**Supplementary Figures**

## $Ca^{2+}$ signaling but not store-operated $Ca^{2+}$ entry (SOCE) is required for the function of macrophages and dendritic cells

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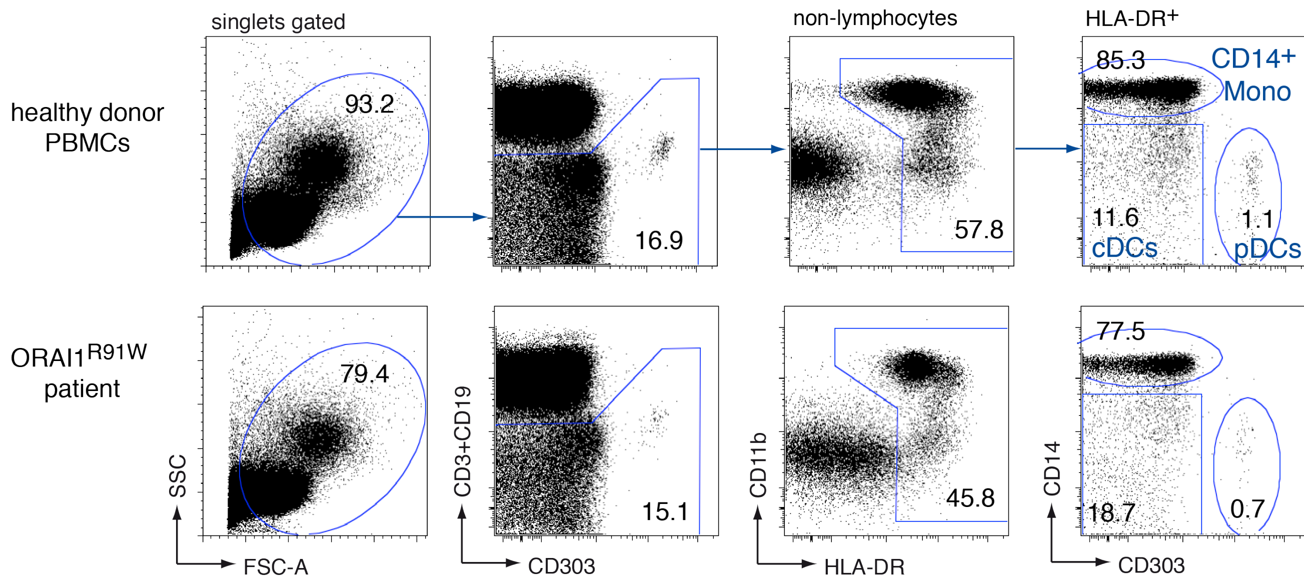


**Figure S1. The loss-of-function ORAI1$^{R91W}$ mutation does not affect the frequency of peripheral blood monocytes and DCs.** Analysis of peripheral $CD14^+$ monocytes, conventional (cDCs), and plasmacytoid dendritic cells (pDCs) of a healthy donor and a patient homozygous for a loss-of-function ORAI$^{R91W}$ mutation (2) by flow cytometry. Singlet PBMC were gated on $CD3^-$ $CD19^-$ non-T and non-B cells that are HLA-$DR^+$. The frequencies and populations of $CD14^+$ $CD303^-$ monocytes, $CD14^-$ $CD303^-$ cDCs, and $CD14^-$ $CD303^+$ pDCs within HD and patient PBMC are indicated.

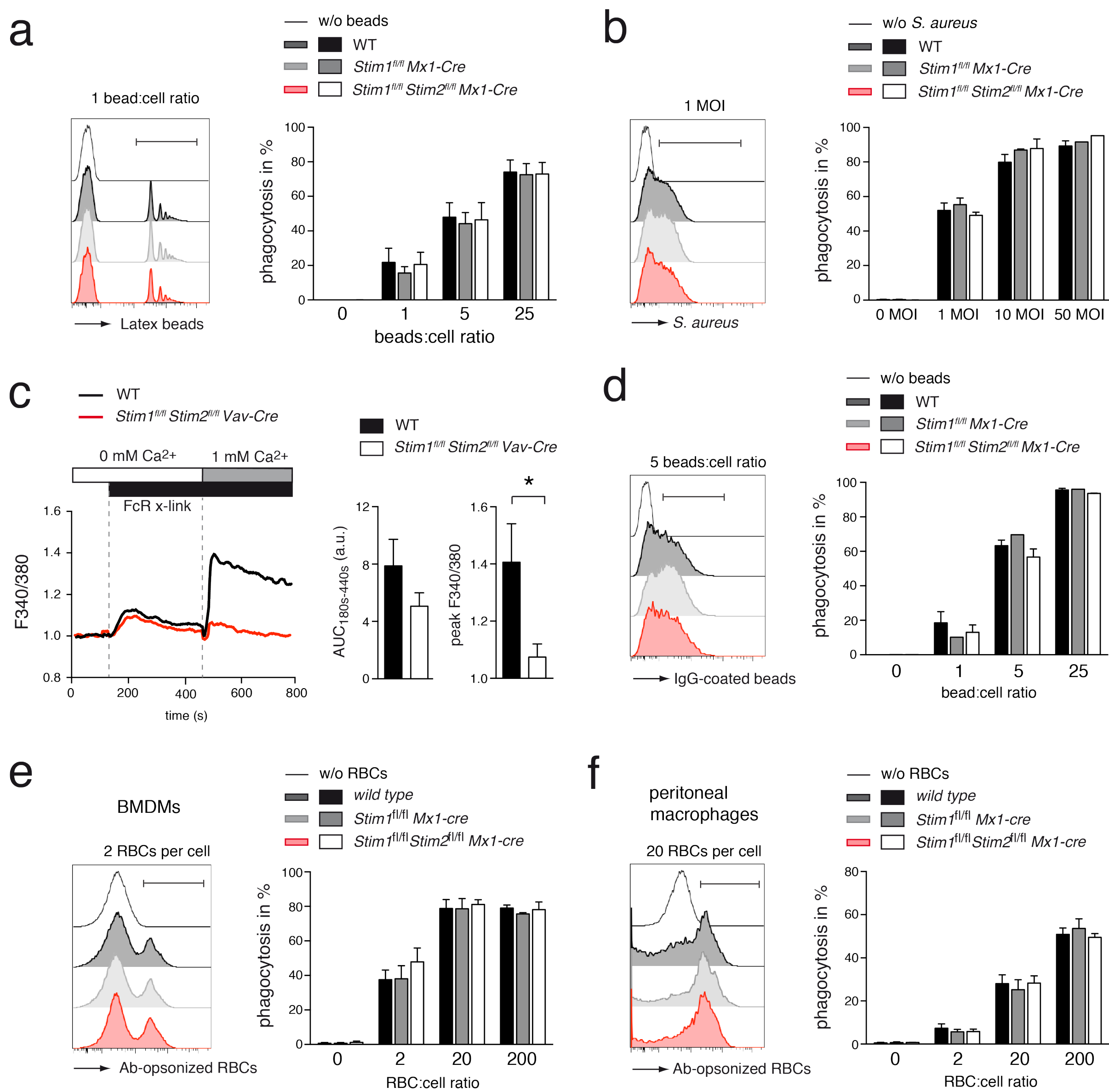


**Figure S2. Intracellular $Ca^{2+}$ signaling but not SOCE is required for FcR-dependent and FcR independent phagocytosis in BMDMs and peritoneal macrophages. (a,b)** Phagocytosis of latex beads and *S. aureus*. BMDMs generated from the BM of poly I:C-treated WT, *Stim1$^{fl/fl}$ Mx1-Cre* and *Stim1$^{fl/fl}$Stim2$^{fl/fl}$ Mx1-Cre* mice were incubated at 1, 5, and 25 bead-to-cell ratios with carboxylate-modified yellow-green microspheres for 2 h **(a)** or 1, 10, and 50 MOI of GFP-expressing *S. aureus* for 30 min **(b)**. Phagocytosis was quantified by flow cytometry. Representative histogram plots (left panels) and averaged data from 3 mice per group (right panels). **(c)** FcR crosslinking induces STIM1/STIM2-dependent SOCE. WT and *Stim1$^{fl/fl}$Stim2$^{fl/fl}$ Vav-Cre* BMDMs were loaded with Fura-2 for 30 min, incubated with 10 µg/ml rat-anti-mouse FcγIIR/FcγIIIR (CD16/CD32) antibody and analyzed for $[Ca^{2+}]_i$ by FlexStation3. Store depletion was induced by FcR crosslinking with 20 µg/ml rabbit-anti-rat antibody in $Ca^{2+}$-free Ringer solution; SOCE was induced by raising the extracellular $[Ca^{2+}]$ to 1 mM. Shown is one representative experiment (left panel); store depletion ($AUC_{180s\text{-}440s}$) and peak $Ca^{2+}$ influx (peak F340/380) were quantified from 5 independent experiments (right panel). **(d)** Phagocytosis of IgG-coated beads. WT, *Stim1$^{fl/fl}$ Mx1-Cre* and *Stim1$^{fl/fl}$Stim2$^{fl/fl}$ Mx1-Cre* BMDMs were incubated at 1, 5, and 25 bead-to-cell ratios with FITC-labeled IgG-coated fluorescent beads and FcR dependent phagocytosis was measured by flow cytometry (left panel); bar graphs show data from 2 individual experiments (right panel). **(e,f)** Phagocytosis of opsonized red blood cells (RBCs). BMDMs **(e)** or primary peritoneal macrophages **(f)** from poly I:C-treated WT, *Stim1$^{fl/fl}$ Mx1-Cre* and *Stim1$^{fl/fl}$Stim2$^{fl/fl}$ Mx1-Cre* mice were incubated for 3 h with mouse RBCs opsonized with an anti-mouse RBC antibody and labeled with anti-Ter119-PE. FcR-dependent phagocytosis was measured using 2, 20, and 200 RBC-to-cell ratios. Representative flow cytometry histogram plots (left panels) and summary of results from 3 mice per group (right panels).

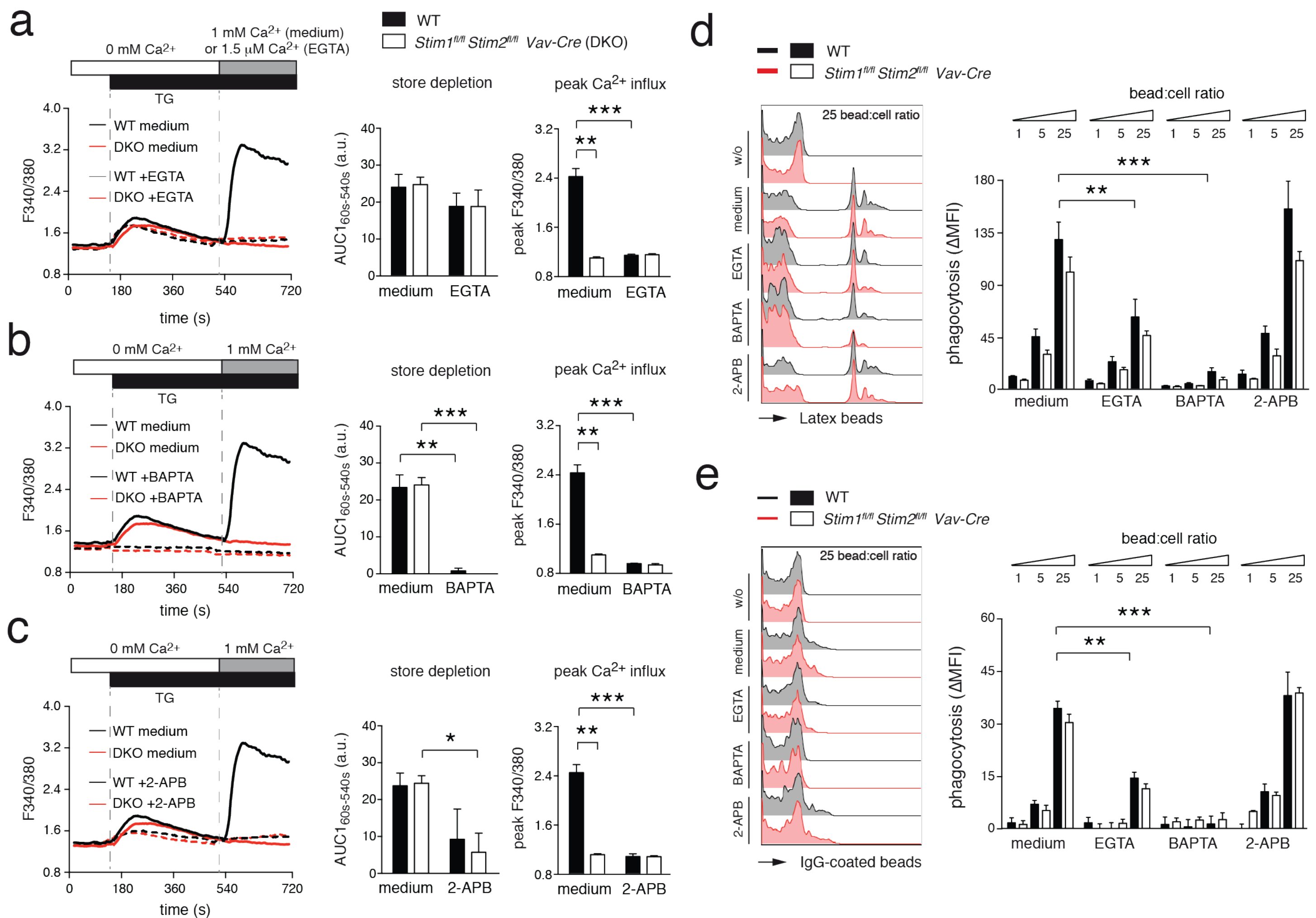


**Figure S3. Phagocytosis by bone marrow-derived DC requires intracellular $Ca^{2+}$ signaling but not SOCE. (a-c)** WT and *Stim1*$^{fl/fl}$*Stim2*$^{fl/fl}$ *Vav-Cre* BMDCs were left untreated (medium) or incubated with 2.2 mM EGTA **(a)**, 10 μM BAPTA-AM **(b)**, or 50 μM 2-APB **(c)** for 30 min and $[Ca^{2+}]_i$ was analyzed using a FlexStation3 plate reader (**a-c**, left panels). Store depletion ($AUC_{60s\text{-}540s}$) after TG stimulation in $Ca^{2+}$-free Ringer solution and peak $Ca^{2+}$ influx (peak F340/380) in 1 mM $Ca^{2+}$ Ringer solution were quantified from 3 individual mice per group (a-c, right panels). **(d,e)** WT and *Stim1*$^{fl/fl}$*Stim2*$^{fl/fl}$ *Vav-Cre* BMDCs were incubated with 1, 5, and 25 bead-to-cell ratios of carboxylate-modified yellow-green microspheres **(d)** or FITC-labeled IgG-coated fluorescent beads **(e)** for 2 h with medium alone or with 2.2 mM EGTA, 10 μM BAPTA-AM, or 50 μM 2-APB as described in (a-c). Phagocytosis was quantified by flow cytometry (left panels) and data from 3 mice per group was compiled as ΔMFI (right panel), where $\Delta MFI = MFI_{beads} - MFI_{no\ beads}$. Bar graphs represent the mean ± SEM. Statistical significance was calculated with an unpaired Student's t test: *, $p<0.05$; **, $p<0.005$; ***, $p<0.001$.

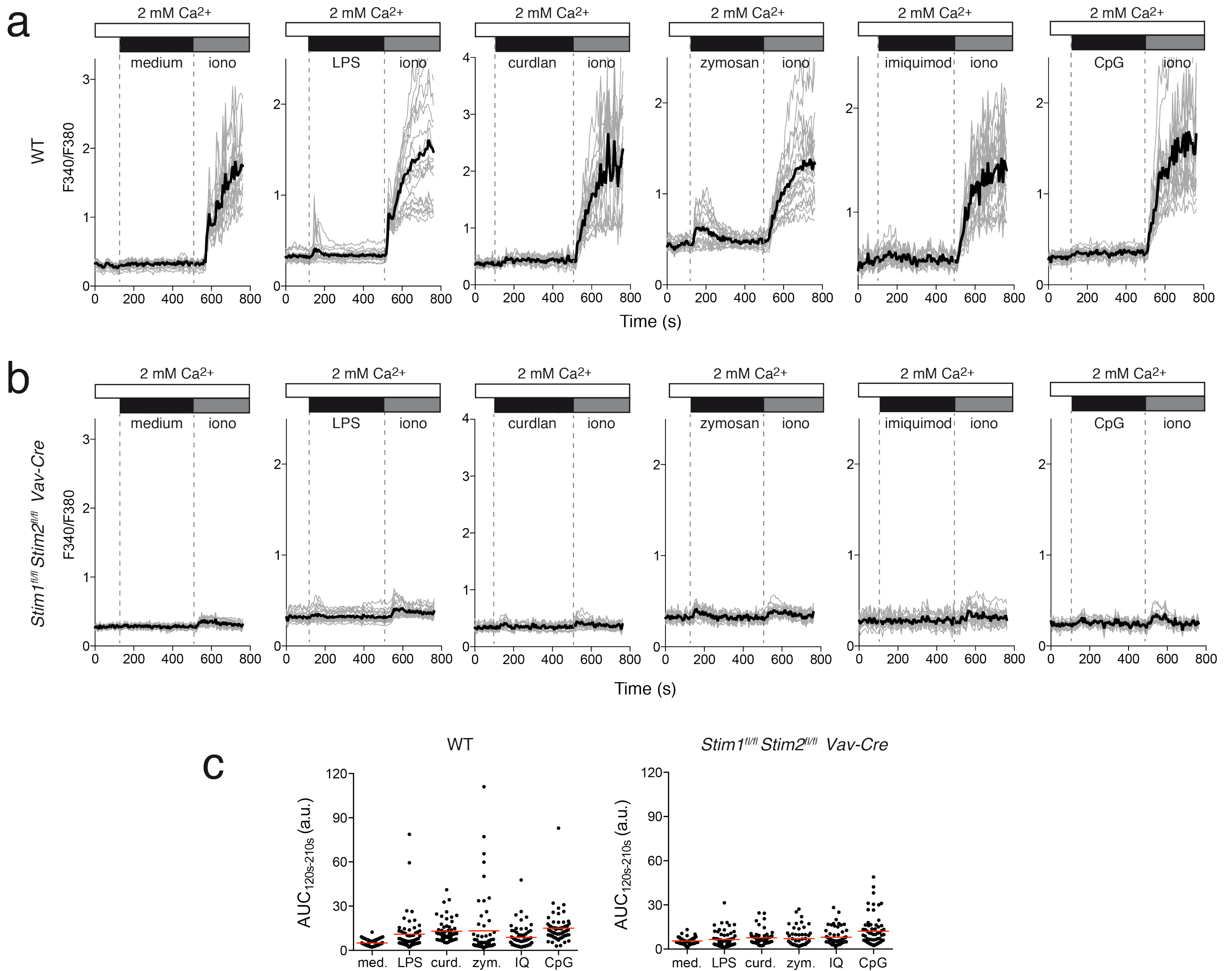


**Figure S4. Intracellular $Ca^{2+}$ signals in BMDCs upon LPS, curdlan, zymosan, imiquimod and CpG stimulation. (a-c)** BMDCs from WT **(a)** and *Stim1*$^{fl/fl}$*Stim2*$^{fl/fl}$ *Vav-Cre* **(b)** mice were loaded with Fura-2 for 30 min and stimulated with 5 µg/ml LPS, 20 µg/ml curdlan, 100 µg/ml zymosan, 1 µg/ml imiquimod, 100 nM CpG or medium alone in 2 mM $Ca^{2+}$ containing Ringer solution. After 420 s, SOCE was induced by addition of 0.2 µM ionomycin. $[Ca^{2+}]_i$ was analyzed by time-lapse microscopy. Graphs in (a) and (b) show individual cell traces (grey lines) and averaged $[Ca^{2+}]_i$ of all cells recorded (bold black line). Dot plots in panel c show the integrated $Ca^{2+}$ signal as the area under the curve (AUC) from the addition to stimulus (120s) to 210s in cells from 3 independent experiments.