SUPPLEMENTARY MATERIALS

METHODS

Vector production: A cosmid was constructed as described[14, 16] to contain the elements of the 29,234 bp HDAd-EBV.1xhRluc sequence diagrammed in Fig. 1a (sequence in Supplementary Materials). Cosmid DNA was digested with PmeI to free the HDAd vector DNA sequence from the bacterial plasmid backbone and purified by phenol/chloroform extraction and ethanol precipitation. 5 μg PmeI cut cosmid DNA, plus, to reduce any possible Cre expression, 5 μg of an anti-Cre shRNA expressing plasmid were co-transfected into two 10 cm plates of 293-FLPe6 cells[20] at 60-70% confluence using calcium phosphate precipitation (Profection kit, Promega, Inc.). The anti-Cre shRNA-expressing plasmid was constructed from the pQCXIN vector (Clonetech) by inserting GGCCCCCGGG ATCAGGGTTAAAGATATTTCAAGAGAATATCTACCCTGATCCCTTTTTG (upper strand) between the NotI and BamHI sties. 48 h later cells were infected with 1.5 $TCID_{50}$ units per cell of the FL-helper virus[20]. Vector was harvested when cytopathic effect was evident, ~48-72 h after infection with the FL-helper. Lysate from the two 10 cm plates prepared by freeze-thawing infected cells was applied to one well of a 6 well tissue culture dish (9.6 cm^2) of 293-FLPe6 cells that were co-infected with FL-helper virus as above. To reduce premature Cre-mediated recombination, the anti-Cre siRNA CGAGUGAUGAGGUUCGCA A + UUGCGA ACCUCAUCACUCG was transfected into these 293FLPe6 cells 48 h prior to infection, using the HiPerFect protocol (Qiagen, Inc.). When CPE was evident (~48-72 h after infection with the FL-helper virus), vector was harvested and the

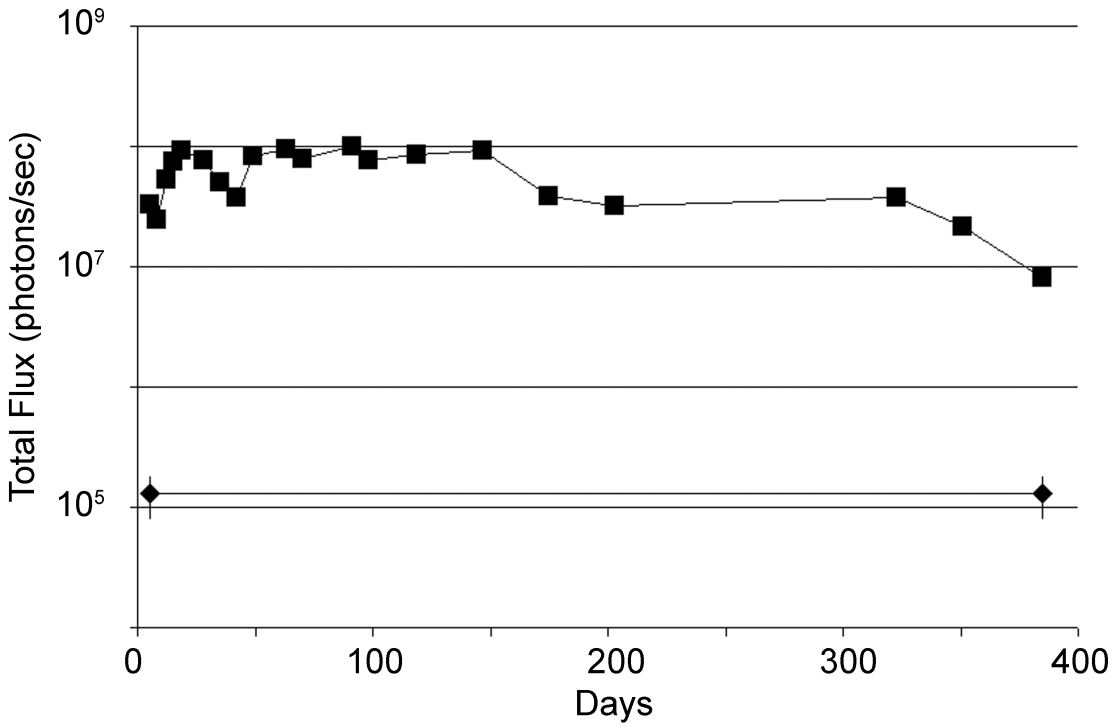
entire lysate was used to infect a fresh 9.6 cm^2 well of si-RNA-transfected 293-FLPe6 cells co-infected with FL-helper as above. Similar cycles of infection in 9.6 cm^2 wells of similarly treated 293-FLPe6 cells were repeated, and the yield of vector and helper from each cycle of infection was determined by infectious genome qPCR assay as described[14]. HDAd infectious DNA was generally not detected until the fifth cycle of infection. Cycles of infection were continued until a total titer of at least 2e7 infectious genome units (IGU) vector was produced. Vector was then expanded by infection of increasing numbers of 293-FLPe6 cells pre-transfected with anti-Cre siRNA at an MOI of 2-5 IGU/cell and simultaneous co-infection with the FL helper at an MOI of 1.5 $TCID_{50}$/cell. Pre-transfection with the anti-Cre siRNA continued until cell numbers reached 4e7, but not when larger numbers of cells were infected. Following two cycles of expansion in the absence of anti-Cre siRNA pre-treatment, vector was harvested from 2e9 293-FLPe6 cells and purified by two successive rounds of CsCl buoyant density step gradient centrifugation as descibed[14]. The collected band of vector virions was concentrated to 0.5 ml using an Amicon Ultra-4 Ultracel-100k centrifugal filter device (Millipore, Inc.), dialyzed into 10 mM Tris-HCl, pH 8.0, made 10% glycerol, frozen in liquid nitrogen, and stored at -80°C. The two HDAd vectors of the two-virus vector system, HDAd.Cre and HDAd-EBV.hRluc, were constructed and propagated as described earlier[14]. For each vector stock, the concentration of Infectious Genome Units (IGU/ml) was determined as described in detail in ref. 14. Briefly, duplicate confluent monolayers of HeLa cells were subjected to infection for 1 h at 37°C with 5 μl of stock diluted into media. Nuclei were isolated

from the infected cells three to six hours p.i., total nuclear DNA was isolated, the number of vector DNA molecules present determined by real-time quantitative PCR, and the number determined used to calculate vector IGU/ml[14].

Titers of vector stocks used in this study determined in this way are shown in the following table:

stock	titer by IGU/ml
immune compromised study	
HDAd-EBV.hRluc	3.3e10
HDAd.Cre	4.4e10
HDAd-EBV.1x.hRluc	6.1e9
immune competent study	
HDAd-EBV.hRluc	2.1e11
HDAd.Cre	3.3e10
HDAd-EBV.1x.hRluc	5.6e10

Animal studies: Animal treatment, vector administration, assay of *Renilla* luciferase expression in the liver by bioluminescence using a Xenogen (Alameda, CA) instrument, and isolation of DNA from liver and assay by real-time quantitative PCR (qPCR) were all as described in detail earlier[14].



Supplementary Figure 1: Extended luciferase expression by the single-virus vector in a C57Bl/6 mouse. A single C57Bl/6 female mouse was administered 1.8 x 10^9 IGU of the single-virus vector by tail vein injection, and luciferase activity in the region of the liver was assayed by bioluminescence at the indicated days post injection (squares). A control mouse that did not receive vector was assayed to establish the background signal in the assay (diamonds).

Supplementary Table 1: Number of nude mice administered the two-virus vector that were imaged at each time point.

week 1 - 9	n=16
week 10-12	n=15
week 13	n=14
week 14-16	n=13
week 17-18	n=12
week 19-21	n=11
week 22	n=10
week 23-24	n=9
week 25-26	n=8
week 27-30	n=6

Sequence of HDAd-EBV.1x.hRluc cosmid:

Hygromycin repeats in bacterial plasmid backbone (443-7605)

Inverted Terminal Repeat and Ψ-packaging sequence (7613-8064)

Bovine growth hormone polyadenylation site (8065-8281)

Cre recombinase coding region (8292-9363)

LoxP site (9364-9397)

Hepatocyte specific promoter (9427-10484)

hORI (10521-31765)

Family of Repeats (31767-32537)

SV40 polyadenylation site (32549-32947)

EBNA-1 coding region (32948-34905)

ECMV IRES (34906-35485)

Renilla luciferase coding region (35502-36438)

LoxP site (36684-36717)

Inverted Terminal Repeat (36721-36842)

Kanamycin resistance (36851-37781)

Cos (37782-38907)

ColE1 *ori* (38370-39250)

Ampicillin resistance (39314-40171)

CTGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGA
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