Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supporting Information

Peptide-Based Delivery Vectors with Pre-defined Geometrical Locks

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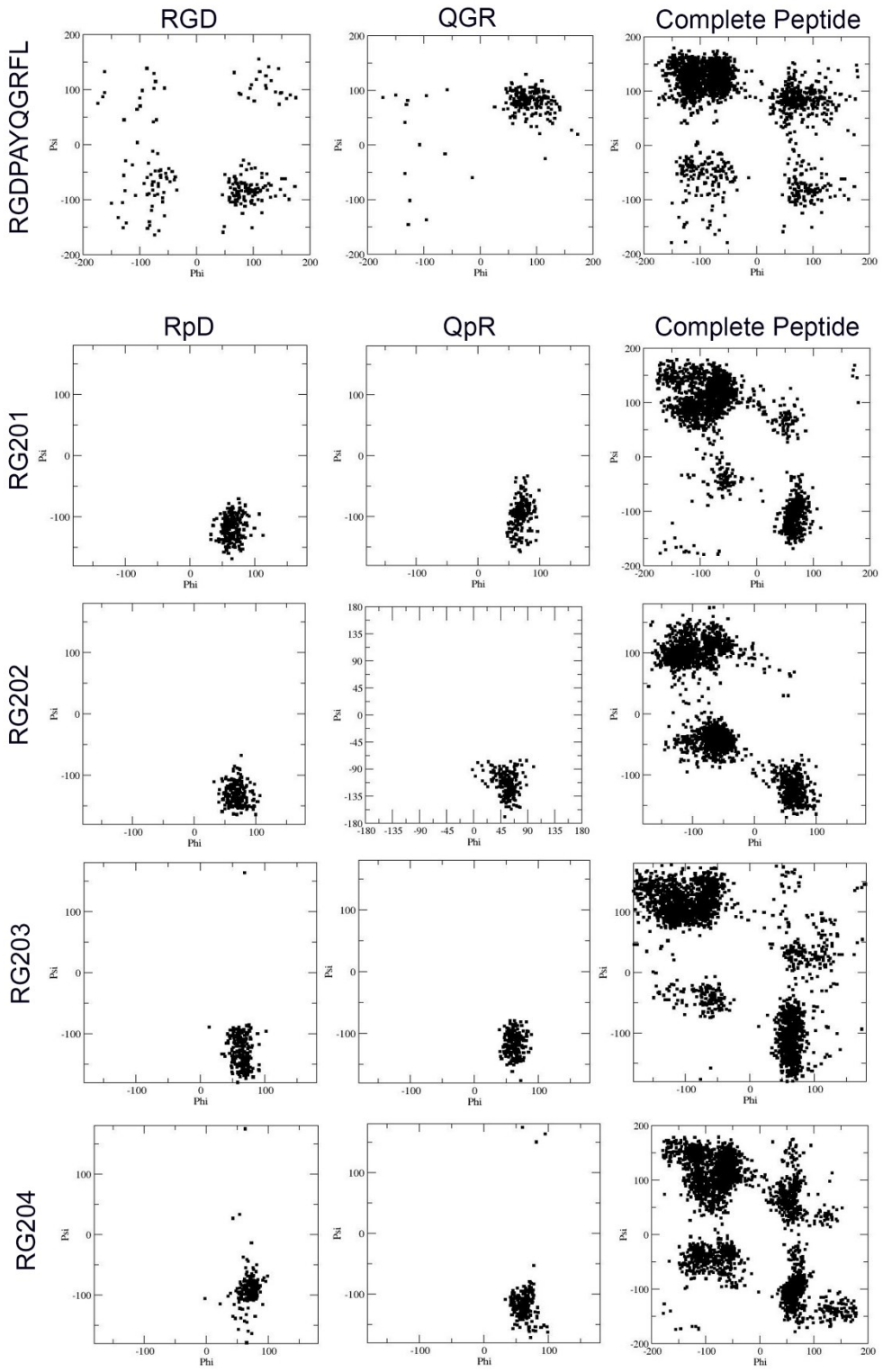

Figure S1. Dihedral angle distribution of the designed peptides. The observed dihedral angle distribution of fixed second and eight residues in the designed peptides (RG201-RG204) along with the complete peptide dihedral distribution using Molecular Dynamics Simulation. RGDPAYQGRFL is a reported tumor homing peptide.[1]

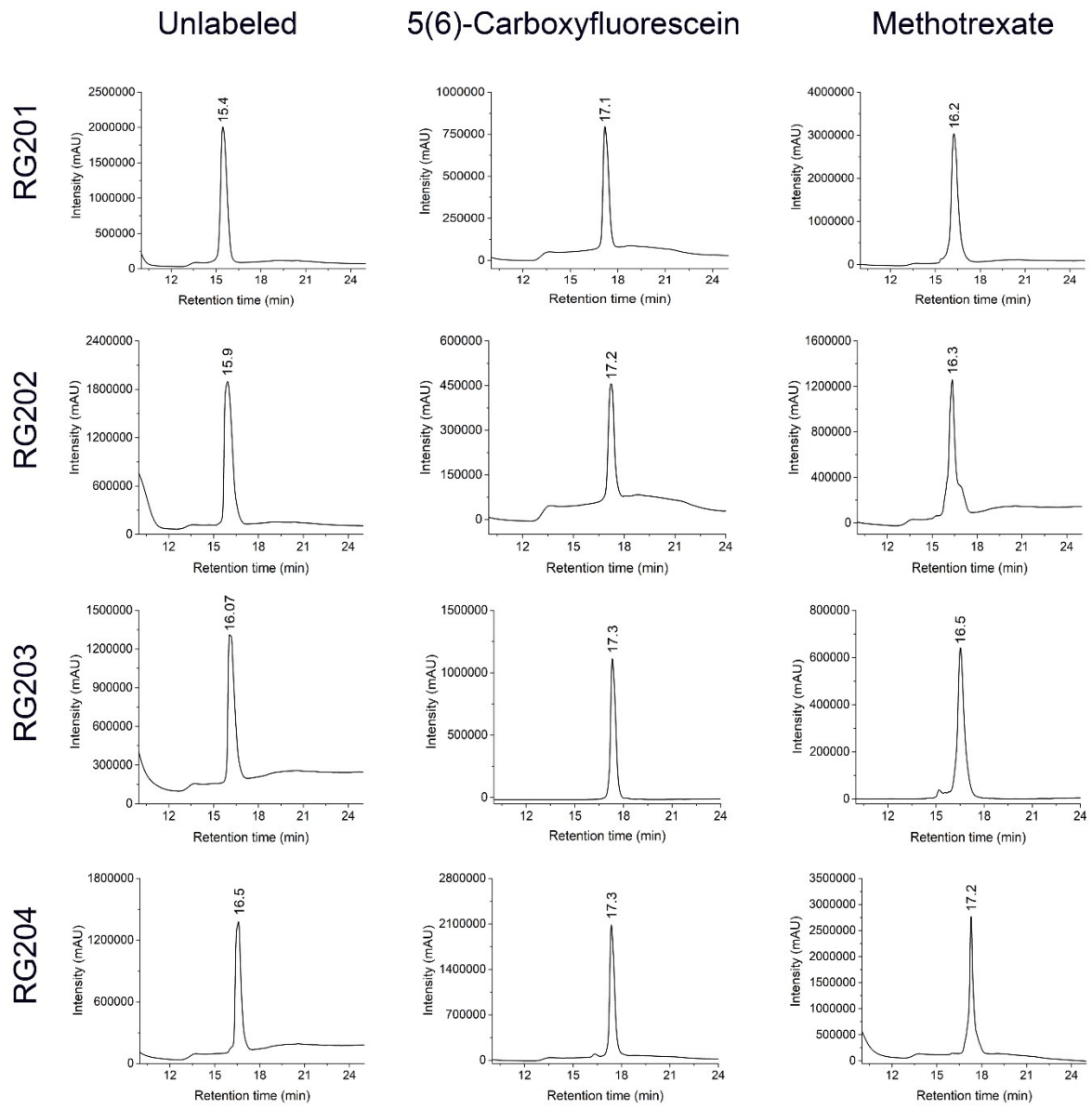

Figure S2. The RP-HPLC chromatograms of the purified peptides and their conjugates. In all plots, absorbance intensity (y-axis) at 210 nm is plotted against retention time (x-axis). Unlabeled represents peptide with free N-terminus. Carboxyfluorescein and methotrexate headlines indicate peptide vectors conjugated with 5(6) carboxyfluorescein and methotrexate, respectively.

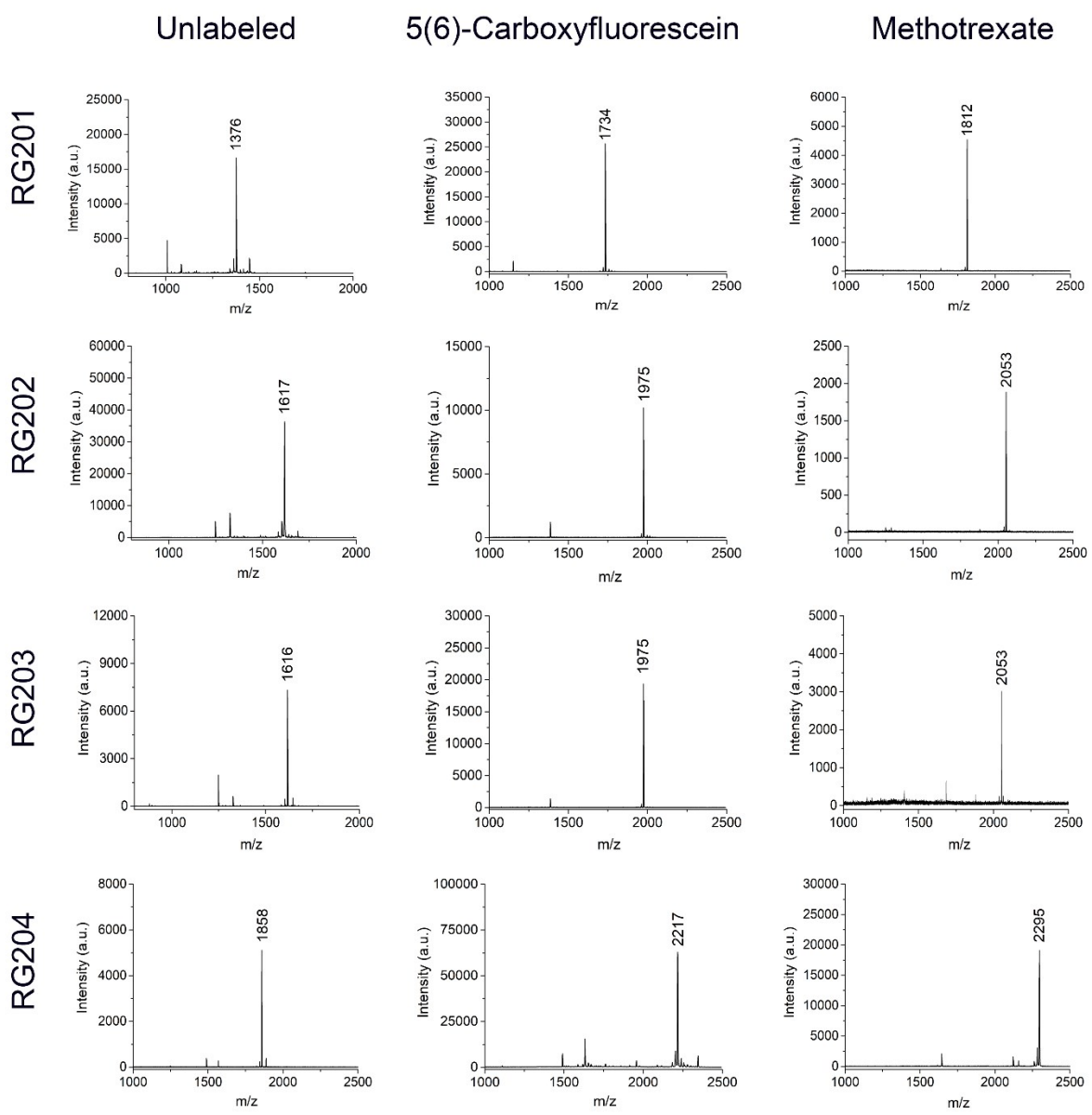

Figure S3. MALDI-MS spectra of peptides and their conjugates. In all plots, intensity (y-axis) is plotted against m/z values (x-axis). Unlabeled represents peptide with free N-terminus. Carboxyfluorescein and methotrexate headlines indicate peptide vectors conjugated with 5(6) carboxyfluorescein and methotrexate, respectively. The observed molecular mass (m/z) values of peptides and their conjugates are $[M+H]^+$, $[M+2H]^+$ or $[M+3H]^+$.

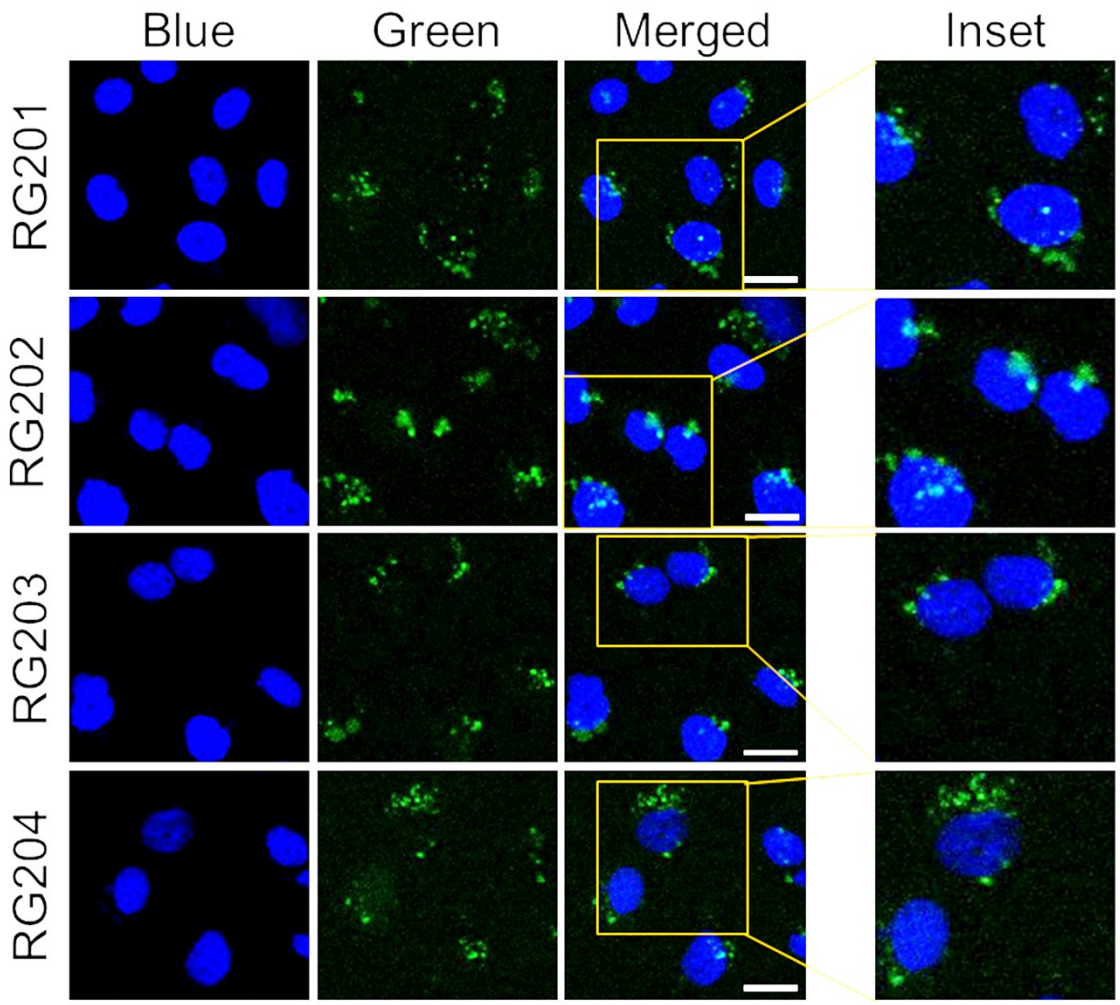

Figure S4. Cellular uptake in MDA-MB-231 cells. The cellular uptake of 5(6)-Carboxyfluorescein-tagged peptides (RG201-RG204) in breast cancer (MDA-MB-231) cells through confocal laser scanning microscopy. After peptide treatment, nuclei were stained with Hoechst 33342. In this figure, blue represents Hoechst staining and green shows CF-tagged peptides. Scale bar corresponds to 50 μm.

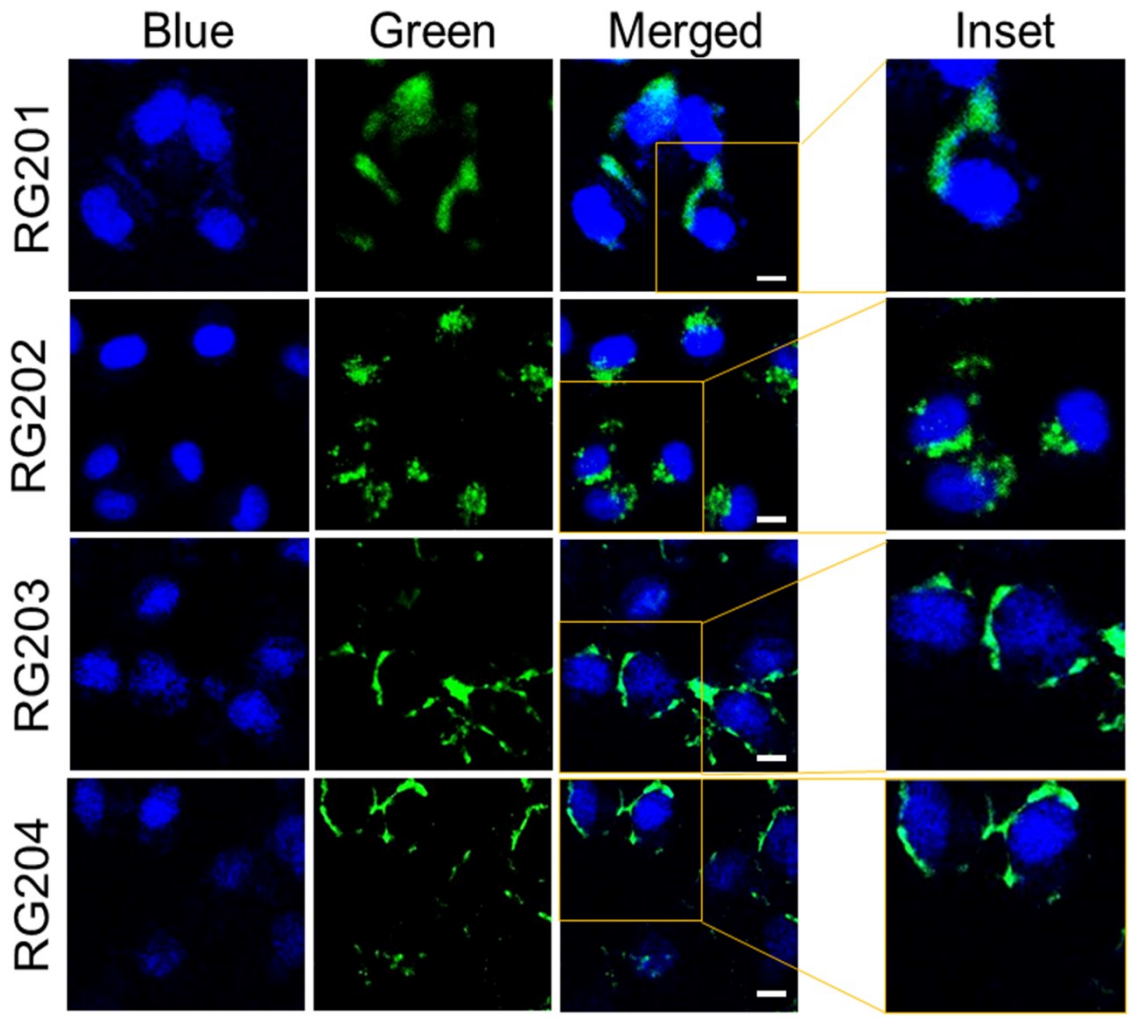

Figure S5. Cellular uptake in HeLa cells. The cellular uptake of 5(6)-Carboxyfluorescein-tagged peptides (RG201-RG204) in cervical cancer (HeLa) cells using confocal laser scanning microscopy. After peptide treatment, nuclei were stained with Hoechst 33342. In this figure, blue represents Hoechst staining and green shows CF-tagged peptide. Scale bar corresponds to 20 μm.

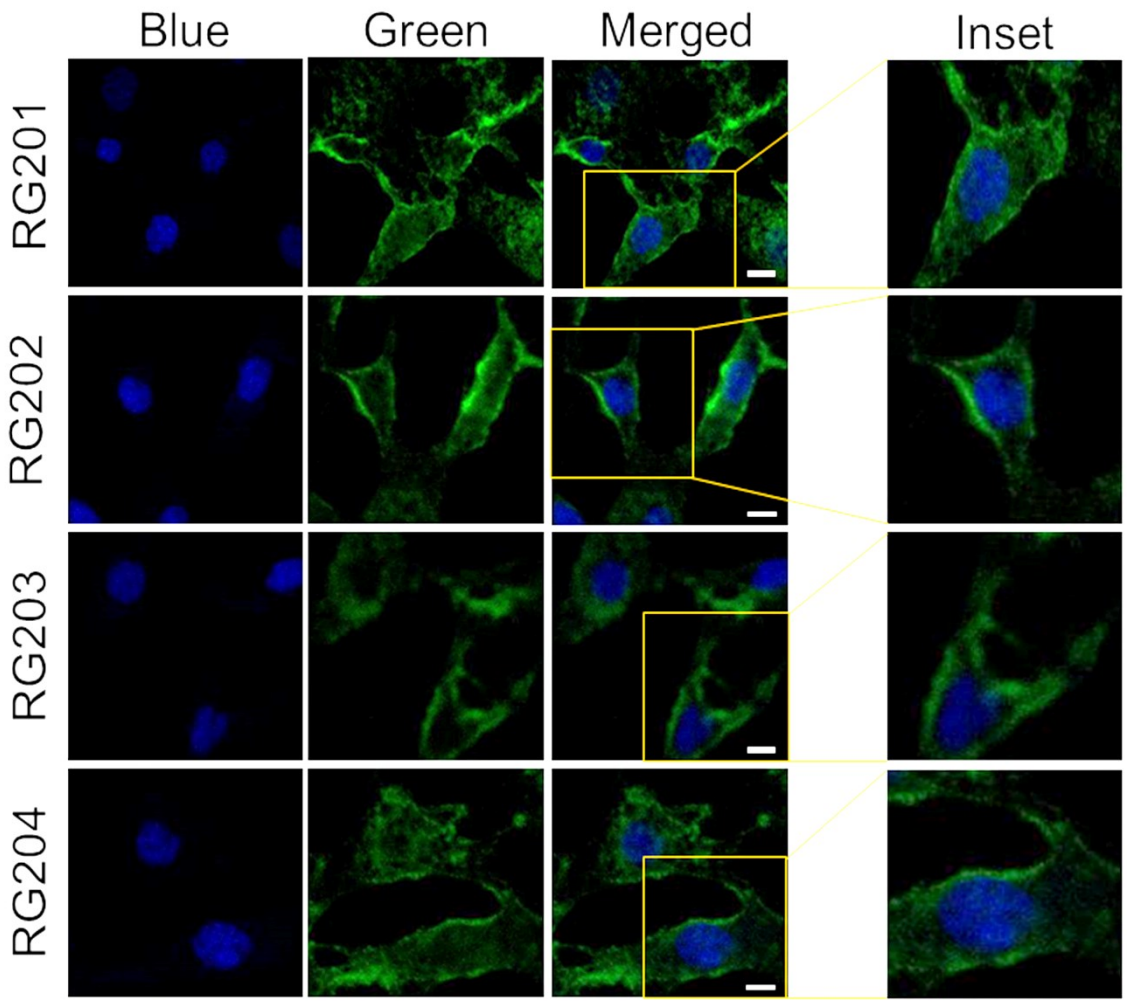

Figure S6. Cellular uptake in MCF-10A cells. The cellular uptake of 5(6)-Carboxyfluorescein tagged peptides (RG201-RG204) in mammary epithelial (MCF-10A) cells through confocal laser scanning microscopy. After peptide treatment, nuclei were stained with Hoechst 33342. In this figure, blue represents Hoechst staining and green shows CF-tagged peptides. Scale bar corresponds to 20 μm.

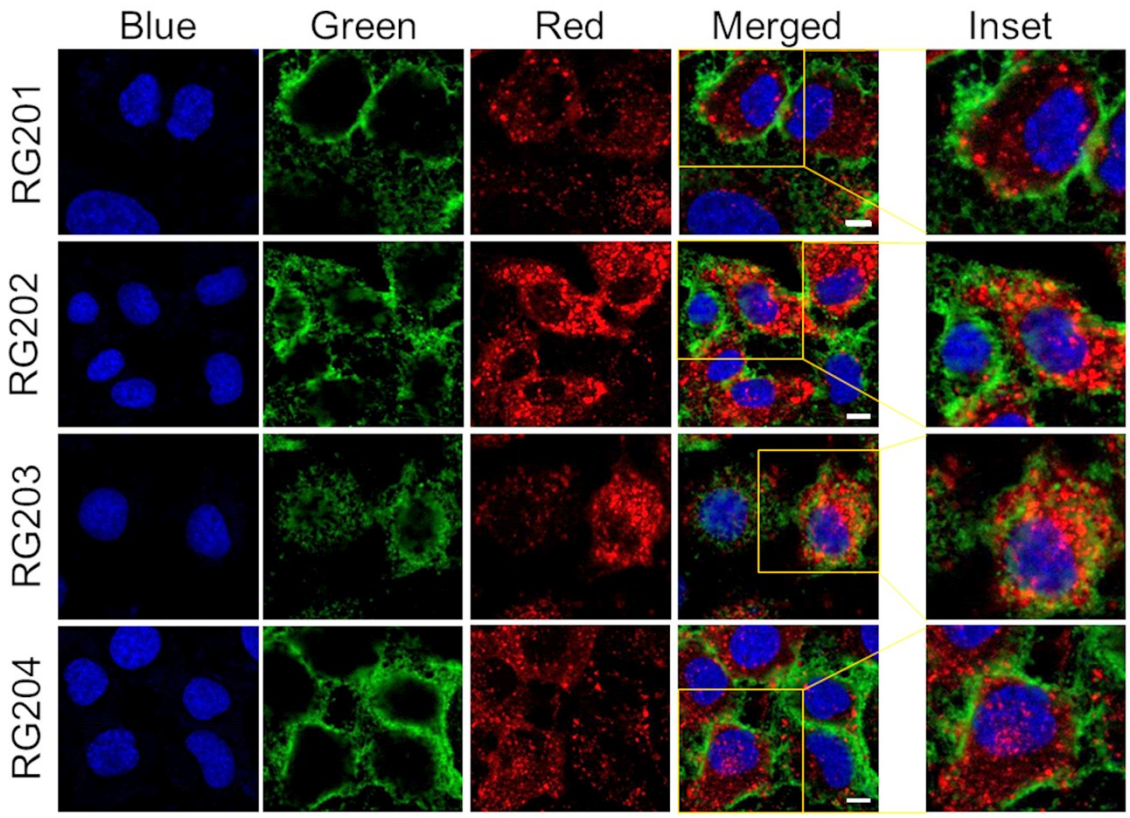

Figure S7. Cellular uptake in U2-OS cells. The cellular uptake of 5(6)-Carboxyfluorescein tagged peptides (RG101-RG108) in LAMP-RFP transfected osteosarcoma (U2-OS) cells through confocal microscopy. After peptide treatment, nuclei were stained with Hoechst 33342. In this figure, blue represents Hoechst staining, green shows CF-tagged peptides and red denotes lysosomes. Scale bar corresponds to 20 μm.

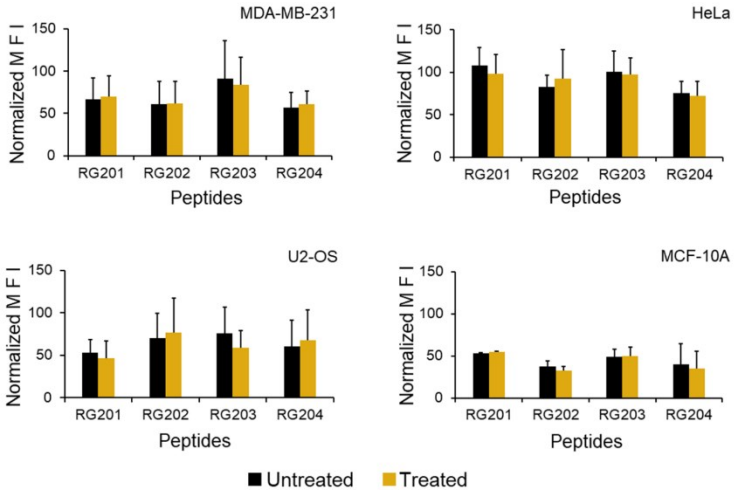

Figure S8. Biocompatibility of peptides. The binding activity of peptides in the presence and absence of serum on MDA-MB-231 cells, HeLa cells, U2-OS cells and MCF-10A cells measured by flow cytometry. The peptide stocks were pre-incubated with fetal bovine serum (FBS) for 1 h at 37°C before cell treatment. Cells were treated with 10 µM of serum untreated and treated CF-tagged peptides (RG201-RG204) for 4 h at 37 °C. The obtained fluorescence intensities were normalized by the intensities of the untreated cells. MFI represents Mean Fluorescence Intensity. Results are presented as mean ± SD of three independent experiments.

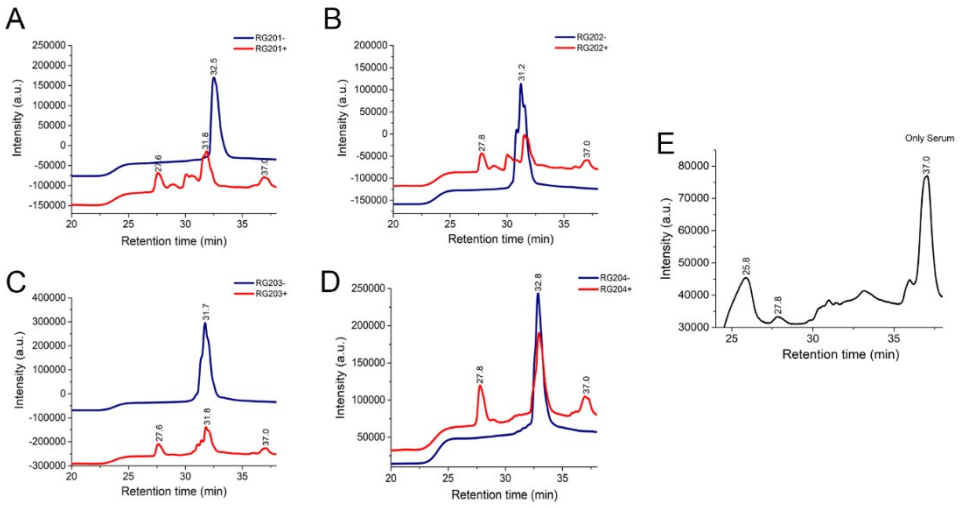

Figure S9. Verification of peptide stability in serum. (A-D) HPLC chromatogram of peptide treated with (+) and without (-) serum. (E) HPLC chromatogram of serum only. This experiment was performed with 1mM stock concentration of peptides. Peptides were incubated with 100% human serum (sterile; Sigma) in 1:1 ratio for 2 h at 37°C in shaking incubator. 0.6% Trichloroacetic acid (TCA) was used for precipitation of serum proteins. After precipitation, the samples were kept at 4°C for 15 min and then centrifuged at 13000 rpm for 5 min. The supernatant was collected and 20 µl of it was injected in HPLC instrument (Shimadzu, LC 20AD). Similar treatment was given to conditions having only serum (black) and only peptides (blue), respectively.

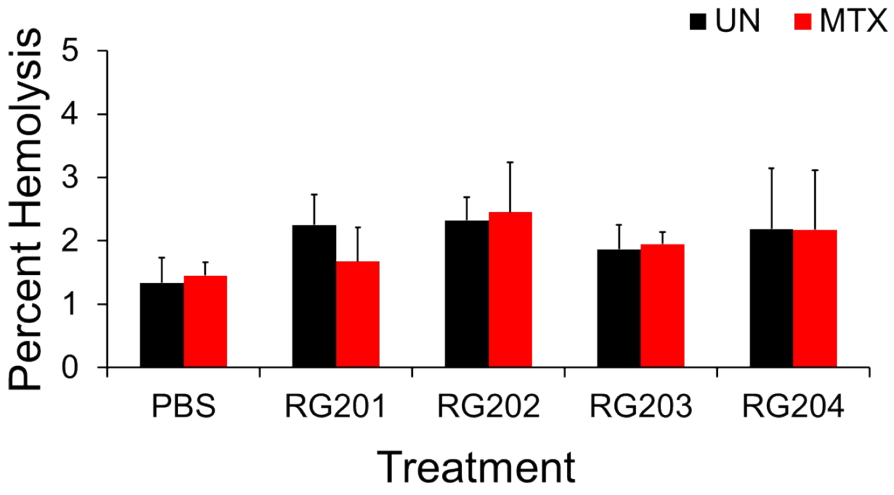

Figure S10. Hemolytic activity of peptides. Hemolytic assay of the designed peptides and their MTX conjugates against human red blood cells (RBCs). Heme release was measured at 540 nm after treating human RBCs with buffer, and 100 μM of peptides (UN) and their MTX conjugates (MTX) for 2 h at 37°C. The maximum heme release was < 3% in all the cases, indicating the almost non-hemolytic nature of peptides and their drug-conjugates even up at 100 μM concentration. The data was normalized by complete lysis with 0.5 % of triton X-100. All results are presented as the mean ± SD of three independent experiments.

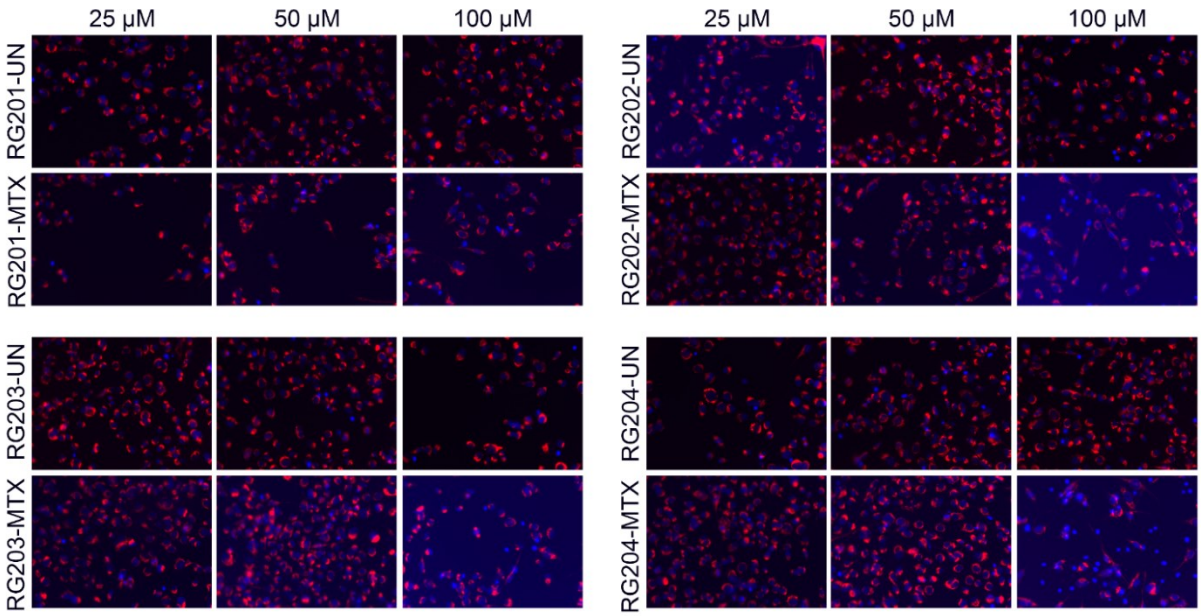

Figure S11. TMRM cytotoxicity assay. Representative images of cytotoxicity of designed peptides and their MTX conjugates on breast cancer MDA-MB-231 cells after 48 h at 25 μM, 50 μM and 100 μM concentrations. The cells with TMRM loss and chromatin condensation were considered undergoing apoptosis and hence, taken for cell death analysis.

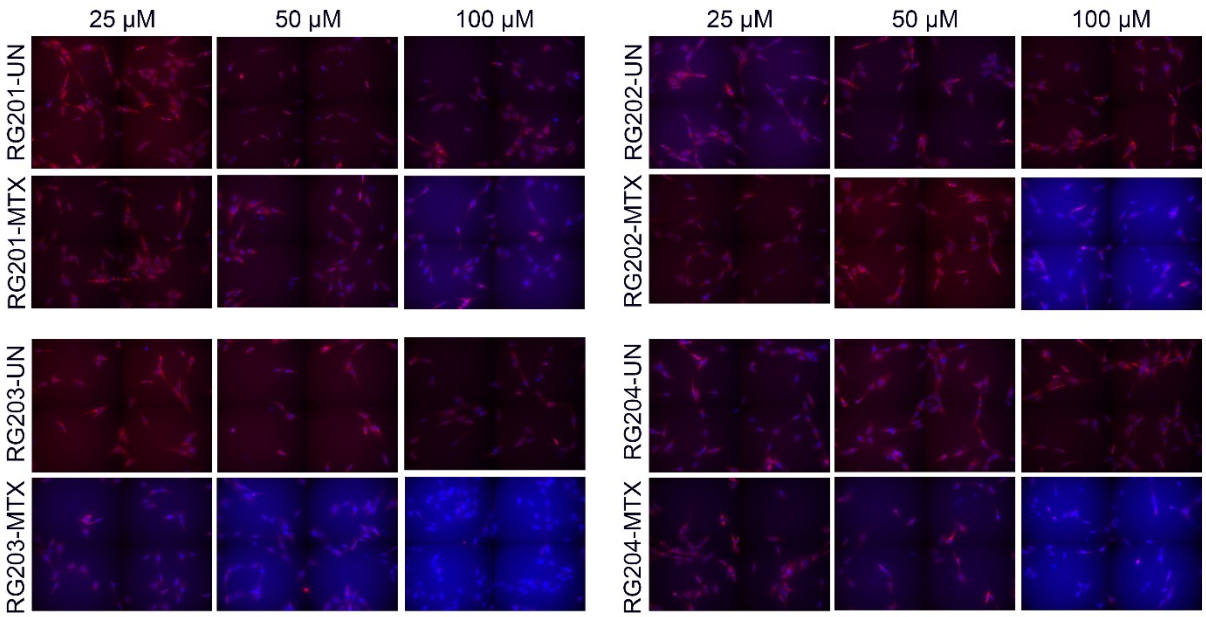

Figure S12. TMRM cytotoxicity assay. Representative images of cytotoxicity of designed peptides and their MTX conjugates on mammary epithelial MCF-10A cells after 48 h at 25 µM, 50 µM and 100 µM concentrations. The cells with TMRM loss and chromatin condensation were considered undergoing apoptosis and hence, taken for cell death analysis.

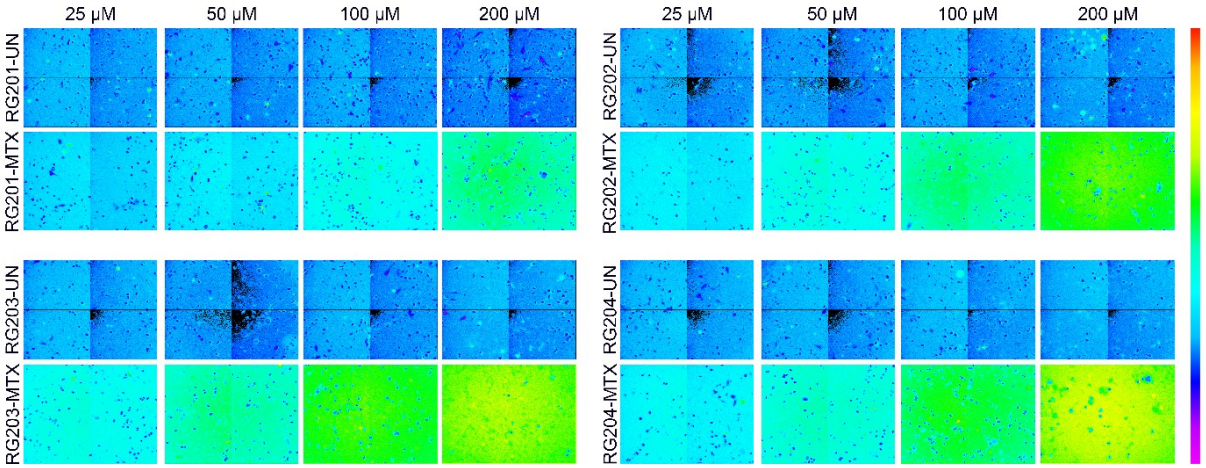

Figure S13. Representative images to show apoptotic cell death. To confirm the cell death by apoptosis, MDA-MB-231 cells having stable expression of CFP-YFP FRET based caspase sensor, DEVD were treated with peptides and peptide-methotrexate conjugates for 48 h at 25 µM, 50 µM and 100 µM concentrations. The loss of FRET in cells was measured in terms of increase in CFP-YFP ratio which corresponds to color change from purple to red on the ratio scale. The images were acquired as 2x2 montages which later stitched as single image for each well (n=4).

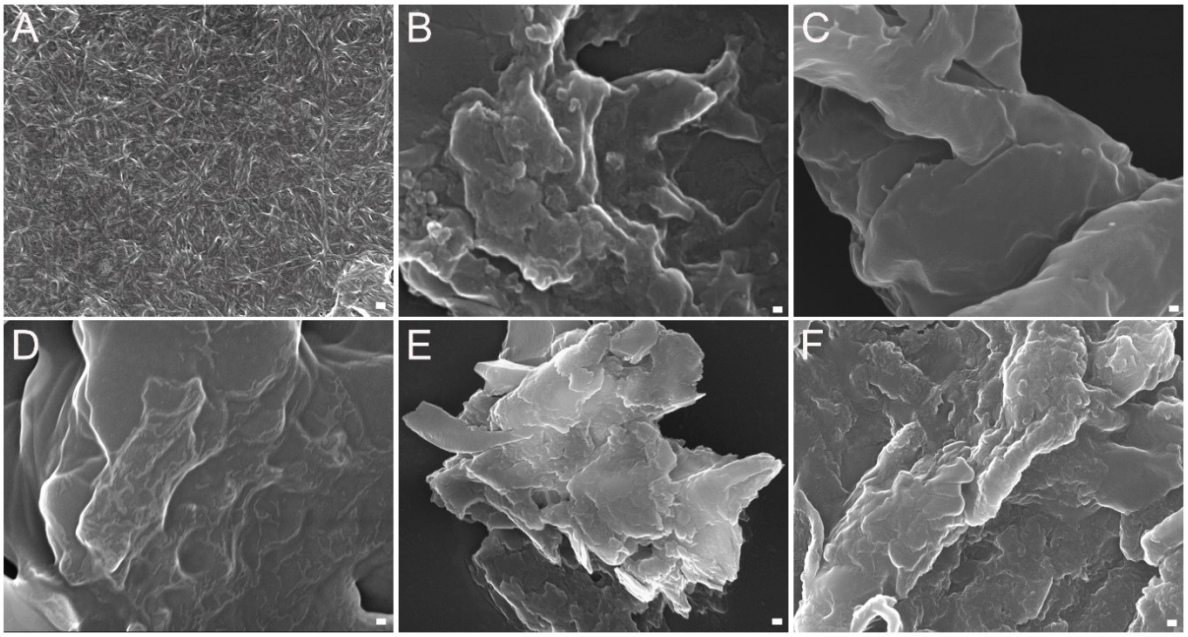

Figure S14. Field emission scanning electron photomicrographs (FESEM) of peptides. (A) PhF6 peptide is taken as positive control for nano-assembly formation (nano fibres).[2] (B) RGDPAYQGRFL peptide is an already reported tumor homing peptide.[1] (C-F) FESEM images of the designed peptides RG201-RG204 showing only amorphous aggregates. Scale bar corresponds to 100 nm.

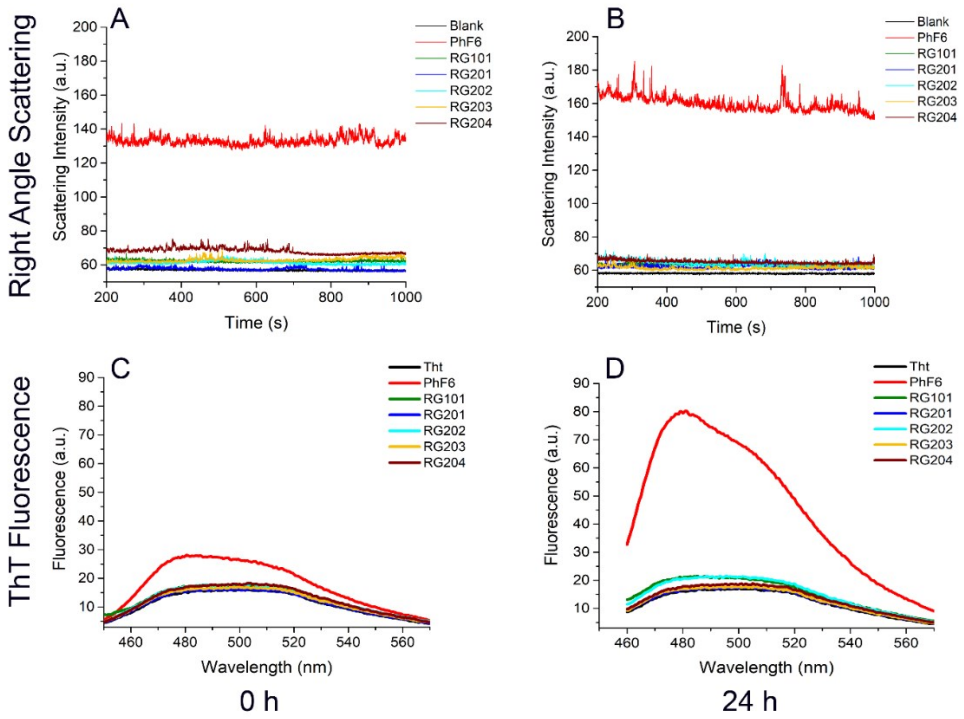

Figure S15. Verification of peptide self-assembly. The self-assembly formation of peptides in solution was verified by Right Angle Scattering (A, B) and ThT Fluorescence Assay (C, D) at two time points 0 h (A, C) and 24 h (B, D).

References

1. Ahmed S, Mathews AS, Byeon N, Lavasanifar A, Kaur K. Peptide Arrays for Screening Cancer Specific Peptides. Analytical Chemistry. 2010;82(18):7533-41.

2. Pandey G, Morla S, Nemade HB, Kumar S, Ramakrishnan V. Modulation of aggregation with an electric field; scientific roadmap for a potential non-invasive therapy against tauopathies. RSC Advances. 2019;9(9):4744-50.