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Supplemental Information

MicroRNA-96 Promotes Schistosomiasis Hepatic Fibrosis in Mice by Suppressing *Smad7*

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Fig. S1. Verification of hepatic fibrosis-relevant miRNA expression profiles. qRT-PCR analysis of miRs expression level between the liver samples infected with 16 S. japonicum cercariae at day 0 (n=3) and day 50 (n=3).

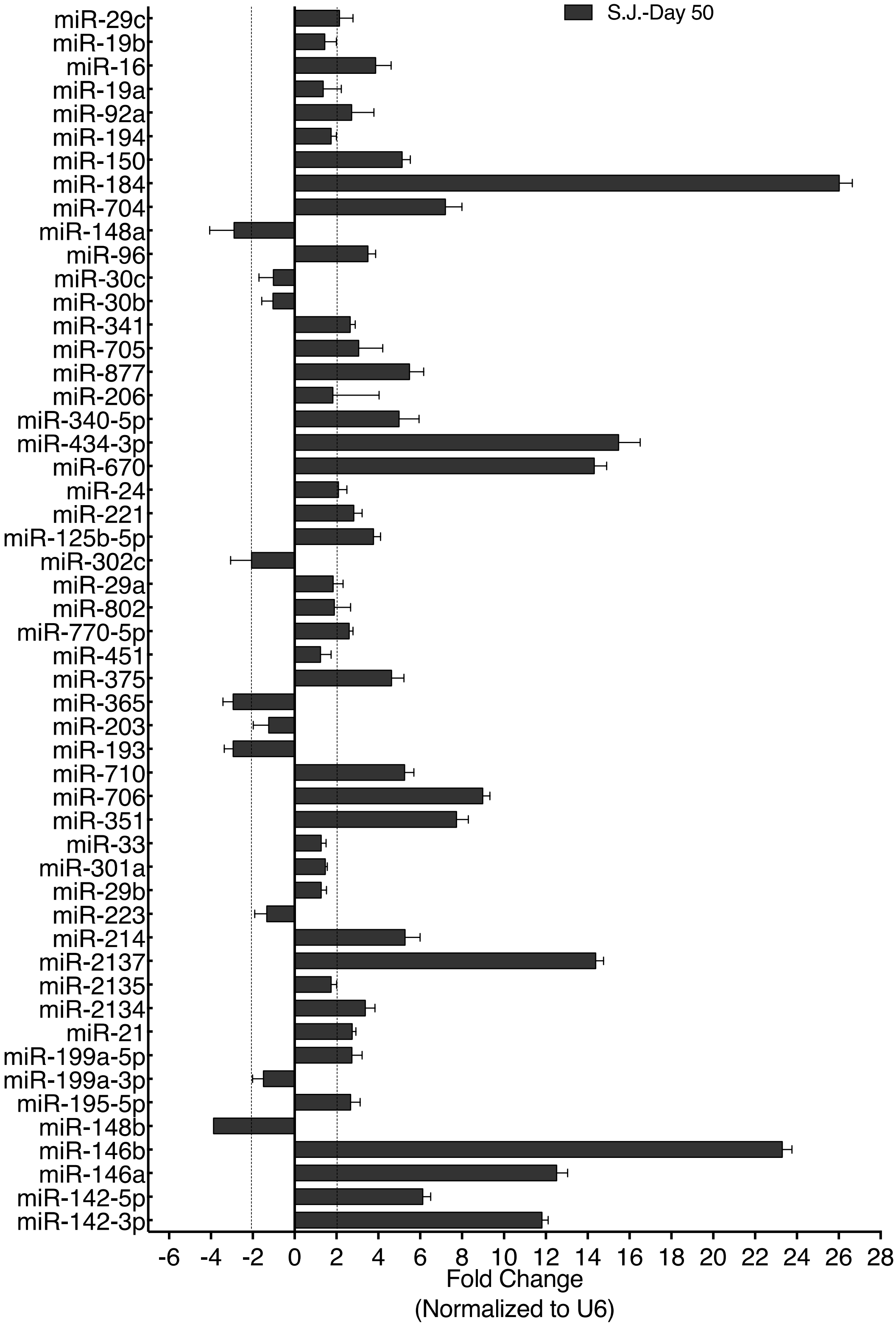


Fig. S2. Verification of altered miRNA expression profiles. qRT-PCR analysis of miRs expression level between the HSCs samples infected with 16 S. japonicum cercariae at day 0 (n=3) and day 50 (n=3).

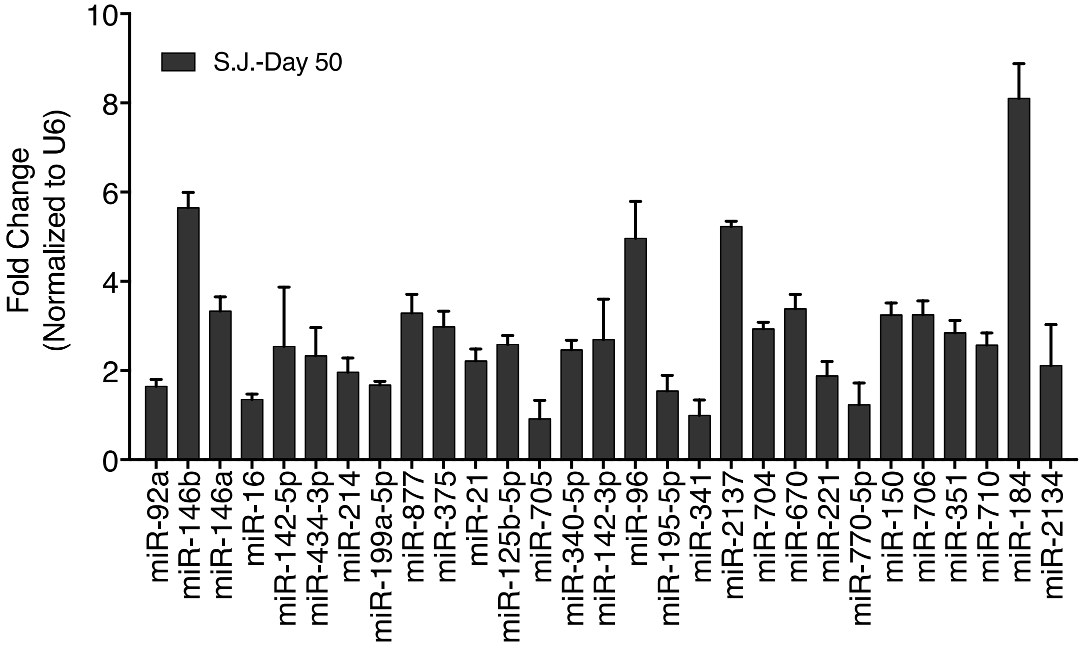
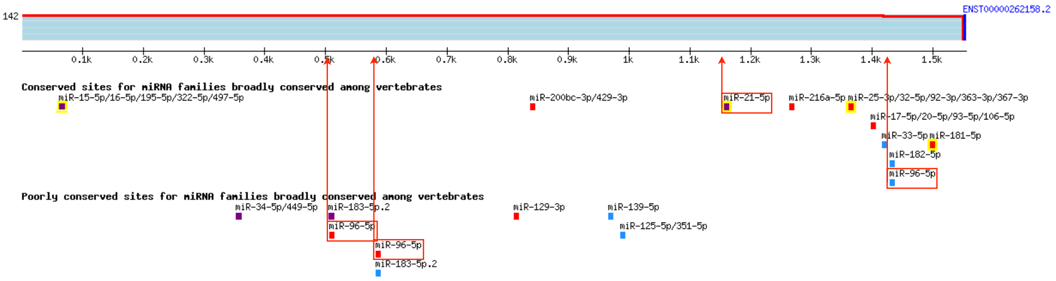


Fig. S3. Predicted miR-96 bind sites in the 3'UTR of *Smad7*. By microRNA targets prediction website: TargetScan, one conserved site of miR-96 (1429-1435) and two poorly conserved sites (507-513, 583-589) of miR-96 were predicted[1].

Mouse SMAD7 ENST00000262158.2 3'UTR length:1554



mmu-miR-96 Position 1429-1435 of SMAD7 3' UTR

```
   MUT:   5'      ...UAAAUGCAAAUAACAAGGCTAAU...
    WT:   5'      ...UAAAUGCAAAUAACAUGCCAAAU...
                      |||||          ||||||
miR-96:   3'     UCGUUUUUACACGAUC---ACGGUUU
```

mmu-miR-96 Position 507-513 of SMAD7 3' UTR

```
   MUT:   5'  ...GCUGAGAGGCUCATTGACCGATG...
    WT:   5'  ...GCUGAGAGGCUCAUAGUGCCAAG...
                             |||||||||
miR-96:   3'     UCGUUUUUACACGAUCACGGUUU
```

mmu-miR-96 Position 583-589 of SMAD7 3' UTR

```
    WT:   5'    ...GCAGACUGGCAGCAGGUGCCAAG...
                                  |||||||
miR-96:   3'       UCGUUUUUACACGAUCACGGUUU
```

Fig. S4. Validation of the target site of miR-96 by the dual-luciferase reporter assays. each histogram shows normalized mean value of relative luciferase activity from three independent experiments. Normalized luciferase activity in the NC-mimics was set to 100. WT: the vector containing the wild type sequence of the target site; Mut: the vector containing the target site mutant.

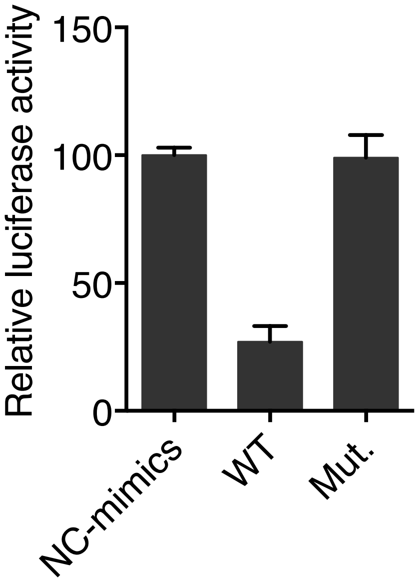


Fig. S5. In vitro validation of rAAV vector plasmids expressing mmu-miR-96. (a) MiR-96 sensor plasmid and pmiCHECK-3x96T was co-transfected with 10 to 500 ng of prAAV-miR-96 plasmids into HEK293 cells. (b) In the presence of 100 ng of prAAV-miR-96 plasmid, 5 to 500 ng of anti-miR-96 TuD was co-transfected with the miR-96 sensor plasmid into HEK293 cells. Twenty-four hours after transfection, β-Gal and Fluc levels were measured in the cellular lysates. The ratio of β-Gal and Fluc activities reflects the active miR-96 in the transfected HEK293 cells.

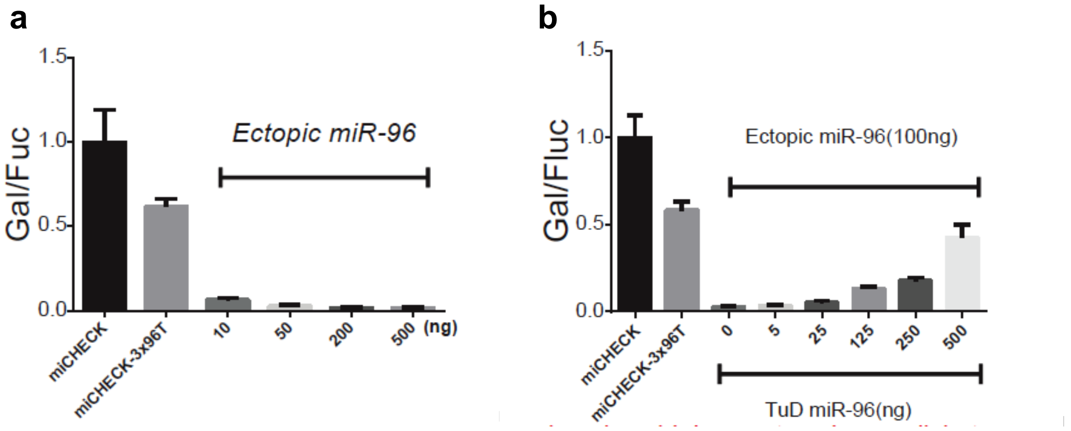
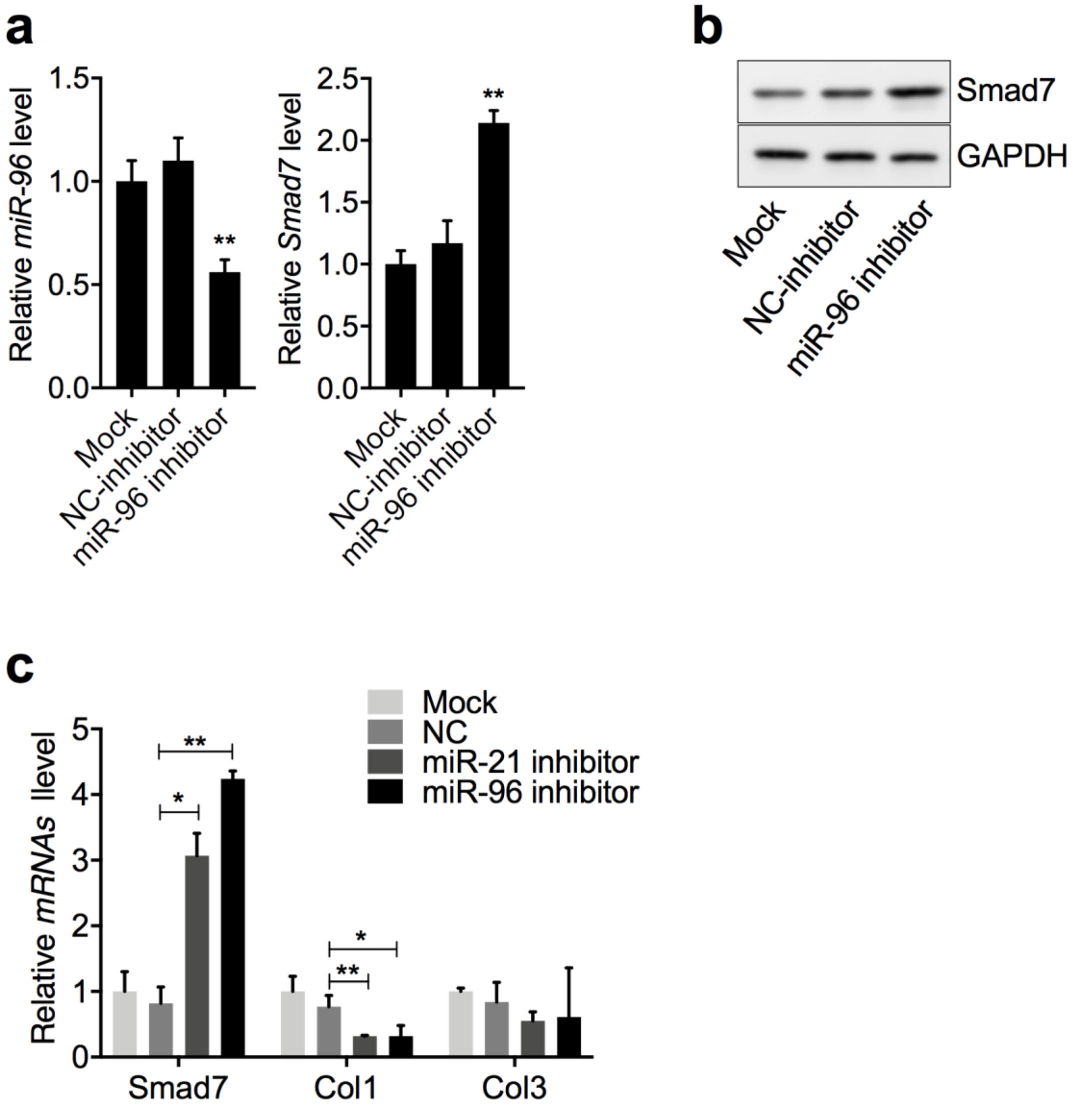


Fig. S6. Validation of the target site of miR-96. (a) RT-PCR analysis of *Smad7* expression in the HSC-T6 cell line transfected with miR-96-inhibitor (160nM), or the NC inhibitor. (b) Western blot analysis of Smad7 expression in the HSC-T6 cell line treated as above. (c) RT-PCR analysis of expressions of *Smad7*, *Col1* and *Col3* in the HSC-T6 cell line transfected with miR-96-inhibitor, miR-21-inhibitor or the NC inhibitor.

Data were represented as mean ± SD. *p < 0.05, **p < 0.01, compared between indicated groups.



References:

1. Agarwal V, Bell GW, Nam J, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. eLife, 4:e05005, (2015).