Supporting Information

Choi et al. 10.1073/pnas.1319955111

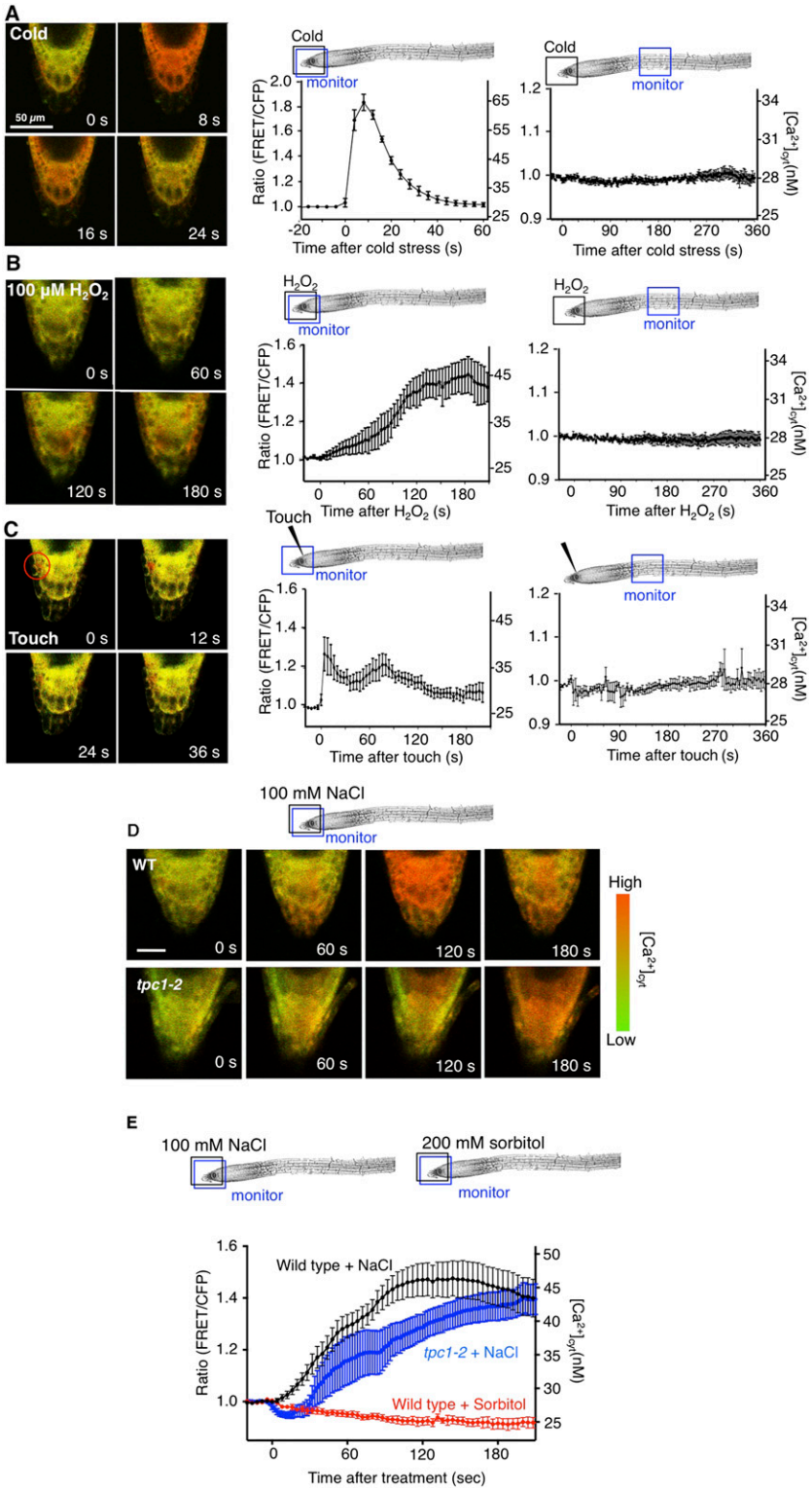

Fig. S1. Cytoplasmic Ca^{2+} changes in response to abiotic stress. (*A*) Cold (4 °C medium), (*B*) 100 μM H_2O_2, (*C*) touch stress, or (*D*) 100 mM NaCl or 200 mM sorbitol as a control were applied to the apical ~50 μm of the root tip of an *Arabidopsis* plant expressing the FRET-based Ca^{2+} sensor YCNano-65. Ca^{2+} changes

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were monitored every 4 s using a confocal microscope and expressed as the FRET/CFP normalized to the ratio for 60 s before stress application. Data are representative ratio images (*Left*) or quantitation of FRET:CFP ratio and corresponding calculated absolute $[Ca^{2+}]$ (*Right* axis, see *Materials and Methods* for $[Ca^{2+}]$ calibration protocols and caveats about absolute values presented) averaged over the apical 100 μm of the root tip or a 100-μm region 500–1,000 μm shootward (blue boxes) of the site of stress application (black box). For touch, the response to the individual touch-stimulated cell is shown. Mean ± SEM; $n = 4$–9. Images taken every 2 s (cold and ROS) or 4 s (touch and NaCl). See also Movies S1–S4. (*D*) Ratio images of Ca^{2+} changes in the root tip of wild type and *tpc1-2* treated with 100 mM NaCl. See also Movies S4 (WT) and S8 (*tpc1-2*). (Scale bar: 50 μm.) (*E*) Quantitative analysis of Ca^{2+} changes from YCNano-65 ratiometric analysis in response to 100 mM NaCl and 200 mM sorbitol monitored over the apical 100 μm. Error bars are SEM of four to seven individual seedlings. Images were taken every 2–4 s. The 200 mM sorbitol response was monitored in the root tip of WT as osmotic control.

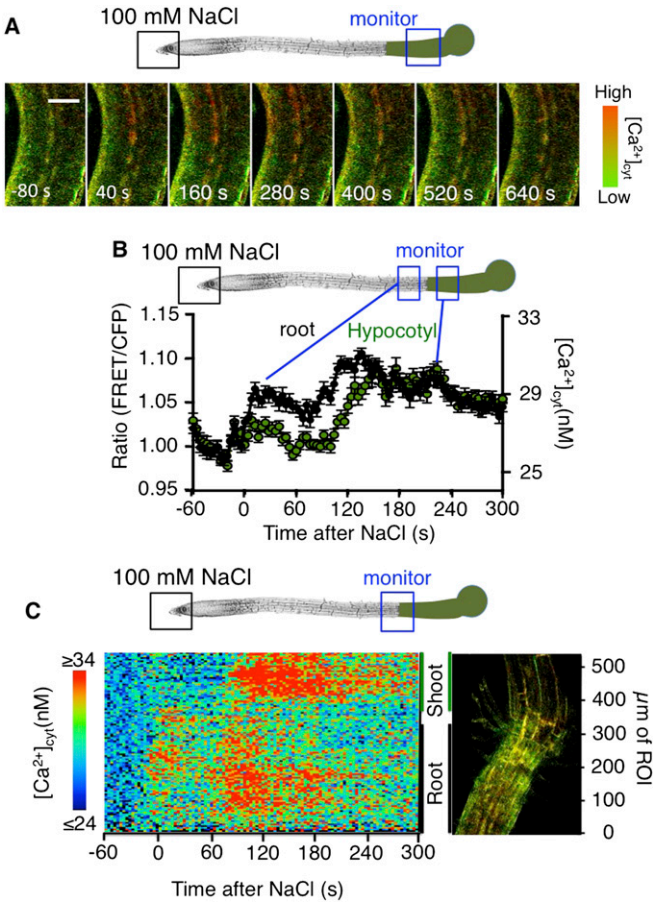


Fig. S2. Salt stress at root tip induces a Ca^{2+} wave, which propagates to the hypocotyl. (*A*) Ca^{2+} imaging using ratiometric confocal imaging for hypocotyl response. Time is seconds after NaCl treatment to the root tip. Images are representative of seven seedlings. (Scale bar: 100 μm.) (*B*) Following addition of salt, the root and hypocotyl of transgenic lines expressing YCNano-65 were imaged for Ca^{2+} changes (blue boxes, 276 × 167-μm root; 264 × 216-μm hypocotyl). Mean ± SEM; representative of three separate experiments. (*C*) Analysis of Ca^{2+} wave propagation through the root/shoot junction. Ca^{2+} levels were quantified from a region of interest (ROI) 5-μm long × 167- to 216-μm wide, scanned along 540 μm of the root/shoot junction as described in the legend of Fig. 1. Representative of $n > 4$.

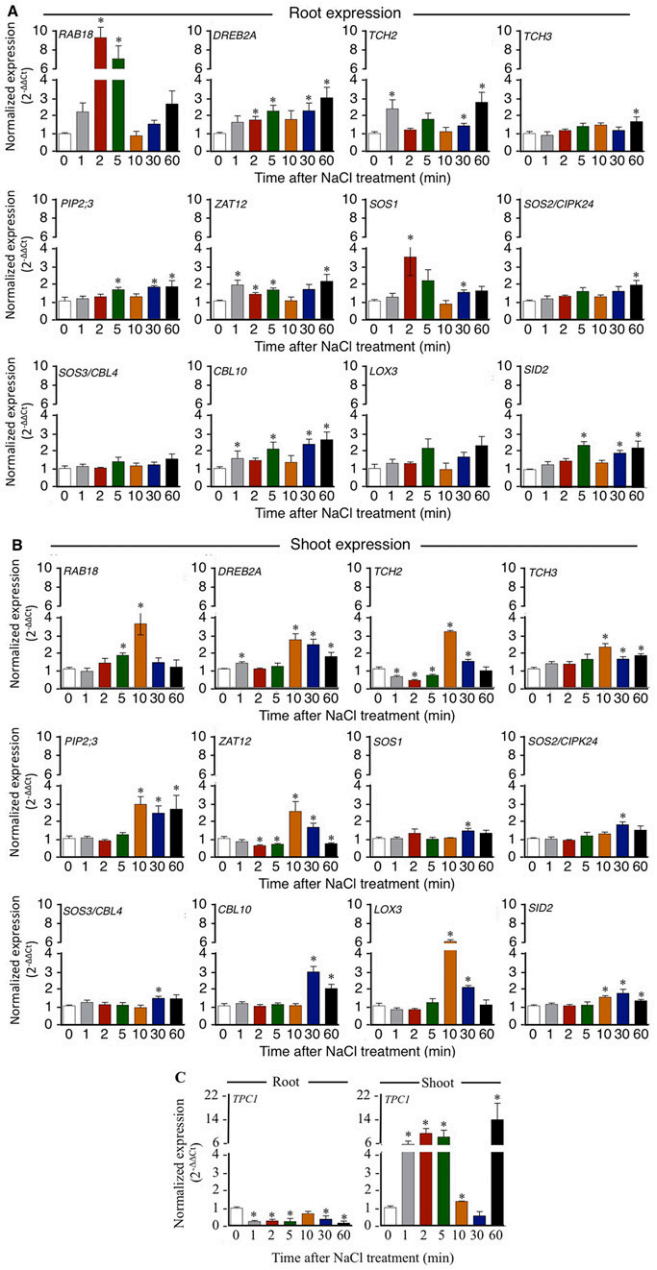


Fig. S3. Quantitative results shown for data in Fig. 3*A* on marker gene expression in response to local root tip salt stimulation. (*A* and *B*) Quantitative real-time PCR (qPCR) analysis of time course of induction of 12 marker genes in root tissues directly responding to 100 mM NaCl (*A*) and in systemically responding shoots (*B*). (*C*) *TPC1* expression in root tissues directly responding to 100 mM NaCl and in systemically responding shoots. Results represent mean ± SEM; $n = 4$–6 from three biological replicates. Each gene is normalized to its own 0-min value. *$P < 0.5$ (*t* test) comparing to the 0-min value for each gene.

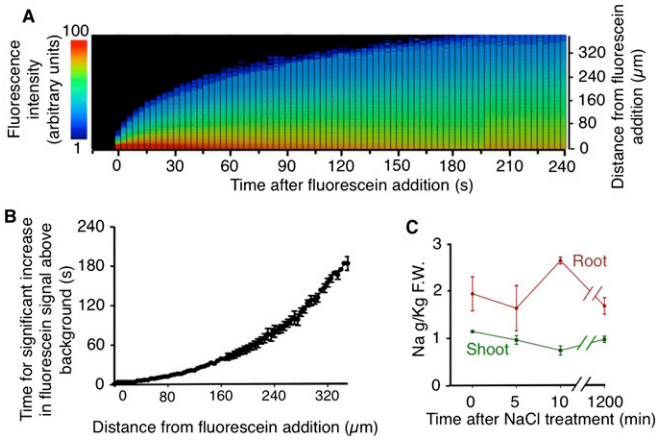


Fig. S4. Passive movement of fluorescein through the growth medium gel and salt movement through the seedling. (*A*) Fifty micromolar fluorescein was added into a window in the medium gel at time 0 s to monitor passive small-molecule movement through the gel. Fluorescein movement was imaged using a Zeiss LSM 510 and quantitative analysis of images performed as described for analysis of ratio images of the YCNano-65 confocal images in Fig. 1. Average fluorescein signal intensity was extracted from a region of interest (ROI) (5-μm long × 128-μm wide) that was sequentially scanned over 380-μm length of the gel medium. Analysis was repeated on images taken every 3.15 s for 4.5 min, and then the data were extracted from four trials averaged and pseudocolor-coded. (*B*) The time for the fluorescein signal to significantly increase above background ($P < 0.05$, *t* test) over distance from point of addition to the gel (mean ± SEM; $n = 4$). (*C*) Time course of Na accumulation in roots responding to local salt stimulation and in systemic shoot tissue. Ten-day-old seedlings were locally stimulated with 100 mM NaCl in the root tip region. Root and leaf (shoot) tissues were harvested (5.1–13.8 mg for root, 28.7–82 mg for shoot) at 0 (control), 5, 10, and 1,200 min (20 h) after salt stress with 100 mM NaCl applied to the root tip. Sodium was analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES). Results are mean (±SD); $n = 8$–12 from three independent experiments.

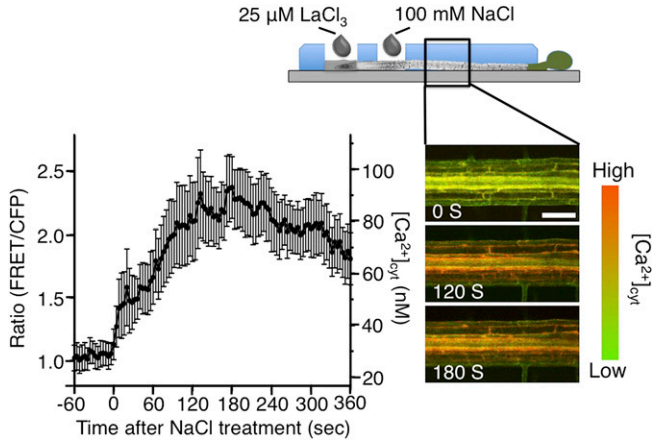


Fig. S5. Site-specific effect of Ca^{2+} channel blocker on salt-induced Ca^{2+} waves. Systemic Ca^{2+} waves were monitored shootward of the 100 mM NaCl-stimulated middle root region. Root tips were treated with 25 μM $LaCl_3$ 30 min before salt stress, which was applied 1,000 μm shootward of the tip. Ca^{2+} levels were then monitored 1,000 μm shootward of the site of NaCl treatment (see *Inset* diagram). Data are representative ratio images (*Left*) and corresponding quantitation of FRET/CFP ratio and calculated absolute [Ca^{2+}] to the *Right*. The speed of the systemic Ca^{2+} wave from these experiments is 397.5 ± 42.1 μm/s (mean ± SEM; $n = 13$).

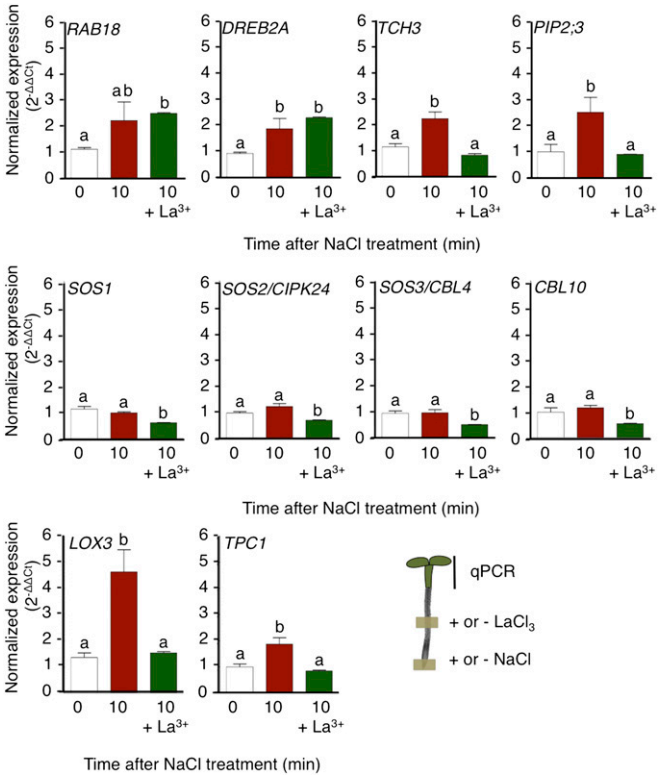


Fig. S6. Effect of blocking the salt stress-induced Ca^{2+} wave on shoot gene expression. qPCR analysis of time course of induction of genes in systemic shoot in response to local 100 mM NaCl stress of the root tip, using 25 μM $LaCl_3$ between root tip and shoot to block Ca^{2+} wave propagation (see *Inset* diagram). Results are mean ± SEM; $n = 4–6$ from three independent experiments. Each gene is normalized to its own 0-min shoot value. The 25 μM $LaCl_3$ blockade was added between root tip and shoot tissues for 30 min before local salt treatment in root tip region. Note that data on *TCH2*, *ZAT12*, and *SID2* are presented in Fig. 3*C*. "a" and "b" denote statistical significance of *t* test to the 0-min control. Same letters show no statistical significance at $P < 0.05$.

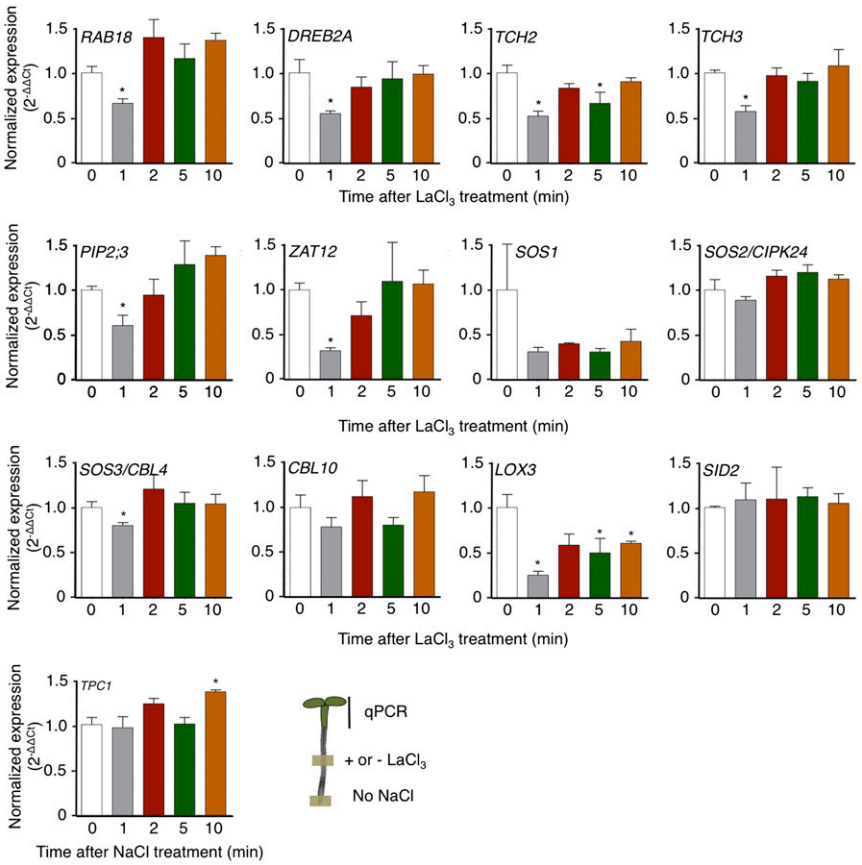


Fig. S7. qPCR analysis of time course of induction of genes in systemic shoot tissues in response to 25 μM $LaCl_3$ (the quantitative data for Fig. 3*B*). $LaCl_3$ was added as for experiments to block NaCl-induced Ca^{2+} wave propagation but no NaCl treatment was applied (see *Inset*). Shoot tissues were harvested, and gene expression was analyzed by qPCR with each gene being normalized to its own 0-min value. Results are mean ± SEM; $n = 4–6$ from three separate experiments. The asterisk (*) represents statistical significance ($P < 0.05$, *t* test) in comparison with each gene's 0 min.

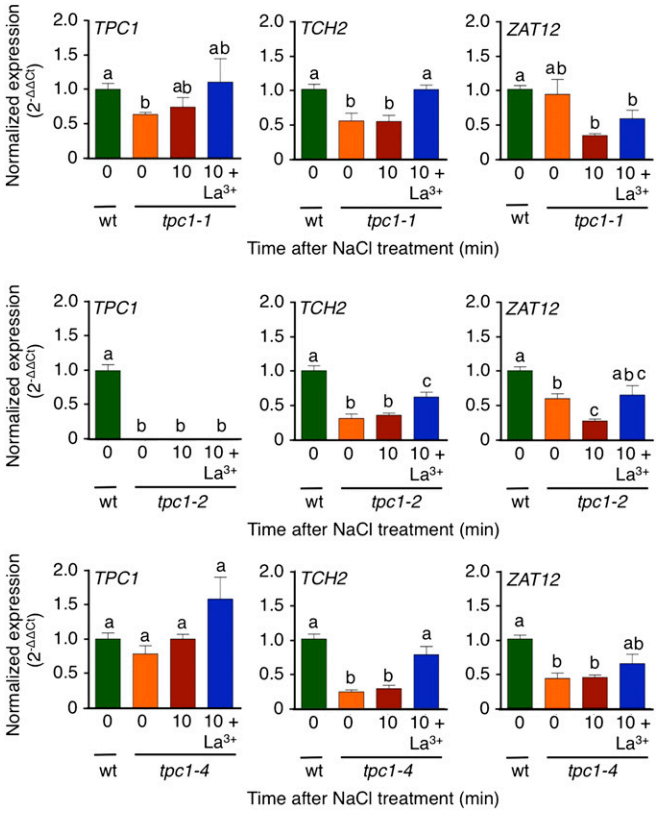


Fig. S8. Gene expression changes in the shoots of *tpc1* mutants after root NaCl treatment. Expression of the early salt stress-responsive genes *TCH2* and *ZAT12* as well as *TPC1* itself was monitored in shoot tissues using qPCR analysis in wild type (wt), *tpc1-1*, *1-2*, and *1-4* mutants. Shoot tissues were sampled from nonstressed plants (0 min), and then 100 mM NaCl was applied to the root tip region for 10 min either in the presence or absence of the 25 μM $LaCl_3$ block in place between the salt-stressed root tip region and the shoot. Results are mean (±SEM); $n = 4$–6 from three biological replicates. Each gene is normalized to its own wild-type shoot 0-min value. "a," "b," and "c" denote statistical significance (*t* test, $P < 0.05$). Bars with the same letters show no statistically significant difference.

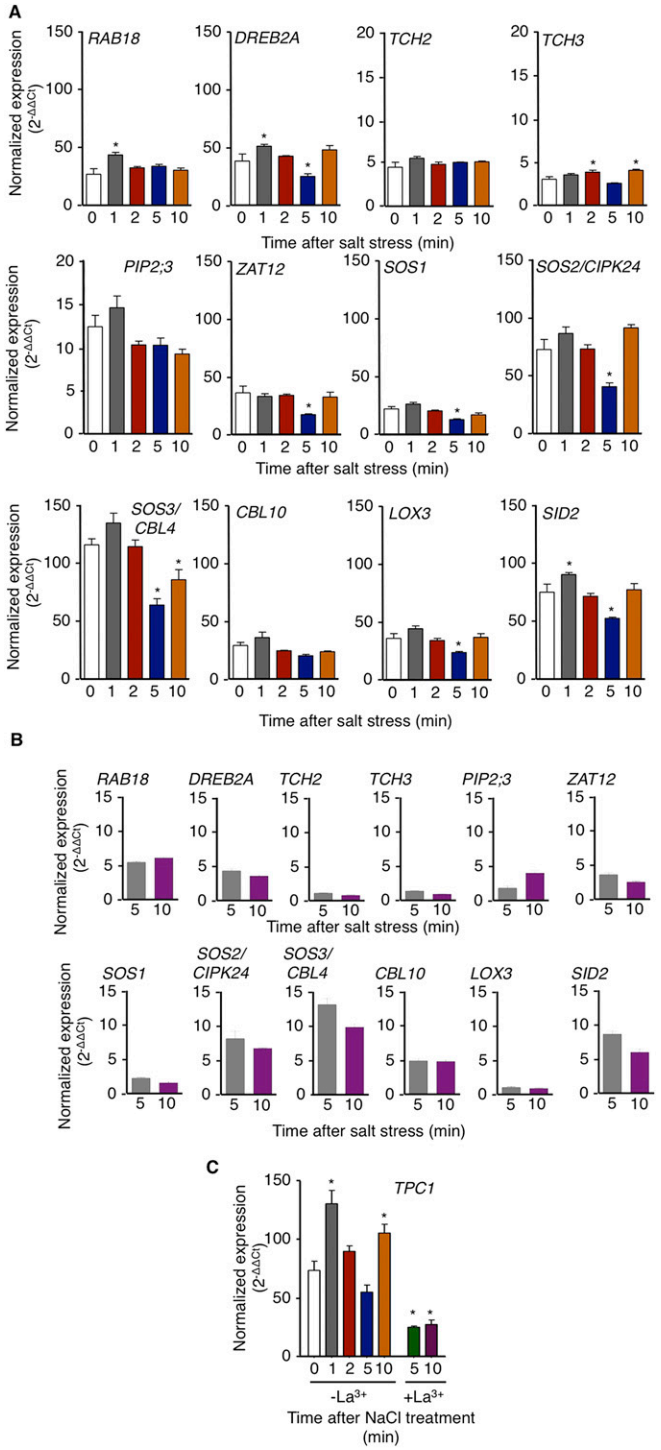


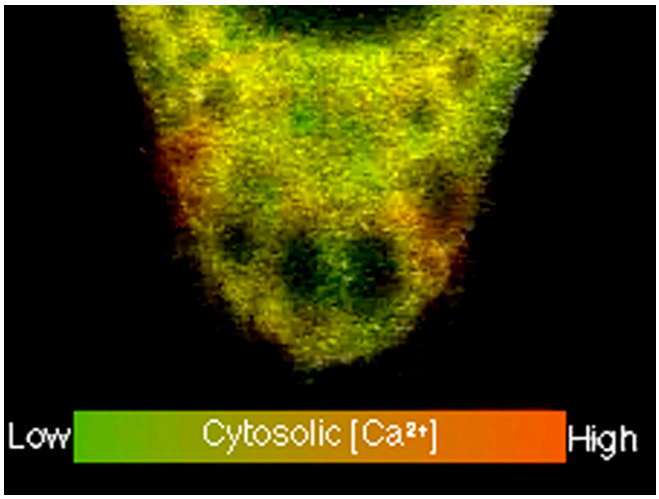
Fig. S9. Expression of marker genes in the shoot tissues of the *OxTPC1* overexpression line in response to local salt stress of the root tip region. (*A*) qPCR analysis of time course of induction of marker genes in shoot tissues responding to 100 mM NaCl added locally to the root tip. Note expanded scale for *TCH2*, *TCH3*, and *PIP2;3*. Each gene is normalized to wild-type shoot 0-min value. (*B*) Effects of $LaCl_3$ blocker on expression of marker genes in the shoot. *OXTPC1* was pretreated with the 25 μM $LaCl_3$ blockade, 30 min before local salt stress in the root tip. Gene expression was then monitored at 5 and 10 min after 100 mM NaCl treatment of the root tip and normalized to wild-type shoot 0-min value. (*C*) Induction of *TPC1* expression by NaCl in *OXTPC1*. Results represent mean ± SEM; $n = 4–6$ from three biological replicates. Each gene is normalized to wild-type shoot 0-min value. The asterisk (*) denotes statistical significance ($P < 0.05$, *t* test) in comparison with the 0-min value for each gene in *A* and *C*. In *B*, all points are significantly lower ($P < 0.05$, *t* test) than the equivalent time points without $LaCl_3$ treatment (*A*).

Table S1. qPCR primers used in this study

Name	Sequence, 5′ to 3′	Target/direction	Length
UBQ10-F	CAC ACT CCA CTT GGT CTT GCG T	*UBQ10*/forward	22
UBQ10-R	TGG TCT TTC CGG TGA GAG TCT TCA	*UBQ10*/reverse	24
RAB18-F	ATA TGA TGC TGG TGG CTA CG	*RAB18*/forward	20
RAB18-R	GAT TGT TCG AAG CTT AAC GGC	*RAB18*/reverse	21
DREB2A-F	CGA GGG AAA GGA TGG TAA TGG	*DREB2A*/forward	21
DREB2A-R	CGT TGT GGG ATT AAG GCA AAT ATC	*DREB2A*/reverse	24
TCH2-F	AGA AGA TGA TGA GTA ATG GTG GTG	*TCH2*/forward	24
TCH2-R	CGC CGT CAC TAA AAT TAA TCT GC	*TCH2*/reverse	23
TCH3-F	CAT AGC GGT CGG GGT TG	*TCH3*/forward	17
TCH3-R	TGT CAG ACC CTA TTG GCA TAA AG	*TCH3*/reverse	23
PIP2;3-F	ACC AAT TCG TTC TAA GGG CC	*PIP2;3*/forward	20
PIP2;3-R	CGT GGC TAA GTT TAA ACG TTG G	*PIP2;3*/reverse	22
Zat12-F	AAC ACA AAC CAC AAG AGG ATC A	*ZAT12*/forward	22
Zat12-R	AAG CAT CAA ACA ATT CGC CG	*ZAT12*/reverse	20
AtSOS1-F	CTT CTT CCT CTG TGT TGT TGC	*SOS1*/forward	21
AtSOS1-R	GAA GAC GAA TCG GTC GCT T	*SOS1*/reverse	19
SOS2-F	AAG CTA TGT TCG AAA CTG GAA AAC	*SOS2*/forward	24
SOS2-R	TGG ATT TAA GTT GGG ATC AAA ACG	*SOS2*/reverse	24
AtSOS3-F	AGA AGG GTG TGT TTG TAT GGG	*SOS3*/forward	21
AtSOS3-R	GAA GCT CGG GAT CCT CAT ATC	*SOS3*/reverse	21
AtCBL10-F	ACG ACA GCA TTC CCA AGT T	*CBL10*/forward	19
AtCBL10-R	CCG GTG TTG CAT GGA CAG	*CBL10*/reverse	18
AtTPC1-F*	GCT CTA TTG GCG TAC AGG TCT TTG	*TPC1*/forward	24
AtTPC1-R*	GAA GAG TGT GAC CAT TCC ATT GG	*TPC1*/reverse	23
LOX3-F	GTA TGA GTT GAT GGC TCC GAG	*LOX3*/forward	21
LOX3-R	ACC ATA CTG CGT CAA AAT CTA ATT ATA T	*LOX3*/reverse	28
SID2-F	TTG CAG TTC ACC AAG TCA ATT G	*SID2*/forward	22
SID2-R	CCC CTT ATC CCC CAT ACA AAC	*SID2*/revese	21

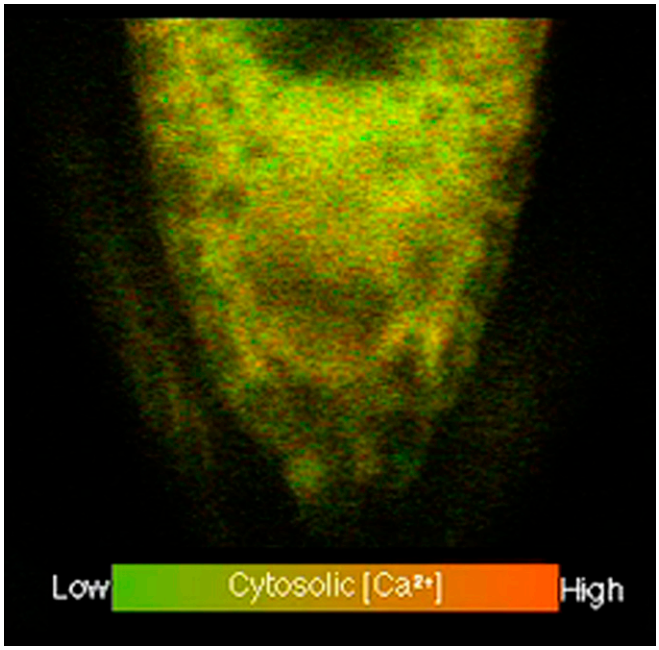
*All primers were designed using the Web tools available at www.IDTDNA.com except the TPC1 primers, which were taken from ref. 1. F, forward; R, reverse.

1. Ulker B, et al. (2008) Getting the most out of publicly available T-DNA insertion lines. *Plant J* 56:665–677.



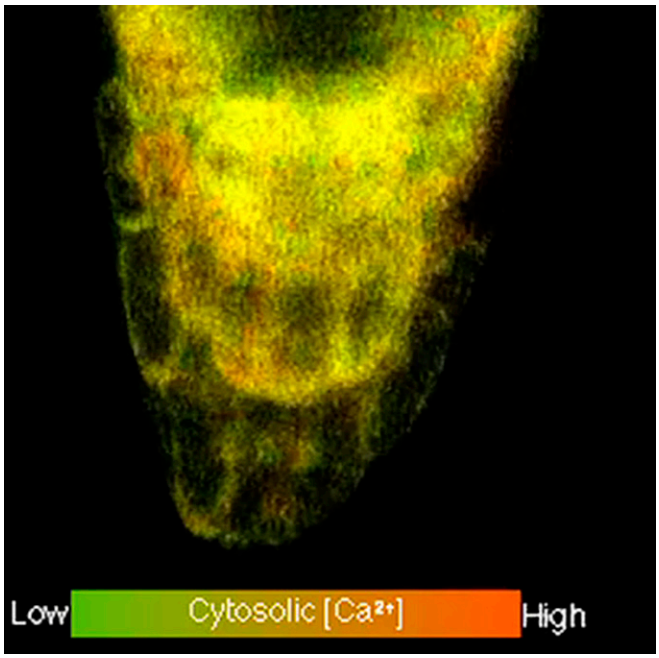
Movie S1. Effect of cold shock on cytosolic Ca^{2+} at the root tip monitored using *Arabidopsis* expressing the YCNano-65 biosensor and confocal ratio imaging. Movie duration, 88 s (4 s per frame, 22 frames).

Movie S1



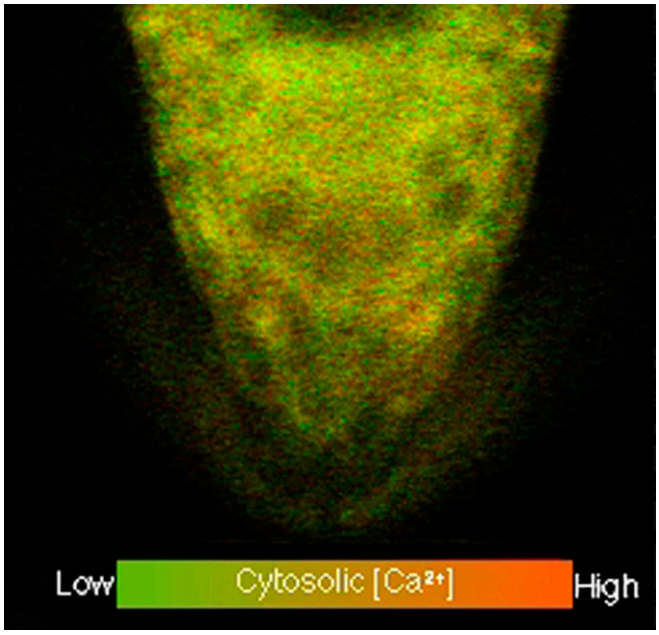
Movie S2. Effect of 100 μM H_2O_2 on cytosolic Ca^{2+} at the root tip monitored using *Arabidopsis* expressing the YCNano-65 biosensor and confocal ratio imaging. Movie duration, 230 s (4 s per frame, 115 frames).

Movie S2



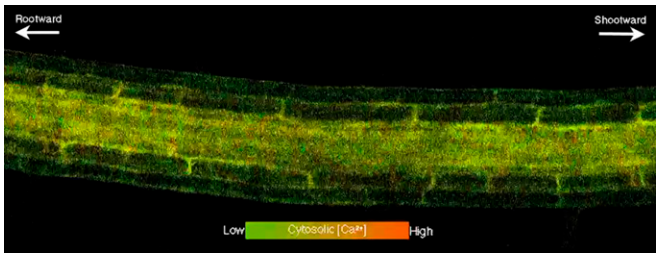
Movie S3. Effect of touch stimulation on cytosolic Ca^{2+} at the root tip monitored using *Arabidopsis* expressing the YCNano-65 biosensor and confocal ratio imaging. Touch was applied by contact with the tip of a glass micropipette. Movie duration, 200 s (4 s per frame, 50 frames).

Movie S3



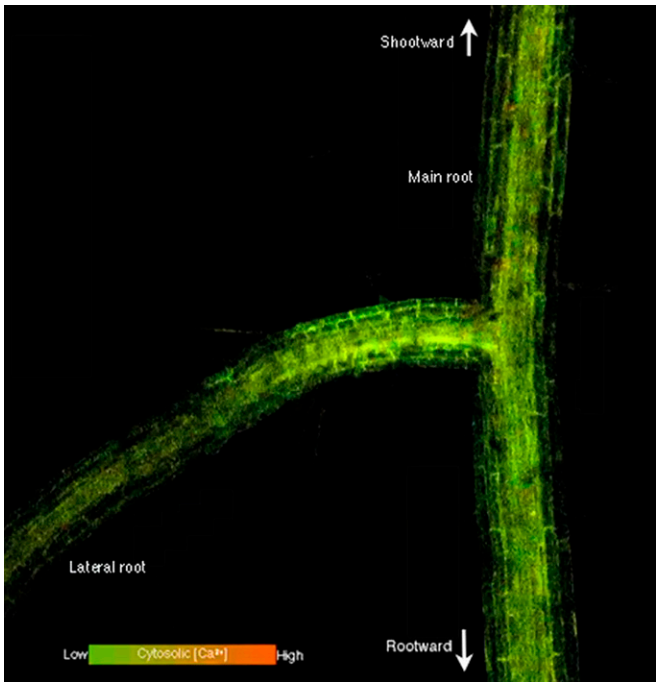
Movie S4. Effect of 100 mM NaCl on cytosolic Ca^{2+} at the root tip monitored using *Arabidopsis* expressing the YCNano-65 biosensor and confocal ratio imaging. Movie duration, 520 s (4 s per frame, 130 frames).

Movie S4



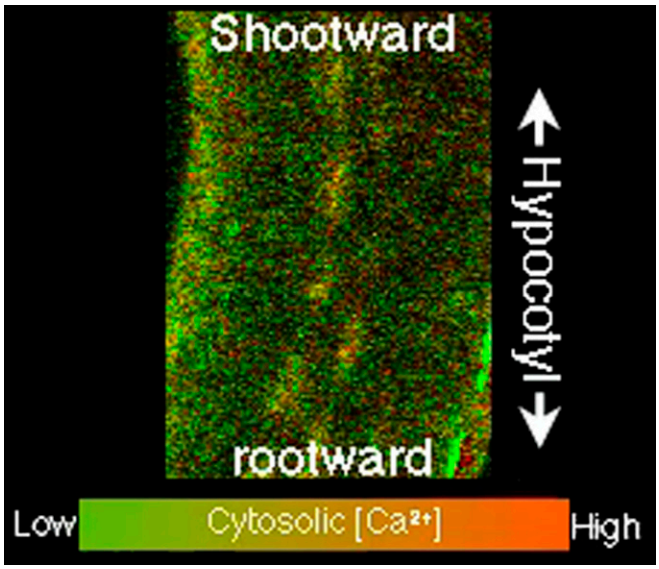
Movie S5. Transmission of Ca^{2+} wave through tissue shootward of the root tip locally treated with 100 mM NaCl. Cytosolic Ca^{2+} was monitored using *Arabidopsis* expressing the YCNano-65 biosensor and confocal ratio imaging. Movie duration, 800 s (4 s per frame, 200 frames).

Movie S5



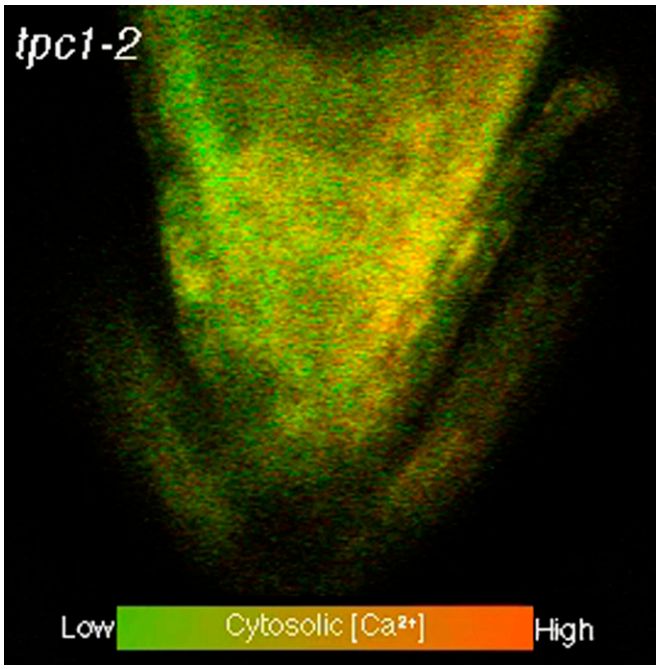
Movie S6. Transmission of Ca^{2+} wave through lateral root to main root after the root tip of the lateral root was locally treated with 100 mM NaCl. Cytosolic Ca^{2+} was monitored using *Arabidopsis* expressing the YCNano-65 biosensor and confocal ratio imaging. Movie duration, 750 s (10 s per frame, 75 frames).

Movie S6



Movie S7. Transmission of Ca^{2+} wave through root tissues and into the hypocotyl shootward of a root tip locally treated with 100 mM NaCl. Cytosolic Ca^{2+} was monitored using *Arabidopsis* expressing the YCNano-65 biosensor and confocal ratio imaging. Movie duration, 320 s (4 s per frame, 80 frames).

Movie S7



Movie S8. Ca^{2+} increase at the root tip of *tpc1-2* locally treated with 100 mM NaCl. Cytosolic Ca^{2+} was monitored using *Arabidopsis* expressing the YCNano-65 biosensor and confocal ratio imaging. Movie duration, 480 s (4 s per frame, 120 frames).

Movie S8