8. Annex

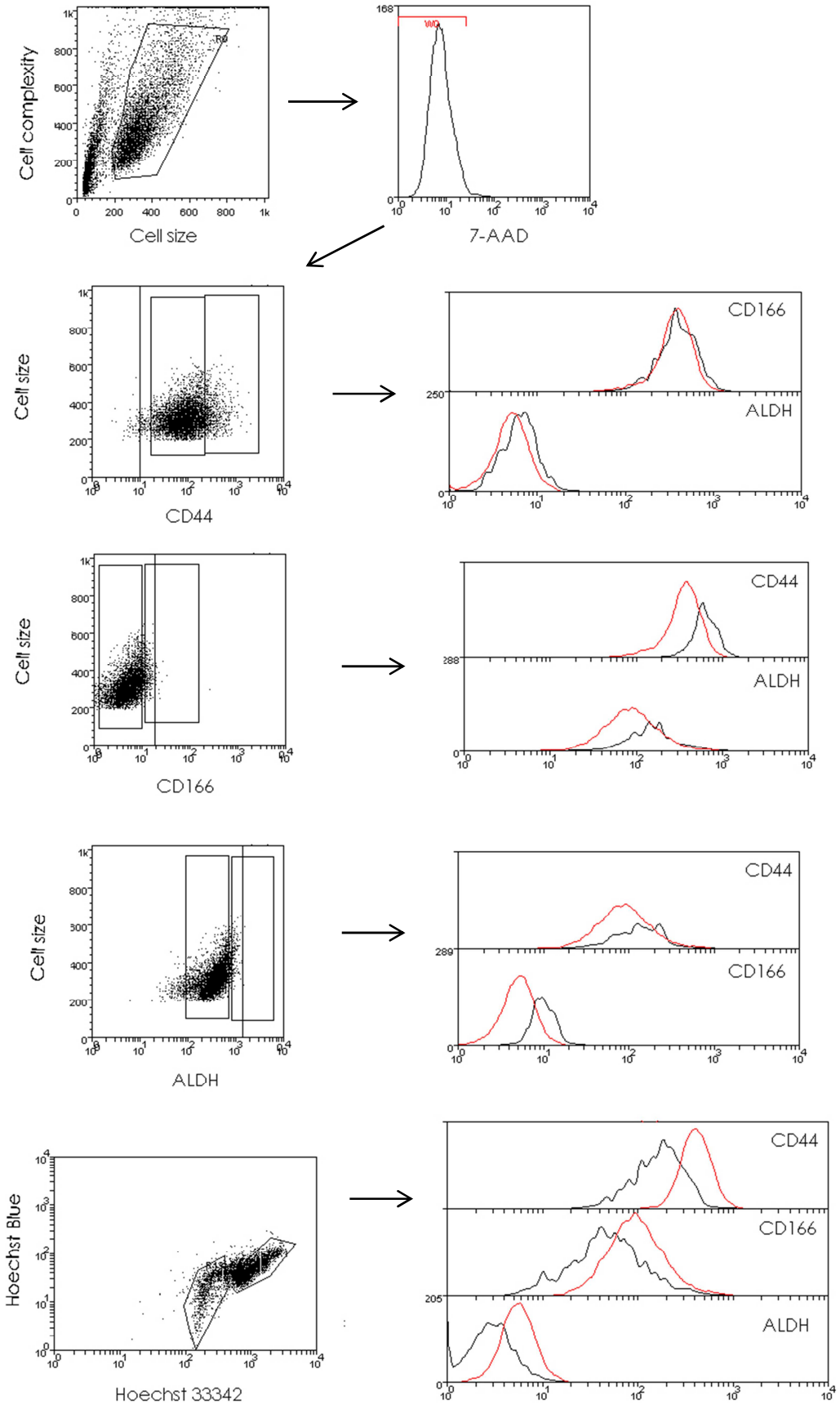


***Figure S1*. Analysis scheme of the correlation between CD44, CD166 and the transcription factors with the ALDH activity and the side population.** First, living cells were gated according to their size and complexity and, over this selection, 7-AAD negative cells were gated again. Using these two gates, living cells were analysed for the expression of the different CSC-related markers: CD44, CD166, ALDH and Hoechst 33342. Finally, cells with high and low expression of these markers were selected and the rest of the markers were analysed over these populations. Results are shown as histograms.

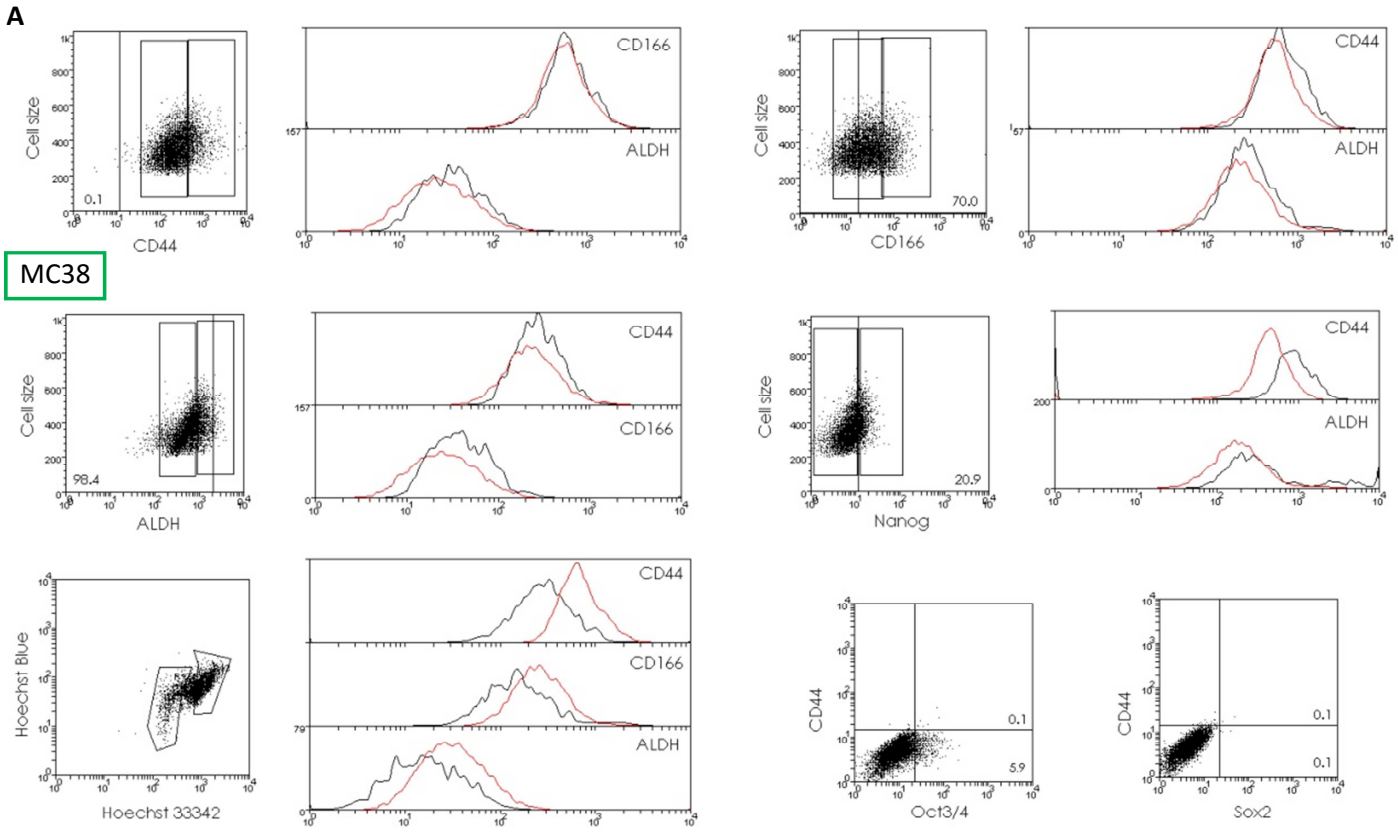
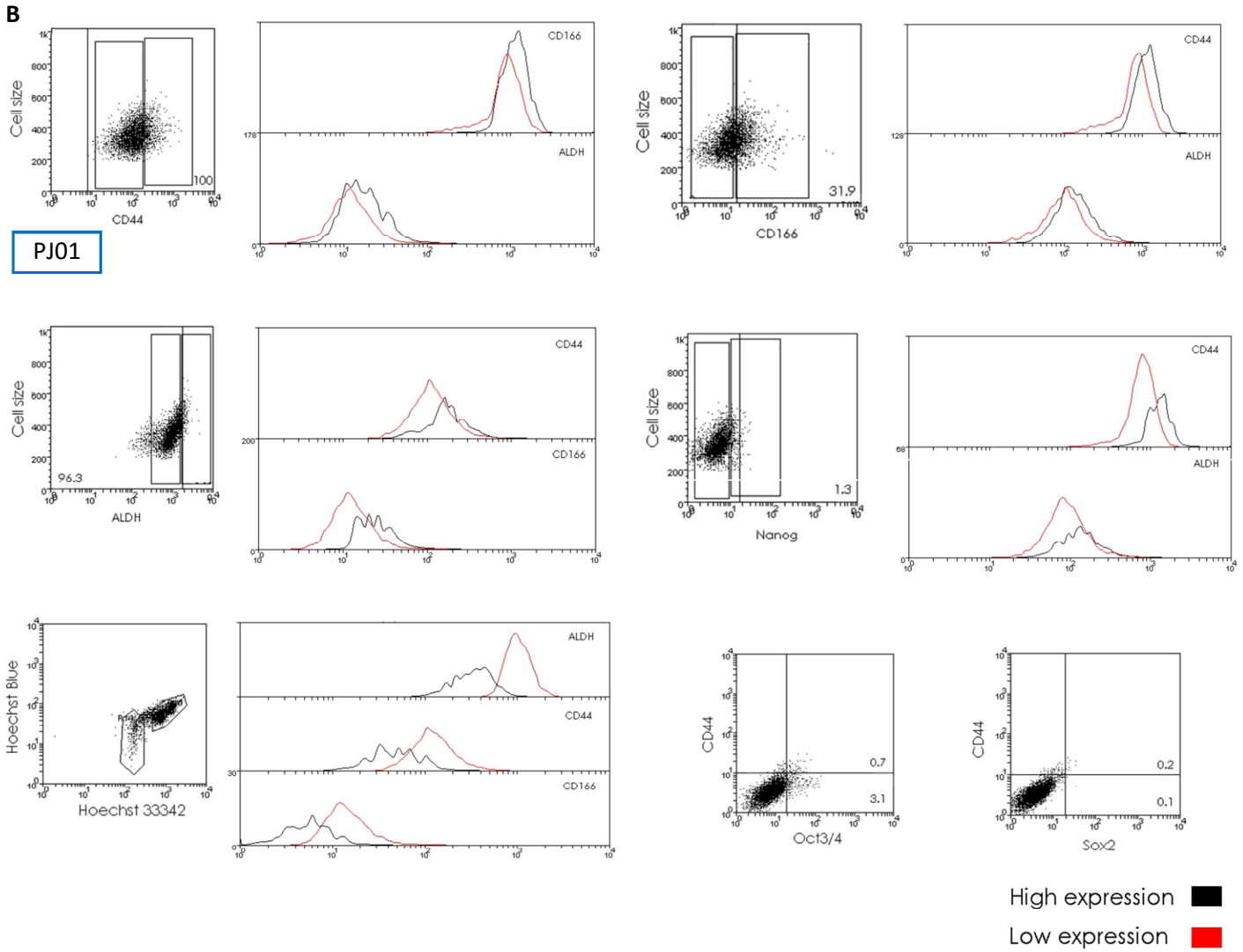


Figure S2. **Correlation between CD44, CD166 and Nanog expression and the ALDH activity and/or the Hoechst 33342 retention in MC38 (A) and PJ01 (B) cell lines.** Cells were incubated with antibodies anti-CD44, anti-CD166 and anti-Nanog and ALDH activity detection as well as Hoechst 33342 retention assays were performed following the protocol descripted in *3.2.3. Materials and Methods* After living cells selection, each marker was analysed by dot plots and their correlation with other markers is shown as histograms.



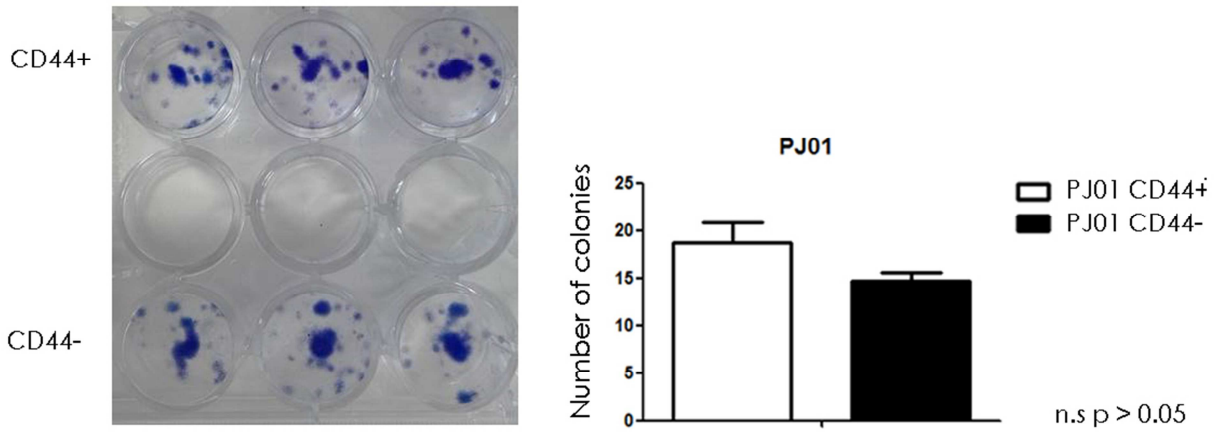
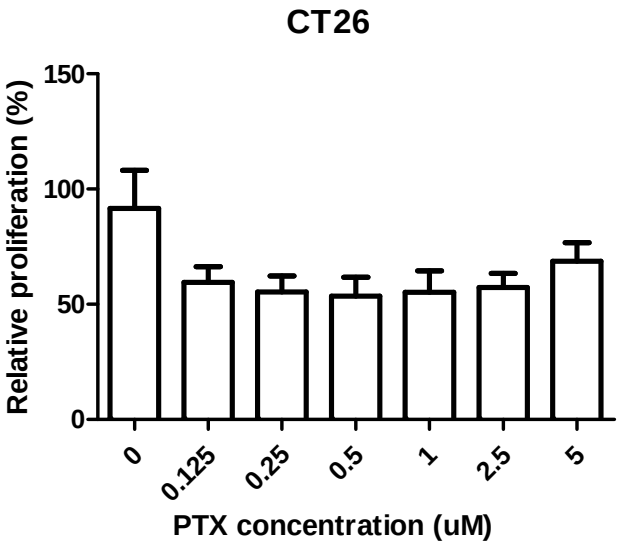
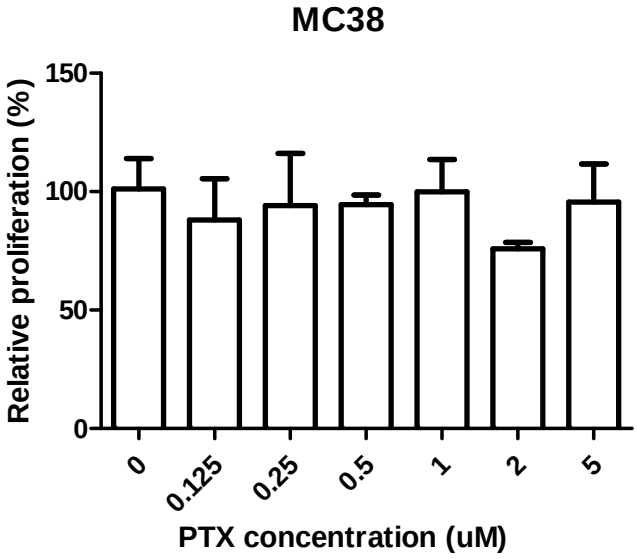


Figure S3. **Results of the clonogenic assay in PJ01 cell line.** Cells were sorted attending their expression of CD44. The top and low 10% of the CD44 expressing cells were sorted in 24-well plate and incubated in complete DMEM media for 10 days. After the incubation, colonies formed were stained using a violet crystal solution which contains 6% of glutaraldehyde in order to visualise them. The number of violet colonies was counted and results are shown as histograms. Statistical analysis was performed using GraphPad Prism software.





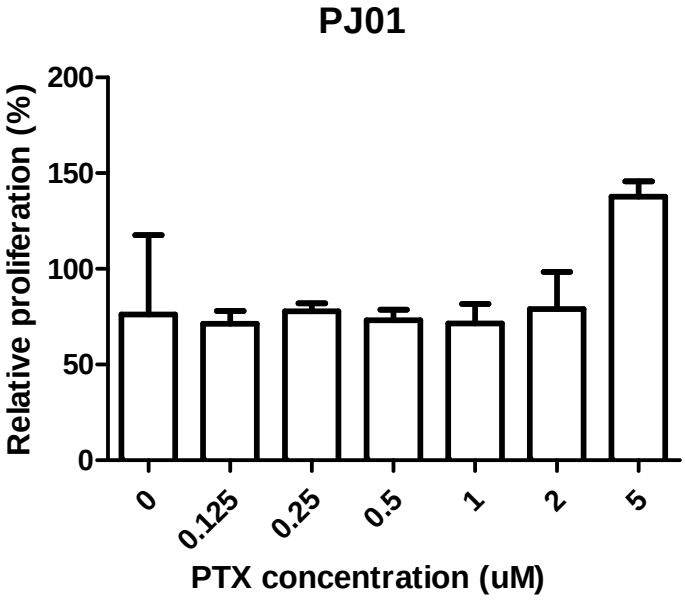


Figure S4. **Results of the MTT assays for Paclitaxel (PTX) in CT26, MC38 and PJ01 cell lines.** 5.000 cells were incubated with different doxorubicin concentrations for 48h and afterwards, MTT assays were performed. Cells were incubated for 1h with MTT reactive which can is reduced by viable cells to formazan. This reactive is insoluble and precipitated inside the cells. After the incubation with MTT, cells were spun down for 30' at 2.850 xg and resuspended in DMSO in order to dissolve the formazan crystals. Absorbance was measured at 540 nm. Results are shown as histograms in which is represented the proliferation relative to the control analysis.

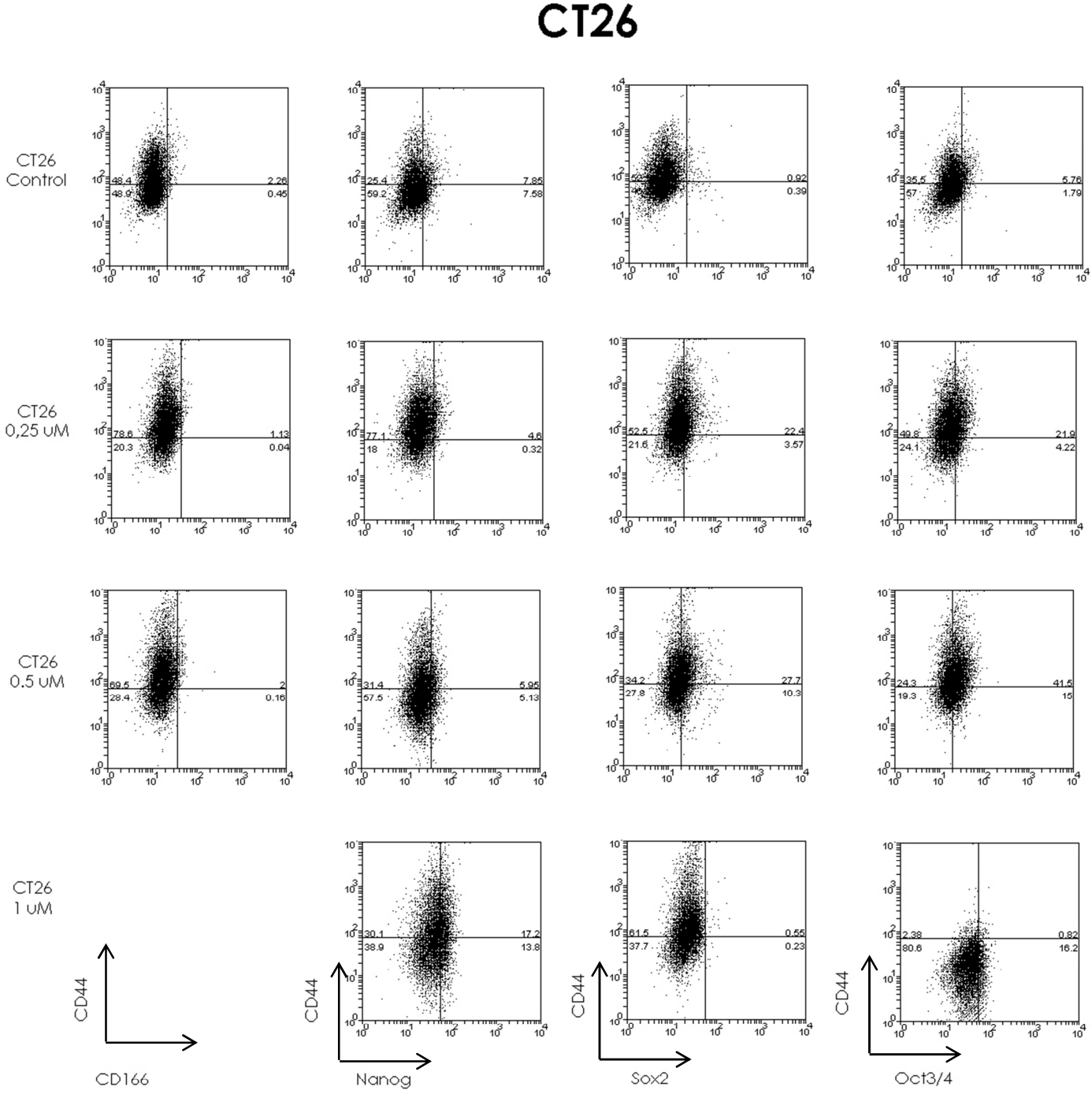


***Figure S5*. Transcription factors and extracellular CSC-related markers after the treatment with different concentrations of the antitumor drug doxorubicin in CT26 cell line.** 100.000 cells were incubated with 0.25, 0.5 or 1 µM of doxorubicin for 48h and afterwards, cells were stained following the extracellular and intracellular staining protocol as indicated in *Materials and Methods.* Results are shown as dot plots in which in indicated the percentage of cell per each quadrant. As anti-CD166, Nanog, Sox2 and Oct3/4 antibodies are conjugated with the same fluorophore, different tubes were used for the analysis but anti-CD44 was added in all of them in order to see differences between replicates.

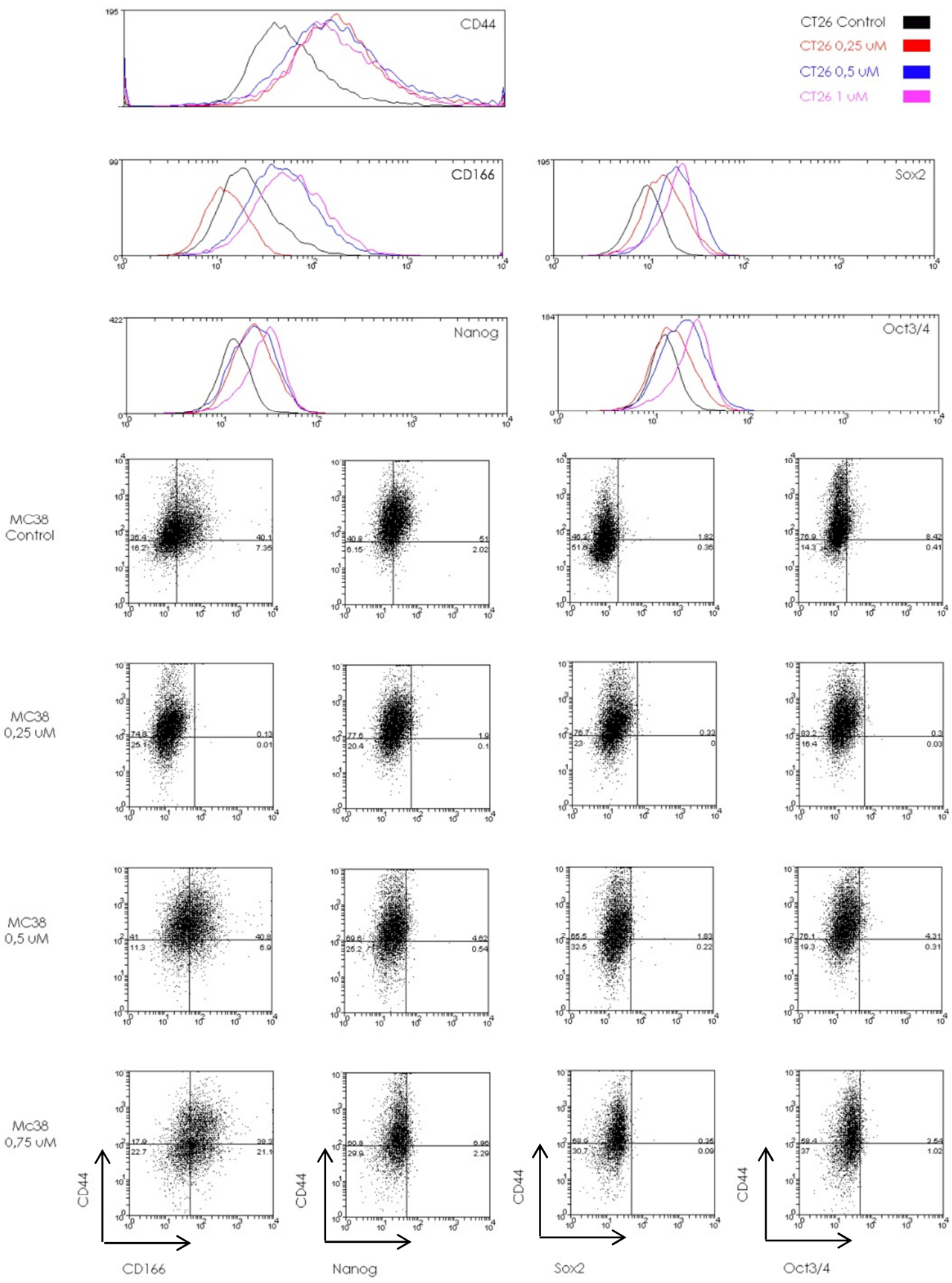


Figure S6. **Transcription factors and extracellular CSC-related markers after the treatment with different concentrations of the antitumor drug doxorubicin in MC38 cell line.** 100.000 cells were incubated with 0.25, 0.5 or 1 µM of doxorubicin for 48h and afterwards, cells were stained following the extracellular and intracellular staining protocol as indicated in *Materials and Methods.* Results are shown as dot plots in which is indicated the percentage of cell per each quadrant as well as histograms. As anti-CD166, Nanog, Sox2 and Oct3/4 antibodies are conjugated with the same fluorophore, different tubes were used for the analysis but anti-CD44 was added in all of them in order to see differences between replicates. CD44 histogram is one representative analysis of the four done.

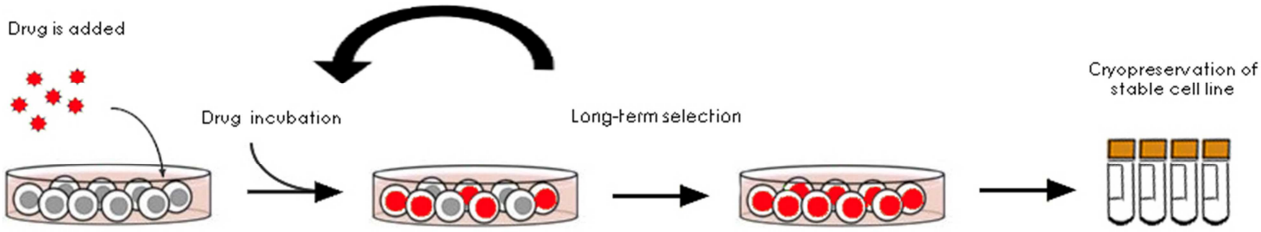


Figure S7. **Procedure scheme of the development of a chemoresistant cell line and cell lines got in this study.** Cells were incubated with a sub-lethal drug concentration for some days. Afterwards, media was replaced by a non-containing drug media for one week in order to allow cells to grow and proliferate. When cells reached the confluence of the flask, the same procedure was repeated for 3 weeks. After this long-term incubation, cells were cryopreserved and drug concentration was increased. The procedure went on until reaching to the desired drug concentration.

	Drug resistance	**Drug resistance concentration (µM)**	**Parental cell line**
CT26-0,5 DoxR	Doxorubicin	0.5	CT26
CT26-1 DoxR	Doxorubicin	1	CT26
CT26-2 DoxR	Doxorubicin	2	CT26
CT26-1 PTXR	PTX	1	CT26
PJ01-50n DoxR	Doxorubicin	0.05	PJ01
MC38-50n DoxR	Doxorubicin	0.05	MC38

Table S1**. Summary of the chemoresistant cell lines generated in this study.** Drug resistance and the concentration at which each cell line is resistant to are shown in the table.