

Supplementary data

T cell engineering by targeted small extracellular vesicles delivering CRISPR/Cas9 system for *PD-1* knock-out

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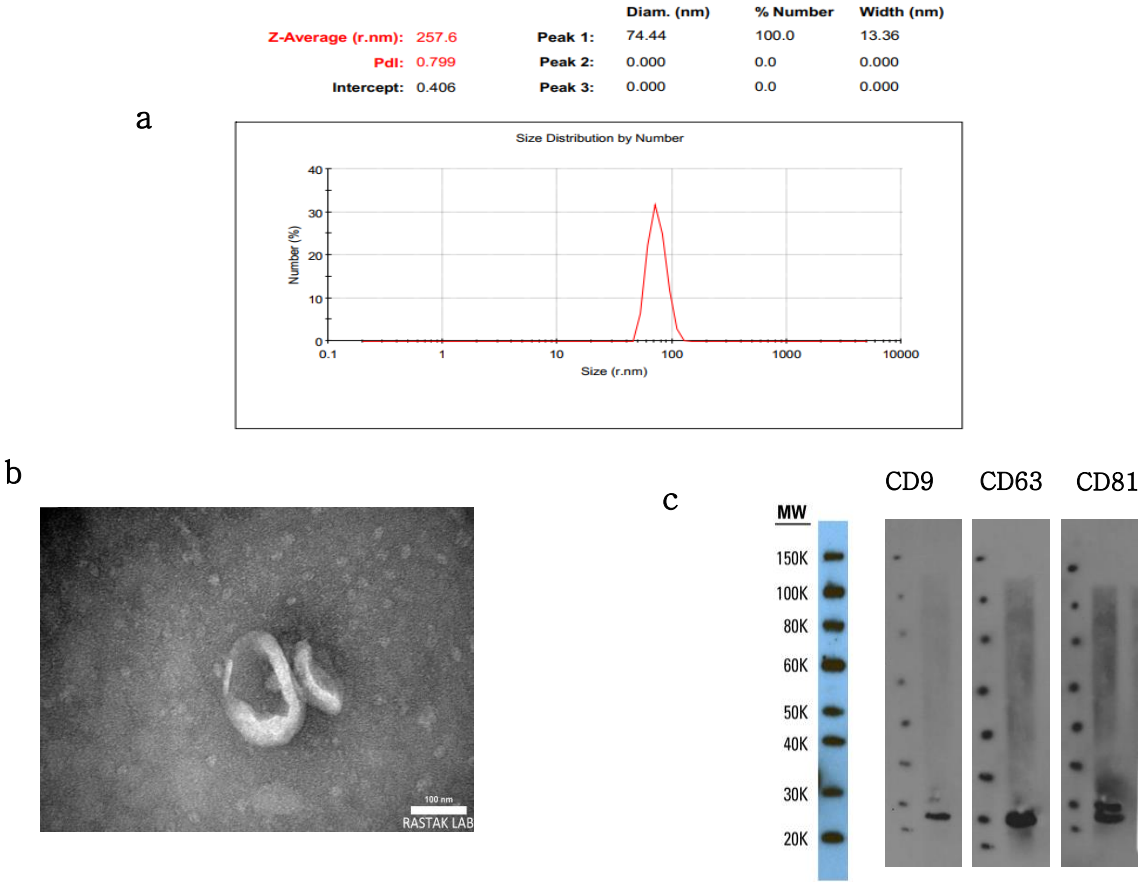


Fig. S1 s-EVs characterization.

a; The size distribution of isolated s-EVs was measured using a zeta sizer. b. TEM image of cup-shaped purified s-EVs. c. western blot for s-EVs characterization, 1: s-EVs were detected by anti-CD9 at ~24 kDa, 2: s-EVs were detected by anti-CD63 at ~26 kDa, 3: s-EVs were detected by anti-CD81 at ~22-26 kDa

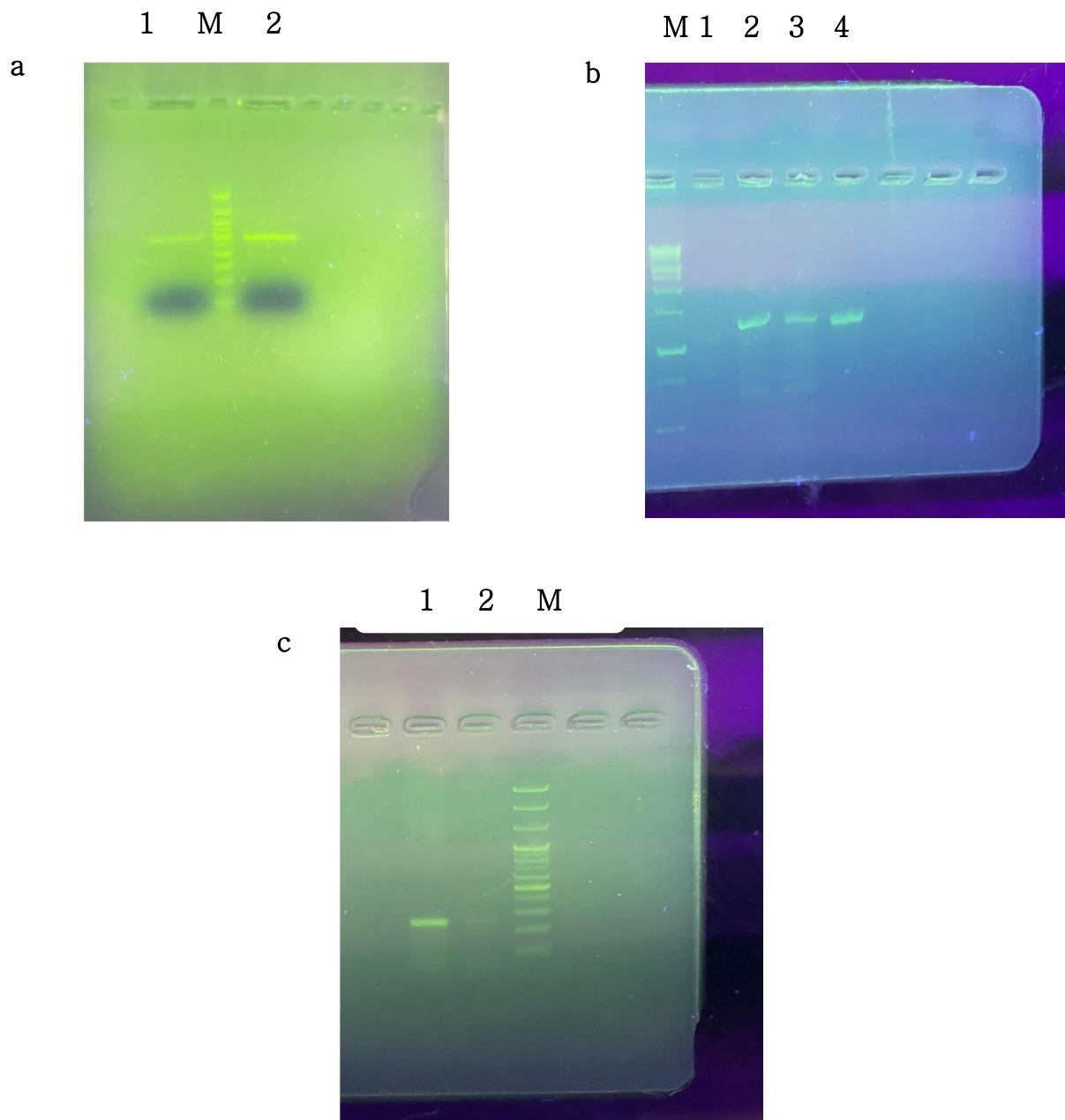


Fig. S2 The original, uncropped gel images for Fig. 4a and Figs. 4d i and ii

a Agarose gel image showing the negative control (lane 1), PCR product generated by primers for gRNA1 on genomic DNA of the KO T cells (lane 2). **b** PCR product generated by primers for both gRNAs on genomic DNA of the KO T cells (transfected by PEI as a positive control) (lane 2), PCR product generated by primers for both gRNAs on genomic DNA of the KO T cells (transfected by s-EVs) (lane 3), and PCR product generated by primers for both gRNAs on genomic DNA of the unmodified control T cells (lane 4). **c** PCR product generated by a primer for the left gRNA and a primer between the two gRNAs on genomic DNA of the unmodified control T cells (lane 1) and PCR product

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generated by a primer for the left gRNA and a primer between the two gRNAs on genomic DNA of the KO T cells (lane 2). M stands for 100bp DNA ladder in all three images

Bulge Type

DNA bulge

RNA bulge

Mismatch

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Bulge Type	Target	Chromosome	Position	Direction	Mismatches	Bulge Size
X	crRNA: GCGTGACTTCCACATGAGCGNGG DNA: GCGTGACTTCCACATGAGCGTGG	chr2	241852725	-	0	0
X	crRNA: CGACACCAACCACCAGGGTTNGG DNA: CGACACCAACCACCAGGGTTTGG	chr2	241852269	+	0	0

Fig. S3 The result of the Cas-OFFinder analysis

Both gRNAs targeting *hPD-1* locus on human chromosome 2, revealed perfect on-target specificity with 0 mismatches, 0 DNA bulges, and 0 RNA bulges