Supporting Information

Interaction of Aβ peptide with tubulin causes inhibition of tubulin polymerization and apoptotic death of cancer cells

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Materials and methods:

Reagents: Beta-amyloid (1-42), scrambled beta-amyliod (1-42), biotinylated beta amyloid (1-42) and beta - amyloid (1 - 42) HiLyte Fluor™ 488-labeled were purchased from AnaSpec. Dimethylsulfoxide (DMSO) and MeOH were purchased from spectrochem. TritonX-100 was purchased from SRL.Diamino-polyethylene glycol with MW 2000Da (NH_2-PEG_{2000}-NH_2) was purchased from Rapp Polymere. 3-Glycidyloxypropyl trimethoxysilane (GOPTS) were purchased from Fluka. EZ-Link NHS Biotin was purchased from Thermo Scientific. 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) was purchased from Himedia. Ethylene-bis(oxyethylenenitrilo)tetraacetic acid (EGTA), 4-Piperazinediethanesulfonic acid (PIPES), bovine serum albumin (BSA), 1,1,1,3,3,3-Hexafluoroisopropanol (HFIP), guanosine-5′-triphosphate sodium salt hydrate (GTP), β-casein, catalase, glucose oxidase, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), dulbecco's modified eagle's medium **(**DMEM), MES, 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI), trypsin-EDTA solution, dimethylsulfoxide for cell culture and formaldehyde solution for molecular biology were purchased from Sigma Aldrich. Penicillin-Streptomycin, neutravidin, alexa fluor 568-carboxylic acid succinimidyl ester and fetal bovine serum (FBS) were purchased from Invitrogen. Rabbit polyclonal anti-Mad2 antibody, rabbit monoclonal anti-alpha Tubulin (EP1332Y) antibody and Goat polyclonal anti-Rabbit IgG H&L (Cy3.5 ®) preadsorbed were purchased from Abcam. Mouse monoclonal anti-BubR1 (human) was purchased from MBL International Corporation. Goat anti-mouse IgG fluorescein conjugated was purchased from Millipore. Goat anti-mouse IgG-RPE conjugated was purchased from Southern Biotech. p53 (F-8) mouse monoclonal IgG, p21 (F-5) mouse monoclonal IgG and annexin V apoptosis detection kit were purchased from Santa Cruz Biotechnology. Nocodazole andbisbenzimide H 33258 (hoechst) were purchased from Calbiochem. Cover glass bottom dishes were purchased from SPL. All compounds were used without further purification.

Cell Culture: We have purchased human breast cancer cell line MCF7, A549, HeLa, WI38 from National Centre for Cell Science (NCCS) Pune, India. Cells were cultured in our lab in a 5% CO_2 humidified atmosphere at 37 ^oC using dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum, kanamycin sulfate (110 mg/L), penicillin (50 units/mL), streptomycin (50 µg/mL) and we have used trypsin-EDTA (1X) solution for cell splitting.

Protein Purification: Tubulin was isolated from pig and goat brain and purified in our lab through two cycles of polymerization and de-polymerization procedure as described before.[1a] We have used MES based buffer for de-polymerization and high molarity PIPES buffer for polymerizationalong with ATP and GTP. We have obtained highly purified concentrated tubulin solution which is free from microtubule

associated proteins and motor proteins. The concentration of tubulin solution was obtained by measuring its absorbance at 280 nm. We have prepared tubulin stocks in BRB80 buffer having concentration 20 mg/mLand stored at -80 ^{o}C using 10% glycerol as a cryo solvent. Tubulin labeling with alexafluor 568-carboxylic acid succinimidyl ester was performed following previously described method in our laboratory.[1b] Prior to every experiment both labeled and unlabeled tubulin were centrifuged at 40000 rpm for 30 min at 4^{o}C.

Aβ42 peptide reconstitution and preparation of solution: Solid peptide was dissolved in 1, 1, 1, 3, 3, 3-hexafluoroisopropanol (HFIP) and stored at -20 ^{o}C. HFIP was removed by nitrogen flash prior to the experiment. For reconstitution we have added 1% NH_4OH solution and supplemented with buffer (BRB80 or serum free media as per experiment). Human scrambled Aβ42 peptide[2] (AIAEGDSHVLKEGAYMEIFDVQGHVFGGKIFRVVDLGSHNVA) was used for control experiments.

Spectrophotometric measurement of tubulin turbidity by light scattering experiment: We have measured the absorbance of tubulin solution at 350 nm using an UV-Spectrophotometer (Cary 60 UV-Vis of Agilent technologies) which is equipped with peltier accessory. We have prepared a 100 µL solution in ice containing 20 µM tubulin, 4 mM GTP in BRB80 buffer. This solution was placed in a quartz cuvette having path length 10 mm and measured the absorbance at 350 nm during 40 minutes at 37^{o}C inside UV-Spectrophotometer. We have found that absorbance of tubulin increased with time. To check the effect of Aβ42 peptide on the tubulin turbidity we have used Aβ42 peptide solutions in BRB80 buffer along with tubulin (experiment was done using tubulin purified from pig and goat brains) and GTP. We have also used scrambled Aβ42 peptide for control. We have prepared three sets of mixture prior to the measurements containingfixed concentration of 20 µM tubulin and 4 mM GTP with variations of Aβ42 peptide such as 20 µM, 40 µM and 80 µM respectively followed by measuring the tubulin turbidity at 350 nm as described before. Interestingly, we have found that turbidity increased significantly compared to tubulin alone. We have also checked the turbidity of 20, 40 and 80 µM of Aβ42 alone in BRB80 buffer in absence of 20 µM tubulin at 350 nm following similar experimental condition as before and we did not observed significant increase of turbidity. The enhancement of turbidity of tubulin alone, Aβ42 alone as well as mixture of tubulin and Aβ42 peptide were compared using a bar diagram. It was observed that turbidity increased significantly only for the mixture of tubulin and Aβ42 peptide compared to tubulin and Aβ42 peptide alone.

Co-sedimentation experiment and SDS-PAGE gel electrophoresis: Interaction between tubulin andAβ42 peptide was studied by this experiment. Here, 20 µM solution of tubulin was incubated with 80 µM Aβ42 peptide at 37 ^{o}C for 40 min in presence of 4 mM GTP in BRB80 buffer. After that the solution was centrifuged at 12000 rpm for 3 minutes. The pellets were re-suspended in BRB80 buffer. The supernatant and pellets were analyzed by 12% SDS-PAGE gel.We have observed bands near 63 KD and 5 KD in lane 2 (pellets) whereas in lane 3 (supernatant) there was only band near 63 KD observed. The bands near 63 KD and 5 KD were corresponding to the tubulin (55 KD) and Aβ42 peptide (4.5 KD) respectively.

Fluorometric measurement of fluorescence intensity of intrinsic tryptophan residue of tubulin: The tryptophan residue of tubulin gives a fluorescence maximum near 330 nm when it was excited by 295 nm wavelength light. Experiments were performed using Quanta Master Spectrofluorometer (QM-40)equipped with peltier for controlling the temperature. We have taken 20 µM solution of tubulin and a mixture of 20 µM solution of tubulin and 40 µM solution of Aβ42 peptide in BRB80 buffer. We have also used 40 µM solution of scrambled Aβ42 peptide for control. Solutions were placed into the

fluorescence cuvette having path length 10 mm. We have set excitation wave length at 295 nm and emission wave length from 310 to 400 nm and fluorescence intensity of both the solutions was measured. It was observed that fluorescence intensity of solution containing Aβ42 peptide was decreased compare to solution without Aβ42 peptide. Therefore, there was quenching of intrinsic tryptophan fluorescence of tubulin due to presence Aβ42 peptide. As we know from literature [3] that quenching of tryptophan fluorescence of tubulin occurs due to interaction of small molecules with tubulin.

Tubulin binding experiment on biotin micropatterned surface: Biotin micropatterned glass surfaces and flow chambers containing this micropatterned glasses were prepared following our previous report.[4,5] Flow chamber was equilibrated with 20 mM HEPES buffer (pH 7.5) followed by β-casein (1 mg/mL). We have loaded 0.3 µM solution of neutravidin in HEPES buffer into the flow chamber and incubated for 7 minutes on ice. Flow chamber was washed with HEPES buffer to remove excess neutravidin and then incubated with 80 µM solution of biotin-labeled amyloid beta (1-42) peptide for 10 minutes on ice. After that we have washed the flow chamber with HEPES buffer (2 times) followed by 3 times with BRB80 buffer (pH 6.9). Pre-cooled flow chamber at 4 ^{o}Cwas loaded with 20 µL solution of BRB80 buffer containing 20 µM tubulin, 3.75 µM alexa fluor-568-labeled tubulin, 4 mM GTP, 25 mM $MgCl_2$, 1 mg/mL glucose oxidase, 0.5 mg/mL catalase and 1mM glucose. Then it was sealed and incubated at 37^{o}C for 30 minutes. Flow chamber was placed on a TIRF microscopy and images were taken using 561 nm laser through 60X objective (Olympus) and an Andor iXon3 897 EMCCD camera. Control experiment was performed in absence of biotin-labeled amyloid beta (1-42). We have found red micropatterns when we used biotin-labeled amyloid beta (1-42) whereas in control we did not observe red micropatterns.

Förster Resonance Energy Transfer (FRET) experiment: FRET[6] is a powerful technique to study macromolecular interaction *in vitro* under physiological condition for determining fluorophore's distance.[7,8] In FRET study, nonradiative energy is transferred from the excited donor molecule to the ground state acceptor molecule. By choosing appropriate FRET pair we can study the interaction between two macromolecules without disturbing their physical property. From, steady state emission intensity we can get a qualitative idea about the efficiency of energy transfer from the donor (hilyte fluor 488) to acceptor (alexa 568).

Since we have found that Aβ42 peptide increases tubulin turbidity, quenches tryptophan fluorescence of tubulin, which means it is interacting with the tubulin molecule, therefore we have studied steady state FRET experiment taking hilyte fluor 488 labeled Aβ42 peptide and alexa 568 labeled tubulin to determine how strong Aβ42 peptide interacting with tubulin and also the distance between the two fluorophores. The loss of donor fluorescence intensity in presence of an acceptor fluor is often quantitated as FRET efficiency.

Förster distance (R_0) between hilyte fluor 488 labeled Aβ42 peptide and alexa 568 labeled tubulin is calculated to be ~ 63±1 Å from the spectral overlap graph (**Figure S5**) of these two donor-acceptor pair.

$$\text{Förster distance } R_0 = 0.211\left[\kappa^2 n^{-4} Q_D J(\lambda)\right]^{1/6}$$

Where, κ^2 is the orientation factor for the relative geometries of donor & acceptor, n is the refractive index of the medium, Q_D is the fluorescence quantum yield of the donor, $J(\lambda)$ is the integrated overlap between the donor emission & acceptor absorption spectra.[6,8,9]

The efficiency of FRET is given by the Equeation, $\varepsilon_{FRET} = \frac{I_A}{I_D + I_A}$

In our FRET studies, as obtained from **Figure S6**, acceptor intensity I_A= 0.2, donor intensity I_D= 0.63 and thus ε_{FRET} = 0.24.

Now the distance (R_{DA}) between hilyte fluor 488 labeled Aβ42 peptide and alexa 568 labeled tubulin may be calculated by the following equation.

$$R_{DA} = R_o \left(\frac{1 - \varepsilon_{FRET}}{\varepsilon_{FRET}} \right)^{\frac{1}{6}}$$

R_{DA} is calculated to be ~ 76 Å.

The value is quite close to the Förster distance (R_0) which means that they are interacting and efficient FRET is occurring between hilyte fluor 488 labeled Aβ42 peptide and alexa 568 labeled tubulin.

Microtubule assembly assay: Fluorescence intensity of DAPI solution increases on binding with microtubule. Therefore, fluorescence intensity of DAPI solution was measured to compare the amount of microtubule formation in absence and in presence of Aβ42 peptide.We have prepared three mixtures containing 35 μM DAPI in BRB80 buffer, containing 100 μM tubulin, 10 mM GTP, 35 μM DAPI in BRB80 buffer and containing 160 μM Aβ42, 100 μM tubulin, 10 mM GTP, 35 μM DAPI in BRB80 buffer. We have measured the DAPI fluorescence using Quanta Master Spectrofluorometer (QM-40) which is equipped with peltier for controlling the temperature during experiment. We have set excitation wavelength at 355 nm and emission from 400 to 600 nm. We have measured the fluorescence at room temperature and then solutions were incubated at 37 ^{o}C and fluorescence was measured at 10 min intervals during incubation at 37 ^{o}C.We have found that fluorescence intensity was decreased when temperature reaches up to 37 ^{o}C from room temperature and after that there was no significant change of fluorescence intensity of 35 μM DAPI alone in BRB80 buffer. But in case of solution containing DAPI, tubulin, GTP and Aβ42 peptide fluorescence intensity was increased when temperature reaches up to 37 ^{o}C from room temperature. Similar observation was observed even in absence of Aβ42 peptide. However, rate of enhancement of fluorescence signal higher in absence of Aβ42 peptide. This result indicates that Aβ42 peptide inhibits tubulin polymerization.

Docking: We have performed docking study using cluspro 2.0 server.[10]The microtubule receptor (1Z2B)[11]was docked with β-sheet Aβ peptide (2LNQ)[12] and partially folded Aβ peptide (2LFM).[13] We have found that Aβ peptide in both forms binds to the vinblastine and GTP/GDP binding pocket of the β-tubulin.

Colchicine binding assay: We have co-incubated 10 μM solution of tubulin and 80 μM solution of Aβ42 peptide in BRB80 buffer at 37 ^{0}C for 60 min. After that 10 μM solution of colchicine was added into the mixture and incubated for another 60 minutes. We have also co-incubated 10 μM solution of tubulin and 10 μM solution of colchicine in BRB80 buffer at 37 ^{0}C for 90 min as a control. We have found fluorescence peak near 432 nm for tubulin-colchicine complex in both cases. Single colchicine and tubulin individually did not show any fluorescence peak near 432 nm. Since we have not found any changes in emission spectra of tubulin and colchicine complex with or without incubation with Aβ42 peptide, therefore we can conclude that Aβ42 peptide does not bind to the colchicine site of tubulin.

Cellular Uptake Study: MCF-7 cells were seeded at a density of 2000 cells in a cover glass bottom dish before 24 hours of treatment. We have prepared 2 μM solution of Hilyte-fluor-488-labelled amyloid beta (1-42) peptide in serum free media and incubated for 4 hours. After that cells were incubated in complete media for 24 hours. Cells were treated with Hoechst 33258 (1 μg/mL) 1 hour before imaging. Cells were washed by PBS and live cell images were captured through an Andor spinning disc confocal microscope with a 60X objective (Olympus) and an Andor iXon3 897 EMCCD camera in bright field, 488 and 405 nm wavelength laser light.

HiLyte Fluor 488-labeled amyloid beta (1-42) peptide and microtubule network of MCF7, A549 and HeLa cells: In this experiment we have shown that Aβ42 peptide interacts with the microtubule network of MCF7, A549 and HeLa cells. MCF-7 cells were seeded at a density of 2000 cells in a cover glass bottom dish before 24 hours of treatment. We have prepared 2 μM solution of HiLytefluor 488-labeled amyloid beta (1-42) peptide in serum free media and cells were treated with peptide solutions for 4 hours. After that cells were incubated in complete media for 12 hours. Then, cells were washed by PBS and treated with 4% paraformaldehyde solution for 30 minutes for cell fixing. We have treated the cells with cell permeable solution (0.1% Triton X-100 in PBS) for 10 minutes. Cells were washed with PBS and incubated with 5% BSA in PBS for 30 minutes to block nonspecific binding sites. Antibody solutions were prepared in PBS. After washing with PBS, cells were incubated with monoclonal anti-α-tubulin IgG [EP1332Y] antibody with dilution 1:300 for 2 hours. After that cells were washed with PBS and incubated with secondary antibody (Cy3.5 pre-absorbed goat anti-rabbit IgG) with dilution 1:600 for 2 hours. Cells were washed with PBS and incubated with Hoechst 33258 (1 μg/mL) for 30 minutes before imaging. Fixed cells were imaged through an Andor spinning disc confocal microscope with a 60X objective (Olympus) and an Andor iXon3 897 EMCCD camera in bright field, 405, 488 and 561 nm wavelength laser lights. We have found that green peptide interacts with the red microtubule network of MCF7, A549 and HeLa cells.

Immunofluorescence microscopy for microtubule cytoskeleton study: MCF-7 cells were seeded at a density of 2000 cells in a cover glass bottom dish before 24 hours of treatment. We have prepared 1 μM and 2 μM solution of Aβ42 peptide in serum free media and cells were treated with peptide solutions for 4 hours. After that cells were incubated with peptide in complete media for 48 hours. Then, cells were washed by PBS and treated with 4% paraformaldehyde solution for 30 minutes for cell fixing. We have treated the cells with cell permeable solution (0.1% Triton X-100 in PBS) for 10 minutes. Cells were washed with PBS and incubated with 5% BSA in PBS for 30 minutes to block nonspecific binding sites. Antibody solutions were prepared in PBS. After washing with PBS, cells were incubated with monoclonal anti-α-tubulin IgG [EP1332Y] antibody with dilution 1:300 for 2 hours. After that cells were washed with PBS and incubated with secondary antibody (Cy3.5 pre-absorbed goat anti-rabbit IgG) with dilution 1:600 for 2 hours. Cells were washed with PBS and incubated with Hoechst 33258 (1 μg/mL) for 30 minutes before imaging. Fixed cells were imaged through an Andor spinning disc confocal microscope with a 60X objective (Olympus) and an Andor iXon3 897 EMCCD camera in bright field, 405, 488 and 561 nmwavelength laser lights. In control sample cells were not treated with Aβ42 peptide. We have found shrinkage of microtubule network when cells were treated with Aβ42 peptide. With increasing the concentration of Aβ42 peptide from 1 to 2 μM the shrinkage was increased.

Immunofluorescence microscopy of Mad2 and BubR1: MCF-7 cells were seeded at a density of 2000 cells in a cover glass bottom dish before 24 hours of treatment. We have prepared 2 μM solution of Aβ42 peptide in serum free media and cells were treated with peptide solutions for 4 hours. After that cells were

incubated with peptide in complete media for 48 hours. Then, cells were washed by PBS and treated with 4% paraformaldehyde solution for 30 minutes for cell fixing. We have treated the cells with cell permeable solution (0.1% Triton X-100 in PBS) for 10 minutes. Cells were washed with PBS and incubated with 5% BSA in PBS for 30 minutes to block nonspecific binding sites. Antibody solutions were prepared in PBS. After washing with PBS, cells were incubated with rabbit polyclonal anti-Mad2 antibodywith dilution 1:400 for overnight at 4^{o}C. After that cells were washed with PBS and incubated with secondary antibody (Cy3.5 pre-absorbed goat anti-rabbit IgG) with dilution 1:400 for 2 hours at 37^{o}C. Cells were washed with PBS and incubated with Hoechst 33258 (1 μg/mL) for 30 minutes before imaging. Fixed cells were imaged through a NiconTi-U eclipse fluorescence microscope with a 40X objective in bright field, 405 and 561 nmwavelength lasers light. In case of positive control we have treated the cells with 500 nM nocodazole whereas in negative control cells were not treated with either nocodazole or Aβ42 peptide. We have found red signal due to Mad2 activation on the nucleus when we have treated the cells with nocodazole and Aβ42 whereas in control we have not found red signal on the nucleus.

In case of immunofluorescence microscopy of BubR1, we have followed the same procedure as like Mad2. Here, we have used mouse monoclonal anti-BubR1 primary antibody (1:400) and goat anti-mouse IgG fluorescence conjugated secondary antibody (1:400). In this case we have used goat anti-mouse IgG-RPE conjugated secondary antibody (1:400) for nocodazole control sample. We have found red and green signal due to BubR1 activation on the nucleus when we have treated the cells with nocodazole and Aβ42 peptide respectively whereas in control we have not found green signal on the nucleus.

AnnexinV-Propidium iodide (PI) assay: MCF7 cells were plated at a density of 2000 cells in a cover glass bottom dish before 24 hours of treatment. We have prepared 2 μM solution of Aβ42 peptide in serum free media and cells were treated with this peptide solution for 4 hours. After that cells were incubated with peptide in complete media for 48 hours. Cells were washed with phosphate buffer (PBS, pH 7.4) and then with assay buffer. Assay buffer was supplied along with the Annexin V apoptosis detection kit (Santa Cruz Biotechnology). We have added 2.5 μL of Propidium iodide (PI) and 2.5 μL of annexin V into the 200 μL of assay buffer and live cells were incubated with this solution for 15 minutes at 37 ^{o}C inside the cell culture incubator. All those reagents were supplied along with the apoptosis detection kit(Santa Cruz Biotechnology). The stock concentration of Propidium iodide and annexin V was 50 μg/mL and 200 μg/mL respectively. Cells were washed with the assay buffer and immediately live cell images were captured through an Andor spinning disc confocal microscope with a 60X objective (Olympus) and an Andor iXon3 897 EMCCD camera in DIC, 488 nm and 561 nmwavelength lasers light.

FACS analysis for apoptosis: Fluorescence activated cell sorting (FACS) experiment was performed for studying the type of cell death. We have studied cell death pathway of MCF7, A549 and HeLa cells in FACS using apoptosis detection kit. Cells were seeded at a density of ~5 X 10^5 cells per well in a 6-well plate before 24 hours of treatment. We have prepared 1 μM and 2 μM solution of Aβ42 peptide in serum free media. Cells were washed with PBS and then incubated with peptide solutions for 4 hours. After that cells were incubated with peptide in complete media for 48 hours. Cells were trypsinized and washed with PBS by help of centrifugation. Cells were incubated in dark at 37 ^{o}C for 15 minutes with a 100 μL solution of assay buffer containing 2.5 μL of Propidium iodide (PI) and 2.5 μL of annexin V. All those reagents were supplied along with the apoptosis detection kit(Santa Cruz Biotechnology). The stock concentration of Propidium iodide and annexin V was 50 μg/mL and 200 μg/mL respectively. After that another 400 μL of assay buffer was added to the cells and these total 500 μL of cell solution was analyzed

by FACS. We have detected the emission of Annexin V and PI in the FITC and PI channels of BD LSRFORTESA flow cytometer using emission filters at 530 and 610 nm respectively. In the represented data, cells in the Q1, Q2 and Q4 quadrants are regarded as necrotic, late apoptotic and early apoptotic cells respectively. Cells in the Q3 quadrant are regarded as normal cells or healthy cells. Data was analyzed using FACS DIVA software.We have found that control cells (cells were not treated with Aβ42 peptide) were mostly populated in Q3 quadrant and population of cells in the Q4 quadrant was increased when cells were treated with Aβ42 peptide. We have compared the percentage of healthy and apoptotic cells after treatment with 1 and 2 μM Aβ42 peptide with the control (untreated cell) using a bar diagram.

Immunofluorescence microscopy of p53 and p21: MCF7 cells were seeded at a density of 2000 cells in a cover glass bottom dish before 24 hours of treatment. We have prepared 2 μM solution of Aβ42 peptide in serum free media and cells were treated with peptide solutions for 4 hours. After that cells were incubated with peptide in complete media for 48 hours. Then, cells were washed by PBS and treated with 4% paraformaldehyde solution for 30 minutes for cell fixing. We have treated the cells with cell permeable solution (0.1% Triton X-100 in PBS) for 10 minutes. Cells were washed with PBS and incubated with 5% BSA in PBS for 30 minutes to block nonspecific binding sites. Antibody solutions were prepared in PBS. After washing with PBS, cells were incubated with mouse monoclonal IgG P53 (F-8)antibodywith dilution 1:300 for 2 hours. After that cells were washed with PBS and incubated with secondary antibody (R-phycoerythrin conjugated goat anti-mouse IgG) with dilution 1:600 for 2 hours. Cells were washed with PBS and incubated with Hoechst 33258 (1 μg/mL) for 30 minutes before imaging. Fixed cells were imaged through a Nicon Ti-U eclipse fluorescence microscope with a 40X objective in bright field, 405 and 561nm wavelength laser lights.

In case of p21, cells were incubated with mouse monoclonal IgG P21 (F-5) antibody with dilution 1:300 for 2 hours. After that cells were washed with PBS and incubated with secondary antibody (R-phycoerythrin conjugated goat anti-mouse IgG)with dilution 1:600 for 2 hours. Cells were washed with PBS and incubated with Hoechst 33258 (1 μg/mL) for 30 minutes before imaging. Fixed cells were imaged through a Nicon Ti-U eclipse fluorescence microscope with a 40X objective in bright field, 405 and 561 nm wavelength laser lights.

Cell Viability assay: We have treated MCF7, A549, HeLa and WI38 cells with the Aβ42 peptide and cellular viability was checked using MTT assay. It is a colorimetric assay where MTT, a yellow tetrazole compound, is reduced to purple formazan by cellular reductase enzymes of live cells. Dead cells cannot do this type of reduction and are not able to develop the purple colour. Therefore, we can get an idea about the amount of MCF-7 cells lived after the treatment of Aβ42 peptide with respect to those cells (control) which are not treated with the peptide. The live cells are called as viable cells and it is denoted as %viability in the Y-axis. We have used four different concentrations of the peptide and it is in the X-axis. We have measured the absorbance at 550 nm and calculated the %viability.

Cells were seeded at a density of 10000 cells per well in 96-well plate before 24 hours of treatment. Aβ42 solutions were prepared in serum free media. Cells were treated with 0.25, 0.5, 1 and 2 μM of Aβ42 peptide solutions and incubated for 4 hours. After that cells were incubated with peptide for 48 hours in complete media. MTT solution (5mg/ml) was prepared in PBS. 50 μL of MTT solution was added into each well and incubated at 37 ^{o}C for 4 hours. Purple colored formazan was dissolved in 1:1 (v/v) DMSO/MeOH and absorbances of the wells were measured at 550 nm by micro-plate ELISA reader. We have calculated the %viability from this absorbance values. Data shows that %viability is decreased with

increasing the concentration of Aβ42 peptide i.e. Aβ42 peptide induces the death of cancer cells. We have also used scrambled Aβ42 peptide for control.

%Viability = [(A_{550}Treated Cells - A_{550} Backgrounds)/ (A_{550}Untreated Cells - A_{550} Backgrounds)]*100.

Data Analysis: Microscopic images were analysed using Image J software.

References:

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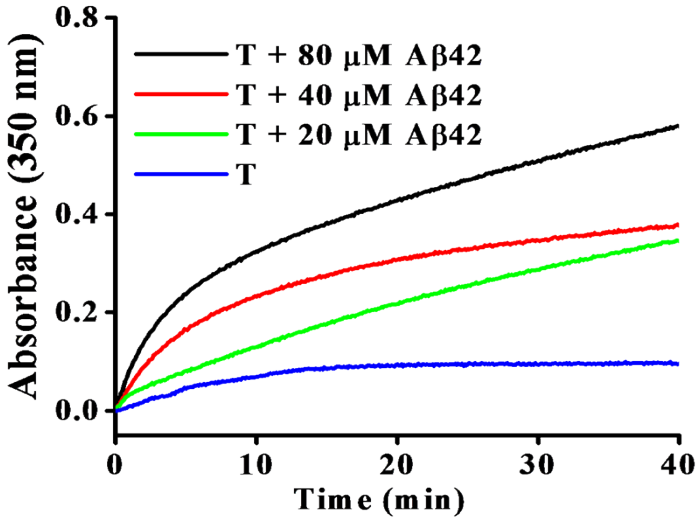

Figure S1. Increase in tubulin (pig brain tubulin) turbidity at 350 nm of 20 µM tubulin in presence of 4 mM GTP (**T**) with different concentrations of Aβ42 peptide.

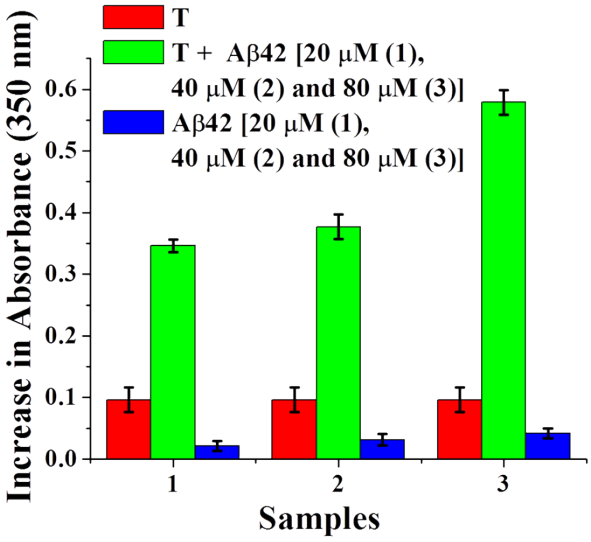

Figure S2. Increase in absorbance at 350 nm of 20 µM tubulin in presence of 4 mM GTP (**T**) solution (Red), "**T**" with different concentrations of Aβ42 peptide (Green) and different concentrations of Aβ42 peptide alone (Blue) in BRB80 buffer containing 4 mM GTP after 40 minutes incubation at 37 ^{o}C. The different concentrations of Aβ42 peptide are indicated by 1, 2, 3 as 20, 40 and 80 µM respectively.

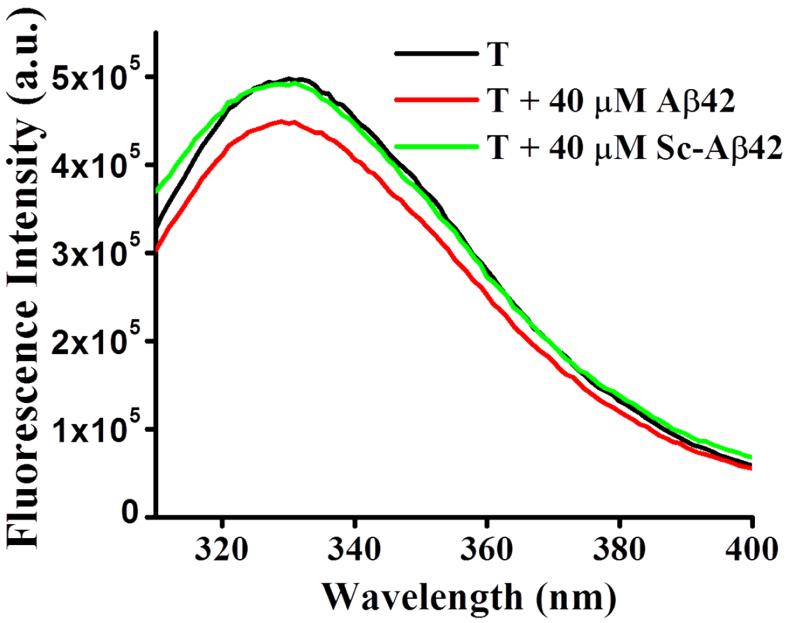

Figure S3. Quenching of intrinsic tryptophan fluorescence of tubulin (T) was found due to Aβ42 peptide.

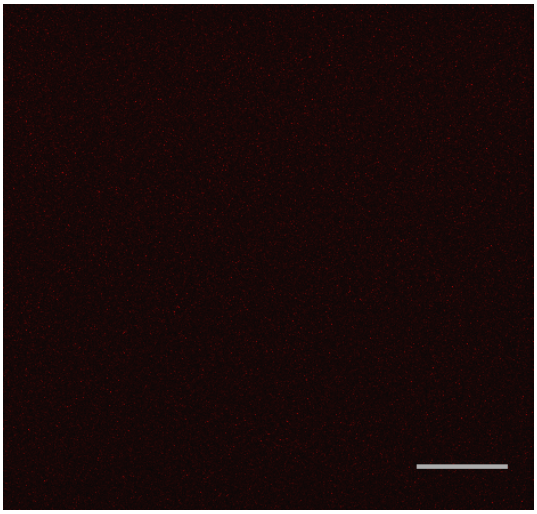

Figure S4. Blank TIRF image indicates that alexa568 labelled tubulin did not bind with biotin micropattern surface in absence of immobilized biotinylated Aβ42 peptide onto the biotin micropatternafter 37 ^{o}C incubation with tubulin. Scale bar corresponds to 20 μm.

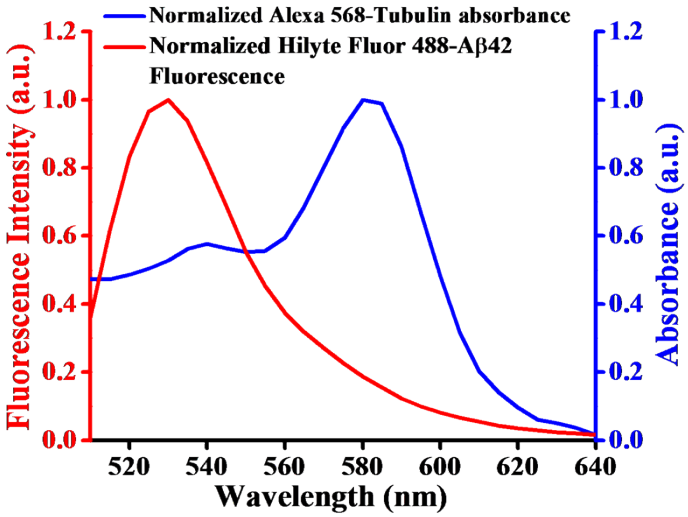

Figure S5. Spectral overlap between donor (hilyte fluor 488 labeled Aβ42 peptide) emission (λ_{ex} = 501 nm) and acceptor (alexa 568 labeled tubulin) absorbance. An R_0 of 63±1 Å is calculated for the above mentioned donor-acceptor pair.

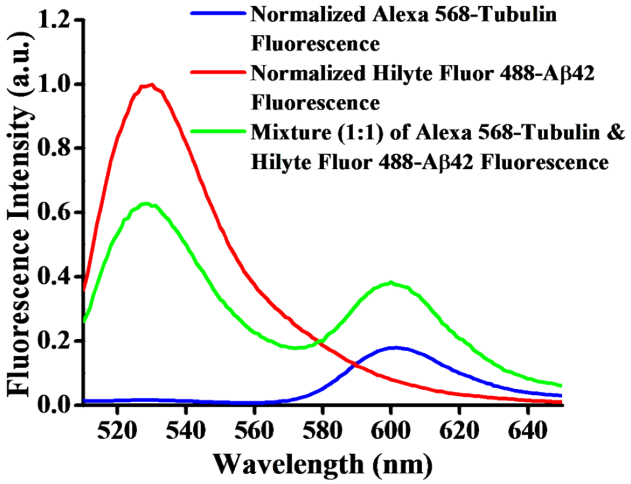

Figure S6. Steady state emission spectra (λ_{ex} = 501 nm) of only hilyte fluor 488 labeled Aβ42 peptide (donor), only alexa 568 labeled tubulin (acceptor) and both hilyte fluor 488 labeled Aβ42 peptide and alexa 568 labeled tubulin (1:1 ratio) in BRB80 buffer media at 4 ^{0}C.

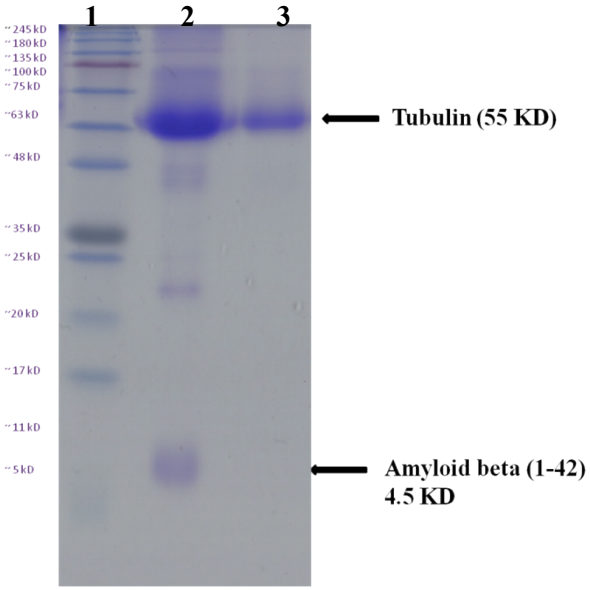

Figure S7. SDS-PAGE gel image shows co-sedimentation of tubulin and Aβ42 peptide in the pellet (lane 2). Lane 1 and lane 3 are ladder and supernatant solution respectively.

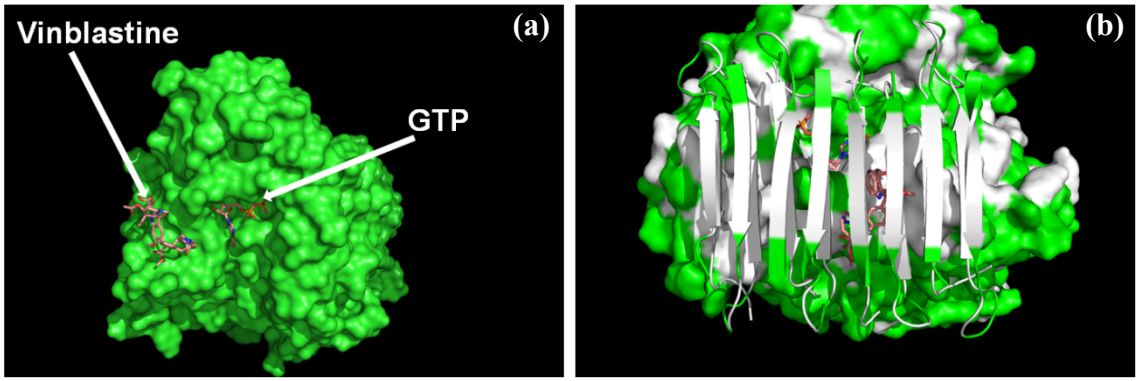

Figure S8. (a) Image shows vinblastine and GTP/GDP binding site of tubulin (green). (b) Docking image reveals hydrophobic-hydrophobic interaction between β-sheet Aβ peptide and tubulin. Green and white colour represents hydrophilic and hydrophobic region respectively of Aβ peptide-tubulin docking complex.

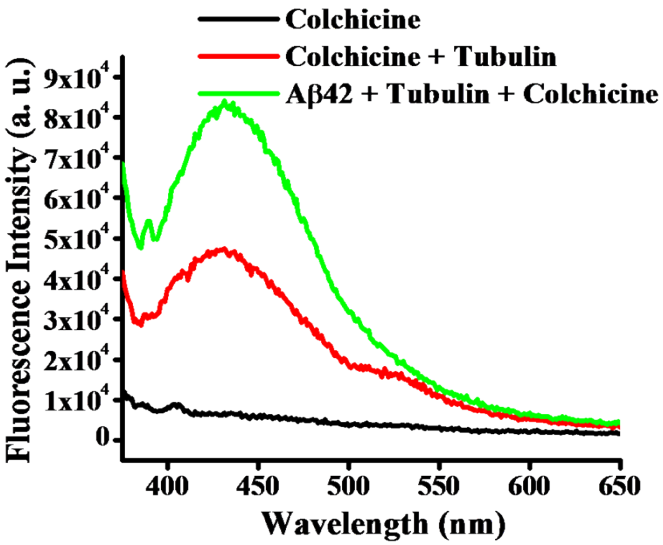

Figure S9. Aβ42 peptide did not bind to the colchicine site of tubulin.

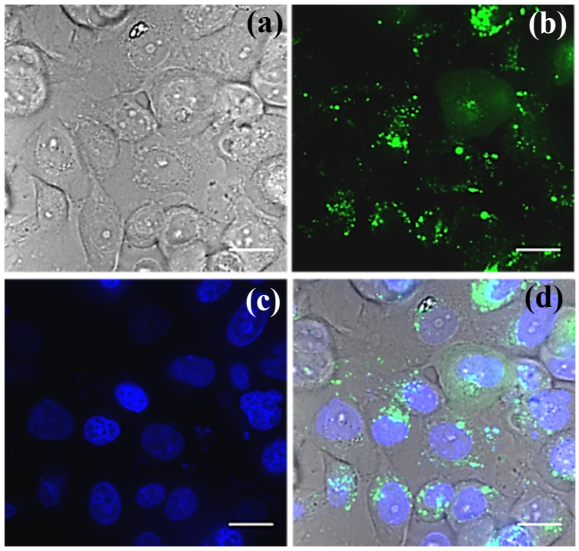

Figure S10. Confocal images of cellular uptake of HiLyte fluor 488-labeled Aβ42 peptide into the MCF7 cells. (a) In bright field, (b) 488 nm channel, (c) 405 nm channel and (d) merged image. Scale bar corresponds to 20 µm.

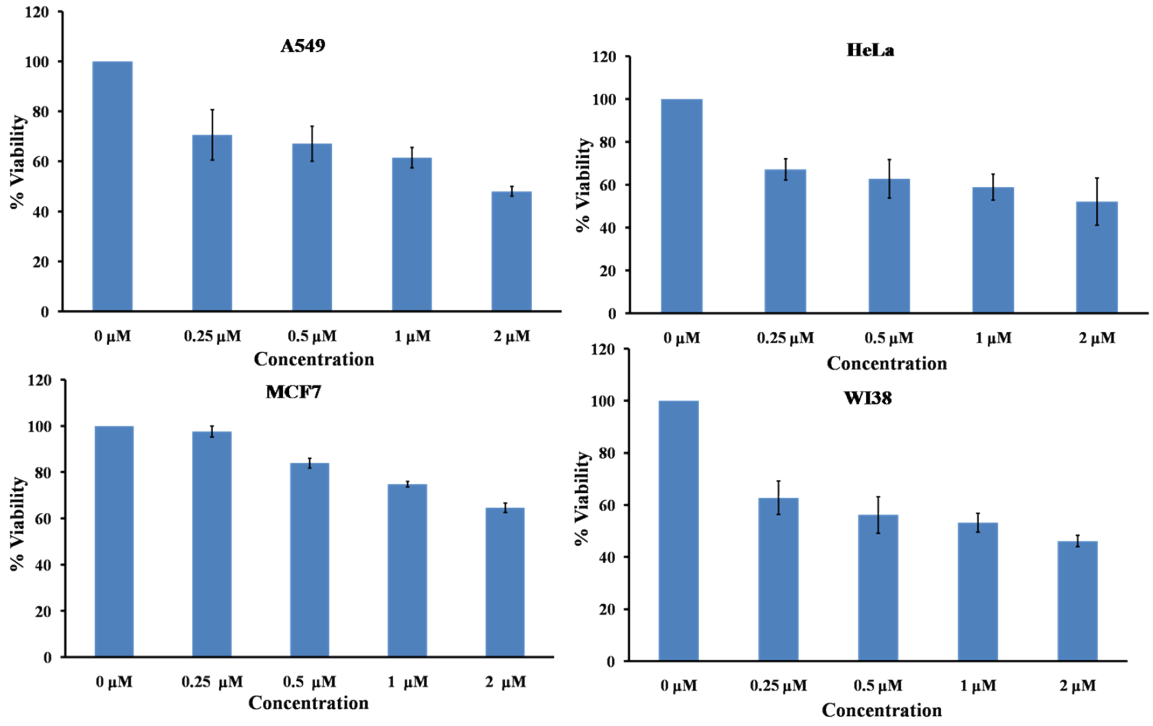

Figure S11. Cell viability (%) decreases with increasing concentrations of Aβ42 peptide ($p < 0.05$).

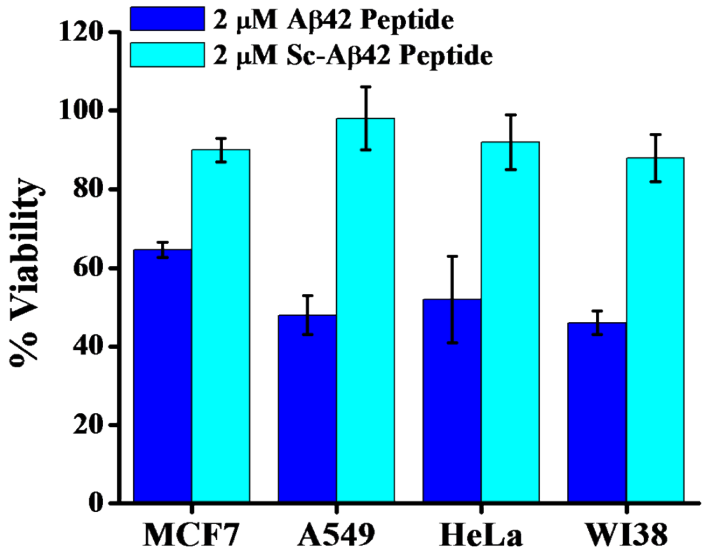

Figure S12. Comparison of cell viability (%) of Aβ42 peptide with scrambled Aβ42 peptide in different cell lines ($p < 0.05$).

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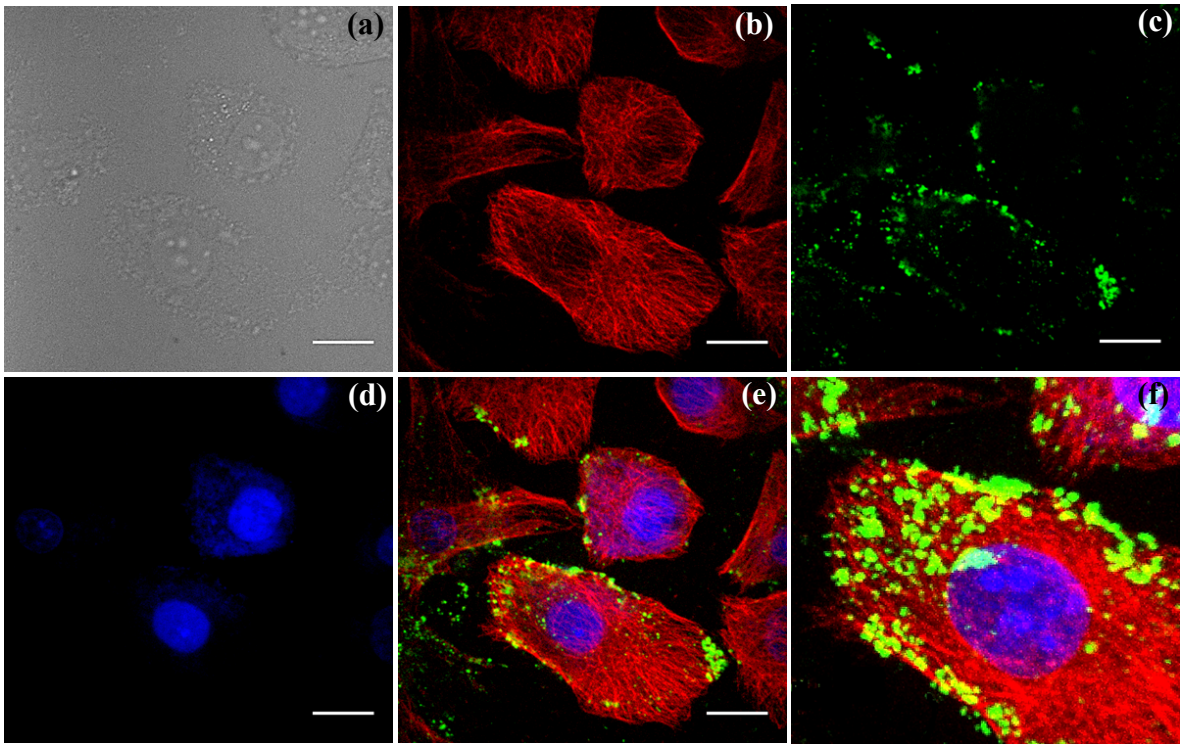

Figure S13. Hilyte fluor 488-labeled Aβ42 interacts with microtubule network of MCF7 cells (red). Nucleus was stained with hoechst (blue). Confocal images in bright field (a), 561 nm channel (b); 488 nm channel (c); 405 nm channel (d); merged image (e) and zoomed image represents yellow colour aggregates of Aβ42 peptide and microtubule (f). Scale bar corresponds to 20 µm.

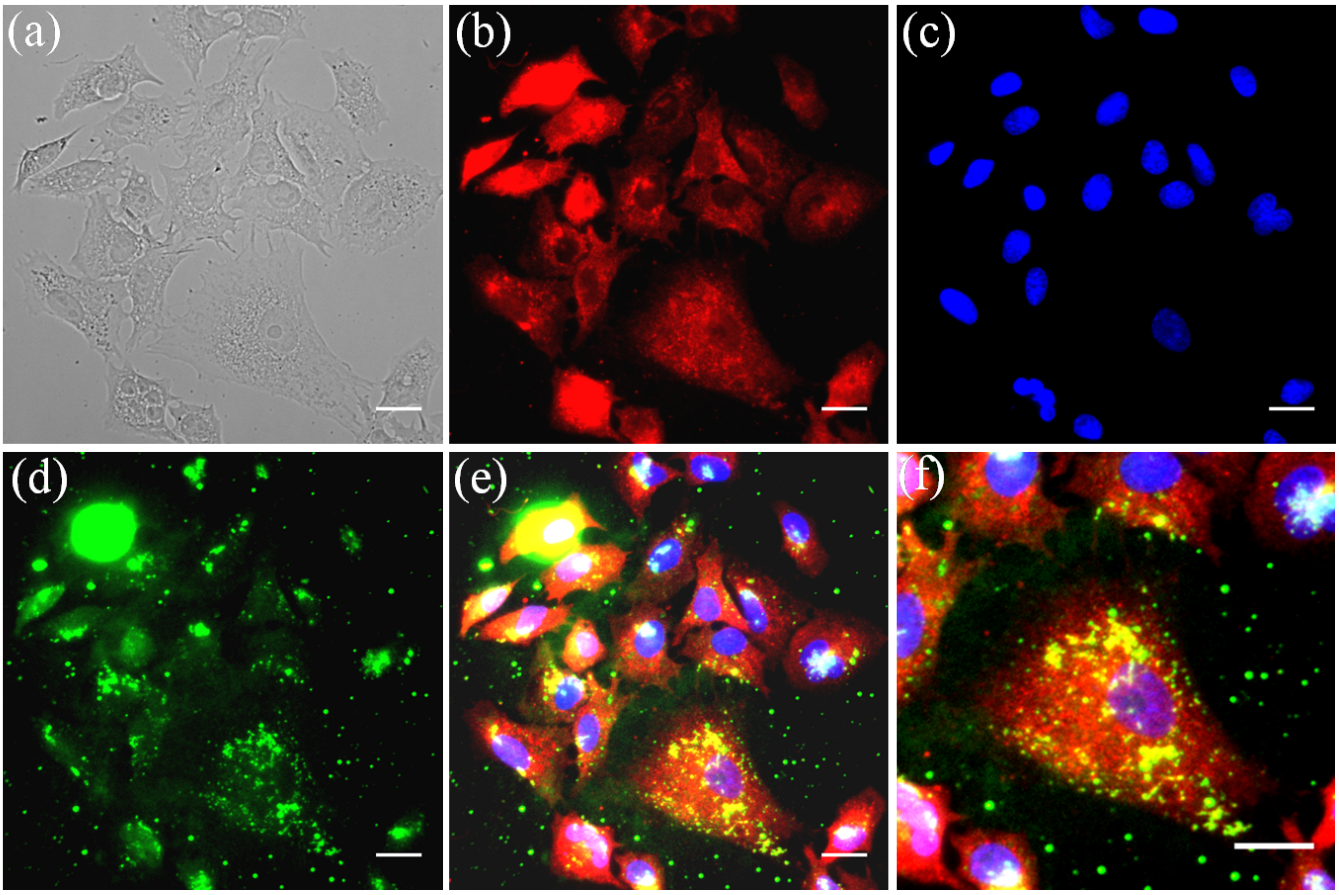

Figure S14. Hilyte fluor 488-labeled Aβ42 interacts with microtubule network of A549 cells (red). Nucleus was stained with hoechst (blue). Confocal images in bright field (a), 561 nm channel (b); 405 nm channel (c); 488 nm channel (d); merged image (e) and zoomed image represents yellow colour aggregates of Aβ42 peptide and microtubule (f). Scale bar corresponds to 20 µm.

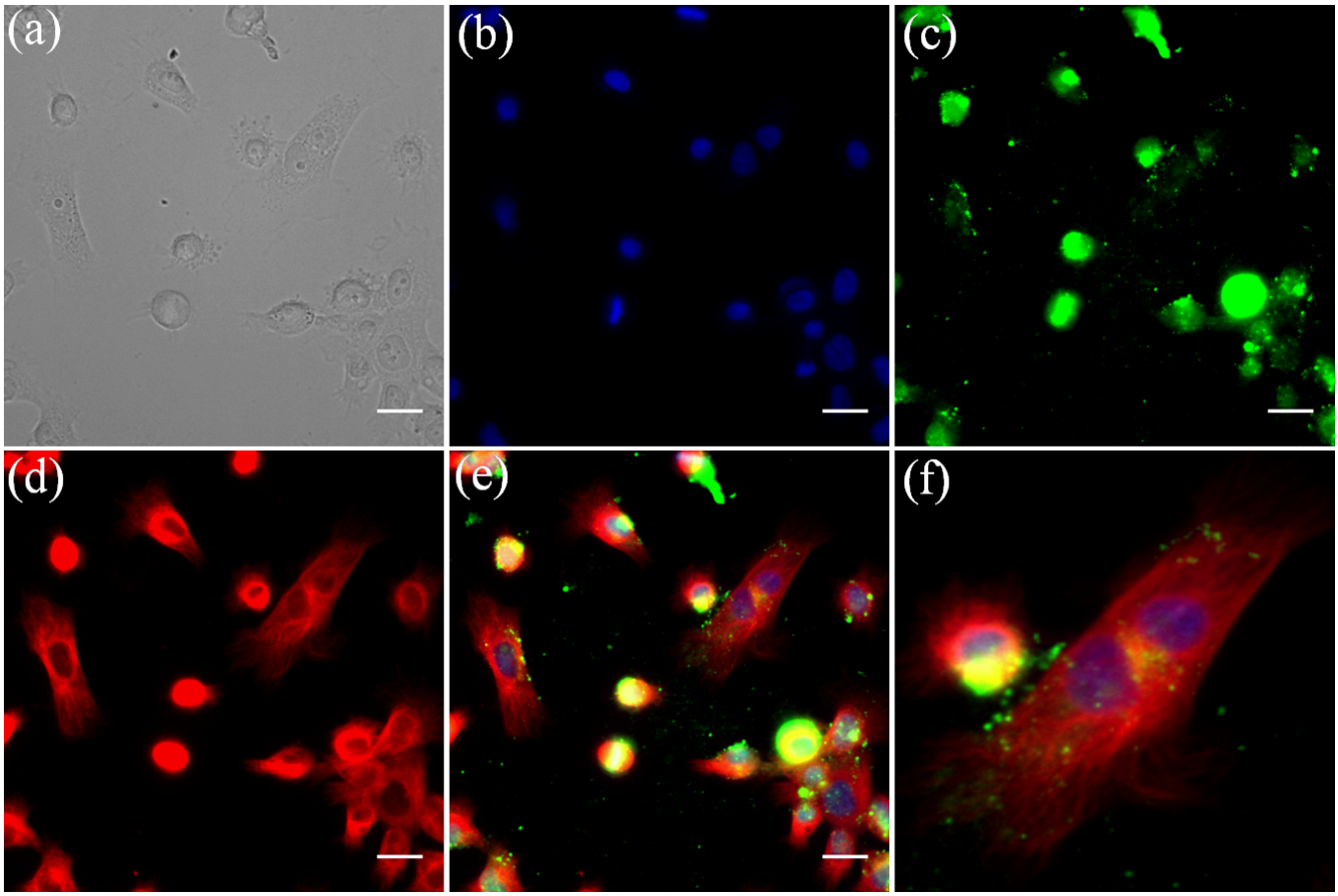

Figure S15. Hilytefluor 488-labeled Aβ42 interacts with microtubule network of HeLa cells (red). Nucleus was stained with hoechst (blue). Confocal images in bright field (a), 405 nm channel (b); 488 nm channel (c); 561 nm channel (d); merged image (e) and zoomed image represents yellow colour aggregates of Aβ42 peptide and microtubule (f). Scale bar corresponds to 20 µm.

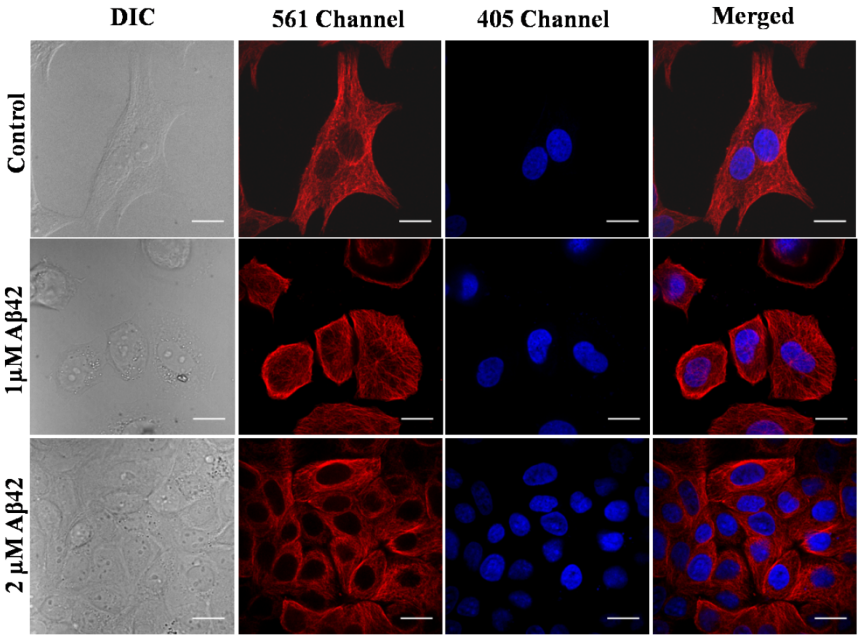

Figure S16. Confocal images show the shrinkage of microtubule network of MCF7 cells after treatment with 1 and 2 μM Aβ42 peptides. Scale bar corresponds to 20 μm.

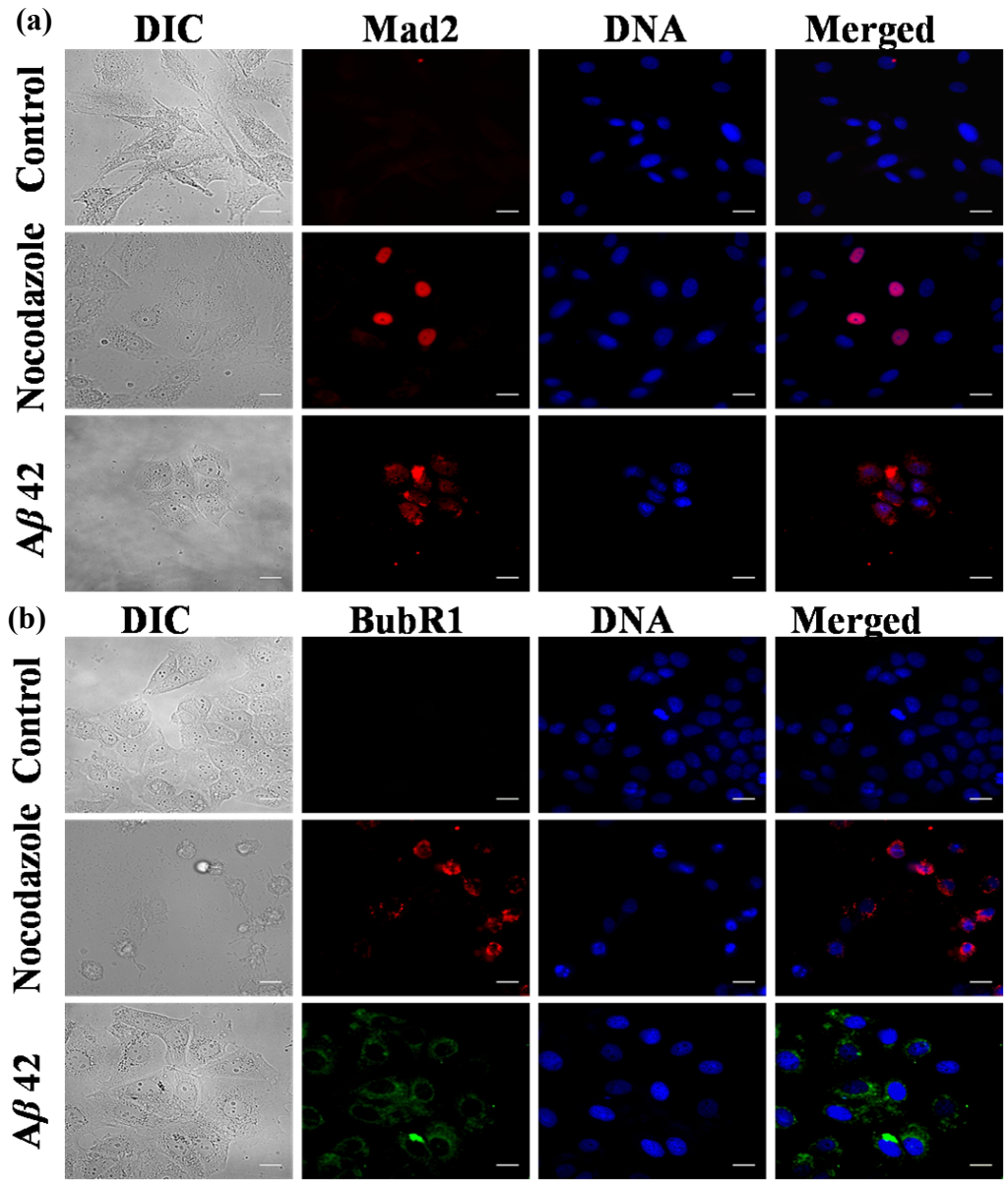

Figure S17. Activation of Mad2 (a) and BubR1 (b) proteins in MCF7 cells after treatment with 2 μM solution of Aβ42 peptide. Scale bar corresponds to 20 μm.

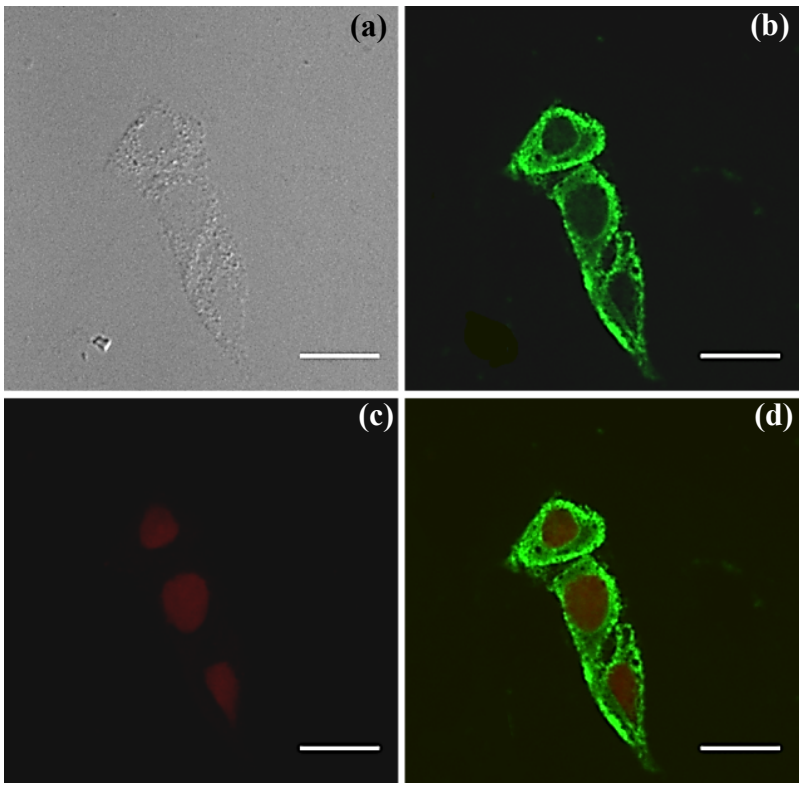

Figure S18. Confocal images of live MCF-7 cells in annexinV propidium iodide assay for apoptosis after treatment with 2 μM Aβ42 peptide. (a) In bright field, (b) 488nm channel, (c) 561 nm channel and (d) merged image of 488 and 561 nm channels. Scale bar corresponds to 20 μm.

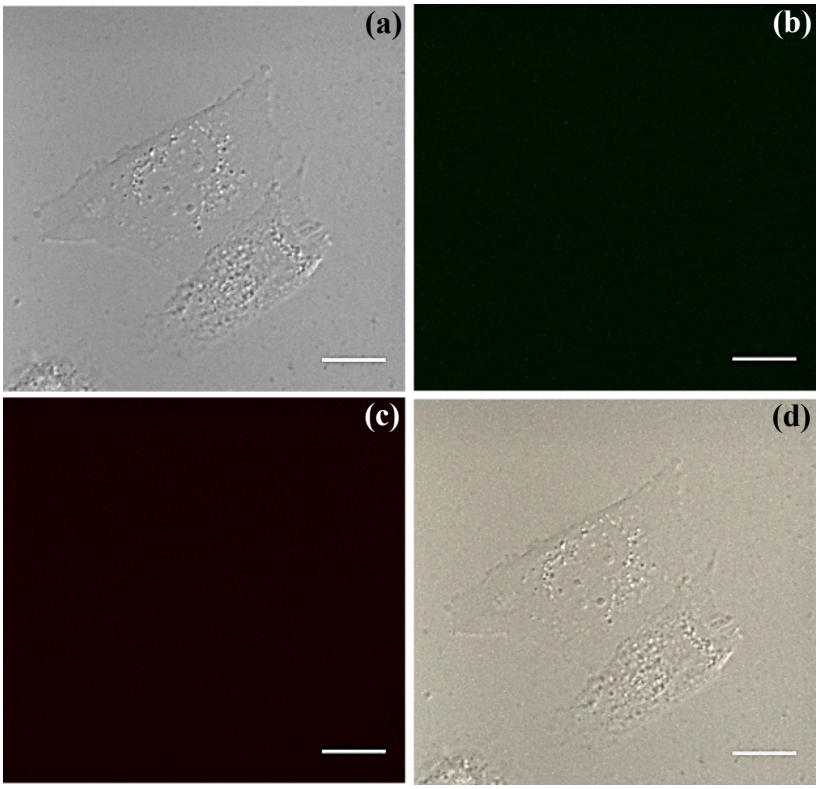

Figure S19. Confocal images of live MCF-7 cells in annexinV propidium iodide assay for apoptosis for control (Cells were not treated with Aβ42 peptide). (a) In bright field, (b) 488 nm channel, (c) 561 nm channel and (d) merged image. Scale bar corresponds to 20 μm.

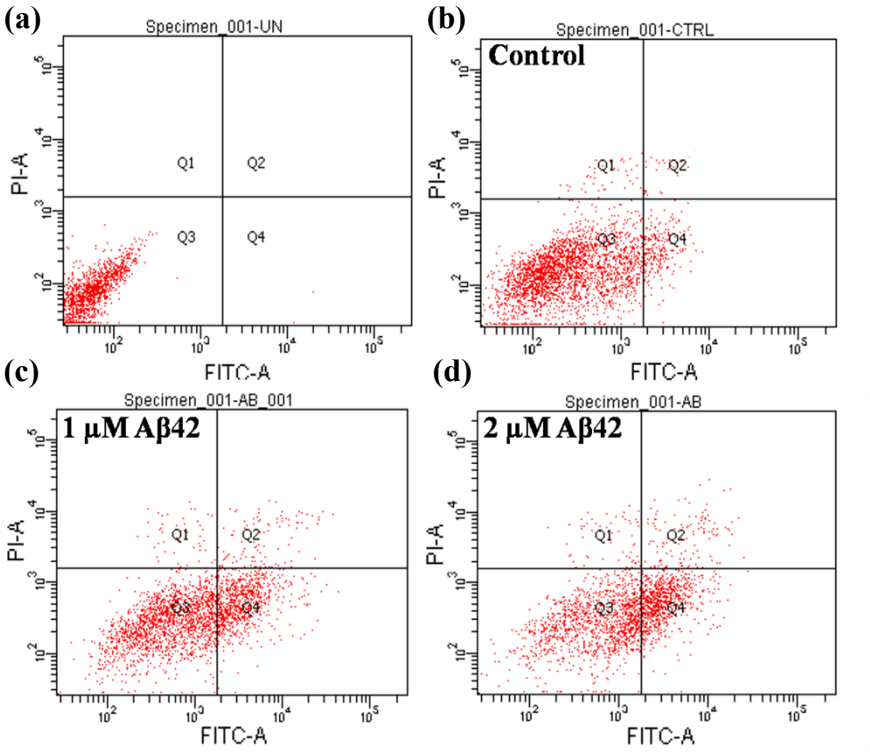

Figure S20. FACS analysis reveals that population of apoptotic MCF7 cells increases with increasing the concentration of Aβ42 peptides. (a) Unstained control cells, (b) Annexin V and PI positive control cells, (c) cells treated with 1 µM Aβ42 peptide, (d) cells treated with 2 µM Aβ42 peptide.

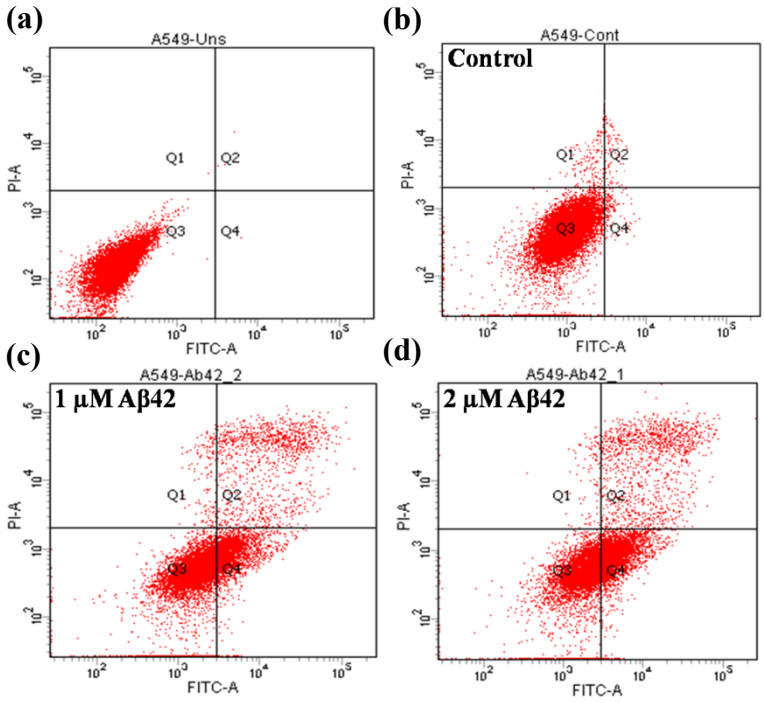

Figure S21. FACS analysis reveals that population of apoptotic A549 cells increases with increasing the concentration of Aβ42 peptide. (a) Unstained control cells, (b) Annexin V and PI positive control cells, (c) cells treated with 1 µM Aβ42 peptide, (d) cells treated with 2 µM Aβ42 peptide.

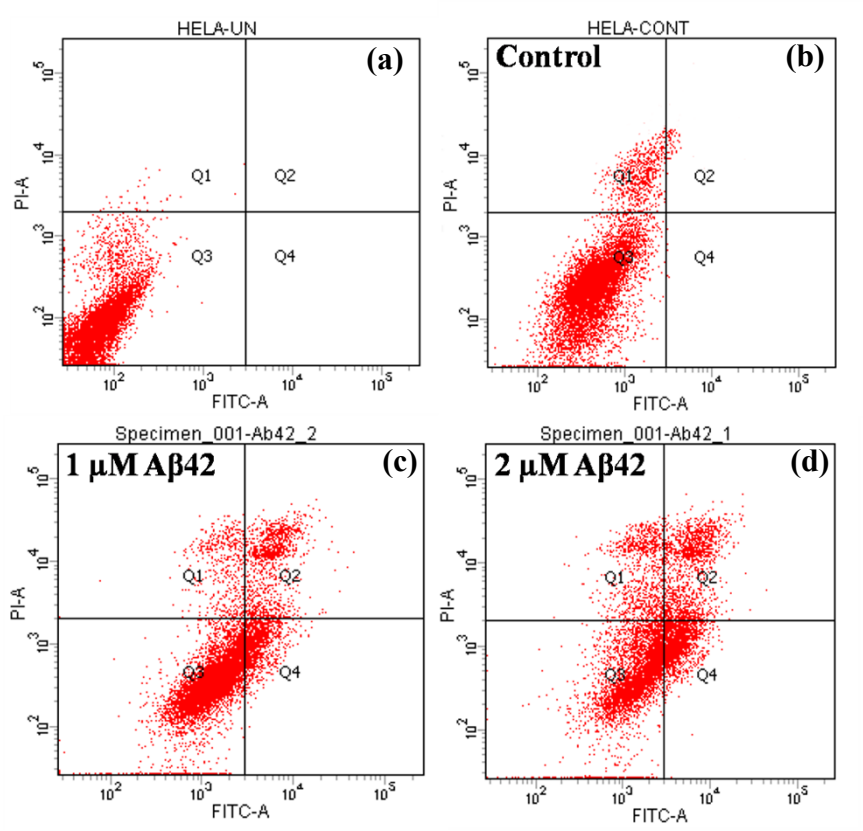

Figure S22. FACS analysis reveals that population of apoptotic HeLa cells increases with increasing the concentration of Aβ42 peptide. (a) Unstained control cells, (b) Annexin V and PI positive control cells, (c) cells treated with 1 µM Aβ42 peptide, (d) cells treated with 2 µM Aβ42 peptide.

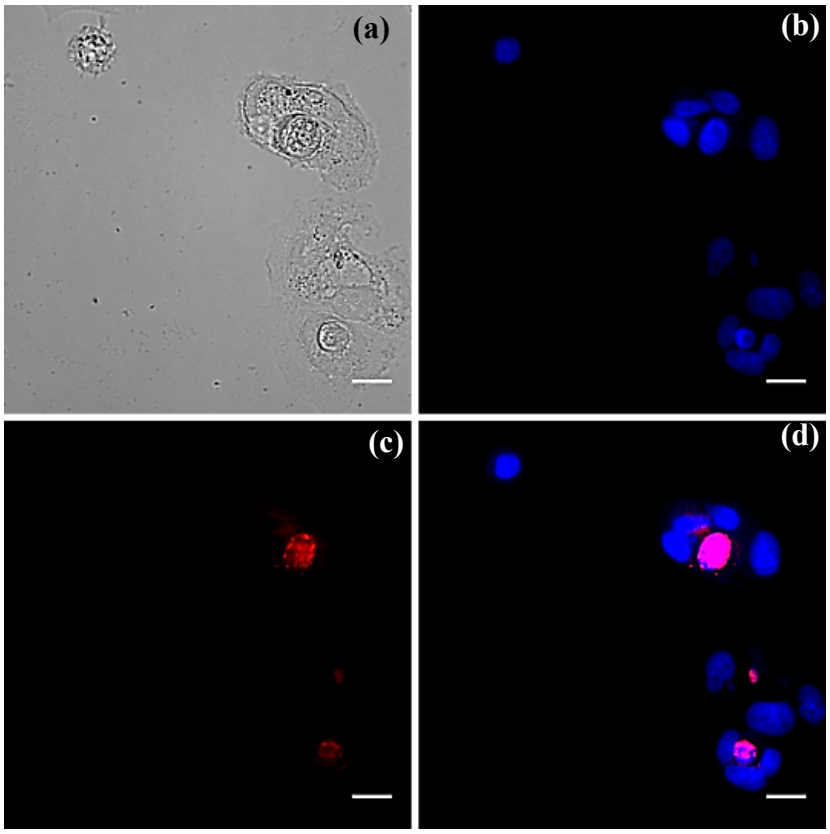

Figure S23. Fluorescence microscopic images show p53 protein activation after treatment with 2 μM Aβ42.Nucleus was stained with Hoechst (blue). Fluorescence microscopic images in bright field (a); 405 nm channel (b); 561 nm channel (c) and merged image (d). Scale bar corresponds to 20 μm.

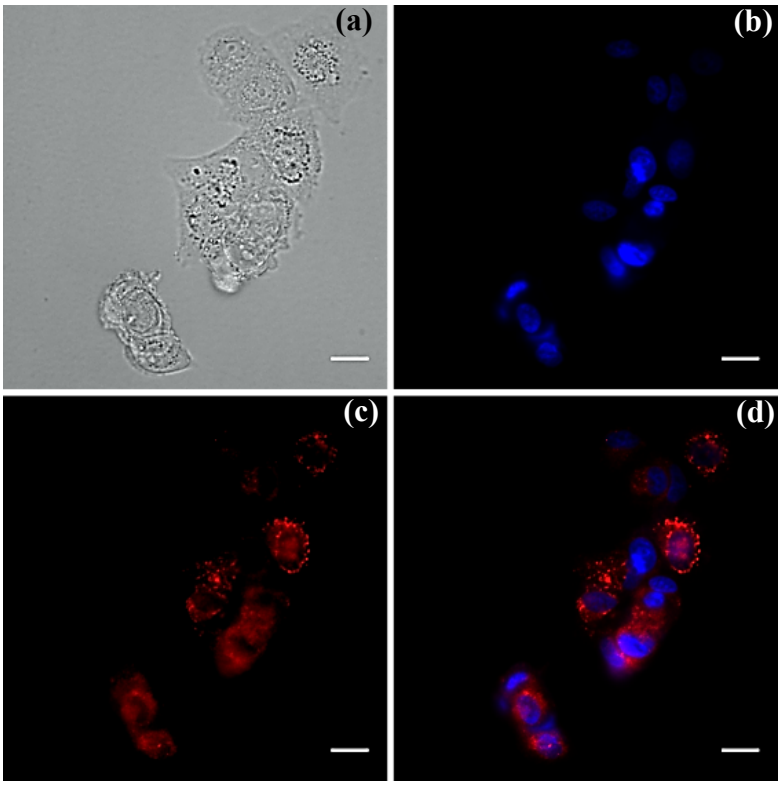

Figure S24. Fluorescence microscopic images show p21 protein activation after treatment with 2 μM Aβ42. Nucleus was stained with Hoechst (blue). Fluorescence microscopic images in bright field (a); 405 nm channel (b); 561 nm channel (c) and merged image (d). Scale bar corresponds to 20 μm.

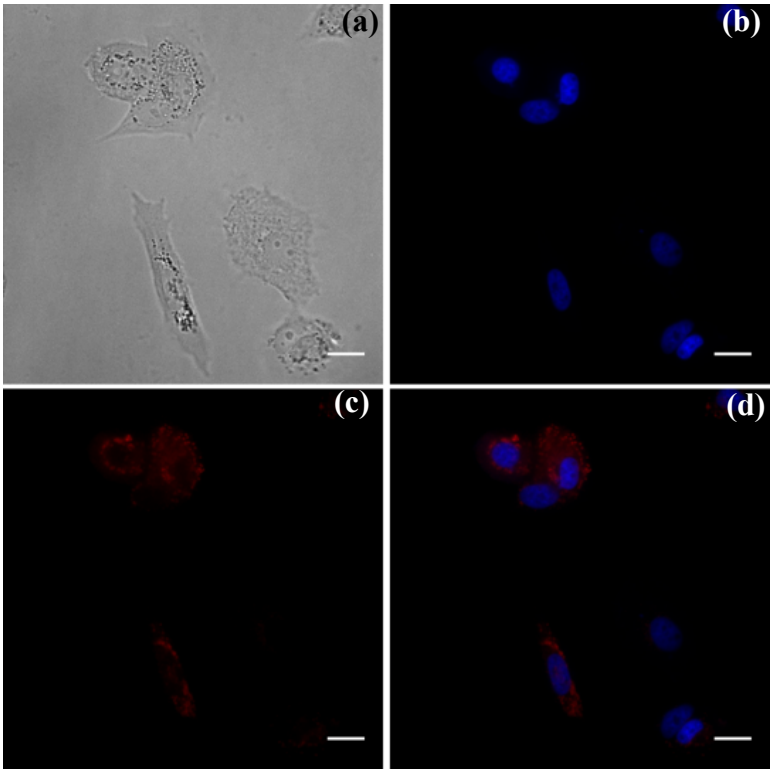

Figure S25. Fluorescence microscopic images of control cells for p53 assay. Nucleus was stained with Hoechst (blue). Fluorescence microscopic images in bright field (a); 405 nm channel (b); 561 nm channel (c) and merged image (d). Scale bar corresponds to 20 μm.

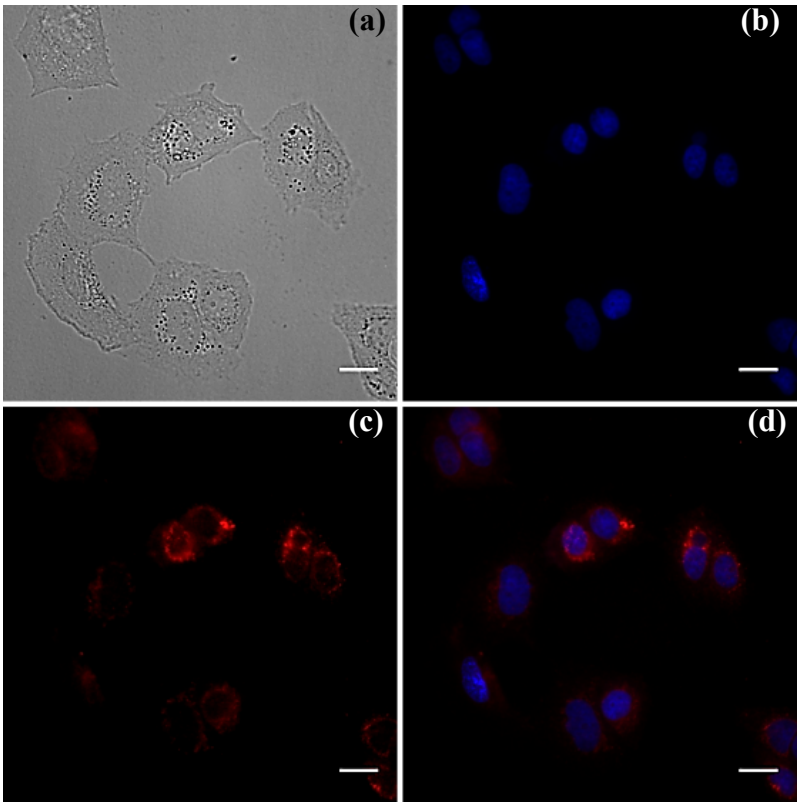

Figure S26. Fluorescence microscopic images of control cells for p21 assay. Nucleus was stained with Hoechst (blue). Fluorescence microscopic images in bright field (a); 405 nm channel (b); 561 nm channel (c) and merged image (d). Scale bar corresponds to 20 μm.

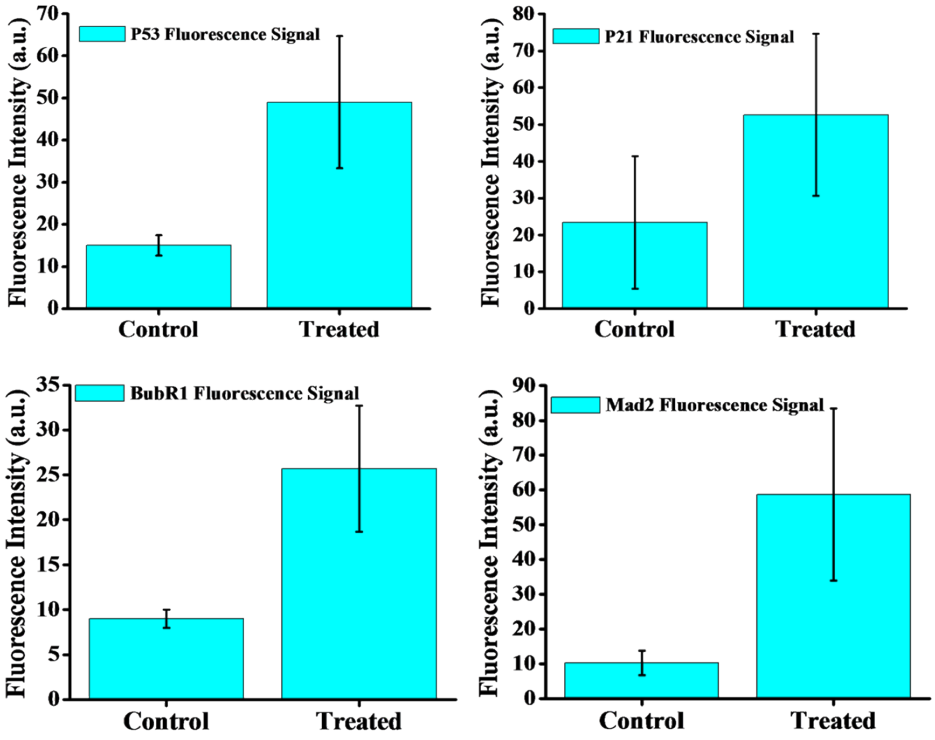

Figure S27. Quantification data of activation of p53, p21, BubR1 and Mad2 proteins from fluorescence microscopic images ($p < 0.05$).