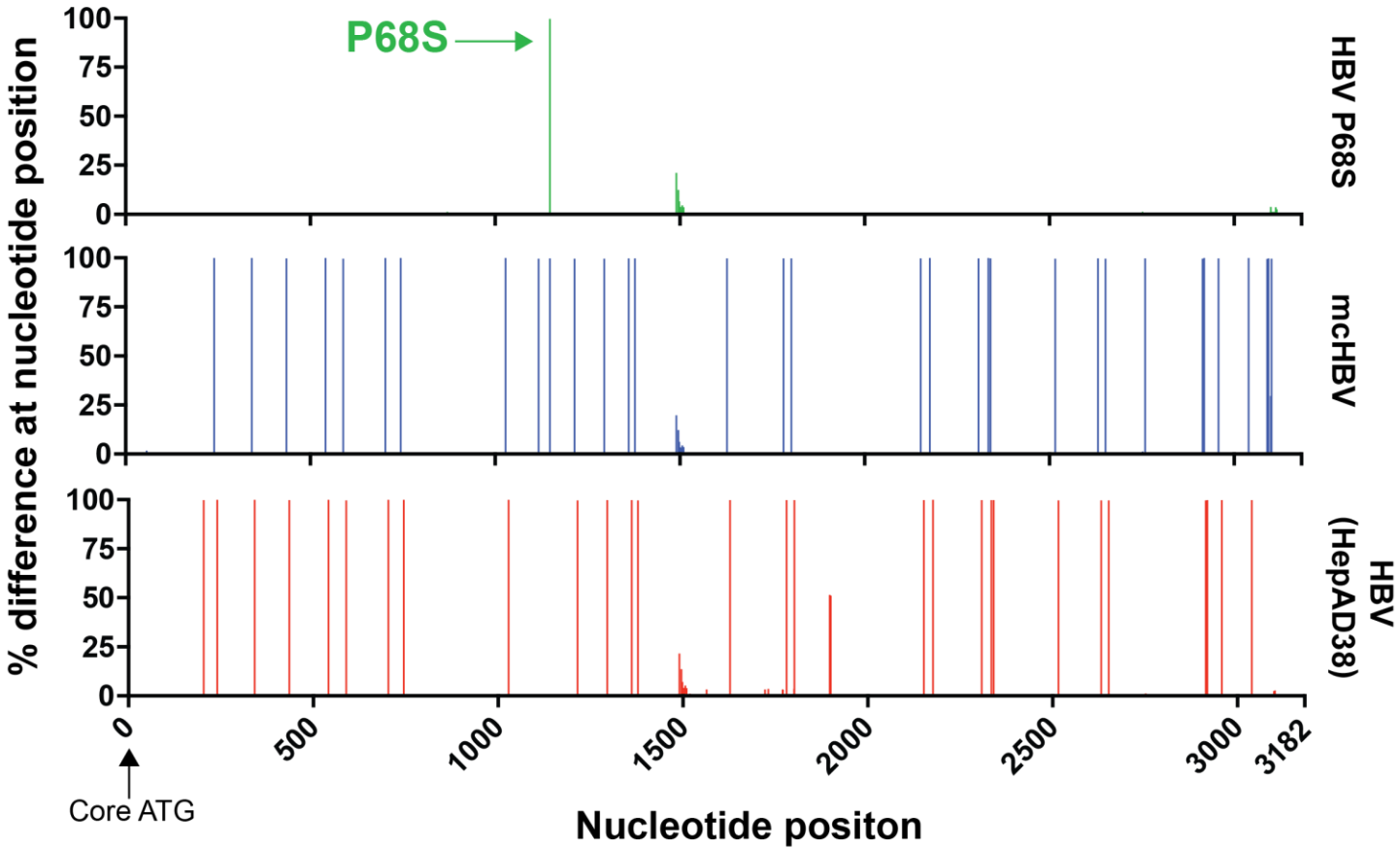


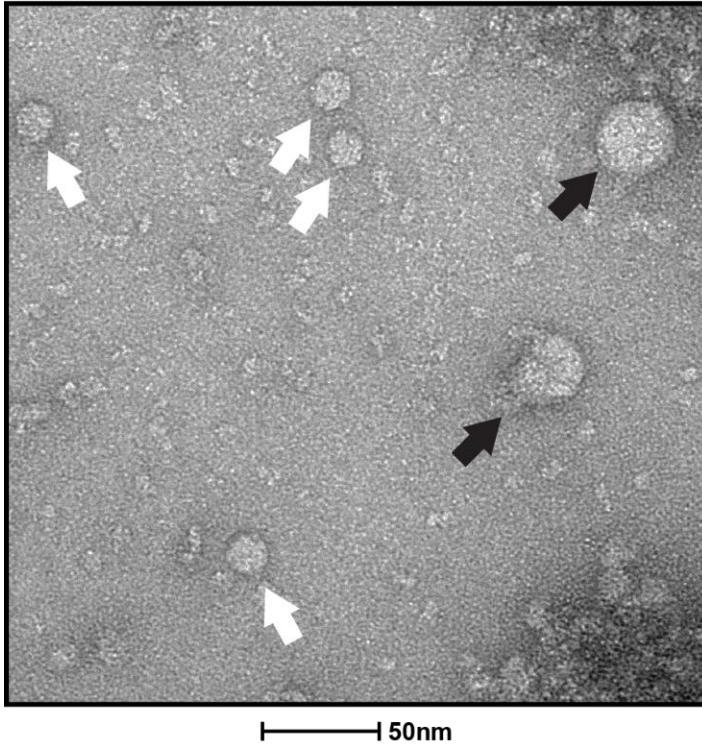
Supplementary Figure 1.



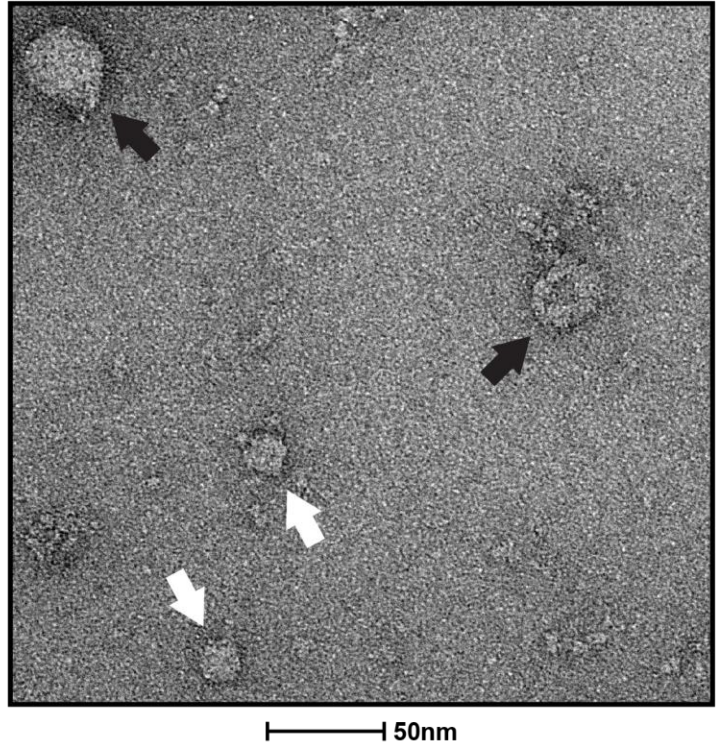
Supplementary Figure 1. Deep sequencing of HBV stocks. HBV stocks were PCR amplified using overlapping amplicons and deep sequenced using an Illumina MiSeq. Reads were aligned against the HBV genotype D serotype ayw described in Pasek *et al.*²⁶. Colored spikes represent the fraction of reads that differ from the reference at each nucleotide position. Nucleotides are numbered beginning with the adenine of the HBV core ATG start codon.

Supplementary Figure 2.

Rhesus macaque PH supernatant - HDAd (10x dilution)

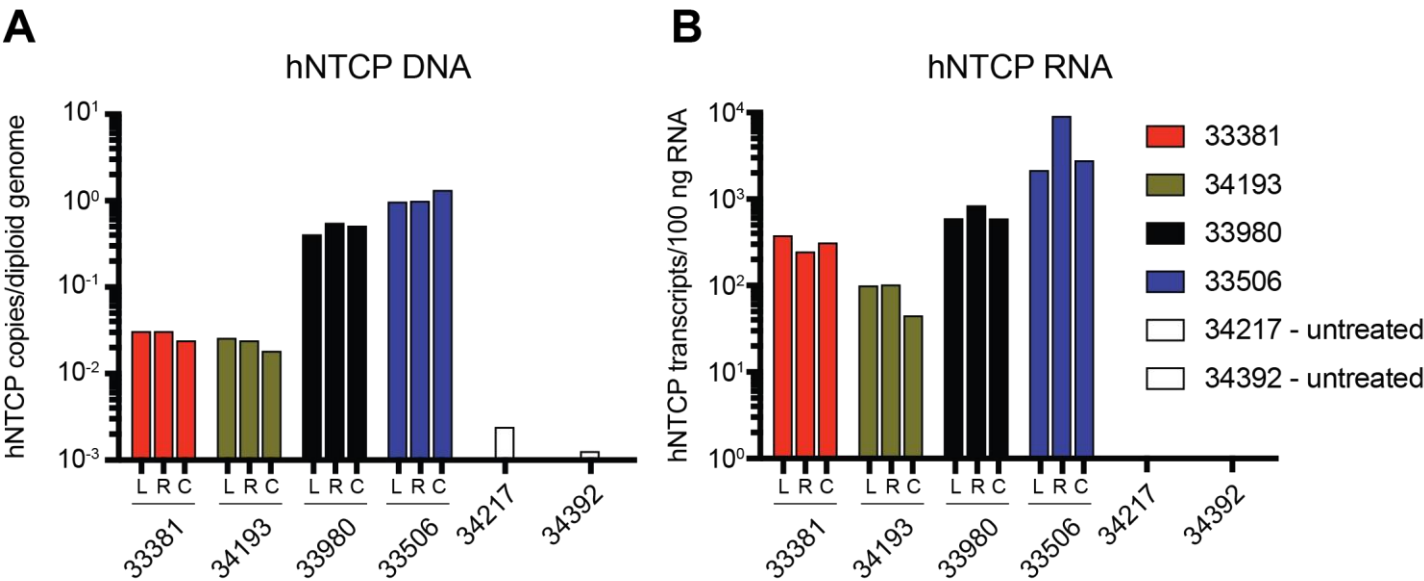


Rhesus macaque PH supernatant - AAV8 (10x dilution)



Supplementary Figure 2. Electron microscopy of Dane particles secreted from HBV-infected rhesus macaque PH. Left - Rhesus macaque PH treated with HDAd-hNTCP. Right - Rhesus macaque PH treated with AAV8-hNTCP. Black arrow = Dane particle. White arrow = Spherical bodies consistent with virion-like HBsAg particles lacking HBV DNA and core.

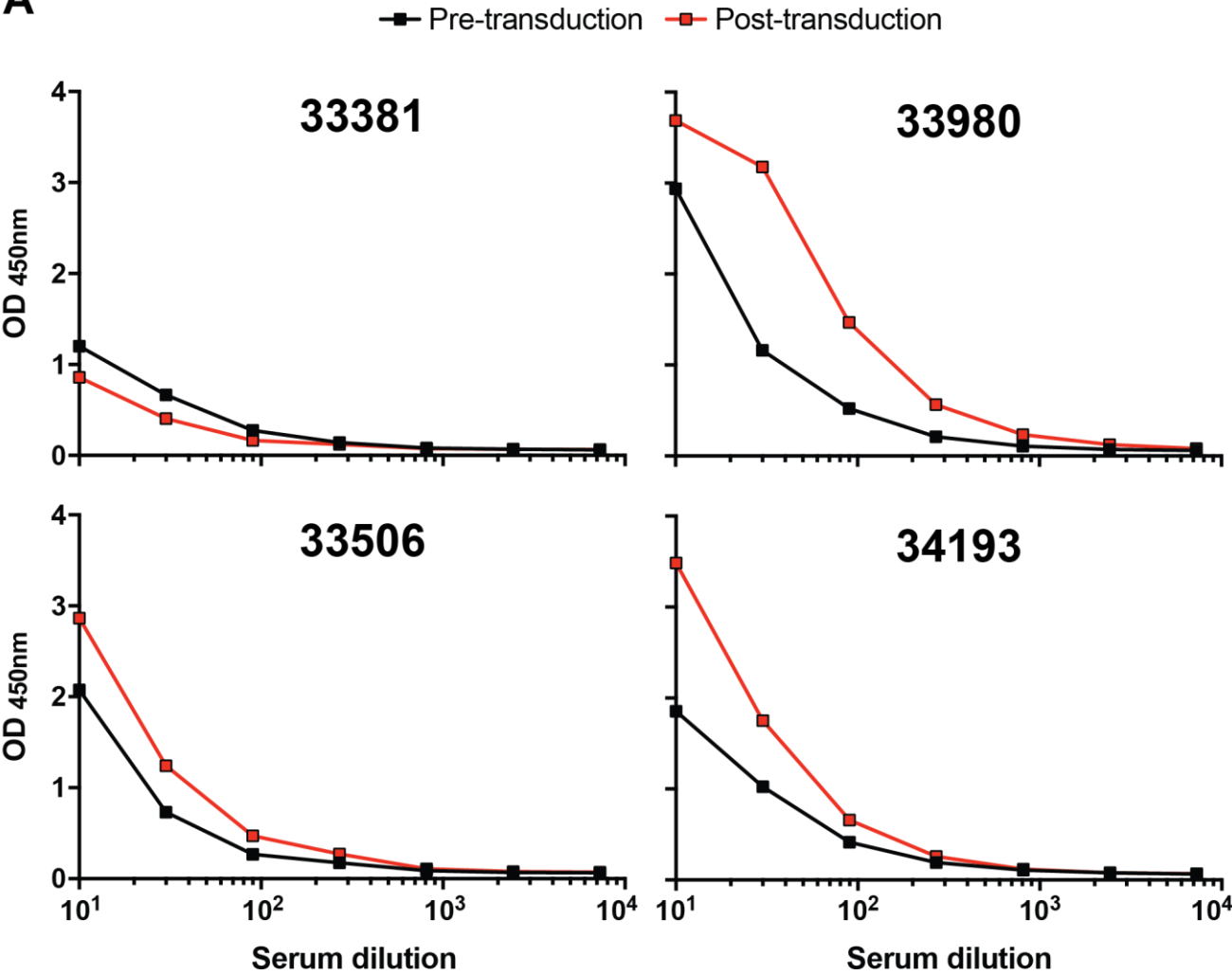
Supplementary Figure 3.



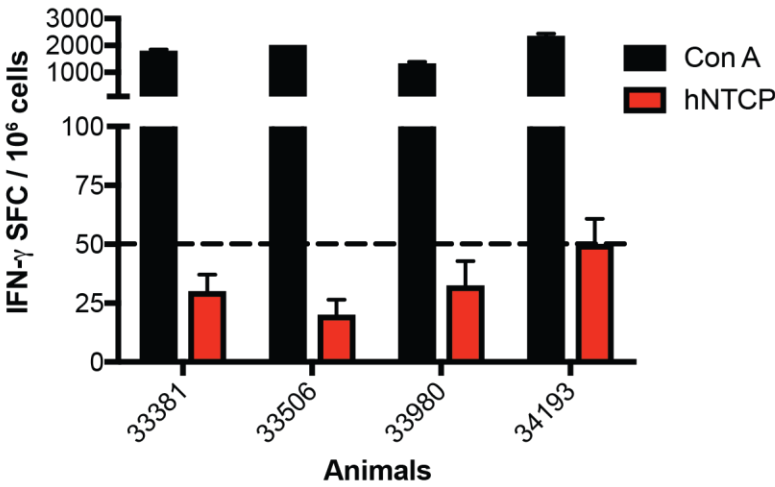
Supplementary Figure 3. Analysis of liver transduction and hNTCP expression. A) qPCR for hNTCP DNA shows low transduction levels as assessed by hNTCP DNA copies per diploid genome. B) qRT-PCR for hNTCP RNA shows expression of hNTCP in all animals in all liver lobes assessed. L = left liver lobe; R = right liver lobe; C = caudate liver lobe.

Supplementary Figure 4.

A



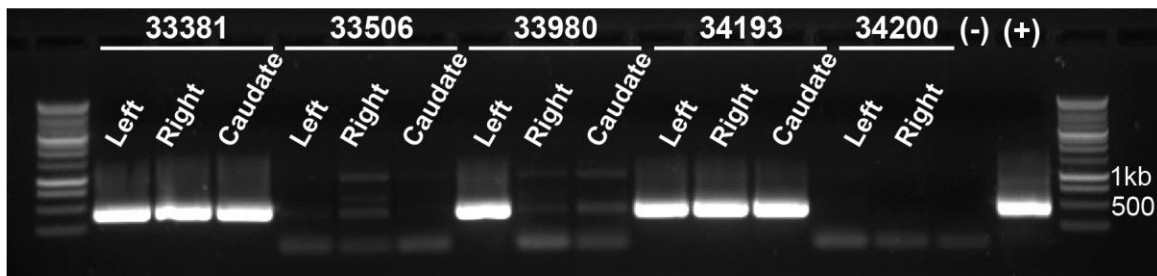
B



Supplementary Figure 4. Measurement of anti-hNTCP immune responses.

A) Anti-hNTCP ELISA using HBV-infected rhesus macaque serum. Positive signal was defined as 3-fold increase in OD 450nm over pre-transduction at each serum dilution. No animals exhibited hNTCP specific humoral responses. B) ELISpot results show no cellular responses against recombinant hNTCP above assay limit of detection (50 IFN- γ SFC/ 10^6 cells). SFC = spot-forming cells. Con A = concanavalin A (positive control). Bars represent standard error of measurement from two ELISpot replicates.

Supplementary Figure 5.



Supplementary Figure 5. Nested PCR shows presence of HBV DNA in liver sections obtained from HBV-infected rhesus macaques. (-) negative control with no template. (+) positive control with HBV DNA template extracted from high-titer stock of HBV.

Supplementary Table 1. Competitive anti-HBc ELISA of MCM cohort at ONPRC

| ONPRC MCM ID | Optical Density (450nm) |
|-----------------|-------------------------|
| 32843 | 1.415 |
| 32844 | 1.507 |
| 32845 | 1.436 |
| 32846 | 0.615 |
| 32847 | 0.734 |
| 32848 | 0.747 |
| 32849 | 0.473 |
| 32850 | 1.077 |
| 32851 | 1.176 |
| 33450 | 0.925 |
| 33454 | 0.848 |
| 33455 | 1.425 |
| 33457 | 0.321 |
| 33460 | 1.403 |
| 33461 | 1.276 |
| 34067 | 0.688 |
| 34068 | 0.417 |
| 34661 | 1.317 |
| 34662 | 1.196 |
| 34663 | 1.234 |
| 34664 | 0.389 |
| 34665 | 1.200 |
| 34666 | 1.414 |
| 34668 | 1.276 |
| 34669 | 1.246 |
| 34670 | 1.073 |
| 34837 | 0.686 |
| Negative (mean) | 1.422 |
| Positive (mean) | 0.010 |
| Cutoff | 0.284 |

| |
|----------|
| negative |
| positive |

Supplementary Table 2. Competitive anti-HBc ELISA of HBV-challenged MCM at ONPRC

| MCM | Optical Density 450nm | |
|---------------------------|-----------------------|--------|
| | Day 0 | Day 56 |
| 33451 (HBV P68S) | 1.332 | 1.351 |
| 33453 (HBV P68S / CD3-IT) | 0.993 | 1.024 |
| 34084 (mcHBV) | n.d. | 1.107 |
| 34079 (mcHBV / CD3-IT) | 1.432 | 1.484 |
| Negative (mean) | 1.680 | |
| Positive (mean) | 0.01 | |
| Cutoff | 0.336 | |

| |
|----------|
| negative |
| positive |

Supplementary Table 3. Competitive anti-HBc ELISA of HBV-challenged rhesus macaques at ONPRC

| | Optical Density 450nm | |
|-----------------|-----------------------|--------|
| Rhesus macaque | Day 0 | Day 56 |
| 33506 (AAV) | 1.832 | 0.055 |
| 33980 (AAV) | 1.488 | 1.434 |
| 33381 (HdAd) | 1.291 | 1.517 |
| 34193 (HdAd) | 1.341 | 1.377 |
| Negative (mean) | 1.680 | |
| Positive (mean) | 0.01 | |
| Cutoff | 0.336 | |

| |
|----------|
| negative |
| positive |

Supplementary Table 4. Serology markers in HBV-challenged rhesus macaques**Anti-HBe measurements (relative detection)**

| Sample | S/CO | Reactivity |
|--------|-------|-------------|
| 33381 | 1.75 | Nonreactive |
| 34193 | 1.90 | Nonreactive |
| 33506 | 2.01 | Nonreactive |
| 33980 | 2.01 | Nonreactive |
| Cutoff | ≤1.00 | - |

Anti-HBs measurements (absolute detection of antibodies)

| Sample | mIU/ml |
|--------|--------|
| 33381 | 0.00 |
| 34193 | 0.00 |
| 33506 | 0.00 |
| 33980 | 0.00 |

HBsAg measurements (relative detection)

| Sample | S/CO | Reactivity |
|--------|-------|-------------|
| 33381 | 0.71 | Nonreactive |
| 34193 | 0.17 | Nonreactive |
| 33506 | 0.15 | Nonreactive |
| 33980 | 0.20 | Nonreactive |
| Cutoff | ≥1.00 | - |