

Supplementary Information: Long-term hepatitis B virus infection of rhesus macaques requires suppression of host immunity

Biswas et al.

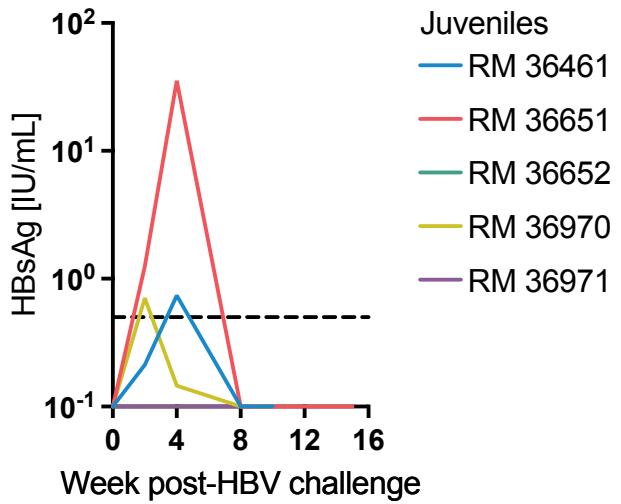


Fig S1. Measurements of HBsAg from the serum of transiently infected RM.

Source data are provided as a Source Data file.

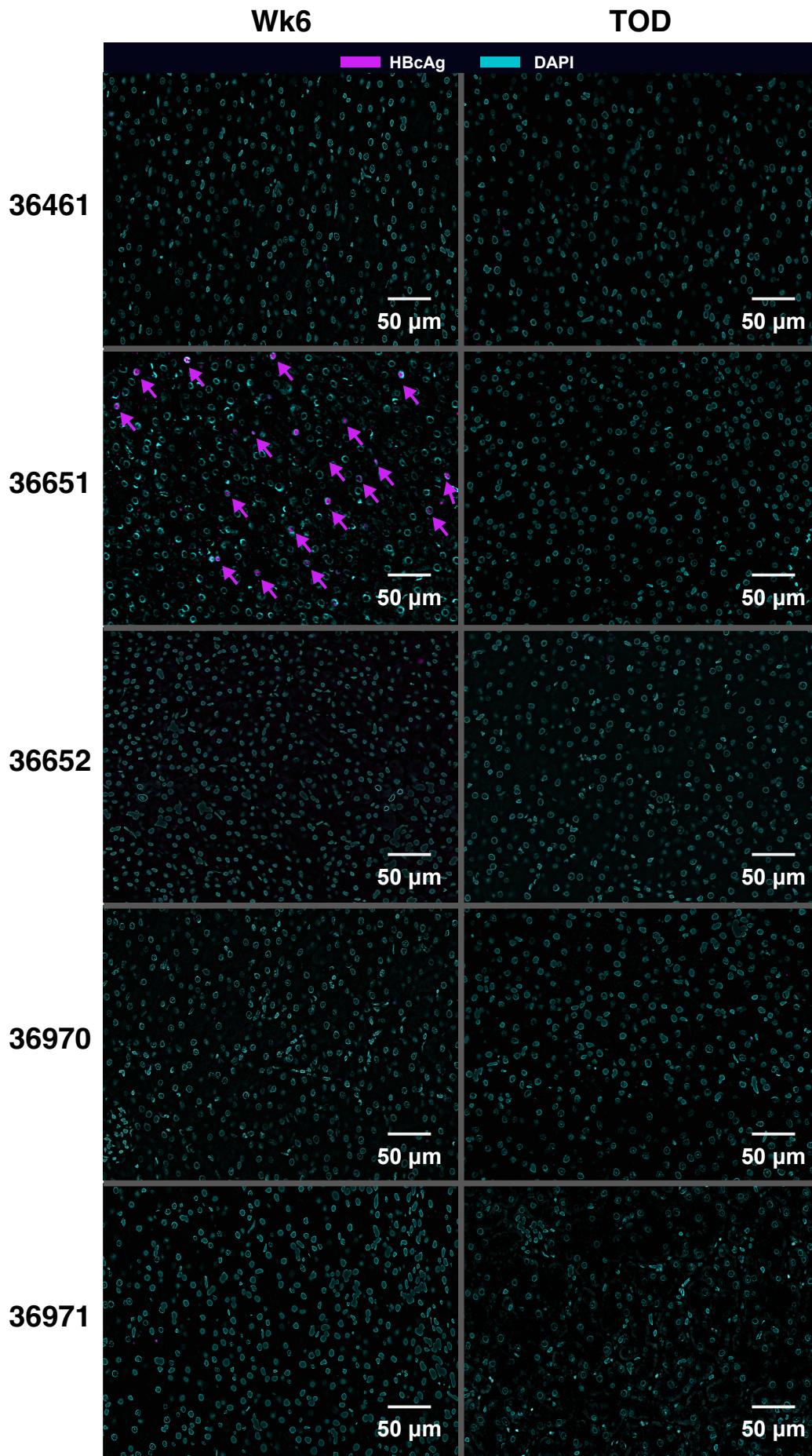


Fig S2. HBcAg staining of liver biopsies from transiently infected RM. Two independent experiments were conducted with similar results. Source data are provided as a Source Data file.

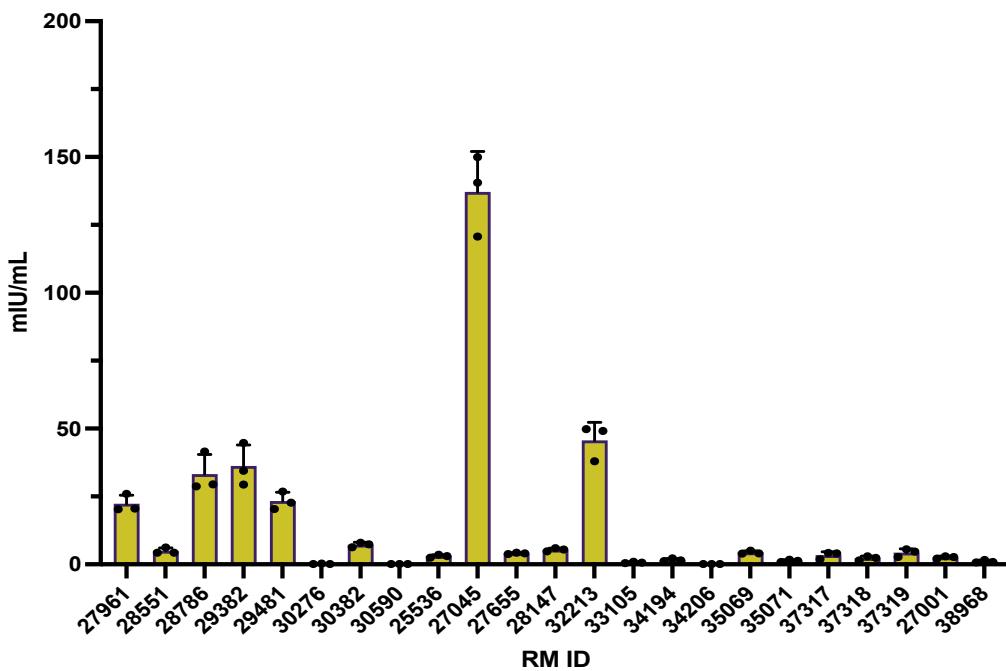


Fig S3. Screening of unvaccinated, HBV-naïve RMs from the ONPRC colony for anti-HBs responses. Data are presented as mean values +/- SD. Source data are provided as a Source Data file.

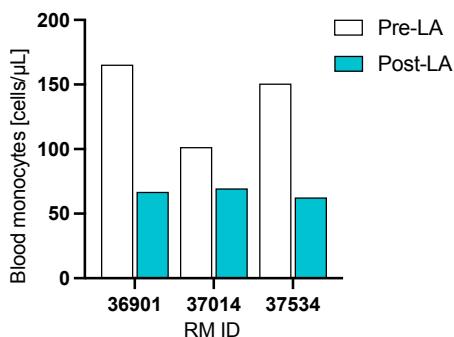
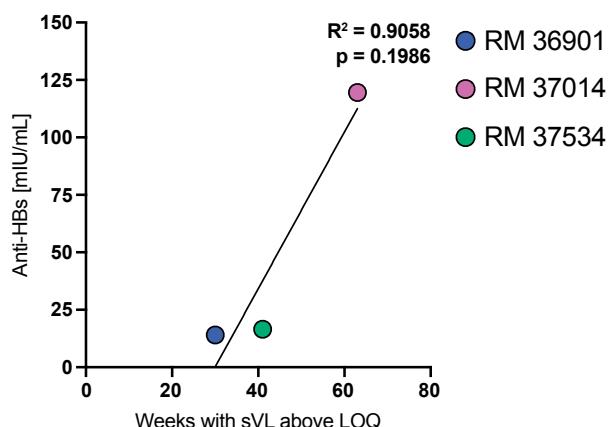
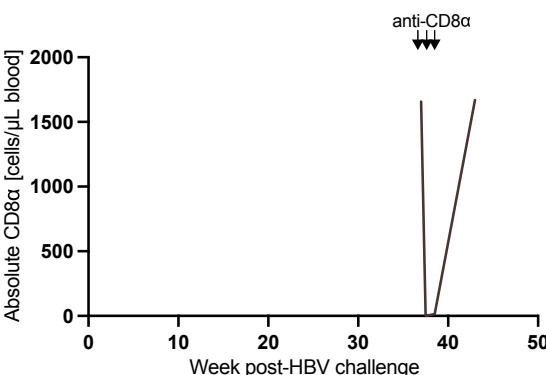
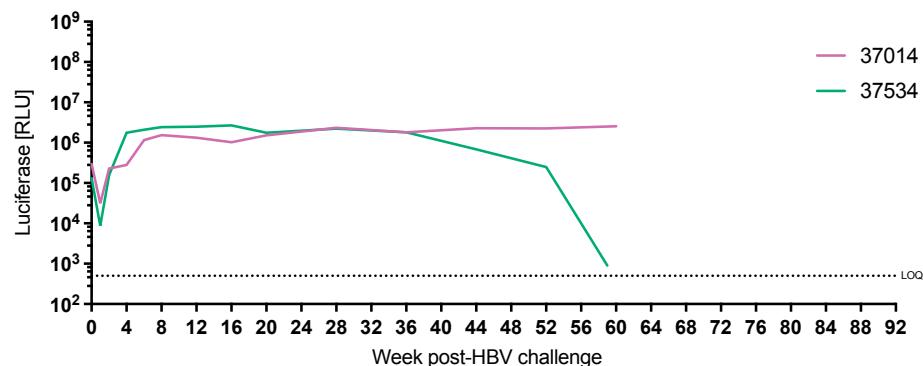
A**B****C****D**

Fig S4. Immune measurements in the blood of RMs. A) Absolute count of monocytes before and after LA administration. B) Correlation between serum anti-HBs levels and the number of weeks with sVL above the limit of quantification. Goodness of fit (R^2) and significance (p -value) were determined by simple linear regression. C) Absolute count of CD8 α expressing cells before and after administration of a CD8 α depleting antibody. D) Neutralization of adenovirus serotype 5 expressing NanoLuc by serum anti-Ad5 antibodies longitudinally in two RM. Source data are provided as a Source Data file.

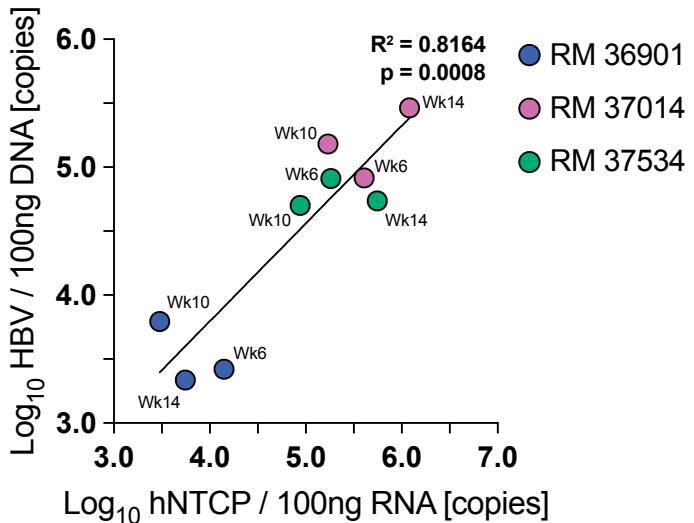


Fig S5. Correlation between HBV DNA and hNTCP RNA in RM livers. Goodness of fit (R^2) and significance (p -value) were determined by simple linear regression. Source data are provided as a Source Data file.

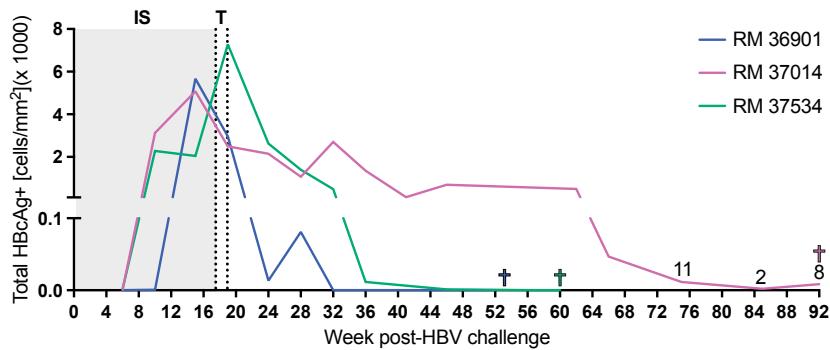
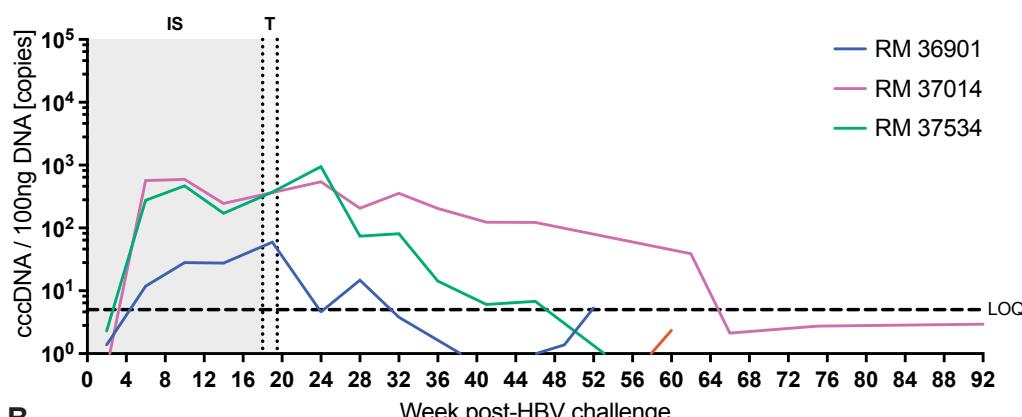
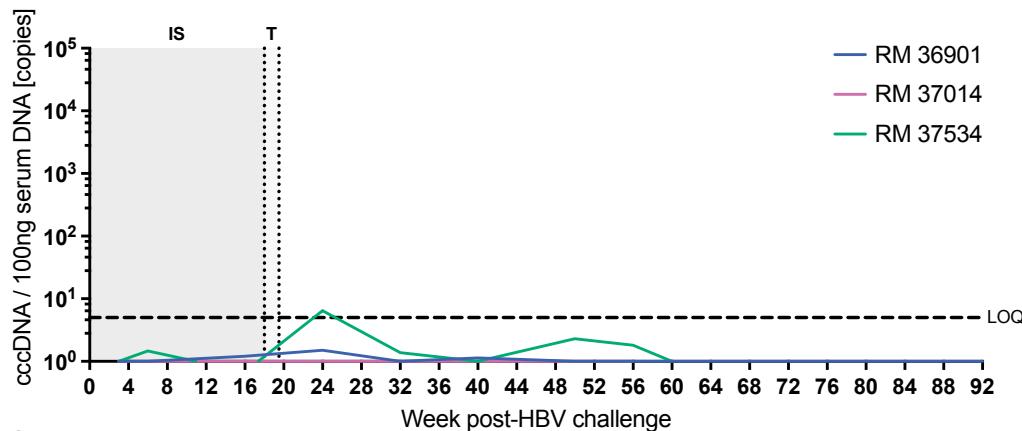


Fig S6. Absolute count of HBcAg+ cells in liver biopsies. Source data are provided as a Source Data file.

A

Week post-HBV challenge

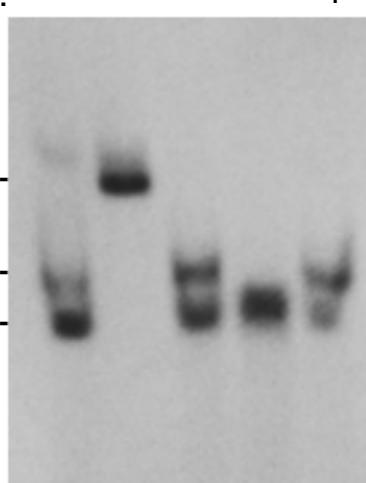
B

Week post-HBV challenge

C

85°C

EcoRI:	-	+	-	+	-
ExoI+III:	-	-	-	-	+



-Dp-rcDNA
-Linearized cccDNA

3.2 kb -
2 kb -
1.7 kb -

-cccDNA
-ssDNA

Fig S7. Detection of cccDNA in RM livers. A) Longitudinal quantification of cccDNA copies in the livers of RM. B) Longitudinal quantification of cccDNA copies in the serum of RM. C) Visualization of various forms of HBV DNA, including cccDNA, in the livers of RM. Two independent southern blot experiments were conducted with similar results. Source data are provided as a Source Data file.

				DPI																						
	Injected	Dose	Route	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	...	-3	...	0	...	5	6	7	8	...	10		
RM 36651	Liposomal alendronate (LA)	1 mg/kg	IV																							
	Dexamethasone	1 mg/kg	IM																							
	Ad-hNTCP	1.70E12 particles/kg	IV																							
	HBV	1.00E9 gc	IV																							
	Tacrolimus	0.04 mg/kg	IM																taper	taper	Stop					
RM 36652	Liposomal alendronate (LA)	1 mg/kg	IV																							
	Dexamethasone	1 mg/kg	IM																							
	Ad-hNTCP	1.70E12 particles/kg	IV																							
	HBV	1.00E9 gc	IV																							
	Tacrolimus	0.04 mg/kg	IM																taper	taper	Stop					
RM 36970	Liposomal alendronate (LA)	1 mg/kg	IV																							
	Dexamethasone	1 mg/kg	IM																							
	Ad-hNTCP	2.40E12 particles/kg	IV																							
	HBV	1.00E9 gc	IV																							
	Tacrolimus	0.04 mg/kg	IM																taper	taper	Stop					
RM 36971	Liposomal alendronate (LA)	1 mg/kg	IV																							
	Dexamethasone	1 mg/kg	IM																							
	Ad-hNTCP	2.40E12 particles/kg	IV																							
	HBV	1.00E9 gc	IV																							
	Tacrolimus	0.04 mg/kg	IM																taper	taper	Stop					
RM 36461	Liposomal alendronate (LA)	1 mg/kg	IV																							
	Dexamethasone	1 mg/kg	IM																							
	Ad-hNTCP	2.40E12 particles/kg	IV																							
	HBV	1.00E9 gc	IV																							
	Tacrolimus	0.04 mg/kg	IM																taper	taper	Stop					
RM 36314	HDAd-hNTCP	1.00E12 particles/kg	IV																							
	HBV	1.00E9 gc	IV																							
RM 36315	HDAd-hNTCP	1.00E12 particles/kg	IV																							
	AAV8-hNTCP	1.73E13 particles/kg	IV																							
	HBV	1.00E9 gc	IV																							
RM 36355	HDAd-hNTCP	1.00E12 particles/kg	IV																							
	AAV8-hNTCP	2.00E13 particles/kg	IV																							
	HBV	1.00E9 gc	IV																							

Table S1. Procedures on juvenile and infant RMs with transient HBV viremia.

Table S2. Procedures on juvenile RMs with long-term HBV infection.

Primer name	Sequence
HBV-RCA1	5'- AATCCTCACAAATACC -3'
HBV-RCA2	5'- GATGGGATGGGAATA -3'
HBV-RCA3	5'- CCTATGGGAGTGGGC -3'
HBV-RCA4	5'- GCAACGGGTAAAGG -3'
HBV-RCA5	5'- ATGCAACTTTTCAC -3'
HBV-RCA6	5'- TCCAAATTCTTATA -3'
HBV-RCA7	5'- TAGAAGAAGAACTCC -3'
HBV-RCA8	5'- AGAATATGGTGACCC -3'

Table S3. Primers for rolling-circle amplification (RCA) of HBV.