

Supplementary information, Data S1 Experimental Procedures

Oocyte Electrophysiology

The full-length cDNAs of *AtCSC1*, *HsCSC1* and *ScCSC1* were subcloned into vector pGEMHE¹. cRNA was synthesized from 1 µg of linearized plasmid DNA template using an mMessage mMachine *in vitro* transcription kit (Ambion, Austin, TX) according to the manufacturer's recommendations. Defolliculated *Xenopus laevis* oocytes were injected with 23 ng and incubated in ND96 at 18 °C for 4 days prior to electrophysiological assays. Oocytes were voltage-clamped at a holding potential of –60 mV using a TEV 200 amplifier (Dagan, Minneapolis, MN) and monitored by computer through a Digidata 1440A/D converter and pCLAMP 10.2 software (Axon Instruments, Foster City, CA). The pipette solution contained 3 M KCl. The oocytes were perfused with a standard ND96 solution containing (in mM) 96 NaCl, 2.0 KCl, 1.0 MgCl₂, 1.8 CaCl₂ and 10 HEPES, pH 7.5, at room temperature. For cation substitution experiments, 96 mM NaCl was replaced with equal molarity KCl or CaCl₂. In calcium-free bath solution, 1.8 mM CaCl₂ was replaced with 0.5 mM EGTA. Hyperosmotic stimuli were applied by perfusion with bath solution containing the indicated concentrations of mannitol.

Cell Lines and Ca²⁺-imaging Experiments

The complete cDNAs of *AtCSC1*, *HsCSC1* and *ScCSC1* were subcloned into eukaryotic expression vector pcDNA3.0 and transfected into CHO-K1 cells using Fugene 6 (Roche Applied Science, Indianapolis, IN). The transfected cells were cultured in DMEM containing 10% FBS (GIBCO, Grand Island, NY) and 1 mg/ml pen-strep (Sigma-Aldrich, St. Louis, MO) in 10% CO₂ and selected with 800 µg/ml G418. Stable clones that expressed the proteins were confirmed by western blot.

Cells were plated on glass-bottom dishes with DMEM culture medium containing 10% FBS (GIBCO, Grand Island, NY) and 1 mg/ml pen-strep (Sigma-Aldrich, St. Louis, MO) in 10% CO₂ and incubated for 48 hours at 37° C. The Ca²⁺-imaging experiments were performed following procedures similar to Liedtke *et al.* (2000)², with minor modifications as described.

After removal of culture medium, cells were loaded with Fura-2 by incubation at room temperature with 10 μ M Fura-2 acetoxymethyl ester dissolved in isosmotic saline solution, which contains (in mM) 130 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 20 D-glucose, 10 HEPES, pH 7.4, and 0.02% pluronic acid (Molecular Probes, Eugene, OR). Following loading with Fura-2, cells were washed four times with saline solution and allowed to recover in 1 mL saline solution for 15 minutes at 37° C. After recovery, cells were imaged at 2-second intervals for 480 seconds in total to monitor cytosolic calcium status. At 120 seconds, hyperosmotic shock was applied by addition of 1 mL saline solution containing 600 mM mannitol (300 mM final mannitol concentration). At 360 seconds, the medium was restored to near-isosmotic conditions by addition of 2 mL H₂O. The Ca²⁺ concentration of all solutions was held constant at 2 mM. Images of Fura-2 loaded cells with the excitation wavelength alternating between 340 nm and 380 nm were captured with a cooled CCD camera. Following subtraction of background fluorescence, the ratio of fluorescence intensity at the two wavelengths was calculated. The ratio of 340/380 nm fluorescence intensity in groups of 10-20 individual cells with the strongest responses were analyzed using MetaFluor (Universal Imaging Corporation). All graphs are the averaged responses from groups of individual cells from representative single experiments.

Mammalian Cell Electrophysiology

CHO cells were plated onto poly-D-lysine-coated coverslips for recording purposes, and recordings were performed 24 h later. Experiments were carried out at room temperature using whole-cell voltage clamp technique, with an Axopatch 2B amplifier filtered at 5 kHz and pClamp suite of software (Axon Instruments, Foster City, CA). Series-resistant compensation was 80% for all experiments, using 2–5 M Ω fire-polished pipettes. Pipette solution contained, in mM, 140 CsCl, 1 EGTA, 10 HEPES, 2 MgATP, pH 7.4 (adjusted with CsOH). The isosmotic bath solution contained (mM) 140 NaCl, 5 KCl, 1 MgCl₂, 2 CaCl₂, 10 HEPES, 10 glucose, pH 7.4 (adjusted with NaOH). During whole-cell recording, cells were maintained in isosmotic bath solution.

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>Arabidopsis_lyrata_D7LUU6

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>Arabidopsis_thaliana_Q9SZT4

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ESASCYLDGDR-
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>Arabidopsis_thaliana_Q9XEA1

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>Aspergillus_niger_A2QDK9

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FKTSMDNSYIWA AVQGILSPA VTSLVYIVLPIIFRRLAIRAGDVTKTSRERHVLNKLYTFFVFNNLI
VFSLSAAWTFVSAVIDAERSLYIKIVNALCQVSPFWVTYLLQRNL-
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>Aspergillus_niger_A2R3B1

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>Aspergillus_niger_A2R6X5

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>Bos_taurus_A7MB88

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>Botryotinia_fuckeliana_A6RS77

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>Canis_familiaris_E2RBB2

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>Chlamydomonas_reinhardtii_XP_001695096.1

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>Chlamydomonas_reinhardtii_XP_001698593.1

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>Chlamydomonas_reinhardtii_XP_001699063.1

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>Chlamydomonas_reinhardtii_XP_001699584.1

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>Chlamydomonas_reinhardtii_XP_001702326.1

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MWQDFGPLSEYLAISSICEV-----NTGRRRYAHP
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KAQKNGRKTHTPCA-Q--EKVDAEQYSELEEKLTDEFNAEKNWI-
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LNILLFLLFFLTTPAIIIVNTMDKFNV-----
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>Debaryomyces_hansenii_B5RSZ8

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>Debaryomyces_hansenii_Q6BLR7

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>Debaryomyces_hansenii_Q6BWA0

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RGFF--

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DIKGV--PPTR----
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>Drosophila_melanogaster_Q6NP91

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>Ricinus_communis_B9S919

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>Volvox_carteri_XP_002949162.1

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>Volvox_carteri_XP_002951035.1

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>Yarrowia_lipolytica_XP_505733.1

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>Zea_mays_B0FSL2

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VKPEEF AVLVRDIPRDETIKDSVDSYFRANTFYRSMV VTDHTKADKIYQEIEGHKQKIARAEVVY
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>Zea_mays_B6SS81

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