

Supplementary Information for

Partial Inhibition of Viral Replication Machinery Enhances Recombination in Herpes Simplex Viruses

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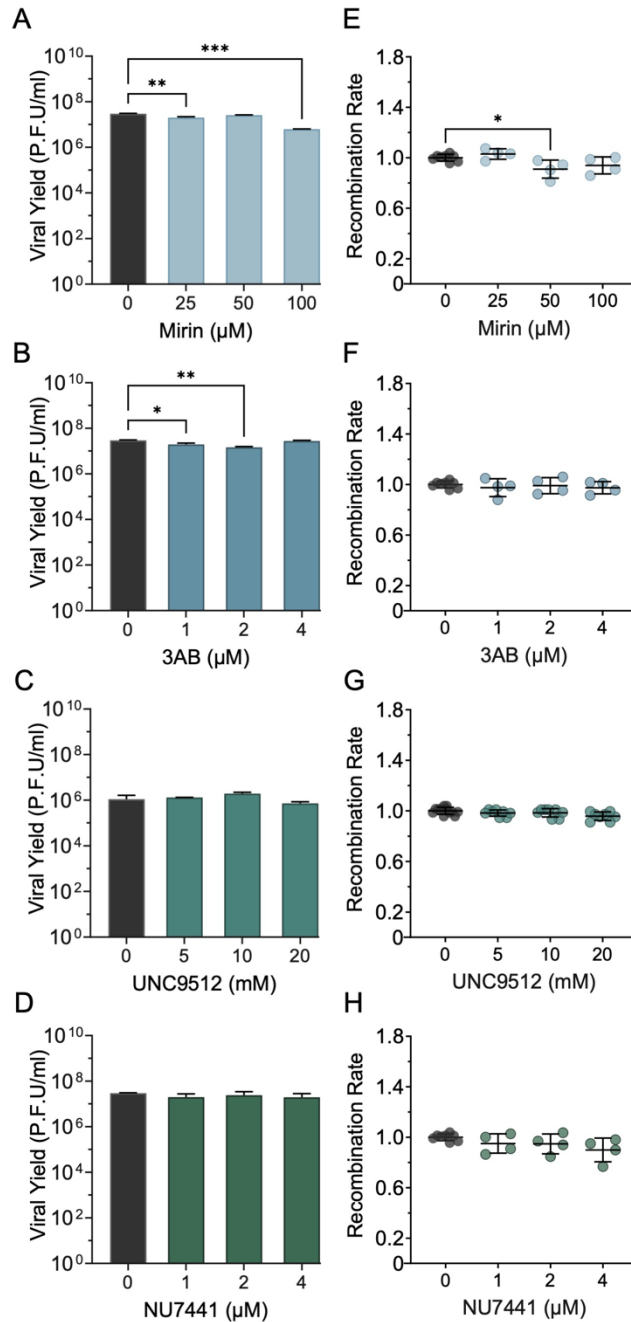


Figure S1. Limited effect of host factors on viral yield and recombination rates.

HFF cells were infected at MOI 20 in the presence of indicated host factor inhibitor:

A,E. Mirin, **B,F.** 3AB, **C,G** UNC9512, and **D,H.** NU7441.

A-D. Viral yield in the presence of indicated host factors. Bars represent the titer mean from one experiment with two biological replicates and four technical replicates each. Error bars indicate standard deviation. **E-H.** Normalized HMR rates for specific inhibitors of host factors. Each circle represents recombination rates normalized to corresponding experiment control mean. Data represent at least two independent experiments with at least two replicates each. Horizontal line represents the mean, and error bars indicate standard deviation. Statistical significance was determined by ordinary one-way ANOVA (* < 0.05 , ** <0.01 and *** <0.001).

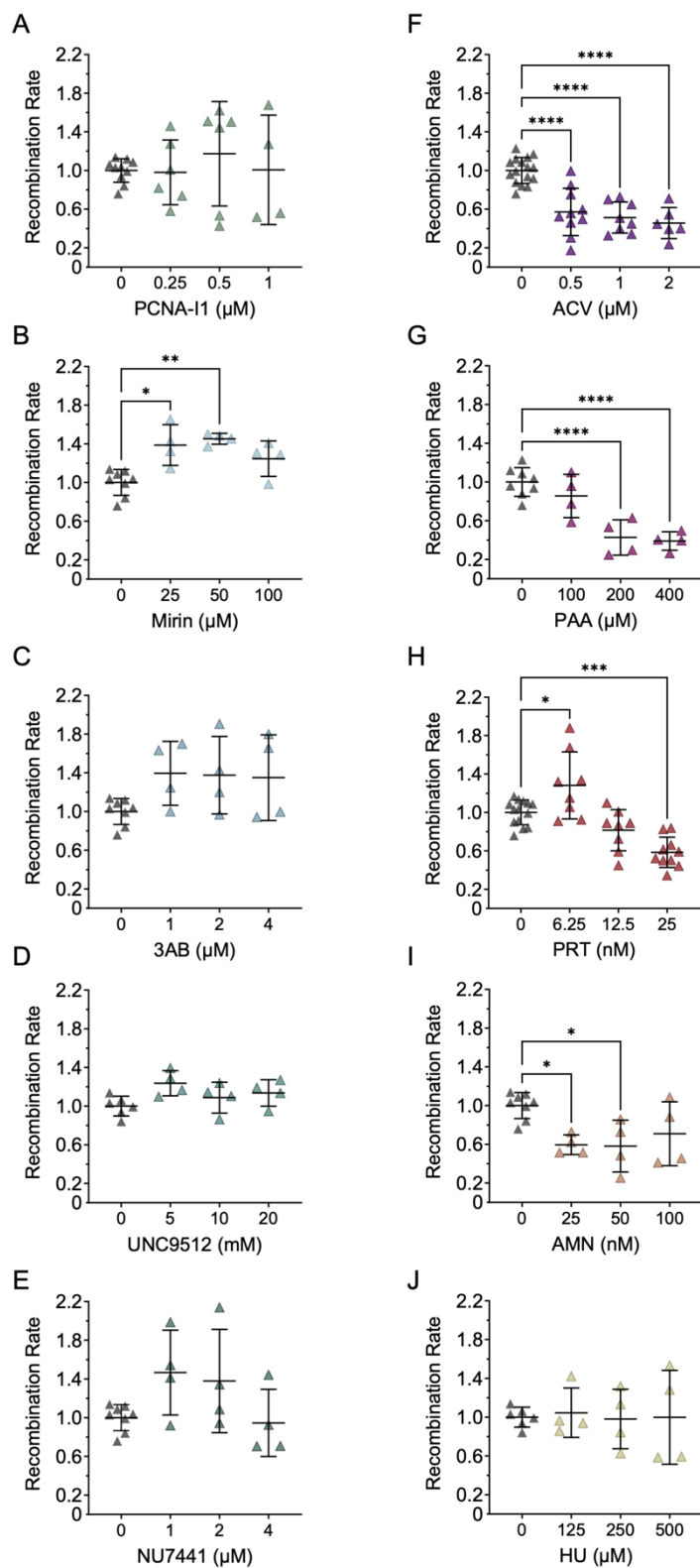


Figure S2. Effect of viral polymerase inhibitors on viral non-canonical recombination. HFF cells were infected at MOI 20 in the presence of inhibitors of viral replication. Normalized NCR rates for specific inhibitors of viral DNA replication: **A.** PCNA-I1, **B.** Mirin, **C.** 3AB, **D.** UNC9512, **E.** NU7441, **F.** Acyclovir, **G.** Phosphonoacetic acid, **H.** Pritelivir, **I.** Amenamevir and **J.** Hydroxyurea. Each triangle represents NCR rates normalized to corresponding experiment control mean. Data represent at least two independent experiments with at least two replicates each. Statistical significance was determined by ordinary one-way ANOVA (* <0.05 , ** <0.01 , *** <0.001 and **** <0.0001). Error bars indicate standard deviation, and horizontal line represents the mean.

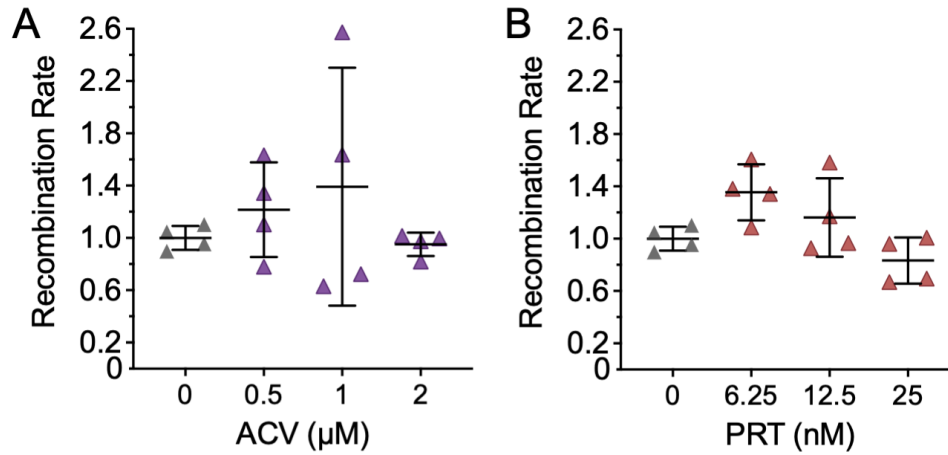


Figure S3. Limited effect of viral polymerase inhibitors on non-canonical recombination in RPE-1 cells. RPE-1 cells were infected at MOI 20 in the presence of inhibitors of viral replication. Normalized NCR rates for A. Acyclovir and B. Pritelivir respectively. Triangles represent repeats of NCR rates normalized to corresponding experiment control mean. Data represent two independent experiments on RPE-1 cells with two replicates each. Statistical significance was determined by ordinary one-way ANOVA. Horizontal line represents the mean, and error bars indicate standard deviation.

Table S1. List of HSV-1 Strain 17 primers for PCR Assays

Gene	Primer Location* (Product Size)	Forward Primer	Reverse Primer
UL1	9600-9733 (134)	CCCAGAAGGCATATTGGGTTA	CGCCTGGCGTATCTCTTTAT
UL5	13368-13501 (134)	GGCGCAGAGACAGAATCTC	CCTGTCGCGGCTCATATTTA
UL9	21430-21564 (135)	GGTCATAGATCCGCCGAAAT	AAATTGTCGCGCTCATGC
UL13	28004-28141 (137)	GTTGGACGTCACCGTATCC	GCACATCCTCACCTCAC
UL18	35840-35971 (132)	GAAGCGCCTTCGGTACG	GAAGGGCGGGTGGTATTC
UL21	42696-42827 (132)	CGCACGGATGTGGTTATCA	CACGCGGTCAATGTGTTTG
UL26	51801-51936 (136)	CCTCCCATTAACAACCAGCTC	GGAGGGTAATGCTGGGAAAG
UL29	61458-61591 (134)	CAACGGGTACACGGGTATG	CCCTCCTGTATCTGGTCGTTA
UL35	70643-70775 (133)	CTTGGCCACCAATAACTCTCAG	GTTTGCGTGGGCCAGAC
UL37	81179-81311 (133)	GCATGCGCCATTACCTCT	CCGTGGCCGAGTTTGTC
UL40	90052-90187 (136)	GTCCCGACATTAACCACCTG	GCGAAGAGGAAGCGGTAAA
UL44	96951-97087 (137)	GACCCTCCGTTGTATTCTGTC	ACCCACGTCCCGTACTC
UL48	104619-104753 (135)	AGAGAGCGCTTCGTAGTAGA	AGCGATGTGGTGGAAATGG
UL54	114960-115093 (134)	GCCTGGACGAACTGTGTT	ACCAAGGGTCGCGTAGT
RL2	3376-3511 122863-122996 (135)	CGTGTGCACGGATGAGAT	TACACCAGCTTGCGGTTG
RS1	127173-127305 150892-151024 (133)	TCAGGTTGTTGCCGTTTATTG	AAGTTGTGGACTGGGAAGG
US1	132786-132922 (136)	AGCCTTGAGTCTGAGGT	CGCCGACATGTCCAGAAA
US3	135308-135443 (136)	CCGGGTGTTTCCTCATGG	GATCTCCTGCAGATGACACAG
US6	138554-138686 (133)	CAAAGACCTTCCGGTCCTG	CTCCAACACGGCGTAGTAAA
US12	144273-144407 (135)	ACAGCACCAGAAAGCACA	CGGGCCAACCAAGAGAC

*- According to NCBI sequence: NC_001806.2

Table S2. List of HSV-2 strain G primers for PCR Assays

Gene	Primer Location* (Product Size)	Forward Primer	Reverse Primer
UL1	9959-10049 (91)	ACTTTGACTACTCACGCACTC	GACGACACAGGCGGTTC
UL5	13716-13813 (97)	AACTCCACCGAGGTCTCC	AGATGGTGTTCGGGTTTCGAC
UL9	21866-21959 (93)	ACGTGCATGCAGTTGAGG	GTACAAGCACTACTTCGAGAG G
UL13	28133-28231 (98)	GGTTGACGGCGAATACGG	GTCCGAGACGGCCATGA
UL18	36049-36141 (93)	AGAAAGACCACTCGACCCT	GGATTGTTTCGAAGCAGACAT C
UL21	42629-42723 (95)	GAATGCCTGGCCGAATACT	AGCTCGATGACCCTGTCT
UL26	51921-52011 (90)	TGCCATGGACACATCCC	AAAGAAGAAGACGACGACGA
UL29	61938-62033 (96)	GATGGTTACCTTGTCATCCC	GCCTTGCTGTATCTGGTGAT
UL35	71302-71394 (93)	GTGACCCACGCCAACAA	GCGCTTAAGACCGAACGA
UL37	81693-81785 (93)	TGATCGGCCGAGCTGAC	GGCCGAGTTCGTTTCAGGA
UL40	90729-90822 (94)	CTTTCTGTTTGCCTTCCTGTC	GTGAAGAATGTCTTCTGTTC G
UL44	97325-97417 (93)	GAGTCCCGCCTCCAGAT	CGACACGTTTACCATCACCTC
UL48	105481-105572 (92)	TCGGGAACCCAGAGAACA	CCGTCTCCTCGACGATCT
UL54	115630-115725 (96)	CTCTACCGCACATTCGCA	CAGGGCCTCGATGAGATTT
RL2	3020-3112 124443-124351 (93)	GATGCTCAACGACATGCAG	GTGGAGTCGCGGTGAAG
RS1	128295–128387 154224-154316 (93)	CAGACGCCCCGATTCCTTC	ACGACGACGGACTGTTTG
US1	134570-134663 (94)	ATCTGTGCGCAGAATCGAC	TCAACCAACCAGCCGTT
US3	137070-137163 (94)	CGGAAACCATCCACCCTT	TCGTCGCTGTGCATCTG
US6	141941-142033 (93)	CCCGAAAGCACACGTACAA	GGCACTCGGTGTATTCCATAA
US12	147469-147563 (95)	CCGAAGCCATTGGGTATGAA	GCCTGTGTGCTCTCAACA

*- According to NCBI sequence: OM370995.1

Table S3. Amount of plaques analyzed by Plaquer, a deep learning-based model. The Amount of total, HMR and NCR plaques analyzed for each condition for the experiments presented in the main text are listed.

Figure	Treatment	Total plaques	HMR plaques	NCR plaques
2 MOI	0.2	12222	1293	156
	2	7395	1433	111
	20	17456	4236	316
3B PCNA-I1 μ M	0	18630	4540	415
	0.25	11062	3099	237
	0.5	18943	4851	539
	1	6590	1591	167
4A ACV μ M	0	31893	7427	965
	0.5	15602	4525	291
	1	24304	7440	282
	2	8753	2556	105
4B PAA μ M	0	16719	3780	541
	100	7733	2300	178
	200	5772	1709	69
	400	8796	2418	112
4C PRT nM	0	28051	6736	669
	6.26	18922	4991	656
	12.5	28649	8380	614
	25	21602	6428	317
4D AMN nM	0	14793	3550	338
	25	6422	2021	93
	50	5461	1708	71
	100	8098	2285	119
4F HU μ M	0	10326	2600	237
	125	8272	2364	189
	250	7939	2313	164
	500	9927	2850	210
5A ACV μ M	0	7873	1795	135
	0.5	8079	2340	169
	1	10838	3307	190
	2	7483	2315	117
5B PRT nM	0	7873	1795	135
	6.26	14049	3770	373
	12.5	16581	4950	334
	25	7200	2098	101