Supplemental Materials

Molecular Biology of the Cell

Giurisato et al.

Supplementary Figure 1. Purified T and B cells isolated from *wt, ksr1*$^{-/-}$and *ksr2* $^{-/-}$ mice were stained for the surface markers CD3ε (T cells) and B220 (B cells). The percentage of T and B cells present in each purification, analyzed by FACS, is reported for each condition tested.

Supplementary Figure 2. Purified T and B lymphocytes, loaded with Fura-2, were resuspended, in a spectrofluorimeter cuvette, in a nominally Ca^{2+} free medium containing 0.2 mM EGTA. A magnification of the Tg and agonist-induced Ca^{2+} elevation is shown for T and B lymphocytes. (A**,** C) Intracellular Ca^{2+} stores were depleted with 0.2 μM thapsigargin (Tg); (B, D) elevation of $[Ca^{2+}]_i$ was induced by anti-CD3 (2C11) followed by anti-IgG antibody (α-hamster) or by anti-IgM stimulation, for T and B lymphocytes, respectively. The area under the curve (AUC) of the average trace obtained after stimulation with specific agonist, from three independent experiments, was measured.

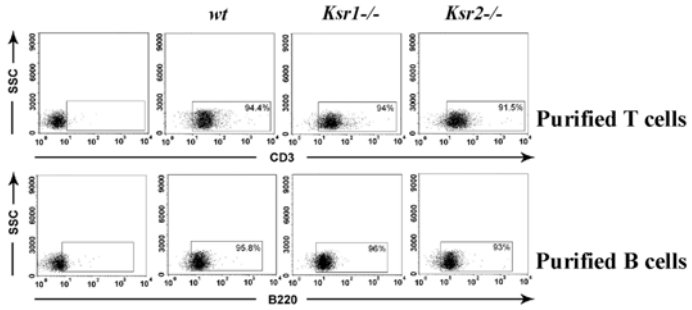
Supplementary Figure 3. A magnification of the TG-induced Ca^{2+}-release, in the absence of external calcium, and the corresponding AUC values, in shRNA control (scr) and shRNA-KSR2 expressing HeLa cells is shown. The traces represent the average from at least three independent experiments: the corresponding AUC values are reported.

Supplementary Figure 4. (A) Immunoblot illustrating coimmunoprecipitation of STIM1 with ORAI1 puncta. ShRNA control and shRNA KSR2 Hela cells overexpressing YFP-STIM1 and ORAI1-RFP fusion proteins were incubated without or with 1 μM Tg for 5 minutes. ORAI1 immunoprecipitates (IP) were prepared and analyzed by immunoblotting using anti-STIM1. The membrane was then stripped and reprobed with anti-ORAI1. One representative experiment out of two is shown. (B) KSR2 protein expression in HeLa and COS-7 cells, ($1x10^6$ cells/lysate). α-Tubulin (α-tub) was used as loading control. Relative molecular mass (kDa) is reported on the left. (C) Intracellular Ca^{2+} concentration was monitored, by video imaging, in COS-7 cells expressing low GFP-KSR2 (0.2 μg, green line), high GFP-KSR2 (0.8 μg, red line) or GFP alone (0.2 μg, black line). Ca^{2+} stores were depleted with 0.5 μM Tg in the presence of 1.5 mM extracellular Ca^{2+}, as evidenced by the peak in Ca^{2+} concentration that remained elevated above baseline. Each trace represents the average response of all cells positive for GFP or GFP-KSR2 fluorescence on a single coverslip. The mean ± SEM of Ca^{2+} influx value, calculated in >10 transfected COS-7 cells from three independent experiments, is reported. Tg induced intracellular Ca^{2+} elevation $[Ca^{2+}]_i$ (nM ± SEM) was monitored in COS-7 cells expressing low GFP-KSR2 (0.2 μg, green line), high GFP-KSR2 (0.8 μg, red line) or GFP (0.2 μg, black line) in the presence of 1.2 mM extracellular Ca^{2+}. Δ $[Ca^{2+}]_i$ =($[Ca^{2+}]_i$ peak after Tg – basal $[Ca^{2+}]_i$). *p = 0.044. **p = 0.0128.

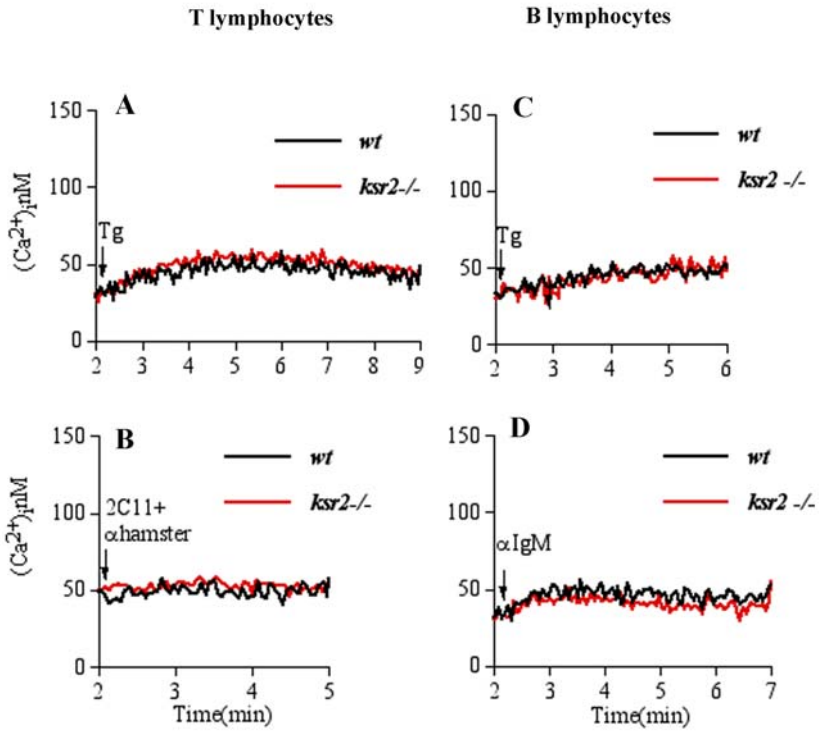
Supplementary Figure 5. SOCE is reduced by cyclosporin A (CsA) pre-treatment only in KSR2 expressing cells. (Left) (A) Jurkat cells expressing KSR2 protein (as shown in the insert), loaded with Fura-2 were analyzed by spectrofluorimeter. Ca^{2+} stores were depleted with 0.2 μM Tg and subsequently, 1.2 mM Ca^{2+} was added to the external medium to reveal Ca^{2+} influx. Black and red traces represent, respectively, cells treated either without or with 5 μM CsA for 30 min at 37°C. (Right) Cells were pre-treated for 30 min at 37°C with the indicated CsA concentration and peak values of Tg dependent Ca^{2+} entry after Ca^{2+} readmission were calculated. Panels show percentage of residual Ca^{2+} influx after CsA treatment, compared to maximal Ca^{2+} peak of their respective vehicle pretreated controls. Values are shown as mean ± SEM of at least three independent experiments.

Supplementary Figure 6. KSR2 deficiency and CN inhibition affects cytoskeleton organization. Fibroblasts isolated from *wt* and *ksr2*$^{-/-}$ mice were grown on gelatin-covered coverslips. After starvation, cells were either stimulated without (basal) or with 1 μM Tg (+Tg). Where indicated, *wt* cells were pretreated with 10 μM CsA for 30 min and stimulated with 1 μM Tg (CsA+Tg). Cells

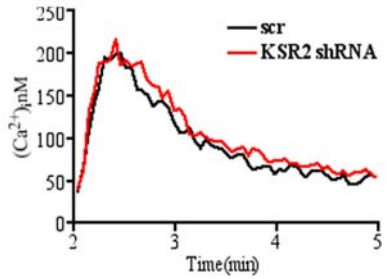
were fixed, permeabilized, and incubated with anti-α-tubulin antibody followed by Cy™-2-labeled secondary antibody (green) and Rhodamine-phalloidin (red). One representative cell out of > 25 for each condition in three independent experiments is shown. Bar, 5 μm.



Suppl. figure 1



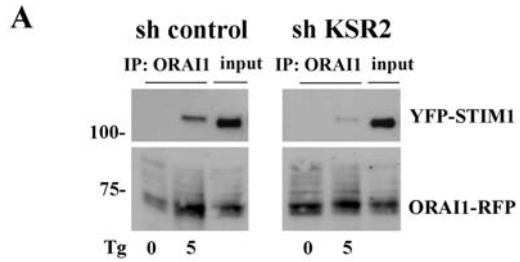
Suppl. figure 2

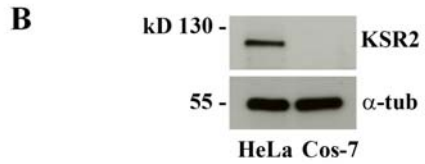


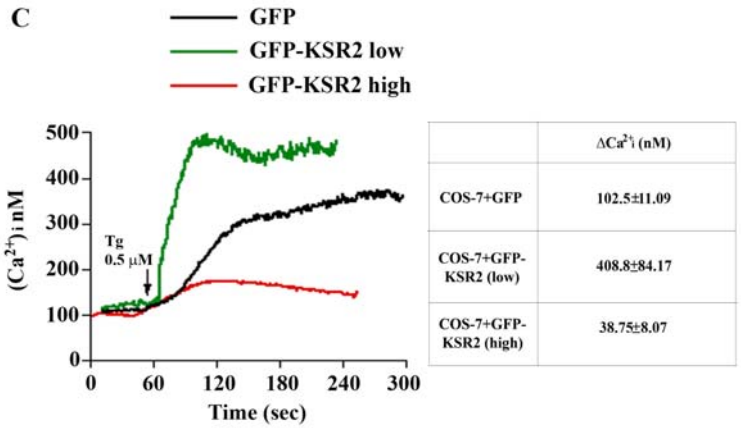
scr: **AUC= 284.7**

KSR2 shRNA: AUC=314.9

Suppl. figure 3

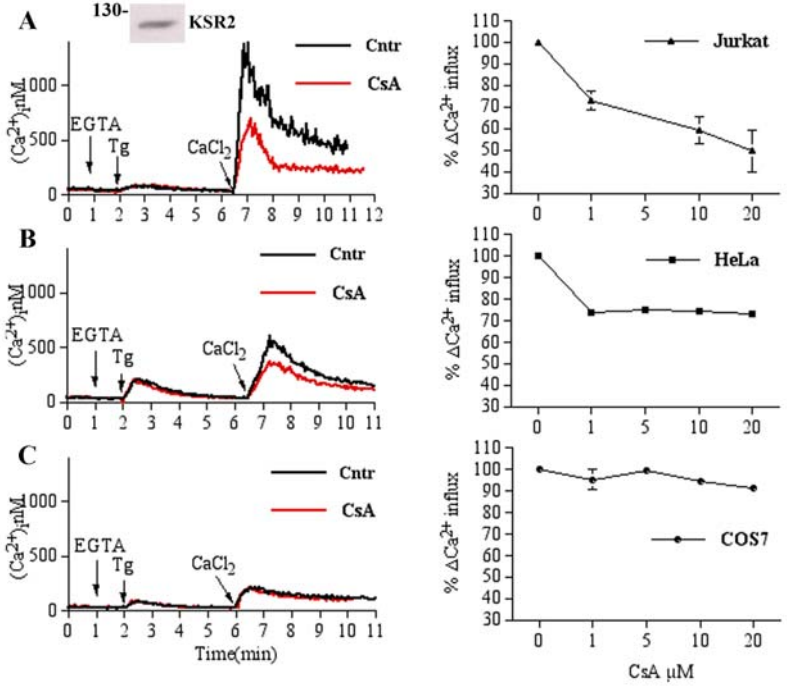


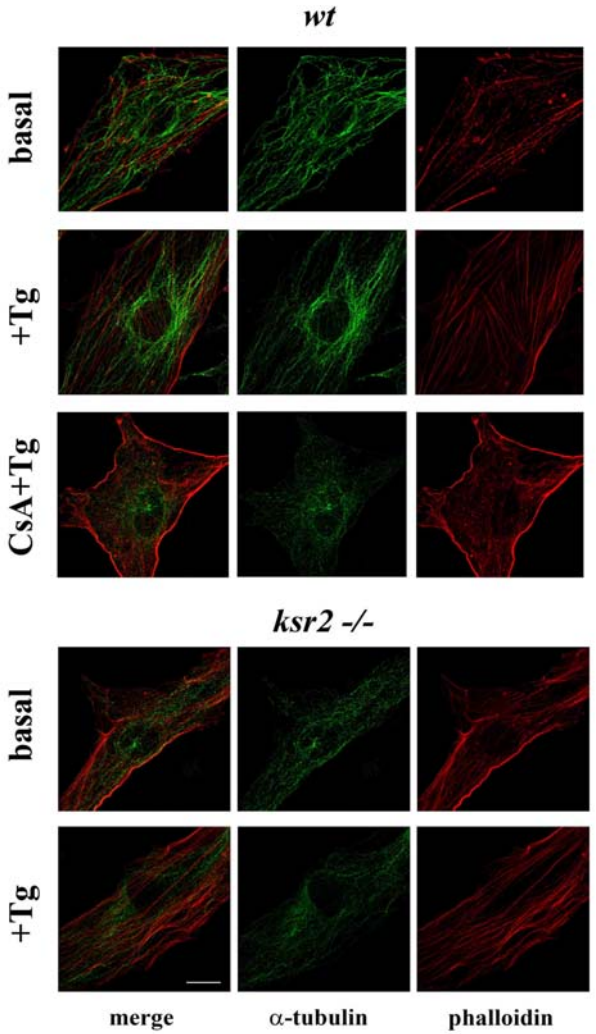




	$\Delta Ca^{2+}i$ (nM)
COS-7+GFP	102.5±11.09
COS-7+GFP-KSR2 (low)	408.8±84.17
COS-7+GFP-KSR2 (high)	38.75±8.07

Suppl. figure 4


A
130-
KSR2
Cntr
CsA
EGTA
Tg
CaCl2
(Ca2+)i nM
B
C
Time(min)
% ΔCa2+ influx
Jurkat
HeLa
COS7
CsA μM



Suppl. figure 6