Figure S1

Anjali Jain

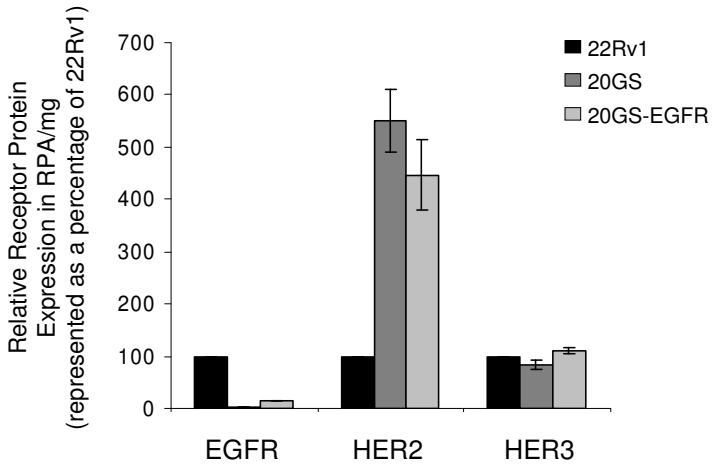


Figure S2

Anjali Jain

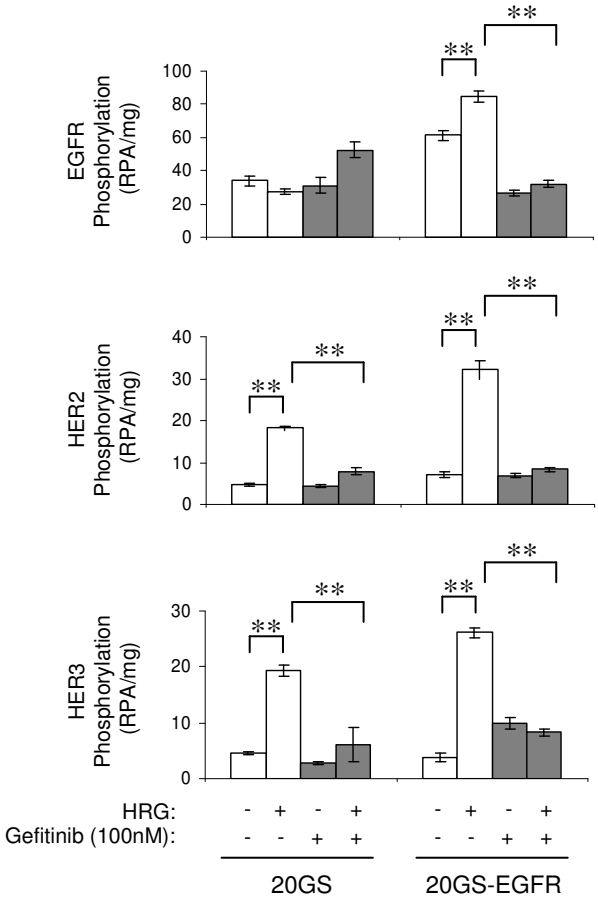
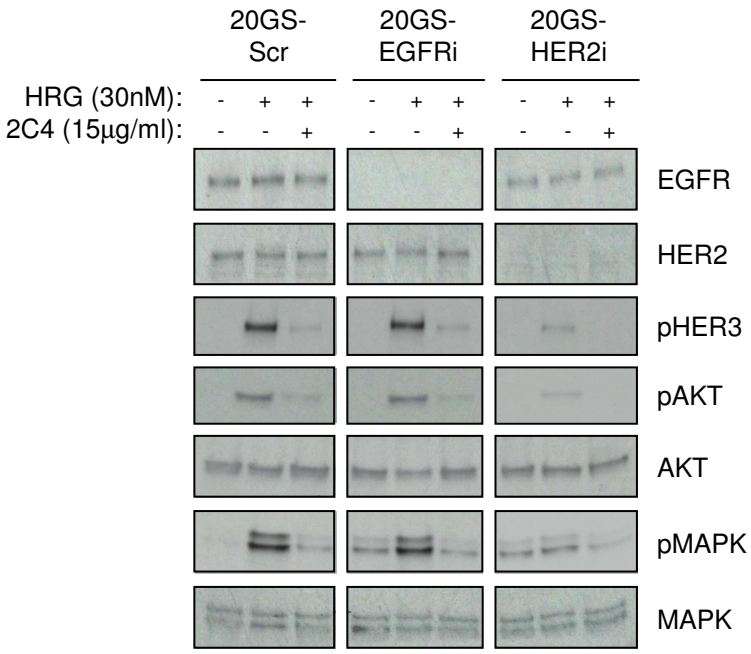


Figure S3

Anjali Jain



Supplementary Figure Legends

Figure S1. Total receptor protein expression measurements using the VeraTag Assay system: Relative peak area/mg total whole cell lysate for the 20GS and 20GS-EGFR cell lines were plotted as a percentage of the 22Rv1 cell line. Using the VeraTag assay, we observed a larger difference in HER2 protein expression between 22Rv1 and 20GS cells (5.5-fold by protein versus 2-fold by mRNA). However, EGFR protein expression level measurements were highly concordant with the mRNA expression i.e., 3% of EGFR protein in the 20GS and ~15% in the 20GS-EGFR relative to EGFR protein expression in the parental 22Rv1 cells.

Figure S2. Receptor phosphorylation measurements using the VeraTag Assay system. 20GS and 20GS-EGFR were serum starved and stimulated with 30nM HRG or pretreated with 100nM gefitinib before HRG stimulation. Relative peak area/mg total whole cell lysate is plotted. ** indicates $p<0.0001$.

Figure S3. HRGβ1-mediated signaling in 20GS cells is dependent on HER2 but not EGFR. Immunoblot analysis of total EGFR, total HER2, phospho-HER3, phospho-AKT, total AKT, phospho-MAPK, and total MAPK in 20GS cells infected with scrambled (Scr), EGFR shRNA (EGFRi) or HER2 shRNA (HER2i) viruses. After viral infection, the cells were serum starved, stimulated with 30nM HRGβ1 for 10 min at 37ºC, or pre-treated with 15μg/ml 2C4 for 2 hours followed by 30nM HRGβ1 as indicated. Inhibition of phosphorylation signals with 2C4 treatment indicated the involvement of HER2 heterodimers.

Supplementary Materials and Methods

VeraTag assays. VeraTag lysate assays were performed using the VeraTag technology previously described (24). Briefly, fluorescent reporter "tags" and streptavidin-conjugated methylene blue (SA-MB: "molecular scissors") beads were synthesized and purified according to protocols described previously (U.S. Patent 7,105,308). Antibody-fluorescent tag and antibody-biotin conjugates were made using sulfo-NHS-LC-LC-biotin (Pierce) linker according to manufacturer's protocol followed by HPLC (Agilent) purification. The lysates version of the VeraTag assay is run as follows: serial dilutions of whole-cell lysates are incubated (1hr, RT) with fluorescent reporter-tagged and biotin-conjugated antibodies in blocking buffer (1X PBS/1% BSA) in pre-blocked Millipore Multiscreen HV filter plates (0.2μM). Subsequently, streptavidin-conjugated methylene blue (SA-MB) beads are added at 1mg/mL in 1M Tris and incubated for 45 minutes at room temperature. Samples are vacuum filtered, and rinsed in 1X PBS/0.5% Triton X-100 and then in 0.005X PBS. Illumination buffer containing 2 pmol/L fluorescein and two CE internal markers (MF and ML) in 0.01X PBS is added and bound reporter tags are released by photo-activated cleavage. Released reporter tags are collected and run on an ABI3100 CE (Applied Biosystems), using the conditions 6kV (Injection Voltage) and 80 sec (Injection time) for 600 seconds. Fluorescent peaks are identified and quantified using software developed at Monogram. The relative peak area (RPA) is measured by normalizing the reporter- tag peak-area to the peak-area of, 2 pmol/L fluorescein.

shRNA studies. EGFR shRNA (validated sequence # TRCN0000121068), HER2 shRNA (validated sequence # TRCN0000039878) and non-target shRNA control (# SHC002) lentiviral plasmid constructs were purchased from SIGMA-ALDRICH. 3μg of the respective viral plasmids were co-transfected with 9μg of ViraPower packaging mix (Invitrogen) into 293T cells using Lipofectamine 2000. 48 hours post-transfection, the viral supernatant was collected and filtered through a 0.45μM cellulose acetate membrane which was then used to infect the 20GS cells at MOI=5. 48 hours post-infection, cells were serum starved for an additional 24 hours before 2C4 and HRGβ1 treatments. Cells were harvested for total protein lysates and analyzed by immunoblots with the respective antibodies using standard procedures. Total RNA was isolated from a parallel set to quantitatively analyze EGFR, HER2, HER3 and HER4 receptor RNA expression levels to ascertain the level and specificity of knockdown.