RESEARCH ARTICLE

Figures and figure supplements

The interferon-inducible GTPase MxB promotes capsid disassembly and genome release of herpesviruses

Manutea C Serrero *et al*

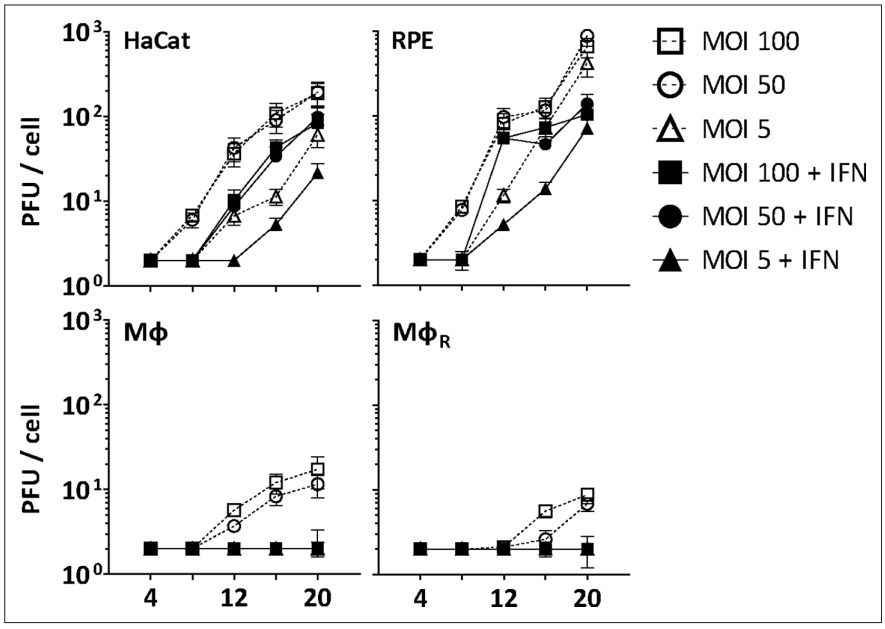

Figure 1. IFN restricts HSV-1 infection in keratinocytes, epithelial cells, and macrophages. HaCat, RPE, Mφ, or $M\varphi_R$ cells were mock-treated or treated with human IFN-α (1000 U/mL) for 16 hr and were infected with HSV-1(17$^+$) Lox at 2.5 × 106 (MOI 5), 2.5 × 107 (MOI 50), or 5 × 107 PFU/mL (MOI 100), and the amount of cell-associated and extracellular virions was titrated on Vero cells. Each data point represents the mean of the three technical replicates of the combined cell-associated and extracellular titers. The error bars represent the standard deviation.

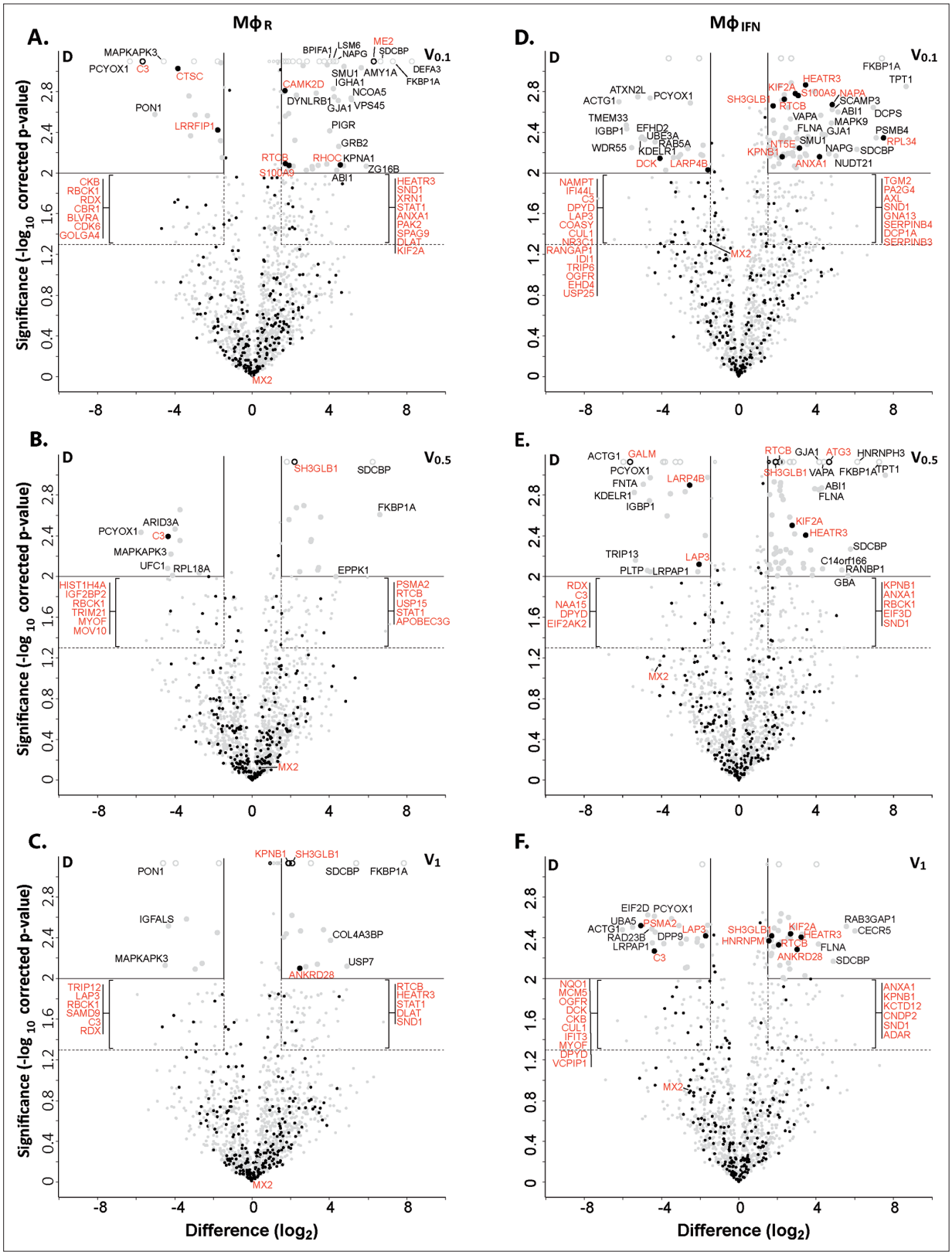

Figure 2. Cytosolic IFN-induced macrophage proteins binding to HSV-1 capsids. Volcano plots of iBAQs counts of proteins identified in capsid-host protein complexes assembled in cytosol from resting THP-1 φ cells (A - C) or treated with interferon-α (D - F) using $V_{0.1}$ (**A, D**), $V_{0.5}$ (**B, E**), or V_1 (**C, F**) capsids in comparison to D capsids. Proteins identified as highly specific interactions are indicated with larger symbols (log_2 difference ≥1.5; Welch's t-test, two-tailed, permutation-based FDR ≤ 0.01); those with a log_2 difference ≥4 are annotated. ISGs (interferome.org) are indicated by filled black circles,

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Figure 2 continued

and are annotated in red if significantly enriched (permutation-based FDR ≤ 0.05, and $\log_2$ difference ≥1.5). Proteins with a q-value = 0 were imputed to - $\log_{10}$ q-value = 3.1 (maximum of the graph), and were indicated with empty circles.

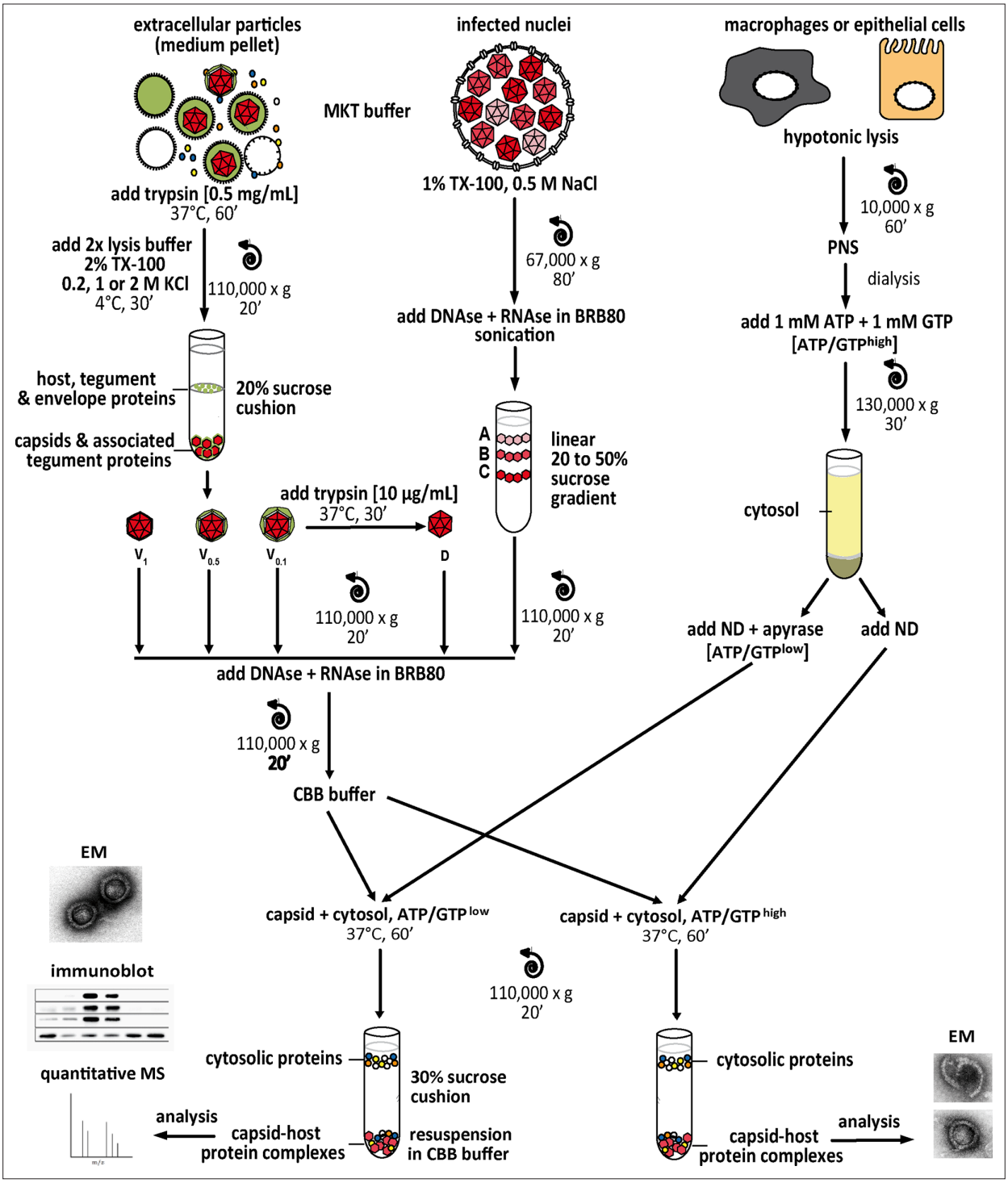

Figure 2—figure supplement 1. Experimental strategy to generate host protein-capsid complexes. Tegumented viral $V_{0.1}$, $V_{0.5}$, or V_1 capsids (red) were isolated from extracellular particles released from BHK-21 cells infected with HSV-1(17$^+$)Lox. They were lysed in 1% Triton X-100 to solubilize the viral envelope, and to extract different amounts of tegument (green) in the presence of 0.1 M, 0.5 M, or 1 M KCl. D capsids were generated from $V_{0.1}$ capsids by mild trypsin digestion. These different capsid types were purified through sucrose cushions. Tegument-free nuclear A, B, and C capsids were isolated from the nuclei of BHK cells infected with HSV-1(17$^+$)Lox by gradient sedimentation. The capsids were resuspended in BRB80 buffer, treated with benzonase to degrade DNA and RNA, sedimented again, and incubated with cytosol fractions (yellow) from control or IFN-induced macrophages (THP-1 φ) or epithelial A549 cells. After sedimentation through sucrose cushions, the capsid-host protein complexes were analyzed by mass spectrometry (MS), immunoblot, or electron microscopy (EM). PNS, post-nuclear-supernatant; ND, nocodazole.

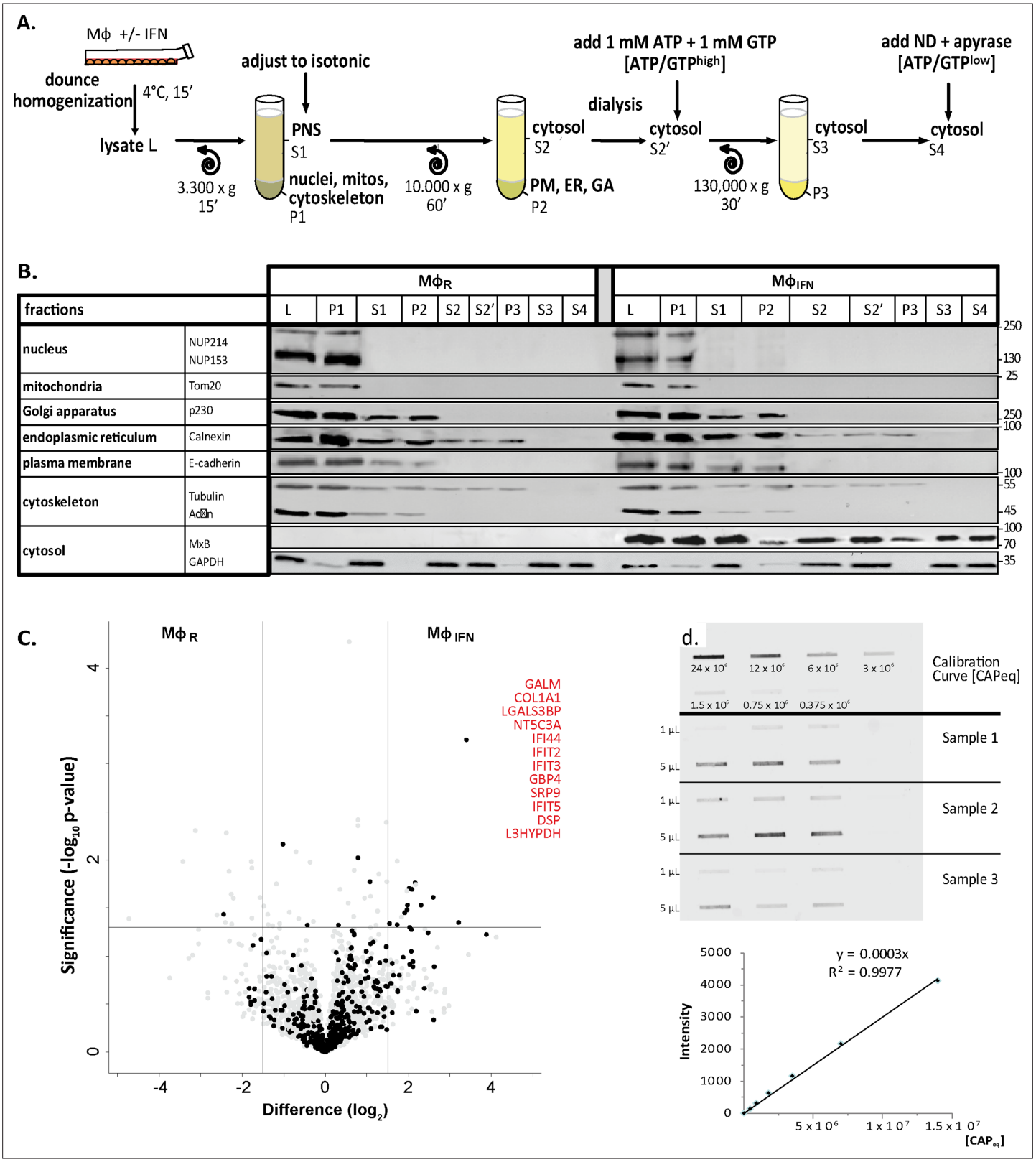

Figure 2—figure supplement 2. Characterization of cytosolic extracts and calibration of capsids. (**A**) Cytosols were prepared from rested $M\varphi_R$ or IFN-induced $M\varphi_{IFN}$ macrophage cells. After swelling in hypotonic buffer, the cells were homogenized (**L**), and nuclei and mitochondria were sedimented (**P1**). The post-nuclear supernatant (**S1**) was adjusted to isotonic salt concentration, and centrifuged to sediment membrane compartments (**P2**), like the PM, ER and GA. To control the nucleotide concentration, the cytosols (**S2**) were dialyzed against a 7 kDa membrane prior to the addition of an

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ATP regeneration system (**S2'**). The remaining actin filaments and microtubules were sedimented in P3 to obtain a soluble cytosol fraction (**S3**). To reduce ATP and GTP levels, some cytosols were treated with 10 U/mL of apyrase (**S4**). Nocodazole (ND) was added to prevent polymerization and sedimentation of microtubules. (**B**) All fractions generated were analyzed by immunoblot for the respective compartment marker proteins as indicated. Nup, nucleoporins. MW, Molecular Weight (kDa). (**C**) Volcano plot summarizing the effect of IFN induction on the cytosol proteome. ISGs associated with the interferomeDB were enriched in cytosol from $M\varphi_{IFN}$ as compared to $M\varphi_{R}$ with an FDR of 7.96×10^{-7} and an FC ≥2 in at least 1 experiment (Fisher's exact test). IFN-inducible proteins are indicated by black circles, and those with an abundance $\log_2$ difference ≥1.5 (vertical lines), and an uncorrected p-value < 0.05 (horizontal line) are labeled in red. (**D**) The slot blot used for the estimation of capsid concentrations (capsids equivalent; CAP_{eq}) of all preparations was labeled with anti-capsid antibodies (rabbit pAb SY4563) and adjusted to a calibration curve of a standard preparation.

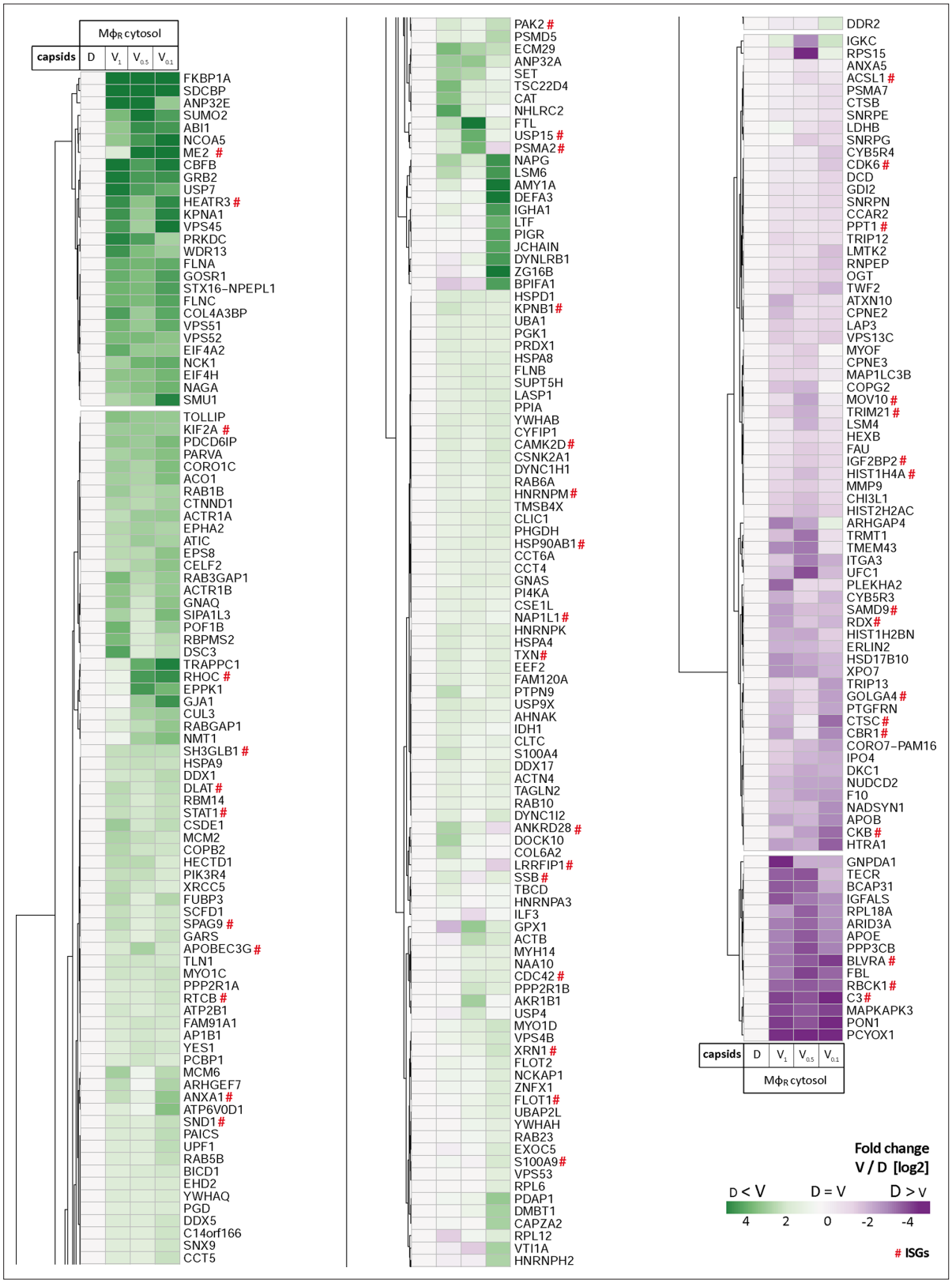

Figure 2—figure supplement 3. HSV-1 capsids interactomes. Unbiased hierarchical clustered heat map showing the $\log_2$ fold changes of host proteins identified from capsids-host protein sediments (c.f. ***Figure 2***; abundance $\log_2$ difference larger than 1; significance permutation-based FDR smaller than 0.05) from (**A**) cytosol of resting Mφ, or (**B**) IFN-induced $M\varphi_{IFN}$ macrophages. For each protein, the fold change was calculated based on their abundance (iBAQs) in V_1, $V_{0.5}$, or $V_{0.1}$ capsids compared to D capsids using a linear scale from violet being the lowest to dark green being the highest.

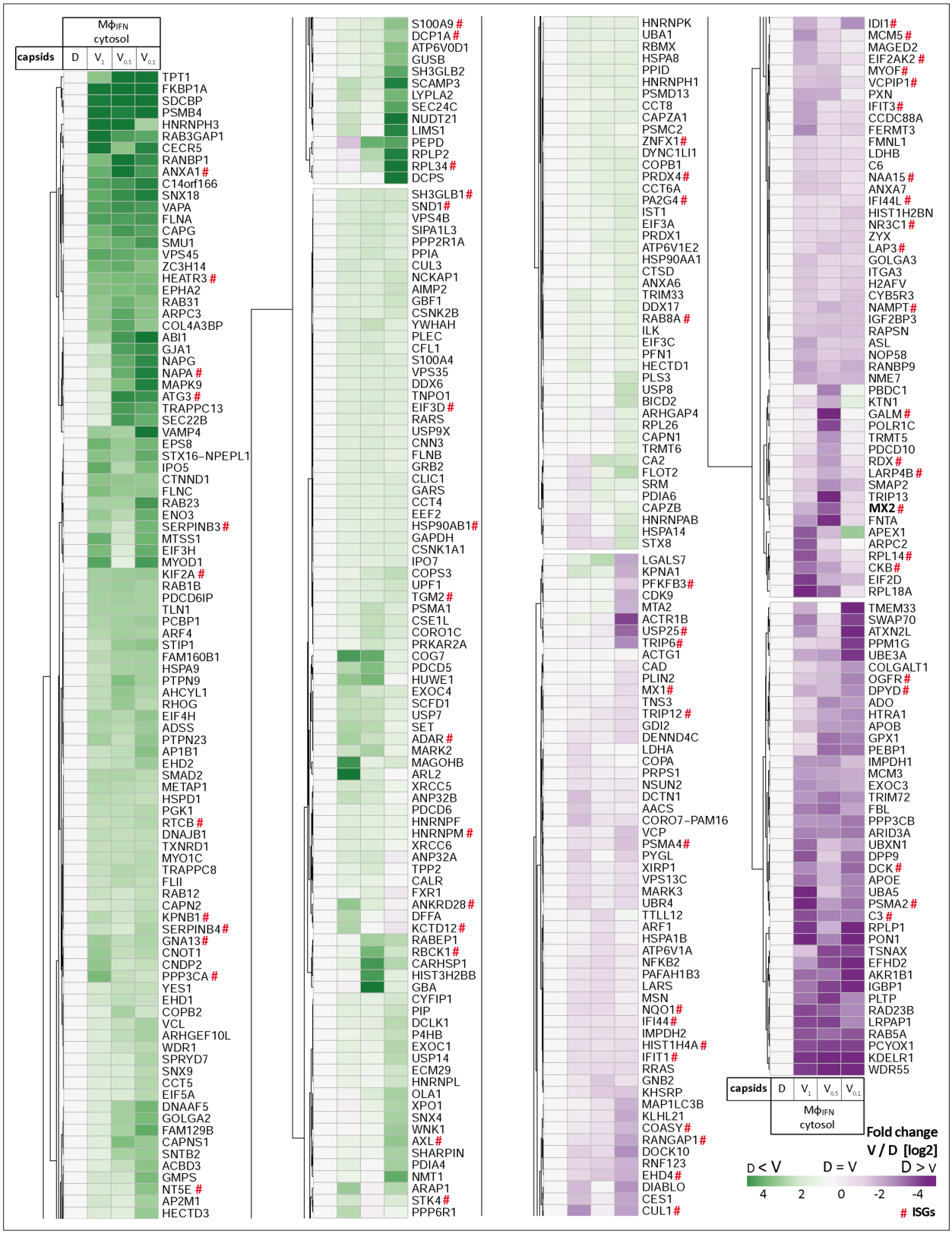

Figure 2—figure supplement 4. HSV-1 capsids interactomes. Unbiased hierarchical clustered heat map showing the $\log_2$ fold changes of host proteins identified from capsids-host protein sediments (c.f. ***Figure 2***; abundance $\log_2$ difference larger than 1; significance permutation-based FDR smaller than 0.05) from (**A**) cytosol of resting macrophages (M_φ), or (**B**) IFN-induced macrophages ($M_{\varphi IFN}$). For each protein, the fold change was calculated based on their abundance (iBAQs) in V_1, $V_{0.5}$, or $V_{0.1}$ capsids compared to D capsids using a linear scale from violet being the lowest to dark green being the highest.

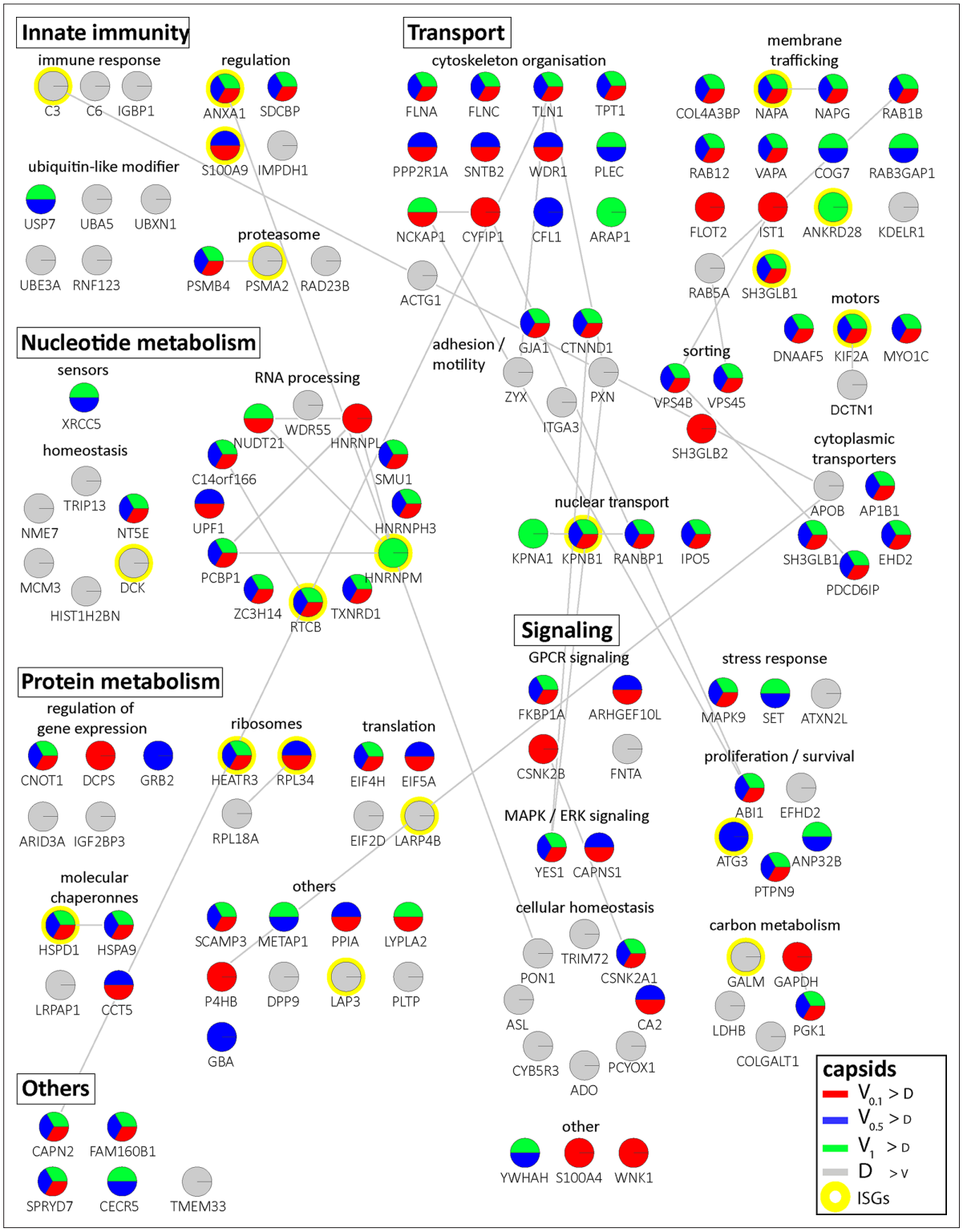

Figure 3. Cytosolic proteins of IFN-induced macrophages binding to HSV-1 capsids. Host proteins from cytosol of IFN-stimulated $M\varphi_{IFN}$ (c.f. D, E, F; abundance $\log_2$ difference larger than 1.5; significance permutation-based FDR smaller than 0.01) interacting with $V_{0.1}$, $V_{0.5}$, V_1, or D capsids were assembled into a functional interaction network of known protein-protein-interactions (gray lines; STRING database, confidence score of 0.7), and grouped according to their known functions (Gene Ontology, Pathway analysis). The Pie chart for each protein indicates its relative enrichment on $V_{0.1}$ (red), $V_{0.5}$ (blue), V_1 (green), or D capsids (gray).

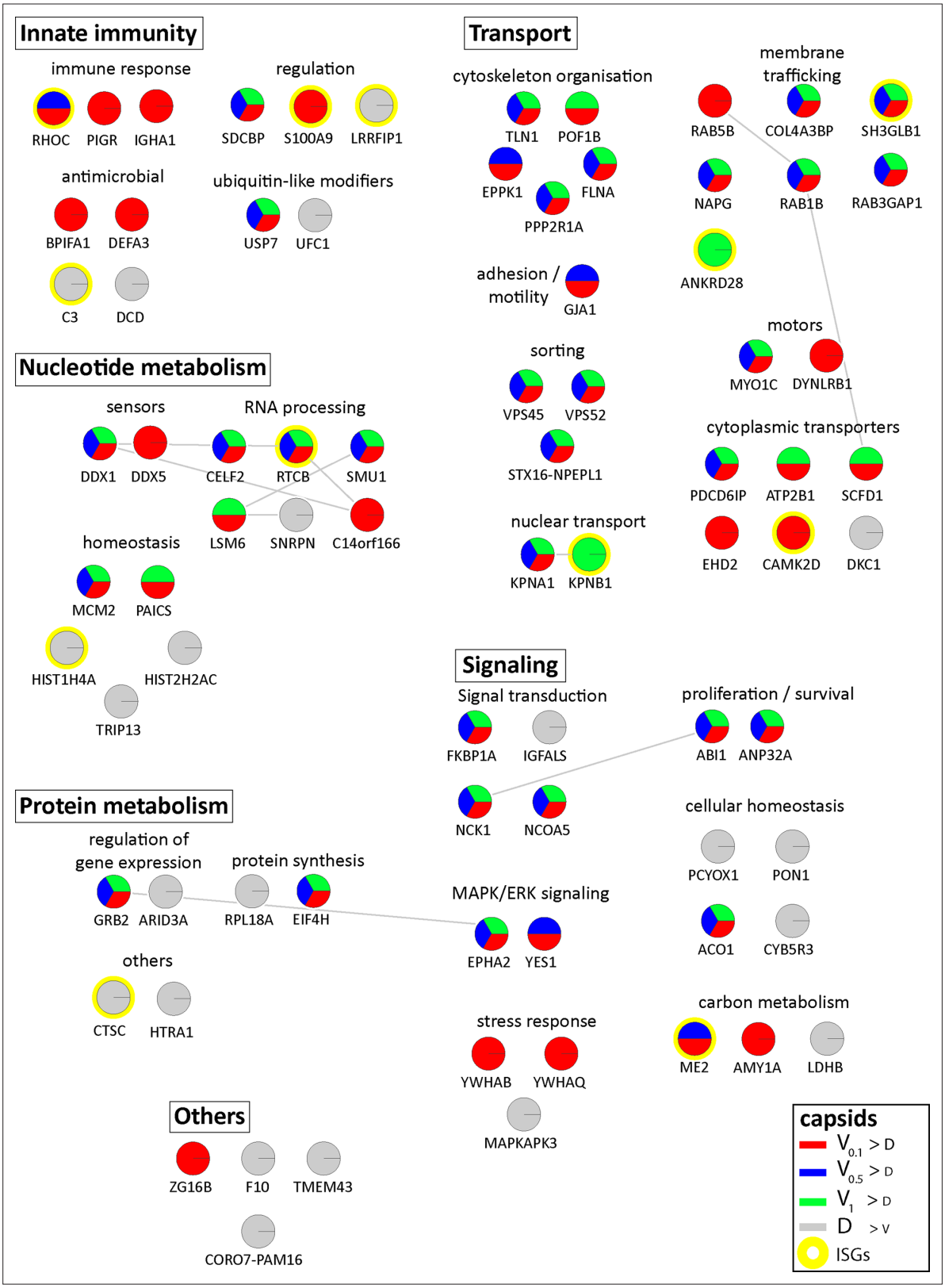

Figure 3—figure supplement 1. Cytosolic proteins of resting macrophage binding to HSV-1 capsids. *Host* proteins from cytosol of resting Mφ (c.f. ***Figure 3A, B and C***; abundance $\log_2$ difference larger than 1.5; significance permutation-based FDR smaller than 0.01) interacting with $V_{0.1}$, $V_{0.5}$, V_1, or D capsids were assembled into a functional interaction network of known protein-protein-interactions (grey lines; STRING database, confidence score of 0.7), and grouped according to their known functions (Gene Ontology, Pathway analysis). The Pie chart for each protein indicates its relative enrichment on $V_{0.1}$ (red), $V_{0.5}$ (blue), V_1 (green), or D capsids (grey).

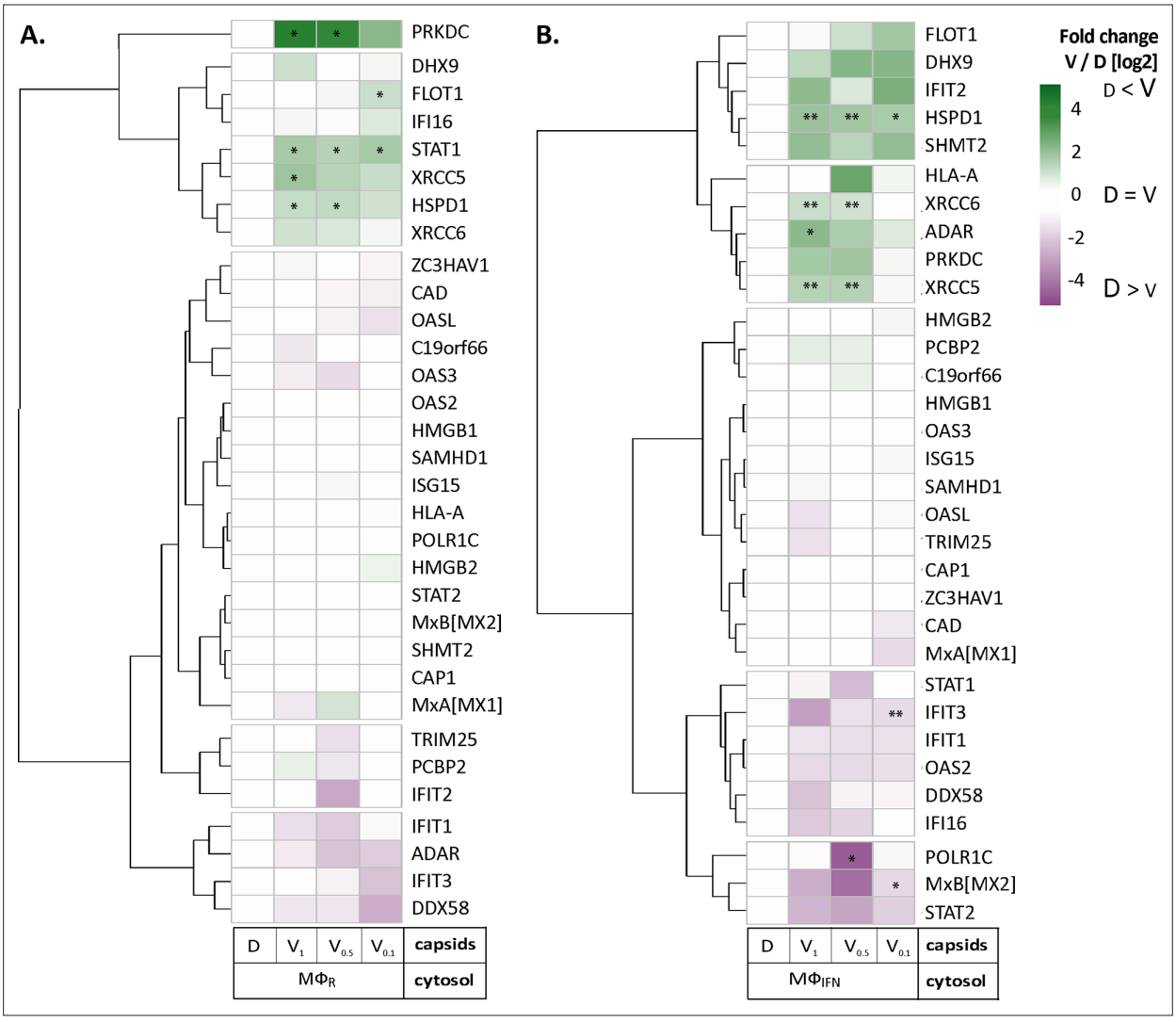

Figure 4. HSV-1 capsids associate with proteins involved in type I IFN response. Unbiased hierarchical clustered heat map showing the $\log_2$ fold changes of IFN-induced proteins (GO type-I IFN) identified from capsids-host protein sediments from cytosol of resting Mφ, or IFN-induced $M\varphi_{IFN}$ macrophages. For each protein, the fold change was calculated based on their abundance (iBAQs) in V_1, $V_{0.5}$, and $V_{0.1}$ capsids as compared to their abundance in D capsids, using a linear scale from violet being the lowest to dark green being the highest. (*) and (**) design the proteins with an FDR corrected p-value ≤ 0.05 and ≤ 0.01, respectively.

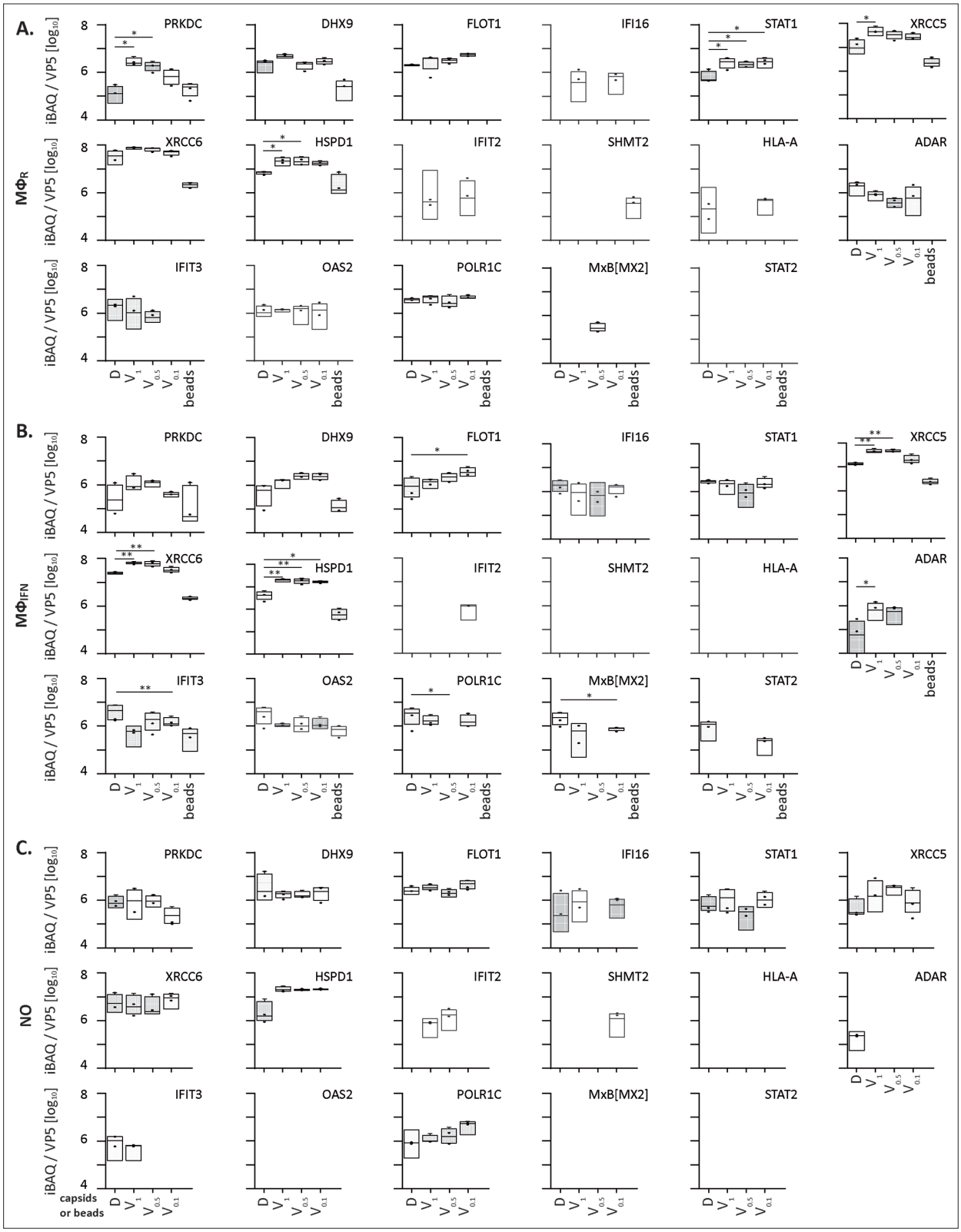

Figure 4—figure supplement 1. HSV-1 capsids binds to a few ISG proteins. Box and whisker plot of iBAQs showing the differential detection of PRKDC, DHX9, FLOT1, IFI16, STAT1, XRCC5, XRCC6, HSPD1, IFIT2, SHMT2, HLA-A, ADAR, IFIT3, OAS2, POLR1C and MxB[MX2] in D, V_1, $V_{0.5}$, and $V_{0.1}$ capsids-host protein sediments after incubation in (**A**) cytosol of resting MφR macrophages, (**B**) IFN-induced MφIFN macrophages or (**C**) no cytosol. (*) design the significant binding to D or V0.1, V0.5, and V1 capsids as assessed by Welch's t-test (two-tailed, permutation-based FDR ≤ 0.05) comparing D vs $V_{0.1}$, $V_{0.5}$, or V_1 capsids in each cytosol separately.

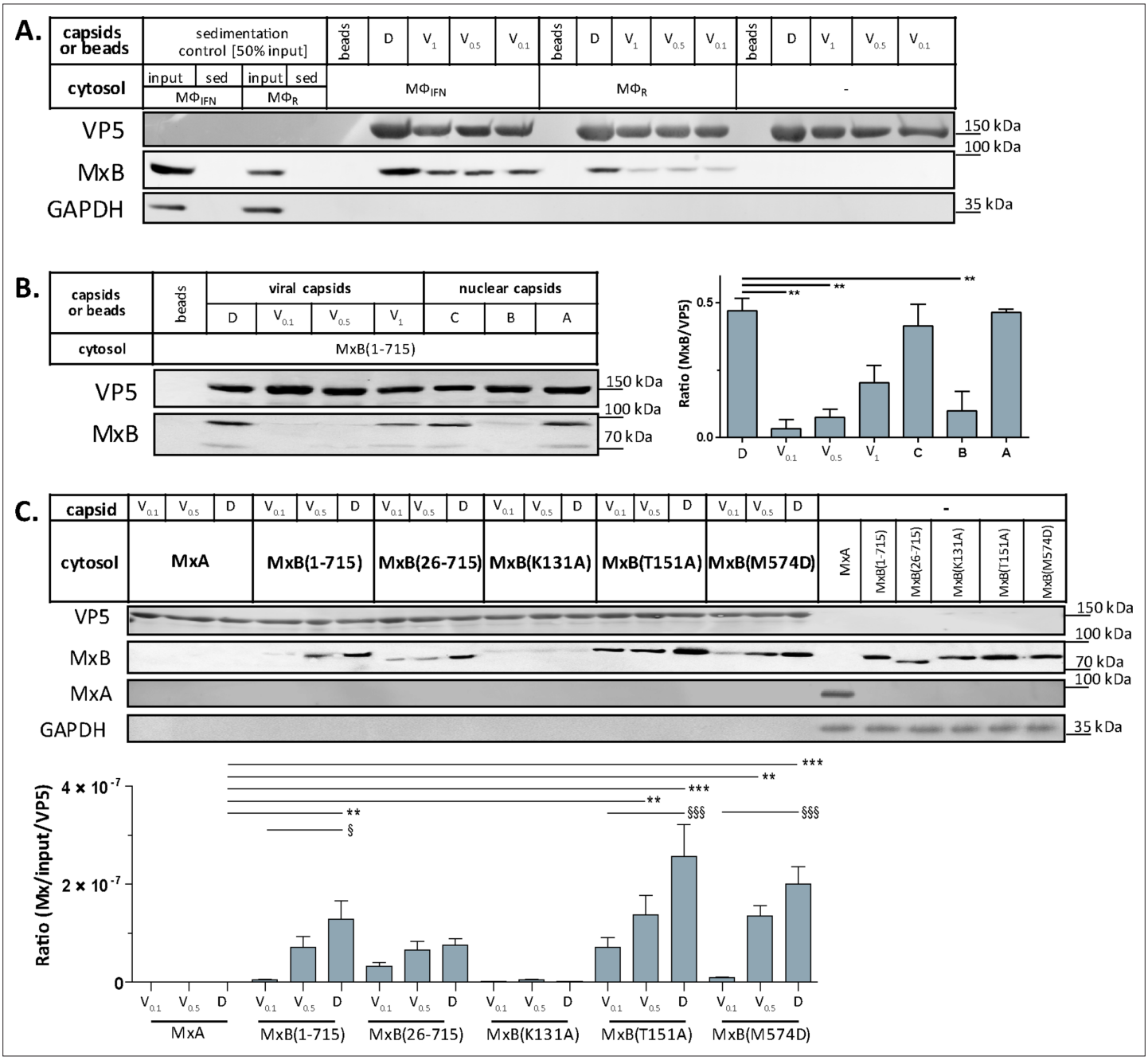

Figure 5. Tegumentation reduces MxB binding to HSV-1 capsids. The binding of MxB to viral $V_{0.1}$, $V_{0.5}$, V_1, or D, or to nuclear A, B, or C capsids was analyzed after incubation in 0.2 mg/mL cytosol prepared from (A; ***Figure 5—source data 1***) THP-1 φ stimulated or not with IFN, or (B-C; ***Figure 5—source data 1***; ***Figure 5—source data 1***) A549 cells stably expressing MxA, MxB(1-715) full length, the short MxB(26-715), or MxB mutants defective in GTP-hydrolysis MxB(T151A), GTP-binding and hydrolysis MxB(K131A), or dimerization MxB(M574D). Sedimented capsid-host protein complexes were then analyzed by immunoblot for VP5 (capsid), MxB, MxA, and GAPDH as a loading control. As control cytosols were sedimented without capsids (A: sed), or with uncoated agarose beads (A, B: beads). The amounts of MxA/MxB found in the capsid-host protein complexes were quantified, and normalized to their respective VP5 levels. Error bars: SEM. summarized from three experiments. One asterisk denotes $p < 0.05$, two asterisks indicate $p < 0.01$ and three asterisks represent $p < 0.001$ as determined by Welch's t-tests comparisons.

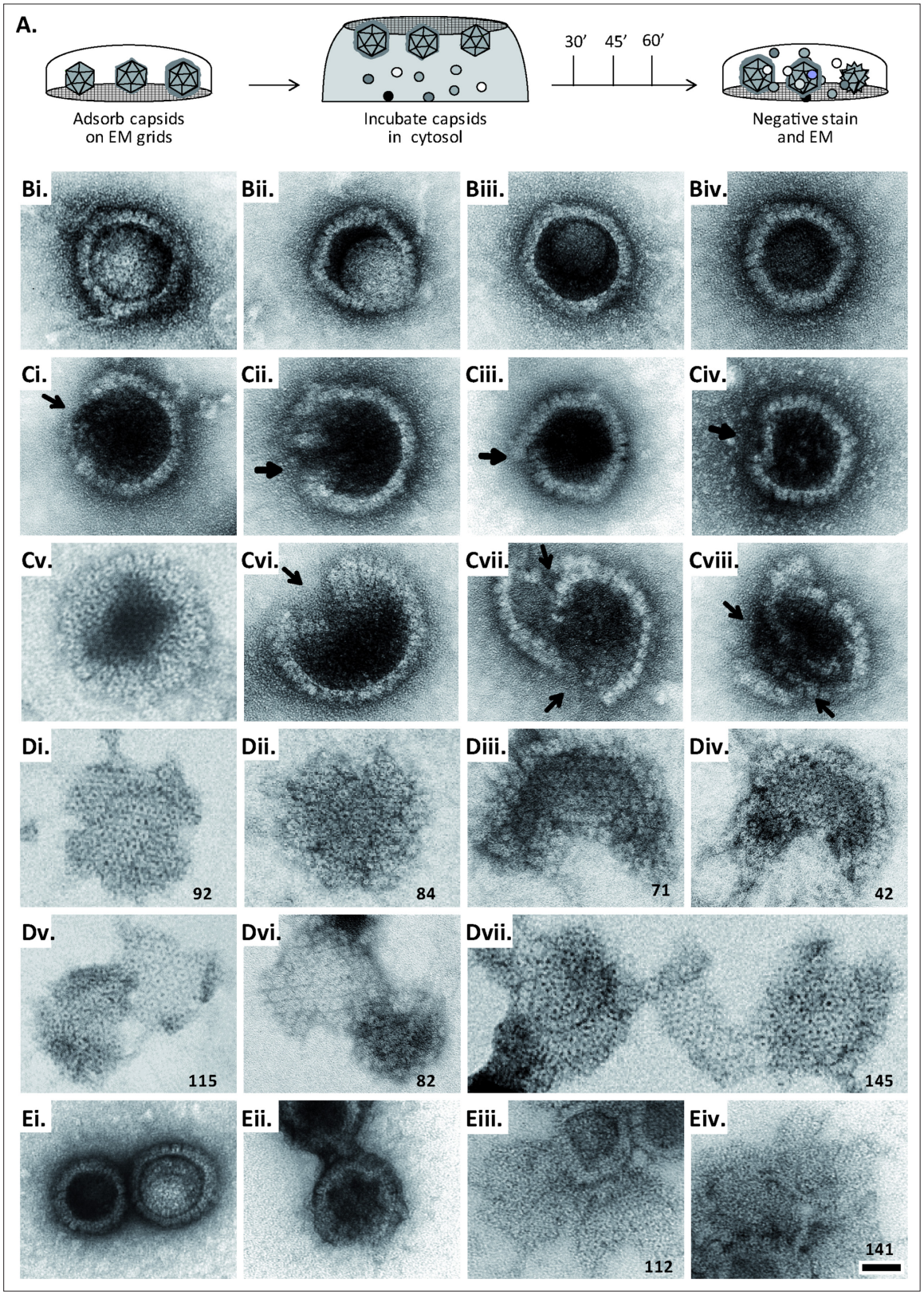

Figure 6. MxB induces disassembly of herpesviral capsids. (**A**) Experimental design: Capsids were adsorbed onto hydrophilic enhanced carbon-coated EM grids for 20 min at RT. The capsids were incubated in cytosol with ATP/GTP^{high}, and the incubation was stopped at different times by extensive washing. The samples were analyzed by EM after negative staining with uranyl acetate. (**B–D**) Capsids after incubation in cytosol derived from rested Mφ or IFN-induced Mφ$_{IFN}$ macrophages, or control or MxB(1-715) A549 expressing cells for 1 hr at 37 °C, and classified as (**B**) intact, (**C**) punched or

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(**D**) disassembled flat phenotypes. The number of capsomers per flat particle was counted, and is displayed at the bottom of each figures. (**E**) Nuclear VZV capsids remain intact (Ei) after incubation in the cytosol of A549 control cells, or but appear punched (Eii) or as flat shells (Eiii, Eiv) after incubation in the cytosol of A549 cells expressing MxB. Scale bar: 50 nm.

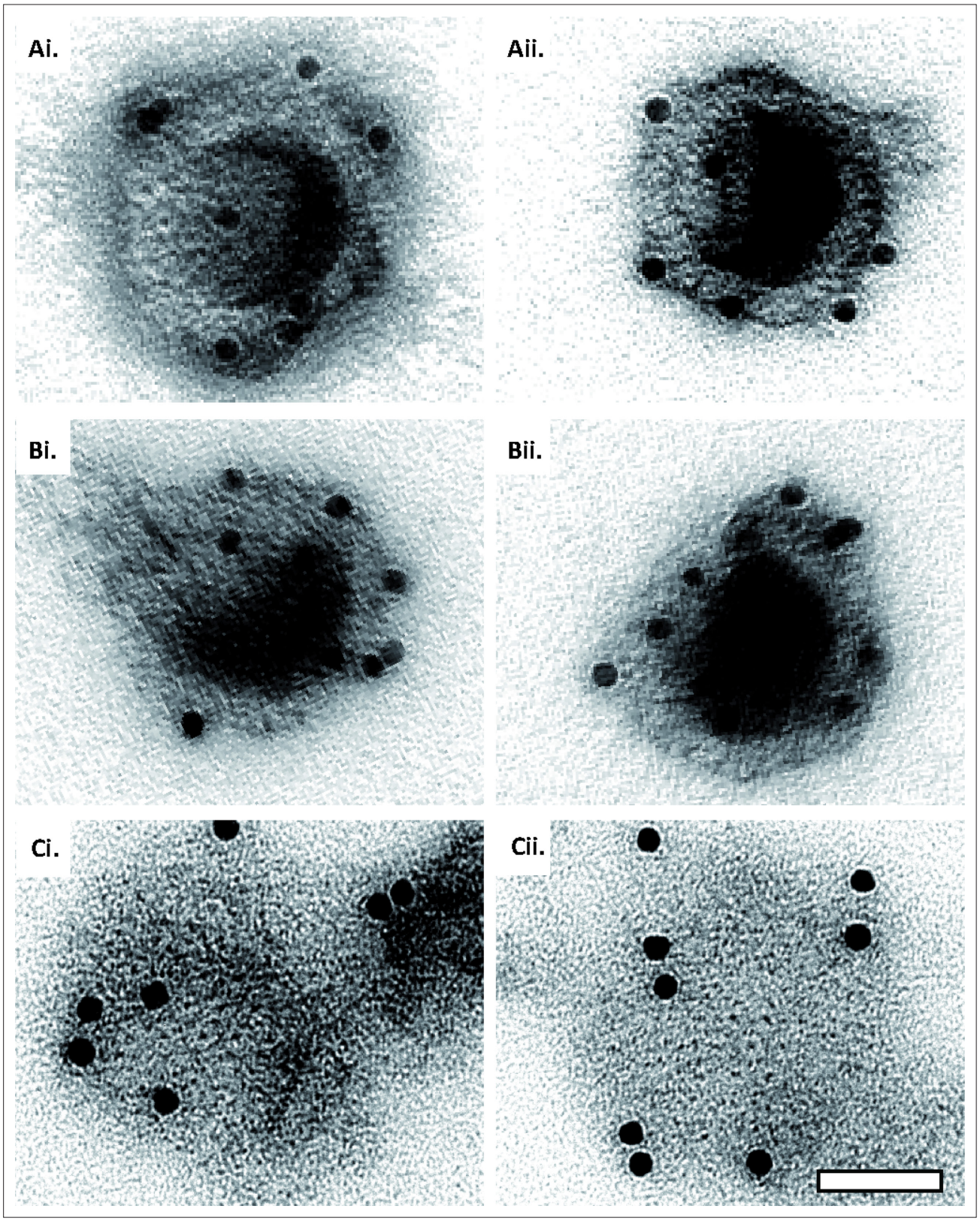

Figure 6—figure supplement 1. Capsid disassembly intermediates by anti-capsid immunoEM. *Images* of capsids after negative staining and labeling with antibodies raised against the major capsid protein VP5 (NC-1), after incubation in ATP-complemented cytosol from A549 control or MxB(1-715) expressing cells for 60 min at 37 °C, and classified as (**A**) *intact*, (**B**) *punched*, or (**C**) *flattened* shells. Scale bar: 50 nm.

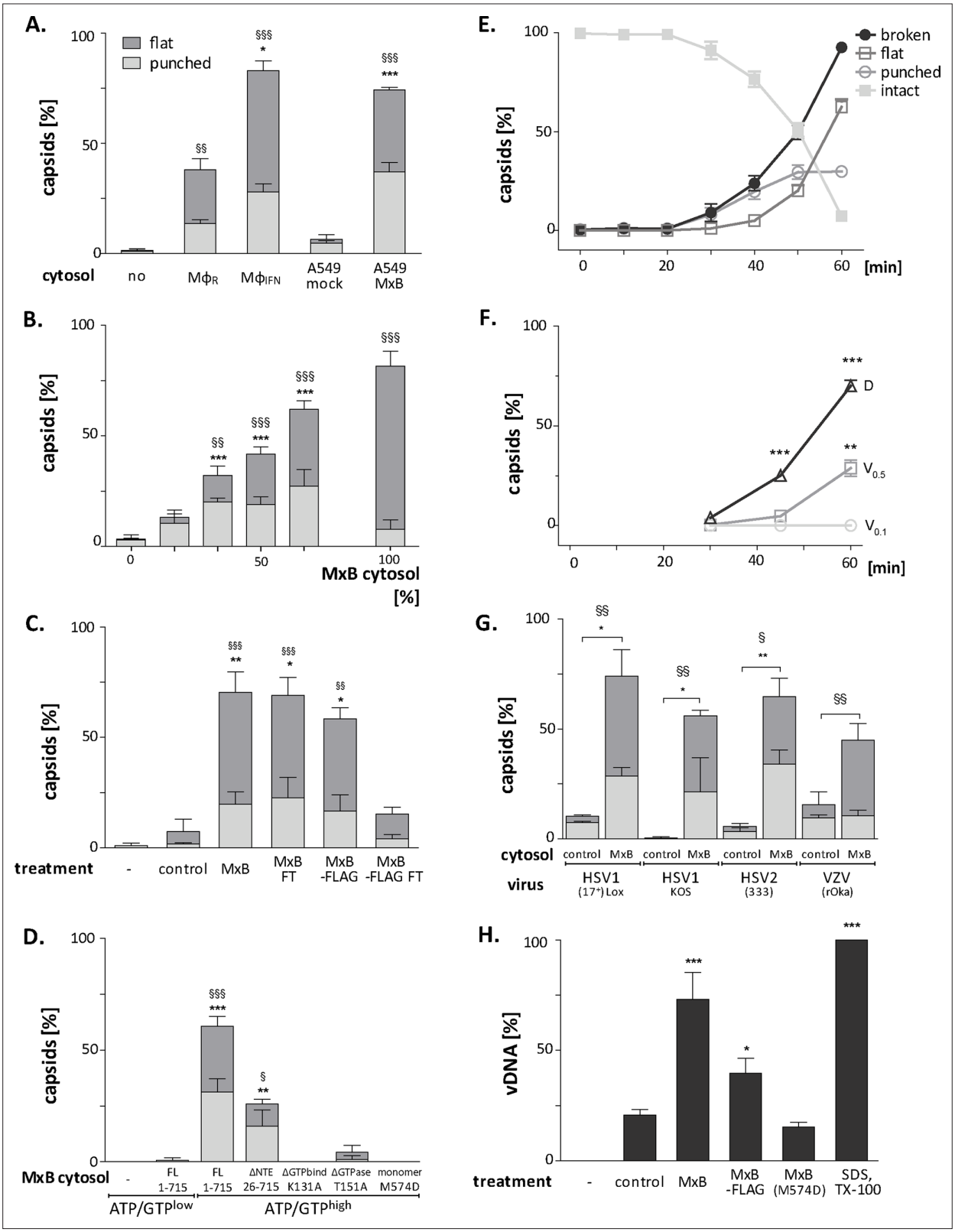

Figure 7. MxB GTP hydrolysis and dimerization required for capsid disassembly and vDNA release of viral genomes. HSV-1 (**A–H**), HSV-2 (**G**) or VZV capsids (**G**) were incubated with cytosol at ATP/GTP^{high} for 1 hr or the indicated time (**E,F**) at 37 °C, and classified into *intact*, *punched* and *flat* capsids by electron microscopy (**A–G**), or the amount of released viral DNA was measured by qPCR (**H**). (**A**) Quantification of *punched* and *flat* D capsid shells in cytosol prepared from rested Mφ or IFN-induced Mφ$_{IFN}$ macrophages, or from control A549 (mock) or A549-MxB(1-715) cells. (**B**) Increasing amounts

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of MxB(1-715) [%] were added to control A549 cytosol, and the amounts of *punched* and *flat* capsids were quantified after incubation in these mixtures. (**C**) Cytosols of A549 cells expressing MxB(1-715) and Mx(25-715) or MxB(1-715)-FLAG and MxB(26-715)-FLAG were incubated with anti-FLAG antibodies coupled to magnetic beads, the flow-through fractions (FT) were harvested, capsids were treated with anti-FLAG treated or control cytosols, and the amount of punched and flat capsids were quantified. (**D**) Capsids were incubated in cytosols prepared from A549 cells expressing full-length (FL) MxB(1-715), MxB(26-715), MxB(K131A), MxB(T151A), or MxB(M574D) at ATP/GTP^{low} or ATP/GTP^{high} levels. (**E**) Time-course of MxB-induced disassembly of capsids pre-adsorbed onto EM grids, incubated with cytosol from A549-MxB(1-715). (**F**) Analysis of D, $V_{0.5}$, or $V_{0.1}$ capsids treated with MxB(1-175) cytosol for *broken* (*punched +flat*) capsids after negative stain and EM as described for panel E. (**G**) Quantification of MxB cytosol disassembly of D capsids of HSV-1(17^{+})Lox, HSV-1(KOS), or HSV-2(333), or nuclear C capsids of VZV, after incubation in cytosol from A549-MxB(1-715) cells. (**H**) D capsids were incubated with different cytosols for 1 hr at 37 °C or treated with 1% SDS and 10% Tx-100 only, and the released DNA not protected by capsid shells was quantified by qPCR. Error bars: SEM from 100 capsids in three biological replicates. One symbol of *or § denotes $p < 0.05$, two $p < 0.01$, and three $p < 0.001$ as determined in One-way analysis of variance with a Bonferroni post-test, and comparing the relative amounts of (*) *punched* and (§) *flat* capsids, or indicating the differences with the mock-treated samples (*).

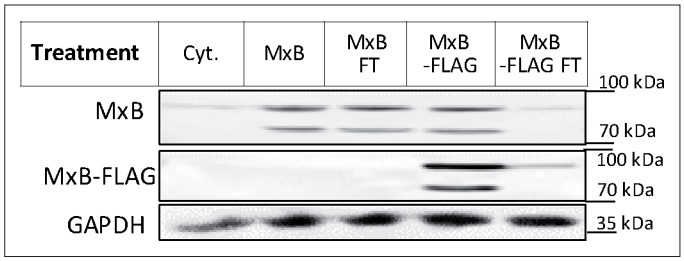

Figure 7—figure supplement 1. Cytosol immunodepleted for MxB. Cytosols prepared from A549-MxB(1-715) and MxB(26-715) expressing MxB(1-715) and MxB(26-715), or A549-MxB-FLAG cells expressing MxB(1-715)-FLAG and MxB(26-715)-FLAG, respectively, were incubated with agarose beads coupled to anti-FLAG antibodies. After immunodepletion with anti-FLAG beads to deplete MxB(1-715)-FLAG and MxB(26-715)-FLAG, the flow through (FT) was harvested. To determine to what extend the FLAG-tagged MxB proteins had been depleted, the starting cytosols (MxB, Mxb-FLAG) as well as the respective FT fractions were probed by immunoblot using antibodies directed against MxB, FLAG, or GAPDH as a loading control. ***Figure 7—figure supplement 1—source data 1***.

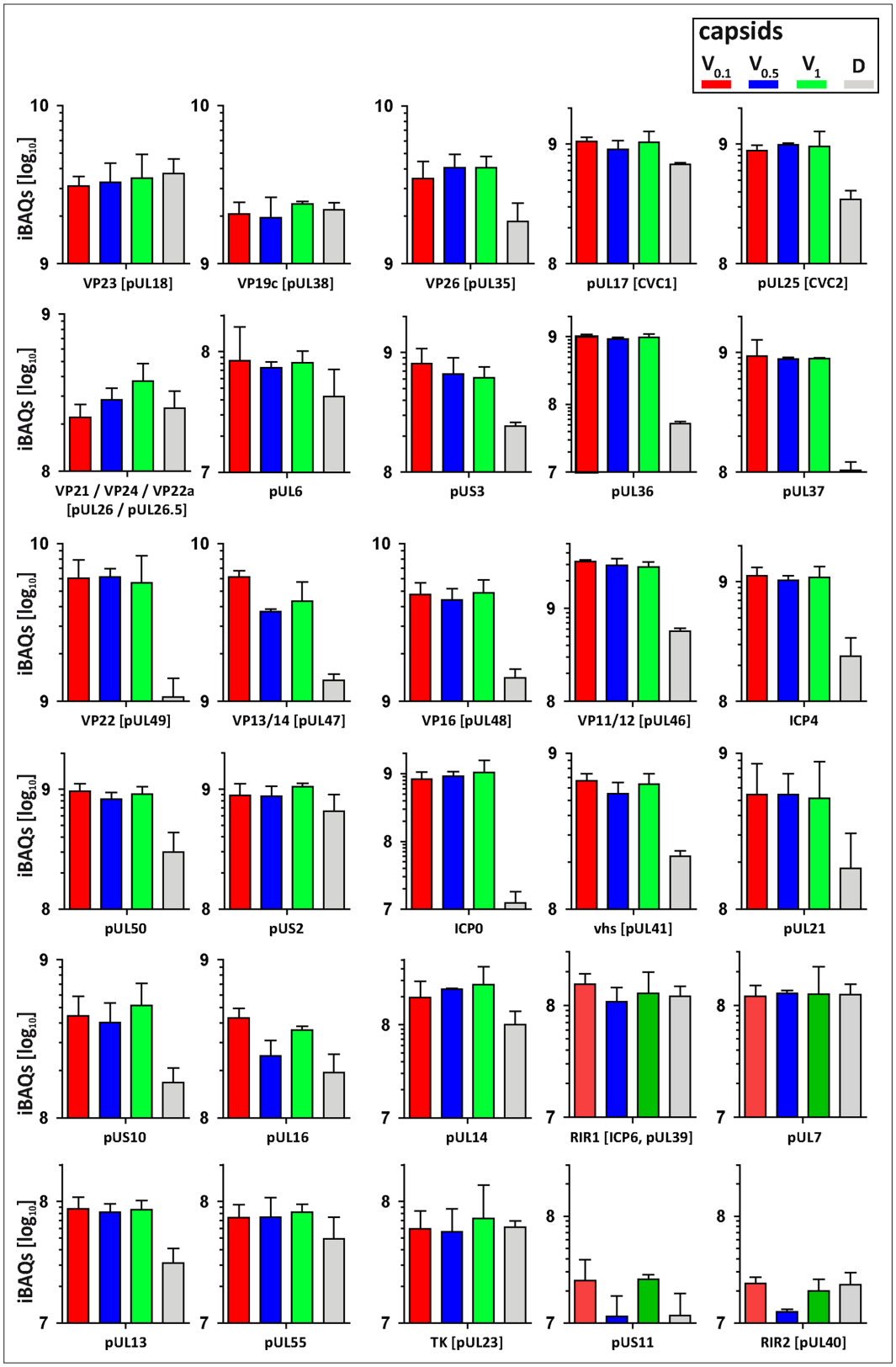

Figure 8. Structural and tegument characterization of $V_{0.1}$, $V_{0.5}$, V_1, and D capsids. The composition of HSV-1(17$^+$) Lox derived $V_{0.1}$ (red), $V_{0.5}$ (blue), V_1 (green), and D (gray) capsids was analyzed by quantitative mass spectrometry in four biological replica. The sum of all the peptides intensities (iBAQ, intensity-based absolute quantification) of each viral protein known to participate in the structure of the capsids was normalized to the one of VP5 and displayed in a bar plot for each viral protein.

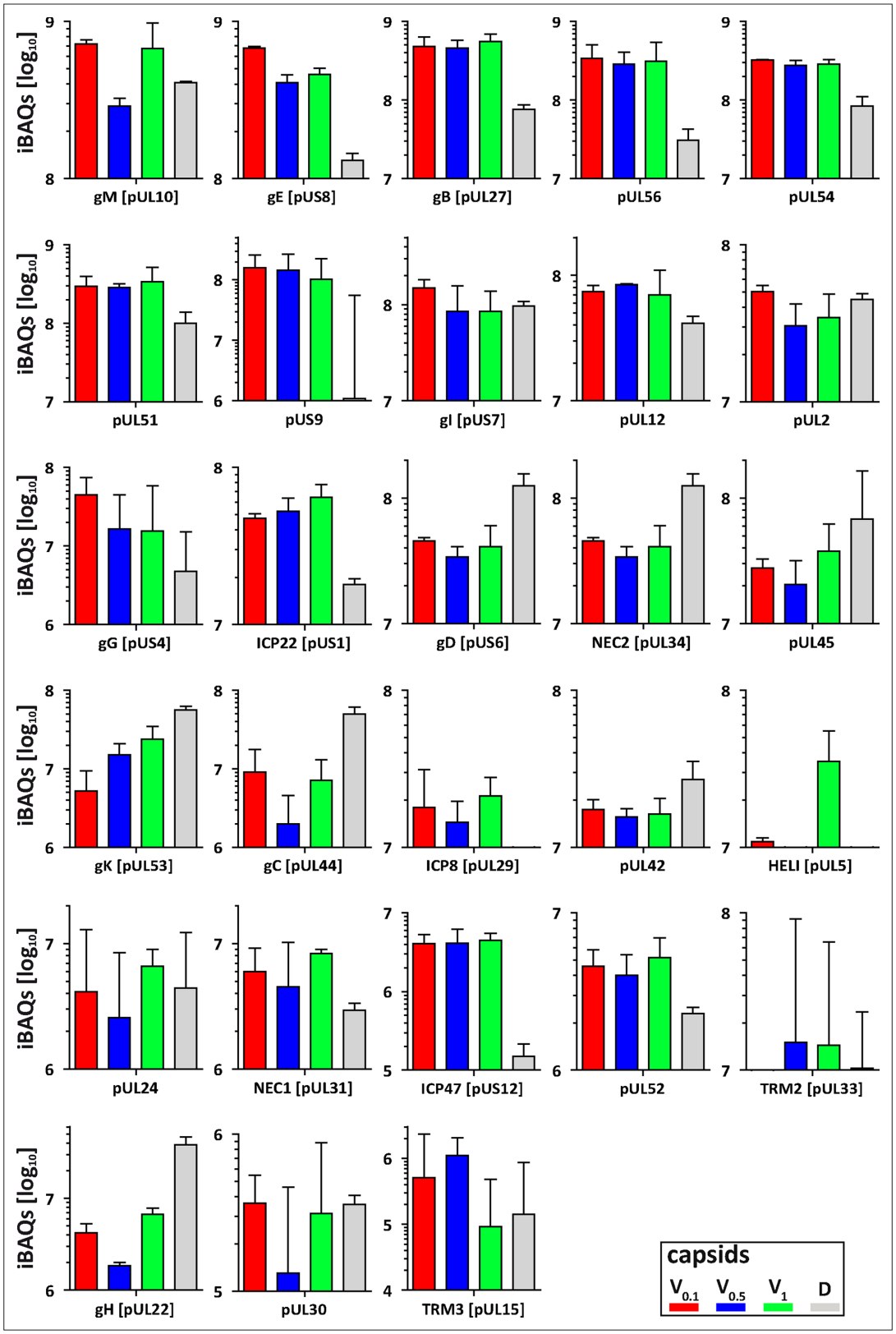

Figure 8—figure supplement 1. Membrane and non-structural proteins on V capsids versus D capsids. The composition of HSV-1 derived $V_{0.1}$ (red), $V_{0.5}$ (blue), V_1 (green), and D (gray) capsids were analyzed by quantitative mass spectrometry in four biological replicates. The sum of all the peptides intensities (iBAQ, intensity-based absolute quantification) of each viral protein unknown to participate in the structure of the capsids was normalized to the one of VP5 and displayed in a bar plot.