**Supplementary Figure S1**

**HER2-positive NCI-N87 Cells Show Lapatinib Responsiveness Compared with HER2-negative Cells.**

**A,** The NCI-N87 human GC cell line shows more responsiveness to lapatinib in all 6 GC cell lines. Cells were treated with lapatinib at the indicated concentrations, and viable cells were measured after 72 hours of treatment. The percentage of viable cells was shown relative to untreated controls. Data points indicate average of replicates of six and bars indicate SEM.

**B,** NCI-N87 cells show HER2 overexpression and activation. Cell analysis by western blot were cultured in full serum. Protein lysates were collected from all 6 gastric cancer cell lines plus 1 gastric epithelial GES cell line and analyzed for HER2 and EGFR expression and activation, as well as key downstream components. β-actin serves as a loading control.

**C,** Lapatinib inhibits HER2 and EGFR activation and blocks downstream signaling through the MAPK and AKT pathways. N87 cells were exposed to increasing concentrations of lapatinib for 3, 6, or 24 hours. Cell extracts were immunoblotted to detect the indicated proteins.

**Supplementary Figure S2**

**Knockdown of Each Gene Was Confirmed by Polymerase Chain Reaction (PCR) or Western Blot Analysis**

NCI-N87 cells were transfected with three independent siRNAs targeting each gene or scrambled siRNA. The indicated gene expression was measured by western blot or PCR after 3 days of 20nM siRNA transfection. MET, HER3, IGF-1R, INSR were significantly knocking down by the indicated siRNA. FLT3 and RET protein expression were undetectable via western blot in this cell line, and mRNA expression were detectable by PCR. FLT3, RET, HGF and IGF-1 gene silencing were confirmed by PCR or real-time PCR. The percentage of IGF-1 gene expression is shown relative to scrambled controls. Column indicate average of replicates of three and bars indicate SEM.

**Supplementary Figure S3**

**TKIs’ Effects on the Corresponding Receptors**

NCI-N87 cells were treated with TKIs for 6 hours, then stimulated with ligands for 30 minutes before whole cell lysates were collected and analyzed by Western blot. PF-02417903, AEW-541, GSK529 shows selective effect on p-MET, p-IGF-1R and p-INSR. AZD8931 shows multi-target effects on p-EGFR, p-HER2 and p-HER3. p-FLT3 and p-RET were undetectable in this line.

**Supplementary Figure S4**

**HER3 Signaling Activation Attenuates Lapatinib-induced Apoptosis and Suppression of cell motility.**

**A**, HER3 signaling activation attenuates lapatinib-induced apoptosis. NCI-N87 cells were treated with lapatinib 0.2 μM for 24 hours, either with NRG1 50 ng/mL, or plus AZD8931 1 μM, and then analyzed by flow cytometry. Columns indicate average of triplicates and bars indicate SD. \*P < 0.0001.

**B,** HER3 signaling activation attenuates lapatinib-induced suppression of cell motility.(Left) Representative images of migration assays. Bars, 50 µm. (Right) Motility assays of SNU-216 cells with indicated treatment. SNU-216 cells were treated with lapatinib 0.05 μM for 30 hours, either with NRG1 50 ng/mL. Data are mean ± SEM (n=6) and are representative of three independent experiments.

**C,** HER3 confers lapatinib resistance by restoring AKT or ERK downstream signaling. Immunoblots showing AKT or ERK re-activation in HER3-positive NCI-N87, SNU-216, and SKBR3 cells. Cells were treated with or without lapatinib for 6 hours, then stimulated with or without NRG1 for 30 minutes before whole cell lysates were collected and analyzed by Western blot.

**Supplementary Figure S5**

**Constitutive Activation of IGF-1R Signaling Promotes Resistance to Lapatinib in HER2-positive N87 Cells.**

**A,** N87 cells were stably transfected with either an empty lentiviral vector, constitutively active CD8-IGFR, or the kinase-inactive CD8-IGFR-YF. Colony formation assays were conducted for about 2 weeks and growth curves were performed according to quantification analysis. The percentage of viable cells was shown relative to untreated vector control. Data points indicate average of replicates of three and bars indicate SEM. \**P* < 0.05 compared with the lapatinib-treated N87-vector control group.

**B** and **C,** Constitutively active CD8-IGFR confers lapatinib resistance by restoring AKT downstream signaling. NCI-N87 cells overexpressing empty vector, CD8-IGFR, or CD8-IGFR-YF were treated with or without lapatinib 0.2 μM for 6 hours before whole cell lysates were collected and analyzed by Western blot.