Supplemental Information

Figure S1. Sir2-Sir4 interactions. (A) Full-length Sir2 with a poly(His)-tag (Sir2-FL) interacts with a long C-terminal fragment of Sir4 (Sir4C, a.a. 745-1358). Lane 1: GST-Sir4C immobilized on glutathione beads forms a complex with Sir2-FL. Lane 2: The Sir4C-Sir2-FL complex eluted from the glutathione column after thrombin cleavage. (B) The interaction between a Sir2 fragment lacking N-terminal 65 residues and a Sir4 fragment encompassing residues 745 to 1083 detected by co-elution from a gel-filtration column. (C) Smaller Sir2 and Sir4 fragments copurified through gel-filtration column chromatography were used for crystallization experiments (the Sir4 fragment used for final structure determination is 4 residues shorter, i.e., a.a. 737-893).

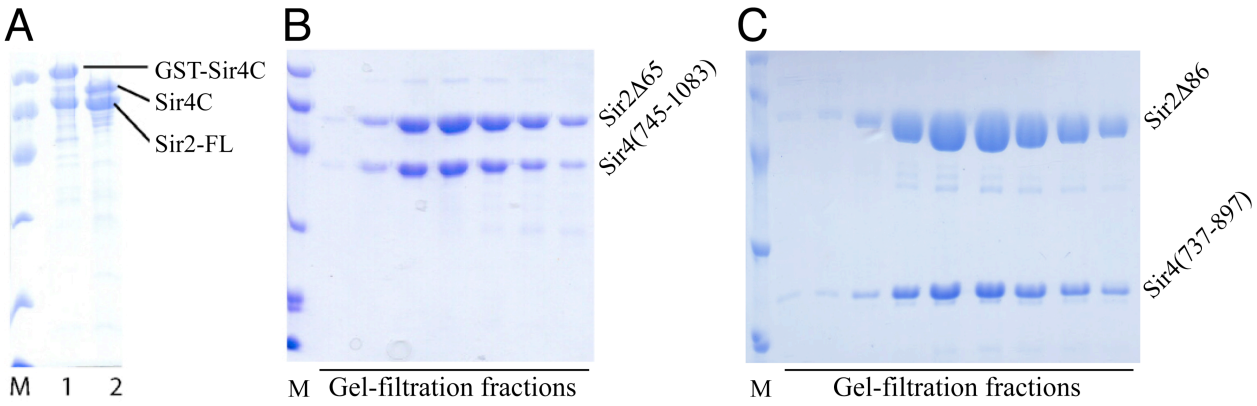

Figure S2. As with longer Sir4 fragments, GST-fused Sir4 segment III (GST_Sir4_840-897) stimulates the activity of a Sir2-Sir4 segment II complex in a concentration dependent manner (Tanny et al., 2004). The chart is shown with the NAD hydrolysis activity of the Sir2-Sir4 segment II complex normalized to 1. The reactions were carried out the same way as that described in the Methods section, and the amount of Sir2 used in each reaction is 100ng.

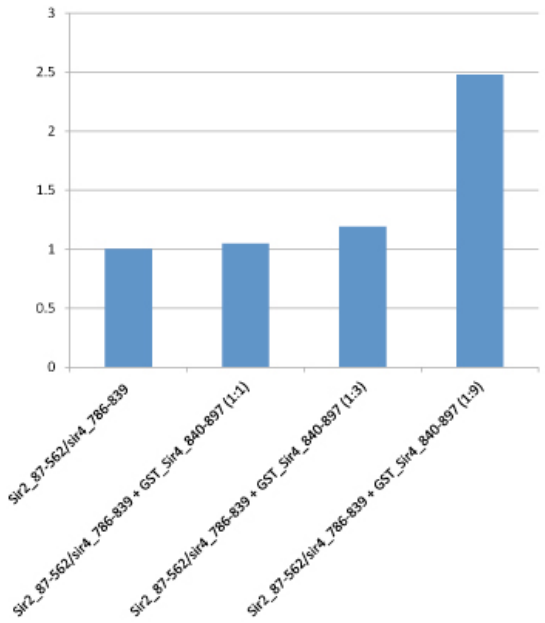

Figure S3. Relative movement of the two Sir2 domains without the Sir4 fragment III. 75 ns molecular dynamics simulation (MDS) with Sir2 and the Sir4 I+II fragment shows that the distance between centers of mass (COM) of the N- and C-terminal domains of Sir2 varies significantly during the course of the simulation. Black and red lines indicate MDS results obtained with the structures of the Sir2-Sir4 complex and that with the Sir4 I+II fragment, respectively. The distances were sampled at 0.1 ns time interval.

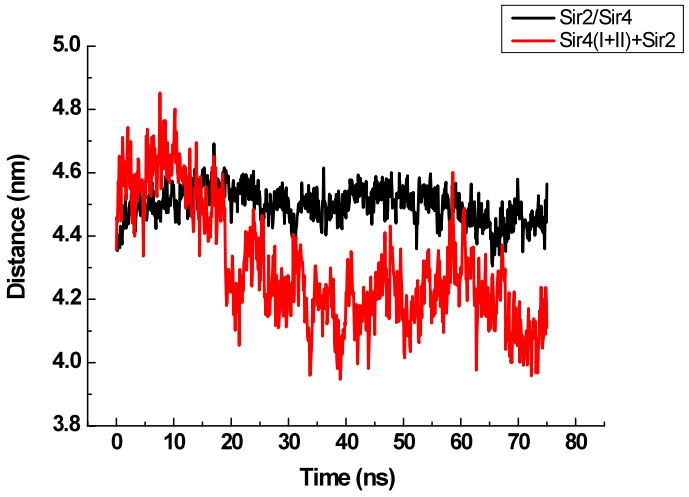

Figure S4. A stereo view of a section of the 2Fo-Fc electron density map contoured at 1.5 σ. A refined model of the Sir2-Sir4^{SID} complex is superimposed.

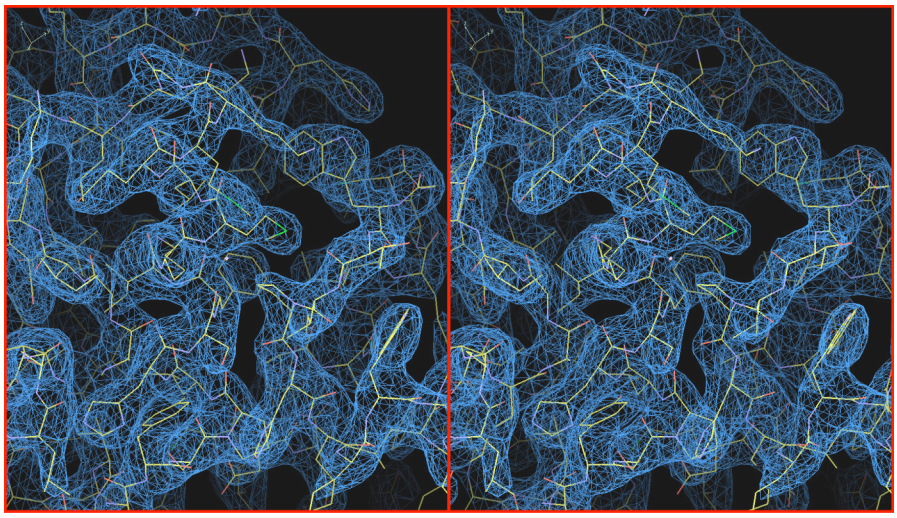

Figure S5. Simulated annealing omit map. The simulated annealing 2Fo-Fc map was calculated with Sir4 molecules omitted and contoured at 1.2 σ. The refined model of the Sir2-Sir4^{SID} complex is superimposed, with the carbon bonds of Sir2 and Sir4 shown in red and green, respectively.