Supplementary Information (SI) for Analyst.

Supplementary Figures

Affinity enrichment of placental extracellular vesicles from unprocessed maternal plasma with magnetic nanowires

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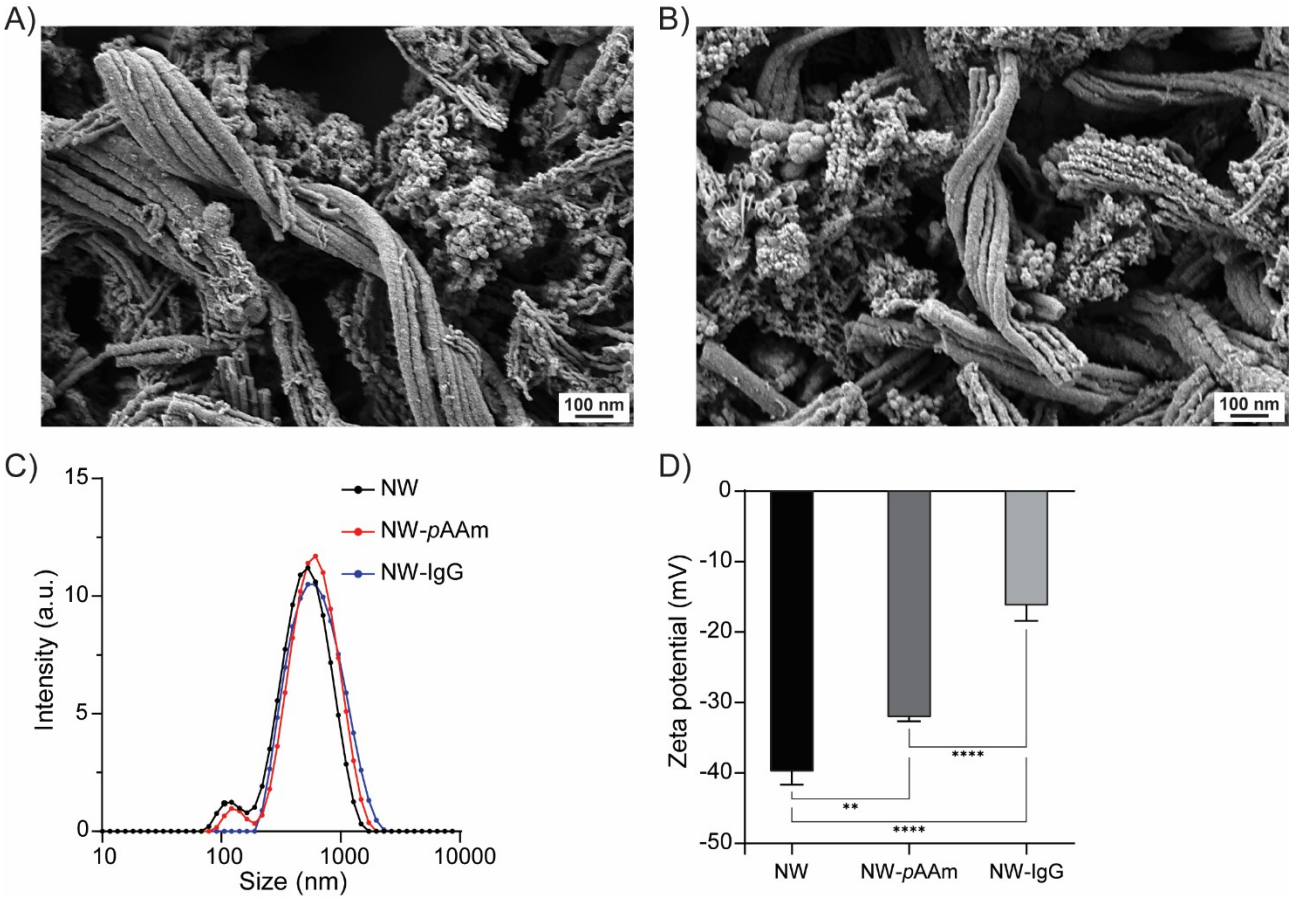

Figure S1. Scanning electron microscopy images showing the twisted structures of A) NW and B) NW-*p*AAm. C) Dynamic light scattering size measurements for NW, NW-*p*AAm and NW-IgG. D) Zeta potentials for NW, NW-*p*AAm and NW-IgG. Data are presented as means + SD (n = 3, one-way ANOVA with Tukey's post-test, $^{**}p < 0.01$ and $^{****}p < 0.0001$).

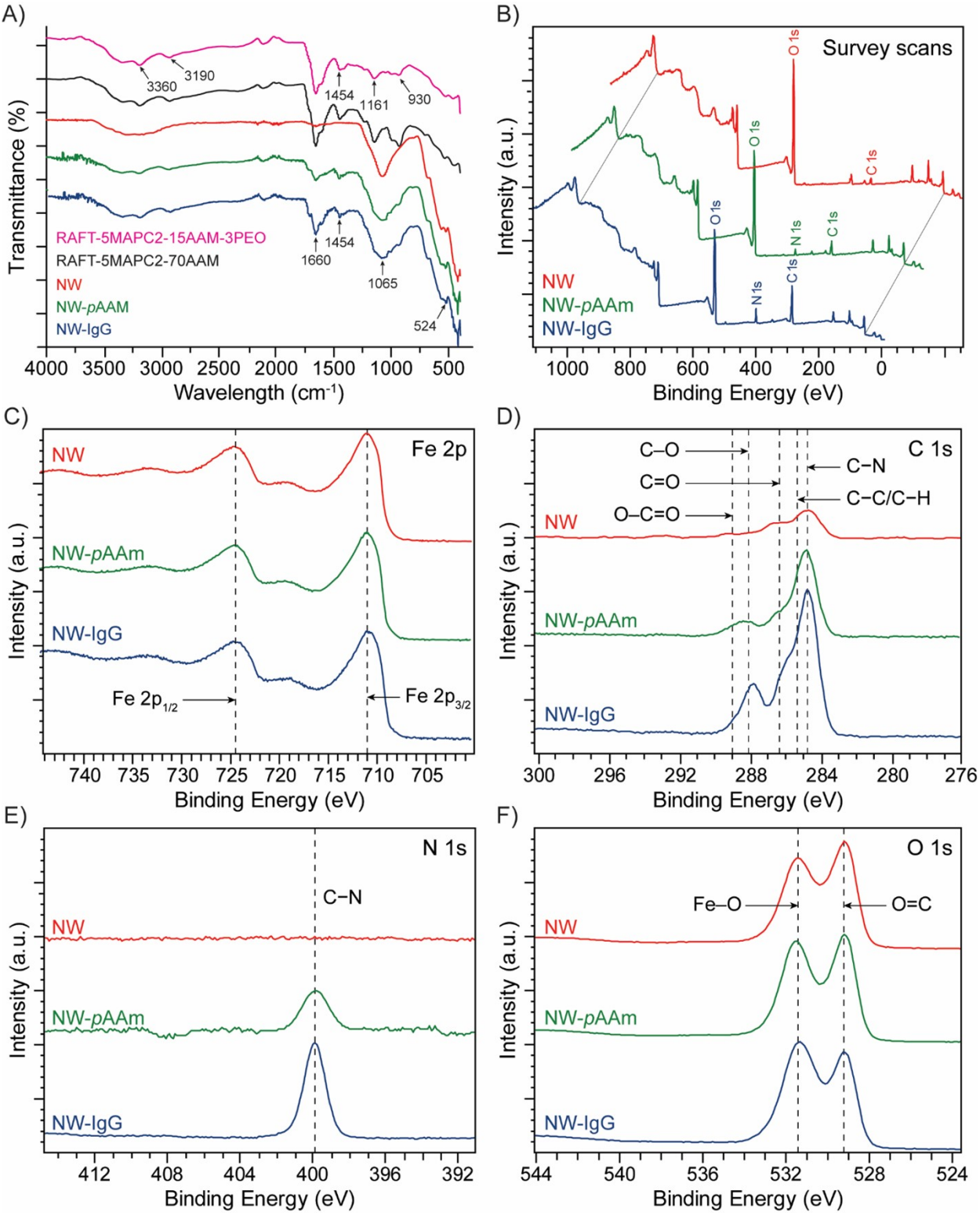

Figure S2. (A) FTIR spectra of RAFT-5MAPC2-15AAM-3PEO and RAFT-5MAPC2-70AAM block copolymers, pristine NW, NW-*p*AAm, and NW-IgG. (B) XPS survey scan spectrum of NW, NW-*p*AAm, and NW-IgG. (C) High-resolution spectra for Fe 2p with dashed lines indicating peaks of Fe $2p_{3/2}$ (711 eV) and Fe $2p_{1/2}$ (724.5 eV). (D) High-resolution spectra for C 1s with dashed lines indicating major peaks of C–C/C–H (284.8 eV), C–N (285.4 eV), C–O (286.3 eV), C=O (288.4 eV), and O–C=O

(289 eV). (D) High-resolution spectra for N 1s with a dashed line indicating the major peak of C–N (399.8 eV). (D) High-resolution spectra for O 1s with dashed lines indicating the major peaks of Fe–O (529.3 eV) and O=C (531.4 eV).

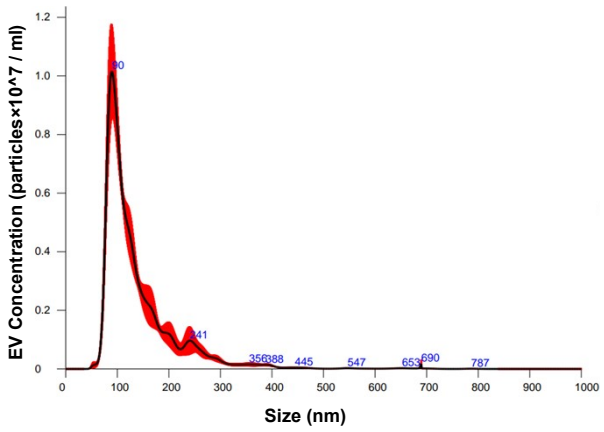

Figure S3. Nanoparticle tracking analyses of eluted PLAP+ve EVs enriched using the NW-PLAP showing the size and concentration. Inset displays a representative image taken with the NTA apparatus

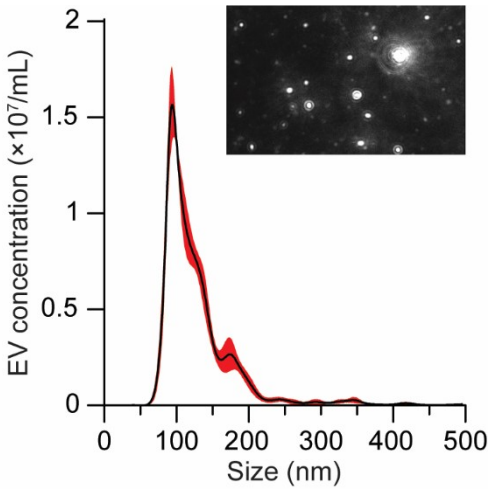

Figure S4. Nanoparticle tracking analyses of eluted PLAP+ve EVs enriched using the NW-PLAP showing the size and concentration. Inset displays a representative image taken with the NTA apparatus.

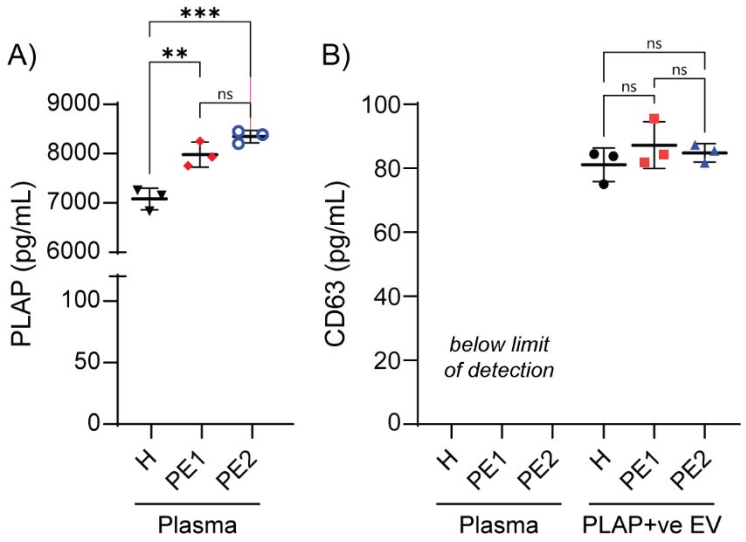

Figure S5. Protein concentrations of (A) PLAP in plasma and (B) CD63 measured in plasma and enriched PLAP+ve EVs in healthy pregnancy (H) and preeclampsia (PE1 and PE2) by ELISA. Data are presented as means ± SD (n = 3, one-way ANOVA with Tukey's post-test, ns: not significant ($p > 0.05$), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$).

Table S1. Raw ELISA values for patient plasma samples (pg/mL).

	EV_H	EV_PE1	EV_PE2	Plasma_H	Plasma_PE1	Plasma_PE2
PLAP	75.1	98.4	103.0	7254.3	8253.2	8204.0
	70.3	94.8	101.7	6835.4	7931.5	8451.4
	71.9	93.9	100.8	7154.5	7753.3	8384.2
PP13	79.7	57.7	50.0	540.5	335.7	429.5
	77.8	41.9	39.4	533.1	334.7	426.7
	80.1	48.1	45.9	512.7	317.3	438.7
NEP	123.4	156.3	141.5	334.8	451.3	419.9
	130.5	148.2	148.4	365.6	483.8	427.2
	129.9	144.6	137.4	326.9	513.2	451.2

Table S2. Placental related proteins identified from liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS) analysis of NW-PLAP enriched EVs from minimally processed plasma.

Gene symbol	Gene name
HTRA1	HtrA serine peptidase
RAP1B	RAP1B, member of RAS oncogene family
ACTN1	Atinin alpha 1
CDH1	Cadherin 1
CFL1	Cofilin 1
CFD	Complement factor D
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GP1BA	Glycoprotein Ib platelet subunit alpha
ITGB3	Integrin subunit beta 3
PAPPA2	Pappalysin 2
PCOLCE	Procollagen C-endopeptidase enhancer
RNASE1	Ribonuclease A family member 1, pancreatic
TLN1	Talin 1
TUBA1B	Tubulin alpha 1b
YWHAZ	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta

LC-MS/MS analysis of NW-PLAP EVs from minimally enriched plasma

Proteomics of NW-PLAP enriched EVs from minimally processed preeclamptic plasma was performed following a previously published method[1]. Analyses of proteins present in the enriched fractions (from plasma sample from PE1) with NW-PLAP and NW-IgG (negative control) indicated respectively 201 and 174 proteins, with “Plasma” and “Serum” as the main protein categories (analysed with DAVID). Of the 39 proteins only present in the NW-PLAP group, 15 were identified as placenta-related, many

of which have a reported association to preeclampsia[45-542-9]. While only preliminary, this data further indicates that the NW-PLAP immuno-enrichment approach is able to enrich placental related EVs. However, the total protein (as measured by BCA) yielded an average of 16.0 µg protein in the NW-PLAP enriched fraction vs 13.9 µg for the NW-IgG fraction. This can be compared to the amount of protein measured in the same plasma sample pre-processed using ultracentrifugation to recover the total EV fractions (2.9 µg for the NW-PLAP, 3.2 µg for NW-IgG). When proteomics was performed, the number of proteins identified in the NW-PLAP and NW-IgG control in the direct enriched samples were higher (201 and 175 proteins, respectively) than after UC (UC + NW-PLAP or NW-IgG, 149 or 43 proteins, respectively). This data indicates as expected, significant non-specific adsorption of plasma protein from the minimally processed plasma.

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