viruses

Supplementary material

Inhibition of p38 Mitogen-Activated Protein Kinase Impairs Mayaro Virus Replication in Primary Human Dermal Fibroblasts and HeLa Cells

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Table S1. List of primers used in this study.

Gene	Primer sequences (5′-3′)	References
MDA5	Forward: GCCATTGCAGATGCAACCAG Reverse: TTGCGATTTCCTTCTTTTGCAG	[1]
ISG15	Forward: GAGAGGCAGCGAACTCATCT Reverse: CTTCAGCTCTGACACCGACA	[2]
MxA	Forward: GGTGGTGGTCCCCAGTAATG Reverse: ACCACGTCCACAACCTTGTCT	[3]
RIG-I	Forward: TGTTCTCAGATCCCTTGGATG Reverse: CACTGCTCACCAGATTGCAT	[4]
OAS2	Forward: AAACCAGGCCTGTGATCTTG Reverse: GGGCTATTTCCAGACAACGC	[1]
AIM2	Forward: ATCAGGAGGCTGATCCCAAAG Reverse: TCTTCATCACTGCAGACACCG	[1]
IL1-β	Forward: AACCTCTTCGAGGCACAAGG Reverse: GTCCTGGAAGGAGCACTTCAT	[1]
TNF-α	Forward: CAGAGGGAAGAGTTCCCCAGGGACC Reverse: CCTTGGTCTGGTAGGAGACGG	[5]
IL-6	Forward: TGTGAAAGCAGCAAAGAGGCACTG Reverse: ACAGCTCTGGCTTGTTCCTCACTA	[4]
RANTES	Forward: GAGGCTTCCCCTCACTATCC Reverse: CTCAAGTGATCCACCCACCT	[4]
IL-8	Forward: CAGCCAAAACTCCACAGTCA Reverse: TTGGAGAGCACATAAAAACATCT	[4]
TLR3	Forward: TGGGACCAAGGCAAAGGAGT Reverse: TTCTCTTGGTTGGGCCACCT	[1]
TLR7	Forward: AATGTCACAGCCGTCCCTAC Reverse: TTATTTTTACACGGCGCACA	[1]
IRF3	Forward: GATGCACAGCAGGAGGATTT Reverse: GATTTTATGTGGGTCGTGAGG	[1]
IRF7	Forward: CAGAGTCTTCTTCCAAGAGCTG Reverse: TGCTATCCAGGGAAGACACA	[1]
IFN-*α*	Forward: GCCTCGCCCTTTGCTTTACT Reverse: CTGTGGGTCTCAGGGAGATCA	[5]
IFN-β	Forward: ATGACCAACAAGTGTCTCCTCC Reverse: GCTCATGGAAAGAGCTGTAGTG	[5]
β-actin	Forward: AGAGCTACGAGCTGCCTGAC Reverse: AGCACTGTGTTGGCGTACAG	[6]

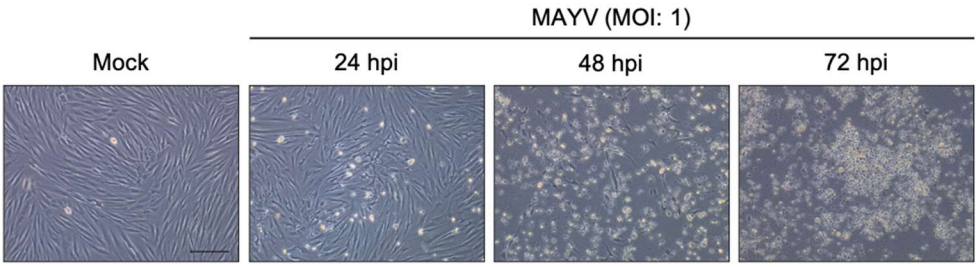

Figure S1. Mayaro virus induces a strong, time-dependent cytopathic effect in primary HDFs. HDFs were infected with MAYV at an MOI of 1. At indicated times after infection, images were captured using an inverted microscope and a MCI70-HD camera (Leica). Scale bar: 100 μm.

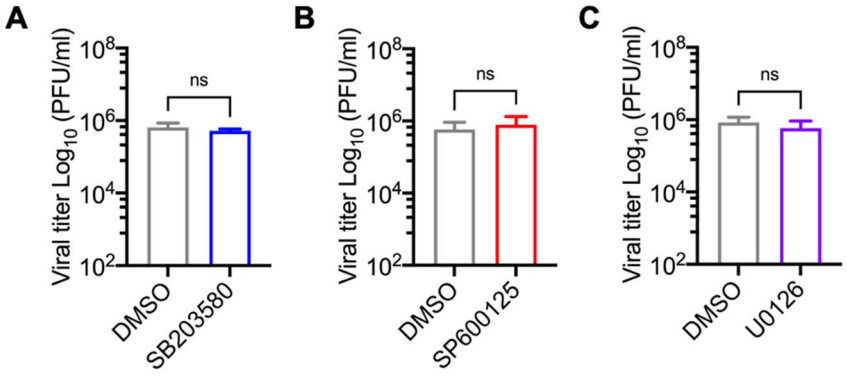

Figure S2. MAPK inhibitors SB203580, SP600125 and U0126 show no virucidal activity against MAYV. 1 x 10^6 PFU of MAYV in serum-free MEM were incubated with SB203580 (A), SP600125 (B) or U0126 (C) at 37 °C for 1 h. Then, the remaining virus for each experimental condition was directly quantified using plaque assay. Statistical differences were analyzed using the Mann-Whitney test: ns: non-significant.

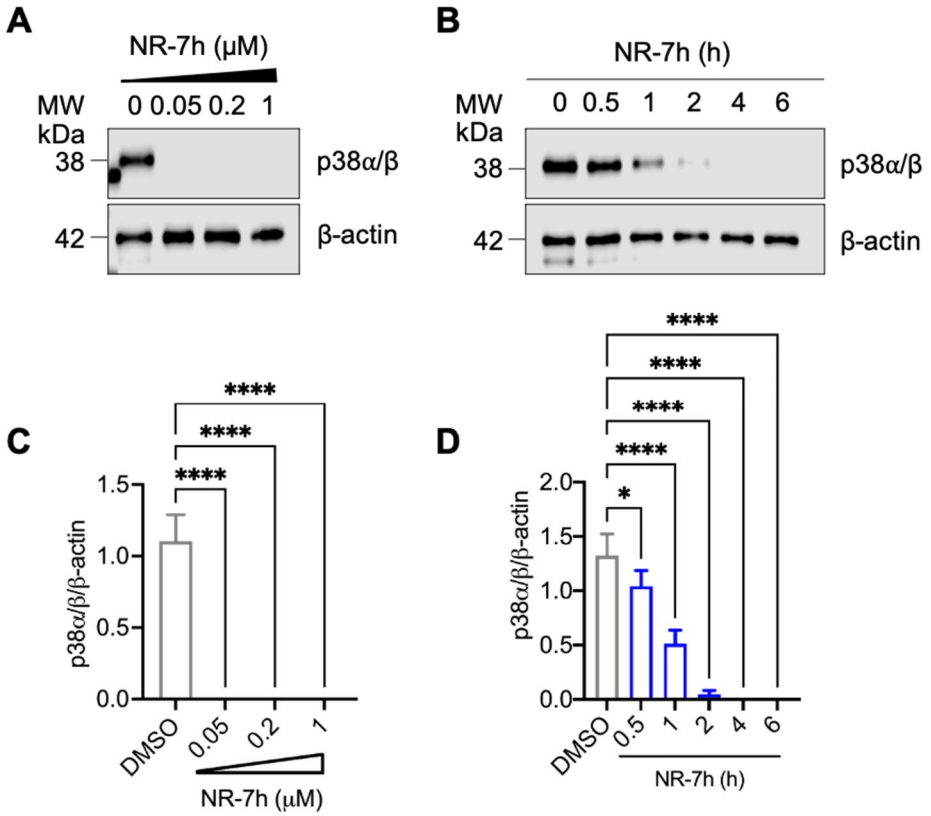

Figure S3. Knockdown of p38α and p38β isoforms using the PROTAC compound NR-7h in HDFs. (**A**) HDFs were treated with increasing concentrations of NR-7h; after 24 h of incubation, p38α/p38β protein levels were analyzed using immunoblot. β-actin protein was used as a loading control. MW: molecular weight. kDa: kilodaltons. (**B**) HDFs were treated with NR-7h at a concentration of 1 μM; at different time points, p38α/p38β protein levels were evaluated as described above. (**C**, **D**) Densitometric analysis of p38α/p38β proteins was performed using ImageJ software and normalized with β-actin protein. Statistical differences were analyzed using the One-way ANOVA test followed by Dunnett's post-test: *$p < 0.05$ and ****$p < 0.0001$.

References

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