Supplemental Figure Legends

Figure S1. Previous VRAC candidates are not by themselves required for hypotonicity-induced iodide influx, Related to Figure 1

(A) Hypotonicity-induced quenching responses (mean ± SEM, *n* = 4) in HEK-YFP cells transfected with scrambled siRNA or siRNA against previous VRAC candidate genes. **(B)** siRNA-mediated gene knockdown in HEK293T cells assayed by qPCR. *GAPDH* was used as the reference gene (black bars). Expression levels were normalized to cells treated with scrambled siRNA, and presented as mean ± SEM (*n* = 2).

Figure S2. LRRC8B-8E are not by themselves required for hypotonicity-induced iodide influx, Related to Figure 2

(A) siRNA-mediated SWELL1 gene knockdown in HEK293T cells assayed by qPCR. *GAPDH* was used as the reference gene. Expression levels were normalized to cells treated with scrambled siRNA, and presented as mean ± SEM (*n* = 2). **(B)** Hypotonicity-induced quenching responses (mean ± SEM, *n* = 4) in HEK-YFP cells transfected with scrambled siRNA or siRNA against *LRRC8B-8E*. **(C)** siRNA-mediated gene knockdown in HEK293T cells assayed by qPCR. *GAPDH* was used as the reference gene. Expression levels were normalized to cells treated with scrambled siRNA (black bars), and presented as mean ± SEM (*n* = 2).

Figure S3. Characteristics of HYPO-induced whole cell currents recorded from HEK293T, HeLa and T lymphocytes are consistent with $I_{Cl, swell}$, Related to Figure 3

(A) Whole cell currents in scrambled siRNA-transfected HEK293T cells elicited by a voltage ramp protocol (right, lower) before (trace 1) and during exposure to 210 mOsm/kg solution (HYPO; trace 2). The currents were rapidly blocked by 20 μM DCPIB (trace 3). Currents (left) at −100 mV and +100 mV were plotted at 15 sec intervals. The gap in the data is due to switching to a voltage step protocol. **(B)** HYPO-induced currents (plotted as in panel A) were inhibited in a voltage-dependent manner by 100 μM DIDS, recovered by washout in HYPO and subsequently inhibited in a voltage-independent manner by 20 μM DCPIB. **(C)** The percent block by 20 μM DCPIB of HYPO-induced currents at +100 and −100 mV is not significant (One sample *t*-test). The numbers of cells are indicated. **(D)** The percent block by 100 μM DIDS of HYPO-induced currents at +100 and −100 mV is significant ($p<0.005$, One sample *t*-test). The numbers of cells are indicated. (**E**) Examples of HYPO-induced currents elicited by the voltage ramp protocol used for data acquisition (lower trace) recorded from HEK293T cells transfected with scrambled siRNA (top) or siRNA targeting *SWELL1* (middle). Current traces acquired every 1 min from the beginning of the stimulus are plotted. R_a and R_m are monitored using the −20 mV step prior to the voltage ramp. **(F)** Whole cell currents in scrambled siRNA-transfected HeLa cells elicited by a voltage ramp protocol (right, lower) before (trace 1) and during exposure to 230 mOsm/kg solution (HYPO; trace 2). The currents were blocked by 20 μM DCPIB (trace 3). The currents recover after washout (trace 4, overlays trace 2 in this case). Currents (left) at −100 mV and +100 mV were plotted at 15 sec intervals. **(G)** Ramp-induced current traces activated by HYPO (blue trace) were

blocked in a voltage dependent manner by 100 μM DIDS (purple trace, 1.5 min exposure). **(H)** The percent block of HYPO-induced currents in HeLa cells by 20 μM DCPIB was not significant (One sample *t*-test). (**I**) The percent block of HYPO-induced currents in HeLa cells by 50-100 μM DIDS at +100 mV vs. −100 mV was significant ($p<0.005$, One Sample *t*-test). **(J)** siRNA-mediated *SWELL1* knockdown in HeLa cells assayed by qPCR. *GAPDH* was used as the reference gene. Expression levels were normalized to cells treated with scrambled siRNA (as 100%), and presented as mean ± SEM ($n = 3$). **(K)** shRNA-mediated *SWELL1* knockdown in T lymphocytes assayed by qPCR. *GAPDH* was used as the reference gene. Expression levels were normalized to cells transduced with lentiviruses expressing control shRNA (as 100%), and presented as mean ± SEM ($n = 3$). (**L**) Families of whole cell currents recorded from human T lymphocytes transduced with lentiviruses expressing shRNAs targeting firefly luciferase (control, top) and SWELL1 (bottom) in isotonic solution (left), 260 mOsm/kg HYPO (middle) and after inhibition by 20 μM DCPIB in 260 mOsm/kg HYPO (right). Voltage steps (840 msec) were applied from −50 mV to voltages between +100 mV and −100 mV in increments of −20 mV every 10 sec (holding voltage, 0 mV).

Figure S4. SWELL1 does not by itself generate larger $I_{Cl,\ swell}$, Related to Figure 4

(A) HEK293T cells transfected 1 day earlier with vector, SWELL1 or SWELL1$^{\Delta 91/+35}$ expression constructs were challenged with HYPO (230 mOsm/kg; applied at the arrow) and the development of current (shown as leak subtracted current density) was determined at −100 and +100 mV. Error bars represent mean ± SEM. **(B)**

Overexpression of FLAG-tagged SWELL1 or SWELL1$^{\Delta 91/+35}$ in HEK293T cells was analyzed by immunoprecipitation with anti-FLAG antibody followed by western blot with antibody against SWELL1. SWELL1 protein sizes are as expected.

Figure S5. Low intracellular ionic strength activates DCPIB-sensitive currents in SWELL1-rescued stable knockdown cells and SCAM analysis of SWELL1 cysteine mutants, Related to Figure 5

(A) A SWELL1-rescued stable knockdown HeLa cell was patch clamped in the ruptured whole cell configuration with low ionic strength intracellular solution in the pipette and isotonic solution in the bath and the standard voltage ramp protocol was begun within 10 sec. Current densities at −100 and +100 mV increase shortly after break in. Low intracellular ionic strength solution has no effect on vector-transfected knockdown cells (see panel B). **(B)** Average current densities at −100 and +100 mV after whole cell access for SWELL1-rescued stable knockdown cells recorded with either low ionic strength (red) or normal ionic strength (black) pipette solutions, and for vector-transfected knockdown cells recorded in the low ionic strength pipette solution (grey). Data are shown as mean ± SEM. **(C)** The percent block of low intracellular ionic strength-induced currents in HeLa cells and SWELL1-rescued stable knockdown cells by 20 μM DCPIB. Error bars represent mean ± SEM. The numbers of cells are indicated. **(D)** Normalized rescue activity (n = 3-4) of hypotonicity-induced quenching response in stable *SWELL1* knockdown HEK-YFP cells expressing RNAi-insensitive WT SWELL1 (normalized as 100%) or SWELL1 mutants with cysteine individually substituted in four putative TM

domains. Red dashed lines indicate a cut-off of 60% (rated as functional in this assay) of WT SWELL1-mediated rescue activity. **(E)** After MTSES block of T44C-mediated rescue currents in stable *SWELL1* knockdown HeLa cells, cells were continuously perfused with HYPO to washout the MTSES reagent (0.1-3.33mM). There was little recovery of currents mediated by both WT and T44C SWELL1 after 5 min. Error bars represent mean ± SEM (n.s., not significant). The numbers of cells are indicated.

Figure S6. The shift of reversal potential of T44C-mediated rescue current in the iodide solution is not dependent on the current density, related to Figure 6

The reversal potential (V_{rev}) of $I_{Cl, swell}$ in extracellular iodide is plotted as a function of current density for stable *SWELL1* knockdown HeLa cells expressing WT or T44C SWELL1 as well as HeLa control cells. V_{rev} for T44C are significantly more negative over the range of current densities observed. Current densities are lower in minimalistic "bi-anionic" conditions (with either external I^- or Cl^-) compared to standard recording conditions, even with the presence of intracellular 4 mM Mg-ATP.

Figure S7. Characterization of cell volumes of control and *SWELL1* siRNA-transfected HeLa cells and HYPO-induced currents in HeLa and SWELL1-rescued stable knockdown cells in taurine vs. chloride-containing external solutions, Related to Figure 7

(A) The average cell volumes of HeLa cells transfected with either scrambled or *SWELL1* siRNA are similar in isotonic solutions. Error bars represent mean ± SEM (n.s., not significant). The numbers of experiments are indicated and the average cell volume of more than 10,000 cells in each experiment was measured. **(B)** Representative HYPO-induced whole cell currents at −100 and +100 mV in HeLa (left) and SWELL1-rescued stable knockdown cells (right) bathed in either taurine- (top) or Cl^--(bottom) containing solutions. Currents were blocked by 20 μM DCPIB. **(C)** Reversal potentials for HYPO-induced currents in both HeLa and SWELL1-rescued stable knockdown cells were shifted dramatically to more positive potentials when cells were bathed in $taurine^-$ compared to Cl^-. The solutions used contained the minimal set of ions that still allowed activation of $I_{Cl,\,swell}$ (see Method). Error bars represent mean ± SEM (n.s., not significant). The numbers of cells are indicated.

Table S1. Composition of hypotonic solutions for the YFP quenching assay, related to Figure 1.

KCl (mM)	HEPES (mM)	Mannitol (mM)	Osmolality* (mOsm/kg)	Final osmolality# (mOsm/kg)
5	20	90	120	215
5	20	120	150	230
5	20	140	170	240
5	20	160	190	250
5	20	180	210	260
5	20	200	230	270
5	20	220	250	280
5	20	280	310	310
*Osmolality is measured by Vapor Pressure Osmometer 5600. #Final osmolality is estimated after equal volume mixing with isotonic solution during the YFP quenching assay.				

Table S2. Electrical and cellular properties of HEK293T, HeLa and human T lymphocytes, related to Figure 3

Parameter	HEK293T, scrambled siRNA	HEK293T, *SWELL1* siRNA	HeLa, scrambled siRNA	HeLa, *SWELL1* siRNA	Lymphocyte control shRNA	Lymphocyte *SWELL1* shRNA
R_a (MΩ)	8.9±0.6 (24)	9.2±1.1 (37)	8.5±0.6 (21)	8.1±0.6 (37)	8.9±0.8 (9)	7.9±0.4 (9)
R_m (MΩ), initial	2860±350 (24)	3070±300 (37)	3110±340 (21)	3500±240 (37)	3320±420 (9)	3910±630 (9)
C_m (pF)	26.2±2.1 (24)	25.6±1.4 (37)	25.4±1.8 (21)	27.0±1.1 (37)	7.2±0.6 (9)	7.6±0.6 (9)
Basal current density @−100mV (pA/pF)	−2.6±0.8 (24)	−2.9±0.7 (37)	−1.8±0.3 (21)	−1.2±0.2 (37)	−7.4±1.3 (9)	−5.8±1.0 (9)

Extended Experimental Procedures

Genome-Wide RNAi Screen

The siRNA library (QIAGEN) was previously described (Zhang et al., 2009). Briefly, it targets 17,631 known and 4837 predicted human genes. Four siRNA constructs were designed for each gene with a pool of two siRNAs per well (two siRNAs/well, two wells/gene) for a total of 89,872 siRNAs. The siRNAs (1 pmol; 0.5 pmol for each siRNA; final concentration of 12.5 nM) were prespotted on 384 well plates. The hypotonicity-induced YFP quenching response was calculated and then normalized to the plate average (set as 1). The average score of two duplicates was determined for each well. The well was considered a hit when its score was 2 standard deviations below the mean. Smartpool siRNAs (Dharmacon) against human *SWELL1* include: #1 GGUACAACCACAUCGCCUA; #2 AGUAUGACCUGGACCGGCA; #3 CAAAGCAGCCAGACGUUGA; #4 GAGCAAGUCUCAAGAGCGC.

Molecular Biology

Myc-FLAG-tagged open reading frame of human SWELL1 (NP_062540) in pCMV-Entry was obtained from OriGene. The coding sequence (2,433 bp) without a tag was PCR amplified and sub-cloned into pIRES2-EGFP (Clontech). For the SWELL1$^{\Delta 91/+35}$ mutant clone, a 2.1 kb PCR amplified fragment from WT SWELL1 coding sequence, and a synthesized 165 bp fragment (IDT) were cloned sequentially into pIRES2-EGFP vector.

The protein sequence of SWELL1$^{\Delta 91/+35}$ is the same as previously reported . Myc (EQKLISEEDL) and FLAG (DYKDDDDK) tags, RNAi-resistant silent mutations, and cysteine mutations were introduced into SWELL1 or SWELL1$^{\Delta 91/+35}$ constructs using QuikChange II XL site-directed mutagenesis kit (Agilent Technologies).

Cell Culture

HEK293T and HeLa cells were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 4.5 mg/ml glucose, 10% fetal bovine serum and 50 units/ml penicillin and 50 μg/ml streptomycin. Primary human CD4+ T lymphocytes were purified from LeukoPac (AllCells) by positive selection with CD4 MicroBeads (Miltenyi Biotec) according to the manufacturer's instruction. The isolated lymphocytes were maintained in Hematopoietic Medium (X-VIVO, Lonza) and stimulated with CD3/CD28 Dynabeads (Life Technologies) for 24 h before lentiviral transduction.

Electrophysiology

The effect of low intracellular ionic strength on whole cell conductance was determined using a method adapted from (Voets et al., 1999). Voltage ramps were applied every 5 sec beginning within 10 sec of patch rupture into the whole cell configuration for up to 8 min. Some cells were subsequently challenged with 20 μM DCPIB. The normal pipette solution contained (in mM) 40 CsCl, 100 Cs-gluconate, 1 $MgCl_2$, 1.93 $CaCl_2$, 5 EGTA, 4 Na_2ATP, 10 HEPES, pH 7.2 with CsOH (305 mOsm/kg). The low ionic strength pipette

solution contained (in mM) 40 CsCl, 30 Cs-gluconate, 1 $MgCl_2$, 1.93 $CaCl_2$, 5 EGTA, 4 Na_2ATP, 10 HEPES, 140 mannitol, pH 7.2 with CsOH (305 mOsm/kg). The ionic strength of these 305 mOsm/kg solutions differed by 70 mM. The osmolality of the standard bath solution was 305 mOsm/kg. The reversal potentials for the low ionic strength-activated currents were −17.6 ± 1.1 mV (n = 9) and −19.9 ± 0.2 mV (n = 8) for SWELL1-rescued stable knockdown cells and HeLa cells, respectively, when corrected for junction potential (−13.3 mV, PClamp LJP calculator; E_{Cl}, −18.5 mV)

The ability of taurine to permeate HeLa cells and SWELL1-rescued stable knockdown cells was examined in essentially bi-ionic conditions similar to those described for iodide permeability experiments; the extracellular solutions were adapted from (Manolopoulos et al., 1997) who investigated this in endothelial cells. The intracellular solution contained 140 CsCl, 4 Mg-ATP, 10 HEPES (pH7.2) and external solutions contained either Cl^- or taurine together with NMDG and HEPES (and mannitol in the isotonic solution). For reversal potential determinations in external Cl^-, isotonic solution contained 120 NMDG, 115 HCl, 10 HEPES and 70 mannitol and hypotonic solution lacked the 70 mannitol (E_{Cl} = 5mV). For reversal potential determinations in taurine, isotonic solution contained 30 NMDG, 210 taurine, 10 HEPES and 70 mannitol (pH8.2, final $taurine^-$ concentration of 35 mM) and hypotonic solution lacked the 70 mannitol. Voltage ramps from −80 to +120 mV were applied every 5 sec. Vector-transfected stable knockdown cells showed no currents reversing at positive potentials, however, small currents reversing at ~−30 mV and/or large currents reversing more negative than −80 mV were observed. The former were observed in SWELL1-rescued stable knockdown cells but they appeared to stabilize prior to the onset of the gradual

development of inward current indicative of $I_{Cl,\ swell}$. At least 5 sweeps were averaged just prior to the increase in inward current and compared to averaged sweeps during the maximum observed HYPO-induced currents to obtain V_{rev}. Similar V_{rev} was observed when 20 μM DCPIB was used block HYPO-induced currents. The solutions used contained the minimal set of ions that still allowed activation of $I_{Cl,\ swell}$.

Quantitative Real-Time PCR

For the analysis of *Swell1* expression profile in mouse tissues, immune cell types (T cells, B cells, and microphages) were sorted using FACS (FACSAria; BD) from single cells prepared from C57BL/6J wild-type mouse spleen using fluorescent dye-conjugated antibodies (anti-CD3, anti-CD19, and anti-F4/80, respectively). Total RNA were isolated using TRIzol reagent (Life Technologies). Total RNA from all other tissues were purchased from Zyagen (San Diego). For the analysis of siRNA or shRNA knockdown efficiency, total RNA were isolated from transfected or transduced cells. 200ng of total RNA was used to generate the 1st strand cDNA using the QuantiTect Reverse Transcription kit (QIAGEN). Taqman qPCR assays for mouse *Swell1* (assay id: Mm.PT.56.12679347), human *SWELL1* (assay id: Hs.PT.49a.14499917), human *ICln* (assay id: Hs.PT.53a.24955799), human *CLCN2* (assay id: Hs.PT.53a.21197454), human *CLCN3* (assay id: Hs.PT.53a.1682211), human *BEST1* (assay id: Hs.PT.53a.23190315), human *LRRC8B* (assay id: Hs.PT.53a.19114225), human *LRRC8C* (assay id: Hs.PT.53a.20916114), human *LRRC8D* (assay id: Hs.PT.53a.15346618), human *LRRC8E* (assay id: Hs.PT.53a.26290551), human *GAPDH* (assay id:

Hs.PT.39a.22214836), and internally designed mouse *Gapdh* assay (forward primer: GCACCACCAACTGCTTAG; reverse primer: GGATGCAGGGATGATGTTC and probe: CAGAAGACTGTGGATGGCCCCTC) were purchased from IDT with a FAM reporter dye and a non-fluorescent quencher. FastStart Universal probe master mix (Rox) from Roche was used. The reaction was run in the ABI 7900HT fast real time system using 0.5 µl of the cDNA in a 10 µl reaction according to the manufacturer's instruction in triplicate. *GAPDH* was used as the reference gene. Calibrations and normalizations were done using the $2^{-\Delta\Delta CT}$ method (Schmittgen and Livak, 2008).

Western Blot Analysis

HEK293T cells were transiently transfected with C-terminal FLAG tagged SWELL1 or SWELL1$^{\Delta 91/+35}$ constructs. Cells were harvested in lysis buffer (50 mM Tris pH 8, 150 mM NaCl, 1% (w/v) n-Dodecyl-β-maltoside, and a cocktail of protease inhibitors). The cell lysates were incubated with 5 mg Dynabeads (Life Technologies) coupled with M2 anti-FLAG antibody (Sigma) at 4°C for 1 h according to the manufacturer's instruction. Following five washes in lysis buffer, immunoprecipitates were eluted with NuPAGE LDS Sampling Buffer and resolved by NuPAGE 4-12% Bis-Tris Gel (Life Technologies). After electrophoresis, the gels were transferred to PVDF membranes. Membranes were blocked with 5% milk in TBS buffer with 0.1% Tween-20, incubated with anti-SWELL1 antibody (1:200; HPA016811, Sigma) at 4°C overnight and followed by peroxidase-conjugated anti-rabbit IgG secondary antibody. Proteins were detected with SuperSignal West Femto Substrate (Thermo Scientific).

Supplemental References

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