Supplemental information

Development of Liver-Targeting $\alpha_v\beta_5^+$ Exosomes as anti-TGF-β Nanocarriers for the Treatment of the Pre-Metastatic Niche

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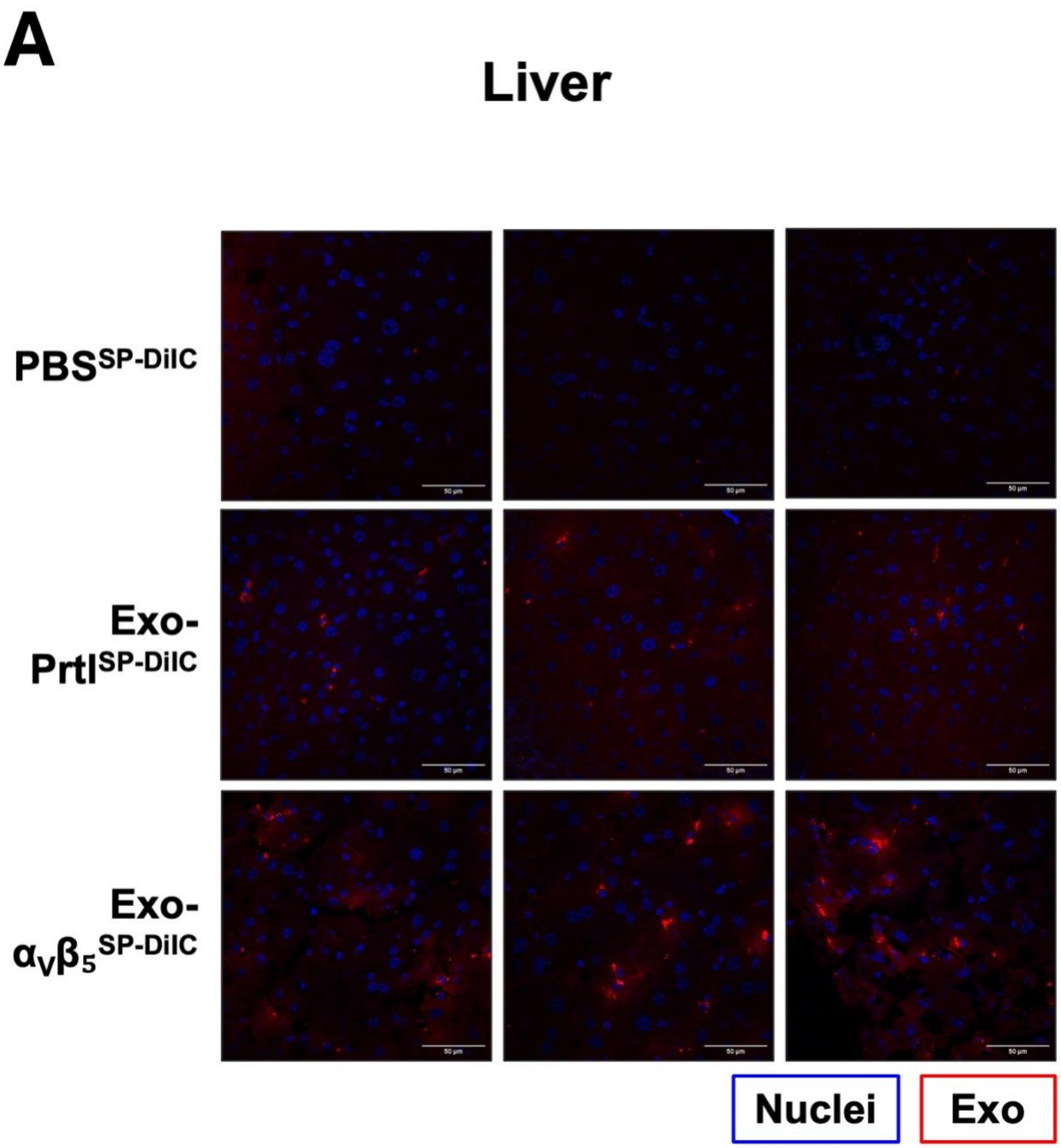
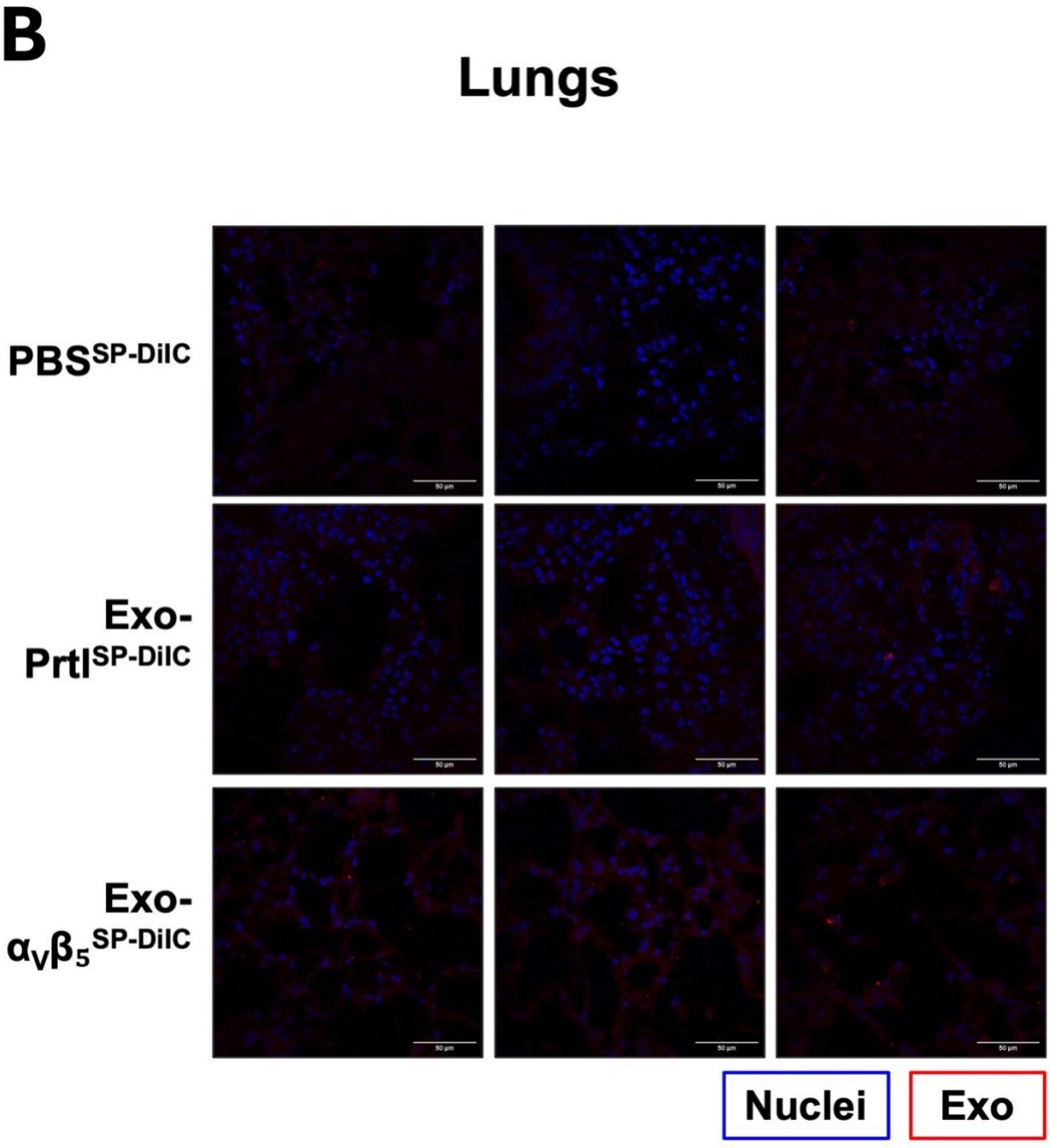
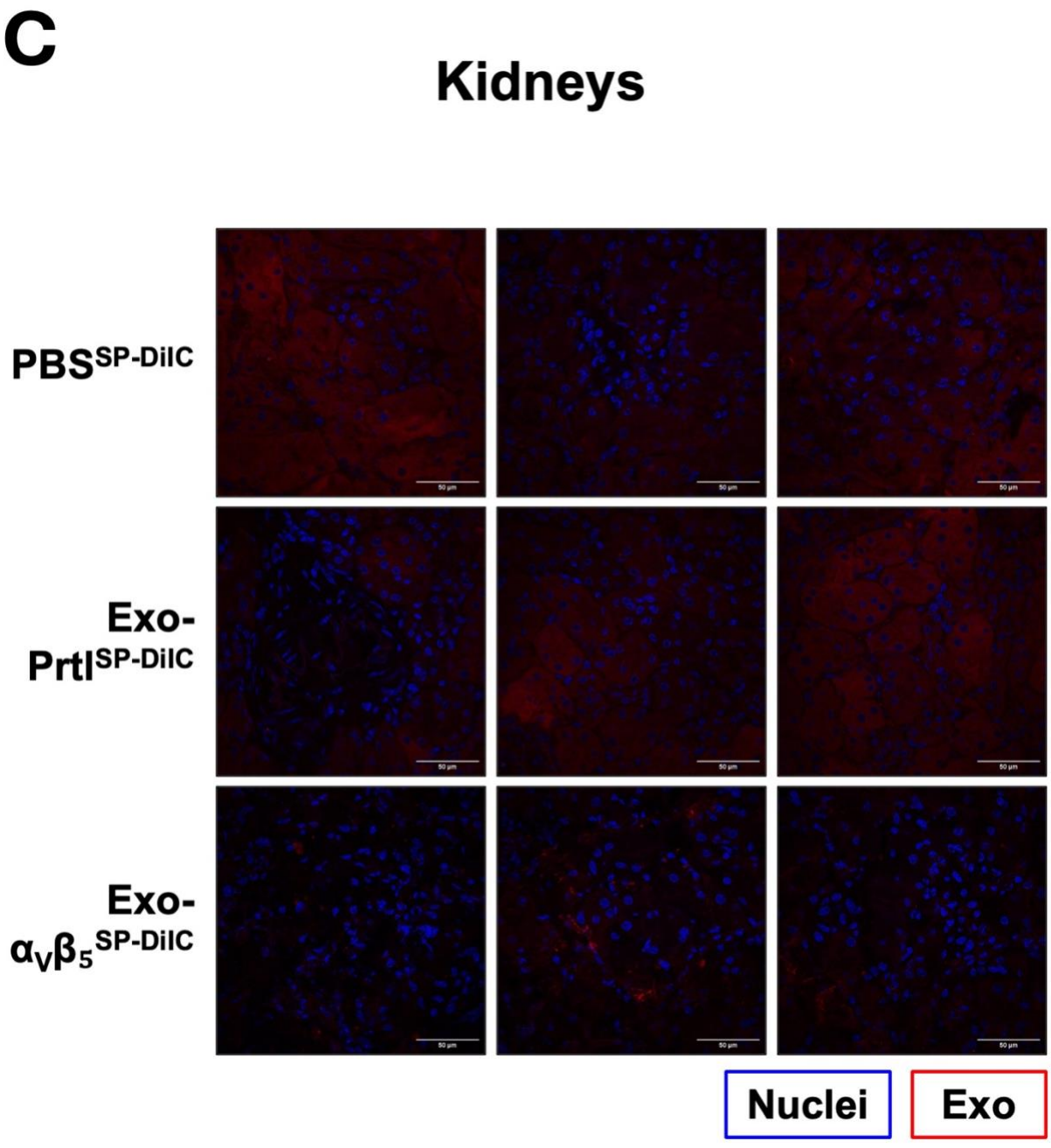
Supplemental Figure S1A. The overexpression of the integrin $\alpha_v\beta_5$ increases exosome accumulation in the liver.



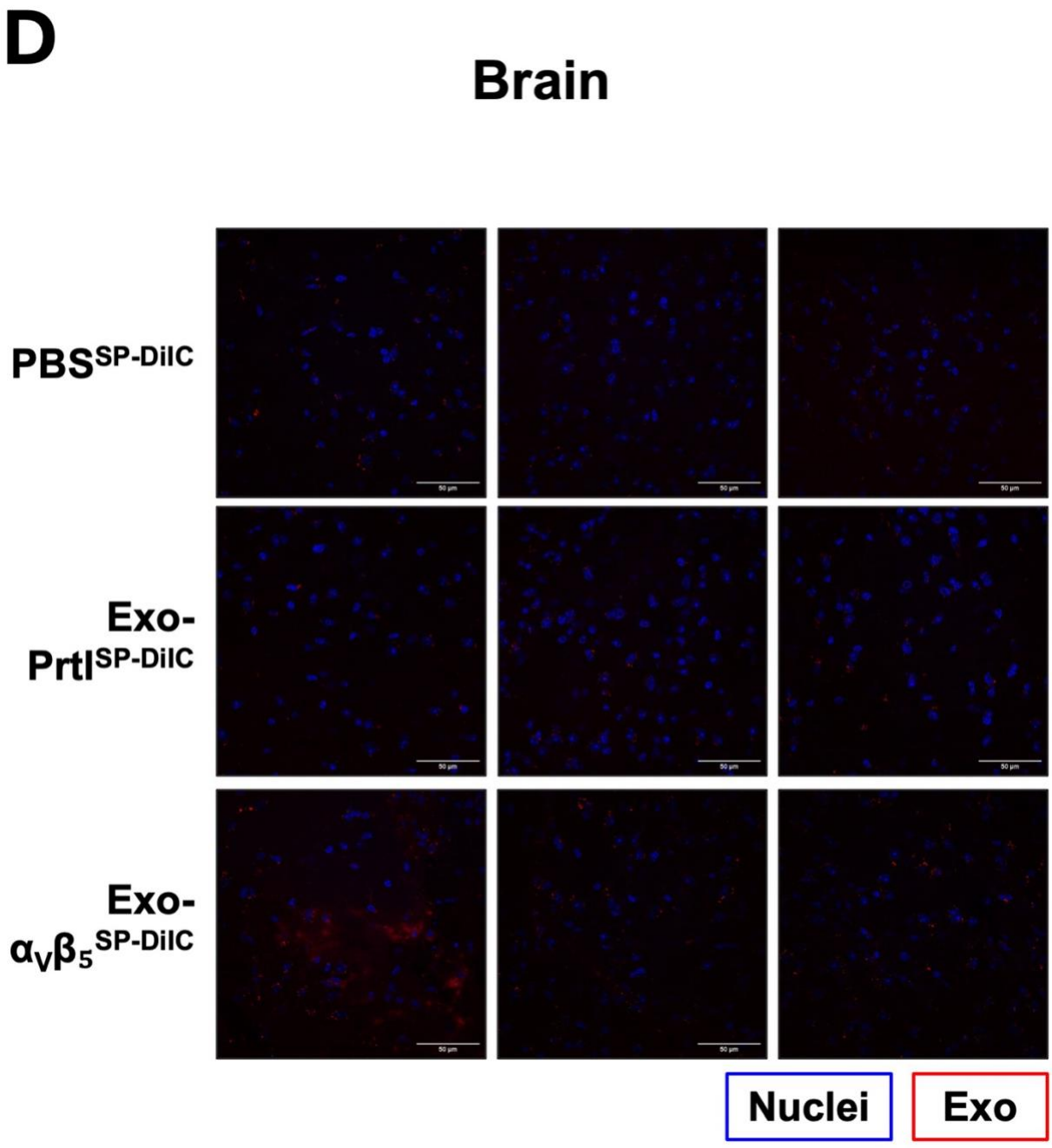
Supplemental Figure S1B. The overexpression of the integrin $\alpha_v\beta_5$ increases exosome accumulation in the liver.



Supplemental Figure S1C. The overexpression of the integrin $\alpha_v\beta_5$ increases exosome accumulation in the liver.

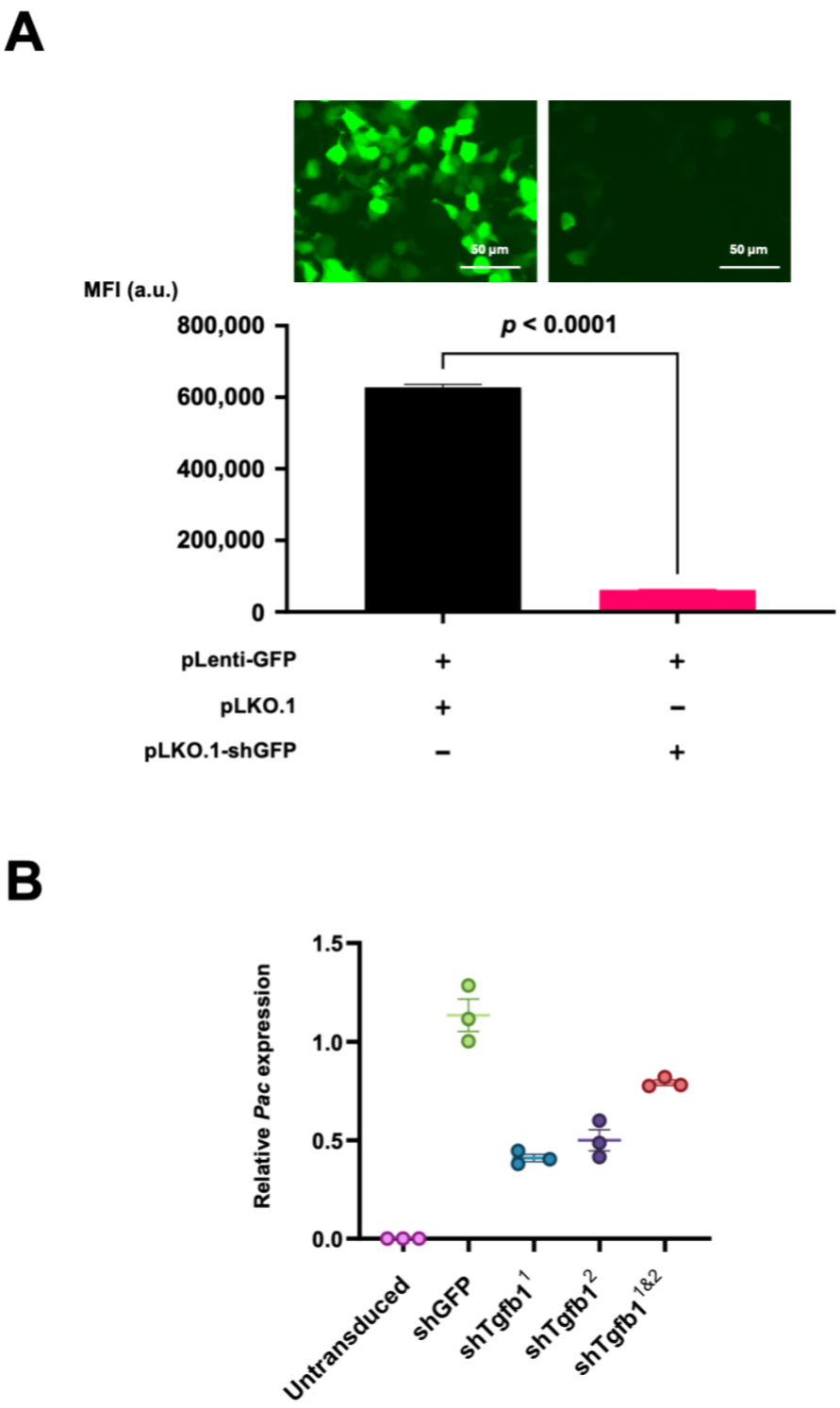


Supplemental Figure S1D. The overexpression of the integrin $\alpha_v\beta_5$ increases exosome accumulation in the liver.



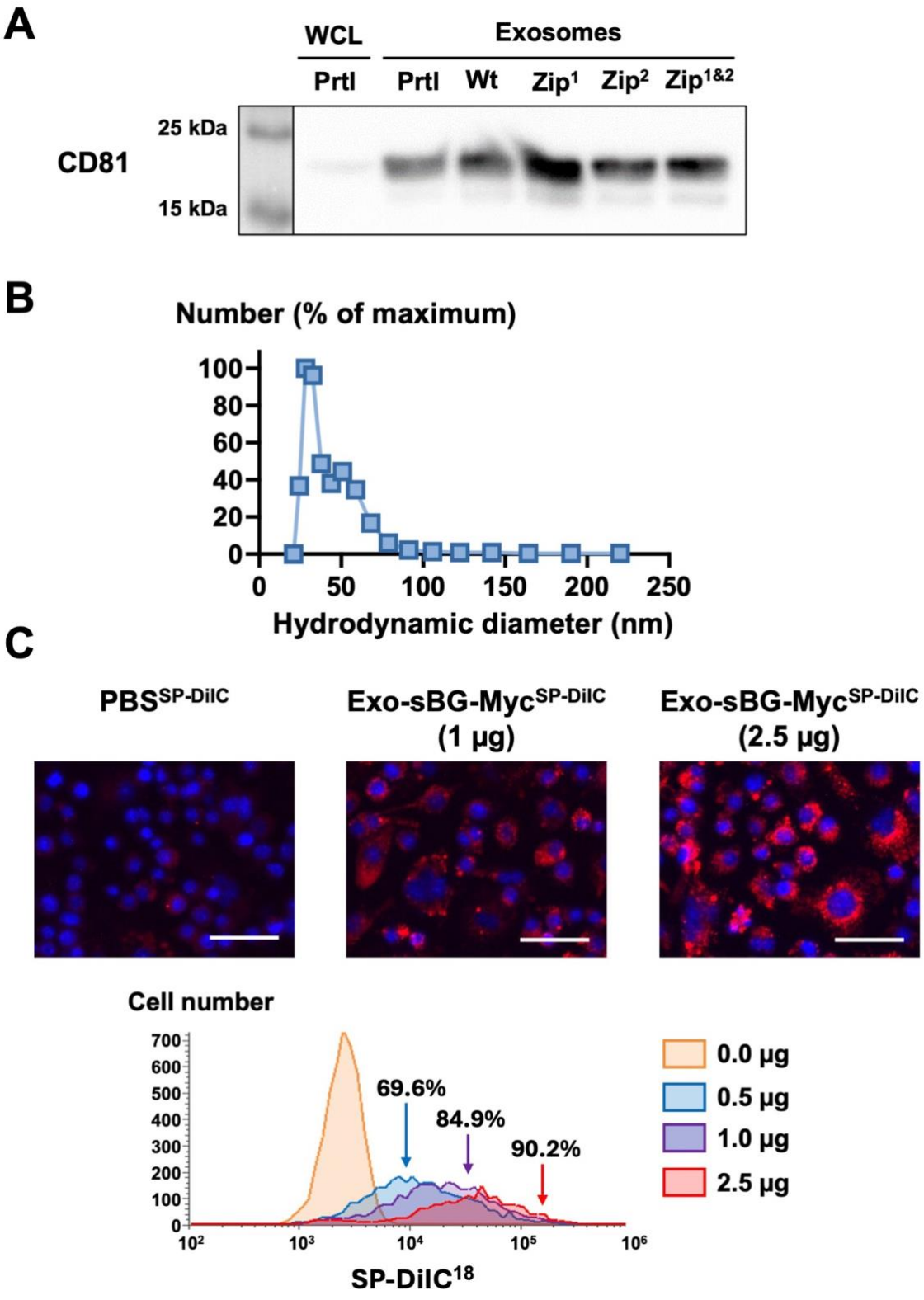
Supplemental Figure S1. The overexpression of the integrin $\alpha_v\beta_5$ increases exosome accumulation in the liver. Mice were inoculated with 40 μg of exosomes from parental 293T cells (Exo-Prtl$^{SP\text{-}DiIC}$) or 293T-$\alpha_v\beta_5^+$ cells (Exo-$\alpha_v\beta_5^{SP\text{-}DiIC}$) stained with SP-DiIC$_{18}$ (red) or with a control solution (PBS$^{SP\text{-}DiIC}$) (5.0 μM SP-DiIC$_{18}$). The next day, the liver, lungs, kidneys, and brain were collected, and cryosections with DAPI counterstaining (blue) were analyzed using a confocal microscope. Results are presented as three images of different areas of the tissue sections of **(A)** liver, **(B)** lungs, **(C)** kidneys, and **(D)** brain where fluorescence was detected. The scale bar indicates 50μm.

Supplemental Figure S2. Transduction of 293T-$\alpha v\beta_5$ cells for expression of shRNA against *GFP* or *Tgfb1*.



Supplemental Figure S2. Transduction of 293T-$\alpha_v\beta_5$ cells for expression of shRNA against GFP or *Tgfb1*. **(A)** 293T-$\alpha_v\beta_5$ cells were co-transfected with the pLenti-CMV-GFP-Puro plasmid for GFP expression and the pLKO.1-puro or pLKO.1-shGFP. Twenty-four hours later, cells were observed under a fluorescence microscope (*upper images*) and analyzed in a flow cytometer to measure the mean fluorescence intensity (MFI) (*lower panel*). a.u., arbitrary unit. **(B)** 293T-$\alpha_v\beta_5$ cells were transduced with pLKO.1-shGFP, pLKO.1-shTgfb1[1], pLKO.1-shTgfb1[2], or both shTgfb1 plasmids (shTgfb1[1&2]) and selected with puromycin. After antibiotic selection, *Tgfb1* expression was evaluated by qRT-PCR in parental and transduced 293T-$\alpha_v\beta_5$ cells.

Supplemental Figure S3. Characterization of exosomes from 293T-$\alpha_v\beta_5$ cells transduced to express *sBG-Myc* mRNA.



Supplemental Figure S3. Characterization of exosomes from 293T-$\alpha_V\beta_5$ cells transduced to express *sBG-Myc* mRNA. **(A)** 293T cells were transduced with pLJM1-sBG-Myc wild-type plasmid or pLJM1-sBG-Myc plasmid modified with exosome zipcode sequences in the 3′ UTR: Zip^1, Zip^2, and $Zip^{1\&2}$. After antibiotic selection, exosomes were isolated and levels of CD81 marker were assessed by western blot. WCL, whole cell lysate. Prtl, parental cells. **(B)** Measurement of the hydrodynamic diameter of the exosomes isolated from 293T-sBG-Zip^1 cells. Results are presented as a histogram of the particle size distribution. **(C)** RAW 264.7 cells were cultured for 24 hours with a control solution ($PBS^{SP\text{-}DiIC}$) or SP-$DiIC_{18}$-stained exosomes (1.0 or 2.5 μg/cm^2, red) isolated from 293T-sBG-Zip^1 cells (Exo-sBG-$Myc^{SP\text{-}DiIC}$) before analyzing the cells by (*upper panel*, the scale bar indicate 50 μM) or flow cytometry (*lower panel*, percentages indicate the amount of SP-$DiIC_{18}^+$ cells).