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online data

SUPPLEMENTARY ONLINE DATA

A drug targeting only p110α can block phosphoinositide 3-kinase signalling and tumour growth in certain cell types

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	% Activity remaining A66 10 μM	PIK75 10 μM	TGX221 10 μM	IC87114 10 μM
MKK1	108	4	84	86
MKK2	116	9	114	105
MKK6	85	55	83	97
ERK1	99	16	103	100
ERK2	92	42	103	105
JNK1	90	19	102	95
JNK2	102	62	86	90
JNK3	106	56	104	101
p38a MAPK	96	98	92	93
p38b MAPK	98	62	99	94
p38g MAPK	90	4	78	100
p38d MAPK	95	2	74	93
ERK8	91	18	102	98
RSK1	84	3	88	99
RSK2	99	4	84	113
PDK1	94	22	99	100
PKBa	97	4	122	103
PKBb	99	70	79	105
SGK1	88	22	103	108
S6K1	98	3	101	104
PKA	89	4	93	94
ROCK 2	100	2	105	97
PRK2	90	1	82	80
PKCa	90	15	103	98
PKCz	110	39	102	106
PKD1	92	36	93	105
MSK1	115	11	90	93
MNK1	91	87	99	94
MNK2	102	47	99	96
MAPKAP-K	105	19	98	98
MAPKAP-K	90	96	83	106
PRAK	85	24	97	98
CAMKKb	93	24	102	116
CAMK1	109	61	92	98
SmMLCK	81	8	98	94
PHK	98	4	87	94

	% Activity remaining A66 10 μM	PIK75 10 μM	TGX221 10 μM	IC87114 10 μM
DAPK1	81	2	102	110
CHK1	97	76	100	146
CHK2	117	34	105	88
GSK3b	95	1	28	98
CDK2-Cyclin	103	2	81	90
PLK1	112	13	89	92
Aurora A	112	13	102	103
Aurora B	109	38	96	98
LKB1	105	10	85	89
AMPK	108	16	98	108
MARK1	97	5	94	96
MARK2	86	36	105	86
MARK3	83	2	99	98
MARK4	87	43	100	103
BRSK1	106	70	185	97
BRSK2	92	30	78	106
MELK	116	4	96	102
NUAK1	81	22	77	100
CK1	95	6	86	90
CK2	91	4	82	111
DYRK1A	96	0	83	94
DYRK2	86	5	89	87
DYRK3	83	-1	85	97
NEK2a	111	9	94	98
NEK6	89	112	95	111
IKKb	114	46	91	95
IKKe	68	8	86	95
TBK1	83	54	93	97
PIM1	111	8	76	102
PIM2	102	13	87	91
PIM3	97	12	64	81
SRPK1	95	83	98	117
EF2K	94	86	92	65
HIPK1	88	8	109	89
HIPK2	100	4	78	98
HIPK3	92	10	99	97
CLK2	20	1	41	89
PAK2	85	14	102	100

	% Activity remaining A66 10 μM	PIK75 10 μM	TGX221 10 μM	IC87114 10 μM
PAK4	100	8	89	71
PAK5	85	29	86	85
PAK6	93	33	105	91
MST2	94	14	101	120
MST4	99	16	103	100
GCK	71	22	121	103
MINK1	100	6	101	106
MEKK1	135	13	92	97
MLK1	83	26	99	100
MLK3	99	13	104	93
TAO1	97	8	73	90
ASK1	93	12	94	94
TAK1	105	6	88	103
IRAK4	67	23	107	105
RIPK2	22	19	76	113
TTK	84	15	74	92
Src	115	19	84	95
Lck	111	6	61	96
CSK	108	22	88	92
YES1	113	19	58	88
BTK	96	6	67	105
JAK2	95	2	99	88
SYK	118	20	101	88
EPH-A2	126	71	76	111
EPH-A4	114	13	63	107
EPH-B1	106	77	99	108
EPH-B2	87	8	84	101
EPH-B3	114	8	115	108
EPH-B4	95	27	73	94
FGF-R1	116	11	86	93
HER4	108	13	111	108
IGF-1R	90	7	105	104
IR	108	4	97	96
IRR	99	12	85	97
TrkA	103	8	70	91
VEG-FR	97	11	78	93

Figure S1 Activity of kinases after addition of A66, PIK-75, TGX-221 or IC87114

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Conflict of interest statement: P.R.S., W.A.D., J.D.K. and G.W.R. have consulted for and own stock in Pathway Therapeutics, a company developing PI3K inhibitors, although none of these compounds are used in the present study.

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Enzyme	% Inhibition by 10 μM A66
ABL1	14
ABL1 E255K	11
ABL1 G250E	8
ABL1 T315I	8
ABL1 Y253F	8
ABL2 (Arg)	-3
ACVR1 (ALK2)	47
ACVR1B (ALK4)	-2
ACVR2B	-2
ADRBK1 (GRK2)	-1
ADRBK2 (GRK3)	-4
AKT1 (PKB alpha)	-1
AKT2 (PKB beta)	-9
AKT3 (PKB gamma)	-13
AMPK A1/B1/G1	-1
AMPK A2/B1/G1	-3
AURKA (Aurora A)	-3
AURKB (Aurora B)	2
AURKC (Aurora C)	12
AXL	14
BLK	6
BMPR1A (ALK3)	12
BMX	3
BRAF	-10
BRAF	4
BRAF V599E	-13
BRAF V599E	7
BRSK1 (SAD1)	-4
BTK	-2
CAMK1 (CaMK1)	29
CAMK1D (CaMKI delta)	-3
CAMK2A (CaMKII alpha)	-2
CAMK2B (CaMKII beta)	-4
CAMK2D (CaMKII delta)	-3
CAMK4 (CaMKIV)	-8
CAMKK1 (CAMKKA)	2
CAMKK2 (CaMKK beta)	14
CDC42 BPA (MRCKA)	-14
CDC42 BPB (MRCKB)	7
CDK1/cyclin B	-1
CDK2/cyclin A	3
CDK5/p25	1
CDK5/p35	-1
CDK7/cyclin H/MNAT1	-20
CDK8/cyclin C	13
CDK9/cyclin K	-1
CDK9/cyclin T1	-16
CHEK1 (CHK1)	19
CHEK2 (CHK2)	-3
CHUK (IKK alpha)	11
CLK1	27
CLK2	58
CLK3	28
CLK4	87
CSF1R (FMS)	9
CSK	7
CSNK1A1 (CK1 alpha 1)	0
CSNK1D (CK1 delta)	3
CSNK1E (CK1 epsilon)	7
CSNK1G1 (CK1 gamma 1)	-5
CSNK1G2 (CK1 gamma 2)	10
CSNK1G3 (CK1 gamma 3)	3
CSNK2A1 (CK2 alpha 1)	7
CSNK2A2 (CK2 alpha 2)	1
DAPK1	-37
DAPK3 (ZIPK)	0
DCAMKL2 (DCK2)	0
DDR1	3
DDR2	2
DMPK	1
DNA-PK	12
DYRK1A	14
DYRK1B	4
DYRK3	-12
DYRK4	-2
EEF2K	-5
EGFR (ErbB1)	0
EGFR (ErbB1) L858R	-3
EGFR (ErbB1) L861Q	-1
EGFR (ErbB1) T790M	7
EGFR (ErbB1) T790M L858R	6
EPHA1	-1
EPHA2	-3
EPHA3	16
EPHA4	19
EPHA5	2
EPHA7	-2
EPHA8	-3
EPHB1	2
EPHB2	4
EPHB3	0
EPHB4	-9
ERBB2 (HER2)	4
ERBB4 (HER4)	-10
FER	-10
FES (FPS)	0
FGFR1	1
FGFR2	9
FGFR3	18
FGFR3 K650E	11

Enzyme	% Inhibition by 10 μM A66
FGFR4	16
FGR	6
FLT1 (VEGFR1)	0
FLT3	12
FLT3 D835Y	4
FLT4 (VEGFR3)	14
FRAP1 (mTOR)	2
FRK (PTK5)	2
FYN	6
GRK4	-8
GRK5	0
GRK6	8
GRK7	4
GSG2 (Haspin)	-14
GSK3A (GSK3 alpha)	-6
GSK3B (GSK3 beta)	-1
HCK	3
HIPK1 (Myak)	-12
HIPK2	4
HIPK3 (YAK1)	-2
HIPK4	30
IGF1R	-9
IKBKB (IKK beta)	-1
IKBKE (IKK epsilon)	0
INSR	1
INSRR (IRR)	1
IRAK1	-18
IRAK4	6
ITK	-7
JAK1	10
JAK2	19
JAK2 JH1 JH2	15
JAK2 JH1 JH2 V617F	3
JAK3	14
KDR (VEGFR2)	1
KIT	29
KIT T670I	0
KIT V654A	8
LCK	2
LIMK1	7
LIMK2	4
LRRK2	35
LRRK2 G2019S	56
LTK (TYK1)	17
LYN A	0
LYN B	-4
MAP2K1 (MEK1)	-3
MAP2K1 (MEK1)	14
MAP2K1 (MEK1) S218D S222D	5
MAP2K2 (MEK2)	-6
MAP2K2 (MEK2)	10
MAP2K3 (MEK3)	-7
MAP2K6 (MKK6)	-10
MAP2K6 (MKK6)	8
MAP2K6 (MKK6) S207E T211E	-7
MAP3K10 (MLK2)	10
MAP3K11 (MLK3)	5
MAP3K14 (NIK)	0
MAP3K2 (MEKK2)	-19
MAP3K3 (MEKK3)	10
MAP3K5 (ASK1)	-5
MAP3K7/MAP3K7IP1 (TAK1-TAB1	15
MAP3K8 (COT)	-17
MAP3K9 (MLK1)	32
MAP4K2 (GCK)	8
MAP4K4 (HGK)	11
MAP4K5 (KHS1)	10
MAPK1 (ERK2)	-2
MAPK10 (JNK3)	-5
MAPK10 (JNK3)	29
MAPK11 (p38 beta)	8
MAPK12 (p38 gamma)	-5
MAPK13 (p38 delta)	-7
MAPK14 (p38 alpha)	15
MAPK14 (p38 alpha) Direct	4
MAPK3 (ERK1)	-10
MAPK8 (JNK1)	14
MAPK8 (JNK1)	6
MAPK9 (JNK2)	6
MAPK9 (JNK2)	18
MAPKAPK2	2
MAPKAPK3	4
MAPKAPK5 (PRAK)	-6
MARK1 (MARK)	-8
MARK2	-19
MARK3	-5
MARK4	-15
MATK (HYL)	0
MELK	11
MERTK (cMER)	1
MET (cMet)	20
MET M1250T	2
MINK1	-3
MKNK1 (MNK1)	-7
MKNK2 (MNK2)	3
MLCK (MLCK2)	16
MST1R (RON)	9
MST4	2
MUSK	-9
MYLK (MLCK)	2

Enzyme	% Inhibition by 10 μM A66
MYLK2 (skMLCK)	-2
NEK1	18
NEK2	10
NEK4	-3
NEK6	2
NEK7	-6
NEK9	-8
NLK	6
NTRK1 (TRKA)	14
NTRK2 (TRKB)	2
NTRK3 (TRKC)	8
NUAK1 (ARK5)	-18
PAK1	2
PAK2 (PAK65)	-1
PAK3	10
PAK4	0
PAK6	10
PAK7 (KIAA1264)	11
PASK	1
PDGFRA (PDGFR alpha)	8
PDGFRA D842V	11
PDGFRA T674I	3
PDGFRA V561D	4
PDGFRB (PDGFR beta)	-3
PDK1	-1
PDK1 Direct	-1
PHKG1	4
PHKG2	-1
PI4KA (PI4K alpha)	-19
PI4KB (PI4K beta)	98
PIK3C2A (PI3K-C2 alpha)	-2
PIK3C2B (PI3K-C2 beta)	77
PIK3C3 (hVPS34)	21
PIK3CA/PIK3R1 (p110 alpha/p85 alp	96
PIK3CD/PIK3R1 (p110 delta/p85 alp	91
PIK3CG (p110 gamma)	86
PIM1	-4
PIM2	-4
PKN1 (PRK1)	-19
PLK1	-3
PLK2	-24
PLK3	3
PRKACA (PKA)	-2
PRKCA (PKC alpha)	2
PRKCB1 (PKC beta I)	3
PRKCB2 (PKC beta II)	-10
PRKCD (PKC delta)	3
PRKCE (PKC epsilon)	9
PRKCG (PKC gamma)	6
PRKCH (PKC eta)	9
PRKCI (PKC iota)	9
PRKCN (PKD3)	7
PRKCQ (PKC theta)	10
PRKCZ (PKC zeta)	3
PRKD1 (PKC mu)	12
PRKD2 (PKD2)	7
PRKG1	-2
PRKG2 (PKG2)	-5
PRKX	-3
PTK2 (FAK)	4
PTK2B (FAK2)	10
PTK6 (Brk)	4
RAF1 (cRAF) Y340D Y341D	-4
RAF1 (cRAF) Y340D Y341D	14
RET	0
RET V804L	-3
RET Y791F	5
RIPK2	66
ROCK1	2
ROCK2	-17
ROS1	11
RPS6KA1 (RSK1)	-1
RPS6KA2 (RSK3)	-4
RPS6KA3 (RSK2)	1
RPS6KA4 (MSK2)	-10
RPS6KA5 (MSK1)	-6
RPS6KA6 (RSK4)	1
RPS6KB1 (p70S6K)	0
SGK (SGK1)	-3
SGK2	4
SGKL (SGK3)	8
SLK	2
SNF1LK2	-5
SPHK1	10
SPHK2	-23
SRC	11
SRC N1	8
SRMS (Srm)	8
SRPK1	2
SRPK2	0
STK16 (PKL12)	-4
STK17A (DRAK1)	11
STK22B (TSSK2)	-12
STK22D (TSSK1)	-7
STK23 (MSSK1)	2
STK24 (MST3)	-2
STK25 (YSK1)	-1
STK3 (MST2)	12
STK33	-5
STK4 (MST1)	11

Enzyme	% Inhibition by 10 μM A66
SYK	8
TAOK2 (TAO1)	0
TAOK3 (JIK)	3
TBK1	-9
TEC	17
TEK (Tie2)	19
TGFBR1 (ALK5)	-2
TNK2 (ACK)	8
TTK	-10
TXK	1
TYK2	-8
TYRO3 (RSE)	5
WEE1	6
WNK2	10
YES1	9
ZAK	5
ZAP70	4

Legend

< 40% Inhibition

40% - 80% Inhibition

≥ 80% Inhibition

Figure S2 Inhibition of kinases by A66

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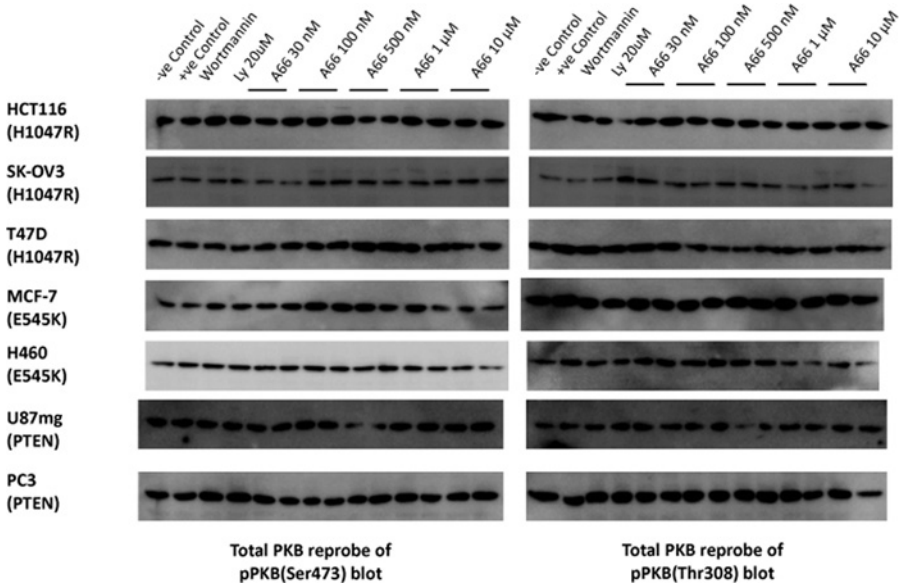

Figure S3 Reprobes of blots in Figure 3 of the main paper with the total PKB antibody

Ly, LY294002.

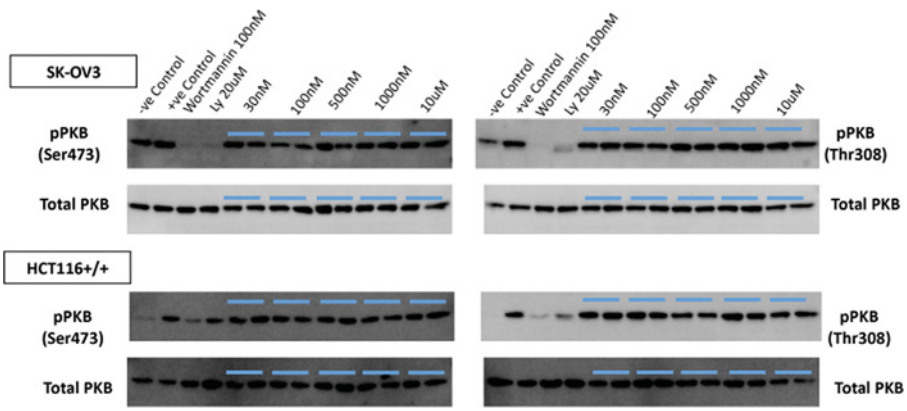

Figure S4 A66 *R* enantiomer does not block signalling to PKB in cells with the *PIK3CA* H1047R mutation

Experiments were performed as in Figure 3 of the main paper, except that the *R* form of A66 was used. Ly, LY294002.

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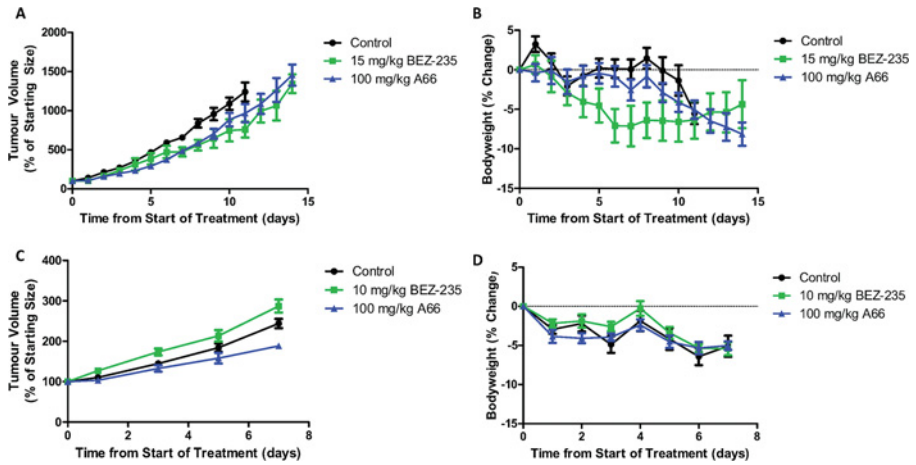

Figure S5 ***In vivo*** **antitumour efficacy and body weight change following treatment with A66 and BEZ-235 in the U87MG and HCT-116 tumour xenograft models**

(**A**) Average tumour volume and (**B**) body weight loss during QD×14 dosing with 100 mg of A66/kg of body weight and 15 mg of BEZ-235/kg of body weight in mice with U87MG tumours. (**C**) Average tumour volume and (**D**) body weight loss during QD×7 dosing with 100 mg of A66/kg of body weight and 10 mg of BEZ-235/kg of body weight in mice with HCT-116 tumours. Bars represent the means ± S.E.M. for five to seven animals.

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