Electronic Supplementary Material (ESI) for Nanoscale.
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Supporting Information

Isolation of circulating exosomes and identification of exosomal PD-L1 for predicting immunotherapy response

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Materials and reagents

PD-L1 aptamer (5'-TACAGGTTCTGGGGGGTGGGTGGGGAACCTGTT-3') was purchased from Sangon Biotech (ShangHai, China). Anti-CD9, anti-CD63, anti-CD81 and anti-PD-L1 were purchased from Abcam (UK), and anti-PD-L1/Cy 5 was obtained from Bioss Antibodies (Beijing, China). 60% w/v iodixanol aqueous solution (1.32 g/mL) was purchased from Sigma (Saint Louis, USA). Series S Sensor Chip SA and HBS-EP+ buffer (10 X) were provided from GE Healthcare (Uppsala, Sweden). PNGase F, glycoprotein denaturing buffer (10X), glycobuffer 2 (10X) and NP-40 were purchased from New England Biolabs (NEB, USA). Asyringe-driven filter unit (0.22 μm) was acquired from Millipore (USA). Triton X-100 was obtained from Solarbio Life Sciences (Beijing, China). 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO) and Hoechst 33342 were purchased from Beyotime Biotechnology CO. Ltd. (Shanghai, China). And the other reagents were analytical grade and all solutions were prepared using deionized water with Milli-Q water (18 MΩ).

Results

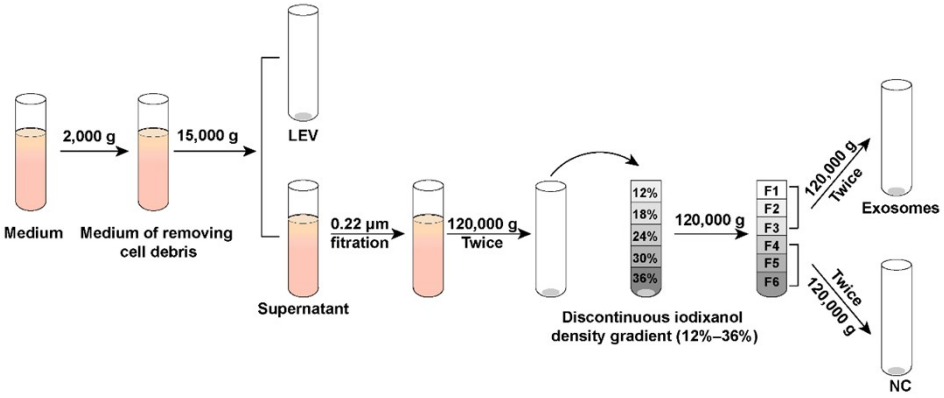

Figure S1. Procedure of separation and purification of exosomes by iodixanol density gradient centrifugation.

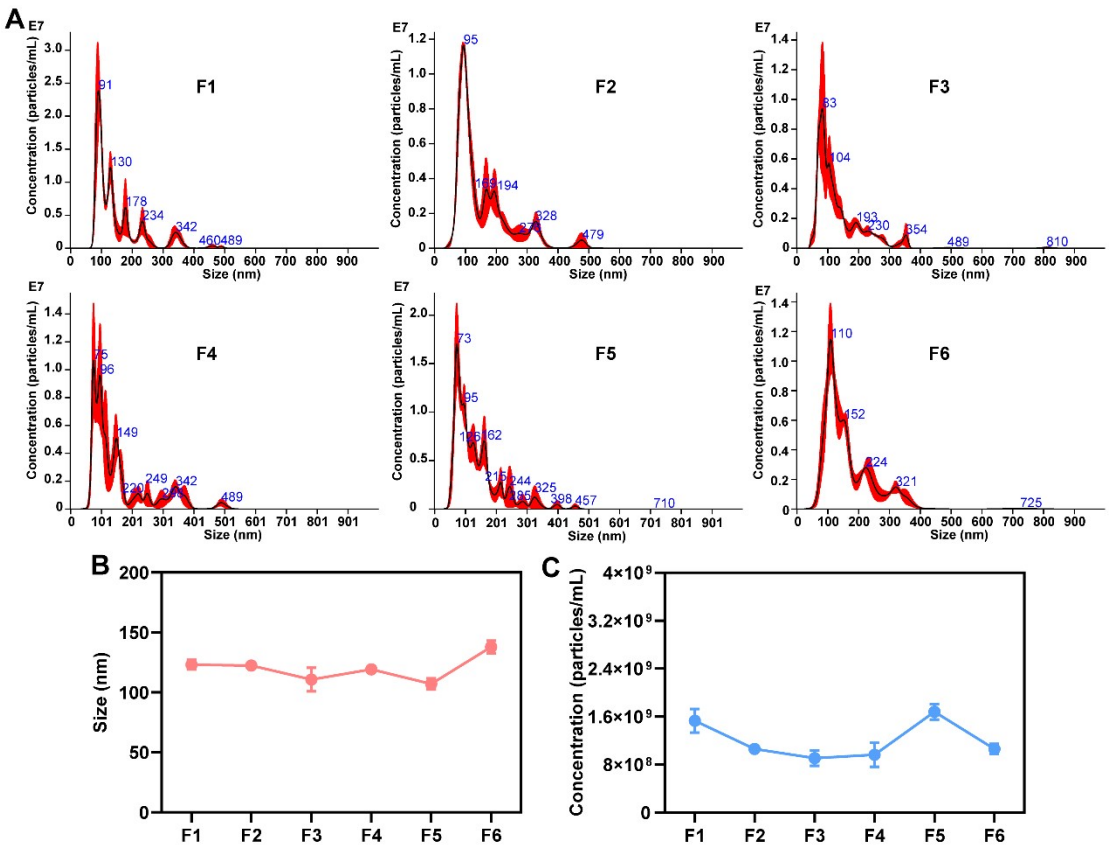

Figure S2. (A) NTA analysis. (B) Size distribution and (C) concentration from six fractions.

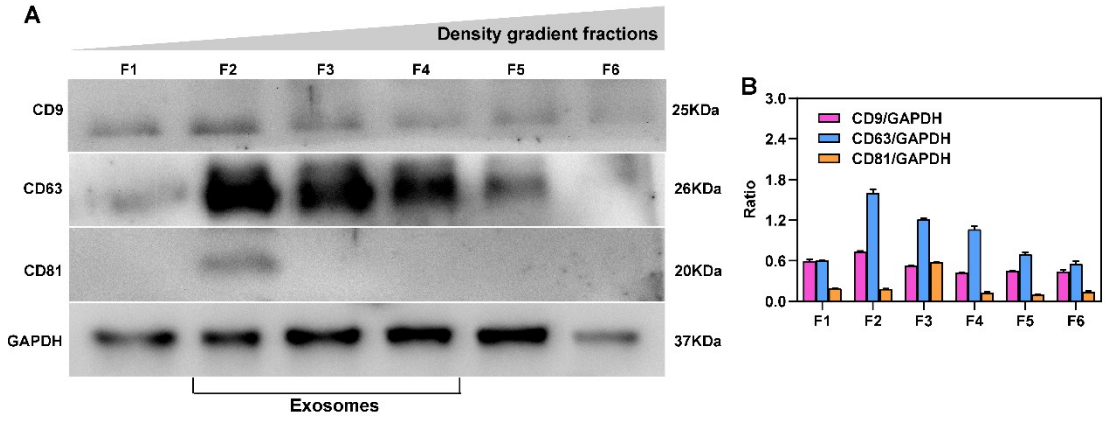

Figure S3. (A) Western blot and (B) semi-quantitative analysis of CD9, CD63 and CD81 from six fractions.

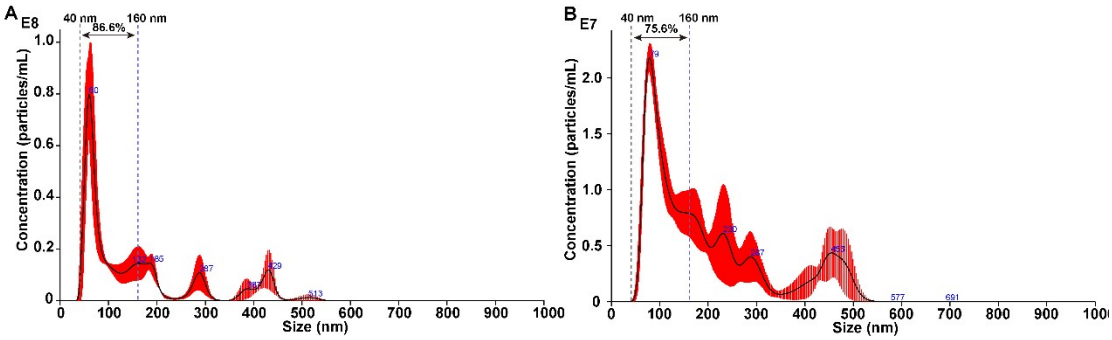

Figure S4. Size distribution of separated exosomes from (A) iodixanol density gradient centrifugation and (B) ultracentrifugation.

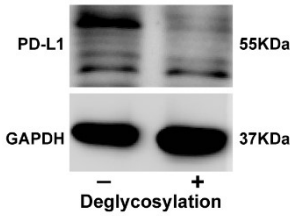

Figure S5. Western blot confirmed the glycosylation of PD-L1 on exosomes.

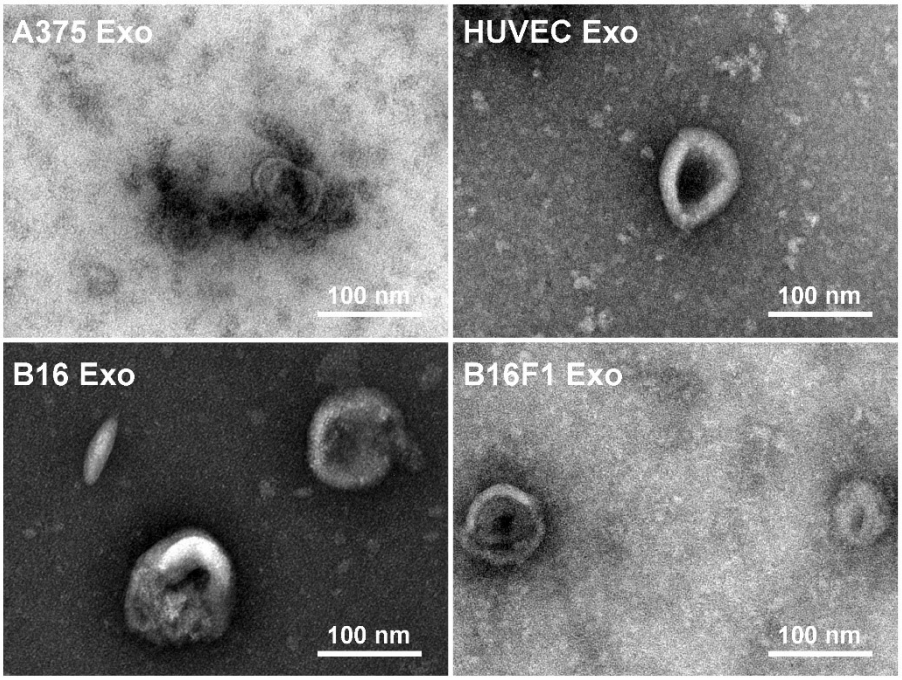

Figure S6. TEM imaging of exosomes from different cell lines.

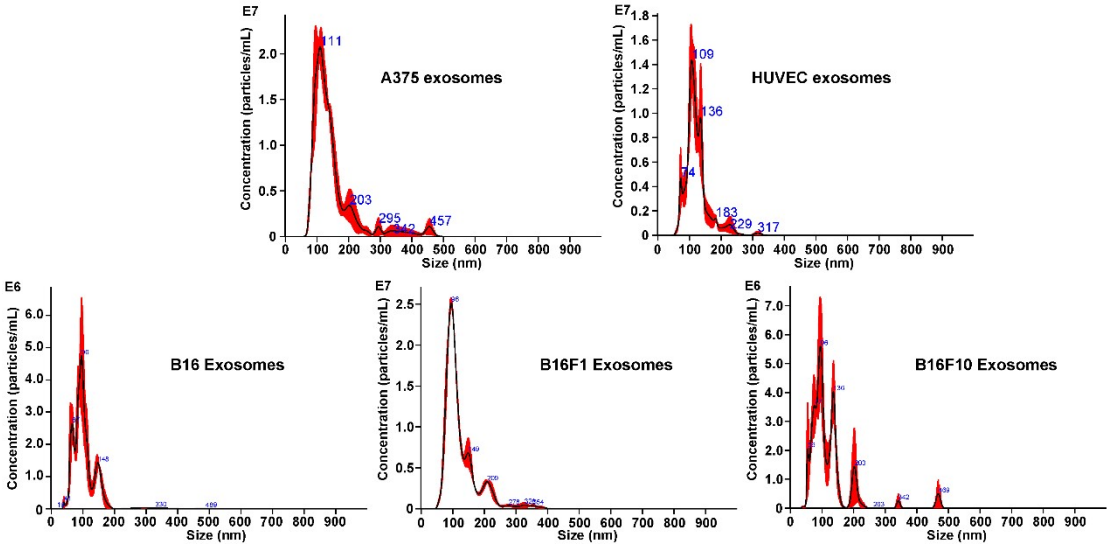

Figure S7. NTA analysis of exosomes from different cell lines.

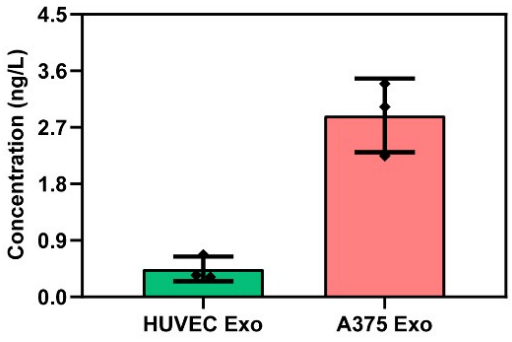

Figure S8. ELISA analysis of PD-L1 of HUVEC exosomes and A375 exosomes.

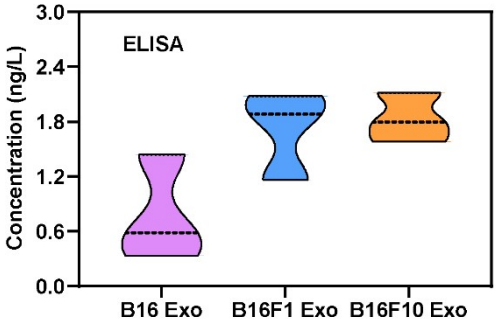

Figure S9. ELISA analysis of PD-L1 from B16, B16F1 and B16F10 exosomes.