Supporting Information

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Fig. S1. Mass spectrometry analysis of K-RAS. HA-tagged G12V K-RAS4B was immunoprecipitated from 293T cells and subjected to LC/MS/MS analysis. Peptides that were identified are highlighted in blue below the primary sequence of K-RAS4B protein.



Fig. 52. Expression of ectopic K-RAS4B in NIH3t3 cells. HA-tagged forms of K-RAS4B were stably expressed in NIH3t3 cells from the pBabe retrovirus. Expression of the ectopic K-RAS4B was detected via Western blotting with anti-HA antibody.



Fig. S3. Effect of K104Q mutation on K-RAS4B localization and activation. (A) Effect of K104 mutations on K-RAS4B localization. K-RAS4B localization in live cells. Various forms of K-RAS4B were fused to mCherry and then transiently transfected into HeLa and COS-1 cells. Protein localization was assessed using confocal microscopy. (B) Effect of the K104Q mutation on K-RAS4B GTP binding. At steady state, in serum-free medium, NIH3t3 cells expressing K104Q K-RAS4B have lower levels of GTP-bound K-RAS4B than cells expressing wild-type K-RAS. Upon addition of serum, GTP binding of K104Q K-RAS4B is stimulated to a lesser extent than wild-type. (C) Effect of the K104Q mutation on activation of MAPK signaling by G12V K-RAS4B. Serum stimulation leads to attenuated ERK activation in NIH3t3 cells expressing G12V/K104Q K-RAS4B. ERK activation was measured as the ratio of phospho-ERK (Thr202/Tyr204) to total-ERK. (D) Effect of the K104Q mutation on activation leads to attenuated AKT activation in NIH3t3 cells expressing G12V/K104Q K-RAS4B. Serum stimulation leads to attenuated AKT activation in NIH3t3 cells expressing G12V/K104Q K-RAS4B. AKT activation was measured as the ratio of phospho-AKT (Thr308) to total AKT.



Movie S1. Structural simulation of K104 RAS. Molecular dynamics simulation of RAS that is not acetylated on lysine 104. The α2 helix of the Switch II domain (highlighted in red) is relatively stable.

Movie S1



Movie S2. Structural simulation of K104Ac RAS. Molecular dynamics simulation of RAS that is acetylated on lysine 104. The α 2 helix of the Switch II domain (highlighted in pink) is highly unstable.

Movie S2