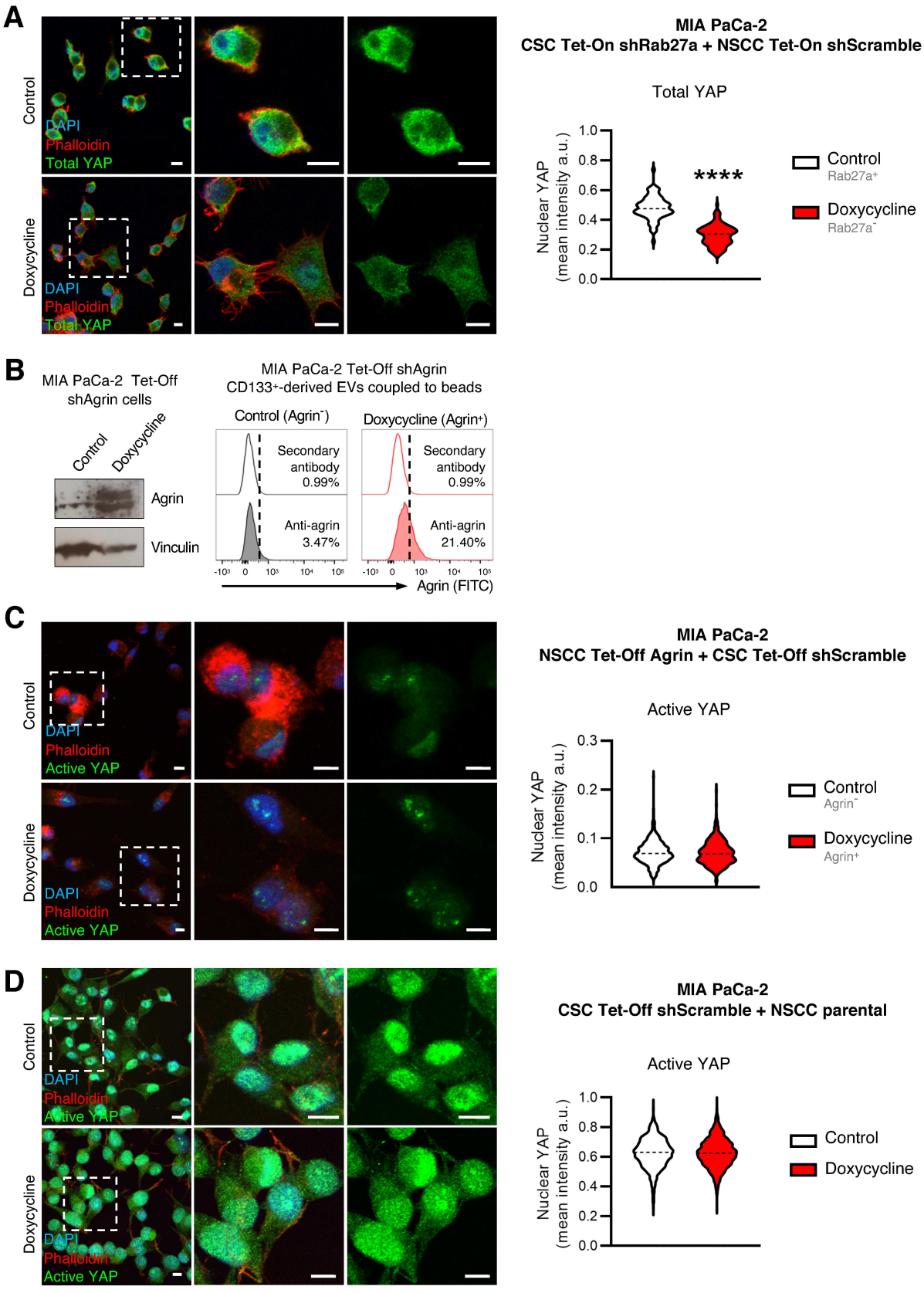
Supplementary Figure 8



Supplementary Figure 8 (related to Figure 6). Agrin positive NSCC extracellular vesicles do not promote YAP nuclear location. (A) Immunofluorescent staining of CSC from MIA PaCa-2 Tet-On shRab27A cultured with NSCC from MIA PaCa-2 Tet-On shScramble (cultured at the same percentages found in the MIA PaCa-2 Tet-On shRab27A). Anti-total YAP staining (green), phalloidin (red) and nuclei (blue) (left), and quantification of YAP nuclear levels (mean intensity per cell; n=2, 6 images per group, unpaired t-test ****p<0.0001). Data are Min to Max. Scale bar 10μm. Dashed lines in violin plot represent median values. **(B)** Western blot of agrin in MIA PaCa-2 Tet-Off shAgrin cells treated with doxycycline or control. Vinculin was used as loading control (left). Representative FACS analysis for Agrin in EVs derived from the $CD133^{+}$ MIA PaCa-2 Tet-Off shAgrin subpopulation. Secondary antibody only was used as control (right). **(C)** Immunofluorescent staining of NSCC from MIA PaCa-2 Tet-Off shAgrin cultured with CSC from MIA PaCa-2 Tet-Off shScramble (cultured at the same percentages found in the MIA PaCa-2 Tet-Off shScramble). Anti-active YAP staining (green), phalloidin (red) and nuclei (blue) (left), and quantification of YAP nuclear levels (mean intensity per cell; n=2, 6 images per group, unpaired t-test). Scale bar 10μm. Data are Min to Max. Dashed lines in violin plot represent median values. **(D)** Immunofluorescent staining of CSC from MIA PaCa-2 Tet-Off shScramble cultured with MIA PaCa-2 NSCC (cultured at the same percentages found in the MIA PaCa-2 Tet-Off shScramble). Anti-active YAP staining (green), phalloidin (red) and nuclei (blue) (left), and quantification of YAP nuclear levels (mean intensity per cell; n= 2, 6 images per group, unpaired t-test). Scale bar 10μm. Data are Min to Max. Dashed lines in violin plot represent median values.