**Regulated Phosphosignaling Associated with Breast Cancer Subtypes and Druggability**

**Supplementary Materials**

**Supplementary Tables**

1. Description of the 77 human breast cancer samples and 24 breast cancer PDXs used in this study, including their PAM50 intrinsic subtypes, ER status, PR status and HER2 status.
2. Count of phosphosites observed in 315 kinases across 9 kinase groups.
3. Known cancer sites from PhosphositePlus observed in breast cancer samples.
4. Significantly associated kinase proteins and *cis*-regulated phosphosites.
5. PDX-validated kinase proteins and *cis*-regulated phosphosites and their regression results in the PDX cohort.
6. Enrichment of kinases with significant *cis* or *trans*-regulated phosphosites in kinase groups.
7. Enrichment of kinases with significant *cis* or *trans*-regulated phosphosites in kinase families.
8. Significantly associated kinase phosphoproteins and *trans*-regulated substrate phosphosites.
9. PDX-validated kinase phosphoproteins and *trans*-regulated phosphosites and their regression results in the PDX cohort.
10. Linear/3D distances and correlations of phosphosite pairs on the same PDB structure.
11. *Cis*-regulated phosphosites forming pairs with PDB phosphosite, active site, or binding sites.
12. *Trans*-regulated phosphosites forming pairs with PDB phosphosite, active site, or binding sites.
13. Counts of outlier *cis* and *trans* kinase-substrate pairs that are potentially druggable across four breast cancer subtypes.
14. Counts of outlier *cis* and *trans* kinase-substrate pairs that are potentially druggable across four breast cancer subtypes in 24 breast cancer PDXs.
15. Counts of druggable events at the mutation, CNV, mRNA, protein and phospho-pair levels.
16. Counts of druggable events at the mutation, CNV, mRNA, protein and phospho-pair levels in 24 breast cancer PDXs.
17. Cascade of druggable kinases and their first and second degree associated substrates.
18. Association of kinase-substrate pairs with pathological stage.
19. Association of kinase-substrate pairs with survival using the Cox model.
20. Association of kinase-substrate pairs with immune score as calculated by the ESTIMATE algorithm.
21. Significantly *cis* and *trans* associations discovered based on phosphosite levels measured through the RPPA data.
22. Frequency of validated cis and trans-regulated phosphosite stratified by *in vitro* or *in vivo* evidence.

**Supplementary Figures**

1. Comparison of standard deviation of known cancer-related phosphosites vs. other phosphosites in human and PDX breast cancer samples.
2. Potentially auto-phosphorylated cis kinase-phosphosite pairs in breast cancer with regression coefficient equal to or greater than 1.
3. Relationship between observed sample size and significance of kinase-substrate coefficient in (A) *cis* and (B) *trans* analysis.
4. Regulation of kinase-substrate pairs identified using various models.  (A) Venn diagrams showing the numbers of cis-regulated phosphosites identified through quantitative association using kinase RNA or protein levels as the independent variable. (B) Comparison of significance level (-log10FDR) of cis-regulated pairs obtained through using the kinase-RNA and kinase-protein models. (C) Venn diagrams showing the numbers of trans-regulated phosphosites identified through quantitative association using kinase RNA, protein or phosphoprotein level as the independent variable. (D) Comparison of significance level (-log10FDR) of trans-regulated pairs obtained through using the kinase-RNA and kinase-Protein models. (E) Comparison of significance level (-log10FDR) of trans-regulated pairs obtained through using the kinase-protein and kinase-phosphoprotein models.
5. Top trans-regulated phosphosites of (A) ATM, (B) ATR, (C) GSK3B, (D) MTOR, and (E) RPS6KB1.
6. Correlation between (A) linear and (B) 3D distances and correlations of phosphosite pairs on the same PDB structure.
7. Landscape of *cis*-regulation of phosphosites in (A) ABL1, (B) PTK2, and (C) RIPK1.
8. Heatmap of regulated kinase-substrate pairs where the kinase or the substrate is a potential druggable target in 24 breast cancer PDX. The sample-pair showing outlier pair event is outlined. Only the top 3 regulated pairs were shown for each kinase when there were more than 3 pairs showing kinase-substrate outliers.
9. Druggability analysis of single and paired events in 24 breast cancer PDX samples. Druggable events identified in the mutation, CNV, RNA, protein and phospho-pair level for breast cancer PDX samples.
10. Druggable events identified in the mutation, CNV, RNA, protein and phospho-pair level showing members of the MAP kinase cascades.
11. Druggable kinase-substrate cascades originating from (A) JAK2 (B) PRKCE and (C) PLK1 in the 77 breast cancer samples. The samples in the heatmap were ordered by the phosphoprotein level of each of the kinase.
12. Landscape of *cis* and *trans*-regulations identified in the breast cancer cohort. For each node of the network diagrams, the color represents the relative level of basal compared to luminal A/B breast cancers, where blue indicates higher level in luminal and red indicates higher level in basal tumors. For the edges, the darkness of the color is scaled by the degree of correlation coefficient and the width is scaled by -log(FDR) of the association.