ELETTRONIC SUPPORTING INFORMATIONS (ESI)

FOR

Amyloid-like Prep1 peptides exhibit reversible blue-green-red fluorescence *in vitro* and in living cells

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Experimental section

Materials. Protected amino acids, coupling agents (HATU, Oxyma) and Fmoc-Rink Amide AM resin used for peptide synthesis were purchased from IRIS Biotech GmbH (Marktrewitz, Germany). Solvents, including acetonitrile (CH_3CN), dimethylformamide (DMF) were purchased from Carlo Erba reagents (Milan, Italy). Other products, such as trifluoroacetic acid (TFA), sym-collidine, diisopropylethylamine (DIPEA), piperidine, were from Sigma-Aldrich (Milan, Italy).

Peptide synthesis, purification and characterization. Peptides were synthesized by solid-phase synthesis as C-terminally amidated molecules following the procedure previously described.[1] HPLC analyses for peptide characterization were performed on an Alliance HT WATERS 2795 system, equipped with a PDA WATERS detector 2996. Preparative purifications were carried out on a WATERS 2545 preparative system (Waters, Milan, Italy) fitted out with a WATERS 2489 UV/Visible detector Peptide characterizations were performed by liquid chromatography–mass spectrometry analysis (LC–MS). MS characterization was performed using an ESI-TOF-MS Agilent 1290 Infinity LC System coupled to an Agilent 6230 time-of-flight (TOF) LC/MS System (Agilent Technologies, Cernusco sul Naviglio, Italy). The LC Agilent 1290 LC module was coupled with a photodiode array (PDA) detector and a 6230 time-of-flight MS detector, and a binary solvent pump with degasser, a column heater and an autosampler. The LC-MS characterization of peptides was performed using a C18 Waters xBridge column (3 µm, 4.6x5.0 mm), applying a linear gradient of CH_3CN/0.05%TFA in water 0.05% TFA from 5 to 70% in 20 min, at a flow rate of 0.2 mL/min. MS analyses were

performed under standard mass spectrometry conditions. The list of all peptides studied in this paper is reported in the **Table S1**. To increase the solubility of the parent peptides, they were conjugated to a polyarginine moiety (R8) via a β-alanine (βAla) as the N-terminal spacer (**Table S1**).

Circular Dichroism (CD) measurements. CD analysis was performed following the procedure previously reported.[2] Briefly, far-UV (190-260 nm) CD spectra of freshly prepared peptides were recorded at 25 °C on a Jasco J-715 spectropolarimeter, equipped with a PTC-423S/15 Peltier temperature controller, in a 0.1 cm path-length quartz cell. Each spectrum is the average of three continuous scans corrected by subtraction of appropriate blank. Peptides were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) to obtain stock solutions at 1.0 mM and used at the working concentrations (25 μM) after dilution with the buffers indicated in the figure legends (sodium phosphate for pH 7.0 and sodium acetate for pH 3.0). The pH-dependent structural transition was monitored by treating 200 μL of the peptide at 25 μM concentration dissolved in the phosphate buffer (pH 7.0) with 3 μL of 0.5 M HCl. The final pH (3.0) was measured with a standard pH meter (pH/mV/°C meter, bench, Eutech pH 700) coupled with a micro-electrode with diameter of 3 mm. For the reverse transition from pH 3.0 to 7.0, 2.0 μL of 0.5 M NaOH were added to the peptide solution solubilized in sodium acetate pH 3.0 at the concentration of 25 μM.

Dynamic Light Scattering (DLS). Mean diameter of the freshly prepared peptide PREP1[117-132] was measured by DLS measurements using a Zetasizer Nano ZS (Malvern Instruments, Westborough, MA) that employs a 173° backscatter detector. Other instrumental settings were: measurement position 4.65 mm; attenuator 9; temperature 25 °C; disposable sizing cuvette as cell. DLS measurements in triplicate were performed on the peptides dissolved in HFIP to obtain stock solutions at 4.0 mM and used at the working concentrations (200 μM) after dilution in phosphate buffer pH 7.0 and after room temperature centrifugation at 13,000 rpm for 20 min.

Intrinsic fluorescence analysis in solution. Measurements of the intrinsic fluorescence emission in solution of peptides were performed on a Jasco Model FP-750 spectrofluorophotometer in a 1.0 cm path length quartz cell. Aqueous solutions of peptides were prepared dissolving peptides with DMSO at 10 mg mL−1 and diluting them in phosphate buffer up to 200 μM. Immediately after the dilution, all samples appeared clear, and the fluorescence emission was recorded in the 500-600 (500-800 nm for the peptide PREP1[117-132], see Fig. S3) range upon excitation in the 400-480 nm range. Emission spectra of the freshly prepared solution of PREP[117-132], as described above, were collected at pH 7.0, upon excitation at 370 nm and 670 nm.

Analysis of peptide fluorescence at the solid-state at confocal microscope. Lyophilized peptides were dissolved at 4.0 mM in 100% HFIP. From these stocks, the following dilutions of the peptides were prepared: 200 μM and 500 μM in 5.0 mM Na-Phosphate buffer pH 7.0, and 200 μM and 500 μM in 5.0 mM Na-Citrate buffer pH 3.0, in a final volume of 100 μL. Finally, 20 μL aliquots of the peptide solution was spread on a glass slide and let to dry for 3 hours before acquisition at the confocal microscope.

Peptide transfection in A549 cells. Lyophilized peptides were dissolved in sterile DMSO to a final concentration of 3.0 mM, then aliquoted and stored at -20 °C until use. A549 cells were seeded on micro-glass coverslips (Prestige), and grown in DMEM (Lonza) supplemented with 10% Fetal Bovine Serum (FBS South America, Euroclone) and 2.0 mM L-glutamine at 37 °C and 5% CO2. When they reached ~60-70% confluence, the growth medium was removed and cells were washed once with PBS and then, the serum-free medium was added. Peptides were administered to cells at a final concentration of 5.0 μM, and incubated in the serum-free medium for 8 h. The medium containing the peptides was removed, cells were washed twice with PBS, and then fixed in 4% paraformaldehyde. Coverslips were mounted on a glass slide with mowiol (Sigma-Aldrich).

GFP expression in A549 cells. A549 cells were infected with the vector pBABE-puro-GFP. The vector pBABE-puro-GFP was obtained by amplifying GFP DNA with oligos GFPSaR (CGTACGTAATGGTGAGCAAGGGCGA) and GFPSnF (GCGTCGACTTACTTGTACAGCTC GTCCA); the PCR product was then inserted into a pBABE-puro vector using SnaBI/SalI restriction sites, as described previously.[3] pBABE-puro was a gift from Hartmut Land & Jay Morgenstern & Bob Weinberg (Addgene plasmid #1764). Phoenix-Ampho cells were transiently transfected with the retroviral vector pBABE-puro-GFP by a standard calcium-phosphate protocol for the retroviral transduction. Cells containing retroviruses were filtered using 0.45 μm filters and employed to perform two rounds of infection. Two days after infection, cells were selected using puromycin (1,1 μg/mL).

Confocal microscopy acquisition. Confocal microscopy acquisitions were performed on a Leica TCS SP8 laser-scanning confocal microscope. For image acquisition of peptides on the glass slide a HC PL APO CS2 20X/075 dry objective was used, while for the acquisition of cells treated with peptides, a HC PL APO CS2 63X /1.40 oil immersion objective was used. Three different laser lines

were used for excitation: 405 nm, 488 nm, and 555 nm. The emitted radiation was recorded using filters with the following wavelength intervals: 436-486, 495-545, and 565-631 nm. The microscope was controlled by the software Leica Application Suite X. Images were acquired with format 1024x1024 pixels and 2x optical zoom. The emission spectrum of PREP1[117-132] peptide was collected using an excitation laser of 488 nm, and recording the emission curve by collecting the signal in a range between 498 nm and 588 nm in 16 steps, using a collection window of 10 nm.

Table S1: Amino acid sequences and theoretical/experimental molecular weights (MW) of peptides characterized in this work. Chemically synthesized peptides have free N- and amidated C-termini. Peptides were conjugated to an Arg_8 moiety (R8) via a β-alanine (βAla) as the N-terminal spacer.

Entry	Sequence	MW (amu)	MW (amu) $[M+H]^+$
PREP1[297-311]	AQTNLTLLQVNNWFI	1772.99	1774.54
PREP1[117-132]	LMVKAIQVLRIHLLEL	1887.22	1888.70
PREP1[117-132]I122A,L125A,L129A, L132A*	LMVKA**A**QV**A**RIH**A**LE**A**	1718.99	1720.68
R8PREP1[297-311]	RRRRRRRRβAlaAQTNLTLLQVNNWFI	3092.79	3093.90
R8PREP1[117-132]	RRRRRRRRβAlaLMVKAIQVLRIHLLE	3207.02	3208.12
R8PREP1[117-132] I122A, L125A, L129A, L132A*	RRRRRRRRβAlaLMVKA**A**QV**A**RIH**A**LE**A**	3038.83	3039.15

*Mutated residues in the peptide PREP1[117-132]$_{I122A,L125A,L129A,L132A}$ and in the R8 variant are shown in bold.

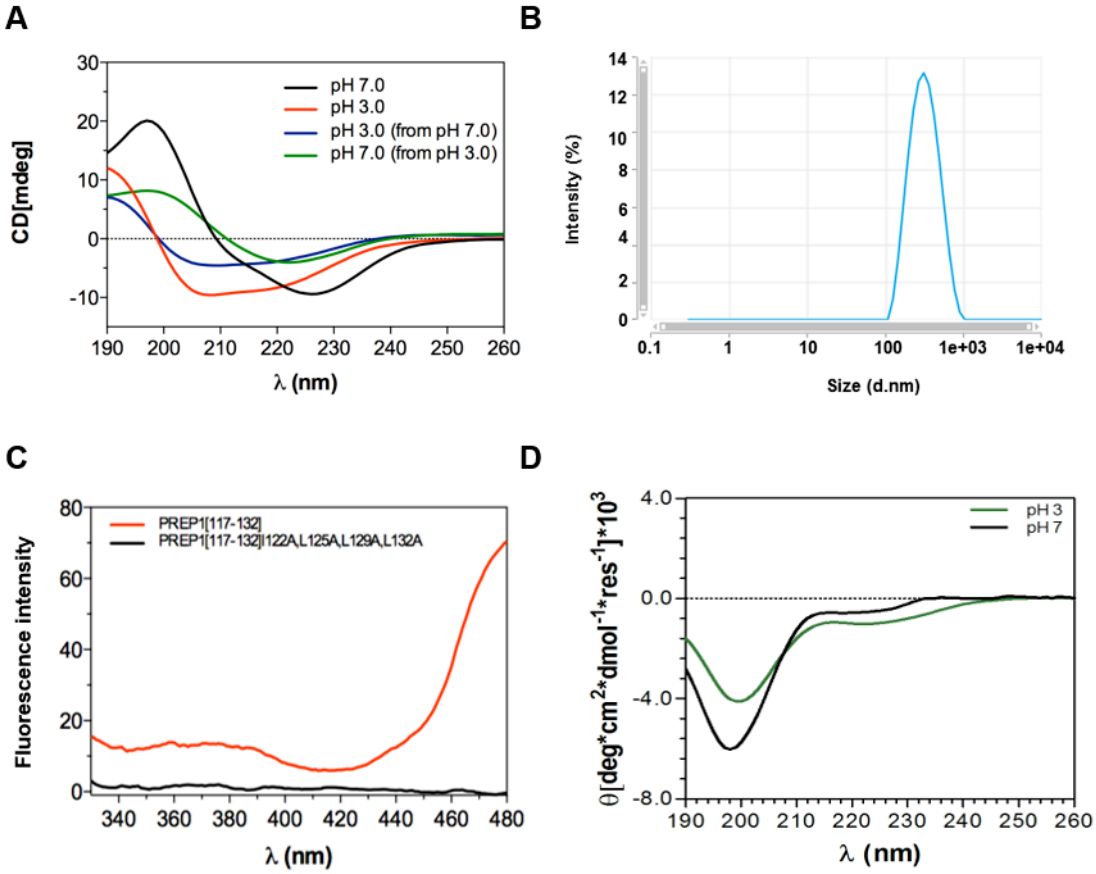

Figure S1. (**A**) Overlay of the CD spectra of PREP1[117-132] at pH 3.0 and pH 7.0 registered at 25 °C. CD analysis was performed using 25 µM of PREP1[117-132] peptide in 5 mM sodium phosphate buffer at pH 7.0 (black line) and in 5 mM sodium citrate at pH 3.0 (red line), at pH 3.0 after pH 7.0 exposure (blue line) and *vice versa* (green line). Peptides were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) to obtain stock solutions at 1.0 mM and used at the working concentrations (25 µM) after dilution with the indicated buffers. (**B**) DLS analysis of PREP1[117-132] in 5 mM sodium phosphate buffer at pH 7.0. Peptides were dissolved in HFIP to obtain stock solutions at 4.0 mM and used at the working concentrations (200 µM) after dilution with the indicated buffers. (**C**) Excitation fluorescence spectra of PREP1[117-132] (red line) and PREP1[117-132]$_{I122A,\ L125A,L129A,L132A}$ (black line) for the emission at 520 nm. Peptides were dissolved in H_2O and 50% (v/v) DMSO, immediately before analysis, to obtain stock solutions at 20 mM and used at the working concentrations (200 µM) after dilution with the indicated buffers. (**D**) Overlay of the CD spectra of PREP1[117-132]$_{I122A,L125A,L129A,L132A}$ at pH 3.0 (green line) and pH 7.0 (black line) registered at 25 °C. CD analyses were performed using 25 µM of the peptide in either 5 mM sodium phosphate buffer at pH 7.0 or 5 mM sodium citrate at pH 3.0. Peptides were dissolved in HFIP, immediately before analysis, to obtain stock solutions at 1.0 mM and used at the working concentrations (25 µM) after dilution with the indicated buffers.

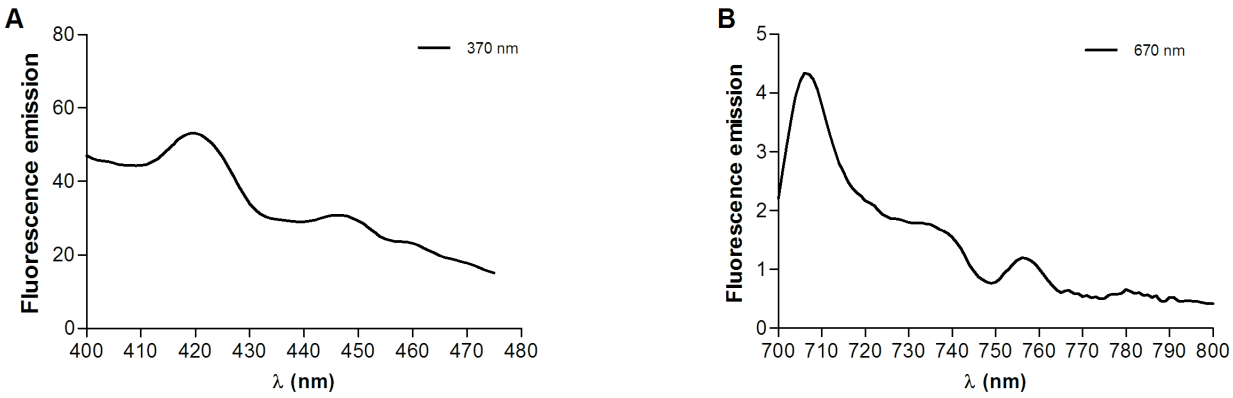

Figure S2. The intrinsic fluorescence emission spectrum of PREP[117-132] at pH 7.0 in the 400-480 nm range upon excitation at 370 nm (**A**) and in the 700-800 nm range upon excitation at 670 nm (**B**).

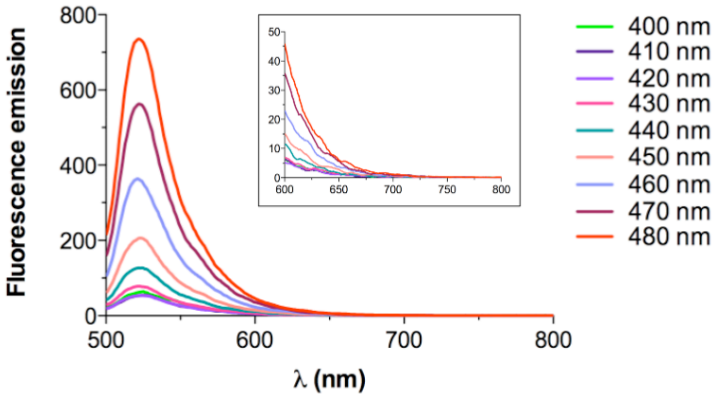

Figure S3. Overlay of intrinsic fluorescence spectra of PREP1[117-132] in the 500-800 nm range upon excitation in the 400-480 nm range in 5 mM sodium phosphate buffer at pH 7.0. A magnified view of curves in the 600-800 nm range is reported in the inset.

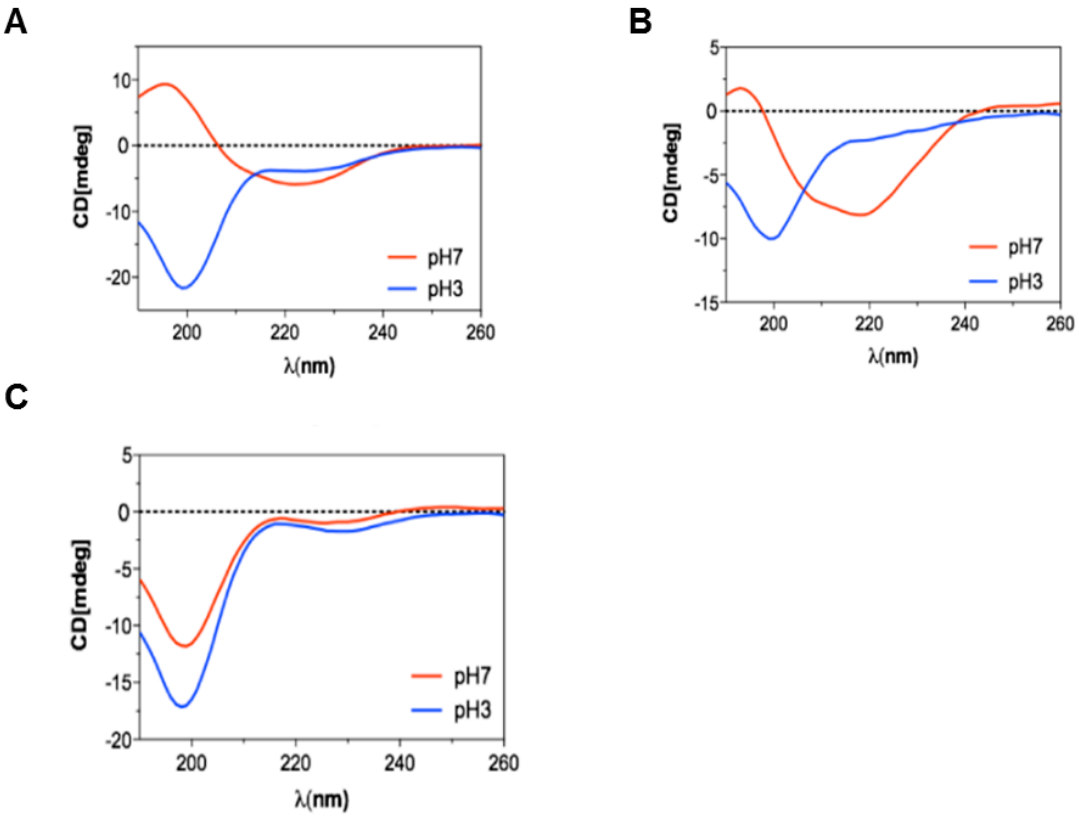

Figure S4. Overlay of the CD spectra of R8PREP1[297-311] (**A**), R8PREP1[117-132] (**B**) and R8PREP1[117-132]$_{I122A,L125A,L129A,L132A}$ (**C**) in sodium phosphate buffer at pH 7.0 (red line) and in sodium acetate at pH 3.0 (blue line). CD analyses were performed using peptides at 25 µM in either 5 mM phosphate buffer (pH 7.0) or 5 mM sodium acetate buffer (pH 3.0). Peptides were dissolved in HFIP to obtain stock solutions at 1.0 mM and diluted at the working concentrations (25 µM) with the indicated buffers.

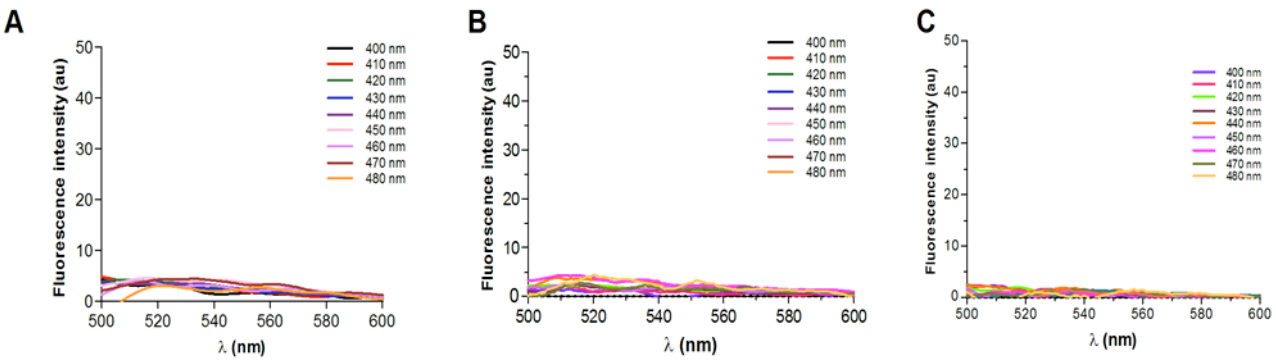

Figure S5. Overlay of the intrinsic fluorescence emission spectra of R8PREP1[297-311] (**A**), R8PREP1[117-132] (**B**), and R8PREP1[117-132]$_{I122A,L125A,L129A,L132A}$ (**C**) in sodium acetate at pH 3.0 in the 500-600 nm range upon excitation in the 400-480 nm range. Peptides were dissolved in 50% H_2O/DMSO (v:v), immediately before the analysis, to obtain stock solutions at 20 mM and diluted at the working concentrations (200 µM) with the above indicated buffers.

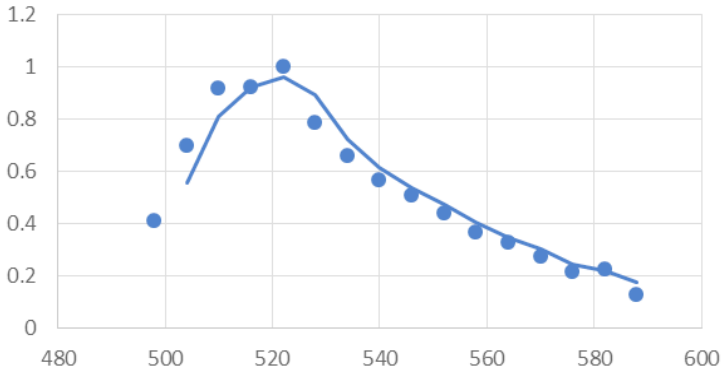

Figure S6. Fluorescence emission spectrum of the PREP1[117-132] peptide at the solid-state. The spectrum was collected using excitation laser of 488 nm, in 16 steps between 498 nm and 588 nm. A collection frame of 10 nm was used.

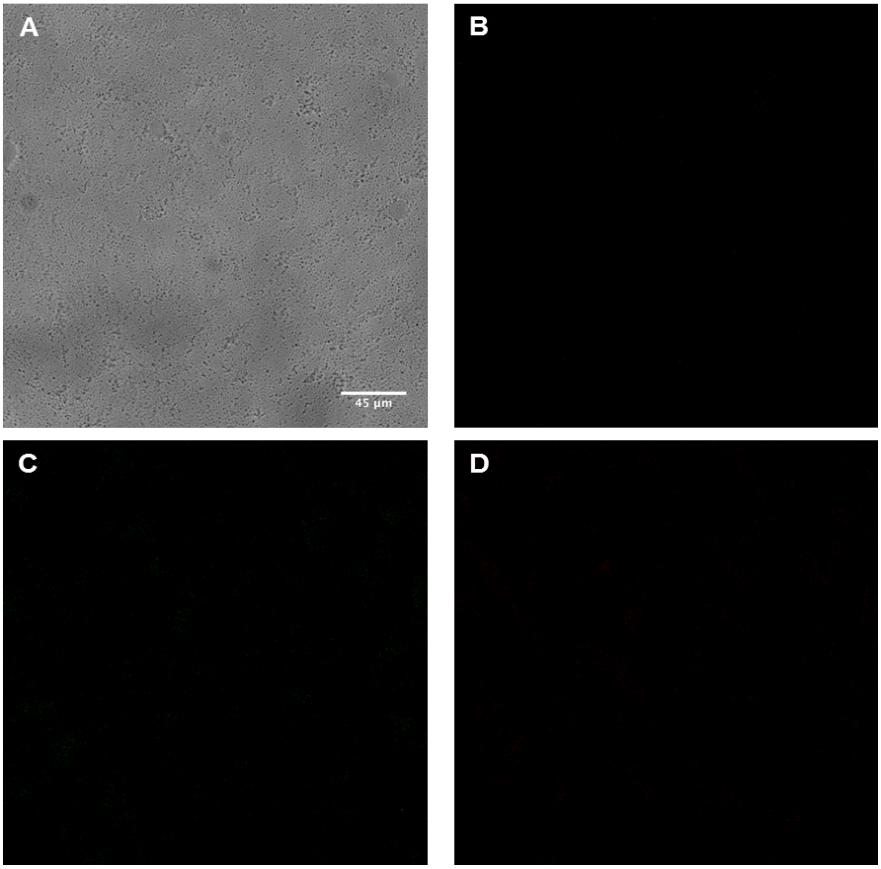

Figure S7. Bright-field (**A**) and fluorescent (**B-D**) confocal microscope images of a dried film of PREP1[117-132]$_{I122A,L125A,L129A,L132A}$. Fluorescent images were obtained by exciting in the UV spectral region of 4',6-diamidino-2-phenylindole (DAPI) (405 nm) (**B**), the green fluorescent protein (GFP) (488 nm) (**C**) and the rhodamine (555 nm) (**D**). Scale bar: 45 µm.

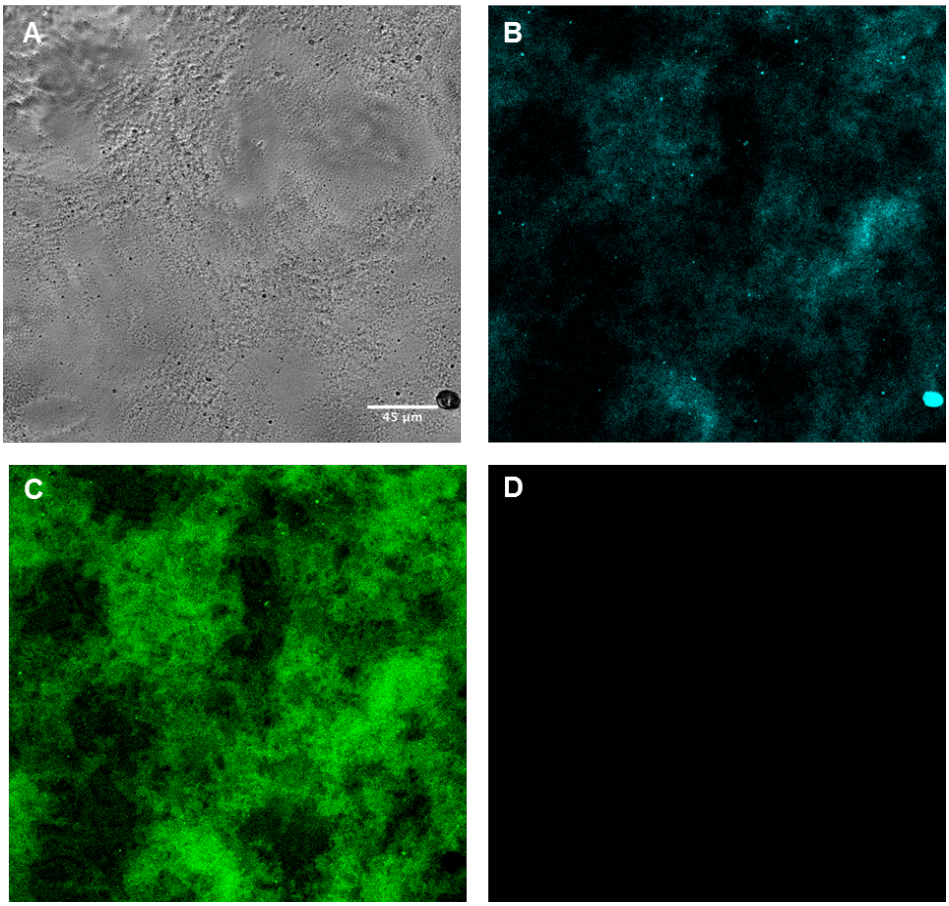

Figure S8. Bright-field (**A**) and fluorescent (**B-D**) confocal microscope images of a dried film of R8PREP1[117-132]. Fluorescent images were obtained by exciting in the UV spectral region of 4',6-diamidino-2-phenylindole (DAPI) (405 nm) (**B**), the green fluorescent protein (GFP) (488 nm) (**C**) and the rhodamine (555 nm) **(D**). Scale bar: 45 µm.

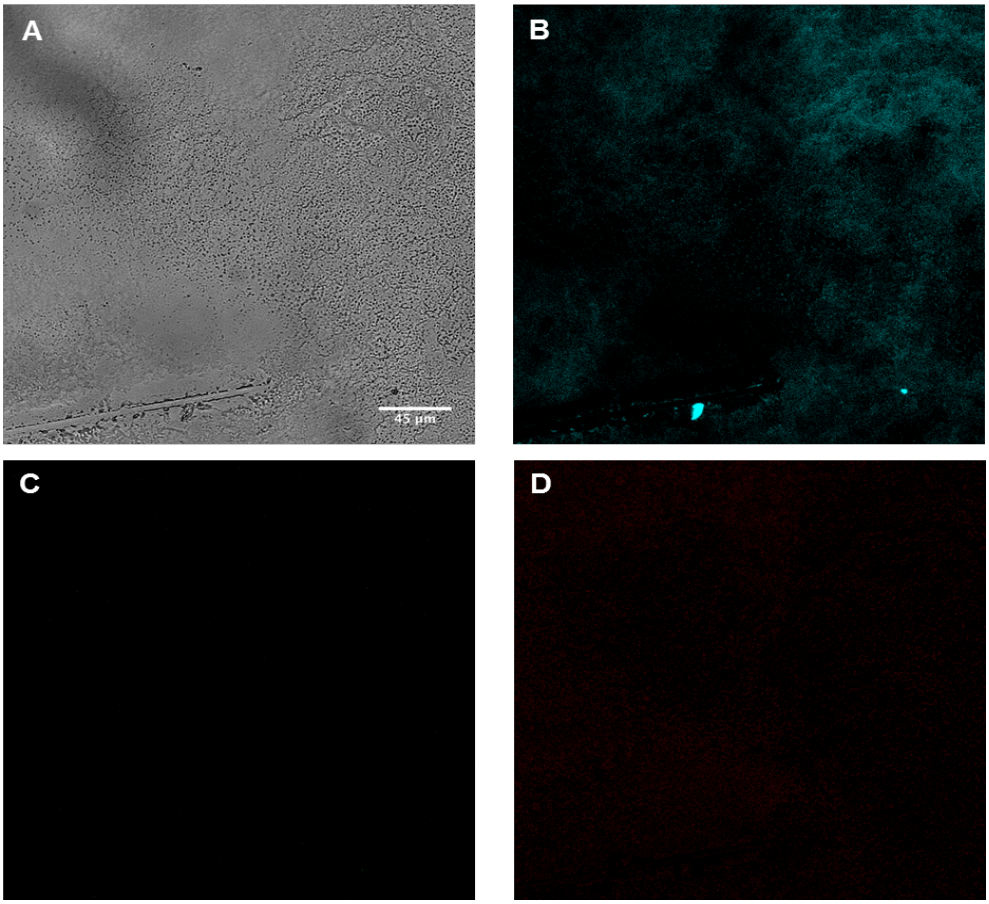

Figure S9. Bright-field (**A**) and fluorescent (**B-D**) confocal microscope images of a dried film of R8PREP1[297-311]. Fluorescent images were obtained by exciting in the UV spectral region of 4',6-diamidino-2-phenylindole (DAPI) (405 nm) (**B**), the green fluorescent protein (GFP) (488 nm) (**C**) and the rhodamine (555 nm) **(D**). Scale bar: 45 µm.

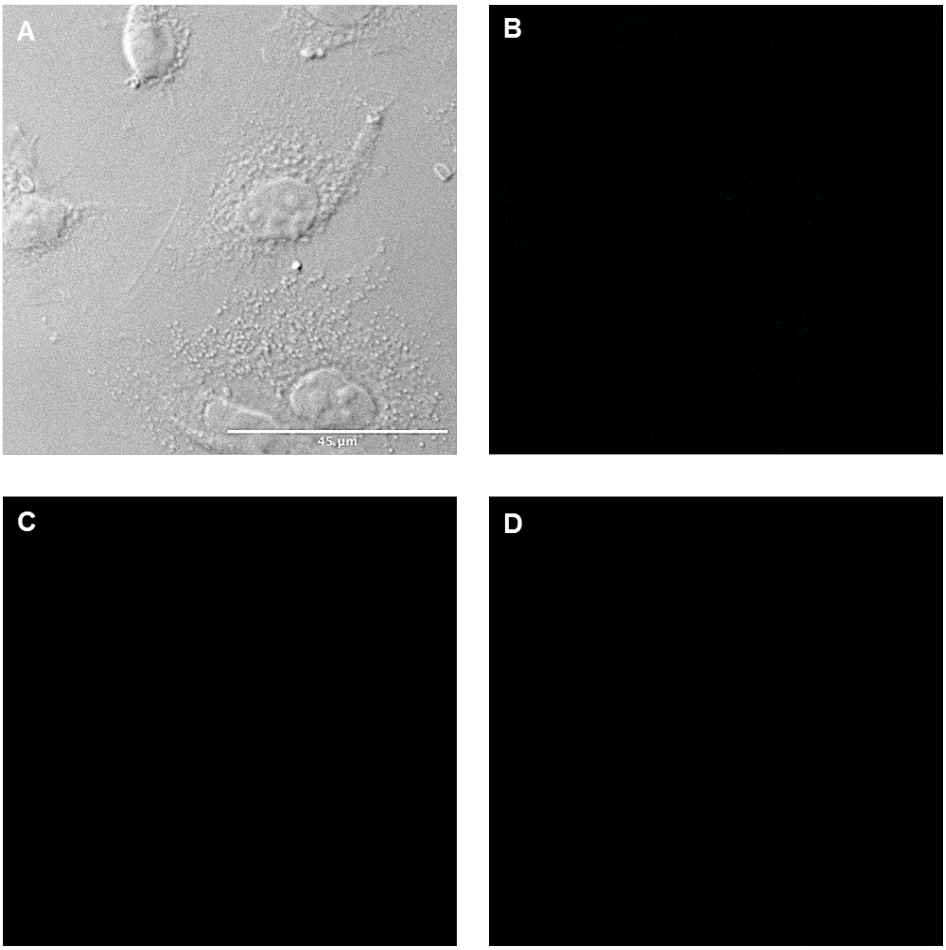

Figure S10. Fixed-cell confocal microscopy images of A549 human lung epithelial cells treated with the R8PREP1[117-132]$_{I122A,L125A,L129A,L132A}$ peptide. Bright-field (**A**) and fluorescent (**B-D**) confocal microscope images. Fluorescent images were obtained by exciting in the UV spectral region of 4',6-diamidino-2-phenylindole (DAPI) (405 nm) (**B**), the green fluorescent protein (GFP) (488 nm) (**C**) and the rhodamine (555 nm) (**D**) after 8 h of incubation. Scale bar: 45 µm.

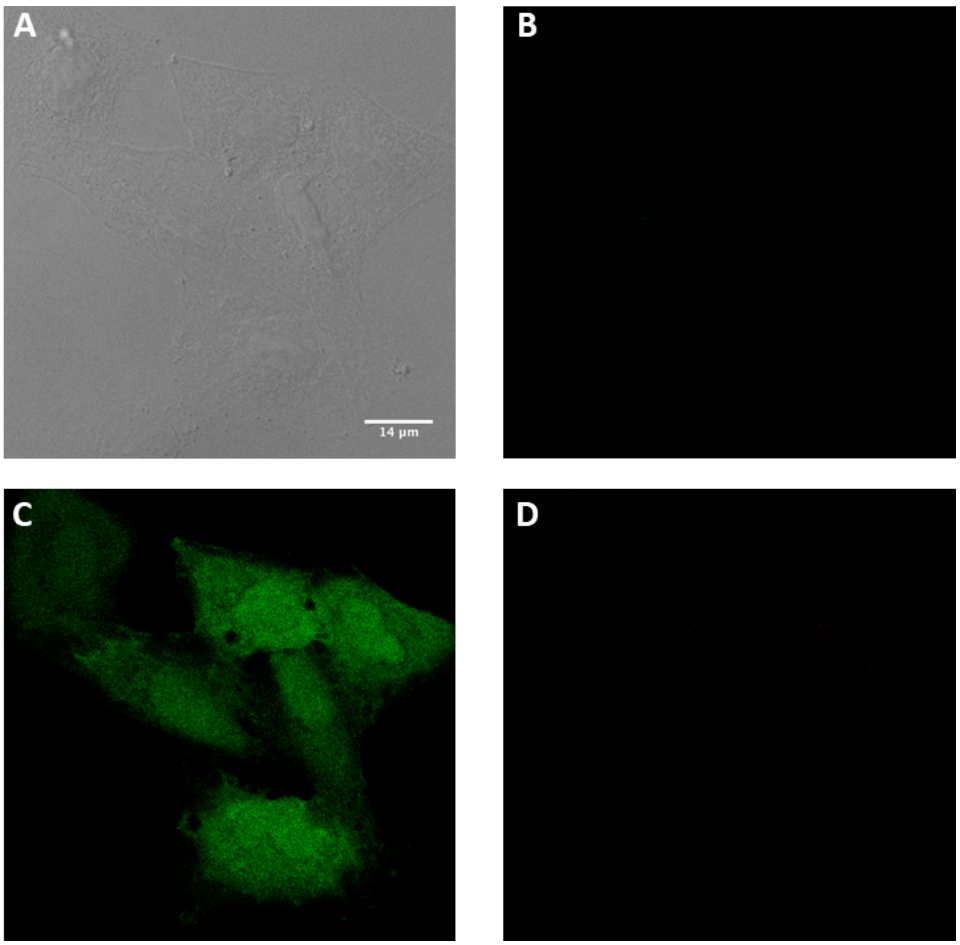

Figure S11. Fixed-cell confocal microscopy images of A549 human lung epithelial cells treated with the pBABE-puro-GFP vector. Bright-field (**A**) and fluorescent (**B-D**) confocal microscope images. Fluorescent images were obtained by exciting in the UV spectral region of 4',6-diamidino-2-phenylindole (DAPI) (405 nm) (**B**), the green fluorescent protein (GFP) (488 nm) (**C**) and the rhodamine (555 nm) (**D**) after 8 h of incubation. Scale bar: 14 µm.

References

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2. N. Doti, A. Monti, C. Bruckmann, L. Calvanese, G. Smaldone, A. Caporale, L. Falcigno, G. D'Auria, F. Blasi, M. Ruvo and L. Vitagliano, *Int J Biol Macromol*, 2020, **163**, 618-629.
3. C. Bruckmann, S. Tamburri, V. De Lorenzi, N. Doti, A. Monti, L. Mathiasen, A. Cattaneo, M. Ruvo, A. Bachi and F. Blasi, *Sci Rep*, 2020, **10**, 16809.