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Supplementary Materials for

SIRT7-mediated ATM deacetylation is essential for its deactivation and DNA damage repair

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This PDF file includes:

Supplementary Materials

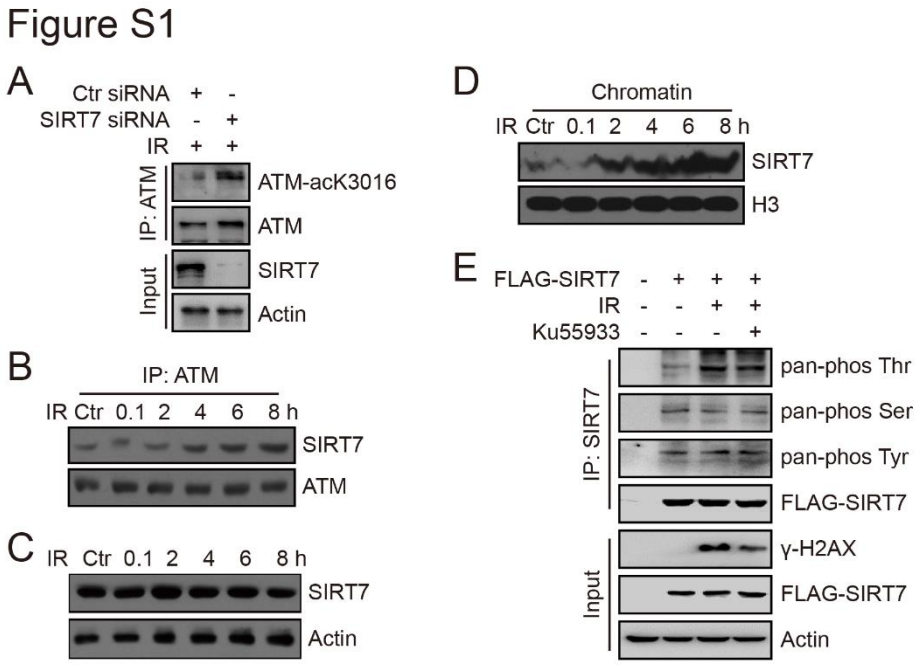

Fig. S1. SIRT7 is recruited onto chromatin upon IR treatment. A. HCT116 cells were transfected with the indicated siRNAs and treated with IR at 10 Gy and released for 8 h. Whole cell extracts were subjected to IP analysis. **B**. LoVo cells were treated with IR at 10 Gy and released for the indicated time periods. Whole cell extracts were subjected to IP analysis. **C**. HCT116 cells were treated with IR at 10 Gy and released for the indicated time periods. Total cell lysates were subjected to immunoblotting. **D**. LoVo cells were treated with IR at 10 Gy and released for the indicated time periods. Chromatin proteins were subjected to immunoblotting. **E**. HCT116 cells were transfected with the indicated plasmids and treated with IR at 10 Gy and released for 2 h with or without Ku55933 at 10 μM. Whole cell extracts were subjected to IP analysis.

Figure S2

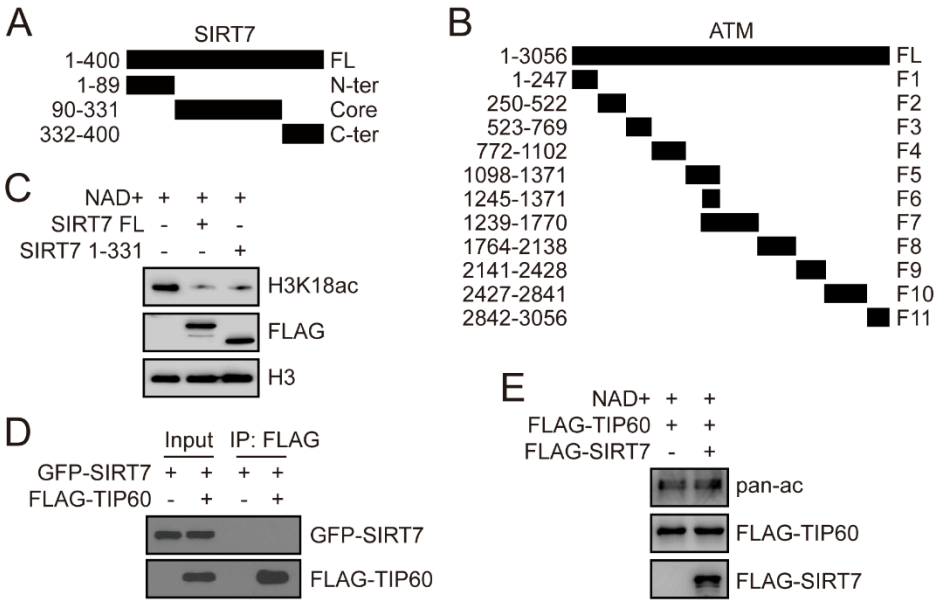

Fig. S2. SIRT7 interacts and deacetylates ATM. A. Fragments of SIRT7. **B**. Fragments of ATM.**C**. Full-length or 1-331 fragment of SIRT7 were purified and subjected to *in vitro* deacetylation assay using free histones as substrates. **D**. HCT116 cells were transfected with the indicated plasmids and whole cell extracts were subjected to co-IP analysis. **E**. FLAG-TIP60 and FLAG-SIRT7 were separately purified and subjected to *in vitro* deacetylation assay.

Figure S3

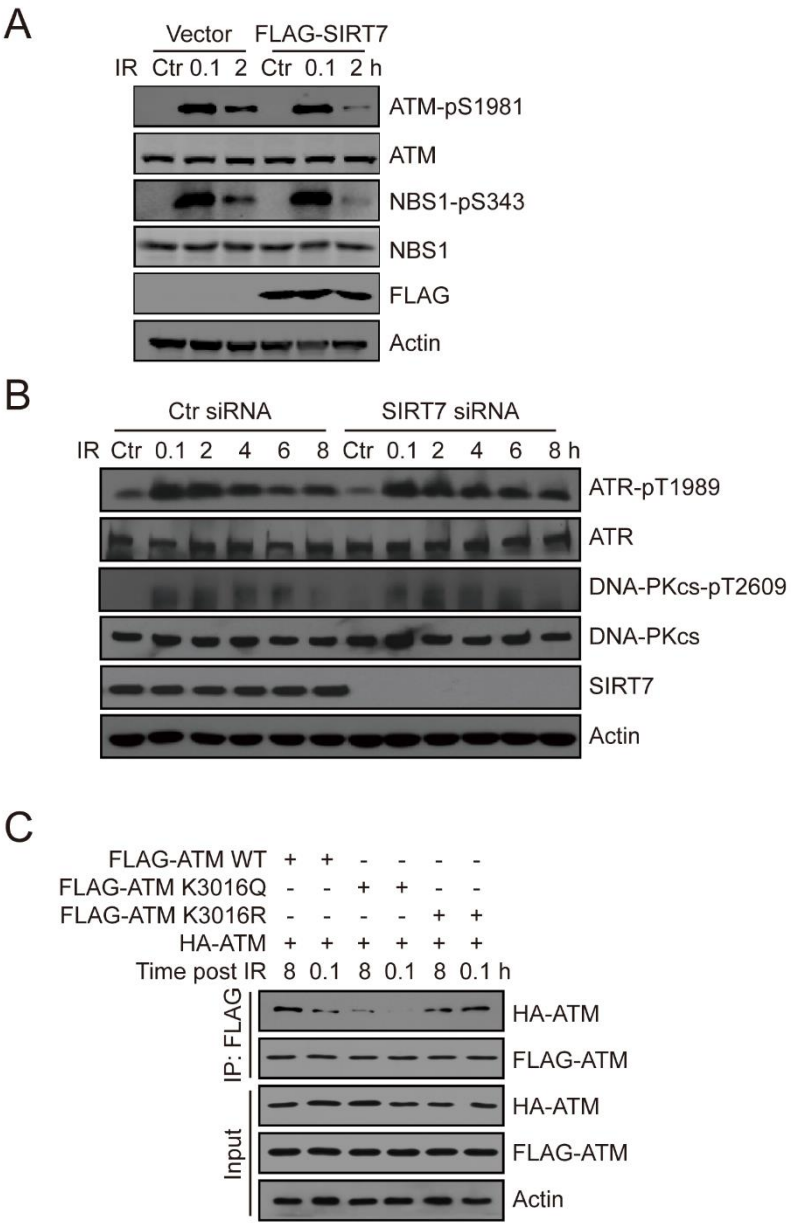
A
Vector
FLAG-SIRT7
IR Ctr 0.1 2 Ctr 0.1 2 h
ATM-pS1981
ATM
NBS1-pS343
NBS1
FLAG
Actin
B
Ctr siRNA
SIRT7 siRNA
IR Ctr 0.1 2 4 6 8 Ctr 0.1 2 4 6 8 h
ATR-pT1989
ATR
DNA-PKcs-pT2609
DNA-PKcs
SIRT7
Actin
C
FLAG-ATM WT + + - - - -
FLAG-ATM K3016Q - - + + - -
FLAG-ATM K3016R - - - - + +
HA-ATM + + + + + +
Time post IR 8 0.1 8 0.1 8 0.1 h
IP: FLAG
HA-ATM
FLAG-ATM
Input
HA-ATM
FLAG-ATM
Actin

Fig. S3. Deletion of SIRT7 had no effects on ATR or DNA-PKcs activation.

A. HCT116 cells were transfected with WT SIRT7 or Vector Control and treated with IR at 10 Gy and released for the indicated time periods. Total cell lysates were subjected to immunoblotting. **B**. HCT116 cells were transfected with the indicated siRNAs and treated with IR at 10 Gy and released for the indicated time periods. Total cell lysates were subjected to immunoblotting. **C**.HCT116 cells were transfected with the indicated plasmids and treated with IR at 10 Gy and released for the indicated time periods. Whole cell extracts were subjected to IP analysis.

Figure S4

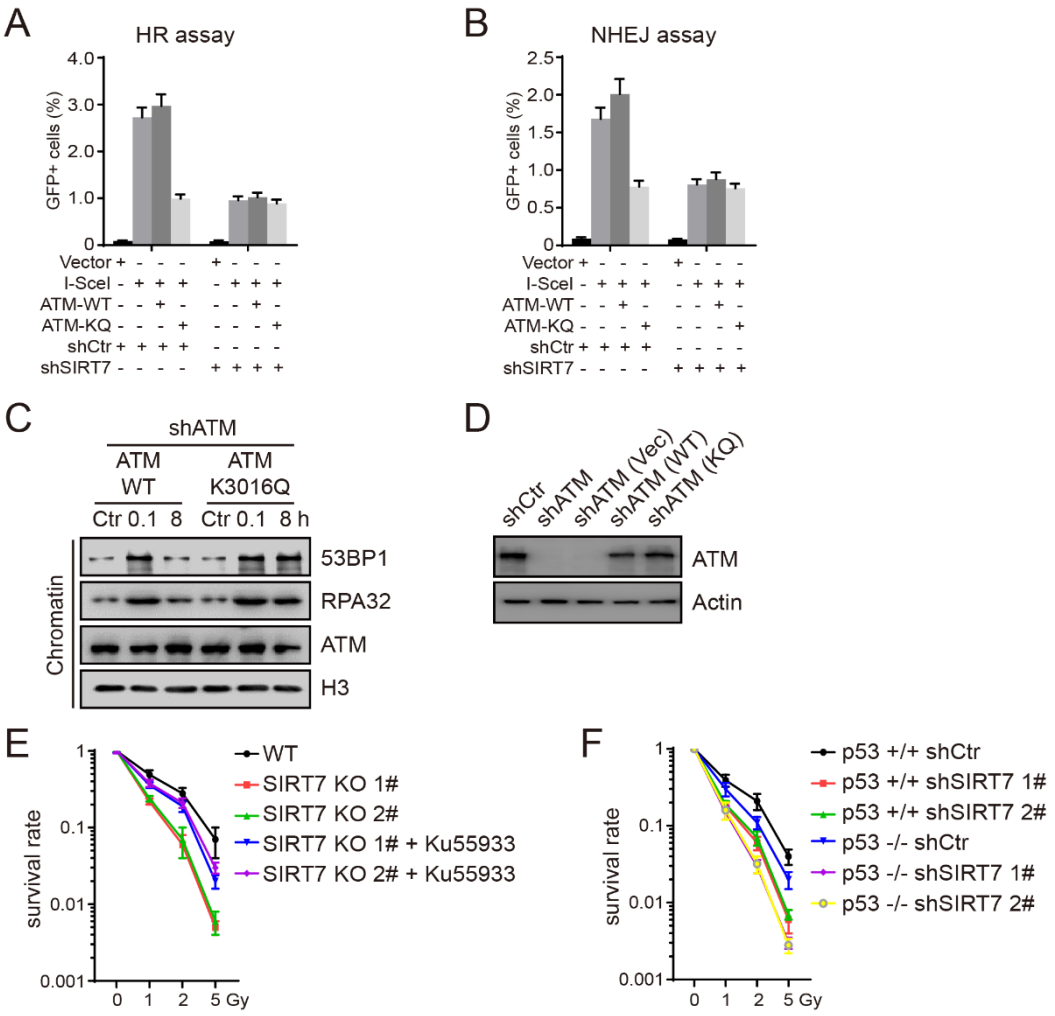

Fig. S4. SIRT7 regulates DNA repair and cell survival through ATM deacetylation. A, **B**. SIRT7 stable knockdown (shSIRT7) or control (shCtr) DR-U2OS and pEJ5-U2OS cells transfected with the indicated plasmids and subjected to HR and NHEJ assays, respectively. All data represent the means ± SD. **C**. ATM stable knockdown cells were transfected with the indicated plasmids and subjected to chromatin fractionation after treating with IR at 10 Gy and released for the indicated time. **D**. Immunoblots for protein levels in Fig. 5**I**. **E**. WT or SIRT7 knockout (1# and 2#) HCT116 cells were subjected to colony formation assay with or without Ku55933 at 10 μM. All data represent the means ± SD. **F**. SIRT7 stable knockdown (shSIRT7) or control (shCtr) $p53^{+/+}$ or $p53^{-/-}$ HCT116 cells were subjected to colony formation assay. All data represent the means ± SD.