Supplementary Materials

Materials and methods

Cell culture

The wild type Madin-Darby canine kidney epithelial cell line (MDCK) was a kind gift from Tilo Eichler (Department of Clinical Medicine, University of Bergen, Norway) and grown in Eagle's Minimum Essential Medium (ATCC, Manassas, VA, USA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific).

Madin-Darby canine kidney epithelial cells (MDCK II) were kindly donated by Professor Marjo Yliperttula (University of Helsinki, Finland). The cells were transduced with the red fluorescent protein mCherry (HIV-based lentiviralvector pGreenFire purchased from System Biosciences Paulo Alto, CA, USA) to also obtain the MDCK II mCherry cell line. The cells were grown in DMEM (Sigma-Aldrich), supplemented with 1% Penicillin/Streptomycin (BioWhittaker), 1% 200 mM L-Glutamine (BioWhittaker) and 5% fetal bovine serum (Thermo Fisher Scientific).

The rat brain endothelial 4 (RBE4) cell line was kindly gifted by Professor Michael Aschner (Vanderbilt University, Nashville, TN, USA). The cells were grown in 1:1 Hams F10 nutrient mix (Thermo Fisher Scientific) and Minimum Essential Mix (Thermo Fisher Scientific), supplemented with 2.5 μL/L human recombinant fibroblast growth factor (Sigma-Aldrich), 5% fetal bovine serum (Thermo Fisher Scientific) and 150 mg geneticin (Thermo Fisher Scientific).

Human cerebral microvascular endothelial cells (hCMEC/D3) were purchased from MerckMillipore (Burlington, MA, USA) and grown in endothelial basal media-2 (Lonza, Köln, Germany) supplemented with SingleQuots (Lonza) and 2% human serum (Sigma-Aldrich).

Human cerebral cortex-derived astrocytes SC-1800 were purchased from Caltag Medsystems (Buckingham, UK) and grown in astrocyte basal media (Lonza) supplemented with SingleQuots (Lonza) and 3% human serum (Sigma-Aldrich).

The H1 and H2 cell lines were established «in house» from biopsies from two different patients with brain metastases from melanoma as previously described (1). Written consent by the Regional Ethical Committee (#013.09) and the Norwegian Directorate of Health (#9634) was obtained before tumor tissue was collected and stored. The H1 cells were transduced with lentiviral vectors encoding the green fluorescent protein variant Dendra and Luciferase to obtain the H1_DL2 cells for *in vivo* experiments. The H1, H1_DL2 and H2 cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich Inc., St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal calf serum (Thermo Fisher Scientific, Waltham, MA, USA), four times the prescribed concentration of non-essential amino acids (BioWhittaker, Verviers, Belgium), 2% L-glutamine (BioWhittaker), penicillin (100 IU/mL, BioWhittaker) and streptomycin (100 µL/mL, BioWhittaker).

All cells were kept in a standard tissue culture incubator at 37 °C with 100% humidity and 5% CO_2. Sub-culturing was done twice a week for the H1, H1_DL2, H2, hCMEC/D3 and SC-1800 cells, whereas this was done every second day for RBE4, MDCK and MDCK II cells. The endothelial cells were grown on collagen-coated dishes, prepared by diluting Collagen, Type I from rat tail (Sigma-Aldrich) in 1 x phosphate-buffered saline (PBS) and distributing this at 7.5 µg/cm^2 culture dish area. All cell lines were routinely tested for mycoplasma using the MycoAlert Mycoplasma Detection Kit (LT07-318, Lonza, Basel, Switzerland) according the manufacturers recommendations and authenticated using short tandem repeat profiling. Upon thawing, the cells were used in experiments for a maximum of ten passages.

K16ApoE peptide synthesis

The linear peptide KKKK-KKKK-KKKK-KKKK-LRVR-LASH-LRKL-RKRL-LRDA-(OH) necessary for this study was synthesized by the solid phase method using the CEM Liberty automatic microwave peptide synthesizer (CEM Corporation Inc., Matthews, NC), employing 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry and commercially available amino acid (Merck

Millipore Novabiochem), single coupling (2× coupling for R). Peptides were cleaved from resin using trifluoroacetic acid (TFA) 95% (v/v); water, 2.5% (v/v); triisopropylsilane (TIS), 2.5% (v/v); two hours and precipitated by addition of ice-cold diethyl ether. The unprotected peptides were purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC). Collected fractions containing purified peptide were pooled and lyophilized. The identity of the peptide was confirmed by high resolution mass spectrometry (HRMS).

Evaluation of the *in vivo* toxicity of K16ApoE

K16ApoE was injected as a bolus over 1 minute into the tail vein of 30 female and 8 male 16 weeks old NOD/SCID mice with an average bodyweight of 22.3 g, using peptide concentrations of 50 µg (n=5), 100 µg (n=11), 200 µg (n=5), 400 µg (n=3), 600 µg (n=3), 800 µg (n=3) or 1,000 µg (n=8). 9 mg/mL NaCl was used to adjust the total injection volume to 100 µL. The animals were then closely observed for the next two hours and immediately sacrificed if they showed any signs of acute toxic symptoms. Surviving animals were then observed over 48 hours to detect any signs of distress such as whisker alterations, cheek bulge, orbital tightening or changes in activity levels.

Eight 12-week-old NOD/SCID mice were injected with either 9 mg/mL NaCl or 1,000 µg K16ApoE in 9 mg/mL NaCl as a bolus over one minute. The cardiac response was monitored by ultrasound for the subsequent three minutes, which was the time it took before the animals in the peptide group died. Blood samples were collected from the left ventricle of the heart and blood smears were stained with eosin to evaluate the morphology. See Supplementary Figure S1A for details.

DCE-MRI

The T_2 weighted spin echo scans prior to DCE-MRI were acquired with a region of interest (ROI) over the mouse brain and neck musculature in coronal positioning (TR/TE: 4,000/48 ms, field of view (FOV): 2.00 cm, matrix size: 256 × 256, slice thickness: 1.00 mm, 7 slices and number of averages (NEX): 4, total scan time 6 minutes 13 seconds). The T_1 weighted scans were acquired with

the same geometry as the T_2 weighted scans (TR/TE 1,000/9 ms, and NEX: 4, total scan time of 3 minutes, 20 seconds). The perfusion scans were acquired in the middle position of the slice package. First, a series of FLASH sequences were acquired (TR/TE: 37.2/2.1 ms, FOV: 2.00 cm, matrix: 256 × 256, slice thickness, 1.00 mm and NEX: 1, total scan time of ~3 minutes), followed by the DCE-MRI sequence: This consisted of 900 repetitions of the FLASH protocol with the same geometry (TR/TE: 15 ms/2.1 ms, NEX: 1, FA: 17, temporal resolution: 1 second and total scan time of 16 minutes 12 seconds).

The perfusion scans were acquired by initiating a series of six fast low angle shot (FLASH) sequences with flip angles (FAs) 5, 10, 15, 20, 25 and 30 before the DCE-MRI scan sequence was acquired. 0.5 mmol/kg Omniscan (GE Healthcare, Little Chalfont, UK) was injected as a bolus through the tail vein using an injection pump (Harvard Apparatus, Holliston, MA, USA) 15 seconds after starting the DCE sequence.

The DCE-MRI data was analyzed using the Extended Tofts model implemented in nordicICE v2.3 (Nordic NeuroLab, Bergen, Norway) using local arterial input functions (AIFs) obtained from an adjacent neck muscle. AIFs were extracted by blind deconvolution (2), using custom developed routines implemented in Matlab 2015b (Mathworks, Natick, MA, USA). These routines are accessible through the online Perfusion Lab tool, available at http://perflab.cerit-sc.cz/. Maps of Area Under the Curve (AUC) and blood-to-tissue transfer constant (K^{trans}) were generated, and ROIs were drawn to cover the whole brain section including muscle tissue, white and grey matter. K^{trans} values were normalized to known values of the extravascular space (V_e) and vascular space (V_b) fractions in a reference muscle tissue, as previously described (3). Mean and standard deviation values were calculated for each ROI, time point and peptide dose, and statistically significant differences between K16ApoE receiving groups and corresponding 9 mg/mL NaCl receiving groups were established using the Mann-Whitney test function.

Flow cytometry

RBE4 cells were seeded in 6-well plates (Corning, New York, USA) at a density of 2.5×10^5 cells per well. After reaching the growth log-phase, the cells were incubated with 5 μg/mL AF647-BSA (Invitrogen Molecular Probes, Waltham, MA, USA) with or without 20 μg/mL rhodamine-conjugated K16ApoE for 45 minutes. For incubation in serum-free conditions, the cells were serum-starved for one hour before incubation in AF647-BSA with or without rhodamine-conjugated K16ApoE for 45 minutes. For endocytosis inhibition experiments, RBE4, MDCK, hCMEC/D3, H1 and H2 cells were seeded in 6-well plates at a density of 2.5×10^5 per well and pre-treated with 80 μM dynasore (a dynamin inhibitor) or 30 μM chlorpromazine (a clathrin inhibitor) for 30 minutes. The cells were then incubated with Alexa Fluor 647 (AF647)-conjugated BSA at a concentration of 5 μg/mL and 20 μg/mL K16ApoE in the continued presence of the endocytosis inhibitors for 45 minutes. For incubation at 4 °C, the RBE4 cells were pre-cooled on ice for 15 minutes, and then incubated with 5 μg/mL AF647-BSA with or without 20 μg/mL rhodamine-conjugated K16ApoE for 45 minutes. After incubation, the cells were washed with PBS, trypsinized and centrifuged twice before they were re-suspended in PBS and taken to the flow cytometer. Rhodamine-conjugated K16ApoE was excited by the blue laser line and detected using the FL2 detector, whereas AF647-BSA was excited by the red laser line and detected with the FL7 detector, using a Beckman Coulter flow cytometer (Beckman Coulter Inc., Brea, CA, USA) or a BD LSR Fortessa (BD Biosciences, San Jose, CA, USA).

***In vitro* cell viability**

5,000 MDCK, MDCK II, RBE4, hCMEC/D3 and H1 cells were seeded in each well in 96-well plates in 100 μL growth medium. After 24 hours of incubation, 100 μL of graded concentrations of K16ApoE (0.1, 1, 20, 40, 60, 80, 100 or 150 μg/mL) diluted in growth medium were added to each well (n = 6). 45 minutes later, the peptide was removed before each well was washed twice with PBS, and 100 μL/well of 0.1 mg/mL resazurin (Sigma-Aldrich) was added. The plates were measured after four hours at 560 nm excitation and 590 nm emission using a VICTOR X3 multilabel plate reader

(Perkin Elmer, Waltham, MA, USA) equipped with WorkOut 2.5 data analysis software. Wells containing 100 µL growth media without cells were used for background corrections. IC_{50} doses were defined as the drug concentration at which 50% of the cell growth was inhibited and was calculated using GraphPad Prism 7 for Mac OS X (GraphPad Software Inc., San Diego, CA, USA). The experiments were done in triplicate.

A Live/Dead assay (Thermo Fisher Scientific) was carried out using 5×10^4 MDCK cells seeded in µ-Slide 4-well glass bottom dishes (Ibidi GmbH, Munich, Germany). The cells were treated with 0, 20, 40 or 80 µg/mL K16ApoE. Immediately after treatment started, solutions of 4 mM Calcein-AM and 2 µM Ethidium Homodimer-1 solutions in the Live/Dead kit were mixed and distributed to the live cells according to the manufacturer's instructions. A time-lapse imaging sequence was obtained on a Leica TCS SP8 STED 3× confocal microscope (Leica, Wetzlar, Germany) using a 20× objective (HC PL APO CS2 20X/0.75 imm) capturing images every minute over a time period of 45 minutes. Calcein-AM was excited at 488 nm and emitted between 500-550 nm, and Ethidium Homodimer-1 was excited at 561 nm and emitted between 570-630 nm. The experiment was carried out once and the confocal images were analysed in FIJI (National Institutes of Health, Bethesda, MA, USA) by measuring the total signal from the red and green channels in all images acquired throughout the time–lapse.

Scanning electron microscopy

MDCK II or RBE4 cells were seeded on collagen coated 24-well plates (Nunc, Roskilde, Denmark) with 12 mm Ø coverslips at a density of 4,000 cells/500 µL growth medium and allowed to reach confluency prior to treatment with 0, 20, 40 or 80 µg/mL K16ApoE for 45 minutes. The cells were fixed using 2.7% glutamic acid aldehyde and then washed twice using 0.1 M sodium cacodylate buffer before post-fixation with 0.1 M OsO_4 in 0.1 M sodium cacodylate buffer and subsequent washing using 0.1 M sodium cacodylate buffer. The cells were then allowed to dry for 15 minutes in graded concentrations of ethanol (30, 50, 70, 96 and 100%). Critical point drying was carried out on

the coverslips before coating with 5 nm Cd-Au alloy, using a JEOL JFC-2300 HR High Resolution Fine Coater (JEOL Ltd., Tokyo, Japan). Microscopic analysis was performed using a Scanning Electron Microscope JEOL JSM-7400F (JEOL Ltd.).

Cell adhesion assay

hCMEC/D3 cells were seeded at 5,000 cells per well in 96-well plates (Greiner Bio-One, Kremsmünster, Austria) and incubated for 24 hours at 37 °C in a 5% CO_2 tissue incubator. K16ApoE at concentrations 0, 20, 40 or 80 µg/mL were added to the wells and incubated for 45 minutes. Subsequently, unattached cells were washed away with PBS and adherent cells were fixed with 4% formaldehyde at 0, 30 and 60 minutes after the peptide was replaced with fresh culture media. Adherent cells were then stained using 0.1% w/v crystal violet. Absorbance of stained nuclei was determined using a plate reader (POLARstar OPTIMA BMG Labtech, Ortenberg, Germany) at 570 nm.

***In vitro* BBB model**

For resistance monitoring using the Electric Cell Substrate Impedance Sensing (ECIS) system, ECIS arrays (8W10E+, 8 wells; Ibidi GmbH) were stabilized with L-cysteine (10nM; 10 minutes), washed in Hank's balanced salt solution (Thermo-Fisher, UK), coated with recombinant Human Endorepellin/Perlecan at 10 µg/mL (R&D systems, Minneapolis, MN, USA) and incubated at 37° C, 5% CO_2 for two hours. After seeding density optimization, 7,500 hCMEC/D3 cells were then seeded into each well and cell resistance monitored at 4,000 Hz using an ECIS Zθ (Applied Biophysics, USA) system for 70 hours at 37 °C with 5% CO_2. 0, 20, 40 or 80 µg/mL of K16ApoE peptide was then added to the system and resistance was further recorded until recovery of the barrier was observed. Resistance values were obtained in Ω. For each experiment, at least three replicates were measured.

For the cell automated sensing system, CellZscope®, 8 µm pore-size at 0.33 cm^2, polycarbonate membrane trans-well inserts (Corning, UK) were used. The transwell inserts were coated with 10 µg/mL human perlecan (R&D systems, Minneapolis, MN, USA) on the apical side of the filter membranes and with 5 µg/mL human fibronectin (Sigma-Aldrich) on the basal side of the filter. 2,500 of SC-1800 cells were seeded on the basal side of the porous filter membrane and left to adhere for two hours in the laminar flow hood before 7.5×10^4 hCMEC/D3 cells were seeded on the apical side of the filter. The cells were then incubated for 72 hours in the incubator at 37 °C and 5% CO_2. The resistance values were recorded using the CellZscope® automated cell monitoring system, CellZscope® (nanoAnalytics GmbH, Münster, Germany). Once the resistance plateaued, 0, 20, 40 and 80 µg/mL of K16ApoE was added and TEER values, expressed in $\Omega.cm^2$, were further recorded in real-time until recovery of the barrier was observed. High TEER values reflected tight junction formation. For each experiment, at least three replicates were measured.

***In vivo* biodistribution of ^{125}I–K16ApoE**

The imidazole ring of histidine in K16ApoE was labelled with ^{125}I using 1,3,4,6-tetrachloro-3α,6α–diphenylglycouril (Iodo-Gen) at pH 9 (4,5). Briefly, 0.1 mg Iodo-Gen (T0656, Sigma-Aldrich) dissolved in 0.05 mL chloroform was dispersed in a 1.8 mL Nunc tube (Nunc). A film of the virtually water-insoluble Iodo-Gen was formed in the Nunc vial by allowing the chloroform to evaporate to dryness under nitrogen. Then, 1 mL 0.05 M phosphate buffer with pH 9.0, containing 2 mg K16ApoE and 2 MBq ^{125}I (Institute for Energy Technology, Kjeller, Norway) was added and the iodinating tube gently agitated for 10 minutes. The reaction was terminated by removing the solution from the Iodo-Gen tube. The stock solution was stored in the dark at 4 °C. Low molecular weight radioactivity accounted for about 40% of the total ^{125}I-activity, and was removed to undetectable levels before use by Pierce™ ^{18}C Spin Columns. ^{125}I-K16ApoE was validated by reversed phase and size exclusion chromatography and found to have the essentially similar elution pattern as unlabeled K16ApoE (Supplementary Figure S6A). Each bolus injection contained about 20 µg ^{125}I-K16ApoE.

Two 18 weeks old NOD/SCID mice were anaesthetized by intramuscular injection of 0.12 mg ketamin and 0.243 µg medetomidine per gram bodyweight dissolved in PBS. The core temperature was maintained at 37 °C with the aid of a heating lamp and a servo-controlled heating pad. A PE-50 catheter (AgnThos AB, Lidingö, Sweden) was inserted into the carotid artery of each mouse to allow blood sampling. 20 µg ^{125}I-K16ApoE diluted in 50 µL 9 mg/mL NaCl was injected into the tail vein of each mouse as a bolus over one minute. Arterial blood samples were then collected at 0.5, 1, 5, 10, 15, 20 and 30 minutes after the ^{125}I-K16ApoE administration was completed. The animals were then sacrificed by injecting saturated KCl into the carotid artery. Immediately after, brain, skin, heart, lung, liver, stomach, spleen, colon, kidneys, muscle tissue and femur were collected and counted for ^{125}I activity using a Wallac Wizard 1470 gamma counter (PerkinElmer, Waltham, MA, USA). The counts were corrected for background values before the peptide distribution was calculated as ^{125}I activity per gram organ or per 10 µL plasma.

***In vivo* treatment study**

Prior to intracardiac injections, the NOD/SCID mice were anesthetized and fixed in a supine position on a heating pad to maintain a core temperature at 37° C. 5×10^5 H1_DL2 cells resuspended in 0.1 mL PBS were injected during 30 seconds into the left cardiac ventricle of each mouse using a 30G insulin syringe (Omnican50, B. Brain Medical AS, Vestskogen, Norway), by ultrasound guidance (Vevo$^{(R)}$ 2100 Imaging System 230 V, Visual Sonics Inc., Toronto, Canada).

Contrast enhanced MRI was carried out four and six weeks after start of the treatment experiment to evaluate the brain metastatic burden. A 7 Tesla small-animal horizontal scanner (Bruker BioSpin GmbH) equipped with a 72 mm quadrature transmit coil and a four-channel mouse brain array receive coil was used. A T_2 weighted scan was performed in coronal orientation (TR/TE: 4,000/48 ms, FOV: 2.00 cm, matrix size: 256 × 256, slice thickness: 1.00 mm, number of slices: 7, NEX: 4, scan time: 6 minutes, 13 seconds) followed by T_1 scans using the same geometrical parameters (TR: 1,000 ms, TE: 9 ms, and NEX: 4, scan time: 3 minutes, 20 seconds) before and after

subcutaneous administration of 0.5 mmol/kg Omniscan (GE Healthcare). The MR images were analysed in OsiriX Lite v.9.5.2 (Pixmeo SARL, Bernex, Switzerland) to determine the tumor numbers and volumes ($4/3\ \pi\ r^3$) as a measure of metastatic burden.

Mass spectrometry

A pilot mass spectrometry experiment was performed by a company provider (ImaBiotech, Loos, France). Briefly, tumor bearing mice treated either with K16ApoE + dabrafenib or saline, were perfused with 4% formaldehyde, their brains were removed and immediately snap-frozen. Thereafter, the brains were embedded into paraffine according to standard protocols. 10 μm thick brain sections were mounted onto indium tin oxide conductive glass slides. 1 μL MALDI matrix (α-cyano-4-hydroxycinnamic acid) was added onto the sections, and the sections were dried under vacuum for 15 minutes. Direct analysis was performed with a 7T-MALDI-FTICR in Full Scan and Continuous Accumulation of Selected Ions positive mode. The data sets were analysed with DataAnalysis 4.1 (Bruker Daltonics, Bremen, Germany).

Labelling of albumin and IgG with ^{18}F

The *in vivo* BBB opening effect of K16ApoE was further evaluated by PET using ^{18}F-albumin from mouse serum (Sigma-Aldrich, cat no A3139) and ^{18}F-IgG from mouse serum (Sigma-Aldrich, cat no I5381). The labelling prosthetic group, [^{18}F]F-Py-TFP (6-[^{18}F]Fluoronicotinic acid 2,3,5,6-tetrafluorophenyl ester), and its precursor, (*N,N,N*-Trimethyl-5-((2,3,5,6-tetrafluorophenoxy)-carbonyl)pyridin-2-aminium trifluoromethanesulfonate) were made according to previously published methods (6). The crude reaction mixture of [^{18}F]F-Py-TFP (from 5 mg precursor) was diluted in 4 mL 20% acetic acid and passed through a preconditioned (5 mL MeCN + 5 mL water) SepPak Light tC18-cartridge. Unreacted precursor was removed with 5 mL 30% MeCN, and the cartridge washed with 5 mL water. [^{18}F]F-Py-TFP was eluted from the tC18-cartridge with 1 mL diethyl ether through a SepPak Dry-cartridge. The ether was removed by evaporation. The freshly

made [^{18}F]F-Py-TFP was used to label both albumin and IgG. The protein labelling was carried out by dissolving 1 mg of the protein in 200 µL 0.05 M phosphate buffer (pH ~ 9) and adding [^{18}F]F-Py-TFP dissolved in small amounts of MeCN. The solution was heated to 40 °C and stirred gently for 15 minutes. The product was purified by a PD MidiTrap G-10 size exclusion column, using 9 mg/mL NaCl solution as an eluent.

Dynamic positron emission tomography/computer tomography (PET/CT) imaging

PET/CT images were acquired on a small-animal nanoScanPC PET/CT scanner (Mediso Medical Imaging Systems, Budapest, Hungary). Healthy (non-tumor bearing) NOD/SCID mice were anesthetized using 3% sevoflurane (Abbott Laboratories Ltd.) mixed in oxygen for the duration of the scans, while monitored for breathing and temperature. CT scans (50 kvP, 300 ms) were performed for anatomical information and attenuation correction of PET images. ~2 MBq of tracer was injected into the tail vein and a dynamic acquisition scan (coincidence 1:5, normal count mode) was started immediately. PET and CT images were co-registered automatically. PET reconstruction was performed by Nucline software (Mediso) from list-mode using the following parameters: Reconstruction algorithm Tera-Tomo 3D (OSEM), energy window 400–600 keV, coincidence mode 1:5, voxel size 0.4 mm, four iterations and six subsets, corrections for random events, detector normalization, decay and dead time. Images were reconstructed to the following time frames: 5 × 2 minutes, 8 × 5 minutes (4 × 5 minutes for the 30 minutes scans). Data analysis was performed using InterView Fusion (version 3.01.004.0000, Mediso).

Four minutes before the PET/CT acquisition, the animals were injected with 200 µg K16ApoE dissolved in 9 mg/mL NaCl. 24 hours later, the same animals were injected with 9 mg/mL NaCl prior to repeating the PET/CT acquisition. Thus, each mouse served as their own negative control. For each animal, an ellipsoid volume of interest (VOI) delineating the skull was drawn to cover the whole brain of each mouse. The mean standard uptake value (SUV_{mean}) was calculated using the following

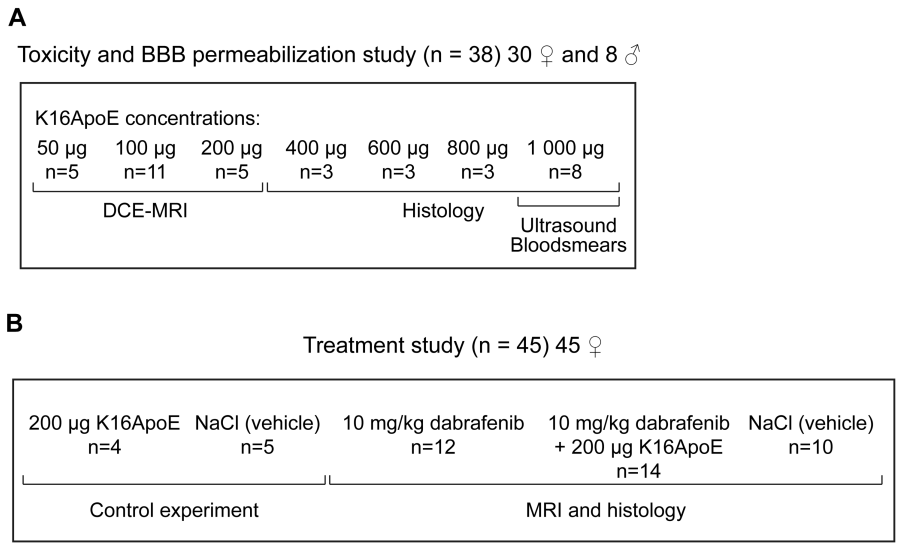
equation: $SUV_{mean} = C_{PET}(T)/(ID/BW)$, where $C_{PET}(T)$ is the mean measured activity in the VOI at time T, ID is injected dose measured in kBq, and BW is the animal weight in kg.

Supplementary Table 1: Mean V_e, K_{ep} and AUC after DCE-MRI.

Group	Minutes	n	V_e (%)	K_{ep} (min-1)	AUC (a.u.)
K16ApoE	10	5	58.98 ± 31.331	0.31 ± 0.203	17.73 ± 5.928
	30	5	43.08 ± 23.384	0.09 ± 0.039	5.78 ± 1.425
	60	6	10.82 ± 8.719	0.20 ± 0.069	2.65 ± 1.525
	120	5	10.42 ± 10.688	0.39 ± 0.343	2.62 ± 0.676
	240	5	2.126 ± 1.973	0.74 ± 0.453	1.68 ± 0.536
Saline	10	8	0.681 ± 0.411	0.59 ± 0.223	1.64 ± 0.809
	30	5	0.872 ± 0.347	0.69 ± 0.255	1.48 ± 0.460
	60	6	0.90 ± 0.819	0.69 ± 0.378	1.42 ± 0.760
	120	5	1.202 ± 0.528	0.69 ± 0.276	1.83 ± 0.534
	240	5	1.22 ± 0.947	0.74 ± 0.406	1.68 ± 0.432

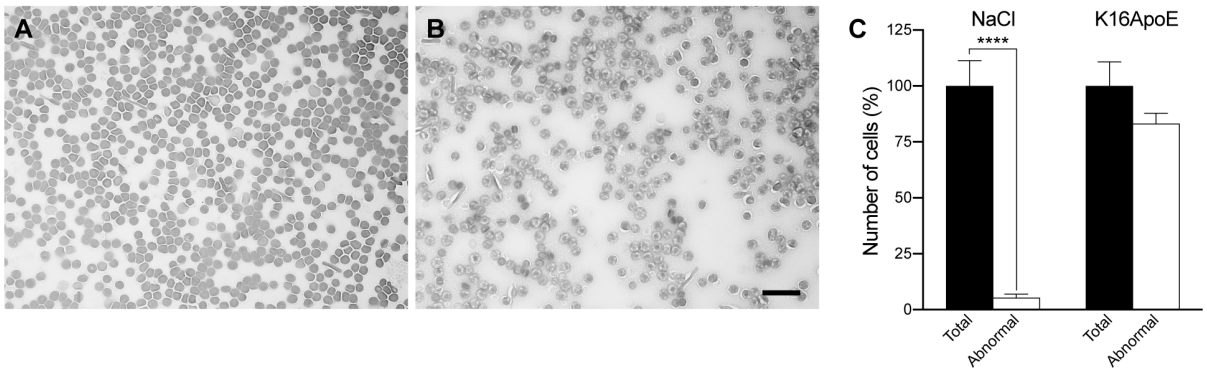
All values are given as mean ± standard deviation. Abbreviations: Min: minutes, V_e: Fractional volume of extravascular extracellular space (EES), K_{ep}: Rate constant from compartment 2 to 1 (reversed K^{trans}), AUC: Area under curve, a.u.: arbitrary units.

Supplementary figures



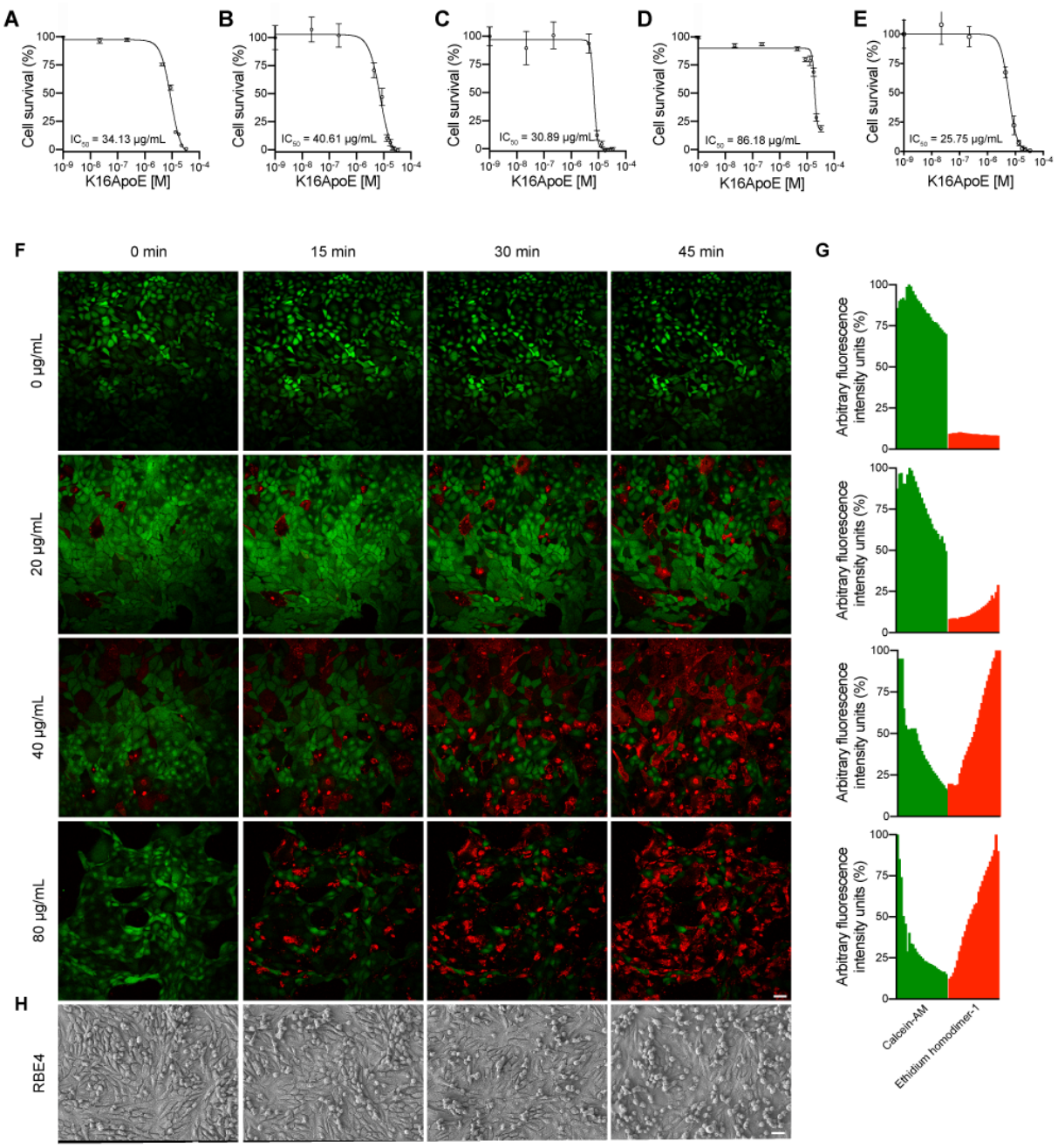
Supplementary Figure S1

In vivo study design. **A,** An illustration of the study design used to evaluate K16ApoE toxicity on a total number of 38 NOD/SCID mice. **B,** An overview of the 45 NOD/SCID mice used in the *in vivo* treatment study shown in Figure 5 and Supplementary Figures S7 and S8.



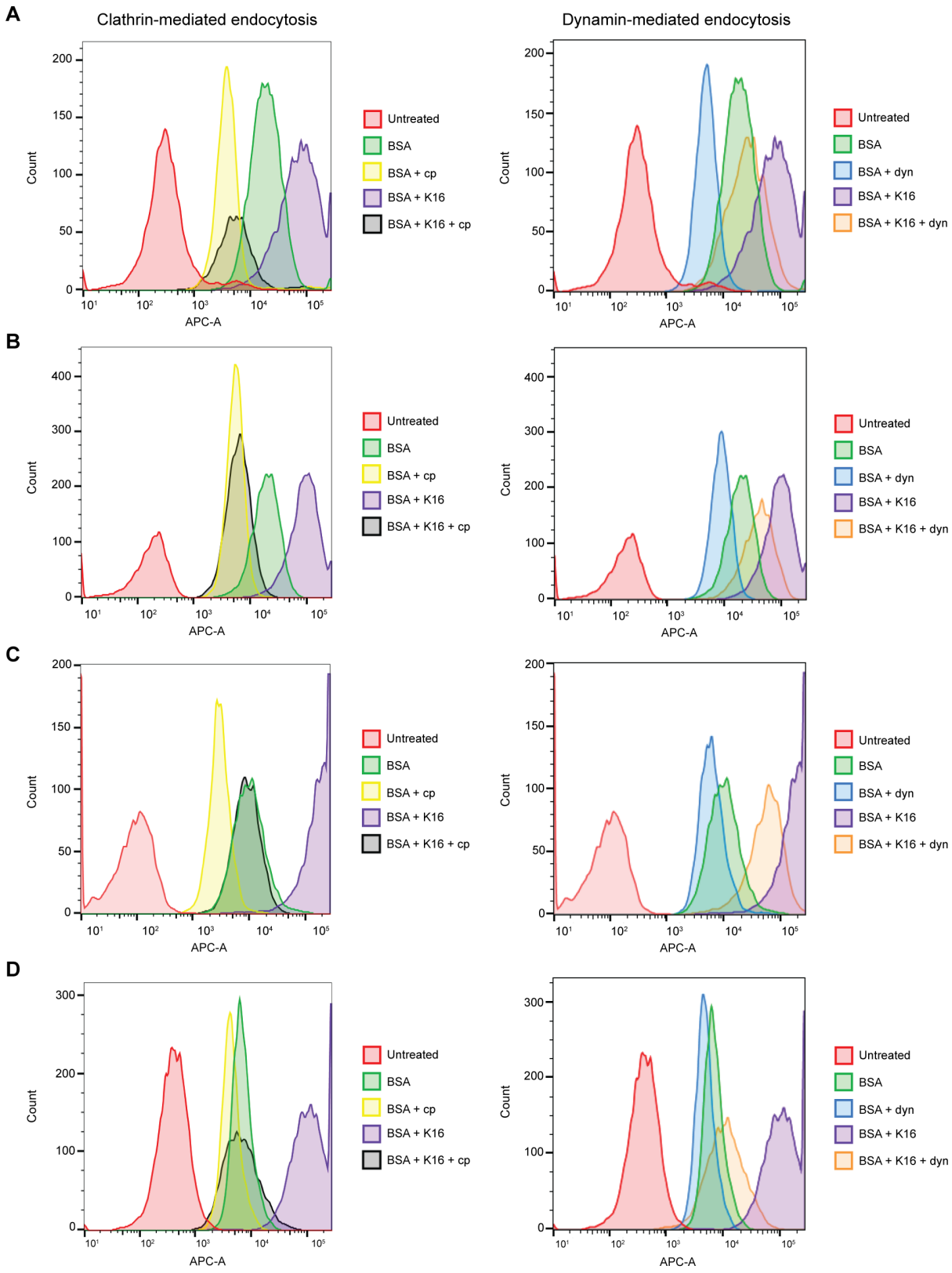
Supplementary Figure S2

Blood smear from mice subjected to 1,000 µg K16ApoE. **A,** A representative phase contrast image of a blood smear sample from a NOD/SCID mouse subjected to 1 000 µg K16ApoE as a bolus injection over approximately one minute. **B,** A representative blood smear from a control NOD/SCID mouse injected with a bolus of 9 mg/mL NaCl. Scalebar 100 µm. **C,** Quantification of the number of erythrocytes with an abnormal morphology as a function of the total cell number per microscope image scaled to 100% (n = 3). Unpaired t-test. Mean ± SEM. Abbreviation: ****: $p<0.0001$.



Supplementary Figure S3

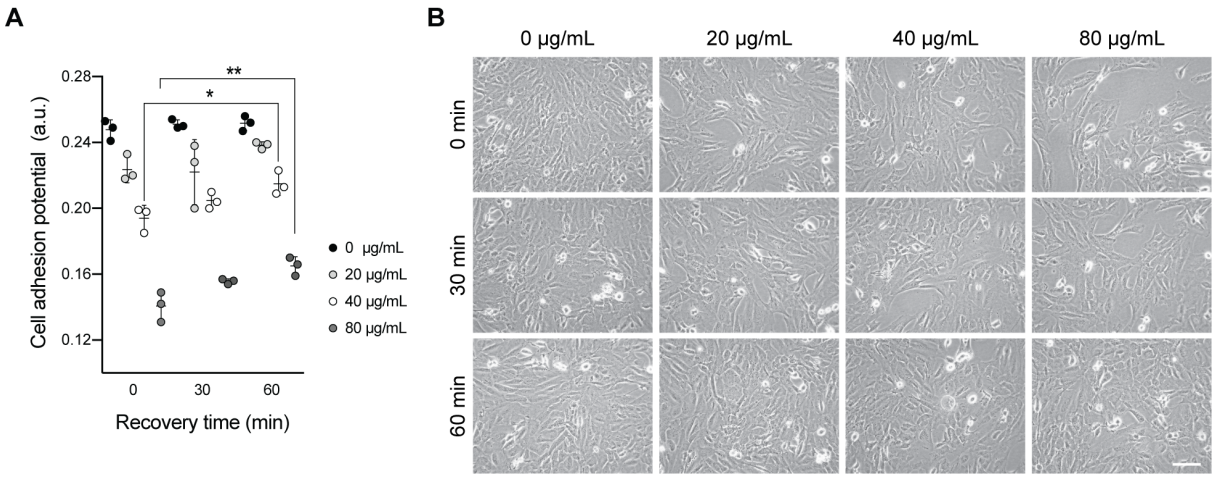
In vitro cell viability. **A,** IC_{50} curves for RBE4 **B,** MDCK **C,** MDCK II **D,** hCMEC/D3 and **E,** H1 cells after incubation with 0, 0.1, 20, 40, 80, 100 or 150 µg/mL K16ApoE for 45 minutes. **F,** Live/Dead staining of MDCK cells incubated with 0, 20, 40 or 80 µg/mL K16ApoE for 45 minutes. Scalebar 50 µm. **G** Quantification of live (green) and dead (red) cells in f. **H** SEM images of RBE4 cells treated with 80 µg/mL K16ApoE. Scalebar 20 µm.



Supplementary Figure S4

Mechanistic studies of the *in vitro* effects of K16ApoE **A**, Flow cytometry data showing the effects of two endocytosis inhibitors; chlorpromazine (left) and dynasore (right) after 30 minutes of incubation on H1, **B,** H2, **C,** hCMEC/D3 and **D,** MDCK cells measured as BSA uptake in the APC-

A channel. Abbreviations: APC-A: allophycocyanin-A, BSA: bovine serum albumin, cp: chlorpromazine, dyn: dynasore, K16: K16ApoE.



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339 **Supplementary Figure S5**

340 *In vitro* BBB model. **A,** Adhesion of hCMEC/D3 cells after incubation with 0, 20, 40 or 80 µg/mL of
341 K16ApoE for 45 minutes as measured by crystal violet. 2way ANOVA statistical test. Mean ± SEM.
342 **B,** Phase contrast images of hCMEC/D3 cells after corresponding incubation times and
343 concentrations with the peptide. Scalebar 50 µm. Abbreviations: *: $p<0.05$, **: $p<0.01$.

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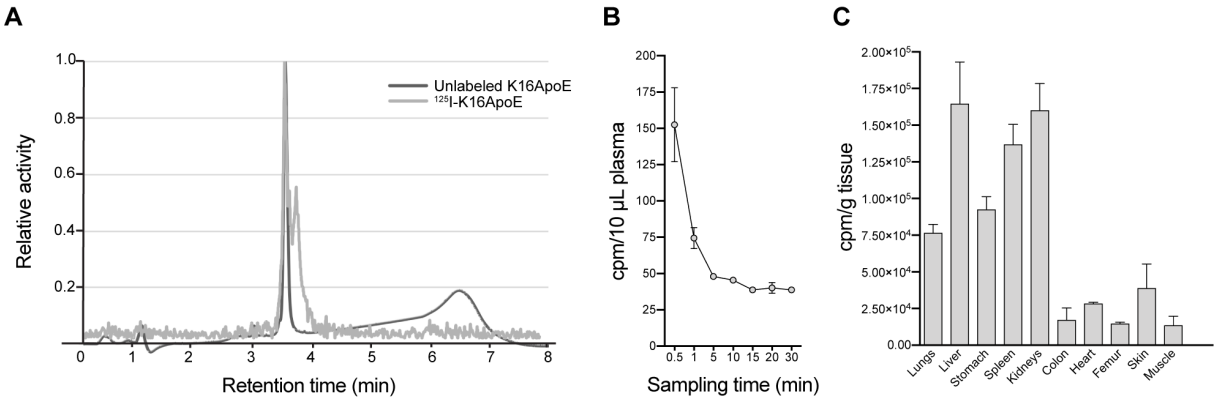
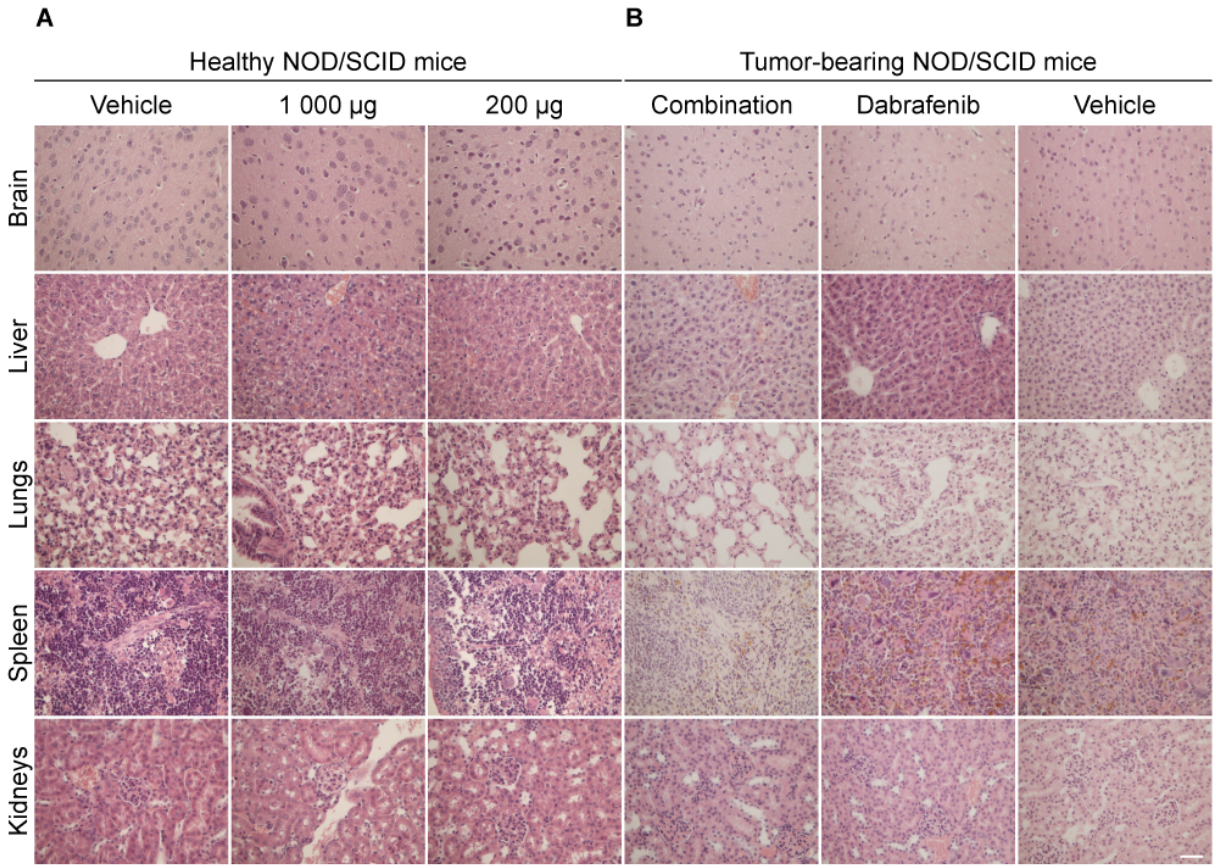


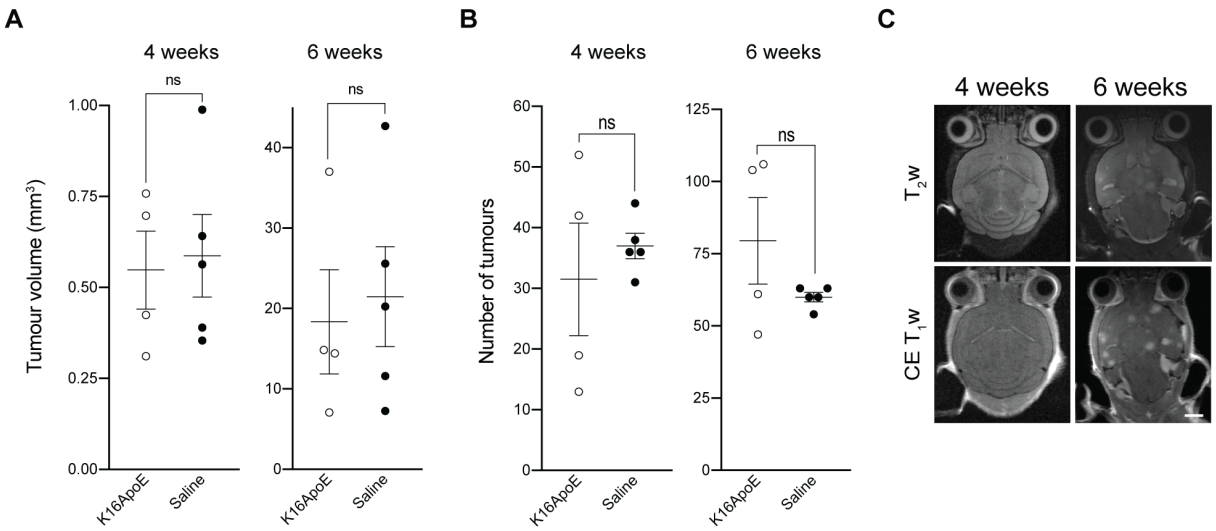
Supplementary Figure S6

Biodistribution of ^{125}I-K16ApoE. **A,** Elution profiles demonstrating native K16ApoE and ^{125}I-K16ApoE. **B,** Temporal elimination of the ^{125}I-K16ApoE from blood plasma (n=3) during 30 minutes. Mean ± SEM. **C**, Accumulation of ^{125}I-K16ApoE within the specified tissues (n=3) after 30 minutes given in cpm/g. Mean ± SEM. Abbreviations: ^{125}I: iodide-125, cpm: counts per minute.



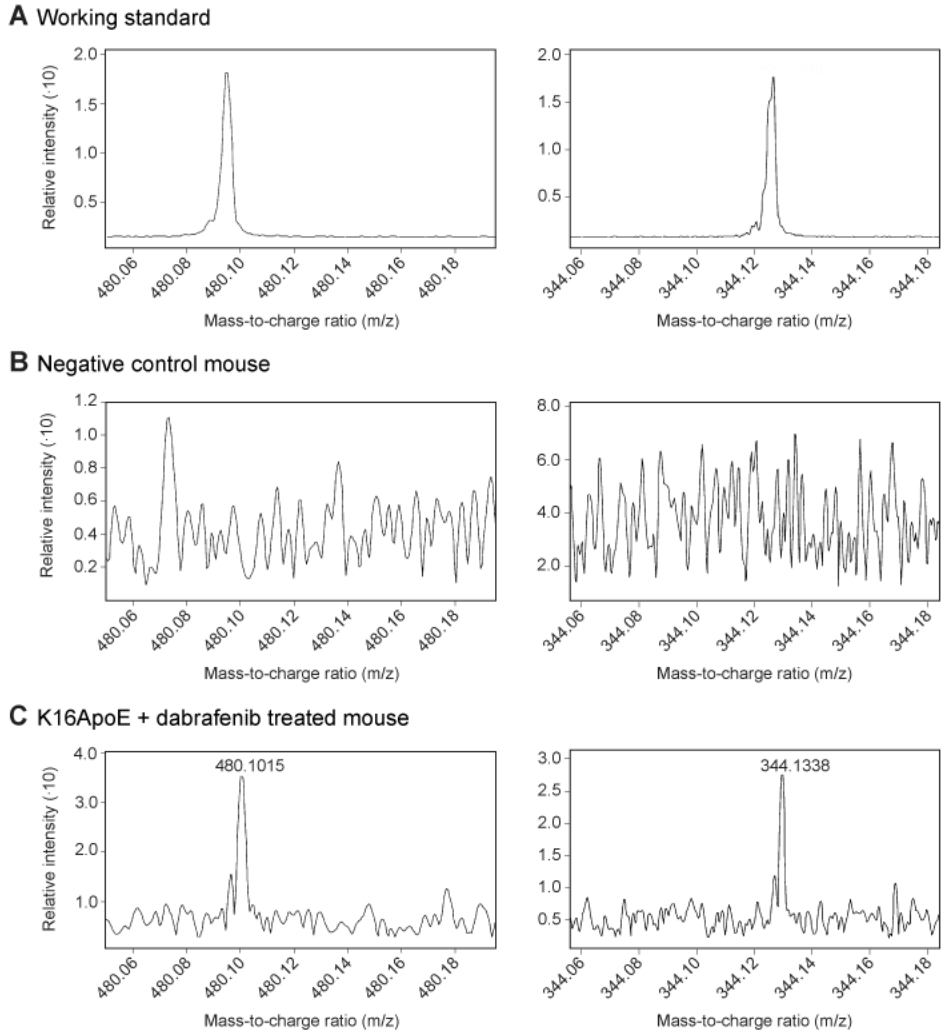
Supplementary Figure S7

Histopathological evaluation of tissue samples after long- and short-term treatment of NOD/SCID mice with the peptide. **A,** Tissue preparations from NOD/SCID mice treated once with the peptide. **B,** Tissue preparations from tumor-bearing NOD/SCID mice treated with the peptide twice a week over six weeks, drug only or vehicle. Scalebar 200 µm.



Supplementary Figure S8

In vivo treatment control experiment. **A,** Mean, total volume of brain metastases four (left) and six weeks (right) after commencing the control experiment, treating mice with saline or K16ApoE only. Mann-Whitney statistical test. Mean ± SEM **B,** Mean, total number of tumors four (left) and six weeks (right) after start of the control experiment. Mann-Whitney statistical test. Mean ± SEM. **C,** Representative contrast enhanced T_1 and T_2 weighted MRI images after four (left) and six (right) weeks. Abbreviations: CE: Contrast enhanced, T_1: T_1 MRI weighting, T_2: T_2 MRI weighting.



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382 **Supplementary Figure S9**

383 Mass spectrometry of treated and untreated brain tissue. **A,** Dabrafenib (m/z 520.108) was fragmented
384 and detected at m/z 480.1 and 344.1, as seen in the working standard. **B,** In the tissue sample
385 originating from the brain of an untreated NOD/SCID mouse, none of these fragments were detected.
386 **C,** In the tissue sample of a NOD/SCID mouse treated with a combination of K16ApoE and
387 dabrafenib, the same fragments as in A were detected.

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Legends to Supplementary Videos

Supplementary Video S1: Sonographic visualization of the axial position of the heart of a NOD/SCID mouse injected with 9 mg/mL NaCl intravenously.

Supplementary Video S2: Sonographic visualization of the axial position of the heart of a NOD/SCID mouse injected with 1 000 µg K16ApoE intravenously.

Supplementary Video S3: A time-lapse series recorded on a Leica TCS SP8 STED confocal microscope, showing a control sample of MDCK cells labeled with Calcein-AM (green), which indicates live cells and Ethidium homodimer-1 (red), indicating dead cells.

Supplementary Video S4: A time-lapse series recorded on a Leica TCS SP8 STED confocal microscope, showing MDCK cells treated with 20 µg of K16ApoE immediately after labelling with calcein-AM, indicating live cells (green) and ethidium homodimer-1, indicating dead cells (red).

Supplementary Video S5: A time-lapse series recorded on a Leica TCS SP8 STED confocal microscope, showing MDCK cells treated with 40 µg of K16ApoE immediately after labelling with calcein-AM, indicating live cells (green) and ethidium homodimer-1, indicating dead cells (red).

Supplementary Video S6: A time-lapse series recorded on a Leica TCS SP8 STED confocal microscope, showing MDCK cells treated with 80 µg of K16ApoE immediately after labelling with calcein-AM, indicating live cells (green) and ethidium homodimer-1, indicating dead cells (red).

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