Supplementary Methods

Antibodies for Immunoblot and Immunoprecipitation. The following primary antibodies were used for immunoblot: anti-Bim (Cell Signaling, #2933); anti-Bcl-2 (for IP, Millipore, #05826; for immunoblot, Neomarker, #MS-123-P1); anti-Bcl-xl (Cell Signaling, #2764); anti-Bcl-w (Cell Signaling, #2724); anti-Mcl-1 (for IP, BD Pharmingen, #559027; for immunoblot, Cell Signaling, #4572); anti-Puma (Cell Signaling, #4976); anti-Noxa (Imgenex, #Img-349A); anti-β-tubulin (Millipore, #MAB3408); anti-Cdc27 (Santa Cruz, #SC-5618).

Transfection. siRNA against human Bcl-2, Bcl-xl, Bcl-w, Mcl-1, A1, Noxa, and the non-targeting control are ON-TARGETplus Smartpool siRNAs (Dharmacon Thermal Scientific). siRNA against human Bim was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Lipofectamine™ RNAiMAX transfection reagent was purchased from Life Technologies (Grand Island, NY).

Protein extraction, immunoblot and immunoprecipitation. For protein extraction RIPA lysis buffer was used. Interactions between BH3-only protein Bim and Bcl-2, Bcl-xl, or Mcl-1 were evaluated by co-immunoprecipitation analysis. For these studies, 3-[(3-cholamidopropyl)-diethylammonio]-1-propanesulfonate (CHAPS) buffer (150 mM NaCl, 10 mM HEPES, pH 7.4, protease inhibitors, and 2% (w/v) CHAPS) was used to lyse the cells. After centrifugation to remove insoluble fraction, supernatant was incubated with 1 μg antibody and rotated at 4°C for 2 hours, protein G sepharose beads were added for further incubation. After washing, the bead-bound protein was eluted by vortexing and boiling in Laemmli sample buffer.

Cell cycle-dependent Mcl-1 protein degradation. Cell synchronization was achieved by culture in medium containing 2 mM thymidine for 18-24 hours, release from the thymidine block with three washes in PBS, followed by culture for 8-12 hours in complete growth media. Cells then underwent a second thymidine block for 16-20 hours, three washes in PBS and release into complete medium containing 330 nM nocodazole.

Cells were washed with cold PBS and lysed in RIPA buffer (supplemented with protease inhibitors and phosphatase inhibitors) at indicated time points.

Database analyses. FBW7 mutation data were obtained from Sanger Institute COSMIC database and Broad Institute Cancer Cell Line Encyclopedia (CCLE) database (23). Criteria for selecting deleterious mutations were: common tumor associated mutation (mostly missense mutations at arginine residues that affect substrate binding), nonsense and frame shift mutations and large deletions. Transcript levels for genes of interest were obtained from the CCLE database. Briefly, raw Affymetrix CEL files were converted to a single value for each probe set using Robust Multi-array Average (RMA) normalization, intensity values are background corrected, $\log_2$ transformed and quantile normalized. DNA copy number data were obtained from CCLE database. Briefly, raw Affymetrix CEL files were converted to a single value for each probe set representing a SNP allele or a copy number probe. Copy numbers were then inferred based upon estimating probe set specific linear calibration curves, followed by normalization by the most similar HapMap normal samples. Segmentation of normalized $\log_2$ ratios (specifically, $\log_2(CN/2)$) was performed using the circular binary segmentation (CBS) algorithm. Drug sensitivity data were obtained either from Supplementary Data 1 of (31), or from internal database of MGH Center for Molecular Therapeutics. Briefly, cells were treated with nine concentrations (two-fold dilutions) of drug for 72 hours before measuring cell number relative to controls. Half-maximal inhibitory concentration (IC_{50}) was calculated subsequently for further analysis as described in (31) .