

## Abstract #3577

The DEAD box RNA helicase DDX5/p68 is aberrantly expressed and post-translationally modified in several cancers. Several studies have indicated that p68 plays important roles in cell proliferation and tumor progression; in particular, p68 that is phosphorylated on Tyr593 has been shown to be associated with cell transformation, epithelial mesenchymal transition and cell migration [1]. Therefore, p68 may be a promising target for novel anti-cancer therapeutics. We previously reported that 1-(3,5-dimethoxyphenyl)-4-[(6-fluoro-2-methoxyquinoxalin-3-yl)aminocarbonyl] piperazine (RX-5902) inhibits the growth cancer cell lines by interacting and interfering with the function of phosphorylated p68 at low nanomolar concentrations, and inhibits tumor growth in several human xenograft models in nude mice [2]. Although p68 is largely a nuclear protein, it has been found to shuttle between the nucleus and cytoplasm in cells, a function that appears to be critical for its role in epithelial-mesenchymal transition. We therefore investigated whether RX-5902 affects the cellular localization of phosphorylated p68 in cancer cell lines. We also examined the effect of RX-5902 on cellular motility in wound healing assays; our data indicate that, apart from affecting cellular proliferation, RX-5902 affects the previously suggested function of phosphorylated p68 in cell migration [3].

## Materials & Methods

### Nuclear/Cytoplasmic Fractionation

Cells were treated with RX-5902 drug for 16h. For Nuclear/Cytoplasmic separation cells were harvested in Cytoplasmic Extract (CE) Buffer (10mM KCl; 5mM MgCl<sub>2</sub>; 50mM TRIS-HCl pH 7.5; 0.5% NP-40; EDTA-free protease inhibitors; phosphatase inhibitor) and collected by centrifugation at 1000g for 5 minutes.

### Proliferation assays

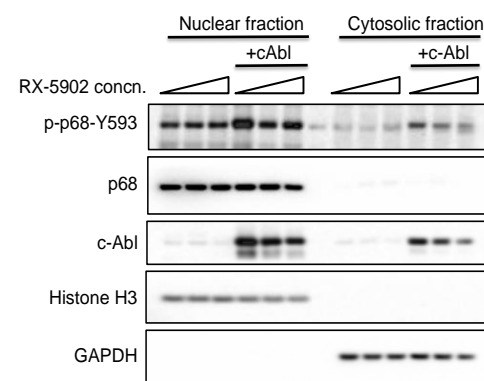
Proliferation of RX-5902 were measured by sulforhodamine (SRB). IC<sub>50</sub> was determined using GraphPad Prism software.

### Migration assay

Cells were treated with RX-5902 for 16h and grown to confluence. 'Scratch wounds' were created and migration was measured in 1% FBS-DMEM to minimize proliferation using the IncuCyte live-cell imager.

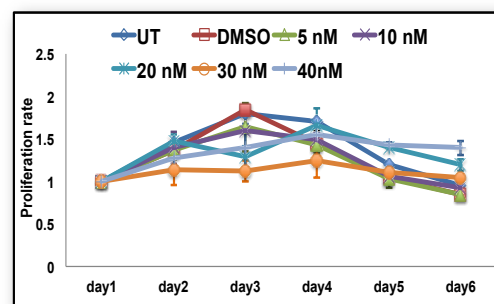
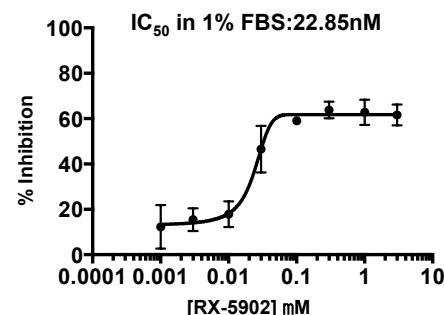
## Results

1. RX-5902 causes a decrease in the level of Y-593 phosphorylated p68, but does not significantly alter total p68 levels or its nucleo-cytoplasmic localization.

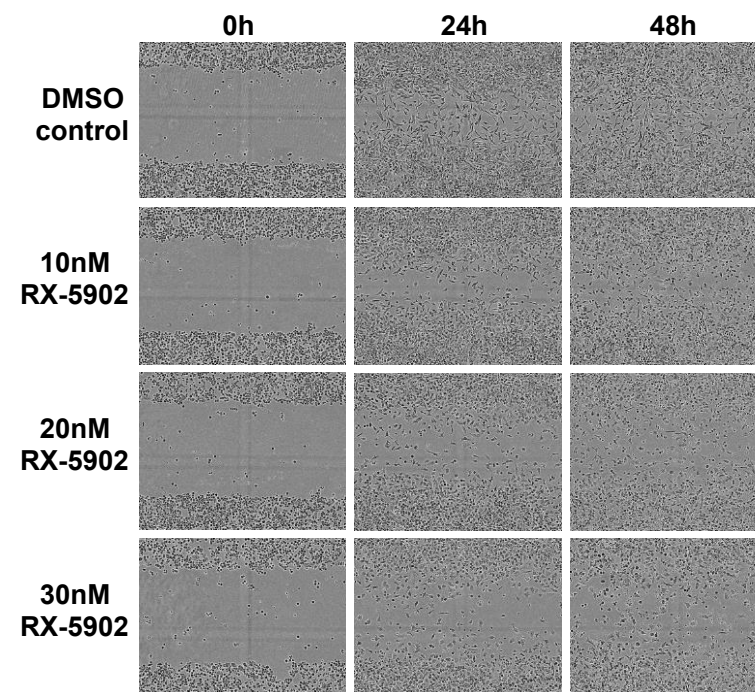
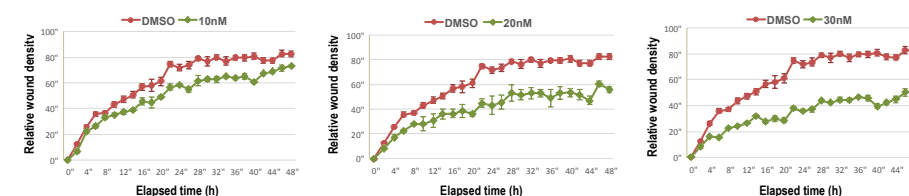


HeLa cells were transfected with constitutively active (nuclear) c-Abl to boost endogenous p68 phosphorylation. Cell lysates were fractionated into nuclear and cytoplasmic fractions to examine whether RX-5902 treatment affected localization of p68.

2. RX-5902 concentrations below IC<sub>50</sub> do not affect proliferation of MDA-MB231 cells cultured at 1% FBS.



3. RX-5902 concentrations below IC<sub>50</sub> (23nM) affect migration of MDA-MB231 cells cultured at 1% FBS.



## Conclusions / References

RX-5902 triggers a decrease in Y-593 phosphorylated p68 levels but has little effect on total p68 levels or localization.

RX-5902 inhibits motility of MDA-MB 231 breast cancer cell lines and could potentially prevent metastasis in cancer.

### References:

1. Yang *et al.* (2006) Cell, 127: 139-155.
2. Kost *et al.* (2015) J. Cell. Biochem. doi: 10.1002/jcb.25113.
3. Wang *et al.* (2013) Nat. Commun. 4: 1354.