De novo design of a biologically active amyloid

Article (Supplemental Material)

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Supplementary Materials

Abbreviations
APR – Aggregation Prone Region
Supplementary Materials and Methods

Bioinformatics
We used the TANGO algorithm for all APR identifications in this manuscript. We used a cutoff on the TANGO score of 5 per residue since this gives a Mathews Correlation Coefficient between prediction and experiment of 0.92(1). The settings of TANGO were Temperature = 298K, pH = 7.5, Ionic Strength = 0.10 M.

Peptides
Peptides were synthesized in-house using an Intavis Multi Pep RSi synthesis robot. Peptides were lyophilized stored at -20 °C prior to use. Stock solutions of each peptide were prepared in 1% w/v ammonium bicarbonate in MiliQ water and filtered through 0.22 μm regenerated cellulose filter (Whatman, USA) and their concentration determined by absorbance at 280 nm using the calculated molar extinction coefficient ε=11380 M⁻¹cm⁻¹.

Cell lines and media
HUVEC cells (Lonza) were grown in EGM2 complete medium (Lonza) in flasks pre-coated with 0.1% gelatin. The cells were never allowed to grow confluent and were only used for experiments between passage p3 and p9. U2-Os and HEK293 cells were maintained in DMEM medium, supplemented with 10% FBS, 1 mM sodium pyruvate, non-essential amino acids and antibiotics (pen/strep). Lipofectamine 2000 (Life Technologies) was used to transiently transfect HEK293 and U2-Os cells with an expression vector for VEGFR2 (pCDNA3). All peptide treatments were done in DMEM/F12 medium without additives. Toxicity of the peptide treatments was evaluated using the CellTiter-Blue Cell Viability Assay according to the instructions of the manufacturer (Promega, USA).

Quantification of growth factor signaling
HUVEC, HEK293VEGFR2 and U2-Os cells were treated with peptide for 4h or overnight. Cells were stimulated with 25 ng/ml recombinant mVEGF (or recombinant hEGF)(R&D Systems) for exactly 5 min at 37°C. Cells were washed twice with ice cold PBS and lysed.
Quantification of VEGFR2 autophosphorylation and ERK phosphorylation was done using an electrochemiluminescence ELISA assay (Meso Scale Discovery).

**Solubility Assay**
HUVEC cells were plated at a density of 300K/well in a 6-well plate the day previous to the experiment. On the day of the experiment, cells were either control treated or treated with FITC-labeled B8 or B8scr3 at a concentration of 20μM. After a 4h treatment, the cells were washed twice with ice cold PBS and lysed in TBS lysis buffer (150 mM NaCl, 50 mM Tris pH 7.6, 1% CHAPS, 1x Complete Inhibitor (Roche), 1U/μl Universal Nuclease (Pierce)) for 30 min. In the SDS-resistance experiment, the lysates were incubated for 1h at RT with the indicated SDS concentration. Centrifugation was used to discriminate between the insoluble and soluble fraction. For this, the lysates were centrifuged at 13400 rpm for 20 min at 4°C. The pellet was washed once with TBS lysis buffer. The fractions were boiled in SDS-Page loading buffer (2% SDS) for 10 minutes. A western blot was done, using anti-VEGFR2 (Cell Signaling, 2479S) antibody.

**High Content Microscopy**
For this analysis cells were plated on 96 well plates at 15K cell/well. Images were acquired using an In Cell Analyzer 2000 instrument (GE Healthcare) equipped with an 60X objective lens. The images were analyzed using the InCell developer Toolbox 1.9.2 software (GE Healthcare).

**Proximity Ligation Assay**
The rabbit/goat Texas Red Duolink kit (Olink Bioscience) was used for this experiment. HUVEC cells were seeded at 15K cells per well in a 96-well plate. Cells were treated with 5 μM FITC-labeled B8K11 peptide for 4h. The cells were fixed with 4% formaldehyde for 1h, and blocked and permeabilized with PBS containing 1% BSA and 0.2% Triton X-100 for 1h at RT. The cells were incubated with rabbit anti-VEGFR2 antibody (2479, Cell signaling, 1:400) for 1h and Goat anti-FITC antibody (ab19224, Abcam, 1:100) for 2h in PBS containing 1% BSA at RT. For the rest of the protocol manufacturer’s instructions were followed.

**Super resolution Microscopy using dSTORM**
HUVEC cells were seeded at 35K cells per well in 8-chamber coverslips (155409, Lab-Tek II, Nunc). Cells were treated for 4h with 0.5 μM Alexa647-labeled peptide. Cells were fixed with 4% formaldehyde for 1h, blocked and permeabilized with PBS containing 1% BSA and 0.2% Triton X-100 for 1h and incubated with primary antibody against VEGFR2 (2479, Cell signaling, 1:800). Two-colour super-resolution microscopy was performed using an inverted total internal reflectance fluorescence (TIRF) microscope custom-built for dSTORM imaging, as described previously (2). Alexa Fluor 647 and 568 dyes were excited with a 640 nm diode laser (Toptica) and 561 nm DPSS laser (Oxxius) respectively, and a 405 nm laser was used as a reactivation source. The fluorescence was collected with a 100X/1.49 NA objective (Nikon) onto an EMCCD camera (iXon3 897, Andor). To induce dye photoswitching the samples were immersed in a switching buffer consisting of 100 mM mercaptoethylamine (Sigma) in PBS at pH 8.2 supplemented with an oxygen scavenger to reduce photobleaching (40 mg/ml glucose, 50 μg/ml glucose oxidase, 1 μg/ml catalase). This buffer achieved similar photo switching performance for both dyes. The red and green channels were imaged sequentially, and for each field of view, a series of 10,000 frames was acquired with 15 ms exposure time and irradiation intensities between 1-5 kW/cm². All image stacks were analysed using rapidSTORM 3.3 (3). The chromatic offset between the two channels was corrected with a localization based image transformation using a custom-written MATLAB routine (4). Transformations were obtained from analysis of randomly distributed multicolour fluorescent microspheres (100 nm Tetraspeck, Invitrogen).

Transmission Electron Microscopy (TEM)

For each sample 7 μL aliquots of peptide solution were adsorbed for 1 minute to formvar film coated on 400-mesh copper grids (Agar Scientific Ltd., England) that were first glow discharged to improve adsorption. Grids were washed by contact with 5 drops of ultrapure water and stained by contact with one drop of uranyl acetate (2% w/v) for 45 sec. The grids were examined using a JEM-2100 transmission electron microscope (Jeol, Japan) at 80 keV.

Biophysical characterization

Dynamic light scattering (DLS) measurements were made at room temperature with a DynaPro DLS plate reader instrument (Wyatt, Santa Barbara, CA, USA) equipped with a
830-nm laser source. Samples (100 µL 300 µM peptide stock) were placed into a flat-bottom 96-well microclear plate (Greiner, Frickenhausen, Germany). The autocorrelation of scattered light intensity at a 90° angle was recorded for 10 s and averaged over 40 recordings to obtain a single data point. The Wyatt Dynamics software was used to calculate the hydrodynamic radius by assuming linear polymer particles. Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR FTIR) was performed using a Bruker Tensor 27 infrared spectrophotometer equipped (Bruker, Germany) with a Bio-ATR II accessory (Harrick Scientific Products, USA). Spectra were recorded in the range of 900 –3500 cm⁻¹ at a spectral resolution of 4 cm⁻¹ by accumulating 120 data acquisitions. The spectrophotometer was continuously purged with dried air. Spectra were corrected for atmospheric vapor interference, baseline-subtracted, and vector normalized in the amide II area (1500 to 1600 cm⁻¹) as implemented in OPUS software (Bruker). Tintorial analysis was performed by incubating vascin or its scrambled version at the concentration indicated on each figure with 20 µM ThT, 20 µM ANS or 0.3 µM h-HTAA and fluorescence emission was recorded in a PolarStar Optima plate reader (BMG labtech, Germany) equipped with 490 nm and 520 nm filters with 10 nm band-pass for excitation and emission, respectively. Emission fluorescence spectra of h-HTAA bound to vascin or scrambled vascin were recorded in a FlexStation 3 (Molecular Devices, USA) at the same concentration of peptide and fluorophore listed above.

ESI–IMS–MS analysis.
A Synapt HDMS quadrupole-time-of-flight mass spectrometer (Micromass UK Ltd) equipped with a Triversa NanoMate (Advion Biosciences) automated nano-ESI (nESI) interface was used for these analyses. The instrument has a travelling-wave IMS device situated between the quadrupole and the time-of-flight analysers. The peptide sample was analysed using positive ionization nESI with a capillary voltage of 1.7 kV and a nitrogen nebulizing gas pressure of 0.8 p.s.i. (55.16 mbar). The following instrumental parameters were used: cone voltage 70 V, source temperature 60 °C, backing pressure 3.95 mbar, ramped travelling wave height 7–20 V, travelling wave speed 300 m s⁻¹, IMS nitrogen gas flow 20 ml min⁻¹ and IMS cell pressure 0.55 mbar. Data were processed by the use of MassLynx v4.1 and Driftscope software supplied with the mass spectrometer. The m/z scale was calibrated with aqueous CsI cluster ions.
**X-ray fiber diffraction**

X-ray fiber diffraction samples were made by allowing a droplet of the stock fibril solution to dry between two wax tipped capillary tubes. X-ray diffraction data was collected using a Rigaku rotating anode (CuKa) with Saturn CCD detector with exposure times of 30-60 seconds and specimen to detector distance of 50 or 100 mm. Reflections were measured using CLEARER (Makin et al., 2007).

**Flow cytometry**

HUVEC cells were grown in EGM2 complete medium for 24h, starved overnight in DMEM/F12 containing 20 µM peptide. Cells were trypsinized from the culture plate, aliquoted at approximately 0.5 million cells per tube, and collected by micro centrifugation at 2500 rpm for 7 min. Non-specific binding sites on the cells were blocked with PBS containing 1% BSA for 15 min. Cells were then incubated with a phycoerythrin-conjugated primary antibody against VEGFR2 (FAB357P, R&D systems) or CD29 (FAB17781P, R&D Systems) or with an isotype control for 1h on ice. Cells were washed once and then resuspended in 300 µl PBS containing 1% BSA. Samples were analyzed using a BD FACSCanto II analyzer.

**Neuronal differentiation hiPSCs**

hiPSCs (purchased from Cellectis) were differentiated toward neural-specific progeny in 6 well plates (NUNC) pre-coated with Matrigel (Corning) diluted in DMEM:F-12 (Invitrogen) for 1 h at 37 °C, in a 21% O2 – 5.0% CO2 – 37°C incubator. hiPSC were dissociated to single cells with Accutase (Gibco, Life Technologies) and incubated at 37°C for 5 minutes. Cells were subsequently collected, centrifuged and resuspended in mTesR1 medium (Stem Cell Technologies, 05850) supplemented with 10 µM Y-27632 (Y-27632; 10 µM Millipore, 688001) and plated at high density. Once the cell culture reached 95% confluence, neural induction was initiated by changing the culture medium to neural induction medium (5) for 12 days resulting in neuroepithelial cells. The neuroepithelial cell layer was then broken up into small clumps using Dispase II and plated in laminin coated plates. The subsequent day, NIM was replaced by neural maintenance media (5) supplemented with basic fibroblast growth factor (20 ng/ml) for the first four days. At this stage neural rosettes started appearing. This Dispase II
passage was repeated 2 to 3. Once a pure population of neural progenitors (NPCs) was reached, cells were passaged with Accutase to expand the NPCs. For final plating in the 384 well plates, NPCs were seeded at a density of 1500 cells/well on matrigel, in NMM with medium change every third day. This density allowed maturation of the NPCs towards cortical neurons. After 21 days the cortical neurons were treated with Vascin peptides along with scrambled peptide overnight followed by live-dead staining with Cell Titer Blue. A cell lysate was obtained from each plate for qRT-PCR characterization of the iPSC progeny. All experiments were done in triplicate (biological replicates). To confirm that NPCs were differentiated to cortical neurons, qRT-PCR was performed. Transcript levels for cortical neuron specific genes (CTIP2, FOXG1, SATB2, TBR1 and TBR2) were induced to the same extent in 384 well plate format compared to 24 well plate format and as described (6).

Quantitative Reverse Transcription PCR
Total RNA was extracted from differentiated iPSCs using the RNeasy Mini kit (Qiagen). Total RNA was reverse transcribed to cDNA using SuperScript® III reverse transcriptase (Life Technologies). Quantitative PCR (qPCR) was performed with primers (as described in table xx) and Platinum SYBR green qPCR Supermix-UDG (Invitrogen) with an Eppendorf Realplex (Eppendorf). All samples were run in duplicate and relative quantification was done using the ΔΔCt method (7) with normalization to GAPDH.

Confocal microscopy
HUVEC cells were seeded at 35K cells per well on 8-chamber coverslips (154534, Lab-Tek II, Nunc). Cells were treated with FITC-labeled B8K11 peptide for 4h. Cells were fixed with 4% formaldehyde for 20 min, permeabilized with PBS containing 0.2% Triton X-100 for 10 min and blocked with PBS containing 1% BSA for 1h. Cells were incubated with primary Abs in blocking buffer: mouse anti-S6 Ribosomal protein Ab (2317S, Cell Signaling, 1:25 for 2h) or rabbit anti-Calnexin antibody (sc-11397, Santa Cruz, 1:100 for 1h). After washing with PBS, cells were stained with the anti-mouse secondary conjugated to the Alexa594 dye (Life Technologies) or the anti-rabbit secondary conjugated to Alexa555 (Life Technologies). Prolong Gold anti-fade with DAPI (Life technologies) was used to mount the slides and stain the nuclei. Confocal fluorescent images were obtained by a SP8X Leica confocal microscope.
**In vivo experiments**

All animal procedures were approved by the local animal ethical committee. Female C57BL/6 mice, 8 weeks of age, were purchased from Janvier (France). The B16.F10 melanoma cell line was obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum. B16 cells (5x10^5 cells/mouse) were implanted subcutaneously in the right dorsal flank of C57BL/6 mice. Starting from 3 days after tumor implantation, mice were randomized in three groups and treated daily by intravenous delivery of vascin or scrambled vascin (10mg/kg), or by oral delivery of PTK787 (75mg/kg). Tumors growth was monitored by caliper measurement every 2–3 days starting from 10 days after tumor injection.

**Short Term Toxicology**

Briefly, 8-week-old C57BL/6N females were divided into 3 groups (5 animals/group) and administered 10 mg/kg of vascin (group A), 10 mg/kg of scrambled (group B) or 200 uL of vehicle only (50 mM Tris pH7.5, group C) once a day for 14 days via IV injection. During the treatment period the following clinical, physiological and behavioral parameters were constantly monitored and recorded: body weight, food and water consumption, body condition score, home cage activity and locomotion. Twenty-four hours after the last administration animals were sacrificed by CO2 asphyxiation. Terminal blood collection was also performed via cardiac puncture. Next, the mice underwent complete necropsy with gross examination and organ weights. The following organs/tissues were finally sampled and immersion fixed in 10% neutral buffered formalin: truncal skin, entire head, spine, salivary glands, esophagus, larynx, trachea, lungs, heart, GI tract, liver and gall bladder, pancreas, kidneys, ovaries, uterus, urinary bladder, lymph nodes (cervical, mesenteric), spleen, thymus, left hindlimb, sternum. Formalin-fixed tissues samples were routinely processed and embedded in paraffin blocks for histopathological examination (Thermo Scientific Excelsior™ AS Tissue Processor and HistoStar™ Embedding Workstation). Five μm thick sections obtained from these blocks (Thermo Scientific Microm HM355S microtome) were then stained with Hematoxylin and Eosin (Leica ST5010 Autostainer XL) and evaluated under a Leica DM 2500 light microscope by a board-certified veterinary pathologist. Trimming and orientation of the various organs/tissues collected were performed
following the RITA guidelines (8-10). Microscopic lesions observed during histopathological examination were classified according to the INHAND system (11-17). Histopathological examination was performed in a blind fashion and details concerning experimental design and tested compounds were revealed only at the end of the study. Whole blood samples were collected in BD Microtainer K2E tubes (ref #365955). Haemathology was performed using the scil Vet abc Plus analyzer. Clinical chemistry was conducted on plasma separated from the whole blood. The analysis included the following parameters: albumin, albumin/globulin ratio, alkaline phosphatase, ALT (SGPT), AST (SGOT), bicarbonate, direct bilirubin, indirect bilirubin, total bilirubin, BUN, BUN/creatinine ratio. Combined thioflavin-S (T1892 SIGMA) and Immunofluorescence for IBA1 (Wako Cat. #019-19741) was also applied on additional brain sections. IBA1 staining was performed using a Ventana Discovery Ultra platform. Slides with brain sections were then incubated in an alcoholic solution of 0.1% thioflavin-S for 5 minutes. After a brief differentiation step in 70% EtOH, the sections were thoroughly rinsed in PBS, counterstained with DAPI (D9542 SIGMA) and finally coverslipped with Mowiol-based mounting medium (324590 ALDRICH).

Fluorescence imaging

Whole-body fluorescence imaging of tumor-baring mice injected with B8-FITC or vehicle was carried out under isoflurane anaesthesia on an IVIS Spectrum system with Living Image software (version 4.5.2, Perkin Elmer, Waltham, Massachusetts) using the excitation/emission 500/540 filter pair in epi-fluorescence mode. After in vivo imaging, mice were sacrificed, tumors dissected and imaged under ex vivo conditions using similar settings. The fluorescence signal intensity of B8-FITC and tumor dilution series in 96-well plates was imaged and quantified after flat field correction for a region of interest covering each well.

Fibered confocal fluorescence microscopy (FCFM)

The CellVizio single band system with 488 nm laser for intravital FCFM was used in combination with the ProFlex z-type probe (PF-2294, Mauna Kea Technologies, Paris, France) providing 49 µm axial resolution, 3.5 µm lateral resolution and a working distance of 93 µm for a probe diameter of 1.8 mm. Software provided by the manufacturer was used for acquisition, visualization and quantification of the data (Image Cell 3.6.0 and IC-viewer 1.1.0). For each sample at least 300 frames were
acquired for which mean fluorescence signal intensity was measured and averaged from 5 randomly positioned regions of interest (ROI) of 150 µm diameter. 

Graphpad Prism (v5.04) was used for data presentation and statistical analysis.

Cross-seeding experiments

Aβ1-40 lyophilized from hexa-fluoro-isopropanol (rPeptide) was resuspended to a concentration of 1 mg/ml (231 µM) in 2mM NaOH and stored in aliquots at -20°C until needed. At time of experiment the peptides were diluted to 10 µM in PBS (140 mM NaCl, 2,7 mM KCl, 10 mM PO₄³⁻, Medicago). His-tagged HuPrP23-231 was purified from E. Coli as previously described (Nyström et al JBC, 2012). Briefly the pelleted cells were lysed by sonication in 6 M GuHCl. The supernatant was applied to NiNta agarose (Qiagen), washed with gradually lower concentration of GuHCl down to 0 M to allow on column refolding to native state. The protein was eluted by imidazole addition prior to SEC using a superdex75 16/60 column and Buffer F (100 mM NaCl, 50 mM KCl, 50 mM Na₂HPO₄). The protein was diluted to 5 µM for the experiments. B8 fibrils were produced as described above. Content of fibrils was verified by ThT fluorescence scan using 50 µM (in respect to monomer) and 2 µM ThT. Unstained fibrils for self-seeding of Aβ and HuPrP90-231 were collected after 24 hours from previous kinetic experiments and were ex situ assayed for amyloidogenicity by addition of 2 µM ThT. For the fibrillation assays, ThT was added to a final concentration of 2 µM to the assayed proteins. 600 µl aliquots were prepared and fibrils were added to reach 1% of the molar concentration of substrate in respect to monomers in the fibril preparations. Corresponding volume of buffer was added to the unseeded samples. The samples were distributed as 100 µl aliquots in Costar 3880 plates (black, non-treated, half area with transparent bottom, Corning) and the plate was sealed with sealing tape. The fibrillation kinetics was monitored by ThT fluorescence at 480 nm through the bottom of the plate every 15 minutes over the time course of the experiment. For Aβ fibrillation the plate was kept stagnant at 37°C between measurements but was shaken for 60 seconds prior to measurement. For HuPrP fibrillation the plate was vigorously shaken at 37°C but allowed to rest during measurement.
Supplementary Text

Supplementary Note 1 – Scrambling as a control for sequence specificity

The total number of (different) permutations of strings with \( n_i \) characters of type \( i \) is given by \( m! / \prod n_i! \) (where \( m \) is the total peptide length and \( n \) the number of instances of amino acid \( i \)). Hence for the sequence of vascin, which has 4 instances of D, L and A and 2 instances of V, W, F, and P, the total number of permutations is \( 4.2 \times 10^{15} \), which is an intractably large number. Given that our purpose was to obtain a sequence composed of the same amino acids that is completely dissimilar to the original, we generated a random selection of 50 scrambled sequences, and selected the one with the least number of conserved positions. This yielded the sequence indicated that does not share any tripeptide segment or larger segment with vascin.

Vascin \[ \text{DLAVLWFDPPDLAVLWFD} \]
Scrambled \[ \text{AADPFDLWVAPADFLVDLLW} \]

Supplementary Note 1 – Proline mutations as a control for aggregation propensity

To probe the importance of the APRs in the vascin design, we designed an additional type of control sequence where we do not rely on permutation, but instead introduced proline substitutions to destroy the beta-sheet potential of the APRs, while maintaining the overall scaffold of the peptide, including the tandem repeat, the linker, the position of the gatekeepers, and the overall hydrophobicity. This particular control better addresses the role of aggregation and beta-sheet formation and through its orthogonality to the scrambled sequence offer additional confidence about the specificity of the activity observed with vascin. We tested four proline substitution mutants in the pERK and pVEGFR2 inhibition assays on HUVEC cells, as well as CellTiter Blue toxicity assays on the same cells. We finally selected the first proline mutant for all subsequent work since we did not observe significant inhibitory effects with any of them.

Vascin \[ \text{DLAVLWFDPPDLAVLWFD} \]
Proline1 \[ \text{DLAPALWFDPPDLAPALWFD} \]
Proline2 \[ \text{DPAPALWFDPPDPAPALWFD} \]
Proline3  DLAVAPWPDPDLAVAPWPD
Proline4  DPAPALWFDPDLAVAPWPD
Supplementary Figures

**Supplementary Figure 1** – TANGO analysis of VEGFR2 and identification of vascin.

**(A)** Shows the TANGO score (red line) per amino acid position of the VEGFR2 sequence as well as an outline of its domain structure. **(B)** Sample data from the screening of the peptides listed in Supplementary Table 2. The image is a western blot of cell lysates Hek293 cells transiently expressing mouse VEGFR2, treated overnight with 20 µM peptide and stimulated for 5 mins with VEGF. The upper bands show phospho ERK staining, whereas the lower bands show total ERK. Vascin is peptide B8 in this screen.
Supplementary Figure 2 – Sequence, purity and mass spectrum of vascin. (A) The aminoterminal region of human and mouse VEGFR2 from which the 7 amino acid sequence fragment was taken for the design of vascin. (B) Schematic representation of the design of vascin, showing the position of the aggregation prone region (APR), gatekeepers and linker residues. (C) HPLC analysis of the raw vascin as obtained after solid phase peptide synthesis. The retention time and relative abundance (in parenthesis) are given for the main peaks. The first non-integrated peak corresponds to
the injection buffer (guanidinium hydrochloride). (D) Mass spectrum of the main peak observed in (C).

Supplementary Figure 3 – Vascin forms oligomers, protofibrils and amyloid fibrils. TEM images of 300 µM vascin in 1% (w/v) NH₄CO₃ after 0, 6 or 24 h incubation at room temperature.
Supplementary Figure 4 – Kinetics of Vascin aggregation. (A) DLS autocorrelation curves recorded during the incubation 300 μM vascin in 1% (w/v) NH₄CO₃. The arrow indicates the progression of the change in characteristic autocorrelation decay time as the aggregation progressed. Autocorrelation curves from Dynamic Light Scattering (DLS) of 300 μM vascin in 1% (w/v) NH₄CO₃. (B) Average particle hydrodynamic radius change over time during vascin aggregation as calculated from the DLS autocorrelation curves. (C) Aggregation kinetics of vascin at concentrations from 0.6 μM (grey dots) to 330 μM (red dots) in 1% (w/v) NH₄CO₃ as monitored by ANS emission fluorescence. The arrow indicates the sense of increase in protein concentration. (D) Emission fluorescence spectra of h-HTAA bound to 300 μM vascin or scrambled vascin. Each spectrum is the average of triplicates and has been blank subtracted.
Supplementary Figure 5 – *vascin* aggregation at 30 uM. (A) DLS autocorrelation curves recorded during the incubation 30 µM scrambled *vascin* in 1% (w/v) NH₄CO₃. (B) Histogram of size distribution of particles calculated from the DLS data recorded in (A) using a linear polymer as particle model. (C) TEM images of 30 µM scrambled *vascin* in 1% (w/v) NH₄CO₃ after 0 h (upper panels) and 24 h (lower panels) incubation at room temperature. (D) Fourier Transform Infrared Spectrum (FTIR) of 30 µM *vascin* in 1% (w/v) NH₄CO₃ monitored over 120 mins.
Supplementary Figure 6 – Scrambled vascin aggregation. (A) DLS autocorrelation curves recorded during the incubation 300 μM scrambled vascin in 1% (w/v) NH₄CO₃. (B) Histogram of size distribution of particles calculated from the DLS data recorded in (A) using a linear polymer as particle model. (C) TEM images of 300 μM scrambled vascin in 1% (w/v) NH₄CO₃ after 0 h (upper panels) and 24 h (lower panels) incubation at room temperature.
**Supplementary Figure 7 – Proline vascin aggregation.** (A) DLS autocorrelation curves recorded during the incubation 300 µM proline vascin in 1% (w/v) NH₄CO₃. (B) Histogram of size distribution of particles calculated from the DLS data recorded in (A) using a linear polymer as particle model. (C) TEM images of 300 µM proline vascin in 1% (w/v) NH₄CO₃ after 4 h (upper panels) and 24 h (lower panels) incubation at room temperature.
Supplementary Figure 8 – Human vascin aggregation. (A) DLS autocorrelation curves recorded during the incubation 300 µM human vascin in 1% (w/v) NH₄CO₃. (B) Histogram of size distribution of particles calculated from the DLS data recorded in (A) using a linear polymer as particle model. (C) TEM images of 300 µM human vascin in 1% (w/v) NH₄CO₃ after 4 h (upper panels) and 24 h (lower panels) incubation at room temperature. (D) Fiber x-ray diffraction pattern of fibrils formed in 300 µM human vascin in 1% (w/v) NH₄CO₃.
Supplementary Figure 9 – *Vascin* decreases cell surface expression of VEGFR2. FACS analysis of (A) non treated HUVEC cells or (B) treated with 20 μM *vascin* or (C) *vascin* scrambled for 4 h analyzed for the abundance of VEGFR2 (upper panels) or Beta1 integrin (lower panels).
Supplementary Figure 10 – Short term toxicology. Mice were treated daily for 14 days with 10 mg/mg of vascin (group A), scrambled control (group B) or vehicle (group C). Five animals were used per group. (A) Overview of the histopathological findings. The most commonly recorded changes (e.g. inflammatory cell foci in different tissues, reactive follicular hyperplasia of spleen and lymph node, scattered tubular hyaline casts in the kidney) represent spontaneous and not treatment-related findings that are often
reported in subacute or chronic toxicology studies. Incidental findings associated with mouse strain (e.g. congenital portosystemic shunts) or experimental manipulation (e.g. hair shaft pulmonary embolism induced by repeated IV injection) were also reported in few cases. (B) Organ weight. R and L stand for Right and Left. (C) Haematology. WBC - White blood cell, RBC - Red blood cells, HGB - Hemoglobin, HCT - Hematocrit, MCV - mean volume of erythrocytes, MCH - Mean content of hemoglobin, MCHC - Mean conc. of hemoglobin in single erythrocytes, PLT – Platelets.

Supplementary Figure 11 – Thioflavin S (green) and GFAP (glial fibrillary acidic protein, red) staining of tissue sections of hippocampus and cortex of the animals from the toxicology study. Mice were treated daily for 14 days with (A) 10 mg/mg of vascin, (B) scrambled control or (C) vehicle. (D) The positive control is a 7-month old female mouse with transgenic expression of mutant APP and PSEN1 [C57BL/6-Tg(Thy1-APPSw,Thy1-PSEN1*L166P)21 (18). The scale bar is 1275 µm.
Supplementary Figure 12: FITC-vascin is present inside B6 tumors. (A)
Photograph (a) and fluorescence image overlay (b) of tumors isolated from a representative mouse injected with B8-FITC (left) or vehicle (PBS, right). (c) Fluorescence images from 2-fold dilution series of tumors isolated from vehicle-injected control mice (n = 3) and B8-FITC-injected mice (n = 5), illustrating the presence of B8-peptide inside the tumors based on FITC-fluorescence. (B) Representative fibered confocal fluorescence microscopy images acquired from tumor tissue samples isolated from different mice injected with B8-FITC (left column) or vehicle (PBS, right column), demonstrating the presence of fluorescent B8-peptide and peptide aggregates inside tumor samples at cellular resolution. Scale bars measure 50 µm.
Supplementary Figure 13: Fluorescence-based estimation of B8-FITC concentration in tumors. Fluorescent signal intensity measured for twofold dilution series of pure B8-FITC (left graphs) and B8-FITC added to tumor tissue (right graphs), quantified from fluorescence imaging (A) and FCFM (B), demonstrating linearity of both fluorescence intensity with peptide concentration and fluorescent signal attenuation by tumor tissue over a wide range of peptide concentrations. R-square and p-values are indicated on each graph. Error bars represent standard deviation of quintuple samples.
Supplementary Figure 14: Effect of vascin on VEGFR2 transiently expressed in Hek293 cells. (A) Western blot analysis of ERK phosphorylation upon VEGF stimulation of HEK293\textsuperscript{VEGFR2} cells treated with vascin. Total ERK levels were assessed on the same samples as loading control. (B) Western blot analysis of the partitioning of VEGFR2 between the soluble and insoluble fraction of HEK293\textsuperscript{VEGFR2} and treated with 20 µM vascin.
Supplementary figure 15: Gene expression of key cortical neuronal genes (CTIP2, FOXG1, SATB2, TBR1 and TBR2) in progeny hiPSC line (n = 3 independent experiments). Error bars indicate mean ± S.D.
Supplementary Tables

Supplementary Table 1: Selected Aggregation prone regions from VEGFR2_MOUSE.

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Supplementary Table 2: Peptide sequences screened for ability to inhibit VEGFR2 signalling in transfected Hek293 cells, which lead to the identification of vascin.

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Supplementary References


