**Supplemental Data JPET #262188**

# Next-Generation Cell-active Inhibitors of the Undrugged Oncogenic PTP4A3 Phosphatase*

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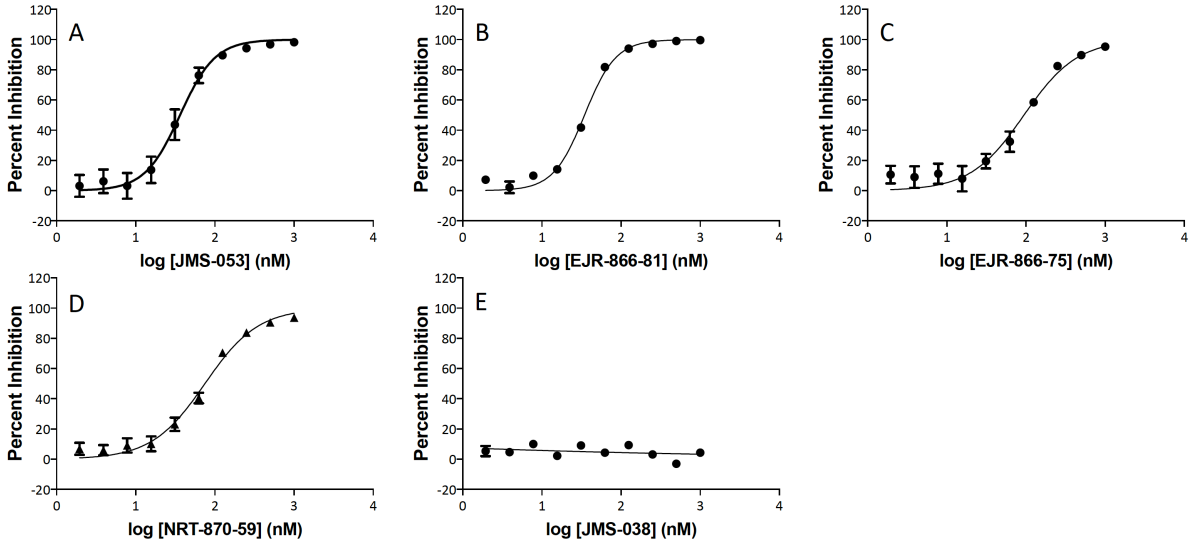
**Supplemental Table 1.** Mutations in breast and ovarian cancer cell lines

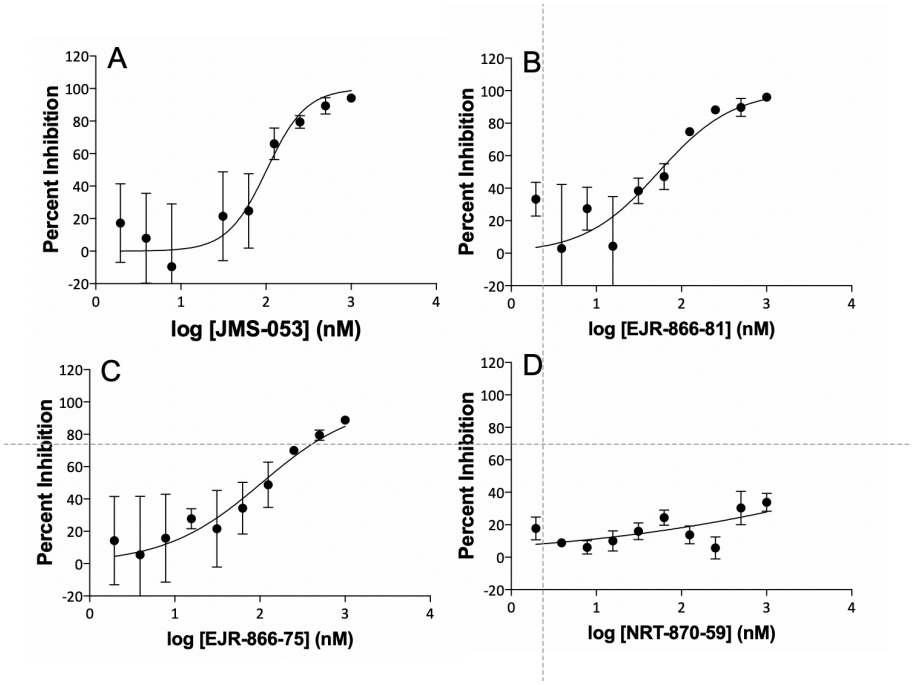
| Cell Type | TP53 | HRAS | KRAS | IL6R |
|---|---|---|---|---|
| MDA-MB-231 | R280K | NR | G13D | NR |
| Hs578T | V157F | G13D | NR | NR |
| OVCAR4 | L130V | NR | NR | NR |
| Kuramochi | D281Y | NR | NR | P431L |

These data were obtained from the Cancer Cell Line Encyclopedia (https://portals.broadinstitute.org/ccle) dated September 2019. NR = none recorded.

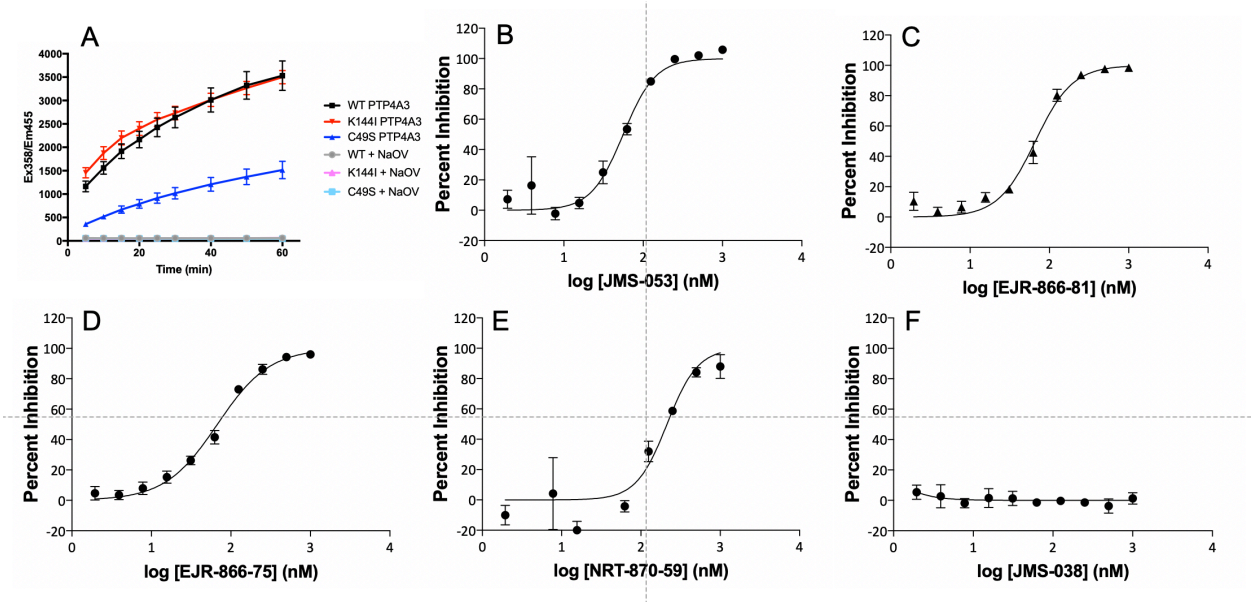
## Supplemental Figures



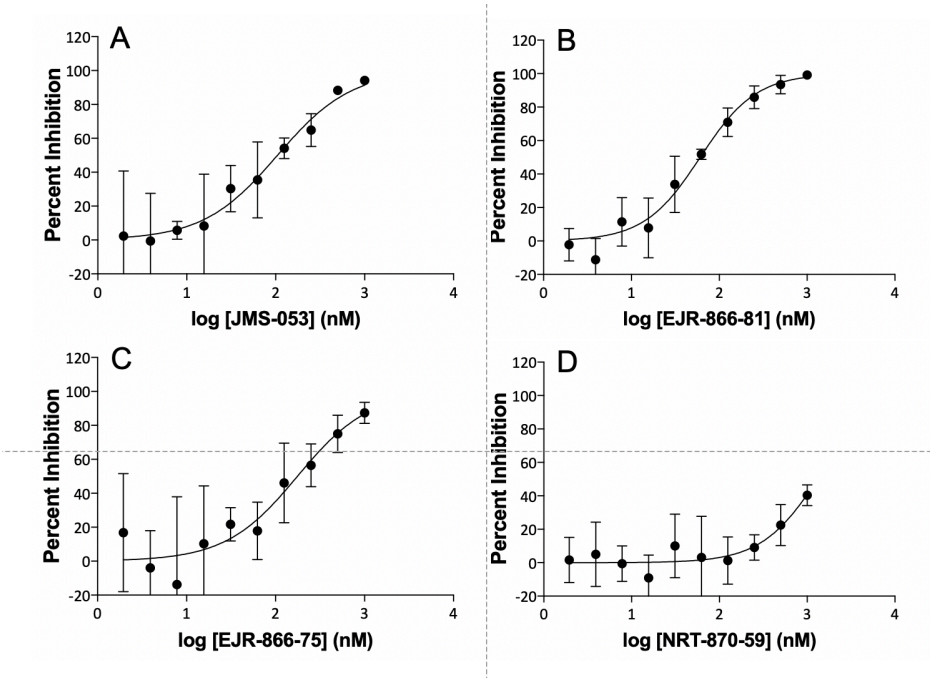

**Supplemental Figure 1.** Small molecule inhibitor PTP4A3 concentration-response curves. Panel A. JMS-053. Panel B. EJR-866-81. Panel C. EJR-866-75. Panel D. NRT-870-59. Panel E. JMS-038. N=3. Bars = SEM unless smaller than the symbol.



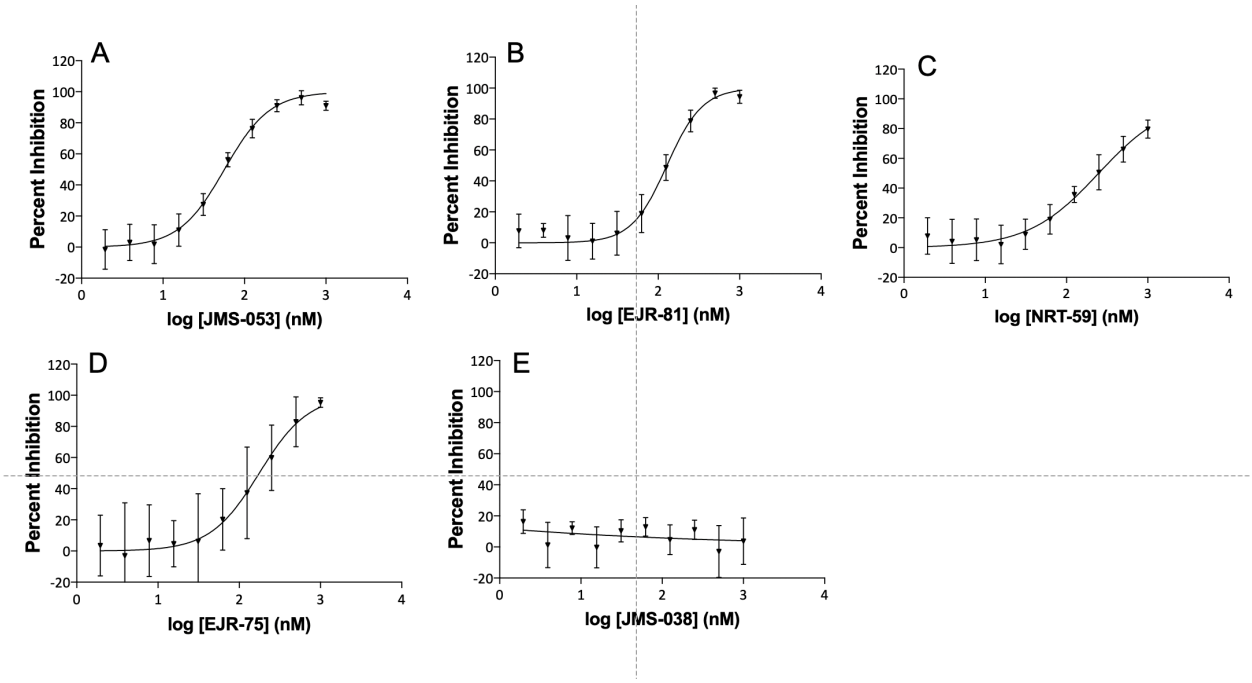

**Supplemental Figure 2.** CDC25B concentration-response curves. Panel A. JMS-053. Panel B. EJR-866-81. Panel C. EJR-866-75. Panel D. NRT-870-59. N=3. Bars = SEM unless smaller than the symbol.



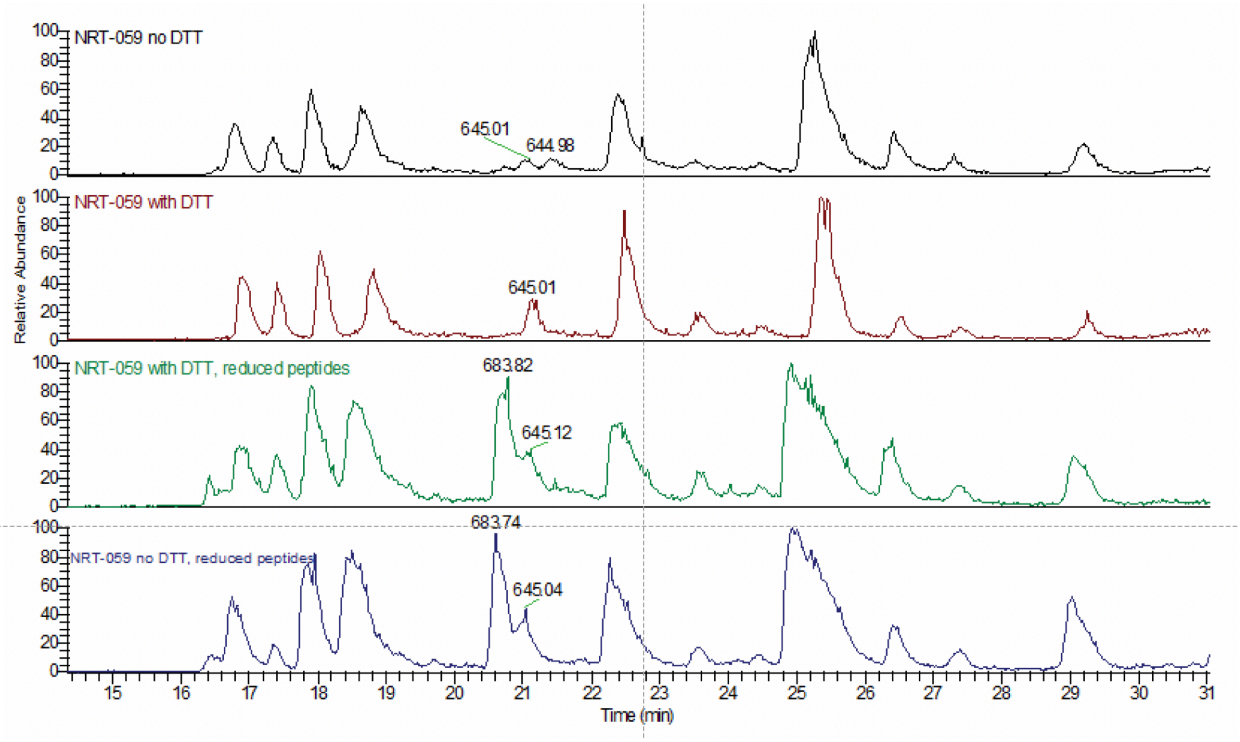

**Supplemental Figure 3.** Mutant enzymatic activity and PTP4A3 K144I mutant analog concentration response curves. Panel A. Enzymatic activity of PTP4A3 mutants and sensitivity to NaOV. ■ - wildtype PTP4A3; ▼ - K144I PTP4A3; ▲ - C49S PTP4A3; ● - wildtype PTP4A3 with NaOV; △ - K144I PTP4A3 with NaOV; □ - C49S PTP4A3 with NaOV. Panel B. JMS-053 inhibition of PTP4A3 K144I. Panel C. EJR-866-81 inhibition of PTP4A3 K144I. Panel D. EJR-866-75 inhibition of PTP4A3 K144I. Panel E. NRT-870-59 inhibition of PTP4A3 K144I. Panel F. JMS-038 lack of inhibition of PTP4A3 K144I. N=3. Bars = SEM unless smaller than the symbol.




**Supplemental Figure 4.** PTP4A3 A111S mutant concentration-response curves. Panel A. JMS-053. Panel B. EJR-866-81. Panel C. EJR-866-75. Panel D. NRT-870-59. N=2. Bars = range unless smaller than the symbol.

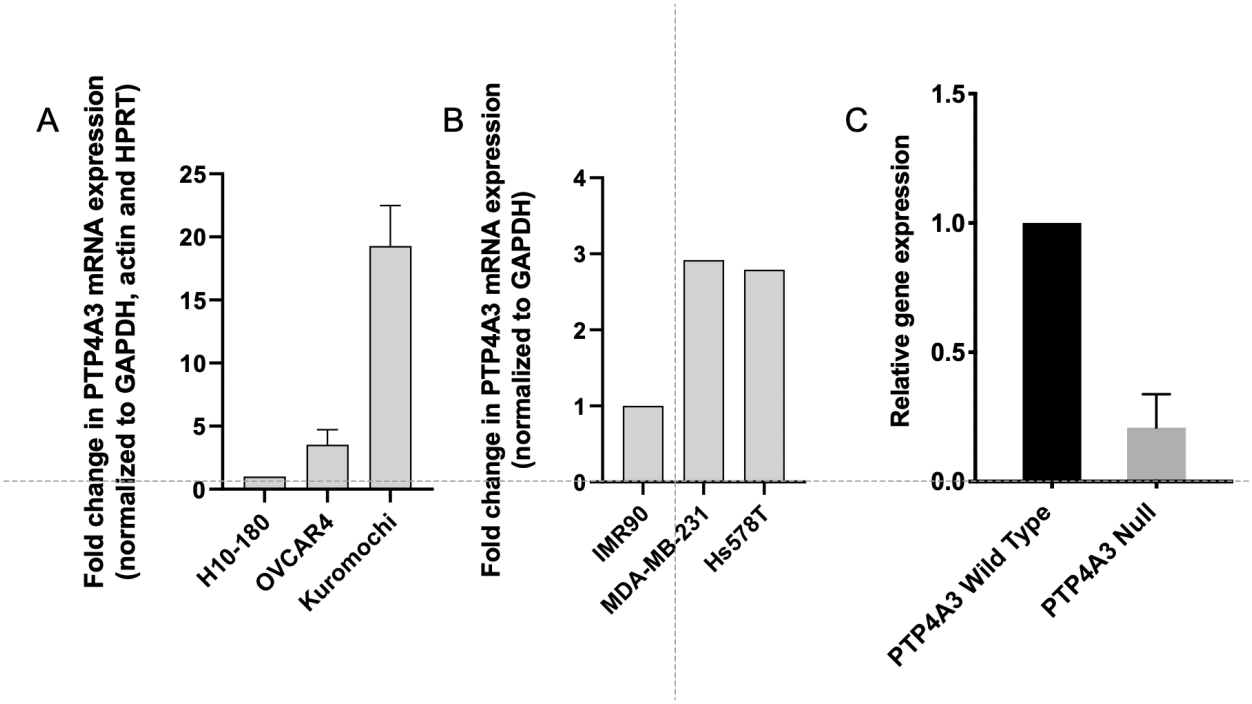



**Supplemental Figure 5.** C49S PTP4A3 concentration-response curves. Panel A. JMS-053. Panel B. EJR-866-81. Panel C. EJR-866-75. Panel D. NRT-870-59. Panel E. JMS-038. N=3. Bars = SEM unless smaller than the symbol.

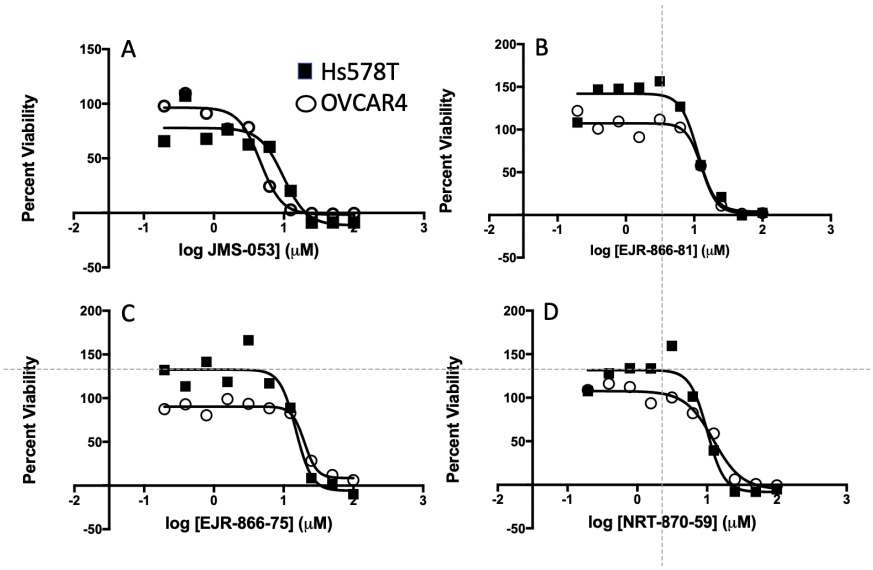



**Supplemental Figure 6.** PTP4A3 treatment with NRT-059 in reducing and non-reducing conditions. After treatment, half the samples were subject to additional reduction and alkylation with iodoacetamide, while the other half only received

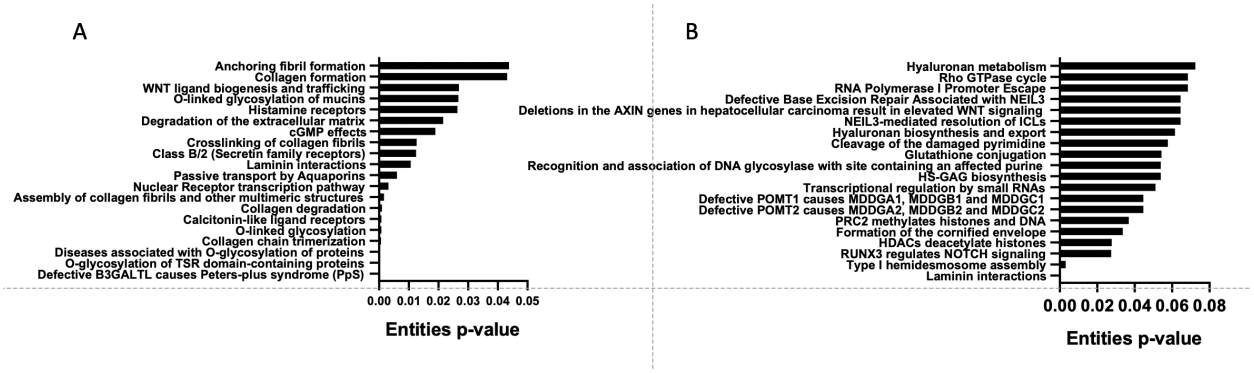
iodoacetamide treatment. MS1 peaks of a mass between 644.90-645.10 were found once again for the peptide containing the catalytic cysteine residue, and no additional peaks were found to indicate a disulfide bond between C49 and C104.




**Supplemental Figure 7.** Expression of PTP4A3 in human and murine cancer cells. PTP4A3 expression was determined by real-time quantitative polymerase chain reaction and normalized to GAPDH, actin and HPRT for human cells and HPRT for murine cells. Panel A. PTP4A3 mRNA levels in nonmalignant human ovarian epithelial H10-180 cells, OVACR4 and Kuramochi ovarian cancer cells. N=3-15 samples; bars = SEM. Panel B. PTP4A3 mRNA levels in nonmalignant human IMR90 fibroblasts. N=1. Panel C. PTP4A3 mRNA levels in mouse PTP4A3 wild type and null cells. N=2, bar = range.




**Supplemental Figure 8.** Cytotoxicity of analogs to breast and ovarian cancer 72 h spheroid assay summary. Hs578T (☐) and OVCAR4 (○). Panel A. JMS-053. Panel B. EJR-866-81. Panel C. EJR-866-75. Panel D. NRT-870-59.




**Supplemental Figure 9.** Gene expression pathway analyses of mouse colon cancer cells after gene deletion or PTP4A3 inhibition. Panel A. Reactome pathway analysis of the mRNA transcripts over- or under-expressed in mouse colon cancer cells that contained or lacked PTP4A3. Panel B. Reactome pathway analysis of the mRNA transcripts over- or under-expressed in mouse colon cancer cells treated with vehicle or 1 μM JMS-053 for 24 h.