Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Picard C, McCarl C-A, Papolos A, et al. *STIM1* mutation associated with a syndrome of immunodeficiency and autoimmunity. N Engl J Med 2009;360:1971-80.

Supplementary Materials

Supplementary Figure Legends

Supplementary Figure 1. Reduced numbers of regulatory T cells in P1. Flow cytometric analysis was performed on permeabilized, age-matched PBMCs from a healthy control (CTRL) and P1 using antibodies to CD3 (APC-conjugated), CD4 (FITC-conjugated) and Foxp3 (PE-conjugated). Percentages of CD4+ Foxp3+ positive cells were calculated after gating on CD3+ T cells (age-appropriate normal range of CD4+ Foxp3+ cells in peripheral blood 2.5% - 6%).

Supplementary Figure 2. P1 is homozygous for two single nucleotide polymorphisms in the 5'UTR of *STIM2*. a, Sequencing of genomic DNA from P1 and P3 revealed two homozygous variations in exon 1 of *STIM2* (C→T and C→G) in P1 at nucleotide positions 391 and 419, respectively of the corresponding mRNA sequence (NM_ 020860). Both nucleotides are located in the 5'UTR of *STIM2* upstream of an unconventional UUG translation start site (nt 478-480)[16]. **b**, Four healthy siblings (V-2, V-3, V-5, V-6) and her parents (IV-1, IV-2) are heterozygous for both variations in the 5'UTR of *STIM2*, whereas P3 (V,7) carries two wild-type alleles like a healthy sister (V-8). **c**, Genomic DNA from 48 individuals of caucasian background unrelated to the kindred were sequenced to assess whether the two variations in the 5'UTR of *STIM2* are common polymorphisms. Fifteen individuals were found to be heterozygous for both variations and 33 individuals had wild-type alleles. None of the controls was homozygous for both variations. Taken together, the two variations are very unlikely to contribute to the disease phenotype and defect in SOCE because (i) both variations occur in the 5'UTR of *STIM2* and not in the coding sequence, (ii) both variations in *STIM2* are common genetic polymorphisms observed in ~ 31% of unrelated controls, (iii) P3 who suffered from the same disease as P1 is wild-type for both variations.

Supplementary Figure 3. STIM1 protein expression is undetectable in P1 fibroblasts.

For Western blot analysis, total cell lysates of fibroblasts from P1, a healthy control (CTRL) and a previously described ORAI1-deficient patient (ORAI1, R91W)[8] were separated by SDS-PAGE, transferred to nitrocellulose membrane and incubated with an affinity purified antibody directed against the C-terminus (CT) of STIM1 (**a**) and a monoclonal antibody directed against the N-terminus (NT, a.a. 25-139) of human STIM1 (**b**). Anti-actin antibody was used to control for protein loading. To control for the specificity of STIM1 detection using anti-STIM1 antibodies, T cells from control mice

(Stim1$^{fl/fl}$) and mice lacking STIM1 expression (Stim1$^{fl/fl}$ CMV-Cre) were included in the experiment[22]. No full-length or truncated STIM1 protein was detectable in fibroblasts from P1 (STIM1-E136X) or in T cells from *Stim1*-deficient mice. Only a weak STIM1 band was detected for the ORAI1-deficient patient (lane 3) because of reduced total protein loaded in lane 3. STIM1 expression was quantified and normalized to Actin expression using ImageJ software (*Right panel* in **b**). Asterisks (*) in **a** denote unspecific bands obtained by incubation with anti-STIM1 antibody.

Supplementary Table 1. Primers used for PCR amplification of genomic DNA and sequencing of human *STIM1* and *STIM2* genes. Reference gene assembly (and gene ID) numbers: STIM1: NT_009237 (6786), STIM2: NT_006316 (57620).

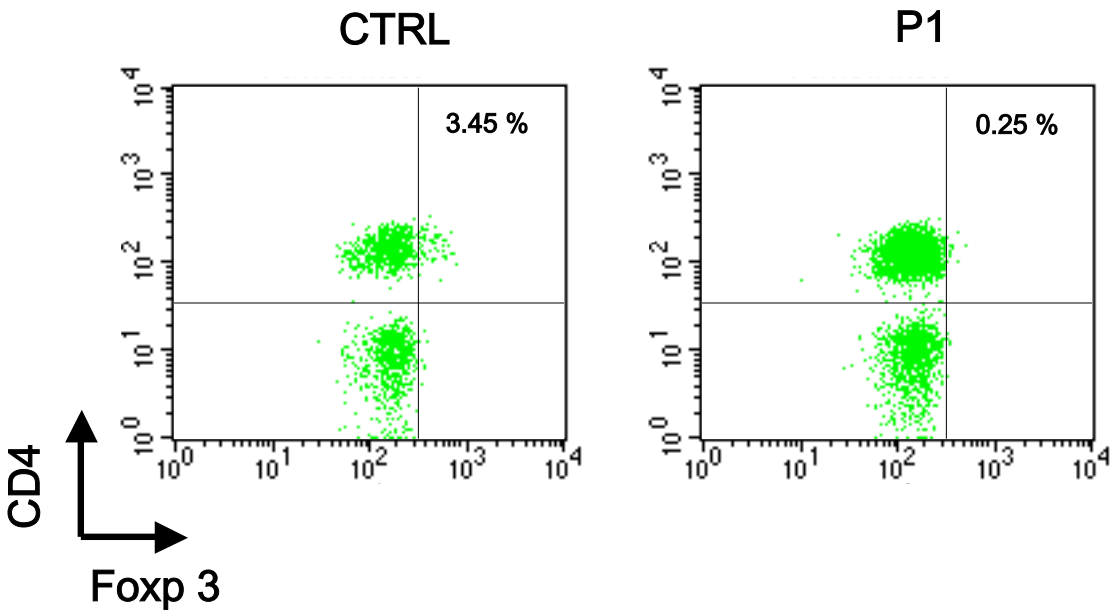
Supplementary Methods

Real-time PCR. Total RNA was isolated from fibroblasts using Trizol reagent (Invitrogen, Carlsbad, CA). cDNA was synthesized from 2-5 mg total RNA using Superscript II cDNA synthesis kit (Invitrogen). Quantitative real-time PCR was performed using an iCycler system (BioRad, Hercules, CA) and SYBR Green dye (Applied Biosystems, Foster City, CA). PCR efficiency was tested by serial dilutions and specificity of amplification was verified by melt curve analysis and agarose gel electrophoresis of PCR products. SYBR green signals were captured at the end of each polymerization step (72 °C). A threshold was set in the linear part of the amplification curve. Threshold cycles (C_T) for genes of interest were normalized to the C_T for GAPDH house keeping gene control obtaining ΔC_T. Expression data were plotted as $0.5^{\Delta Ct} * 10^6$, where $1x10^6$ arbitrary units equal GAPDH cDNA expression levels. The following primers were used for amplification of STIM1, STIM2 and GAPDH (Integrated DNA Technologies): STIM1 Ex9 5' AGAAACACACTCTTTGGCACC, STIM1 Ex10 3' AATGCTGCTGTCACCTCG; STIM2 Ex10 5'AGTCTCCATTCCACCCTATCC, STIM2 Ex10 3' CCAGGAGGTTTAGCCATGG; GAPDH Ex8 5' TCTCCTCTGACTTCAACAGC, GAPDH Ex9 3' CTGTTGCTGTAGCCAAATTCG. Primers were designed to straddle an intervening intronic sequence to prevent amplification of genomic DNA.

Cell extracts and immunoblotting. Immunoblotting was done as described using standard protocols[14]. For immunoblotting, protein was extracted from fibroblasts using a lysis buffer containing 250 mM NaCl, 20 mM TrisHCl (pH 7.5), 10 mM $MgCl_2$, 1% nonidet-P40, 0.5% sodium deoxycholate, 0.1% SDS (all from Sigma) and protease inhibitors PMSF (1 mM), aprotinin (25ng/ml), leupeptin (25 ng/ml, all from Sigma). Proteins were separated by 8-14% SDS-PAGE and electrotransferred onto nitrocellulose membranes. Antibody incubations were performed in 1x TBS (10 mM Tris-HCl pH 8.0, 150 mM NaCl) plus 5% non-fat dry milk, blots were washed with TBS containing 0.05% Tween-20 and bands detected with Western Lightning Chemiluminescence Reagent

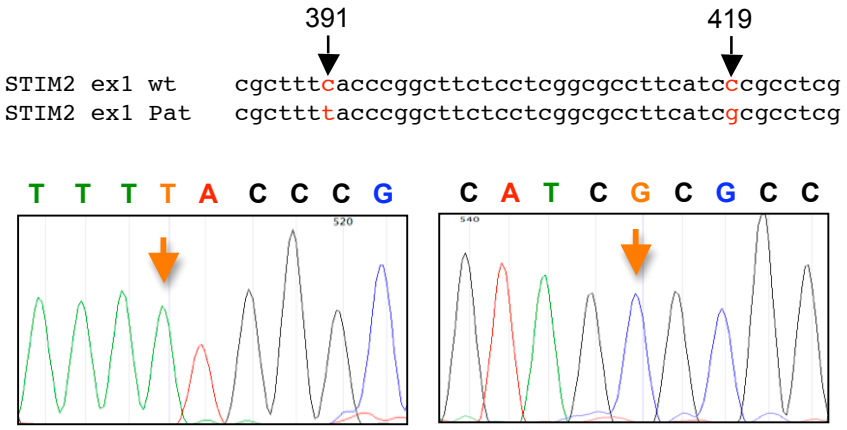
Plus (PE Life Sciences, Boston, MA) and HyBlot CL autoradiography film (Denville Scientific). To assess equal loading, blots were reprobed with anti-actin antibody.

Single-cell calcium imaging. Measurements of intracellular Ca^{2+} concentrations were done as described[8]. Briefly, fibroblasts were grown directly on UV-sterilized coverslips and loaded with 3 μM fura-2/AM. Cells were analyzed by time-lapse videoimaging on a IX81 epifluorescence microscope (Olympus) using Slidebook imaging software v4.2 (Olympus). The standard extracellular Ringer's solution contained (in mM): 155 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose, and 5 Na-Hepes (pH 7.4). Ca^{2+}-free Ringer's was prepared by substituting 2 mM $MgCl_2$ for $CaCl_2$. Cells were stimulated with thapsigargin (1 μM, EMD Biosciences, San Diego, CA).

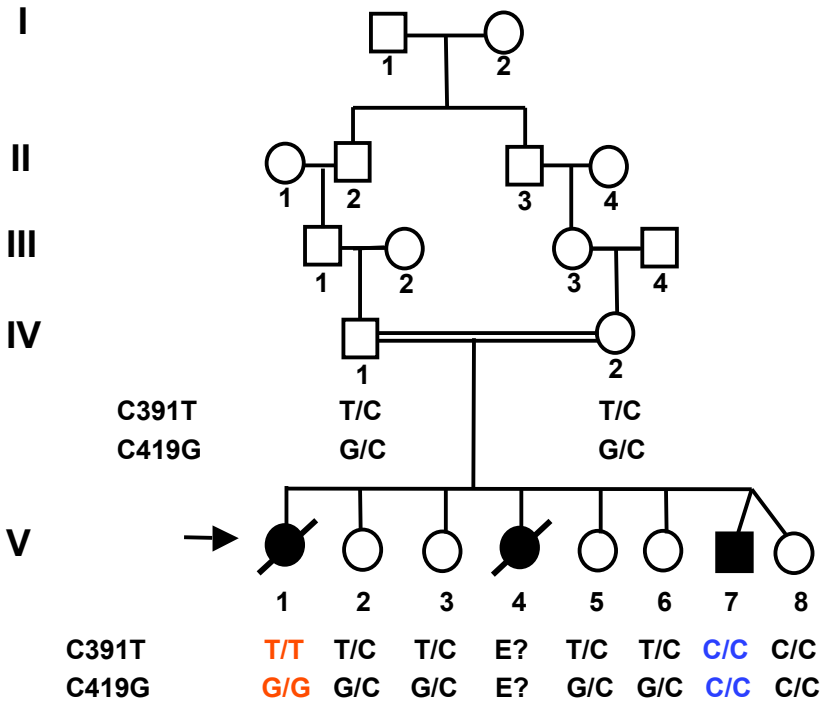


Supplementary Figure 1

a



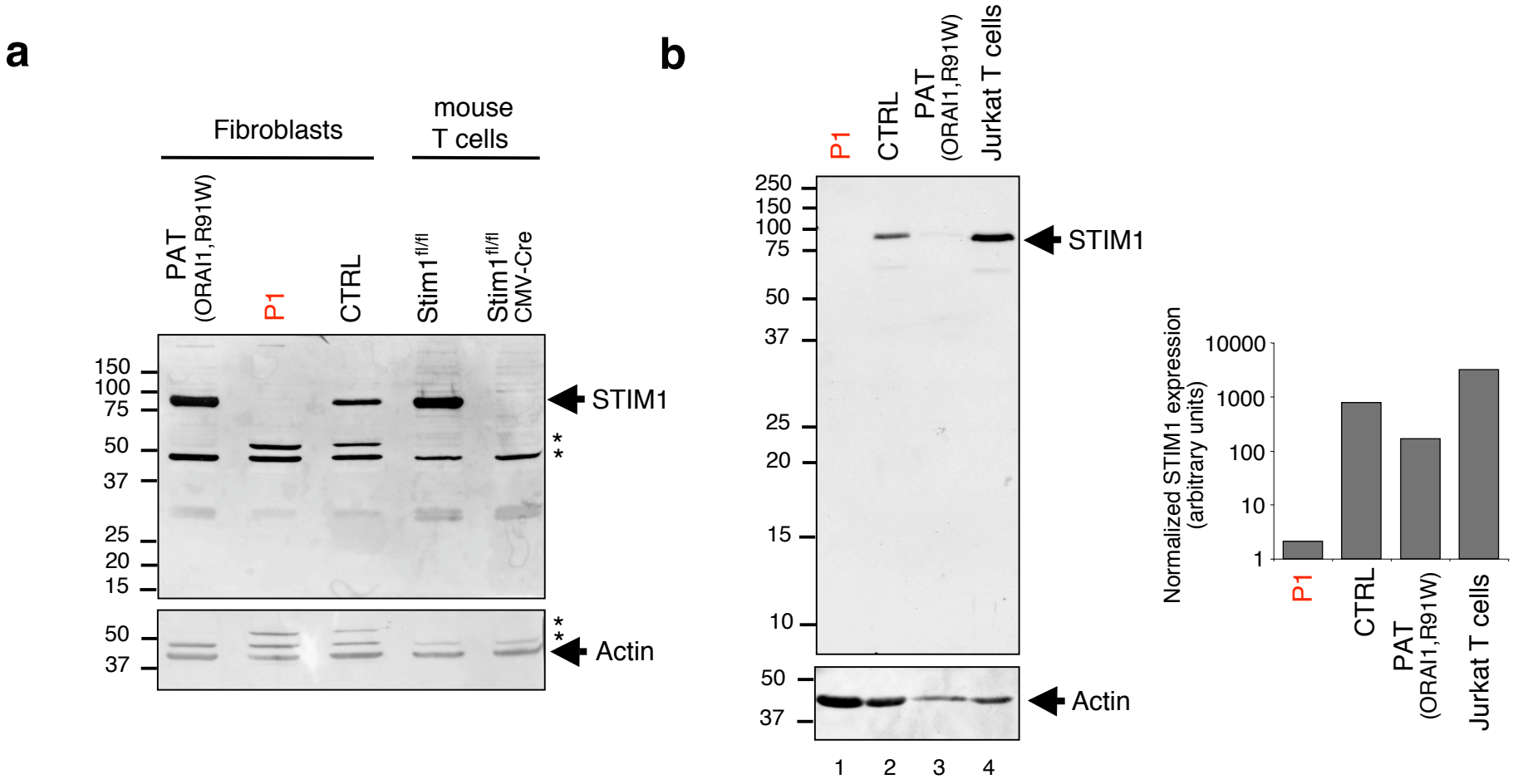
b



c

	C/C	C/T	T/T	n
C391	33	15	0	48
	C/C	**C/G**	**G/G**	**n**
C419	33	15	0	48

Supplementary Figure 2



Supplementary Figure 3

	5’ Primer sequence		3’ Primer sequence	
STIM1				
Exon 1	PCR+SEQ	AAGCTGGGACTTGATCCTTTG	PCR+SEQ	CATGTACAAACCTAGATTTCAACTTGC
Exon 2	PCR+SEQ	ATGCTAGAAGCTAAGGATGCTG	PCR+SEQ	GAAGAGGCTGTCTAAGTAGC
Exon 3	PCR+SEQ	GAATGTGTTATGGCTAGCTAGAG	PCR+SEQ	ATGTTCTTCTAAGGCCAAGTTGC
Exon 4	PCR+SEQ	ATGTGGTAAATATTAAGGTCAGCATGAC	PCR+SEQ	TTAACTGGCCAGAGCAATCTG
Exon 5	PCR+SEQ	CAATCACCAAGAGCTAGAAGTG	PCR+SEQ	TGGAAATAGTATAGGTGCTATCTTGC
Exon 6	PCR+SEQ	TCTGTTATGGAAGGCTTCATAGAG	PCR+SEQ	AGTTTTGGAAGGATGATGCAGC
Exon 7	PCR+SEQ	AGCTGTCATTTTCCTCTTTGATGC	PCR+SEQ	TGACTCTAGAACATAGTCTTTGGATC
Exon 8	PCR+SEQ	AAAGCAGATAAGAAGTCTGAGTTCTG	PCR+SEQ	ACCACCAGGATATCTCTTCAC
Exon 9/10	PCR+SEQ	GCCTTTCTCATTTATTCCATTCTCG	PCR+SEQ	CATCTGCTGTTTAAGCACAACAG
Exon 11	PCR+SEQ	ATTCTCCAGATTGGCATTAGAGG	PCR+SEQ	CTTCAGAACTGAAAGACTGTCC
Exon 12	PCR+SEQ	TCCTTGTCTTCTCGTGTTGTC	PCR+SEQ	AACAGCAACTAAGACATGCACTG
STIM2				
Exon 1	PCR	F2: AAGACGCCGTACCTTTCTACC	PCR	R1: TGAAAAGGAAAGACGTCCGG
	SEQ	F4: TAACCGGAACCAATGAACGC	SEQ	R4: AGATGCTGACCTCTGCACG
Exon 2	PCR+SEQ	ACATAACTCCTGGTTGTAGTTGC	PCR+SEQ	GTTGAAGATGAAGGCAATGAGC
Exon 3	PCR+SEQ	GGTGGCGTTACGATTAGTAGC	PCR+SEQ	AACTTTAGGCTCTCAGACATGC
Exon 4	PCR+SEQ	GGCTTTCTTCTTCTCATAGAGTGC	PCR+SEQ	GATTGGCCAAAAGTTGACCC
Exon 5	PCR+SEQ	CTAAGTTTGCACTGAAGAGG	PCR+SEQ	CTGTTAGGCTCTATTGCTTCATGC
Exon 6	PCR+SEQ	GAATTTTAAAAGGCTAGAGCTTGTGC	PCR+SEQ	GGTTCAAATGACAGGGAAAGC
Exon 7	PCR+SEQ	GCTTTCCCTGTCATTTGAACC	PCR+SEQ	AAGACAGTGAAGATGGCAAGG
Exon 8	PCR+SEQ	AAGATGCACTTGAAGCTCAGC	PCR+SEQ	ACCTGAATCAGATATGAAGCAGC
Exon 9	PCR+SEQ	TTGGAATGCAGGGATATCTTGG	PCR+SEQ	CAATACATGAACAGACACTGGC
Exon 10	PCR+SEQ	CATGTATTGCCTTTTTTCAGTGCC	PCR+SEQ	AACCCAATTTTTTCTCACAGATTTCG
Exon 11	PCR+SEQ	TCAGTAAAGGGAGATGAAACAGTG	PCR+SEQ	GTCTGTGGTACCTTGATATGTAGC
Exon 12	PCR+ SEQ	F1: CAGCATTGAGTTTTGAGAAGCC	PCR+ SEQ	R1: GCCCCAATGGAGTTACATTCC
	PCR+ SEQ	F2: GTGAACTGGCTGACTTGATGG	PCR+ SEQ	R2: ATGCAGTTCAAGAAGCTTCCC
	PCR+ SEQ	F3: GAAAACCCGCGCTTTTATTATGG	PCR+ SEQ	R3: TGTTCATCCAAACATCCATCTGC
	PCR+ SEQ	F4: CATAAGTGATTTGGTTACTGCAATGC	PCR+ SEQ	R4: GGATAGTAGTATTTGACCTGCTTGC

Supplementary Table 1