Supplemental information:

- Supplemental Experimental Procedures
- Supplemental References

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasmids

The doxycycline (dox)–inducible system was modified from the Tet-On 3G system (Clontech). Briefly, the doxycycline-dependent transactivator Tet-On 3G was subcloned from pRetroX-Tet3G (Clontech) into the retroviral vector pMXs-IRES-Blasticidin (Cell Biolabs, Inc). The resistance cassette of pRetroX-TRE3G (Clontech) was switched from puromycin to zeocine to generate the retroviral vector pRetroX-TRE3G-Zeo. To generate the Venus^{2A} inducible system, the Venus fluorescent protein was cloned in frame with the T2A sequence (GAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAGGAG AATCCTGGCCCA) (Szymczak-Workman et al., 2012) and full-length BAX, BAK, or full-length and truncated/modified BOK into pRetroX-TRE3G-Zeo. The dox-inducible Venus-BOK protein fusion expression system was generated by cloning the Venus fusion protein directly in the N-terminal of BOK into pRetroX-TRE3G-Zeo. The following truncated/modified versions of BOK were used: BOK$^{FL\ or\ WT}$ (full-length murine BOK), BOK$^{6K\text{-}R}$ (K25R, K32R, K123R, K160R, K177R, K203R), BOK$^{\Delta C\ or\ Cyto}$ (amino acids (aa) 1-184), BOK^{Mito} (aa 1-184 of BOK fused to the 26 C-terminal residues of Bcl-xL: ERFNRWFLTGMTVAGVVLLGSLFSRK), BOK^{ER} (aa 1-183 of BOK fused to the 35 C-terminal residues of cytochrome b5: ITTVESNSSWWTNWVIPAISALVVALMYRL YMAED). For tandem affinity purification and immunoprecipitation experiments, a N-terminal SF-TAP tag (composed of a FLAG-M2 tag and a Twin-Strep-tag® (Gloeckner et al., 2009)) was cloned in frame with BOK^{FL} and BOK$^{\Delta C}$ into pRetroX-TRE3G-Zeo. FLAG-tagged BCL-2, BCL-xL, and MCL-1 were cloned into pMX-IRES-Cerulean

(pMICII), and BOK^{WT} and $BOK^{6K\text{-}R}$ were cloned into pMX-IRES-GFP (pMIGII).

All transient expression constructs were cloned into pcDNA3.1. BCL-2 family members were cloned with an N-terminal FLAG-M2-tag, including BIM-S, BAD, NOXA, BCL-2, BCL-w, BCL-xL, MCL-1, BAX, BAK, and BOK.

For live cell imaging, Venus or Cerulean fluorescent proteins were cloned in N-terminals of BOK^{FL}, BOK^{Mito}, BOK^{ER}, and $BOK^{\Delta C/Cyto}$ in pcDNA3.1. To generate Cerulean- CT^{BOK} and Venus-CT^{BOK}, Cerulean and Venus were cloned in frame with the 29 C-terminal residues of BOK (aa 185-213). Cerulean fluorescent protein was cloned in the C-terminal of gp78 in pcDNA3.1. ER-RFP (pCMV6-AC-RFP-Calreticulin) and pDsRed1-mito were purchased from OriGene and Clontech, respectively. The pBabe Omi-mCherry vector has been described previously (Tait et al., 2010).

For bacterial expressions, BCL-$2^{\Delta C}$ (aa 1-217), BCL-$xL^{\Delta C}$ (aa 1-211), MCL-$1^{\Delta C}$ (aa 1-328), and bacterial codon–optimized $BOK^{\Delta C}$ (aa 1-183) were cloned with a C-terminal 8-histidine motif (8xHIS) into pRL574. pGEX4T1-n/cBID, pTYB1-BAX have been described previously (Kuwana et al., 2002; Suzuki et al., 2000).

Protein expression and purification

BCL-$2^{\Delta C}$, BCL-$xL^{\Delta C}$, MCL-$1^{\Delta C}$, $BAK^{\Delta C}$, and $BOK^{\Delta C}$ were expressed as C-terminal 8xHis-tagged fusion proteins, using the pRL574 plasmid in BL21* *E. coli* (Invitrogen). Cells were resuspended in 50 mM NaH_2PO_4 pH 8.0, 300 mM NaCl plus cOmplete

protease inhibitor cocktail (Roche) and lysed by sonication. After centrifugation, cell lysates were affinity purified on a cOmplete His-Tag Purification Resin (Ni^{2+}-NTA; Roche) and proteins were eluted with 250 mM imidazole. Proteins were further purified by fast protein liquid chromatography (FPLC) on an S200 gel filtration column (GE Healthcare). Proteins were exchanged in 20 mM HEPES, 2 mM DTT, concentrated on Amicon® Ultra-15, 10 kDa Centrifugal Filter Units (Millipore), flash frozen in liquid nitrogen, and stored at –80°C. Thrombin-cleaved recombinant BID (n/cBID) was purified as described previously (Kuwana et al., 2002). Recombinant full-length BAX was purified as an intein/chitin-binding domain (CBD) fusion as described previously (Suzuki et al., 2000). Recombinant BAK-ΔC was purified as described previously (Moldoveanu et al., 2006).

Biotinylation of MCL-1 for surface plasma resonance (SPR)

MCL-1 (N-terminal his6-tagged Δ1-151–MCL-1–ΔTM) was minimally biotinylated by reaction with EZ-Link Sulfo-NHS-LC-LC-Biotin (Thermo Scientific). The biotin reagent was added to the protein at a 0.5:1 molar ratio, and the reaction was incubated on ice overnight. Unconjugated biotin was removed by processing the samples through 2 Zeba Spin Desalting Columns (Thermo Scientific) that had been equilibrated with storage buffer (20 mM Tris pH 7.5, 150 mM NaCl, 10 mM dithiothreitol, 20% glycerol). Bovine serum albumin was added to the reaction mixture at a final concentration of 0.1 mg/mL immediately before processing through the spin columns to improve recovery (Papalia and Myszka, 2010). The biotinylated protein dispensed into aliquots, flash-frozen, and stored at –80°C for use in subsequent binding experiments.

Kinetic/equilibrium affinity analysis of compound binding by SPR

SPR experiments were conducted at 25°C, using a Biacore T200 optical biosensor (GE Healthcare). Neutravidin (Thermo Scientific) was covalently immobilized on a carboxymethyl dextran hydrogel–coated gold surface (CM4 chip; GE Healthcare), using routine amine coupling chemistry in immobilization buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 0.005% Tween 20). Carboxyl groups on the hydrogel were activated with N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide, and neutravidin was injected in 10 mM sodium acetate pH 5.0 until immobilization levels of ~4800-5500 RU were achieved. The remaining active sites were blocked by reaction with ethanolamine. The instrument was primed with the binding buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1 mM TCEP, 0.01% Triton X-100, 5% glycerol, 3% DMSO), and biotinylated MCL-1 was injected until ~580 RU of protein was captured.

BH3 peptides were prepared in the binding buffer as a 3-fold dilution series starting at 2 μM for BID-BH3 (Ac-EDIIRNIARHLAQVGDSMDRSI-NH2) and at 20 μM for BOK-BH3 (Ac-PVPGRLAEVCAVLLRLGDELEMIRPSVY-NH2) and were injected in triplicate at a flow rate of 75 μL/min. A series of buffer-only (blank) injections was included throughout the experiment to account for instrumental noise. Data were processed, double-referenced, solvent corrected, and analyzed (Myszka, 1999) using the software package Scrubber2 (version 2.0c, BioLogic Software). The equilibrium dissociation constants (K_D) were determined by fitting the data to a 1:1 (Langmuir) interaction model. For the BOK-BH3 peptide, the K_D was determined by equilibrium affinity analysis as the concentration that gave 50% occupancy of the chip (i.e,. response

= ½ R_{max}). For the BID-BH3 peptide, the K_D was determined by kinetic analysis as the quotient of the kinetic rate constants, k_d/k_a.

Cross-linking and sodium carbonate extraction

For crosslinking experiments, MEF cells were digitonin-permeabilized with 50 μg/mL digitonin (Sigma-Aldrich) in PBS for 10 min on ice. Cells were washed twice in PBS incubated with 250 μM of bismaleimidohexane (Life Technologies) for 30 min at room temperature. The reaction was stopped with a quenching solution of DTT at a final concentration of 50 mM for 5 min at room temperature. Cross-linked cells were then permeabilized in CHAPS lysis buffer (50mM Tris-Cl pH 7.4, 150 mM NaCl, cOmplete® protease inhibitors cocktail (Roche), and 2% CHAPS (Sigma-Aldrich)) and analyzed by SDS-PAGE and Western blot.

Alkali extraction of LUV proteins was performed by resuspending the LUV reaction mixture in 0.1 M Na_2CO_3 (pH 11.5) and incubated for 20 min on ice. Membranes were then pelleted by centrifugation (100,000 *g* for 30 min at 4°C) and analyzed by SDS-PAGE and Western blot.

Western blotting

Cells were lysed in cell lysis buffer (50mM Tris-Cl pH 7.4, 150 mM NaCl, cOmplete® protease inhibitors cocktail (Roche), and 0.5% Nonidet P-40). To detect phosphorylation or ubiquitylation, the cell lysis buffer was supplemented with phosSTOP® phosphatase inhibitor cocktail (Roche) or 10 mM iodoacetamide (Sigma-Aldrich) and 10 mM N-

ethylmaleimide (Sigma-Aldrich), respectively. Protein concentration in cell lysates was measured by the BCA assay (Pierce) and systematically normalized before Western blotting.

The following antibodies were used for Western blotting: BAK (G-23; sc832; Santa Cruz), BAX (N-20; sc-493; Santa Cruz), FLAG-M2 (F1804; Sigma-Aldrich), GFP (7.1 and 13.1; 11814460001; Roche), ubiquitin (P4D1; Santa Cruz), gp78 (AMFR; 16675-1-AP; ProteinTech), Erlin-1 (17311-1-AP; ProteinTech), Erlin-2 (SPFH2; ab129207; Abcam), UBAC2 (ab103857; Abcam), UBXD8 (FAF2; SAB4200475, Sigma-Aldrich), UBAC2 (SAB2103837; Sigma-Aldrich), VCP (p97; 2648S; Cell Signaling), PERK (C33E10; 3192; Cell Signaling), Phospho-Thr980-PERK (16F8; 3179; Cell Signaling), human BOK (ab186745; Abcam), or (LS-C98850; LS Bio). Murine BOK was detected using a mouse monoclonal antibody (see below).

Lysates and immunoprecipitates were resolved by SDS-PAGE. The proteins were transferred to supported Hybond C nitrocellulose (Amersham Bioscience) and immunodetected by using appropriate primary and peroxidase-coupled secondary antibodies (Amersham Bioscience). Proteins were visualized by enhanced chemiluminescence (ECL, Amersham Bioscience).

Size exclusion chromatography

Size exclusion chromatography was performed by FPLC on a Superdex 200 10/300 GL column (GE Healthcare) in 1% CHAPS, 150 mM NaCl, 20 mM HEPES pH 7.5, 5 mM

DTT, and 1% glycerol (Dai et al., 2011). For calibration, molecular markers (Pierce) were run through the same column.

Immunoprecipitation

Immunoprecipitations were performed as previously described (Llambi et al., 2011). Briefly, cells were lysed in cell lysis buffer (see above), and clarified lysates were immunoprecipitated with anti-FLAG®-M2 affinity gel (A2220, Sigma-Aldrich) or a combination of primary antibody and Protein A/G PLUS-Agarose (sc-2003, Santa Cruz) incubated for at least 2 h at 4°C, and complexes were washed 5 times in cell lysis buffer. Immunoprecipitates were eluted from the anti-FLAG®-M2 affinity gel by incubating with the FLAG® Peptide (100 µg/mL; F3290, Sigma-Aldrich).

Tandem affinity purification

Twin-Strep-tag®-FLAG® tandem affinity purification was performed as previously described (Gloeckner et al., 2009). Briefly, cells were lysed in cell lysis buffer (see above), and clarified lysates were precipitated with Strep-Tactin® Superflow® (2-1206-010; IBA Lifesciences), washed in cell lysis buffer, and eluted with 2.5 mM D-desthiobiotin (2-1000-002; IBA-Lifesciences). FLAG immunoprecipitation was performed as described above. The final FLAG eluate was concentrated using Amicon® Ultra-4, 3 kDa Centrifugal Filter Units (Millipore).

LC-MS/MS analysis

SF-TAP-BOK and SF-TAP-BOK$^{\Delta C}$ were purified by tandem affinity purification, as described above, and briefly subjected to SDS-PAGE. After Coomassie-blue staining, the total protein fraction was excised and subjected to dithiothreitol reduction, iodoacetamide alkylation, and in-gel digestion (12.5 ng/μL trypsin overnight). Resulting tryptic peptides were concentrated and analyzed by C18 capillary reversed-phase liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), using an LTQ Orbitrap Elite (Thermo Fisher Scientific) mass spectrometer operating in the optimized high-resolution mode (Pagala et al., 2015; Xu et al., 2009). The acquired MS/MS data were searched against the Uniprot Mouse database, using the SEQUEST algorithm (v28 revision 13) (Eng et al., 1994) in our in-house data-processing pipeline (Wang et al., 2014) by using a target/decoy search strategy (Elias and Gygi, 2007). Sites modified by ubiquitylation were identified by using the PEAKS software (PEAKS7, build 20140321, Bioinformatics Solutions, Inc.) (Ma et al., 2003), by dynamically assigning a mass addition of +114.0429 Da for modified lysine residues. All matched MS/MS spectra were filtered by mass accuracy and matching scores to reduce the protein false discovery rate to less than 1%. Peptide and modified residue assignments were further validated by independent de novo sequencing of raw spectra and confirmed on the basis of the unambiguous assignment of characteristic site-specific fragment ions.

Generation of a mouse monoclonal anti-BOK antibody

Bok$^{-/-}$ (C57BL/6) mice 6–8 weeks of age (see below) were immunized (200 μL/mouse i.p.) with 50 μg of the KLH-conjugated peptide (AEIMDAFDRSPTDKEL) dissolved in PBS per animal in an emulsion (1:1 v/v) with complete Freund's adjuvant (F5881; Sigma)

for the initial injection and with incomplete Freund's adjuvant (F5506; Sigma) for subsequent boosts. Mice were bled 2 weeks after each boost, and sera antibody titers were tested by ELISA against the unconjugated peptides. The animal having the highest titers for the peptide was selected for fusion. Four weeks after the last boost, a final boost with 50 μg/200 μL of the peptide in PBS was split between i.v. and i.p injections. Four days later, the mouse was euthanized and a splenectomy was performed. A single-cell suspension of splenocytes was mixed with an equal number of X63-Ag-8.653 mouse myeloma cells and fused by using an electrofusion system (Cyto Pulse Sciences, Inc). Fused cells were selected using HAT media (StemCell Technologies). Single-cell cloning was performed, and clone 1-04.1 was selected for further analyses. Spent media from the hybridoma was filtered by using a 0.2-μm filter and passed over a 5-mL HiTrap Protein G HP column (17-0405-01; GE Healthcare Bio-Sciences Corp) to purify the antibody as per manufacturer's instructions.

Cell culture

All adherent cell lines were maintained at 37°C/5% v/v CO_2 in a humidified incubator in DMEM (GIBCO) supplemented with 10% FBS, 2 mM L-glutamine (GIBCO), and 100 U/mL penicillin-–streptomycin (GIBCO). MEF culture medium was supplemented with 1mM sodium pyruvate (GIBCO), nonessential amino acids (GIBCO), and 55 μM β-mercaptoethanol (GIBCO). Cell lines U266 and H929 were maintained in RPMI Medium (GIBCO) supplemented as above. To induce amino acid starvation, cells were incubated in HBSS (GIBCO) alone. Cancer cell lines T98G, A172, ES-2, U118, H1299, HT29, CaoV-3, PC3, PA-1, SK-OV-3 SW-626, M059, U266, and H929 were obtained

from ATCC. WT and *bax*$^{-/-}$*bak*$^{-/-}$ HCT116 cell lines were a gift from Richard Youle (Wang and Youle, 2011). MEFs were either immortalized with the SV40 large T antigen (pBabe-Neo-SV40-LTA) or transformed with H-Ras^{G12V} and 12S E1A (pWZL-Hygro-12S-E1A and pBabe-Puro-H-Ras^{G12V}). Clonogenic survival was assessed after methylene blue staining.

Transient transfection and retroviral transduction

DNA and siRNA transient transfection was performed over 48 h using Lipofectamine™ 2000 and Lipofectamine™ RNAiMAX transfection reagents, respectively, as per the manufacturers' instructions (Invitrogen). All siRNA oligos used were ON-TARGETplus siRNA pools of 4 oligos purchased from Dharmacon. U266 cells were transfected by electroporation, using the Amaxa® Cell Line Nucleofector® Kit C and a Nucleofector® following Lonza's optimized protocol for U266 cells. Transfection efficiency was assessed by flow cytometry 48 h after electroporation of the pmaxGFP® vector.

All stable cell lines were generated by retroviral transduction. Briefly, Phoenix amphotropic virus producer cells were transfected with the appropriate plasmid by using Lipofectamine™ 2000 for 48 h. Target cells were infected with filtered virus containing the culture medium from packaging cells supplemented with 5 μg/mL polybrene. Stable transductants were selected after adding 200 μg/mL Zeocin™ (Invitrogen), 20 μg/mL blasticidin (Invitrogen), 200 μg/mL Geneticin® (GIBCO), 40 μg/mL hygromycin B (Invitrogen), or 0.2 μg/mL puromycin (Sigma-Aldrich) or were sorted by flow cytometry for Cerulean-, Venus-, GFP-, or mCherry-positive cells.

Apoptosis induction and chemical inhibitors

Unless indicated otherwise, all cell lines stably expressing a Tet-On3G–based system were preincubated with 300 ng/mL of doxycycline (Clontech) for 16 h before apoptotic stimulation. The following chemicals were used: MG132 (10 μM; Sigma-Aldrich), carfilzomib (CZ; 1 μM; Selleckchem), brotezomib (BZ; 1μM; Selleckchem), eeyarestatin I (ESI; 1 μM; Santa Cruz), NMS-873 (10 μM; Selleckchem), 4μ8C (IRE1αI; 50 μM; Selleckchem), GSK2656157 (PERKi; 500 nM; Millipore), PF 429242 (S1Pi; 10 μM; Sigma-Aldrich), salubrinal (Sal; 100 μM; Sigma-Aldrich), thapsigargin (TG; 1 μM; Sigma-Aldrich), tunicamycin (TN; 1 μg/mL; Sigma-Aldrich), actinomycin D (ActD; 1 μM; Sigma-Aldrich), staurosporine (STS; 1 μM; Sigma-Aldrich), etoposide (Etop; 100 μM; Sigma-Aldrich), human-TNFα (50 ng/mL; PeproTech), cycloheximide (CHX; 1 μg/mL for MEFs and 2 μg/mL for HCT116 cells; Sigma-Aldrich), Q-VD-OPh (40 μM; MP Biomedicals), chlorothalonil (2 μM; Sigma-Aldrich), quinacrine dihydrochloride (20 μM; Sigma-Aldrich), anthothecol (11-acetoxycedrelone; MicroSource Discovery Systems, Inc.), obtusaquinone (MicroSource Discovery Systems, Inc.), and pomiferin (MicroSource Discovery Systems, Inc.). Amino acid deprivation (-A.A) was done by incubating cells in HBSS medium (GIBCO). UV irradiation (10 mJ/cm^2) was done using a Stratagene UV cross-linker. ABT-737 (30 μM in HCT116) was obtained from AbbVie.

qRT-PCR

mRNA for qRT-PCR were extracted using the RNeasy Mini Kit (Qiagen). Reverse-transcription reactions were preformed with M-MLV reverse transcriptase (Invitrogen)

following the manufacturer's protocol and using random hexamers. Real-time PCR was performed with SYBR™ Green and a 7500 Fast Real-Time PCR System (Applied Biosystems).

Intracellular staining

For intracellular staining, HCT116 cells were fixed and permeabilized by using the Cytofix/Cytoperm™ Kit (BD Bioscience) and stained with anti-BOK antibodies (ab186745, Abcam or LS-C98850, LS Bio) and a secondary goat anti-rabbit antibody conjugated to FITC.

Live confocal microscopy

Cells were plated on 4-well glass chamber slides (Mattek) coated with fibronectin (100 μg/mL in PBS). To mitigate phototoxicity and pH changes, cells were imaged in a medium supplemented with 55 μM 2-mercaptoethanol and 20 mM HEPES. Cells were maintained at 37°C and 5% CO_2 in an environmental control chamber (Solent Scientific, UK). Confocal microscopy was performed using a Marianas spinning disk confocal imaging system (Intelligent Imaging Innovations/3i) consisting of a CSU-22 confocal head (Yokogowa Electric Corporation, Japan); solid-state diode-pumped laser launch (3i) with wavelengths of 445 nm, 473 nm, 523 nm, 561 nm, and 658 nm; and a Carl Zeiss Axiovert 200M motorized inverted microscope equipped with a precision motorized XY stage (Carl Zeiss MicroImaging) and spherical aberration correction optics (3i). Images were acquired with either a Zeiss Plan-Neofluar 40× 1.3 NA DIC objective or a Zeiss Plan-Apochromat 63x 1.4 NA DIC objective and a Cascade II 512 EMCCD camera

(Photometrics), using the SlideBook 6 software (3i). Colocalization was quantitatively assessed by drawing regions around individual cells and computing the Pearson's correlation coefficient by using the SlideBook 6 software (3i). Live-microscopy movies and confocal-microscopy pictures are representative of at least 3 independent experiments.

High-Throughput Screening (HTS)

HTS was performed using an automation system (HighRes Biosolutions) with integrated Wellmate (Matrix Technologies) for reagent dispensing and Pin Tool (HighRes Biosolutions) with surface-coated pins (V&P Scientific) for compound transfer. In the primary screen, 8904 (4282 unique) compounds with known biologic activity from the bioactive collection at St. Jude Children's Research Hospital, as previously described, (Walters et al., 2014) were tested. Briefly, *bax*$^{-/-}$*bak*$^{-/-}$ MEFs stably expressing dox-inducible Venus^{2A}BOK were plated into white, solid-bottom, tissue culture–treated 384-well plates (Corning) at a density of 2500 cells per well in 25 μL of media containing 0.3 μM doxycycline. After 20 h, 30 nL of compound (10 mM in DMSO), MG-132 (positive control, Sigma), or DMSO (negative control, Fisher Scientific) was transferred into each well with a final concentration of 12 μM, 2.5 μM, and 0.12%, respectively. Assay plates were incubated for 16 h at 37°C, 5% CO_2, and 95% relative humidity. Then, the Caspase-Glo 3/7 Assay (Promega) was performed and the luminescence signal was detected with the Envision plate reader (PerkinElmer). The relative caspase 3/7 activity of a given compound was calculated as follows: % Activity = (sample – negative control) / (positive control – negative control)) × 100. In the confirmation screen, the top 59 hits (>15%

relative caspase activity) from the primary screen were tested in a 10-point 1-to-3 dilution series (final compound concentration 56 μM to 2.8 nM; final DMSO concentration 0.56%) in quadruplicate. Data processing and visualization were performed using in-house custom programs written in the Pipeline Pilot platform (Accelrys, v8) and the R program (Morfouace et al., 2014).

Generation of *Bok*$^{-/-}$ Mice

The *Bok* targeting vector was engineered by using gap-repair technology as described previously (Liu et al., 2003). A 20.3-kb fragment from the BAC clone VG10047 (Knockout Mouse Project (KOMP)), in which the *Bok* gene (exons 2 to 5; encompassing all coding sequences) was replaced by the ZEN-UB1 cassette (KOMP), was subcloned into pBR322-DTA (Pelletier et al., 2012). The ZEN-UB1 cassette contains a promoter-less *LacZ* gene encoding β-galactosidase and a selection cassette encoding the neomycin resistance gene whose expression is driven in eukaryotic cells by the promoter of the human ubiquitin C and in prokaryotic cells by the EM7 promoter. The selection cassette is flanked by 2 LoxP sites (Fig. S1A).

Gene targeting by homologous recombination was performed in EmbryoMax® mouse embryonic stem cell line CMTI1 (129/SVEV, Millipore). Positive clones were identified by Southern blot, using external 5′ and 3′ probes (Fig. S1B), and single integration events were confirmed by using a neo probe. Two ES cell clones were used to generate *Bok* knock-out mice. The mice were back-crossed on a C57BL/6N Tac background for at least 10 generations.

Mouse genotyping was performed by PCR on genomic DNA obtained from toe or tail

biopsies. By using the primers *Bok*-F2 (5'-CTGGAGGCGGTCCGGTCTGA-3'), *Bok*-R3 (5'- TGCTCCTCCAGAGCTTTCTGTGT-3'), and *Bok*-R52 (5'-ACACAGAAAGCTCTGGAGGAGCA-3') PCR fragments of 658 bp and 415 bp, corresponding to WT and null alleles, respectively, were obtained (Fig. S1C).

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