Supplementary Materials for:

Sotorasib is a pan-RAS^{G12C} inhibitor capable of driving clinical response in *NRAS*^{G12C} cancers

Douglas A. Rubinson[1]*, Noritaka Tanaka[2]*, Ferran Fece de la Cruz[2], Kevin S. Kapner[1,3], Michael H. Rosenthal[4], Bryanna L Norden[2], Haley Barnes[2], Sara Ehnstrom[2], Alvin A. Morales-Giron[2], Lauren K. Brais[1], Christopher T. Lemke[3], Andrew J. Aguirre[1,3#], Ryan B. Corcoran[2#]

* Denotes equal contribution

Co-corresponding authors

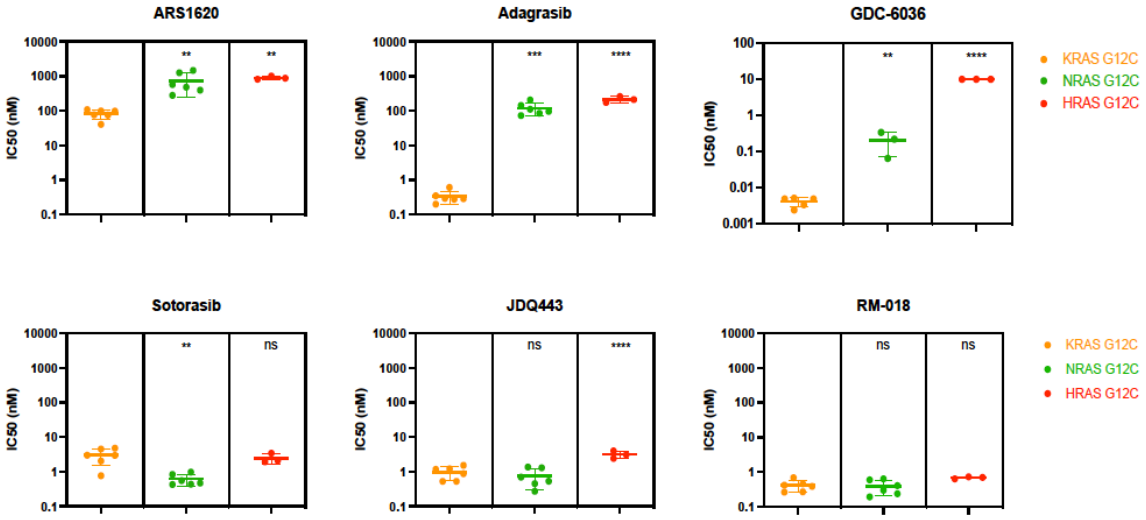
[1] Dana Farber Cancer Institute and Department of Medicine, Harvard Medical School, Boston, MA USA

[2] Massachusetts General Hospital Cancer Center and Department of Medicine, Harvard Medical School, Boston, MA USA

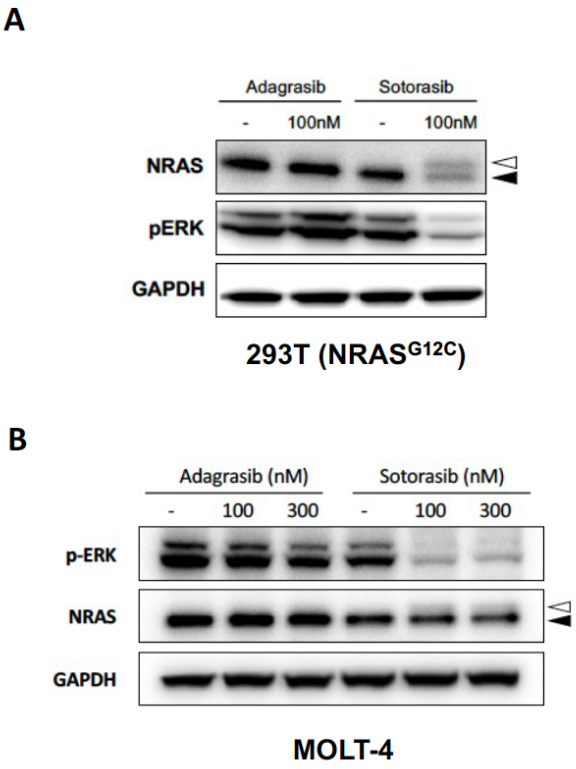
[3] The Broad Institute of Harvard and MIT, Cambridge, MA

[4] Dana Farber Cancer Institute and Brigham and Women's Hospital, Department of Radiology, Boston, MA USA; Harvard Medical School, Boston, MA USA

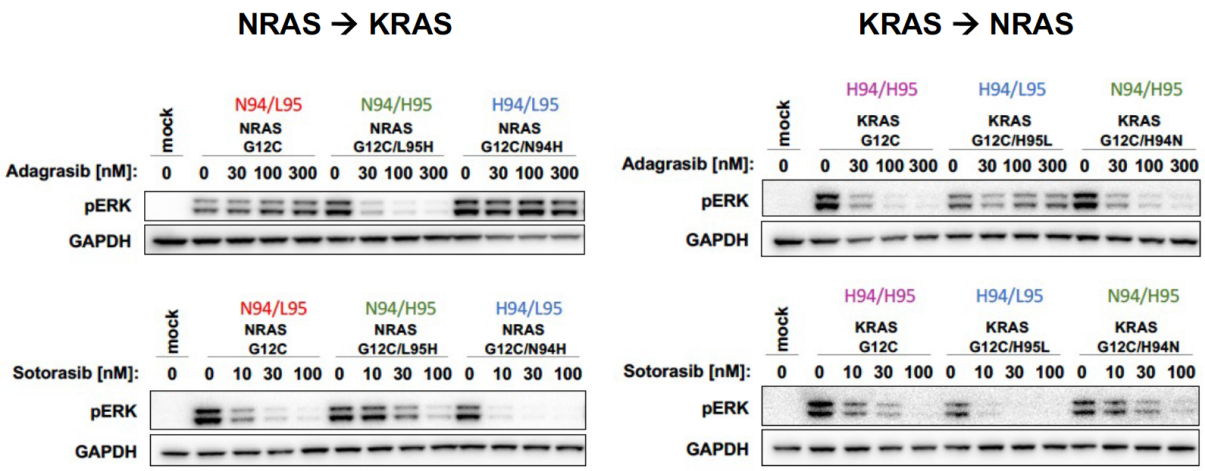
Contents:



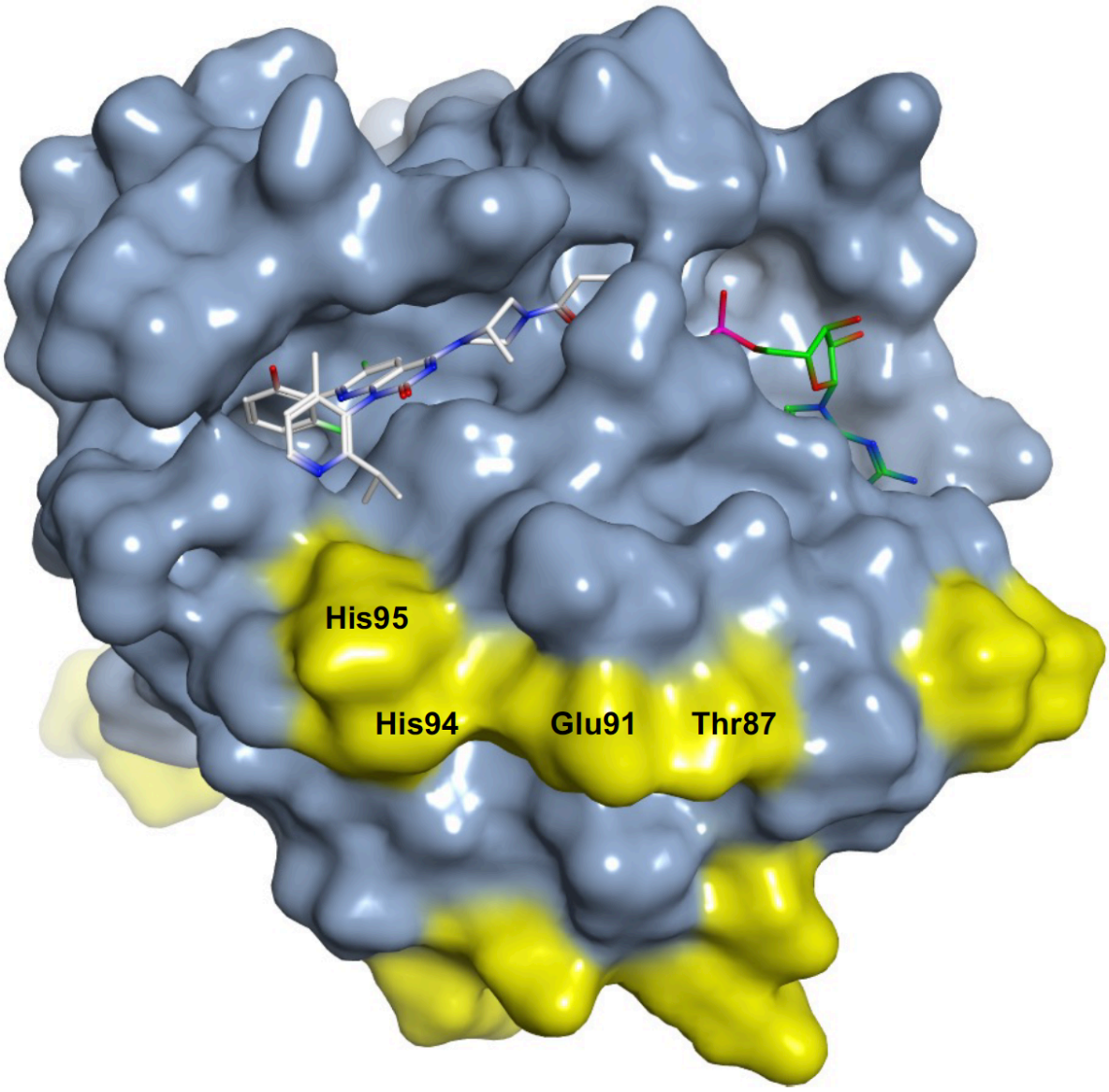
Supplementary Figure S1: IC50 values of KRAS^{G12C} inhibitors for KRAS^{G12C}, NRAS^{G12C}, HRAS^{G12C}. IC50 values were calculated from the experiments in Figure 1, in which Ba/F3 cells engineered to express either KRAS^{G12C}, NRAS^{G12C}, or HRAS^{G12C} were treated for 72 hours in the absence of IL-3 with various concentrations of the indicated inhibitors. The individual values from multiple biological replicates and their averages are shown. ns = not significant, ** = p<0.01, *** = p<0.001, **** = p <0.0001.



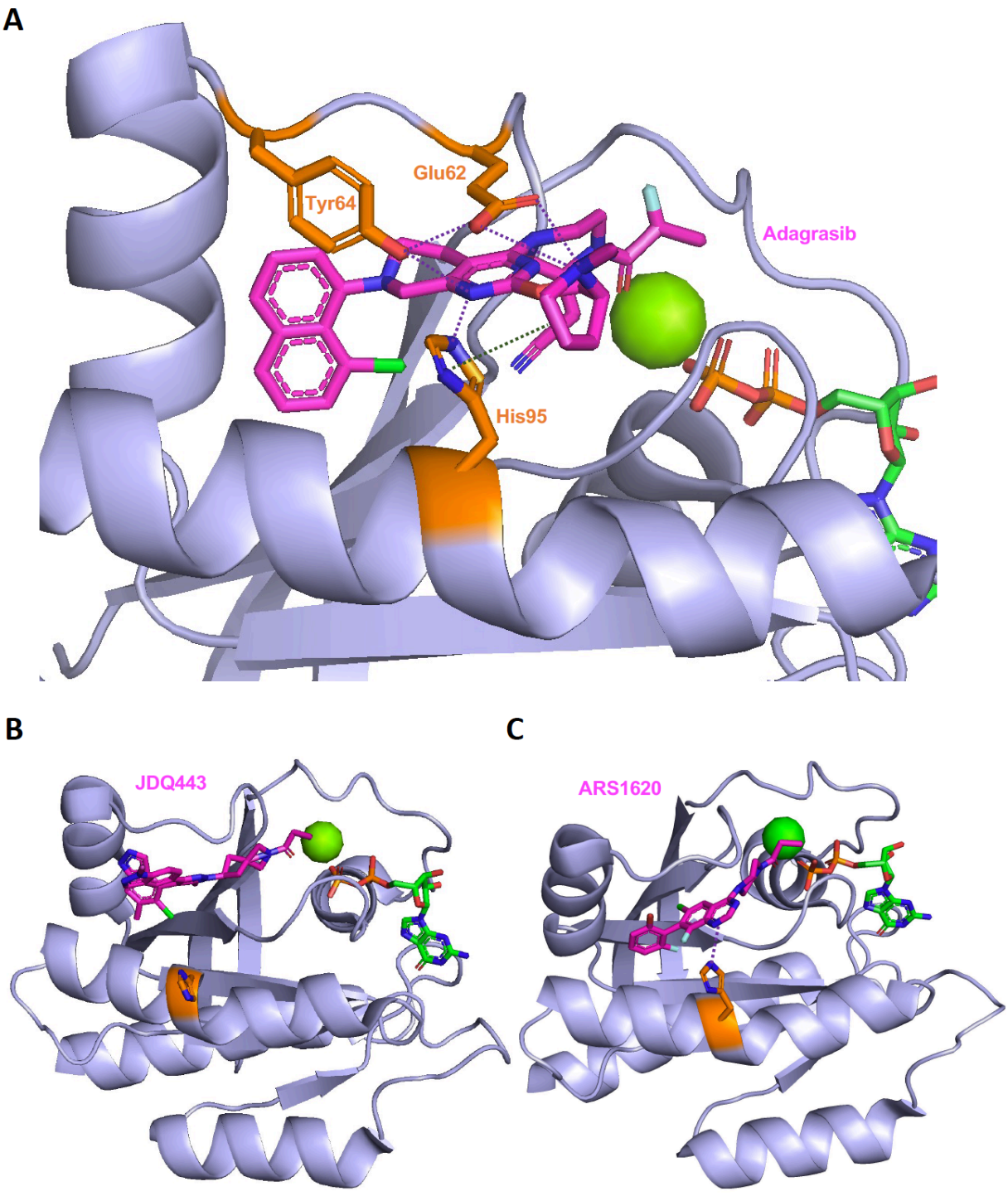
Supplementary Figure S2: Inhibition of NRAS^{G12C} by sotorasib in 293T and MOLT-4 cells. (A) 293T cells engineered to express NRAS^{G12C} or (B) MOLT-4 cells which harbor an endogenous NRAS^{G12C} allele were treated for 4 hours with the indicated concentrations of adagrasib or sotorasib prior to lysis and western blotting. In blots performed with an NRAS-specific antibody, black arroheads indicate unmodified NRAS and white arrowheads indicate covalently modified NRAS^{G12C}, which runs at a higher molecular weight.



Supplementary Figure S3: Effect of amino acid substitutions in NRAS^{G12C} or KRAS^{G12C} on signaling in response to sotorasib and adagrasib. Reciprocal mutagenesis studies were performed to explore the effects of substituting the amino acids at the 94 and 95 positions within the switch-II binding pocket, with NRAS amino acids substituted into KRAS^{G12C} or KRAS amino acids substituted into NRAS^{G12C}. Constructs were expressed in 293T cells and treated for 4 hours with the indicated inhibitors prior to lysis and western blotting.

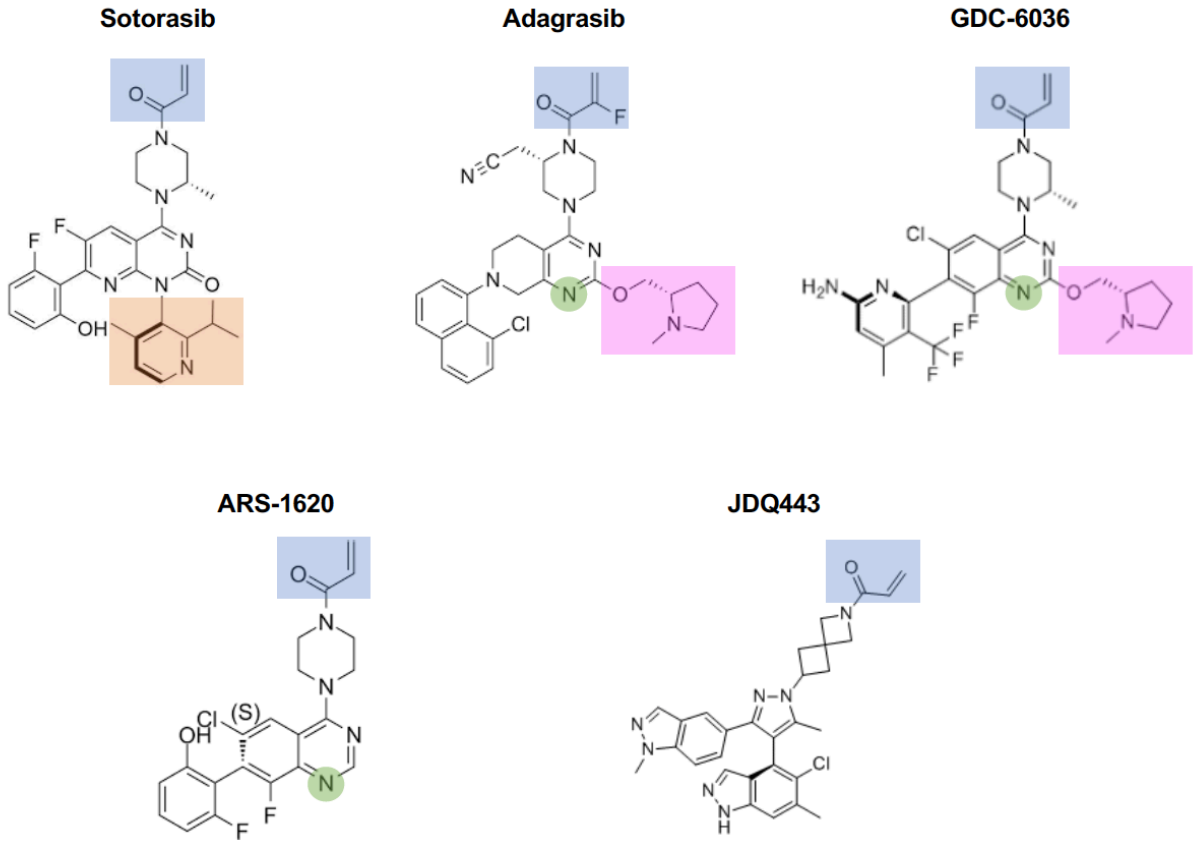


Supplementary Figure S4: Location of isoform-variable residues relative to Switch-II binders. Surface representation of sotorasib (white) and GDP (green) bound to $KRAS^{G12C}$ (PDB 6OIM). Yellow patches indicate surfaces corresponding to isoform variable residues. Labels indicate approximate positions of the key residues evaluated in this study.

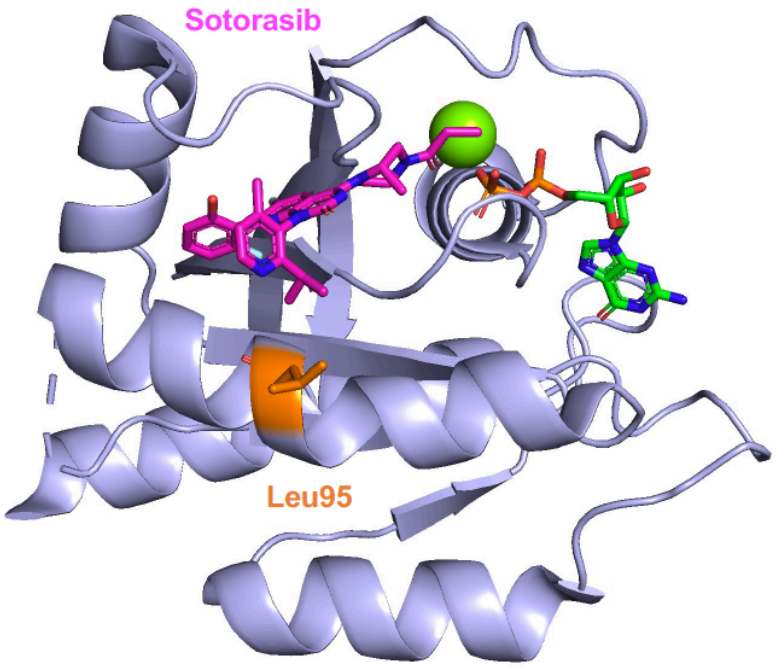


Supplementary Figure S5: Crystal structures of KRAS^{G12C} inhibitors and interactions with His95. (A) Specific interactions between adagrasib (magenta) and His95 are shown, with key amino acids shown in orange. Hydrogen bonding network

between adagrasib and His95, Glu62, and Tyr64 is shown in dark violet and other interactions are shown in dark green. GDP and a conserved Mg ion are shown in green. (B, C) Crystal structures of JDQ443 (B, 7R0M) and ARS1620 (C, 5V9U) bound to $KRAS^{G12C}$ are shown with distinct interactions with His95 (orange). Each inhibitor is colored magenta, and GDP and a conserved Mg ion are shown in green, and key hydrogen bonds are shown in purple.



Supplementary Figure S6: Chemical structures of switch-II pocket-binding KRAS^{G12C} inhibitors. The chemical structures of each of the five switch-II pocket inhibitors are shown with colored labels to highlight shared structural features. The blue rectangles indicate the acrylamide-based covalent warheads that undergo irreversible reaction with the mutant cysteine. The green circle highlights the unsubstituted core nitrogen capable of interacting directly with His95 that is present in the three inhibitors that form direct hydrogen bonds with His95. The magenta rectangle indicates the shared N-methyl-pyrrolidine substituent shared by adagrasib and GDC-6036, which contrasts with the isopropyl-pyridine substituent of sotorasib highlighted in orange. The structure of JD443, which is highly unrelated to the other inhibitors tested is also shown.



Supplementary Figure S7: Structural model of sotorasib interaction with Leu95. Molecular modeling of sotorasib (magenta) bound to $KRAS^{G12C}$ (derived from 6OIM) with the Leu95 residue present in NRAS introduced in place of His95, indicated in orange, illustrating a potential hydrophobic interaction between Leu95 and the isopropyl-pyridine substituent of sotorasib. GDP and a conserved Mg ion are shown in green.