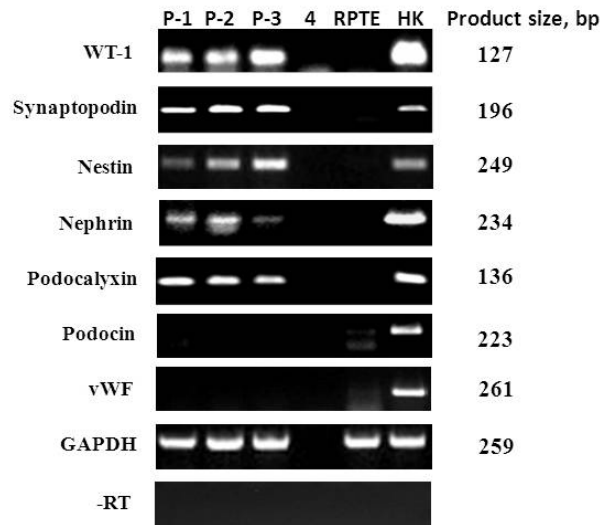


Supplemental Figure 1

A



B

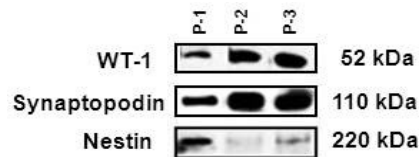


Figure S1 Characterization of podocyte markers by RT-PCR and Western blots in cultured podocytes harvested from the urine of children with HIVAN. A. Primary podocytes (P-1), and two podocyte cell lines (P-2 and P-3), generated from cells isolated from the urine of two children with HIVAN, were expanded and tested for the expression of podocyte markers by RT-PCR. Lanes loaded with water (4), RNA derived from human renal proximal tubular epithelial cells (RPTE), or a human kidney tissue sample (HK), served as negative and positive controls respectively. The mRNA expression of WT-1, synaptopodin, nestin, nephrin, and podocalyxin was detected in all podocyte clones (P1, P2, P3) and the HK, but not in RPTE cells. mRNA for podocin and the endothelial cell marker Von Willebrand factor (vWF), were detected only in the HK. GAPDH served as an internal control. Reaction for GAPDH without RT (-RT) was performed to rule out the possibility of genomic DNA contamination. B. Western blots showing WT-1, synaptopodin and nestin protein expression in the podocyte clones P-1, P-2, and P-3.

Supplemental Table 1 Pairs of primers used for the characterization of human podocytes in Figure S1.

Gene	Primer sets for RT-PCR	Expected size (bp)
WT1	Forward: 5'-ATAACCACACAACGCCCATC-3' Reverse: 5'-TCAGATGCCGACCGTACAAG-3'	127
Nestin	Forward: 5'-CTGGAGCAGGAGAAACAGG-3' Reverse: 5'-TGGGAGCAAAGATCCAAGAC-3'	249
Podocin	Forward: 5'-GGGAATCAAAGTGGAGAGAATAG-3' Reverse: 5'-CAGAGACTGAAGGGTGTGGAG-3'	223
Nephrin	Forward: 5'-CGCAGGAGGAGGTGTCTTATTC-3' Reverse: 5'-CGGGTCCAGAGTGTCCAAG-3'	234
Synaptopodin	Forward: 5'-GCCTGCCACTTTCTAGCATC-3' Reverse: 5'-TCTCTGGAGAAGGTGCTGGT-3'	196
Podocalyxin	Forward: 5'-CTCCCAGAGGAAGGACCAG-3' Reverse: 5'-CCCGTTGAGGCTGACCAC-3'	136
vWF	Forward: 5'-TGCCTCAAAGGGCTGTATC-3' Reverse: 5'-CACCCTGT TCTCCACTGCTC-3'	261
GAPDH	Forward: 5'-ACAGTCAGCCGCATCTTCTT-3' Reverse: 5'-GACAAGCTTCCCGTTCTCAG-3'	259

Supplemental Figure 2

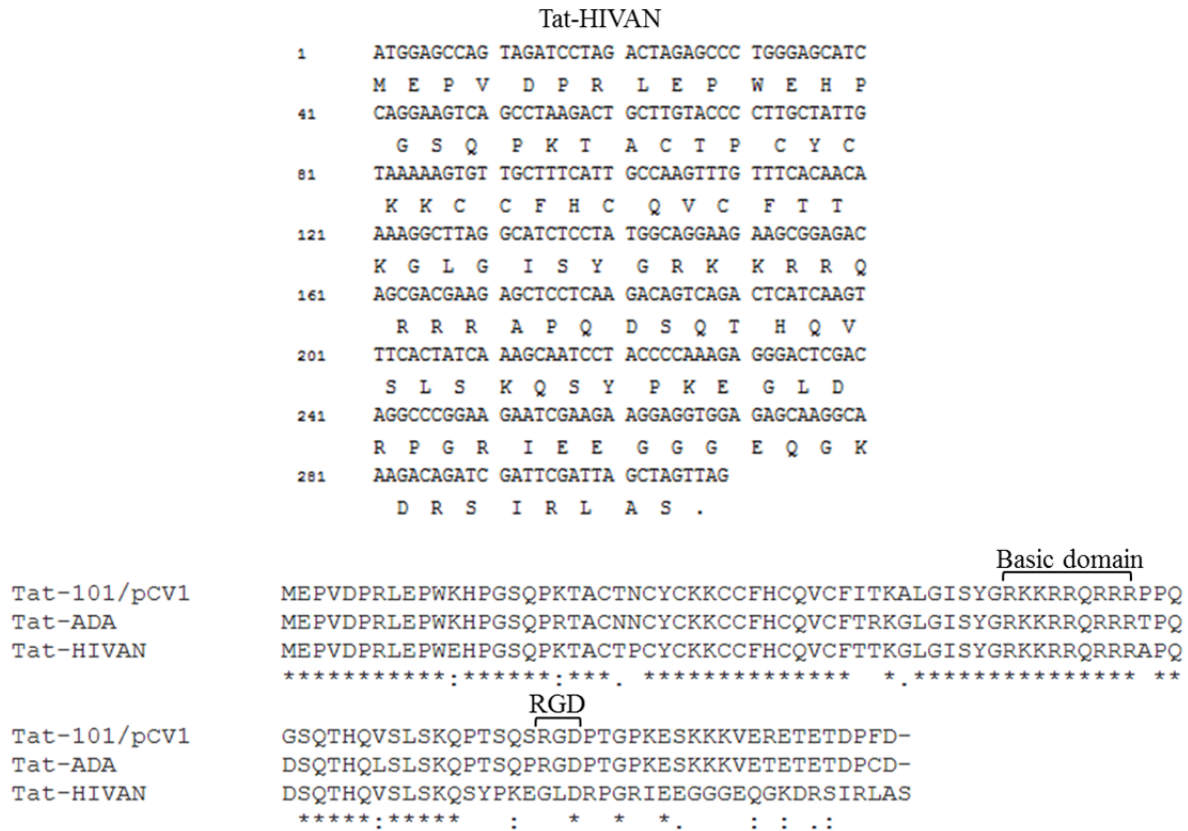


Figure S2 Sequence information corresponding to the *Tat* gene cloned from cultured renal epithelial cells infected with HIV-1, and used to generate the adenoviral Tat-WT vector. The upper panel shows the DNA sequence of the *Tat* gene cloned from cultured primary renal epithelial cells harvested from the urine a child with HIVAN, and infected with primary HIV-1 isolates corresponding to the same child, as previously described.²⁹ In the lower panel, the protein sequence of the Tat-HIVAN gene is aligned with other *Tat* genes, including pcDNA3.1-Tat(101), which was subcloned from pcDNA3.1/Tat101-Flag (PEV280, NIH AIDS Research and Reference Reagent Program), and originally derived from the HIV-1 cDNA clone pCV1, and the monocyte-tropic HIV-1 isolate ADA. We used the Clustal Omega multiple sequence alignment program (<http://www.ebi.ac.uk/Tools/msa/clustalo/>). The basic domain and RGD motif are indicated in brackets. The *Tat* sequencing procedure was repeated three times, to rule out the possibility of a sequencing error. Other *Tat* clones generated from the same cells, also showed a similar RGD motif deletion, suggesting that the Tat-HIVAN sequence is unlikely derived from an inactive quasispecies.

Supplemental Figure 3

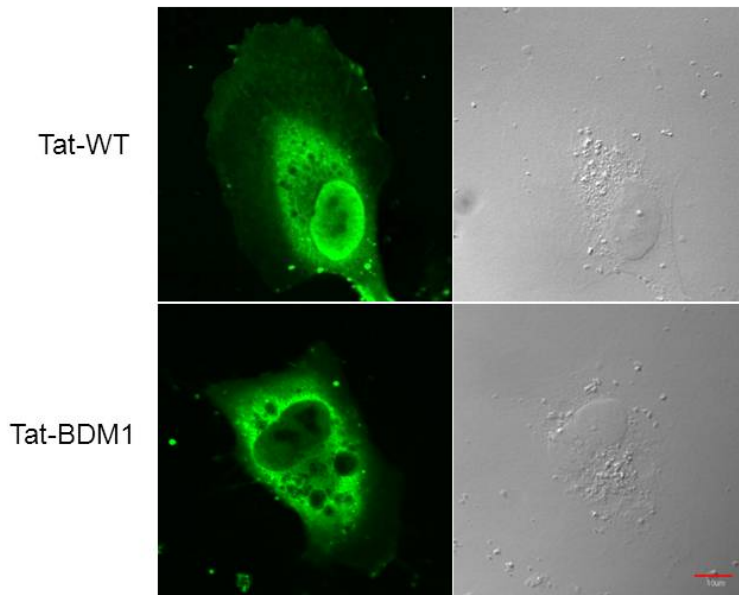


Figure S3 Translocation of Tat-HIVAN to the nucleus of cultured podocytes. Podocytes were transfected with cDNA vectors corresponding to either the wild type Tat-HIVAN, described in Figure S2, or Tat-HIVAN carrying a mutation in the basic domain (Tat-BDM1). Thirty six hours later, the cells were fixed with 4% paraformaldehyde, and stained with an anti-Flag antibody followed by anti-mouse IgG Alexa Flour 488, to detect Tat-Flag. The intracellular distribution of Tat was assessed by confocal microscopy. The left panels show the fluorescence microscopy studies, and the right panels show phase contrast microscopy. Tat-WT was detected in the cytoplasm and the nucleus of cultured podocytes, while Tat-BDM1 remained localized in the cytoplasm of these cells. These findings confirm that the wild type Tat-HIVAN can translocate to the nucleus of cultured podocytes, and demonstrate the critical role of the Tat basic domain in this process. The scale bar is 10 μm .

Supplemental Figure 4

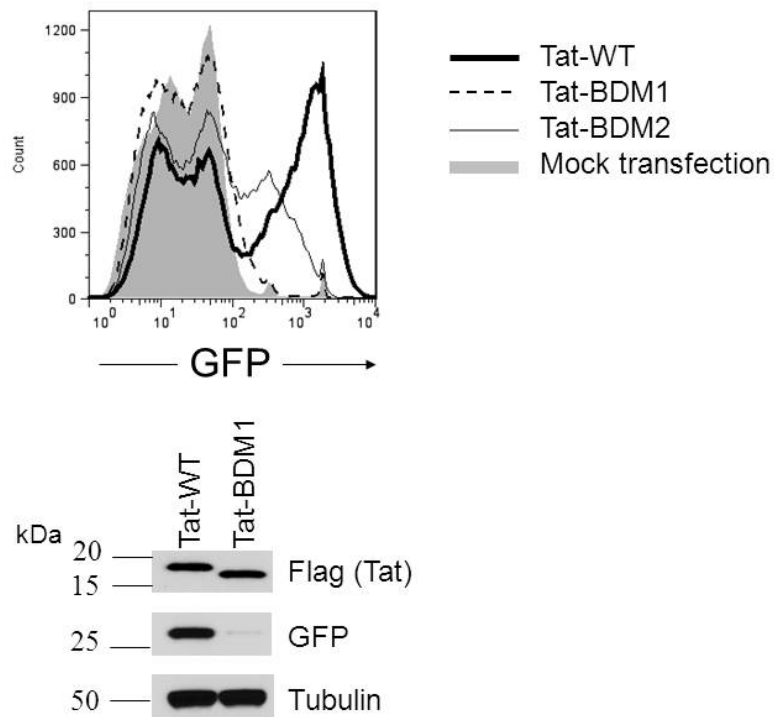


Figure S4 The wild type Tat-HIVAN, unlike the Tat-HIVAN carrying a mutation in the basic domain, induces transactivation of the HIV-LTR in GHOST cells. GHOST cells were transiently transfected with wild type Tat-HIVAN (Tat-WT) or Tat-HIVAN carrying mutations in the basic domain (Tat-BDM1 or Tat-BDM2). Thirty six hours later, all cells were harvested for FACS analysis (upper panel) or Western blotting (lower panel). As discussed in methods, in this reporter system, the expression of GFP is under the control of the HIV-LTR and can be used as readout system to assess the transactivation activity of Tat.

Supplemental Figure 5

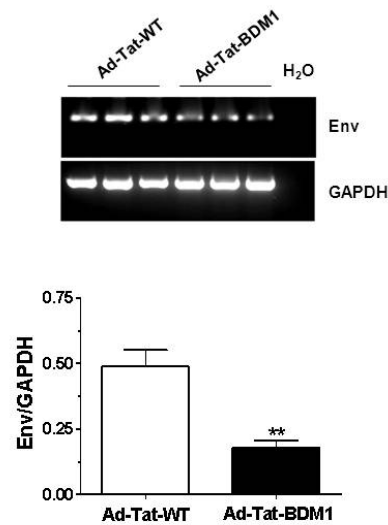


Figure S5 Induction of HIV-*envelope* (*Env*) gene expression in the kidney of young HIV-Tg₂₆ mice infected with Ad-Tat-WT. Newborn HIV-Tg₂₆ mice (n =3 mice per group) were infected with adenoviral vectors carrying wild type Tat-HIVAN (Ad-Tat-WT) or Tat-HIVAN carrying a mutation in the basic binding domain (Ad-Tat-BDM1) as previously described.³⁶ Seven days later, all mice were sacrificed, and their kidneys were harvested and processed for the RT-PCR studies. The upper panel shows representative RT-PCR results corresponding to HIV-*env* mRNA expression in the kidney of young HIV-Tg₂₆ mice infected with Ad-Tat-WT or Ad-Tat-BDM1. The graph summarizes the results corresponding to three mice per group (unpaired *t* test; ** p = 0.011).

Supplemental Figure 6

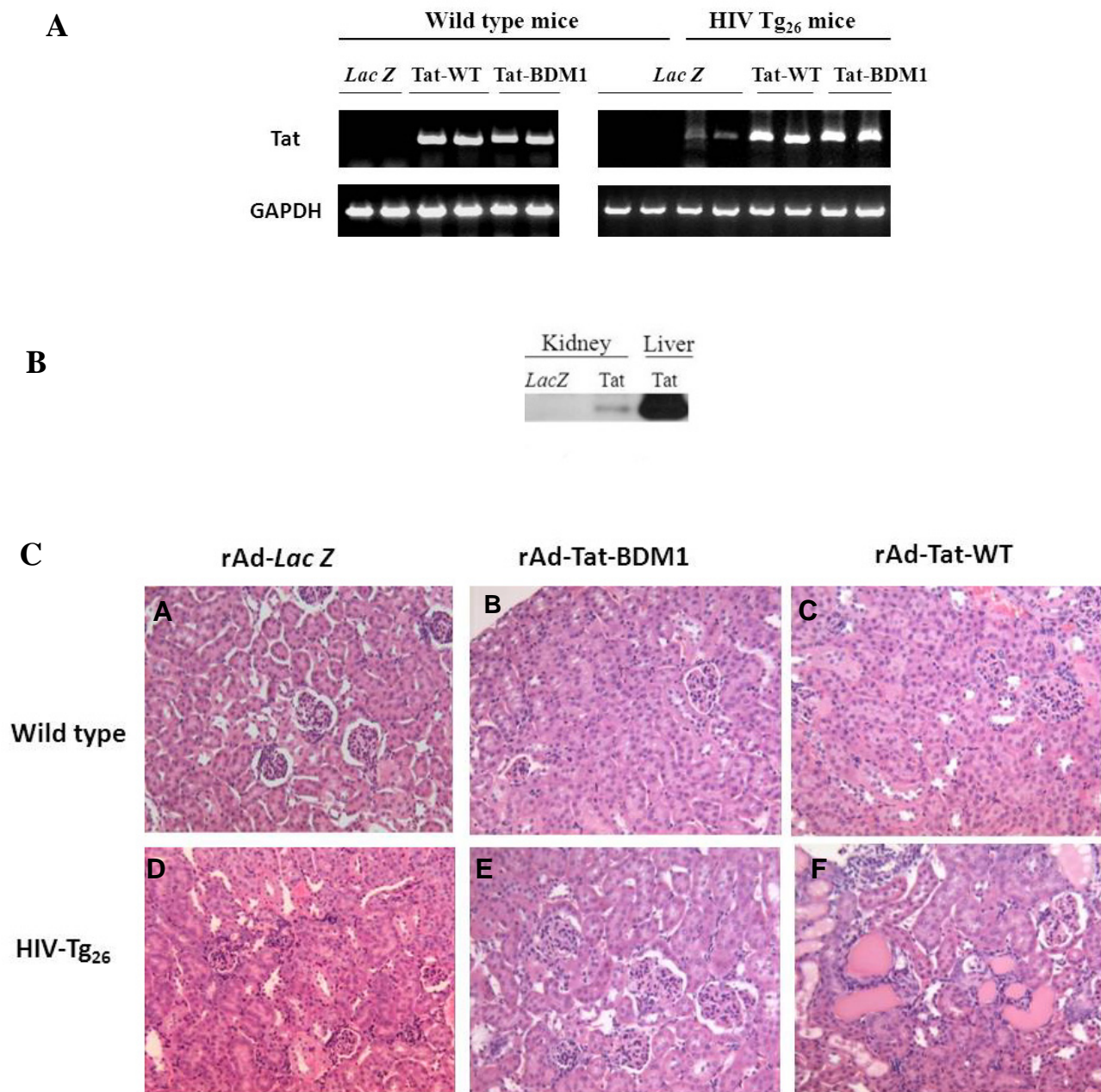


Figure 6S Adenoviral vectors carrying WT-Tat-HIVAN precipitated the development of HIVAN in young HIV-Tg₂₆ mice. Newborn heterozygous HIV-Tg₂₆ mice were infected with adenoviral vectors carrying

either, *Lac Z* (*rAd-LacZ*), Tat-HIVAN with a mutation in the basic binding domain (rAd-Tat-BDM1), or wild type Tat-HIVAN (rAd-Tat-WT) as previously described.³⁶ Seven days later, all mice were sacrificed, and the kidneys were processed for the RT-PCR studies. The upper panel shows representative RT-PCR results for Tat mRNA in kidney samples collected from wild type (WT) and HIV-Tg₂₆ mice infected with rAd-*LacZ*, rAd-Tat-BDM1, or rAd-Tat-WT (n = 4 - 8 mice per group). The 301 bp Tat amplification product shown in panel A, was sequenced to confirm the expression of Tat in the kidney. Panel B shows a representative Western Blot of the immunoprecipitated Tat protein expressed in the kidney and liver of WT mice infected with rAd-Tat-WT-Flag vector. Tat was detected with an anti-Flag antibody as described in the methods section. Panel C shows representative renal sections stained with hematoxylin and eosin. All mice were infected at birth with the corresponding adenoviral vectors and sacrificed at 35 days. At this time point, fifty per cent (4/8) of the HIV-Tg₂₆ mice infected with rAd-WT-Tat developed the complete renal histological HIVAN phenotype (F). These findings represent a significant acceleration of the timing for developing HIVAN in our HIV-Tg₂₆ mouse colony. In contrast, none (0/8*) of the HIV-Tg₂₆ mice infected with rAd-*LacZ* (D) or rAd-Tat-BDM1 (E) developed the complete renal histological HIVAN phenotype at this time point. In addition, none (0/12**) of the WT mice infected with rAd-*LacZ*, rAd-Tat-BDM1, or rAd-Tat-WT, showed significant renal histological lesions at 35 days of life. (Chi-square test, * p < 0.02; ** p < 0.006). (A-F, original magnification x 100)

Supplemental Figure 7

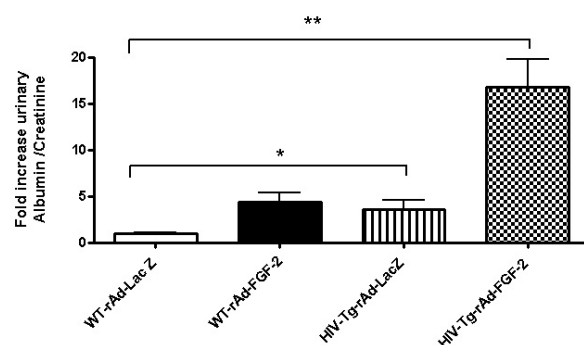


Figure7S Circulating FGF-2 induced albuminuria in wild type and HIV-Tg₂₆ mice. Wild type and HIV-Tg₂₆ mice (4 weeks old), without pre-existing renal disease were infected with adenoviral viral vectors carrying either a secreted form of human recombinant FGF-2 or the *Lac-Z* gene (n = 5 mice per group), as described in the methods section.³⁷ Urine samples were collected 28 days after the adenoviral infection. FGF-2 induced significant albuminuria in both WT and HIV-Tg₂₆ mice, although these changes were more significant in HIV-Tg₂₆ mice (* p< 0.01; ** p< 0.001, ANOVA).