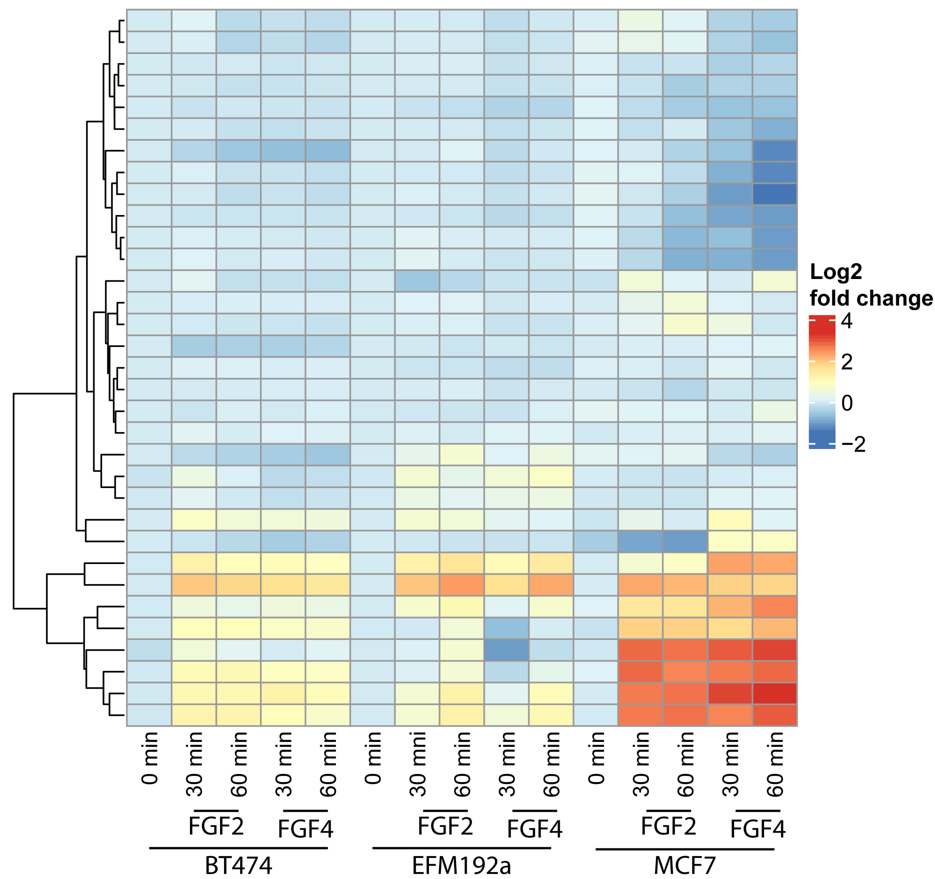
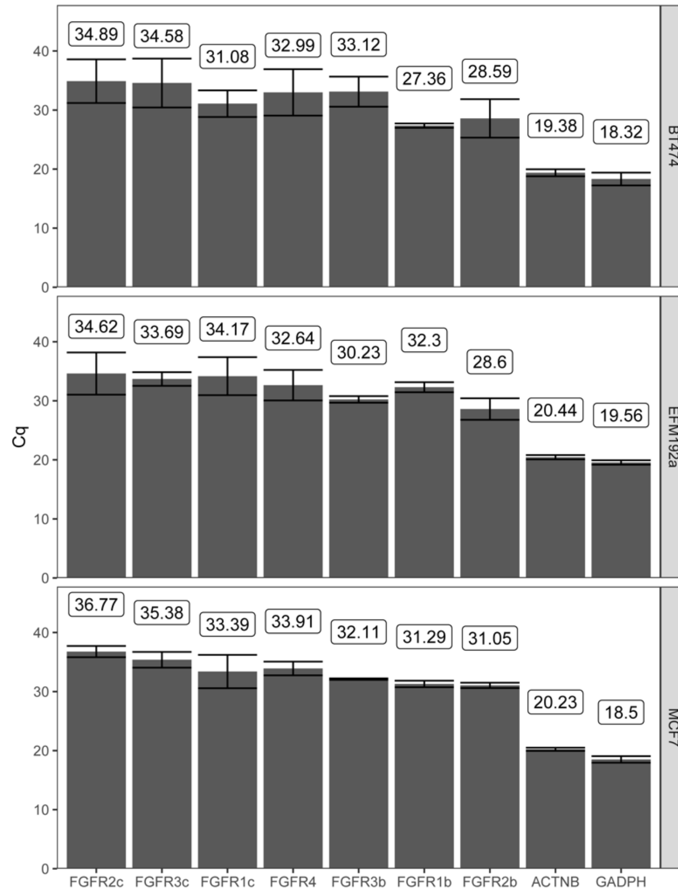
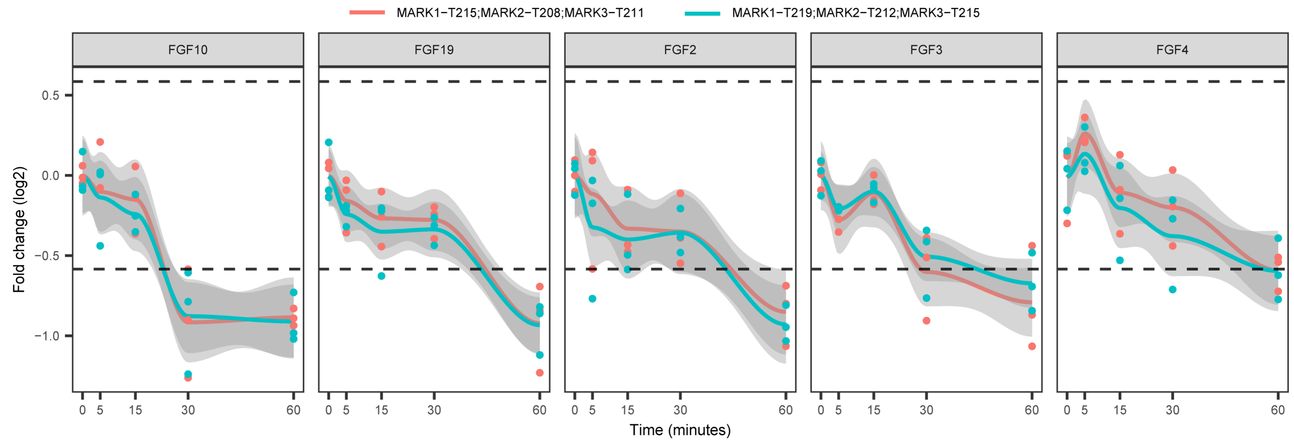
Supplemental figures



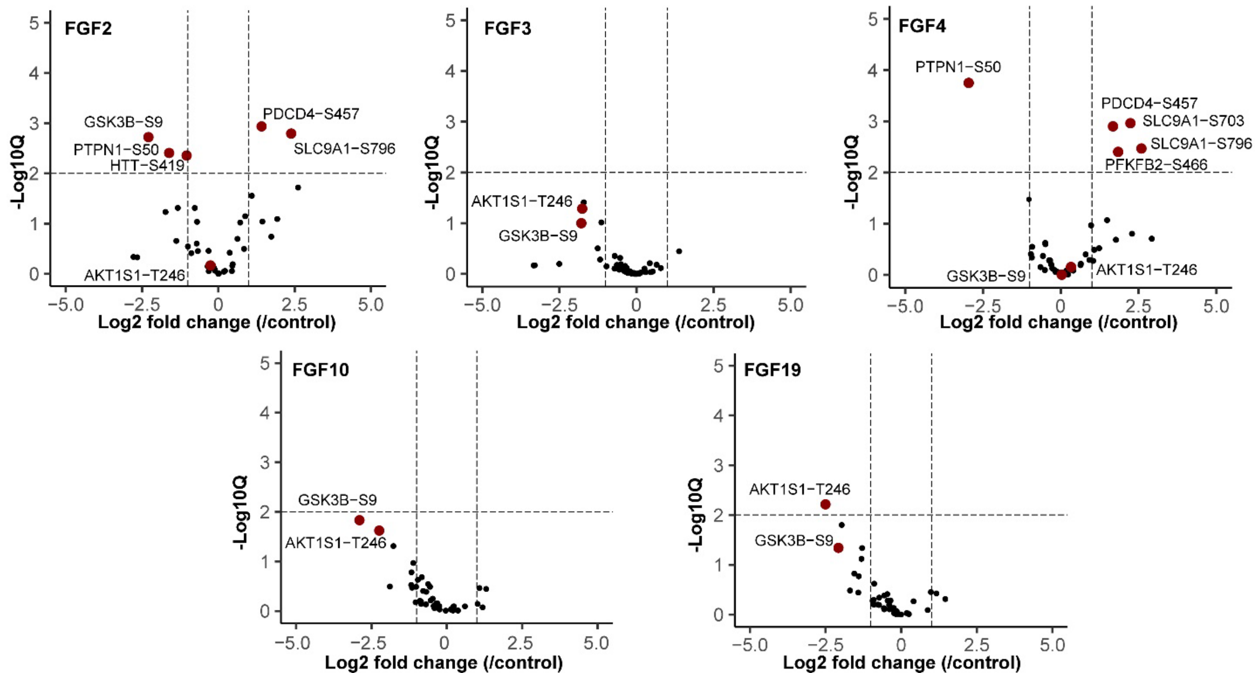
Supplementary Figure 1: Kinome activity after FGF stimulation in breast cancer cells. MCF-7, BT-474, and EFM-192a cells were stimulated with 50ng/mL of either FGF2 or FGF4 supplemented with 5µg/mL of heparin. After 0, 30, and 60 minutes, the cells were harvested and subjected to measurement using the targeted kinome assay. The heatmap shows the quantified activation-determining phosphorylated sites on the kinases (biological triplicates). Only significantly changing values are shown (ANOVA $p < 0.05$).



Supplementary Figure 2: qPCR quantification of FGFR abundance. FGFR expression levels were quantified using qPCR in MCF-7, BT-474, and EFM-192a cells using FGFR subtype-specific primers (triplicate measurements) (**Supplementary table 5**). Beta-actin and Glyceraldehyde-3-phosphate dehydrogenase was quantified to enable normalization across cell types. Reported values are the quantitation cycles (Cq) that negatively correlates with the RNA expression levels.

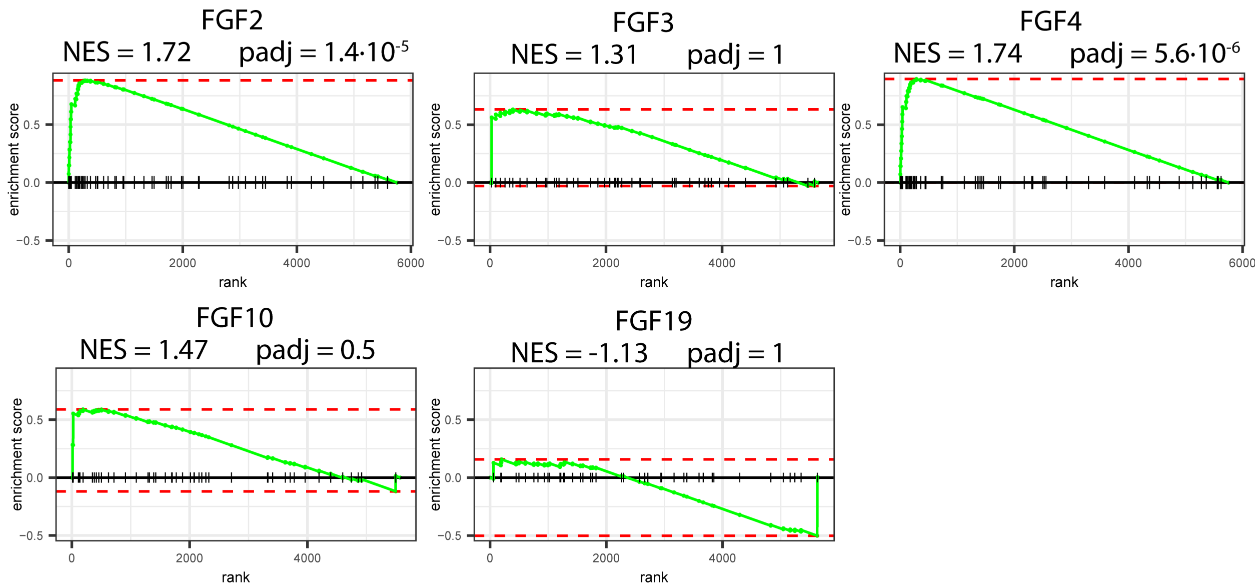


Supplementary Figure 3: FGF-induced MARK kinase regulation. Activity-determining phosphorylation in the activation loop of MARK1, MARK2, and MARK3 was quantified in MCF-7 treated with different FGF ligands. Line plots show these quantified phosphorylated sites (biological triplicates). Values are represented in log2 and the 1.5 fold-change is represented using the dashed line.

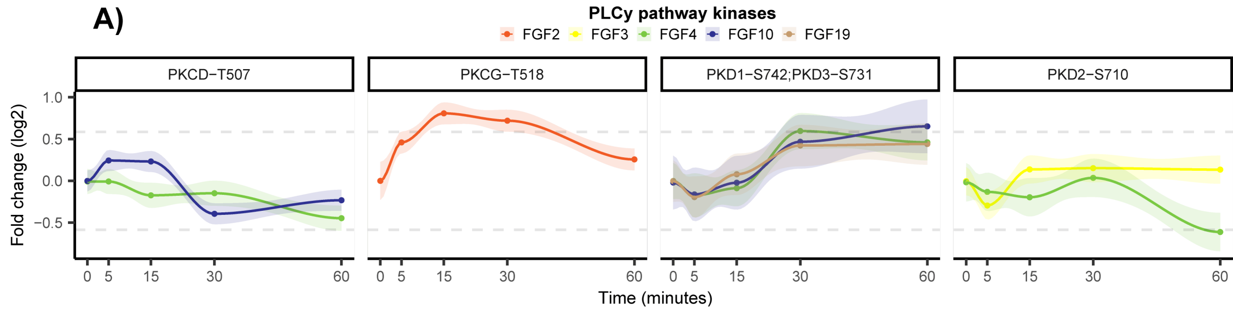


Supplementary Figure 4: quantification of AKT1/2 activity. Known substrate phosphorylation sites from AKT1/2, as identified by PhosphositePlus, were extracted from

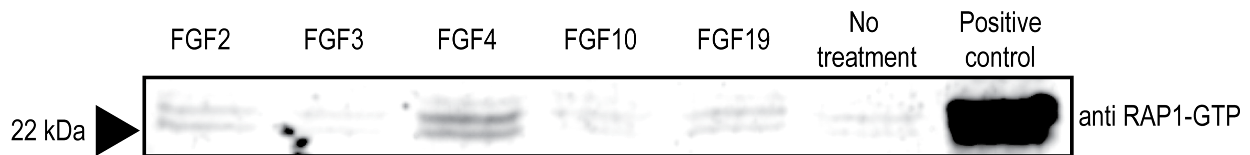
the phosphoproteomics dataset. The cutoffs were set to a Q-value < 0.01 (two-sided t-test, FDR), and a fold change of < -2 or >2. The resulting phosphorylated sites and commonly used phosphorylated sites for AKT1/2 activity inferences are represented in red.



Supplementary Figure 5: proteome derived EMT signature. MCF-7 cells treated were treated for 24 hours with the FGF ligands and their proteomes quantified. Subsequently, GSEA analysis on these proteomes (biological triplicates) was performed using the MsigDB signature “HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION”. The normalized enrichment score (NES) along with the adjusted p-value is reported.



Supplementary Figure 6: Regulation of kinases implicated in PLCγ signaling A) Kinome activity was quantified of MCF-7 cells treated with 50ng/mL of either FGF2, FGF3, FGF4, FGF10, or FGF19, together with 5µg/mL of heparin. Only the activity dynamics of significantly regulated kinases (biological triplicates, ANOVA $p < 0.05$) from the PLCγ pathway are plotted. Grey lines represent a 1.5 fold-change, and 90% confidence intervals are presented per quantified phosphorylated peptide.



Supplementary Figure 7: RAP1 pulldown on FGF-stimulated cells. MCF-7 cells were stimulated with either FGF2, FGF3, FGF4, FGF10, and FGF19. Also, a no-stimulation control and a positive control were included. The negative control constituted unstimulated MCF-7 cells. The positive control constituted MCF-7 cell lysate with activated RAP1 by incubating the lysate with GTPγS, which activates all RAP1 in the lysate. The assay consisted of a pulldown of GTP-bound (active) Rap1 of equal amounts of proteins, followed by a western blot using an α–RAP1-GTP antibody.