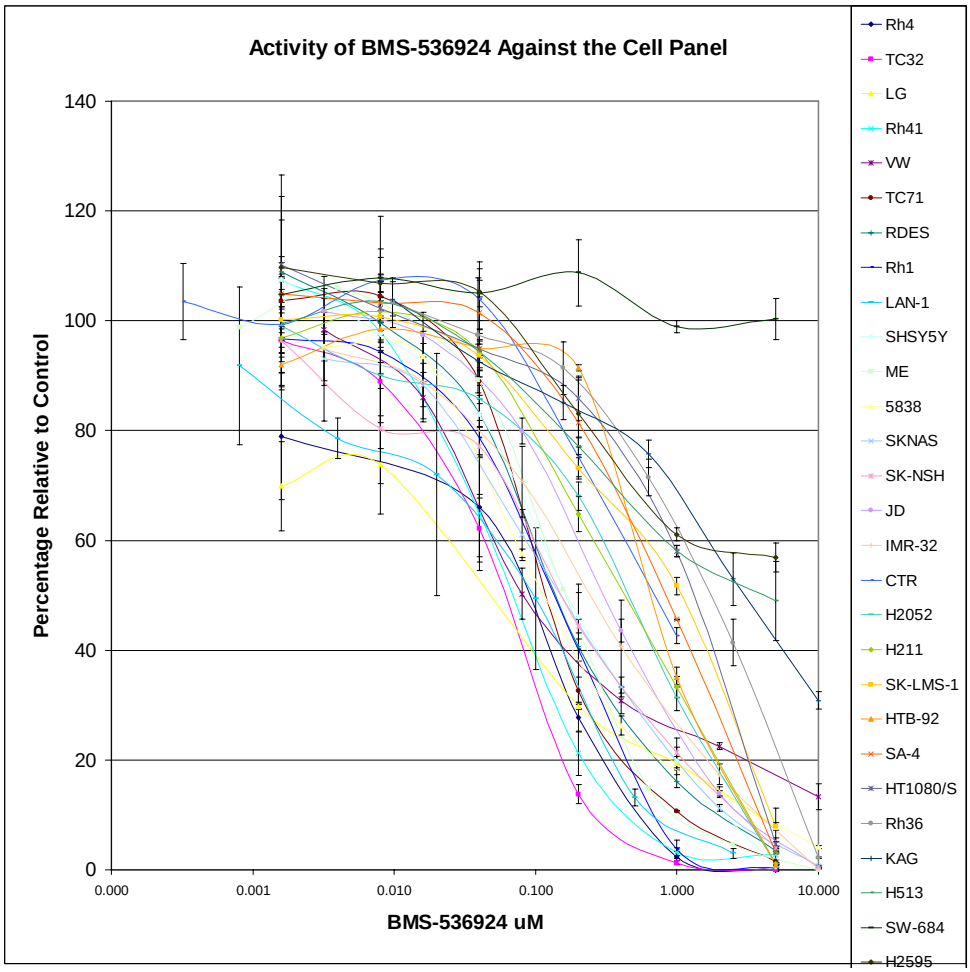
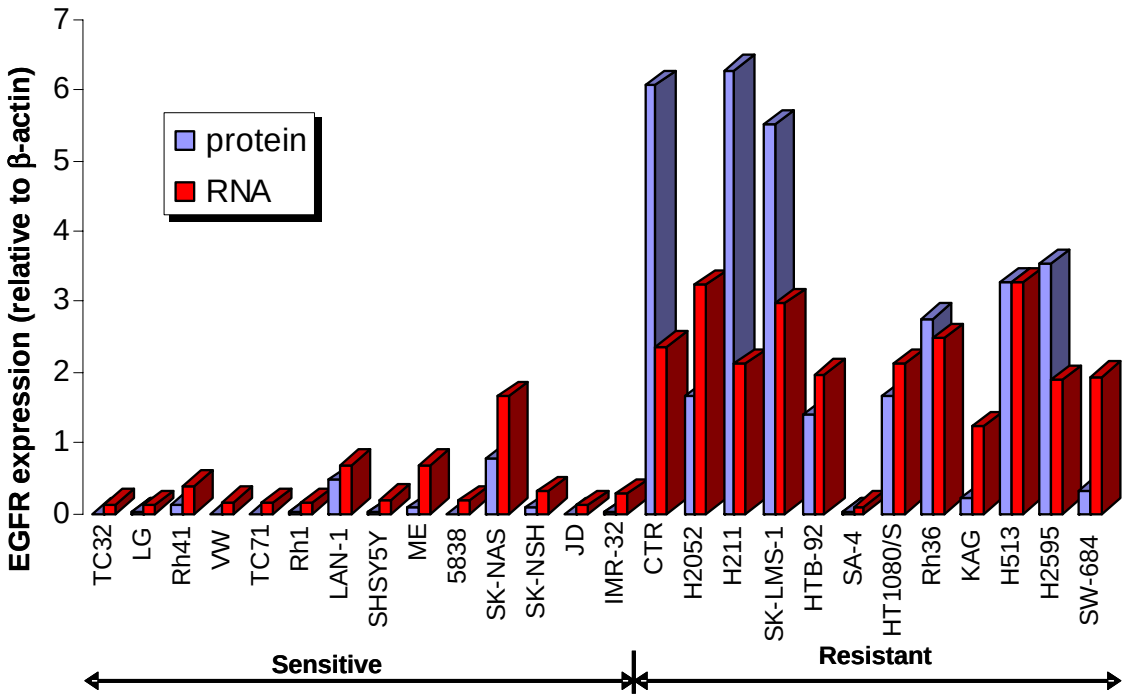
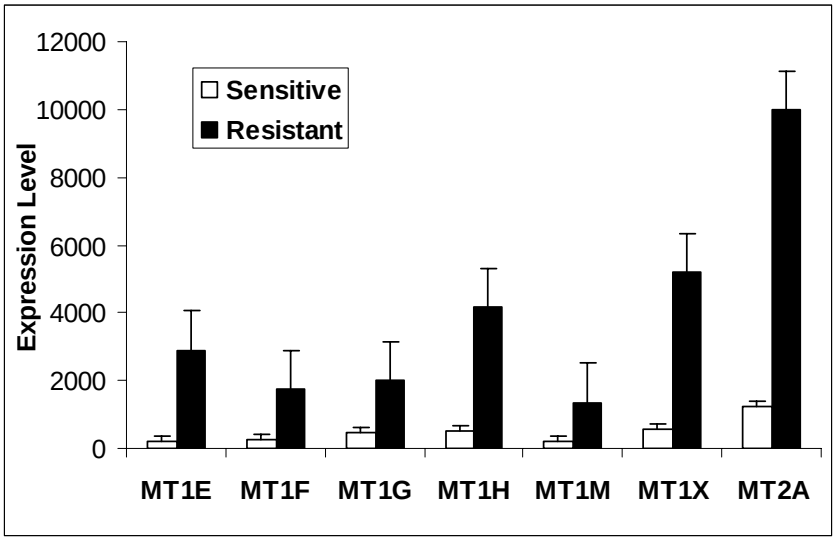
Supplemental Figure 1: The representative results of cell proliferation assay on the panel of 28 cell lines. Cell proliferation was evaluated by [3H]-thymidine incorporation after exposure to BMS-536924 for 72 hours. Cells were plated at an optimized density in 96-well plates, incubated overnight at 37°C, and then exposed to a serial dilution of the drug. After 72 hours incubation, cells were pulsed with 4μCi/ml [3H]-thymidine (Amersham Pharmacia Biotech, UK) for 3 hours, trypsinized, harvested onto UniFilter-96 GF/B plates (PerkinElmer, Boston, MA); scintillation was measured on a TopCount NXT (Packard, CT). The values on y axis represent the cell prolifersation rates when treated with BMS-536924 relative to untreated control cells. X axis represents BMS-536924 concentrations in μM. The standard deviations are shown.



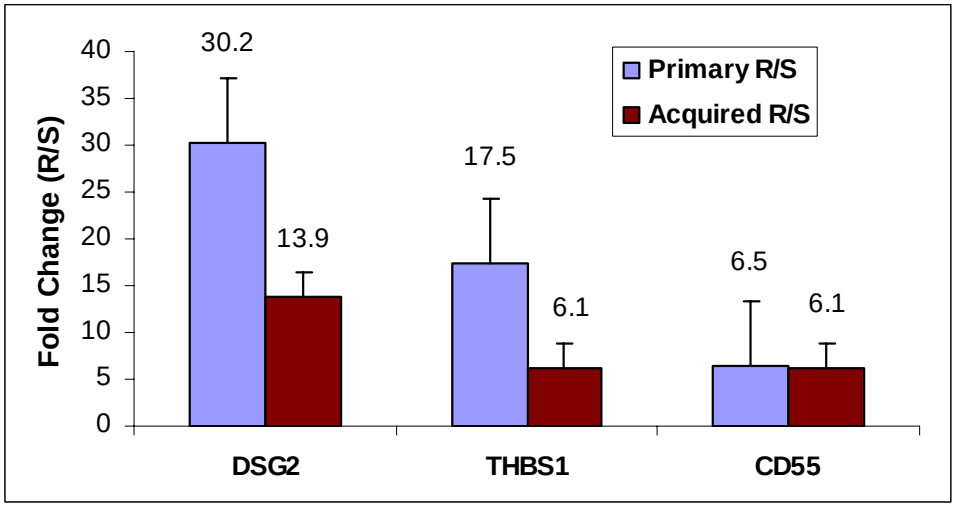
Supplemental Figure 2: Both EGFR RNA and protein are higher expressed in BMS-536924 primary resistant cell lines than in sensitive cell lines. RNA levels are measured by Affymetrix Genechip; protein levels are measured by Western blots and the integrated signal intensity was quantified using Odyssey Infrared Imaging System (Li-Cor Biosciences). Both data are normalized to β-actin. High correlation (p=0.00001) between EGFR expression pattern on RNA and protein is observed.



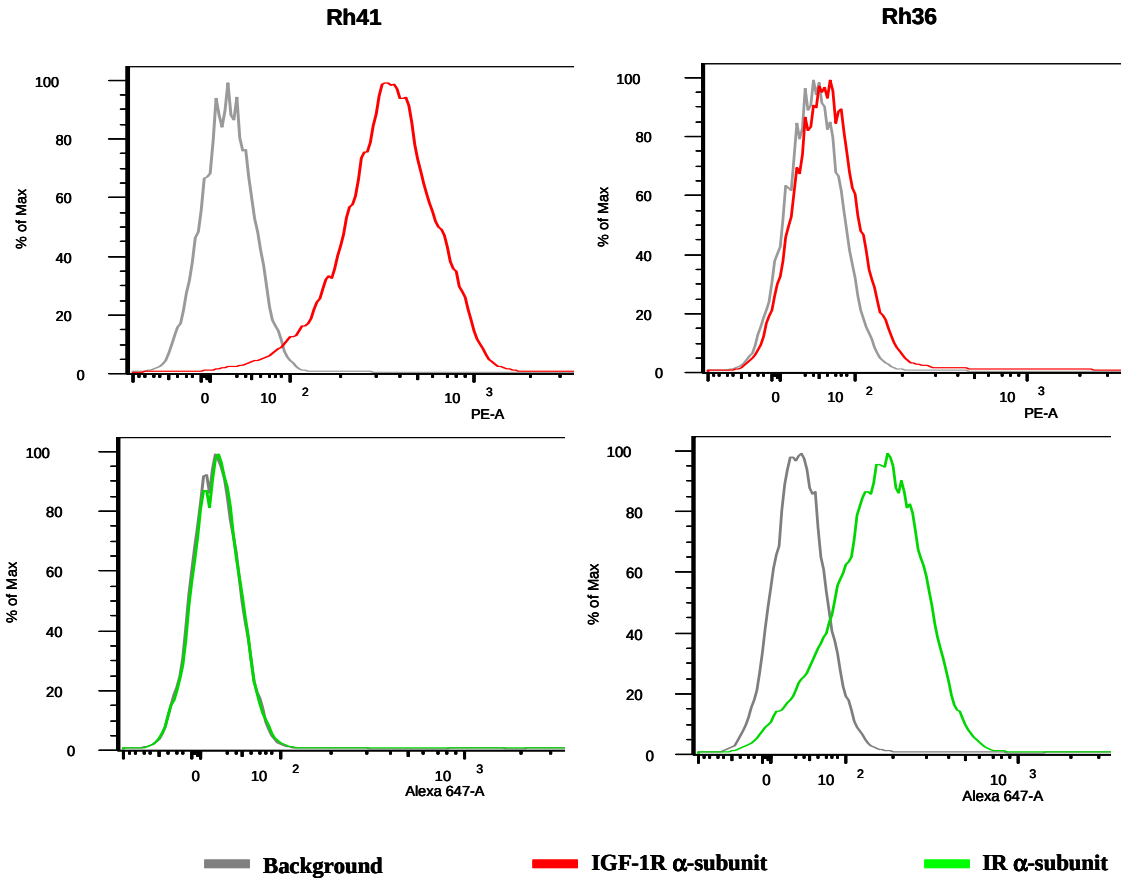
Supplemental Figure 3: Metallothionein family members are over-expressed in BMS-536924 primary resistant cell lines. The expression data were detected by Affymetrix Genechip; the average expression values (y axis) of the 16 sensitive and the 12 resistant cell lines with standard errors are shown.



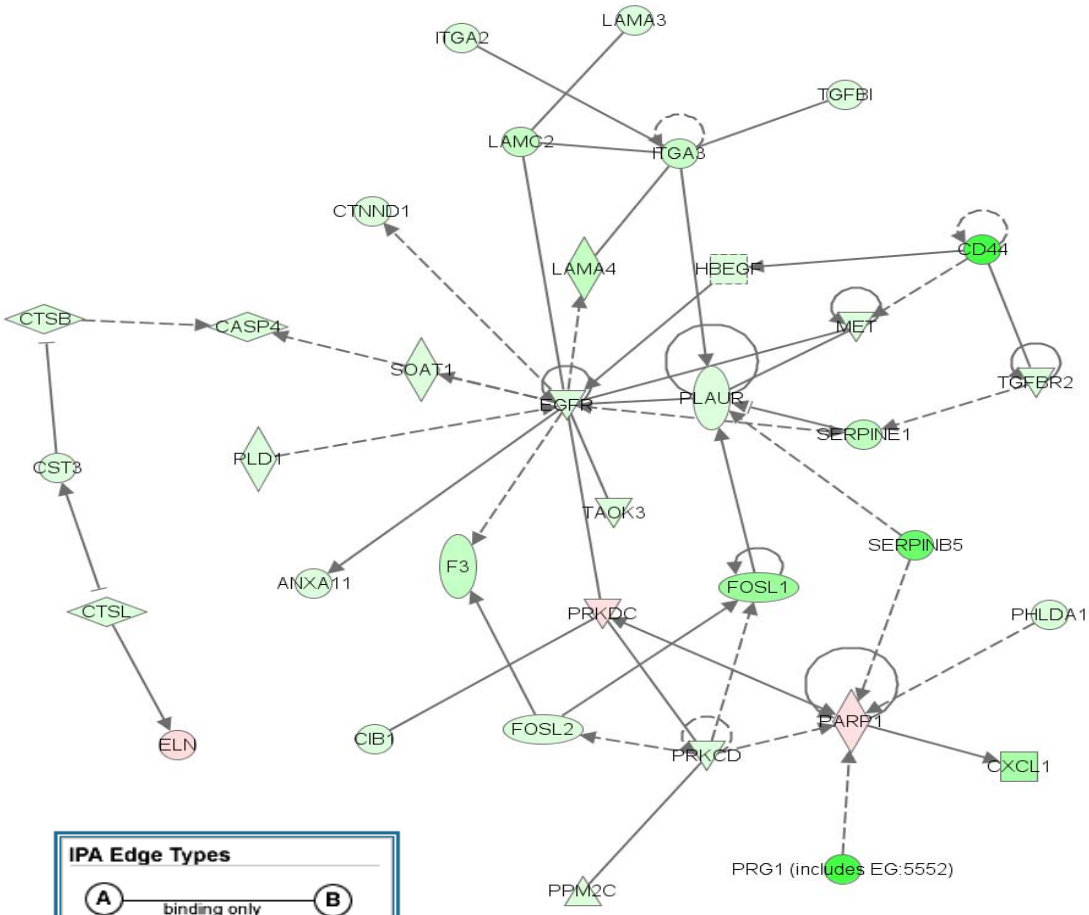
Supplemental Figure 4: DSG2, THBS1 and CD55 are involved in both primary and acquired resistance to BMS-536924 with higher expression in resistant cell lines. The fold changes between the 12 resistant cell lines and 16 sensitive cell lines are indicated for primary resistance. The fold changes between BMS-536924 acquired resistant RD-1R and the sensitive parental RD-1S are indicated for acquired resistance. The standard errors and fold changes between resistant and sensitive cell lines are shown.



Supplemental Figure 5: Differential expression of IGF-1R and IR in sensitive Rh41 and resistant Rh36 cell lines as measured by Flow cytometry analyses. Fluorescence intensity (x-axis) is directly correlated with the amount of IGF-1R (red) or IR (green).



Supplemental Figure 6: Ingenuity Pathway Analysis. 386 probe sets (Supplemental Table 1) whose basal expression level correlated with the sensitivity to BMS-536924 in 28 cell lines were used to conduct pathway analysis (http://www.ingenuity.com) to generate network maps. One of Network Maps generated by Ingenuity Pathway Analysis highlighting over-expression of multiple signaling pathways and cross-talk between them in resistant cell lines. The genes higher expressed in resistant cell lines are highlighted by green, and genes higher expressed in sensitive cell lines are highlighted by red.



IPA Edge Types

- A — binding only — B
- A — inhibits — B
- A — acts on — B
- A — inhibits AND acts on — B
- A — leads to — B
- A — translocates to — B

Note: "Acts on" and "Inhibits" edge may also include a binding event.

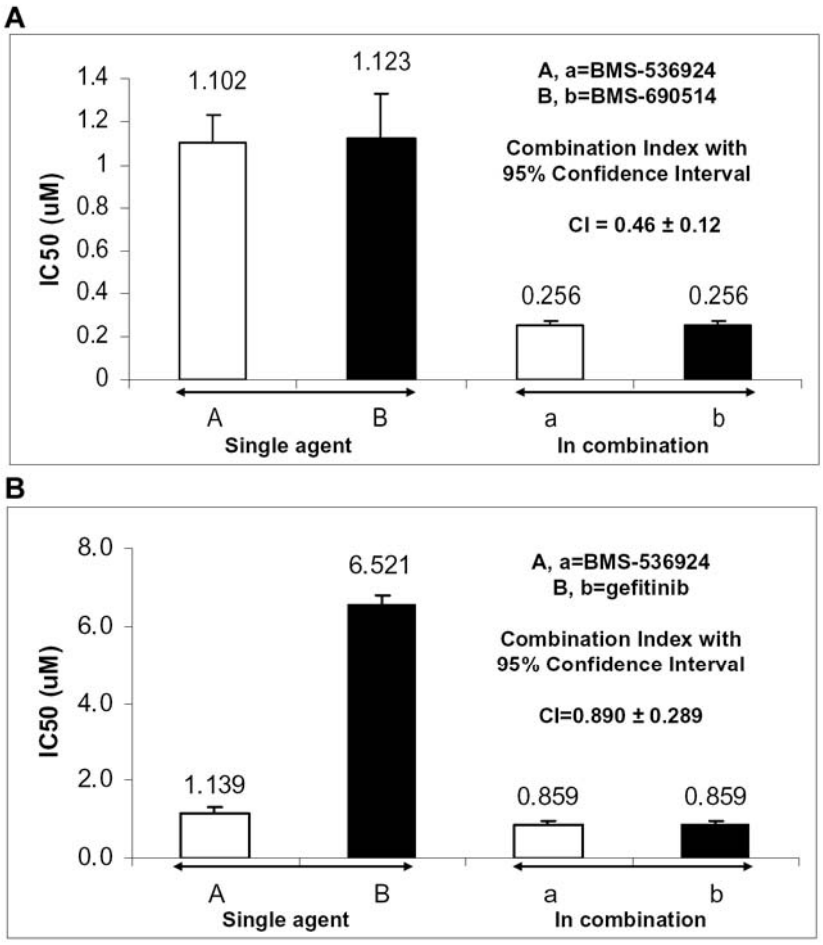
direct interaction

indirect interaction

IPA Node Types

- Growth Factor
- Kinase
- Peptidase
- Phosphatase
- Transcription Regulator
- Cytokine
- Enzyme
- Transmembrane Receptor
- Other

Supplemental Figure 7: Enhanced anti-proliferative activities observed in combination studies in vitro for Rh36 cell line. A dilution of ratios drug combination method was used in cellular proliferation assays to test the IC50 values of single agent as well as in combination. IC50 values are shown with standard error, data representative of n=3. Results are derived from 1:1 ratio of two drugs in combination. Combination Index = (a/A + b/B). *A,* Synergistic effort of IGF-1R inhibitor BMS-536924 in combination with BMS-690514, a Her1/Her2 inhibitor. Combination Index ± 95% confidence interval (CI=0.460 ± 0.122) less than 1 indicates synergy. *B,* Additive effort of BMS-536924 in combination with gefitinib. Combination Index ± 95% confidence interval (CI=0.890 ± 0.289) greater than 1 indicates additivity.



Supplemental Figure 8: Effects of BMS-536924 and Erbitux treatment, singly or in combination, on the growth of the GEO human colon carcinoma xenograft model in nude mice. The tumor-borne mice were treated with either control, 270 mg/kg of BMS-536924 (PO, QD x 17), Erbitux (0.125 mg/mouse, IP, Q3D x 6), or in combination of Erbitux and different dose levels of BMS-536924 as indicated. The median tumor weight was indicated. Enhanced activity observed when mice treated with combination BMS-536924 and Erbitux.