Supplementary Figure 1

(**A**) Analysis of the relative abundance of MCF7 infected cells recovered after selection with Blasticidin. Data are normalized and depicted as blasticidin treated versus untreated cells. The data are the average of two independent infections. (**B**) Summary of ORF kinase screen results for MCF7 and BT474 cell lines treated with BKM120 or BEZ235. (++) signifies greater than 3 standard deviations above the mean, (+) signifies greater than 2 standard deviations above the mean.

Supplementary Figure 2

(**A**) BEZ235 dose response of MCF7 cells expressing indicated ORFs (n=3). Cell number was determined by Cell Titer-Glo. Graph represents mean percentage relative to controls (mean ± SD). (**B**) Colony formation assay of MCF7 cells overexpressing RSK and S6K family members treated with 100 nM BEZ235 for 14 days. Lower panel shows western blot analysis of MCF7 cells overexpressing V5 tagged RSK family members. (**C**) MCF7 cells expressing GFP, AKT1or RSK4 were treated with BEZ235 at increasing concentrations for 24 hours. The levels of the indicated proteins were determined by immunoblotting.

Supplementary Figure 3

Cell cycle analysis of MCF7 cells expressing GFP, RSK3 or RSK4 after 24 hours of treatment with BEZ235 (100 nM), BEZ235 (250 nM), BKM120 (750 nM), or GDC0941 (1 uM).

Supplementary Figure 4

(**A**) Panel of breast cancer cell lines assessed for levels of phospho-RSK-359/363, phospho-RSK-380, RSK3 and RSK4. (**B**) MCF7, MDA-MB-231, and AU565 cells were treated with BEZ235 (100 nM), BKM120 (1 uM) or pp242 (1 uM) for 24 hours. The levels of the indicated proteins were determined by immunoblotting. Asterisk indicates non-specific band. (**C**) MCF7, MDA-MB-231, and AU565 cells were treated with BEZ235 (100 nM), BKM120 (1 uM) or the mTOR kinase inhibitor MLN-128 (100 nM) for 24 hours. The levels of the indicated proteins were determined by immunoblotting.

Supplementary Figure 5

Quantification of crystal violet staining of (**A**) RSK3 or (**B**) AKT1 overexpressing MCF7 cells treated with BEZ235 (200 nM), BKM120 (750 nM), GDC-0941 (1 uM), or MK-2206 (2 uM) in combination with either MEK162 (1 uM) or BI-D1870 (5 uM) for 8 days. Bars represent fold increase relative to treated GFP controls (mean ± SD of 3 independent experiments) .

Supplementary Figure 6

(**A**) MCF7 cells expressing GFP or RSK4 were treated with BEZ235 and/or AZD6244. The levels of the indicated proteins were determined by immunoblotting. (**B**) Proliferation of breast cancer cells treated with BEZ235 and/or BI-D1870 for 24 hours, assessed by Cell Titer-Glo. Bars represent relative proliferation compared to untreated controls (mean ± SD of 3 independent experiments). (**C**) Sub-G1 analysis of breast cancer cells treated with BEZ235 (100 nM) and/or BI-D1870 (10 uM) for 24 hours. Bars represent mean percentage of apoptosis (mean ± SEM of 3 independent experiments). (**D**) Western blot analyses of AU565 cells transfected with siRNA against RSK4. Whole cell extracts were probed with the indicated antibodies. (**E**) Western blot analyses of HCC1143 cells transfected with siRNA against RSK4. Whole cell extracts were probed with the indicated antibodies.

Supplementary Figure 7

(**A**) Sub-G1 analysis of AU565 breast cancer cells transfected with siRNA against RSK4 treated with DMSO, BEZ235 (100 nM) and/or MEK162 (1 uM) for 24 hours. Bars represent mean percentage of apoptosis (mean ± SEM of 3 independent experiments). (**B)** Sub-G1 analysis of HCC1143 breast cancer cells transfected with siRNA against RSK4 treated with DMSO, BEZ235 (100 nM) and/or MEK162 (1 uM) for 24 hours. Bars represent mean percentage of apoptosis (mean ± SEM of 3 independent experiments). (**C)** MCF7 cells overexpressing RSK4 were treated for 24 hours with indicated PI3K inhibitors in combination with MEK162 (1 uM) or (**D**) BI-D1870 (10 uM) prior to labeling new protein synthesis with ^{35}S. Figures represent quantification of newly synthesized protein (mean ± SD of 3 independent experiments).

Supplementary Figure 8

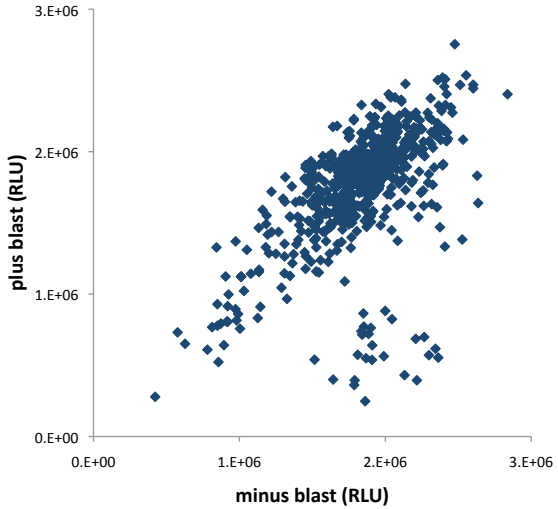
(**A**) Box plots of mouse xenografts of MCF7 cells overexpressing RSK4 or GFP control. Mice

were treated 6 times per week with single agent BEZ235 (30 mg/kg), BKM (30 mg/kg), or MK2206 (100 mg/kg). Boxes represent tumor volume variation; lines represent mean tumor volume; bars represent SE. A two-tailed student T test compares the two treated populations. *P<0.05 (mean ± SEM). (**B**) H-Score quantification of immunohistochemical analysis of $pERK^{202/204}$, of tumors from Figure 6C. A two-tailed student T test compares the two treated populations. **P<0.01 (mean ± SEM). (**C**) Tumors from Figure 6C were analyzed by immunohistochemistry for phospho-4EBP1-37/46. Representative images are shown. (**D**) H-Score quantification of immunohistochemical analysis of phospho-4EBP1-37/46.

Supplementary Figure 9

(A) Reverse phase protein assay (RPPA) of PDX tumors following 21 days of treatment of BKM120. (**B**) Analysis of RPPA data across breast tumor intrinsic subtypes for RSK phosphorylated at Thr359/Ser363, across a panel of breast invasive tumors from the TCGA tumor bank (http://tcga-data.nci.nih.gov/tcga/). FDR values: across all tumor type (4.64%), between Basal-like and HER2-enriched tumors (7.71%), between Basal-like and the rest of tumor samples (1.08%). Lines represent average phospho-RSK expression levels across tumor samples. (**C**) Analysis of AKT1, AKT2, AKT3, RSK3, and RSK4 copy number alteration (CNA) or upregulation of mRNA across a panel of 59 breast cancer cell lines from the Cancer Cell Line Encyclopedia (CCLE) (http://www.broadinstitute.org/ccle/home).

A



B

	MCF7 BKM120	MCF7 BEZ235	BT474 BKM120	BT474 BEZ235	Validated
AKT1	++		++		YES
AKT3	++	+			YES
ALK	+				N.D.
BTK	++				N.D.
CDK5R1			++		N.D.
CRKL	+				N.D.
DYRK1B			++	+	N.D.
ERBB2	++	++			YES
IGF1R	++	++			YES
NLK		++			NO
NME7		++			NO
PIM2	++	++			YES
PRKACA		++			YES
RAF1	++	++			NO
RPS6KA2		+			YES
RPS6KA6		++		++	YES
YES1	++				N.D.

Figure S1

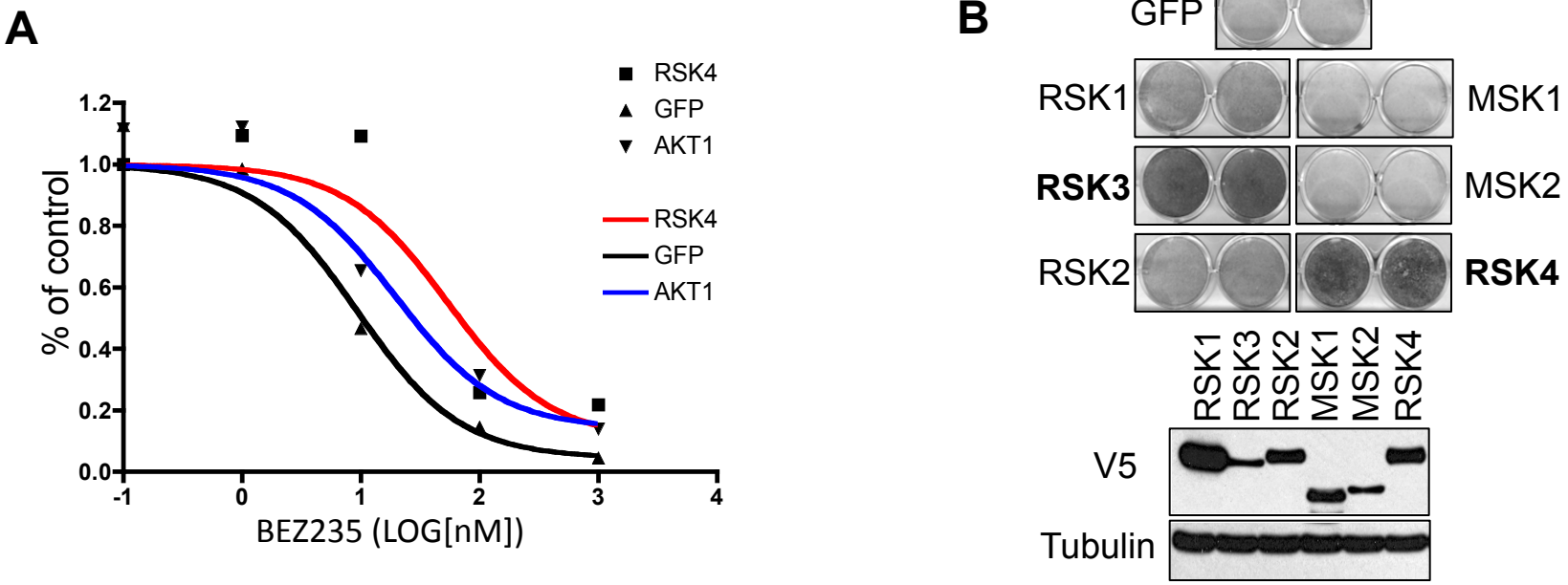

A
RSK4
GFP
AKT1
RSK4
GFP
AKT1
% of control
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-1
0
1
2
3
4
BEZ235 (LOG[nM])
B
GFP
RSK1
MSK1
RSK3
MSK2
RSK2
RSK4
RSK1
RSK3
RSK2
MSK1
MSK2
RSK4
V5
Tubulin
C
GFP
RSK4
AKT1
0
100
250
1000
0
100
250
1000
0
100
250
1000
BEZ(nM)
PARP
pS6-235/236
Tubulin

Figure S2

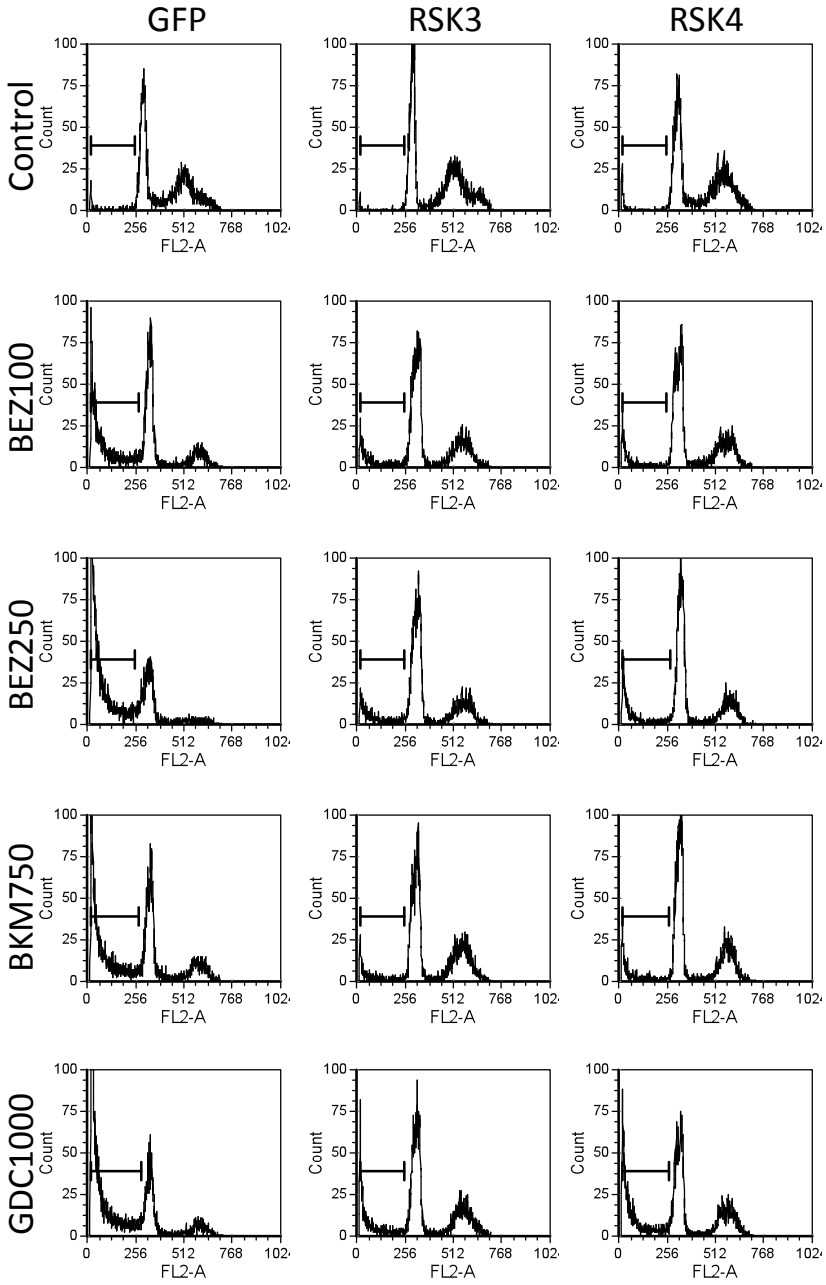

GFP
RSK3
RSK4
Control
BEZ100
BEZ250
BKM750
GDC1000
Count
FL2-A
0
25
50
75
100
256
512
768

Figure S3

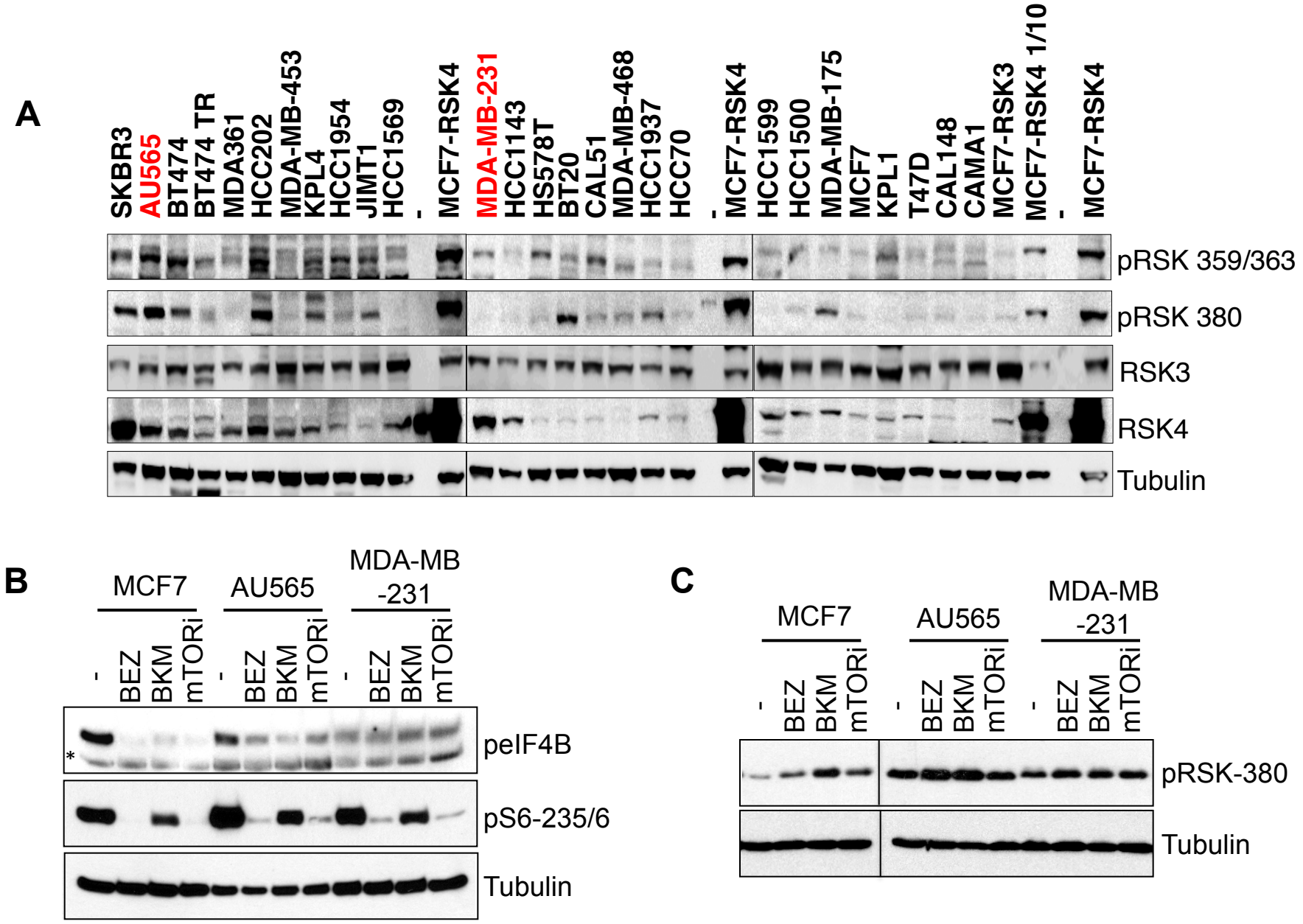

A
SKBR3
AU565
BT474
BT474 TR
MDA361
HCC202
MDA-MB-453
KPL4
HCC1954
JIMT1
HCC1569
-
MCF7-RSK4
MDA-MB-231
HCC1143
HS578T
BT20
CAL51
MDA-MB-468
HCC1937
HCC70
-
MCF7-RSK4
HCC1599
HCC1500
MDA-MB-175
MCF7
KPL1
T47D
CAL148
CAMA1
MCF7-RSK3
MCF7-RSK4 1/10
-
MCF7-RSK4
pRSK 359/363
pRSK 380
RSK3
RSK4
Tubulin
B
MCF7
AU565
MDA-MB -231
-
BEZ
BKM
mTORi
peIF4B
pS6-235/6
Tubulin
C
MCF7
AU565
MDA-MB -231
-
BEZ
BKM
mTORi
pRSK-380
Tubulin

Figure S4

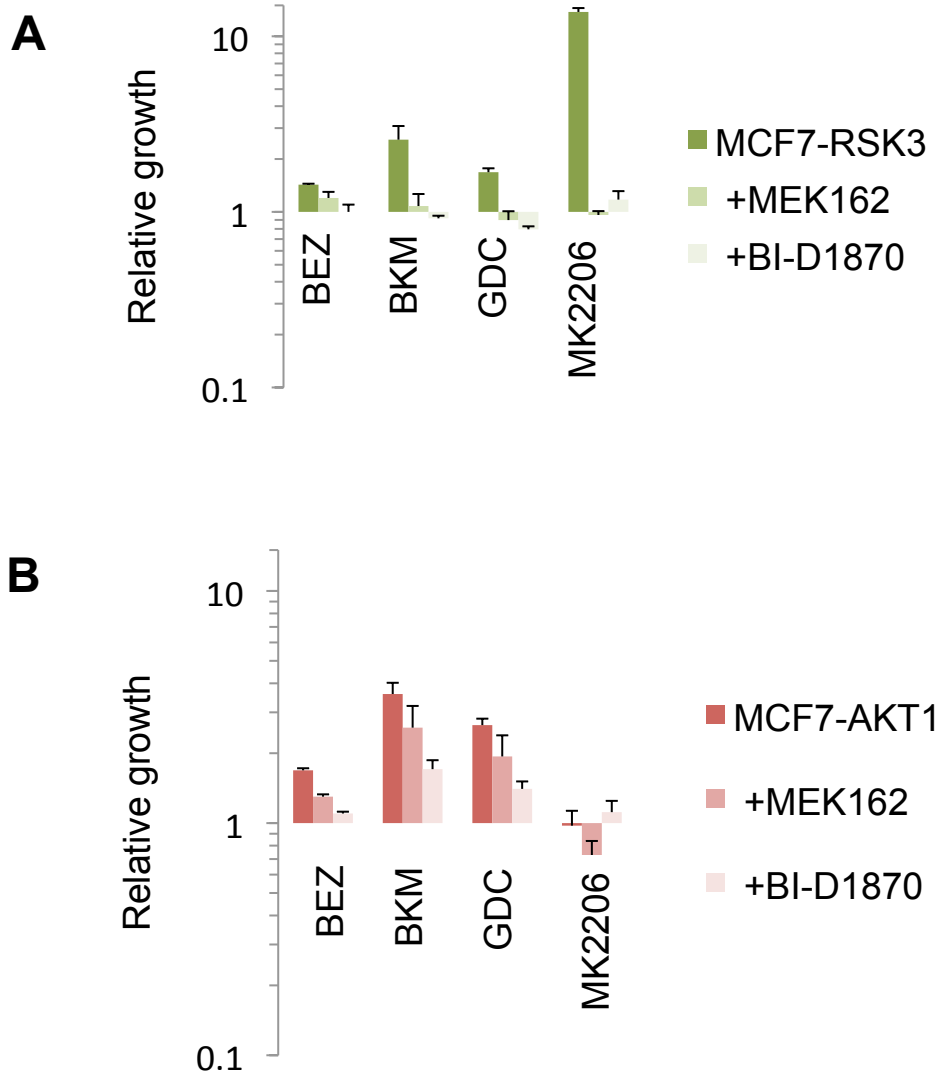

A
Relative growth
10
1
0.1
BEZ
BKM
GDC
MK2206
MCF7-RSK3
+MEK162
+BI-D1870
B
Relative growth
10
1
0.1
BEZ
BKM
GDC
MK2206
MCF7-AKT1
+MEK162
+BI-D1870

Figure S5

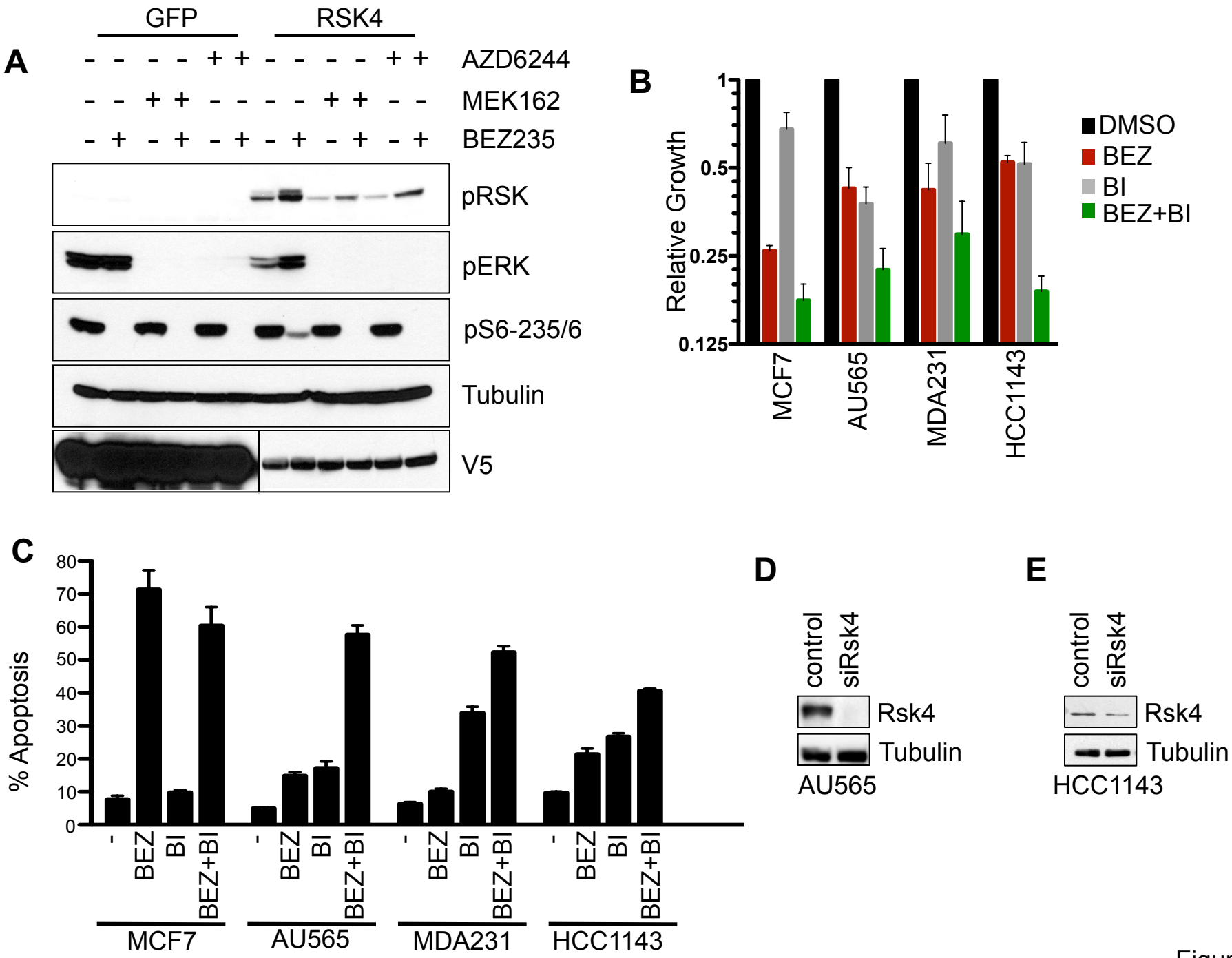

A
GFP
RSK4
AZD6244
MEK162
BEZ235
pRSK
pERK
pS6-235/6
Tubulin
V5
B
Relative Growth
1
0.5
0.25
0.125
DMSO
BEZ
BI
BEZ+BI
MCF7
AU565
MDA231
HCC1143
C
% Apoptosis
80
70
60
50
40
30
20
10
0
-
BEZ
BI
BEZ+BI
MCF7
AU565
MDA231
HCC1143
D
control
siRsk4
Rsk4
Tubulin
AU565
E
control
siRsk4
Rsk4
Tubulin
HCC1143

Figure S6

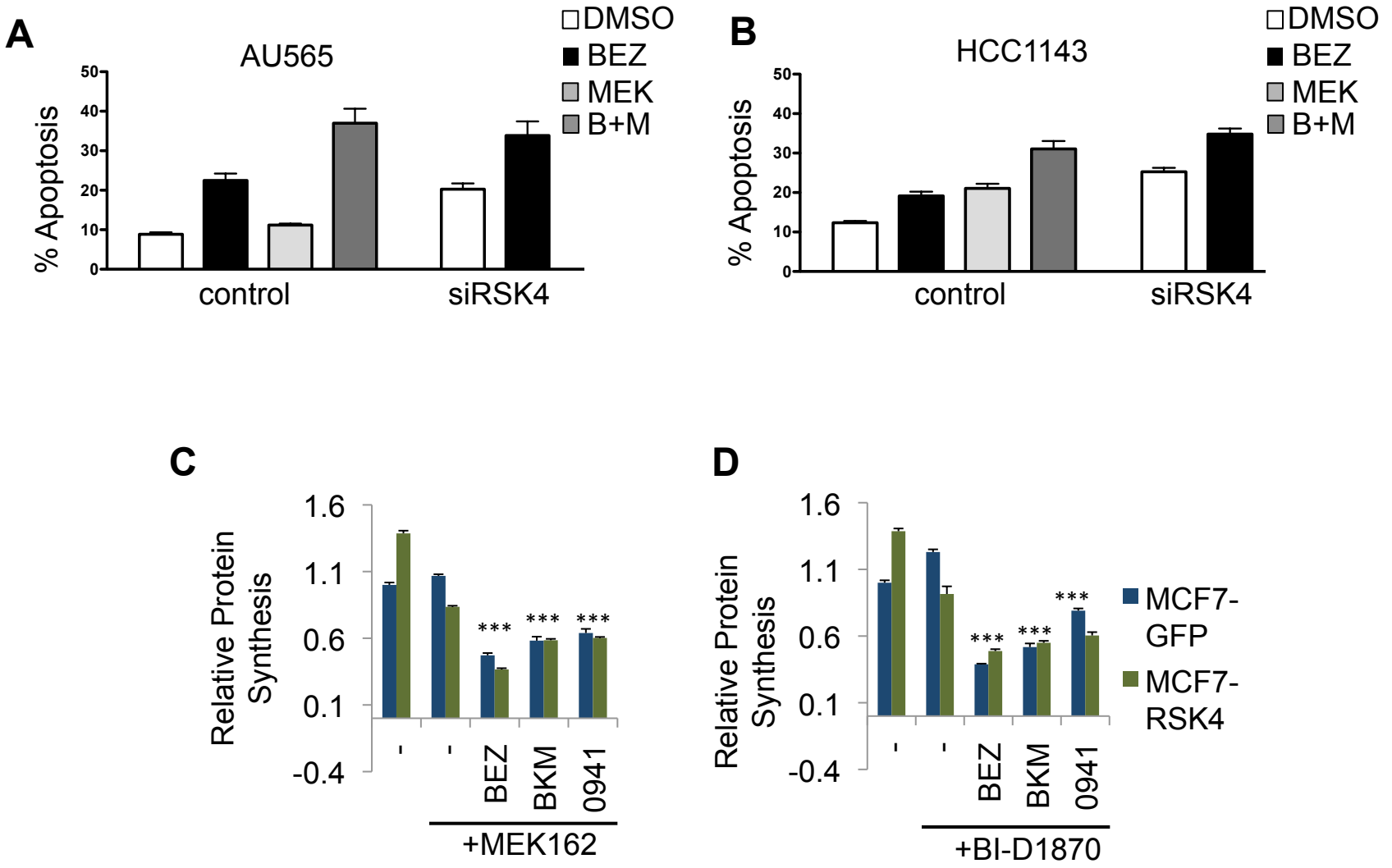

A
AU565
% Apoptosis
50
40
30
20
10
0
control
siRSK4
DMSO
BEZ
MEK
B+M
B
HCC1143
% Apoptosis
50
40
30
20
10
0
control
siRSK4
DMSO
BEZ
MEK
B+M
C
Relative Protein Synthesis
1.6
1.1
0.6
0.1
-0.4
***
***
***
-
-
BEZ
BKM
0941
+MEK162
D
Relative Protein Synthesis
1.6
1.1
0.6
0.1
-0.4
***
***
***
-
-
BEZ
BKM
0941
+BI-D1870
MCF7-GFP
MCF7-RSK4

Figure S7

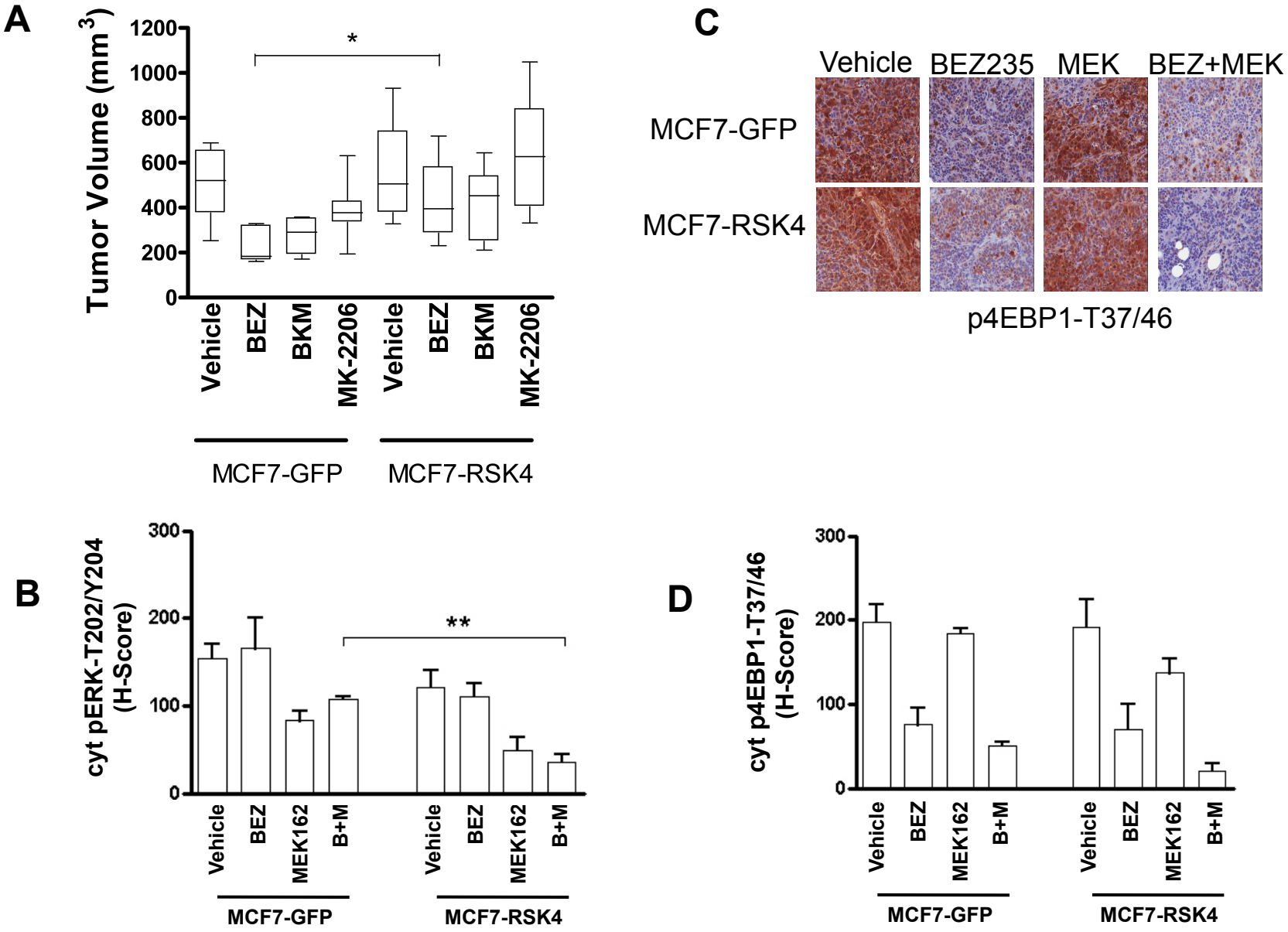
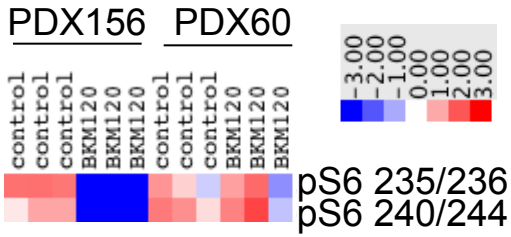

A
Tumor Volume (mm3)
1200
1000
800
600
400
200
0
*
Vehicle
BEZ
BKM
MK-2206
Vehicle
BEZ
BKM
MK-2206
MCF7-GFP
MCF7-RSK4
C
Vehicle
BEZ235
MEK
BEZ+MEK
MCF7-GFP
MCF7-RSK4
p4EBP1-T37/46
B
cyt pERK-T202/Y204 (H-Score)
300
200
100
0
**
Vehicle
BEZ
MEK162
B+M
Vehicle
BEZ
MEK162
B+M
MCF7-GFP
MCF7-RSK4
D
cyt p4EBP1-T37/46 (H-Score)
300
200
100
0
Vehicle
BEZ
MEK162
B+M
Vehicle
BEZ
MEK162
B+M
MCF7-GFP
MCF7-RSK4

Figure S8

A



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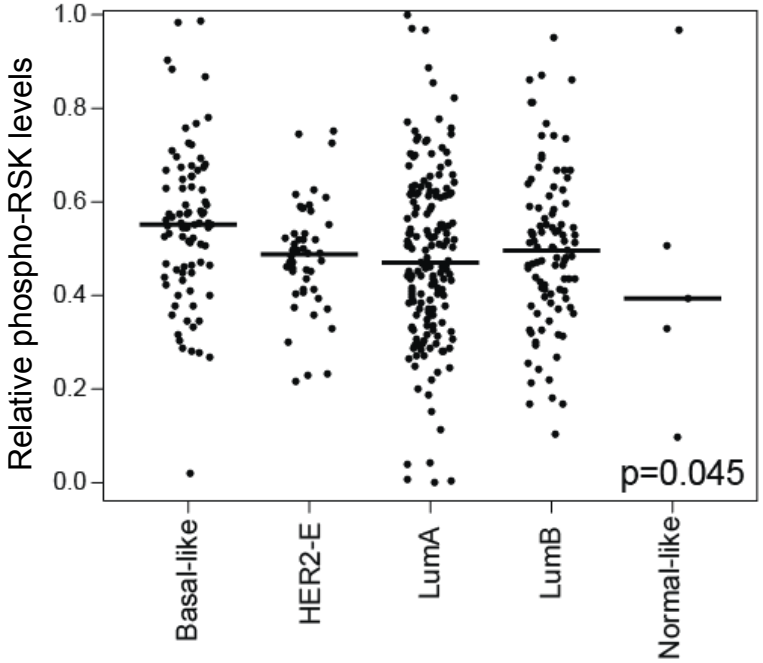

C

	Gain CN>2	Upregulated Expr>2
AKT1	2%	20%
AKT2	2%	3%
AKT3	7%	0%
RSK3	0	8%
RSK4	2%	46%

Figure S9