**Supplemental Materials**

# Histone deficiency and accelerated replication stress in T cell aging

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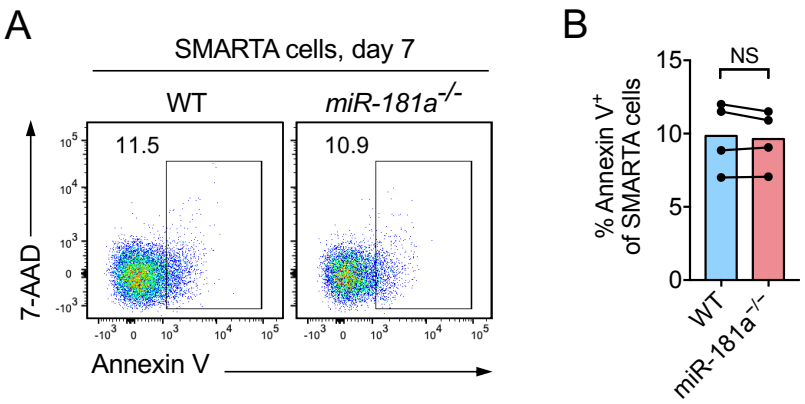
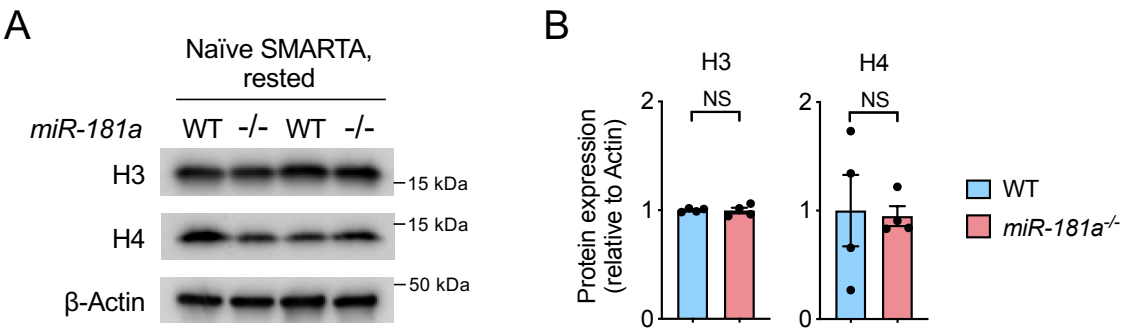
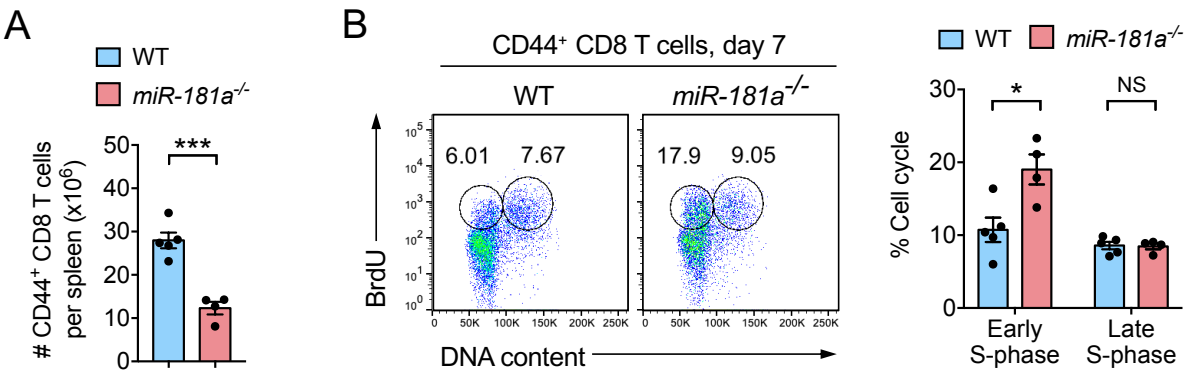


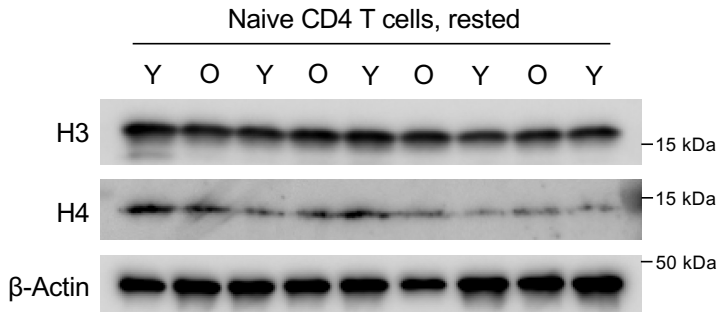

**Supplemental Figure 1. Reduced expansion of miR-181a-deficient T cells is not due to increased cell death. Related to Figure 1A.** Congenically marked WT and *miR-181a*$^{-/-}$ SMARTA cells were co-transferred into B6 mice before LCMV infection. (**A**) Representative flow plots of Annexin V and 7-AAD staining of SMARTA cells on day 7 after LCMV infection. (**B**) Summary data of the percentages of Annexin V$^{+}$ apoptotic cells (mean, two-tailed paired t test). Data are representative of two experiments with 4-5 mice per group. NS, not significant.



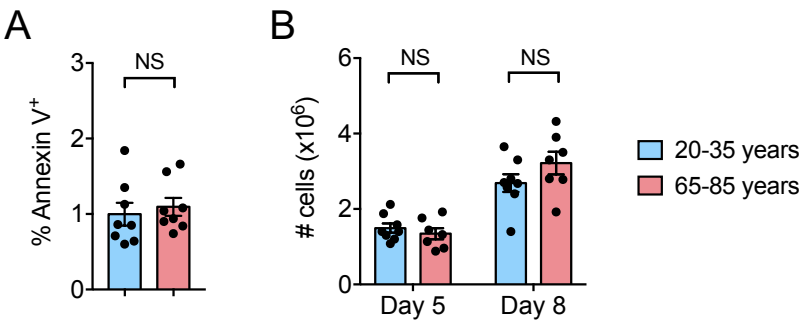

**Supplemental Figure 2. Comparison of histone expression in unstimulated naïve WT and miR-181a-deficient T cells. Related to Figure 1D.** (**A**) Immunoblots for histone H3 and H4 proteins from unstimulated naïve SMARTA cells. (**B**) Summary data of mean normalized intensities (n=4, mean ± SEM, two-tailed unpaired t test). NS, not significant.



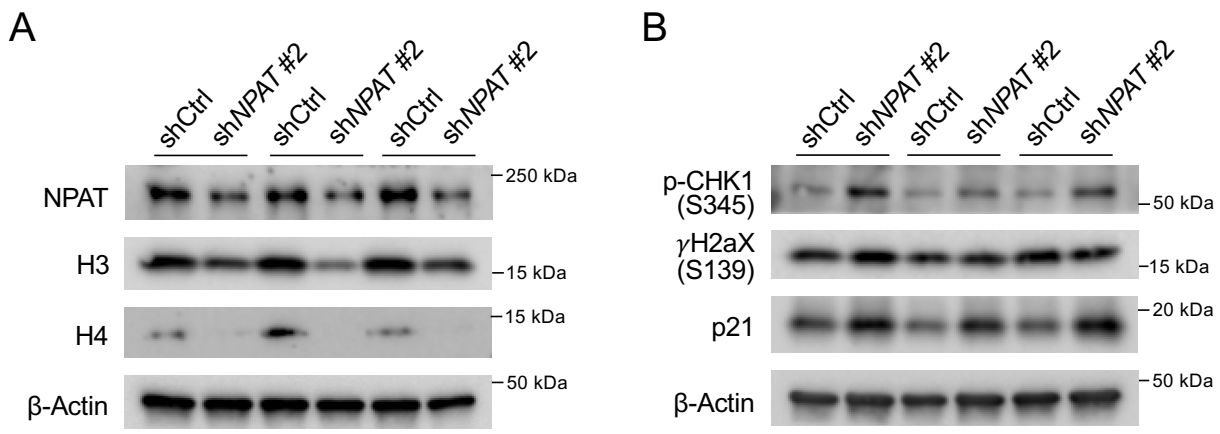

**Supplemental Figure 3. Proliferative defect and accumulation of *miR-181a*$^{-/-}$ CD8 T cells in the early S-phase of the cell cycle**. **Related to Figure 1, F and G.** On day 7 after LCMV infection, WT and *miR-181a*$^{-/-}$ mice were injected with BrdU for 1 hour prior to spleen harvest. (**A**) Numbers of CD44$^{+}$ CD8 T cells (mean ± SEM, two-tailed unpaired t test). (**B**) Representative flow plots of BrdU incorporation and DNA content in CD44$^{+}$ CD8 T cells (left) and summary of frequencies (right, mean ± SEM, two-tailed unpaired t test). Data are representative of two experiments with 3-6 mice per group. *P<0.05, ***P<0.001, NS, not significant.



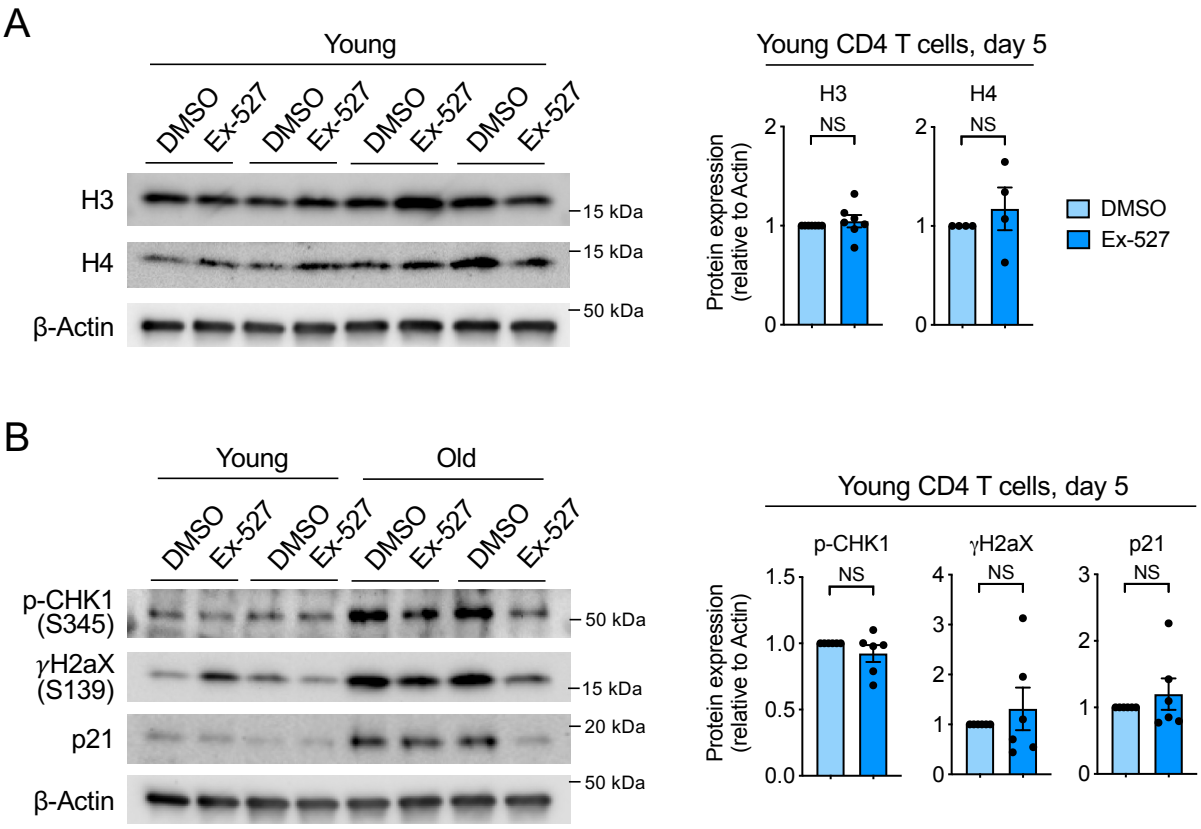

**Supplemental Figure 4. Comparison of histone expression in unstimulated naïve CD4 T cells from young and old adults. Related to Figure 2C.** Immunoblots for histone H3 and H4 in unstimulated naïve CD4 T cells from 5 young and 4 old individuals.




**Supplemental Figure 5. Proliferative responses of naïve CD4 T cells from young and older individuals. Related to Figure 2F.** Naïve CD4 T cells from healthy young and old individuals were activated with beads coated with anti-CD3/anti-CD28 antibodies. (**A**) Summary graph of frequencies of annexin $V^+$ apoptotic cells on day 5 from 8 young and 8 old adults (mean ± SEM, two-tailed unpaired t test). (**B**) Summary data of the number of cells on day 5 and day 8 after activation from 8 young and 7 old individuals (mean ± SEM, two-way ANOVA followed by Tukey's post-hoc test). NS, not significant.

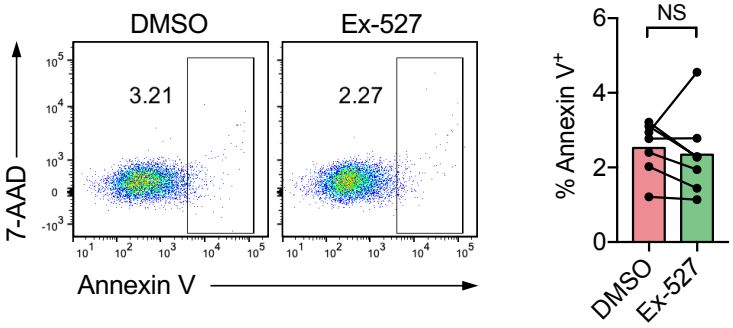



**Supplemental Figure 6. Reduced histone expression promotes replication stress. Related to Figure 3.** Naïve CD4 T cells from three young adults were activated with anti-CD3/anti-CD28 beads and transduced with control shRNA (shCtrl) or *NPAT* shRNA (sh*NPAT* #2) lentivirus for 6 days. Immunoblots for NPAT, histone H3 and H4 (**A**) and p-CHK1 (S345), γH2aX (S139) and p21 (**B**) in lentivirally-transduced GFP$^+$ cells.

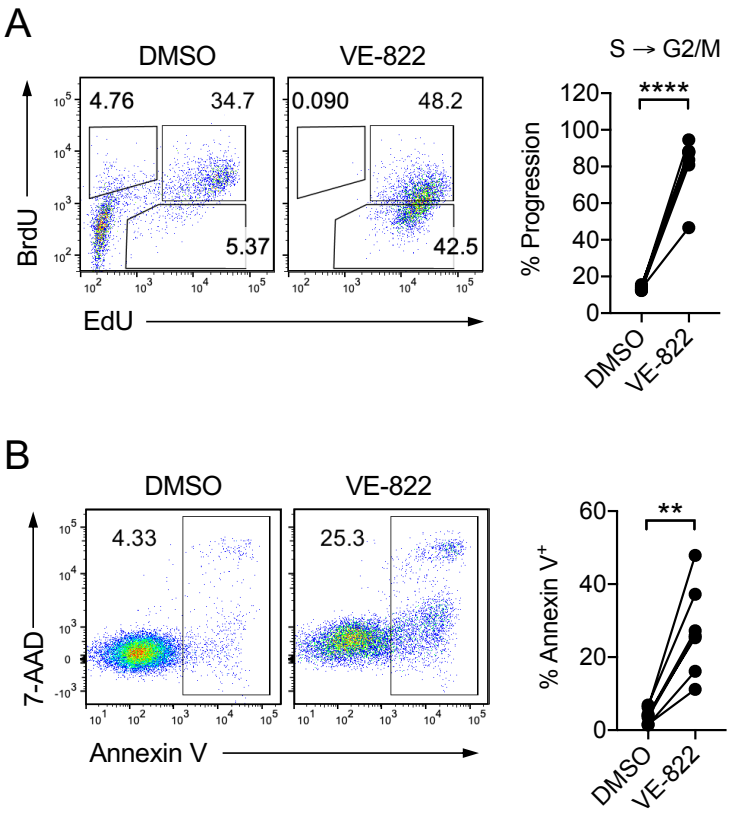



**Supplemental Figure 7. Inhibition of SIRT1 activity in T cell response of young adults.**

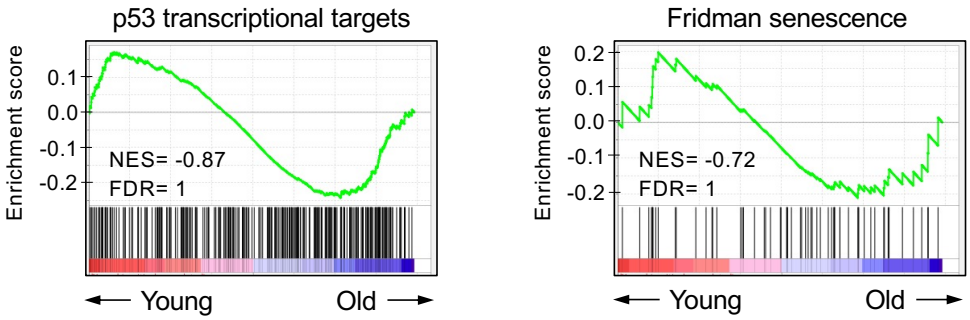
Naïve CD4 T cells from young or older individuals were activated with either DMSO or Ex-527 for 5 days. (**A**) Immunoblots of histone H3 and H4 in cycling cells (left) and summary data from 4-7 young adults (right, mean ± SEM, two-tailed paired t test). Related to Figure 5G. (**B**) Immunoblots of indicated proteins in cycling young and old cells (left) and summary data from 6 young adults (mean ± SEM, two-tailed paired t test). Related to Figure 6B. NS, not significant.



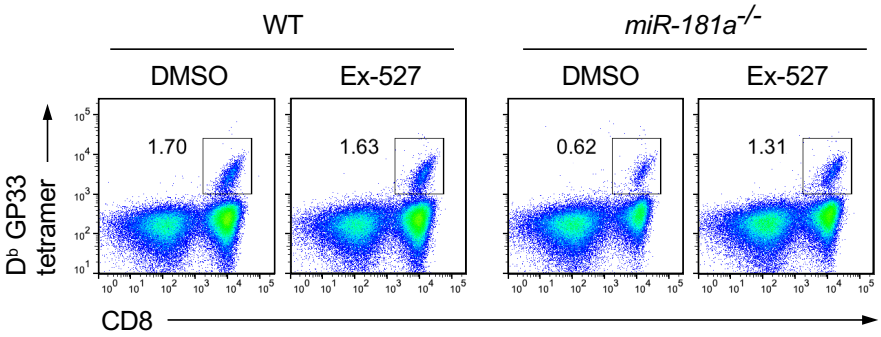

**Supplemental Figure 8. Cell survival after inhibition of SIRT1 activity during T cell activation. Related to Figure 6F.** Naïve CD4 T cells from six old individuals were activated with anti-CD3/CD28 beads for 5 days in the presence of either DMSO or Ex-527. Representative flow plots of Annexin V and 7-AAD staining (left) and summary data of the percentages of Annexin $V^+$ apoptotic cells (right, mean, two-tailed paired t test). NS, not significant.




**Supplemental Figure 9. Abrogation of ATR checkpoint accelerates cell cycle progression and increases cell death. Related to Figure 6.** Naïve CD4 T cells from six old individuals were activated with anti-CD3/CD28 beads for 5 days. The ATR inhibitor VE-822 was added to the culture on day 4. (**A**) On day 5, cells were pulsed with EdU for 2 hours, followed by BrdU for 1 hour. Representative flow plots of BrdU and EdU incorporation (left) and summary graphs of the percentages of $BrdU^{-}$ $EdU^{+}$ G2/M-phase cells among $EdU^{+}$ cells (right, two-tailed paired t test). (**B**) Representative flow plots of Annexin V and 7-AAD staining (left) and summary data of the percentages of Annexin $V^{+}$ apoptotic cells (right, two-tailed paired t test). **P<0.01, ****P<0.0001.




**Supplemental Figure 10. GSEA of the transcriptome of unstimulated naïve CD4 T cells from young and old individuals. Related to Figure 7A.** GSEA of p53 transcriptional target genes and genes related to senescence in the transcriptome of unstimulated young naïve CD4 T cells relative to the expression in old naïve CD4 T cells (accession number SRA: PRJNA638216).




**Supplemental Figure 11. SIRT1 inhibition augments antigen-specific T cell responses in vivo. Related to Figure 8D.** WT and *miR-181a*$^{-/-}$ mice were infected with LCMV and given DMSO or Ex-527 daily starting the day after the infection . Representative flow plots of splenic $D^b$ LCMV GP33 tetramer$^+$ CD8 T cells on day 7. Summary data of number of tetramer$^+$ CD8 T cells is shown in Figure 8D. Data are representative of two experiments with 5 mice per group.

**Supplemental Table 1. Oligonucleotide primer sets used in this study.**

| Gene | Forward | Reverse |
|---|---|---|
| *HIST1H1B* | CTAAGGAGCGCAATGGCCTTT | CTTCGGAGTCTTCTTCACTGC |
| *HIST1H2AE* | CTACTCCGAACGAGTCGGG | GATGGTCACGCGACCTAGAAG |
| *HIST2H2AB* | CCATCTGCAACTAGCCGTGAG | CAGGCTTGTGACTCTCCGT |
| *HIST1H2BF* | ACCTGCTAAGTCCGCTCCT | CTACGCTTGCGCTTCTTACCA |
| *HIST1H3A* | ACTGCTCGGAAGTCTACTGGT | GCGCTGGAAAGGTAGTTTACGA |
| *HIST1H3B* | ATGGCTCGTACTAAACAGACAGC | TTCCGAATCAGCAACTCGGTC |
| *HIST1H3D* | CCATTCCAGCGTCTAGTCCG | TCTGAAAACGCAGATCAGTCTTG |
| *HIST1H4B* | AAGGCGGTAAAGGTTTGGGTA | GGAAATTCGCTTAACCCCACC |
| *HIST1H4E* | CCGTAAGGTCCTGCGAGATAA | AGTCACAGCATCACGAATCAC |
| *HIST1H4H* | GAGGAGCTAAGCGTCATCGC | ACACCTTCAGAACACCACGAG |
| *TIPIN* | AGAATGGCGTGATTGACCTACC | CCAGTGCTCCATGTGTCTGATTA |
| *CDC6* | TGTTCTCCTCGTGTAAAAGCC | GGGGAGTGTTGCATAGGTTGT |
| *CLSPN* | TGGAGAGTGGGGTCCATTCAT | CCGGGGTTTACGTTTGAAGAAA |
| *RFC2* | GTGAGCAGGCTAGAGGTCTTT | TGAGTTCCAACATGGCATCTTTG |
| *RFC4* | TTGGGCCTGAACTTTTCCGAT | AGCGACTTCCTGACACAGTTA |
| *RAD51* | CAACCCATTTCACGGTTAGAGC | TTCTTTGGCGCATAGGCAACA |
| *BHLHE40* | GACGGGGAATAAAGCGGAGC | CCGGTCACGTCTCTTTTTCTC |
| *LIF* | CCAACGTGACGGACTTCCC | TACACGACTATGCGGTACAGC |
| *DDIT4* | TGAGGATGAACACTTGTGTGC | CCAACTGGCTAGGCATCAGC |
| *AEN* | CTTCCAGGCGCTCAAGTATGT | GGGCCAGGTCCTTTAGAGAGA |
| *SDC4* | GGACCTCCTAGAAGGCCGATA | AGGGCCGATCATGGAGTCTT |
| *FOSL1* | CAGGCGGAGACTGACAAACTG | TCCTTCCGGGATTTTGCAGAT |
| *CSF1* | TGGCGAGCAGGAGTATCAC | AGGTCTCCATCTGACTGTCAAT |
| *CSF2* | TCCTGAACCTGAGTAGAGACAC | TGCTGCTTGTAGTGGCTGG |
| *CCL3* | AGTTCTCTGCATCACTTGCTG | CGGCTTCGCTTGGTTAGGAA |
| *CXCL8* | ACTGAGAGTGATTGAGAGTGGAC | AACCCTCTGCACCCAGTTTTC |
| *IFIT1* | TTGATGACGATGAAATGCCTGA | CAGGTCACCAGACTCCTCAC |
| *IFIT2* | AAGCACCTCAAAGGGCAAAAC | TCGGCCCATGTGATAGTAGAC |
| *MX1* | GTTTCCGAAGTGGACATCGCA | CTGCACAGGTTGTTCTCAGC |
| *IRF5* | GGGCTTCAATGGGTCAACG | GCCTTCGGTGTATTTCCCTG |
| *IRF7* | CCCACGCTATACCATCTACCT | GATGTCGTCATAGAGGCTGTTG |
| *RSAD2* | CAGCGTCAACTATCACTTCACT | AACTCTACTTTGCAGAACCTCAC |
| *ISG15* | CGCAGATCACCCAGAAGATCG | TTCGTCGCATTTGTCCACCA |
| *OAS3* | TCTGAGACTCACGTTTCCTGA | CACTGTTGAGGAGGGTAGAGTA |
| *USP18* | CCTGAGGCAAATCTGTCAGTC | CGAACACCTGAATCAAGGAGTTA |
| *IFI27* | TGCTCTCACCTCATCAGCAGT | CACAACTCCTCCAATCACAACT |
| *ACTB* | ATGGCCACGGCTGCTTCCAGC | CATGGTGGTGCCGCCAGACAG |