Supplementary data

Supplementary Figure S1. Resistant tumor growth is evident for osimertinib and JNJ-61186372 monotherapy in NSCLC cell line xenografts.

Supplementary Figure S2. Multiplex TMT-labeling scheme and results from MS analysis of NSCLC xenograft tumors.

Supplementary Figure S3. Evaluation of *in vivo* target efficacy in HCC827 and HCC827-ER1 xenografts after 6-hour treatment with osimertinib and JNJ-61186372 alone or in combination.

Supplementary Figure S4. Phosphopeptide correlation and heterogeneous phosphoprotein network upregulation in resistant tumors.

Supplementary Figure S5. Kinase substrate motif enrichment analyses of resistant tumors.

Supplementary Figure S6. Combined EGFR and SFK inhibition results in superior growth inhibition of DR-HCC827 cells.

Supplementary Table S1: Summary of data from vehicle-treated tumors.

Supplementary Table S2: Summary of data from target efficacy evaluation.

Supplementary Table S3: Summary of data from drug resistant tumors.

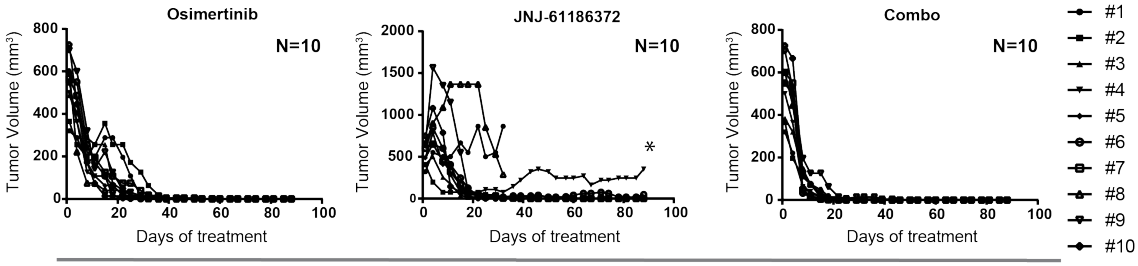
A

Cell lines: H1975, H1975-HGF, HCC827, HCC827-ER1

Experiment	Type	N	Treatment	Dose (mg/kg)	Dosing route	Doses administered
Vehicle	*Control*	5	PBS	0	p.o.	Single dose
Target efficacy	*Single drug*	5	Osimertinib	5	p.o.	Single dose
Target efficacy	*Single drug*	5	JNJ-61186372	10	i.p.	Single dose
Target efficacy	*Combo*	5	Osimertinib JNJ-61186372	5 10	p.o. i.p.	Single dose Single dose
Therapy resistance	*Single drug*	10	Osimertinib	5	p.o.	QD x 60 days
Therapy resistance	*Single drug*	10	JNJ-61186372	10	i.p.	BIW x 12 weeks
Therapy resistance	*Combo*	10	Osimertinib JNJ-61186372	5 10	p.o. i.p.	QD x 80 days BIW x 12 weeks

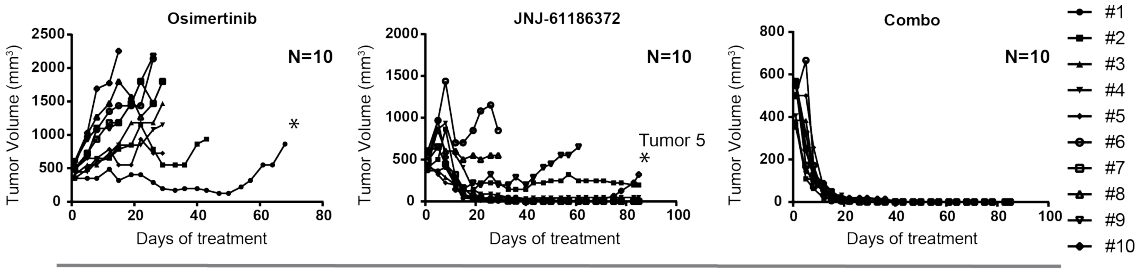
BIW; biweekly, QD; once daily

B



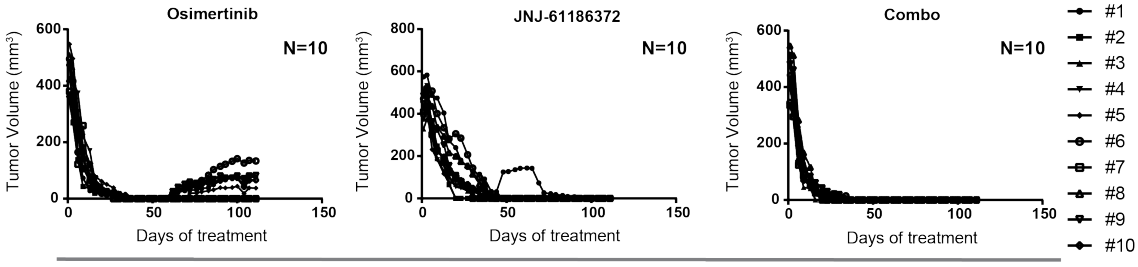
H1975

C



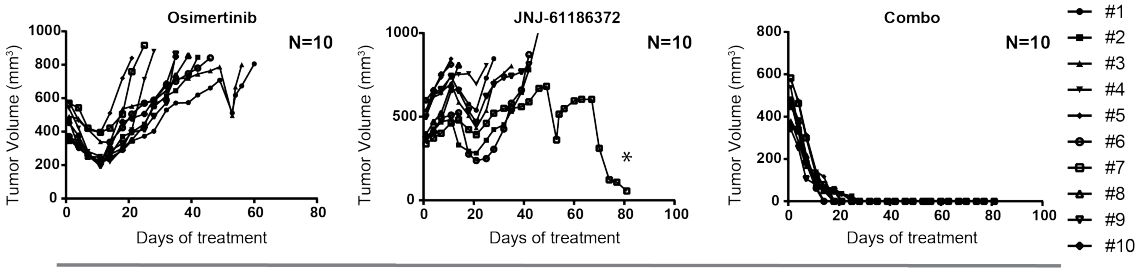
H1975-HGF

D



HCC827

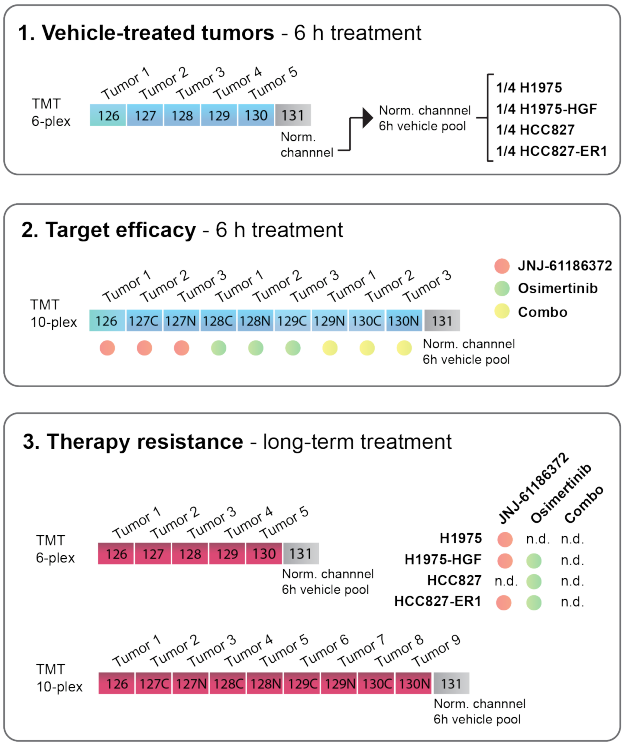
E



HCC827-ER1

Supplementary Figure S1. Resistant tumor growth is evident for osimertinib and JNJ-61186372 monotherapy in NSCLC cell line xenografts. A Experimental design and dosing scheme for *in vivo* analysis of variation among vehicle-treated tumor, target efficacy evaluation and drug resistance. Cell lines (H1975, H1975-HGF, HCC827 and HCC827-ER1) were established as xenografts in nude mice. Mice were treated with osimertinib and JNJ-61186372 alone or in combination according to the scheme. N, number of mice per group, i.p., intraperitoneal injection, p.o., *per os* (oral). **B-E** Tumor growth curves displaying tumor volume (in mm^3) measured every second day after treatment initiation. Results from four xenograft models (H1975, H1975-HGF, HCC827 and HCC827-ER1) upon mono- and combination therapy with osimertinib and JNJ-61186372 are displayed. 10 mice (N=10) were included per treatment group.

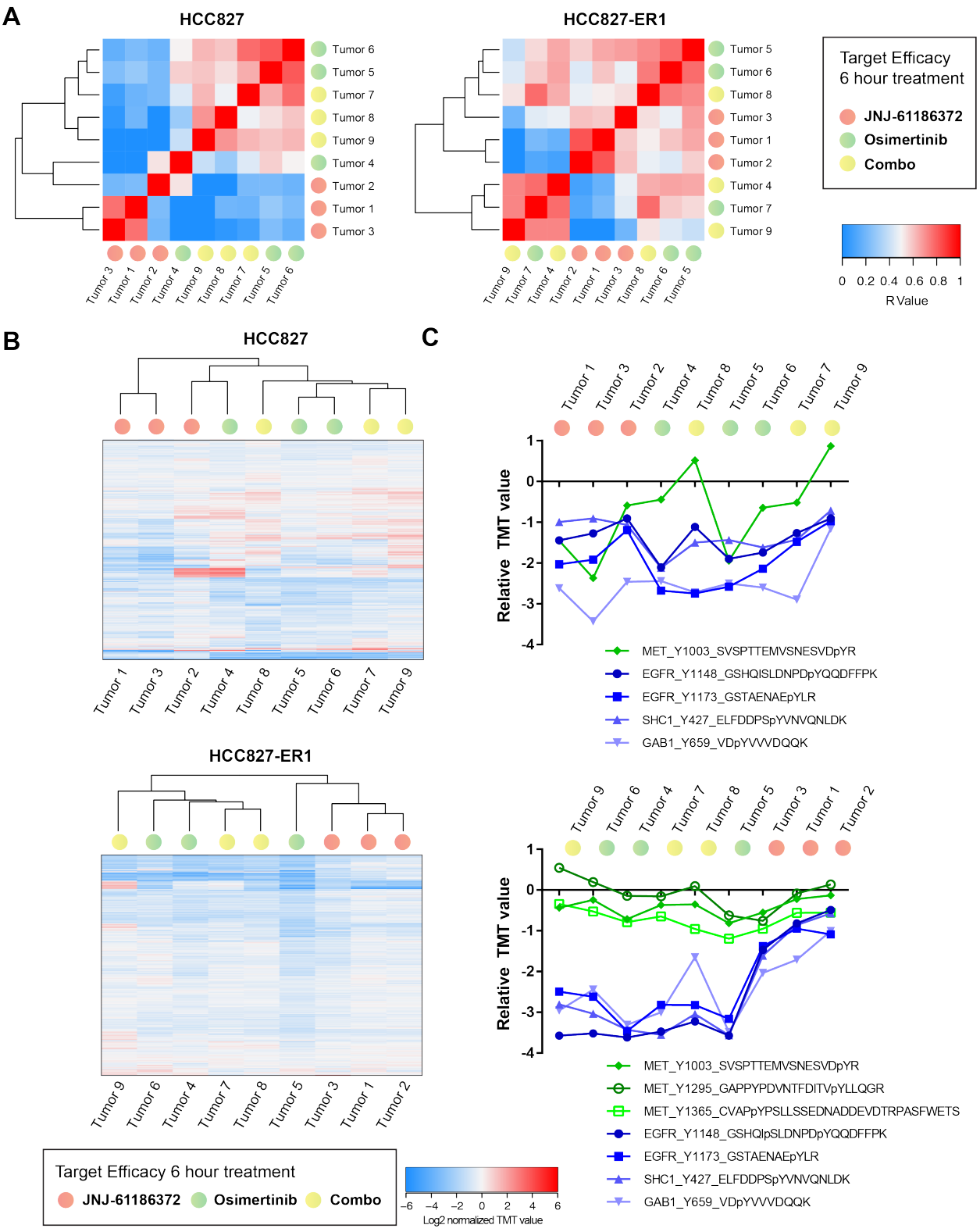
A



B

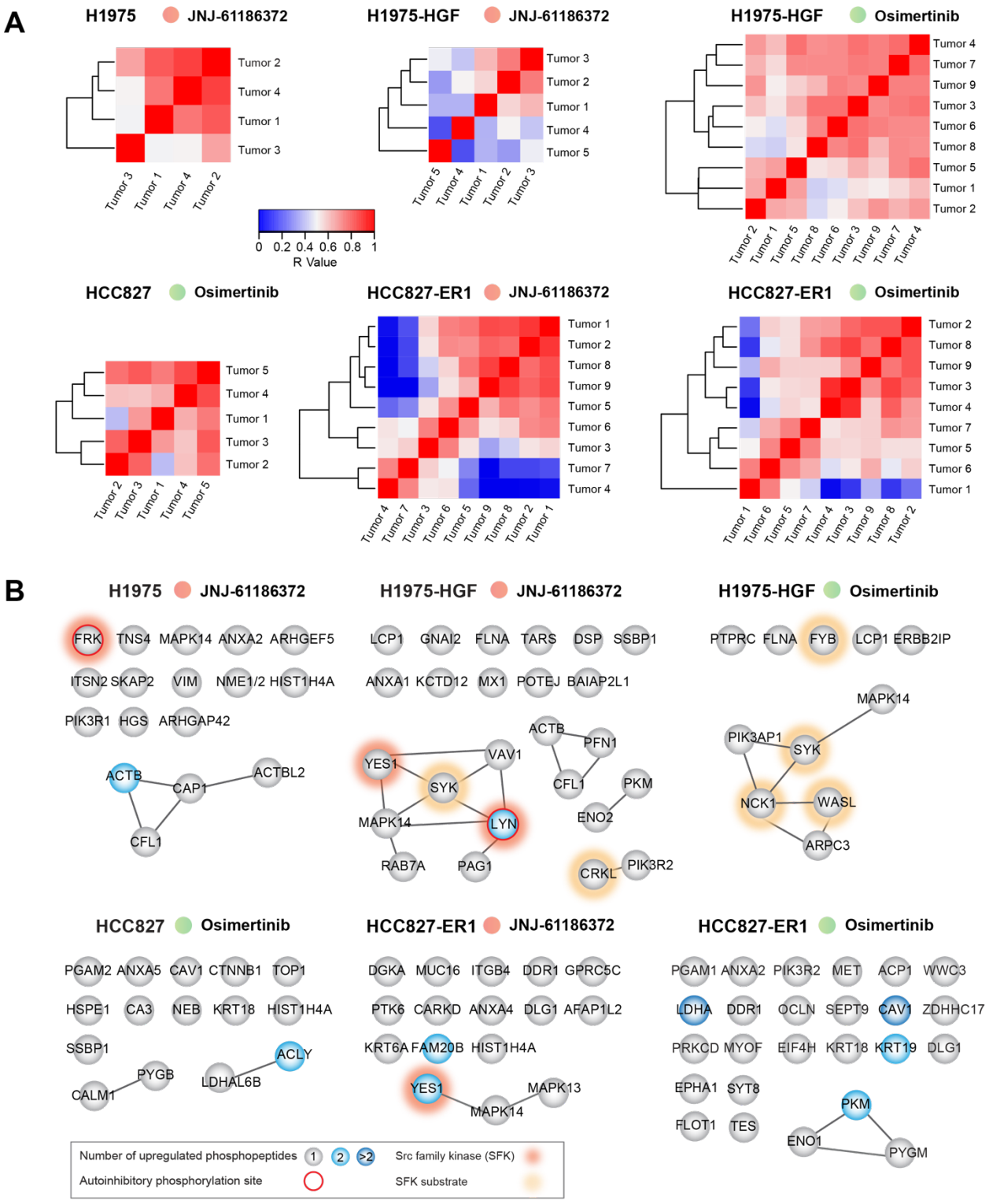
Experiment	Compound	Duration	Description	MS identified phosphopeptides
H1975 cell line				
Vehicle	PBS	6 hours	Same group	234
Target efficacy	Osimertinib	6 hours	3 from each group	118
	JNJ-61186372			
	Combo			
Therapy resistance	JNJ-61186372	Long-term	Same group	407
H1975-HGF cell line				
Vehicle	PBS	6 hours	Same group	517
Target efficacy	Osimertinib	6 hours	3 from each group	616
	JNJ-61186372			
	Combo			
Therapy resistance	Osimertinib	Long-term	Same group	335
Therapy resistance	JNJ-61186372	Long-term	Same group	328
HCC827 cell line				
Vehicle	PBS	6 hours	Same group	315
Target efficacy	Osimertinib	6 hours	3 from each group	445
	JNJ-61186372			
	Combo			
Therapy resistance	Osimertinib	Long-term	Same group	253
HCC827-ER1 cell line				
Vehicle	PBS	6 hours	Same group	513
Target efficacy	Osimertinib	6 hours	3 from each group	559
	JNJ-61186372			
	Combo			
Therapy resistance	Osimertinib	Long-term	Same group	643
Therapy resistance	JNJ-61186372	Long-term	Same group	614

Supplementary Figure S2. Multiplex TMT-labeling scheme and results from MS analysis of NSCLC xenograft tumors. A Overview of the setup for TMT labeled samples for quantitative mass spectrometry. Three types of analyses were performed to evaluate tumor variation in the vehicle-treated group, target efficacy and therapy resistance. 1. For each cell line, vehicle-treated tumors were measured as a TMT-6-plex including a normalization channel consisting of a pool of equal amounts of peptides from all 6 h vehicle-treated mice. 2. Target efficacy was measured as a TMT10plex for each cell line including peptides from 3 tumors of each treatment group (osimertinib, JNJ-61186372 and combo (osimertinib + JNJ-61186372)) together with a cell line specific pool of peptides from vehicle-treated tumors. 3. Therapy resistance was measured for each cell line as either a TMT-6-plex or TMT-10-plex depending on the number of resistant tumors. n.d., not detected. **B** Overview of phosphoproteomic data presenting the number of identified phosphorylated peptides resulting from analyses of vehicle-treated tumors, target efficacy and therapy resistance as indicated.

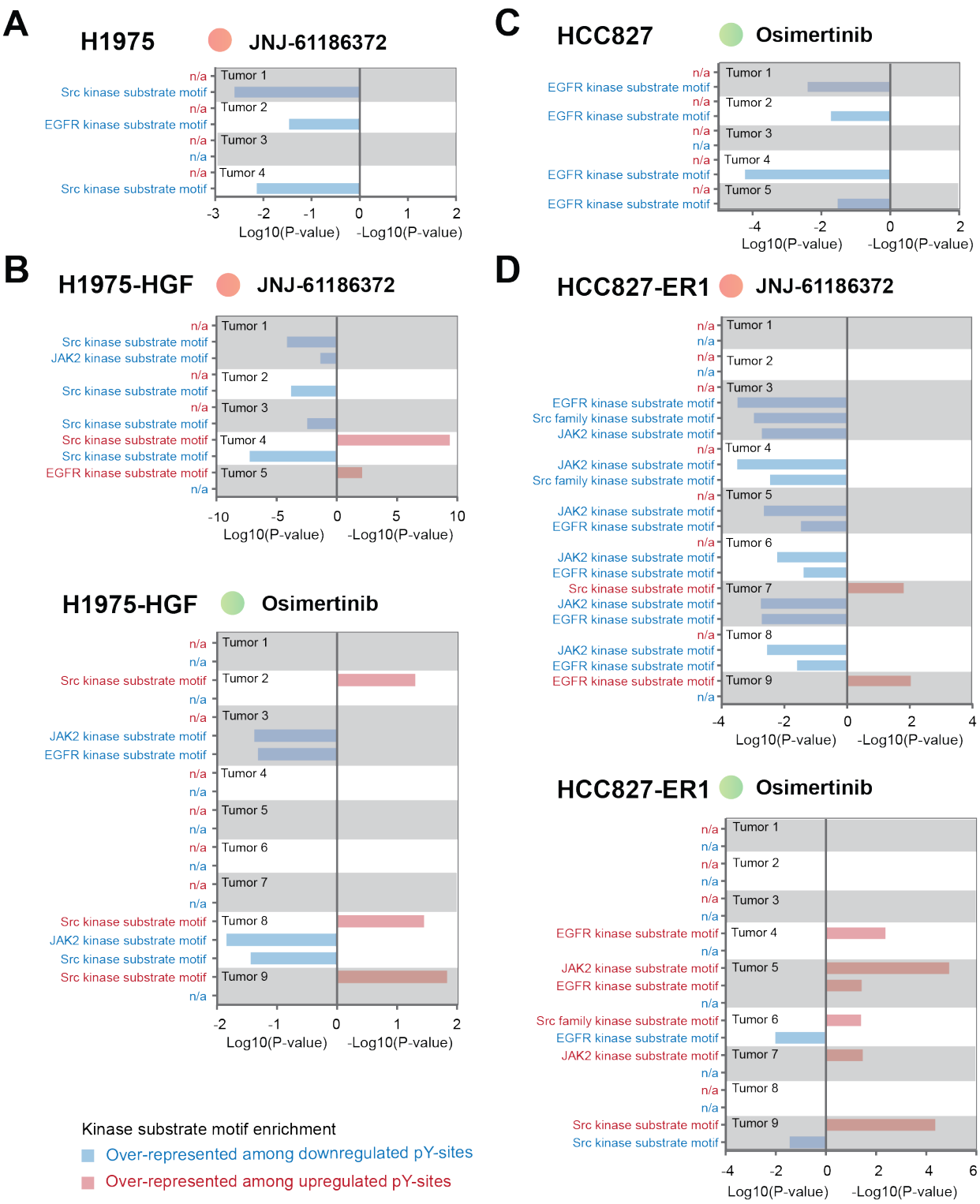


Supplementary Figure S3. Evaluation of *in vivo* target efficacy in HCC827 and HCC827-ER1 xenografts after 6-hour treatment with osimertinib and JNJ-61186372 alone or in combination. A Heatmaps of Pearson's correlation coefficient (R-value) from HCC827 (left) and HCC827-ER1 (right) xenograft models for evaluation of target efficacy upon 6 hours of treatment with osimertinib and JNJ-61186372 alone or in combination. (**B** and **C**) Hierarchical clustering (Euclidian distance) of relative

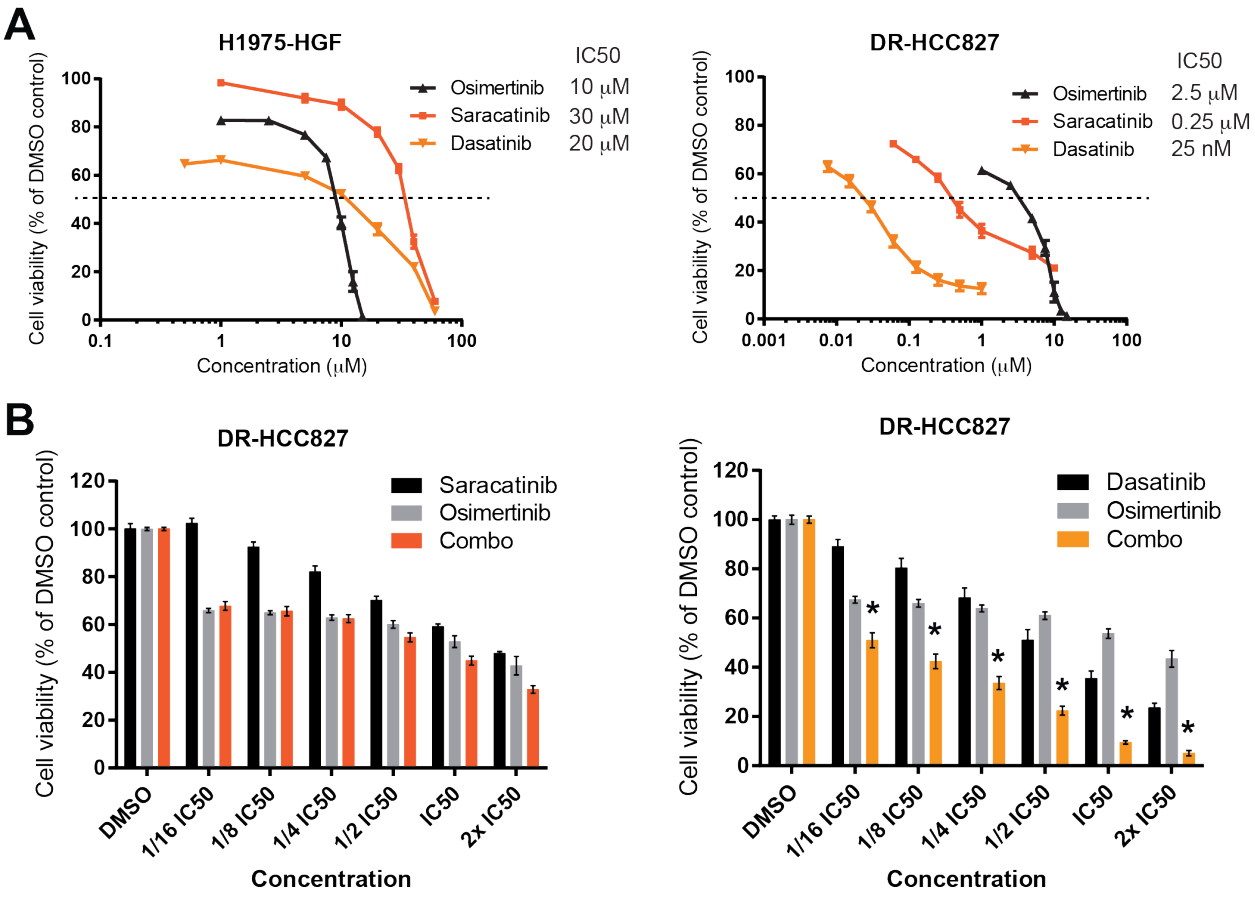
phosphopeptide changes (**B**) and extracted phosphotyrosine profiles from selected proteins (EGFR, Shc1 and Gab1) (**C**) as indicated to evaluate *in vivo* target efficacy. Results are presented for tumors from HCC827 (upper panels) and HCC827-ER1 (lower panels) xenografts. p, phosphorylated.



Supplementary Figure S4. Phosphopeptide correlations in resistant tumors reveal heterogeneous tumor profiles. A Heatmaps of Pearson's correlation coefficient (R-value) of phosphopeptide quantitation values from MS analyses of resistant xenograft models (H1975, H1975-HGF, HCC827 and HCC827-ER1) after long-term treatment with osimertinib and JNJ-61186372 monotherapy. **B** Phosphoprotein interaction networks for each group of resistant tumors as indicated. All proteins are represented by their gene name and have at least one phosphopeptide with a relative ratio >1.4 compared to the 6 h vehicle-treated tumors for minimum 5 of 9 tumors (TMT-10-plex) or 3 of 5 tumors (TMT-6-plex). Networks were generated using STRING.



Supplementary Figure S5. Kinase substrate motif enrichment analyses of resistant tumors. Kinase substrate motif enrichment analysis was done independently for each treatment resistant tumor. Motifs within up- and downregulated pool of phosphotyrosines (pY) were compared to the non-regulated pool of phosphotyrosines (Fisher's exact test, $P<0.05$).



Supplementary Figure S6. Heterogeneous phosphoprotein network upregulation in resistant tumors and combined EGFR and SFK inhibition results in superior growth inhibition of DR-HCC827 cells. **A** and **B** Cell viability of H1975-HGF (left) and DR-HCC827 (right) cells treated with the indicated concentrations of osimertinib, saracatinib and dasatinib. The approximate IC50 values are indicated to the right of each graph. Data are means ± SEM of three experiments. **C** Cell viability of DR-HCC827 cells treated with osimertinib and saracatinib (left) or dasatinib (right) alone or in combination. The compounds were used at the indicated concentrations. For the combination (combo) treatment a constant ratio was applied for the individual compounds (IC50; osimertinib: 2.5 μM, saracatinib: 0.25 μM, dasatinib: 25 ̈ M). Data are means ± SEM of three experiments. * denotes a synergistic combination effect (ΔI>0).

Supplementary Table S1: Summary of data from vehicle-treated tumors. List of identified and quantified phosphopeptides. Table is provided as an Excel file in the online additional supplementary materials.

Supplementary Table S2: Summary of data from target efficacy evaluation. List of identified and quantified phosphopeptides. Table is provided as an Excel file in the online additional supplementary materials.

Supplementary Table S3: Summary of data from drug resistant tumors. List of identified and quantified phosphopeptides. Table is provided as an Excel file in the online additional supplementary materials.