

# Supporting Information

## **KRAS Switch Mutants D33E and A59G Crystallize in the State 1 Conformation**

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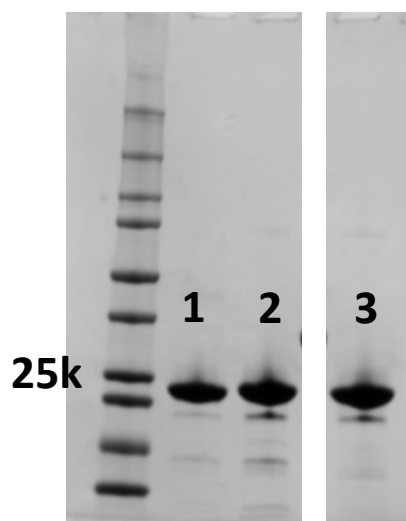
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### Author Contributions

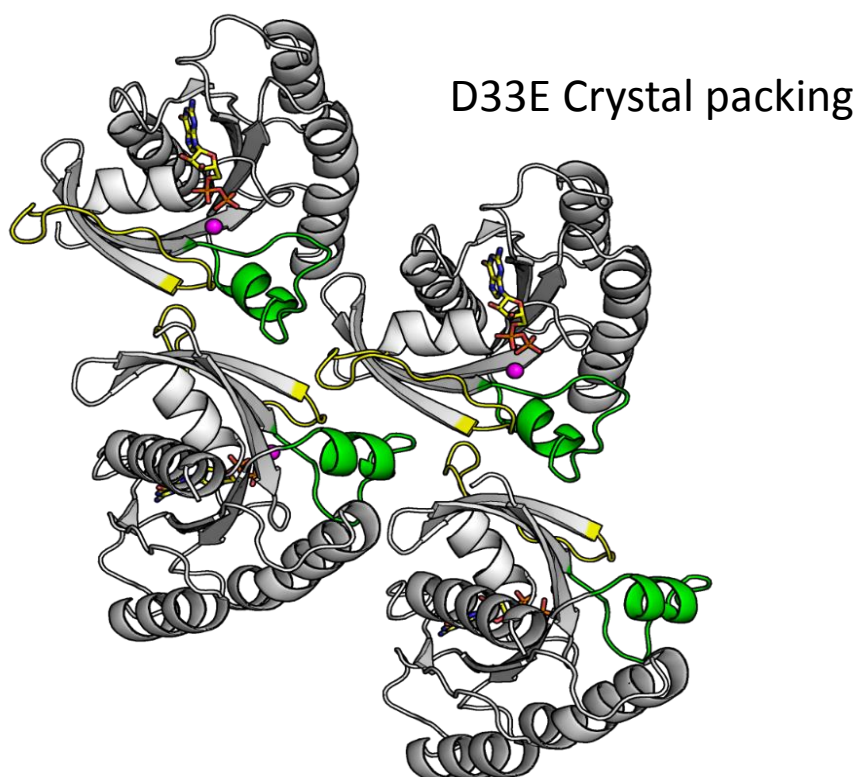
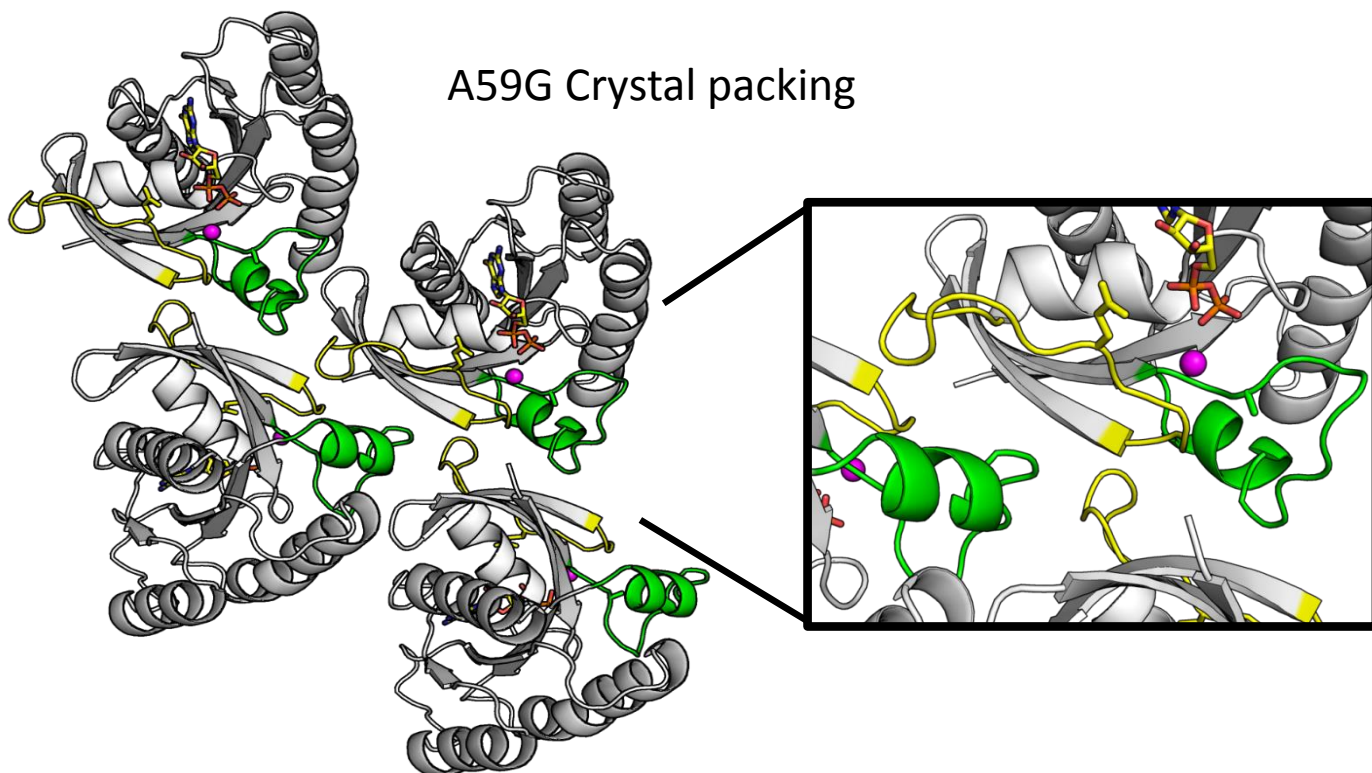
The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. <sup>#</sup>These authors contributed equally.

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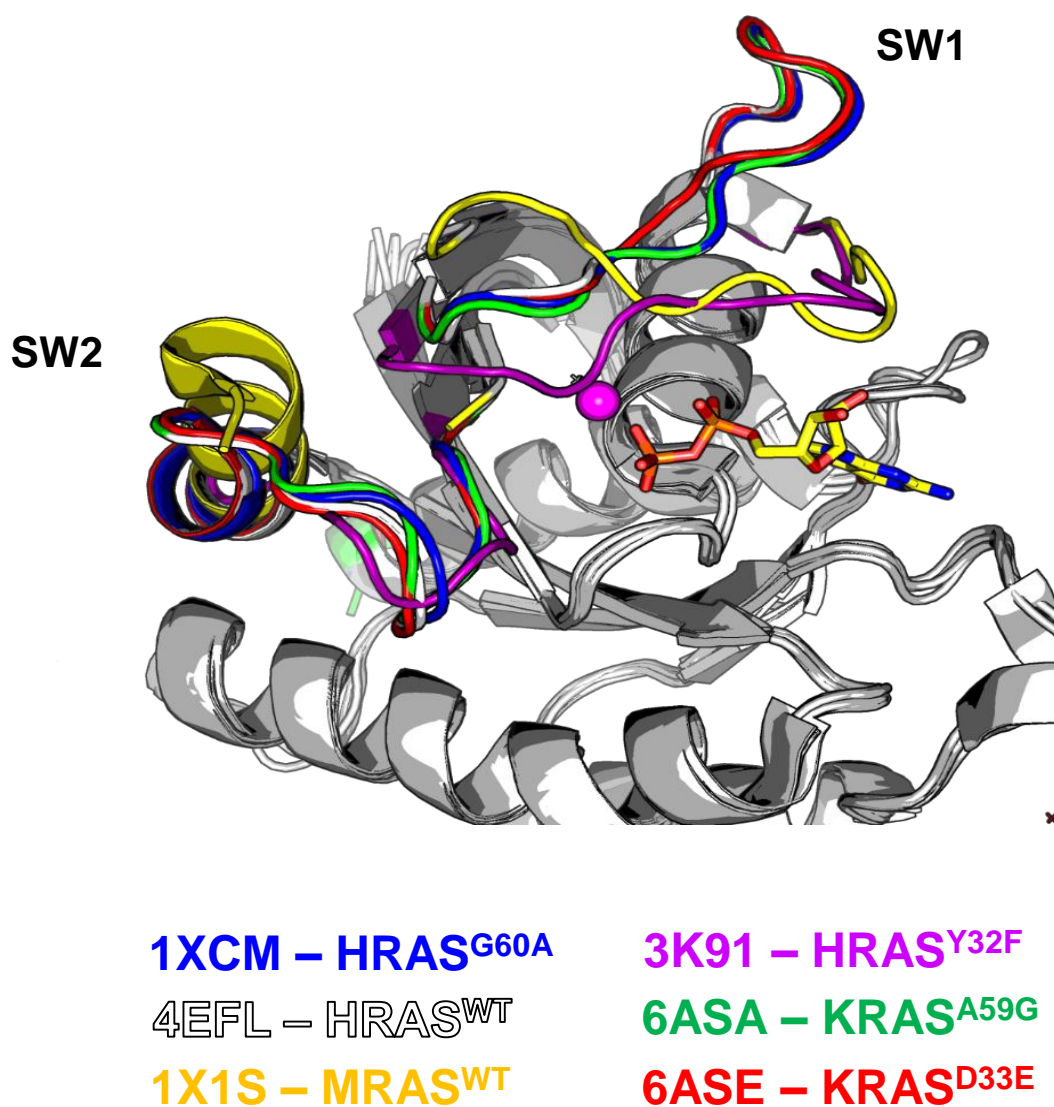
Email: [kenneth.westover@utsouthwestern.edu](mailto:kenneth.westover@utsouthwestern.edu)



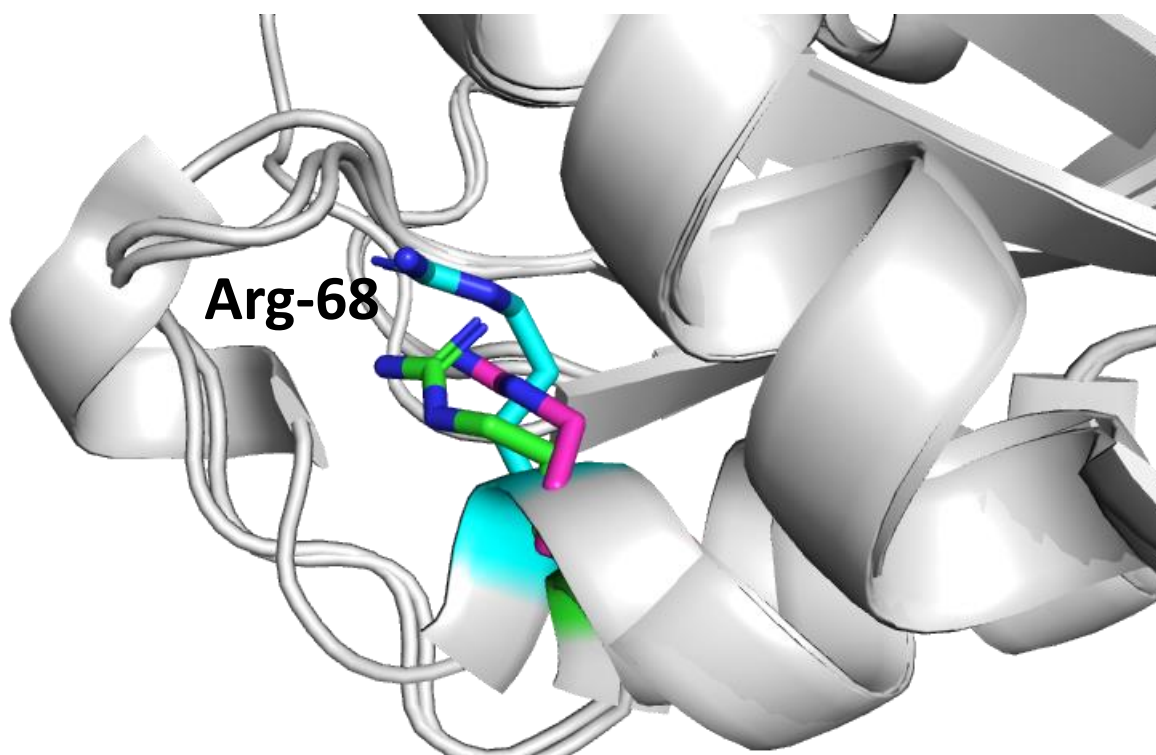
**Figure S1.** Coomassie stained SDS-PAGE gel (4-20%) of purified KRAS proteins used for structural and biochemical studies: **(1)** WT; **(2)** A59G; **(3)** D33E.



**Figure S2.** Crystal packing of KRAS A59G (top) and D33E (bottom). SW1 is in yellow and SW2 is in green. Residue A59G (green) and D33E (yellow) are shown in stick in the zoom-in window.

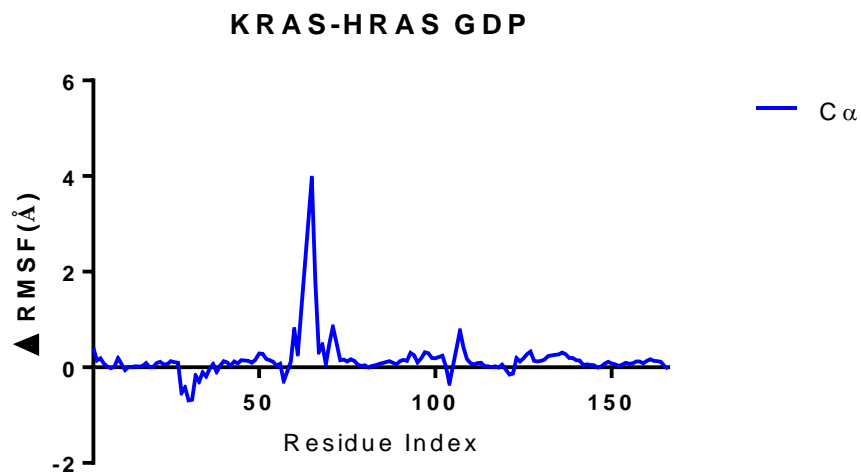


**Figure S3.** Superimposition of HRAS and KRAS state 1 crystal structures. SW1 and 2 of each structure are color-coded responding to the legend. The nucleotide is shown in stick and magnesium ion is shown in magenta sphere.

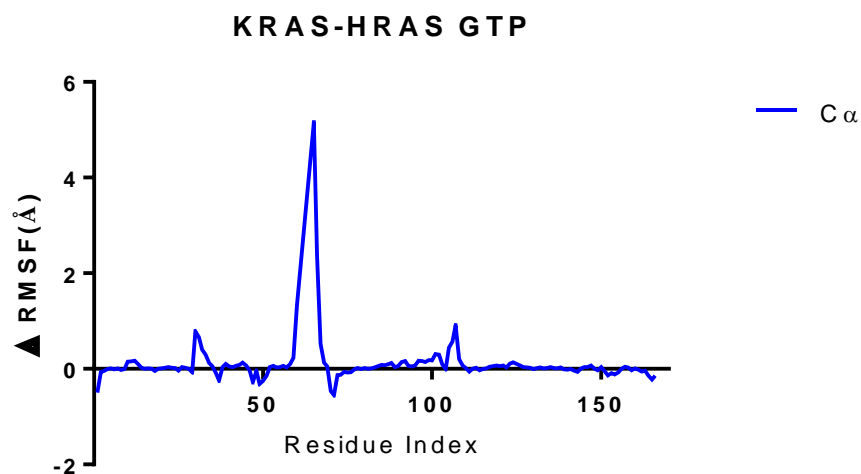


**Figure S4.** Superposition of residue 68 in HRAS<sup>WT</sup> GTP bound State 1 (green), state 2 (cyan) and KRAS<sup>A59G</sup> GDP state (magenta). Arg68 is shown in stick.

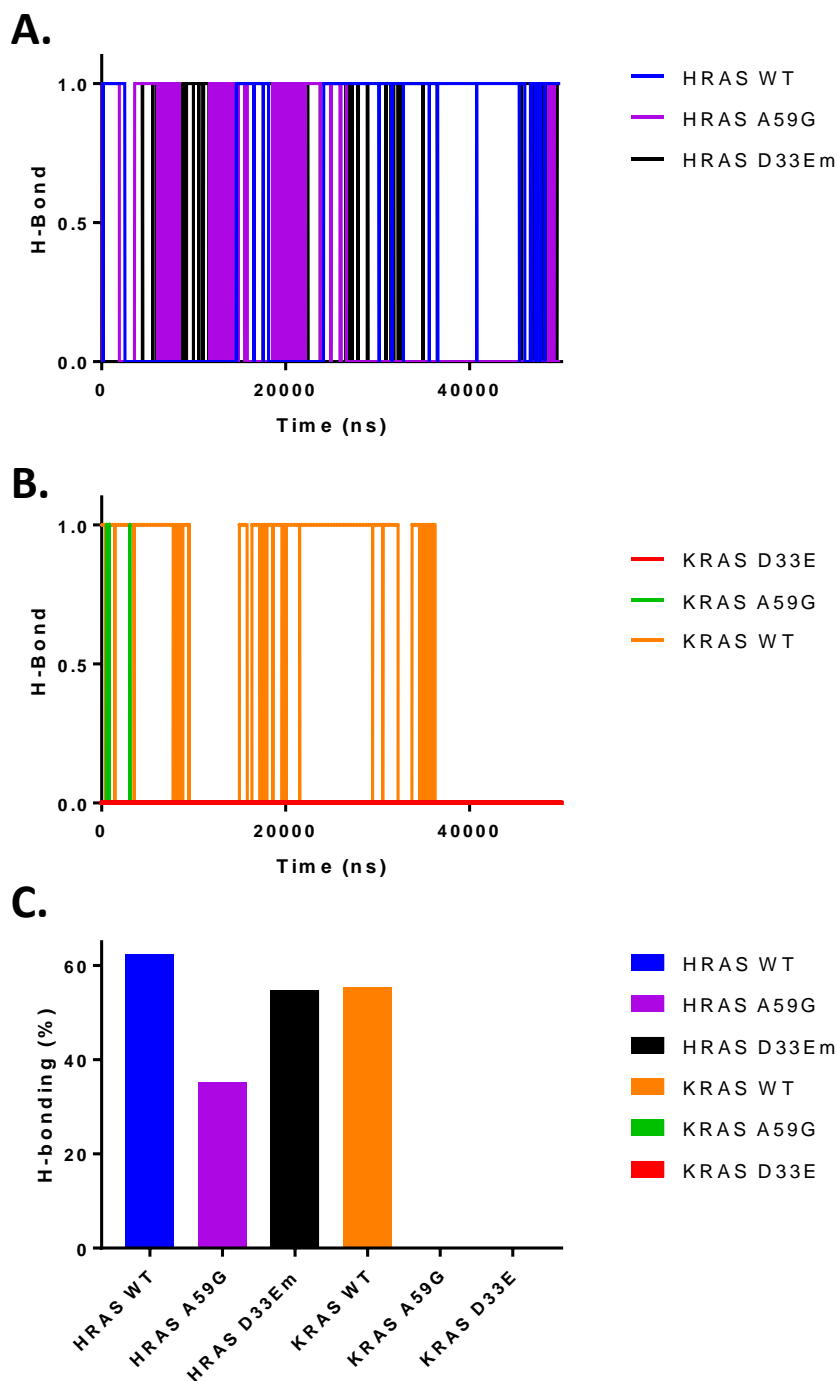
**A.**



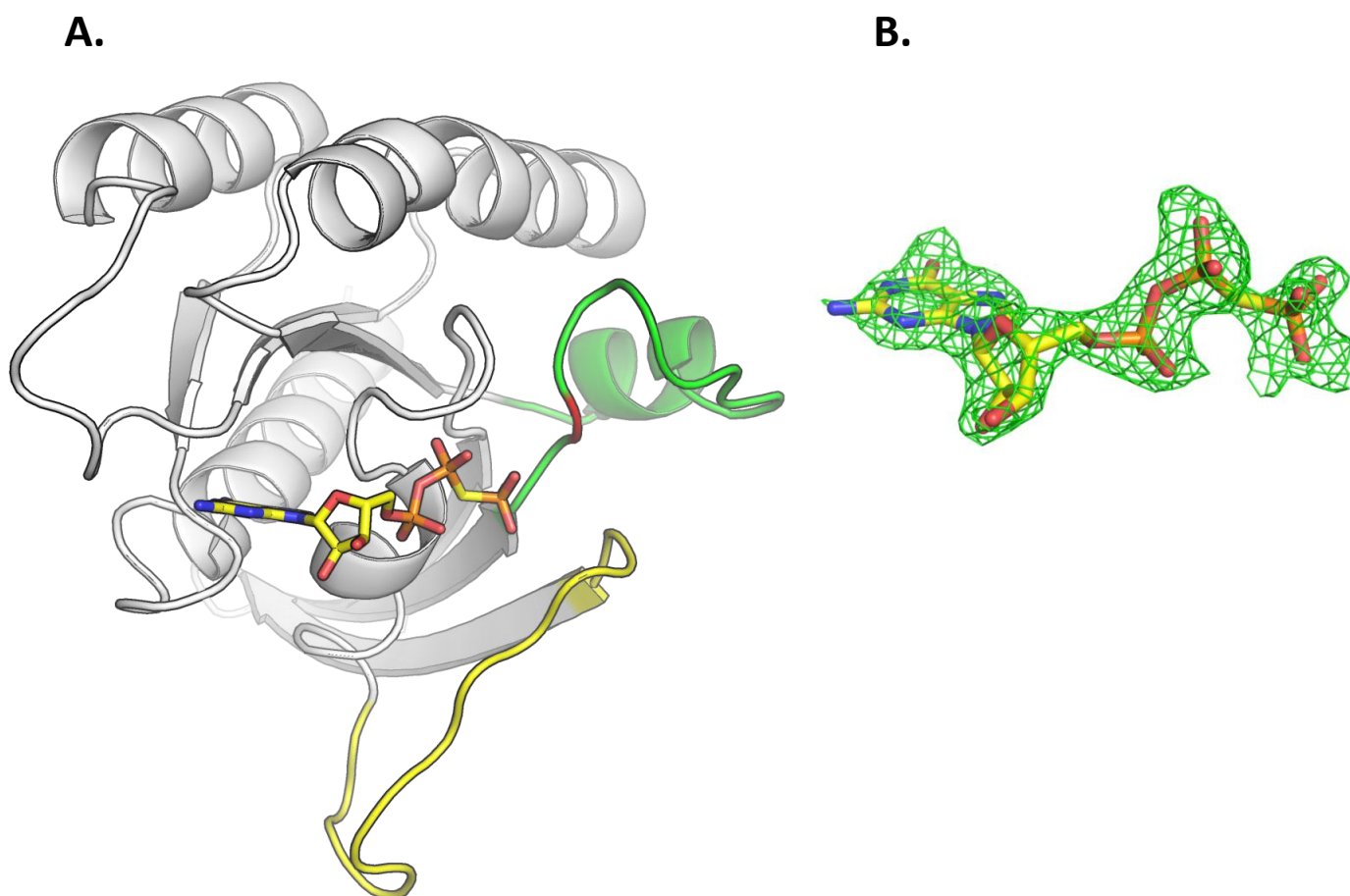
**B.**



**Figure S5.** MD simulations. Differences in RMSF ( $\Delta$ RMSF, in Å) are calculated to indicate the difference between KRAS and KRAS when bound to GDP (**A**), or GTP (**B**). Positive values indicate higher dynamic behavior.

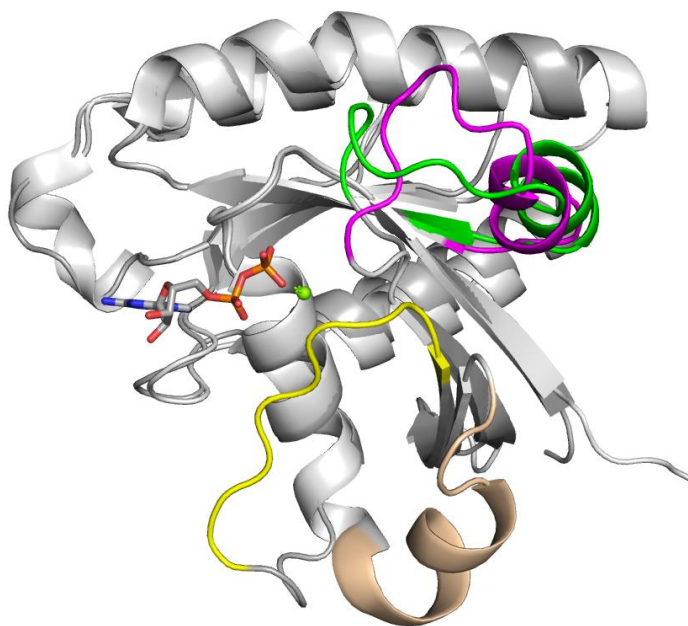


**Figure S6.** Hydrogen bonding between side chains of residues 33 and 35 were plotted during the MD simulation (50 ns) in HRAS **(A)** and KRAS **(B)**. **(C)** Summary statistics of panels A and B. HRAS and KRAS WT maintain hydrogen bonding relative to KRAS A59G and D33E during the simulation.

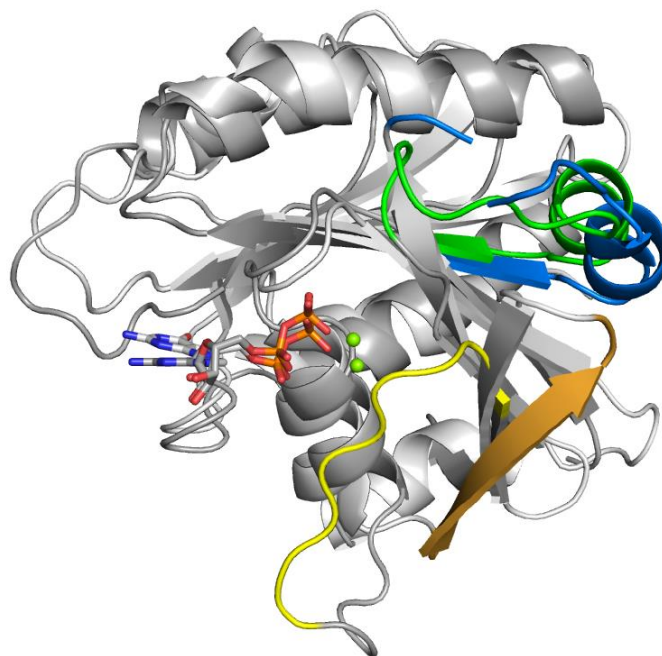


**Figure S7. (A).** Crystal structure of KRAS A59G in bound to GppCp. Gly59 in red. **(B).** Fo-Fc map of GppCp contoured at  $3\sigma$ .





A59G with RAB28A



A59G with ARF1

**Figure S8.** Superposition of KRAS A59G and RAB28A, ARF1.

A59G: Switch I in yellow; L2 (Switch II) in green

RAB28A: Switch I in wheat; L2 (Switch II) in magenta (2HXS)

ARF1: Switch I in orange; L2 (Switch II) in marine (1HUR)

			<u>G2</u>	<u>G3</u>
RAS Family	HRAS	-----	YDPTIEDSY	----- LDILDTAGQ
	NRAS	-----	YDPTIEDSY	----- LDILDTAGQ
	KRAS2B	-----	YDPTIEDSY	----- LDILDTAGQ
	ERAS	-----	YDPTIEDSY	----- LNVLDTAGQ
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RHO Family	RAC1	-----	YIPTVFDNY	----- LGLWDTAGQ
	RHOJ	-----	YVPTVFDHY	----- LGLYDTAGQ
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RAB Family	RAB28	-----	YKQTIGLDF	----- LQIWDIGGQ
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ARF Family	ARF1	-----	TIPTIGFNV	----- FTVWDVGGQ

**Figure S9.** Alignment of RAS superfamily members. NPT and DXXG motifs are highlighted in red and green respectively.