**SUPPLEMENTAL INFORMATION**

**Supplemental figure legends**

**Supplemental Figure 1.**  **Association of** **IKBKE level with overall survival and activation of IKBKE only by constitutively active EGFR mutants in NSCLC.** (**A**) Elevated levels of IKBKE are associated with poor overall survival. (**B**) *In vitro* IKBKE kinase assay was performed with myc-IKBKE immunoprecipitates prepared from HEK293 cells co-transfected with myc-IKBKE and indicated EGFRs.

**Supplemental Figure 2. IKBKE interacts with EGFR.** (**A**) H1299 cells were transfected with the indicated plasmids. Following treatment with and without EGF for 15 minutes, IKBKE was immunoprecipitated using Myc (for IKBKE) antibody. EGFR bound to IKBKE was detected with EGFR antibody. pEGFR and EGFR expressions were shown in panels 3 and 4. (**B** and **C**) Co-IP shows the interaction of endogenous IKBKE with EGFR in H1975 cells (B), which was not affected by EGFR inhibitor afatinib treatment in HCC827 cells (C). (**D**) H292 cells were treated with Afatinib (200nM) for 16 hrs and the IKBKE was immunoprecipitated with IKBKE antibody and then immunoblot with EGFR antibody. (**E**) Confocal microscopy of co-localization of IKBKE and EGFR in H1299 cells transfected GFP-IKBKE and EGFRL858R/T790M and immuno-stained with EGFR antibody. (**F**) Following *in vitro* incubation of recombinant EGFR and GST-IKBKE, immunoprecipitation was performed with IKBKE antibody and then detected with indicated antibodies. (**G**) *In vitro* GST-pulldown assay by incubating GST-EGFR or GST-vector with lysates from HEK293T cells expressing myc-IKBKE.

**Supplemental Figure 3. Identification of IKBKE tyrosine residue(s) phosphorylated by EGFR.** (**A**)Mass spectrometry analysis of EGFR phosphorylation of tyrosine residues in IKBKE. (**B**) Following *in vitro* incubation of recombinant EGFRL858R/T790M withindicated GST-fusion IKBKE peptides spanning each of the phosphorylation tyrosine residues, the GST-fused IKBKE peptides were immunoblotted with pTyr antibody. (**C**) *In vitro* EGFR kinase assay was carried out by incubation of recombinant EGFRL858R/T790M with GST-fusion N-terminal and C-terminal domains of IKBKE. The GST-IKBKE-NT and GST-IKBKE-CT were then blotted with pTyr antibody. (**D**) Indicated myc-IKBKE plasmids were co-expressed with EGFRL858R/T790M. After IP with myc antibody, myc-IKBKEs were immunoblotted with indicated antibodies. (**E** and **F**) Characterization of pIKBKE-Y153 antibody. Following *in vitro* EGFR kinase assay by incubation of recombinant EGFRL858R/T790M with GST-fusion peptides spanning IKBKE tyrosine 153 (as shown in panel B), the reaction was immnoblotted with indicated antibodies (E). Western blot analysis was performed with pY153-IKBKE antibody in H1975 cells that were transfected with IKBKE siRNA or control siRNA and the lysates from control siRNA were further treated with calf intestinal phosphatase (lane 1) prior to SDS-PAGE (F).

**Supplemental Figure 4. EGFR mutant NSCLCs express high levels of pIKBKE-Y153.** (**A**) Western blot analysis of 3 wild type and 3 mutant EGFR NSCLC cell lines with indicated antibodies.(**B**) Representative images of immunohistochemical staining of NSCLC TMAs using pY153-IKBKE antibody. EGFR mutated NSCLC specimen exhibited intensive staining (right) when compared to wild type EGFR tumor (left).

**Supplemental Figure 5. Effect of EGFR phosphorylation of IKBKE-Y153/179 on EGFR-induced cell phenotype. (A**-**C)** Indicated EGFR mutant cells, were transfected with IKBKE shRNA and control shRNA and then were assayed for cell viability (A) colony formation (B) and cell invasion (C). **(D** and **E**) H1299 cells were transfected with indicated plasmids and then assayed for migration (D) and invasion (E). \* p<0.05 and \*\* p<0.005

**Supplemental Figure 6. IKBKE inhibitor amlexanox induces G1 arrest and pERK.** (**A**)H1975 cells were treated with 50µM amlexanox for indicated times. Cell cycle analysis was performed using PI staining. (**B**) HCC827 cells were treated with amlexanox for 16 hrs and then immunoblotted with indicated antibodies.

**Supplemental Figure 7. Amlexanox synergizes with AZD6244 in EGFR-mutated HCC827 cells.**  (**A**) HCC827 cells were treated with indicated concentrations of amlexanox and AZD6244 for 48 hours. Cell viability was measured using MTT assay. (**B**) Combination indexes were calculated using Compusyn software. (**C**) Colony growth assay was performed by treating HCC827 cells for 9 days with indicated concentrations of amlexanox or/and AZD6244.

**Supplemental Figure 8. Effects of inhibitor of IKBKE and MEK on HCC827 xenograft growth.** (**A** and **B**) (**A** and **B**) Tumor growth (A) and tumor weight (B) of HCC827 xenograft mice. (**C**) Immunoblot analysis of HCC827 xenograft tissues with indicated antibodies. ( (**D**) Body weight of mice bearing H1975 xenografts at the end point of the studies.