Supporting Information

Hofmann *et al.* 10.1073/pnas.0803746106

SI Materials and Methods

Lentiviral Vector Production. The SIN lentiviral vector (CMV-EGFP) (1) was cloned from the previously described PGK-EGFP vector (2). Recombinant lentivirus (3) and fluorescent lentiviral particles (pCHIV.eGFP) (4) were produced as previously described. The titer of virus preparations was measured in 2 ways to obtain the biological and physical virus titer. For determination of the biological virus titer, HEK293T cells were infected by a serial dilution of the virus preparation, the number of EGFP-positive cells was quantified by flow cytometry, and the quantity of infectious particles was calculated. To determine the physical virus titer, the content of reverse transcriptase in the virus preparation was measured by ELISA (Roche). For experiments with the CMV-EGFP vector, we used 50 infectious virus particles (biological virus titer) per cell for standard infections. For fluorescent lentiviral particles, we measured the physical virus titer of these preparations by ELISA and compared the values to the physical virus titer of CMV-EGFP preparations.

MNP-Assisted Lentiviral Transduction of Cells. HUVECs (no. 354151; BD Biosciences) were cultured in endothelial cell growth medium (C-22010; PromoCell). CM and TM nanoparticles were obtained from Chemicell. The standard LV/MNP mixture, which contained a relative amount of ≈50 LVs per cell and ≈27.6 pg of MNPs per virus particle (equal to ≈1,380 pg of MNPs per cell), was incubated in a total volume of 800 μL of HBSS (no. 14025–050; Gibco) for 20 min at room temperature and applied to the cells. For hypothermic transduction, cells were positioned onto magnetic plates (Chemicell) and placed into a refrigerator for 30 min. For transduction under hydrodynamic flow stress, a single NdFeB magnet (1-mm diameter, 10-mm length) was positioned central under the plate during transduction. Thereafter, cells were washed with PBS and cultured for 3 days.

MNP-Assisted Lentiviral Transduction of Aortas. Aortas of 8-week-old mice were cut into small squares (≈2 × 2 mm) and pinned with needles onto 24-well plates (with the endothelial layer to the top). In a total volume of 800 μL of HBSS, 52.5 μg of MNPs were incubated with ≈1×10^8 LVs for 20 min at room temperature. Then, the mixture was applied to the wells, and an NdFeB magnet (5-mm diameter, 14-mm length) was placed under the aortas. After incubation for 30 min at 4 °C, the supernatant was removed, and aortas were cultivated in DMEM [no. 41965; (Gibco) supplemented with 20% FCS] for 6 days at 5% CO_2 and 37 °C.

Ex Vivo Perfusion of Mouse Aortas. Descending aortas of 6- to 8-week-old mice were attached to cannulae on both sides. Intercostal arteries were cauterized. Perfusion was performed at room temperature with 12 mL of DMEM by using a peristaltic pump (Minipuls 3; Gilson) at a mild pressure of ≈30 mmHg with a flow rate of 4–5 mL/min. A total of 33.75 μg of MNPs was mixed with 1.7×10^8 LVs in a total volume of 800 μL of HBSS and incubated for 20 min at room temperature. During perfusion (30 min), 2 small NdFeB magnets (5-mm diameter, 14-mm length) were placed in close proximity to one side of the aorta (Fig. 2*A*). Then, aortas were cultured for 7 days in DMEM (supplemented with 20% FCS) at 5% CO_2 and 37 °C.

Magnetic Positioning of HUVECs. LV/MNP-transduced HUVECs were trypsinized 48 h after transduction and transferred to 1.5-mL reaction tubes, and a magnet was placed at one side (MagnaRack; Invitrogen). After 15 min, the supernatant was removed, and the adhered cells were quantified by sulforhodamine B stainings. For cell positioning, transduced HUVECs were transferred to 6-well plates and placed onto an aluminum plate containing magnets at defined positions (Fig. S1). The complete setup was placed onto a compensator (at 5% CO_2) and cultured overnight while being shaken slowly (20 rpm). The next morning, cells were stained with sulforhodamine B. For the positioning of HUVECs to vessels, small aortic squares (≈2 × 2 mm) were pinned onto 6-well plates, and LV/TM-transduced HUVECs were added. Small NdFeB magnets (5-mm diameter, 14-mm length) were positioned under the aortas, and the complete setup was placed onto a compensator (at 5% CO_2) and cultured overnight while being shaken slowly (20 rpm). For the positioning of HUVECs to vessels by ex vivo perfusion, LV/TM-transduced HUVECs were introduced into the perfusion system. After 30 min, the perfusion was stopped. The aortas were cut off the cannulae and cultured in HUVEC medium for 24 h at 5% CO_2 and 37 °C to allow for attachment of the cells.

Western Blot Analyses. HUVECs were lysed in protease-inhibiting solution (0.5% Triton X-100, 150 mM NaCl, 2 mM $CaCl_2$, and protease inhibitors). EGFP levels were quantified as described previously (5).

Imaging of Cells and Tissues. Images were taken with inverted microscopes (*i*) Axiovert 200 (Zeiss) equipped with a slider module (ApoTome), and (*ii*) DMIL 4000 (Leica), and a stereomicroscope (MZ 16F; Leica) equipped with EGFP filter (GFP3 Ultra Leica MZ16 F/FA).

Magnetorelaxometry. HUVECs were fixed with 4% paraformaldehyde, resuspended in PBS, and transferred to polystyrene microtiter vials. In addition, a serial dilution of MNPs was freeze-dried and used as standard curve to quantify the amount of MNPs per cell. Magnetorelaxometry was performed as described previously (6). In brief, the magnetic moments of MNPs are first polarized by a static magnetic field, and after switching off the magnetizing field the relaxation of the magnetic moment is measured by a superconducting quantum interference device. The amount of incorporated MNPs is given by the initial relaxation amplitude. Because the relaxation curves of freeze-dried MNP reference samples and LV/MNP-transduced cell samples showed similar shapes, we conclude that the MNP size distribution was not significantly altered in the cell samples.

Calculation of Magnetic Flux Density. The magnetic flux density fields were calculated by means of numeric field simulation with the CST EM Studio software (CST).

Prussian Blue and Sulforhodamine B Staining of Cells. For Prussian Blue stainings, a mixture of 2% hydrochloric acid and 2% potassium hexacyanoferrat-(II) solution (1:1) was applied to the cells for 1 h. For sulforhodamine B stainings, cells were fixed with 10% trichloric acid for 1 h at 4 °C, incubated for 30 min at room temperature with 0.4% sulforhodamine B solution (in 1% acetic acid), washed 3 times with 1% acetic acid, and dried. For the quantification of cells, the residual blue dye was solved in Tris solution (10 mM, pH 10.5) and measured in a microplate photometer (Tecan) at 570 nm.

Immunohistochemistry. Cryosections (10 μm) were permeabilized with 0.2% Triton-X (Roth) and blocked with 5% donkey serum (Vector Laboratories). Primary antibodies anti-CD31murine (rat, 1:800; PharMingen) and anti-CD31human (mouse, 1:300, BioGenex) were incubated for 2 h; secondary antibodies anti-mouse-IgG (Cy3-coupled, 1:400; Dianova) and anti-rat-IgG (Cy3-coupled, 1:400; Dianova) were incubated for 1 h at room temperature. For nuclear staining, Hoechst-33258 (1 μg/mL; Sigma) was applied to the sections for 15 min.

Calculation of EGFP and CD31 Colocalization. To quantify the extent of colocalization between EGFP and human CD31 in the images of Fig. 4 *B* and *D*, the corresponding fluorescence pictures were overlaid and analyzed by the "histogram" function of Photoshop CS2 Software (Adobe). First, the EGFP-positive spots in each image were enframed, and the amount of green signal in the defined areas was measured. Then, the human CD31-positive spots in the same picture were enframed, and the amount of green signals in these areas was also measured. Thereby, 2 parameters were determined in each area: the total size of the area (measured in pixels) and the green intensity (measured as green/pixel). To calculate the total amount of green signal, these factors were multiplicated for each area (size area $\times$ green intensity), and the resulting values of all areas were summarized. Finally, the degree of colocalization between EGFP and human CD31 was evaluated by comparing the total amount of green signal in the EGFP-positive spots versus the amount of green signal in CD31-positive (red) spots per image.

Analysis of Cell Seeding Efficiency. Cells were transduced with LV/TM and LV/CM complexes and transferred to new cell culture plates 24 h after transduction. After an additional 24 h, the number of attached cells was quantified by sulforhodamine B stainings.

Analysis of Cytotoxicity. At 24 h after transduction with LV/TM and LV/CM complexes, the viability of the cells was measured by the WST-1 cell proliferation reagent (Roche) according to the manufacturer's instructions. In addition, potential cytotoxic effects of the used nanoparticles were measured in LV/TM- and LV/CM-transduced cells at the same time point by an LDH release assay (Roche).

In Vivo Targeting of Lentiviral Vectors. A total of 100 μg of MNPs was incubated with $\approx 2 \times 10^8$ LVs in a total volume of 500 μL of HBSS for 20 min at room temperature. LV/CM complexes were injected via catheter (that was advanced toward the aortic arch) into the carotid artery of anesthetized (isoflurane) and intubated mice. To analyze the natural biodistribution of LV/CM complexes in vivo, the mice (n = 3) were killed 6 days after injection, and the organs (lung, liver, spleen, heart, and kidney) were harvested for DNA extraction and quantitative real-time PCR analyses. To alter the native biodistribution, strong NdFeB magnets [N42 cuboid (40 $\times$ 40 $\times$ 20 mm)/N45 cuboid (60 $\times$ 60 $\times$ 30 mm) + N45 disk (45 $\times$ 30 mm)] were placed at the right abdominal wall close to the liver during LV/CM injection and were kept in place for 30 min (n = 5).

Real-Time PCR Analyses and Calculation of Biodistribution. Genomic DNA from transduced cells was isolated by using classical phenol/chloroform extraction methods. To isolate genomic DNA from transduced organs, the tissues were completely homogenized, and 30–50 mg of the homogenate was used for extraction. To quantify the number of viral integrants per genome, quantitative real-time PCR analyses were performed as described previously (7). In brief, we used a 6-FAM-labeled probe for the integrated lentiviral DNA and a Texas Red-labeled probe for the Burkitt lymphoma receptor 1 (BLR1) gene (7). For HUVECs, we used human BLR1 primer/probe sets (forward, TCCTCTCCATCCACATCACC; reverse, GCGTTTCTGCTTGGTTCTC; probe Texas Red-AACAACTCCCTGCCACGTTGCACCTT-BHQ1). The absolute copy number per genome was calculated by comparison of the cycle threshold (ct) values from the virus-specific probe versus the probe of the BLR1 single-copy gene (standard with 1 copy per genome per cell). To calculate the in vivo biodistribution of lentiviral vectors in the organs of mice, the measured copy numbers per genome per cell were normalized to the relative weight of the different organs. Finally, the total values of the organs analyzed were added and set to 100% to calculate the relative distribution of integrated vectors in single organs.

In Vivo Positioning of HUVECs. To position MNP-labeled cells in noninjured murine vessels in vivo, $\approx$80,000 LV/TM-transduced HUVECs were injected into the carotid arteries of mice via a catheter (that was advanced toward the aortic arch). Small NdFeB magnets (1-mm diameter, 10-mm length) were positioned close to the abdominal aorta (3 magnets) or iliac artery (2 magnets). After 20 min, the vessels were sealed and dissected free of connective tissue. Subsequently, the presence of EGFP-expressing cells in vessels was analyzed by a fluorescence stereomicroscope.

Arterial injury model: Carotid artery injury was induced as described previously (8). In brief, the bifurcation of the left carotid artery was exposed via a midline incision of the ventral side of the neck. After temporary occlusion of the external carotid artery, internal carotid artery, and common carotid artery (CCA) with ligatures, a transverse arteriotomy was performed by passing a 0.13-mm curved flexible wire 3 times along the CCA in a rotating manner. After restoration of the blood flow, LV/MNP-transduced HUVECs ($\approx$40,000 in 400 μL of medium) were injected into the external carotid artery while 3 small magnets (1-mm diameter, 10-mm length) were placed in close proximity to the ventral side of the CCA. Ten minutes after restoring blood flow, in vivo EGFP expression was monitored by using a fluorescence stereomicroscope. Control experiments were performed under the same conditions without placing magnets at the CCA.

1. Pfeifer A, *et al.* (2001) Transduction of liver cells by lentiviral vectors: Analysis in living animals by fluorescence imaging. *Mol Ther* 3:319–322.
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8. Lindner V, Fingerle J, Reidy MA (1993) Mouse model of arterial injury. *Circ Res* 73:792–796.

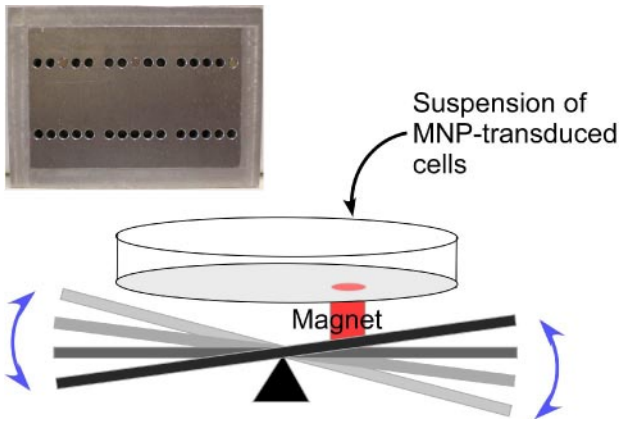

Fig. S1. Setup for nanomagnetic cell positioning on 6-well plates. To position small magnets at defined areas under a 6-well plate, a specially designed aluminum plate with drill holes was used (*Upper*). The cell culture dish was put on top of the aluminum plate, and both were placed onto a compensator, which was shaking slowly (*Lower*).

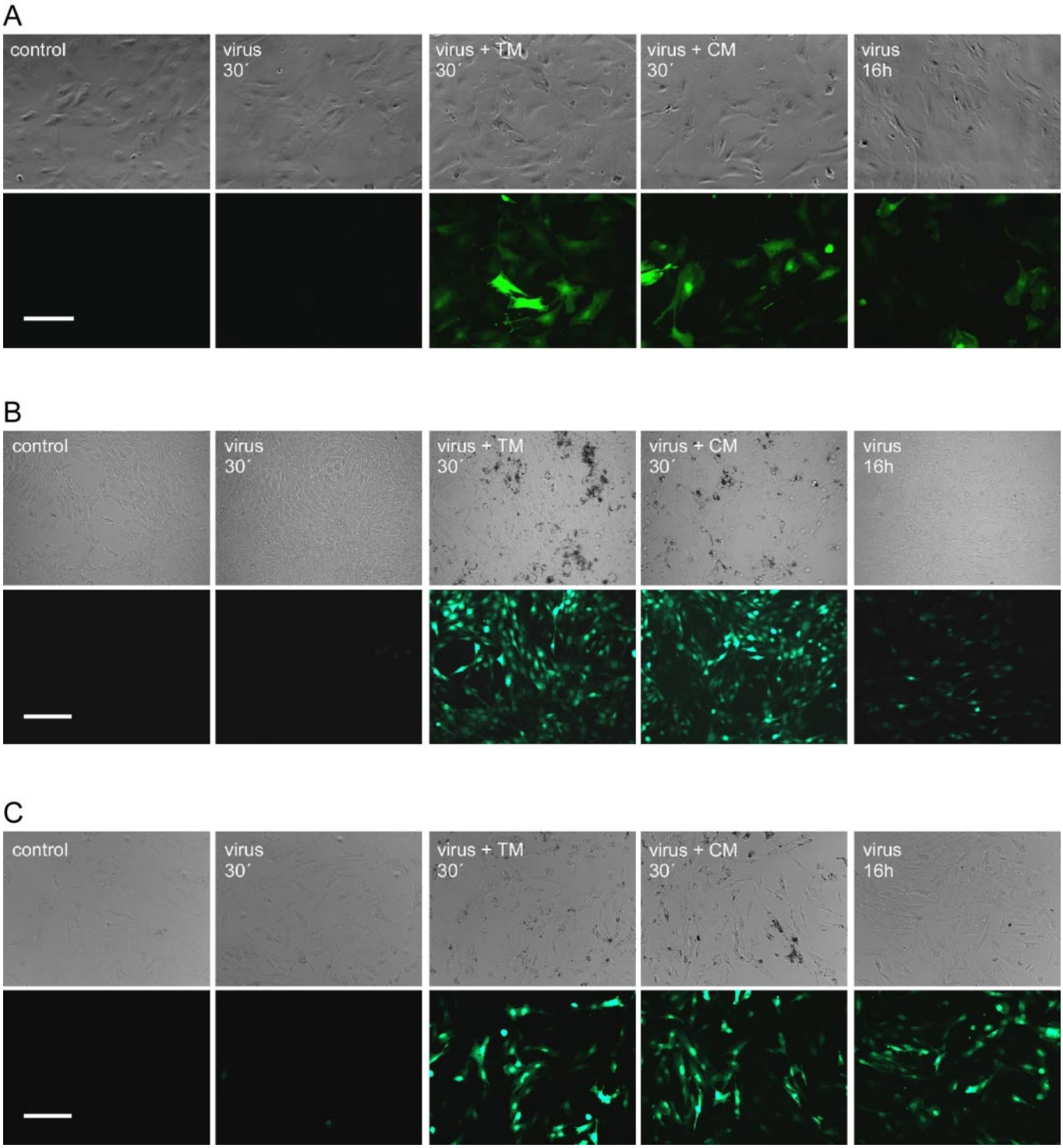

Fig. S2. Analyses of MNP-assisted transduction under nonpermissive conditions in various cell types. Primary rat endothelial cells (*A*), murine NIH 3T3 fibroblasts (*B*), and immortalized porcine skin fibroblasts (*C*) were hypothermically transduced with LV/TM (virus + TM 30′) and LV/CM (virus + CM30 ′). Shown are bright-field (*Upper*) and fluorescence images (*Lower*) 72 h after transduction. Control indicates untreated cells; virus 30′, cells transduced without MNPs; virus 16h, overnight at 37 °C infected cells. (Scale bars: 100 μm.)

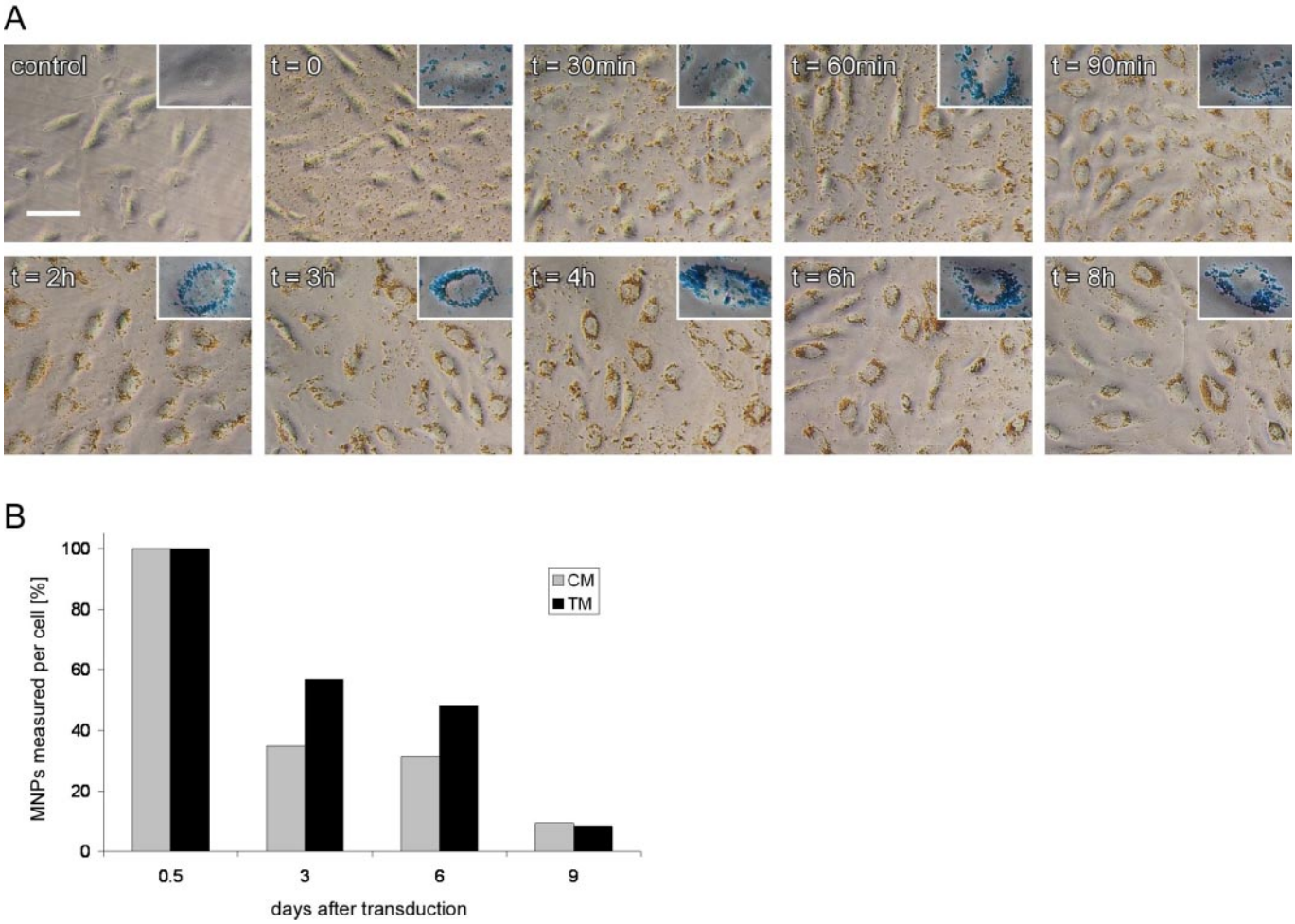

Fig. S3. Analysis of LV/TM uptake and persistence. (*A*) Analysis of cellular MNP uptake. Shown are light microscopy images and Prussian blue stainings (*Insets*) of HUVECs transduced with LV/TM complexes at defined time points after transduction (30 min to 8 h). (Scale bar: 100 μm.) (*B*) Analysis of MNP persistence in LV/MNP-transduced cells. Relative amount of incorporated MNPs in HUVECs at defined points after transduction (0.5, 3, 6, and 9 days) as measured by magnetorelaxometry. Values at 0.5 days were set to 100%. TM indicates cells transduced with LV/TM for 30 min; CM, cells transduced with LV/CM for 30 min.

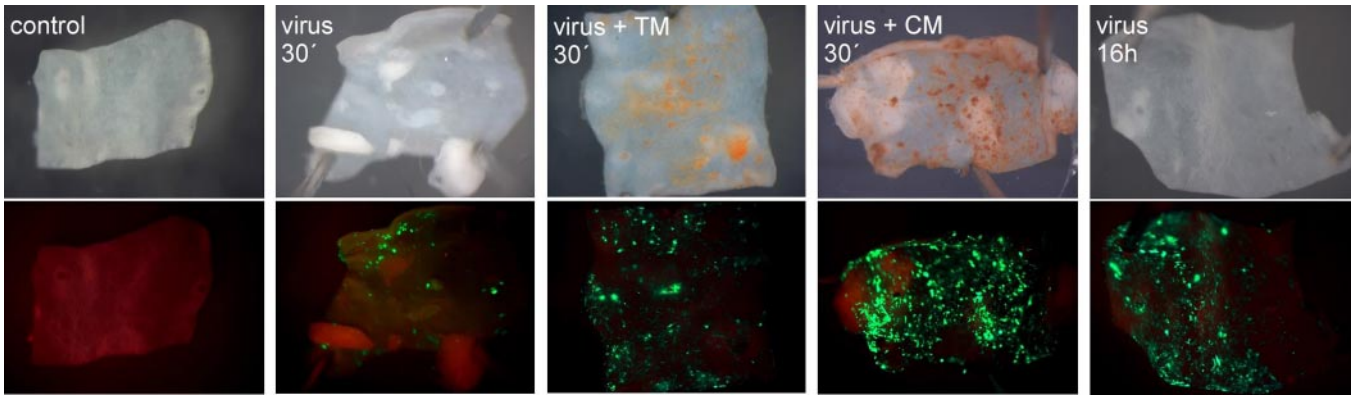

Fig. S4. Aortic strips were transduced for 30 min at 4 °C with LV/TM (virus + TM 30′) and LV/CM (virus + CM 30′) complexes. Incubation for 30 min without MNPs (virus 30′) resulted only in low EGFP expression. Shown are also an overnight infected aorta (virus 16h) and an untreated control (control).

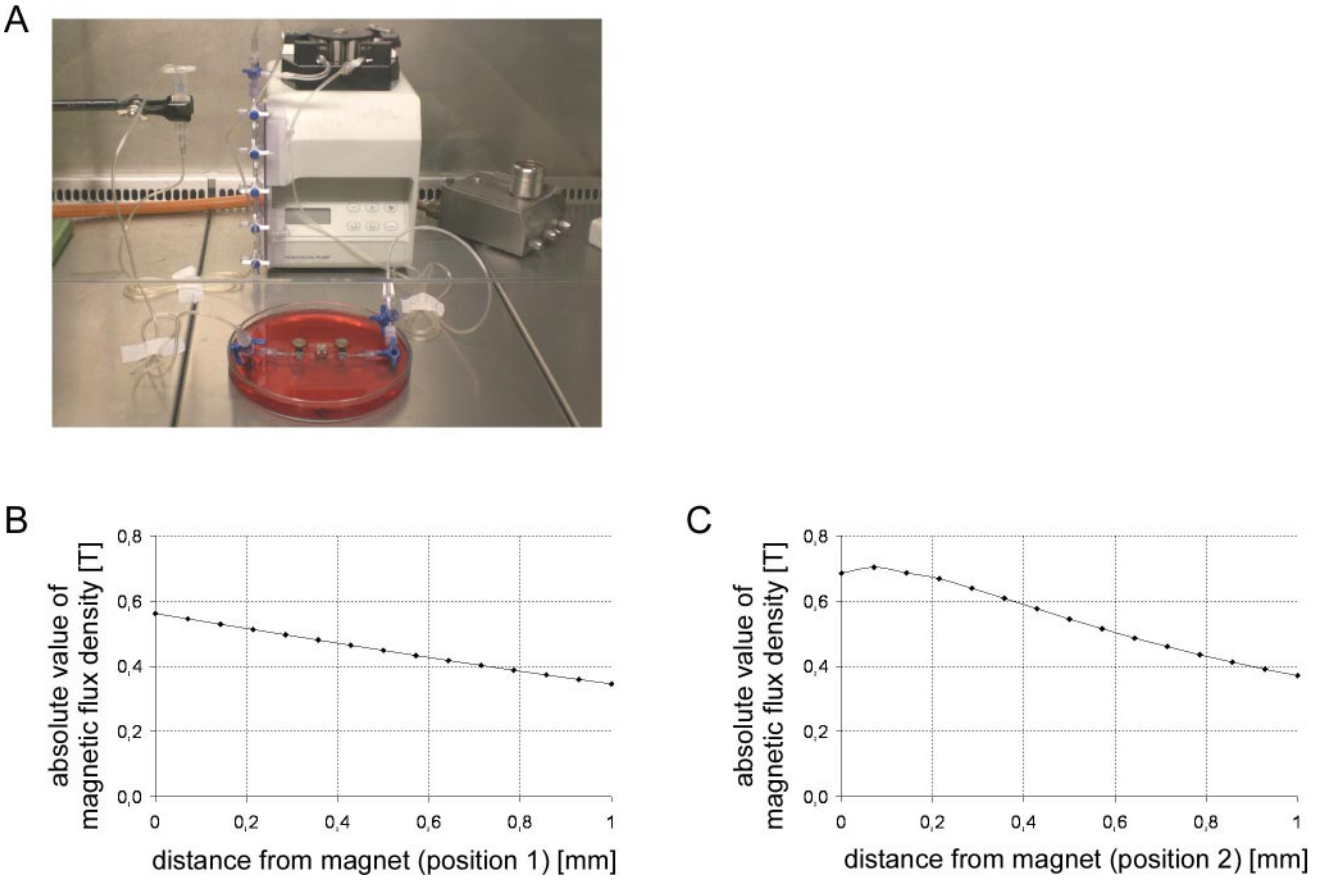

Fig. S5. Setup for ex vivo aorta perfusion and calculation of magnetic flux densities. (*A*) Explanted aortas were attached to 2 cannulae and perfused ex vivo for 30 min with LV/MNP complexes. (*B*) Absolute magnetic flux density versus distance from the poles (indicated as 1 in Fig. 2*A*) of the magnets. (*C*) Absolute magnetic flux density versus distance from the gap between the 2 magnets (indicated as 2 in Fig. 2*A*).

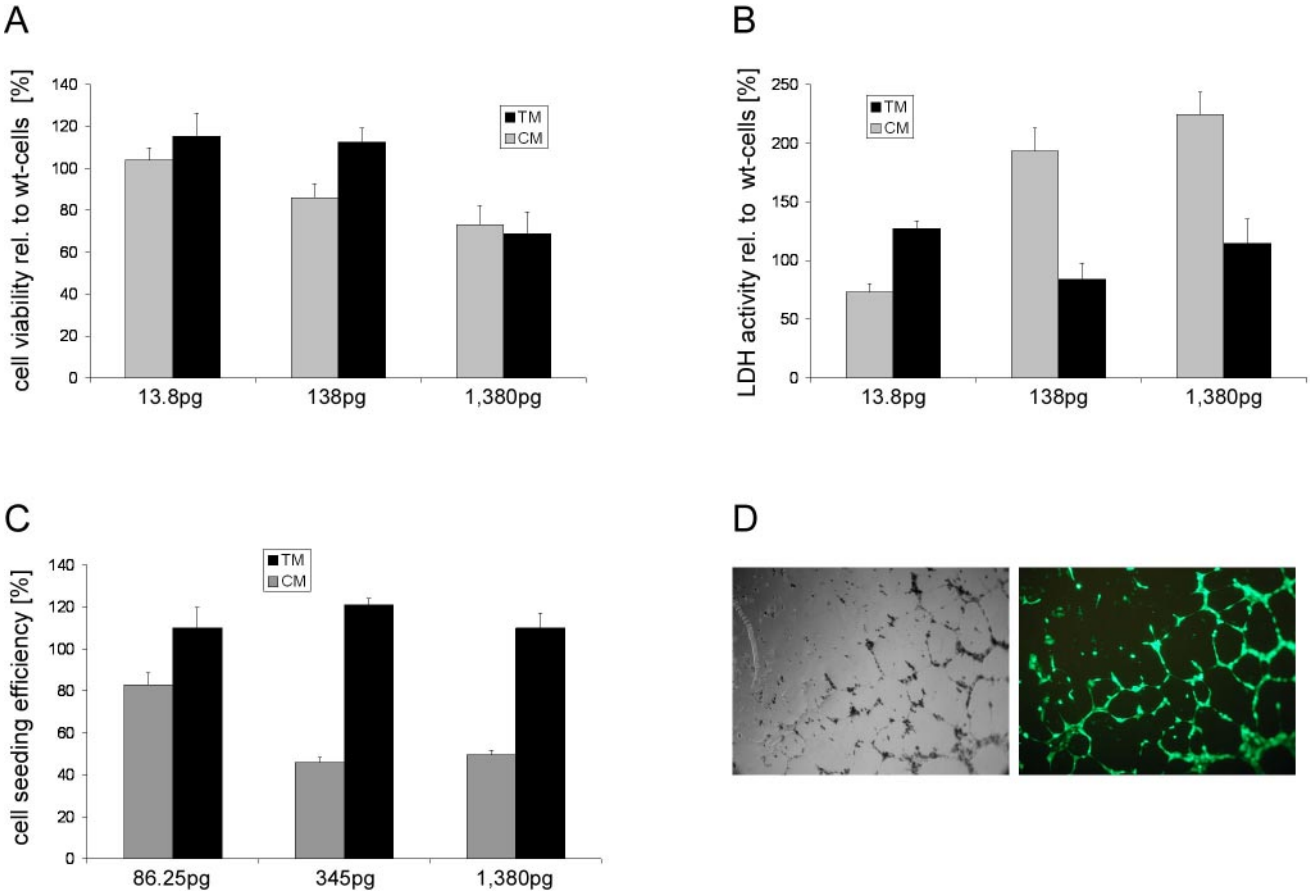

Fig. S6. Analysis of MNP toxicity in LV/MNP-transduced HUVECs. (*A*) Analysis of cell viability by a WST-1 assay (measures the activity of mitochondrial enzymes in LV/TM- and LV/CM-transduced cells). (*B*) Analysis of MNP cytotoxicity by an LDH assay (measures the release of cytoplasmatic LDH from dead or damaged cells after LV/TM and LV/CM transduction). Note that only 7.5% of the transduced cells (1,380 pg, LV/CM) were lysed. (*C*) Seeding efficiency: LV/TM- and LV/CM-transduced HUVECs were trypsinized and transferred to new cell culture plates. After 24 h, the number of attached cells was quantified by sulforhodamine B stainings. (*D*) LV/TM-transduced HUVECs (1,380 pg of MNPs per cell) were transferred to Matrigel-coated cell culture dishes. Twenty-four hours later, formation of GFP-positive vascular networks was visible. Shown are a bright-field (*Left*) and a fluorescence (*Right*) image. The MNP doses (picograms of nanoparticles that were applied per cell) are indicated below the graphs in *A*–*C*. All values are mean ± SEM of $n = 3$.

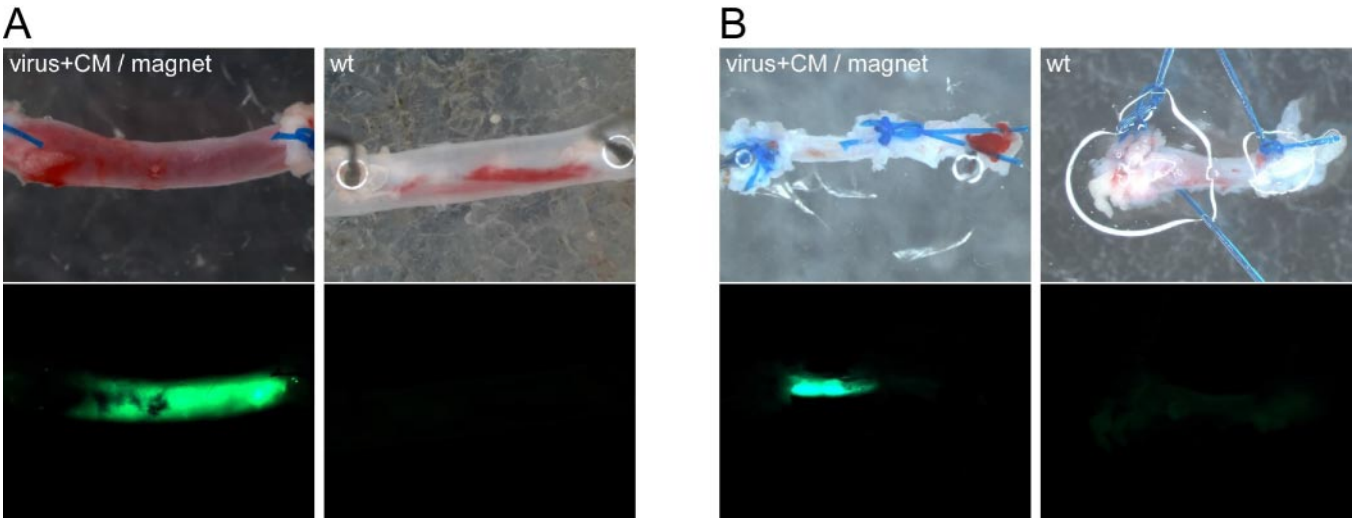

Fig. S7. In vivo positioning of systemically administered, MNP-labeled endothelial cells. LV/TM-transduced HUVECs were injected into the thoracic aorta (via a catheter), and a magnet was positioned at the abdominal aorta (*A*) and the iliac artery (*B*). The images show vessels after dissecting free of connective tissues. Shown are bright-field (*Upper*) and fluorescence images (*Lower*). (Magnification: 2.5×.)