Supporting Online Material for

BIM expression in treatment naïve cancers predicts responsiveness to kinase inhibitors

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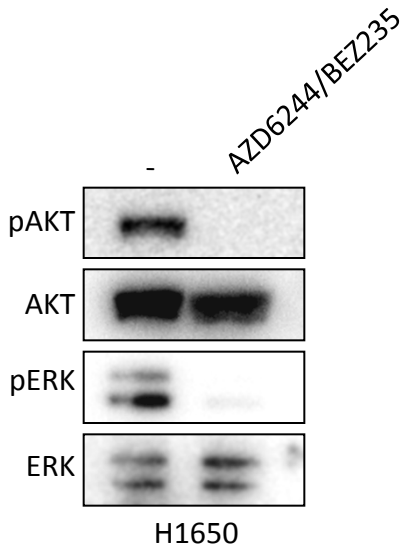

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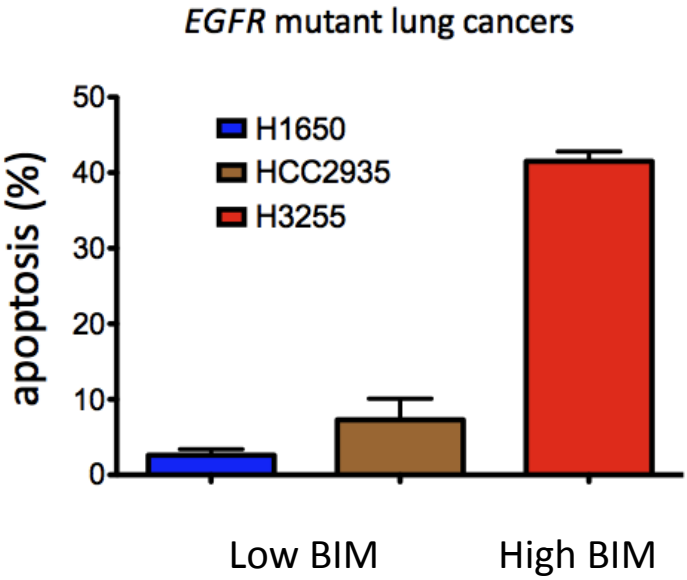

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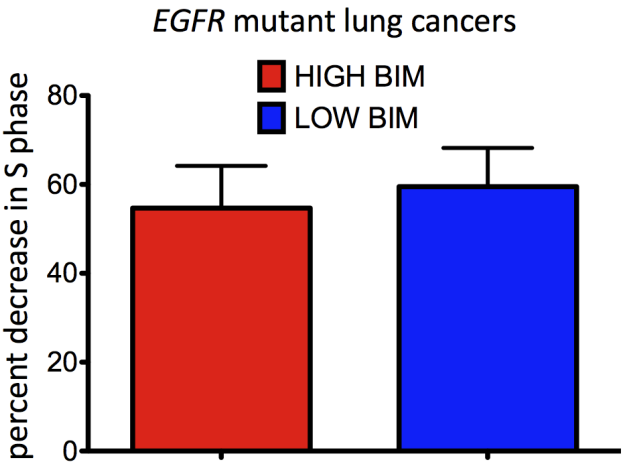

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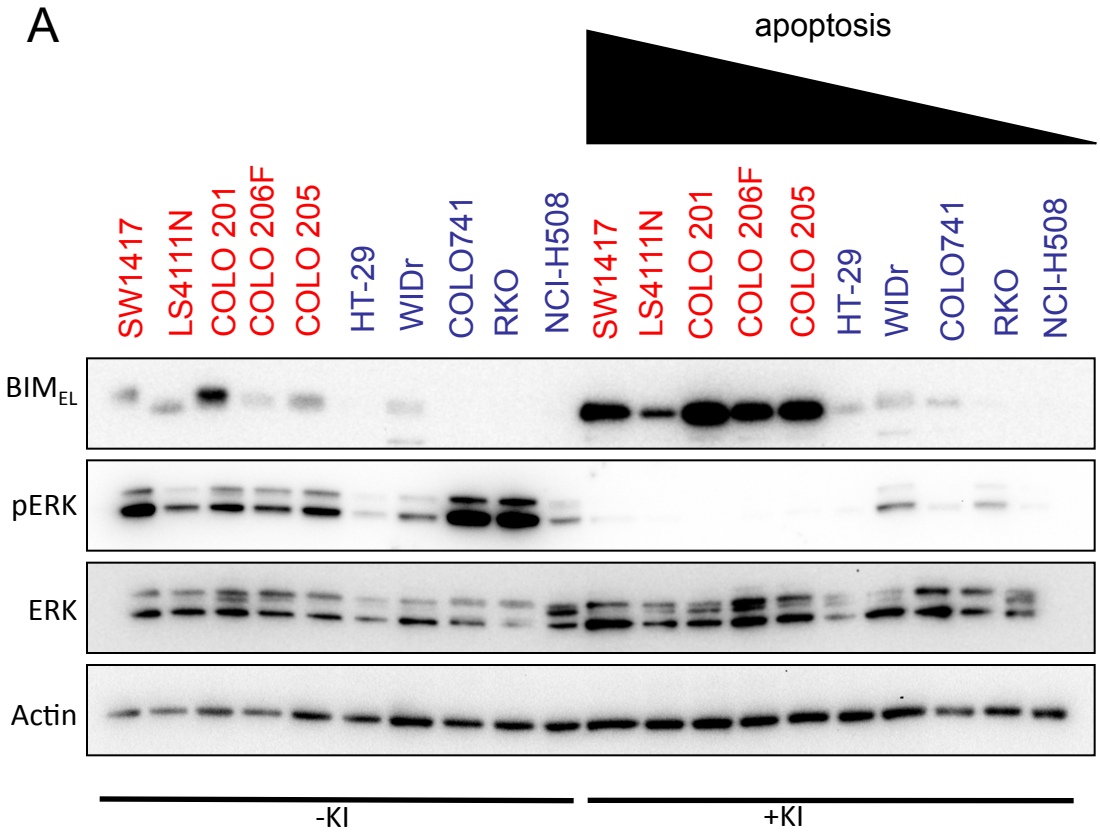

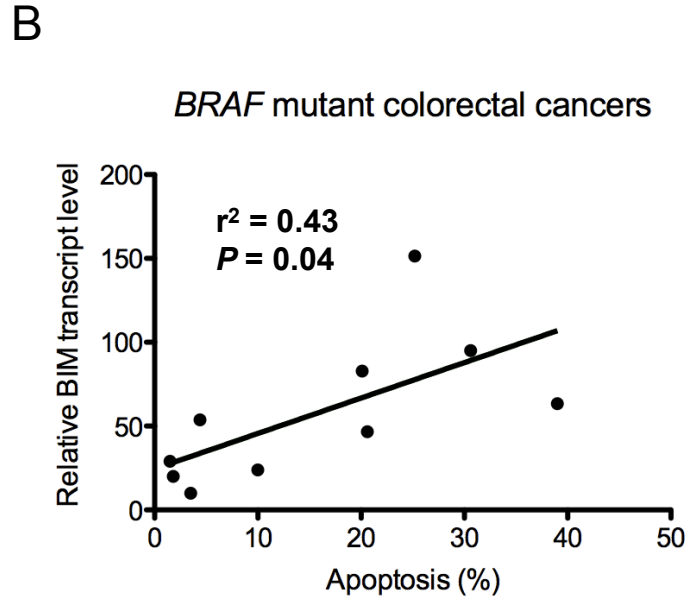

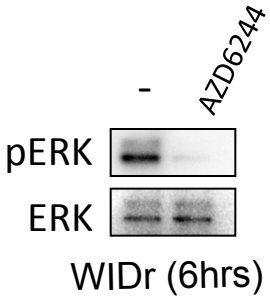

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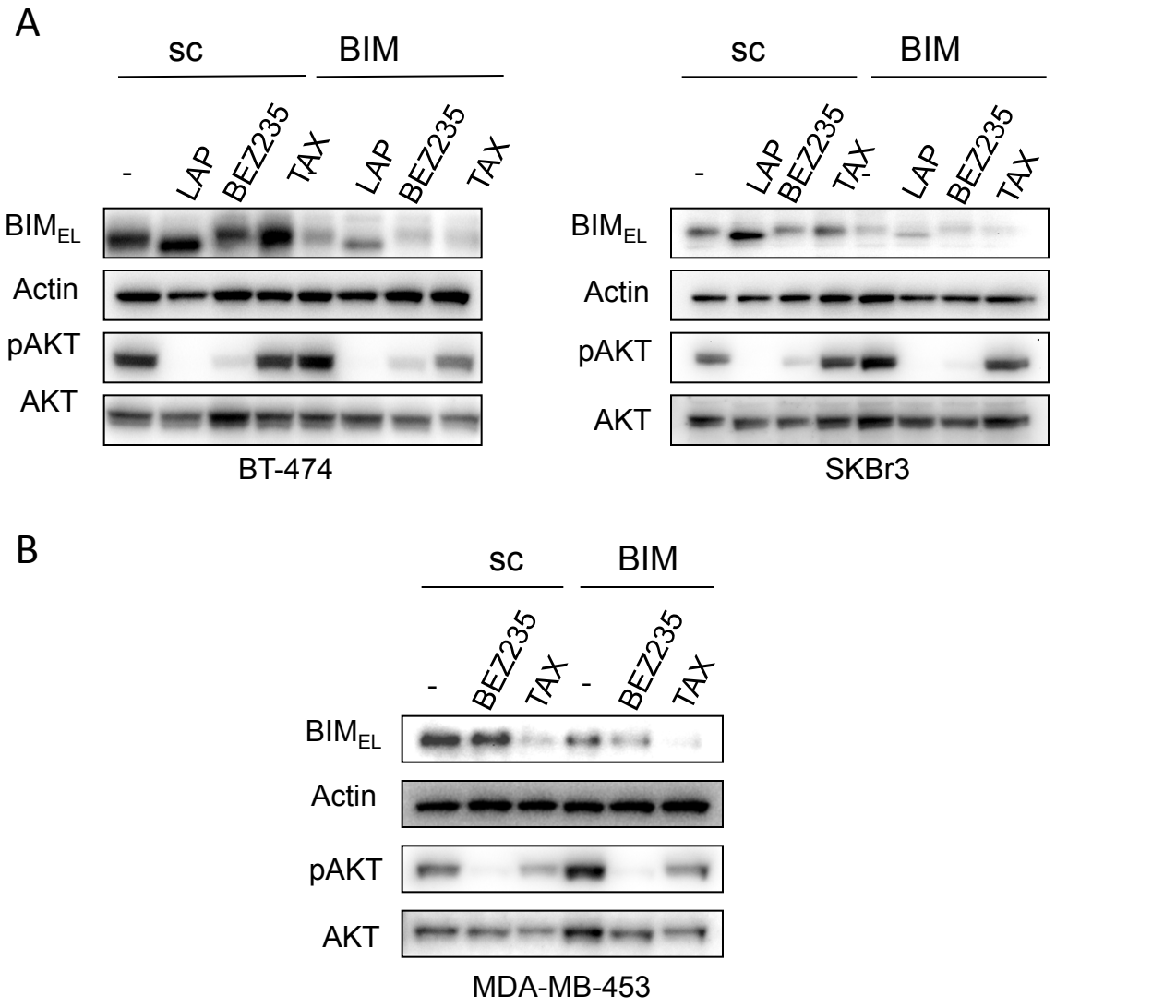

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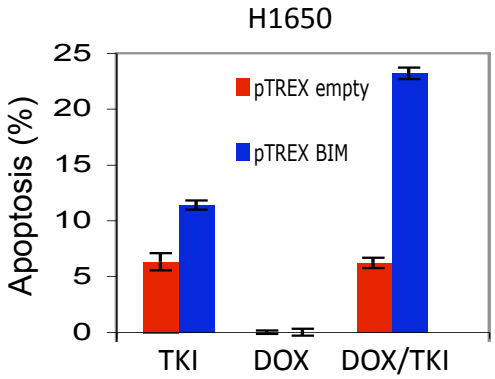

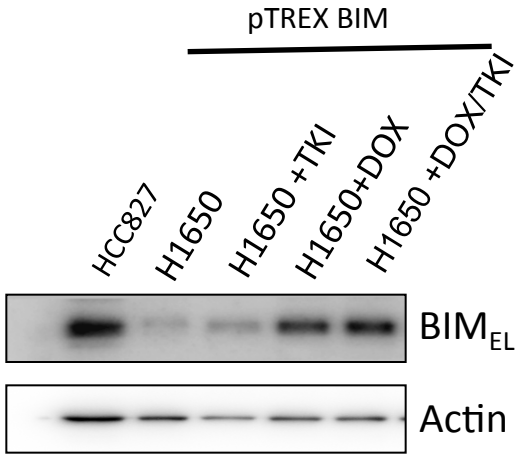

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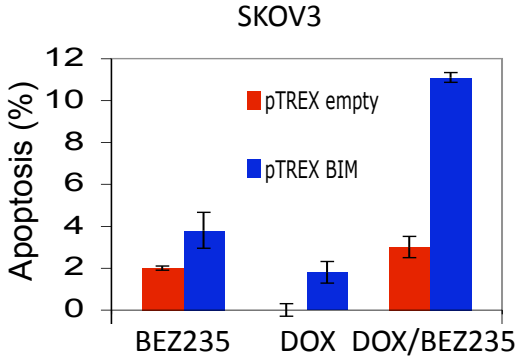

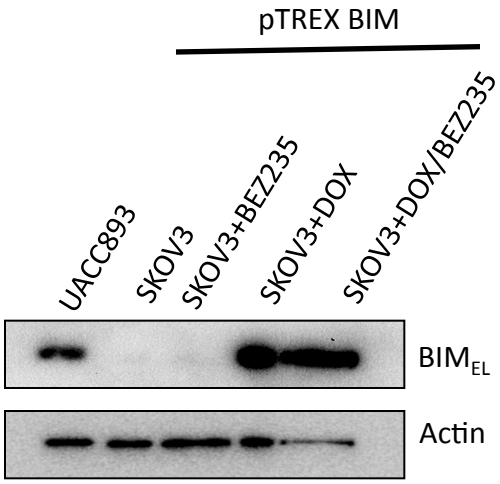

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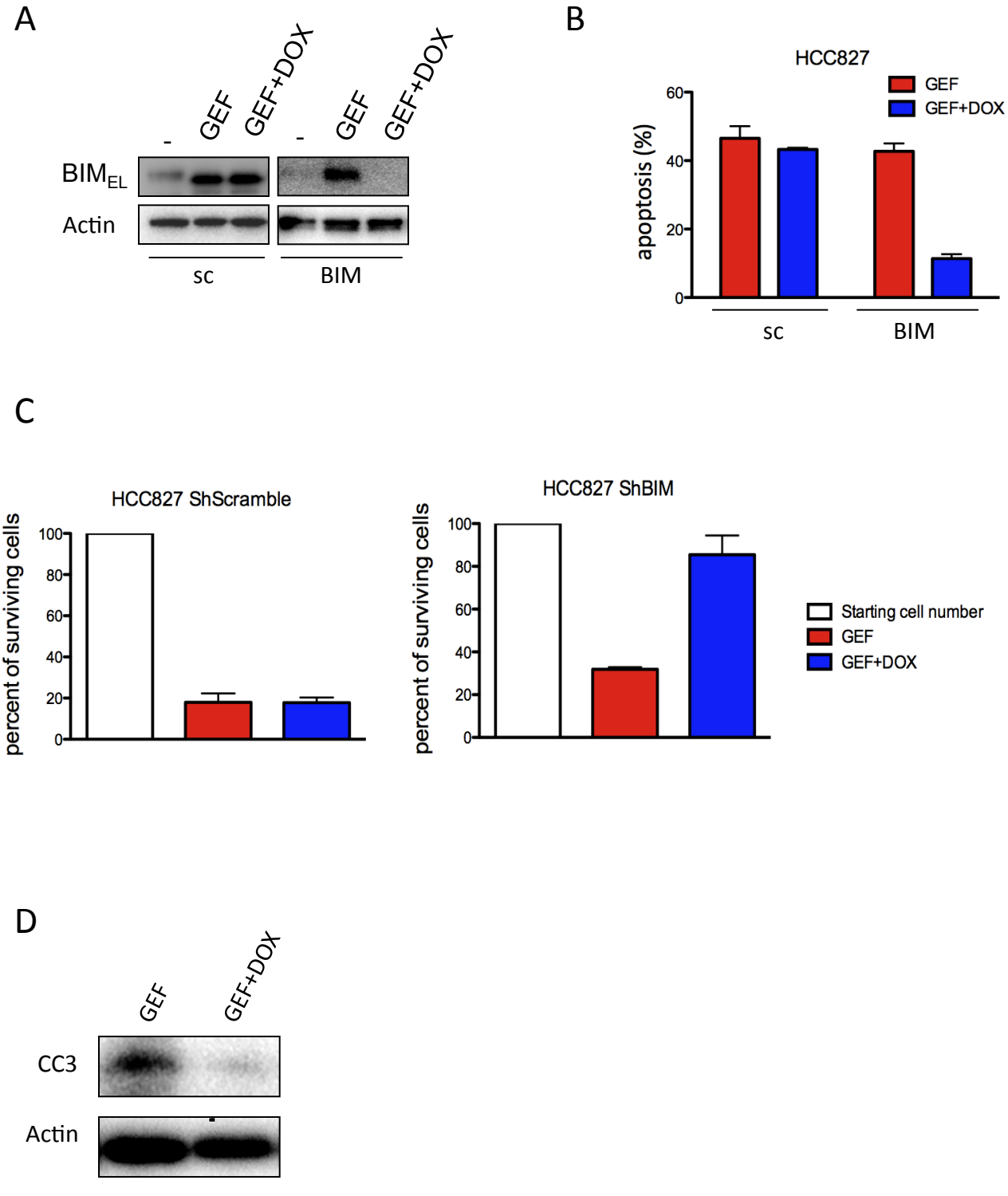

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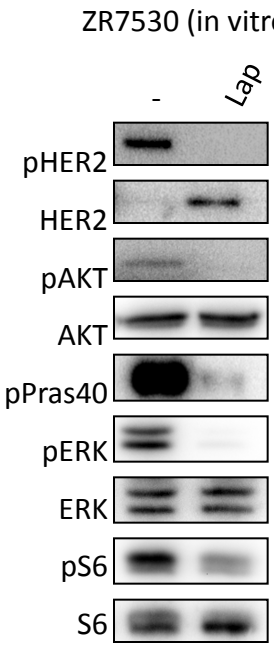

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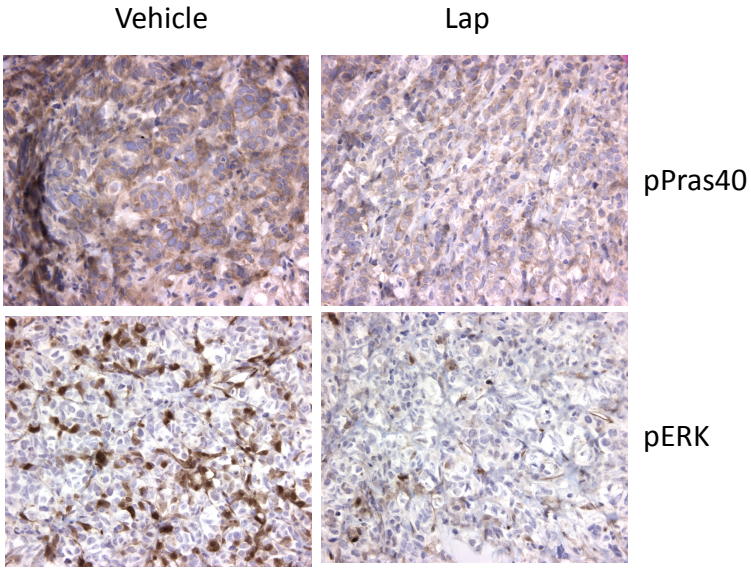

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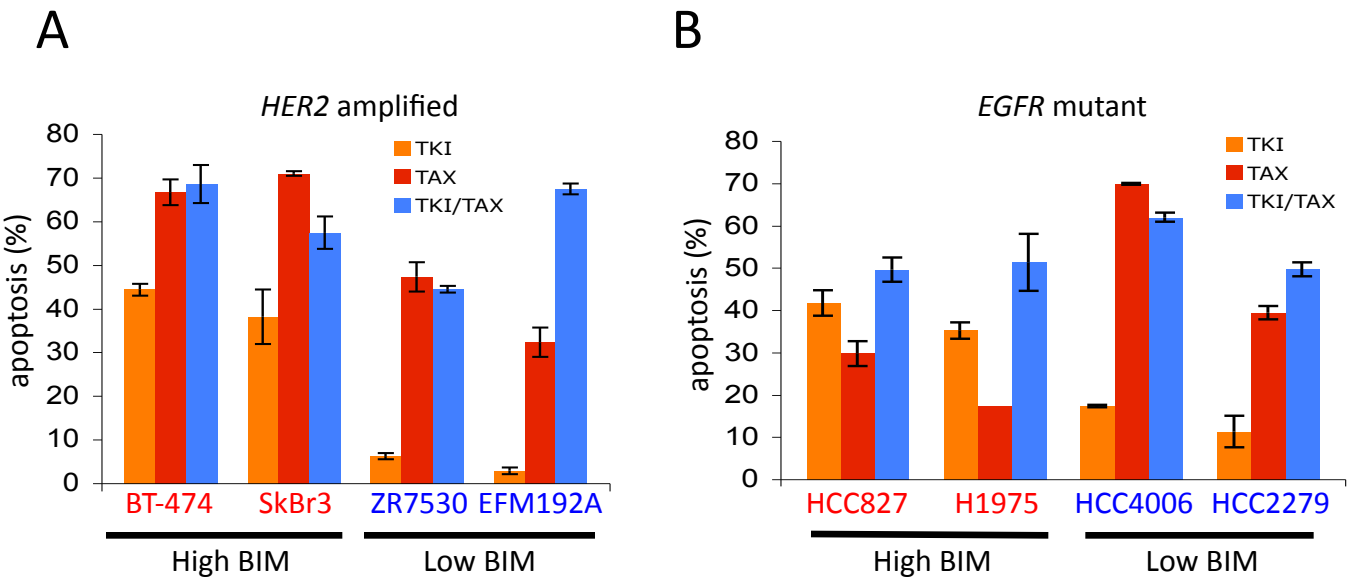
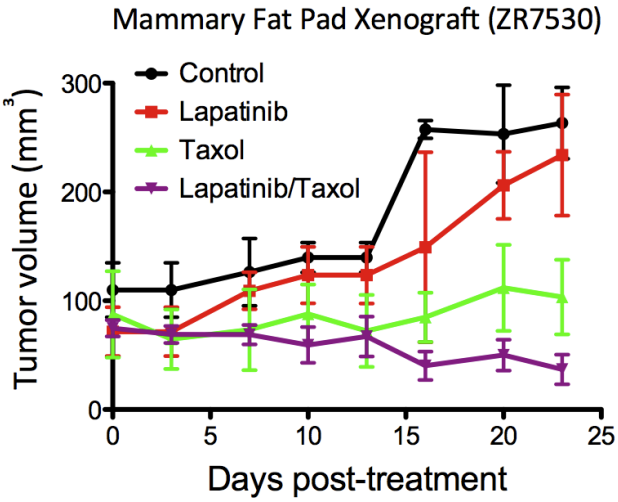
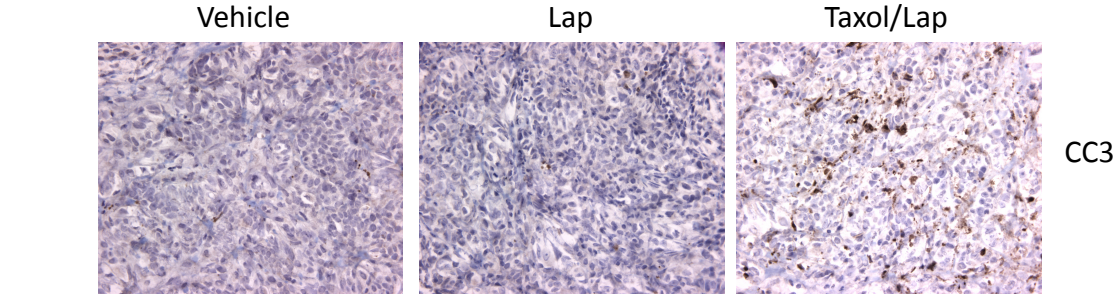






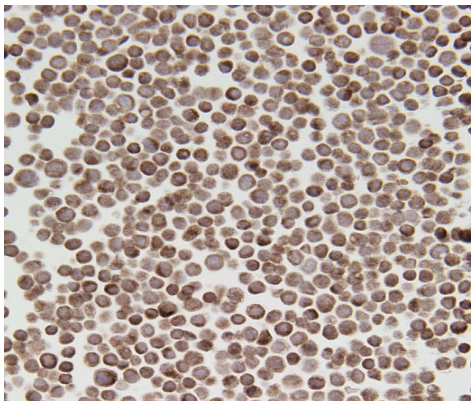
Sup. Figure 7, Faber et al.

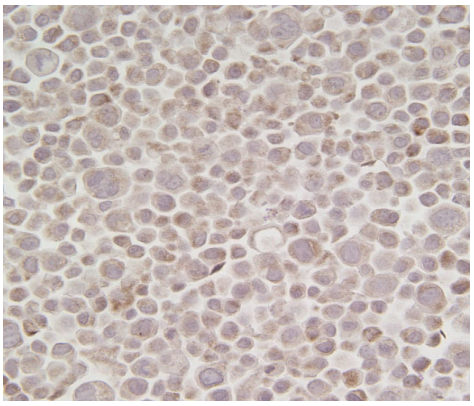
High BIM

Low BIM

EGFR mutant cancers

PC9

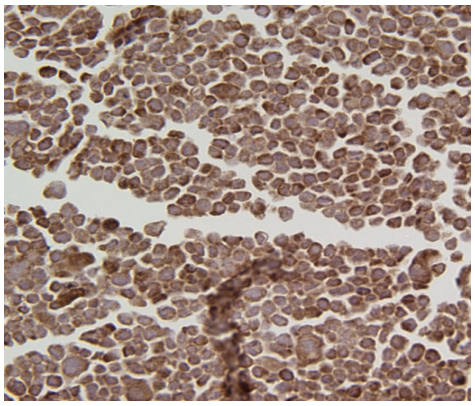


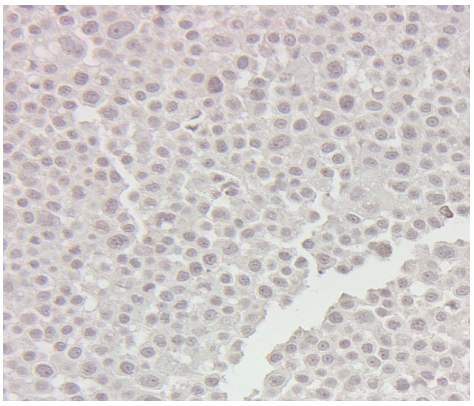


HCC4006

HER2 amplified cancers

BT-474





H1819

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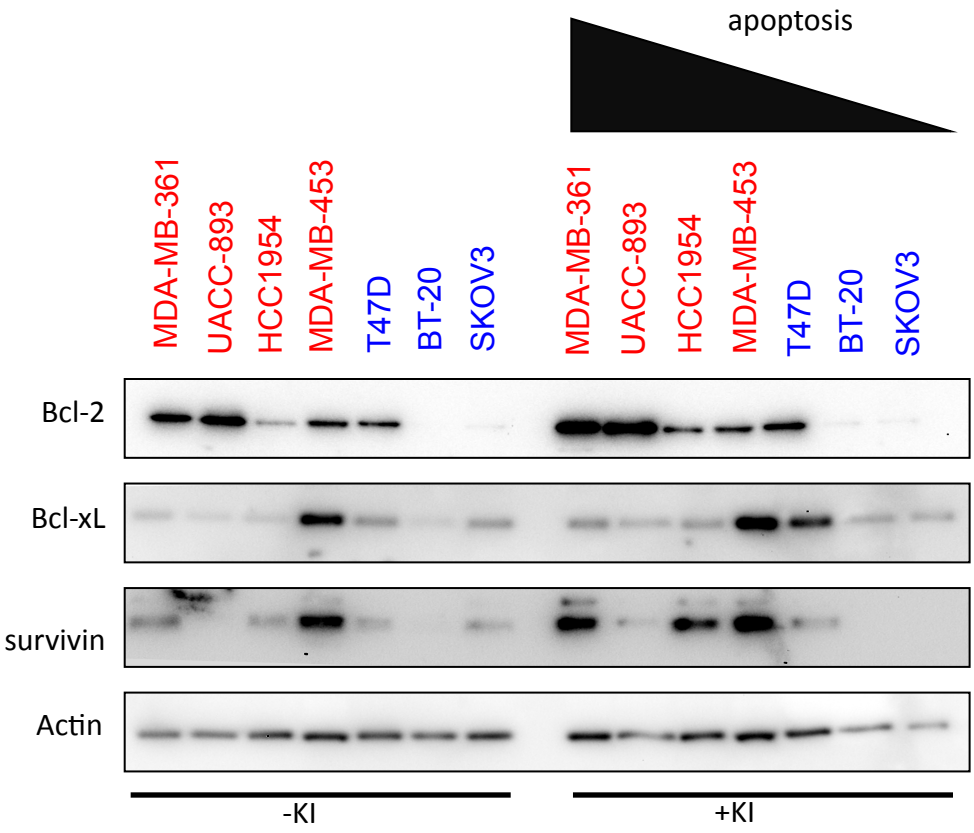

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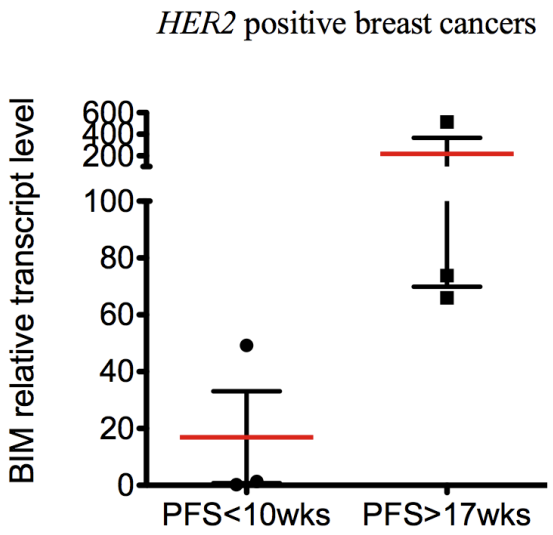
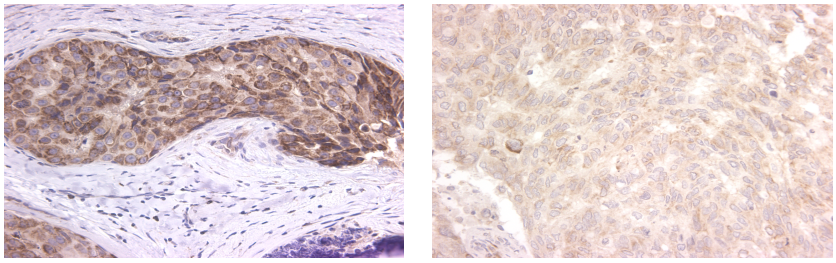


B

Patient #	Relative BIM RNA levels (to β-Actin)	Progression-Free Survival (PFS) in weeks
1	0.2	8
2	1.3	9
3	49.2	9
4	66.0	18
5	514.3	40
6	73.8	75

C



75 weeks 8 weeks

Sup. Figure 10, Faber et al.

Sup. Table 1. Characteristics of the cell lines involved in this study.

EGFR mutant lung cancers	Apoptosis	Relative BIM mRNA levels	Mutation
PC9	66.0+/-1.0	112.3+/-20.3	Exon 19 deletion
H1975	64.1+/-2.5	58.5+/-10.8	L858R/T790M
HCC827	58.2+/-1.7	85.1+/-27.3	Exon 19 deletion
H3255	48.8+/-3.0	77.6+/-18.6	L858R
HCC2935	23.5+/-1.0	15.2+/-2.9	Exon 19 deletion
HCC4006	19.2+/-1.6	10.3+/-0.9	Exon 19 deletion
H1650	8.9+/-0.7	19.2+/-1.7	Exon 19 deletion
HCC2279	6.7+/-0.6	8.0+/-0.9	Exon 19 deletion
HER2 amplified cancers			Copy Number
BT474	65.3+/-10.6	95.7+/-14.3	17 (2)[#]
OE-19	56.8+/-3.6	64.7+/-5.1	8.2 (3)
SkBr3	54.8+/-2.6	80.8+/1.8	12.4 (5)
AU565	53.8+/-4.3	75.6+/-7.1	13.4 (2)
NCI-N87	30.1+/-1.8	58.7+/-5.0	8.6 (3)
HCC1419	17.1+/-3.5	18.6+/-1.3	49.9 (2)
HCC2170	16.7+/-0.5	20.5+/-0.4	8.9 (4)
Calu-3	4.3+/-0.7	23.9+/-4.5	10.2 (3)
ZR7530	1.4+/-0.9	27.0+/-2.1	31.2 (2)
EFM-192A	0.9+/-0.9	14.3+/-1.8	28.5 (2)
H1819	0.6+/-2.2	4.9+/-0.7	8 (6)
PIK3CA mutant cancers			Mutation
MDA-MB-361*	64.2+/-2.0	83.5+/-20.1	E545K
UACC-893*	59.5+/-2.7	398.1+/-34.1	H1047R
HCC1954*	31.3+/-1.8	32.0+/-0.5	H1047R
MDA-MB-453*	25.1+/-5.2	74.0+/-0.9	H1047R
T47D	6.6+/-1.6	14.5+/-2.7	H1047R
BT-20	6.1+/-0.5	20.7+/-0.3	H1047R
SKOV3*	2.7+/-1.0	16.3+/-6.5	H1047R
***BRAF* mutant colorectal cancers**			Mutation
SW1417	39.0+/-3/9	63.4+/-13.1	V600E
LS411N	30.6+/-9.6	95.0+/-15.4	V600E
Colo201	25.2+/-3.4	151.4+/-27.6	V600E
Colo206F	20.6+/-1.5	46.7+/-23.6	V600E
Colo205	20.1+/-0.3	82.8+/-30.1	V600E
HT-29	10.0+/-1.6	23.9+/-4.5	V600E
WiDr	4.4+/-3.9	53.7+/-10.6	V600E
Colo741	3.5+/-0.9	10.0+/-3.0	V600E
RKO	1.8+/-0.4	20.0+/-4.1	V600E
H508	1.5+/-1.8	29.0+/-5.2	G596R

Table 1. The cell lines used in this study are shown. The amount of apoptosis induced by the corresponding kinase inhibitor +/- SD from three individual experiments are displayed. Relative BIM mRNA levels +/- SD are and the genetic mutation in each cell line are shown. Degree of apoptosis in the *EGFR* mutant cell lines did not correlate with epithelial or mesenchymal phenotype (1). * indicates concurrent *HER2* amplification. # indicates the reference cited for the *HER2* copy number.

Sup. Table 2. Kinetics of apoptosis to EGFR TKI in *EGFR* mutant cell lines.

Apoptosis (%)	PC9	HCC2279
24 hrs	8.3+/-0.4	0.6+/-1.5
48 hrs	23.0+/-1.4	4.2+/-3.1
72 hrs	66.0+/-1.0	6.7+/-0.6

Table 2. *EGFR* mutant PC9 and HCC2279 cells were treated with or without EGFR TKI (gefitinib) for the indicated times and apoptosis was measured. The amount of apoptosis induced by EGFR TKI +/- SD from three individual experiments are displayed.

Supplemental References.

1. Takeyama Y, Satu M, Horio M, Hase T, Yoshida K, Yokoyama T, et al. Knockdown of ZEB1, a master epithelial-to-mesenchymal transition (EMT) gene, suppresses anchorage-independent cell growth of lung cancer cells. Cancer Lett 2010;2:216-24.

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3. Kao J, Salari K, Bocanegra M, La Choi Y, Girard L, Gandhi J, et al. Molecular Profiling of Breast Cancer Cell Lines Defines Relevant Tumor Models and Provides a Resource for Cancer Gene Discovery. Plos One 2009;7: e6146.

4. McDermott U, Sharma SV, Dowell L, Greninger P, Montagut C, Lamb J, et al. Identification of genotype-correlated sensitivity to selective kinase inhibitors by using high-throughput tumor cell line profiling. PNAS 2007;50:19936-41.

5. Huang J, Wei W, Chen J, Zhang J, Liu G, Di X, et al. CARAT: a novel method for allelic detection of DNA copy number changes using high density oligonucleotide arrays. BMC Bioinformatics 2008;7:83e.

6. Zhao X, Weir BA, LaFramboise T, Lin M, Beroukhim R, Garraway L, et al. Homozygous Deletions and Chromosome Amplifications in Human Lung Carcinomas Revealed by Single Nucleotide Polymorphism Array Analysis. Cancer Res 2005;65:5561-60.

Supplemental Figure Legends.

Figure 1**. Low BIM expressing *EGFR* mutant lung cancer cells are resistant to apopotosis induced by PI3K/MEK inhibitors combinations but undergo similar growth arrest following EGFR TKI treatment.** (A) H1650 cells were treated with AZD6244 (2 μM)/NVP-BEZ235 (200 nM) for 24 hours and lysates were probed with the indicated antibodies. (B) Low BIM expressing HCC2935 and H1650 cells and high BIM expressing H3255 cells were treated with the PI3K/MEK inhibitor combination as in (A) for 72 hours, stained with propidium iodide and annexin V, and analyzed by FACS to quantify annexin positive cells. Data is presented as percent of cells undergoing apoptosis over vehicle-treated control cells. Error bars are -/+ the S.D. of the mean of three experiments. (C) High BIM expressing *EGFR* mutant lung cancers (PC9, H1975, HCC827 and H3255) and low BIM expressing *EGFR* mutant lung cancers (HCC2935, HCC4006, H1650 and HCC2279) were treated vehicle or EGFR TKI for 24 hours and cell cycle distribution was determined by propidium iodide staining followed by FACS analyses. Bars indicate S phase decrease caused by EGFR TKI. Error bars are -/+ S.E.M. of the mean percent S phase reduction caused by gefitinib in the two groups.

Figure 2. **Superior induction of apoptosis following AZD6244 correlates with higher basal BIM expression across a panel of *BRAF* mutant colorectal cancers**. (A) The indicated *BRAF* mutant colorectal cancers were treated with (+ KI) or without (- KI) 1uM AZD6244 for 24 hours and lystates were probed with the indicated antibodies. (B) BIM RNA levels were quantified by qRT-PCR, normalized to β-Actin, and plotted against the amount of apoptosis induced by the AZD6244 (over vehicle-control) as determined by annexin FACS. Both RNA values and apoptosis are the mean of at least three experiments (see Table 1). The r^2 value was 0.43 with a significance of P= 0.04. (C) Of note, WiDr cells did not demonstrate suppression of P-ERK in (A). The WiDr cells were treated with or

without 1uM AZD6244 for 6 hours and lystates were probed with the indicated antibodies. Please note that there was suppression of P-ERK at 6 hours.

Figure 3. **BIM siRNA reduces levels of BIM in oncogene-addicted cancer cell lines.** (A) *HER2* amplified cancers or (B) *PIK3CA* mutant MDA-MB-453 cancers were transiently transfected with either scrambled (SC) or BIM siRNA for 24 hours, trypsonized and re-seeded for the experiments indicated in Fig. 6. Identically treated plates were treated with vehicle, lapatinib, NVP-BEZ235 (BEZ235) or the microtubule poison taxol (TAX) for 24 hours and lysates were probed with the indicated antibodies. (C) *HER2* amplified SkBr3 or *PIK3CA* mutant HCC1954 cancer cell lines were transiently transfected with either scrambled (SC) or BIM siRNA for 24 hours and then split for the drug treatments. The next day cells were treated with vehicle control, or the microtubule poison taxol (TAX, 200nM) for 24 hours or 48 hours and stained with propidium iodide and annexin V and analyzed by FACS. Bars represent average percent of apoptosis drug treatment over vehicle control. Error bars are -/+ S.D. of the mean of three experiments. * indicates significance ($P < 0.05$) between two treatments as determined by a Student's *t* test and NS = not significant. (D) *HER2* amplified SkBr3 or *PIK3CA* mutant HCC1954 cells were transiently transfected with either scrambled (SC) or BIM siRNA for 24 hours, trypsonized and re-seeded. The next day cells were treated with vehicle control, 500nM gemcitabine (GEM) or 10 μM cisplatin (CDDP) for 24 hours (taxol treatments when indicated) or 48 hours (remainder of the treatments) and stained with propidium iodide and annexin V and analyzed by FACS. Bars represent average percent of apoptosis drug treatment over vehicle control. Error bars are -/+ S.D. of the mean of three experiments. * indicates significance ($P < 0.05$) between two treatments as determined by a Student's *t* test and NS = not significant.

Figure 4. **Induction of BIM expression in low BIM expressing cancers sensitizes to targeted therapies.** Low BIM expressing *EGFR* mutant H1650 cells (A) or *PIK3CA* mutant SKOV3 cells (B) infected with lentivirus expressing doxycycline-inducible BIM (pTREX BIM) or control (pTREX empty) were treated with DOX for 24 hours and subsequently treated with the indicated kinase inhibitors (1uM gefitinib for H1650 cells, 200nM NVP-BEZ235 for SKOV3). *Left panels,* After 72 hours, apoptosis was quantified by propidium iodide and annexin V FACS. Data is presented as apoptosis of treatment. Error bars are -/+ the standard deviation of three experiments. *Right panels*, Lysates were prepared and probed with the indicated antibodies 24 hours following drug treatments.

Figure 5. **BIM knockdown protects cells from apoptosis and leads to increased numbers of total viable cells in long-term assays in vitro.** (A and B) High BIM expressing HCC827 *EGFR* mutant cancer cells were infected with a doxycycline (DOX)-inducible short hairpin (SH) targeting BIM or scramble (SC) control. The cells were pre-treated with 10ng/ul of doxycycline for 48 hours and then treated with 1μM gefitinib for 24 hours and lysates were prepared and probed with the indicated antibodies. (B) Cells were treated as above for 72 hours and apoptosis was measured using propidium iodide and annexin V FACS. Bars represent percent of apoptosis, and error bars are +/- S.D. (C) HCC827 *EGFR* mutant cancer cells infected with BIM and SC shRNA were plated for long-term growth assays in vitro. One plate of cells was quantified the next day (white bars) and the cells in other plates were cultured in the presence or absence of 10ng/ul of doxycycline and gefitinib for 10 days. Cells were replenished with fresh media (with doxycycline and gefitinib as indicated) every 3 days. Cell viability was determined by staining with the nuclear acid stain, SYTO60. (D) Tumors from the in vivo experiment in Fig. 7A and B were probed with the indicated antibodies to assess induction of apoptosis. Tumors were from mice (+ or – DOX) following 3 days of gefitinib treatment.

Figure 6. **Lapatinib suppresses intracellular signaling in ZR7530 cells.** (A) ZR7530 cells were treated with lapatinib (1μM) or vehicle control for 24 hours and lysates were probed with the indicated antibodies. (B) Immunohistochemistry (IHC) from ZR7530 mammary fat pad tumors grown and treated with either vehicle or lapatinib for 3 days. Please note Pras40 is phosphorylated by AKT at T246 and thus serves as a readout for PI3K/AKT activity.

Figure 7. **Adding taxol to TKIs in low BIM expressing cells induces apoptosis and shrinks tumors in vivo.** (A and B) The indicated high and low BIM expressing *HER2* amplified breast cancer (A) and EGFR mutant lung cancer (B) cells were treated with TKI (1μM lapatinib for breast cancers, 1μM gefitinib for lung cancers), taxol (TAX, 200nM), or TKI plus taxol (1μM/200nM) for 72 hours. Apoptosis was quantified by propidium iodide and annexin V FACS. Percent of cells undergoing apoptosis +/- the S.D. of the mean of three experiments are shown. (C) ZR7530 *HER2* amplified breast cancer cells were injected into the mammary fat pad of mice following ovariectomy and were treated with vehicle or 100mg/kg lapatinib (qd), 10mg/kg paclitaxel (taxol) (q wk), or the combination of 10mg/kg paclitaxel (taxol) (q wk), and 100mg/kg lapatinib (qd). Tumor volumes were measured for ~ 30 days and the mean tumor volume +/- SEM of the different treatment groups are shown. (D) Immunohistochemistry (IHC) from ZR7530 mammary fat pad tumors grown and treated with either vehicle (qd for 3 days), lapatinib (qd for 3 days), or lapatinib (qd for 3 days) plus taxol (day 1 and day 3) combination. Note staining for cleaved caspase 3 (CC3), a marker for active apoptosis, is enhanced by the addition of taxol.

Figure 8. **Immunohistochemistry (IHC) staining of BIM correlates to RNA levels in cell lines.** High BIM expressing PC9 *EGFR* mutant NSCLC cells and BT-474 *HER2* amplified breast cancer cells and low BIM expressing H4006 *EGFR* mutant cells and *HER2* amplified Calu-3 cells were assayed by IHC for BIM as described in materials and methods.

Figure 9. ***PIK3CA* mutant cancers do not consistently downregulate anti-apoptotic proteins following NVP-BEZ235 treatment**. (A) The indicated *PIK3CA* mutant cancers were treated with (+ KI) or without (- KI) 200nM NVP-BEZ235 for 24 hours and lystates were probed with the indicated antibodies. Note high BIM expressors are in red and low BIM expressors are in blue.

Figure 10. **Patients with *HER2* positive breast cancers and low BIM expression have poorer progression-free survival (PFS).** (A) Six *HER2* positive cancers from patients treated with monotherapy lapatinib were assayed for pre-treatment BIM RNA expression (relative to β-Actin, average of three measurements) and grouped according to progression-free survival (PFS) (less than 10 weeks or greater than 17 weeks). Red Bars represent average relative BIM RNA levels of both groups. (B) Table of results. (C) Immunohistochemistry (IHC) of BIM in *HER2* positive breast cancer from patients included in the qRT-PCR analyses in (A). The numbers below the images correspond to progression-free survival (PFS) of the patient whose tumor is stained.