Supplementary Figures

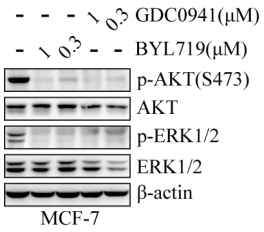

Figure S1: PI3K inhibitors down-regulate phosphorylated both AKT and ERK. MCF-7 cells were treated with BYL-719 or GDC0941 for 1 h followed by immunoblotting for indicated proteins. Data shown are representative from three independent experiments.

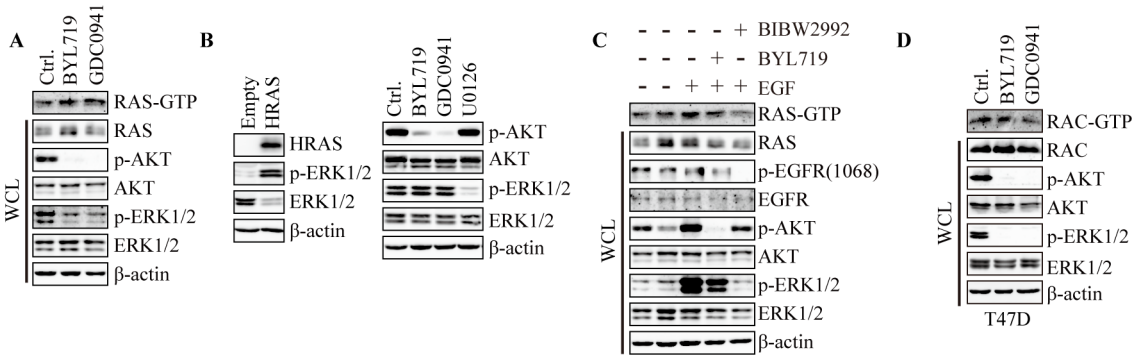

Figure S2: Hyper-activation of RAS abrogates PI3K-regulated ERK phosphorylation. (A) MCF-7 cells were treated with BYL-719 (1 μM) or GDC0941 (1 μM) for 1 h. RAS-GTP was pulled down using GST-RAF1-RBD agarose. (B) MCF-7 cells stably expressing HRAS(G12V) were treated with BYL-719 (1 μM) or GDC0941 (1 μM) for 1 h. (C) FBS-starved MCF-7 cells were treated with BYL-719 (1 μM) or BIBW2992 (1 μM) for 1 h and then stimulated with EGF (50 ng/ml) for 15 min. (D) T47D cells were treated with BYL-719 (1 μM) or GDC0941 (1 μM) for 1 h. Representative immunoblots of indicated proteins from two or three independent experiments are presented.

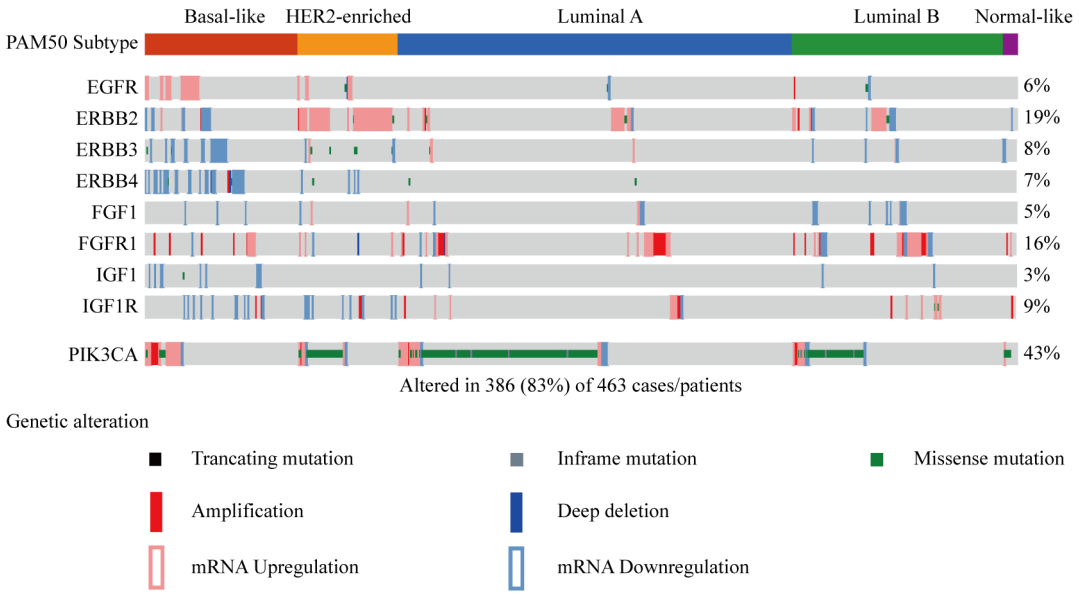

Figure S3: Alterations of receptor tyrosine kinases and *PIK3CA* in breast cancer. Data were adapted from previous study (1).

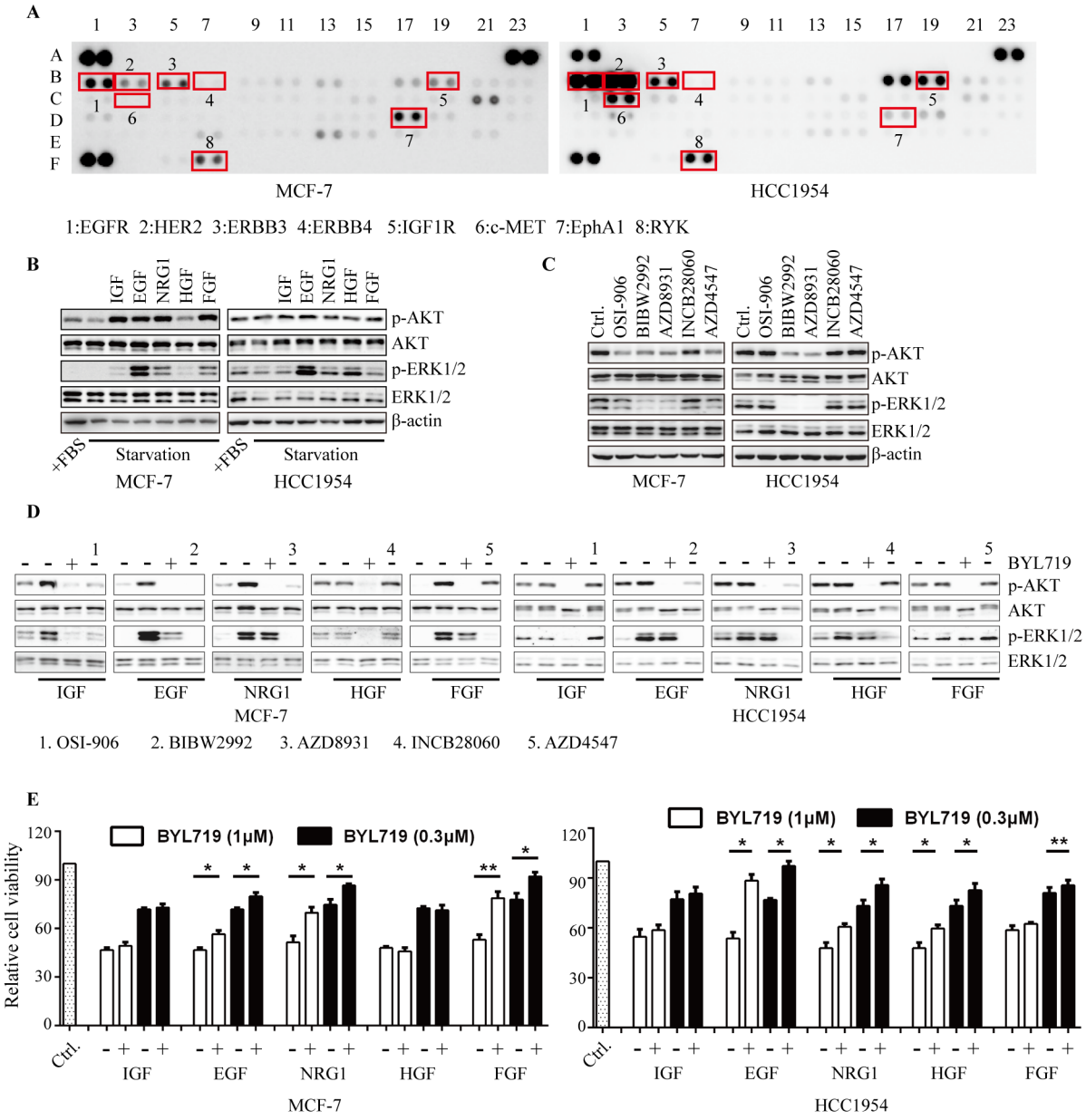

Figure S4: The growth factors distinctly determine PI3K-regulated ERK phosphorylation in breast cancer cells. (A) Cell lysate of MCF-7 or HCC1954 cells was applied to phospho-RTK array. MCF-7 or HCC1954 cells were stimulated with indicated growth factors (50 ng/mL) for 15 min (B) or treated with indicated inhibitors of receptor tyrosine kinases (1 μM) for 1 h (C). (D) Starved MCF-7 or T47D cells were treated with BYL-719 (1 μM) or respective inhibitor of RTKs (1 μM) for 1 h and then stimulated with indicated growth factors (50 ng/mL) for 15 min. Representative immunoblots of indicated proteins from two or three independent experiments are presented (A-D). (E) Cell viability of MCF-7 or HCC1954 cells treated with BYL-719 alone or concurrently with indicated growth factors (50 ng/mL) for 72 h was measured via SRB assay. Bars, mean ± SEM. * $P < 0.05$; ** $P < 0.01$, compared with respective group in the absence of growth factors using paired *t* test.

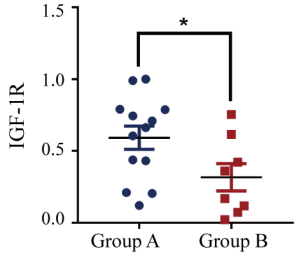

Figure S5: The difference of IGF1R expression between Group A and Group B cells. Bars, mean ± SEM. Unpaired *t* test was performed.

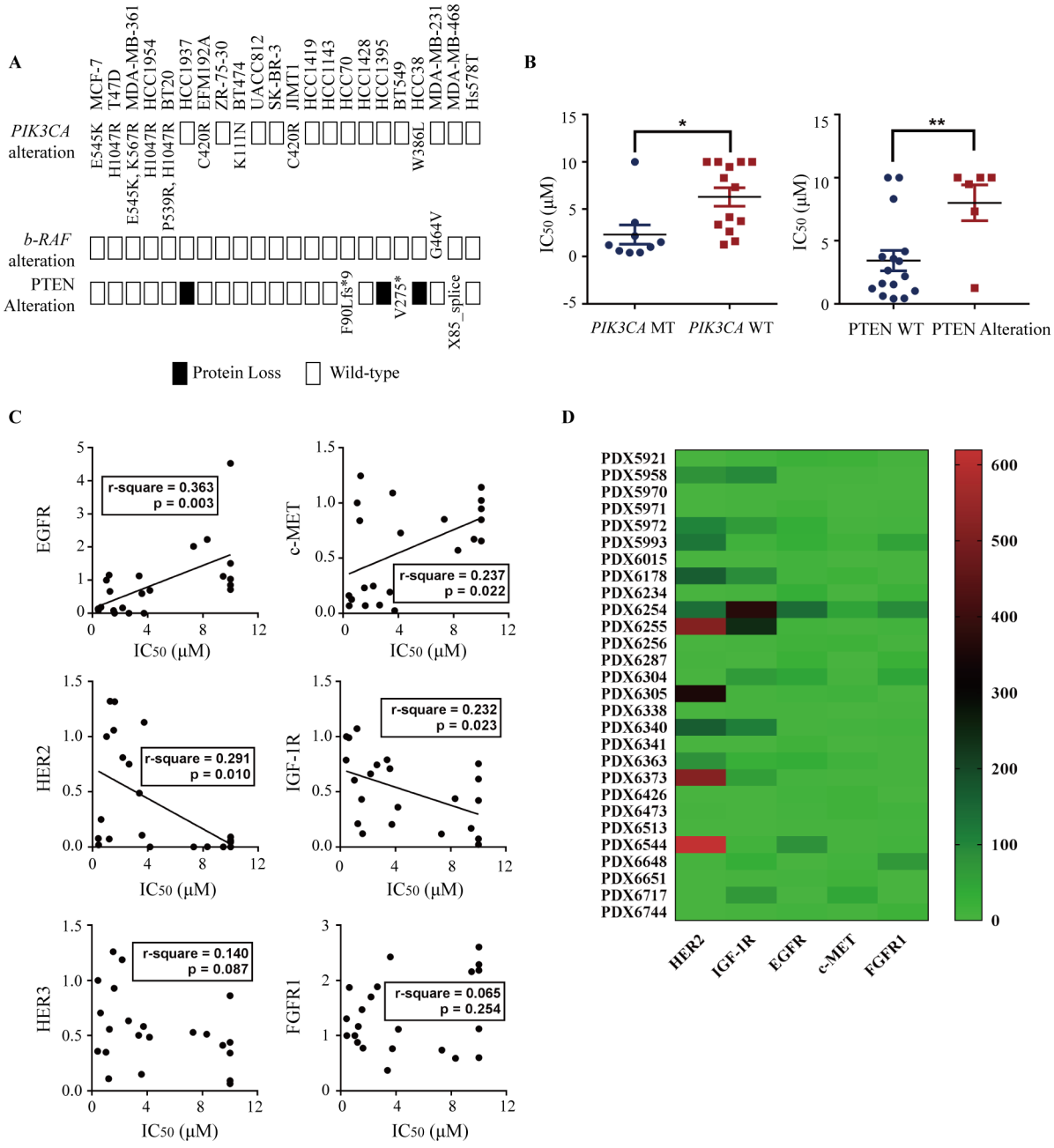

Figure S6: The expression profile of RTKs determines efficacy of PI3K inhibitors in breast cancer. (A) The alteration of PIK3CA, b-RAF and PTEN in 22 breast

cancer cell lines. Alteration of PIK3CA and b-RAF were from the database of CCLE and COSMIC. Expression status of PTEN was obtained from CCLE and previous studies (2, 3). (B) Scatter plot showing IC_{50}s (BYL719) in different breast cancer cells as indicated. Bars, mean ± SEM. Unpaired *t* test was performed. * $P < 0.05$; ** $P < 0.01$. (C) Correlation of expression of respective RTK and IC_{50}s (BYL719) in 22 breast cancer cell lines. (D) Quantitative heatmap showing expression level of indicated RTKs in 28 PDX tissues via IHC. The expression of RTKs was quantified by normalizing the optical density of respective RTK with that of nucleus staining using Image-Pro Plus Software.

References

1. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490:61-70.
2. Stern HM, Gardner H, Burzykowski T, et al. PTEN Loss Is Associated with Worse Outcome in HER2-Amplified Breast Cancer Patients but Is Not Associated with Trastuzumab Resistance. Clinical Cancer Research. 2015;21:2065-74.
3. O'Brien C, Wallin JJ, Sampath D, et al. Predictive Biomarkers of Sensitivity to the Phosphatidylinositol 3 ' Kinase Inhibitor GDC-0941 in Breast Cancer Preclinical Models. Clinical Cancer Research. 2010;16:3670-83.