Supporting Information

Lindqvist et al. 10.1073/pnas.1406425111

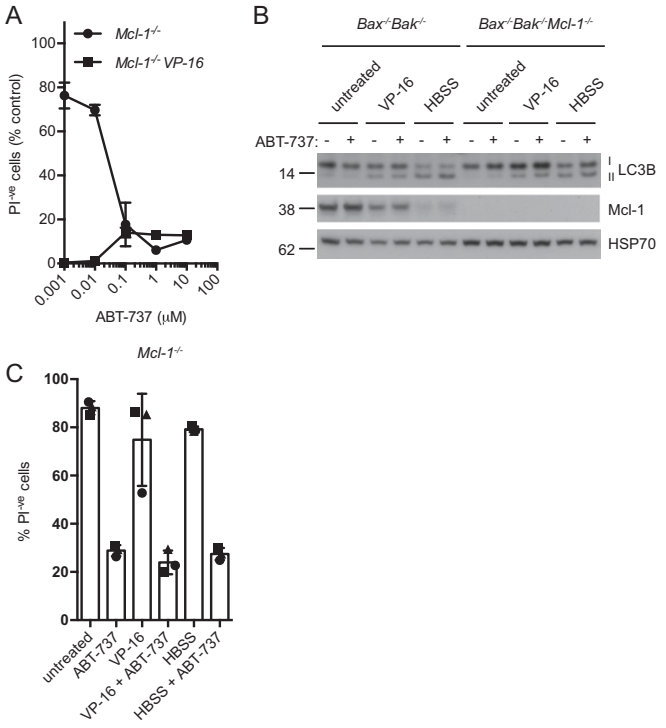

Fig. S1. (*A*) Mouse embryonic fibroblasts (MEFs) were cotreated with 34 μM etoposide (VP-16) and the indicated concentrations of ABT-737. After 96 h, viability was measured by the absence of propidium iodide (PI) uptake. The data for myeloid cell leukemia 1 (Mcl-1)-null MEFs in the absence of VP-16 is also shown in Fig. 1*B* for comparison. The means ± SD of two independent lines tested on two different occasions is shown ($n = 4$). (*B*) Microtubule-associated protein 1 light chain 3 beta (LC3B) lipidation is unaltered by ABT-737 in fibroblasts lacking Bcl-2–associated X (Bax) and Bcl-2 homologous antagonist/killer (Bak). Western blot of MEFs after a 4-h treatment with 34 μM VP-16, HBSS, and 1 μM ABT-737 where indicated. (*C*) Viability of MEFs with functional Bax and Bak but lacking Mcl-1 was measured via exclusion of PI after a 4-h treatment with 1 μM ABT-737, 34 μM VP-16, and HBSS (two independent *Mcl-1*$^{-/-}$ lines) in parallel with *B*. The mean ± SD of three independent experiments are indicated by squares, circles, and triangles.

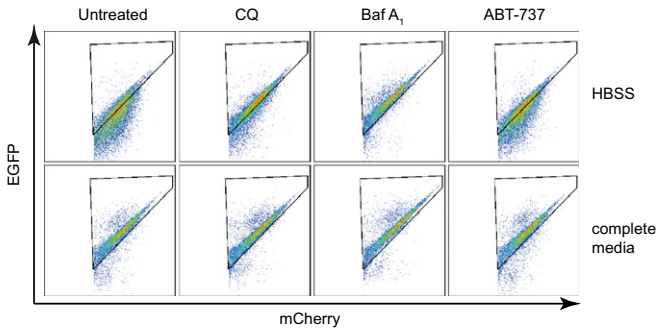

Fig. S2. Autophagolysosome function is unaltered by ABT-737. *Bax*$^{-/-}$*Bak*$^{-/-}$*Mcl-1*$^{-/-}$ MEFs expressing the fusion protein mCherry-EGFP-LC3B were treated with 1 μM ABT-737, 50 μM CQ, and/or 0.1 μM bafilomycin A_1 in complete media or under starvation conditions (HBSS) to induce autophagy. After 24 h, cells were harvested and fluorescence was measured by flow cytometry. Representative experiment of mCherry^{+} cells illustrating the gating used to determine GFP^{high}.

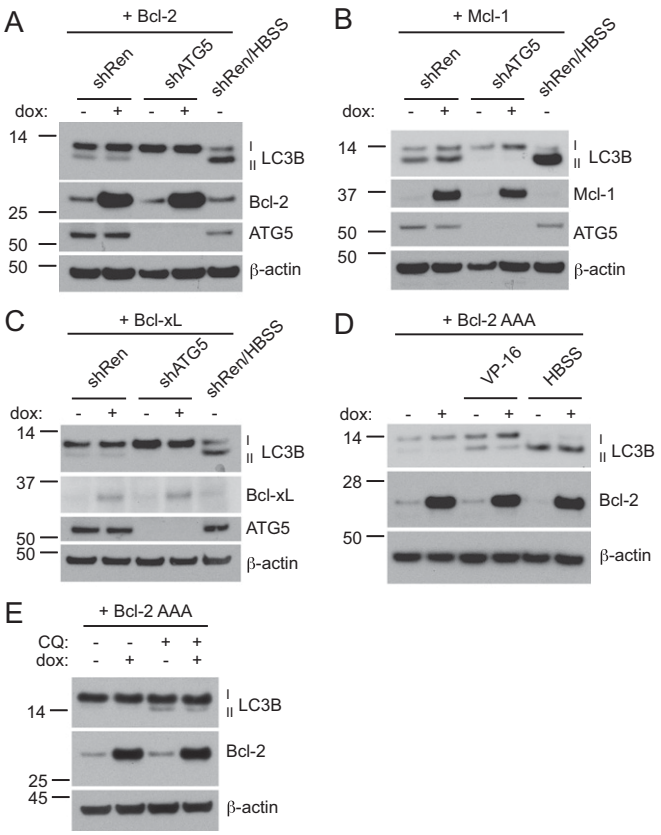

Fig. S3. Overexpression of murine B-cell lymphoma 2 (Bcl-2), Mcl-1, or B-cell lymphoma-X large (Bcl-xL) does not affect autophagy in fibroblasts lacking Bax and Bak. (*A*–*C*) Western blot of *Bax*$^{-/-}$*Bak*$^{-/-}$ MEFs expressing a constitutive shRNA to Renilla (negative control) or autophagy related 5 (ATG5) engineered to overexpress (*A*) Bcl-2, (*B*) Mcl-1, or (*C*) Bcl-xL in a dox-inducible manner. Cells were treated with 1 μg/mL dox for 24 h prior or a 4-h culture in HBSS where indicated. (*D*) *Bax*$^{-/-}$*Bak*$^{-/-}$ MEFs were infected with a dox-inducible Bcl-2 mutant, which cannot be phosphorylated by JNK1 (T69A/S70A/S84A). Cells were treated with 1 μg/mL dox for 24 h before addition of 34 μM VP-16 or culture in HBSS for 4 h to induce autophagy. (*E*) LC3B-II turnover is unchanged by prosurvival Bcl-2 AAA overexpression. Western blot after 48 h of 1 μg/mL dox to overexpress Bcl-2 (T69A/S70A/S84A) followed by 10 μM CQ for 4 h to inhibit LC3B-II degradation.

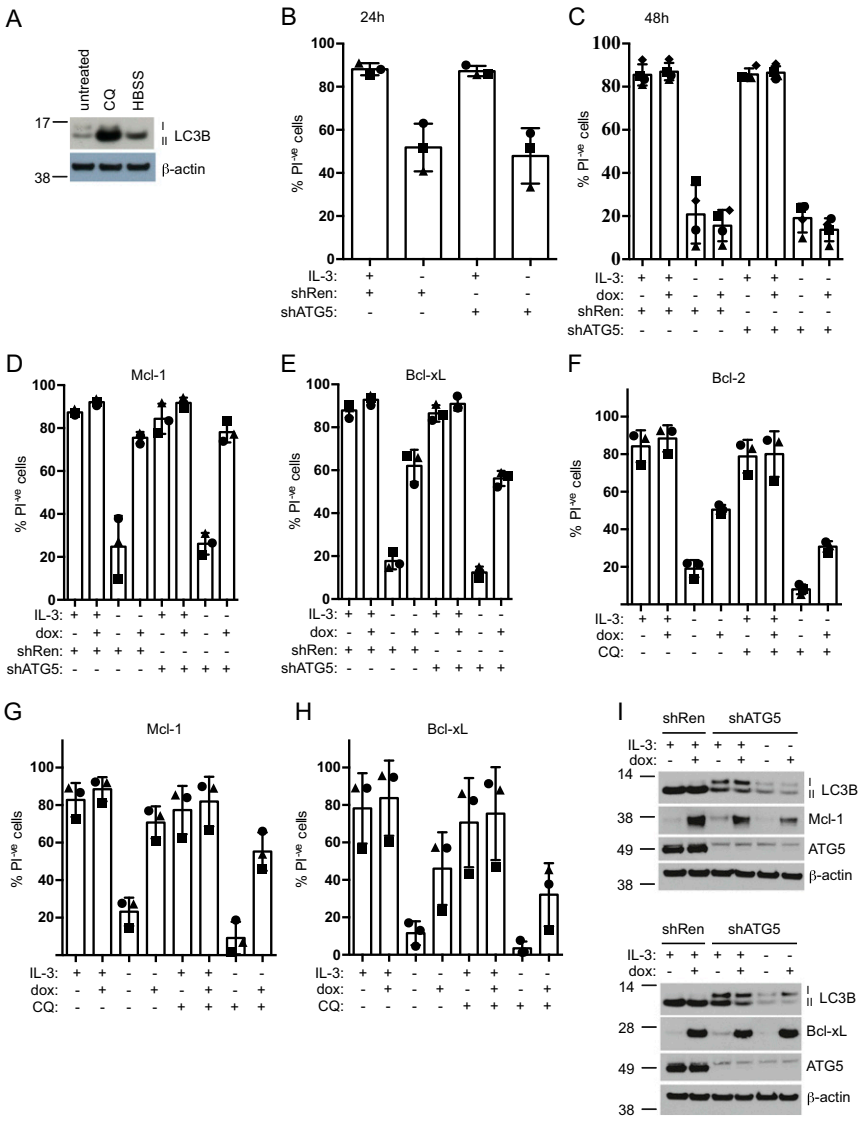

Fig. S4. (*A*) Western blot of $Bax^{-/-}Bak^{-/-}$ MEFs after treatment with 10 μM chloroquine (CQ) for 16 h or 4 h culture in HBSS were performed in parallel with Fig. 4*C*. (*B* and *C*) The viability of wild-type interleukin-3 (IL-3)–dependent (factor-dependent) myeloid (FDM) cells expressing an shRNA against ATG5 or Renilla was analyzed after (*B*) 24 h or (*C*) 48 h IL-3 withdrawal (two independent cell lines). (*D* and *E*) IL-3–dependent FDM cells were infected with dox-inducible (*D*) Mcl-1 or (*E*) Bcl-xL constructs. The viability was determined after 48 h dox (1 μg/mL) treatment and/or IL-3 withdrawal. (*F*–*H*) Viability of IL–3 dependent FDM cells after 48 h treatment with 10 μM chloroquine (CQ) and 1 μg/mL dox to induce (*F*) Bcl-2, (*G*) Mcl-1, or (*H*) Bcl-xL overexpression. (*I*) LC3B lipidation is unaffected by elevated levels of prosurvival Bcl-2 family members in IL-3–dependent FDM cells lacking Bax and Bak. IL-3–dependent $Bax^{-/-}Bak^{-/-}$ FDM cells were infected with a plasmid expressing inducible Mcl-1 or Bcl-xL, and an shRNA against ATG5 where indicated. IL-3 was removed, and 1 μg/mL dox was added 4 d before cells were lysed for Western blot analysis. All graphs shown the mean of the individual experiments and error bars represent the SD.

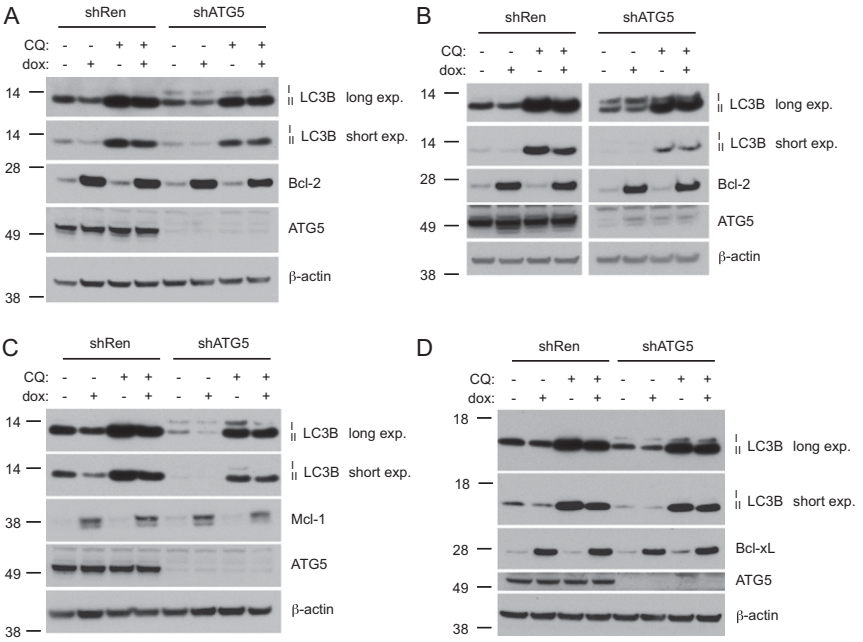

Fig. S5. (*A–D*) LC3B-II turnover is unchanged by prosurvival Bcl-2 family overexpression in *Bax*$^{-/-}$*Bak*$^{-/-}$ FDM cells. Western blot after 48 h of dox treatment to induce (*A*) wild-type Bcl-2, (*B*) Bcl-2 (T69A/S70A/S84A), (*C*) Mcl-1, or (*D*) Bcl-xL followed by 10 μM chloroquine (CQ) for 4 h to inhibit autophagolysosomal function.

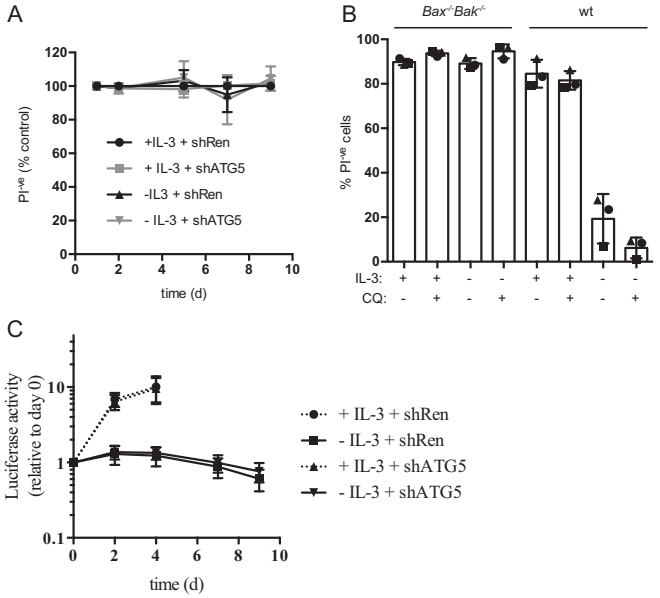

Fig. S6. (*A*) Viability of IL-3–dependent *Bax*$^{-/-}$*Bak*$^{-/-}$ FDMs expressing either shRNA against Renilla (negative control) or ATG5 was measured via PI exclusion ($n = 3$). (*B*) Wild-type and *Bax*$^{-/-}$*Bak*$^{-/-}$ IL-3 dependent FDMs were treated with 10 μM chloroquine (CQ) for 48 h before viability was measured using the vital dye PI. (*C*) Concentration of viable IL-3–dependent FDM cells lacking Bax and Bak was determined using the CellTiter-Glo luminescent cell viability assay ($n = 9$). The mean and SD is shown in all graphs.