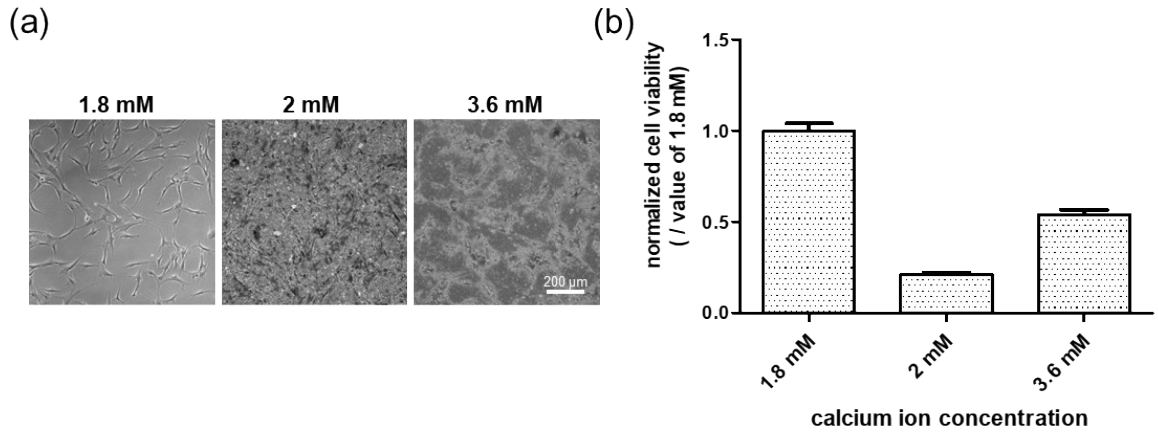
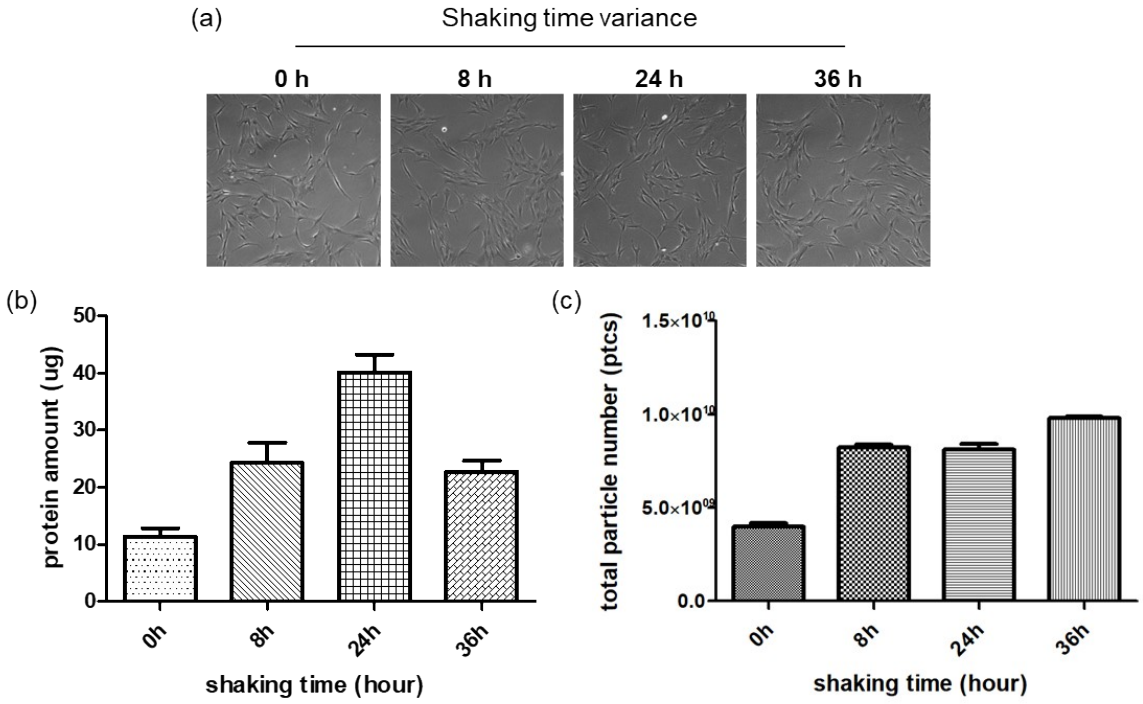
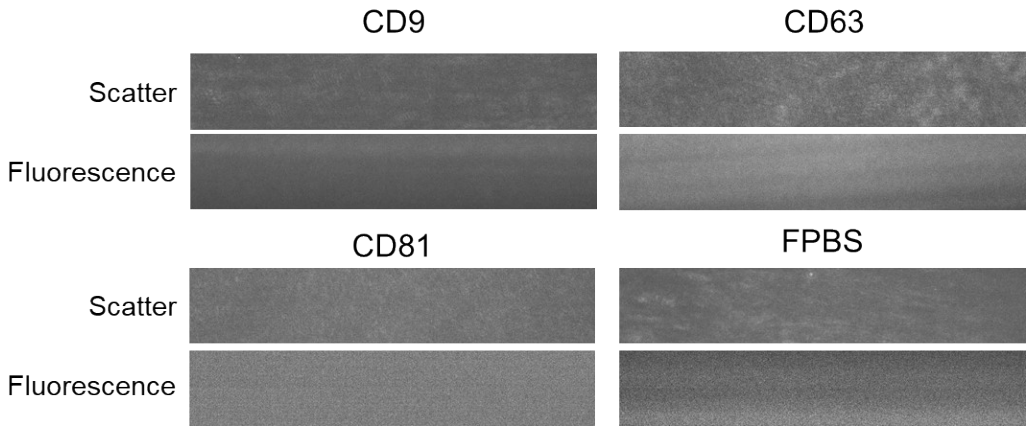
Supplementary Information (SI) for Nanoscale.



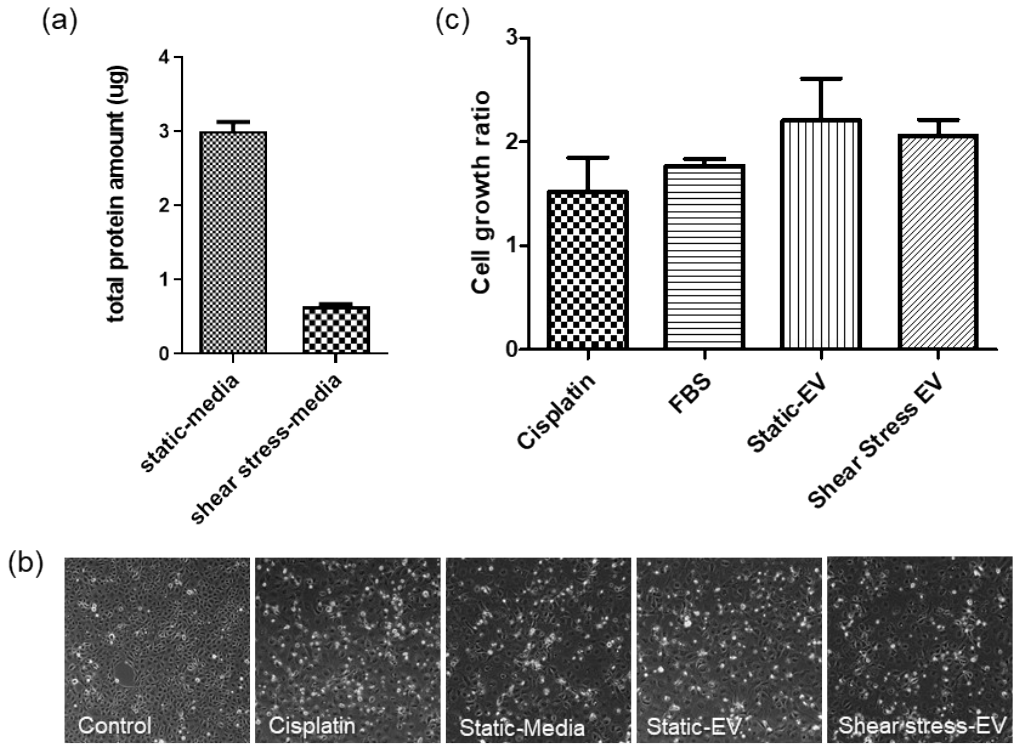
Supplementary Figure. 1 Cell condition under various calcium ion concentrations in the culture media exerting shear stress of 0.05 dyne/cm^2 to all experimental groups for 6 hours. (a) Cell morphology was shown according to the calcium ion concentration. After 6 hours, cells with higher calcium ion concentrations exhibited completely different morphology under the microscope compared to the normal concentration group. In 2mM and 3.6 mM calcium ion concentration groups, some of cells were attached, but a lot of precipitated salts were observed with the cells. Those precipitated salts appeared to more dispersed at 2mM than 3.6 mM, where 3.6mM of calcium ion concentration group showed more aggregated salts clustered around the cells. (b) Cell viability was quantitively analyzed using MTT assay under calcium ion concentration changes. Following twice of washing with PBS, MTT solution was added to the cells according to the instruction. The results of the cell viability assay indicated that cells cultured in 1.8 mM calcium ion concentration, w hich maintained normal morphology in the images, showed the highest viability. Conversely, higher calcium ion concentrations caused cell damage and decreased viability.



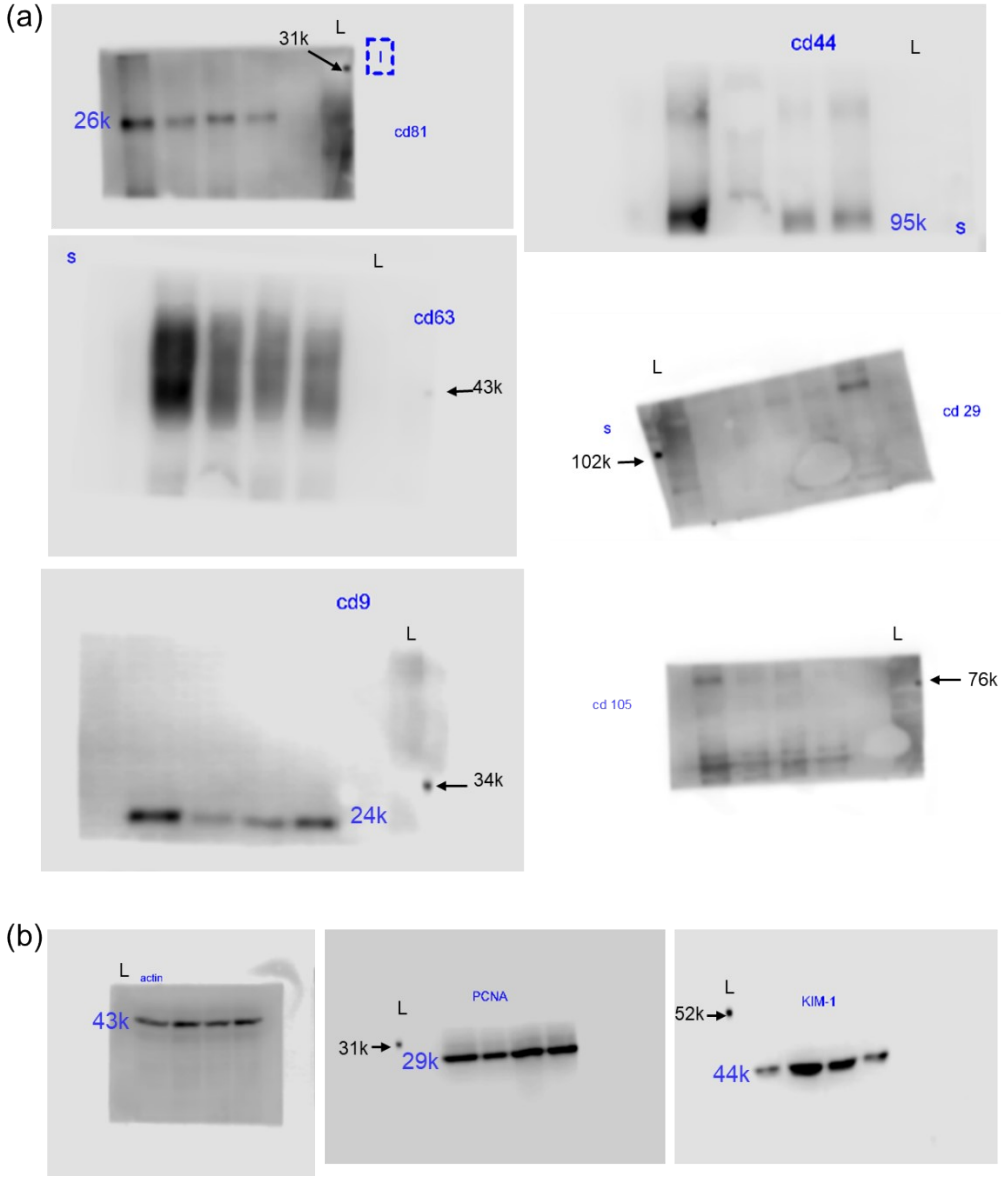
Supplementary Figure. 2 Yield difference of EVs under different incubation times of shaking culture conditions. The number of EVs increased when shear stress was applied compared to conditions with no shear stress (0 rpm) (a) Cell morphology after different exposure time of shaking culture was shown in images captured 10x magnification. Under microscopic observation, cells remained attached to the cell culture dish even after 36 hours of shaking at 50 rpm (0.05 dyne/cm^2). (b) Total protein amounts in different shaking time groups were shown. (c) The total particle number was analyzed using Nanoparticle Tracking Analysis (NTA) tool under different shaking time experimental groups. The 8-hour shaking incubation yielded almost the same value of the particle number as the 24-hour shaking groups, while the 36-hour shaking groups showed a slightly increased particles number.



Supplementary Figure. 3 The images of Nanoparticle Tracking Analysis (NTA) using a blank buffer with staining and washing protocols of EV samples**.** In case of CD9, CD63, and CD81 imaging, each antibody without EVs was put at a concentration of 5ug/ml. The incubation process occurred at 37'C for 2 hours. Then, the samples underwent the same washing procedure at 15,000g 1h for twice, followed by staining with 2nd antibody. 2nd antibody was washed at 15000g for 1h and images under NTA. The FPBS sample followed the same incubation and washing procedures but without the addition of any antibody.



Supplementary Figure. 4 The therapeutic effect of EVs and isolated media was verified using AKI model in vitro. The medium with depleted FBS was shacked or left static, similar to the conditions used in static or shaking cell culture systems and underwent the same isolation process as for EVs. Each 60ml of media was pre-cleaned and ultracentrifuged for twice, and then eluted in 100 μl of PBS. (a) Amount of protein from ultracentrifugation-isolated static and shear stress media. The protein amount of isolated 180 ml of media corresponding to the media volume from 5×10^6 cells, was analyzed using the Bradford assay. (b) Cell images of AKI *in vitro* experimental groups; control(non-treated), cisplatin, static-media, static EV, and shear stress EV treated groups. (c) Cellular growth ratio result of the experimental groups.



Supplementary Figure. 5 Raw images of western blot. (a) The raw image of the western blot result in figure 3. The order of samples is as follows, from the lane closest to the molecular marker to the end: static EV with calcium free media, static EV with normal media, shear stress EV with calcium free media, and shear stress EV with normal media. (b) The raw image of western blot result in figure 5 is shown. The order of samples is as follows, from the lane closest to the molecular marker to the end: non-treated group(control), cisplatin-treated group, static EV-treated group, and shear stress EV-treated group. The black colored arrow and letters indicate the information of molecular mass markers, and the blue-colored letters indicate the information of the protein that are analyzed.