**SUPPLEMENTARY FIGURE LEGEND:**

**Supplementary Figure 1. NSCLC harboring EGFR kinase domain mutation develops EGFR TKI resistance with mesenchymal phenotype.**

A. annexin V assay showing HCC4006 cells but not mesenchymal HCC4006Ge-R cells undergo significant (\*p=0.0348, Student’s t-test) apoptosis upon gefitinib treatment. (Left) representative flow histogram of Annexin V apoptosis assays. (Right) Quantification of apoptosis assay by Annexin V assay. Average of three independent assays. Bars, S.D. B. 72 hours cell viability assay with EGFR inhibitor (gefitinib) or AXL inhibitor (R428) or a combination. Note HCC4006Ge-R that shows activated AXL in RTK (Fig.1C) is resistant to the combination treatment. Data points are average of duplicate wells from two independent assays. Error bars, S.D. C. 72 hours cell viability assay with irreversible EGFR inhibitor (CL-387,785). Note mass culture of NCI-H1975 cells grown resistant to increasing concentration of CL-387,785 (NCI-H1975CLR) are resistant to CL-387,785. Data points are average of duplicate wells from two independent assays. Error bars, S.D. D. immunoblot demonstrating that NCI-H1975CLR mass culture shows upregulation of mesenchymal markers (N-Cadherin and vimentin) and downregulation of epithelial marker (E-Cadherin). E. GSEA of ERC4 cells. The normalized enrichment score (NES) and the nominal p values are indicated.

**Supplementary Figure 2. Chronic exposure of HCC4006 cells to TGFβ1 promotes EMT and gefitinib resistance.**

A. (Left) immunoblot showing the HCC4006 cells undergoes epithelial to mesenchymal transition (EMT) and mesenchymal to epithelial transition (MET) with TGFβ1 exposure for approximately 30 days and with TGFβ1 withdrawal for approximately 30days, respectively. (Middle) quantification of apoptosis by Annexin V assay showing the HCC4006 cells but not HCC4006 cells exposed to TGFβ1 for 30days undergo significant (p<0.05, One way ANOVA) apoptosis upon gefitinib treatment. Note that withdrawal of TGFβ1 for 30days significantly re-sensitized the cells to gefitinib compared to its TGFβ1-induced mesenchymal state (p<0.05, One way ANOVA). Average of two independent assays. Bars, S.D. (Right) representative flow histogram of Annexin V apoptosis assays. B. immunoblot demonstrating that HCC4006 cells exposed to TGFβ1 for 30days are mesenchymal with upregulation of mesenchymal markers (N-Cadherin and vimentin) and downregulation of epithelial marker (E-Cadherin). C. 72 hours cell viability assay with gefitinib. Note HCC4006 cells exposed to TGFβ1 for 30days are resistant to gefitinib. Data points are average of duplicate wells from two independent assays. Error bars, S.D. D. number of HCC4006 cells and established HCC4006TGFβ1 cells (cultured in TGFβ1 from more than 30days) after 5 days of culture. Equal numbers of cells (9.0X105 cells) were seeded. HCC4006 cells were allowed to grow in the absence of TGFβ1 and HCC4006TGFβ1 cells were cultured in the media supplemented with 10ng/ml TGFβ1 for 5 days and viable cells were counted. Results are average of three independent assays. Error bars, S.D.

**Supplementary Figure 3. Chronic EGFR inhibition in *EGFR* mutant NSCLC cells promotes TGFβ1 secretion and SMAD pathway activation.**

A. 100nM erlotinib-treated HCC827 cells (above) secrete TGFβ1 into the media in a time dependent manner. In contrast, 100nM gefitinib treatment does not significantly promote TGFβ1 secretion in NCI-H1734 cells (below, *EGFR* wild-type, kras mutant). The concentration of TGFβ1 in the media was normalized to 5X105 cells. (\* p<0.05, \*\* p<0.01, \*\*\* p<0.001) Error bars, S.D. B. equal concentration (100nM) of gefitinib or erlotinib similarly promotes TGFβ1 secretion from HCC827 or HCC4006 cells in 72 hours (\*p<0.05 \*\*\*p<0.0001, Student’s t-test). Error bars, S.D. C. immunoblot demonstrating SMAD2 phosphorylation upon treatment of HCC4006 cells with gefitinib (100nmol/L). The SMAD2 phosphorylation was diminished with a presence of TGFβ receptor inhibitor, SB431542 (1µmol/L). Lysates were harvested at indicated times and 40µg of the lysates were subjected to Western blot with indicated antibodies. Representative blots from more than two independent experiments are shown. D. NCI-H1734 cells harboring wild-type EGFR were treated with DMSO, erlotinib (100nmol/L), SB431532 (1µmol/L) or combination of erlotinib and SB431542 for 72 hours. Viable cells were lysed and equal amounts of protein were subjected to a Luminex assay to quantify the SMAD2 and SMAD3 activities. Fold increase of phosphorylated proteins was calculated using mean fluorescent intensity (MFI) that is proportional to the amount of phosphorylated protein in the cell. The results represent an average of two independent assays run in duplicate samples. Error bars: S.D. E. immunoblot showing the depletion of EGFR by shRNA in HCC827 cells generates subclones with epithelial and mesenchymal phenotypes. Note Clone 9 shows strong mesenchymal phenotype with E-Cadherin downregulation and vimentin upregulation. In contrast, subclone 10 is epithelial with slight increase in MET expression. F. *MET* copy number analysis. HCC827-GR5 is resistant to gefitinib with *MET* amplification and used as a positive control (4). Average of three independent experiments ran in triplicates are shown. Error bars, S.E.M. G. a phospho-RTK array reveals that HCC827shEGFR clone9 cells show no phosphorylation of 42 RTKs in the presence of 10µmol/L gefitinib. Duplicate spots in the corners are phospho-tyrosine controls.

**Supplementary Figure 4. Concurrent EGFR and TGFβR inhibition prevents mesenchymal transition but does not avert emergence of EGFR TKI resistance.**

A. immunoblot showing mesenchymal transition in HCC827 cells treated with gefitinib over the course of 3 to 6 months. The presence of SB431542 prevents mesenchymal transition induced by continuous gefitinib treatment in HCC827 cells. Lysates were made at indicated times and they were subjected to Western blot with antibodies indicated. B. immunoblot showing epithelial (E-Cadherin) and mesenchymal (N-Cadherin, Vimentin, and CD44) markers in HCC4006 parental and resistant cells. Ge-R cells exhibit strong mesenchymal phenotype while GeSB-R cells remain epithelial. C. 72 hours cell viability assay with gefitinib or afatinib. Note HCC4006Ge-R cells are resistant to gefitinib and afatinib; however, HCC4006GeSB-R cells treated with afatinib show reduced viability. Data points are average of duplicate wells from three independent assays. Error bars, S.D. D. annexin V assay showing HCC4006GeSB-R cells undergo statistically significant (\*p=0.0385, Student’s t-test) apoptosis upon afatinib treatment (Right). (Left) representative flow histogram of Annexin V apoptosis assays. Quantification of apoptosis assay by Annexin V assay is from average of three independent assays. Bars, S.D.

**Supplementary Figure 5. AZD9291 treatment induces significant apoptosis in HCC4006GeSB-R cells with acquired T790M mutation.**

A. irreversible EGFR inhibitor AZD9291 suppresses EGFR activity and its downstream signaling in HCC4006GeSB-R cells harboring secondary T790M mutation. HCC4006, HCC4006Ge-R, and HCC4006GeSB-R cells were treated with DMSO (-) or 500nmol/L AZD9291 (+) for 2 hours and lysates were made. Lysates were subject to Western blot with antibodies indicated. Representative blots from more than three independent experiments are shown. B. 72 hours cell viability assay with gefitinib or AZD9291. Note HCC4006Ge-R cells are resistant to gefitinib and AZD9291; however, HCC4006GeSB-R cells treated with afatinib show reduced viability. Data points are average of duplicate wells from three independent assays. Error bars, S.D.

**Supplementary Figure 6. Distribution of cells harboring T790M in PC9 and HCC4006 cells and STR analysis for HCC4006 cells and HCC4006 gefitinib resistant cells.**

A. two-dimensional analysis from ddPCR. Droplet digital PCR using genomic DNA from HCC4006 cells and PC9 cells showed a subclonal T790M mutation not detected by Sanger sequencing. FAM (blue) droplets indicate the presence of T790M mutant EGFR DNA, VIC (green) droplets indicate the presence of T790 wild type *EGFR* DNA, and orange droplets indicate the presence of both T790M mutant and T790 wild type DNA. NTC: Non-Template Control.