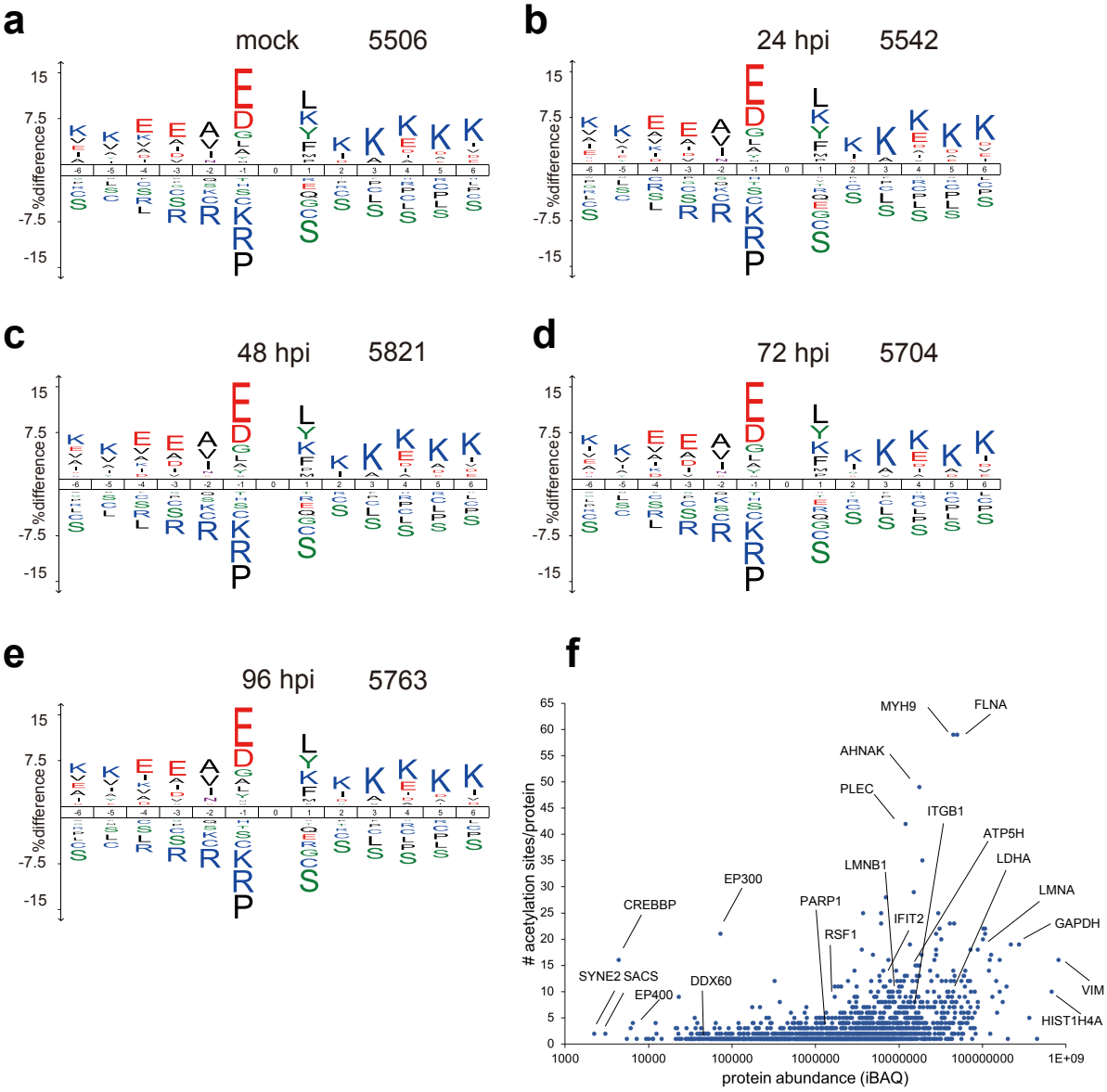
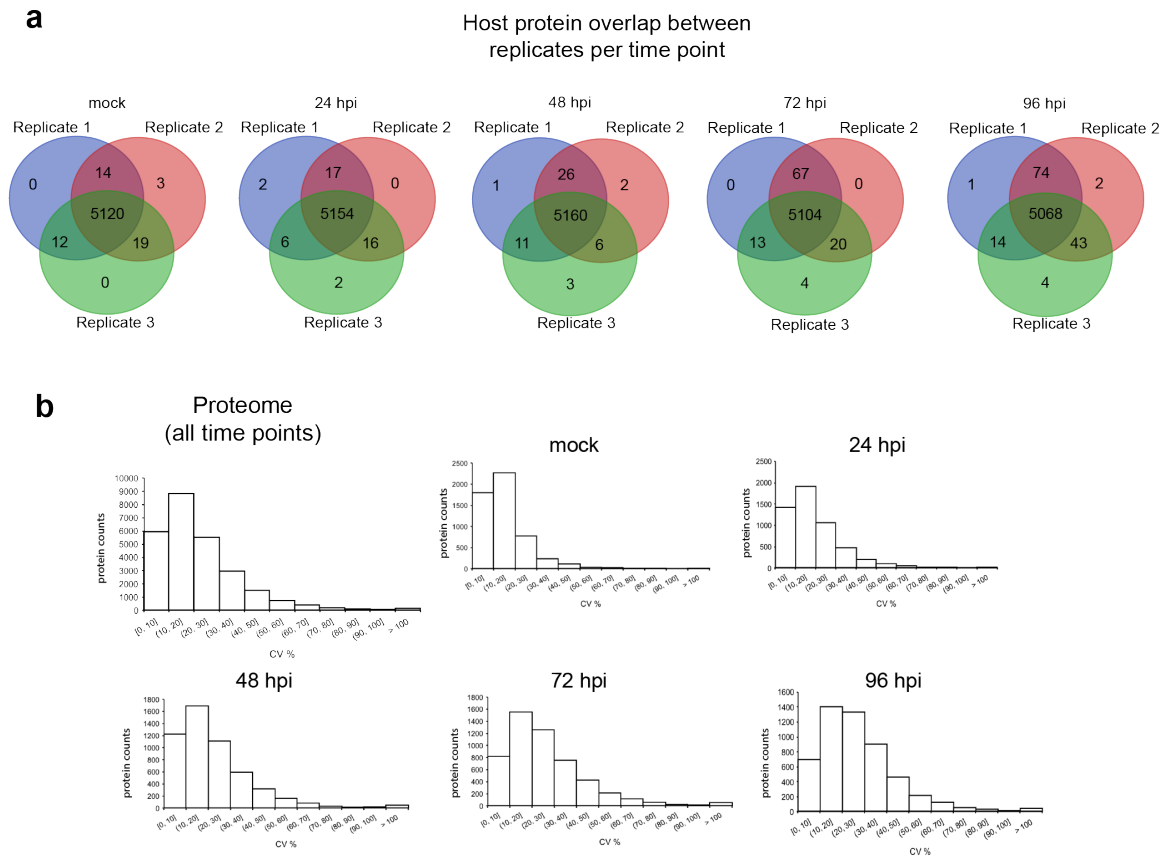
Orchestration of protein acetylation as a toggle for cellular defense and virus replication

Murray, et al.

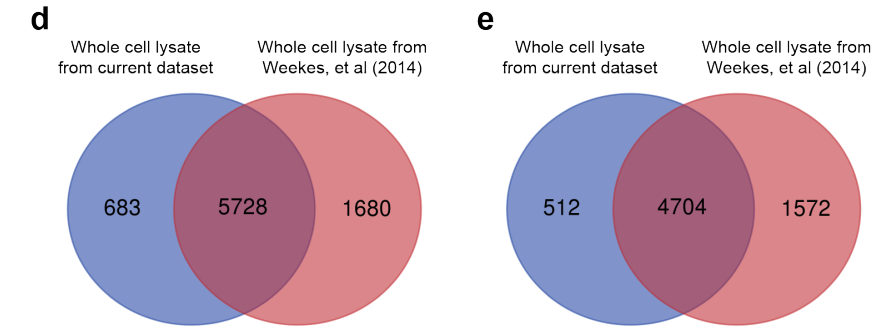


Supplementary Figure 1. Analysis of acetylated cellular peptides from uninfected and infected cells. Motifs from **a** uninfected and infected cells at **b** 24 hpi, **c** 48 hpi, **d** 72 hpi, and **e** 96 hpi were analyzed by IceLogo. Data represent results from a percentage difference scoring method with a P-value cut-off equal to 0.01 for the six amino acids flanking the acetylated lysine. The height of the letter at each position represents the difference in the amino acid residue frequency between the experimental set and the reference set. Numbers on the top indicate the number of peptides used for generating IceLogos. **f** Relationship between number of acetylation

sites and corresponding protein abundance. Protein quantification was performed using the iBAQ algorithm.

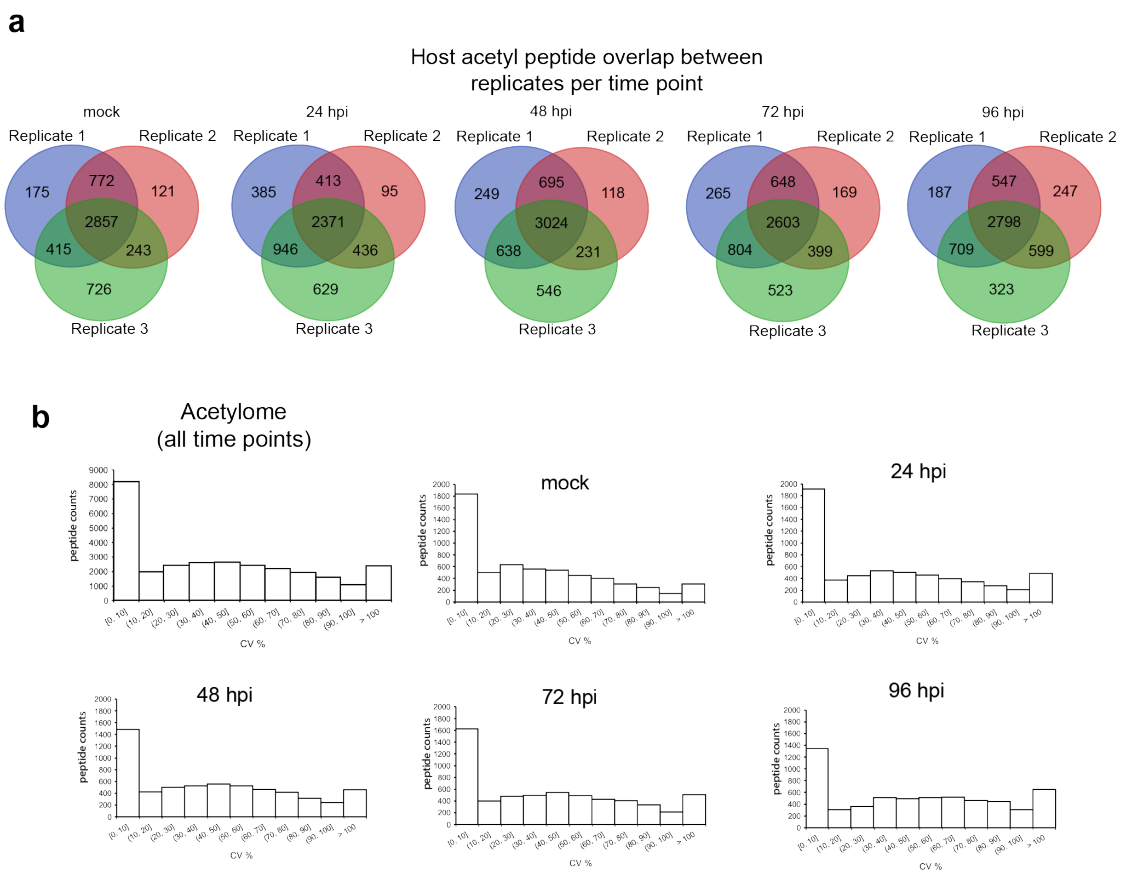


Replicate	mock rep1	mock rep2	mock rep3	24 hpi rep1	24 hpi rep2	24 hpi rep3	48 hpi rep1	48 hpi rep2	48 hpi rep3	72 hpi rep1	72 hpi rep2	72 hpi rep3	96 hpi rep1	96 hpi rep2	96 hpi rep3
# proteins	5146	5156	5151	5179	5187	5178	5198	5194	5180	5184	5191	5141	5157	5187	5129
# peptides detected	45438	48270	47449	47040	48908	47798	47383	48943	47381	46934	48476	46169	44233	48311	45768
# peptides quantified	43618	46188	45532	45098	46777	45746	45454	46803	45319	45015	46500	44068	42327	46273	43663



Supplementary Figure 2. Comparison of the proteome datasets from the biological replicates, as well as with prior literature. a Venn diagrams showing the overlap in host proteins quantified between three biological replicates at each time point during infection. **b** Distribution of the coefficient of variation (CV %) of all time points combined, and individual

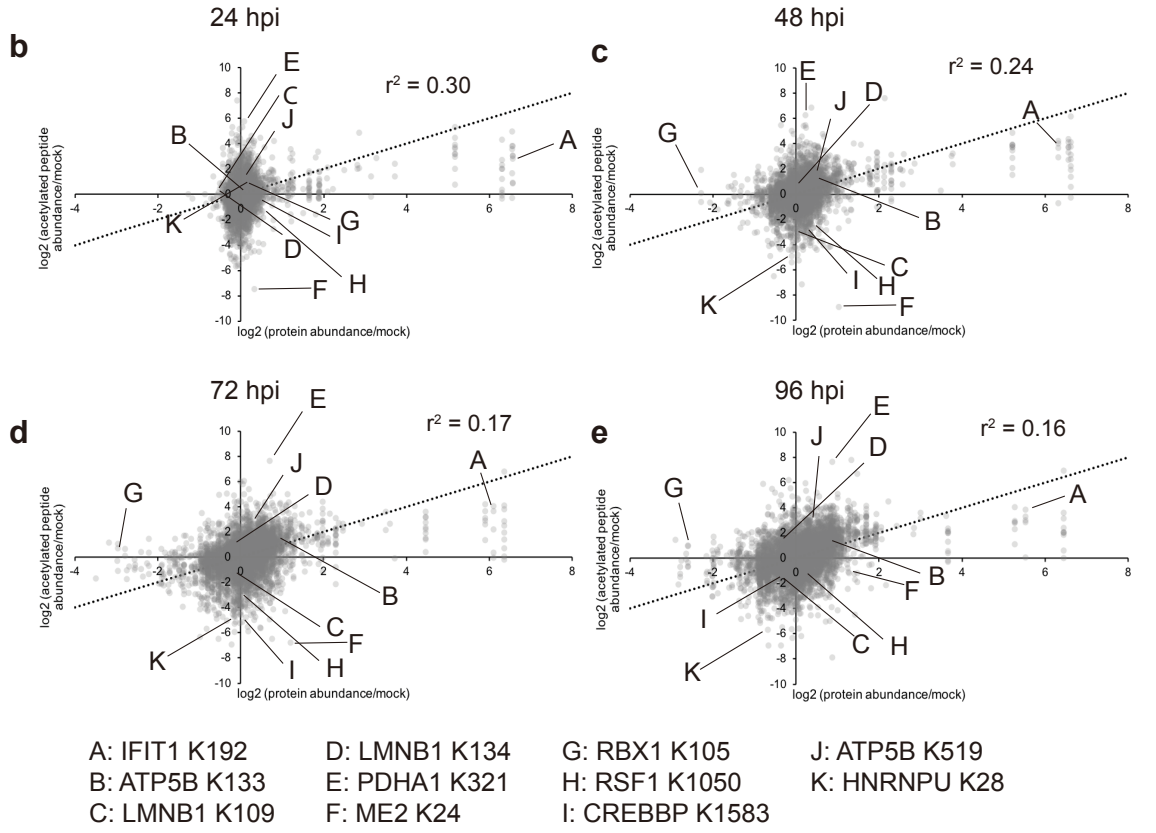
time points for host whole proteome. **c** Comparison of number of host proteins and number of corresponding detected and quantified unmodified peptides across all three biological replicates in all time points. **d, e** Comparison of the number of identified proteins from the current proteome dataset and a previous study (Weekes, et al 2014). **d** Number of identified proteins with more one or more unique peptides (not including isoforms). **e** Number of identified proteins with two or more unique peptides (not including isoforms).



Supplementary Figure 3. Comparison of the acetylome datasets from the biological replicates. a Venn diagrams showing the overlap in host acetylated peptides filtered and used in the quantification analysis between three biological replicates at each time point during infection. **b** Distribution of the coefficient of variation (CV %) of all time points combined, and individual time points for host peptides in the acetylome. CV percentages do not include imputed values.

a

Replicate	mock rep1	mock rep2	mock rep3	24 hpi rep1	24 hpi rep2	24 hpi rep3	48 hpi rep1	48 hpi rep2	48 hpi rep3	72 hpi rep1	72 hpi rep2	72 hpi rep3	96 hpi rep1	96 hpi rep2	96 hpi rep3
# acetylated peptides	4219	3993	4241	4115	3315	4382	4606	4068	4439	4320	3819	4329	4241	4191	4429
# acetylated proteins	1608	1563	1604	1606	1367	1631	1692	1559	1639	1610	1502	1593	1566	1590	1602
% imputed acetyl peptide abundance (only used for trend analysis)	27	31	26	29	42	24	20	29	23	25	34	25	26	27	23

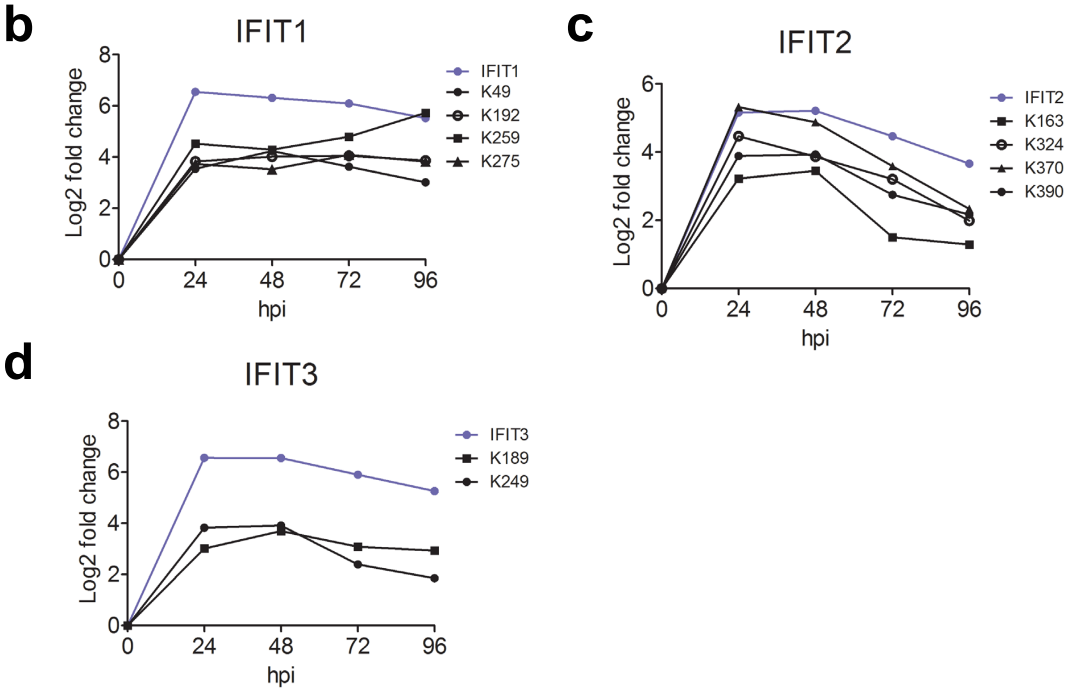


Supplementary Figure 4. Assessment of correlation between changes in acetylated peptide abundance and changes in protein abundance for host proteins. a Comparison of host acetylated peptides, the corresponding acetylated proteins, and the percentage of imputed values (used only in trend analyses in Figure 2b, Figure 3a, and Supplementary Figure 4b-e) across all three acetyl-IP biological replicates in all time points. These numbers represent the dataset of acetylated peptides that were filtered and used for quantitative analysis. **b-e** Dot plots of the log2 fold change of acetylated peptide abundance (normalized to mock) and corresponding protein abundance (normalized to mock) at **b** 24, **c** 48, **d** 72, and **e** 96 hpi. Each dot represents one

acetylated peptide, and a number of peptides of interest are marked. The dotted line indicates a slope equal to 1.

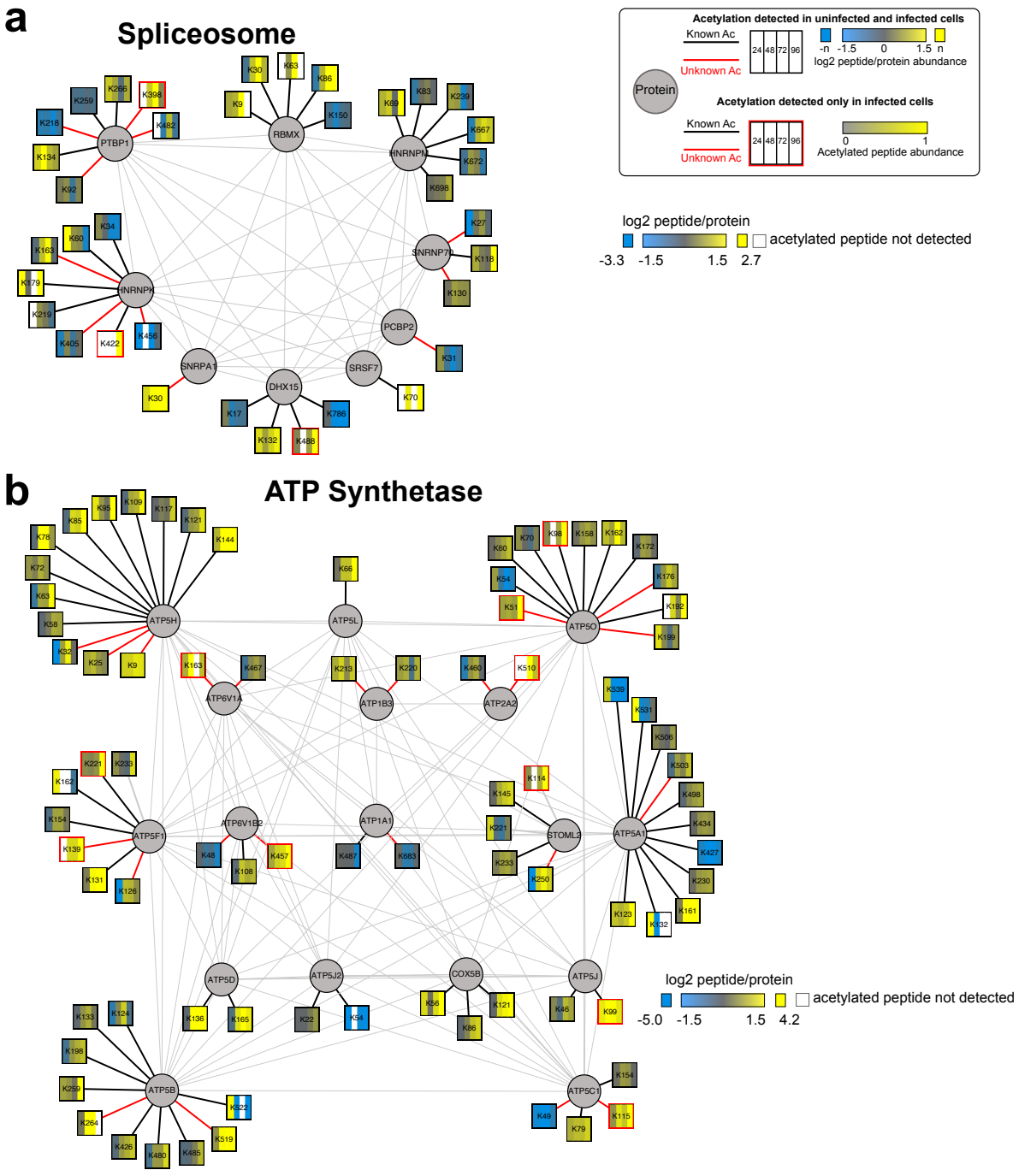
a

Cluster	# Proteins	Enriched GO Biological Processes	Representative Proteins	p-value	FDR
1	234	TCA cycle, response to virus, type I interferon signaling, gluconeogenesis	DLD, IFIT1, STAT1, GOT1	3.89E-14, 1.18E-05, 1.19E-05, 6.10E-05	5.11E-11, 1.74E-03, 1.74E-03, 4.45E-03
2	551	TCA cycle, glyoxylate metabolic process, tRNA aminoacylation for protein translation, canonical glycolysis, oxidative phosphorylation	PDHA, DLAT, MARS, ENO1, NDUFB6	2.22E-16, 8.40E-9, 5.11E-08, 1.03E-6, 1.82E-95	2.46E-13, 3.92E-06, 1.03E-05, 1.13E-04, 1.22E-03
3	641	translation, rRNA processing, cell-cell adhesion, TCA cycle, nucleosome assembly, glyoxylate metabolic process	RPL5, LDHA, ATP5O, SMARCA5, OGDH	1.11E-16, 1.11E-16, 1.11E-16, 4.28E-12, 1.19E-11, 5.57E-05	3.94E-14, 3.94E-14, 3.94E-14, 1.33E-09, 3.28E-09, 3.95E-03
4	833	translation, RNA processing, cell-cell adhesion, antigen processing and presentation, regulation of ubiquitin-protein ligase activity, NIK/NF-kappaB signaling, protein folding, viral process, canonical glycolysis, nucleosome assembly	RPL4, RUVBL1, MYO6, PSMD8, HSP90AB1, CREBBP, ENO1, KAT6B	1.11E-16, 1.11E-16, 1.11E-16, 1.11E-16, 1.11E-16, 5.77E-15, 2.93E-14, 2.49E-13, 9.94E-13, 6.69E-10, 1.27E-07, 5.85E-05	4.09E-14, 4.09E-14, 4.09E-14, 4.09E-14, 4.09E-14, 1.83E-12, 7.83E-12, 5.23E-11, 1.79E-10, 9.37E-08, 1.13E-05, 3.10E-03
5	662	translation, viral transcription, protein targeting to membrane, cell-cell adhesion, nucleosome assembly, mRNA splicing, histone H3 acetylation, protein stabilization	RPL4, FLNB, HIST2H2BF, HNRNPU, JADE1, HSP90AA1	1.11E-16, 1.11E-16, 1.11E-16, 1.11E-16, 2.67E-12, 1.59E-10, 8.56E-07, 5.24E-05	3.91E-14, 3.91E-14, 3.91E-14, 3.91E-14, 8.23E-10, 4.35E-08, 1.32E-04, 3.76E-03
6	329	cell-cell adhesion, translation, protein targeting to membrane, viral transcription, nucleosome assembly, rRNA processing, cell-junction assembly, regulation of mRNA stability	PFN1, EIF4A1, RPS9, H3F3A, ACTB, HNRNPD	2.19E-14, 2.13E-12, 2.82E-12, 7.14E-12, 3.00E-07, 4.77E-07, 6.45E-07, 2.52E-05	3.59E-11, 1.54E-09, 1.54E-09, 2.93E-09, 7.02E-05, 9.78E-05, 1.06E-04, 3.22E-03
7	230	beta-catenin-TCF complex assembly, nucleosome assembly	CREBBP, RSF1	7.34E-08, 6.81E-07	8.06E-05, 3.98E-04



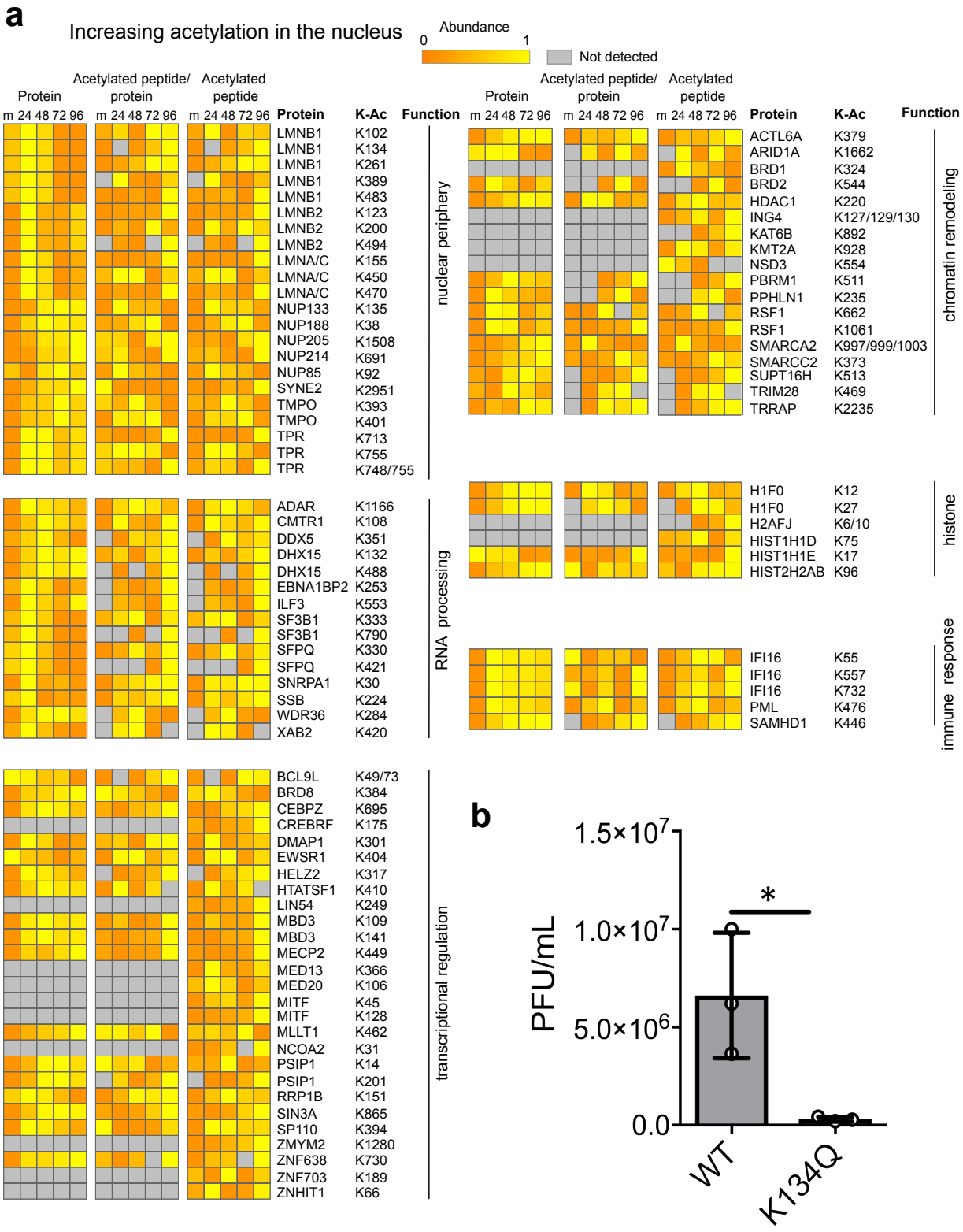
Supplementary Figure 5. Distinct acetylation abundance trends and different enriched GO Biological Processes. a Enriched GO Biological Processes with p-values ≤ E-05 were determined for each acetyl trend cluster through the Reactome plugin in Cytoscape. The p-value

and FDR for each term are shown in the same order as the terms are shown. **b-e** Comparison of log2 fold change of protein abundance to acetylated peptide abundance for IFIT proteins. IFIT protein abundance (normalized to mock) is shown in blue. Abundance of each acetylated peptide that was detected in both mock and infection time points is shown in black (normalized to mock). **b** IFIT1, **c** IFIT2, **d** IFIT3.

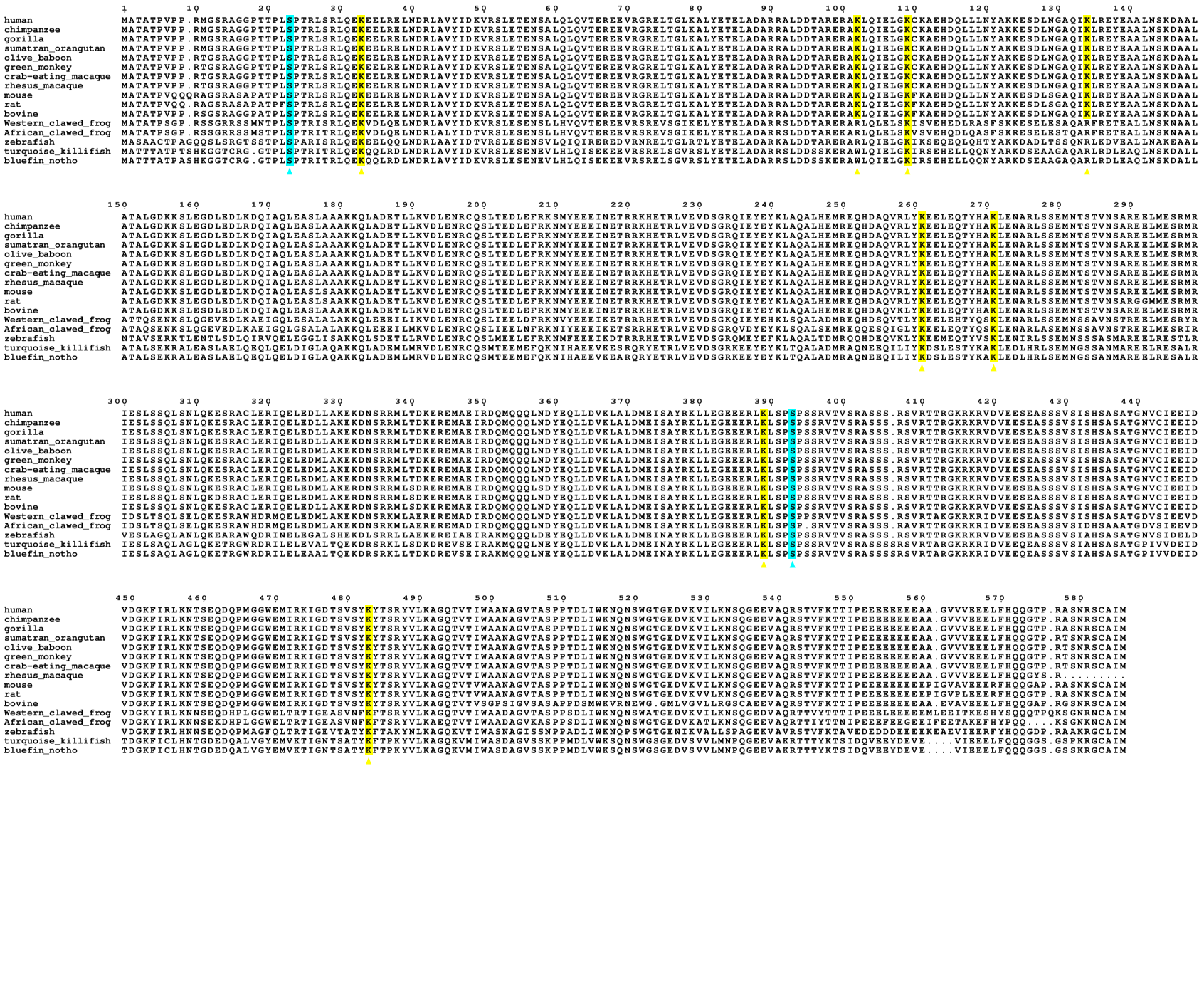


Supplementary Figure 6. Site-specific acetylation trends are observed on mRNA splicing and ATP synthetase proteins. a, b All detected acetylation sites on proteins from the indicated biological function representing different site-specific trends in acetylation during infection.

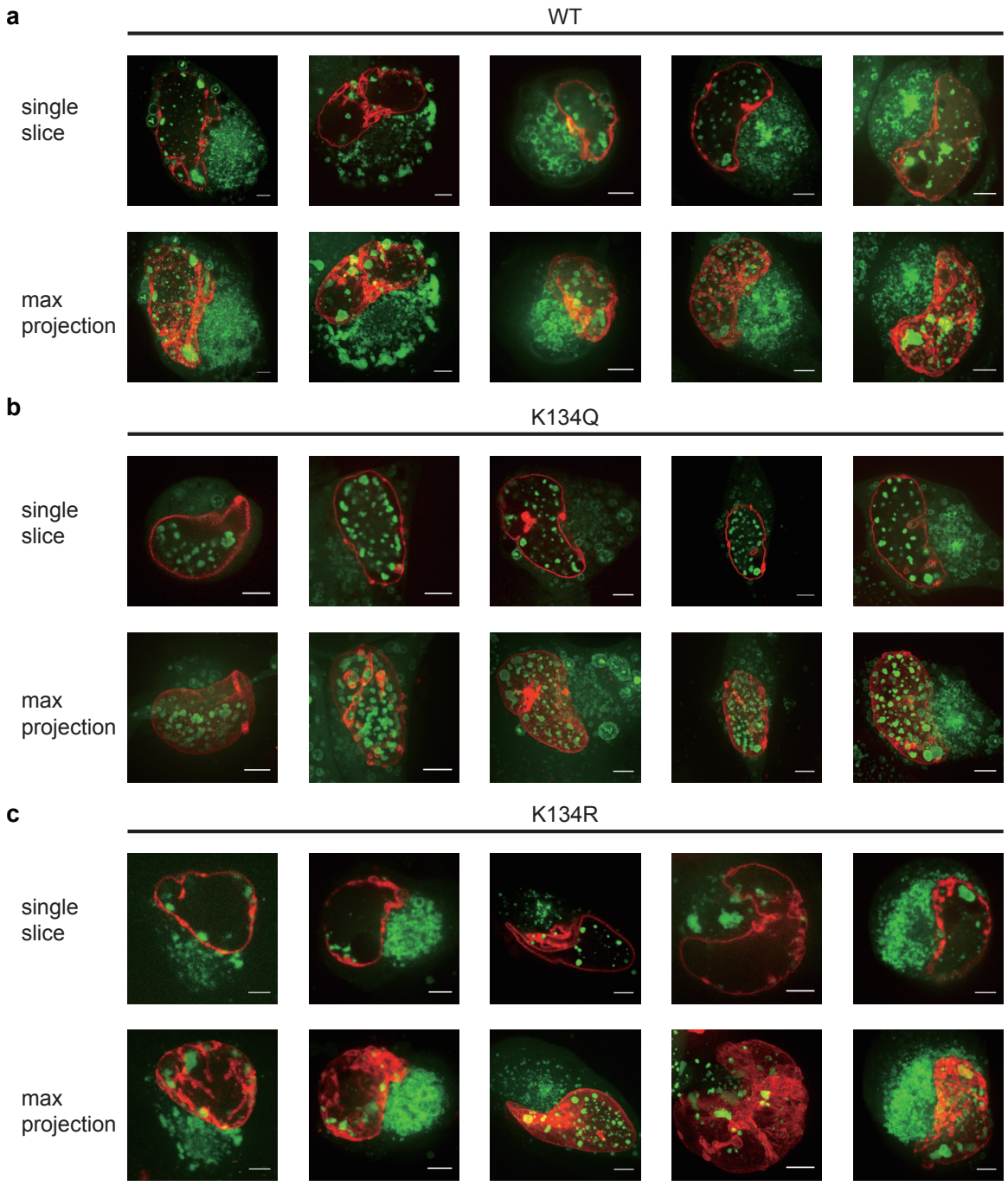
Log2 peptide/protein values are shown unless the acetylated peptide was not identified in mock, in which case the acetylated peptide abundances during infection are shown scaled 0 to 1. **a** Spliceosome **b** ATP synthetase complex. Circles, proteins; squares, acetylation sites; red lines, previously unknown acetylation sites; black lines, known acetylation sites; black boxes, detected in mock; red boxes, only detected during infection. If a peptide was not quantified in a given time point, the corresponding column within the acetyl box is white.


a
Increasing acetylation in the nucleus
Abundance
0
1
Not detected
Protein
Acetylated peptide/ protein
Acetylated peptide
m 24 48 72 96
Protein
K-Ac
Function
LMNB1 K102
LMNB1 K134
LMNB1 K261
LMNB1 K389
LMNB1 K483
LMNB2 K123
LMNB2 K200
LMNB2 K494
LMNA/C K155
LMNA/C K450
LMNA/C K470
NUP133 K135
NUP188 K38
NUP205 K1508
NUP214 K691
NUP85 K92
SYNE2 K2951
TMPO K393
TMPO K401
TPR K713
TPR K755
TPR K748/755
nuclear periphery
ADAR K1166
CMTR1 K108
DDX5 K351
DHX15 K132
DHX15 K488
EBNA1BP2 K253
ILF3 K553
SF3B1 K333
SF3B1 K790
SFPQ K330
SFPQ K421
SNRPA1 K30
SSB K224
WDR36 K284
XAB2 K420
RNA processing
BCL9L K49/73
BRD8 K384
CEBPZ K695
CREBRF K175
DMAP1 K301
EWSR1 K404
HELZ2 K317
HTATSF1 K410
LIN54 K249
MBD3 K109
MBD3 K141
MECP2 K449
MED13 K366
MED20 K106
MITF K45
MITF K128
MLLT1 K462
NCOA2 K31
PSIP1 K14
PSIP1 K201
RRP1B K151
SIN3A K865
SP110 K394
ZMYM2 K1280
ZNF638 K730
ZNF703 K189
ZNHIT1 K66
transcriptional regulation
ACTL6A K379
ARID1A K1662
BRD1 K324
BRD2 K544
HDAC1 K220
ING4 K127/129/130
KAT6B K892
KMT2A K928
NSD3 K554
PBRM1 K511
PPHLN1 K235
RSF1 K662
RSF1 K1061
SMARCA2 K997/999/1003
SMARCC2 K373
SUPT16H K513
TRIM28 K469
TRRAP K2235
chromatin remodeling
H1F0 K12
H1F0 K27
H2AFJ K6/10
HIST1H1D K75
HIST1H1E K17
HIST2H2AB K96
histone
IFI16 K55
IFI16 K557
IFI16 K732
PML K476
SAMHD1 K446
immune response
b
PFU/mL
1.5×10^7
1.0×10^7
5.0×10^6
0.0
*
WT
K134Q

Supplementary Figure 7. Acetylation is increased on a subset of nuclear proteins, including those at the nuclear periphery such as LMNB1. a Sites on nuclear proteins with increased levels of acetylation during infection clustered by function. Abundances are scaled from 0 to 1 (left, protein abundance; middle, acetylated peptide abundance normalized to protein abundance; right, acetylated peptide abundance). **b** $TCID_{50}$ assessment of the effect of LMNB1 K134Q mutant on infectious extracellular virus produced. Average of three replicates ± SD. One-sided Student's t-test was used. * $p < 0.05$.

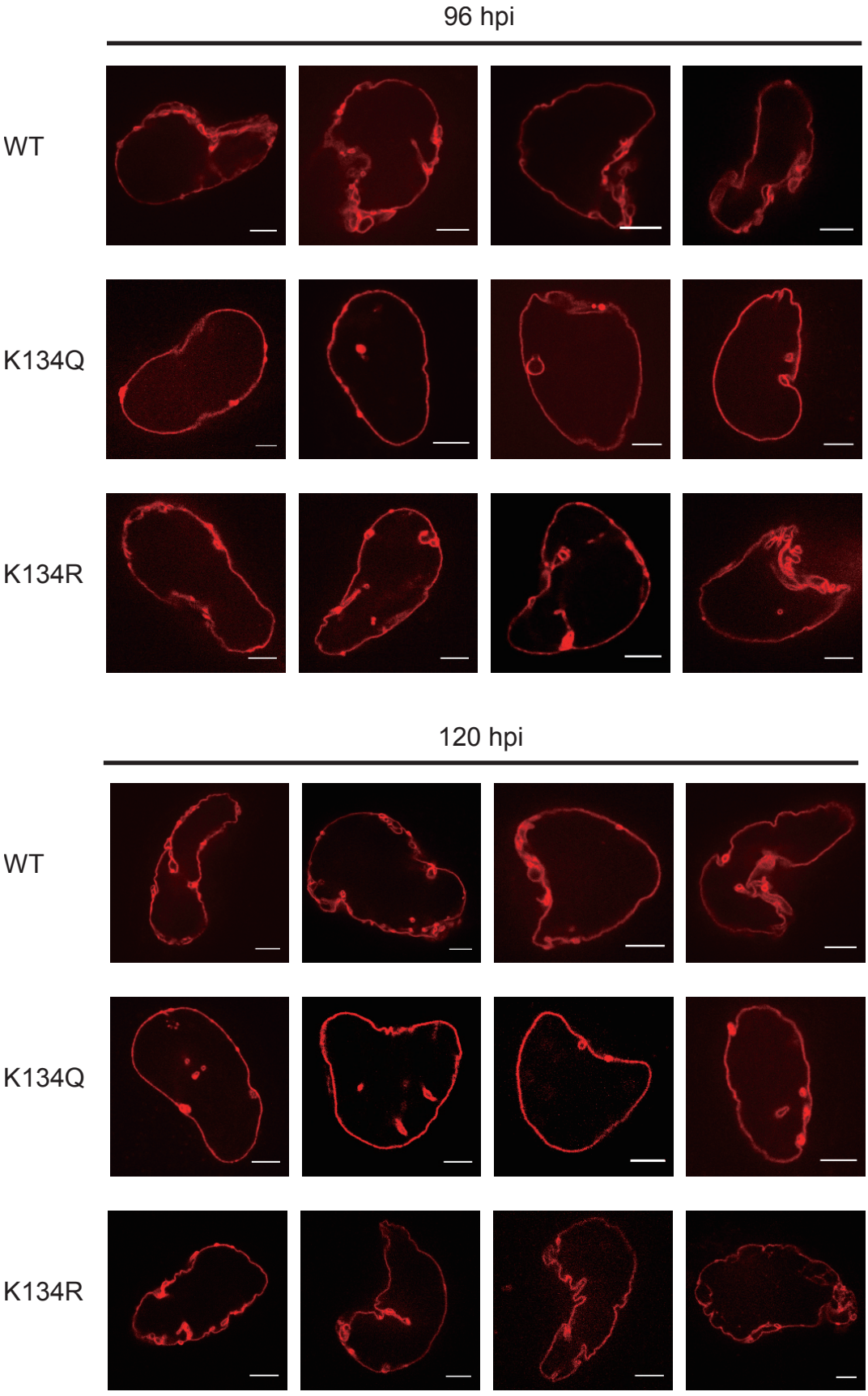

human
chimpanzee
gorilla
sumatran_orangutan
olive_baboon
green_monkey
crab-eating_macaque
rhesus_macaque
mouse
rat
bovine
Western_clawed_frog
African_clawed_frog
zebrafish
turquoise_killifish
bluefin_notho

Supplementary Figure 8. Sequence alignment of identified LMNB1 acetylated lysines and regulatory phosphorylated serines from different vertebrate species. Protein amino acid sequences from 16 species were retrieved from Uniprot, aligned by MultAlin, and colored by ESPript3. The conserved acetylated lysine sites detected in this study are highlighted in yellow and conserved phosphorylated serine sites of known function are highlighted in cyan.



Supplementary Figure 9. Acetylation of LMNB1 impedes nuclear capsid egress. Additional examples of live cell confocal fluorescence microscopy images of cells transfected with **a** mCherry-LMNB1 WT, **b** K134Q, or **c** K134R (red) and infected with HCMV AD169 UL32-

GFP (green) at 120 hpi. Five representative images for each construct are shown as a single slice through the center of the Z-stack (upper row) and as a maximum projection of the Z-stack (bottom row). The rightmost images for each construct are the single slice and maximum projection that correspond to the images shown in Figure 5a; scale bar = 5 μm.


96 hpi
WT
K134Q
K134R
120 hpi
WT
K134Q
K134R

Supplementary Figure 10. Acetylation at K134 on LMNB1 decreases nuclear periphery disruption. Cells transfected with mCherry-LMNB1 WT, K134Q, or K134R (red) and infected with HCMV were imaged by live cell confocal fluorescence microscopy at 96 and 120 hpi. Four representative images for each LMNB1 construct per time point are shown as a single slice through the center of the Z-stack; scale bar = 5 μm.

a

Replicate	24 hpi rep1	24 hpi rep2	24 hpi rep3	48 hpi rep1	48 hpi rep2	48 hpi rep3	72 hpi rep1	72 hpi rep2	72 hpi rep3	96 hpi rep1	96 hpi rep2	96 hpi rep3
# acetylated peptides	17	10	19	15	13	27	18	13	29	24	16	31
# acetylated proteins	12	8	13	11	9	17	13	9	19	14	11	19

b

Replicate	24 hpi rep1	24 hpi rep2	24 hpi rep3	48 hpi rep1	48 hpi rep2	48 hpi rep3	72 hpi rep1	72 hpi rep2	72 hpi rep3	96 hpi rep1	96 hpi rep2	96 hpi rep3
# proteins	75	73	77	79	79	88	83	85	90	89	90	91
# peptides detected	376	380	482	527	528	791	680	766	944	787	917	1007
# peptides quantified	349	354	406	504	504	584	660	739	767	761	885	912

c

Capsid

Protein	Domain region (aa)	Domain region function	Protein length (aa)	Temporal expression	Acetylated lysine(s)
UL46			290	L	25, 270
UL85			306	L	15, 23, 166
UL86	1-59	N-lasso	1370	L	24, 85, 118,
	60-189, 234-290, 363-397, 1032-1106	Johnson fold			165, 220, 377, 413, 415, 439, 513, 1257, 1280, 1336
	190-233	helix-hairpin			
	291-362	dimerization			
	398-480, 1322-1370	channel			
	481-1031	upper region			
	1107-1321	buttress			

Envelope

Protein	Domain region (aa)	Domain region function	Protein length (aa)	Temporal expression	Acetylated lysine(s)
gB	1-31	signal peptide	906	DE	230, 370, 633, 669, 699
	92-111	disintegrin-like domain			
	152-158	involved in fusion and/or binding to host membrane			
	237-244	involved in fusion and/or binding to host membrane			
	411-447	required to assemble an antigenic region			
	696-748	hydrophobic membrane proximal region			
	894-897	internalization motif			
gM (UL100)	1-13	intravirion	372	L	8, 108, 331
	14-34	helical			
	35-79	virion surface			
	80-100	helical			
	101-126	intravirion			
	127-147	helical			
	148-151	virion surface			
	152-172	helical			
	173-200	intravirion			
	201-221	helical			
	222-239	virion surface			
	240-260	helical			
	261-264	intravirion			
	265-285	helical			
	286-298	virion surface			
	299-319	helical			
	320-372	intravirion			
	300-372	interacts with FIP4			
	1-118	region that binds gN			
gL	1-30	signal peptide	278	L	248
gH (UL75)	24-270	virion surface	743	L	131, 327, 405
	218-281	interacts with gL			
	721-741	helical			
	742-743	intravirion			
gO (UL74)			466	L	188
IRL10	80-100	helical	171	DE	35
IRL11	24-294	virion surface	345	DE	175
	295-317	helical			
	318-345	intravirion			

Tegument

Protein	Domain region (aa)	Domain region function	Protein length (aa)	Temporal expression	Acetylated lysine(s)
UL25			656	L	609
UL26	1-34	important for viral growth	222	DE	203
	184-222	essential for replication			
UL32	1-275	capsid (SCP) binding region	1048	DE	237, 373, 394, 805, 903, 1000
	982-1048	C terminal interacts with UL96			
UL35			640	DE	118
UL43			423	L	135
UL44	1-309	needed for function as a processivity factor	433	DE	101, 288, 338, 376
	1-290	self-dimerization			
	162-174	highly flexible loop, involved in DNA binding			
	425-431	NLS			
	40-433	interacts with UL112/113			
	291-433	interacts with UL114			
	129-140	connector loop, interacts with UL54			
	1-290	interacts with UL84			
	1-200, 313-433	interacts with Ubc9			
UL45			906	DE	523, 614
UL47	474-983	interaction with UL48	983	L	281
UL48	1-238	deubiquitination activity	2241	DE	439, 465, 481, 595, 649, 809, 1001, 1003, 1019, 1175, 1261, 1288, 1478
	322-754	interacts with UL47			
UL50	52-60, 125-139	heterodimerization with UL53	397	DE	20, 132
	1-169	conserved			
UL54	379-421, 492-588, 696-742, 771-790, 805-845, 905-919, 962-970, 978-988	conserved with other DNA-dependent DNA polymerases (labeled in aa order): (IV, δ-region C, II, VI, III, I, VII, V); I, II, III: binding of deoxynucleoside triphosphates, chelating the Mg2+ ion, and interacting with primer and template; IV and δ-region C: 3' to 5' exonuclease function	1242	DE	500
	1153-1159	NLSA			
	1222-1227	NLSB			
	1220-1242	interaction with UL44			
	1-1212	interaction with UL114			
UL69	17-30	UAP56 interaction	744	L	275, 580
	35-46, 122-139	RNA binding domain			
	387-501	zinc finger CHC2-type			
	596-624	NES			
UL82			559	L	91, 376
UL83	1-386	pyrin association domain	561	L	154, 176, 191, 215, 283, 378, 509, 538, 557
	387-470	linker			
	471-561	conserved C terminal domain and NLS			
UL84	1-68	association with pUL44 and viral DNA replication	587	DE	94, 339, 436
	101-200	interaction with CK2, essential for DNA replication			
	161-170	nuclear localization signal			
	229-238	nuclear export signal 1			
	360-367	nuclear export signal 2			
UL94			345	DE	306, 316, 324
UL96	1-101	interaction with UL32	127	DE	10
	122-127	critical for virus maturation			
UL97	48-110	NLS	707	DE	586
	292-707	important for phosphorylation activity			
	337-345	ATP binding/ Domain I (sim to PK)			
	352-360	Domain II (sim to PK)			
	373-381	Domain III (sim to PK)			
	433-450	Domain VIa (sim to PK)			
UL122	200-208	non-covalent SUMO1 binding region	580	IE	21, 161, 337, 421
US22			576	DE	197, 305, 356

Supplementary Figure 11. Viral acetylome. **a** Comparison of the number of viral acetylated peptides and the corresponding acetylated viral proteins across all three acetyl-IP biological replicates in all infection time points. **b** Comparison of the number of viral proteins and the number of corresponding detected and quantified unmodified peptides across all three biological

replicates in all infection time points from the whole proteome dataset. **c** Functional domains, temporal expression, and acetylated lysine sites detected in this study on HCMV proteins known to be part of the capsid, envelope, and tegument. All domain information is annotated from Uniprot and primary literature.