Supplemental Data

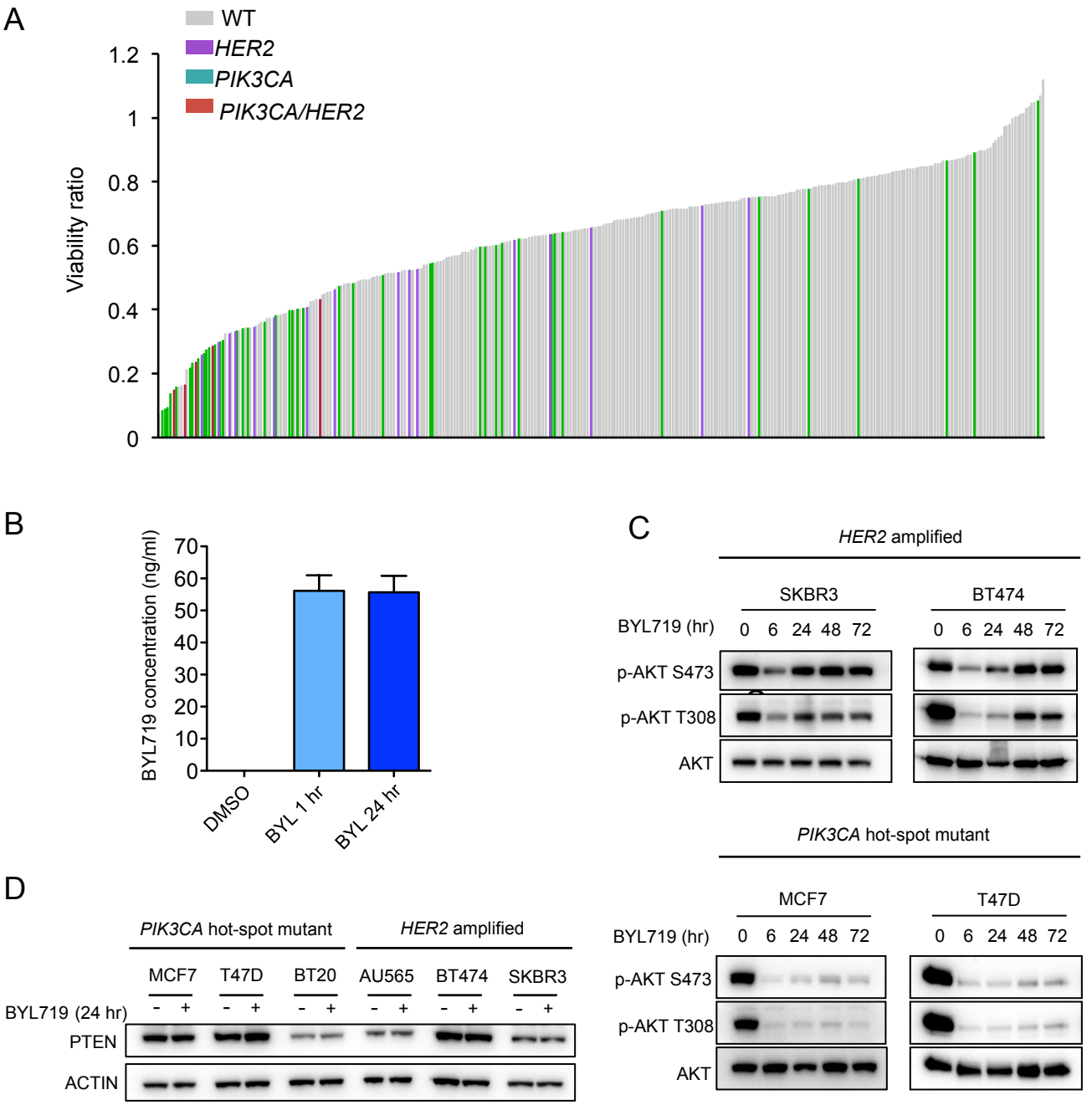

Figure S1 (Related to Figure 1). Phospho-AKT signaling is restored only in *HER2* amplified breast cancer after 48 hours of treatment with BYL719.

(A) Effects of BYL719 on proliferation of a panel of 321 cancer cell lines. *HER2* amplified versus *HER2* WT, Fishers test p=0.0002; *PIK3CA* mutant versus *PIK3CA* WT, Fishers test p<0.0001.

(B) BT474 cells were harvested following treatment with 1 μM BYL719 for the indicated periods of time and the intracellular concentration of BYL719 was measured. Each data point is the average±SEM.

(C, D) The indicated cell lines were treated with 1 μM BYL719 for the different periods of time. Lysates were immunoblotted to detect the indicated proteins.

Table S1 (Related to Figure 1)

Cell Line	Viability ratio
MCF7	0.0837
HCC1954	0.1483
MDA-MB-453	0.1638
KPL-1	0.2168
EFM-19	0.2454
T47D	0.2628
UACC-893	0.2839
ZR-75-30	0.2963
SK-BR-3	0.3309
CAL-51	0.3606
JIMT-1	0.3734
MDA-MB-175-VII	0.4021
EFM-192A	0.4057
HCC202	0.4314
AU565	0.4602
HCC1419	0.5224
CAL-85-1	0.5789
ZR-75-1	0.6161
BT-549	0.6298
HCC1569	0.6342
Hs 578T	0.6785
MDA-MB-134-VI	0.7123
BT-483	0.7144
CAL-120	0.7519
HCC70	0.7595
HCC1395	0.782
MDA-MB-415	0.7864
HCC1143	0.7869
MDA-MB-157	0.7966
MDA-MB-436	0.8399
MDA-MB-231	0.8493
HCC1937	0.8578
BT-20	0.8645
HCC1806	0.8922
MDA-MB-468	1.0041
CAMA-1	1.0445

Cell lines included in the screening presented in Figure 1A

Table S2 (Related to Figure 1)

Cell Line	Viability ratio	Cell Line (2)	Viability ratio (2)	Cell Line (3)	Viability ratio (3)	Cel lLine (4)	Viability ratio (4)	Cell Line (5)	Viability ratio (5)
DOK	0.0717	FaDu	0.4925	MEWO	0.6472	647-V	0.7639	SiHa	0.9367
MCF7	0.0837	SKG-IIIa	0.4996	HuH-7	0.6493	NCI-H520	0.7663	NCI-H2347	0.9428
ME-180	0.0873	RD	0.5019	NUGC-3	0.6513	CAL-62	0.7669	NCI-H810	0.9716
OAW42	0.0924	COLO 205	0.5032	MFE-296	0.6521	CFPAC-1	0.7728	CAL-54	0.9736
HGC-27	0.1373	COR-L23	0.5032	OE33	0.6555	CAL-72	0.7739	NCI-H1693	0.978
HCC1954	0.1483	NCC-IT-A3	0.5069	CHL-1	0.6569	RPMI-7951	0.7743	NCI-H1581	0.997
CAL-33	0.1565	MHH-ES-1	0.5116	NCI-H1651	0.6575	RT4	0.7746	C3A	1.0014
NB69	0.1572	HT55	0.5118	TYK-nu	0.6584	J82	0.7762	MDA-MB-468	1.0041
AN3CA	0.1614	SCC-25	0.5119	COLO 741	0.6597	HOS	0.7778	C-4 II	1.0115
MDA-MB-453	0.1638	AsPC-1	0.5136	HT-3	0.6633	NCI-H1573	0.7812	HMVII	1.0135
A2780	0.212	OE19	0.5151	ABC-1	0.6688	HCC1395	0.782	SW 1116	1.0291
KPL-1	0.2168	BHT-101	0.5151	Calu-1	0.669	MDA-MB-415	0.7864	PFSK-1	1.0328
HSC-2	0.2316	SNU-449	0.5203	BFTC-905	0.6704	HCC1143	0.7869	CAMA-1	1.0445
SK-OV-3	0.2342	CaR-1	0.5223	KYSE-140	0.6767	WM-115	0.7876	SW 1990	1.0466
EFM-19	0.2454	HCC1419	0.5224	Hs 578T	0.6785	NCI-H441	0.7884	NCI-H2342	1.0523
KYSE-410	0.2565	H4	0.5229	SK-N-AS	0.6799	LN-405	0.7886	U-251 MG	1.068
T47D	0.2628	OVCAR-5	0.5231	G-361	0.6801	PC-14	0.7887	PC-3	1.1173
MKN1	0.2737	NCI-H2170	0.525	RO82-W-1	0.6814	SW-1710	0.7934		
MFE-280	0.2809	KOSC-2 cl3-43	0.5256	NCI-H2291	0.6829	NCI-H1650	0.7951		
UACC-893	0.2839	NCI-H358	0.5277	NCI-H1648	0.6845	COLO-680N	0.796		
NCI-H596	0.2883	MG-63	0.5375	CAL-39	0.6868	DoTc2 4510	0.7965		
ZR-75-30	0.2963	HUP-T4	0.5406	SNU-387	0.6873	MDA-MB-157	0.7966		
AGS	0.2989	TCCSUP	0.5417	NCI-H1755	0.688	NCI-H727	0.8003		
LU99A	0.3028	KYSE-510	0.5446	769-P	0.6893	CCF-STTG1	0.8036		
ChaGo-K-1	0.3225	Capan-2	0.5455	COLO-678	0.69275	SW 1417	0.8061		
NCI-N87	0.3242	NCI-H838	0.5495	M-14	0.6979	C-33 A	0.8064		
SW 48	0.3289	NCI-H1666	0.5499	SW 1463	0.6987	NCI-H2030	0.8066		
SK-BR-3	0.3309	SW 780	0.5532	SK-LU-1	0.7	HLE	0.8108		
GP5d	0.3318	Panc 10.05	0.56	A431	0.7013	PA-1	0.811		
KYSE-180	0.3334	SK-MES-1	0.5623	MEL-HO	0.7043	NCI-H661	0.8138		
SW 1573	0.3395	RMG-I	0.5656	HT-1197	0.7066	HUP-T3	0.8139		
NCI-H23	0.3411	NCI-H2009	0.5681	VM-CUB1	0.7079	SHP-77	0.8167		
HT-29	0.3423	BxPC-3	0.5683	HO-1-N-1	0.7102	EGI-1	0.8174		
A-204	0.3425	NCI-H630	0.5706	MDA-MB-134-VI	0.7123	Panc 08.13	0.8216		
Calu-3	0.3441	CAL-85-1	0.5789	B-CPAP	0.7128	A-427	0.822		
NCI-H1703	0.3479	SAS	0.5795	SW837	0.7131	Saos-2	0.8231		
DV-90	0.3532	COLO-679	0.58	SW 13	0.7133	ACHN	0.8238		
HuO9	0.3593	HN	0.586	BT-483	0.7144	U-2 OS	0.8273		
CAL-51	0.3606	5637	0.5863	SW620	0.7145	786-O	0.8303		
IGROV-1	0.3722	MES-SA	0.5937	SK-MEL-30	0.7148	BHY	0.8326		
SCC-9	0.3729	Ca Ski	0.5948	HSC-4	0.7198	SK-HEP-1	0.8327		
JIMT-1	0.3734	SK-CO-1	0.595	NCI-H1793	0.7199	Panc 03.27	0.8346		
KYSE-70	0.3799	HEC-1	0.5957	Ca9-22	0.72	NCI-H2452	0.8353		
Lu-135	0.3812	GMS-10	0.5971	T98G	0.7205	KP-4	0.8384		
NCI-H1623	0.3818	DMS 273	0.5973	OVCAR-8	0.7227	IGR-1	0.839		
BPH-1	0.3841	HPAF-II	0.5989	SW 1783	0.7257	MDA-MB-436	0.8399		
CHP-212	0.3864	ESS-1	0.6007	RPMI 2650	0.7271	SW 626	0.8416		
Detroit 562	0.3954	HuCCT1	0.6019	T24	0.7285	UACC-62	0.8434		
HCT-15	0.3955	22RV1	0.6054	A673	0.7293	LoVo	0.8467		
RT-112	0.3963	NCI-H1792	0.6098	OAW28	0.7301	SW756	0.847		
T84	0.4019	NIH:OVCAR-3	0.6111	SNU-423	0.7314	RVH-421	0.847		
MDA-MB-175-VII	0.4021	IPC-298	0.6122	HT 1376	0.7321	MIA PaCa-2	0.8492		
SW-948	0.4035	MKN7	0.6157	UM-UC-3	0.7336	MDA-MB-231	0.8493		
EFM-192A	0.4057	ZR-75-1	0.6161	SN-12C	0.7345	HSC-3	0.8548		
G-401	0.4085	NCI-H1048	0.6193	NCI-H2087	0.7355	A-375	0.8548		
A549	0.4229	CAL-12T	0.6201	YKG-1	0.7362	HCC1937	0.8578		
H292	0.4254	GCT	0.6205	CAL 27	0.7371	Capan-1	0.8639		
COLO 792	0.4308	NCI-H2122	0.621	FTC-133	0.7396	BT-20	0.8645		
HCC202	0.4314	H28	0.6238	EFO-21	0.7402	H2052	0.8648		
SJCRH30	0.4446	U-118 MG	0.6262	SW 1088	0.7447	C2BBe1	0.867		
23132/87	0.4488	Daoy	0.6283	PLC/PRF/5	0.747	EFO-27	0.8672		
NCI-H1993	0.4532	BT-549	0.6298	NCI/ADR-RES	0.7475	KU-19-19	0.869		
KYSE-150	0.4559	Caov-4	0.6301	SNU-475	0.7475	NY	0.872		
AU565	0.4602	KYSE-520	0.6311	COR-L 105	0.75	SBC-5	0.8739		
NCI-H2405	0.4655	MC-IXC	0.6317	DU 145	0.7506	DBTRG-05MG	0.8765		
LS174T	0.4725	HCC1569	0.6342	639-V	0.751	HT 1080	0.8825		
8305C	0.4742	BFTC-909	0.6345	NCI-H1299	0.7518	A2058	0.8831		
NCI-H2228	0.4795	HCT 116	0.6371	CAL-120	0.7519	SNG-M	0.8902		
NCI-H1734	0.4798	KYSE-450	0.6374	KYSE-270	0.7523	HCC1806	0.8922		
SW 900	0.4803	UO-31	0.6374	EFE-184	0.7524	A172	0.8953		
NCI-H460	0.4806	DK-MG	0.6402	Caki-1	0.7533	HTC-C3	0.8965		
G-402	0.483	SCC-4	0.6406	SK-MEL-28	0.7536	S-117	0.8967		
NCI-H1437	0.4873	HuO-3N1	0.6413	VMRC-RCZ	0.7566	SF-295	0.9008		
LK-2	0.4909	GAMG	0.6432	NCI-H650	0.7569	M059J	0.9055		
LCLC-103H	0.4914	NCI-H522	0.6463	HCC70	0.7595	UMC-11	0.92		
MKN45	0.4915	MEL-JUSO	0.647	MKN28	0.761	DMS 53	0.9293		

Cell lines included in the screening presented in Figure S1A

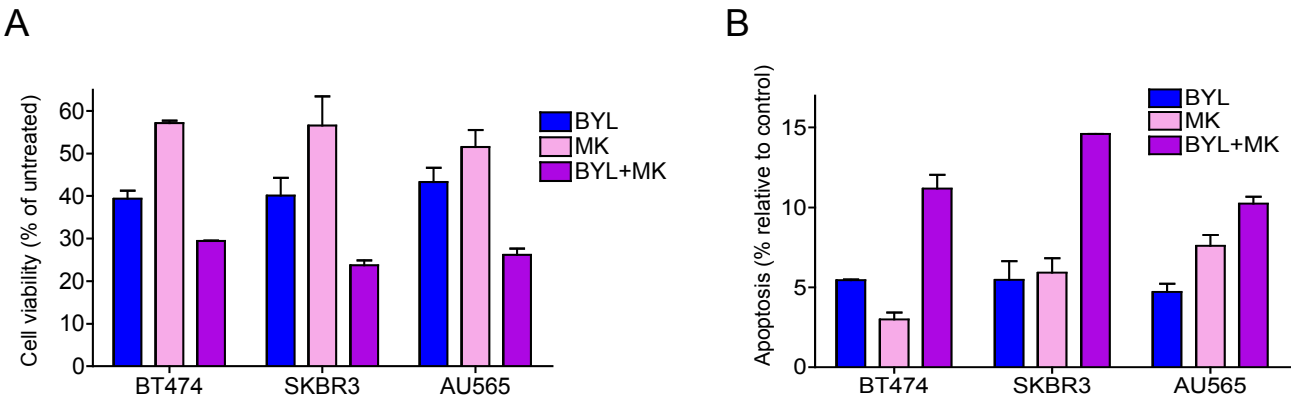

Figure S2 (Related to Figure 2). Rebound of AKT signaling controls cell survival and proliferation in *HER2* amplified cells.

(A) Cells were treated with 1 μM BYL719, 156 nM MK2206 or the combination for 3 days, and cell viability was determined by CellTiter-Glo.

(B) Cells were treated with 1 μM BYL719, 1 μM MK2206 or the combination for 72 hr. The percentage of cells undergoing apoptosis, as measured by annexin V positivity, is shown relative to control cells.

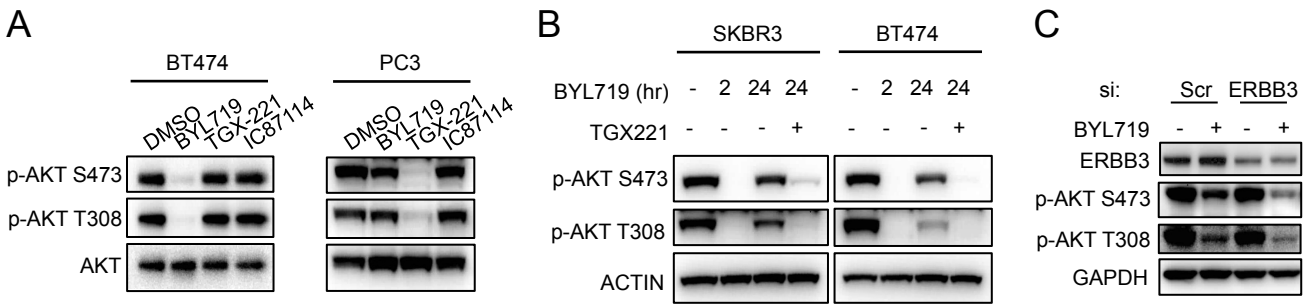

Figure S3 (Related to Figure 3). Feedback activation of AKT following BYL719 is triggered by p110β.

(A) The indicated cells were treated with p110α inhibitor BYL719 (1 μM), p110β inhibitor TGX-221 (1 μM) and p110δ inhibitor IC87114 (1 μM) for 6 hr. Lysates were immunoblotted to detect the indicated proteins.

(B) The indicated cell lines were treated with 1 μM TGX-221 for 9 hr following 24 hr treatment with 1 μM BYL719. Lysates were immunoblotted to detect the indicated proteins.

(C) SKBR3 cells were transfected with control (Scr) or ERBB3-targeted siRNA for 48 hr, followed by treatment with 1 μM BYL719 for an additional 24 hr. Lysates were immunoblotted to detect the indicated proteins.

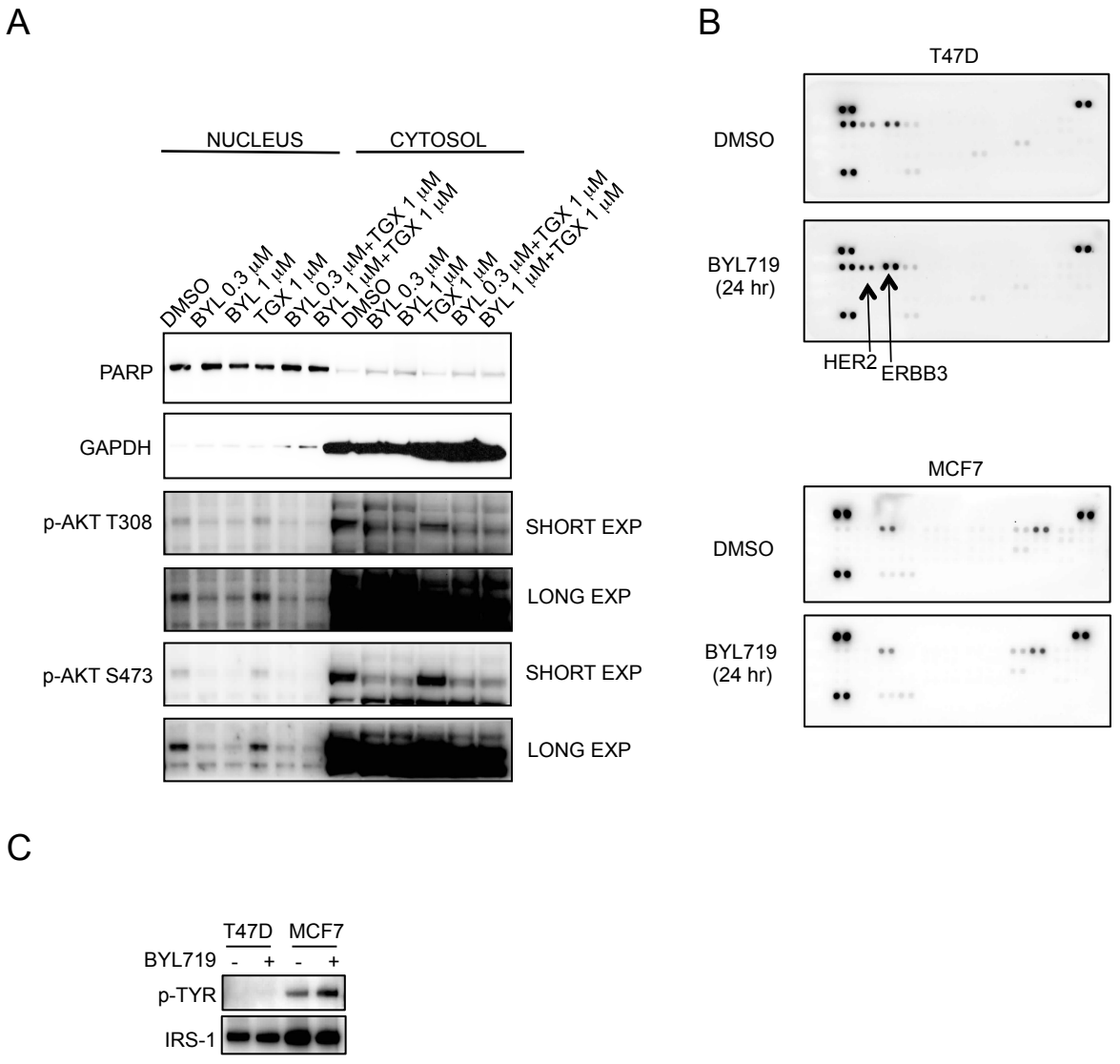

Figure S4 (Related to Figure 4). BYL719 treatment induces activation of different RTKs in *PIK3CA* mutant cells.

(A) MCF7 cells were treated with the indicated drug(s) for 24 hr. Equal amounts of cytoplasmic, and nuclear protein fractions were immunoblotted to detect the indicated proteins.

(B) Cells were treated as indicated with DMSO control or 1 μM BYL719 for 24 hr. Cell lysates were prepared and incubated with pRTK arrays (R&D Biosystems). Arrows indicate RTKs whose phosphorylation was increased upon treatment with BYL719.

(C) Cells were treated for 24 hr with 1 μM BYL719 and lysates were immunoprecipitated with IRS1 antibody. Immunoprecipitates were analyzed by Western blot analyses to detect the indicated antibodies.

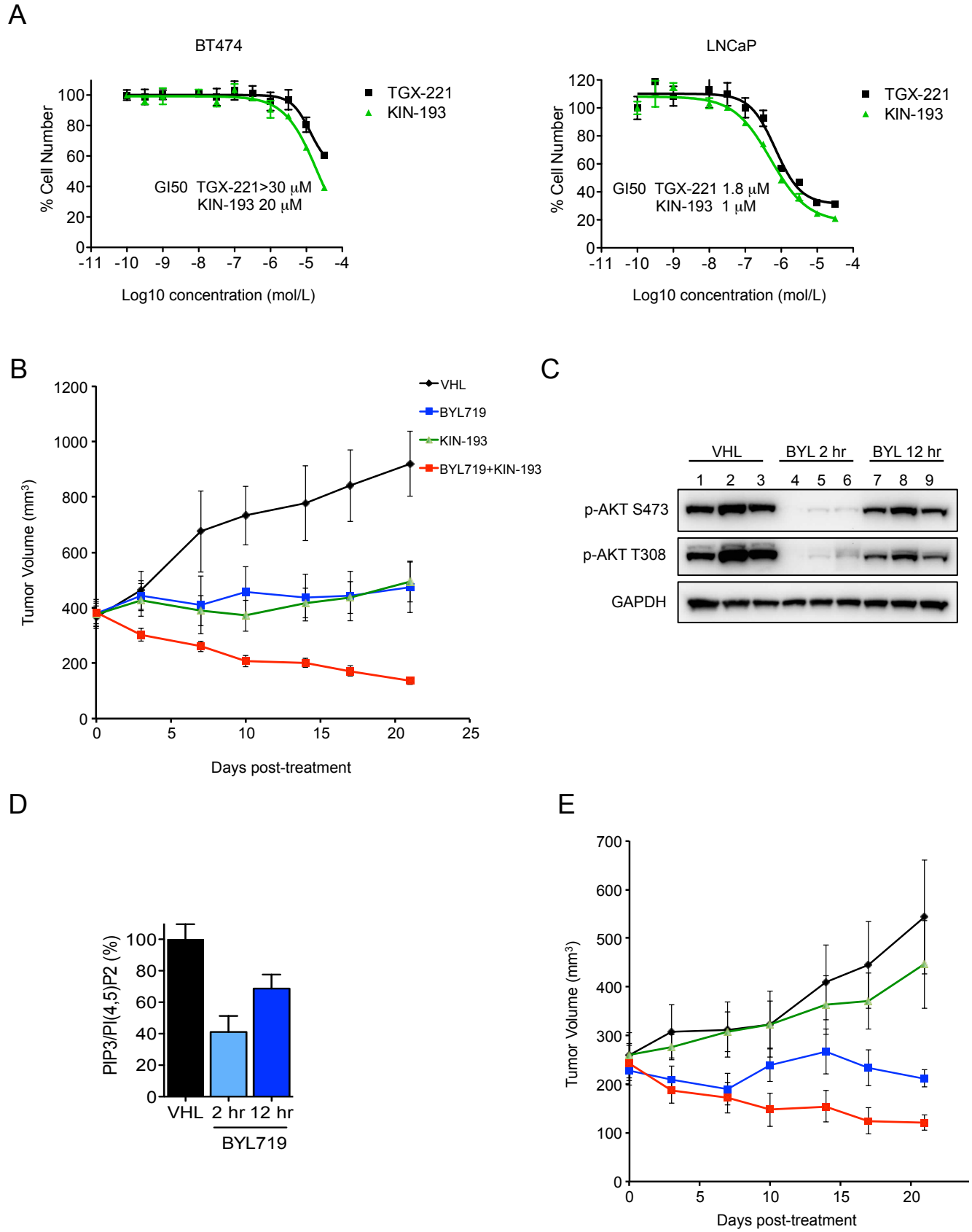
A
BT474
% Cell Number
Log10 concentration (mol/L)
TGX-221
KIN-193
GI50 TGX-221>30 μM
KIN-193 20 μM
LNCaP
GI50 TGX-221 1.8 μM
KIN-193 1 μM
B
Tumor Volume (mm3)
Days post-treatment
VHL
BYL719
KIN-193
BYL719+KIN-193
C
VHL
BYL 2 hr
BYL 12 hr
p-AKT S473
p-AKT T308
GAPDH
D
PIP3/PI(4,5)P2 (%)
VHL
2 hr
12 hr
BYL719
E
Tumor Volume (mm3)
Days post-treatment

Figure S5 (Related to Figure 5). In vivo rebound of PIP_3 and phospho-AKT after BYL719 treatment in *HER2* amplified tumors.

(A) The indicated cell lines were treated with the increasing doses of TGX-221 or KIN-193 for 72 hr. Cell viability was measured in sextuplicate using CellTiter-Glo.

(B) BT474 cells were grown as xenograft tumors in Nu/Nu mice, and, when tumors were approximately 400 mm^3, mice were randomized into treatment cohorts that received vehicle (VHL), BYL719 (BYL) 25 mg/kg per oral gavage daily, KIN-193 (KIN) 20 mg/kg per IP injection twice daily, and the combination. Tumor measurements were performed two times per week, and the average tumor volume for each cohort is displayed. BYL719 versus BYL719+KIN-193 $p < 0.05$ by t student test.

(C) Mice harboring BT474 tumors were administered with vehicle (VHL), BYL719 (BYL) 25 mg/kg per oral gavage daily for 3 days. Mice were sacrificed 2 and 12 hr after the last treatment. Lysates were prepared and blotted with the indicated antibodies.

(D) Phospholipids were isolated from tumors treated as in (C) and PIP_3 and $PI(4,5)P_2$ levels were quantified by ELISA.

(E) MCF7 cells were grown as xenograft tumors in Nu/Nu mice. When tumors were approximately 250 mm^3, mice were randomized into treatment cohorts and monitored as described in (B). BYL719 versus BYL719+KIN-193 $p < 0.05$ by t student test.

All error bars in the figure represent ± SEM.

Supplemental Experimental Procedures

Measurement of compound concentration

Cultured cells were washed with PBS twice prior to be switched to compound free medium. Two millions of cells were collected from untreated, 1 and 24 hr post compound wash out and stored at -80^{o}C. Calibration standards were prepared at 1, 2, 5, 10, 50, 250, 1000, 2500, 5000, 10000, and 15000 ng/mL in acetonitrile/water (50/50, v/v) from BYL719 primary stock solution (1mg/mL in DMSO).The frozen cell pellet samples were thawed and centrifuged for 10 minutes at 3300rpm. Aliquot 10 µL of matrix and control blank (50:50 Acetonitrile:Water), calibration standard, and cell samples into 96 well plate. 50 µL internal standard (Alprazolam) was added into all samples, except control blank, while 50 µL of acetonitrile was added into the control blank. The 96-well plate was covered and vortexed, then centrifuged for 10 minutes at 3300 rpm. 40 µL of supernatants were transferred into a clean 96-well plate. 40 µL of Milli-Q water was added into all wells. The plate was covered, vortexed again. All samples were subject to LC/MS analysis. Perkin Elmer PE 200 Series Micropumps was used for LC analysis. An 1.50 min gradient was utilized going from 99% of Mobile phase A and 1% Mobile phase B to 5% of Mobile phaseA and 95% of Mobile Phase B with a total run time of 3.50 minutes at a flow rate of 0.6 ml/min. Mobile phase A was 0.1% formic acid in water and Mobile phase B was 0.1 % formic acid in acetonitrile. All the chromatographic measurements were performed at room temperature and the autosampler was maintained at 4^{o}C. The quantitation of the samples was achieved against a calibration curve with a linear regression analysis by API4000 LC-MS/MS in positive ion Multiple Reaction Monitoring (MRM) mode.

Nuclear and cytoplasmic fractionation and proteome profiler human phospho-RTK arrays.

Cells were incubated for 10 min at 4°C in hypotonic buffer (20 mM HEPES at pH 7.6, 20% glycerol, 10 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.1% NP-40). Following centrifugation at 5000 rpm for 1 min, supernatants were used for the cytoplasmic fraction. The pelleted nuclei were washed with hypotonic buffer and incubated for 30 min at 4°C in nuclear extraction buffer (hypotonic buffer containing 500 mM NaCl) and cleared by centrifugation at 13500 rpm. The pRTK arrays were performed according to the manufacturer`s instruction.