

Inside the Experiment

How researchers induced gut dysbiosis, injected E0771.Br cells, and mapped the immune system

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With their precision brain metastasis tool — the mCherry-labeled E0771.Br cells derived from the CH3 Biosystems [E0771](#) — ready, the researchers designed a carefully staged experiment to map the brain's immune response during tumor growth.

Phase 1: Creating the Gut Dysbiosis Model

Adult female C57BL/6 mice were divided into two groups. One received a broad-spectrum antibiotic cocktail (metronidazole, vancomycin, ampicillin, and neomycin) for 14 days, inducing severe gut dysbiosis confirmed by 16S rRNA sequencing of stool samples. The control group received vehicle only.

Phase 2: Injecting E0771.Br Cells

On Day 0, exactly 300,000 mCherry-labeled [E0771](#).Br cells were injected into the left internal carotid artery of each mouse. This precise injection method — intra-carotid injection — combined with a standardized cell number from the characterized CH3 Biosystems [E0771](#) stock — ensured that any differences in tumor growth could be attributed to the gut microbiome manipulation, not to variation in tumor seeding.

Phase 3: Mapping the Immune Response with CITE-seq

At four time points — Day 0, 4, 8, and 14 — brains were extracted and immune cells were profiled using CITE-seq: simultaneous single-cell RNA sequencing and surface protein measurement. Three biological replicates per condition per time point produced tens of thousands of individually profiled immune cells — the most detailed time-lapse of brain immunity during breast cancer metastasis ever created.

Coming up in Part 4: The four major discoveries the data revealed about gut health, immunity, and tumor growth.

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