Published in final edited form as:

Proteomics. 2015 June ; 15(12): 1957–1967. doi:10.1002/pmic.201500020.

Proteomic Analysis of the Herpes Simplex Virus 1 Virion Protein 16 Transactivator Protein in Infected Cells

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Abstract

The herpes simplex virus 1 VP16 tegument protein forms a transactivation complex with the cellular proteins HCF-1 and Oct-1 upon entry into the host cell. VP16 has also been shown to interact with a number of virion tegument proteins and viral glycoprotein H to promote viral assembly, but no comprehensive study of the VP16 proteome has been performed at early times post-infection. We therefore performed a proteomic analysis of VP16-interacting proteins at 3 hours post-infection. We confirmed the interaction of VP16 with HCF-1 and a large number of cellular Mediator complex proteins, but most surprisingly, we found that the major viral protein associating with VP16 is the ICP4 immediate-early transactivator protein. These results raise the potential for a new function for VP16 in associating with the immediate-early ICP4 and playing a role in transactivation of early and late gene expression, in addition to its well-documented function in transactivation of immediate-early gene expression.

Keywords

Herpes simplex virus; Infected; VP16; ICP4

1 Introduction

Multiple approaches have been used to define viral protein function, including genetic, biochemical, and proteomic analyses. Genetic analysis involves the isolation of mutant viruses and the definition of the phenotype of the mutant virus, which can define the function(s) of the mutant viral protein. The phenotype of a mutant virus, however, can be due to direct or indirect effects of the absence of the viral gene product. Biochemical analysis can identify enzymatic or other biochemical properties of a viral protein, but this does not always define the function of the protein. Proteomic analysis involves the identification of the proteins that associate directly or indirectly with a specific viral protein, and this analysis can provide new information about the function of the viral gene product. Viral proteomics has illuminated several new protein functions including: tumor suppressor proteins such as Rb and p53, which interact with simian virus 40 (SV40) [1-4], adenovirus [5-7], and papilloma virus [8, 9] tumor antigens; adaptor proteins such as the E6AP protein that links a target protein to an E3 ubiquitin ligase [10]; and the role of host cell DNA repair

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The authors have declared no conflict of interest.

and recombination proteins in viral DNA replication [11]. Viral proteomic studies are often conducted in cells over-expressing a viral protein, but the activities of proteins when over-expressed may not be the same as in infected cells. For example, the herpes simplex virus (HSV) infected cell protein (ICP) 27 protein shows repressive activities in transfected cells [12-14], while genetic studies in infected cells have largely revealed transactivator functions for ICP27 [15-17]. Therefore, it is important to study the proteomics of viral proteins in infected cells where the authentic functions of the viral proteins are being carried out. Proteomic studies of viral proteins in infected cells have been very informative and raised new roles for viral and cellular proteins in viral replication [11, 18].

HSV has a double-stranded DNA genome that is transcribed by the cellular RNA polymerase II in the infected cell nucleus and utilizes host transcription mechanisms for its gene expression. Transcription of eukaryotic protein-coding genes is catalyzed by the RNA polymerase II holoenzyme, which requires, at the minimum, multiple general transcription factors (RNA polymerase II, TFIIA, TFIIB, TFIID, TFIIIE, TFIIF, and TFIIH). These proteins are assembled into the pre-initiation complex (PIC). However, the PIC requires diverse transcriptional cofactors to regulate individual gene transcription including gene specific activators and the Mediator complex. Initially, the Mediator complex was considered to provide a connection between the activators and the PIC for their communication and stability of the DNA-PIC [19-23]. A wealth of evidence from recent studies has expanded our understanding of the functional roles of the Mediator to include epigenetic regulation [24-27], super enhancer formation [28, 29], transcriptional elongation [30-32], transcription termination [33], and mRNA processing [33, 34]. Furthermore, multiple subunits of Mediator are known to be targets of diverse signal transduction pathways [35-37], and mutations in genes encoding Mediator subunits have been related to human diseases (refs in [38]). Therefore, the Mediator complex plays the role of a control hub to regulate transcription in diverse biological situations.

HSV gene expression occurs in a cascade fashion with immediate-early (IE) gene expression leading to early (E) gene expression, which leads to DNA replication, which leads to late (L) gene expression [39]. During lytic infection, efficient IE gene transcription is critical for the subsequent E and L gene transcription [40]. IE gene transcription is transactivated by complexes of viral and host proteins binding to IE gene promoter elements involving the HSV virion protein 16 (VP16) binding to the cellular proteins host cell factor-1 (HCF-1) and octamer-binding transcription factor-1 (Oct-1) to form a transactivator complex, which binds to the *cis*-regulatory consensus sequence, TAATGARAT (where R is purine), in the upstream sequences of the HSV IE genes. VP16 interacts directly with transcriptional activators and interacts indirectly with epigenetic factors through HCF-1. VP16 has a core region and C-terminal transcriptional activation domain (TAD). The core region is sufficient for VP16-induced transactivator complex formation [41, 42], and the TAD is known to be critical for activation of transcription. This transactivation is induced by interactions with multiple general transcription factors including TATA-binding protein (TBP) [43], transcription factor IIA (TFIIA) [44], TFIIB [45], TFIID [46, 47], and TFIIH [48]. In addition, the VP16 TAD interacts with subunits of Mediator [49-51], implying a direct role in the regulation of the RNA polymerase II machinery. HCF-1 contributes to epigenetic

modifications for IE gene transcription by recruiting many epigenetic regulating factors [40]. Upon entry of the HSV genome into the nucleus, histones are associated with the viral genome, and initial viral gene transcription depends on cellular epigenetic regulation. To facilitate IE gene transcription, HCF-1 recruits histone methyltransferase (SETD1A and MLLs) [52], demethylases (KDM1A and KDM4s) [53, 54], and histone chaperone HIRA [55], and Asf1s [56]. The HSV IE ICP0 then promotes the reduction in chromatin loading, the removal of heterochromatin marks [57] (Raja, Lee and Knipe, manuscript in preparation), and the addition of euchromatin marks on the E and L genes [58]. The HSV ICP4 transactivator promotes the assembly of the PICs on viral E and L promoters [59, 60] and transcription of E and L genes [61-63]. ICP4 interacts with components of the basal TFIID and the Mediator complex to promote the formation of the transactivational PIC [59, 64, 65].

The VP16 TAD has been shown to interact with three subunits (MEDs) of Mediator, MED15, MED17, and MED25 [49-51, 66, 67]. However, these studies used the VP16 TAD domain alone, which could limit the binding partners that are identified. Furthermore, these studies were performed using purified or overexpressed tagged TAD, and none of these studies identified VP16 binding partners during the HSV lytic infection. In addition, little is known about which host factors are associated with VP16 in the cytoplasm during HSV lytic infection. Therefore, we hypothesized that full-length VP16 might interact with more diverse cellular proteins during the HSV lytic infection, which could provide insight into other functions of VP16.

Epitope tags have been used to facilitate affinity purification of proteins [68]. Green fluorescence protein (GFP) has also been established as a tag to study protein interactions [69] and recombinant viruses expressing GFP-fused viral proteins have been used to characterize virus-host and virus-virus protein interactions using mass spectrometry [70-72] demonstrating GFP can be an effective tool for proteomic studies. Furthermore, in our case, fusion of GFP to VP16 has little or no effect on HSV-1 lytic infection [73, 74] implying that the GFP-fused VP16 is similar to the native VP16.

We therefore performed mass spectrometry analysis on VP16-GFP immunoprecipitates from HSV-infected cells at early times post-infection. We confirmed the interaction of VP16 with the Mediator complex, but, most surprisingly, the major viral protein found in the immunoprecipitates was ICP4, the major IE transactivator. This observation raises the possibility that VP16 plays a role in the transactivation of E and L genes as well as IE gene expression.

2 Materials and Methods

2.1 Cells and viruses

U2OS, human foreskin fibroblast (HFF), and Vero cells were obtained from the American Type Culture Collection (Manassas, VA). U2OS cells were maintained in DMEM (GIBCO) supplemented with 5% (v/v) FBS, 5% (v/v) bovine calf serum (BCS) and 2 mM L-glutamine in 5% CO₂. The HSV-1 KOS wild-type (WT) strain [75] and VP16-GFP fusion HSV-1 DG1 virus [73] were titrated on Vero cells. Viruses were diluted in PBS containing 0.1% glucose

(wt/vol), 0.1% BCS (vol/vol) and incubated with cells for 1 h with shaking at 37°C followed by replacing media with DMEM containing 1% BCS and incubation at 37°C.

2.2 Immunoprecipitation

U2OS cells (1×10^8) or HFF cells (2×10^7) were infected with WT HSV-1 or DG-1 at a multiplicity of infection (MOI) of 50, washed with PBS twice at 3 hours post-infection (hpi), and harvested in PBS supplemented with protease cocktail inhibitor (Roche). The cells were collected by centrifugation at $1000 \times g$ at 4°C for 5 min. The cells were lysed by incubation on ice for 30 min in 2 mL of NP-40 lysis buffer (0.5% NP-40, 10% glycerol, 50 mM Tris pH 7.5, 50 mM NaCl, 50 mM NaF, 0.5 mM dithiothreitol, and 1×Complete protease inhibitors cocktail (Roche)). The lysates were clarified by centrifugation at $1000 \times g$ at 4°C for 10 min and the cytoplasmic fraction was saved on ice. The pellets were resuspended in 0.8 mL of NP-40 lysis buffer again, sonicated to disrupt the nuclei using a Bioruptor (Diagenode) for 30 sec at maximum amplitude, and clarified by centrifugation at maximum speed at 4°C for 10 min. The cytoplasmic and soluble nuclear fractions were combined, and immunoprecipitation was performed by incubation of 0.5 mL of μ MACS anti-GFP MicroBeads (MACS) or anti-GFP mAb-Magnetic beads (MBL) with the lysate at 4°C for 4 h followed by 4 washes with NP-40 lysis buffer. Proteins were eluted from the beads using Laemmli sample buffer, boiled for 10 min, and resolved by SDS-PAGE.

2.3 SDS-PAGE, Silver staining, and Immunoblotting

For initial characterization of the immunoprecipitates, one tenth of the immunoprecipitated samples were resolved in NuPAGE 4–12% Bis-Tris Gels (Invitrogen), and silver staining was performed using the Pierce Silver Stain Kit for Mass Spectrometry (Thermo Fisher Scientific) following the manufacturer's protocol. For immunoblotting, resolved samples were transferred to nitrocellulose membranes (Bio-Rad). The membranes were blocked with Odyssey Blocking Buffer (LI-COR) and incubated with antibodies specific for HSV ICP4 (1:4000, mAb 58S [76], HSV ICP8 (1:5000, rabbit serum 3-83 [77]), GFP (1:2000, rabbit serum, Abcam), VP5 (1:2000, mAb, Eastcoast), and GAPDH (1:10000, mAb, Abcam). The membranes were incubated with secondary antibodies IRDye 680RD and IRDye 800 (LI-COR) for 45 min and near-infrared fluorescence was detected using Odyssey (LI-COR).

2.4 Mass spectrometry

Immunoprecipitated samples were resolved in SDS-PAGE gels, and proteins were stained with Coomassie Brilliant Blue G-250 (PIERCE). The lane of the gel was divided into two sections, one larger than 75kDa and the other smaller than 75kDa, and subjected to a modified in-gel trypsin digestion [78]. The gel pieces were washed and dehydrated with acetonitrile for 10 min and completely dried. The gel pieces were then rehydrated with trypsin solution (50 mM ammonium bicarbonate and 12.5 ng/ μ L modified sequencing-grade trypsin (Promega)) at 4°C for 45min, and the trypsin solution was replaced with sufficient fresh trypsin solution to cover the gel pieces. The gel pieces were incubated at 37°C overnight. The trypsin digest solution was collected, and the gel pieces were washed with washing solution (50% acetonitrile and 1% formic acid) once. The peptide extracted trypsin solution and the wash were combined and lyophilized in a speed-vac. The dried samples were reconstituted in HPLC solvent A (2.5% acetonitrile and 0.1% formic acid), and

peptides were eluted using a fused silica capillary with an increasing concentration of gradient solvent B (up to 97.5% acetonitrile and 0.1% formic acid). The eluted peptides were detected using a LTQ Velos ion-trap mass spectrometer (ThermoFisher) and peptide sequences were analyzed to identify proteins using protein databases with the acquired fragmentation pattern by the software program, Sequest (ThermoFisher) [79] at the Taplin Biological Mass Spectrometry Facility, Harvard Medical School.

2.5 Bioinformatic analysis

We prepared the VP16-interacting protein network using BioGrid and VirHostNet databases. The VP16-interacting protein networks were merged and re-constructed including our hits using Cytoscape 3.2.0. [81]. We performed gene ontology analysis with our hits using ClueGO databases [80] in Cytoscape. Default parameters were used to analyze gene ontology of molecular function.

3 Results

3.1 Identification of VP16-interacting proteins using an HSV-1 recombinant virus expressing VP16-GFP

To identify VP16-interacting proteins at early times post-infection, we performed proteomic analysis using the HSV-1 DG1 virus that expresses VP16-GFP for infection of U2OS cells. U2OS cells were used in the initial studies because they show a strong dependence on VP16 for viral gene expression [82]. In two independent experiments, we infected U2OS cells with DG1 virus at an MOI of 50 and harvested the infected cells at 3 hpi. We have used this MOI in previous studies to allow detection of input virion VP16 by immunoblotting and immunofluorescence [83]. The cells were lysed, and immunoprecipitation was performed with an anti-GFP antibody as described in the Materials and Methods section. To validate the immunoprecipitates, aliquots (one tenth) of the immunoprecipitated samples were resolved by SDS-PAGE, and protein bands were visualized using silver staining. DG1-virus infected cell immunoprecipitates showed a specific band of VP16-GFP and a number of other bands not observed in the WT virus-infected lysate (Figure 1). To identify the VP16-GFP-interacting proteins, the remaining portions of the samples were analyzed by microcapillary liquid chromatography-tandem Mass spectrometry as described in Materials and Methods. This analysis identified over 200 proteins from the immunoprecipitates of DG1 virus-infected cell lysates. To define the most significant hits, we considered only proteins that were detected in VP16-GFP immunoprecipitates from each of the two biological duplicate experiments and as at least two unique peptides in one of the immunoprecipitates. Table 1 and Figure 2A show 38 proteins that were defined as hits. We found previously identified VP16-interacting proteins, including HCF-1 [84] and Mediator proteins. Previous studies had identified multiple MEDs as VP16 TAD-interacting proteins, and we also found 21 MEDs: seven MEDs previously reported (MED1, MED6, MED15, MED17, MED23, MED25, and CCNC) [49-51, 67, 85-89], and 14 novel MEDs (MED4, MED10, MED12, MED13, MED14, MED16, MED18, MED20, MED22, MED24, MED26, MED27, MED30, and MED31). We also found O-glycosyl transferase (OGT), which is known to interact with HCF-1 [90], two vesicle-associated proteins (VAPA and VAPB), three 14-3-3 proteins (YWHAB, YWHAQ, and YWHAZ), and one heterogeneous

ribonucleoprotein, hnRNP3A. Therefore, the analysis confirmed the interaction of VP16 with the Mediator complex and identified one hnRNP protein, raising a potential role for VP16 in loading hnRNP proteins on nascent transcripts for export from the nucleus.

To search for HSV proteins that interacted with VP16, we used the Uniprot database (ID # 10298) to identify HSV peptides in the immunoprecipitates. Using the criteria of more than two unique peptides in the VP16-GFP immunoprecipitates in both experiments but none in the control immunoprecipitates, we identified 19 HSV proteins including 7 previously identified proteins (Table 2 and Figure 2A). The majority of these were virion structural proteins, four capsid proteins (U_L19/VP5, U_L18/VP23, U_L38/VP19C, and U_L25), ten tegument proteins (U_L49/VP22, U_L36/VP1/2, U_L41/vhs, U_L47/VP13/14, U_L46/VP11/12, U_S10, U_L13, U_S2, U_L55, and U_S3), and one assembly protein (U_L55). Nonstructural proteins included ICP6, the U_L12 nuclease, ICP0, and thymidine kinase (TK), but, surprisingly, the most abundant protein was ICP4, the major IE transactivator. Therefore, the analysis in the infected cells at early times post-infection showed a new viral protein-protein interaction, that of VP16 with ICP4.

We performed functional analysis of VP16-interacting proteins based on gene ontology (GO) annotations using ClueGO database (Figure 2B). The analysis classified functional roles of VP16 in transcriptional activity, nuclear receptor binding activity, and acetyltransferase activity, which confirmed the previously known functions of VP16-associated complexes. Therefore, this analysis validates our proteomic approach to identify VP16-interacting proteins.

3.2 Validation of VP16-interacting proteins

To validate the viral protein hits, we performed immunoprecipitation and immunoblotting with extracts from a different human cell type, human foreskin fibroblasts (HFFs). HFF cells were infected with DG1 virus at an MOI of 50 and harvested at 3 hpi. VP16-GFP was immunoprecipitated with anti-GFP antibody, and ICP4, VP5, and ICP8 were detected using their cognate antibodies. ICP4 and VP5 were detected in the VP16-GFP immunoprecipitates (Figure 3A). However, ICP8 did not co-immunoprecipitate with VP16 (Figure 3A), demonstrating the specificity of the co-immunoprecipitation. Reciprocal immunoprecipitation experiments also showed that VP16 co-immunoprecipitated with ICP4 (Figure 3B). Therefore, these results confirmed that analysis of VP16 interactors at early times post-infection identified novel VP16-interacting proteins.

4 Discussion

Viral proteins can show different activities in infected cells as compared with cells in which the proteins are over-expressed in short-term transfected or stably transformed cells; therefore, it is important to study the properties and functions of viral proteins in infected cells where there are authentic levels of viral proteins. The HSV-1 VP16 protein has been shown to bind to the cellular HCF-1 and Oct-1 proteins to form a transactivator complex that binds in the promoter/regulatory regions of viral IE genes and activate their transcription. At early times post-infection, VP16 is known to interact with the Mediator complex and TFIID subunits to promote IE gene transcription. At late times, newly expressed VP16 is known to

interact with other tegument proteins and assemble into progeny virions. Thus, there is some information about proteins that interact with VP16; however, there has not been a comprehensive analysis of proteins interacting with VP16, in particular at very early times post-infection. In this study we examined the cellular and viral proteins interacting with VP16 at 3 hpi. We found the cellular Mediator proteins and viral tegument proteins previously shown to interact with VP16. We also found capsid proteins co-precipitating with VP16, possibly via incompletely uncoated capsids co-precipitating with anti-VP16 antibody. Most significantly, we found that the major viral protein co-precipitating with VP16 at 3 hours postinfection was ICP4, the viral major IE transactivator protein. This raises the new idea that VP16 may cooperate with ICP4 to transactivate E gene expression at these very early times.

4.1 Interaction of VP16 with virion proteins

VP16, as a component of the tegument layer of the HSV virion, is known to interact with a number of virion proteins including the VP11/12 tegument proteins [91], the VP13/14 tegument proteins [91], vhs/U_L41 tegument protein [92, 93], and VP22/U_L49 tegument protein [93] as well as glycoprotein H in the envelope [94]. Based on the interactions of VP16 with both tegument proteins and gH, VP16 has been proposed to play a role with VP22 in budding of the nucleocapsid through the nuclear membrane [95]. In this study we also saw capsid proteins co-precipitating with VP16 but no gH. This difference may be due to the early time that we studied, at which time we may be detecting the disassembling input virions rather than assembling virions at the later times used in other studies [94]. These results suggest that VP16 may interact with directly or indirectly with the capsid.

4.2 Interaction of VP16 with the Mediator complex

Previous studies had shown that VP16 or portions of it interact with Mediator complex subunits [49-51, 66, 67]. These earlier studies were done with the isolated VP16 transactivation domain or in transfected or stably transformed cells. Thus, it is important that we have confirmed this interaction in HSV-infected cells, showing that VP16 interacts with the Mediator complex in infected cells, because this shows it is an authentic interaction in infection.

4.3 Interaction of VP16 with ICP4

The most surprising observation was that the major HSV protein co-precipitating with VP16 at 3 hours post-infection is ICP4, the IE transactivator of E and L gene transcription [61-63]. VP16 is known to promote the expression of ICP4, but there is no evidence that they function together in any way; however, the interaction observed in our studies argues that they may function together to transactivate E and L gene expression. We hypothesize that ICP4 may bind to viral DNA and tether VP16 to the viral E gene promoters, promoting their transcription. ICP4 is known to associate with the Mediator complex [65, 96]; thus, the interaction of VP16 with ICP4 may be indirect through Mediator. Alternatively, ICP4 and Mediator may each interact directly with VP16.

Further studies are needed to determine if VP16 interacts directly with ICP4. If so, mapping of the portions of the two proteins needed for the interaction will be informative. Co-

transfection studies will be performed to determine if the two viral proteins cooperate in transactivation of E promoters such as the *ICP8/U_L29* gene promoter as observed previously for ICP4 and ICP0 [97]. This may provide a new target for antivirals that block HSV replication and/or reactivation. Thus, screens for small molecules that inhibit the cooperation between VP16 and ICP4 could lead to new antiviral inhibitors of HSV.

Acknowledgements

We thank Steve Triezenberg for providing the HSV-1 DG1 virus, Jeho Shin for technical assistance, and Patrick T. Waters for assistance in preparation of the manuscript. This work was supported by National Institutes of Health grant AI063106 to DMK.

Abbreviations

BCS	bovine calf serum
E	early
GFP	green fluorescence protein
HCF-1	host cell factor 1
HFF	human foreskin fibroblast
HSV	herpes simplex virus
ICP	infected cell protein
IE	immediate-early
L	late
MOI	multiplicity of infection
Oct-1	octamer-binding transcription factor 1
OGT	O-glycosyl transferase
PIC	pre-initiation complex
SV40	simian virus 40
TAD	transcriptional activation domain
TBP	TATA-binding protein
TF	transcription factor
TK	thymidine kinase
VAPA	(vesicle-associated membrane protein)-associated protein A
VP16	virion protein 16
WT	wild-type

5 References

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Figure 1. Co-immunoprecipitation of cellular and viral proteins with VP16-GFP

Proteins immunoprecipitated from VP16-GFP HSV-1 (DG1) or WT HSV-1 infected U2OS cells using anti-GFP-conjugated magnetic beads were resolved in an gradient (4–12%) SDS-PAGE gel and stained with silver stain reagents. The identified VP16-interacting HSV proteins are indicated. The molecular masses of marker protein in kDa are shown in the lane labeled M at the right.

Figure 2. Schematic diagram and functional enrichment analysis of VP16-interacting proteins
 A. VP16-interacting cellular (ovals) and viral (rectangles) proteins identified in previous studies (boxes with dashed bars) and in this study (boxes with bold bars) are grouped. B. Enrichment of protein functions among VP16-interacting cellular proteins was analyzed using ClueGO in Cytoscape. Genes (small circles) are connected their functional groups (large circles) and numbers indicate the number of genes in the individual functional groups.

Figure 3. Validation of the hits by co-immunoprecipitation

Immunoprecipitations were performed using mock, WT, or DG1 infected HFF cells with anti-GFP-conjugated magnetic beads (A) or anti-ICP4 antibody-magnetic beads (B). A. ICP4, VP5, ICP8, VP16-GFP, and GAPDH were detected using antibodies specific for the indicated proteins. B. ICP4, VP16-GFP, and GAPDH were detected using antibodies specific for the indicated proteins.

Table 1

Analysis of Cellular Proteins in VP16-GFP Immunoprecipitates

Gene name	# of Unique peptides in 1st IP	# of total peptides in 1st IP	Sequence coverage (%) in 1st IP	# of Unique peptides in 2nd IP	# of total peptides in 2nd IP	Sequence coverage (%) in 2nd IP
HCFC1	40	114	24%	27	44	17.50%
OGT	16	23	16.50%	9	9	11.30%
MED23	10	12	8.10%	19	28	14.50%
EIF4H	9	20	34.70%	11	19	36.30%
MED4	8	16	28.50%	8	11	32.20%
MED12	7	8	3.90%	23	32	13.10%
MED24	7	8	10.10%	13	22	14.60%
MED16	7	9	9.40%	9	17	11.30%
YWHAB	7	13	27.20%	1	1	3.30%
MED1	6	6	4.20%	22	27	15.30%
MED17	6	6	11.20%	9	12	14.90%
MED14	5	6	3.90%	26	41	22.80%
MED20	5	5	19.80%	4	6	14.60%
PRDX1	5	8	23.10%	2	2	10.10%
VAPA	4	8	14.50%	12	41	44.20%
MED15	4	5	4.30%	10	15	10.50%
MED27	4	6	19.90%	7	9	33.80%
RPS11	4	5	17.70%	4	4	22.80%
MED6	4	6	12.20%	3	3	15.90%
CCNC	4	5	14.10%	2	2	6.70%
YWHAZ	4	5	18%	1	1	7.30%
VAPB	3	3	18.10%	13	34	45.70%
MED13	3	3	1.90%	11	14	5.80%
MED25	3	3	5%	5	7	9.10%
MED22	3	6	10%	3	3	17%
RPL10L	3	6	10.70%	3	3	10.70%
YWHAQ	3	3	18.40%	2	2	10.60%
MED18	2	3	9.60%	3	5	13.50%
MED10	2	2	16.30%	2	2	21.50%
C1QBP	2	3	12.10%	1	1	7.10%
CALU	2	2	6%	1	1	3.50%
HNRNPA3	2	3	2.60%	1	1	2.60%
MED30	2	3	11.80%	1	1	6.70%
PHB2	2	2	7.70%	1	1	4%
SNX9	1	1	1.50%	6	6	11.40%

Gene name	# of Unique peptides in 1st IP	# of total peptides in 1st IP	Sequence coverage (%) in 1st IP	# of Unique peptides in 2nd IP	# of total peptides in 2nd IP	Sequence coverage (%) in 2nd IP
MED26	1	1	1.70%	4	5	6.50%
DECR1	1	1	3.90%	2	2	10.40%
MED31	1	1	13%	2	2	22.10%

Table 2

Analysis of HSV Proteins in VP16-GFP Immunoprecipitates

Gene – protein name	Function	# of Unique peptides in 1st IP	# of total peptides in 1st IP	Sequence coverage (%) in 1st IP	# of Unique peptides in 2nd IP	# of total peptides in 2nd IP	Sequence coverage (%) in 2nd IP
<i>R_S1</i> – ICP4	IE transactivator (transcriptional regulator)	61	281	42.60%	86	704	65.90%
<i>U_L19</i> – VP5	Major capsid protein	53	244	39.10%	25	34	21.10%
<i>U_L36</i> – VP1/2	Tegument protein	52	68	17.50%	5	5	2.10%
<i>U_L41</i> – vhs	Tegument protein	18	65	25.60%	24	83	47.90%
<i>U_L39</i> – ICP6 (RR1)	Ribonucleoside Reductase large subunit	33	57	25.50%	21	25	18.70%
<i>U_L18</i> – VP23	Capsid protein	15	42	46.20%	6	6	14.20%
<i>U_L47</i> – VP13/14	Tegument protein	17	36	20.30%	35	186	51.50%
<i>U_L12</i>	Nuclease	12	31	23%	3	3	8%
<i>U_L38</i> – VP19C	Capsid triplex protein	14	29	38.50%	4	4	9.70%
<i>U_L25</i>	Capsid protein	16	28	28.80%	3	4	6.20%
<i>U_L46</i> – VP11/12	Tegument protein	12	26	17.80%	6	23	7%
<i>U_S10</i>	Tegument protein	13	26	39.10%	10	13	32.10%
<i>U_L13</i>	Tegument kinase	9	18	17.40%	11	20	28.80%
<i>U_L23</i> – TK	Thymidine kinase	7	16	16.20%	5	6	14.10%
<i>U_S2</i>	Tegument protein	3	11	14.40%	4	6	22.30%
<i>R_L2</i> – ICP0	E3 ubiquitin ligase	7	7	12%	7	8	12.10%
<i>U_L49</i> – VP22	Tegument protein	2	5	5.60%	4	14	15.30%
<i>U_L55</i>	Assembly, tegument protein	3	4	19.40%	3	5	19.40%
<i>U_S3</i>	Protein kinase, tegument protein	2	2	4%	5	12	14.10%