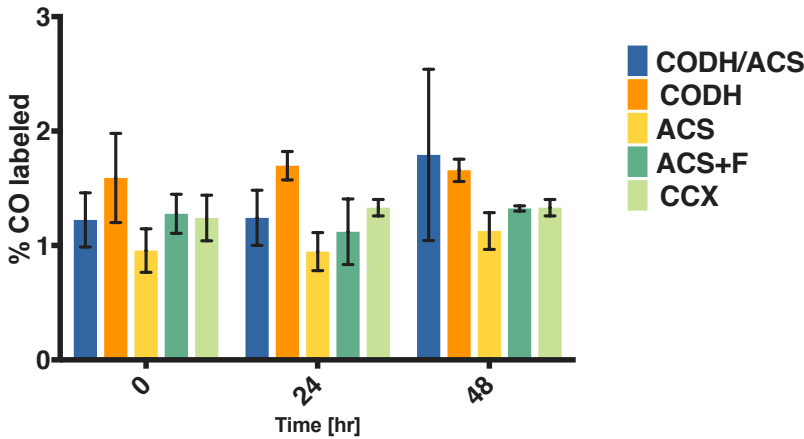


1 SUPPLEMENTAL FIGURES



2

3 **Fig. S1. Probing the ACS catalytic activity via the acetyl-CoA-exchange reaction using ¹³C-**

4 **acetate.** A) Strains harboring the CODH/ACS, CODH, ACS, ACS and the nickel-accessory

5 protein AcsF (blue, orange, yellow, green, respectively) (displayed left to right) were grown in

6 defined medium with 40 g/L glucose and containing 30 mM sodium 1-¹³C-acetate. *C.*

7 *carboxidivorans* (CCX) was used as a native acetogen control (light green). CO concentration

8 was measured in the headspace for all strains of *C. acetobutylicum* after 24 and 48 hours of

9 growth. Even though the CODH/ACS strain exhibited an increase in ¹³C labeling in CO, it is

10 similar to the labeling pattern of strain expressing CODH alone. The ¹³C labeling in CO derives

11 from labeled CO₂. ¹³C isotope labeling in the presence ¹³C-acetate (carbonyl carbon) results in

12 acetyl-CoA labeling at the carbonyl position. The carbonyl carbon of acetyl-CoA can exchange

13 with CO using ACS. The acetyl-CoA can also be converted to acetone (releasing ¹³CO₂), and the

14 CO₂ can be converted to CO using the CODH.

15