Materials and Methods:

Antibodies and Reagents

Anti-Bim antibodies were purchased from Enzo Life Sciences (rat monoclonal 3C5) or Stressgen (rabbit polyclonal). Anti-HSP70 antibody was a kind gift from Dr R Anderson (Peter MacCallum Cancer Centre, Melbourne, Australia) and anti-Bcl2 antibody (mouse monoclonal, Bcl2-100) was a kind gift from Dr. L Oreilly (Walter and Eliza Hall Institute, Melbourne, Australia). Anti-Mcl-1 antibody was purchased from Santacruz Biotechnology (SC819). Phospho-(Ser/Thr) PKA substrate antibody was purchased from Cell Signalling (#9612). ERK inhibitor U0126 was purchased from Cell Signalling. Forskolin, Akt Inhibitor VIII, H 89 and Isoproterenol were purchased from Calbiochem. Rolipram and IBMX were purchased from Sigma. AKAP binding peptide was synthesized with the following sequence: GRKKRRQRRRGGGFEELAWKIAKMIWSDVFQQ (Hundsrucker et al, Biochem. Soc. Trans. 34:472).

Cell culture, Transfection, Lentiviral Infection and RNAi

MCF7 and mouse embryonic fibroblasts (MEFs) were cultured in DMEM supplemented with 10% fetal calf serum (Invitrogen) at 37°C in a humidified 10% CO₂ incubator. Wild type and bim−/− MEFs were generated from E15 embryos in accordance with standard procedures and were infected with SV40 large T antigen expressing lentivirus. To generate lentiviral particles, 293T cells were transfected with packaging constructs pCMV δR8.2 and VSVg and the relevant lentiviral plasmid at a ratio of 1:0.4:0.6 using Fugene 6.0™ transfection reagent (Roche) following the manufacturer’s instructions. The virus containing supernatants were harvested, filtered (0.8 µM), and supplemented with Polybrene (4 µg/mL). Target cells were infected with virus supernatant as described by Vince et al (Vince et al, 2007). The RNAi construct for stable repression of Bim expression has previously been described (Bouillet et al, 2005).
Supplementary Figure Legends

Figure S1
Binding of PRKAR1A to Bim\textsubscript{EL} is not mediated through the AKAP binding domain and S83A mutation does not affect Bim\textsubscript{EL} folding.
A. EE-PRKAR1A and Bim\textsubscript{EL} were co-expressed in 293T cells. PRKAR1A was immunoprecipitated with anti-EE antibodies in the presence or in the absence of AKAP binding peptide and bound Bim\textsubscript{EL} was detected by Western blot analysis. Lower panels show protein expression in the whole cell lysates.
B. Positive control for AKAP binding peptide. MEFs were fractionated into cytosolic or mitochondrial fractions in the presence of either AKAP binding peptide (TAT-tagged, added to culture at 1 µM concentration) or the control peptide. Samples were probed for PRKAR1A and cytochrome C.
C. HEK 293T cells were transfected with the indicated constructs and 48 h later lysates were subjected to immunoprecipitation with Bim specific antibodies. Captured proteins were size-fractionated by SDS-PAGE, transferred to membranes and analyzed by Western blotting using the indicated antibodies. The lower panels show the Western blot analysis of the whole cell lysates for the expression levels of the indicated proteins.

Figure S2
Beta-adrenergic receptor signalling induced apoptosis is bim dependant.
A. MCF7 WT, clones expressing PRKAR1A (#35 and 37) or polyclonal population expressing bim RNAi were treated with Isoproterenol (10 µM), Rolipram (10 µM) and ERK inhibitor (U0126, 10 µM) and cell death was measured at indicated times. The error bars represent means +/- SEM from 3 independent experiments and the p values were determined by one tailed, type 1 Student’s t test.
B. Western blot analysis of MCF7 (WT) or PRKAR1A#35 were treated as above and cell lysates were analysed by Western blots for PARP cleavage and Bim expression.

Figure S3
Mouse embryonic fibroblasts from bim\textsuperscript{-/-} mice expressing comparable levels of WT and S83A mutant Bim\textsubscript{EL} and PKA inhibition increases Bim\textsubscript{EL} turnover.
A. MEF clones expressing either the WT or the S83A mutant form of Bim\textsubscript{EL} were induced with 4-OHT for 24 hours and total RNA was subjected to Northern blot analysis for expression levels of bim and actin (loading control).
B. MEF clones were induced with 4-OHT as above and protein lysates were analysed by Western blot using different antibodies as shown.
C. MEFs expressing the WT protein were treated with CHX+/- PKA inhibitor (Myristoylated 14-22 amide at 1 µM) and samples harvested at the indicated time points and subjected to Western Blot analysis for the indicated proteins. UT, untreated.

References:
Moujalled et al, Suppl. Fig.3

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- bim
- actin

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- BimEL
- Cleaved Casp 3
- HSP70

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- BimEL
- pCREB
- HSP70