Discovery of Antivirals Targeting Sars-CoV2 Viral Helicase

Viral helicase as a novel antiviral target

Antiviral Drug Discovery (AViDD) Open Science Forum
7/17/2024

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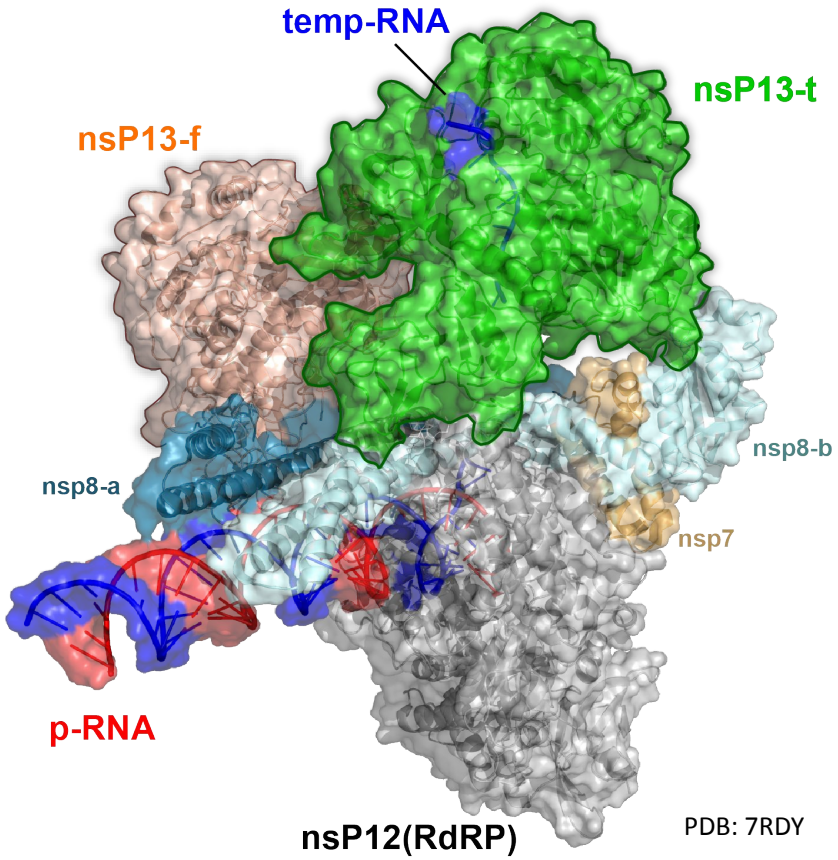
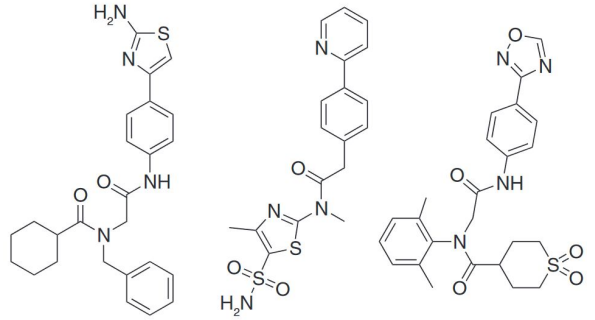
Viral helicases:

Novel, understudied antiviral target class.



- Part of the viral replicase complex
  - unwinding of dsRNA
- Limited antiviral examples of targeting viral helicases

Anti-HSV compounds targeting the viral helicase/primase (UL5/UL52)



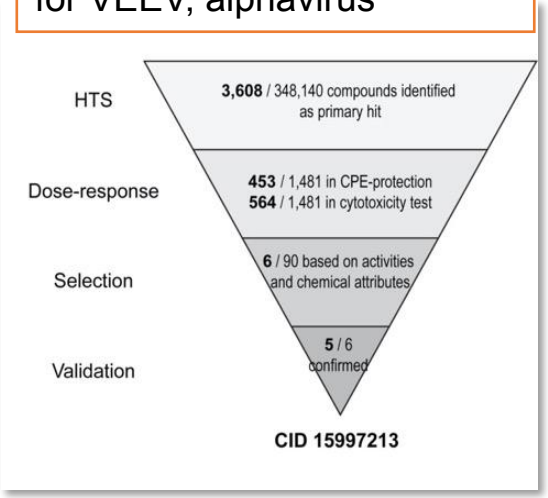
William R. Shadrick et al. DOI: 10.1177/1087057113482586

Chen et al : Nature Structural & Molecular Biology 2022

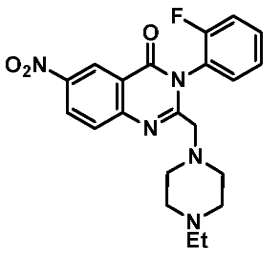
Project 5 of Midwest AViDD

Alphavirus nsP2 and SARS-CoV2 nsP13 are functional and structural homologs : Superfamily I helicase

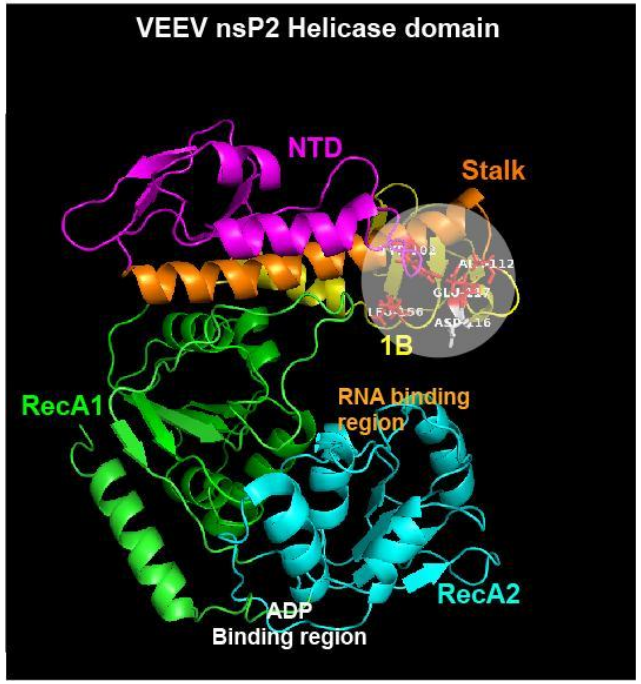
HTS of 348K MLSMR library for VEEV, alphavirus

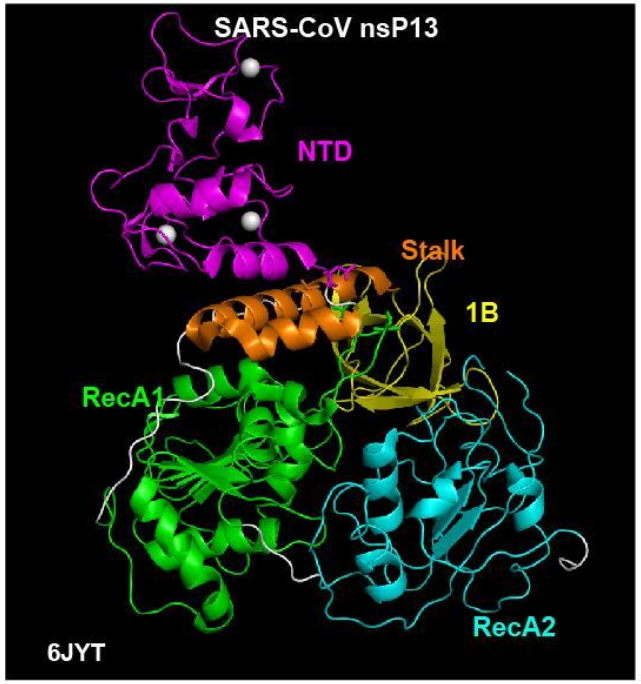


quinazolinone hit
CID 15997213



VEEV EC_{50} = 840 nM
VERO76 CC_{50} > 50 µM





ML336 → EC_{50} =30-40 nM

BDGR-4 → EC_{50} = 30-40 nM, improved virus yield reduction

BDGR-49 → EC_{50} = 1-10 nM, 100% protection with a 2 days delay of treatment in vivo

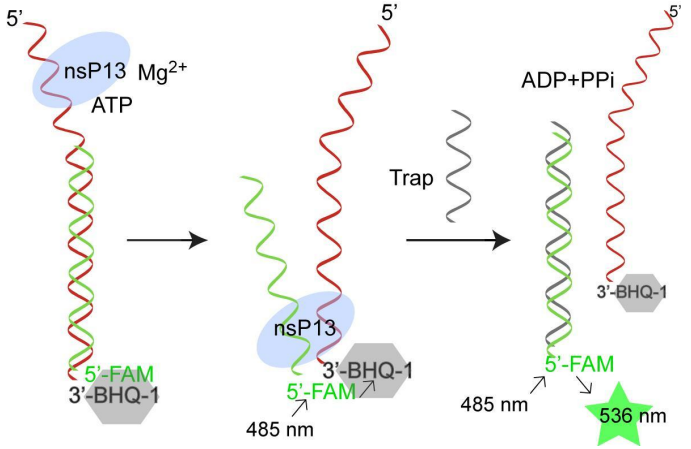
Cao et al. : Sci. Transl. Med. 2023

Table 1. Comparison of viral helicases proposed in this proposal

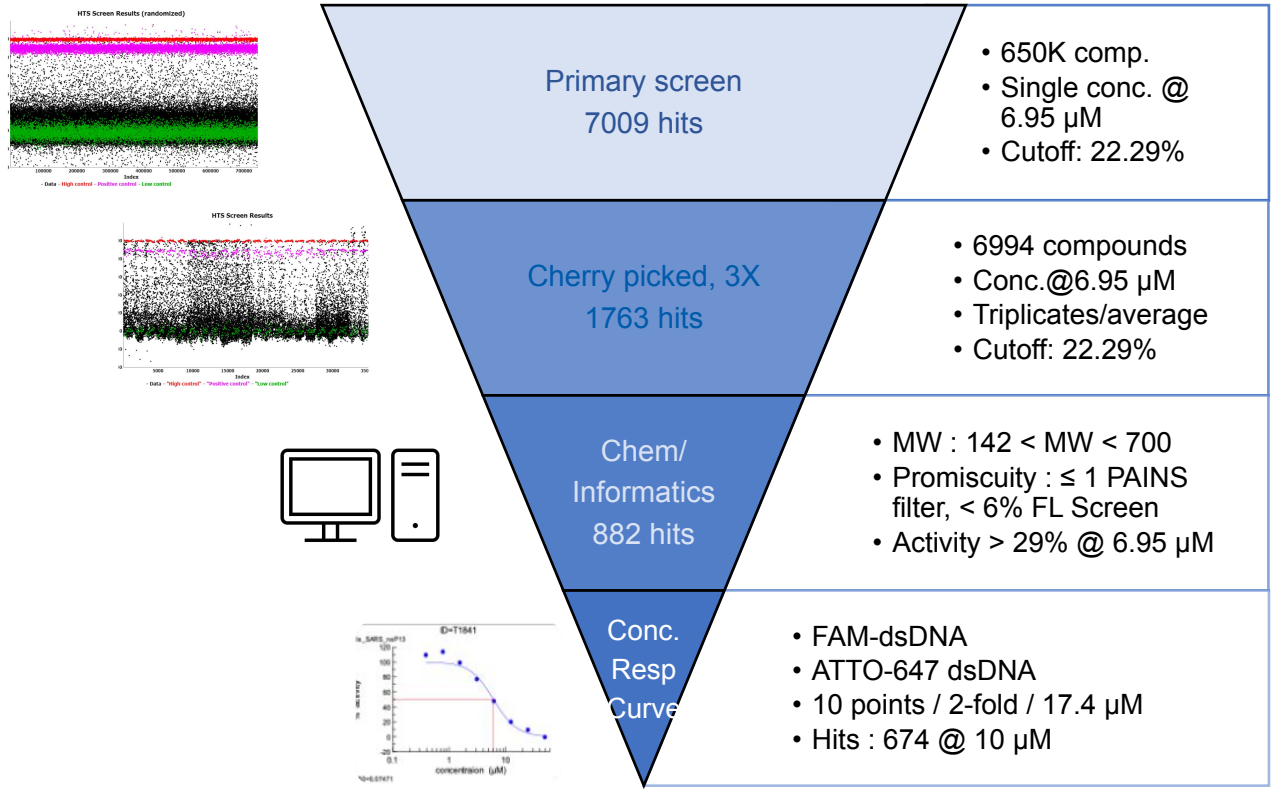
	Sars-CoV2	Alphavirus	Flavivirus	Human orthologs
Gene	nsP13	nsP2	nsP3	RecQ4L
Helicase superfamily	SF1	SF1	SF2	SF2
Substrate	DNA or RNA	RNA	DNA or RNA	DNA
Translocation direction	5′–>3′	5′–>3′	3′ –> 5′	3′–>5′

HTS-SCV2 helicase uHTS

Midwest AViDD Core B / UF-Scripps

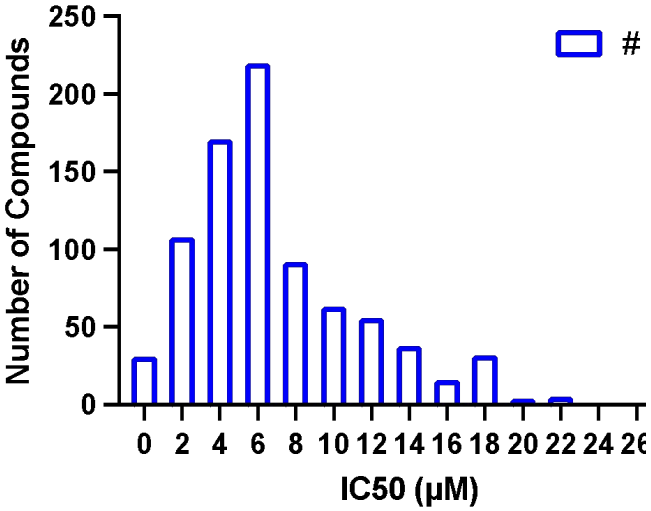


1536-well plate format



Screen	No. of plates	Ave Z'	Ave Z	Ave S:B	Hit cutoff	Hit rate
Primary (n=650K)	522	0.86 ± 0.05	0.61 ± 0.72	5.14 ± 0.55	22.29%	1.1%
Confirmatory (n=6994)	24	0.86 ± 0.03	0.24 ± 0.21	4.45 ± 0.11	22.29%	25.2%
CRC (n=881)	24	0.79 ± 0.05	-0.02 ± 0.10	4.19 ± 0.17		

HTS Hit identification



- A total of 217, active, curated compounds
  /Anti-viral helicase library/

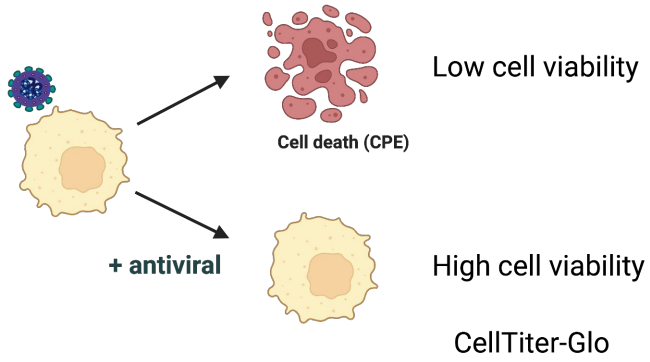
Criteria	No. of selected
Total	881
Non-PAINS	746
Max>50%/DR	677
IC50 < 10 µM	565
IC50 < 5 µM	266
IC50 < 1 µM	29

Parallel executions of

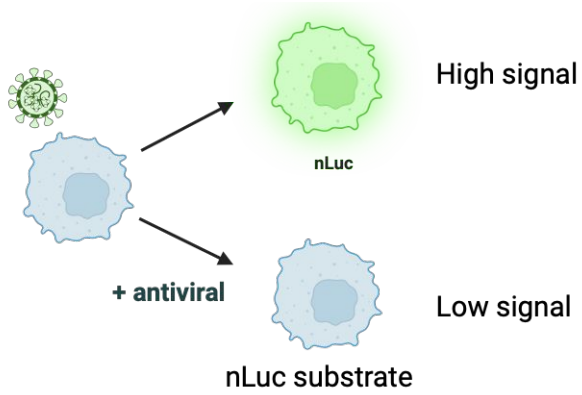
In vitro biochemical			Cell-based antiviral Specificity					Target engagement (BioPhysics)			Pharm
rSARS-CoV 2-helicase	Tm (counter)	rHuWRN (counter)	SARS-CoV2 wt virus (EC50, µM)	Other viruses (specificity)			Cytotox (counter)	TSA/ BioCore	NMR	Co-crystal	In vitro ADME
dsDNA-FAM/647 (dsRNA)	dsDNA	dsDNA	CPE-SARS 2	nLuc-SARS2	Anti-ZIKV	Anti-CHIKV	Anti-VSV (counter)	A549-hACE2	Kd	Kd	Binding site	Stability

Frontline assays: anti-viral activity

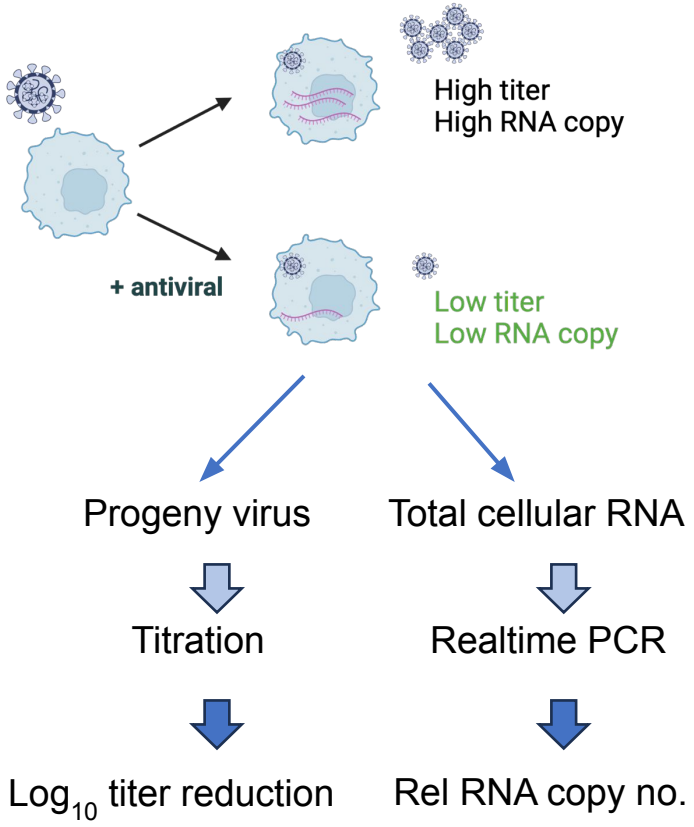
SARS-CoV-2 CPE
/A549-hACE2

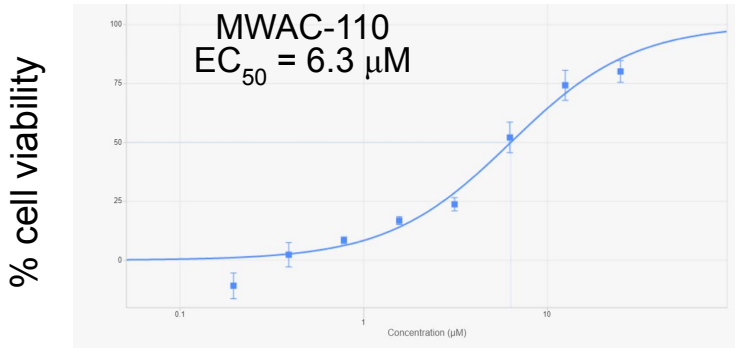


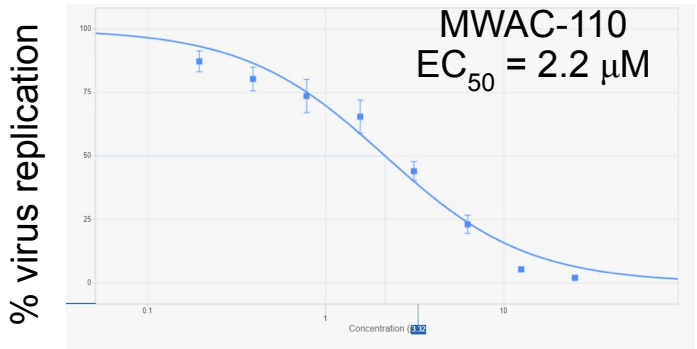
SARS-CoV-2 nLuc
/A549-hACE2



Virus yield reduction





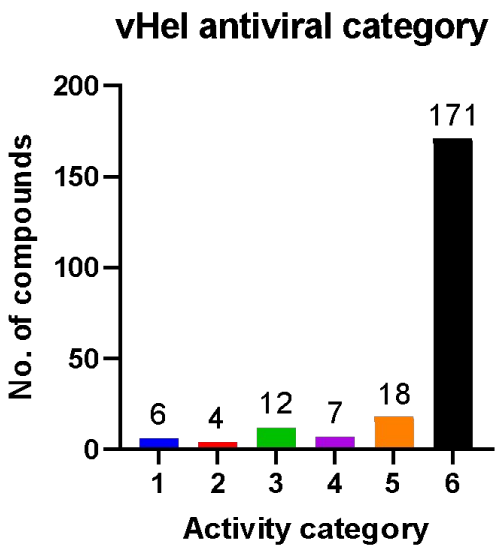


SARS-CoV-2 WT-1/A549-hACE2

Antiviral activities of uHTS-hit scaffold compounds

vHel library (~ 220 compounds)

Cell-based antiviral assays
Anti-SARS-CoV2, CHIKV, Cytotoxicity



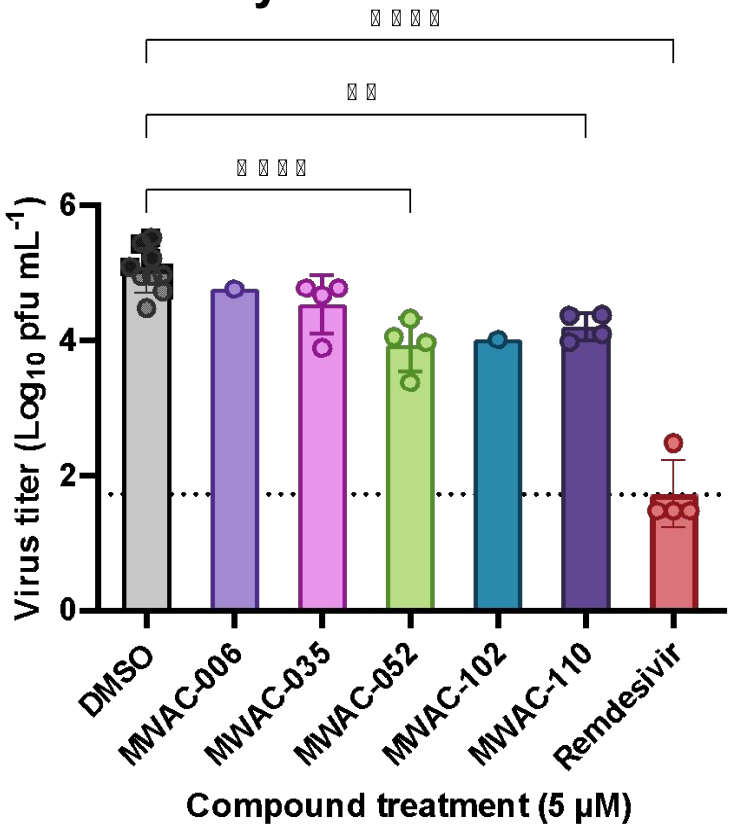
#1 : SARS-CoV2 specific
#2 ; SARS-CoV2 specific with a low SI
#3 : Anti-nLuc only-unknown mechanism
#4 : Low SI50 - antiviral due to cytotoxicity
#5 : Cytotoxic
#6 : No antiviral activity

	EC_{50}-nLuc (SARS-CoV2-nLuc)	EC_{50}-CPE (SARS-CoV2 WT)	CC50 (A549-ACE2)	SI50
MWAC-002	8.5	3.83	22.4	2.6
MWAC-032	14.72	27.3	>50	>3.4
MWAC-046	16.11	30.6	>50	>3.1
MWAC-052	4.15	1.9	>50	>12.1
MWAC-102	2.8	6.02	>50	>17.9
MWAC-110	3.31	8.06	>50	>15.1
MWAC-140	3.83	4.49	17	4.4
MWAC-142	2.5	2	16	6.4

Units

HTS Hit selection based on viral assays

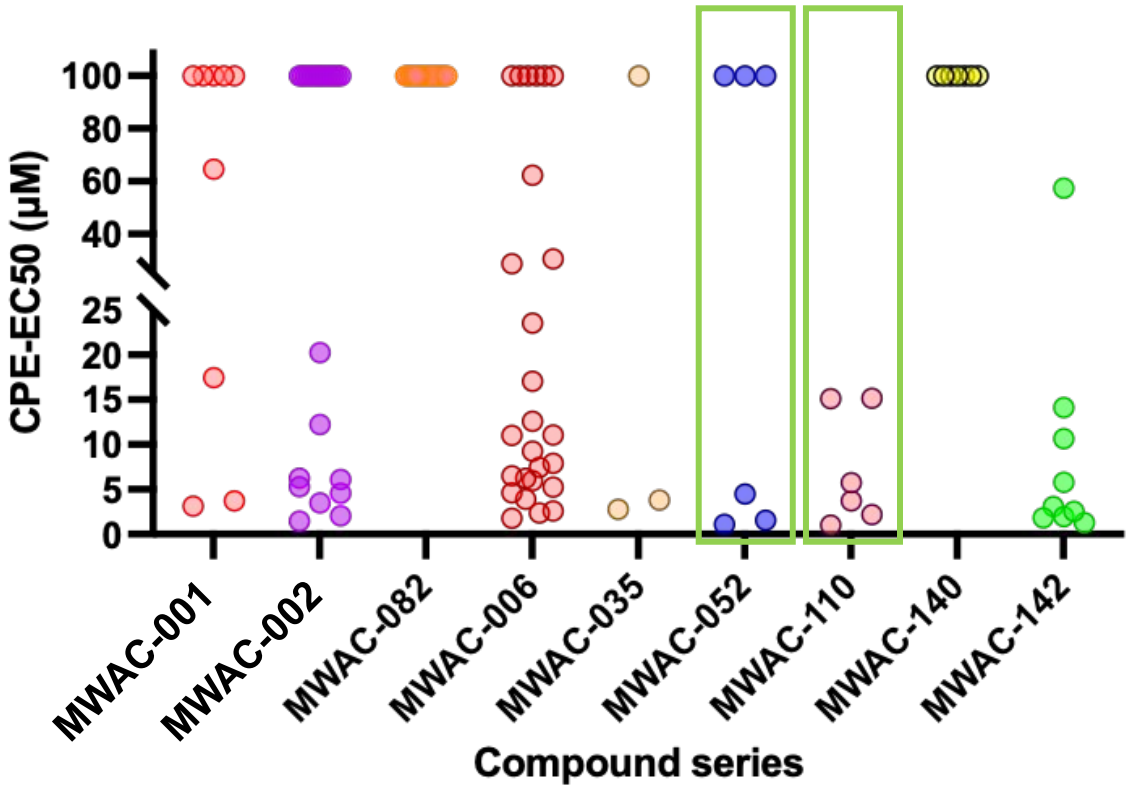
Virus titer reduction activity



Virus : SARS-CoV2/2009/WT-1
Cells : A549-hACE2

Remdesivir at 1 µM

Performance of structural analogs



Medicinal chemistry of the two leading series

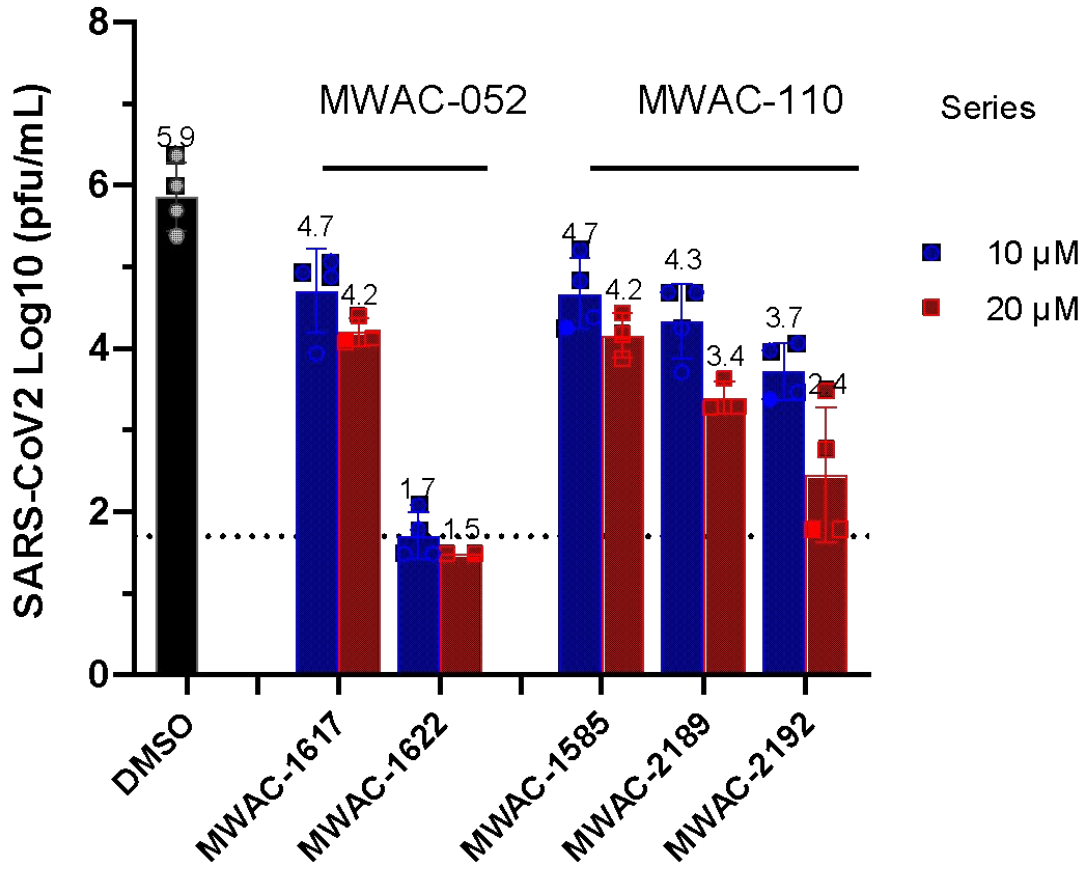
	MWAC-052 series					MWAC-110 series				
	MWAC-052	**MWAC-16**	**MWAC-16**	**MWAC-28**	**MWAC-27**	**MWAC-01**	**MWAC-15**	**MWAC-15**	**MWAC-15**	**MWAC-21**
	2	**18**	**22**	**43**	**74**	**10**	**85**	**86**	**87**	**92**
SARS-CoV2 EC_{50}-CPE	1.9	4.5	**0.32**	**0.11**	1.0	5.3	2.2	3.7	**1.0**	**1.1**
SARS-CoV2 EC_{50}-nLuc	4.2	8.1	**0.50**	**0.39**	0.45	2.8	1.1	2.0	**0.59**	**0.66**
ZIKV EC_{50}-CPE	> 50	> 50	> 50	>50	> 25	> 50	> 50	> 50	> 50	> 50
CC_{50}	>50	>50	> 50	51	32	>25	> 25	22	9.5	> 50
SI	>12	>6.2	**> 100**	**131**	32	>9	> 22	11	**16**	**> 75**

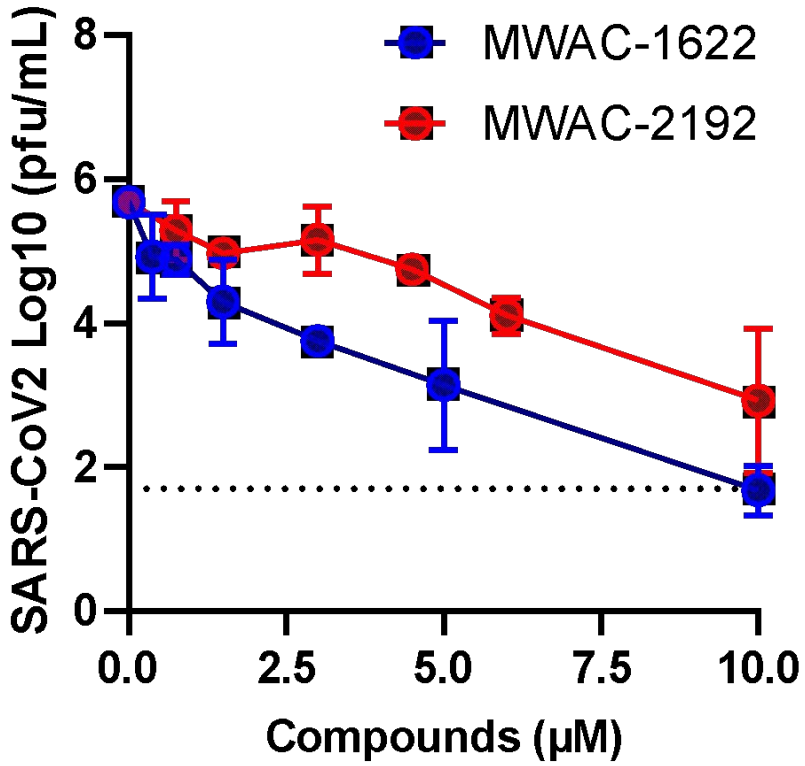
>35 analogs tested to date

EC50 and CC50 (µM), Virus: SARS-CoV-2 WT-WA1 ;
CC_{50}: in A549-hACE2 cells

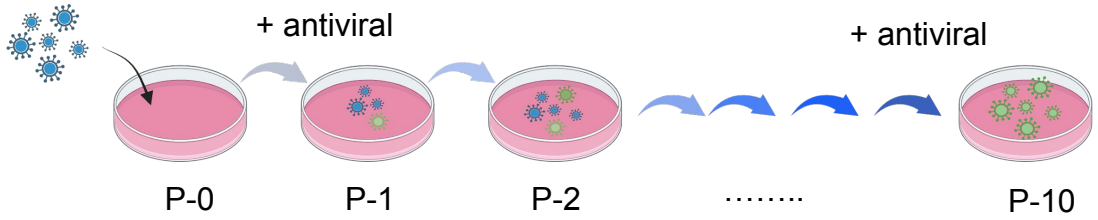
Dr. Bannister group

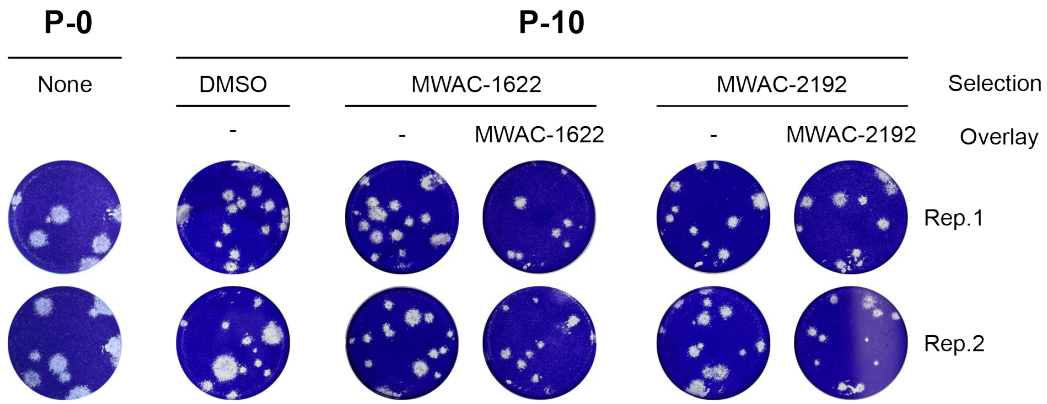
The two series showed anti-'VIRAL' activity



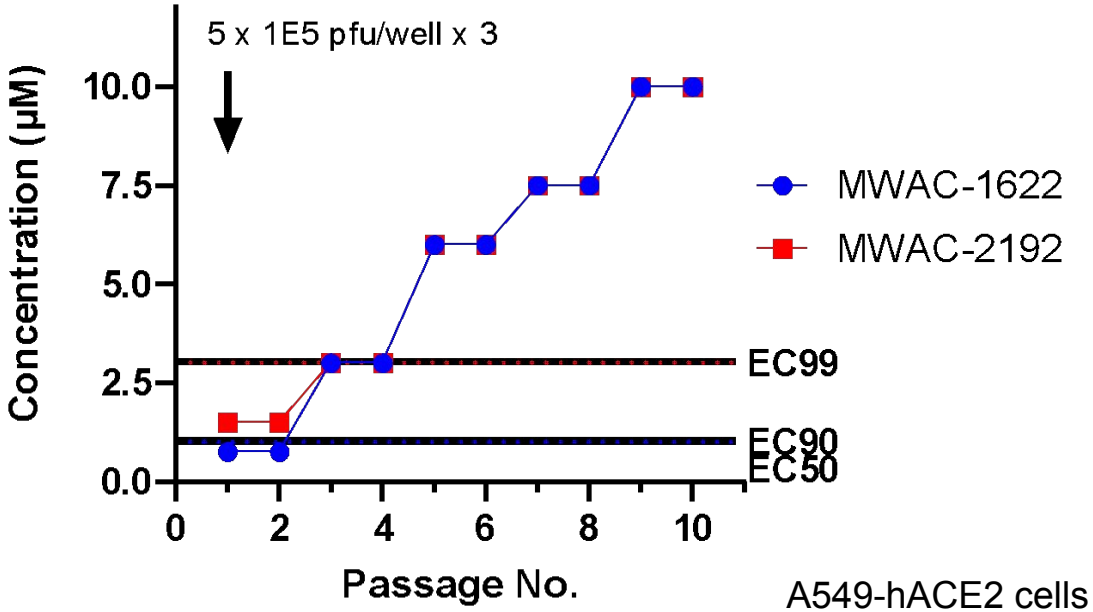


Genetic approach: Isolation of resistant virus

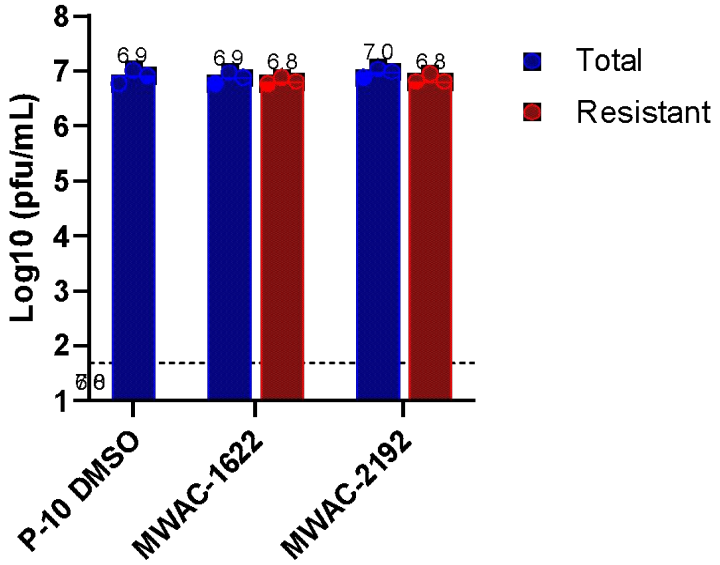




Clear CPE was noticeable.

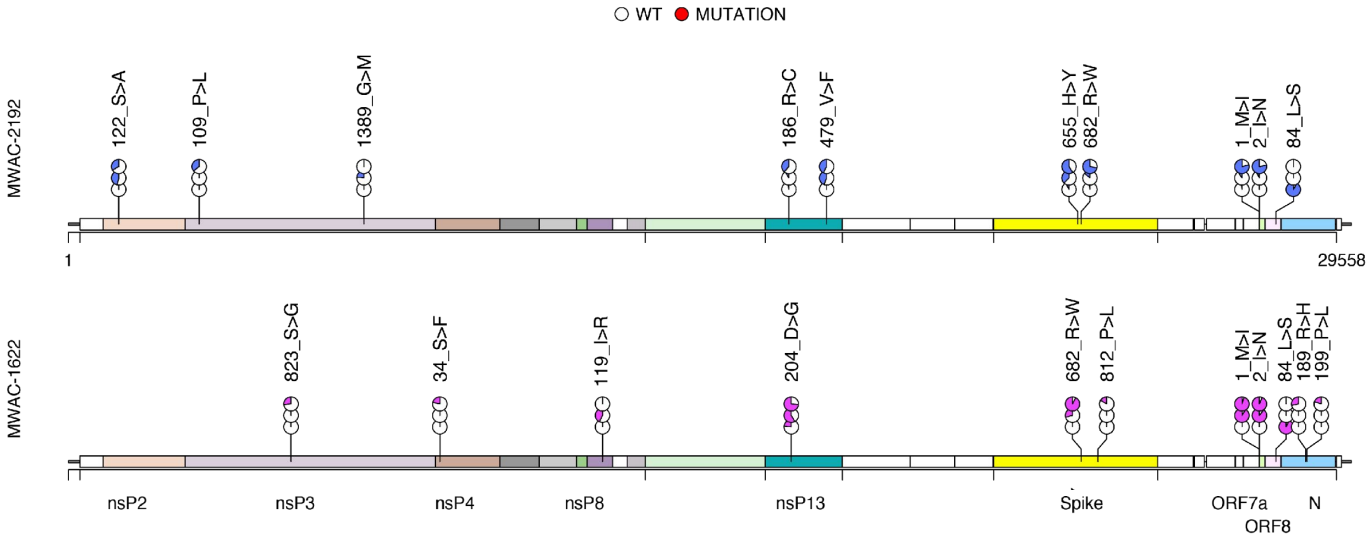


A549-hACE2 cells



Mutations identified in serially-passaged, resistant populations

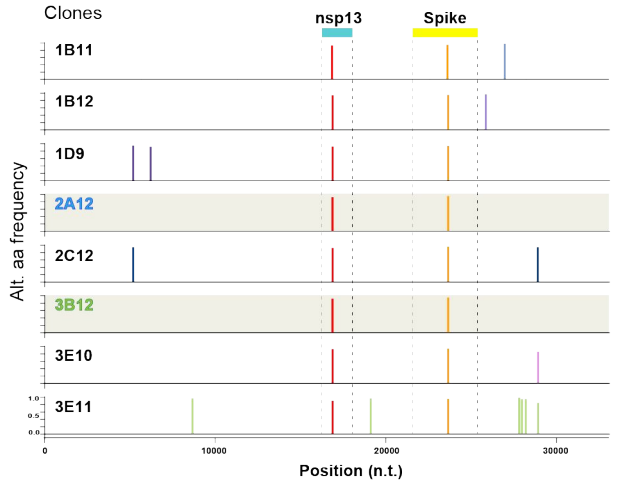
Total population sequencing



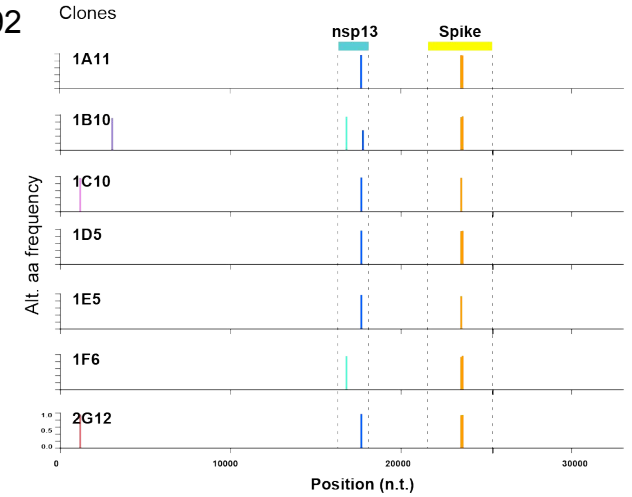
P-10	nsP2		nsP3		nsP4		nsP13			S			ORF7b		Np	
Mutations	122_S>V	109_P>L	823_S>G	34_S>F	186_R>C	204_D>G	479_V>F	655_H>Y	682_R>W	812_P>L	1_M>Y	2_I>N	189_R>H	199_P>L
MWAC-1622			0.26	0.21		0.74			0.93	0.16	0.96	0.94	0.28	0.20
MWAC-2192	0.34	0.36			0.39		0.43	0.59	0.72		0.79	0.78		

Individual isolates

MWAC-1622



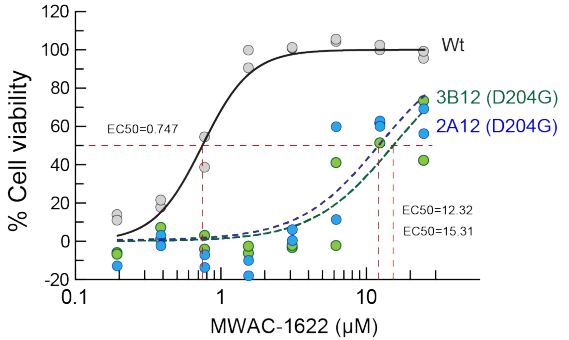
MWAC-2192

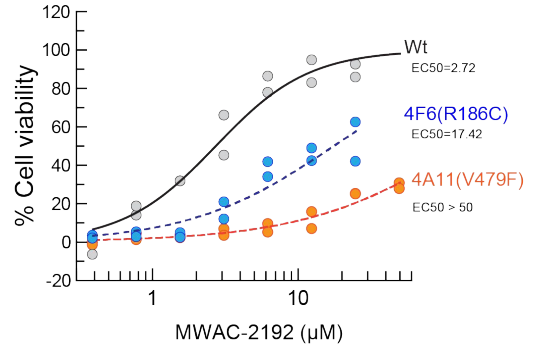


Phenotypic resistance of MWAC-1622 and 2192 resistant clones

Serial 10-passages + compound → Single pfu isolation → EC_{50}, Sequencing

Selection compound	Genotype	Remdesivir		MWAC-1622		MWAC-2192	
		EC50 (μM)	Fold-increase	EC50 (μM)	Fold-increase	EC50 (μM)	Fold-increase
	Wt	0.10	-	0.75	-	2.72	-
MWAC-1622	**nsP13 D204G***	0.2 ± 0.1	x 2	**13.8**	**x 18.4**	8.5	x 3
MWAC-2192	**nsP13 R186C****	0.04	x 0.4	1.29	1.7	**17.4**	**6.4**
	nsP13 V479F**	0.10 ± 0.07	x 1	3.55 ± 1.9	4.7	**> 50**	**> 18**

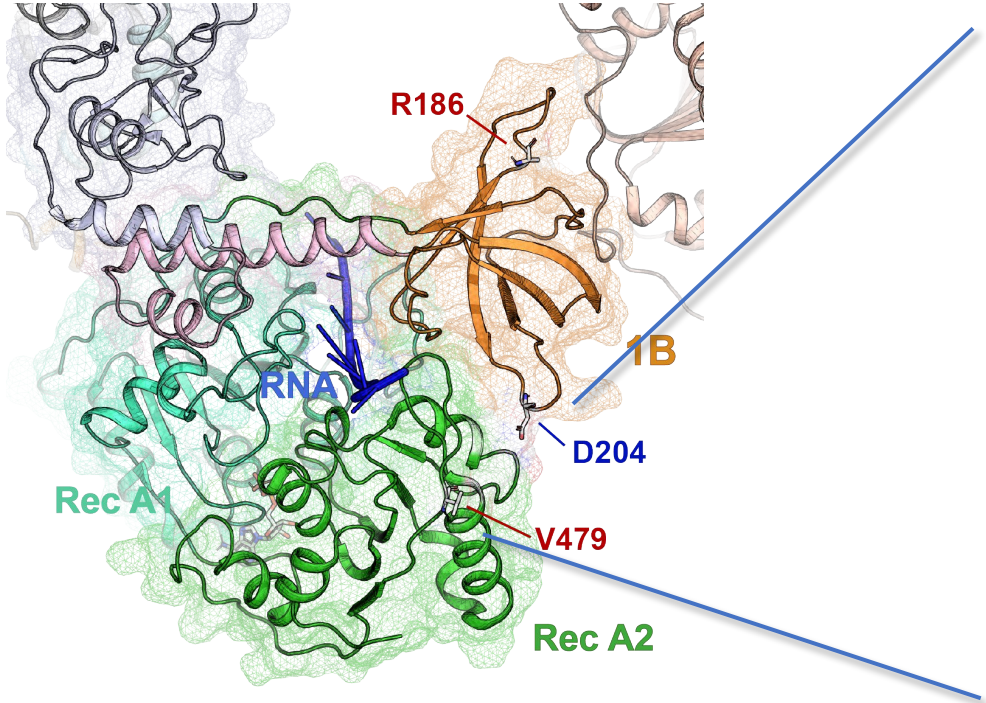


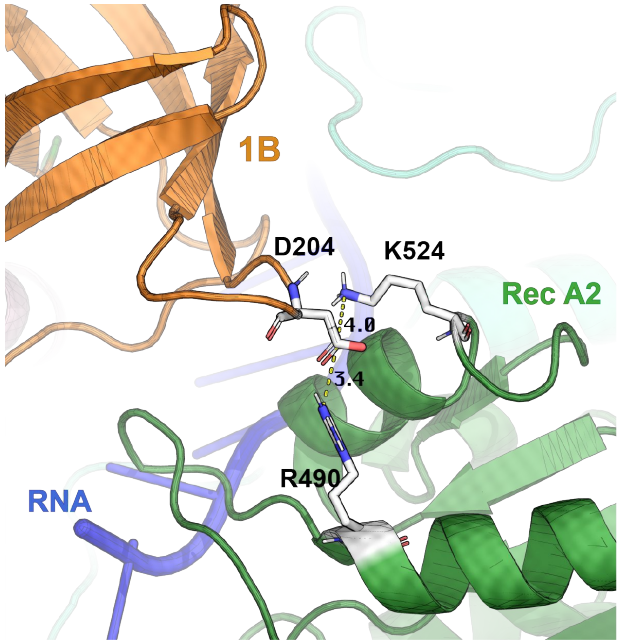


** Spike R682W; ** Spike H655Y/R682W were also found.*

Confirmed nsP13 as the target.

Molecular insights into the mechanism

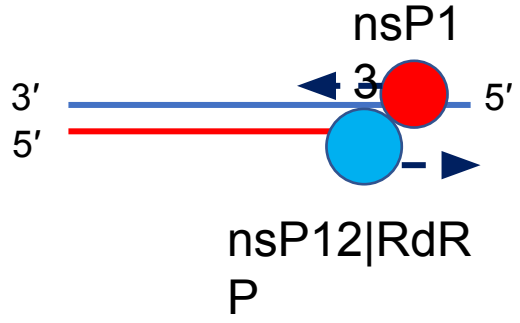


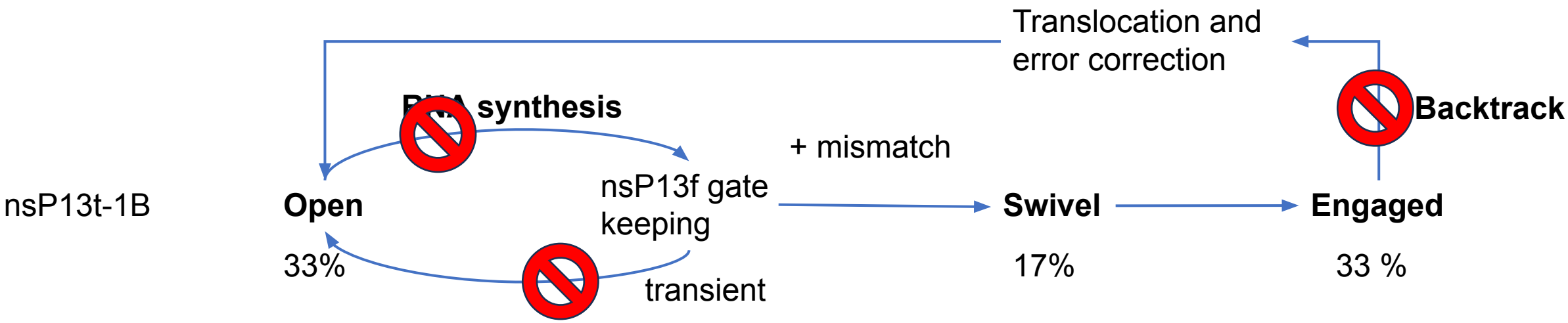


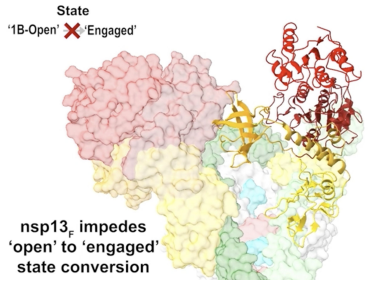
Selection	Genotype	Location
MWAC-1622	nsP13 D204G*	1B
MWAC-2192	nsP13 R186C**	1B
	nsP13 V479F**	RecA2

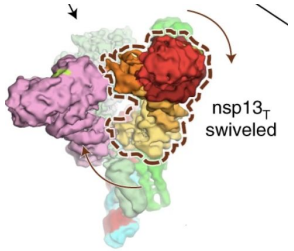
Interaction between 1B domain and Rec A2 by a salt bridge via D204

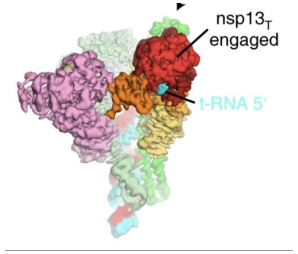
nsP13 conundrum : a tug war











Interaction of 1B domain

?

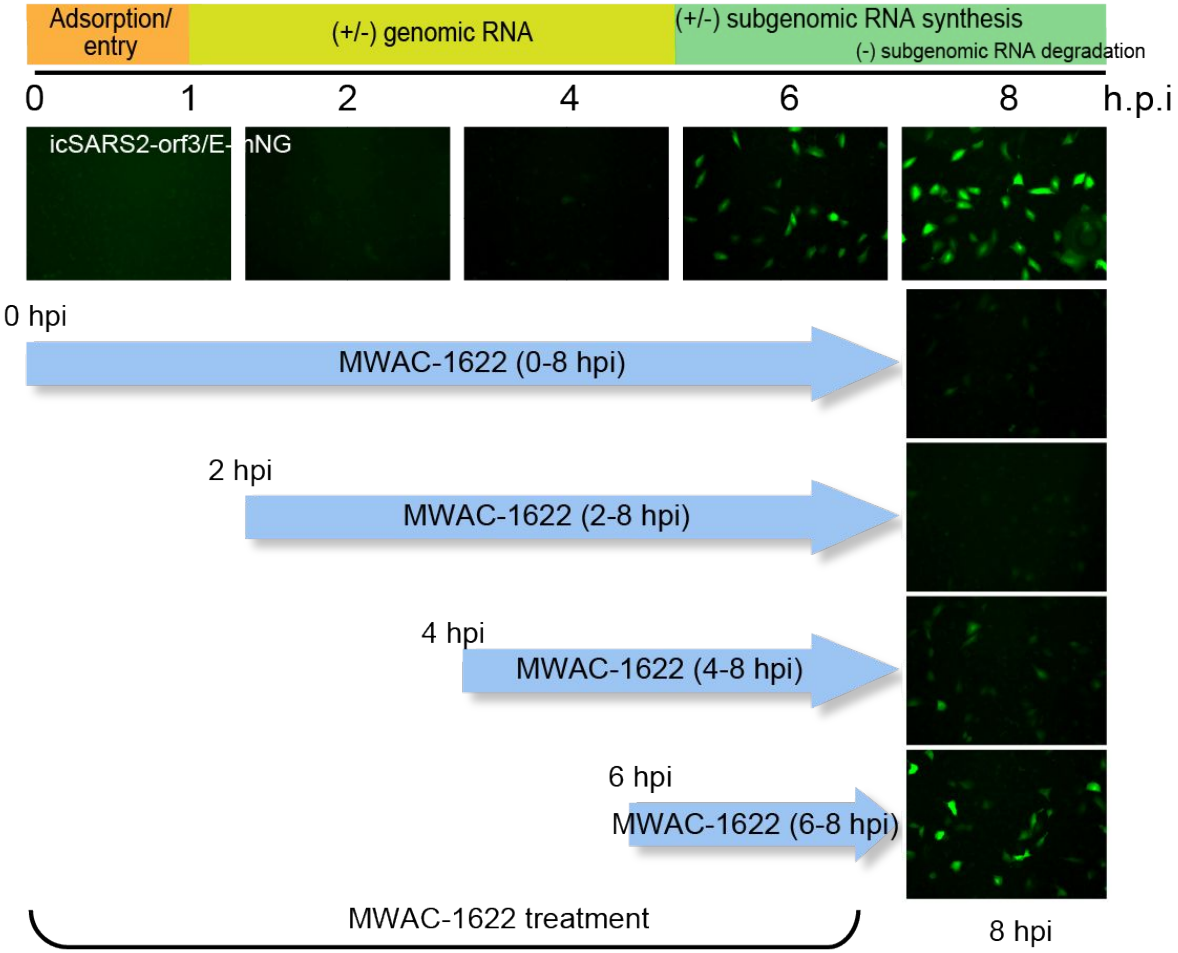
nsP13f

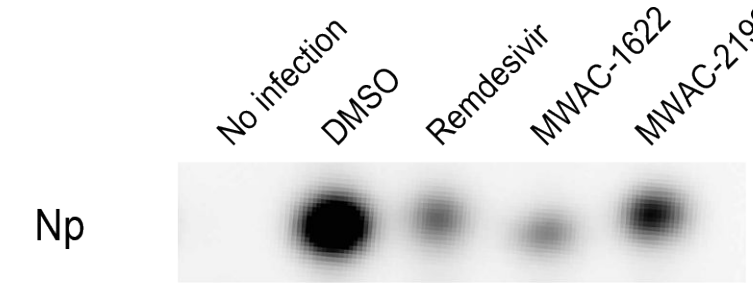
None?

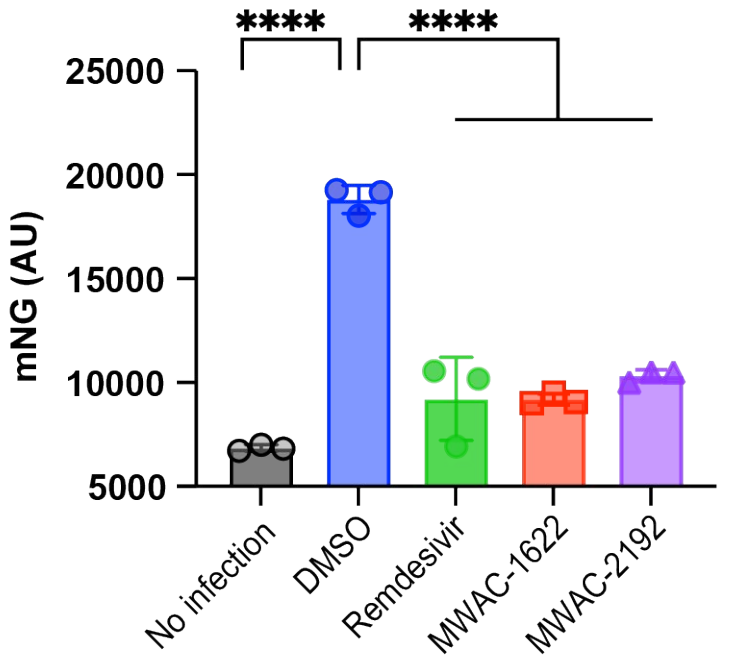
nsP13t-RecA2

https://doi.org/10.1038/s41594-022-00734-6

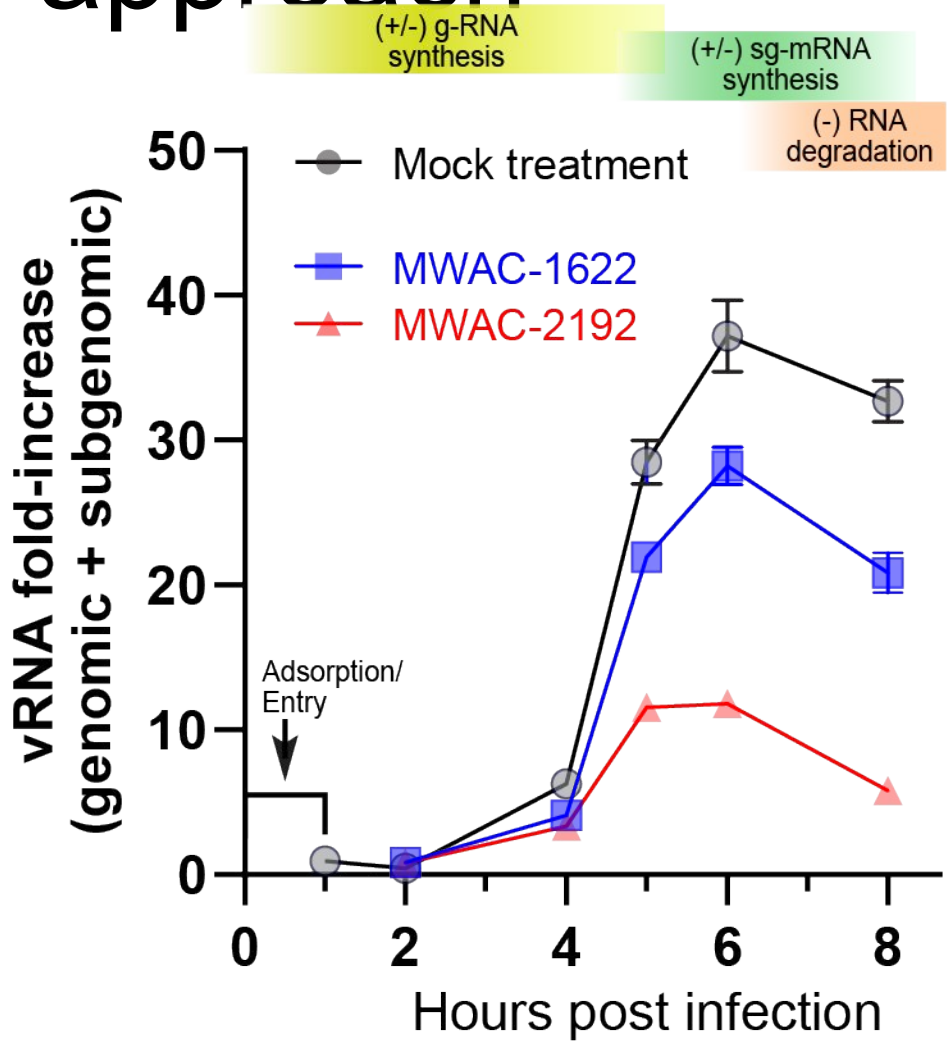
Hit characterization : Virological approaches

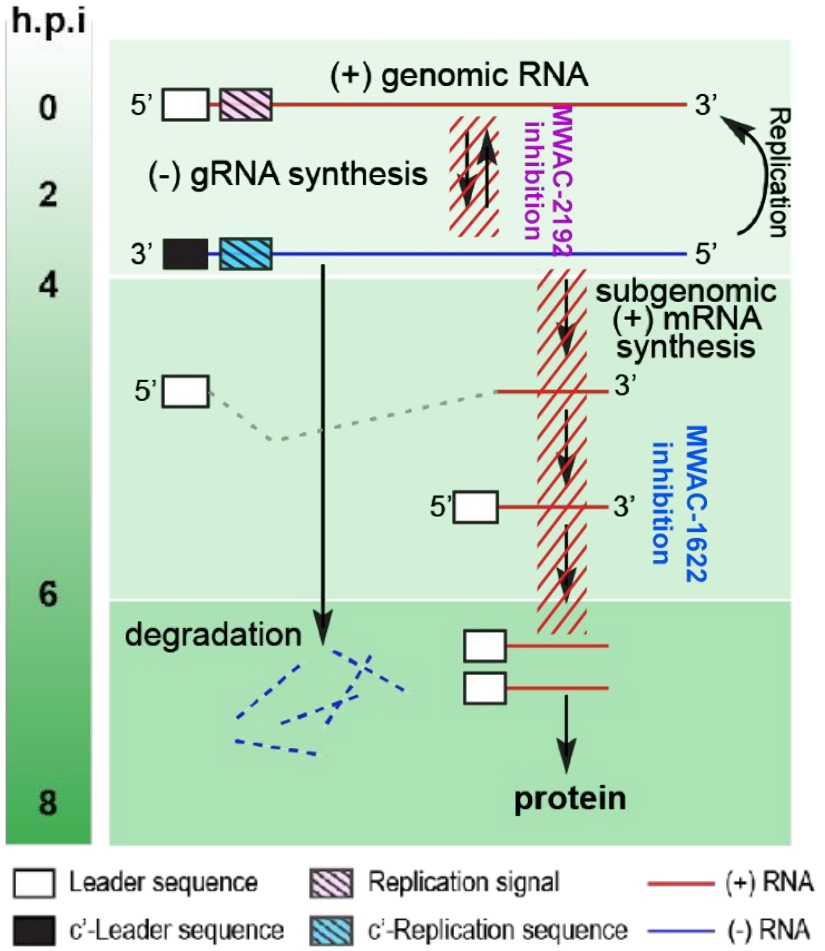




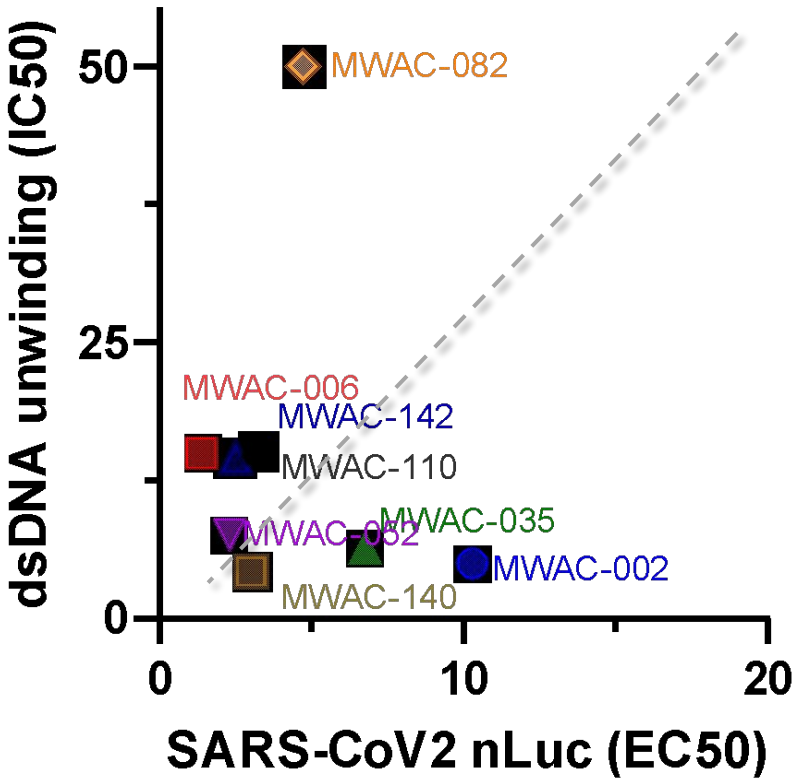


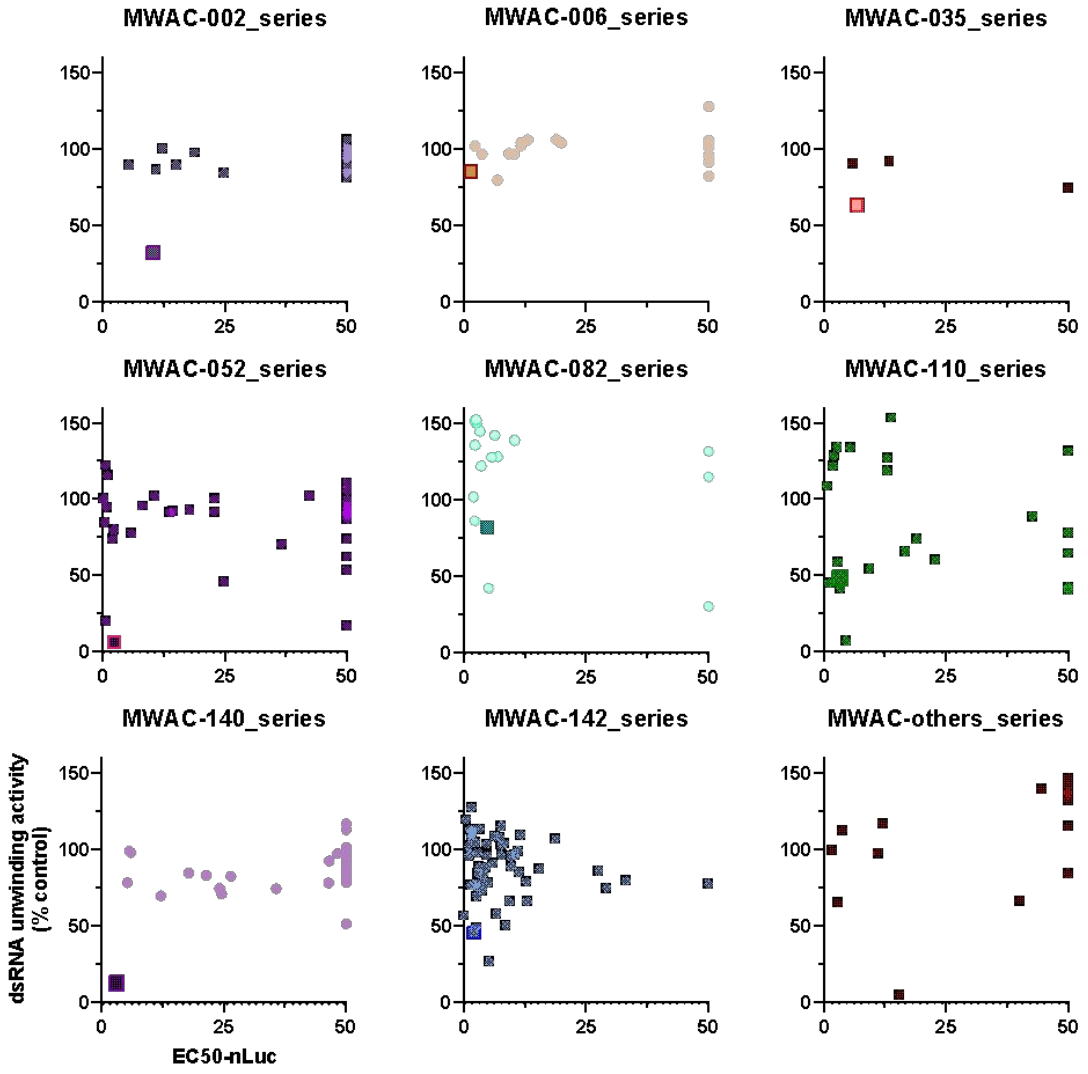
Mechanism of action : Virological approach





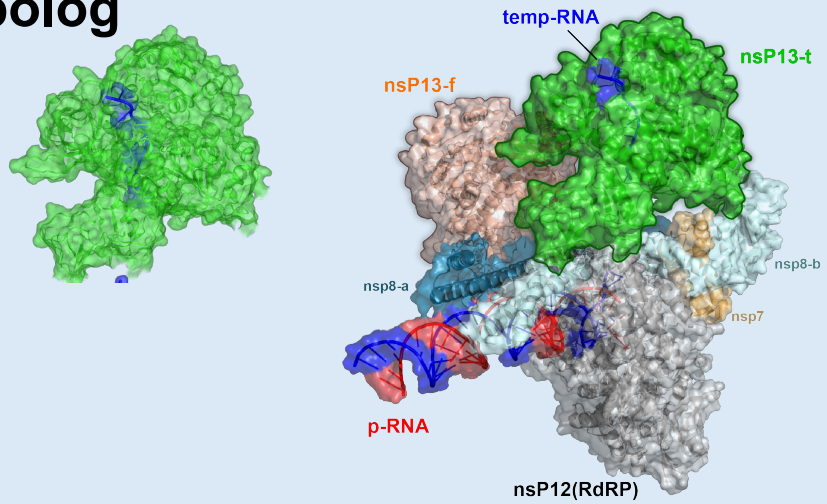
Anti-helicase vs. Anti-viral





Nothing exists alone: nsP13 vs virus

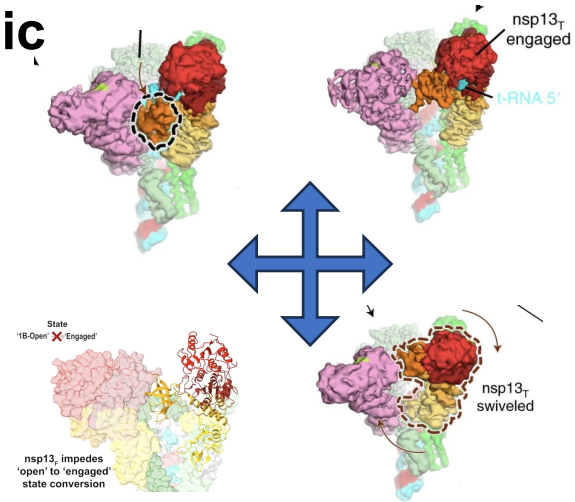
Topology



Biochemistry

- Translocation/ATPase - 700 – 800 nt(ATP) /s
- Mg^{2+} vs Fe-S cluster
  - *Nunziata Maio et al. PNAS, 2023*
- dsDNA > dsRNA

Dynamics



Virological issues

- Location of the replication complex (e.g., DMV)
- How much is enough to show phenotypic changes (e.g., viral titer difference)
- Etc.

Summary

- uHTS identified ~300 compounds with a good anti-nsP13 activity.
- 7 compound series were confirmed with SARS-CoV2-specific antiviral activity.
- Two leading series are being advanced with medicinal chemistry.
- The resistant mutation studies have identified mutations conferring resistance within the nsP13.
- NsP13 may be more than a “dsRNA unwinding engine”.

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Questions?