Supporting Information

Ilic et al. 10.1073/pnas.1108237108

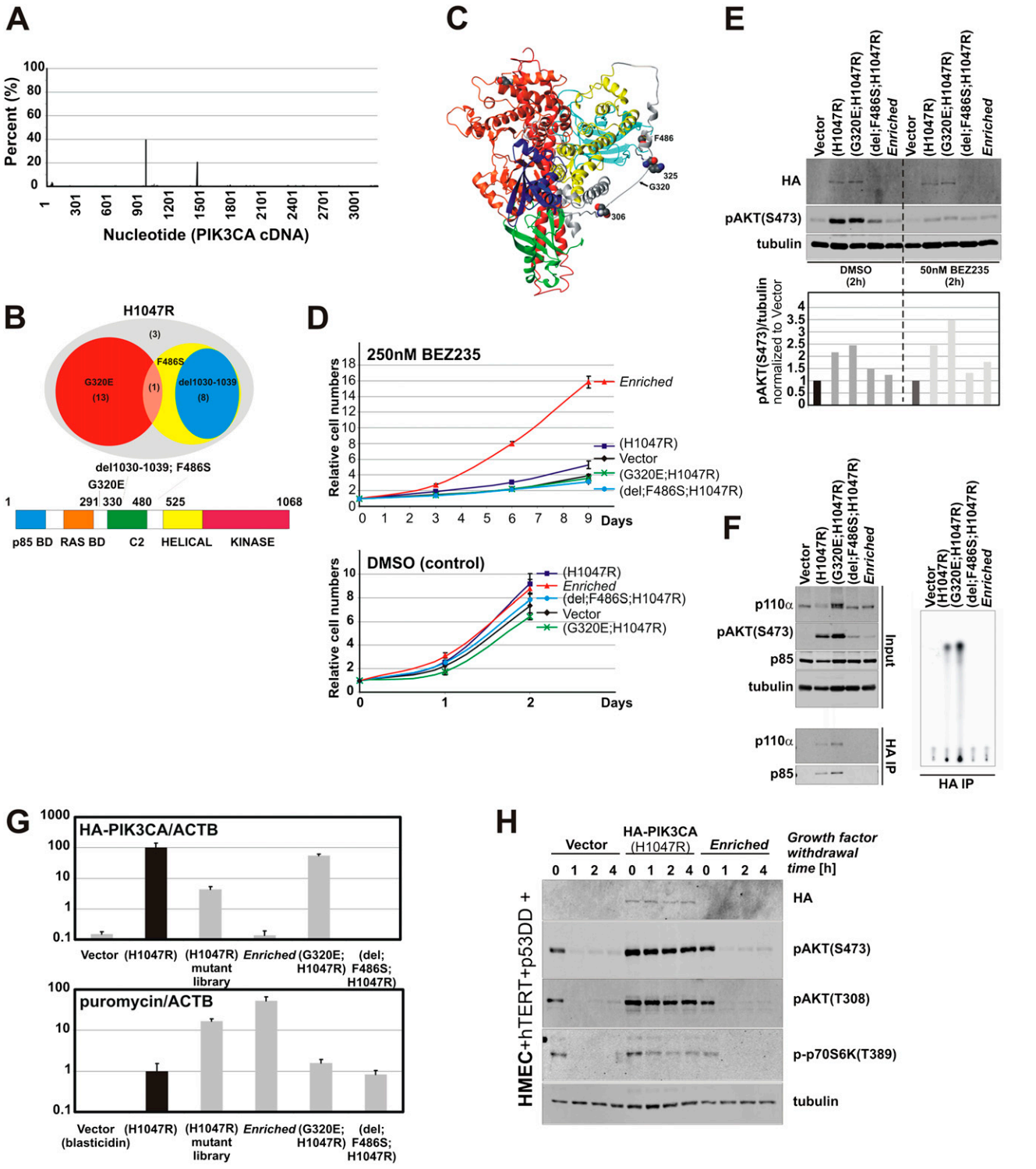

Fig. S1. Mutational analysis of BEZ235-resistant cells (related to Fig. 2). (*A*) SOLiD deep sequencing analysis of the exogenous PIK3CA(H1047R) alleles expressed in the BEZ235-resistant *Enriched* cells indicating the presence of two point mutations: G320E and F486S substitutions. (*B*) Results of manual sequencing of TOPO (Invitrogen) subclones of 28 individual exogenous PIK3CA(H1047R) alleles from the *Enriched* cells indicating preponderance of two alleles—G320E and

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del;F486S *in trans*. (*C*) Published crystal structure of p110α (1) with two positions of our two identified mutations positioned indicated. (*D*) Growth curves of human mammary epithelial cells (HMECs) expressing vector control, PIK3CA alleles—H1047R, G320E;H1047R, del;F486S;H1047R, or the *Enriched* cells in the presence of DMSO (vehicle) or 250 nM BEZ235. (*E*) Western blot analysis of the cell lines in *D* in the absence or presence of 50 nM BEZ235. (*F*) HA-tag immunoprecipitation (IP) and Western blot analysis of cell lines in *D*; matching IP samples were used for lipid kinase assays to measure the exogenous alleles' lipid kinase activity. (*G*) Real-time quantitative PCR for ectopic PIK3CA (HA-PIK3CA) and puromycin expression within the indicated genotypes of HMECs—vector (control), PIK3CA(H1047R), PIK3CA(H1047R) mutant library (preselection), *Enriched*, PIK3CA(G320E;H1047R), or PIK3CA(del;F486S;H1047R) normalized to beta-actin (ACTB). (*H*) Western blot analysis comparing HMECs expressing vector control, PIK3CA(H1047R), and *Enriched* cells after withdrawal from growth factors from the media for the indicated time periods. (Data are represented as mean ± SEM.)

1. Huang C-H, et al. (2007) The structure of a human p110α/p85α complex elucidates the effects of oncogenic PI3Kα mutations. *Science* 318:1744–1748.

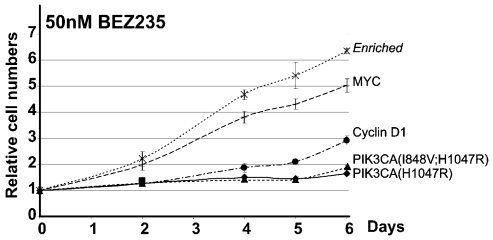

Fig. S2. Neither Cyclin D1 nor PIK3CA gatekeeper mutant I848V confers resistance to BEZ235 (related to Fig. 3). Growth curve in 50 nM BEZ235 of HMECs expressing PIK3CA alleles (H1047R or I848V;H1047R), Cyclin D1, MYC, and *Enriched* cells. (Data are represented as mean ± SEM.)

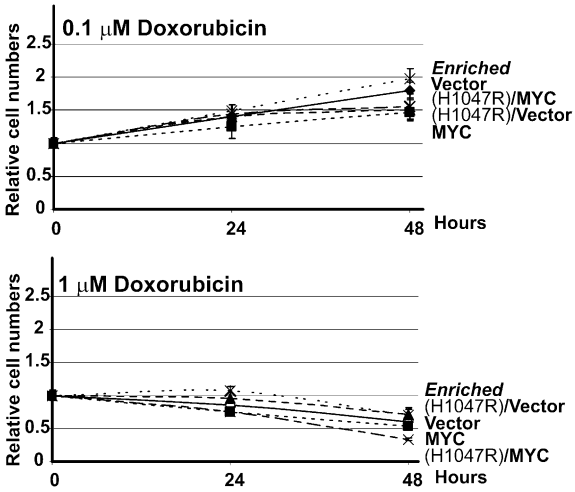

Fig. S3. MYC does not confer resistance to Doxorubicin (related to Fig. 4). Growth curves of HMECs expressing empty vector, PIK3CA(H1047R), MYC, PIK3CA (H1047R)+MYC, or the *Enriched* cells grown in 0.1 or 1 μM Doxorubicin. (Data are represented as mean ± SEM.)

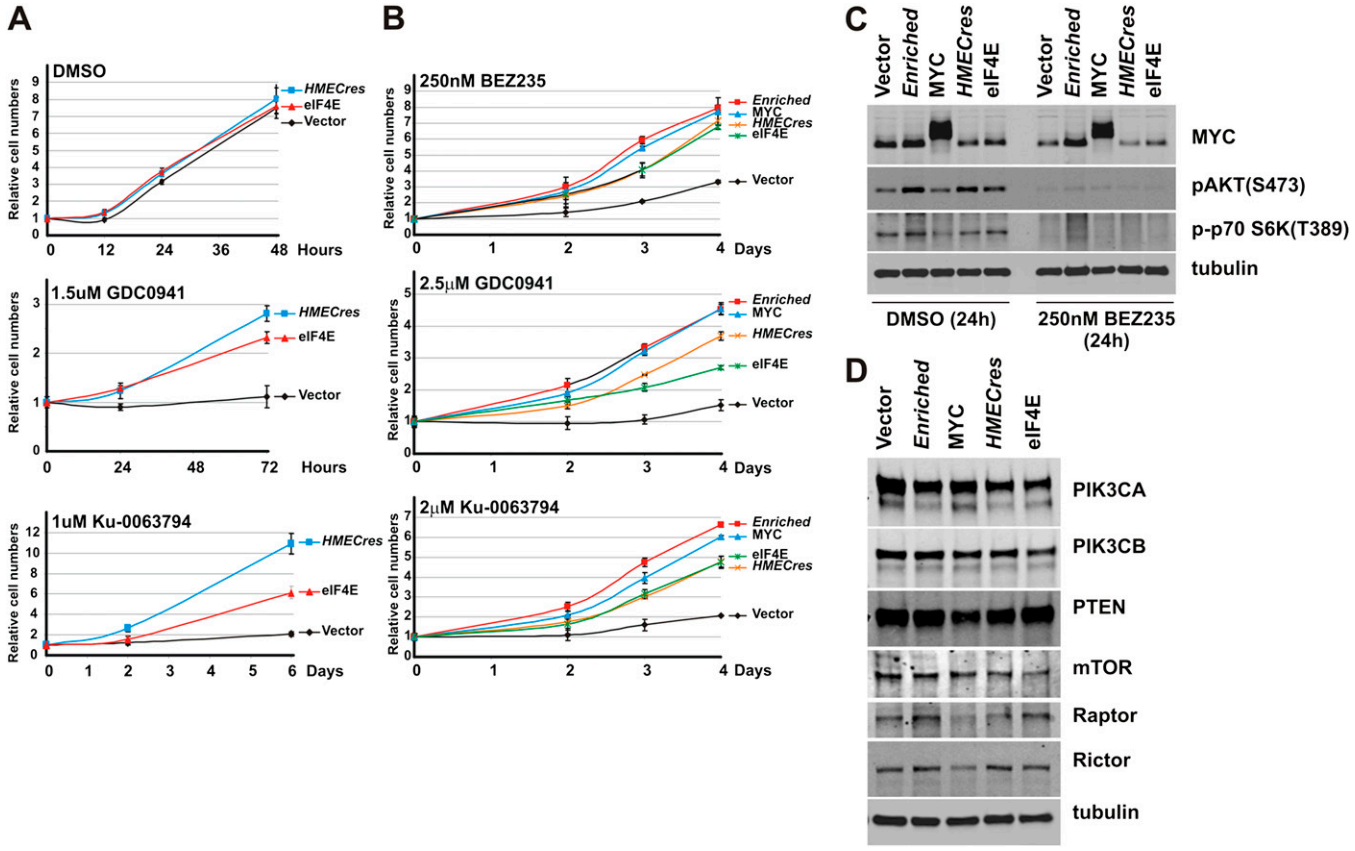

Fig. S4. eIF4E overexpression confers resistance to PI3K-mTOR inhibition (related to Fig. 6). (*A*) Growth assays in the presence of DMSO (control), 1.5 μM GDC0941, and 1 μM Ku-0063794 of parental HMECs transduced with vector (control), eIF4E, or *HMECres*. (*B*) Growth assays in the presence of 250 nM BEZ235, 2.5 μM GDC0941, or 2 μM Ku-0063794 of parental HMECs transduced with vector (control), *Enriched* cells, MYC, *HMECres*, or eIF4E. (*C*) Western assay of the cell lines in *B* in the absence or presence of 250 nM BEZ235. (*D*) Western assay of parental HMECs transduced with vector (control), *Enriched* cells, MYC, *HMECres*, or eIF4E.

Table S1. Genes contained within the chromosome 8 amplicon in the *Enriched* BEZ235-resistant cells (related to Fig. 2)

Abbreviation	Gene
RSPO2	R-spondin 2 homolog (*Xenopus laevis*)
EIF3E	Eukaryotic translation initiation factor 3, subunit E
TTC35	Tetratricopeptide repeat domain 35
TMEM74	Transmembrane protein 74
TRHR	TSH-releasing hormone receptor
NUDCD1	NudC domain containing 1
EBAG9	Estrogen receptor binding site-associated antigen 9
KCNV1	Potassium channel, subfamily V, member 1
CSMD3	CUB and Sushi multiple domains 3
TRPS1	Trichorhinophalangeal syndrome
EIF3H	Eukaryotic translation initiation factor 3, subunit H
RAD21	RAD21 homolog (*Schizosaccharomyces pombe*)
SLC30A8	Solute carrier family 30 (zinc transporter), member 8
MED30	Mediator complex subunit 30
EXT1	Exostoses (multiple) 1
SAMD12	Sterile α-motif domain containing 12
TNFRSF11B	TNF receptor superfamily, member 11b
MAL2	Mal, T-cell differentiation protein 2
NOV	Nephroblastoma overexpressed gene
TAF2	RNA polymerase II, TATA box binding protein (TBP) -associated factor, 150 kDa
COL14A1	Collagen, type XIV, α1
MRPL13	Mitochondrial ribosomal protein L13
HAS2	Hyaluronan synthase 2
ZHX2	Zinc fingers and homeoboxes 2
DERL1	Der1-like domain family, member 1
FAM83A	Family with sequence similarity 83, member A
WDYHV1	WDYHV motif containing 1
ANXA13	Annexin A13
C8orf78	Chromosome 8 ORF 78
TMEM65	Transmembrane protein 65
RNF139	Ring finger protein 139
ZNF572	Zinc finger protein 572
TRIB1	Tribbles homolog 1 (*Drosophila*)
FAM84B	Family with sequence similarity 84, member B
MYC	V-myc myelocytomatosis viral oncogene homolog (avian)
CCDC26	Coiled-coil domain containing 26
GSDMC	Gasdermin C
ASAP1	ArfGAP with SH3 domain, ankyrin repeat and PH domain 1
ADCY8	Adenylate cyclase 8 (brain)

Table S2. Genes contained within the chromosome 4 amplicon in the *HMECres* BEZ235-resistant cells (related to Fig. 6)

Abbreviation	Gene
BMPR1B	Bone morphogenetic protein receptor, type Ib
UNC5C	UNC5, *Caenorhabditis elegans*, homolog of c
PDHA2	Pyruvate dehydrogenase, e1-α polypeptide, testis-specific form
C4orf37	Chromosome 4 ORF 37
RAP1GDS	RAP1, GTP-GDP dissociation stimulator 1
TSPAN5	Tetraspanin 5
EIF4E	Eukaryotic translation initation factor 4E
METAP1	Methionyl aminopeptidase 1
ADH5	Alcohol dehydrogenase 5 (class III), χ-polypeptide
ADH4	Alcohol dehydrogenase 4 (class II), π-polypeptide
ADH6	Alcohol dehydrogenase 6 (class V)
ADH1A	Alcohol dehydrogenase 1A (class I), α-polypeptide
ADH1B	Alcohol dehydrogenase 1B (class I), β-polypeptide
ADH7	Alcohol dehydrogenase 7 (class IV), μ- or σ-polypeptide
C4orf17	Chromosome 4 ORF 17
RG9MTD2	RNA (guanine-9-) methyltransferase domain containing 2
MTTP	Microsomal triglyceride transfer protein
DAPP1	Dual adaptor of phosphotyrosine and 3-phosphoinositides
LAMTOR3	Late endosomal/lysosomal adaptor, MAPK and MTOR activator 3
DNAJB14	DnaJ (Hsp40) homolog, subfamily B, member 14
H2AFZ	H2A histone family, member Z
DDIT4L	DNA damage-inducible transcript 4-like
EMCN	Endomucin