Supporting Information

Hydrophilic magnetic MOF for consecutive enrichment of exosomes and exosomal phosphopeptides

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Experiment section

Chemicals. 20×TBS and 1×PBS were purchased from Solarbio. CD63 polyclonal antibody, anti-TSG101 polyclonal antibody and goat anti-rabbit IgG (HRP conjugated) were purchased from MultiSciences (Lianke) Biotech. CD9 polyclonal antibody, EPCAM monoclonal antibody and HRP-conjugated affinipure goat anti-mouse IgG were purchased from Proteintech. Sequencing grade trypsin was purchased from Promega. 1,1'-dioctadecyl-3,3,3',3'tetramethylindocarbocyanine perchlorate (DiI) were purchased from Meilun Biotechnology Company. Phosphotungstic acid hydrate were purchased from Alfa Aesar. Dithiothreitol (DTT), iodoacetamide (IAA), urea, thiourea, phenylmethanesulfony fluoride (PMSF), zircomiun tetrachloride ($ZrCl_4$), 2-aminoterephthalic acid (H_2BDC-NH_2), N,N'-dimethylformamide (DMF), dopamine hydrochloride, tris(hydroxymethyl)-aminomethane (Tris), and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich. Iron chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ethylene glycol, sodium acetate anhydrous and concentrated ammonia solution (28 wt%) were purchased from Guoyao Chemical Reagent Company. Acetonitrile (ACN) was purchased from Merck. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Thermo Scientific.

Synthesis of Fe_3O_4@PDA@UIO-66-NH_2. Generally speaking, $FeCl_3 \cdot 6H_2O$ and CH_3COONa were dispersed in 75 mL ethylene glycol to form Fe_3O_4 nanoparticles via the solvo-thermal reaction. Then, the obtained Fe_3O_4 was added into Tris buffer incorporating dopamine hydrochloride and Fe_3O_4@PDA was formed at room temperature. Finally, Fe_3O_4@PDA@UIO-66-NH_2 was synthesized by the one-pot reaction at 120 °C for 1 h, in

which Fe_3O_4@PDA, as the MOF precursor, was mixed with $ZrCl_4$ (9 mM) and H_2BDC-NH_2 (9 mM) in 75 mL DMF.

Cell culture. MCF-7 cells were cultured in petri dishes (100 mm diameter, 20 mm height) with 10% FBS, 89.5% DMEM and 0.5% penicillin-streptomycin and set in a humidified incubator with 5% CO_2 at 37 ℃. The above supernatant was instead by condition media without FBS since 80% bottom area of the petri dish was covered by MCF-7 cells. After incubation for 24 h, the late supernatant was collected to capture exosomes.

Preparation of exosomes samples from MCF-7 cells and urine. The collected supernatants of MCF-7 cells were filtered with filter (0.22 μm) to remove apoptotic blebs, cell debris, and cells. Then the filtrates were concentrated through an ultrafiltration tube (Merck Millipore, 100 kDa) and some proteins and peptides were removed together. Afterwards, the concentrate were dispersed in 1×TBS and stored at -80 ℃ for further use.

The urine samples were collected from healthy donors who provided written informed consent with prior approval of the ethics committee of the Shanghai Zhongshan Hospital. Firstly, urine samples were collected from 4 healthy volunteers (24-28 years old, 2 males and 2 females), and second morning mid-stream urine (approximately 100 mL) of each healthy volunteer was collected into 50-ml centrifuge tubes storing in the ice for an hour to promote some salt impurities precipitating. Secondly, the above samples were centrifuged at 2,000 *g* for 15 min and the supernatants were treated subsequently as same as above procedures of cell samples.

Exosomes isolation. First of all, 6.0 mg Fe_3O_4@PDA@UIO-66-NH_2 were incubated with

1 mL pre-treated MCF-7 cell samples at 4 ℃ for 10 min. Secondly, the supernatants were removed with the effect of magnet and the sediments were washed three times with PBS. Finally, the enriched exosomes were eluted by 0.4 M ammonia aqueous solution for 10 min at 4 ℃ and the solvents were substituted by PBS with ultrafiltration tubes to keep exosomes activity. In addition, urinary exosomes were purified with the same process as above.

Exosomes dye. 40 μg purified exosomes secreted by MCF-7 cells was incubated in 1×TBS buffer containing 10 μM DiI and placed on the shaker at 37 ℃ for 15 min in the dark. After reacting with Fe_3O_4@PDA@UIO-66-NH_2, the fluorescence intensity of supernatants and eluents were detected by fluorescence spectrometer (Hitachi, F-7000) at excitation and emission wavelengths of 545 nm and 570 nm to obtain the materials capture efficiency and exosomes recovery.

Characterization of isolated exosomes. Transmission electron microscopy. Exosomes suspended in PBS were dropped onto a 200-mesh formvar carbon coated copper grid and standing for 10 min. The excess solution was absorbed at the edge of the copper grid with the filter paper. Next, 2% phosphotungstic acid was dropped onto the above grid and incubated for 3 min to stain the exosomes samples. Then the grid was dried under the incandescent lamp and observed using a transmission electron microscope at 120 KV(Hitachi, HT7700).

Western blot analysis. The protein concentration of samples were measured with Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific). Next, The 5× SDS-PAGE

protein loading buffer was added in the exosomes proteins samples separately and the samples were heated at 100 ℃ for 5 min to make proteins denatured. Then the proteins were resolved by electrophoresis using SDS-PAGE (WSHTbio, Hepes-Tris gel) and transferred onto a polyvinylidene fluoride (PVDF) microporous membrane (Merck Millipore Ltd., Immobilon-P Transfer Membrane) through the wet membrane transfer device. The PVDF membrane was rinsed and blocked with western blocking agent (Dalian Meilun Biotechnology Co. Ltd., China) for 1 h at room temperature. Next, the PVDF membrane was incubated with different antibody separately at 4 ℃ overnight. After rinsing, PVDF membrane was incubated with the secondary antibody labeled with HRP for 2 h. Lastly, blots were presented through ChemiDocTM Touch Imaging System (Bio-Rad Laboratories, USA).

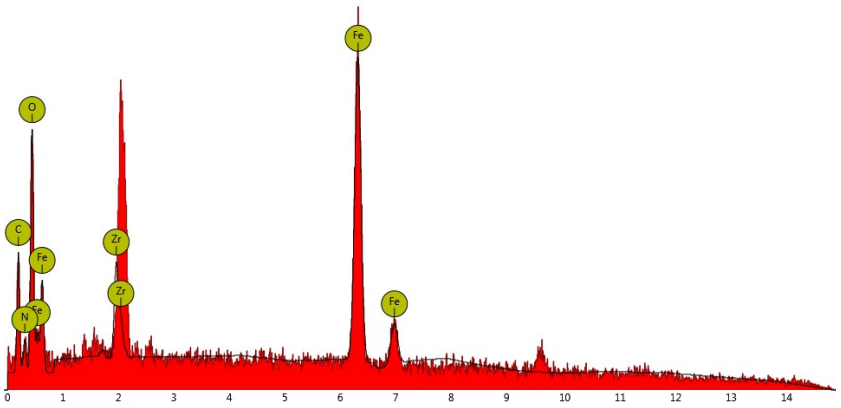
Exosomes lysis and digestion. Isolated exosomes were suspended in the urea lysis buffer containing 8 M urea, 2 M thiourea and 1 mM PMSF and ultrasonicated for 15 min. After centrifugation at 10,000 *g* for 5 min, the supernatant were substituted by 50 mM NH_4HCO_3 buffer using ultrafiltration tubes (3 kDa). Following by heating for 10 min at 90 ℃, the proteins were reduced by 10 mM DTT at 56 ℃ for 1 h and alkylated by 25 mM IAA in the dark at 37 ℃ for 0.5 h. Then trypsin was added in the samples (trypsin: protein=1:40, w/w), and incubated at 37 ℃ for 16 h. Finally, the peptides were collected after desalting and lyophilizing.

Enrichment of urinary exosomes phosphopeptides. Urinary exosomes phosphopeptides were dispersed in 100 μL 50%ACN/0.1%TFA (v/v) solution and 600 μg

96 Fe_3O_4@PDA@UIO-66-NH_2 were incubated in the mixture at 37 ℃ for 1 h to capture
97 phosphopeptides. After washing by the above loading buffer to remove impurities, the goal
98 peptides were eluted by 0.4 M ammonia aqueous solution for 45 min. Finally, through
99 desalting and lyophilizing, eluents were analyzed by LC-MS/MS.
100 **LC-MS/MS analysis.** The peptides were resuspended with 10 μL solvent A respectively
101 (A: water with 0.1% formic acid; B: ACN with 0.1% formic acid), separated by nano-LC
102 and analyzed by on-line electrospray tandem mass spectrometry. The experiments were
103 performed on an EASY-nLC 1000 system (Thermo Fisher Scientific, Waltham, MA)
104 connected to an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, San Jose,
105 CA) equipped with an online nano-electrospray ion source. 5 μL peptide sample was loaded
106 onto the analytical column (Acclaim PepMap C18, 75 μm × 25 cm), and subsequently
107 separated with a linear gradient from 2% B to 90% B in 120 min. The column was re-
108 equilibrated at initial conditions for 10 min. The column flow rate was maintained at 300
109 nL/min. The electrospray voltage of 2.0 kV versus the inlet of the mass spectrometer was
110 used.
111 The Orbitrap Fusion mass spectrometer was operated in the data-dependent mode to
112 switch automatically between MS and MS/MS acquisition. Survey full-scan MS spectra
113 (m/z 350–1600) were acquired in Orbitrap with a mass resolution of 60,000 at m/z 200.
114 The AGC target was set to 500,000, and the maximum injection time was 50 ms. MS/MS
115 acquisition was performed in Orbitrap with 3 s cycle time, the resolution was 15,000 at m/z
116 200. The intensity threshold was 50,000, and the maximum injection time was 100 ms. The

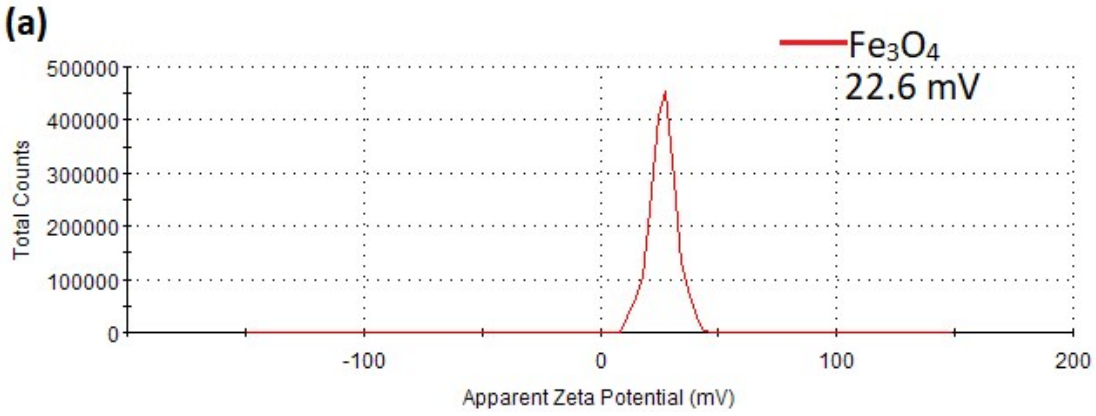
AGC target was set to 150,000, and the isolation window was 2 m/z. Ions with charge states 2+, 3+, and 4+ were sequentially fragmented by higher energy collisional dissociation (HCD) with a normalized collision energy (NCE) of 30%, fixed first mass was set at 110. In all cases, one microscan was recorded using dynamic exclusion of 30 s.

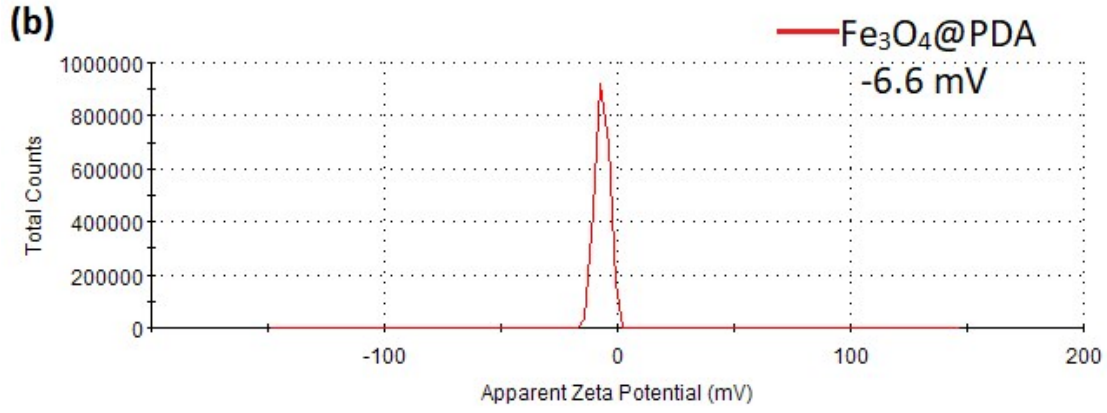
MS/MS spectra were extracted by the Proteome Discoverer software (Thermo Fisher Scientific, version 1.4.0.288) with the MASCOT searching engine version 2.3.2. And the database was the Human UniProtKB/Swiss-Prot database (release 2018-01-26, with 20,245 sequences). Raw files generated by the Orbitrap Fusion were searched directly using a 10-ppm precursor mass tolerance and a 20-ppm fragment mass tolerance. The enzyme specificity with trypsin was used. Up to two missed cleavages were allowed and peptides with at least six amino acids were retained. Carbamidomethyl on cysteine was set as a fixed modification. Oxidation on methionine and deamidation on asparagine were set as variable modifications. The FDRs were estimated by the program from the number and quality of spectral matches to the decoy database. For all data sets, the FDRs at spectrum, peptide, and protein level were < 1%.



Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
6	C	Carbon	37.37	18.56
8	O	Oxygen	27.71	18.33
26	Fe	Iron	21.09	48.70
7	N	Nitrogen	11.82	6.85
40	Zr	Zirconium	2.01	7.57

Fig. S1. Energy dispersive X-ray (EDX) spectrum data of Fe_3O_4@PDA@UIO-66-NH_2.





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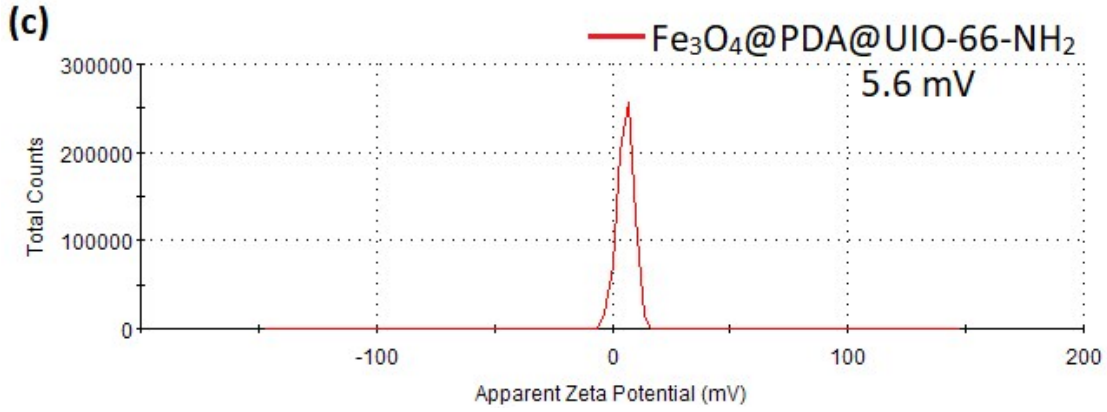

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142 **Fig. S2.** Zeta potential of Fe_3O_4 (a), Fe_3O_4@PDA (b) and Fe_3O_4@PDA@UIO-66-NH_2 (c).

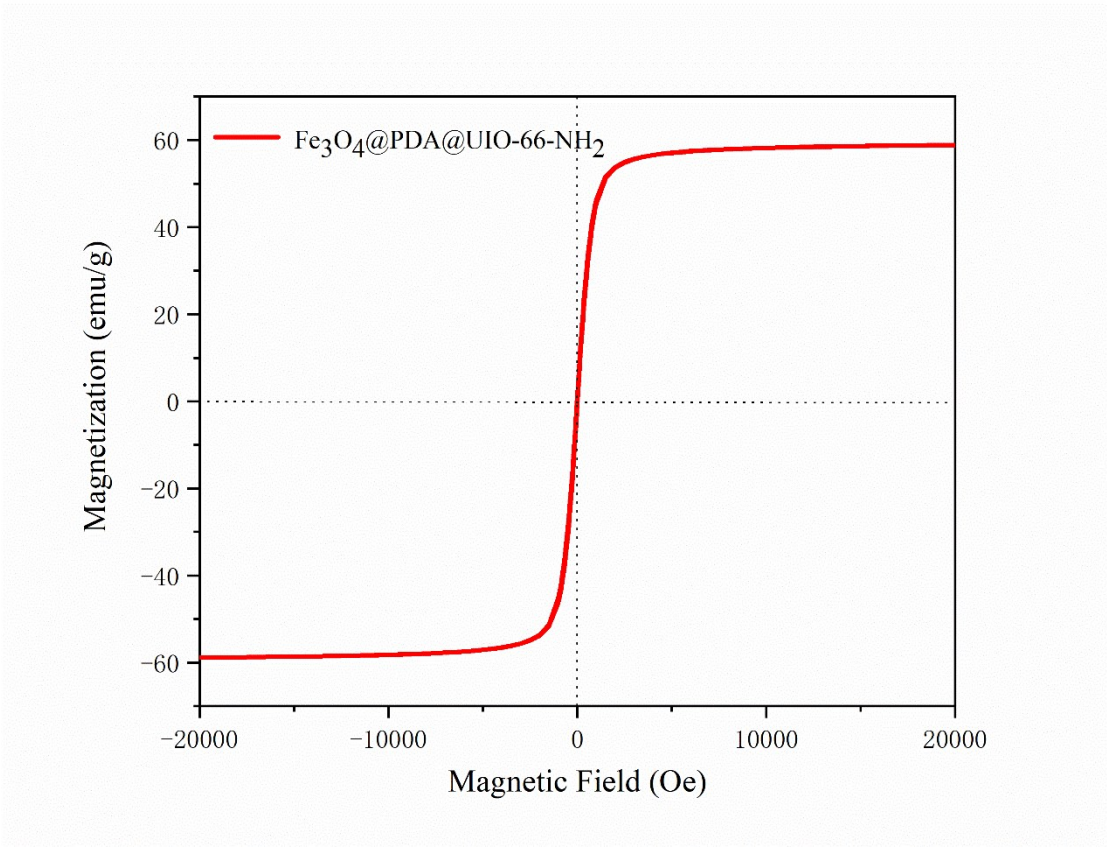


Fig. S3. Magnetic hysteresis curves of Fe_3O_4@PDA@UIO-66-NH_2.

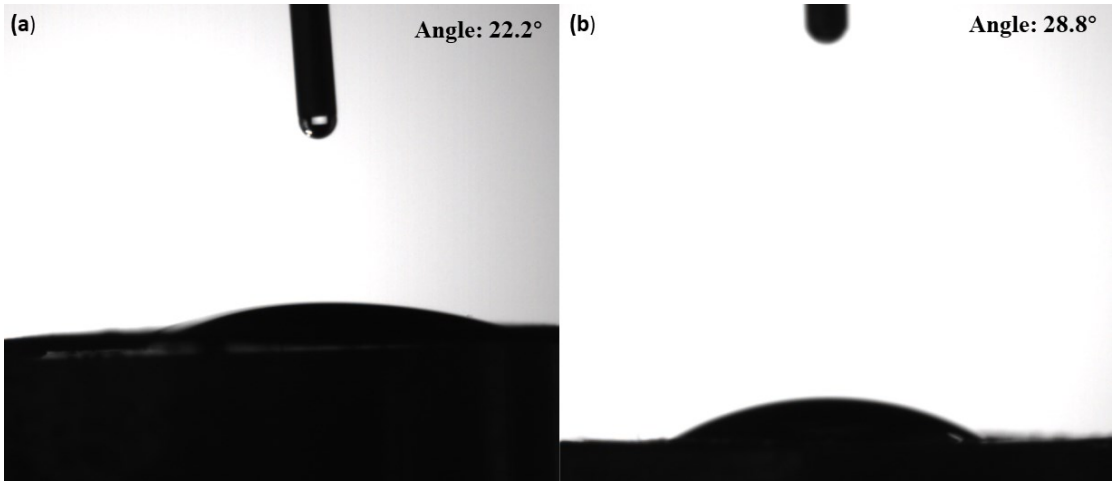
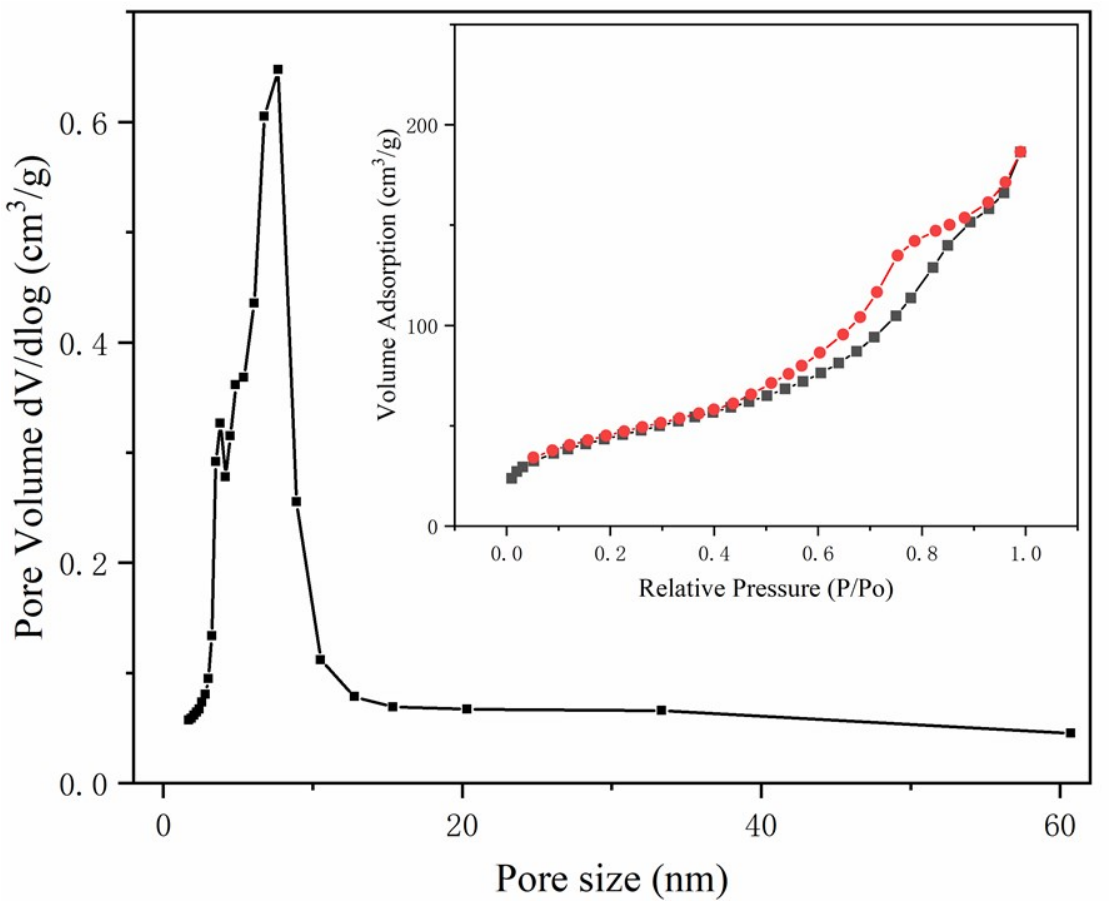


Fig. S4. Contact angle of (a) Fe_3O_4@PDA@UIO-66-NH_2 and (b) Fe_3O_4@PDA@UIO-66.



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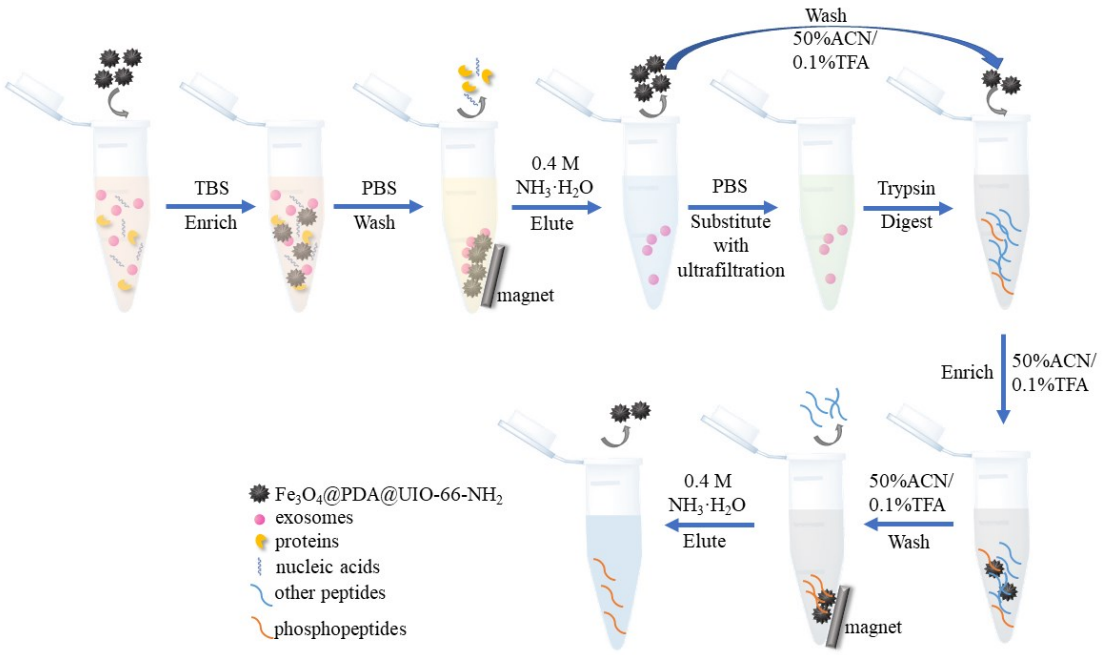
148 **Fig. S5**. Nitrogen sorption isotherms and pore size distribution of Fe_3O_4@PDA@UIO-66-

149 NH_2.



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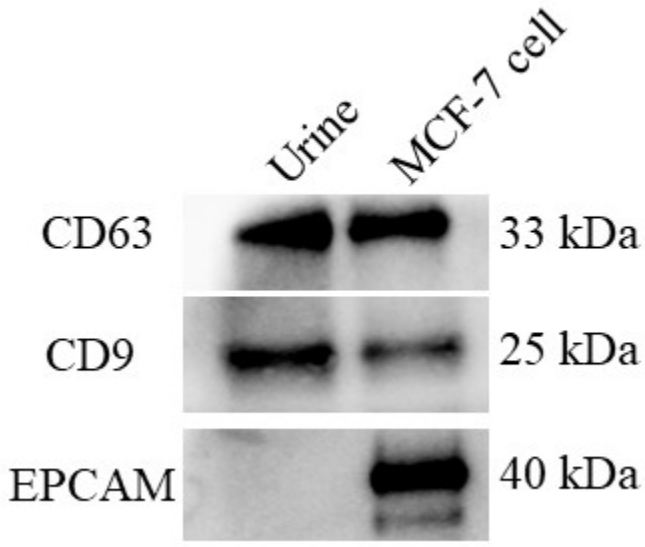
151 **Fig. S6**. Workflow of exosomes and phosphopeptides consecutive enrichment from

152 biological samples using Fe_3O_4@PDA@UIO-66-NH_2.



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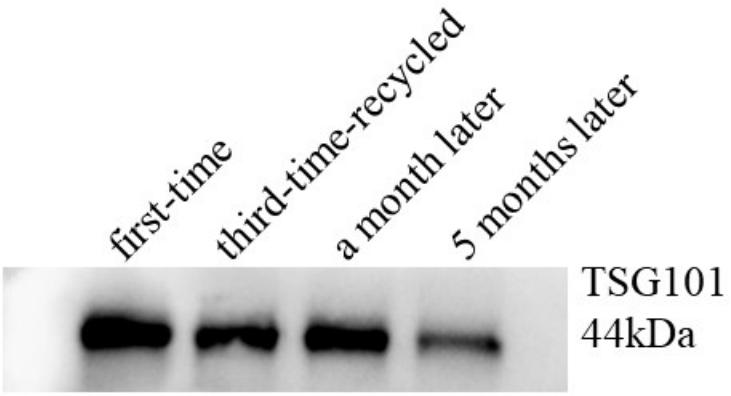
154 **Fig. S7**. Western blot results of three marker proteins (CD63, CD9 and EPCAM) in urinary

155 exosomes and MCF-7 cells derived exosomes.



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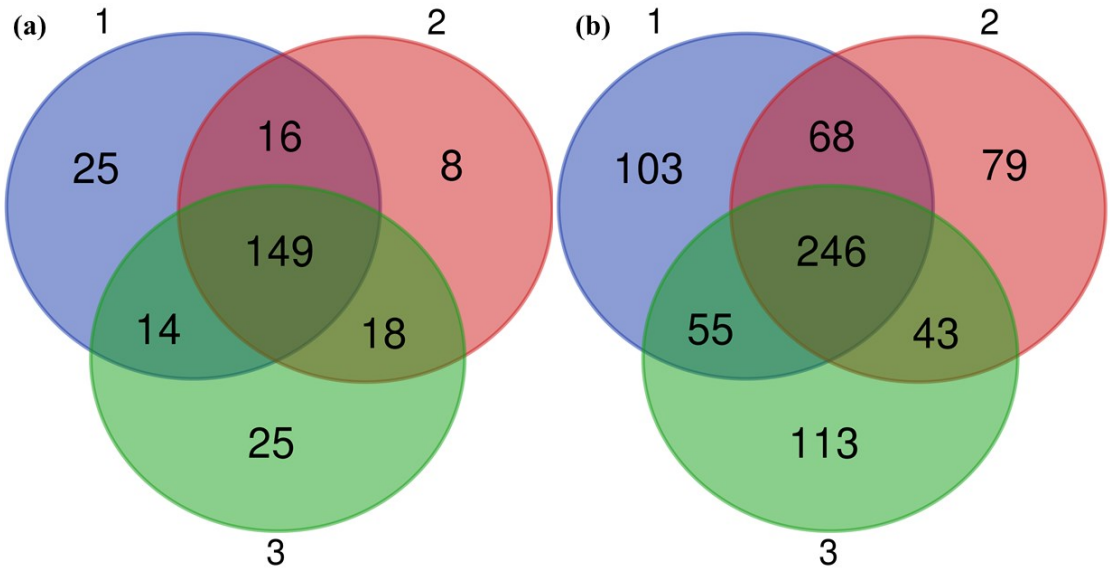
157 **Fig. S8**. Western blot results of TSG101 protein in isolated urinary exosomes with first

158 time / third time recycled Fe_3O_4@PDA@UiO-66-NH_2 and with Fe_3O_4@PDA@UiO-66-

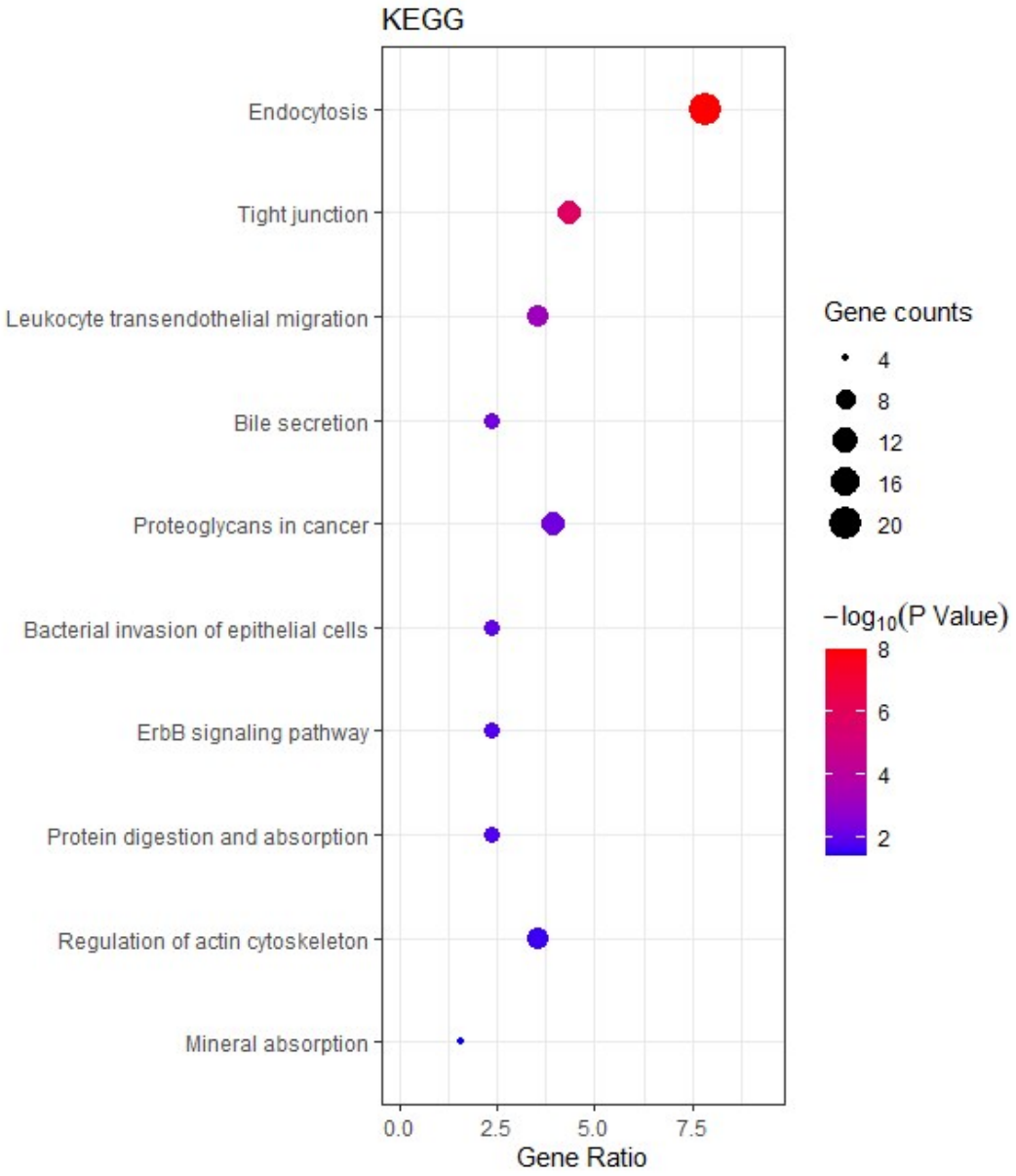
159 NH_2 storing for a month / 5 months.



160 **Fi**

161 **g. S9**. Venn diagram of three parallel enrichment experiments of urine exosomes

162 phosphoproteins result (a) and phosphopeptides statistics (b).



163

164 **Fig. S10**. The bubble chart about KEGG pathways of phosphoproteins from urine

165 exosomes. The closer the color is to red, the smaller the corresponding P value, and the

166 bigger the bubble, the more protein it contains.