

## **Expanded View Figures**

## Figure EV1. SYNB8802 in vitro evaluation.

- A SYNB8802 vs. Oxalobacter formigenes. Left y-axis: <sup>13</sup>C<sub>2</sub>-oxalate in mM, <sup>13</sup>C-formate in mM, x-axis: time in hours. Oxalobacter formigenes (black, triangle, solid line: oxalate, dotted line: formate). SYNB8802 (light blue, square, solid line: oxalate, dotted line: formate). The control EcN (pink, circle, solid line: oxalate, dotted line: formate). Biological triplicates were run and plotted separately.
- B Scaae3 is needed for oxalate degradation in EcN. Y-axis shows oxalate in mM. X-axis: time in hours. Wild type EcN (pink, circle). EcN expressing the OF genes *oxdC*, *oxlT*, and *frc* on p15a (orange, square). EcN expressing the OF genes including *scaaE3* on p15a (light blue, triangle). Biological duplicates were run and plotted separately.
- C SYNB8802 vs. SYN1388. Y-axis shows oxalate in mM. X-axis: time in minutes. Wild type EcN (pink, circle). Prototype strain SYN1388 (blue, square). SYNB8802 (light blue, triangle). Biological duplicates were run and plotted separately.
- D Overexpression of individual pathway components. *Y*-axis shows oxalate in mM. *X*-axis: time in hours. SYNB8802 (blue, circle). SYNB8802, with added plasmid-based expression of *oxlT* (blue, triangle). SYNB8802, with added plasmid-based expression of *scaaE3* (pink, triangle). SYNB8802, with added plasmid-based expression of *frc* (black, diamond). Biological duplicates were run and plotted separately.



## Figure EV2. EcN and SYNB8802 intestinal distribution and fecal clearance in mice.

A ECN and SYNB8802<sup>AbxR</sup> in vivo kinetics. Abundance of ECN or SYNB8802<sup>AbxR</sup> in gut effluents of healthy mice following single oral dose of bacteria. Data represented as

 a ECN and STNB8802<sup>AbxR</sup>
mean ± SEM, n = 4/group/timepoint. Full lines represent ECN and hatched lines represent SYNB8802<sup>AbxR</sup>.
B EcN and SYNB8802<sup>AbxR</sup> fecal clearance. Abundance of EcN or SYNB8802<sup>AbxR</sup> in fecal pellets of healthy mice following single oral dose of bacteria. Data represented as mean  $\pm$  SEM, n = 5/group. Dark blue curve represents EcN and light blue curve represent SYNB8802<sup>AbxR</sup>.



Figure EV3. SYNB8802 pH inhibition in vitro simulation.

SYNB8802 activity as a function of exposure time to medium at pH ranging from 2.0 to 7.0. Points and error bars in black represent *in vitro* measurements (n = 3 replicate cultures per group; mean  $\pm$  SD). Blue curves represent exponential decay models fit to in vitro measurements for each pH level.



## Figure EV4. Agarose gel of PCR verification for SYNB8802.

Lane 1 shows insertion of *agal/rsml*:: P<sub>Fnrs</sub> -scaaE3oxdC-frc. Lane 2 shows insertion of *exo/cea*::P<sub>Fnrs</sub>-oxlT. Lane 3 shows knock out *thyA*.