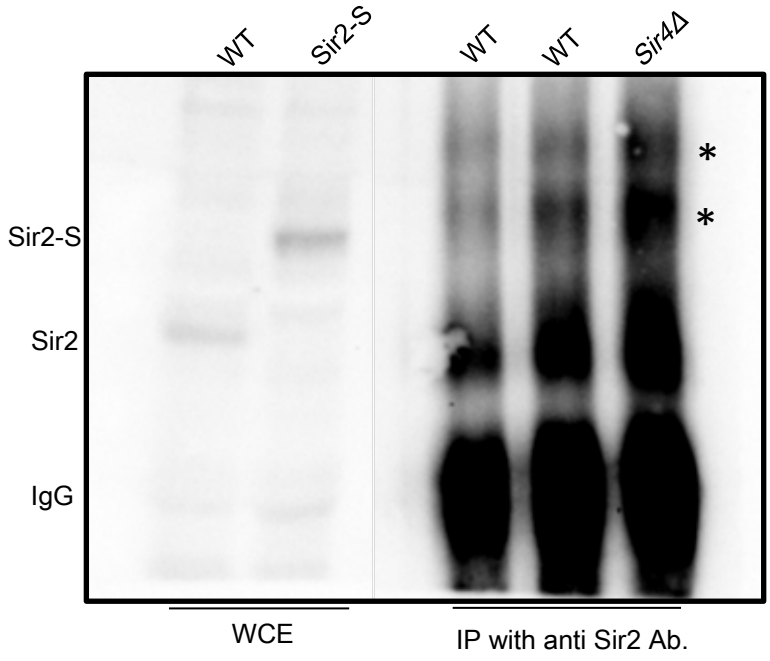
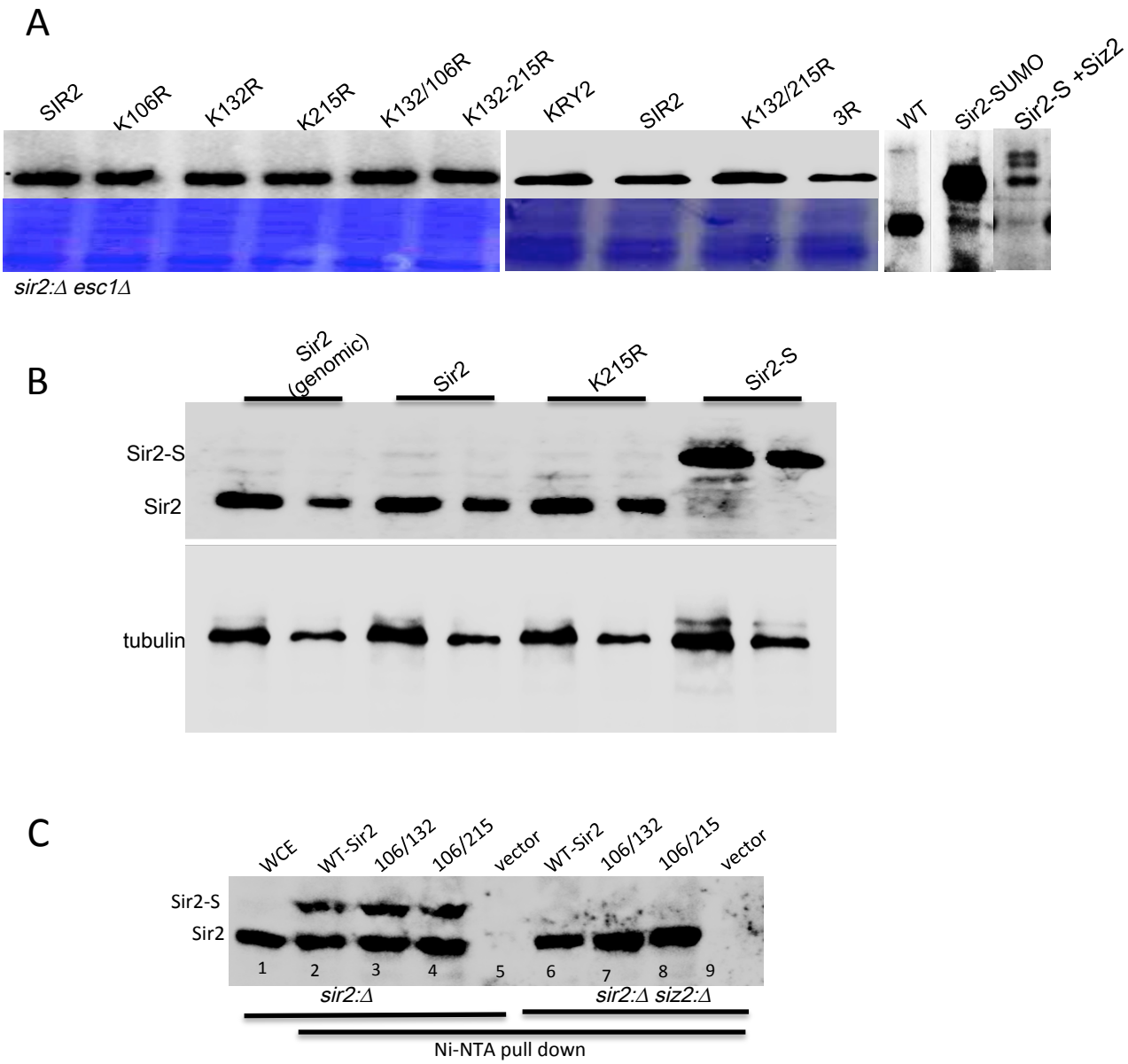
Supplementary Figure 1

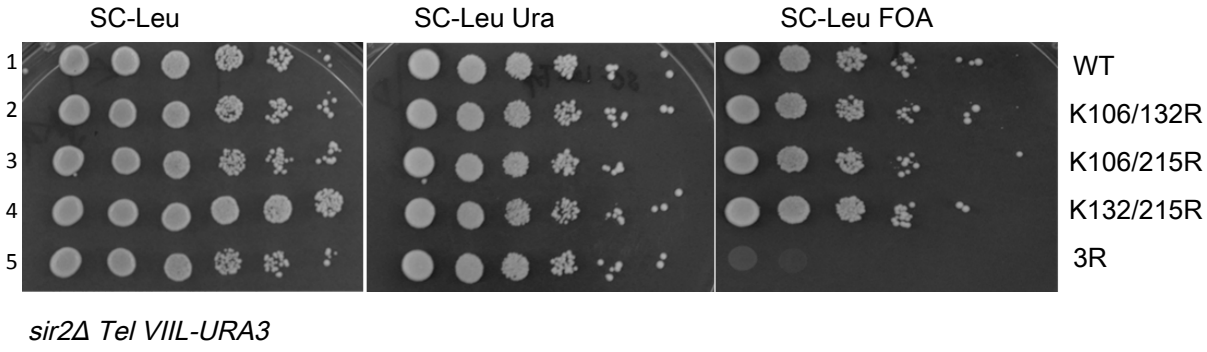


Sup. Fig. 1: Sir2 is sumoylated in vivo. Total protein extracts from untagged strains were immunoprecipitated with anti-Sir2 antibodies. Slower moving sumoylated Sir2 can be detected upon loading in the ratio of 1/100 and longer exposure of film. It is also more prominent in *sir4*Δ.

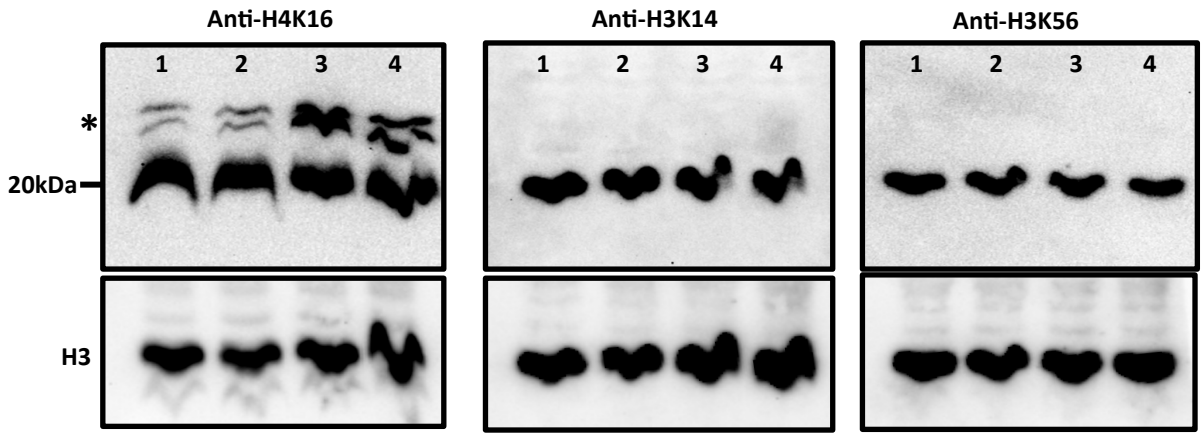
Supplementary Figure 2

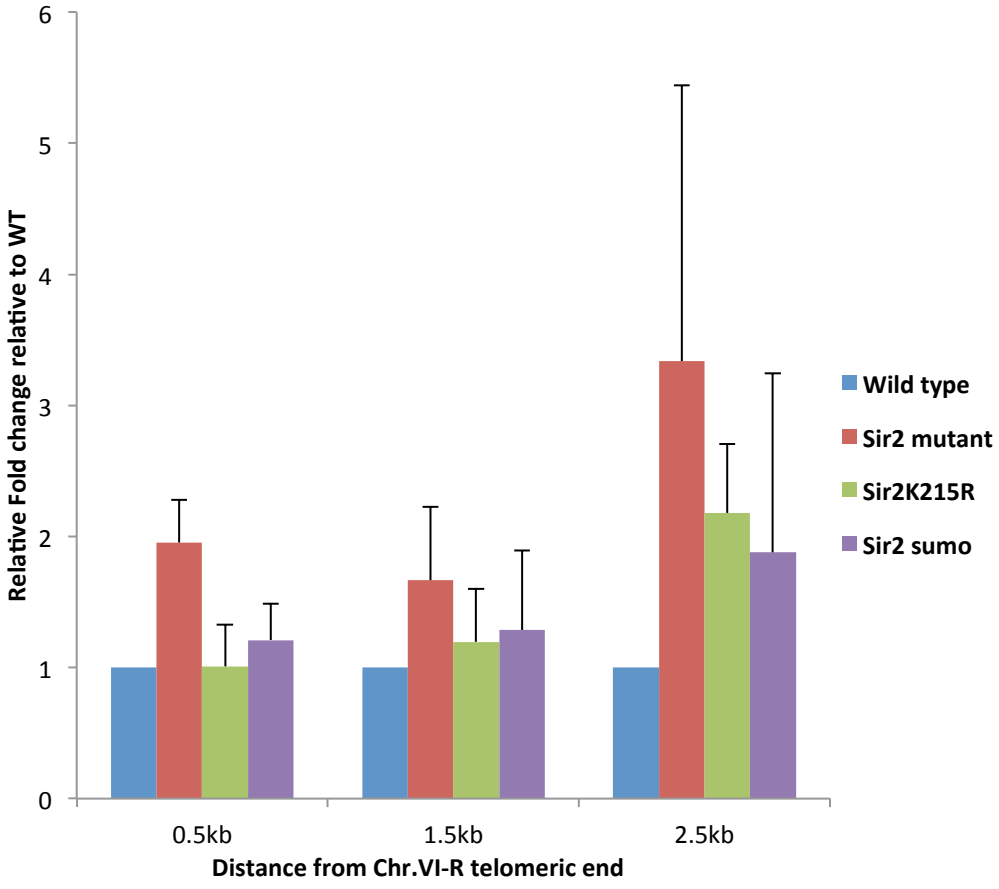


Sup. Fig. 2a: WT Sir2, point mutants and SUMO fusion are expressed stably. KRY 788 was co-transformed with indicated plasmids and *SIZ2* and total protein was extracted and western blots were performed with anti-Sir2 antibodies. Similar amounts of Sir2 were detected in all strains. **Sup. Fig. 2b**: **Expression of Sir2 and all modified forms of Sir2 encoded by plasmids is comparable to genomic copy**. Total protein was extracted from indicated strains and western blots with Sir2 antibody were performed. WCE along with 2 fold dilution of each sample was loaded; same blot was developed with anti-tubulin antibodies as loading control. **Sup.Fig. 2c: Sumoylation of Sir2 is Siz2 dependent.** Ni-NTA pull down from *sir2*Δ (KRY758; lanes 2-5) or *sir2*Δ*siz2*Δ (KRY878; lanes 6-9) expressing the indicated plasmids was performed. The slow moving sumoylated Sir2 band is detected only in strains carrying wild type Siz2. In addition, since double mutants have only one sumoylatable site and that modification cannot be detected in *siz2*Δ confirms that the modification is sumoylation. Whole cell extract from WT and strains expressing Si2r-SUMO are shown for comparison

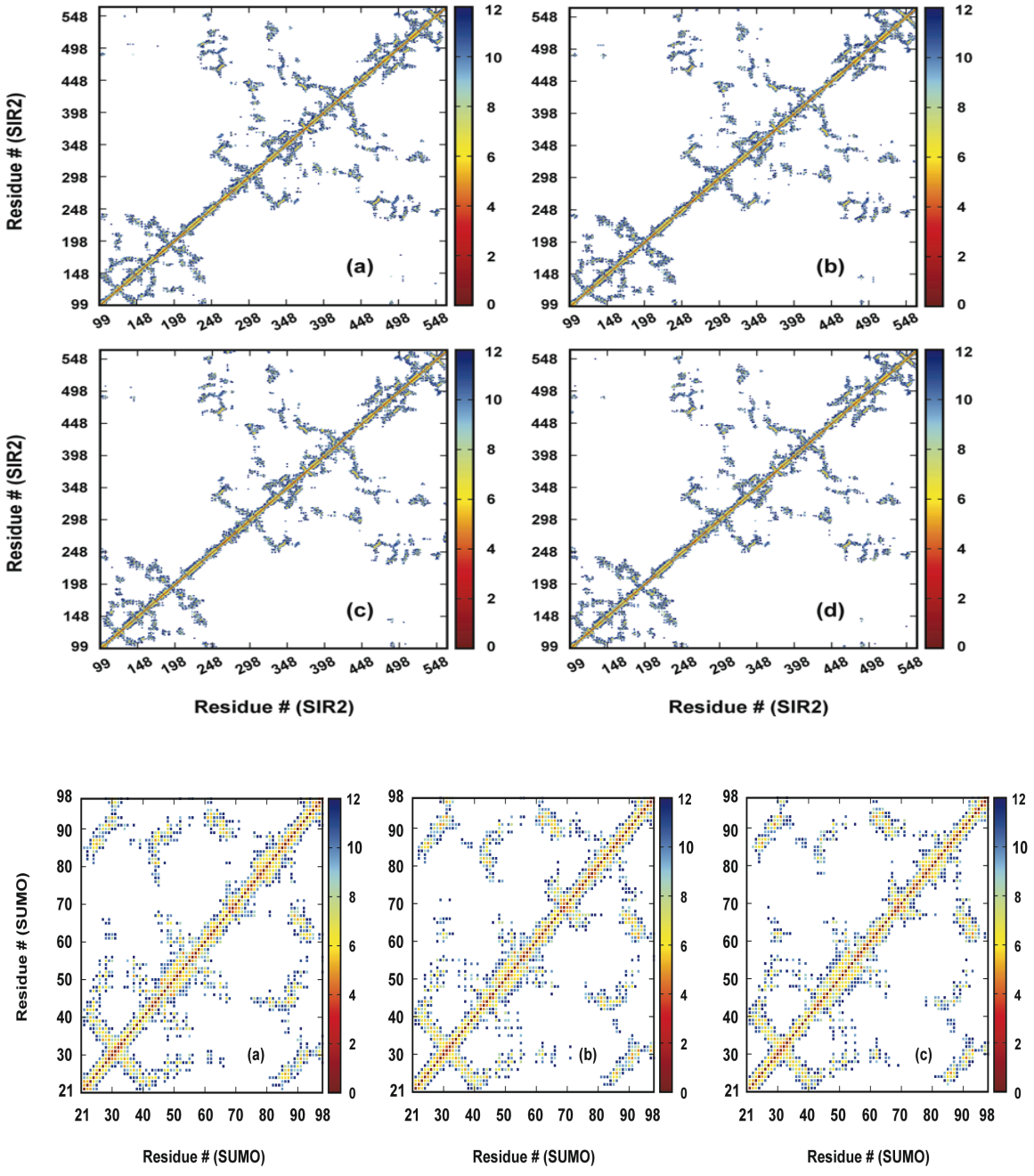


Sup. Fig. 3: SIR2-3R is non-functional for telomere position effect in unperturbed cells. *sir2*Δ (KRY 793) was transformed with either WT or Sir2 double mutants or Sir2-3R and telomere position effect was analyzed. Sir2-3R was unable to establish silencing at telomeres even in unperturbed cells while the double mutants were indistinguishable from WT.





Sup. Fig 4: Histone deacetylase activity of Sir2 is not affected A) Total protein extracts from *sir2*Δ (1), Sir2 (2) Sir2K216R (3), Sir2-SUMO (4) were separated by SDS-PAGE and western blot developed with the indicated antibodies. No significant change was observed. antiH3 was used as loading control. **B)** ChIP for H4K16 acetyl was performed in strains expressing vector alone or Sir2 wild type or Sir2K215R or Sir2-SUMO. No significant difference between WT and Sir2K215R or Sir2-SUMO was detected while *sir2*Δ had higher levels of acetylated H4K16.



Sup. Fig. 5: A) Contact maps showing the tertiary interactions among the residues in the SUMO protein attached to Sir2 sumoylated at K106 (a), K132 (b), and K215 (c). **B)** Contact maps showing the tertiary interactions among the residues in the SUMO protein attached to Sir2 sumoylated at K106 (a), K132 (b), and K215 (c). Those distances above 12 Å are not plotted for clarity.