Exosomes from M1-polarized macrophages enhance paclitaxel antitumor activity by activating macrophages-mediated inflammation

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Keywords

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Supplementary Table

Table S1 Primer used for quantitative RT-PCR.

Gene	Primer sequence (5’-3’)
GAPDH	F:GGTCGGTGTGAACGGATTTGG
	R:GCCGTGGGTAGAGTCATACTGGAAC
iNOS	F:CCTTCCGAAGTTTCTGGCAGCAGC
	R:GGCTGTCAGAGCCTCGTGGCTTTGG
IL-10	F:TTCTTTCAAACAAAGGACCAGC
	R:GCAACCCAAGTAACCCTTAAAG
IL-6	F:CTCCCAACAGACCTGTCTATAC
	R:CCATTGCACAACTCTTTTCTCA
IL-12b	F:TGAGAAGTATTCAGTGTCCTGC
	R:CTGTGAGTTCTTCAAAGGCTTC
IL-4	F:TACCAGGAGCCATATCCACGGATG
	R:TGTGGTGTTCTTCGTTGCTGTGAG
TNF-α	F:ATGTCTCAGCCTCTTCTCATTC
	R:GCTTGTCACTCGAATTTTGAGA
NF-kB	F:CCTCTGGCGAATGGCTTTAC
	R:GCCTGGTCCCGTGAAATACA
IkB	F:ACTTCTCCTGAAGCCGGTG
	R:TGGTTGTCAGGTCTGCAATTT

Figure S1 The uptake of Exos

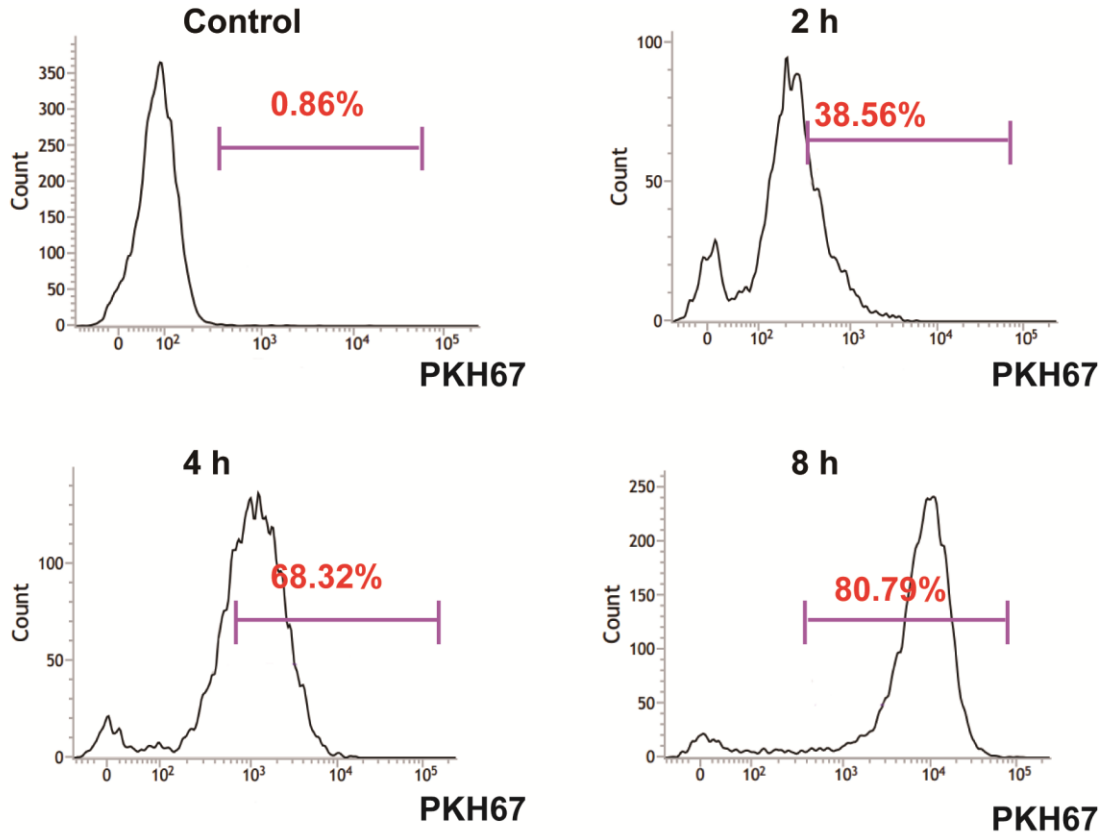
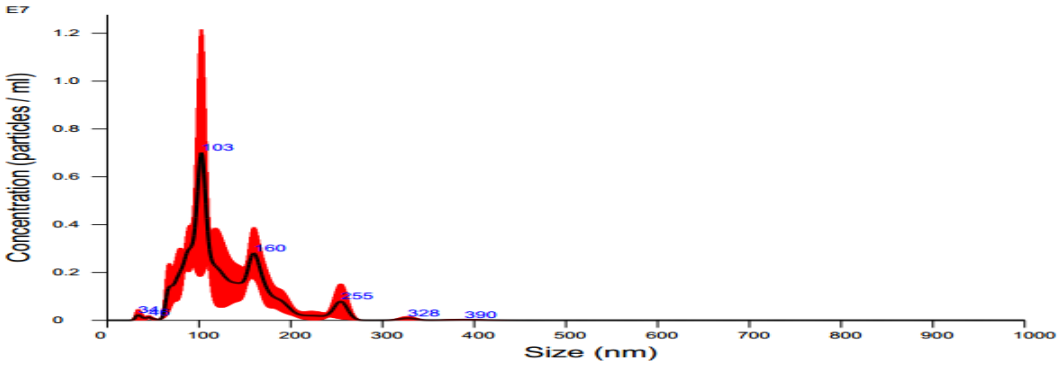


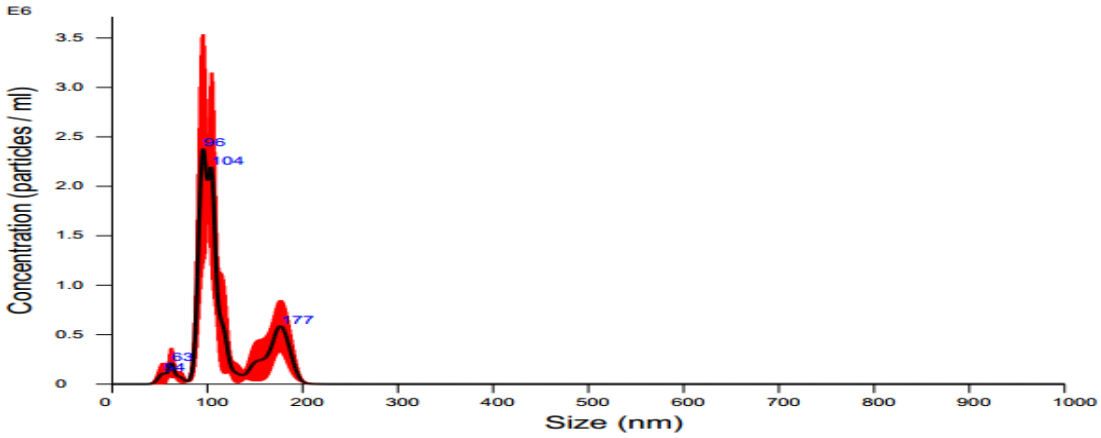
Figure S1. Flow cytometry was used to analyze the fluorescence transfer of PKH67-labeled Exos for 2 h, 4 h, and 8h.

Figure S2 NTA was used to detect the size of Na ïve M1-Exos, Sonicated M1- Exos and PTX-M1- Exos

A Na ïve M1-Exos



B Sonicated M1- Exos



C PTX-M1-Exos

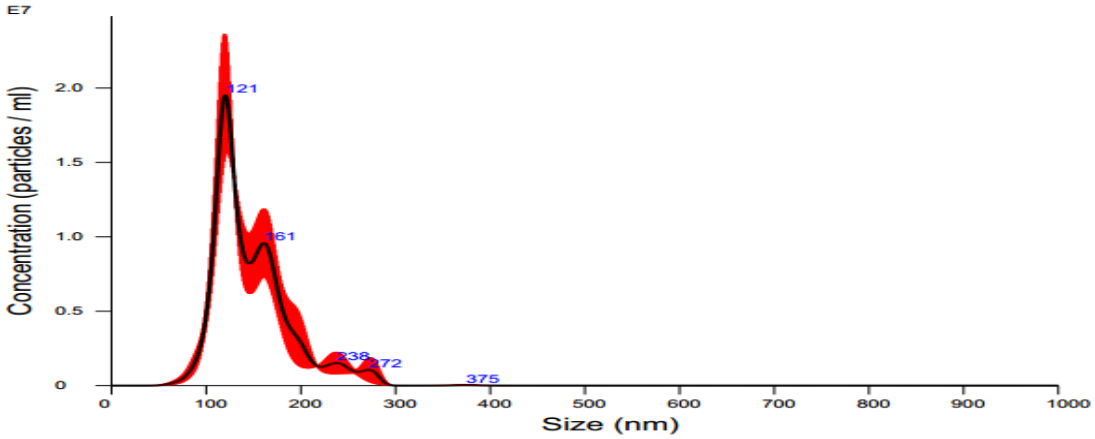
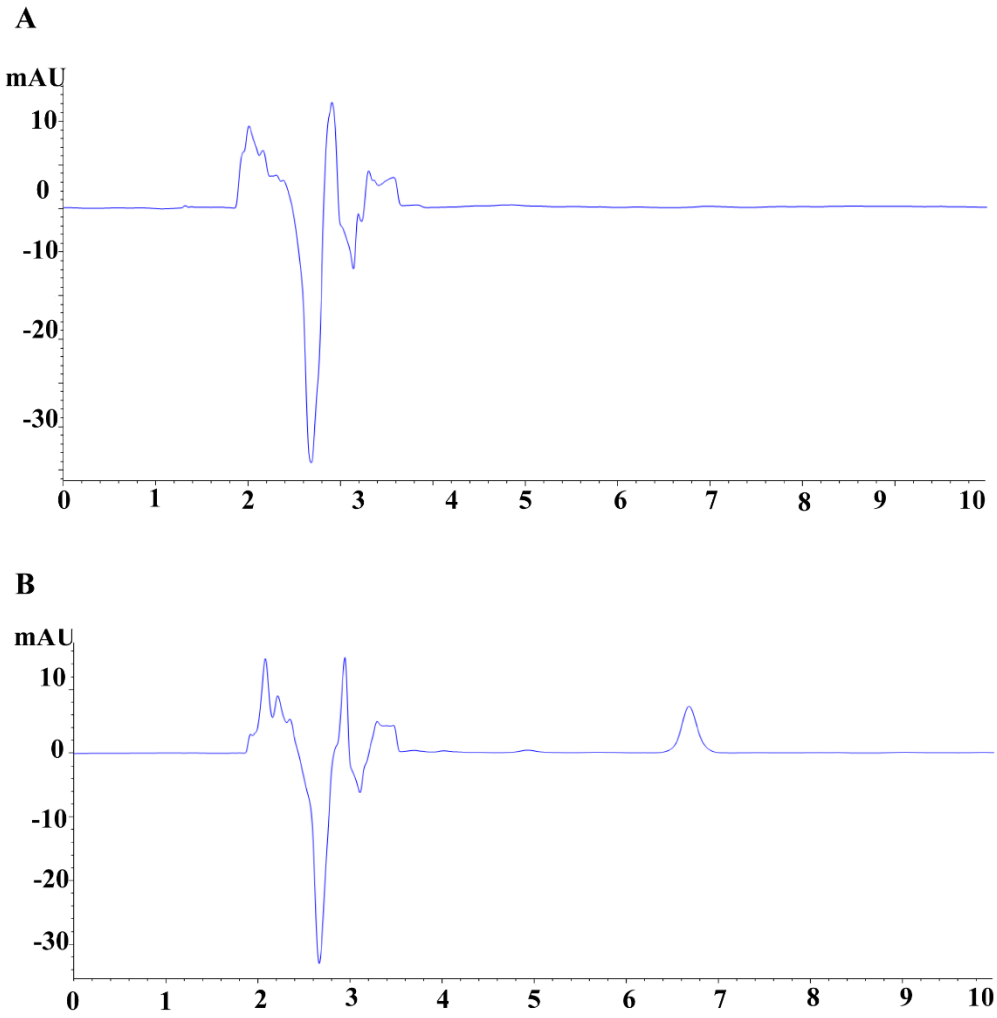


Figure S2. NTA demonstrates the size distribution and concentration of Naïve M1-Exos (S 2A), Sonicated M1- Exos (S 2B) and PTX-M1- Exos (S 2C).

Drug loading analysis

PTX was quantified by using a Waters 1260 HPLC system on a C_{18} column, in isocratic mode using acetonitrile and 0.01% phosphoric acid solution (65: 35, v/v) as mobile phase and detected at 227 nm (Figure S3). Samples concentrations were calculated according to the standard curve.

Figure S3 HPLC was used to determine the concentration of PTX



The loading capacity of PTX-M1- Exos formulation was analyzed according to previous study [1]. The encapsulation efficiency could be calculated as follows:

$$LC(\%)=W_E/W_T \times 100\%$$

Where W_E, and W_T, were the weight of PTX entrapped in the M1- Exos, the weight of total PTX, respectively.

Figure S4 MTT assay

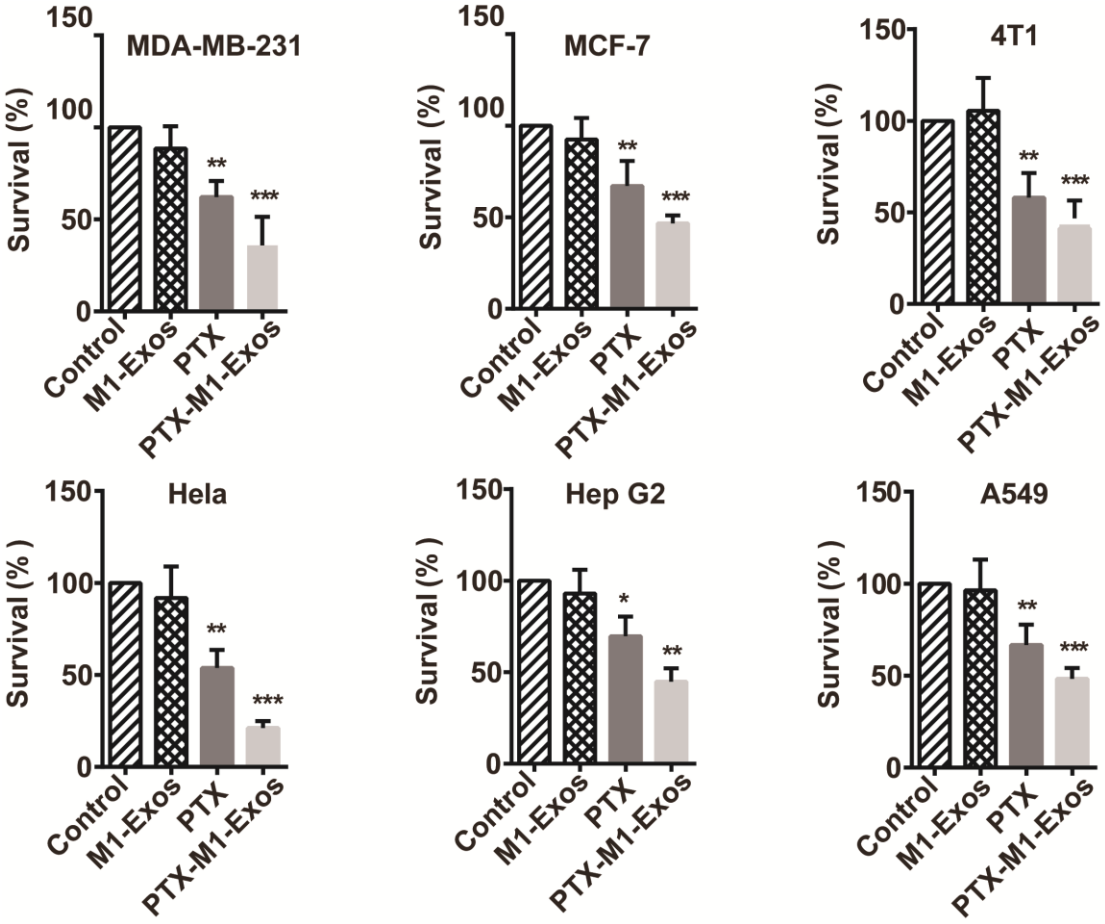


Figure S4. MDA-MB-231, MCF-7, 4T1, A549, HepG2 and Hela cells were incubated with medium (Control), M1- Exos (40 μg/mL), 15 μg/mL free PTX, and PTX-M1-Exos (at a dose of PTX 15 μg/mL) for 24 h. The inhibitory effects were detected by MTT assay. (n=6; **P*<0.05 ***P*<0.01, *** *P*<0.001vs. the control group)

Figure S5

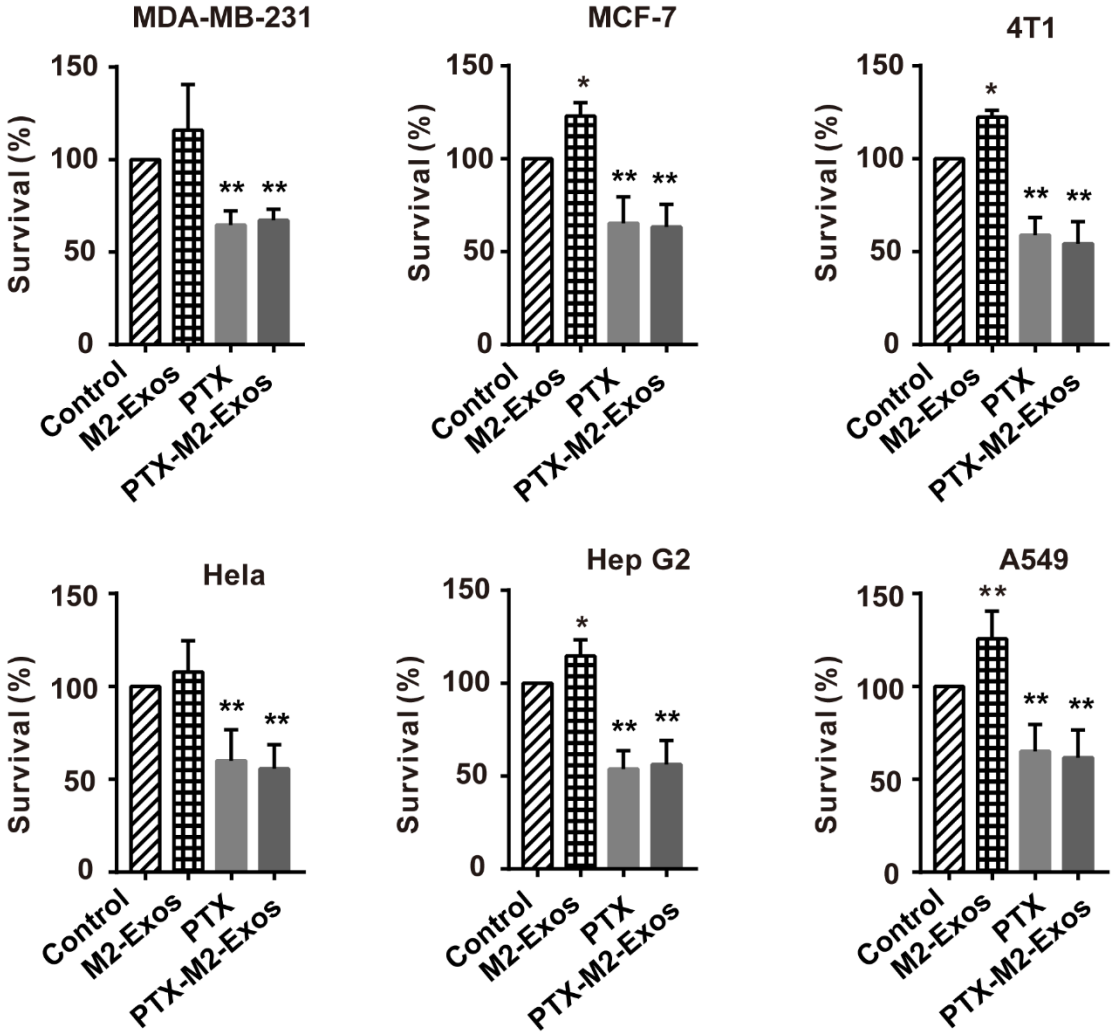


Figure S5.MDA-MB-231, MCF-7, 4T1, A549, HepG2 and Hela cells were incubated with medium (Control), M2- Exos (40 μg/mL), 15 μg/mL free PTX, and PTX-M2-Exos (at a dose of PTX 15 μg/mL) for 24 h. The inhibitory effects were detected by MTT assay. (n=6; **P*<0.05 ***P*<0.01, *** *P*<0.001vs. the control group)

Figure S6

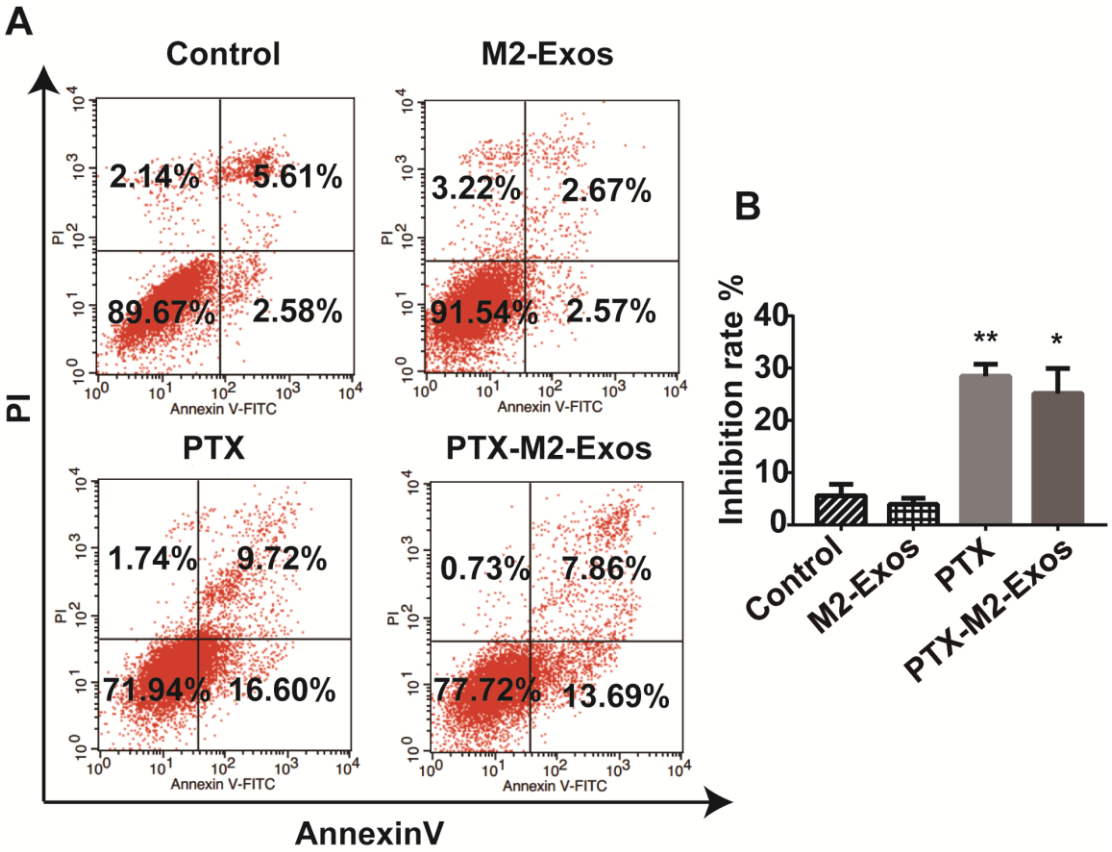


Figure S 6. (A) Cell apoptosis was detected by flow cytometry at 24 h in each group, and (B) Statistical analysis of data. The data shown are mean ± SD. (n=3; **P*<0.05 ***P*<0.01, *** *P*<0.001vs. the control group)

Figure S7

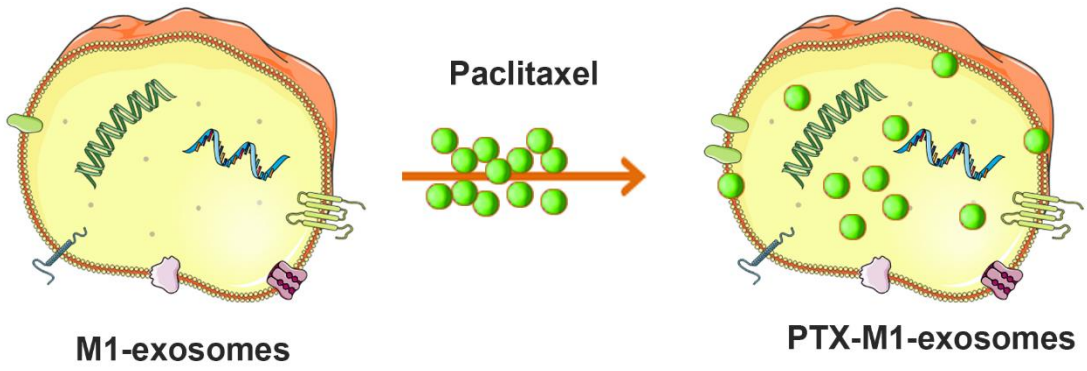


Figure S7. Model of exosomes loading PTX.