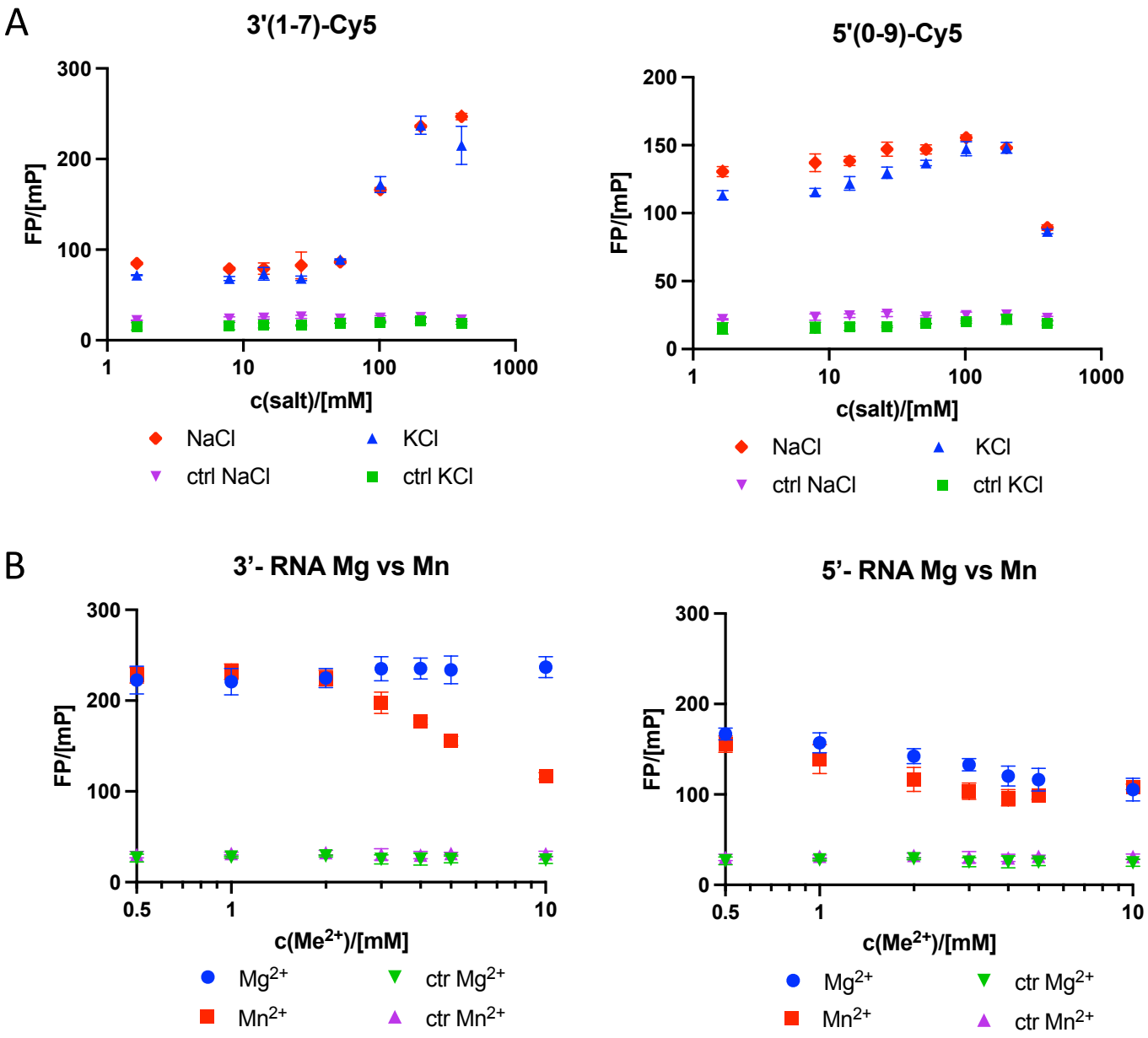
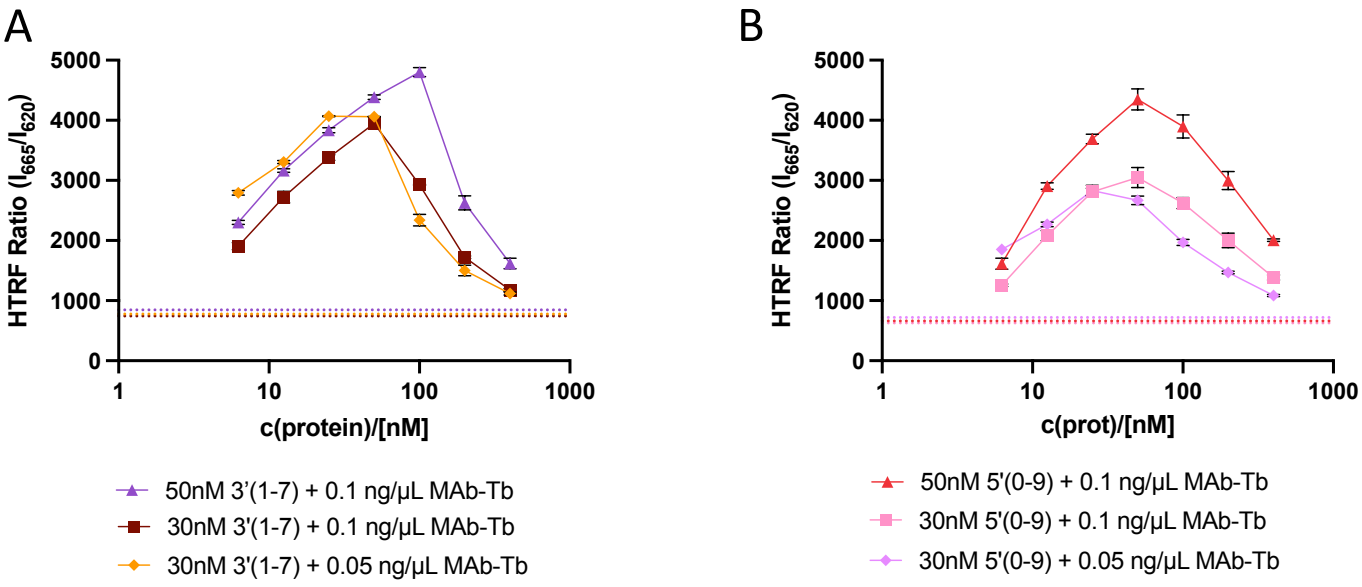
Supplement

Supplementary Figure S1



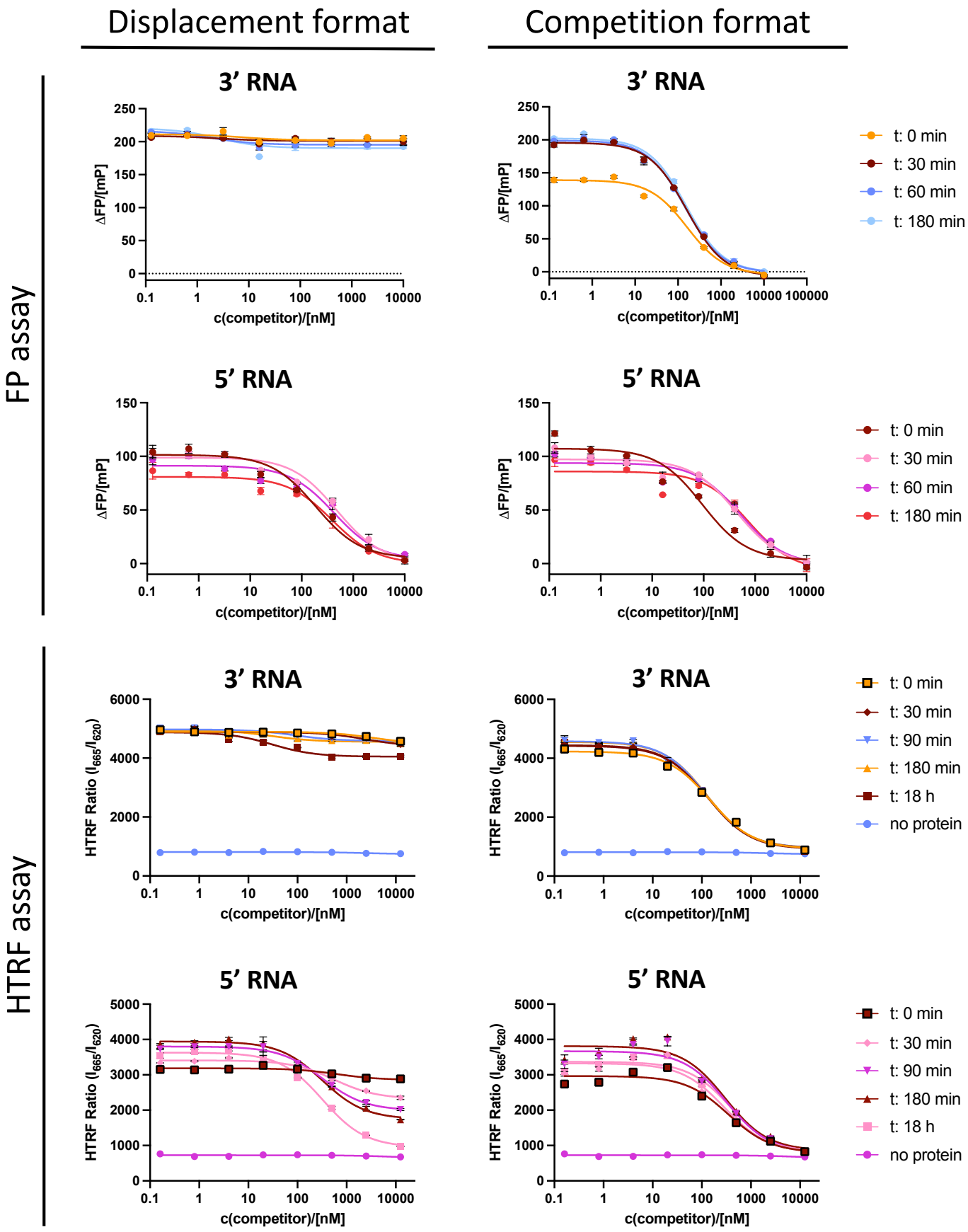
Supplementary Figure S1: Influence of (A) NaCl and KCl ions and (B) Mg^{2+} and Mn^{2+} on tracer binding. The influence of respective ions on the FP signal of the complex of L protein and 3' (left panels) or 5' (right panels) RNA was assessed. L protein was mixed with increasing amounts of **(A)** NaCl, KCl, **(B)** $MgCl_2$ or $MnCl_2$ as indicated before the addition of 3'(1-7)-Cy5 or 5'(0-9)-Cy5 tracers. Final concentrations of assay components were 25 nM L protein, 10 nM 3'(1-7)-Cy5 or 5'(0-9)-Cy5 in 10 mM HEPES(NaOH), pH 7.0, CHAPS (0.1% (w:v)) as well as either NaCl or KCl (1.6-400 mM as indicated), or 200 mM NaCl and either $MgCl_2$ or $MnCl_2$ (0.5-10 mM as indicated). A single nucleotide with the same label (pCp-Cy5) was used as a non binding dye control. FP signals were measured after 90 min at RT. Data points are shown as mean ± SD (n=3).

Supplementary Figure S2



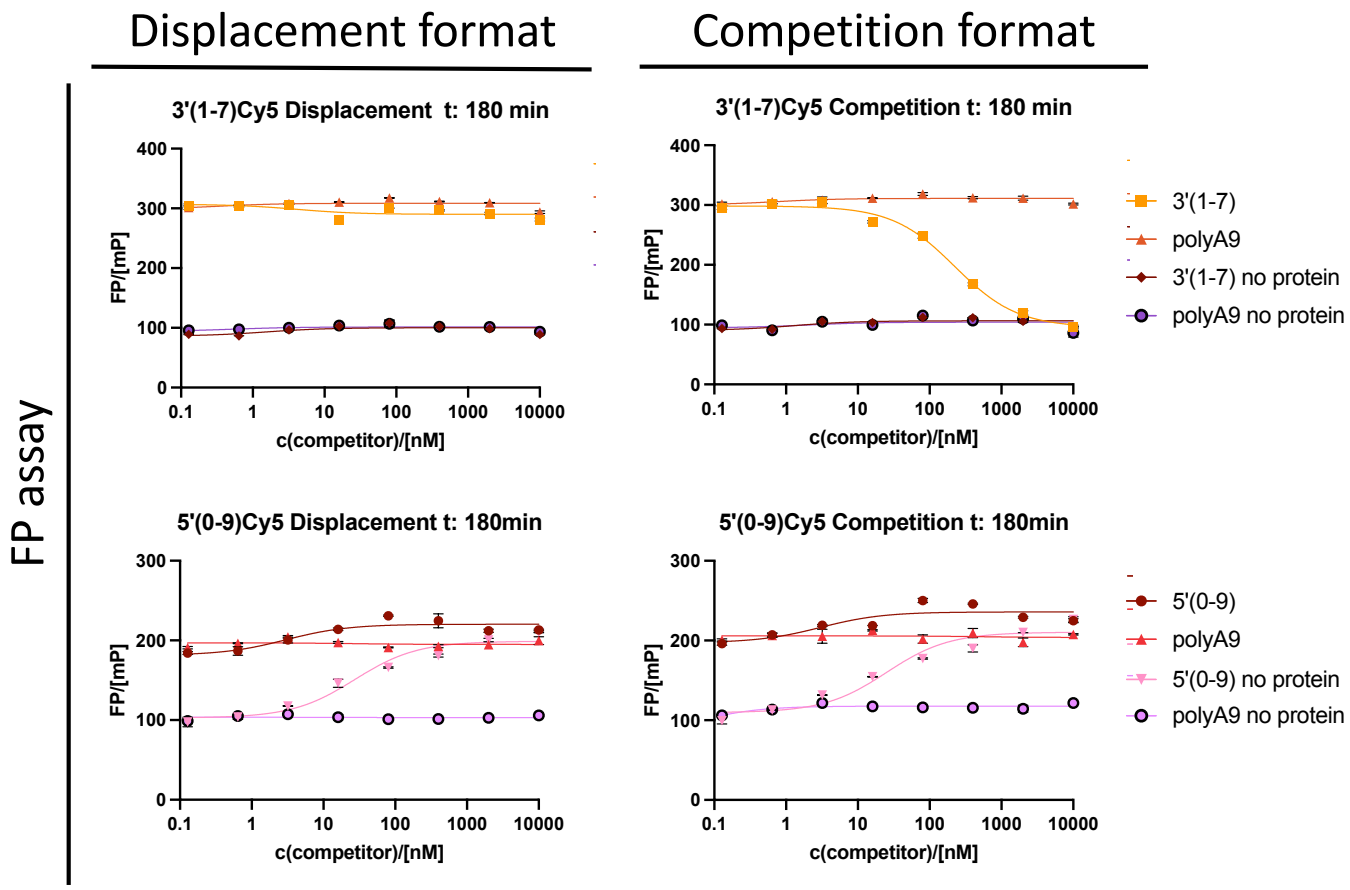
Supplementary Figure S2: Influence of L protein and FRET donor concentrations on the HTRF signal of the (A) 3' RNA assay and the (B) 5' RNA assay. Increasing concentrations of LASV L with C-terminal His-StrepII-tag was first mixed with 0.05 or 0.1 ng/µL anti His-MAb-Tb cryptate as FRET donor for 15 min. HTRF signal was measured 60 min after addition of 50 or 30 nM Cy5-RNA as FRET acceptor. Binding reactions were conducted at RT in 10 mM HEPES(NaOH), pH 7.0, 200 mM NaCl. The dotted lines indicate the background HTRF signal of donor and acceptor in absence of L protein as negative controls. Signals were measured after 60 min at RT. Data points are shown as mean ± range (n=2).

Supplementary Figure S3



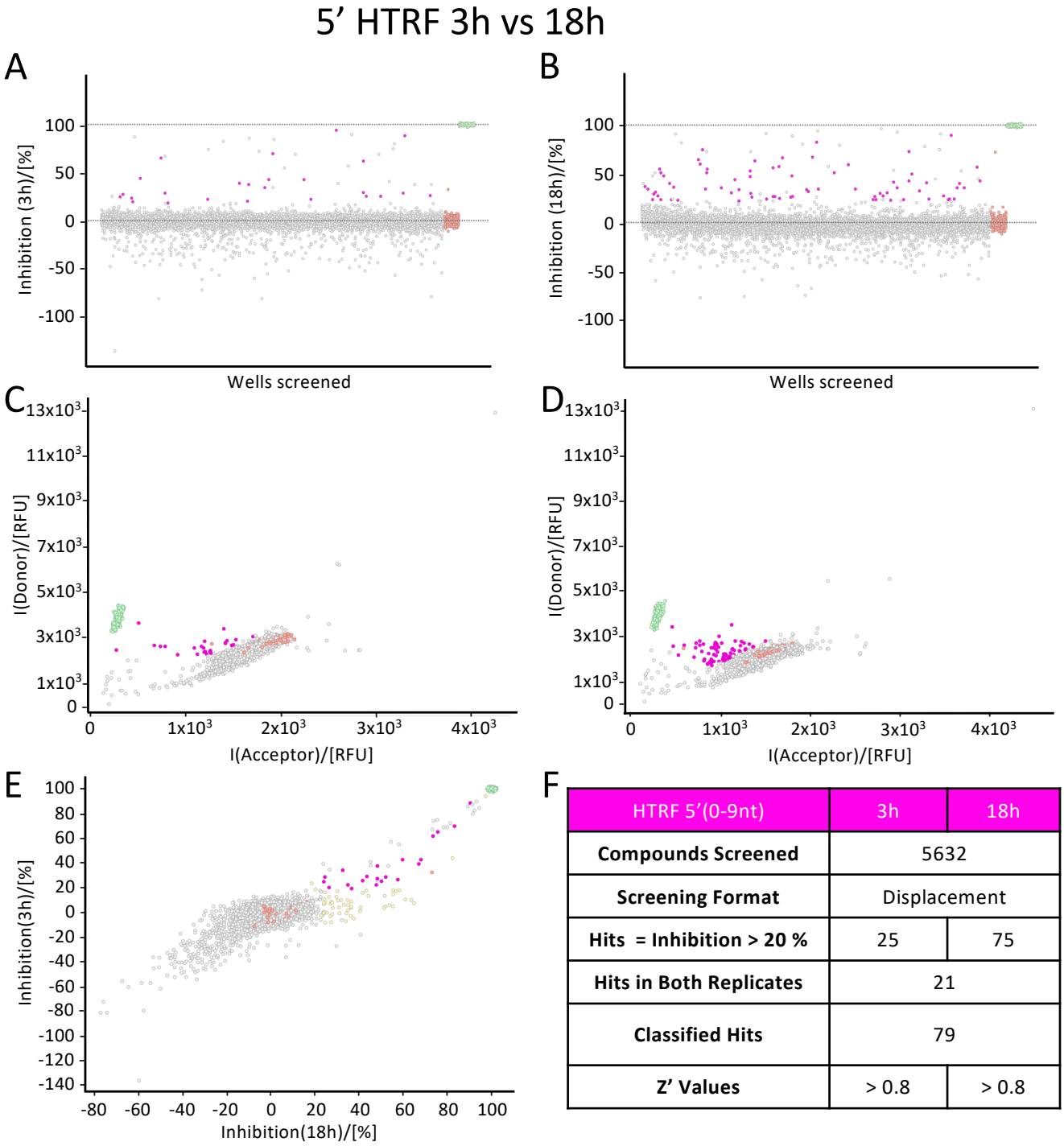
Supplementary Figure S3: Time-course experiments of displacement and competition format. Measurements of FP (upper panels) and HTRF (lower panels) signals were performed after different incubations times at RT (see legends) in displacement (left panels) and competition (right panels) formats for 3'(1-7)-Cy5 and 5'(0-9)-Cy5 RNAs and the non-labelled RNAs as competitors. Buffer composition was 10 mM HEPES(NaOH), pH 7.0, 200 mM NaCl, and CHAPS (0.1% (w:v)). Curves were fitted using Prism (version 10.6) according to a inhibition vs. response model (three parameters). Data points are shown as mean ± range (n=2).

Supplementary Figure S4



Supplementary Figure S4: Uncorrected FP signals in displacement and competition format for 3'(1-7)-Cy5 and 5'(0-9)-Cy5. The FP signal from LASV L bound to 3'(1-7)-Cy5 is only slightly reduced after the addition of the unlabelled 3'(1-7) RNA (orange rectangle) in the displacement format (left). In the competition format (right), the signal (orange rectangle) drops to the level of the control without protein (purple circle). For 5'(0-9)-Cy5, the addition of 5'(0-9) leads to an increase in the FP signal in the displacement format (left) as well as in the competition format (right), both with (dark red circle) and without L protein (red triangle). For this reason, the difference (ΔFP) between the FP signals with L (bound) and without (free tracer) was used for further evaluation. None of the signals are altered upon addition of the unspecific poly-A9 RNA (pink triangle). Signals were measured after 3h incubation at RT in 10 mM HEPES(NaOH), pH 7.0, 200 mM NaCl, CHAPS (0.1% (w:v)). Curves were fitted using Prism (version 10.6) according to a inhibition vs. response model (three parameters). Data points are shown as mean ± range (n=2).

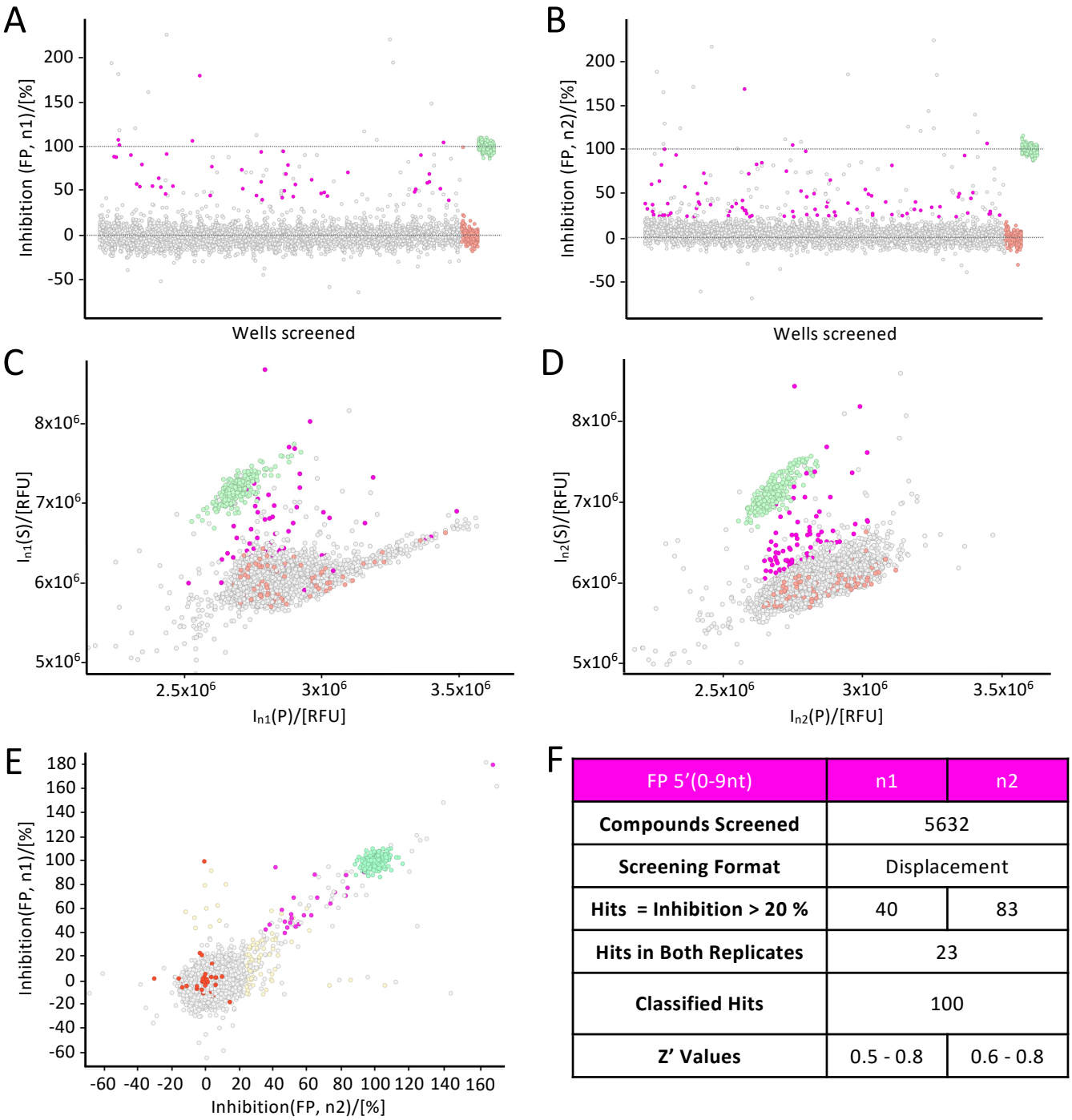
Supplementary Figure S5



HTRF 5’(0-9nt)	3h	18h
Compounds Screened	5632	
Screening Format	Displacement	
Hits = Inhibition > 20 %	25	75
Hits in Both Replicates	21	
Classified Hits	79	
Z’ Values	> 0.8	> 0.8

Supplementary Figure S5: Screening of the Fraunhofer Repurposing collection for inhibition of 5’ RNA binding by LASV L protein using the HTRF assay. Dot plot representations of the whole screening data sets of HTRF with screened compounds colored in light grey, identified hits in magenta, positive and negative controls in green and red. Positive or negative controls for inhibition contained 10 nM 5’(0-9)-Cy5 and 0.05 ng/µL mAb α-6HIS Tb with or without 50 nM LASV L, respectively. Results of the measurement after 3 h (A and C) and 18 h (B and D) are shown. **A**) and **B)** depict the inhibition calculated for each compound screened, **C)** and **D)** display the measured intensities of the donor versus the acceptor emission. **E)** Shows the correlation of the hits selected at each time point. Shared hits between the two time points are highlighted in magenta, additional hits from both screens selected for profiling are colored in light yellow. **F)** Key data of both screens are summarized in the table.

Supplementary Figure S6

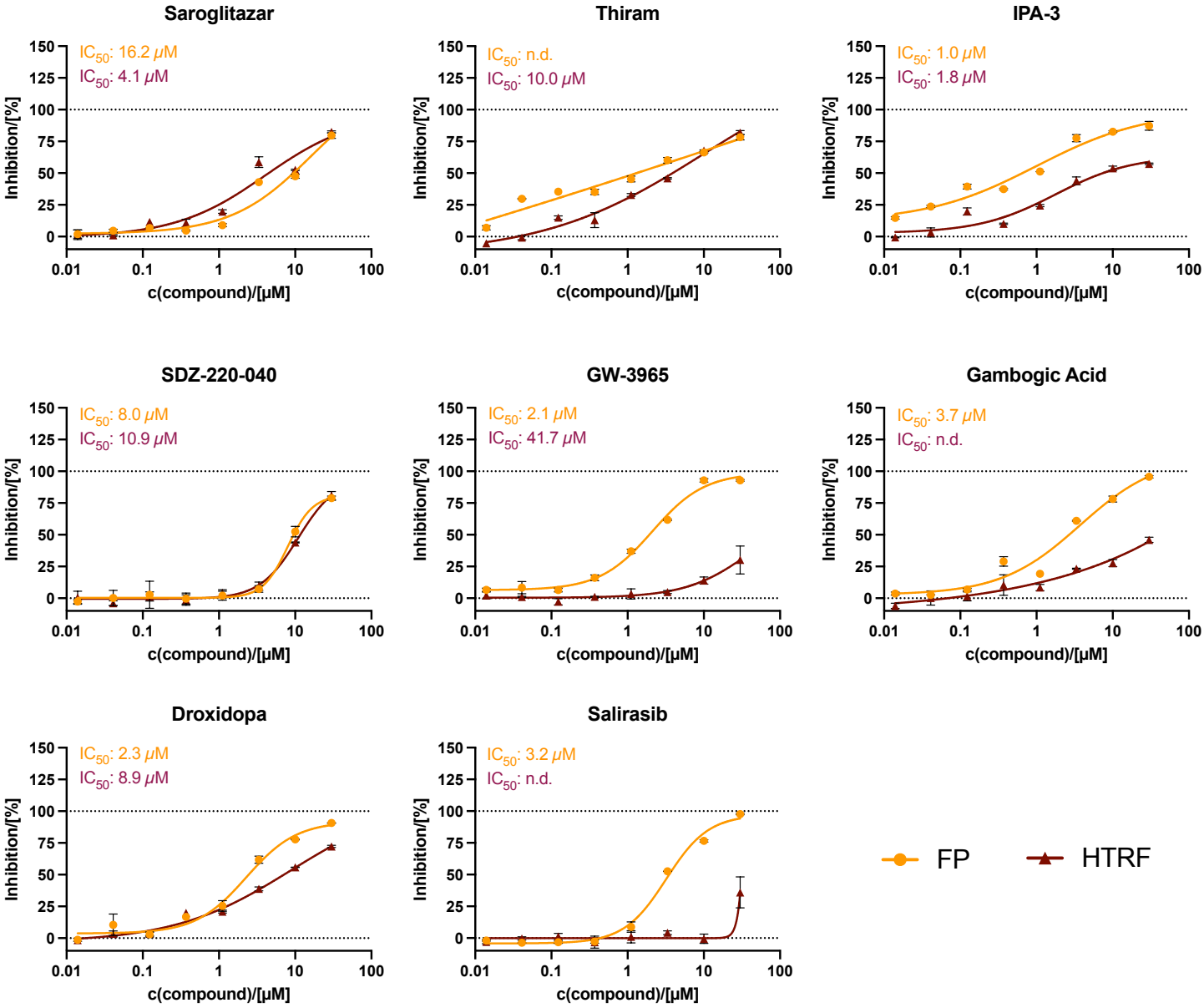


F

FP 5'(0-9nt)	n1	n2
Compounds Screened	5632	
Screening Format	Displacement	
Hits = Inhibition > 20 %	40	83
Hits in Both Replicates	23	
Classified Hits	100	
Z' Values	0.5 - 0.8	0.6 - 0.8

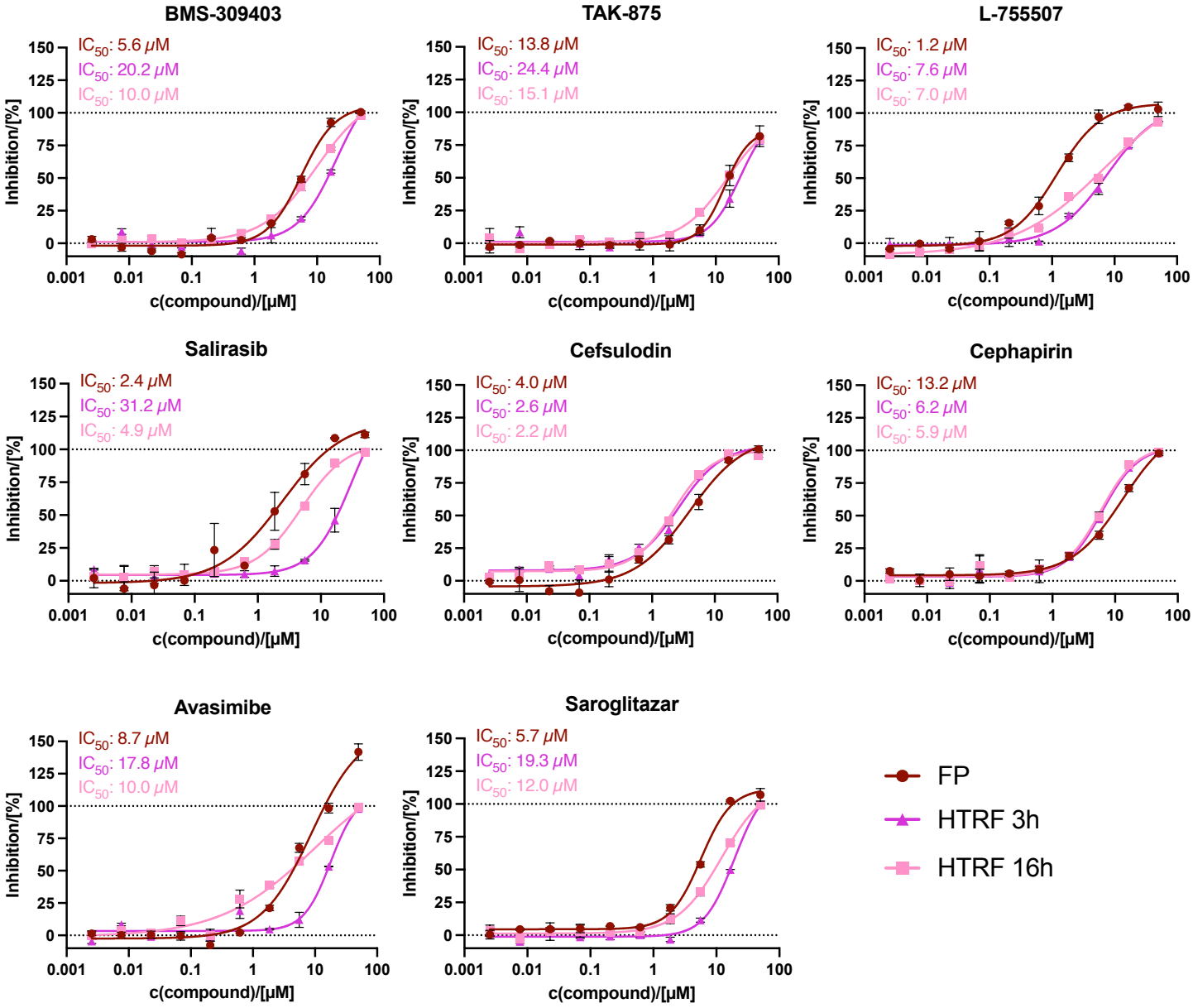
Supplementary Figure S6: **Screening of the Fraunhofer Repurposing collection for inhibition of 5' RNA binding by LASV L protein using the FP assay.** Dot plot representations of the inhibition calculated for the whole FP-based screening data sets of the two replicates n1 and n2 with screened compounds colored in light grey, identified hits in magenta, positive controls in green (tracer only) and negative controls (tracer plus L protein) in red. **A**) and **B)** depict the inhibition calculated for each compound screened in replicate n1 and n2, respectively. **C)** and **D)** display the measured intensities in the P versus the S channel. **E)** The correlation of the hits selected in the FP replicates n1 and n2 is shown. Shared hits between n1 and n2 are highlighted in magenta, additional hits from both screens selected for profiling are colored in light yellow. **F)** Key data of both screens are summarized in the table.

Supplementary Figure S7



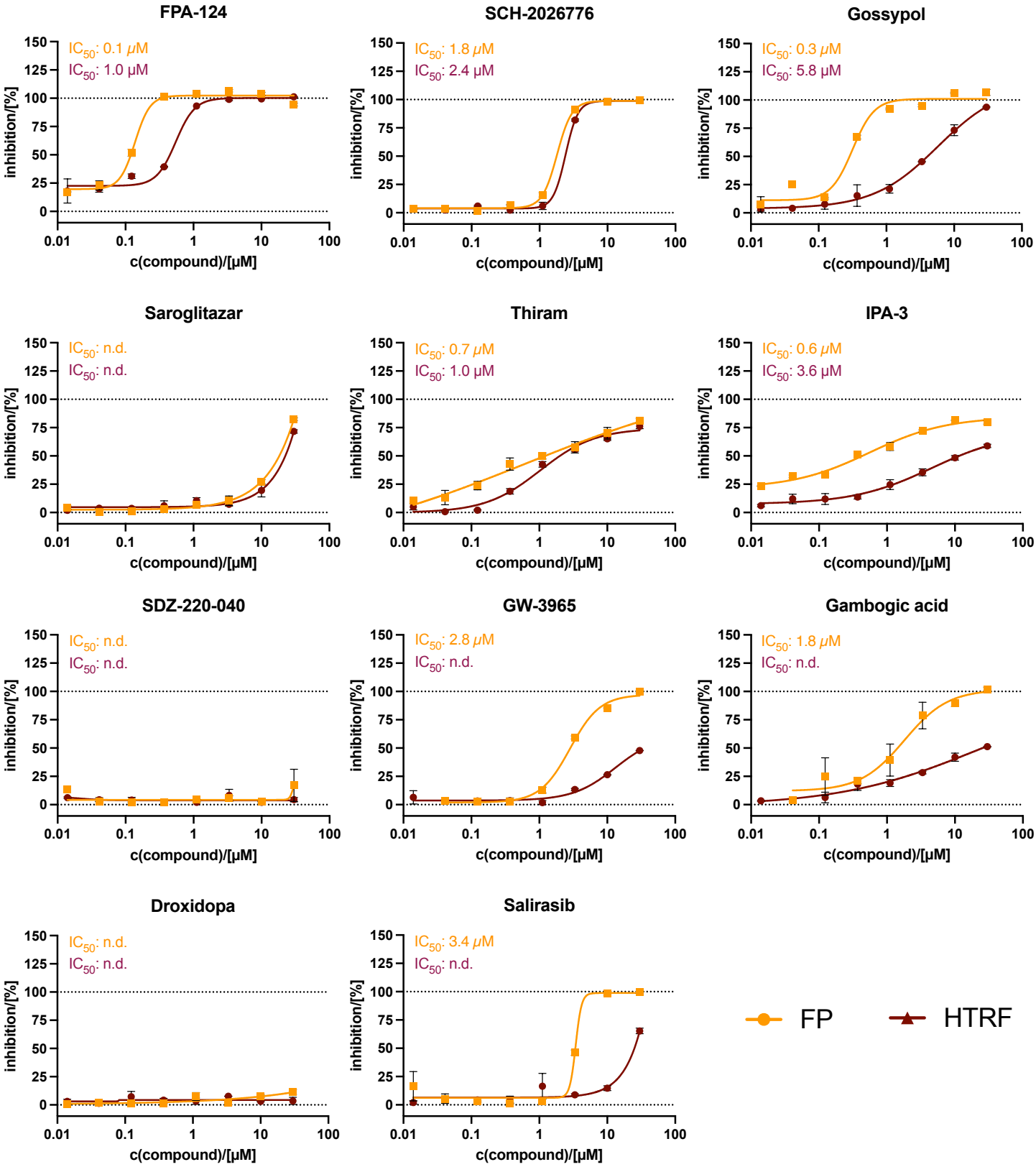
Supplementary Figure S7: **Dose-response curves of selected hits from the 3' RNA screening.** Hit molecules (see labels of panels) of the 3' RNA screening were tested in the FP (orange curve/dots) and HTRF (brown curve/triangles) assays at a concentration range of 14 nM to 30 µM of the respective compound, 6 nM 3'(1-7)-Cy5, 13 nM LASV L and 0.05 ng/µL mAb-Tb in case of HTRF. Signals were detected after 2h at RT. All dose-response experiments were carried out in competition format (preincubation of L and small molecules for 30 min before addition of Cy5-3'RNA) in 10 mM HEPES(NaOH), pH 7.0, 200 mM NaCl, and CHAPS (0.1% (w:v)). Compounds were picked from the screening plates. Curves were fitted using Prism (version 10.6) according to an inhibition vs. response model (four parameter, variable slope). Data points are shown as mean ± range (n=2).

Supplementary Figure S8



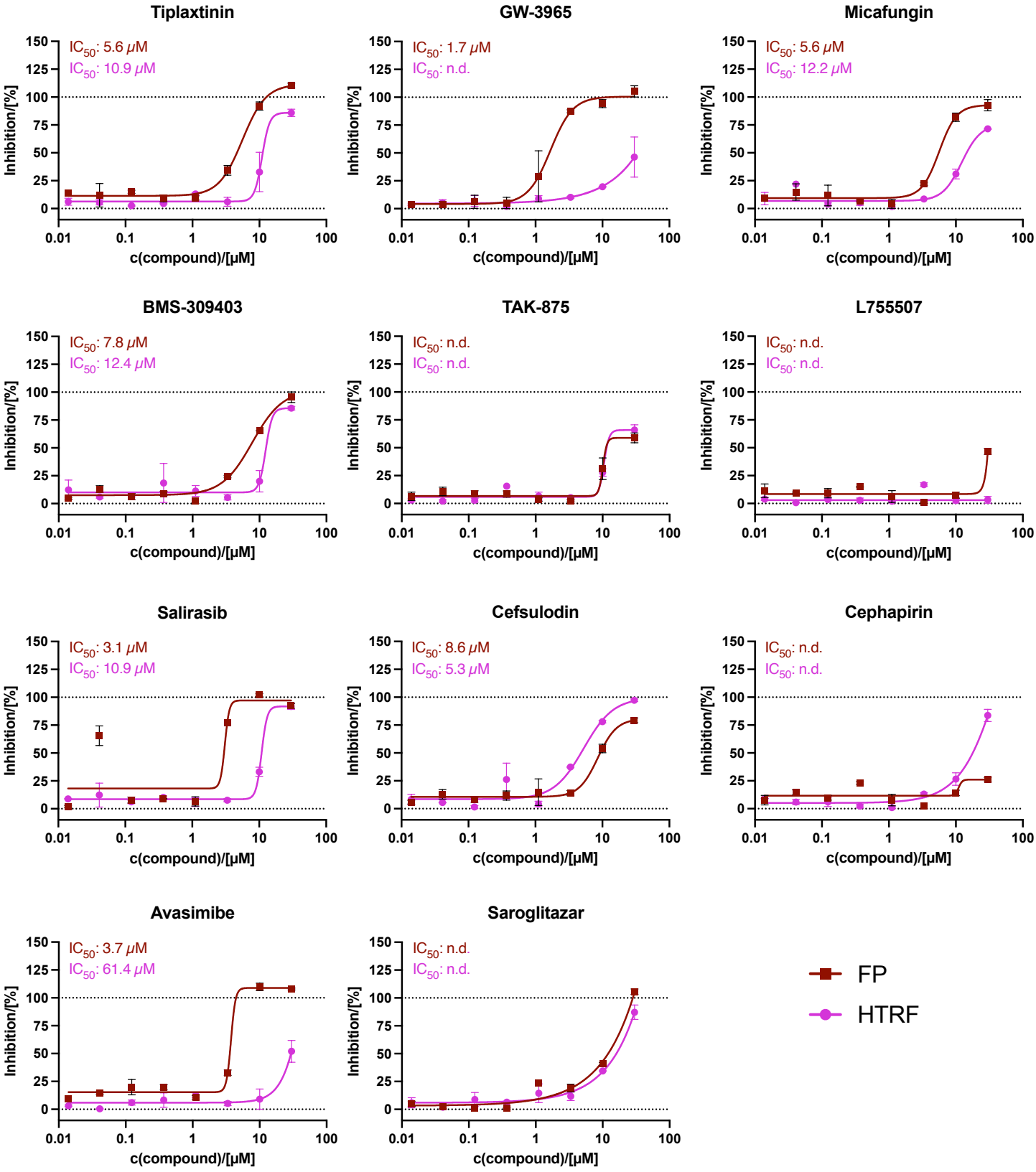
Supplementary Figure S8: **Dose-response curves of selected hits from the 5' RNA screening.** 5' RNA screening hit molecules (see labels of panels) were tested in the FP (brown curve/dots) and HTRF (magenta curve/triangles, pink curve/squares) assays at concentrations between 2.5 nM and 50 µM of the respective compound. For FP, 10 nM 5'(0-9)-Cy5 and 50 nM LASV L were used and signals were detected after 3h at RT. For the HTRF assay, 25 nM 5'(0-9)-Cy5, 25 nM LASV L and 0.05 ng/µL MAb-Tb were used with signals detected after 3h (magenta curve/triangles) and 16h pink curve/squares) at RT. All dose-response experiments were carried out in competition format (preincubation of L and small molecules for 30 min before addition of Cy5-5'RNA) in 10 mM HEPES(NaOH), pH 7.0, 200 mM NaCl, and CHAPS (0.1% (w:v)). Compounds were picked from the screening plates. Curves were fitted using Prism (version 10.6) according to an inhibition vs. response model (four parameter, variable slope). Data points are shown as mean ± range (n=2).

Supplementary Figure S9



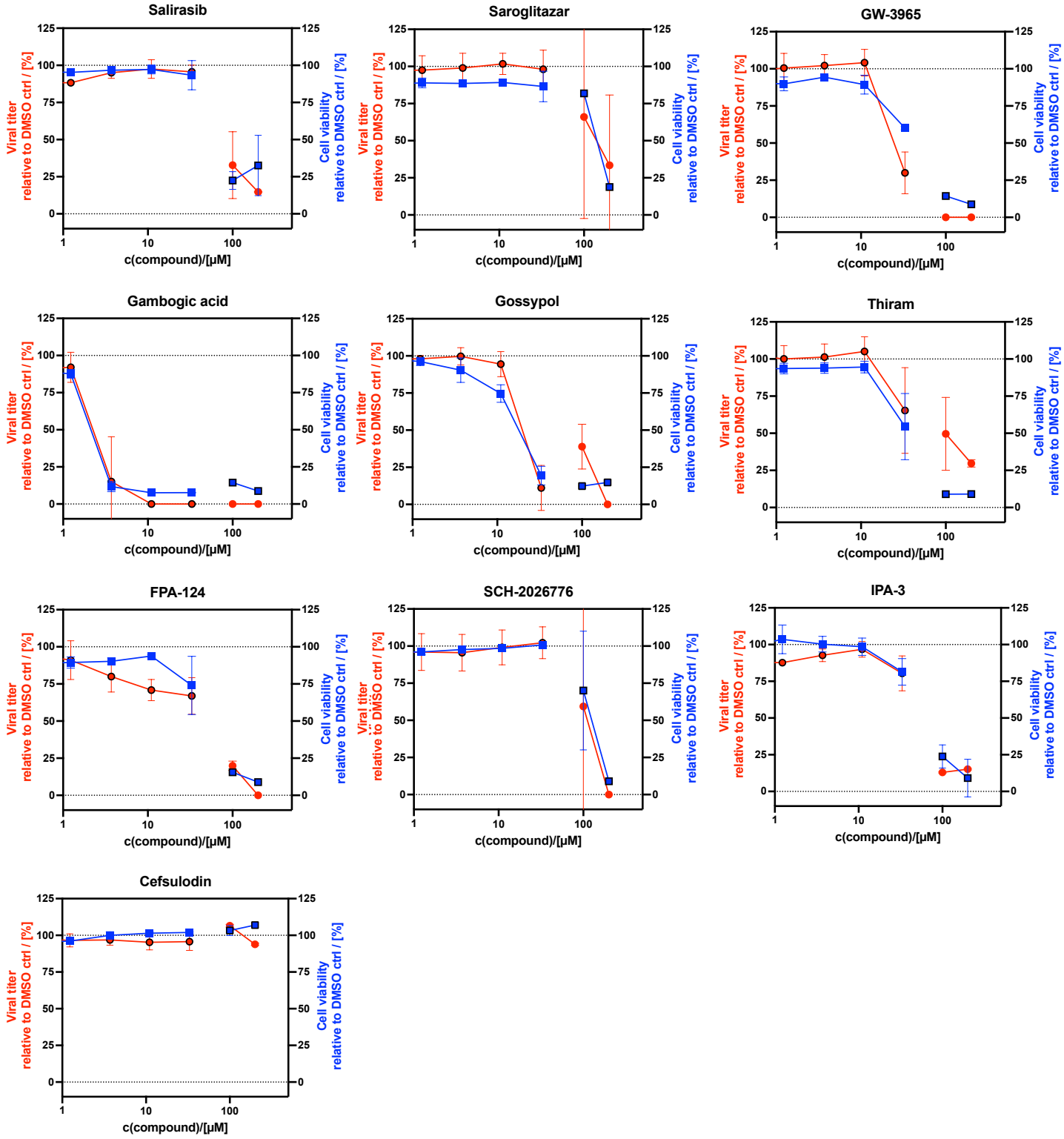
Supplementary Figure S9: **Dose-response curves of selected hits from the 3' RNA screening.** Hit molecules (see labels of panels) of the 3' RNA screening were tested in the FP (orange curve/dots) and HTRF (brown curve/triangles) assays at a concentration range of 14 nM to 30 µM of the respective compound, 6 nM 3'(1-7)-Cy5, 13 nM LASV L and 0.05 ng/µL mAb-Tb in case of HTRF. Signals were detected after 2 h at RT. All dose-response experiments were carried out in competition format (preincubation of L and small molecules for 30 min before addition of Cy5-3'RNA) in 10 mM HEPES(NaOH), pH 7.0, 200 mM NaCl, and CHAPS (0.1% (w:v)). Compounds were prepared freshly from new synthesis batches. Curves are normalized to the average of positve (100%) and negative (0%) controls and fitted using an inhibitor vs. response (four parameter, variable slope) model using Prism (version 10.6). Data points are shown as mean ± range (n=2).

Supplementary Figure S10



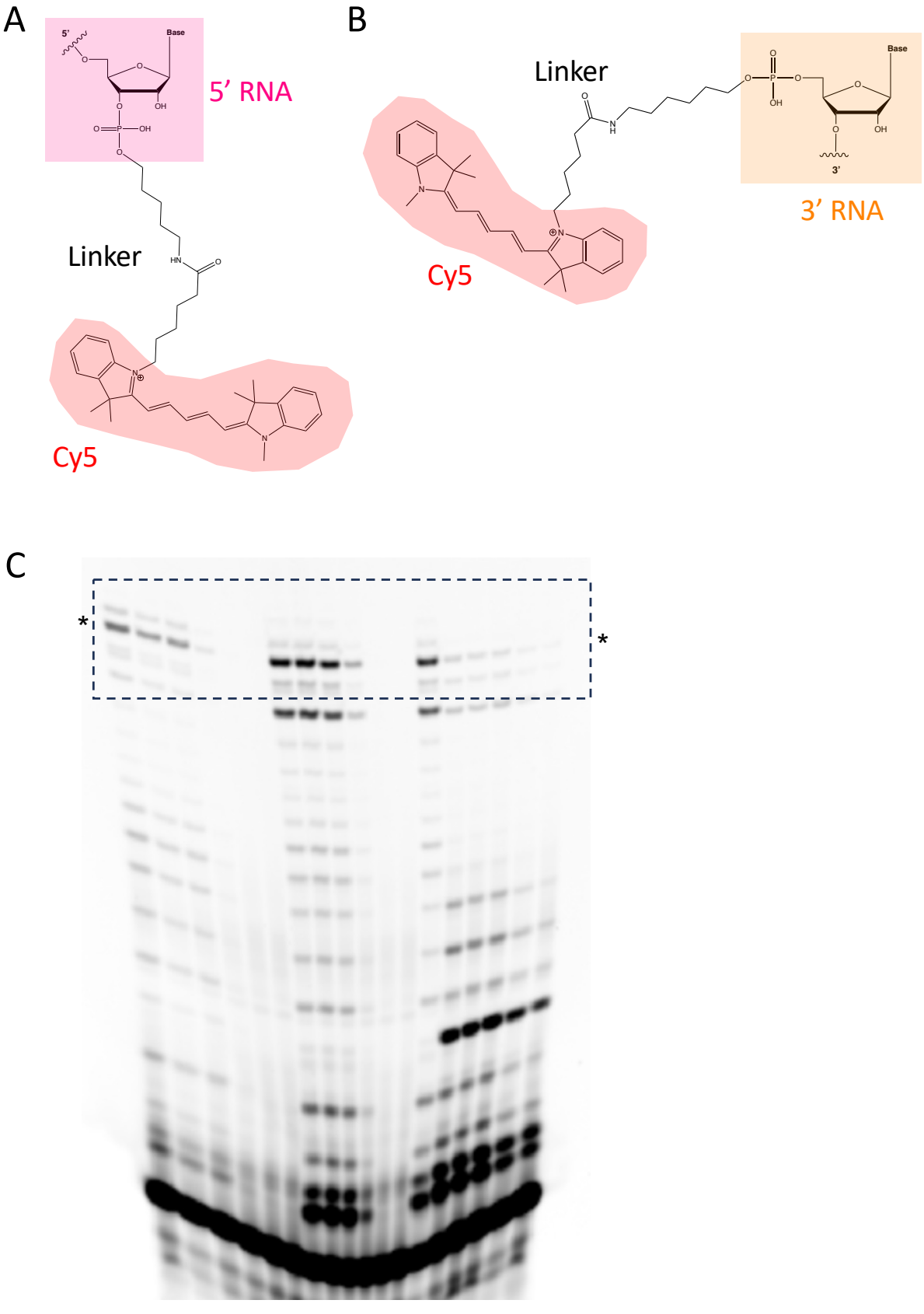
Supplementary Figure S10: **Dose-response curves of selected hits from the 5' RNA screening.** 5' RNA screening hit molecules (see labels of panels) were tested in the FP (brown curve/sqares) and HTRF (magenta curve/dots) assays at concentrations between 2.5 nM and 50 µM of the respective compound. For FP, 10 nM 5'(0-9)-Cy5 and 50 nM LASV L were used and signals were detected after 3 h at RT. For the HTRF assay, 25 nM 5'(0-9)-Cy5, 25 nM LASV L and 0.05 ng/µL MAb-Tb were used with signals detected after 16 h pink (magenta curve/dots) at RT. All dose-response experiments were carried out in competition format (preincubation of L and small molecules for 30 min before addition of Cy5-RNA oligos) in 10 mM HEPES(NaOH), pH 7.0, 200 mM NaCl, and CHAPS (0.1% (w:v)). Compounds were picked from the screening plates. Curves are normalized to the average of positve (100%) and negative (0%) controls and fitted using an inhibitor vs. response (four parameter, variable slope) model using Prism (version 10.6). Data points are shown as mean ± range (n=2).

Supplementary Figure S11



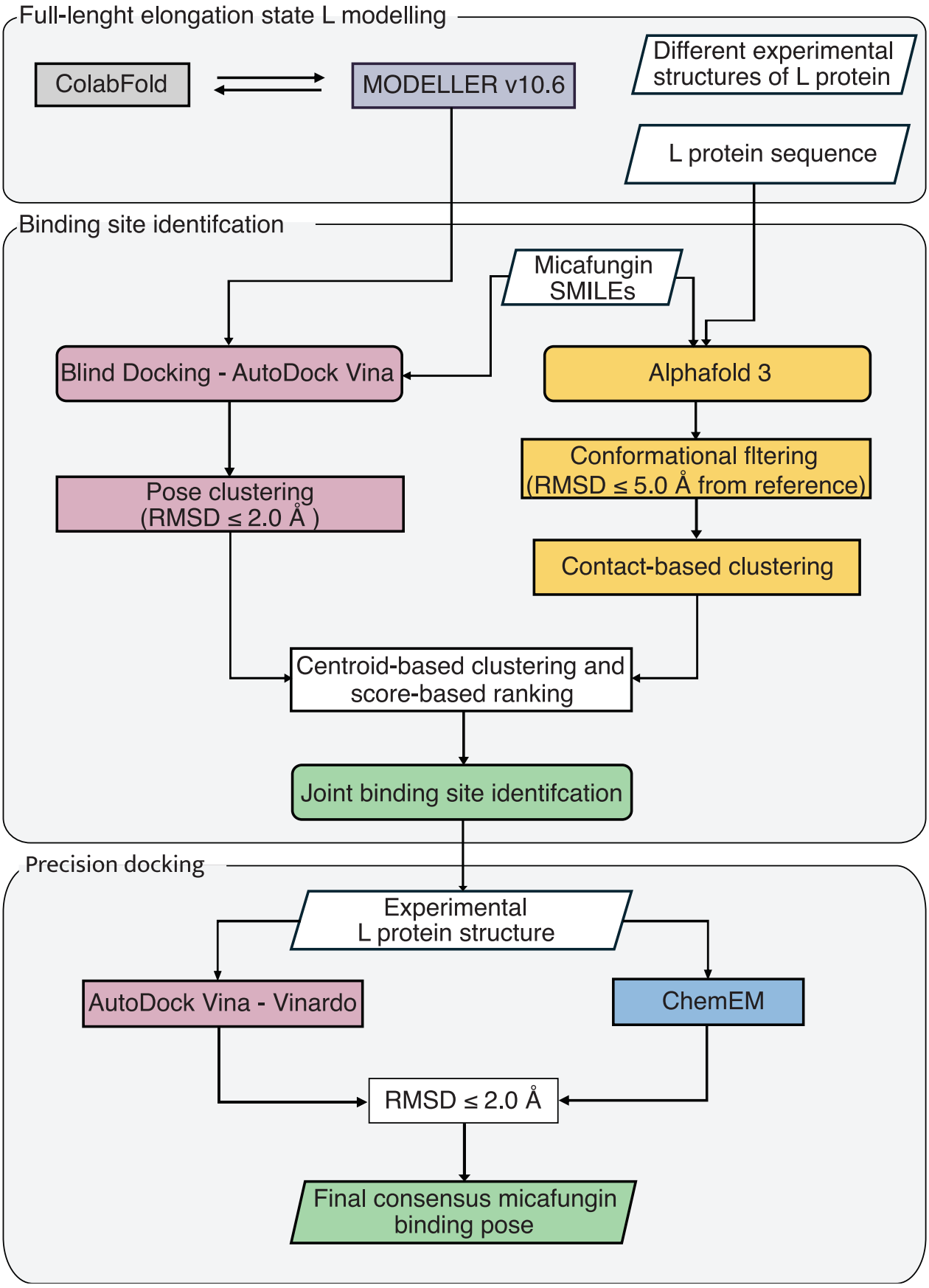
Supplementary Figure S11: Cell-based testing. The effect of selected compounds (see panel labels) on LASV amplification in A549 cells was assessed. Ribavirin (RBV) and T705 controls are presented in Fig. 6. Cells were infected in a 96 well format with LASV Ba366 at an MOI of 0.1 for 1h. The virus inoculum was removed and replaced with 100 µL compound containing medium (0.5% DMSO) or the solvent control. Compound concentrations of 20 nM – 33 µM and in a second step also 100 and 200 µM were tested (interrupted curves for transparency). Supernatants containing LASV were harvested 72 h post infection for determination of viral titres by a titration series and an immuno-focus assay (IFA, all samples in technical duplicates). Cell viability was determined using an MTS assay. Data points are shown as the normalized mean of three biological replicates ± SD (n=3).

Supplementary Figure S12



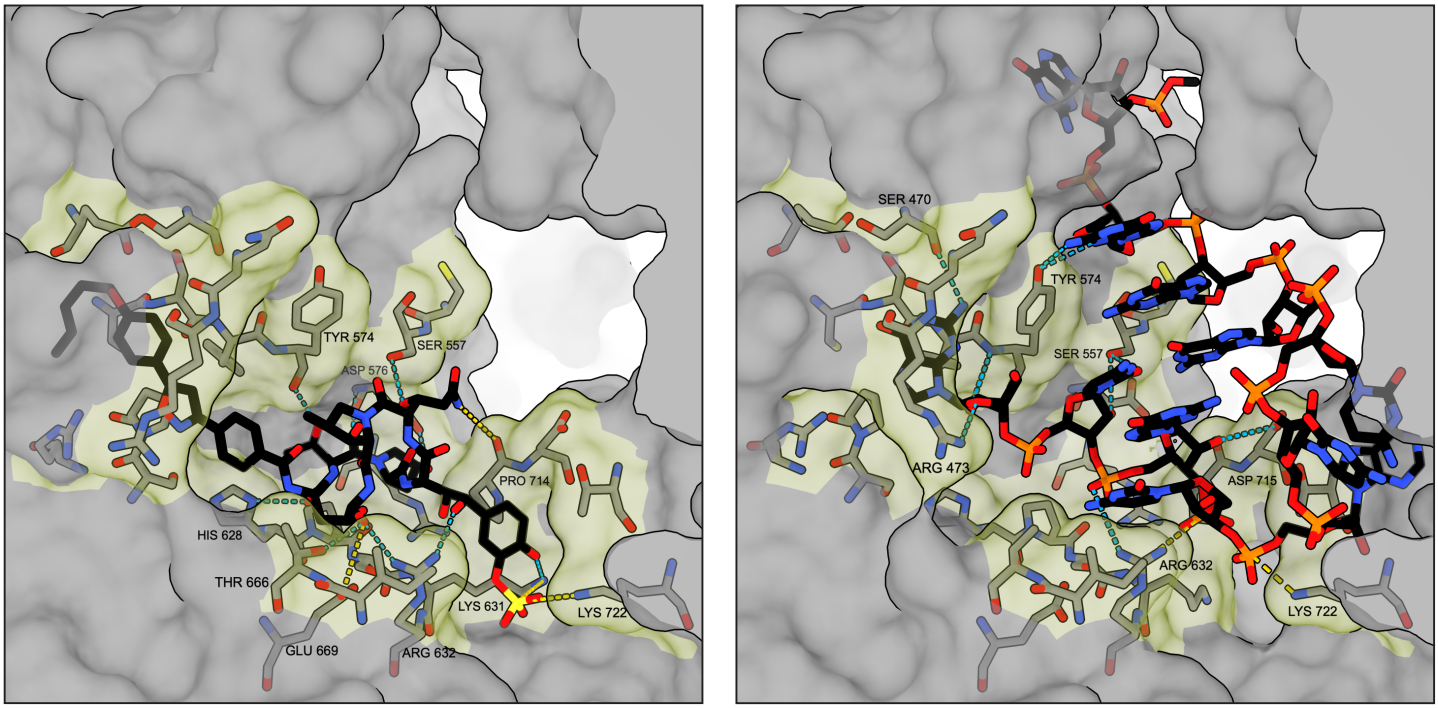
Supplememtary Figure S12: Linkers used in the tracers and uncropped polymerase assay gel. A) Linker used for the 5'(0-9)-Cy5 tracer. **B)** Linker used for the 3'(1-7)-Cy5 tracer. Structures were drawn with Chemdraw. **C)** Uncropped polymerase assay gel presented in Fig. 7, the selected area for Fig. 7 was marked with a dashed rectangle, asterisks indicate the expected product size of 21 nt.

Supplementary Figure S13



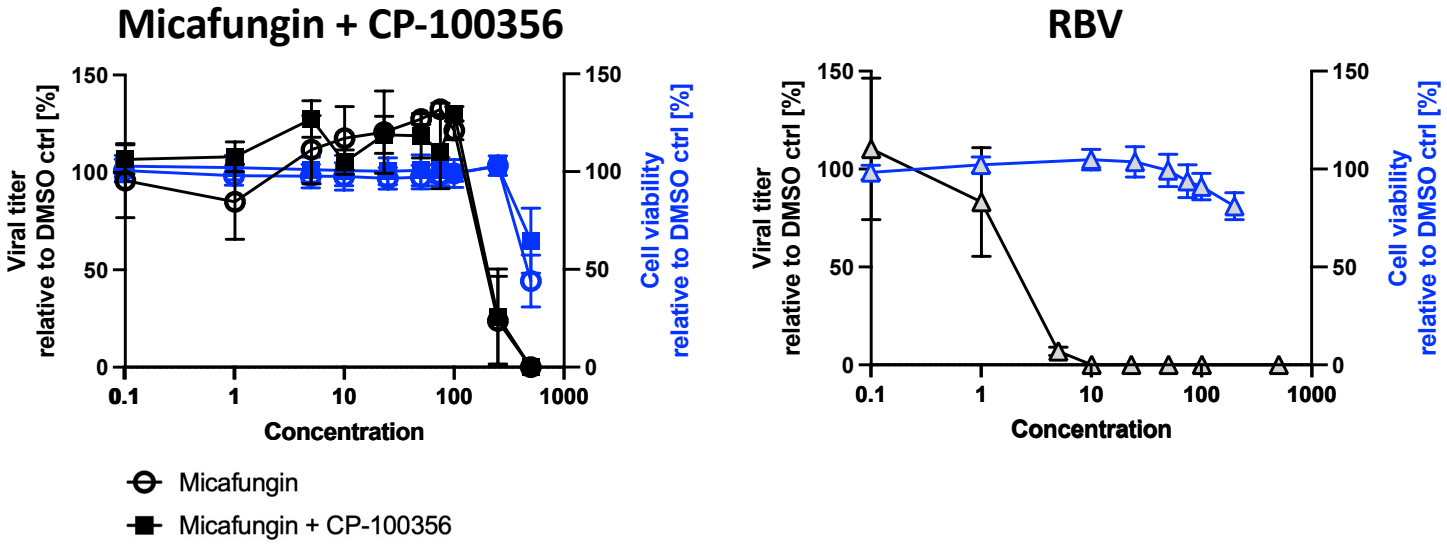
Supplementary Figure S13: Computational pipeline for micafungin binding site and pose prediction. The workflow consists of three sequential phases integrating structure-based modelling, docking, and AI-driven complex prediction. The arrows show the flow of information between the following steps: (i) Modelling of the full-length L protein structure using MODELLER and ColabFold from the sequence, experimental structures and missing domain information. (ii) Binding site prediction using a consensus-based clustering and ranking of Autodock Vina and Alphafold 3 results. (iii) Consensus precision docking applied to the experimental L protein structure using AutoDock Vina (Vinardo scoring function) and ChemEM, resulting in the final predicted pose of micafungin.

Supplementary Figure S14



Supplementary Figure S14: Shared interaction footprint of micafungin and the 5' RNA on the L protein. The figure highlights the amino acids (shown as grey sticks with atom coloring in a pale green surface) involved in the interaction of both the micafungin (left panel) and the 5' RNA (right panel) with the L protein shown as semi-transparent grey surface. Micafungin and the RNA are shown as black sticks withelement-specific heteroatom coloring. Key interactions such as H-bonds (cyan dotted lines) and salt bridges (yellow dotted lines) are highlighted based on PLIP analysis (Philipp Schake, Sarah Naomi Bolz, Katja Linnemann, Michael Schroeder, PLIP 2025: introducing protein–protein interactions to the protein–ligand interaction profiler, *Nucleic Acids Research*, Volume 53, Issue W1, 7 July 2025, Pages W463–W465, https://doi.org/10.1093/nar/gkaf361)

Supplementary Figure S15



Supplementary Figure S15: Combination treatment of micafungin and p-Glycoprotein inhibitor CP-100356. The effect of a combination treatment with micafungin and p-Glycoprotein inhibitor CP-100356 on LASV amplification in A549 cells was assessed. Cells were infected in a 96 well format with LASV Ba366 at an MOI of 0.1 for 1 h. The virus inoculum was removed and replaced with 100 µL compound containing medium (0.5% DMSO) or the solvent control. Compound concentrations of 2 µM for CP-100356 and from 100 nM to 500 µM for micafungin were tested (left panel). Ribavirin (RBV, right panel) served as a positive control. Supernatants containing LASV were harvested 72 h post infection for determination of viral titers by a titration series and an immuno-focus assay (IFA, all samples in technical duplicates, black curves). Cell viability was determined using an MTS assay (blue curves). Data points are shown as the normalized mean of three biological replicates ± SD (n=3).

Supplementary Table S1: Summary of the precision docking parameters. The table lists the name and associated SMILEs string along with the centre of the binding site and size of the grid box. Additional docking parameters (for Vina and ChemEM) are included to specify the settings applied in the simulations.

Name	SMILEs	Center	Size	Vina Parameters	ChemEM Parameters
Micafungin full	CCCCCOC1=CC=C(C=C1)C2=CC(=NO2)C3=CC=C(C=C3)C(=O)N[C@H]4C[C@H]([C@H](NC(=O)[C@@H]5[C@H]([C@H](CN5C(=O)[C@@H](NC(=O)[C@@H](NC(=O)[C@@H]6C[C@H](CN6C(=O)[C@@H](NC4=O)[C@@H](C)O)O)[C@@H]([C@H](C7=CC(=C(C=C7)O)OS(=O)(=O)O)O)O)[C@@H](CC(=O)N)O)C)O)O)O	X -35.9 Y 4.1 Z 10.9	X 30 Y 30 Z 30	--exhaustiveness 64, --energy_range 1, --scoring vinardo	--dock, --no-map, --minimize-docking, --rescore, -fr, --energy-cutoff 5.0, --cluster-docking 1.0, --repulsion-cap-0 4.0, --repulsion-cap-1 10.0, --repulsion-cap-nm 20.0, --repulsion-cap-polish 30.0
Micafungin Tail	(CCCCCOC1=CC=C(C=C1)C2=CC(=NO2)C3=CC=C(C=C3)C(=O)N[C@H])	X -35.9 Y 4.1 Z 10.9	X 30 Y 30 Z 30	--exhaustiveness 32, --energy_range 3, --scoring vinardo	--dock, --no-map, --minimize-docking, --rescore, -fr, --bias-radius 9.0, --energy-cutoff 5.0, --cluster-docking 1.0, --repulsion-cap-0 6.0, --repulsion-cap-1 15.0, --repulsion-cap-nm 30.0, --repulsion-cap-polish 45.0