

Fig. 1: Effect of miR-301a on viral replication. (A) BV2 cells were transfected with either miR-301a mimic (Mimic-miR-301a) or inhibitor (Anti-miR-301a) along with their respective negative controls (Mimic-Con or Anti-miR-Con) followed by JEV infection for 24 hours. Viral titres in the culture supernatants were detected by plaque assay. All data are means \pm SD of three biological replicates. Statistical analysis of the data by one-way ANOVA followed by Bonferroni's post hoc test showed that there was no statistically significant difference.

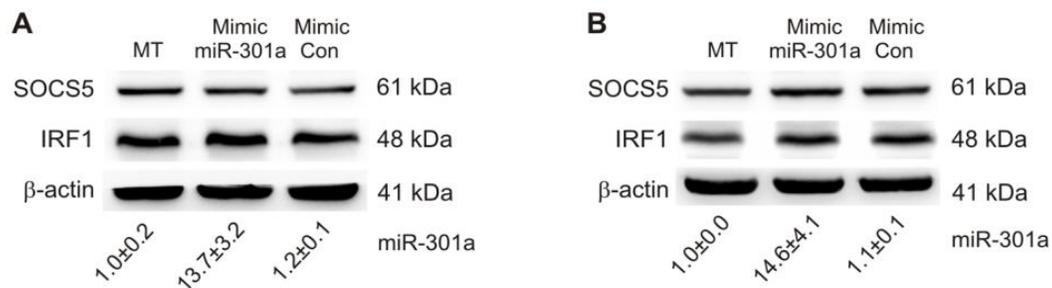


Fig. 2: Abundances of SOCS5 and IRF1 in miR-301a overexpression. (A and B) Following mock transfection (MT) or transfection with either miR-301a mimic (Mimic-miR-301a) or negative control (Mimic-Con) for 24 hours, CHME3 (A) and BV2 (B) cells were subjected to Western blotting for analysis of the abundances of SOCS5 and IRF1 proteins. β -actin was used as loading control. The relative abundance of miR-301a as determined by qRT-PCR analysis of each set of cells is shown below the blots to confirm effective transfection. Western blots are representative of three independent experiments.

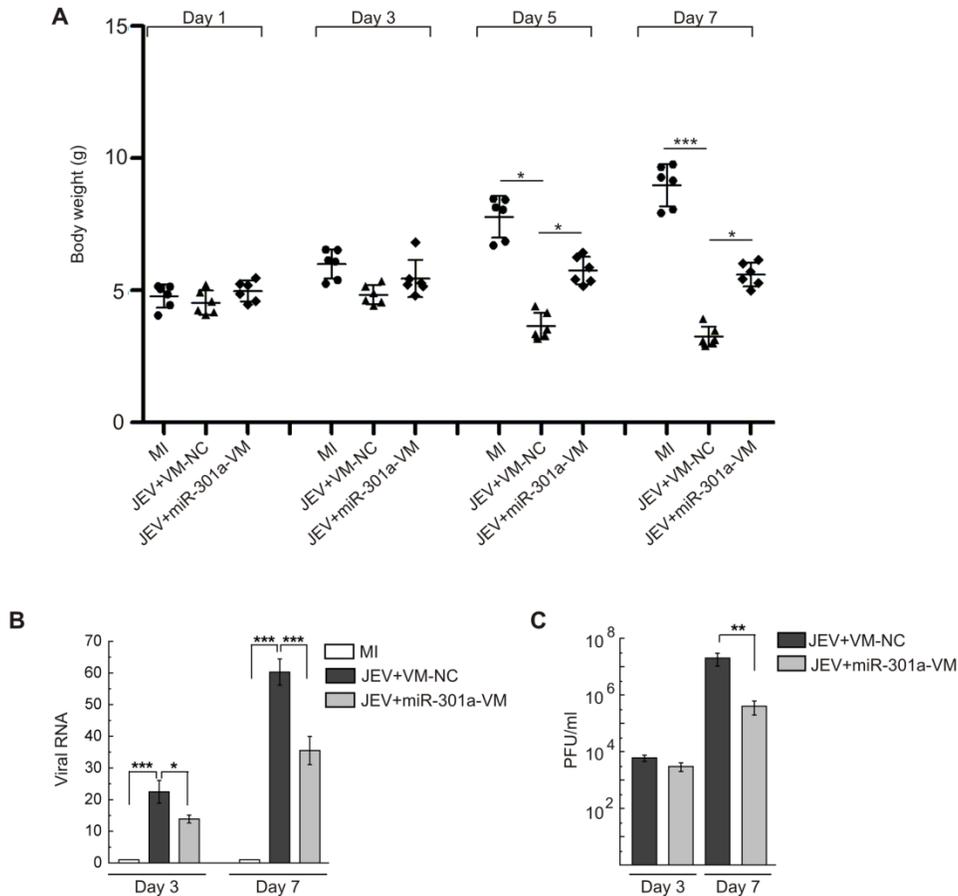


Fig. 3: Effect of Anti-miR-301a in JEV-infected mice. (A) BALB/c mice were mock-infected (MI) with PBS or were infected with JEV (3×10^5 PFU). Following 24 hours of infection, they were treated intracranially with either miR-301a Vivo-Morpholino (JEV + miR-301a-VM) or scrambled Vivo-Morpholino (JEV + VM-NC) and were monitored for changes in body weight for 7 days. Data are means \pm SD of six mice from each group. (B and C) The abundance of viral RNA and viral titre in mice brain treated as mentioned in (A) were evaluated by qRT-PCR analysis (B) and plaque assay (C), respectively. Data are means \pm SD of four mice from each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, as calculated by one-way ANOVA followed by Bonferroni's multiple comparisons.

Table 1. Primer sequences

Primer	Forward (5'-3')	Reverse (5'-3')
Real-Time assay		
NKRF (Human)	AGAAAGATGGGTTGGACT	CTGTGTGGCTCTCGGA
NKRF (Mouse)	GTAGCAAGAATGGGTCCAGAG	CACAAGGTCCTCTCCGATTATG
IL1- β (Human)	CTGCGTGTTGAAAGATGATAAG	CCACATTCAGCACAGGACTC
IL1- β (Mouse)	TGGAAAAGCGGTTTGTCT	ATAAATAGGTAAGTGGTTGCC
TNF- α (Human)	GGAGAAGGGTGACCGACTCA	CTGCCCAGACTCGGCAA
TNF- α (Mouse)	GGCAGGTCTACTTTGGAGTCATTGC	ACATTTCGAGGCTCCAGTGAATTCGG
IL-4 (Human)	ACTTTGAACAGCCTCACAGAG	TTGGAGGCAGCAAAGATGTC
IL-4 (Mouse)	TCCTCACAGCAACGAAGAACAC	GAAGCCCTACAGACGAGCTCA
IL-10 (Human)	GCCTAACATGCTTCGAGATC	TGATGTCTGGGTCTTGGTTC
IL-10 (Mouse)	GGTTGCCAAGCCTTATCGGA	ACCTGCTCCACTGCCTTGCT
CCL5 (Human)	CTGTCATCCTCATTGCTACTGC	ATGTACTCCGAACCCATTCT
CCL5 (Mouse)	TGCCACGTCAAGGAGTATTTC	AACCCACTTCTTCTCTGGGTTG
CD68 (Human)	GCTACATGGCGGTGGAGTACAA	ATGATGAGAGGCAGCAAGATGG
CD68 (Mouse)	TCCAAGATCCTCCACTGTTG	ATTTGAATTTGGGCTTGGAG
Arginase-1 (Human)	GGCAAGGTGATGGAAGAAAC	AGTCCGAAACAAGCCAAGGT
Arginase-1 (Mouse)	GGAACCCAGAGAGAGCATGA	TTTTTCCAGCAGACCAGCTT
CD86 (Human)	GTATTTTGGCAGGACCAGGA	GCCGCTTCTTCTTCTTCAT
CD206 (Human)	CGAGGAAGAGGTTTCGGTTCACC	GCAATCCCGGTTCTCATGGC
CCL2 (Human)	CCCCAGTCACCTGCTGTTAT	TGGAATCCTGAACCCACTTC
IFN- γ (Human)	CCAACGCAAAGCAATACATGA	CCTTTTTCGCTTCCCTGTTTTA
JEV (GP78)	CAGGGAAGAGATCAGCCATTAG	GGAGCATGTACCCATAGTGAAG
GAPDH (Human)	GCAAATTCCATGGCACCGT	TCGCCCCACTTGATTTTGG
GAPDH (Mouse)	ATGGCAAGTTCAAAGGCACAGTCA	TGGGGGCATCAGCAGAAGG
pMIR luciferase reporter vector		
NKRF Luc	cggactagtGTAAATGCCTAATGAGGCAG	cggacgcgtTTCTGATCAACTTTGGCTCC
NKRF Luc Mut	GACAATAAGCCTCATACTACACGTAAATGTCC	TACGTGTAGTATGAGGCTTATTGTCAAACATT
pcDNA3.1 (+) expression vector		
NKRF cds	cccaagcttGCCACGCTTGATGGAAAAA	cgcggatccTCAATTTGCTTGAGGCATAA