Supporting Information

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SI Methods

Case Reports. The ORAI1-deficient patient was clinically asymptomatic until 16 wk of age, when he received a transplant because of a history of SCID in an older brother who had a similar immunological phenotype (1–3). Both brothers carried a homozygous missense mutation in *ORAI1* (c.271C > T, p.R91W) associated with a lack of CRAC channel function, SOCE, and severely impaired T-cell activation (2, 4). The patient received a bone marrow transplant without conditioning from an aunt with identical HLA. Mixed T-cell chimerism was documented 6 mo later. At the time of analysis, the patient was 14 y of age, with moderate muscular weakness and mild bronchiectasis presumably favored by an impaired capacity to cough. He had normal Ig levels, specific antibody responses, normal lymphocyte subsets (including naive T cells), and normal proliferative responses to mitogens. In the course of this study, we also identified a 5-y-old girl with combined immunodeficiency, mild myopathy, and abnormalities of tooth enamel. The patient carried a homozygous missense mutation in *STIM1* (c.1285C > T, p.R429C) resulting in absent Ca^{2+} influx in T cells following thapsigargin treatment. The severe clinical course of this patient precluded a more extensive analysis at this time point. A detailed description of the molecular, clinical, and immunological consequences of this *STIM1* mutation will be given in a future report.

Cells. *Drosophila* Schneider 2 (S2) cells were maintained in Schneider's medium supplemented with 10% (vol/vol) FCS (Invitrogen). S2-cell transfectants expressing human intercellular adhesion molecule-1, CD48, and ULBP1, or combinations thereof have been described (5, 6).

Antibodies. The following antibodies were used for flow functional analysis of NK cells and T cells: anti-CD3 (clone SK7), anti-CD8 (clone SK1), anti-CD16 (clone 3G8), anti-CD56 (clone NCAM 16.2), anti-CD62L (clone Dreg56), anti-CD107a (clone H4A3), anti–MIP-1β (clone D21-1351), anti–IFN-γ (clone 25723.11; all from BD Bioscience), and anti–TNF-α (clone MP6-XT22; eBioscience). Conformational specific and activating anti-CD18 (clones 327C and 240Q, respectively) were provided by D. Staunton (ICOS Corporation, Seattle, WA) (7). For cell stimulation, purified anti-CD16 (clone 3G8) and anti-CD3 (clone UCHT1; both from BD Bioscience) were used. For cross-linking, secondary goat $F(ab')_2$ anti-mouse IgG (Jackson ImmunoResearch) was used.

The following fluorochrome-conjugated antibodies were used for phenotypical analysis of NK cells: anti-CD3 (clone SK7), anti-CD2 (clone RPA-2.10), anti-CD7 (clone M-T701), anti-CD8 (clone SK1), anti-CD16 (clone 3G8), anti-CD56 (clone NCAM 16.2), anti-CD57 (clone NK-1), anti-CD62L (clone Dreg56), anti-CD226 (DNAM-1; clone DX11), anti-CD314 (NKG2D; clone 1D11), antiperforin (clone δG9; all from BD Bioscience), anti-CD11a (clone 25.3), anti-CD158a (KIR2DL1; clone EB6), anti-CD85j (LIR-1; clone HP-F1), anti-CD158b (KIR2DL2, KIR2DL3; clone GL183), anti-CD159a (NKG2A; clone Z199), anti-CD244 (2B4; clone C1.7), anti-CD335 (NKp46; clone BAB281), anti-CD337 (NKp30; clone Z25; all from Beckman Coulter), anti-CD27 (clone M-T271; DAKO Cytomation), and anti-KLRG1 [clone 13F12F2 (8)]. A rabbit serum against S2 cells was raised by immunizations with S2-cell membranes (9).

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2. Feske S, et al. (1996) Severe combined immunodeficiency due to defective binding of the nuclear factor of activated T cells in T lymphocytes of two male siblings. *Eur J Immunol* 26:2119–2126.
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5. Barber DF, Long EO (2003) Coexpression of CD58 or CD48 with intercellular adhesion molecule 1 on target cells enhances adhesion of resting NK cells. *J Immunol* 170: 294–299.
6. Bryceson YT, Ljunggren HG, Long EO (2009) Minimal requirement for induction of natural cytotoxicity and intersection of activation signals by inhibitory receptors. *Blood* 114:2657–2666.
7. Beals CR, Edwards AC, Gottschalk RJ, Kuijpers TW, Staunton DE (2001) CD18 activation epitopes induced by leukocyte activation. *J Immunol* 167:6113–6122.
8. Marcolino I, et al. (2004) Frequent expression of the natural killer cell receptor KLRG1 in human cord blood T cells: Correlation with replicative history. *Eur J Immunol* 34: 2672–2680.
9. Bryceson YT, March ME, Barber DF, Ljunggren HG, Long EO (2005) Cytolytic granule polarization and degranulation controlled by different receptors in resting NK cells. *J Exp Med* 202:1001–1012.

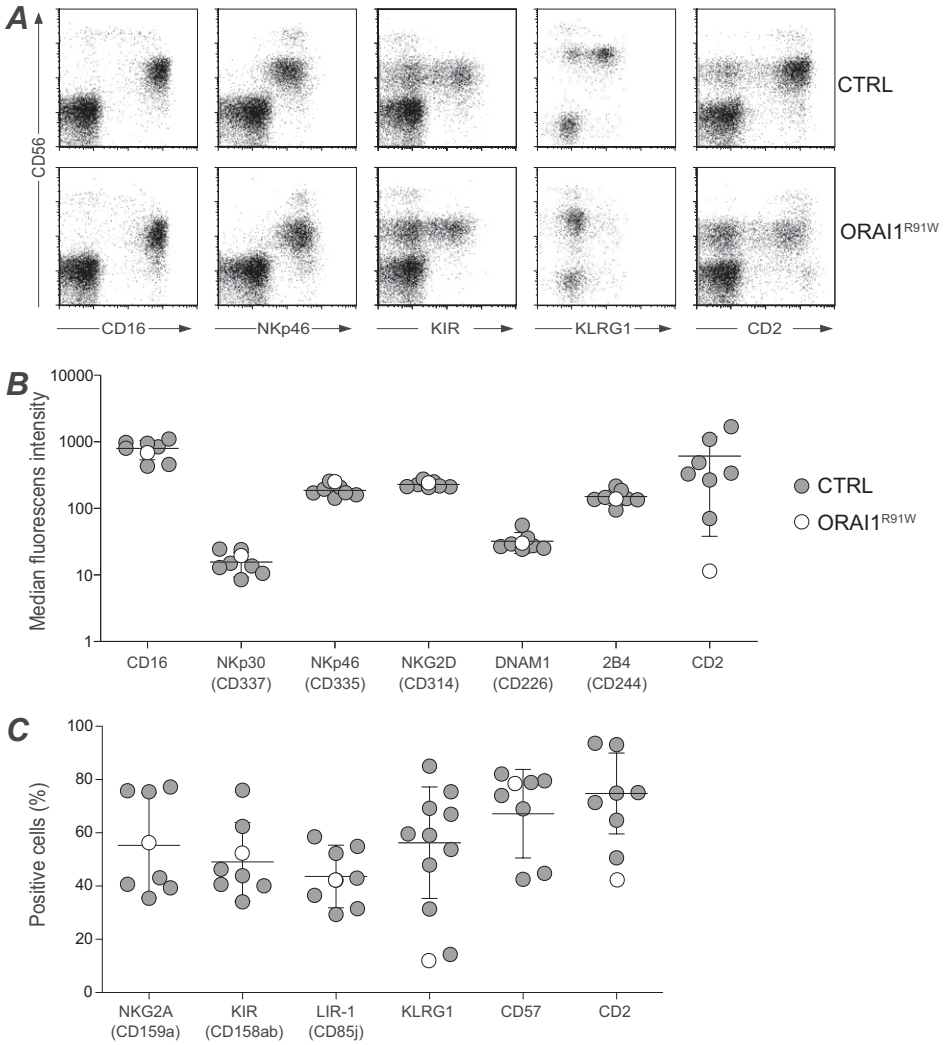

Fig. S1. Phenotype of ORAI1-deficient NK cells. PBMCs were stained with fluorochrome-conjugated anti-CD3, anti-CD56, and antibodies to different NK cell receptors. Lymphocytes were gated on forward scatter/side scatter characteristics. (*A*) Plots show expression of CD56 and CD16, NKp46, KIR, KLRG1, or CD2 on $CD3^-$ lymphocytes from a healthy donor (CTRL) and ORAI1-deficient patient ($ORAI1^{R91W}$) as indicated. (*B*) Expression levels of NK cell-activating receptors on $CD3^-CD56^{dim}$ NK cells from CTRLs (n = 7) and the ORAI1-deficient patient. Bars indicate mean ± SD. (*C*) Frequency of $CD3^-CD56^{dim}$ NK cells from CTRL and ORAI1-deficient patient expressing NK cell inhibitory receptors and maturation markers as indicated. Bars indicate mean ± SD. Healthy CTRLs were age-matched (10–26 y). One representative staining is shown of two experiments performed 6 mo apart. CTRL, control.

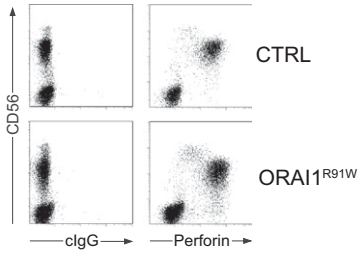

Fig. S2. Intracellular perforin expression in ORAI1-deficient ($ORAI1^{R91W}$) NK cells. PBMCs were surface-stained with fluorochrome-conjugated anti-CD3 and anti-CD16 mAbs, fixed, permeabilized, and stained intracellularly with fluorochrome-conjugated antiperforin mAb. Lymphocytes were gated on forward scatter/side scatter characteristics. Perforin vs. CD56 expression is plotted on $CD3^-$ lymphocytes. CTRL, control.

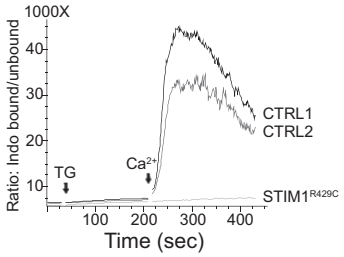

Fig. S3. STIM1-deficient T cells have impaired SOCE influx. Negatively isolated $CD3^+$ T cells from the STIM1-deficient patient ($STIM1^{R429C}$) and two healthy donors (CTRL) were incubated with TG, followed by the addition of 2 mM $CaCl_2$. The graph shows the ratio of unbound to bound indo-1-AM as a measure of Ca^{2+} influx during the course of the experiment. The assay was performed twice with similar results. CTRL, control; TG, thapsigargin.

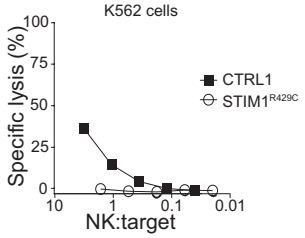

Fig. S4. STIM1-deficient NK cells show impaired natural cytotoxicity. Cytotoxicity was assessed in a standard ^{51}Cr release assay using PBMCs from the STIM1-deficient patient ($STIM1^{R429C}$) and a healthy donor (CTRL1) as effector cells on K562 cell targets. Effector/target (NK:target) ratios were calculated based on the frequency of NK cells among PBMCs as determined by flow cytometry. Results are representative of two independent experiments. CTRL, control.

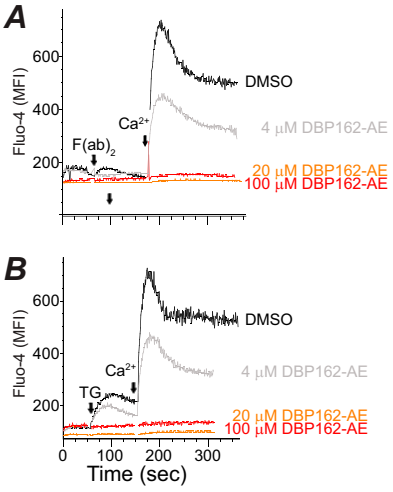

Fig. S5. Effect of ORAI1 pharmacological inhibitors on SOCE in NK cells. (*A* and *B*) NK cells were purified from the peripheral blood of healthy donors by negative selection. (*A*) NK cells were preincubated with anti-CD16 mAb in Ca^{2+}-free PBS, followed by cross-linking and the addition of 2 mM $CaCl_2$ as indicated. (*B*) TG was added to NK cells, followed by the addition of 2 mM $CaCl_2$. The graphs show the Fluo-4 mean fluorescence intensity as a measure of Ca^{2+} influx during the course of the experiment. One representative donor of two is shown. The assay was performed twice with similar results. TG, thapsigargin.

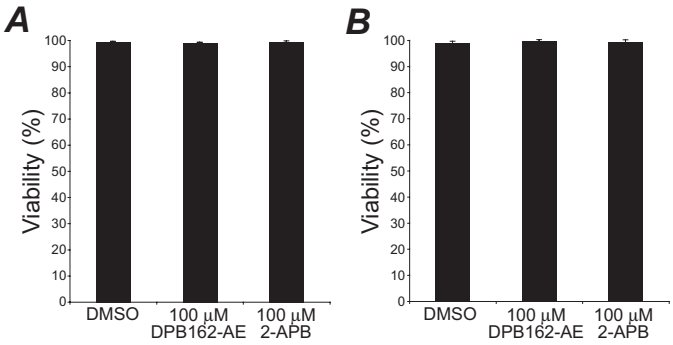

Fig. S6. Viability of NK cells or PBMCs following treatment with pharmacological inhibitors of SOCE. PBMCs (*A*) or NK cells (*B*) purified from the peripheral blood of healthy donors by negative selection were treated with vehicle only (DMSO) or with the indicated concentrations of pharmacological inhibitors of SOCE for 4 h at 37 °C. Viability was determined by trypan blue exclusion. Values with error bars represent mean ± SD of three donors.

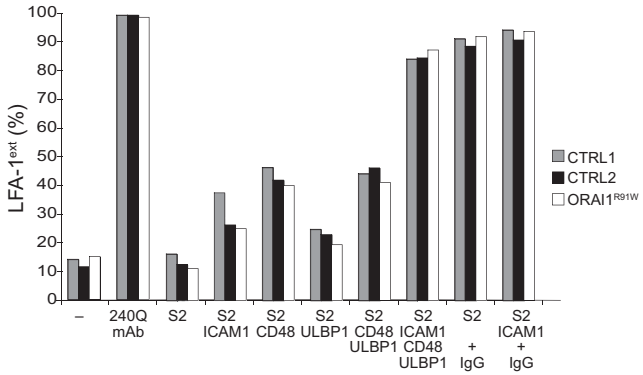

Fig. S7. Signals for LFA-1 activation are ORAI1-independent. PBMCs from an ORAI1-deficient patient (ORAI1^{R91W}) or healthy donors (CTRL) were mixed with target cells as indicated. Where indicated, S2 cells were preincubated with a rabbit anti-S2 serum (+ IgG). Cells were incubated for 5 min at 37 °C; stained with lineage marker and conformation-specific, biotinylated, anti–LFA-1 mAbs; washed; and stained with fluorochrome-conjugated streptavidin. Lymphocytes were gated on forward scatter/side scatter characteristics. The percentage of CD3$^-$CD56^{dim} NK cells with 327C^{high} expression (LFA-1^{ext}), indicating LFA-1 in the extended ligand-binding conformation, is presented. One representative experiment of two is shown. CTRL, control.

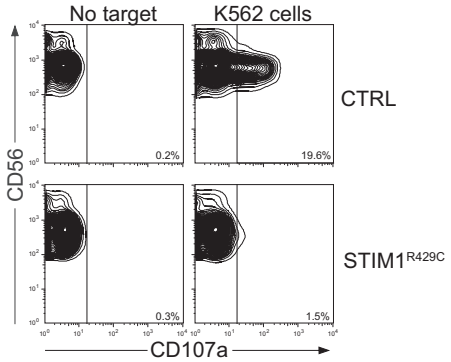

Fig. S8. Target cell-induced NK cell degranulation requires STIM1. (*A* and *B*) PBMCs from the STIM1-deficient patient (STIM1^{R429C}) and healthy donors (CTRL) were stimulated with K562 cells for 2 h at 37 °C and thereafter stained with fluorochrome-conjugated lineage markers and anti-CD107 anti-mAbs. The plots show CD56 vs. CD107a expression of CD3$^-$CD56$^+$ lymphocytes. One representative experiment of three is shown. CTRL, control.

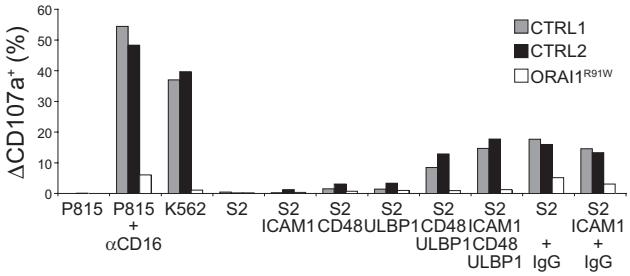

Fig. S9. Cytotoxic lymphocyte degranulation requires ORAI1. PBMCs from an ORAI1-deficient patient (ORAI1^{R91W}) or healthy donors (CTRL) were stimulated with target cells as indicated. Where indicated, S2 cells were preincubated with a rabbit anti-S2 serum (+ IgG). After 2 h of incubation at 37 °C, the cells were stained with fluorochrome-conjugated lineage markers and anti-CD107 anti-mAbs. Lymphocytes were gated on forward scatter/side scatter characteristics. The percent increase of CD3^{-}CD56^{dim} NK cells expressing surface CD107a after incubation with target cells relative to CD3^{-}CD56^{dim} NK cells expressing surface CD107a without target cells (ΔCD107a^{+}) is presented. One representative experiment of four is shown. CTRL, control.