Electronic Supplementary Information (ESI):

Directional Molecular Sliding Mvement in Peptide Hydrogels Accelerates Cell Proliferation

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CONTENTS

1. Structure and Characterization of Peptides

General Materials. Rink amide 4-methyl-benzhydrylamine (MBHA) resin (Loading density: 0.436 mmol / g) and all Fmoc-protected amino acids used in solid-phase peptide synthesis were obtained from Bide Pharmatech Co., Ltd. (China). Organic solvents for peptide synthesis and purification were provided by Tianjin Concord Technology Co., Ltd. Water used in all experiments was purified by Arium Pro Ultrapure water systems (Sartorius, 18.2 MΩ). Hydrogen peroxide (30% H_2O_2) and Tris (2-Carboxyethyl) Phosphine Hydrochloride (TCEP·HCl) were purchased from J&K Scientific LTD.

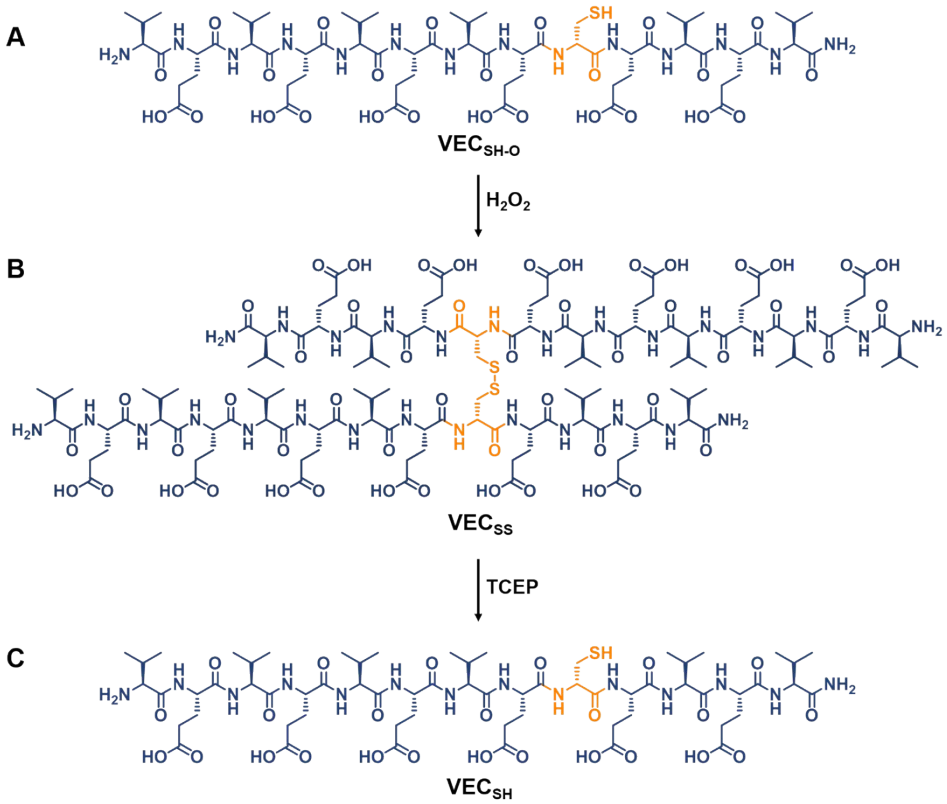

Figure S1. Chemical structures and the synthetic route of peptides **VEC$_{SH-O}$** (A), **VEC$_{SS}$** (B), and **VEC$_{SH}$** (C).

The structure and purity of the synthesized peptides **VEC$_{SH-O}$**, **VEC$_{SS}$**, and **VEC$_{SH}$** were characterized by analytical HPLC (Agilent 1260) and MALDI-TOF mass spectroscopy (Autoflex III TOF/TOF200), respectively.

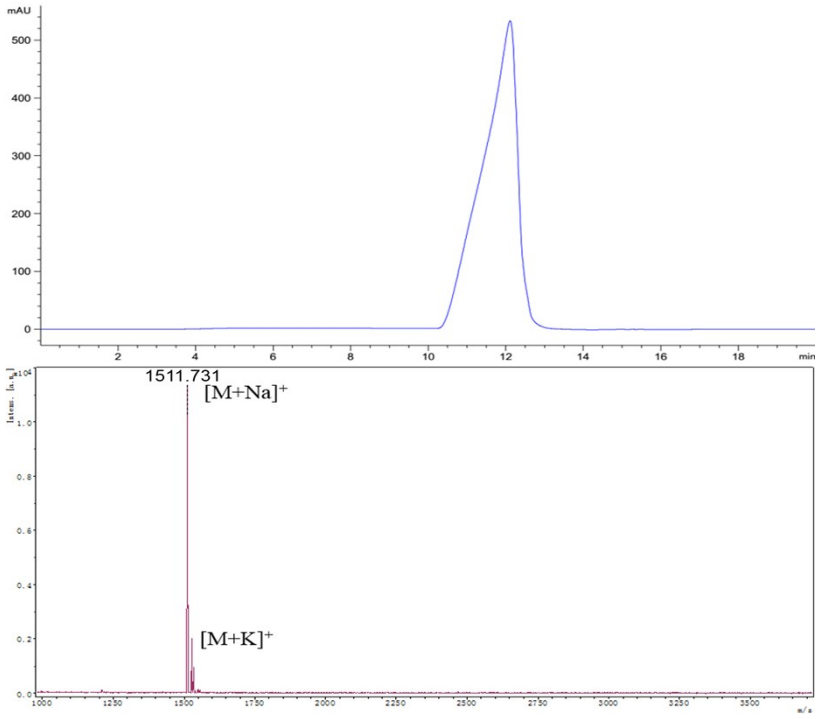

Figure S2. Analytic HPLC trace and MALDI-TOF mass spectrum of peptide **VEC$_{SH-O}$**.

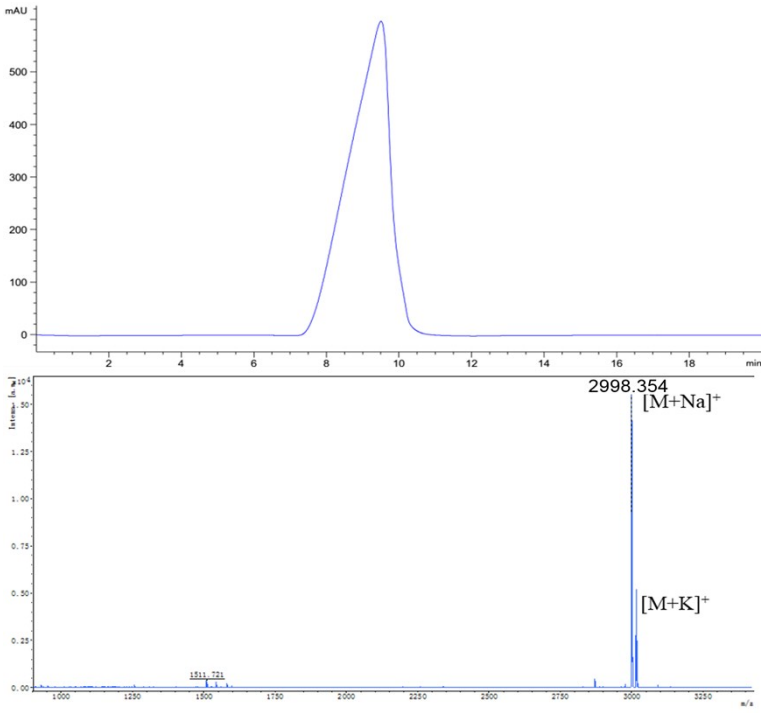

Figure S3. Analytic HPLC trace and MALDI-TOF mass spectrum of peptide $\mathbf{VEC_{SS}}$.

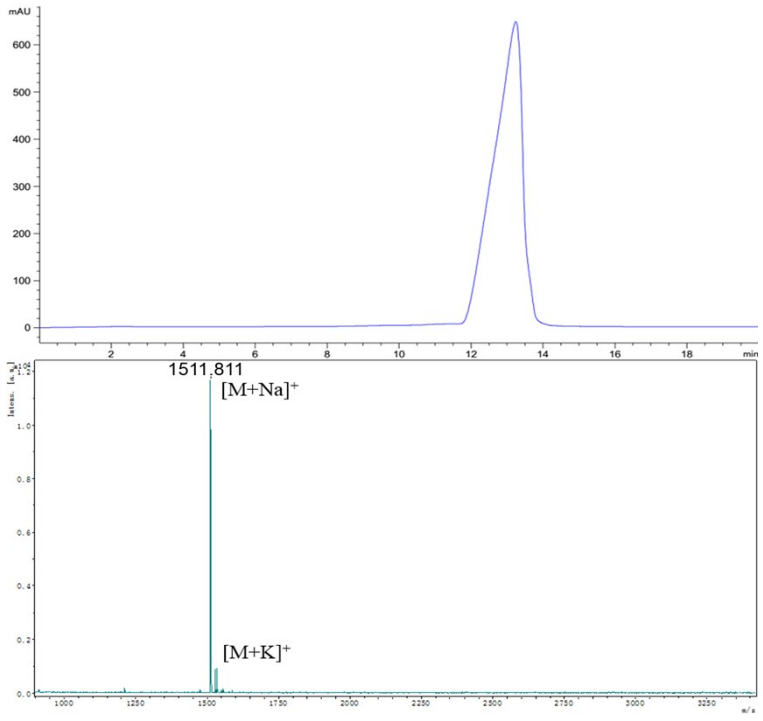

Figure S4. Analytic HPLC trace and MALDI-TOF mass spectrum of peptide $\mathbf{VEC_{SH}}$.

2. Characterization of Peptide Conformation and Assemblies

All the samples for the conformational and assembly characterization of peptides **VEC_{SH-O}**, **VEC_{SS}**, and **VEC_{SH}** were prepared from the stock solution of peptides. Annealing peptide **VEC_{SS}** solution led to significant oxidation of disulfide bonds to sulfinic or sulfonic acids (-SO2H / -SO3H) potentially due to the presence of remained trace amount of H_2O_2. Hence all the stock solutions of peptides were prepared by aging the fresh solution for 2 days to achieve the thermodynamic equilibrated assemblies under a constant condition. To maintain an identical mass concentration for different peptides, the concentration of the stock solution of peptides **VEC_{SH-O}**, **VEC_{SH}**, and **VEC_{SS}** was maintained as 4, 4, and 2 mM, respectively.

2.1 Circular Dichroism (CD) Spectroscopy.

CD experiments were performed on a spectrometer (Biologic MOS-500) using 0.2 cm quartz cuvettes. All scans were recorded with a wavelength interval of 1.0 nm and an acquisition time of 1 s at 25 °C. To maintain an identical mass concentration for different peptides, CD samples at a concentration of 50 μM for **VEC_{SH}** or **VEC_{SH-O}** and 25 μM for **VEC_{SS}** were prepared by diluting the stock peptide solution (4 mM for **VEC_{SH-O}** or **VEC_{SH}** and 2 mM for **VEC_{SS}**)

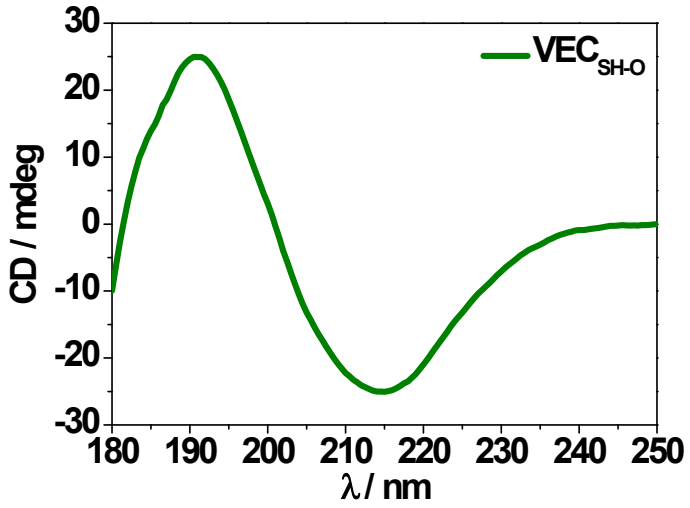

Figure S5. CD spectrum of **VEC$_{SH-O}$** showed the signal features for *β*-sheets.

2.2 Fourier Transform Infra-Red Spectroscopy (FTIR).

FTIR experiments were carried out on a Tensor II FTIR spectrometer (Brucker). All FTIR samples were prepared by dropping the stock solutions (4 mM for **VEC$_{SH-O}$** or **VEC$_{SH}$** and 2 mM for **VEC$_{SS}$**) on the quartz detector under the light source. The FTIR signals were collected at room temperature in a wavenumber region from 4000 to 400 cm^{-1}, while only the region corresponding to the absorption of amide I vibration was shown in the Figures for clarity.

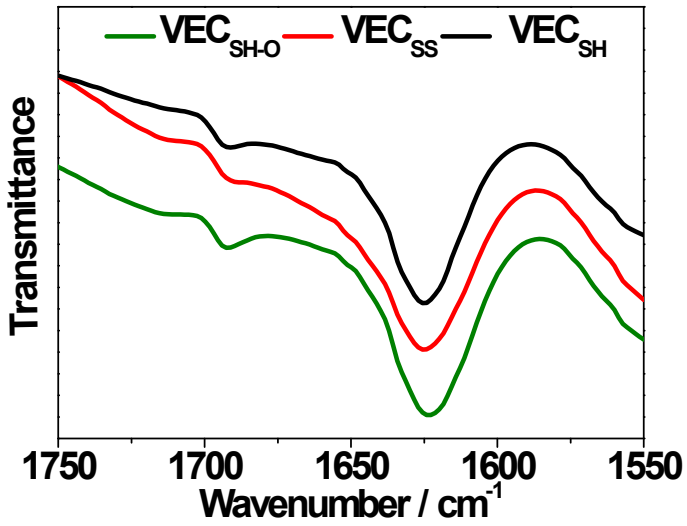

Figure S6. FTIR spectra of peptides **VEC$_{SH-O}$**, **VEC$_{SS}$**, and **VEC$_{SH}$**. All peptides showed two transmission bands in the regions of 1640-1620 cm^{-1} and 1695-1690 cm^{-1}, which are associated with amide I vibration within anti-parallel β-sheets.

2.3 Wide-Angle X-Ray Scattering (WAXS).

WAXS experiments were carried out on the Xeuss WAXS system (Xenocs, France) at The National Center for Nanoscience and Technology in Beijing. The powders of samples were obtained by lyophilizing the stock solution of peptides (4 mM for **VEC$_{SH-O}$** or **VEC$_{SH}$** and 2 mM for **VEC$_{SS}$**) and were scanned under conventional condition for 1 h.

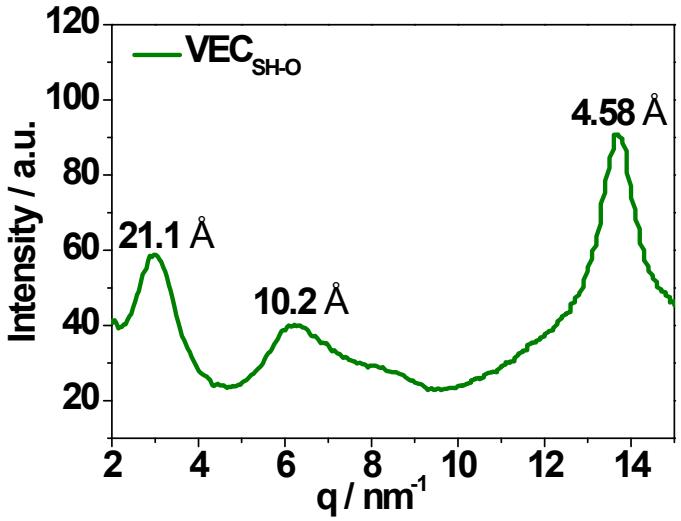

Figure S7. Wide-angle X-ray scattering profile of the powder of peptide **VEC$_{SH-O}$**.

2.4 Critical Aggregation Concentration (CAC).

The CAC values of peptide **VEC$_{SS}$** and **VEC$_{SH}$** were estimated using Nile Red as the fluorescent probe based on the emission shift of Nile Red associated with the microenvironment.[1] The solution of peptides (2 mL) with different concentrations were prepared by diluting from the stock solutions (4 mM for **VEC$_{SH-O}$** or **VEC$_{SH}$** and 2 mM for **VEC$_{SS}$**) in small vials. Subsequently, 2 μL of the stock solution of Nile Red (100 μM) in ethanol was added to each vial, leading to a concentration of 100 nM for Nile Red in the solution of peptides. The peptide samples with Nile Red were aged overnight prior to measurement. Fluorescence spectra were recorded on an Agilent Cary Eclipse fluorescence spectrophotometer with the excitation of 550 nm. Plotting the wavelength of the maximal fluorescent emission of Nile Red as a function of the concentration of peptides results in the curve for estimation of CAC values.

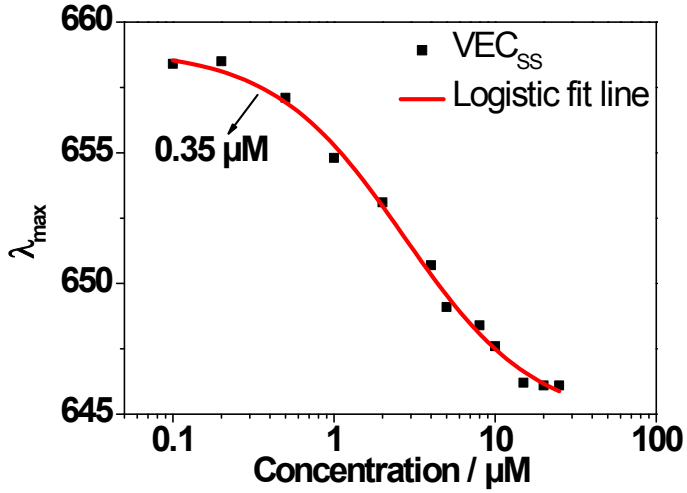

Figure S8. The wavelength of the maximal emission (λ_{max}) of Nile Red in the presence of peptide $\mathbf{VEC_{SS}}$ at a concentration in the range of 0.1 and 25 μM. Based on the CAC determination, peptide $\mathbf{VEC_{SS}}$ starts to aggregate below the concentration of 0.35 μM.

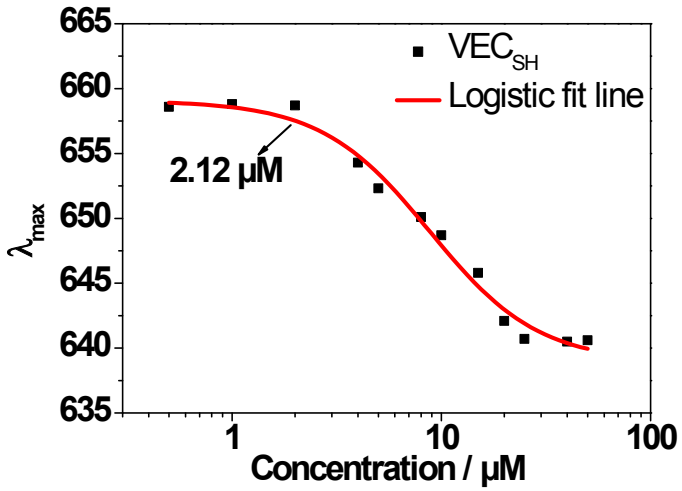

Figure S9. The wavelength of the maximal emission (λ_{max}) of Nile Red in the presence of peptide $\mathbf{VEC_{SH}}$ at a concentration in the range of 0.5 and 50 μM. Based on the CAC determination, peptide $\mathbf{VEC_{SH}}$ starts to aggregate below the concentration of 2.12 μM.

2.5 Transmission Electron Microscopy (TEM).

TEM images were taken by a Tecnai G2 F20 microscope at 100 kV accelerating

voltage. TEM samples were prepared by diluting the stock solution of peptides to 100 μM for **VEC$_{SH-O}$** or **VEC$_{SH}$** and 50 μM for **VEC$_{SS}$**. The diluted peptide solution was pipetted onto the surface of carbon-coated copper grid and allowed to stand for 4 min, then blotted by filter paper. Subsequently a drop of 2 wt % uranyl acetate was placed on the grid for staining and blotted by filter paper after 2 min. The grid was dried in a desiccator prior to measurement.

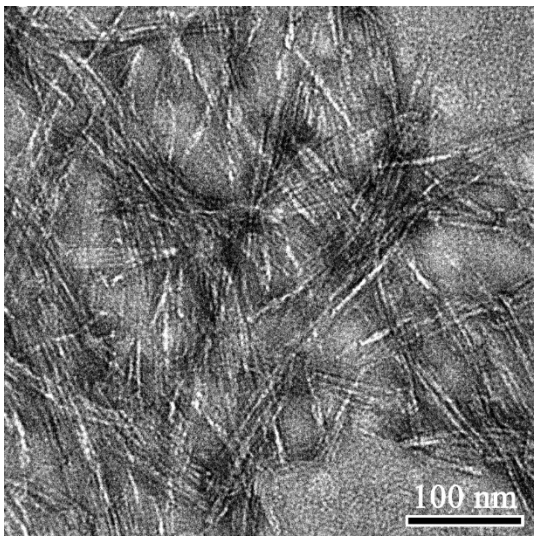

Figure S10. TEM image of peptide **VEC$_{SH-O}$**.

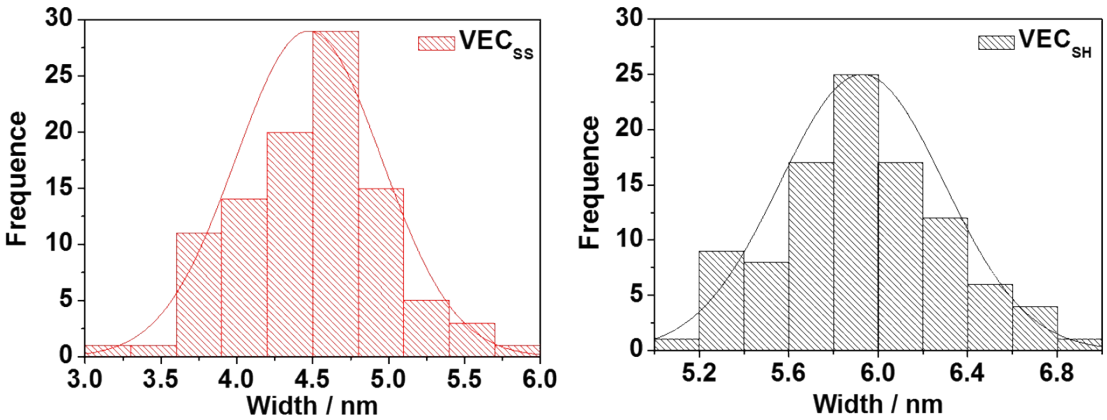

Figure S11. The histogram distribution of the widths of the nanofibers formed by

peptides **VEC$_{SS}$** and **VEC$_{SH}$** based on the measurements (N=100) of the nanofibers in the TEM images shown in Figure 3A and B, which provided an average width of 4.47 and 5.93 nm for the nanofibers formed by peptides **VEC$_{SS}$** and **VEC$_{SH}$**, respectively.

2.6 Atom Force Microscopy (AFM).

AFM studies were carried out on a Bruker ICON instrument under the tapping mode. AFM samples were prepared by diluting the stock solution of peptides to a concentration of 25 μM for **VEC$_{SS}$** and 50 μM for **VEC$_{SH-O}$** or **VEC$_{SH}$** and depositing on the freshly cleaved mica surfaces for 2 min. The retained solution on mica was removed by filter papers and the samples were dried in a desiccator prior to AFM experiments.

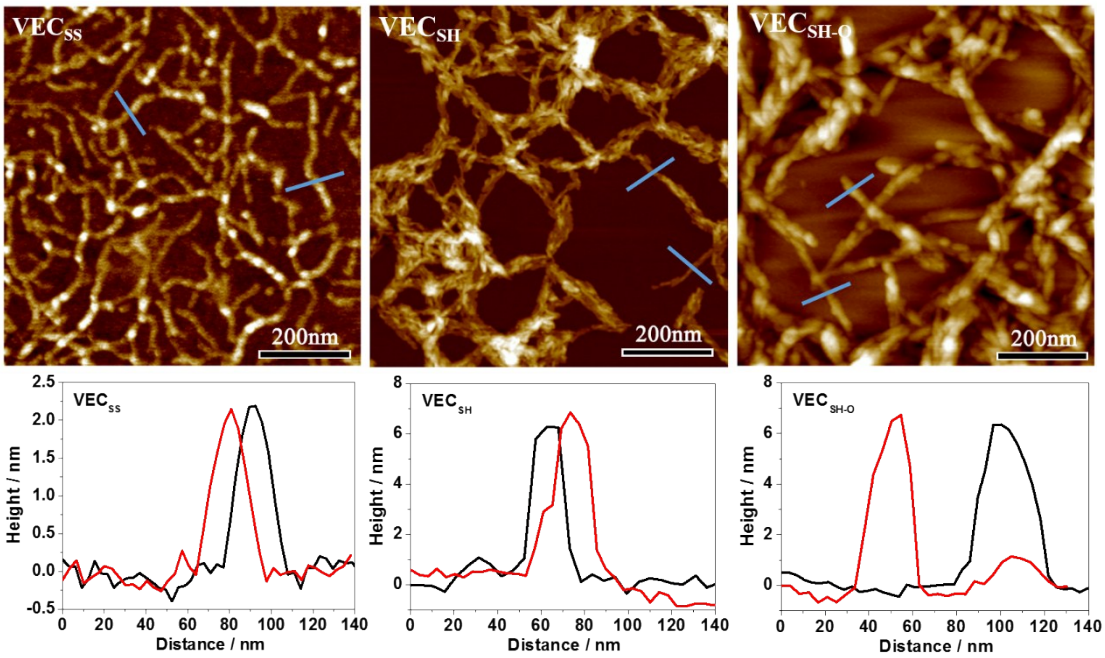

Figure S12. AFM images (Top: left to right) and the height profiles (Bottom: left to right) formed by **VEC$_{SS}$**, **VEC$_{SH}$**, and **VEC$_{SH-O}$**, respectively.

3. Molecular Models of Peptides Building Blocks

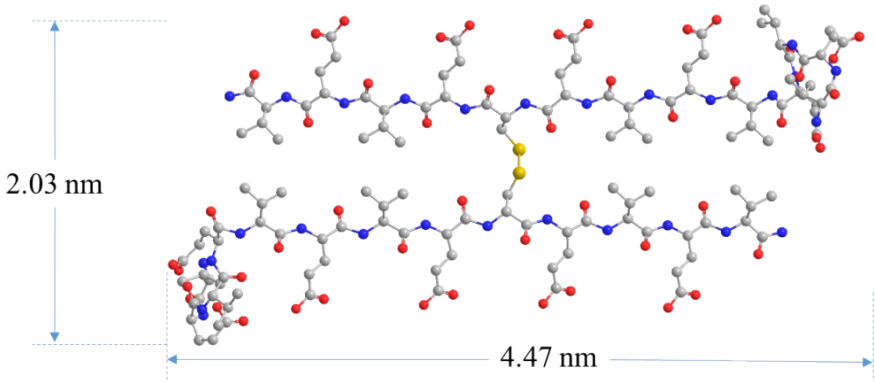

Figure S13. Molecular model of the monomer within the assemblies formed by peptide **VEC$_{SS}$** with a partial hydrophobic collapse (ca. eight residues within the constrained hydrophobic interfaces) and two appended disordered segments, in which the hydrogen atoms were omitted for clarity.

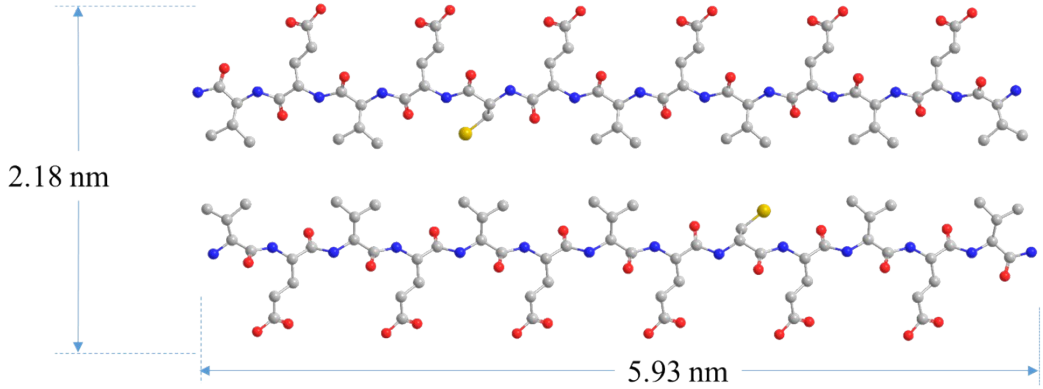

Figure S14. Molecular model of the dimers within the assemblies formed by peptide **VEC$_{SH}$** with a full hydrophobic collapse involving all the residues (twelve residues and one cysteine residue), in which the hydrogen atoms were omitted for clarity.

4. Hydrogel Characterization

4.1 Rheological Experiments.

Rheological measurements were conducted on a TA Instruments AR 1500ex rheometer operating in oscillatory mode. Peptide samples were loaded on a 40 mm parallel plate equipped with a solvent trap covered by silicon oil for prevention of solvent evaporation. Corresponding peptide solutions (2 wt%, 380 mL) introduced in EXPERIMENTAL SECTION were transferred to the stage (400 μm gap), whereas calcium chloride ($CaCl_2$) solutions (2 M, 20 μL) were homogeneously loaded on the top cone for hydrogelation of peptides. Peptide hydrogels were immediately subjected to a 2 h time sweep test. After reaching a well-defined equilibrium, a series of frequency sweep and strain amplitude sweep tests were subsequently carried out. Dynamic time sweep experiments were performed with a frequency and strain amplitude of 1 rad/s and 0.1 %, respectively. The dynamic frequency sweep tests were studied over a range of frequencies from 0.1 to 100 rad/s at 0.1 % constant strain to measure the linear viscoelastic storage (G ′) and loss modulus (G″), respectively. The oscillatory strain amplitude sweep tests were investigated over a range of strain amplitude from 0.1 % to 10 % at a fixed frequency of 1 rad/s.

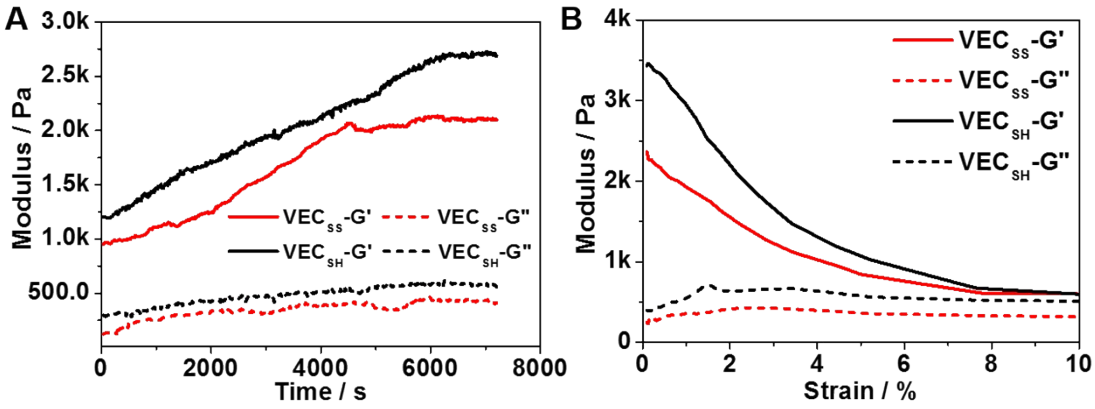

Figure S15. Storage modulus (G′) and loss modulus (G″) of static hydrogels **VEC$_{SS}$** and **VEC$_{SH}$** as a function of time (A) and strain (B), respectively.

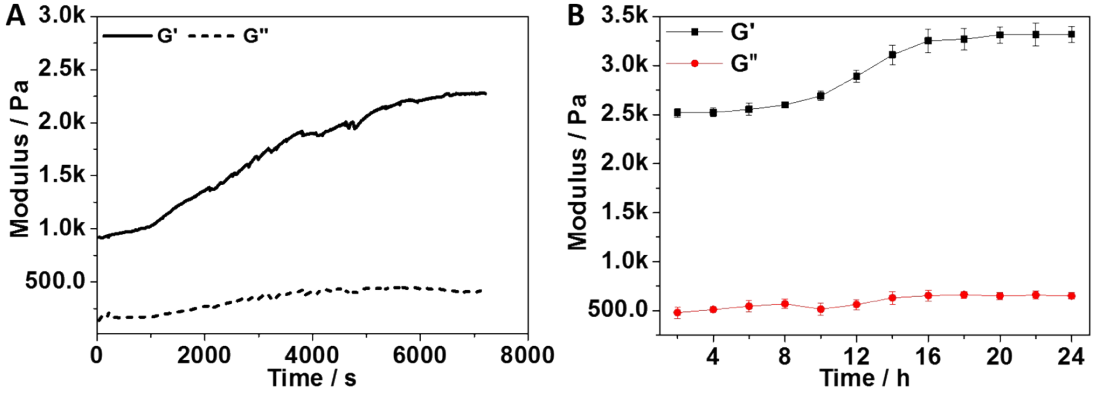

Figure S16. (A) Storage modulus (G′) and loss modulus (G″) of hydrogel **VEC$_{SH}$-MD** as a function of time. (B) Time-dependent frequency sweep storage modulus (G′) and loss modulus (G″) of hydrogel **VEC$_{SH}$-MD** with a 2-hour interval between each sample. The initial and terminal storage moduli of hydrogel **VEC$_{SH}$-MD** were estimated to be 2520 and 3319 Pa, which are close to that of static hydrogels **VEC$_{SS}$** and **VEC$_{SH}$**, respectively.

4.2 Time-Dependent CD Spectroscopy

Time-dependent CD studies of non-equilibrated peptide **VEC$_{SH}$** bilayer assemblies in both hydrogel **VEC$_{SH}$-MD** (Figure S17) and solution **VEC$_{SH}$-MD** (Figures S18-19)

were carried out. Corresponding CD samples were prepared by diluting hydrogel **VEC$_{SH}$-MD** or solution **VEC$_{SH}$-MD** to 50 μM. The time interval of each sample is 2 h.

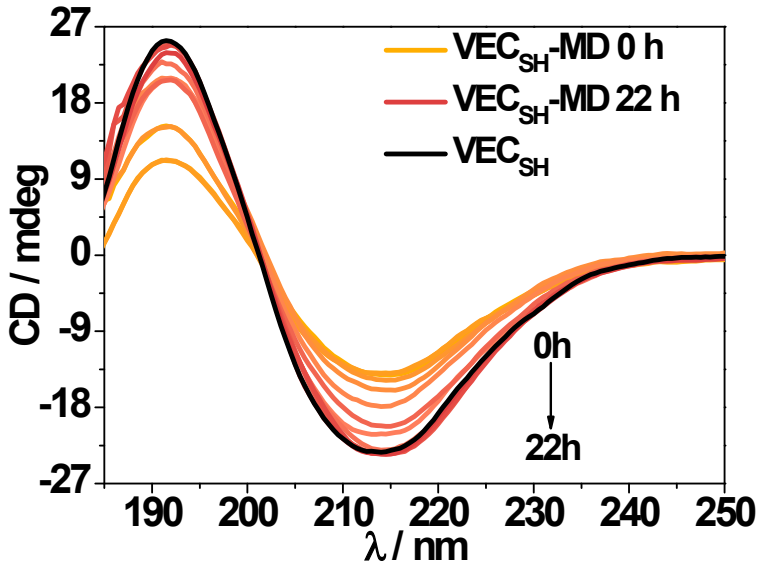

Figure S17. Time-dependent CD spectra of hydrogel **VEC$_{SH}$-MD** with a 2-hour test interval, in which the spectra of hydrogel **VEC$_{SH}$** was shown (black line).

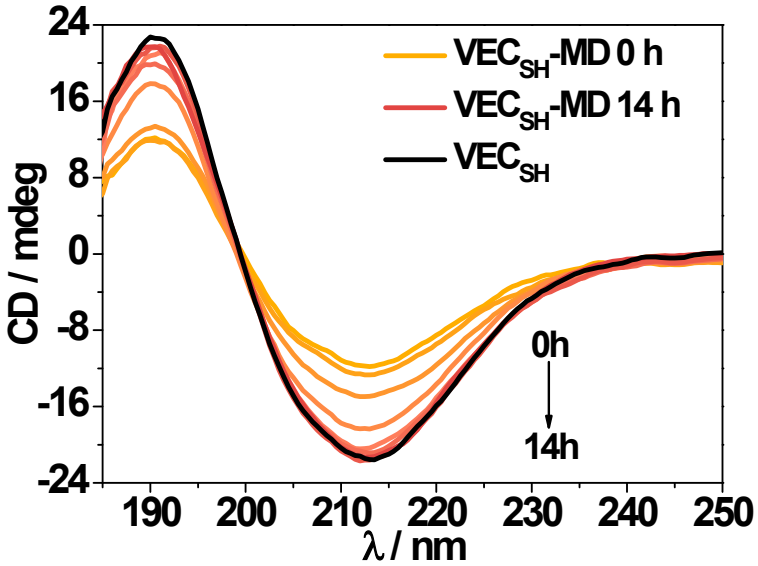

Figure S18. Time-dependent CD spectra of solution **VEC$_{SH}$-MD** with a 2-hour test interval, in which the spectra of solution **VEC$_{SH}$** was shown (black line).

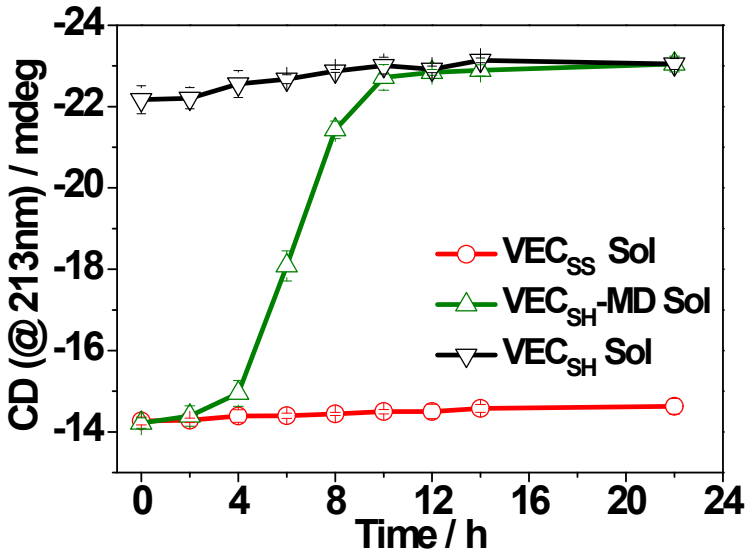

Figure S19. The intensities of CD signals (@213 nm) of the solutions of **VEC$_{SH}$-MD**, **VEC$_{SH}$**, and **VEC$_{SS}$** as a function of time with a 2-hour time interval.

5. Cell Proliferation Assay

5.1 Cell Culture on Hydrogels.

The cell culture medium was prepared by addition of 10 % FBS (fetal bovine serum), 100 units/mL of penicillin, and 100 mg/mL streptomycin to DMEM. Prior to being cultured on the hydrogel surfaces, LO2 (human hepatocyte) or A10 cells (rat aortic vascular smooth muscle cells) were trypsinized using the trypsinase (0.25 %) – EDTA (0.02 %) solution and centrifuged at 1000 rpm for 3 min to remove the media. Eventually 60 μL of the cell suspension solution with a cell density of 16.7×10^4 / mL was added to each well covered with peptide hydrogel and incubated under the atmosphere of 5 % CO_2 at 37 °C for 4, 16, 36, and 48 h, respectively. The process for preparation of different peptide hydrogels and cell culture on the surface of different hydrogels were illustrated in *Figure 6*.

5.2 Live-Dead Assay.

The viability of incubated cells on the surfaces of peptide hydrogels was determined by a Live-Dead assay. A 60 μL aliquot of serum-free DMEM containing ethidium homodimer-1 (EthD-1, 4 μM) and calcein AM (2 μM) was pipetted into each well. After incubated at 37 ° C for 30 min, the cells were imaged by a fluorescence microscope (Nikon Eclipse TE2000-U) equipped with excitation filters of 450-490 nm (Calcein AM, green) or 510-560 nm (EthE-1, red).

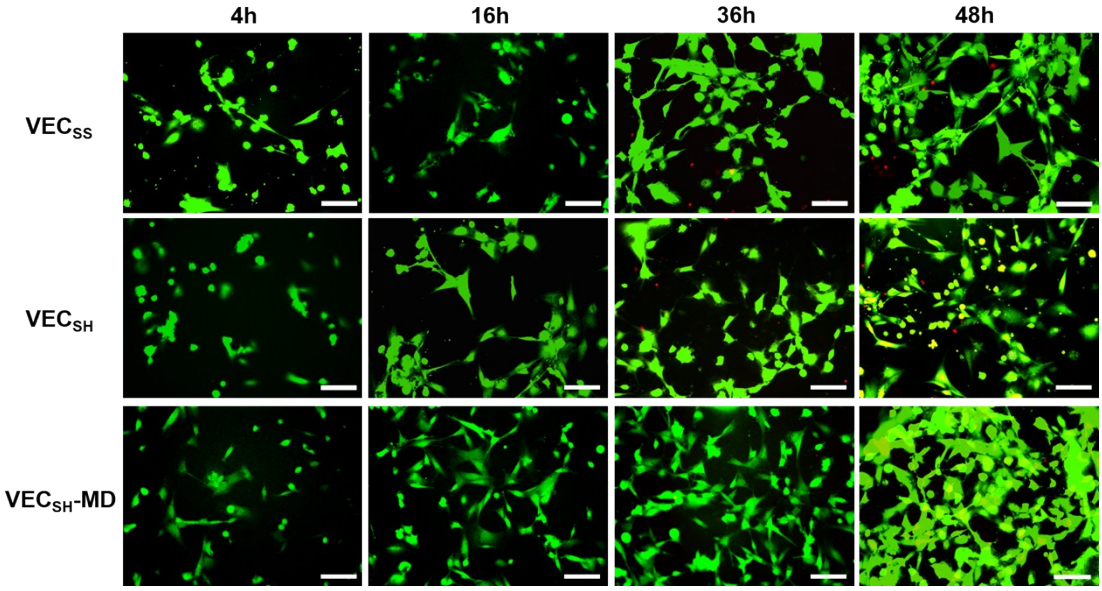

Figure S20. Fluorescence images of A10 cells with Live-Dead staining treatment after incubation on the surfaces of static hydrogels **VEC$_{SS}$** and **VEC$_{SH}$** and mechanically dynamic hydrogel **VEC$_{SH}$-MD** for 4, 16, 36, and 48 h. Scale bars: 50 μm.

5.3 CCK-8 Assay.

To quantify cell proliferation on the hydrogel surfaces, a CCK-8 assay was performed.

The 384-well plate was loaded with a mixed solution of CCK-8 and cell medium (10 : 90, in volume) and incubated under the atmosphere of 5 % CO_2 at 37 °C for 4 h. The absorption intensity was determined by a microplate reader (Thermo scientific, USA) at a wavelength of 450 nm.

5.4 EdU Assay.

EdU assay was further carried out for the cells cultured on the surface of peptide hydrogels to confirm cell proliferation. A thymidine derivative 5-ethynyl-2'-deoxyuridine (EdU) can be incorporated into cellular nucleus during cell proliferation and DNA replication. The incorporated EdU was detected via labeling with a fluorescent dye connected through a click reaction between ethynyl groups in EdU and azide groups in Alexa Fluor 488.[2] EdU staining was conducted using EdU Cell Proliferation Kit with Alexa Fluor 488 (Beyotime, China) based on the standard protocol provided by the manufacturer. The cells cultured on the surface of hydrogels were incubated with EdU (5 μM) for 4 h and fixed with 4% paraformaldehyde in PBS for 15 min. The fixed cells were permeablized with 0.5 % Triton X-100 in PBS for 15 min and stained with Alexa Fluor 488 and DAPI for 10 min under dark condition, in which DAPI was used as the nucleus-tracker. Afterwards, the labeled cells were imaged by a fluorescence microscope and the proportion of EdU-containing cells was quantified using Image J.

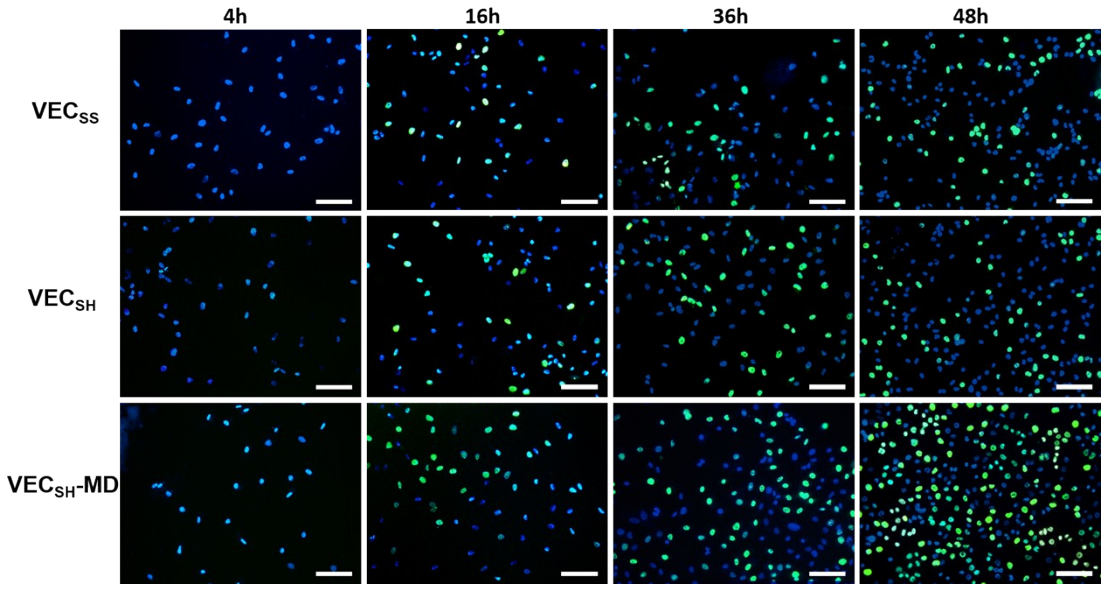

Figure S21. Fluorescence images of A10 cells with DAPI and Alexa Fluor 488 co-staining treatment after incubation on the surfaces of static hydrogels **VEC$_{SS}$** and **VEC$_{SH}$** and mechanically dynamic hydrogel **VEC$_{SH}$-MD** for 4, 16, 36, and 48 h. Blue: DAPI; Green: Alexa Fluor 488; Cyan: merged DAPI and Alexa Fluor 488. Scale bars: 50 μm.

5.5. YAP-GFP OE LO2 Cell Culture on Hydrogel VEC$_{SH}$-MD.

The YAP-GFP overexpression LO2 cells were prepared by incubating the original LO2 cells in the solution of the pEGFP-YAP plasmid-lipofectamine 3000 complexes for 24 h. The solution of the pEGFP-YAP plasmid-lipofectamine 3000 complexes was prepared via mixing the solution of the pEGFP-YAP plasmid (500 ng) in P3000 (1 μL) and Opti-MEM medium (25 μL) and the solution of lipofectamine 3000 (0.75 μL) in Opti-MEM medium (25 μL) and incubating for 15 min. After culturing the YAP-GFP OE cells on hydrogels for 36 h, the nucleus and F-actin of the cells was stained by DAPI and Alexa Fluor 647-phalloidin, respectively. The cells were imaged by a confocal laser

scanning microscope (NIKON A1+). The statistical analysis of the localization of YAP-GFP in LO2 cells was carried out by calculating over 500 cells for each hydrogel.

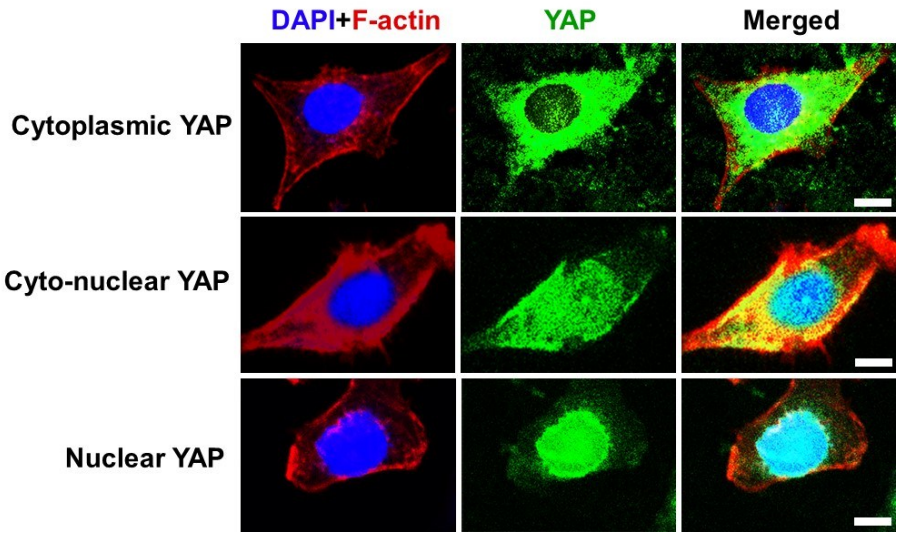

Figure S22. Representative CLSM images of YAP-GFP OE LO2 cells with cytoplasmic, cyto-nuclear, and nuclear YAP, respectively, as the judgment criteria for data statistics in *Figure 9C*. Blue: DAPI (nucleus); Red: Alexa Fluor 647-phalloidin (F-actin); Green: GFP labeled YAP. Scale bar: 10 μm.

References

1. M. C. Stuart, J. C. van de Pas and J. B. Engberts, *J. Phys. Org. Chem.*, 2005, **18**, 929-934.

2. F. Chehrehasa, A. C. Meedeniya, P. Dwyer, G. Abrahamsen and A. Mackay-Sim, *J. Neurosci.*, 2009, **177**, 122-130.