Supplementary Figure Legends

Figure S1 Caspase 9, but not caspase 8, is required for paclitaxel killing. (a) MCF-7 cells were treated with paclitaxel (100 nM) for 0-48 h. Total cell lysates were analyzed for the activation of caspase-9 and caspase-8 by immunoblotting. Activation of caspase-9 and caspase-8 was also evaluated by fluorometric caspase activation assays (lower panel, left). Data were expressed as relative fluorescence units (RFU) (mean ± SEM, n = 4). MCF-7 cells were pretreated with 20 μM pancaspase inhibitor (Z-VAD-FMK), 20 μM caspase-9 inhibitor (Z-LEHD-FMK) and 20 μM caspase-8 inhibitor Z-IETD-FMK before treatment with paclitaxel (100 nM) for 48 h. The percent apoptotic response was evaluated by Annexin V staining (mean ± SEM, n = 4) (lower panel, right). **(b)** MCF-7 cells were transiently transfected with Bim siRNA, Bmf siRNA or Scrambled siRNA for 48 h. Untransfected and siRNA-transfected cells were treated with paclitaxel (100 nM), ABT-737 (100 nM) or paclitaxel plus ABT-737 (100 nM) for 48 h and apoptosis was evaluated by using Annexin V staining (mean ± SEM, n = 4, *p< 0.05, **p < 0.01 by two-tailed t test). **(c)** MCF-7 cells were transiently transfected with Bad siRNA or Scrambled siRNA for 48 h. The interaction of Bim with Bcl-2 following paclitaxel treatment (100 nM, 12 h) was evaluated by coimmunoprecipitation assays. The efficiency of Bad knockdown was monitored by immunoblot analysis. Input for coimmunoprecipitation was also subjected to immunoblot analysis and actin was probed as a loading control. **(d)** T47D, **(e)** MDA-MB-468 and **(f)** BT20 cells were treated with paclitaxel (100 nM) for 0-48 h. Total cell lysates were analyzed for the activation of caspase-9, caspase-8 and caspase-3 by immunoblotting. Activation of caspase-9, caspase-

8 and caspase-3 was also evaluated by fluorometric caspase activation assays (lower panels, left). Data were expressed as relative fluorescence units (RFU) (mean ± SEM, n = 4). Cells were pretreated with 20 μM pancaspase inhibitor (Z-VAD-FMK), 20 μM caspase-9 inhibitor (Z-LEHD-FMK) and 20 μM caspase-8 inhibitor Z-IETD-FMK before treatment with paclitaxel (100 nM) for 48 h. The percent apoptotic response was evaluated by Annexin V staining (mean ± SEM, n = 4) (lower panels, right).

Figure S2 Bim is localized to membranes. (a) MCF-7 cells were treated with paclitaxel (100 nM) for 0, 4, 8, and 12 h and the interaction of Bim with Bcl-xL and Mcl-1 was detected by coimmunoprecipitation assays. Immunoprecipitations were also probed for Bcl-xl and Mcl-1. **(b)** Total cell lysates from MCF-7 were sequentially immunoprecipitated with Bcl-2, Bcl-xL and Mcl-1 antibodies. Pre-immunoprecipitation and post-immunoprecipitation supernatants were subjected to immunoblot analysis with anti-Bim antibody. Actin was probed as loading control. Protein expression levels were semiquantitatively determined by densitometry using ImageJ software and expressed as a ratio of Bim/Actin (left panel). MCF-7 cells were treated with paclitaxel (100 nM) for 12 h and total cell lysates from MCF-7 were sequentially immunoprecipitated with Bcl-2, Bcl-xL and Mcl-1 antibodies. Pre-immunoprecipitation and post-immunoprecipitation supernatants were subjected to immunoblot analysis with anti-Bim antibody. Actin was probed as loading control. Protein expression levels were semiquantitatively determined by densitometry using ImageJ software and expressed as a ratio of Bim/Actin (middle panel). MCF-7 cells were treated with paclitaxel (100 nM) for 12 h. Heavy membrane (HM), light membrane (LM) and S-100 fractions were isolated and Bim was detected by immunoblot analysis. CoxIV was probed as a loading control for HM fractions and

BiP/Grp78 was probed as a loading control for LM fractions (right panel). **(c)** Only Bmf increases its interaction with Bcl-2 after paclitaxel treatment. MCF-7 cells were stably transfected with either pCI-Neo.FlagBcl-2 (MCF-7 Bcl-2). We immunoprecipitated Bcl-2 using anti-FLAG mAb-conjugated agarose (M2-agarose; Sigma) and probed for Bim, Bmf, Bax, Bak, Puma and Bad after treatment with paclitaxel for 12 h by using immunoblot analysis. **(d)** MCF-7 cells were treated with paclitaxel (100 nM) for 12 h and the interaction of Bax or Bak with Bcl-2, Bcl-xL and Mcl-1 was detected by coimmunoprecipitation assays.

Figure S3 Paclitaxel consistently causes cytochrome c release from mitochondria. (a) T47D, **(b)** MDA-MB-468 and **(c)** BT20 cells were treated with paclitaxel (100 nM) for 0-48 h. Cytosolic and mitochondrial fractions were immunoblotted for cytochrome *c*. CoxIV was probed as a loading control for mitochondrial fractions.

Figure S4 Paclitaxel consistently causes Bax and Bak activation. Activation of Bax and Bak in **(a)** T47D, **(b)** MDA-MB-468 and **(c)** BT20 cells treated with paclitaxel (100 nM) for 0-48 h was analyzed by immunoprecipitation with active conformation-specific anti Bax (6A7) and anti-Bak (Ab-2) antibodies followed by immunoblot analysis of Bax and Bak. Inputs for immunoprecipitations were also detected immunoblot analysis. Actin was probed as a loading control.

Figure S5 Puma is not required for paclitaxel-induced cell death in other breast cancer cells. (a) MDA-MB-468, **(b)** BT20 and **(c)** MCF-7 cells were transfected with

Puma siRNA or Scrambled siRNA for 48 h. The efficiency of knockdown was monitored by immunoblots. Untransfected and siRNA-transfected cells were treated with paclitaxel (100 nM) for 48 h and apoptosis was evaluated by using Annexin V staining.