Supplementary Figure Legends

Suppl. Figure S1: Genotypic match of the UM-HMC cell lines with the human mucoepidermoid carcinoma specimens from which the cell lines were generated. Table depicting the short tandem repeat (STR) profile of the human tumor specimen (*i.e.* reference DNA), as compared to UM-HMC-1, UM-HMC-3A, and UM-HMC-3B cell lines at lower and high passages. Table also depicts the data on mycoplasma contamination at high passage. Of note, mycoplasma levels were measured periodically, but not at the exact passage as the lower passages used here for STR profiling.

Suppl. Figure S2: p53 protein levels regulate the fraction of mucoepidermoid carcinoma cancer stem cells *in vitro*. A, C, F, Graphs depicting cell density measured through sulforhodamine B (SRB) in MEC cell lines. Data was normalized against vehicle controls. *Graph inserts:* correspond to half-maximal inhibitory concentration (IC_{50}) per time point. Ambiguous=values not accurately measurable for the tested dose range (A). MEC cells were treated with increasing doses of MI-773 for 24 to 72 hours (A) or APG-115 for 48 hours (C). **B,** Graphs depicting the band density of the Western blots presented in Figure 1B for UM-HMC-1 and UM-HMC-3A treated with MI-773 for 24 or 48 hours. Band density data (pooled together was both cell lines) was normalized initially by GAPDH and then by respective untreated controls. **D,** Western blots of MEC cells treated with APG-115, a 2nd generation small molecule inhibitor of the MDM2-p53 interaction. **E,** Fraction of cancer stem cells ($ALDH^{high}CD44^{high}$) measured using flow cytometry in MEC cell lines treated with APG-115 for 72 hours. **F,** Growth curves and doubling time (DT) calculations in vector control and p53-silenced cells based on SRB measurements. **G,** Dot plots of flow cytometry analysis for ALDH and CD44 in vector control and p53-silenced cells treated with MI-773 for 72 hours. All results are representative of at least two independent experiments. Data was analyzed by two-tailed student's *t*-test (α=0.05). * *P*<0.05, ns=not significant.

Suppl. Figure S3: Analyses of apoptosis and cell cycle in bulk MEC cells. A, Flow cytometry plots indicating the impact of 0-1 µM MI-773 on the cell cycle of MEC cells (UM-HMC-1, UM-HMC-3A). **B-E,** Bar graphs depicting the percentage of cells in G1, S, and G2 phase (B,D) and in S phase (C,E) of the cell cycle. **F,** Western blot depicting the impact of increasing concentrations of MI-773 on the expression of apoptosis-related proteins (*i.e.* PUMA, BIM, MDM2 and p53).

Suppl. Figure S4: The p53-p21 signaling axis does not interfere with salisphere formation or Bmi-1 regulation. A, Unsorted cells were plated in sphere conditions and treated the following day with APG-115. Graph depicting number of primary salispheres formed 7 days after treatment with APG-115. **B,** Representative micrographs of primary salispheres generated with UM-HMC-3A cells transduced with p21 shRNA or scrambled vector control. **C,** Western blot showing effects of p21 knockdown on Bmi-1 protein levels in primary salispheres. **D,** Quantification of primary salispheres formed from shRNA-p21 or vector control UM-HMC-3A cells 7-9 days after treatment with vehicle or MI-773 (1 µM). All results are representative of at least two independent experiments. Means not sharing any lower-case letters are significantly different from each other by one-way ANOVA followed by post-hoc Tukey (α=0.05).

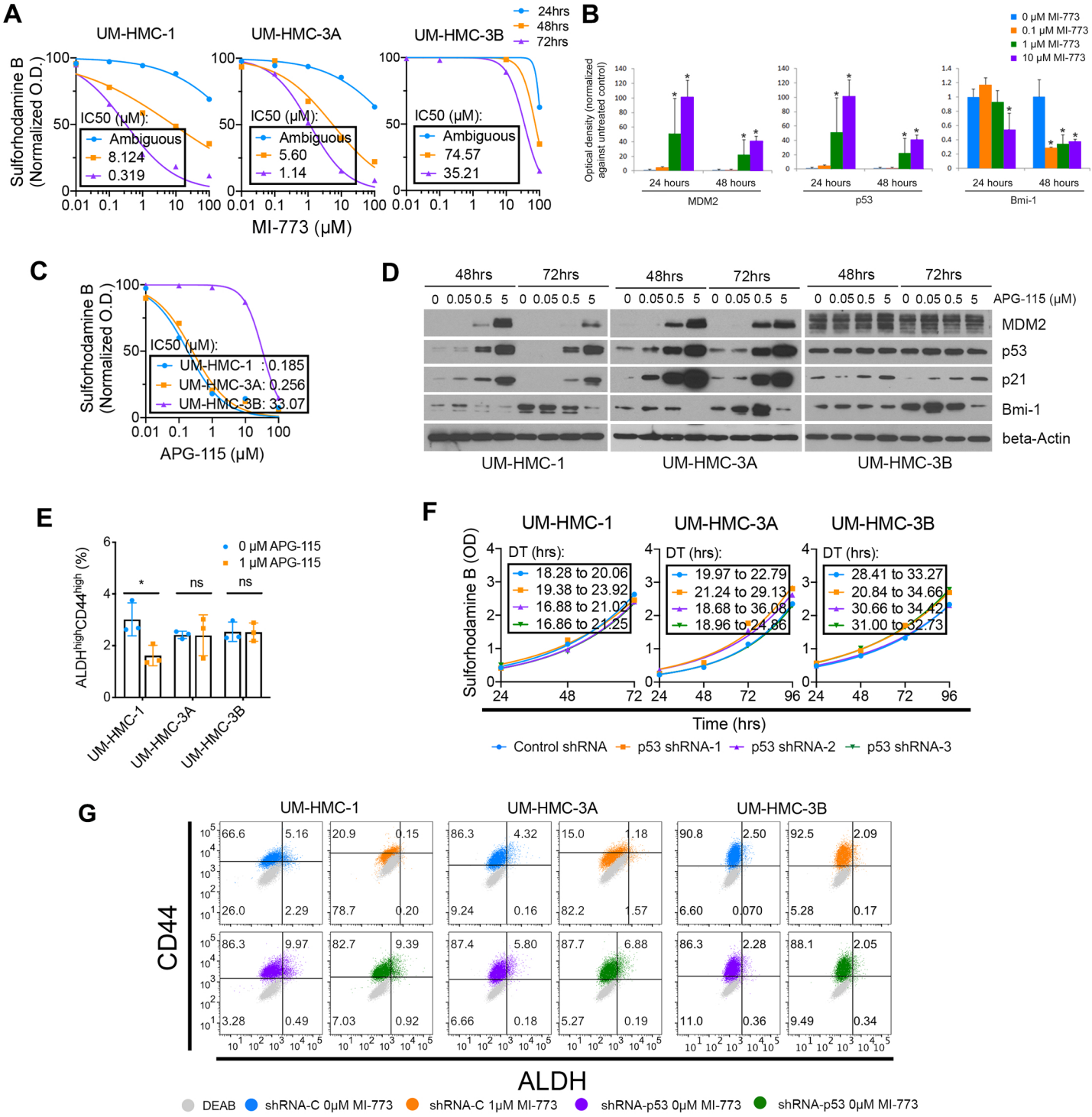
Suppl. Figure S5: Regulation of Bmi-1 expression. A, Western blot showing Bmi-1 protein expression in UM-HMC-3A cells pre-treated for 3 hours with 10 µM MI-773 followed by 50 µg/mL cycloheximide (CXH) to inhibit protein synthesis. **B,** Western blot showing protein accumulation over time of UM-HMC-1 cells treated with the proteosome inhibitor MG132 (10 µM).

Suppl. Figure S6: Bmi-1 inhibition with PTC596 results a decrease in the fraction of cancer stem cells in mucoepidermoid carcinoma cancer stem cells *in vitro*. A, Graphs depicting the fraction of cancer stem cells ($ALDH^{high}CD44^{high}$) in MEC cells (UM-HMC-1, UM-HMC-3A, UM-HMC-3B) exposed to increasing concentrations (0-200 nM) the small molecule inhibitor of Bmi-1 (PTC596) for 24 hours. **B,** Dot plots of flow cytometry analysis for ALDH and CD44 in MEC cells (UM-HMC-1, UM-HMC-3A, UM-HMC-3B) exposed to 0-200 nM PTC596 for 24 hours. * P<0.05.

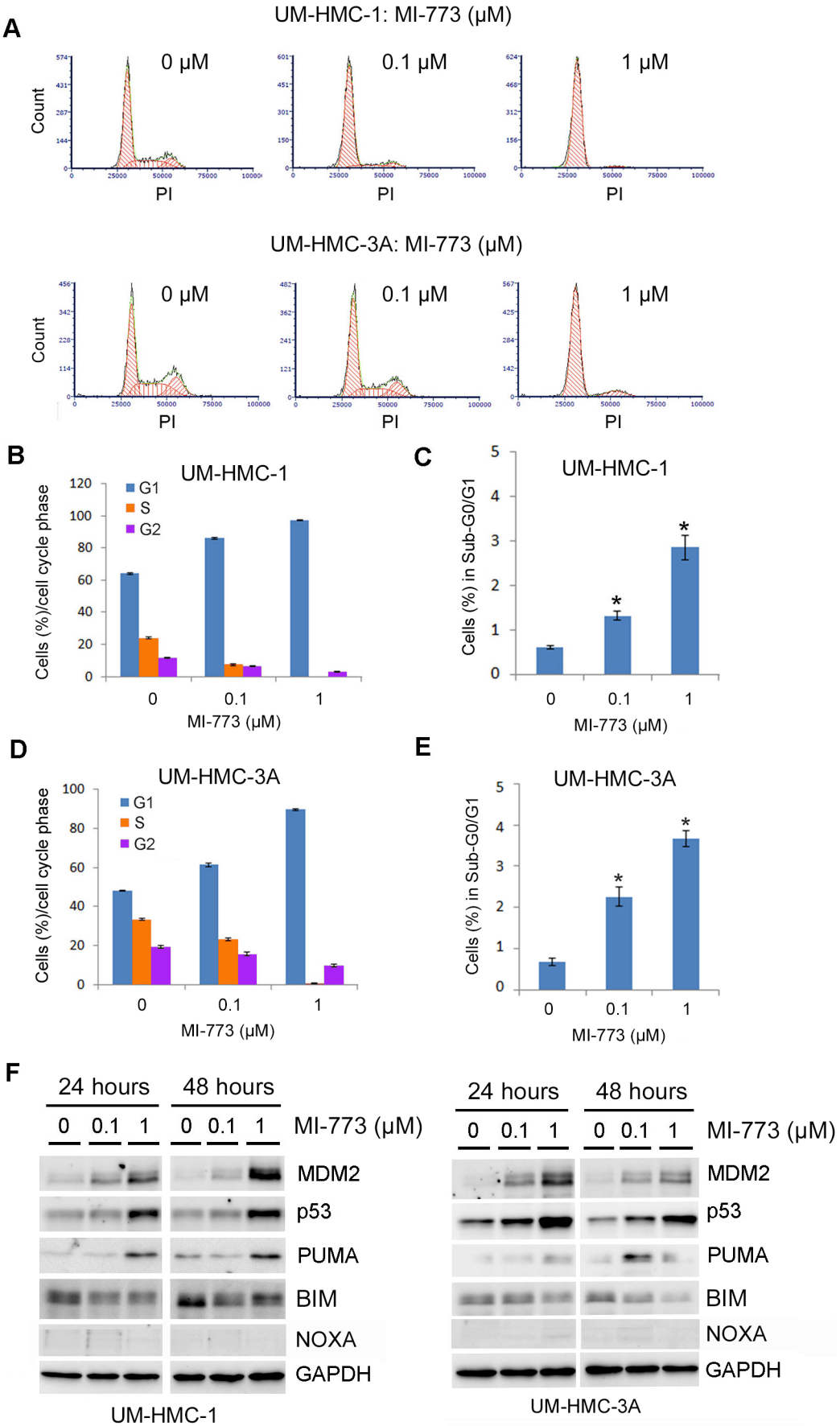
	D3S1358	D7S820	vWa	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D16S539	TH01	TPOX	CSF1PO	AMEL	Penta D	Penta E	% Match	Mycoplasma
Reference (Patient-1)	15, 16	11, 12	16	18.2, 20	15	30, 31	12, 17	10, 13	12, 13	11	7, 9	6, 9	7, 11	X, Y	6, 13	9, 10	100	Not tested
UM-HMC-1 (p # 88)	15, 16	11, 12	16	18.2, 20	15	30, 31	12, 17	10, 13	12, 13	11	7, 9	6, 9	7, 11	X, Y	6, 13	9, 10	100	Not tested
UM-HMC-1 (p # 186)	15, 16	11, 12	16	18.2, 20	15	30, 31	12, 17	10, 13, 14	12, 13	11	7, 9	6, 9	7, 11	X, Y	6, 13	9, 10	96.7	Negative

	D3S1358	D7S820	vWa	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D16S539	TH01	TPOX	CSF1PO	AMEL	Penta D	Penta E	% Match	Mycoplasma
Reference (Patient-3)	14	8, 9	15	20, 21	13	29, 32.2	12, 16	11 ,12	8, 11	12, 13	8, 9	9, 12	12	X	10, 14	9, 17	100	Not tested
UM-HMC-3A (p # 89)	14	8, 9	15	20, 21	13	29, 32.2	12, 16	11 ,12	8, 11	12, 13	8	9, 12	12	X	10, 14	9, 17	100	Not tested
UM-HMC-3A (p # 157)	14	8, 9	15	20, 21	13	29, 32.2	12, 16	11 ,12	8, 11	12, 13	8	9, 12	12	X	10, 14	9, 17	100	Negative
UM-HMC-3B (p # 90)	14	8, 9	15	20, 21	13	29, 32.2	12, 16	11 ,12	11	12, 13	8, 9	9, 12	12	X	10, 14	9, 17	100	Not tested
UM-HMC-3B (p # 156)	14	8, 9	15	20, 21	13	29, 32.2	12, 16	11 ,12	11	12, 13	8, 9	9, 12	12	X	10, 14	9, 17	100	Negative

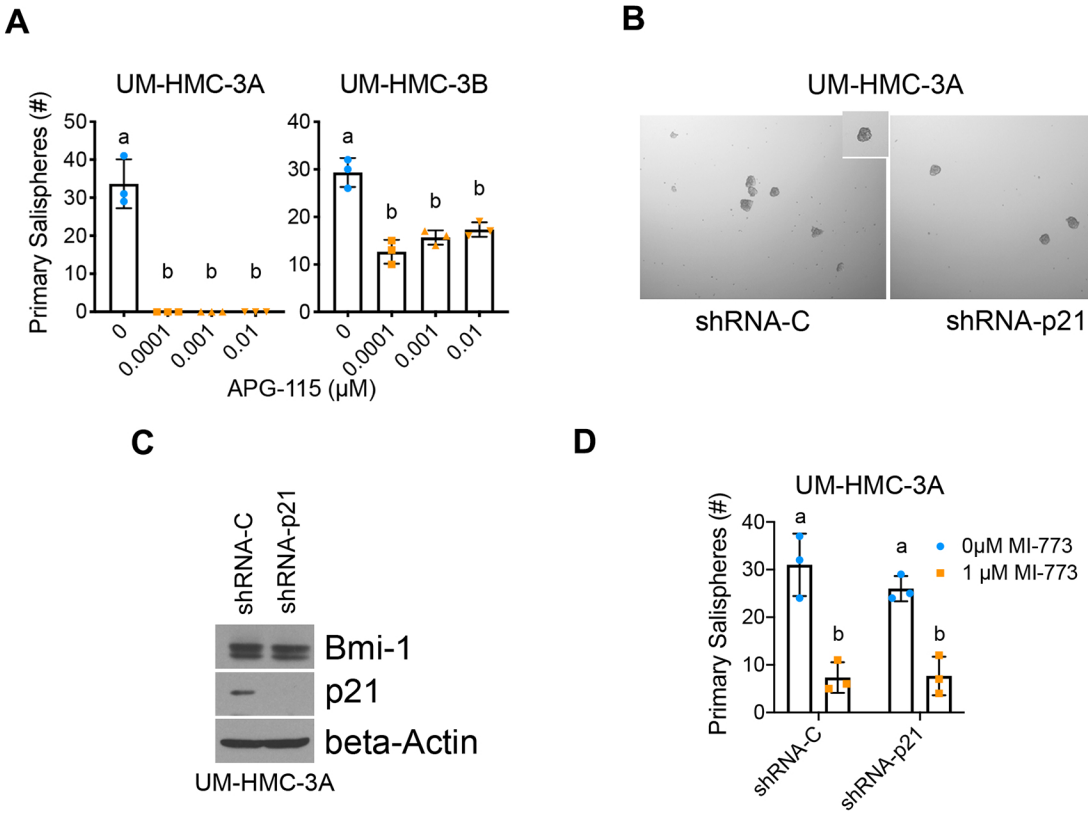
Suppl Figure 1 - Rodriguez-Ramirez et al



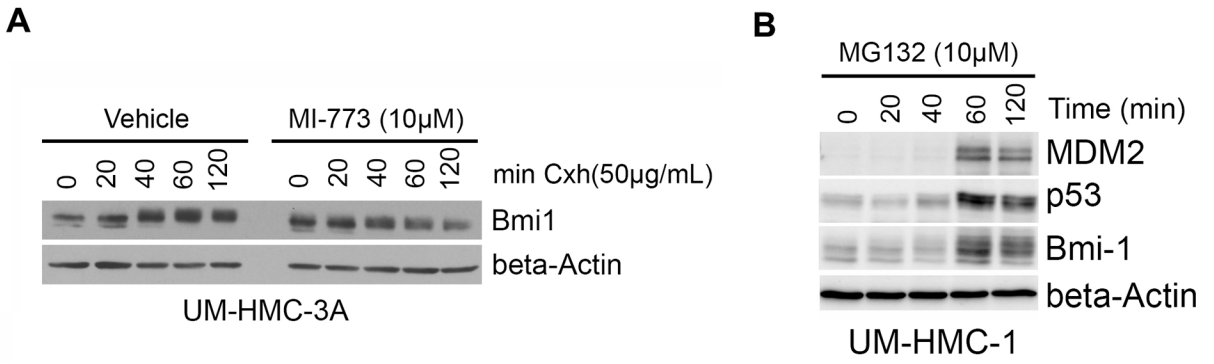
Suppl Fig 2 - Rodriguez-Ramirez et al



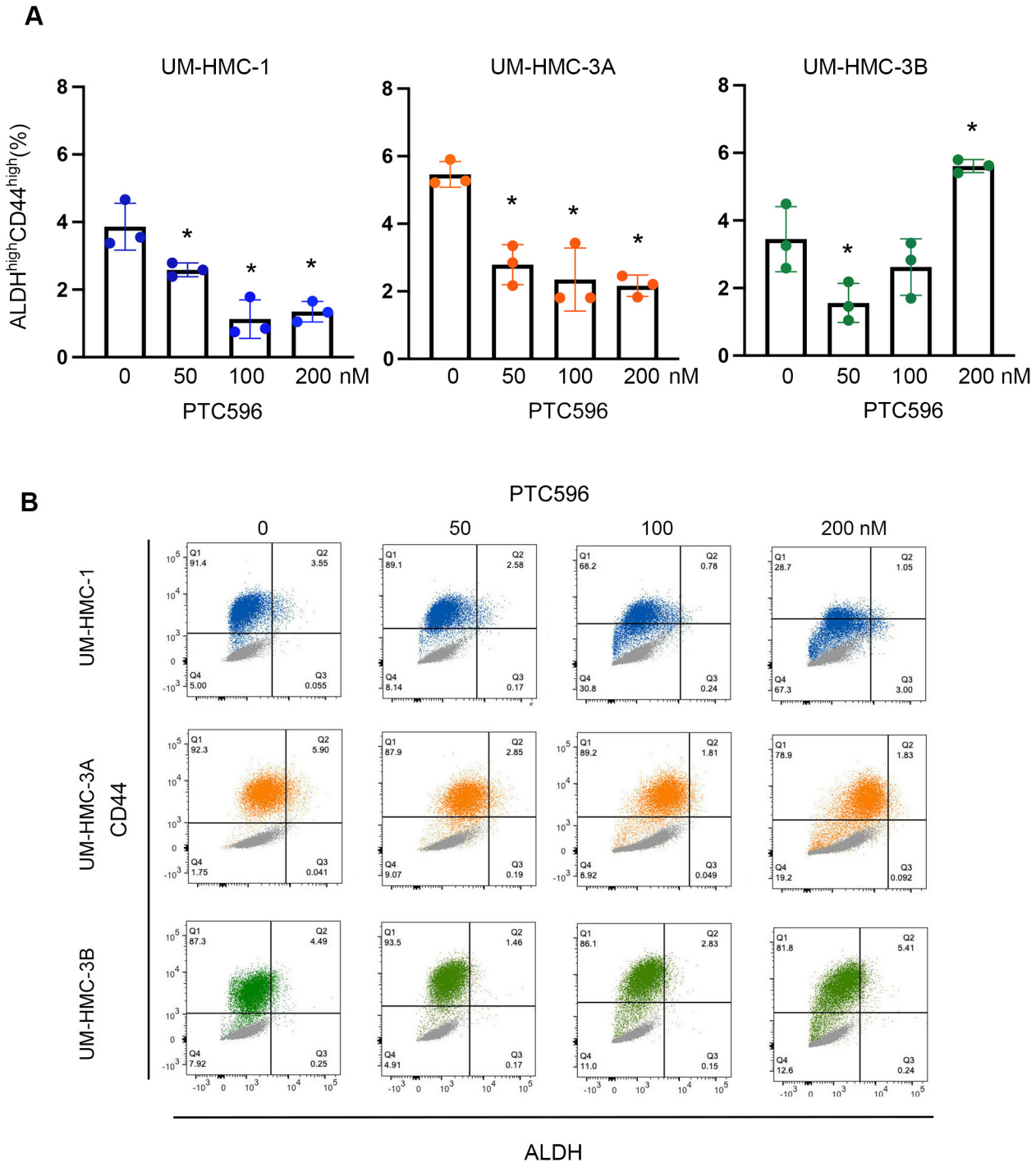
Suppl Fig 3 - Rodriguez-Ramirez et al



Suppl Figure 4 - Rodriguez-Ramirez et al



Suppl Fig 5 - Rodriguez-Ramirez et al



Supplementary Fig. 6 - Rodriguez-Ramirez et al