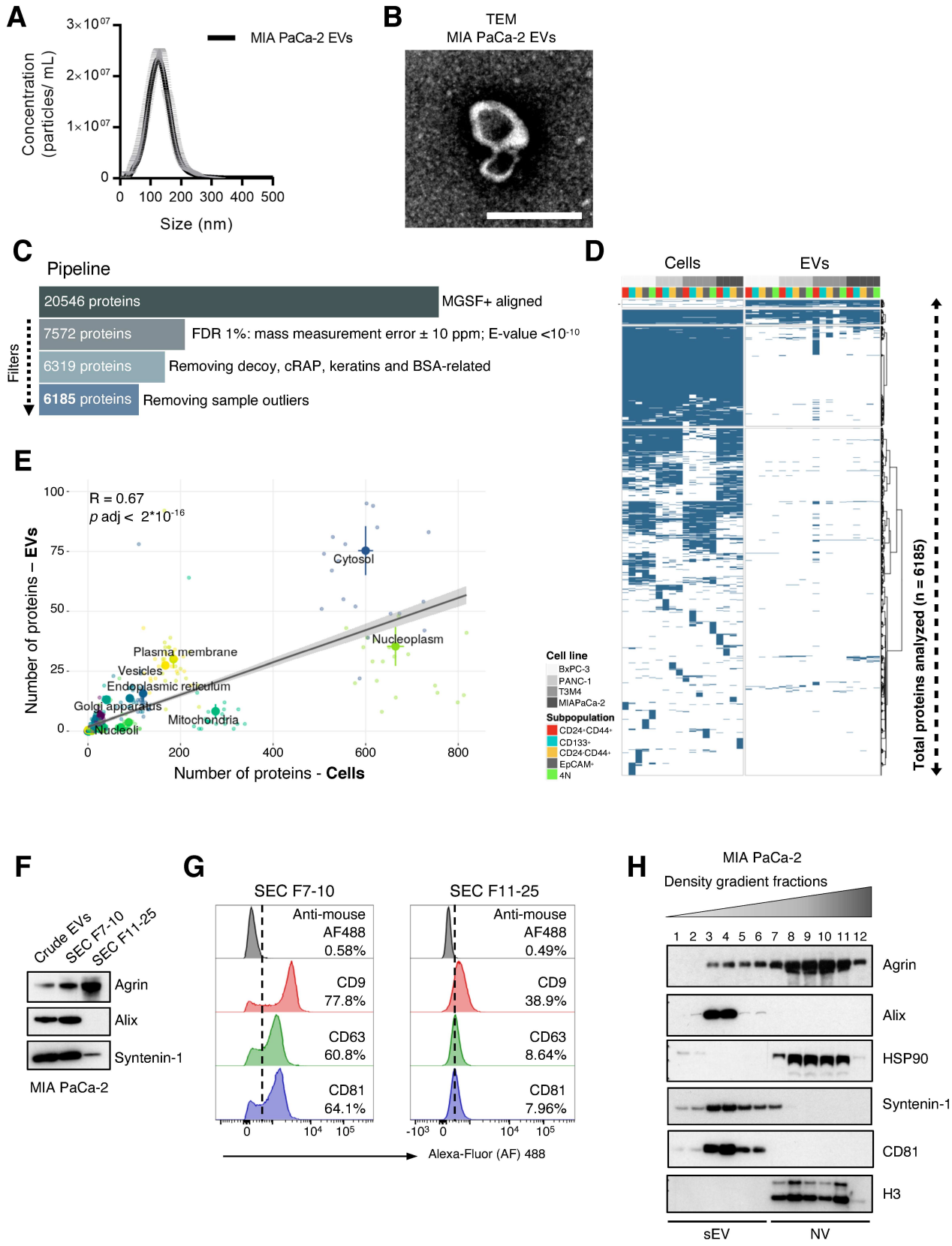
Supplementary Figure 7



Supplementary Figure 7 (related to Figure 5). Agrin is present in extracellular vesicles and non-vesicular content derived from PDAC cells. (A-B) Representative nanoparticle tracking analysis **(A)** and transmission electron microscopy micrograph **(B)** of MIA PaCa-2 EVs isolated by ultracentrifugation. Scale bar 500nm. **(C)** Pipeline used for analysis of the LC/ESI-MS/MS data. Global number of proteins detected in all samples at every step during the distinct filtering steps. FDR: False Discovery Rate. **(D)** Heatmap depicting total proteins identified in PDAC subpopulations and respective EVs in four cell lines (BxPC3, PANC-1, T3M4 and MIA PaCa-2). The heatmap depicting only the protein clusters of this full heatmap is represented in Figure 5B. **(E)** Comparison of subcellular location of detected proteins in cells and EVs (PCC=0.67), based on Human Protein Atlas database. EVs samples were found enriched in *cytosol*, *plasma membrane, vesicles* and *endoplasmic reticulum* proteins. **(F)** Western blot of agrin in MIA PaCa-2 crude EVs (protein extracted from EVs isolated by ultracentrifugation at 100000g overnight after being filtered through a 200nm filter) and in fractions 7-10 (vesicular content) and fractions 11-25 (non-vesicular content) after size exclusion chromatography (SEC) of MIA PaCa-2 EVs. Alix and Syntenin-1 were used as markers to identify fractions containing vesicular content. **(G)** Flow cytometry analysis of CD9, CD63 and CD81 in fractions 7-10 (vesicular content) and fractions 11-25 (non-vesicular content) after SEC of MIA PaCa-2 EVs coupled to beads. Secondary antibody only was used as control. **(H)** Western blot of agrin in density gradient fractions of MIA PaCa-2 EVs. EVs fractions were characterized by the expression of CD81, Syntenin-1 and Alix and the non-vesicular fractions by the presence of HSP90 and Histone H3 .