Supporting Information

Carrington et al. 10.1073/pnas.1005256107

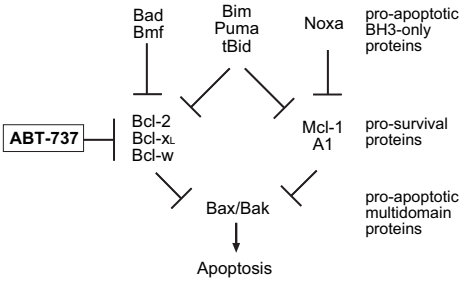

Fig. S1. Selective interactions between Bcl-2 family members, or their mimetics, regulate cytochrome-c–dependent apoptosis. Under steady-state conditions, prosurvival Bcl-2 family proteins Bcl-x_L, Bcl-2, Bcl-w, Mcl-1, and A1 restrain proapoptotic multidomain proteins Bax and Bak and prevent the induction of apoptosis. After an apoptotic stimulus, proapoptotic BH3-only proteins are up-regulated and engage prosurvival proteins, allowing release of Bax and Bak and induction of apoptosis. Under physiological conditions, BH3-only proteins Bim, Puma, and tBid are potent death inducers as they can engage all known prosurvival proteins, whereas the remaining BH3-only proteins engage only select prosurvival proteins. For example, Bad and Bmf only interact with Bcl-2, Bcl-x_L, and Bcl-w. Noxa can only engage A1 and Mcl-1. The BH3-only mimetic ABT-737 engages only Bcl-2, Bcl-x_L, and Bcl-w and thus displays a similar specificity profile to Bad or Bmf.

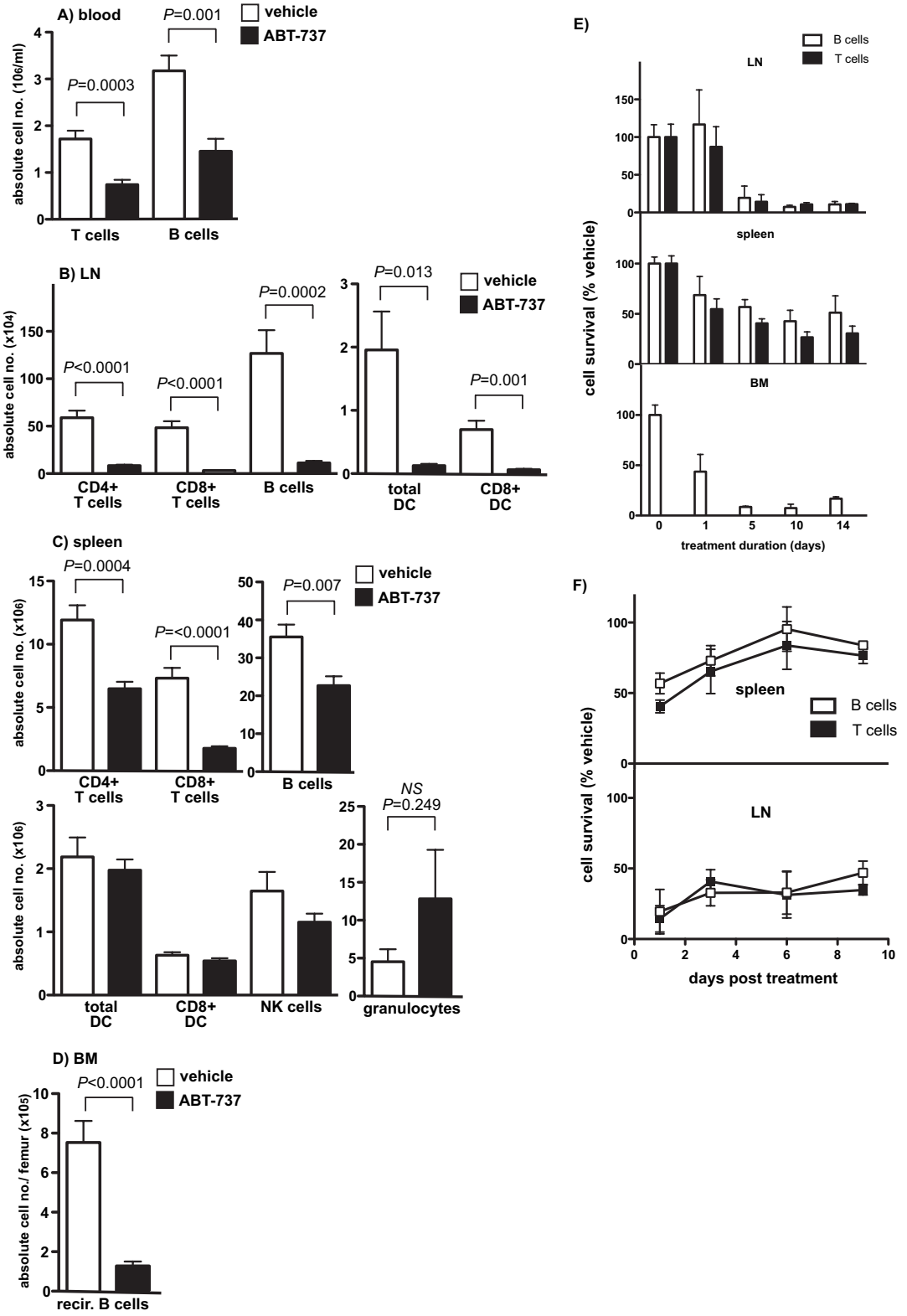

Fig. S2. In vivo ABT-737 treatment leads to a selective reduction in peripheral immune cell subsets. B6 mice were treated for 14 consecutive d with either ABT-737 (75 mg/kg) or vehicle control. After treatment, blood (*A*) lymph node (*B*), spleen (*C*), and bone marrow (*D*) were recovered, and an absolute number of T cells ($CD4^+$, CD8+), B cells, DC, NK cells, and granulocytes were determined by flow cytometry. All data are expressed as mean ± SE from individual blood (blood T and B cells, $n = 8–9$; lymph node T and B cells, $n = 9–11$; spleen T and B cells $n = 8–11$; spleen DC, $n = 6$; spleen NK, $n = 3$; spleen granulocytes, $n = 5$). (*E*) B6 mice were treated with ABT-737 (75 mg/kg) or vehicle control for 1, 5, 10, or 14 consecutive d ($n = 6$). (*F*) B6 mice were treated with ABT-737 (75 mg/kg) or vehicle control for 5 consecutive d ($n = 3$). After treatment (*E*), or in the days after cessation of treatment (*F*), lymph node, spleen, and bone marrow were recovered, and the proportion of T cells ($CD4^+$, CD8+), B cells, and DC in organs were enumerated by using flow cytometry. All data are shown as a proportion (%) of the average number of cell subset isolated from vehicle-treated mice, with mean ± SE from individual drug-treated mice.

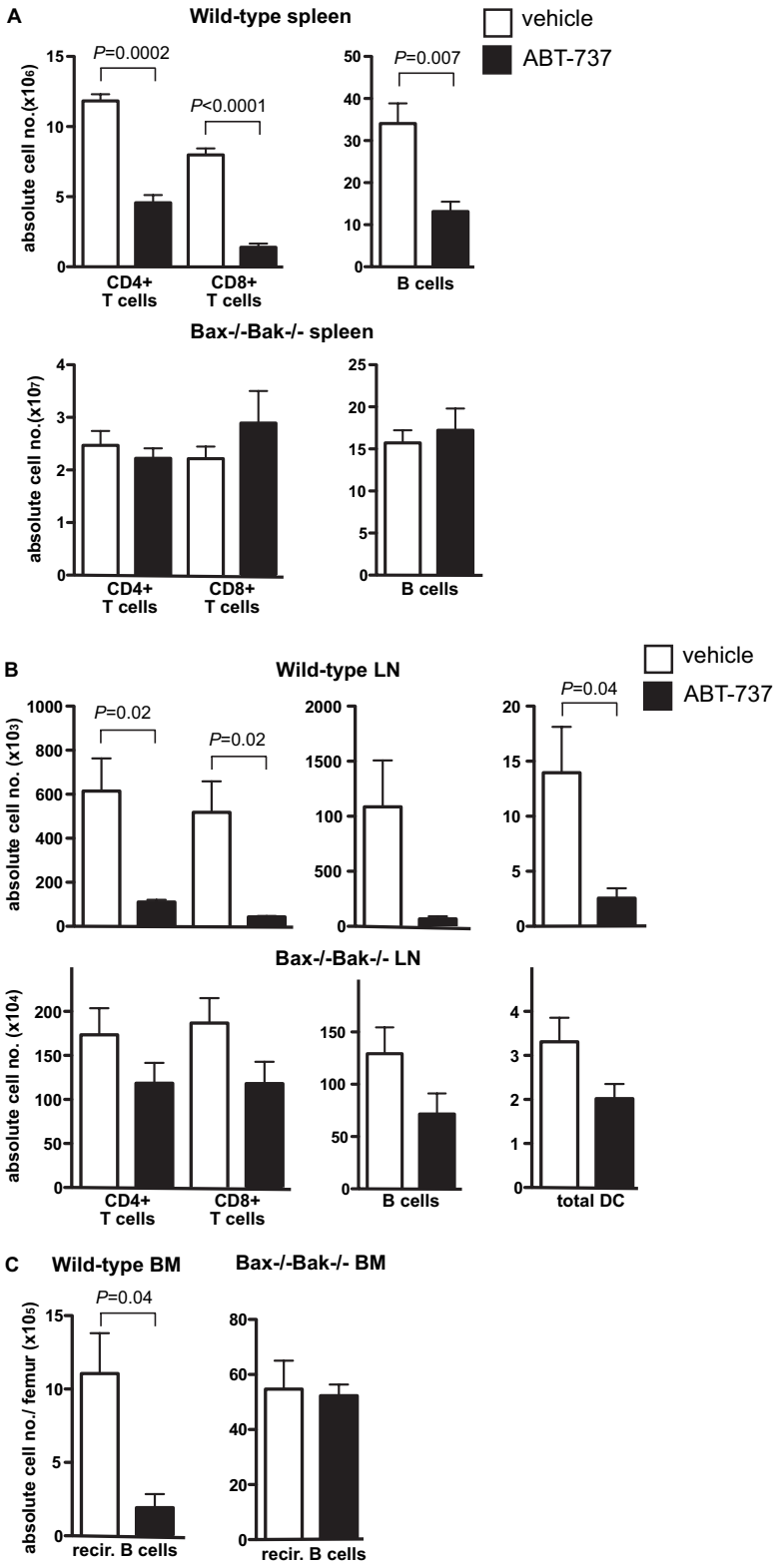

Fig. S3. Loss of proapoptotic death mediators Bax and Bak protect against sensitivity to in vivo ABT-737 treatment. (*A*) $Bax^{-/-}Bak^{-/-}$ reconstituted congenic B6 (CD45.1) (n = 5–6) or wild-type mice (n = 3–4) were treated daily for 14 consecutive d with either ABT-737 (75 mg/kg) or vehicle control. After treatment, lymph node and spleen (*A*), lymph nodes (*B*), and bone marrow (*C*) were recovered. Absolute number of T cells ($CD4^+$,$CD8^+$), B cells, and DC were determined by flow cytometry. All data are expressed as the mean ± SE from individual mice where applicable.

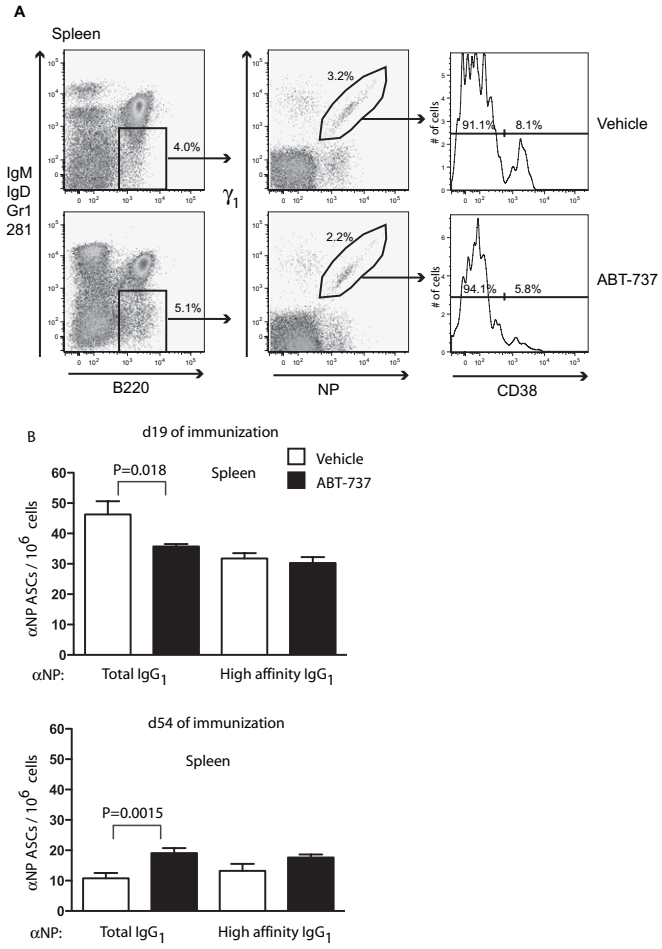

Fig. S4. In vivo B cell responses to exogenous antigen are inhibited by ABT-737 treatment. B6 mice were immunized with NP-KLH in alum and then treated with ABT-737 (75 mg/kg) or vehicle control for 14 consecutive d starting either day 5 or 40 after immunization. (*A*) Spleens were analyzed for NP-specific germinal center (CD38lo) or memory (CD38hi) B cells on day 19 after immunization revealing a diminished representation of NP-specific memory B cells. (*B*) Frequencies of total (NP20) and high affinity (NP2) NP-specific IgG_1 ASCs in spleen. Data are the mean ± SE of between six and eight mice for each group at each time point.