

¹ Supplementary Materials: Elucidating the Transcriptome of
² Turkey Hemorrhagic Enteritis Virus

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⁴ **Running Title:** Novel Insights into Turkey Hemorrhagic Enteritis Virus Transcriptome

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¹⁶ **Supplementary Table 1A**

Table 1: Table 1a: Most Transcriptionally Active Regions of THEV at 12h.p.i

Time	Region	Strand	Total Reads	Percentage
12hpi	MLP	+	235	38.8%
12hpi	E2	-	161	26.6%
12hpi	E3	+	104	17.2%
12hpi	E4	-	40	6.6%
12hpi		-,+/-	40	6.6%
12hpi	E1	+	20	3.3%
12hpi	IM	-	5	0.8%

¹⁷ **Supplementary Table 1B**

Table 2: Table 1b: Most Transcriptionally Active Regions of THEV at 24h.p.i

Time	Region	Strand	Total Reads	Percentage
24hpi	MLP	+	52,589	45.7%
24hpi	E3	+	29,209	25.4%
24hpi	E2	-	27,833	24.2%
24hpi	E1	+	2,724	2.4%
24hpi		-,+/-	1,312	1.1%

Time	Region	Strand	Total Reads	Percentage
24hpi	IM	-	744	0.6%
24hpi	E4	-	664	0.6%

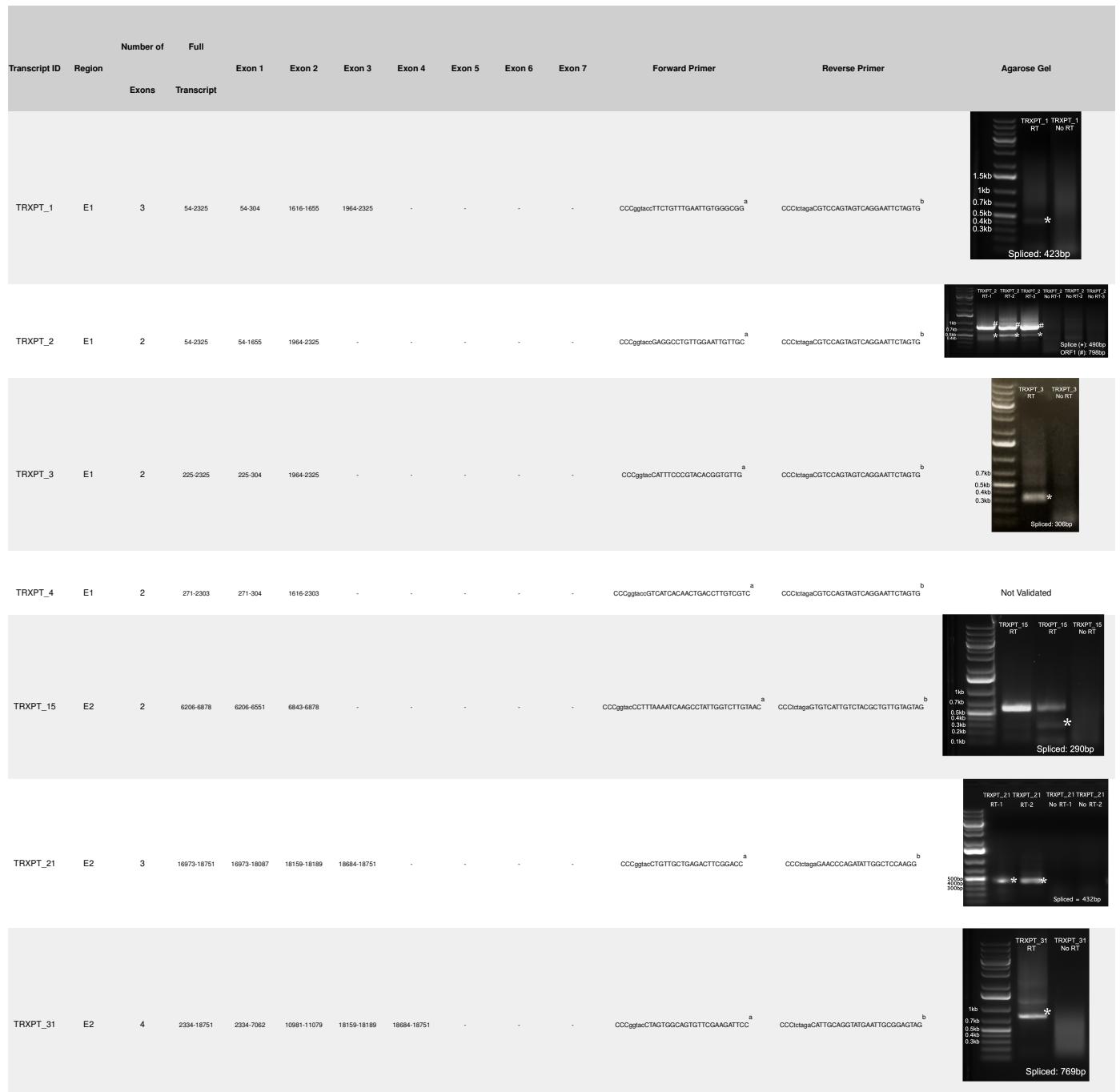
¹⁸ **Supplementary Table 1C**

Table 3: Table 1c: Most Transcriptionally Active Regions of THEV at 72h.p.i

Time	Region	Strand	Total Reads	Percentage
72hpi	MLP	+	1,437,273	67.4%
72hpi	E2	-	304,274	14.3%
72hpi	E3	+	271,392	12.7%
72hpi	E1	+	74,135	3.5%
72hpi		-,+/-	27,680	1.3%
72hpi	IM	-	14,484	0.7%
72hpi	E4	-	3,568	0.2%

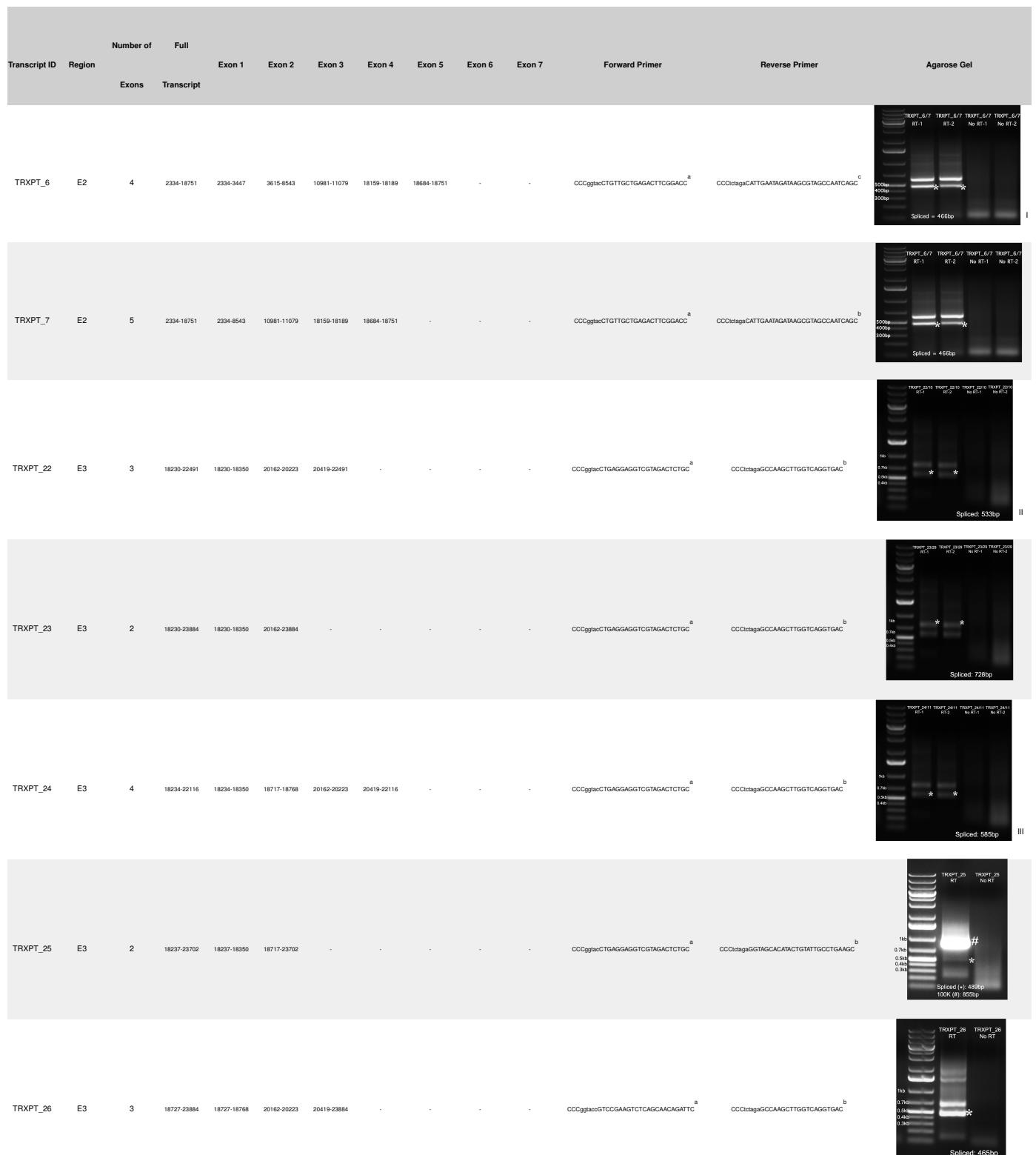
19 **Supplementary PCR Methods**

Table 4: Table 1: Agarose Gels Showing PCR Amplification of THEV cDNA With Gene-Specific Primers



^a Primer binds inside first exon; ^b Primer binds inside terminal exon; ^c Primer binds inside fourth exon; ^d Agarose gel identical to TRXPT_7 due to identical splicing; ^e Agarose gel identical to last 3 exons of TRXPT_10 due to identical splicing; ^f Agarose gel identical to last 4 exons of TRXPT_11 due to identical splicing; ^g Agarose gel identical to TRXPT_23 due to identical splicing; ^h Agarose gel identical to TRXPT_9 due to identical splicing; ⁱ Agarose gel identical to TRXPT_14 due to identical splicing;

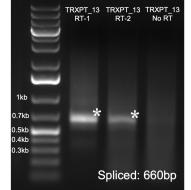
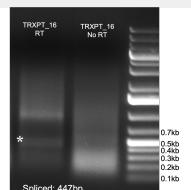
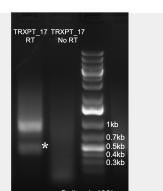
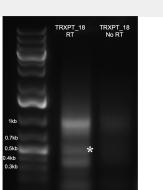
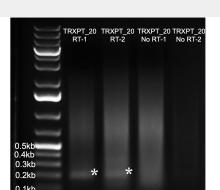
^{IV} due to identical splicing; ^V Agarose gel identical to TRXPT_23 due to identical splicing; ^{VI} Agarose gel identical to TRXPT_9 due to identical splicing; ^{VI} Agarose gel identical to TRXPT_14 due to identical splicing;



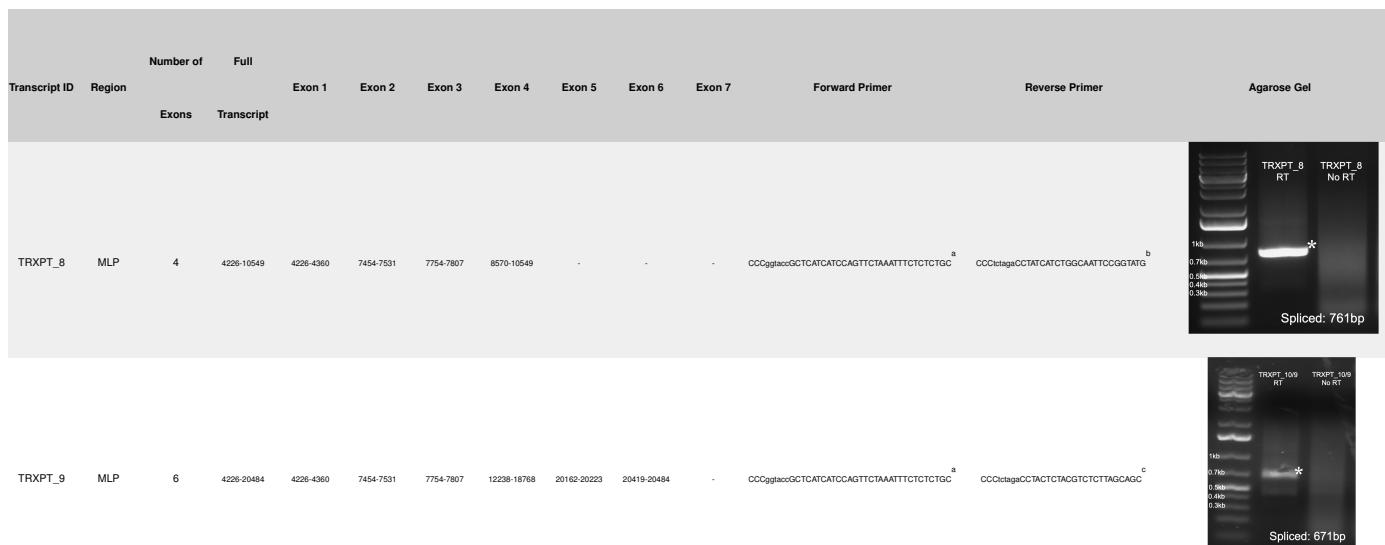
^a Primer binds inside first exon; ^b Primer binds inside terminal exon; ^c Primer binds inside fourth exon; ^I Agarose gel identical to TRXPT_7 due to identical splicing; ^{II} Agarose gel identical to last 3 exons of TRXPT_10 due to identical splicing; ^{III} Agarose gel identical to last 4 exons of TRXPT_11 due to identical splicing.

^{IV} Agarose gel identical to TRXPT_23 due to identical splicing; ^V Agarose gel identical to TRXPT_9 due to identical splicing; ^{VI} Agarose gel identical to TRXPT_14 due to identical splicing;



Transcript ID	Region	Number of Exons		Full Transcript							Forward Primer	Reverse Primer	Agarose Gel
		Exon 1	Exon 2	Exon 3	Exon 4	Exon 5	Exon 6	Exon 7					
TRXPT_13	MLP	6	4279-22116	4279-4360	7454-7531	7754-7807	18717-18768	20162-20223	20419-22116	-	CCGgtaccGCTCATCACAGTTCTAAATTCTCTCTGC ^a	CCClctagaGCCAAGCTGGTCAGGTGAC ^b	
TRXPT_14	MLP	4	4304-16870	4304-4360	7454-7531	7754-7807	13610-16870	-	-	-	CCGgtaccGCTCATCACAGTTCTAAATTCTCTCTGC ^a	CCClctagaGCCCTAGTATTAGCAGCTGACAACC ^b	
TRXPT_16	MLP	4	6934-12709	6934-6969	7454-7531	7754-7807	9430-12709	-	-	-	CCGgtaccGGATCTCCAGATTCTGGTCTGTG ^a	CCClctagaGCCTGTCCAACACCTGC ^b	
TRXPT_17	MLP	4	6934-12709	6934-6969	7454-7531	7754-7807	11001-12709	-	-	-	CCGgtaccGGATCTCCAGATTCTGGTCTGTG ^a	CTCCCCATCTAGACCTTTCATCTAAC ^b	
TRXPT_18	MLP	4	6934-12709	6934-6969	7454-7531	7754-7807	12238-12709	-	-	-	CCGgtaccGGATCTCCAGATTCTGGTCTGTG ^a	CCClctagaGTTCTCGTCTCTACGTCGTG ^b	
TRXPT_19	MLP	2	7401-7836	7401-7531	7754-7836	-	-	-	-	-	-	-	N/A
TRXPT_20	MLP	2	7765-16856	7765-7807	12466-16856	-	-	-	-	-	CCGgtaccGAGGATTGAAGCCAATTCCCTCAACG ^a	CCClctagaCTGCAGGCCAACACAGGTG ^b	

^a Primer binds inside first exon; ^b Primer binds inside terminal exon; ^c Primer binds inside fourth exon; ^d Agarose gel identical to TRXPT_7 due to identical splicing; ^e Agarose gel identical to last 3 exons of TRXPT_10 due to identical splicing; ^f Agarose gel identical to last 4 exons of TRXPT_11 due to identical splicing; ^g Agarose gel identical to TRXPT_23 due to identical splicing; ^h Agarose gel identical to TRXPT_9 due to identical splicing; ⁱ Agarose gel identical to TRXPT_14 due to identical splicing;



^aPrimer binds inside first exon; ^bPrimer binds inside terminal exon; ^cPrimer binds inside fourth exon; ^IAgarose gel identical to TRXPT_7 due to identical splicing; ^{II}Agarose gel identical to last 3 exons of TRXPT_10 due to identical splicing; ^{III}Agarose gel identical to last 4 exons of TRXPT_11 due to identical splicing; ^{IV}Agarose gel identical to TRXPT_23 due to identical splicing; ^VAgarose gel identical to TRXPT_9 due to identical splicing; ^{VI}Agarose gel identical to TRXPT_14 due to identical splicing;

- 20 In the table above, the restriction sites in the primer tails are shown in lowercase letters. All the primer
 21 melting temperatures (TMs) are 58-60°C using a hot start Taq DNA polymerase. The PCR reaction mix
 22 was done per manufacturer's instructions. The PCR cycling conditions were as follows: Initial denaturation
 23 – 95°C for 1 minute; cyclical denaturation – 95°C for 30 seconds, annealing – variable temperature (at least
 24 53°C) for 30 seconds, primer extension – 68°C for variable time, and final elongation – 68°C for 5 minutes.
 25 We used 35 cycles of amplification.

26 Supplementary Computational Analysis

- 27 Snakemake v7.24.0 was used to manage our entire workflow. A graph of the main steps in our pipeline
 28 generated with Snakemake is shown below. Our trimmed RNA-seq reads were mapped to the genome of *M.*
gallopavo (with THEV's genome as one of its chromosomes) using Hisat2, to generate the alignment (BAM)
 30 files and StringTie used to assemble the transcriptome with a GTF annotation file containing the predicted
 31 THEV ORFs as a guide. The GTF annotation file was derived from a GFF3 annotation file obtained from
 32 NCBI using Agat – version 1.0.0, a program for converting between many different file formats used in
 33 bioinformatics. However, the NCBI GFF3 annotation file itself was first modified to remove all unimportant
 34 features, leaving only the ORFs.

35 StringTie was also used to estimate the normalized expression levels (FPKM) of all the transcripts and
36 Ballgown in R was used to perform statistical analysis and comparisons of the transcript expression levels,
37 which instructive in understanding the temporal regulation THEV gene expression.

38 In these steps above, each sample (replicate of each time point) was processed independently and merged
39 only in the final transcriptome assembly or during analysis with Ballgown. In the subsequent steps de-
40 scribed below, all samples for each time point were processed together.

41 We used RegTools to extract and analyze the splice junctions in the BAM files. The command regtools
42 junctions extract provides a wealth of information about all the splice sites in the BAM file provided
43 such as: the start and end positions, the strand, and number of reads supporting the splice junctions.

44 The command regtools junctions annotate gives even more information such as: the splice site
45 donor-acceptor sequences and transcripts/genes that overlap the junction. These information was the
46 basis for estimating and comparing the splicing activity of different regions (TUs) of THEV over time.

47 Also, Samtools was also used to count the total sequencing reads for all replicates at each time point.

