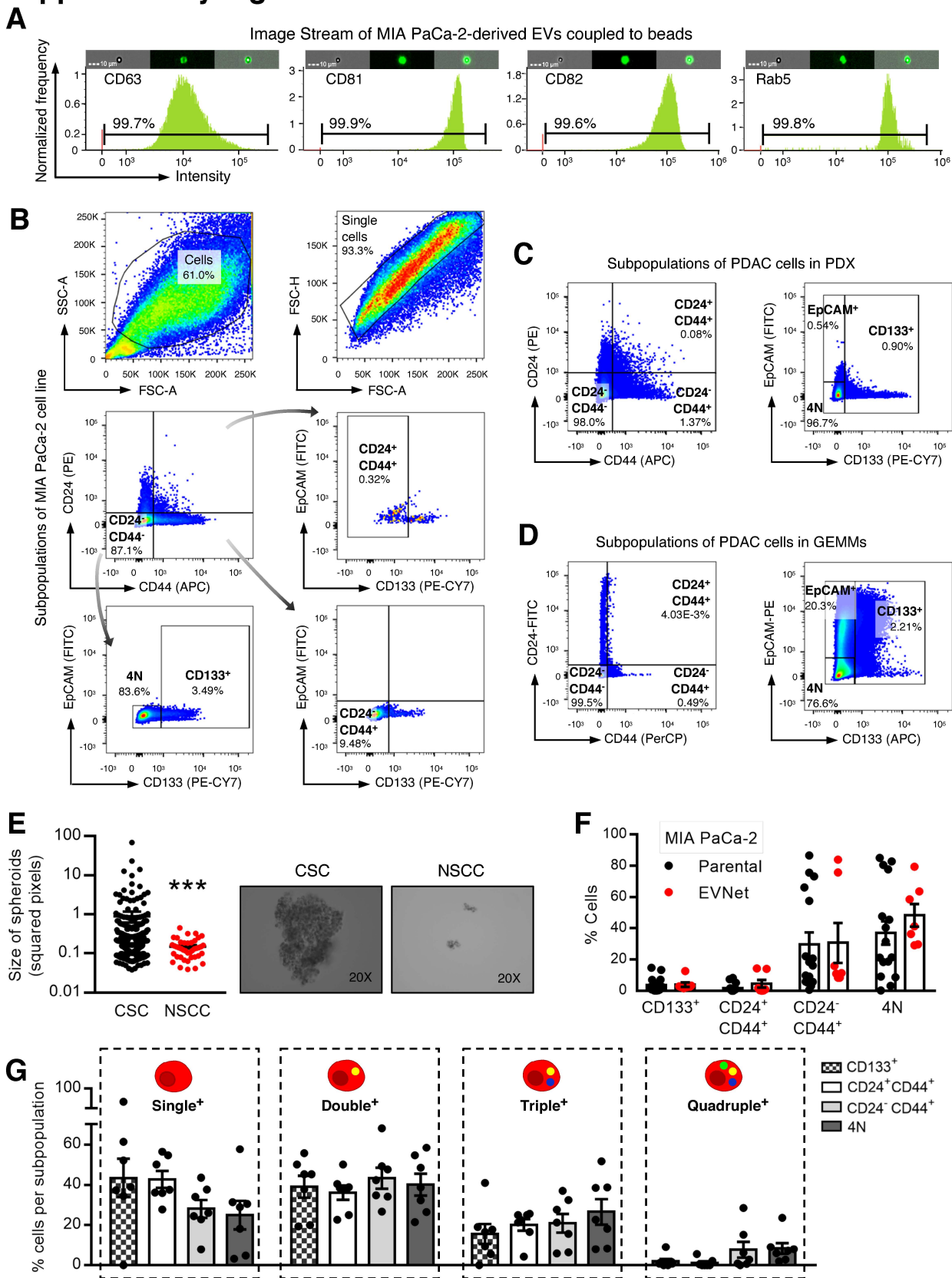


Supplementary Figure 1



Supplementary Figure 1 (related to Figure 1). Characterization of extracellular vesicles markers and PDAC subpopulations. (A) Image stream analysis of a panel of EVs markers (CD63, CD81, CD82 and Rab5) in EVs isolated from the human pancreatic cancer cell line, MIA PaCa-2. Scale bar 10 μ m. **(B)** Gating strategy for the isolation of the PDAC subpopulations: CD24⁺CD44⁺CD133⁻EpCAM⁻; CD133⁺CD24⁻CD44⁻EpCAM⁻; CD24⁻CD44⁺CD133⁻EpCAM⁻; CD24⁻CD44⁻CD133⁻EpCAM⁻ and CD24⁻CD44⁻CD133⁻EpCAM⁺. Percentages of subpopulations are relative to single cells gating. **(C)** Representative FACS plots of the isolation of PDAC subpopulations in a PDX pancreas tumor. Percentages of subpopulations are relative to viable cells gating and negative to CD31. The same gating strategy described in (B) was used. **(D)** Representative FACS plots of the isolation of PDAC subpopulations in the PDAC GEMM KPC. Percentages of subpopulations are relative to viable cells gating and negative to CD31. The same gating strategy described in (B) was used. **(E)** Size of spheroids originated from MIA PaCa-2 subpopulations (CSC - CD24⁺CD44⁺ and CD133⁺; NSCC - CD24⁻CD44⁺ and 4N) (left, Mann-Whitney test, *** $p=0.0009$) and representative pictures (right). **(F)** Distribution of PDAC subpopulations, in MIA PaCa-2 parental (n=16) or in MIA Paca-2 clones expressing CD63-turboGFP, CD82-eYFP, CD81-tdTomato and Rab5-mPlum (n=7) used in EVNet experiments (Figure 1A) (Sidak's multiple comparisons test, ns). **(G)** Quantification of the percentage of single, double, triple and quadruple positive cells found in each subpopulation in the MIA Paca-2 intermix cultures (Figure 1A; n=7). Data are mean \pm SEM.