

## Supplemental material

### **Escape mutations in NS4B render dengue virus insensitive to the antiviral activity of the paracetamol metabolite AM404**

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**Table S1.** Primer sequences used for qRT-PCR-based quantification of DENV RNA in the culture supernatant of infected Hela cells. RNA was isolated from 100 µl of supernatant using Isol-RNA Lysis Reagent (5 PRIME) and cDNA synthesis was performed using TaqMan Reverse Transcription Reagents (Life Technologies). DENV RNA levels were analyzed by qPCR using GoTaq qPCR Master Mix (Promega) on a LightCycler 480 instrument (Roche). Genome copies were calculated based on standard curves with DENV NS1 plasmids.

<b>Serotype and strain</b>	<b>Primer name</b>	<b>Sequence</b>
DENV1 (16007)	DENV1-NS1 Fw	5'-GGGAAGTAGAGGACTATGGATTTG-3'
	DENV1-NS1 Rv	5'-GTGGTCACATACTTGGGTGTAG-3'
DENV2 (NGC)	DENV2-NS1 Fw	5'-AGAACTGAAGTGTGGCAGTGGGAT-3'
	DENV2-NS1 Rv	5'-TGCCCTCTTCATGAGCTTTCTGGA-3'
DENV4 (H241)	DENV4-NS1 Fw	5'-ACTTGGGCAAATTGGAGATAGA-3'
	DENV4-NS1 Rv	5'-CAGTAGTGGTCCTCAAAGATGG-3'

**Table S2.** Effect of Cox inhibitors in the NIH Clinical Collection on DENV2 replication and cell viability. Luciferase activity and cell viability of Hela DENV2 replicon cells were determined after 48 h of culture in the presence of compounds (10  $\mu$ M). Data represent percentages of the DMSO control; means and standard deviations of 3 biological replicates are reported. Ribavirin was included as a positive control.

Compound	NCC structure ID	PubChem CID	PubChem SID	Luciferase activity (%)		Cell viability (%)	
				Mean	Stdev	Mean	Stdev
AM404	CPD000449274	6604822	46386792	48.4	1.6	102.9	6.3
Ampiroxicam	CPD000469285	2176	46386688	87.4	11.3	100.2	4.6
Flurbiprofen	CPD000042823	3394	46386788	98.6	7.3	97.2	2.4
Indomethacin	CPD000449290	23581802	46386809	103.5	1.0	96.7	4.0
Naproxen sodium	CPD000058746	169117	46386948	97.3	5.8	99.4	2.4
Parecoxib sodium	CPD000469594	15895902	46386785	86.7	15.3	102.6	3.5
Piroxicam	CPD000449291	54705052	46386918	100.5	3.2	100.6	4.0
Rofecoxib	CPD000466331	5090	46386634	69.1	12.4	97.8	4.0
Valdecoxib	CPD000466327	119607	46386621	93.9	6.1	96.2	3.3
Ribavirin		37542	24278685	19.1	0.6	89.1	1.4

NCC structure ID, unique structure identifier assigned by Molecular Libraries Small Molecule Repository (MLSMR); PubChem CID, compound identifier; PubChem SID, substance identifier.