

Supplementary Information

Factors Influencing Measurement of the Secretion Rate of Extracellular Vesicles

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Table S1: Comparison of FBS-derived protein in isolated EV pellet at 24-h and 48-h time point. The abundance of top 25 mutual proteins in two groups detected by NanoLC-MS/MS.

Number	Gene Symbol	24h	48h
1	TF	5992171533	4049531142
2	SERPINA1	5060952805	4754967731
3	LOC516742	2704526056	3787793034
4	AHSG	1263138730	2300543760
5	ORM1	877064629	621098210
6	A2M	664351507	5758086477
7	FN1	528704378	763152712
8	HBG	495271691	239849813
9	SERPINA3-1	437556096	232233685
10	APOA1	418448965	899699452
11	TTR	404208000	261339084
12	HBA1; HBA	386268438	104756687
13	KRT82	338361090	45237912
14	SLC25A5	335138636	67894668
15	MYL12A	328632726	178744184
16	SERPINC1	285473956	292272596
17	VDAC1	214416576	67855503
18	C3	201519671	594763061
19	A1BG	201295558	169431025
20	VTN	196885699	242074079
21	ADIPOQ	189945389	127302270
22	SERPINA3-7	189016775	224630050
23	HIST1H1D	188326525	122002662
24	HSPA9	169749260	55243761
25	RPL7A	164606223	142173231

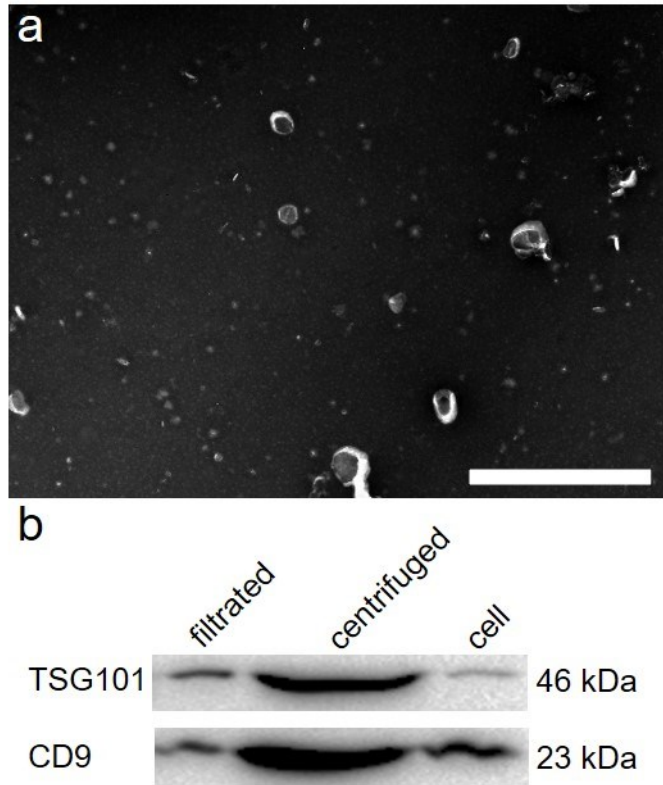


Figure S1. Characterization of EVs in centrifuged supernatant. **a**, TEM image shows MDA-MB-231 cell-derived EVs in supernatant processed by 20,000 g centrifugation. Scale bar represents 1 μm . **b**, TSG101 and CD9 proteins were extracted and identified from EVs in 20,000 g centrifuged supernatant, parental cells, and EVs in filtrated supernatant. Supernatant was lyophilized followed by lysis and western blot analysis.

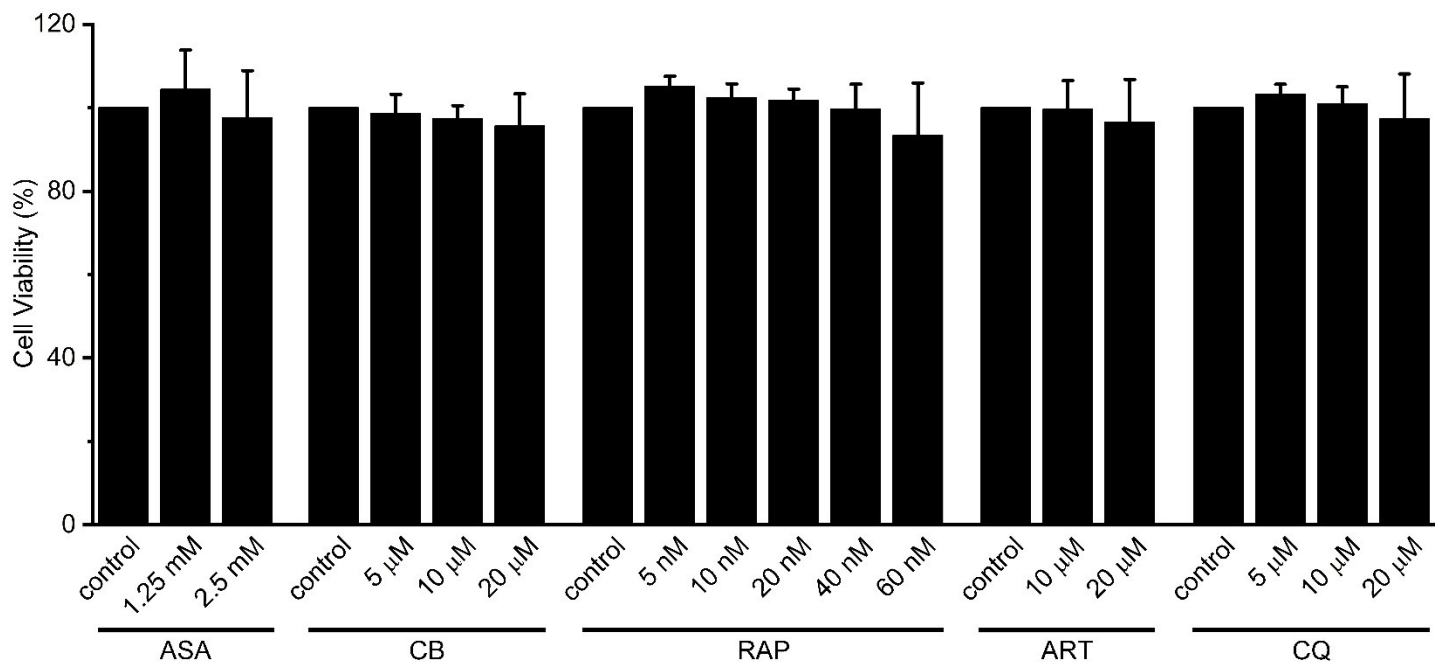


Figure S2. The viability of MDA-MB-231 cells treated with different doses of acetylsalicylic acid (ASA), celecoxib (CB), rapamycin (RAP), artemisinin (ART), and chloroquine (CQ) for 24 h. Cell viability was determined by CCK-8 assay.