Supplementary Tables

Supplementary Table 1. Summary output PFU/ml

Psg	Osel (μM)	Favi (μM)	MOI	Output PFU/ml					
				Comb 1	Comb 2	Comb 3	Osel 1	Osel 2	Osel 3
3	0	0	0.01*						
4	0.1	1	0.01	2.0E+06	1.1E+07	1.1E+07	4.0E+06	3.0E+07	3.6E+07
5	0.2	2	0.01	4.0E+04	1.3E+06	7.0E+05	2.6E+06	3.2E+07	3.3E+07
6	0.4	4	0.01	1.0E+04	1.4E+06	1.1E+06	6.0E+06	8.0E+06	2.1E+07
7	0.8	8	0.01	2.8E+06	3.0E+05	1.0E+05	8.6E+06	4.0E+06	9.0E+06
8	1.6	16	0.01	8.0E+04	1.1E+05	2.8E+05	7.0E+06	2.0E+06	6.0E+06
9	3.2	32	0.01	2.0E+04	9.0E+04	3.0E+05	9.0E+05	3.0E+06	3.0E+06
10	6.4	64	0.01#	3.0E+02	8.0E+03	2.4E+04	6.0E+05	5.0E+06	7.0E+06

* MOI of 0.0103 in comb1, comb2, osel1 and osel2

MOI of 0.005 in comb1 and osel1

Supplementary Table 2. Summary information on the number of segregating sites exceeding 40% frequency

Replicate	Number of sites with DAF> 40%
Comb 1	14
Comb 2	14
Comb 3	24
Osel 1	6
Osel 2	8
Osel 3	5

Supplementary Table 3. Putatively neutral and deleterious mutations segregating with DAF >40% in the combined drug replicates

Seg	Position	Seg name	Ref base	Mut base	Ref AA	Mut AA	Type	AA number	SNP	Dataset
seg1	46	PB2	C	T	L	L	S	7	L7L	comb3
seg1	212	PB2	G	A	R	K	N	62	R62K	comb3
seg1	780	PB2	G	A	R	R	S	251	R251R	comb3
seg1	867	PB2	A	G	L	L	S	280	L280L	comb2
seg1	1650	PB2	C	T	G	G	S	541	G541G	comb3
seg1	2078	PB2	C	T	A	V	N	684	A684V	comb3
seg1	2132	PB2	G	A	R	K	N	702	R702K	comb3
seg2	730	PB1	C	T	L	L	S	236	L236L	comb3
seg2	1089	PB1	C	T	Y	Y	S	355	Y355Y	comb1
seg2	2058	PB1	C	T	S	S	S	678	S678S	comb3
seg3	323	PA	C	T	A	V	N	100	A100V	comb1
seg3	1431	PA	G	A	L	L	S	469	L469L	comb3
seg3	1854	PA	G	A	E	E	S	610	E610E	comb2
seg3	2175	PA	C	T					?	comb1
seg4	210	HA	C	T	L	L	S	60	L60L	comb1
seg4	767	HA	C	T	Y	Y	S	245	Y245Y	comb3
seg4	1261	HA	G	A	G	D	N	410	G410D	comb2
seg4	1332	HA	A	G	I	V	N	434	I434V	comb2
seg4	1551	HA	G	A	E	K	N	507	E507K	comb2
seg5	210	NP	G	A	R	R	S	55	R55R	comb1
seg5	815	NP	C	T	T	I	N	257	T257I	comb3
seg5	1023	NP	C	T	S	S	S	326	S326S	comb3

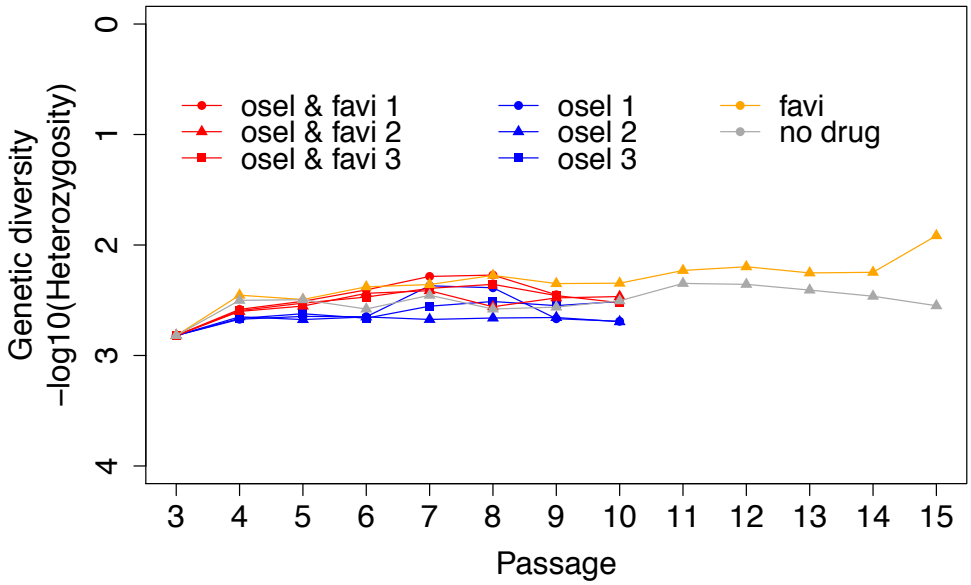
seg5	1327	NP	G	A	A	T	N	428	A428T	comb2
seg5	1369	NP	G	A	A	T	N	442	A442T	comb2
seg5	1500	NP	A	G	G	G	S	485	G485G	comb3
seg7	208	MP1	A	G	G	G	S	61	G61G	comb3
seg7	309	MP1	G	A	R	K	N	95	R95K	comb2
seg7	314	MP1	G	A	V	I	N	97	V97I	comb2
seg7	370	MP1	A	G	I	M	N	115	I115M	comb3
seg7	426	MP1	G	A	R	K	N	134	R134K	comb1
seg7	607	MP1	A	G	G	G	S	194	G194G	comb3
seg8	290	NS1	G	A	R	R	S	88	R88R	comb3
seg8	613	NS2	G	A	M	I	N	19	M19I	comb1
seg8	640	NS1	G	A	S	N	N	205	S205N	comb1

Supplementary Table 4. Matrix of pairwise correlations between mutations segregating in excess of 40% DAF

Pairwise correlations are shown in the attached excel spreadsheets, with the cluster groups that are derived using the hierarchical cluster analysis (Fig. 5, see methods for more details) highlighted in the corresponding colors. Results confirm high pairwise correlations within cluster groups containing NA H275Y, NA A454V, NA E128G and HA E78G, and lower pairwise correlations outside of the cluster groups.

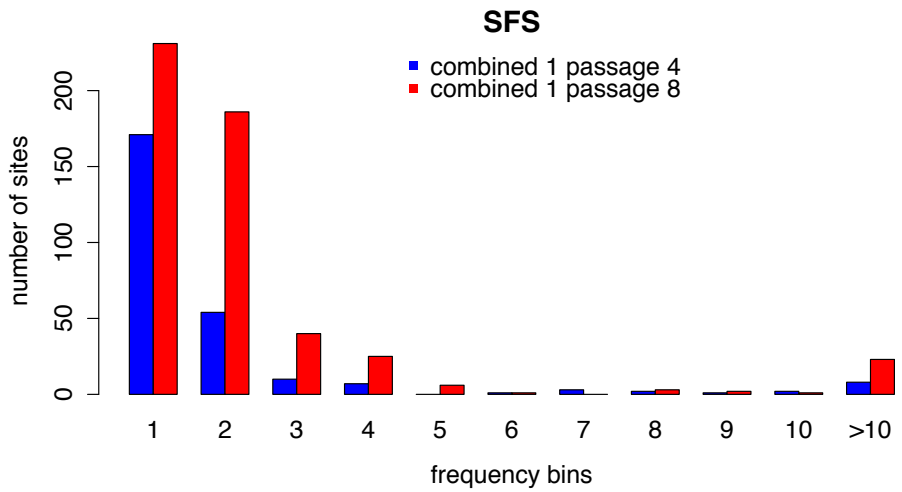
Supplementary Figures

Supplementary Figure 1. Genetic diversity



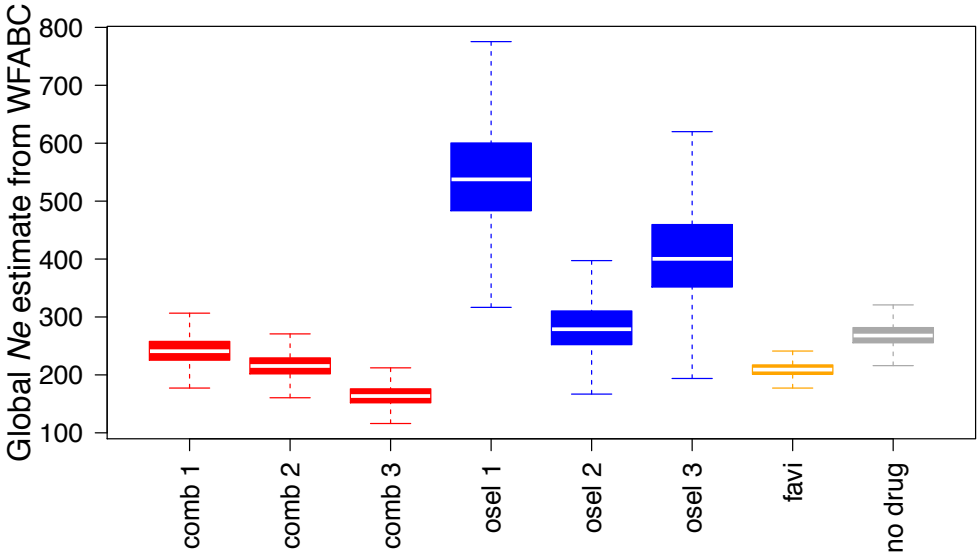
We calculated genetic diversity as the average expected heterozygosity (Nei 1973) in passages 4 to 10 for the combined drug replicates (red) and the oseltamivir-only replicates (blue), and in passages 3-15 for the favipiravir-only (yellow) and no-drug (grey) populations. Genetic diversity was consistently low for all replicates, although it was slightly lower for the oseltamivir-only replicates (2.6×10^{-3} average for replicate 1, 2.5×10^{-3} for replicate 2, 2.0×10^{-3} for replicate 3) compared to the combination drug replicates. (3.6×10^{-3} for replicate 1, 3.2×10^{-3} for replicate 2, 3.0×10^{-3} for replicate 3).

Supplementary Figure 2. Site Frequency Spectrum



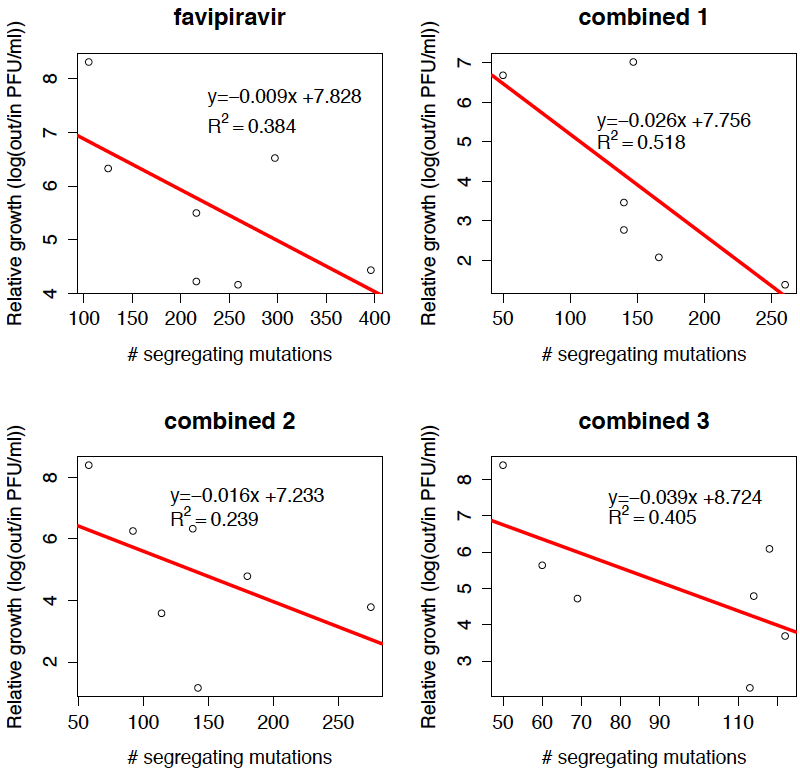
For each site, counts were randomly (hypergeometrically) downsampled to 100 after filtering (based on frequency > 1% in one of the passages to reduce sequencing error estimated at 1%). The site frequency spectra are shown for combined drug replicate 1 at passage 4 (in blue) and passage 8 (in red). There is a left bias to the SFS, likely reflecting the strength of purifying selection throughout all passages. Counts in the low frequency bins have increased by passage 8 because of the mutagenic impact of favipiravir on the number of segregating mutations.

Supplementary Figure 3. WFABC Global Effective Population sizes



Calculated from the variance in allele frequencies between time points. Estimates for the combined drug and oseltamivir only replicates are calculated over 8 passages (passages 3-10). Estimates for the favipiravir and no drug populations are calculated over 13 passages (passages 3-15).

Supplementary Figure 4. Correlations between growth rate and mutational load



The negative correlation between relative growth ($\log_{10}$(output/input PFU/ml)) and the number of segregating sites (R^2=0.239-0.518) supports the impact of favipiravir in driving high mutational load. To ensure comparability, the graphs include only data from passages 3-10 for each of the four replicates of interest (favipiravir and combined drug treated replicates).