

Fig. S3. Activities of succinate—CoA ligase (ADP-forming) and acetate—CoA ligase (ADP-forming) in *Selenomonas ruminantium* HD4. Values are mean \pm SEM. Activity of the former enzyme differed from 0 ($P = 0.014$) ($n = 3$), whereas the latter did not ($P = 0.644$) ($n = 6$). Previous assays (Michel and Macy, 1990) found activity of an acetate—CoA ligase (ADP-forming), but these assays were biased by generating succinyl-CoA as a byproduct, which in turn would be a substrate for succinate—CoA ligase (ADP-forming). Our assay does not generate succinyl-CoA and does not have this bias. Assay mixtures contained 50 mM KPO_4 (pH = 7.2), 2.5 mM MgCl_2 , 1.25 mM EDTA (pH = 8), 0.1 mM acetyl- or succinyl-CoA, 1 mM ADP, 1 mM DTNB, 0.45 U/mL citrate synthase, and 50 $\mu\text{L}/\text{mL}$ cell extract. The reaction was initiated by adding cell extract and monitored by following release of CoA, which forms of TNB^{2-} (extinction coefficient = 14,150 $\text{M}^{-1} \text{cm}^{-1}$ at 412 nm). Activities are corrected for controls without 1) cell extract or 2) acetyl- or succinyl-CoA. No activity was found in absence of ADP.

Figure S3

