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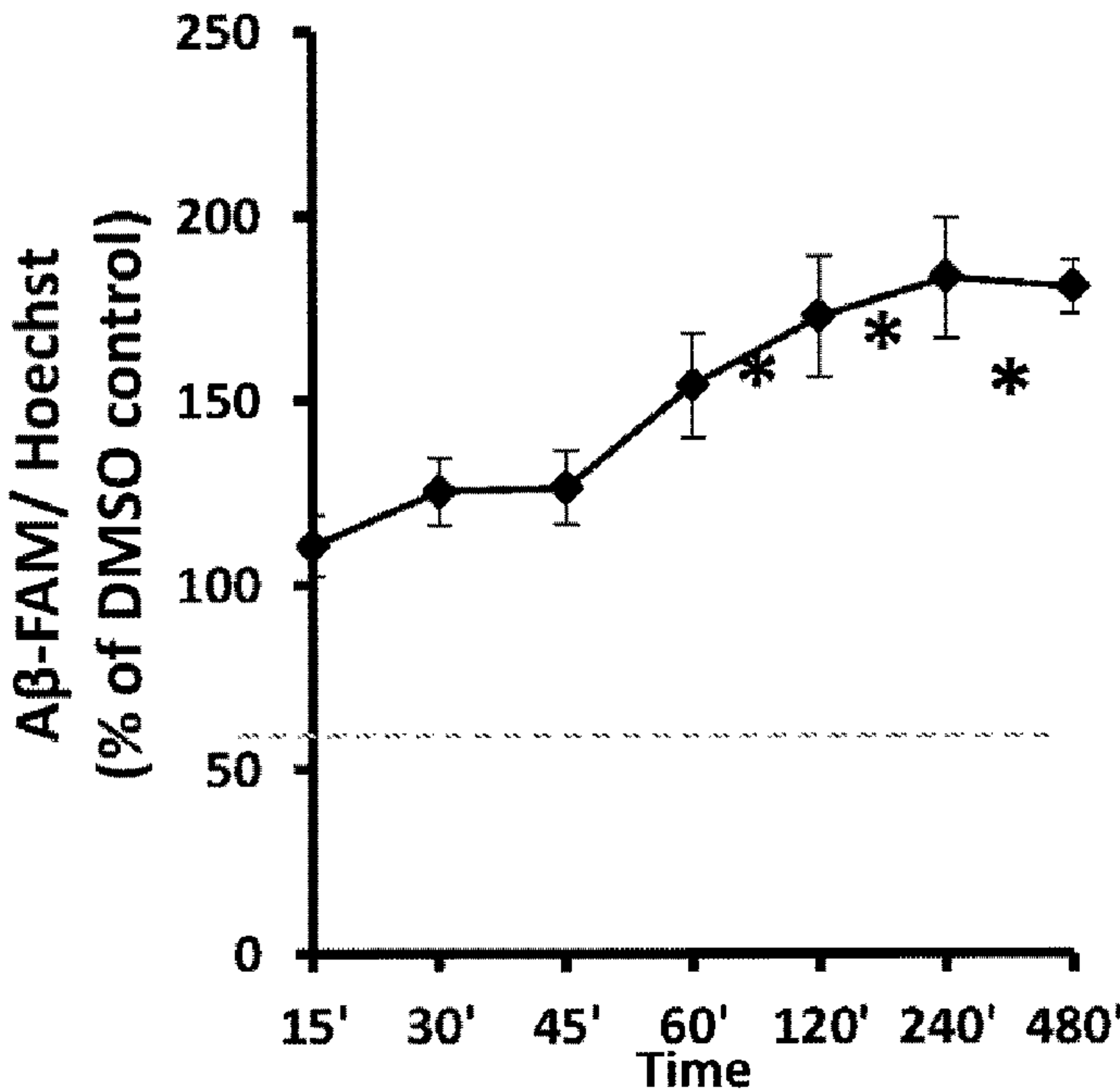
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(71) Demandeur/Applicant:
RUSH UNIVERSITY MEDICAL CENTER, US
(72) Inventeurs/Inventors:
PAHAN, KALIPADA, US;
GHOSH, ARUNAVA, US
(74) Agent: ROBIC

(54) Titre : COMPOSITION ET PROCEDES POUR STIMULATION DE CLAIRANCE DE PROTEINE β -AMYLOIDE
(54) Title: COMPOSITION AND METHODS FOR STIMULATING CLEARANCE OF AMYLOID- β PROTEIN

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(57) **Abrégé/Abstract:**
The present invention generally relates to compositions and methods for stimulating astroglial uptake and degradation of amyloid- β protein aggregates. One aspect of the invention provides a method of preventing or treating Alzheimer's disease including administering a clinically effective amount of combination of vitamin A or a derivative thereof and an agonist of proliferator-activated receptor α ("PPAR α ") to a human or veterinary subject in need of such treatment.

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(71) Applicant: RUSH UNIVERSITY MEDICAL CENTER
[US/US]; 1653 West Congress Parkway, Chicago, IL 60612 (US).

(72) Inventors: PAHAN, Kalipada; Rush University Medical Center, 1653 West Congress Parkway, Chicago, IL 60612 (US). GHOSH, Arunava; Rush University Medical Center, 1653 West Congress Parkway, Chicago, IL 60612 (US).

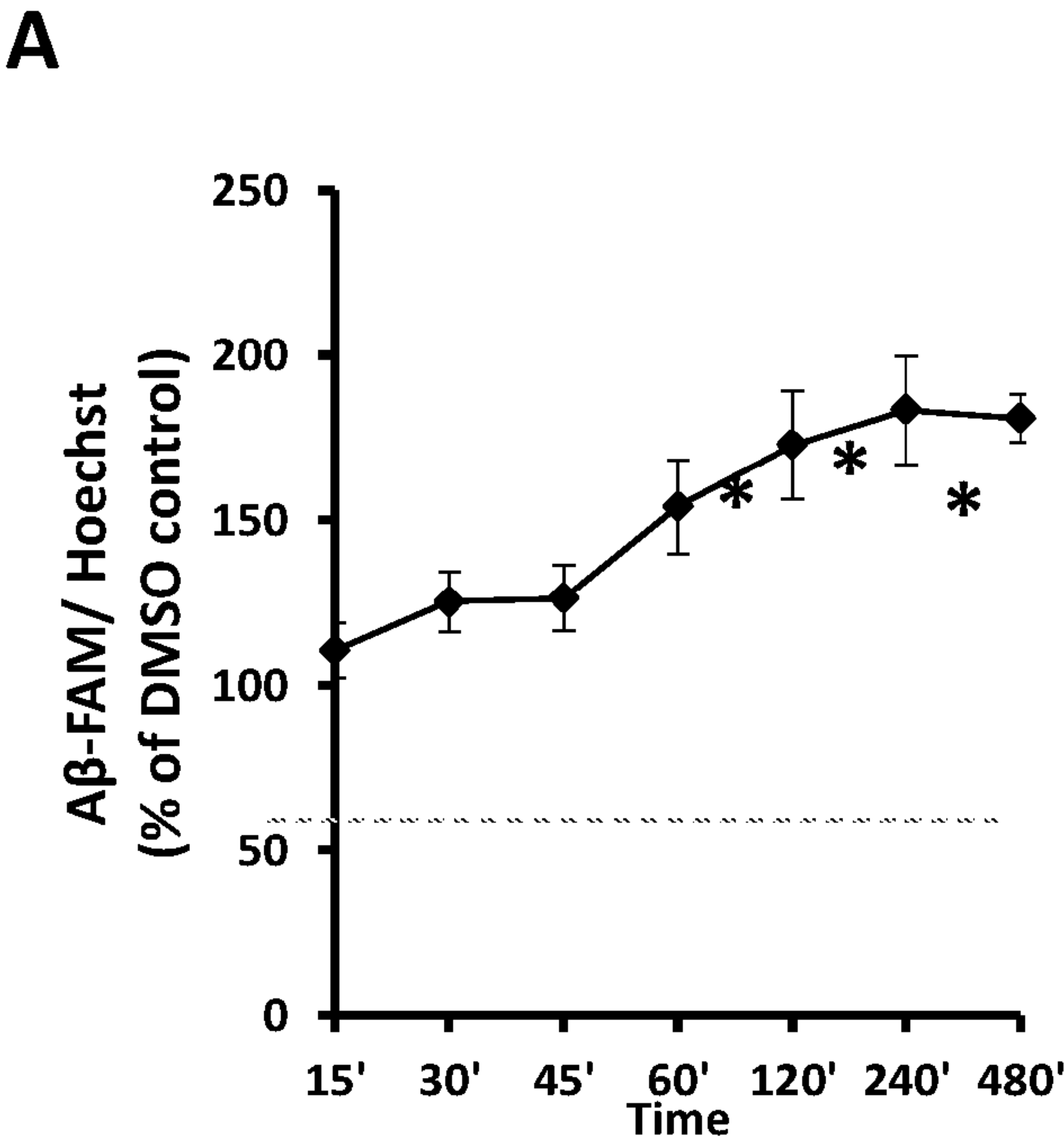
(74) Agent: MURRAY, John; Brinks Gilson & Lione, P.O. Box 10087, Chicago, IL 60610 (US).

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(54) Title: COMPOSITION AND METHODS FOR STIMULATING CLEARANCE OF AMYLOID- β PROTEIN



(57) Abstract: The present invention generally relates to compositions and methods for stimulating astroglial uptake and degradation of amyloid- β protein aggregates. One aspect of the invention provides a method of preventing or treating Alzheimer's disease including administering a clinically effective amount of combination of vitamin A or a derivative thereof and an agonist of proliferator-activated receptor α ("PPAR α ") to a human or veterinary subject in need of such treatment.

TITLE: COMPOSITIONS AND METHODS FOR STIMULATING CLEARANCE OF AMYLOID- β PROTEIN

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] The present patent application claims the benefit of the filing date of U.S. Provisional Patent Application No. 62/308,374, filed March 15, 2016, the contents of which is hereby incorporated by reference.

TECHNICAL FIELD

[002] The present invention generally relates to compositions and methods for stimulating astroglial uptake and degradation of amyloid- β protein aggregates. One aspect of the invention provides a method of preventing or treating Alzheimer's disease including administering a clinically effective amount of combination of vitamin A or a derivative thereof and an agonist of proliferator-activated receptor α ("PPAR α ") to a human or veterinary subject in need of such treatment.

BACKGROUND

[003] Alzheimer's disease is progressive neurodegenerative disease with classic memory impairment and cognitive disorder. The pathological hallmarks of Alzheimer's disease are presence of senile plaques (SPs), composed of oligomeric amyloid beta (A β 40/42) and formation neurofibrillary tangles (NFTs), originating from Tau hyper-phosphorylation, in the cortex and hippocampus of brain (1,2). The abnormal accumulation of A β and formation NFTs induces neuro-inflammation and subsequent neuronal loss, which is the primary cause of Alzheimer's disease (3).

[004] Aggregate prone A β 40/42 fragments are generated by the sequential activity of β - and γ -secretase on amyloid precursor protein (APP), whereas the action of α -secretase produces soluble APP (sAPP) fragments

that are not prone to aggregation (4,5). α -secretase is mainly associated to the plasma membrane, whereas majority of β -secretase is present in the endosomal-lysosomal compartments (6,7). Additionally, some of the cathepsins (D and E) could exhibit β -/ γ - secretase like activity (8). The processing of APP could happen in either secretory pathway or endosomal-lysosomal pathway. Newly synthesized APP could be either be delivered to plasma membrane where it is processed mainly by α -secretase (secretory pathway), or occasionally the APPs are recycled back into endosomes by endocytosis, where it could be processed by β - and γ -secretase (endosomal-lysosomal pathway) producing A β fragments (9,10).

[005] Under normal conditions, further cleavage by other proteases (mainly Cathepsin B) in the lysosomes degrade the A β fragments into even smaller non-toxic fragments, which are recycled or expunged from the cell (11). Also both *in vitro* and *in vivo* conditions, extracellular A β could also be endocytosed and degraded in the lysosomes (12). Decline in lysosomal function due to ageing or other pathological condition may result in abnormal accumulation of A β fragments inside the lysosome and increase the lysosomal load. This may lead to rupture of lysosomal membrane, which not only releases the toxic A β into the cytosol, but also trigger lysosomal membrane permeability (LMP) that can initiate necrotic or apoptotic cell death (13). Therefore, it is imperative that enhanced lysosomal function could be a possible therapeutic mechanism of A β clearance in Alzheimer's disease.

SUMMARY OF THE PREFERRED EMBODIMENTS

[006] In one aspect, the present invention provides a method for reducing amyloid- β protein aggregates in the brain of a subject including administering to the subject in need of such treatment a composition including a therapeutically effective amount of a combination of an agonist of proliferator-activated receptor α ("PPAR α ") and vitamin A or a derivative thereof. In one

embodiment, the agonist is an amphipathic carboxylic acid. In another embodiment the agonist is clofibrate, gemfibrozil, ciprofibrate, bezafibrate, clonofibrate or fenofibrate. The composition can also include at least one pharmaceutically acceptable carrier.

[007] In another embodiment, the therapeutically effective amount is an amount that stimulates the uptake of amyloid- β protein by astrocytes present in the brain. In yet another embodiment, the therapeutically effective amount is an amount that stimulates degradation of amyloid- β protein by such astrocytes.

[008] The subject may be a human subject, for example, a human subject exhibiting symptoms of Alzheimer's disease. The composition may be administered orally. Alternatively, the composition is administered by a subcutaneous, intra-articular, intradermal, intravenous, intraperitoneal or intramuscular route. In another embodiment, the composition is administered directly to the central nervous system.

[009] Another aspect of the invention provides a method for reducing amyloid- β protein aggregates in the brain of a subject's brain including administering a composition including a therapeutically effective amount of an agonist of proliferator-activated receptor α ("PPAR α ") to the subject. In certain embodiments the agonist is an amphipathic carboxylic acid. The agonist may be, for example, clofibrate, gemfibrozil, ciprofibrate, bezafibrate, clonofibrate or fenofibrate..

BRIEF DESCRIPTION OF THE DRAWINGS

[010] Figures 1(A-B) illustrate that GFB and RA treatment enhances A β uptake in mouse primary astrocytes: In Figure 1(A), mouse primary astrocytes were treated for 24hrs with GFB and RA, followed by incubation with 500nM oligomeric FAM-tagged A β (1-42) for 15', 30', 45' 1hr, 2hr, 4hr, 8hr. A β uptake assay was performed as described herein. Data is represented a percentage change compared to DMSO treated control. Figure 1(B) shows microscope

photographs showing mouse primary astrocytes treated with GFB and RA and incubated with 500nM HF-A β (1-42) and 75nM LysoTracker Red before observation. Scale bar = 20 μ M. $p^* < 0.05$ w.r.t DMSO treated control. All data are representative of the mean \pm SEM of three independent experiments.

[011] Figures 2 (A-C) illustrate the effect of HSPG, TFEB and LDLR on GFB and RA mediated A β uptake in mouse primary astrocytes. Figure 2(A): Mouse primary astrocytes were treated with DMSO or GFB-RA, followed by treatment with diluent of Heparin (100 μ g/ml) and further incubated in 500nM FAM-A β for 4hrs. A β uptake assay was performed and data is represented as percentage change w.r.t untreated control. Figure 2(B): Mouse primary astrocytes were transfected with scrambled siRNA, Tfeb siRNA or LDLR siRNA, treated with GFB-RA, followed by incubation in 500nM FAM-A β for 4hrs. Data from A β uptake assay is represented as percentage change w.r.t DMSO and scrambled siRNA treated control. Figure 2(C) Quantitative RT-PCR was performed to measure the effectivity of LDLR silencing by siRNA and levels of LDLR in PPAR α (-/-) cells. Figure 2(D) Quantitative RT-PCR was performed to measure the effect of TFEB silencing by siRNA. $p^* < 0.05$ w.r.t control; ns-not significant. All data are representative of the mean \pm SEM of three independent experiments.

[012] Figures 3(A-D) illustrate that GFB and RA treatment enhances A β degradation in mouse primary astrocytes: Figure 3(A): Mouse primary astrocytes were treated for 24hrs with GFB and RA, followed by incubation with 500nM oligomeric FAM-tagged A β (1-42) for 4hr and allowed to grow in A β -free media for 15', 30', 1hr, 2hr, 4hr, 6hr and 8hr. A β degradation assay was performed as described in Methods section. Data was represented as percentage change compared to unwashed control. Figure 3(B): Mouse primary astrocytes treated with GFB and RA were incubated with 500nM HF-A β (1-42), washed for 4h and 6h, further incubated with 75nM LysoTracker Red and observed under microscope. Scale bar = 20 μ M. $p^* < 0.05$ w.r.t unwashed control.

Figure 3(C): Mouse primary astrocytes were treated with GFB and RA for 24hrs, followed by treatment with 100nM Bafilomycin A1 for 45mins, followed by incubation with 500nM FAM-A β , washed in A β free media for 6hrs and degradation assay was performed. Data is represented as percentage change w.r.t unwashed controls. Figure 3(D): A β degradation assay was done in mouse primary astrocytes which were either transfected with scrambled siRNA or Tfeb siRNA, prior to treatment with DMSO or GFB-RA. Data is compared to DMSO treated, scrambled siRNA transfected controls. $p^* < 0.05$ w.r.t control; ns-not significant. All data are representative of the mean \pm SEM of three independent experiments. All data are representative of the mean \pm SEM of three independent experiments.

[013] Figure 4(A-E) illustrates the role of PPAR α and PPAR β in A β uptake and degradation in mouse primary astrocytes. Figure 4(A): Mouse primary astrocytes isolated from PPAR α (-/-), PPAR β (-/-) and WT animals were isolated, treated with GFB-RA or DMSO, followed by incubation with 500nM FAM-A β and subjected to A β uptake assay. Data was compared to DMSO-treated WT control and represented as percentage change. $p^* < 0.05$ w.r.t control; ns-not significant. Figure 4(B): Mouse primary astrocytes isolated from PPAR α (-/-), PPAR β (-/-) and WT animals were isolated, treated with GFB-RA, followed by incubation with 500nM FAM-A β for 4hrs, washed in A β free media for 6hrs and subjected to A β degradation assay. Analysis of data is described in detail in Discussion. $p^* < 0.05$ w.r.t control; ns-not significant. Mouse primary astrocytes isolated from WT (Figure 4(C)), PPAR α (-/-) (Figure 4(D)) and PPAR β (-/-) (Figure 4(E)) animals were isolated, treated with DMSO, followed by incubation with 500nM HF-647-A β for 4hrs and 75nM LysoTracker for 45mins (*first panel*), treated with GFB-RA, followed by incubation with 500nM HF-647-A β for 4hrs and 75nM LysoTracker for 45mins (*second panel*), treated with GFB-RA, followed by incubation with 500nM HF-647-A β for 4hrs and washed in A β -free media for 6hrs and incubated in 75nM LysoTracker for 45mins (*third*

panel) and observed under microscope. Scale bar = 20 μ m. All data are representative of the mean \pm SEM of three independent experiments.

[014] Figures 5 (A-F) illustrate that GFB and RA treatment increased lysosomal activity as well as autophagic flux: Figures 5(A-B) Mouse primary astrocytes were either untransfected, transfected with scrambled siRNA or Tfeb siRNA and treated with DMSO or GFB and RA. Whole cell extract was prepared and subjected to cathepsin assay for CtsB (A) and CtsD (B) (described in detail herein). Figure 5(B) Whole cell extract from cells treated with DMSO or GFB-RA was used to perform immunoblot for the levels of CtsB and CtsD. Figure 5(D) Densitometric analysis of the immunoblot, normalized to β -Actin. Figure 5(E) Mouse primary astrocytes were treated with DMSO or GFB-RA, in presence of absence of 100nM Bafilomycin A1, or 500nM oligomeric A β . Whole cell extract was subjected to immunoblot for the levels of LC3 and p62. Figure 5(F) Densitometric analysis of the immunoblot, normalized to β -Actin. $p^* < 0.05$ w.r.t control; ns-not significant. All data are representative of the mean \pm SEM of three independent experiments.

[015] Figure 6 shows a test for bleed through signals in different IF channels. Mouse primary astrocytes were cultured in DMEM/F12 media and stained separately with LysoTracker Red (*top panel*) and HF647-A β (*bottom panel*). Dapi is used to stain nuclei. The cells were observed under IF Microscope in DAPI, Cy2, Cy3 and Cy5 channels.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions

[016] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to

those described herein can be used in the practice or testing of the present invention.

[017] The uses of the terms "a" and "an" and "the" and similar references in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as", "for example") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[018] The term "therapeutic effect" as used herein means an effect which induces, ameliorates or otherwise causes an improvement in the pathological symptoms, disease progression or physiological conditions associated with or resistance to succumbing to a disorder, for example the aggregation of amyloid- β protein in the brain of a human or veterinary subject. The term "therapeutically effective amount" as used with respect to a drug means an amount of the drug which imparts a therapeutic effect to the human or veterinary patient.

Methods for Stimulating Clearance of Amyloid- β Protein

[019] Alzheimer's disease is the most common human neurodegenerative disease, resulting in progressive neuronal death and

memory loss. Neuropathologically, the disease is characterized by the presence of both neurofibrillary tangles and neuritic plaques composed of aggregates of amyloid- β ($A\beta$) protein, a 40-43 amino acid proteolytic fragment. In the Alzheimer's disease brain, while neurons die, glial cells like astrocytes and microglia do not die. Astrocytes are the major cell type in the brain, and may be utilized for breaking down amyloid plaques even in advanced Alzheimer's disease.

[020] The administration of a composition including a therapeutically effective amount of an agonist of proliferator-activated receptor α ("PPAR α ") stimulates the uptake and degradation of amyloid- β ($A\beta$) in astrocytes. The composition may also include vitamin A or a derivative thereof. The agonist can be an amphipathic carboxylic acid. In certain embodiments the agonist is clofibrate, gemfibrozil, ciprofibrate, bezafibrate, clinofibrate or fenofibrate. In one preferred embodiment, the composition includes a combination of gemfibrozil and retinoic acid.

[021] Gemfibrozil, an FDA-approved lipid-lowering, and vitamin A derivative retinoic acid stimulate the uptake and degradation of amyloid- β ($A\beta$) in astrocytes. Low density lipoprotein receptor (LDLR) plays an Important role in the uptake, whereas, TFEB mediated induction in lysosomal activity is critical for the degradation. Gemfibrozil and retinoic acid treatment also increased the autophagic flux and lysosomal activity in astrocytes as observed from increased LC3-11 formation and Increased cathepsin (B/D) activity, respectively.

[022] Furthermore, the effect of gemfibrozil and retinoic acid on $A\beta$ uptake/degradation is abrogated in absence of peroxisomal proliferator activated receptor α (PPAR α), which plays a key role in gemfibrozil-retinoic acid mediated induction of TFEB. These results identify PPAR α as an important regulator of astroglial uptake and degradation of $A\beta$ via enhancement of lysosomal $A\beta$ clearance and suggest that combination of vitamin A derivative retinoic acid and gemfibrozil or other PPAR α agonists may reduce $A\beta$ plaque load in Alzheimer's disease patients.

[023] Gemfibrozil, an agonist of peroxisome proliferator-activated receptor α (PPAR α) alone and in conjunction with all-trans-retinoic acid (RA) is capable of enhancing TFEB in brain cells. Retinoid X receptor (RXR α) and PPAR α , but not PPAR β and PPAR γ , are involved in GFB-mediated upregulation of TFEB. Reporter assay and chromatin immunoprecipitation studies confirmed the recruitment of RXR α , PPAR α , and PPAR γ co-activator 1 α (PGC1 α) on the PPAR binding site on Tfeb promoter. Subsequently, the drug mediated induction of TFEB caused increase in certain lysosomal proteins and the lysosomal proliferation in cell (14). These findings were in accordance with another study that also showed transcriptional regulation of TFEB by recruitment of PGC1 α on TFEB promoter (15, 16).

[024] Enhanced activity of lysosomes in A β uptake and degradation by mouse astrocytes was evaluated using an *in vitro* A β uptake and degradation assay, supported by microscopic observation of intracellular A β load. Significant increases were observed in both uptake and degradation of A β in WT and PPAR β (-/-), but not in PPAR α (-/-) cells, when stimulated with GFB-RA. Silencing of LDLR by LDLR siRNA, reduced the rate of A β uptake, whereas gene silencing of TFEB, reduced the degradation rate. Furthermore, significant changes were observed in autophagic flux and lysosomal activity that could be mediated by GFB-RA treatment, by monitoring levelsof Cathepsin B/D (CtsB/D), LC3-I/II and p62. Our data indicates that TFEB upregulation (and increase in lysosomal biogenesis) by PPAR α :RXR α :PGC1 α activation leads to increased uptake of A β and subsequent degradation of endocytosed A β in the lysosomes in mouse primary astrocytes.

Pharmaceutical Compositions

[025] Another aspect of the present invention provides pharmaceutical compositions including an agent that is proliferator-activated receptor α ("PPAR α ") or an agonist of PPAR α . In one embodiment, the agonist is an

amphipathic carboxylic acid. For example, the agonist may be clofibrate, gemfibrozil, ciprofibrate, bezafibrate, clinofibrate or fenofibrate. Yet another aspect of the invention provides pharmaceutical compositions including a combination of an agent that is proliferator-activated receptor α ("PPAR α ") or an agonist of PPAR α and vitamin A or a derivative thereof. In one preferred embodiment, the pharmaceutical composition includes gemfibrozil and retinoic acid.

[026] The pharmaceutical compositions can be in the form of, for example, tablets, pills, dragees, hard and soft gel capsules, granules, pellets, aqueous, lipid, oily or other solutions, emulsions such as oil-in-water emulsions, liposomes, aqueous or oily suspensions, syrups, alixiers, solid emulsions, solid dispersions or dispersible powders. In pharmaceutical compositions for oral administration, the agent may be admixed with commonly known and used adjuvants and excipients, for example, gum arabic, talcum, starch, sugars (such as, e.g., mannitolose, methyl cellulose, lactose), gelatin, surface-active agents, magnesium stearate, aqueous or non-aqueous solvents, paraffin derivatives, cross-linking agents, dispersants, emulsifiers, lubricants, conserving agents, flavoring agents (e.g., ethereal oils), solubility enhancers (e.g., benzyl benzoate or benzyl alcohol) or bioavailability enhancers (e.g. GELUCIRE). In the pharmaceutical composition, the agent may also be dispersed in a microparticle, e.g. a nanoparticulate, composition.

[027] For parenteral administration, the agent or pharmaceutical compositions of the agent can be dissolved or suspended in a physiologically acceptable diluent, such as, e.g., water, buffer, oils with or without solubilizers, surface-active agents, dispersants or emulsifiers. As oils for example and without limitation, olive oil, peanut oil, cottonseed oil, soybean oil, castor oil and sesame oil may be used. More generally, for parenteral administration the agent or pharmaceutical compositions of the agent can be in the form of an aqueous, lipid, oily or other kind of solution or suspension or even administered

in the form of liposomes or nano-suspensions.

Modes of Administration

[028] The pharmaceutical composition may be administered by any method that allows for the delivery of a therapeutic effective amount of the agent to the subject. Modes of administration can include, but are not limited to oral, topical, transdermal and parenteral routes, as well as direct injection into a tissue and delivery by a catheter. Parenteral routes can include, but are not limited to subcutaneous, intradermal, intra-articular, intravenous, intraperitoneal and intramuscular routes. In one embodiment, the route of administration is by topical or transdermal administration, such as by a lotion, cream, a patch, an injection, an implanted device, a graft or other controlled release carrier. Routes of administration include any route which directly delivers the composition to the systemic circulation (e.g., by injection), including any parenteral route. Alternatively, administration can be by delivery directly to the central nervous system.

[029] One embodiment of the method of the invention includes administering the composition in a dose, concentration and for a time sufficient to prevent the development of, or to lessen the extent of Alzheimer's disease. In another embodiment, the invention includes administering the composition in a dose, concentration and for a time sufficient to reduce amyloid- β protein aggregates in the brain of a subject. In yet another embodiment, the invention includes administering the composition in a dose, concentration and for a time sufficient to stimulate the uptake of amyloid- β protein aggregates by astrocytes in the brain of a subject. In another embodiment, the invention includes administering the composition in a dose, concentration and for a time sufficient to stimulate degradation of amyloid- β protein aggregates by astrocytes in the brain of a subject.

[030] Certain embodiments include administering systemically the composition in a dose between about 0.1 micrograms and about 100 milligrams per kilogram body weight of the subject, between about 0.1 micrograms and about 10 milligrams per kilogram body weight of the subject, between about 0.1 micrograms and about 1 milligram per kilogram body weight of the subject. In practicing this method, the composition can be administered in a single daily dose or in multiple doses per day. This treatment method may require administration over extended periods of time. The amount per administered dose or the total amount administered will be determined by the physician and will depend on such factors as the mass of the patient, the age and general health of the patient and the tolerance of the patient to the compound.

[031] Embodiments of the invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

Example 1 - Isolation of Primary Mouse Astroglia:

[032] Astroglia were isolated from mixed glial cultures as described (17,18) according to the procedure of Giulian and Baker (19). Briefly, on day 9, the mixed glial cultures were washed three times with Dulbecco's modified Eagle's medium/F-12 and subjected to shaking at 240 rpm for 2 h at 37°C on a rotary shaker to remove microglia. After 2 days, the shaking was repeated for 24 h for the removal of oligodendroglia and to ensure the complete removal of all nonastroglial cells. The attached cells were seeded onto new plates for further studies.

Example 2 – Semi-Quantitative Reverse Transcriptase-Coupled Polymerase Chain Reaction (RT-PCR):

[033] Total RNA was isolated from mouse primary astrocytes and human primary astrocytes using RNA-Easy Qiagen kit following manufactures protocol. Semi-quantitative RT-PCR was carried out as described earlier (20)

using oligo (dT) 12–18 as primer and moloney murine leukemia virus reverse transcriptase (MMLV-RT, Invitrogen) in a 20µl reaction mixture. The resulting cDNA was appropriately amplified using Promega Master Mix and the primers for murine genes. Tfeb primer: Fwd: 5'-*aacaaaggcaccatcctcaa*-3' SEQ ID NO.: 1; Rev: 5'-*cagctcggccatattcacac*-3' SEQ ID NO.: 2 Ldlr primer was purchased from SantaCruz Biotechnology (Cat. No. sc-35803-PR). Amplified products were electrophoresed on 2% agarose gels and visualized by ethidium bromide staining. Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) mRNA was used as a loading control to ascertain that an equivalent amount of cDNA was synthesized from each sample.

Example 3 – Quantitative Real-Time PCR:

[034] The mRNA quantification was performed using the ABI-Prism7700 sequence detection system using SYBR Select master mix. The mRNA expression of the targeted genes was normalized to the level of *Gapdh* mRNA and data was processed by the ABI Sequence Detection System 1.6 software.

Example 4 – Immunoblotting:

[035] Western blotting was conducted as described earlier (21,22) with modifications. Briefly, cells were scraped in 1X RIPA buffer, protein was measured using Bradford reagent and sodium dodecyl sulfate (SDS) buffer was added and electrophoresed on NuPAGE® Novex® 4-12% Bis-Tris gels (Invitrogen) and proteins transferred onto a nitrocellulose membrane (Bio-Rad) using the Thermo-Pierce Fast Semi-Dry Blotter. The membrane was then washed for 15 min in TBS plus Tween 20 (TBST) and blocked for 1 hr in TBST containing BSA. Next, membranes were incubated overnight at 4°C under shaking conditions with the following 1° antibodies; CtsB (Cell Signalling Technology, 1:1000), CtsD (Cell Signalling Technology, 1:1000), LC3 (Novus, 1:500), p62 (Abcam, 1:500) and β-actin (Abcam, 1:1000). The next day,

membranes were washed in TBST for 1 hr, incubated in 2° antibodies against 1° antibody hosts (all 1:10,000; Jackson ImmunoResearch) for 1 hr at room temperature, washed for one more hour and visualized under the Odyssey® Infrared Imaging System (Li-COR, Lincoln, NE).

Example 5 - Amyloid beta uptake assay:

[036] Mouse primary astrocytes were plated in black 96-well plates. After appropriate treatment, the wells were incubated at 37°C with 500nM oligomeric FAM-tagged A β (1-42) for appropriate time-points. Finally the A β -containing medium was removed and wells were gently washed with normal media, followed by quenching of extracellular A β with 100 μ l 0.2% trypan blue in PBS for 2mins. After aspiration the fluorescence was measured Ex./Em. of 485/535 in Victor X2 microplate reader (Perkin Elmer). The wells were further incubated with 100 μ l 50 μ g/ml Hoechst 33342 dye in PBS for 30mins and fluorescence was measured Ex./Em. of 360/465nm (23). The A β fluorescence was normalized to Hoechst fluorescence to account for cell number variability if any.

Example 6 - Amyloid beta degradation assay:

[037] Mouse primary astrocytes were plated, treated and then incubated for 4 hrs with FAM-tagged A β (1-42). After incubation, A β containing media was removed and after a single gentle wash, the plates were incubated with normal media at 37°C for different time points. The measurement of A β and Hoechst fluorescence was measure as mentioned above.

Example 7 - Immunocytochemistry for amyloid beta uptake/ degradation:

[038] Mouse primary astrocytes were cultured on square coverslips placed in 6 well plates. After treatment cells were incubated with 500nM of oligomeric HF-647-tagged A β (1-42). For degradation study, the cells were

further allowed to grow in normal media, after removal of A β containing media. After incubation, cells were further incubated in media containing 75nM LysoTracker Red DND99 for 30mins. The cells were then washed, fixed on glass slides and observed under BX41 fluorescence microscope (23).

Example 8 - Cathepsin Assay:

[039] Mouse primary astrocytes were cultured, treated and lysed in 100 mM sodium acetate, pH 5.5, with 2.5 mM EDTA, 0.01% Triton X-100, and 2.5 mM DTT.

[040] For Cathepsin B assay, the supernatant was incubated for 30mins at pH 6.0 with 100 μ M Z-Arg-Arg-AMC. 7-amino-4-methylcoumarin, AMC was used as standard. The fluorescence was measured at Ex./Em. of 355/460nm in Victor X2 microplate reader.

[041] For Cathepsin D assay, the supernatant was incubated 10 μ M substrate 7-methoxycoumarin-4-acetyl-(Mca)-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys-2,4 nitrophenyl (Dnp)-D-Arg-NH₂ at pH 4.0 for 30mins. Mca-Pro-Leu-OH was used as standard. The fluorescence was measured at Ex./Em. of 320/420nm.

[042] The fluorescence readings of the samples were compared to the respective standard to measure the amount of product obtained. Cathepsin activity (in Units) was calculated per mg of cell extract, considering 1Unit of enzyme activity released 1nmole of product per hour at 37°C (24,25).

Example 9 - Densitometric Analysis:

[043] Protein blots were analyzed using ImageJ (NIH, Bethesda, MD) and bands were normalized to their respective β -actin loading controls.

Immunofluorescence quantification data are representative of the average fold change with respect to control for at least 25 different images per condition from three independent set of experiments.

Example 10 - Statistical Analysis:

[044] Values are expressed as means \pm SEM of at least three independent experiments. Statistical analyses for differences were performed via Student's T-test. This criterion for statistical significance was $p < 0.05$.

Example 11 - GFB and RA treatment enhancement of A β uptake in mouse primary astrocytes:

[045] Lysosomal activity is crucial for the clearance of A β in the Alzheimer's disease brain. Therefore, we explored the effect of GFB and RA, which enhances lysosomal biogenesis, on the uptake of extracellular A β by mouse primary astrocytes. We performed both quantitative *in vitro* assay and qualitative microscopic analysis to measure the alterations in the levels of A β in taken up by the cells.

[046] The *in vitro* assay is a robust technique that can quantitatively measure the signal intensity of FAM-tagged A β (1-42) from inside the cell. The cells were treated with GFB-RA and further incubated with FAM-A β (1-42) for various time points (15', 30', 45', 1hr, 2hr, 4hr and 8hr). The signal intensity of A β was first normalized to that of Hoechst, to account for the variability in cell number in each well, if any. Then the normalized A β signals of GFB-RA treated samples were compared to their DMSO treated counterparts and percentage change in the A β signal was calculated for each time point.

[047] After 2hrs of incubation in A β containing media, the amount of A β inside the GFB-RA treated cells were ~60% more compare to the DMSO treated cells. At 4hrs, the A β signal in treated cells were about ~80% higher than the control (Fig 1A). However further incubation up to 8hrs did not yield any further increase in the A β content in treated cells, indicating that 4hrs of incubation would be the optimum time point for the assay. Therefore, for further uptake assays, this time point of A β incubation was selected.

[048] Fluorescence microscopy was performed by incubating the cells with HF-647-tagged A β (1-42) for 2hrs and 4hrs followed by incubation with LysoTracker Red. We observed increased punctate signal of HF-647-A β in both 2hrs and 4hrs in GFB-RA treated cells compared to DMSO control. Furthermore, the A β signal co-localized with the LysoTracker signal, indicating that the A β taken up by the cells were residing in the acidic vesicles inside the cell (late endosomes or lysosomes) (Fig. 1B). Since the patterns of A β signal and LysoTracker signal were expected to be similar, we incubated cells separately with LysoTracker and HF-647-A β and tested all channels for any bleed through signals. As expected, only LysoTracker showed slight signal overlap between CY2 and CY3 channels, but there was no significant bleed through signal in any other channel for HF-647-A β apart from its true signal in CY5 channel (Fig. 6).

Example 12 - Effect of LDLR and TFEB on GFB-RA mediated uptake of A β :

[049] A β could be taken up through micropinocytosis assisted by heparan sulfate proteoglycans (HSPGs) (26). Therefore, to elucidate the mechanism of GFB-RA mediated enhancement of A β uptake, we performed A β uptake assay in presence of Heparin (inhibitor of HSPGs) first. Cells treated with GFB-RA in presence of heparin showed ~40% increase in A β uptake compare to ~80% in GFB-RA treated cells in absence of heparin (Fig. 2A). Although, this reduction in the uptake level is statistically significant, but still there was about 40% uptake even in presence of heparin, which indicates that other factors may also be responsible for the uptake process. We transfected the cells with Tfeb siRNA, and observed slight decrease (not statistically significant, $p=0.59$) in the uptake level of A β in Tfeb siRNA transfected cells compared to scrambled siRNA transfected cells (Fig 2B). The efficacy of TFEB silencing is shown in Fig. 2D. These data further enforced the idea that neither HSPGs nor TFEB alone is responsible for the enhance uptake of A β . Recent

reports suggest that lipoprotein receptors like LDLR and LRP1 also facilitate the internalization of A β in glial cells. Interestingly, there are reports that hepatic expression of LDLR is induced by fenofibrate (FF) by a PPAR α dependent mechanism involving Akt phosphorylation and transcriptional activation of SREBP2 (27,28). Therefore, we further transfected cells with Ldlr siRNA and observed that the effect of GFB-RA on A β uptake is attenuated in absence of LDLR (Fig. 2B). The efficiency of LDLR silencing is evident from Fig. 2C. Also, treatment with GFB, a fibrate, along with RA induced the expression of LDLR in WT astrocytes, but not in PPAR α (-/-) cells (Fig. 2C). Taken together, this set of data, indicates that GFB-RA promotes uptake of A β in astrocytes via LDLR mediated endocytosis.

Example 13 - GFB and RA treatment enhances degradation of A β in mouse primary astrocytes:

[050] We have observed colocalization of A β with the LysoTracker, a lysosomal dye (Fig. 1B). So it is imperative that there will be degradation of A β inside the lysosome, provided there is proper functioning of the organelle. We wanted to observe, whether induction of TFEB (and subsequent induction of lysosomal genes & lysosomal biogenesis) could accelerate the process of degradation A β in the lysosome. We deployed the same in vitro assay for intracellular A β content, but this time, after incubation with A β for 4 hrs, the cells were allowed to grow for different time points (15', 30', 1hr, 2hr, 4hr, 6hr and 8hr) in A β -free media. The normalized A β signal (normalized to Hoechst signal) for DMSO treated cells and GFB-RA treated cells were compared to their respective counterparts which were not allowed to grow in A β free media (termed as "0min wash"). As expected, the basal level of lysosomal processing of A β caused reduction in signal intensity of intracellular A β by ~20% within 6-8hrs compared to 0' wash cells. On the other hand, cells treated with GFB-RA showed an accelerated clearance rate, with a reduction of signal by ~40%

within 6-8hrs (Fig. 3A). The data showed optimal degradation at 6hrs, hence that time point was used for further degradation assays (termed as “6hr wash”). We also visualized reduced puncta of HF-647-A β after 6hrs of wash under the microscope (Fig. 3B). To determine whether the loss of A β signal was due to lysosomal processing, we incubated the cells with Bafilomycin A1 (BafA1) that inhibits lysosomal acidification, thereby reducing its activity. The presence of BafA1 arrested the accelerated loss of A β as observed in GFB-RA treated cells, and rate of A β degradation was almost similar in both DMSO and GFB-RA treated cells, in presence of BafA1 (Fig. 3C). Furthermore, transfection of cells with Tfeb siRNA also attenuated the GFB-RA mediated accelerated lysosomal degradation of A β (Fig. 3D). Collectively, these data indicates that, GFB-RA treatment mediated induction of lysosomal biogenesis could accelerate the process of lysosomal A β degradation.

Example 14 - Role of PPAR α and PPAR β in GFB-RA mediated A β uptake and degradation:

[051] PPAR α plays a key role mediating the transcriptional activation of TFEB and subsequent enhancement in lysosomal biogenesis. We tested the absence of PPAR α and PPAR β affects the regulation of A β uptake and degradation in mouse primary astrocytes. Cells isolated from WT, PPAR α (-/-) and PPAR β (-/-) animals were treated with GFB and RA and further incubated with FAM-A β (1-42) (for in vitro assay) and HF-647-A β along with LysoTracker Red (for microscopy). As before, the A β signals were normalized to Hoechst signal to account for any variability in cell number.

[052] The A β uptake assay, after 4hrs of incubation with A β , showed prominent increase in the A β content (measured by FAM-A β signal intensity) inside the cell, in both WT and PPAR β (-/-) cells, but not in PPAR α (-/-) (Fig. 4A). The signal intensity for all cells was compared to DMSO-treated WT controls. Although there was a slight increase in the levels of A β in PPAR α (-/-) cells

treated with GFB-RA (~20%), it was not significant compared to the ~80% and ~70% increase in GFB-RA treated WT and PPAR β (-/-) cells, respectively (Fig. 4A). In order to assess the role of PPARs in A β degradation, the cells from WT and both knockout animals were treated, incubated with A β and further allowed to grow in A β -free media for 6hrs. The normalized A β signal for GFB-RA treated astrocytes for each of the cell types, either with or without the 6hr wash, were first compared to their respective DMSO-treated controls. Then percentage change of intracellular A β signals in 6hr washed GFB-RA treated cells (previously normalized to their DMSO treated controls) were calculated with respect to 0min washed GFB-RA treated cells (previously normalized to their DMSO-treated controls) for each cell types. We observed ~60% reduction in the levels of A β both in WT and PPAR β (-/-) cells, but only 30-35% loss in signal in case of PPAR α (-/-) (Fig. 4B). Although, as observed earlier, A β was differentially taken up by these three cell types, but comparing the percentage change with respect to A β content pre- and post- 6hr wash (*derivations described in detail in Discussion*), accounted for the variability of A β uptake and provided an absolute measure for A β degradation.

[053] Furthermore, the observations from microscopy, also revealed reduced signal intensity of both A β and LysoTracker in PPAR α (-/-) cells compared to PPAR β and WT cells (Figs. 4C,4D & 4E). This was in agreement with our previous finding that absence of PPAR α abrogates the GFB-RA mediated enhancement of lysosomal proliferation as well as attenuates the expression of LDLR, a key component of A β uptake.

[054] Microscopic analysis also revealed reduced puncta of HF-647-A β in WT and PPAR β (-/-) post 6hr wash, but not a significant change in PPAR α (-/-) cells (Figs. 4C,4D & 4E). Collectively, these data indicate that PPAR α has a dual role - by regulating the expression of LDLR, it could facilitate the uptake A β and by enhancing lysosomal biogenesis via TFEB, it induces accelerated degradation of A β in the lysosomes.

Example 15 - GFB and RA treatment enhanced lysosomal activity and autophagic flux:

[055] The enhancement of lysosomal degradation of A β led us to investigate the markers for lysosomal activity and autophagy. We assessed the GFB-RA treatment on cathepsin B (CtsB) & cathepsin D (CtsD), the two important cathepsins involved in degradation of A β fragments in the lysosomes. The cathepsin activity assay was performed as described in Materials and methods, in cells treated with GFB-RA both in presence and absence of Tfeb siRNA, to determine whether any alteration in the activity due to GFB-RA treatment is mediated via TFEB. Our data indicates an increase in the activity both the cathepsins upon treatment with GFB-RA. Silencing of TFEB by siRNA abrogated the effect of the drugs on cathepsins activity (Fig. 5A & 5B). The protein levels of both cathepsins were found to increase by about 2-3 fold in cells treated with GFB-RA (Fig. 5C & 5D). This is in accordance with the findings that CtsB and CtsD are direct targets of TFEB and enhancement of TFEB activity subsequently induces the levels and activity of cathepsins as well.

[056] It has been reported that deficiency in autophagy or blockage of autophagic pathway, result in abnormal accumulation of A β in autophagic vacuoles inside the cell and is one of the main cause for A β induced neurotoxicity (9). Therefore, we observed the changes in autophagic flux in GFB-RA treated cells, by monitoring the levels of LC3 (LC3-I/LC3-II) and p62/SQSTM1. GFB-RA treatment increased the levels of the LC3-II, the phosphatidylethanolamine conjugated form of LC3-I (Fig. 5E & 5F). The conversion of LC3-I to LC3-II is a hallmark of autophagy induction. We further blocked lysosomal activity by using BafA1 and observed further accumulation of LC3-II (Fig. 5E & 5F). In accordance of previous studies, we also observed

reduced levels of p62 in conditions where there is accumulation of LC3-II, further enforcing the enhancement of autophagic flux (fig 5E & 5F).

[057] Taken together, these data validates our hypothesis, that increase in lysosomal biogenesis via TFEB, mediated by GFB-RA, in a PPAR α -dependant manner could induce the A β uptake and subsequent degradation in the lysosomes by mouse primary astrocytes.

Example 16 – Discussion of Experimental Protocols

[058] The role of lysosomal activity in A β production, uptake and clearance has been well established in the past few years (1, 2, 7, 11, 23). Here, we observed the enhanced lysosomal biogenesis by GFB-RA in A β uptake and degradation by mouse primary astrocytes. An *in vitro* assay using FAM-tagged A β (1-42) was performed in 96-well microplates, allowing for assessment of multiple samples/ treatment at the same time. Only intracellular A β signal from live cells are detected at 485/535nm (Ex./Em.) by quenching the extracellular signal and signal from dead cells by using Trypan blue. Furthermore, normalizing the A β signal with Hoechst 33342 signal from the same well at 360/465nm accounts for the cell number variability.

[059] Depending on the type of experiment, the data can be compared to appropriate controls and represented as fold change or percentage change of the A β uptake/degradation. WT and PPAR β (-/-) cells showed significant increase in A β uptake upon treatment, whereas PPAR α (-/-) cell did not show much increase. The co-localization of HF-647-A β signal and LysoTracker observed under microscope showed that internalized A β indeed ended up in the lysosomes. The reduced intracellular A β signal in presence of Heparin and LDLR siRNA indicated the role of HSPGs and LDLR in micropinocytosis and endocytosis of extracellular A β by astrocytes. Although the link between PPAR α or TFEB activation with micropinocytosis is not fully understood, it

appears that increased lysosomal biogenesis enhances the turnover of pinocytic vesicle, thereby resulting in increased HSPGs mediated uptake of A β .

[060] The role of LDLR in A β uptake and degradation has been well established. Previous studies showed that overexpression of LDLR inhibited A β deposition and enhanced clearance of extracellular A β (29). The effect could be mediated with or without the involvement of Apolipoprotein E (ApoE), one of the strongest genetic risk factors for Alzheimer's disease (27-29). Furthermore, LDLR overexpression has been also been shown to facilitate the rate of brain-to-blood transport of cerebral A β , thereby enhancing clearance of pathologic A β from brain (30). Also, when LDLR is deleted in 5XFAD mouse model of Alzheimer's disease (5XFAD/LDLR^{-/-}), there was evidence of increased amyloid beta deposition and reduced glial inflammatory response, which indicate the role the LDLR in gliosis and A β clearance, independent of ApoE (31). Interestingly, expression of LDLR, another candidate for A β uptake is also regulated via SREBP2 by activation of PPAR α . Fenofibrate (FF), another fibrate that belongs to the same class as gemfibrozil has been shown to upregulate hepatic LDLR expression in a PPAR α - SREBP2 mediated pathway (32). In our study treatment with GFB, which is a well known activator of PPAR α , also increased LDLR expression in WT cells but not in PPAR α (^{-/-}) cells. Also, knockdown of LDLR in astrocytes, attenuated the enhancement of A β uptake. Based on this data, we revealed a novel role of PPAR α (as well as its activator, GFB) in facilitating the uptake of A β in vitro in mouse primary astrocytes in LDLR-dependant manner.

[061] The degradation assay was also performed in similar fashion. Only this time, the cells were allowed to grow in A β -free media for various time points prior to the measurement of signal. Inhibition of lysosomal activity by BafA1 or silencing of TFEB showed reduced degradation of A β , which reinforced the role of lysosome in degradation of A β (1-42). However, when we assayed for A β degradation in WT, PPAR α (^{-/-}) and PPAR β (^{-/-}) cells, the

calculations were a bit more complicated. In this case, we had three different cell types (WT, PPAR α (-/-) and PPAR β (-/-)) which respond differentially to GFB-RA treatment in terms of A β uptake. So, for proper assessment of degradation, the levels of A β , post 6hr wash, had to be compared with the fold change in A β prior to wash (0' wash), individually, for each type of GFB-RA treated cells.

[062] *1st order derivation:*

A β signal normalized to Hoechst signal = A β_{norm} (for all conditions)

[063] *2nd order derivation:*

A β_{norm} (Tx, 0' wash) normalized to A β_{norm} (DMSO, 0' wash) = A β_{fold} (Tx, 0' wash)

A β_{norm} (Tx, 6hr wash) normalized to A β_{norm} (DMSO, 6hr wash) = A β_{fold} (Tx, 6hr wash)

[064] *3rd order derivation:*

{A β_{fold} (Tx, 6hr wash) / A β_{fold} (Tx, 0' wash)} * 100 = % change

[065] This third order derivation of the A β signal allowed us to compare between the net reduction in A β content in the cell compared to the net uptake of A β by the same cells prior to wash.

[066] Finally, the activity of lysosome was measured by monitoring the activity of two of its hydrolases, Cathepsin B and D. CtsB and CtsD are two well-known direct targets of TFEB, so as expected we observed increased activity and levels of the enzymes. In the endosomal-lysosomal pathway of A β production, the beta-amyloid fragments generated by lysosomal hydrolases are subsequently degraded by the cathepsins (CtsB & D) (8). Inhibition of cathepsins cause a rapid buildup of A β fragments, on the other hand it has been shown that increased cathepsin activity results in effective degradation of A β and reduction in A β plaques (33). Also, it has been shown that enhanced autophagy results in lysosomal degradation of A β and protects neurons from A β induced neurotoxicity (34).

[067] Abnormal or improper processing of A β by autophagic process also causes secretion of toxic A β fragments to extracellular space and deficiency in autophagy results in accumulation of A β in lysosomes, thereby causing LMP (13). Therefore, we also checked the alteration in autophagic flux by monitoring the conversion of LC3-I to LC3-II and its associated protein p62. Microtubule associated protein 1 (MAP1) light chain 3 (MAP-LC3 or simply LC3) exists as a free soluble form (LC3-I), which is covalently conjugated to phosphatidylethanolamine (LC3-II) by the enzymatic action of Atg4 (35,36). Signals leading to induction of autophagy trigger the conversion. LC3-II remains bound to the autophagosome membrane and is essential for the *de novo* production of autophagic vacuole (37,38). Monitoring the changes in the levels of LC3-I/II is considered to be a simple and effective way to monitor autophagy induction (10). However, mere increase in the levels of LC3-II does not necessarily indicate complete autophagy. LC3-II itself is degraded in the later stages of autophagic degradation, which makes the interpretation of LC3 immunoblot results more complex.

[068] Therefore, monitoring LC3-I/II levels both in presence of activators and inhibitors of autophagy has been proposed to be a better way to interpret the data (39,40). The increased accumulation of LC3-II under lysosomal inhibitory condition is due to constant increase of autophagic flux, but reduced clearance of LC3-II by lysosomal degradation. On the other hand, another marker for autophagy, p62, also known as sequestosome1 (SQSTM1), which delivers LC3-II to the autophagosome and majority of p62, is degraded in the early stages of autophagosome formation (39-42). The expected negative correlation of p62 and LC3, as observed in our data as well as by other groups, indicated increased autophagic flux in cells treated with GFB-RA.

[069] The role of autophagy in APP processing, A β production and degradation has been extensively studied. The endosomal-lysosomal pathway of APP processing, as discussed earlier, contributes significantly in regulating the generation of pathologic A β fragments (7,9). Because APP is processed in

the lysosomes, disruption of lysosomal function as well as suppression of A β degradation and secretion results in accumulation of intra- and extra-lysosomal A β (43,44). In neuroblastoma cell line, N2a, inhibition of glycogen synthase kinase 3 (GSK3) promotes lysosomal biogenesis and facilitate A β degradation in lysosomes (45). Also, LDLR-related protein 1 (LRP1) mediates A β internalization and degradation in neurons (46,47). A wide array of receptors, like complement receptor 1 (CR1), scavenger receptors (SR-A), CD36, receptor for advanced glycosylation endproducts (RAGE), toll-like receptors (TLRs), transforming growth factor beta1 (TGF-beta1), triggering receptor expressed on myeloid cells 2 (TREM2), etc. has been identified on the surface of microglia that interacts with extracellular A β and induces signalling mechanisms leading to A β uptake and degradation (48-51). The role of astrocytes in A β clearance and degradation is also considered beneficial, as astrocytes internalize ApoE-A β complexes from extracellular space and subsequently degrades them or secretes them in perivascular spaces (52-54). Astrocytes are also capable of degrading A β by enzymatic action of NEP, matrix metalloproteinase-9 (MMP-9), or insulin-degrading enzyme (IDE) (55-60). However, prolonged exposure to pathologic A β , renders astrocytes incapable of handling such huge amount of A β cargo and results in A β accumulation in astrocytes (61,62). In the past few years, enhancement of lysosomal biogenesis has been shown to play a critical role in A β internalization and degradation. Adeno-associated virus (AAV) carrying TFEB gene driven by glial fibrillary acidic protein (GFAP) promoter or CMV-promoter was administered by stereotactic injection into hippocampus of APP/PS1 mice, which are specifically targeted to astrocytes or neurons, respectively. TFEB overexpression leads to induction in lysosomal biogenesis and eventually results in enhanced uptake and clearance of A β from the interstitial fluids by astrocytes and enhanced processing of APP by neurons, that reduces A β production (23,63). These studies underscore the importance of astrocytic clearance of A β in Alzheimer's disease, however, drug mediated enhancement of A β clearance by inducing lysosomal biogenesis has not been well studied so far.

[070] In summary, activation of PPAR α by fibrates leads to enhanced uptake and clearance of A β by mouse primary astrocytes. The outcome of this investigation highlights previously unknown properties of PPAR α , provides a new treatment option for Alzheimer's disease, as well as lysosomal storage disease, and reveals a more dynamic regulation of TFEB.

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[0134] Although the invention has been described and illustrated with reference to specific illustrative embodiments thereof, it is not intended that the invention be limited to those illustrative embodiments. Those skilled in the art will recognize that variations and modifications can be made without departing from the true scope and spirit of the invention as defined by the claims that follow. It is therefore intended to include within the invention all such variations and modifications as fall within the scope of the appended claims and equivalents thereof.

We claim:

1. A method for reducing amyloid- β protein aggregates in the brain of a subject, the method comprising administering to the subject in need of such treatment a composition comprising a therapeutically effective amount of a combination of vitamin A or a derivative thereof and an agonist of proliferator-activated receptor α ("PPAR α ").
2. The method of claim 1, wherein the therapeutically effective amount is an amount that stimulates the uptake of amyloid- β protein by astrocytes.
3. The method of claim 1, wherein the therapeutically effective amount is an amount that stimulates degradation of amyloid- β protein by astrocytes.
4. The method of claim 1, wherein the agonist is an amphipathic carboxylic acid.
5. The method of claim 4, wherein the agonist is selected from the group consisting of clofibrate, gemfibrozil, ciprofibrate, bezafibrate, clinofibrate and fenofibrate.
6. The method of claim 5, wherein the agonist is gemfibrozil.
7. The method of claim 1, therein the composition comprises retinoic acid.

8. The method of claim 1, wherein the composition further comprises at least one pharmaceutically acceptable carrier.
9. The method of claim 1, wherein the composition is administered orally.
10. The method of claim 1, wherein the composition is administered by a route selected from the group consisting of the subcutaneous, intra-articular, intradermal, intravenous, intraperitoneal and intramuscular routes.
11. The method of claim 1, wherein the composition is administered directly to the subject's central nervous system.
12. The method of claim 1, wherein the subject is a subject exhibiting symptoms of Alzheimer's disease.
13. The method of claim 1, wherein the subject is a human subject.
14. A method for reducing amyloid- β protein aggregates in a subject's brain, the method comprising administering a composition comprising a therapeutically effective amount of an agonist of proliferator-activated receptor α ("PPAR α ") to the subject.

15. The method of claim 14, comprising administering the agonist of PPAR α , wherein the agonist is an amphipathic carboxylic acid.
16. The method of claim 15, wherein the agonist is selected from the group consisting of clofibrate, gemfibrozil, ciprofibrate, bezafibrate, clinofibrate and fenofibrate.
17. The method of claim 16, wherein the agonist is gemfibrozil.
18. The method of claim 14, wherein the composition further comprises vitamin A or a derivative thereof.
19. The method of claim 14, wherein the composition further comprises retinoic acid.
20. The method of claim 19, wherein the composition further comprises at least one pharmaceutically acceptable carrier.
21. The method of claim 14, wherein the subject is a human subject.
22. A composition comprising vitamin A or a derivative thereof, an agonist of proliferator-activated receptor α ("PPAR α ") and least one pharmaceutically acceptable carrier.

23. The composition of claim 22, wherein the agonist of proliferator-activated receptor α ("PPAR α ") is gemfibrozil and a vitamin A derivative, wherein the vitamin A derivative is retinoic acid.

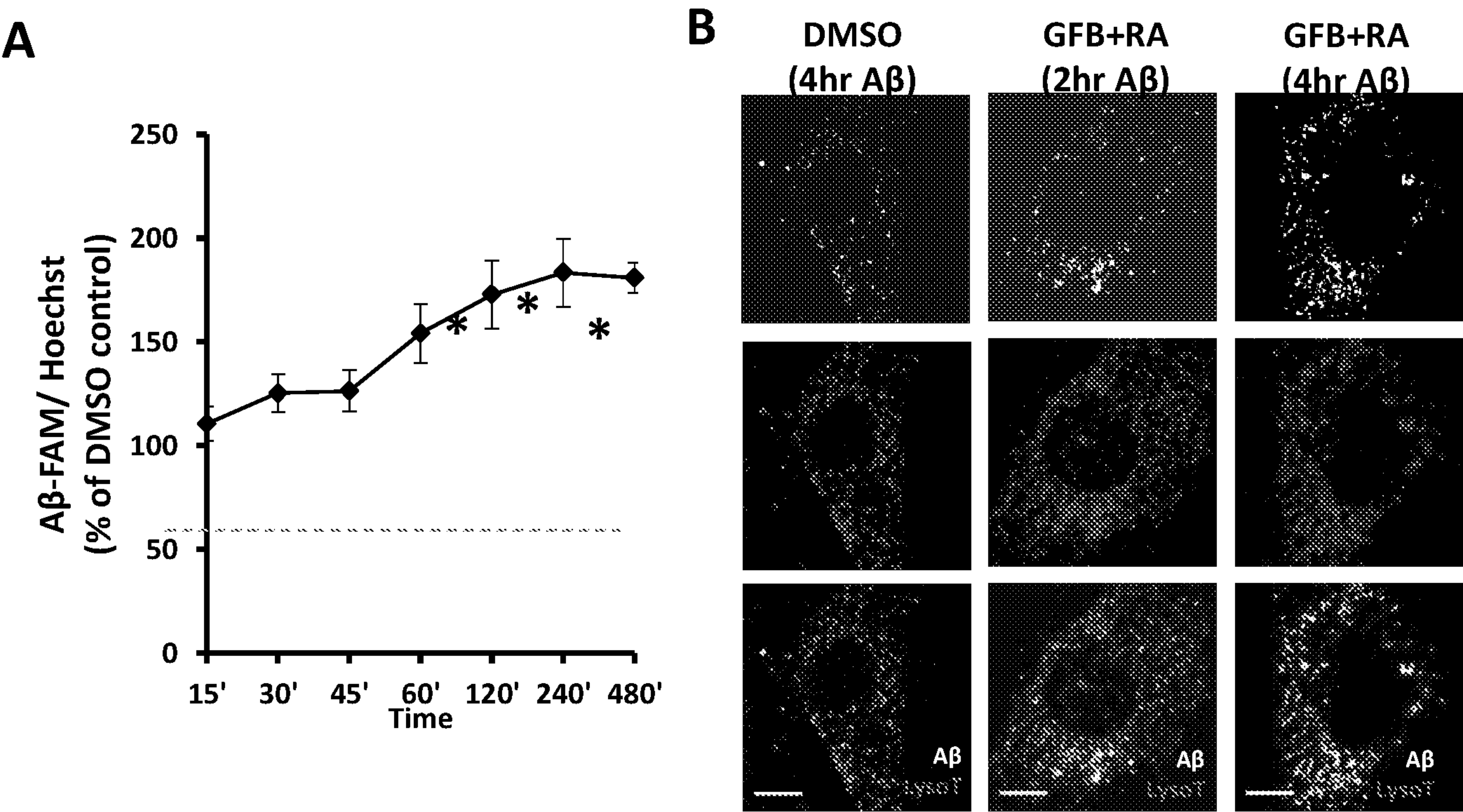


Figure 1(A-B)

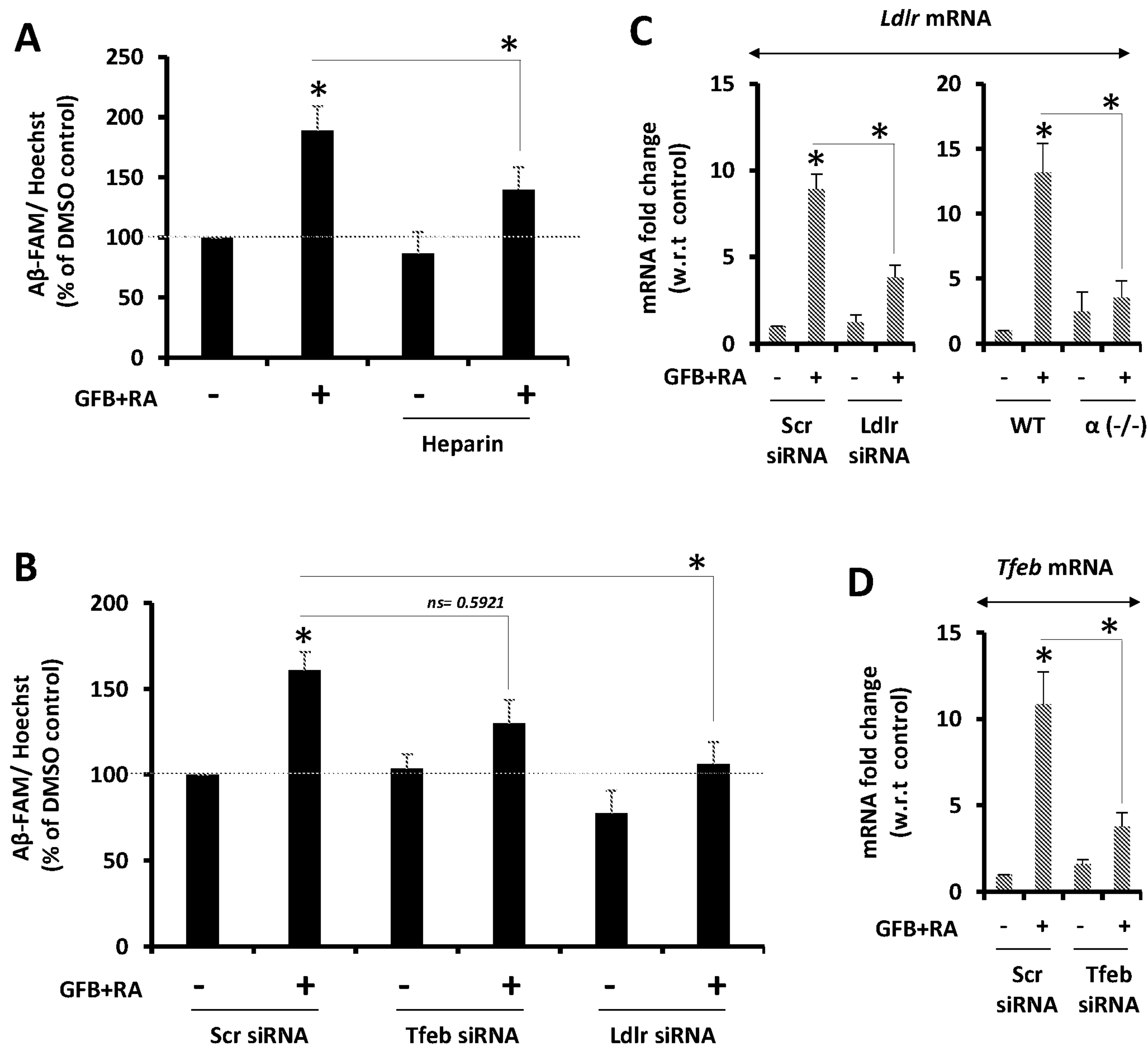


Figure 2(A-D)

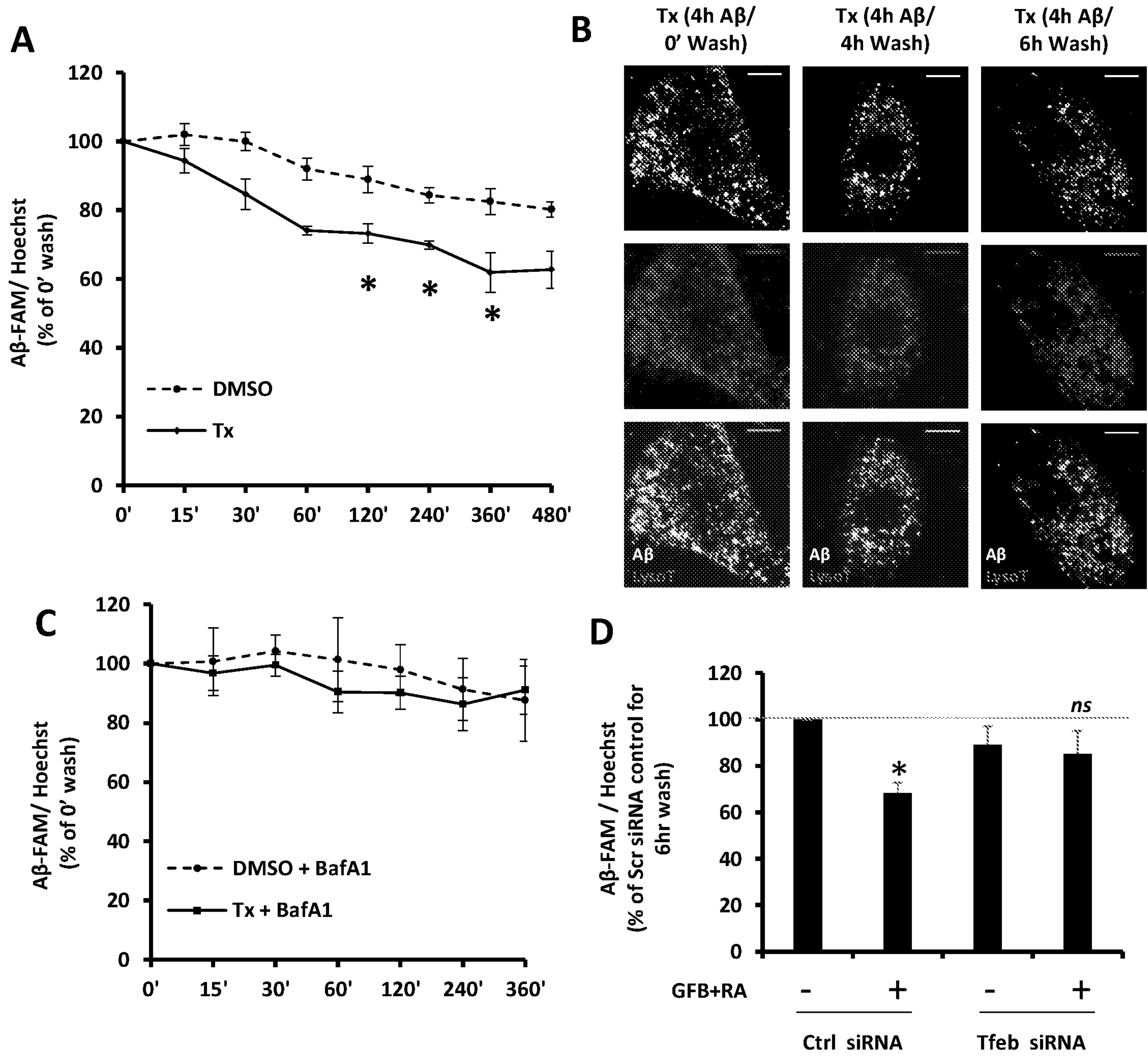


Figure 3(A-D)

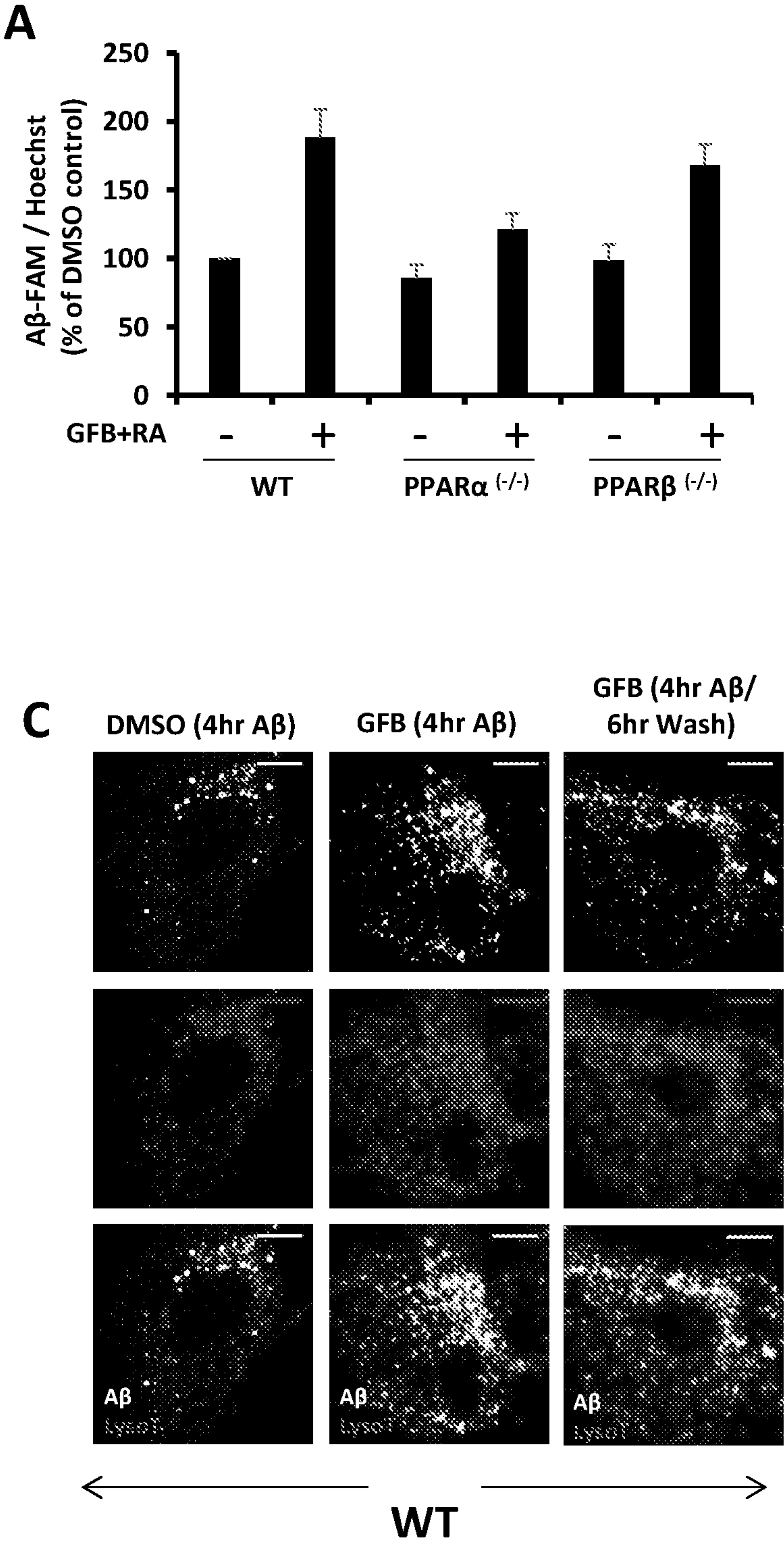
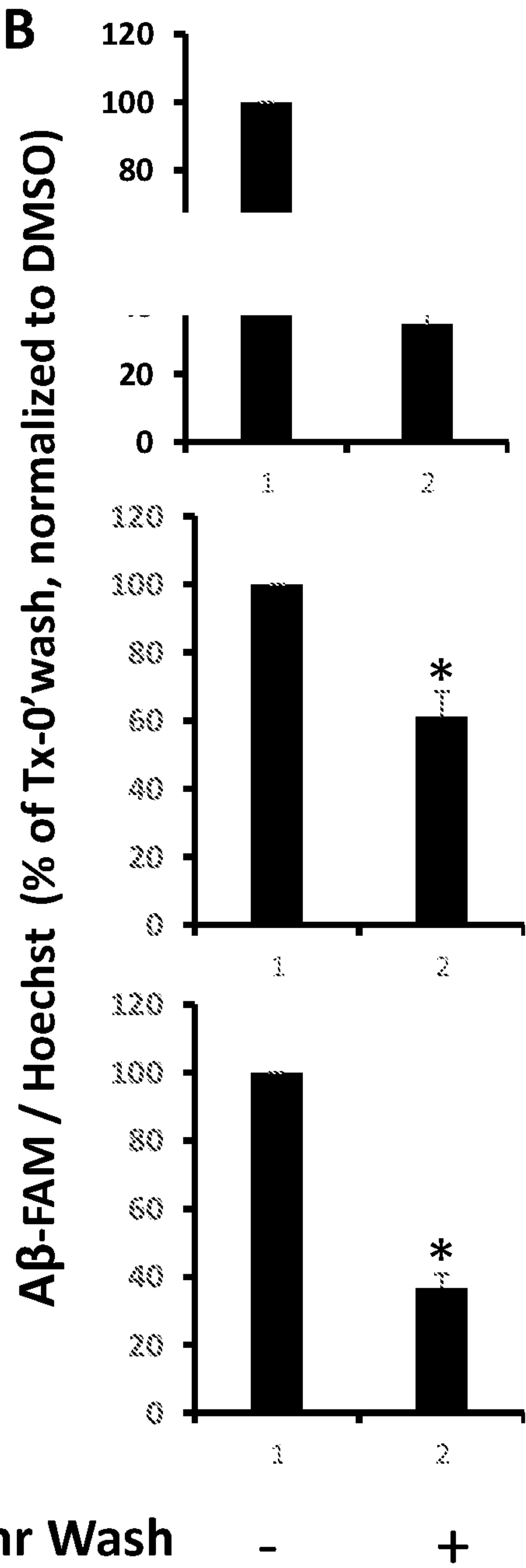


Figure 4(A-C)



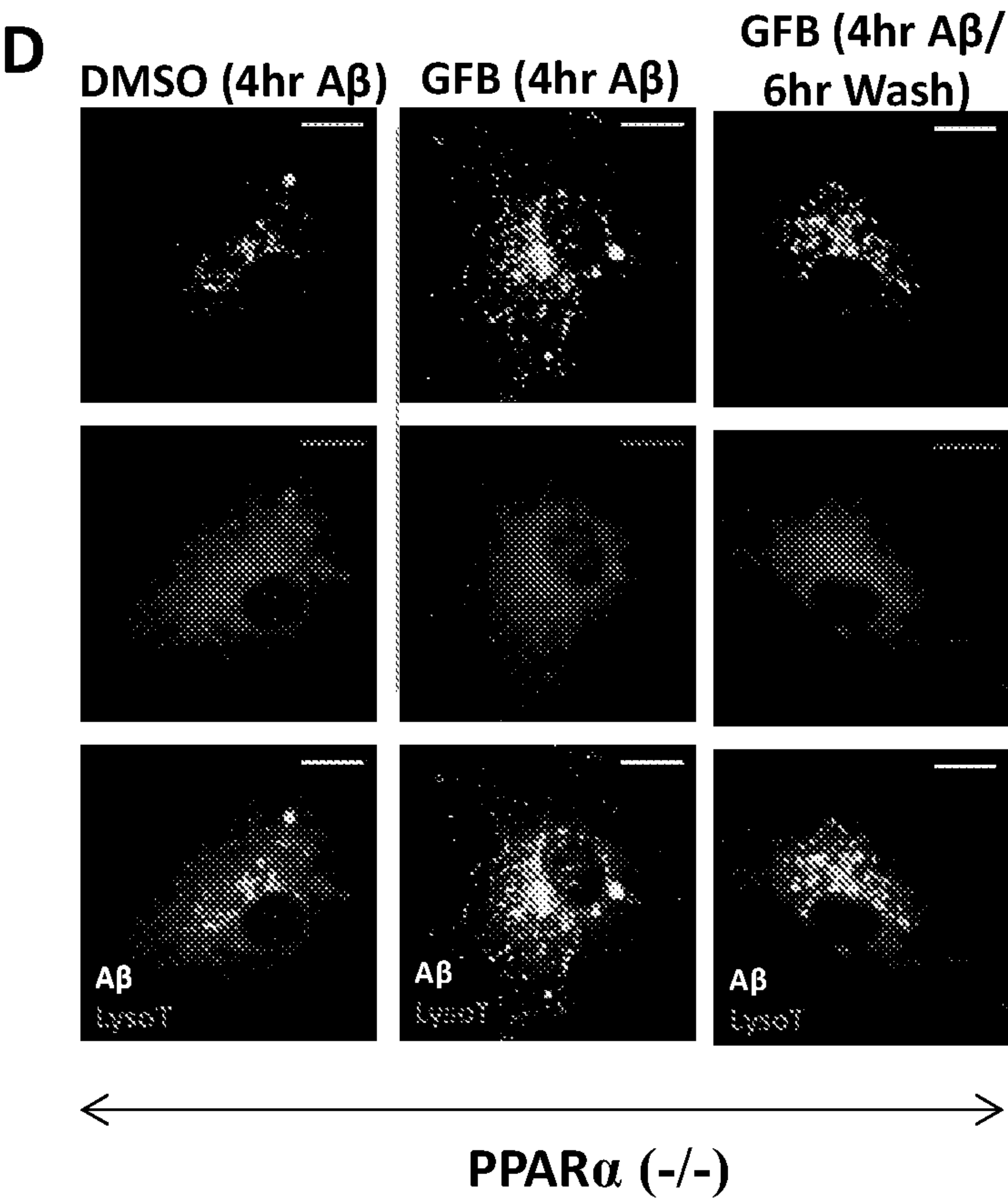
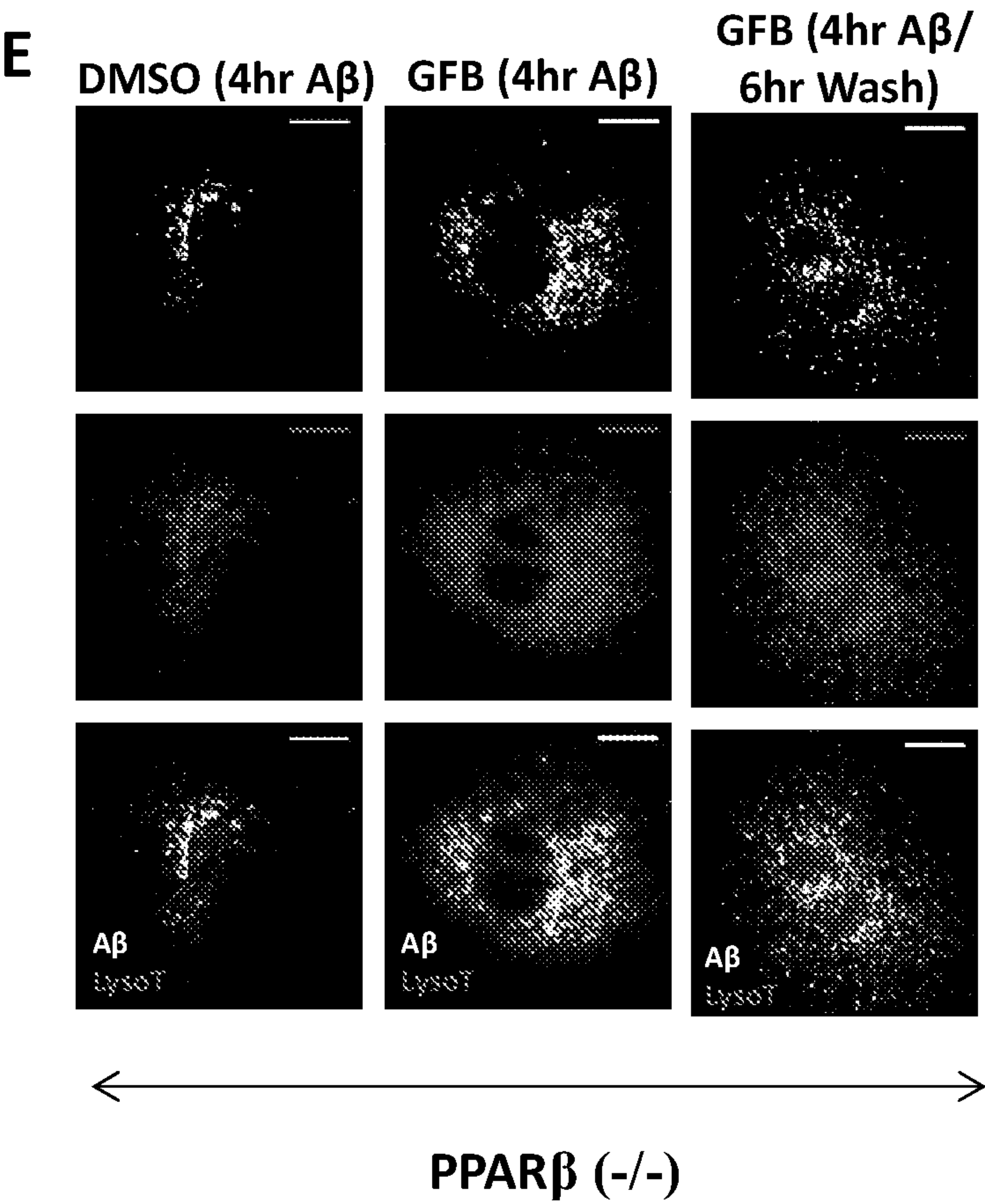


Figure 4(D-E)



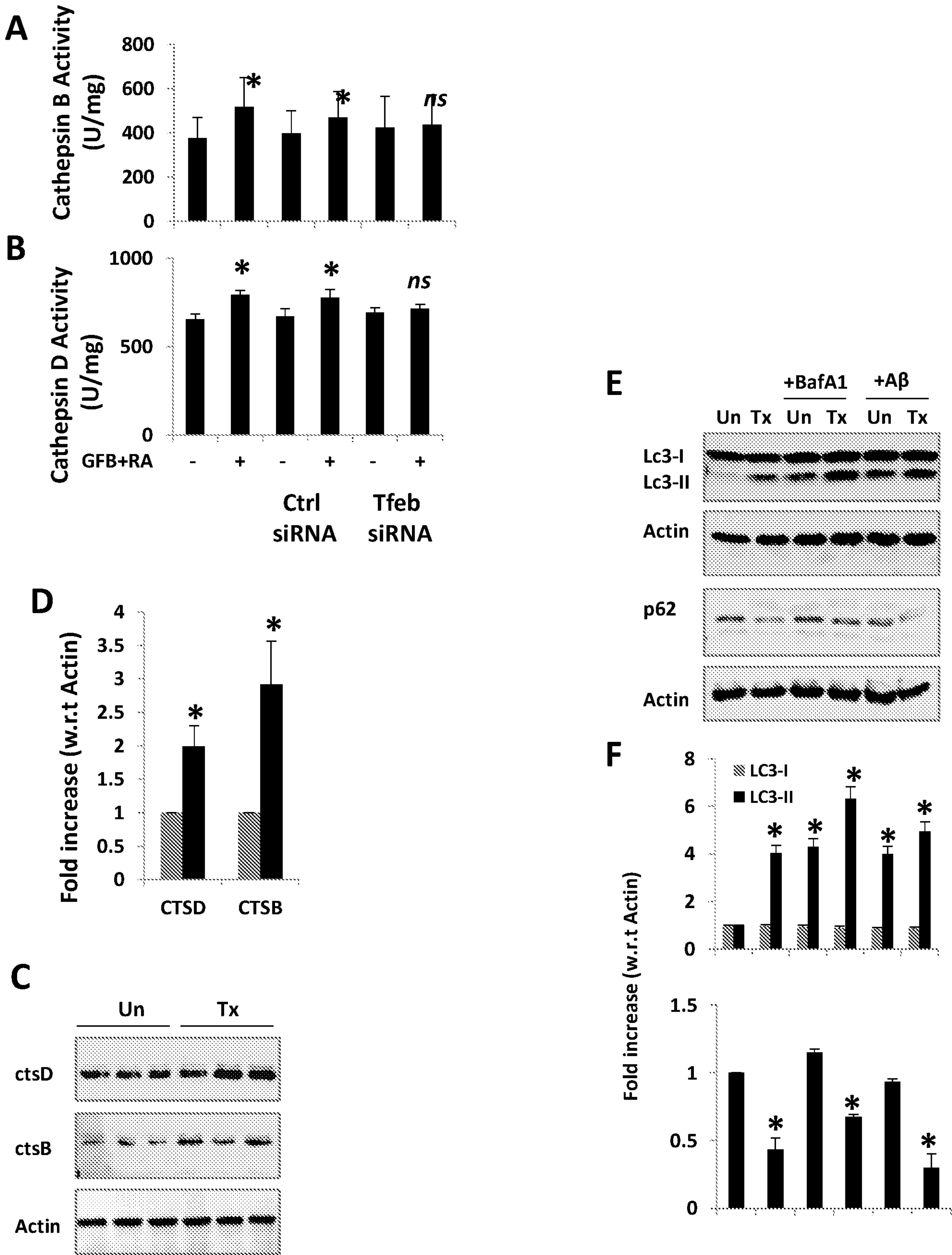
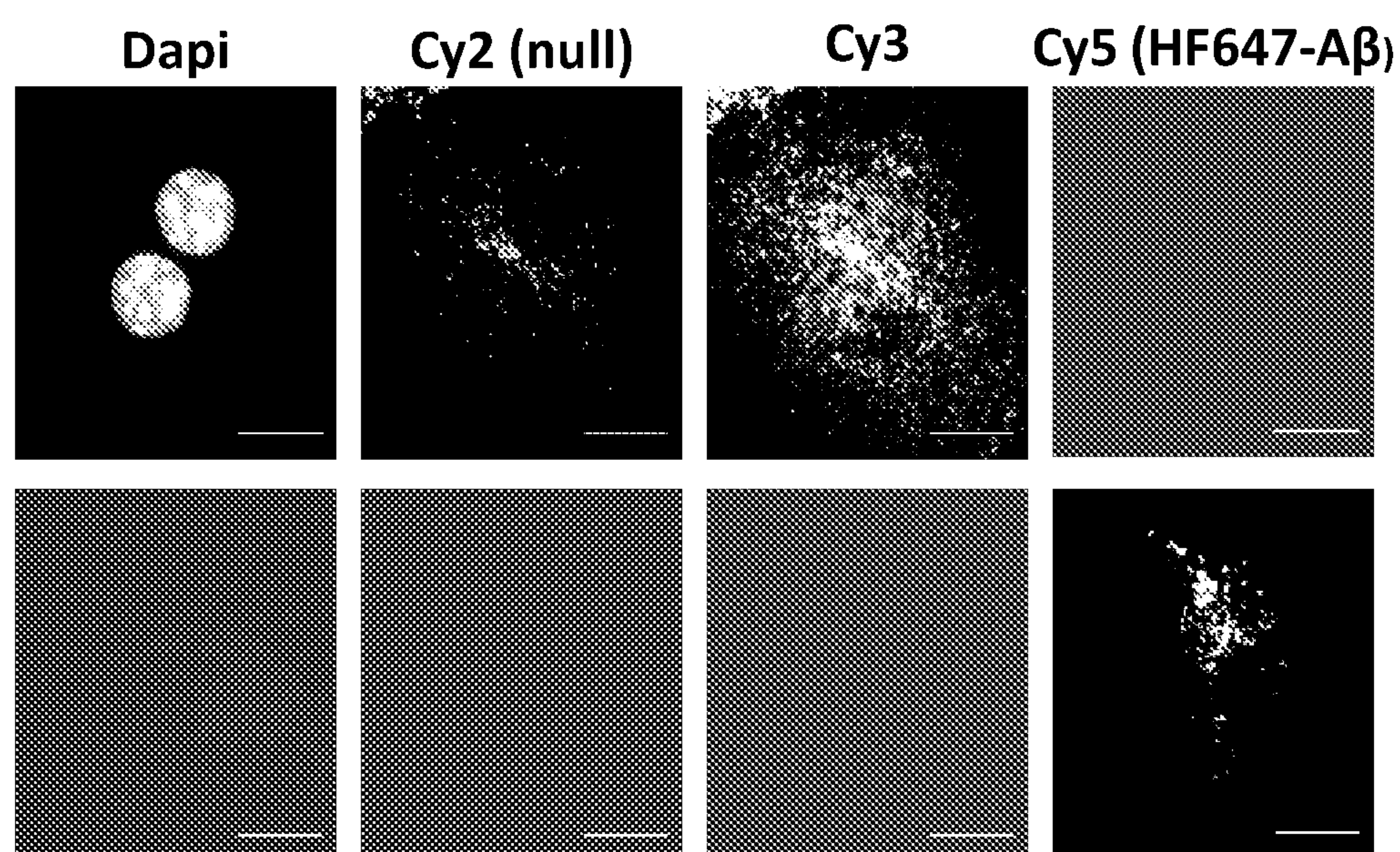


Figure 5(A-F)

**Figure 6**

A

