Supplemental Material

Material and Methods

RTK-antibody array profile: Whole cell extracts were made in lysis buffer containing 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% NP-40, 10% glycerol, 2 mM EDTA, 1 mM sodium orthovanadate, 1xprotease inhibitor cocktail (complete mini-EDTA from Roche scientific), and 1 mM DTT. Following 30 min incubation on ice, cells were scraped and cleared by centrifugation at 14000 rpm for 10 min at 4°C. Protein concentrations were determined using Bio-Rad protein assay reagent. Either 200 µg (Fig 1A) or 500 µg (Fig 2A) were used as suggested by the manufacturer.

Immunoprecipitation and immunoblot: For the immunoprecipitation (IP) experiment, cells were incubated with either vehicle (0.5% DMSO) or with indicated concentrations of GW2974 in 0.5% DMSO containing cell media for 2 hr. Cells were immediately washed in cold PBS. Whole cell extracts were made as in RTK-antibody array profile. EGFR and Her2 complex were immunoprecipitated using mouse anti-EGFR or mouse anti-Her2 antibody with total of 1 mg of protein. Protein A sepharose beads (Amersham Bioscience) were used to capture the complex and were washed in IP buffer and eluted by boiling in 1x SDS-PAGE loading buffer for 5 min. 50% of eluted proteins from each IPs were used to blot with antibody against Met. Another 25% of eluted protein was used to perform WB using anti-phospho-tyrosine antibody and the remaining 25% was used to determine the total EGFR (for EGFR IP) or Her2 (for Her2 IP) protein level. Proteins were separated in NuPage 4-12% gradient Bis-Tris gels, transferred to nitrocellulose, blocked in 5% non-fat dry milk in TBST and blotted with antibodies as indicated. Proteins were detected using secondary antibody either goat anti-mouse-HRP or goat anti-rabbit-HRP. Whole cell
extracts were also separated, transferred and detected for total and phosphorylated proteins using appropriate antibodies.

**HGF activation:** Cells were plated in 24-well plate in 750 µl of starvation media (no FBS and no IL-3 containing WEHI media) at a density of 5x10^6 cells/well for 32D/Met cells. After 2 hr of starvation, compounds were serially diluted into cell starvation media as 2x stocks containing 1% DMSO and 750 µl of each dilution was added to the wells after 2 hr of starvation. Additional 2 hr incubation with compound in starvation media, cells were activated with 50 ng/ml of HGF for 5 min. Cells were collected immediately in 2 ml eppendorf tubes and spun down for 2 min at 4°C, washed once with PBS followed by addition of 100 µl of lysis buffer in each tube. After 30 min of incubation at 4°C the lysates were cleared by centrifugation at 14000 rpm for 10 min at 4°C. Protein estimation was performed and equal amounts of proteins were separated on NuPage 4-12% gradient Bis-Tris gels as mentioned above. Total Met and phospho-Met levels were determined by WB using appropriate antibodies. Beta-tubulin was used as loading control.

**Wound healing assay:** For wounding assay, confluent cells were scraped using a sterile pipette tip, washed once with PBS to remove all floating dead cells. Immediately treated or left untreated (0.5% DMSO control) with appropriate concentration of compound in 10% FBS media containing 0.5% DMSO. Wound healing was monitored by photographing each scratch at different time intervals. Each treatment was done at least in triplicate and repeated several times.
**Figure Legend.**

Figure S1. Same RTK array as in Figure 2A, but a different exposure.

Figure S2. Gefitinib does not inhibit the phosphorylation of Met in 32D/Met cells. Western blot analysis of same samples as in Figure 3B lanes 2, 3 and 19.

Figure S3. GW2974 inhibits wound healing in H441 cells. A-H. Confluent layer of cells were wounded by scratch followed by treatment with DMSO (A-C) or with GW2974 (25 µM, D-H) and photographed at indicated times. Representative data are shown from results of multiple wounds.

Figure S4. Confluent layer of H441-pLKO.1 and H441-Met shRNA cells were wounded by scratch and photographed at indicated times. Representative data are shown from the results of multiple wounds. A-B. H441-pLKO.1; and C-D. H441-Met shRNA cells.