SUPPLEMENTARY MATERIAL

MicroRNA-302a suppresses influenza A virus-stimulated interferon regulatory factor-5 expression and cytokine storm induction

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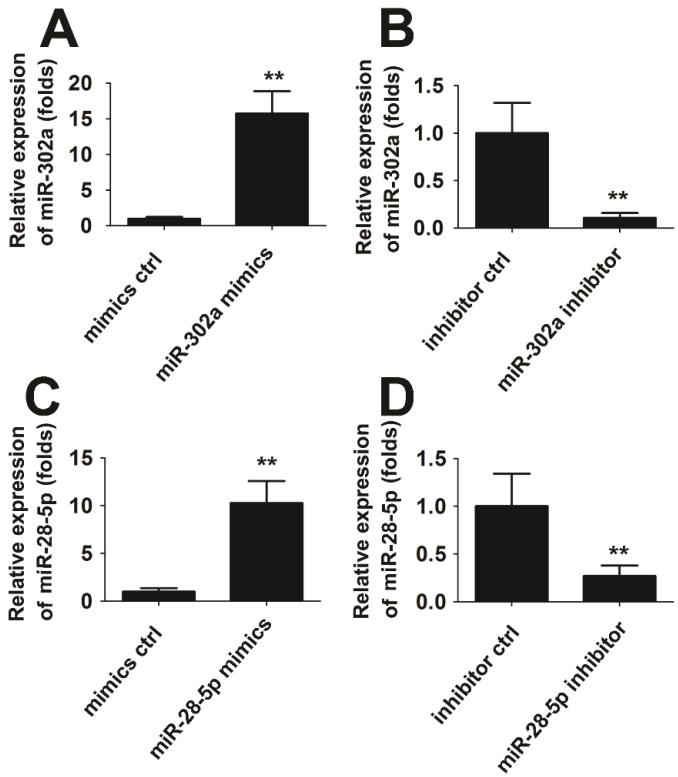
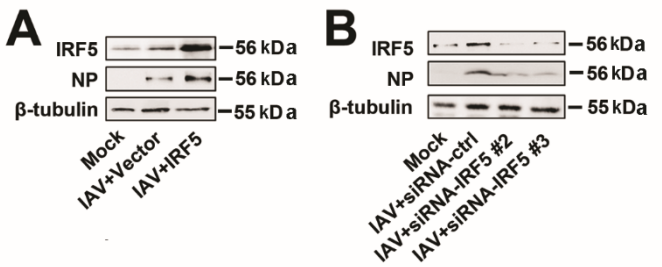
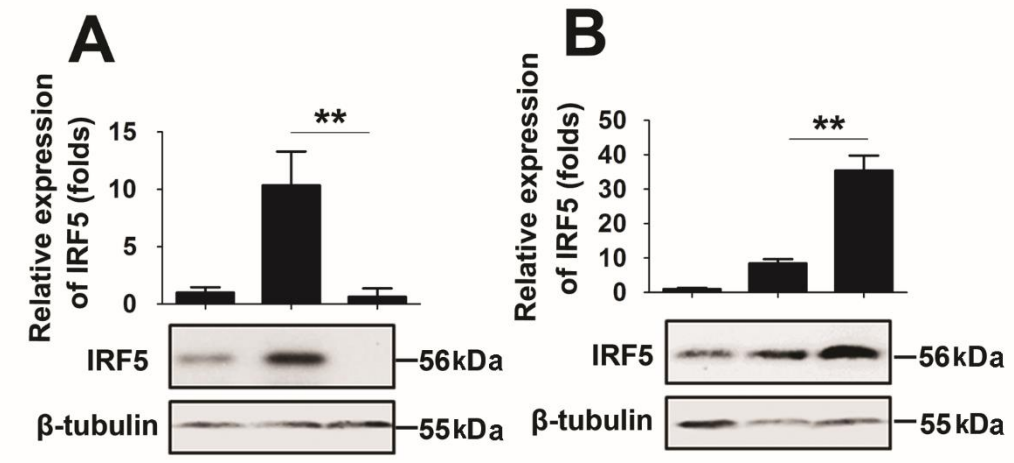
Supplemental Figure



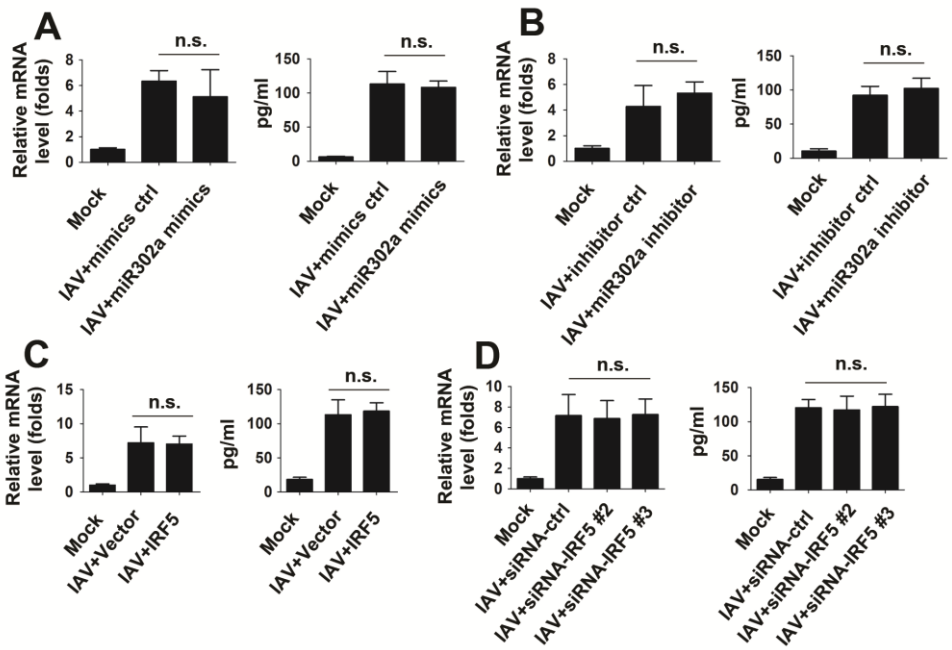
Supplemental Figure 1. Determination of the efficiency of miRNA mimics and inhibitor. (A) A549 cells were transfected with miRNA mimics control or miR-302a mimics for 48 h prior to real-time RT-PCR assays. (B) Experiments were performed as in A, except inhibitor control or miR-302a inhibitor were used. (C and D) Experiments were performed as described in A and B, except that miR-28-5p mimics or inhibitor was used. In the real-time RT-PCR experiments, the control was designated as 1. Bar graphs present means ± SD, n=3 (**P < 0.01; *P < 0.05).



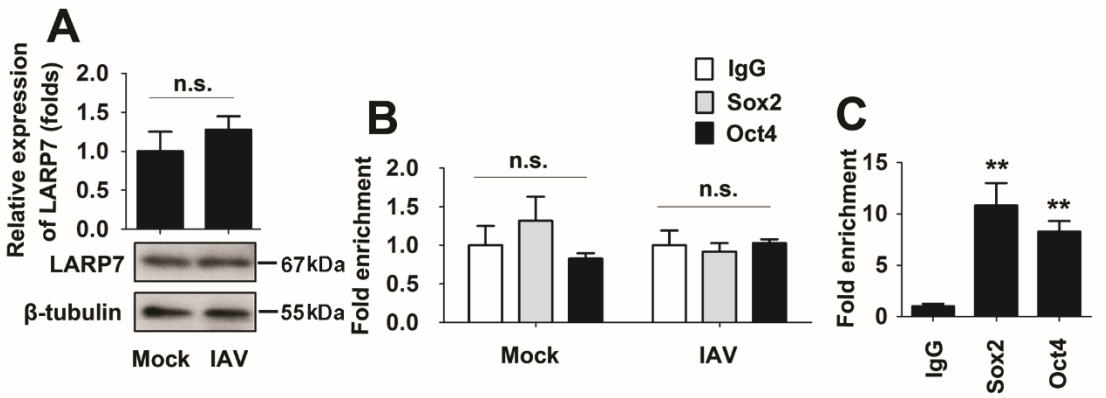
Supplemental Figure 2. Determination of the efficiency of transfection and infection in Figure 3. (A and B) Experiments were performed as in Fig 3 B and E except protein levels of IRF5 and NP were quantified using Western blot. All experiments were repeated at least three times with consistent results.



Supplemental Figure 3. IAV induces IRF-5 expression via miR-302a in MLE-12 cells. (A and B) Experiments were performed as described in Fig 5 A and B, except that MLE-12 cells were used. All experiments were repeated at least three times with consistent results. In the real-time RT-PCR experiments, the control was designated as 1. Bar graphs present means ± SD, n=3 (**P < 0.01; *P < 0.05).



Supplemental Figure 4. The role of miR-302a/IRF-5 axis in IAV-induced IFNα production. Experiments were performed as in Fig 3, except the expression of IFNα was analyzed. All experiments were repeated at least three times with consistent results. Bar graphs present means ± SD, n=3 (**P < 0.01; *P < 0.05), n.s., not significant.



Supplemental Figure 5. IAV infection did not regulate miR-302 expression at a transcriptional level. (A) A549 cells infected with the WSN virus (MOI=1) or mock infected for 24 h prior to real-time RT-PCR (upper panel) and Western blot (lower panel) analyses. (B) A549 cells were infected with WSN virus (MOI=1) or mock infected for 24 h. ChIP assays were performed with anti-Sox2, -Oct4 or IgG-conjugated agarose. Promoter sequences in the input DNA and the DNA recovered from antibody-bound chromatin segments were detected using real-time RT-PCR. (C) Experiments were performed as in B, except hESCs were used. All experiments were repeated at least three times with consistent results. In the real-time RT-PCR experiments, the control was designated as 1. Bar graphs present means ± SD, n=3 (**P < 0.01; *P < 0.05).

Supplemental Table 1 Primers Used in Real-time RT-PCR.

Gene name	5'primer	3'primer
IRF-5 (h)	5'-GCCATGAACCAGTCCATCCCAGTG-3'	5'-CCACGCCTTCGGTGTATTTCCCT-3'
IFN-β (h)	5'-AAAGAAGCAGCAATTTTCAGC-3'	5'-CCTTGGCCTTCAGGTAATGCA-3'
TNF-α (h)	5'-CTTCTCGAACCCCGAGTGAC-3'	5'- ATGAGGTACAGGCCCTCTGA-3'
IL-6 (h)	5'-TGGTGGATGTTCCCCCCGAG-3'	5'-TCCTGGGAATACTGGCACGG-3'
IL-8 (h)	5'-GGTGCAGTTTTGCCAAGGAG-3'	5'-TTCCTTGGGGTCCAGACAGA-3'
CCL2 (h)	5'-CTCATAGCAGCCACCTTCA-3'	5'-CACAGCTTCTTTGGGACAC-3'
CCL5 (h)	5'-CCCTCGCTGTCATCCTCAT-3'	5'-ACACTTGGCGGTTCTTTCG-3'
β-actin (h)	5'-GGACTTCGAGCAAGAGATGG-3'	5'-AGGAAGGAAGGCTGGAAGAG-3'
U6	5'-GCTTCGGCAGCACATATACTAAAAT-3'	5'- CGCTTCACGAATTTGCGTGTCAT-3'
miR-302a	5'- CGGGCATAAGTGCTTCCA-3'	5' -CAGTGCAGGGTCCGAGGT -3'