Supplemental Figure Legends

Supplemental Figure 1. Localization of EPHA2 in erlotinib resistant cells. (A) EPHA2 localization was assessed in PC-9/ERC16 cells following a timecourse of erlotinib withdrawal. EPHA2 receptor (green) was found to be localized to the cell border after erlotinib was removed from the culture media and was maintained there during the duration of the timecourse. Scale bar: 20µm  (B) 3D reconstructions from Z-stack images of EPHA2 staining (green) in PC-9/ERC16 cells at 72 and 96 hours post erlotinib withdrawal displayed EPHA2 on the cell surface as indicated by the white arrowheads.

Supplemental Figure 2. Validation of EPHA2 antibody specificity in patient tumor specimens
Immunohistochemistry was performed similarly as in Figure 1F by probing adjacent tissue sections with control human IgG in EGFR TKI resistant tumor samples compared to pre-treatment tumor specimens from 3 patients. Scale bar: 50µm.

Supplemental Figure 3. EPHA2 kinase activity is required in maintaining cell viability of erlotinib resistant lung cancer (A) Wild-type EPHA2 or mutant EPHA2^{D739N} were expressed in cells in which endogenous EPHA2 was knocked down using shRNA directed at the 3’ untranslated region. Expression of EPHA2 were analyzed by western blot analysis. (B) The effects of wild-type EPHA2 and mutant EPHA2^{D739N} on cell viability were assessed by the MTT assay. Data from two independent experiments were pooled and presented as the mean ± SEM. *p<0.05; **p<0.005; two way ANOVA with Bonferroni post hoc analysis. n.s., not significant.

Supplemental Figure 4. Biochemical analysis of TKI-resistant tumors from EGFR^{L858R+T790M} EPHA2^{+/−} and control mice (A) A Myc-tagged full length recombinant EPHA2, an N-terminal (1-427 amino acid) fragment, or a C-terminal (430-976 amino acid) fragment of EPHA2 were expressed in 293T cells. Cell lysates were subjected to western blot analysis using antibodies against either Myc or the N-terminal region of EPHA2. The blot was stripped and re-probed for β-tubulin to serve as a loading control. (B) Tumor lysates from freshly isolated lung tumors from EGFR^{L858R+T790M} EPHA2^{+/−}, or EGFR^{L858R+T790M} EPHA2^{−/−} mice were subjected to western blot analysis. The blot was stripped and re-probed for β-tubulin to serve as a loading control. The N-terminal EPHA2 antibody recognized the full-length EPHA2 protein in tumors from EPHA2 wild-type mice, but did not detect any full length or truncated versions of EPHA2 in tumors from EPHA2 targeted mice. Each mouse number represents an independent tumor sample from
a unique mouse. (C) Signaling studies of cell lysates from lung tumors of \( \text{EGFR}^{L858R+T790M}\text{EphA2}^{+/+} \) or \( \text{EGFR}^{L858R+T790M}\text{EphA2}^{-/-} \) mice. Shown are representative immunoblots in which signaling molecules were detected with the indicated antibodies. Each mouse number represents an independent tumor sample from a unique mouse.

Supplemental Figure 5. Signaling analysis in EPHA2 knockdown cells by siRNA-mediated gene silencing. Signaling analysis on cell lysates from two pairs of isogenic erlotinib sensitive and resistant cell lines were treated with \( \text{siControl} \), \( \text{siEPHA2#1} \), \( \text{siEPHA2#2} \) for 72 hours. Shown are representative immunoblots in which signaling molecules were detected with the indicated antibodies.

Supplemental Figure 6. Localization of EPHA2 upon ligand or EPHA2 inhibitor treatment (A) HCC827 cells were treated for 6 hours with 1µM erlotinib, ALW-II-41-27, or DMSO. Cells were then fixed, permeabilized, and stained for EPHA2 (green). Scale bar: 15µm. (B) HCC827/ER cells were treated as in A, and additionally treated for 15 minutes with EPHRIN-A1-Fc (EFNA1-Fc) before cells were fixed, permeabilized, and stained for EPHA2 (green). White scale bar: 30µm; Yellow scale bar: 15µm. EFNA1-Fc induced EPHA2 endocytosis (arrow), which was blocked by ALW-II-41-27, but not erlotinib.