Electronic Supplementary Material (ESI) for Analyst.

Effective Isolation of Exosomes by Polyethylene Glycol from Cell Culture Supernatant for In-depth Proteome Profiling

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Results

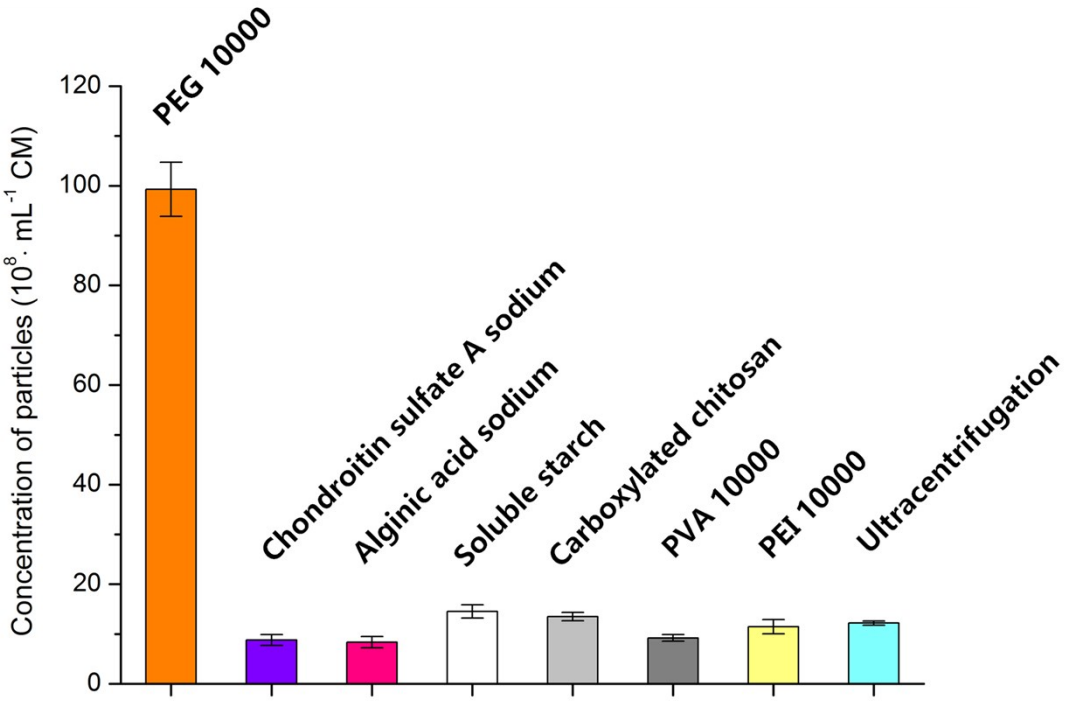

Fig. S1 Concentration of particles isolated by using different polymers or macromolecules. Other conditions: PEG, 10% (g/mL) 10 kDa; chondroitin sulfate A sodium, 8% (g/mL) (saturated); alginic acid sodium, 6% (g/mL) (saturated); soluble starch, 4% (g/mL) (saturated); carboxylated chitosan, 8% (g/mL) (saturated); PVA, 10% (g/mL) 10 kDa; PEI, 10% (g/mL) 10 kDa; ultracentrifugation. All results were measured in three technical replicates and showed as mean ± S.D..

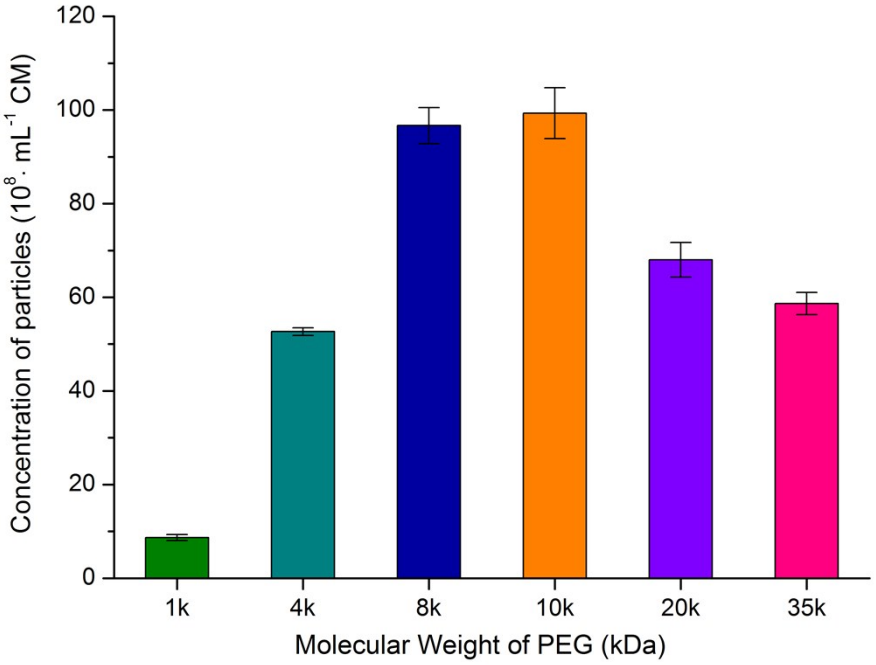

Fig. S2 Concentration of particles isolated by PEG with different Mw. Other conditions: 10% (g/mL) PEG without any salts. All results were measured in three technical replicates and showed as mean ± S.D..

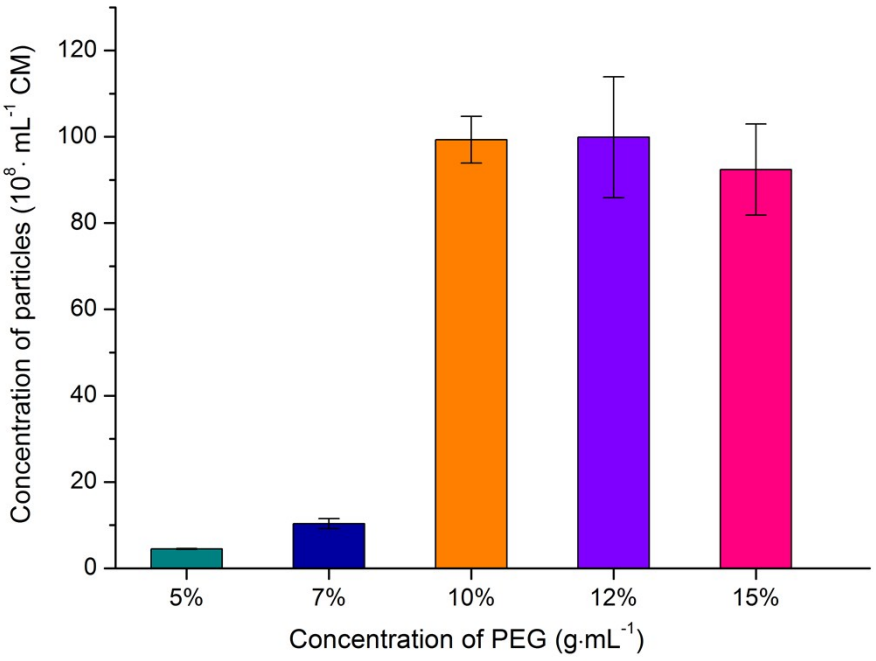

Fig. S3 Concentration of particles isolated by PEG with different concentrations. Other conditions:

PEG (10 kDa) without any salts. All results were measured in three technical replicates and showed as mean ± S.D..

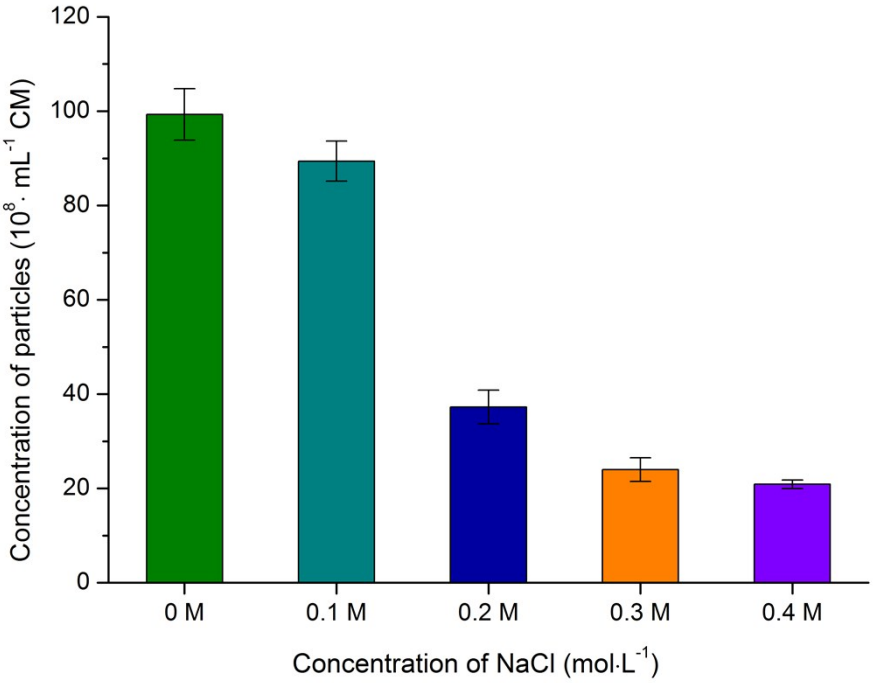

Fig. S4 Concentration of particles isolated by using PEG with different NaCl concentrations. Other conditions: 10% (g/mL) PEG with Mw of 10 kDa. All results were measured in three technical replicates and showed as mean ± S.D..

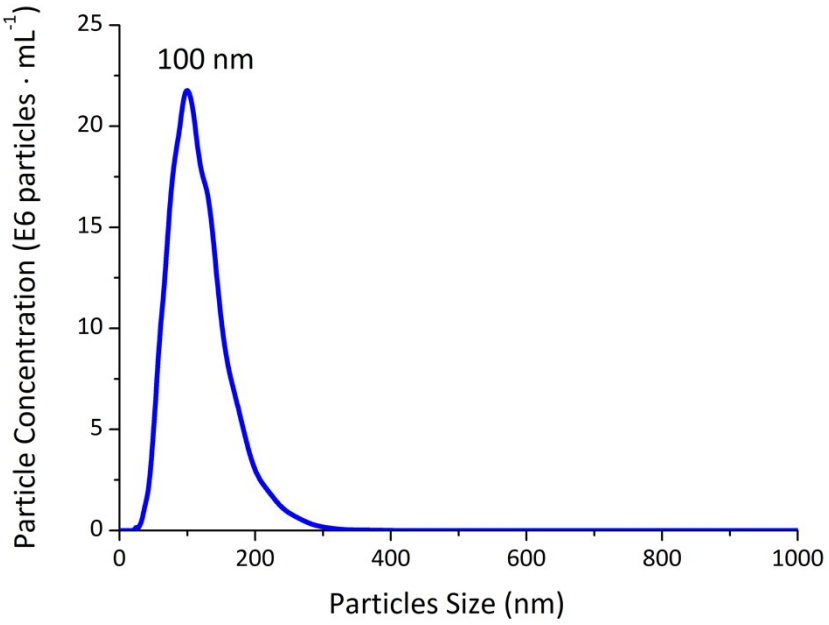

Fig. S5 Size distribution by NTA analysis of particles isolated from HeLa cell culture supernatant by

optimized PEG-based approach.

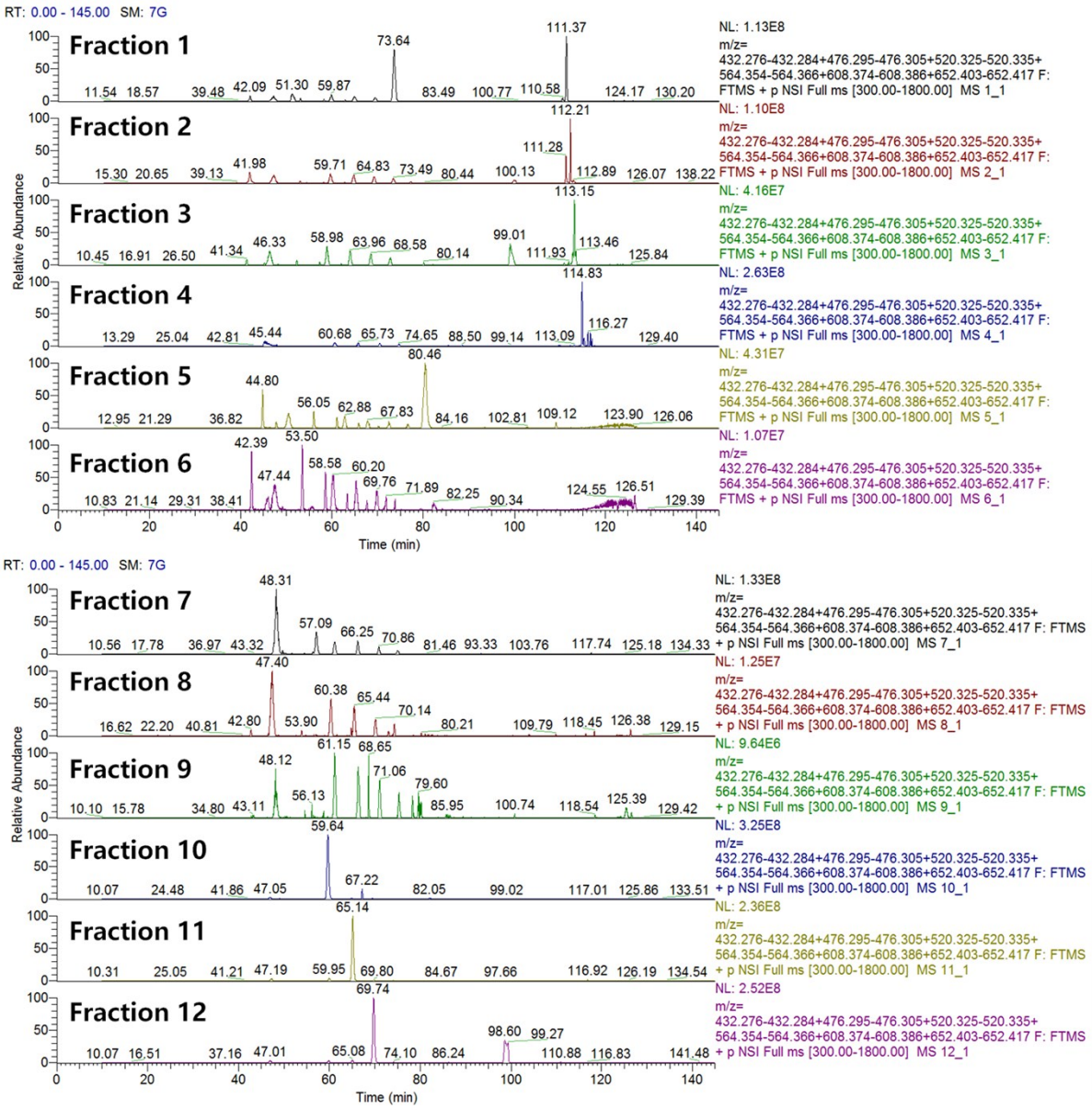

Fig. S6 Extracted ion chromatograms (XICs) of PEG ions (m/z 432.28, 476.30, 520.33, 564.36, 608.38, 652.41) in each fraction with mass tolerance of 10 ppm.

Table S1 Number and ratio of identified peptides, neo-N termini and original N-termini in a technical replicate.

Investigation / Samples	all peptides	neo-N termini	original N-termini[a]	ratio of neo-N termini	ratio of original N-termini	$\frac{neo-N\ termini}{original\ N-termini}$
Exosome isolated from HeLa cells	30670	4470	353	14.6%	1.2%	**12.66**
HeLa cells	13946	788	321	5.7%	2.3%	**2.45**
Human erythrocytes[1]	N/A[b]	883	435	N/A	N/A	**2.03**

[a]: original N-termini: starts with intact or removed initiator-Met

[b]: N-terminal peptides were enriched using terminal amino isotopic labeling of substrates (TAILS),[2] thus, most peptides were removed, which resulted in the missing of information of all peptides.

Reference

1. Lange, P. F.; Huesgen, P. F.; Nguyen, K.; Overall, C. M., Annotating N termini for the human proteome project: N termini and Nα-acetylation status differentiate stable cleaved protein species from degradation remnants in the human erythrocyte proteome. *J. Proteome Res.* **2014,** *13* (4), 2028-2044.

2. Kleifeld, O.; Doucet, A.; auf dem Keller, U.; Prudova, A.; Schilling, O.; Kainthan, R. K.; Starr, A. E.; Foster, L. J.; Kizhakkedathu, J. N.; Overall, C. M., Isotopic labeling of terminal amines in complex samples identifies protein N-termini and protease cleavage products. *Nat. Biotechnol.* **2010,** *28* (3), 281-288.