**Supplemental Methods, posted to GitHub as a text file**

***Media and Growth conditions***

*Bacteroides fragilis* strains P207, NCTC9343 and 638R were all grown in supplemented brain heart infusion (BHIS) media composed of Bacto BHI (BD, Franklin Lakes, NJ), 1% w/v yeast extract (Fisher Scientific, Waltham, MA) and 0.1% w/v L-cysteine (Sigma-Aldrich, St. Louis, MS). We also added filter sterilized 0.5% w/v hemin (Sigma-Aldrich, prepared in 0.1 N NaOH) and 0.1% vitamin K1 (Sigma, prepared in 100% ethanol) immediately before culture inoculation, due to decreased bacterial viability if added when media was initially autoclaved (data not shown) (6). M9S media is composed of the following components: M9 salts solution (Na2HPO4x7H2O, KH2PO4, NaCl, NH4Cl; prepared together at 5x stock concentration), MgSO4 (prepared at a 1 M stock), CaCl2 (prepared in a 1 M stock), D-glucose (20% w/v stock), FeSO4x7H2O (2 mg/mL stock), L-cysteine (16 mg/mL stock), hemin (5 mg/mL stock in 0.1 N NaOH), vitamin K1 (1 mg/mL stock in EtOH), and vitamin B12 (5 ug/mL stock in EtOH) with 1% w/v casamino acids (Fisher) supplemented as indicated. *Escherichia coli* strains EC100D and WM3064 (gift of W. Metcalf) as well as others were grown in Miller LB (BD) supplemented with antibiotics for selection as necessary: carbenicillin (100 μg/mL) and kanamycin (50 μg/mL) as appropriate. Diaminopimelic acid (DAP) was used, where appropriate at 5 µM concentrations. All growth was conducted at 37oC and all *B. fragilis* strains were grown in a Coy Anaerobic chamber in an atmosphere composed of 2-4% H2 and 5% CO2, with a balance of N2. Growth curves were conducted in a 96-well plate format on a Tecan Infinite M Nano plate reader (Tecan, Switzerland), with analysis performed in R (v. 4.3.3) with packages: ggplot2, tidyr, data.table and readxl among others (21-26).

***Construction of vectors***

pMKS01 was generated by cloning in a modified MCS from pCOLADuet1 (ordered as a geneblock from IDT, Coralville, IA) into pLGB13 (Garcia-Bayona et al.) by restriction enzyme cloning. pMKS66-67 have a constitutively active promoter from *Bacteroides thetaiotaomicron* (RpoD sigma factor, *bt\_1311/*BT\_RS06635) (27). pMKS66-71 were generated by cloning in the inducible selection promoter systems from pLGB13 and pLGB30 to replace the constitutive promoter in pErmInt and pErmRep (6). The 3xFLAG tag was obtained from pMM018. mNeonGreen and mScarlet-I were cloned from pPTM056 and pMM018, respectively. pGEX iLOV was a gift from John Christie (Addgene plasmid #26587; <http://n2t.net/addgene:26587>) (28). For plasmid conjugation into *B. fragilis*, see work done by Garcia-Bayona et al with the following modifications (6). To conjugate into *B. fragilis* P207,we transformed vectors into chemically competent *E. coli* WM3064, a DAP auxotroph. As described previously, we back-diluted *B. fragilis* P207 to very low OD600 (~0.005) and let grow to a barely optically perceptible OD600 before combining with log phase *E. coli* WM3064 (OD600 0.3-0.8). We then spotted mixtures onto BHIS plates with 5 µM DAP (6). Mating spots were streaked for isolation on BHIS with 5 ug/mL erythromycin before counter-selecting using the *Bacteroides-*specific toxin ssbfe1 induced with 100 ng/mL aTC. Sequences were validated by Sanger sequencing or long read sequencing of whole plasmid (Plasmidsaurus).

***Fluorescence microscopy***

Anaerobic fluorescence recovery was performed after establishing log phase *B. fragilis* cultures in anaerobic conditions and then removing *B. fragilis* from the chamber and incubating aerobically while shaking at 37oC for 1 hour (16). Fluorescence measurements were taken on a Tecan Spark 20M plate reader at 506 nm (excitation) and 517 nm (emission) for mNeongreen, 569 nm (excitation) and 593 nm (emission) for mScarlet-I, and 476 nm (excitation) and 510-550 nm (emission) for iLOV (28-30). Cells were visualized in phase contrast on a Leica inverted DMI6000 B microscope with a Hamamatsu ORCA-R2 10600 camera with a 63x Plan Apo objective.

***Minimal media construction and auxotrophy prediction***

To identify putative *B. fragilis* P207 auxotrophies, we used KBase’s jupyter notebook interface (18). We uploaded the *B. fragilis* P207 genome, reannotated it using the “Annotate Microbial Genome” function from RASTtk v 1.73 to apply annotations that are consistent with the metabolic modelling pipeline (17, 31). We then built a metabolic model using fba\_tools (v2.0.0) and then used “Model Characterization” from fba\_tools to identify auxotrophies (19).