Inventory of Supplemental Items

Figure S1: Control experiments and bioinformatics analysis for Murine gammaherpesvirus 68 tiled array results.

A) Mock infected samples have low signal on the tiled array.

B) Spliced viral gene ORF-57 is detected by the tiled array.

C) Genome-wide RNA expression in γHV68 using a 2 standard deviation threshold.

D) Comparison of γHV68 RNA expression at 8 hpi versus 18 hpi.

E) Translated regions conserved within EGRs are restricted to those contained in highly conserved antisense protein genes.

F) Gene predictions in γHV68 using GeneMarkS-EV detects 3 potential genes in Expressed Genomic Regions.

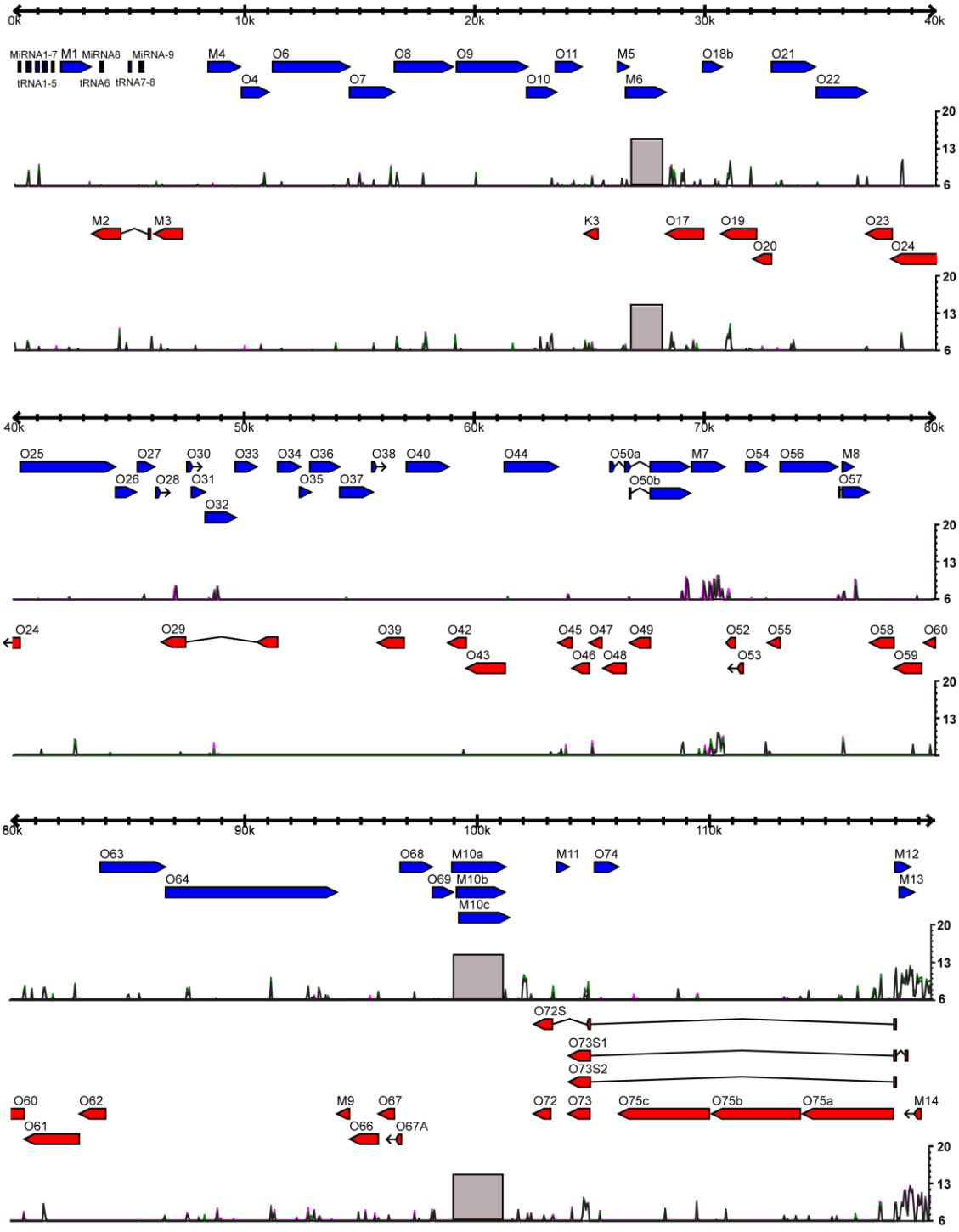
G) Transposon mutants mapped to Expressed Genomic Regions.

Table S1: Conservation of peptide fragments within Expressed Genomic Regions.

Figure S5: Fold inhibition of viral DNA synthesis by cidofovir.

Figure S1

A)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O9
O11
M5
O18b
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O4
O7
O10
M6
O22
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13
6
M2
M3
K3
O17
O19
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O74
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M13
M10c
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O73S1
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O60
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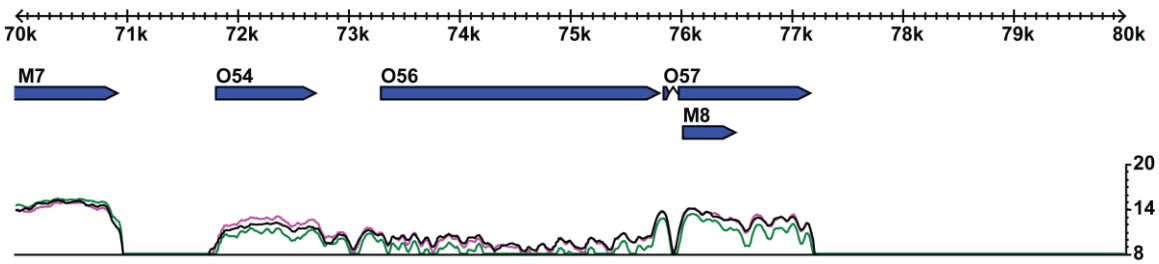
O66
O67A

Figure S1

B)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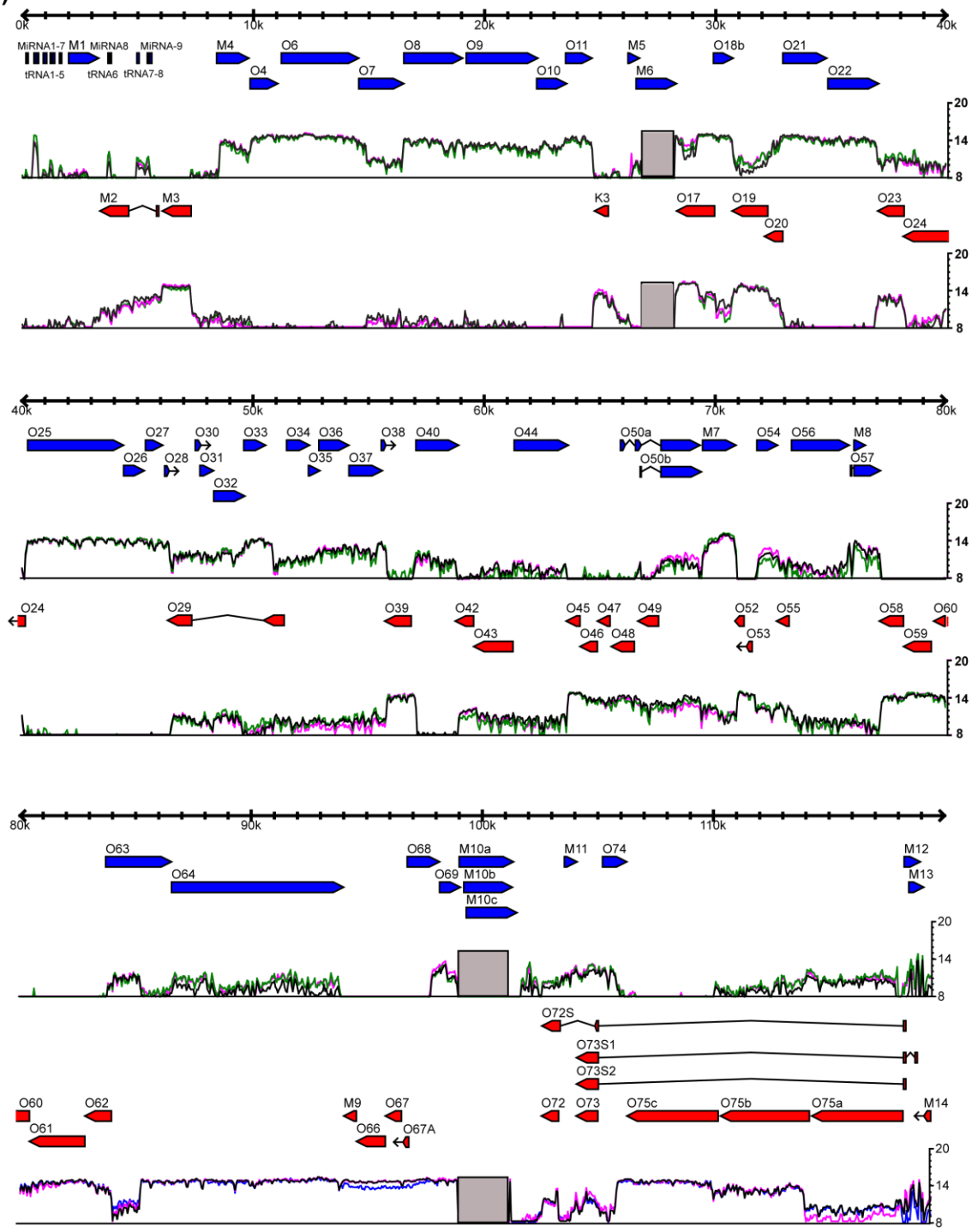
8

Figure S1

C)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O18b
O21
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O7
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M2
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O69
M10b
M13
M10c
O72S
O73S1
O73S2
O60
O62
M9
O67
O72
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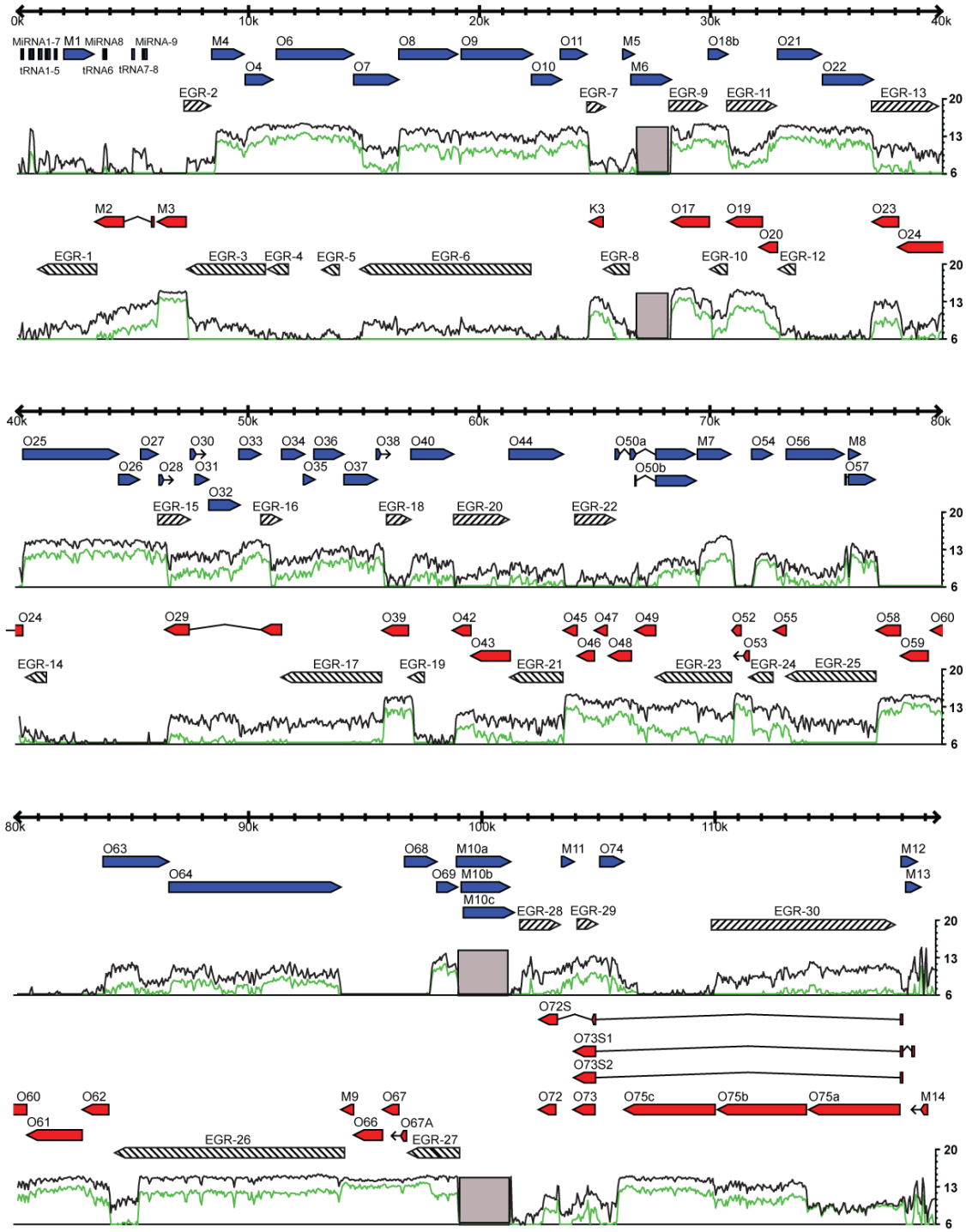
O75b
O75a
M14
O61
O66
O67A

Figure S1

D)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O6
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O9
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O18b
O21
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O7
O10
M6
O22
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M2
M3
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O24
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O57
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EGR-20
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M13
M10c
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EGR-29
EGR-30
O72S
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O73S2
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M9
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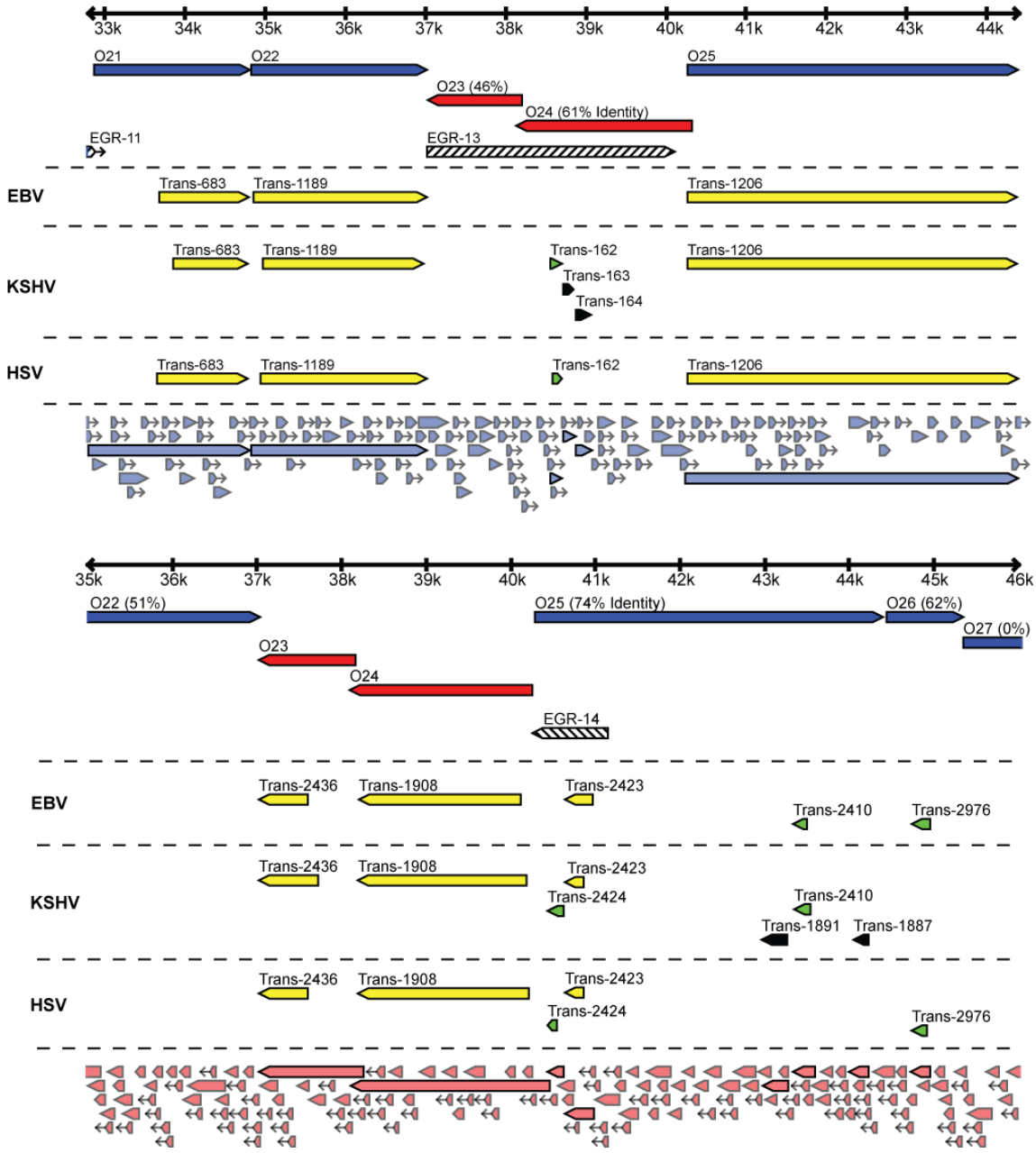
EGR-26
EGR-27

Figure S1

E)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O24 (61% Identity)
EGR-11
EGR-13
EBV
Trans-683
Trans-1189
Trans-1206
KSHV
Trans-683
Trans-1189
Trans-162
Trans-1206
Trans-163
Trans-164
HSV
Trans-683
Trans-1189
Trans-162
Trans-1206
35k
36k
37k
38k
39k
40k
41k
42k
43k
44k
45k
46k
O22 (51%)
O25 (74% Identity)
O26 (62%)
O27 (0%)
O23
O24
EGR-14
EBV
Trans-2436
Trans-1908
Trans-2423
Trans-2410
Trans-2976
KSHV
Trans-2436
Trans-1908
Trans-2423
Trans-2424
Trans-2410
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HSV
Trans-2436
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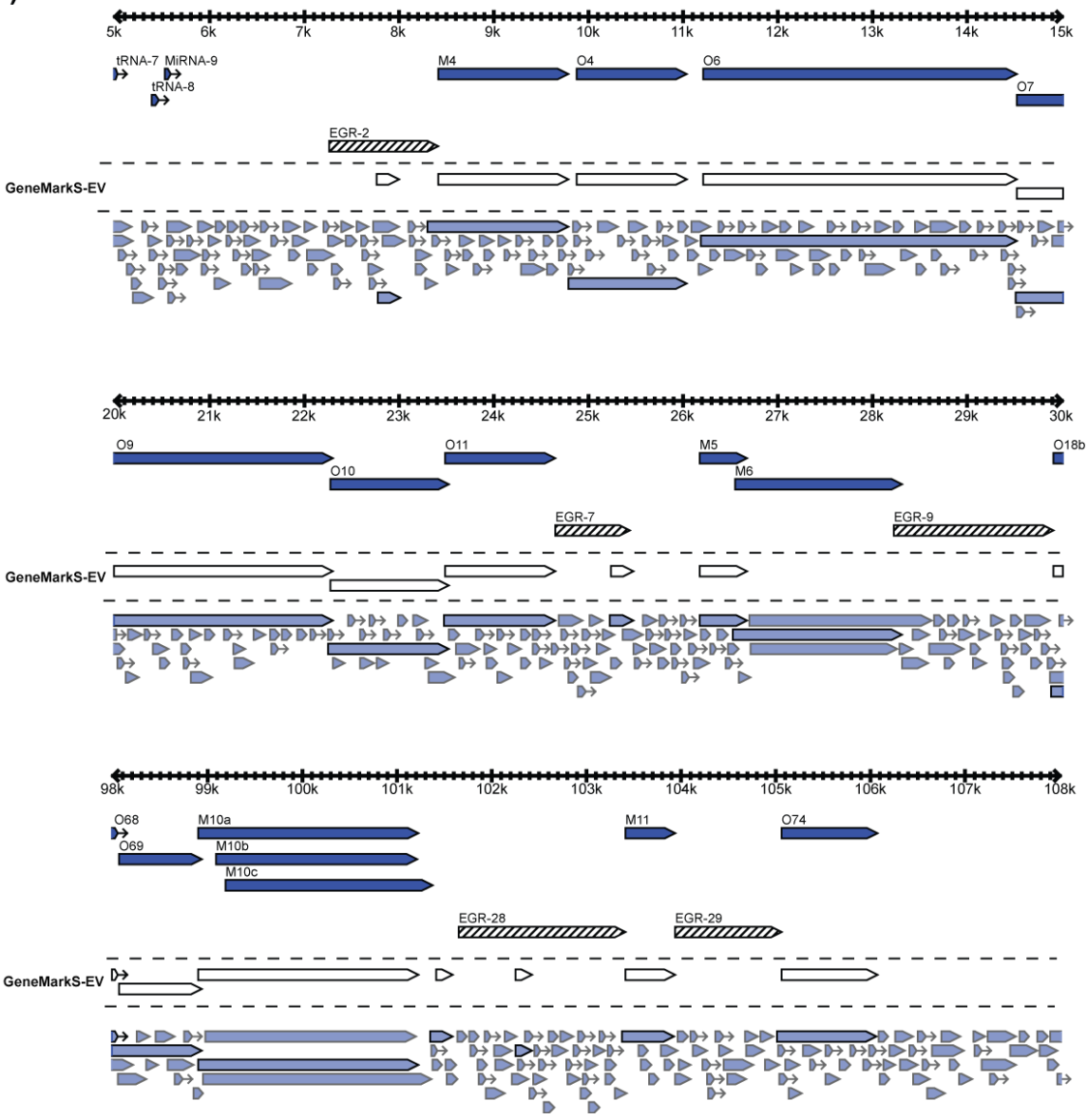
Trans-2976

Figure S1

F)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tRNA-8
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O4
O6
O7
EGR-2
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24k
25k
26k
27k
28k
29k
30k
O9
O10
O11
M5
M6
O18b
EGR-7
EGR-9
GeneMarkS-EV
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99k
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101k
102k
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104k
105k
106k
107k
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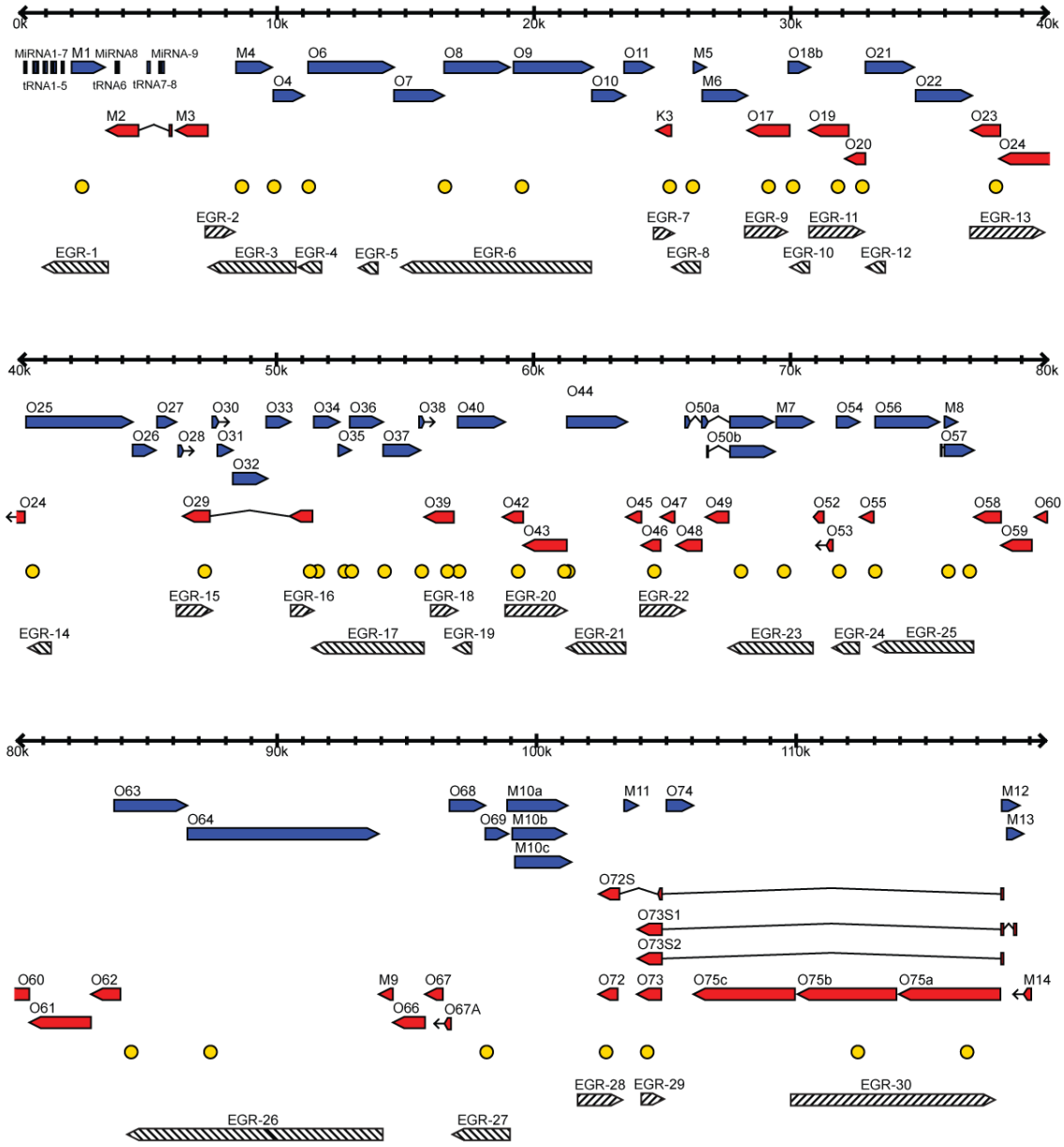
EGR-28
EGR-29
GeneMarkS-EV

Figure S1

G)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M3
K3
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EGR-9
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90k
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110k
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O73S2
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O75c
O75b
O75a
M14
O61
O66
O67A
EGR-28
EGR-29
EGR-30
EGR-26
EGR-27

Figure S1: Control experiments and bioinformatic analyses for Murine gammaherpesvirus 68 tiled array. A) Mock infected samples have low signal on the tiled array. 3T12 cells were mock infected, total RNA was harvested at 18 hpi, labeled, and hybridized to the γHV68 tiled array. The mean fluorescence of probes overlapping each nucleotide position is plotted on a $\log_2$ scale underneath the annotated gene positions. The three colored lines represent the data from three replicate mock infections. Blue or red arrows represent annotated genes on the positive or negative strand of the genome, respectively. Gray boxes represent the two internal repeat regions of the γHV68 genome that are excluded from our analysis. B) Detection of the spliced viral gene ORF-57. The transcriptional signals from three replicate infections in the region of ORF 57 are shown as colored lines. There is a clear drop in signal corresponding to the splice site in ORF-57. C) Genome-wide RNA expression in γHV68 using a 2 standard deviation threshold. 3T12 cells were infected with γHV68 at an MOI=10. The three colored lines represent the data from three replicate infections that are expressed two standard deviations above the mean mock-infected signal. D) Comparison of γHV68 RNA expression at 8 hpi versus 18 hpi. γHV68 RNA expression at 8 hpi, green lines, versus 18 hpi, black lines The results from the different timepoints were normalized to each other using spike-in RNA. EGRs 1-30 are shown as diagonal-filled arrows. With the exception of ORF-75a, the pattern of expression at the two time points is similar, however the overall signal level is significantly higher at 18 hours after infection. E) Conservation of peptide fragments within Expressed Genomic Regions 13 and

14. The γHV68 genome was translated in the six possible reading frames without the requirement of an initiator-methionine. Translated regions (Trans) from γHV68, light blue (positive strand) or light red (negative strand) arrows, were compared at an amino acid level with those from EBV, KSHV, and Herpesvirus saimiri (HVS). 8/30 EGRs contain translated regions with $P<1e^{-8}$. Two example regions are EGR13 and EGR14 (For a full list see Table S1). Translated regions conserved between 1, 2, or 3 gammaherpesviruses are colored black, green, or yellow, respectively. Percent identities of protein genes on the opposite are shown in parentheses. Translated regions conserved within EGRs are restricted to those contained in highly conserved antisense protein genes. F) Gene predictions in γHV68 using GeneMarkS-EV. GeneMarkS-EV gene predictions are shown as black outlined arrows. 69/80 γHV68 annotated ORFs were detected by GeneMark. In addition to these, GeneMark predicted 5 small novel protein-coding genes in the γHV68 genome. Three of these predictions were contained within EGR2, EGR7, and EGR28. G) Transposon mutants mapped to Expressed Genomic Regions. Mutant locations from a transposon mutagenesis study in γHV68 mapped to Expressed Genomic Regions. Open reading frames disrupted by these mutants were characterized as essential (red dots), attenuated (yellow dots), or non-essential (green dots). Since these transposon mutants disrupt the positive and negative strands, these ORF designations need to be reassessed in light of the Expressed Genomic Regions identified in this study.

Table S1

		γHV68 Coords		Region of Homology with EBV		Region of Homology with KSHV		Region of Homology with HSV	
EGR	**Trans**	**Start**	**Stop**	**Start**	**Stop**	**Start**	**Stop**	**Start**	**Stop**
EGR6	Trans-3096	18024	17893	NA	NA	18024	17920	18024	17908
EGR6	Trans-3092	18777	18574	NA	NA	18741	18574	18765	18574
EGR6	Trans-3093	18495	18322	NA	NA	NA	NA	18495	18349
EGR6	Trans-2508	20713	20297	20557	20300	NA	NA	20644	20508
EGR6	Trans-3080	21264	21100	21264	21100	21264	21100	21264	21124
EGR6	Trans-3081	20850	20701	NA	NA	NA	NA	20850	20740
EGR6	Trans-2505	21490	21131	21484	21131	NA	NA	NA	NA
EGR6	Trans-2506	21124	20867	NA	NA	21115	20889	NA	NA
EGR6	Trans-2508	20713	20297	20557	20388	NA	NA	20644	20508
EGR6	Trans-3078	21993	21481	21843	21480	21696	21481	NA	NA
EGR13	Trans-162	38560	38708	NA	NA	38560	38700	38581	38700
EGR13	Trans-163	38722	38874	NA	NA	38722	38851	NA	NA
EGR13	Trans-164	38878	39078	NA	NA	38878	39069	NA	NA
EGR14	Trans-2423	40978	40634	NA	NA	40855	40634	40858	40640
EGR14	Trans-2424	40630	40433	NA	NA	40630	40433	40534	40433
EGR16	Trans-1236	50739	50978	NA	NA	50739	50975	NA	NA
EGR17	Trans-2344	54925	54731	NA	NA	54898	34734	NA	NA
EGR20	Trans-258	60112	60480	60235	60480	60286	60445	NA	NA
EGR21	Trans-1802	61712	61587	NA	NA	NA	NA	61709	61587
EGR21	Trans-1799	62435	62109	NA	NA	62435	62245	NA	NA
EGR29	Trans-945	104483	104686	NA	NA	104498	104683	NA	NA

Table S1: Conservation of peptide fragments within Expressed Genomic Regions. Coordinates of translated regions from γHV68 conserved between EBV, KSHV and HVS. All start and stop coordinates refer to γHV68 genomic positions.

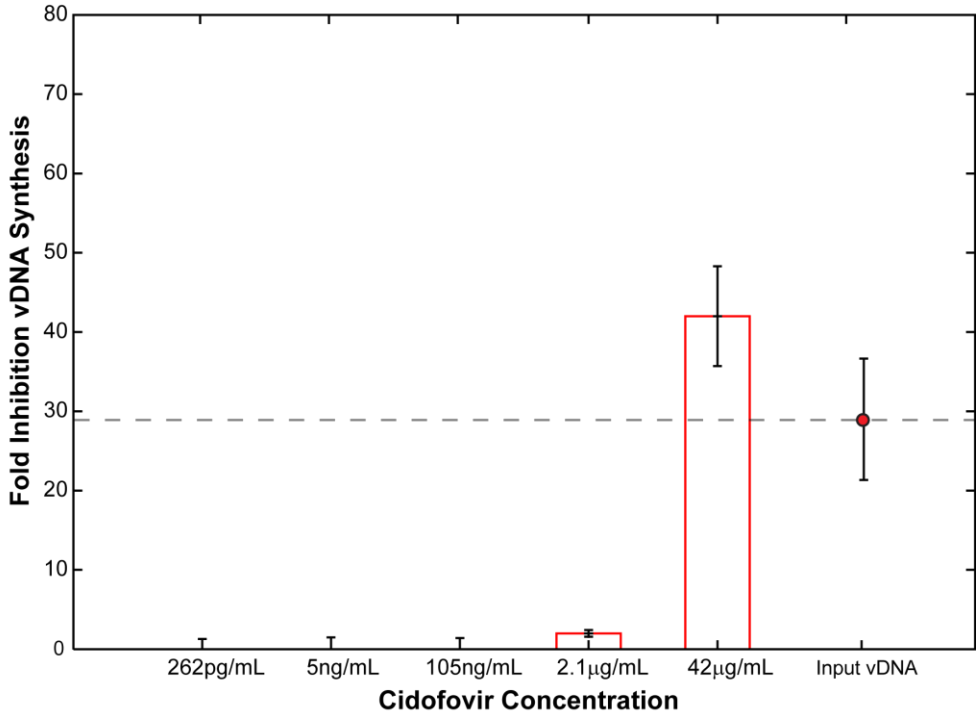

Figure S5: Fold inhibition of viral DNA synthesis by cidofovir. Three replicate sets of 3T12 cells were infected with γHV68 at an MOI=10 in the presence of 0-42µg/ml of cidofovir. Genomic DNA was harvested at 18 hpi and viral DNA level was assessed using primers for ORF-50. The amount of viral genomic DNA detected in untreated cells was used to set the baseline 0-fold inhibition. Viral DNA harvested at 0 hpi was used as a measure of input virus DNA (red circle, dashed line). Viral DNA levels detected using 42µg/ml cidofovir, used in our experiments, was comparable to input virus DNA amount.