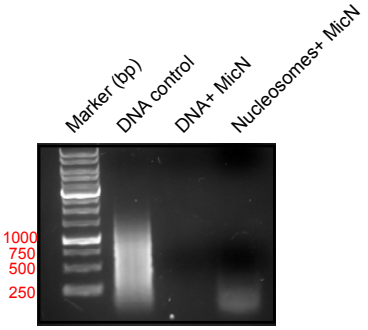
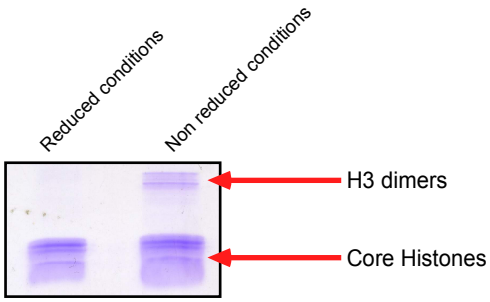
Supplemental figures

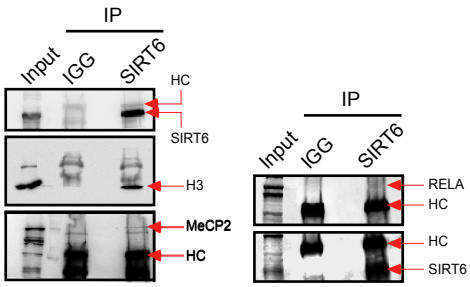
1



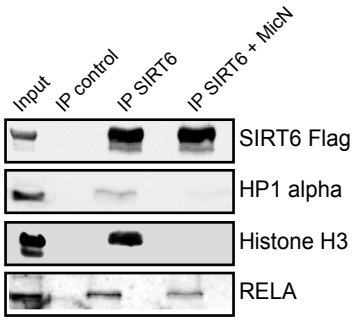
2



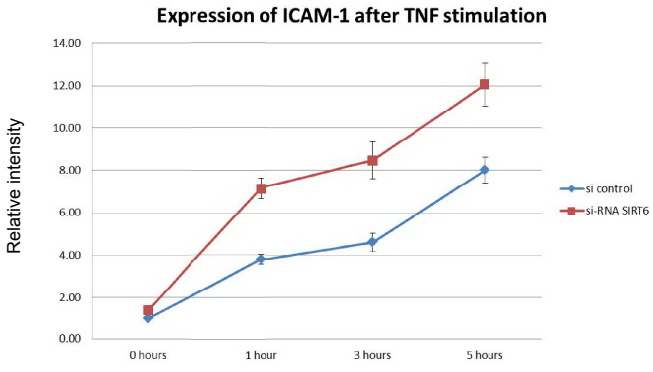
3



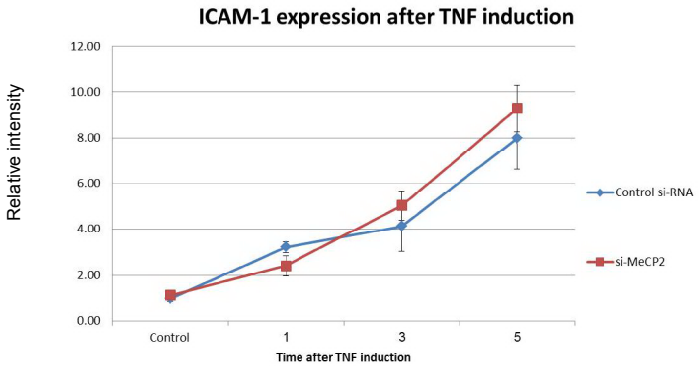
4

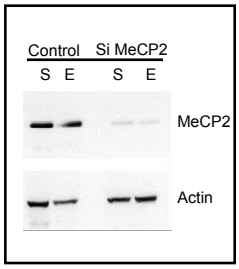


5

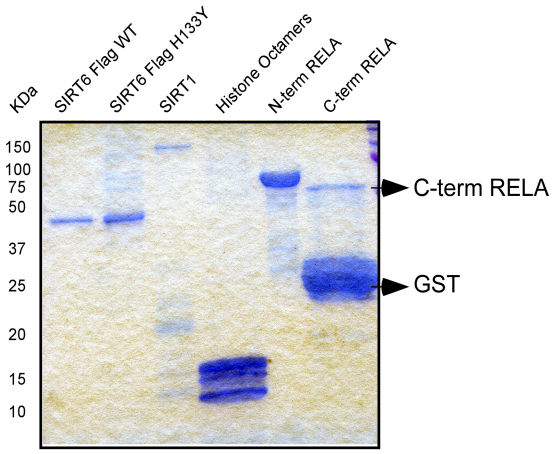


S- At start of the experiment
E- At end of the experiment

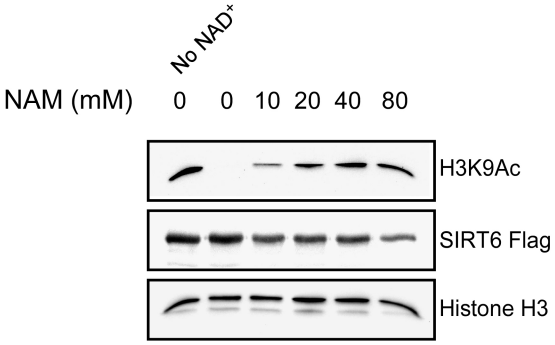




6


KDa
SIRT6 Flag WT
SIRT6 Flag H133Y
SIRT1
Histone Octamers
N-term RELA
C-term RELA
150
100
75
50
37
25
20
15
10
C-term RELA
GST

7


No NAD+
NAM (mM)
0
0
10
20
40
80
H3K9Ac
SIRT6 Flag
Histone H3

Supplementary information

Figure S1. Integrity analysis of the reconstituted nucleosomes. 1µg of free DNA and 0.5µg of nucleosomal DNA were treated with MicN (0.1 kunitz units) for 30 minutes.

Figure S2. Integrity analysis of histone octamers. Histone octamers treated with Laemmli buffer with (right lane) or without (left lane) DTT. Samples were loaded on a 15% SDS PAGE and coomassie stained.

Figure S3. Endogenous SIRT6 binds Histone H3, RELA and MeCP2.

Figure S4. SIRT6 interacts with chromatin-related proteins in a DNA dependent manner. Disruption of DNA dependent interactions by MicN shows that *in vivo* SIRT6 associations with Hp1α and Histone H3 are DNA dependent. RELA was used as a positive control.

Figure S5. **Knockdown of MeCP2 has no effect on RELA regulated genes.** si-RNA against SIRT6 but not against MeCP2 enhanced ICAM-1 expression upon TNFα stimulation, as measured by quantitative real time PCR. The knockdown efficiencies are shown on the right.

Figure S6. Coomassie staining of recombinant SIRT6-Flag, SIRT6-Flag H133Y, SIRT1, histone octamers, and truncated amino terminus (N-term a.a 1-313) or carboxyl terminus (C-term a.a. 334-551) of RELA.

Figure S7. Nicotinamide (NAM) blocks SIRT6 deacetylase activity. SIRT6-Flag deacetylation of H3K9Ac, bound in a nucleosome, under increasing concentrations of NAM.