

**Figure S1.** Fluorescent microscope image (40×) in CT-26 and HCT-116 EpCAM positive and HEK-293 EpCAM-negative cell line; A: Free FITC: A clear green fluorescence signal was observed inside the cells was indicated internalization of the FITC in cancerous cell lines and in normal HEK-293 (as EpCAM negative) cell line confirm the non-specific uptake. B: Ap-FITC-NPs: Show relatively further uptake of FITC-loaded NPs into cancerous cell lines compared to EpCAM negative cell lines.



**Figure S2.** Determination of relative binding affinity and specificity by flow cytometry of selected Cy3-labelled aptamers for A: HEK-293 (as EpCAM negative cell line), B and C: CT-26 and HCT-116 (as EpCAM positive cell line) respectively. The fluorescence shifts in the selected Cy3-labelled-aptamers cell lines compared to the unstained each control cell lines exhibited high affinity of aptamers to the CT-26 and HCT-116 cancer cell lines, while the aptamers showed no binding affinity to the HEK-293 EpCAM negative cell line.



**Figure S3.** Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. ALT and AST levels in serum were assayed using a commercially available test kits according to the manufacturer's instructions. As sown in Figure 5-FU and FU-NPs treated groups have significant elevated ALT and AST levels than other mice groups. Data are presented as mean  $\pm$  SD. (n = 3; \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001, ns: no significant).