



Supporting Figure 3. Hemolysis evaluation of NEP1-40-loaded PLGA-CTX/LEX NPs. Results suggested that NEP1-40-loaded PLGA-CTX/LEX NPs did not have detectable hemolytic effects at the tested concentrations.

Mouse hemolysis evaluation was performed according to a method previously reported.¹ Briefly, mouse blood was collected into a heparin tube and centrifuged at 1200g for 10 min to harvest erythrocytes. Erythrocytes were washed for 4 times, resuspended in 0.9% saline saline at 5%(v/v) and plated into a 24-well plate with 400 uL suspension per well. Then, 100 uL NP suspension were added to make final concentrations of NPs at 1, 5, 25, and 125 ug/ml, respectively. Tween 80 0.01%(v/v) and Triton X-100 0.1%(v/v) were used as a negative control and a positive control, respectively. The plate was then incubated at 37°C/300 rpm for 30 min. After incubation, suspension from each well was transferred into a 1.5 mL Eppendorf tube and spun at 10,000 g for 5 minutes. Lastly, 100 uL of supernatant was aliquoted into a 96 well plate and the ODs were measured using a microplate reader (Synergy 2, Bioteck, USA).

[1] Nemmar A, Beegam S, Yuvaraju P, Yasinb J, Shahinc A, and Ali BH. Interaction of amorphous silica nanoparticles with erythrocytes in vitro: role of oxidative stress. *Cellular Physiology and Biochemistry*, 2014, 34(2): 255-265.