Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Designing aromatic N-cadherin mimetic short peptide based bioactive scaffolds for controlling cellular behaviour

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Table S1. LC-MS analysis data of different peptides from Chemdraw (Ultra 12.0) and ESI-MS.

Peptide amphiphile (Chemical formula)	**Calculated m/z value (M+1) (Chem Draw Ultra 12.0)**	**Found m/z value (M+1) (ESI-MS)**
HAVDI ($C_{24}H_{40}N_8O_7$)	553.31	553.54
Fmoc-HAVDI ($C_{39}H_{50}N_8O_9$)	775.37	775.66
Nap-HAVDI ($C_{36}H_{48}N_8O_9$)	734.36	734.54
Fmoc-AVIDH ($C_{39}H_{50}N_8O_9$)	775.37	775.62
Nap-AVIDH ($C_{36}H_{48}N_8O_9$)	736.36	734.66
Myristyl-HAVDI ($C_{38}H_{66}N_8O8$)	762.99	763.14

Table S2. Quantitative comparison of biological studies perfomed on N-cadherin mimetic peptide hydrogel with that of already reported laminin-based peptide hydrogles (*ACS Biomater. Sci. Eng.* 2020, **6**, 2832−2846).

Bioactivity studies	**Laminin based peptide (Fmoc-IKVAV and Fmoc-YIGSR)**	**N-cadherin based peptide (Fmoc-HAVDI)**
Proliferation study (Alamar assay) with C6 cell line after	On hydrogel of peptides Fmoc-IKVAV and Fmoc -YIGSR	On hydrogel of peptide Fmoc-HAVDI
i)48 h of cell seeding	Relatively less proliferation on hydrogel than on uncoated surface.	No significant difference in cellular proliferation on hydrogel and on uncoated surface.
ii)5days of cell seeding	Similar to that of control cells	Similar to that of control cells
Neurite extension (after 24 h)	~74 ± 16 μm (Fmoc IKVAV) and ~87 ± 27μm (Fmoc-YIGSR)	~90 ± 10 μm

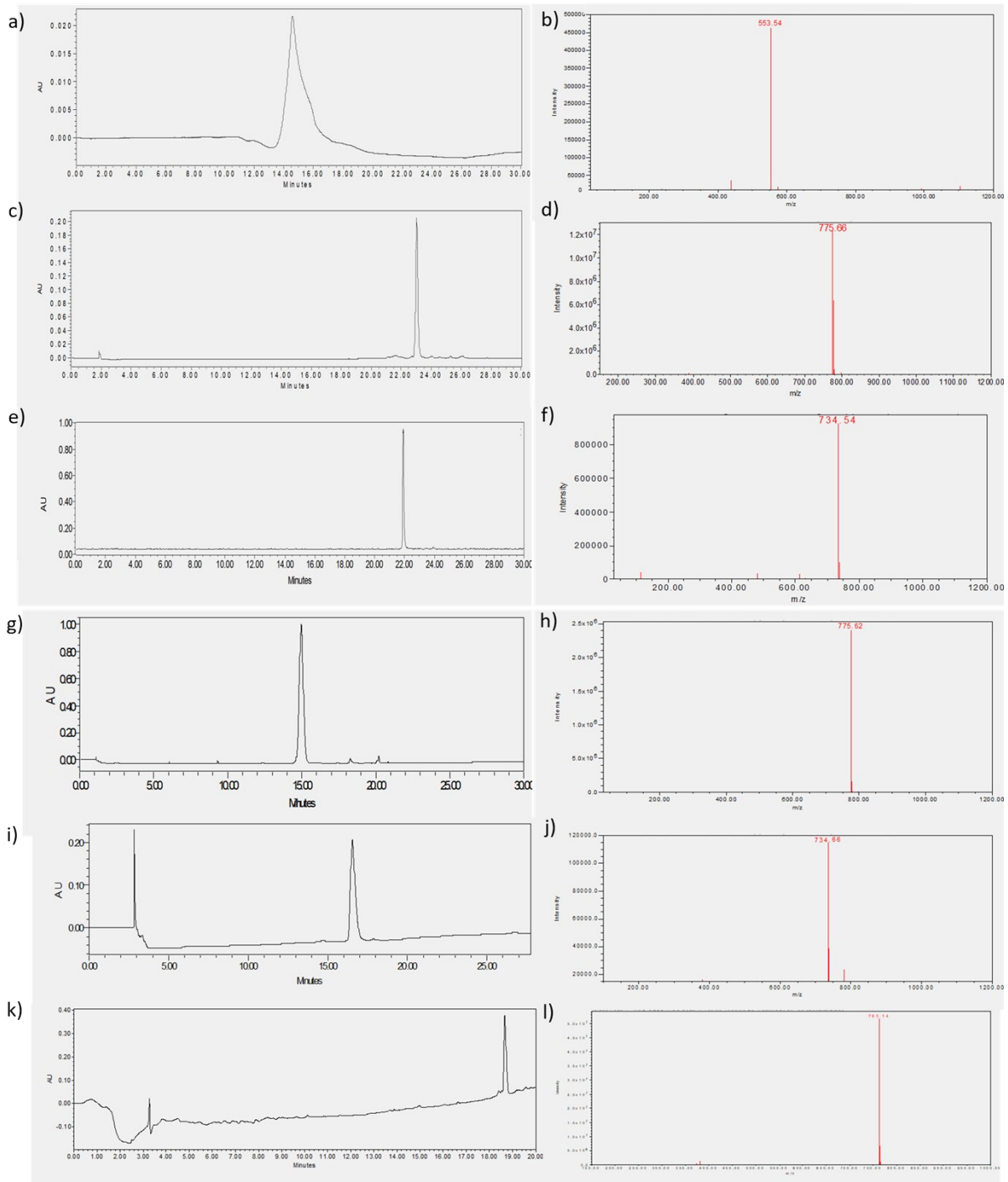

Figure S1: Reverse phase HPLC and Mass spectra of N-cadherin mimetic peptides (a, b) HAVDI, (c, d) Fmoc-HAVDI, (e, f) Nap-HAVDI, (g, h) Fmoc-AVIDH, (i, j) Nap-AVIDH and (k, l) Myristyl-HAVDI.

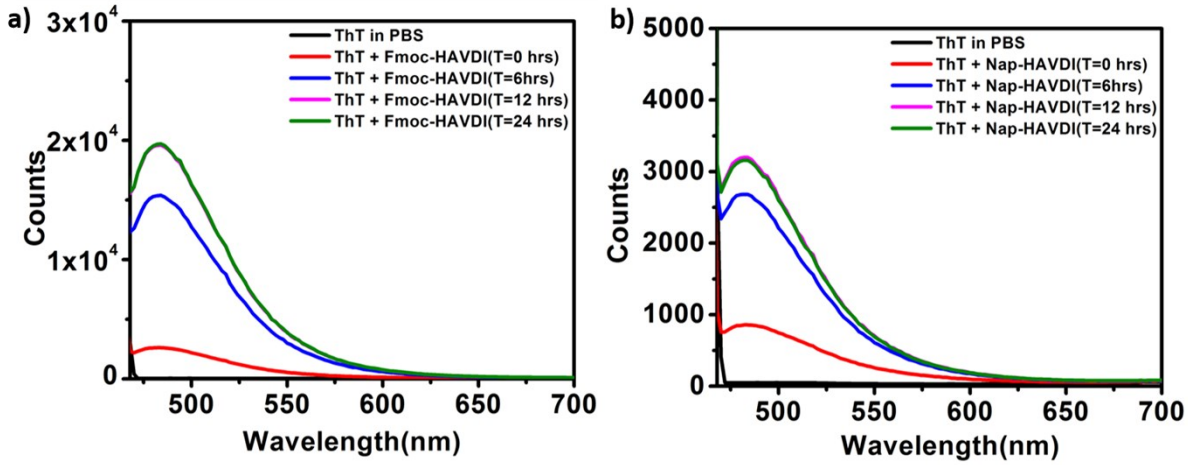

Figure S2: Assessment of change in viscosity of N-cadherin mimetic peptides (a) Fmoc-HAVDI and (b) Nap-HAVDI upon gelation using Thioflavin T assay, which has been reported as molecular rotor.

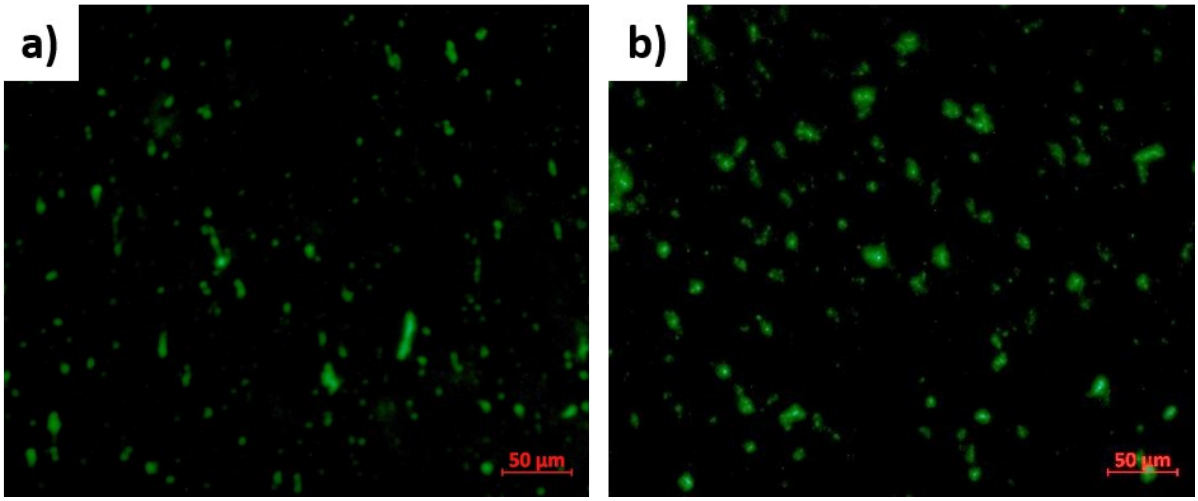

Figure S3: Fluorescence microscopic images of Thioflavin T binding to the nanostructures present in the N-cadherin mimetic peptides (a) Fmoc-HAVDI and (b) Nap-HAVDI hydrogels. Scale bar is 50 µm.

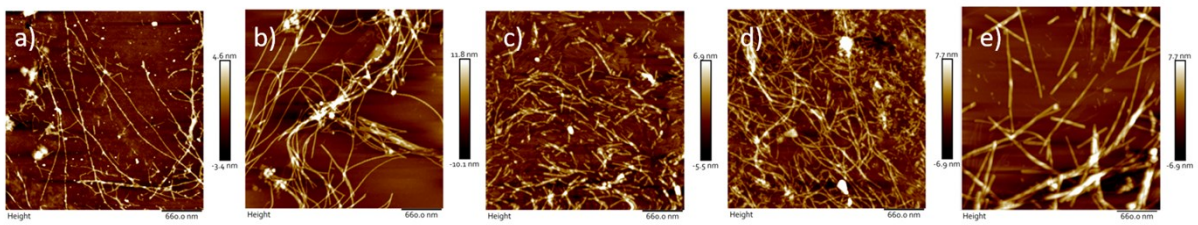

Figure S4: AFM images of nanostructures formed in hydrogels of (a) Fmoc-FF, (b) Nap-FF, (c) Fmoc-AVIDH, (d) Nap-AVIDH and e) Myristyl-HAVDI.

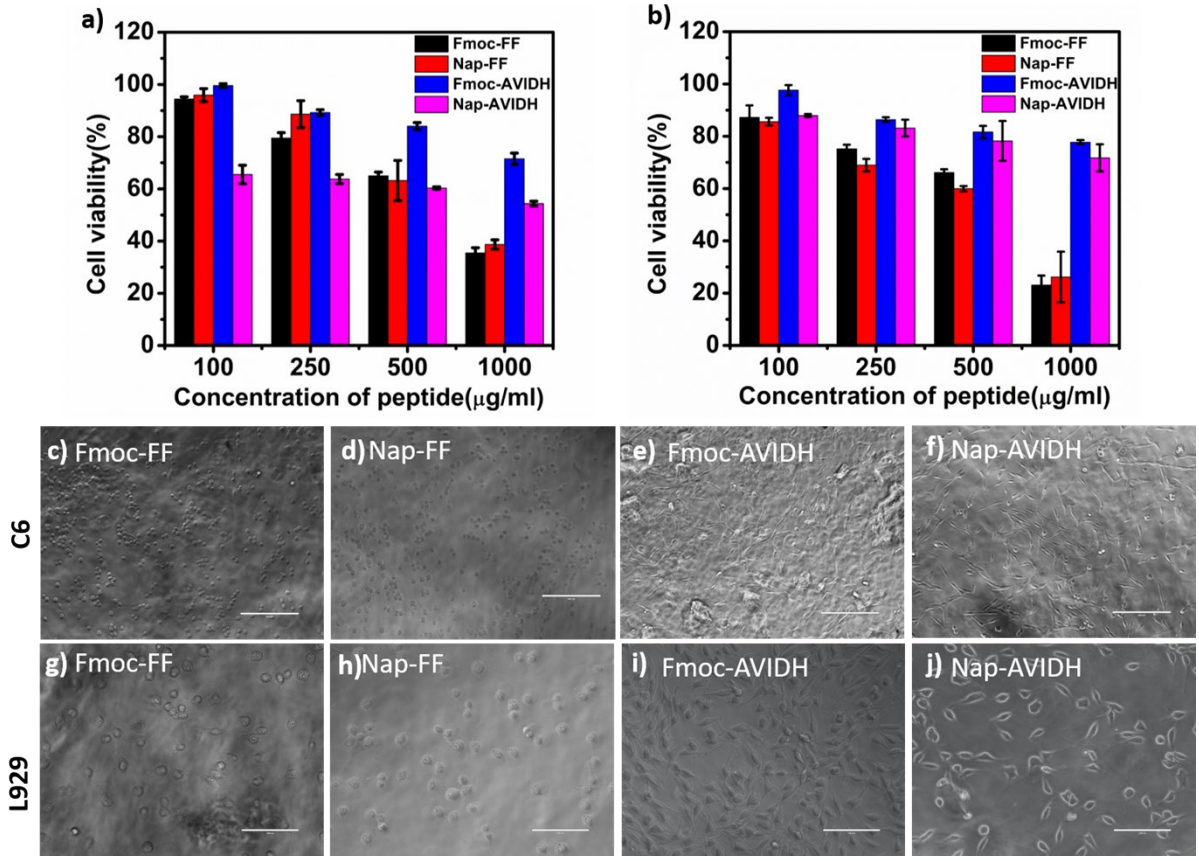

Figure S5: Evaluation of biocompatibility of N-cadherin mimetic peptides using MTT assay on (a) C6 and b) L929 cell lines. Optical images of C6 cells treated with 1000 μg/ml of control peptides (c) Fmoc-FF, (d) Nap-FF, (e) Fmoc-AVIDH and (f) Nap-AVIDH after 4 h of incubation period. Optical images of L929 cells treated with 1000 μg/ml of control peptides (g) Fmoc-FF, (h) Nap-FF, (i) Fmoc-AVIDH and (j) Nap-AVIDH after after 4 h of incubation period. Scale bar is 100 μm.

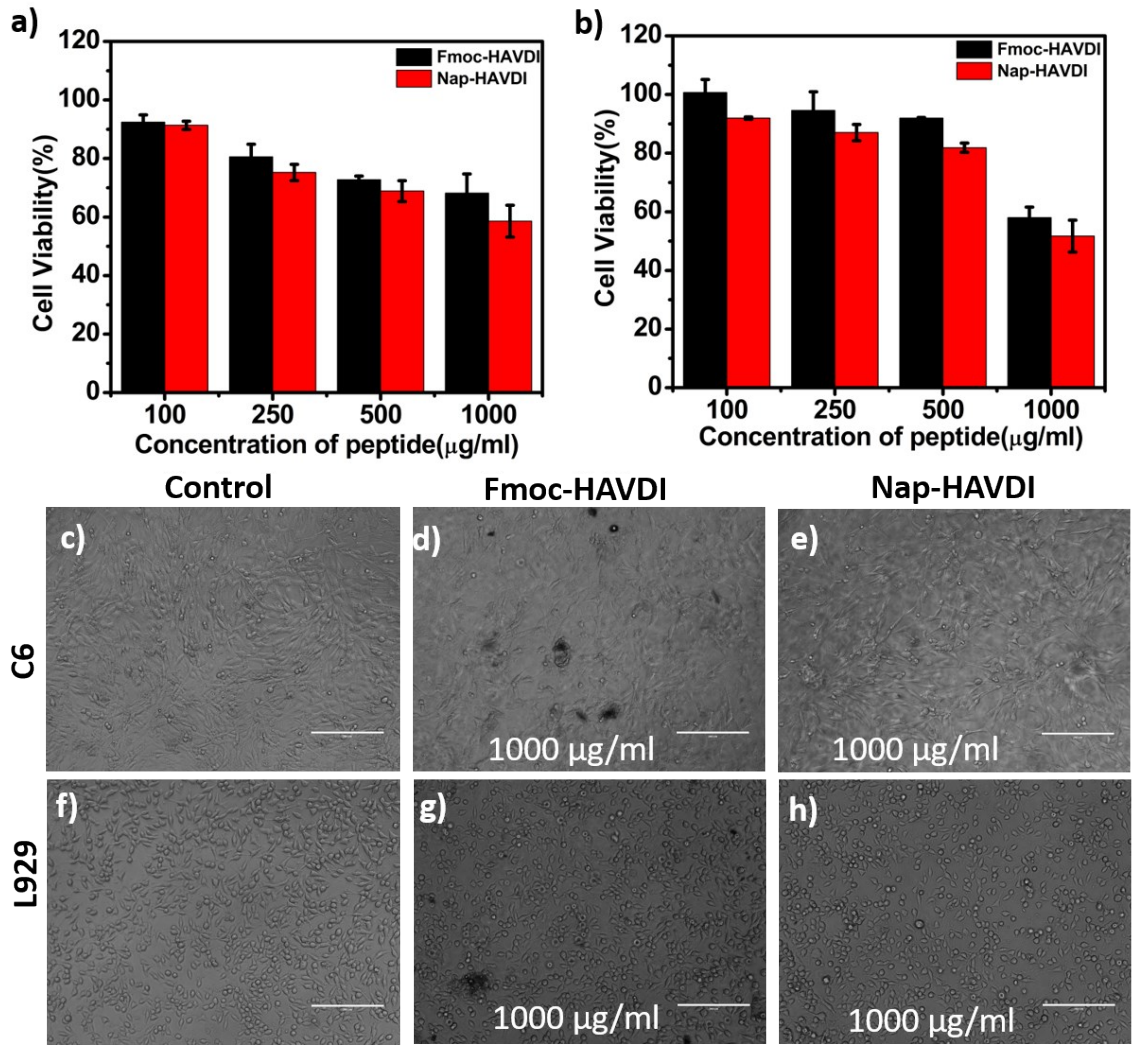

Figure S6: Evaluation of biocompatibility of N-cadherin mimetic peptides using MTT assay on (a) C6 and b) L929 cell lines. Optical images of C6 cells (c) control, (d) treated with 1000 μg/ml of Fmoc-HAVDI, and (e) treated with 1000 μg/ml of Nap-HAVDI after 24 h of incubation period. Optical images of L929 cells, (f) control, (g) treated with 1000 μg/ml of Fmoc-HAVDI, and 1000 μg/ml of Nap-HAVDI after 24 h of incubation period. Scale bar is 200 μm.

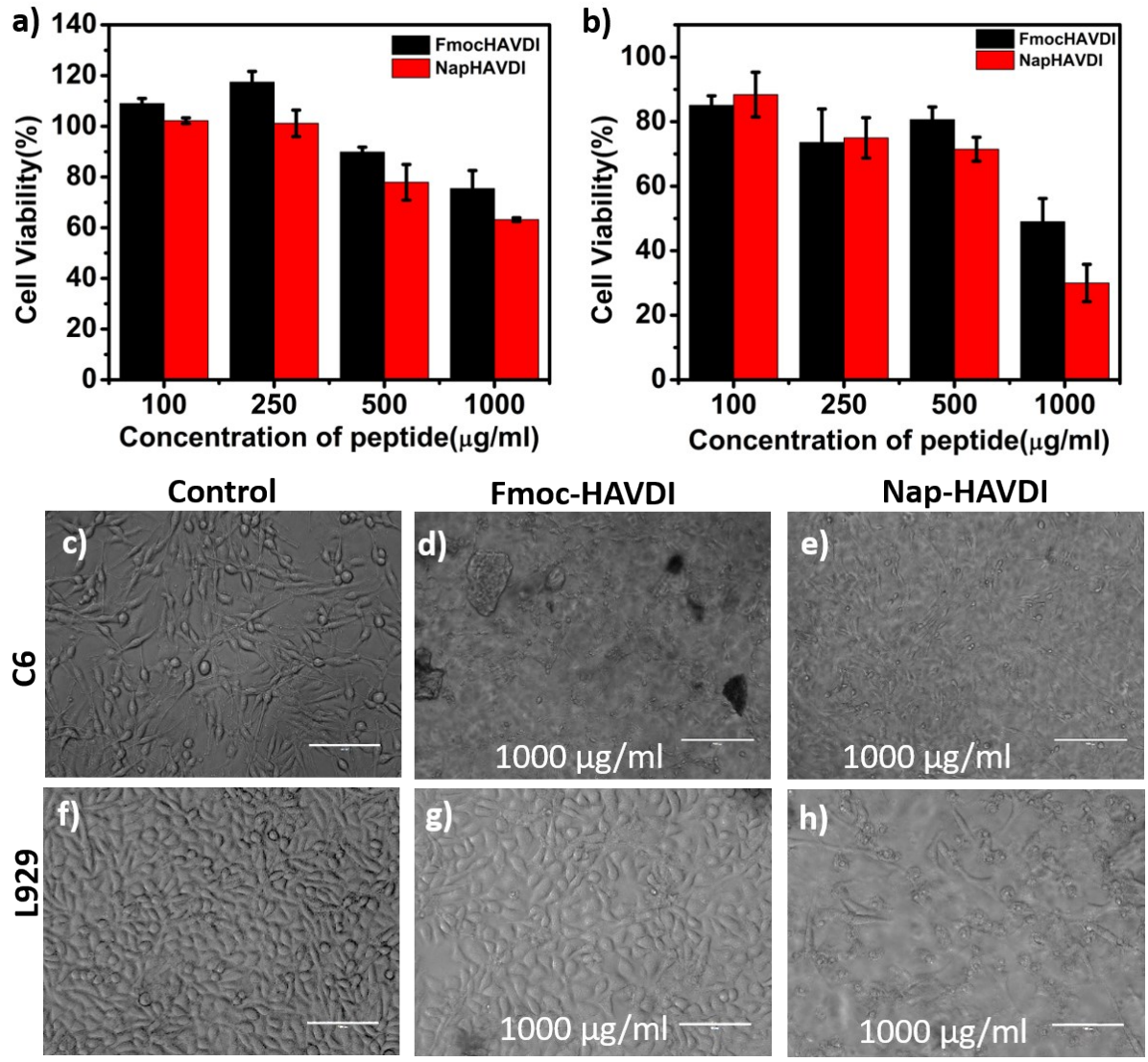

Figure S7: Evaluation of biocompatibility of N-cadherin mimetic peptides using MTT assay on (a) C6 and b) L929 cell lines. Optical images of C6 cells (c) control, (d) treated with 1000 μg/ml of Fmoc-HAVDI, and (e) treated with 1000 μg/ml of Nap-HAVDI after 48 h of incubation period. Optical images of L929 cells, (f) control, (g) treated with 1000 μg/ml of Fmoc-HAVDI, and 1000 μg/ml of Nap-HAVDI after 48 h of incubation period. Scale bar is 100 μm.

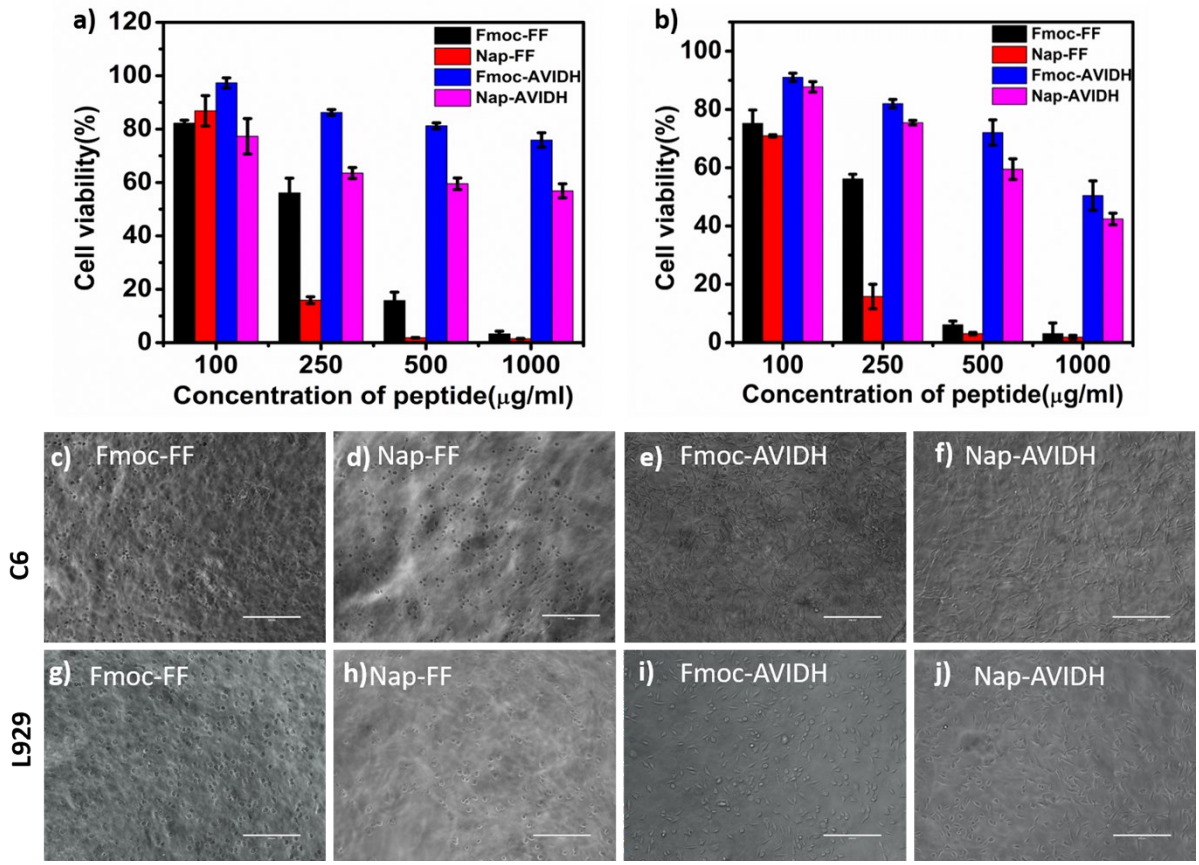

Figure S8: Evaluation of biocompatibility of control peptides using MTT assay on (a) C6 and b) L929 cell lines. Optical images of C6 cells treated with 1000 μg/ml of control peptides (c) Fmoc-FF, (d) Nap-FF, (e) Fmoc-AVIDH and (f) Nap-AVIDH after 24 h of incubation period. Optical images of L929 cells treated with 1000 μg/ml of control peptides (g) Fmoc-FF, (h) Nap-FF, (i) Fmoc-AVIDH and (j) Nap-AVIDH after after 24 h of incubation period. Scale bar is 100 μm.

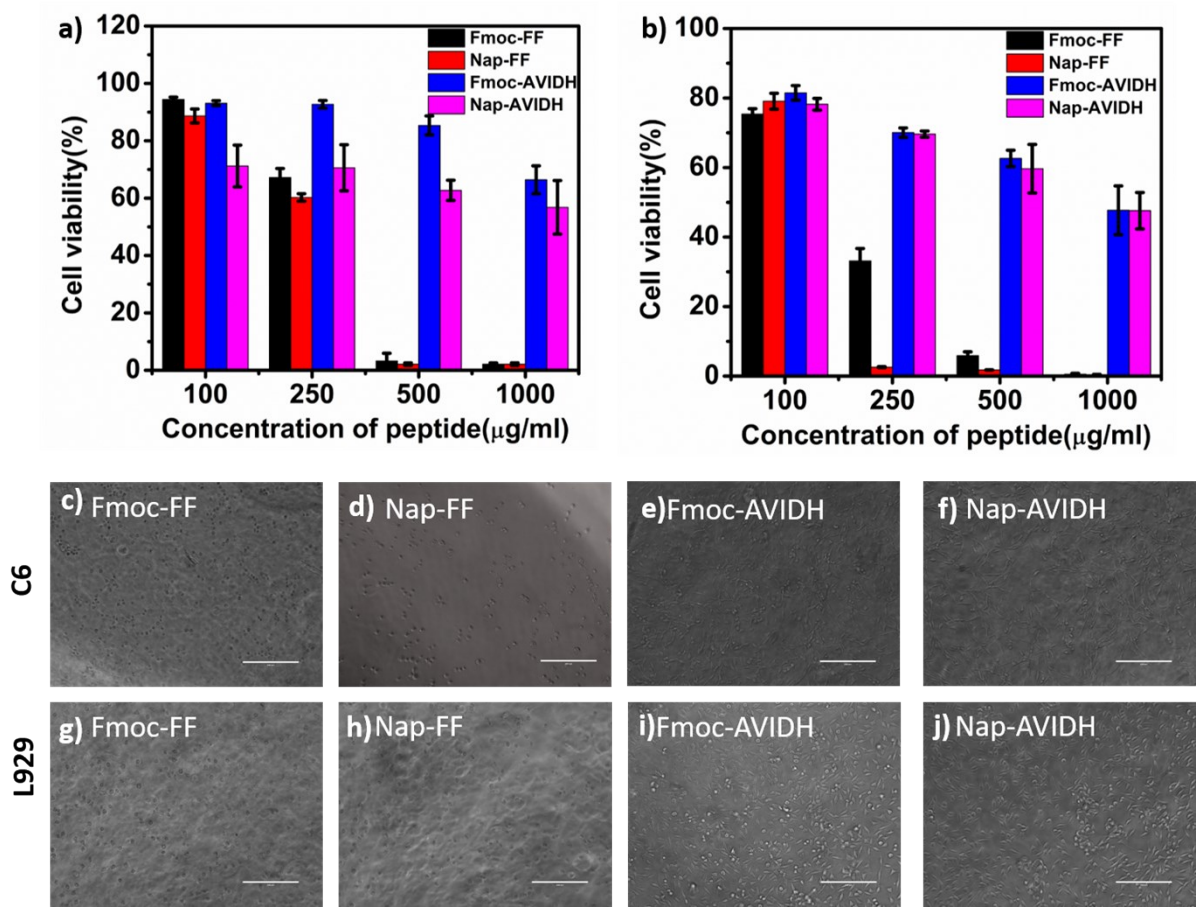

Figure S9: Evaluation of biocompatibility of control peptides using MTT assay on (a) C6 and b) L929 cell lines. Optical images of C6 cells treated with 1000 μg/ml of control peptides (c) Fmoc-FF, (d) Nap-FF, (e) Fmoc-AVIDH and (f) Nap-AVIDH after 48 h of incubation period. Optical images of L929 cells treated with 1000 μg/ml of control peptides (g) Fmoc-FF, (h) Nap-FF, (i) Fmoc-AVIDH and (j) Nap-AVIDH after after 48 h of incubation period. Scale bar is 100 μm.

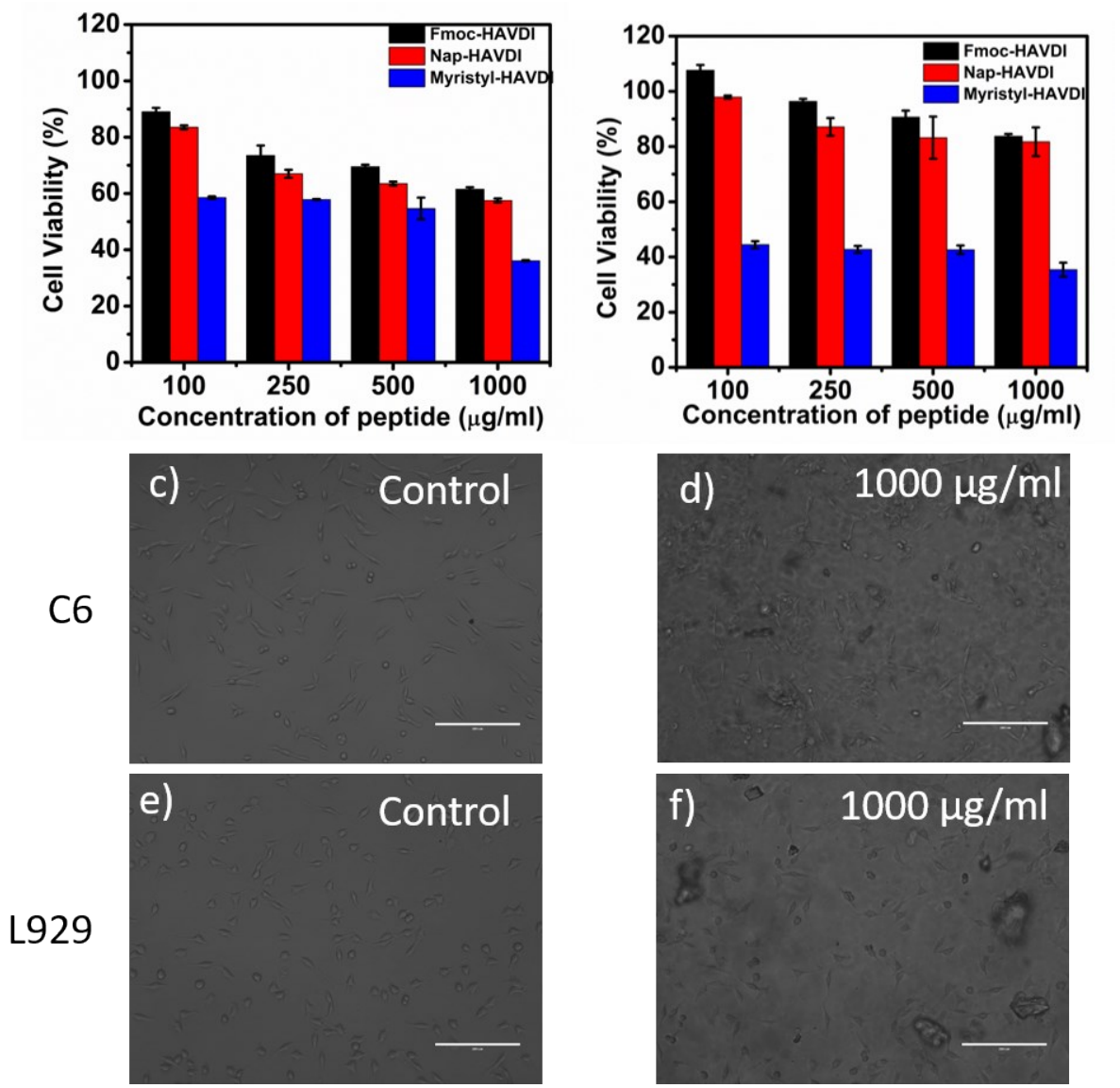

Figure S10: Evaluation and comparison of biocompatibility of Myristyl-HAVDI with Fmoc-HAVDI and Nap-HAVDI using MTT assay on (a) C6 cell line and (b) L929 cell line. Optical images of C6 cells (c) control, (d) treated with 1000 µg/ml of Myristyl-HAVDI after 4 h of incubation period. Optical images of L929 cells, (e) control, (f) treated with 1000 µg/ml of Myristyl-HAVDI after 4 h of incubation period. Scale bar is 200 µm.

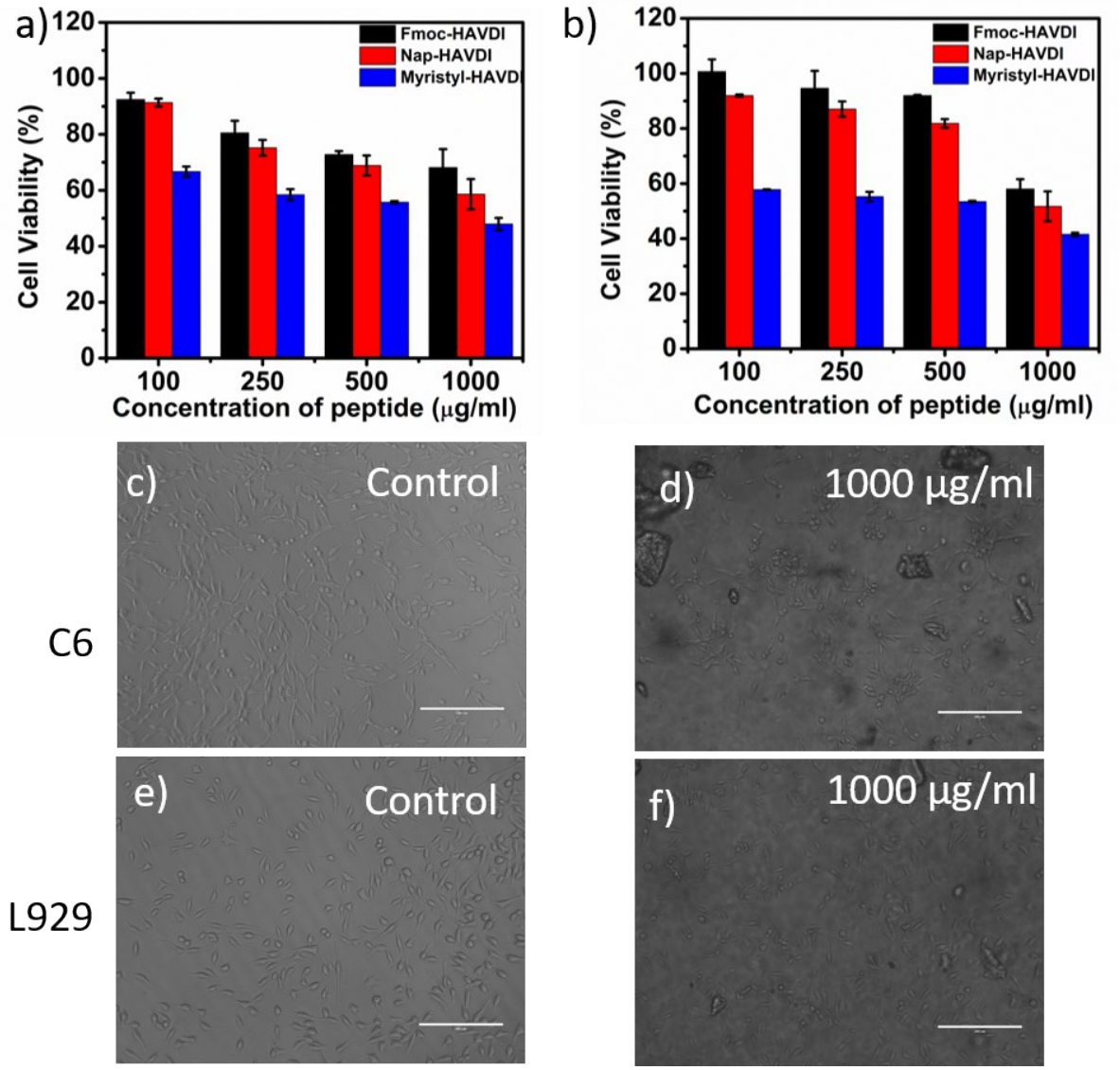

Figure S11: Evaluation and comparison of biocompatibility of Myristyl-HAVDI with Fmoc-HAVDI and Nap-HAVDI using MTT assay on (a) C6 cell line and (b) L929 cell line. Optical images of C6 cells (c) control, (d) treated with 1000 μg/ml of Myristyl-HAVDI after 24 h of incubation period. Optical images of L929 cells, (e) control, (f) treated with 1000 μg/ml of Myristyl-HAVDI after 24 h of incubation period. Scale bar is 200 μm.

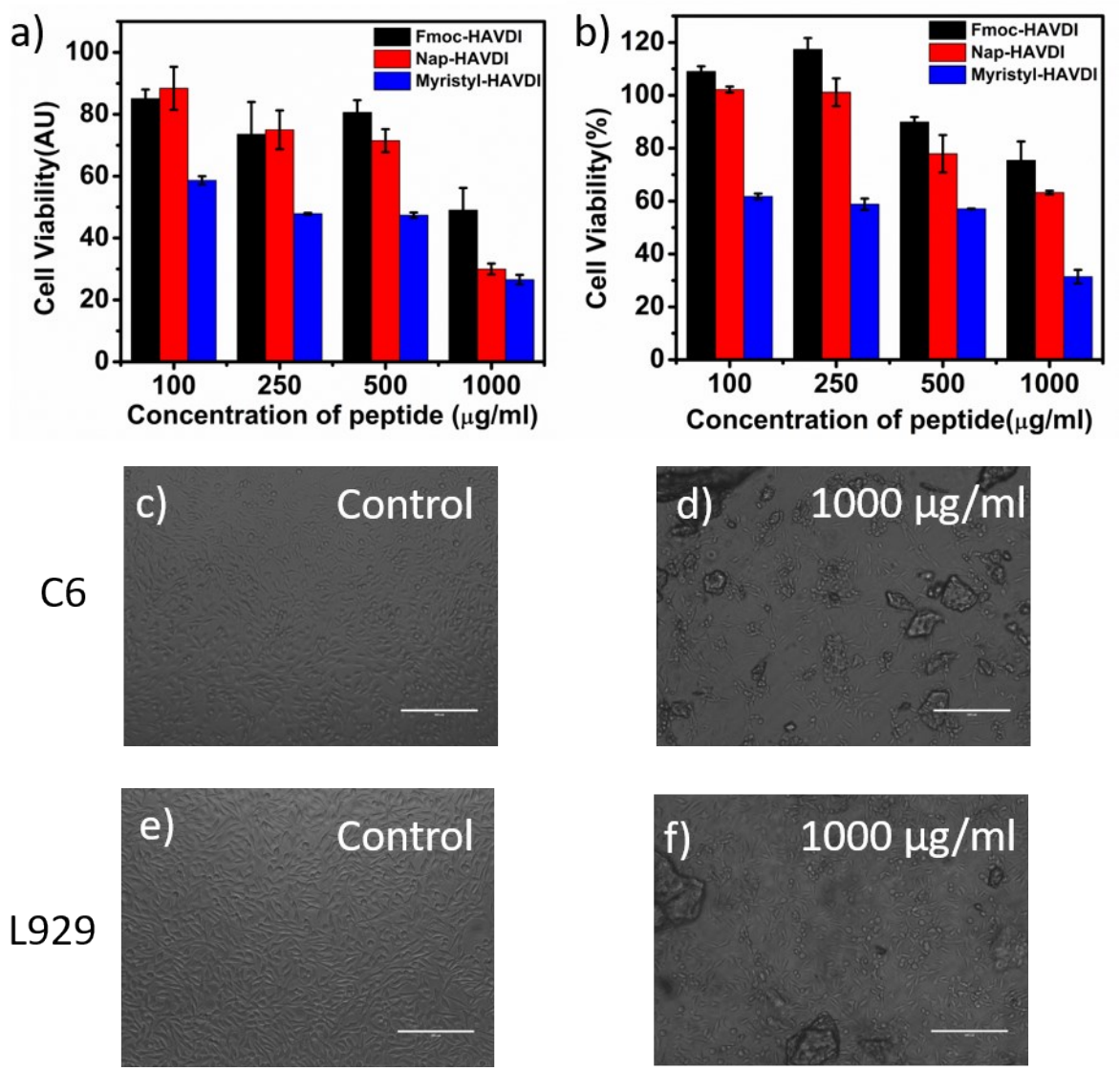

Figure S12: Evaluation and comparison of biocompatibility of Myristyl-HAVDI with Fmoc-HAVDI and Nap-HAVDI using MTT assay on (a) C6 cell line and (b) L929 cell line. Optical images of C6 cells (c) control, (d) treated with 1000 μg/ml of Myristyl-HAVDI after 48 h of incubation period. Optical images of L929 cells, (e) control, (f) treated with 1000 μg/ml of Myristyl-HAVDI after 48 h of incubation period. Scale bar is 200 μm.

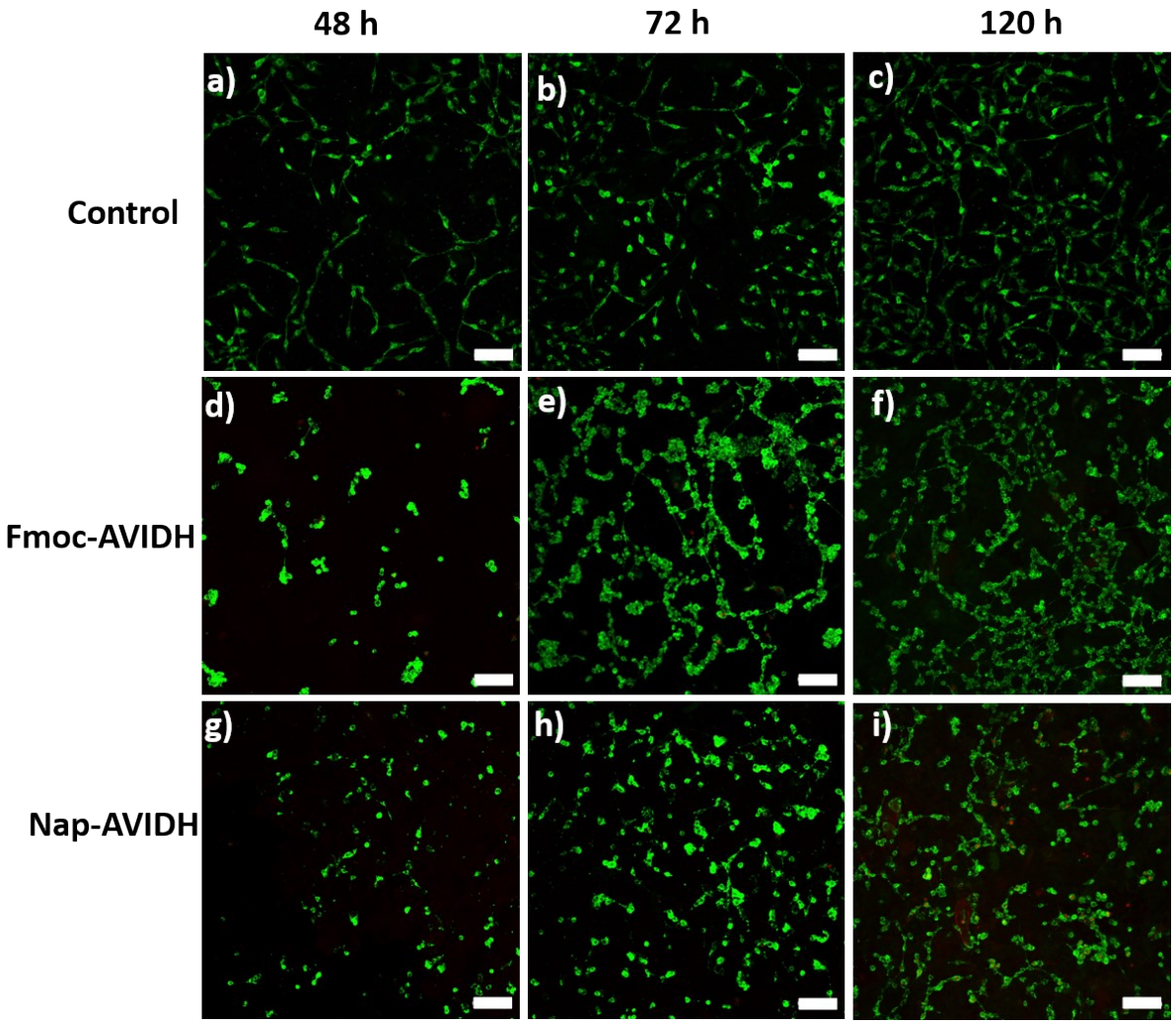

Figure S13: 2D cell culture study of C6 cell line on control scrambled N-cadherin peptide hydrogels. Analysis of adhesion and proliferation of rat glioma cell line, C6 on N-cadherin mimetic peptide hydrogels by Live/Dead staining. Confocal images of live and dead C6 cells labelled with DiOC18(3) and PI, respectively showing the cellular viability, adhesion, and proliferation on (a-c) control (uncoated coverslip), and (d-f) Fmoc-AVIDH hydrogel and (g-i) Nap-AVIDH hydrogel after 48 h, 72 h and 120 h, respectively. Scale bar is 100 μm.

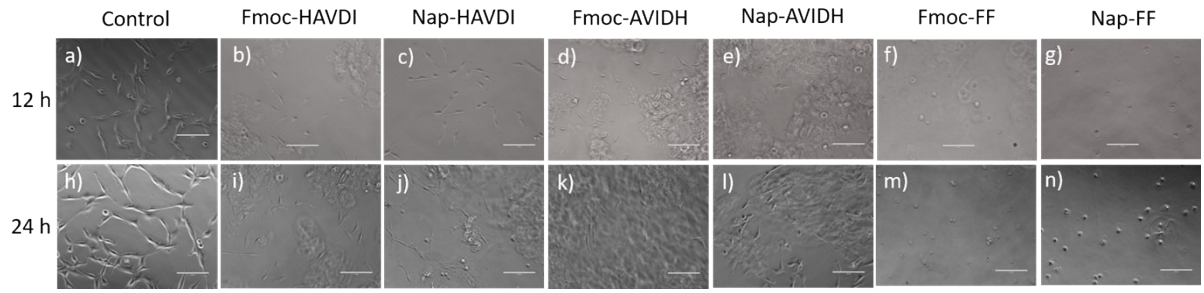

Figure S14: Optical images of the C6 cell line on N-cadherin mimetic peptide and control peptide hydrogels. Morphological analysis of rat glioma cell line, C6 on N-cadherin mimetic peptide and control peptide hydrogels by optical imaging. Optical images of C6 cells on the (a, h) culture dish, (b, i) Fmoc-HAVDI, (c, j) Nap-HAVDI, (d, k) Fmoc-AVIDH, (e, l) Nap-AVIDH, (f, m) Fmoc-FF and (g, h) Nap-FF hydrogels after 12 h, and 24 h, respectively. Scale bar is 100 µm.

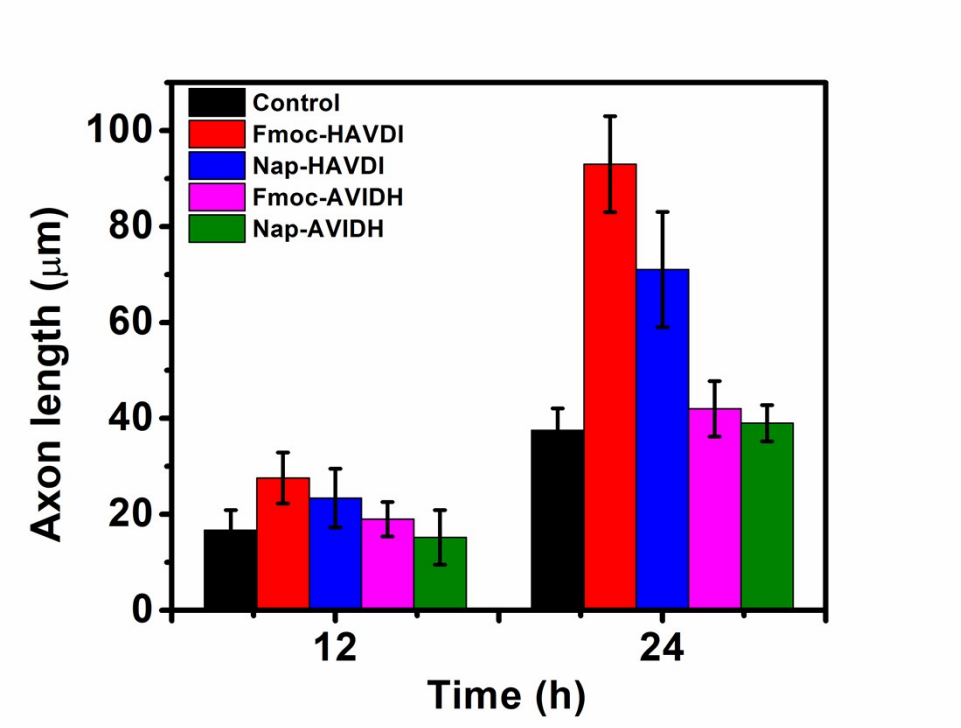

Figure S15: Average lengths of axons of C6 cells measured using ImageJ software by measuring 10 cells cultured on the control (culture dish), Fmoc-HAVDI, Nap-HAVDI, Fmoc-AVIDH, and Nap-AVIDH, hydrogels after 12 h, and 24 h.

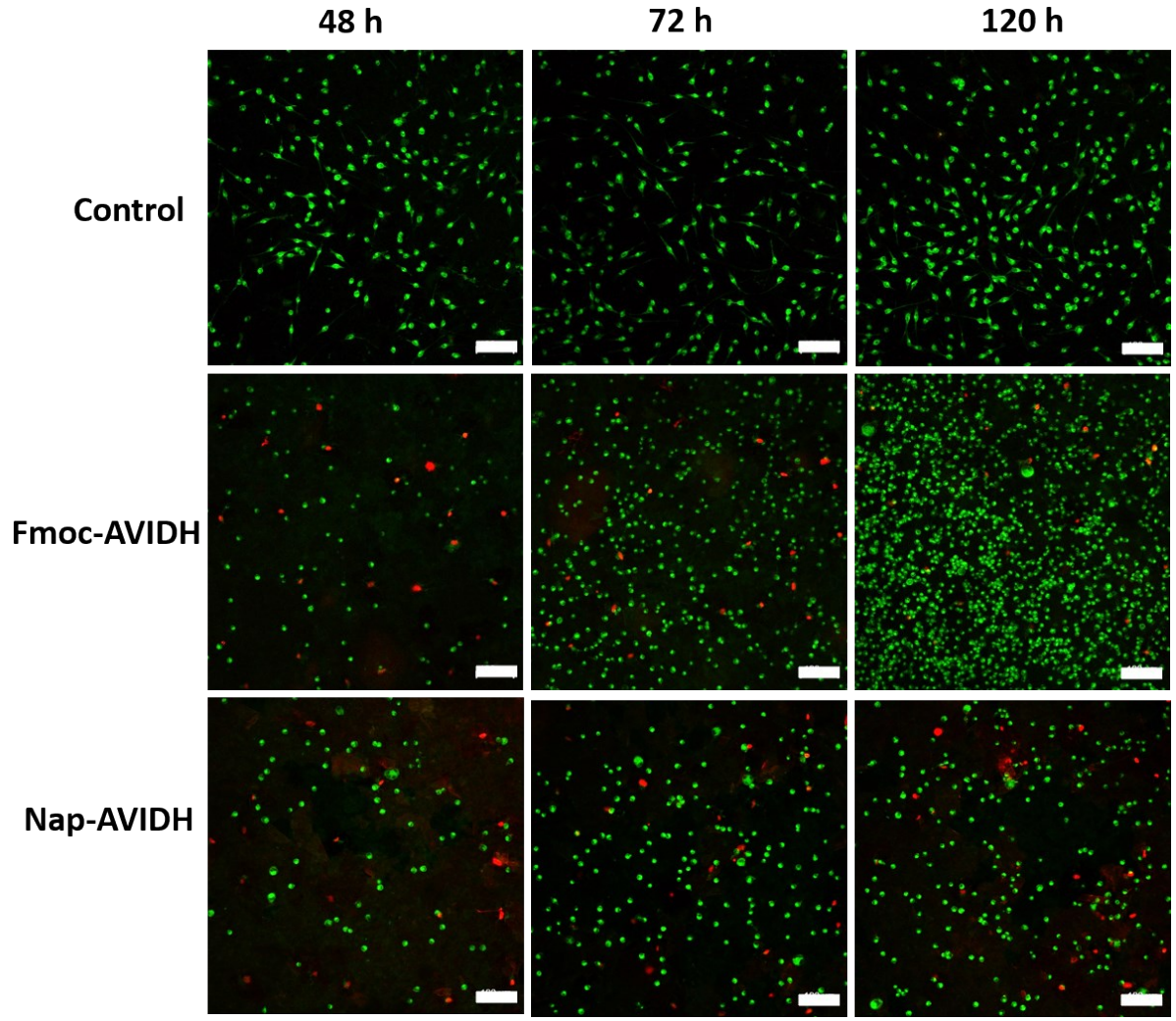

Figure S16: 2D cell culture study of L929 cell line on control scrambled N-cadherin peptide hydrogels. Analysis of adhesion and proliferation of mouse fibroblast cells, L929 on N-cadherin mimetic peptide hydrogels by Live/Dead staining. Confocal images of live and dead L929 cells labelled with DiOC18(3) and PI, respectively showing the cellular viability, adhesion, and proliferation on (a-c) control (uncoated coverslip), and (d-f) Fmoc-AVIDH hydrogel and (g-i) Nap-AVIDH hydrogel after 48 h, 72 h, and 5 days, respectively. Scale bar is 100 μm.

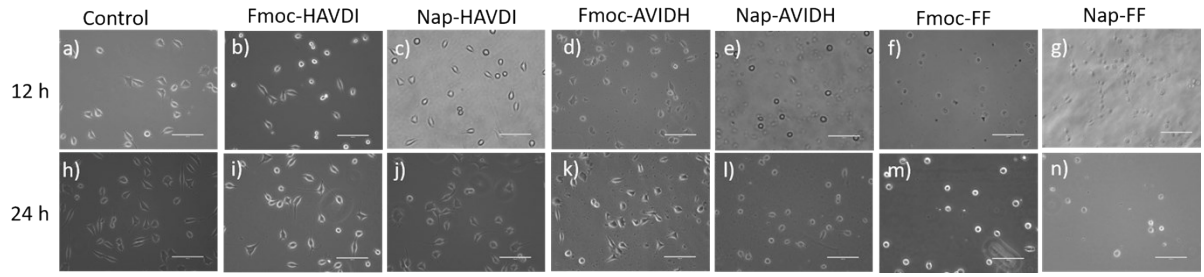

Figure S17: Optical images of the L929 cell line on N-cadherin mimetic peptide and control peptide hydrogels. Morphological analysis of mouse fibroblast cells, L929 on N-cadherin mimetic peptide and control peptide hydrogels by optical imaging. Optical images of L929 cells on the (a, h) culture dish, (b, i) Fmoc-HAVDI, (c, j) Nap-HAVDI, (d, k) Fmoc-AVIDH, (e, l) Nap-AVIDH, (f, m) Fmoc-FF and (g, h) Nap-FF hydrogels after 12 h, and 24 h, respectively. Scale bar is 100 µm.

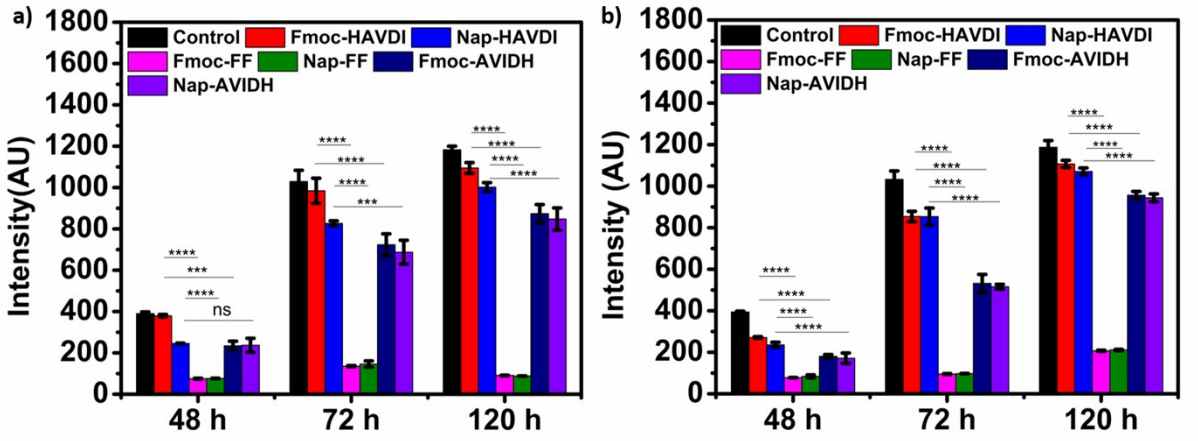

Figure S18: Quantification of cellular viability and proliferation of (a) rat glioma cell line, C6 and (b) mouse fibroblast cell line, L929 on N-cadherin mimetic peptide Fmoc-HAVDI, Nap-HAVDI, control peptide Fmoc-FF, Nap-FF, Fmoc-AVIDH, and Nap-AVIDH peptide hydrogels using alamar blue assay at di□erent time points. Data are represented as mean ± SD. * represents P-value ≤ 0.05, ** P-value ≤ 0.01, and **** P-value ≤ 0.0001 and "ns" stands for nonsignificant di□erence (two-way ANOVA, Bonferroni's multiple comparisons tests).

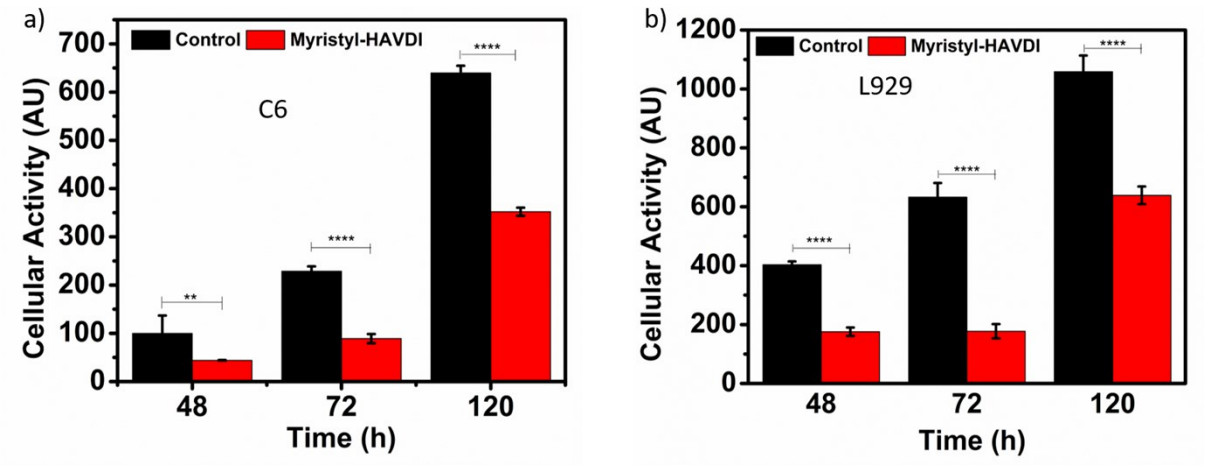

Figure S19: Quantification of cellular viability and proliferation of (a) rat glioma cell line, C6 and (b) mouse fibroblast cell line, L929 on Myristyl-HAVDI peptide hydrogels using alamar blue assay at di□erent time points. Data are represented as mean ± SD. * represents P-value ≤ 0.05, ** P-value ≤ 0.01, and **** P-value ≤ 0.0001 and "ns" stands for nonsignificant di□erence (two-way ANOVA, Bonferroni's multiple comparisons tests).

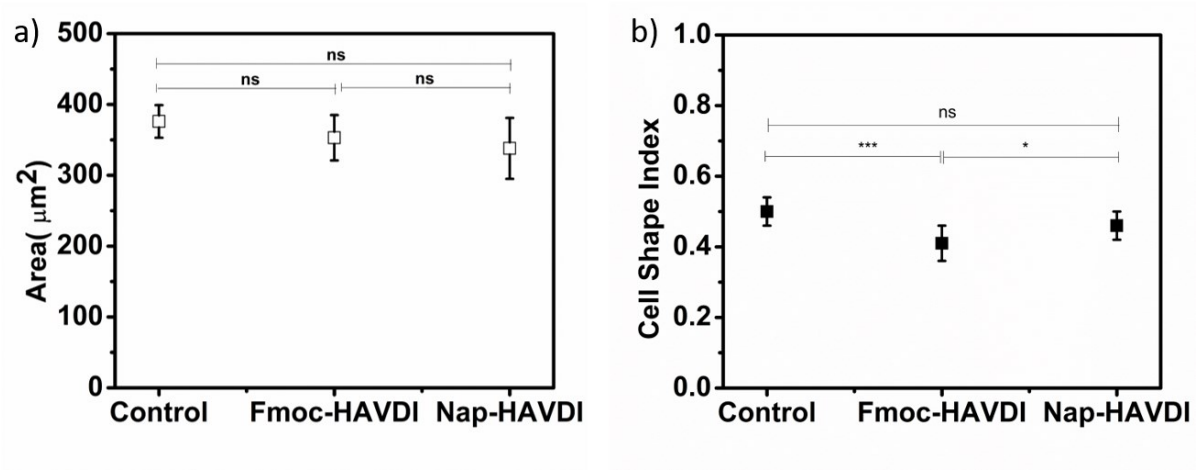

Figure S20: Quantification of (a) cells spreading area and (b) cell shape index of rat glioma cell line, C6 on uncoated coverslip and N-cadherin mimetic peptide Fmoc-HAVDI and Nap-HAVDI. Data are represented as mean ± SD. * represents P-value ≤ 0.05, ** P-value ≤ 0.01, and **** P-value ≤ 0.0001 and "ns" stands for nonsignificant di□erence (one-way ANOVA, Bonferroni's multiple comparisons tests).

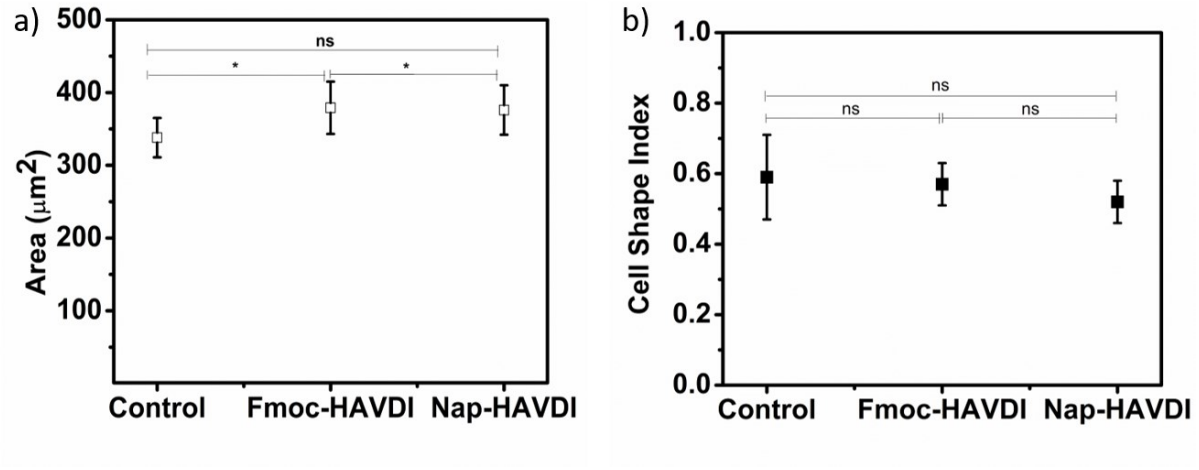

Figure S21: Quantification of (a) cells spreading area and (b) cell shape index of rat mouse fibroblast cell line, L929 on uncoated coverslip and N-cadherin mimetic peptide Fmoc-HAVDI and Nap-HAVDI. Data are represented as mean ± SD. * represents P-value ≤ 0.05, ** P-value ≤ 0.01, and **** P-value ≤ 0.0001 and "ns" stands for nonsignificant di□erence (one-way ANOVA, Bonferroni's multiple comparisons tests).

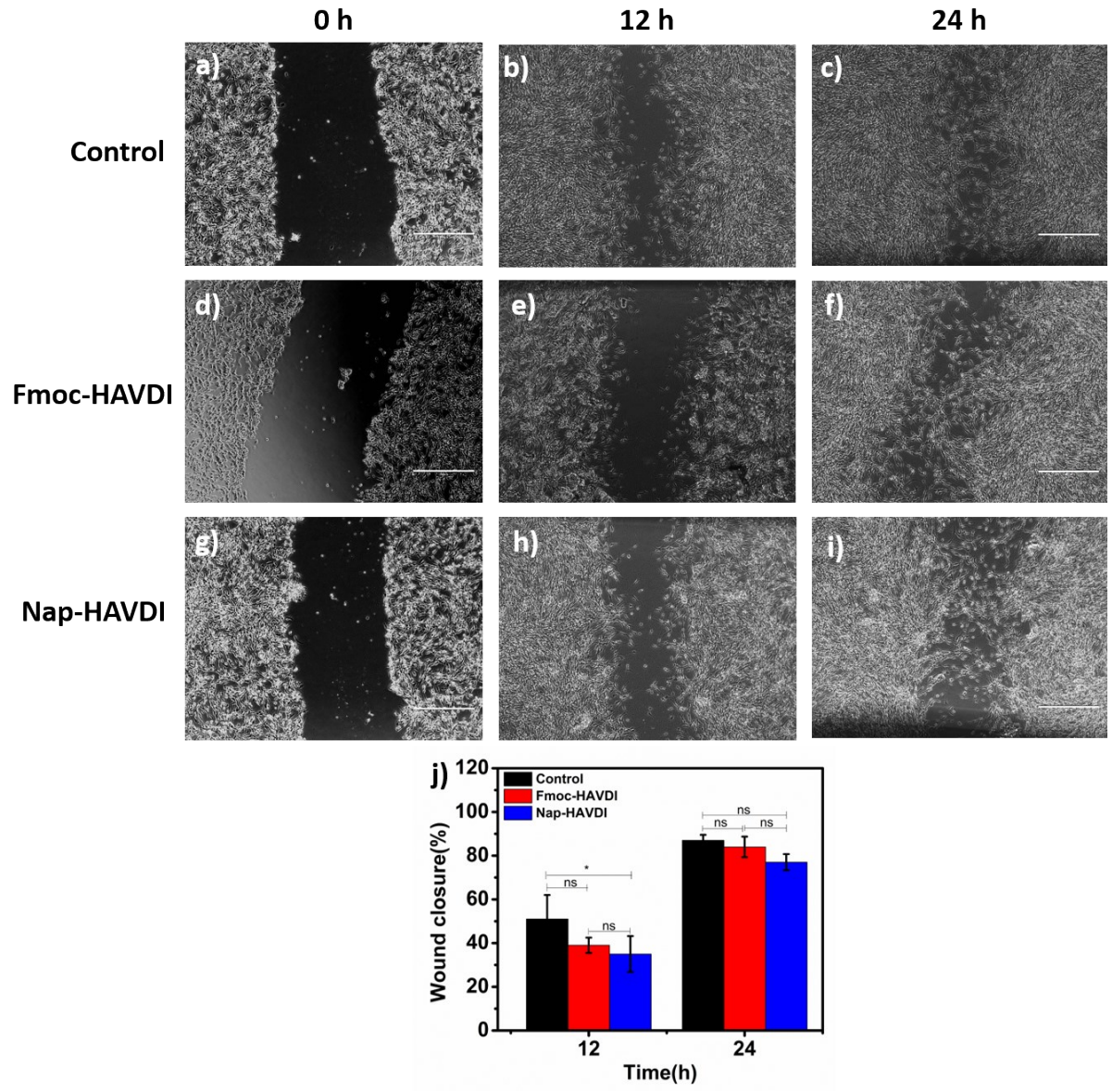

Figure S22: Scratch wound assay to analyse the cellular migration of C6 cells in presence of the peptide treatment. Scratch closure in (a-c) TCP, (d-f) Fmoc-HAVDI and (g-i) Nap-HAVDI at 0, 12 and 24 hours. Scale bar is 400 µm. (j) Quantification of the wound closure with time i.e., after 12h and 14 h.

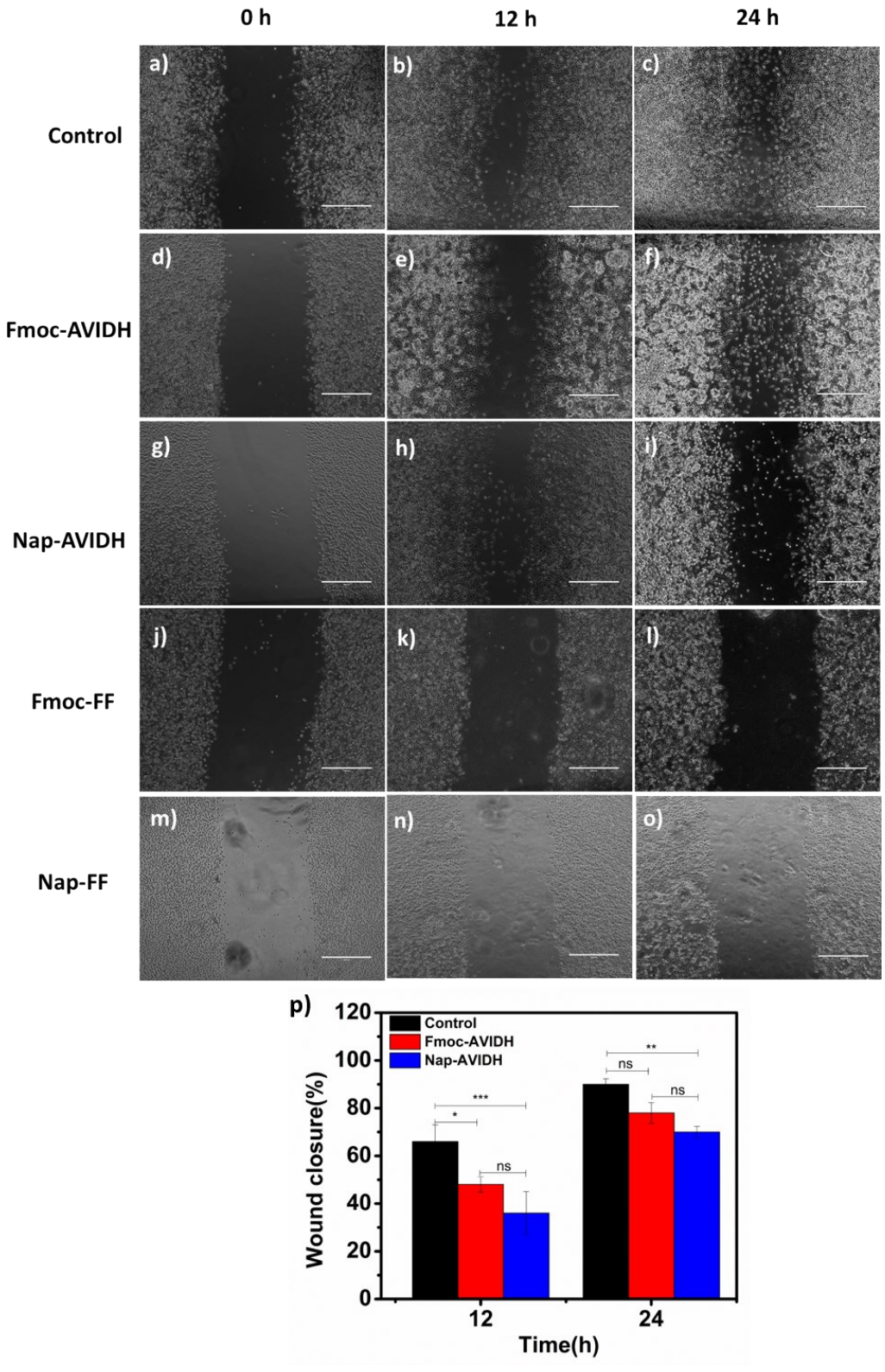

Figure S23: Scratch wound assay to analyse the cellular migration of C6 cells in presence of the peptide treatment. Scratch closure in (a-c) TCP, (d-f) Fmoc-AVIDH, (g-i) Nap-AVIDH, (j-l) Fmoc-FF and (m-o) Nap-FF at 0, 12 and 24 hours. Scale bar is 400 μm. (p) Quantification of the wound closure with time i.e., after 12h and 14 h.

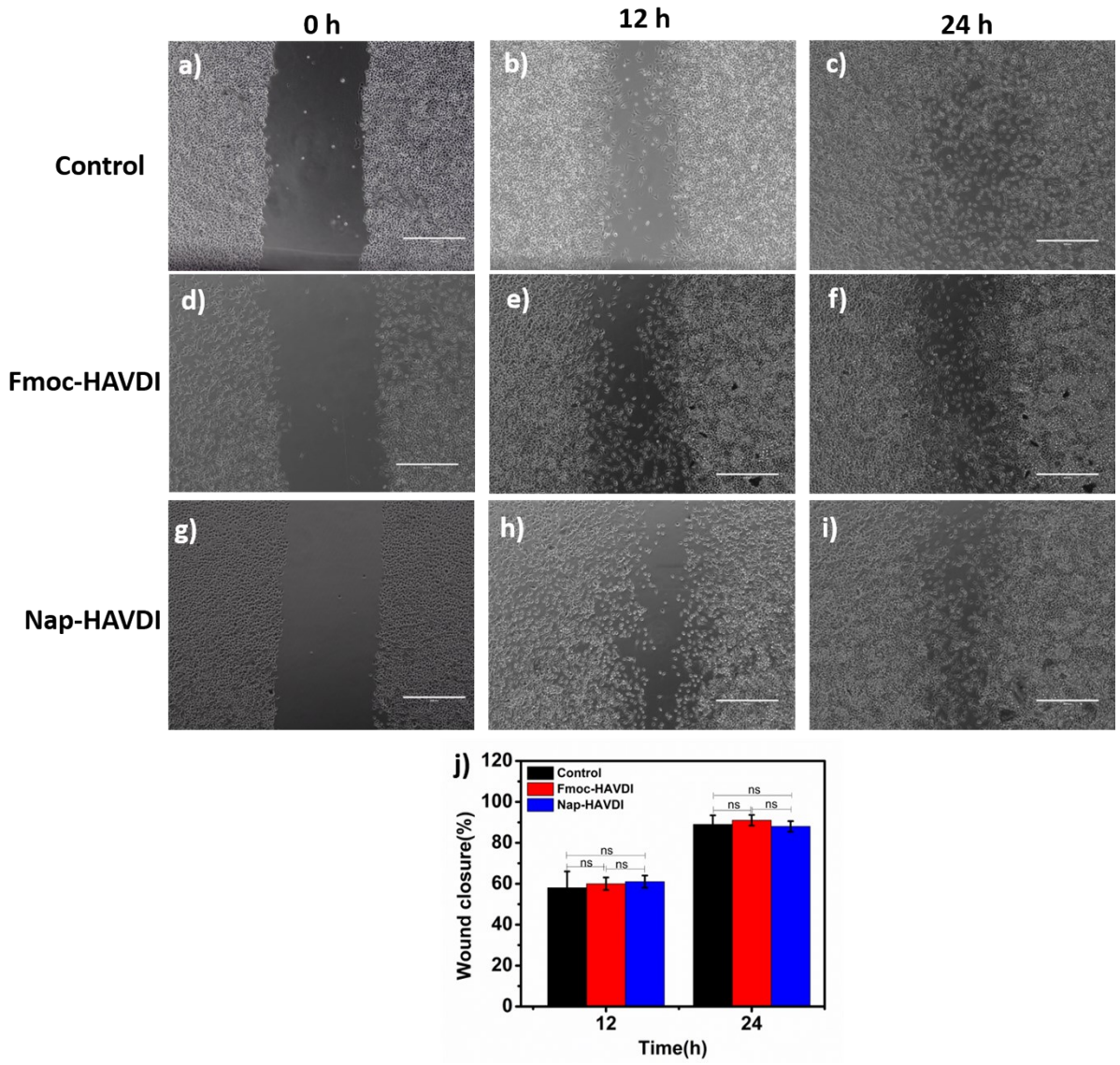

Figure S24: Scratch wound assay to analyse the cellular migration of L929 cells in presence of the peptide treatment. Scratch closure in (a-c) TCP, (d-f) Fmoc-HAVDI and (g-i) Nap-HAVDI at 0, 12 and 24 hours. Scale bar is 400 µm. (j) Quantification of the wound closure with time i.e., after 12h and 14 h.

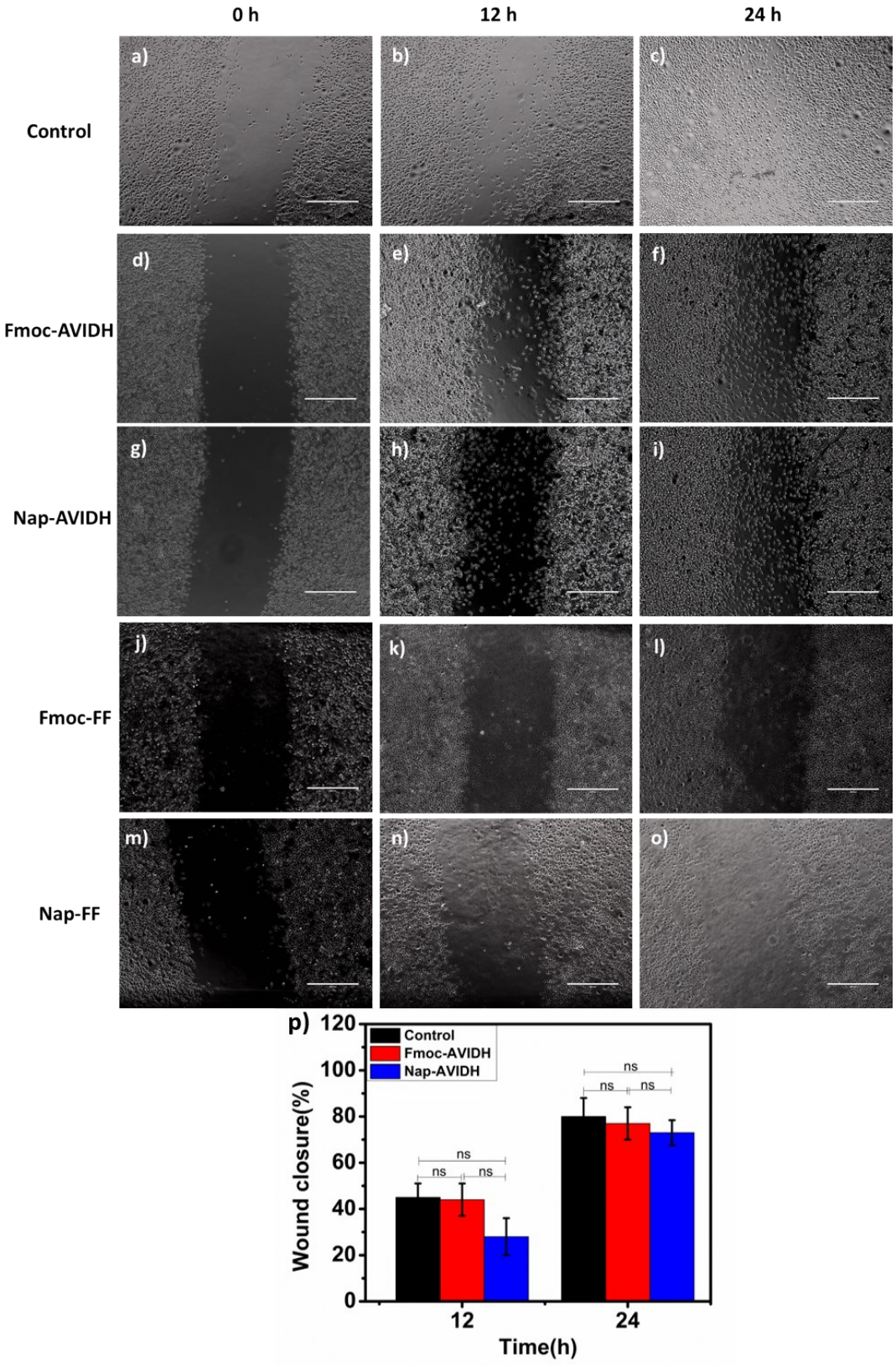

Figure S25: Scratch wound assay to analyse the cellular migration of L929 cells in presence of the peptide treatment. Scratch closure in (a-c) TCP, (d-f) Fmoc-AVIDH, (g-i) Nap-AVIDH, (j-l) Fmoc-FF and (m-o) Nap-FF at 0, 12 and 24 hours. Scale bar is 400 µm. (p) Quantification of the wound closure with time i.e., after 12h and 14 h.