**Reviewer #1**: *The authors addressed most of my previous concerns, however there are some issues, which require more detail/precision in the manuscript.*

*In the order of appearance in the text:*

*- "we used an in-house developed R pipeline." -> Here, they should include the link to the github repository. The link in the data availability section is not working though.*

Thank you for noticing, we have corrected the link, it should work now.

*- "The CAGEfightR [35] package was used to determine TSS positions. The TSS clusters within a 10 nucleotides window were termed identical." Does TSS cluster refer to the TSS positions outputted by CAGEfightR or are these the clusters obtained after merging TSS within 10 nt.*

TSS clusters are groups of transcription start sites (TSSs) identified and merged within a 10-nucleotide window by CAGEfightR, rather than individual raw TSS positions. The software first detects individual TSSs (CTSSs) and then groups them into clusters based on the specified distance threshold.

*- "Reference transcript counting": It is unclear what is compared against the reference annotation here. The reads from dcRNA-seq? This needs to be clarified.*

We introduced a term “transfrags” to facilitate understanding of the workflow. We refer transfrags to a list of unique alignments of the dcDNA reads to the viral genome (considering both exons aka. matches in the alignment and introns – appearing as Ns, at the nucleotide level). Their characteristics and distribution throughout the dcDNA dataset was used as a basis for the downstream analysis.

We have clarified that reference transcript counting was performed by comparing the list of the assembled transfrags (from dcDNA-Seq) to our previously annotated transcript dataset using GFF-compare. The comparison was thus not performed using individual sequencing reads but rather by aligning the transfrags to our reference transcripts list to quantify the reference transcripts presence in each sample.

*- nucleotide vs. nt-s: The authors use both terms, but should only use one consistently*

We have standardized the terminology and now consistently use "nucleotide" throughout the revised version of the manuscript.

*- "The clusters were merged with the dcDNA-Seq dataset" -> The TSS clusters?*

Yes, this refers to the TSS clusters obtained from CAGEfightR. We have explicitly stated "TSS clusters" in the revised text for clarity.

*- ". , which contains transcript identities based on the alignment of 5′-ends" -> I don't understand what this is supposed to mean or refer to. The TSSs or the dcRNA-seq, if the latter why would only the 5'end be aligned. This needs to be rephrased to be unclear*

This refers to the TSS clusters obtained from CAGEfightR and the transfrags (obtained from the dcDNA data), and means that the two data was merged based on the genomic position (start, end and strand) of the former and 5′-end position (and strand) of the latter. This was made more clear (by the usage of the term transfrags) in the revised article.

*- "Transcripts were reconstructed by pairing validated TSS peaks": what are validated TSS peaks? peaks confirmed by dcRNA-seq in the previous paragraph? But this already merged transcripts (from dcRNA-seq? unclear) with TSS, why do you need to do it again? Also transcripts are from the dcRNA-seq data or where do they come from?*

CAGE TSS clusters were sometimes broad, exceeding 150 nucleotides in some cases. To improve precision, we refined these TSSs using the 5′-end counts of transfrags obtained from dcDNA-Seq. For each TSS cluster, a peak analysis was conducted to identify validated TSS peaks, which were not identical to the original CAGE TSS clusters but were supported by both CAGE-Seq and dcDNA-Seq data.

This step ensured that only high-confidence TSSs were used for novel transcript reconstruction. Some transfrags could not be assigned to reference transcripts from our previous study, but their strong TSS signals in both dcDNA-Seq and CAGE-Seq suggested they represented previously unannotated transcripts.

The full workflow has been clarified in the Methods section, and the correct terminology is dcDNA-Seq, not dcRNA-Seq.

*- "their 3′-ends overlapped a known TES" -> known means from the previous annotation?*

Yes, "known" refers to TESs from our previous transcriptome annotation. This has been explicitly stated in the revised text.

*- "This approach enabled the integration of the CAGE-Seq and dcDNA-Seq datasets to annotate TSSs."-> but you did this already in the previous section? Why did you do this again?*

This was referring to the previous section. We have clarified this distinction in the text.

*- "Newly assembled transcripts were integrated with our prior annotation"-> how?*

From the gff-compare output, we analyzed how each novel transcript is related to the reference list. If the novel transcripts met the validation criteria (TSS-TES pairing, expression threshold, and support by multiple sequencing methods), they were incorporated into the updated annotation. We also inspected the newly identified transcripts manually and compared them to our previous transcript list.

*- "Validated transcripts"-> when is a transcript considered validated?*

A novel transcript (not found in the reference list with the ± 10 nucleotide wobble) was considered validated if it met all of the following criteria:

1. Supported by at least three independent dcDNA-Seq reads.
2. The TSS was within ±10 nt of validated TSS peak (within a CAGE-Seq TSS cluster).
3. The TES overlapped a known TES within ±10 nt.
4. Correct 5′- and 3′-adapter sequences were detected.

This definition has been explicitly added to the Methods section.

*- "To further filter TSS transcripts" -> what are TSS transcripts, it has not been defined to which transcripts this term refers to.*

This was a typo, we was referring to “To further filter the TSS of transcripts …”. This means that we were extra strict to accept the 5′-truncated transcripts that contain 5′-truncated ORFs, in order to filter out sequencing artefacts, where the reverse transcription step in the dcDNA protocol was not complete. This was corrected.

*- "To identify groups of TSSs, TESs, and transcripts with similar temporal expression patterns, we performed de novo clustering on normalized gene expression data. [..] For gene clustering, .." -> gene clustering aims to identify groups of transcripts with similar temporal expression patterns? If not how are these groups of transcripts identified. This needs to be clarified.*

Yes, transcripts were grouped into clusters based on their temporal expression profiles. The terms gene expression and transcript expression were used interchangeably, but we have clarified that this analysis specifically refers to transcript expression, as only canonical transcripts were counted and clustered. Unlike gene-level clustering, which includes all transcript isoforms, this approach avoids ambiguity in assigning isoforms to specific genes. Differential transcript expression, including isoform-specific patterns, was analyzed separately in the sections "Dynamics of transcriptional isoform switching in selected genes" and "Dynamics of spliced transcript expression." These distinctions have now been clarified in the Methods section.

*- "Our findings reinforced that ORF64 is the sole IE gene in EHV-1" -> It needs to be explicitly mentions that ORF64 is the only gene with significant expression levels after CHX treatment.*

We have revised the manuscript to explicitly state that ORF64 is the only gene with significant expression levels after CHX treatment. Statistical analyses, including t-tests, Z-score analysis, coefficient of variation (CV), and interquartile range (IQR) outlier detection, confirm that ORF64 is the sole significantly expressed viral gene under CHX treatment, while other detected transcripts likely represent background noise. This reinforces ORF64’s classification as the only true immediate-early (IE) gene in EHV-1.

*- "Among the examined transcripts, 251 received the highest level of support (\*\*\*) - indicating robust validation - while 47 had medium support (\*\*) and 51 showed the lowest level of support (\*) (see Methods for details)." -> Methods only talks about assigning confidence to TSS. Are you talking about TSS here? You need to be more precise with your terms!*

We refer here to transcripts; specifically, how many received certain confidence categories in our CAGE-based TSS analysis. As described in the Methods (‘TSS Clusters Validation’), the ‘\*’, ‘’, and ‘’ levels apply exclusively to TSS clusters identified via CAGE-Seq. We then merged these clusters with dcDNA-Seq transfrags, based on their 5′-position overlaps, and to which reference transcripts were already assigned (through gff-compare). This is now clarified in “*Transcript Merging and TSS Refinement*”. The reported numbers – 251 (\*\*\*), 47 (\*\*), and 51 (\*) – reflect the reference (previously annotated) transcripts inheriting TSS confidence ratings from CAGE-Seq.

However, if a transfrag did not align to any known reference transcript but showed a strong CAGE signal (and a correct dcDNA 5′-adapter signal), we assembled a novel transcript from that transfrag. This is now detailed in the “*Transcript Assembly and Validation”* section.

*- OriS = Oris? If yes, please use a consistent notation, if not explain.*

OriL and OriS are the correct terms and we have corrected the article accordingly.

*- Supplementary Figure S1 needs to indicate the location of raRNA, Ori-L and the other genes/mRNAs mentioned in this section, otherwise it cannot be understood.*

In Supplementary Figure S1, the aligned nucleotides of the NOIR (A) and CTO (B) genes in the three viral strains were shown along with their orientation. As this was a gene multiple sequence alignment, the inclusion of surrounding genomic regions and/or transcripts would not be feasible.

However to enhance the clarity of this section, we have created two additional figures. The first, Supplementary Figure S12, shows the replication origin region of EHV-1 at high resolution, while the second, Supplementary Figure S13, displays the same for the PRV. These figures clearly illustrate where the NOIR and CTO genes and transcripts and replication origins are located within the given region. In the figures, the molecules overlapping the replication origin are shown in green, the molecules transcribed from the forward strand in red, and those transcribed from the reverse strand in green.

*- noir = NOIR? If yes, please use a consistent notation, if not explain.*

Any usage of “noir” was omitted, we now use “NOIR” throughout the manuscript.

*- Legend to Supplementary Figure S2 and Supplementary Figure S5 need to state the scale of the y-axis is determined independently for each time-point*

We have revised the legends for both Supplementary Figure S2 and Supplementary Figure S5 to explicitly note that each facet’s y-axis is scaled independently (via ggplot’s scale = "free\_y"). This ensures the y-axis range adapts to the data in each time-point facet, allowing for clearer visualization of expression profiles.

*- Supplementary Figure S4 should be before Supplementary Figure S3 und referenced in the previous paragraph, which discusses the traditional kinetic clusters*

The reviewer is absolutely correct again, we have switched the ordering of Supplementary Figure S3 and S4. In addition, following the same logic, we switched the ordering of Supplementary Figure S6 and S7 as well.

*- Figure 7 should be a Supplementary Figure as it is too large for the main manuscript. The text will be unreadable if it is scaled to fit in the main manuscript.*

*Zsolt, a bíráló kéri, hogy a Torma IGV-s ábrája (most fig 7) legyen supplementary mert nem látszódnak rendesen a dolgok. Teljesen igaza van. Pontosan ezt javasoltam Karácsony előtt, de nem mentél bele (nem értettem akkor se, miért)*

We agree. Figure S7 is now Supplementary Figure S11 in order to enhance visibility the annotations and other texts.

*- "By integrating multiple data sources (dcDNA-Seq, dRNA-Seq, and CAGE-Seq) and using different tools - including LoRTIA for dcDNA-Seq libraries and NAGATA for dRNA-Seq data" -> where is the dRNA-seq data integrated? This did not become clear in the methods (see also my above questions).*

We used our previous dRNA-Seq dataset and the NAGATA software to 1.) validate introns and 2.) to confirm the presence of newly identified TSSs, with the focus on 5-prime truncated ORF-carrying “putative transcripts” – mainly in order to reduce the possibility of annotating sequencing artifacts resulting from the stalling of the enzyme during reverse transcription, which is a known issue in dcDNA-Seq. These steps were taken in order to complement and validate both the sequencing methods used in this study (dcDNA and CAGE-Seq) and the bioinformatic approach.

Specifically, in the Methods section (see “Filtering 5′-truncated ORF-carrying Transcripts” and now “*Transcript Assembly and Validation” also*), we explain how we re-analyzed our earlier dRNA-Seq data with NAGATA software to ensure that 5′-truncated ORF isoforms—and the introns discovered in dcDNA-Seq—were also supported by dRNA reads. Specifically:

* Intron Validation: We only accepted introns in the dcDNA data if they also appeared in our prior dRNA-Seq dataset.
* TSS Cross-Checking: Any novel TSS identified in dcDNA-Seq and CAGE-Seq had to be detectable (within a 25 nt window) in the dRNA-Seq-NAGATA output as well, to confirm its authenticity.

This integrated approach helped eliminate potential artifacts and strengthened our confidence in newly discovered TSSs and alternative isoforms.

*- "The R codes used to perform the analysis and generate the plots are available at: https://github.com/Balays/EHV-1-dynamic" -> the link does not exist.*

The provided link [*https://github.com/Balays/EHV-1-dynamic*](https://github.com/Balays/EHV-1-dynamic) is now working and the scripts that were used to generet the analysis and figures are now accessible.