Supporting Information

HPRR-Based Diatomic Catalyst Electrochemistry Biosensor for Detecting Cancer-Related Extracellular Vesicles

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Culture of cells and isolation of extracellular vesicles (EVs). HL-60 (human acute myeloid leukemia) cells were inoculated with 80% IMDM medium and 20% fetal bovine serum (FBS). The cells were cultured in an aseptic saturated humidity incubator containing 5%CO_2 at 37 ℃. When the cell density reached 70% - 80%, it was washed twice with PBS, and cultured in exosome-depleted fetal bovine serum (EDFBS) medium for 48 hours. Collect the supernatant of the cells.

The EVs were separated by the ultracentrifugation method. The collected supernatant was transferred to a new centrifuge tube at 200 × g at 4 °C for 5 min. The supernatant was transferred to a new centrifuge tube and centrifuged twice at 2000 × g at 4 ℃ for 15 min. The supernatant was carefully transferred to a new centrifuge tube and centrifuged again at 10000 × g 4 ℃ for 40 min to remove the large vesicles. The supernatant was transferred to a new centrifuge tube and the overspeed rotor was selected at 4 ℃ at 100000 × g for 70 min. The collected EVs was suspended in 1 × PBS buffer and stored at -20 ℃. The EVs isolated by the ultracentrifugation method were characterized by TEM, NTA and western blot.

Chemicals. Phosphate buffer saline (PBS, 0.01M, pH=7.4) was purchased from Thermo Fisher Biochemical Products (Beijing) Co., Ltd. Hydrogen peroxide (30 wt %), potassium ferricyanide ($K_3Fe(CN)_6$), potassium hexacyanoferrate(II) trihydrate ($K_4Fe(CN)_6 \cdot 3H_2O$), potassium chloride (KCl), methanol and ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. Copper acetate monohydrate ($Cu(OAc)_2 \cdot H_2O$), zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) 2,6-diformyl-4-methylphenol, hexadecyl trimethyl ammonium bromide (CTAB), 1,3-diaminopropane

and 2-methylimidazole were purchased from Aladdin (Shanghai, CN). Anti-CD63 Antibody (RFAC4), Anti-CXCR4 Antibody (12G5), Nafion-117, BMPO, foetal bovine serum (FBS) and ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) were purchased from Sigma (Shanghai, CN), All analytical grade chemicals were used as received without further purification. Indium Tin Oxide eletrodes (ITO) was purchased from XuJue Ltd. (Luoyang, CN). Human Promyelocytic Leukemia Cells (HL-60) was purchased from Cellverse Bioscience Technology Co., Ltd. (Shanghai, CN). Deionized water was used for all the experiments.

Aapparatus. The crystal structures of the nanozymes in the 2θ range of 10–80° were obtained by using a D8 Advance Polycrystalline X-ray diffractometer (XRD, AXS, BRUKER) with a scan rate of 0.1° min^{-1}. The binding energy and the valence state of iron of the samples were analyzed by using an Thermo escalab 250XI X-ray Photoelectron Spectroscopy (XPS) instrument (Thermo Scientific, USA) with Al Kα X-ray radiation. The CAT-like activity of nanozymes was measured by PreSens OXY-1 ST (PreSens, Germany). The structures of the DACs were carefully identified using aberration-corrected high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) by a Talos F200x (Thermo Scientific, USA). All electrochemical experiments were performed using a CHI 760E electrochemical workstation (CH Instruments, Shanghai, CN) by using a platinum coil electrode as the auxiliary electrode.

Optimization of experimental conditions

Times of cycles for electrodeposited Au. Au nanoparticles (Au NPs) were deposited onto the electrode surface using an electrodeposition method performed by cyclic voltammetry. The parameters for electrodeposition were set as follows: a high potential of −0.1 V, a low potential of −0.7 V, a scan rate of 50 mV s^{-1}, and 5, 10, 15 and 20 cycles respectively. Then the CV scans were performed for different electrodes in a buffer consisted of, 0.1 M KCl, and 5 mM $Fe(CN)_6^{3-/4-}$ in a 1:1 ratio, find out the electrode with the largest current response.

The concentration of anti-CXCR4 antibodies. The ITO/Au electrode was immersed in a PBS solution containing various concentration of anti-CXCR4-specific antibody (12G5, 10, 15, 20 and 25 μg mL^{-1}) and incubated for 4 hours at 4°C to allow for the immobilization of the antibody on the electrode surface via Au-S bonds. Then the CV scans were performed for different electrodes in a buffer consisted of, 0.1 M KCl, and 5 mM $Fe(CN)_6^{3-/4-}$ in a 1:1 ratio, find out the electrode with the lowest current response.

The incubation time of EVs. ITO@Au@anti-CXCR4-antibody were placed in 1.5 mL centrifuge tubes and then 1 mL of phosphate-buffered saline (PBS) solutions containing EVs (1000000 particles mL^{-1}) were added to the tubes. The tubes were incubated at 37°C for 15, 30, 45 and 60 minutes in a thermostatic incubator, respectively. Then the CV scans were performed for different electrodes in a buffer consisted of, 0.1 M KCl, and 5 mM $Fe(CN)_6^{3-/4-}$ in a 1:1 ratio, find out the electrode with the lowest current response.

The concentration of anti-CD63 antibodies. 1 mL of a Fe/Cu diatomic nanozyme

solution (0.15 mg mL^{-1}), modified with various concentration of anti-CD63 antibodies (5, 10, 15 and 20 μg mL^{-1}). The maximum coupling rate of Fe/Cu DACs to antibodies was detected using the Coomassie Brilliant Blue.

The amount of H_2O_2 used. The ITO@Au modified with 50 μg Fe/Cu DACs was detected in buffer comprised 0.01 M PBS (pH=7.4) various concentration (1, 2, 3, 5, 10 and 20 mM) of H_2O_2.

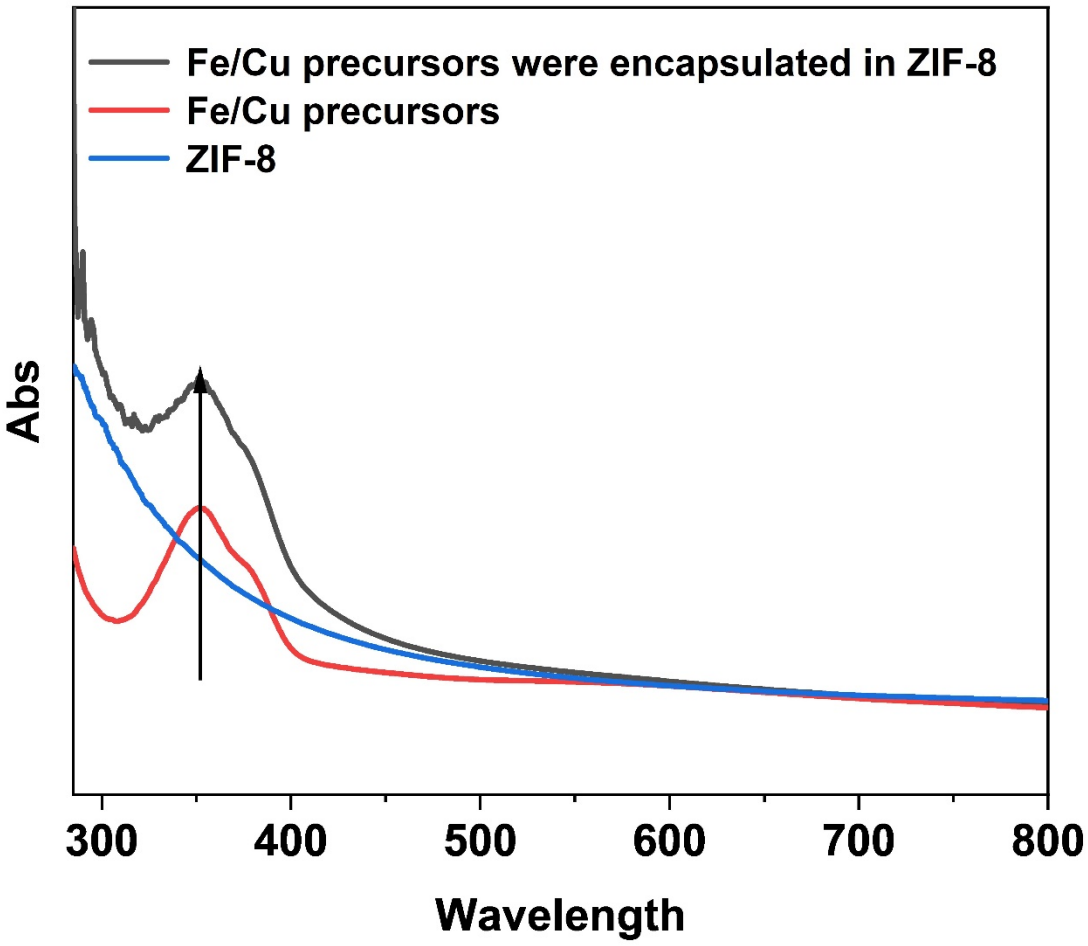


Figure. S1 UV-VIS spectra of Fe/Cu precursors, ZIF-8 and Fe/Cu precursor within the ZIF-8.

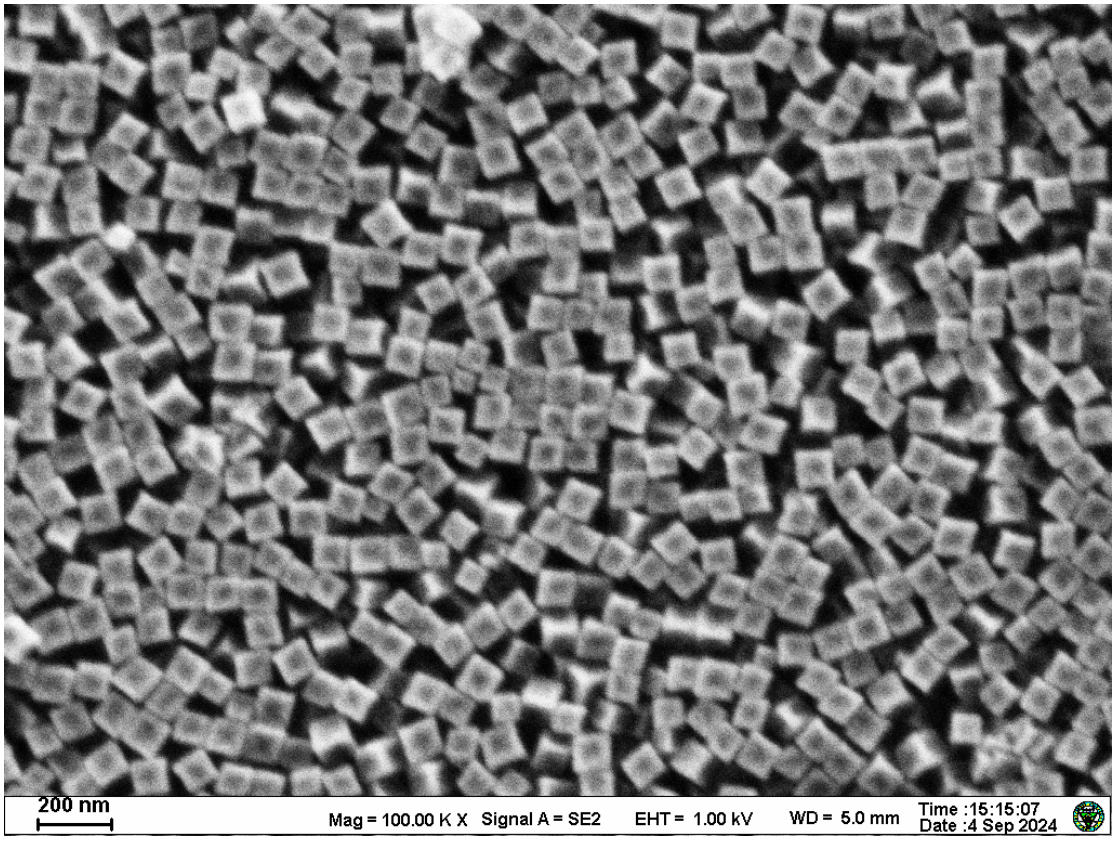


Figure. S2 The SEM image of Fe/Cu DACs.

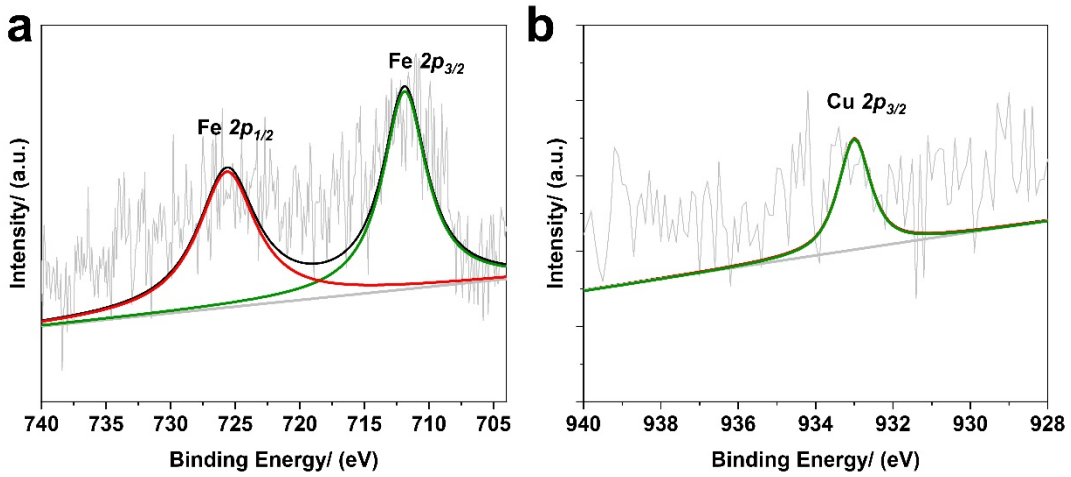
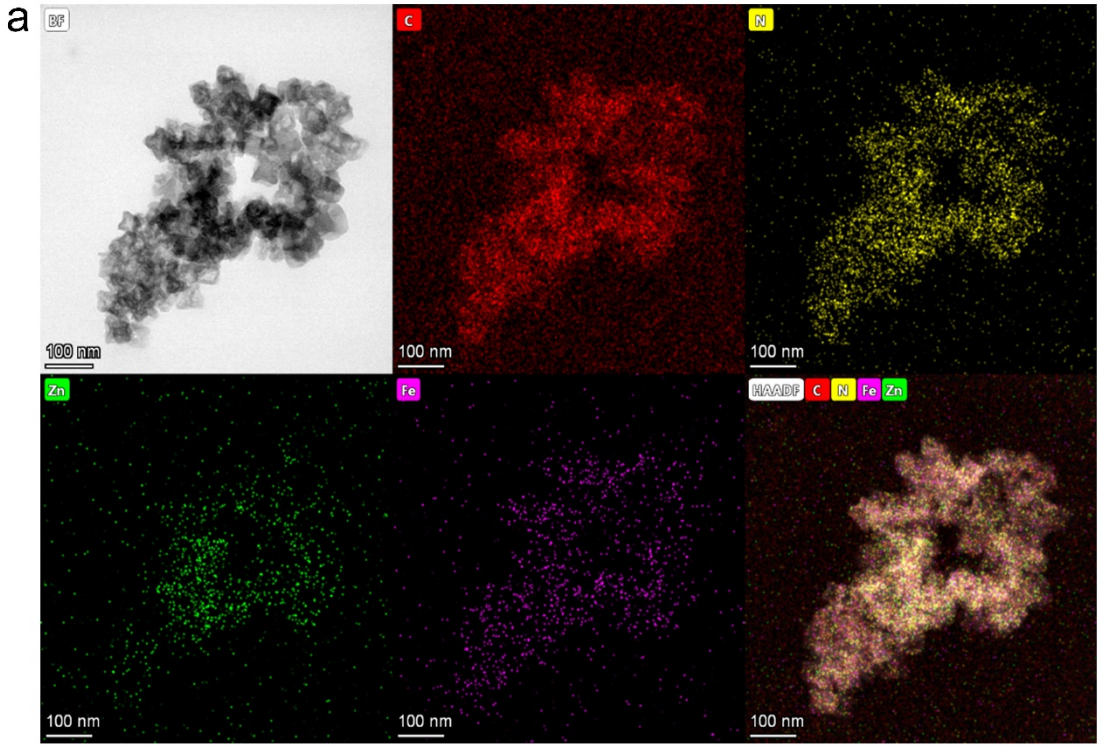
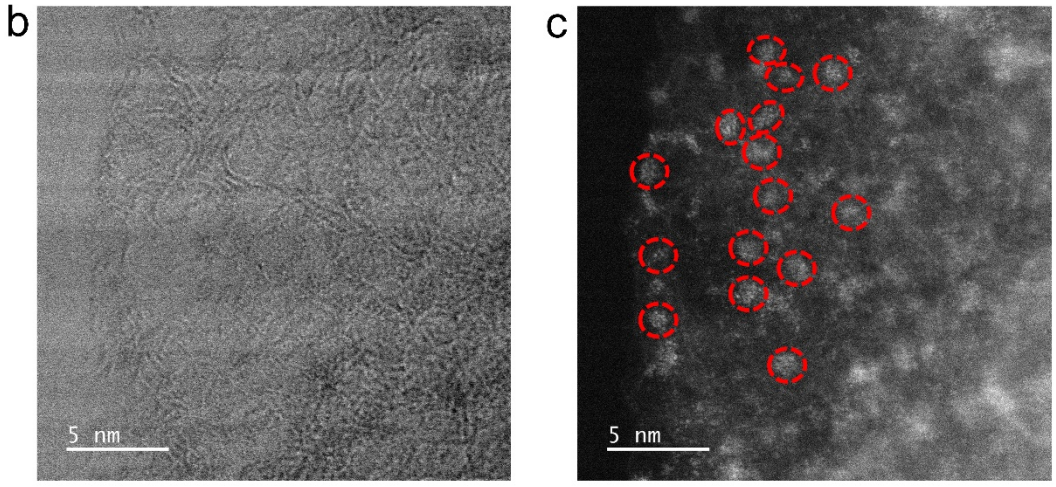


Figure. S3 The XPS patterns of Fe (a) and Cu (b).

Table. S1 Metal contents for Fe SACs and Fe/Cu DACs as determined by ICP-OES

Catalysts	Metal content (wt%)
Fe SACs	Fe 0.52
Fe/Cu DACs	Fe 0.81 Cu 0.78


a
BF
100 nm
C
100 nm
N
100 nm
Zn
100 nm
Fe
100 nm
HAADF C N Fe Zn
100 nm


b
5 nm
c
5 nm

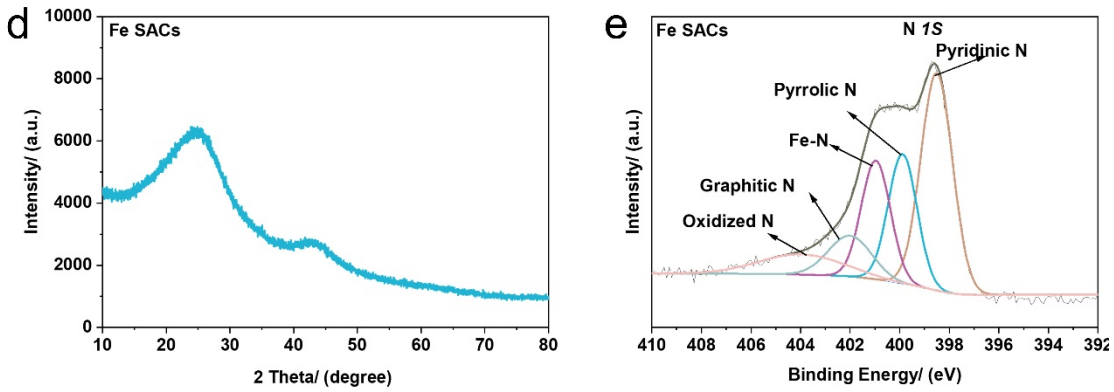

d
Fe SACs
Intensity/ (a.u.)
2 Theta/ (degree)
e
Fe SACs
N 1S
Pyridinic N
Pyrrolic N
Fe-N
Graphitic N
Oxidized N
Intensity/ (a.u.)
Binding Energy/ (eV)

Figure. S4 Characterization of Fe SACs. (a) TEM image and EDX elemental mapping images of Fe SACs. (b, c) HRTEM image (b) and HAADF-STEM (c) images of Fe/Cu DACs, the scale bar was 5 nm. (d, e) The XRD (d) and XPS (e) patterns of Fe SACs.

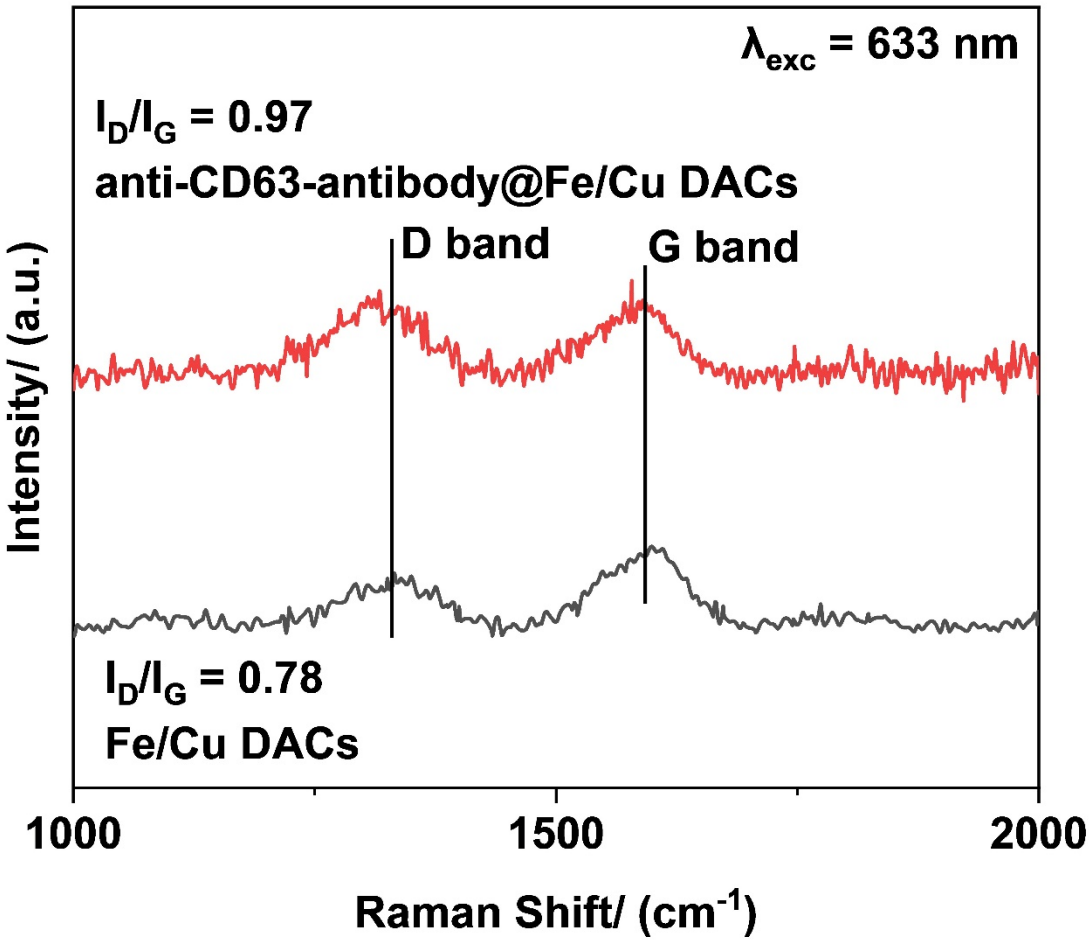


Figure. S5 Raman spectra of Fe/Cu DACs and anti-CD63-antibody@Fe/Cu DACs.

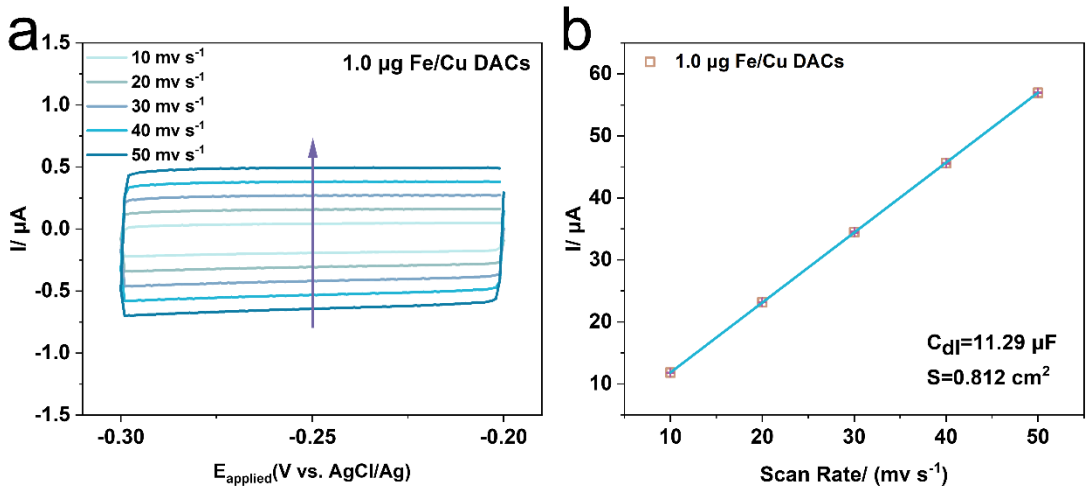


Figure. S6 The ECSA mesurement of Fe/Cu DACs.

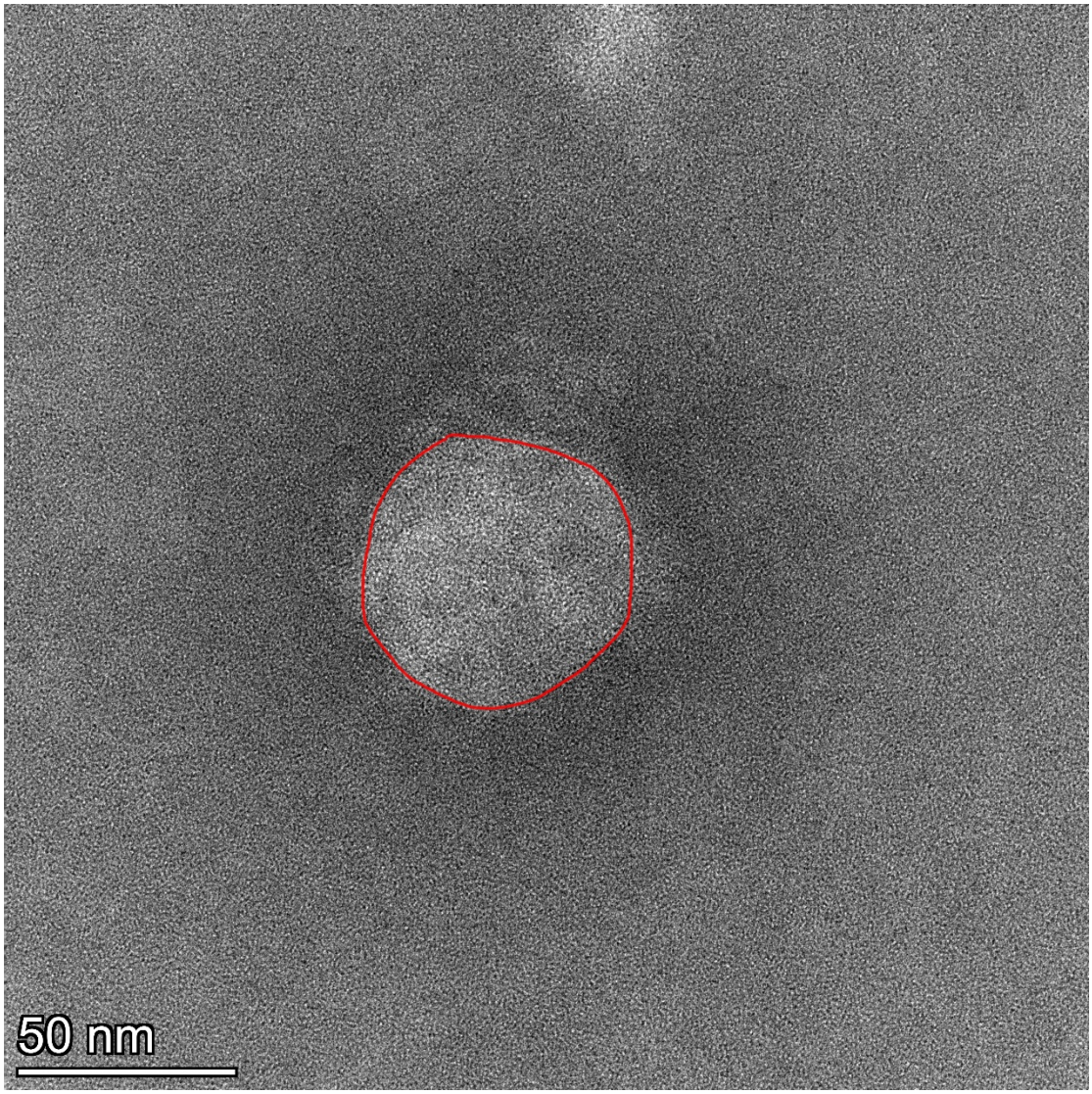


Figure. S7 The TEM image of EV.

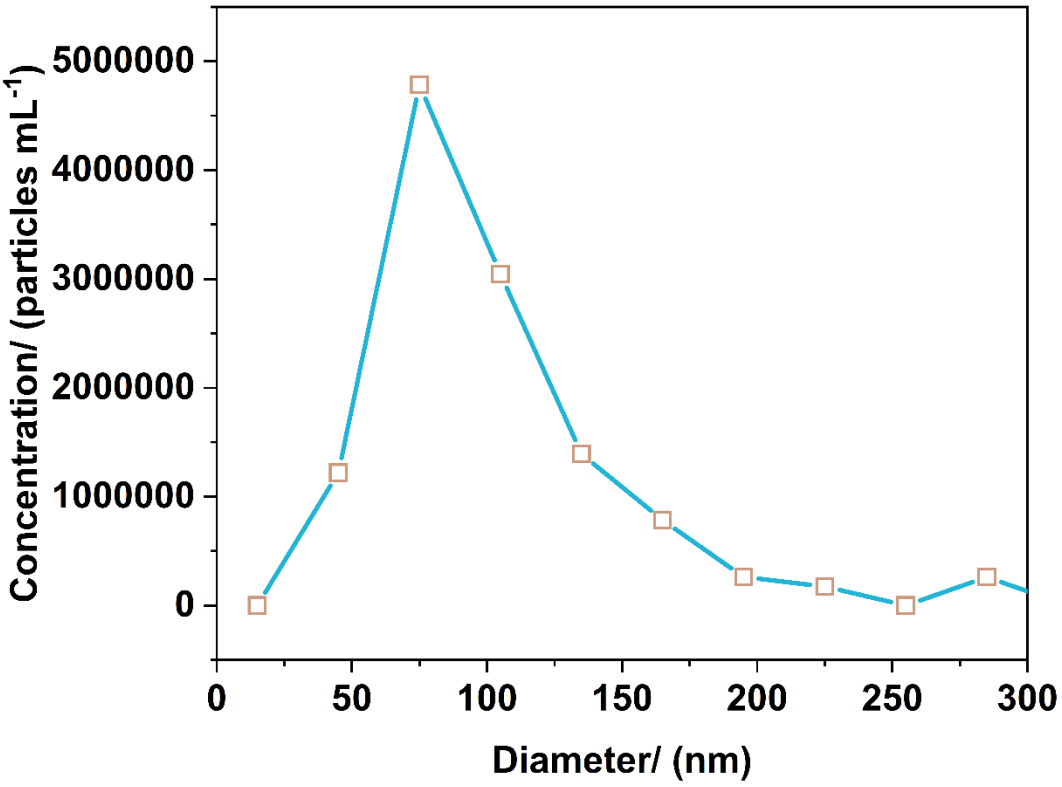


Figure. S8 Particles size analysis of EVs by NTA.

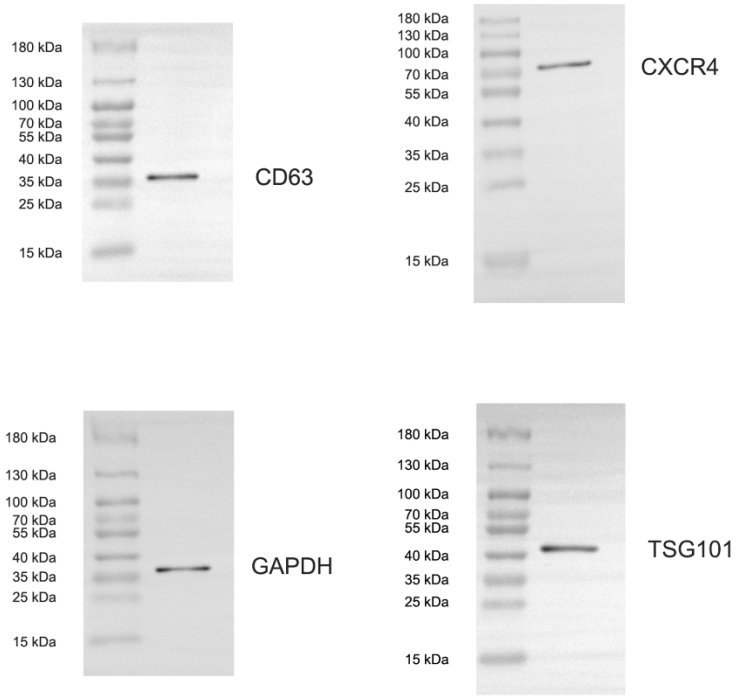


Figure. S9 Western blot images of CD63, CXCR4, GAPDH and TSG101 proteins from cancer plasma-derived EVs.

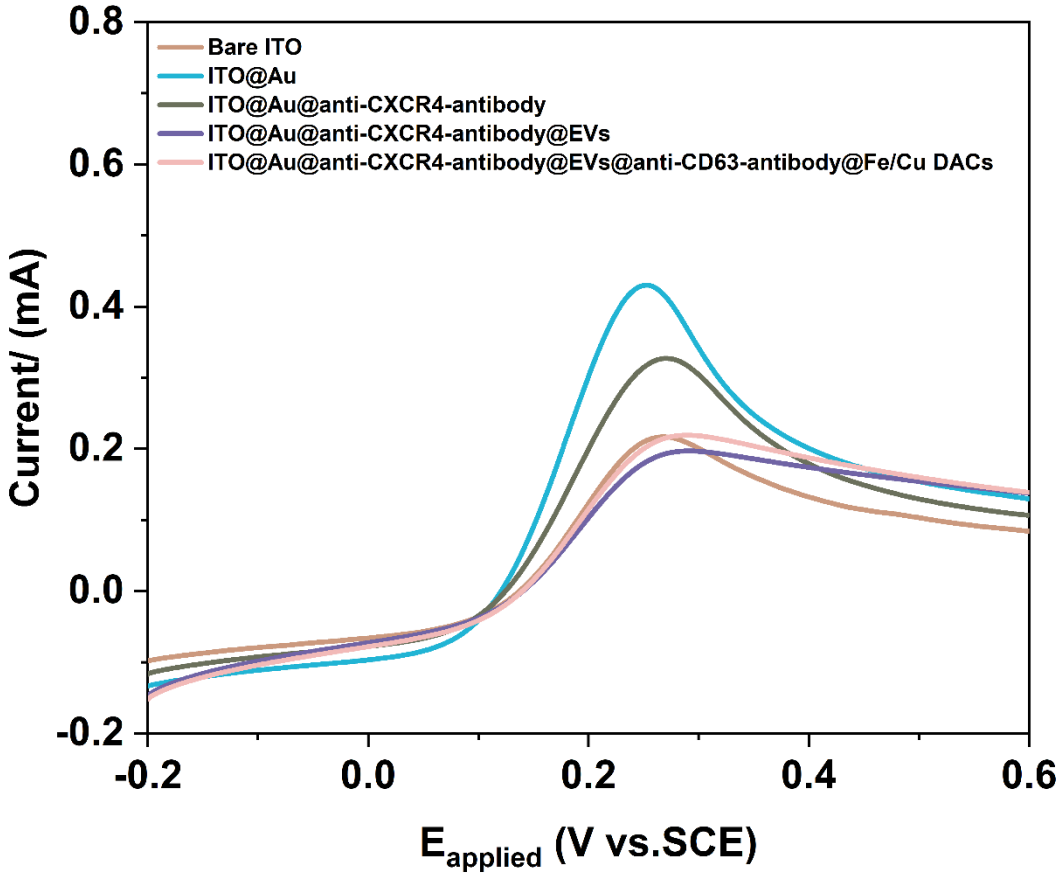


Figure. S10 Current changes of electrodes in a 0.1 M KCl, and 5 mM $Fe(CN)_6^{3-/4-}$ solution during modification process.

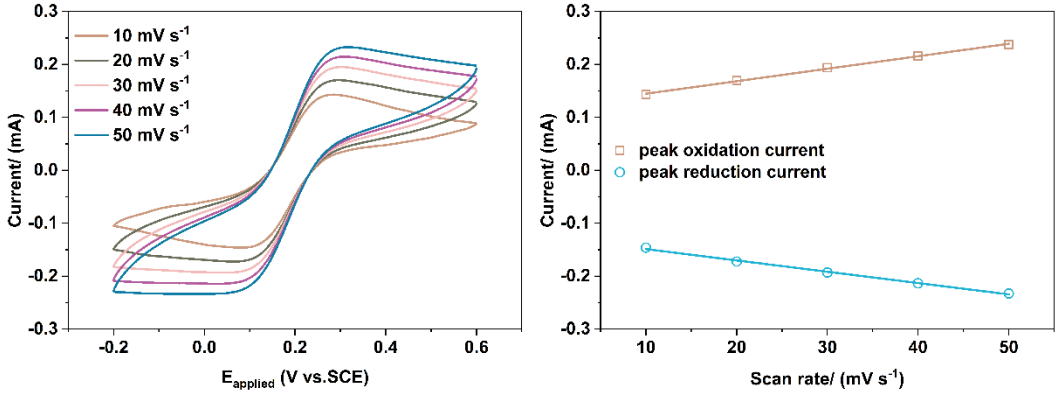


Figure. S11 The redox peak currents of the electrode at various sweep speeds.

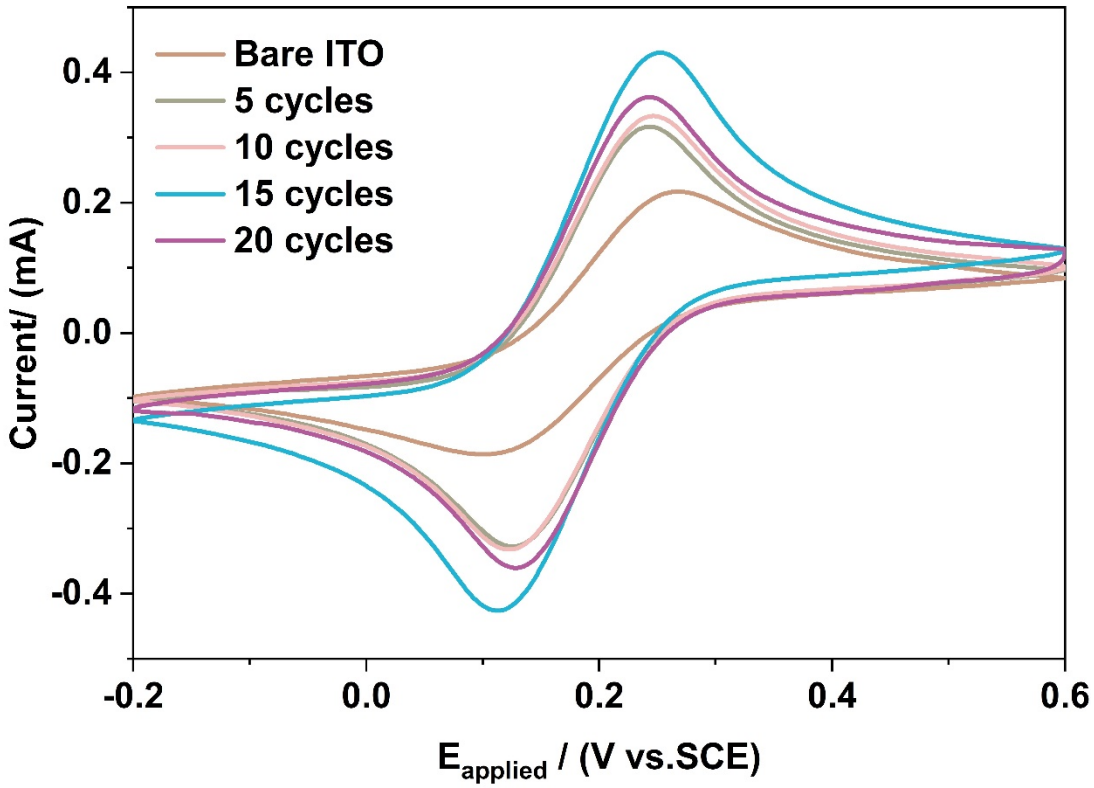


Figure. S12 Current changes of times of cycles for electrodeposited Au.

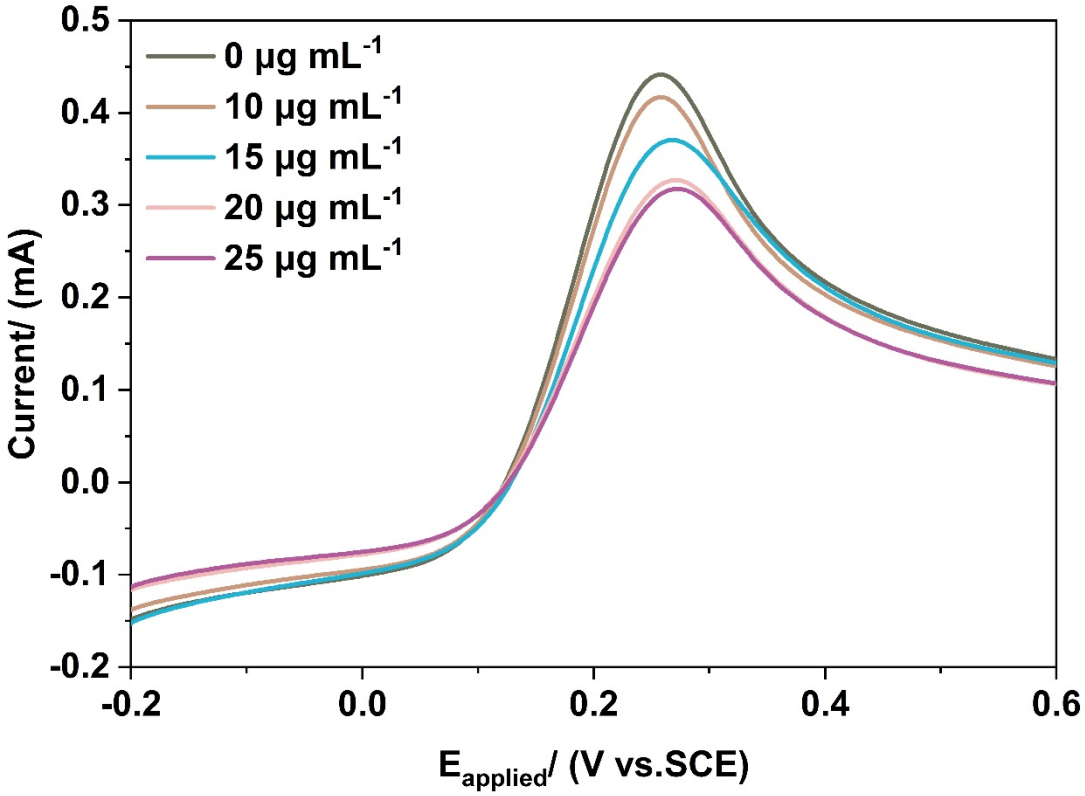


Figure. S13. Current changes of electrode modified with various concentration of anti-CXCR4 antibodies.

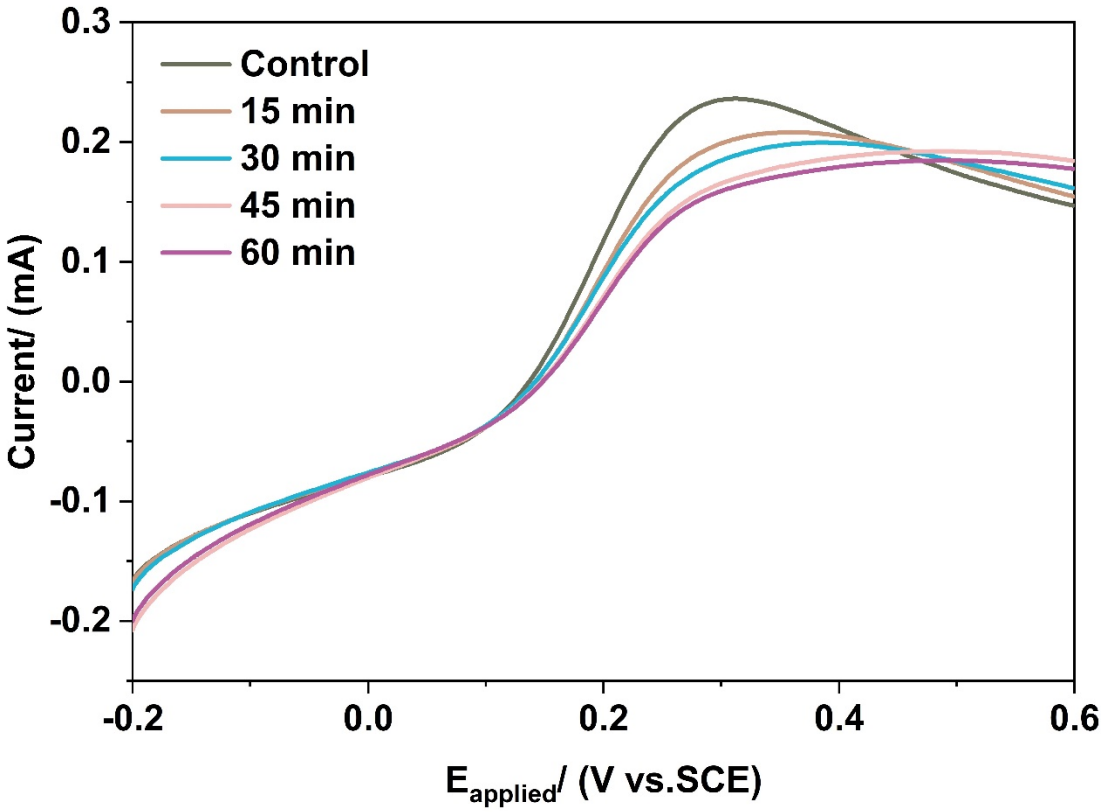


Figure. S14 Current changes of the various incubation time of EVs.

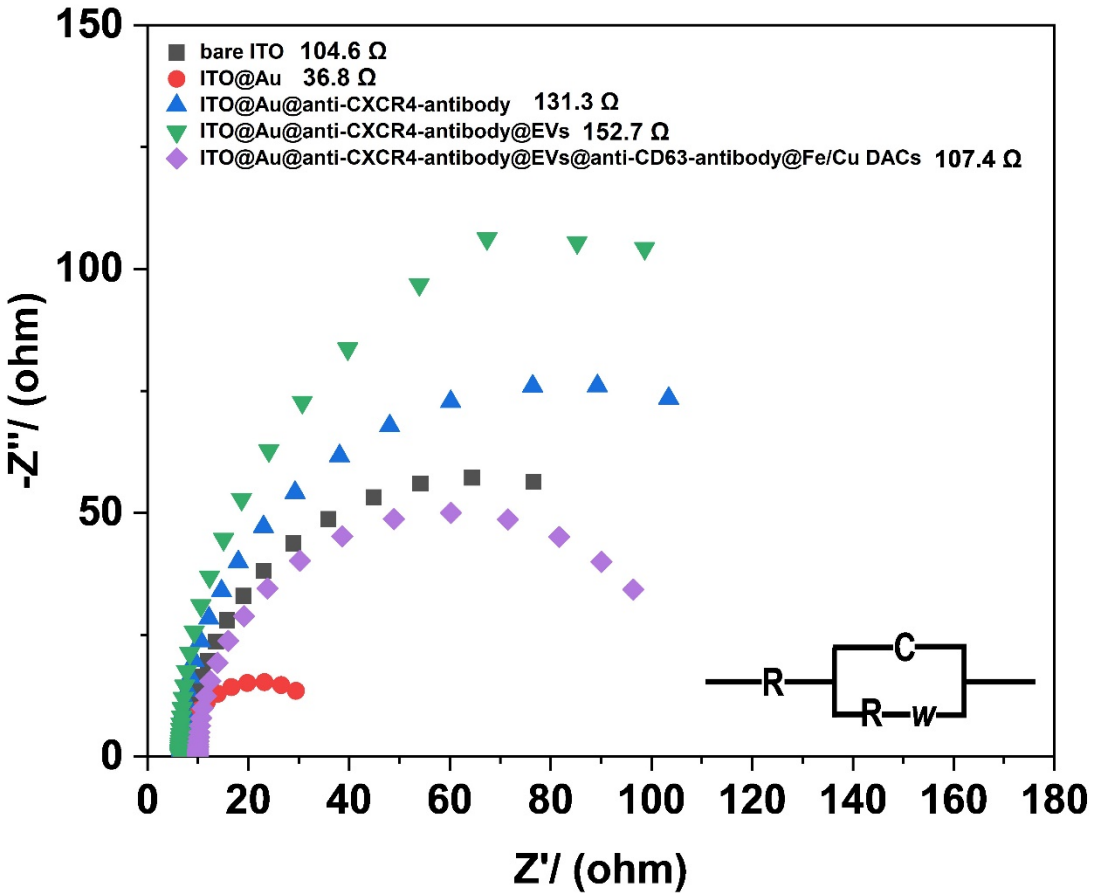


Figure. S15 EIS of bare ITO, ITO@Au, ITO@Au@anti-CXCR4-antibody, ITO@Au@anti-CXCR4-antibody@EVs and ITO@Au@anti-CXCR4-antibody@EVs@anti-CD63-antibody@Fe/Cu DACs.

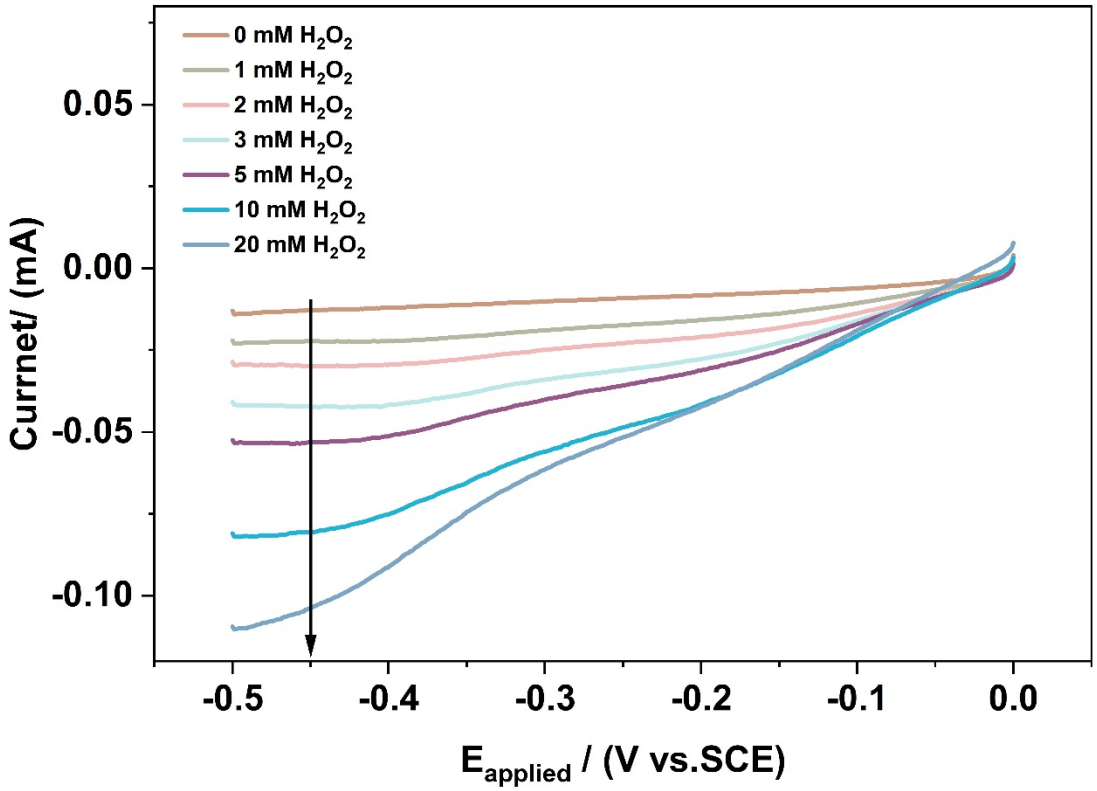


Figure. S16 Current changes of various amount of H_2O_2 used.