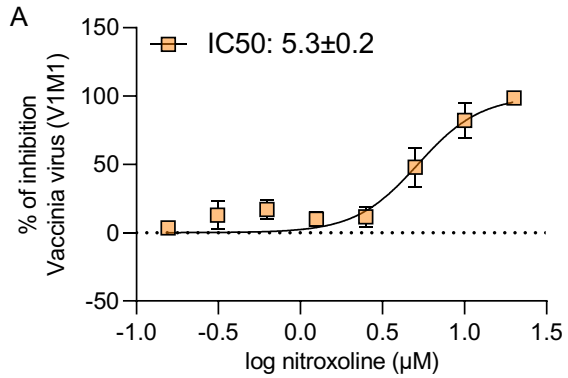
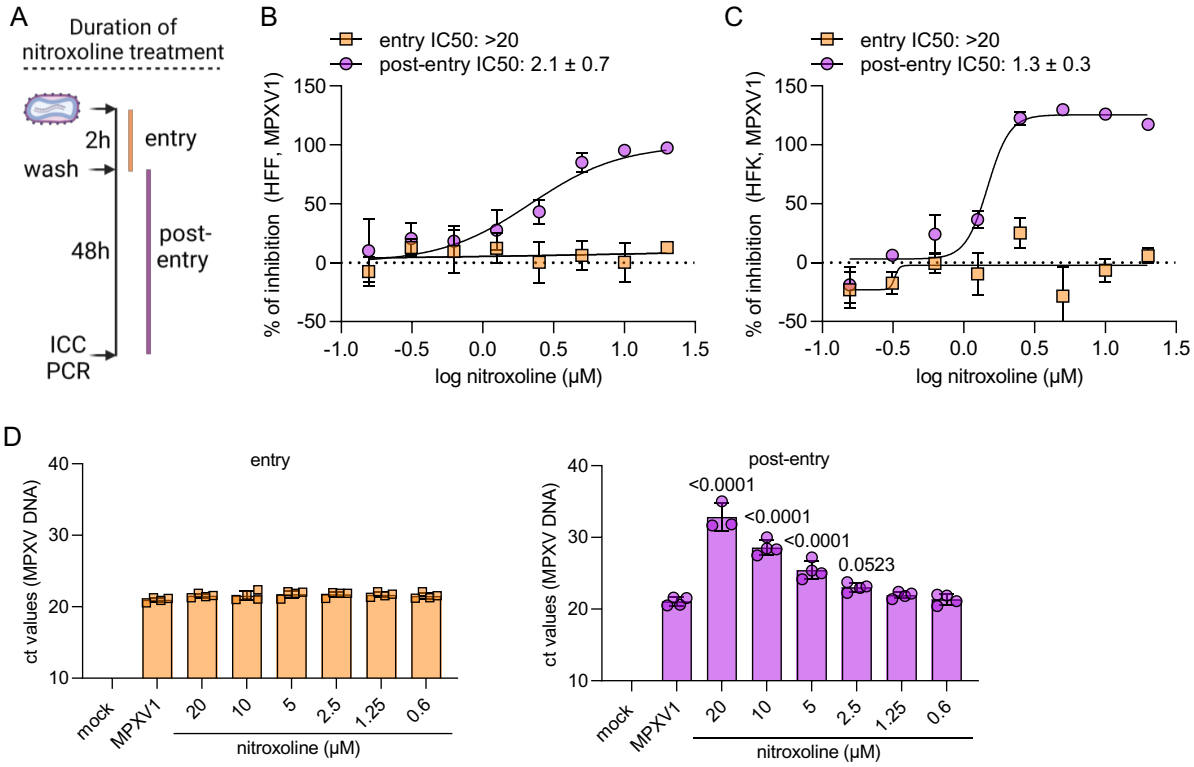
Suppl Fig 1



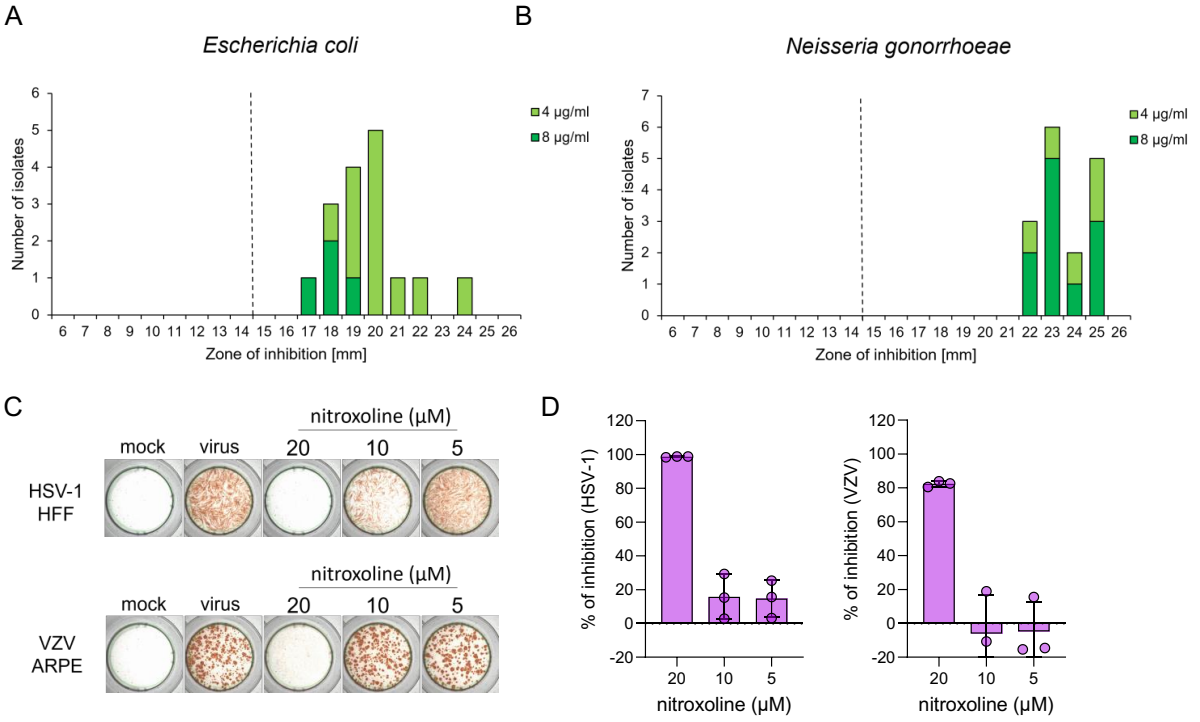
Suppl. Figure 1. Effects of nitroxoline on vaccinia virus (V1M1) replication in primary human fibroblasts (HFF). Concentration-dependent effects of nitroxoline on V1M1 virus infection in HFF, as indicated by immunostaining. IC50 = concentration that inhibits V1M1 virus infection by 50% as indicated by immunostaining.

Suppl Fig 2



Suppl. Figure 2. Effects of nitroxoline on mpox virus (MPVX) replication in primary human fibroblasts (HFF) and keratinocytes (HFK). A) Design of time-of-addition experiments. B-D) Effects of nitroxoline on mpox virus (MPXV1) infection and replication in HFFs when exclusively administered during (entry) or after (post-entry) the 2h virus adsorption period as indicated by immunostaining (B,C) or qPCR for genomic mpox virus DNA (D).

Suppl Figure 3



Suppl. Figure 3. Effects of nitroxoline on pathogens that are commonly transmitted together with mpox virus. Antimicrobial susceptibility to nitroxoline was determined by disc diffusion for *Escherichia coli (A)* and *Neisseria gonorrhoeae* (B). C,D) Effects of nitroxoline against herpes simplex virus type 1 (HSV-1) and Varicella zoster virus (VZV). Effects on HSV-1 were determined in McIntyre strain (MOI 0.01)-infected human foreskin fibroblasts by immunostaining 24h post infection. Effects on VZV were detected in ARPE cells infected with a clinical isolate [Schmidt-Chanasit et al., 2008] at MOI 0.1 by immunostaining 48h post infection.

Suppl. Table 1. Nitroxoline concentrations that inhibit infection of primary human foreskin fibroblasts (HFF) and keratinocytes (HFK) with clinical mpox virus (MPXV) isolates (MOI 0.01) by 50% (IC50) as indicated by immunostaining 48h post infection.

Mpox virus isolate	HFF[1]	HFK[1]
MPXV1	4.19 ± 1.24	0.68 ± 0.12
MPXV2	4.60 ± 0.12	0.69 ± 0.09
MPXV3	3.75 ± 0.51	0.50 ± 0.25
MPXV4	3.74 ± 0.71	0.91 ± 0.06
MPXV5	3.70 ± 0.05	0.85 ± 0.03
MPXV6	3.43 ± 0.74	0.96 ± 0.09
MPXV7	3.09 ± 0.81	0.69 ± 0.05
MPXV8	3.64 ± 0.39	0.76 ± 0.04
MPXV9	4.56 ± 1.28	0.52 ± 0.03
MPXV10	2.49 ± 0.67	0.50 ± 0.08
MPXV11	4.53 ± 0.59	1.55 ± 0.01
MPXV12	2.90 ± 0.61	1.07 ± 0.13

[1] mean ± S.D.

Suppl. Table 2. Amino acid substitutions in the tecovirimat-adapted MPXV1 substrain MPXV1^{r}TECO as determined by complete virus genome sequencing relative to a reference genome (ON563414.2). Sequence changes in MPXV1^{r}TECO relative to MPXV1 are highlighted in bold.

ID Frankfurt	Total reads	Reads mapped against MPX [a]	Mean genome coverage	Coding sequence coverage with >10 reads	Amino acid substitutions (compared to outbreak reference sequence [a])
MPXV1	10,096,514	3,247,443	2470.9	100% [b]	gp10: 913 nt deletion (affecting AA221-525, new stop codon); gp157: D4N
MPXV1^{r}TECO	16,421,220	5,874,779	4462.0	100% [b]	gp10: 913 nt deletion (affecting AA221-525, new stop codon); gp44: **D549N**; gp45: **N267D**, **I372N**; gp157: D4N; **gp161:S306L**

[a] ON563414.2 - Monkeypox virus isolate MPXV_USA_2022_MA001, 2022

[b] 913 nt deletion in gp010

Suppl. Table 3. Effects of nitroxoline on *Escherichia coli* determined using clinical isolates and the reference strain ATCC 25922 by disc diffusion and agar dilution assay.

Method		Disc diffusion	Agar dilution	Broth microdilution
		Inhibition zone (mm)	MIC (μM)	MIC (μM)
E.coli (isolates)	2652	19	21	21
	2966	22	21	21
	3513	20	21	21
	3719	20	21	21
	3726	17	42	42
	4041	20	21	21
	4559	20	21	21
	4954	18	42	21
	5173	17	42	42
	5301	19	21	21
	5752	19	21	21
	5762	18	21	21
	5763	16	42	42
	7975	21	21	21
	8667	20	21	21
	ATCC 25922	24	21	10.5

Suppl. Table 4. Effects of nitroxoline on *Neisseria gonorrhoeae* determined using clinical isolates and the reference strain ATCC ATCC49226 by disc diffusion and agar dilution assay.

Method		Disc diffusion	Agar dilution
		Inhibition zone (mm)	MIC [µM]
N. gonorrhoeae (isolate)	428	25	42
	429	24	42
	432	22	42
	436	25	42
	439	23	42
	440	25	42
	441	23	42
	442	23	42
	444	23	42
	520	25	21
	550	23	42
	570	23	21
	600	25	21
	690	24	21
	740	22	21
	ATCC49226	22	42

Reference

Schmidt-Chanasit J, Bleymehl K, Rabenau HF, Ulrich RG, Cinatl J Jr, Doerr HW. In vitro replication of varicella-zoster virus in human retinal pigment epithelial cells. J Clin Microbiol. 2008 Jun;46(6):2122-4. doi: 10.1128/JCM.00122-08.