**SUPPLEMENTAL MATERIAL**

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**Figure S1.** Hematopoietic analysis of Bak/Bax BM chimeric mice. (a) Western blot of platelet protein lysates from FLC-reconstituted mice 8 wk after transplant. Tubulin was included as loading control. (b) Platelet counts in lethally irradiated mice reconstituted with FLCs lacking Bak and Bax, 8 wk after transplantation. Data represent mean ± SD. n = 24–60 mice per group. (c) Platelet survival curves in lethally irradiated mice reconstituted with FLCs lacking Bak and Bax, 8 wk after transplantation. Platelets were labeled via intravenous injection of NHS-biotin. Data represent mean ± SD. n = 7 mice per group. (d) Platelet survival curves. FLC-reconstituted mice were injected with NHS-biotin 8 wk after transplant, and then labeled platelets were purified and transfused into wild-type recipients. Platelet clearance was measured by flow cytometric analysis at the indicated time points. Data represent mean ± SEM. n = 6–7 mice per group. ***, P < 0.0001. (e) Peripheral blood lymphocyte numbers in FLC-reconstituted mice 8 wk after transplant. Data represent mean ± SEM. n = 24–60 mice per genotype. ***, P < 0.0001. (f) Spleen weight in FLC-reconstituted mice 8 wk after transplant. Data represent mean ± SEM. n = 5 male mice per genotype. ***, P < 0.0001. (g) Morphologically recognizable MGKs in H&E-stained sections of BM. Each symbol represents the mean number per field of view (200x) from 6 fields per individual mouse. Data represent overall mean ± SD. (h) Megakaryocyte ploidy distribution profiles in FLC-reconstituted mice 8 wk after transplant. Bak+/− Bax−/− mice were not FLC-reconstituted. Data represent mean ± SD. n = 3–8 mice per genotype. ***, P = 0.0009. (i) Serum thrombopoietin levels in FLC-reconstituted mice 8 wk after transplant. Each symbol represents an individual mouse. Data represent mean ± SEM. *, P < 0.05. (j) Hematopoietic progenitor cell numbers in FLC-reconstituted mice 8 wk after transplant. 25,000 BMCs were cultured with stem cell factor, IL-3, and erythropoietin in semisolid agar for 7 d. MGK, megakaryocyte. Non-MGK colonies represent the total of blast, granulocyte, mixed granulocyte/macrophage, macrophage, and eosinophil colonies. Data represent mean ± SD. n = 3 Bak+/− Bax+/− and 3 Bak−/− Bax−/−; n = 2 Bak+/− Bax+/+. *, P = 0.0138; **, P = 0.0055; ***, P = 0.0005.
Figure S2.  Conditional deletion of Bcl-x in the megakaryocyte lineage. (a) Peripheral blood smears from adult mice stained with May Grunwald Giemsa. Platelets are indicated by arrowheads. Representative images from five independent experiments. Bars, 20 μm. (b) Cell surface profile of platelets in PRP. Data represent 3–4 mice per genotype. (c) Platelet survival in vivo assessed by flow cytometric analysis of PRP. Platelets were labeled via intravenous injection of a DyLight 488-conjugated anti-GP Ibα (CD42c) antibody. Blood samples were collected 0.25, 1, 3, 5 h, and after that once daily. Data represent mean ± SEM, n = 4 Bcl-x+/+, 8 Bcl-x+/−/+Ptf4, 3 Bcl-x+/−/−, 2 Bcl-x−/+, Bcl-x−/+Ptf4. P < 0.0001 at 5, 24 and 48 h when comparing Bcl-x−/+Ptf4 to Bcl-x−/+. P = 0.004 at 48 h when comparing Bcl-x−/+Ptf4 to Bcl-x−/+. (d) Platelet survival in vivo assessed by flow cytometric analysis of whole blood. Platelets were labeled in vivo as above and blood collected 0.5, 1, 3, 5, 8 h, and after that once daily. Data represent mean ± SEM. n = 4 animals per genotype. (e) H&E-stained sections of BM from adult mice. MGKs are indicated by arrowheads. Scale bar: 50 μm. (f) Western blot of protein lysates from fetal liver-derived MGKs. FLCs were cultured in serum free media in TPO for 5 d, then large MGKs were purified from a BSA gradient. Actin was used as control for protein loading. (g) Detection of the recombined Bcl-x locus in MGKs. FLCs were cultured in serum free media in TPO for 1, 2, or 3 d. At each stage, CD41+ cells were sorted by flow cytometry, DNA extracted and subjected to PCR. (h) Reticulated platelet fraction in adult conditional knockout mice. Thiazole orange positive platelets were measured by flow cytometry. Each symbol represents an individual mouse. Data represent mean ± SEM. ***, P < 0.0001. (i) Expression of intrinsic pro and anti-apoptotic proteins in MGKs. Shown are Western blots of protein lysates from fetal liver-derived MGKs derived as in (g). Negative controls were Bak−/−, Bax−/− MGKs, and Bcl-x−/− or Mcl-1−/− mouse embryonic fibroblasts (MEFs). Actin was used as a protein loading control.
Figure S3. Transmission electron microscope images of BM megakaryocytes lacking Bcl-xL. (a) Bcl-xLfl/fl BM megakaryocyte. Bar, 10 μm. (i and ii) High magnification of two areas showing the megakaryocyte ultrastructure demarcation membrane system (DMS). Bars, 1 μm. (b) Bcl-xLfl/fl BM sinus containing platelets. Bar, 5 μm. (i and ii) High magnification of two areas showing platelet ultrastructure. Bars, 1 μm. Red blood cell (R). (c) Bcl-xPf4fl/H9004/Pf4fl/H9004 BM sinus containing megakaryocytic fragments and an actively shedding megakaryocyte. Bar, 10 μm. (i and ii) High magnification of two areas showing the megakaryocytic ultrastructure. Bars, 1 μm. (d) Bcl-xPf4fl/H9004/Pf4fl/H9004 BM sinus containing two megakaryocytic fragments. Bar, 10 μm. (i and ii) High magnification of two areas from the same fragment showing the megakaryocytic ultrastructure. Bars, 1 μm. Nucleus (N).
Figure S4. Deletion of Bak and Bax protects BM derived megakaryocytes from death signals. Viability of Bak- and Bax-deficient MGKs in response to (a) ABT-737, (b) etoposide and (c) STS. Mature BM-derived MGKs were purified from a BSA gradient and cultured in serum-free media with TPO and either ABT-737 (5 μM), Etoposide (50 μM), STS (5 μM), vehicle controls DMSO (0.05% ABT-737, 0.5% STS), or ethanol (EtOH) 0.27%. Viability was measured 24 h after reseeding using the CellTiter-Glo assay system. DMSO controls were set as 100%. Data represent mean ± SEM. (a) n = 3–6 independent experiments except for Bak−/− Bax+/−, n = 1. (b) n = 2–4 independent experiments except for Bak−/− Bax+/−, n = 1. (c) n = 3–4 independent experiments except for Bak−/− Bax+/−, n = 1.

Video 1. Proplatelet formation by Bcl-xfl/fl control megakaryocytes.

Video 2. Proplatelet formation by Bcl-xFlΔFvFlΔ megakaryocytes.
Video 3. Proplatelet formation by Bcl-x<sup>+/+</sup> control megakaryocytes. PS exposure visualized by Annexin V-Alexa Fluor 488 in green.

Video 4. Proplatelet formation by Bcl-x<sup>Pf4/H9004/Pf4/H9004</sup> megakaryocytes. PS exposure visualized by Annexin V-Alexa Fluor 488 in green.