Supplemental figure legends

Figure 1. The lung, skin, liver and intestine of transgenic mice with Egfr exon 19 deletion mutation and EGFR exon 21 L858R point mutation after 4 weeks administration of vehicle or afatinib. Vehicle or afatinib (5 mg/kg/day) were given once daily, 5 days per week as a suspension by gavage. The sections were stained with hematoxylin-eosin.

Figure 2. A. CT images of lungs from transgenic mice with Egfr exon 19 deletion mutation pretreatment (day 0), after 1 week and 4 weeks administration of vehicle or gefitinib (5mg/kg/day) or afatinib (5mg/kg/day). Arrows: tumors. B. Changes of tumor size. The average longest diameter of three tumors was measured before treatment and at 1 week and 4 weeks administration of each drug. Bars, SD, *, p<0.05, left upper and right upper for vehicle versus gefitinib, left lower and right lower for gefitinib versus afatinib.

Figure 3. Xenograft model
Growth curves of (A) RPC-9 and (B) H1975 xenograft tumors in mice receiving oral administration of 10 mg/kg/day afatinib or 10 mg/kg/day gefitinib 5 times per week or i.p. administration of 5 mg/kg bevacizumab twice a week. Difference in tumor volume was compared using Student’s t-test. Bars, SD, *, p<0.05, for vehicle alone versus combination of afatinib or gefitinib with bevacizumab.

Figure 4. A. Western blot analysis of the signal transduction pathway. Frozen tumors were obtained from RPC-9 xenograft models after a week treatment of each drug. GAPDH was used as a loading control. Phosphorylated or total forms of EGFR and AKT and cleaved caspase 3 were examined. B. Following a week of treatment with afatinib or bevacizumab alone or combination of bevacizumab with afatinib, tumor specimens were immunostained for the endothelial cell marker CD31. The numbers of CD31-positive blood vessels per field (x100) were counted. Ten fields per tumor specimen were counted and the bar graphs represented the average. Brown is CD31-positive blood vessel in the histology of the tumors. Bars, SE, *, p<0.01.