**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure 1: Effects of EGFR antagonists, FAK or PYK2/FAK inhibition/depletion on basal-like TNBC growth either alone or in combination. (A)** Effect of Gefitinib and Erlotinib on EGFR phosphorylation. MDA-MB-468 and BT-20 cells were incubated with the indicated concentrations of Gefitinib and Erlotinib for 72 h, and their effect on EGFR phosphorylation was assessed by Western blotting (WB) using the indicated antibodies **(B)** Effects of FAK inhibitor on survival of basal-like TNBC cell lines in the absence or presence of Gefitinib. The indicated basal-like TNBC cell lines were treated with increasing doses of PF228, Gefitinib or combinations of both for 72h and cell viability was assessed by MTT assay. IC50 values and synergism (CI<1) are shown in the table. Representative matrices of combined drug treatment of MDA-MB-468 and BT-20 cells are shown in the middle panels and numbers indicate percentage of viable cells as compared to non-treated control cells. Color gradient displays fraction of surviving cells (dark blue; high, dark red; low). Right panels show representative pictures of crystal violet staining of MDA-MB-468 and BT-20 cells, 72 h following treatment with the indicated concentrations of Gefitinib and/or PF228. **(C)** Effect of Erlotinib and/or PYK2/FAK dual inhibitor on basal-like TNBC survival. The indicated basal-like TNBC cell lines were treated with increasing doses of PF396, Erlotinib or combinations of both for 72 h and cell viability was assessed by MTT assay. IC50 values and synergism (CI<1) are shown in the table. **(D)** Effect ofdouble knockdown of both PYK2 and FAK in HCC1937, HCC1143 and HCC38 on the IC50 values of Gefitinib (upper panel) as well as on cell growth (lower panel). PYK2/FAK-depleted cells were treated with Gefitinib for 72 h and IC50 values were determined by MTT assay. Mean values ± s.d. from three independent experiments are shown. Knockdown efficiency was assessed by WB using anti-PYK2 and anti-FAK antibodies. The lower panel shows the effects of PYK2 or FAK knockdown as well as the double PYK2 and FAK knockdown on cell growth compared to control cells as determined 48 h after seeding by MTT assay. Mean values ± s.d. from three independent experiments are presented. **(E)** Effect of Gefitinib either with cMet inhibitor (EMD12140; left table) or MEK inhibitor (GSK112021; right table) on the survival of MDA-MB-468 and BT-20 cells. Survival was assessed by MTT assay, as described above. IC50 values and synergism (CI<1) are shown in the tables. **(F)** Influence on anchorage-independent growth of MDA-MB-468 and BT-20 cells treated with a combination of Gefitinib and PF396. Representative images of cell colonies grown in soft agar for two weeks followed by a three weeks treatment with Gefitinib, PF396 or their combination (MDA-468: 1 M each, BT-20: 5 M and 1 M, respectively). Scale bar, 100 m. Quantitative evaluation shows number of colonies, colony size and area as percentage of non-treated control.

**Supplementary Figure 2: Analysis of PYK2 and EGFR gene expression in human breast cancer samples.** **(A, B)** Cutoff selection for EGFR mRNA expression in 1096 TNBC samples. **(A)** Two probesets for EGFR (211607\_x\_at and 210984\_x\_at) displayed a strong correlation in 1096 TNBC samples. Samples with expression values below -0.005 for both probesets were defined as “EGFR low” (blue dots). **(B)** The distribution of the mean values of both probesets is displayed by green and blue colors for high and low EGFR, respectively. **(C)** Cutoff selection for PYK2 mRNA expression based on the distribution in 1096 TNBC samples.We used Cutoff Finder (1) to fit a mixture model of two Gaussian distributions (red lines) to the mRNA expression data of PYK2 (Affymetrix probeset 203111\_s\_at). The optimal cutoff from the mixture model (-0.007 in the graph) was selected to stratify samples into high and low PYK2 expression. **(D)** Kaplan-Meier analysis of event free survival of TNBC patients according to EGFR expression.Survival analysis was performed for 471 patients with follow-up information from the cohort of 1096 TNBC. Patients were stratified into groups with ‘high’ or ‘low’ EGFR expression according to the combined cutoff from Affymetrix probesets (211607\_x\_at and 210984\_x\_at) for EGFR. **(E)** Quantitative analysis of 77 TNBC tissue samples for PYK2 and EGFR expression was carried out applying H-score as described in the Methods section, and accordingly, samples were grouped into low (0-2.5) and high (2.5 (+)-4(+)) staining intensity.

**Supplementary Figure 3:** **Combined inhibition of PYK2/FAK and EGFR enhances pro-apoptotic signals and reduces pro-survival signals. (A)** Effect of PYK2/FAK inhibitors in the five basal-like TNBC cell lines on the protein levels and activation states of the indicated signaling proteins. Depicted are representative Western blotting (WB) results of reproducible experiments using antibodies against the specified proteins and their phosphorylated form. **(B)** Effects of PYK2/FAK and EGFR inhibition on survival/proliferative pathways in BT-20 cells treated with Gefitinib (IC25), PF431396 (0.5 M) or combination of both for 24 h. The influence of these treatments on the indicated signaling pathways was assessed by WB. The effects were quantitated based on protein bands intensities using ImageJ software and are presented in the graph as fold changes of non-treated control. **(C)** Effects of PYK2 or FAK knockdown as well as PYK2/FAK dual inhibitor PF396, with or without Gefitinib treatment on the activation of major pro-apoptotic proteins as determined by PARP and Caspase 9 cleavage. For MDA-MB-468 and BT-20 knockdown cells, we used 0.5 M and 10 M Gefitinib, respectively. For drug combinations, we used 1 M and 5 M Gefitinib for MDA-MB-468 and BT-20, respectively, whereas 1µM PF396 was used for both the cell lines. Cells were treated with drugs for 24 h following WB analysis.

**Supplementary Figure 4:** **PYK2 interacts with HER3 in a kinase dependent manner and suppresses NDRG1-induced HER3 proteasomal degradation.** **(A)** PYK2 and FAK suppress NDRG1-induced proteasomal degradation of HER3 in a kinase dependent manner. HEK293 cells expressing HER3, Myc-NDRG1, HA-tagged PYK2, FAK or their kinase deficient mutants PKM and FKM were treated with either MG132 (3 M) for 24h or with PF396 (3 M) or PF228 (2.5 M) for 12h before harvesting as indicated in the panels. Cells were lysed and the levels of the indicated total and phospho-proteins were determined by WB using the specified antibodies. **(B)** PYK2 interacts with HER3 in a kinase activity dependent manner. HEK293 cells were transfected with DNA constructs encoding V5-HER3, HA-tagged wild-type PYK2 or FAK and their corresponding kinase deficient mutants PKM and FKM as indicated. The cells were treated with the PYK2/FAK dual inhibitor PF396 (3 M) or with the FAK specific PF228 (2.5 M) inhibitor for 12 h, as indicated. HER3 was immunoprecipitated (IP) by anti-V5 antibody and its interaction with PYK2, PKM, FAK or FKM was determined by immunoblotting (IB) with anti-HA antibody.