**Online Supplement Figure Captions**

**Figure O1: Dye Diffusion Plate layout**

(A) The raw optical density readings from the diffusion experiment are shown. Red – phenol red, Purple – bromocresol purple. (B) The layout of the experiment on the BioMe plate is shown.

**Figure O2: Dye Calibration Plate layout**

(A) The raw optical density readings from the dye calibration are shown. Red – phenol red, Purple – bromocresol purple. (B) The layout of the experiment on the BioMe plate is shown.

**Figure O3: Metabolite diffusion curves fit to exponential function**

The experimental data from the chemical dye diffusion experiment was used to plot the value on the left hand side of the equation (Supplemental Text S1 A) (red – phenol red, purple – bromocresol purple). An exponential function was then fit to this data using the form of the right hand side of the equation (Supplemental Text S1 A) (dotted black line).

**Figure O4: Drosophila gut microbe interactions - plate layout**

(A) The raw experimental optical density measurements for all cultures are shown for co-culture experiments of A. oryzifermentans (purple), L. plantarum (yellow), and L. brevis (orange). (B) The layout of the experiment on the BioMe plate is shown. Two outliers for the A. oryzifermentans v.s. self-co-cultures were left out of the analysis done in figure 3, these are the curves from the interacting wells E7 and E8.

**Figure O5: E. coli ΔIle auxotroph amino acid diffusion experiments - plate layout**

(A) The raw optical density readings for the E. coli ΔIle auxotroph isoleucine diffusion experiment are shown. Green – ΔIle, Red – negative controls, Gray – positive controls. (B) The layout of the experiment on the BioMe plate is shown.

**Figure O6: E. coli ΔLys auxotroph amino acid diffusion experiments - plate layout**

(A) The raw optical density readings for the E. coli ΔLys auxotroph lysine diffusion experiment are shown. The layout of the experiment on the BioMe plate is the same as that shown for the ΔIle experiment, with ΔLys replacing ΔIle and lysine replacing isoleucine. Blue – ΔLys, Red – negative controls, Gray – positive controls.

**Figure O7: Initial growth curves for ΔLys and ΔIle in LB medium**

Initial cultures for experiments were diluted from the sampling point shown by the dotted black line. Four replicates for both ΔLys and ΔIle. The strains were grown in a 12 well microplate with 5mL of LB+Chloramphenicol broth at 30oC in a static incubator for 72hrs.

**Figure O8: E. coli auxotroph co-culture experiments - plate layout**

(A) The raw optical density measurements for the E. coli auxotroph co-culture experiment are shown. Dotted lines indicate cross-over of auxotrophs as indicated in Supplemental Figure S7. Blue – ΔLys, Green – ΔIle, Red – negative controls, Gray – positive controls. (B) The layout of the experiment on the BioMe plate is shown.

**Figure O9: Simulation of E. coli amino acid auxotroph experiments**

Different experiments can be simulated by changing the initial conditions and parameters of the model. (A) The legend describes the variables in the dynamic model. These variables appear in the schematic above each experiment, describing the layout of the experiment performed. (B) Chemical diffusion: glucose is added to one side of the device, and diffuses across the membrane until the concentration is equal on both sides. (C&D) Amino acid diffusion: Auxotrophic E. coli strains are placed on one side of the membrane with their auxotrophy amino acid on the opposite side (C – Lysine, D – Isoleucine). Growth is observed as the amino acid diffuses across the membrane and is taken up by E. coli. (E, F) Auxotroph co-culture: Auxotrophic E. coli strains are placed on the opposite (E) or same (F) sides of the device with no initial amino acid. Growth is observed to be much faster in the same well case (F) than the opposite well case (E).

**Figure O10: Sampling of diffusion and leakage parameters with noise**

All previously fixed parameters (excluding volume) were randomly sampled from a log uniform distribution that varied by 1 order of magnitude around the originally fixed literature estimated value. (A) Sampled space of diffusion and leakage parameters. (B) Applying an approximate Bayesian computation approach the posterior distribution of the leakage stoichiometry was determined (black – prior distribution, gold – posterior distribution).

**Figure O11: Sampling of unequal leakage parameters with noise**

All previously fixed parameters (excluding volume) were randomly sampled from a log uniform distribution that varied by 1 order of magnitude around the originally fixed literature estimated value. The sampled space of unequal leakage parameters is shown. Applying an approximate Bayesian computation approach the posterior distribution of the leakage stoichiometries were determined (black – prior distribution, gold – posterior distribution).