Appendix

Title: MiR-342 controls Mycobacterium tuberculosis susceptibility by modulating inflammation and cell death

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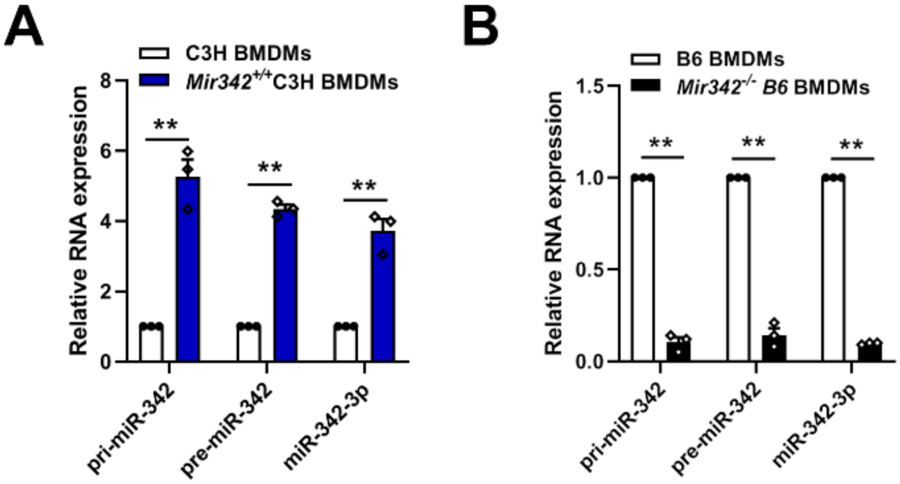
Appendix Figures and Figure Legends

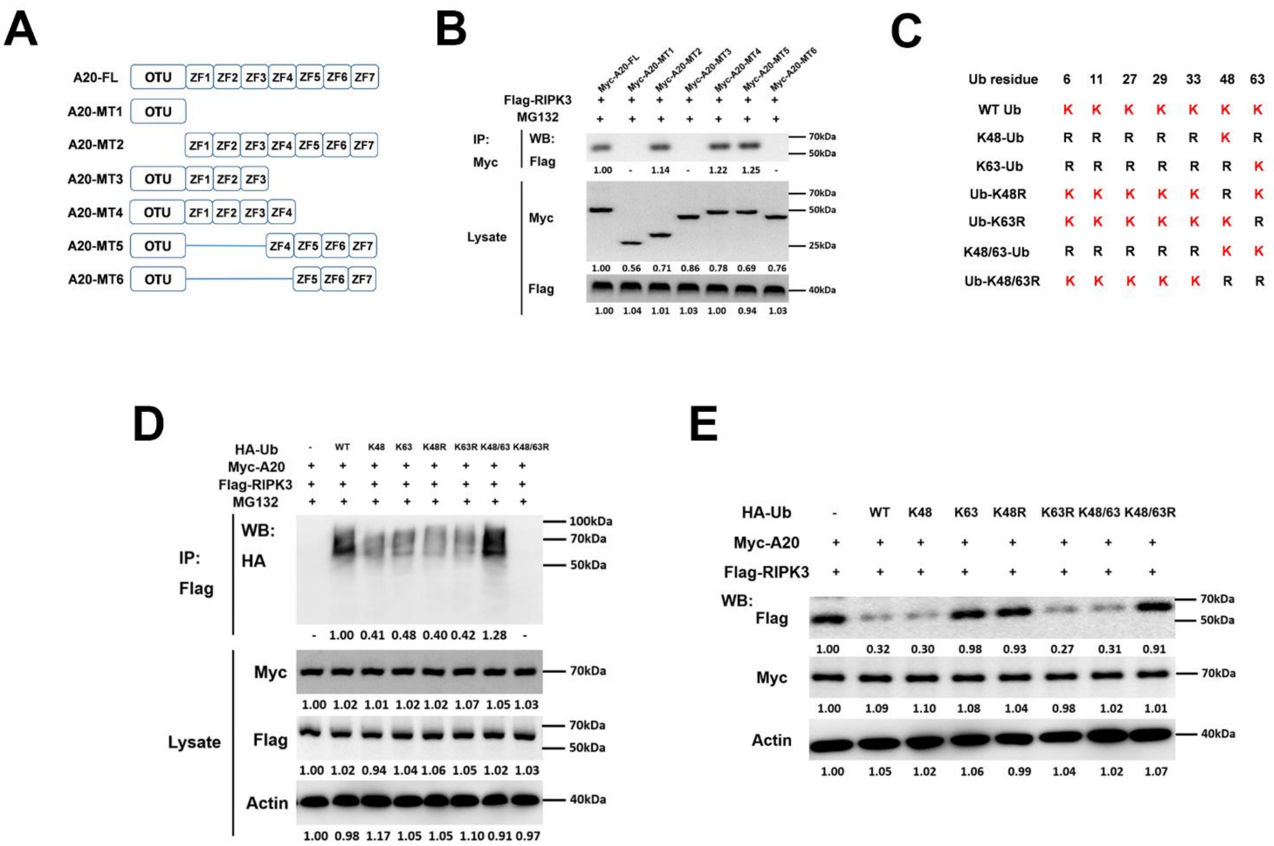
Appendix Figure S1 **Validation of *Mir342*$^{+/+}$C3H and *Mir342*$^{-/-}$B6 mice**



(A, B) Relative expressions of miR-342-3p in BMDMs from *Mir342*$^{+/+}$C3H (A) and *Mir342*$^{-/-}$B6 (B) mice were analyzed by qRT-PCR. Data are shown as the mean ± s.e.m. of n = 3 biological replicates.

Data information: ANOVA followed by Bonferroni post *hoc* test was used for data analysis. **, $p < 0.01$.

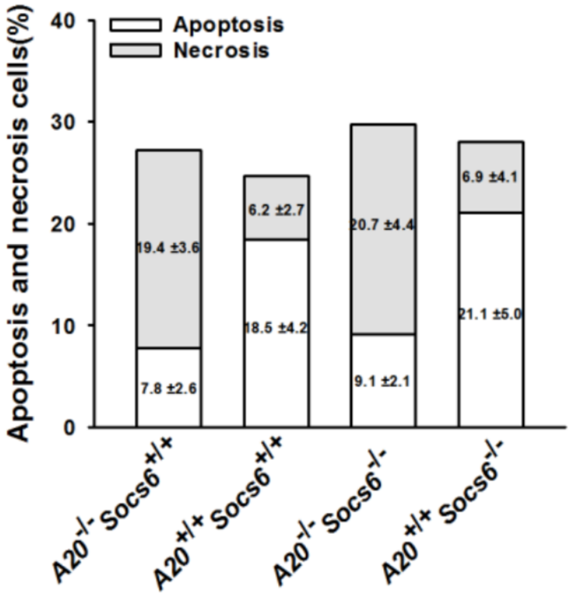
Appendix Figure S2 **A20 promotes K48-linked polyubiquitination of RIPK3**



(A, B) Schematic of full-length or truncated A20 (A) and results from immunoprecipitation assays showing the binding regions between A20 and RIPK3 (B, representative blots from n = 3 biological replicates are shown).

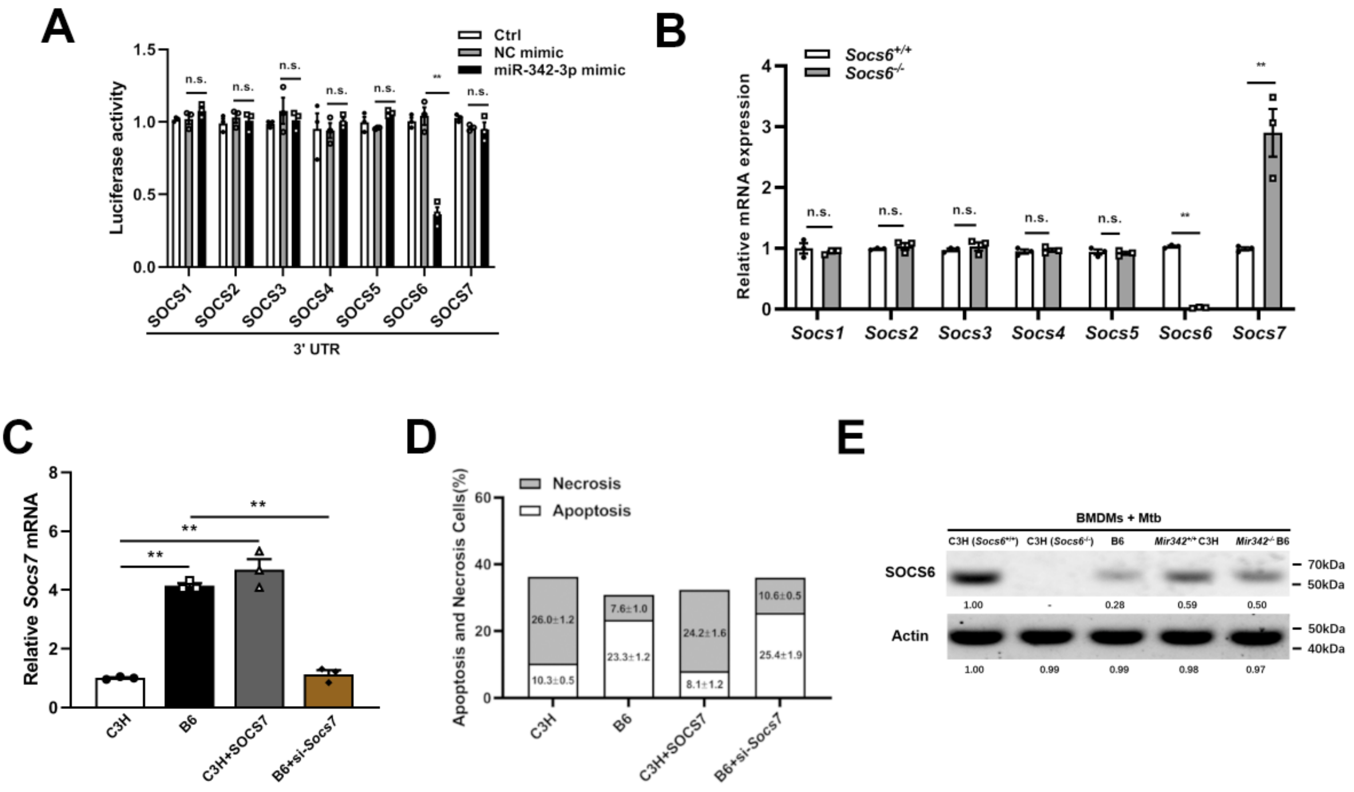
(C-E) Schematic of wild-type or mutated ubiquitin (K, Lysine; R, Arginine) (C), and results from immunoprecipitation assays showing the specific lysine-linked ubiquitin chains of RIPK3 (D, E, representative blots from n = 3 biological replicates are shown). The lower panels of blots represent the immunoblot analysis of whole cell lysates.

Appendix Figure S3 **A20-mediated cell death mechanism is independent of inflammatory responses**



Cell death mechanisms of *A20*$^{-/-}$*Socs6*$^{+/+}$, *A20*$^{+/+}$*Socs6*$^{+/+}$, *A20*$^{-/-}$*Socs6*$^{-/-}$ and *A20*$^{+/+}$*Socs6*$^{-/-}$ BMDMs stimulated with Mtb. Representative data (from n = 3 biological replicates) are shown as the mean ± s.e.m. of technical replicates.

Appendix Figure S4 **The effect of miR-342-3p and SOCS6 on SOCS and STAT family members**



(A) HEK-293T fibroblasts were co-transfected with miR-342-3p mimic and luciferase reporter construct containing UTRs of SOCS family members. After 24 hours, cells were collected for luciferase assays. Data are shown as the mean ± s.e.m. of n = 3 biological replicates.

(B) Expression levels of SOCS family members in *Socs6*$^{+/+}$ or *Socs6*$^{-/-}$ BMDMs were examined by qRT-PCR. Data are shown as the mean ± s.e.m. of n = 3 biological replicates.

(C) Relative expressions of *Socs7* in BMDMs from C3H, B6, C3H supplemented with SOCS7-overexpressing vector or B6 supplemented with *Socs7* siRNA were analyzed by qRT-PCR. Data are shown as the mean ± s.e.m. of n = 3 biological replicates.

(D) Cell death mechanisms of BMDMs from C3H, B6, C3H supplemented with

SOCS7-overexpressing vector or B6 supplemented with *Socs7* siRNA, followed by Mtb infection for 36 hours. Representative data (from n = 3 biological replicates) are shown as the mean ± s.e.m. of technical replicates.

(E) SOCS6 expression levels in C3H (*Socs6*$^{+/+}$), C3H (*Socs6*$^{-/-}$), B6, *Mir342*$^{+/+}$C3H and *Mir342*$^{-/-}$B6 mice were detected by western blotting. Representative blots from n = 3 biological replicates are shown.

Data information: ANOVA followed by Bonferroni post *hoc* test (A-C) was used for data analysis. **, $p < 0.01$. Abbreviations: n.s., not significant.

Appendix Tables

Appendix Table S1 Gross pathology of mice infected with Mtb

Animal Group	**Lung Score**	**Mean ± s.e.m.***
B6	0	0
	0	
	0	
Mir342$^{-/-}$ B6	12	11.33±1.20
	13	
	9	
C3H	14	13.67±2.02
	10	
	17	
Mir342$^{+/+}$ C3H	4	5.00±1.53
	8	
	3	
Mir342$^{-/-}$ B6+si-*Socs6*	5	5.67±0.67
	5	
	7	
Mir342$^{+/+}$ C3H+*Socs6*	14	11.67±1.20
	11	
	10	

***Median values per group (n = 3)**

Appendix Table S2 Primers used for plasmids construction

Name	Forward	Reverse
psicheck-2-Socs6 3’UTR WT	AATCCTCGAGTCTTGCACTTTGGGGTTC	GCAAGCGGCCGCCCAGGGTTATCTTACTTTTG
psicheck-2-Socs6 3’UTR site1 mut	TTATTCGAGGTAGTCATCA	GATGACTACCTCGAATAA
psicheck-2-Socs6 3’UTR site2 mut	AGAATCGAGGTTTATCTTTC	AAGATAAACCTCGATTCTAA
Myc-Ago2	AACGGAATTCCCACCATGTACTCGGG	TCAAGCGGCCGCATTAAAGTGTTTTAA
Myc-RIPK3	AACTGAATTCCGACGATGTCTTCTGTC	TCAAGCGGCCGCTCCAACTGTCCTCA
Myc-RIPK3 K51A	TGTAGCAGTCGCGATCGTGA	GTTCACGATCGCGACTGCTA
Myc-A20	AATCGAATTCGGACCATGGCTGAACA	TCAAGCGGCCGCCAGGCTGACCCTGAC
Myc-A20 C103A	TGATGGAAACGCCCTCATGC	TGCATGAGGGCGTTTCCATC
Myc-A20 C609/612A	AGGGCTTTGCCACTCTAGCTTTCAT	CGATGAAAGCTAGAGTGGCAAAGCC
pcDNA3.1(+)-SOCS6	AATCGAATTCAAAATGAAGAAAATCA	TTAAGCGGCCGCAATCTGGTCATTC
Lenti-SOCS6	AATCGAATTCAAAATGAAGAAAATCA	TTAAGCGGCCGCAATCTGGTCATTC
Flag-RIPK3	AATAGCGGCCGCGACGATGTCTTCTGTCA	CTAAGAATTCAGTTTGGGTGTAGGTCCAAC
Flag-RIPK3 K51A	AGCAGTCGCGATCGTGAAC	TTCACGATCGCGACTGCTA

Appendix Table S3 Primers used for qRT-PCR

Name	Forward	Reverse
pri-miR-342	TTGGTTGGCTGGGTTCAGTT	GCTCATGCATGCACCACAAA
pre-miR-342	GAAAATGGGCTCAAGGTGAGGGGTG	TCAGCAGGCCAAGGTGACGGGTGCG
miR-342-3p	TCTCACACAGAAATCGCACCCGT	Universal Primer
miR-342-5p	AGGGGTGCTATCTGTGATTGAG	Universal Primer
Socs6	CCTTCAGTACACCGTGCCTT	GGTCCTGGTCCACATGACTG
Socs1	CAACGGAACTGCTTCTTCGC	AGTCACGGAGTACCGGGTTA
Socs2	CAGCTGGACCGACTAACCTG	GTTGGTAAAGGCAGTCCCCA
Socs3	TCACCCACAGCAAGTTTCCC	CCTCACACTGGATGCGTAGG
Socs4	GCTTCGTGTACAGGTGGTCA	GGAACAAGGCAGTGGACGTA
Socs5	CAGGCGGAACCAAAACTGTG	GAGTGGCTTTGACTGCTTGC
Socs7	TGATATCAGTGGGACGCTGC	CCATCTGGCTTCCCCTTCAG
Ccl5	CTCACCATATGGCTCGGACA	GCACTTGCTGCTGGTGTAGA
Cxcl10	CCAAGTGCTGCCGTCATTTT	CTCAACACGTGGGCAGGATA
Icam1	CTGGGCTTGGAGACTCAGTG	CCACACTCTCCGGAAACGAA
Caspase 3	GTCATCTCGCTCTGGTACGG	CACACACACAAAGCTGCTCC
Caspase 7	CGGAATGGGACGGACAAAGA	GAGTTGCTGTGGTCCTCCTC
Caspase 8	CAGGAGACCATCGAGGATGC	CCCACCGACTGATGTGGAAA
Caspase 2	AGGAGGAGCAGGATTTTGGC	AGGGCTTCACAGAAGGCATC
Caspase 9	ATTCAGCAGGCAGGATCTGG	ACCAGGTGGTCTAGGGGTTT
β-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT