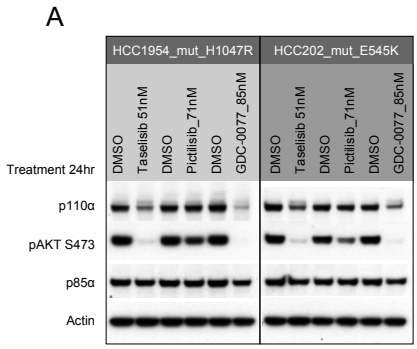
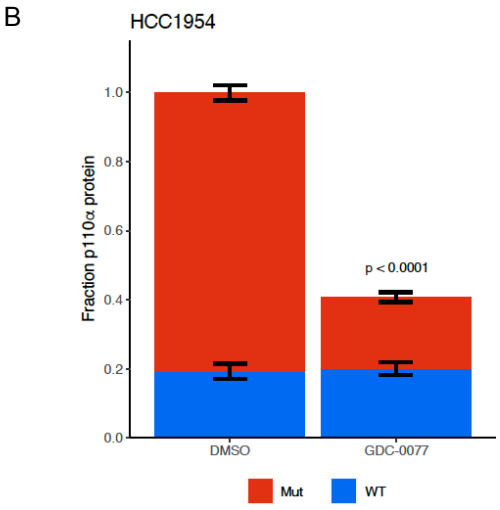
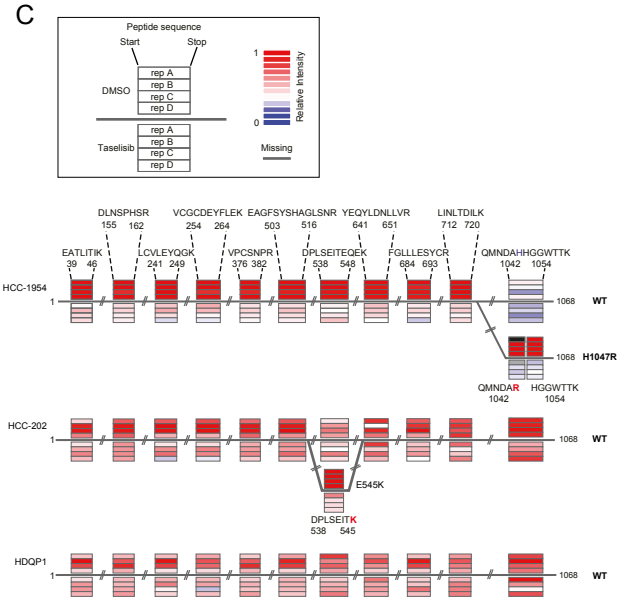
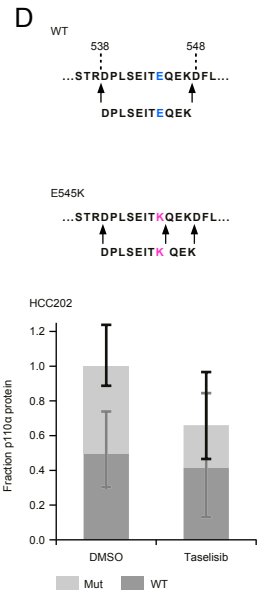
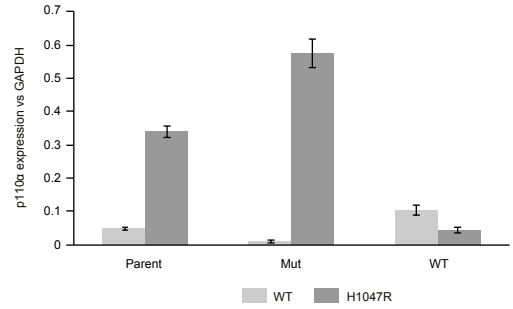
Figure S4

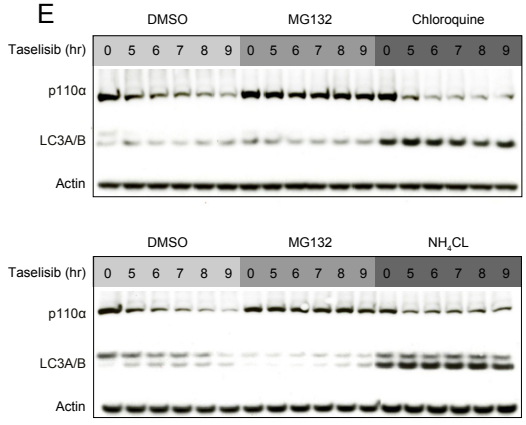

A
HCC1954_mut_H1047R
HCC202_mut_E545K
DMSO
Taselisib 51nM
DMSO
Pictilisib_71nM
DMSO
GDC-0077_85nM
DMSO
Taselisib 51nM
DMSO
Pictilisib_71nM
DMSO
GDC-0077_85nM
Treatment 24hr
p110α
pAKT S473
p85α
Actin

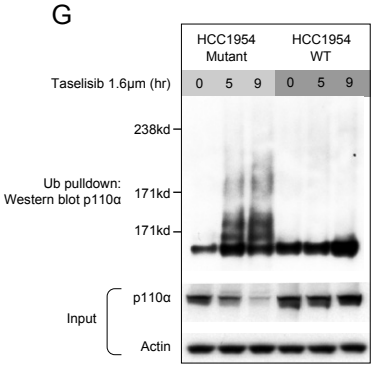

B
HCC1954
Fraction p110α protein
p < 0.0001
DMSO
GDC-0077
Mut
WT


C
Peptide sequence
Start
Stop
DMSO
rep A
rep B
rep C
rep D
Taselisib
Relative Intensity
Missing
HCC-1954
HCC-202
HDQP1
WT
H1047R
E545K


D
WT
...STRDPLSEITEQEKDFL...
DPLSEITEQEK
E545K
...STRDPLSEITKQEKDFL...
DPLSEITK QEK
HCC202
Fraction p110α protein
DMSO
Taselisib
Mut
WT


F
DNA Allele frequency (%) by NGS
1047 allele
HCC1954 parental
HCC1954_mutant
HCC1954_mutant
H (WT)
12%
0%
85%
R (mutant)
88%
100%
15%
p110α expression vs GAPDH
Parent
Mut
WT
WT
H1047R


E
DMSO
MG132
Chloroquine
Taselisib (hr)
p110α
LC3A/B
Actin
DMSO
MG132
NH4CL
Taselisib (hr)
p110α
LC3A/B
Actin


G
HCC1954 Mutant
HCC1954 WT
Taselisib 1.6μm (hr)
Ub pulldown: Western blot p110α
238kd
171kd
171kd
Input
p110α
Actin

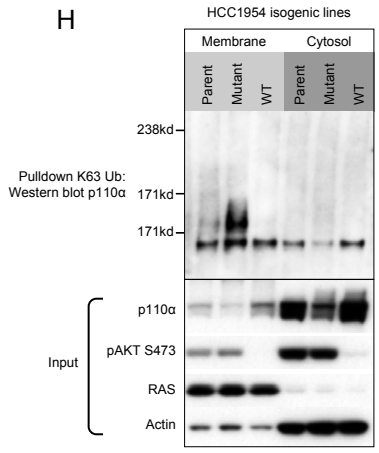

H
HCC1954 isogenic lines
Membrane
Cytosol
Parent
Mutant
WT
Parent
Mutant
WT
Pulldown K63 Ub: Western blot p110α
238kd
171kd
171kd
Input
p110α
pAKT S473
RAS
Actin

Figure S4

Taselisib depletes mutant p110α protein through ubiquitin and proteasome mechanism in a dose and time dependent manner

(A) PIK3CA-mutant cells were treated with PI3K inhibitors at concentrations relevant to plasma concentrations achieved with clinically administered doses for pictilisib (GDC-0941) (330 mg), taselisib (6 mg), and GDC-0077 (9 mg).

(B) Mass spectrometry of HCC1954 cells treated for 24 hours with 500 nM GDC-0077. A neo tryptic peptide generated from PIK3CA H1047R was used to assess mutant protein levels compared to wild-type protein in the same lysate.

(C) Quantitative mass spectrometry analysis of p110α levels in HCC1954, HCC202 and HDQP1 cells treated with either DMSO or 500 nM taselisib for 24 hours (n=4). For each cell line, the relative abundance of peptides from loci spanning the protein are compared between DMSO (above line) and taselisib-treated (below line) cells. For mutant loci (H1047R in HCC1954 cells, E545K in HCC202 cells), the protein sequence is split to depict the wild-type and mutant-specific peptides in parallel.

(D) Mass spectrometry analysis of peptides representing wild-type and E545K mutant p110α from HCC202 breast cancer cells treated for 24 hours with either DMSO or 500 nM taselisib. The neo trypic peptide generated from p110α E545K was used to assess mutant protein levels compared to wild-type protein in the same lysate (n=4).

(E) Lysosomotropic agents chloroquine and ammonium chloride (NH_4Cl) do not rescue p110α degradation induced by 1.6 μM taselisib in HCC1954 cells.

(F) NGS and qRT-PCR for allele-specific mRNA expression in HCC1954 parental and isogenic HCC1954_mutant and HCC1954_wild-type cells. Error bars are standard deviation of triplicates.

(G) HCC1954_mutant and HCC1954_wild-type isogenic cells were treated with 1.6 μM taselisib for up to 9 hours. Pull down of ubiquitinated protein was followed by western blotting with anti p110α antibody.

(H) Subcellular fractionation of HCC1954_parental and isogenic HCC1954_mutant and HCC1954_WT cells. Pull down of ubiquitinated protein was followed by western blotting with anti-p110alpha .