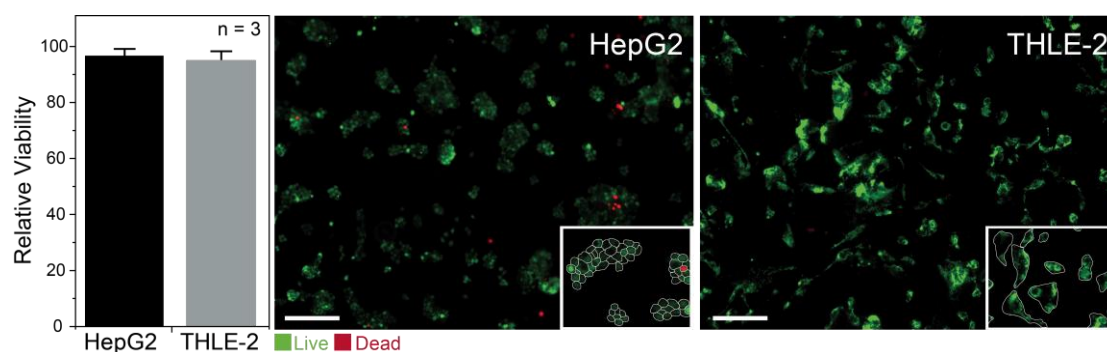


Title: A novel microfluidic co-culture system for investigation of bacterial cancer targeting

Authors: Jung Woo Hong, Sukhyun Song and Jennifer H. Shin

[Supplementary Fig. S-3]



Supplementary Fig. S-3 Cell viability in isolated chambers. After 60 hours in culture chambers (48hrs in serum-supplemented followed by 12hrs in serum-free media), both cells reach their confluency of approximately 80%. Because dying cells may attract *S.typhimurium*, we checked the viability of cells to rule out the possibility of dead cells' contribution in preferential accumulation of *S.typhimurium* at HepG2. For viability assay, cells were thoroughly washed with sterile DPBS, before analyzing the survival rate using a viability test kit from Molecular Probes which utilizes calcein AM (2 μ M) and ethidium homodimer-1 (4 μ M) to distinguish live and dead cells, respectively. Live cells are stained by intracellular esterase activity, visualized the enzymatic conversion of the nonfluorescent dye calcein AM to the green fluorescent calcein. Ethidium homodimer-1 enters cells with damaged membranes and binds to nucleic acids, producing a bright red fluorescence in dead cells (Papadopoulos et al., J Immunol Methods, 1994) within 30 minutes. The numbers of live and dead cells were counted from the images acquired with fluorescence microscope (Axiovert 200M, Carl Zeiss), and the ratio of live cells over total cells were normalized for comparison (Scale bars represent 100 μ m). After 60 hours of culture, we confirmed that more than 90% of both cell types survived 60 hours of culture with no significant difference between two cell types.