

Supplementary Table 1 Assessment of the natural replication capacity of HBV strains

Viral strain	Mean $\pm$ SD (IU/mL)	<i>P</i> value*
rtWT (wild-type)	1.35E+06 $\pm$ 1.92E+04	–
rtWT (lab)	1.24E+06 $\pm$ 2.84E+04	0.68
rtCYE	7.95E+05 $\pm$ 2.90E+04	0.11
rtCYEI	5.64E+05 $\pm$ 3.24E+04	0.00002
rtCYE+M204I	4.16E+05 $\pm$ 2.38E+04	0.0002
rtCYE+L80I+L180M+M204I	5.25E+05 $\pm$ 3.83E+04	0.001
rtCYEI+M204I	3.81E+05 $\pm$ 1.94E+04	0.0005
rtCYEI+L80I+L180M+M204I	6.72E+05 $\pm$ 2.94E+04	0.03
rtCYEI+A181V	4.16E+05 $\pm$ 4.68E+04	0.002
rtCYEI+N236T	4.92E+05 $\pm$ 5.24E+04	0.008

*Comparison with rtWT (lab). Experiments were performed at least three times independently. WT, wild-type; rtCYE, rtS106C+H126Y+D134E; rtCYEI, rtS106C+H126Y+D134E+L269I; (lab), laboratory-modified.

Supplementary Table 2 Drug susceptibility analysis of representative HBV strains

Viral strain	T0	T0.78	T3.125	T12.5	T50	T200	EC50
rtWT (wild-type)	1.35E+06±37.35	1.20E+06±185.27	8.32E+05±1191.15	4.57E+05±3596.84	1.73+05±286.99	3.14E+04±2866.64	5.79
rtWT (lab)	1.46E+06±204.45	1.35E+06±281.15	1.01E+06±512.99	3.93E+05±480.95	1.36E+05±389.78	2.49E+04±318.37	5.90
rtCYE	7.95E+05±148.12	6.80E+05±4063.59	6.87E+05±1678.12	2.05E+05±3777.63	5.51E+04±291.05	1.14E+04±411.81	7.41
rtCYEI	4.91E+05±1585.51	4.24E+05±5518.44	3.24E+05±4341.91	2.00E+05±2029.12	4.49E+04±1582.97	2.08E+04±1836.76	8.51
rtCYE+M204I	1.91E+06±43.32	1.56E+06±125.62	1.28E+06±63.68	7.91E+05±61.09	2.59E+05±295.54	3.25E+04±139.07	6.95
rtCYE+L80I+L180M+M204I	5.90E+05±63.03	4.60E+05±300.44	4.16E+05±185.17	2.41E+05±394.96	7.29E+04±711.20	9.34E+03±31.10	6.92
rtCYEI+M204I	5.25E+05±3224.49	3.81E+05±299.21	3.63E+05±63.78	2.62E+05±343.75	1.34E+05±388.13	4.36E+04±31.67	8.92
rtCYEI+L80I+L180M+M204I	7.03E+05±1304.17	5.84E+05±1641.64	5.60E+05±1371.25	3.38E+05±1110.09	8.66E+04±763.93	4.17E+04±638.51	10.23
rtCYEI+A181V	4.16E+05±3924.15	3.89E+05±115.66	3.64E+05±421.36	2.23E+05±571.05	2.05E+05±127.69	1.17E+05±250.18	36.15
rtCYEI+N236T	8.47E+05±1485.90	7.71E+05±3364.85	6.91E+05±414.90	4.90E+05±2190.48	4.43E+05±360.37	3.32E+05±642.84	57.33

*Comparison with rtWT. EC₅₀, half maximal effective concentration. Fold, the EC₅₀ of mutant/the EC₅₀ of wild-type. Experiments were performed at least three times independently. WT, wild-type; rtCYE, rtS106C+H126Y+D134E; rtCYEI, rtS106C+H126Y+D134E+L269I; (lab), laboratory-modified.