

SUPPLEMENTARY FIGURES

Differential effects of KRAS G-domain and hypervariable region mutants on cancer phenotypes

James Allen D. de Borja¹, Kim Denyse J. Hao Lin¹, Daniel Angelo R. Mirador^{1,†}, Alexandra Isabelle D. Ang^{1,†}, Sidney Allen A. Chua^{1,†}, Joanne Marie D. Sytangco^{1,†}, Dennis L. Sacdalan^{1,2}, and Reynaldo L. Garcia^{1,*}

¹Disease Molecular Biology and Epigenetics Laboratory, National Institute of Molecular Biology and Biotechnology, University of the Philippines Diliman, Quezon City 1101, Philippines

²Division of Medical Oncology, Department of Medicine, University of the Philippines, Manila City, 1000, Philippines

[†] These authors contributed equally to this work

*Correspondence: rlgarcia1@up.edu.ph

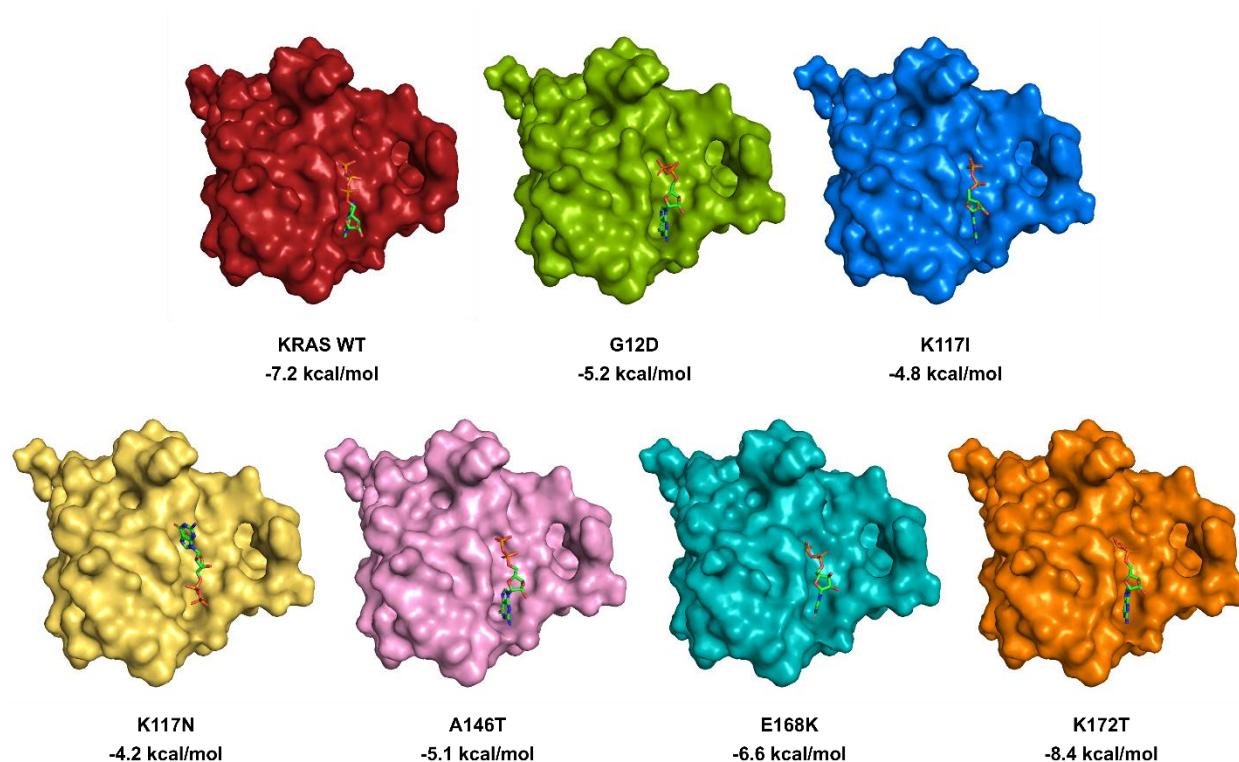


Figure S1. K-Ras-GDP docking. Generated homology structures of the K-Ras mutants are used as receptors to dock the GDP ligand using AutoDock Vina. Docked configurations represent GDP binding to the nucleotide binding pocket as initially present in the published structure of wild type K-Ras (PDB ID: 5TAR). Indicated below are the calculated ΔG values for each structure. Docked configuration for K117N only show an inverted GDP. Colores: K-Ras WT – red, G12D – green, K117I – blue, K117N – yellow, A146T – pink, E168K – cyan, K172T – orange.

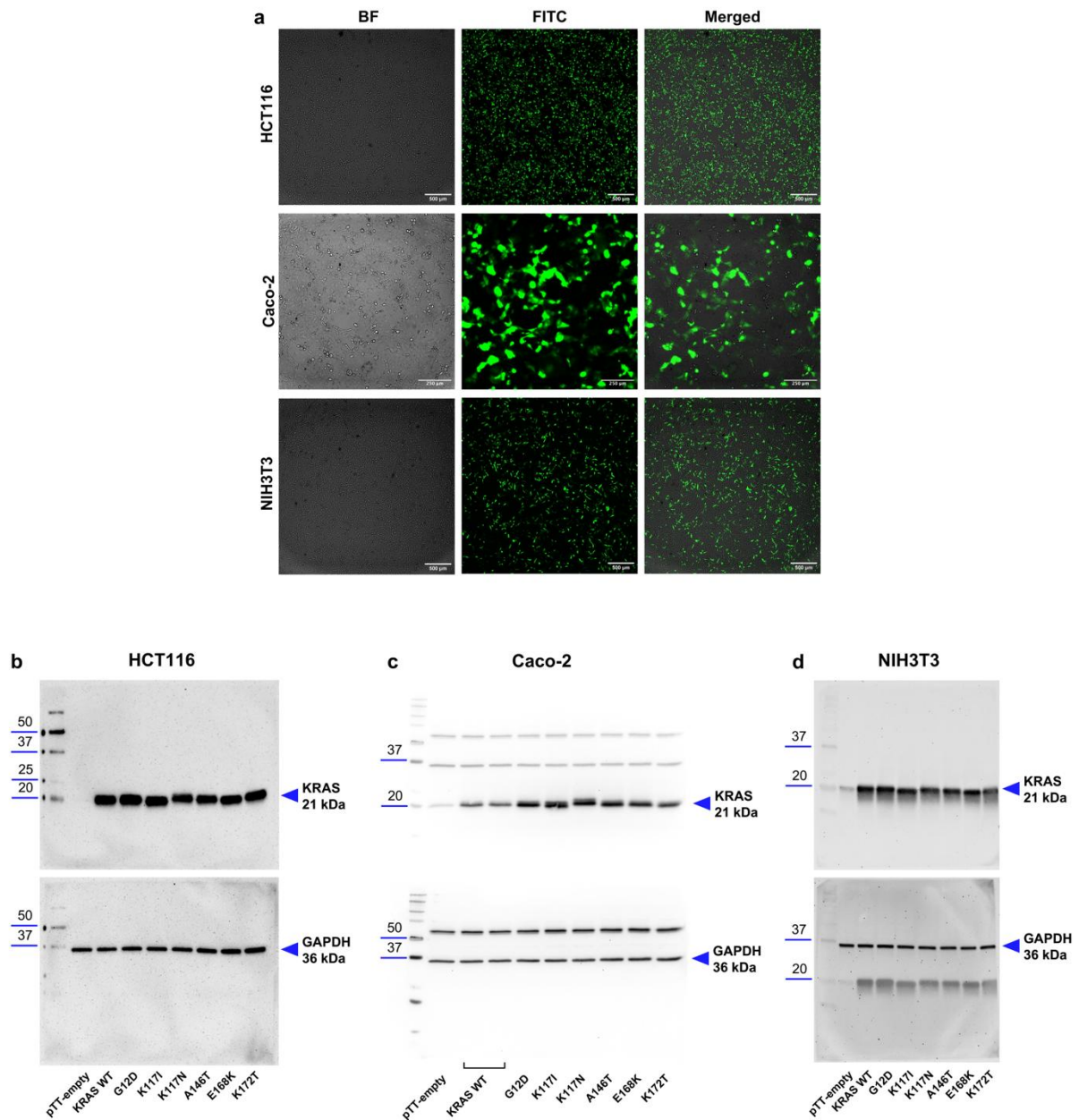


Figure S2. Verification of KRAS expression via western blot analysis. (a) ZsGreen1 expression (FITC) in HCT116, Caco-2, and NIH3T3 cells was used to estimate transfection efficiency through a parallel transfection setup (brightfield, BF). Photomicrographs for transfection efficiency were taken 24 h post-transfection and were obtained under 4X magnification for HCT116 and NIH3T3 and 10X magnification for Caco-2. Scale bar: 500 μ m for HCT116 and NIH3T3, 250 μ m for Caco-2 (b) KRAS is a 21 kDa monomer detected at 48 h post-transfection in HCT116, Caco-2, and NIH3T3 cell lines. The blot was also probed for GAPDH, which served as an internal loading control.

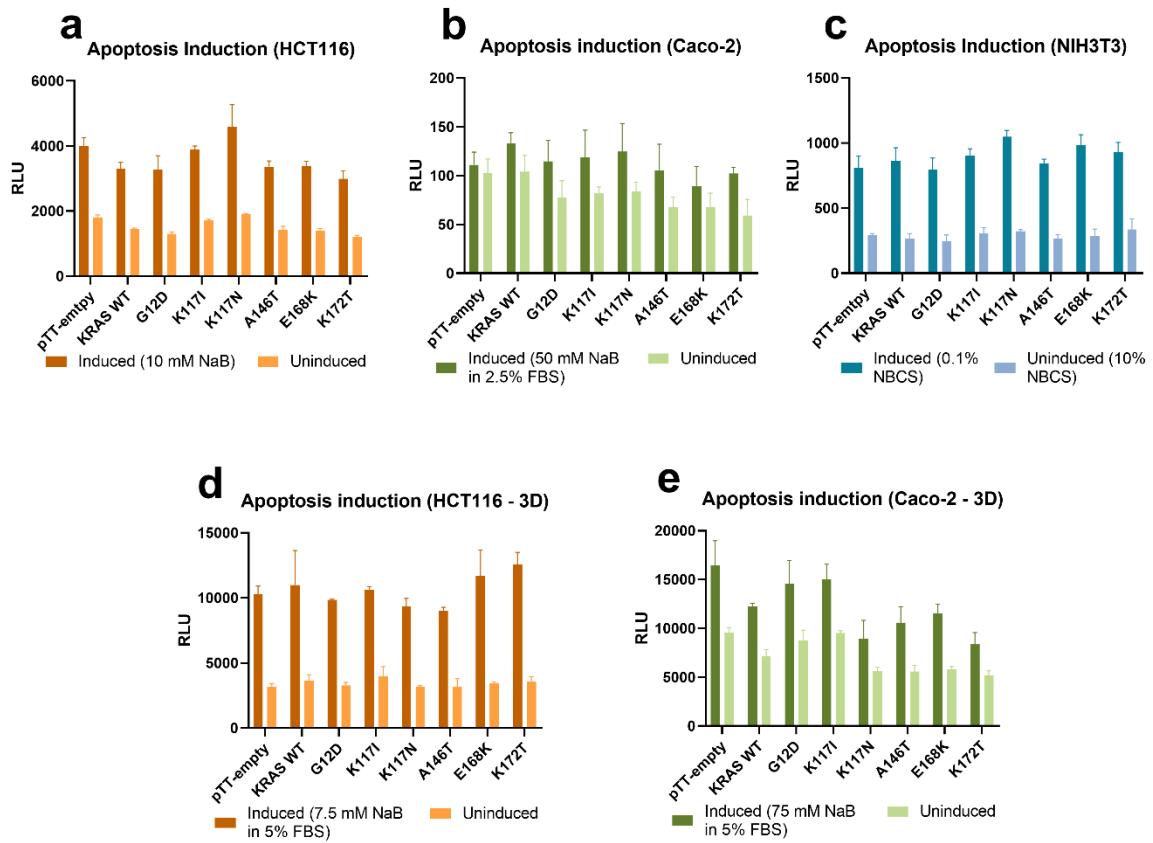


Figure S3 Successful apoptosis induction was determined by an increase in luminescence signal in induced setups versus uninduced setups. In the 2D apoptosis assays, apoptosis induction was performed by treatment with (a) 10 mM NaB to HCT116 cells, (b) 50mM NaB with serum reduction to 2.5% FBS to Caco-2 cells, and (c) serum reduction to 0.1% NBCS to NIH3T3 cells. For the 3D apoptosis assays, apoptosis induction was done by treatment of (d) 7.5mM NaB with serum reduction to 5% FBS to HCT116 spheroids and (e) 75mM NaB with serum reduction to 5% FBS to Caco-2 spheroids. Shown are representatives from three independent trials performed, each done in triplicate. NaB: sodium butyrate.

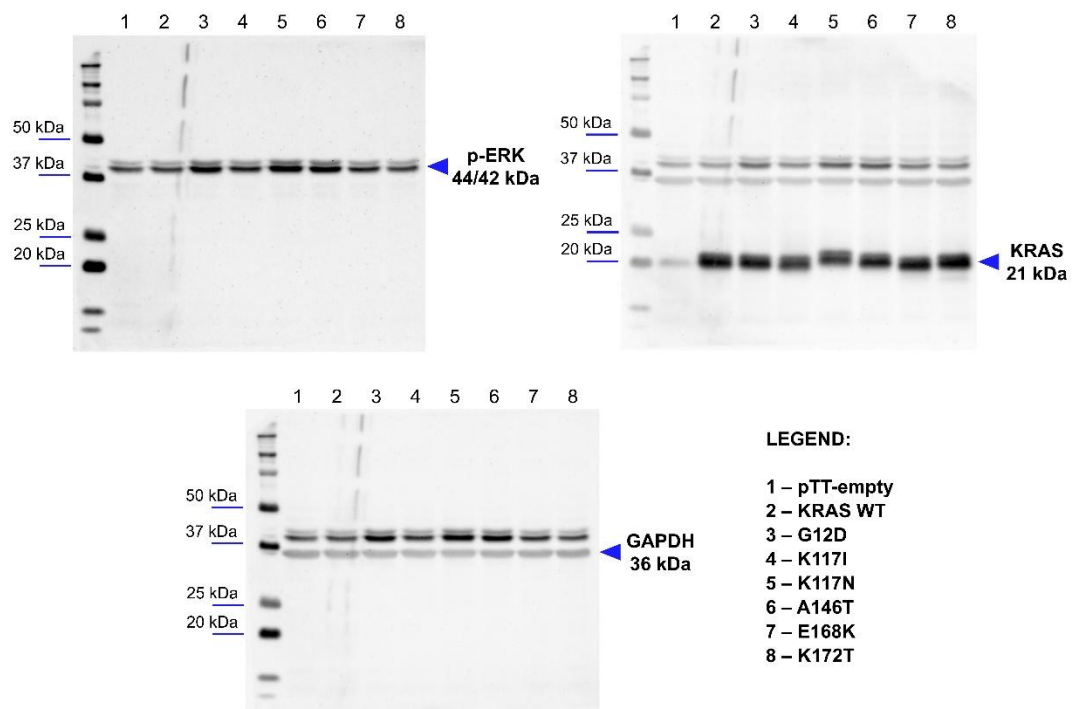
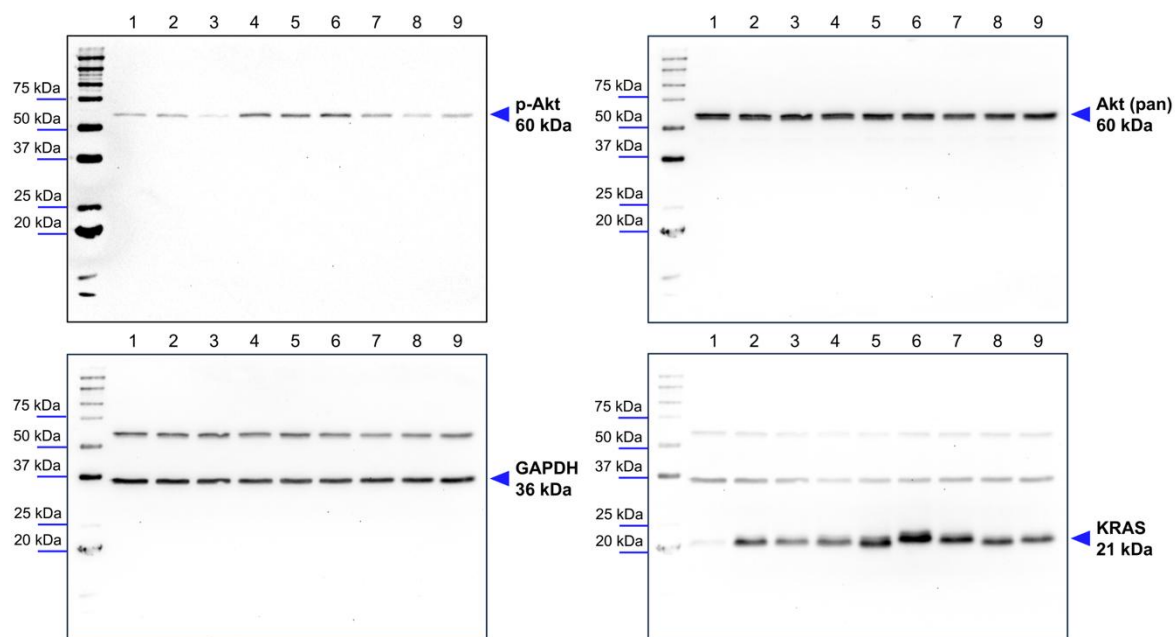


Figure S4. Phospho-ERK (p-ERK) detection by western blot analysis. Approximately 30 μ g of protein from total HCT116 lysates was run 48 h post-transfection. p-ERK and KRAS was detected at 44/42 kDa and at 21 kDa, respectively. GAPDH (36kDa) was also probed as an internal loading control.



LEGEND:

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|------------------------|-----------|
| 1 – pTT-empty | 6 – K117N |
| 2 – KRAS WT stimulated | 7 – A146T |
| 3 – WT unstimulated | 8 – E168K |
| 4 – G12D | 9 – K172T |
| 5 – K117I | |

Figure S5. Phospho-Akt (p-Akt) detection by western blot analysis. After transfection, Caco-2 cells were starved for 24 h and treated with 10 ng epidermal growth factor (EGF) for 10 minutes before proceeding with protein extraction. Approximately 30 μ g of protein from total Caco-2 lysates was loaded for each setup. Bands for p-Akt and Akt (pan) were detected at 60kDa while KRAS were detected at 21 kDa. GAPDH (36 kDa) was probed as an internal loading control. A KRAS WT unstimulated setup was added as an additional control to ensure EGF stimulation was successful.