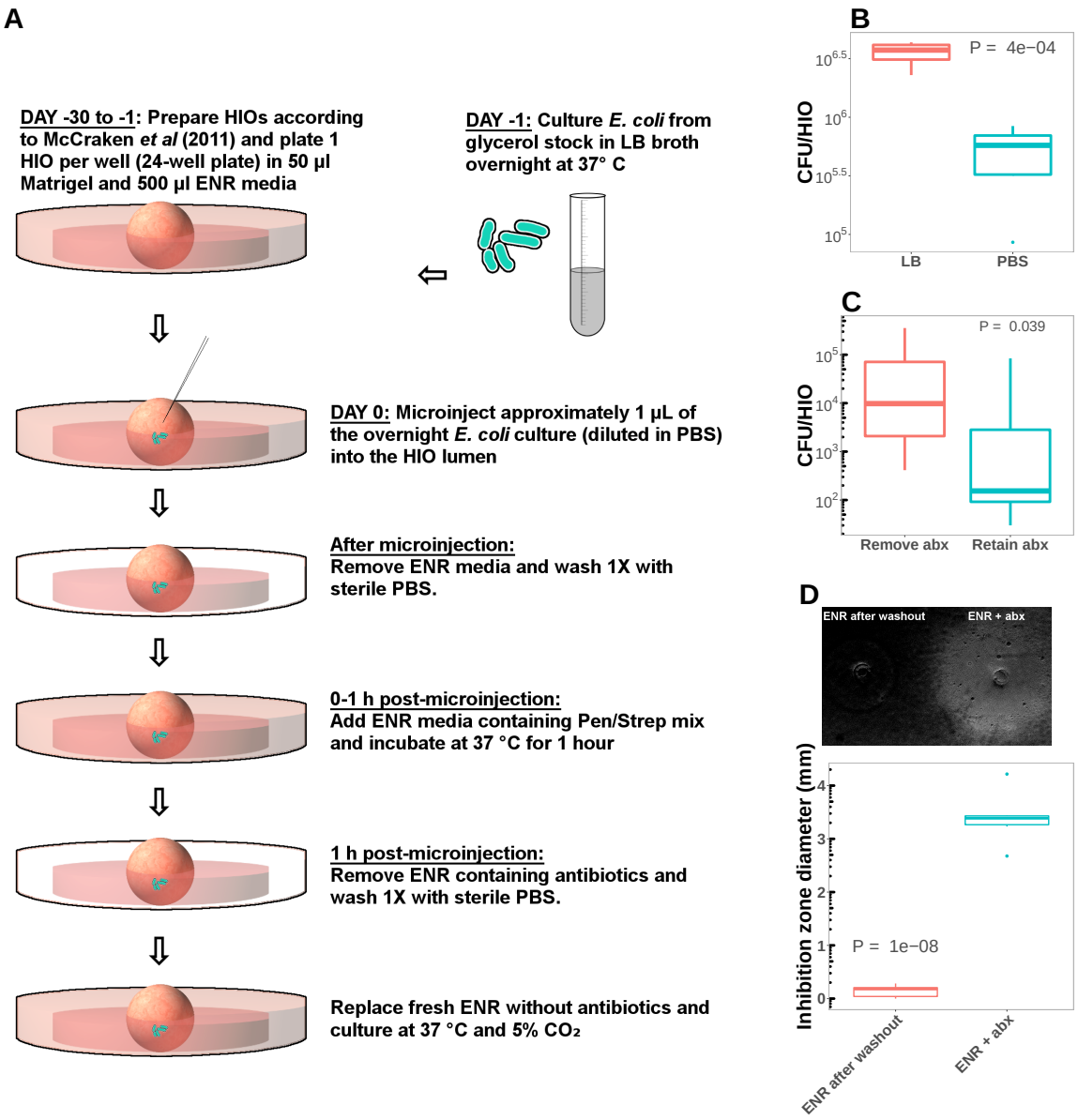

Figure 1 - Supplement 3. **A** Schematic representation of the microinjection of HIOs with live *E. coli*. See Materials and Methods for additional details. **B** A comparison of CFU/HIO at 24 h post-microinjection of HIOs microinjected with 10×10^3 CFU live *E. coli* diluted in sterile PBS or fresh LB broth. *N* = 5 HIOs per condition. All experiments presented in the main paper represent *E. coli* diluted in PBS. *C* To test for the effect of antibiotic carryover of *E. coli* growth in the HIO lumen, we compared CFU/HIO in HIOs cultured in antibiotic free media ("Remove abx") or media containing penicillin and streptomycin at 24 h post-microinjection with 10^3 CFU live *E. coli*. All experiments presented in the main paper represent HIOs cultured in antibiotic-free media due to the apparent effect of antibiotics in suppressing *E. coli* growth within the HIO lumen. *N* = 5 HIOs per condition. *D* In several experiments, bacterial translocation was measured by samplign the external HIO culture media (Figures 1 and 8). To evaluate the potential influence of antibiotic carryover in our estimates of bacterial translocation, we measured growth inhibition in *E. coli* cultures plated as a lawn of LB agar and treated with 1 μl samples of HIO media collected during the 1 h antibiotic wash step or after HIO culture washout with PBS and replacement with fresh antibiotic free media (see panel A). *N* = 6 replicates per culture condition. All *P*-values represent the results of unpaired one-tailed Student's *t*-test comparisons.