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Supplemental information

Natural history of Ebola virus disease in rhesus monkeys shows viral variant emergence dynamics and tissue-specific host responses

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Supplemental Figures

Supplemental figures for “Natural history of Ebola virus disease in rhesus monkeys shows viral variant emergence dynamics and tissue specific host responses” by Normandin et al.

Figures Contents

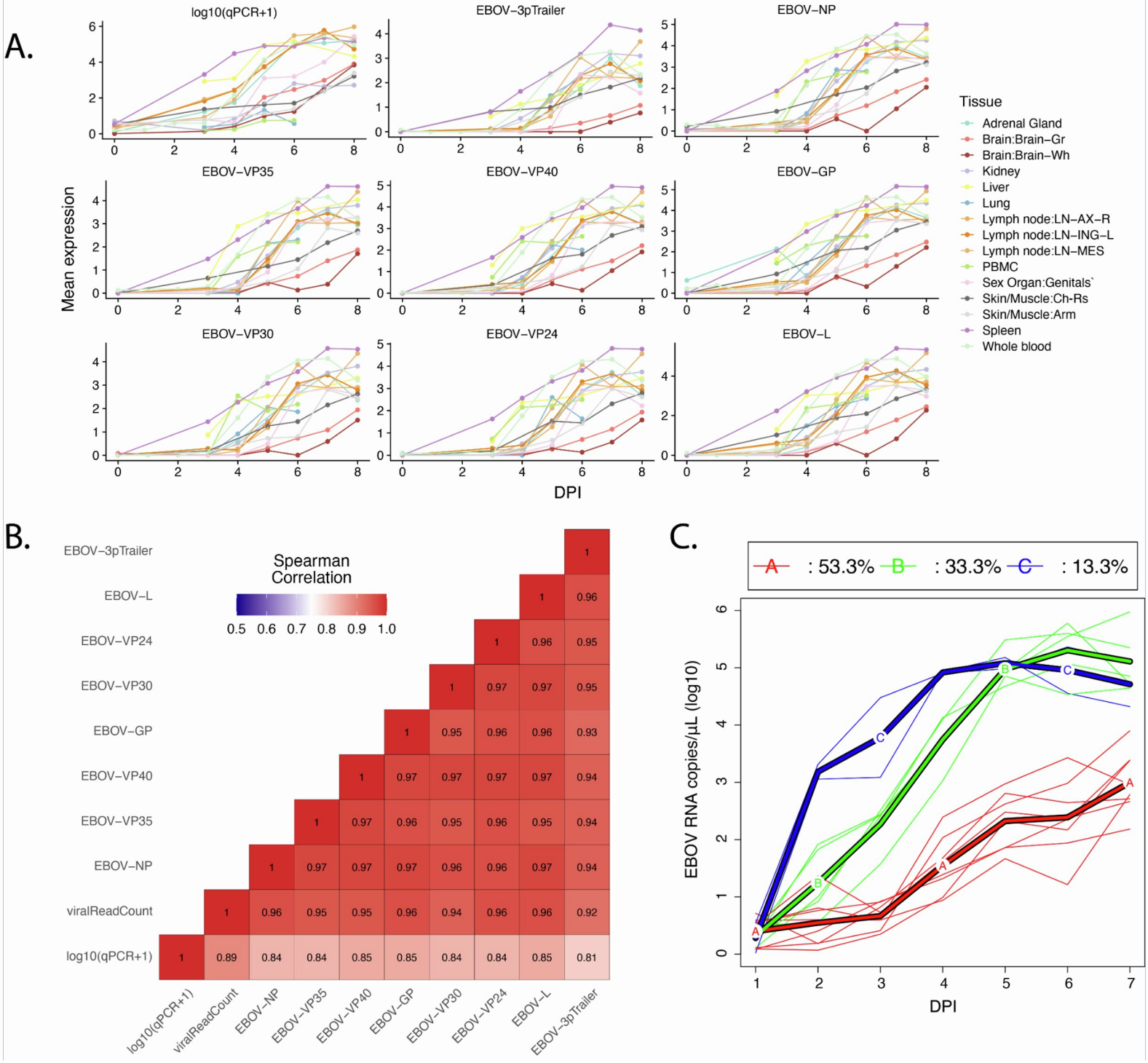


Figure S1. qPCR quantification compared to viral read counts, related to figure 1. (A) Mean expression of viral genes across tissues and DPI (B) Heatmap showing spearman correlations between each viral gene, the total viral read count and the qPCR quantification of GP RNA. (C) Longitudinal K-mean clustering of qPCR data.

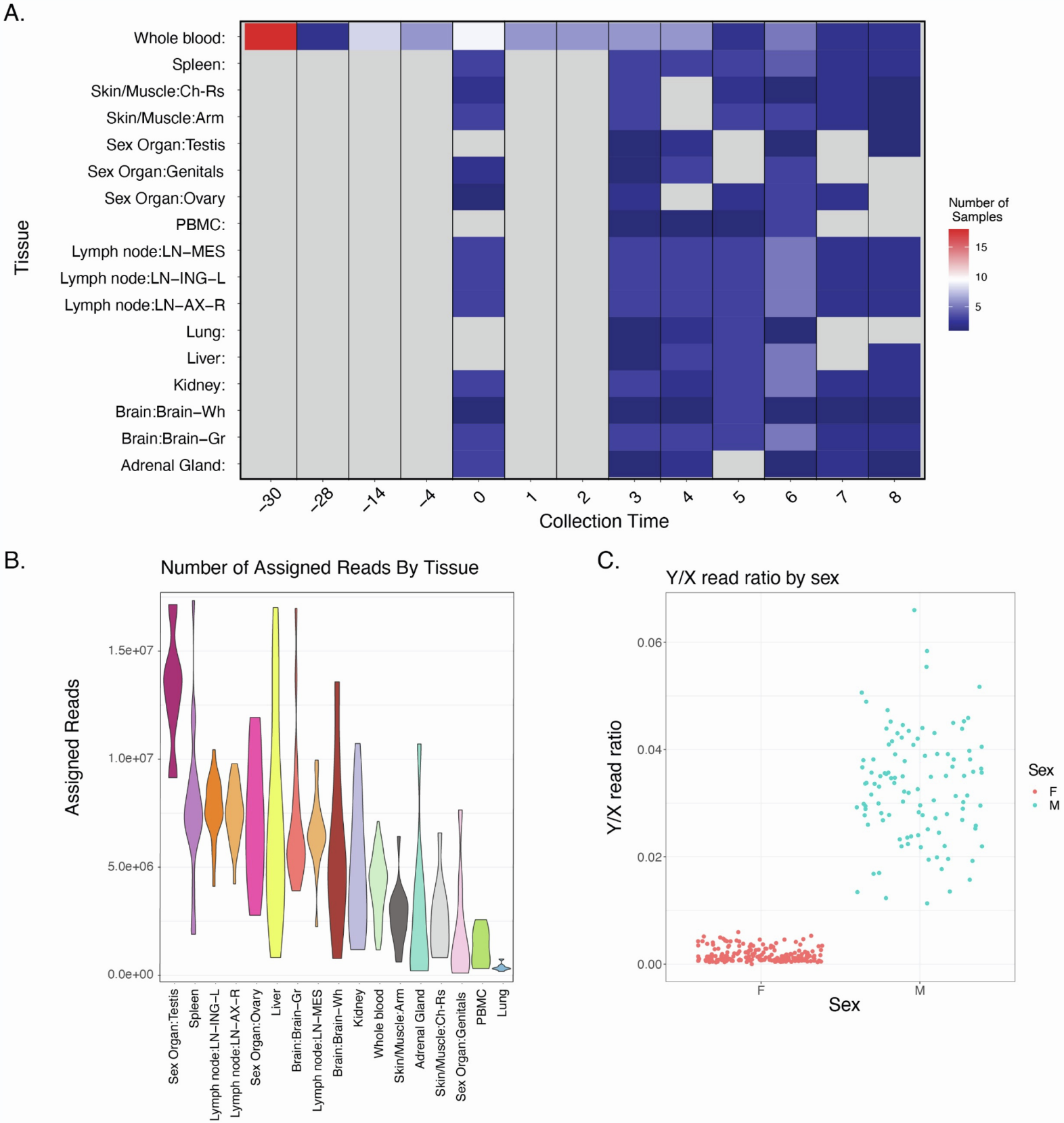


Figure S2. Overview of samples profiled, related to figure 1. (A) Sequenced sample count per tissue and collection time (B) Violin plots of total assigned reads per tissue. (C) Chromosome X and Y ratio per sample.

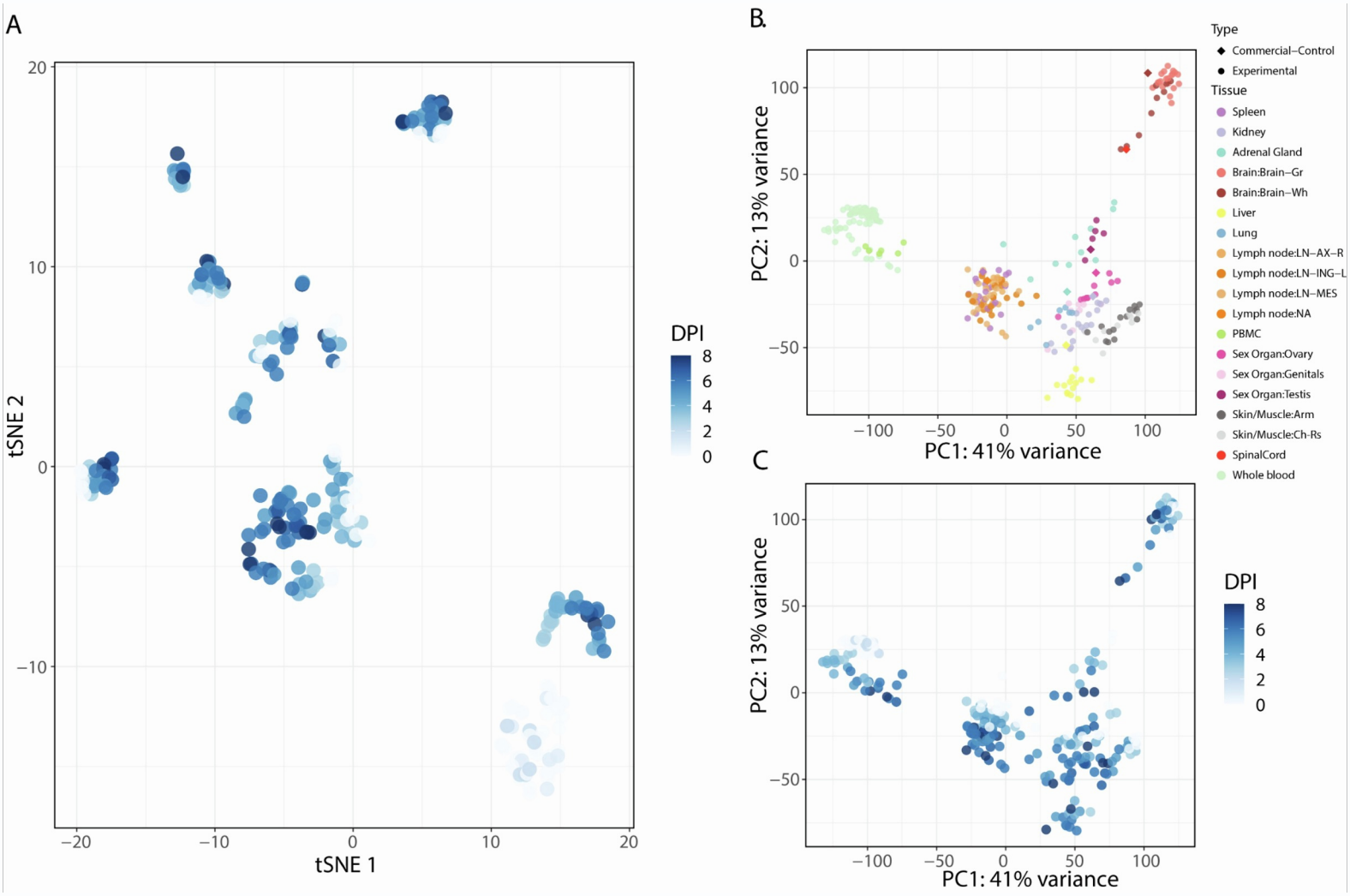
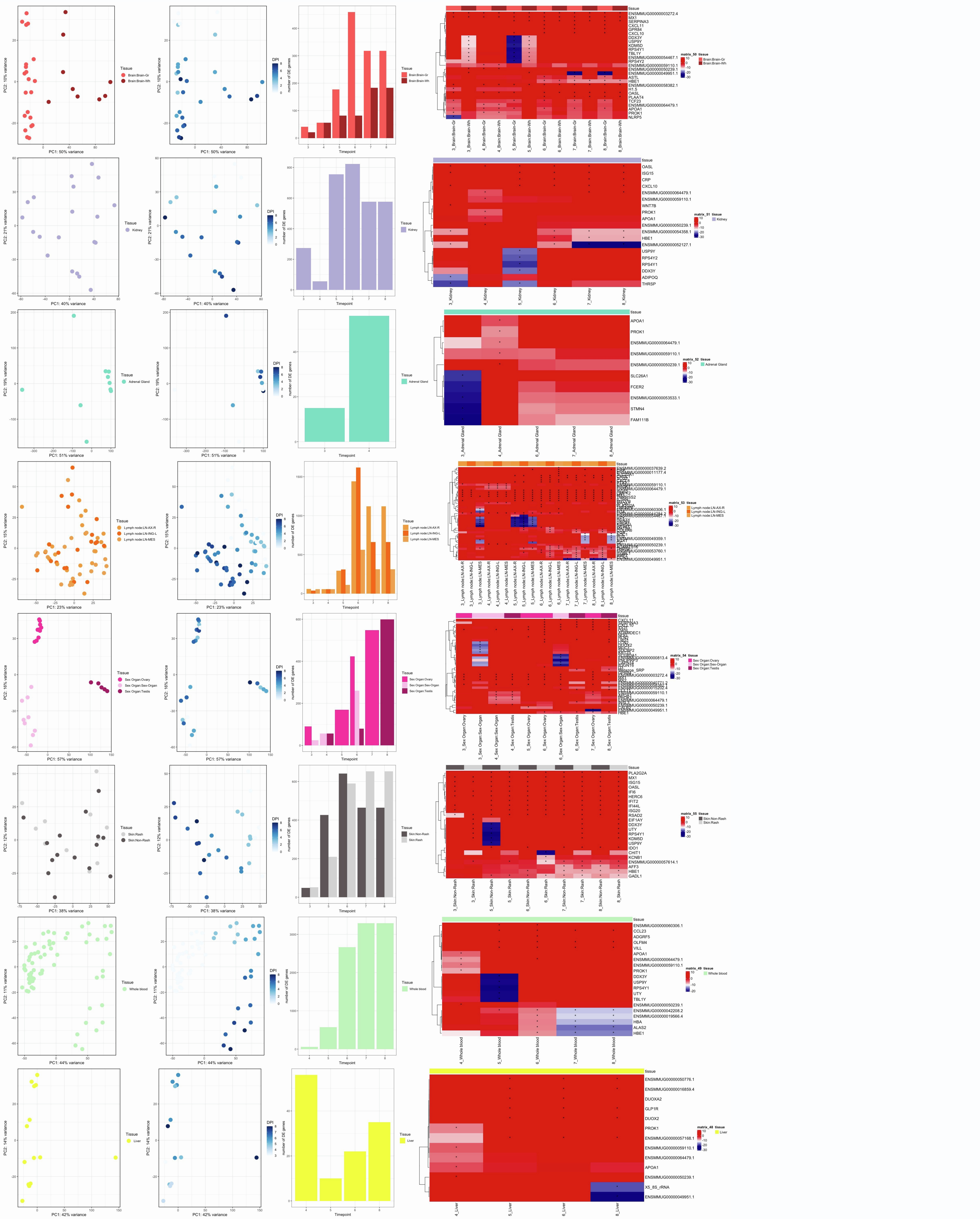


Figure S3. Host transcriptome dimensionality reduction, related to figure 1. (A) tSNE plot of transcriptional signatures colored by DPI. PCA of transcriptional profiles colored by (B) sample type and (C) DPI.



PC1: 50% variance
PC2: 10% variance
Tissue
Brain:Brain-Gr
Brain:Brain-Wh
DPI
number of DE genes
Timepoint
tissue
ENSMMUG00000003272.4
MX1
SERPINA3
CXCL11
GPR84
CXCL10
DDX3Y
USP9Y
KDM5D
RPS4Y1
TBL1Y
ENSMMUG00000054467.1
RPS4Y2
ENSMMUG00000059110.1
ENSMMUG00000050239.1
ENSMMUG00000049951.1
ASTL
HBE1
ENSMMUG00000058382.1
H1.5
OASL
PLAAT4
TCF23
ENSMMUG00000064479.1
APOA1
PROK1
NLRP5
matrix_50
PC1: 40% variance
PC2: 21% variance
Kidney
OASL
ISG15
CRP
CXCL10
ENSMMUG00000064479.1
ENSMMUG00000059110.1
WNT7B
PROK1
APOA1
ENSMMUG00000050239.1
ENSMMUG00000054358.1
HBE1
ENSMMUG00000052127.1
USP9Y
RPS4Y2
RPS4Y1
DDX3Y
ADIPOQ
THRSP
matrix_51
PC1: 51% variance
PC2: 19% variance
Adrenal Gland
APOA1
PROK1
ENSMMUG00000064479.1
ENSMMUG00000059110.1
ENSMMUG00000050239.1
SLC26A1
FCER2
ENSMMUG00000053533.1
STMN4
FAM111B
matrix_52
PC1: 23% variance
PC2: 15% variance
Lymph node:LN-AX-R
Lymph node:LN-ING-L
Lymph node:LN-MES
matrix_53
PC1: 57% variance
PC2: 16% variance
Sex Organ:Ovary
Sex Organ:Sex-Organ
Sex Organ:Testis
matrix_54
PC1: 38% variance
PC2: 12% variance
Skin:Rash
Skin:Non-Rash
PLA2G2A
MX1
ISG15
OASL
IFI6
HERC6
IFIT2
IFI44L
ISG20
RSAD2
EIF1AY
DDX3Y
UTY
RPS4Y1
KDM5D
USP9Y
IDO1
CHIT1
KCNB1
ENSMMUG00000057614.1
AFF3
HBE1
GADL1
matrix_55
PC1: 44% variance
PC2: 11% variance
Whole blood
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CCL23
ADGRF5
OLFM4
VILL
APOA1
ENSMMUG00000064479.1
ENSMMUG00000059110.1
PROK1
DDX3Y
USP9Y
RPS4Y1
UTY
TBL1Y
ENSMMUG00000050239.1
ENSMMUG00000042208.2
ENSMMUG00000019566.4
HBA
ALAS2
HBE1
matrix_49
PC1: 42% variance
PC2: 14% variance
Liver
ENSMMUG00000050776.1
ENSMMUG00000016859.4
DUOXA2
GLP1R
DUOX2
PROK1
ENSMMUG00000057168.1
ENSMMUG00000059110.1
ENSMMUG00000064479.1
APOA1
ENSMMUG00000050239.1
X5_8S_rRNA
ENSMMUG00000049951.1
matrix_48

Figure S4. Expression profiles during infections across tissues related to figure 3. Right, PCA of transcriptional profiles of each tissue colored by sample type and DPI. Center, Number of differentially expressed genes between non-infected and each time point. Heatmap of Fold-changes of Top DE genes in each time point and tissue

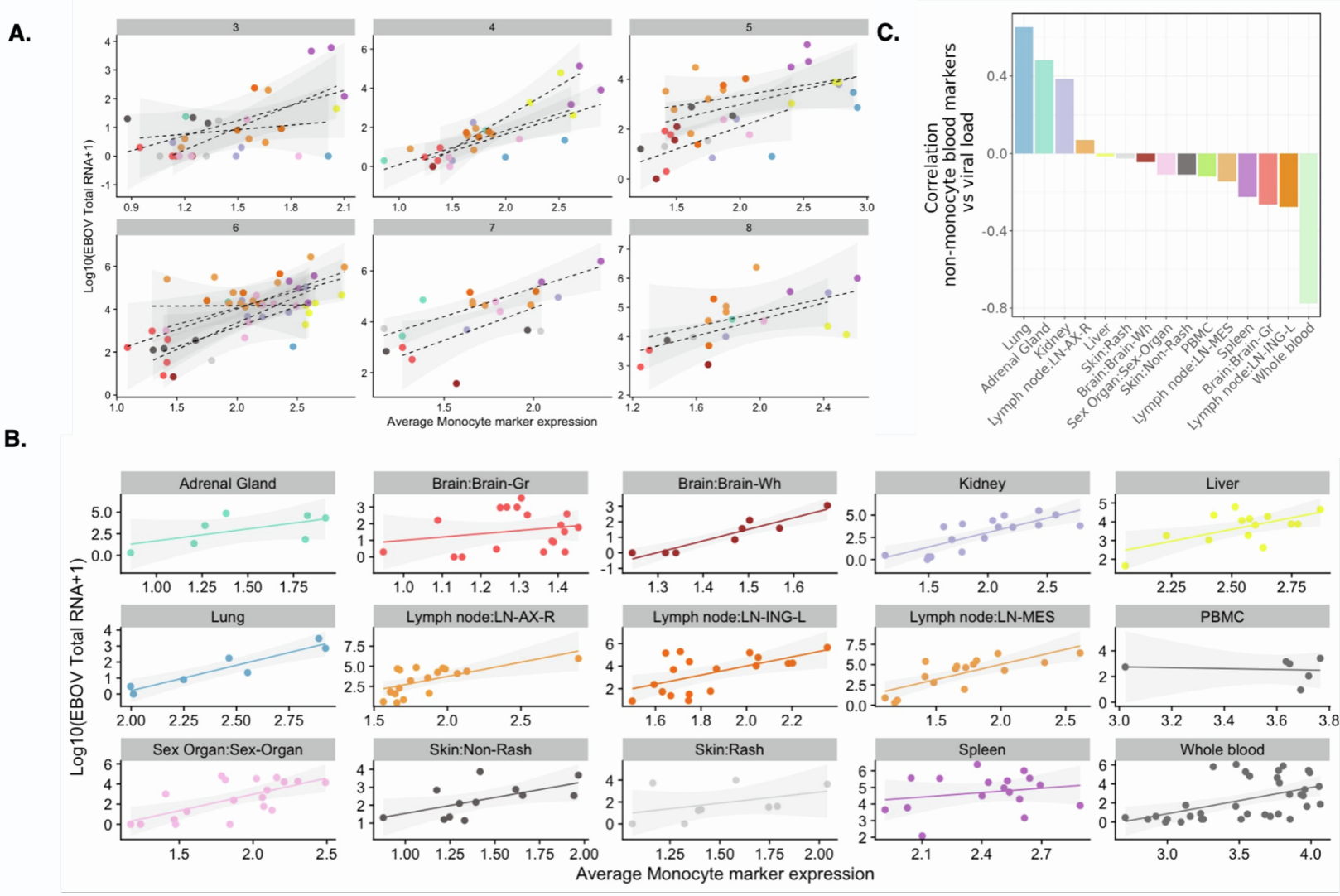

Figure S5. Viral load correlates with monocyte markers, related to figure 2. (A) Total viral counts compare to the average expression of canonical monocyte markers (*CTSS, VCAN, FCN1, CD14, S100A9*) across each time by individual (A) and by tissue (B). (C) Correlation between viral load and non-monocyte blood markers (*CD3D,HBA, SELL, PPBP, HBA,CD8A, GNLY, CD4*) expression across each tissue.

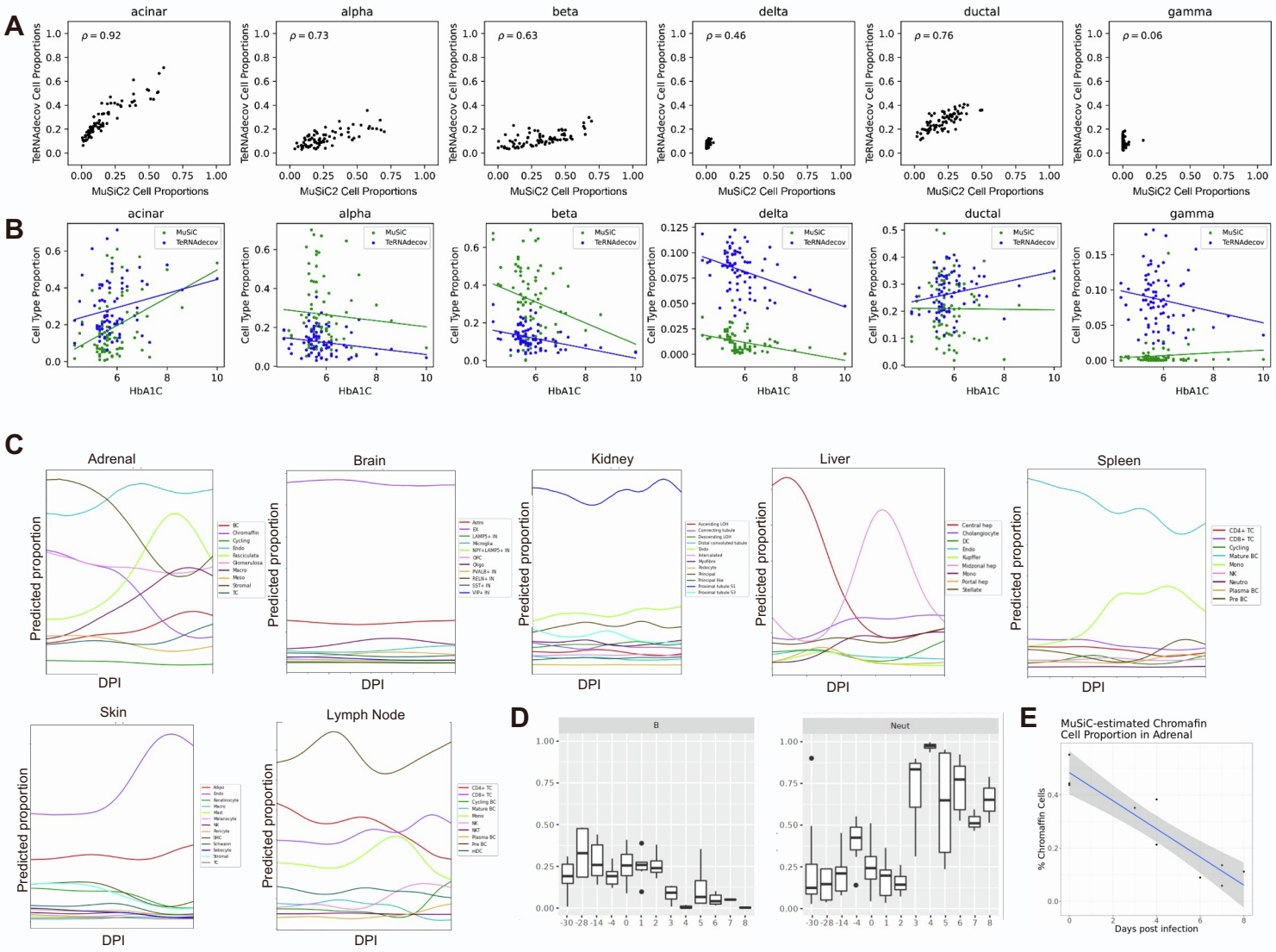

Figure S6. Cell type deconvolution of Bulk RNA-seq, related to figure 2. A) Predicted cell type proportion for pancreatic islet bulk RNA-seq data from Fadista et al using ternaDecov and MuSiC2. B) Correlation of HbA1c level and cell-type composition C) Deconvolution of predicted cell types changes across time for each tissue based on a single-cell RNAseq reference of *Macaca fascicularis*. (D) Deconvolution of Whole Blood with MuSiC confirms Neutrophil peak at 4 DPI. (E) Deconvolution of adrenal tissues using MuSiC confirms the reduction of the relative proportion of Chromaffin cells during infection.

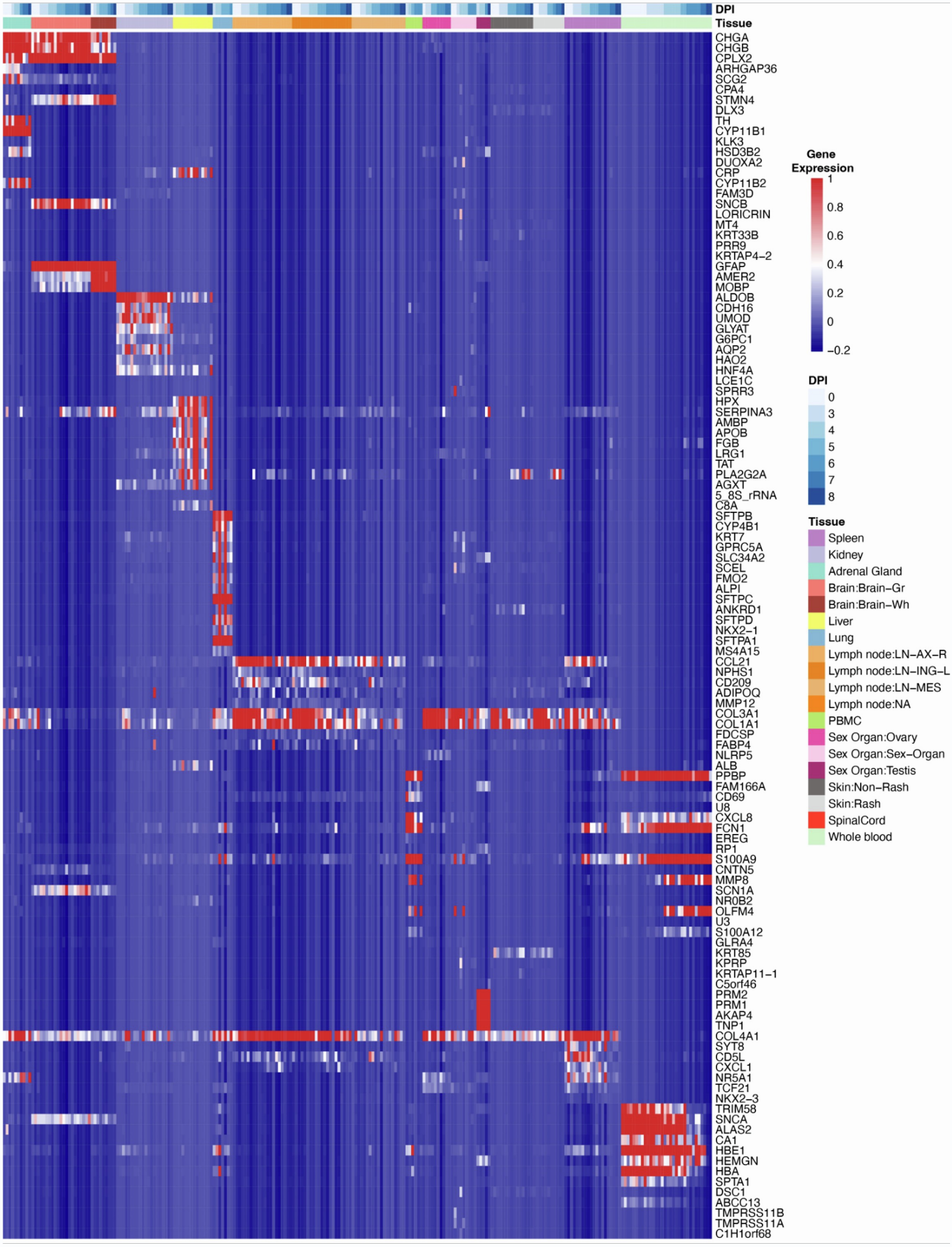

Figure S7. Tissue specific marker genes, related to figure 3. Heatmap of top specific tissue marker genes across time points.

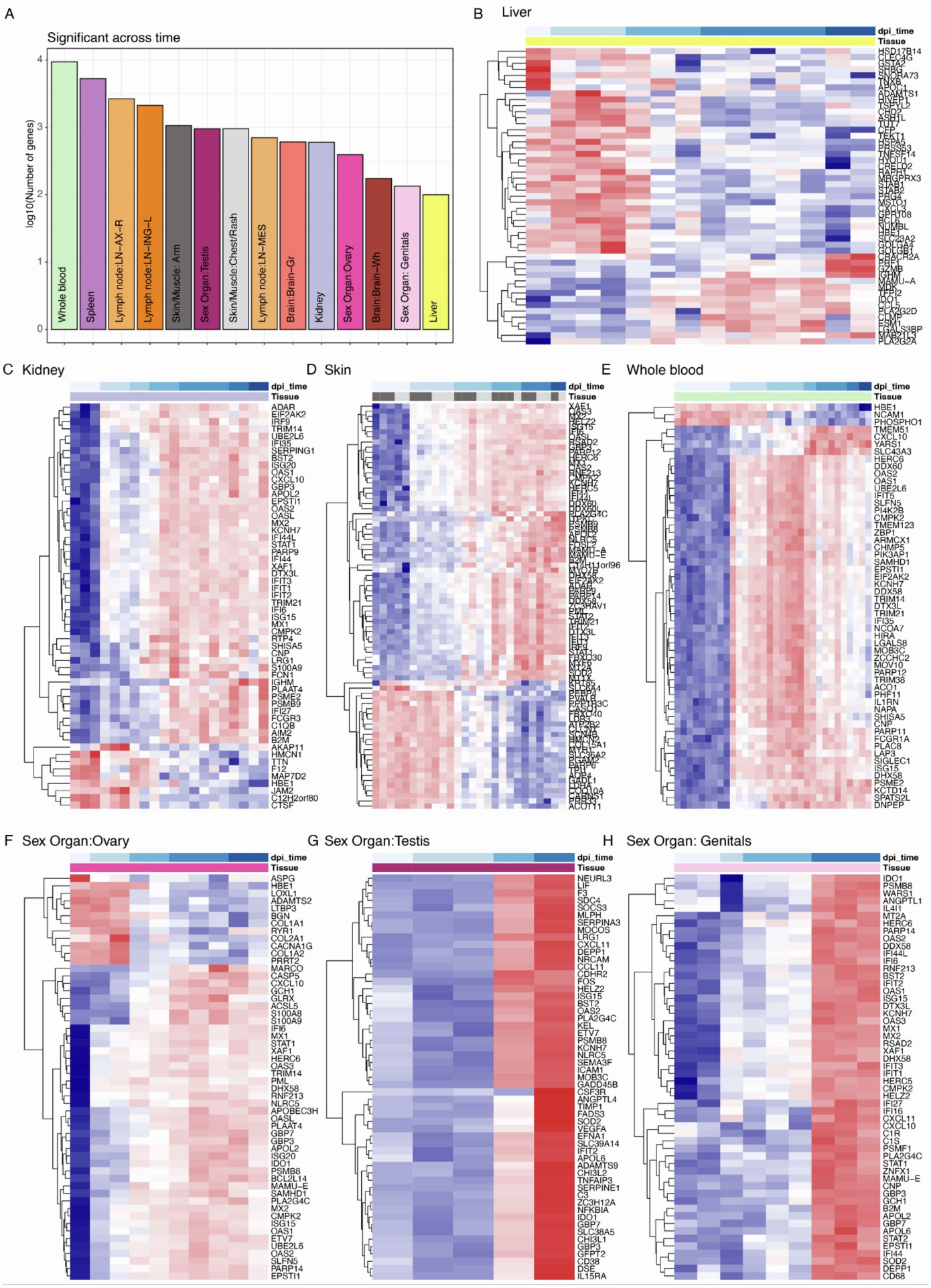

Figure S8. Genes change across time, related to figure 3. (A) Number of differentially expressed genes across time, tissues with more than 5 DE genes are shown in the plot. Heatmap of genes changing significantly across time for (B) Liver (C) Kidney (D) Skin (E) Whole Blood (F) Ovary (G) Testis and (H) Genitals

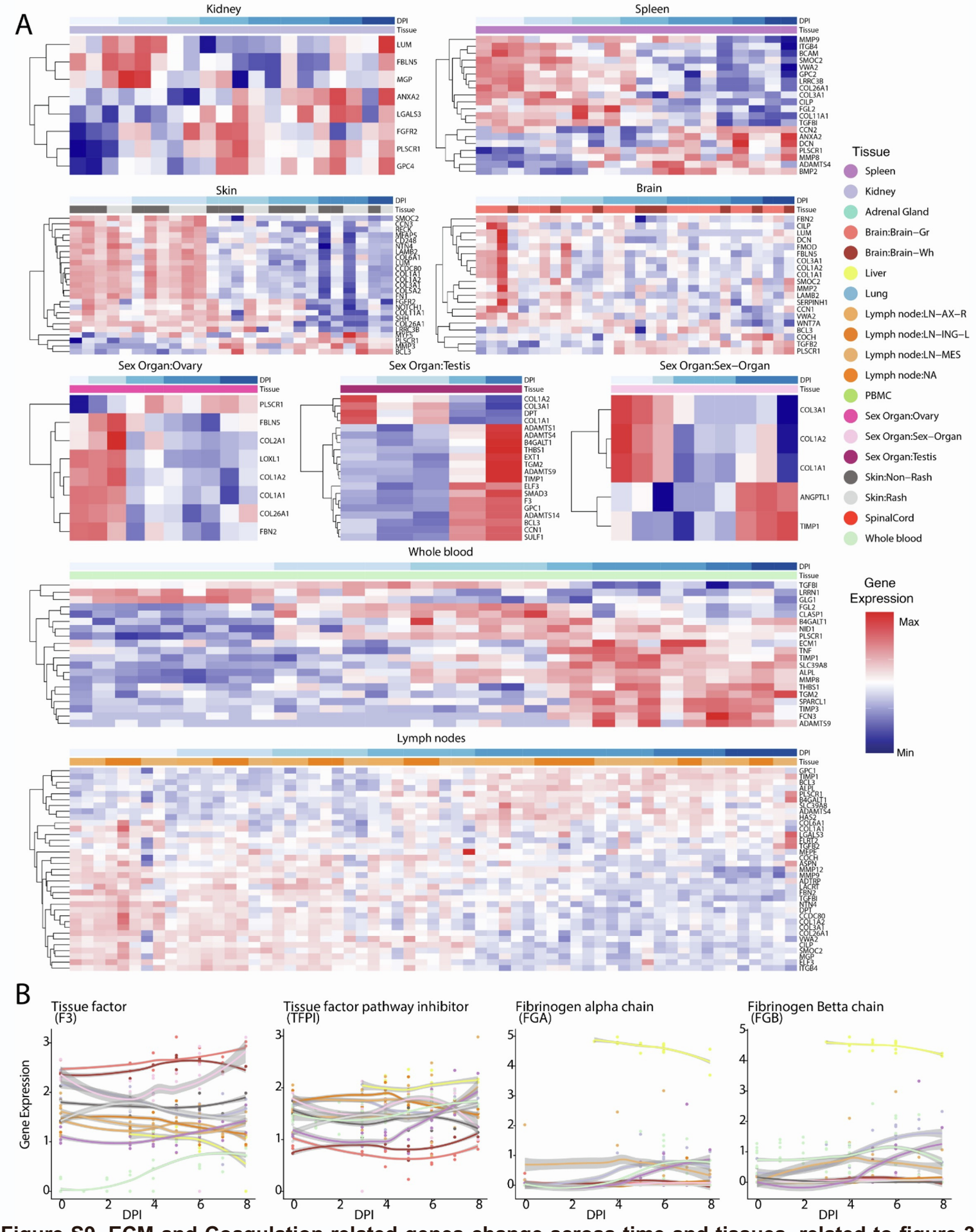

Figure S9. ECM and Coagulation related genes change across time and tissues, related to figure 3. (A) Heatmap of ECM genes that significantly change across time for each tissue (ImpulseDE2 Adjusted P-Value < 0.05). (B) Selected coagulation related genes expression.

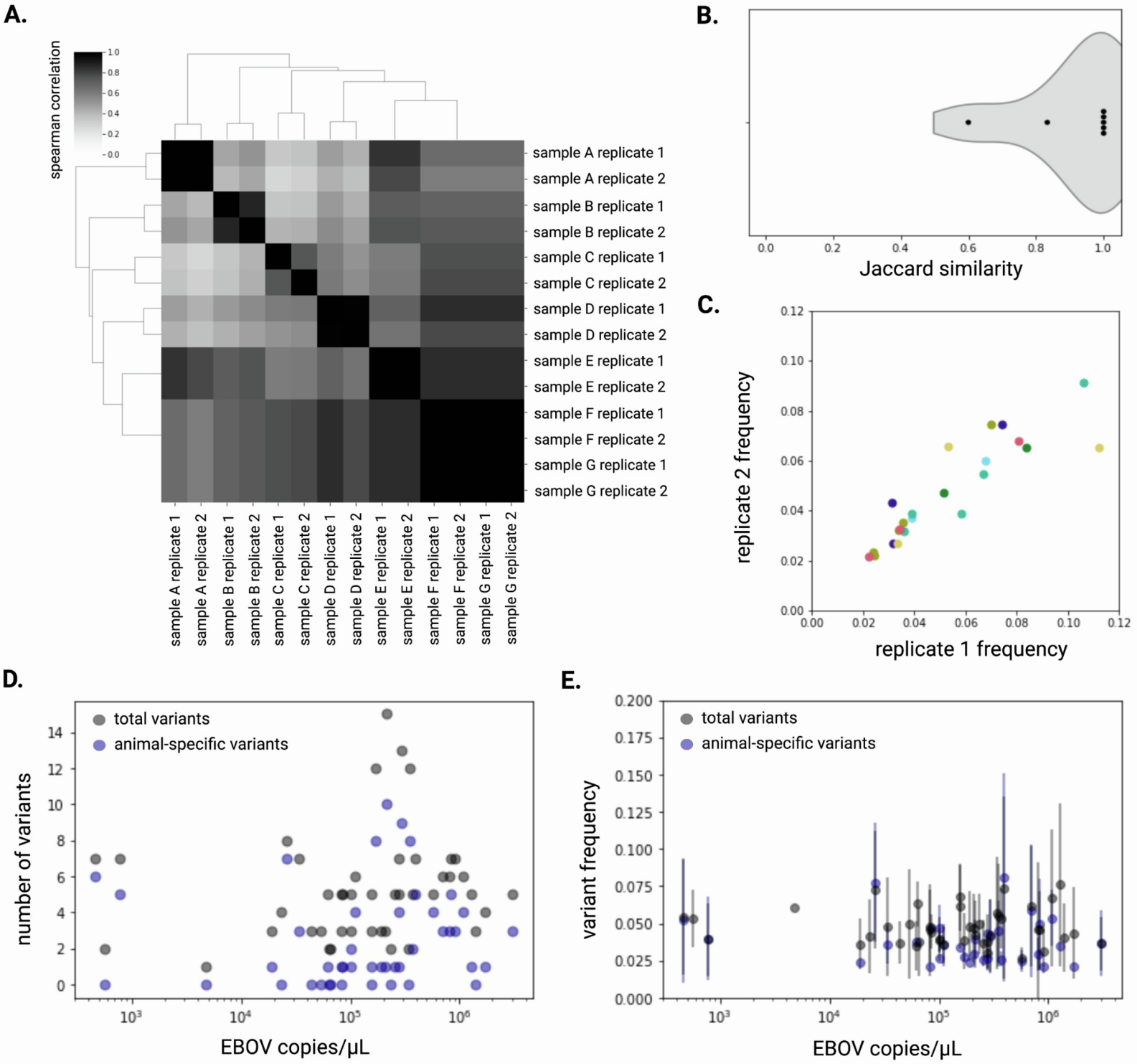

Figure S10. Reliability of minor variant calling methodology, related to figure 4. (A) Spearman correlation of variant profiles of 7 select samples with duplicate sequencing. (B) Violin plot of Jaccard similarities of variants identified in duplicate libraries from 7 samples. (C) For variants identified in two replicates, comparison of the frequency at which the variant was identified in each replicate. Each of the 7 samples is represented by a different color. (D) Total (black) and animal-specific (blue) variants identified versus viral load in each sample profiled. (E) Mean variant frequency versus viral load in each sample profiled. Error bars represent standard deviation of variant frequency.

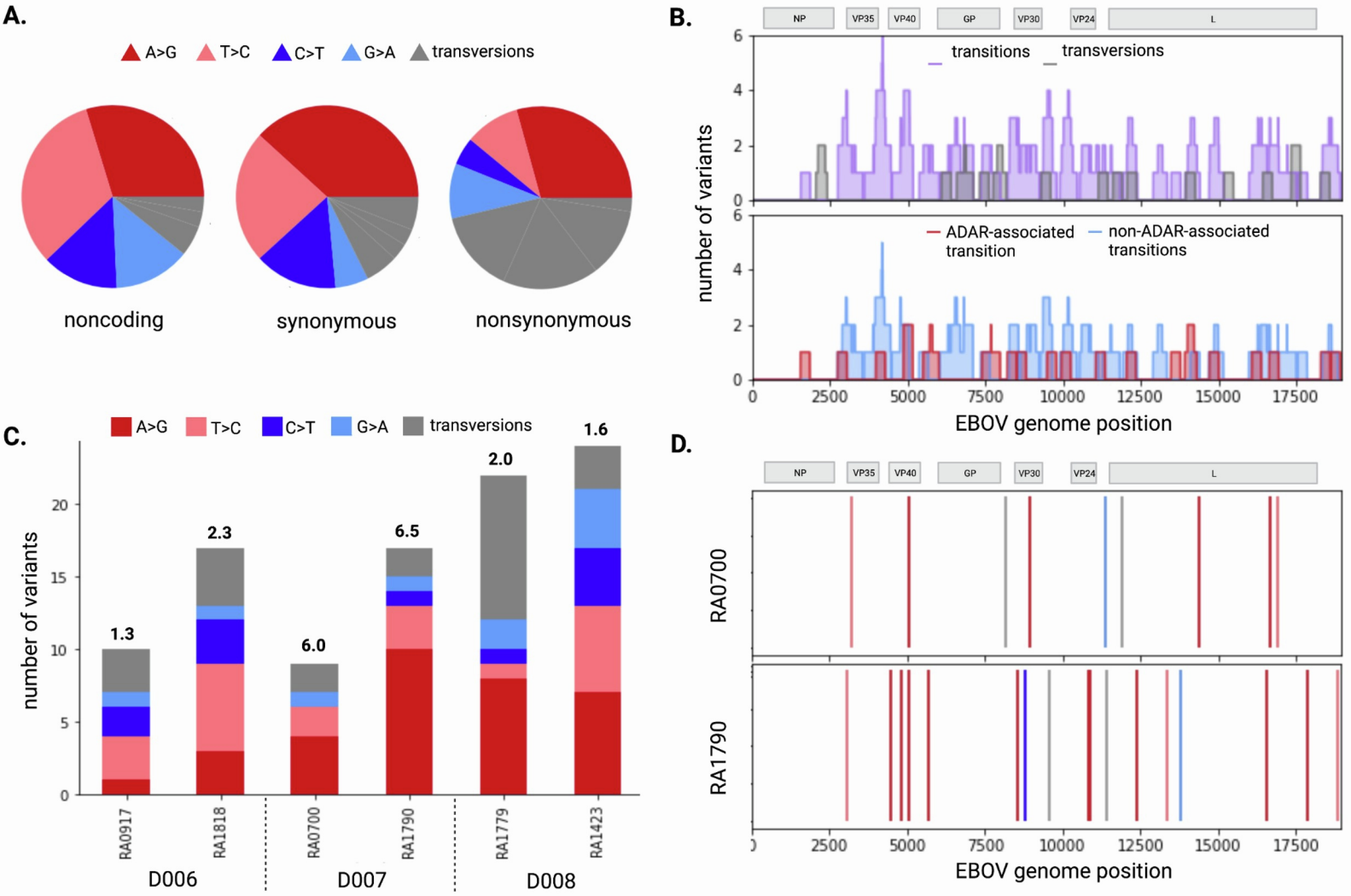

Figure S11. Mutation types across the sample set, related to figure 4. (A) Pie charts show relative amounts of each type of transition (labeled by color) and transversions (gray), separated by the mutation type. (B) Number of mutations that are transitions (purple) or transversions (gray) quantified by a 300 base pair sliding window across the EBOV genome. An EBOV gene map is above. (C) Stacked bar plots show the number of mutations that were each type of transition (labeled by color) and transversions (gray), separated by animal, ordered by DPI cohort. The bold number above shows the ratio of A-to-G and T-to-C mutations to C-to-T and G-to-A mutations, a marker of host RNA editing enzyme activity. (D) For two animals with the highest ratio of host RNA editing-associated mutations (RA0700 and RA1790), each mutation is represented by a vertical line along the EBOV genome on the x-axis. An EBOV gene map is above.

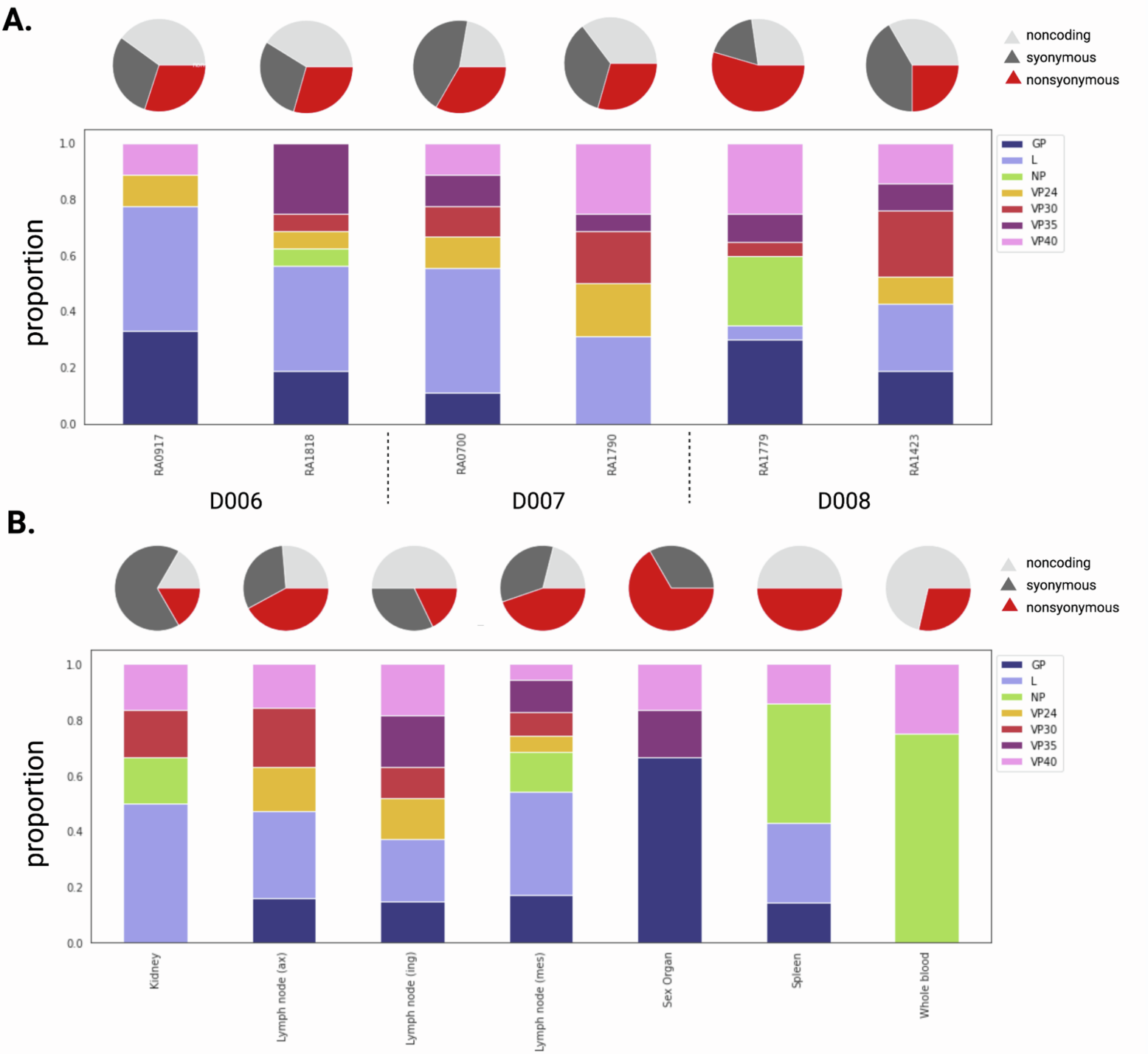

Figure S12. Mutations across animals and tissues, related to figure 4. (A) Proportion of nonsynonymous (red), synonymous (dark gray), and noncoding (light gray) variants across animals, ordered by cohort (top). Proportion of variants falling into each EBOV gene in each animal, ordered by cohort (bottom). (B) Proportion of nonsynonymous (red), synonymous (dark gray), and noncoding (light gray) variants within each tissue (top). Proportion of variants falling into each EBOV gene within each tissue (bottom).

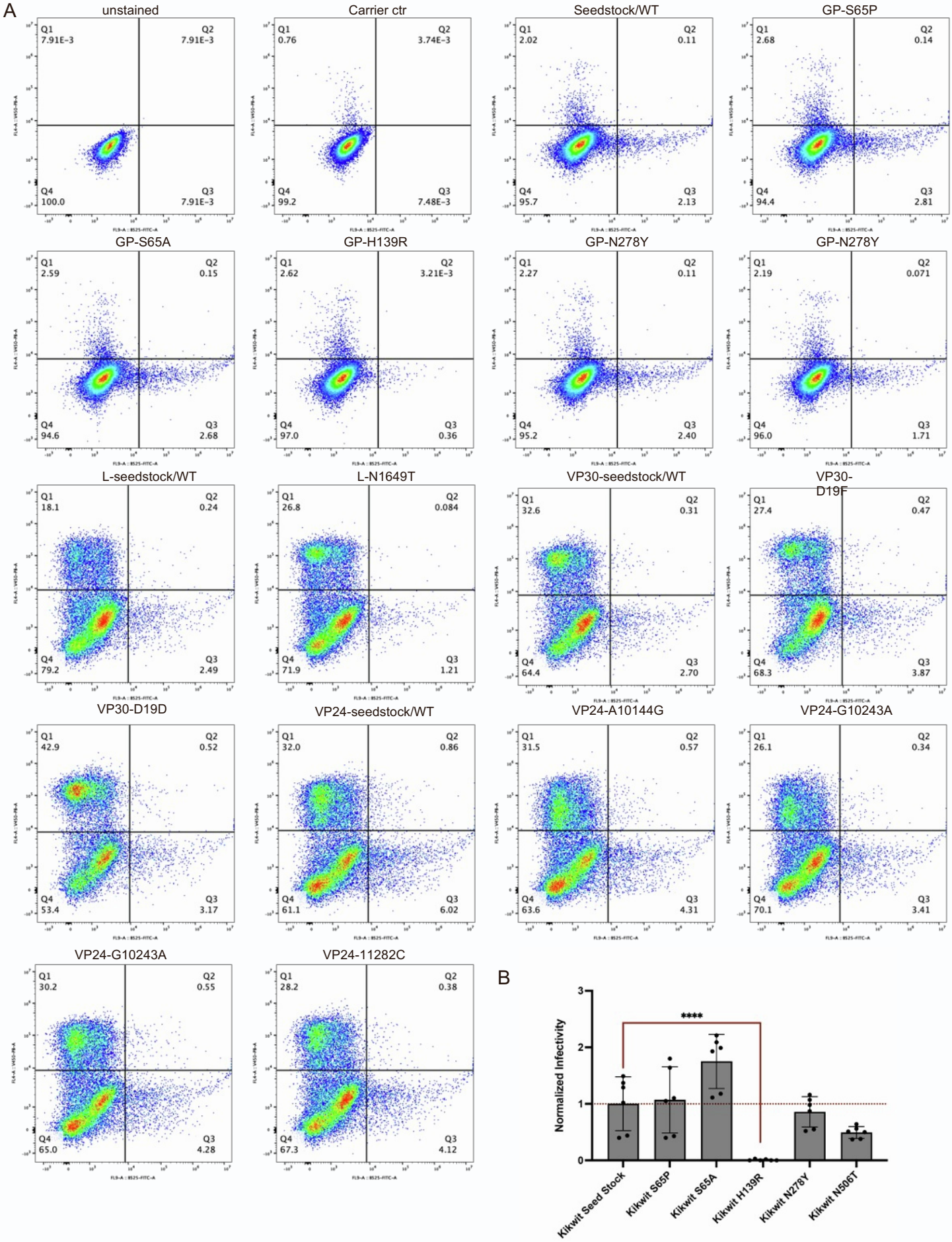

Figure S13. Functional characterization of viral variants, related to figure 5 (A) Gating strategy for GFP+ cells EBOV minigenome expression in HEK293T cells, representative data for n=2 independent biological replicates. (B) Quantification of fold difference in mCherry mRNA expression in HEK293T cells transduced with lentiviral virions pseudotyped with EBOV GP bearing the viral seed stock sequence or variants. Error bars represents standard deviation.

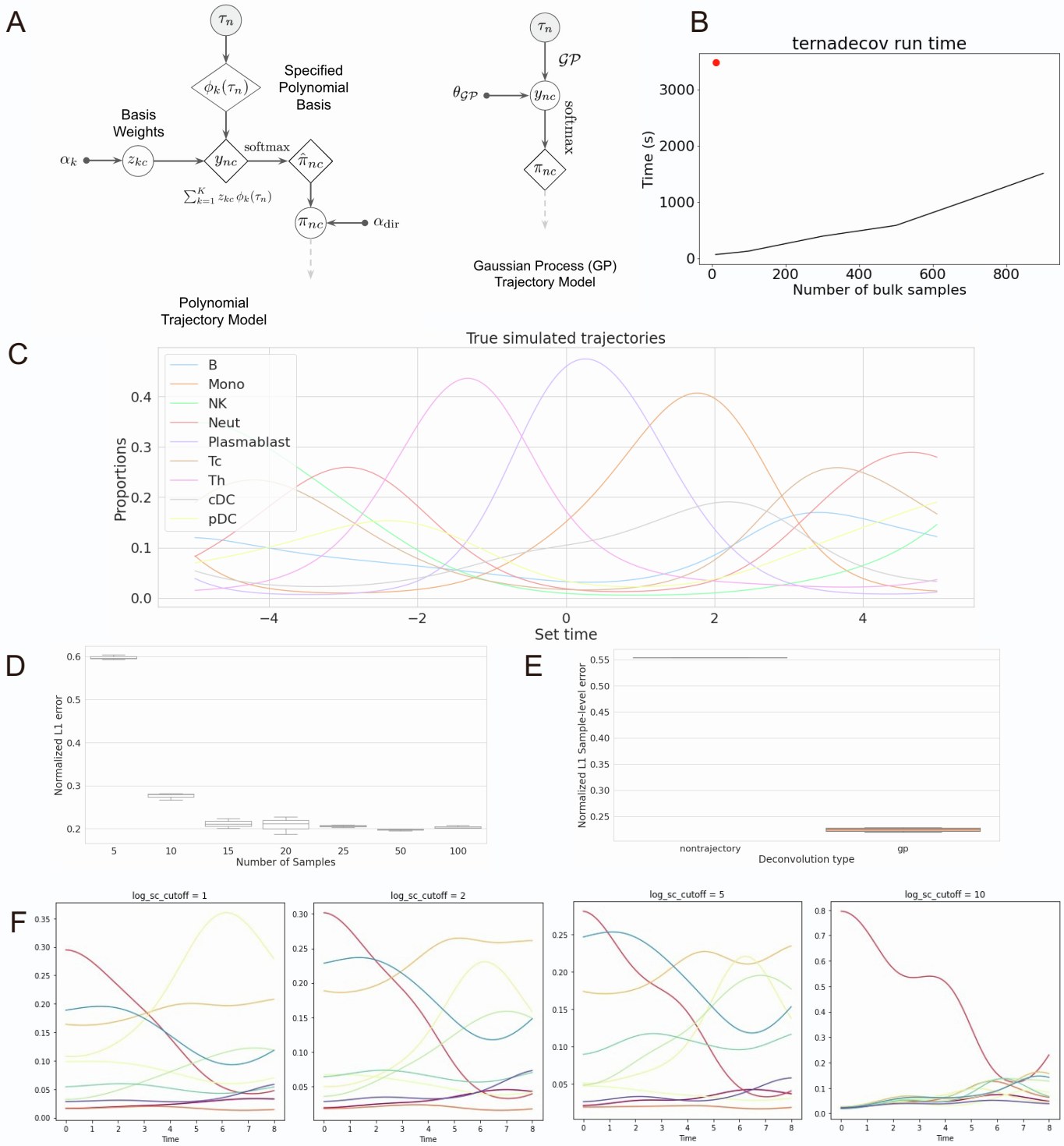

Figure S14. Utilizing Time-series Covariate Information for Enhanced RNA-seq Deconvolution, related to STAR methods (A) The parametric polynomial basis trajectory model (left), and the non-parametric Gaussian process trajectory model (right). The models are drop-in replacements as sub-models inside ternaDecov (see Figure 2D) (B) Execution time of ternaDecov (5,000 iterations, no GPU acceleration) for varying number of simulated samples using the GP trajectory module. The red point indicates the time required for deconvolution of the 10 samples from adrenal glands using MuSiC, shown for validation in the main text (C) Random simulated trajectory used for assessment of improvement in trajectory estimation as the number of sampled points increases (D) Normalized L1 trajectory error for trajectory estimation (at 1000 fixed points) using GP method from variable number of samples (3 replicates per point). Trajectory estimation improves as the number of samples increases (E) Normalized L1 sample-level error for the GP and non-trajectory (naive) model indicates that imposition of trajectory improves our ability to deconvolve the sample composition (F) Deconvolution results for adrenal tissues with increasing values of the log_sc_cutoff parameter (abundance cutoff for gene selection of the single-cell data) indicates robustness to parameter values.

Data S1: Pentacistronic Minigenome Assay plasmid sequences, related to STAR methods.

1. EBOV/Kikwit 5MG

CGGACACACAAAAAGAAAGAAGAATTTTTAGGATCTTTTGTGTGCGAATAACTATGAGGAAGATTAATAATTTTCC
TCTCATTGAAATTTATATCGGAATTTAAATTGAAATTGTTACTGTAATCACACCTGGTTTGTTTCAGAGCCACATCA
CAAAGATAGAGAACAACCTAGGTCTCTGAAGGGAGCAAGGGCATCAGTGTGCTCAGTTGAAAATCCCTTGTCAA
CATCTAGGTCTTATCACATCACAAGTCCCACCTCAGACTCTGCAGGGTGATCCAACAACCTTAATAGAAACATTAT
TGTTAAAGGACAGCATTAGTTCACAGTCAAACAAGCAAGATTGAGAATTAACCTTGGTTTTGAACTTGAATACTTA
GAGGATTGGAGATTCAACAACCCTAAAGCTTGGGGTAAAACATTGGAAATAGTTAAAAGACAAATTGCTCGGAAT
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ACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGA
CCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTGACCTACGG
CGTGCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGC
TACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG
GCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAA
GCTGGAGTACAACTACAACAGCCACAAGGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGTGAACT
TCAAGACCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGG
CGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAG
AAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACA
AGTAAATGAGCATGGAACAATGGGATGATTCAACCGACAAATAGCTAACATTAGGTAGTCAAGGAACGAAAACAG
GAAGAATTTTTGATGTCTAAGGTGTGAATTATTATCACAATAAAAGTGATTCTTATTTTTGAATTTAAAGCTAGCTT
ATTATTACTAGCCGTTTTTCAAAGTCCAATTTGAGTCTTAATGCAAATAGGCGTTAAGCCACAGTTATAGCCATAA
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2. EBOV/Kikwit NP-P2A-VP35

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3. EBOV/Kikwit L

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4. EBOV/Kikwit VP30

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