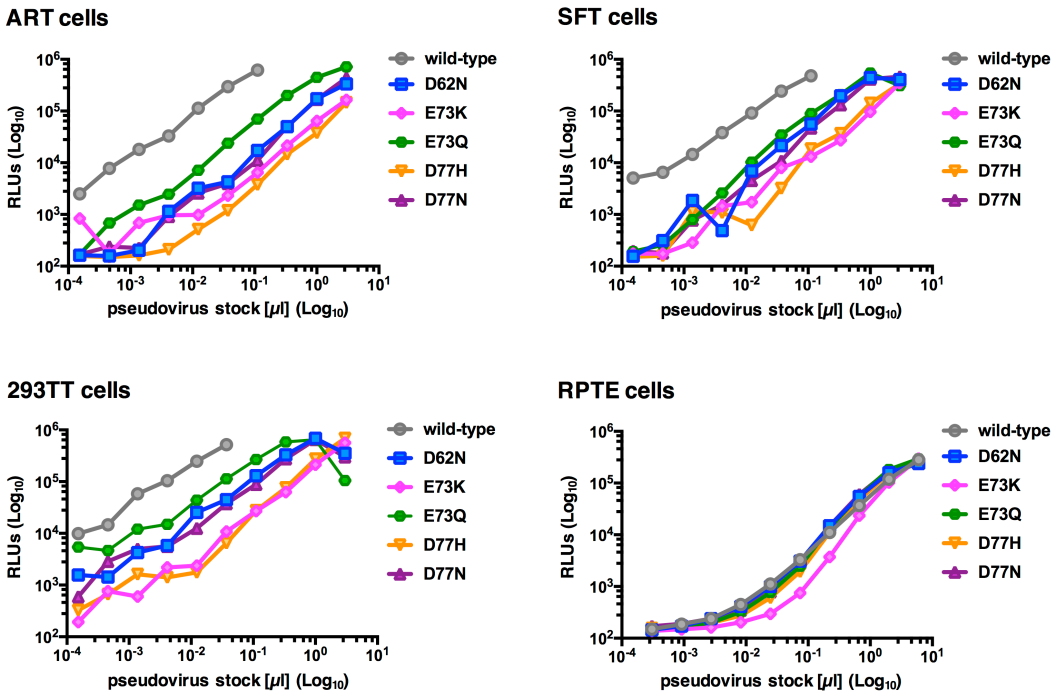

Figure S1, related to Figure 2. Titration of patient-specific BKV reporter pseudovirus stocks in the four cell lines used in neutralization assays.

To determine specific working dilutions, serial 3-fold dilutions of each stock were tested on each cell line under the same conditions used in neutralization assays. Luminometric reading was performed at a gain of 2200 after 72 h (ART, SFT and 293TT cells) or 6 days (RPTE cells).

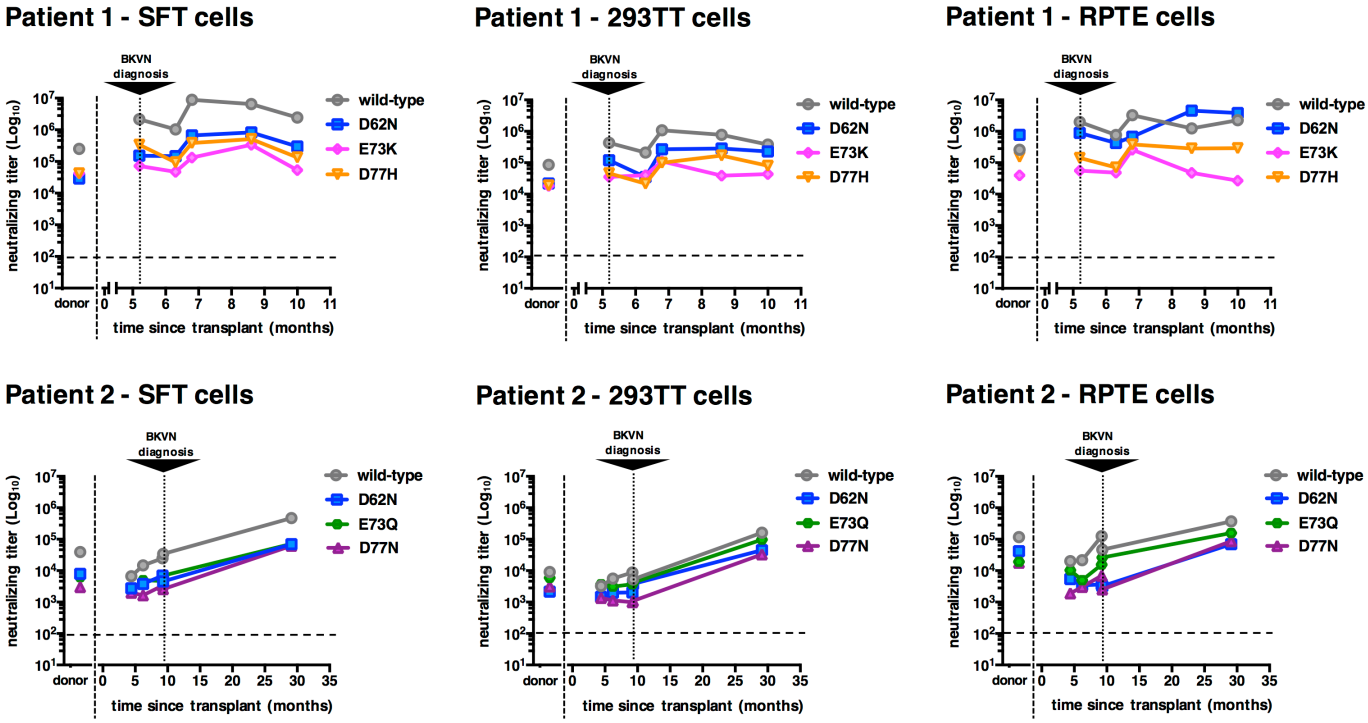

Figure S2, related to Figure 2. Neutralization patterns of patient-cognate VP1 BC loop variants.
Patient sera harvested at different time points after transplant (x-axis) and donor sera were serially diluted and tested using neutralization assays specific to the indicated patient-cognate variants. Neutralizing titers (EC_{50} values) for each serum are shown (y-axis). The results shown were obtained from experiments performed on three different cell lines.

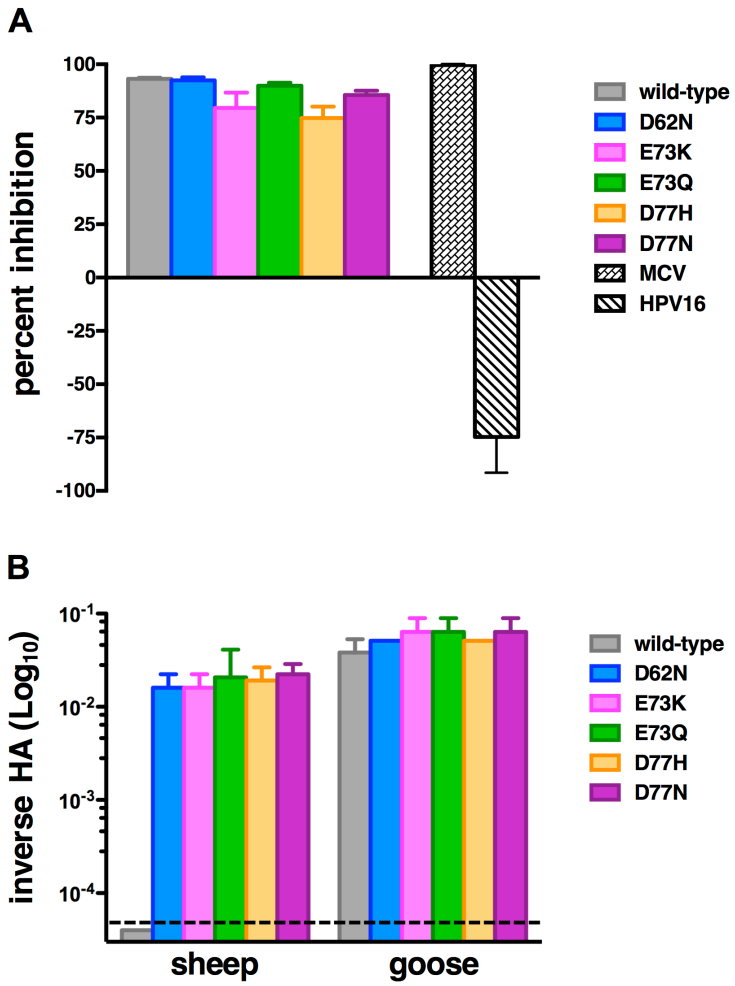

Figure S3, related to Figure 2. Relative glycan receptor binding properties of patient-specific VP1 BC loop variants.
(A) Effect of treatment with sialyltransferase inhibitor 3Fax on the transducibility of target cells with reporter pseudoviruses representing patient-specific variants. Pseudoviruses were applied to SFT cells cultured either with or without 3Fax. Results are expressed as percent inhibition, calculated on the basis of reporter signals measured after transduction of 3Fax-treated over mock-treated cells. Merkel cell polyomavirus (MCV), which is known to require sialylated glycan co-receptors for infectious entry (Schowalter et al., 2011), was used as a positive control for 3Fax activity. HPV16, which does not require sialylated glycans for infectious entry, was used as a control for cell health in the presence of 3Fax treatment. Results are an average of quintuplicate values. Error bars represent standard error of the mean (SEM). Similar results were obtained in two independent experiments.
(B) Hemagglutination patterns of pseudovirions representing patient-specific variants. Bars express, in Log_{10} scale, the inverse of the lowest amount (in ng/ml) of VP1 that hemagglutinated a 1% solution of sheep or goose red blood cells (RBCs). The dotted line indicates the highest VP1 dose tested (20,000 ng/ml). Results are an average of four independent experiments. Error bars represent standard error of the mean (SEM). Hemagglutination patterns similar to sheep RBCs were observed with llama RBCs and hemagglutination patterns similar to goose RBCs were observed with human, chicken and turkey RBCs. HA: hemagglutination.

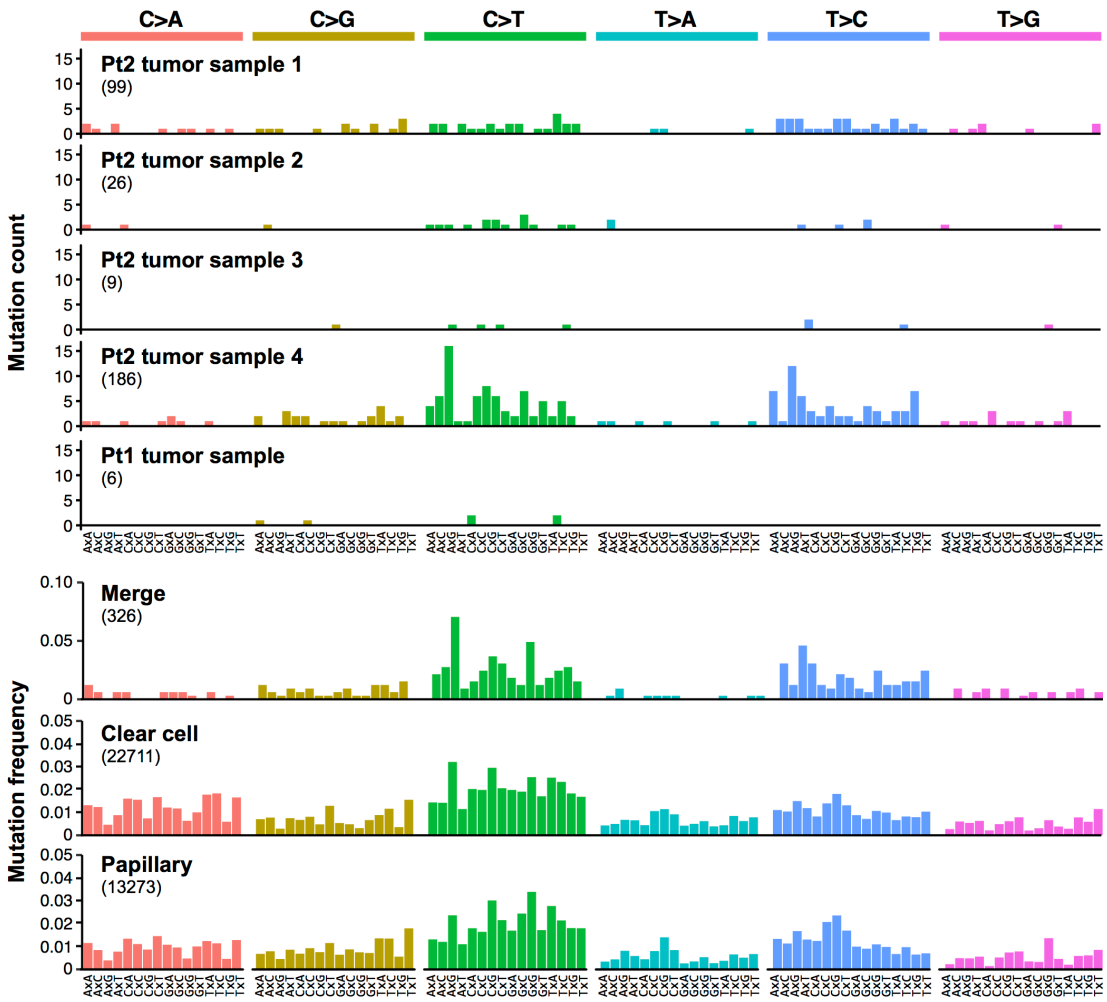

Figure S4, related to Figure 1. Mutational signature analysis of kidney tumor exome sequencing.
Exome sequencing was performed on tumor (clear cell renal cell carcinoma) or adjacent healthy tissue specimens from Patient 1 and Patient 2, respectively. The distribution of mutations for each analyzed tumor sample is shown (upper diagram). The merged mutation profile for all samples compared to broader groups of kidney tumors (clear cell and papillary renal cell carcinomas, respectively) previously reported in The Cancer Genome Atlas (TCGA) database is also shown for reference (lower diagram). The mutational spectrum of each tumor sample was deduced using the R package SomaticSignatures.

VP1 variant	ART RLUs X 10^3	SFT RLUs X 10^3	293TT RLUs X 10^3	RPTE RLUs X 10^3
wild-type	206.6	165.3	481.3	4.2
D62N	16.2	58.0	78.5	11.2
E73K	8.4	12.5	27.0	6.7
E73Q	50.7	42.1	117.5	5.6
D77H	2.8	12.1	22.5	6.4
D77N	13.3	45.6	65.3	5.9

Table S2, related to Figure 2. Relative infectivity of patient-specific BKV reporter pseudovirus stocks in the four cell lines used in neutralization assays, normalized to VP1 amount.

Luminometric values for indicated cell cultures treated with pseudoviruses standardized to 0.5 ng (ART, SFT and 293TT cells) or 20 ng (RPTE cells) of VP1 were interpolated using Prism Software (GraphPad) by fitting a linear (ART, SFT and 293TT cells) or non-linear (RPTE cells) regression curve to values from Figure S1.

VP1 variant	VP1 stock concentration (ng/µl)	working dilution	VP1 dose (ng/well)	ART RLUs x 10^3	SFT RLUs x 10^3	293TT RLUs x 10^3	RPTE RLUs X 10^3
wild-type (stock 1)	15	1:10,000	0.1	195.4 ± 14.2	38.9 ± 3.9	72.0 ± 7.7	NA
wild-type (stock 2)	200	1:10,000	1.6	NA	NA	NA	10.1 ± 1.0
D62N (stock 1)	6	1:300	1.6	259.9 ± 23.0	129.0 ± 7.5	240.5 ± 11.8	NA
D62N (stock 2)	100	1:10,000	0.8	NA	NA	NA	3.8 ± 0.6
E73K (stock 1)	4	1:300	1.1	111.8 ± 7.1	39.6 ± 5.6	78.2 ± 4.9	NA
E73K (stock 2)	70	1:4,000	1.4	NA	NA	NA	1.8 ± 0.3
E73Q (stock 1)	8	1:1,000	0.6	223.1 ± 11.5	59.4 ± 9.1	139.6 ± 3.3	NA
E73Q (stock 2)	150	1:10,000	1.2	NA	NA	NA	3.2 ± 0.5
D77H (stock 1)	6	1:300	1.6	87.0 ± 7.7	61.8 ± 5.5	65.1 ± 2.9	NA
D77H (stock 2)	120	1:8,000	1.2	NA	NA	NA	3.0 ± 0.4
D77N (stock 1)	6	1:300	1.6	266.3 ± 17.0	123.2 ± 6.8	253.6 ± 12.1	NA
D77N (stock 2)	150	1:10,000	1.2	NA	NA	NA	3.2 ± 0.4

Table S3, related to Figure 2. Concentration, amount and typical luminometric signals of patient-specific BKV reporter pseudovirus stocks used in neutralization assays.

Stock concentrations were determined by SDS-PAGE and protein gel staining. Reporter pseudoviruses alone ("no serum" control in neutralization assays) were applied to cells in the amounts reported and luminometric reading was performed at a gain of 2200 (ART, SFT and 293TT cells) or 3500 (RPTE cells) after 3 days (SFT and 293TT cells) or 7 days (ART and RPTE cells). Background luminometric values ("no pseudovirus" control in neutralization assays) typically ranged between 100 and 200 RLUs. Results are an average of ten replicate values from a single representative experiment.

	plasma/serum				urine			
Patient identifier	**BKV genotypes (abundance)**	**mutations (trinucleotide)**	**mutations (aminoacid)**	**mutations (abundance)**	**BKV genotypes (abundance)**	**mutations (trinucleotide)**	**mutations (aminoacid)**	**mutations (abundance)**
SS-01	PCR neg	NA	NA	NA	PCR neg	NA	NA	NA
SS-02	Ib2 (100%)	G1911C (TGA → TCA) [b] C2341T (GCT → GTT)	- A264T	~100% 2.8%	Ib2 (100%)	G1911C (TGA → TCA) [b]	-	~100%
SS-03	Ib2	-	-	-	PCR neg	NA	NA	NA
SS-04	PCR neg	NA	NA	NA	IV (100%)	G1953T (TGT → TTT)	Q134K	15.2%
SS-05	Ib2 (~85%) IV (~15%)	G1911C (TGA → TCA) [b] A2628G (TAG → TGG) C2635G (ACC → AGC) -	- - V362L -	68.9% 23.1% 33.3% -	PCR neg	NA	NA	NA
SS-06	Ib2 (~85%) IV (~15%)	G1911C (TGA → TCA) [b] -	- -	4.7% -	Ib2 (~95%) IV (~5%)	A2628G (TAG → TGG) C2635G (ACC → AGC) -	- V362L -	4.2% 4.4% -
SS-07	PCR neg	NA	NA	NA	PCR neg	NA	NA	NA
SS-08	Ib2 (100%)	G1797T (TGT → TTT) G2628A (TGG → TAG) [b] G2635C (AGC → ACC)	D82E [a] - L362V	2.8% 2.3% 2.3%	Ib2 (100%)	-	-	-
SS-09	PCR neg	NA	NA	NA	PCR neg	NA	NA	NA
SS-10	Ib2 (100%)	-	-	-	Ib2 (100%)	G2562T (TGC → TTC)	-	4.2%
SS-11	PCR neg	NA	NA	NA	Ib2 (100%)	G1797T (TGT → TTT) G2628A (TGG → TAG) [b] G2635C (AGC → ACC)	D82E [a] - L362V	23.5% 22.1% 20.9%
SS-12	Ib2 (~95%) IV (~5%)	C2219A (CCC → CAC) -	G223V -	2.4% -	Ib2 (100%)	-	-	-
SS-13	Ib2 (~95%) IV (~5%)	C2046A (GCA → GAA) A2628G (TAG → TGG) -	- - -	5.8% 10.6% -	PCR neg	NA	NA	NA
SS-14	PCR neg	NA	NA	NA	PCR neg	NA	NA	NA
SS-15	PCR neg	NA	NA	NA	PCR neg	NA	NA	NA
SS-16	Ib2 (~80%) IV (~20%)	G1911C (TGA → TCA) [b] A2628G (TAG → TGG) C2635G (ACC → AGC) -	- - V362L -	33.3% 32.3% 32.3% -	IV (100%)	-	-	-
SS-17	IV (100%)	T1909C (CTC → CCC)	E119G	4.7%	PCR neg	NA	NA	NA
SS-18	IV (~55%) Ib2 (~35%) Ib1 (10%)	T1909C (CTC → CCC) - -	IV: E119G - -	9.0% - -	PCR neg	NA	NA	NA
SS-19	Ib2 (100%)	G1811A (GGG → GAG)	P87L [a]	3.1%	PCR neg	NA	NA	NA
SS-20	PCR neg	NA	NA	NA	PCR neg	NA	NA	NA
SS-21	Ib1 (~50%) Ib2 (~50%)	- T1707A (TTA → TAA) G1911C (TGA → TCA) [b] A2628G (TAG → TGG) C2635G (ACC → AGC)	- - - - V362L	- 29.0% ~50% 2.9% 2.9 %	Ib1 (~95%) Ib2 (~5%)	- -	- -	- -
SS-22	PCR neg	NA	NA	NA	Ib2 (~95%) IV (~5%)	T1632C (GTA → GCA) T1707C (TTA → TCA) G1911C (TGA → TCA) [b] C2570T (TCT → TTT) [b] A2628G (TAG → TGG) C2635G (ACC → AGC) -	- - - R340K - V362L -	43.8% 9.5% ~100% 15.0% 35.0% 35.9% -
SS-23	Ib2 (~75%) IV (~15%) Ib1 (~10%)	T1707A (TTA → TAA) G1911C (TGA → TCA) [b] C2061T (TCC → TTC) - -	- - - - -	13.0% 20.4% 60.0% - -	Ib2 (100%) [c]	-	-	-
HF-01	Ib2 (100%)	C1768G (TCA → TGA) [b] C2061T (TCC → TTC)	E73Q [a] -	14.8% ~100%	NA	NA	NA	NA

[a] aminoacid substitutions mapping to the BC loop of VP1
[b] possible APOBEC3B signature (see main text)
[c] this sample revealed a mixture of reads specific to BKV-Ib2 (~35%) and JCV (~65%)

Table S4, related to Table 1. BKV genotypes and VP1 mutations revealed by deep sequencing of PCR products spanning the VP1 open reading frame from blood and urine samples of 24 kidney transplant patients.

Kidney transplant patients were diagnosed with BKV viremia (SS-01 to SS-23) or biopsy-proven nephropathy (HF-01). Overall, multiple genotypes were observed in 8 plasma samples and 3 urine samples. In cases where both plasma and urine samples from the

same patient yielded PCR products, genotypes were generally concordant between plasma and urine. In total, 10 distinct non-silent VP1 mutations were detected (8 mutations in genotype background Ib2 + 2 mutations in genotype background IV), distributed among 10 plasma/serum samples and 4 urine samples. At least some of these mutations have been previously reported (Boldorini et al., 2009; Krautkramer et al., 2009), and thus may represent recurrent mutational hotspots in VP1. Numbering for nucleotide positions is based on isolate FIN-10 (GenBank: AB260032) for genotype Ib2 and on isolate LAB-33 (GenBank: AB301097) for genotype IV, set with the start base of large T antigen open reading frame (lying on the negative DNA strand) as wrap point. A rough relative abundance is reported for genotypes and mutations as a percentage of reads.