Supplementary Material. Bacchiega et al.

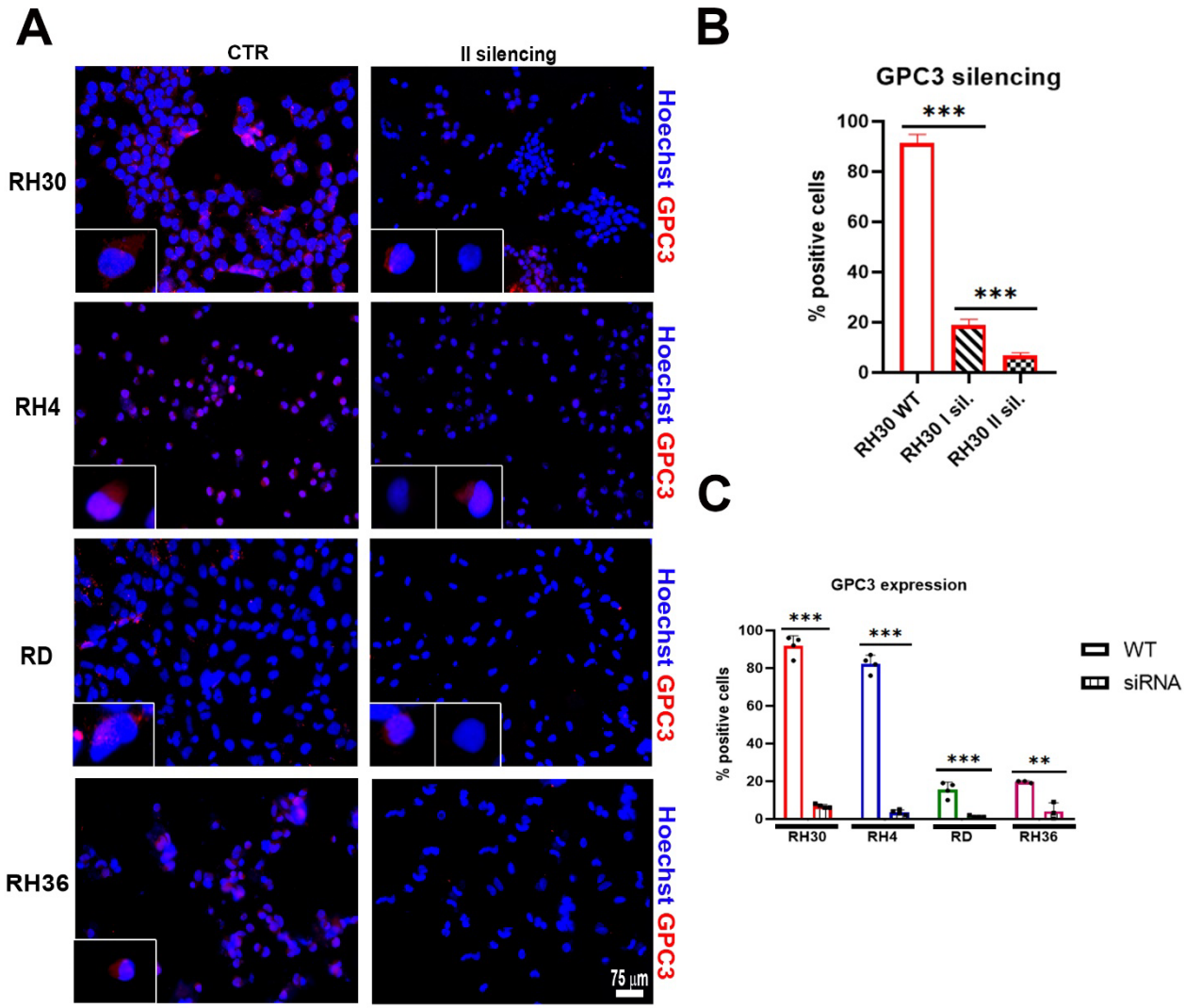

Fig S1. Evaluation of GPC3 expression after silencing. (**A**). Immunostaining of GPC3 in different cell lines, after the second siRNA treatment. (**B**). In RH30, the most aggressive cell line, after a single siRNA treatment, there was a lower level of proteoglycan expression, but to have better significance, a second treatment was done. (**C**). GPC3 expression in WT cells and siRNA-treated cells: the GPC3 level becomes significantly lower after the second treatment (Mann-Whitney test; **:p<0,001; ***:p<0,0001).

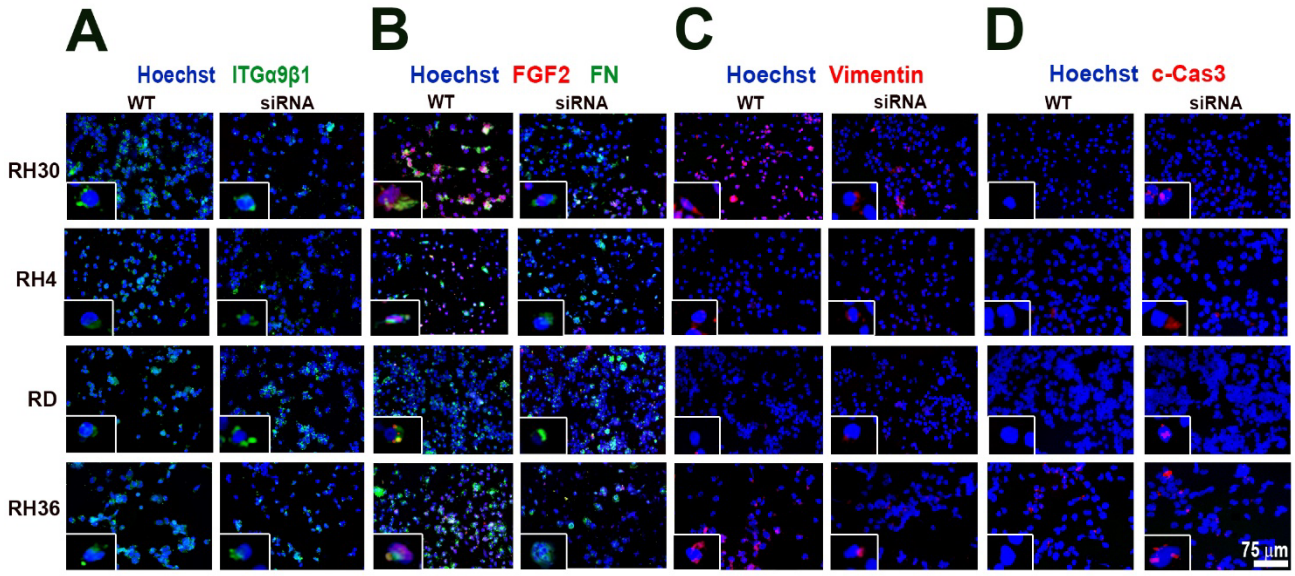

Fig S2. Immunofluorescence of the cell lines. (**A).** Immunostaining in WT cells and siRNA-treated cells for ITGα9β1: there was a lower expression of ITGα9β1in siRNA-treated cells, demonstrating the link between GPC3 and the integrin. (**B)**. Immunostaining in WT cells and siRNA-treated cells for FGF2 and FN: in ARMS cell lines, FN expression decreases in siRNA-treated cells, while in ERMS cell lines its expression is increased. FGF2 expression decreases in all cell lines because it is bound to GPC3 heparan sulfate chains. (**C)**. Immunostaining in WT cells and siRNA-treated cells for Vimentin: GPC3 is implicated in Vimentin expression, so it is involved in cell structure maintenance. (**D)**. Immunostaining in WT cells and siRNA-treated cells for cleaved Caspase 3: cells do not die only with siRNA treatment.

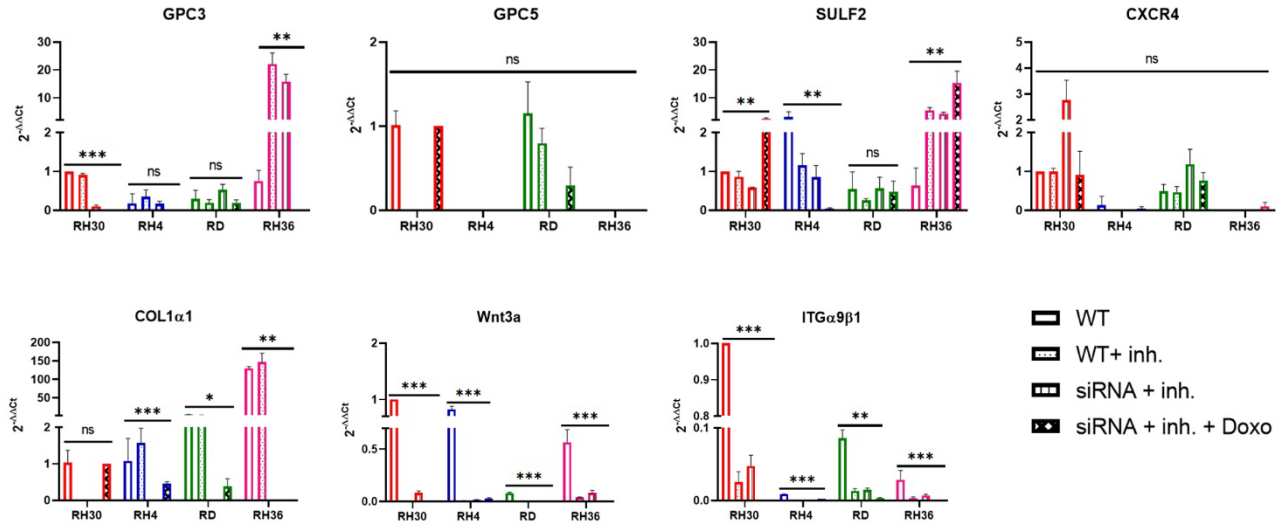

Fig S3. qRT-PCR after different treatments. GPC3, GPC5, SULF2, CXCR4, COL1α1, Wnt3a and ITGα9β1gene expression in the different conditions of cells cultured in 2D model.

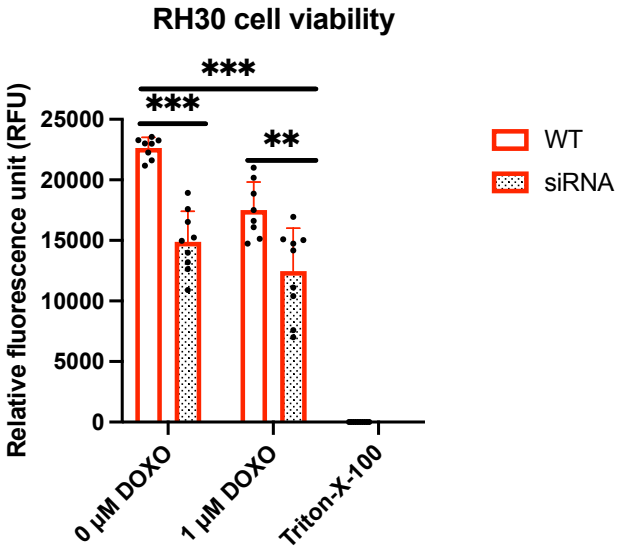

Fig S4. RH30 cell viability without and with DOXO. RH30 cell WT and after GPC3 siRNA were treated with DOXO. Cells after DOXO and GPC3 siRNA died significantly more than WT after DOXO treatment

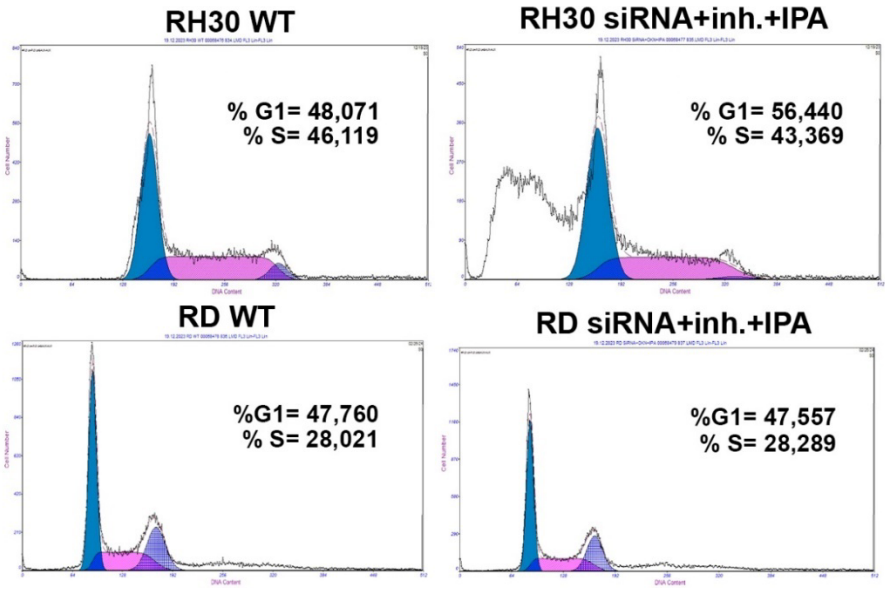

Fig S5. **Cell cycle analysis**. Cell cycle of RH30 and RD WT and siRNA+SULF2-inhibitor+Ipafricept treatment showed that change in S phase of the cells after treatment. The change was marked in RH30 cells, the more aggressive RMS.

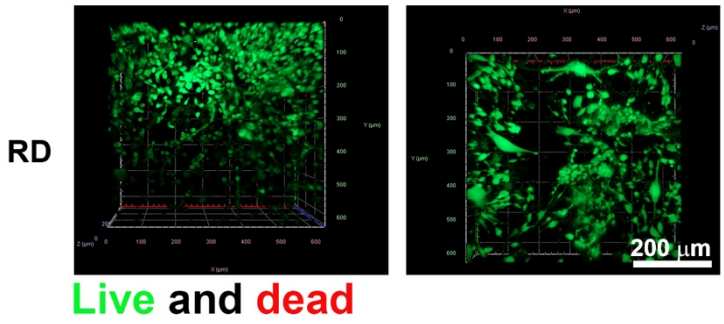

Fig S6. RD hydrogel embedded. Live and dead assay of RD cells.

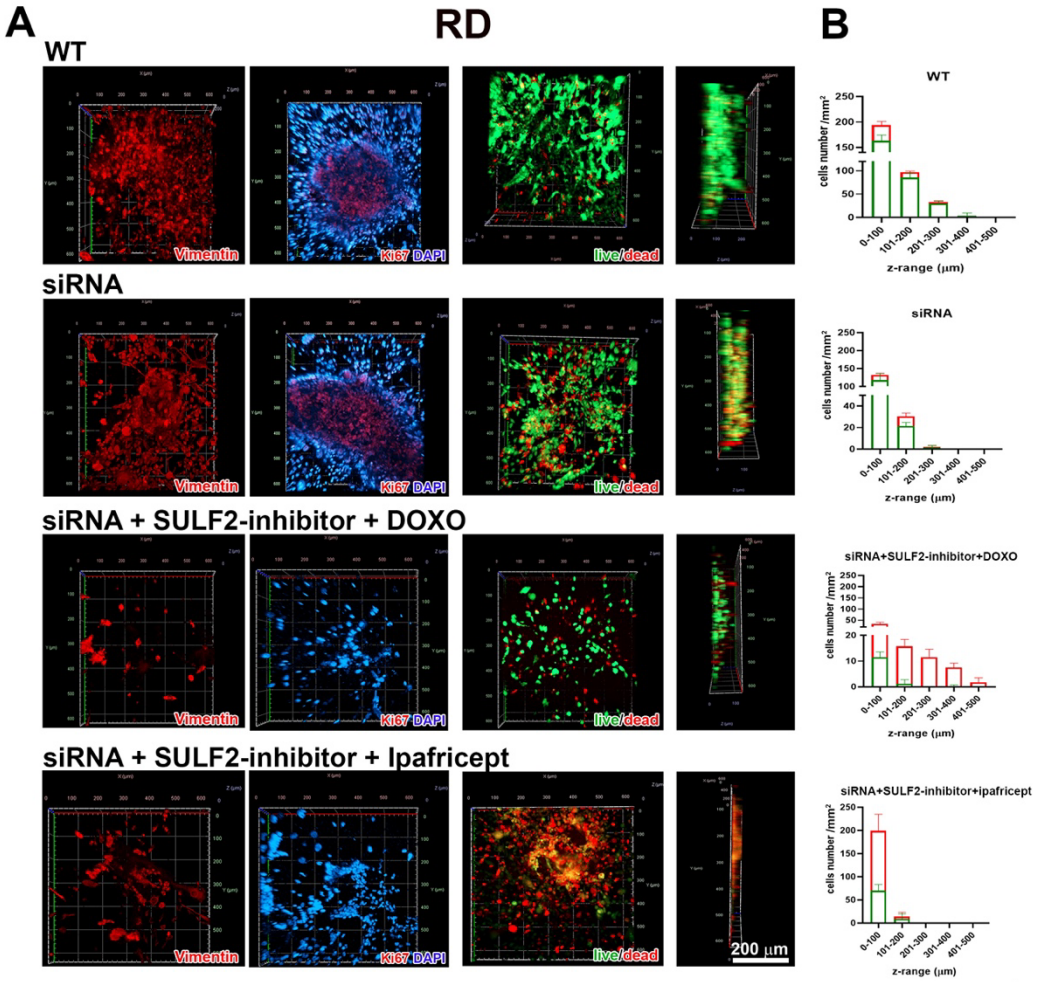

Fig S7. 3D models under different treatment conditions in the RD cell line. Distribution, viability and proliferation. (A). First row. WT cells have better migration, viability, and proliferation than other conditions. Second row. The addition of siRNA against GPC3 decreased cell mobility and proliferation, Third and forth row. The addition of SULF2-inhibitor and drugs strongly decreased cell proliferation and distribution, indicating a key role of GPC3 in the above mentioned functions. Scale bar: 200 µm. (**B).** Histograms of the different cell condition showing cell distribution calculated as the number of cells per volume of 10 mm^3 across the z-axis (z-range). µ

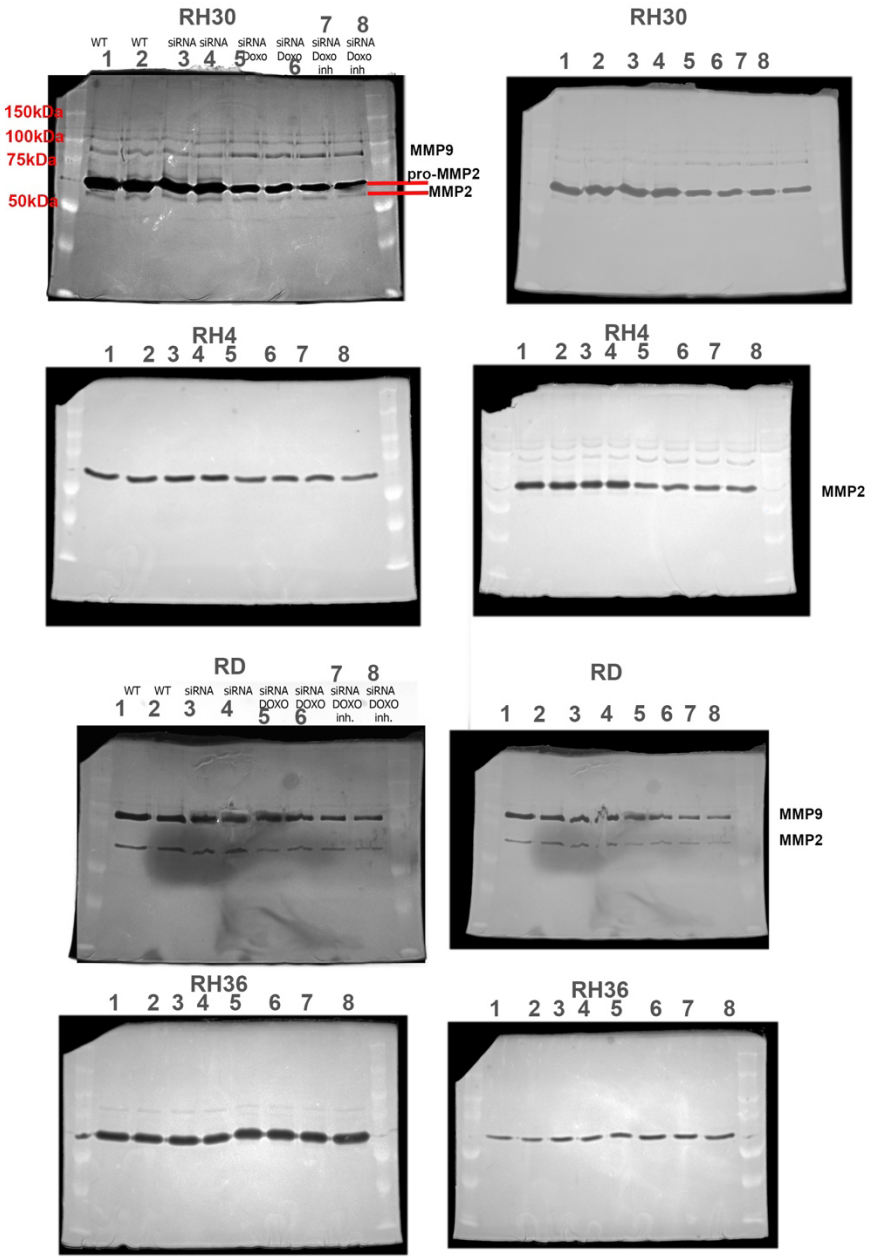

Fig S8. Original membranes of the zymography of RH30, RH4 (ARMS) and RD, RH36 (ERMS) cells. N= 4 technical replicates of all cell lines, 2 different experiments for RH4 and RH36, 3 for RH30 and RD cell lines.