Expanded View Figures

Figure EV1. rs350845 (chr19:4174953:A:G) is a *cis*-eQTL for *SIRT6* upregulation across multiple tissues.

A GTEx tissue types are shown sorted by m-value, which is the posterior probability that an eQTL exists for *SIRT6* in a specific tissue from a cross-tissue meta-analysis. NES = Normalized Effect Size based on single-tissue analysis; *P*-value is from a t-test comparing observed NES to a null within a single tissue.

B Single-tissue eQTL *P*-value vs. m-values.

C Violin plots showing *SIRT6* expression differences across three example tissues with m-value = 1. Reference allele is G and alternate allele is A, and each panel shows the normalized expression of *SIRT6* in a different tissue. Values beneath genotypes represent a number of individuals.

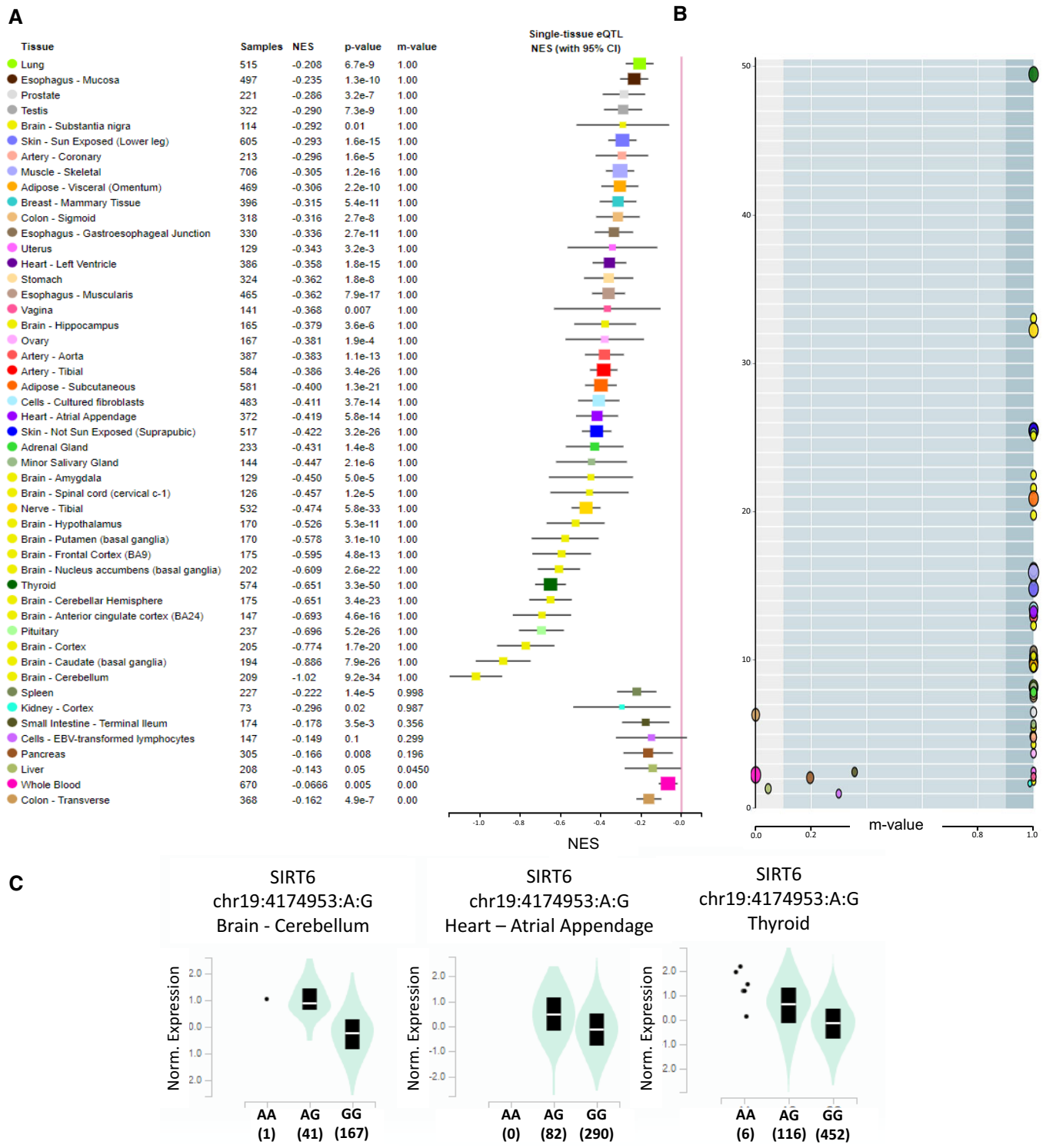

Figure EV1.

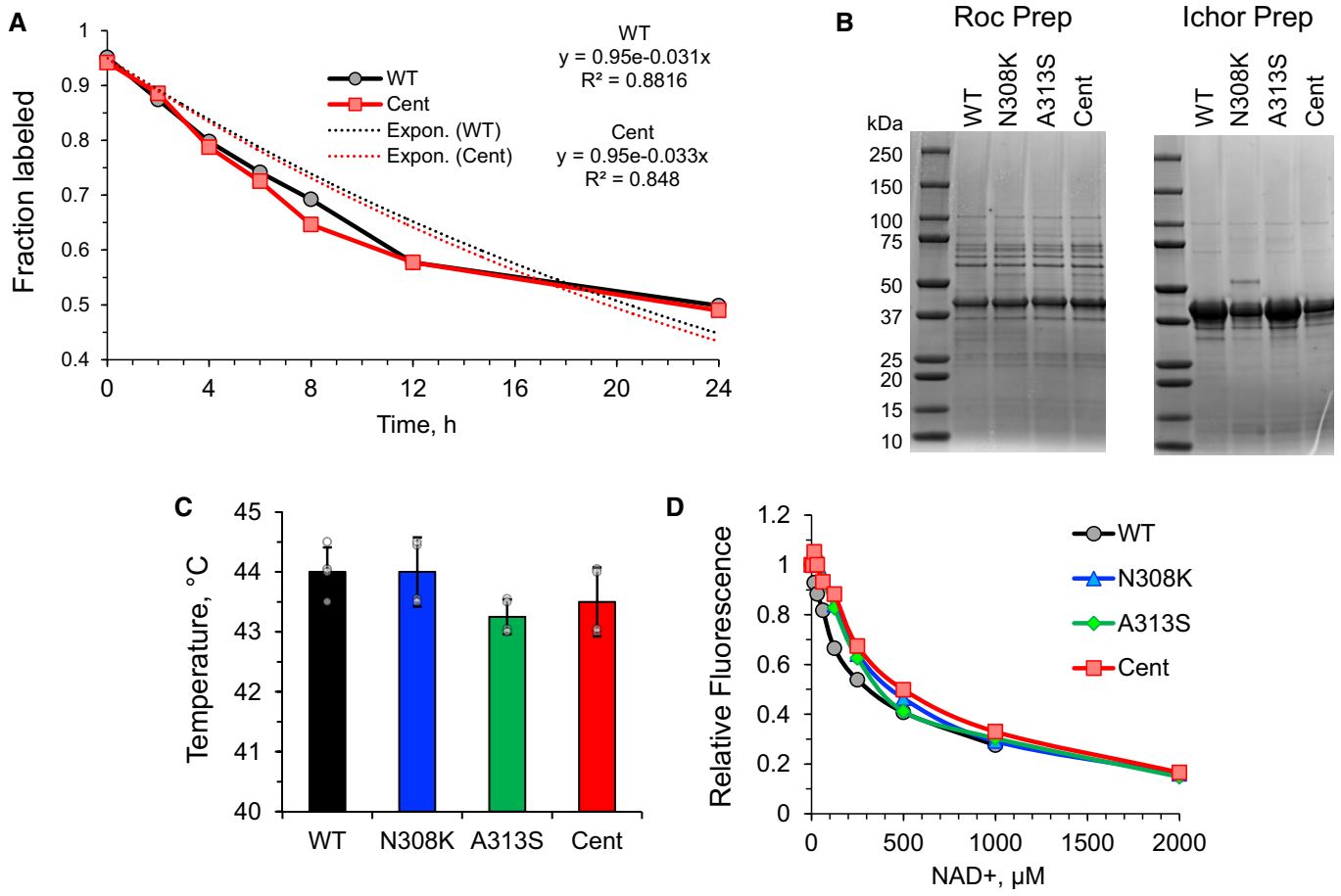

Figure EV2. Turnover rate and biochemical properties of purified SIRT6 proteins.

A Protein turnover rate. SILAC analysis on HEK293 cells expressing SIRT6 variants.

B Coomassie staining of SIRT6 purified from Rosetta-Gami *E. coli* cells. Proteins were purified at the University of Rochester (Roc) and the Ichor Therapeutics facility in Ithaca. All *in vitro* experiments were performed with both protein preparations and the data was consistent between the two preps.

C Thermostability of purified SIRT6 proteins. Data represent two replicates with two technical replicates each using SIRT6 from the Roc and Ichor preps. $n = 4$ TR; error bars = SD. Student's *t*-test, two-tailed.

D Tryptophan fluorescence curves for SIRT6 variants with titrated concentrations of NAD^+.

Figure EV3. HeLa histone deacetylation by recombinant SIRT6, and SIRT6 expression in cumate-inducible cell lines.

A *In vitro* deacetylation rates of purified SIRT6 proteins with histones purified from HeLa cells. H3K9ac, H3K18ac, and H3 are immunoblots, SIRT6 loading Coomassie stain. Control lanes, from the same gels, were repositioned for consistency. Uncut gels are shown in Appendix Fig S1. Asterisks indicate $P < 0.05$ compared with WT. $n = 3$ TR; error bars = SD. Student's *t*-test, two-tailed.

B SIRT6 expression in cumate-inducible telomerase-immortalized HCA2 human fibroblast cell lines. SIRT6 alleles were integrated into the genome of SIRT6 knockout HCA2 cells using PiggyBac Transposon Vector System. Different dosages of cumate and resulting SIRT6 abundance were used to determine the dose needed to achieve equivalent SIRT6 expression for each cell line. Cells were normalized by count and total protein. Subsequent experiments utilizing cumate-inducible SIRT6 fibroblasts were controlled for SIRT6 abundance using these data. For each cell line, the red box indicates the corresponding cumate dosage for equivalent expression (WT = 60 μg/ml, N308K = 30 μg/ml, A313S = 30 μg/ml, and Cent = 7.5 μg/ml). These concentrations were used in experiments with these cells.

C qRT–PCR expression analysis of SIRT6 using standardized dosages of cumate (colored bars are cells treated with cumate, and empty bars are uninduced controls). Cumate dosage corresponds to red boxes in Appendix Fig S2B). Actin was used for standardization and samples were normalized to uninduced WT. $n = 3$ BR; error bars = SD. Student's *t*-test, two-tailed.

D Apoptosis assay of HCA2 cells treated with varying doses of cumate. $n = 3$ TR; error bars = SD. Student's *t*-test, two-tailed.

E Population doubling rate of HCA2 cells treated with different doses of cumate to assess the impact on cell division.

F Coomassie-stained SDS–PAGE of histone preparations for quantitative mass spectrometry.

Source data are available online for this figure.

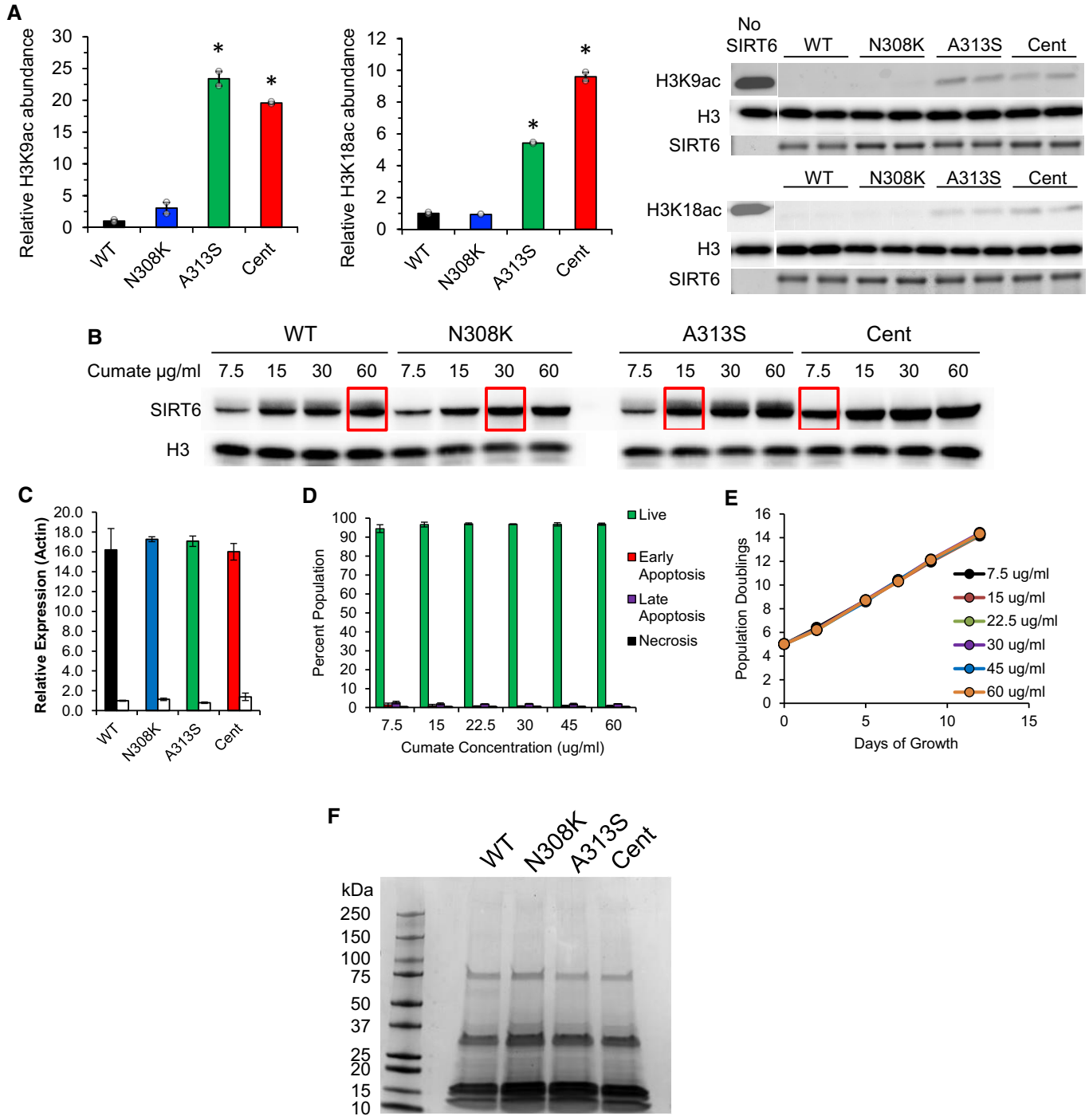

Figure EV3.

Figure EV4. Quantification of SIRT6 expression and FACS gating for DNA repair assays.

A Representative images of FACS analysis for DNA DSB repair assays (Fig 4D and E).
B Representative images of basal γH2AX immunostaining (Fig 4F). Dashed outline denotes nucleus borders. Scale bar 10 µm.
C Representative images of γH2AX immunostaining after γ-irradiation (Fig 4G). Scale bar 10 µm.
D Representative images of FACS traces for paraquat sensitivity assay (Fig 4H and I).

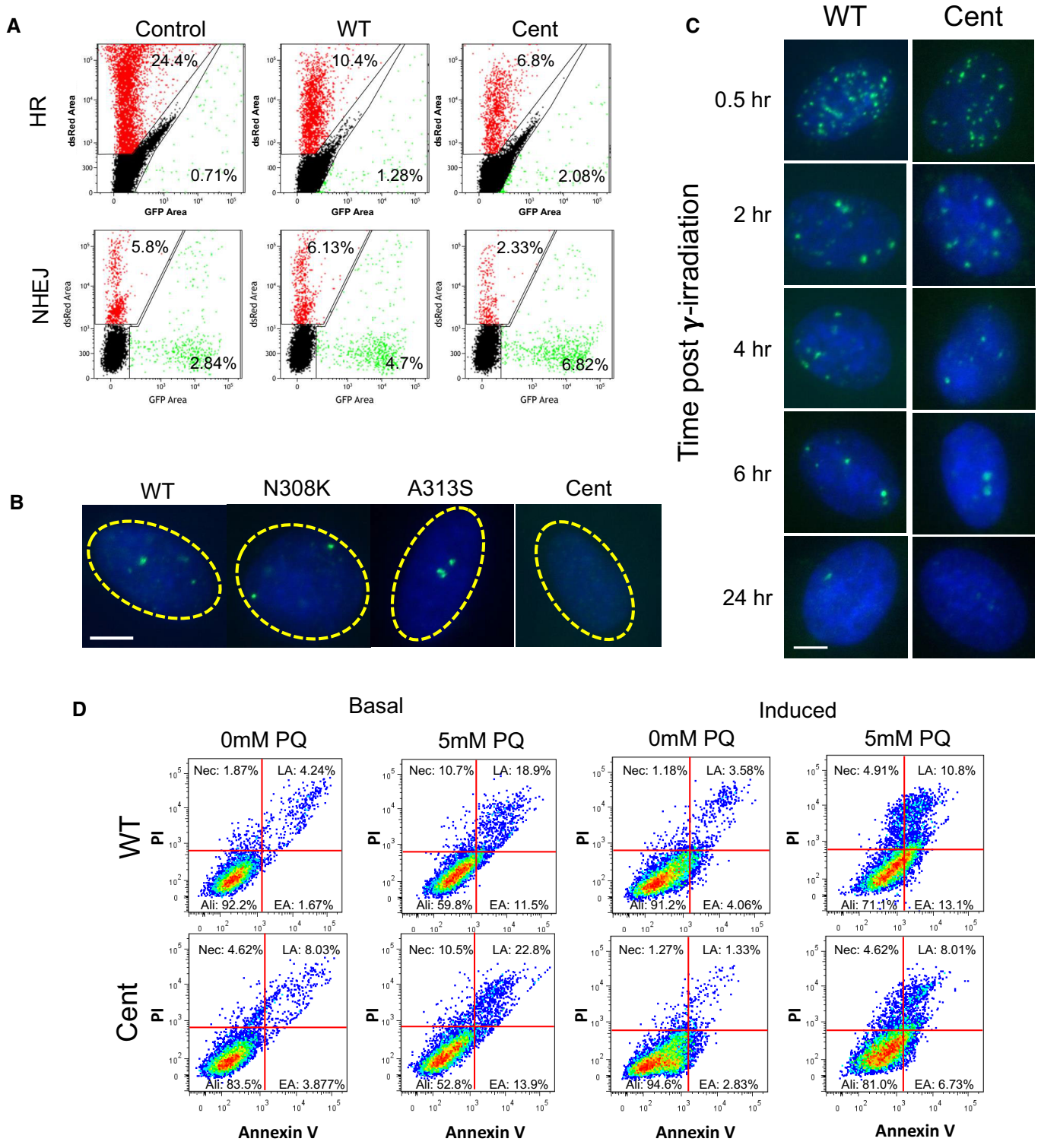
A
Control
WT
Cent
HR
NHEJ
24.4%
0.71%
10.4%
1.28%
6.8%
2.08%
5.8%
2.84%
6.13%
4.7%
2.33%
6.82%
dsRed Area
GFP Area
B
WT
N308K
A313S
Cent
C
WT
Cent
0.5 hr
2 hr
4 hr
6 hr
24 hr
Time post γ-irradiation
D
Basal
Induced
0mM PQ
5mM PQ
WT
Cent
PI
Annexin V
Nec: 1.87%
LA: 4.24%
Ali: 92.2%
EA: 1.67%
Nec: 10.7%
LA: 18.9%
Ali: 59.8%
EA: 11.5%
Nec: 1.18%
LA: 3.58%
Ali: 91.2%
EA: 4.06%
Nec: 4.91%
LA: 10.8%
Ali: 71.1%
EA: 13.1%
Nec: 4.62%
LA: 8.03%
Ali: 83.5%
EA: 3.877%
Nec: 10.5%
LA: 22.8%
Ali: 52.8%
EA: 13.9%
Nec: 1.27%
LA: 1.33%
Ali: 94.6%
EA: 2.83%
Nec: 4.62%
LA: 8.01%
Ali: 81.0%
EA: 6.73%

Figure EV4.

Figure EV5. Analysis of CRISPR/Cas9-edited human MSC cell lines.

A, B Double strand repair efficiency in wild-type and centSIRT6 hMSCs. DSB repair reporter constructs were integrated into hMSCs. After 72 h recovery, reactivation of the GFP reporter was measured by flow cytometry. Stimulation of NHEJ or HR was calculated as the ratio of $GFP^+/DsRed^+$ positive cells. Asterisks indicate $P < 0.05$. $n = 3$ BR; error bars = SD. Student's *t*-test, two-tailed.

C Cell viability in MMS-treated wild-type and centSIRT6 hMSCs. hMSCs were treated with MMS for 48 h and cell viability was evaluated by MTS assay. Data were normalized to the control group (0 mM). Asterisks indicate $P < 0.05$. $n = 6$ BR; error bars = SD. Student's *t*-test, two-tailed.

D Immunofluorescence staining of 53BP1 in wild-type and centSIRT6 hMSCs under MMS treatment. Numbers of 53BP1 foci in the nuclei of wild-type and centSIRT6 hMSCs with or without MMS (0.25 mM) treatment were quantified. >600 nuclei from 10 TR images were scored. Asterisks indicate $P < 0.05$. Error bars = SD. Student's *t*-test, two-tailed.

E qRT–PCR of NRF2 stress response pathway genes in wild-type and centSIRT6 hMSCs. $n = 3$ BR; error bars = SEM. Student's *t*-test, two-tailed.

F RNAseq expression of NRF2 stress response pathway genes in wild-type and centSIRT6 hMSCs. $n = 4$ BR; error bars = SEM. Student's *t*-test, two-tailed.

G Western blot analysis of SIRT6 in wild-type and centSIRT6 hMSCs. β-Actin was used as a loading control and used to normalize quantification. Asterisks indicate $P < 0.05$. $n = 2$ BR; error bars = SD. Student's *t*-test, two-tailed.

Source data are available online for this figure.

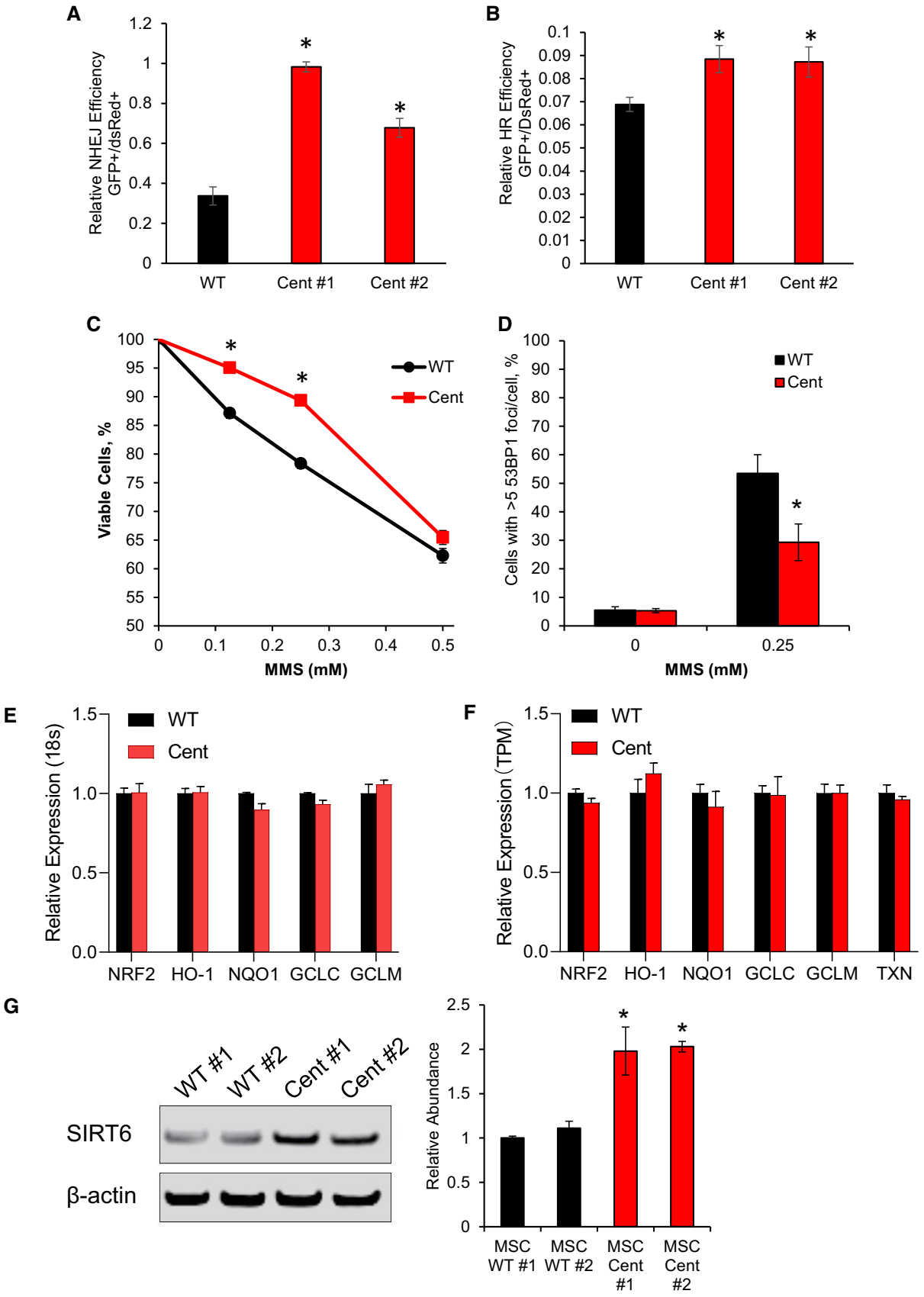
A
Relative NHEJ Efficiency GFP+/dsRed+
1.2
1
0.8
0.6
0.4
0.2
0
*
*
WT
Cent #1
Cent #2
B
Relative HR Efficiency GFP+/DsRed+
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
*
*
WT
Cent #1
Cent #2
C
Viable Cells, %
100
95
90
85
80
75
70
65
60
55
50
0
0.1
0.2
0.3
0.4
0.5
MMS (mM)
WT
Cent
*
*
D
Cells with >5 53BP1 foci/cell, %
100
90
80
70
60
50
40
30
20
10
0
0
0.25
MMS (mM)
WT
Cent
*
E
Relative Expression (18s)
1.5
1.0
0.5
0.0
WT
Cent
NRF2
HO-1
NQO1
GCLC
GCLM
F
Relative Expression (TPM)
1.5
1.0
0.5
0.0
WT
Cent
NRF2
HO-1
NQO1
GCLC
GCLM
TXN
G
WT #1
WT #2
Cent #1
Cent #2
SIRT6
β-actin
Relative Abundance
2.5
2
1.5
1
0.5
0
*
*
MSC WT #1
MSC WT #2
MSC Cent #1
MSC Cent #2

Figure EV5.