Viral and Cellular *N*^{6}-Methyladenosine (m^{6}A) and *N*^{6}, 2′-O-Dimethyladenosine (m^{6}Am) Epitranscriptomes in KSHV Life Cycle

Brandon Tan[1], Hui Liu[1,2,7], Songyao Zhang[3,4,7], Suzane Ramos da Silva[1], Lin Zhang[2,3], Jia Meng[5], Xiaodong Cui[3], Hongfeng Yuan[1], Océane Sorel[1], Shaowu Zhang[4], Yufei Huang[3,*], Shou-Jiang Gao[1,6*]

[1]Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA, 90033, USA; [2]School of Information and Electrical Engineering, China University of Mining and Technology, Xuzhou, Jiangsu 221116, China; [3]Department of Electrical and Computer Engineering, University of Texas at San Antonio, San Antonio, TX, 78249, USA; [4]School of Automation, Northwestern Polytechnic University, Xi’an, Shaanxi, 710072, China; [5]Department of Biological Sciences, Xi'an Jiaotong-Liverpool University, Suzhou, Jiangsu 215123, China; [6]Laboratory of Human Virology and Oncology, Shantou University Medical College, Shantou, Guangdong 515041, China. [7]Equal contribution. *Corresponding authors.

Supplementary Figures and Tables

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Supplementary Figure 6: Illustration and validation of selected cellular m^{6}A/m peaks in four pairs of uninfected cells and cells latently infected by KSHV.

Supplementary Figure 7: Clustering analysis of different types of cells latently infected by KSHV, and uninduced and induced KiSLK and BCBL1-R cells.

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Supplementary Figure 9: Schematic illustration of features of viral and cellular m^6A/m epitranscriptomes in KSHV latent and lytic infection, and a model of YTHDF2-mediated inhibition of KSHV lytic replication.

Supplementary Figure 10: Uncropped images of Western-blots.

Supplementary Table 1: Latent KSHV m^6A/m peaks in five types of cells latently infected by KSHV.

Supplementary Table 2: Latent and lytic KSHV m^6A/m peaks in KiSLK and BCBL1-R cells.

Supplementary Table 3: Summary of cellular m^6A/m peaks from three biological replicates of all cell types.

Supplementary Table 4: Differential cellular methylation and gene expression in uninfected cells and cells latently infected by KSHV.

Supplementary Table 5: Significantly enriched pathways of hypomethylated and hypermethyated cellular genes following latent KSHV infection.

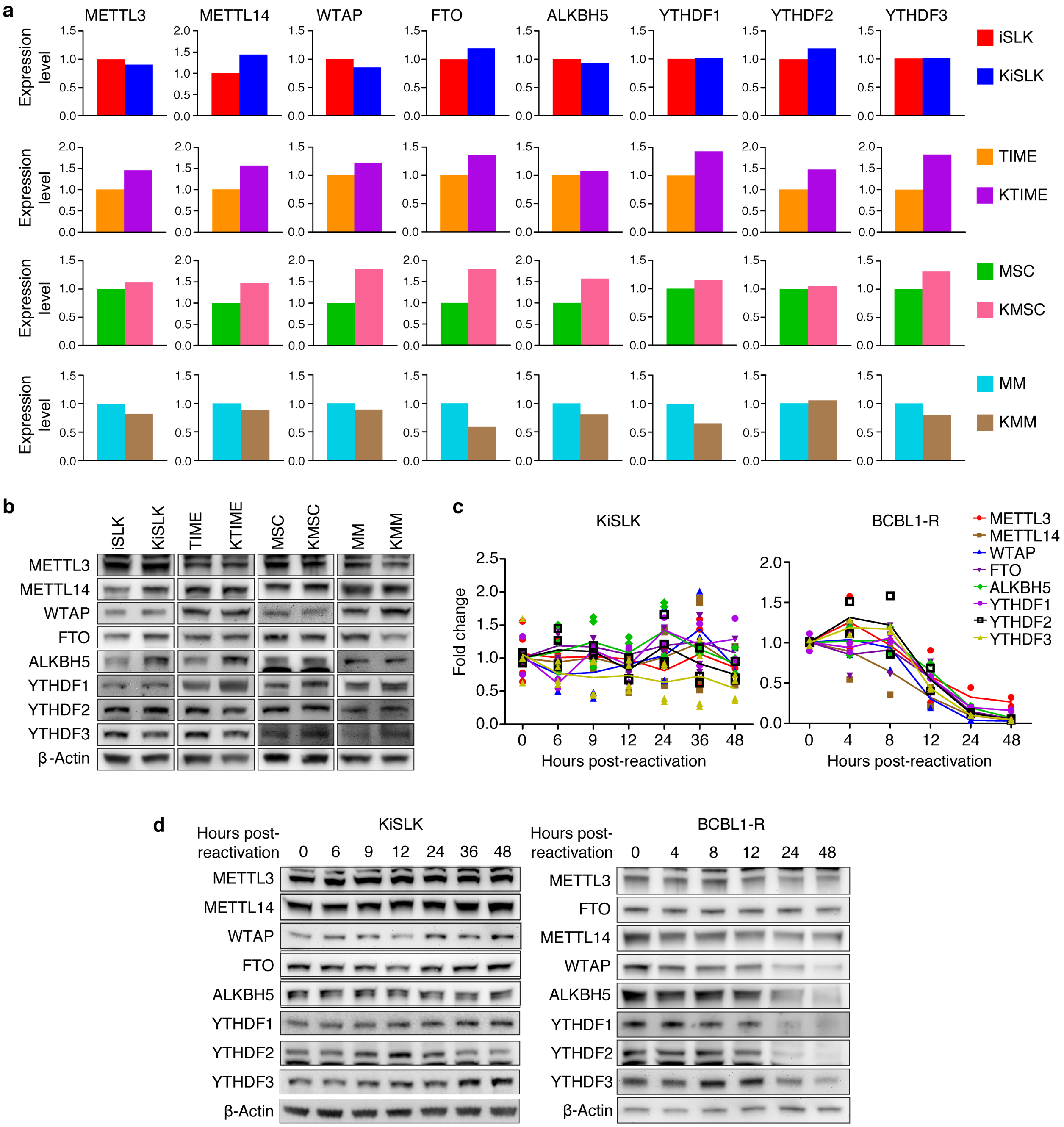
Supplementary Table 6: Differential cellular methylation and gene expression in KSHV-infected cells induced for lytic replication for 48 h compared to uninduced cells in KiSLK and BCBL1-R cells.

Supplementary Table 7: Significantly enriched pathways of 5’UTR hypomethylated and 3’UTR hypermethyated cellular genes in KSHV-infected cells induced for lytic replication for 48 h compared to uninduced cells in KiSLK and BCBL1-R cells.

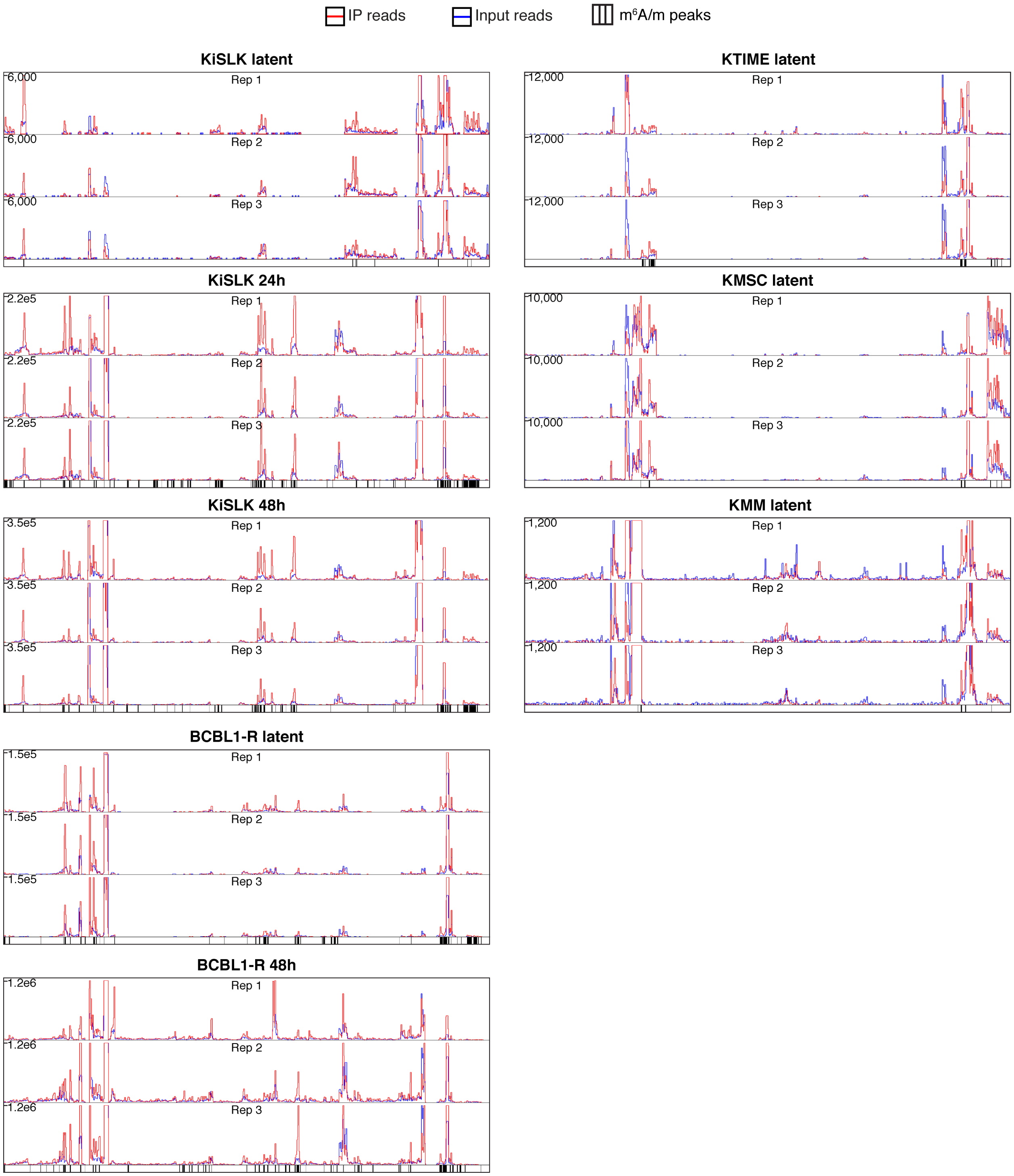
Supplementary Table 8: Summary of the numbers of mapped cellular and viral sequencing reads in different samples.

Supplementary Figures

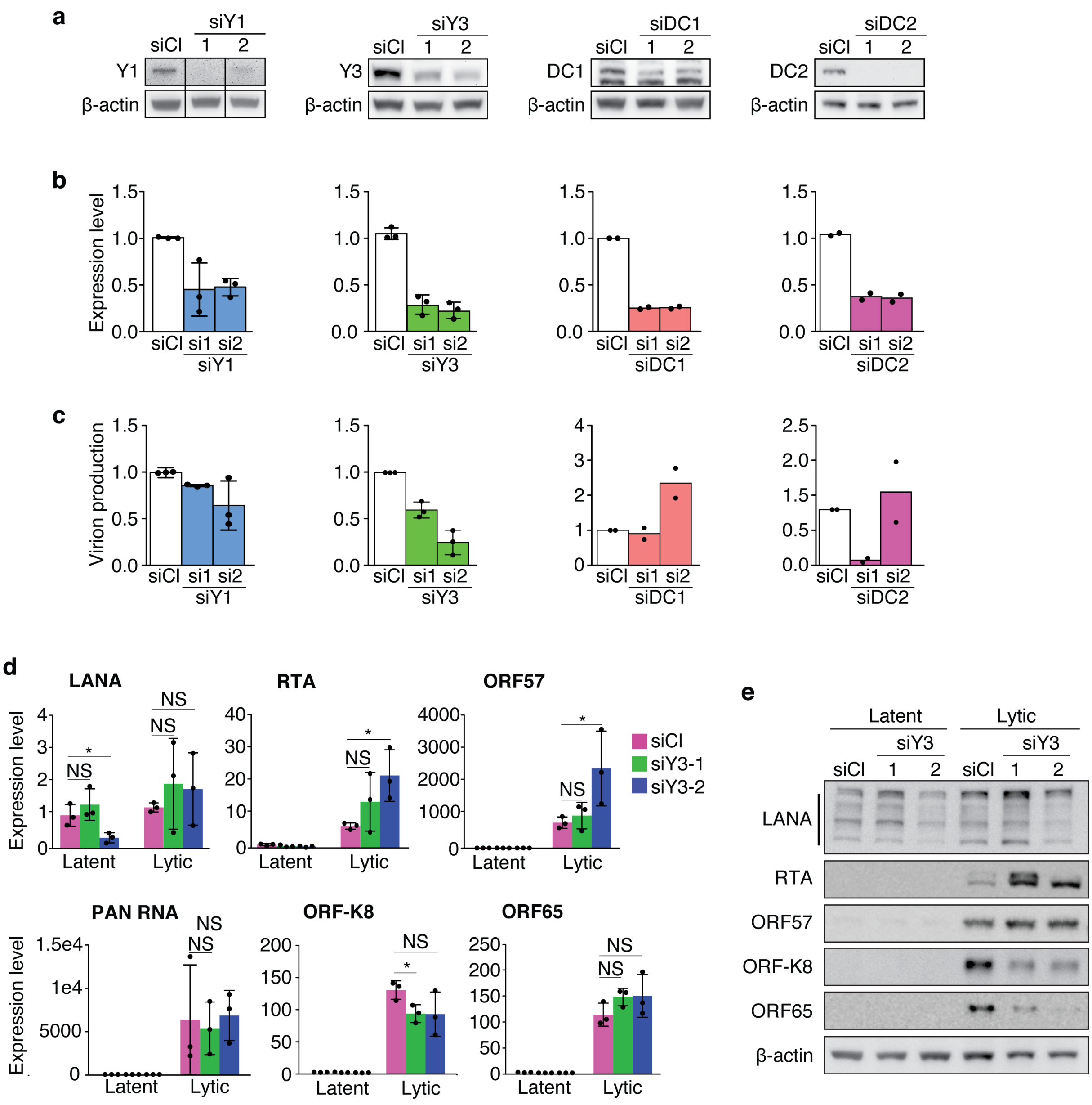
Supplementary Figure 1. Expression of m^{6}A/m-related enzymes in uninfected cells, cells latently infected by KSHV, and cells induced for lytic replication. a,b, Transcript (**a**) and protein (**b**) levels of m^{6}A/m-related proteins in uninfected cells and cells latently infected by KSHV examined by RT-qPCR and Western-blotting, respectively. Results are from a single experiment. **c,d,** Transcript (**c**) and protein (**d**) levels of m^{6}A/m-related proteins in KiSLK (left) and BCBL1-R (right) cells induced for lytic replication examined by RT-qPCR and Western-blotting, respectively. The experiments were independently repeated two times. RT-qPCR results are presented as mean. Western-blots from one representative experiment are presented.



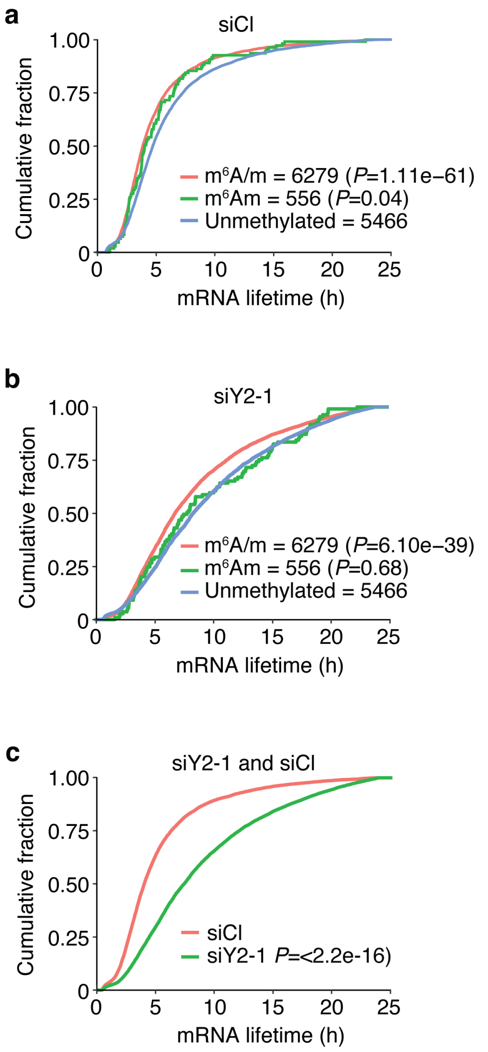
Supplementary Figure 2. Read tracks and m^{6}A/m peaks of the KSHV genome in different cell types from three biological replicates. Read tracks indicating IP (red) and input (blue) reads, and m^{6}A/m peaks of the KSHV genome from three biological replicates of KiSLK (latent, 24 h, and 48 h), BCBL1-R (latent and 48 h), KTIME (latent), KMSC (latent), and KMM (latent) cells.



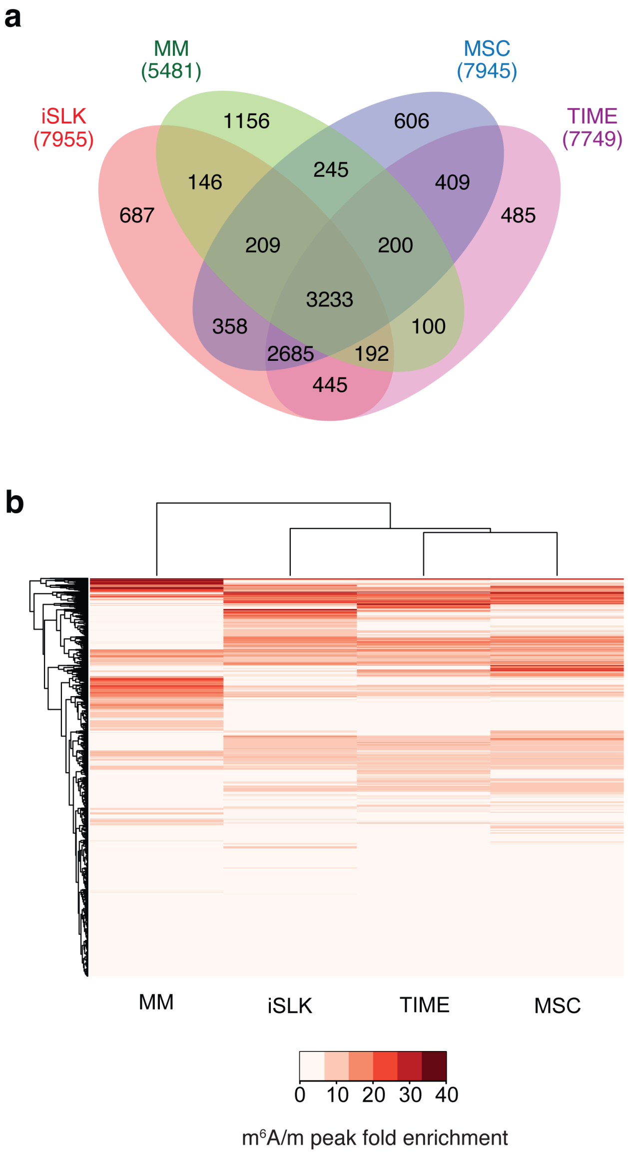
Supplementary Figure 3. Effect of YTHDF1, YTHDF3, YTHDC1, or YTHDC2 knockdown on KSHV lytic replication. a,b, Examination of knockdown efficiency of each reader by Western-blotting (**a**) and RT-qPCR (**b**) on day 2 post-transfection with the respective siRNA. **c**, Quantification of KSHV virions in culture supernatant by qPCR at day 3 after induction of lytic replication. For YTHDF1 (Y1) and YTHDF1 (Y3), experiments were repeated three times and results are presented as mean +/- SD from the three experiments (**b,c**). For YTHDC1 (DC1) and YTHDC1 (DC2), experiments were repeated twice and results are presented as mean (**b,c**). Western-blotting results are representative results from one experiment (**a**). **d,e,** Levels of viral transcripts (**d**) and proteins (**e**) were examined by RT-qPCR and Western-blotting, respectively, at day 3 after induction of lytic replication. Experiments were repeated three times and results are presented as mean +/- SD from the three experiments. NS = not significant, * p<0.05, ** p<0.01, *** p<0.001. Western-blotting results are representative results from one experiment (**e**). Gel image for Y1 (a) was cropped from a single blot.


a
siCl
siY1
1
2
Y1
β-actin
siY3
Y3
siDC1
DC1
siDC2
DC2
b
Expression level
1.5
1.0
0.5
0.0
si1
si2
c
Virion production
4
3
2
1
0
2.5
2.0
d
LANA
RTA
ORF57
Expression level
NS
*
Latent
Lytic
siCl
siY3-1
siY3-2
40
30
20
10
4000
3000
2000
1000
PAN RNA
ORF-K8
ORF65
1.5e4
1e4
5000
200
150
100
50
250
e
Latent
Lytic
LANA
RTA
ORF57
ORF-K8
ORF65
β-actin

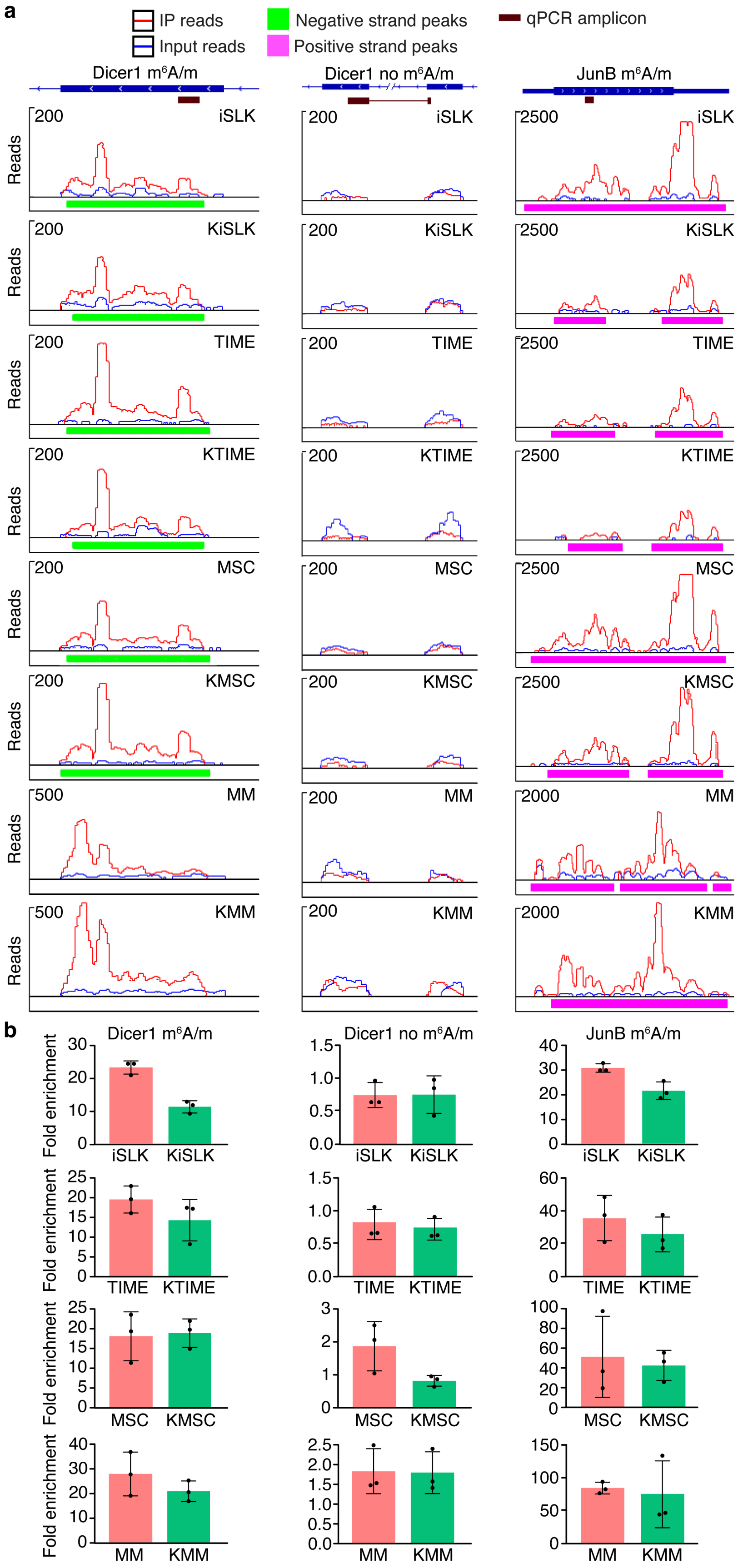
Supplementary Figure 4. mRNA lifetimes of m^6A/m, m^6Am and unmethylated transcripts in KiSLK cells treated with a control siRNA (siCl) or a siRNA to YTHDF2 (siY2-1). a, Cellular mRNA lifetimes of m^6A/m, m^6Am, and unmethylated mRNA in siCl cells. **b**, Cellular mRNA lifetimes of m^6A/m, m^6Am, and unmethylated mRNA in YTHDF2 knockdown cells. **c**, mRNA lifetimes of cellular transcripts in siCl and YTHDF2 knockdown cells. Results are from two independent experiments.



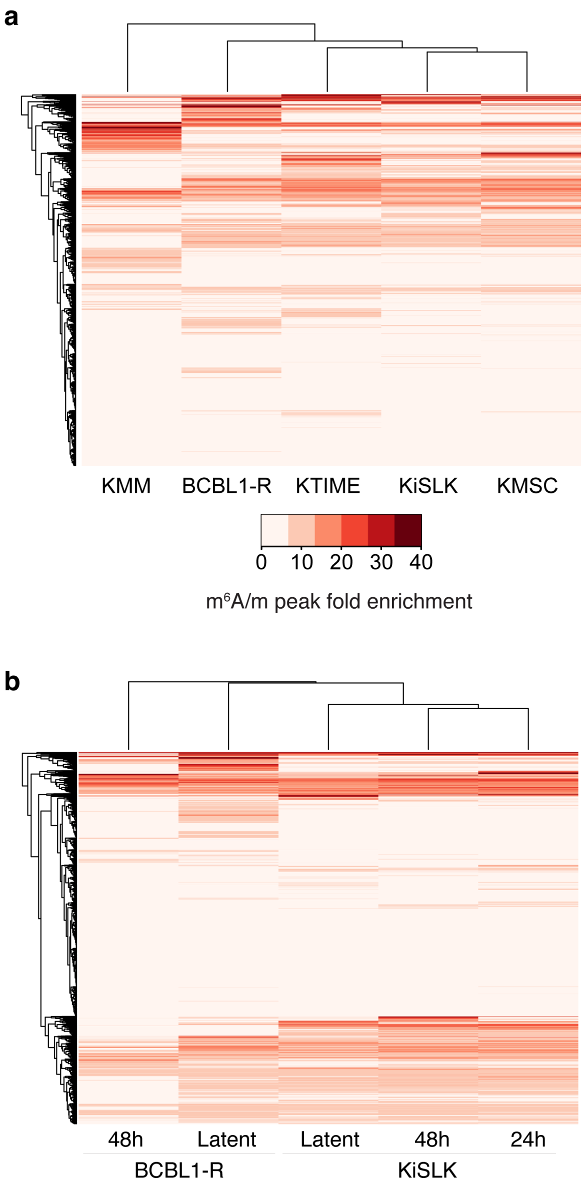
Supplementary Figure 5. Analyses of conserved and unique methylated cellular genes among four different uninfected cells. a, Venn diagram showing the overlaps of methylated cellular genes in four types of uninfected cells. **b**, Cluster analysis of m^{6}A/m methylated cellular genes of four types of uninfected cells. The results are from three biological replicates.



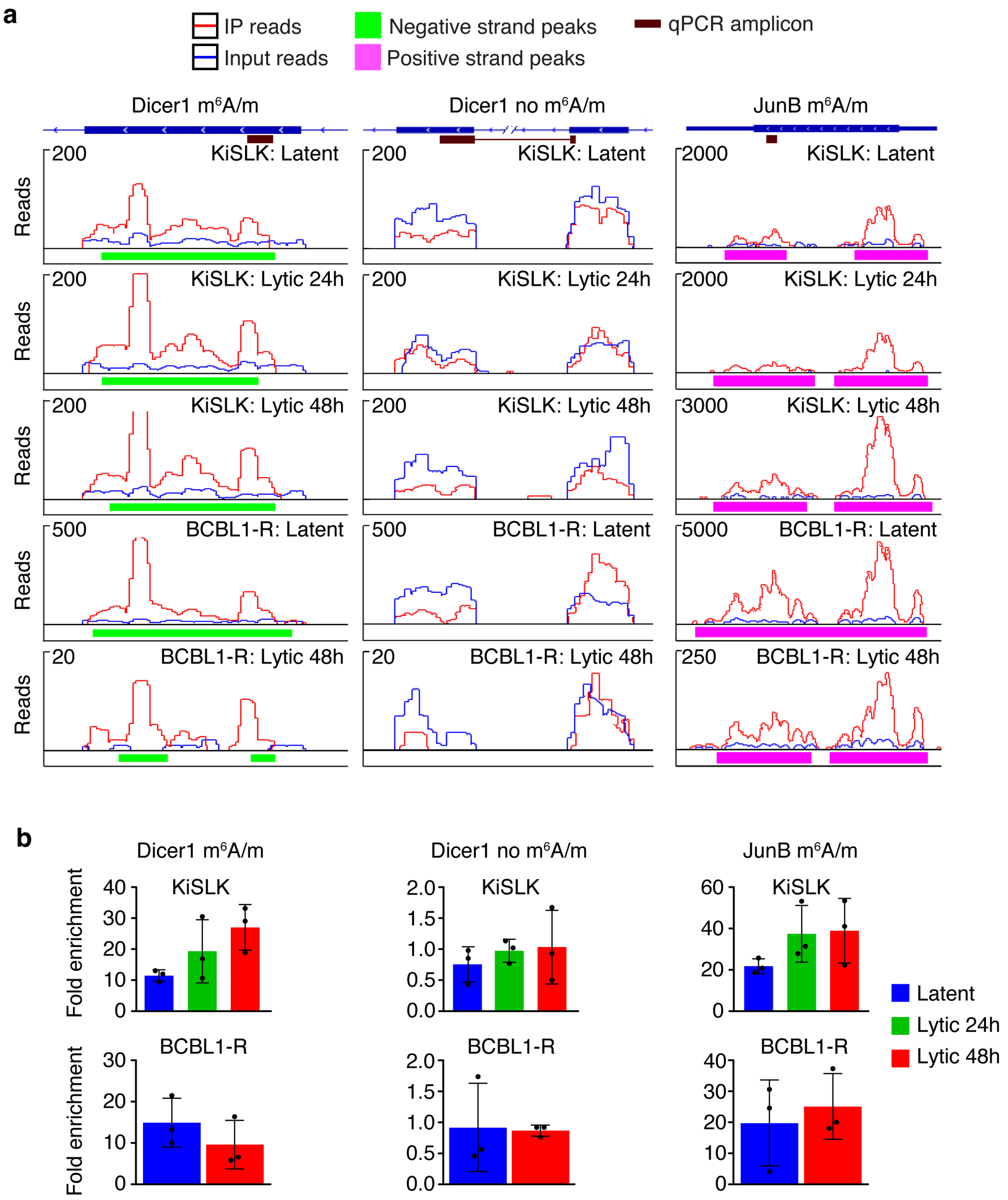
Supplementary Figure 6. Illustration and validation of selected cellular m^{6}A/m peaks in four pairs of uninfected cells and cells latently infected by KSHV. a, Regions of Dicer1 with and without an m^{6}A/m peak and JunB containing an m^{6}A/m peak with qPCR amplicons indicated. For KMM and MM cells, no m^{6}A/m peak was detected by exomePeak in Dicer1 as the gene was not annotated by the rn5 rat genome. **b**, Validation of cellular m^{6}A/m peaks shown in (**a**) by MeRIP-qPCR. Experiments were independently repeated three times, and results are presented as mean +/- SD from the three experiments.


a
IP reads
Input reads
Negative strand peaks
Positive strand peaks
qPCR amplicon
Dicer1 m⁶A/m
Dicer1 no m⁶A/m
JunB m⁶A/m
Reads
200
2500
2000
500
iSLK
KiSLK
TIME
KTIME
MSC
KMSC
MM
KMM
b
Fold enrichment
Dicer1 m⁶A/m
Dicer1 no m⁶A/m
JunB m⁶A/m
30
20
10
0
1.5
1.0
0.5
0.0
40
25
15
5
60
3
2
1
100
80
150
50
2.5
2.0
iSLK
KiSLK
TIME
KTIME
MSC
KMSC
MM
KMM

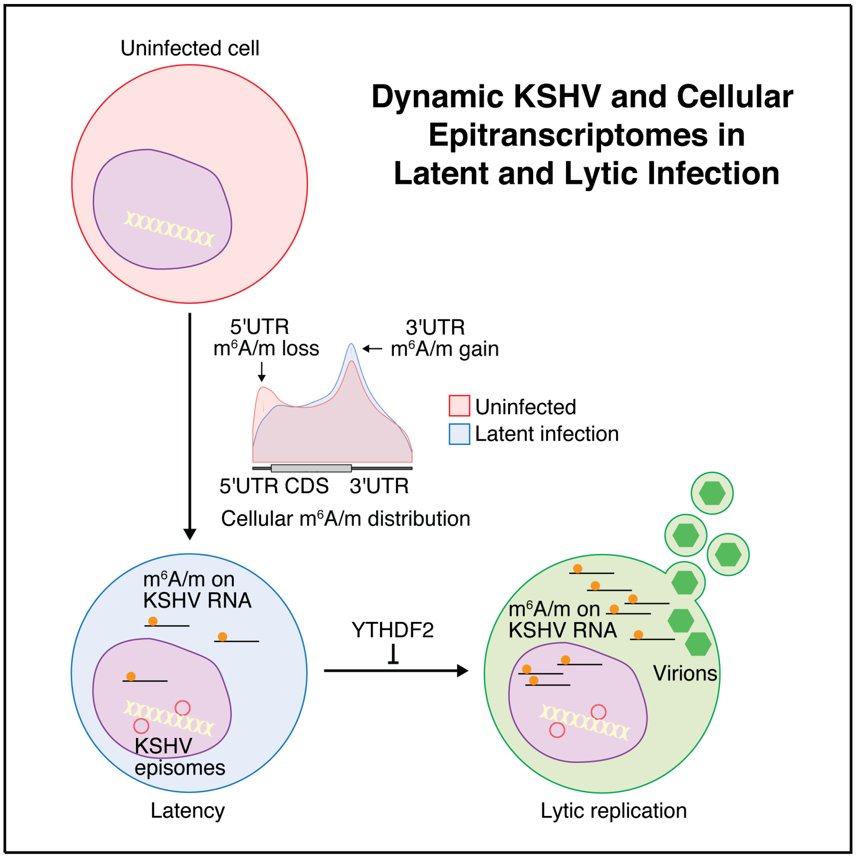
Supplementary Figure 7. Clustering analysis of different types of cells latently infected by KSHV, and uninduced and induced KiSLK and BCBL1-R cells. a, Cluster analysis of m^{6}A/m methylated cellular genes of five types of cells latently infected by KSHV. **b**, Cluster analysis of m^{6}A/m methylated cellular genes of uninduced and induced KiSLK and BCBL1-R cells. The results are from three biological replicates.



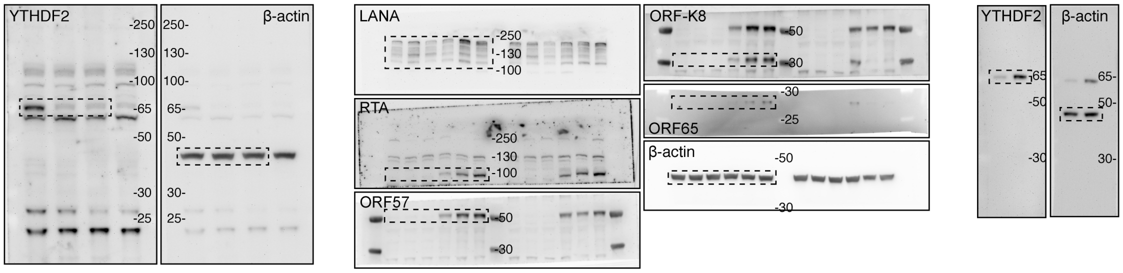
Supplementary Figure 8. Illustration and validation of selected cellular m^{6}A/m peaks in uninduced cells and cells induced for lytic replication. **a**, Regions of Dicer1 with and without a m^{6}A/m peak and JunB containing a m^{6}A/m peak with qPCR amplicons indicated. **b**, Validation of cellular m^{6}A/m peaks shown in (**a**) by MeRIP-qPCR. Experiments were independently repeated three times, and results are presented as mean +/- SD from the three experiments.



Supplementary Figure 9: Schematic illustration of features of viral and cellular m^{6}A/m epitranscriptomes in KSHV latent and lytic infection, and model of YTHDF2-mediated inhibition of KSHV lytic replication.



Supplementary Figure 10: Uncropped images of Western-blots.

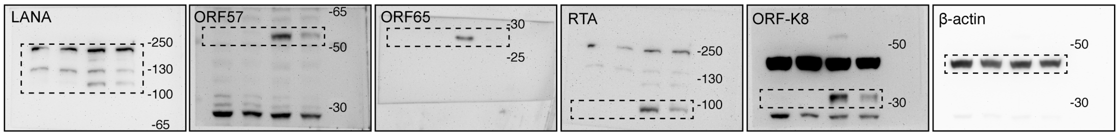


a. Corresponding to Figure 3A

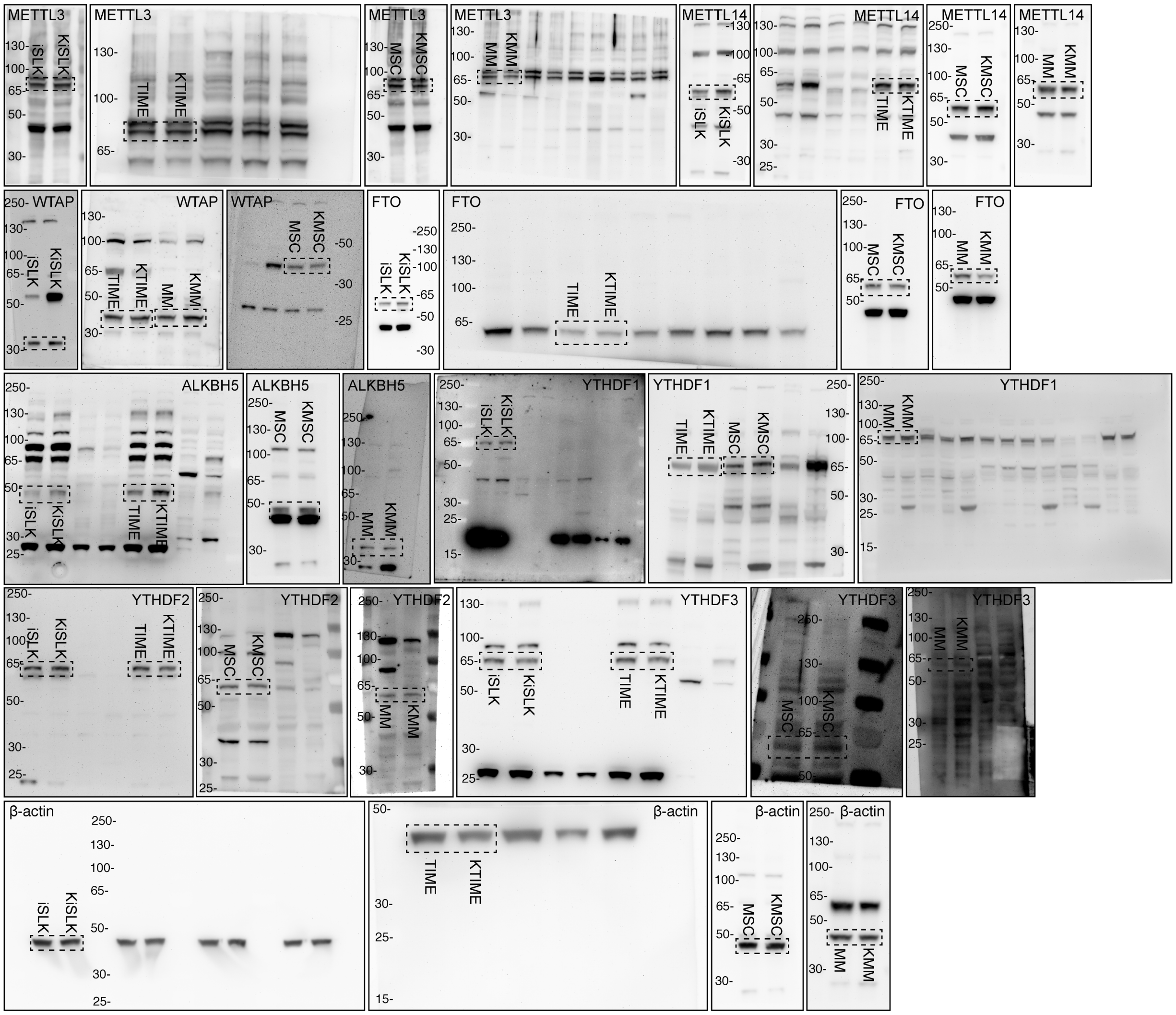
b. Corresponding to Figure 3E

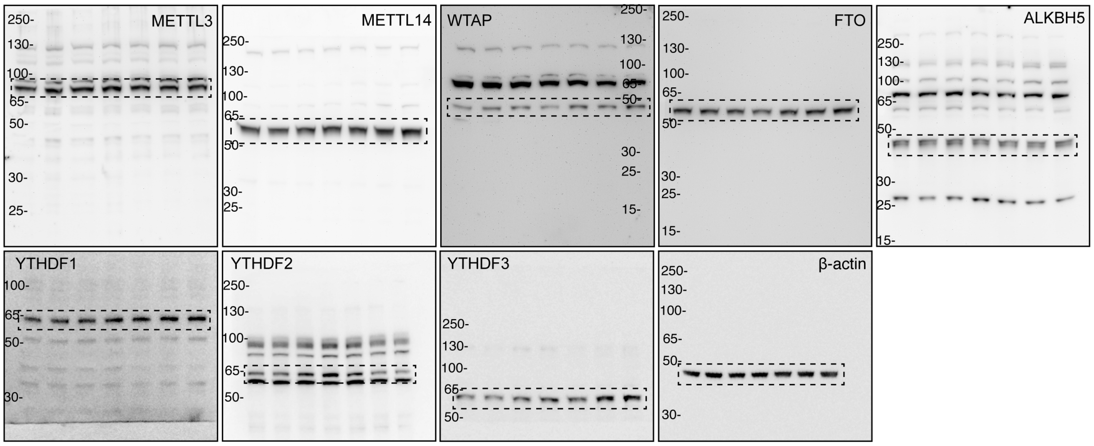
c. Corresponding to Figure 3F

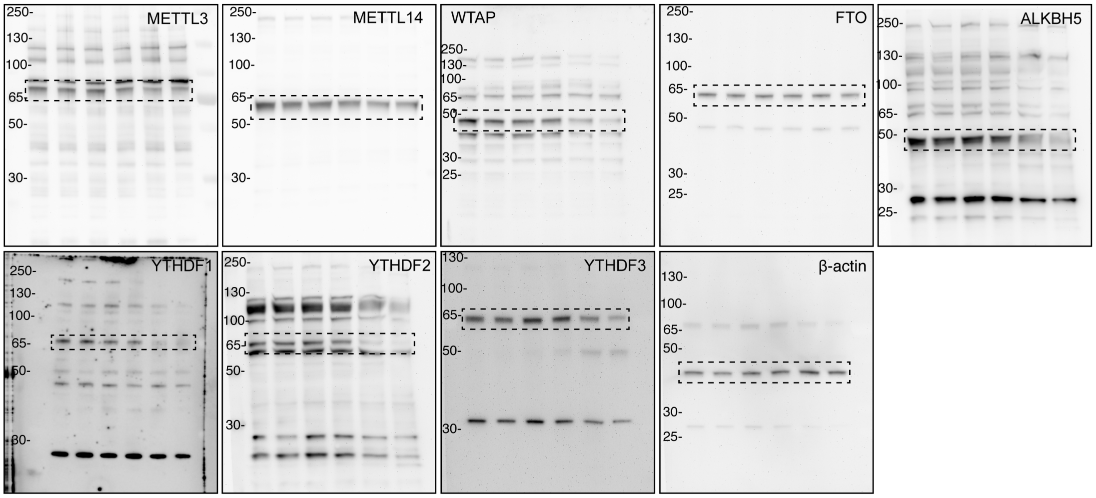
d. Corresponding to Figure 3H

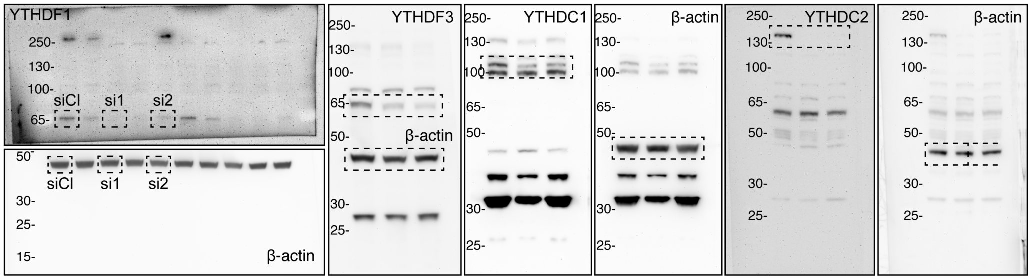


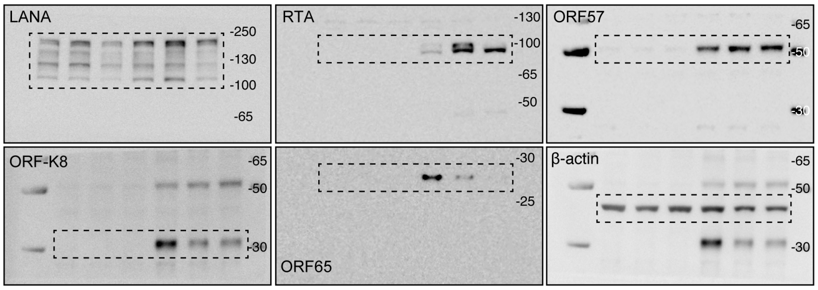
e. Corresponding to Supplementary Figure 1B




f. Corresponding to Supplementary Figure 1D (KiSLK)


g. Corresponding to Supplementary Figure 1D (BCBL1-R)


h. Corresponding to Supplementary Figure 3A


i. Corresponding to Supplementary Figure 3E

Supplementary Tables

Supplementary Table 1. Latent KSHV m^{6}A/m peaks in five types of cells latently infected by KSHV.
See Supplementary Dataset 1.

Supplementary Table 2. Latent and lytic KSHV m^{6}A/m peaks in KiSLK and BCBL1-R cells. The latent KiSLK and BCBL1-R data is the same as in Supplementary Table 1 and are included here for ease of comparison.
See Supplementary Dataset 2.

Supplementary Table 3. Summary of cellular m^{6}A/m peaks from three biological replicates of all cell types.

Sample name	# peaks			# overlapping peaks				
	Replicate 1	Replicate 2	Replicate 3	Replicates 1 & 2	Replicates 1 & 3	Replicates 2 & 3	Replicates 1 & 2 & 3	Three replicates combined
KiSLK, 24hr	18027	17294	15896	13607	13115	14634	12204	14349
KiSLK, 48hr	18989	15096	16374	12666	13803	13566	11750	13720
KiSLK, latent	17762	17410	15085	12515	12194	13562	11102	13271
iSLK	20211	16816	15359	14908	14050	14039	13317	15367
KMSC, latent	23166	15056	15693	13297	13660	13510	12299	13835
MSC	23453	16401	16919	14694	15395	14571	13668	15051
KTIME, latent	19500	17071	17232	14808	15111	15466	13931	16326
TIME	20076	15324	14817	13947	13348	13341	12552	14569
BCBL1-R, 48hr	10759	8440	11573	5858	7647	7169	5630	6269
BCBL1-R, latent	22205	17203	23431	15942	18011	15726	14571	18649
KMM, latent	11835	12173	11626	9814	9300	10641	8340	9589
MM	12823	9087	12114	8575	10565	8803	7778	8663

Supplementary Table 4. Differential cellular methylation and gene expression in uninfected cells and cells latently infected by KSHV.
See Supplementary Dataset 3.

Supplementary Table 5. Significantly enriched pathways of hypomethylated and hypermethyated cellular genes following latent KSHV infection.
See Supplementary Dataset 4.

Supplementary Table 6. Differential cellular methylation and gene expression in KSHV-infected cells induced for lytic replication for 48 h compared to uninduced cells in KiSLK and BCBL1-R cells.
See Supplementary Dataset 5.

Supplementary Table 7. Significantly enriched pathways of 5'UTR hypomethylated and 3'UTR hypermethyated cellular genes in KSHV-infected cells induced for lytic replication for 48 h compared to uninduced cells in KiSLK and BCBL1-R cells.
See Supplementary Dataset 6.

Supplementary Table 8: Summary of the numbers of mapped cellular and viral sequencing reads in different samples.

Sample	Cellular reads					
	Replicate 1		Replicate 2		Replicate 3	
	IP	Input	IP	Input	IP	Input
iSLK	47259226	45371211	61471530	41234673	51757410	44279942
KiSLK, latent	51077525	49414760	46870298	36598637	39848756	25708141
KiSLK, 24h	46941244	33704057	47175748	37129864	38870071	23149150
KiSLK, 48h	42600259	36427970	28355590	34569118	52739535	29175970
BCBL1-R, latent	60904961	51178970	70324105	34964036	58419322	65588251
BCBL1-R, 48h	8982177	11135640	13561807	11486260	23616859	17104239
KMSC, latent	66873093	46392153	39779820	30369603	47039116	38341770
MSC	66423259	47493604	52700167	35094806	52699578	33535825
KTIME, latent	45408410	43870273	58983797	25871277	60897505	28616979
TIME	58775750	37985952	55671973	28000760	56725250	27487994
KMM, latent	59707262	67310734	37019671	31486490	22074030	31486490
MM	53009118	42845232	27776700	25062040	37420391	35441818

Sample	Viral reads					
	Replicate 1		Replicate 2		Replicate 3	
	IP	Input	IP	Input	IP	Input
KiSLK, 24h	1909503	1647644	4408403	3124248	3237258	1937454
KiSLK, 48h	2980397	3108333	3743536	3798058	8904549	3621940
KiSLK, latent	92214	43348	214004	56788	139498	28325
BCBL1-R, latent	1127138	763352	1605556	442130	1378019	954780
BCBL1-R, 48h	34086056	35133738	19314942	17007273	31057727	17935625
KMSC, latent	214097	45195	124565	28162	165219	41329
KTIME, latent	21470	15657	82365	18774	77561	20560
KMM, latent	177673	62401	201293	34612	106457	27068