SUPPLEMENTAL INFORMATION

c-Raf, but not B-Raf, is essential for development of K-Ras oncogene driven non small cell lung carcinoma

Rafael B. Blasco[¶], Sarah Francoz[¶], David Santamaría, Marta Cañamero, Pierre Dubus, Jean Charron, Manuela Baccarini and Mariano Barbacid

INVENTORY

Figure S1 is related to Figure 1.
Figure S2 is related to Figure 2.
Figure S3 is related to Figure 3.
Figure S4 is related to Figure 4.
Figure S5 is related to Figure 5.
Figure S6 is related to Figure 7.
Figure S7 is related to Figure 6.
Figure S8 is related to Figure 8.

SUPPLEMENTAL INFORMATION

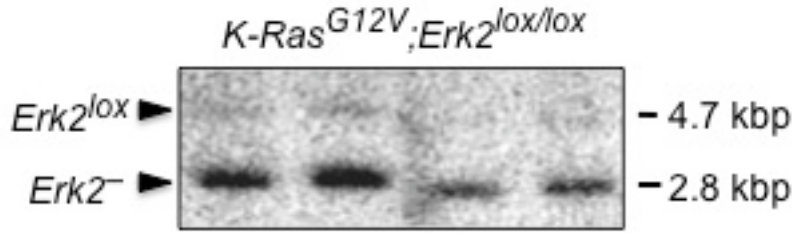

Figure S1. Related to Figure 1. Southern blot analysis of DNA isolated from individual tumors obtained from *K-Ras*$^{+/G12V}$*;Erk2*$^{lox/lox}$ mice infected with Ad-Cre particles at 8 weeks of age.

Tumor DNAs were digested with KpnI. The sizes of the diagnostic DNA fragments for *Erk2*^{lox} and *Erk2*$^{-}$ alleles are indicated.

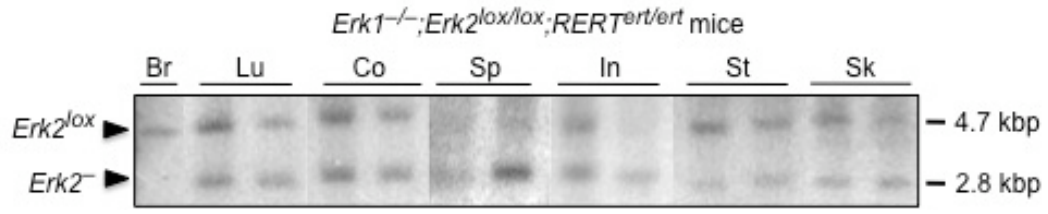

Figure S2. Related to Figure 2. Systemic ablation of *Erk* alleles is not compatible with life in adult mice.

Southern blot analysis of DNA isolated from tissues of *Erk1*$^{-/-}$;*Erk2*$^{lox/lox}$;*RERT*$^{ert/ert}$ mice fed *ad libitum* with a tamoxifen diet since P30. Mice were sacrificed at humane end-point. Migration of the unrecombined *Erk2*^{lox} allele and the ablated *Erk2*$^{-}$ allele is indicated by arrowheads. Tissues analyzed include brain (B), lung (Lu), colon (Co), spleen (Sp), small intestine (In), stomach (St) and skin (Sk).

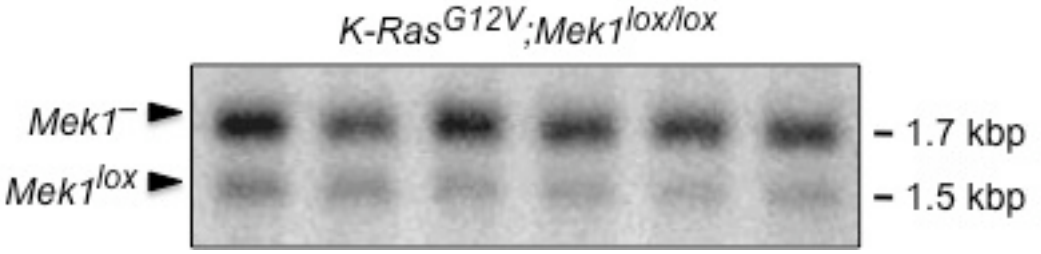

Figure S3. Related to Figure 3. Southern blot analysis of DNA isolated from individual tumors obtained from *K-Ras*$^{+/G12V}$;*Mek1*$^{lox/lox}$ mice infected with Ad-Cre particles at 8 weeks of age.

Tumor DNAs were digested with HindIII. The sizes of the diagnostic DNA fragments for *Mek1*^{lox} and *Mek1*$^{-}$ alleles are indicated.

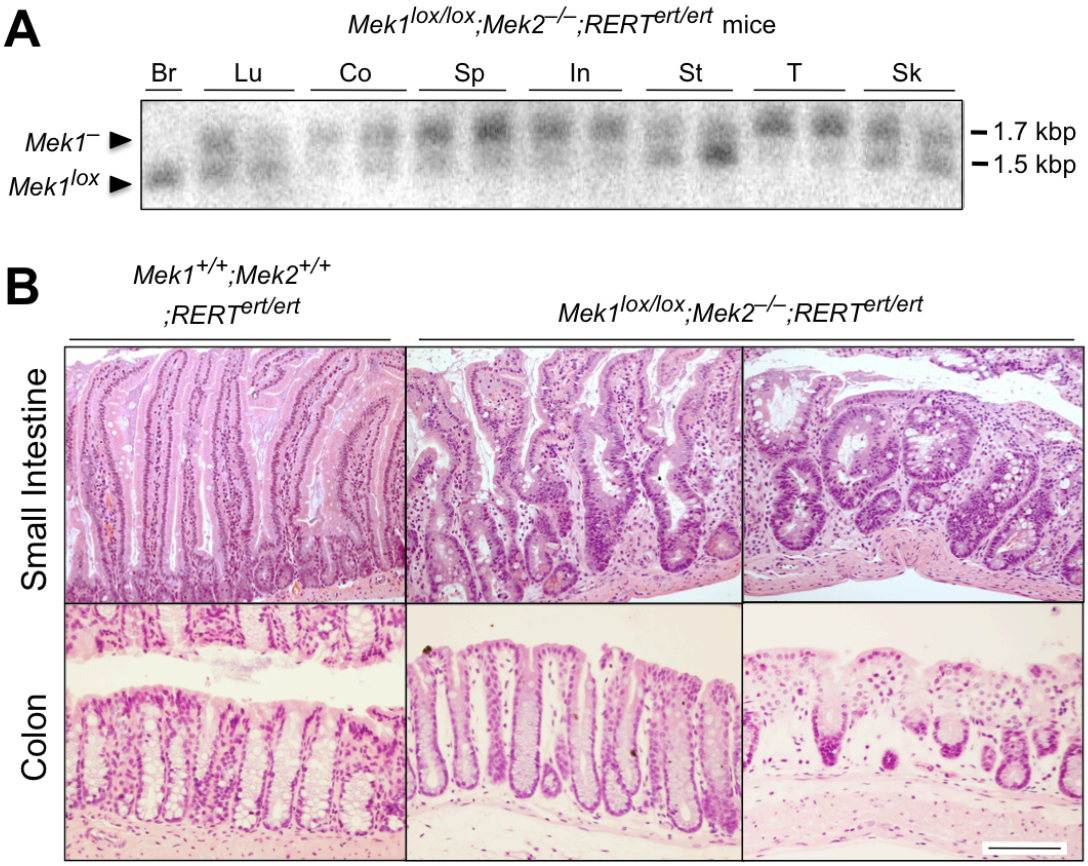

Figure S4. Related to Figure 4. Systemic ablation of *Mek* alleles is not compatible with life in adult mice.

(A) Southern blot analysis of DNA isolated from tissues of *Mek1*$^{lox/lox}$;*Mek2*$^{–/–}$;*RERT*$^{ert/ert}$ mice fed *ad libitum* with a tamoxifen diet since P30. Mice were sacrificed at humane end-point. Migration of the unrecombined *Mek1*^{lox} allele and the ablated *Mek1*$^{–}$ allele is indicated by arrowheads. Tissues analyzed include brain (B), lung (Lu), colon (Co), spleen (Sp), small intestine (In), stomach (St), tail (T) and skin (Sk).

(B) H&E staining of paraffin sections from small intestine and colon tissue obtained from *Mek1*$^{+/+}$;*Mek2*$^{+/+}$;*RERT*$^{ert/ert}$ and *Mek1*$^{lox/lox}$;*Mek2*$^{–/–}$;*RERT*$^{ert/ert}$ mice fed *ad libitum* with a tamoxifen-containing diet to activate the knocked in CreERT2 recombinase encoded by the *RERT*^{ert} alleles. Scale bar, 100μm.

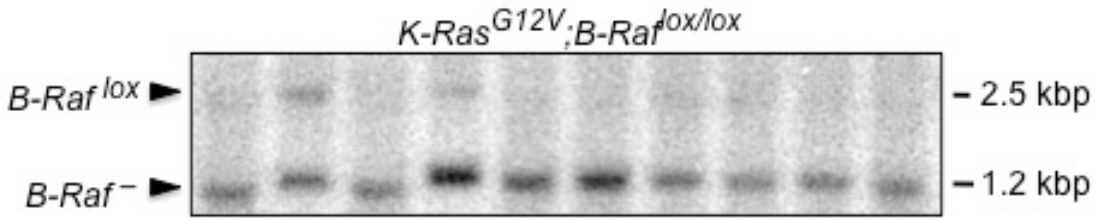

Figure S5. Related to Figure 5. Southern blot analysis of DNA isolated from individual tumors obtained from *K-Ras*$^{+/G12V}$;*B-Raf*$^{lox/lox}$ mice infected with Ad-Cre particles at 8 weeks of age.

Tumor DNAs were digested with HindIII. The sizes of the diagnostic DNA fragments for *B-Raf*^{lox} and *B-Raf*$^{-}$ alleles are indicated.

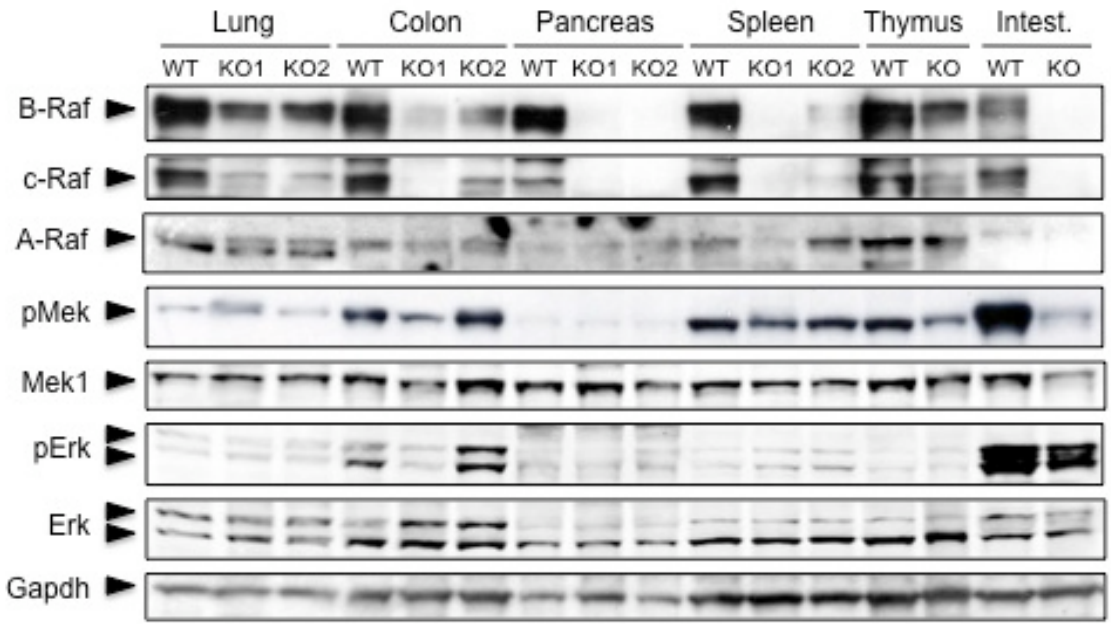

Figure S6. Related to Figure 6. Expression of the Mek and Erk proteins in B-Raf/c-Raf partially ablated adult tissues.

Western blot analysis of B-Raf, c-Raf, A-Raf, pMek, Mek1, pErk1/2 and Erk1/2 expression in lysates derived from tissues isolated from *B-Raf*$^{+/+}$;*c-Raf*$^{+/+}$;*RERT*$^{ert/ert}$ (WT lanes) and *B-Raf*$^{lox/lox}$;*c-Raf*$^{lox/lox}$;*RERT*$^{ert/ert}$ mice (KO lanes) fed *ad libitum* with a tamoxifen diet for three months (P30 to P120). Gapdh was used as loading control. Migration of the corresponding proteins is indicated by arrowheads.

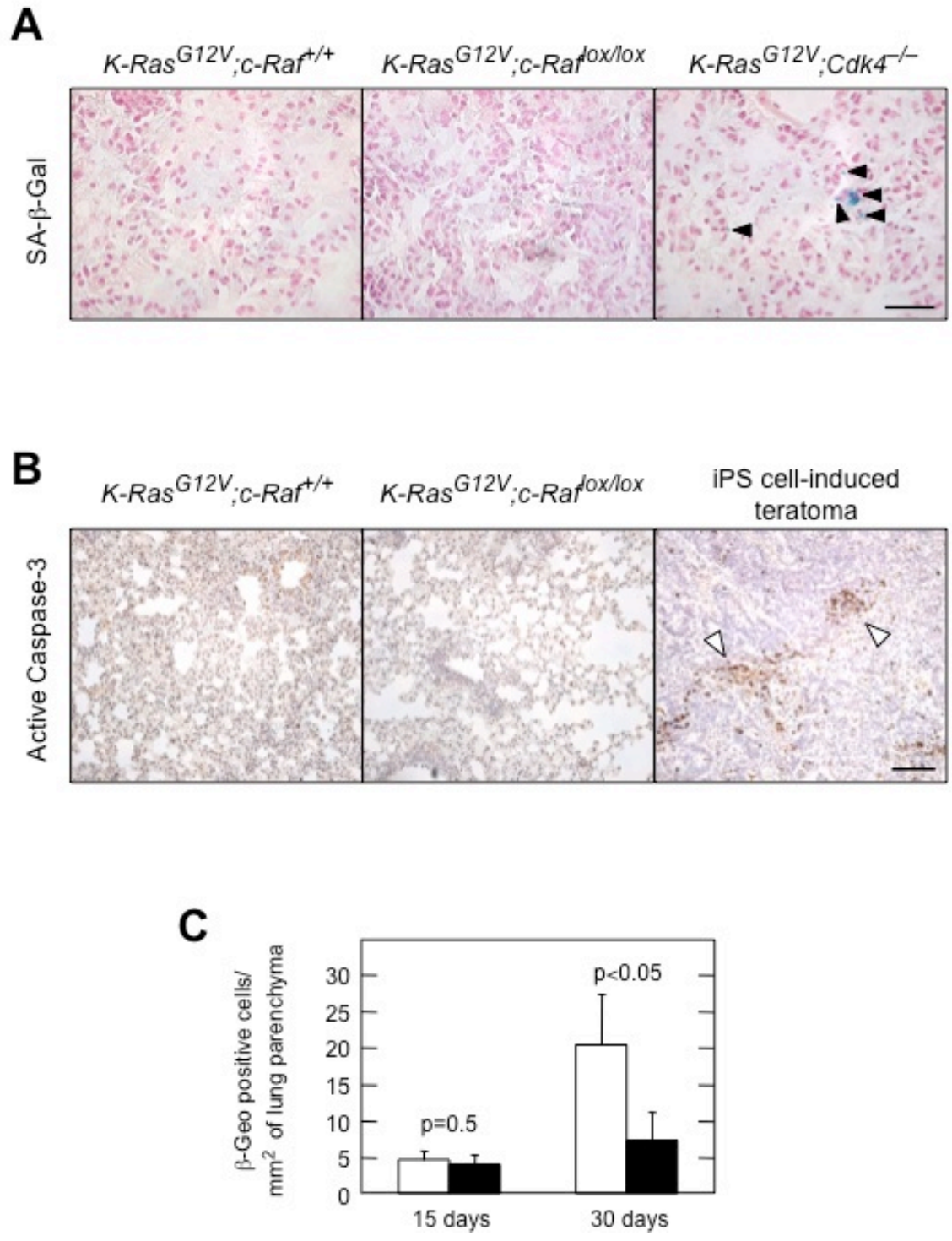

Figure S7. Related to Figure 7. Loss of c-Raf in K-Ras^{G12V}-expressing lung cells does not induce senescence or apoptosis.

(A) SA-β-Gal staining of lung sections obtained from (**left**) *K-Ras$^{+/G12V}$;c-Raf$^{+/+}$* and (**center**) *K-Ras$^{+/G12V}$;c-Raf$^{lox/lox}$* mice four weeks after Ad-Cre treatment. (**Right**) SA-β-Gal staining of a lung section obtained from *K-Ras$^{+/G12V}$;RERT$^{ert/ert}$;Cdk4$^{-/-}$* mice four

weeks after exposure to 4OHT was used as positive control.

Scale bar 50 μm.

(B) Active Caspase 3 staining of lung sections obtained from (**left**) *K-Ras*$^{+/G12V}$*;c-Raf*$^{+/+}$ and (**center**) *K-Ras*$^{+/G12V}$*;c-Raf*$^{lox/lox}$ mice four weeks after Ad-Cre treatment. (**Right**) Active Caspase 3 staining of a section obtained from a teratoma induced by injecting iPS mouse cells in irradiated nude mice (Li et al., 2009) was used as positive control.

Scale bar 100 μm.

(C) Quantification of K-Ras^{G12V} expressing cells by X-Gal staining (based on the expression of the surrogate β-Geo marker) on lung cryosections, of (open bars) *K-Ras*$^{+/G12V}$*;RERT*$^{ert/ert}$*;c-Raf*$^{+/+}$ (n=3) and (solid bars) *K-Ras*$^{+/G12V}$*;RERT*$^{ert/ert}$*;c-Raf*$^{lox/lox}$ (n=3) mice. Animals were injected once intraperitoneally with 4OHT (2 mg) at 8-10 weeks of age. Samples were collected 15 days and 30 days after 4OHT treatment.

Error bars indicate +/- SD of the mean.

p values were calculated according to Student's t test

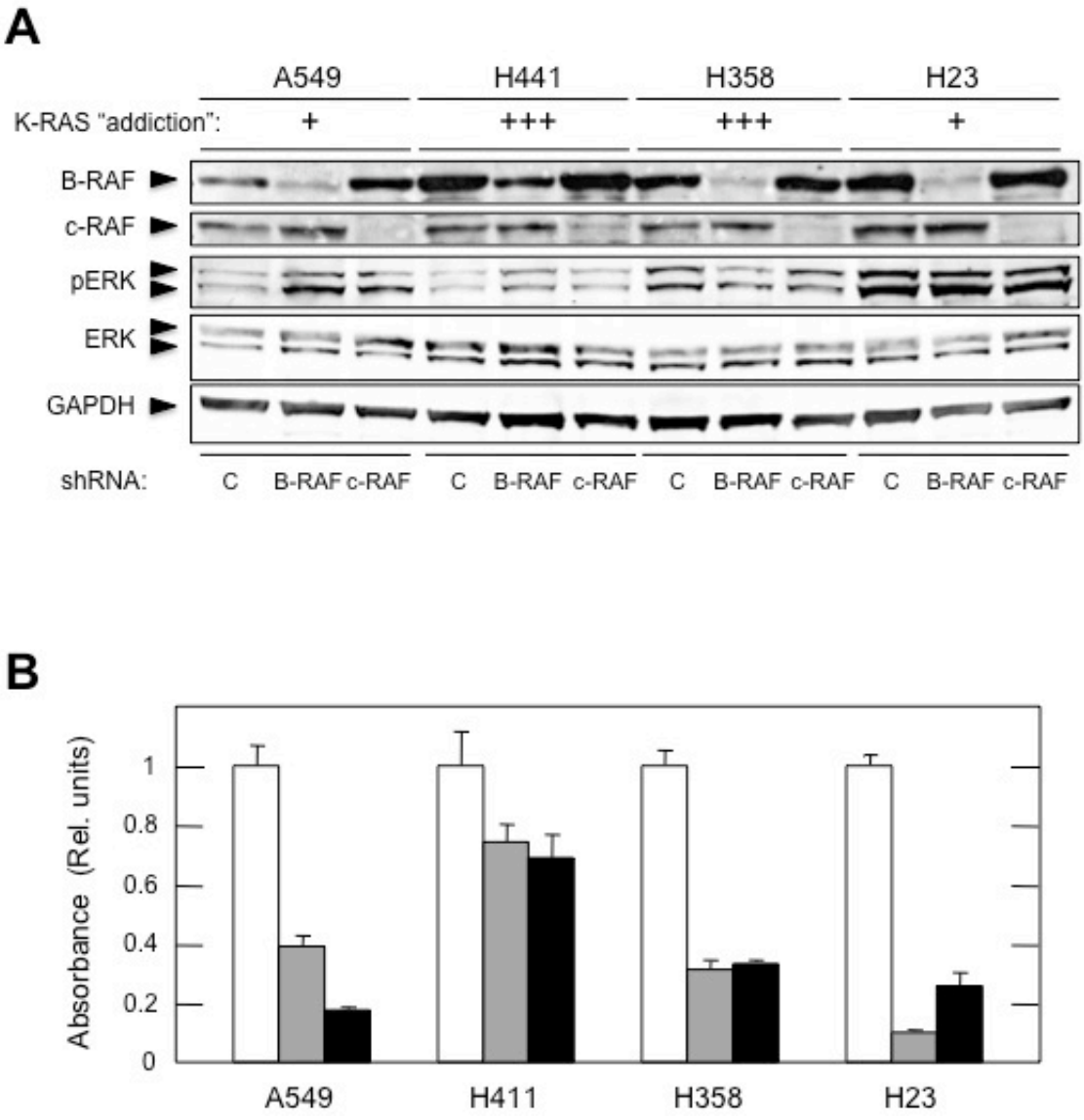

Figure S8. Related to Figure 8. Effect of B-RAF and c-RAF knock down on the proliferation of human NSCLC cell lines with different "addiction" levels to K-RAS oncogenes.

(A) Western blot analysis of B-RAF, c-RAF, pERK and ERK expression in human NSCLC cell lines carrying K-RAS oncogenes and infected with lentiviral vectors carrying control shRNAs (C) or shRNAs specific for *B-RAF* and *c-RAF* sequences. K-RAS "addiction" according to Singh et al., (2009) is indicated for each cell line.

GAPDH was used as loading control. Migration of the corresponding proteins is indicated by arrowheads.

(B) Relative proliferation rates of the indicated human NSCLC cell lines infected with lentiviral vectors carrying control shRNA (open bars) or shRNAs specific for B-*RAF* (grey bars) or c-*RAF* (solid bars) sequences and allowed to proliferate for 6 days. Data shown represent mean +/- SD, n=4.