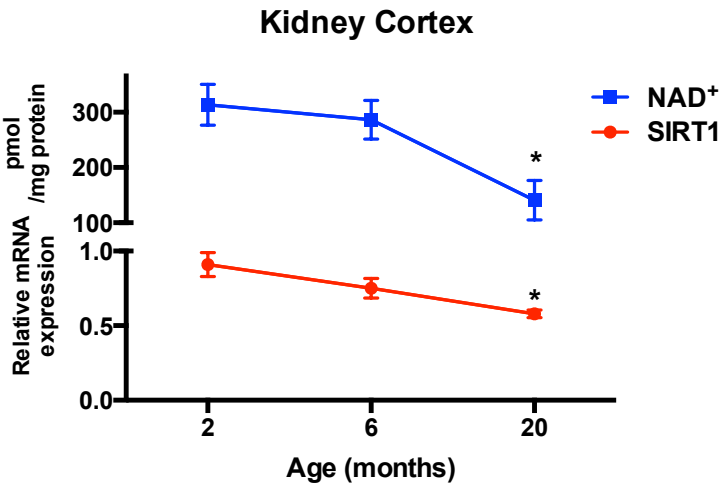
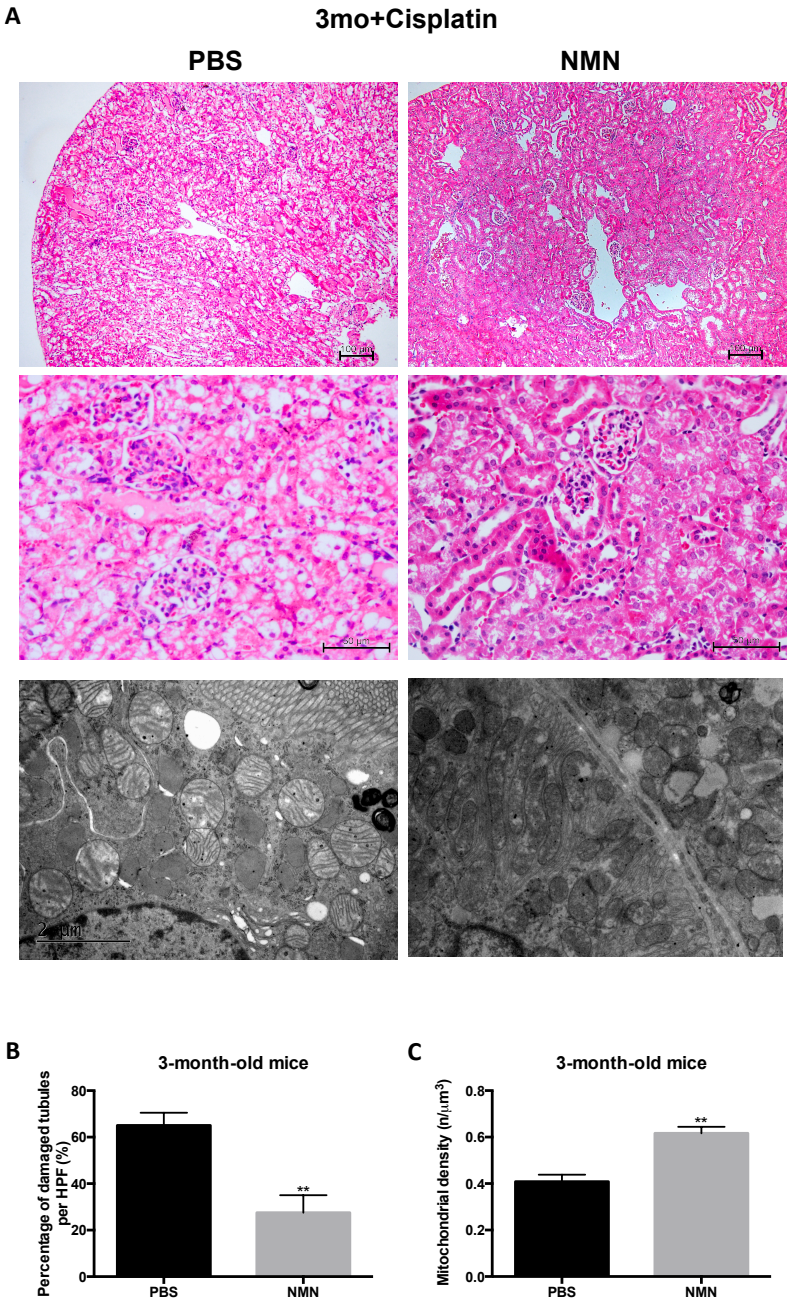


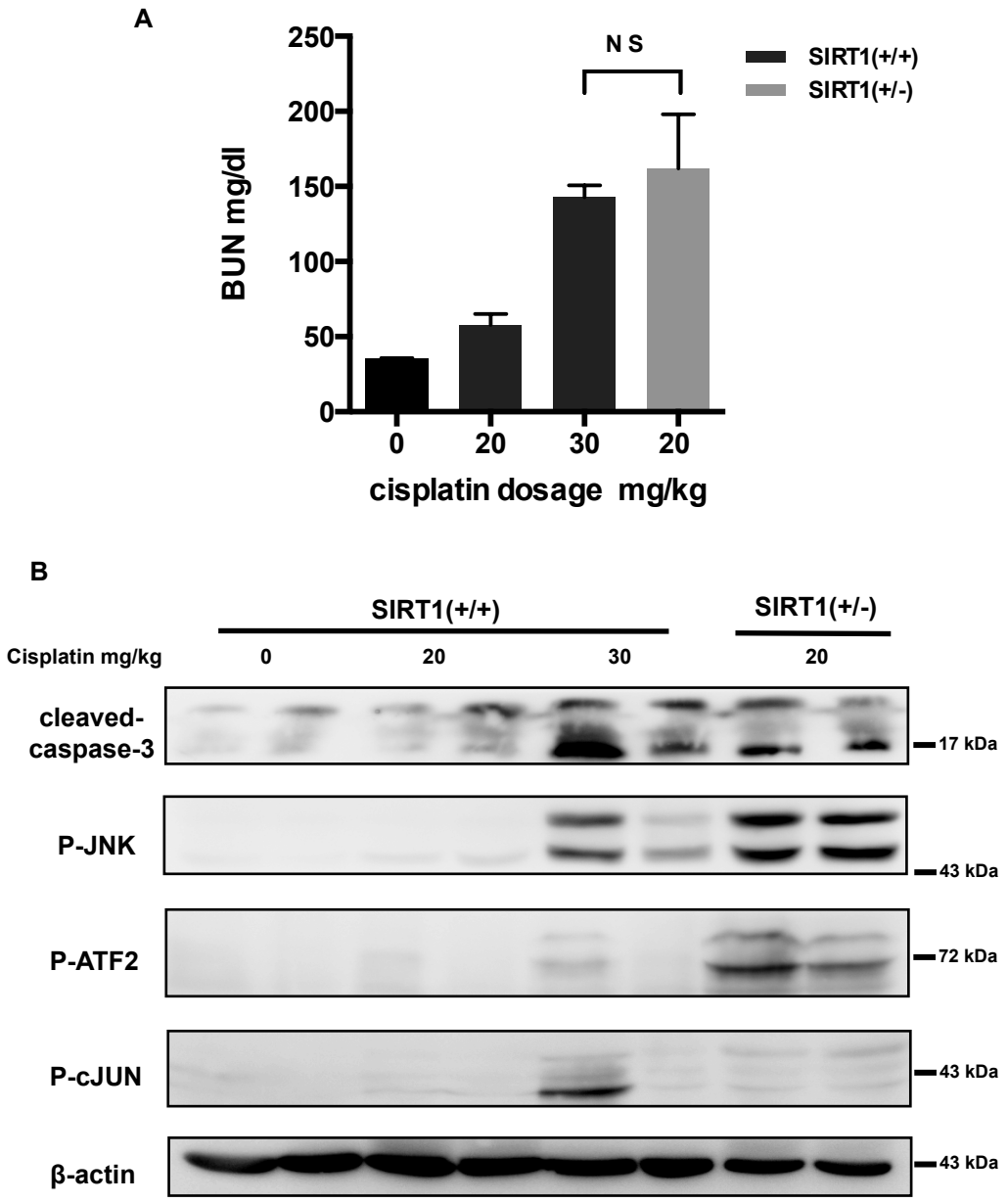
Sup1. SIRT1 expression and NAD^+ metabolism are decreased during the aging process in C57 mouse kidney

NAD^+ levels (upper panel) and relative mRNA expression of SIRT1 (lower panel) in the kidney cortex of 2-, 6-, and 20-month-old C57 mice. (n=6) Data were expressed as mean ± SEM and analyzed using ANOVA followed by post-tests. *P<0.05, significantly different from 2-month-old mice.

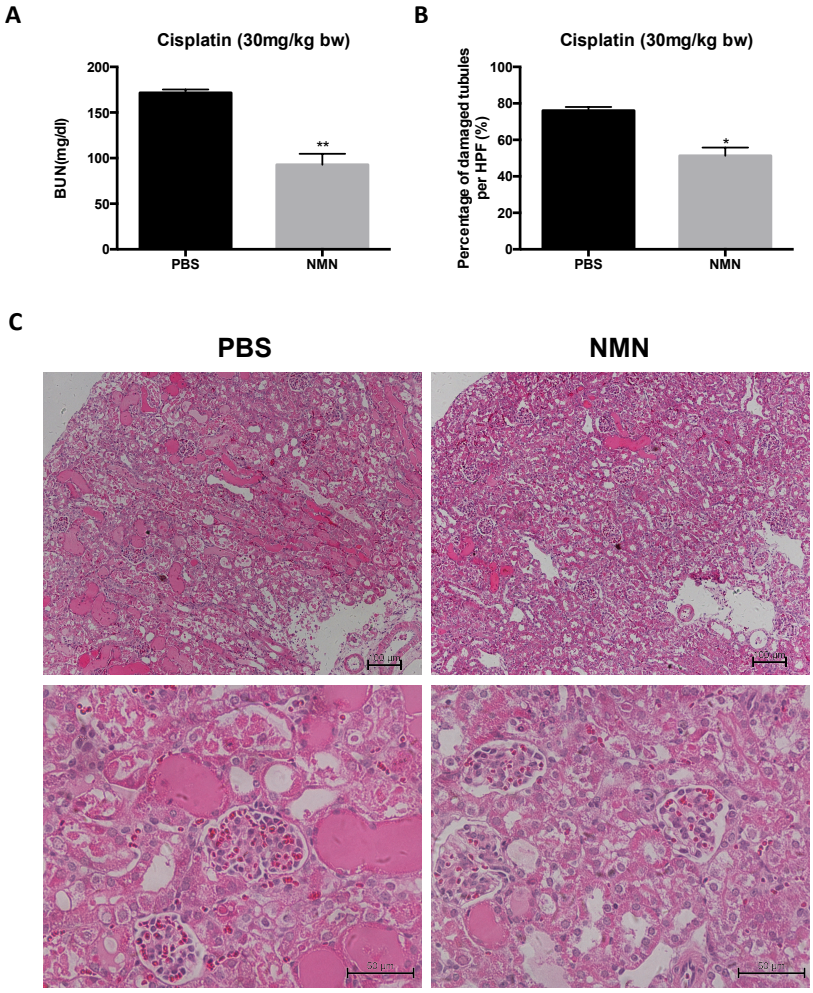


Sup2. The effect of NMN on cisplatin-induced AKI in 3-month-old mice.

(A) Representative H&E–stained kidney sections and EM of representative renal PTCs (last panel, scale bars: 2 μm) are shown at 72 hours after being subjected to cisplatin with or without NMN treatment in 3-month-old mice. **(B,C)** Tissue injury evaluated by percentage of damaged tubules and mitochondrial density. Data were expressed as mean ± SEM and analyzed by Student's t-test. **P<0.01.



Sup3. JNK activation is enhanced by SIRT1 deficiency.
(A) Renal function was evaluated by BUN. **(B)** Wild-type and SIRT1 heterozygotes were subjected to different different doses of cisplatin as indicated. Apoptosis and JNK activation were examined by Western blotting. Data were expressed as mean ± SEM and analyzed using ANOVA followed by post-tests. NS means no statistical significance.



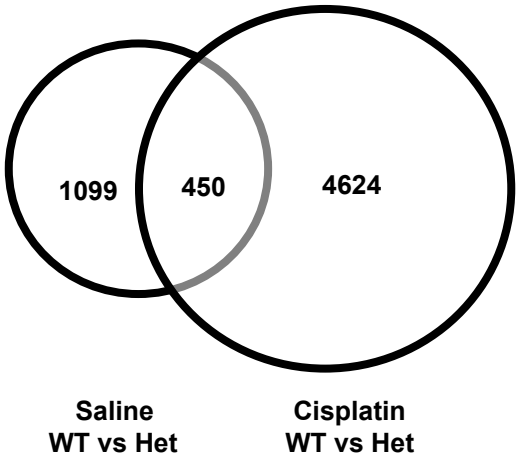
Sup4. The effect of NMN on severe AKI induced by cisplatin.

As indicated by Sup4, 30mg/kg bw cisplatin induced equivalent severe renal damage in wild-type mice to 20mg/kg in SIRT1 heterozygotes (mice strain:C57BL/6). NMN rescued wild-types from severe renal injury induced by 30mg/kg cisplatin, demonstrated by **(A)** decreased renal function evaluated by BUN and **(B,C)** improved histology manifestation. (n=3) Data were expressed as mean ± SEM and analyzed by Student's t-test. *P<0.05, **P<0.01.

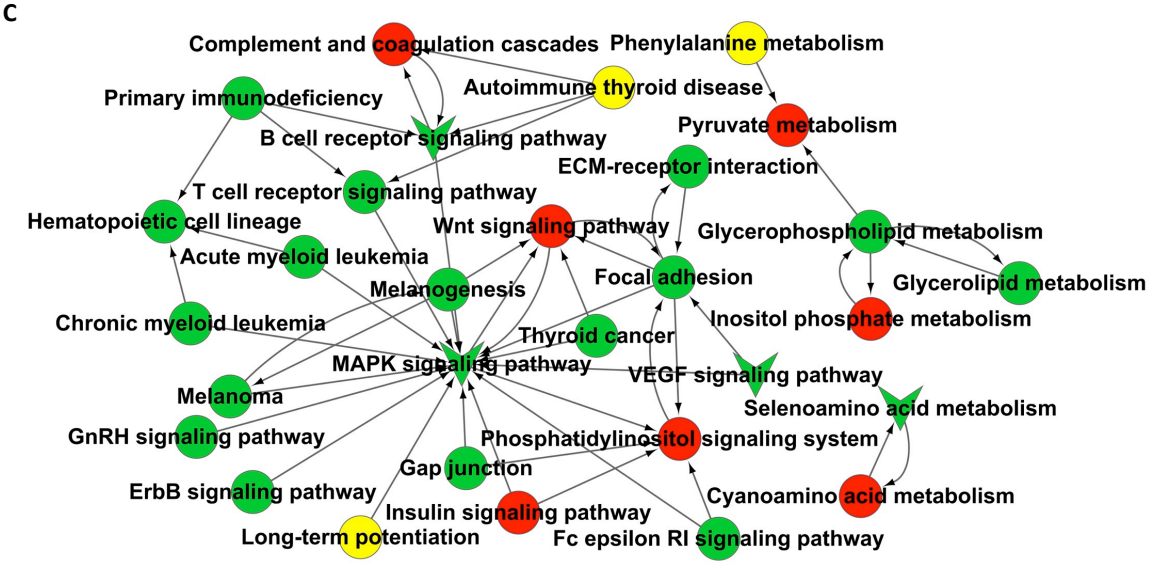
A

Groups	N
SIRT1(+/+) + Saline	3
SIRT1(+/-) + Saline	3
SIRT1(+/+) + Cisplatin	3
SIRT1(+/-) + Cisplatin	3

B

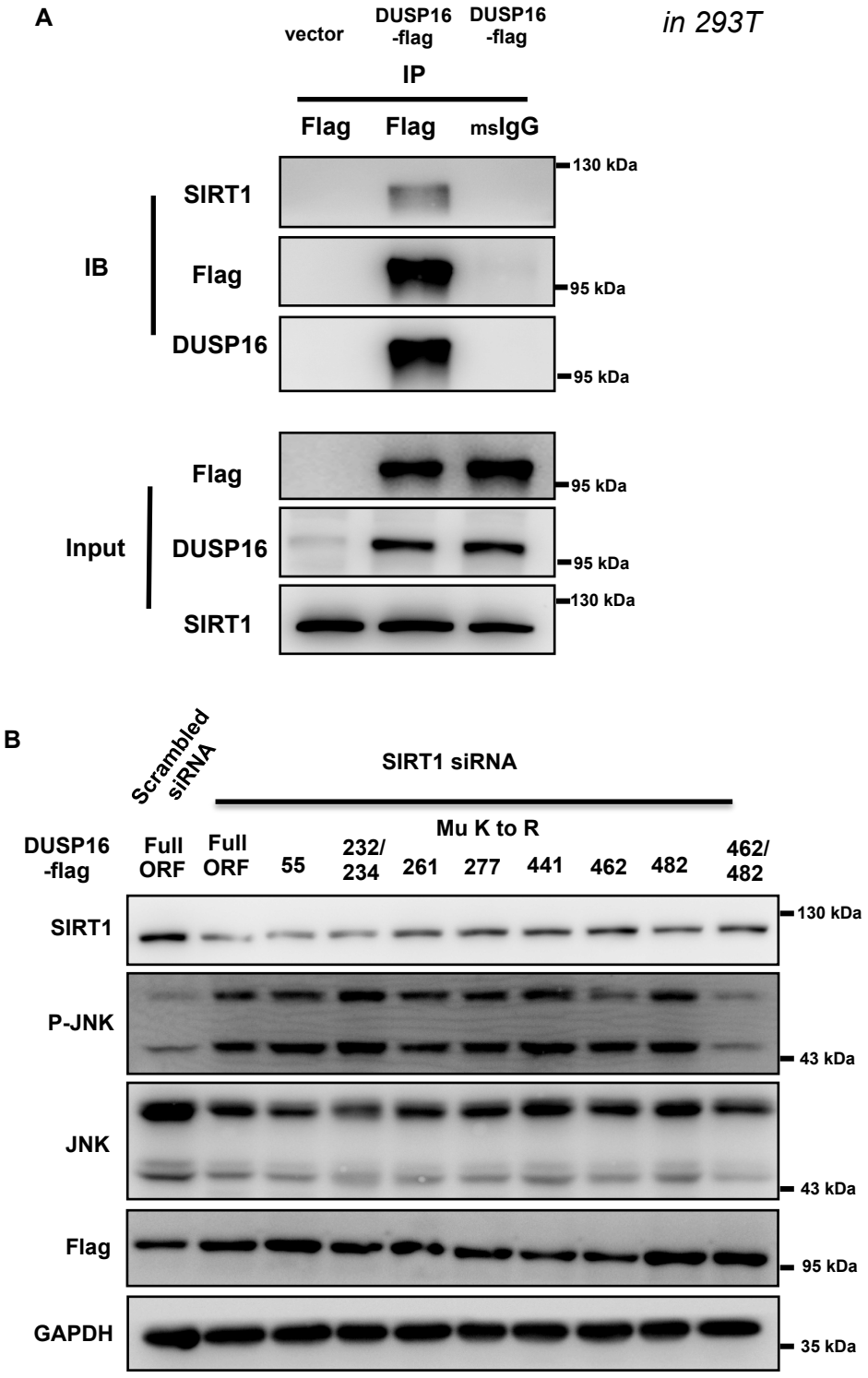


C



Sup5. Analysis of the mRNA array.

(A) Kidney samples admitted to the microarray study are listed. **(B)** Venn diagram representation of the subset of genes significantly altered (1.5-fold) with saline or cisplatin treatment in wild-type and SIRT1-deficient mice. **(C)** Pathway-Act-Network analysis based on the top 50 enriched pathway terms annotated by the overlapped genes in Venn diagram. Red represents the pathway involved with the up-regulated genes, green represents the pathway involved with the down-regulated genes, yellow represents the pathway involved with both the up-regulated and down-regulated genes and the arrow represents the salient pathway. (the saline group compared to cisplatin group)



Sup6. Interaction between SIRT1 and DUSP16.

(A) The interaction between SIRT1 and DUSP16 was determined by immunoprecipitation of flag in 293T cells transfected with flag-tagged DUSP16 plasmid. **(B)** HK2 cells were co-transfected with siRNA (scrambled or anti-SIRT1) and flag-tagged DUSP16 plasmid (full ORF or K to R mutant) as indicated. JNK phosphorylation was induced by SIRT1 deficiency and deterred by Lys^{462} and Lys^{482} of DUSP16 mutation to Arg. Efficiency of transfection was determined by Western blotting of SIRT1 and Flag. GAPDH was used as loading control. Data are representative of three independent experiments.