SUPPLEMENTAL MATERIAL

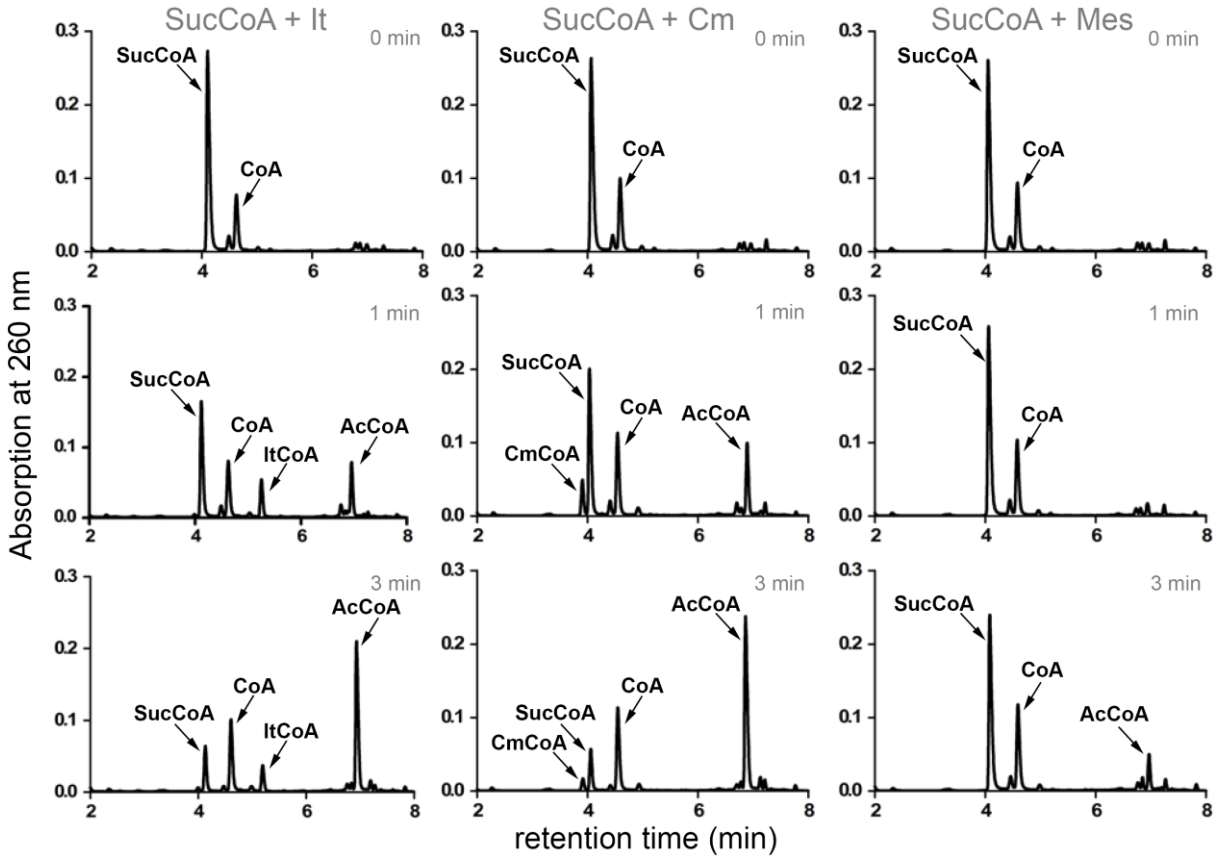

Fig. S1. Analysis of the products of succinyl-CoA dependent itaconate, citramalate and mesaconate conversions in cell extracts of itaconate-grown *B. xenovorans*. The samples were analyzed by reverse-phase C_{18} UPLC to follow CoA esters (at 260 nm). The reaction mixture contained 1.5 mg protein ml^{-1} in the assay. It, itaconate; Cm, (*S*)-citramalate; Mes, mesaconate; SucCoA, succinyl-CoA; ItCoA, itaconyl-CoA; CmCoA, (*S*)-citramalyl-CoA; AcCoA, acetyl-CoA.

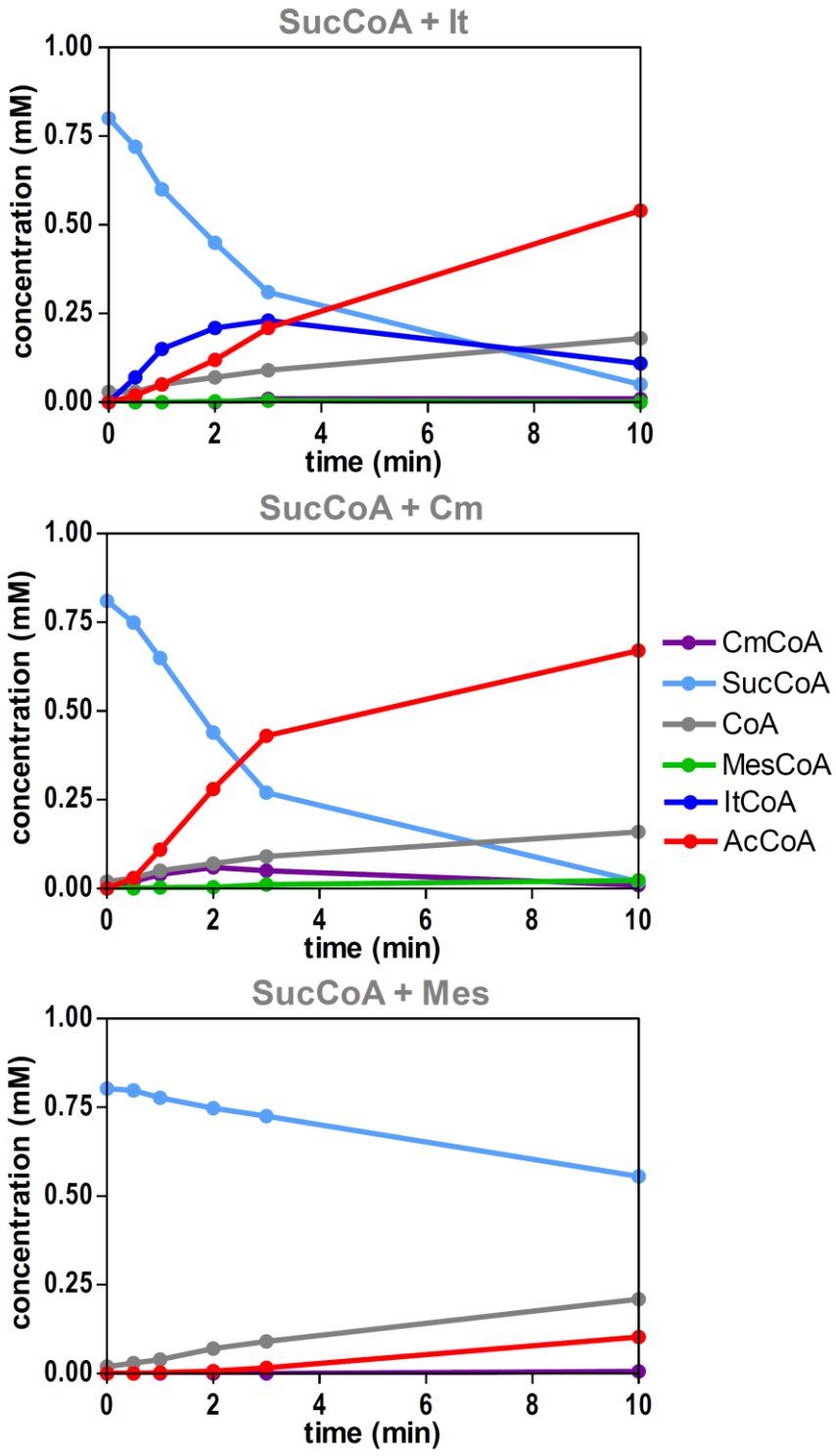

Fig. S2. Time-course of succinyl-CoA conversion and CoA-ester formation in the presence of itaconate, (*S*)-citramalate and mesaconate in cell extracts of **itaconate-grown** *B. xenovorans*. The reaction mixture contained 0.67 mg protein ml^{-1}. SucCoA, succinyl-CoA; It, itaconate; Cm, (*S*)-citramalate; Mes, mesaconate; CmCoA, (*S*)-citramalyl-CoA; MesCoA, mesaconyl-C4-CoA; ItCoA, itaconyl-CoA; AcCoA, acetyl-CoA.

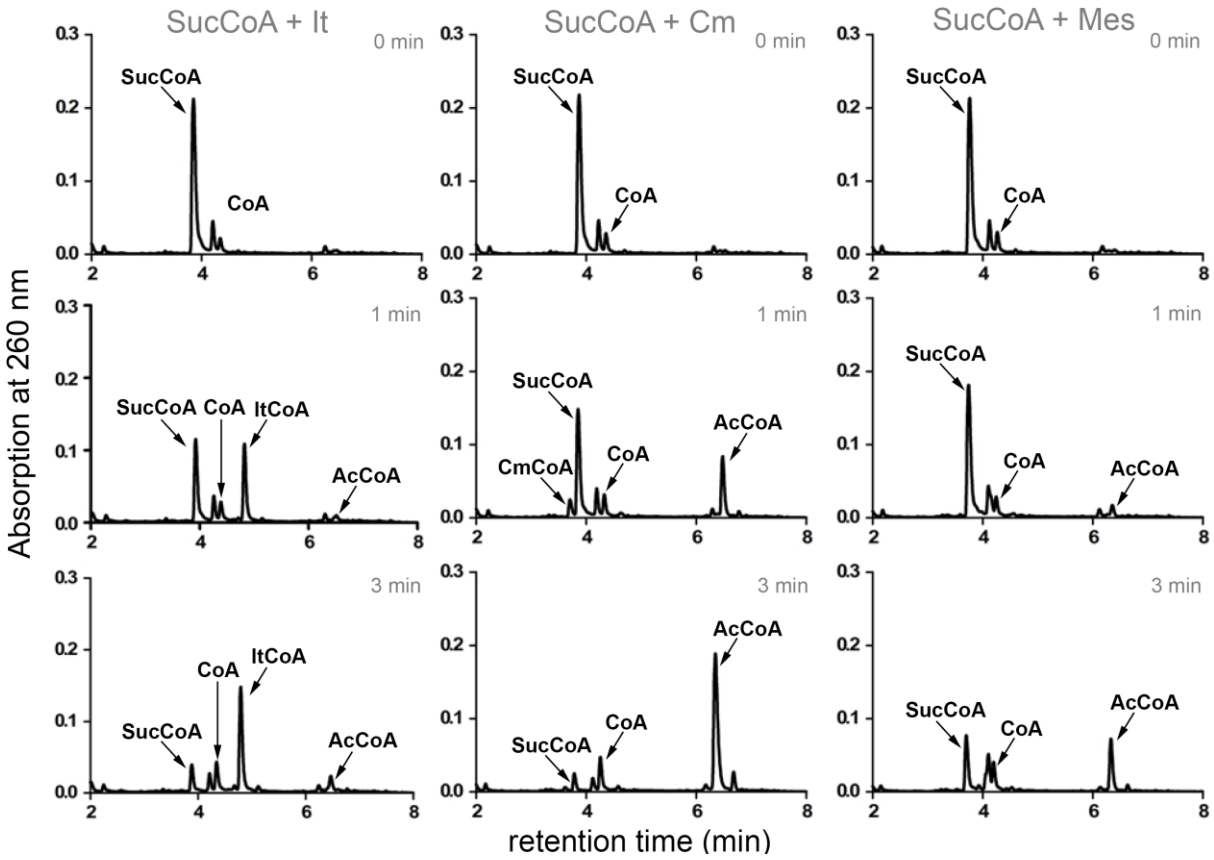

Fig. S3. Analysis of the products of succinyl-CoA dependent itaconate, citramalate and mesaconate conversions in cell extracts of mesaconate-grown *B. xenovorans*. The samples were analyzed by reverse-phase C_{18} UPLC to follow CoA esters (at 260 nm). The reaction mixture contained 1.4 mg protein ml^{-1} in the assay. It, itaconate; Cm, (*S*)-citramalate; Mes, mesaconate; SucCoA, succinyl-CoA; ItCoA, itaconyl-CoA; CmCoA, (*S*)-citramalyl-CoA; AcCoA, acetyl-CoA.

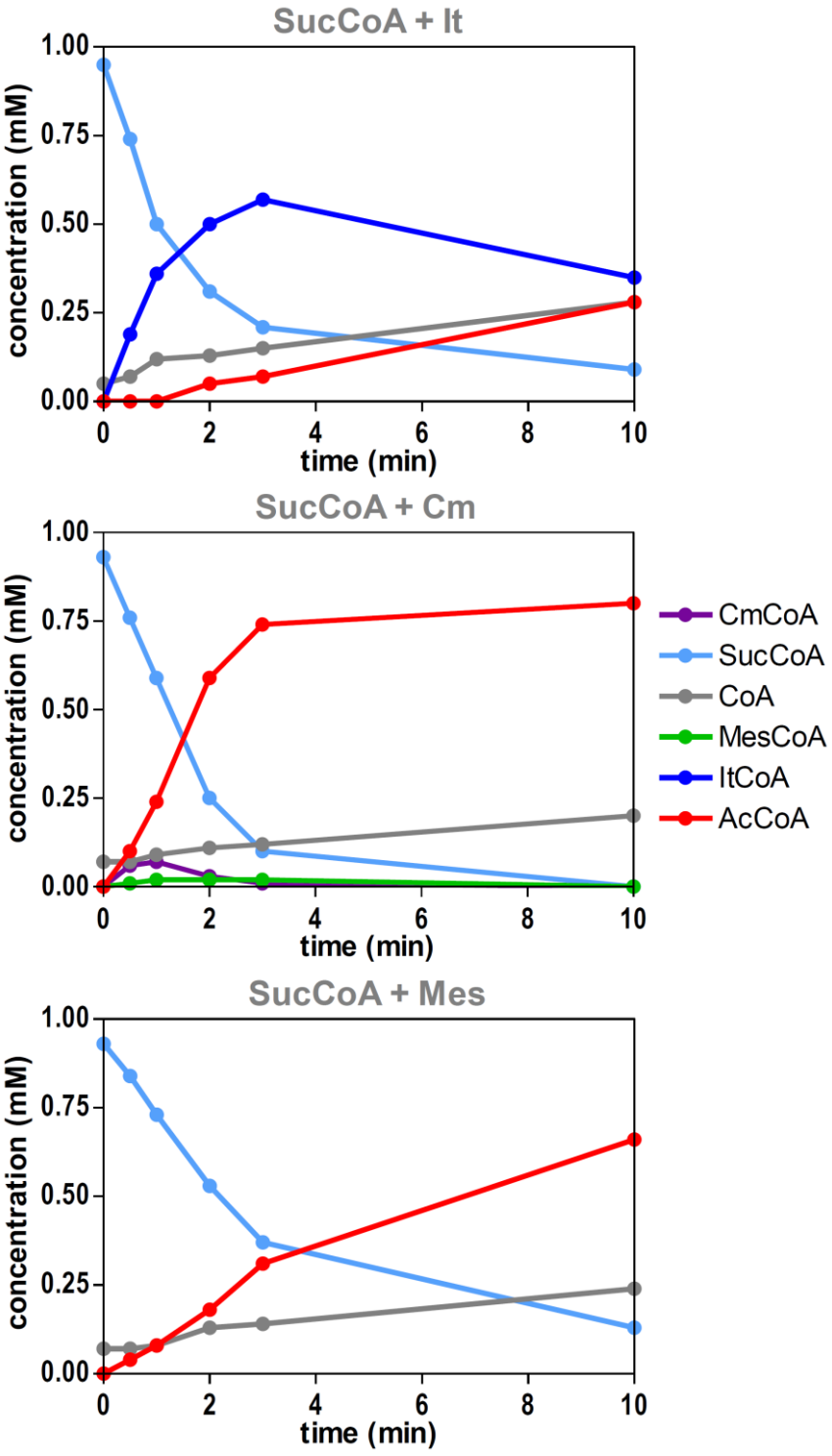

Fig. S4. Time-course of succinyl-CoA conversion and CoA-ester formation in the presence of itaconate, (*S*)-citramalate and mesaconate in cell extracts of **mesaconate-grown** *B. xenovorans*. The reaction mixture contained 1.4 mg protein ml^{-1}. SucCoA, succinyl-CoA; It, itaconate; Cm, (*S*)-citramalate; Mes, mesaconate; CmCoA, (*S*)-citramalyl-CoA; MesCoA, mesaconyl-C4-CoA; ItCoA, itaconyl-CoA; AcCoA, acetyl-CoA.

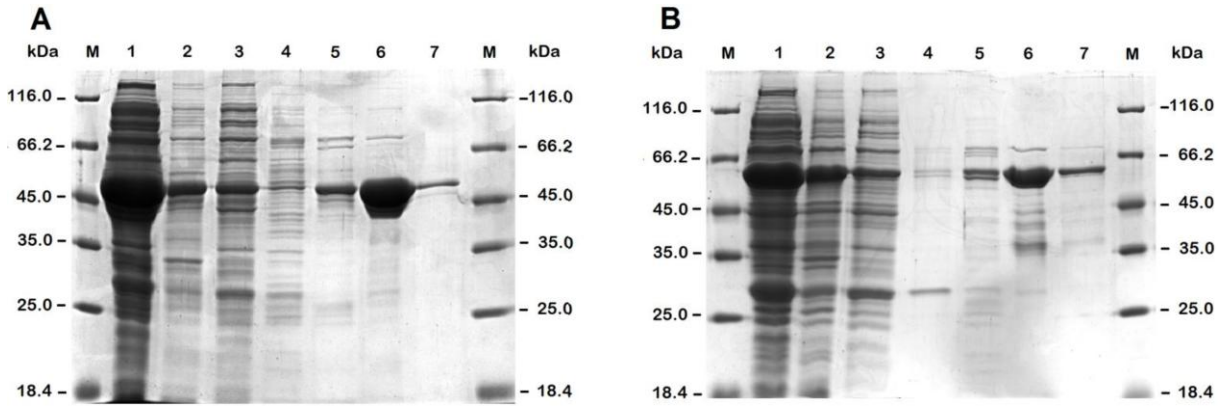

Fig. S5. SDS-PAGE (12.5%) of fractions obtained during purification of recombinant fumarate hydratase Bxe_A1038 (**A**) and Bxe_A3136 (**B**) from *B. xenovorans*. Lane 1, molecular mass standard proteins; lane 2, cell extract of *E. coli* producing the corresponding proteins; lane 3, flow through after Ni-NTA column; lane 4, elution with 25 mM imidazole; lane 5, elution with 50 mM imidazole; lane 6, elution with 300 mM imidazole; lane 7, elution with 500 mM imidazole. The fraction eluted with 300 mM imidazole was used for the following enzyme characterization. Proteins were stained with Coomassie blue. The predicted molecular mass of Bxe_A1038 is 49.7 kDa, that of Bxe_A3136 55.9 kDa.