Supporting Information

Nesin et al. 10.1073/pnas.1312520111

SI Materials and Methods

Whole-Exome Sequencing. Exome enrichment was performed by using the Truseq Exome Enrichment kit (Illumina) per the manufacturer's protocol. Sequences were generated by using an Illumina HisEq 2000 instrument with paired-end, 100-bp reads. Sequences were aligned to the human reference genome (hg19) by using the Burrows–Wheeler Aligner (3). Local realignment around indels followed by empirical base quality score recalibration was performed using the Genome Analysis Tool Kit (4). Exonic variants were identified for each patient by using the Genome Analysis Tool Kit Unified Genotyper program. Variants were excluded from analysis if they did not meet the following criteria: alignment quality score of 30 or greater, a read depth of at least 8, a quality by depth score of at least 2.5, presence of a homopolymer run of 5 bases or less, a strand bias score of less than −10.0, and a map quality score greater than 25. Putatively functional variants were identified by using the program Annovar by using a filtering strategy (5). Synonymous variants were first identified and removed, followed by removal of variants not within highly conserved regions or those within genomic segmental duplications. Variants were then removed if they were previously identified in either the 1000 Genomes Project pilot study or the dbSNP130 database. Variants remaining after this filtering strategy were mapped back to their respective genes. Gene lists for each patient were generated and compared between patients. Effects of each variant, whether benign or damaging, were predicted by using Sorts Intolerant From Tolerant (SIFT) (6). Identified variants were confirmed by inspection of raw aligned reads using the Integrated Genome Viewer (7) followed by bidirectional Sanger sequencing of the relevant exon(s).

Ratiometric Single-Cell Ca^{2+} Imaging. Lymphocytes (B-cell lineage) were derived from a healthy individual, a patient with Stormorken syndrome (female proband; Fig. 1*A*, arrow), and a patient with congenital miosis and tubular-aggregate myopathy (female proband; Fig. 4*A*, arrow) and immortalized by EBV in vitro as described previously (8). No mutations in STIM1 or ORAI1 were detected in cells from the healthy individual by whole-exome sequencing. Cells were plated onto glass coverslips and loaded with 2 μM Fura-2/AM in extracellular solution (ECS) containing 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 10 mM glucose, and 15 mM Hepes, pH 7.4 ($[Ca^{2+}]_o$: 1.8 mM), in the presence of 0.05% Pluronic F-127 for 45 min at room temperature. Cells were washed twice in ECS and incubated for 15 min in 37 °C before intracellular imaging. Cells were incubated in a Ca^{2+}-free solution (same as ECS but without $CaCl_2$) and stimulated with 2 μM thapsigargin at the indicated time. Ca^{2+} influx in response to store depletion was determined by the readdition of ECS containing 2 mM $[Ca^{2+}]_o$. Individual cells were excited by the DeltaRam XTM monochromator (Photon Technology International), and emission images were collected by a high-definition imaging scientific complementary metal-oxide semiconductor camera driven by the EasyRatioPro software (Photon Technology International). Fluorescence ratios of 340/380 were taken every 5 s using 100-ms exposure time. Intracellular Ca^{2+} concentration was expressed as 340/380 ratio.

Site-Directed Mutagenesis. cDNAs encoding mouse STIM1 (BC021644) or human ORAI1 (BC015369) were obtained from Open Biosystems. QuikChange II XL site-directed mutagenesis kit (Agilent Technologies) was used to generate STIM1_R304W, STIM1_K(384-6)Q, STIM1_D76A, or ORAI1_P245L by using the following forward primers, respectively:

5′-GCGGCTGAAGGAGCTGTGGGAGGGTACTGAGAA-TG-3′,

5′-GAGGGGGCTGAGAAAATACAACAGCAGAGAAAC-ACGCTTTTTGG-3′,

5-CATCCATAAGCTGATGGCTGACGATGCCAATGGT-G-3′, or

5′-CTCGACCACCATCATGGTGCTCTTCGGCCTGATCT-TTATCG-3′.

For the in vivo experiments, WT STIM1 (NM_003156.3) and WT ORAI1 (NM_032790) were cloned into the pENTR221 vector (Invitrogen). For the production of the capped human mRNA, the STIM1_WT, STIM1_D76A, STIM1_R304W, STIM1_K(384-6)Q, STIM1_R304W plus K(384-6)Q, ORAI1_WT, and ORAI1_P245L inserts were transferred to pCS2+ by using the Gateway technology (Invitrogen). The mutations were introduced to STIM1_WT and ORAI1_WT by site-directed mutagenesis. All constructs were fully sequenced by using exonic primers to verify the sequence.

Transient Transfections. HEK293 cells were transfected in 35-mm dishes by using Lipofectamine 2000 (Invitrogen). A ratio of 2:1:01 (in micrograms plasmid DNA per dish) for ORAI1:STIM1:CD8α was used throughout the study. In the case of STIM1_WT/STIM1_R304W transfection, 0.5 μg of each plasmid was used. CD8α was used as a transfection marker. Cells were allowed to recover for 24 or 48 h following transfection, and $CD8\alpha^+$ cells were identified by magnetic beads coated with anti-CD8α (Dynabeads; DYNAL) and processed for electrophysiology.

Electrophysiology. Whole-cell patch-clamp experiments were performed in voltage-clamp tight-seal configuration at room temperature. Recordings were acquired by using the Warner PC-505B amplifier (Warner Instruments) and pClamp9.2 software (Axon Instruments). Pipettes were pulled from borosilicate glass capillaries (Warner Instruments) and polished to a final resistance of 2–4 MΩ. Voltage ramps of 100-ms duration spanning a range of −100 to +100 mV were delivered from a holding potential of 0 mV at a rate of 0.5 Hz with an interramp interval of 10 s. Currents were filtered at 2 kHz and digitized at 100-μs intervals. Capacitive currents were determined and corrected before each voltage ramp. Standard external solution (bath) was as follows (in mM): NaCl 120, KCl 2.8, CsCl 10, $MgCl_2$ 2, $CaCl_2$ 10, Hepes 10, 10 mM tetraethylammonium, and glucose 10, pH 7.2, with 300 mOsm NaOH. Standard internal solution (pipette) was as follows (in mM): Cs-methanesulfonate 120, NaCl 8, EGTA or BAPTA 10, $MgCl_2$ 3, Hepes 10, pH 7.2, with 300 mOsm CsOH. Traces recorded after application of 20 μM La^{3+} were used as templates for leak subtraction. Step currents were obtained by a voltage step protocol applying 200-ms hyperpolarizing pulses from +30 mV to −80, −100, and −120 mV potentials at 200 s after "break-in." Whole-cell currents in EBV-immortalized lymphocytes were measured as described earlier in transfected cells, except for the concentration of $MgCl_2$ in the pipette solution, which was raised from 3 to 8 mM to block possible contamination by endogenous TRPM7 currents. Step currents were obtained at 400 s after break-in.

Zebrafish Experiments. Zebrafish embryos and adults were maintained and mated according to standard procedures (9) and all experiments were carried out with the approval of the institutional

animal care and use committee. Embryos at 24 h post fertilization (hpf) were raised in 0.2 mM 1-phenyl-2-thio-urea (Sigma-Aldrich) to prevent pigment formation and allowed to develop for 48 hpf before o-dianisidine staining. Tg(CD41:GFP) embryos were allowed to develop for 72 hpf, which is the time point at which thrombocyte progenitors are visible. Capped mRNAs for WT or mutant forms of human *STIM1* or *ORAI1* were prepared using the SP6 Message Machine Kit (Ambion). Capped mRNA (100 pg) was used for embryo injection.

o-Dianisidine Staining. Live embryos were dechorionated and stained for 15 min in 0.6 mg/mL o-dianisidine (Santa Cruz), 0.01 M NaAc (pH 4.6), 0.65% H_2O_2, and 40% (vol/vol) EtOH in the dark. Stained embryos were cleared by two washes with 40% EtOH and scored for spontaneous bleeding.

Formation of Caudal Vein. The Tg(Fli1:GFP) line shows expression of GFP in endothelial cells. After 24 hpf, embryos were raised in 0.2 mM 1-phenyl-2-thio-urea (Sigma-Aldrich) to prevent pigment formation and allowed to develop for an additional 24 h before scoring. Capped mRNAs for WT or mutant forms of human STIM1 were prepared by using the SP6 Message Machine Kit (Ambion), and 100 pg of capped mRNA was used for embryo injection. Injected embryos were dechorionated and scored for GFP expression in the caudal vein at 48 hpf.

Isolation of Human Primary Skin Fibroblasts. Informed consent was obtained for 2-mm punch skin biopsy under local anesthesia from a healthy individual and the patient with Stormorken syndrome from family 1 (Fig. 1*A*). Tissue was minced and cells were released by trypsinization. Cells were cultured in a medium containing 70% (vol/vol) α-MEM, 10% (vol/vol) M-199, and 20% (vol/vol) heat-inactivated FBS and supplemented with nonessential amino acids, sodium pyruvate, L-glutamine, penicillin/streptomycin, and gentamycin (50 μg/mL). Fibroblast outgrowth started at day 3–7, and cultures were allowed to grow up to 70% confluence before splitting.

1. Böhm J, et al. (2013) Constitutive activation of the calcium sensor STIM1 causes tubular-aggregate myopathy. *Am J Hum Genet* 92(2):271–278.
2. Shahrizaila N, Lowe J, Wills A (2004) Familial myopathy with tubular aggregates associated with abnormal pupils. *Neurology* 63(6):1111–1113.
3. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25(14):1754–1760.
4. McKenna A, et al. (2010) The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 20(9):1297–1303.
5. Wang K, Li M, Hakonarson H (2010) ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 38(16):e164.
6. Kumar P, Henikoff S, Ng PC (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 4(7):1073–1081.
7. Robinson JT, et al. (2011) Integrative genomics viewer. *Nat Biotechnol* 29(1):24–26.
8. Amoli MM, Carthy D, Platt H, Ollier WE (2008) EBV Immortalization of human B lymphocytes separated from small volumes of cryo-preserved whole blood. *Int J Epidemiol* 37(suppl 1):i41–i45.
9. Westerfield M, ed (2000) *The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (Danio rerio)* (Univ Oregon Press, Eugene, OR), 4th Ed.

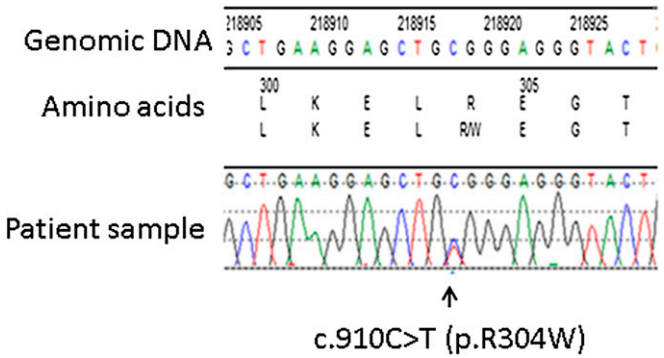

Fig. S1. Sequence chromatogram showing a heterozygous mutation in STIM1, with affected members having a C/T genotype.

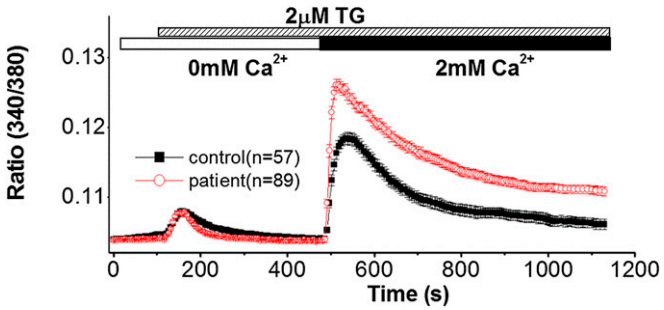

Fig. S2. STIM1_R304W enhances Ca^{2+} release-activated Ca^{2+} (CRAC) channel activity. Store-operated Ca^{2+} entry in skin fibroblasts obtained from an unaffected individual (control; black, $n = 57$ cells) or a patient with Stormorken syndrome (patient; red, $n = 89$) by using single-cell Ca^{2+} imaging. Cells were loaded with 2 μM Fura-2/AM and placed in ECS containing 0 mM Ca^{2+}. Stores were depleted with 2 μM thapsigargin (TG), and Ca^{2+} influx was stimulated by the addition of 2 mM Ca^{2+} in the ECS.

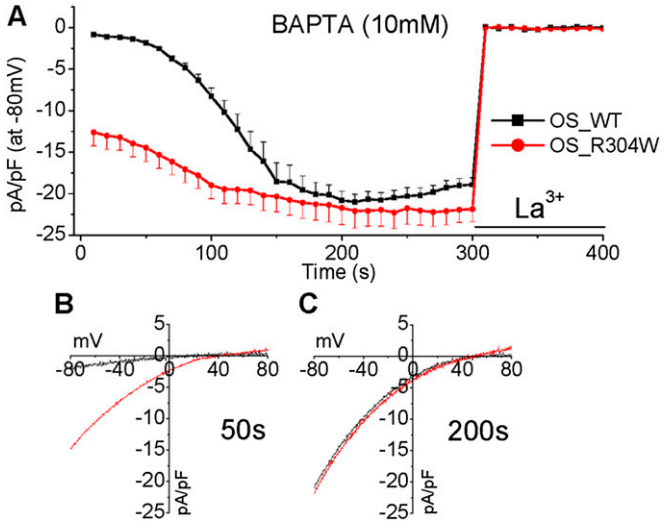

Fig. S3. Constitutive activation of I_{CRAC} by STIM1_R304W by using BAPTA. (*A*) Time course of I_{CRAC} in HEK 293 cells transiently cotransfected with ORAI1 plus WT STIM1 (OS_WT; black, $n = 5$) or ORAI1 plus STIM1_R304W (OS_R304W; red, $n = 7$) induced by 10 mM BAPTA and suppressed by 20 μM La^{3+}. (*B* and *C*) Current–voltage curves taken at 50 (*B*) or 200 s (*C*) after break-in in cells transfected with OS_WT (black, $n = 5$) or OS_R304W (red, $n = 7$).

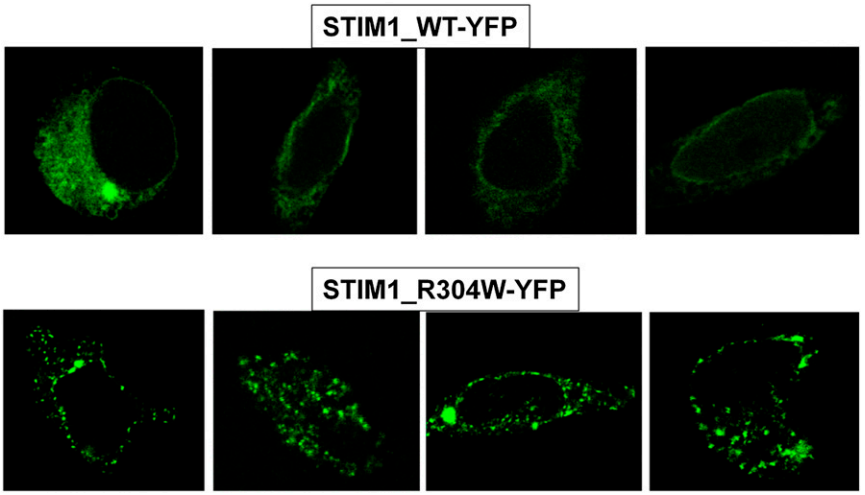

Fig. S4. STIM1_R304W accumulates in preformed puncta in resting cells. HEK 293 cells were transiently transfected with YFP-tagged WT STIM1 (STIM1_WT-YFP; 1 μg of plasmid) or STIM1_R304W-YFP (1 μg of plasmid) and WT ORAI1 (2 μg of plasmid). Images of four representative resting cells from each transfection taken by an Olympus inverted confocal microscope and processed with Fiji software are shown.

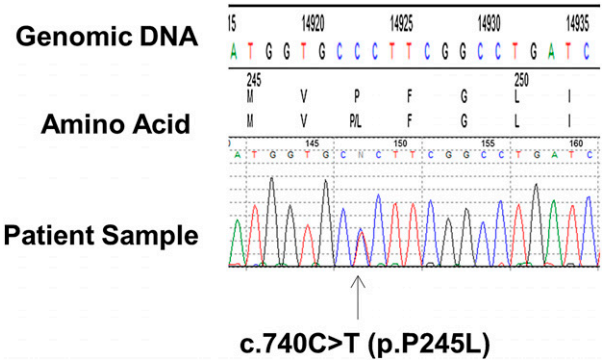

Fig. S5. Sequence chromatograms provide evidence of a heterozygous mutation in *ORAI1*, with affected members having a C/T genotype.

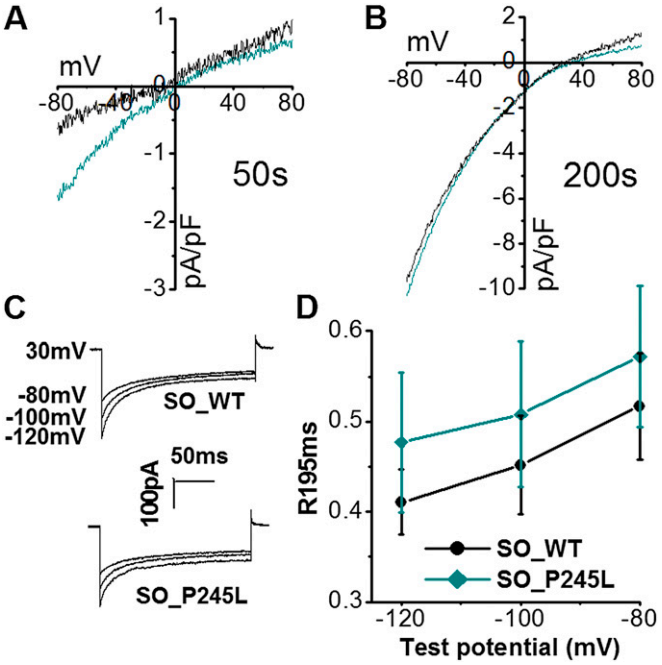

Fig. S6. Orai1_P245L forms CRAC channels with normal fast Ca^{2+}-dependent inactivation. (*A and B*) Current–voltage curves taken at 50 (*A*) or 200 s (*B*) after break-in in cells transfected with SO_WT (black, $n = 5$) or SO_P245L (dark cyan, $n = 8$) (*C*) Representative step currents generated from hyperpolarizing pulses at the indicated test potentials at 200 s following break-in in cells transfected with SO_WT or SO_P245L in 10 mM extracellular Ca^{2+}. Duration of the pulse was 200 ms. (*D*) Quantification of inactivation as determined by the ratio (R195ms) of the peak current at the beginning of the pulse (I_0) to tail current at the end of the pulse (I_{195}) in cells transfected with the indicated plasmids.

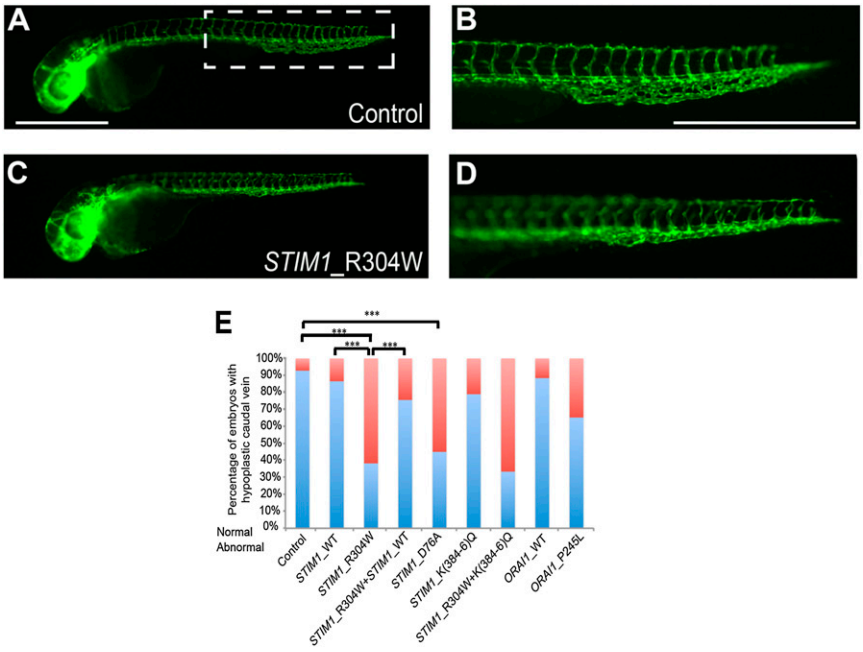

Fig. S7. Overexpression of STIM1_R304W causes hypoplastic caudal vein formation in developing zebrafish embryos. (*A–D*) Lateral views of a control (*A*) or *STIM1_R304W* (*C*)-injected Tg(Fli1:GFP) transgenic embryo expressing GFP in endothelial cells and hence allowing the visualization of developing blood vessels 48 hpf. *B* and *D* are magnified views of the boxed area in *A* in the respective embryos, showing that the caudal vein in the STIM1_R304W injected embryos (*C*) appears hypoplastic compared with the controls (*B*). (*E*) Percent distribution of normal vs. abnormal embryos as determined by phenotypic aberrations of the caudal vein (**$P < 0.001$, ***$P < 0.0001$).

Table S1. Relevant physical and laboratory findings in the two probands with Stormorken syndrome, and the two distantly related probands with Stormorken-like syndrome

	Gene/mutation				
Finding	STIM1 p.R304W		ORAI1 p.P245L		
Probands	Case 1	Case 2	Case 1	Case 2	Normal range
Creatine kinase level	1,668	3,819	800	500	60–365 IU/L
Platelet count	99	83	Normal	Normal	150–400 K/mm^3
Bleeding diathesis	+	+	–	–	
Asplenia	+	+	–	–	
Weakness	+	+	+	+	
Miosis	+	+	+	+	

Table S2. Phenotypic spectrum and effect of mutations in *STIM1* and *ORAI1* on the CRAC channel in Stormorken and Stormorken-like syndromes

	Gene/mutation		
Effect	STIM1 (mutations in EF hand)*	ORAI1 p.P245L[†]	STIM1 (mutations in Cα2 helix)[‡]
Muscle pathology	Tubular myopathy	Tubular myopathy	Tubular myopathy
Effect on CRAC channel	Constitutive activation, normal fast CID, reduced slow CID	No constitutive activation, normal fast CID, reduced slow CID	Constitutive activation, reduced fast CID, reduced slow CID
Creatine kinase level	Elevated	Elevated	Elevated
Platelet count	Normal	Normal	Reduced
Bleeding diathesis	–	–	+
(Functional) asplenia	–	–	+
Weakness	+	+	+
Miosis	–	+	+

CID, Ca2+-dependent inactivation.
*Böhm et al. (1).
[†]Shahrizaila et al. (2) and this paper.
[‡]This paper.