Supplementary Materials

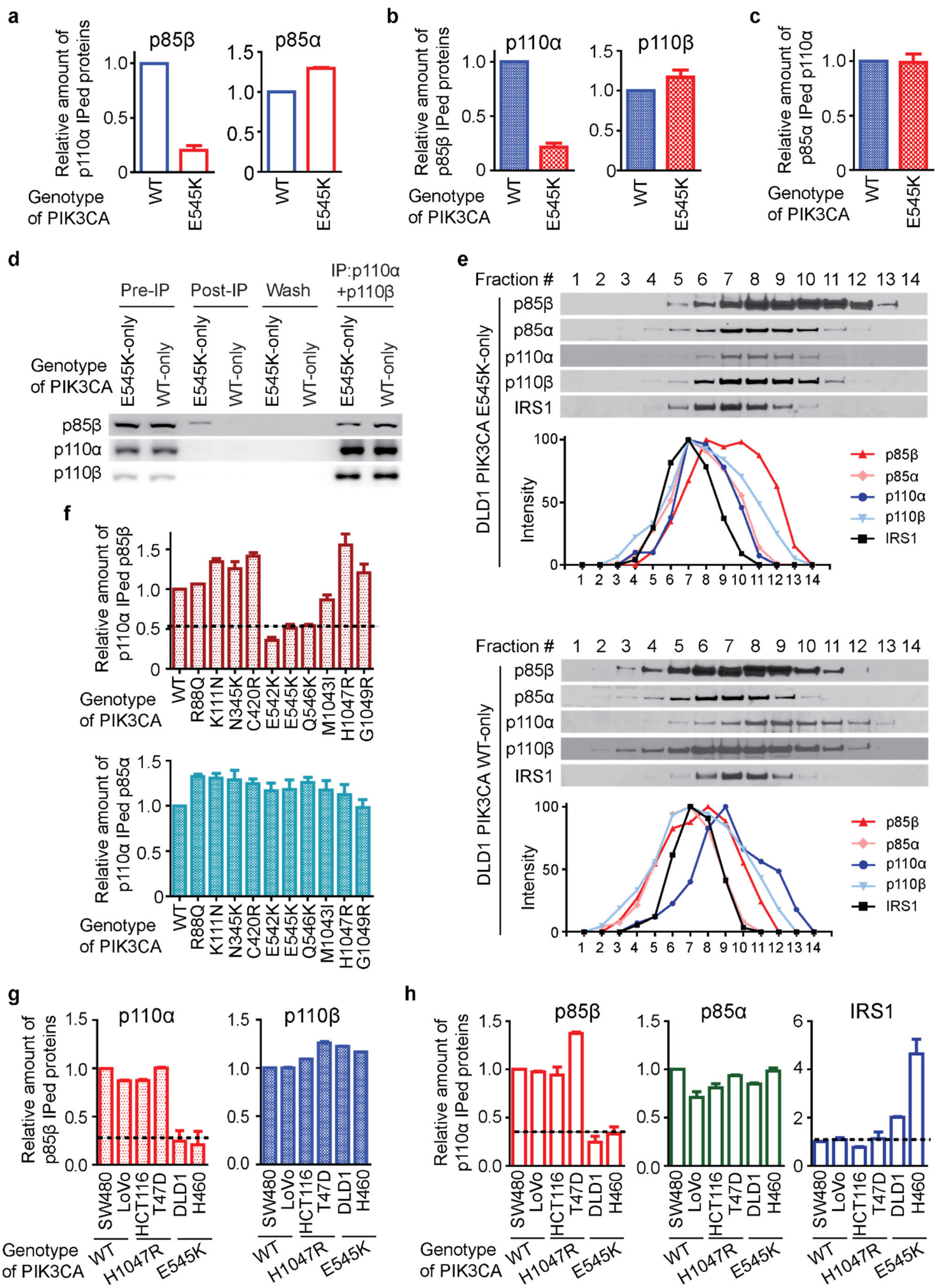
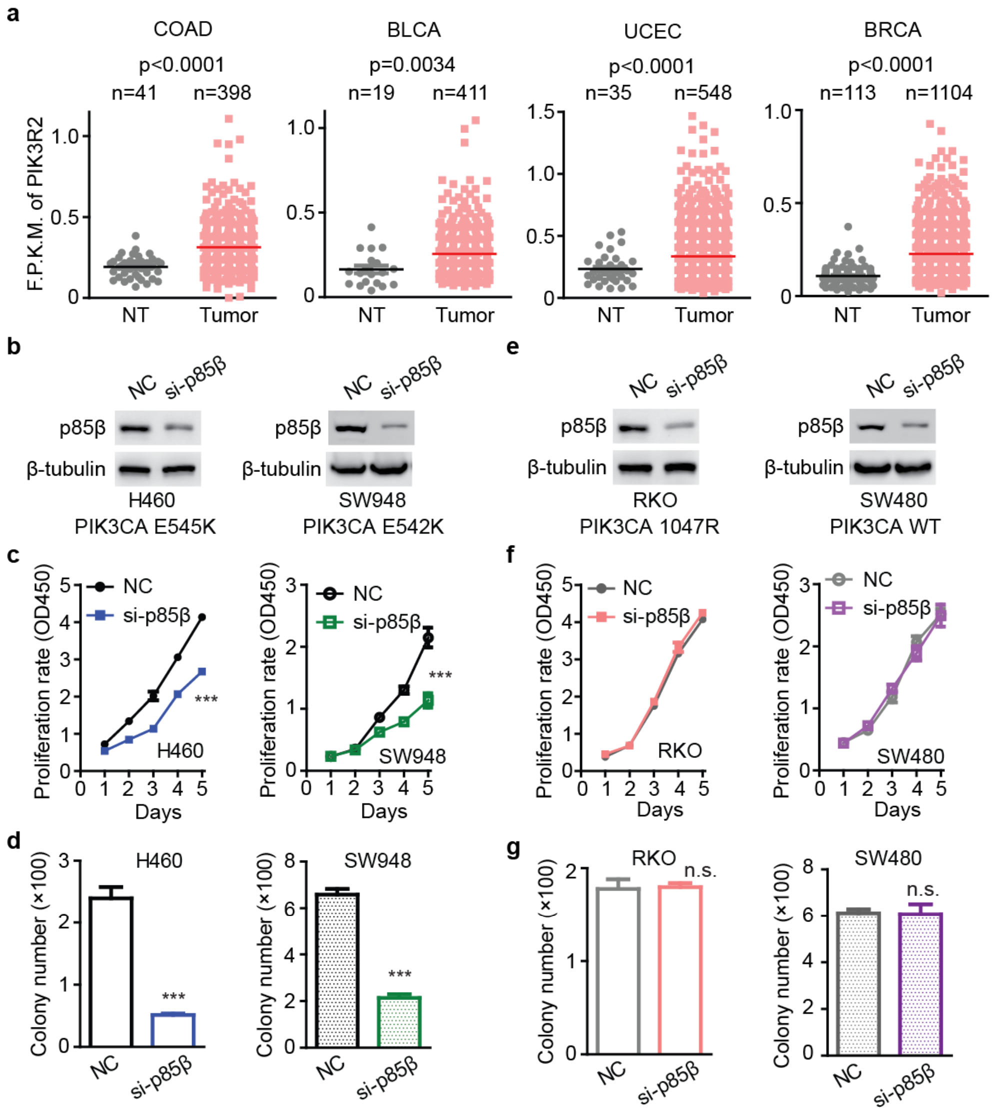


Figure S1. p85β disassociates from p110α helical domain mutated PI3K complex
(a) Quantification of Western blots shown in Fig 1b by Image J.
(b) Quantification of Western blot shown in Fig 1c by Image J.
(c) Quantification of Western blot shown in Fig 1d by Image J.
(d & d) PIK3CA helical domain mutant cells have more p110-free p85β. (d) Cell lysates were immunoprecipitated with both anti-p110α and anti-p110β antibodies and blotted with indicated antibodies. Pre-IP indicates cell lysates before immunoprecipitation; Post-IP indicates cell lysates after immunoprecipitation; Wash indicates buffers after washing beads; IP: p110α+p110β indicates immune complexes on the beads after immunoprecipitation. (e) Indicated cell lysates were fractionated with a Sepharose 6B column. The indicated elution fractions were blotted with the indicated antibodies, and the intensities of each protein at the indicated fractions were quantified with the Image J software.
(f) Quantification of Western blot shown in Fig 1f by Image J.
(g) Quantification of Western blot shown in Fig 1g by Image J.
(h) Quantification of Western blot shown in Fig 1h by Image J.



h

Cell lines / Gene mutations	DLD1 E545K-only	MB-361	H460	SW948	DLD1 WT-only	T47D	RKO	SW480
PIK3CA	E545K	E545K	E545K	E542K	WT	H1047R	H1047R	WT
PTEN	WT	NA	WT	NA	WT	WT	WT	WT
K-Ras	G13D	WT	Q61H	Q61L	G13D	WT	WT	G12V
BRAF	WT	WT	WT	NA	WT	WT	V600E	WT
TP53	WT	NA	WT	T118Qfs*5	WT	L194F	WT	R273H
CTNNB1	WT	NA	WT	NA	WT	WT	K312K	NA

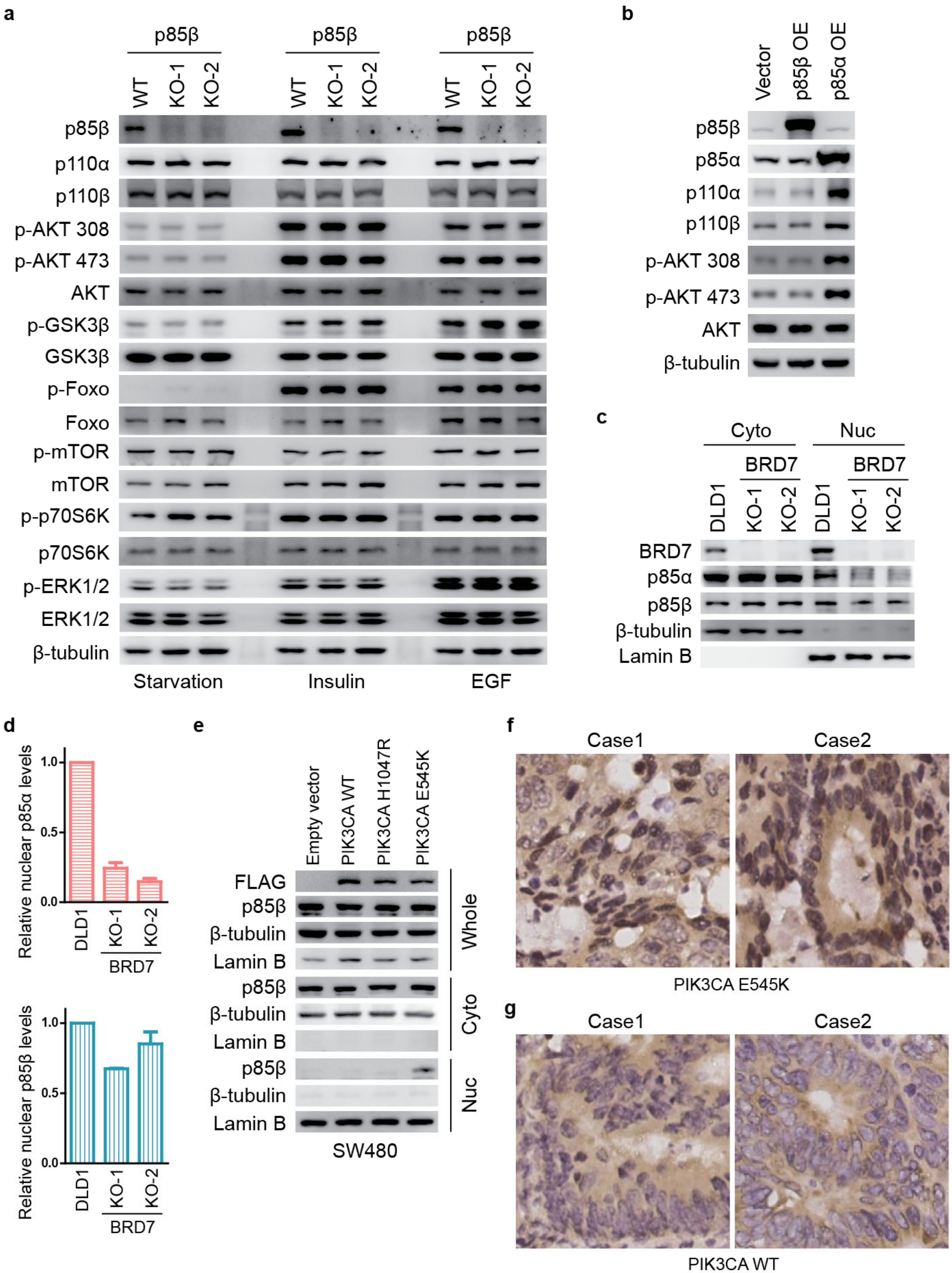
Figure S2. Depletion of p85β specifically impairs the growth of cancer cells with a PIK3CA helical domain mutation.

(a) PIK3R2 expression levels are higher in tumors compared to corresponding non-tumor tissue (NT). The RNA-seq data of tumors and matched non-tumor tissue were downloaded from the TCGA website. COAD: colon adenocarcinoma; BLCA: bladder carcinoma; UCEC: endometrial carcinoma; BRCA: breast cancer; FPKM: fragments per kilobase of exon per million reads mapped.

(b-g) Attenuating of p85β impair the growth of cancer cells a PIK3CA E545K mutation, but not cells with a PIK3CA H1047R mutation or wild-type PIK3CA. p85β was knocked down with siRNA in the indicated cell lines, and the cells were assayed for: Western blot analyses of p85β protein (a, e); cell proliferation (c, f); colony formation (d, g).

(h) Mutation status of the indicated genes in cell lines used in this study. The data were obtained from COSMIC. NA: the indicated information is not available.

The student's *t*-test was used for statistical analyses. Data are presented as mean ± SEM of three independent experiments. *** $p < 0.001$; n.s., not significant.



a
p85β
WT
KO-1
KO-2
p85β
p110α
p110β
p-AKT 308
p-AKT 473
AKT
p-GSK3β
GSK3β
p-Foxo
Foxo
p-mTOR
mTOR
p-p70S6K
p70S6K
p-ERK1/2
ERK1/2
β-tubulin
Starvation
Insulin
EGF
b
Vector
p85β OE
p85α OE
p85β
p85α
p110α
p110β
p-AKT 308
p-AKT 473
AKT
β-tubulin
c
Cyto
Nuc
BRD7
DLD1
KO-1
KO-2
BRD7
p85α
p85β
β-tubulin
Lamin B
d
Relative nuclear p85α levels
Relative nuclear p85β levels
1.0
0.5
0.0
DLD1
KO-1
KO-2
BRD7
e
Empty vector
PIK3CA WT
PIK3CA H1047R
PIK3CA E545K
FLAG
p85β
β-tubulin
Lamin B
Whole
Cyto
Nuc
SW480
f
Case1
Case2
PIK3CA E545K
g
Case1
Case2
PIK3CA WT

Figure S3. PIK3CA E545K mutation promotes the nuclear translocation of p85β.

(a & b) Depletion or overexpression of p85β have no impact on p110 stability and AKT signaling. The parental and p85β KO cells were grown in 6-well plates. After serum starvation (16 hours), cells were treated with insulin (1μg/ml for 15 minutes) or EGF (200 ng/ml for 15 minutes). Cell lysates were blotted with the indicated antibodies (a). Overexpression of p85α, but not p85β, stabilizes p110α protein and increases phosphorylation of AKT. DLD1 PIK3CA E545K-only cells were transfected with HA-tagged p85β or p85α. Three days after transfection, lysates from indicated cell lines were harvested and blotted with indicated antibodies (b).

(c) Knockout of BRD7 abolishes nuclear translocation of p85α but not p85β. BRD7 gene was knocked out in DLD cells using the CRISPR/Cas9 system. DLD1 cells and BRD7 KO cells were fractionated and blotted with indicated antibodies.

(d) Quantification of Western blots shown in Figure S3C by Image J.

(e) SW480 was transfected with FLAG-tagged wild-type PIK3CA, PIK3CA H1047R, or E545K mutant construct. Three days post-transfection, cell lysates were fractionated and blotted with indicated antibodies.

(f & g) Immunohistochemistry images of p85β staining in colorectal tumors with PIK3CA E545K mutation (f) or wild-type PIK3CA (g).

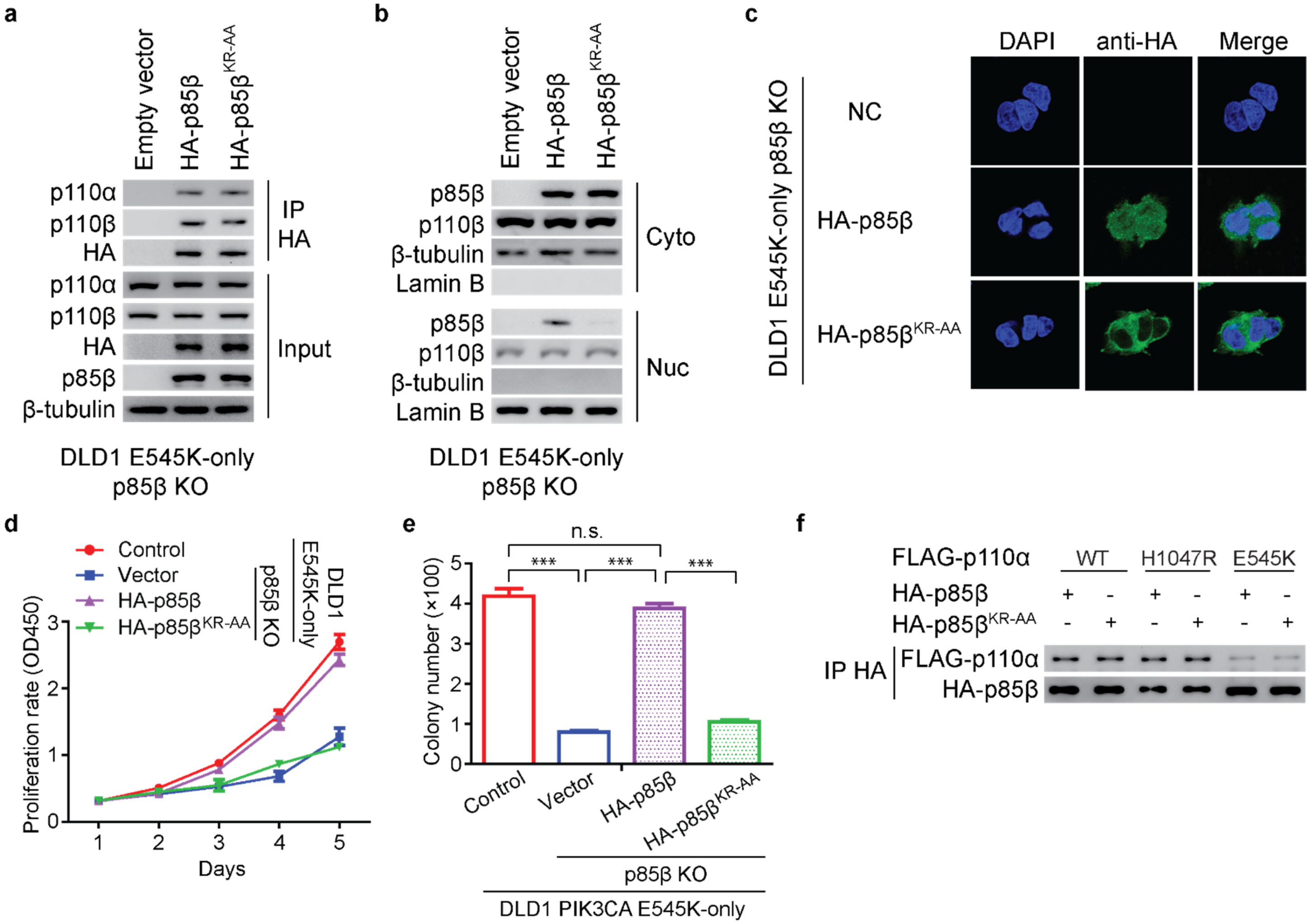


Figure S4. Nuclear localization signal is critical for nuclear translocation of p85β.

(a) The p85β NLS mutant does not impact its interaction with p110α and p110β. The DLD1 PIK3CA E545K-only p85β KO cells were transfected with either HA-tagged p85β or HA-tagged p85β^{KR-AA}. Stable expression clones were selected. Cell lysates were immunoprecipitated with anti-HA agarose and blotted with the indicated antibodies.

(b & c) NLS of p85β is critical for its nuclear translocation. The indicated cells were fractionated and blotted with indicated antibodies (b). The p85β subcellular localization of the indicated cell lines was evaluated by immunofluorescence assay (c).

(d & e) Reconstitution of wild-type p85β, but not the p85β^{KR-AA} mutant, rescues growth defects caused by p85β depletion in DLD1 PIK3CA E545K cells. The growth curve (d) and colony formation (e) of indicated cell lines are shown.

(f) KR-AA mutation has no impact on p85β-p110α interaction. The indicated FLAG-tagged p110α and HA-tagged p85β constructions were co-transfected into 293T cells. Cell lysates were immunoprecipitated with anti-HA agarose and then blotted with indicated antibodies.

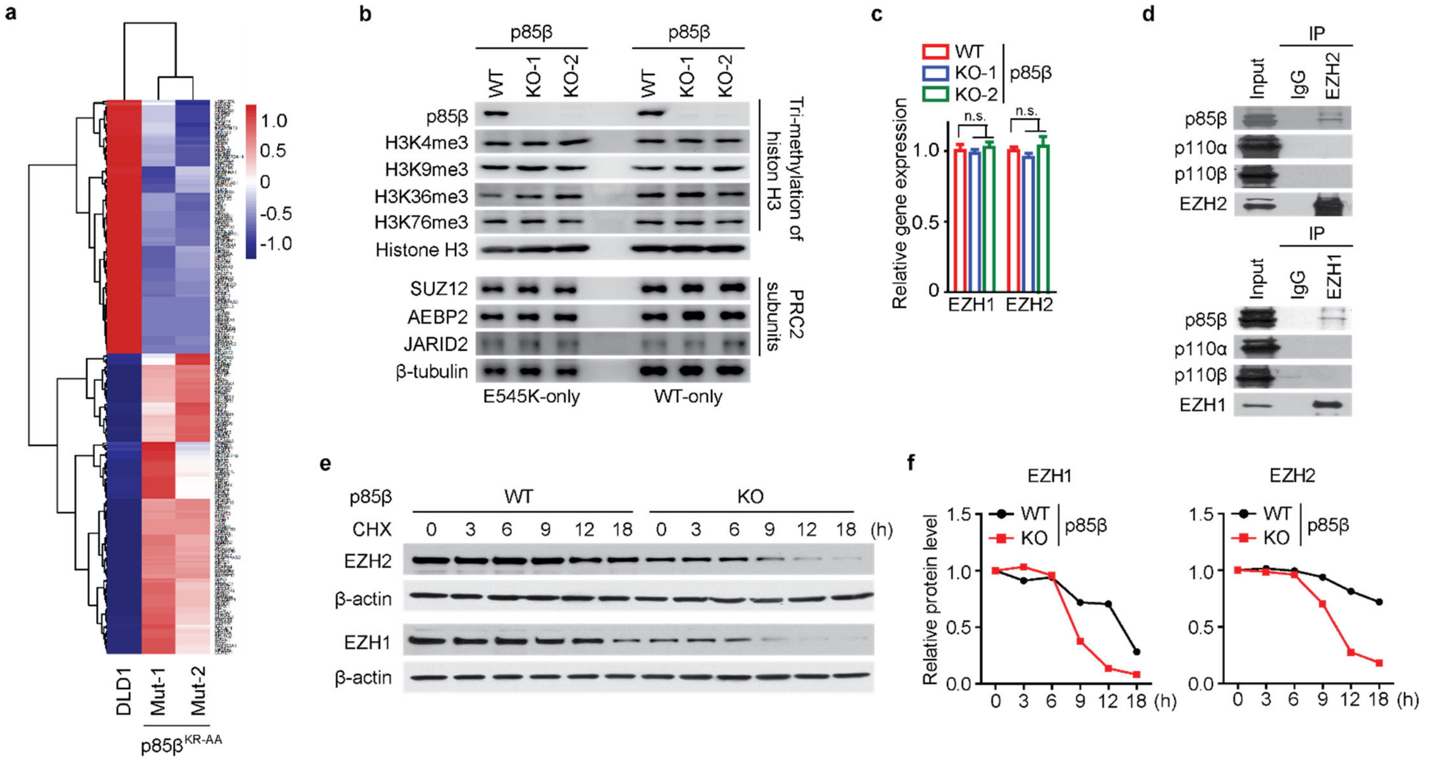


Figure S5. Nuclear p85β increases tri-methylation of H3K27 by stabilizing EZH1/2 proteins.

(a) Heat map analysis of microarray data showing differentially expressed genes between DLD1 and p85β^{KR-AA} mutant cells.

(b) Nuclear p85β have no impact on trimethylation of other sites of histone H3 (except H3K27me3 as shown in Fig. 5 a to c) and protein levels of other subunits of PRC2 (except EZH1 and EZH2 as shown in Fig. 5 a to c). Cell lysates of p85β KO and their control cells were blotted with indicated antibodies.

(**c**) EZH 1 and EZH2 proteins are less stable in p85β knockout E545K-only cells. DLD1 E545K-only and its p85β knockout derivative cells were treated with cycloheximide (CHX) for the indicated times. Cell lysates were blotted with the indicated antibodies. Western blots are shown in the left panel, and quantification of EZH1 and EZH2 protein levels are shown in the right panel.

(d) Knockout of p85β has no effect on mRNA levels of EZH1 and EZH2. Expression of EZH1 and EZH2 in DLD1 PIK3CA E545K-only and DLD1 PIK3CA E545K-only p85β KO cells were analyzed by quantitative RT-PCR.

(e) EZH1 and EZH2 bind to p85β, but not p110α and p110β. EZH1 and EZH2 were immunoprecipitated from DLD1 cell lysates, respectively, and blotted with the indicated antibodies.

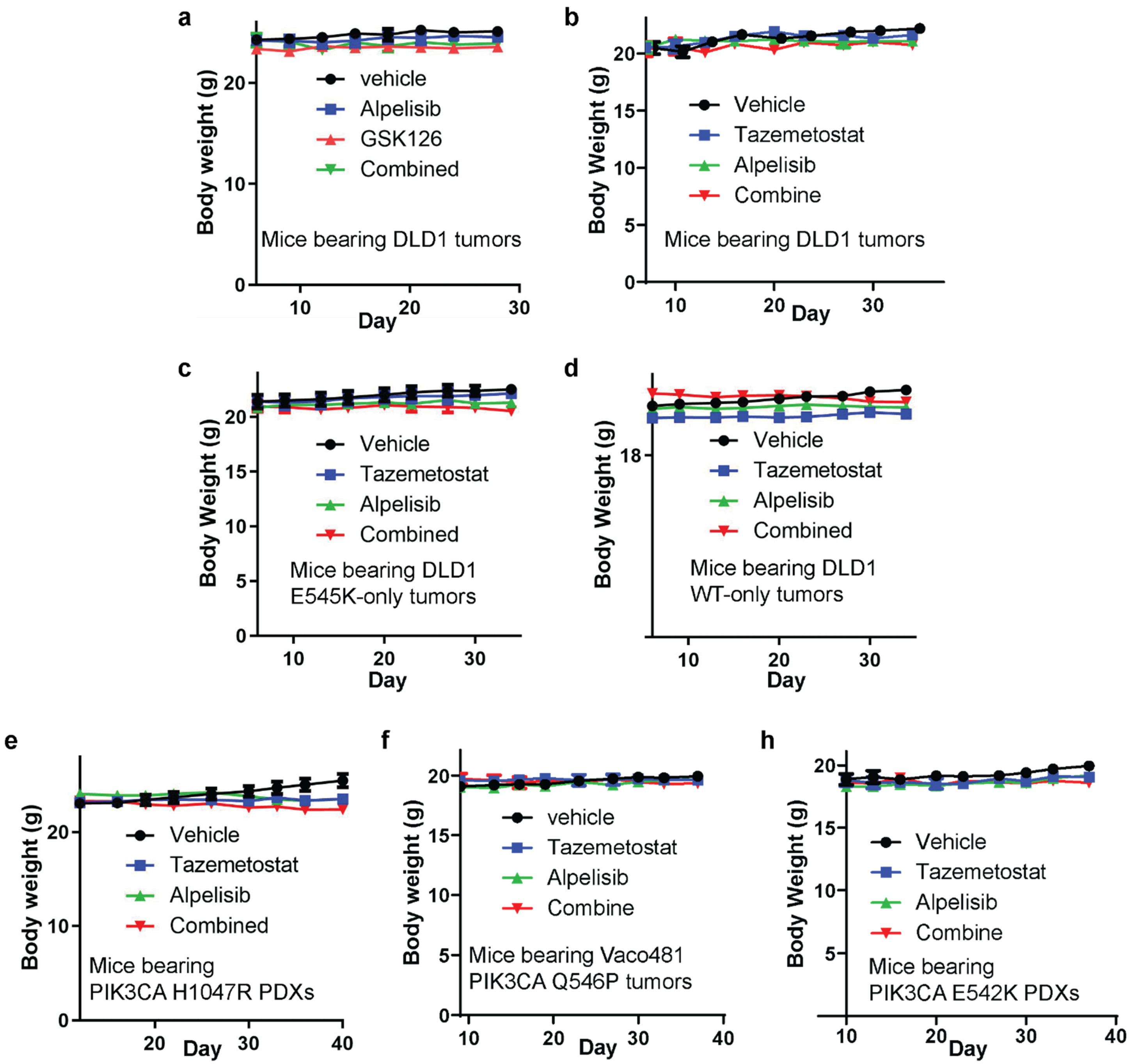


Figure S6. **Body weights of mice treated were maintained during the course of treatment.**

Table S1. List of reagents

Antibodies and Reagents	Source	Identifier
Antibodies		
Mouse monoclonal antibody anti-FLAG	Sigma-Aldrich	Cat# F1804, RRID:AB_262044
Mouse monoclonal antibody anti-Myc	Santa Cruz	Cat# sc-40, RRID:AB_627268
Mouse polyclonal antibody anti-HA	Santa Cruz	Cat# sc-805, RRID:AB_631618
Rabbit monoclonal antibody anti-p110 alpha	Cell Signaling Technology	Cat# 4249, RRID:AB_2165248
Rabbit monoclonal antibody anti-p110 beta	Cell Signaling Technology	Cat# 3011, RRID:AB_2165246
Rabbit monoclonal antibody anti-p85 alpha	Abcam	Cat# ab191606
Rabbit monoclonal antibody anti-p85 alpha pY607	Abcam	Cat# ab182651, RRID:AB_2756407
Rabbit monoclonal antibody anti-p85 beta	Abcam	Cat# ab180967
Rabbit monoclonal antibody anti-p85 beta pY464	Abcam	Cat# ab138364
Rabbit polyclonal antibody anti-IRS1	Proteintech	Cat# 17509-1-AP, RRID:AB_10596914
Rabbit polyclonal antibody anti-BRD7	Proteintech	Cat# 51009-2-AP, RRID:AB_2259226
Rabbit polyclonal antibody anti-β-tubulin	Bioss	Cat# bs-4511R, RRID:AB_11114300
Rabbit monoclonal antibody anti-Lamin B	Bioss	Cat# bsm-33010M
Rabbit monoclonal antibody anti-AKT pT308	Cell Signaling Technology	Cat# 13038, RRID:AB_2629447
Rabbit monoclonal antibody anti-AKT pS473	Cell Signaling Technology	Cat# 4060, RRID:AB_2315049
Rabbit monoclonal antibody anti-AKT	Cell Signaling Technology	Cat# 9272, RRID:AB_329827
Rabbit polyclonal antibody anti-GSK-3β pS9	Cell Signaling Technology	Cat# 9336, RRID:AB_331405
Rabbit monoclonal antibody anti-GSK-3β	Cell Signaling Technology	Cat# 9315, RRID:AB_490890
Rabbit monoclonal antibody anti-FoxO1 pT24	Cell Signaling Technology	Cat# 9464, RRID:AB_329842
Mouse monoclonal antibody anti-FoxO1	Millipore	Cat# 3012276
Rabbit monoclonal antibody anti-mTOR pS2448	Cell Signaling Technology	Cat# 5536, RRID:AB_10691552
Rabbit monoclonal antibody anti-mTOR	Cell Signaling Technology	Cat# 2983, RRID:AB_2105622
Rabbit monoclonal antibody anti-p70 S6 kinase pS371	Cell Signaling Technology	Cat# 9208, RRID:AB_330990
Rabbit monoclonal antibody anti-anti-p70 S6 kinase	Cell Signaling Technology	Cat# 2708, RRID:AB_390722
Rabbit monoclonal antibody anti-Erk1/2 pT202, pT204	Cell Signaling Technology	Cat# 4370, RRID:AB_2315112
Rabbit monoclonal antibody anti-Erk1/2	Cell Signaling Technology	Cat# 4695, RRID:AB_390779
Rabbit monoclonal antibody anti-H3K4me3	Cell Signaling Technology	Cat# 9783, Tri-Methyl Histone H3 Antibody Sampler Kit
Rabbit monoclonal antibody anti-H3K9me3	Cell Signaling Technology	Cat# 9783, Tri-Methyl Histone H3 Antibody Sampler Kit
Rabbit monoclonal antibody anti-H3K27me3	Cell Signaling Technology	Cat# 9783, Tri-Methyl Histone H3 Antibody Sampler Kit
Rabbit monoclonal antibody anti-H3K36me3	Cell Signaling Technology	Cat# 9783, Tri-Methyl Histone H3 Antibody Sampler Kit
Rabbit monoclonal antibody anti-H3K79me3	Cell Signaling Technology	Cat# 9783, Tri-Methyl Histone H3 Antibody Sampler Kit
Rabbit monoclonal antibody anti-H3	Cell Signaling Technology	Cat# 9783, Tri-Methyl Histone H3 Antibody Sampler Kit
Rabbit monoclonal antibody anti-EZH1	Cell Signaling Technology	Cat# 62083, PRC2 Antibody Sampler Kit
Rabbit monoclonal antibody anti-EZH2	Cell Signaling Technology	Cat# 62083, PRC2 Antibody Sampler Kit
Rabbit monoclonal antibody anti-SUZ12	Cell Signaling Technology	Cat# 62083, PRC2 Antibody Sampler Kit
Rabbit monoclonal antibody anti-EED	Cell Signaling Technology	Cat# 62083, PRC2 Antibody Sampler Kit
Rabbit monoclonal antibody anti-JARID2	Cell Signaling Technology	Cat# 62083, PRC2 Antibody Sampler Kit
Rabbit monoclonal antibody anti-AEBP2	Cell Signaling Technology	Cat# 62083, PRC2 Antibody Sampler Kit
Rabbit polyclonal antibody anti-IgG	Cell Signaling Technology	Cat# 2729, RRID:AB_1031062
Chemicals and reagents		
Anti-Flag Affinity Gel	Bimake	Cat# B23101

Anti-Myc tag Mouse mAb conjugated Agarose Beads	Engibody Biotechnology	Cat# AT0080
Anti-HA tag Mouse mAb conjugated Agarose Beads	Engibody Biotechnology	Cat# AT0079
EGF	Sigma-Aldrich	Cat# E5036
Insulin	Sigma-Aldrich	Cat# I2643
Alpelisib	Selleck Chemicals	Cat# S2814
GSK126	Selleck Chemicals	Cat# S7061
Tazemetostat	Selleck Chemicals	Cat# S7128
MG132	Selleck Chemicals	Cat# S2619
DAPI	Sigma-Aldrich	Cat# D9542
Critical Commercial Assay Kits		
USER cloning system	NEB	Cat# #M5505L
Site-Directed Mutagenesis Kit	Agilent	Cat# 200523
EnVision-HRP kit	Dako	Cat# K4001
PrimeScript RT Reagent Kit	TAKARA	Cat# RR037A
Mycoplasma Detection Kit	Yeasen	Cat# 40601ES20

Table S2. List of primers.

Primer name	Primer sequences（5' to 3'）	
Primers for qRT-PCR		
PIK3R2	Forward	AAAGGCGGGAACAATAAGCTG
	Reverse	CAACGGAGCAGAAGGTGAGTG
EZH1	Forward	ATGCGACTTCGACAACTTAAACG
	Reverse	GGCTTCATTGACTGAACAGGTT
EZH2	Forward	AATCAGAGTACATGCGACTGAGA
	Reverse	GCTGTATCCTTCGCTGTTTCC
β-tubulin	Forward	GGCCAAGGGTCACTACACG
	Reverse	GCAGTCGCAGTTTTCACACTC
Primers for sgRNAs and siRNAs		
PIK3R2	Forward	CACCGCCGGCCTCAACGAGCGCACA
sgRNA-1	Reverse	AAACTGTGCGCTCGTTGAGGCCGGC
PIK3R2	Forward	CACCGAGAACTGCTCGGGCAAGTCG
sgRNA-2	Reverse	AAACCGACTTGCCCGAGCAGTTCTC
PIK3R2	Forward	CACCGCCCACTGATCCACGTCGCTC
sgRNA-3	Reverse	AAACGAGCGACGTGGATCAGTGGGC
BRD7	Forward	CACCGTCGGACAAACACCTCTACG
sgRNA-1	Reverse	AAACCGTAGAGGTGTTTGTCCGAC
BRD7	Forward	CACCGAAGTCACCGAACTCTCCAC
sgRNA-3	Reverse	AAACGTGGAGAGTTCGGTGACTTC
BRD7	Forward	CACCGGAGGCAAGTCTAATCTCAC
sgRNA-3	Reverse	AAACGTGAGATTAGACTTGCCTCC
p85β	Forward	GGCUGGACAGCGAAUCUCAdTdT
siRNA-1	Reverse	UGAGAUUCGCUGUCCAGCCdTdT
p85β	Forward	GGAACAAUAAGCUGAUCAAdTdT
siRNA-2	Reverse	UUGAUCAGCUUAUUGUUCCdTdT
Subcloning primers		
FLAG-p110α	Forward	GGTCCCA/ideoxyU/TGCCTCCACGACCATCATCAG
WT	Reverse	GGCATAG/ideoxyU/TCAGTTCAATGCATGCTGTT
FLAG-p110α	Forward	GATGAAACAAGACAACTTTGTGACCTTCGG
R88Q	Reverse	CCGAAGGTCACAAAGTTGTCTTGTTTCATC
FLAG-p110α	Forward	CAACCGTGAAGAAAACATCCTCAATCGAGA
K111N	Reverse	TCTCGATTGAGGATGTTTTCTTCACGGTTG
FLAG-p110α	Forward	GCAACCTACGTGAAAGTAAATATTCGAGAC
N345K	Reverse	GTCTCGAATATTTACTTTCACGTAGGTTGC
FLAG-p110α	Forward	GCTAAAGAGGAACACCGTCCATTGGCATGG
C420R	Reverse	CCATGCCAATGGACGGTGTTCCTCTTTAGC
FLAG-p110α	Forward	CGAGATCCTCTCTCTAAAATCACTGAGCAG
E542K	Reverse	CTGCTCAGTGATTTTAGAGAGAGGATCTCG
FLAG-p110α	Forward	CTCTCTGAAATCACTGCGCAGGAGAAAGAT
E545K	Reverse	ATCTTTCTCCTGCGCAGTGATTTCAGAGAG
FLAG-p110α	Forward	CTGAAATCACTGAGAAGGAGAAAGATTTTC
Q546K	Reverse	GAAAATCTTTCTCCTTCTCAGTGATTTCAG
FLAG-p110α	Forward	CATGAAACAAATTAATGATGCACATCATGG
M1043I	Reverse	CCATGATGTGCATCATTAATTTGTTTCATG
FLAG-p110α	Forward	CAAATGAATGATGCACTTCATGGTGGCTGG
H1047L	Reverse	CCAGCCACCATGAAGTGCATCATTCATTTG
FLAG-p110α	Forward	GAATGATGCACATCATCGTGGCTGGACAAC
G1049R	Reverse	GTTGTCCAGCCACGATGATGTGCATCATTC
HA-p85α	Forward	CCGGAATTCATGAGTGCTGAGGGGTACCAG
	Reverse	ACGCGTCGACTCATCGCCTCTGCTGTGCATA
HA-p85β	Forward	CCGGAATTCATGGCGGGCCCTGAGGGCTTC
	Reverse	CCGCTCGAGTCAGCGGGCGGCAGGCGGCG
HA-p85β KR477,478AA	Forward	CTCCCAGGAGCTGCAGATGGCGGCTACTGCAATTG AGGCCTTC

	Reverse	GAAGGCCTCAATTGCAGTAGCCGCCATCTGCAGCTCCTGGGAG
	Left Arm forward	GGGAAAG/ideoxyU/CACAATGGCTCAAGCCTGTA
	Left Arm reverse	GGAGACA/ideoxyU/CCTGGGACTCCCCAAAAGGC
	Right Arm forward	GGTCCCA/ideoxyU/AGGTGCTGAGCTGCGCC
	Right Arm reverse	GGCATAG/ideoxyU/CTCTCATGGATCTCGGCAAT
	KR477, 478AA of p85b for genomic DNA and PAM mutation for gRNA, forward	GCGCCCACTCCTCCAGGAGCTGCAGATGGCGGCTACTGCAATT
p85β KR477, 478AA Knockin vector	KR477, 478AA of p85b for genomic DNA and PAM mutation for gRNA, reverse	AATTGCAGTAGCCGCCATCTGCAGCTCCTGGAGGAGTGGGCGC
	P1 for AAV and p85b screening, forward	TTTTGTCACTCAAGGACTGTGC
	P2 for AAV and p85b screening, reverse	TATGGAGCCGCCACTTACAC
	gRNA for p85b genomic DNA, forward	CACCGTCATCTGCAGCTCCTGGAGG
	gRNA for p85b genomic DNA, reverse	AAACCCTCCAGGAGCTGCAGATGAC