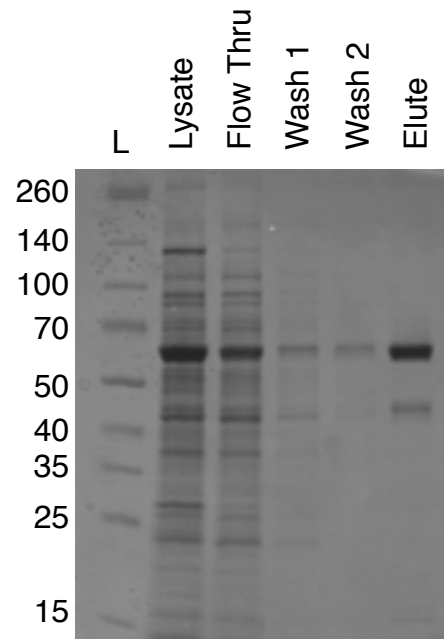

Figure S1: Sample partial purifications of C-terminally His_6-tagged FadD. Lanes contain: (L) Spectra Multicolor Broad Range Protein Ladder (Thermo Scientific) with the molecular weight (kDa) of each band in the ladder next to the gel, (Lystate) lysate fraction from Ni-NTA partial purification (Materials and Methods), (Flow Thru), flow thru fraction from the Ni-NTA partial purification (Materials and Methods), (Wash 1 and 2) wash fractions from the Ni-NTA partial purification (Materials and Methods), and (Elute) pooled elutant fractions from the Ni-NTA partial purification of wild-type FadD (Materials and Methods).

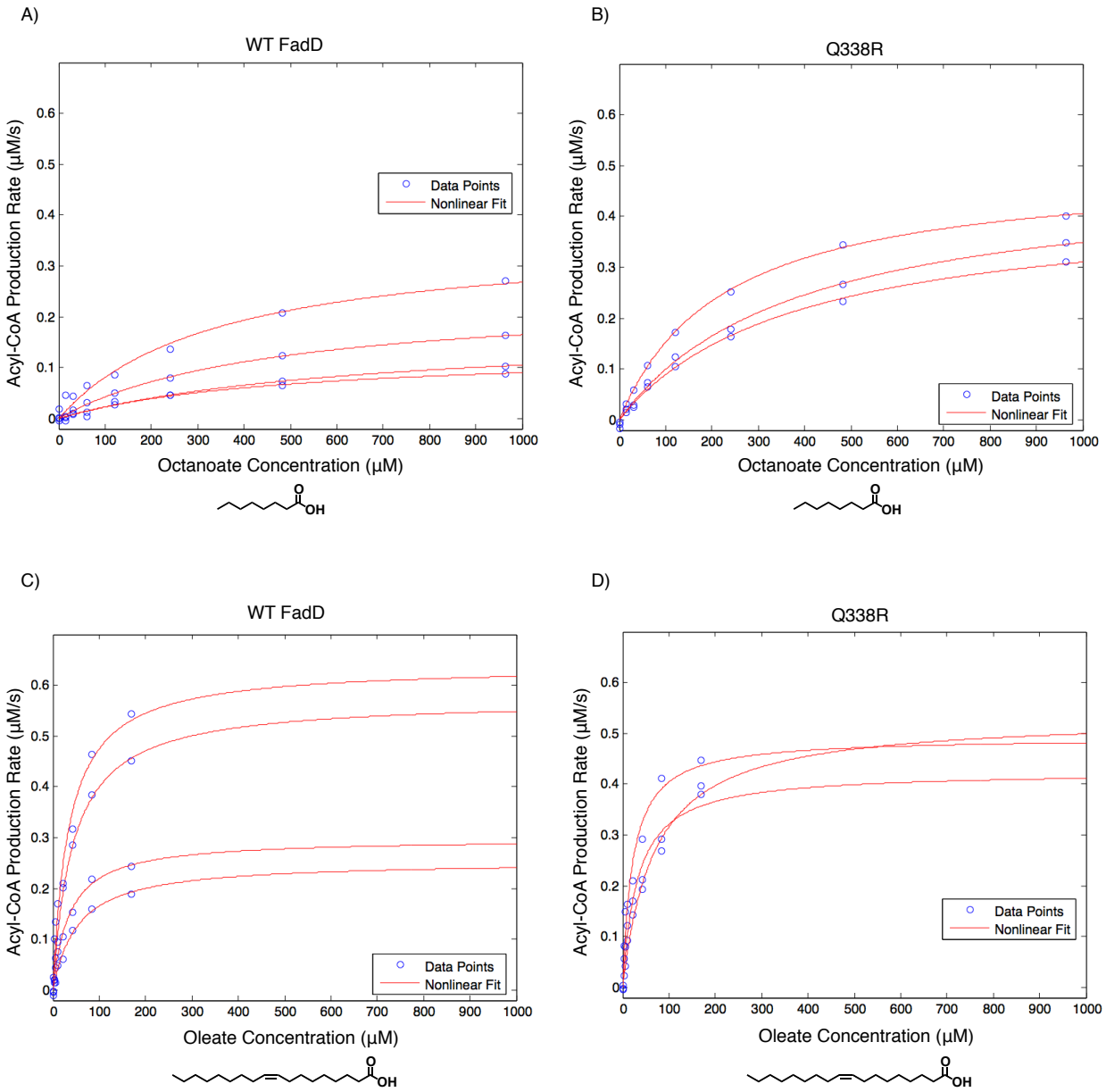

Figure S2: Sample rate vs. substrate concentration curves for the AMP production assay. Blue circles indicate rates determined from independent purifications and red lines are nonlinear fits to the Michaelis Menten equation for wild-type FadD and mutant Q338R using octanoate (A,B) and oleate (C,D) as substrates.

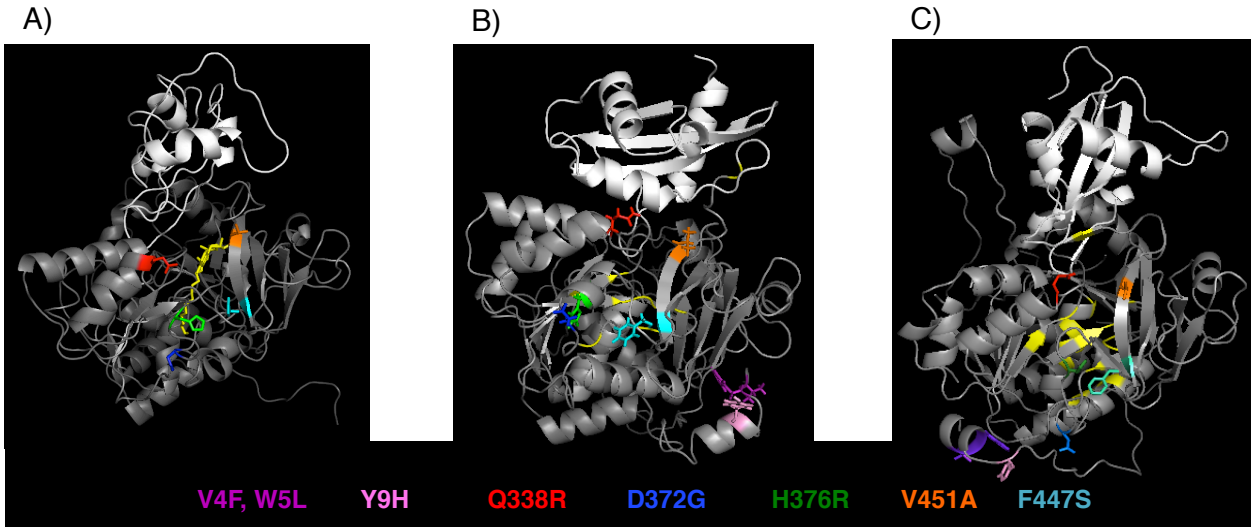

Figure S3. FadD Homology Modeling. Three separate FadD homology models were generated using (i) The SWISS-MODEL Homology modeling server (Arnold et al. 2006; Benkert et al. 2011; Biasini et al. 2014) and the *Thermus thermophilus* structure as the template, (ii) the I-TASSER server (Roy et al. 2010; Yang et al. 2015; Zhang 2008), and (iii) SAM-T08 (Karchin et al. 2004; Karchin et al. 2003; Karplus 2009; Karplus & Hu 2001; Karplus et al. 2001; Karplus et al. 2003; Karplus et al. 2005; Shackelford & Karplus 2007). Models were visualized in PyMOL with large N-terminal domain in gray, smaller C-terminal domain in white, and fatty acid binding pocket or myristoyl-AMP (overlayed from the *Thermus Thermophillus* structure) in yellow (Hisanaga et al. 2004; Shackelford & Karplus 2007). Residues whose mutation results in increased growth rate on octanoate are color-coded according to the identity of the mutation (text below models).

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