

Supplementary Figure Legends

Supplementary Figure 1: Effect of Raf deficiency on K-Ras^{G12D}-mediated transformation of MEFs. (A) Increase in cell number of Ad-Cre infected MEFs of the indicated genotypes relative to Ad-Mock infected isogenic control cells. Cells were analyzed in 2% serum. The fold increase of proliferation of Ad-Cre infected cells compared to Ad-Mock infected isogenic control cells is shown. (B) Focus formation assay. B-Raf or C-Raf deficiency does not impair the ability of K-Ras^{G12D} MEFs to form foci (arrows). (C) Western blot analysis of MAPK activation in response to K-Ras^{G12D} expression. M, Ad-Mock infected cells; C, Ad-Cre infected cells. (D) Western blot for pERK. K, BBK, and CCK MEFs were serum starved overnight and re-stimulated with 10% FCS for the indicated time points.

Supplementary Figure 2: Individual proliferation curves of K, BBK, and CCK MEFs grown in 10% and 2% fetal calf serum. B-Raf or C-Raf deficiency does not prevent increased proliferation of MEFs expressing K-Ras^{G12D}.

Supplementary Figure 3: Cell cycle and signaling in B-Raf^{fl/fl} and C-Raf^{fl/fl} BMKs following gene ablation. (A) Cell cycle analysis of Ad-Mock and Ad-Cre infected C-Raf^{fl/fl} BMKs demonstrate a G2-M block following C-Raf deletion. (B) Western blot for expression of pERK and cyclin D1 is shown. (C) C-Raf deficiency does not result in apoptosis of BMK cells. Western blot for the apoptotic markers cleaved caspase 3 and cleaved PARP is shown. (D) Knock-down of Cyclin D1 results in diminished proliferation in K and BBK BMK cells. Percentage of cells with incorporated BrdU is shown. Cyclin D1 knock-down efficiency was >90% (not shown).

Supplementary Figure 4: MAPK activation and proliferation of lung tumors in C-Raf^{fl/fl}; K-Ras^{LSL} mice. (A) Similar MAPK activation assessed by pERK content in tumors at endpoint. (B) Similar proliferation of tumors at 12 weeks post Ad-Cre infection.