**METHODS**

**Treatments of patient-derived triple negative breast cancer xenograft models**

 Tumor volumes were measured every 2-3 days with a caliper. Response metrics were adapted from Heather et al. [1]. Low values (≤ 0.1 cm3) were truncated and growth curves were log2 transformed before slopes were calculated and the difference in slopes was measured between treatment and control groups for each animal using the formula: Treatment/Control rate based growth = 2(µTreatment−µControl) \* days treatment. For biomarker studies, tumor bearing mice were divided into 5 treatment groups (n=2 mice per group, 2 tumors each mice from the bilateral 4th mammary fat pads: vehicle 2h (vehicle on day 1 at hour 0), buparlisib 2h (buparlisib 30/kg IP on day 1 at hour 0), vehicle 50h (vehicle daily on days 1-3), buparlisib 50h (buparlisib 30mg/kg IP on days 1, 2 and 3), washout (buparlisib 30mg/kg IP on days 1 and 2, followed by vehicle on day 3). Mice were sacrificed with xenografts harvested 2 hours after the last dose of treatment. Xenograft tumors from the same mice and in the same treatment group were pooled for subsequent processing and analyses.

**Reversed phase protein arrays**

Fluorescence-labeled slides were scanned on a GenePix AL4200 scanner, each slide, along with its accompanying negative control slide, is scanned at an appropriate PMT to obtain optimal signal for this specific set of samples. The images were analyzed with GenePix Pro 7.0 (Molecular Devices). Total fluorescence signal intensities of each spot were obtained after subtraction of the local background signal for each slide and were then normalized for variation in total protein, background and non-specific labeling using a group-based normalization method as described. For each spot on the array, the background-subtracted foreground signal intensity was subtracted by the corresponding signal intensity of the negative control slide (omission of primary antibody) and then normalized to the corresponding signal intensity of total protein for that spot. Each image, along with its normalized data, was carefully evaluated for quality through manual inspection and control samples. Antibody slides that failed the quality inspection were either repeated at the end of the staining runs or removed before data reporting. Total 203 antibodies remained in the list. Please see link for a complete list of validated antibodies: https://www.bcm.edu/centers/cancer-center/research/shared-resources/cprit-cancer-proteomics-and-metabolomics/reverse-phase-proteinarray. The median of the triplicate experimental values (normalized signal intensity) is taken for each sample for subsequent statistical analysis. We determined significantly changed proteins between experimental groups by employing Student’s t-test (significant for p ≤ 0.05) and requesting a fold change of at least 1.5 times.

**Global proteome and phosphoproteome**

*Protein extraction, digestion and TMT labeling of peptides*

Tumors from patient derived xenograft tumors (n = 2 tumors per treatment and animal) were cryo-pulverized. Circa 200 mg (w/w) of tissue powder was lysed at 4°C in 1,200 uL lysis buffer (8 M urea, 75 mM NaCl, 1 mM EDTA in 50 mM Tris HCl (pH 8), 10 mM NaF, phosphatase inhibitor cocktail 2 (1:100; Sigma, P5726) and cocktail 3 (1:100; Sigma, P0044), 2 µg/mL aprotinin (Sigma, A6103), 10 µg/mL Leupeptin (Roche, 11017101001), and 1 mM PMSF (Sigma, 78830). Lysates were spun at 20,000 rcf for 10 min and supernatant (containing extracted proteins) was transferred to a clean microcentrifuge tube. Protein concentrations were determined using the Pierce BCA assay at a dilution of 1:10 with distilled water. Protein lysates were reduced with 5 mM dithiothreitol (Thermo Scientific, 20291) for 45 min at room temperature and alkylated with 10 mM iodoacetamide (Sigma, A3221) for an additional 45 min. Protein digests were diluted 1:4 with 50 mM Tris HCl (pH 8) before digestion with LysC (Wako, 100369-826) for 2 h and with trypsin (Promega, V511X) overnight. Both lysis steps were performed at a 1:50 enzyme-to-protein ratio and at room temperature. Digested samples were acidified with formic acid (FA; Fluka, 56302) to a final concentration of 1% (final pH of < 3), and then centrifuged at 2,000 rcf for 5 min to clear precipitated urea. Peptide lysates were desalted on C18 SepPak columns (Waters, 100mg/1cc) and 666 ug peptide aliquots were dried down using a SpeedVac. Desalted peptides were then labeled with 6-plex tandem mass tag (TMT [2]) reagents (lot# PI203521) according to the manufacturer’s instructions. Briefly, TMT reagent powder was reconstituted in anhydrous acetonitrile (MeCN) while dried peptides were reconstituted in 50 mM HEPES buffer (pH 8.5; Alfa Aesar). Labeling of peptides was performed at room temperature for 1 h with shaking. 5 µg of material was removed and desalted on stage tips (Empore C18) for label control analyses while the remaining bulk of the material was stored in -80°C. A labeling of ≥ 95 % of fully labeled spectra was considered a pass. Labeling was quenched using 5 % hydroxylamine (8 µL per 100 µg peptides) at room temperature for 15 min with shaking. All 6 TMT channels per PDX tumor were combined and desalted on C18 SePak columns (Waters, 500mg/6cc).

For the purpose of linking quantitative values between TMT6-plexes, we created a physical common reference sample/internal reference. This sample was comprised of equal amounts of digested peptide material from each sample (n=30). In detail, 133.4 ug was taken from each sample and pooled to a 4 mg sample that was mixed and aliquoted as 666 ug aliquots to each plex. The internal reference was set to constantly occupying the last, 131 channel of each plex. This internal reference sample was subsequently used as a common denominator for each other sample in the plex, allowing for a comparison between all 5 TMT6-plexes and across all 30 samples, regardless of which plex they initially belonged to.

*Offline fractionation and preparation of proteome and phosphoproteome samples*

Sample complexity was reduced by high pH reversed phase (RP) fractionation as described earlier [3]. Desalted TMT labelled peptides were reconstituted in 900 µL 20 mM ammonium formate (pH 10) and 2 % MeCN, loaded on a 4.6 mm x 250 mm column RP Zorbax 300 A Extend-C18 column (Agilent, 3.5 µm bead size), and separated on an Agilent 1100 Series HPLC instrument using basic reversed-phase chromatography. Eighty-four fractions were collected and subsequently concatenated as described earlier into 24 fractions [4]. Five percentage of the total material from each fraction was dried down and resuspended in 3 % MeCN/0.1 % FA to a peptide concentration of 1 µg/µL for LC-MS/MS analyses of the proteome.

The remaining 95 % of material was further combined into 12 fractions and enriched for phosphopeptides using immobilized metal affinity chromatography as previously described [4]. For phosphopeptide enrichment, Fe3+ nitrilotriacetic acid agarose beads were mixed with peptides in an 80 % MeCN/0.1 % TFA (trifluoroacetic acid) solution for 30 min. Beads were washed by centrifugation on a table top centrifuge using the same buffer and then loaded onto C18 Empore stage tips. Beads were further washed with 80 % MeCN/0.1 % TFA and 1 % FA before trans-eluted off from the beads to the C18 resin using 500 mM dibasic sodium phosphate (pH 7.0, Sigma S9763). One additional wash with 1 % FA preceded a final elution using 60 µL 50 % MeCN/0.1 % FA. Phosphopeptides were dried down and re-suspended in 9µL 50 % MeCN/0.1 %FA for LC-MS/MS analysis.

*Analysis of tumor samples by high performance liquid chromatography tandem mass spectrometry (LC-MS/MS)*

Online fractionation was performed using a nanoflow Proxeon EASY-nLC 1000 UHPLC system (Thermo Fisher Scientific) and separated peptides were analyzed on a benchtop Orbitrap Q Exactive mass spectrometer (Thermo Fisher Scientific) equipped with a nanoflow ionization source (James A. Hill Instrument Services, Arlington, MA). In-house packed columns (20 cm x 75 μm diameter C18 silica picofrit capillary column; 1.9 μm ReprosIl-Pur C18-AQ beads, Dr. Maisch GmbH, r119.aq; Picofrit 10 μm tip opening, New Objective, PF360-75-10-N-5). Mobile phase flow rate was 200 nL/min, comprised of 3 % acetonitrile/0.1 % formic acid (Solvent A) and 90 % acetonitrile /0.1 % formic acid (Solvent B). The 110 min LC-MS/MS method consisted of a 10 min column-equilibration procedure; a 20 min sample-loading procedure; and the following gradient profile: (min: % B) 0:2; 1:6; 85:30; 94:60; 95;90; 100:90; 101:50; 110:50 (the last two steps at 500 nL/min flow rate). Data-dependent acquisition was performed using Xcalibur QExactive v2.1 software in positive ion mode at a spray voltage of 2.00 kV. MS1 Spectra were measured with a resolution of 70,000, an AGC target of 3e6 and a mass range from 300 to 1800 m/z. Up to 12 MS/MS spectra per duty cycle were triggered at a resolution of 17,500, an AGC target of 5e4, an isolation window of 2.5 m/z, a maximum ion time of 120 msec, and a normalized collision energy of 27. Peptides that triggered MS/MS scans were dynamically excluded from further MS/MS scans for 20 sec. Charge state screening was enabled to reject precursor charge states that were unassigned, 1, or >6. Peptide match was enabled for monoisotopic precursor mass assignment.

*Protein-peptide identification, phosphosite localization, and quantification*

MS data was interpreted using the Spectrum Mill software package v5.0 pre-release (Agilent Technologies, Santa Clara, CA) co-developed by Dr. Karl Clauser of the Carr lab. MS/MS spectra were merged if they were acquired within +/- 45 sec of each other with the same precursor m/z. Also, MS/MS spectra that did not having a sequence tag length > 0 (i.e., minimum of two masses separated by the in chain mass of an amino acid) or did not have a precursor MH+ in the range of 750-6000 were excluded from searching. MS/MS spectra searches were performed against a concatenated human and mouse RefSeq database containing 31,767 human proteins, 24,821 mouse proteins, and 85 additional contaminants (RefSeq release 60, 2013/7/27-2013/7/30). ESI-QEXACTIVE-HCD-v2, for whole proteome datasets, and ESI-QEXACTIVE-HCD-v3, for phosphoproteome datasets were set as scoring parameters. Spectra were allowed +/- 20 ppm mass tolerance for precursor as well as product ions, 40% minimum matched peak intensity, and “trypsin allow P” was set as enzyme specificity with up to 4 missed cleavages allowed. Carbamidomethylation at cysteine was set as fixed modification together with TMT6 isobaric labels at lysine residues (N-termini would be considered regardless if it was TMT labelled). Acetylation of protein N-termini, oxidized methionine, deamidation of asparagine, pyroglutamic acid at peptide N-terminal glutamine, and pyro-carbamidomethylation at peptide N-terminal cysteine were set as variable modifications with a precursor MH+ shift range of -18 to 64 Da for the proteome searches. For the phosphoproteome searches the precursor MH+ shift range was set to 0 to 272 Da and variable modifications of phosphorylation of serine, threonine, and tyrosine, while disallowing deamidation.

Identities interpreted for individual spectra were automatically designated as confidently assigned using the Spectrum Mill autovalidation module to use target-decoy based false discovery rate (FDR) estimates to apply score threshold criteria. For the whole proteome datasets thresholding was done at the spectral and protein levels. For the phosphoproteome datasets thresholding was done at the spectral and phosphosite levels.

*Single-shot LC-MS/MS analysis of vehicle treated WHIM tumors and the WHIM 12 derived cell line*

WHIM 12 derived cells were cultivated as described under main Materials and Methods section: In vitro validations; WHIM3 and WHIM12 patient-derived xenograft cell lines. A snap frozen cell line pellet was lysed using the same lysis protocol as used for the WHIM tumor samples. One micro-gram of desalted peptides for all vehicle treated WHIM tumors (2 hours treatments; WHIM 12 both 2 hours and 50 hours vehicle treatments), as well as from the WHIM 12 derived cell line was analyzed using LC-MS/MS. Online fractionation was performed using a nanoflow Proxeon EASY-nLC 1000 UHPLC system (Thermo Fisher Scientific) and separated peptides were analyzed on a benchtop Orbitrap Q Exactive mass spectrometer (Thermo Fisher Scientific) equipped with a nanoflow ionization source (James A. Hill Instrument Services, Arlington, MA). In-house packed columns (20 cm x 75 μm diameter C18 silica picofrit capillary column; 1.9 μm ReprosIl-Pur C18-AQ beads, Dr. Maisch GmbH, r119.aq; Picofrit 10 μm tip opening, New Objective, PF360-75-10-N-5). Mobile phase flow rate was 200 nL/min, comprised of 3 % acetonitrile/0.1 % formic acid (Solvent A) and 90 % acetonitrile /0.1 % formic acid (Solvent B). The 110 min LC-MS/MS method consisted of a 10 min column-equilibration procedure; a 20 min sample-loading procedure; and the following gradient profile: (min: % B) 0:2; 1:6; 85:30; 94:60; 95;90; 100:90; 101:50; 110:50 (the last two steps at 500 nL/min flow rate). Data-dependent acquisition was performed using Xcalibur QExactive v2.1 software in positive ion mode at a spray voltage of 2.00 kV. MS1 Spectra were measured with a resolution of 70,000, an AGC target of 3e6 and a mass range from 300 to 1800 m/z. Up to 12 MS/MS spectra per duty cycle were triggered at a resolution of 17,500, an AGC target of 5e4, an isolation window of 2.5 m/z, a maximum ion time of 120 msec, and a normalized collision energy of 27. Peptides that triggered MS/MS scans were dynamically excluded from further MS/MS scans for 20 sec. Charge state screening was enabled to reject precursor charge states that were unassigned, 1, or >6. Peptide match was enabled for monoisotopic precursor mass assignment.

In average, 4,507 proteins were quantified by LC-MS/MS (searched using Max Quant [REF]) per sample.

**Mass spectrometry-based kinome profiling**

*Preparation of tumor lysates for kinase enrichment*

Frozen tumor tissues from each of the 30 experimental conditions were cryo-pulverized into a powder, pooled and aliquoted (~ 150 mg) as previously described [3]. Aliquots from the same pools of cryo-pulverized tissue were used for all analyses. This included global proteomic and phosphoproteomic analysis, relative kinase levels using multi-inhibitor bead (MIB) enrichment [5] and RPPA. Aliquots of tumor powder were solubilized on ice with 800 µL of MIB lysis buffer (50 mM HEPES, pH 7.5) containing 0.5% Triton X-100, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA (Sigma), 2.5 mM Sodium orthovanadate, 10 mM NaF, 1X protease inhibitor cocktail (Roche), and 1% each of phosphatase inhibitor cocktails 2 and 3 (Sigma) (MIB lysis buffer). Each extraction (800 µL) was transferred to a glass tube (12 x 12 mm) for ultra-sonication in the Covaris S220X instrument. The transfer from the tubes containing the tissue powder was accomplished with 2-3 lysis buffer extractions. The following settings were used: Peak Incident Power: 100; Duty Factor: 10%; Cycles/Burst: 500; Time: 2 min; 4°C. The sonicated lysates were transferred to 1.7 mL Eppendorf tubes and spun at 15,000 x g for 10 min at 4°C (Eppendorf 5424R). The supernatants were filtered (Costar, Part No. 8163 0.45 µ) at 4°C and combined in a 15 mL Falcon tube. The protein content was determined from an aliquot (10 µL; Advanced Protein Assay from Cytoskeleton). Standards (BSA) were prepared by serial dilution (1:2) in deionized water (7 µg to 2000 µg/mL). The sonicated lysates were diluted (1:50) and duplicate aliquots (10 µL) were analyzed at 590 nm in a Thermo-BioMate 3 spectrophotometer (1 mL cuvette). The lysates aliquots (1 mg) were stored at -80°C.

*Multi-inhibitor kinase bead enrichment and peptide preparation*

The tissue lysates (1 mg in 200 µL) were thawed on ice for ~ 30 min. The MIB slurry (50%) was prepared by combining individual suspension of beads in the following proportions: 11.7% of Shokat, PP58, Purvalanol B, UNC-21474 and 17.7% VI-16832, CTx-0294885 and Buparlisib. The combined aliquots were washed with 50 mM HEPES (pH 7.5), 0.5% TritonX-100, 1 M NaCl, 1 mM EDTA, and 1 mM EGTA,. The lysate and bead mixtures (70 µL) were tumbled overnight on a Mini Lab roller (Labnet) at 4°C. After incubation, the lysate and bead mixtures were separated by centrifugation at 1000 x g for 2 min and the supernatants were removed. The MIB beads were then washed twice with buffer containing 50 mM Hepes (pH 7.5), 1 M NaCl, 1 mM EDTA, 1 mM EGTA, 10 mM NaF, 2.5 mM NaVO4 and phosphatase inhibitor cocktails 2 and 3 (Sigma P5762 and Sigma P0044), both inhibitor cocktails were diluted 100-fold. The washed beads were resuspended in 60 µL of MIB elution buffer containing 2% SDS, 100 mM DTT and 100 mM Tris, pH 7.6 and mixed (ThermoFisher, Thermomixer) at 1000 rpm for 30 min at 57°C. The MIB eluates were transferred to a new tube after centrifugation at 1000 x g for 2 min. The samples were digested as previously described using the enhanced FASP method [6]. The MIB eluates (60 µL) were mixed with 300 µL of 100 mM Tris-HCL buffer, pH 8.5 containing 8 M urea and transferred to the 30K filter unit (Millipore, MRCF0R030). The units were spun in a microcentrifuge (Eppendorf 5424) at 14,000 x g for 15 min. An additional 200 µL of 100 mM Tris-HCL buffer, pH 8.5 containing 8 M urea was added to the top compartment of the filter apparatus and was spun at 14,000 x g for 15 min. The flow through was then discarded and the proteins were alkylated with 50 mM iodoacetamide (100 µL) and mixed in the dark at 550 rpm at room temperature for 20 min. The filter was spun at 14000 x g for 10 min and the flow through discarded. Unreacted iodoacetamide was spun through the filter with two 200 µL wash-spin (10 min) cycles of the 100 mM Tris-HCL buffer containing 8 M urea. The urea-containing buffer was exchanged with the digest buffer (100 mM ammonium bicarbonate, pH 8) using two 200 µL wash-spin cycles. The filter units were then transferred to a new collection tube and 100 µL of digest buffer containing 1 µg of trypsin (Sigma T6567) was added to the upper chambers. The samples were digested overnight at 37°C in a humidity chamber, after which an additional 1 µg of trypsin was added with incubation for 4 h at 37°C. The filter units were spun at 14,000 x g for 15 min to collect the peptides in the lower chambers. The filters were washed with 50 µL of 0.5 M sodium chloride and centrifuged into the lower chamber containing the peptides. Residual Triton X-100 was removed using a phase transfer procedure [6]. In an Eppendorf tube (1.7 mL), ethyl acetate (J.T.Baker 9260-02), saturated with water, was added (1 mL) to each digest, vortexed (1 min) and centrifuged at 16,000 x g (1 min). The extraction was repeated twice and the ethyl acetate was removed by heating the samples 65º C (15 min). The extracted digests were acidified (5% formic acid), transferred to PCR tubes (0.5 mL) and positioned in 96 well holders for robotic solid phase extraction (SPE). Each digest was extracted sequentially with one C4 tip (Glygen BIOMEK NT3C04) and one porous graphite carbon micro-tip (Glygen BIOMEK NT3CAR) with the following auto-pipetting steps: i) wet tips with MeCN/FA (60%/1%) (10 x 25 µL); ii) equilibrate tips with MeCN/FA (1%/1%) (10 x 25 μL); iii) extract peptides with repetitive aspirations of the digest (50 x 60 µL); iv) wash loaded tips with MeCN/FA (1%/1%) (10 x 25 µL); v) elute peptides with MeCN/FA (60%/1%) (5 x 35 µL) and vi) wash tips with MeCN/FA (60%/1%) (1 x 25 µL). The SPE eluants and wash were pooled and dried in a SpeedVac centrifuge (SVC) and dissolved in MeCN/FA (1%/0.1%) (15 µL). A peptide assay was performed (10%) using the Pierce Quantitative Fluorometric Peptide Assay Kit (Prod# 23290). The remaining peptides were stored at -80ºC. For TMT-6 labeling, aliquots (1 µg) were transferred to PCR tubes (0.5 mL), dried (SVC) and dissolved in 12 µL of labeling buffer (50 mM HEPES, pH 8.0). The TMT reagents 6-plex (Cat#: 90064, Pierce) were allowed to come to room temperature for 30 min and then centrifuged. Each of the TMT-6-plex reagents was dissolved in 90 µL of anhydrous acetonitrile. The TMT reagents (4 µL) were added to each peptide sample and the reference pool consisting of peptides from all 30 samples. The reference pool was labeled with TMT tag 126. After incubation for 2 h at room temperature, the reactions were quenched with 1 µl of 5% hydroxylamine in 200 mM TEAB. For each PDX model, the five labeled treatment samples (15 µL) and reference pool were combined into a PCR tube (0.5 mL) and dried (SVC). The pooled peptides were dissolved in MeCN/FA (1%/5%) and desalted using the robotic solid phase extraction method, as described above. The desalted, labeled peptides were dried and dissolved in MeCN/FA (1%/1%) and transferred to autosampler vials (Sun-SRI, Cat No. 200046) for LC-MS analysis.

*LC/MS Analysis*

LC-ESI/MS/MS analysis was performed using a Q-Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Plus mass spectrometer (ThermoFisher Scientific) coupled to an EASY-nanoLC 1000 system (ThermoFisher Scientific). The samples were loaded (2.5 µL) onto a 75 µm i.d. × 50 cm Acclaim™ PepMap™ 100 RP column (Thermo-Fisher Scientific). The peptides were eluted at a flow rate of 300 nL/min with an acetonitrile gradient in aqueous formic acid (0.1%) as mobile phase A. After isocratic elution with 5% B for 1 min the acetonitrile proportion was increased linearly to 17% with solvent B (100% MeCN, 0.1% FA) over 127 min, followed by sequential increases in B to 27% in 112 min, 35%B in 40 min, 70%B in 6 min, isocratic at 70%B for 6 min, 95% B over 2 min and an isocratic wash at 95% B for 6 min. Full-scan mass spectra were acquired by the Orbitrap™ mass analyzer in the range of *m/z*= 375 to 1500 and with a mass resolving power set to 70,000. Twelve data-dependent high-energy collisional dissociations (HCD) were performed with a mass resolving power set to 35,000, a fixed first *m/z* 100, an isolation width of 1.2 *m/z*, and the normalized collision energy setting of 32. The maximum injection time was 50 ms for parent-ion analysis and 105 ms for product-ion analysis. Target ions already selected for MS/MS were dynamically excluded for 30 sec. An automatic gain control (AGC) target value of 3e6 ions was used for full MS scans and 1e5 ions for MS/MS scans. Peptide ions with charge states of one or greater than six were excluded from MS/MS acquisition. The tandem mass spectra were processed using Matrix Science Distiller version 2.5 without charge state deconvolution and deisotoping. The processed files were used for protein database searches using Mascot (Matrix Science, London, UK; version 2.5.1). Searches were performed against a concatenated human and mouse RefSeq database containing 31,767 human proteins, 24,821 mouse proteins, and 85 additional contaminants (RefSeq release 60, 2013/7/27-2013/7/30). Peptide identifications were matched to their respective human genes for inference, and peptides matching only mouse or contaminant database entries were removed. Peptides that matched two or more human genes were excluded from quantification, resulting in 5,139 quantified human genes from 41,379 unique peptides. For each gene, PSMs from each TMT sample channel were summed, and expression values were represented as the ratio of the summed sample channel intensity to the summed reference pool channel. Identifications were excluded from quantification if either the sample channels or the reference pool had a summed intensity value of 0. Data from all TMT 6-plexes were aligned, log transformed, and normalized using a two component Gaussian mixture model.

 **One-sample moderated *t*-test**

A one-sample moderated *t*-test was used to identify markers that are regulated in the same direction across all tumors after buparlisib treatment. The two-tailed one-sample *t*-test was performed using r-package the limma R-package [7]. Buparlisib treated tumors were normalized to respective controls. Phosphosites were additionally normalized to the proteome before normalization to controls. P-values were adjusted for multiple hypothesis testing using the Benjamini-Hochberg method. It should be noted that after Benjamini-Hochberg adjustment many markers are significant with at an conventional adjusted p-value of less than 0.05, however, since we had few samples (n=6) and large biological variability between the tumors at baseline and after treatment (Supplementary Fig. 5), we chose to relax significance to ≤ 0.1 to capture more changes, and for inclusion for bioinformatic analyses (such as pathway enrichment) to ≤ 0.2. Relaxation of adjusted p-values comes with rigorous cross-referencing to the literature and/or known pathways and gene-sets. For the pathway enrichment analyses we additionally applied a stringent p-value filtering with a Bonferroni correction of ≤ 0.05, and we therefore believe that an initial adjusted p-value cut-off of ≤ 0.2, on marker level, can be tolerated.

**Two-sample moderated *t*-test**

To identify markers that differentiate between the most sensitive and resistant PDX tumors we used a two-tailed two-sample moderated *t*-test the limma R-package [7]. Buparlisib treated tumors were normalized to respective controls. Phosphosites were additionally normalized to the proteome before normalization to controls. P-values were adjusted for multiple hypothesis testing using the Benjamini-Hochberg method. Phosphosites were in a second round of analyses collapsed to phospho-genes and keeping the TMT based quantitative values from the most varying site (performed using R [8]). This additional step to roll-up/collapse phosphosites was performed to get a list of unique gene names from the global phosphoproteomic data to perform subsequent pathway enrichment analyses (see previous section *GeNets pathway enrichment analyses*).

**PEST motifs identifier**

To identify PEST motifs (rich in proline [P], glutamic acid [E], serine [S], and threonine [T]) we used the online tool: emboss.bioinformatics.nl/cgi-bin/emboss/epestfind (epestfind). This algorithm predicts PEST motifs from amino acid sequences (≥ 12 amino acids) and scores them based on their inclusion of amino acids P, E, S and T, as well as expected enrichment for additional negatively charged amino acid residues. The epestfind algorithm also excludes positively charged amino acids R, H and K, however, requires putative PEST motifs to be flanked by positively charged residues. A threshold score of ≥ 5 was kept as default for calling a PEST motif as *identified*. Protein FASTA sequences were collected from the Uniprot.org repository (http://www.uniprot.org/).

**GeNets pathway enrichment analyses**

Pathway enrichment analyses for significantly regulated transcripts, proteins or phosphoproteins were performed using the GeNets online tool developed at The Broad Institute (https://apps.broadinstitute.org/genets). Markers used were collapsed to HGNC symbols and the top 250 significant markers were included in each analyses. For the analyses in main figure 3b we chose to relax significance to ≤ 0.2 for what we called a *significant marker* before pathway enrichment. The pathway enrichment works through a hypergeometric test against the MSigDB (C2:CP) with Bonferroni corrections of p-values. A significant pathway was called as enriched if it had a Bonferroni corrected p-value ≤ 0.05.

**Kinase outlier analysis**

Kinase outlier analysis of the global phosphoproteome was performed as described earlier [9]. In brief, log2 normalized phosphosite data from all samples/conditions was used for this analysis. Distributions for each phosphosite were created using log2 normalized phosphoproteomics data from 19 basal TCGA tumors, and aberrantly activated kinases in the buparlisib data were identified as those with normalized phosphosite expression greater than 1.5 interquartile ranges (IQR) from the median. We felt confident in using the TCGA tumors to create our base distribution after comparing those distributions to a set of distributions from PDX tumors using a Kolmogorov-Smirnov test and finding that they differed in less than 95% of cases tested. Additionally, data from both this study and the TCGA proteomic analysis7 contained a common reference, allowing for cross-comparison. Aberrant kinases were then classified as being enriched in a specific sample or resistance group (resistant: WHIM12 and WHIM2, in-between: WHIM6 and WHIM21, sensitive: WHIM30 and WHIM4) with > 50% of samples containing a phosphosite outlier and < 5% FDR using Benjamini-Hochberg-adjusted p-values. Outliers were collapsed across treatment types for each sample for display purposes.

**Pearson correlation of mRNA and protein levels and gene-set enrichment analysis**

Human markers that were identified in both the global transcriptome and proteome, as defined by HGNC symbols (n = 7,009), were used to estimate the correlation between mRNA and protein levels in this study. Pearson correlations were used calculated for all these markers for all 30 samples. The resulting Pearson *r* values were then used to rank all markers from highest correlation (0.99) to most anti-correlated marker (-0.90). This rank was then used to perform a pre-ranked gene-set enrichment analysis (GSEA) [10]. The GSEA was weighted, escluding smaller gene-sets than ≤ 15 and larger than ≥ 500; normalized-enrichment scores were based on a 1,000 iterations and the database queried was the MSiGDB (C2:CP).

**Principle component analyses**

Principal component analyses were performed on vehicle treated PDX tumors using the global transcriptome, proteome and phosphoproteome data separately. Analyses were performed with the r-package in R using ‘prcomp’ function of the base package.

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