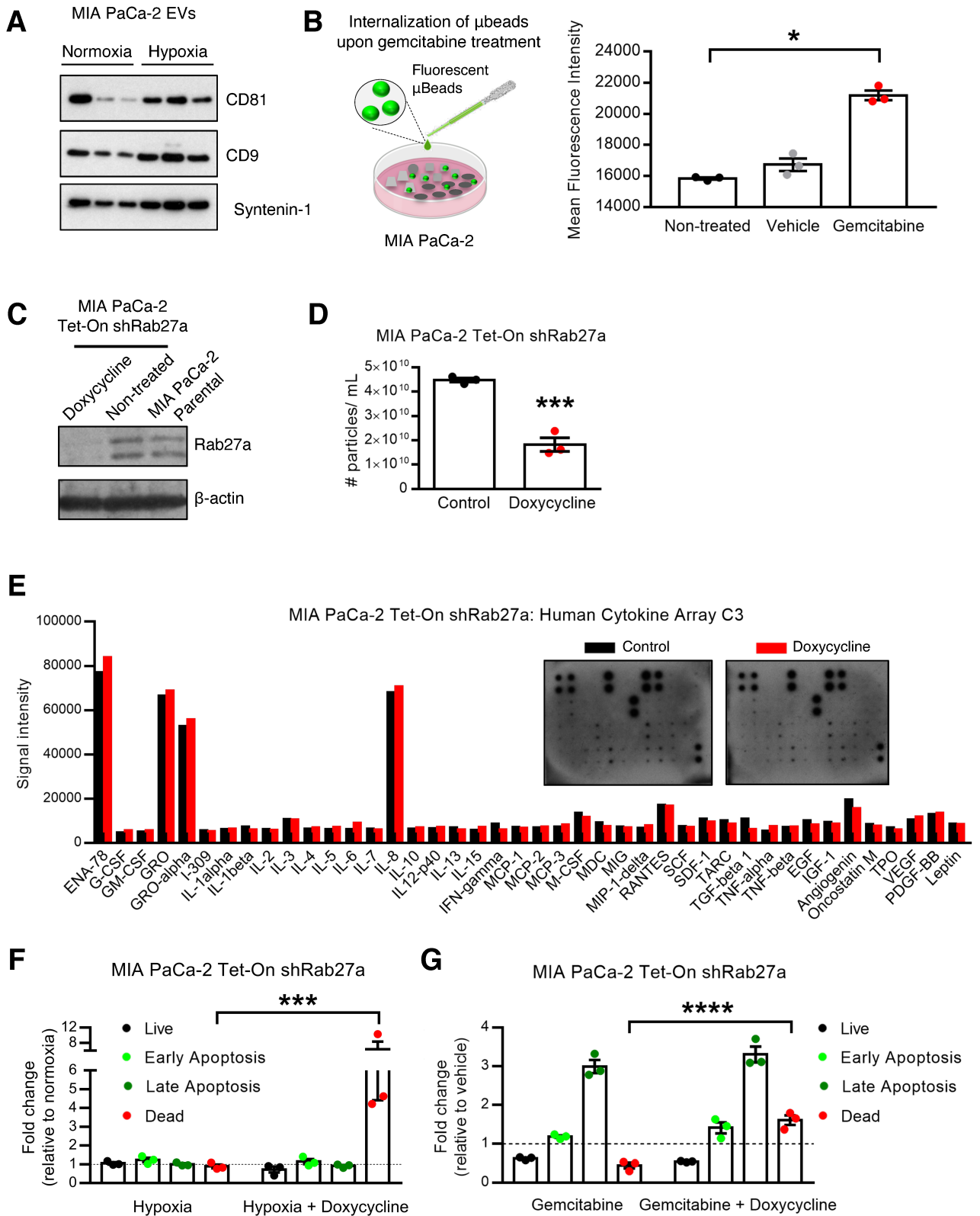


## Supplementary Figure 2



**Supplementary Figure 2 (related to Figure 2). Hypoxia and gemcitabine promote changes in extracellular vesicles release and uptake. (A)** Western blot of EVs from MIA PaCa-2 cells upon normoxia (21% O<sub>2</sub>) or hypoxia (1% O<sub>2</sub>) isolated by ultracentrifugation at 100000 g and stained with CD81, CD9 and Syntenin-1 (equal volume was loaded for each sample). **(B)** Experimental setup (left): MIA PaCa-2 was treated with gemcitabine and fluorescent microbeads, and analysed through flow cytometry for fluorescent microbeads uptake. Quantification of mean fluorescent intensity (MFI) of cells showing uptake of fluorescent microbeads upon non-treatment, vehicle (H<sub>2</sub>O) or gemcitabine (1 μM) (right, n=3, one-way anova \**p*<0.05). Data are mean±SEM. **(C)** Representative Rab27a western-blot in the Mia PaCa-2 Tet-On shRab27a cell line cultured without treatment or with doxycycline. Mia PaCa-2 parental cell line was included as control. Beta-actin was used as a loading control. **(D)** Quantification of the number of particles secreted by the Mia PaCa-2 Tet-On shRab27a cell line cultured without treatment or with doxycycline measured by nanoparticle tracking analysis (n=3, unpaired t-test \*\*\**p*<0.001). **(E)** Representative images of a cytokine array on MIA PaCa2 Tet-On shRab27a conditioned media from non-treated or doxycycline treated (Rab27a KD) cells (top). Quantification of the 42 cytokine array targets detected in conditioned media from MIA PaCa2 Tet-On shRab27a cells untreated or treated with doxycycline (bottom). **(F)** Quantification of live, early apoptosis, late apoptosis or dead MIA PaCa-2 Tet-On shRab27a cells under hypoxia (1% O<sub>2</sub>) untreated (Rab27a WT n=3) or treated with doxycycline (Rab27a KD n=3). Results are fold change to normoxia (Sidak multiple comparison test \*\*\**p*=0.0002). **(G)** Quantification of live, early apoptosis, late apoptosis or dead MIA PaCa-2 Tet-On shRab27a cells treated with gemcitabine (1 μM) (Rab27a WT n=3) or treated with gemcitabine (1 μM) and doxycycline (Rab27a KD; n=3). Results are fold change to vehicle (Sidak multiple comparison test \*\*\*\**p*<0.0001). Data are mean±SEM.