Supplementary Materials

Supplemental Experimental Procedures

Cell Lines

ART, SFT, and SNBT cells are "NCI-60" cell lines ADR-RES (an ovarian tumor line), SF-539 (a gliosarcoma line), and SNB-75 (a glioblastoma line), respectively, that were stably transfected with an SV40 Large T antigen expression plasmid, pTIH (Buck et al., 2004). Parent cell cultures were obtained from the NCI DTP Tumor Repository, National Cancer Institute at Frederick, MD. These cells have been previously described (Ray et al., 2015) and were chosen due to their infectability by a wide range polyomaviruses, including JCV, BKV, SV40, and MCV. ART, SFT, and SNBT cells were maintained in R5 medium, which consists of RPMI medium (Invitrogen) supplemented with 5% FBS (Sigma), Glutamax-I (Invitrogen) and 170, 50, or 25 μg/ml hygromycin (Invivogen), respectively. Another NCI-60 cell line, A549, was also maintained in R5. 293TT cells (Buck et al., 2004; Shaw et al., 2002) were maintained in DMEM (Invitrogen) supplemented with 10% FBS, nonessential amino acids (Invitrogen), Glutamax-I and 250 μg/ml hygromycin (D10). 293FT cells were purchased from Thermo Fischer (R70007) and maintained in D10. HSC is an SV40-immortalized human Schwann cell line that was a generous gift from Ahmet Höke (Lehmann et al., 2012). The HSC line was maintained in D10.

Pseudovirus and VLP Production

Detailed maps for all expression plasmids used to generate the pseudoviruses and VLPs from this study are available at our lab website, http://home.ccr.cancer.gov/LCO/. PML patient isolates 5228w (wild-type) and 5228m (S269F mutant), described in Ray et al. (Ray et al., 2015), were chosen as a representative JCV genotype 2 VP1 wild-type and mutant pair. In the current study, these strains are referred to as JCV2 and JCV2-269F, respectively. Additionally, a wild-type JCV genotype 3 VP1 and mutant JCV3-L55F were chosen for comparison. All JCV pseudoviruses and VLPs used consensus VP2 and VP3 minor capsid proteins, which are identical to those of JCV

isolate 313B (accession AAK28470). BKV pseudoviruses and VLPs used in this study include a previously described BKV genotype Ia wild-type VP1 (accession JF894228)(Pastrana et al., 2013) and a F76W mutant that was incorporated into the BKV Ia VP1 open reading frame by PCR mutagenesis. Both BKV pseudoviruses and VLPs used the BKV-IV VP2 and VP3 sequences described in Pastrana et al. (Pastrana et al., 2013). SV40 pseudovirus was produced using the previously described pCAG-SV40 plasmid encoding VP1, VP2, and VP3 (Nakanishi et al., 2008). MCV pseudovirus and VLPs were produced using VP1 and VP2 plasmids described in Pastrana et al. (Pastrana et al., 2009).

The reporter plasmids used for pseudovirus production encode a secreted version of NanoLuc luciferase (Hall et al., 2012), derived from pNL1.3 (Promega). The secreted NanoLuc ORF was moved from pNL1.3 and used to replace HPV16 L1 in the plasmid p16L1-GFP (Buck et al., 2005) to create phsNuc, which encodes secreted NanoLuc under the control of a minimal human EF1α promoter. Likewise, the secreted NanoLuc ORF was cloned into pEGFN-1 (Clontech) to replace EGFP and create pcsNuc, which encodes NanoLuc under the control of the CMV immediate early promoter. Two separate promoters were used in hope of maximizing reporter gene expression in different cell lines.

Pseudoviruses were produced using previously described methods (Buck et al., 2004; Pastrana et al., 2009; Ray et al., 2015). All pseudoviruses were prepared using the "Revised Production" method. Briefly, 21 million 239TT cells were pre-plated overnight in a T225 flask then transfected with a mixture of plasmids encoding codon modified VP1, VP2, VP3, and the reporter plasmids pcsNuc and phsNuc at a 2:1:1:1:1 ratio, respectively. The transfection mixture was left on cells for roughly 16 hours, then removed and replaced with fresh medium. Forty-eight hours after transfection, cells were trypsinized, collected by centrifugation, and washed once in Dulbecco's PBS with calcium and magnesium supplemented with an additional 9.5 mM $MgCl_2$ and Pen-Strep-Fungizone (PSF, Invitrogen)(DPBS-Mg). Cells were resuspended in DPBS-Mg at

1.4 cell pellet volumes. Neuraminidase V (Sigma N2876) was then added at a final concentration of 2 U/ml and the cell suspension was incubated at 37ºC for 30 minutes. Triton X-100 or Brij-58 was added at 0.5% (v/v) of cell suspension volume and the mixture was incubated at 37ºC for 30 minutes. Finally, a 1M pH 9 stock of ammonium sulfate was used to adjust the lysate to 25 mM ammonium sulfate and an RNAse A/T1 cocktail (Ambion) was added at 0.1% of lysate volume. The buffered lysates were incubated at 37ºC overnight to allow capsid maturation. Lysates were clarified by low speed centrifugation, the pellet was re-extracted with additional PBS, and pooled clarified material was purified either by ultracentrifugation over an Optiprep gradient (JCV, MCV, and HPV16) or by agarose gel filtration (BKV and SV40), as previously described (Buck et al., 2008; Cardone et al., 2014). Stocks were characterized by quantification of reporter plasmid by qPCR (pseudovirions) using primers targeting NanoLuc (AGATTTCGTTGGGGACTGGCG and CCGCTCAGACCTTCATACGGG). In some instances, VP1 content of the stock was characterized by comparison to BSA standards run on Novex SDS-PAGE gels stained with SYPRO Ruby dye (Invitrogen). Pseudovirus doses used for each figure are shown in Supplemental Table 2.

VLPs were produced in the same manner as pseudoviruses except the NanoLuc reporter plasmids were omitted from the transfection step and, instead of RNAse, lysates were treated with Benzonase (Sigma) and PlasmidSafe (Epicentre) nucleases. VLPs were purified using Optiprep gradients.

Synthesis of 3Fax-Peracetyl Neu5Ac

The identity and purity of 3Fax was validated by NMR and mass spectrometry. 3Fax was dissolved in DMSO at 50 mg/ml and stored at -80 ºC. The efficacy of the inhibitor was verified by culturing cells in a dilution series of 3Fax and using flow cytometry (see below) to evaluate the

binding of biotinylated lectins *Sambucus nigra* (SNA, 2 μg/ml) and *Maackia amurensis II* (MAL-II, 5 μg/ml) (Vector Laboratories).

VLP Binding Assay with 3Fax and Heparinase I/III Treatment

SFT cells were cultured in R5 with 200 μM 3Fax or the equivalent volume of DMSO (mock treatment) for 2 days, then trypsinized and replated in a 10 cm^2 tissue culture dish in R5 with 200 μM 3Fax or DMSO. After 2 days, cells were dislodged from the plate using PBS with 10 mM EDTA and resuspended in wash medium (DPBS with 1% FBS, 10 mM HEPES, and PSF). Cells were resuspended at 50,000 cells/0.1 ml and both DMSO and 3Fax treated cells were either mock treated with enzyme buffer (20 mM Tris pH 7.5, 50 mM NaCl, 4 mM CaCl2, 0.01% BSA) or 0.02 units of heparinase I/III (Sigma H3917) dissolved in enzyme buffer. All conditions were incubated at 37 ºC for 1 h in a water bath with gentle mixing every 10 minutes. Following the incubation, 100 μl of cell suspension from each treatment condition was transferred to a 96-well plate and washed once in wash medium. Cells were incubated with 50 ng of VLPs for 1 h at 4 ºC with rocking and, after two washes in wash medium, incubated with primary antibody (1:1000) for 30 min at 4 ºC. Primary antibodies used include: mouse-anti-JCV polyclonal sera (Ray et al., 2015), mouse-anti-BKV polyclonal sera (Pastrana et al., 2013), rabbit-anti-MCV polyclonal sera (Pastrana et al., 2009), mouse-anti-HPV polyclonal sera (a generous gift from Hanna Seitz and John Schiller, NCI). Additionally, anti-heparan sulfate mAb HS20 (0.5 μg/ml) or a combination of biotinylated SNA (2 μg/ml) and MAL-II (5 μg/ml) lectins were added to measure the effect of the 3Fax treatment on surface expression of heparan sulfate and sialic acid, respectively. After two washes, cells were incubated with the appropriate Alexa Fluor 488 conjugated secondary antibody (Invitrogen) or, for the lectins, with streptavidin conjugated to Alexa Fluor 488 (Invitrogen). After two final washes, cells were resuspended in 225 μl of wash medium and Alexa

Fluor 488 signal was measured by flow cytometry using a BD FACSCanto II. Cytometry was analyzed using FlowJo software.

Heparin Inhibition Assay

ART, SFT, or 293TT cells were trypsinized and plated in 96-well plates at 5,000 cells/well in 100 µl, avoiding usage of the evaporation-prone outer wells of the plate. Heparin (Sigma H4784) was reconstituted in PBS and a 1:2 dilution series of heparin at 4x the desired final concentration was diluted in R5 media, and added to cells in 50 µl in quintuplicate. The pseudovirus inoculum was added to cells in 50 µl of R5 medium, bringing the final volume in each well to 200 µl. After incubation at 37 ºC for five days (BKVs) or six days (JCVs), 25 µl of supernatant was transferred to white luminometry plates (Perkin-Elmer), mixed with 50 µl of NanoLuc working substrate diluted 1:5 in PBS and signal was read using a POLARstar Optima luminometer (BMG), typically with gain set at 3200.

The heparin inhibition assay was also performed with 3Fax-treated cells. SFT cells were cultured in 200 µM 3Fax or an equivalent volume of DMSO for two days, trypsinized and replated with 3Fax or DMSO in 96-well plates at 5,000 cells/well. For both 3Fax and DMSO treated cells, virus was added (Supplemental Table 2) in 50 µl to all cells, and either 50 µl R5 or a single dose of heparin (100 µg/ml) was added, to bring the final volume to 200 µl. After six days, supernatant was harvested and NanoLuc signal was measured as described above.

HS20 Neutralization Assay

Purified HS20 was a generous gift from M. Ho and W. Gao (Antibody Therapy Section, Laboratory of Molecular Biology, NCI). ART or SFT cells were trypsinized and plated in 96-well plates at 5,000 cells/well in 50 µl, avoiding usage of the evaporation-prone outer wells of the plate. A 1:3 dilution series of HS20 at 4x the desired final concentration was made in R5 media,

and added to cells in 25 µl in quintuplicate. Virus was diluted in R5 and added to cells in 25 µl. After incubation at 37ºC for four days, 25 µl of supernatant was transferred to white luminometry plates and luminescent signal read as described for the heparin inhibition assay.

Transduction of Sodium Chlorate-Treated A549

A549 cells were cultured in R5 supplemented with or without 50 mM sodium chlorate (Sigma) for three days, trypsinized, and replated in R5 with or without 50 mM sodium chlorate at 5,000 cells/well in 100 µl. Virus was diluted in R5 and added in 25 µl in quadruplicate to cells. Infection was allowed to proceed for six days, and then 25 µl of supernatant was transferred to a white luminometry plate and luminescent signal read as described for the heparin inhibition assay.

Cell Binding Assay with H2B-NanoLuc (H2BN) VLPs

JCV VLPs carrying an encapsidated histone H2B-NanoLuc fusion protein (H2BN) were generated by inclusion of plasmid pH2BN in the VLP transfection mix (see above). The H2BN ORF was generated using PCR to recombine segments of pNL1.1CMV (Promega), plasmid pwM (Tolstov et al., 2009), and pBOS-H2BGFP (BD Pharmingen) (Kanda et al., 1998). Sequencing revealed that the H2B gene in pBOS-H2BGFP contained two non-silent mutations (D26G and V119I). The errors were reverted to wild-type by overlap PCR.

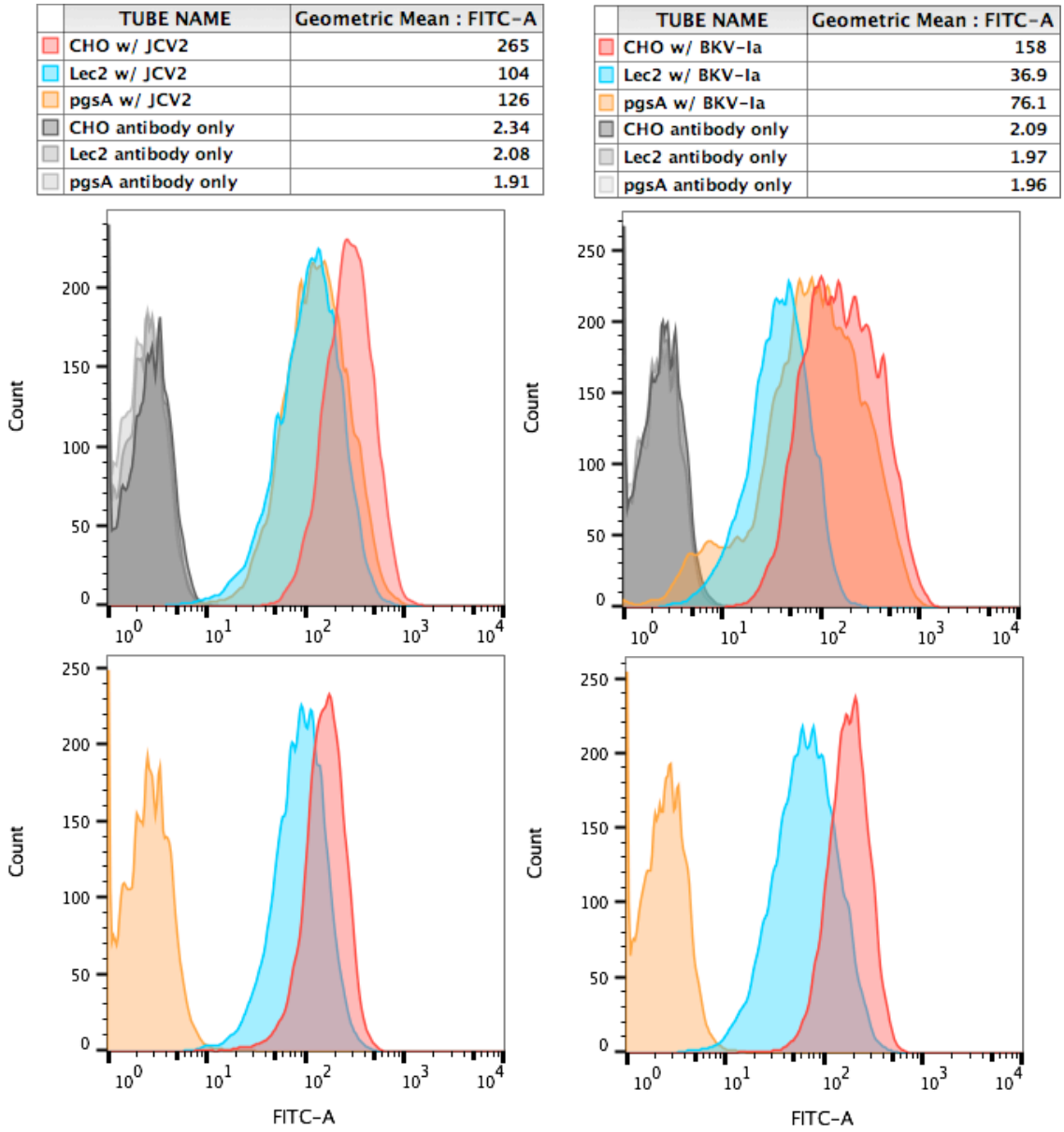
Hemagglutination Assay

JCV-1A VLPs were diluted to 0.1 ng/µl in PBS and added to pre-chilled 96-well round bottom wells in 20 µl volume. A 1:5 dilution series of anti-JCV VP1 mAbs was made in PBS starting at 50 ng/µl and 20 µl of each antibody dilution was added to the round-bottom wells. Type O+ red blood cells (RBCs) were washed three times in ice cold PBS. Ten µl of a 5% (v/v) suspension of RBCs in chilled DPBS was added to pre-chilled 96-well round bottom wells containing the

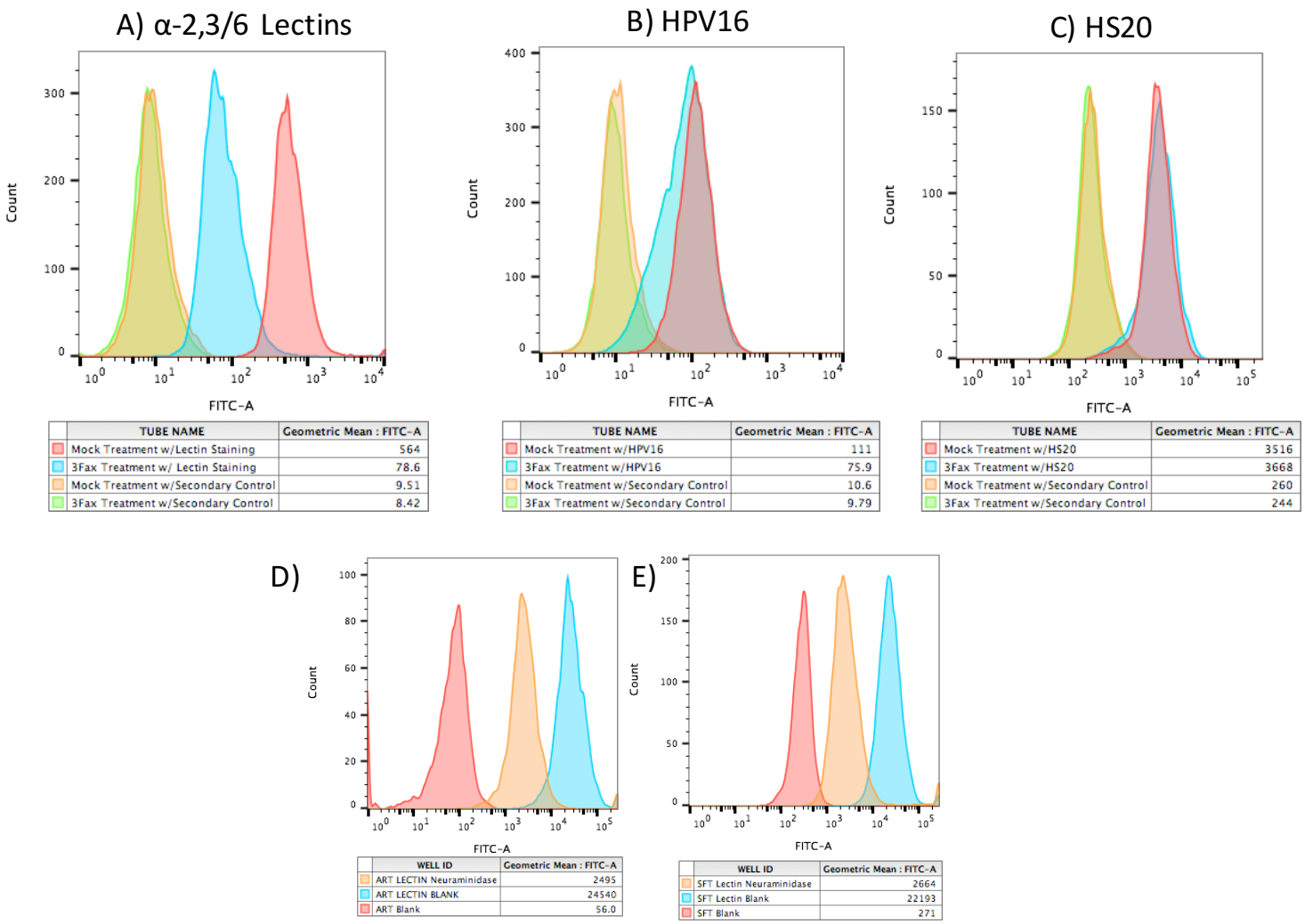
VLP/antibody mixtures. RBCs were resuspended by trituration and plates were placed on ice overnight.

Statistical Analysis

Graphpad Prism software was used to estimate EC_{50} values by fitting a sigmoidal dose-response curve with variable slope. The top and bottom of the curve were fixed at 100% neutralization or 0% neutralization, respectively.



Supplemental Figure 1. Binding of JCV and BKV VLPs to CHO-Based Cell Lines. Related to Figure 1. JCV and BKV VLPs were incubated with CHO, Lec2 (which lack sialic acid), or pgsA-745 (which lack GAGs) and binding was detected using anti-VP1 antibodies. Results are representative of three independent experiments.



TUBE NAME	Geometric Mean : FITC-A
Mock Treatment w/Lectin Staining	564
3Fax Treatment w/ Lectin Staining	78.6
Mock Treatment w/Secondary Control	9.51
3Fax Treatment w/Secondary Control	8.42

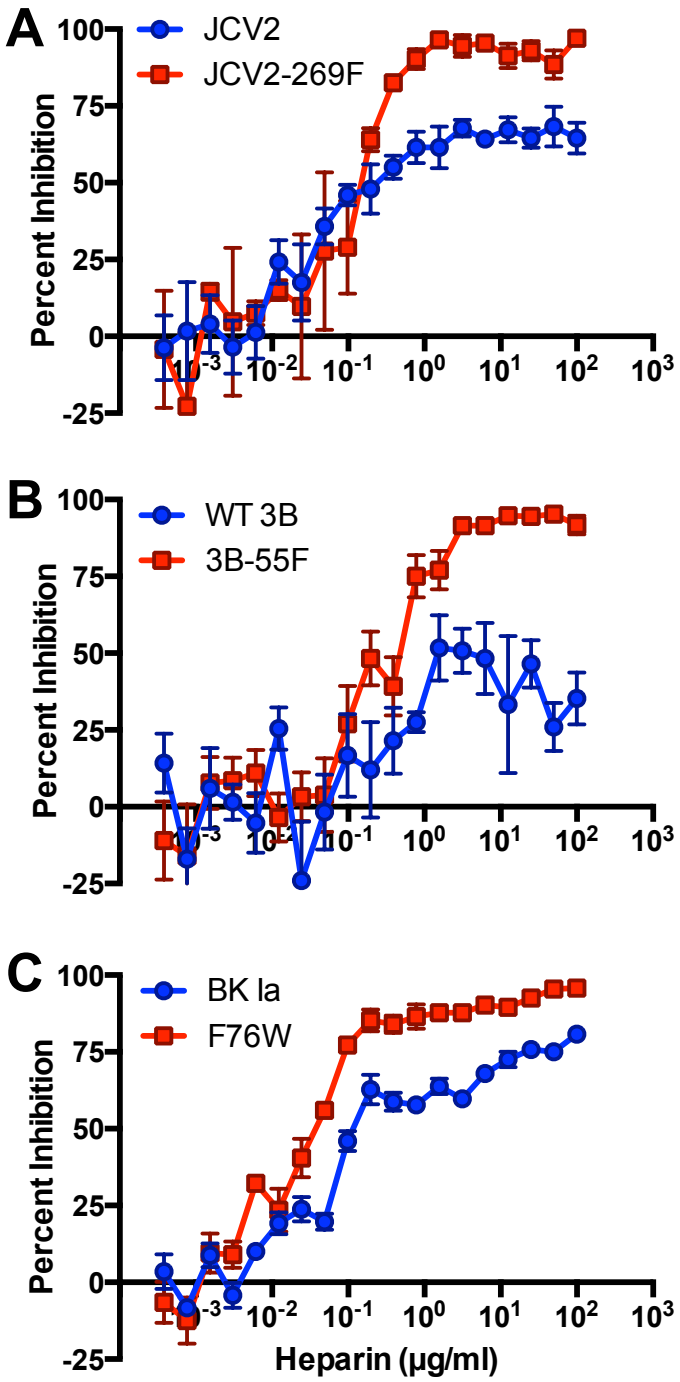
TUBE NAME	Geometric Mean : FITC-A
Mock Treatment w/HPV16	111
3Fax Treatment w/HPV16	75.9
Mock Treatment w/Secondary Control	10.6
3Fax Treatment w/Secondary Control	9.79

TUBE NAME	Geometric Mean : FITC-A
Mock Treatment w/HS20	3516
3Fax Treatment w/HS20	3668
Mock Treatment w/Secondary Control	260
3Fax Treatment w/Secondary Control	244

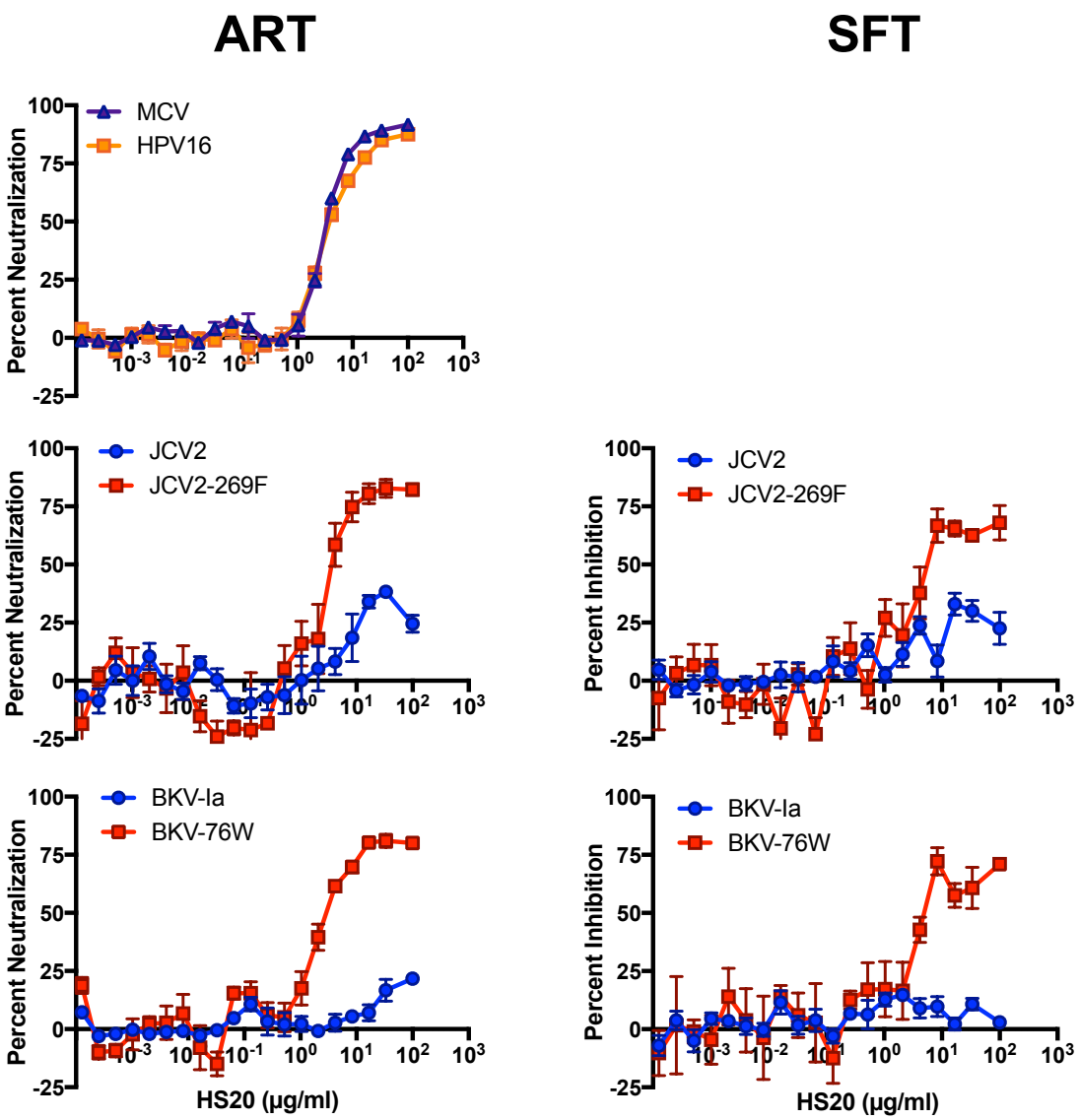
WELL ID	Geometric Mean : FITC-A
ART LECTIN Neuraminidase	2495
ART LECTIN BLANK	24540
ART Blank	56.0

WELL ID	Geometric Mean : FITC-A
SFT Lectin Neuraminidase	2664
SFT Lectin Blank	22193
SFT Blank	271

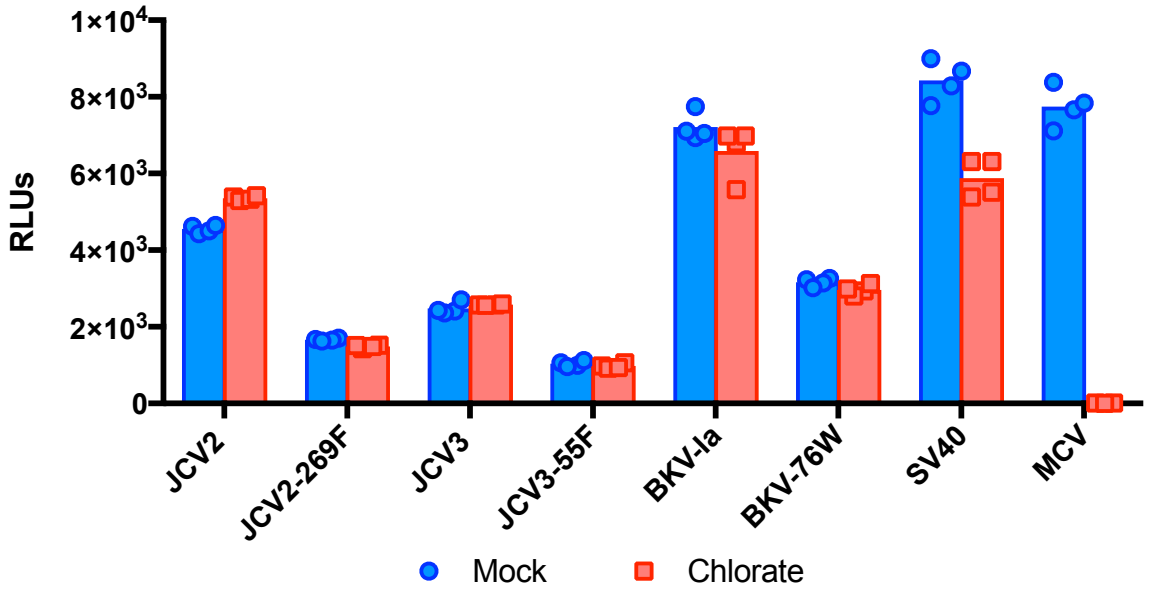
Supplemental Figure 2. Flow cytometric analysis of 3Fax- and neuraminidase-treated cells. Related to Figure 1 and 3, and Supplemental Figure 6. Panels (**A-C**): SFT cells were cultured in DMSO (mock treatment) or 3Fax for 3 days and then incubated with (**A**) biotinylated lectins specific for α-2,3- and α-2,6-linked sialic acids, (**B**) HPV16 VLPs, or (**C**) the anti-heparan sulfate mAb HS20. Panels (**D**) and (**E**): ART or SFT cells were incubated with neuraminidase for one hour and stained with lectins. Secondary-only controls (Alexa Fluor 488-labeled streptavidin without lectins or Alexa Fluor 488 goat-anti-mouse secondary without anti-HPV16 primary, or without HS20) are shown. Results are representative of three independent experiments.



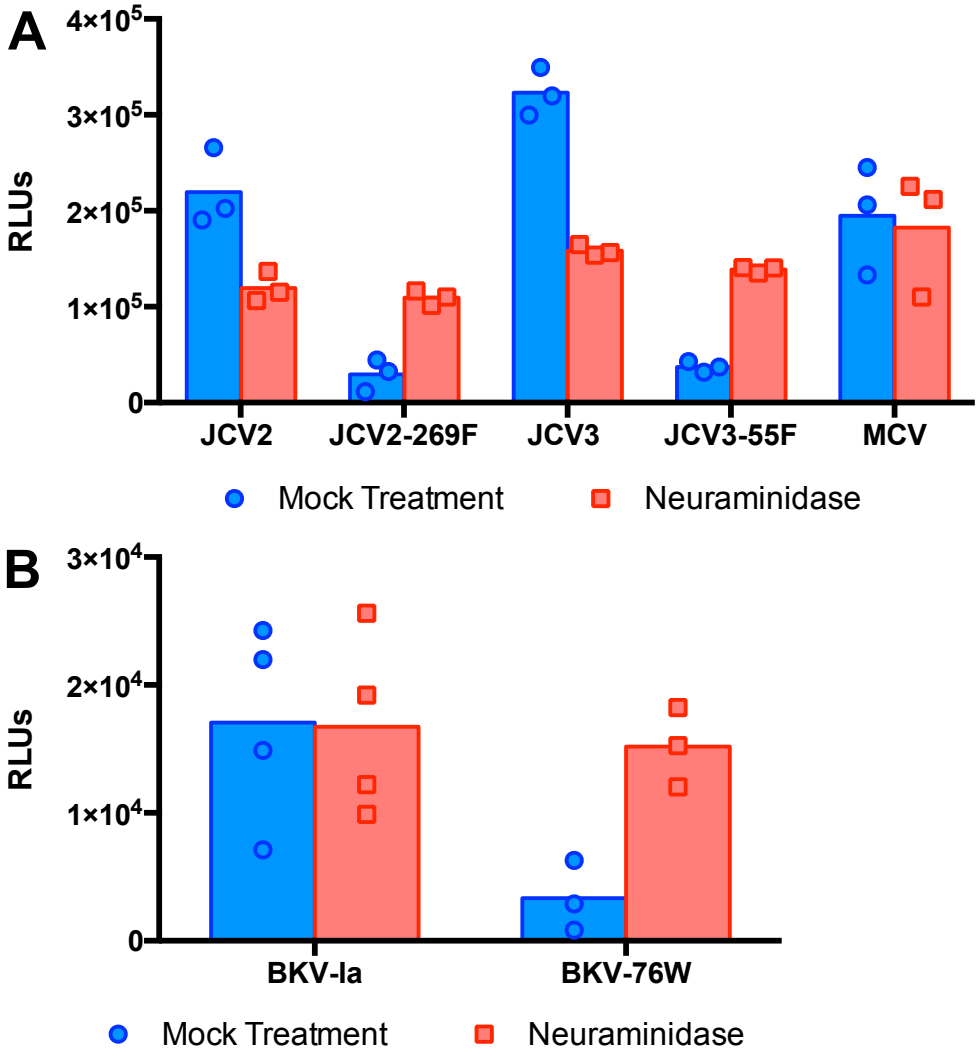
Supplemental Figure 3. SA-mutants are more sensitive to heparin inhibition than wild-type JCV or BKV. Related to Figure 2. Infectivity of indicated pseudoviruses was measured in the presence of a two-fold dilution series of heparin on SFT cells. Results are normalized to a "no heparin" control and are an average of quintuplicate values. Error bars represent SEM.



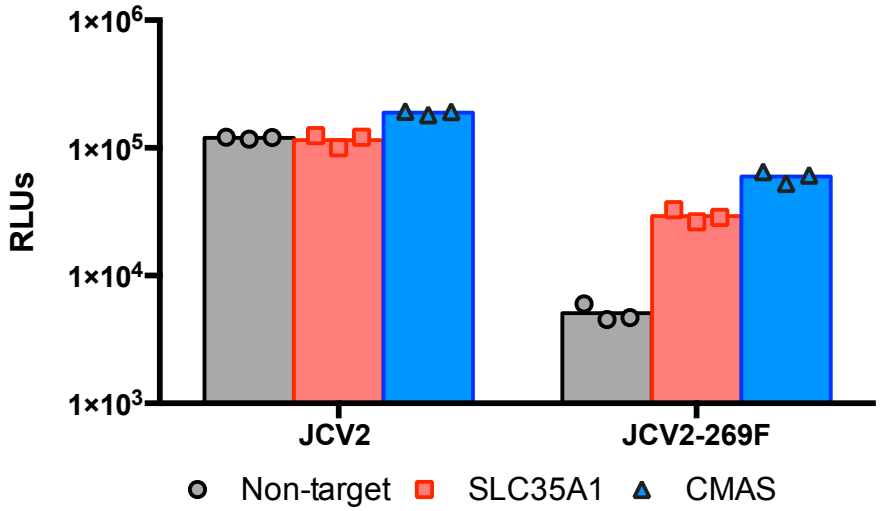
Supplemental Figure 4. SA-mutants are more sensitive to inhibition by the GAG-blocking mAb HS20 than wild-type JCV or BKV. Related to Figure 2. A complementary approach to establishing the GAG-dependency of polyomavirus infectious entry used HS20, a mAb originally identified based on its ability to bind heparan sulfate chains displayed on the liver cancer antigen glypican-3 (Gao et al., 2014; Gao et al., 2015a; Gao et al., 2015b). HS20 effectively blocked the infectivity of MCV and human papillomavirus type 16 (HPV16), both of which are known to require GAGs for cell attachment (row A). The SA-mutants were likewise almost completely neutralized by HS20 (red points, rows B and C). In contrast, the infectivity of wild-type JCV2 and wild-type BKV were only modestly affected by HS20, even at the highest tested concentrations (blue points, rows B and C). The results, performed on both ART (left panels) and SFT (right panels) cells, confirm that SA-mutant viruses depend on engagement of GAGs for infectious entry and that wild-type JCV and BKV can remain infectious when GAG engagement is blocked. The infectivity of indicated pseudoviruses was measured in the presence of a three-fold dilution series of HS20 (x-axes). Background signal (no virus) was subtracted from all results and quintuplicate values were averaged and expressed as percent inhibition compared to untreated virus control. Error bars represent SEM. Results are representative of three independent experiments.



Supplemental Figure 5. JCV and BKV pseudovirus transduction is unaffected by hypo-sulfation. Related to Figure 2. MCV infectious entry is known to require the presence of sulfate modifications both on the GAGs the virus uses to attach to cells and on the sialylated co-receptor glycans required for post-attachment entry steps (Schowalter et al., 2011). To determine whether JCV or BKV infectivity depends on sulfate modifications, cells were treated with sodium chlorate, which serves as a competitive inhibitor of both N- and O-sulfation (Baeuerle and Huttner, 1986). The infectivity of an MCV pseudovirus was used as a control for the effectiveness of chlorate treatment for blocking GAG sulfation. By this measure, A549 cells (but not ART, SFT, SNBT, or 293TT cells) were confirmed (Schowalter et al., 2011) to be sensitive to chlorate treatment. The infectivity of all tested JCV and BKV pseudoviruses was unaffected by the chlorate treatment of A549 cells. The results suggest that, in contrast to MCV, JCV and BKV do not require interactions with sulfate groups. It is unclear whether this reflects an insensitivity to the presence of sulfate groups or the two viruses are instead specifically interacting with non-sulfated segments of these complex glycans. A459 cells were cultured in sodium chlorate for three days and the infectivity of a panel of pseudoviruses was measured and compared to untreated cells. Similar results were observed in a total of three independent experiments.



Supplemental Figure 6. Neuraminidase treatment of cells enhances SA-mutant infectivity. Related to Figures 3 and 4. To confirm that the enhancement of PML-mutant infection observed on 3Fax-treated SFT cells was due to the production of hypo-sialylated glycans (as opposed to an unknown off-target effect of 3Fax), cells were treated with a neuraminidase to enzymatically remove sialic acid residues from existing surface glycans. The enzymatic treatment was effective, as measured by decreased lectin staining (Supplemental Figure 1A). The neuraminidase treatment modestly inhibited the infectivity of wild-type pseudoviruses and enhanced the infectivity of SA-mutant pseudoviruses by roughly 3-fold. SFT cells were treated with neuraminidase or mock-treated for 1 hour at 37º C and then plated in R5 and infected with indicated pseudoviruses. Reporter signal was measured after six days. Results are representative of three independent experiments. Similar results were observed for ART cells (data not shown).



Supplemental Figure 7. siRNA suppression of sialylation enhances the infectivity of a PML-mutant pseudovirus on ART cells. Related to Figures 3 and 4. To further confirm that suppression of sialylation enhances PML-mutant infectivity, siRNAs were used to target two genes in the sialyl-glycan biosynthetic pathway, SLC35A1 and CMAS . Attempts at silencing these genes in ART cells resulted in a modest (~40%) reduction of lectin staining (data not shown). Despite the relatively modest reduction in sialylated glycan synthesis, the infectivity of JCV2-269F was enhanced by an average of 4.5 fold when SLC35A1 was silenced and 8.5 fold when CMAS was silenced. The infectivity of the wild-type JCV2 pseudovirus was unaffected by either siRNA, presumably reflecting the ability of JCV2 to utilize the remaining sialylated glycans at the cell surface. Cells were transfected twice with siRNAs targeting either SLC35A1 or CMAS, and then replated and transduced with JCV2 or JCV2-269F pseudoviruses. Similar results were obtained in two independent experiments.

Cell line	Putative tissue of tumor origin	Percent reduction in lectin staining	JCV2-269F (fold increase)	JCV3-55F (fold increase)
ART	Ovary	14%	1.4	nt
293FT	Neuronal	57%	3.9	5.1
293TT	Neuronal	57%	3.9	5.6
HSC	Schwann cell	63%	7.2	11.1
SFT	Gliosarcoma	81%	14.9	20.5
SNBT	Glioblastoma	86%	4.1	3.1

Supplemental Table 1. Effect of 3Fax on various cell lines. Related to Figure 3. Cell lines were cultured in medium with 200 μM 3Fax or the equivalent volume of DMSO for 3 days, then dislodged from tissue culture plates with PBS with 10 mM EDTA. Cells were stained with a mixture of lectins recognizing alpha-2,3 and alpha-2,6 sialylated glycans and flow cytometry was performed to determine the percent decrease in lectin staining compared to mock-treated cells. 3Fax-treated cells were also used to evaluate the effect of decreased sialylated glycan expression on the infectivity of PML-mutant JCV strains. For the infectivity experiment, 3Fax- or mock-treated cells were trypsinized and replated in 96-well plates at 5,000 cells/well with the appropriate amount of 3Fax or DMSO. Replated cells were then inoculated with pseudovirions carrying secreted NanoLuc reporter plasmids. NanoLuc signal in the culture supernatant was measured six days after inoculation. The fold enhancement of luminometric signal for JCV2-269F and JCV3-55F (PML-mutant) pseudovirions on 3Fax-treated cells relative to DMSO-treated control cells was determined. nt = not tested.

Pseudovirus	Figures 2-3, S. Figure 3-5	Figure 4	Figure 5	S. Figure 6	S. Figure 7	S .Table 1
JCV2	8.2E+04	6.0E+03	4.63E+04	9.5E+04	4.6E+04	-
JCV2-269F	5.0E+05	4.4E+05	8.76E+04	9.4E+05	4.4E+05	1.8E+06
JCV3	3.0E+04	9.3E+03	2.86E+04	6.2E+04	-	-
JCV3-55F	3.7E+05	3.7E+05	1.18E+05	1.1E+06	-	1.5E+06
JCV-IA	-	-	6.03E+04	-	-	-
BKV-Ia	2.2E+04	-	-	1.0E+05	-	-
BKV-76W	1.2E+05	-	-	2.4E+05	-	-
SV40	5.4E+04	-	-	-	-	-
MCV	6.2E+05	-	-	6.2E+05	-	-
HPV16	6.2E+05	-	-	-	-	-

Supplemental Table 2. Characteristics of pseudovirus stocks. Related to Figures 2-5, Supplemental Figures 3-7, Supplemental Table 1. The table shows the number of copies of NanoLuc reporter gene (in the form of purified pseudovirions) used for each 96-well of cells. Pseudovirus stocks collected using Triton X-100 were used for Figures 2-3 and Supplemental Figures (SF) 3-5, stocks collected using Brij-58 were used for Figure 4, Figure 5, and Supplemental Figures 6-7, and Supplemental Table 1.

	Figure legend title	BKV Ia and F76W	JCV2 and JCV2-269F	JCV3 and JCV3-55F	SV40	MCV	HPV16	Other	Cell lines
Figure 1	SA-mutant VLPs require GAGs for binding to SFT gliosarcoma cells.	+	+	-	-	+	-		SFT; ART not responsive to 3Fax
Figure 2	SA-mutant pseudoviruses are more sensitive to heparin inhibition than wild-type pseudoviruses.	+	+	+	+	-	-		ART, SFT (Supplemental Figure 2), SNBT and 293TT (data not shown)
Figure 3	JCV and BKV pseudovirions make facultative use of either sialylated glycans or by heparin-like GAGs for infectious entry.	+	+	+	+	+	+		SFT and SNBT (data not shown); ART not responsive to 3Fax
Figure 4	Addition of asialo-GM1 enhances the infectivity of PML-mutant JCV strains.	-	+	+	+ (SFT - data not shown)	-	-		ART and SFT (data not shown)
Figure 5	Neutralization properties of anti-JCV mAbs on 3Fax-treated SFT cells.	-	+	+	-	-	-	Mad1	3Fax treated SFT; untreated SFT and ART (data not shown)
Figure 6	Neutralizing mAbs do not prevent JCV attachment to cells.	-	+	+	-	-	-	Heparin and HS20	ART (data not shown) and SFT
Figure 7	Model for infectious entry of JCV.	-	-	-	-	-	-		
Supplemental Figure 1	Binding of JCV and BKV VLPs to CHO-Based Cell Lines.	+	+	-	-	-	-		CHO, Lec2 (no sialic acid), pgsA (no GAGs)
Supplemental Figure 2	Flow cytometric analysis of 3Fax- and neuraminidase-treated cells.	-	-	-	-	-	+	Lectins and HS20	ART and SFT
Supplemental Figure 3	SA-mutants are more sensitive to heparin inhibition than wild-type JCV or BKV.	+	+	+	-	-	-		SFT
Supplemental Figure 4	SA-mutants are more sensitive to inhibition by HS20 than wild-type JCV or BKV.	+	+	-	-	ART Only	ART Only		ART and SFT
Supplemental Figure 5	JCV and BKV pseudovirus transduction is unaffected by reduced sulfation.	+	+	+	+	+	-		A549
Supplemental Figure 6	Neuraminidase treatment of cells enhances SA-mutant infectivity.	+	+	+	-	+	-		SFT and ART (data not shown)
Supplemental Figure 7	siRNA suppression of sialylation enhances the infectivity of a PML-mutant pseudovirus on ART cells.	-	+	-	-	-	-	Lectins	ART; SFT not responsive to siRNA knowdown
Supplemental Table 1	Effect of 3Fax on various cell lines.	-	JCV2-269F Only	JCV3-55F Only	-	-	-		ART, 293TT, 293FT, SFT, SNBT, HSC

Supplemental Table 3. Experimental Summary Table. Related to all figures and tables. The table lists all reported experiments and summarizes which viruses and cell lines were used in each experiment.

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