Supplemental information

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Predicting treatment outcome using kinome activity profiling in HER2+ breast cancer biopsies

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## Supplementary Table 1. Patient information

| Patient | Histology | Tumour grade | ER (%) | PR (%) | HER2 score | Treatment outcome | Tumour (%) |
|---------|-----------|--------------|--------|--------|------------|--------------------|------------|
| 1       | ID        | 2            | 95     | 70     | 3+         | pCR                | 80         |
| 2       | ID        | 2            | 100    | 0      | 3+         | pCR                | 80         |
| 3       | ID        | 3            | 20     | 0      | 3+         | pCR                | 80         |
| 4       | ID        | 2            | 80     | 5      | 3+         | pCR                | 70         |
| 5       | IL        | 3            | 100    | 40     | 3+         | npCR               | 80         |
| 6       | ID        | 2            | 40     | 0      | 3+         | pCR                | 60         |
| 7       | ID        | 2            | 100    | 1      | 2+         | pCR                | 70         |
| 8       | ID        | 3            | 80     | 70     | 3+         | npCR               | 70         |
| 10      | ID        | 2            | 0      | 0      | 3+         | pCR                | 70         |
| 11A*    | ID        | 2            | 0      | 0      | 3+         | pCR                | 60         |
| 11B*    | ID        | 2            | 0      | 0      | 3+         | pCR                | 60         |
| 12      | ID        | 3            | 0      | 0      | 3+         | pCR                | 70         |
| 13      | ID        | 2            | 0      | 0      | 3+         | pCR                | 60         |
| 14      | ID        | 3            | 60     | 5      | 2+         | npCR               | 60         |
| 15      | ID        | 3            | 0      | 0      | 3+         | pCR                | 60         |
| 16      | IL        | 3            | 90     | 0      | 3+         | pCR                | 80         |
| 17      | ID        | 3            | 90     | 100    | 3+         | npCR               | 70         |
| 18      | ID        | 3            | 100    | 20     | 3+         | No pCR             | 80         |
| 20      | ID        | 3            | 90     | 40     | 3+         | pCR                | 60         |
| 21      | ID        | 3            | 99     | 1      | 2+         | No pCR             | 80         |
| 22      | ID        | 3            | 100    | 60     | 2+         | No pCR             | 80         |
| 23      | ID        | 2            | 70     | 70     | 3+         | pCR                | 60         |
| 24      | ID        | 3            | 10     | 0      | 2+         | pCR                | 80         |
| 25      | ID        | 3            | 100    | 100    | 3+         | pCR                | 70         |
| 26      | ID        | 2            | 50     | 100    | 3+         | No pCR             | 70         |
| 27      | ID        | 3            | 100    | 5      | 3+         | No pCR             | 80         |
| 28      | ID        | 3            | 100    | 90     | 3+         | No pCR             | 70         |
| 30      | ID        | 2            | 100    | 40     | 3+         | No pCR             | 60         |
| 32      | ID        | 3            | 100    | 1      | 3+         | npCR               | 60         |
| 33      | ID        | 3            | 100    | 0      | 2+         | pCR                | 80         |
| 34      | ID        | 2            | 100    | 0      | 3+         | npCR               | 70         |
| 36      | ID        | 3            | 100    | 5      | 3+         | No pCR             | 80         |
| 37      | IL        | 2            | 100    | 0      | 2+         | No pCR             | 80         |

ID: invasive ductal  
IL: invasive lobular  
*Workflow replicate
Supplementary Figures

**Supplementary Figure 1.** Histological/H&E image representation of the three treatment outcome groups, related to Figure 1. Representative examples of pre-treatment HER2+ biopsies are shown, with respectively a pCR, npCR and no pCR post treatment. Pathology response measurement was done by a breast pathologist during the microscopic inspection of the surgical resection specimen at routine diagnostics.

**Supplementary Figure 2, related to Figure 1.** Characterisation of quantified T-loops and kinases in this study. A) Total number of quantified kinases, phosphopeptides, phosphosites and T-loops across all biopsies. B) Average kinase T-loop abundance of the endogenous signals, coloured by kinase family. Amongst the most highly abundant kinases are family members of the CMGC-family (GSK3A and CDKs). Kinase class abbreviations: CAMK, Ca^{2+}/calmodulin-dependent kinase; STE, serine/threonine protein kinases; CMGC, CDK, MAPK, GSK and CDK-like protein kinases; AGC, protein kinase A, G, and C families; TKL, tyrosine kinase-like kinases. C) Boxplot of T-loop standard abundance per individual runs. D) Correlation plot of all quantified kinase T-loops in this study. Kinase names are coloured according to the kinase family. Pearson correlation was used. Hierarchical clustering method was Ward’s. E) Kinase mutation frequency in breast cancer, reported by TCGA (The Cancer Genome Atlas). Many detected kinases show a very high mutation frequency in breast cancer, comparable to other well-known breast cancer associated genes such as BRCA and ESR1. F) Number of references in PhosphoSitePlus for each endogenously detected T-loop phosphosite. Although many (but not all) sites have been identified often in high throughput studies (HTS), the biological relevance has been mostly understudied, as evidenced by the low number of low throughphut studies (LTS).

**Supplementary Figure 3.** Boxplots of kinase T-loops, related to Figure 3. A) Boxplots of differentially regulated kinase T-loops. * p-value < 0.05; ** p-value < 0.001. B) Boxplots of kinase T-loops of p38A that were not significantly changing between treatment outcome groups.

**Supplementary Figure 4.** Detected oxidised and non-oxidised kinase T-loops, related to Figure 3. A) Correlation plots, comparing the abundance of the oxidised and non-oxidised T-loop versions. Good linear correlations show the rate of oxidation is similar between samples. Moreover, oxidised versions are >10-fold lower in abundance. B) The number of quantified T loops is lower for the oxidised T loop phosphopeptides.
Supplementary Figure 1.
Supplementary Figure 3.
Supplementary Figure 4.

A

CAMKID [T180]

\[ R^2 = 0.8750 \]
\[ Y = 1.134X + 0.3689 \]

p38A [T180/Y182]

\[ R^2 = 0.7151 \]
\[ Y = 0.7464X + 3.132 \]

PAK4 [S474]

\[ R^2 = 0.9379 \]
\[ Y = 0.9956X + 2.006 \]

RSK1 [T573]

\[ R^2 = 0.8937 \]
\[ Y = 1.150X + 0.2168 \]

B

Coomassie Blue- stained proteins

| Protein    | Oxidized | Doubly phosphorylated |
|------------|----------|-----------------------|
| CAMKID [T180] | 30       | 40                    |
| p38A [T180/Y182] | 20       | 30                    |
| PAK4 [S474] | 10       | 10                    |
| RSK1 [T573]  | 40       | 50                    |

T-loop detection frequency