Supplementary Materials and Methods

ExoView

The PD-L1 expression of a single exosome was analyzed with an ExoView tetraspanin kit and an ExoView (NanoView Biosciences, Boston, MA, USA) according to the manufacturer's instructions. In brief, the isolated exosomes were diluted for the recommended concentration (1-3 x 10⁸ particles in total) upon NTA measurement, and 35 µl of samples (mixed 1:1 with incubation solution) were added on the chip and incubated at RT for 16 h. The incubated samples were labeled with fluorescently labelled antibodies (anti-CD9; HI9A; CF488A, anti-CD63; H5C6; CF647, anti-CD81; JS81; CF555 provided in the kit, anti-PD-L1; MIH1; APC; cat#17-5983-42, Invitrogen, Carlsbad, CA, USA, anti-Alix; Abcam; cat#ab76608, Cambridge, UK), followed washing and imaging. For the Alix analysis, the diluted exosomes were added and incubated on the chip, following the fixation and permeabilization process to label the internal protein. The obtained data were analyzed using the NanoView analysis software version 2.9, and further analysis was performed using Matlab (MathWorks).