

Figure S1. A. ACADM is essential for Glioblastoma tumor growth in vivo. Five-day growth curve of GSC 8.11 cells upon shRNA ACADM silencing (shACADM1 or shACADM2). Day 0 was defined as 48 hours post puromycin selection. Values represent the mean \pm SD of three independent experiments. P values were generated using Kruskal–Wallis ANOVA. Dunn test for comparison among groups. *, $P \leq 0.02$. B. MRI images of tumor progression after implantation of GSCs 8.11 at 4 and 8 weeks. The orange line indicates the outline of the tumors. Puca F. et. al. Cancer Discovery (2021) 11 2904-2923

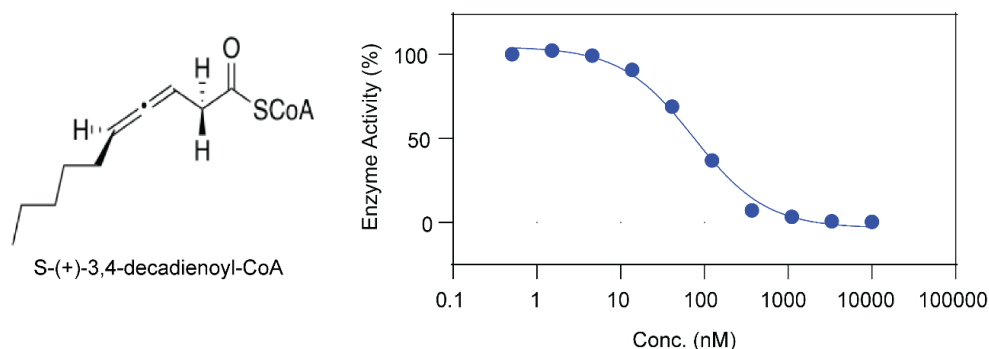


Figure S2. Inhibition of Human ACADM holoenzyme by S-(+)-3,4-decadienoyl-CoA with an IC_{50} of 77 nM. Enzyme activity was measured using a rapidfire mass spectrometry assay that directly detects the amount of enzyme substrate and product.

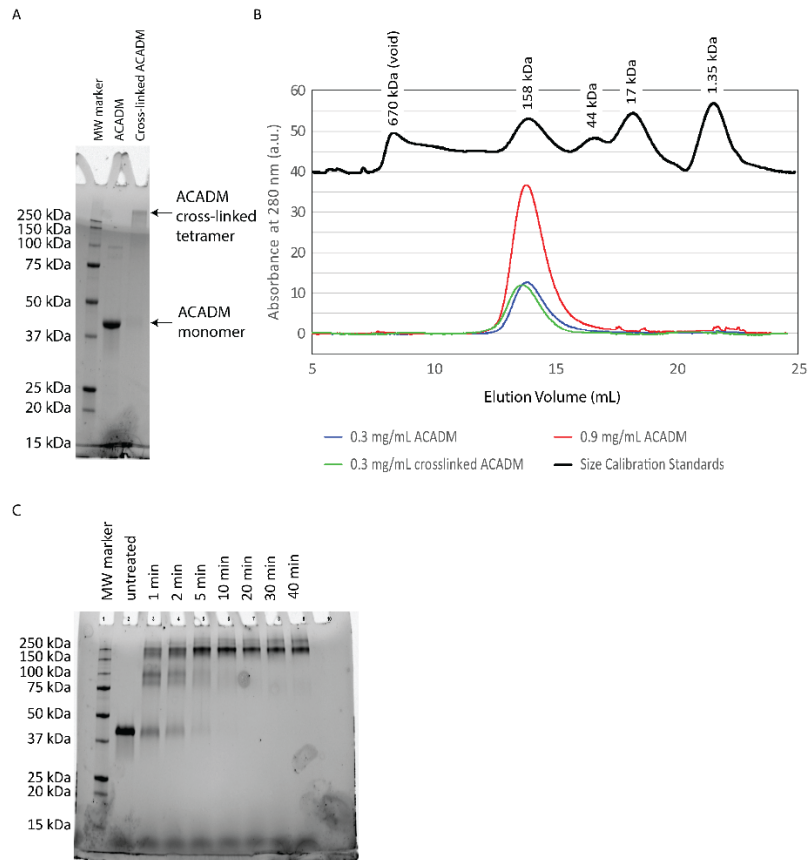


Figure S3. A. SDS PAGE gel analysis for purified Human ACADM holoenzyme (residues 26-421). B. Size exclusion chromatography chromatogram for 100 μ L of ACADM protein loaded at 20 μ M (-) and 7 μ M (-) concentrations. The elution profile of a protein mixture containing Thyroglobulin (670 kDa), γ -globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa) and Vitamin B12 (1.35 kDa) is shown above for comparison. The ACADM protein elutes as a homotetramer ($MW_{calc} = 173$ kDa). C. Time-course experiment for a glutaraldehyde cross-linking reaction using 1 mg/mL ACADM and 0.2% (v/v) glutaraldehyde. 5 min appears optimal for this cross-linking reaction.