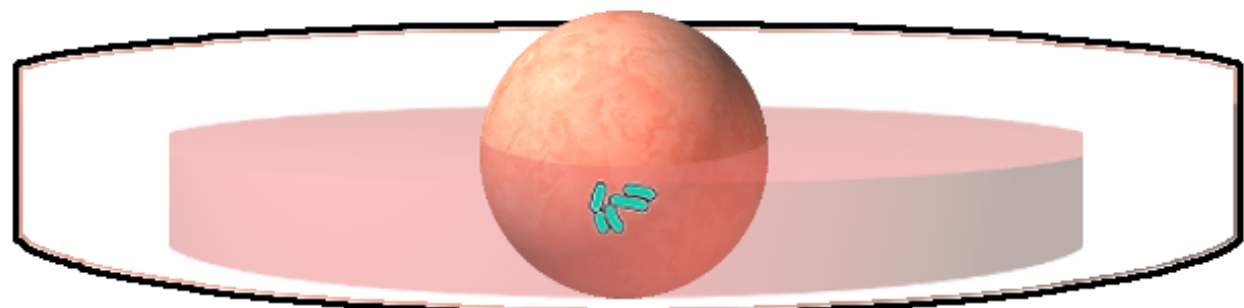
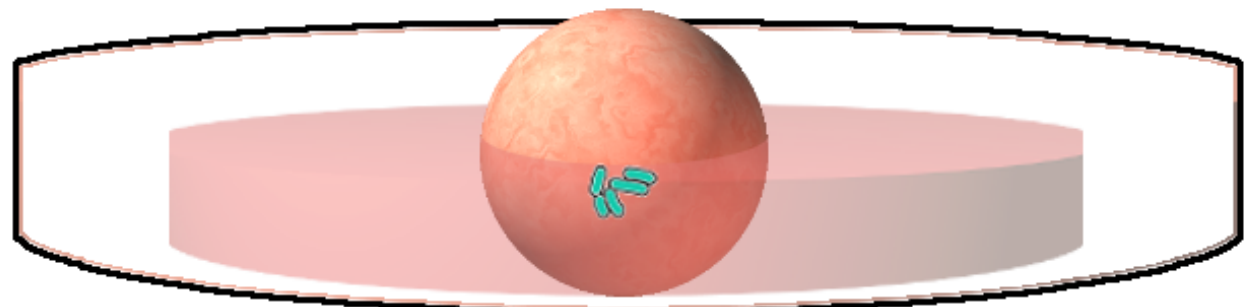
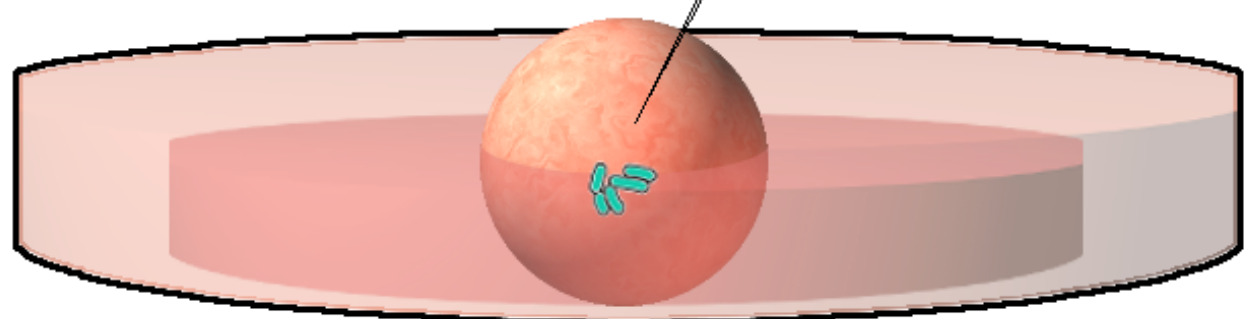
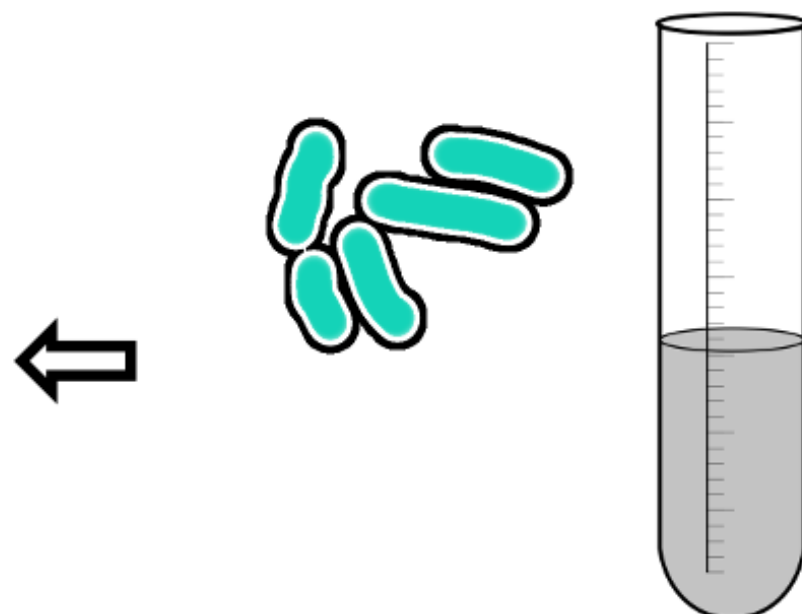


A

DAY -30 to -1: Prepare HIOs according to McCracken *et al* (2011) and plate 1 HIO per well (24-well plate) in 50 μ l Matrigel and 500 μ l ENR media



DAY -1: Culture *E. coli* from glycerol stock in LB broth overnight at 37° C



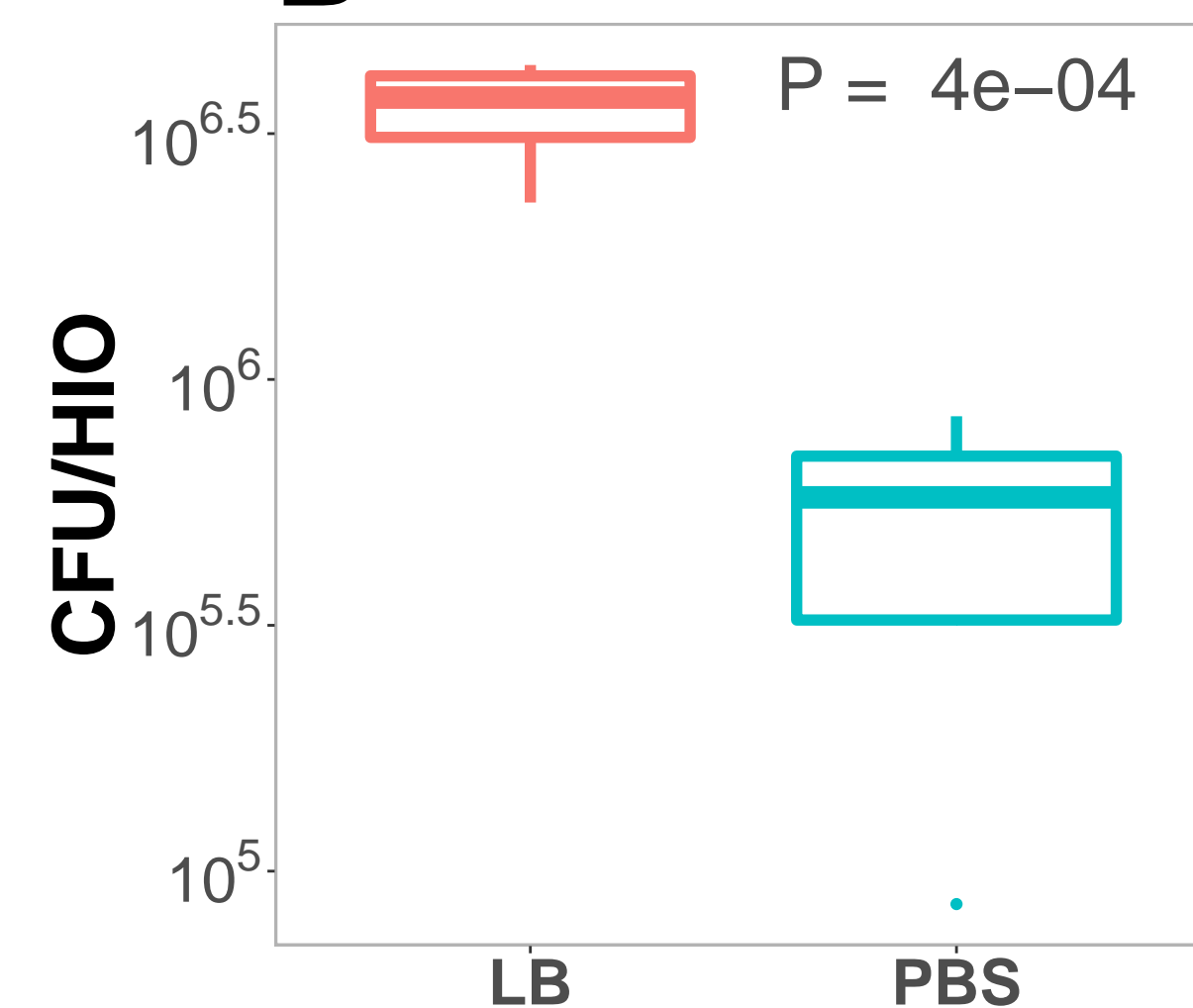
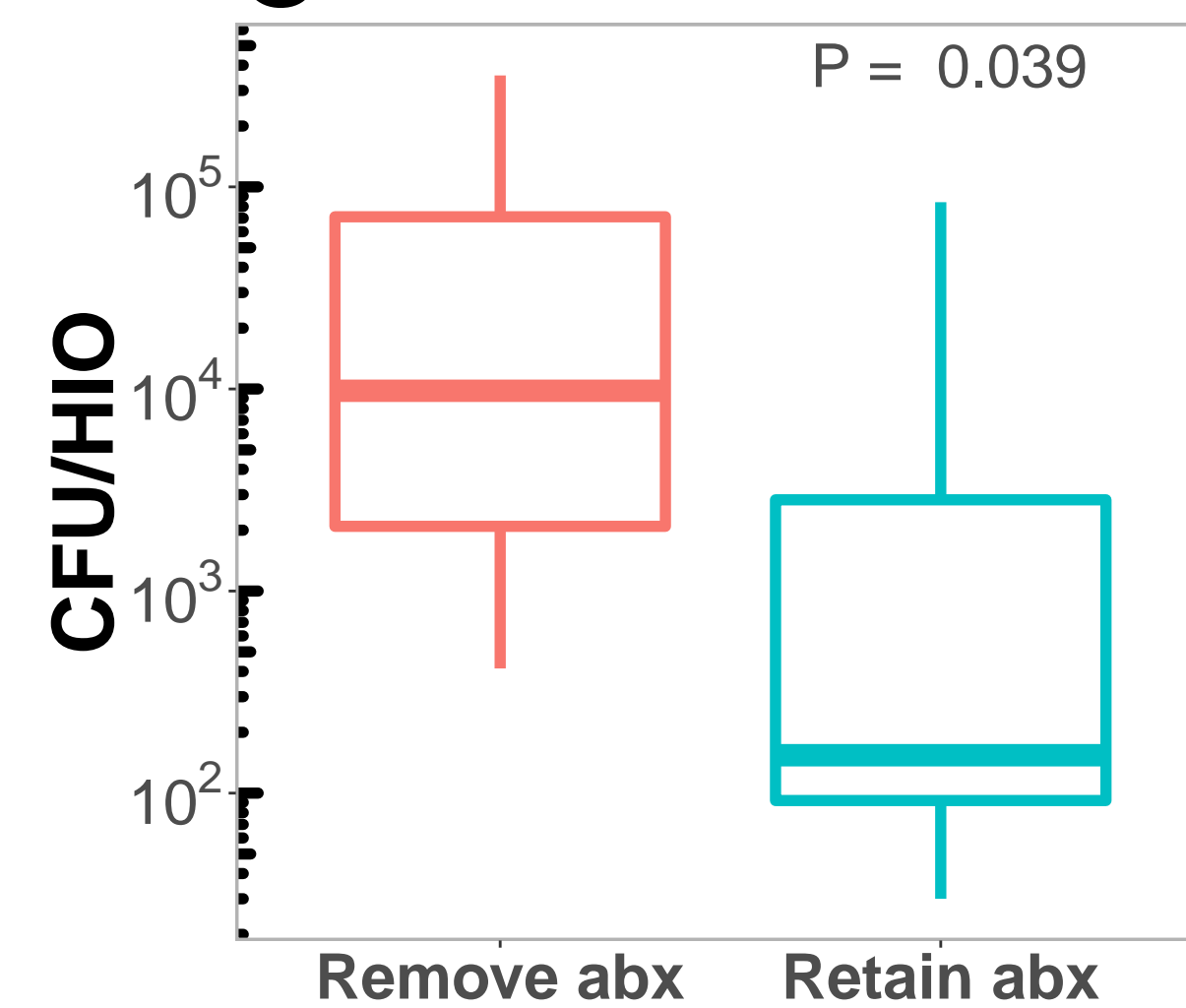
DAY 0: Microinject approximately 1 μ L of the overnight *E. coli* culture (diluted in PBS) into the HIO lumen

After microinjection:
Remove ENR media and wash 1X with sterile PBS.

0-1 h post-microinjection:
Add ENR media containing Pen/Strep mix and incubate at 37 °C for 1 hour

1 h post-microinjection:
Remove ENR containing antibiotics and wash 1X with sterile PBS.

Replace fresh ENR without antibiotics and culture at 37 °C and 5% CO₂

B**C****D**