Table of Contents

Appendix Material and Methods…………………………………………………………………………………2

Appendix Table S1: Sequences printed in Aβ sliding window membranes…………………………6

Appendix Table S2: Sequences printed in PLAK/AD membranes………………………………………7

Appendix Figure S1: Aβ sliding window controls……………………………………………………………8

Appendix Figure S2: Binding of Aβ oligomers in peptide microarrays through 3 different randomizations ……………………………………………………………………………………………………..9

Appendix Figure S3: Normalized ThT kinetics of Aβ in presence and absence of homologue peptides derived from human proteins……………………………………………………………………10

Appendix Figure S4: ThT kinetics of 10μM homologue peptides in absence of Aβ……………11

Appendix Figure S5: TEM images of Aβ fibrils made in presence of homologous peptides…12

Appendix Figure S6: TEM images of peptides in absence of Aβ………………………………………13

Appendix Figure S7: Length distribution of Aβ fibrils in presence or absence of peptides as measured from TEM……………………………………………………………………………………………….14

Appendix Figure S8: Normalized pFTAA fluorescence spectrum of rAβ1-42 fibrils made in presence or absence of homologue peptides………………………………………………………………15

Appendix Figure S9: Normalized Curcumin fluorescence spectrum of rAβ1-42 fibrils made in presence and absence of homologues peptides……………………………………………………………16

Appendix Figure S10: Inducing Aβ aggregation by Aβ reverse seeds…………………………………17

Appendix Figure S11: FRAP of aggregates induced by constructs………………………………………18

Appendix Figure S12: Effect of expression of construct 9 in aggregation of Aβ…………………..19

Appendix Figure S13: Effect of expression of Aβ aggregation inducing constructs in other biosensor cell lines…………………………………………………………………………………………………….20

Appendix Material and Methods.

Staining of Aβ sliding window membranes with Aβ antibodies

The membranes were blocked overnight in 1% BSA in PBS-T, then were incubated with Anti-β-amyloid 6E10 (Biolegend, 803001) or 4G8 (Biolegend, 800701) or 12F4 (Biolegend, 805509) in 1% BSA in PBS-T for 1h. Membranes were washed three times and were incubated with anti-mouse HRP (Promega). Finally, membranes were washed in PBS-T for three times 5 min and developed through chemiluminescence using a ChemiDoc XRS (Bio-Rad).

Seeding and transfection assay of Sup35NM and Tau biosensor cell line

The N2A NM-GFP (Hofmann *et al*, 2013) or Tau RD P301S FRET Biosensor (ATCC-CRL-3275) (Sanders *et al*, 2014) cell line was cultured in DMEM medium, supplemented with 10% FBS at 37°C, and a 5% CO_2 atmosphere.

Briefly, the assay was performed in 96-well plate (PerkinElmer), previously coated for 30' with poly-L-lysine at 37°C and washed three times with PBS. Adhered cells were passed twice through a 22G needle and plated at 15.000 cells/well and 5h later were transfected with 100ng of DNA per well, using Lipofectamine 3000 (Invitrogen) according to the manufacturer. GFP+seeds was transfected with 100ng of DNA and after 17h with 100nM sup35NM seeds or Tau seeds.

After 41h of DNA transfection, the cells were fixed with 4% formaldehyde in PBS for 10 minutes. Cells were washed with PBS, block and permeabilize with 1%BSA, 0.2% TritonX-100 in PBS for 1hour. Cells were stained with 1:1000 HA-antibody (C29F4) Rabbit mAb (Cell signaling 3724) in 1%BSA in PBS for 1h. Cells were washed and stained with secondary Alexa Fluor 647 goat anti-rabbit (ThermoFisher A-21245) in 1%BSA in PBS for 1h. Cells were nuclei stained with 1:5000 DAPI (ThermoFisher D1306) in 1%BSA in PBS for 1h. Cells were washed and plates were imaged using Operetta CLS. For NM-GFP cells: For each well 17 fields were imaged by using the channels Digital Phase Contrast, Alexa647 (Ex:615-645, Em:655-760), EGFP (Ex:460-490, Em: 500-550), DAPI (Ex:355-385, Em: 430-500). The images were analyzed by Operetta CLS. Nuclei was detected with DAPI, Cytoplasm with Digital Phase Contrast. Spots (GFP) measured on ROIs: Nuclei and Cell. For Tau biosensor: For each well 17 fields were imaged by using the channels Digital Phase Contrast, Alexa647 (Ex:615-645, Em:655-760), DAPI (Ex:355-385, Em: 430-500), Tau YFP (Ex: 490-515, Em: 525-580). The images were

analyzed by Operetta CLS. Nuclei was detected with DAPI, Cytoplasm with Digital Phase Contrast. Spots (YFP) measured on ROIs: Nuclei and Cell. Alexa647 intensity was measured for each cell identified. The baseline Alex647 was calculated in PBS treated cells. Every cell with higher fluorescence was identified as transfected with our plasmids. The number of spots were identified in cells with and without Alexa647 fluorescence. Number of spots per cell was calculated from Number of spots/ number of cells for Alexa647 positive and negative cells. Statistical significance was calculated using Ordinary one-way ANOVA with Dunett multiple comparison correction. GraphPad was used for statistics and graphs.

Transfection with amyloidogenic proteins of biosensor Aβ1-42 cell line

The biosensor mcherry-Aβ1-42 HEK293T cell line was cultured in DMEM medium, supplemented with 10% FBS at 37ºC, and a 5% CO_2 atmosphere.

Briefly, the assay was performed in 96-well plate (PerkinElmer), previously coated for 30' with poly-L-lysine at 37°C and washed three times with PBS. Adhered cells were passed twice through a 22G needle and plated at 15.000 cells/well and 5h later were transfected with 100ng of DNA per well, using Lipofectamine 3000 (Invitrogen) according to the manufacturer. Plasmids for 6,9,10 and Tau were produced from Twist Bioscience. pcDNA6 asyn WT was a gift from Hilal Lashuel (Addgene plasmid # 107425 ; http://n2t.net/addgene:107425 ; RRID:Addgene_107425). Generation of pCDNA4-SOD1-Myc-His6 A4V was described before (Claes *et al*, 2019).

After 41h of DNA transfection, the cells were fixed with 4% formaldehyde in PBS for 10 minutes. Cells were washed with PBS, block and permeabilize with 1%BSA, 0.2% TritonX-100 in PBS for 1hour. Cells were stained with 1:1000 HA-antibody (C29F4) Rabbit mAb (Cell signaling 3724) for staining 6,9,10 constructs, 1:1000 tau antibody (Agilent, A002401-2) for tau construct, α-synuclein antibody (14H2L1, Thermo 701085) for α-synuclein, c-Myc monoclonal antibody (9E10, ThermoFisher 13-2500) for SOD1 construct in 1%BSA in PBS for 1h. Cells were washed and stained with secondary Alexa Fluor 647 goat anti-rabbit (ThermoFisher A-21245) or Alexa Fluor 647 goat anti-mouse (ThermoFisher A-21235) in 1%BSA in PBS for 1h. Cells were nuclei stained with 1:5000 Dapi (ThermoFisher D1306) in 1%BSA in PBS for 1h. Cells were washed and plates were imaged using Operetta CLS. For transfected cells: For each well 17 fields were imaged by using the channels Digital Phase Contrast, mCherry (Ex:530-560, Em:570-650), DRAQ7 (Ex:615-645, Em: 655-705), Alexa647

(Ex:615-645, Em:655-760). The images were analyzed by Operetta CLS. Nuclei was detected with DRAQ7, Cytoplasm with Digital Phase Contrast. Spots measured on ROIs: Nuclei and Cell. Alexa647 intensity was measured for each cell identified. The baseline Alexa647 was calculated in PBS treated cells. Every cell with higher fluorescence was identified as transfected with our plasmids. The number of spots were identified in cells with and without Alexa647 fluorescence. Number of spots per cell was calculated from Number of spots/ number of cells for Alexa647 positive and negative cells. Statistical significance was calculated using Ordinary one-way ANOVA with Dunett multiple comparison correction and unpaired t-test for comparison between transfected/nontransfected cells. GraphPad was used for statistics and graphs.

Subcellular localization

For the list of plaque proteins identified by Xiong et al(Xiong *et al*, 2019a, b), we retrieved subcellular location information from Uniprot (UniProt, 2008). Specifically, we used the information listed in Uniprot's "Subcellular location [CC]" feature. The subcellular location annotations were then filtered to only retain the location terms in Uniprot's standardized subcellular location vocabulary. To reduce complexity and increase interpretability of the annotations, subcellular location terms were grouped thusly:

Cytoplasmic proteins:

Cytoplasmic vesicle, Centrosome, T-tubule, Cell projection, Preautophagosomal structure, Microsome, Endomembrane system, cis-Golgi network, Cytolytic granule, Lysosome lumen, Rough endoplasmic reticulum, Melanosome, trans-Golgi network, Lysosome membrane, Cytoplasmic vesicle membrane, Midbody ring, Autolysosome, Lysosome, Endoplasmic reticulum membrane, Nucleoplasm, trans-Golgi network membrane, Midbody, Vacuole, Nucleus speckle, Cajal body, Cytoplasmic granule, Nucleus membrane, COPII-coated vesicle, Cleavage furrow, Zymogen granule, Endoplasmic reticulum-Golgi intermediate compartment, Nucleus, Early endosome membrane, Endoplasmic reticulum, Cortical granule, Perikaryon, Lipid droplet, Nucleus matrix, Recycling endosome, Cytoplasm, Mitochondrion, PML body, Sarcoplasm, Late endosome, Z line, P-body, Photoreceptor inner segment, Mitochondrion matrix, Mitochondrion outer membrane, Stress granule, Golgi stack, Golgi outpost, Golgi apparatus, Early endosome, Chromosome, Endosome, Endosome membrane, Cytosol

Membrane proteins:

Membrane raft, Tight junction, Cell membrane, Cell surface, Cell junction, Apical cell membrane, Basolateral cell membrane, Lateral cell membrane, Membrane

Secreted/extracellular proteins:

Secreted, Postsynaptic density

Proteins were then grouped into one of 7 categories, consisting of the combinations between the three classifications above: Strictly cytoplasmic, strictly membrane, strictly extracellular, cytoplasmic and membrane, cytoplasmic and extracellular, membrane and extracellular, and cytoplasmic, membrane and extracellular combined. For each of these groups, the proportions in the list of proteins identified by Xiong et al were calculated.

Claes F, Rudyak S, Laird AS, Louros N, Beerten J, Debulpaep M, Michiels E, van der Kant R, Van Durme J, De Baets G *et al* (2019) Exposure of a cryptic Hsp70 binding site determines the cytotoxicity of the ALS-associated SOD1-mutant A4V. *Protein Eng Des Sel* 32: 443-457

Hofmann JP, Denner P, Nussbaum-Krammer C, Kuhn PH, Suhre MH, Scheibel T, Lichtenthaler SF, Schatzl HM, Bano D, Vorberg IM (2013) Cell-to-cell propagation of infectious cytosolic protein aggregates. *Proc Natl Acad Sci U S A* 110: 5951-5956

Sanders DW, Kaufman SK, DeVos SL, Sharma AM, Mirbaha H, Li A, Barker SJ, Foley AC, Thorpe JR, Serpell LC *et al* (2014) Distinct tau prion strains propagate in cells and mice and define different tauopathies. *Neuron* 82: 1271-1288

UniProt C (2008) The universal protein resource (UniProt). *Nucleic Acids Res* 36: D190-195

Xiong F, Ge W, Ma C (2019a) Quantitative proteomics reveals distinct composition of amyloid plaques in Alzheimer's disease. *Alzheimers Dement* 15: 429-440

Xiong F, Ge W, Ma C (2019b) Quantitative proteomics reveals distinct composition of amyloid plaques in Alzheimer's disease. [DATASET]. *Alzheimers Dement* 15: 429-440

Appendix Table S1

Row	Column	Name	Sequence
A	1	>abeta1	DAEFRHDSGYEVGGS
A	2	>abeta2	AEFRHDSGYEVHGGS
A	3	>abeta3	EFRHDSGYEVHHGGS
A	4	>abeta4	FRHDSGYEVHHQGGS
A	5	>abeta5	RHDSGYEVHHQKGGS
A	6	>abeta6	HDSGYEVHHQKLGGS
A	7	>abeta7	DSGYEVHHQKLVGGS
A	8	>abeta8	SGYEVHHQKLVFGGS
A	9	>abeta9	GYEVHHQKLVFFGGS
A	10	>abeta10	YEVHHQKLVFFAGGS
B	1	>abeta11	EVHHQKLVFFAEGGS
B	2	>abeta12	VHHQKLVFFAEDGGS
B	3	>abeta13	HHQKLVFFAEDVGGS
B	4	>abeta14	HQKLVFFAEDVGGGS
B	5	>abeta15	QKLVFFAEDVGSGGS
B	6	>abeta16	KLVFFAEDVGSNGGS
B	7	>abeta17	LVFFAEDVGSNKGGS
B	8	>abeta18	VFFAEDVGSNKGGGS
B	9	>abeta19	FFAEDVGSNKGAGGS
B	10	>abeta20	FAEDVGSNKGAIGGS
C	1	>abeta21	AEDVGSNKGAIIGGS
C	2	>abeta22	EDVGSNKGAIIGGGS
C	3	>abeta23	DVGSNKGAIIGLGGS
C	4	>abeta24	VGSNKGAIIGLMGGS
C	5	>abeta25	GSNKGAIIGLMVGGS
C	6	>abeta26	SNKGAIIGLMVGGGS
C	7	>abeta27	NKGAIIGLMVGGGGS
C	8	>abeta28	KGAIIGLMVGGVGGS
C	9	>abeta29	GAIIGLMVGGVVGGS
C	10	>abeta30	AIIGLMVGGVVIGGS
D	1	>abeta31	IIGLMVGGVVIAGGS
D	2	>abeta12_F9P	VHHQKLVFPAEDGGS
D	3	>abeta15_F6P	QKLVFPAEDVGSGGS
D	4	>abeta24_I9P	VGSNKGAIPGLMGGS
D	5	>abeta26_I7P	SNKGAIPGLMVGGGS
D	6	>abeta11_scrambled	VKEFHEQALFHVGGS
D	7	>abeta14_scrambled	FGDLEVQAKVHFGGS
D	8	>abeta25_scrambled	IKGALNVSIGMGGGS
D	9	>abeta27_scrambled	INIGKLGGAVMGGGS
D	10	>abeta29_scrambled	IVGAGMGGLVVIGGS

Appendix Table S1: Sequences printed in Aβ sliding window membranes

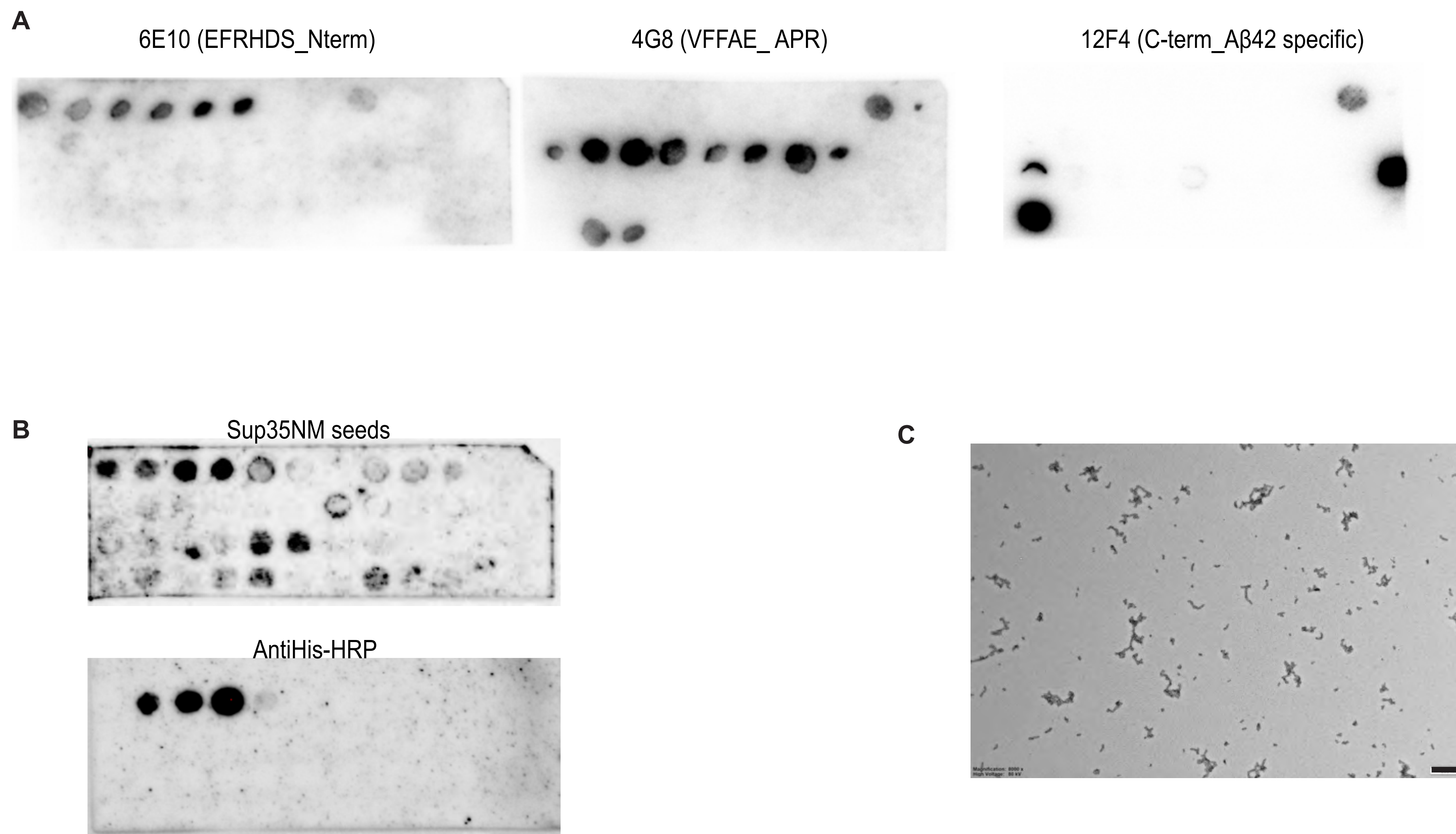
Appendix Table S2

PLAK			
Row	Column	Name	Sequence
A	1	O00322-1;O00322-2	SGLSLFAETIWGGS
A	2	Q92985-2;Q92985-4	DFRVFFQELVEGGS
A	3	random121	TSSQRFCDSQDAGGS
A	4	random405	ERSQQQEPLVCAGGS
A	5	Q86X55-2;A6NN38	SGILSFFAAQAGGS
A	6	random185	EHPQSCTLSAEEGGS
A	7	random457	ESLKKELDTDRPGGS
A	8	random258	ERSYGCGECGKSGGS
A	9	O00322-1;O00322-2	SGLSLFAETIWGGS
A	10	random240	CLPKTQEQCQAKGGS
A	11	random84	TSRPPENSASAQGGS
A	12	P16144-1;P16144-2;P16144-5;B4E3N0	HLLVFSTESAFGGS
A	13	random165	QGGQVDCGEFQDGGS
A	14	O60412	FLLLGFAEDSDGGS
A	15	random505	EENNRKIAEAQAGGS
B	1	Q86WR7-1;A6NDS2	DVLLFFEETIDGGS
B	2	random114	STQKCSGQLSQEGGS
B	3	Q9BY11	KRLVFLKEVLLGGS
B	4	random124	QAPTAARSEGDCGGS
B	5	Q92581;A8K160	ELLNFLAENFIGGS
B	6	random203	KLYPESQGSDTAGGS
B	7	random448	EQQEGQVKHLEKGGS
B	8	Q68DE7	LPLTFFTELEKGGS
B	9	random224	GMDLINRETVHEGGS
B	10	Q8TCJ0-1;B4DYA3	EWKLMYFALQKGGS
B	11	random211	LQEQKDSQCLHVGGS
B	12	O95273-1	LRKLVRGATLDGGS
B	13	O15354	LPLVIFHELTKGGS
B	14	random126	DSEAAINRQINLGGS
B	15	random451	EDAEGRLMEARKGGS
C	1	P10323	DWRLVFGAKEIGGS
C	2	Q6ZNZ2	LLKLLFFNESPGGS
C	3	random218	SREDTICLLQNEGGS
C	4	random416	EQEELRRELAKVGGS
C	5	O75110-1;O75110-2	ASLVFLHEFIDGGS
C	6	random406	PPTTPQNEEIRAGGS
C	7	random424	DQLREQRKTLEQGGS
C	8	O60412	FLLLGFAEDSDGGS
C	9	P16144-1;P16144-2;P16144-5;B4E3N0	HLLVFSTESAFGGS
C	10	O95273-1	LRKLVRGATLDGGS
C	11	random225	MSYCEEHARSDPGGS
C	12	Q8IZU8	TLKLHFFQEVLGGS
C	13	Q68DE7	LPLTFFTELEKGGS
C	14	random113	STGSTYGSSQKEGGS
C	15	random418	LREEKVSGDRKPGGS
D	1	B4DWA5;B4DWN0	ILLRLFAEDGGS
D	2	random125	DQANCRWAATEQGGS
D	3	random447	QSEEKRGITAREGGS
D	4	random426	EMWRQEEKIREQGGS
D	5	O95497	KGTVFFDEFTFGGS
D	6	Q96QZ0	HLDVFFQEEFSGGS
D	7	random374	ITEQQEVAQQISGGS
D	8	Q8IZU8	TLKLHFFQEVLGGS
D	9	random427	ATCKDEKGKQEMGGS
D	10	P23786	AVLRFFNEVFKGGS
D	11	random417	EAERLQVEKERLGGS
D	12	P23786	AVLRFFNEVFKGGS
D	13	random	REAQAREVRCREGGS
D	14	Q8TCJ0-1;B4DYA3	EWKLMYFALQKGGS
D	15	random446	ERLTQQQDIRKDGGS
E	1	random495	ESELGRQKAENNGGS
E	2	random438	SEEERAKAKHLAGGS
E	3	random519	AQARQAEKEQQHGGS
E	4	random466	LNQQLEKRKEMEGGS
E	5	random500	ESQDHTPGQKREGGS
E	6	random494	QLQQQKNKEMEQGGS
E	7	random480	EQEQRQALEQARGGS
E	8	random423	KCEECEKSFKQRGGS
E	9	random508	IPEKDMDERRRLGGS
E	10	random439	EVARRKLQEIEDGGS
E	11	random454	KAKIGRCETEERGGS
E	12	random441	GRDGSEKKIRECGGS
E	13	random515	VSENLRKEMEQKGGS
E	14	random455	EKVKEQLEAAKPGGS
E	15	random501	ADAAEKIRKENPGGS

AD			
Row	Column	Name	Sequence
A	1	random426	EMWRQEEKIREQGGS
A	2	random446	ERLTQQQDIRKDGGS
A	3	random427	ATCKDEKGKQEMGGS
A	4	random457	ESLKKELDTDRPGGS
A	5	random505	EENNRKIAEAQAGGS
A	6	random121	TSSQRFCDSQDAGGS
A	7	random417	EAERLQVEKERLGGS
A	8	random424	DQLREQRKTLEQGGS
A	9	random418	LREEKVSGDRKPGGS
B	1	random495	ESELGRQKAENNGGS
B	2	random500	ESQDHTPGQKREGGS
B	3	O15354	LPLVIFHELTKGGS
B	4	random494	QLQQQKNKEMEQGGS
B	5	random	REAQAREVRCREGGS
B	6	O00322-1;O00322-2	SGLSLFAETIWGGS
B	7	Q92985-2;Q92985-4	DFRVFFQELVEGGS
B	8	random405	ERSQQQEPLVCAGGS
B	9	random423	KCEECEKSFKQRGGS
C	1	random439	EVARRKLQEIEDGGS
C	2	Q68DE7	LPLTFFTELEKGGS
C	3	random185	EHPQSCTLSAEEGGS
C	4	Q8TCJ0-1;B4DYA3	EWKLMYFALQKGGS
C	5	random258	ERSYGCGECGKSGGS
C	6	Q92581;A8K160	ELLNFLAENFIGGS
C	7	random240	CLPKTQEQCQAKGGS
C	8	Q9BY11	KRLVFLKEVLLGGS
C	9	random84	TSRPPENSASAQGGS
D	1	random165	QGGQVDCGEFQDGGS
D	2	O60412	FLLLGFAEDSDGGS
D	3	P16144-1;P16144-2;P16144-5;B4E3N0	HLLVFSTESAFGGS
D	4	O95273-1	LRKLVRGATLDGGS
D	5	random114	STQKCSGQLSQEGGS
D	6	P10323	DWRLVFGAKEIGGS
D	7	random124	QAPTAARSEGDCGGS
D	8	Q96QZ0	HLDVFFQEEFSGGS
D	9	random203	KLYPESQGSDTAGGS
E	1	random224	GMDLINRETVHEGGS
E	2	Q8IZU8	TLKLHFFQEVLGGS
E	3	random211	LQEQKDSQCLHVGGS
E	4	P23786	AVLRFFNEVFKGGS
E	5	random416	EQEELRRELAKVGGS
E	6	B4DWA5;B4DWN0	ILLRLFAEDGGS
E	7	Q6ZNZ2	LLKLLFFNESPGGS
E	8	random447	QSEEKRGITAREGGS
E	9	random441	GRDGSEKKIRECGGS

Appendix Table S2: Sequences printed in PLAK/AD membranes
Homologue peptides and unrelated peptides to Aβ KLVFFA and LVFFAE that printed in PLAK/AD membranes

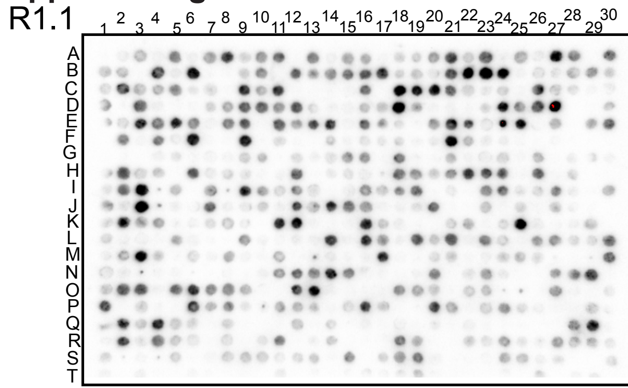
Appendix Figure S1

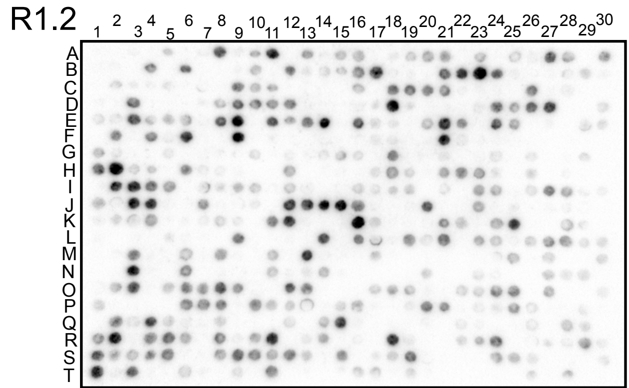


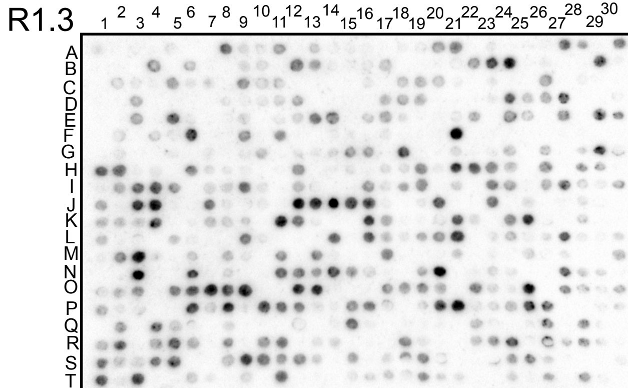
Appendix Figure S1. Aβ sliding window Controls

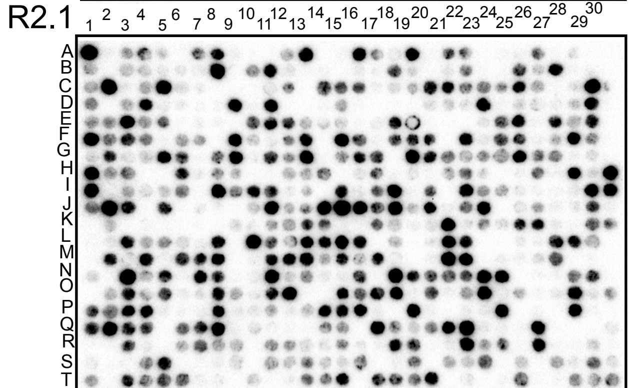
A.Staining of the membranes with Aβ antibodies (6E10, 4G8, 12F4) confirms the presence of Aβ sequence on the membranes. 6E10 recognizes EFRHDS sequence, 4G8 the VFFAE and 12F4 the c-terminus of Aβ1-42.
B.Incubation of Aβ sliding windows with Sup35NM seeds (top membrane). High signal in N-terminal may come from His-HRP antibody used for detection. Bottom membrane shows binding of His-HRP in the membrane in absence of Sup35NM seeds.
C. TEM image of Sup35NM seeds. Scale bar: 500nm

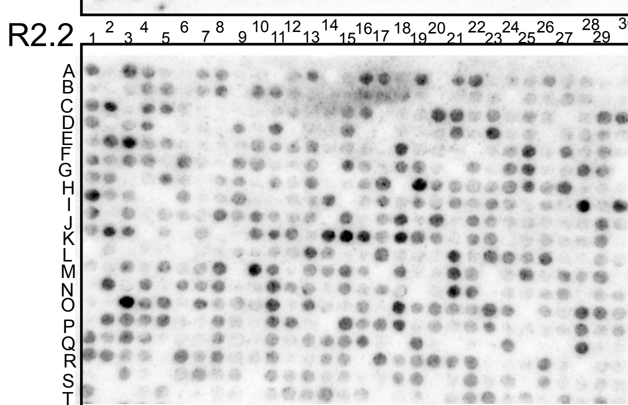
Appendix Figure S2

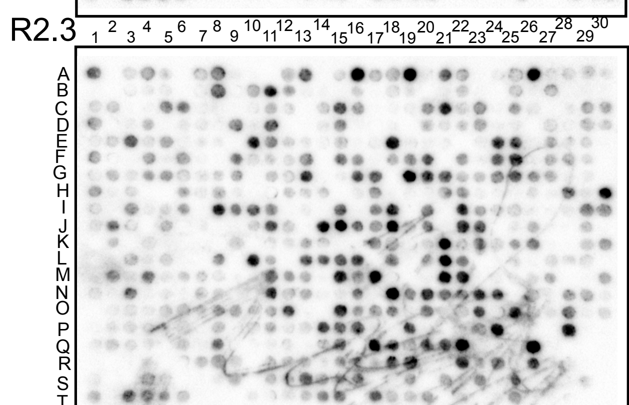


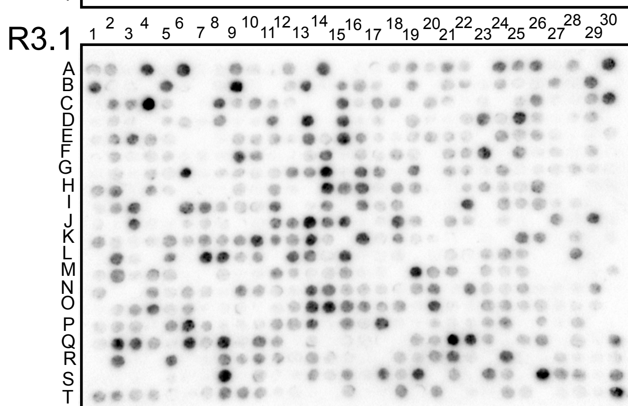


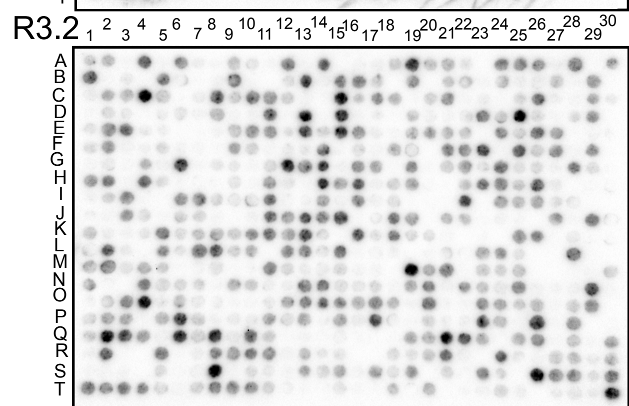






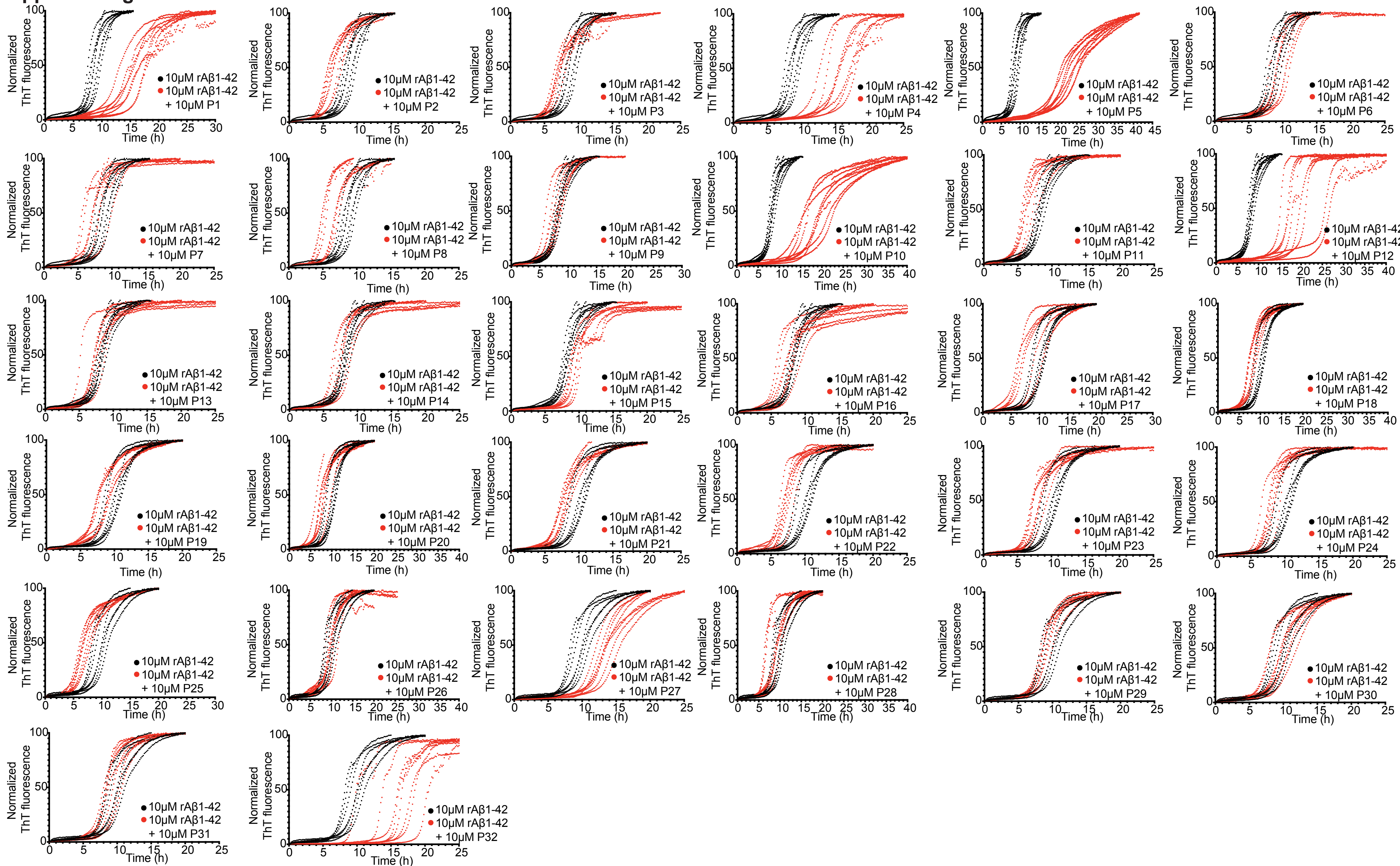






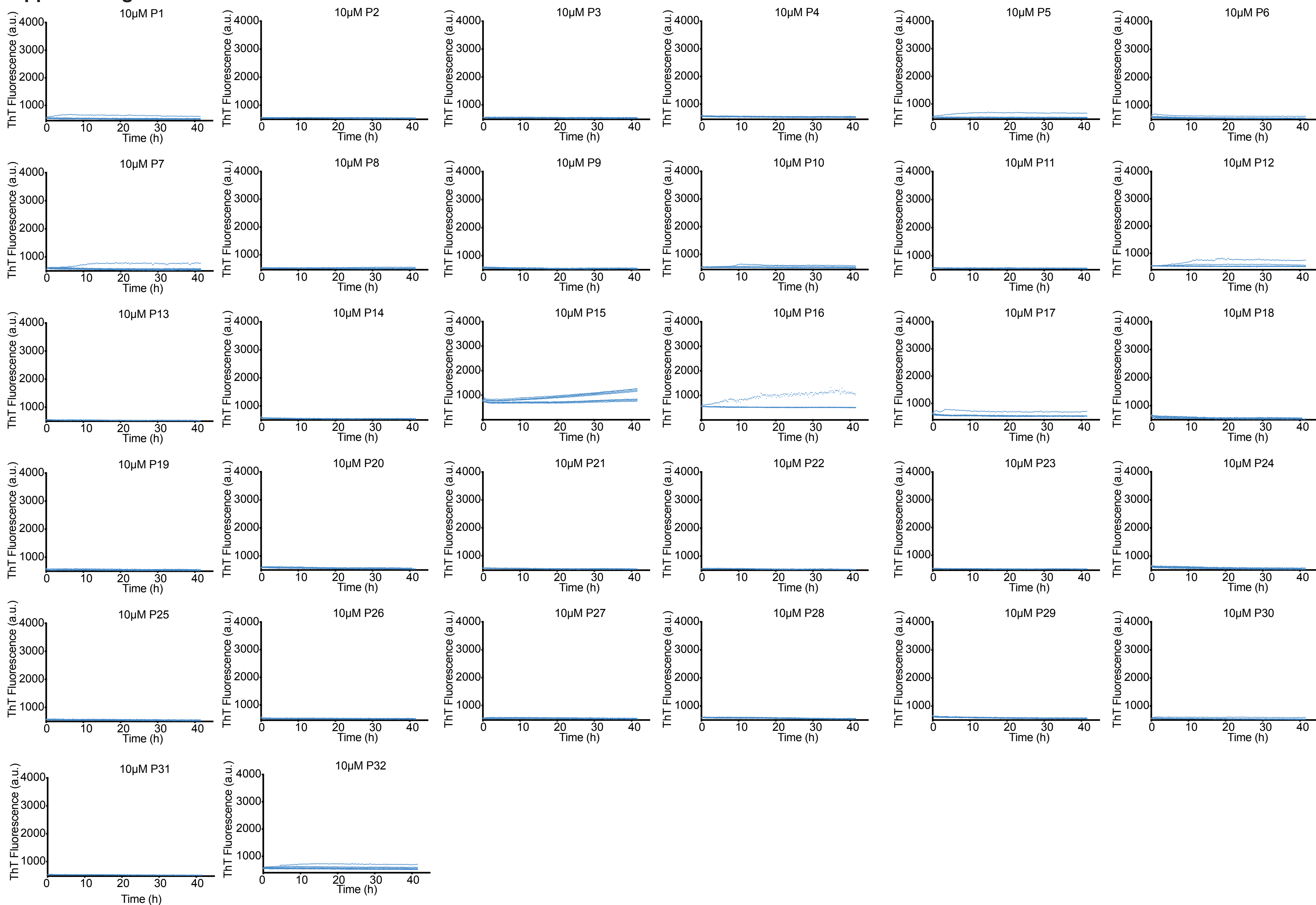
Appendix Figure S2. Binding of Aβ oligomers in peptide microarrays through 3 different randomizations. R1,2,3 are membranes with the same peptides in different order. 3 repeat was done for R1 and 2, 3 repeats for R3. Sequences in DatasetEV1.

Appendix Figure S3



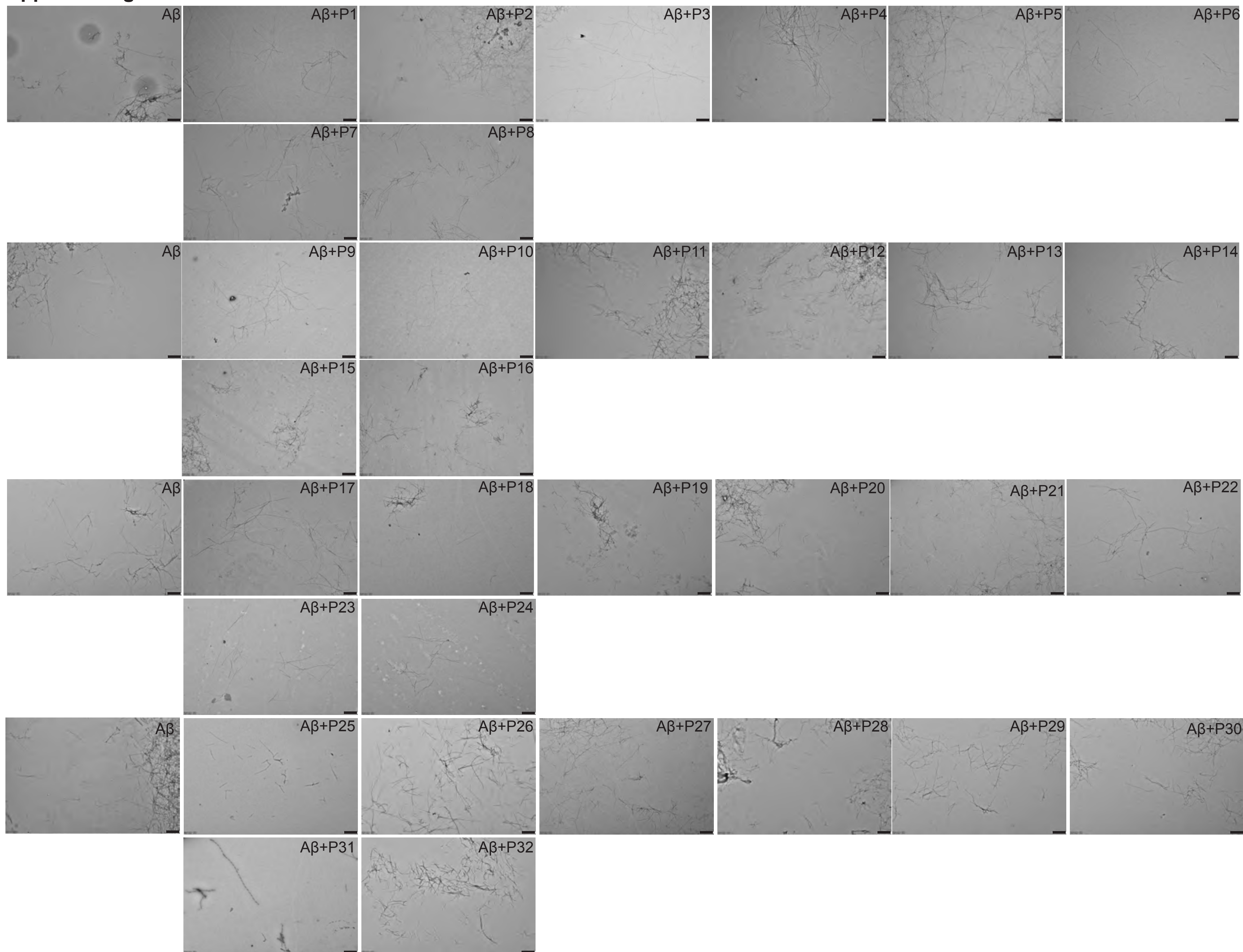
Appendix Figure S3. Normalized ThT kinetics of Aβ in presence and absence of homologue peptides derived from human proteins.
Black: 10µM rAβ1-42. Red: 10µM rAβ1-42+10µM peptide. n= 2 independent experiments with 4 repeats

Appendix Figure S4



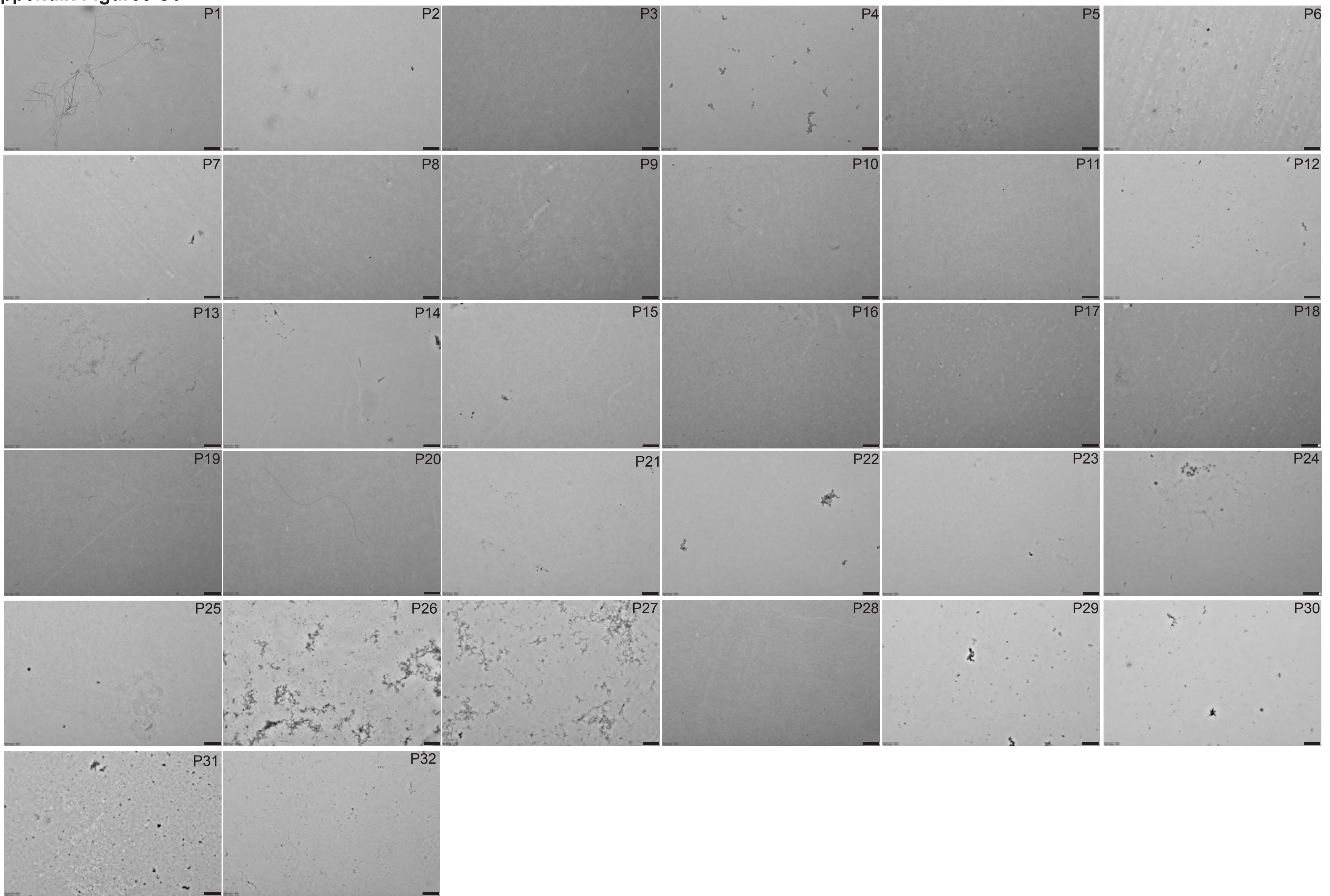
Appendix Figure S4: ThT kinetics of 10μM homologue peptides in absence of Aβ. n=2 independent experiments with 4 repeats

Appendix Figure S5



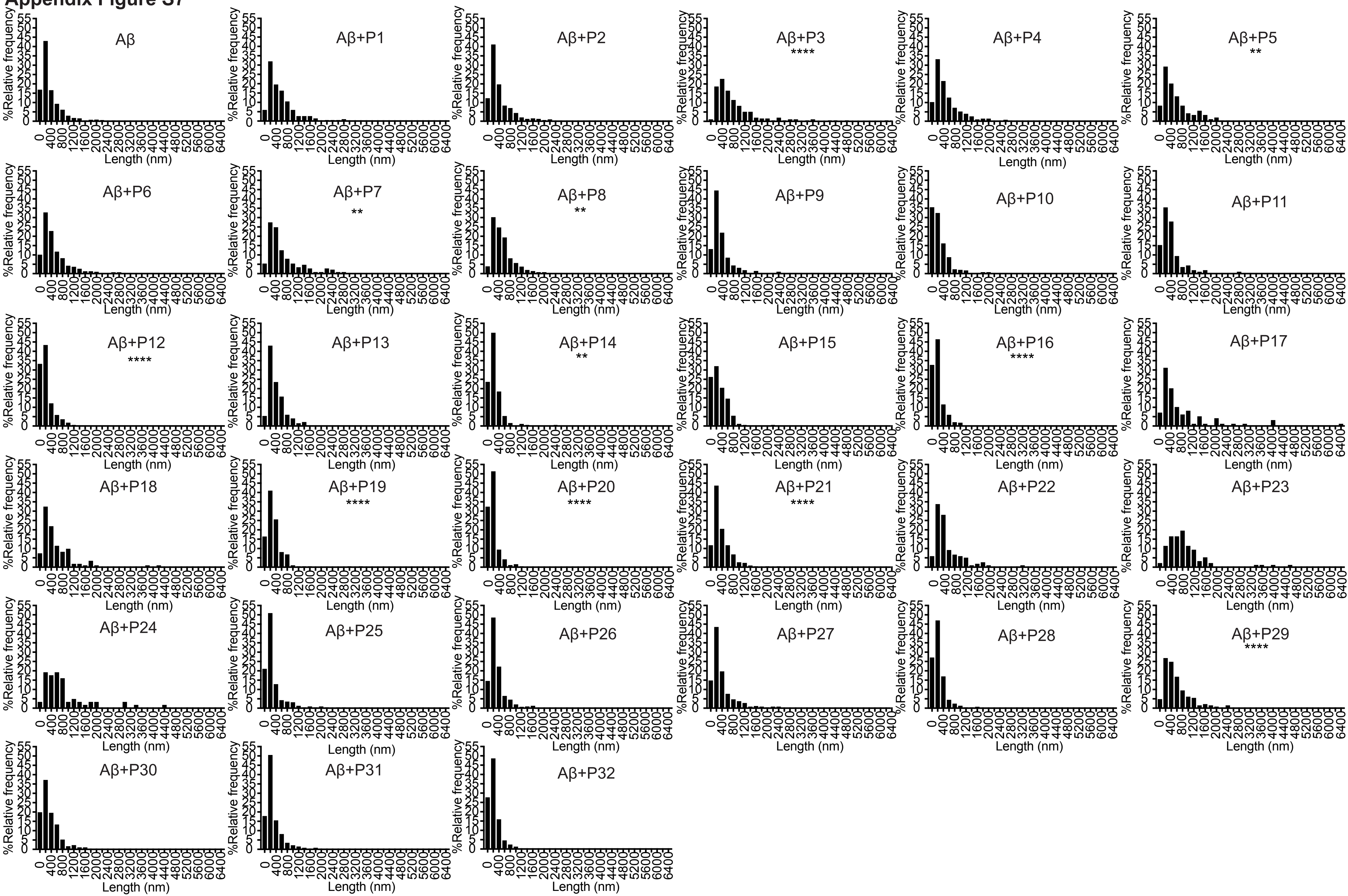
Appendix Figure S5: TEM images of Aβ fibrils made in presence of homologous peptides. (10µM rAβ1-42+10µM peptides). Scale bars: 500nm

Appendix Figures S6



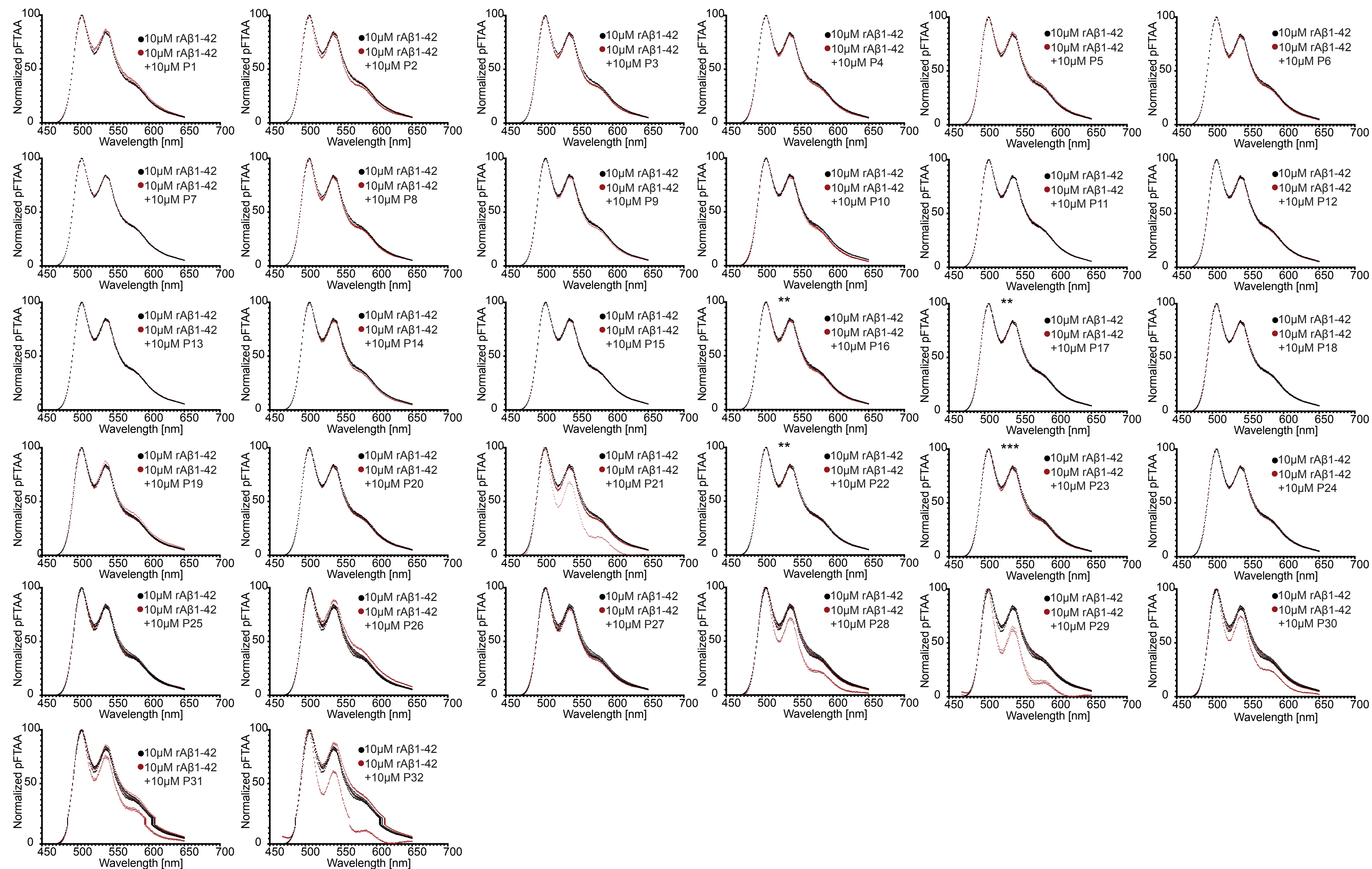
Appendix Figure S6: TEM images of peptides in absence of Aβ. Scale bars: 500nm

Appendix Figure S7



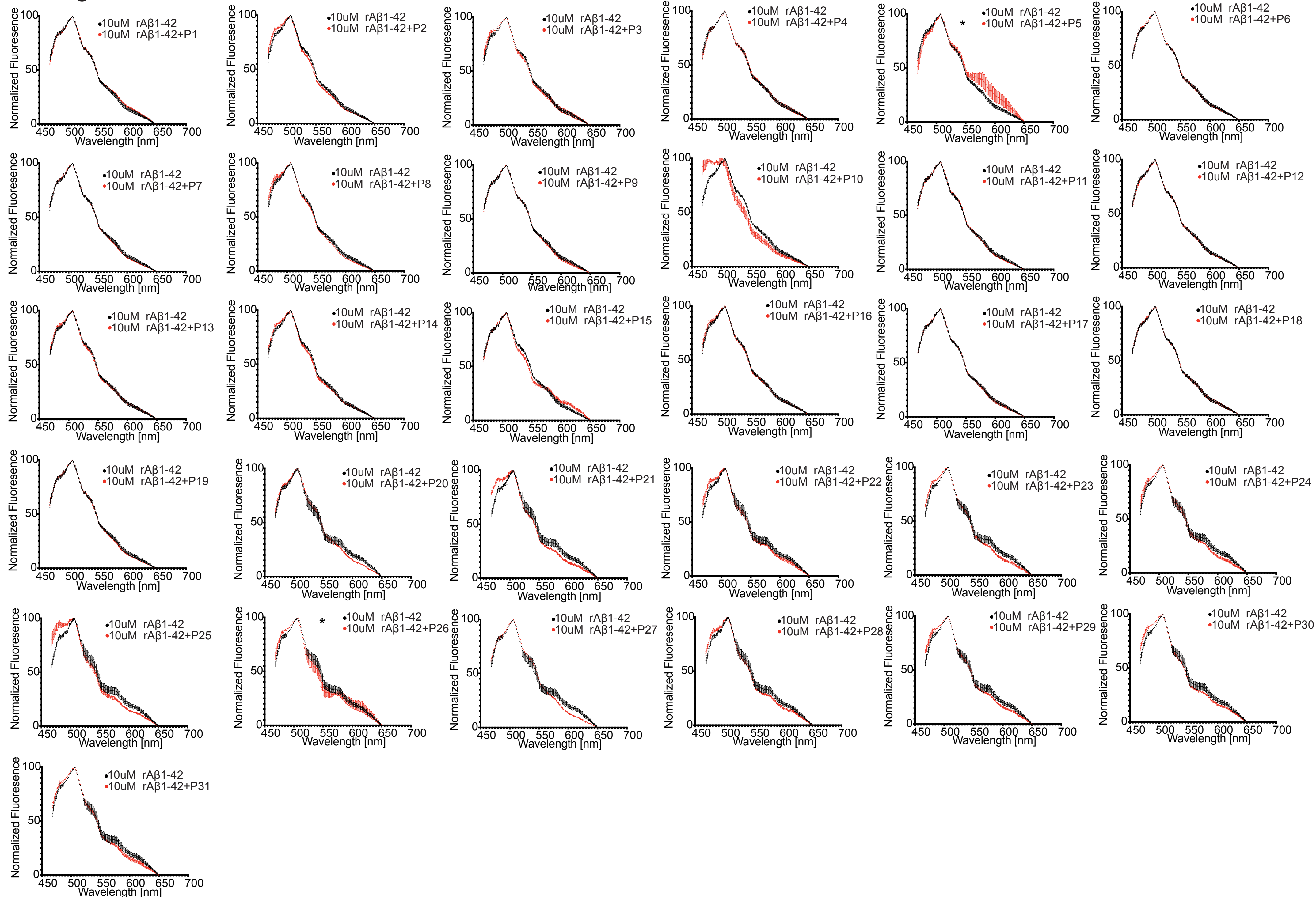
Appendix Figure S7: Length distribution of Aβ fibrils in presence or absence of peptides as measured from TEM.
Statistics: Brown-Forsythe and Welch Anova test with Games-Howell multiple comparison correction). * P ≤0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001.

Appendix Figure S8



Appendix Figure S8: Normalized pFTAA fluorescence spectrum of rAβ1-42 fibrils made in presence or absence of homologue peptides. Black:10μM rAβ1-42, Red: 10μM rAβ1-42+10μM peptide. n=2 independent experiments, 3 repeats, Statistics: Brown-Forsythe and Welch ANOVA test with Dunnett T3 multiple comparisons correction. * P ≤0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001

Appendix Figure S9



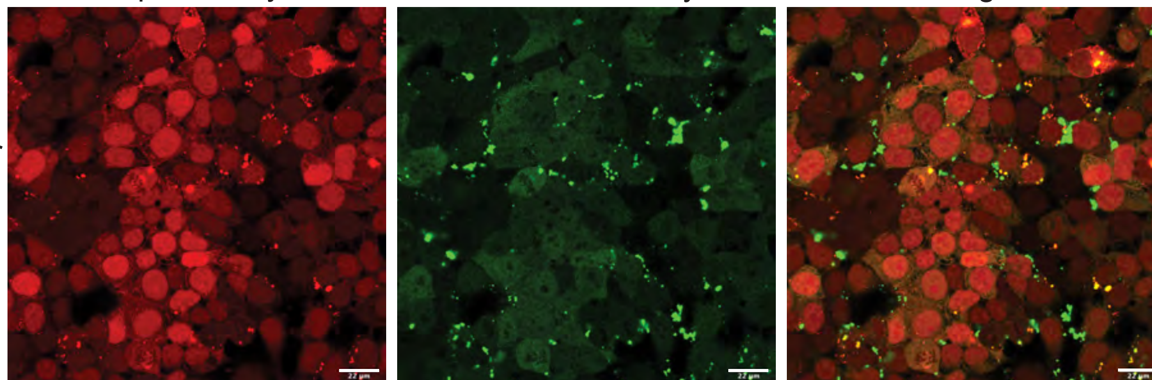
Appendix Figure S9: Normalized Curcumin fluorescence spectrum of rAβ1-42 fibrils made in presence and absence of homologues peptides. Black: 10μM rAβ1-42. Red: 10μM rAβ1-42+ 10μM peptide. n=2 independent experiments, at least 4 repeats. Statistics: Kolmogorov-Smirnov test. Graph: mean±SD * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

Appendix Figure S10

A

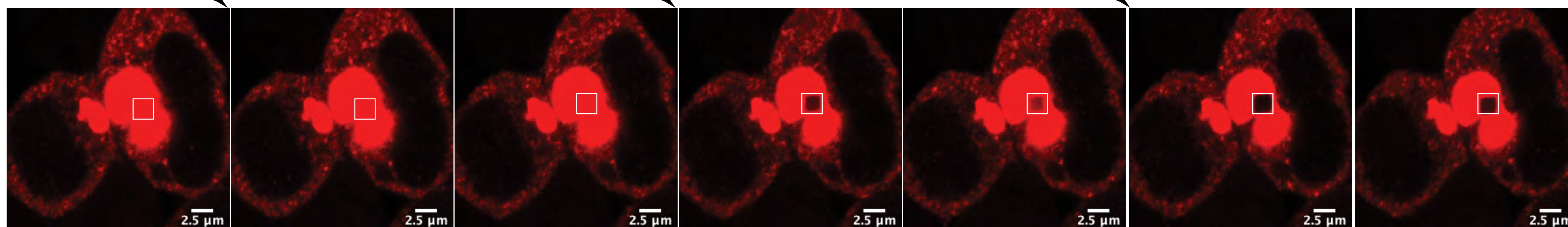
Aβ-mcherry | 6E10 antibody | Merged

Aβ biosensor seeded



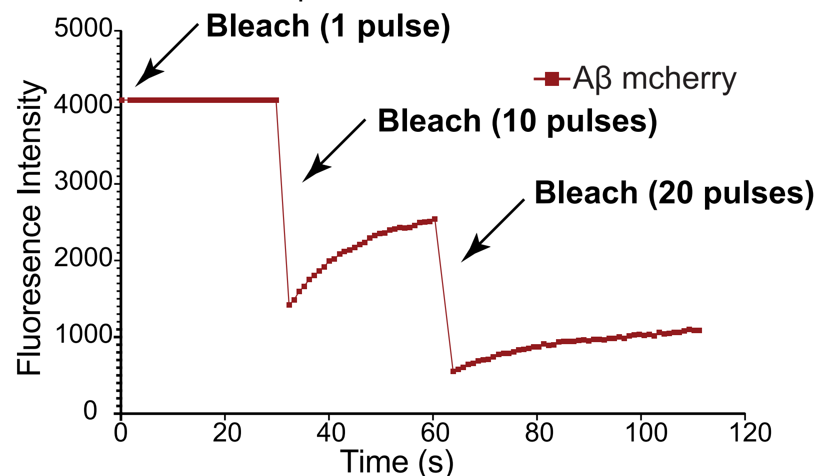
B

Bleach (1 pulse) | Bleach (10 pulses) | Bleach (20 pulses)



C

Aβ biosensor seeded

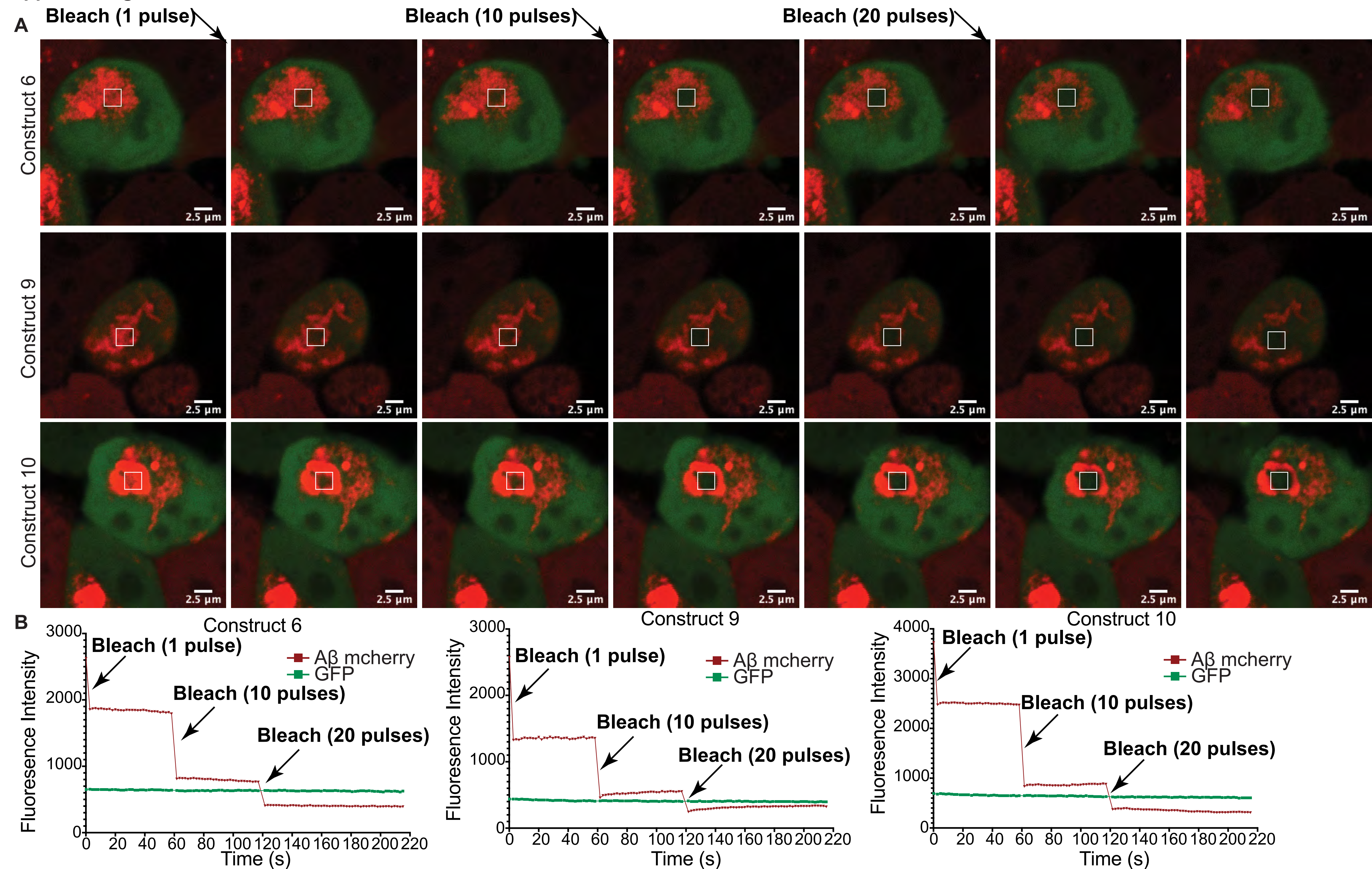


Appendix Figure S10: Inducing Aβ aggregation by Aβ reverse seeds.

A. Immunostaining of Aβ biosensor with 6E10 Aβ antibody. Aβ biosensor was transfected with rAβ1-42 reverse seeds. Red: Aβ expressed by the cell line. Green: 6E10 antibody, binds both Aβ expressed and seeds. Merged:Colocalization. Scale bars: 22μM.

B. FRAP experiment in aggregates induced by reverse seeds. White box on images indicates the area of bleaching. Scale bar 2.5μm.

C. Plot of the recovery of fluoresence after photobleaching. Arrows indicate bleaching time (1,10,20 pulses)

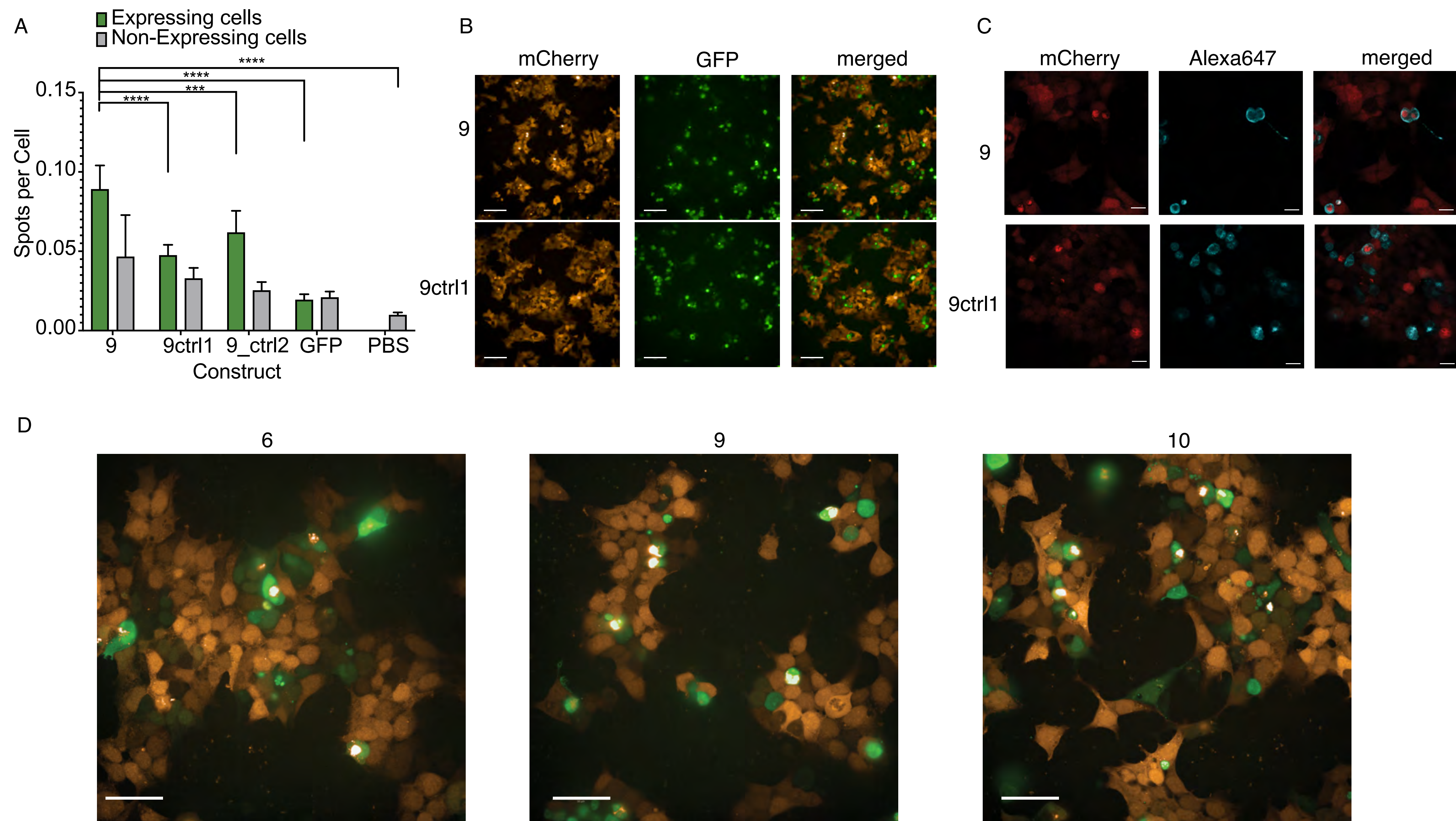


Appendix Figure S11: FRAP of aggregates induced by constructs.

A. FRAP analysis of Aβ aggregates in cells expressing constructs 6,9,10. Green: The reporter GFP. Red: Aβ mcherry. White box on images indicates the area of bleaching. Scale bar 2.5μm.

B. Fluoresence intensity measumerment before and after bleahing.Arrows indicate bleching time (1, 10,20 pulses)

Appendix Figure S12



Appendix Figure S12: Effect of expression of construct9 in aggregation of Aβ.

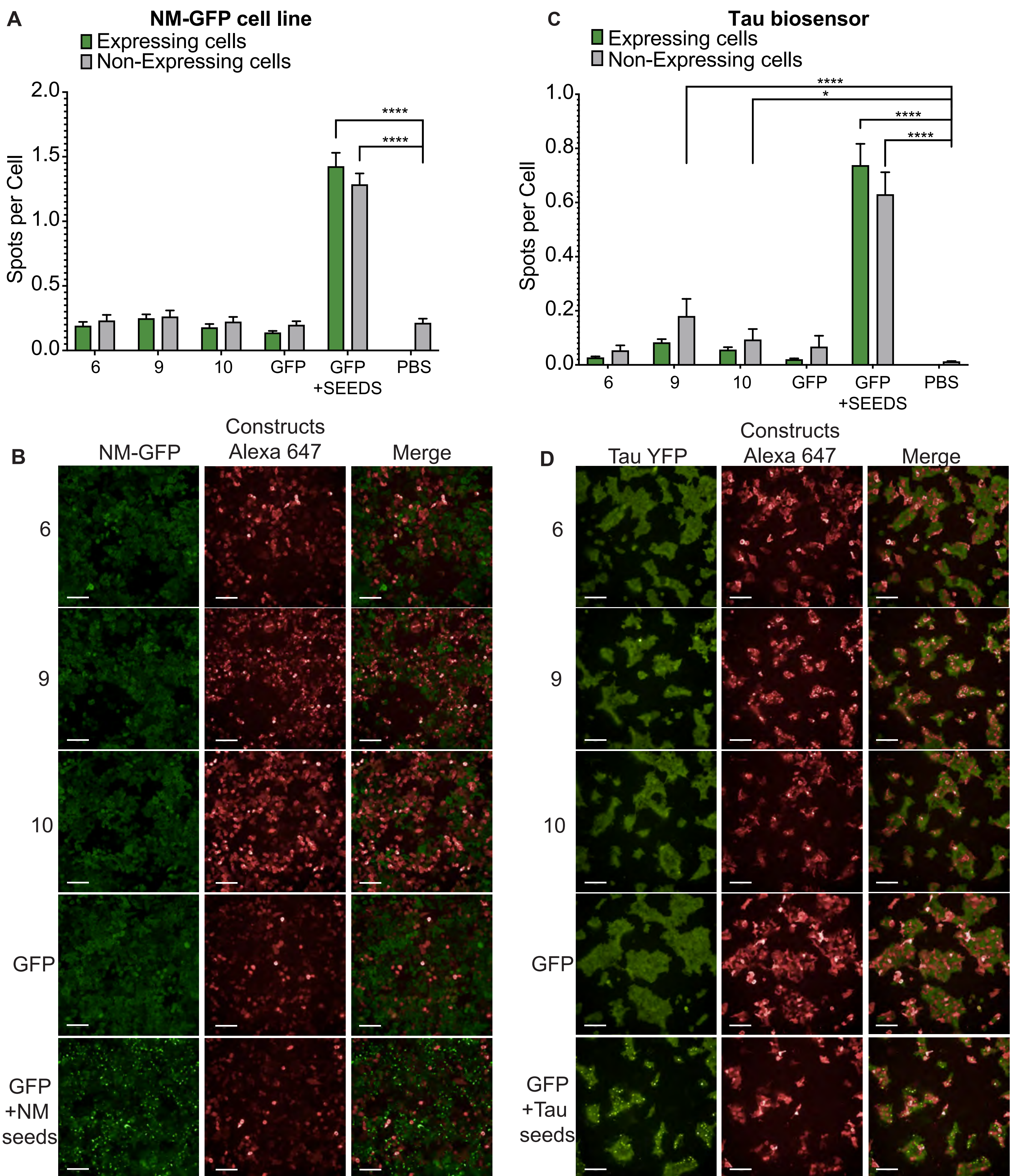
A. Quantification of spots per cell for construct 9 and its controls. Graph: mean with 95%CI. (n=4 independent experiments, statistics: ordinary one-way Anova).* P ≤0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001

B. Representative images of 9 and 9ctrl1, mcherry indicates the Aβ, GFP the transfection reporter. Scale bars: 100μm

C. Construct 9 and 9ctrl1 are not colocalizing with Aβ aggregates, but exist in the same cells. mCherry indicates Aβ, Alex647 the constructs. Scale bar: 22μm

D. 40x magnification of cells expressing constructs 6,9,10, showing clear colocalization of spots in green cells. Scale bar: 50μm

Appendix Figure S13



Appendix Figure S13: Effect of expression of Aβ aggregation inducing constructs in other biosensor cell lines.

A. Quantification of spots per cell for aggregation inducer constructs (6,9,10) in NM-GFP cell line. GFP+seeds indicate the cells transfected both with GFP construct and sup35-NM seeds.Graph: mean with 95%CI. (n=6 independent experiments, statistics: ordinary one-way Anova). * P ≤0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001

B. Representative images of cells expressing the different constructs, mcherry indicates the Aβ, Alexa647 the constructs. Scale bar:100μm

C. Quantification of spots per cell for aggregation inducer constructs (6,9,10) in Tau biosensor cell line. GFP+seeds indicate the cells transfected both with GFP construct and Tau seeds.Graph: mean with 95%CI. (n=4 independent experiments, statistics: ordinary one-way Anova). * P ≤0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001

D. Representative images of cells expressing the different constructs, mcherry indicates the Aβ, Alexa647 the constructs. Scale bars:100μm