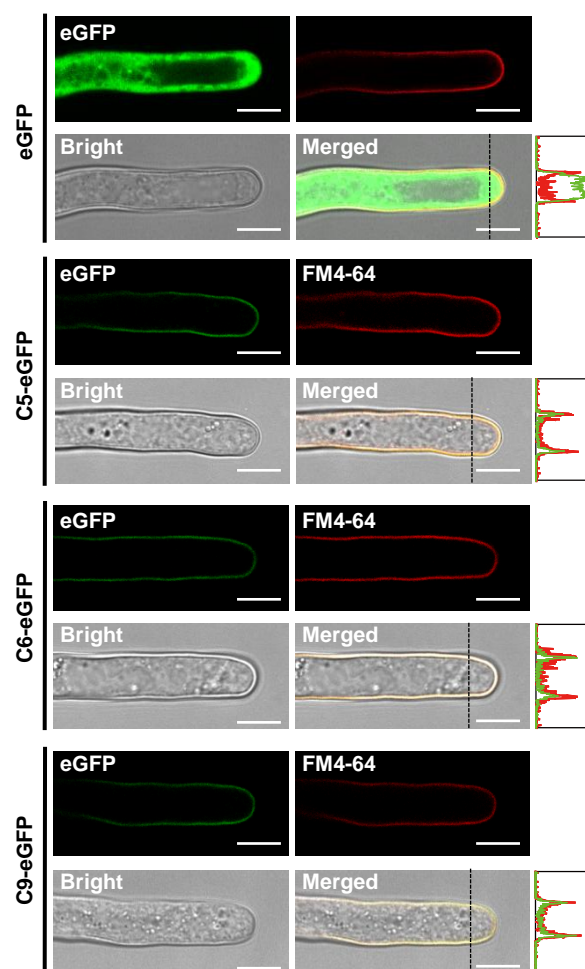
(A) Images of typical roots and hairs of Arabidopsis plants. See Figure 1B for wild type. (B) Statistical analysis of RH density. (C) Average diameters of RH trunks close to RH tips. (D) Average diameters of RH initiation area. (E) Diagram showing RHZ for RH phenotypic analysis, including RH length, branching rate, rupturing rate and density, and RHIZ for time-lapse analysis of RH initiating and elongating growth. *c5-1*, *c6-2*, *c9-1*, *c14-1*, *c5-1c6-2*, *c5-1c9-1*, *c6-2c9-1*, *C5-COM2*, *C5-COM5*, *C6-COM1*, *C6-COM2*, *C9-COM15*, *C9-COM23*, *C5-OE35*, *C5-OE42*, *C6-OE1*, *C6-OE27*, *C9-OE37* and *C9-OE42* denote *cngc5-1*, *cngc6-2*, *cngc9-1*, *cngc14-1*, *cngc5-1cngc6-2*, *cngc5-1cngc9-1*, *cngc6-2cngc9-1*, *CNGC5-COM2*, *CNGC5-COM5*, *CNGC6-COM1*, *CNGC6-COM2*, *CNGC9-COM15*, *CNGC9-COM23*, *CNGC5-OE35*, *CNGC5-OE42*, *CNGC6-OE1*, *CNGC6-OE27*, *CNGC9-OE37* and *CNGC9-OE42*, respectively, for Supplemental Figure 1 and Figure 1. Scale bars equal to 0.1 mm in (A). No less than 30 roots and 200 RHs (7-10 RHs per root) were counted for RH density analysis, and n.s. denotes no significant difference between samples with  $P > 0.05$  (Kruskal-Wallis one-way ANOVA analysis) in (B). Letter n denotes the numbers of RHs analyzed for RH diameters, and \*\* denotes significant difference with  $P < 0.01$  versus wild type (Mann-Whitney Rank Sum Test) in (C) and (D). Error bars depict means  $\pm$  SEM.

## Supplemental Figure 4



**Supplemental Figure 4.** Time-Lapses of RH Growth in Air in Arabidopsis. Merged pictures from a set of optical sections are shown. The step for optical section is 22.5  $\mu\text{m}$ . A set of optical sections is composed of 15 sections for *shrh1* and 21 sections for wild type. The numbers of roots tested are 7 for wild type and 4 for *shrh1* mutant. Scale bars equal to 0.1 mm.

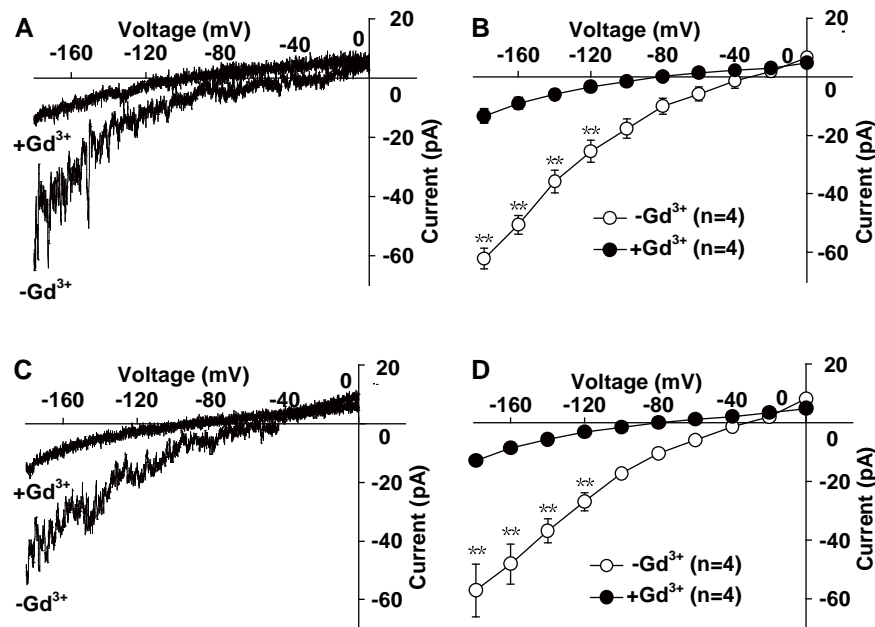
## Supplemental Figure 5



**Supplemental Figure 5.** CNGC5, 6 and 9 Are Localized in the Plasma Membrane of RHs. eGFP fluorescence, FM4-64 fluorescence, bright field, merged images and relative fluorescent intensity of eGFP (green) and RFP (red) along the dashed lines across the merged photos in RHs are shown. The number of RHs tested is 14 for CNGC5, 11 for CNGC6, 18 for CNGC9 and 15 for eGFP control. *C5-eGFP*, *C6-eGFP* and *C9-eGFP* denote *CNGC5-OE4*, *CNGC6-OE12* and *CNGC9-OE18*, respectively. Scale bars equal to 10  $\mu\text{m}$ .



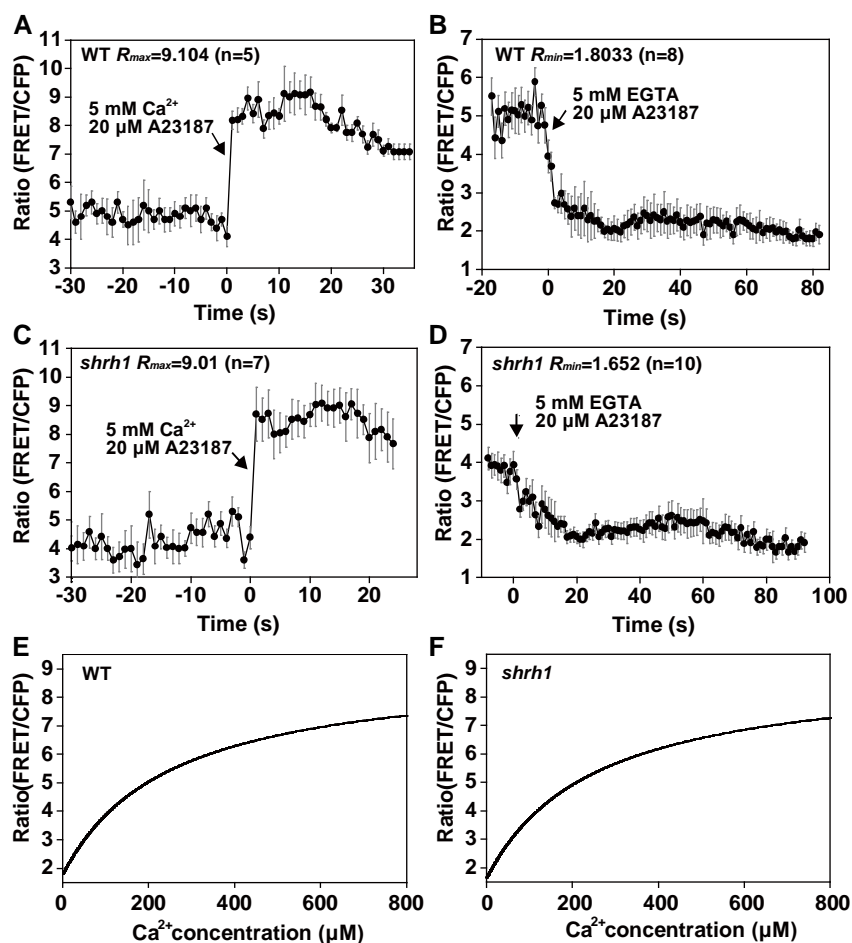
## Supplemental Figure 6



**Supplemental Figure 6.** Inward Channel Currents Recorded in HEK293T Cells Expressing either CNGC5 or CNGC6 Are Mainly Carried by Ca<sup>2+</sup>, Not Obviously by Na<sup>+</sup>. (A) and (B) Typical whole-cell recordings (A) and average current-voltage curves of steady-state whole-cell currents (B) recorded in HEK293T cells expressing *CNGC5*. (C) and (D) Typical whole-cell recordings (C) and average current-voltage curves of steady-state whole-cell currents (D) recorded in HEK293T cells expressing *CNGC6*. Letter n denotes the numbers of HEK293T cells tested, and \*\* denotes significant difference between Gd<sup>3+</sup>-free and +100  $\mu$ M Gd<sup>3+</sup> conditions at the same voltage with  $P < 0.01$  (Kruskal-Wallis one-way ANOVA analysis). Error bars depict means  $\pm$  SEM.



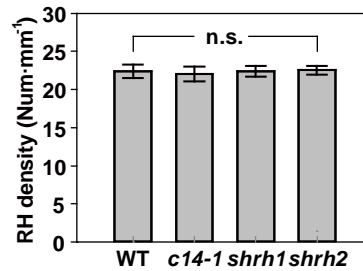
## Supplemental Figure 7



**Supplemental Figure 7.** *In vivo* Calibration of FRET/CFP Ratio of YC3.6 to  $[Ca^{2+}]$  in RHs.

Equation  $[Ca^{2+}] = (K_d[R-R_{min}]/[R_{max}-R])(F_{min}/F_{max})$  is used to analyze the cytosolic  $[Ca^{2+}]$  level as described in Material and Method. **(A)** and **(B)** Relative average FRET/CFP ratio of YC3.6 in wild type. **(C)** and **(D)** Relative average FRET/CFP ratio of YC3.6 in *shrh1* mutant.  $R_{max}$  was recorded upon the application of 20  $\mu M$   $Ca^{2+}$  ionophore Br-A23187 and 5 mM  $Ca^{2+}$  (**A** and **C**), and  $R_{min}$  was recorded upon the application of 20  $\mu M$  Br-A23187 and 5 mM EGTA (**B** and **D**). **(E)** and **(F)** Diagram of cytosolic  $[Ca^{2+}]$  level calibration in the RHs of wild type (**E**) and *shrh1* (**F**). Letter n denotes the numbers of RHs tested. Error bars depict means  $\pm$  SEM.

## Supplemental Figure 8



**Supplemental Figure 8.** Statistical Analysis of RH Density of RHs Embedded in Solid Medium.

No less than 30 roots and 200 RHs (7-10 RHs per root) were counted for each line. n.s. denotes no significant difference between samples with  $P > 0.05$  (Kruskal-Wallis one-way ANOVA analysis). Error bars depict means  $\pm$  SEM.

**Supplemental Table 1.** Relative Changes of RH Length and Branching.

Genotype	Treatment ( $\mu\text{M}$ )	Relative changes of RH length (%) $\pm$ SE	Relative change of RH branching rate $\pm$ SE
WT	0.05 IAA	+ 37.43 $\pm$ 4.19	+13.68 $\pm$ 1.75
<i>shrh1</i>		+ 33.24 $\pm$ 8.3	
<i>C9-COM15</i>		+ 35.79 $\pm$ 3.63	
<i>C9-OE42</i>		+ 9.78 $\pm$ 2.13	
WT	50 yucasin	-65.71 $\pm$ 1.4	-60.12 $\pm$ 7.3
<i>shrh1</i>		-69.51 $\pm$ 1.3	
<i>C9-COM15</i>		-66.2 $\pm$ 1.89	
<i>C9-OE42</i>		-63.97 $\pm$ 1.64	

+ denotes increase, and – denotes decrease relative to control for the same genotype.

**Supplemental Table 2.** Restriction sites and primers for plant genotyping and vector construction.

Primer name	Sequence(5' to 3')	Use
CNGC5-LP	GAGCTTTCTGGTTAAGCCGTC	Genotyping of T-DNA insertion mutants
CNGC5-RP	CACGCTCCCTAAGATCTTGTG	
CNGC6-LP	GTGGCAAGCCTTACTCCAACAGTCGTT	
CNGC6-RP	GAAGTATCAGAGAAGAAGATATTTGATCCACA	
CNGC9-LP	ATTTGCAGCAAACCTTGAAGC	
CNGC9-RP	TGTTTATGGTGGGGACTTCAG	
CNGC14-LP	TCGGAACAATTGGCAGAATAC	
CNGC14-RP	CACCTGCTTGTAAGCAAAGG	
LBb1.3	ATTTTGCCGATTTCCGGAAC	
pCNGC1-F-XbaI	CTAG TCTAGA ATGAATCATGATAGAAATGAGCTGA	Vectors for the generation of transgenic Arabidopsis lines for GUS staining analysis
pCNGC1-R-NcoI	CATGCCATGG CCACGAAAGTCACAAACTTTTG	
pCNGC2-F-XbaI	CTAG TCTAGA ACTTTGGTCCCTACGGTTTTT	
pCNGC2-R-NcoI	CATGCCATGG AGTGATTGGGAGAGAGAAGAGTGAT	
pCNGC3-XbaI-F	TT TCTAGA GACCCTCTCAGGCCATTTGA	
pCNGC3-NcoI-R	CATGCCATGG CGCTGATTGTTTTCTTCCACAGAT	
pCNGC4-F-SalI	ACGC GTCGAC GTGAATGACCAAAAATTTAGGGA	
pCNGC4-R-NcoI	CATGCCATGG TTAATTATGAAGAAGAATTTTCT	
pCNGC5-F-XbaI	CTAG TCTAGA CTCCAAATCACAACAGAAGCGTT	
pCNGC5-R-NcoI	CATGCCATGG GATTGCTTACAAGGAATTCAGAACT	
pCNGC6-F-SalI	ACGC GTCGAC TATATATAAAGCAGTGGCACCAACC	
pCNGC6-R-NcoI	CATGCCATGG CTCTTTTAACTCATGAATCCTAAAAA	
pCNGC7-F-XbaI	CTAG TCTAGA AGCTAGAGATGTTGCTGGAAGCT	
pCNGC7-R-NcoI	CATGCCATGG TCCAGTCGCAGGAGAAGAACT	
pCNGC8-F-XbaI	CTAG TCTAGA ATTAAAACATCTGAAGATGTT	
pCNGC8-R-NcoI	CATGCCATGG TTGAAACCCTCGAATCTGTATCA	
pCNGC9-F-XbaI	CTAG TCTAGA GTCATGTTGAACCTTTGTGAAAGAAT	
pCNGC9-R-NcoI	CATGCCATGG GCTTTTTCTTGTGACTCAACTC	
pCNGC10-F-XbaI	CTAG TCTAGA TTGTTCTCTTCTAAACCCAGCTT	
pCNGC10-R-NcoI	CATGCCATGG CAGCCAAACCCTTGATATTTCA	
pCNGC11-F-SalI	ACGC GTCGAC TTTGAAGGAGAGATCATTTTTGC	
pCNGC11-R-NcoI	CATGCCATGG GTTTTTATCTGTCAATCTTCAATATTTT	
pCNGC12-F-SalI	ACGC GTCGAC TGCTCAAAAAGGTAACCTTTTATTT	
pCNGC12-R-NcoI	CATGCCATGG TTAGTATATGTCGTCAATCTGCAATAT	
pCNGC13-F-XbaI	CTAG TCTAGA GATCTCTTGCTTCTTAGACAGCTTT	
pCNGC13-R-NcoI	CATGCCATGG GAGTCAAGCTTTTTTTTGCTATAACG	
pCNGC14-F-XbaI	CTAG TCTAGA ACCATACAACAGCATAACTTTCCAG	
pCNGC14-R-NcoI	CATGCCATGG GGCAAGTGCATTGAGAATTGTAT	
pCNGC15-F-SalI	ACGC GTCGAC TTTCGATGAAGTGAACAATATCGTA	
pCNGC15-R-NcoI	CATGCCATGG TCTTCTCTTTATCAAATCCTCTGAC	
pCNGC16-F-SalI	ACGC GTCGAC GTTACTTTTGTCTGAAATCCATGAA	
pCNGC16-R-NcoI	CATGCCATGG TTGGGGGTTGCGTGTGTT	
pCNGC17-F-XbaI	CTAG TCTAGA AACCCAAAACCTGAACCCTATTTTAT	
pCNGC17-R-NcoI	CATGCCATGG TAGAAGAAGCTCAAGCTCAAGCTC	
pCNGC18-F-KpnI	GGGGTACC TAGGGTCCCAAATGATTAATATGTT	
pCNGC18-R-EcRI	CC GAATTC TCTTCCATGGCGGATTCC	
pCNGC19-F-SacI	CT GAGCTC TGTGAAAGTGGTCATCTCTCATCTT	
pCNGC19-R-BmI	TT GGATCC TTTTATTTTTCAGAAACCCAAAATCT	
pCNGC20-F-SalI	ACGC GTCGAC TTTTTTCAGAGAGTTAAGAAATGCA	
pCNGC20-R-NcoI	CATGCCATGG TCTTCTCTTCGTAGCCAGGT	

Primer name	Sequence(5' to 3')	Use
eGFP-F-XmaI	TCCC CCCGGG ATGGTGAGCAAGGGCGAGGAGC	Vectors for the generation of COM and OE Arabidopsis lines
eGFP-R-XbaI	CTAG TCTAGA CTTGTACAGCTCGTCCATGCC	
pCNGC5-F-XmaI	TCCC CCCGGG CTCCAAATCACAACAGAAGCGTT	
pCNGC5-R-XbaI	CTAG TCTAGA GATTGCTTACAAGGAATTCAGAACT	
CNGC5-F-SalI	ACGC GTCGAC ATGGCAGGGAAAAGAGAAAACTTTG	
CNGC5-R-PstI	AA CTGCAG GTCAGCAGTCAAATCAGGCTCAGGAG	
pCNGC6-F-SacI	CT GAGCTC TATATATAAAGCAGTGGCACCAACC	
pCNGC6-R-XmaI	TCCC CCCGGG ACTCTTTTAACTCATGAATCCTAAA	
CNGC6-F-XbaI	CTAG TCTAGA ATGTTTCGATACTTGTGGCC	
CNGC6-R-PstI	GC CTGCAG GTGATCTTCAGCAGAGAAA	
pCNGC9-F-SacI	CT GAGCTC GTCATGTTGAACTTTGTGAAAGAAT	
pCNGC9-R-XmaI	TCCC CCCGGG GCTTTTTCCTTGTGACTCAACTC	
CNGC9-F-SalI	ACGC GTCGAC ATGTTAGACTGTGGCAAAAAGC	
CNGC9-R-PstI	AA CTGCAG ACTAGTATCATCAGCAGAGAAATCAGG	
CNGC5-qRT-F	AGAAAATGGAAGAGGCAGAAGC	
CNGC5-qRT-R	TCACCAAATCCCTAATCTTTGTGT	
CNGC6-qRT-F	ATAACACTTGCTATTGCTGGACT	
CNGC6-qRT-R	CAATGGAACCTTTCGGACTAACG	
CNGC9-qRT-F	ATTCAGATTCTACTCGCAACAA	
CNGC9-qRT-R	CTTTGCTTCAATACGGTTCTT	
18S-qRT-F	CGGCTACCACATCCAAGGAA	
18S-qRT-R	TGTCACTACCTCCCCGTGTCA	
CNGC5-F-NheI	TCTA GCTAGC ATGGCAGGGAAAAGAGAAAAC	Vectors for transient expression of CNGCs in HEK293T cells
CNGC5-R-EcoRI	CC GAATTC GTCAGCAGTCAAATCAGGCTCAGGAG	
CNGC6-F-NheI	TCTA GCTAGC ATGTTTCGATACTTGTGGCC	
CNGC6-R-EcoRI	CC GAATTC GTGATCTTCAGCAGAGAAA	
CNGC9-F-XbaI	CTAG TCTAGA ATGTTAGACTGTGGCAAAAAGC	
CNGC9-R-SalI	ACGC GTCGAC ACTAGTATCATCAGCAGAGAAATCAG	