nature cell biology

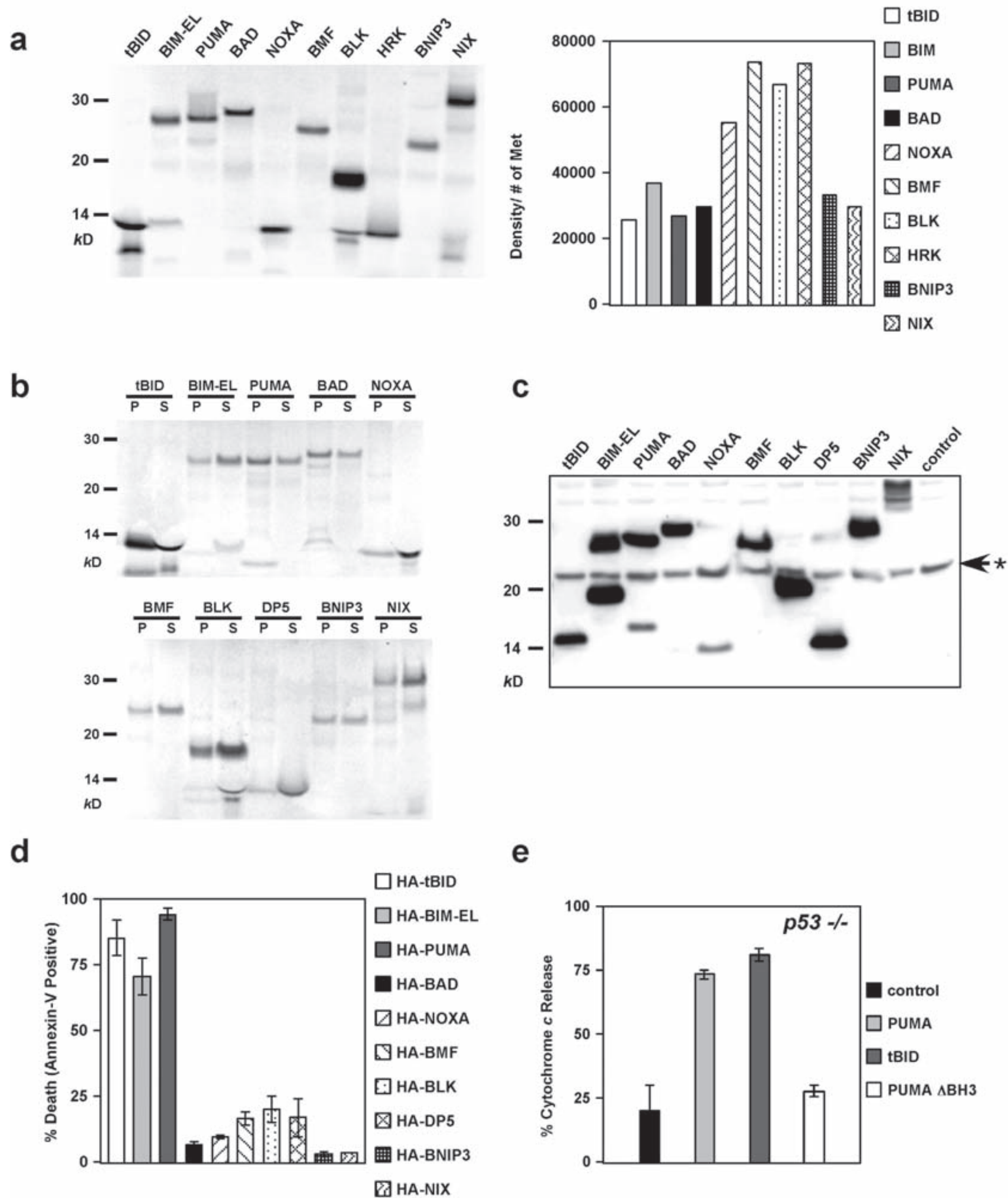

Figure S1 (a) Autoradiogram of BH3-only proteins translated in vitro in the presence of [^{35}S]methionine. The quantity of each protein was determined by densitometry (Fuji, ImageGauge) then divided by the number of methionine present in each protein. (b) Autoradiogram of in vitro targeting of indicated BH3-only proteins to mitochondria. P denotes mitochondrial pellet; S, mitochondrial supernatant. **(c)** Anti-HA Western blot of indicated HA-tagged BH3-only molecules transduced in *Bax*, *Bak* DKO cells by retrovirus. Asterisk indicates a cross-reactive band, serving as a loading control. **(d)** Wild type MEFs were infected with retrovirus expressing indicated HA-tagged BH3-only molecules. Death is presented as mean ± 1 SD of Annexin-V positive cells at 24 hr from three independent experiments. **(e)** Mitochondria isolated from p53 null MEFs were incubated with IVTT wild type or mutant PUMA or truncated BID proteins and cytochrome *c* release was quantitated by ELISA assays. Values shown are mean ± 1 SD of three independent experiments.

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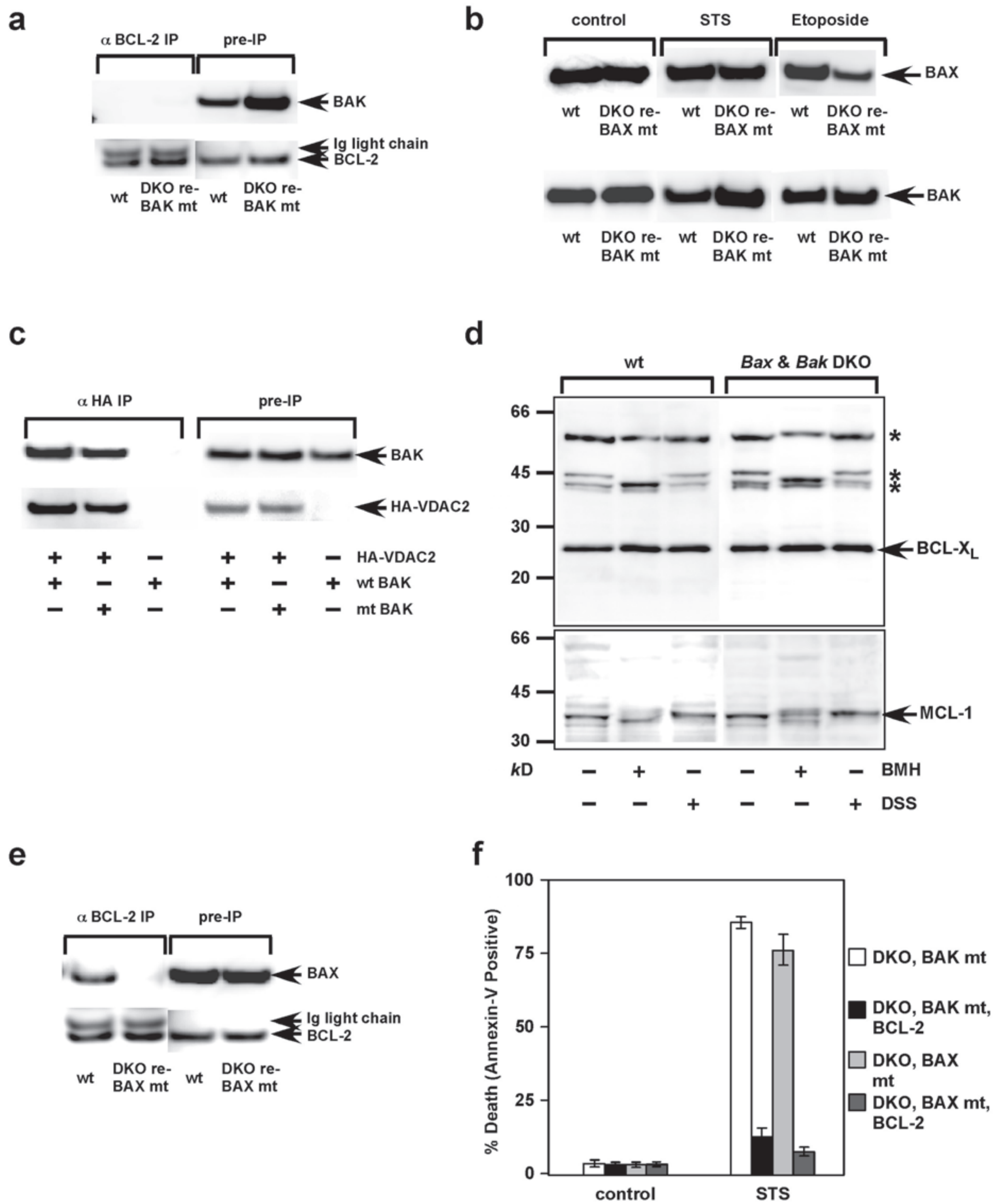

Figure S2 **(a)** Wild type MEFs or *Bax, Bak* DKO MEFs reconstituted with mutant BAK (I82A & N83A) were lysed in 0.2% NP-40 lysis buffer, immunoprecipitated with anti-BCL-2 antibody, and analyzed by anti-BAK or anti-BCL-2 Western blots. **(b)** Anti-BAX and anti-BAK Western blots of wild type MEFs or *Bax, Bak* DKO MEFs reconstituted with either mutant BAX (L70A & D71A) or BAK (I82A & N83A) before or after treatment with staurosporine (STS) for 6 hours or etoposide for 12 hours. **(c)** Wild-type or mutant BAK (I82A & N83A) was transiently co-expressed with HA-tagged VDAC2 in *Bax*, *Bak* DKO MEFs. Cells lysed in 1% CHAPS lysis buffer, were immunoprecipitated with anti-HA antibody and analyzed by anti-BAK or anti-VDAC2 Western blots. **(d)** Wild type MEFs or *Bax, Bak* DKO MEFs were treated with 10 mM BMH or DSS crosslinker and analyzed by anti-BCL-X_L (upper panel) or anti-MCL-1 (lower panel) Western blots. No discernable differences following cross-linking were observed between wild type and *Bax* & *Bak* DKO cells, indicating the lack of cross-linking between BCL-X_L/MCL-1 and BAX/BAK. Asterisks denote cross-reactive bands. **(e)** Wild type MEFs or *Bax, Bak* DKO MEFs reconstituted with mutant BAX (L70A & D71A) were lysed in 0.2% NP-40 lysis buffer, immunoprecipitated with anti-BCL-2 antibody, and analyzed by anti-BAX or anti-BCL-2 Western blots. **(f)** *Bax, Bak* DKO MEFs reconstituted with either mutant BAK (I82A & N83A) or mutant BAX (L70A & D71A) were transduced with control retrovirus (MSCV) or retrovirus expressing BCL-2, then treated with 1 µM staurosporine (STS) to induce apoptosis. Death is presented as mean ± 1 SD of Annexin-V positive cells at 24 hr from three independent experiments.

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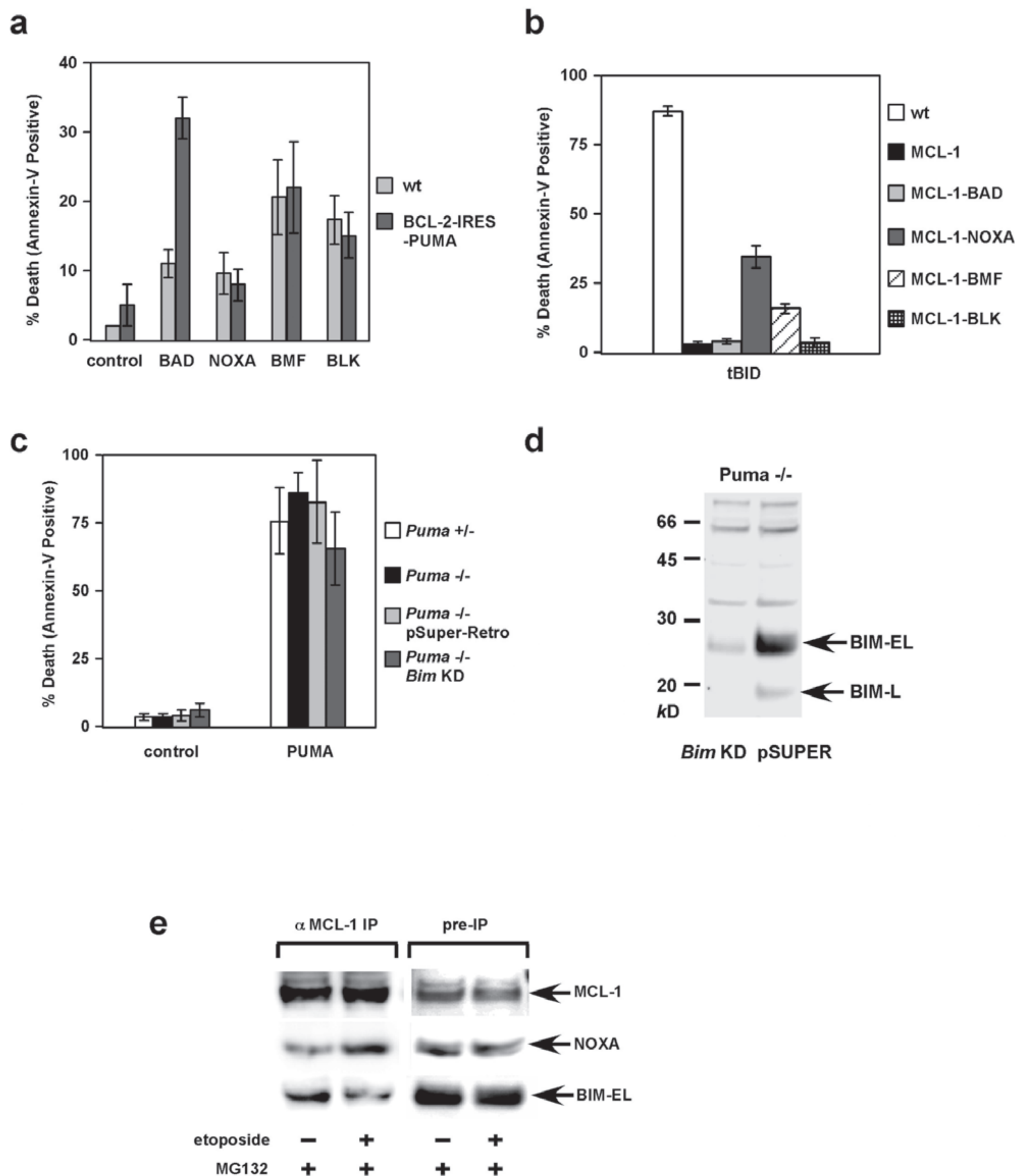

Figure S3 **(a)** Wild type MEFs or MEFs stably expressing BCL-2-IRES-PUMA were transduced with indicated BH3-only molecules by retrovirus. Death is presented as mean ± 1 SD of Annexin-V positive cells at 24 hr from three independent experiments. **(b)** Wild type MEFs or MEFs stably expressing MCL-1, or MCL-1 and BAD, MCL-1 and NOXA, MCL-1 and BMF, or MCL-1 and BLK were transduced with retrovirus expressing truncated BID. Death is presented as mean ± 1 SD of Annexin-V positive cells at 24 hr from three independent experiments. **(c)** *Puma* +/-, *Puma* -/- MEFs, or *Puma* -/- MEFs stably expressing shRNA against *Bim* using pSUPER-RETRO system were transduced with control MSCV retrovirus or PUMA expressing retrovirus. Death is presented as mean ± 1 SD of Annexin-V positive cells at 24 hr from three independent experiments. **(d)** Anti-BIM Western Blot of *Puma* -/- MEFs stably expressing shRNA against *Bim*. **(e)** SV40 transformed *Apaf* -/- MEFs with or without etoposide (10 μg/ml) and MG132 (10 μM) treatment for 18 hours, were lysed in 0.2% NP-40 lysis buffer, immunoprecipitated with anti-MCL-1 antibody, and analyzed by anti-MCL-1, anti-NOXA, or anti-BIM Western blots.

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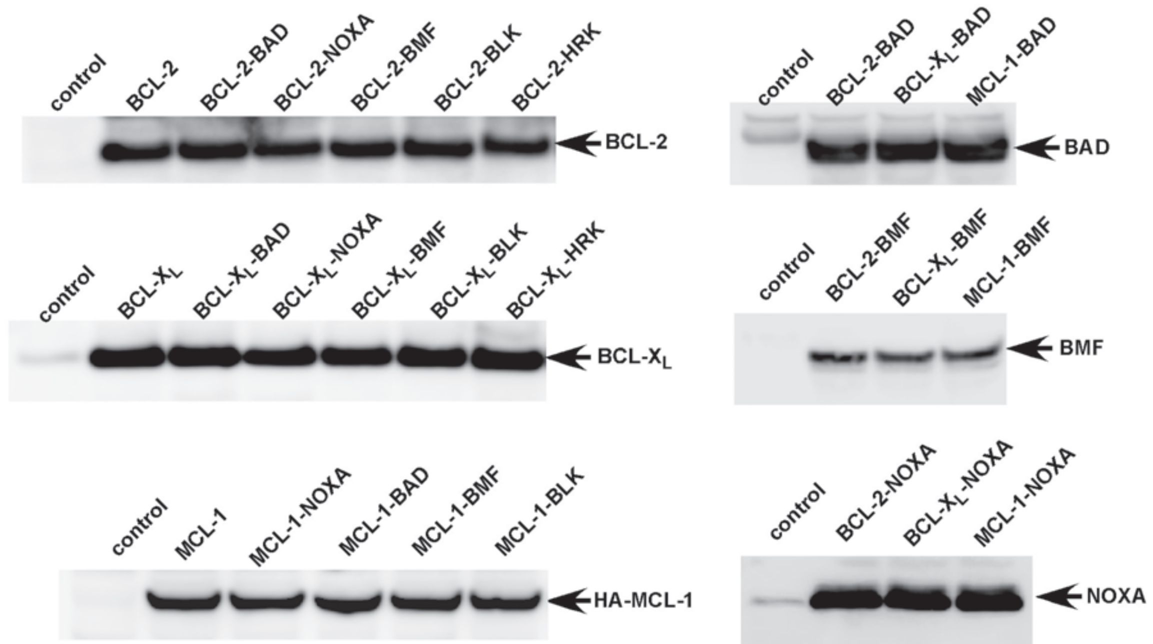

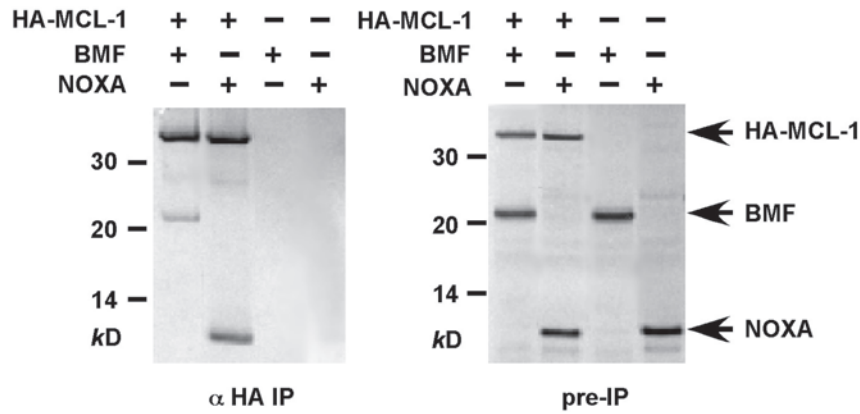

Figure S4 **(a)** Anti-BCL-2, anti-BCL-X_L, anti-HA, anti-BAD, anti-BMF, and anti-NOXA Western blots of indicated cell lines used for viability assays shown in Fig. 7. **(b)** N-terminal HA-tagged MCL-1 was co-translated with BMF or NOXA in vitro in the presence of [^{35}S]methionine. IVTT proteins of BMF and NOXA alone served as negative controls. Reticulocytes in 0.2% NP-40 lysis buffer were immunoprecipitated with anti-HA antibody and analyzed in 10% Nu-PAGE gels and autoradiography.

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