

Supplemental Material:

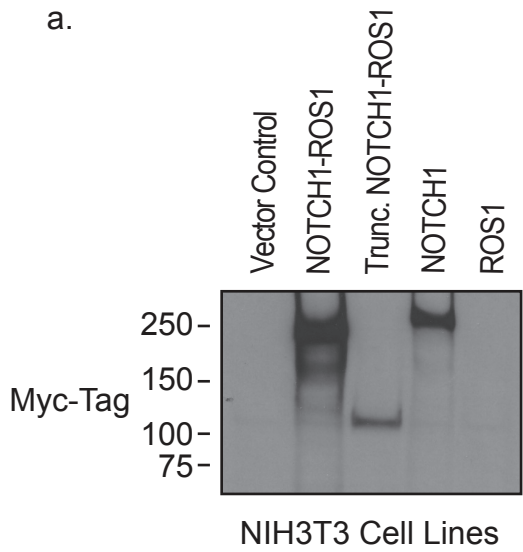
Supplementary Figure 1: NOTCH1-ROS1 fusion protein expressed in heterologous cell models show membrane localization and protein dimerization, neither of which are essential for oncogenicity. **(a)** Western blot showing stable expression of Myc-tagged vector control, NOTCH1-ROS1 and trunc. NOTCH1-ROS1 in NIH3T3 using anti-Myc tag antibody (top panel) and ROS1 antibody (bottom panel, high exposure of tROS1 panel in figure 3c.). **(b)** Western blot showing protein expression levels in BA/F3 cell model. **(c)** Biochemical membrane-cytosol fractionation to show localization of NOTCH1-ROS1 and Trunc. NOTCH1-ROS1 protein. ◀ represents NOTCH1-ROS1 (256 kDa); ◄ represents Trunc. NOTCH1-ROS1 (118 kDa); ◀◀ represents Cleaved NOTCH1-ROS1 (~75 kDa). **(d, e)** Co-immunoprecipitation assay to show homo-dimerization and hetero-dimerization of NOTCH1-ROS1 fusion and Trunc-NOTCH1-ROS1 proteins in HEK293 cells.

Supplementary Figure 2: Gene expression analysis using Nanostring PanCancer Pathways Panel. **(a)** Pathway Scoring analysis that clusters samples in an unbiased hierarchy. **(b)** NOTCH pathway specific gene signature map to show differential clustering of NOTCH1-ROS1 and Trunc. Notch1-ROS1. NIH3T3 cell models analyzed with and without serum, n=1 each.

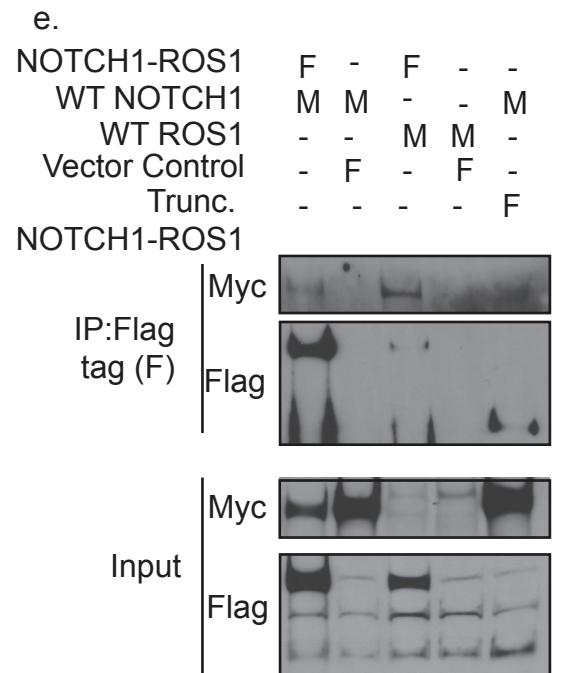
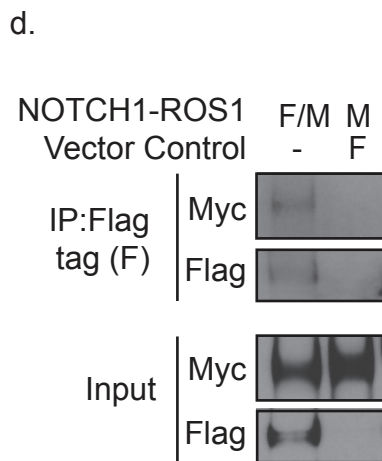
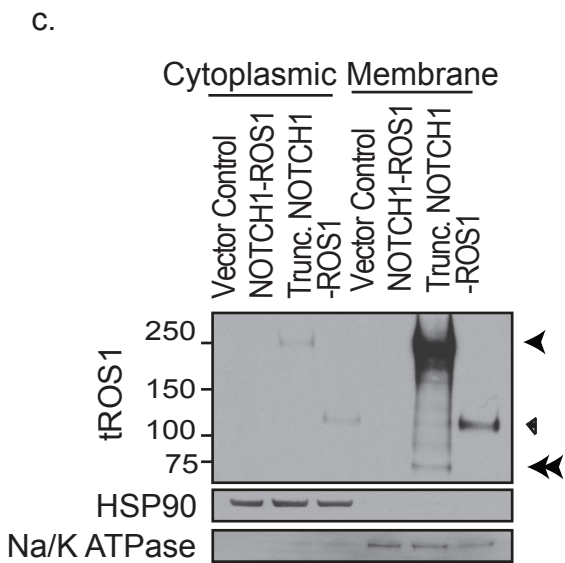
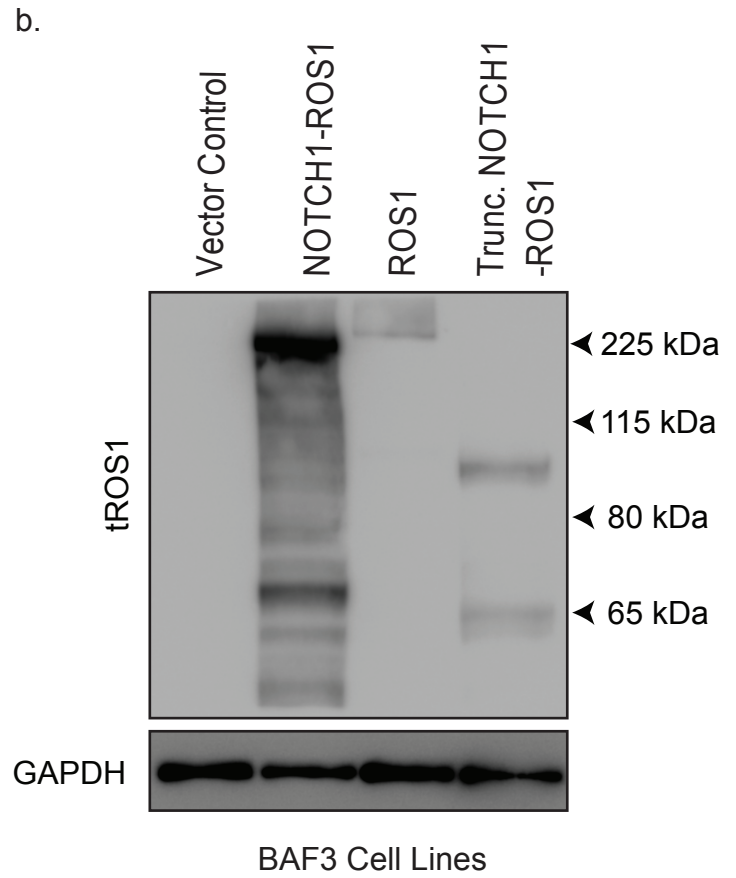
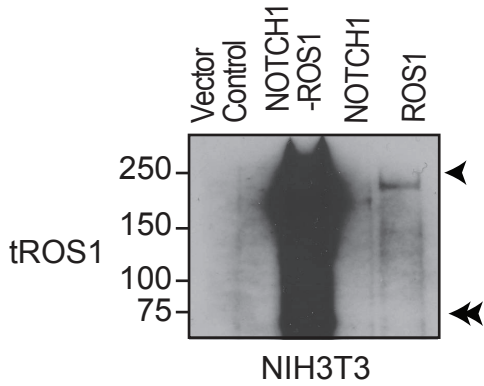
Supplementary Figure 3: Membrane localization of NOTCH1-ROS1 fusion in BA/F3 cells. Western blot analysis of the neutravidin pulldown of proteins expressed at the cell surface (after biotinylation). ◀ represents NOTCH1-ROS1 (256 kDa); ◀◀ represents Cleaved NOTCH1-ROS1 (~75 kDa).

Supplementary Figure 4: NOTCH1 inhibitors do not suppress NOTCH1-ROS1 signaling in NIH3T3 cell models. Western blot showing response of signaling pathways in NIH3T3 cells treated with (a) gamma secretase inhibitor, RO4929097 (b) ADAM 10/17 inhibitor, GW280264X , and (c) gamma secretase inhibitor, semagacestat.

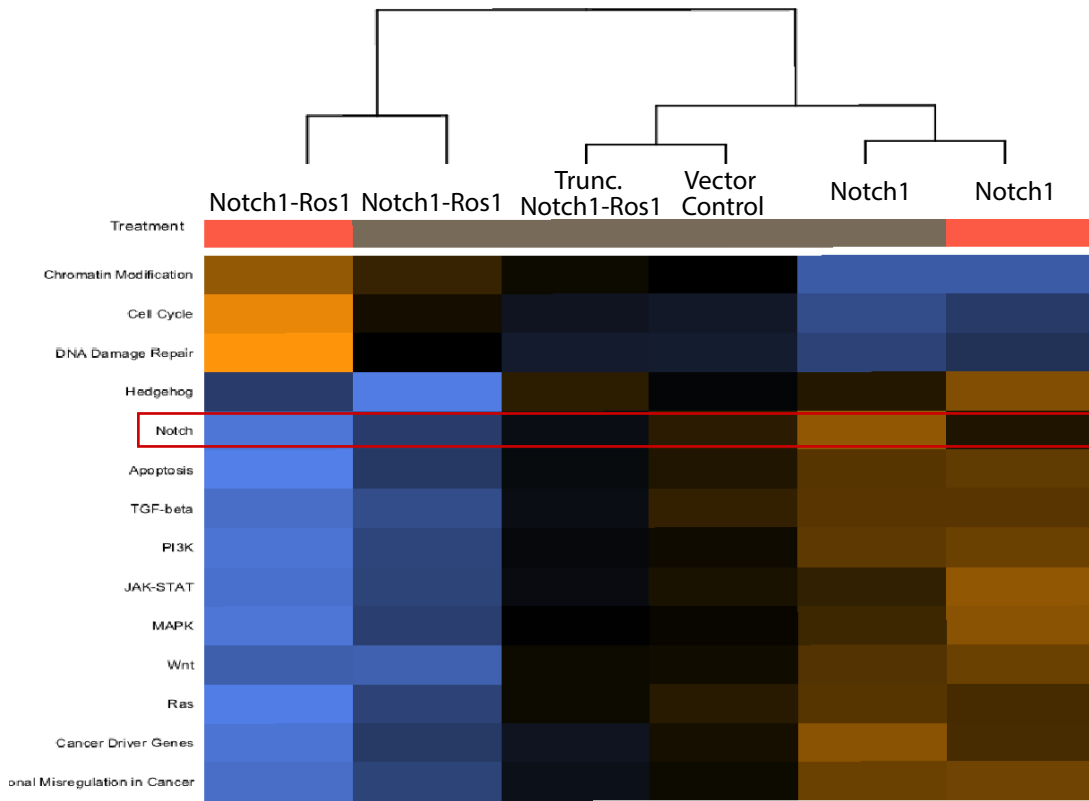
Supplementary Figure 5: ROS1 inhibitor Entrectinib tested in NOTCH1-ROS1 flank xenograft mouse model. (a) Kaplan Meier survival curves for NSG mice with NOTCH1-ROS1 expressing NIH3T3 and vector control cells and treatment with Entrectinib. (b) Pharmacokinetic (PK) study for Entrectinib.



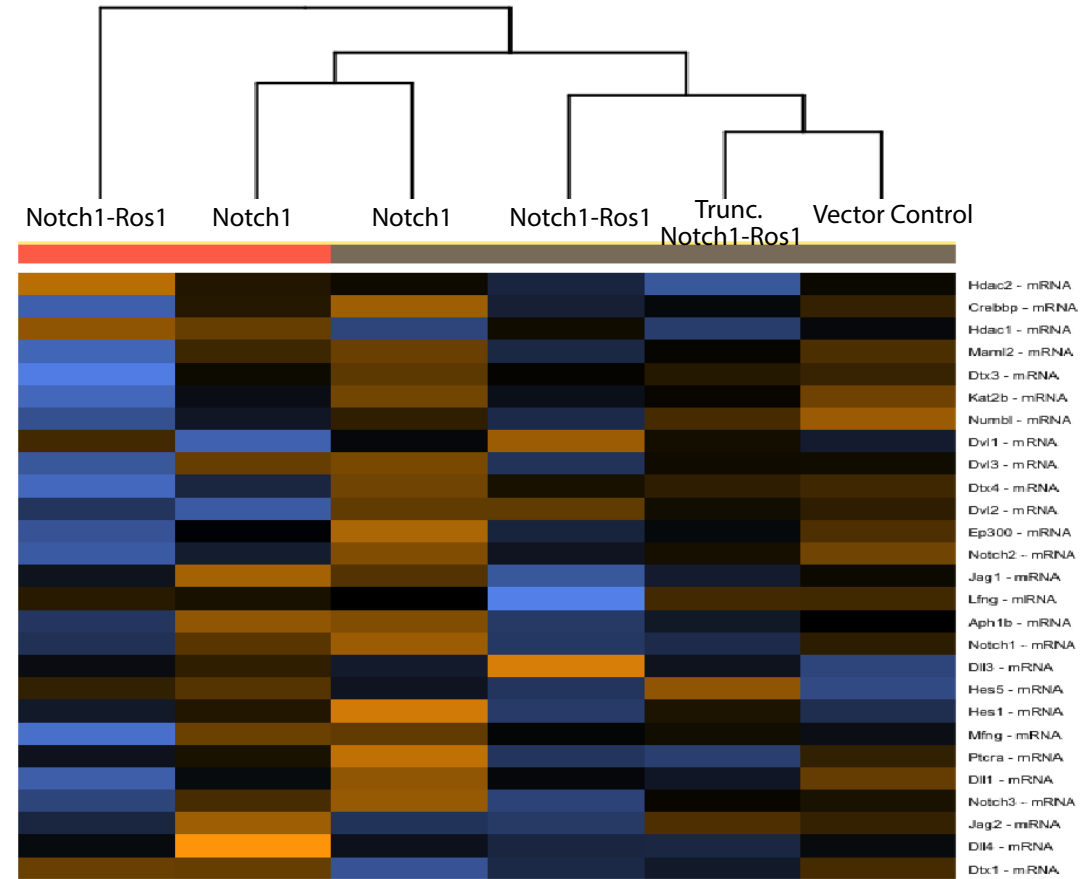
High exposure of figure 3c:



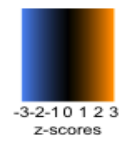
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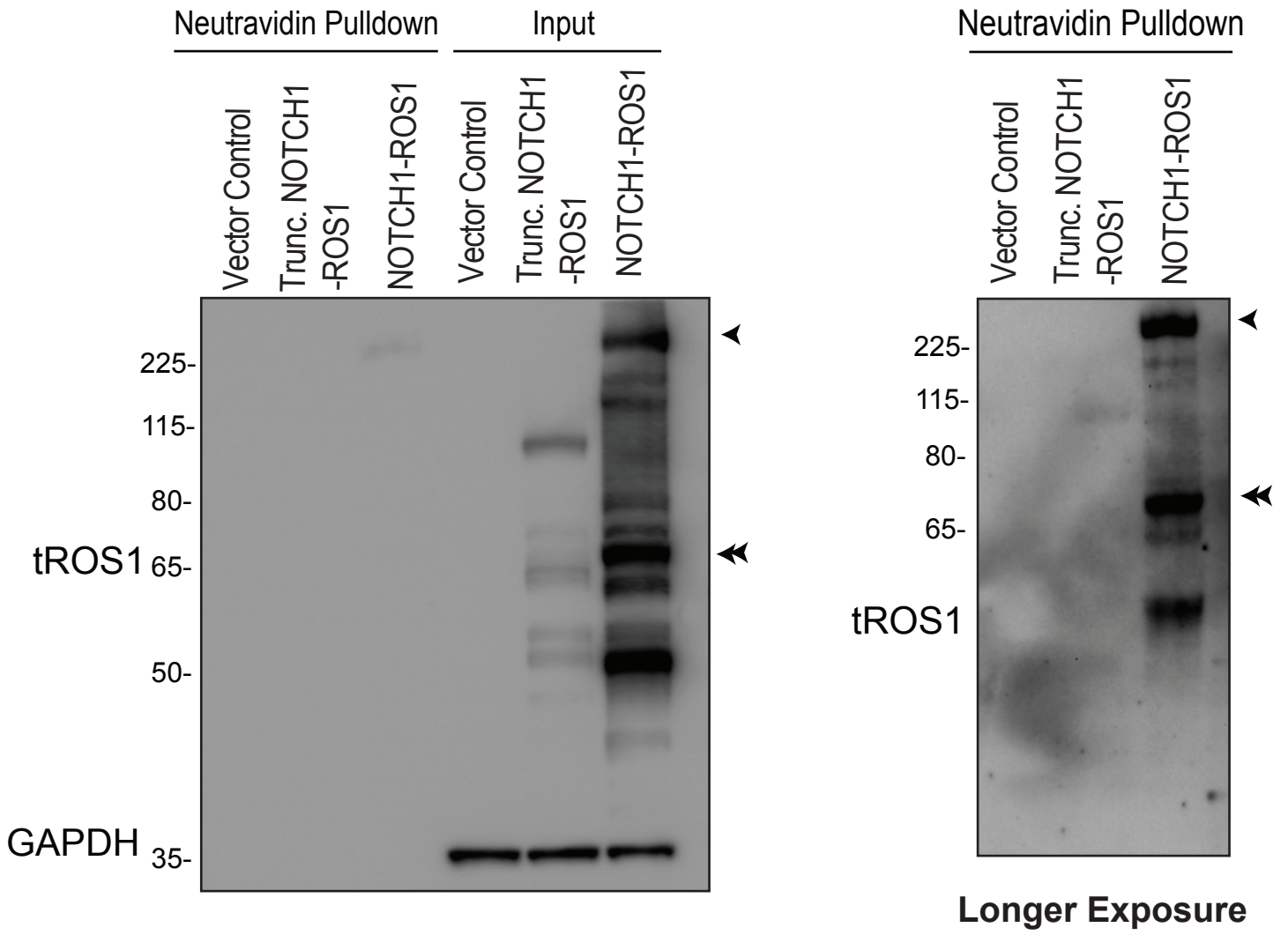
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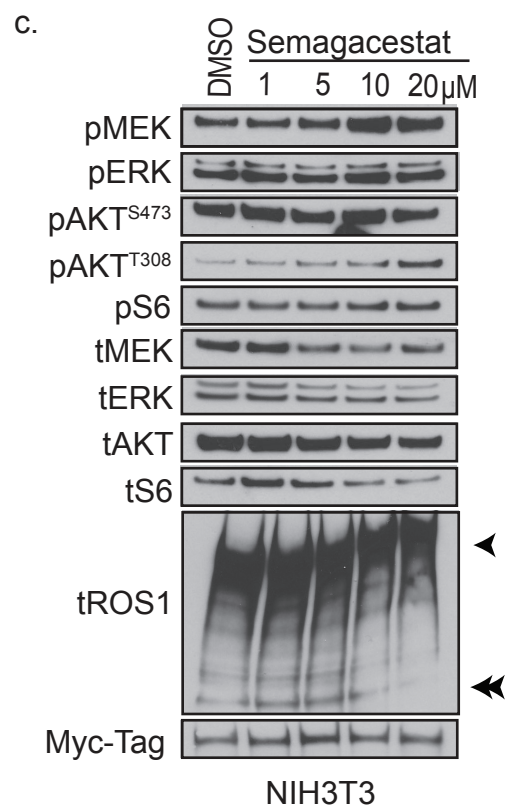
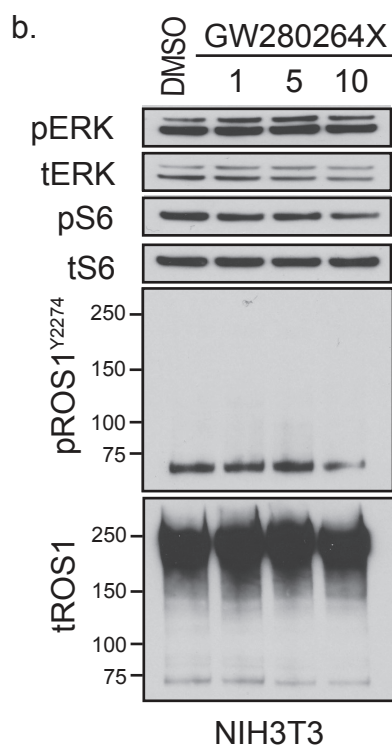
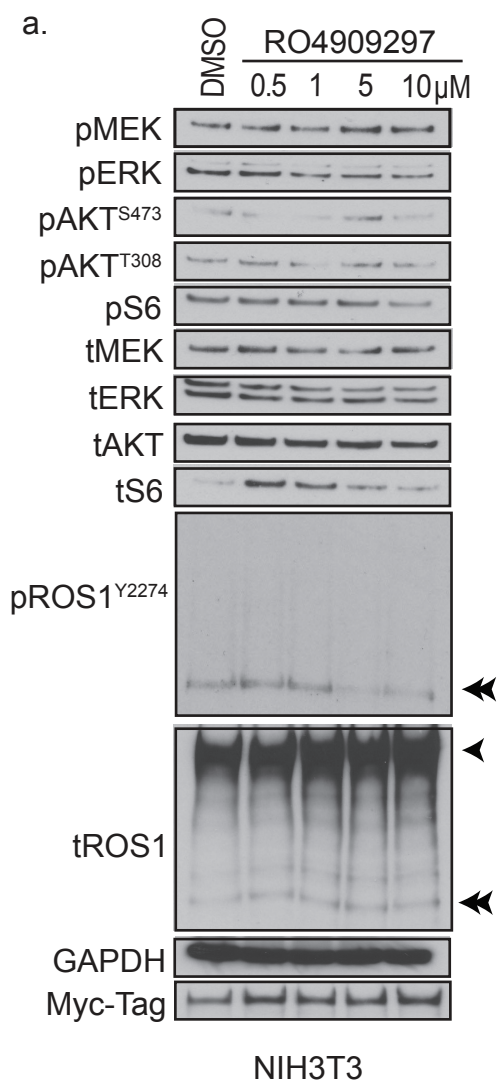


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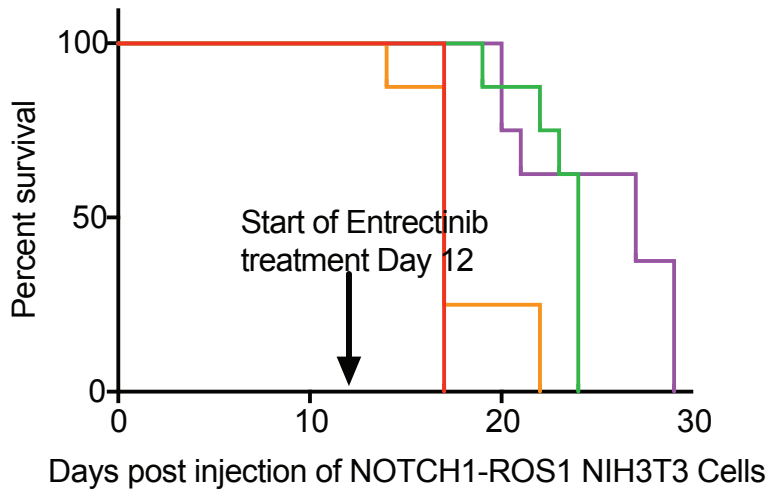


a.





a.



- Vehicle Control
- 15 mg/kg Entrectinib
- 30 mg/kg Entrectinib
- 60 mg/kg Entrectinib

b.

Treatment Group	Entrectinib Steady State Trough (ng/ml)
15 mg/kg	23.8 (17.3-43.5)
30 mg/kg	320 (91.7-1290)
60 mg/kg	805 (576-4080)