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## The sirtuin SIRT6 deacetylates H3 K56Ac *in vivo* to promote genomic stability

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Sirtuin deacetylases promote longevity in invertebrates. Mammals possess seven sirtuin homologs, which are involved in a diverse array of processes including development, metabolism, and stress resistance<sup>1</sup>. Among these, the sirtuin SIRT6 plays a particularly important role in the maintenance of cellular and organismal homeostasis. Mouse embryonic fibroblasts (MEFs) and embryonic stem (ES) cells lacking SIRT6 show poor growth, sensitivity to genotoxins (MMS, hydrogen peroxide, and ionizing radiation), and an increased frequency of genomic aberrations, including chromosomal gaps, breaks, and fusions<sup>2</sup>. The molecular basis for the genomic instability observed in SIRT6 deficiency remains obscure. SIRT6 is tightly associated with nuclear chromatin<sup>2</sup>. It has been reported that in human cells, SIRT6 maintains telomeric stability via deacetylation of histone H3 acetylated on lysine 9 (H3 K9Ac)<sup>3</sup>; and that SIRT6 promotes recruitment of DNA-PKcs, a protein involved in the non-homologous end-joining (NHEJ) DNA repair pathway, to DNA lesions<sup>4</sup>. However, SIRT6-deficient mice show neither telomeric instability, nor defects observed in NHEJ mutants<sup>5</sup>. Therefore we sought to define alternative mechanisms by which SIRT6 maintains chromosomal integrity. Acetylation of histone H3 on lysine 56 (H3 K56Ac) is a recently described chromatin modification linked to genomic stability and genotoxin resistance. H3 K56Ac is deacetylated by the sirtuins Hst3 and Hst4 in yeast<sup>6</sup>, and SIRT1 and SIRT2 in mammalian cells<sup>7, 8</sup>. Based on knockdown experiments, it has been reported that SIRT6 does not play a significant role in H3 K56Ac deacetylation<sup>7, 9</sup>. In yeast and mammals, failure to regulate H3 K56Ac levels, or mutations mimicking such a defect, leads to impaired cell cycle progression, genotoxin sensitivity and spontaneous DNA damage<sup>6, 7</sup>, all phenotypes associated with SIRT6 deficiency in mammalian cells. To examine a potential role for SIRT6 in deacetylating H3 K56Ac in the context of SIRT6 null mutants, levels of this modification were assessed in SIRT6-deficient MEFs and littermate controls. Levels of H3 K56Ac were dramatically increased in SIRT6 knockout cells (Fig. 1A, left panel). H3 K56Ac levels were also elevated in two independent SIRT6-deficient ES cell lines compared with a wild-type control (Fig. 1A, middle panel). Reconstitution with wild-type SIRT6, but not a catalytically inactive SIRT6 mutant (H133Y)<sup>3, 10</sup>, rescued H3 K56 hyperacetylation in SIRT6-deficient fibroblasts (Fig. 1A, right panel), indicating that H3 K56 hyperacetylation in these cells is a direct consequence of loss of SIRT6 function. For assessment of H3 K56Ac levels in individual cells, immunofluorescence analysis was performed on SIRT6-deficient primary MEFs and littermate controls. H3 K56Ac staining was markedly more intense in SIRT6-deficient MEFs, and was also present in a much greater fraction of cells (Fig. 1B, left panel). H3 K56Ac was detectable in 97% of SIRT6-deficient cells, but in only 22% of controls (Fig. 1B, right panel). To test the role of SIRT6 in deacetylating H3 K56Ac *in vivo*, H3 K56Ac levels were assessed in tissues from SIRT6-deficient mice and littermate controls. We focused on thymus and brain, both sites of prominent SIRT6 expression<sup>2</sup>. SIRT6 deficiency was associated with dramatically

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higher levels of H3 K56Ac in both tissues (Fig. 1C). In order to rule out the possibility that hyperacetylation of H3 K56 in SIRT6 deficiency might represent an indirect effect of the genomic instability in this mutant background, the ability of SIRT6 to deacetylate H3 K56Ac *in vitro* was assessed. Wild-type SIRT6, but not the SIRT6 catalytic mutant, robustly deacetylated H3 K56Ac, a reaction blocked by the sirtuin inhibitor nicotinamide (Fig. 1D). We conclude that SIRT6 directly deacetylates H3 K56Ac to regulate global levels of this histone modification *in vivo*. H3 K56Ac levels are tightly regulated in yeast, flies, and mammals; increased levels of this modification lead to genomic instability and genotoxin sensitivity<sup>6, 7</sup>. In this context, there are conflicting reports regarding the impact of DNA damage induction on H3 K56Ac levels in metazoans<sup>7–9</sup>. In MEFs, we do not observe any major effect of DNA damaging agents on global H3 K56Ac levels (Fig. S1). Precise mechanisms by which H3 K56Ac modulates genomic stability and genotoxin sensitivity in mammals are unclear, and likely multi-factorial. The ability of SIRT6 to deacetylate H3 K56Ac likely represents a principal means by which this sirtuin promotes genomic stability.

## Supplementary Material

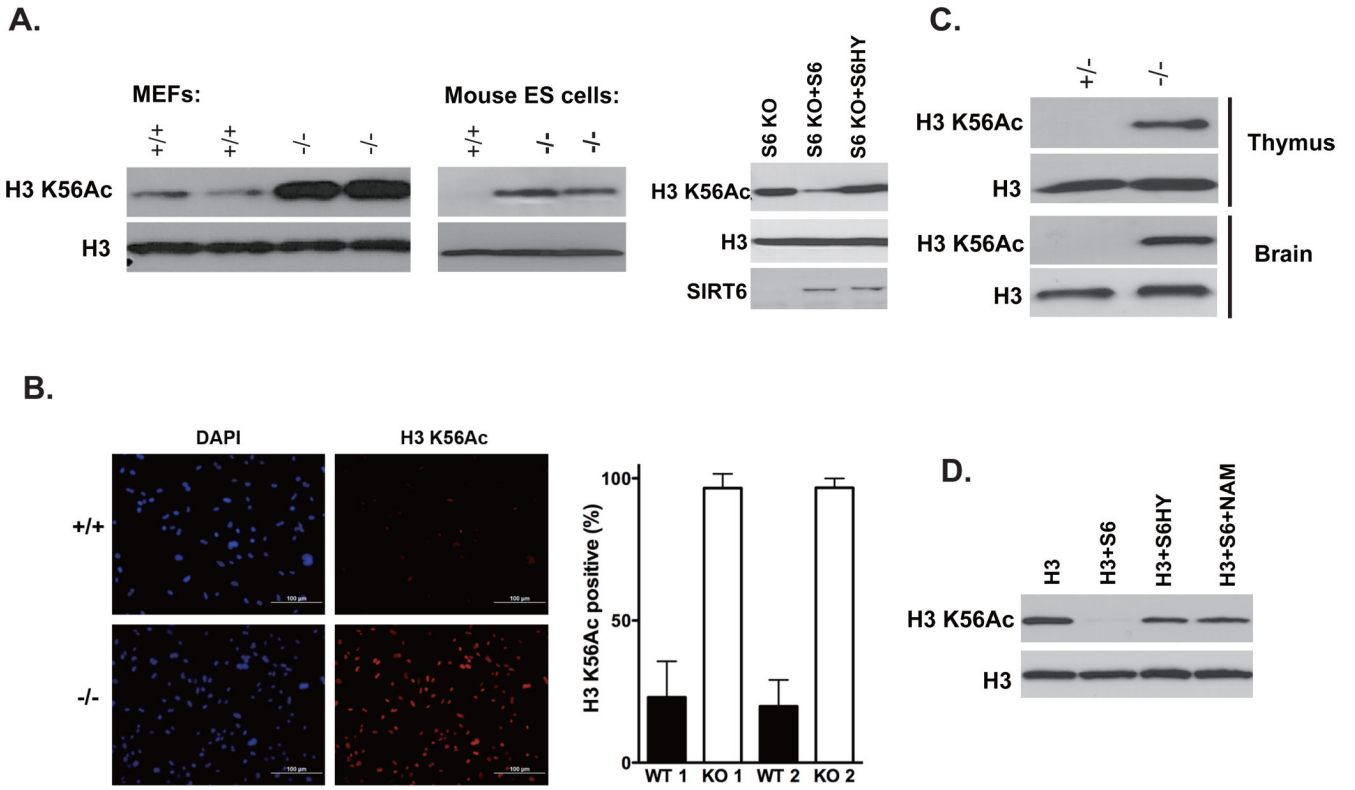
Refer to Web version on PubMed Central for supplementary material.

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**Fig. 1.** SIRT6 deacetylates H3 K56Ac. (A) Hyperacetylation of H3 K56Ac in the absence of SIRT6. (*Left panel*) Hyperacetylation of H3 K56Ac in SIRT6-deficient MEFs. (*Middle panel*) Hyperacetylation of H3 K56Ac in SIRT6-deficient ES cells. (*Right panel*) Reconstitution of a SIRT6-deficient fibroblast cell line with catalytically active SIRT6 reduces H3 K56Ac levels. (B) Immunofluorescence analysis of SIRT6-deficient MEFs and littermate controls. (C) Hyperacetylation of H3 K56Ac in thymus (*top panels*) and brain (*bottom panels*) of a SIRT6-deficient mouse and littermate control. (D) Recombinant SIRT6 deacetylates H3 K56Ac *in vitro*.