# Appendix

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Appendix Figure S1: AM gating strategy used for testing efficiency of SIRT1 inactivation by Cripsr/Cas9
A: FACS gating strategy showing enrichment of BFP<sup>hi</sup> AM. Alveolar macrophages were harvested by bronchoalveolar lavage from Rosa26-Cas9p2aGFP transgenic mice (Chu et al, 2016) and transduced with lentiviral supernatant, then selected with puromycin. Selected alveolar macrophages were subjected to FACS to enrich for BFP<sup>hi</sup> macrophages. PE detection = dump channel.
B Quantitative PCR showing efficiency of SIRT1 deletion (2 gRNA tested) in AM after puromycin selection.
C Tide analysis on sorted AM expressing Cas9 infected with lentivirus expressing gRNA sirt1 (2 gRNA tested). Tide analysis was performed as described (Brinkman et al, 2014).

Appendix Figure S2: Peritoneal macrophage gating strategy for testing efficiency of NAM inhibition of SIRT1
A: FACS gating strategy using F480, CD64, TIM4, Ly6C staining markers. F480<sup>hi</sup>, CD64<sup>+</sup>, Tim4<sup>-</sup> and F480<sup>hi</sup>, CD64<sup>+</sup>, Tim4<sup>+</sup> represent resident peritoneal macrophages. F480<sup>lo/-</sup>, CD64<sup>+</sup>, Ly6C<sup>+</sup> represent monocyte-derived macrophages.
B Percentage of Ki67<sup>+</sup> cells in indicated macrophage population of a representative experiment done twice.

Appendix Figure S3: AM gating strategy used for testing efficiency of NAM inhibition of SIRT1
FACS gating strategy. Single lived cells with SiglecF<sup>+</sup>, Cd11C<sup>+</sup> Cd11b<sup>-</sup> are tested for Ki67 staining.

Appendix Figure S4: No Impact on E2F and DP transcription factor mRNA expression after NAM treatment in Maf-DKO macrophages
Heatmap showing the normalized expression values of E2F and DP family transcription factors in microarray samples of Maf DKO macrophages treated or not with NAM for 1h or 10h.

Appendix Figure S5: GSEA analysis of apoptosis and p53 gene sets upon NAM mediated SIRT1 inhibition.
A GSEA analysis of untreated versus 10h NAM treated Maf-DKO macrophages with apoptosis-related gene set.
B GSEA analysis of untreated versus 10h NAM treated Maf-DKO macrophages with p53 pathway gene sets a from transcription factor targets collection V$P53-_DECAMER-_Q2 and V$P53-_02 gene sets.

Appendix Figure S6: SIRT3 knockdown reduces self-renewal ability in Maf-DKO macrophages
A Quantitative PCR for the expression of SIRT3 comparing shRNA-infected Maf-DKO macrophages to non infected cells Maf-DKO and wild type (WT) macrophages. Shown are average normalized to HPRT and standard error of the mean of two independent experiments.
B Effect of SIRT3 down-regulation on the colony formation potential of Maf-DKO macrophages. Phase contrast magnification X10. Each condition was done in duplicate and results shown are representative of two independent experiments.
C Quantification of panel C. Data shown are pooled from 2 independent experiments. Error bars indicate the standard error of the mean.
Appendix Figure S1

A

B

C

Total eff. = 60.9%

Total eff. = 44.7%
Appendix Figure S2

A

B

Tim4− PM macrophages

Tim4+ PM macrophages

Monocyte-derived macrophages

Ki67\% cells

-NAM

+NAM

-NAM

+NAM

-NAM

+NAM

-NAM

+NAM
Appendix Figure S3

Isotype control with the same Gating strategy
Appendix Figure S4
Appendix Figure S5

A

APOPTOSIS_GO

B

PS1_DECAMER

PS3

NE1: -0.21
mean absolute value: 0.36
STD: 0.47

NE1: -0.79
mean absolute value: 0.55
STD: 0.80

NE1: -0.65
mean absolute value: 0.77
STD: 0.66
Appendix Figure S6

A

Sirt3 expression

Fold change compared to WT BMM

B

no shRNA  LacZ

Sirt3 #1  Sirt3 #2

C

CFU-M / 500 cells

DKO no shRNA  DKO LacZ  DKO Sirt3 #1  DKO Sirt3 #2