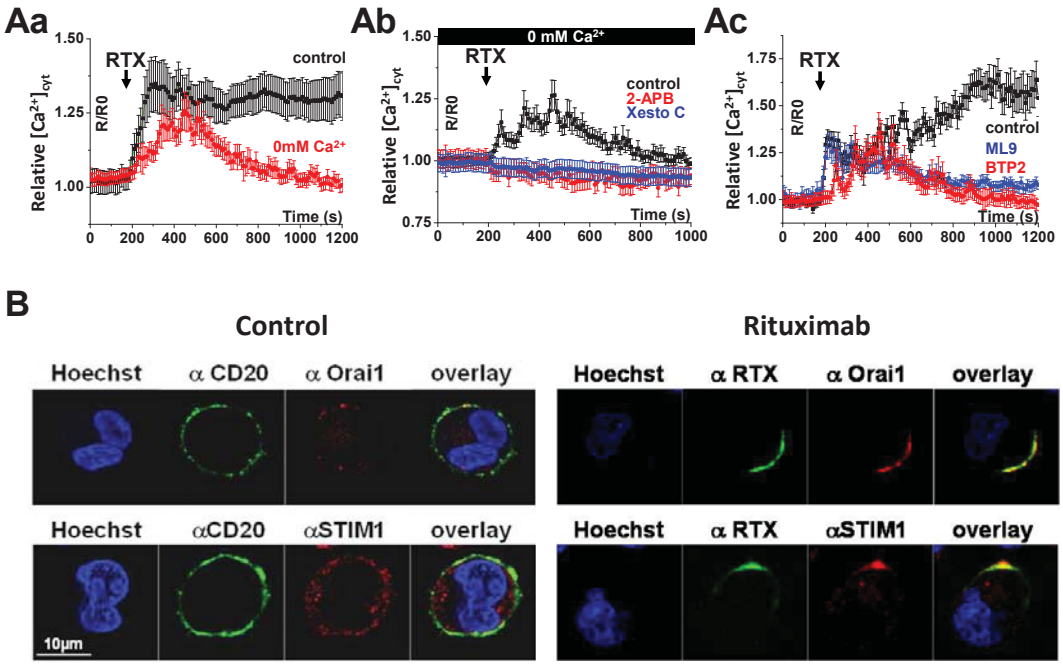
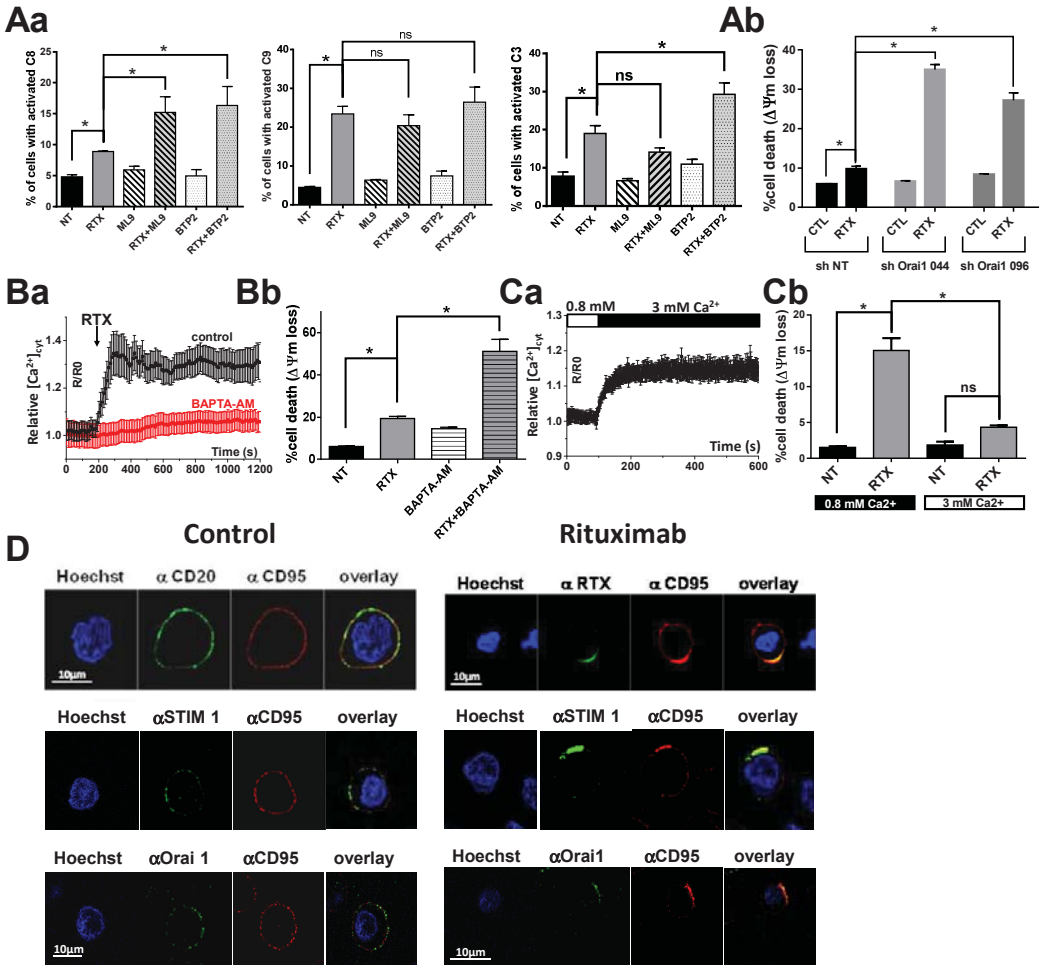
Supplemental Figure 1



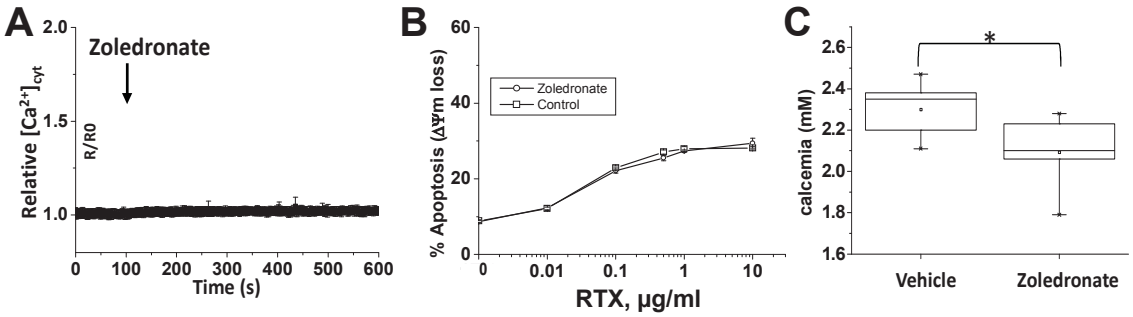
Supplemental figure 1: Rituximab induced intracellular calcium response and colocalization of Orai1, STIM1, and CD20 in the Raji cell line. A: RTX-mediated mobilization of ER-stored Ca^{2+} and Ca^{2+} influx through CRAC channels. Ca^{2+} responses to RTX were measured ratiometrically in Fura-PE3-loaded Raji cells. Recordings were performed using a conventional videomicroscopy set-up (Olympus IX-70 microscope, objective x40). Data were processed using OriginPro 7.5 software (Origin Lab). The data represent mean±SD of three independent experiments. The addition of RTX (10µg/ml) is indicated by the black arrow. The data represent mean±SE of three independent experiments. **Aa**: Cells were recorded in extracellular medium containing 2 mM Ca^{2+} (control, n=24, in black) or in Ca^{2+}-free medium (0 mM Ca^{2+}, n=41, in red). **Ab**: Cells were preincubated or not (control, n=41, in black) with Xestospongin C (360 nM, n=38, in blue) or 2-APB (44 µM, n=14, in red) for 15 min and recorded in Ca^{2+}-free medium. **Ac**: Cells were preincubated or not (control, n=59, in black) with ML9 (50 µM, n=40, in blue) or BTP2 (10 µM, n=22, in red) for 15 min and recorded in extracellular medium containing 2 mM Ca^{2+}. **B:** Immunostaining showing colocalization of Orai1 and STIM1 with CD20-capping in Raji cell line. Cells were incubated in presence or absence of RTX (10 µg/ml) at 37°C for 15 min. Cells were fixed and stained with anti-CD20-FITC or anti-RTX-FITC, anti-Orai1 and anti-STIM1 revealed by donkey anti-rabbit Ab coupled to Alexa 594. Nuclei are depicted in blue. Images were acquired with a Zeiss LSM510 confocal microscope using an Apoplan 63X objective.

Supplemental Figure 2



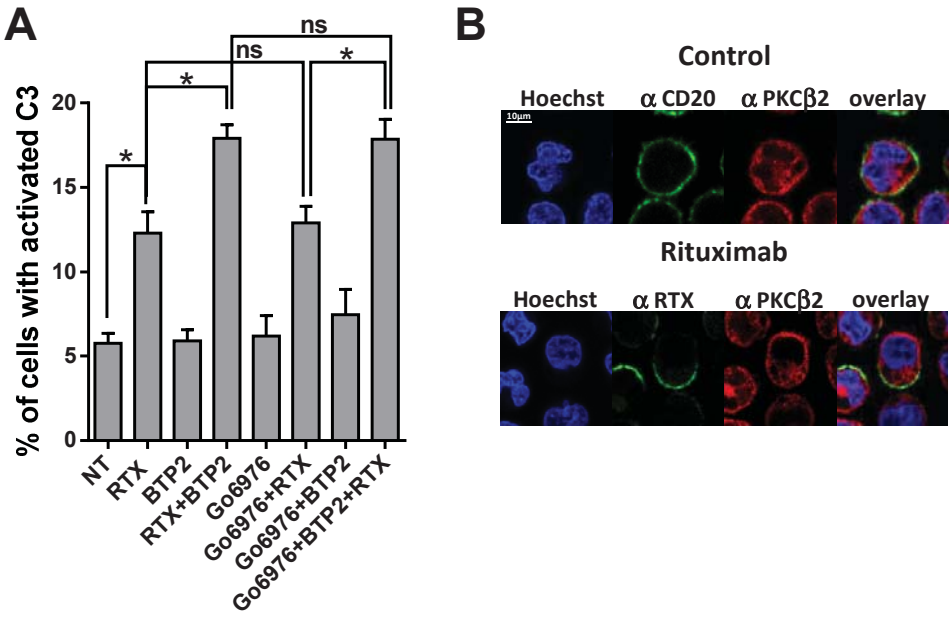
Supplemental figure 2: Calcium influx inhibited RTX-induced apoptosis in NHL cell lines. A: SOCE inhibition increased RTX-induced apoptosis. **Aa:** Raji cells were incubated with RTX (10 µg/ml) in presence or not of BTP2 (10 µM) or ML9 (50 µM) for 24h. Active caspase 8, 9, and 3, were detected by FAM-FLICA *in vitro* caspase detection kit and analyzed by flow cytometry. **Ab:** SUDHL4 cells expressing sh RNA NT or sh RNA Orai1 were incubated with RTX (10 µg/ml) for 24h. Cell death was measured by detecting mitochondrial membrane potential (ΔΨm) loss using TMRM as mitochondrial membrane potential dye. Cells were analyzed by flow cytometry. The data represent mean±SE of three independent experiments. (*P<0.05)**. B:** Intracellular Ca^{2+} chelation potentiated RTX-induced cell death in Raji cell line. **Ba:** Cells were pre-incubated or not (control, n=24, in black) with BAPTA-AM (5µM, n=40, in red) for 30 min, then Ca^{2+} responses to RTX (10µg/ml) were recorded in extracellular medium containing 2 mM Ca^{2+}. Ca^{2+} recordings were performed as described in supplemental figure 1. The data represent mean±SE of three independent experiments. **Bb:** Intracellular Ca^{2+} chelation potentiated RTX-induced cell death. Cells were pre-incubated or not with BAPTA-AM (5µM) for 30 min, and then treated with RTX (10µg/ml) for 24h. Cell death was assessed by measuring the decrease in ΔΨm using TMRM (tetramethyl rhodamine methyl ester) as fluorescent dye and analyzed by flow cytometry. **C:** Basal Ca^{2+} influx increased with extracellular Ca^{2+} concentration and impaired Rituximab-induced apoptosis . **Ca:** Raji cells were placed in an extracellular medium containing 0.8 mM Ca^{2+} then the extracellular medium was changed to 3 mM Ca^{2+} (n=5). $[Ca^{2+}]i$ was measured ratiometrically in an indo-1AM loaded Raji cell population. Recordings were performed at 37°C with a Hitachi F-2500 spectrophotometer. Data were processed using OriginPro 7.5 software (Origin Lab). The data represent mean±SE of three independent experiments. **Cb:** Raji cells were incubated with RTX (10µg/ml) in standard medium (containing 0.8 mM Ca^{2+}) or in medium containing 3 mM Ca^{2+} for 24h. Cell death was assessed as described in Bb. **D:** Immunostaining showing colocalization of CD95 with CD20-capping, Orai1, and STIM1 in Raji cell line. Cells were incubated in presence or absence of RTX (10 µg/ml) at 37°C for 15 min. Cells were fixed and stained with anti-CD95 revealed by donkey anti-mouse Ab coupled to Alexa 594 and anti-CD20-FITC or anti-RTX-FITC or anti-STIM1 or anti-Orai1 revealed by donkey anti-rabbit Ab coupled to Alexa 488. Nuclei are depicted in blue. Images were acquired with a Zeiss LSM510 confocal microscope using an Apoplan 63X objective.

Supplemental Figure 3



Supplemental figure 3: Zoledronate had no direct effect on $[Ca^{2+}]i$ or apoptosis of Raji cells but provoked hypocalcemia in mice. A: Zoledronate had no effect on $[Ca^{2+}]i$ *in vitro*. Cells were recorded in extracellular medium containing 2 mM Ca^{2+}. Zoledronate (10µM) addition is indicated by the black arrow. Ca^{2+} responses to Zoledronate were measured as described in supplemental figure 1. Data represent mean±SE of three independent experiments (n=58). **B**: Zoledronate had no effect on RTX-induced apoptosis. Cells were incubated with RTX with or without Zoledronate (10µM) for 24h. Apoptosis was assessed by measuring the decrease in $\Delta\Psi m$ using TMRM (tetramethyl rhodamine methyl ester) as fluorescent dye and analyzed by flow cytometry. Data represent mean±SE of two independent experiments. **C:** Zoledronate reduced calcemia in mice. Female Ragγ2C$^{-/-}$ mice (n=6) were treated intraperitoneally with Zoledronate (1mg/kg). Two hours after injection, a blood sample was taken and calcemia was measured using COBAS 400 technology (Roche).

Supplemental Figure 4



Supplemental figure 4: PKCβ2 is not the downstream effector of RTX-induced Ca^{2+} influx in the Raji cell line. A: PKCα,β inhibitor had no effect on RTX-induced apoptosis. Cells were incubated with RTX (10 µg/ml) in the presence or absence of Gô6976 (250 nM) and/or BTP2 (10 µM) for 24h. Active caspase 3 was detected by the FAM-FLICA *in vitro* caspase detection kit and analyzed by flow cytometry. The data represent mean±SE of three independent experiments (*P<0.05). **B:** No PKCβ2 redistribution in CD20-capping was induced by RTX. SUDHL4 cells were treated or not with RTX (10 µg/ml) for 15 min. Cells were fixed and stained with anti-CD20-FITC or anti-RTX-FITC and anti-PKCβ2 revealed by donkey anti-rabbit Ab coupled to Alexa 594. Nuclei are depicted in blue. Images were acquired as described in supplemental Fig 2.