

# The novel thienopyrimidine derivative, RP-010, produces its efficacy in prostate cancer cells by inducing the fragmentation of $\beta$ -catenin

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**Running title:** *RP-010 anti-prostate cancer efficacy and safety.*

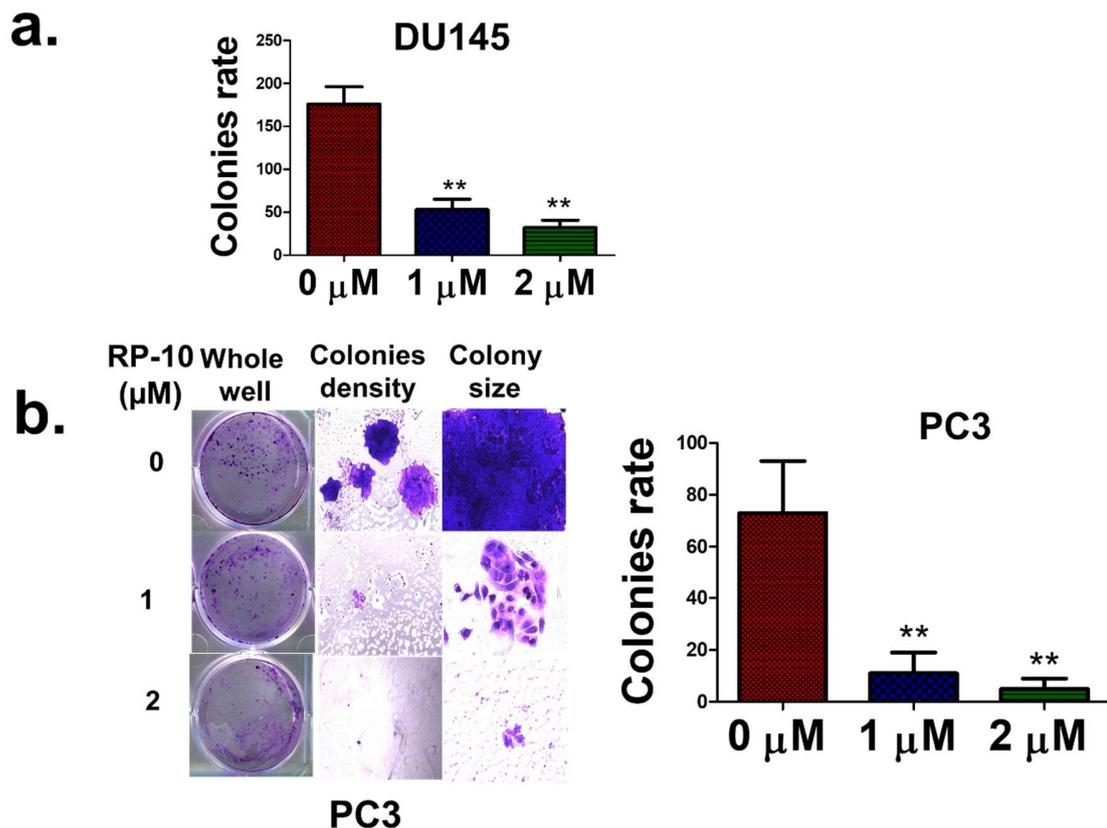
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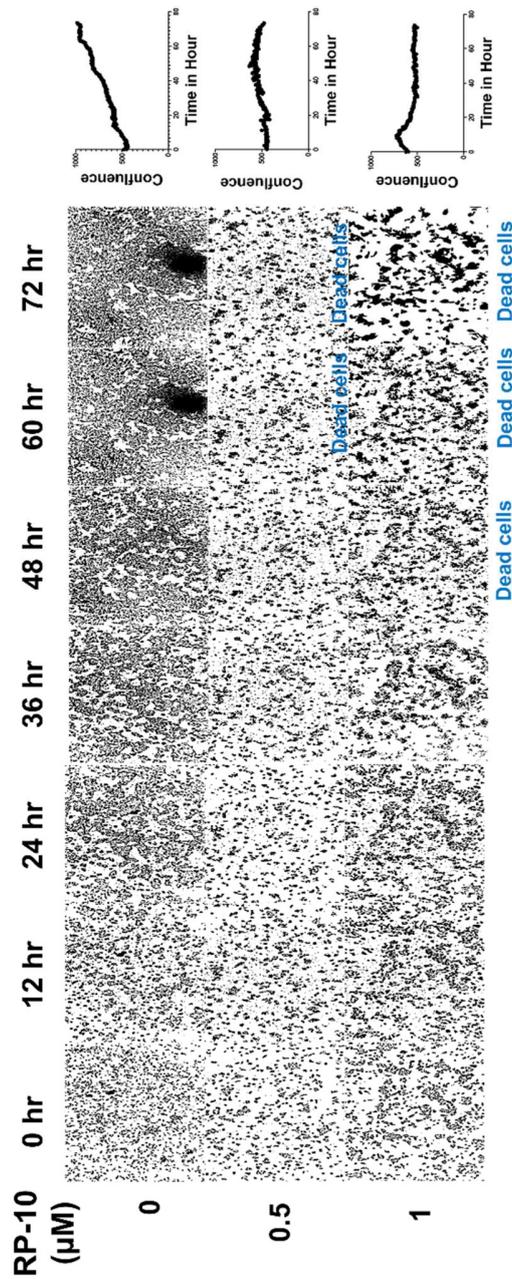
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**Figure 1S:** The effect of RP-010 on the colony formation rate of prostate cancer cells. **(a)** A histogram showing the effect of RP-010 (1 or 2  $\mu\text{M}$ ) and vehicle on the number of colonies formed in DU145 cells. **(b)** The effect of RP-010 (1 or 2  $\mu\text{M}$ ) and vehicle on colony density (10x) and colony size (20x) of PC3 cells and a histogram quantitating the results. All results are presented as the means  $\pm$  SD of three independent experiments.  $**p < 0.01$ .

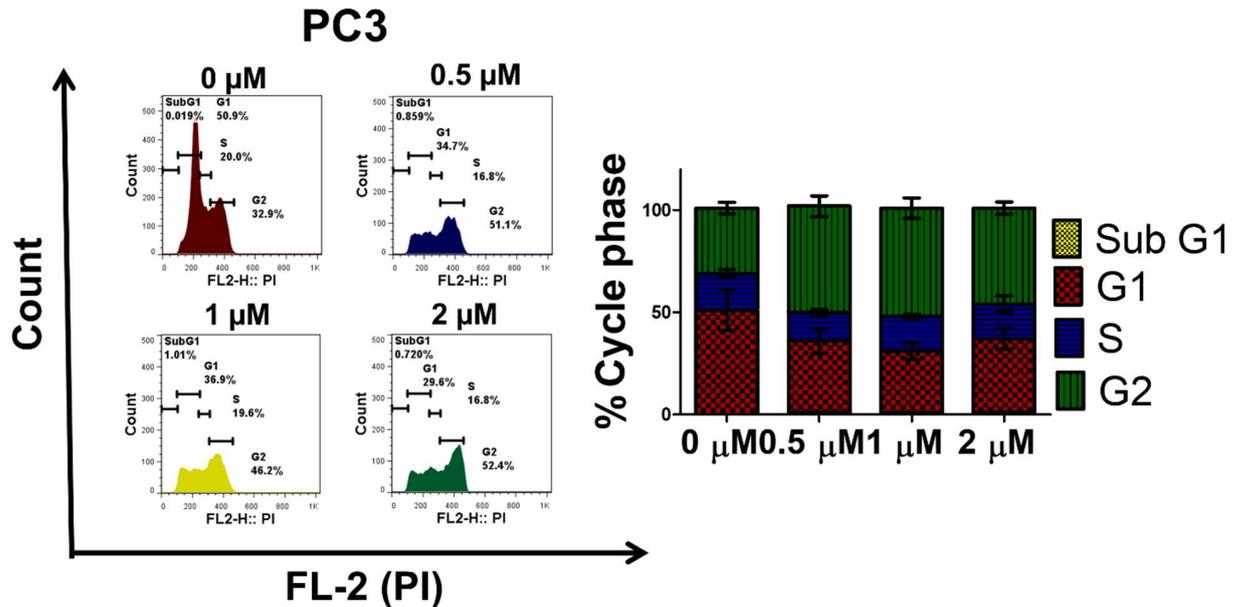




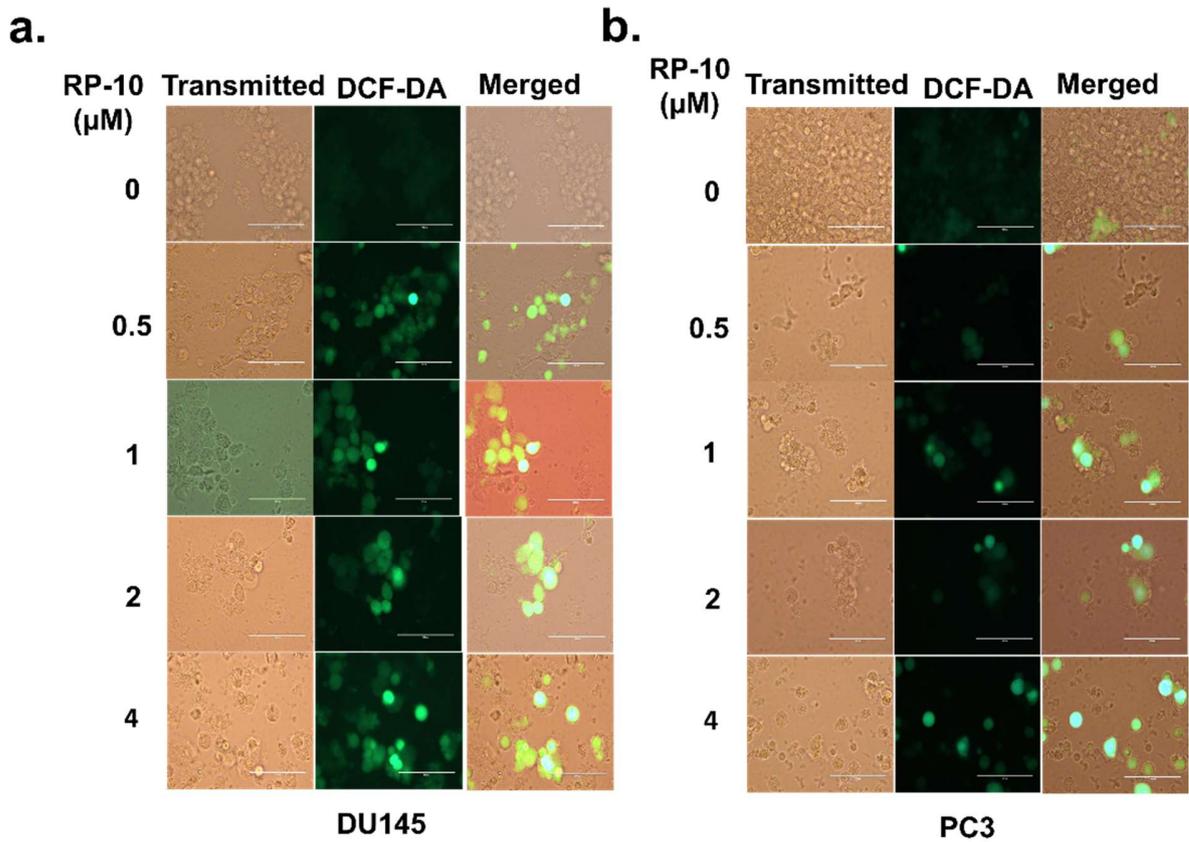
**Figure 2S:** The effect of RP-010 on cell confluence and viability over time in DU-145 cells. DU-145 cells were incubated with RP-010 (0.5 or 1  $\mu$ M) or vehicle for 72 hrs. The images represent cell confluence over time. The graphs adjacent to the pictures represent the quantification of cell confluence over time. Dead cells are those that were detached and clearly floating.



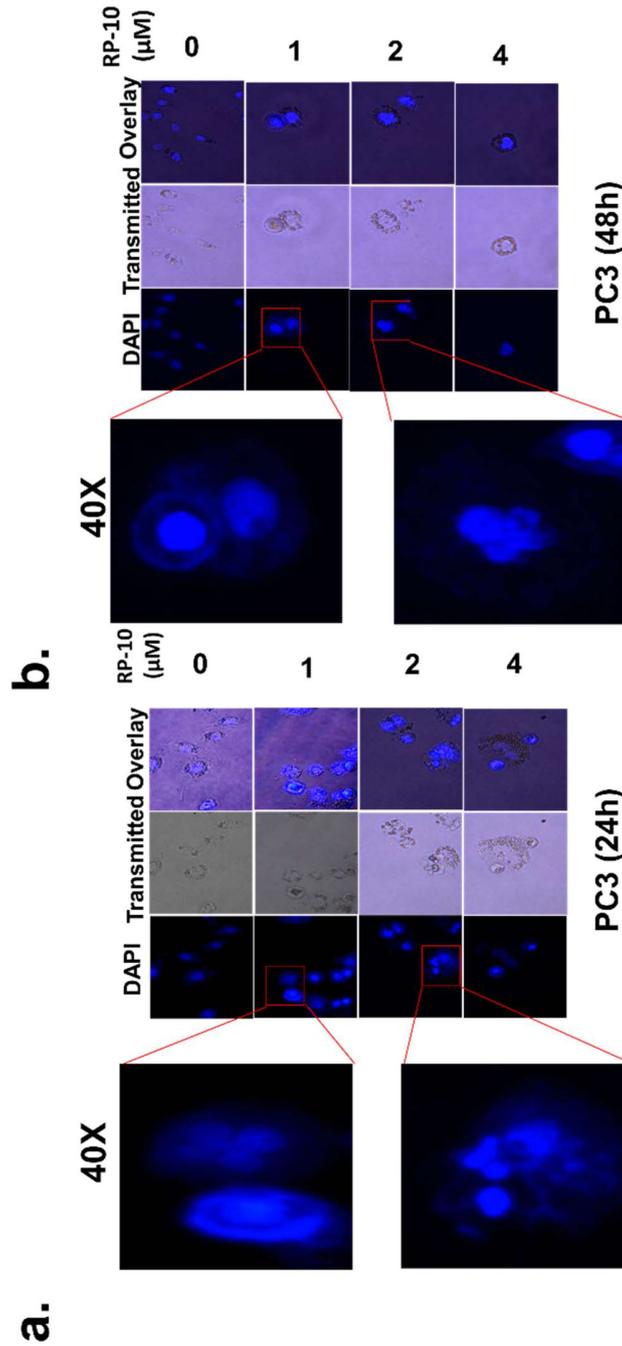
**Figure 3S:** RP-010-induced alterations in the cell cycle in PC3 cells. The analysis of RP-010 (0.5, 1 or 2 $\mu$ M) - induced alterations in the cell cycle using flow cytometric assay (P1 on Y axis and cell count on x axis). A graph showing the percent change for each phase of the cell cycle for RP-010.



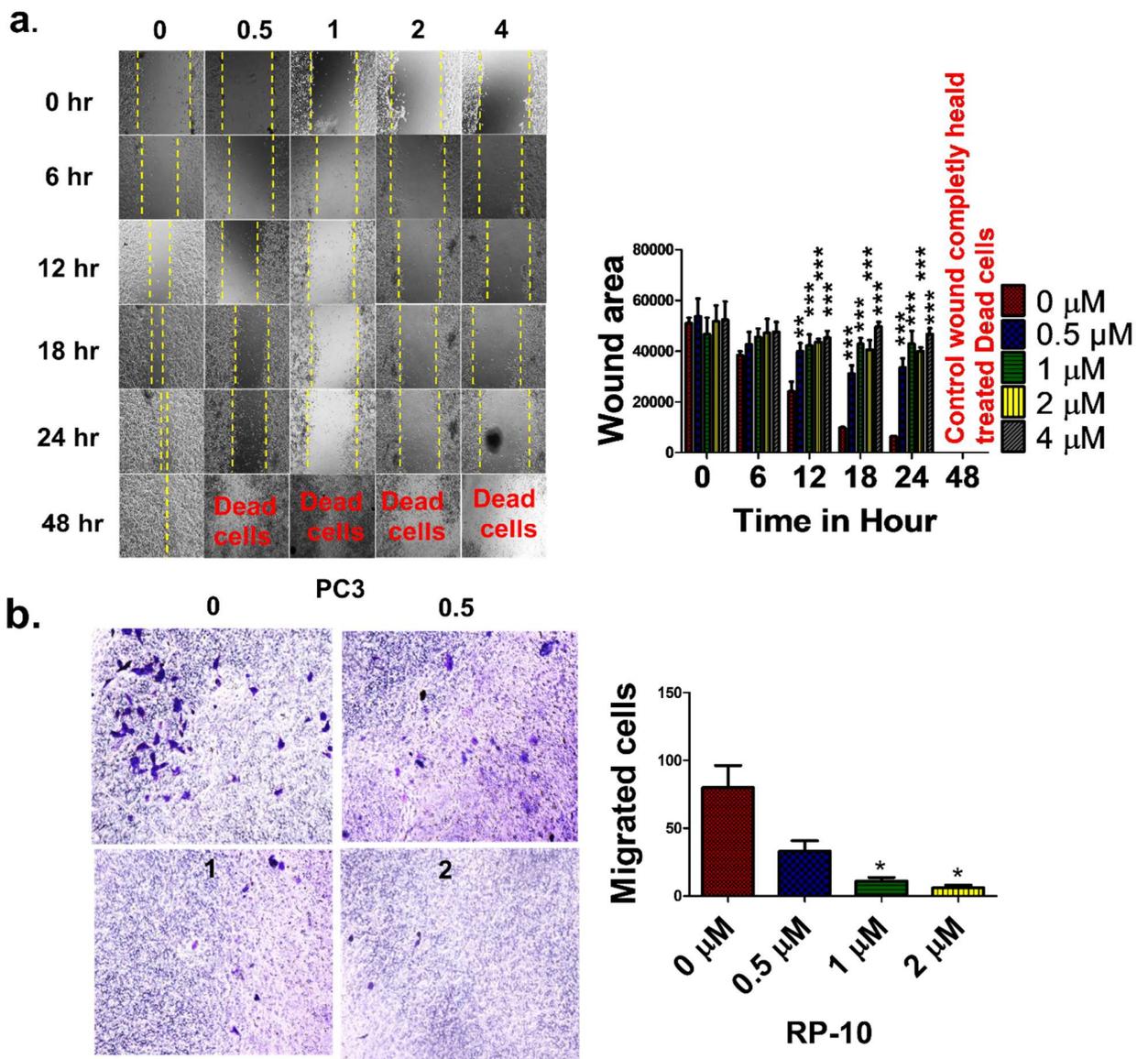
**Figure 4S:** The effect of RP-010 on oxidative stress in DU-145 and PC3 cells. (A, B) Representative images of the DCF fluorescence after incubation with 0.5, 1, 2 or 4  $\mu\text{M}$  of RP-010 or vehicle (0  $\mu\text{M}$ ) for 24 h in DU-145 and PC3 cells, respectively. The results are relative to the control. The experiments were repeated in triplicate.



**Figure 5S:** The effect of RP-010 on nuclear events in PC3 cells. The effect of RP-010 (1, 2 or 4  $\mu\text{M}$ ) or vehicle on the nucleus of PC3 cells after incubation for 24 or 48hrs, respectively. Both chromatin condensation and mitotic catastrophe can be seen.

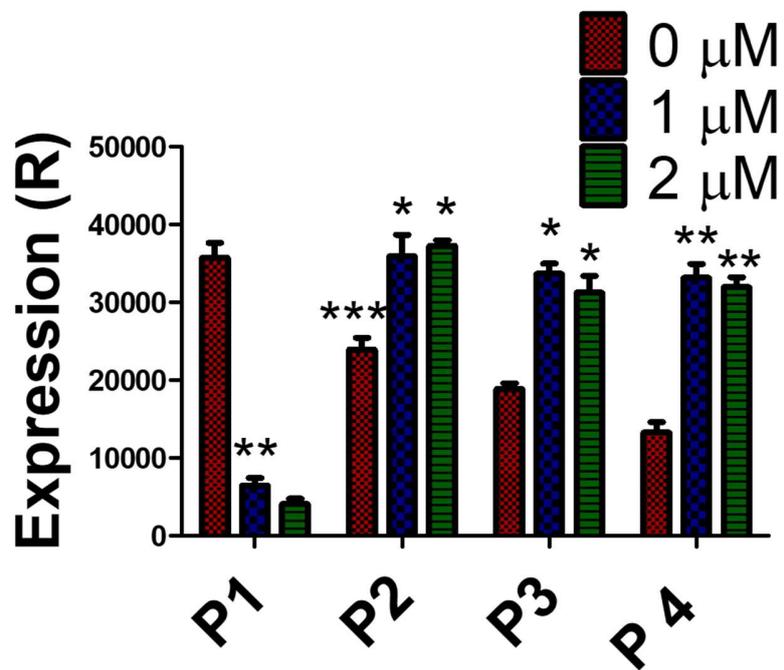


**Figure 6S:** RP-010 significantly reduces cell migration and invasiveness in PC3 cells **(a)** The pictures show the results of the wound healing assay after incubation with RP-010 (0.5, 1, 2 or 4 $\mu$ M) or vehicle. The results are also summarized in the adjacent histogram **(b)** the pictures show the transwell migration of cells after incubation with RP-010 (0.5, 1 or 2  $\mu$ M) or vehicle. The results are summarized in the adjacent histogram



**Figure 7S:** RP-010 induces significant changes in Wnt/ $\beta$ -catenin signaling. The effect of RP-010 (1 or 2  $\mu$ M) or vehicle on  $\beta$ -catenin fragmentation. (R) = relative.

## $\beta$ -catenin fragmentation



**Figure 8S:** The effect of RP-010 (1 or 2  $\mu\text{M}$ ) or vehicle on the expression of E-cadherin, N-cadherin,  $\alpha$ -tubulin, and HSP90. RP-010 was added at 0, 1 or 2  $\mu\text{M}$ .  $\beta$ -actin was used as a reference protein. (R) = relative.

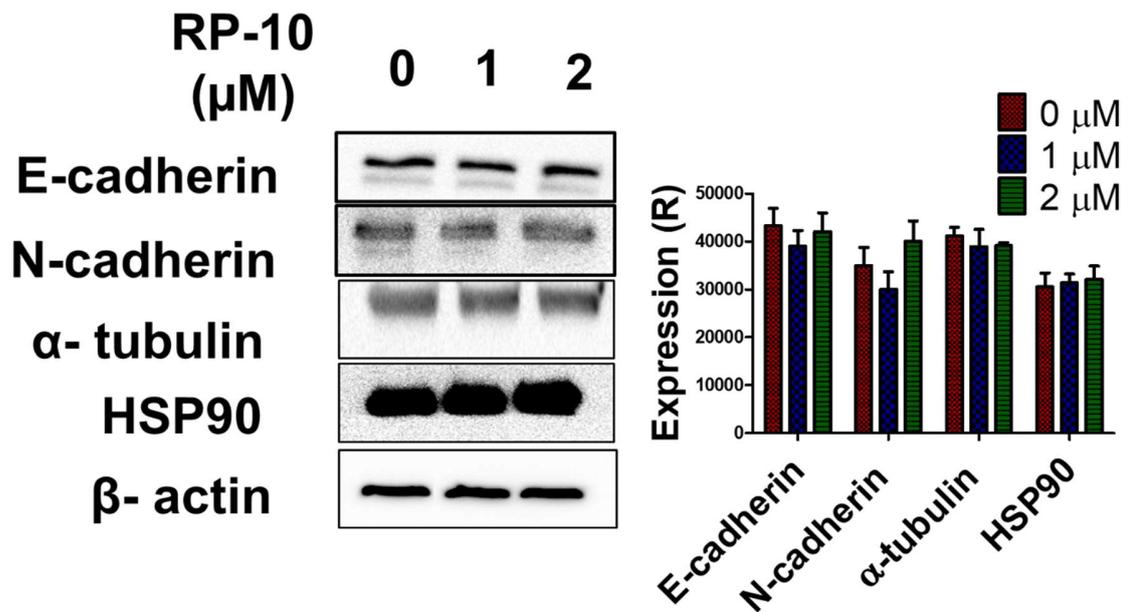


Figure 9S: The effect on the heart rate of zebra fish exposed for up to 48 h with (0.3, 1, 3, 6 or 10  $\mu\text{M}$ ) or without (0  $\mu\text{M}$ ) RP-010.

