Supplementary materials

Supplementary Methods

Vector release kinetics

Thirty two pieces of stainless steel foil (approximately 7x7 mm) were modified with PABT, PEI-PDT, PrG, anti-AAV2 antibody and $AAV2_{iNOS}$ as specified in the Vector Immobilization Protocol section. The samples were PBS-washed and 4 foils (as 2 duplicates of 2 samples) were removed, placed in individual vials and stored at -20°C (zero time point samples). The rest of the foil coupons were incubated in 5% bovine serum albumin/DMEM with 70 rpm shaking at 37°C (release phase). On days 1, 2, 5, 9, 15, 21, and 31, the medium was aspirated, the foils were washed in PBS, and 2 pairs of foils were placed in respectively labeled vials and stored at -20°C. The release medium was then replenished and the release was resumed. The experiment was terminated at the 31-day time point.

The amount of $AAV2_{iNOS}$ vector genome associated with the foil samples at each time point was determined by Pico Green assay, as described above. The relative fluorescence intensity values were converted to the vector genome numbers using a calibration curve constructed with the serial dilutions of free $AAV2_{iNOS}$ of a known titer. The surface areas of individual samples were derived from their weights presuming a 50-µm thickness and a 7.95 g/cm^3 specific density of the foil material. Finally, the VG numbers were normalized by the surface area of individual foil coupons and plotted as a function of time.

Immunohistochemistry and dual immunofluorescence

Formalin-fixed, paraffin-embedded destented arterial sections were rehydrated and boiled in epitope retrieval buffer (pH 6) in a pressure cooker for 10 min, followed by permeabilization in 0.5% Tween-20/PBS for 15 min and blocking with 10% horse serum for 15 min. Primary antibodies (anti-DYKDDDDK Tag, Cell Signaling Technology #14793; anti-CD68, BioRad

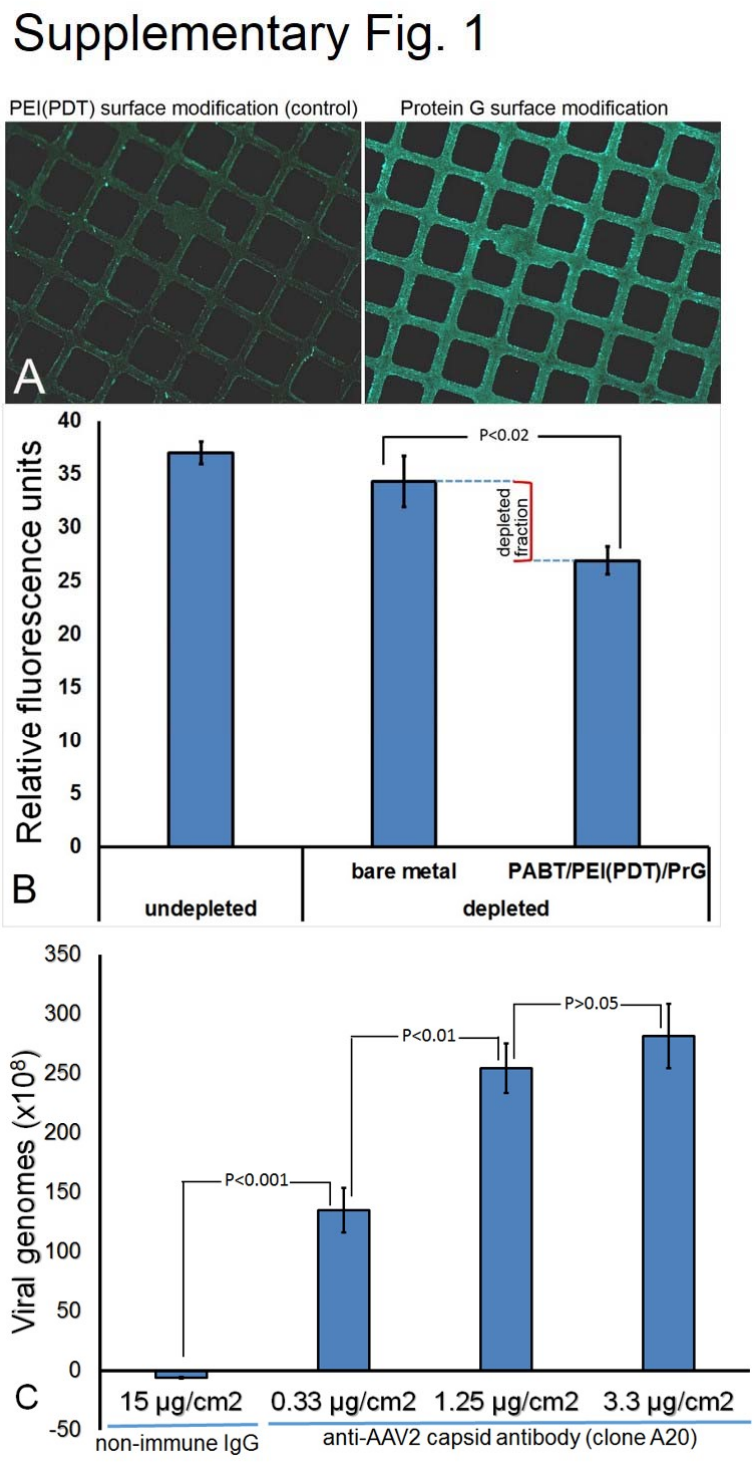
#MCA341) were applied in a 1:100 dilution at 4°C overnight. Secondary biotinylated anti-mouse (BA-2001) and anti-rabbit (BA-1100) antibodies from Vector Labs were used in a 1:100 dilution at room temperature for 1 hour, followed by the application of the ABC Vectastain system and ImmPACT DAB peroxidase substrate. The slides were further counterstained with Gill 3 hematoxylin.

For dual immunofluorescence staining, rabbit-made anti-FLAG antibody was applied to the slides concurrently with mouse-made anti-cell type marker antibodies (either anti-CD68, or anti-alpha smooth muscle actin), followed by co-treatment with secondary DyLight488-labeled anti-rabbit antibody and biotinylated anti-mouse antibody. Finally, the slides were incubated with DyLight549-labeled avidin and mounted.

PrG/Ab-mediated Ad vector immobilization and transduction

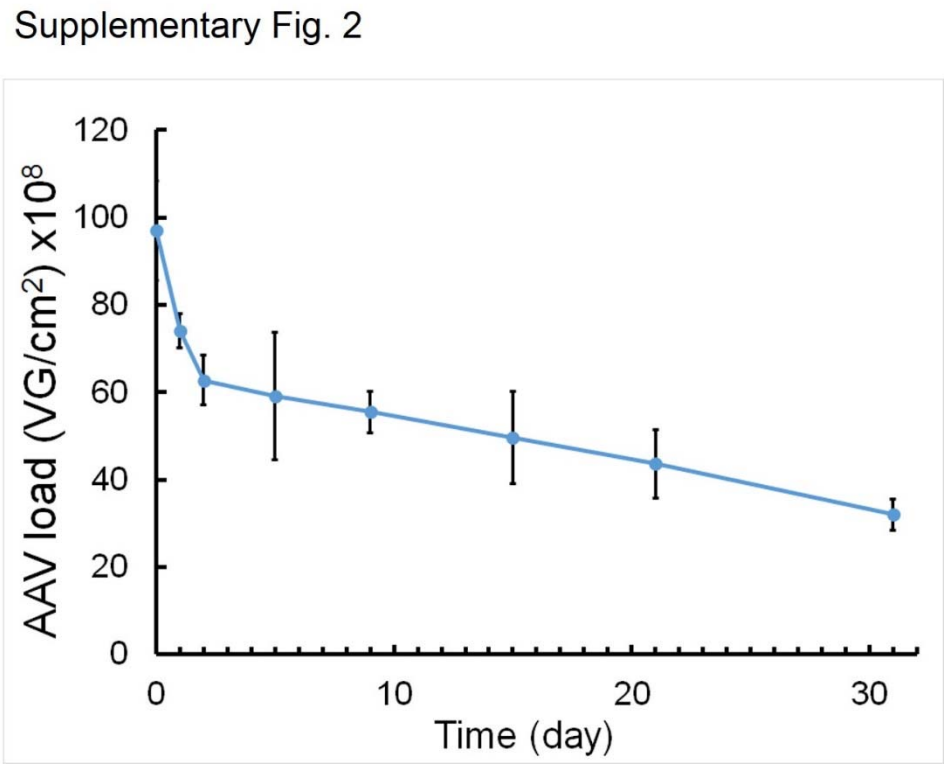
Stainless steel mesh disks were modified with PABT, PEI-PDT, PrG followed by anti-Ad hexon antibody as delineated in the Vector Immobilization Protocol section, prior to the exposure of the samples to $5x10^{10}$ plaque forming units of Ad_{eGFP}. The resulting vector loading on the meshes amounted to $1.2x10^{8}$ VG of Ad_{eGFP}, as determined by qPCR. Control meshes were formulated omitting anti-Ad hexon antibody attachment step and were exposed to the same Ad_{eGFP} suspension as the properly formulated samples. Following PBS washing, the meshes were individually placed in the wells of 96-well plates with subconfluent cultures rat aortic smooth muscle cells. Transduction was assessed 3 days after mesh placement by fluorescence microscopy and fluorimetry.

Supplementary Figure 1. The effects of PrG and AAV2-binding antibody on AAV2 immobilization on model metal surfaces.



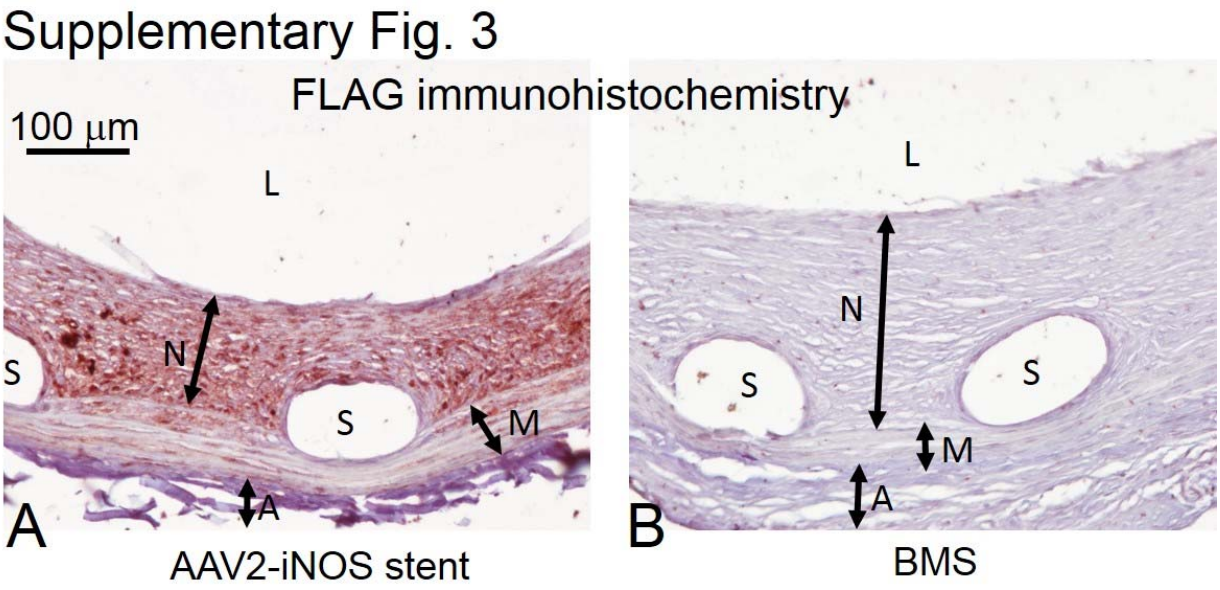
Functionalization of the metal surface with protein G is essential for the subsequent attachment of AAV2-binding Ab as determined by A) fluorescence microscopy (100x original magnification; FITC filter set) of the stainless steel mesh disk treated (right) or untreated (left) with thiolated protein G prior to the exposure to FITC-labeled mouse anti-rabbit antibody ((#31584 Thermo Fisher Scientific; 10 µg/ml in BSA) and B) fluorimetry (485/538 nm) of an undepleted solution of FITC-labeled mouse anti-rabbit antibody 100 ng/ml in 1% BSA) or the same antibody solution depleted with protein G-functionalized and bare metal foil samples. C) The effectiveness of AAV2 immobilization as determined by Pico Green assay was shown to be dependent on the amount of antibody provided in the reaction with the PrG-functionalized surface.

Supplementary Figure 2. Release kinetics of the vector *in vitro*



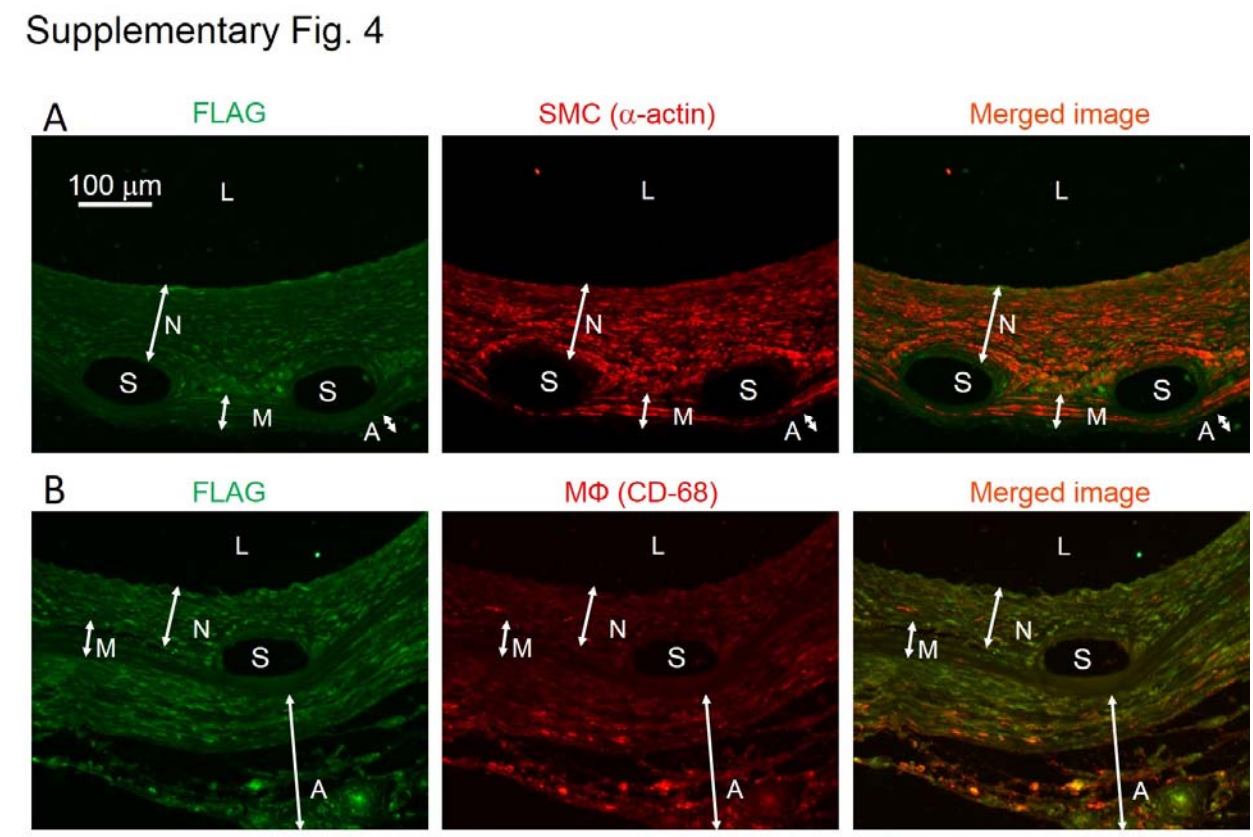
Residual amount of AAV2$_{iNOS}$ vector genomes associated with PrG/A20-tethered foil coupons following incubation in 5% BSA/DMEM at 37°C with shaking (70 RPM) plotted as a function of time.

Supplementary Figure 3. Immunohistochemical detection of FLAG epitope expression in the arteries treated with AAV2-iNOS stents



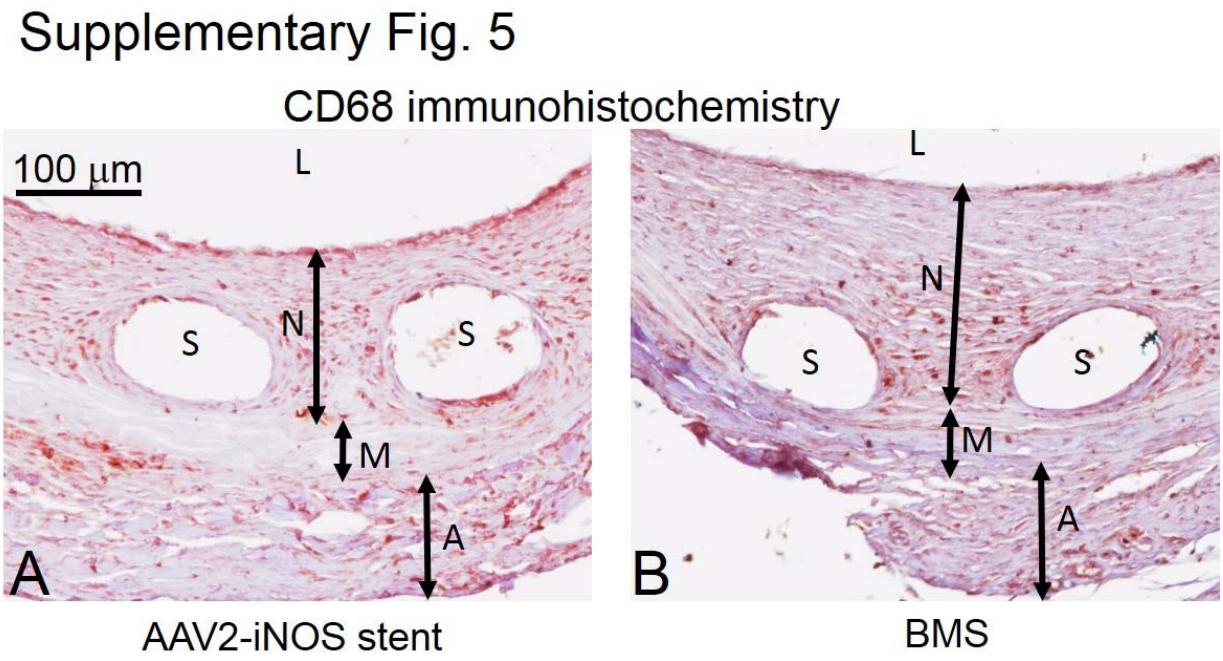
Representative arterial sections from animals treated with AAV2-iNOS stent (A) and BMS (B) for 14 days. Destented sections were stained using an anti-DYKDDDK (FLAG) tag antibody that reveals the FLAG epitope co-expressed with human iNOS. DAB was used to detect peroxidase activity. L, N, M, A and S denote lumen, neointima, media, adventitia and spaces left by dissolved stent struts, respectively. Original magnification is 200x.

Supplementary Figure 4. Dual immunofluorescence for detection of transduced cell types in AAV2-iNOS stent-treated artery



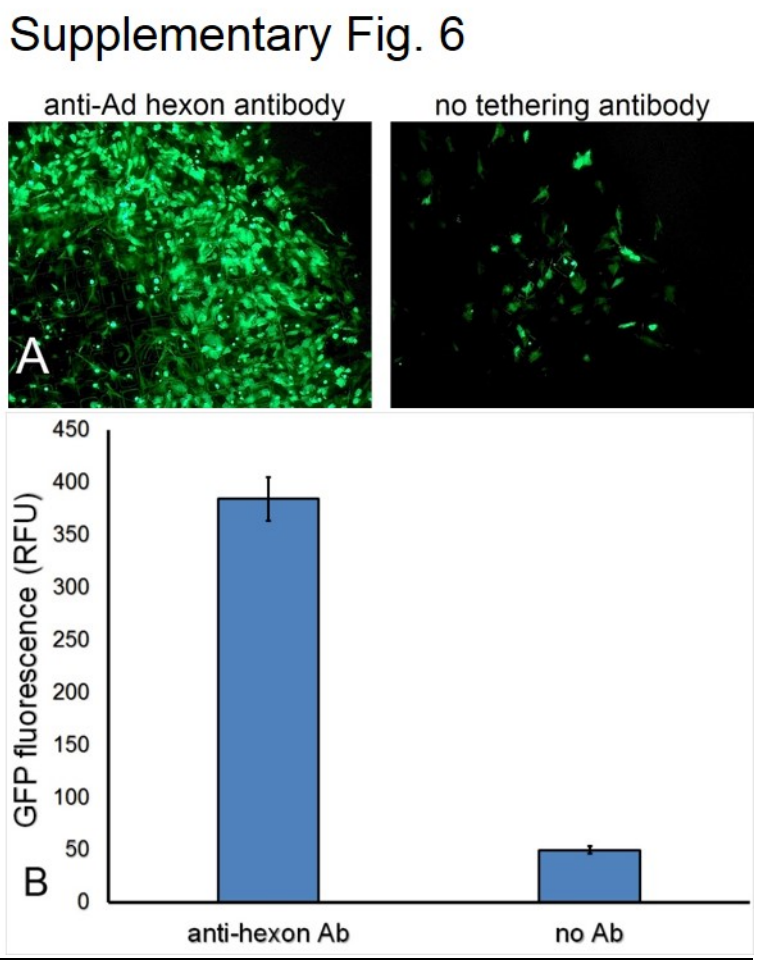
Representative immunofluorescence results obtained by co-staining arterial sections from AAV2-iNOS GDS-treated animals with anti-FLAG antibody and either anti-SMC (A) or anti-macrophage (B) antibodies. DyLight488-labeled anti-rabbit antibody (left column) and biotinylated anti-mouse antibody followed by DyLight549-labeled avidin (middle column) were used to label transduced cells. The cells binding both types of antibodies appear orange in the merged image (left column). L, N, M, A and S denote lumen, neointima, media, adventitia and spaces left by dissolved stent struts, respectively. Original magnification is 200x.

Supplementary Figure 5. Macrophage prevalence in stented carotid arteries of rats treated with AAV2-iNOS GDS and BMS



Representative images of CD68 immunohistochemistry of AAV2-iNOS stent-treated (A) and BMS-treated (B) rat carotid arteries showing comparable prevalence of CD68-positive macrophages in the neointima, media and adventitia of stented vessels. L, N, M, A and S denote lumen, neointima, media, adventitia and spaces left by dissolved stent struts, respectively. Original magnification is 200x.

Supplementary Figure 6. Cell transduction with mesh-immobilized Ad_{eGFP} tethered via PrG/anti-Ad hexon antibody pair.



(A) Representative fluorescence microscopy images and (B) quantitative fluorimetric data of primary rat aortic smooth muscle cells transduced with Ad_{eGFP} vector immobilized on bare stainless steel meshes with PrG/anti-Ad hexon tether (A, left) or with similarly Ad_{eGFP}-treated meshes formulated without the tethering antibody. Original magnification is 200x.