

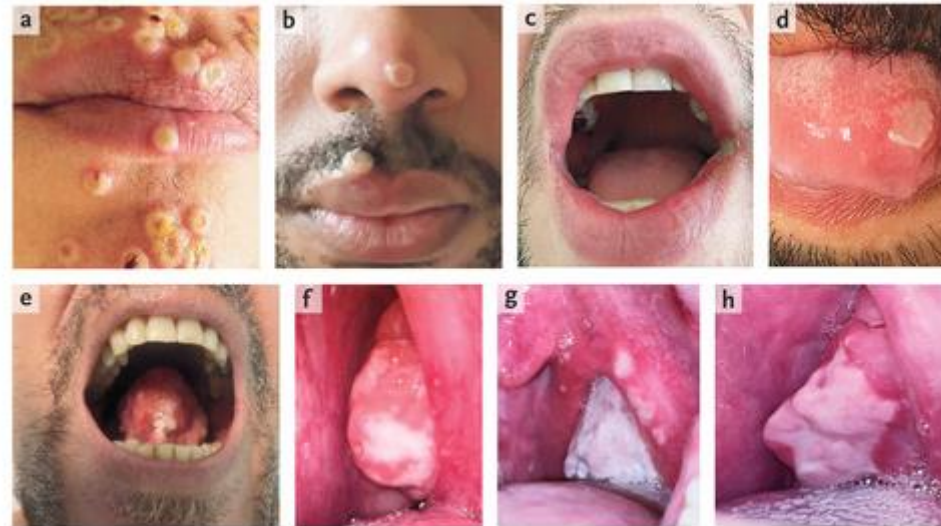
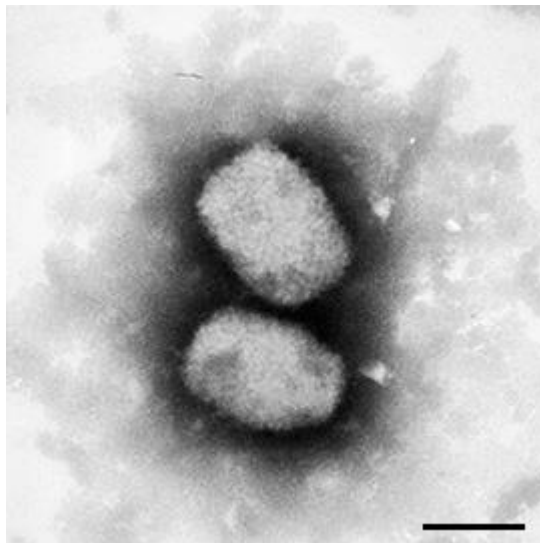
Characterization of Mpox virus genomes and patient isolates from Franconia (Bavaria, Germany) and antiviral testing of patient isolates

Dr. Arne Cordsmeier

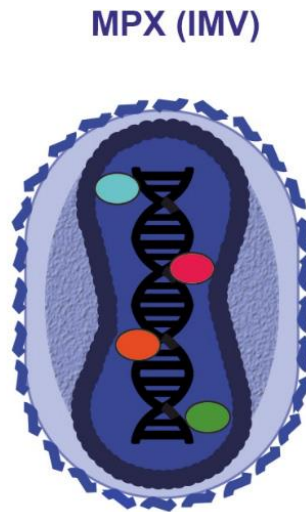
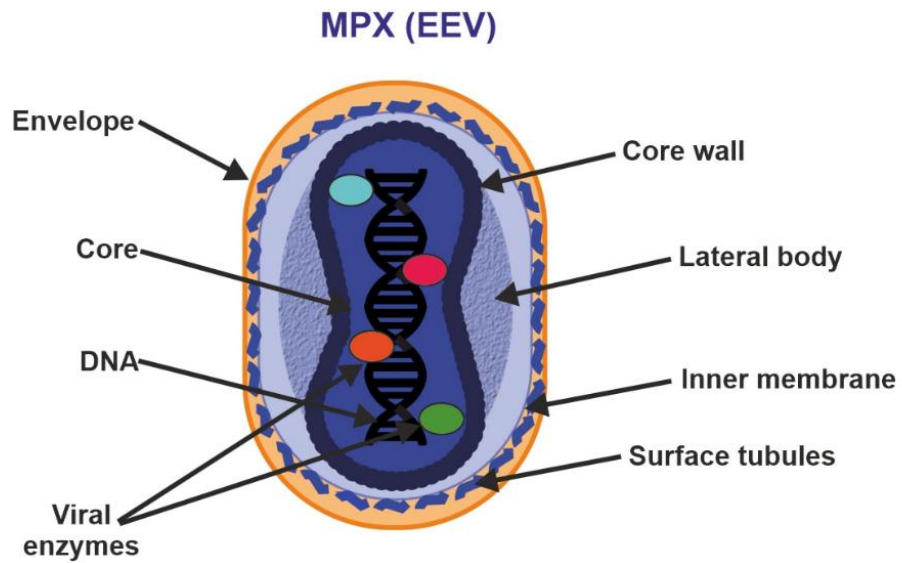


Mpox virus

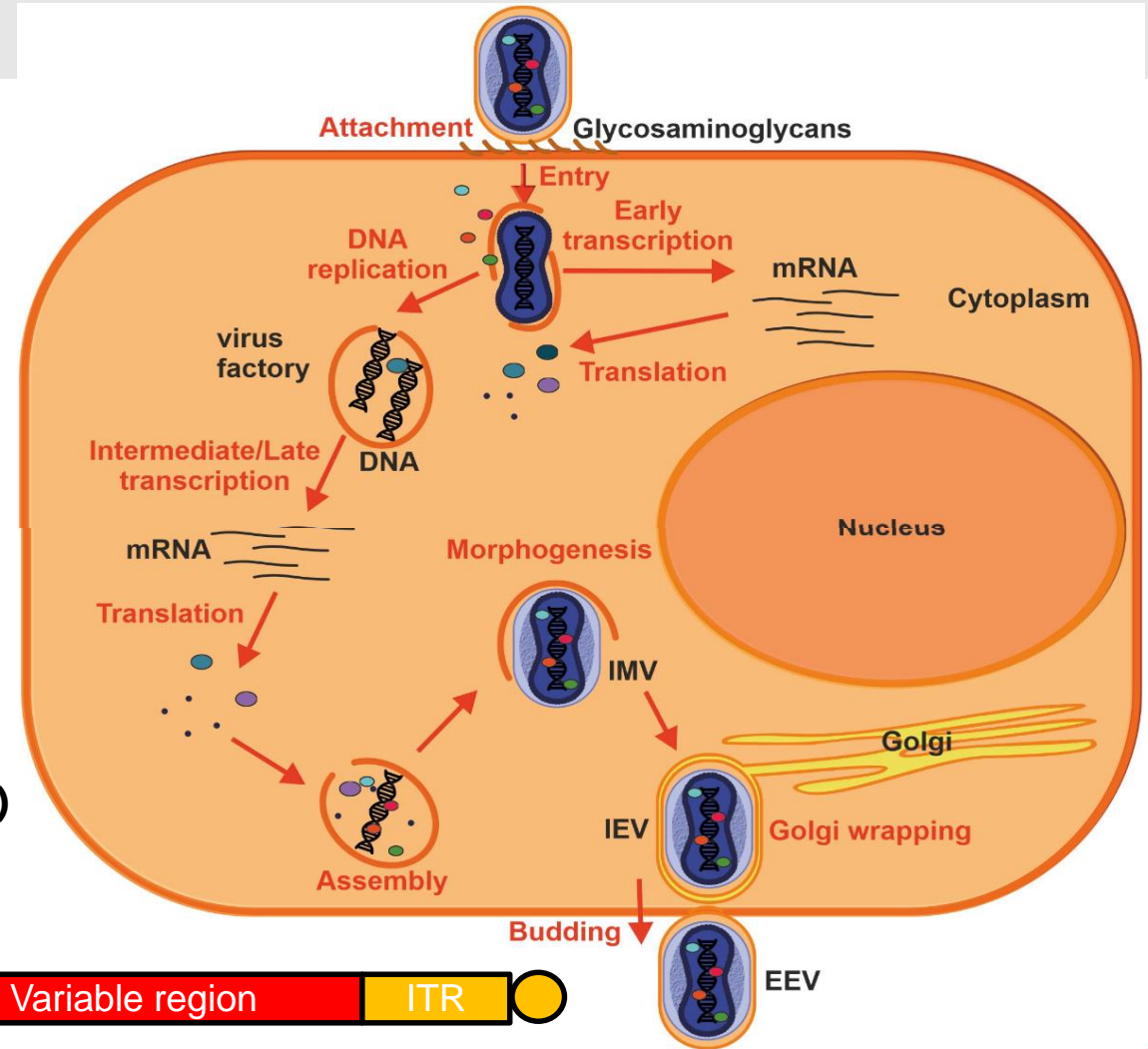
- first identified 1958 from macaques; first human case detected in 1970 in Democratic Republic of Congo
- also detected in squirrels, pigs, and shrews
- enveloped dsDNA virus belonging to the orthopoxviruses
- 197 kbp genome encoding 190 ORFs; sequence identity to Variola virus (smallpox) ~96 %
- 3 known clades: Clade 1 endemic in central Africa with a mortality rate of >10 %; Clade 2 and 3 endemic in West Africa with a mortality rate of <1 %
- treatment of orthopoxviruses with Cidofovir and Tecovirimat (ST-246) whereas upcoming resistance is likely



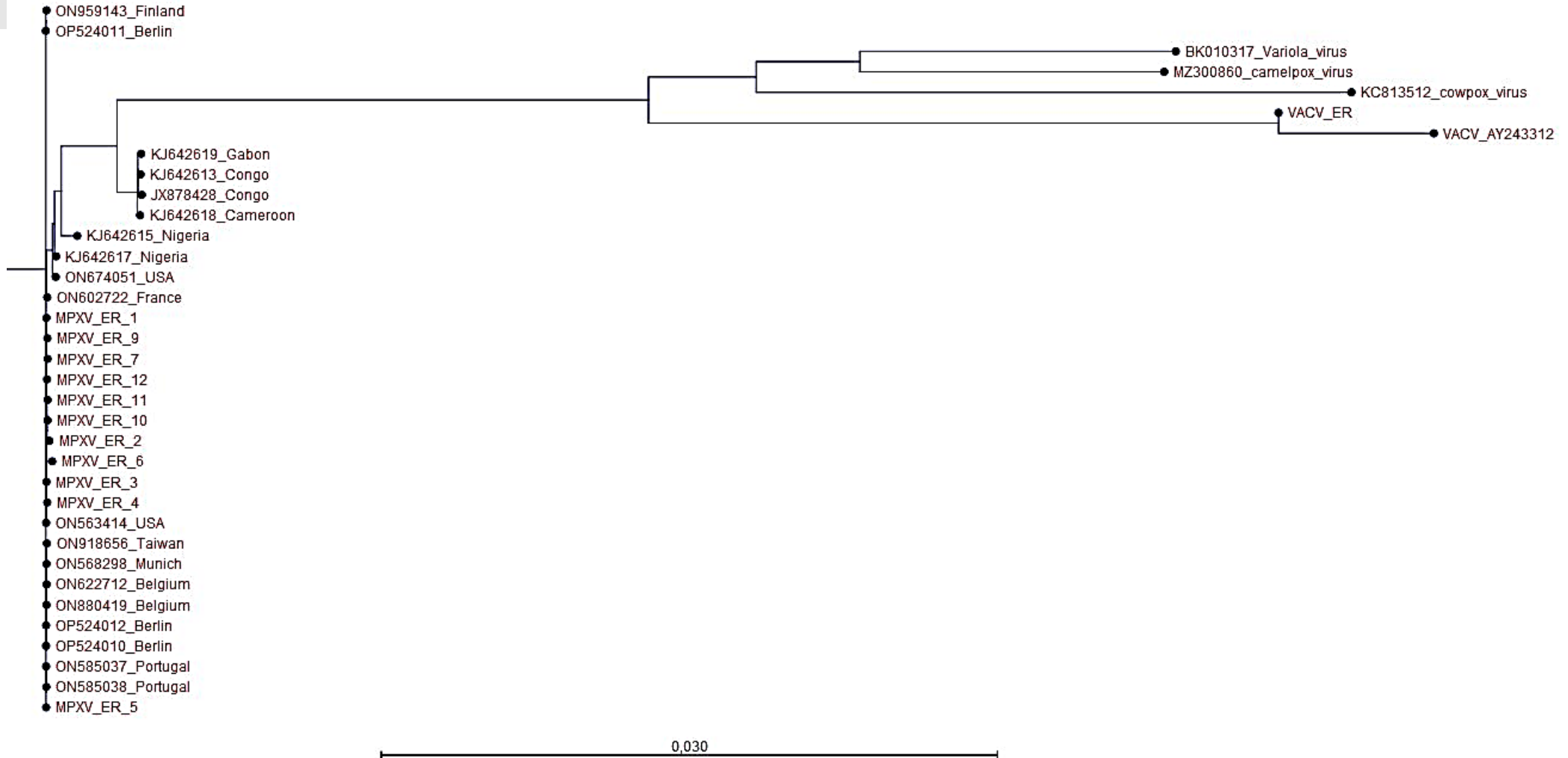
Mpox virus replication cycle



Kmieciak & Kirchhoff (2022)



Phylogenetic relationships



Mutational profile of Mpox virus outbreak in Franconia, Bavaria

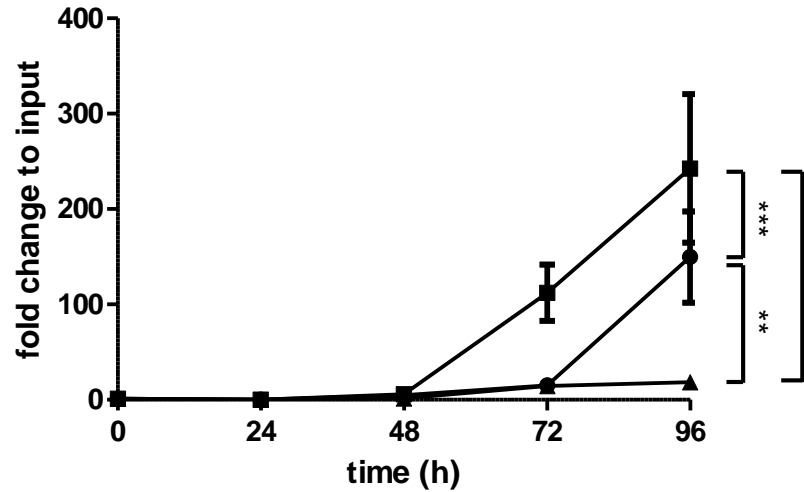
Sample	Gene (new)	Gene (old)	Mutation	Frequency [%]	Coverage
AP2	OPG066	F4L	S237L	100	27
AP3	OPG025	D9L	D539N	100	63
	OPG176	A47R	D190N	100	67
AP4	OPG038	M2L	E141K	100	119
	OPG093	G9R	R194H	100	109
	OPG116	E4R	E196K	100	129
	OPG152	A27L	S291F	100	197
AP5	OPG066	F4L	S237L	100	64
AP7	OPG093	G9R	R194H	100	147
AP10	OPG025	D9L	D71N	100	25
	OPG085	G1L	S424L	100	44
AP11	OPG037	O1L	G351S	100	147
	OPG204	B16R	D188N	100	28
AP12	OPG133	A8L	M63I	100	23
	OPG133	A8L	D29N	100	48
	OPG016	N3R	M51L	78,5	56

→ The analyzed MPXV genomes show unique mutations

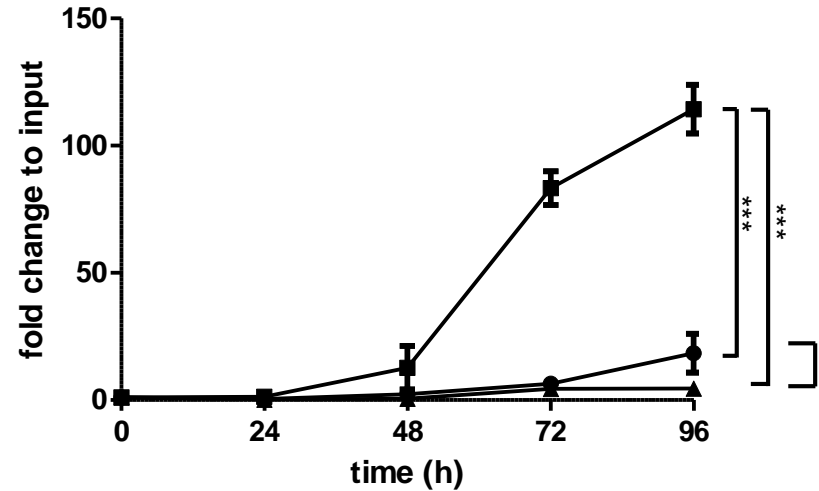


Replication of three different Mpox isolates

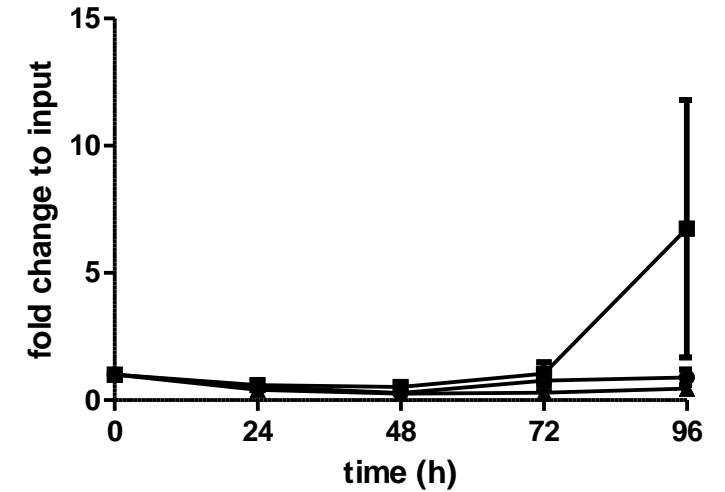
Vero E6 cells



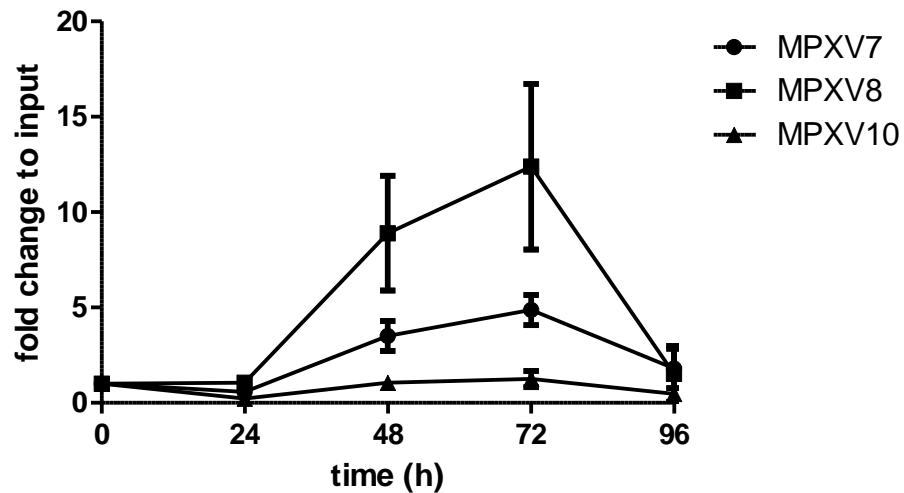
HEK293T cells



CaCo-2 cells

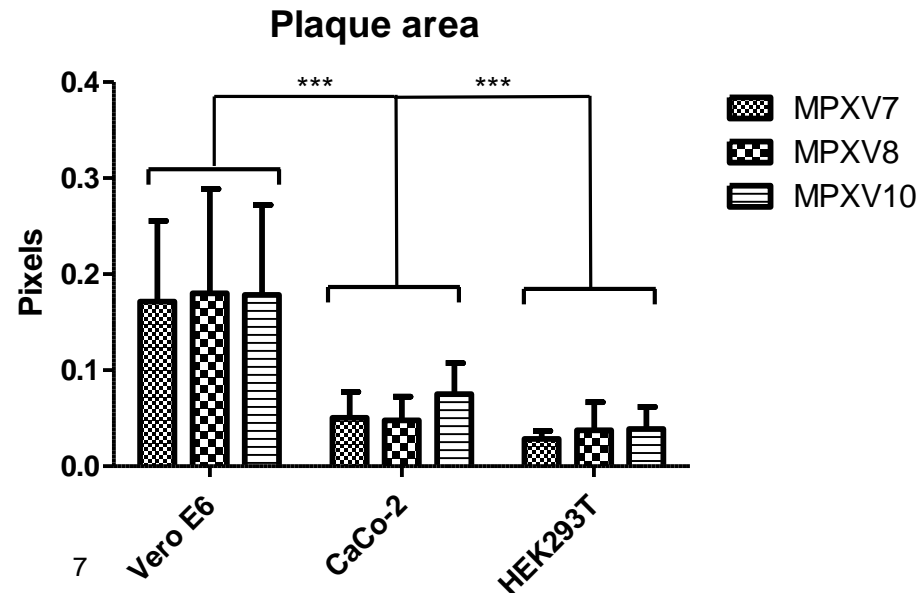
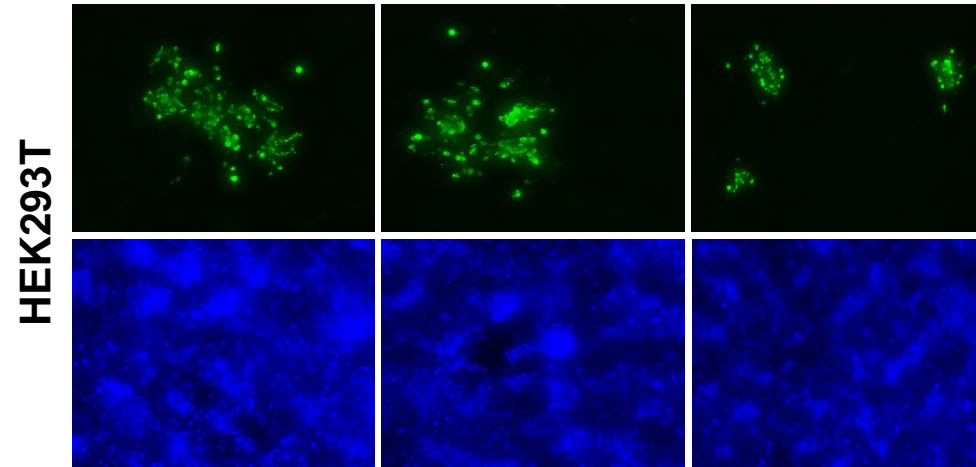
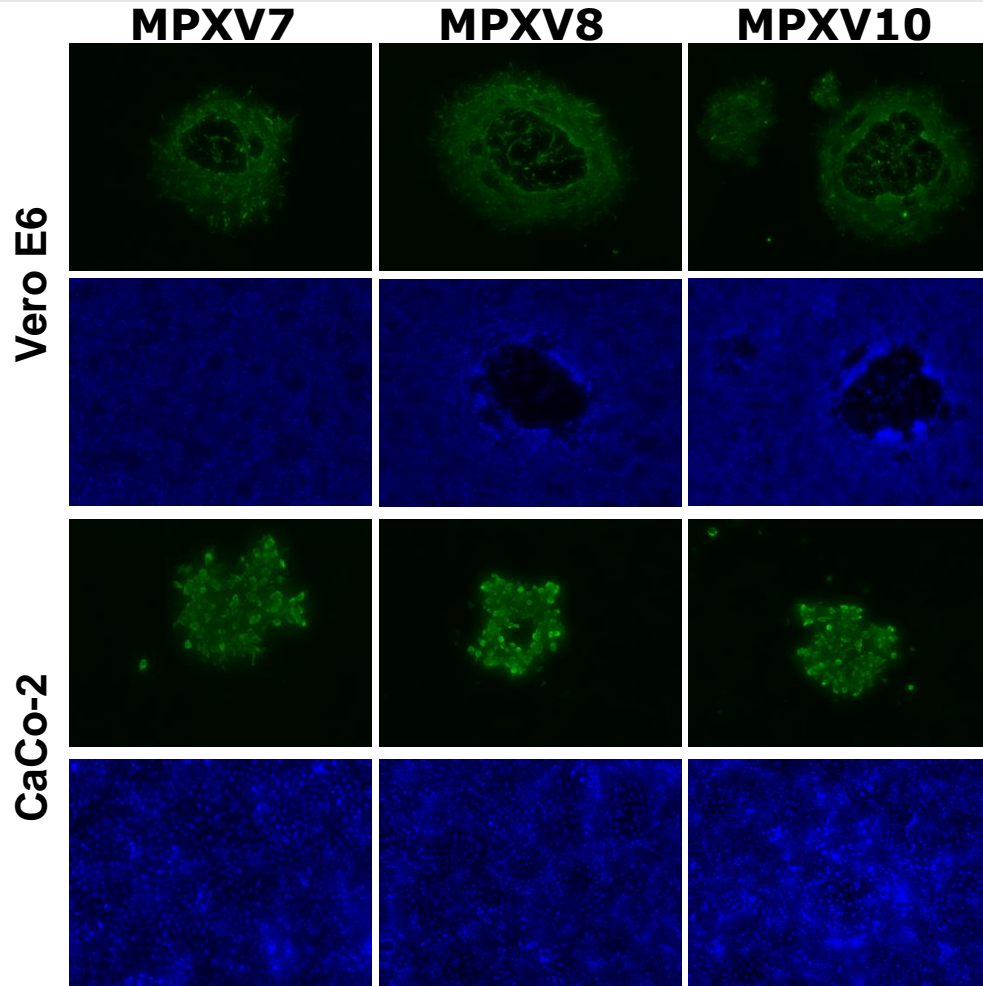


CaCo-2 cells cells



→ **MPXV8 replicates faster and to higher viral loads**

Plaque morphology and size of three different Mpox isolates



→ MPXV causes larger plaques in Vero E6 cells



Genomic analysis of our Mpox isolates

Sample	Gene (new)	Gene (old)	Mutation	Frequency [%]	Coverage
MPXV7	OPG038	O2L	E141K	100	147
	OPG094	G10R	R194H	100	147
	OPG153	A28L	D385 duplication	56	121
MPXV8	intergenic	intergenic	non-coding	100	19
	OPG089	G5R	synonymous	100	28
	OPG136	A11L	P616L	100	23
	intergenic	intergenic	non-coding	55	18
MPXV10	OPG038	O2L	E141K	100	199
	OPG079	I3L	synonymous	100	191
	OPG094	G10R	R194H	100	198
	OPG153	A28L	D385 duplication	59,7	144

- MPXV isolates show non-synonymous mutations
- MPXV8 differs from the other isolates

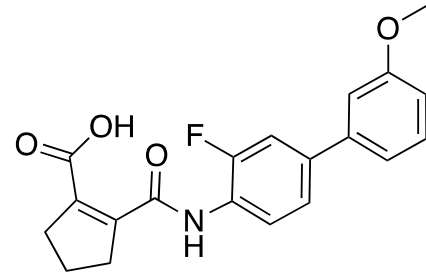


DHODH inhibitors

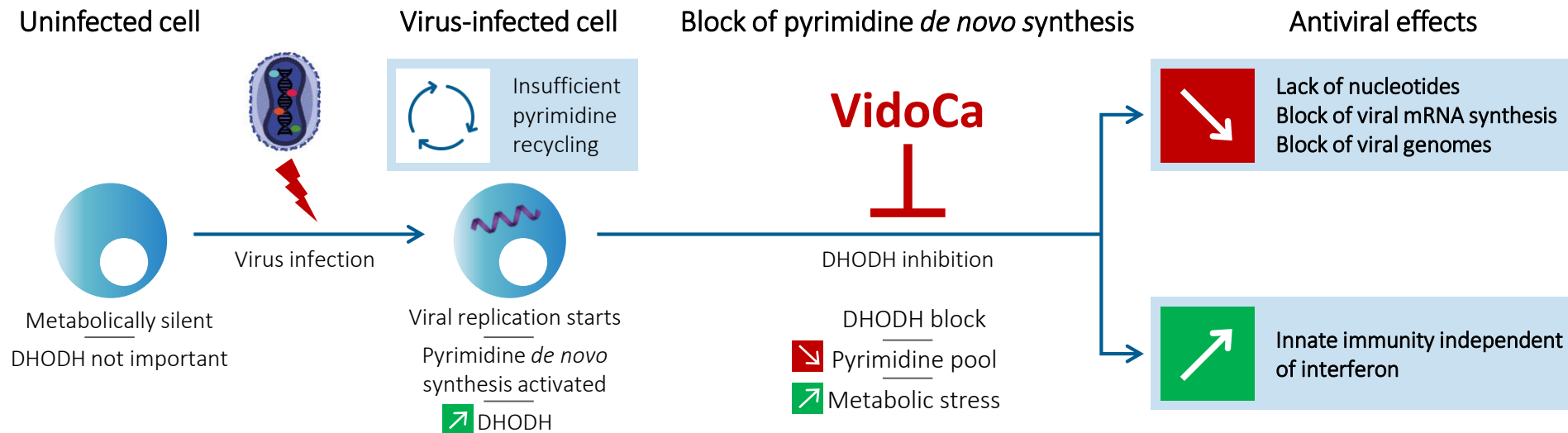
- Dihydroorotate dehydrogenase (DHODH) is involved in *de novo* pyrimidine synthesis
- Depletion leads to stimulation of ISG expression
- Critical in cells with higher requirement for nucleotides



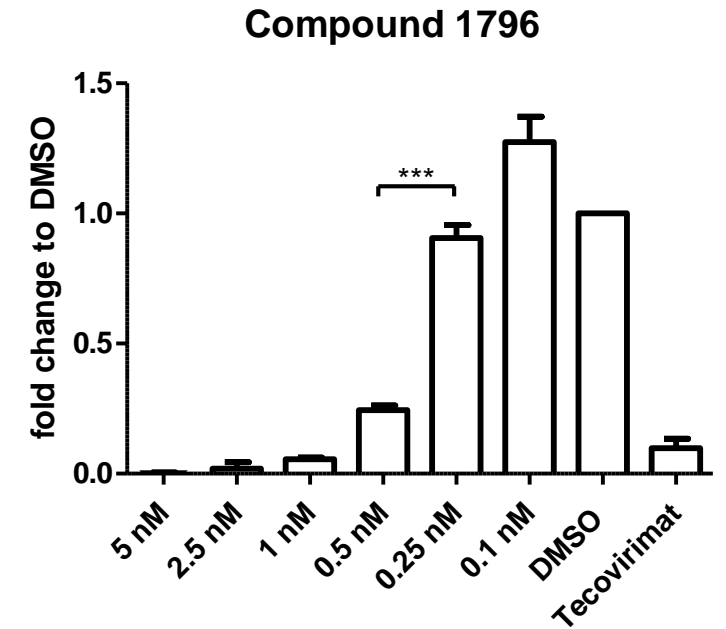
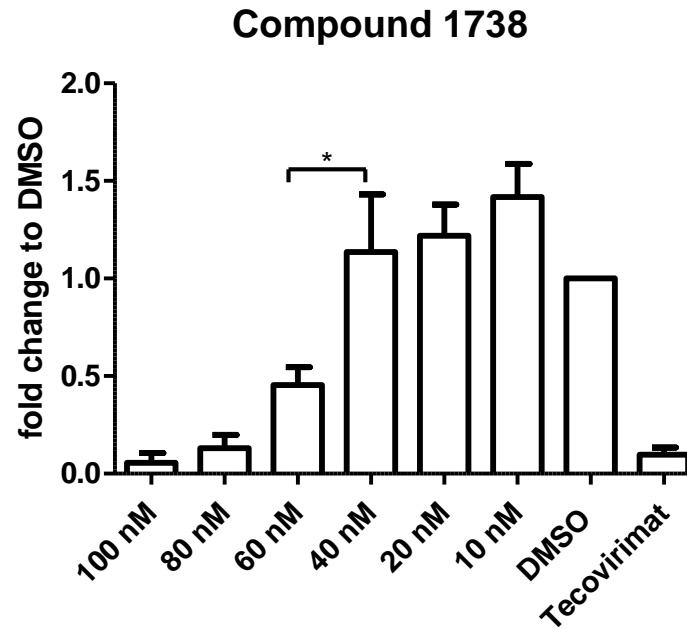
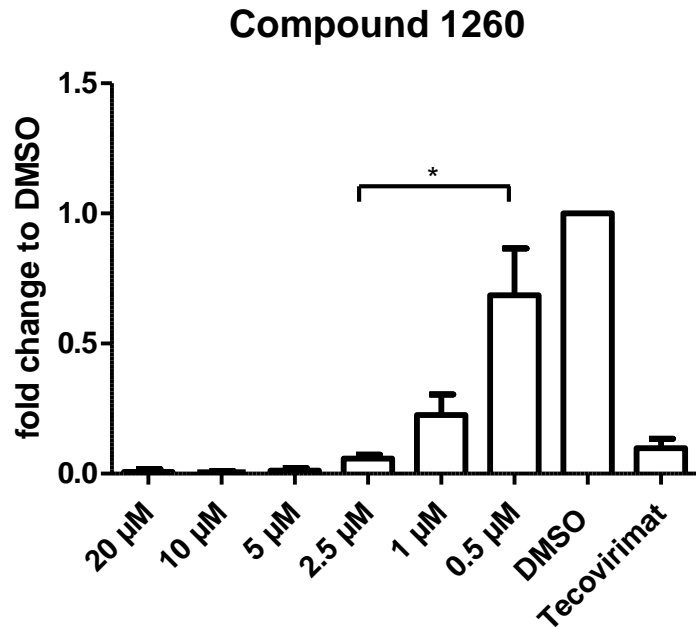
DHODH inhibitors – mode of action



IMU-838/Vidofludimus Calcium (VidoCa)



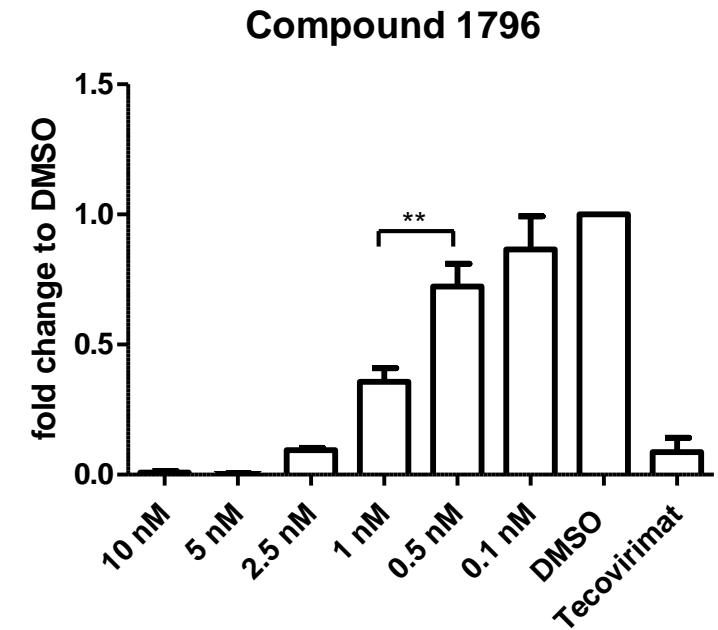
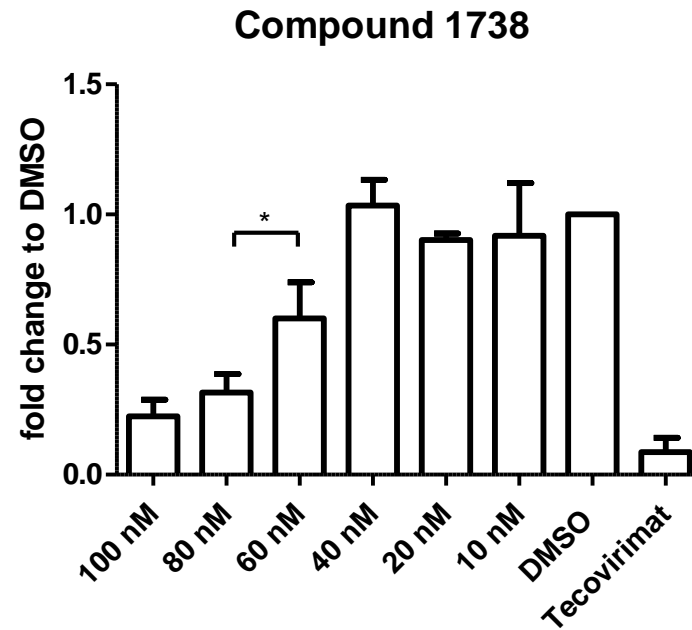
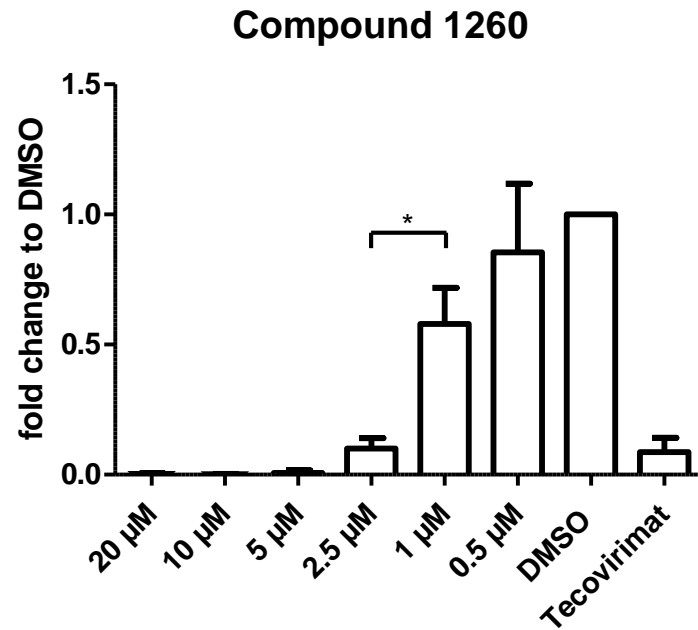
Antiviral activity against Mpox virus



Compound	EC50 [nM]	CC50 [µM]
1260	649.3	170.9
1738	58.2	>200
1796	0.38	94.2

→ DHODH inhibitors are active against MPXV

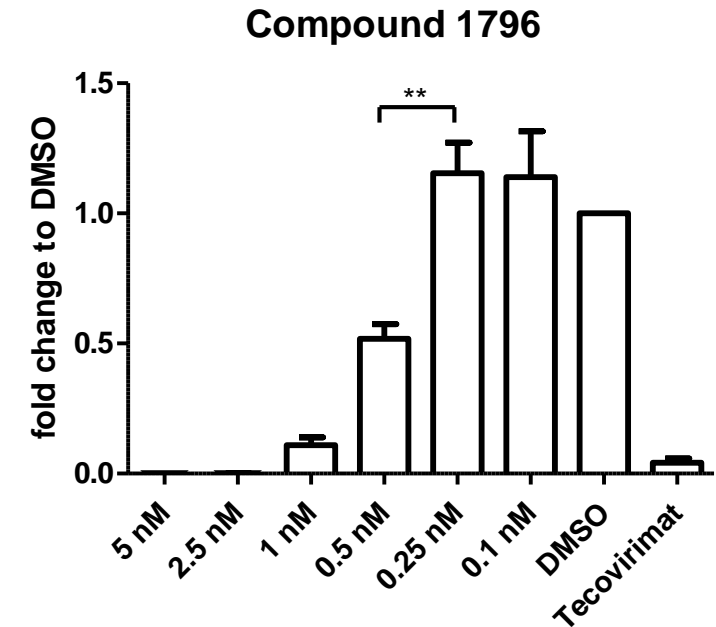
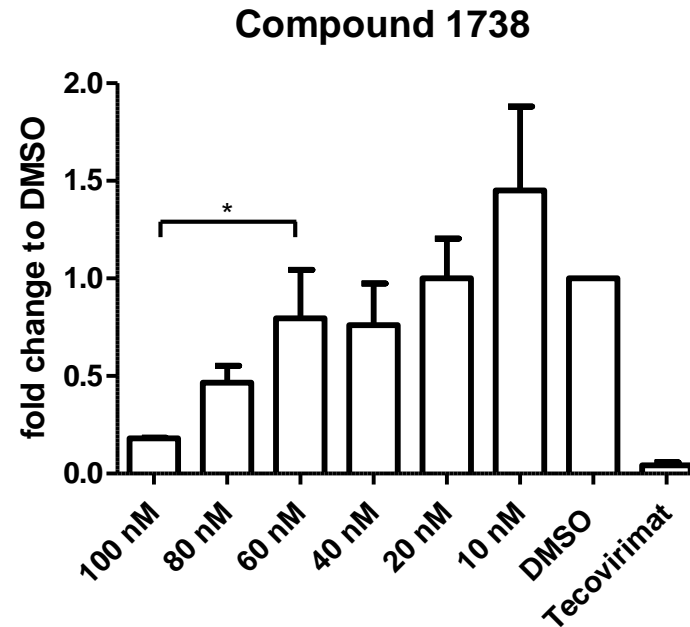
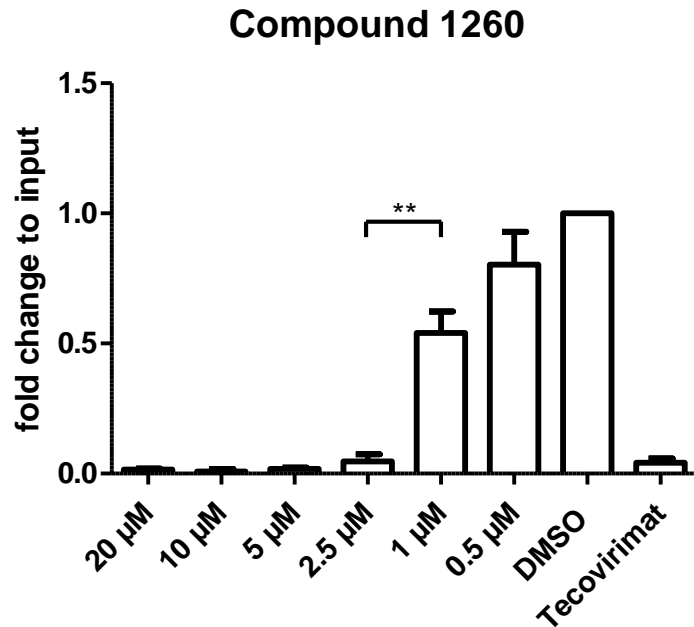
Antiviral activity against *Vaccinia virus*



Compound	EC50 [nM]	CC50 [µM]
1260	1150.4	170.9
1738	65.7	>200
1796	0.38	94.2

→ DHODH inhibitors are active against VACV

Antiviral activity against Cowpox virus



Compound	EC50 [nM]	CC50 [µM]
1260	1065.1	170.9
1738	77.2	>200
1796	0.52	94.2

→ DHODH inhibitors are active against CPXV

Summary

- Franconian MPXV genomes of the outbreak are highly related, but also show unique mutations
- MPXV8 isolate replicates faster and to higher viral loads and has a specific non-synonymous mutation in comparison to the other isolates
- The tested DHODH inhibitors are active against orthopoxvirus infection at low nanomolar concentrations



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