nature cell biology

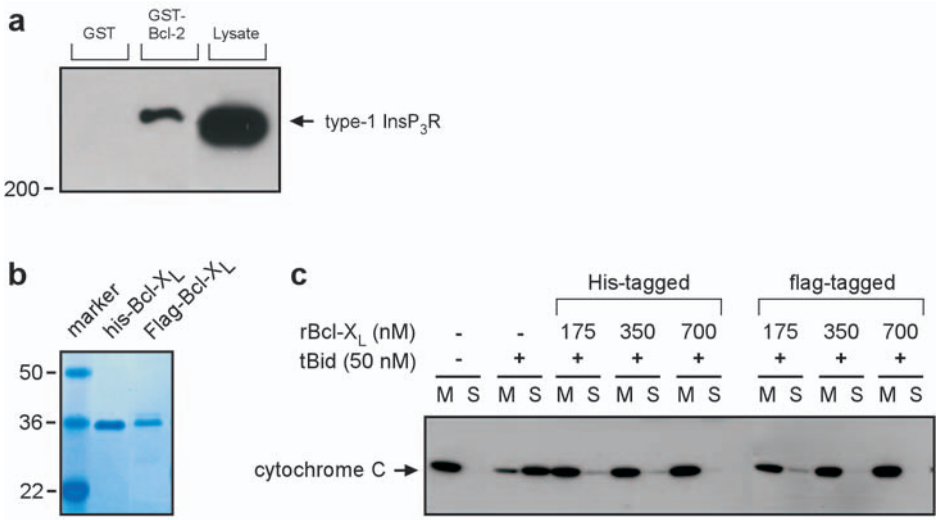

Figure S1 Interaction of Bcl-2 with type-1 $InsP_3R$ and Inhibition of cytochrome C release from mitochondria under *in vitro* apoptotic conditions is inhibited by recombinant Bcl-X_L. **a,** Lysates from DT40-$InsP_3R$-KO cells stably expressing rat type-1 $InsP_3R$ were incubated with GST-Bcl-2 and bound $InsP_3R$ was detected with isoform-specific antibody. **b,**. His-hBcl-XL and Flag-hBcl-X_L proteins were purified from bacterial cells or insect cells, respectively, and their purity examined by Coomassie Blue staining. **c,** Bcl-X_L inhibits tBid-mediated cytochrome C release. Mitochondria were isolated from a $Bak^{-/-}Bax^{-/-}$ hematopoietic cell line in which Bax was re-expressed. In the absence of tBid cytochrome C was detected exclusively in the mitochondrial fraction (M). After incubation with tBid cytochrome C was released from mitochondria and detected in the supernatant (S). Addition of Bcl-X_L blocked the release of cytochrome C in the presence of tBid.

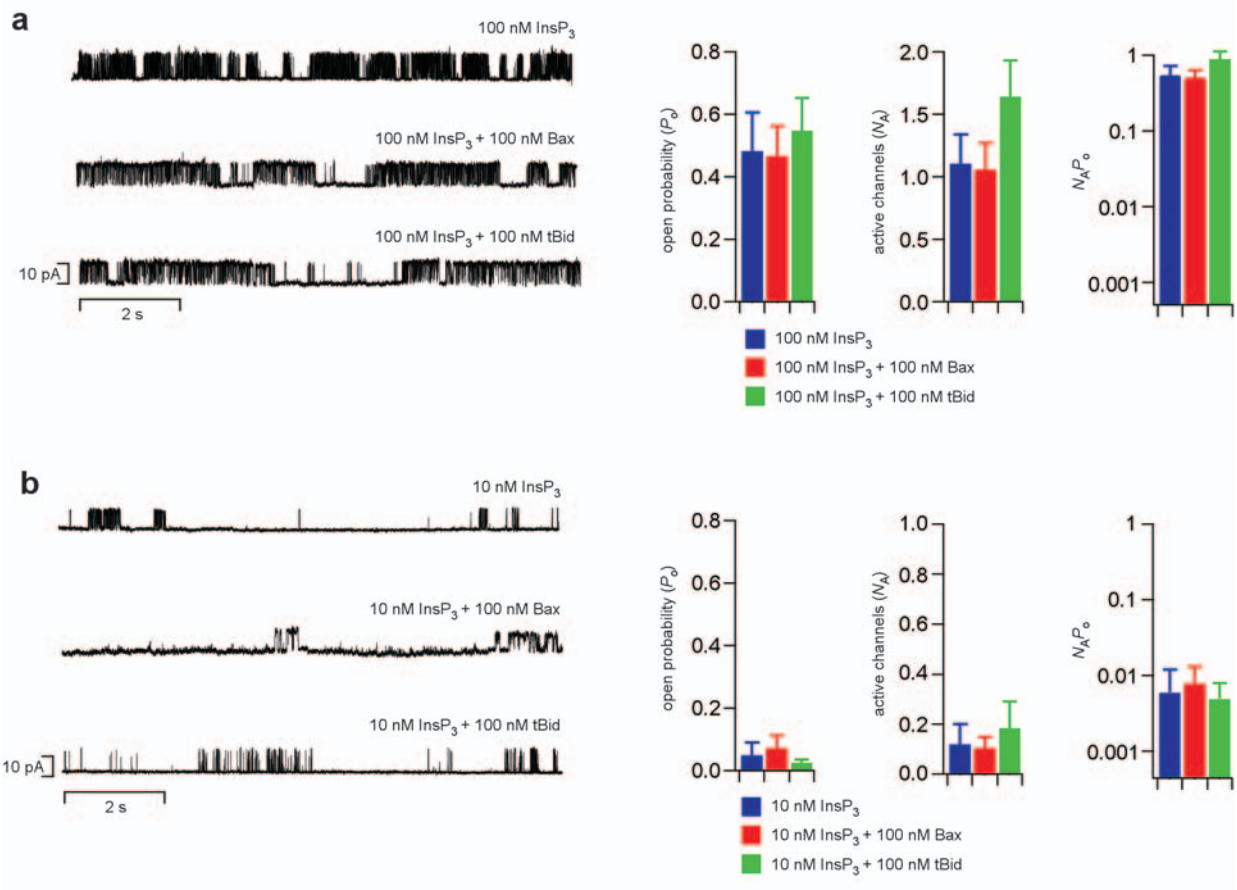

Figure S2 $InsP_3R$ activity in the absence of Bcl-X_L and presence of Bax or tBid. **a**, Typical $InsP_3R$ current traces in the presence of 100 nM $InsP_3$ in Sf9 nuclei, pipette $[Ca^{2+}]$ was 1 μM. Addition of 100 nM tBid or Bax along with 100 nM $InsP_3$ to the pipette solution had no effect on P_o, N_A or N_AP_o, ($P>0.05$, unpaired *t*-test). **b**, Similarly, in the presence of 10 nM $InsP_3$, pipette $[Ca^{2+}]$ 0.85 μM, addition of 100 nM Bax or tBid had no effect on channel P_o, N_A or N_AP_o, ($P>0.05$, unpaired *t*-test).

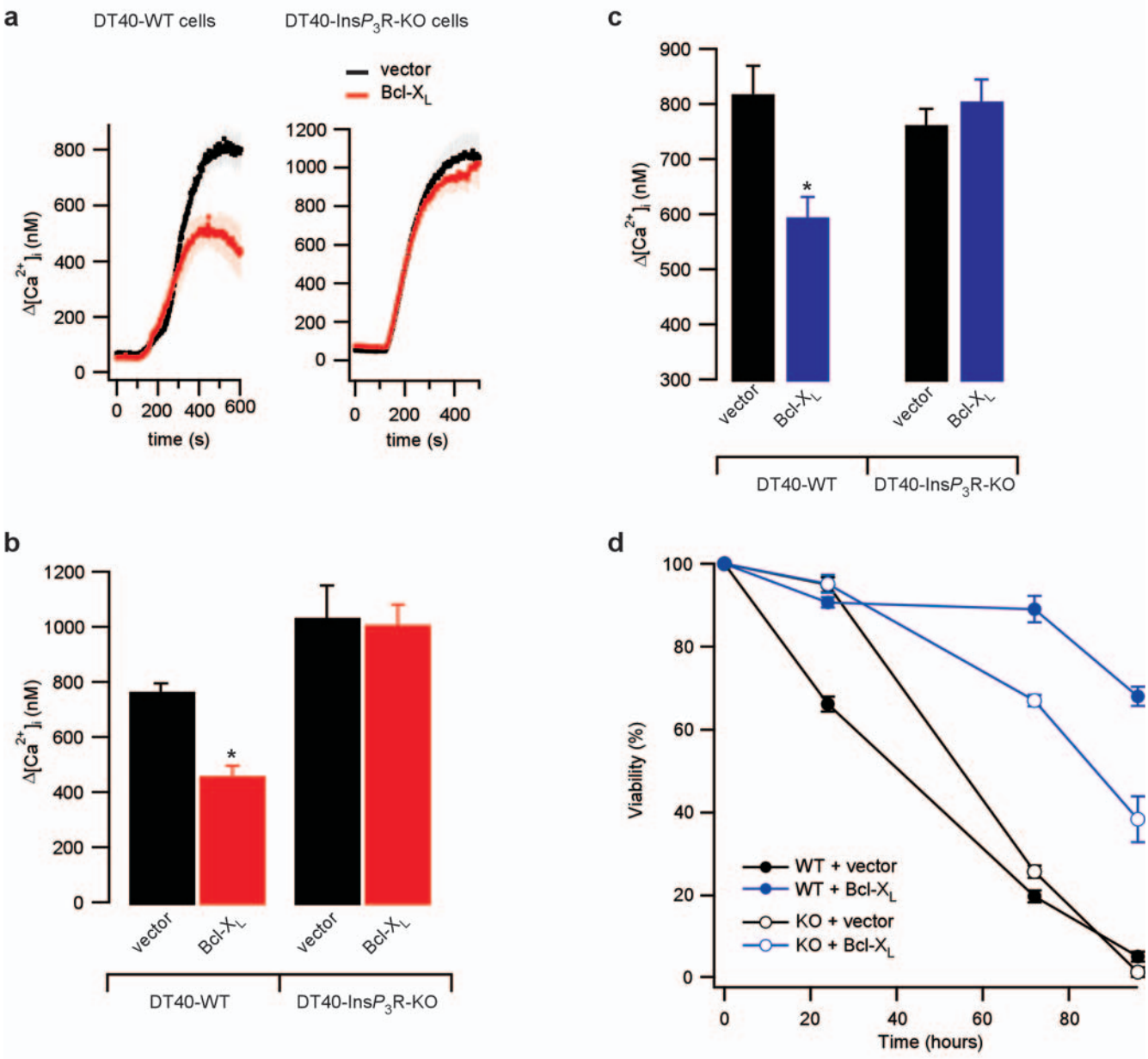

Figure S3 Interaction of Bcl-X_L with $InsP_3R$ is essential for Bcl-X_L effects on ER Ca^{2+} regulation and apoptosis. **a**, Effects of Bcl-X_L transient expression on $Ca^{2+}{}_{ER}$. Typical records depicting change in cytoplasmic $[Ca^{2+}]$ ($[Ca^{2+}]_i$) in response to application of 1 μM thapsigargin in DT40-WT and DT40-$InsP_3R$-KO cells transiently transfected with either Bcl-X_L or vector alone. Each trace represents the mean ± SEM of at least six individual cells within the image field. **b**, Summary of the effects of Bcl-X_L transient expression on $Ca^{2+}{}_{ER}$. Thapsigargin-induced increases in $[Ca^{2+}]_i$ in vector alone and Bcl-X_L-expressing cell lines. Data represent mean ± SEM for at least 30 individual cells in two independent transfections. Asterisk indicates $P<0.05$, ANOVA. **c**, Effects of Bcl-X_L expression on ER Ca^{2+} regulation in independent clones. Summary of the effects of stable Bcl-X_L expression on $Ca^{2+}{}_{ER}$. Thapsigargin-induced increases in $[Ca^{2+}]_i$ in vector alone and Bcl-X_L-expressing cell lines. Data represent mean ± SEM for at least 30 individual cells in multiple trials. Asterisk indicates $P<0.05$, ANOVA. **d**, Cell viability after treatment with 20 μg/ml α-IgM (time 0) of DT40-WT (solid symbols) and DT40-$InsP_3R$-KO (open symbols) cells stably expressing Bcl-X_L (Blue) or vector alone (Same clones as in c).

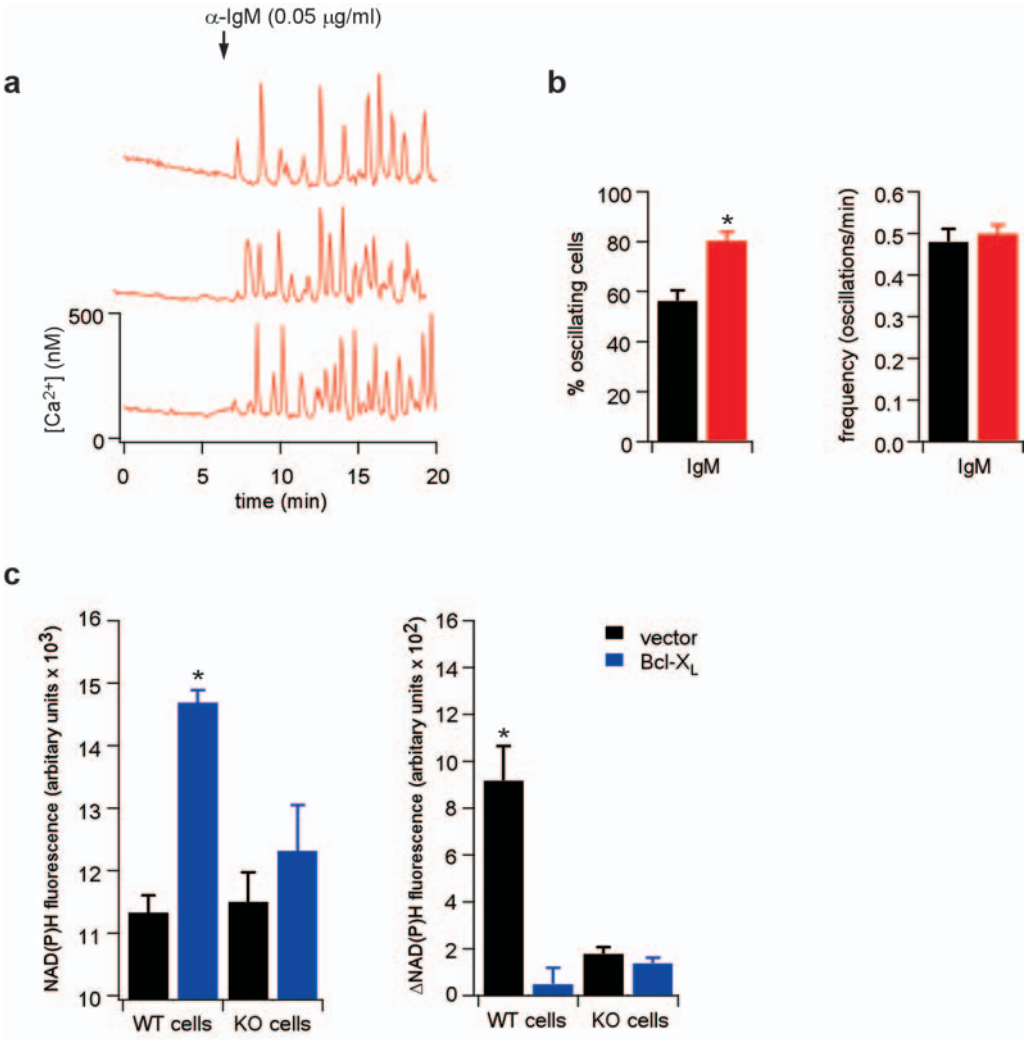

Figure S4 Receptor stimulation is more sensitively coupled to calcium signalling in Bcl-X_L expressing cells and modulates mitochondrial NADH levels. **a**, $[Ca^{2+}]_i$ measurements in DT40-WT cells stably expressing Bcl-X_L. Receptor stimulation with low concentration of α-IgM evokes Ca^{2+} oscillations in many cells that were initially quiescent, examples of three responsive cells are shown. **b**, The percentage of cells that respond to α-IgM stimulation in this way, and the oscillation frequency is summarised as the mean ± SEM for at least 80 individual cells in no less than two independent trials. Asterisk indicates P<0.0001, unpaired *t*-test. **c,** The Bcl-X_L - $InsP_3R$ interaction modulates mitochondrial NADH levels. The average (± SEM) resting NAD(P)H fluorescence and the change in NAD(P)H fluorescence in response to α-IgM stimulation for three replicate experiments are plotted. Asterisk indicates P<0.001, ANOVA.