Expanded View Figures

Figure EV1. TRIM21 is a binding partner for SIRT5.

A Indirect immunofluorescence assay of 293T cells transfected with indicated constructs. Scale bar: 10 μm.

B BMDM cells were fractionated. Total cell lysates from BMDM cells, and cytoplasmic, mitochondria and nuclear protein extracts were used for IP assay with anti-TRIM21 antibody and analyzed by Western blot using indicated antibodies. GAPDH, COXIV and total H3 were used as fraction and loading controls respectively.

C Immunoblot (IB) analysis of whole-cell lysates (WCLs) and immunoprecipitates (IPs) derived from 293T cells transfected with Flag-TRIM21 together with HA-SIRT5 constructs. Cells were treated with 10 μM MG132 for 4 h before harvesting.

D Purified HA-SIRT5 protein were incubated with purified recombinant Flag-TRIM21 proteins as indicated for 90 min at 30°C, followed by pull down with anti-HA antibody and immunoblot analysis.

E (top panel) Scheme for generation of the expression constructs encoding the serial deletions of SIRT5 as indicated. (bottom panel) IB analysis of WCLs and IPs derived from 293T cells transfected with Flag-TRIM21 together with the indicated SIRT5 deletion constructs. Cells were treated with 10 μM MG132 for 4 h before harvesting.

F (top panel) Scheme for generation of the expression constructs encoding the serial deletions of TRIM21 as indicated. (bottom panel) IB analysis of WCLs and IPs derived from 293T cells transfected with HA-SIRT5 together with the indicated TRIM21 deletion constructs. Cells were treated with 10 μM MG132 for 4 h prior to harvesting.

G IB analysis of WCLs and immunoprecipitates derived from 293T cells transfected with Flag-TRIM21 together with HA-SIRT5 constructs in the presence or absence of 3 mM NAD^+ or NADH as indicated. Cells were treated with 10 μM MG132 for 4 h before harvesting.

Data information: All IP and WB data in this work otherwise indicated are representative of at least three independent experiments.

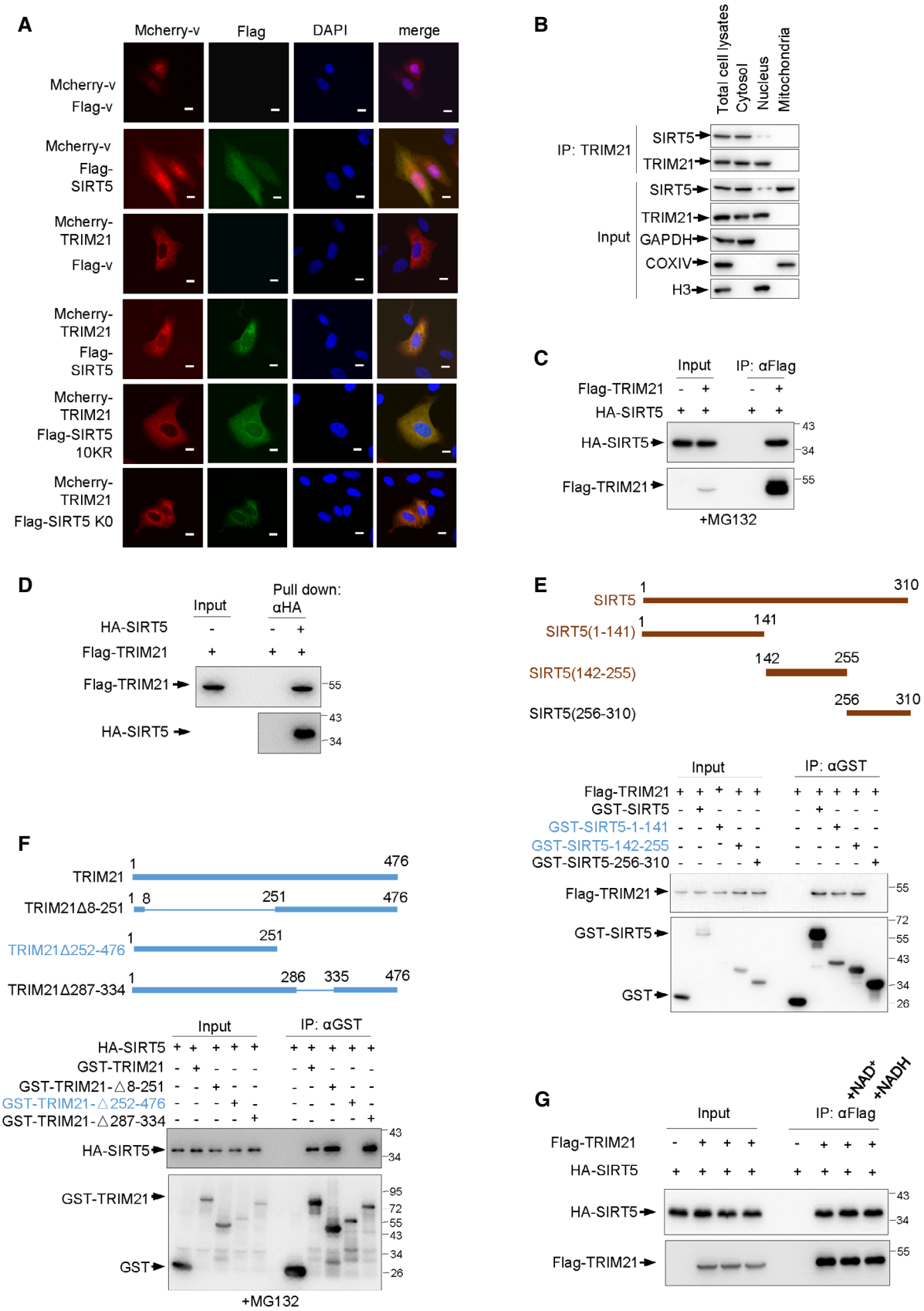

A
Mcherry-v
Flag
DAPI
merge
Mcherry-v
Flag-v
Mcherry-v
Flag-SIRT5
Mcherry-TRIM21
Flag-v
Mcherry-TRIM21
Flag-SIRT5
Mcherry-TRIM21
Flag-SIRT5 10KR
Mcherry-TRIM21
Flag-SIRT5 K0
B
Total cell lysates
Cytosol
Nucleus
Mitochondria
IP: TRIM21
SIRT5
TRIM21
Input
SIRT5
TRIM21
GAPDH
COXIV
H3
C
Input
IP: αFlag
Flag-TRIM21
HA-SIRT5
HA-SIRT5
Flag-TRIM21
43
34
55
+MG132
D
Input
Pull down: αHA
HA-SIRT5
Flag-TRIM21
Flag-TRIM21
HA-SIRT5
55
43
34
E
SIRT5
SIRT5(1-141)
SIRT5(142-255)
SIRT5(256-310)
1
310
141
142
255
256
Input
IP: αGST
Flag-TRIM21
GST-SIRT5
GST-SIRT5-1-141
GST-SIRT5-142-255
GST-SIRT5-256-310
Flag-TRIM21
GST-SIRT5
GST
55
72
43
34
26
F
TRIM21
TRIM21Δ8-251
TRIM21Δ252-476
TRIM21Δ287-334
476
8
251
286
335
Input
IP: αGST
HA-SIRT5
GST-TRIM21
GST-TRIM21-△8-251
GST-TRIM21-△252-476
GST-TRIM21-△287-334
HA-SIRT5
GST-TRIM21
GST
95
72
55
43
34
26
+MG132
G
+NAD+
+NADH
Input
IP: αFlag
Flag-TRIM21
HA-SIRT5
HA-SIRT5
Flag-TRIM21

Figure EV1.

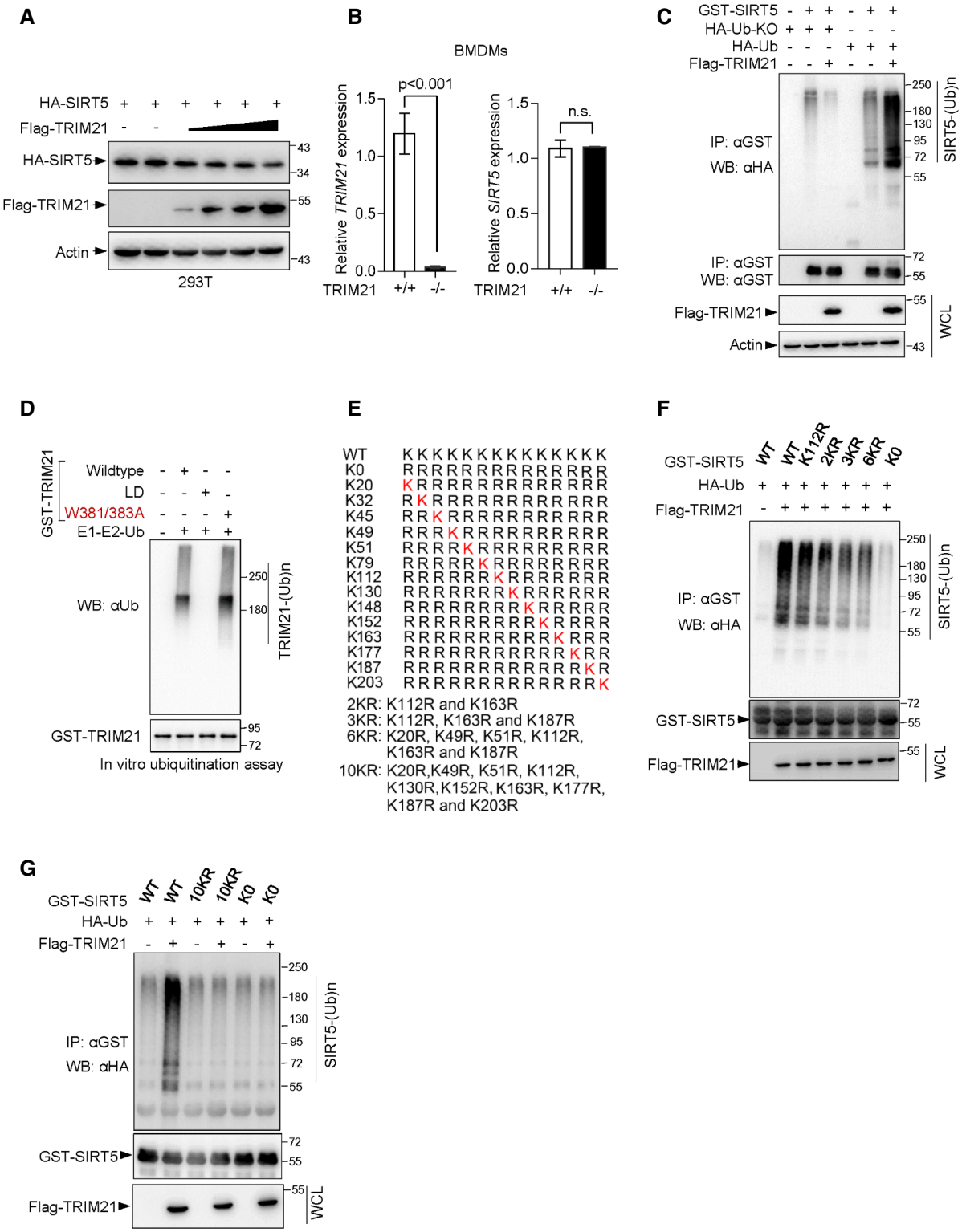


Figure EV2.

Figure EV2. Identification of the lysine residues in SIRT5 that are responsible for TRIM21 ubiquitination.

A IB analysis of WCLs from 293T cells transfected with HA-SIRT5 together with increasing amounts of Flag-TRIM21 constructs.
B mRNA levels of TRIM21 and SIRT5 in *TRIM21*$^{+/+}$ and *TRIM21*$^{-/-}$ BMDMs were analyzed by qRT-PCR. Data are means ± SD. $n = 3$ biological replicates. *P*-values were determined by unpaired two-tailed Student's *t*-tests. ns, not significant.
C IB analysis of WCLs and anti-GST immunoprecipitated products derived from 293T cells transfected with indicated constructs. Cells were treated with 10 μM MG132 for 4 h before harvesting.
D Purified TRIM21 and its mutant proteins as indicated were incubated with purified E1, E2 (UBE2D2) and ubiquitin at 30°C for 90 min. TRIM21 ubiquitination and protein levels were analyzed by Western blotting.
E Mutants of human SIRT5 in which various lysine residues (K) are substituted with arginine (R).
F, G *In vivo* ubiquitination assay analysis of WCLs and anti-GST immunoprecipitated products derived from 293T cells transfected with the indicated plasmids. 10 μM MG132 was added for 4 h before harvesting.

Data information: All IP and WB data in this work otherwise indicated are representative of at least three independent experiments.

Figure EV3. HAUSP physically interacts with SIRT5.

A IB analysis of WCLs and immunoprecipitates derived from 293T cells transfected with Flag-HAUSP together with HA-SIRT5 constructs. Cells were treated with 10 μM MG132 for 4 h before harvesting.
B Purified GST-SIRT5 proteins were incubated with purified recombinant Flag-HAUSP proteins as indicated for 90 min at 30°C, followed by pull down with anti-GST antibody and Western blot analysis.
C IB analysis of WCLs and IPs derived from 293T cells transfected with Flag-HAUSP together with the indicated SIRT5 deletion constructs. Cells were treated with 10 μM MG132 for 4 h before harvesting.
D IB analysis of WCLs and immunoprecipitates derived from 293T cells transfected with indicated constructs. Cells were treated with 10 μM MG132 for 4 h before harvesting.
E IB analysis of WCLs and immunoprecipitates derived from 293T cells transfected with Flag-HAUSP together with HA-SIRT5 constructs in the presence or absence of 3 mM NAD^+ or NADH as indicated. Cells were treated with 10 μM MG132 for 4 h before harvesting.
F IB analysis of WCLs and immunoprecipitates derived from 293T cells transfected with Flag-HAUSP together with HA-SIRT5, HA-SIRT5-K0, HA-SIRT5-10KR as indicated. Cells were treated with 10 μM MG132 for 4 h before harvesting.
G 293T cells were transfected with increasing amounts of Flag-HAUSP construct and endogenous SIRT5 expression was analyzed by Western blotting.
H IB analysis of WCLs and immunoprecipitates derived from 293T cells transfected with indicated constructs. Cells were treated with 10 μM MG132 for 4 h before harvesting.
I mRNA levels of HAUSP and SIRT5 in BMDMs transfected with control siRNA (−) or HAUSP siRNA for 48 h were measured by qRT-PCR. Data are means ± SD. $n = 3$ biological replicates. *P*-values were determined by unpaired two-tailed Student's *t*-tests. ns, not significant.

Data information: All IP and WB data in this work otherwise indicated are representative of at least three independent experiments.

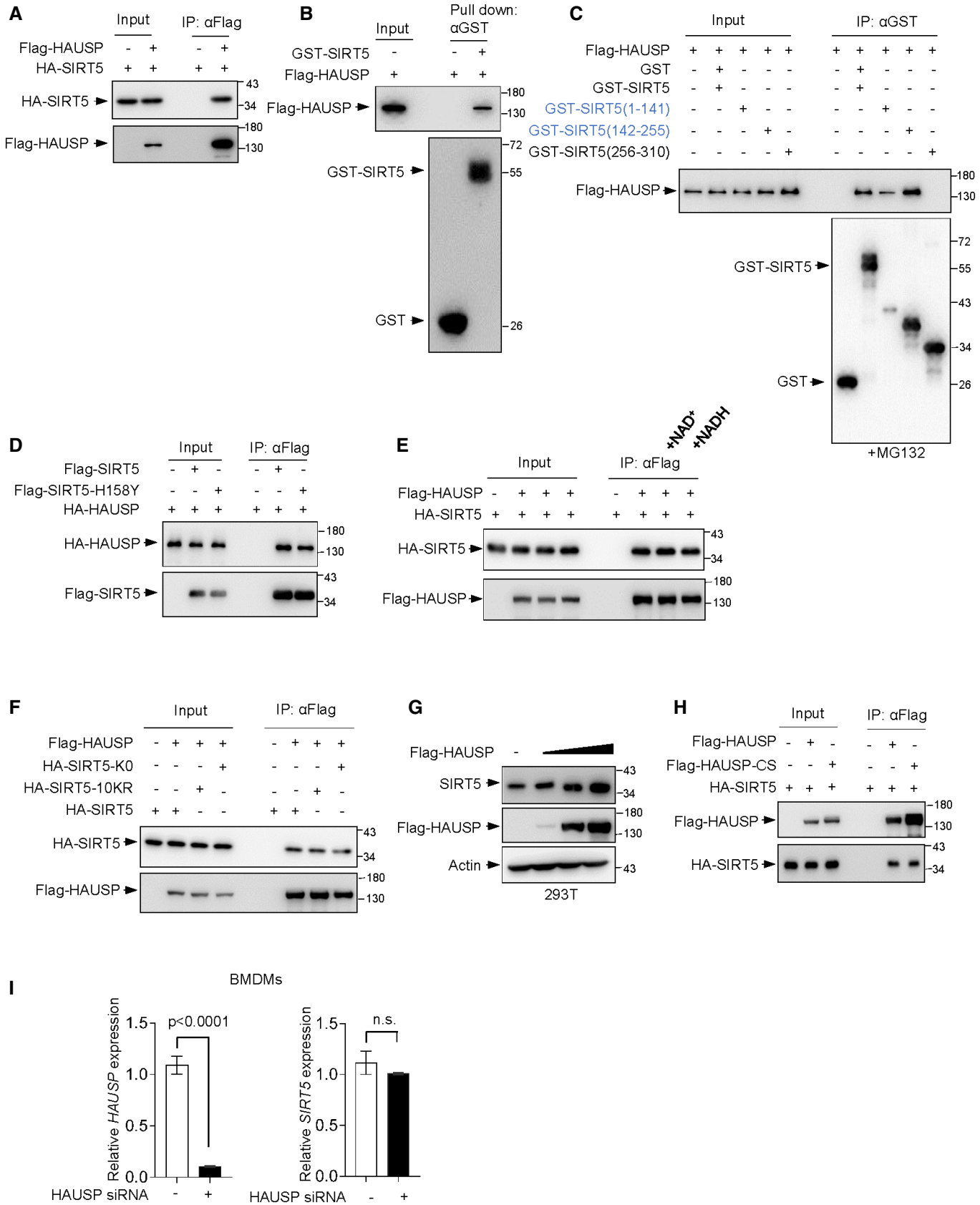


Figure EV3.

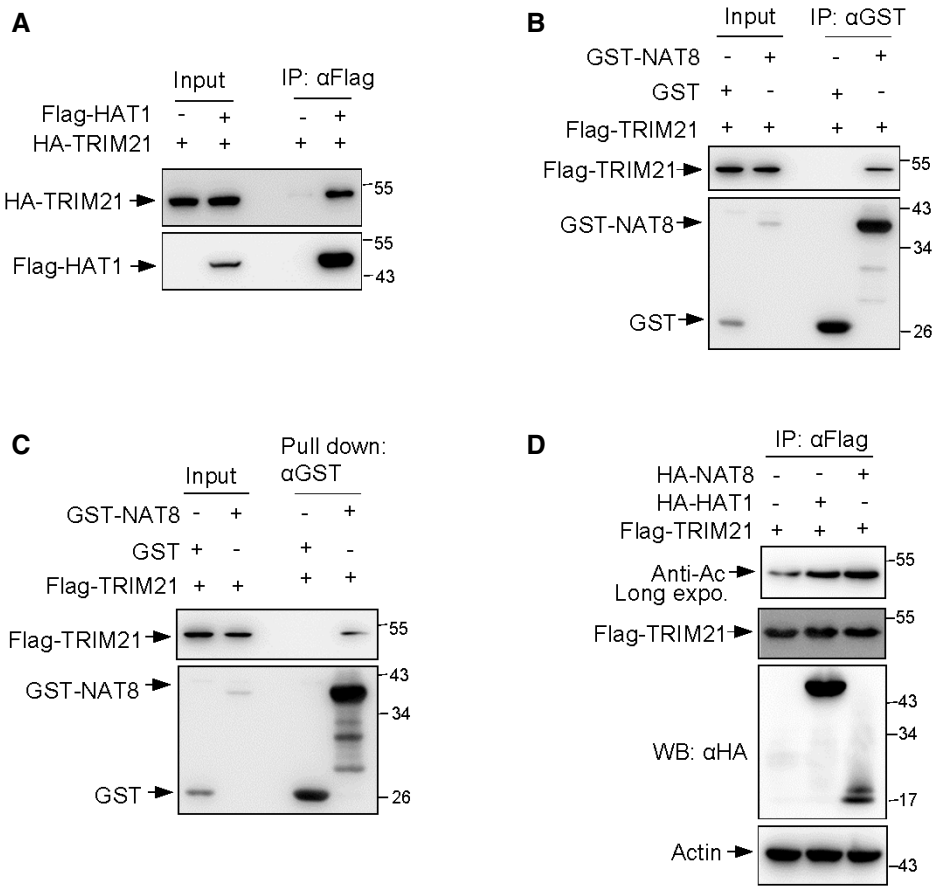


Figure EV4. Both HAT1 and NAT8 can bind to TRIM21.

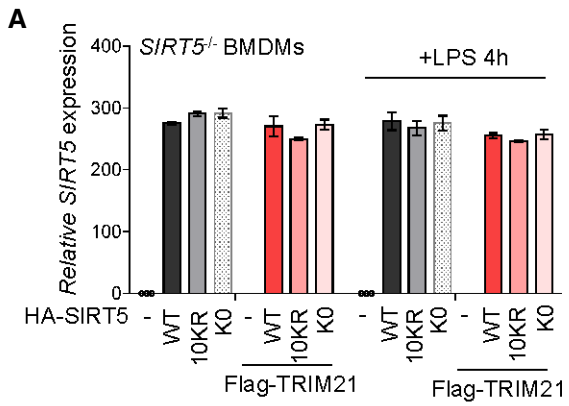
A IB analysis of WCLs and immunoprecipitates derived from 293T cells transfected with Flag-HAT1 together with Flag-TRIM21 constructs.

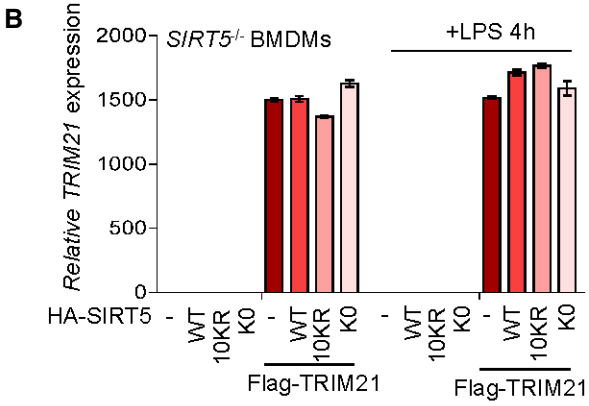
B IB analysis of WCLs and anti-GST immunoprecipitated products derived from 293T cells transfected with indicated constructs.

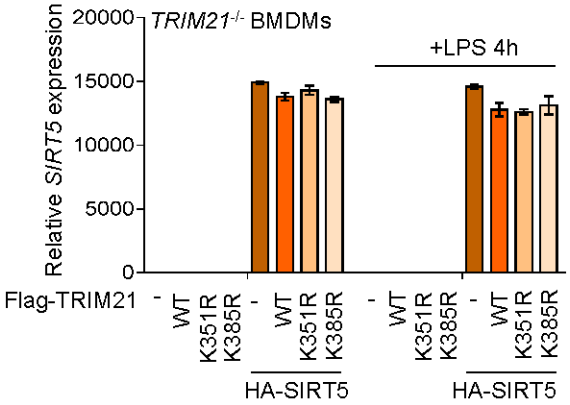
C Purified Flag-TRIM21 proteins were incubated with purified GST or GST-NAT8 proteins as indicated for 90 min at 30°C, followed by pull down with anti-GST antibody and Western blot analysis.

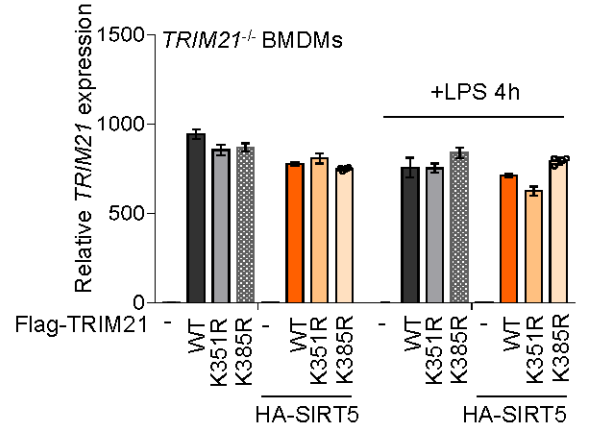
D IB analysis of WCLs and anti-Flag immunoprecipitated products derived from 293T cells transfected with the indicated plasmids. 10 μM MG132 was added for 4 h before harvesting.

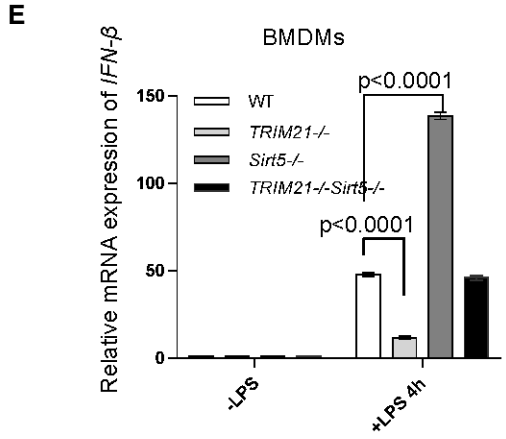
Data information: All IP and WB data in this work otherwise indicated are representative of at least three independent experiments.











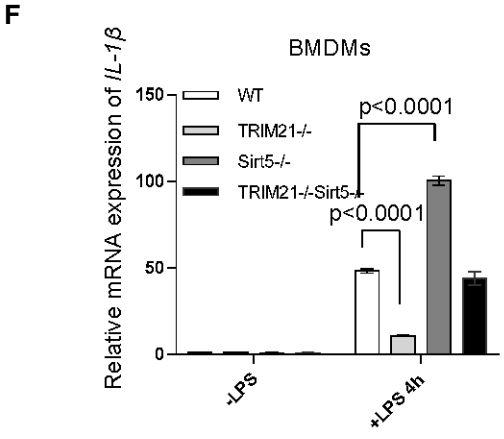


Figure EV5.

Figure EV5. Effect of SIRT5 and TRIM21 mutual regulation on macrophage pro-inflammatory cytokine production.

A, B mRNA expression of SIRT5 (A) and TRIM21 (B) in LPS-treated and untreated *SIRT5*$^{-/-}$ BMDMs expressing Flag-TRIM21 together with HA-SIRT5 or various HA-tagged SIRT5 mutants as indicated was determined by qRT-PCR analysis and normalized to β-actin. Macrophages were stimulation with 100 ng/ml LPS for 4 h.

C, D mRNA expression of SIRT5 (C) and TRIM21 (D) in LPS-treated and untreated *TRIM21*$^{-/-}$ BMDMs stably expressing HA-SIRT5 together with Flag-TRIM21 or various Flag-tagged TRIM21 mutants as indicated was determined by qRT-PCR analysis and normalized to β-actin. Macrophages were stimulation with 100 ng/ml LPS for 4 h.

E, F mRNA expression of IFN-β (E) and IL-1β (F) in LPS-treated and untreated BMDMs derived from wild-type (WT), *TRIM21*$^{-/-}$, *SIRT5*$^{-/-}$ and *TRIM21*$^{-/-}$*SIRT5*$^{-/-}$ mice. Macrophages were stimulation with 100 ng/ml LPS for 4 h.

Data information: Data are means ± SD. In (A–F), $n = 3$ biological replicates. *P*-values were determined by unpaired two-tailed Student's *t*-tests. ns, not significant.