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Supplementary Materials for

Systemic analysis of tyrosine kinase signaling reveals a common adaptive response program in a HER2-positive breast cancer

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Other Supplementary Material for this manuscript includes the following:

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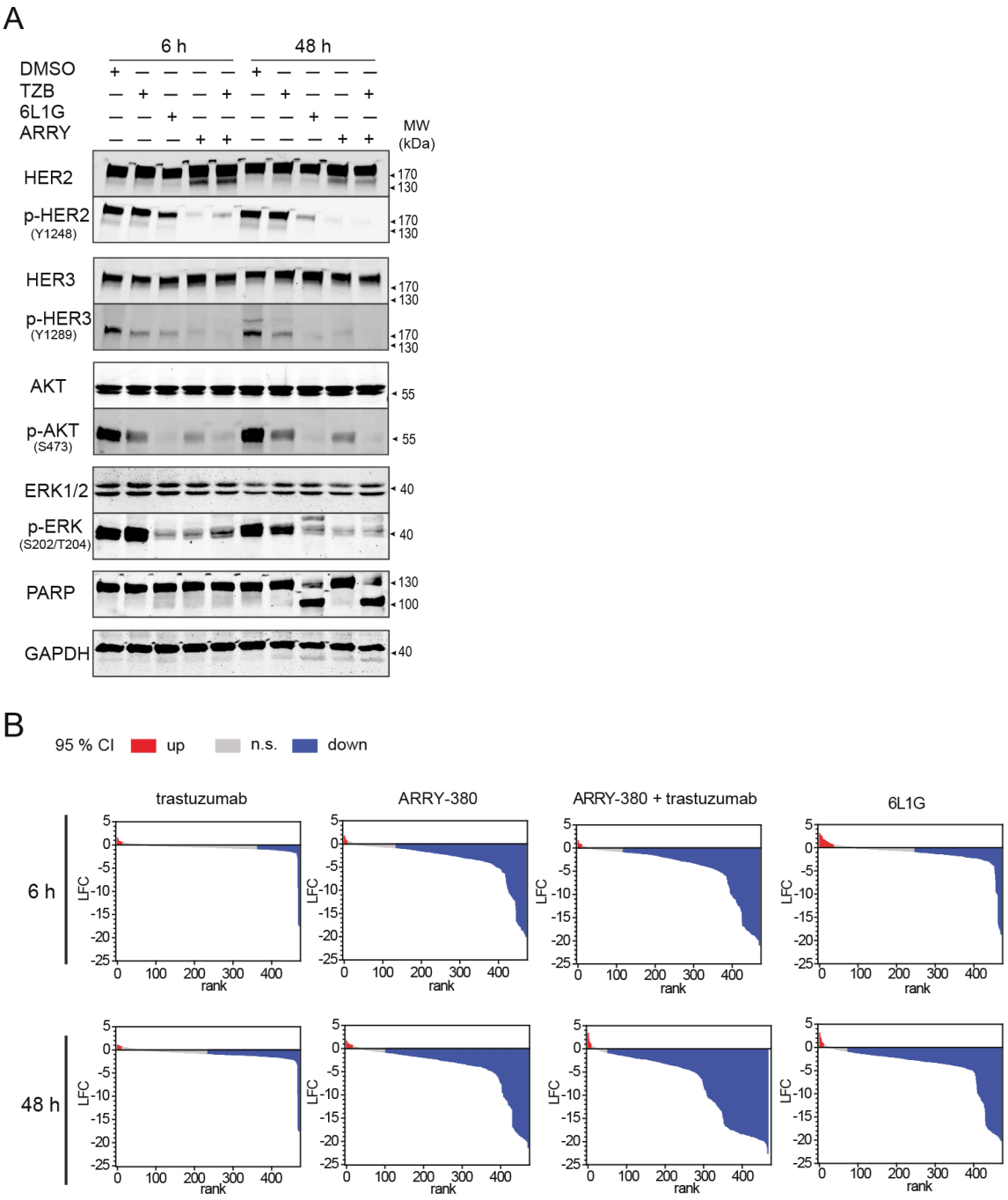

Fig. S1. HER2/HER3 signaling after anti-HER2 treatments and the distribution of phosphopeptides in the TMT LC-MS/MS dataset and peptide signature. (A) Western blot analysis of HER2 downstream signaling in BT474 cells after 6 and 48 hours of treatment with 10 µM ARRY-380 (ARRY), 100 nM trastuzumab (TZB), 100 nM biparatopic DARPin (6L1G) or indicated combinations. After 48 hours of treatment, significant PARP cleavage was found after 6L1G and ARRY+TZB treatment. Blots are representatives from 2 independent experiments. **(B)** Bar diagram of $\log_2$ fold change (LFC) distribution of 471 p-Tyr peptides from TMT LC-MS/MS dataset. 95% confidence interval (CI) was determined on the distribution of LFC values after the 48-hours' DMSO treatment (Fig. 1B). An upper threshold of 0.506 and lower threshold of -0.757 was calculated. Data represent mean values of two biological replicates.

[*Figure S1 continues next page*]

C	Protein ID	UNIPROT	Sequence
1	ACK1	Q07912	kPTyDPVSEDQDPLSSDFk
2	APLP2	Q06481	yLEQmQI
3	CI078	Q9NZ63	nAEDcLyELPENIR
4	CK052	Q96A22	hVHLENATEyATLR
5	CSK	P41240	vMEGTVAAQDEFyR
6	CTND1	O60716	hYEDGYPGGSDNyGsLSR
7	CTND2	Q9UQB3	aSYAAGPASNyADPYR
8	CTND2	Q9UQB3	aLQSPEHHIDPIyEDR
9	DDR1	Q08345	nLyAGDyYR
10	DSC2	Q02487	hAQDYVLTYNyEGR
11	DYR1B	Q9Y463	iYQyIQSR
12	EPHB4	P54760;P54753	vYIDPFTyEDPNEAVR
13	EPHB4	P54760	fLEENSSDPTyTSSLGGk
14	ERBB2	P04626	lLDIDETEYHADGGkVPIkWmALEsILR
15	ERBB3	P21860	hSLLTPVtPLSPPGLEEEDVNGYVMPDTHLk
16	ERBB3	P21860	eGTLSSVGLSSVLGTEEEDEDEEyEYMNR
17	ERBB3	P21860	dGGGPGGDYAAMGAcPASEQGyEEMR
18	FAK1	Q05397	ymEDSTYYk
19	GNAL	P38405	qyELL
20	HIPK2	Q9H2X6	aVcSTyLQSR
21	HS90B	P08238	eDQTEyLEER
22	ICK	Q9UPZ9	skPPYtDyVSTR
23	INADL	Q8NI35	eQEDLPLyQHQATR
24	K1C19	P08727	dYSHyYTTIQDLR
25	K2C7	P08729	lSSARPGGLGSSsLyGLGASRPR
26	KPCD	Q05655	rSDSASSEPVGIyQGFEk
27	KPCD	Q05655	tGVAGEDMQDNSGTyGk
28	LAP4B	Q86VI4	ePPPPyVSA
29	LDLR	P01130	tTEDEVHIcHNQDGySYPSR
30	LPP	Q93052	nDSDPtYGQQGHPNTWk
31	PAR3L	Q8TEW8	gLLDYATGAIGSVYDMDDDEMDPNyAR
32	PARD3	Q8TEW0	eRDyAEIQDFHR
33	PAXI	P49023	fIHQQPQSSsPVyGSSAk
34	PKHA5	Q9HAU0	gGNRPNTGPLyTEADR
35	PKHA6	Q9Y2H5	lPPRSEDIyADPAAYVMR
36	PKP4	Q99569	tVHDMEQFGQQQyDIYER
37	SG223	Q86YV5	eATQPEPIyAESTk
38	SHB	Q15464	dkVTIADDySDPFDAk
39	SHB	Q15464	lDycGGSGEPGGVQR
40	SHB	Q15464	aGkGESAGyMEPYEAQR
41	SHB	Q15464	vTIADDYsDPFDAk
42	SHB	Q15464	lPQDDDRPADEyDQPWEWNR
43	SKT	Q5T5P2	nVyYELNDVR
44	SPIT2	O43291	nTyVL
45	SRCN1	Q9C0H9	nVFyELEDVRDIQDR
46	SRSF1	Q07955	dGyDYDGYR
47	SSH2	Q76I76	tTNPFYNtm
48	T106B	Q9NUM4	nGDVSQFPyVEFTGR
49	T106C	Q9BVX2	eQEEAIAQFPyVEFTGR
50	TAB1	Q15750	vEPyVDFAEFYR
51	TM1L2	Q6ZVM7	kTVTyEDPQAVGGLASALDNR
52	TYK2	P29597	lLAQAEGEPcyIR
53	XYLK	O75063	dHVVEGEPyAGYDR
54	ZDHC5	Q9C0B5	ePsPVRyDNLSR

Fig. S1, continued: **(C)** Consensus of 54 persistently phosphorylated Tyr peptides from 46 different proteins between the two non-apoptotic treatments ARRY and TZB after 48 hours in TMT dataset (n=2). [*Figure S1 continues next page*]

D

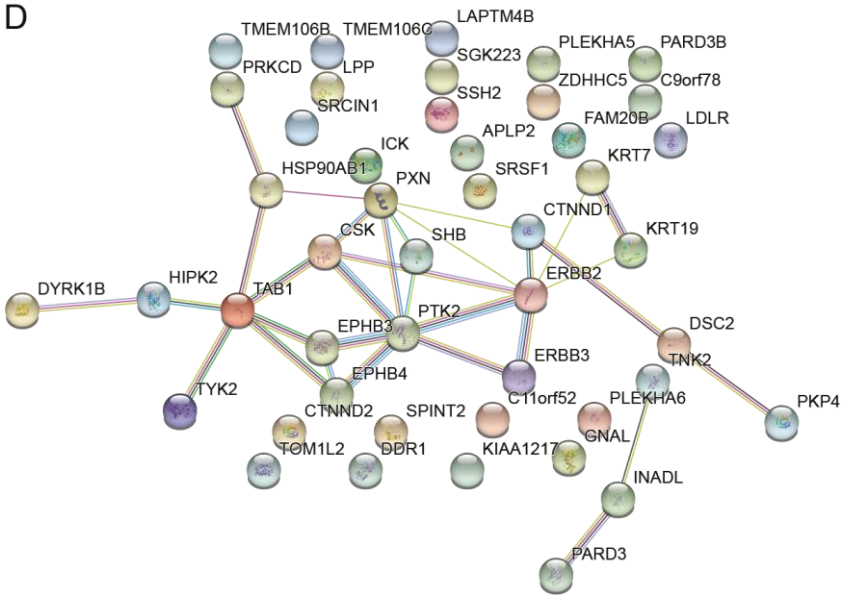

Fig. S1, continued: **(D)** Functional protein interaction network from string-db.org using the 54 persistently phosphorylated Tyr peptides from TMT dataset (see C). PTK2 (FAK1) was found to be a major signaling hub in this subset of phospho-peptides, which are directly linked to the adaptive anti-drug response (n=2).

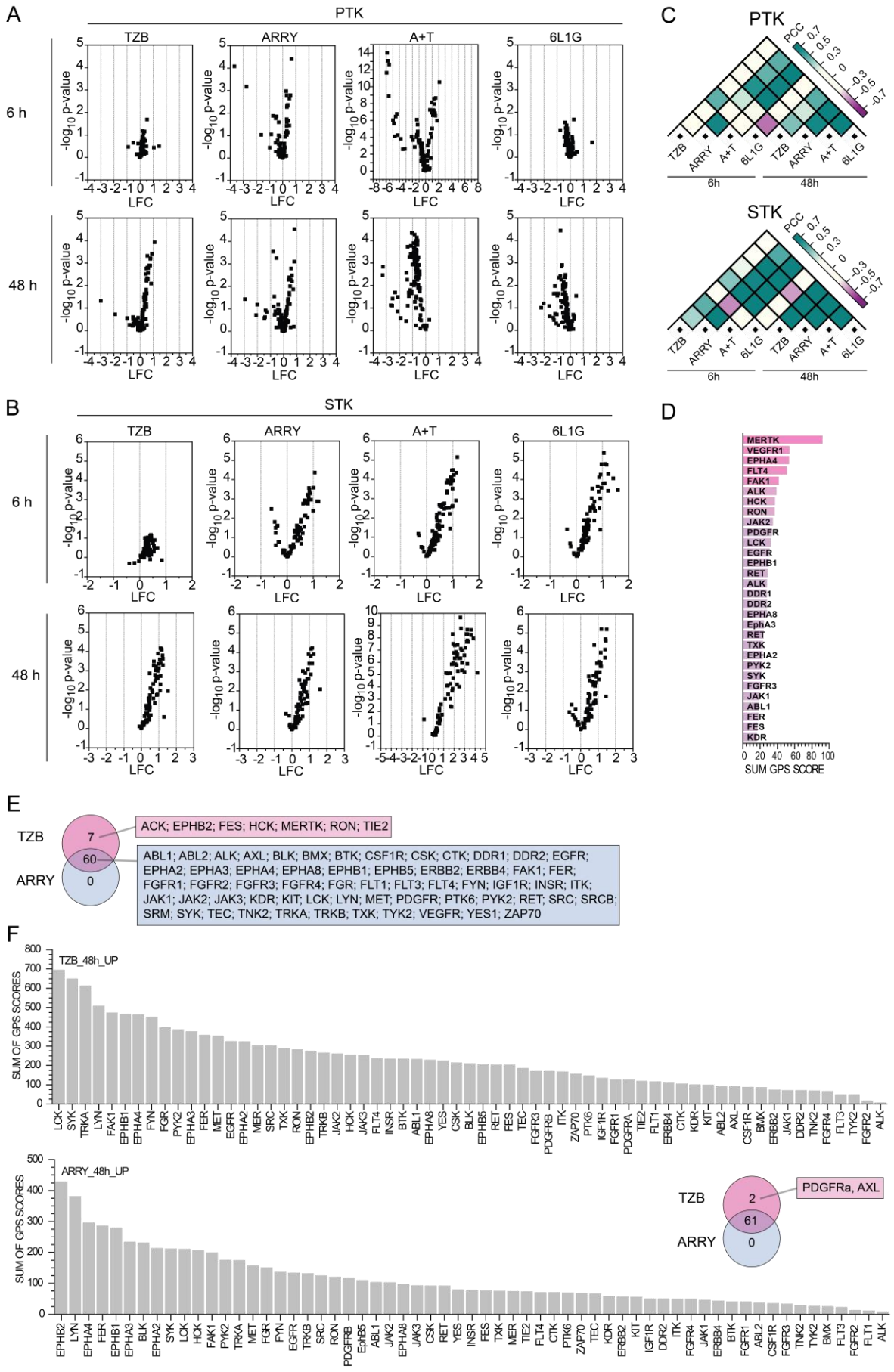

Fig. S2. Peptide chip array and kinase predictions. (A and B) Individual volcano plots of the phospho-tyrosine peptide chip array (PTK) and the phospho-serine/threonine peptide chip array (STK). The p-

values were calculated versus the corresponding DMSO treatment from 4 biological replicates by ANOVA and post-hoc Dunnett's test in the BioNavigator software. **(C)** Pairwise comparison of Tyr (PTK) or Ser/Thr (STK) phosphorylation pattern from peptide chip array by Pearson correlation coefficient (PCC) (n=4). **(D)** Sum of scores from group-based prediction system (GPS) based on significantly ($p < 0.05$) phosphorylated Tyr peptides from PTK array after 6 hours of treatment with the trastuzumab and ARRY-380 combination treatment (A+T; Fig. 3A) (n=4). **(E)** Comparison of active Tyr-kinases, which were predicted by a group-based prediction system on significantly ($p < 0.05$) phosphorylated Tyr peptides from the PTK array after 48 hours of treatment with trastuzumab (TZB) or ARRY-380 (ARRY) (n=4). **(F)** Sum of scores from group-based prediction system of active Tyr-kinases from persistently phosphorylated peptides from the TMT dataset after 48 hours of treatment with trastuzumab or ARRY-380. Predicted cognate kinases were compared by a Venn diagram between both non-apoptotic treatments (n=4).

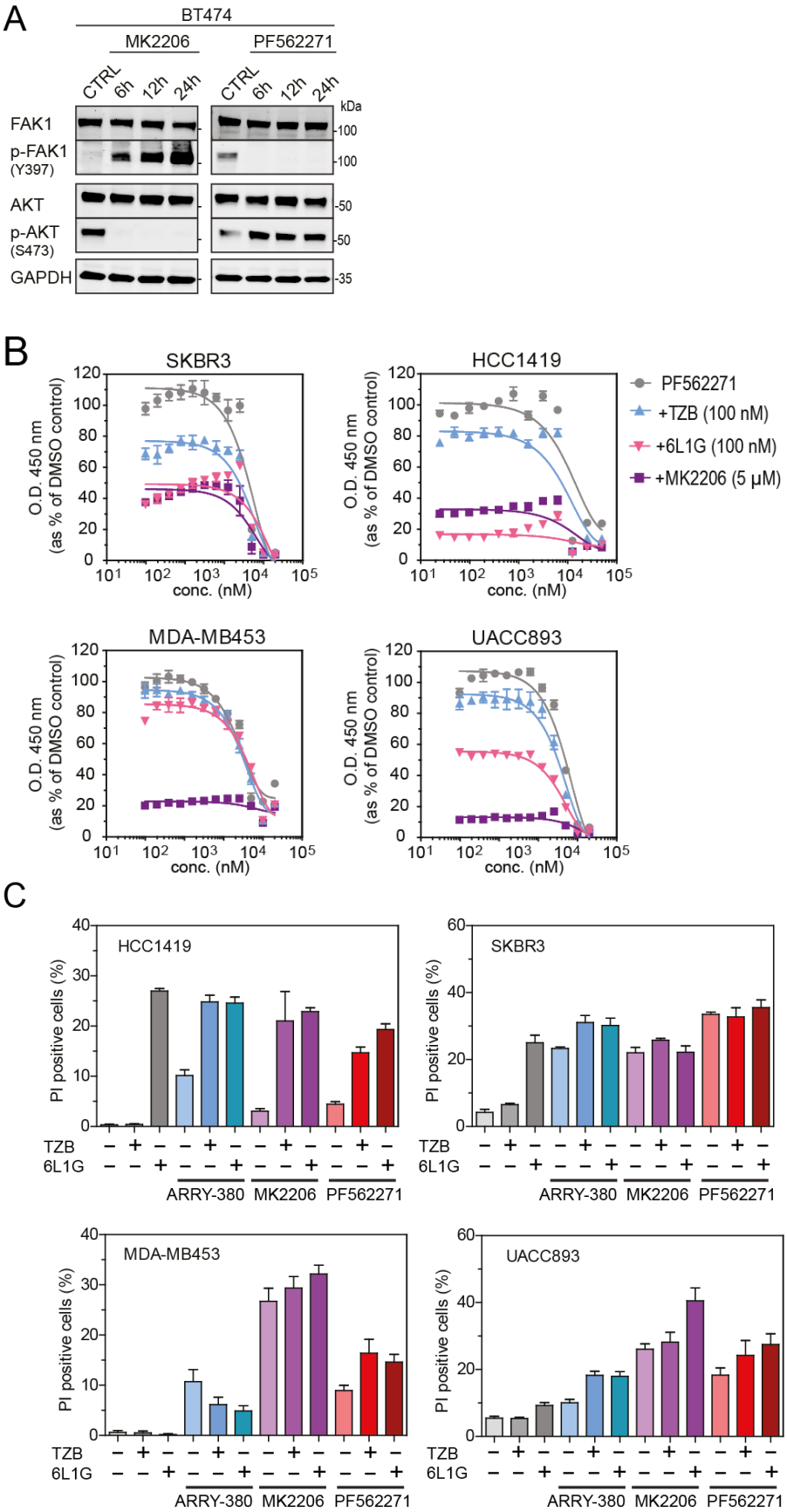

Fig. S3. Counter-activation of FAK1-AKT1 signaling and the effect of combination treatments. (A) Western blot time series follows the activation of p-FAK1 and p-AKT in BT474 cells after treatment with 5 μM MK2206 or 10 μM PF562271 for indicated times. Blots are representatives from 2 independent experiments. **(B)** XTT cell proliferation assays with HER2-positive breast cancer cell lines

with different *PIK3CA* point mutations or wt form. SKBR3 (WT), HCC1419 (WT), MDA-MB453 (H1047R), and UACCC893 (H1047R) were treated for 4 days with the indicated titration of PF562271 and subsequently (less than 5 min) with trastuzumab (TZB; 100 nM), biparatopic DARPin (6L1G; 100 nM) or MK2206 (5 µM), as described for BT474 (K111N) and MDA-MB361 (E345K) cells shown in Fig. 4C. (n= 3 ± SD). **(C)** High-throughput microscopy analysis of PI/Hoechst-33342 counterstained breast cancer cells to detect membrane-permeable dead cells versus total number cells, respectively. Cells were continuously treated for 3 days with ARRY-380 (10 µM) or PF562271 (10 µM) and subsequently (less than 5 min) with TZB (100 nM) or 6L1G (100 nM) at day 0 (n=5 ± SD).

Table S1. LFC of peptide abundance from TMT LC-MS/MS dataset. The table contains mean LFC values from two biological replicates of 471 unique p-Tyr peptides from the TMT LC-MS/MS dataset. BT474 cell were treated in the following scheme: DMSO_6h (0.0001 % v/v, used for normalization of all treatments), TZB_6h (100 nM trastuzumab treatment for 6 hours), 6L1G_6h (100 nM biparatopic DARPin treatment for 6 hours), ARRY_6h (10 µM ARRY-380 treatment for 6 hours), A + T_6h (10 µM ARRY-380 + 100 nM trastuzumab treatment for 6 hours), DMSO_48h (0.0001 % v/v), TZB_48h (100 nM trastuzumab treatment for 48 hours), 6L1G_48h (100 nM biparatopic DARPin treatment for 48 hours), ARRY_48h (10 µM ARRY-380 treatment for 48 hours), A + T_48h (10 µM ARRY-380 + 100 nM trastuzumab treatment for 48 hours). Table is related to the data in Figs. 1, 2, and 3 and is provided as an '.xls' file in the online supplementary materials.

Table S2. LFC and *P* values for Tyr peptide phosphorylation from kinase activity profiling. The table contains mean LFC values and p-values from four biological replicates. BT474 cell were treated in the following scheme: DMSO_6h (0.0001 % v/v, used for normalization of all treatments), TZB_6h (100 nM trastuzumab treatment for 6 hours), 6L1G_6h (100 nM biparatopic DARPin treatment for 6 hours), ARRY_6h (10 µM ARRY-380 treatment for 6 hours), A + T_6h (10 µM ARRY-380 + 100 nM trastuzumab treatment for 6 hours), DMSO_48h (0.0001 % v/v), TZB_48h (100 nM trastuzumab treatment for 48 hours), 6L1G_48h (100 nM biparatopic DARPin treatment for 48 hours), ARRY_48h (10 µM ARRY-380 treatment for 48 hours), A + T_48h (10 µM ARRY-380 + 100 nM trastuzumab treatment for 48 hours). Table is related to the data in Fig. 3 and is provided as an '.xls' file in the online supplementary materials.

Table S3. LFC and *P* values for Ser/Thr peptide phosphorylation from kinase activity profiling. The table contains mean LFC values and p-values from four biological replicates. BT474 cell were treated in the following scheme: DMSO_6h (0.0001 % v/v, used for normalization of all treatments), TZB_6h (100 nM trastuzumab treatment for 6 hours), 6L1G_6h (100 nM biparatopic DARPin treatment for 6 hours), ARRY_6h (10 µM ARRY-380 treatment for 6 hours), A + T_6h (10 µM ARRY-380 + 100 nM trastuzumab treatment for 6 hours), DMSO_48h (0.0001 % v/v), TZB_48h (100 nM trastuzumab treatment for 48 hours), 6L1G_48h (100 nM biparatopic DARPin treatment for 48 hours), ARRY_48h (10 µM ARRY-380 treatment for 48 hours), A + T_48h (10 µM ARRY-380 + 100 nM trastuzumab treatment for 48 hours). Table is related to the data in Fig. 3 and is provided as an '.xls' file in the online supplementary materials.