**SUPPLEMENTARY EXTENDED MATERIALS AND METHODS**

**Antibodies, Reagents and Chemicals**

The following antibodies were purchased from Santa Cruz Biotechnology(Santa Cruz, CA): rabbit polyclonal antibodies against pPYK2 (Y402, sc-101790, 1:1,000 WB, 1:250 IF), STAT3 (C-20, 1:1,000 WB), FAK (sc-558, 1:1,000 WB), ERK1/2 (sc-93, 1:1,000 WB), pERK1/2 (pT202/ pY204.22A, sc-136521, 1:500 WB), AKT (sc-8312, 1:1,000 WB), HER3 (sc-285, 1:500 WB, 1:100 IF), pHER3 (Y1328, sc-135654, 1:500 WB), NEDD4-1 (sc-25508, 1:1,000 WB, 1:100 IF), cMet (sc-10, 1:1000 WB), caspase 9 (sc-8355, 1:1,000 WB) and PCNA (sc-7907, 1:100 IHC), as well as mouse monoclonal antibodies against HER3 (sc-415, 1:100 IF), monoclonal NDRG1 (sc-100786, 1:75 IF), anti-hemagglutinin (HA, sc-57592, 1:500 WB) and anti-myc (sc-40, 1:500 WB). Protein A/G PLUS-Agarose beads (sc-2003) were also purchased from Santa Cruz Biotechnology (Santa Cruz, CA).  Rabbit polyclonal antibody against PYK2 (P3902, 1:50) used in IHC, mouse monoclonal antibodies against α-Tubulin (T6074, 1:10,000 WB) and pPYK2 (1:500 WB, 1:300 IF) were purchased from Sigma-Aldrich Israel (Rehovot Israel). Antibodies against pAKT (T308, #2965 and S473, #9271, 1:1,000 WB), pSTAT3 (Y705, #9145, 1:1,000 WB), pc-Met (Y1234/5, #3077, 1:1,000 WB), pFAK (Y397, #8556, 1:1,000 WB), pEGFR (Y1068, #2234, 1:1,000 WB), pNDRG1 (T346, #5482, 1:1,000 WB, 1:250 IF), cleaved PARP (D214, #9541, 1:1,000 WB) and cleaved caspase-3 (Asp175, #9661, 1:100 IHC) were purchased from Cell Signaling Technologies (Danvers, MA, USA). Rabbit polyclonal antibodies NEDD4-2 (ab46521, 1:1,000 WB, 1:500 IF) and EEA1 (ab2900, 1:1,000 IF) were purchased from Abcam (Cambridge, MA, USA). Monoclonal antibodies against EGFR (clone 111.6, 1:5,000 WB, 1:1,000 IF, 1:50 IHC) and against V5-tag (Hybridoma, 1:100 WB) were kindly provided by Prof. Y. Yarden and Prof. Elior Peles, respectively, Weizmann Institute of Science, Rehovot, Israel. Anti-NDRG1 (rabbit polyclonal) was a generous gift from Prof. T. Commes, University of Montpellier. Polyclonal anti-PYK2 (1:500 WB) antibody was prepared as described previously (2). Furthermore, monoclonal antibodies EEA1 (610457, 1:300 IF) and RAB11 (610657, 1:100 IF) were obtained from BD Transduction Laboratories (San Jose, CA, USA), LAMP-1 (1D3B, 1:50 IF) from DSHB (University of Iowa) and anti-ubiquitin (MAB1510, 1:100 IF) from Chemicon (Merck Millipore, USA). Rabbit polyclonal anti-Rab11 (1:300 IF) was kindly provided by Prof. Benjamin Aroeti, Hebrew University, Israel. Anti-Alexa 488 donkey anti-mouse and anti-rabbit IgGs were purchased from Invitrogen (Carlsbad, CA). Cyanine Cy3-conjugated goat anti-rabbit and goat anti-mouse IgGs were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Hoechst 33342, PF431396 (PZ0185), Chloroquine (C6628) and Dp44MT (SML0186) were obtained from Sigma-Aldrich Israel (Rehovot Israel). PF573228 (324878) and MG132 (474790) were purchased from Calbiochem (Merck Millipore, USA) and Gefitinib (G-4408) and Erlotinib (10483-250) from LC Laboratories (Woburn, MA, USA) and Cayman Chemical Company (Ann Arbor, MI USA) respectively. EMD1214063 (A3388) and GSK 1120212B (1187431-43-1) were purchased from Apexbio Technology, Houston and Pekag Chemicals International Corp., China respectively.

**Cell Culture**

MDA-MB-468, HCC-1937, HCC-1143, HCC-38, MDA-MB-231, BT-549 and Hs578T cells were grown in RPMI (Gibco BRL; Grand Island, NY, US). BT549 medium was supplemented with Insulin (0.023 IU ml-1) and Hs578T medium with 2 mM L-glutamine. BT-20 cells were grown in Eagle’s Minimum Essential Medium (MEM-Eagle’s) supplemented with 1 mM sodium pyruvate and 2 mM L-glutamine. HEK293 cells were grown in DMEM (Gibco BRL; Grand Island, NY, US). SUM159PT cells were cultured in DMEM/F12 Ham’s Mixture (1:1) (Gibco BRL; Grand Island, NY, US) medium supplemented with 5% FCS, Insulin (0.144 IU ml-1) and hydrocortisone (5 µg ml-1). All media preparations were supplemented with 10% fetal bovine serum (Gibco BRL, Grand Island, NY, US), and a penicillin–streptomycin mixture (100 U ml−1; 0.1 mg ml−1; Beit Haemek, IL) unless specified.

**Establishment of Gefitinib and Erlotinib resistant cell lines**

Gefitinib and Erlotinib resistant cells were established as described elsewhere (3). In brief, cells were exposed to increasing concentrations of Gefitinib or Erlotinib starting with IC10-25. Medium containing drug was changed every three days with an increase of 10 to 25% in concentration. Cells were designated as drug resistant once they grew exponentially in the presence of high concentrations of the drugs. The Gefitinib and Erlotinib resistant MDA-MB-468 were established within 1.5 months and were continuously grown in the presence of 2 or 4 µM of Gefitinib and 4.5 µM of Erlotinib.

**DNA constructs lentivirus production and infection**

The pLX302-HER3 over-expression lentiviral construct was kindly provided by Moshe Elkabetz (Ben-Gurion University of the Negev, Be'er Sheva, IL). To knockdown NDRG1 expression two previously described shRNA sequences were used; 1-GCACATTGTGAATGACATGAA, 2- GCACATTGTGAATGACATGAA (4). The NDRG1 shRNAs were cloned into the pLKO.1-puro lentiviral vector. Lentivirus production and infection were performed as described previously (5)**.** HA-tagged wild-type PYK2 and kinase dead PYK2 mutant (PKM) were subcloned into the pRK5 vector where as wild type FAK-HA was subcloned in pcDNA3 vector. A pcDNA3 vector containing HA-tagged-kinase dead mutant of FAK (FKM) was a kind gift form Prof. Benjamin Geiger (Weizmann Institute of Science, Rehovot). NDRG1-myc was established by subcloning the NDRG1 cDNA from the pCMV-NDRG1-DsRed vector (kindly provided by Sushant Kachhap, Johns Hopkins University School of Medicine, US) into the pcDNA3.1-myc-his vector. pcDNA3-V5-hNEDD4-WT and pcDNA3-V5-hNEDD4L-WT were kindly provided by Daniela Rotin (University of Toronto, CAN). pcDNA3-HER3-HA was generously provided by Mara Steinkamp (University of New Mexico, US).

**Cell viability and Proliferation**

For cell viability assays, cells were plated at 7,000 cells per well were plated in 96-well plates in triplicates. Cell viability was assessed 72 h later by: (a) MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) colorimetric assay. The cell were incubated with medium containing MTT solution (0.5 mg/ml; Sigma) for 2 h at 37°C. Cell were lysed with 100 μl lysis buffer (0.4% NP-40 in 0.04 mol/l HCl-isopropanol), and absorbance was measured at 570 nm with a 680 nm reference wavelength using ELISA microplate reader (Corning, NY, US). Cell viability is depicted as percentage of control. Data is represented as the mean values of three independent biological replicates. (b) Crystal Violet staining. The cells were washed with PBS, and 50µl of 0.2% crystal violet in 4% formalin was added to each well for 10 min. The cells were then washed with distilled water, dried, and plates were scanned using HP Scanjet G4010. Pictures are representative of three independent experiments.

**Soft agar colony formation assay**

Cells were suspended in complete medium containing 0.3% agar and seeded in 24-well plates pre-coated with 0.5% agar (5,000 cells/well). The cells were grown for up to 5 weeks. When indicated, drugs were added two weeks post seeding and replaced every 3-4 days. Photographs were taken by Nikon Eclipse TS100 microscope. Images are representative of two biological replicates. Quantitation was performed by ImageJ software (NIH, USA).

**Breast Cancer Mouse Xenografts**

All procedures for mouse xenograft experiments were carried out in accordance with the Guidelines for the Care and Use of Research Animals at the Weizmann Institute. A suspension of 3 x 106 luciferase-expressing MDA-MB-468 breast cancer cells infected with shRNA-Control or shRNA-PYK2 lentiviruses in PBS was implanted into the fourth inguinal mammary gland of female Nu/Nu mice of matching age, 4- to 6-week-old (N=48). Six weeks later, mice were randomly divided into two equally sized groups before oral administration of either vehicle or Gefitinib. Gefitinib was dissolved in 0.5% Methylcellulose/0.2% Tween-80, and administered by oral gavage for 39 days. Tumor size was monitored weekly; the gross tumor dimensions were measured by caliper and bioluminescence images were acquired by the IVIS instrument with the Living Image 3.0 software (Xenogen Caliper Life Sciences) once weekly. Tumor volumes were calculated (width2 × length/2), and 11.5 weeks post implantation, mice were sacrificed. Tumors were excised and processed for immunohistochemistry and protein extraction. Student’s *t*-test was applied for statistical analysis.

**Western Blotting**

Performed as described previously (5,6). Briefly, cells were lysed in cold lysis buffer (0.1% Triton-X-100, 50 mM Hepes pH 7.5, 100 mM NaCl, 1 mM MgCl2, 50 mM NaF, 0.5 mM NaVO3, 20 mM β-glycerophosphate, 1 mM phenylmethylsulphonyl fluoride, 10 μg ml−1 leupeptin, 10 μg ml−1 aprotinin), vortexed for 30 seconds and incubated on ice for 15 min. Cleared cell extracts were obtained by centrifuging at 14,000 rpm for 20 min at 4 °C. Protein concentration in each sample was estimated by Bradford assay (Bio-Rad, Hercules, CA) and equal protein amounts (40–60 μg) were analyzed by SDS–polyacrylamide gel electrophoresis and WB using standard procedures. Blocking buffer containing 5% nonfat dry milk in TBS-Tween (0.05%) was used. For densitometric analysis, the intensity of protein bands was measured using the Image J software (NIH, USA).

**Immunofluorescence staining**

Immunofluorescence (IF) staining was performed as described previously (7). Briefly, cells were grown on coverslips in 24-well plates and cultured for 48 h. After washing with PBS, cells were fixed with 4% paraformaldehyde (PFA) in PBS for 20 min at room temperature. The cells were then incubated for 15 min in 0.1 M glycine in PBS, followed by 30 min incubation in blocking buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 10% goat serum, 2% bovine serum albumin in Tris-buffered saline and 0.1% Triton-X-100). Primary antibody (dilution prepared in blocking buffer) was applied for 1 h at room temperature and cells were washed 3 times with PBS and subsequently incubated with fluorescence-labelled secondary antibodies for 1 h. Cells were washed with PBS and incubated for 5 min with 2 ng μl-1 Hoechst 33342, washed again and then mounted on microscopic slides using mounting media (10 mM phosphate buffer, pH 8.0, 16.6% w/v Mowiol 4–88 and 33 % glycerol). A confocal laser-scanning microscope (LSM 510; Carl Zeiss) equipped with a 63 Å/1.4 oil differential interference contrast M27 objective lens (Plan Apochromat; Carl Zeiss) was used to analyze the IF staining using the 488-, 543- and either 405- or 633-nm excitation for fluorescein, Cy3 epifluorescence and either 4,6-diamidino-2-phenylindole (Hoechst) or Cy5, respectively. Images were acquired using the LSM 510 software.

**Immunoprecipitation**

Immunoprecipitation studies were performed as described previously (5). Briefly, cells were washed with cold PBS and lysed using cold lysis buffer described above, centrifuged at 15,000 g for 20 minutes to obtain cleared lysates. Protein concentration was estimated for each sample using Bradford reagent and subsequently, 90% of the supernatants were incubated for 3 h at 4°C with the indicated primary antibody bound to protein A/G Sepharose beads and the remaining lysates were used as inputs. The beads were then washed three times with cold lysis buffer. Pulled down proteins were released by adding 15 µl 3 x SDS sample buffer and boiled for 5 min. The resulting samples (excluding beads) were loaded directly into protein SDS-PAGE gels and subject to Western blot as described above.

**Immunohistochemical staining and analysis**

Tissue samples of invasive breast cancer cases were obtained with institutional review board approval (Ethik-Kommission Fachbereich Medizin der Goethe-Universität Frankfurt, DE) and written informed consent from patients undergoing surgical resection at the Department of Gynecology and Obstetrics at the Goethe-University in Frankfurt am Main (DE). TNBC samples were identified according standard pathological criteria, including estrogen receptor, progesterone receptor and HER2 status. Formalin-fixed paraffin-embedded sections were mounted on Superfrost Plus slides. The TNBC as well as the mouse xenograft tumor tissue sections were processed for immunohistochemistry as previously described (8). The intensity of the immunohistochemistry staining of the TNBC sections was evaluated semiquantitatively and classified as low (0 – 2.5) and high (2.5+ - 4+) intensity. H-Score was calculated for each sample essentially as we described previously (7,8). Tumor grade was evaluated according to clinical and pathological data. Statistics were performed using *χ2*-analysis.

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