Oncogenic *NTRK3* mutations exhibit differential sensitivity to tropomyosin receptor kinase inhibitors in patients with acute myeloid leukemia

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NTRK-Experiment-19. All other data are provided in the manuscript.

SUPPLEMENTAL DOCUMENT:

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SUPPLEMENTAL FIGURE & TABLE LEGENDS:

Supplemental Figure 1: *NTRK3* mutations lie in regions that are relatively conserved in receptor tyrosine kinases. A. The sequence conservation among receptor tyrosine kinases was ascertained using ConSurf-DB[1], a repository for the evolutionary conservation patterns of a majority of PDB proteins. The analysis was performed using the Ntrk3_human (Uniprot ID: Q16288) and was displayed on the protein structure (PDB ID: 6KZD) using PyMol. Tube thickness indicates the highest conservation score. P792 is relatively more conserved than L568. **B-C.** Larotrectinib (**B**) and repotrectinib (**C**) were docked *in silico* on the active conformation (Aspartate-Phenylalanine-Glycine (DFG)-in) of the Wild type (WT) TrkC receptor to study extent of local interactions. Purple boxes at bottom right corner indicate aggregate interaction scores. A higher score correlates with enhanced interactions between TrkC receptor and respective inhibitor. Four different interactions are highlighted: hydrophobic (green), cation- π (blue), π-π (red), and ionic (pink). The cation-π interaction is formed by the proximity of a positively charged residue (Lys, Arg) with aromatic residues (Phe, Trp, and Tyr).

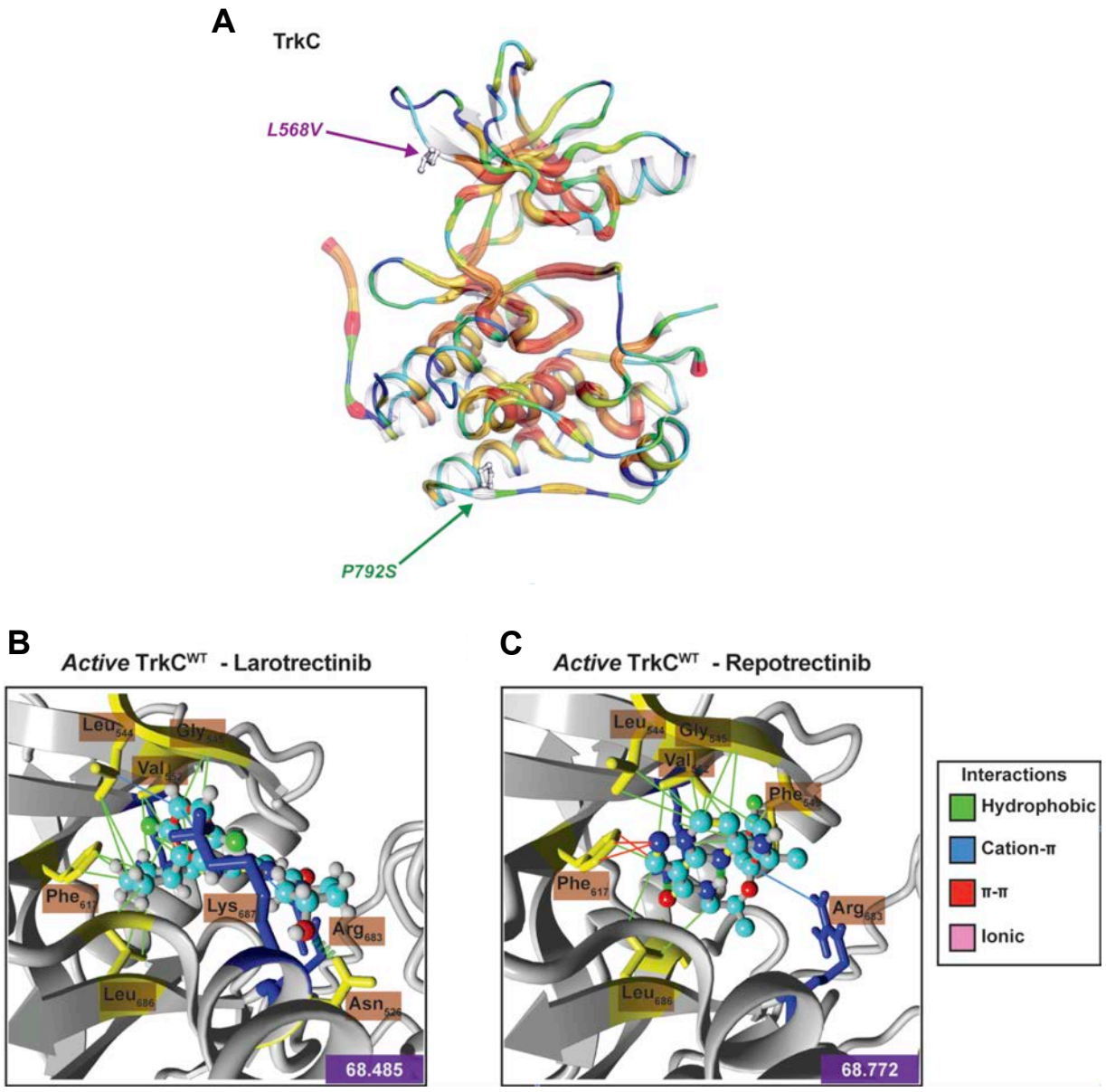
Supplemental Figure 2: GO Biological Process annotation of up and down regulated pathways identified in NTRK3 mutant expressing cells. Enrichment scores of the top eight Go Biological Processes (GOBP) identified as uniquely upregulated or downregulated in $NTRK3^{L568V}$ (**A**), and $NTRK3^{P792S}$ (**B**) expressing cells when peptides from $NTRK3^{WT}$ expressing cells have been removed. Significance = adjusted p-value.

Supplemental Table 1: Tab 1: Patient characteristics. *NTRK3* mutation information, VAF, Variant Allele Frequency; CLIA, Clinical Laboratory Improvement Amendment; **Tab 2: Global Summary**. Total unique genes identified in differential protein expression analysis comparing *NTRK3* mutants to *NTRK* wildtype and empty vector control; **Tab 3: Inhibitor Summary Screen**. Summary of IC_{50} values for inhibition of *NTRK3* mutant-transformed Ba/F3 cell growth by small-molecule inhibitors calculated from data presented in **Figures 3B-F**; **Tab 4: Dissociation Constants for Docking Experiment.** Summary of estimated dissociation constants between larotrectinib and repotrectinib for inactive and active conformations of $TrkC^{WT}$, $TrkC^{L568V}$, and $TrkC^{P792S}$. **Tabs 5 to 9: Full Proteomic Data from Ba/F3 Engineered Cell Lines.** Proteomic data from Ba/F3 cell lines engineer to express pMX-IRESpuro vector control, *NTRK3* wild type ($NTRK3^{WT}$), $NTRK3^{L568V}$, or $NTRK3^{P792S}$. **(Please, see Excel file)**

REFERENCES:

1. Ben Chorin A, Masrati G, Kessel A, et al. ConSurf-DB: An accessible repository for the evolutionary conservation patterns of the majority of PDB proteins. Protein Sci. 2020;29(1):258-267.

Supplemental Figure 1



Supplemental Figure 2