Supporting Information

Peptide Handling and Storage

RADA16 peptides were custom-made and purchased from Genscript (Piscataway, NJ, USA) and Auspep (Tullamarine, Victoria, Australia). For ease of handling, 500µl RADA16 peptide stock solutions (1% w/v) were prepared by dissolving in 0.1% Trifluroacetic acid (TFA) solution. Dilutions of 0.5%, 0.1% and 0.05% were made from the 1% stock solution and subsequently stored at -20^{o}C. Prior to use in tissue culture experiments, frozen peptide solutions were defrosted at room temperature and sonicated for 20 min.

Atomic Force Microscopy (AFM)

Atomic force microscopy utilized two different sample preparations: a "drop and dry" preparation and a "wet gel" preparation. The "drop and dry" preparation first involved depositing 10µl of 1% peptide solution onto a clean piece of silicon wafer. After 30s, the peptide solution was rinsed with deionised water and left to dry until no visible water droplets were seen whereupon imaging was immediately undertaken. For "wet gel samples", 10µl of 1% peptide solution was deposited on a clean piece of silicon wafer. 10µl of cell culture media was gently added onto the peptide solution to induce gelation. The gel was left for 15mins in ambient air and then immediately scanned. Images were obtained in tapping mode on the Dimension 3100 microscope (Digital Instruments). Silicon nitride tips with spring constants of 50 Nm^{-1} (Mikro Mash) were used and imaging was carried out at a scan rate of 1 Hz. The Root Mean Square (RMS) roughness value for the respective samples was determined using Gwyddion SPM software (version 2.9).

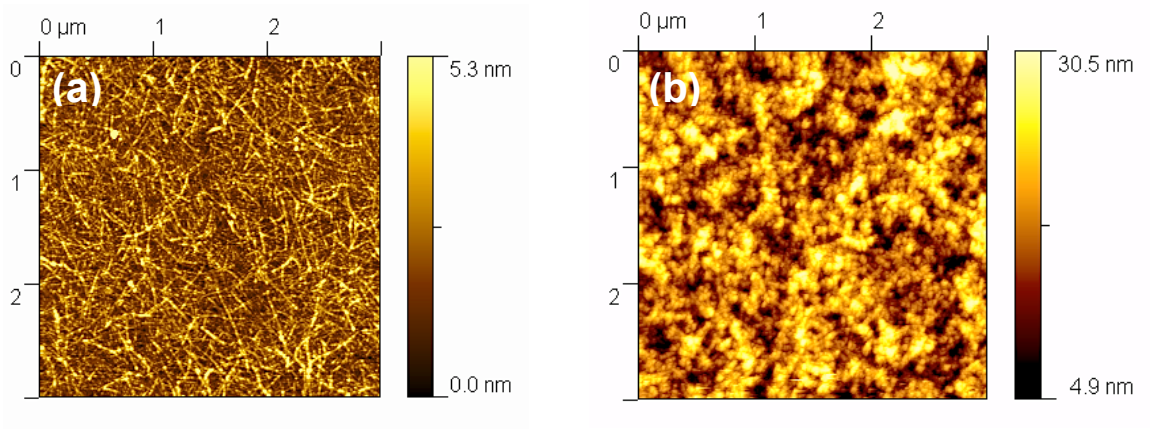

Fig S1. AFM height images of (a) "drop and dry" and (b) "wet gel" samples. Morphological differences between samples were attributed to the dry and hydrated states respectively. RMS values of 0.98nm and 2.94nm in the dry and hydrated states respectively also reflect a change in topography.

Scanning Electron Microscopy (SEM)

Prior to SEM imaging, samples with and without cells were fixed in 2.5% glutaraldehyde for 2 h at 4^{o}C and dehydrated. Samples were washed with deionized water and dehydrated in a microwave in serial concentrations of ethanol (50%, 70% and 90% once then 3x in absolute ethanol), before critical point drying with carbon dioxide for 1h and then coating with 5nm of platinum. Peptide concentrations of 1%, 0.5%, 0.1% and 0.05% were imaged using a Zeiss 1555 VP-FESEM.

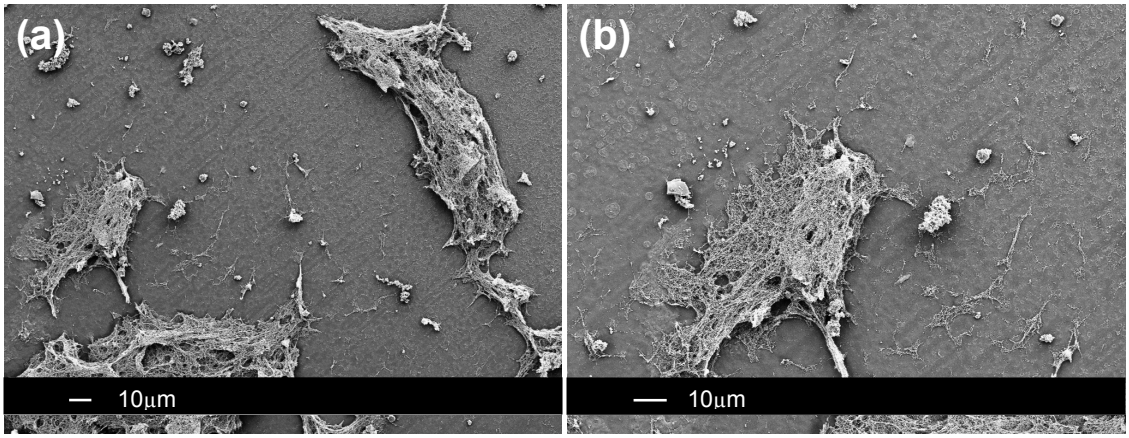

Fig S2. SEM micrographs of RADA16 SAPNS which formed "clumps"at 0.05% .(a) 1.3k x and (b) 2.3k x magnification.

Preparation of Cell Culture Plates

Mixed retinal cells were cultured on 10mm glass coverslips (ProSciTech) in 12-well cell culture plates (Costar). Prior to culture, coverslips were coated with 1 mg ml^{-1} Poly-L-Lysine (PLL, Sigma) in sterile distilled water overnight at 37^{o}C, dried at room temperature and 30µl of peptide solution deposited onto the centre of the coverslips. Gelation and scaffold formation was facilitated by the addition of 30µl Neurobasal + 10% foetal calf serum (growth medium, Gibco) onto the peptide. Once gelation had occurred, the scaffold was washed twice with growth media with the final wash remaining in the wells until cells were ready to be plated.

Isolation and Culture of Retinal Cells

Retinae were dissected from 1-3 day old PVG rat pups (supplied by Animal Resources Centre, WA Australia). All procedures were conducted in accordance with ethical guidelines and were approved by The University of Western Australia Animal Ethics Committee.

Dissected retinae were enzymatically digested using 165U papain (Worthington) activated with cysteine (Sigma) at 5mg ml^{-1} in HBSS at 37^{o}C for 30min. Retinae were gently agitated in solution every 10min during the digestion and then tissue was dissociated by gentle tituration in growth media using a sterile Pasteur pipette.
The resultant cell suspension was filtered through sterile nylon gauze (20µm diameter, Setar filter specialists) and gently re-titurated. Cell numbers were adjusted to 6.65 x 10^5 cells/cm^2 and plated onto coverslips on which the RADA16 had previously been gelled in 12 well culture plates in 0.5ml of growth medium.

After 24hr incubation at 37^{0}C, 5% CO_2, the cells and gels were fixed. 250µl of growth medium was replaced with 250µl 4% paraformaldehyde (Merck) in PBS. The resultant growth medium/paraformaldehyde mixture was then replaced by 500µl of 4% paraformaldehyde in PBS. Cells and gels were left for 20min for full fixation. whereupon the paraformaldehyde was replaced with PBS/Sodium Azide (Sigma) buffer. Cell culture plates were stored at 4^{o}C until immunohistochemical staining.

Immunohistochemical Staining

Mixed retinal cultures were immunohistochemically stained for retinal ganglion cells (RGCs: β-III tubulin) and astrocytes (GFAP). The PBS/Sodium Azide buffer was removed and the wells washed 3 times with PBS. Cells were permeabilised with PBS containing 0.2% Triton X-100 for 20mins whereupon the cells and gels were incubated with 200µl of primary antibodies (anti-β-III tubulin 1:1000, Jomar Bioscience; anti-

GFAP 1:1000, DAKO) for 2h at room temperature on a shaker followed by 48h at 4^{o}C. Following PBS washes, antibody binding was visualised with anti-mouse Alexa fluor 488 (1:400. Molecular Probes) and anti-rabbit Alexa fluor 546 (1:400, Molecular Probes) following incubation for 24h at room temperature. Coverslips were mounted on glass slides covered with Fluoromount G (Southern Biotechnology Associates, Inc.). All experiments included triplicate cultures for each peptide concentration and each experiment was performed three times.

Confocal and Fluorescence Microscopic Analysis

Immunohistochemically stained samples were analysed using confocal and fluorescence microscopy. An area toward the centre of the coverslips was selected for analysis, Due to low astrocyte numbers, fluorescence microscopy at a lower magnification was used for quantification.

Confocal microscopy was carried out using a Leica TCS SP2 AOBS Multiphoton Confocal microscope (magnification 40x objective) and fluorescence microscopy with a Diaplan fluorescence microscope (magnification 10x objective). Control sections stained only with secondary antibodies were included in all experiments and showed no, or only minimal, fluorescence (data not shown).

Image and Statistical Analysis

To determine the effect of RADA16 SAPNS on mixed retinal cell cultures, 3 outcomes were measured: (a) the length of the longest neurite on all RGCs in all images, assessed using Image J analysis software (version 1.42q, NIH), (b) the numbers of RGCs in each

field of view (40x objective) and (c) the numbers of astrocytes in each field of view (10x objective). All immunohistochemical analyses were conducted by a single investigator, ensuring constant selection criteria, and results expressed as means ± SEM.

Data were analysed using Statview data management software to conduct ANOVA on groups of data. Statistically significant differences between each treatment were determined using Bonferroni/Dunn *post hoc* tests ($p \leq 0.05$).