Title: A novel microfluidic co-culture system for investigation of bacterial cancer targeting

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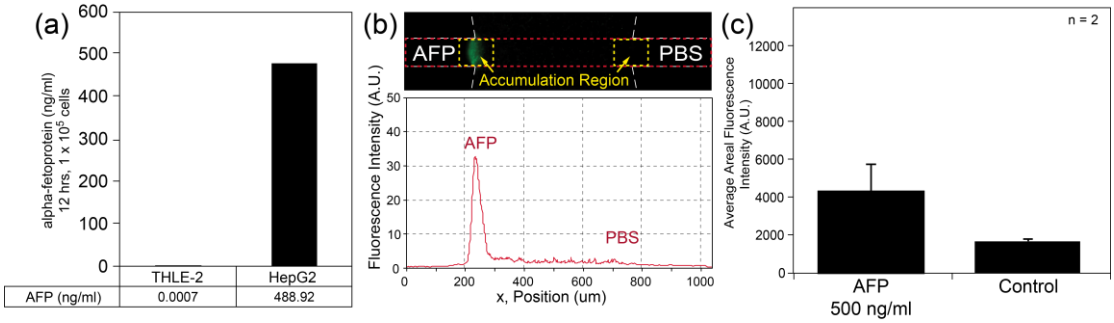
[Supplementary Fig. S-5]



Supplementary Fig. S-5 The cell conditioned media were prepared under conditions identical to procedure described in the manuscript and stored at -80°C before use. The conditioned media were mixed with reagents in the Quantikine Human AFP kit (R&D Systems) and incubated for recommended time (Assay Diluent: 2 hours, AFP Conjugate: 2 hours, Substrate Solution: 30 minutes) at room temperature Optical density (OD) values were measured at 450 nm using a spectrophotometer and AFP concentration of each sample was calculated using a standard curve, which was determined using an AFP standard basic solution. (a) Quantification of AFP expression in normal and cancer hepatocytes revealed that HepG2 cultured media expressed approximately 500 ng/ml of AFP where as none was detected for THLE-2 cultured media. (b) Preference analysis of *S.typhimurium* for 500 ng/ml of AFP against PBS revealed evident preferential accumulation of *S.typhimurium* at the AFP side in two independent sets of experiment, showing a significantly high peak in the intensity profile at AFP (measured across the red box). (c) The average of total intensity in the accumulation regions marked by yellow dotted-rectangles indicates evidently high number of bacteria at the AFP site.