**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.**

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**Gene Primer sequences (5’-3’) References**

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Reverse: TTGCGATTTCCTTCTTTTGCAG

*ISG15* Forward: GAGAGGCAGCGAACTCATCT [2]

Reverse: CTTCAGCTCTGACACCGACA

*MxA* Forward:GGTGGTGGTCCCCAGTAATG [3]

Reverse: ACCACGTCCACAACCTTGTCT

*RIG-I* Forward: TGTTCTCAGATCCCTTGGATG [4]

Reverse: CACTGCTCACCAGATTGCAT

*OAS2* Forward: AAACCAGGCCTGTGATCTTG [1]

Reverse: GGGCTATTTCCAGACAACGC

*AIM2* Forward: ATCAGGAGGCTGATCCCAAAG [1]

Reverse: TCTTCATCACTGCAGACACCG

*IL1-β*  Forward: AACCTCTTCGAGGCACAAGG [1]

Reverse: GTCCTGGAAGGAGCACTTCAT

*TNF-⍺* Forward: CAGAGGGAAGAGTTCCCCAGGGACC [5]

Reverse: CCTTGGTCTGGTAGGAGACGG

*IL-6* Forward: TGTGAAAGCAGCAAAGAGGCACTG [4]

Reverse: ACAGCTCTGGCTTGTTCCTCACTA

*RANTES* Forward: GAGGCTTCCCCTCACTATCC [4]

Reverse: CTCAAGTGATCCACCCACCT

*IL-8* Forward: CAGCCAAAACTCCACAGTCA [4]

Reverse: TTGGAGAGCACATAAAAACATCT

*TLR3* Forward: TGGGACCAAGGCAAAGGAGT [1]

Reverse: TTCTCTTGGTTGGGCCACCT

*TLR7*  Forward: AATGTCACAGCCGTCCCTAC [1]

Reverse: TTATTTTTACACGGCGCACA

*IRF3* Forward: GATGCACAGCAGGAGGATTT [1]

Reverse: GATTTTATGTGGGTCGTGAGG

*IRF7* Forward: CAGAGTCTTCTTCCAAGAGCTG [1]

Reverse: TGCTATCCAGGGAAGACACA

IFN-*⍺* Forward: GCCTCGCCCTTTGCTTTACT [5]

Reverse: CTGTGGGTCTCAGGGAGATCA

*IFN-β* Forward: ATGACCAACAAGTGTCTCCTCC [5] Reverse: GCTCATGGAAAGAGCTGTAGTG

*β*-actin Forward: AGAGCTACGAGCTGCCTGAC [6]

Reverse: AGCACTGTGTTGGCGTACAG

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**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. Aproximately 1 x 106 PFU of MAYV in serum-free MEM were incubated with SB203580 (A), SP600125 (B) or U0126 (C) at 37 ºC 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 p38⍺ and p38β isoforms using the PROTACs compound NR-7h in HDFs. (**A**) HDFs were treated with increasing concentrations of NR-7h; after 24 h of incubation, p38⍺/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, p38⍺/p38β protein levels were evaluated as described above. (**C**, **D**) Densitometric analysis of p38⍺/p38β proteins was performed using ImageJ software and normalized with β-actin protein. Statistical differences were analyzed using the One-way ANOVA test: \*p < 0.05 and \*\*\*\*p < 0.0001.

**References**

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