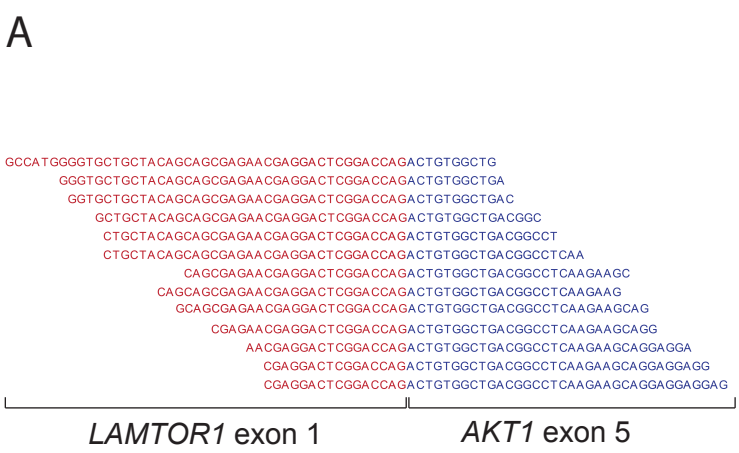
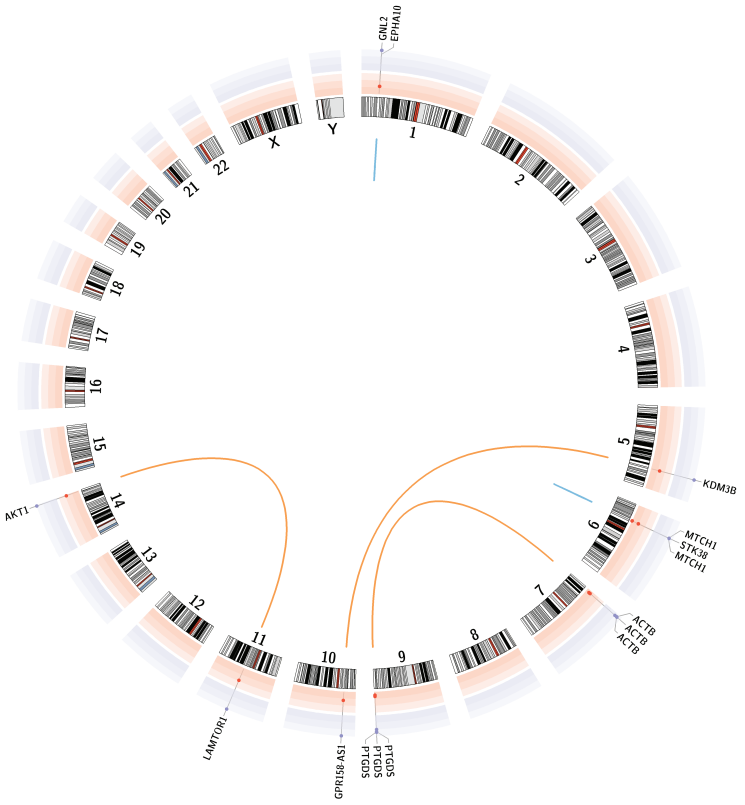
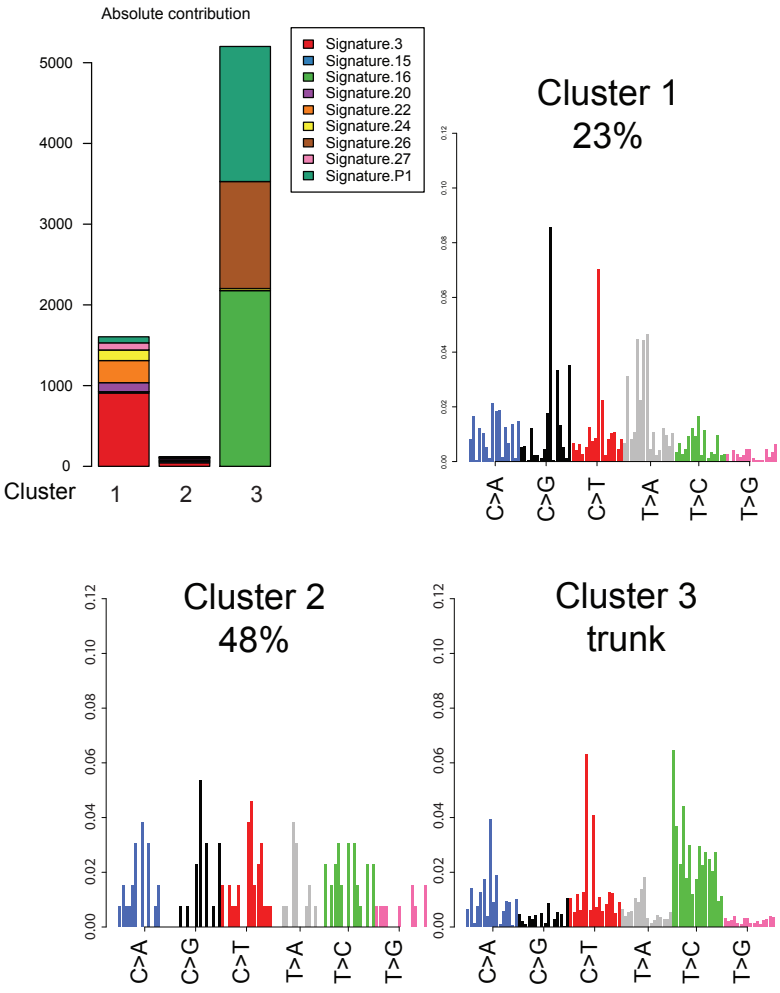
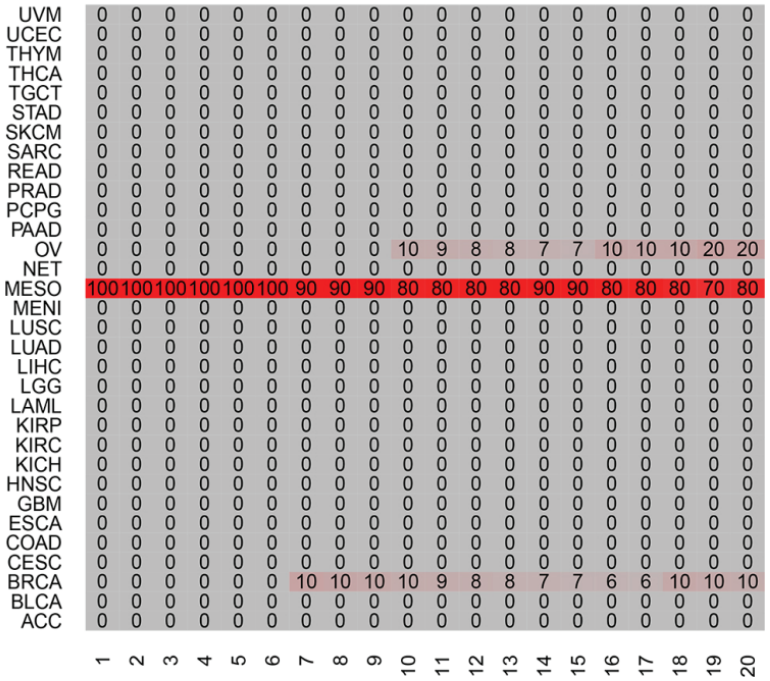
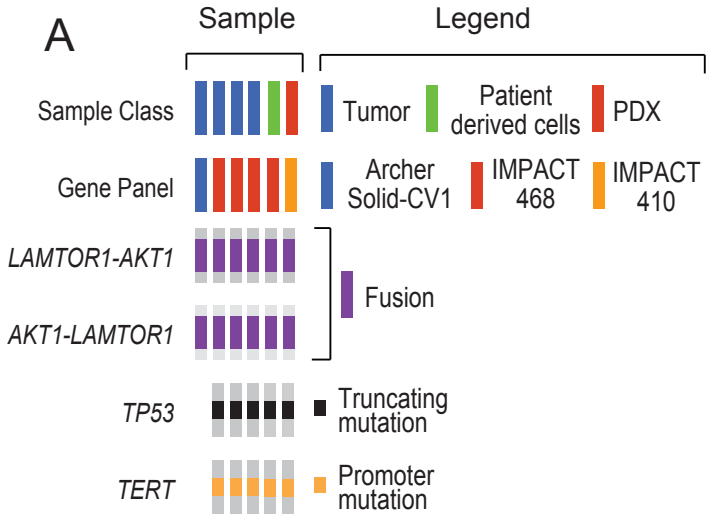

A
GCCATGGGGTGCTGCTACAGCAGCGAGAACGAGGACTCGGACCAGACTGTGGCTG
GGGTGCTGCTACAGCAGCGAGAACGAGGACTCGGACCAGACTGTGGCTGA
GGTGCTGCTACAGCAGCGAGAACGAGGACTCGGACCAGACTGTGGCTGAC
GCTGCTACAGCAGCGAGAACGAGGACTCGGACCAGACTGTGGCTGACGGC
CTGCTACAGCAGCGAGAACGAGGACTCGGACCAGACTGTGGCTGACGGCCT
CTGCTACAGCAGCGAGAACGAGGACTCGGACCAGACTGTGGCTGACGGCCTCAA
CAGCGAGAACGAGGACTCGGACCAGACTGTGGCTGACGGCCTCAAGAAGC
CAGCAGCGAGAACGAGGACTCGGACCAGACTGTGGCTGACGGCCTCAAGAAG
GCAGCGAGAACGAGGACTCGGACCAGACTGTGGCTGACGGCCTCAAGAAGCAG
CGAGAACGAGGACTCGGACCAGACTGTGGCTGACGGCCTCAAGAAGCAGG
AACGAGGACTCGGACCAGACTGTGGCTGACGGCCTCAAGAAGCAGGAGGA
CGAGGACTCGGACCAGACTGTGGCTGACGGCCTCAAGAAGCAGGAGGAGGAG
CGAGGACTCGGACCAGACTGTGGCTGACGGCCTCAAGAAGCAGGAGGAGGAG
LAMTOR1 exon 1
AKT1 exon 5

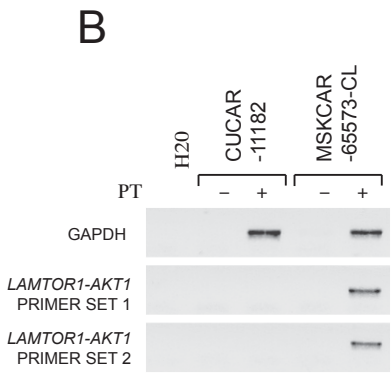

B
GNL2
EPHA10
KDM3B
MTCH1
STK38
MTCH1
ACTB
ACTB
ACTB
PTGDS
PTGDS
PTGDS
GPR158-AS1
LAMTOR1
AKT1

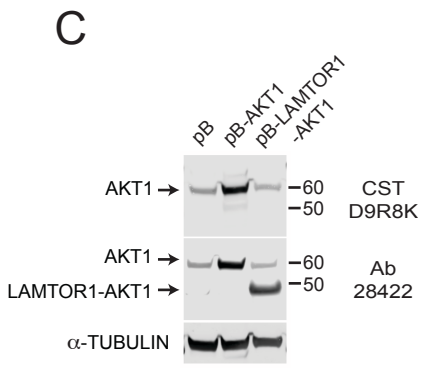

C
Absolute contribution
Signature.3
Signature.15
Signature.16
Signature.20
Signature.22
Signature.24
Signature.26
Signature.27
Signature.P1
Cluster
Cluster 1
23%
Cluster 2
48%
Cluster 3
trunk
C>A
C>G
C>T
T>A
T>C
T>G

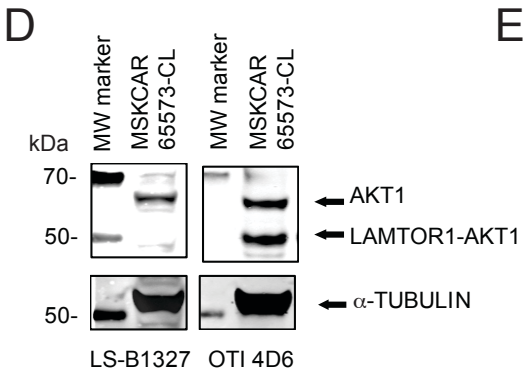

D
UVM
UCEC
THYM
THCA
TGCT
STAD
SKCM
SARC
READ
PRAD
PCPG
PAAD
OV
NET
MESO
MENI
LUSC
LUAD
LIHC
LGG
LAML
KIRP
KIRC
KICH
HNSC
GBM
ESCA
COAD
CESC
BRCA
BLCA
ACC

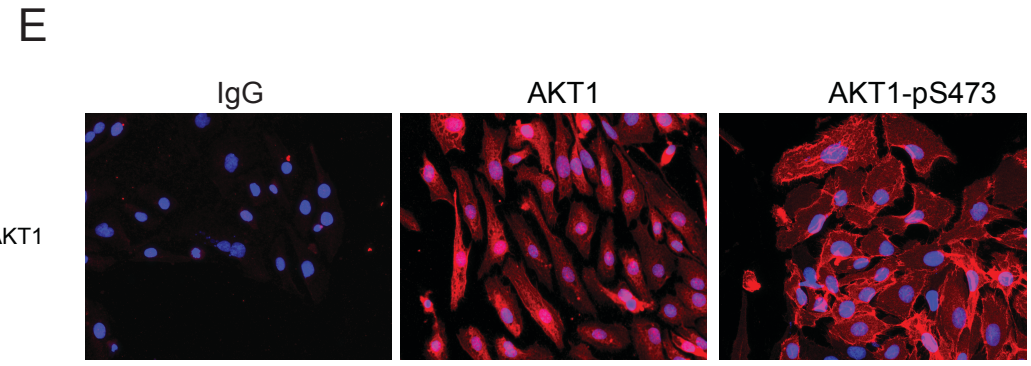
Supplementary Figure 1. A) RNA-sequencing reads spanning the junction between LAMTOR1 exon 1 and AKT1 exon5 **B)** Circos plot representing fusion events confirmed via RNA sequencing **C)** Mutational signature analysis of independent tumor clones using deconstructSigs. **D)** Heatmap representing the percentage of each tumor type in the k (from 1 to 20) nearest neighbors space of the patient sample using spearman correlation as distance; tumor type abbreviations: ACC adrenocortical carcinoma, BLCA bladder urothelial carcinoma, BRCA breast carcinoma, CESC cervical carcinoma, COAD colon adenocarcinoma, ESCA esophageal carcinoma, GBM glioblastoma multiforme, HNSC head and neck carcinoma, KICH kidney chromophobe, KIRC clear cell carcinoma of the kidney, KIRP renal papillary cell carcinoma, LAML acute myeloid leukemia, LGG low grade glioma, LIHC hepatocellular carcinoma, LUAD lung adenocarcinoma, LUSC lung squamous cell carcinoma, MENI meningioma, MESO mesothelioma, NET gastrointestinal neuroendocrine tumor, OV ovarian carcinoma, PAAD pancreatic adenocarcinoma, PCPG pheochromocytoma and paraganglioma, PRAD prostate adenocarcinoma, READ rectal adenocarcinoma, SARC sarcoma, SKCM cutaneous melanoma, STAD gastric adenocarcinoma, TGCT testicular germ cell tumor, THCA thyroid carcinoma, THYM thymoma, UCEC uterine corpus endometrial carcinoma, UVM uveal melanoma

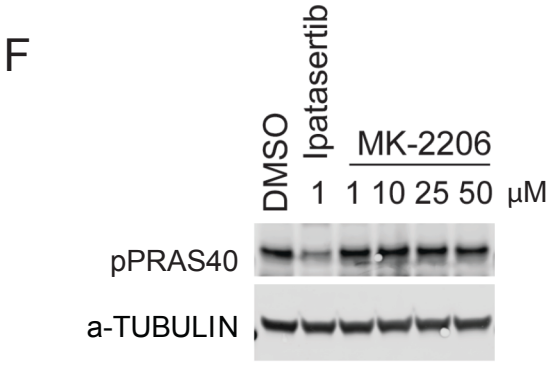

A
Sample
Legend
Sample Class
Tumor
Patient derived cells
PDX
Gene Panel
Archer Solid-CV1
IMPACT 468
IMPACT 410
LAMTOR1-AKT1
AKT1-LAMTOR1
Fusion
TP53
Truncating mutation
TERT
Promoter mutation

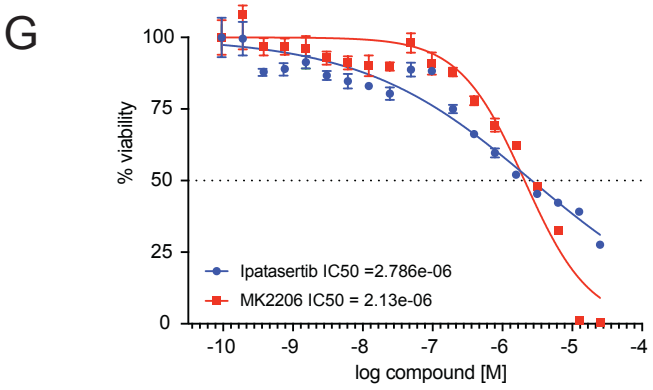

B
H20
CUCAR -11182
MSKCAR -65573-CL
PT
– + – +
GAPDH
LAMTOR1-AKT1 PRIMER SET 1
LAMTOR1-AKT1 PRIMER SET 2


C
pB
pB-AKT1
pB-LAMTOR1 -AKT1
AKT1
60
50
CST D9R8K
AKT1
LAMTOR1-AKT1
60
50
Ab 28422
α-TUBULIN

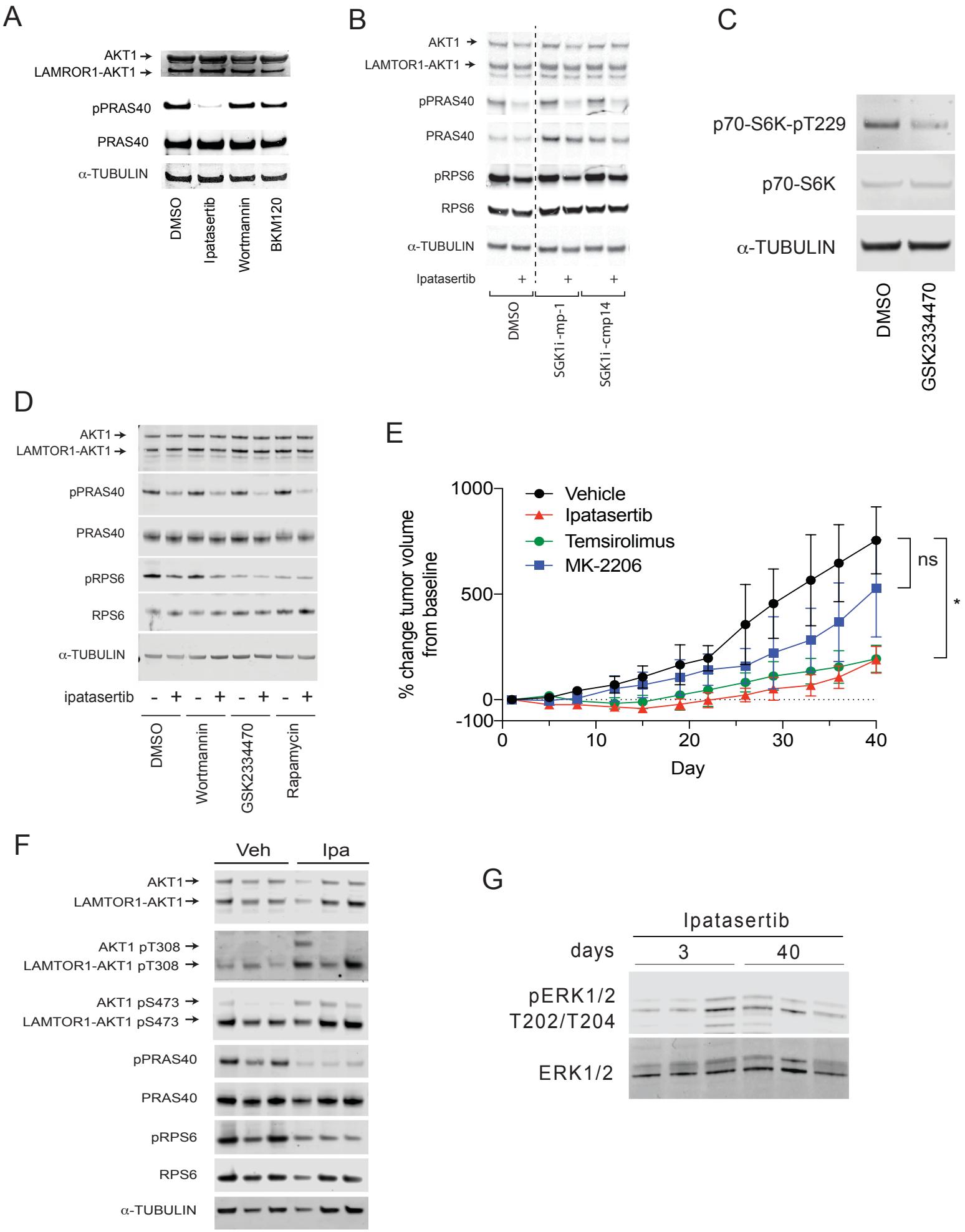

D
kDa
MW marker
MSKCAR 65573-CL
MW marker
MSKCAR 65573-CL
70-
50-
50-
AKT1
LAMTOR1-AKT1
α-TUBULIN
LS-B1327
OTI 4D6


E
IgG
AKT1
AKT1-pS473


F
DMSO
Ipatasertib
MK-2206
1 1 10 25 50 μM
pPRAS40
a-TUBULIN

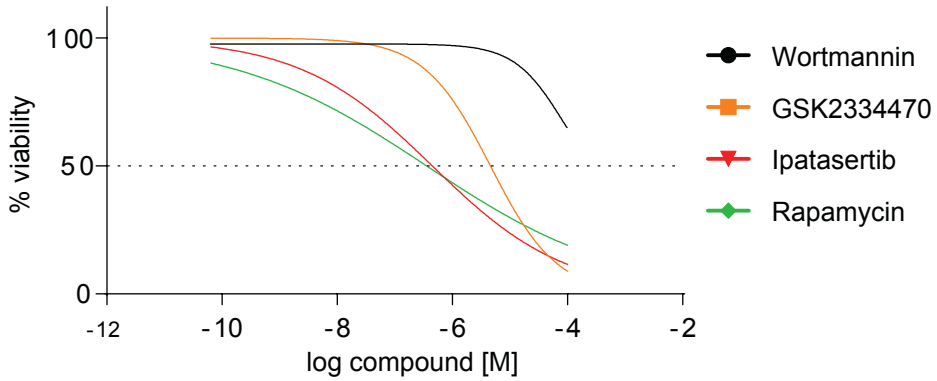

G
% viability
100
75
50
25
0
-10
-9
-8
-7
-6
-5
-4
log compound [M]
Ipatasertib IC50 =2.786e-06
MK2206 IC50 = 2.13e-06

Supplementary Figure 2. A) cBioPortal Oncoprint representation of genomic resemblance between patient tumors (blue), patient derived cells (green) and xenograft (PDX) (red) samples via RNA-seq (Archer Solid-CV1), and IMPACT^{TM} 410 and 468 sequencing platforms **B)** RT-PCR showing expression of LAMTOR1-AKT1 RNA in MSKCAR-65573-CL cells **C)** Transient expression of AKT1 and LAMTOR1-AKT1 in HEK-293T cells confirming the molecular weight of the LAMTOR1-AKT1 fusion protein **D)** Immunoblotting analysis showing detection of AKT1 and LAMTOR1-AKT1 by C-terminal AKT1 antibody (OTI 4D6) (also see Figure 3A and 3C) and exclusive detection of AKT1 by an N-terminal AKT1 antibody (LS-B1327) **E)** Immunofluorescence analysis of AKT1 localization using LS-B1327 antibody when compared with the AKT1-pS473 localization **F)** Phosphorylation levels of PRAS40 upon ipatasertib or MK2206 treatment, showing the inability of MK2206 to inhibit LAMTOR1-AKT1 activity at any dose tested in MSKCAR-65573-PDCL **G)** Evaluation of Ipatasertib and MK-2206 efficacy in vitro confirming MK-2206 efficacy on A204 cells


A
AKT1
LAMROR1-AKT1
pPRAS40
PRAS40
α-TUBULIN
DMSO
Ipatasertib
Wortmannin
BKM120
B
AKT1
LAMTOR1-AKT1
pPRAS40
PRAS40
pRPS6
RPS6
α-TUBULIN
Ipatasertib
+
+
+
DMSO
SGK1i -mp-1
SGK1i -cmp14
C
p70-S6K-pT229
p70-S6K
α-TUBULIN
DMSO
GSK2334470
D
AKT1
LAMTOR1-AKT1
pPRAS40
PRAS40
pRPS6
RPS6
α-TUBULIN
ipatasertib
- + - + - + - +
DMSO
Wortmannin
GSK2334470
Rapamycin
E
% change tumor volume from baseline
1000
500
0
-100
0
10
20
30
40
Day
Vehicle
Ipatasertib
Temsirolimus
MK-2206
ns
*
F
Veh
Ipa
AKT1
LAMTOR1-AKT1
AKT1 pT308
LAMTOR1-AKT1 pT308
AKT1 pS473
LAMTOR1-AKT1 pS473
pPRAS40
PRAS40
pRPS6
RPS6
α-TUBULIN
G
Ipatasertib
days
3
40
pERK1/2
T202/T204
ERK1/2

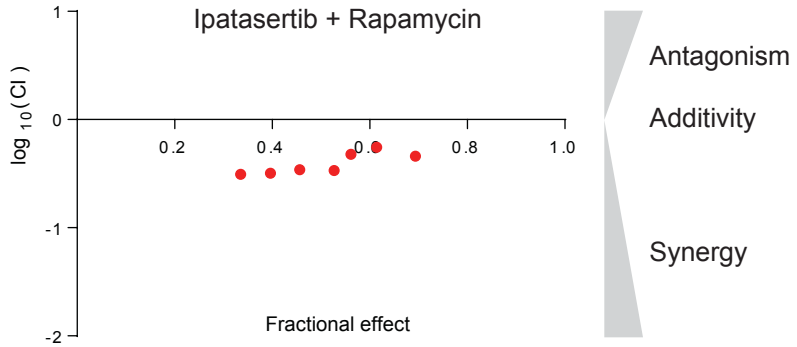
Supplementary Figure 3. A) Expression and phosphorylation levels of indicated proteins in MSKCAR-65573-CL cells upon treatment with PI3K inhibitors wortmannin or BKM120 **B)** Expression levels and phosphorylation levels of indicated proteins in MSKCAR-65573-PDCL upon treatment with SGK1 inhibitors alone or in combination with ipatasertib; all lanes come from a unique gel and exposure and lanes 3-6 were shifted in proximity to lanes 1-2 for presentation **C)** Phosphorylation levels of p70-S6K T-229 in response to PDK1 inhibition **D)** Expression and phosphorylation levels of indicated proteins in MSKCAR-65573-PDCL upon treatment with the specified inhibitors alone or in combination with ipatasertib **E)** Growth curve plot representation of the PDX *in vivo* testing comparing continuous vehicle, ipatasertib, MK-2206 and temsirolimus treatments up to 40 days using area under the curve (AUC) analysis; *=$p<0.05$ **F)** Expression levels and phosphorylation levels of indicated proteins after 40 days of continuous ipatasertib treatment **G)** Expression levels and phosphorylation levels of ERK1/2 Thr202/204 cells on samples continuously treated with ipatasertib for 3 or 40 days, showing no upregulation of the MEK/ERK pathway

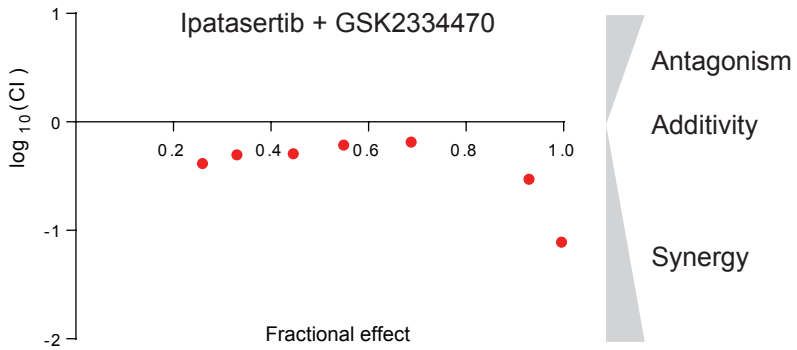
A



	Wortmannin	GSK2334470	Ipatasertib	Rapamycin
IC50	N/A	4.603e-006	4.514e-007	3.61e-007

B





Supplementary Figure 4. A) Dose response curve of MSKCAR-65573-CL cells treated with the indicated inhibitor for 72 hours **B)** Analysis of drug combination using the Chou-Talalay method CI =combination index, fractional effect =fraction of cells affected; each dot represents a different dose of the combined drugs