Supporting Information

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SI Materials and Methods

Virus Design and Rescue. The splice acceptor site in the NS1 ORF (521 5′tcttccaggacat3′ 533) was mutated to prevent splicing (521 5′ tctCccGggacat3′ 533) of NS mRNA at this site by site-directed mutagenesis using the primers 5′-CCATTGCCTTCTCTCCC-GGGACATACTGCTGAGGATGTC-3′ and 5′- GACATCCTC-AGCAGTATGTCCCGGGAGAGAAGGCAATGG-3′. The fragment corresponding to the NS1 ORF, along with the 3′ noncoding region of vRNA (nucleotides 1–716), was amplified from this splice acceptor site mutant NS segment with primers carrying SapI and XhoI sites (5′-GATCGCTCTTCTGGGAGCAAAAGCAGG-5′ and 5′-CCCCTCGAGTCAAACTTCTGACCTAATTGTTCCC-5′. The fragment corresponding to the NEP/NS2 and the 5′-noncoding region of vRNA (nucleotides 508–890 in the WT NS segment) was amplified from an NS plasmid using primers carrying XhoI and SapI sites (5′-cgCTCGAGCACCATTGCCTTCTCTT-CCAGG-3′ and 5′-CATCGCTCTTCTATTAGTAGAAACAAG-G-3′). The NS1 and NEP/NS2 fragments were digested with SapI and XhoI and ligated into a previously established influenza A virus rescue vector described elsewhere (1). The recombinant viruses were rescued by using previously described reverse genetic techniques (1, 2). Briefly, 0.5 μg of each of the 8 influenza A virus-encoding plasmids were transfected into 293T cells. After 24 h, the 293T cells with supernatants were injected into 8-d-old eggs. The recombinant virus was harvested from the allantoic fluid at 48 h postinfection. After plaque purification, the modified NS segment was confirmed by sequencing the RT-PCR product of vRNA. A ClaI restriction site was further introduced into the intergenic region of the NS vRNA by performing standard site-directed mutagenesis. The ClaI insertion site was used to ligate the miR-124-2 murine locus (chr3: 17,695,454–17,696,037) or four copies of miR-142-3p targets, as previously described (3).

qPCR Primers. qPCR and RT primers included in this study were as follows: PB2, 5′-ATCGGAATCGCAACTAACGA-3′ and 5′-TTTGCGGACCAGTTCTCTCT- 3′; canine tubulin, 5′-GGTTC-GAGTTCTGGAAGCAG-3′ and 5′-GGGGATGTAGTGCTCA-TCGT-3′; NEP/NS2, 5′-CACTGTGTCAAGCTTTCAGGACAT-ACTG-3′ and 5′-CTCGTTTCTGTTTTGGAGTGAGTG-3′; NS1 (for standard RT), 5′-GGGCTTTCACCGAAGAGGGAGC-3′ and 5′-GTGGAGGTCTCCCATTCTCA-3′; NS, 5′ cRNA 5′-GA-CCAAGAACTAGGCGATGC-3′ and 5′-CGCTCCACTATCTG-CTTTCC-3′; NS 3′cRNA, 5′-AAGGGTGAGACACAAACTGA-AGGT-3′ and 5′-AGTAGAAACAAGGGTGTTTTTTAT-3′; NS loop, 5′-CCATCGATGAGCTCCAAGAGAGGGTGAA-3′ and 5′-CCATCGATTCTCCCCACCCTTCCTAACT-3′; murine tubulin 5′-TGCCTTTGTGCACTGGTATG-3′ and 5′-CTGGAG-CAGTTTGACGACAC-3′.

1. Hoffmann E, Neumann G, Kawaoka Y, Hobom G, Webster RG (2000) A DNA transfection system for generation of influenza A virus from eight plasmids. *Proc Natl Acad Sci USA* 97:6108–6113.
2. Fodor E, et al. (1999) Rescue of influenza A virus from recombinant DNA. *J Virol* 73: 9679–9682.
3. Brown BD, et al. (2007) Endogenous microRNA can be broadly exploited to regulate transgene expression according to tissue, lineage and differentiation state. *Nat Biotechnol* 25:1457–1467.

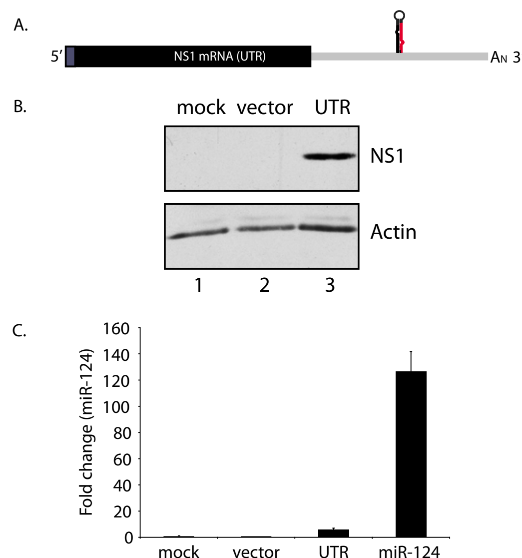

Fig. S1. miR-124 is not produced from NS1 UTR. (*A*) Diagram of plasmid expressing NS1 with a mir-124 hairpin in the 3′ UTR. (*B*) Western blot analysis of mock, scbl, and UTR transfected cells. Fibroblasts were harvested 24 h posttransfection. Blots depict NS1 protein and actin. (*C*) qRT-PCR analysis of miR-124 levels of samples from *B* plus full-length NS1/NEP-124, standardized with small nucleolar RNA-202.