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13. SUPPLEMENTARY NOTES

14. ABSTRACT
One of the cancer-causing effects of cigarette smoke is a specific genetic mutation in the KRAS gene, which results in changes in the KRAS protein at codon 12 from glycine to cysteine (G12C). KRAS G12C mutations are a major driver of cigarette smoke-associated lung cancers, occurring in ~23,000 new cases of lung cancer per year. The Westover lab and collaborators previously developed small molecule inhibitors such as SML-8-73-1 that, in a test tube, irreversibly attach to cysteine 12 and inactivate KRAS G12C protein; however, these molecules have poor pharmacological properties. The overall goal of the current project is to improve on these results by developing KRAS G12C inhibitors with pharmacological properties that would allow advancement into preclinical animal models and clinical studies.

15. SUBJECT TERMS
KRAS, small molecule inhibitors, lung cancer, smoking

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1. INTRODUCTION:

This research seeks to discover tool compounds that target a leading genetic driver of lung cancer, KRAS G12C. Such compounds will be considered for advancement to preclinical testing as possible therapeutic agents.

2. KEYWORDS:

KRAS, small molecule inhibitor, lung cancer, smoking

3. ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1: Structural evaluation of KRAS G12C in complex with covalent fragments
Major Task 1: Solve x-ray crystal structures
Planned: Months 1-18
Completion (%): 85%
Projected completion date: Month 30

Subtask 1: Express and purify KRAS G12C in large scale for x-ray crystallography
Subtask 2: Label purified KRAS G12C with covalent fragments and verify labeling by mass spectrometry
Subtask 3: Screen for crystallization conditions for labeled KRAS G12C and once found produce crystals and freeze for diffraction.
Subtask 4: X-ray diffraction and structure solution by molecular replacement
Subtask 5: Determine the effects of fragments on RAS dimerization.

Specific Aim 2: Use 3D structures to design hybrid compounds containing elements of guanosine and fragments
Major Task 2: Design hybrid guanosine-covalent fragment compounds
Subtask 1: Molecular docking and computer-aided modeling
Subtask 2: Synthesis of SML analogues
Planned time: Months 6-24
Completion (%): 100%
Projected completion date: Month 30

Specific Aim 3: Evaluate evolved compounds
Major Task 3: Progress promising compounds through a flowchart of assays
Planned time: Months 9-24
Completion (%): 75%
Projected completion date: Month 30
Subtask 1: Test for covalent labeling of purified KRAS G12C by MS
Subtask 2: Prioritize compounds for further development using biochemical assays (RAS:RBD, Kinetic GDP displacement, Kinact/KI)
Subtask 3: Test high priority compounds for cell permeability
Subtask 4. Test high priority compounds for impacts on RAS dimerization and antiproliferative activity
Subtask 5: Test high priority compounds selectivity using MS selectivity profiling

Milestone: Development of cell permeable inhibitors of KRAS G12C; publication of 1-2 peer reviewed papers

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Aim 1. Subtask 1: Express and purify KRAS G12C in large scale for x-ray crystallography
During the current period we performed several purification runs of KRAS G12C for biochemical study and x-ray crystallography and obtained ~100 mg of pure protein.

Aim 1. Subtask 2: Label purified KRAS G12C with covalent fragments and verify labeling by mass spectrometry.
This was completed in the prior period and no work was done in GY2.

Aim 1. Subtask 3: Screen for crystallization conditions for labeled KRAS G12C and once found produce crystals and freeze for diffraction.
Several of the fragments identified in subtask 2 were chosen for crystal screens which were performed, but no suitable crystallization conditions were obtained. We therefore turned our efforts toward development of other assay methods to detect stabilization of KRAS with ligands, such as DSF.

Aim 1. Subtask 4: X-ray diffraction and structure solution by molecular replacement
Dependent on Subtask 3 which is still in progress, so no work done.
Aim 1. Subtask 5: Determine the effects of fragments on RAS dimerization.
During GY2 we recognized that RAS dimerization is critical to RAS function in many contexts. In particular, we recognized that allosteric KRAS G12C inhibitors have a high likelihood of interacting with RAS dimerization. We therefore dedicated effort to characterization of RAS dimerization with the intent of studying the effect of KRAS G12C inhibitors on RAS dimerization.

It has been known for several years that wild-type KRAS acts as a tumor suppressor in *KRAS*-mutant cancer cells (Singh et al., 2005). These tumor growth-restraining functions in LUAD are removed when the wild-type *KRAS* allele is lost during tumor progression (To et al., 2008; Zhang et al., 2001). Alternatively, wild-type *KRAS* inhibitory effects can also be overcome by copy number gains of the oncogenic form resulting in allelic imbalance (Westcott et al., 2015). Furthermore, loss of wild-type *KRAS* has recently been shown to enhance tumor fitness in *KRAS*-mutant acute myeloid leukemia (AML) and colorectal cancer (CRC) cell lines while concomitantly resulting in increased sensitivity to MEK inhibition (Burgess et al., 2017). How wild-type KRAS exerts its growth inhibitory function is still largely unknown. Proposed molecular mechanisms include competition for proper membrane localization, shared regulators, downstream mediators or activation of parallel signaling pathways (Young et al., 2013).

Numerous observations suggest that RAS functions as a dimer (Güldenhaupt et al., 2012; Lin et al., 2014; Muratcioglu et al., 2015; Spencer-Smith et al., 2017; Zhou and Hancock, 2015). Additionally, activation of RAF, a known RAS effector, requires RAF dimerization, which may be facilitated by RAS dimerization (Lavoie and Therrien, 2015). Indeed, artificially forced dimerization of RAS has been shown to enhance activation of the MAPK pathway (Nan et al., 2015). However, definitive proof that dimerization of RAS is essential for its biological functions is lacking.

Loss of wild-type *KRas* accelerates cell proliferation and increases mutant KRAS-GTP levels *in vitro* and *in vivo*

To study the impact of wild-type KRAS on oncogenic KRAS, we used an inducible system generated from *Ras*-less mouse embryonic fibroblasts (MEFs) (Drosten et al., 2010). Endogenous *HRas* and *NRas* alleles are constitutively knocked out, whereas the $KRas^{lox/lox}$ alleles are under the control of a resident 4-hydroxytamoxifen (4OHT)-dependent CRE^{ERT2} recombinase. We transduced $HRas^{-/-}$; $NRas^{-/-}$; $KRas^{lox/lox}$ MEFs with different human HA-tagged *KRAS* mutants including the most common mutations detected in human LUAD (G12C, G12D and G12V). Treatment with 4OHT abolished expression of endogenous wild-type KRas, thus allowing characterization of a loss of heterozygosity (LOH) phenotype in isogenic cell lines expressing different KRAS mutants (herein referred to $KRas^{lox}$ $KRAS^{MUT}$) (Figure 1A). Remarkably, elimination of wild-type *KRas* significantly increased the growth rate across all $KRas^{lox}$ $KRAS^{MUT}$ cells (Figure 1B). Moreover, after loss of wild-type *KRas*, $KRas^{lox}$ $KRAS^{MUT}$ cells acquired a strong spindle-shaped transformed phenotype. Interestingly, protein levels of mutant KRAS were upregulated upon elimination of resident wild-type *KRas* alleles (Figure 1A). To rule out the possibility that growth acceleration after 4OHT was not from concomitant increases in mutant KRAS expression due to positive selection during 4OHT treatment, we evaluated the growth rate of $KRas^{lox}$ $KRAS^{MUT}$ cells upon acute infection with adenoviral particles carrying the CRE recombinase (AdCRE). Under these conditions, which led to a deletion of wild-type *KRas* alleles within 48 hours, expression levels of wild-type and mutant KRAS were comparable; nevertheless proliferation rates remained markedly accelerated in the absence of wild-type *KRas* alleles (data not shown). We next assessed the impact of wild-type *KRas* on KRAS-GTP levels. EGF stimulation resulted in a small increase in GTP-bound $KRAS^{G12C}$ but no increase in $KRAS^{G12D}$ or $KRAS^{G12V}$ GTP-bound mutants (Figure 1C). Consistent with the cell phenotype, ablation of the *KRas* wild-type allele resulted in a marked increase in the KRAS-GTP-bound fraction of all three oncogenic mutants that was further enhanced upon EGF stimulation in $KRAS^{G12C}$ but not the others.

To investigate the contribution of wild-type *KRas in vivo*, we used a mouse model that allows selective deletion of the wild-type *KRas* allele in lung epithelial cells expressing the mutant KRas oncogene (Puyol et al., 2010). In this model, the wild-type *KRas* allele is replaced by a conditional $KRas^{lox}$ allele in $KRas^{+/LSLG12V}$ mice (Guerra et al., 2003). Intratracheal infection of $KRas^{lox/LSLG12V}$ mice with AdCRE virus led to expression of the $KRas^{G12V}$ oncogene with a concomitant deletion of the wild-type *KRas* allele, reproducing a *bona fide* LOH condition. The lifespan of AdCRE infected $KRas^{lox/LSLG12V}$ mice was significantly shorter than those of AdCRE infected $KRas^{+/LSLG12V}$ mice, which retained expression of the wild-type *KRas* allele. While $KRas^{lox/LSLG12V}$ mice reached a median survival of 32 weeks after induction of $KRas^{G12V}$ expression, those mice also expressing the wild-type *KRas* allele had a median survival of 40 weeks (Figure 1D). Histological analysis of $KRas^{lox/LSLG12V}$ mice 6 months following AdCRE infection revealed

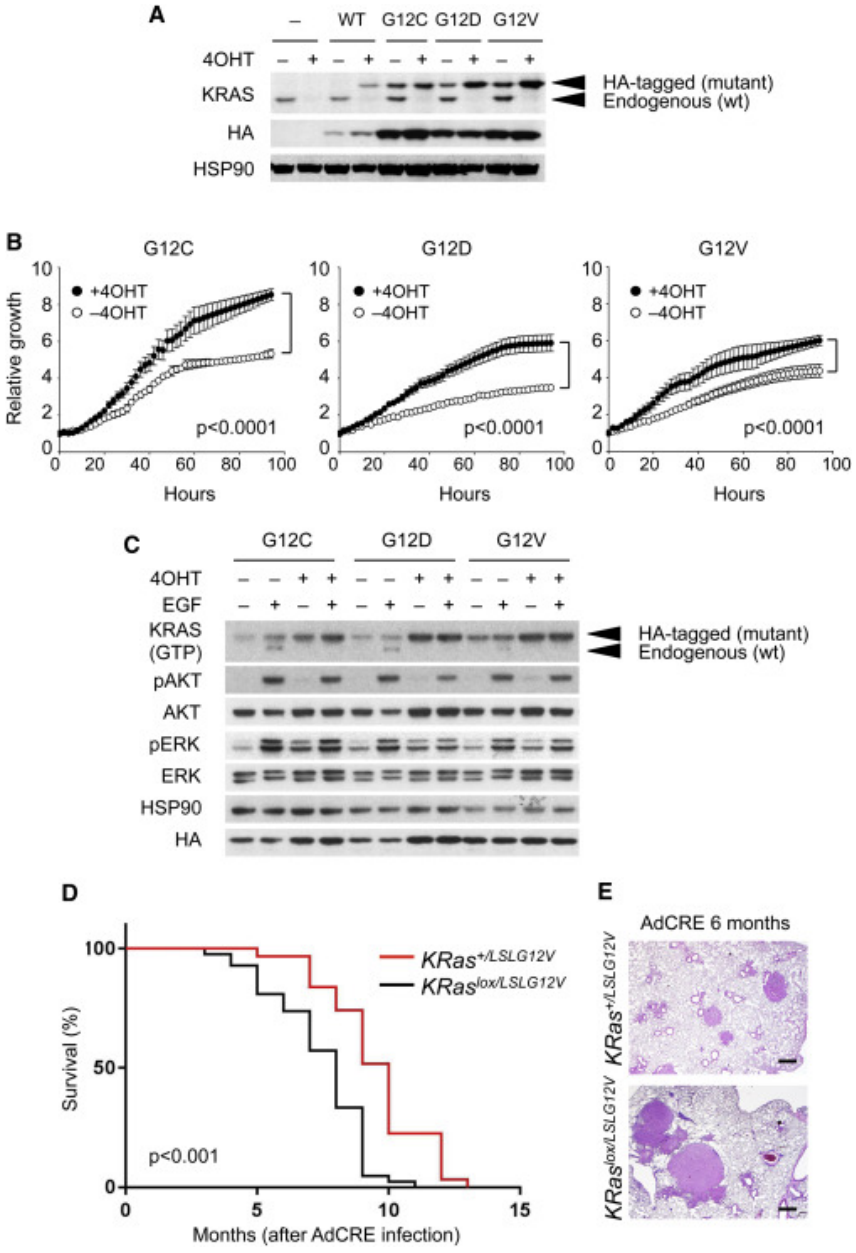

Figure 1. Loss of Wild-Type KRas Accelerates Cell Proliferation and Increases Mutant KRASGTP Levels

(A) $HRas^{-/-}$; $NRas^{-/-}$; $KRas^{lox/lox}$ cells stably transduced with human HA-tagged $KRAS^{WT}$, $KRAS^{G12C}$, $KRAS^{G12D}$, or $KRAS^{G12V}$ ($KRas^{lox}KRAS^{MUT}$ cells) were cultured in absence or presence of 4OHT and analyzed by western blot to measure both endogenous KRas and exogenous KRAS expression. Results are representative of one of three similar experiments.

(B) Growth rates of $KRas^{lox}KRAS^{G12C}$, $KRAS^{G12D}$, or $KRAS^{G12V}$ cells in presence (−4OHT, empty circles) or absence (+4OHT, solid circles) of endogenous wild-type KRas alleles in 10% fetal bovine serum (FBS) medium as assessed by IncuCyte measurements ($p < 0.0001$ by unpaired Student's t test). Results are representative of one of three similar experiments.

(C) Ras-GTP levels and activation of downstream signaling in $KRas^{lox}KRAS^{G12C}$, $KRAS^{G12D}$, or $KRAS^{G12V}$ cells in presence (−4OHT) or absence (+4OHT) of endogenous wild-type *KRas* alleles in 0. 1% FBS medium upon stimulation with EGF (50 ng/mL). Results are representative of one of three similar experiments.

(D) Kaplan-Meier analysis demonstrates shorter survival of $KRas^{lox/LSLG12V}$ mice (n = 42, black line) compared to $KRas^{+/LSLG12V}$ mice (n = 31, red line) after intra-tracheal instillation of 10^6 pfu/mouse AdCRE virus ($p < 0.001$; log-rank test [Mantel-Cox]).(E) H&E staining of representative lung sections obtained from $KRas^{lox/LSLG12V}$ and $KRas^{+/LSLG12V}$ mice 6 months after AdCRE (scale bar: 500μm).

increased tumor number and size compared to *KRas*$^{+/LSLG12V}$ mice (Figure 1E), as previously reported (Puyol et al., 2010).

Wild-type KRas impairs response to MEK inhibition in *KRAS*-mutant cells

Responses of *KRAS*-mutant cancer cells (Solit et al., 2006) and human tumors (Blumenschein et al., 2015; Jänne et al., 2017) to MEK inhibitor treatment are variable and the contingencies are unclear. However, our model system is ideally suited to ask whether the presence of wild-type *KRAS* contributes to MEK inhibitor sensitivity in *KRAS*-mutant lung cancer. Ablation of wild-type *KRas* by 4OHT dramatically increased sensitivity of *KRas*^{lox} *KRAS*^{MUT} cells to the MEK1/2 inhibitor selumetinib (Figure 2A). *KRas* wild-type loss also increased sensitivity to trametinib, a more potent FDA approved drug with superior pharmacological properties due to its ability to prevent feedback reactivation of ERK (Lito et al., 2014) (Figure 2A). Interestingly, the morphology of *KRas*^{lox} *KRAS*^{MUT} cells, which had a transformed phenotype upon *KRas* LOH, reverted to a non-transformed phenotype upon selumetinib treatment but only in the absence of endogenous wild-type *KRas*. Evaluation of MAPK signaling in this context showed that in the presence of wild-type KRas, selumetinib treatment resulted in an incomplete inhibition of the MAPK pathway in EGF-stimulated serum-starved cells expressing KRAS^{G12C} or KRAS^{G12D} (Figure 2B). Similarly, under the same experimental conditions, full pERK inhibition upon

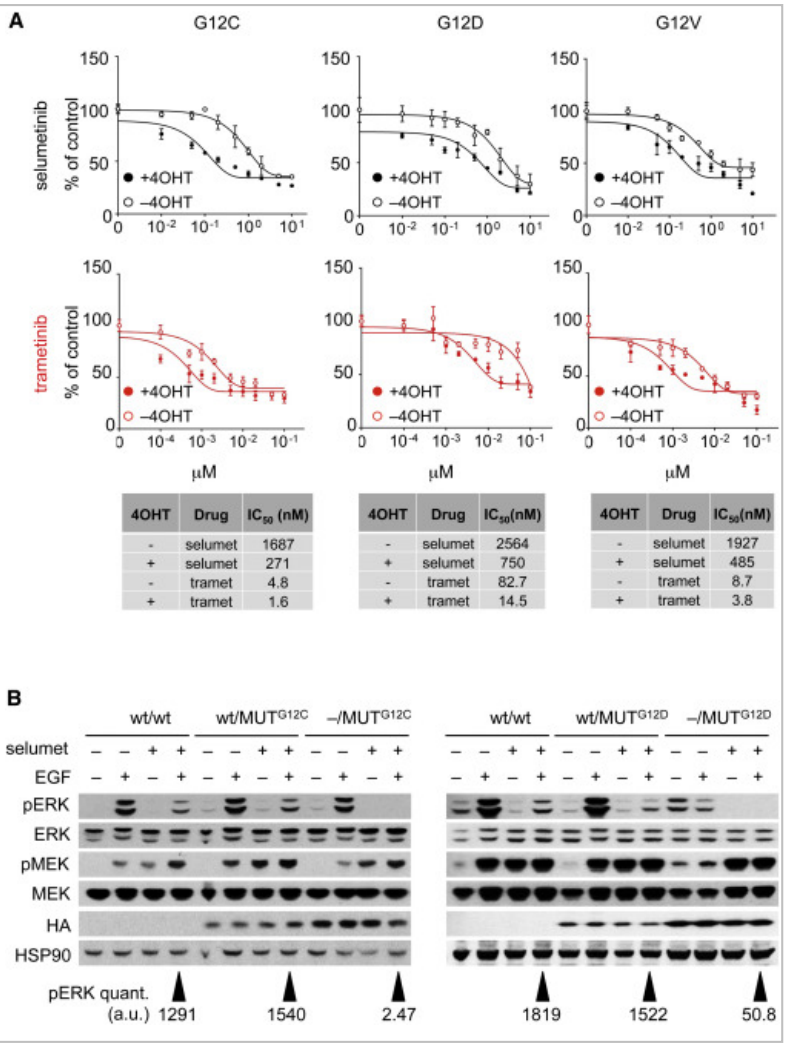

Figure 2. Wild-Type KRas Impairs Response to MEK Inhibition in KRAS Mutant Cells (A) Comparison of IC_{50} values to MEK inhibitors selumetinib (top) or trametinib (bottom) between *KRas*^{lox}MEFs expressing exogenous HA-tagged KRAS^{G12C}, KRAS^{G12D}, or KRAS^{G12V} in presence (−4OHT, open circles) or absence (+4OHT, solid circles) of endogenous wild-type *KRas* alleles. Error bars represent mean ± SD of cell lines belonging to each group. Results are representative of one of three similar experiments.

(B) Western blotting showing *KRas*^{lox}*KRAS*^{G12C}*and* *KRas*^{lox}*KRAS*^{G12D} cells in presence (−4OHT, indicated as wt/MUT^{G12}) or absence (+4OHT, indicated as –/MUT^{G12}) of endogenous wild-type *KRas*alleles. Cells were maintained in 0.1% FBS medium for 12 hr with or without selumetinib (1 μM) and stimulated with EGF (50 ng/mL) as indicated. Parental *KRas*^{lox} cells are referred to as wt/wt. The arrows indicate residual phosphorylation of ERK in response to selumetinib treatment upon EGF stimulation (densitometric quantification of western blot bands values are shown below). Results are representative of one of three similar experiments

treatment with either trametinib or CH5126766, a dual MEK/CRAF inhibitor, was only achieved in *KRas*^{lox} *KRAS*^{MUT} cells lacking wild-type *KRas* (data not shown).

Charge-reversal D154Q mutation impairs KRAS dimerization

Prior evidence in support of RAS dimerization led us to speculate that the genetic interactions we observed between wild-type and mutant KRAS could be explained on the basis of physical, RAS dimer interactions. To test this hypothesis, we sought to identify a *KRAS* mutation that could efficiently disrupt KRAS dimers. We evaluated KRAS crystal structures which we and others had previously solved (Hunter et al., 2014, 2015; Xiong et al., 2016), searching for those with crystal packing interactions meeting criteria we considered important for biologically relevant dimer formation. These included (1) positioning of C-terminus of both dimer members in the same direction as would be expected for simultaneous insertion of prenylated CAAX motifs into the cell membrane; (2) positioning of the GNP nucleotide binding pocket towards the center of the cell to maximally expose the pocket towards the cellular store of GNP nucleotide where exchange would be most efficient; and (3) an overall configuration that would allow binding of KRAS effectors simultaneously to both dimerized KRAS protomers without significant steric clashes. Interestingly, in our crystal structure of wild-type KRAS bound to GTP we noted an interaction involving the α4-α5 interface meeting these conditions (Figure 3A). Of note, this model shared the same interface compared to a model wherein HRAS crystal structures were used to identify α4-α5 as the dimer interface (Spencer-Smith et al., 2017) and is also consistent with biophysical measurements evaluating NRAS dimerization (Güldenhaupt et al., 2012). In this model, residue D154 forms a salt bridge with R161 from the opposing monomer. We thus hypothesized that a charge reversal mutation in this residue (D154Q) could potentially lead to disruption of dimerization by repulsion at the interface.

To directly test this hypothesis, we designed a cell-based FRET system using CFP (donor) and YFP (acceptor) fusions of KRAS to measure protein-protein interactions between KRAS dimers (Figure 3B-D). When we co-expressed CFP-KRAS^{WT} and YFP-KRAS^{WT} proteins (Figure

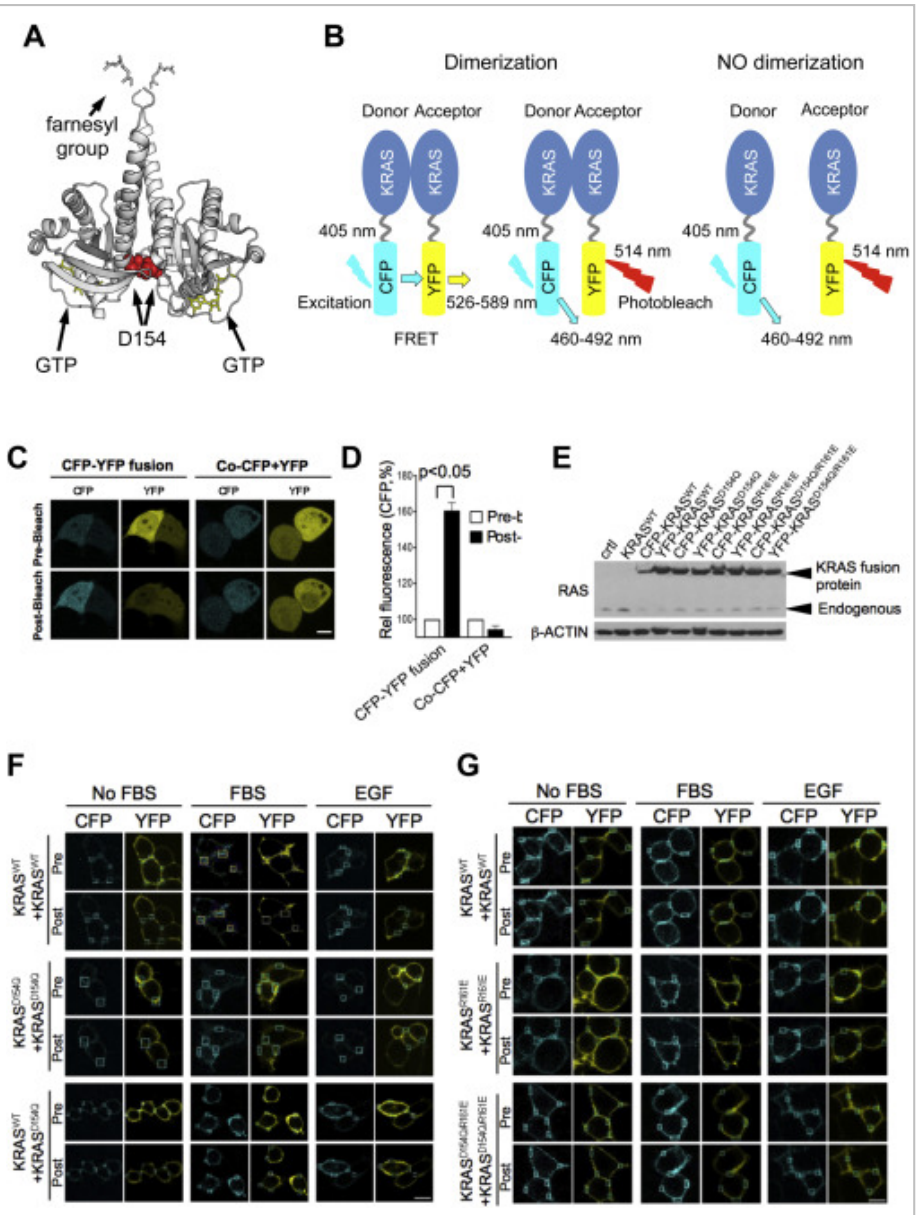

Figure 3. Photobleaching FRET Assay Principle and Validation of Impaired KRAS Dimerization in the Presence of the D154Q Mutation, Related to Figure 3

(A) GTP-bound crystal structure of a KRAS dimer showing the localization of the D154 residue.

(B) Schematic diagrams showing KRAS dimerization and no dimerization using acceptor photobleaching FRET assay. CFP (donor) and YFP (acceptor) are fused to the N terminus of KRAS. In the dimerized form, CFP is excited and energy transferred to YFP; CFP emission increases due to loss of energy absorption by YFP while photobleaching the acceptor. In the not-dimerized form, no alteration in emission from CFP is observed due to absence of energy transfer from CFP to YFP, with or without photobleaching.

(C) Representative images demonstrating the photobleaching FRET assay principle. HEK293T cells were transfected with CFP-YFP plasmid or co-transfected with CFP and YFP plasmids for 36-48 hours and subjected to acceptor photobleaching. Magnification: 40X oil; Scale bar: 10 μM.

(D) Quantification of CFP emission after photobleaching YFP in CFP-YFP plasmid transfected cells. Error bars represent mean ± SD ($p < 0.001$; unpaired Student's t test).

(E) Expression levels of KRAS mutants. HEK293T cells were transfected with $KRAS^{WT}$, CFP-$KRAS^{WT}$, YFP-$KRAS^{WT}$, CFP-$KRAS^{D154Q}$, YFP-$KRAS^{D154Q}$, CFP-$KRAS^{R161E}$, YFP-$KRAS^{R161E}$, CFP-$KRAS^{D154Q/R161E}$, or YFP-$KRAS^{D154Q/R161E}$, and cell lysates were analyzed by western blotting assay.

(F) Representative images showing the CFP and YFP signals for $KRAS^{WT}$ and $KRAS^{D154Q}$ proteins. HEK293T cells were co-transfected with CFP-$KRAS^{WT}$ and YFP-$KRAS^{WT}$, or CFP-$KRAS^{D154Q}$ and YFP-$KRAS^{D154Q}$, or CFP-$KRAS^{WT}$ and YFP-$KRAS^{D154Q}$. 24 hours post-transfection, cells were serum starved for 22 hours, followed by EGF (10 ng/ml) stimulation for 30 minutes. Magnification: 40X oil; Scale bar: 10 μM.

(G) Representative images showing the CFP and YFP signals for $KRAS^{WT}$, $KRAS^{R161E}$, and $KRAS^{D154Q/R161E}$ proteins. HEK293T cells were co-transfected with CFP-$KRAS^{WT}$ and YFP-$KRAS^{WT}$, CFP-$KRAS^{R161E}$ and YFP-$KRAS^{R161E}$, or CFP-$KRAS^{D154Q/R161E}$ and YFP-$KRAS^{D154Q/R161E}$. 24 hours post-transfection, cells were serum starved for 22 hours, followed by EGF (10 ng/ml) stimulation for 30 minutes. Magnification: 40X oil; Scale bar: 10 μM.

3E), under conditions where KRAS is expected to form dimers such as serum supplementation or EGF stimulation, we observed an increased CFP signal after YFP bleaching (Figures 4A). In

contrast, wild-type KRAS proteins containing a D154Q mutation did not show a significant increase in CFP emission following bleaching, suggesting a lack of $KRAS^{D154Q}$-$KRAS^{D154Q}$ interaction. Notably, the $KRAS^{WT}$-$KRAS^{D154Q}$ heterodimer also exhibited a decreased CFP signal, although to a lesser degree (Figures 4A). To further confirm that KRAS dimers utilize the α4-α5 interface via a D154-R161 salt bridge, we evaluated KRAS proteins with an arginine to glutamic acid mutation in codon 161 (R161E) and demonstrated a relative loss of CFP signal after photobleaching, consistent with loss of KRAS-KRAS dimerization (Figures 4B). Furthermore, the $KRAS^{D154Q/R161E}$ double charge reversal mutation was able to restore dimerization (Figures 4B). These effects were not related to alterations in protein expression (data not shown). Collectively, these data demonstrate that KRAS dimerizes in cells through the α4-α5 interface in an EGF/mitogen dependent manner utilizing a salt bridge between D154 and R161.

To exclude the possibility that D154Q alters the biochemical properties, and therefore the activation state, of KRAS we measured GTPase, GDP exchange and RAS-RBD binding activities. Both $KRAS^{WT}$ and $KRAS^{D154Q}$ exhibited similar intrinsic and GAP-stimulated GTP hydrolysis (data not shown). Also, $KRAS^{WT}$ and $KRAS^{D154Q}$ showed similar GDP dissociation rates (data not shown). We further evaluated whether D154Q could change the ability of KRAS to engage with CRAF, a requirement for activation of KRAS-dependent MAPK signaling (Lavoie and Therrien, 2015). We first tested the interaction between recombinant KRAS proteins with CRAF using a

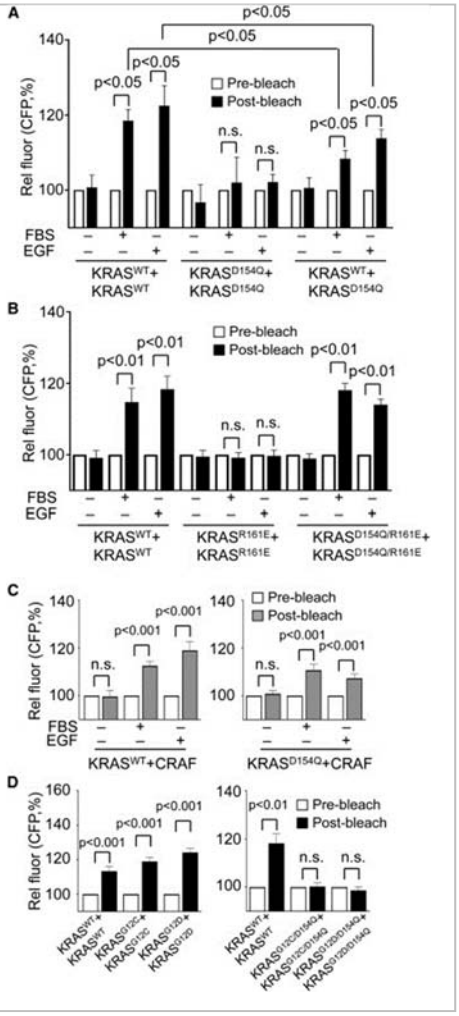

Figure 4. D154Q Mutation Abrogates KRAS Dimerization (A) CFP emission for $KRAS^{WT}$ and $KRAS^{D154Q}$. HEK293T cells were co-transfected with CFP-$KRAS^{WT}$and YFP-$KRAS^{WT}$, CFP-$KRAS^{D154Q}$, and YFP-$KRAS^{D154Q}$ or CFP-$KRAS^{WT}$ and YFP-$KRAS^{D154Q}$ serum starved and stimulated with EGF (10 ng/mL) or serum. Error bars represent mean ± SD ($p < 0.05$ by unpaired Student's t test).
(B) CFP emission for $KRAS^{WT}$, $KRAS^{R161E}$, and $KRAS^{D154Q/R161E}$. HEK293T cells were co-transfected with CFP-$KRAS^{WT}$ and YFP-$KRAS^{WT}$, CFP-$KRAS^{R161E}$, and YFP-$KRAS^{R161E}$ or CFP-$KRAS^{D154Q/R161E}$and YFP-$KRAS^{D154Q/R161E}$ serum starved and stimulated with EGF (10 ng/mL) or serum. Error bars represent mean ± SD ($p < 0.05$; unpaired Student's t test).
(C) CFP emission for $KRAS^{WT}$ and $KRAS^{D154Q}$. HEK293T cells were co-transfected with CFP-$KRAS^{WT}$and YFP-CRAF, or CFP-$KRAS^{D154Q}$ and YFP-CRAF, serum starved and stimulated with EGF (10 ng/mL) or serum. Cells were subjected to confocal microscopic examination. Error bars represent mean ± SD ($p < 0.05$; unpaired Student's t test).
(D) CFP emission for $KRAS^{WT}$, $KRAS^{G12C}$, $KRAS^{G12D}$, $KRAS^{G12C/D154Q}$, and $KRAS^{G12D/D154Q}$. HEK293T cells were co-transfected with CFP-$KRAS^{WT}$ and YFP-$KRAS^{WT}$, CFP-$KRAS^{G12C}$, and YFP-$KRAS^{G12C}$; CFP-$KRAS^{G12D}$ and YFP-$KRAS^{G12D}$, CFP-$KRAS^{G12C/D154Q}$, and YFP-$KRAS^{G12C/D154Q}$; or CFP-$KRAS^{G12D/D154Q}$ and YFP-$KRAS^{G12D/D154Q}$. Cells were subjected to confocal microscopy upon stimulation with serum. Error bars represent mean ± SD ($p < 0.05$; unpaired Student's t test).

quantitative protein-protein interaction assay we previously reported (Hunter et al., 2015). $KRAS^{D154Q}$ exhibited similar RAF-RBD binding affinity to $KRAS^{WT}$. Of note, we observed similar findings when we compared $KRAS^{G12D}$ to $KRAS^{G12D/D154Q}$ (data not shown). In addition, we conducted FRET experiments to examine KRAS-CRAF interactions. We co-transfected HEK293T cells with CFP-$KRAS^{WT}$ or CFP-$KRAS^{D154Q}$ in combination with YFP-CRAF. Under conditions of serum supplementation or EGF stimulation, but not under conditions of serum starvation, we observed an increase in CFP signal after YFP bleaching for both $KRAS^{WT}$ and $KRAS^{D154Q}$ expressing cells suggesting a protein-protein interaction between KRAS and CRAF (Figure 4C). Consistent with the FRET results, we further noted that $KRAS^{WT}$ and $KRAS^{D154Q}$ recruited CRAF from the cytosol to the plasma membrane upon serum supplementation. Together, our observations support the conclusion that $KRAS^{D154Q}$ does not impair the interaction between KRAS and CRAF and that KRAS dimerization is not required for CRAF-KRAS interactions.

Finally, we evaluated whether oncogenic mutations might impact KRAS dimerization. We introduced the D154Q mutation *in cis* with either G12C or G12D mutants fused to CFP or YFP. Similar to wild-type, $KRAS^{G12C}$ and $KRAS^{G12D}$ showed increased CFP signal after YFP bleach consistent with dimer formation upon serum stimulation, but not in the presence of D154Q (Figures 4D). D154Q had no impact on GTP hydrolysis in $KRAS^{G12D}$. Collectively, these data

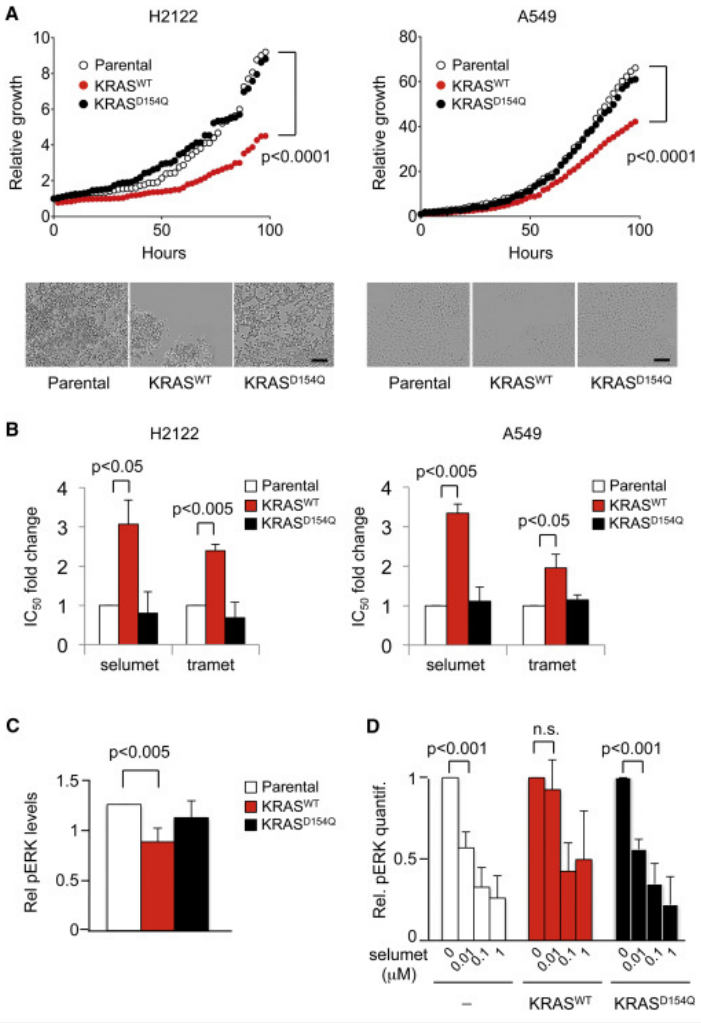

Figure 5. Impaired Dimerization Abolishes the Growth Inhibitory Effect of Wild-Type KRAS on Mutant KRAS while Restoring Sensitivity to MEK Inhibitors

(A) Growth rates of parental H2122 and A549 cells (white circles) compared to the same cell lines stably expressing exogenous $KRAS^{WT}$ (red circles) or $KRAS^{D154Q}$ (black circles) ($p < 0.0001$; unpaired Student's t test). Representative pictures at the end point are shown in the bottom panels (scale bar: 50μm). Results are representative of one of three similar experiments.

(B) IC_{50} fold-changes to the MEK inhibitors selumetinib and trametinib of parental H2122 and A549 cells (white bars) compared to the same cell lines stably expressing exogenous $KRAS^{WT}$ (red bars) or $KRAS^{D154Q}$ (black bars). Error bars represent mean ± SD of cell lines belonging to each group. Results are representative of one of three similar experiments.

(C) Quantification of basal phosphorylated ERK relative to total ERK levels from A549, H2122, SKLU1, H2030, H1792, H23, and H358 western blots (Figure S5E and data not shown; untreated conditions). Error bars represent mean ± SD ($p < 0.001$; unpaired Student's t test).

(D) Quantification of phosphorylated ERK relative to total ERK levels from A549, H2122, SKLU1, H2030, H1792, H23, and H358 western blots (Figure S5E and data not shown). Error bars represent mean ± SD ($p < 0.001$; unpaired Student's t test).

show that D154Q abrogates both wild-type and mutant KRAS dimerization without influencing intrinsic GTPase activity, GEF or GAP sensitivity, or CRAF binding.

Impaired wild-type/mutant KRAS dimerization abolishes growth inhibitory effects by wild-type KRAS and increases sensitivity to MEK inhibitors *in vitro* and *in vivo*

To evaluate the impact of KRAS dimer disruption via the $KRAS^{D154Q}$ mutation on the efficacy of MEK inhibitors in human lung cancers, we examined a panel of lung cancer cell lines with different *KRAS* mutations and *KRAS* allelic frequencies both at the DNA and cDNA levels (Table S1). Two of these cell lines, A549 and H2122, did not express any endogenous wild-type KRAS thus allowing us to evaluate the impact of reintroducing wild-type. We transduced the panel of lung cancer cell lines with either $KRAS^{WT}$ or $KRAS^{D154Q}$ (data not shown). Interestingly, we noted both a longer average time required to select $KRAS^{WT}$ cell lines compared to those expressing $KRAS^{D154Q}$ and a high number of clones with low/undetectable expression of exogenous $KRAS^{WT}$ (data not shown) demonstrating expression of $KRAS^{WT}$ is a negative selection factor. Accordingly, the presence of $KRAS^{WT}$ reduced growth rates in H2122 and A549 cell lines and in several other *KRAS*-mutant cell lines. In contrast, the $KRAS^{D154Q}$ mutant did not affect cell proliferation (Figures 5A). As with MEFs, the presence of $KRAS^{WT}$ was also uniformly associated with resistance to selumetinib and trametinib, but the effect was eliminated by the D154Q mutation (Figures 5B). Of note, cells infected with empty vector did not show any changes in cell proliferation or drug sensitivity relative to parental lines (data not shown). Consistent with this phenotype, *KRAS*-mutant lung cancer cells expressing $KRAS^{WT}$, but not $KRAS^{D154Q}$ maintained ERK phosphorylation at low selumetinib concentrations, although basal levels of ERK phosphorylation were lower in WT (Figures 5C, 5D).

Recent studies suggest that robust MAPK pathway suppression, with >80-90% inhibition of ERK signaling, must be achieved in order to obtain therapeutic efficacy (Albeck et al., 2013; Chapman et al., 2014). Therefore, we considered that the residual MAPK activity attributable to the wild-type *KRAS* allele might be critical for shifting the therapeutic window and determine responses to MEK inhibition *in vivo*. We hypothesized that *KRAS*-mutant lung cancer cells with high expression of wild-type KRAS have a fitness disadvantage in the absence of treatment, but nevertheless may be intrinsically more resistant to MEK inhibition *in vivo*. To test this, we developed murine lung cancer cell lines from primary $KRas^{G12V}$ or $KRas^{G12V};p53^{-/-}$ tumors lacking the wild-type *KRas* allele (Ambrogio et al., 2014) and introduced either $KRAS^{WT}$ or $KRAS^{D154Q}$ (data not shown). The presence of the $KRAS^{WT}$, but not the $KRAS^{D154Q}$ mutant, diminished the growth rate in both $KRas^{G12V}$ and $KRas^{G12V};p53^{-/-}$ cell lines (data not shown) and increased the IC_{50} of MEK inhibitors (data not shown). Similar to the human cancer cell lines, both $KRas^{G12V}$ and $KRas^{G12V};p53^{-/-}$ cells expressing $KRAS^{WT}$, but not $KRAS^{D154Q}$ displayed sustained ERK phosphorylation upon selumetinib treatment (data not shown). Tail vein injection of these cell lines into mice, followed by tumor establishment and a 7 day exposure to selumetinib showed results consistent with cell culture. We detected a strong reduction in ERK phosphorylation over time in both parental and $KRAS^{D154Q}$-expressing $KRas^{G12V};p53^{-/-}$ tumors, but not in $KRAS^{WT}$-expressing $KRas^{G12V};p53^{-/-}$ tumors (Figure 6A). Interestingly, feed-back reactivation of CRAF-S338 phosphorylation was faster and more robust in $KRAS^{WT}$-expressing $KRas^{G12V};p53^{-/-}$ tumors than parental and $KRAS^{D154Q}$-expressing $KRas^{G12V};p53^{-/-}$ tumors (Figure 6A). Consistently, evaluation of a six gene-signature of MEK output (Brant et al., 2017), demonstrated a significant downregulation following selumetinib treatment in both parental and $KRAS^{D154Q}$-expressing $KRas^{G12V};p53^{-/-}$ tumors, but not tumors expressing $KRAS^{WT}$ (data not shown).

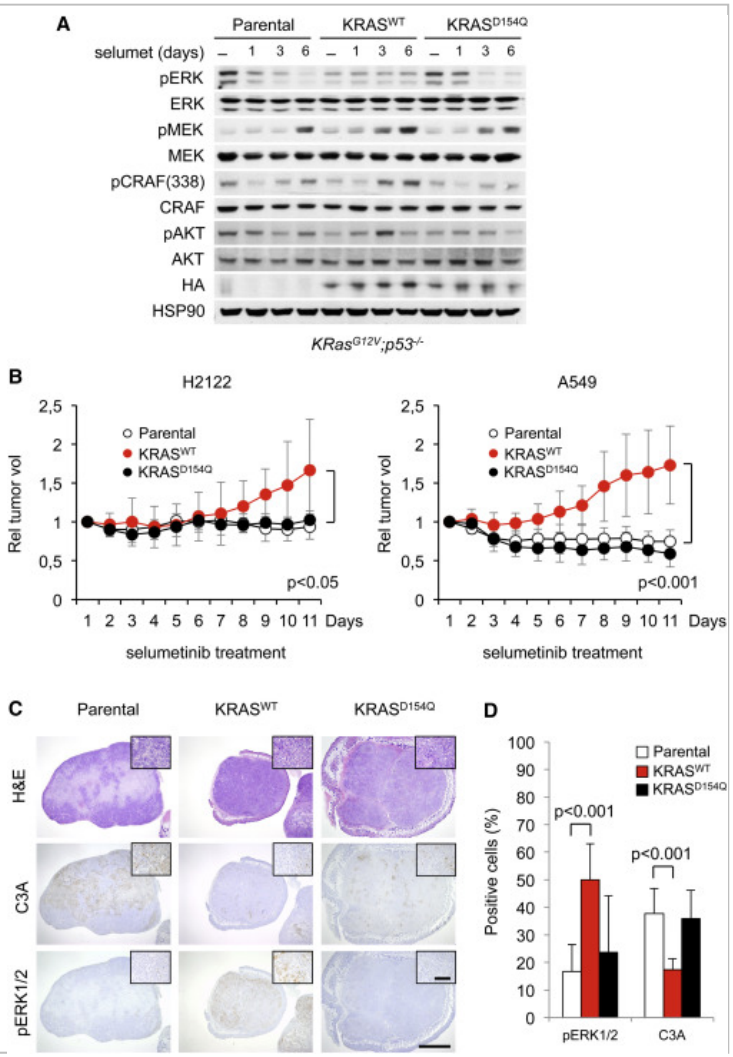

Figure 6. Impaired KRAS Dimerization Restores Sensitivity to MEK Inhibition in KRAS Mutant Lung Cancer Cells In Vivo

(A) Parental murine *KRas^{G12V};p53$^{-/-}$* lung cancer cells or the same cell lines stably expressing exogenous KRAS^{WT} or KRAS^{D154Q} were injected intravenously into nude mice. After one week, animals were treated with selumetinib (50 mg/kg daily) and sacrificed at the indicated time points. Lungs were lysed and analyzed by western blot with the indicated antibodies.

(B) Parental H2122 and A549 cells (white circles) or the same cell lines stably expressing exogenous KRAS^{WT} (red circles) or KRAS^{D154Q} (black circles) were injected subcutaneously into nude mice. Once tumors reached a size of 240–300 mm^3, animals were treated with selumetinib (50 mg/kg daily) for 12 days. The mean fold-change in tumor volume relative to initial tumor volume is shown. Error bars represent mean ± SD ($p < 0.05$ and $p < 0.001$; unpaired Student's t test in H2122 and A549 xenografts, respectively).

(C) H&E, Caspase3A (C3A) and pERK staining of sections from representative parental, KRAS^{WT}, or KRAS^{D154Q} xenografts from H2122 cells treated with selumetinib during 12 days. Insets display high-magnification images. Scale bars: 2 mm and 50 μm (insets).

(D) Quantification of C3A and pERK in tumors (n = 8) from mice carrying xenografts shown in (C). Error bars represent mean ± SD ($p < 0.001$; unpaired Student's t test).

To extend the *in vivo* analysis to human cancer, we generated nude mice xenografts from A549 or H2122 cells expressing either KRAS^{WT} or KRAS^{D154Q}, and compared them to parental cell lines. Tumors expressing KRAS^{WT}, but not KRAS^{D154Q}, grew significantly slower than their parental controls (data not shown). Selumetinib treatment inverted this trend in both A549 and H2122 xenografts with the KRAS^{WT} tumors achieving a significant growth advantage after 10 days of treatment. In contrast, introduction of D154Q did not alter the sensitivity to selumetinib (Figure 6B). Histopathological evaluation revealed that selumetinib-treated, tumors expressing KRAS^{WT}, but not KRAS^{D154Q}, displayed significantly lower apoptotic death compared to control tumors. The fraction of cells harboring residual pERK staining following selumetinib treatment was significantly higher in KRAS^{WT} tumors than control or KRAS^{D154Q} tumors (Figures 6C and 6D). Consistent with higher levels of pERK, expression of ERK transcriptional target genes was minimally altered in KRAS^{WT} tumors. In contrast, both parental and KRAS^{D154Q} tumors showed a significant reduction in expression of ERK transcriptional target genes after selumetinib treatment compared to KRAS^{WT} tumors (data not shown).

Dimerization of oncogenic KRAS is essential for activation of downstream signaling and cell growth *in vitro* and *in vivo*

Our findings demonstrate that wild-type KRAS dimerization with mutant KRAS modulates KRAS biology. However, dimerization may also be essential for the full functions of oncogenic KRAS itself. We generated a panel of *KRas*^{lox} *KRAS*^{MUT} cells by introducing the D154Q mutation *in cis* with G12 oncogenic mutations (G12C, G12D or G12V). In presence of 4OHT, *KRas*^{lox} *KRAS*$^{MUT/D154Q}$ cells only expressed monomeric mutant KRAS, allowing us to evaluate the importance of dimerization for its oncogenic functions. Interestingly, the growth rate of *KRas*^{lox} *KRAS*$^{MUT/D154Q}$ cells was severely impaired relative to *KRas*^{lox} *KRAS*^{MUT} (Figure 7A). When challenged with low serum or low glucose containing media, *KRas*^{lox} *KRAS*$^{MUT/D154Q}$ cells showed increased levels of apoptosis as detected by active Caspase3 (data not shown). This phenotype was not attributable to detectable changes in essential biological functions of double KRAS$^{MUT/D154Q}$ mutants including membrane localization, binding to downstream mediators or total amount of KRAS-GTP, which was similar in the presence or absence of the D154Q mutation (Figure 7B).

Because RAF activation by KRAS requires RAF dimerization (Lavoie and Therrien, 2015), and each RAF monomer has the potential to bind KRAS (Simanshu et al., 2017), we hypothesized that defective oncogenic KRAS dimerization could result in inefficient formation of BRAF/CRAF heterodimers leading to reduced downstream signaling. We observed a decrease in BRAF/CRAF heterodimers by co-immunoprecipitation (IP) in *KRas*^{lox} *KRAS*$^{MUT/D154Q}$ cells compared to *KRas*^{lox} *KRAS*^{MUT} cells, accompanied by attenuated CRAF-S338 phosphorylation (Figure 7C). This is in

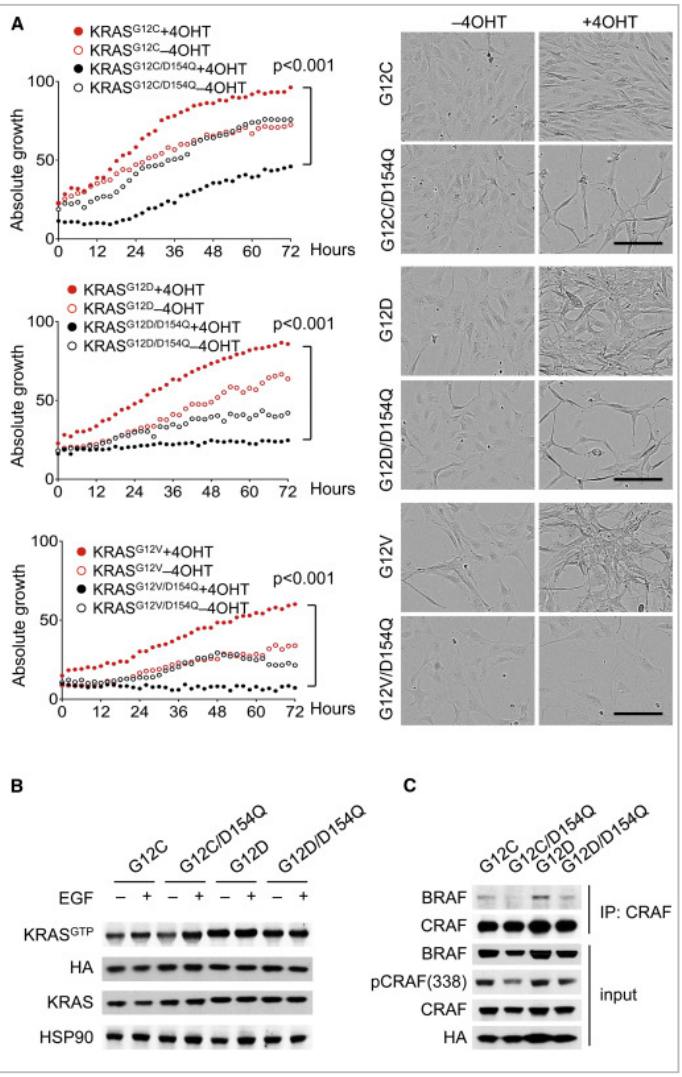

Figure 7. Dimerization of Oncogenic KRAS Is Essential for Cell Growth In Vitro (A) Growth rates of *KRas*^{lox} MEFs expressing exogenous HA-tagged KRAS^{G12C}, KRAS^{G12D}, KRAS^{G12V}(red circles) or KRAS$^{G12C/D154Q}$, KRAS$^{G12D/D154Q}$, KRAS$^{G12V/D154Q}$ (black circles) in presence (−4OHT, empty circles) or absence (+4OHT, solid circles) of endogenous wild-type KRas alleles in 0.5% FBSmedium as assessed by IncuCyte ($p < 0.001$; unpaired Student's t test). Results are representative of one of three similar experiments. Right panels show phase-contrast images of the corresponding *KRas*^{lox}*KRAS*^{MUT} or *KRas*^{lox}*KRAS*$^{MUT/D154Q}$ cells in presence (−4OHT) or absence (+4OHT) of endogenous *KRas* alleles at 72-hr time point (scale bar: 20 μm).

(B) Ras-GTP levels in *KRas*^{lox} MEFs expressing exogenous HA-tagged KRAS^{G12C}, KRAS$^{G12C/D154Q}$, KRAS^{G12D}, or KRAS$^{G12D/D154Q}$ cells in absence (+4OHT) of endogenous wild-type *KRas* alleles in 0.1% FBS medium upon stimulation with EGF (50 ng/mL). Results are representative of one of three similar experiments.

(C) Lysates from *KRas*^{lox} MEFs expressing exogenous HA-tagged KRAS^{G12C}, KRAS$^{G12C/D154Q}$, KRAS^{G12D}, or KRAS$^{G12D/D154Q}$ cells in absence (+4OHT) of endogenous wild-type *KRas* alleles were immunoprecipitated for CRAF and immunoblotted with the indicated antibodies. Total cell lysates (input) were analyzed by western blot with the indicated antibodies. Results are representative of one of three similar experiments.

agreement with recent findings describing that disrupted RAS dimerization and nanoclustering, using a monobody, led to blockade of CRAF/BRAF heterodimerization (Spencer-Smith et al., 2017).

We further evaluated the impact of dimerization-deficient mutant KRAS *in vivo* by generating *KRas*^{lox} *KRAS*$^{MUT/D154Q}$ allografts and measuring their growth proficiency. When compared to control *KRas*^{lox} *KRAS*^{MUT} cells expressing either KRAS^{G12C} or KRAS^{G12D}, paired *KRas*^{lox} *KRAS*$^{MUT/D154Q}$ cells were completely unable to form tumors *in vivo* (Figure 8A). Histology and immunostains revealed that large tumors generated by *KRas*^{lox} *KRAS*^{MUT} cells were composed of a dense cellular infiltrate with high pERK levels and proliferation rate, whereas *KRas*^{lox} *KRAS*$^{MUT/D154Q}$ tumors showed bland morphology with regressive areas associated with lower pERK levels and proliferation rates (Figures 8B). Interestingly, phosphorylation levels of CRAF-S338, MEK, ERK and S6 were strongly decreased in *KRas*^{lox} *KRAS*$^{MUT/D154Q}$ compared to *KRas*^{lox} *KRAS*^{MUT} tumors (Figure 8C). Consistently, the expression of ERK transcriptional target genes was significantly decreased in *KRas*^{lox} *KRAS*$^{MUT/D154Q}$ tumors (Figure 8D), demonstrating an impairment of the activation of the MAPK pathway when oncogenic KRAS is forced to function as a monomer *in vivo*.

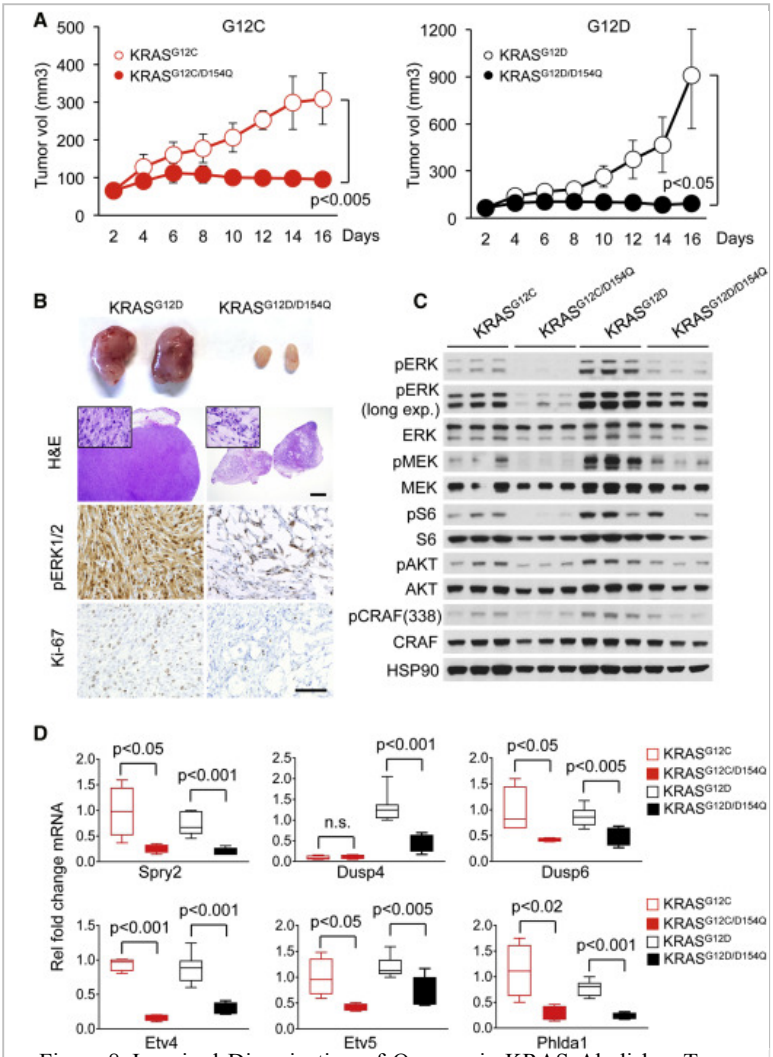

Figure 8. Impaired Dimerization of Oncogenic KRAS Abolishes Tumor Growth In Vivo. (A) *KRas*^{lox} cells expressing KRAS^{G12C} and KRAS$^{G12C/D154Q}$ (left) or KRAS^{G12D} and KRAS$^{G12D/D154Q}$(right) grown in presence of 4OHT were injected subcutaneously into nude mice. Tumor growth was followed over time. Error bars represent mean ± SD. (B) Representative images of the indicated *KRas*^{lox} *KRAS*^{G12D} and *KRas*^{lox}*KRAS*$^{G12D/D154Q}$ allografts at day 16 after tumor implantation (top) with respective H&E, pERK, and Ki-67 stainings of sections (bottom). Insets display high-magnification H&E images. Scale bars: 2 mm for H&E and 50 µm for immunostains. See also Figure S7D. (C) Tumor explants (n = 3 each genotype) as in (A) were lysed and analyzed by western blot with the indicated antibodies.
(D) qRT-PCR analysis of ERK transcriptional targets in allografts from *KRas*^{lox} cells expressing KRAS^{G12C} and KRAS$^{G12C/D154Q}$ (red) or KRAS^{G12D} and KRAS$^{G12D/D154Q}$ (black). Error bars represent mean ± SD.

Aim 2. Subtask 1: Molecular docking and computer-aided modeling
Completed in GY 1. No Work.

Aim 2. Subtask 2: Synthesis of SML analogues
Remaining work related to manuscript publication claimed in GY1 (proofing, etc.) completed.

Aim 3. Subtask 1: Test for covalent labeling of purified KRAS G12C by MS
Completed in GY1.

Aim 3. Subtask 2: Prioritize compounds for further development using biochemical assays (RAS:RBD, Kinetic GDP displacement, Kinact/KI)
No testing in current period.

Aim 3. Subtask 3: Test high priority compounds for cell permeability.
No new compounds appropriate for testing in current period.

Aim 3. Subtask 4. Test high priority compounds for impacts on RAS dimerization and antiproliferative activity.
Using groundwork laid by work on Aim 1, Subtask 5, we have preliminary data suggesting that G12C inhibitors prevent RAS dimerization. This work will be the major focus of the NCE.

Aim 3. Subtask 5: Test high priority compounds selectivity using MS selectivity profiling.
No new compounds appropriate for testing in current period.

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What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Dr. Westover spoke at the November 2017 Forbec Forum as one of 15 RAS investigators representing the "cream of oncology expertise from around the world".
Dr. Westover will speak at the 2018 AACR RAS meeting in San Diego.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

We published 1 manuscript during the reporting period.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Nothing to report.

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Establishment of a structural model for RAS dimerization provides an additional fundamental layer of understanding regarding how RAS is regulated. It also provides another measurable parameter against which small molecule inhibitors might be measured.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Our report on the RAS dimer may have far-reaching impacts on multiple branches of biology. We hypothesize that RAS dimerization will not only have implications for how KRAS G12C function is regulated, but also for how many other RAS forms may be differentially regulated.

Assay methods we developed could have implications for other drug development projects.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Assay methods we reported have been adopted by other research labs pharmaceutical companies

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. CHANGES/PROBLEMS: The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

UT Southwestern updated their accounting system in September of 2018, but the transition has not been smooth. We experienced problems with accessing established grand funding for many months because of the system update. As a result we requested a no-cost extension.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

UT Southwestern updated their accounting system in September of 2018, but the transition has not been smooth. We experienced problems with accessing established grand funding for many months because of the system update. As a result we requested a no-cost extension.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

- Ambrogio C, Köhler J, Zhou ZW, Wang H, Paranal R, Li J, Capelletti M, Caffarra C, Li S, Lv Q, Gondi S, Hunter JC, Lu J, Chiarle R, Santamaría D, Westover KD, Jänne PA. KRAS Dimerization Impacts MEK Inhibitor Sensitivity and Oncogenic Activity of Mutant KRAS. Cell. 2018 Feb 8;172(4):857-868.e15. doi: 10.1016/j.cell.2017.12.020. Epub 2018 Jan 11. Published, acknowledged - yes

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other* publications*, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Assay to detect binding of compounds to switch 2 pocket of KRAS G12C Assay to detect protein dynamics of switch 2 of KRAS Assay to detect shifts in thermal stability of KRAS G12C upon binding to small molecule inhibitors

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Kenneth Westover – No Change.

Name / Project Role: *Sudershan Gondi / Research Scientist*
Nearest person month worked: *1*
Contribution to Project: *Assisting with the production and purification of protein and crystallography.*

Name / Project Role: *Zhiwei Zhou / Postdoc*
Nearest person month worked: *6*
Contribution to Project: *Conducting biochemical assays.*

Name/ Project Role: *Yihe Huang / Postdoc*
Nearest person month worked: *3*
Contribution to Project: *Assisting with the expression, purification, and crystallization of KRAS G12C complexes.*

Name / Project Role: *Asim Bera / Research Associate*
Nearest person month worked: *2*
Contribution to Project: *Assisting with the expression, purification, and crystallization of KRAS G12C complexes, replaced Yihe Huang.*

Name / Project Role: *Emily Campbell*
Nearest person month worked: *Assisted with cell culture.*
Contribution to Project:

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

American Cancer Society Research Scholar Grant has been awarded with an effective start date of 07/01/2018. Dr. Westover has 0.6 calendar months effort on his new ACS grant, which will not impact his effort on this project.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Organization Name: Dana Farber Cancer Institute
Location of Organization: Boston, MA
Partner's contribution to the project : Collaboration

Organization Name: Northeastern University
Location of Organization: Boston, MA
Partner's contribution to the project : Collaboration

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: N/A

9. APPENDICES: N/A