

Supplemental Information

SIRT3 Deacetylates Mitochondrial 3-Hydroxy-3-Methylglutaryl CoA Synthase 2 and Regulates Ketone Body Production

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Human	1	MQRLLTPVKR	ILQLTRAVQE	TSLTPARLLP	VAHQRFSTAS	AVPLAKTDTW	PKDVGILALE	60
Mouse	1	MQRLLAPARR	VLQVKRAMQE	TSLTPAHLLS	AAQQRFSSTIP	PAPLAKTDTW	PKDVGILALE	60
Human	61	VYFPAQYVDQ	TDLEKYNVVE	AGKYTVGLGQ	TRMGFCSVQE	DINSLCLTVV	QRLMERIQLP	120
Mouse	61	VYFPAQYVDQ	TDLEKFNNVE	AGKYTVGLGQ	TRMGFCSVQE	DINSLCLTVV	QRLMERTKLP	120
Human	121	WDSVGRLEVG	TETIIDKSKA	VKTVLMELFQ	DSGNTDIEGI	DTTNACYGGT	ASLFNAANWM	180
Mouse	121	WDAVGRLEVG	TETIIDKSKA	VKTVLMELFQ	DSGNTDIEGI	DTTNACYGGT	ASLFNAANWM	180
Human	181	ESSWDGRYA	MVVCGDIAYV	PSGNARPTGG	AGAVAMLIGP	KAPLALERGL	RGTHMENVYD	240
Mouse	181	ESSYWDGRYA	LVVCGDIAYV	PSGNARPTGG	AGAVAMLIGP	KAPLVLEQGL	RGTHMENAYD	240
Human	241	FYKPNLASEY	PIVDGKLSIQ	CYLRALDRCY	TSYRKKIQNQ	WKQAGSDRPF	TLDDLOYMIF	300
Mouse	241	FYKPNLASEY	PLVDGKLSIQ	CYLRALDRCY	AAYRKKIQNQ	WKQAGNNQPF	TLDDVQYMIF	300
Human	301	HTPFCRMVQK*	SLARLMFNDF	LSASSDTQTS	LYKGLEAFGG	LKLEDYTNK	DLDKALLKAS	360
Mouse	301	HTPFCRMVQK*	SLARLMFNDF	LSSSSDKQNN	LYKGLEAFRG	LKLEETYNK	DVDKALLKAS	360
Human	361	QDMFDKKTKA	SLYLSTHNGN	MYTSSLYGCL	ASLLSHHSAQ	ELAGSRIGAF	SYGSGLAASF	420
Mouse	361	LDMFNQKTKA	SLYLSTNNGN	MYTSSLYGCL	ASLLSHHSAQ	ELAGSRIGAF	SYGSGLAASF	420
Human	421	FSFRVSODAA	PGSPLDKLVS	STDLPKRLA*	SRKCVSPEEF	TEIMNQREQF	YHKVNFSPPG	480
Mouse	421	FSFRVSKDAS	PGSPLEKLVV	SVSDLPKRLD	SRRRMSPEEF	TEIMNQREQF	YHKVNFSPPG	480
Human	481	DTNSLFPGTW	YLERVDEQHR	RKYARRPV*				509
Mouse	481	DTSNLFPGTW	YLERVDEMHR	RKYARCPV*				509

■ Acetylation Site
* Acetylation Sites Regulated by SIRT3

Figure S1, related to Figure 2. Human and Murine HMGCS2 Homology

Analysis of the acetylated lysines of murine mitochondrial HMGCS2 and human HMGCS2. The three acetylation sites that are studies here (K310, K447, and K473) are indicated by an asterisk.

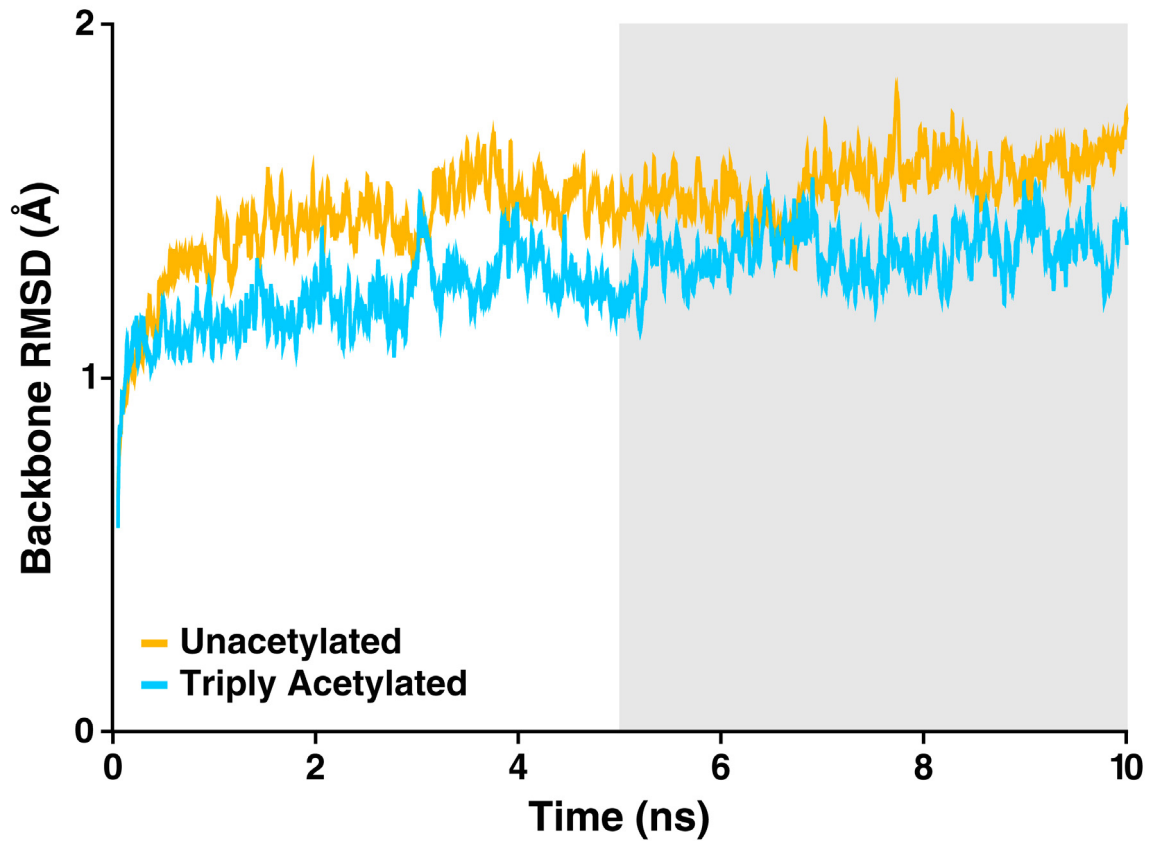


Figure S2, related to Figure 3. Backbone Root-Mean-Squared Deviation (RMSD) Was Stable over the Last 5 ns of a 10-ns Simulation

Statistical analyses of molecular dynamic simulations averaged over the last 5 ns of a 10-ns simulation (gray); data shown for the three acetylated states (unacetylated and triply acetylated) as a function of time (ns).

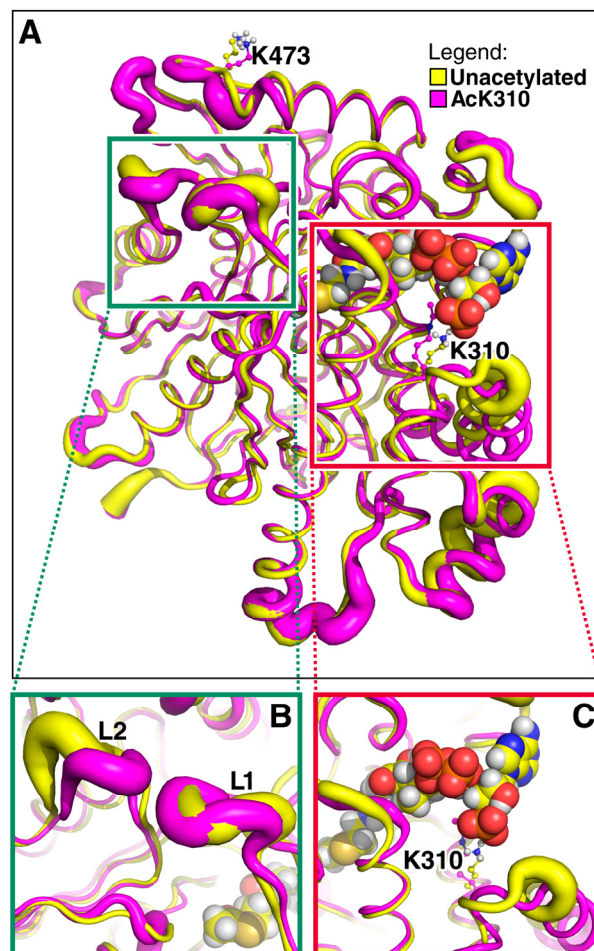


Figure S3, related to Figure 4. Molecular Dynamic Simulation of Unacetylated and Singly Acetylated (K310) HMGCS2

(A) Overview of the entire structure for the unacetylated (yellow) and singly acetylated K310 (magenta) mouse HMGCS2. Acetyl-CoA in the model is shown using spheres, whereas K310, K447, K473 and their acetylated forms are shown as balls and sticks. The tubes represent the average backbone conformation of the protein over the last 5 ns of five independent simulations, and the widths of the tubes represent the fluctuations from the average conformation (i.e., thicker lines indicate increased conformational flexibility).

(B) Closer view of the region around a distal portion of the active site [the green box in (A)]. The two loops showing significant conformational and dynamical changes are labeled L1 (residues 242–251) and L2 (residues 131–140).

(C) Closer view of the region around K310 [the red box in (A)].

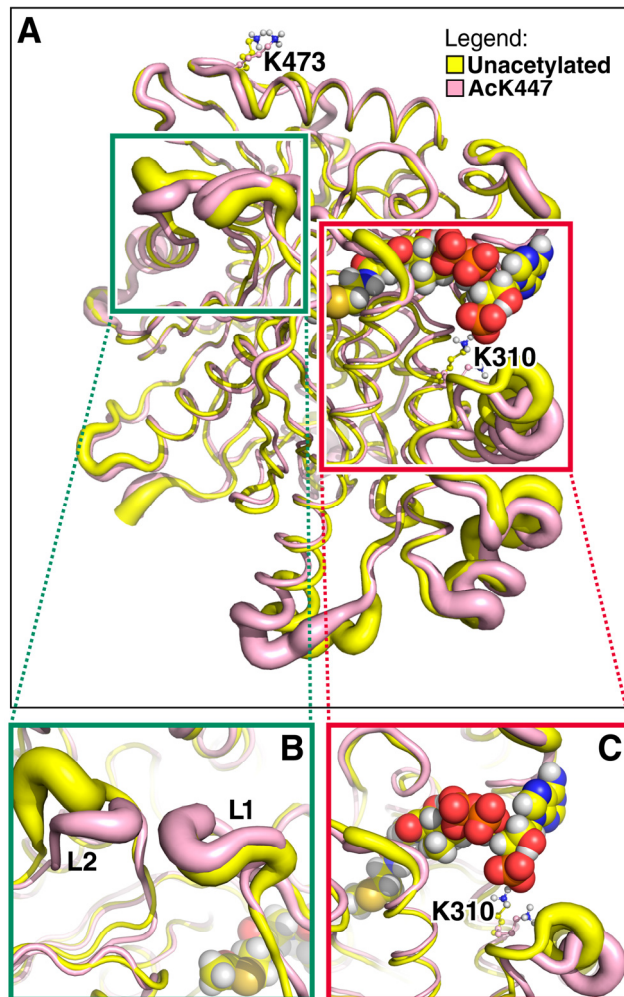


Figure S4, related to Figure 4. Molecular Dynamic Simulation of Unacetylated and Singly Acetylated (K447) HMGCS2

(A) Overview of the entire structure for the unacetylated (yellow) and singly acetylated K447 (pink) mouse HMGCS2. Acetyl-CoA in the model is shown using spheres, whereas K310, K447, K473 and their acetylated forms are shown as balls and sticks. The tubes represent the average backbone conformation of the protein over the last 5 ns of five independent simulations, and the widths of the tubes represent the fluctuations from the average conformation (i.e., thicker lines indicate increased conformational flexibility).

(B) Closer view of the region around a distal portion of the active site [the green box in (A)]. The two loops showing significant conformational and dynamical changes are labeled L1 (residues 242–251) and L2 (residues 131–140).

(C) Closer view of the region around K310 [the red box in (A)].

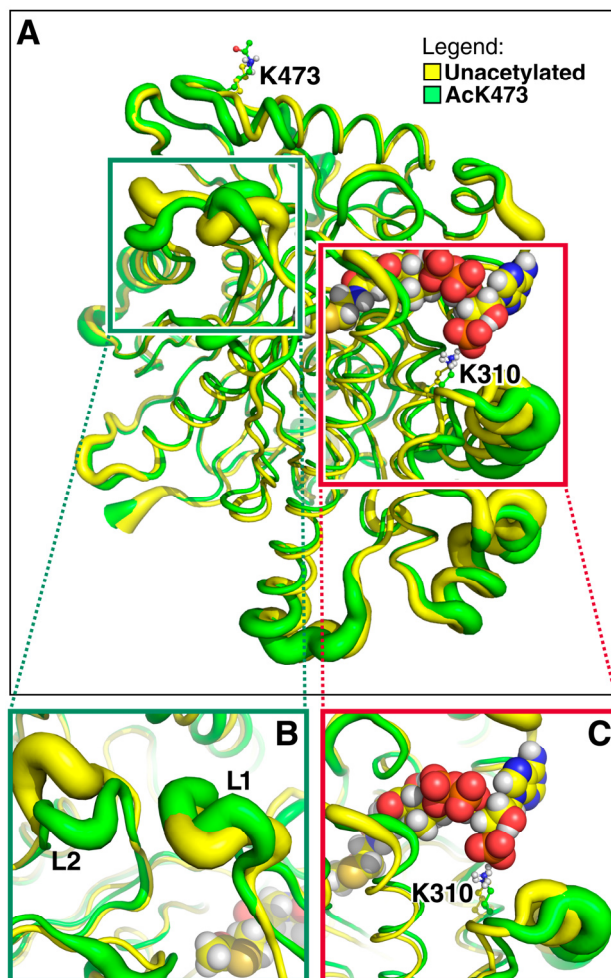


Figure S5, related to Figure 4. Molecular Dynamic Simulation of Unacetylated and Singly Acetylated (K473) HMGCS2

(A) Overview of the entire structure for the unacetylated (yellow) and singly acetylated K473 (lime) mouse HMGCS2. Acetyl-CoA in the model is shown using spheres, whereas K310, K447, K473 and their acetylated forms are shown as balls and sticks. The tubes represent the average backbone conformation of the protein over the last 5 ns of five independent simulations, and the widths of the tubes represent the fluctuations from the average conformation (i.e., thicker lines indicate increased conformational flexibility).

(B) Closer view of the region around a distal portion of the active site [the green box in (A)]. The two loops showing significant conformational and dynamical changes are labeled L1 (residues 242–251) and L2 (residues 131–140).

(C) Closer view of the region around K310 [the red box in (A)].

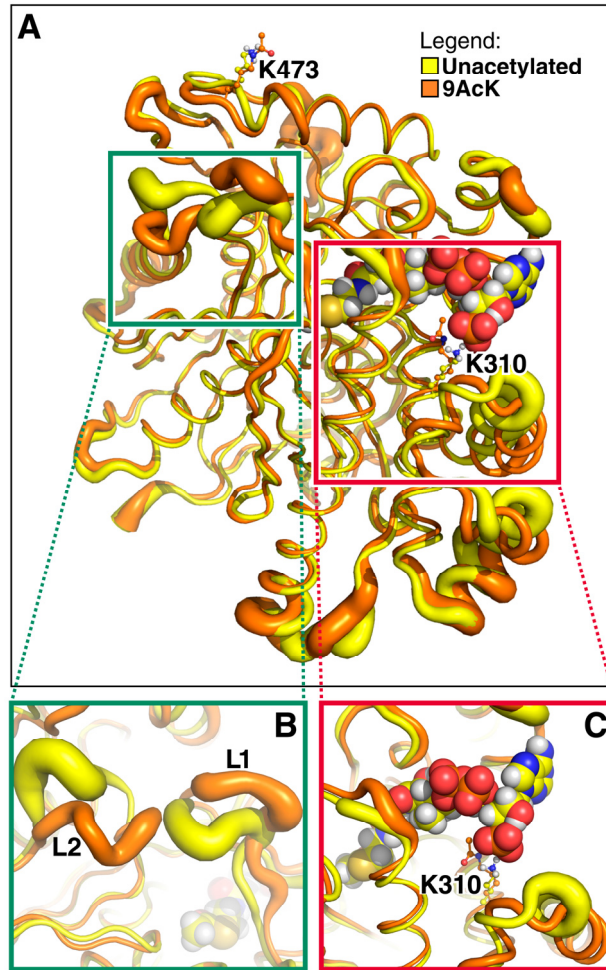


Figure S6, related to Figure 4. Molecular Dynamic Simulation of Unacetylated and Fully Acetylated (Nine Acetylated Lysines) HMGCS2

(A) Overview of the entire structure for the unacetylated (yellow) and fully acetylated (orange) mouse HMGCS2. Acetyl-CoA in the model is shown using spheres, whereas K310, K447, K473 and their acetylated forms are shown as balls and sticks. The tubes represent the average backbone conformation of the protein over the last 5 ns of five independent simulations, and the widths of the tubes represent the fluctuations from the average conformation (i.e., thicker lines indicate increased conformational flexibility).

(B) Closer view of the region around a distal portion of the active site [the green box in (A)]. The two loops showing significant conformational and dynamical changes are labeled L1 (residues 242–251) and L2 (residues 131–140).

(C) Closer view of the region around K310 [the red box in (A)].