SUPPLEMENTARY INFORMATION

Affinity-based protein profiling of the antiviral natural product Nanchangmycin

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I. Supplementary figures

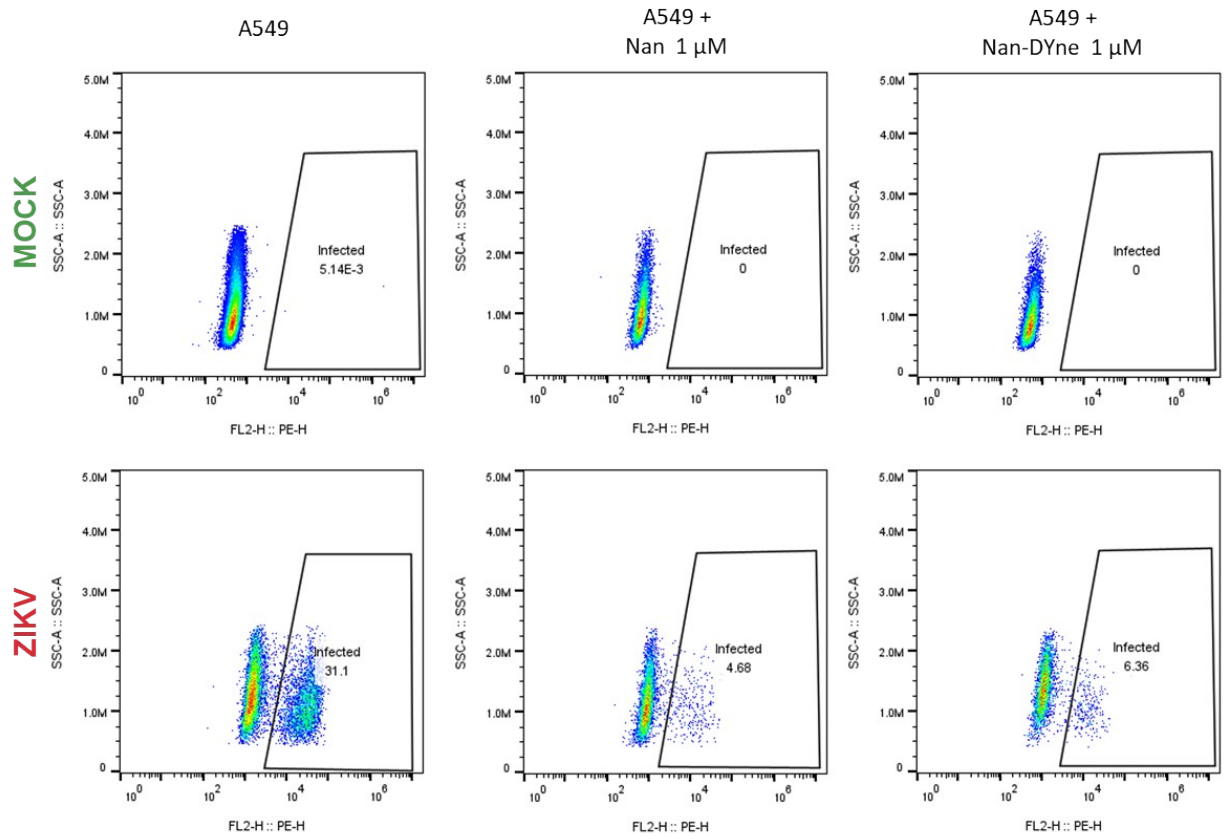


Figure S1. Flow cytometry identification of ZIKV-infected A549 72 h post-ZIKV infection (MOI = 1). Cells were fixed, permeabilised, stained with NS1-Alexa647 and analysed by flow cytometry. Representative dot plots showing the side scatter (SSC) as a function of the fluorescence. Infected cells were gated with respect to cells exposed to non-infected A549 controls (MOCK). The plots shown were obtained with one biological replicate. They are representative of three independent experiments and were used in Figure 1C.

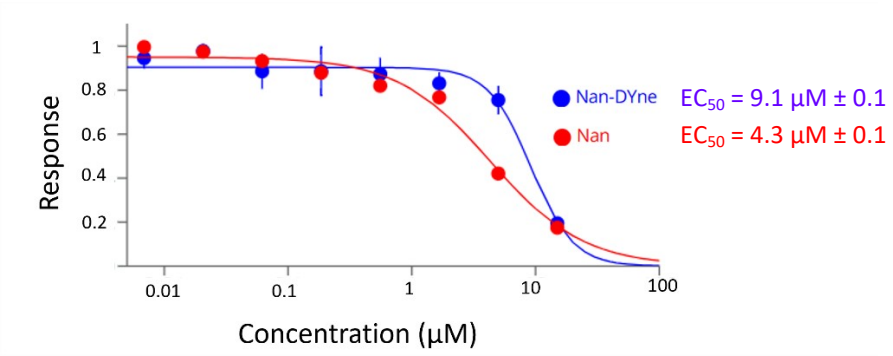


Figure S2. *MTS assay in HeLa cells treated with inscreasing concentrations of Nan or Nan-DYne for 72 h (n = 6). Data were plotted using the AAT Bioquest online tool.* [1] *Half-maximal effective concentrations (EC_{50}) are indicated.*

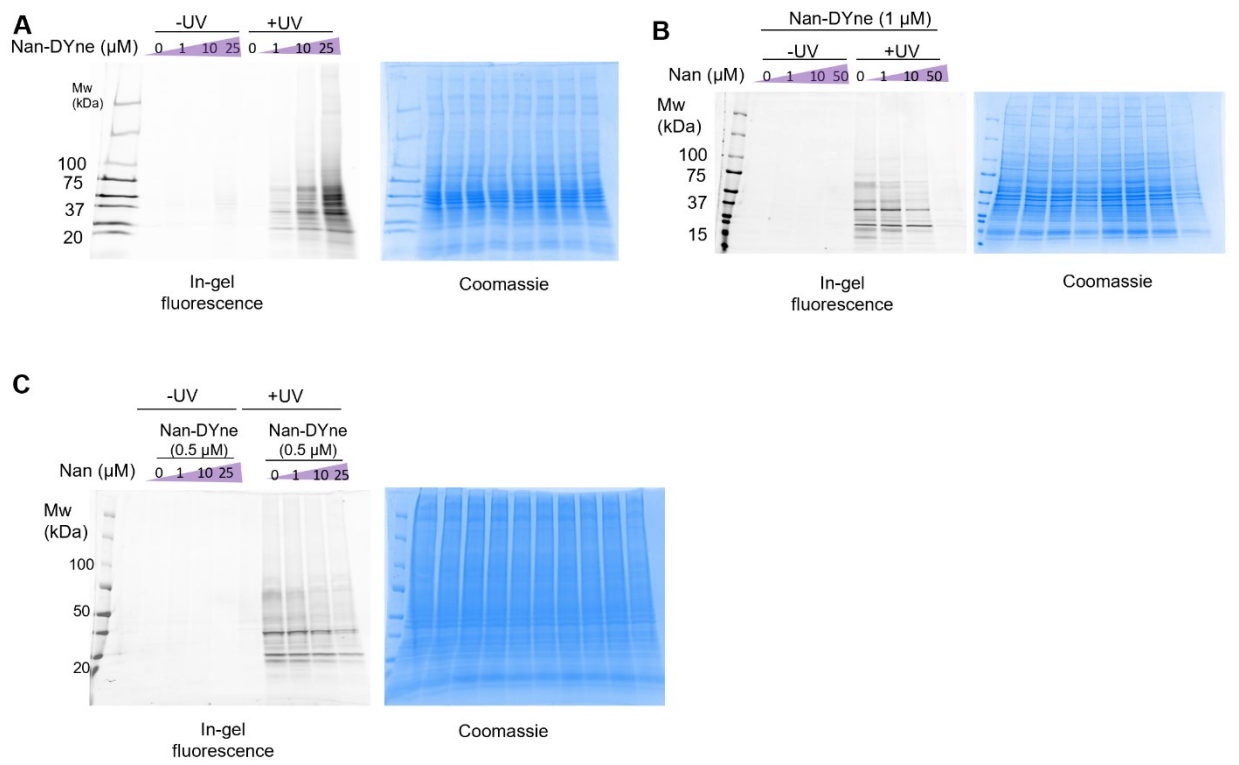


Figure S3. Nan-DYne labelling is UV-dependant, increases with concentration and is sensitive to competition with the natural product Nanchangmycin (Nan). Cells were treated with Nan or DMSO, then with Nan-DYne for 1h, irradiated or not with UV, lysed. Nan-DYne-labelled proteins were coupled to azido-TAMRA via CuAAC and separated by SDS-PAGE gel. **A.** Cells were treated with increasing concentrations of Nan-DYne. **B-C.** Cells were treated with increasing concentrations of Nan for the competition and labelled with 1 μM (B) or 0.5 μM (C) of Nan-DYne.

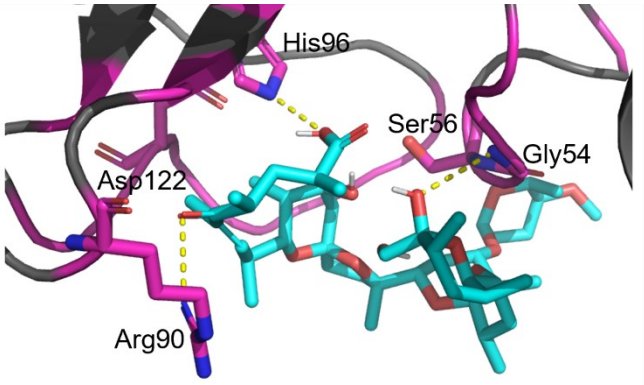


Figure S4. Molecular docking of Nan in SEC11A (PDB: 7p2p)[2] with SwissDock.[3,4] Nan is shown in blue, SEC11A in pink. The residues of the active site are Ser56, His96, Asp122.[2] Nan engages in multiple interactions, including hydrogen bonds with Gly54 His96 and Arg90. The docking analysis reveals a favourable binding mode for nanchangmycin within the active sites of SEC11A with a calculated affinity of -6.4 kcal/mol.

II. Chemical methods

1. General chemical methods

Commercially available reagents were used without further purification. Solvents and chemicals were purchased from Sigma-Aldrich and VWR. Nanchangmycin was obtained from MedChemExpress. 2-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)ethan-1-amine was purchased from BLDPharm.

Reaction monitoring was performed by thin-layer chromatography (TLC) on Merck aluminium plates pre-coated with silica gel 60 F254. Visualisation of spots was performed using a UV lamp (at 254 nm) or by TLC plate staining using potassium permanganate or ninhydrin.

2. Synthesis of Nan-DYne

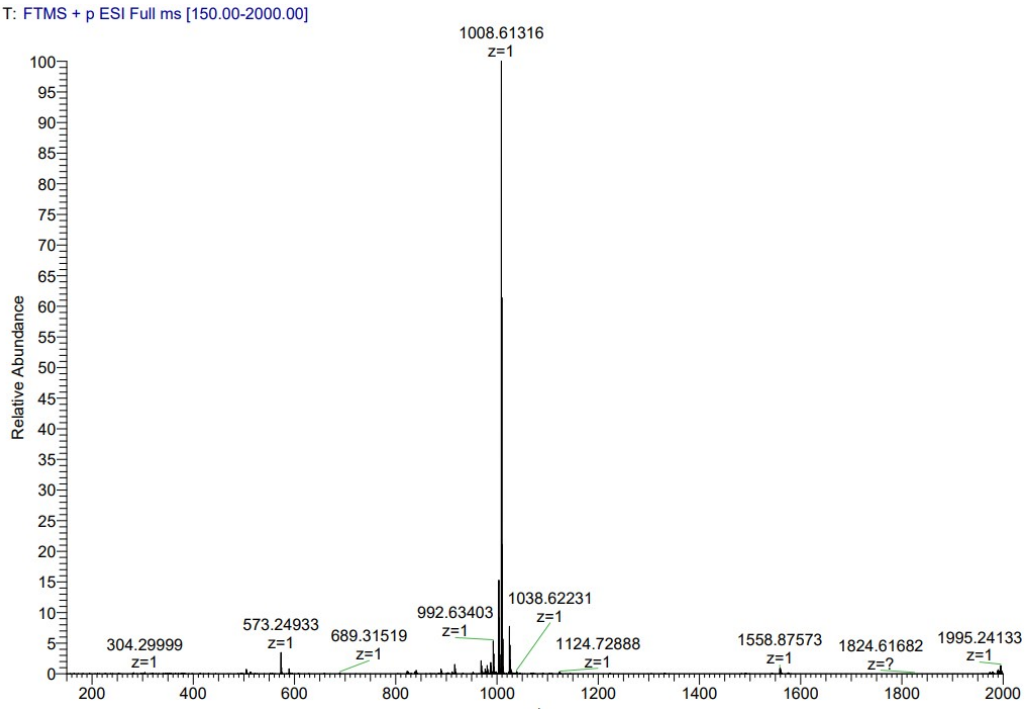
In a Schlenk flask, 10 mg (11.25 mmol, 1 eq) of Nanchangmycin (NAN-COOH) (**1**) were dissolved in 2 mL of anhydrous DMF, together with 6 µL (34.44 mmol, 3 eq) diisopropylethylamine (DIPEA) and 6.5 mg (16.88 mmol, 1.5 eq) 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU). After 30 minutes, 3.10 µL (22.50 mmol, 2 eq) 2-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)ethan-1-amine (Amino diazirine alkyne) were added at 0 °C, allowed to warm to room temperature and stirred overnight. DMF was removed under vacuum and the product dissolved in 50 mL EtOAc. The organic phase was washed with water (3 x 20 mL), dried over Na_2SO_4 and the solvent removed under vacuum, affording Nan-DYne as a white powder (0.0097 g, 87%). Rf Hex:EtOAc 1:9 = 0.56

^{1}H NMR (800 MHz, DMSO) δ 7.92 (t, *J* = 5.6 Hz, 1H), 6.41 (d, *J* = 9.9 Hz, 1H), 4.80 (s, 1H), 4.57 (d, *J* = 9.2 Hz, 1H), 4.30 (d, *J* = 6.4 Hz, 1H), 4.01 (d, *J* = 10.2 Hz, 1H), 3.98 (d, *J* = 7.0 Hz, 1H), 3.88 (d, *J* = 3.7 Hz, 1H), 3.78 (q, *J* = 8.2 Hz, 1H), 3.62 (dd, *J* = 6.9, 3.6 Hz, 1H), 3.50 (d, *J* = 11.8 Hz, 1H), 3.30 – 3.25 (m, 5H), 3.22 (dd, *J* = 11.0, 5.4 Hz, 2H), 3.18 (q, *J* = 6.8 Hz, 2H), 3.01 (dq, *J* = 13.1, 6.5 Hz, 1H), 2.92 (dq, *J* = 12.9, 6.4 Hz, 1H), 2.81 – 2.76 (m, 2H), 2.64 – 2.60 (m, 1H), 2.49 – 2.44 (m, 3H), 2.26 (q, *J* = 7.1 Hz, 1H), 2.18 – 2.11 (m, 1H), 2.03 (q, *J* = 8.9 Hz, 3H), 1.99 (td, *J* = 7.5, 2.5 Hz, 2H), 1.92 – 1.87 (m, 2H), 1.84 (t, *J* = 10.7 Hz, 1H), 1.81 – 1.74 (m, 3H), 1.73 (s, 3H), 1.72 – 1.69 (m, 1H), 1.65 (dt, *J* = 16.1, 7.6 Hz, 1H), 1.60 (m, 5H), 1.53 (t, *J* = 6.9 Hz, 3H), 1.49 (d, *J* = 11.8 Hz, 1H), 1.41 (dtd, *J* = 30.6, 12.1, 4.8 Hz, 2H), 1.32 (dd, *J* = 13.4, 4.3 Hz, 1H), 1.25 (s, 4H), 1.21 (t, *J* = 13.7 Hz, 1H), 1.16 (d, *J* = 6.2 Hz, 3H), 1.04 (d, *J* = 6.5 Hz, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.95 (dd, *J* = 12.7, 6.6 Hz, 3H), 0.91 (d, *J* = 6.9 Hz, 3H), 0.82 (d, *J* = 6.7 Hz, 3H), 0.76 (d, *J* = 6.6 Hz, 3H), 0.70 (d, *J* = 7.0 Hz, 3H).

^{13}C NMR (201 MHz, DMSO) δ 204.82, 175.10, 143.13, 134.30, 109.20, 106.66, 100.67, 96.61, 86.49, 83.04, 79.70, 79.48, 78.34, 77.87, 73.62, 72.80, 71.69, 69.75, 65.47, 55.97, 39.99, 39.60, 38.62, 37.71, 37.38, 37.30, 37.24, 36.32, 36.19, 35.69, 35.58, 34.67, 33.61, 33.22, 33.08, 32.39, 32.03, 31.29, 30.26, 27.22, 26.47, 25.01, 23.06, 18.66, 18.35, 18.28, 17.19, 17.04, 16.90, 16.29, 13.23, 12.71, 11.48, 10.53.

MS(ESI-LC-MS): m/z calculated for $[C_{54}H87N_3O_{13}+Na]^+$ 1008.6131; $[M+H]^+$: found 1008.6132

3. HRMS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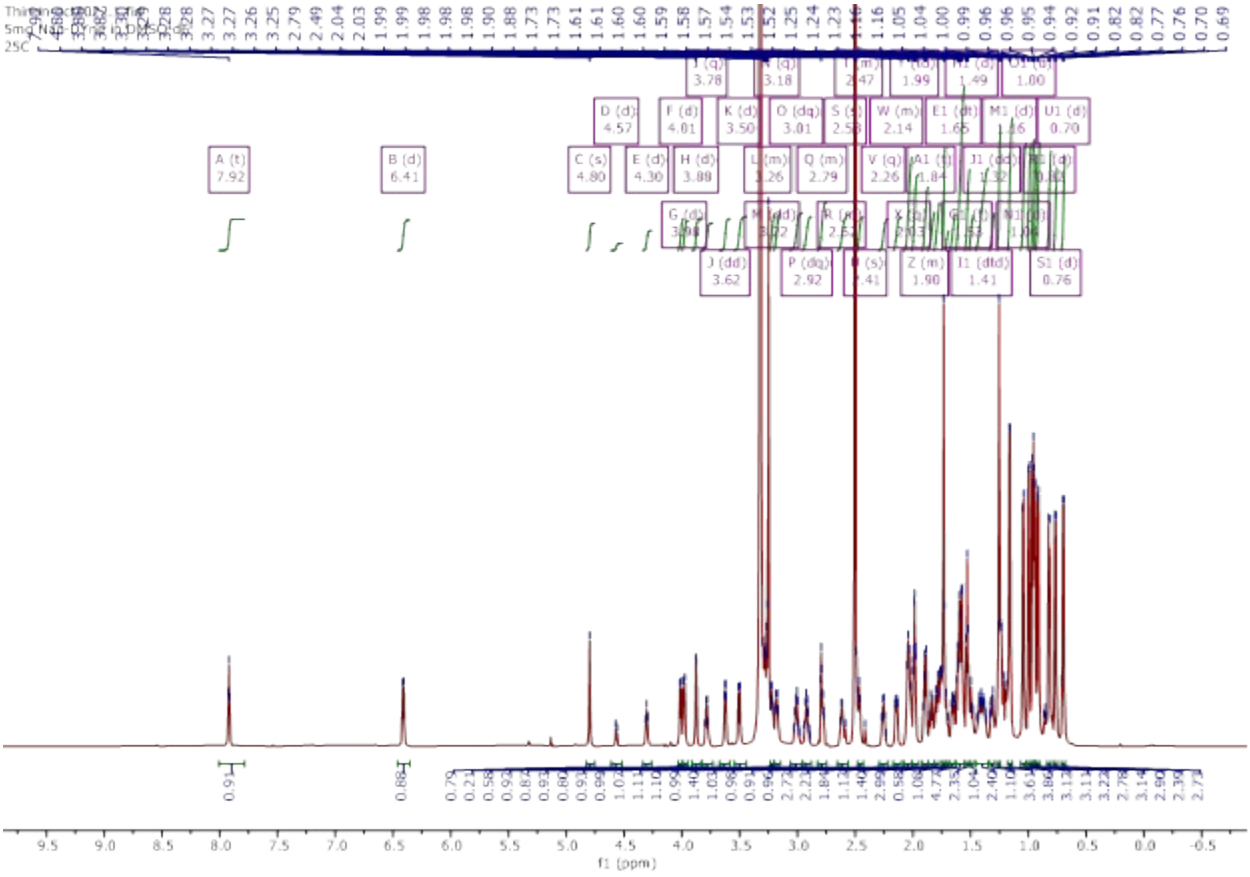
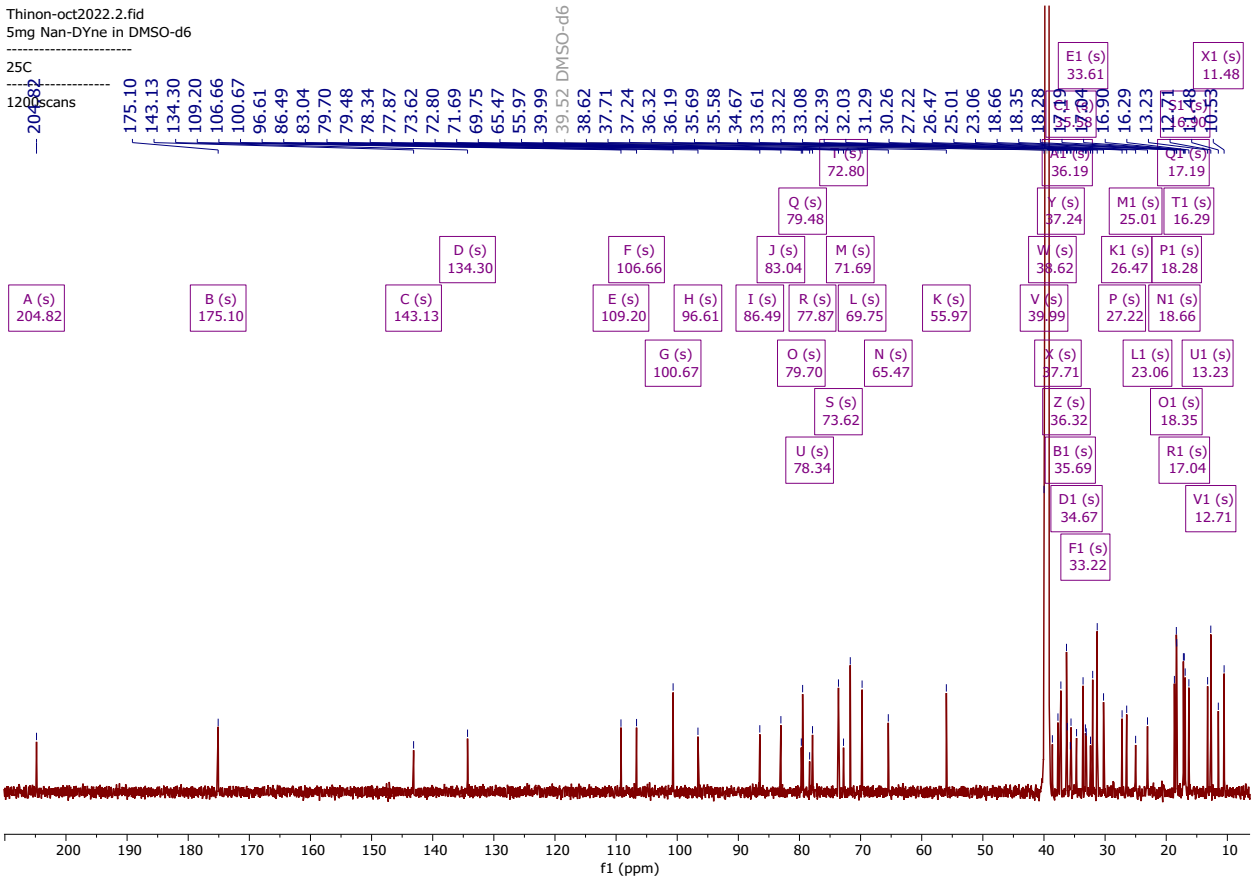
4. NMR spectra

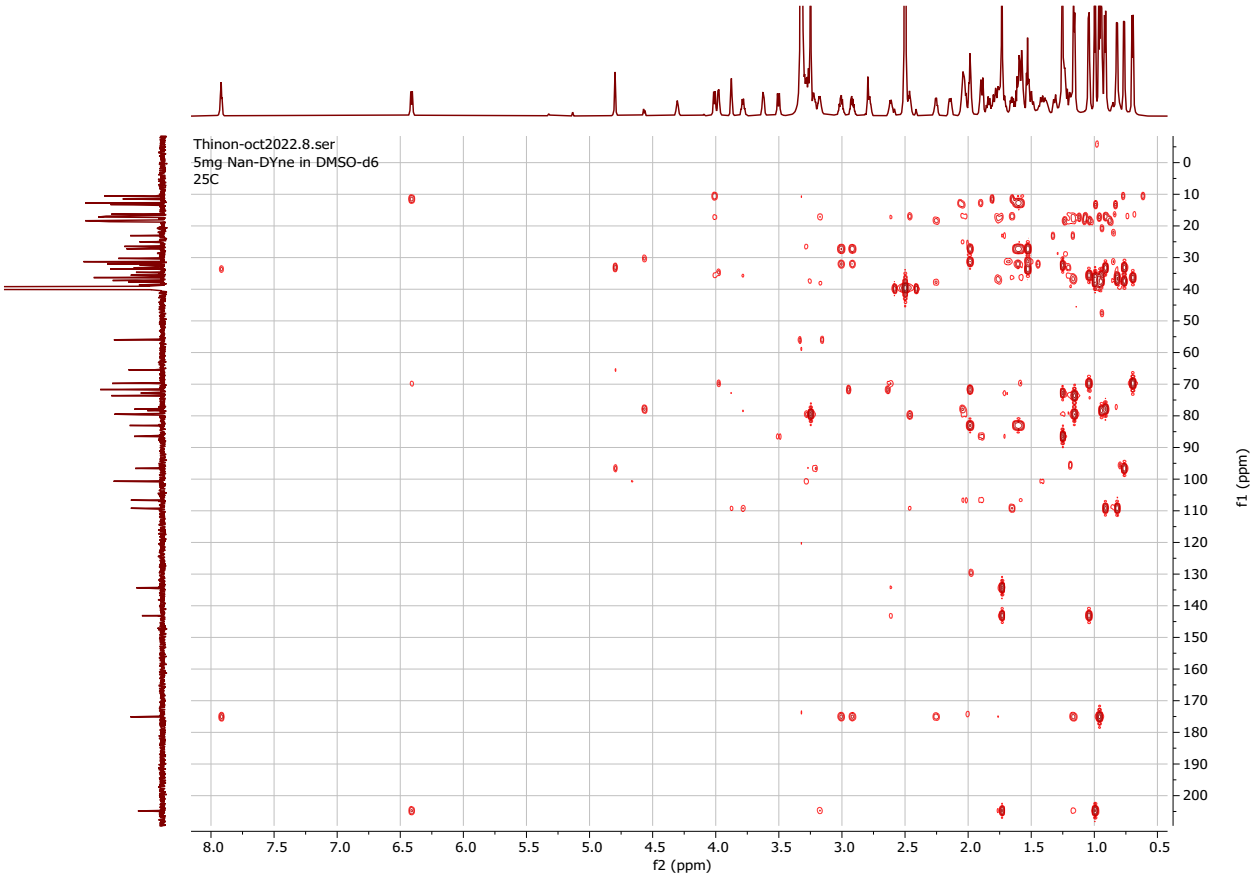
^{1}H NMR



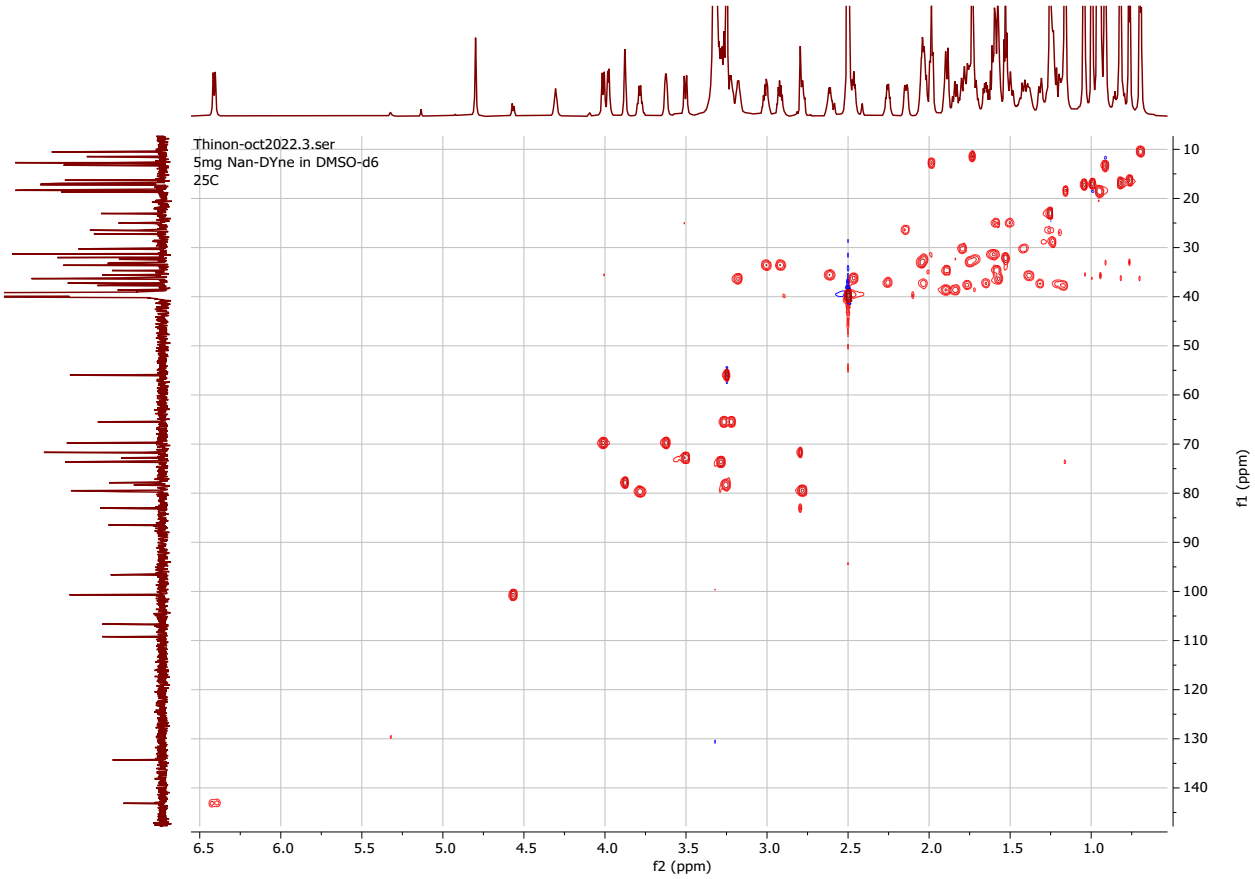
^{13}C NMR


Thinon-oct2022.2.fid
5mg Nan-DYne in DMSO-d6
25C
1200scans
DMSO-d6
A (s) 204.82
B (s) 175.10
C (s) 143.13
D (s) 134.30
E (s) 109.20
F (s) 106.66
G (s) 100.67
H (s) 96.61
I (s) 86.49
J (s) 83.04
K (s) 55.97
L (s) 69.75
M (s) 71.69
N (s) 65.47
O (s) 79.70
P (s) 27.22
Q (s) 79.48
R (s) 77.87
S (s) 73.62
T (s) 72.80
U (s) 78.34
V (s) 39.99
W (s) 38.62
X (s) 37.71
Y (s) 37.24
Z (s) 36.32
A1 (s) 36.19
B1 (s) 35.69
C1 (s) 34.67
D1 (s) 34.67
E1 (s) 33.61
F1 (s) 33.22
G1 (s) 33.08
H1 (s) 32.39
I1 (s) 32.03
J1 (s) 31.29
K1 (s) 26.47
L1 (s) 23.06
M1 (s) 25.01
N1 (s) 18.66
O1 (s) 18.35
P1 (s) 18.28
Q1 (s) 17.19
R1 (s) 17.04
S1 (s) 16.90
T1 (s) 16.29
U1 (s) 13.23
V1 (s) 12.71
X1 (s) 11.48
f1 (ppm)

2D ^{1}H-^{13}C HMBC



2D ^{1}H-^{13}C HSQC



III. Biological Methods

1. General biological methods

EDTA-free protease inhibitor was purchased from Roche Applied Science and Benzonase from EMD Millipore. Protein concentration was measured with the BCA protein assay (Thermo Scientific Pierce).

Cell Lines

HeLa, A549, Vero E6 and HEK-293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% Fetal Bovine Serum (FBS, Gibco), L-Glutamine (2 mg/mL, Gibco) and Penicillin-Streptomycin (100 U/mL, Gibco) and incubated at 37°C in a humidified incubator with 5% CO_2. Cells were transfected using Lipofectamine 3000 (Life Technologies) with a 3:1 ratio of transfection reagent/DNA according to the manufacturer's protocol in cell growth media.

SDS-page gels

4-20 % gradient sodium dodecyl sulphate-polyacrylamide (SDS-PAGE) gel (0.75 mm thick) were prepared in-house (with a 6% stacking gel on top) using Acrylamide: Bis-Acrylamide 37.5:1 (Serva). Pre-stained protein ladders (Precision plus protein all blue standard and precision plus dual color standards) were purchased from Bio-Rad Laboratories.

Samples containing 1x sample loading buffer (4x Laemmli:BME 1:0.1) were boiled for 5 min and separated by SDS-PAGE.

Typically, to 10 µL of a sample, 3.33 µL of 4x Loading Buffer (Laemmli:β-mercaptoethanol 9:1) were added, and the samples were boiled at 95°C for 5 min, centrifuged for 1 min at 17,000 x g, then loaded on a gel. The migration was carried out at 100 V for the first 5-10 minutes and then 200 V until the end. The gel was briefly washed for 5 min in water. The gel was washed twice with deionised water before in-gel fluorescence or stain-free scanning or staining with Coomassie (InstantBlue, Expedeon).

Stain-free scanning were performed using a BioRad ChemiDoc Imaging System. In-gel fluorescence was visualised on a TyphoonTM FLA 9500 imager (GE Healthcare).

For western blotting, SDS-PAGE gels that were run (and not Coomassie stained) were transferred onto a nitrocellulose membrane (GE Healthcare Amersham Protran Premium 0.2 µm NC) using a semi-dry transfer set-up using a BioRad Trans-Blot Turbo Transfer System (Standard SD protocol) and the following transfer buffer: 25 mM Tris (base), 192 mM glycine, supplemented with 20% (v/v) MeOH. To check for protein loading and confirm that the wet transfer was successful, the membrane was stained by Ponceau S stain (Sigma-Aldrich) for 5 mins at r.t.

Western Blot Analysis

Primary antibodies used were anti-HA (901501, Biolegend), rabbit anti-V5 tag primary antibody (14440-1-AP, Proteintech, 1:1000). Secondary antibodies used were HRP-conjugated goat anti-rabbit IgG secondary antibody (111-035-003, Jackson ImmunoResearch, 1:5000), and HRP conjugated goat anti-mouse IgG (115-035-003, Jackson ImmunoResearch, 1:5000).

Membranes were blocked in 5% non-fat dry milk (Sigma-Aldrich) in TBS-T (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Tween-20) for 1 h at room temperature and incubated overnight at 4°C with the primary antibody in blocking buffer. After three washes with TBS-T, membranes were incubated with the appropriate secondary antibody (1:5000, Jackson ImmunoResearch) for 1 h at room temperature. Following three additional washes with TBS-T, signal detection was performed using Immobilon® Crescendo Western HRP substrate (Merck) according to the manufacturer's instructions and protein

bands were visualised using the ChemiDoc XRS+ Imaging System (Bio-Rad). Band intensities were determined using the Fiji ImageJ software.

2. Zika infection

Viruses

The ZIKV virus ATCC ® VR-1843™ was a gift of Vincent Calvez, Hospital Pitié-Salpêtrière, Paris, France. Agreement to work with infectious ZIKV was obtained in a Class II Biosafety Cabinet under BSL-3 conditions at the UB'L3 facility (TransBioMed core, University of Bordeaux, France).

Viral stock production

The ZIKV strain was propagated by infecting Vero E6 cells at a multiplicity of infection (MOI) of 0.1. Infected cells were incubated at 37 °C and 5% CO_2 in a humidified incubator for 72 hours. Then, the culture supernatant was clarified by centrifugation (5 min at 1500 rpm) and aliquots were stored at 80 °C.

Viral infections

A549 cells previously cultured in a 12-well plate were treated with either a vehicle control, Nan (1 µM), or Nan-DYne (1 µM) for 1 hour. Following treatment, the cells were infected with ZIKV at a multiplicity of infection (MOI) of 1. The infection medium consisted of the appropriate dilution of the ZIKV stock in Opti-MEM medium (Gibco), supplemented with either the vehicle control, Nan (1 µM), or Nan-DYne (1 µM). After 1 hour, the infection medium was removed and replaced with growth medium supplemented with the respective treatments. Non-infected control cells (Mock) were subjected to the same treatment conditions, except that fetal bovine serum (FBS) was used in place of the ZIKV stock. Both infected and Mock-treated cells were incubated at 37 °C with 5% CO_2 in a humidified incubator for 72 hours.

Flow Cytometry

Flow cytometry assays were performed to assess ZIKV infection in A549 cells. After 72 hours post infection, culture supernatants were collected for RT-qPCR and $TCID_{50}$ assays and cells were washed with 1 mL of PBS. Then, cells were harvested by treating each well with 400 µL of 0.05% trypsin solution and incubating at 37°C for 10 minutes. Trypsinization was stopped by adding 600 µL of DMEM supplemented with 10 % FBS. Cells suspensions were homogenised by pipetting up and down. Cells were collected by centrifugation at 300 × g for 5 minutes and washed twice with 500 µL of PBS. After resuspension in 500 µL of PBS, fixation was performed by adding 500 µL of 8% paraformaldehyde in PBS and incubating at room temperature for 15 minutes. Fixed cells were collected by centrifugation at 300 × g for 5 minutes and washed twice with 500 µL of PBS. Permeabilization was carried out using 500 µL of 0.1% Triton X-100 in PBS for 10 minutes at room temperature, followed by two additional washes with 500 µL of PBS. Blocking was performed by incubating the cells with 500 µL of 5% FBS 0.01% Triton X-100 in PBS for 30 minutes at room temperature. Cells were then incubated with an anti-Flavivirus Envelope Protein antibody conjugated with Alexa Fluor™ 647 (BD Biosciences) diluted 1:300 in 300 µL of 5% FBS 0.01% Triton X-100 in PBS for 30 minutes at room temperature. After incubation, cells were collected by centrifugation at 300 × g for 5 minutes and washed twice with 500 µL of 5% FBS 0.01% Triton X-100 in PBS. Finally, cells were resuspended in 250 µL of 2% FBS in PBS and analysed by flow cytometry using a BD Accuri C6 Plus Flow Cytometer (BD biosciences) to assess the number of ZIKV infected cells.

Viral RNA purification and RT-qPCR analysis

Viral RNA was purified from 200 µl of cell culture supernatant after 72 hours post infection, using High Pure Viral Nucleic Acid Kit (Roche Diagnostics) according to the manufacturer protocol. RNA was eluted

into 50 µL of elution buffer. Each replicate of RT-qPCR assays was performed using 5 µL of RNA elution and GoTaq® 1-Step RT-qPCR System (Promega) in a CFX Opus 96 Real Time PCR System (Bio-Rad). ZIKA RNA was amplified using forward ZFB 1086 (CCGCTGCCCAACACAAG) and reverse ZRB1162 (CCACTAACGTTCTTTTGCAGACAT) primers. These primers are localised in a region between M gene and NS1 gene. The thermocycling conditions were: one retro-transcription step for 30 minutes at 50 °C, followed by 10 minutes at 95°C and 40 cycles of 15 seconds at 95°C, 15 seconds at 60°C and 15 seconds at 72°C.

TCID measurement

Viral titers were determined by adding serial dilutions of the viral suspensions (viral stock or supernatant from infected cells conditions) to A549 cells previously cultivated in a 96-well plate. Four replicates were performed. Plates were incubated at 37°C for 72 hours and examined for cytopathic effect. Quantification of cytopathic effect was determined using the CellTox™ Green Cytotoxicity Assay (Promega) according to manufacturer instructions. The level of fluorescence associated with the cytopathic effects were measure using a Victor Nivo plate reader (Perkin-Elmer). The TCID50/ml values were calculated according to the method of Reed and Muench.[5]

3. Cell cytotoxicity assay

HeLa cells were seeded in a 96-well plate 24 h before treatments by adding 50 µL of cell suspension to each well (50 000 cells/mL). 24 h after seeding, 50 µL of growth media containing DMSO, Nan, Nan-DYne or Puromycin (4 µg/mL, 50 µL, final concentration in the plate 2 µg/mL) were added to the cells. 7 concentrations of inhibitor were prepared (triple dilutions starting from 30 µM (final concentration in the plate starting from 15 µM, n= 6 replicates). 72 h later, the MTS reagent (Promega) was prepared according to the supplier's protocol and 20 µL of this reagent were added to each well of the 96-well plate. Absorbance was measured at 490 nm. The average of absorbance values of the negative control (cells treated with Puromycin) was subtracted from each value. The metabolic activity was calculated as a percentage relative to the positive control. EC50s were calculated by fitting the data to the IC_{50} function using AAT Bioquest online tool. [1]

4. Nan-DYne labelling

Nan-DYne treatment and UV irradiation

HeLa cells were plated 24 h before treatment. Cells were labelled with Nan-DYne (0.5 µM unless otherwise stated) for 1 h. For competition experiments, cells were pre-treated for 15 min with nanchangmycin (25 µM unless otherwise stated), or DMSO as a vehicle control.

Cells were washed with ice-cold PBS (2x) and ice-cold PBS was added. Cells were placed on ice and irradiated with UV for 10 min using an UVP Crosslinker CL-3000L 365nm, 230 (Analytik Jena). Cells were harvested, flash-frozen and stored at −80 °C.

CuAAC click labelling and in-gel fluorescence

Frozen protein pellet was lysed in 4% SDS, 150 mM NaCl, 50 mM Hepes pH 7.4, benzonase, EDTA-free protease inhibitor (Roche). Lysates (100 µg) were diluted with 150 mM NaCl, 50 mM Hepes pH 7.4 to 94 µL and 6 µL of freshly prepared CuAAC reactant solution [azido-TAMRA-biotin (1 µL, 10 mM stock solution in DMSO, click chemistry tools), $CuSO_4$ (2 µL, 50 mM in H_2O), TCEP (2 µL, 50 mM in H_2O)), TBTA (1 µL, 10 mM stock solution in DMSO, Sigma] were added and vortexed for 1 h at room temperature (final protein concentration: 1 mg/mL, final SDS concentration < 0.5%). EDTA (2 µL of a 0.5 M solution in H_2O) was added to stop the CuAAC reaction. Samples were briefly vortexed and proteins were precipitated by

$CHCl_3$/MeOH precipitation (4 volumes (400 µL) MeOH, 1 volume (100 µL) $CHCl_3$, 3 volumes (300 µL) H_2O). The protein pellets were allowed to air-dry for 5-10 min, resuspended in 37.5 µL of 4% SDS, 150 mM NaCl, 50 mM Hepes pH 7.4, diluted with 12.5 µL 4× sample loading buffer (4xLaemmli:BME 1:0.1), boiled for 5 min and separated by SDS-PAGE.

5. Proteomics

LC-MS grade H_2O, LC-MS grade solvent and filtered tips were used to prevent any contamination. Eppendorf® LoBind microcentrifuge tubes were used. Mass spectrometry grade trypsin was purchased from Promega.

Sample preparation for proteomics

Lysates were prepared as described above. 500 µg of cell lysates / sample were used. CuAAC reaction was scaled up and performed as described above. Following the last $CHCl_3$/MeOH protein precipitation, protein pellets were air-dried for 10 min and resuspended in 500 µL 0.4% SDS, 50 mM Hepes pH 7.4, 150 mM NaCl and added to 15 µL of high-capacity Neutravidin beads (Thermo scientific) (pre-washed three times with 0.4 % SDS, 50 mM Hepes pH 7.4, 150 mM NaCl). Samples were incubated at room temperature with end-over-end rotation for 90 min. The beads were washed with 1 mL 1 % SDS in PBS (3 x 5 min), 1 mL 4 M urea in PBS (2 x 5 min), 1 mL AMBIC (ammonium bicarbonate) (5 x 2 min).

Protein samples were solubilised in Laemmli buffer, and samples were deposited in triplicate onto SDS-PAGE gel. After colloidal blue staining, each lane was cut out from the gel and was subsequently cut in 1 mm × 1 mm gel pieces. Gel pieces were destained in 25 mM ammonium bicarbonate 50% ACN (acetonitrile), rinsed twice in ultrapure water, and shrunk in ACN for 10 min. After ACN removal, gel pieces were dried at RT, reduced in 10 mM DTT for 30 min at 56°C before alkylation in 100 mM Iodoacetamide for 30 min at room temperature. After being dried again, gel pieces were covered with the trypsin solution (10 ng/µl in 50 mM NH_4HCO_3), rehydrated at 4°C for 10 min, and finally incubated overnight at 37°C. Spots were then incubated for 15 min in 50 mM NH4HCO3 at RT with rotary shaking. The supernatant was collected, and an H2O/ACN/HCOOH (47.5:47.5:5) extraction solution was added onto gel slices for 15 min. The extraction step was repeated twice. Supernatants were pooled and dried in a vacuum centrifuge. Digests were finally solubilised in 0.1% HCOOH.

LC-MS/MS analysis

Peptide mixtures were analysed on an Ultimate 3000 nanoLC system (Dionex, Amsterdam, The Netherlands) coupled to an Electrospray Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA). The peptide digests were resolubilised in 2 % ACN 0.5 % TFA then diluted by 1/6. Ten microliters of peptide digests dilution were loaded onto a 300-µm-inner diameter x 5-mm C18 PepMapTM trap column (LC Packings) at a flow rate of 10 µL/min.

The peptides were eluted from the trap column onto an analytical 75-µm id x 50-cm C18 Pep-Map column (LC Packings) with a separation flow rate was set at 300 nL/min.

The peptides were separated with a 4–40% linear gradient of solvent B in 91 min (solvent A was 0.1% FA and solvent B was 0.1% FA in 80% ACN) followed by a 25 min gradient from 40% to 90% solvent B.

The mass spectrometer operated in positive ion mode at a 2-kV needle voltage. Data were acquired using Xcalibur software in a data-dependent mode. MS scans (m/z 375-1500) were recorded in the Orbitrap at a resolution of R = 120,000 (@ m/z 200) and an AGC target of 4 x 105 ions collected within 50 ms. Dynamic exclusion was set to 60 s and top speed fragmentation in HCD mode was performed over a 3 s cycle. MS/MS scans were collected in the Orbitrap with a resolution if 30,000 and maximum fill time of 54 ms. Only +2 to +7 charged ions were selected for fragmentation. Other settings were as follows: no sheath nor auxiliary gas flow, heated capillary temperature, 275 °C; normalised HCD collision energy of 30 %,

isolation width of 1.6 m/z, AGC target of 5 x 104 and normalised AGC target of 100%. Monoisotopic precursor selection (MIPS) was set to Peptide and an intensity threshold was set to 2.5 x 10^4.

Data processing
A FASTA database was downloaded from www.uniprot.org[6] using a search for "9606" as "Taxonomy [OC]" at UniProtKB and "reviewed" on 04/09/2024
("uniprotkb_taxonomy_id_9606_AND_reviewed_2024_09_04.fasta", called "UniProt database" in the protocols below). Decoy and contaminants were added with FragPipe[7] for data analysis.
Data were processed using the FragPipe computational platform, the FragPipe interface version 21.1[7] was used with MSFragger version 4.0[8], IonQuant version 1.10.27[9]. For all data analysis using Perseus, Perseus 2.0.6.0[10] was used.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD062176.

Data processing and analysis with FragPipe and Perseus
In FragPipe, a custom LFQ workflow was loaded, based on FragPipe LFQ-MBR provided workflow. Briefly, in MSFragger, precursor mass tolerance was set between -20 and 20 ppm. Fragment mass tolerance was set as 20 ppm. Mass calibration, parameter optimization was chosen and isotope error set at 0/1/2. The corresponding protein digestion enzyme was entered. Missed cleavages were allowed up to 2. Peptide length was set 7 - 50 and peptide mass range was set 500 - 5000. Met oxidation, N-terminal acetylation and Cys carbamidomethylation were searched as variable modifications (15.9949 on M with max 3 occurrences, 42.0106 on [^ with max 1 occurrence and 57.0215 on C with 3 max occurrences). The mass corresponding to the probe/capture reagent exact mass was added as a variable modification, with the site set to "C" and Max occurrences to "3". All mods used in first search was unchecked. No fixed modification was entered. The mass offset was set to 0. Other MSFragger parameters were left as default. In the Validation panel, MSBooster was abled. PSM validation was checked and Percolator was run with a min probability of 0.5 and the command "--only-psms --no-terminate --post-processing-tdc". PTMProphet was disabled. ProteinProphet was allowed with the command "--maxppmdiff 2000000". The protein-level summary was generated with the filter "--sequential --prot 0.01". In PTM-sherpherd was disabled. In MS1 Quanti, LFQ was run with MaxLFQ checked and MaxLFQ min ions set at 2. Re-quantify was enabled. MBR and Normalization were disabled. Min scans were set to 1, Min isotopes to 1, m/s tolerance to 10 ppm, RT tolerance to 0.4 min, Top N ions to 3, Min Freq to 0.5 and min site localization probability to 0.75.
In "Database", the FASTA file "Uniprot database" with decoys and contaminants was added and the Decoy tag was auto-detected.

The FragPipe output data were processed with Perseus. The data were transformed to log2. Categorical annotation rows were added to give each experiment the same name (Nan-DYne or Nan-Dyne + competition). The data were filtered to a minimum of two valid values per group and a volcano plot was generated.

6. Plasmid

Sec11A-HA (with BamHI added as the 5' restriction site and NotI as the 3'restriction site) was synthesised by Eurofins in a pEx-A128 vector. The plasmid was digested with BamHI/NotI and inserted onto a pcDNA3.1(+) plasmid.

7. IP/click (immunoprecipitation/click)

HeLa cells were transfected with Sec11A-HA for 24h and treated with Nan-Dyne as described above. Cells were irradiated with UV as described above and lysed in 25 mM Tris pH7.3, 150 mM NaCl, 1% Triton x--100, 0.5% sodium deoxycholate, 0.1% SDS, EDTA-free protease inhibitor cocktail. The lysate was kept on ice for 15 min and centrifuged at 500 × g for 10 min at 4 ˚C to remove cellular debris. 10% of samples were kept for "whole cell lysate". HA-tagged proteins were immunoprecipitated using anti-HA magnetic beads (Pierce). Following 1 h incubation at room temperature with end-over-end rotation, the beads were washed three times with 1 mL of ice-cold "IP wash buffer" (1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 25 mM Hepes pH 7.4). The beads were resuspended in 22 µL of IP wash and 3 µL of CuAAC reactant solution [azido-TAMRA-biotin (0.5 µL, 10 mM stock solution in DMSO), $CuSO_4$ (1 µL, 50 mM in H_2O), TCEP (1 µL, 50 mM in H_2O)), TBTA (0.5 µL, 10 mM stock solution in DMSO] were added. Beads were incubated at room temperature for 1 h and EDTA (1 µL of a 0.5 M solution in H_2O) was added to stop the CuAAC reaction. Beads were washed 3 x 5 min with "IP wash buffer". Proteins were eluted by boiling the beads for 5 minutes in 1X Laemmli sample buffer (4x Laemmli:BME: 4% SDS 50 mM TEA, 150 mM NaCl pH 7.3, 1:0.1:3) and separated by SDS-PAGE. The gel was washed twice with deionised water before in-gel fluorescence detection. The gel was then transferred to a nitrocellulose membrane to do an anti-HA western blot as described above.

8. CETSA

To evaluate drug target interactions in cells, the cellular thermal shift assay (CETSA) was performed as previously described by Xiong Tan et al. [11]

Typically, 2x 5 cm dish of HeLa were transfected for 24 h with Sec11A-HA. On the day of the experiment, the cells were washed with 10 mL of ice-cold PBS (two times) and harvested in 500 µL of cold lysis buffer (0.5% DDM in PBS + protease inhibitor). The cells were lysed by three freeze–thaw cycles in liquid nitrogen and passed through a 25G needle. The lysed cells were centrifuged at 14,000g for 20 min at 4 °C. The supernatant was divided into two tubes and the protein amount was measured and adjusted to 1 mg/mL protein concentration. The lysates were incubated with DMSO or with 2 µM nanchangmycin for 15 min at 4 °C with end-over-end rotation. This suspension was aliquoted into a series of microcentrifuge tubes (nanchangmycin vs DMSO) after which they were subjected to a 3 min heat shock to the appropriate temperature (40-75 °C) for generating melt curves followed by rapid cooling to 0 °C and the cell lysates were centrifuged at 14,000g for 20 min at 4 °C to pellet the cell debris together with precipitated and aggregated proteins. The supernatants were carefully removed without disturbing the pellets. The supernatants were analysed by SDS-PAGE followed by western blot analysis.

The temperature range of 40 to 75 °C was selected based on previously published CETSA protocols.[12,13] The range was appropriate to observe the melting temperature of Sec11A-HA.

9. Activity of the SPC complex

Cell Transfection and HPG pulse-labelling

Hek293T cells were cultured in 6-well plates to 80% confluency and transfected with a plasmid encoding ZIKV 2K-NS4B tagged with a V5 peptide (pcDNA-2K-NS4B-V5, kindly provided by Prof. Dr. Jan Rehwinkel, MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK) using Lipofectamine 3000 (Thermo Fisher Scientific) following the manufacturer's instructions. After 24 h, the culture medium was replaced, cells were washed with PBS and then incubated for 60 min in methionine-free DMEM medium (Gibco) supplemented with either vehicle control (DMSO), Nan, or Nan-Dyne at variable concentrations depending on the experimental conditions, in order to deplete intracellular methionine reserves. Subsequently, cells were incubated for 30 min in methionine-free DMEM supplemented with 100 µM L-homopropargylglycine (HPG), 200 µM L-cysteine, 4 mM L-glutamine and either vehicle control (DMSO), Nan, or Nan-DYne at their respective concentrations. Cells were then washed twice with PBS and incubated for 12 h in complete DMEM supplemented with 10% FBS, 4 mM glutamine and either vehicle

control (DMSO), Nan, or Nan-DYne at their respective concentrations. Subsequently, cells were washed twice with PBS and lysed in 2% SDS HEPES buffer (pH 7.4) supplemented with EDTA-free protease inhibitor (Roche).

Click chemistry and protein enrichment
Cell lysates were diluted in HEPES buffer (pH 7.4) to reduce SDS concentration to <0.5%. For each sample, 100 µg of protein was subjected to a click chemistry reaction in freshly prepared buffer containing 100 µM Azide-PEG3-Biotin (Sigma) ,1 mM $CuSO_4$ (Acros organics), 100 µM Tris(2-carboxyethyl)phosphine hydrochloride (TCEP, Pierce Themo), and 1 mM Tris(3-hydroxypropyltriazolylmethyl)-amine (TBTA, Sigma) in HEPES buffer (pH 7.4). The reaction was incubated at room temperature for 1 h, then quenched by adding EDTA to a final concentration of 10 mM. Proteins were precipitated using the chloroform/methanol method, pelleted by centrifugation at 20,000 g for 10 min, washed with ice-cold methanol and air-dried for 5 min. Pellets were resuspended in 20 µL of 2% SDS HEPES buffer (pH 7.4) and further diluted in HEPES buffer (pH 7.4) to reduce SDS concentration to <0.2%. Proteins labelled by HPG and coupled to biotin were enriched using NeutrAvidin Agarose Beads (Pierce™ NeutrAvidin™, Thermo Fisher Scientific). Beads were washed (3x HEPES buffer) and proteins were eluted in 50 µL of 2× Laemmli Sample Buffer (Bio-Rad). SDS-Page gels and western blot analyses were performed as described above.

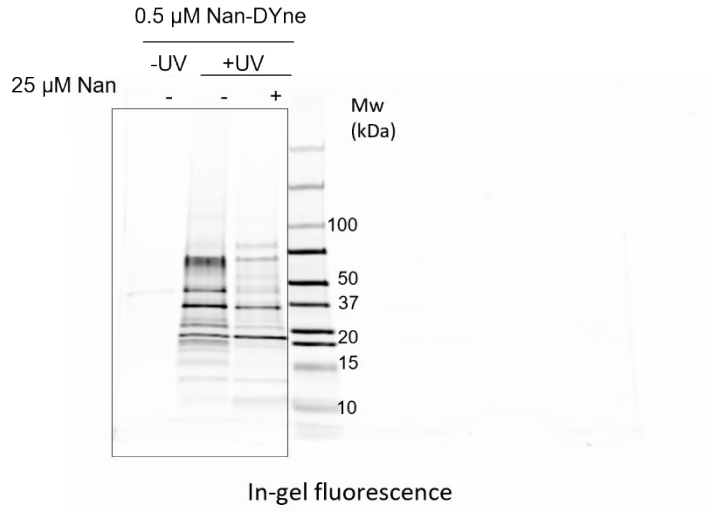
10. Docking

Molecular docking was performed using the AutoDock Vina engine, accessed through the SwissDock platform. We selected the "Docking with AutoDock Vina" option provided in the current version of SwissDock (2024) (https://www.swissdock.ch/),[3] which allows direct use of the Vina algorithm within a web-based environment. Nanchangmycin was docked onto SEC11A using the PDB 7P2P using the following parameters:
Ligand:
CO[C@H]1CC[C@@H](O[C@H]2C[C@H](O[C@]3(O[C@H](C[C@@H]3C)[C@@H]3O[C@@](O)(CO)[C@H](C)C[C@@H]3C)[C@@H]2C)[C@]2(C)CC[C@]3(C[C@H](O)[C@@H](C)[C@H](O3)[C@@H](C)\C=C(/C)C(=O)[C@H](C)C[C@H](C)C(O)=O)O2)O[C@H]1C
Target: receptor.pdb
Box center: 70 - 80 - 112
Box size: 30 - 30 - 30
Docking method: Vina
Sampling exhaustivity: 2

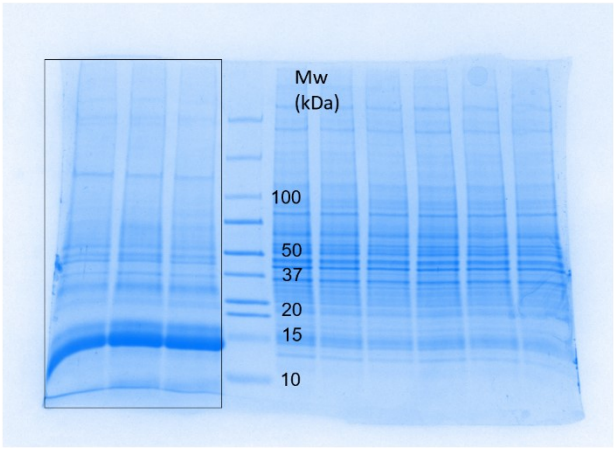
IV. Uncropped and unprocessed gel and Western blot images

Boxes indicate gel areas shown in the main figures and/or used for quantification.

Full gel images related to Figure 2B

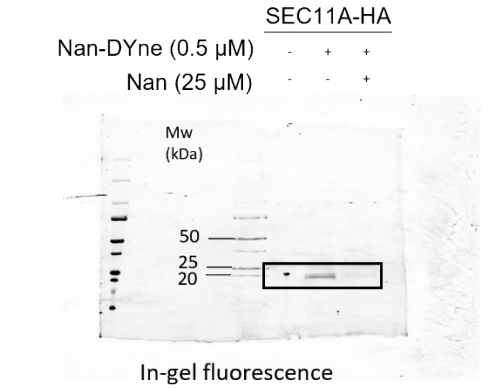


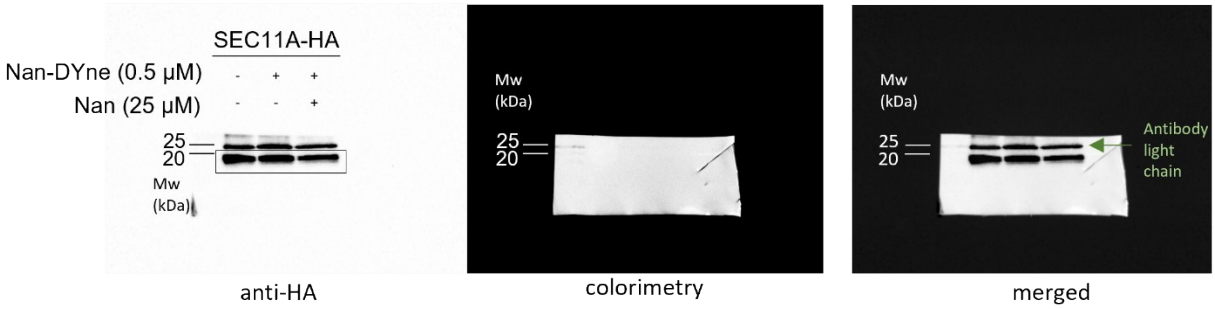
In-gel fluorescence



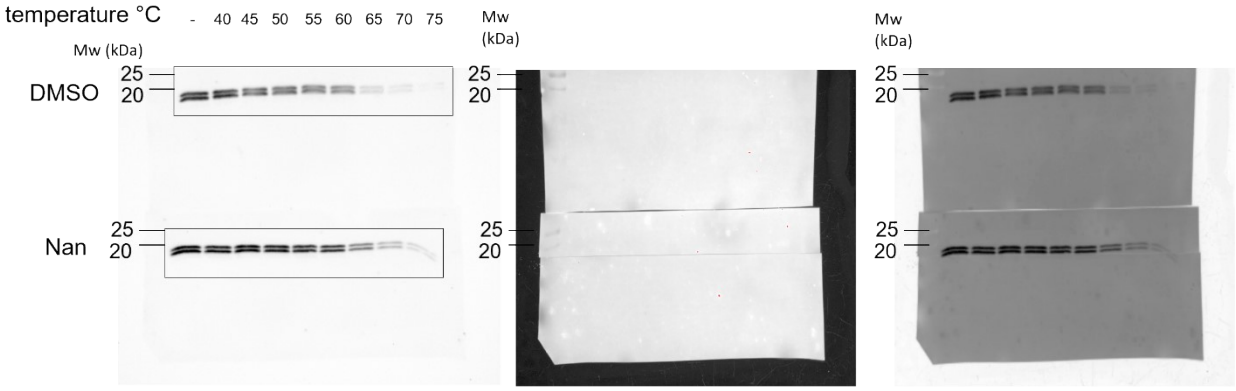
coomassie

Full gel images related to Figure 3A

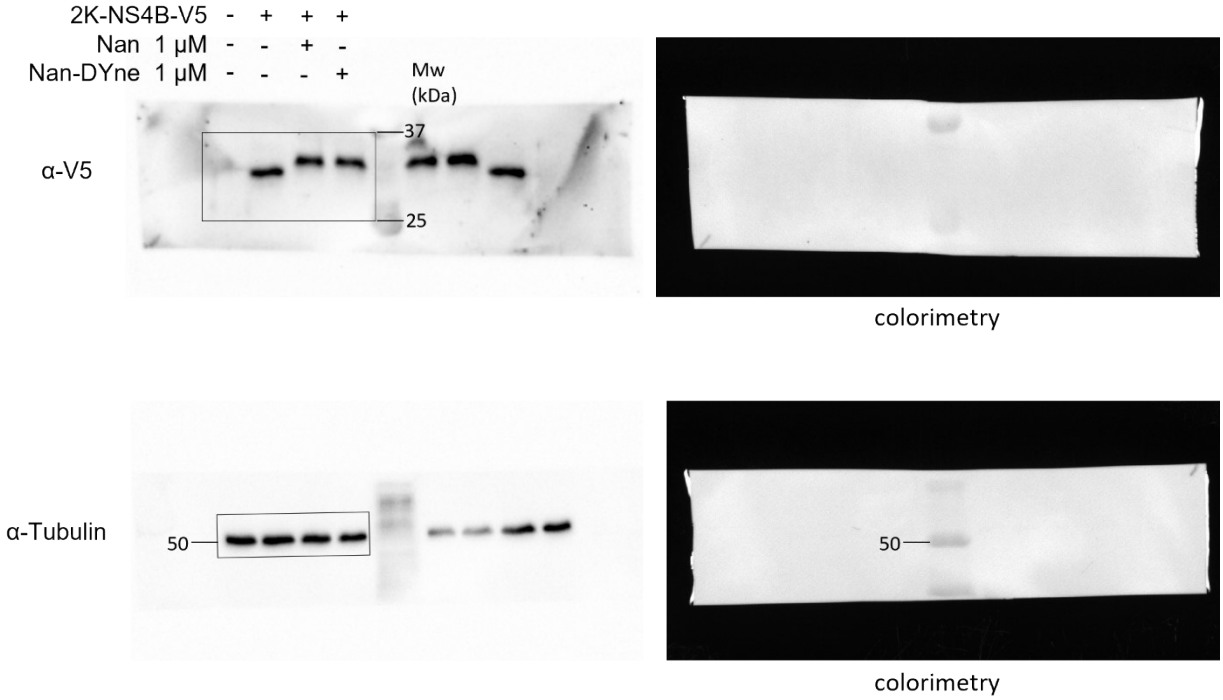




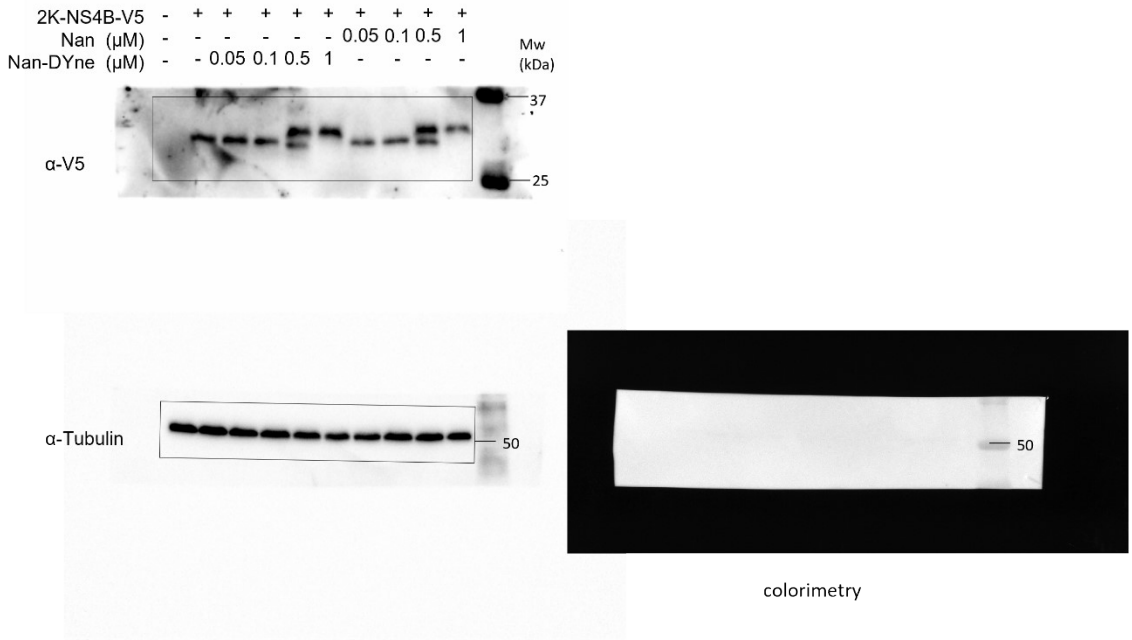
Full gel images related to Figure 3B



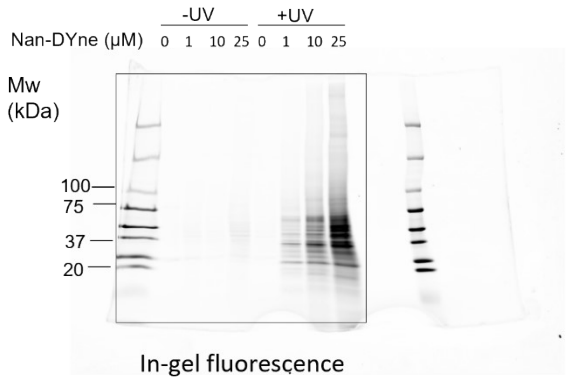
Full gel images related to Figure 4A



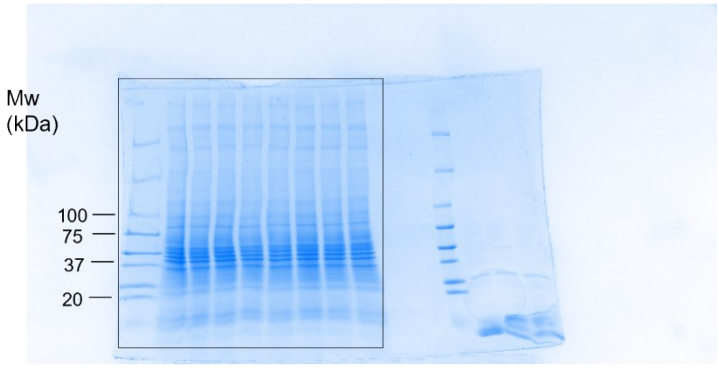
Full gel images related to Figure 4B



Full gel images related to Figure S2A

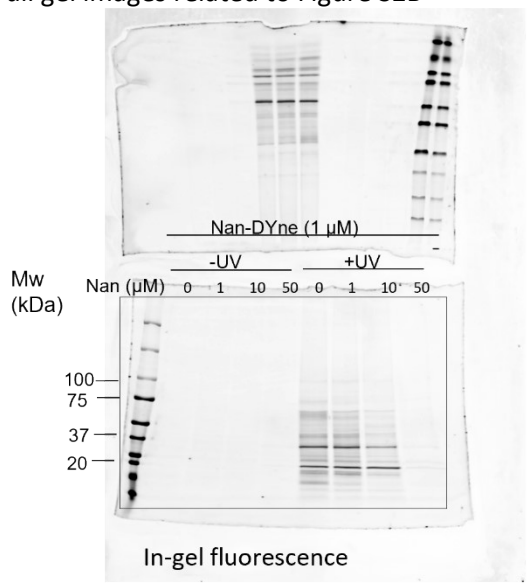


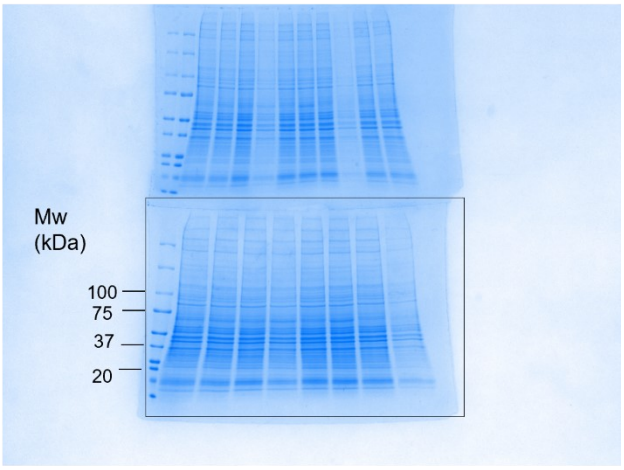
In-gel fluorescence



coomassie

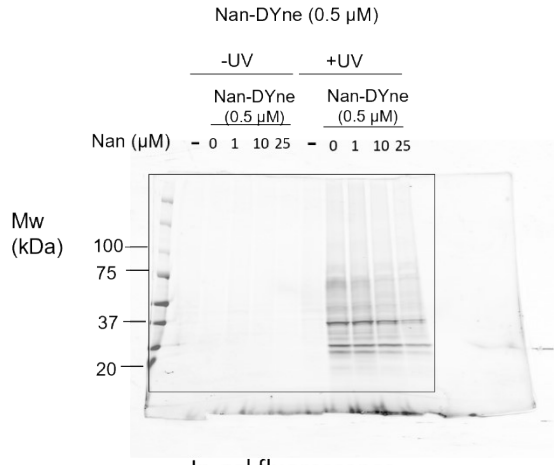
Full gel images related to Figure S2B



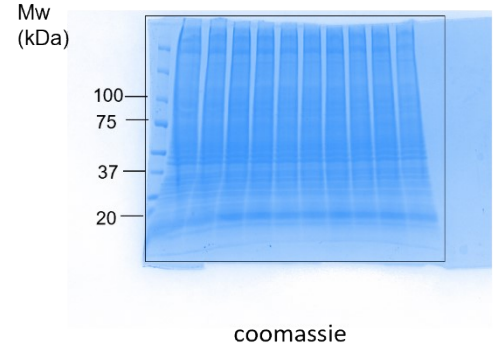


coomassie

Full gel images related to Figure S2C



In-gel fluorescence



coomassie

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