The cyclic nucleotide-gated channels, AtCNGC11 and 12, are involved in Ca2+ dependent physiological responses beyond pathogen defence

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Supplementary material
Figure S1

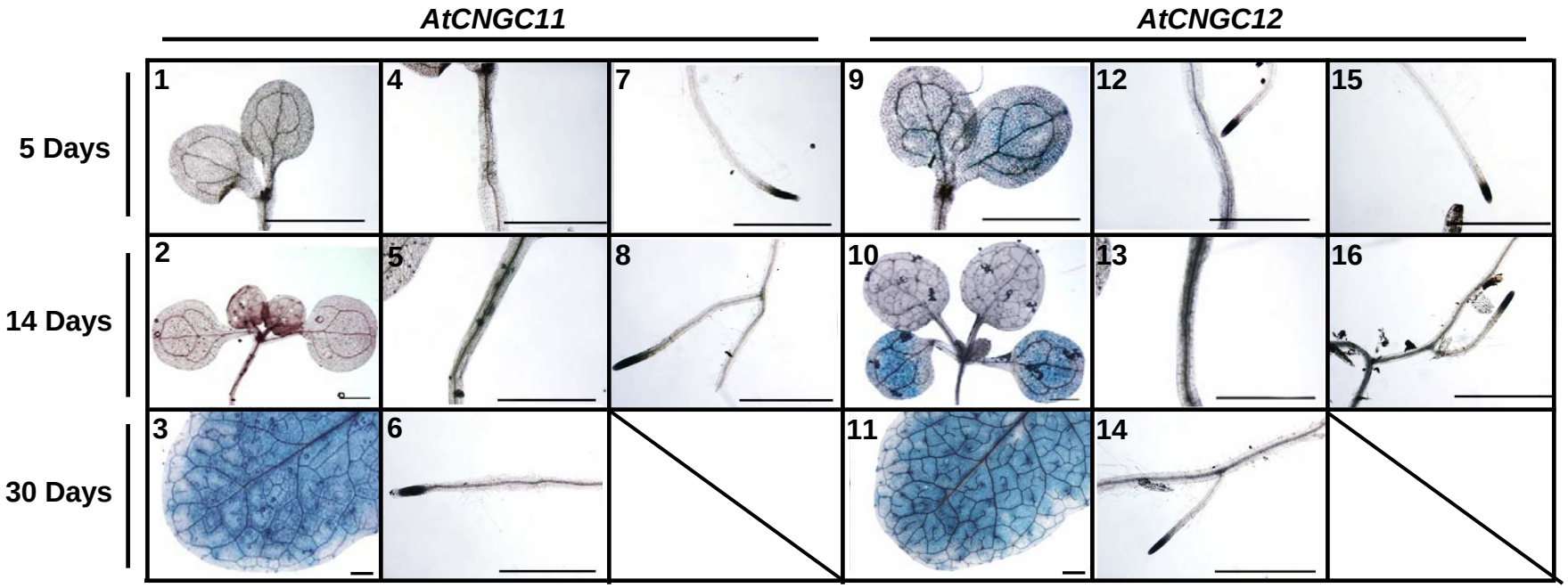

Figure S1. Tissue specific expression analysis of *AtCNGC11* and *12* using soil grown transgenic *Arabidopsis* plants carrying promoter:GUS reporter constructs. The tissue was stained with X-Gluc solution for 1.5 hours and then cleared with ethanol/acetic acid. *AtCNGC11* expression is undetectable in both shoots and roots in 5 day old seedlings (1, 4 and 7). *AtCNGC11* expression is very low in 14 day old seedling leaves (2) and is absent in the roots (8), but became stronger as plants aged (3). *AtCNGC12* expression is ubiquitous throughout the leaves at all levels of developmental stages (9, 10 and 11) but it became stronger when the plants aged (11). In the roots, expression of both genes was not detectable in 5 day and 14 day old plants (7, 8, 15 and 16), but very weak expression was observed in 30 day old plants (6 and 14). Bar = 0.1cm

Supplementary material
Figure S2

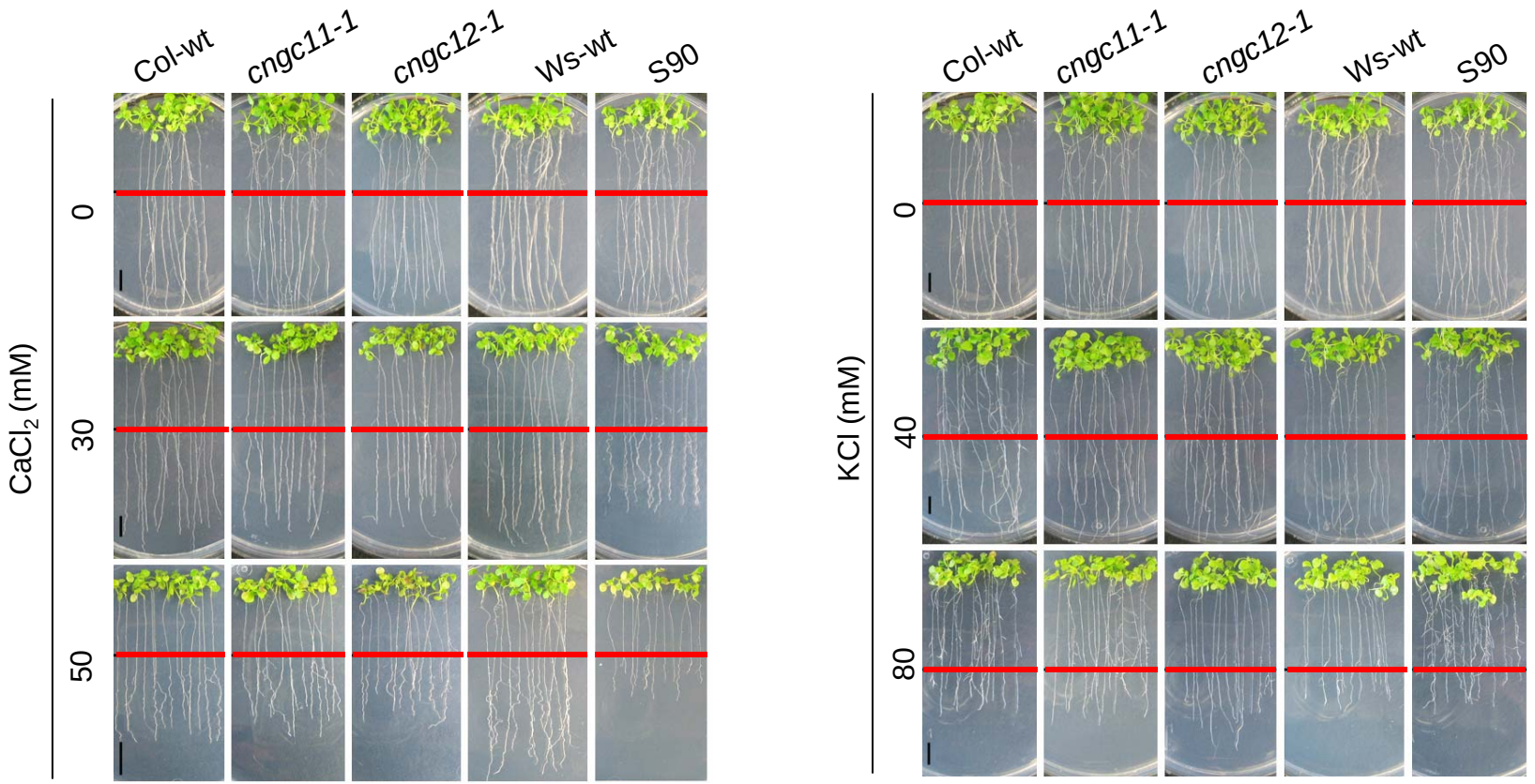

Figure S2. Root growth during K^+ or Ca^{2+} stress. *cngc11-1* and *cngc12-1*, S90 and their respective wild type seeds were grown on 0.5 X MS agar medium for 7 days before being transferred to 0.5 X MS agar medium with indicated concentrations of $CaCl_2$ or KCl. Root lengths were measured 7 days after transferring the seedlings to plates with elevated levels of K^+ or Ca^{2+}. Red lines indicate the position of root tips when they were transferred.

Supplementary material
Figure S3

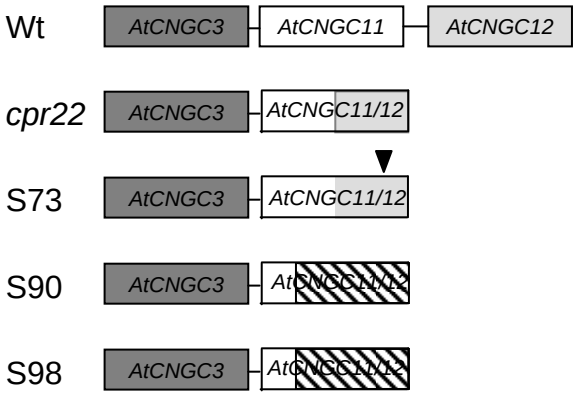

Figure S3. Configuration of *AtCNGC3*, *AtCNGC11* and *AtCNGC12* in wild type, *cpr22*, and its suppressor mutants, S73, S90 and S98. *cpr22* has an approximate 3kb deletion between *AtCNGC11* and *AtCNGC12*, creating an in-frame chimeric *AtCNGC11/12* gene (Yoshioka *et al*., 2006). S73 has a single amino acid substitution in the cyclic nucleotide binding domain resulting in loss of channel function (Baxter *et al*., 2008). S90 and S98 both have premature stop codons before the pore region. Filled triangle shows mutation position in S73. Hatched boxes show the area after the premature stop codons.

Table S1. Gravitropic bending in S98 and S73[a]

	Ca^{2+} Concentration (mM)	
Lines	0	50
Ws (Wt)	75.4 +/- 8.4	32.9 +/- 5.5
S98	26.0 +/- 5.3 *	46.7 +/- 2.8 *
S73	20.4 +/- 4.9 *	50.6 +/- 5.1 *

[a] Degree of root curvature +/- SE at 6 hours post rotation. The experiment was repeated more than 3 times with similar results.

* Denotes those values that are statistically different from their wild type (Student T test $p<0.05$)