SUPPLEMENTARY FIGURE AND TABLE LEGEND

Supplementary Figure 1. Generation of mitochondrially targeted and ER-targeted Bcl-x_L mutants. (A) Scheme of the Bcl-x_L mutants. The C-terminal transmembrane domain of Bcl-x_L was deleted (ΔC) or replaced with the mitochondria-targeting sequence of ActA or the ER-targeting sequence of cb5. **(B)** The indicated GFP and Bcl-x_L fusion proteins were transiently expressed in Bcl-x-KO MEFs. The mitochondrial protein Tom20 was detected as organelle marker. The fluorescence was visualized by confocal microscopy. Scale bar, 10 μm. **(C)** Confocal microscopy images of GFP-tagged Bcl-x_L proteins expressed in Bcl-x-KO MEF cells. The ER protein calreticulin was used as organelle marker. Scale bar, 10 μm. **(D)** Intracellular localization of GFP-tagged Bcl-x_L mutants in live cells. The indicated fusion proteins of GFP and Bcl-x_L were transiently expressed in Bcl-x-KO MEFs and imaged using confocal microscopy. The ER or mitochondria were visualized by co-transfection with plasmids carrying either ER-targeted DsRed or mitochondrial-targeted RFP. Scale bar, 20 μm.

Supplementary Figure 2. Bcl-x_L localized on mitochondria protects against apoptotic insults more effectively than ER-targeted Bcl-x_L. (A) Viability of the indicated MEF cells was measured in the presence of the following death stimuli: 5.0 μM etoposide, 1.0 μM doxorubicin, 0.2 μg/μl actinomycin D, 5.0 nM staurosporine and 0.4 mM H_2O_2. Mean ± standard deviation of triplicate experiments were shown. Experiments were performed independently three times. **(B)** Caspase 3/7 activity was measured 7 hours following actinomycin D treatment, 9 hours following H_2O_2 treatment or 12 hours after the treatment with etoposide, doxorubicin, or staurosporine using fluorometric assay. Values are normalized to the values of untreated cells. Data shows mean ± standard deviation of triplicate experiments, which have been repeated three times.

Supplementary Figure 3. All three subtypes of $InsP_3R$ are expressed in wild type and Bcl-x-KO MEFs. Western blot analysis for the three different $InsP_3R$ subtypes was performed on two wild type and two Bcl-x-KO MEF cell lines as indicated in Figure 1C.

Supplementary Table 1. Lack of Bcl-x_L expression does not alter the expression of BH3-only Bcl-2 proteins. mRNA levels of BH3-only Bcl-2 proteins in two wild-type (WT) and two Bcl-x knock-out (KO) cell lines were determined by microarray analysis. Data are shown as fold-changes in mean mRNA value of triplicate RNA samples for each cell line. Negative numbers indicate down-regulation.

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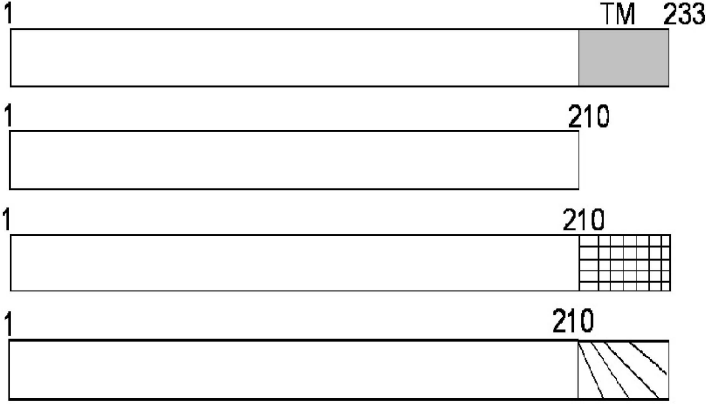
A



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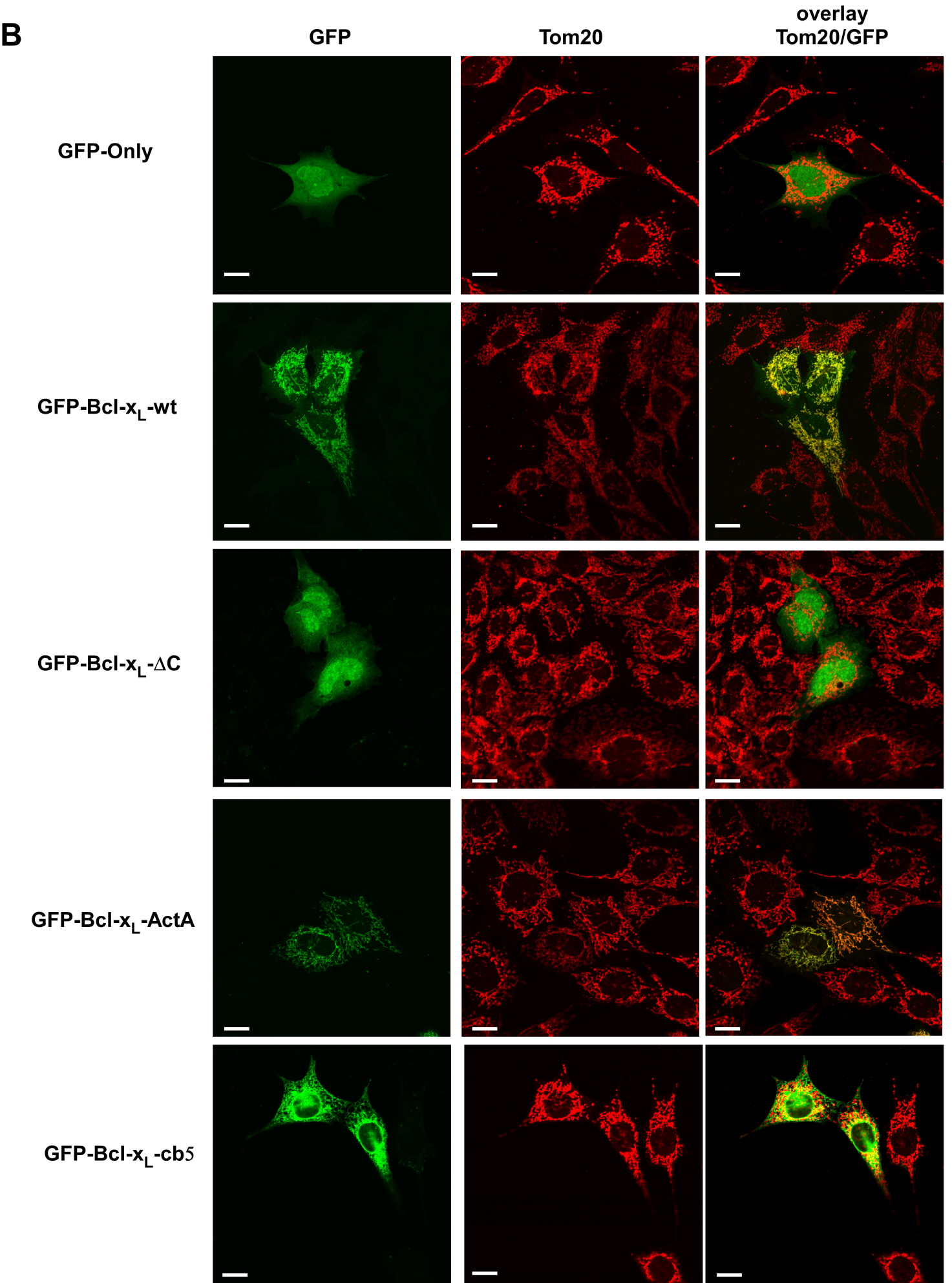

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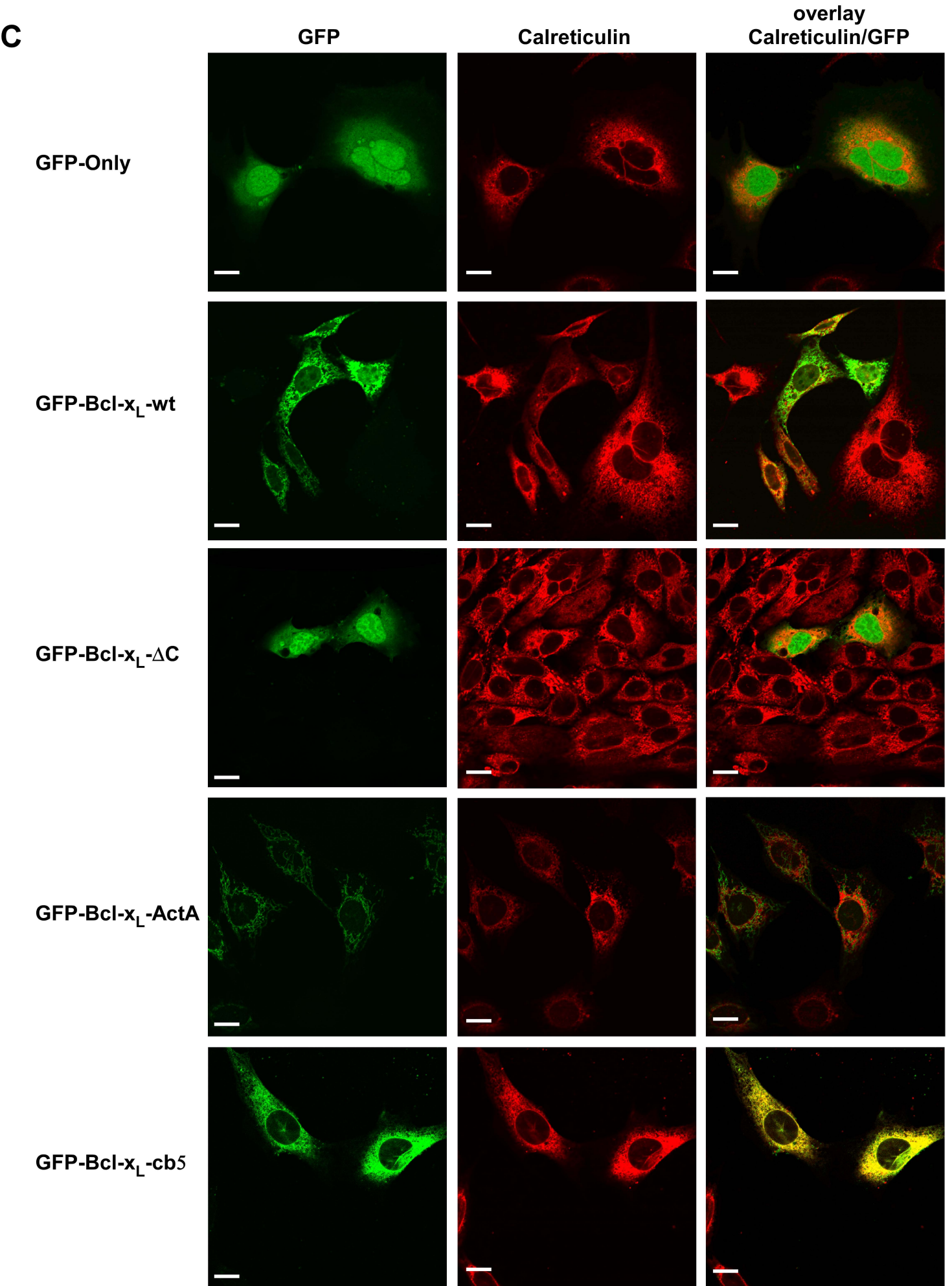

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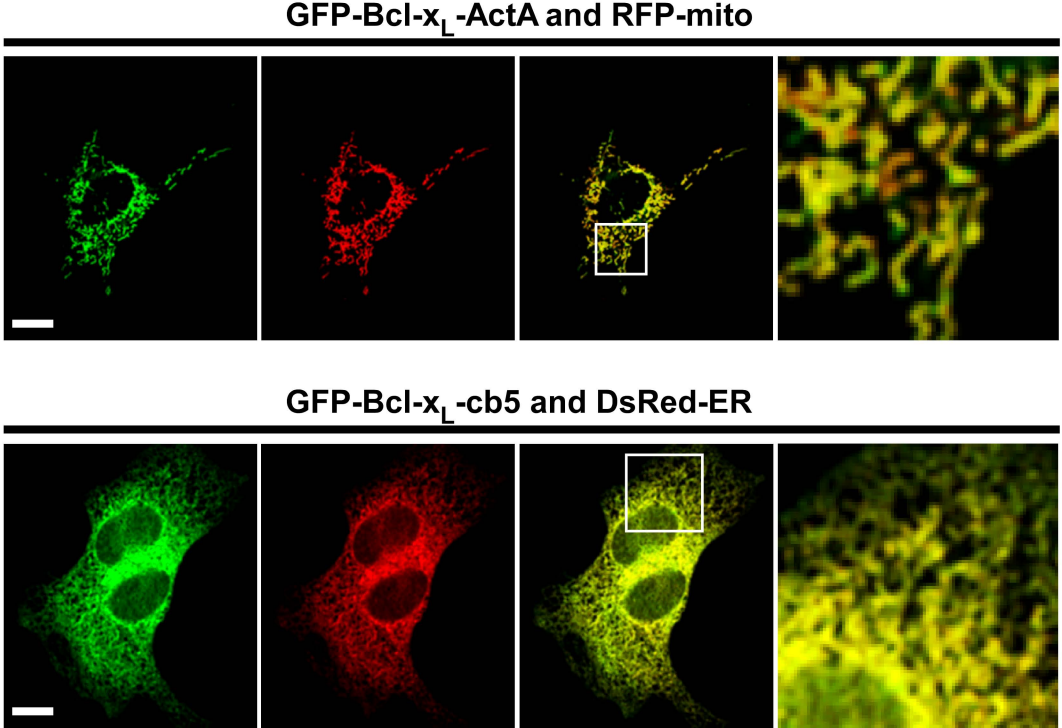
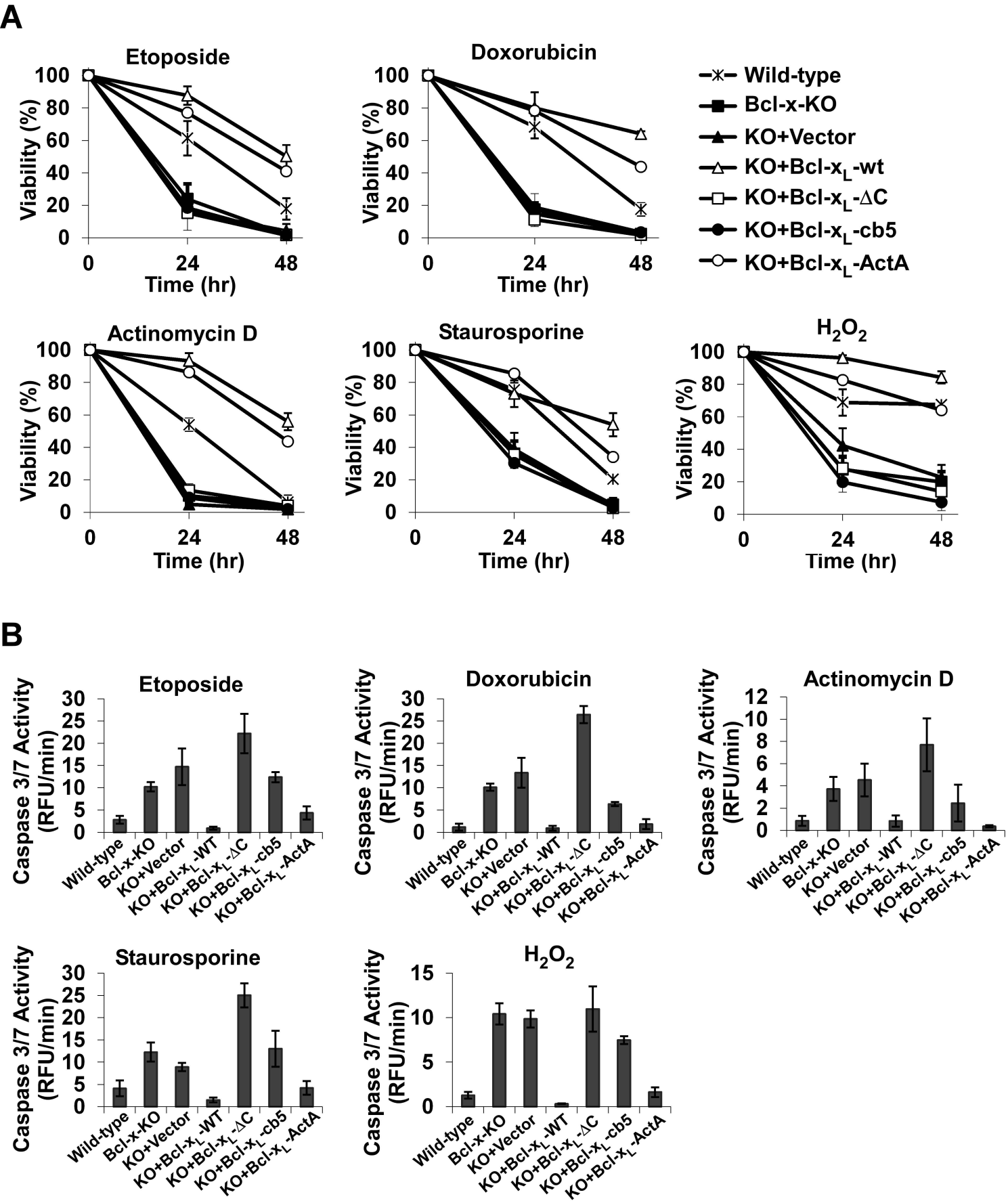
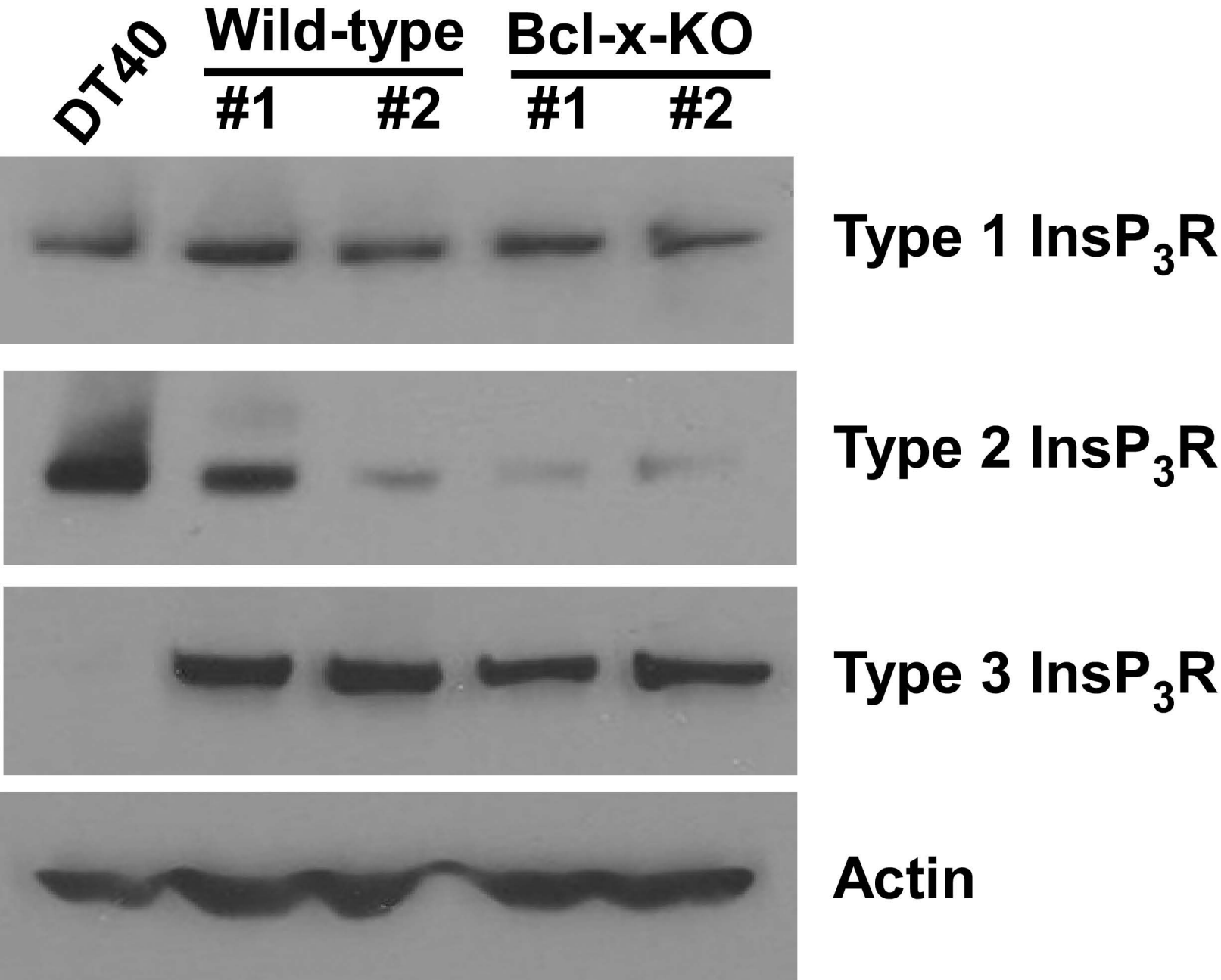
D



Eno et al., Supplementary Figure 2



Eno et al., Supplementary Figure 3



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Gene Description	Fold changes (KO/WT)	p value
Bcl-2-interacting killer (Bik)	1.29	0.001245
Harakiri, Bcl-2 interactin protein (Hrk)	1.12	0.497068
Bcl-2 modifying factor (Bmf)	1.09	0.086236
Bcl-2 binding component 3 (Puma)	1.05	0.045677
Bcl-2-like 11 (Bim)	-1.37	0.000303
Bcl-2 associated agonist of cell death (Bad)	1.02	0.610081
Bcl-2 /adenovirus E1B Interacting protein 3 (Bnip3)	1.12	0.497.68
Phorbol-12-myristate-13-acetate-induced protein1(Pmaip1, Noxa)	1.43	0.071210
BH3 interacting domain death agonist (Bid)	1.33	0.011095
Bcl-2 /adenovirus E1B Interacting protein 3-like (Bnip3l, Nix)	1.04	0.381809