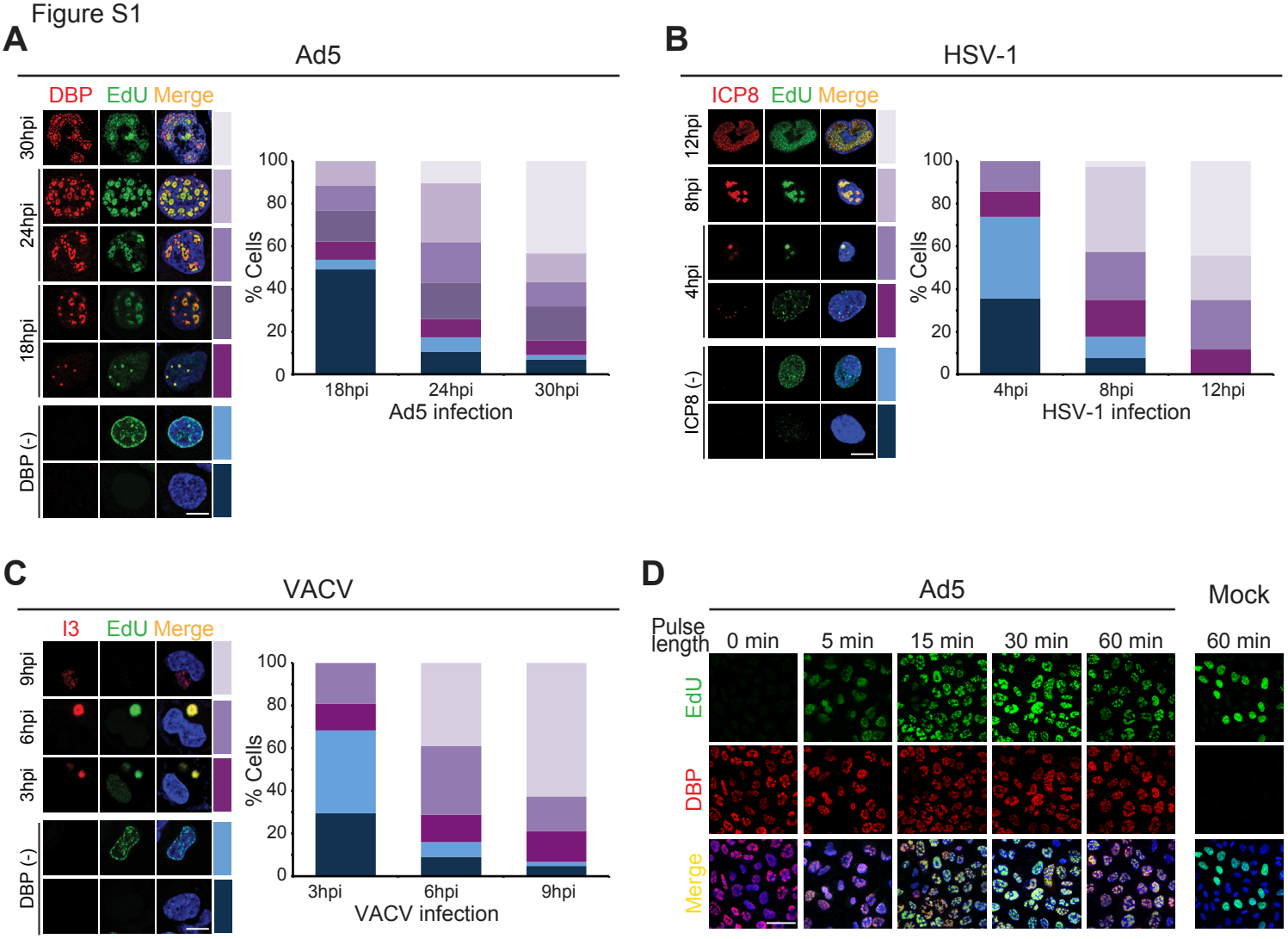
Figure S1



E

Sample	Total number of seq reads:	% Human genome aligned seq reads:
Ad5.A	3,450,184	975277 (28.3%)
Ad5.B	2,303,238	657890 (28.6%)
HSV-1.A	1,850,756	860723 (46.5%)
HSV-1.B	2,212,039	991055 (44.8%)
VACV.A	1,753,384	705001 (40.2%)
VACV.B	1,526,139	713756 (46.8%)
Host.A	1,276,662	1053422 (82.5%)
Host.B	1,711,688	1354412 (79.1%)

Figure S2

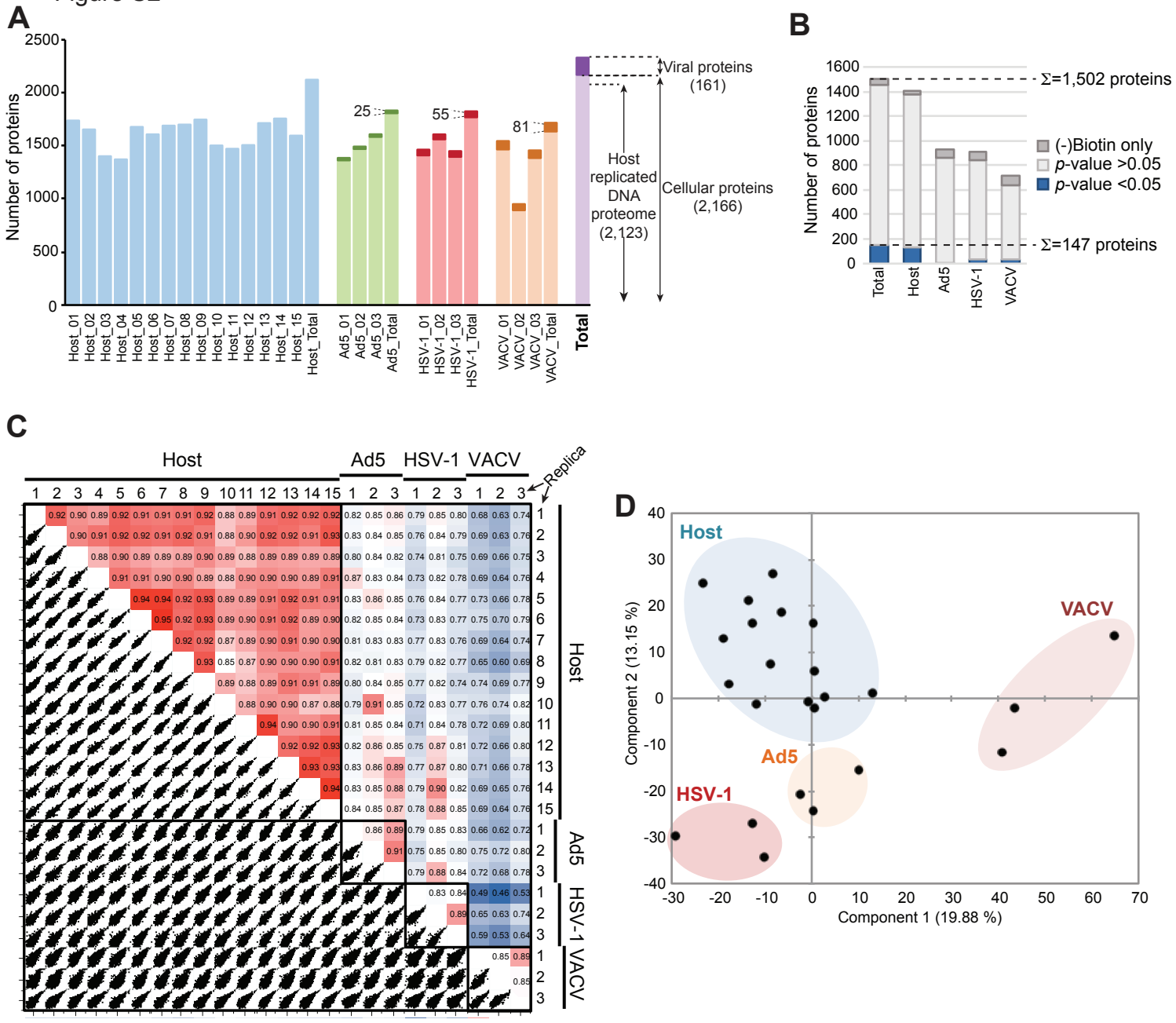
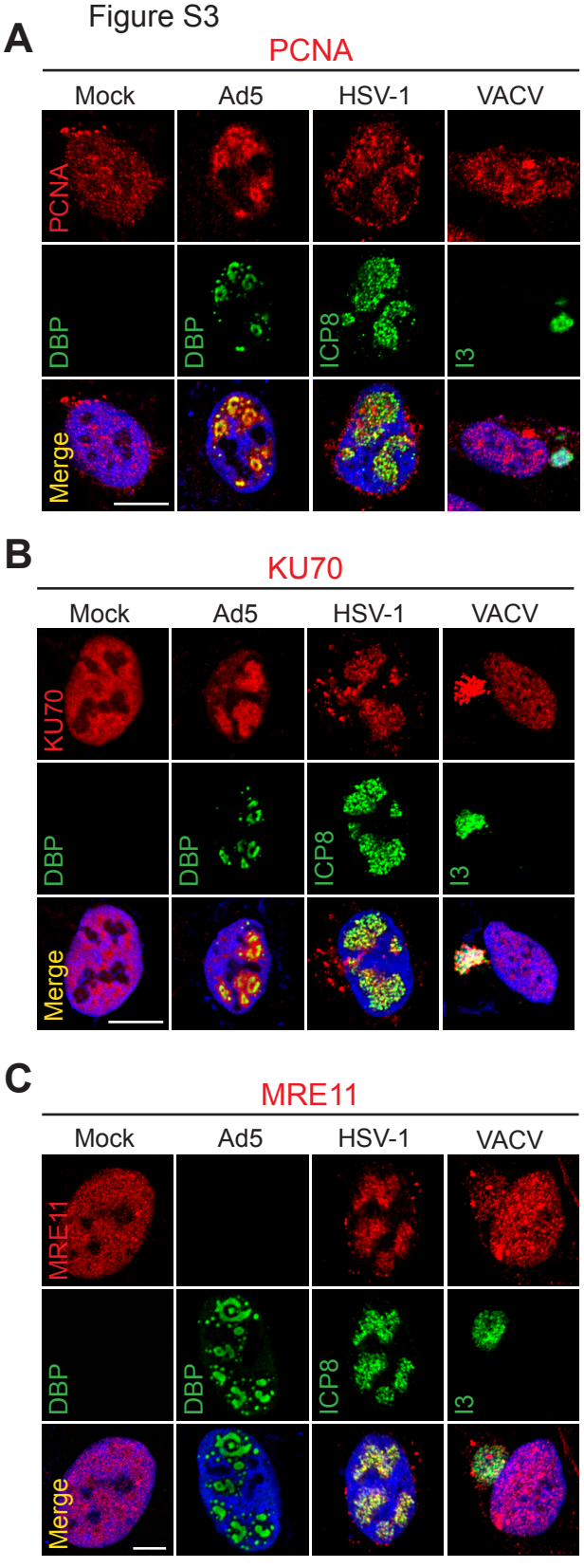

A
Number of proteins
2500
2000
1500
1000
500
0
25
55
81
Viral proteins (161)
Host replicated DNA proteome (2,123)
Cellular proteins (2,166)
Host_01 Host_02 Host_03 Host_04 Host_05 Host_06 Host_07 Host_08 Host_09 Host_10 Host_11 Host_12 Host_13 Host_14 Host_15 Host_Total
Ad5_01 Ad5_02 Ad5_03 Ad5_Total
HSV-1_01 HSV-1_02 HSV-1_03 HSV-1_Total
VACV_01 VACV_02 VACV_03 VACV_Total
Total
B
Number of proteins
1600
1400
1200
1000
800
600
400
200
0
Σ=1,502 proteins
Σ=147 proteins
(-)Biotin only
p-value >0.05
p-value <0.05
Total
Host
Ad5
HSV-1
VACV
C
Host
Ad5
HSV-1
VACV
Replica
D
Host
Ad5
HSV-1
VACV
Component 2 (13.15 %)
Component 1 (19.88 %)

Figure S3



Normalized Protein iBAQ Intensities				Gene Names
Average Host (+)	Average Ad5 (+)	Average HSV-1 (+)	Average VACV (+)	
8.3028	0.1957	7.2201	4.1028	**RAD50**
5.7444	**0.1897**	**3.7472**	**2.4215**	**MRE11A**
1.9468	0.0701	1.2593	0.7740	**NBN**

Figure S4

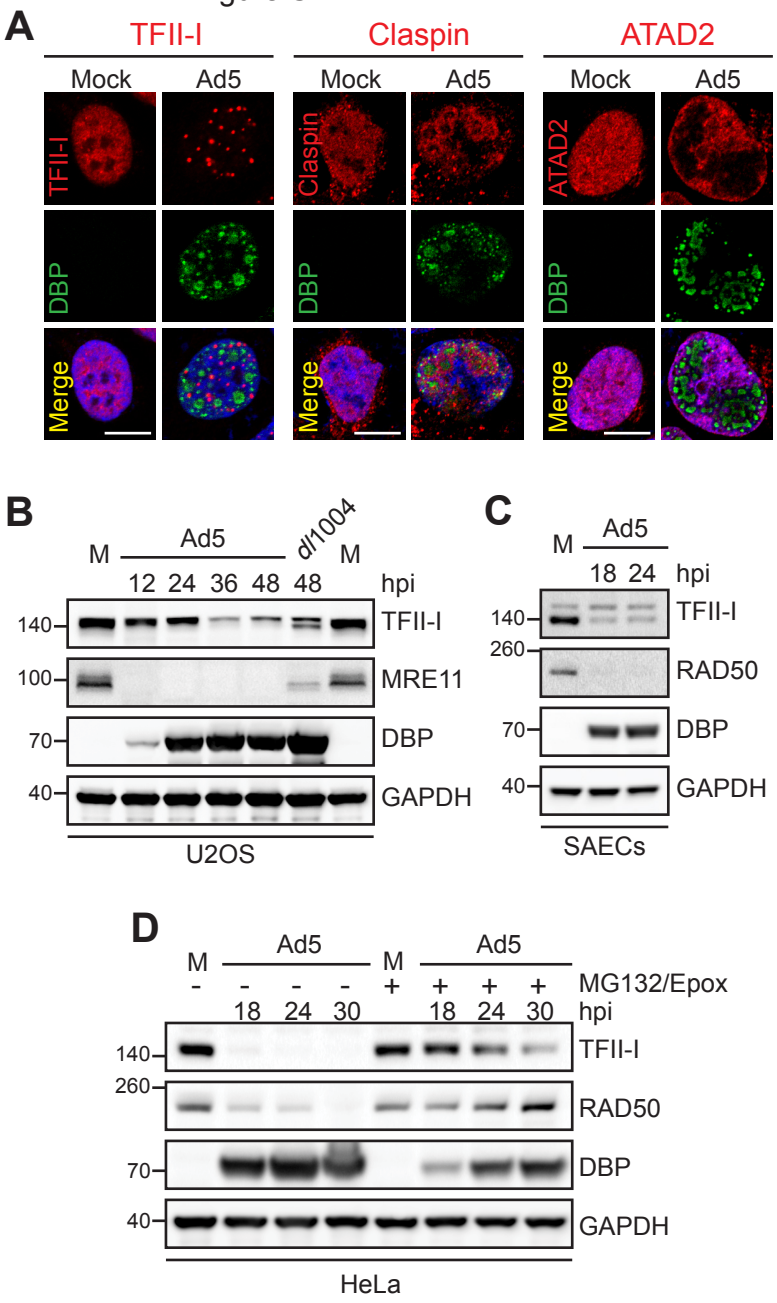
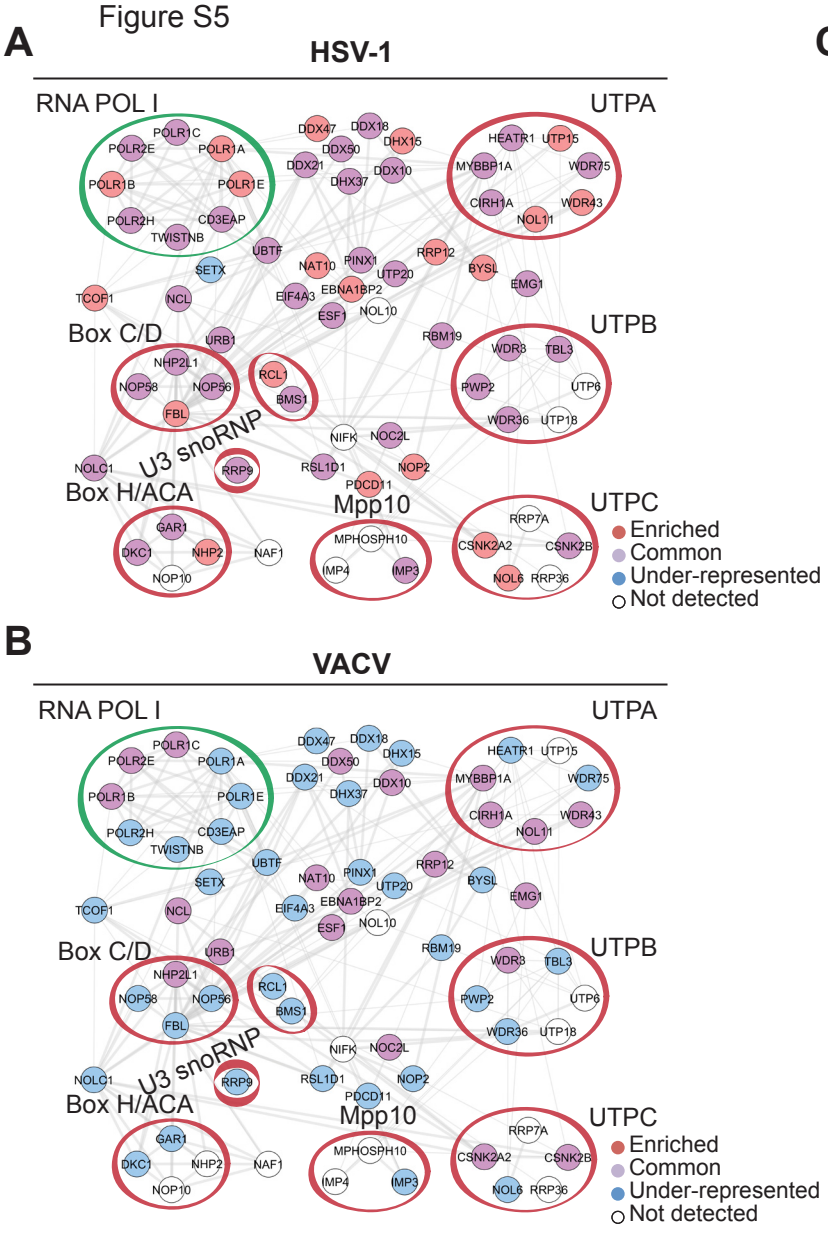

A
TFII-I
Claspin
ATAD2
Mock
Ad5
Mock
Ad5
Mock
Ad5
TFII-I
DBP
Merge
Claspin
DBP
Merge
ATAD2
DBP
Merge
B
M
Ad5
dl1004
M
12 24 36 48 48
hpi
140
TFII-I
100
MRE11
70
DBP
40
GAPDH
U2OS
C
M
Ad5
18 24
hpi
140
TFII-I
260
RAD50
70
DBP
40
GAPDH
SAECs
D
M
Ad5
M
Ad5
- - - - + + + +
MG132/Epox
18 24 30 18 24 30
hpi
140
TFII-I
260
RAD50
70
DBP
40
GAPDH
HeLa

Figure S5


A
HSV-1
RNA POL I
UTPA
Box C/D
UTPB
U3 snoRNP
Box H/ACA
Mpp10
UTPC
Enriched
Common
Under-represented
Not detected
B
VACV
RNA POL I
UTPA
Box C/D
UTPB
U3 snoRNP
Box H/ACA
Mpp10
UTPC
Enriched
Common
Under-represented
Not detected

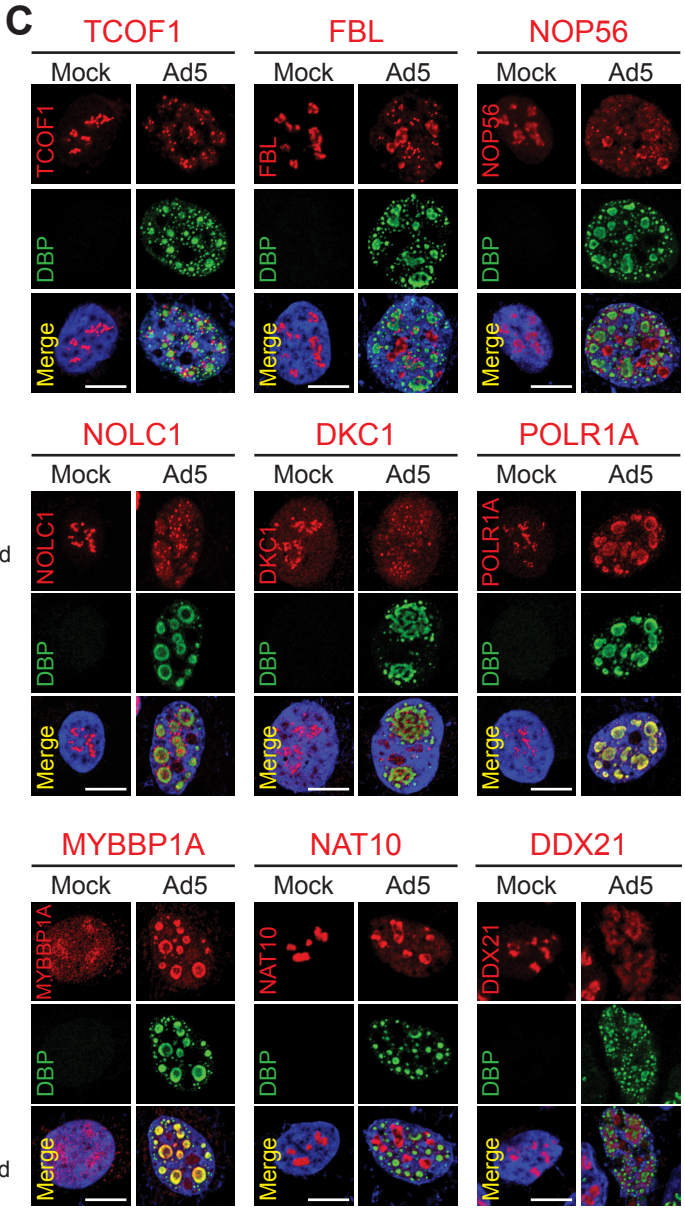
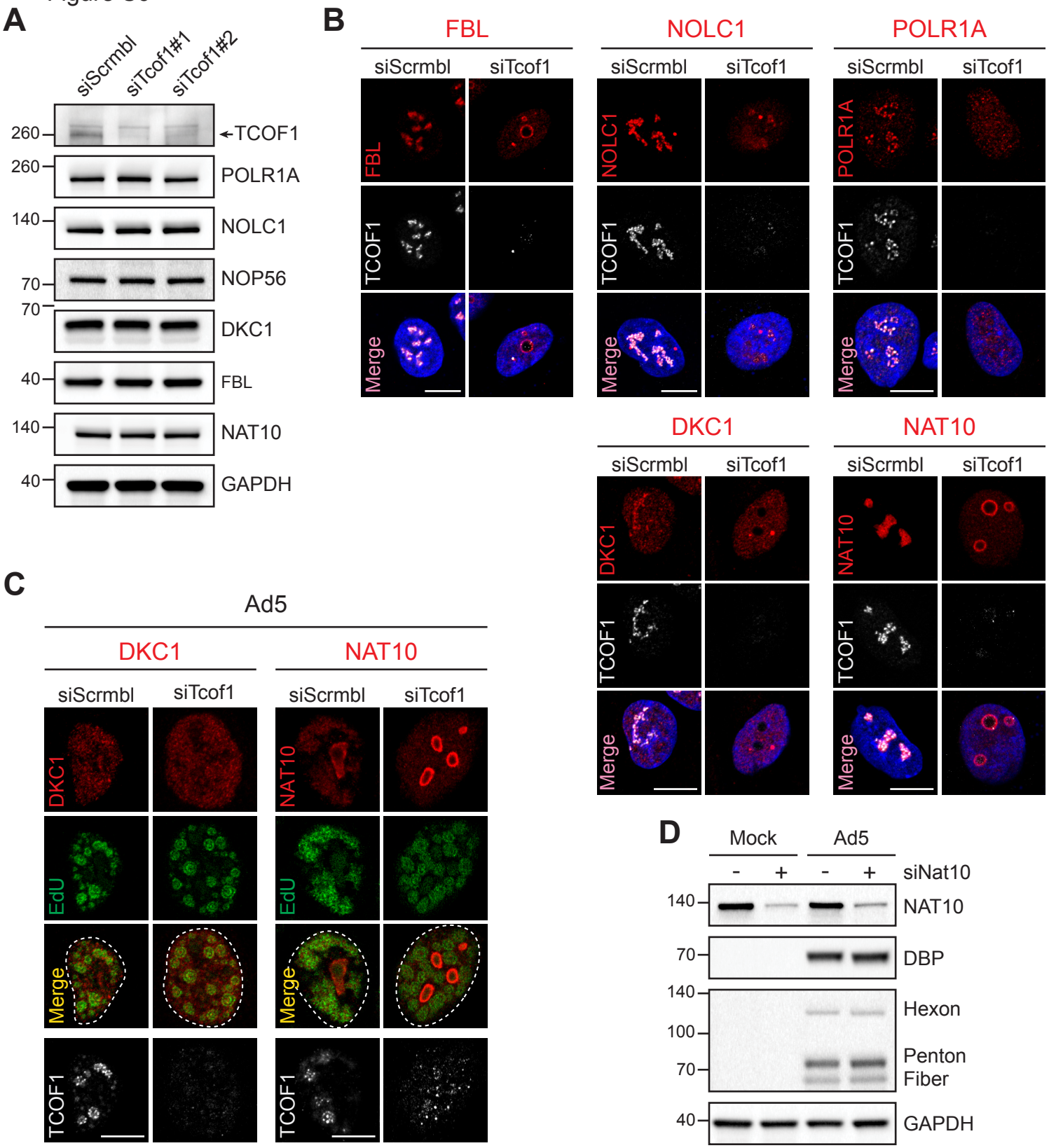

C
TCOF1
FBL
NOP56
NOLC1
DKC1
POLR1A
MYBBP1A
NAT10
DDX21
Mock
Ad5
DBP
Merge

Figure S6


A
siScrmbl
siTcof1#1
siTcof1#2
260
TCOF1
260
POLR1A
140
NOLC1
70
NOP56
70
DKC1
40
FBL
140
NAT10
40
GAPDH
B
FBL
NOLC1
POLR1A
siScrmbl
siTcof1
FBL
NOLC1
POLR1A
TCOF1
Merge
DKC1
NAT10
C
Ad5
DKC1
NAT10
EdU
D
Mock
Ad5
siNat10
NAT10
DBP
Hexon
Penton
Fiber
GAPDH

SUPPLEMENTAL FIGURE LEGENDS

Figure S1, related to Figure 1.

Optimization of viral DNA labeling with EdU. (A-C) Representative patterns of EdU incorporation in virus infected cells. U2OS cells were infected with (**A**) Ad5 (MOI 25), (**B**) HSV-1 (MOI 3) or (**C**) VACV (MOI 7) and pulsed with EdU (10 μM) for 15 min at the indicated times. EdU was visualized by conjugating AlexaFluor 488-azide via click chemistry. VRCs are marked by immunostaining for DBP, ICP8 and I3 as indicated. DAPI stains nuclei. EdU patterns observed over the time course of infection were quantified. DAPI positive cells were classified into two main groups, VRC negative (blue shades) and VRC positive (purple shades). VRC negative cells displayed either undetectable EdU signal or dispersed, pan-nuclear EdU staining distinctive of cellular replication. VRC positive cells were further clustered based on distinct VRC structures representative of the infection stages observed throughout the time course: 5 structures in Ad5 infections, 4 in HSV-1 and 3 in VACV. In general, cells with early stage VRCs (small foci) showed pan-nuclear EdU staining with strong accumulation at VRCs. Cells with middle stage VRCs (medium foci) had EdU staining almost exclusively accumulated on VRCs. In cells with late stage VRCs, EdU staining was still accumulated mainly on the large VRCs except for VACV where the EdU signal was lost. At least 50 cells were counted per time point. (**D**) U2OS cells were uninfected (Mock) or infected with Ad5 (MOI 5) and labeled with EdU (10 μM) at 24 hpi for the indicated pulse length. EdU was visualized as in (**A**) and DBP antibody marked Ad5 VRCs. Scale bar, 10 μm (**A-C**) or 50μm (**D**). EdU incorporation into replicating viral DNA is affected by the time at which pulse is given during infection and the length of pulse. (**E**) Table

summarizing the percentage of total reads of DNA recovered by iPOND from each infection condition that aligned to the human genome. Host samples represent DNA recovered from uninfected cells.

Figure S2, related to Figures 1, 2 and Table S1, S2 and S3.

Composition and quality of proteomes associated with replicated DNA recovered from uninfected and infected cells. (**A**) Bar chart showing the number of proteins identified on replicated DNA recovered from (+)Biotin samples across uninfected (*Host*) and infected (Ad5, HSV-1, and VACV) conditions. Each bar represents quantification of the indicated replica sample. A total of 2327 proteins were identified across all conditions, and of these 161 were viral (dark shade) and 2166 were cellular proteins (light shade). Only 2123 cellular proteins were included in our *Host* nascent DNA proteome (43 proteins excluded due to excessively variable quantification). (**B**) Bar chart showing the number of proteins identified within proteomes recovered from (-)Biotin samples (background samples). A total of 1,502 proteins were identified in the background samples. Of these, 147 were considered contaminants as they were significantly enriched (*t*-test; *p*-value <0.05, blue) in proteomes from iPOND minus over plus biotin. Proteins identified exclusively in (-)Biotin samples (dark gray) were also excluded from further analysis. Proteins not significantly enriched (*t*-test; *p*-value >0.05, light gray) in iPOND minus over plus biotin were included in all further analysis. Statistical analysis was performed on at least three biological replicates, except for VACV (-)Biotin samples where $n_{biological}$=2. (**C**) The heat map shows Pearson correlation coefficients and corresponding matrix of 264 correlations plots between proteomes from

(+)Biotin replicates of similar or different infection condition. The color code from red to blue indicates descending correlation values. Replica number is indicated at the top of each column and at the right side of each row. (**D**) Principal component analysis (PCA) of proteomes enriched on cellular or viral DNA. The proteomes were segregated based on component 1 and component 2, which accounted for 19.88% and 13.15% of variability, respectively.

Figure S3, related to Figures 3 and 4.

Effect of infection with DNA viruses on subcellular localization of host DNA replication and repair factors. (**A-C**) Differential localization of host DNA replication and repair factors with respect to VRCs of DNA viruses. U2OS cells mock-infected or infected with Ad5 (MOI 25), HSV-1 (MOI 3), or VACV (MOI 3) and fixed at 24, 8 or 6 hpi, respectively. Cells were immunostained with antibodies to (**A**) PCNA, (**B**) Ku70, or (**C**) MRE11. VRCs were marked by immunostaining with antibodies to DBP for Ad5, ICP8 for HSV-1 and I3 for VACV. DAPI stains nuclei. Scale bar, 10 μm. PCNA and KU70 signal accumulates at VRCs of all three viruses. In contrast, MRE11 signal is lost in Ad5-infected cells, but localizes to VRCs in cells infected with HSV-1 and VACV. The table shows the average normalized protein IBAQ intensities calculated for RAD50, MRE11 and NSB1 in the replicated DNA proteomes isolated from cells uninfected (Host) and infected with Ad5, HSV-1 and VACV in presence of biotin (+).

Figure S4, related to Figure 5.

Effect of Ad5 infection on localization of potential host targets. (**A**) Immunofluorescence analysis reveals TFII-I, Claspin and ATAD2 do not localize to VRCs during Ad5 infection. A549 cells were mock-infected or infected with Ad5 (MOI 25), fixed at 24 hpi and immunostained as indicated. VRCs were marked by DBP, and nuclei were stained with DAPI stains. Scale bar, 10 μm. The dispersed nucleoplasmic signal of all three host proteins is altered during Ad5 infection. TFII-I signal is reduced and concentrated into small foci away from VRCs during Ad5 infection. In contrast, Claspin signal accumulates at the periphery of VRCs without overlapping with DBP. ATAD2 signal disappears in areas occupied by VRCs. (**B-C**) Targeting of TFII-I by early viral proteins. Lysates from U2OS cells (**B**) or SAECs (**C**) infected with wild-type Ad5 (MOI 25) or E4-deleted mutant *dl*1004 (MOI 25) were harvested at the indicated time and probed for TFII-I. The MRE11 and RAD50 proteins serve as controls for E4-dependent degradation. GAPDH controls for loading and DBP shows progression of infection. (**D**) HeLa cells were infected with Ad5 (MOI 25) with or without treatment MG132 (40 μM) and epoxomicin (4 μM) at 10 hpi as indicated. Cell lysates were prepared at 18, 24 or 30 hpi and immunoblotted as indicated. GAPDH controls for loading and DBP shows progression of infection.

Figure S5, related to Figure 6 and Table S2.

SSU processome components associate with viral DNA of nuclear-replicating viruses. (**A-B**) Protein network analysis of RNA polymerase I and SSU processome components as enriched in HSV-1 (**A**) or VACV (**B**) DNA proteomes. Proteins in the indicated sub-complexes are encircled. Proteins are colored by enrichment status. (**C**)

Immunofluorescence analysis confirms localization of representative SSU processome components to Ad5 VRCs during infection. U2OS cells mock-infected or infected with Ad5 (MOI 25) were fixed at 24 hpi and co-stained with DBP (green) and TCOF1 (red), FBL (red), NOP56 (red), NOLC1 (red), DKC1 (red), POLR1A (red), MYBBP1A (red), NAT10 (red), and DDX21 (red). DBP marks Ad5 VRCs and DAPI stains nuclei. Scale bar, 10 μm.

Figure S6, related to Figure 6.

Effect of TCOF1 depletion on protein level and subcellular localization of SSU processome components. (**A**) Protein levels of SSU processome components are not affected by TCOF1 depletion. A549 cells transfected with the indicated siRNA. Cell lysates were prepared 72 h post-transfection and immunoblotted as indicated. GAPDH controls for loading. (**B**) TCOF1 depletion alters sub-cellular localization of SSU protein components. A549 cells were transfected with the indicated siRNA. Cells were fixed 96 h post-transfection and co-stained for TCOF1 (gray), and FBL (red), NOLC1 (red), POLR1A (red), DKC1 (red) or NAT10 (red). DAPI stains nuclei. Scale bar, 10 μm. (**C**) DKC1 and NAT10 localization to Ad5 VRCs is not affected by TCOF1 depletion. A549 were transfected with the indicated siRNA and infected with Ad5 (MOI 25) 72 h post-transfection. Cells were fixed 24 hpi and co-stained for TCOF1 (gray), and DKC1 (red) or NAT10 (red). Viral replication compartments were observed with EdU. Dotted white line shows nucleus. Scale bar, 10 μm. (**D**) Ad5 late protein production is not affected by NAT10 depletion. A549 cells transfected with indicated siRNA and infected as in (**C**). Cell lysates were prepared at 24 hpi and immunoblotted as indicated.

SUPPLEMENTAL TABLE LEGENDS

Table S1, related to Figure 1 and S2.

Viral proteins identified within proteomes recovered from DNA replicated during infection with Ad5, HSV-1 or VACV. Columns labeled "(-) Biotin Rep#" indicate iBAQ intensity for each viral protein quantified in control replicates in which biotin was excluded from the click chemistry reaction. Columns labeled "(+) Biotin Rep#" show iBAQ intensity for each viral protein quantified in replicates in which biotin was included. Viral proteins are presented by virus in individual worksheets labeled Ad5, HSV-1 or VACV.

Table S2, related to Figure 2 and S2.

Host factors considered as background in *Host* and *Virus* nascent DNA proteomes. Worksheet 1 (147 background) shows quantification and statistical analysis for 147 cellular proteins found significantly enriched (*t*-test *p*-value <0.05) in proteomes from (-)Biotin over (+)Biotin replicates. Proteins isolated exclusively in the absence of biotin were considered contaminants, and thus removed from the list of identified proteins (Figure S2A and B). Worksheet 2 (Unique to *Virus* nasc DNA prot) lists 43 cellular proteins identified exclusively in (+)Biotin replicates from cells infected with Ad5, HSV-1 or VACV, but not considered for further analysis as their quantification was estimated as excessively variable (*t*-test *p*-value vs *Host* >0.05).

Table S3, related to Figures 2, 3, 4, 5, 6, S2, and S5.

Cellular proteins identified within proteomes from DNA replicated in uninfected (*Host*) and infected (*Virus*) cells. Worksheet 1 (Host Proteins_Summary) shows relevant values that summarized the proteomics quantification and statistics of *Virus-Host* comparisons for all cellular proteins identified within (+)Biotin replicates from cells uninfected (Mock) or infected with Ad5, HSV-1 or VACV. The average relative abundance of each host protein within each replicated-DNA proteome profiled is indicated by the $\log_2$ z-normalized protein iBAQ intensity value (Mock = yellow, Ad5 = blue, HSV-1 = green and VACV = orange). For each *Virus-Host* replicated-DNA proteome comparison (color-coded as before), we indicate *t*-test *p*-value, $\log_2$ Fold Change and Enrichment. Enrichment column indicates the enrichment classification assigned for each protein in the replicated-DNA proteome of the indicated virus. Rows highlighted in light gray indicate cellular proteins identified exclusively in (+)Biotin replicates from cells infected with Ad5, HSV-1 or VACV. Worksheet 2 (Host Proteins_Full) includes normalized protein iBAQ intensities for each (+)Biotin replicate included in the analysis.

Table S4, related to Figures 3, 4, and S3.

Abundance and enrichment of DNA replication and DNA repair host proteins in replicated-DNA proteomes of Host, Ad5, HSV-1 and VACV. Worksheet 1 (Replication Factors) lists all cellular DNA replication proteins as in Figure 3A. Worksheet 2 (Repair Factors) lists all cellular DNA repair/damage proteins as in Figure 4A. Both worksheets provide values for each protein's abundance (Normalized Protein iBAQ Intensities) and enrichment over background (iPOND (+Biotin)/(-Biotin)) within the

proteome isolated under each infection condition (Host = yellow, Ad5 = blue, HSV-1 = green and VACV = orange). For each *iPOND(+) - iPOND(-)* comparison (color-coded as before), we indicate *t*-test *p*-value, $\log_2$ Fold Change.

Guide to Navigating Supplementary Tables

Supplementary tables can be searched using Uniprot ID, gene name or protein name. We recommend using the Uniprot ID linked to the protein of interest as this entry accounts for all the different gene and protein names that have been attributed to a specific protein. If a specific Uniprot ID is not found in our tables, one can be confident that the associated protein was not identified by our analysis. The Uniprot ID of a specific protein can be obtained from the UniprotKB database (www.uniprot.org).

Table S1 lists the viral proteins found within proteomes associated with DNA replicated during virus infection from experiments including or excluding biotin. The highlighted columns report "*t*-test *p*-value" and "$\log_2$ Fold Change" values calculated for each protein. The *p*-value indicates whether the difference in abundance for a specific protein between the compared proteomes is statistically significant (*t*-test *p*-value <0.05). In turn, the $\log_2$ Fold Change value shows the magnitude and direction (sign) of the change in abundance of a specific protein in the (+)Biotin compared to the (-)Biotin proteome ($\log_2$ FC >0, more in (+)Biotin; $\log_2$ FC <0, less in (+)Biotin). The lack of a $\log_2$ Fold Change value indicates that the given protein was uniquely identified in one of the compared proteomes. To find out in which proteome this protein was uniquely identified, one must look at the columns listing the protein iBAQ intensity values for each replicate

under each condition (e.g. “(+)Biotin Rep1”). For example, our data show enrichment of the Ad5 protein DBP (Uniprot ID P03265) in (+)Biotin over (-)Biotin Ad5 proteomes since this protein has a *p*-value <0.05 (0.027) and $\log_2$ FC >0 (7.49). This suggests Ad5 DBP is associated with replicated Ad5 DNA. Similarly, our data suggest Ad5 protein E1b55K (Uniprot ID P03243) is also associated with replicated Ad5 DNA since this protein was uniquely found within the (+)Biotin Ad5 proteome (no FC) with a *p*-value = 0.009.

Table S2 lists the cellular proteins considered as contaminants within replicated-DNA proteomes. The highlighted columns report “*t*-test *p*-value” and “$\log_2$ Fold Change” values calculated for each protein after comparing (+) and (-)Biotin proteomes for each condition (*Host*, Ad5, HSV-1 and VACV). The *p*-value indicates whether the difference in abundance for a specific protein between the compared proteomes is statistically significant (*t*-test *p*-value <0.05). The $\log_2$ Fold Change value shows the magnitude and direction (sign) of the change in abundance of a specific protein in the (-)Biotin compared to the (+)Biotin proteome (log2 FC >0, more in (-)Biotin; $\log_2$ FC <0, less in (-)Biotin). All 147 cellular proteins listed in the worksheet “147 background”, were identified as significantly abundant in (-)Biotin over (+)Biotin proteomes and thus excluded from out analysis. The cellular proteins listed in the worksheet “Removed 43 protein” had a highly variable quantification and thus were also excluded from our analysis.

Table S3 lists all cellular proteins identified on replicated DNA recovered from (+)Biotin samples across all conditions. The tab “Host Proteins_Summary” (blue) presents a summary of our data analysis for each identified protein including $\log_2$ z-normalized protein iBAQ intensities. This parameter represents the normalized abundance for each protein within each proteome (Host, Ad5, HSV-1 and VACV). Values in these columns compare the abundance of a specific protein relative to the mean protein abundance within the specified proteome. In other words, the $\log_2$ z-normalized protein iBAQ intensity value indicates whether a protein has an abundance that falls above, below or within the mean protein abundance in that specific proteome. The columns grouped by Virus-Host comparison, e.g “Ad5(+)/Host(+)”, show “*t*-test *p*-value” and “$\log_2$ Fold Change”, show values resulting from comparing protein abundance between each *Virus* proteome to the *Host.* The *p*-value indicates whether the difference in abundance for a specific protein between the compared proteomes is statistically significant (*t*-test *p*-value <0.05). In turn, the $\log_2$ Fold Change value shows the magnitude and direction (sign) of the change in abundance of a specific protein in the *Virus* proteome (Ad5, HSV-1 or VACV) compared to the *Host* proteome ($\log_2$ FC >0, more in (+)Biotin; $\log_2$ FC <0, less in (+)Biotin). The lack of a $\log_2$ Fold Change value indicates that the given protein was uniquely identified in one of the compared proteomes. To find out in which proteome this protein uniquely identified, one must look at the protein iBAQ intensity columns, e.g. $\log_2$ z-normalized Host(+)”. Cellular proteins identified uniquely within a *Virus* proteome are highlighted in gray.

Within each *Virus-Host* comparison, a column was also included to show the "Enrichment" for each protein based on our classification. The "Enrichment" column can be used as a first indicator for whether a specific host protein is expected to associate with replicated viral DNA during infection. Cellular proteins classified as "Common" show a *p*-value >0.05 and their abundance is either significantly not changing in *Virus* compare to *Host* proteomes or is too variable to determine a significant change. Proteins classified as "Enriched" or "Under-represented" display a significant $\log_2$ Fold Change (*p*-value <0.05) with "Enriched" proteins having a $\log_2$ FC >0 and "Under-represented" proteins having a $\log_2$ FC <0). We expect that "Common" and "Enriched" proteins within each are associated with viral DNA during virus replication and thus may be potentially exploited by the specific virus. Regarding proteins classified as "Under-represented", we anticipate these proteins being more likely to be associated with replicated viral DNA when the $\log_2$ FC and $\log_2$ z-normalized iBAQ intensity is closer to zero within a given *Virus* proteome. Those "Under-represented" proteins with a $\log_2$ FC and a $\log_2$ z-normalized iBAQ intensity significantly less than zero or uniquely identified in the *Host* proteome are likely to be excluded from replicating viral DNA. This last group of "Under-represented" proteins may include potential substrates of early viral proteins. For example, the host proteins RPA1 (Uniprot ID P27694) and ATAD2 (Uniprot ID Q6PL18) are classified as "Under-enriched". However, RPA1 has a $\log_2$ FC= -0.94 and a $\log_2$ z-normalized IBAQ intensity= 1.39, while ATAD2 shows a $\log_2$ FC= -6.93 and a $\log_2$ z-normalized IBAQ intensity= -1.09. Based on these observations, we could hypothesize that RPA1 associates with viral DNA replicated during Ad5 infection, but ATAD2 does not.

Table S4 lists all cellular proteins involved in DNA replication and DNA repair/ DNA damage signaling present on replicated DNA recovered from (+)Biotin samples of uninfected cells (Host). The tabs “Replication Factors” (green) and “Repair Factors” (yellow) show values for “Normalized Protein iBAQ Intensities”, which represents the average abundance of each listed protein within the indicated iPOND(+) proteome (replicates: 15 Host, 3 Ad5, 3 HSV-1 and 3 VACV). Empty cells indicate a specific protein was not identified in the indicated iPOND(+) proteome. Values for “*t*-test *p*-value” and “$\log_2$ Fold Change” resulting from comparing average protein abundance of each listed factor in iPOND(+) to iPOND(-) proteomes isolated under each infection condition is also provided here. The *p*-value indicates whether the difference in abundance for a specific protein between the compared proteomes is statistically significant (*t*-test *p*-value <0.05). The $\log_2$ Fold Change value shows the magnitude and direction (sign) of the change in abundance of a specific protein in the iPOND proteome recovered when biotin was included (iPOND(+)) compared to the iPOND proteome isolated without biotin ($\log_2$ FC >0, more in (+)Biotin; $\log_2$ FC <0, less in (+)Biotin). A protein unique the iPOND(+) proteome of a given infection condition will have a value for under the “Normalized Protein iBAQ Intensity” column, but not under the “$\log_2$ Fold Change” column of the given condition.

SUPPLEMENTAL MATERIALS & METHODS

Antibodies

Primary antibodies were purchased from Abcam (ATM ab32420, DDX21 ab49335, Fibrillarin ab5821, MYBBP1A ab54160, TFII-I ab129025, Topoisomerase I ab3825), Abnova (ERCC6L H00054821, BTBD12 [SLX4] H00084464-B01P), Bethyl Laboratories (MCM2 A300-191A, NOP56 A302-720A) GeneTex (GAPDH GTX100118, RAD50 GTX70228, Topoisomerase I GTX63013), Millipore (PCNA NA03), Novus Biologicals (BTBD12 [SLX4] NBP1-28680, Claspin NB100-247, MRE11 NB100-142, Timeless NB100-40853), Proteintech (NAT10 13365-1-AP), Santa Cruz Biotechnology (Dyskerin sc48794, KU70 sc9033, NOPP140 [NOLC1] sc374033, PCNA sc7907, RPA194 [POLR1A] sc48385, Treacle [TCOF1] sc-374536, HSV ICP0 sc-53070, HSV thymidine kinase [TK] sc-28037), and Sigma-Aldrich (ATAD2 HPA029424, TCOF1 HPA038237, FLAG F3165). Antibodies to Ad proteins DBP and E1b55K were gifts from A. Levine, E4orf3 from T. Dobner, and adenoviral late proteins from J. Wilson. Antibodies to HSV-1 protein ICP8 were from D. Knipe and ATCC (Hybridoma), and the antibody to HSV-1 protein VP21 was from G. Cohen. Antibody to VACV I3 was from D. Evans.

Plasmids, siRNAs, and transfections

Plasmids expressing Ad5 proteins E4orf6, E4orf3 and E1b55K have been described previously (1-3). Silencer® Select siRNAs targeting TCOF1 (s13919 and s13921), NAT10 (s30492) and Negative Control No. 1 (non-targeting) were obtained from Life Sciences. Plasmid DNA and siRNA transfections were carried out using Lipofectamine 2000 and Lipofectamine RNAiMax (Invitrogen) respectively, following the manufacture's instructions. Virus infection was performed 72 h after transfection of siRNAs.

Native iPOND

We modified our iPOND protocol for native conditions as follows. Four 15 cm culture dishes containing U2OS were used per condition. Cell infections and EdU pulse were carried out as before at the indicated period of time. After EdU pulse, cells were washed in ice-cold PBS, scraped and harvested by pelleting together cells from the same condition. Cell pellets were resuspended in 2 ml of nuclear fractionation buffer (15 mM Tris pH.7.5, 60 mM KCl, 15 mM NaCl, 5 mM MgCl2, 1 mM CaCl2, 250 mM sucrose) containing 0.5% NP-40 and supplemented with 1 mM DTT, 2 mM Na3VO4 and protease inhibitors (Complete protease inhibitor cocktail tablets [Roche] and 1 mM phenylmethylsulfonyl fluoride) followed by incubation 10 min on ice. Resulting nuclei were pelleted, washed in fractionation buffer without detergent and subjected to the click reaction as previously described except incubation was carried out at 4˚C. Cell lysis, sonication and capture with streptavidin beads were performed as before except sonication time was reduced to 15 min. Beads were washed four times in wash buffer and once in PBS. Captured proteins were eluted in 45 µl 1X LDS sample buffer (10% DTT) by boiling at 95˚C for 10 min.

Preparation of iPOND samples for Next Generation Sequencing (NGS)

To isolate EdU-labeled genomes, iPOND was performed under native conditions on U2OS cells uninfected or infected with Ad5, HSV-1, and VACV following the previously described method with a modified elution step. DNA lo-bind tubes (Eppendorf) were used for sample preparation. Captured DNA from each sample was eluted in 100 µl preheated elution buffer (10mM Tris, 1mM EDTA, 1% SDS) at 95˚C for 5 minutes,

vortexing briefly at the 2.5 min mark. After elution, beads were separated using a magnet and the supernatant was transferred to a new tube. DNA was isolated using the Minelute PCR Purification kit (Qiagen) according to manufacturers instructions, with the following adaptations: 1) Columns were washed 2x with pre-warmed EB, 2) DNA was eluted from purification 2x using 10 µl pre-warmed (55˚C) EB, for a total volume of 20 µl. NGS library preparation was performed using NEBNext DNA Library Master Mix Set for Illumina (NEB). The end-preparation, adaptor ligation, cleanup of adaptor ligated DNA, PCR amplification, and cleanup of PCR amplification were performed according to NEBNext kit manufacturers instructions. 2.5 ng DNA from each replicate sample was used for library preparation. NGS libraries from each sample were assigned unique index primers to facilitate multiplexed Illumina sequencing reactions.

Downstream bioinformatics analyses

All MS data were mapped to gene symbols for comparison to the previous studies on nascent chromatin proteome (4-6). GO biological process (BP) information ranked by *p*-value enrichment score was obtained from GeneGo's MetaCore pathways analysis package (Thomson Reuters) with FDR <5%. Perseus software (7) was used to visualize data principal component analysis plot (PCA), and the matrix of correlation plots. PCA plot and matrix of correlation plots were performed on the normalized iBAQ protein intensity values. Protein interaction network was extracted from STRING database (8), Ingenuity Pathway Analysis (IPA; Ingenuity Systems) and GeneGo's MetaCore software. The Cytoscape software (9, 10) was used for protein interaction network visualization.

Immunoblotting

Infected cells were washed once with PBS and lysed in 1X LDS sample buffer and equal amounts of cell lysates were separated by SDS-PAGE. Western blot analysis was carried out using standard methods. Proteins were visualized with Pierce ECL Western Blotting Substrate (Thermo) and detected using a G:Box imaging system (Syngene).

Immunofluorescence

Cells on glass coverslips were infected with Ad5 (MOI 25), HSV-1 (MOI 3) or VACV (MOI 1-5). Cells were grown on glass coverslips in 24-well plates and either mock infected or infected with the indicated virus. Cells were washed in PBS, fixed in paraformaldehyde and blocked with BSA before staining with the indicated antibodies according to standard procedures. For all HSV-1 infections, cells were pre-extracted in CL buffer (20 mM HEPES, pH7.9, 20 mM NaCl, 5 mM $MgCl_2$, 0.5% NP-40) supplemented with 1 mM DTT, 2 mM Na_3VO_4 and protease inhibitors (Complete protease inhibitor cocktail tablets [Roche] and 1 mM phenylmethylsulfonyl fluoride) by incubating 12 min on ice before fixation. Pre-extraction of cells mock-infected and infected with Ad5, HSV-1 and VACV was carried out for immunofluorescence with the SLX4 and ERCC6L antibodies. For EdU labeling, cells were pulsed with 10 μM EdU for 15 min, washed and fixed as above. EdU was detected by conjugating Alexa Fluor 488 (Life Technologies) at a final concentration of 20 μM in a click reaction cocktail (PBS, 10 mM sodium ascorbate, 2 mM $CuSO_4$). We stained nuclear DNA with 4',6-diamidino-2-phenylindol (DAPI). Coverslips were mounted using ProLong Gold Antifade Reagent

(Life Technologies) and immunofluorescence was visualized using a Zeiss LSM 710 Confocal microscope (Cell and Developmental Microscopy Core at UPenn) and ZEN 2011 software. Images were processed using ImageJ and assembled with Adobe CS6.

SUPPLEMENTAL REFERENCES

1. Cathomen, T., and Weitzman, M. D. (2000) A functional complex of adenovirus proteins E1B-55kDa and E4orf6 is necessary to modulate the expression level of p53 but not its transcriptional activity. *J.Virol.* 74, 11407–11412

2. Stracker, T. H., Lee, D. V., Carson, C. T., Araujo, F. D., Ornelles, D. A., and Weitzman, M. D. (2005) Serotype-specific reorganization of the Mre11 complex by adenoviral E4orf3 proteins. *J. Virol.* 79, 6664–6673

3. Schwartz, R. A., Lakdawala, S. S., Eshleman, H. D., Russell, M. R., Carson, C. T., and Weitzman, M. D. (2008) Distinct requirements of adenovirus E1b55K protein for degradation of cellular substrates. *J. Virol.* 82, 9043–9055

4. Lopez-Contreras, A. J., Ruppen, I., Nieto-Soler, M., Murga, M., Rodriguez-Acebes, S., Remeseiro, S., Rodrigo-Perez, S., Rojas, A. M., Mendez, J., Muñoz, J., and Fernandez-Capetillo, O. (2013) A proteomic characterization of factors enriched at nascent DNA molecules. *Cell Rep.* 3, 1105–1116

5. Dungrawala, H., Rose, K. L., Bhat, K. P., Mohni, K. N., Glick, G. G., Couch, F. B., and Cortez, D. (2015) The replication checkpoint prevents two types of fork collapse without regulating replisome stability. *Mol. Cell* 59, 998–1010

6. Alabert, C., Bukowski-Wills, J.-C., Lee, S.-B., Kustatscher, G., Nakamura, K., de Lima Alves, F., Menard, P., Mejlvang, J., Rappsilber, J., and Groth, A. (2014) Nascent chromatin capture proteomics determines chromatin dynamics during DNA replication and identifies unknown fork components. *Nat. Cell Biol.* 16, 281–293

7. Tyanova, S., Temu, T., Sinitcyn, P., Carlson, A., Hein, M. Y., Geiger, T., Mann, M., and Cox, J. (2016) The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nat. Methods*,

8. Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., Simonovic, M., Roth, A., Santos, A., Tsafou, K. P., Kuhn, M., Bork, P., Jensen, L. J., and Mering, von, C. (2015) STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.* 43, D447–52

9. Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., and Ideker, T. (2003) Cytoscape: a software environment for

integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498–2504

10. Smoot, M. E., Ono, K., Ruscheinski, J., Wang, P.-L., and Ideker, T. (2011) Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics* 27, 431–432