



**S5 Fig. A.** The crystal structure of RMCAH (C46S/C162A/C218V/G53C/E235C) with the domain-2 locked in a partially open conformation, overlaid with the 2mFo-DFc electron density contoured at 1.0  $\sigma$ . Blue mesh highlights electron density for the disulfide bond. A citrate molecule occupies the active site but electron density for the citrate is omitted for clarity. **B** and **C.** Activities of the re-engineered *Moorella* CAH (RMCAH) and its double mutant RMCAH-CC (G53C/E235C) tested in reducing (10 mM dithiothreitol) and non-reducing (no dithiothreitol) conditions.