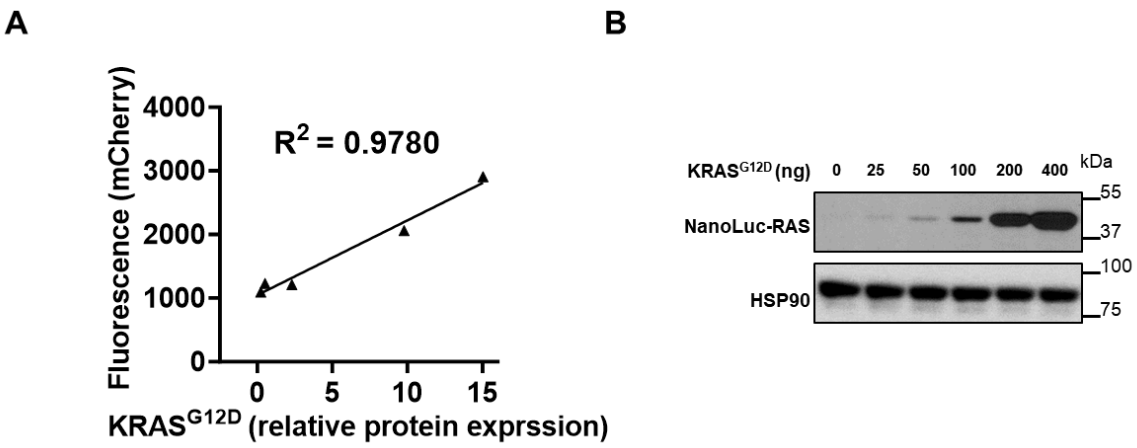
Supporting information

The experimental variables that determine the outcome of RAS oligomerization

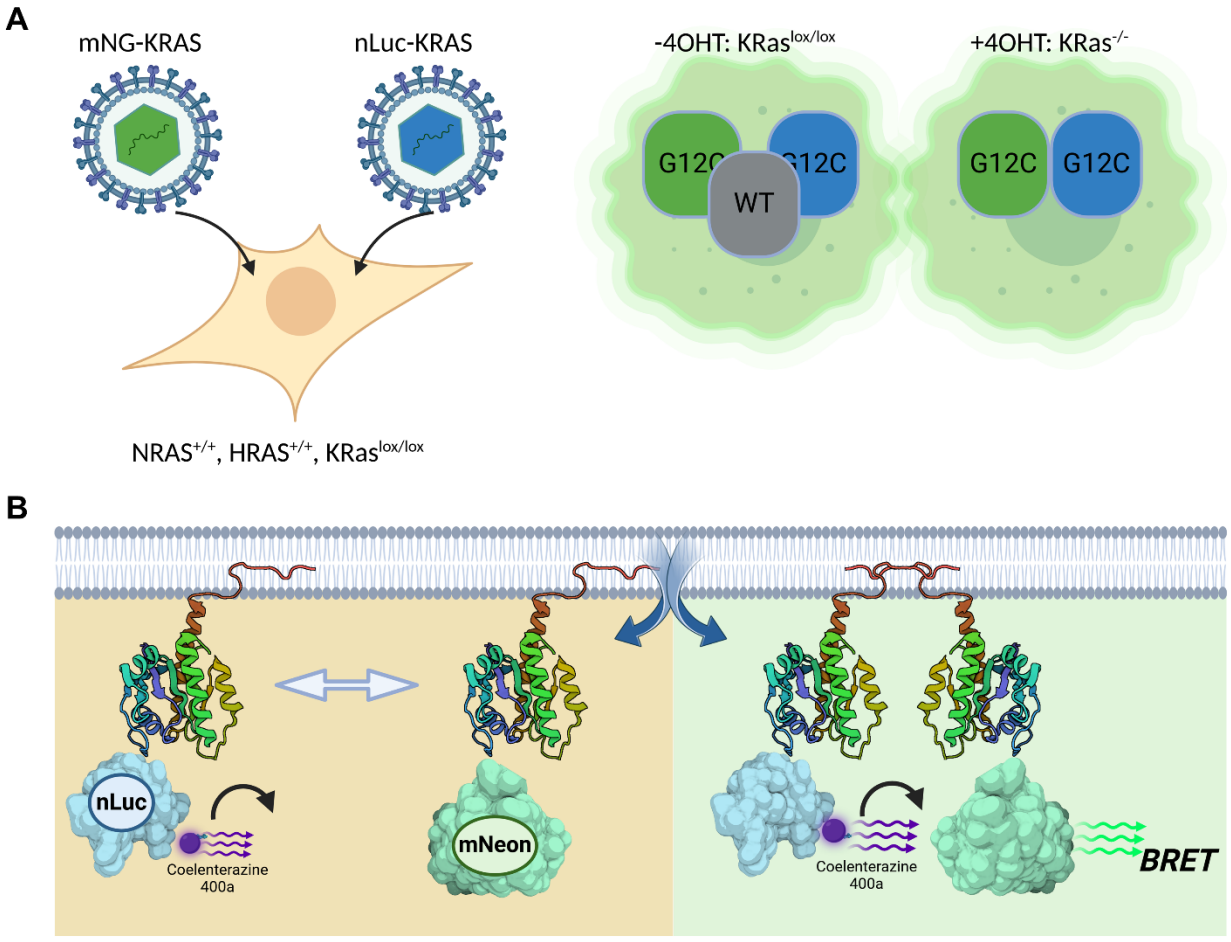
Zhiwei Zhou[1], Tra Ly Nguyen[2], Xingxiao Li[1], Christel Poujol[3], Ewa Berlinska[4], Sandra Vietti Michelina[4], Jonas N. Kapp[5], Andreas Plückthun[5], Monte M. Winslow[6], Chiara Ambrogio[4], Yibing Shan[7], David Santamaría[8,9], Kenneth D. Westover[1,*]

[1]Departments of Biochemistry and Radiation Oncology, The University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
[2]BoRdeaux Institute of onCology (BRIC), INSERM U1312, Univ. Bordeaux, 33000, Bordeaux, France
[3]University of Bordeaux, CNRS, INSERM, Bordeaux Imaging Center, BIC, UAR 3420, US 4, Bordeaux, France
[4]Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Torino, Torino, Italy
[5]Department of Biochemistry, University of Zurich, Zurich, Switzerland
[6]Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA 94305
[7]Antidote Health Foundation for Cure of Cancer, Cambridge, MA, USA 02139
[8]University of Bordeaux, INSERM U1218, ACTION Laboratory, IECB, Pessac, France
[9]Centro de Investigación del Cáncer, CSIC-Universidad de Salamanca, Salamanca, Spain

*Address correspondence to: K.W. (Kenneth.Westover@UTSouthwestern.edu)

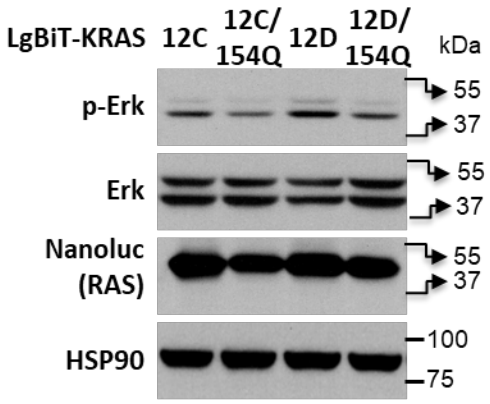


Supplementary figure 1 The correlation between RAS protein expression level and fluorescent signal. (A) The linear relationship between RAS protein expression level and fluorescent signal. HEK293T cells were transfected with pcDNA-CMV-mCherry-H2B-P2A-NanoLuc-tagged KRASG12D plasmid at 25, 50, 100, 200, and 400 ng for 48 h. Signals were measured using a plate reader. (B) RAS expression level determined via Western blot.

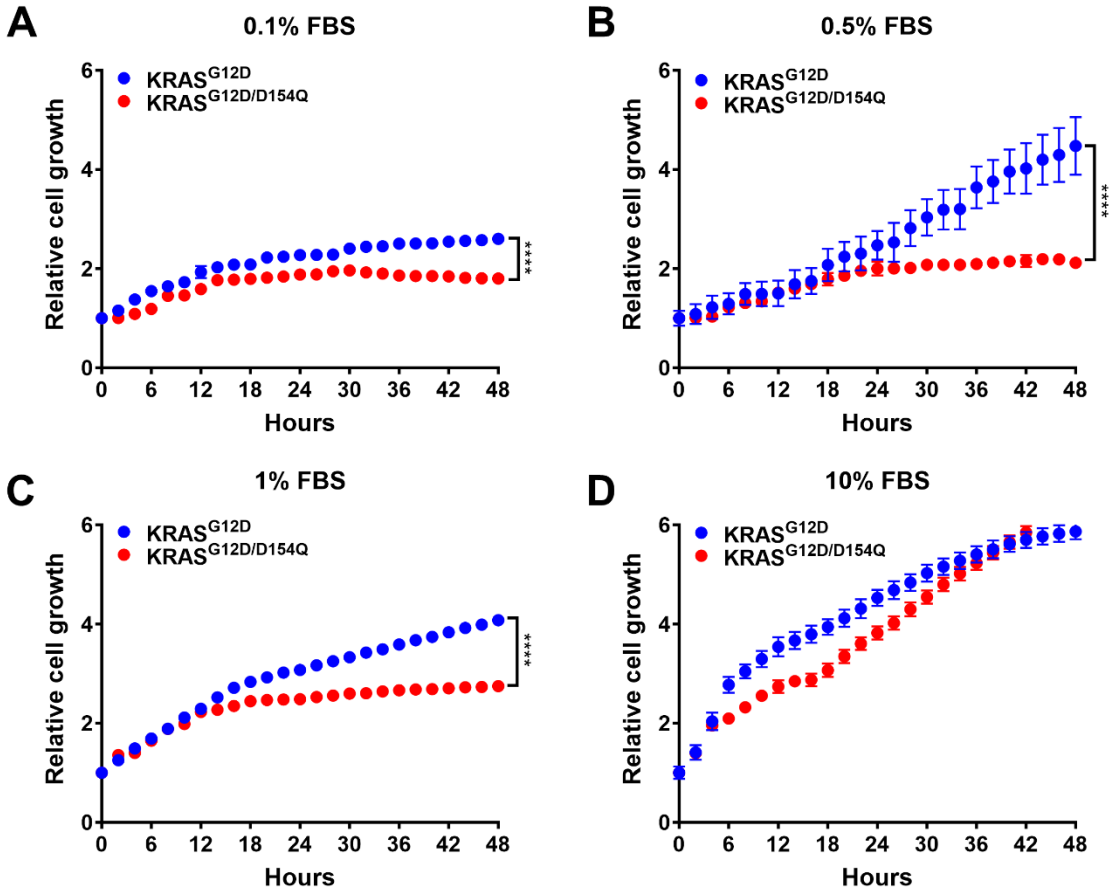


Supplementary figure 2. Schematic of BRET system generated in *Ras*-less MEFs.

(A) *Ras*-less MEFs were transduced with mNeongreen-fused KRAS^{G12C} (mNG-KRAS^{G12C}) and nanoLuciferase-fused KRAS^{G12C} (nLuc-KRAS^{G12C}). (B) BRET signal was generated when two KRAS^{G12C} protomers interact.

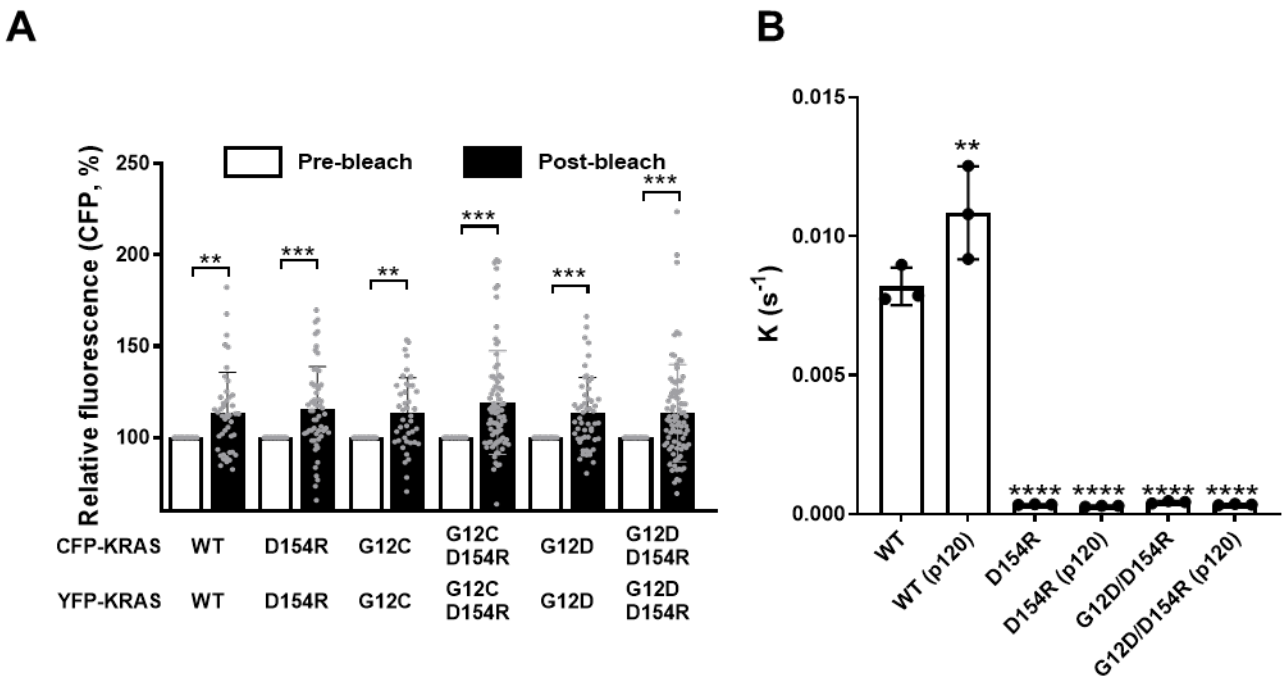


Supplementary figure 3. **Western blot confirms even expression of LgBiT-tagged RAS**. HEK293T cells were transiently transfected with LgBiT-tagged KRAS G12C, G12C/D154Q, G12D, or G12D/D154Q for 48 hrs in the presence of 10% FBS. The ERK signal was measured by western blot. The blots are representative of one of four similar experiments.



Supplementary figure 4. FBS concentration counteracts the inhibitory effect of D154Q on G12D KRAS in Rasless MEFs.

Growth rates of *KRas*^{lox} MEFs expressing exogenous HA-tagged KRAS^{G12D} (blue circles), or KRAS$^{G12D/D154Q}$ (red circles), in the absence (+4-OHT) of endogenous wild-type KRas alleles in different concentrations of FBS medium as assessed by IncuCyte. (A) Cells were cultured in 0.1% FBS medium; (B) Cells were cultured in 0.5% FBS medium; (C) Cells were cultured in 1% FBS medium; (D) Cells were cultured in 10% FBS medium. ($p < 0.001$; unpaired Student's t test). Results are representative of one of three similar experiments.



Supplementary figure 5. **KRAS^{D154R} does not produce RAS-RAS association defect.** (A) CFP emission after photobleaching indicates multimerization between CFP and YFP-fused KRAS. All KRAS mutants retain the ability to dimerize, including the charge altering mutant D154R in the background of G12C or G12D. HEK293T cells were co-transfected with CFP-KRAS and YFP-KRAS constructs and subject to confocal microscopy for FRET assay. WT, wild type. Data are presented as mean ± standard deviation (SD). ** $p<0.01$ and *** $p<0.001$ via two-way ANOVA. (B) GTP hydrolysis rate evaluations for KRAS variants. KRAS D154R and G12D/D154R have a slow GTP hydrolysis rate and are not responsive to p120 stimulation. Data are presented as mean ± standard deviation (SD). ** $p<0.01$ and **** $p<0.0001$ via one-way ANOVA.

Table S1. Key resources table.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal, Anti-KRAS (F234)	Santa Cruz Biotech	Cat#sc-30
Rabbit monoclonal, Anti-RAS (27H5)	Cell Signaling	Cat#3339
Rabbit polyclonal, Anti-HSP90 (H114)	Santa Cruz Biotech	Cat#sc-7947
Mouse polyclonal, Anti-HSP90	Santa Cruz Biotech	Cat#sc-13119
Rabbit monoclonal, Anti-phosphorylated ERK	Cell Signaling	Cat#4370
Rabbit monoclonal, Anti-ERK	Cell Signaling	Cat#4695
Rabbit monoclonal, Anti-BRAF	Cell Signaling	Cat#14814
Mouse monoclonal, Anti-beta actin	Santa Cruz	Cat#SC-47778
Mouse monoclonal, Anti-Nanoluc	Bio-Techne	Cat#MAB10026
Anti-rabbit IgG, HRP-linked secondary antibody	Cell Signaling	Cat#7074S
Anti-mouse IgG, HRP-linked secondary antibody	Cell Signaling	Cat#7076S
Chemicals, Peptides, and Recombinant Proteins		
4OHT ((Z)-4-Hydroxytamoxifen)	Sigma Aldrich	Cat#H7904
DOX (Doxycycline)	Thermo Fisher	Cat#446061000
Dabrafenib	AbMole	Cat#M1855
Vemurafenib	Selleckchem	Cat#S1267
Critical Commercial Assays		
CellTiter 96® AQueous Non-Radioactive	Promega	Cat#G1112
One Shot™ Stbl3™ Chemically Competent E. coli	Invitrogen	Cat#C737303

Gibson Assembly Master Mix	NEB	Cat#E2611S/L
EnzChek Phosphate Assay Kit	Life Technologies	Cat#E6646
*Pfu*Ultra II Hotstart PCR Master Mix	Agilent	Cat#600850-51
Experimental Models: Cell Lines		
Ras-less Mouse Embryonic Fibroblasts (MEFs)	Drosten et al., 2010	N/A
KRas^{lox} *KRAS*^{MUT} MEFs	This paper	N/A
HEK293T cell line; see main text and detailed methods for the specific lines	American Type Culture Collection (ATCC)	N/A
Recombinant DNA		
pBABE human KRAS G12C HA-tagged	This paper	N/A
pBABE human KRAS G12D HA-tagged	This paper	N/A
pBABE human KRAS G12C/D154Q HA-tagged	This paper	N/A
pBABE human KRAS G12D/D154Q HA-tagged	This paper	N/A
pCMV-VSV-G	Addgene	Cat#8454
pcDNA3-CFP-KRAS WT	This paper	N/A
pcDNA3-YFP-KRAS WT	This paper	N/A
pcDNA3-CFP-KRAS D154R	This paper	N/A
pcDNA3-YFP-KRAS D154R	This paper	N/A
pcDNA3-CFP-KRAS G12C	This paper	N/A
pcDNA3-YFP-KRAS G12C	This paper	N/A
pcDNA3-CFP-KRAS G12C/D154R	This paper	N/A

pcDNA3-YFP-KRAS G12C/D154R	This paper	N/A
pcDNA3-CFP-KRAS G12D	This paper	N/A
pcDNA3-YFP-KRAS G12D	This paper	N/A
pcDNA3-CFP-BRAF WT	This paper	N/A
pcDNA3-CFP-BRAF D287H	This paper	N/A
pcDNA3-CFP-BRAF V459L	This paper	N/A
pcDNA3-CFP-BRAF G466A	This paper	N/A
pcDNA3-CFP-BRAF G446V	This paper	N/A
pcDNA3-CFP-BRAF F595L	This paper	N/A
pcDNA3-CFP-BRAF V660E	This paper	N/A
pcDNA3-GFP-H2B-P2A-SmBiT KRAS WT	This paper	N/A
pcDNA3-mCherry-H2B-P2A-LgBiT KRAS WT	This paper	N/A
pcDNA3-GFP-H2B-P2A-SmBiT KRAS D154Q	This paper	N/A
pcDNA3-mCherry-H2B-P2A-LgBiT KRAS D154Q	This paper	N/A
pcDNA3-GFP-H2B-P2A-SmBiT KRAS R161E	This paper	N/A
pcDNA3-mCherry-H2B-P2A-LgBiT KRAS R161E	This paper	N/A
pcDNA3-mCherry-H2B-P2A-LgBiT KRAS G12C	This paper	N/A
pcDNA3-mCherry-H2B-P2A-LgBiT KRAS G12D	This paper	N/A
pcDNA3-mCherry-H2B-P2A-LgBiT KRAS G12C/D154Q	This paper	N/A

pcDNA3-mCherry-H2B-P2A-LgBiT KRAS G12D/D154Q		This paper	N/A
pcDNA3-mCherry-H2B-P2A-NanoLuc KRAS G12D		This paper	N/A
psPAX2		Addgene	Cat#12260
pMD2.G		Addgene	Cat#12259
Ef1a_Large T-antigen_Ires_Puro		Addgene	Cat#18922
pLenti PGK Hygro DEST (w530-1)		Addgene	Cat#19066
pLenti EF1a mNG-KRAS G12C		This paper	N/A
pLenti PGK nLuc-KRAS G12C		This paper	N/A
Softwares and Algorithms			
GraphPad Prism		GraphPad Software, Inc.	http://www.graphpad.com/scientific-software/prism/
ImageJ ZEN		IMAGEJ ZEISS	https://imagej.net/Fiji/