OMTM, Volume *24*

Supplemental information

Prednisolone reduces the interferon response to AAV in cynomolgus macaques and may increase liver gene expression

Lili Wang, Claude C. Warzecha, Alexander Kistner, Jessica A. Chichester, Peter Bell, Elizabeth L. Buza, Zhenning He, M. Betina Pampena, Julien Couthouis, Sunjay Sethi, Kathleen McKeever, Michael R. Betts, Emil Kakkis, James M. Wilson, Samuel Wadsworth, and Barbara A. Sullivan

Supplemental Data

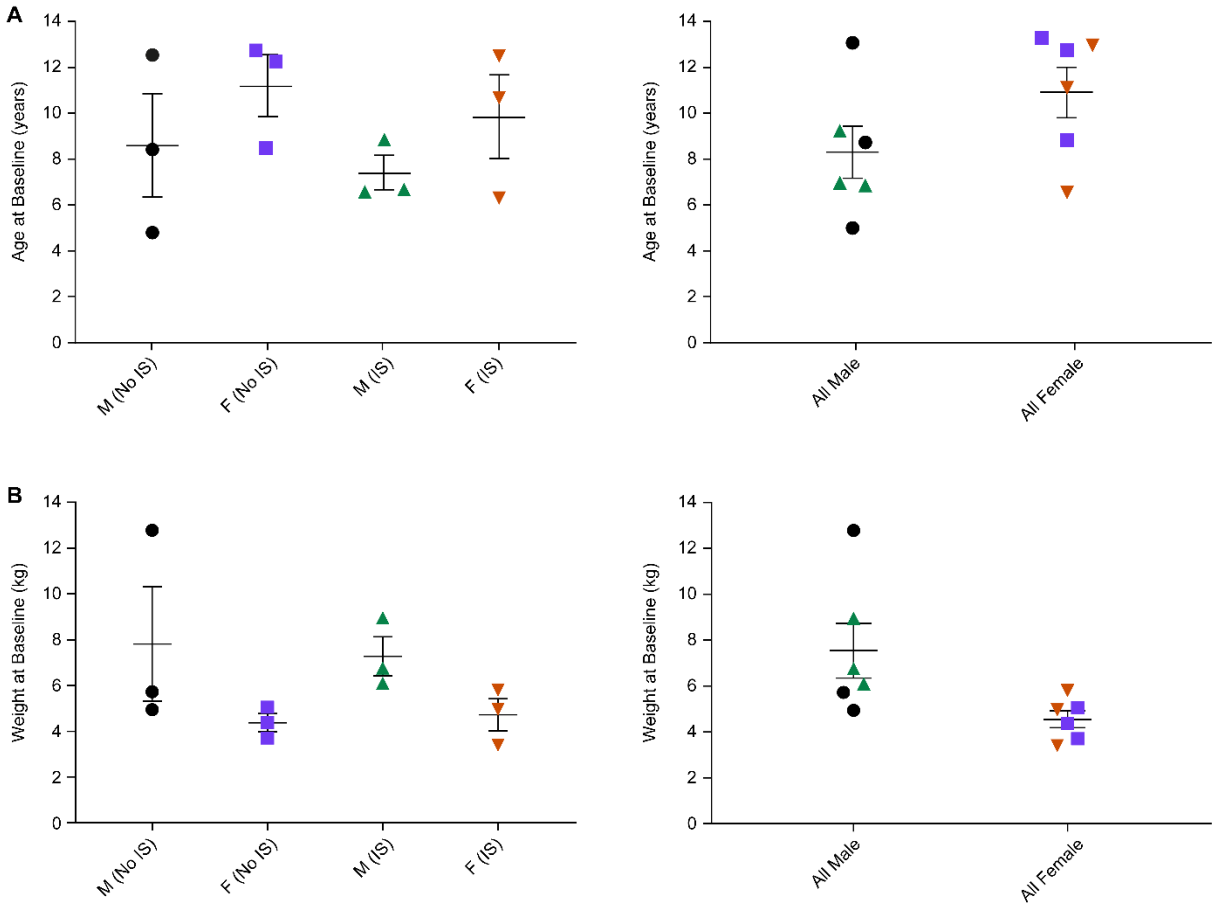


Figure S1. Age and weight at baseline according to study group and sex. (A) Age and (B) weight at baseline for individual macaques is shown per study group (left panels) and by sex (right panels). Data are represented as individual values for each animal with mean ± SEM. F, female; IS, immunosuppression; M, male.

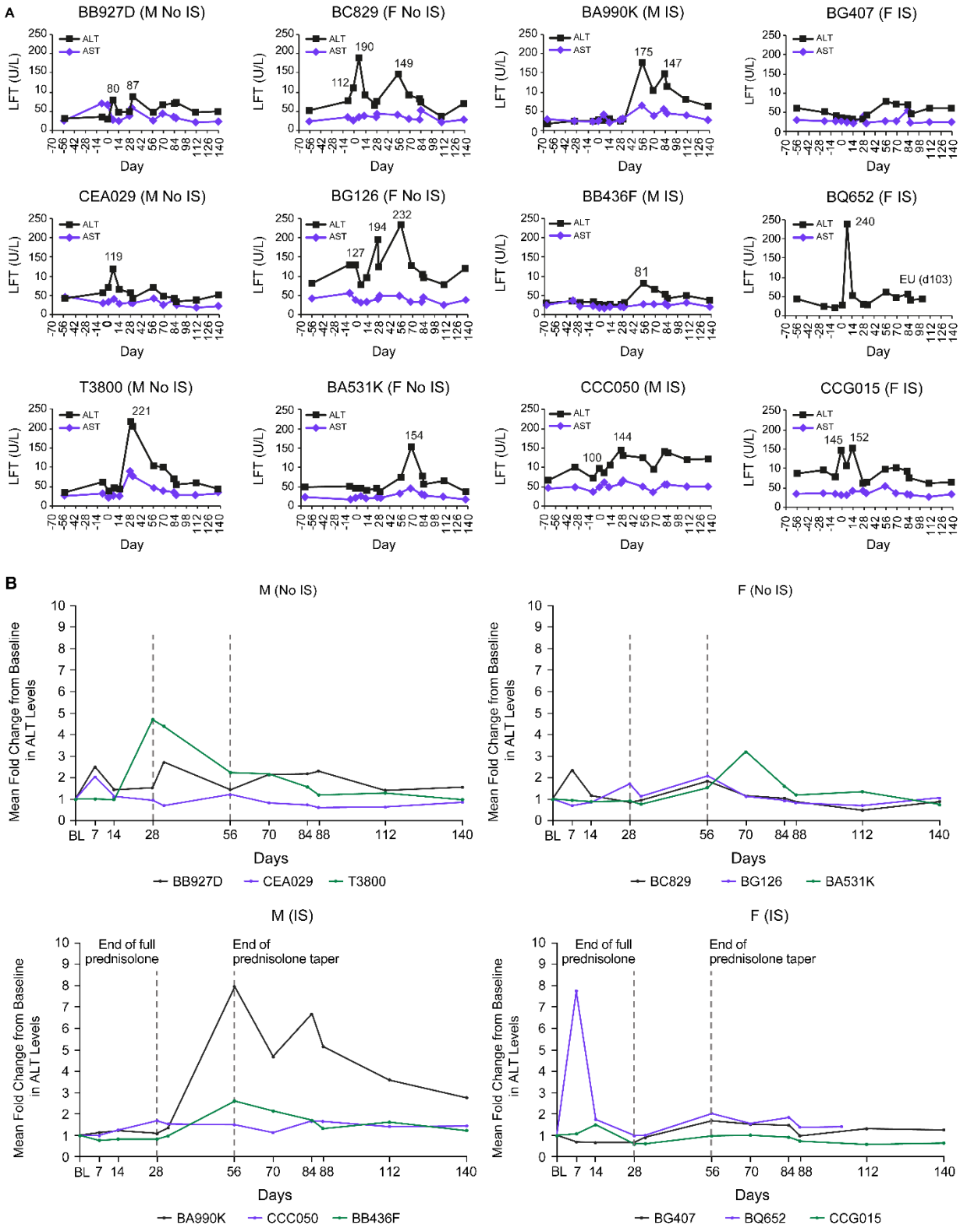


Figure S2. Alanine aminotransferase and aspartate aminotransferase levels in non-human primates (NHPs) following DTX301 administration. (A) Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were quantified in individual macaques at the indicated time points. (B) ALT levels shown as fold change

from baseline for individual cynomolgus macaques by treatment group. F, female; IS, immunosuppression; LFT, liver function test; M, male.

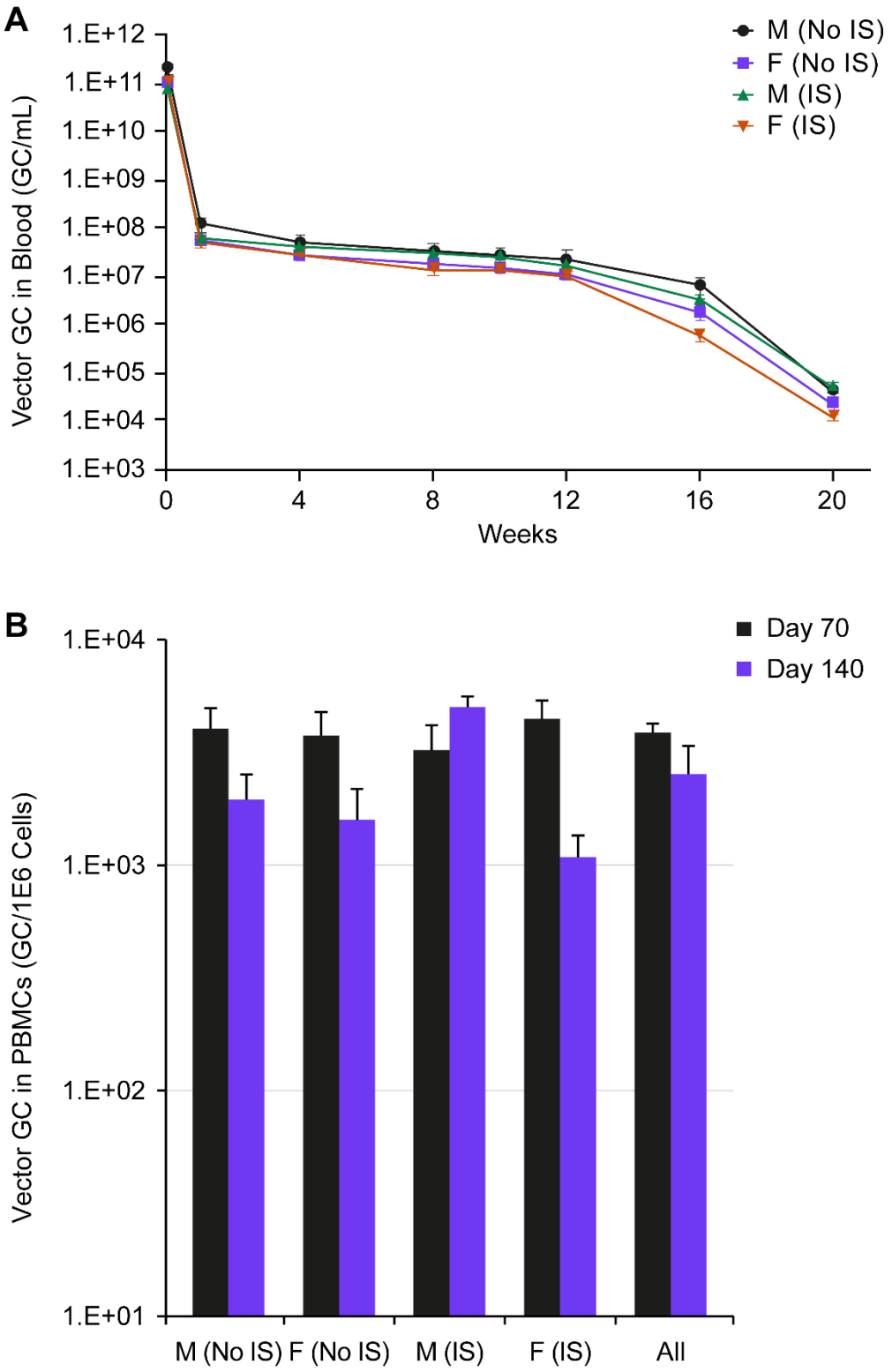


Figure S3. Vector DNA expression in the blood. Vector DNA levels were quantified in the blood of individual macaques by quantitative polymerase chain reaction (qPCR) at the indicated time points. (A) Vector DNA in whole blood. Data are represented as the mean ± SEM for each treatment group. (B) Vector DNA in peripheral blood mononuclear cells (PBMCs). Data are represented as mean ± SEM for each treatment group and for all animals. F, female; GC, genome copies; IS, immunosuppression; M, male.

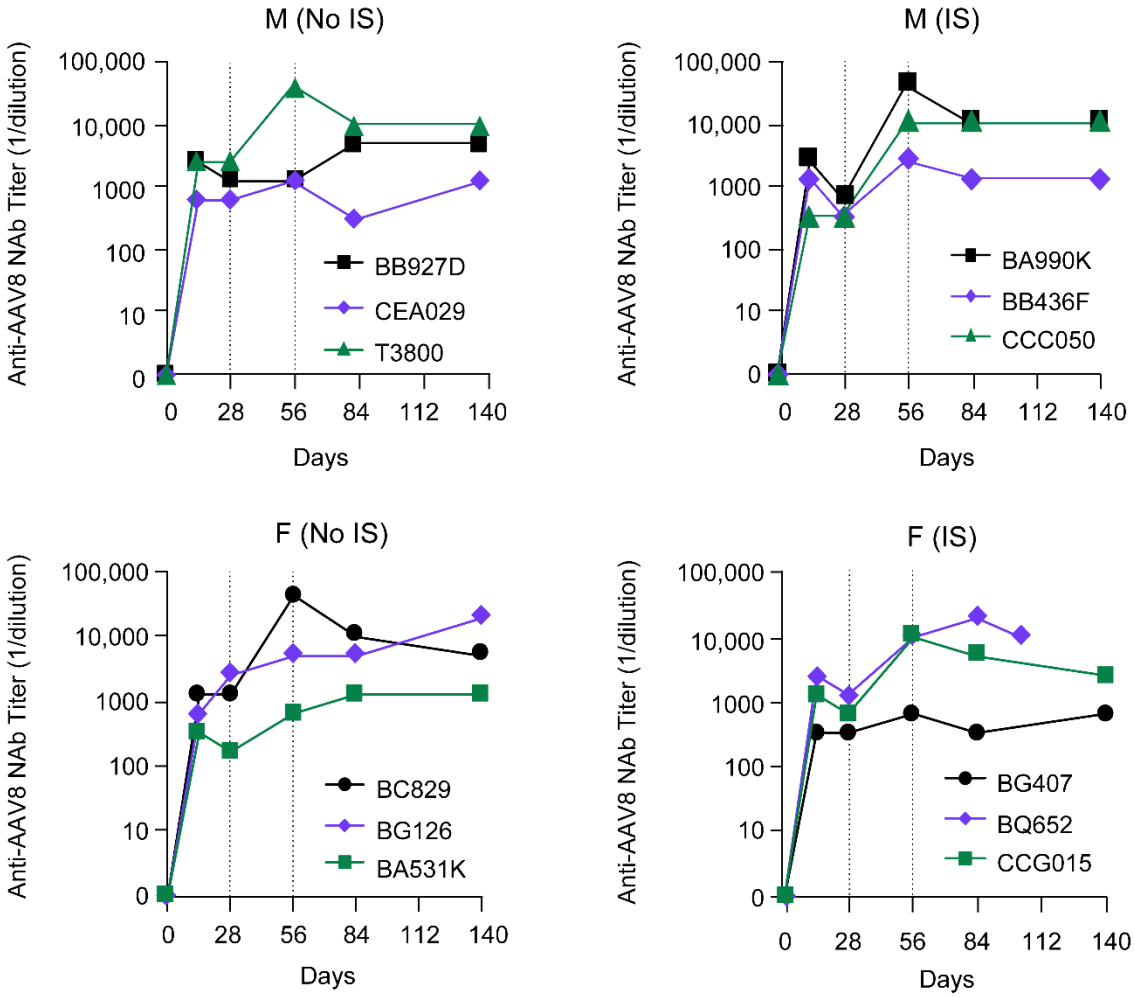


Figure S4. Neutralizing antibody assay responses following DTX301 administration in NHP with or without immunosuppression. Serum anti-AAV8 neutralizing antibody (NAb) titers were evaluated in individual cynomolgus macaques at the indicated time points using a cell-based neutralization assay. The NAb titer values are reported as the reciprocal of the highest serum dilution at which AAV transduction is reduced 50% compared to the negative control. F, female; IS, immunosuppression; M, male.

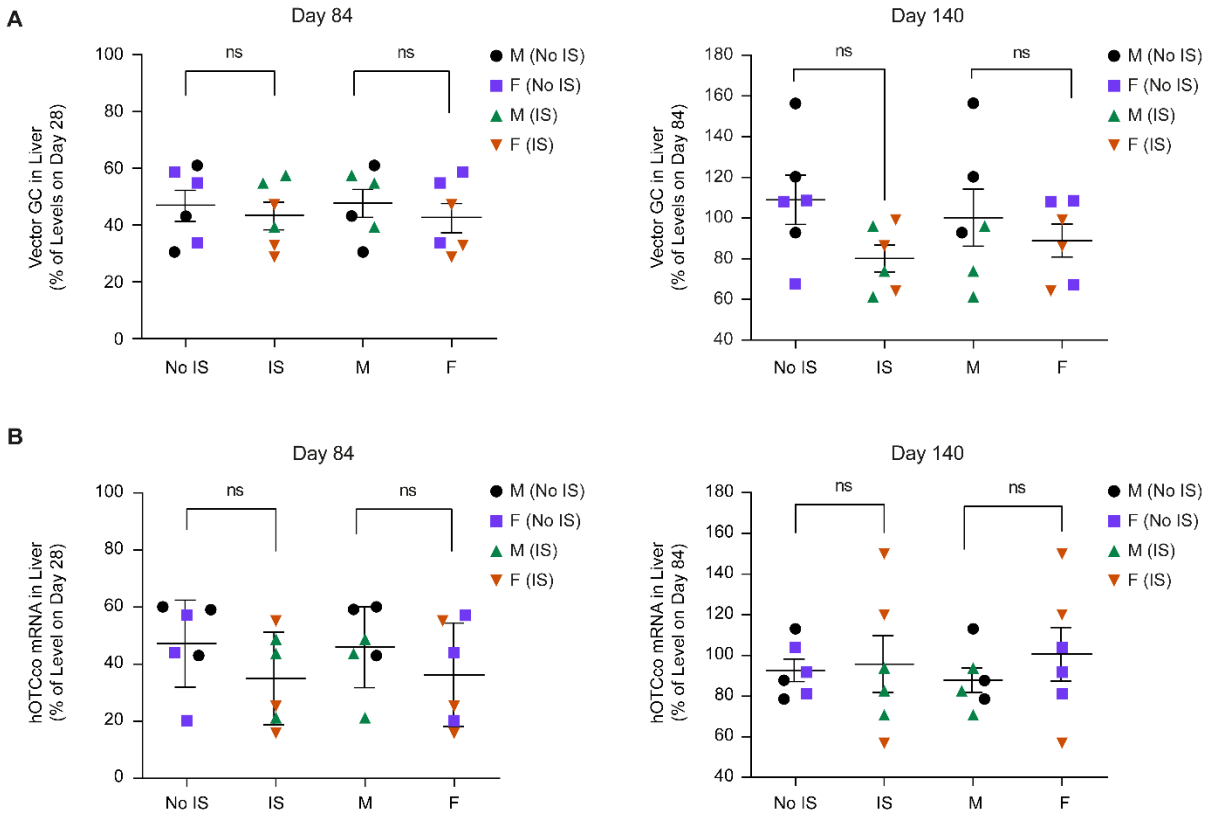
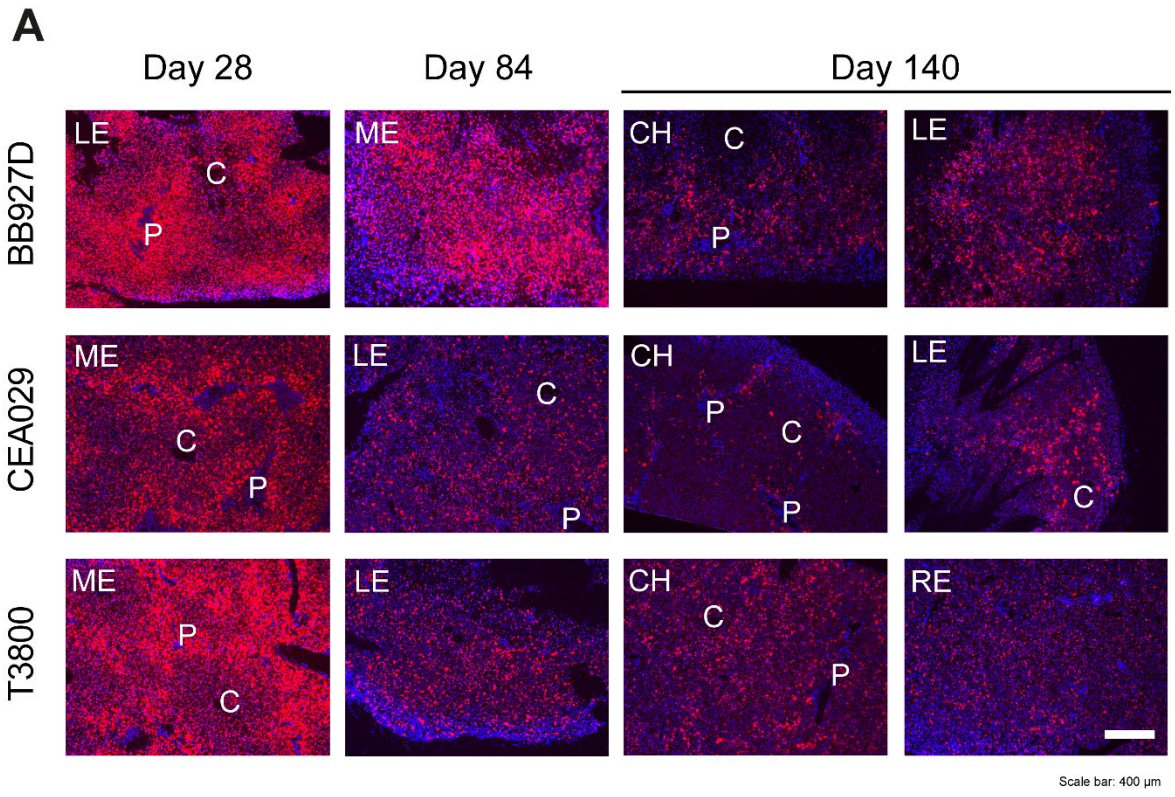
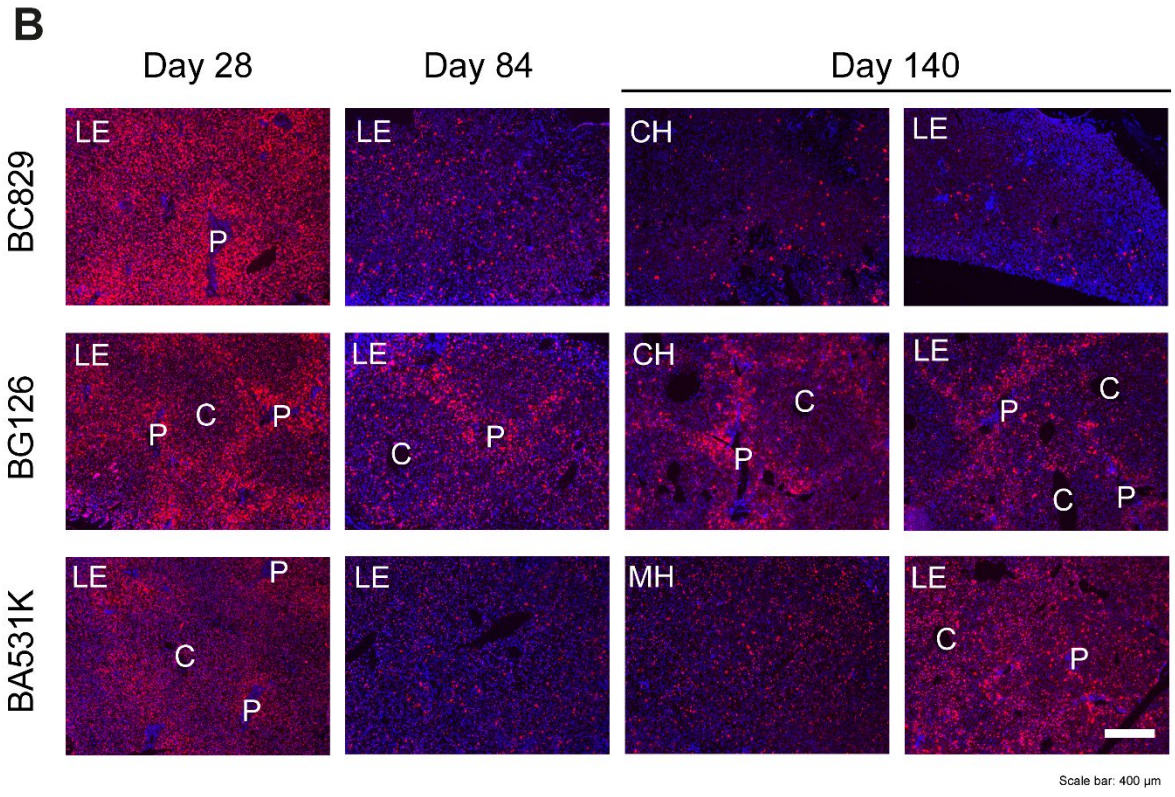
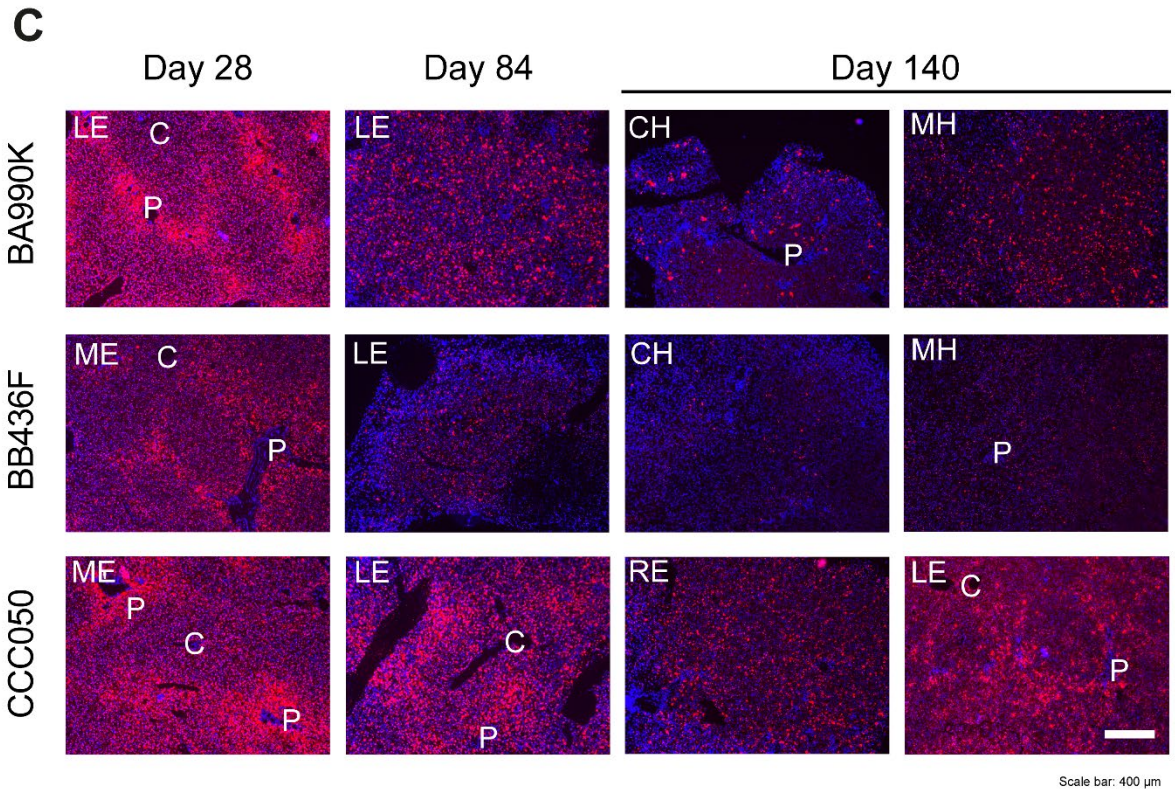


Figure S5. Change of vector genome copies and human codon-optimized OTC messenger RNA levels over time in liver. (A) Vector DNA and (B) human codon-optimized OTC (hOTCco) messenger (m)RNA levels were quantified in liver biopsies of individual cynomolgus macaques at day 84 and day 140. For each NHP, vector genome copies (GC) and hOTCco mRNA levels in liver on day 84 (left panel) and day 140 (right panel) are presented as the proportion of the levels on the previous time points (day 28 and day 84, respectively). Data are represented as individual values for each animal with mean ± SEM. Statistical analysis was performed using a Mann-Whitney test. ns, not significant. F, female; GC, genome copies; IS, immunosuppression; M, male. See also Figure 2.


A
Day 28
Day 84
Day 140
BB927D
CEA029
T3800
LE
ME
CH
RE
C
P
Scale bar: 400 µm


B
Day 28
Day 84
Day 140
BC829
BG126
BA531K
LE
CH
MH
C
P
Scale bar: 400 µm


C
Day 28
Day 84
Day 140
BA990K
BB436F
CCC050
LE
C
P
ME
CH
MH
RE
Scale bar: 400 µm

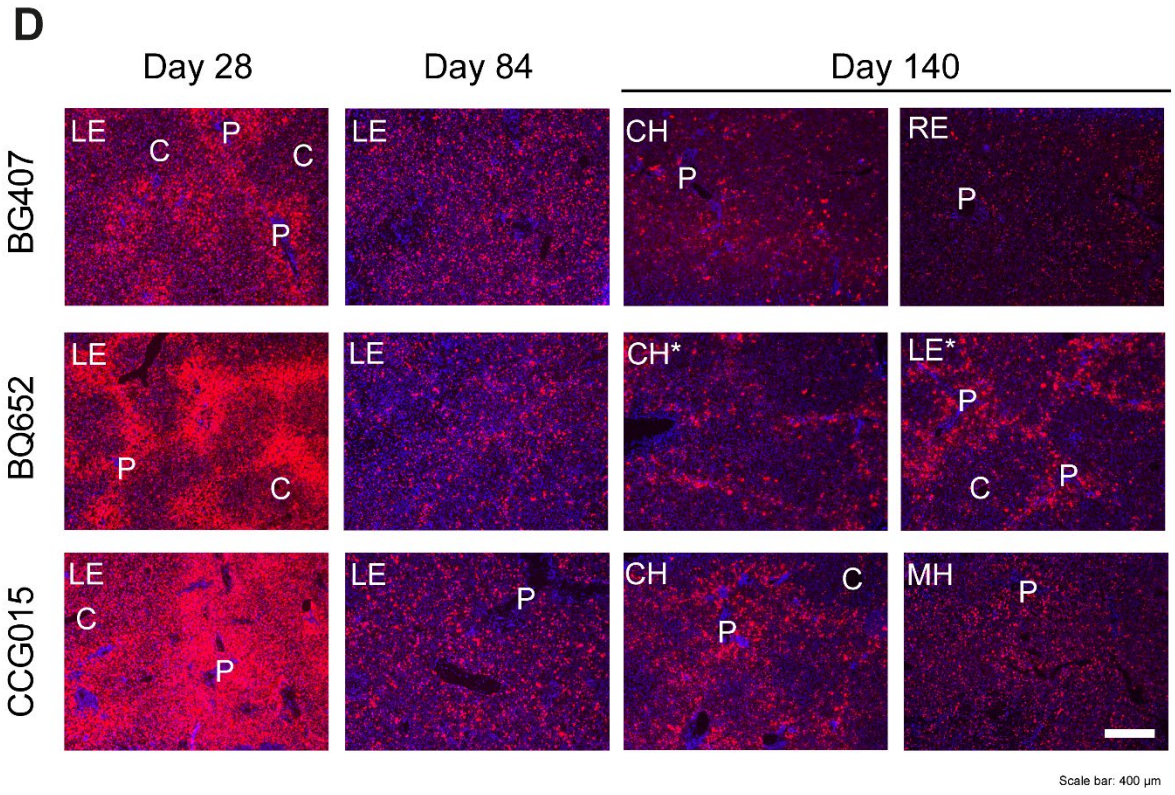
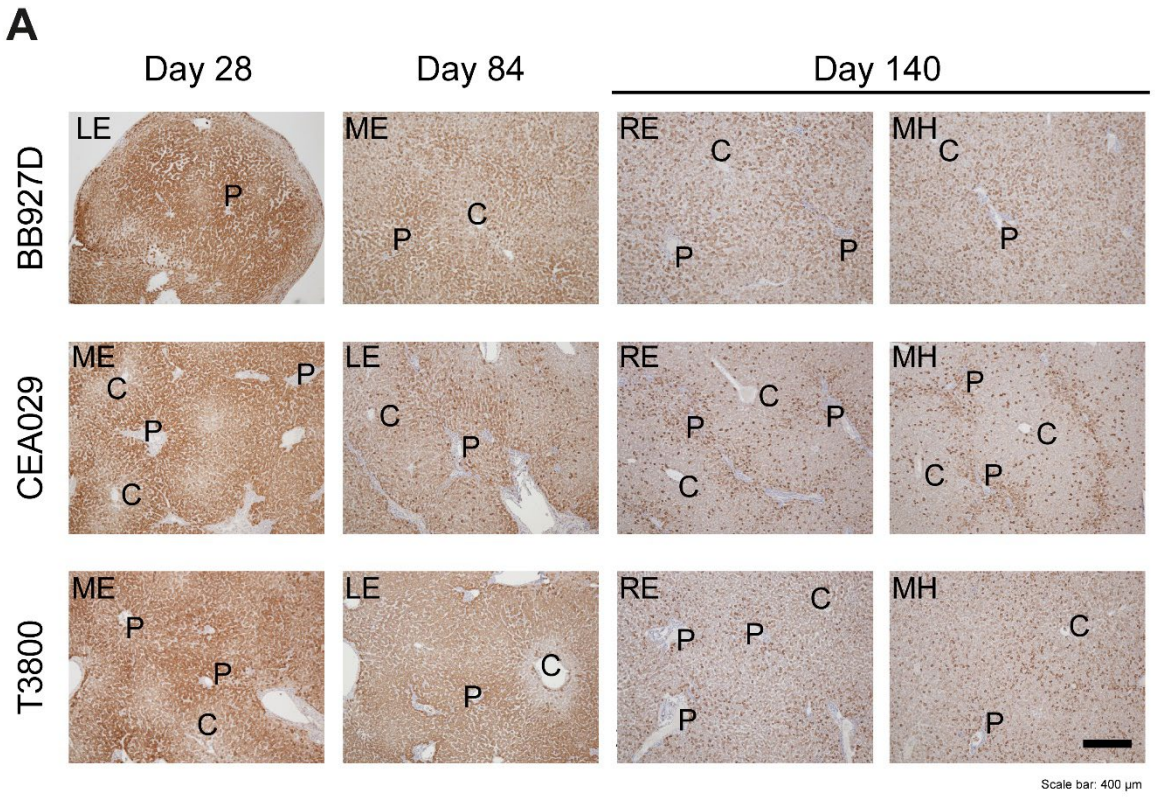
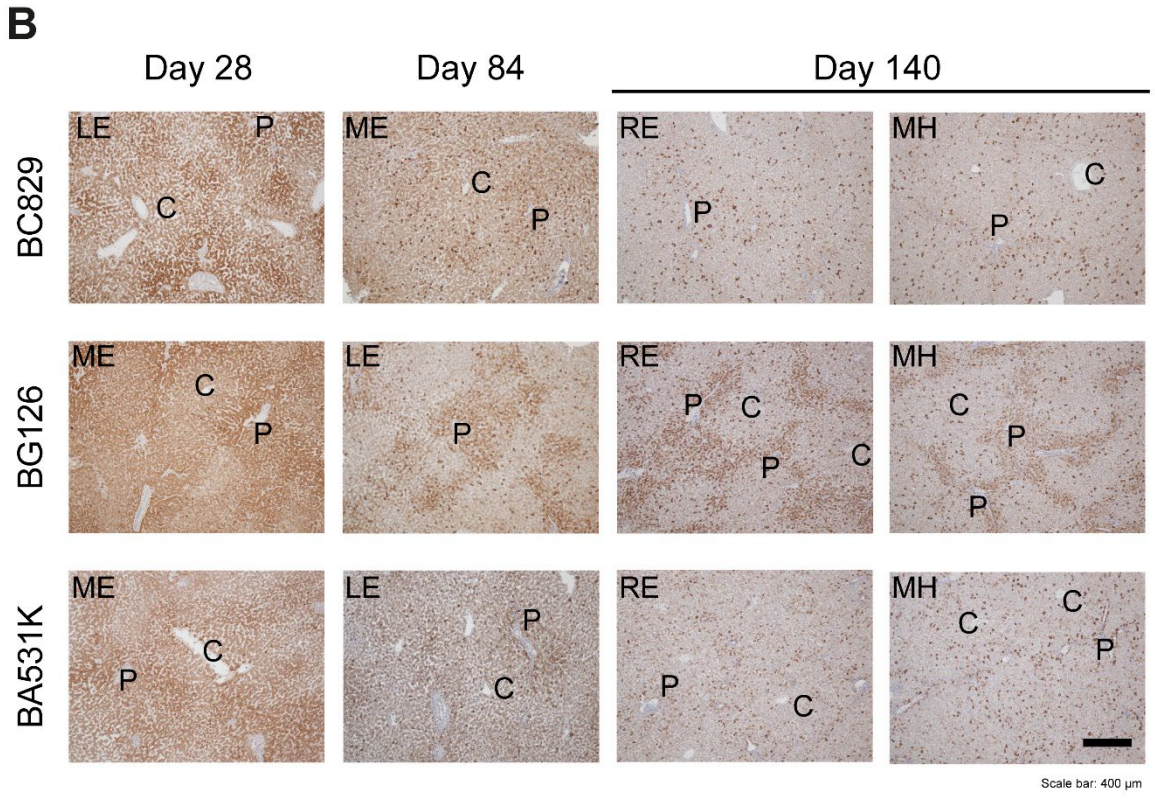
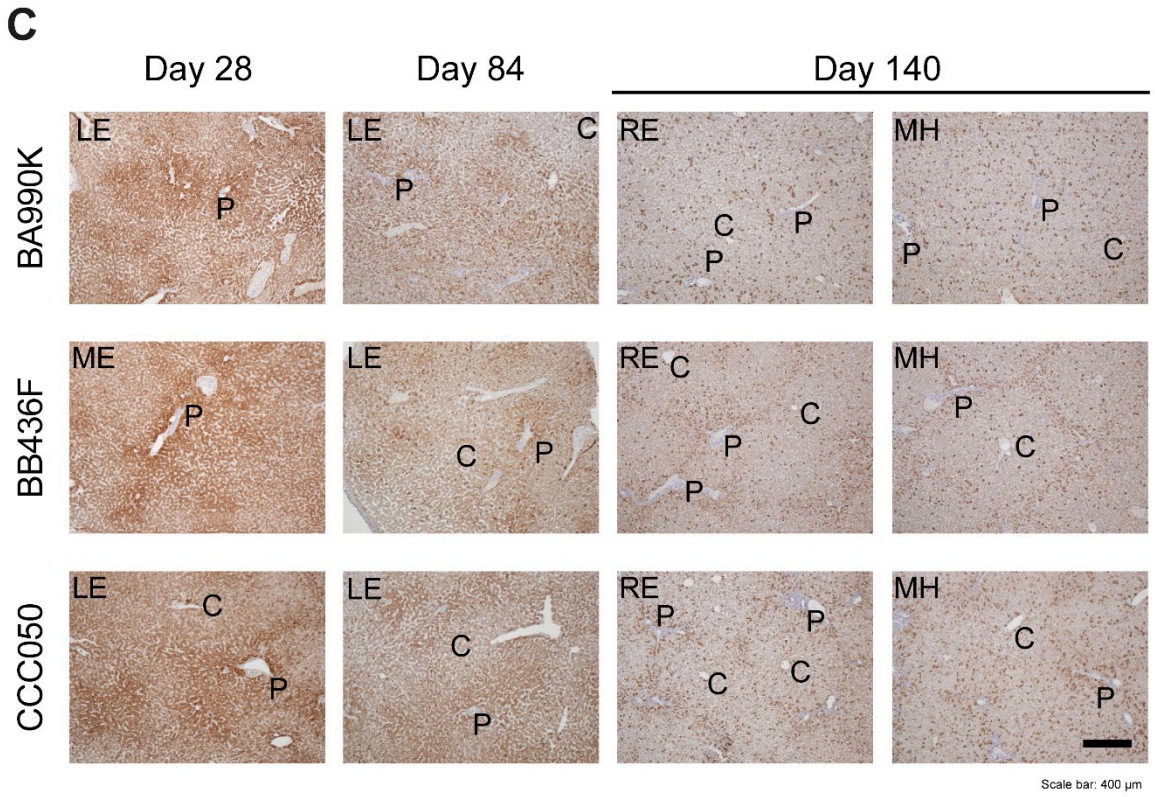

D
Day 28
Day 84
Day 140
BG407
BQ652
CCG015
LE
C
P
CH
RE
CH*
LE*
MH
Scale bar: 400 µm

Figure S6. hOTCco expression in the liver by In Situ Hybridization. Transgene expression by hepatocytes was analyzed in liver sections of individual cynomolgus macaques for (A) M (No IS), (B) F (No IS), (C) M (IS) and (D) F (IS) by performing In Situ Hybridization (ISH) at day 28, day 84 and day 140. ISH images (4x) are shown for each animal. Central areas are labelled with C and portal regions are labelled with P. Scale bar indicates 400 μm. *ISH images for animal BQ652 are from day 103 when this animal was necropsied. CH, caudate hilus; F, female; IS, immunosuppression; LE, left edge; M, male; ME, medial edge; MH, medial hilus; RE, right edge. See also Figure 3 and Figure S8.


A
Day 28
Day 84
Day 140
BB927D
CEA029
T3800
LE
ME
RE
MH
P
C
Scale bar: 400 µm


B
Day 28
Day 84
Day 140
BC829
BG126
BA531K
LE
ME
RE
MH
P
C
Scale bar: 400 µm


C
Day 28
Day 84
Day 140
BA990K
BB436F
CCC050
LE
ME
RE
MH
P
C
Scale bar: 400 µm

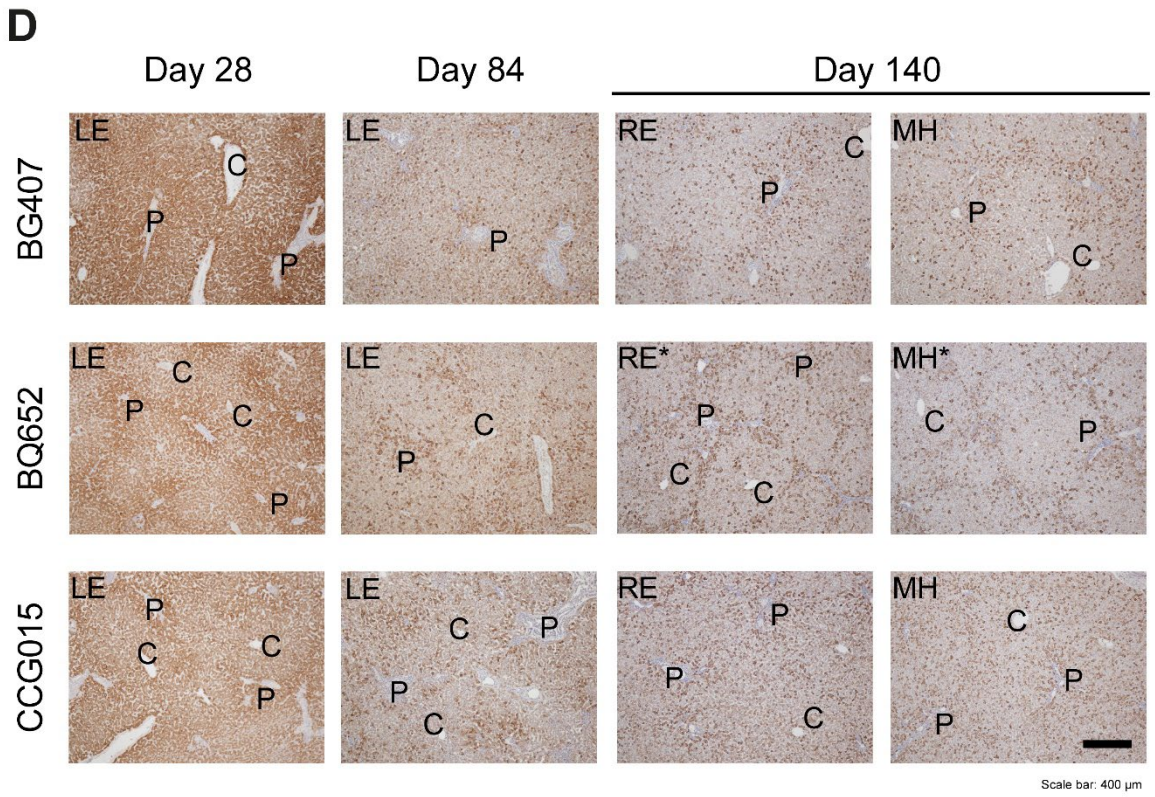

D
Day 28
Day 84
Day 140
BG407
BQ652
CCG015
LE
RE*
MH*
P
C
Scale bar: 400 µm

Figure S7. hOTC expression in the liver by immunohistochemistry. Transgene expression by hepatocytes was analysed in liver sections of individual cynomolgus macaques for (A) M (No IS), (B) F (No IS), (C) M (IS) and (D) F (IS) by performing IHC) at day 28, day 84 and day 140. IHC images (4x) are shown for each animal. Central areas are labelled with C and portal regions are labelled with P. Scale bar indicates 400 μm. *IHC images for animal BQ652 are from day 103 when this animal was necropsied. F, female; IS, immunosuppression; LE, left edge; M, male; ME, medial edge; MH, medial hilus; RE, right edge. See also Figure 4.

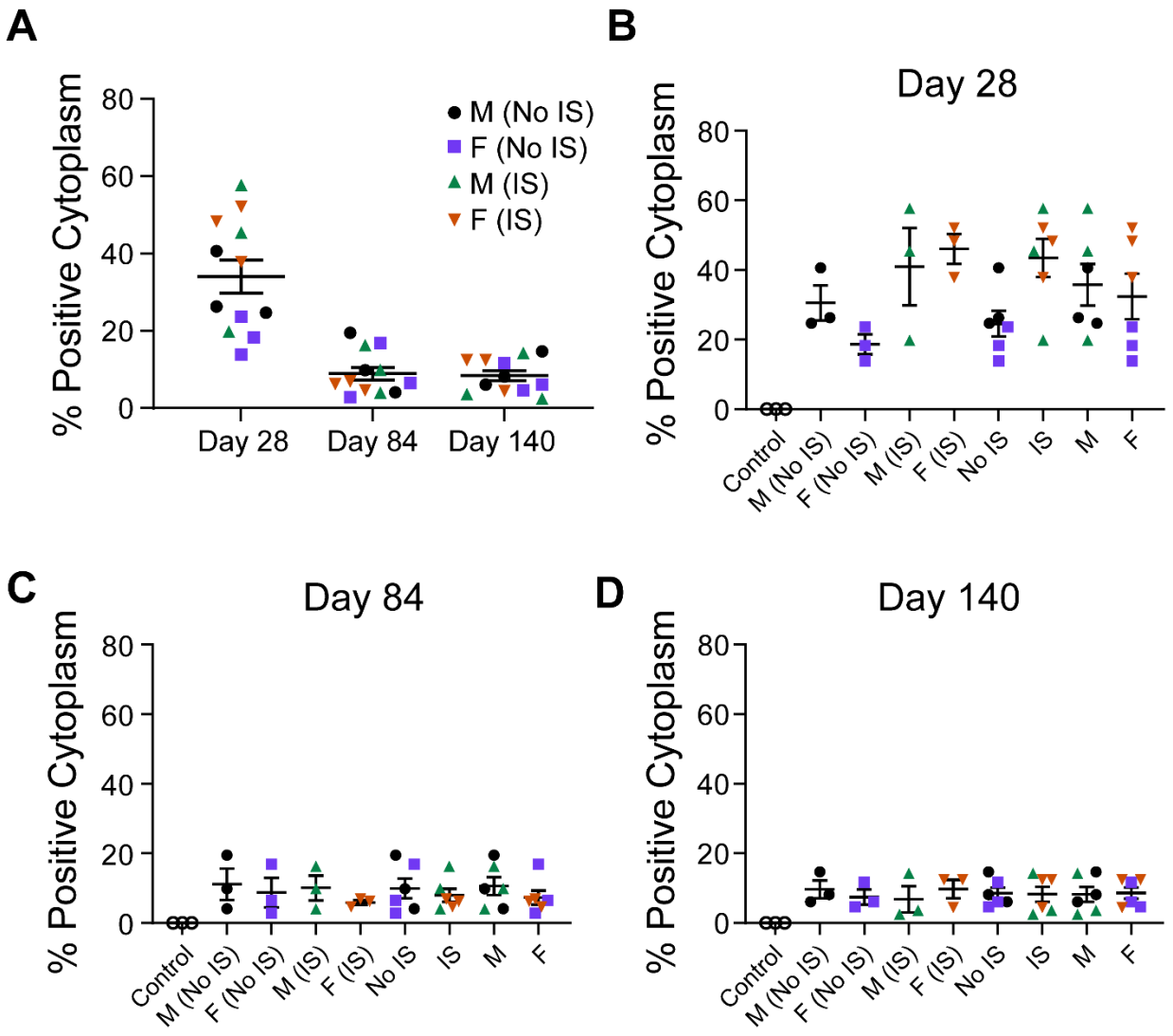


Figure S8. hOTCco expression in the liver at different time points by In Situ Hybridization. Transgene expression by hepatocytes was analyzed in liver sections of individual cynomolgus macaques by performing In Situ Hybridization (ISH) at day 28, day 84 and day 140. hOTCco expression was analyzed using computer morphology analysis. Proportion of hepatocytes with positive cytoplasm is shown by day (A) and for individual groups (B – D). For day 28 and day 84, data are represented as individual values for each animal with mean ± SEM. For day 140, data are represented as the mean of 2 lobe values for each animal with mean ± SEM. F, female; IS, immunosuppression; M, male. See also Figure 3 and Figure S6.

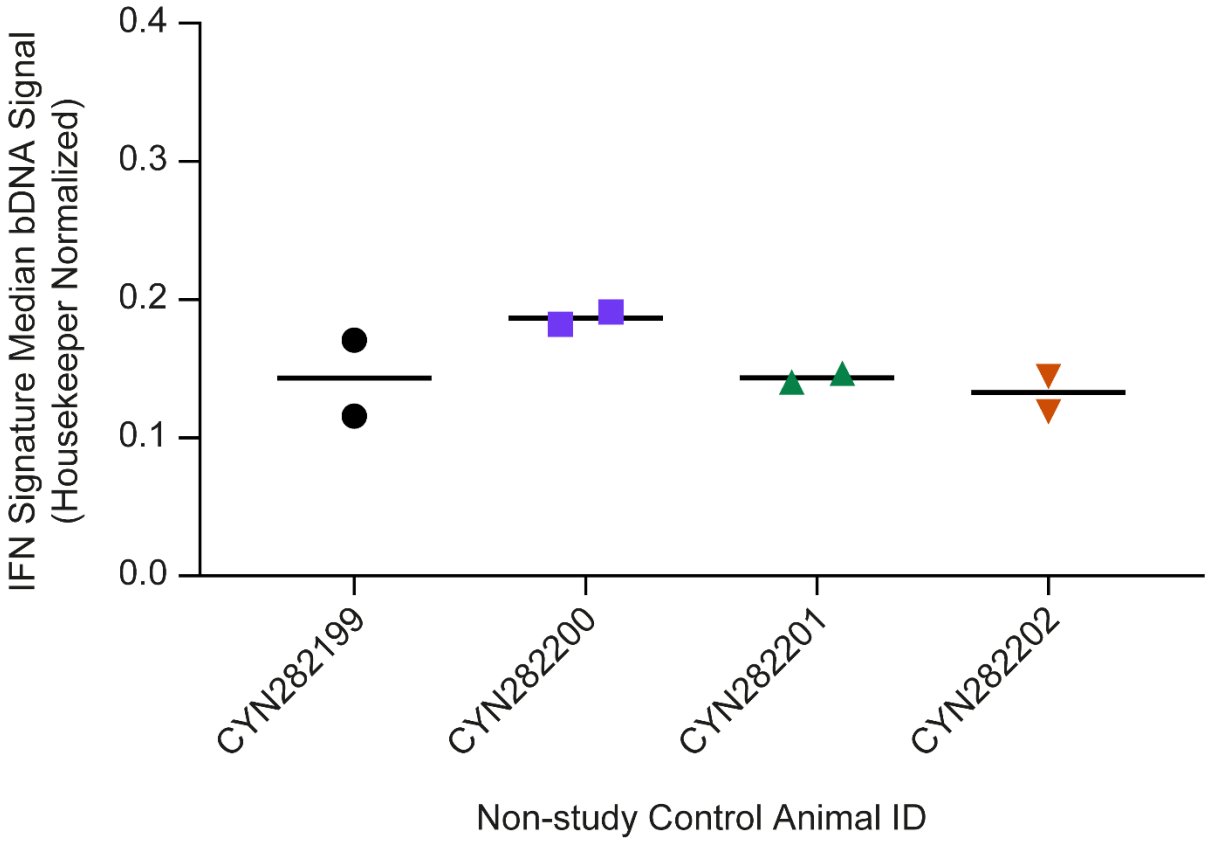


Figure S9. Inter-lobe IFN signature concordance within control liver samples. The hepatic IFN gene signature was evaluated in 4 control cynomolgus macaques by determining the expression of 21 IFN-driven genes in liver lysates using a branched (b)DNA assay. Shown is the median IFN signature bDNA signal normalized to expression of housekeeping genes for the left and right liver lobes (mean shown as horizontal lines). See also Figure 5.

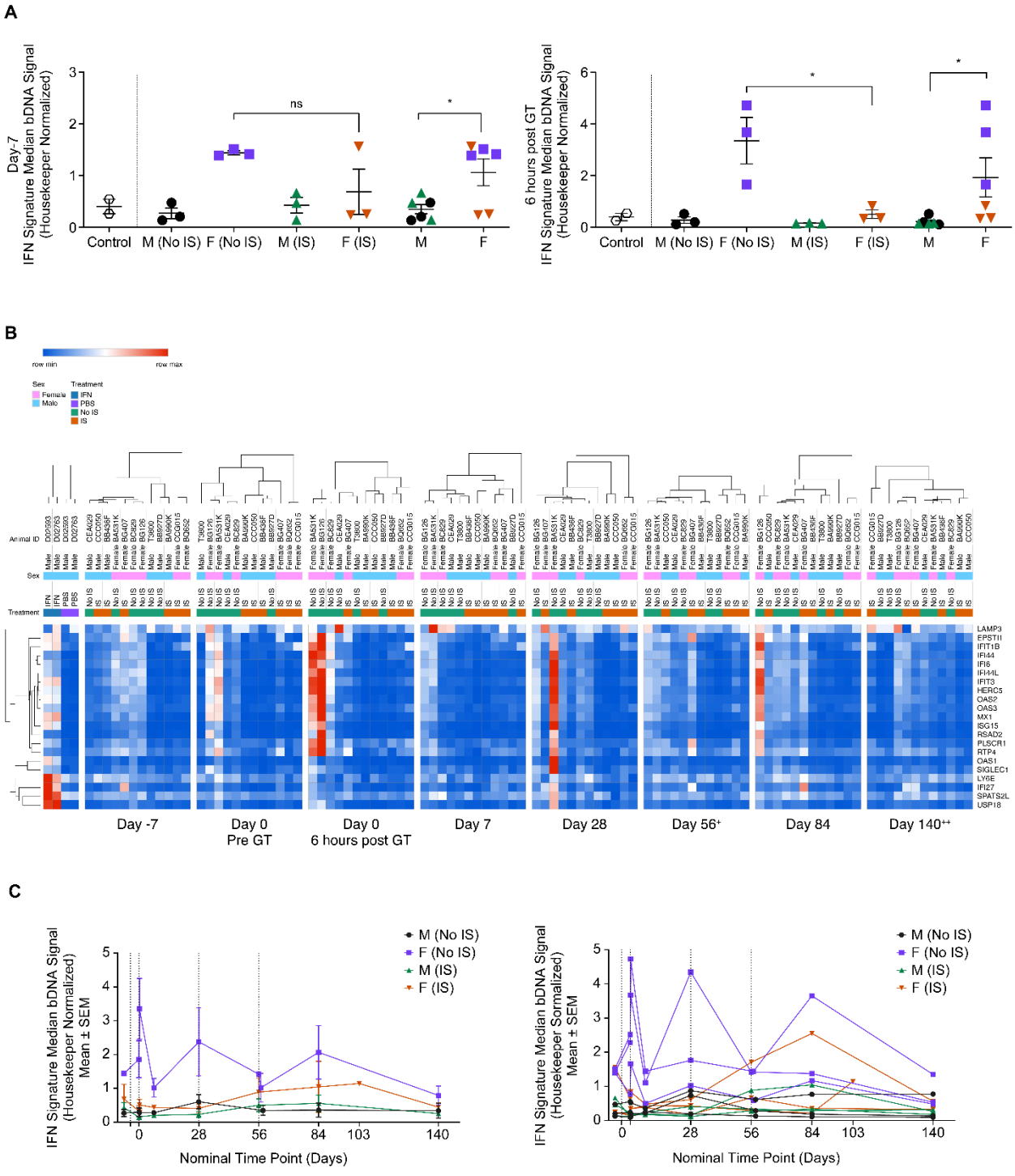


Figure S10. Analysis of blood IFN signature. The blood IFN gene signature was evaluated in individual cynomolgus macaques by determining the expression of 21 IFN-driven genes in whole blood using a bDNA assay. (A) Blood IFN gene signature by group at day -7 (left panel) and day 0 (6 hours post-vector administration) (right panel) for the study animals alongside non-study control blood samples. Data are represented as mean ± SEM of the median IFN signature bDNA signal normalized to expression of housekeeping for each animal. (B) Blood IFN gene signature heatmaps showing all 21 genes at day 0 (pre- and 6 hours post-vector administration), day 7, day 28, day 56, day 84 and day 140. [+]Animal BG126 and BC829 evaluated at day 57; animal CEA029, T3800 and BB927D

evaluated at day 58. ++Animal BQ652 evaluated at day 103. (C) Blood IFN gene signature at the indicated time points. Left panel: data are represented as mean ± SEM of the median IFN signature bDNA signal for each group. Right panel: median IFN signature bDNA signal for individual animals. Statistical analysis was performed using an unpaired t test. ns, not significant, *$P < 0.05$. F, female; GT, gene therapy; IS, immunosuppression; M, male. See also Figure S11.

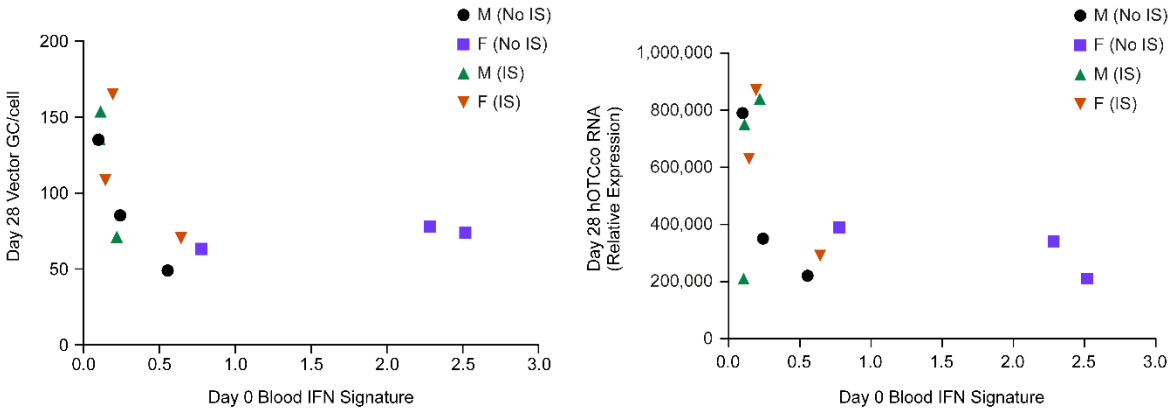


Figure S11. Analysis of baseline blood IFN signature with liver vector GC/cell and hOTCco mRNA at day 28. The blood IFN gene signature of individual macaques at baseline (day 0) was plotted against vector DNA (left panel) and hOTCco mRNA (right panel) at day 28. See also Figure S9. F, female; GC, genome copies; IS, immunosuppression; M, male. See also Figure S10.

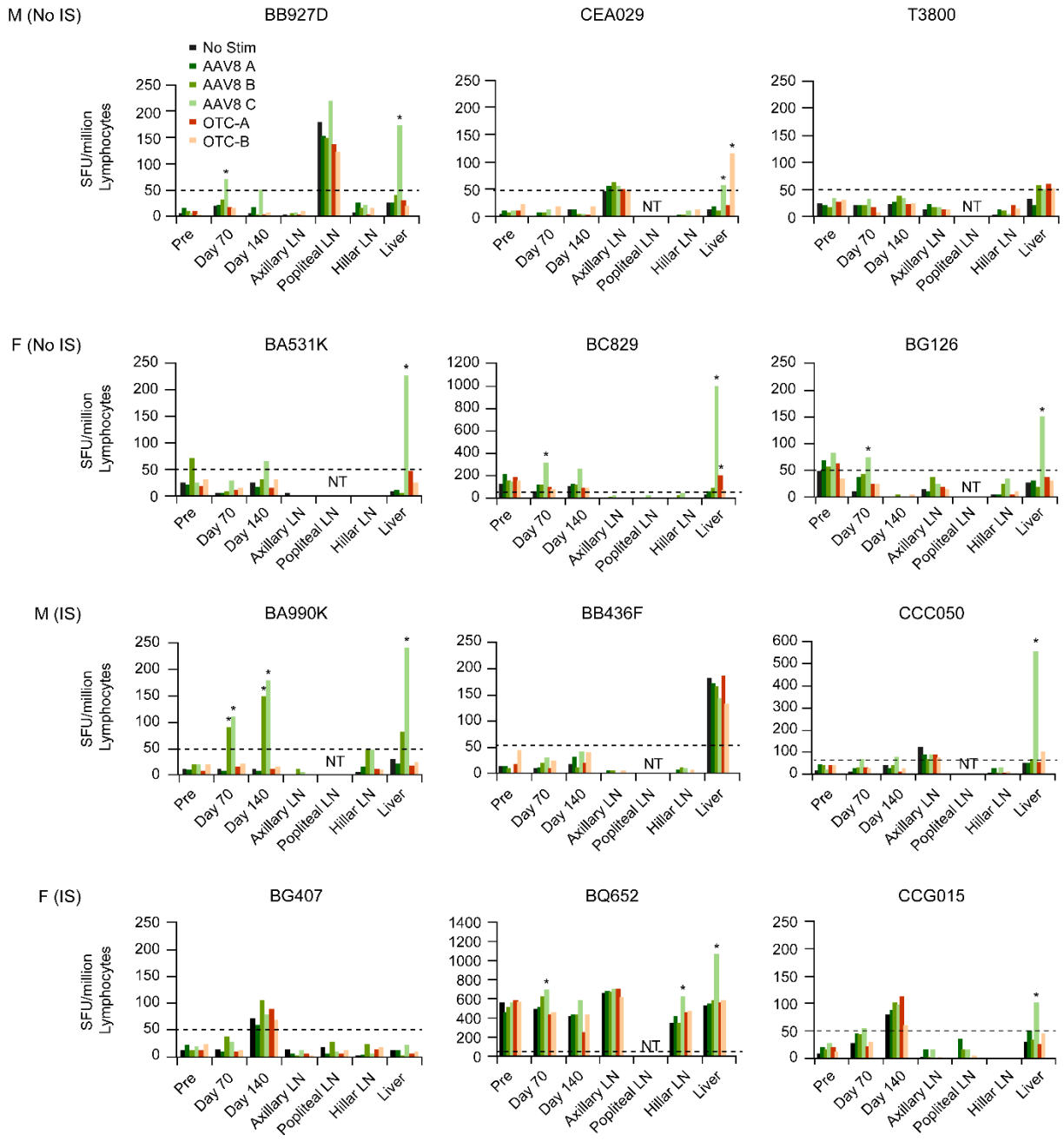


Figure S12. ELISpot analysis of liver lymphocytes and control tissues. The IFN γ cellular response to AAV8 capsid and hOTC protein was evaluated in PBMCs, lymph nodes (axillary, popliteal, and hilar), and the liver of individual cynomolgus macaques by IFN γ ELISpot. PBMCs were analysed at baseline (day -7), day 70 and day 140; lymph nodes and liver were analysed at day 140 (animal BQ652 was euthanized before study end and was evaluated at day 103). The AAV8 capsid peptide library was divided into 3 peptide pools (A, B, and C) and hOTC was split into 2 peptide pools (A and B). * indicates positive response which is arbitrarily defined as greater than 55 spot-forming units (SFU) per million cells and at least 3 times greater than the non-stimulated control values. F, female; IS, immunosuppression; LN, lymph node; M, male; NT, not tested.

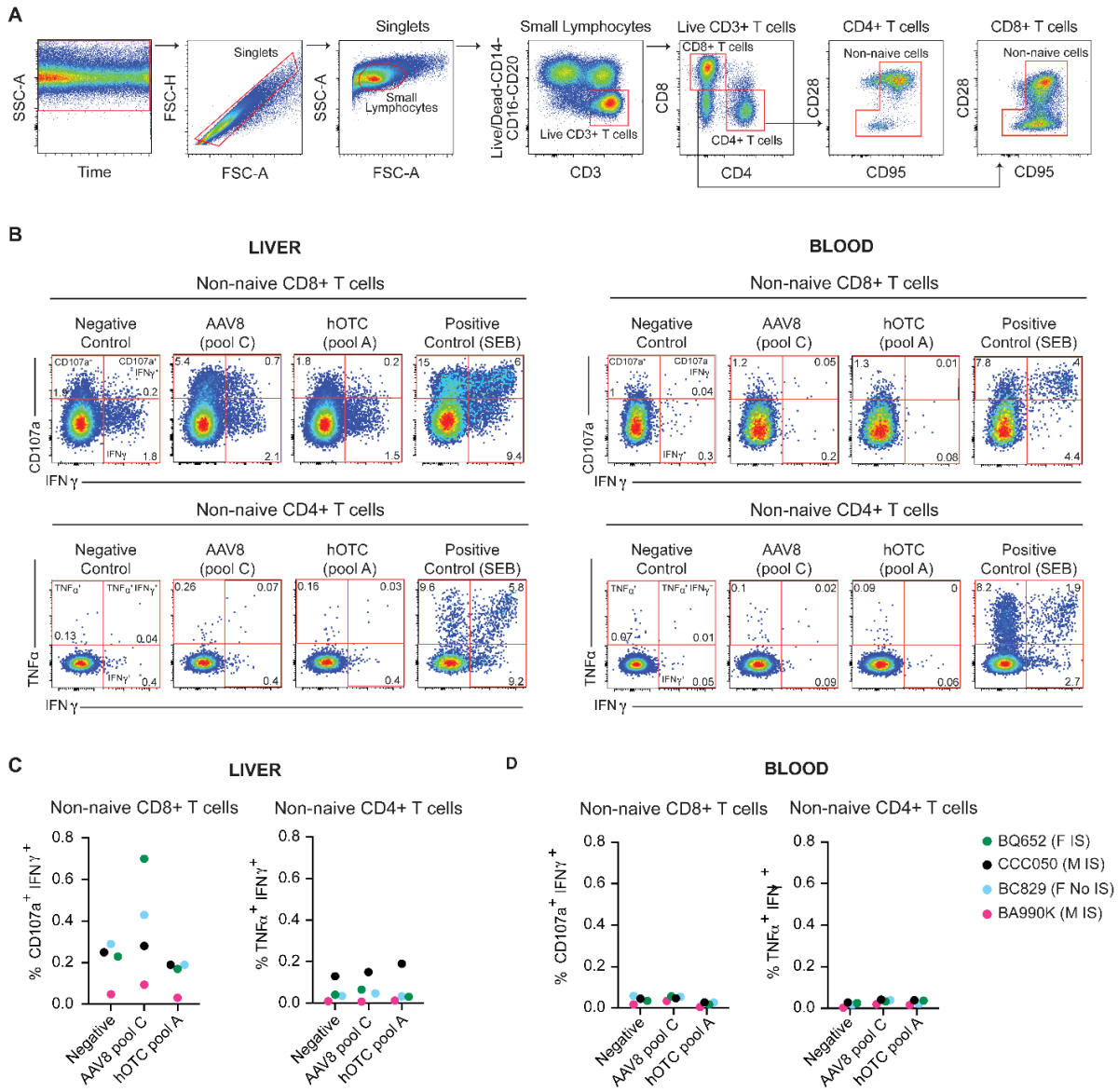


Figure S13. Flow cytometric analysis of liver and blood T cells from animals showing elevated ELISpot counts. (A) Representative flow plots showing the gating strategy used to analyze T cell responses. The most stable acquisition was first selected. Forward scatter height (FSC-H)-versus-forward scatter area (FSC-A) and side scatter area (SSC-A)-versus-FSC-A plots were used to exclude doublets and focus on singlet small lymphocytes. Dead cells were excluded by gating on cells negative for the viability marker Aqua Blue. Monocytes, B cells and NK cells were excluded via the CD14/20/16 dump gate. CD4+ and CD8+ T lymphocytes were gated within CD3+ cells. To determine the memory phenotype, CD28 versus CD95 were used, and naïve T cells (CD28+CD95-) were excluded from the analysis. (B) Flow cytometry plots from cynomolgus BQ652 showing, for each condition, the frequency of CD107a+ or IFNγ+ or double positive CD107a+IFNγ+ cells within non-naive CD8+ T cells; and TNFα+ or IFNγ+ or double positive TNFα+IFNγ+ cells within non-naïve CD4+ T cells in liver or blood at day 103. The numbers indicate the frequency within the parent population. (C) Collated data showing the CD8+ and CD4+ T cell responses against AAV8 peptide pool C and hOTC peptide pool A in liver at day 103 for cynomolgus BQ652 or at day 140 for BA990K, BC829 and CCC050. (D) Collated data showing the CD8+ and CD4+ T cell responses against AAV8 peptide pool C and hOTC peptide pool A in blood at day 103 for cynomolgus BQ652 or at day 140 for BA990K, BC829 and CCC050. SEB, Staphylococcus Enterotoxin B.

Table S1: Cellular composition of liver samples at Days 28, 84, and 140.

Biopsy 1	**Day 28**			
Immunosuppression	Control (none)		Prednisolone	
Sex	Male	Female	Male	Female
Number of animals evaluated per group	3	3	3	3
Liver				
No. examined	3	3	3	3
No. abnormalities detected	0	0	2	0
Infiltrate; mononuclear cell				
Grade 1	-	3	1	2
Grade 2	2	-	-	1
Necrosis; individual hepatocellular, periportal				
Grade 1	1	-	-	-
Hypertrophy; hepatocellular, with dissociation, diffuse				
Grade 1	-	-	-	1
Multinucleation; hepatocellular, centrilobular, focal				
Grade 1	-	-	-	1
Fibrosis; capsule, regional				
Grade 1	-	1	-	1
Grade 2	1	-	-	-
Fibrosis; capsule and subcapsular, regional				
Grade 3	-	1	-	-
Decreased, portal and centrilobular areas; focally extensive				
Grade 2	1	-	-	-
Biopsy 2	**Day 84**			
Immunosuppression	Control (none)		Prednisolone	
Sex	Male	Female	Male	Female
Number of animals evaluated per group	3	3	3	3
Liver				
No. examined	3	3	3	3
No. abnormalities detected	0	0	0	0
Infiltrate; mononuclear cell				
Grade 1	1	3	3	-
Grade 2	1	-	-	1
Grade 3	-	-	-	1
Necrosis; individual hepatocellular, periportal				
Grade 1	-	-	-	1
Multinucleation; hepatocellular, multifocal				
Grade 2	1	-	-	2
Fibrosis; capsule, regional				
Grade 1	-	-	1	-
Fibrosis; capsule, diffuse				
Grade 2	2	-	1	-
Fibrosis; capsule and subcapsular, regional				

Grade 3	-	-	-	1
Decreased, portal and centrilobular areas; focally extensive				
Grade 4	1	-	-	1
Decreased, lobular size; subcapsular, regional				
Grade 2	-	-	-	1
Necropsy	**Day 140**[a]			
Immunosuppression	Control (none)		Prednisolone	
Sex	Male	Female	Male	Female
Number of animals evaluated per group	3	3	3	3[a]
Liver (Necropsy)				
No. liver lobes examined (hilus and edge sections for caudate, left, middle, right)	24	24	24	24
No. abnormalities detected	0	0	0	0
Infiltrate; mononuclear cell				
Grade 1	5	1	4	-
Grade 2	19	23	20	24[a]
Multinucleation; hepatocellular, multifocal				
Grade 2	1	-	6	-
Fibrosis/fibrovascular tissue; capsule +/- subcapsular				
Grade 1	5	9	4	10
Grade 2	3	2	1	5[a]
Grade 3	1	-	-	-
Inflammation; pigmented macrophages, capsule, focally extensive (note: with intracellular foreign material, hemosiderophages and multinucleated cells)				
Grade 2	-	1	-	-
Atypical proliferation; epithelial, subcapsular, focal				
Grade 2	-	1	-	-

[a]Animal BQ652 was necropsied at day 103 due to procedural-related complications.