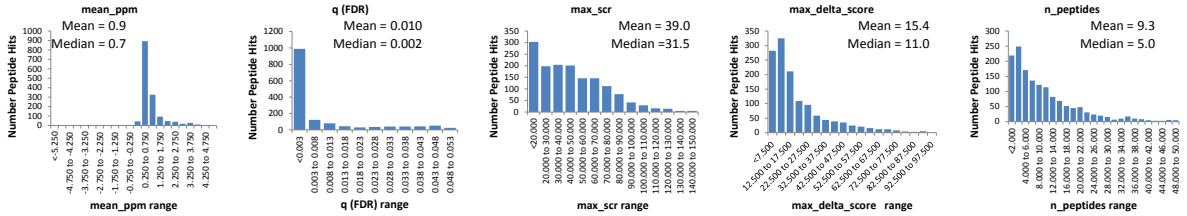
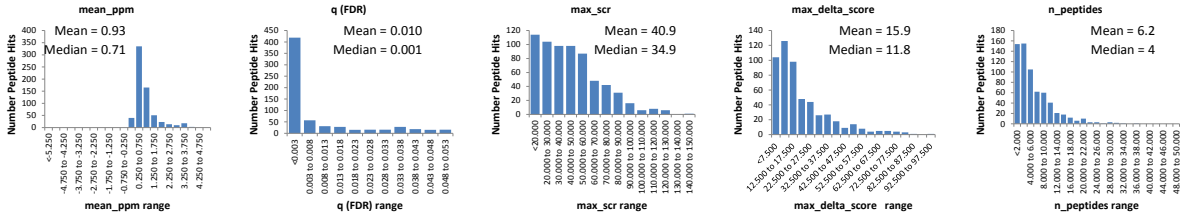
Cross-species proteomics reveals specific modulation of signaling in cancer and stromal cells by PI3K inhibitors

Supplementary Materials

Phosphoproteomics human



Phosphoproteomics mouse



Proteomics human

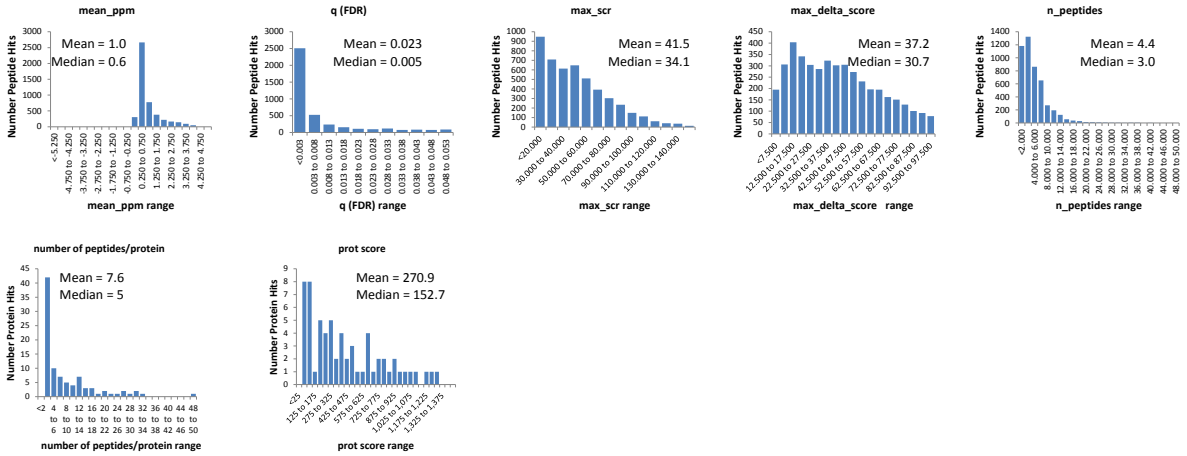
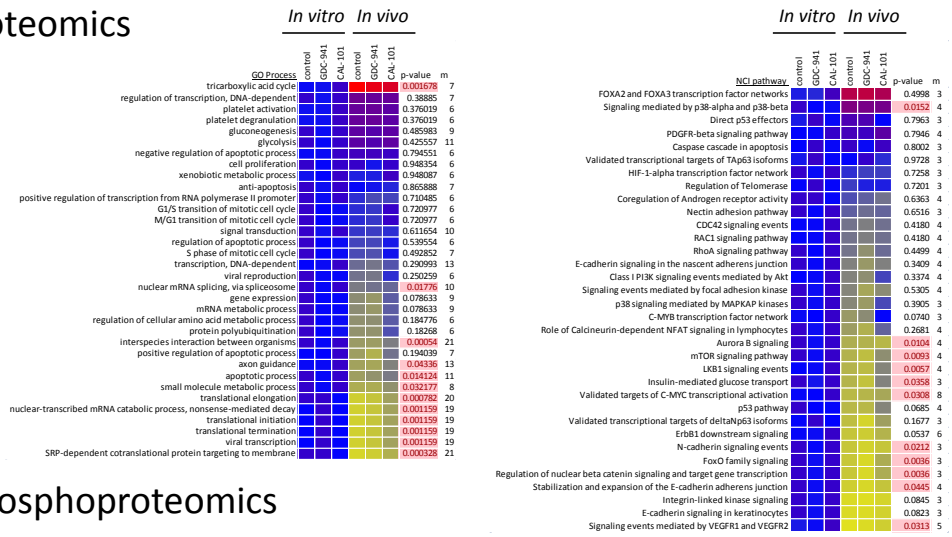


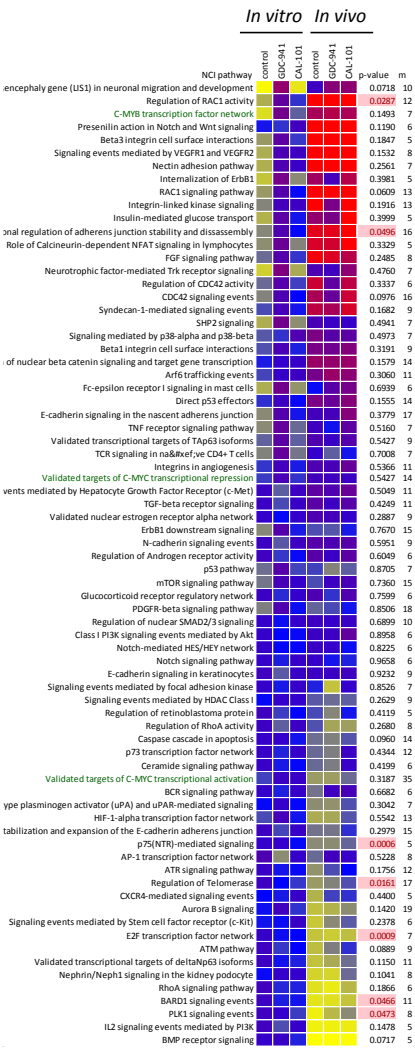
Figure S1. Descriptive statistics of data quality for peptide and phosphopeptides identifications from MS/MS data

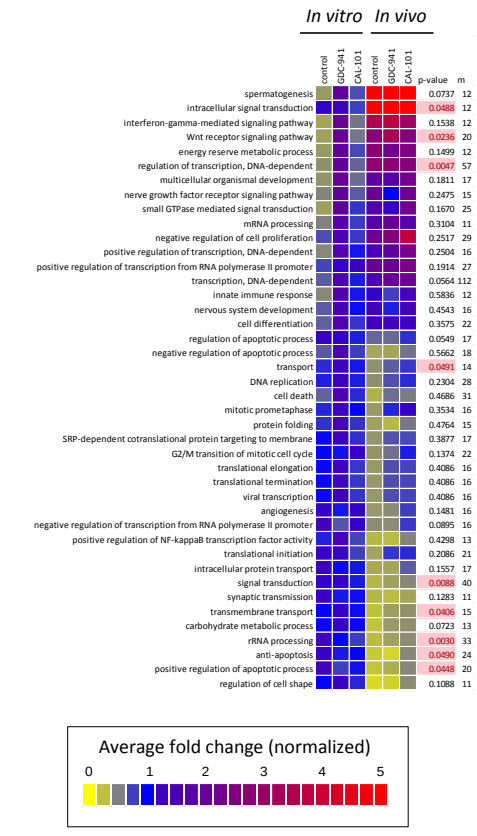
Parameters returned by Mascot searchers against the Uniprot database are shown as frequencies for human phosphoproteins, mouse phosphoproteins and human proteins. Graphs show the following measures of data quality: Mean_ppm, average mass error between calculated and experimental m/z values for a given peptide spectral match; q(FDR), the ratio of positive identifications between searchers against forward and reversed databases at a given sets of windows for mass tolerances and score threholds; max_scr, largest Mascot score of all MS/MS spectra matching the same peptide; max_delta_score, largest difference in Mascot score between first and second possibility of all MS/MS spectra matching the same peptide; n_peptides, number of MS/MS spectra matching a single peptide.

Proteomics



Phosphoproteomics





m, number of phosphopeptides in the named pathway or ontology

Figure S2. Gene ontologies and pathways matched to human proteins and phosphopeptides sequences.

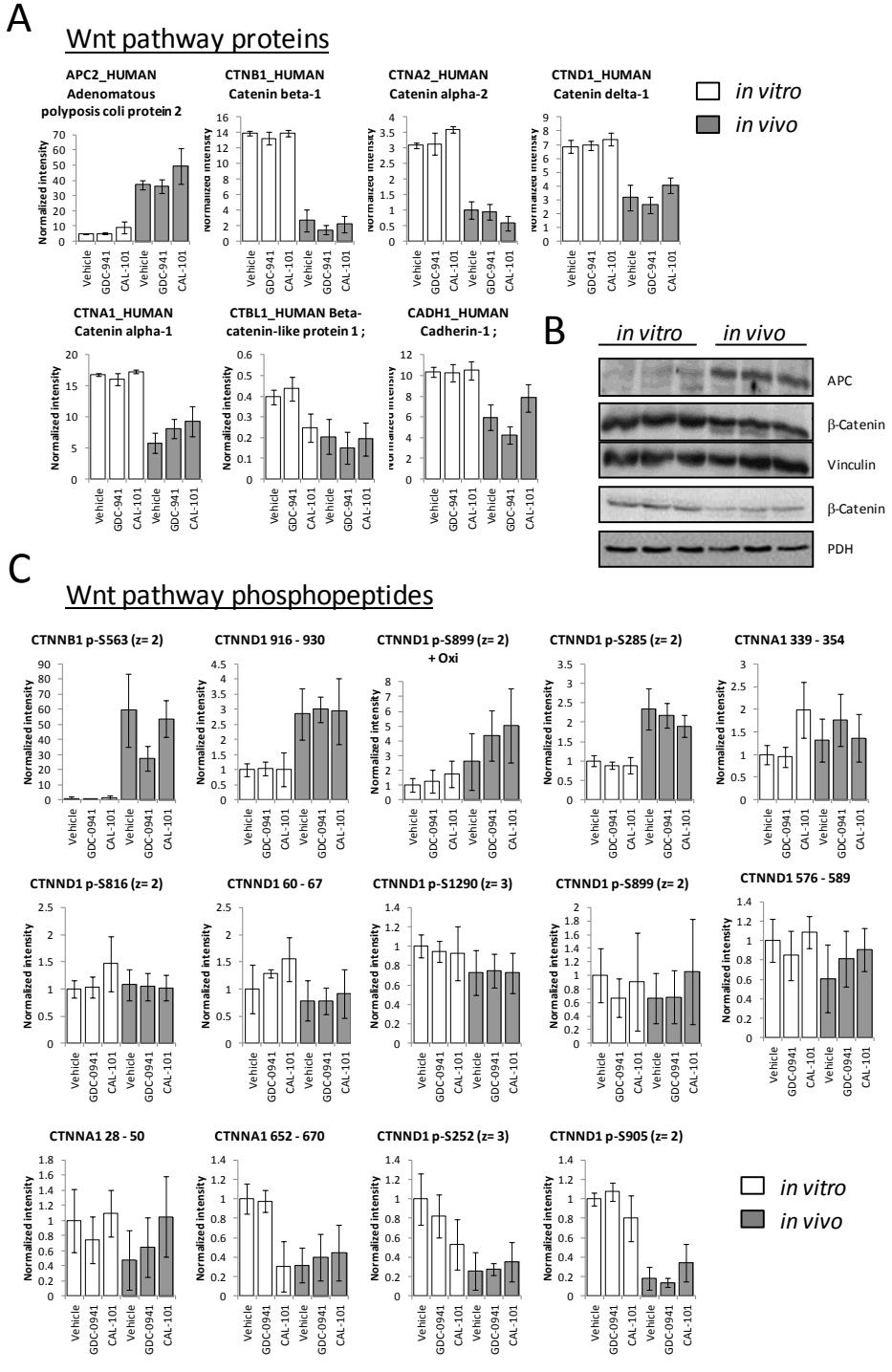


Figure S3. The tumor microenvironment induced changes in the abundance of proteins and phosphoproteins in the Wnt signaling pathway

(A) Expression of Wnt/catenin/cadherin proteins in cancer cells grown *in vitro* or *in vivo.* (B) WB shows the analysis of three biological replicates of untreated cells per conditions. The expression of β-catenin is shown in two blots at different exposure times. APC, Adenomatous polyposis coli; PDH, pyruvate dehydrogenase. (C) Fold changes of phosphopeptides on catenin sequences modulated in

cells grown *in vivo* relative to those grown *in vitro*. Values shown in bar charts are mean fold-change over control ± SD (n=6).

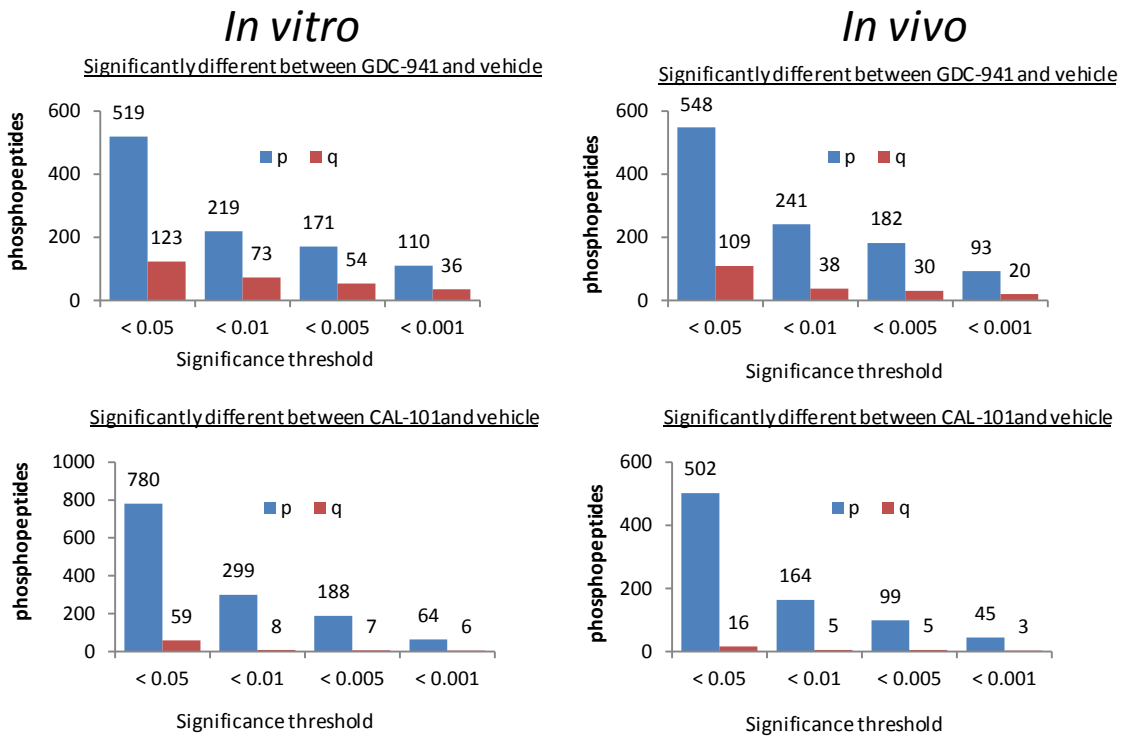


Figure S4. Number of human phosphopeptides significantly modulated by PI3K inhibitors *in vivo* and *in vitro* at different significance thresholds.

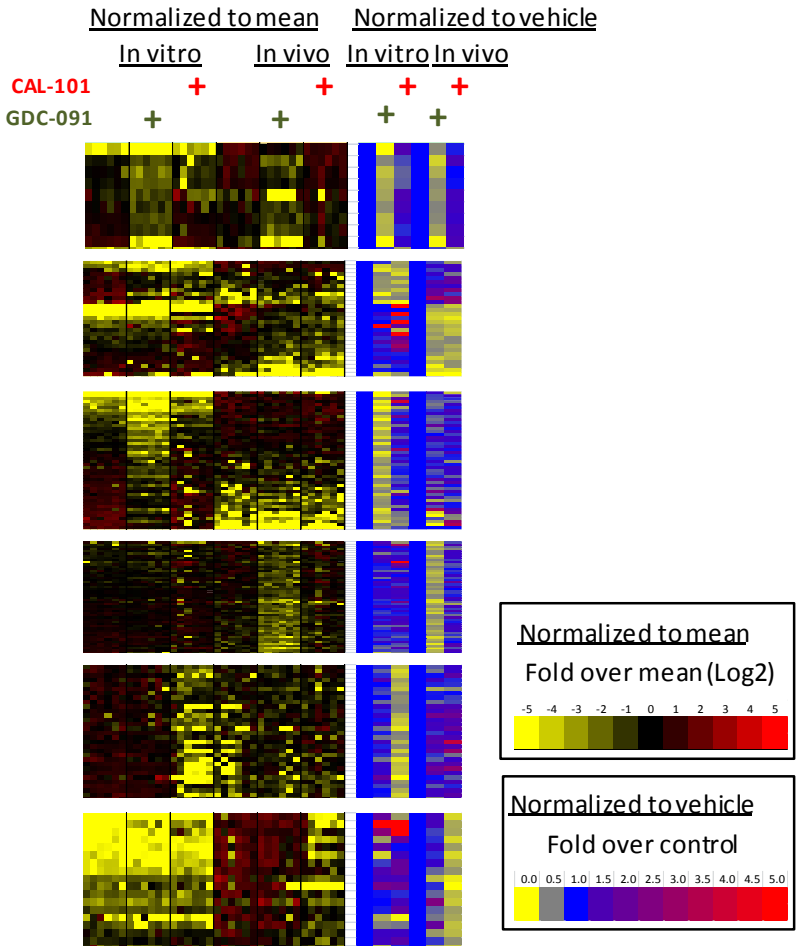


Figure S5. Clusters of drug-modulated phosphorylation sites. Phosphorylation sites significantly inhibited by CAL-101, GDC-0941 in vivo or in vitro were classified by patterns of inhibition using supervised methods.

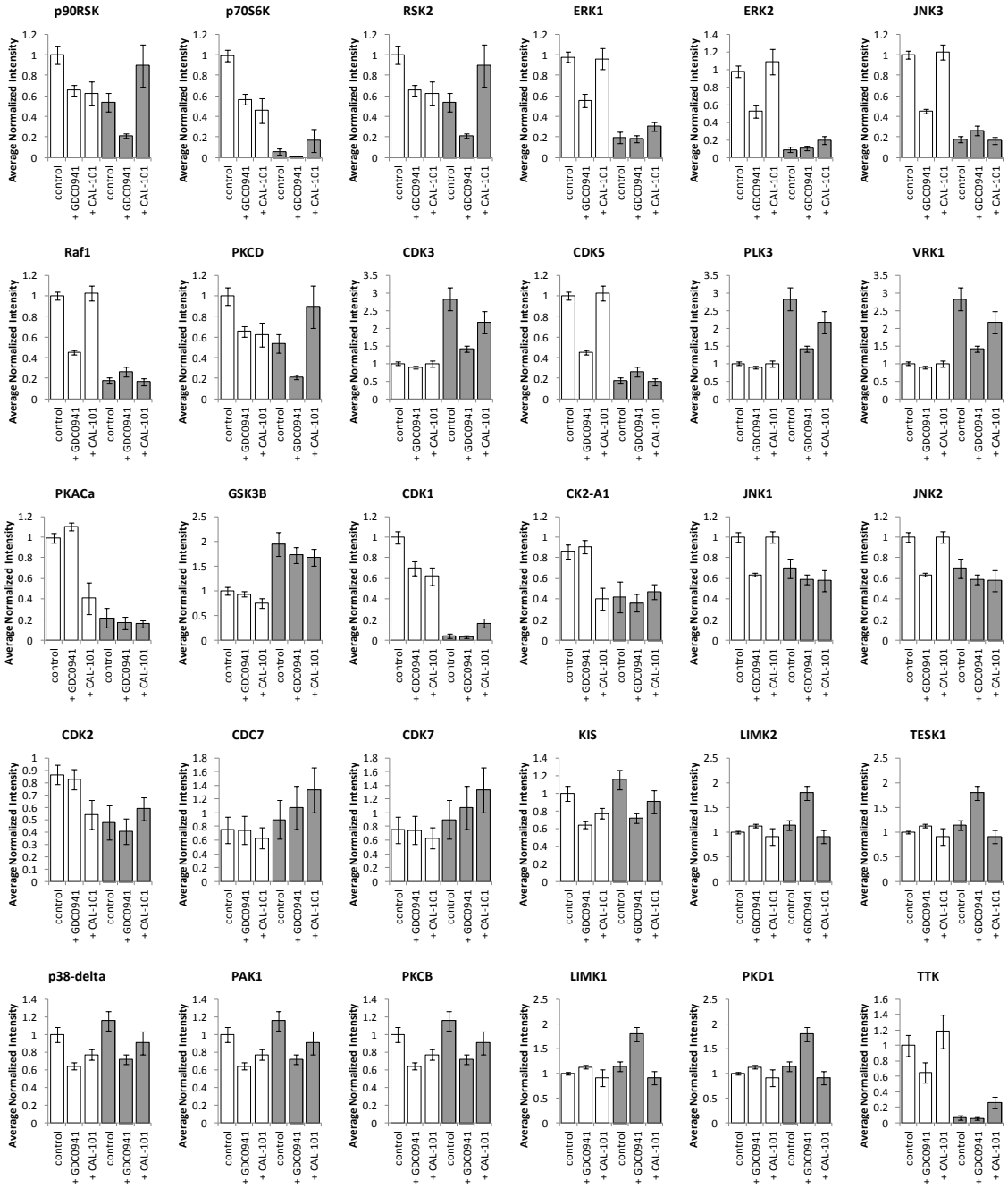


Figure S6. Kinase Substrate Enrichment Analysis of drug treated cells grown in tumors or *in vitro*. The normalized intensities of peptides containing phosphorylation sites linked to the named kinases were combined to give values of substrate group enrichment as described previously (Casado et al 2013). KSEA values reflect kinase activities.

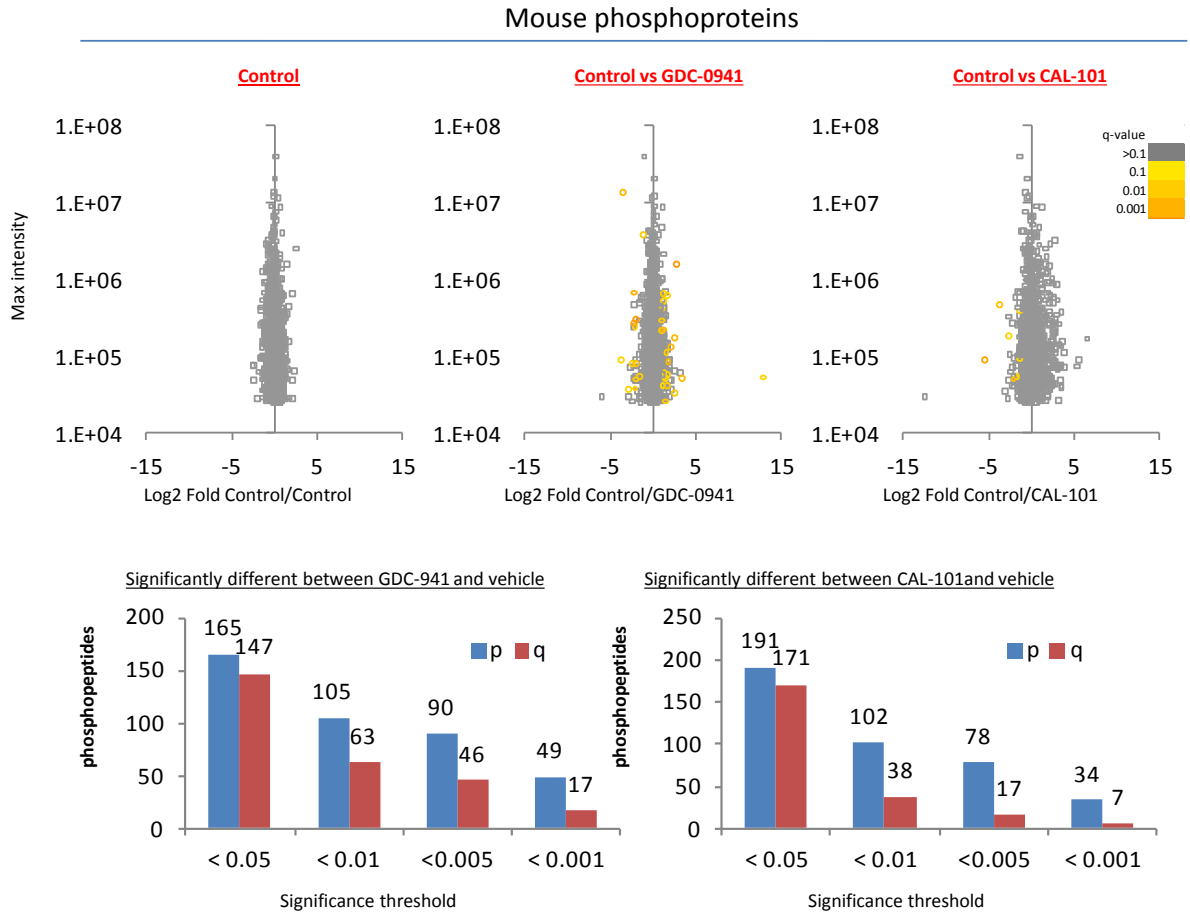


Figure S7. Number of mouse phosphopeptides significantly modulated by PI3K inhibitors *in vivo* at different significance thresholds.