Supplementary Information

Supplementary Tables

Table S1. List of primers used for cloning *sirt2* and site-directed mutation

Primer	Sequence (5’-3’)
Sirt2-F50BamHI	CGCGGATCCAGCCTGGGCAGCCAGAAG
Sirt2-R356XhoI	CCGCTCGAGTCACGACTGGGCATCTATGCT
sirt2-FF96A	GCAGGCATCCCCGACGCGCGCTCTCCATCCACC
sirt2-RF96A	GTCGGGGATGCCTGC
sirt2-FH187A	GACTTGGTGGAGGCGGCGGGCACCTTCTACACATC
sirt2-RH187A	CGCCTCCACCAAGTC

Supplementary Figures

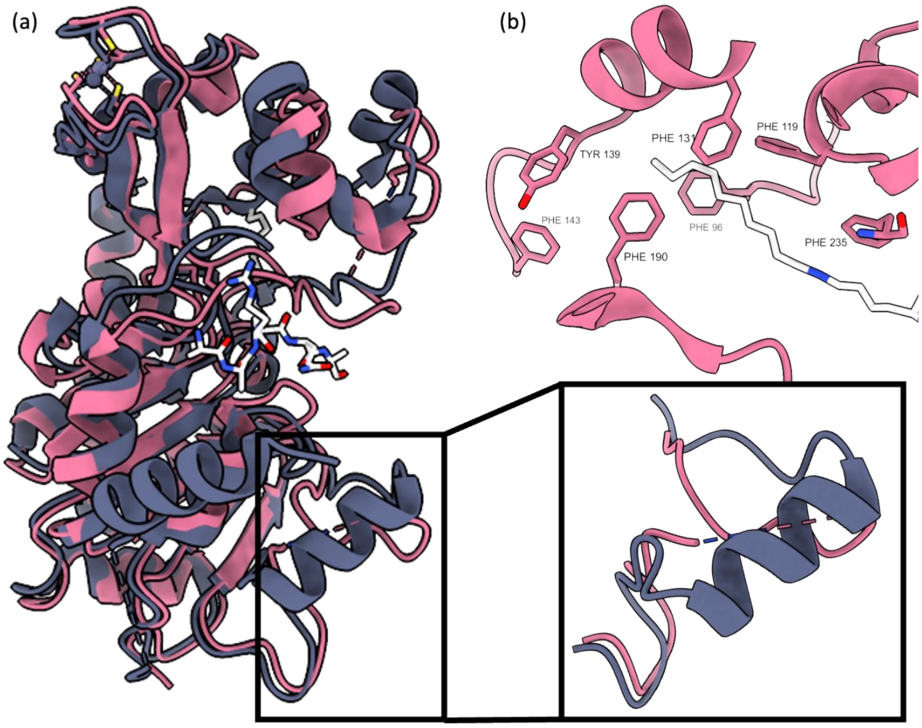

Fig. S1. Structural comparison of apo SIRT2 and the SIRT2/H3K18myr complex. (a) Superimpose of apo SIRT2 and SIRT2/H3k18myr complex, cyan: apo SIRT2; pale violet red: SIRT2/H3K18myr complex. The α-helix of the Rossmann fold exhibits increased flexibility upon myristoylated peptide binding. (b) Hydrophobic ligand binding pocket, highlighting the myristoyl chain accommodation site.

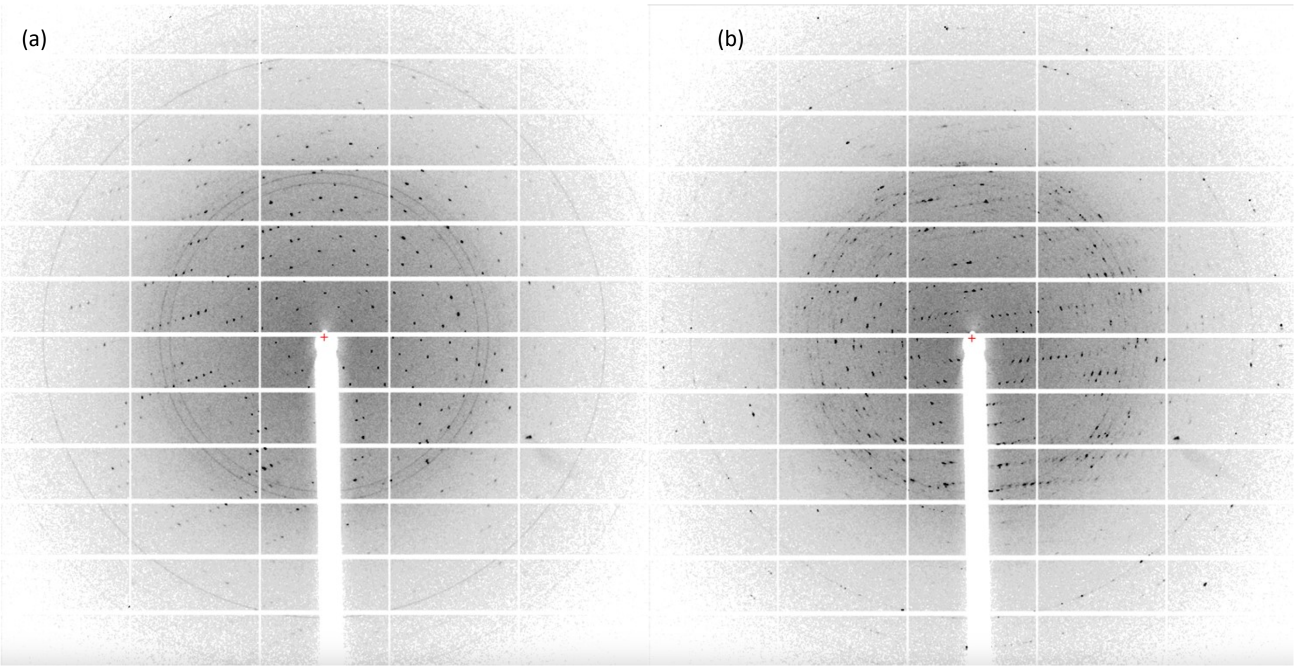

Fig. S2. Comparison of diffraction spots of SIRT2-H3K18myr crystals socking NAD$^+$ with diffraction time. (a) crystal with socking time of 11 min. (b) crystal with socking time less than 10 mins. Crystals with socking time less than 10 min are blurry because of zinc-binding domain shift.

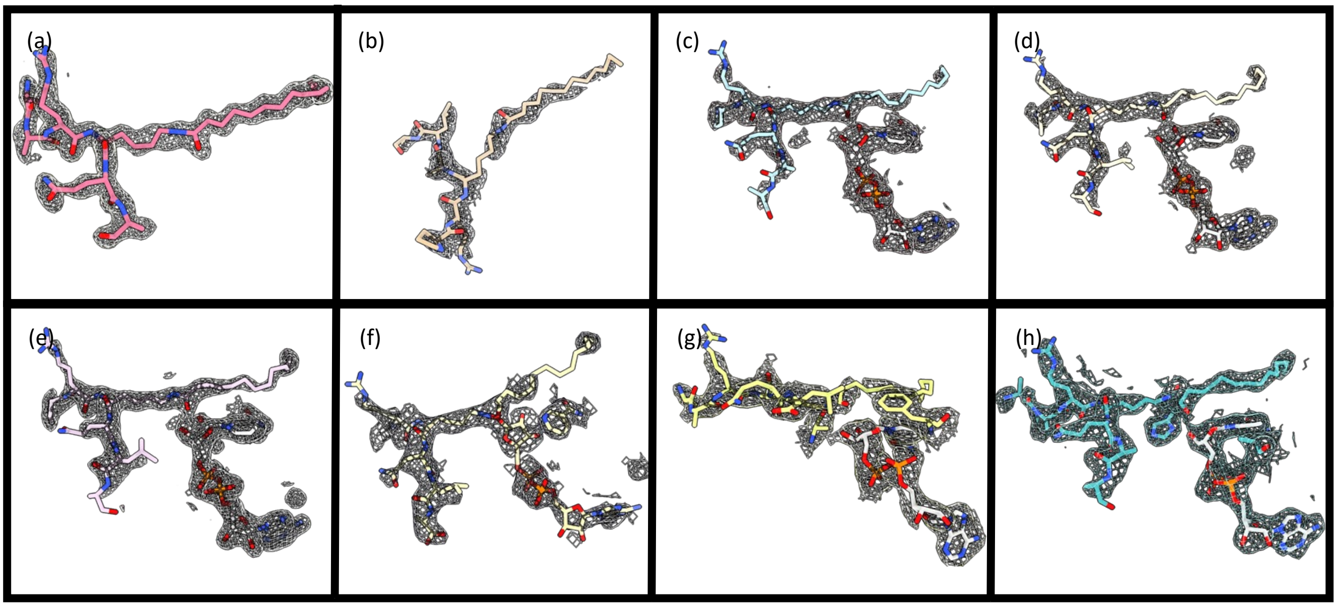

Fig. S3. 2Fo-Fc electron density map contoured at 1σ of myristoylated peptide substrate and NAD⁺.(a) 9VG0 (SIRT2 structure in complex with H3K18myr peptide) ;(b) 9VG3 (SIRT2 structure in complex with H3K18myr peptide: pre-binding state) ;(c) 9VEM (SIRT2 structure in complex with H3K18myr peptide and native NAD⁺: pre-catalysis state 1) ;(d) 9VEW (SIRT2 structure in complex with H3K18myr peptide and native NAD⁺: pre-catalysis state 2) ;(e) 9V7W (SIRT2 structure in complex with H3K18myr peptide and native NAD⁺ : pre-catalysis state 3) ;(f) 9VGE (SIRT2 demyristoylation intermediate I structure) ; 9VH0 (SIRT2-H187A structure in complex with H3K18myr peptide and native NAD⁺) ; 9VGZ (SIRT2-F96A structure in complex with H3K18myr peptide and native NAD⁺)

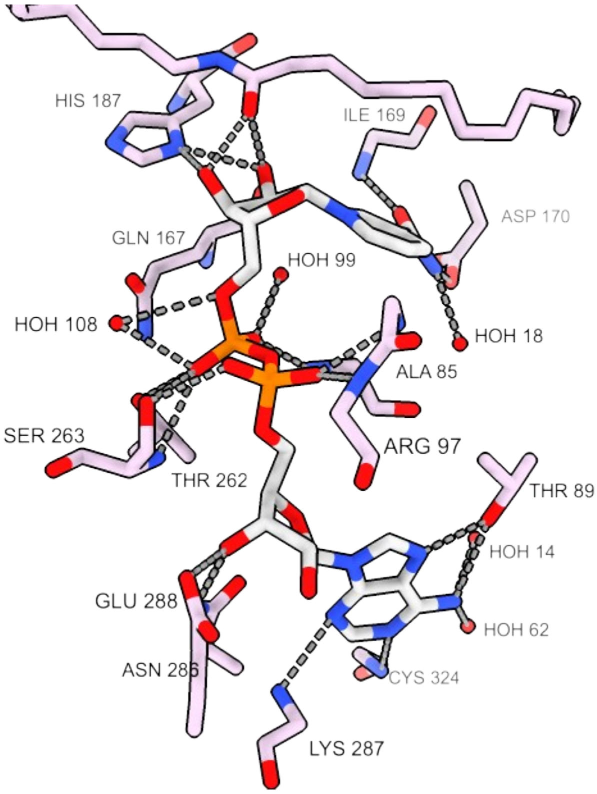

Fig. S4 Overall hydrogen bond interaction between SIRT2 (thistle sticks), H3K18myr and NAD⁺ (white sticks.). Hydrogen bonds: gray dashed lines. Water: red balls. N: blue, O: red, P: orange.

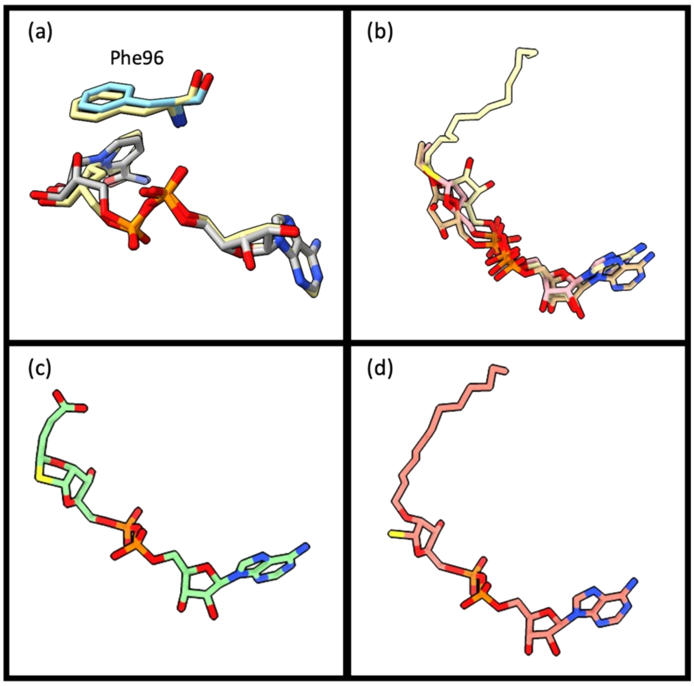

Fig. S5 Catalytic mechanism shown on structural basis. (a) Pre-catalytic state captured by hSIRT2/H3K18myr/NAD$^+$ complex (light blue: SIRT2, light grey: NAD$^+$, PDB: 9VEM) and hSIRT2/BHJH-TM1/carba-NAD$^+$ complex (pale yellow: SIRT2 and carba NAD$^+$, PDB: 4X3P). (b) Intermediate I captured by hSIRT2 (Pale goldenrod, PDB: 9VGE), Sir2Tm (light pink, PDB: 3D81) and hSIRT3 (burly wood, PDB:4BVG). (c) Bicyclic intermediate II captured by hSIRT5 (light green, PDB: 4F56). (d) Intermediate III captured in hSIRT2 (salmon, PDB: 4X3O).