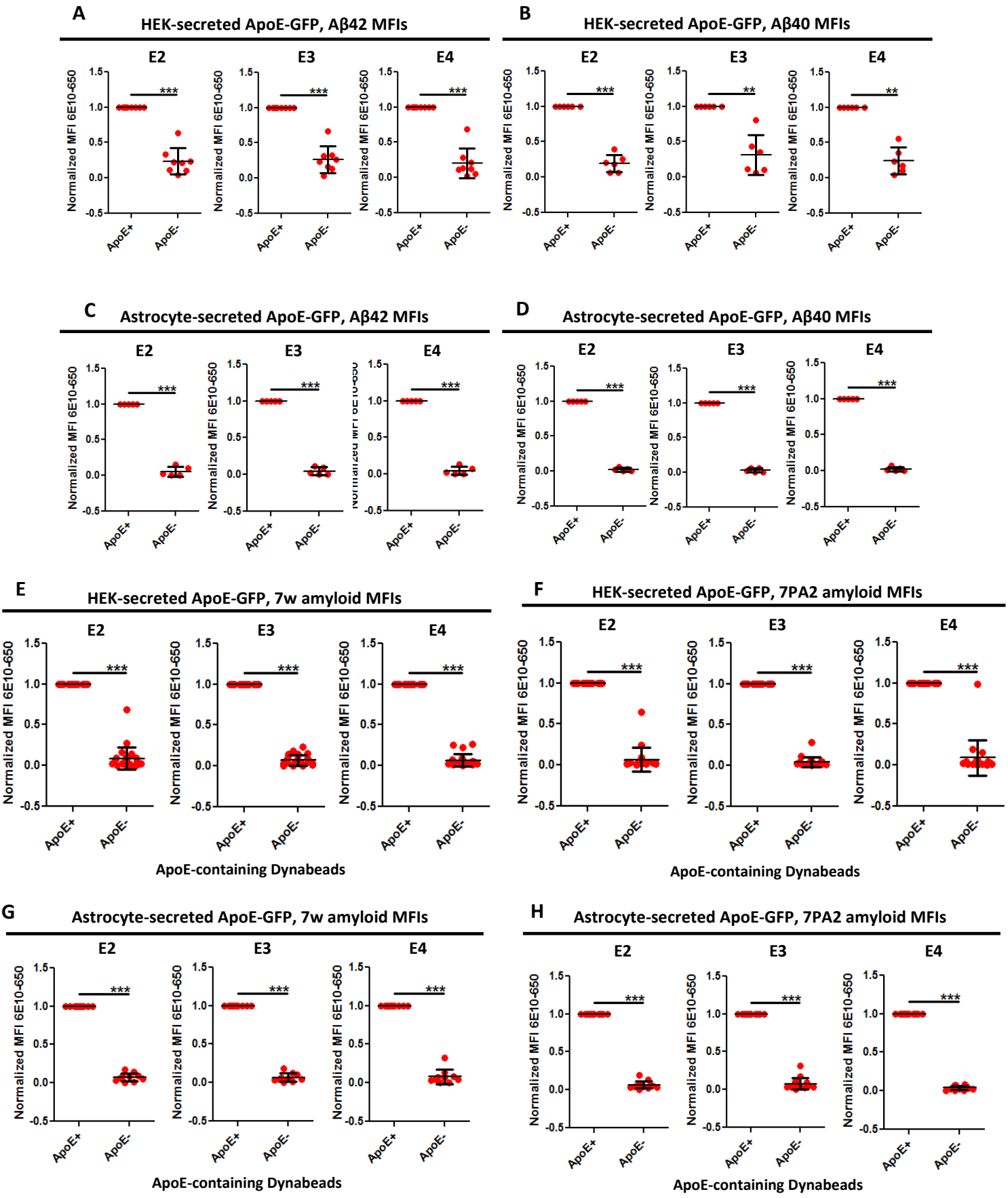
Supplementary information

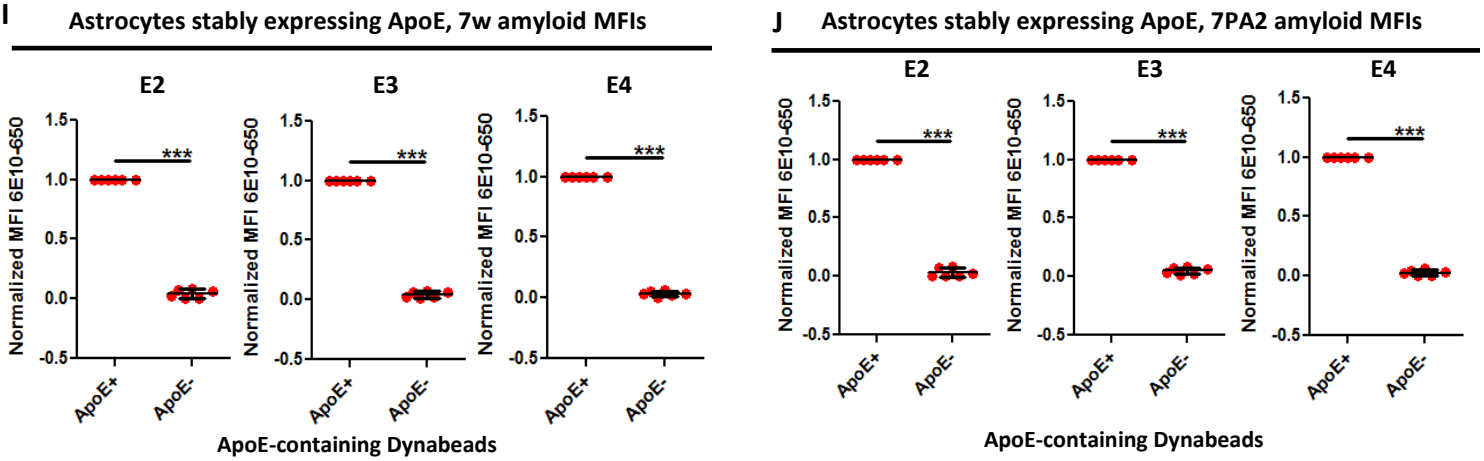
A novel flow cytometry interaction assay reveals the formation of isoform- and cell type-specific Apolipoprotein E-amyloid beta complexes in vitro

Eleanna Kara*, Jordan D Marks*, Allyson D Roe#, Caitlin Commins#, Zhanyun Fan, Maria Calvo-Rodriguez, Susanne Wegmann, Eloise Hudry, Bradley T Hyman[$]

Supplementary table 1: Concentrations of ApoE and Aβ used in the experiments as determined by ELISA. For ApoE and CHO-secreted Aβ three independent measurements were performed. The concentrations of synthetic Aβ were not measured by ELISA, but were determined through the dilution of a stock solution with a known concentration. A single batch of Tg2576 conditioned medium was used for the experiments, therefore ELISA measurements were performed on technical replicates from that batch, and the concentrations of each were averaged.

	Stock concentrations (nmol/L)		Final concentrations (nmol/L)	
	mean	SD	mean	SD
HEK ApoE				
E2	71,38588566	8,086465	53,53941	6,064848
E3	70,89108827	11,37235	53,16832	8,529265
E4	35,23146089	20,46795	26,4236	15,35096
Astrocyte ApoE				
E2	20,06834517	0,83755	15,05126	0,628162
E3	11,87246938	2,42198	8,904352	1,816485
E4	7,280623566	1,712784	5,460468	1,284588
synthetic Ab42	221,5281008	NA	6,645843	NA
synthetic Ab40	221,5281008	NA	6,645843	NA
Tg2576	5	NA	1,25	NA
7w Ab42	0,009202256	0,006287	0,002301	0,001572
7w Ab40	0,3886385	0,534358	0,09716	0,13359
7PA2 Ab42	0,902284849	0,772533	0,225571	0,193133
7PA2 Ab40	9,122778677	11,75813	2,280695	2,939531


A
HEK-secreted ApoE-GFP, Aβ42 MFIs
B
HEK-secreted ApoE-GFP, Aβ40 MFIs
C
Astrocyte-secreted ApoE-GFP, Aβ42 MFIs
D
Astrocyte-secreted ApoE-GFP, Aβ40 MFIs
E
HEK-secreted ApoE-GFP, 7w amyloid MFIs
F
HEK-secreted ApoE-GFP, 7PA2 amyloid MFIs
G
Astrocyte-secreted ApoE-GFP, 7w amyloid MFIs
H
Astrocyte-secreted ApoE-GFP, 7PA2 amyloid MFIs
E2
E3
E4
Normalized MFI 6E10-650
ApoE+
ApoE-
***
**
ApoE-containing Dynabeads



Supplementary Figure 1 (continued from page S-2):

A-D. Normalized MFI levels of the 647Hilyte-Aβ42 and 647Hilyte-Aβ40 in ApoE+ and ApoE- populations in HEK- and astrocyte-secreted ApoE. In all samples, the ApoE+ population had significantly higher 647Hilyte MFI in comparison to the ApoE- population. Number of independent experiments for both synthetic Aβ42 and Aβ40: E2: 5; E3: 5; E4: 5.

E. Normalized MFI levels of the 650Dylight-6E10 in ApoE+ vs ApoE- Dynabead populations for HEK-secreted ApoE samples incubated with 7w Aβ, indicating the presence of a significant interaction. Number of independent experiments: E2: 24; E3: 24; E4: 23.

F. Normalized MFI levels of the 650Dylight-6E10 in ApoE+ vs ApoE- Dynabead populations for HEK-secreted ApoE samples incubated with 7PA2 Aβ shows a significant interaction. Number of independent experiments: E2: 20; E3: 20; E4: 20; Aβ42-E3-GFP: 5.

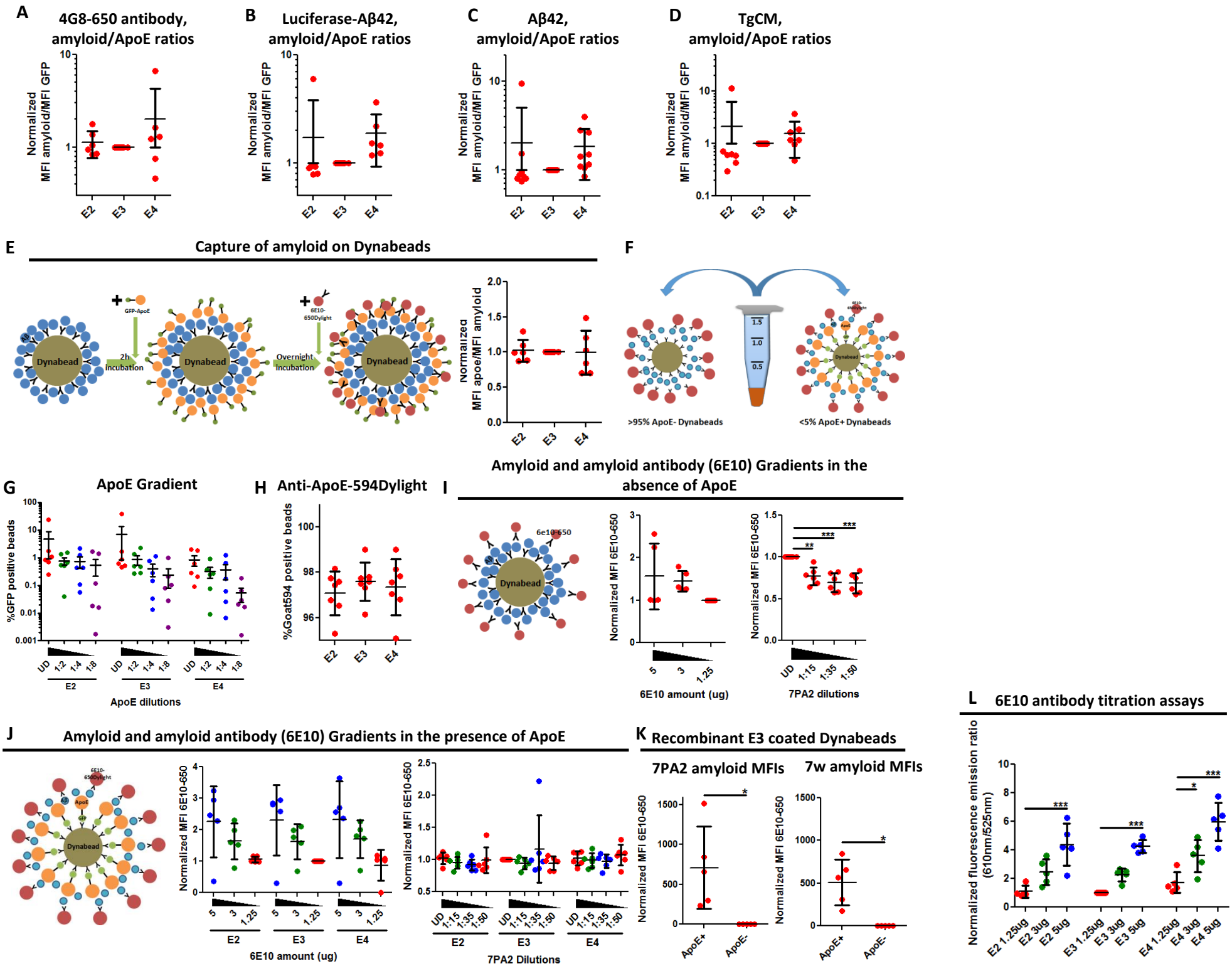
G. Normalized MFI levels of the 650Dylight-6E10 in ApoE+ vs ApoE- Dynabead populations for astrocyte-secreted ApoE samples incubated with 7w Aβ indicating the presence of a significant interaction. Number of independent experiments: E2: 10; E3: 10; E4: 10.

H. Normalized MFI levels of the 650Dylight-6E10 in ApoE+ vs ApoE- Dynabead populations for astrocyte-secreted ApoE samples incubated with 7PA2 Aβ indicating the presence of a significant interaction. Number of independent experiments: E2: 16; E3: 16; E4: 16.

I. Normalized MFI levels of the 650Dylight-6E10 in ApoE+ vs ApoE- Dynabead populations for astrocyte-secreted untagged ApoE samples incubated with 7w Aβ. Number of independent experiments: E2: 6; E3: 6; E4: 6.

J. Normalized MFI levels of the 650Dylight-6E10 in ApoE+ vs ApoE- Dynabead populations for astrocyte-secreted untagged ApoE samples incubated with 7PA2 Aβ. Number of independent experiments: E2: 6; E3: 6; E4: 6.

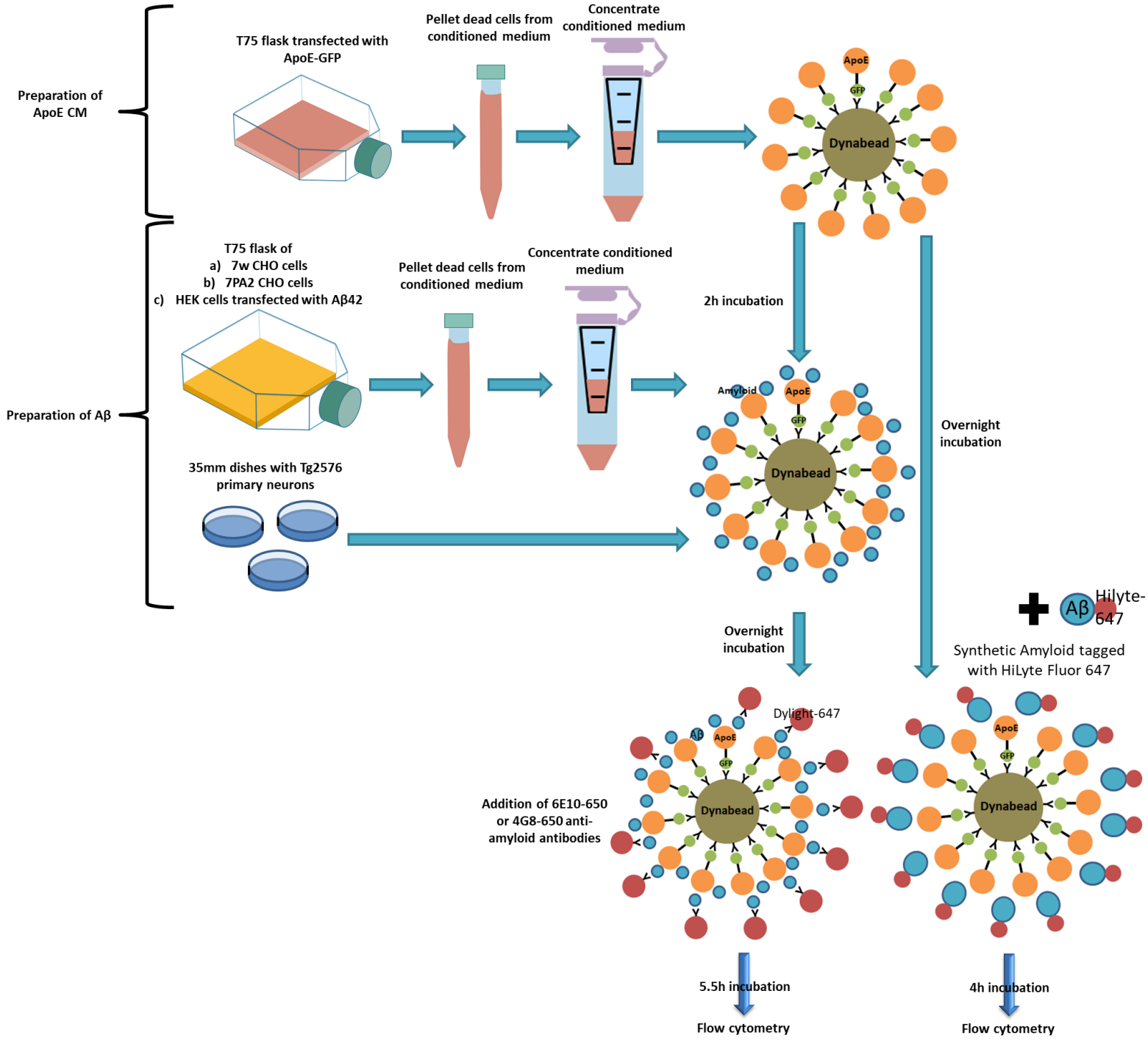
The asterisks on the graph indicate *P* values as follows: *** $P \leq 0.001$; ** $P \leq 0.01$.


A
4G8-650 antibody, amyloid/ApoE ratios
B
Luciferase-Aβ42, amyloid/ApoE ratios
C
Aβ42, amyloid/ApoE ratios
D
TgCM, amyloid/ApoE ratios
Normalized MFI amyloid/MFI GFP
E2
E3
E4
E
Capture of amyloid on Dynabeads
Dynabead
GFP-ApoE
2h incubation
6E10-650Dylight
Overnight incubation
Normalized MFI apoe/MFI amyloid
F
>95% ApoE- Dynabeads
<5% ApoE+ Dynabeads
G
ApoE Gradient
%GFP positive beads
UD
1:2
1:4
1:8
ApoE dilutions
H
Anti-ApoE-594Dylight
%Goat594 positive beads
I
Amyloid and amyloid antibody (6E10) Gradients in the absence of ApoE
6e10-650
Normalized MFI 6E10-650
6E10 amount (ug)
7PA2 dilutions
J
Amyloid and amyloid antibody (6E10) Gradients in the presence of ApoE
6E10 amount (ug)
7PA2 Dilutions
K
Recombinant E3 coated Dynabeads
7PA2 amyloid MFIs
7w amyloid MFIs
ApoE+
ApoE-
L
6E10 antibody titration assays
Normalized fluorescence emission ratio (610nm/525nm)
E2 1.25ug
E2 3ug
E2 5ug
E3 1.25ug
E3 3ug
E3 5ug
E4 1.25ug
E4 3ug
E4 5ug

Supplementary figure 2 (previous page): Interactions between ApoE and Aβ can be detected robustly and specifically by flow cytometry.

A. Plot indicating the Aβ/ApoE ratios for HEK-secreted ApoE incubated with 7PA2 CM. Aβ was detected by using the 4G8-650 antibody. There are no statistically significant differences between isoforms, though E4 has a trend for stronger interaction with Aβ compared to E2 and E3. Number of independent experiments: E2: 6; E3: 6; E4: 6.
B. Plot indicating the Aβ/ApoE ratios for HEK-secreted ApoE incubated with luciferase- Aβ42 CM generated after transfection of HEK cells. Aβ was detected using an anti-luciferase antibody tagged with 650Dylight. E4 has a trend to interact more strongly than E2 and E3 with Aβ. Number of independent experiments: E2: 6; E3: 6; E4: 6.
C. Plot indicating the Aβ/ApoE ratios for HEK-secreted ApoE incubated with Aβ42 in CM from HEK cells that were transfected with a plasmid encoding pure Aβ42. E4 has a trend to interact more strongly than E2 and E3 with Aβ. Number of independent experiments: E2: 8; E3: 9; E4: 9.
D. Plot indicating the Aβ/ApoE ratios for HEK-secreted ApoE incubated with transgenic CM (TgCM) from primary neurons generated from transgenic mice expressing the APP Swedish mutation. There are no statistically significant differences between the isoforms. Number of independent experiments: E2: 7; E3: 7; E4: 7.
E. Aβ was captured on Dynabeads using the 4G8 antibody. 2h later, HEK-secreted ApoE-GFP was added to each sample for overnight incubation. The following day, the Aβ was detected by using the 6E10-650 antibody. The plot showing the Aβ/ApoE ratios suggests that there are no statistically significant differences between isoforms.
F. Cartoon explaining that less than 5% of Dynabeads were positive for ApoE and more than 95% were negative for ApoE; therefore, the ApoE-negative population was used as an internal negative control in the assay.
G. Plot indicating the percentage of ApoE+ Dynabeads in various concentrations of ApoE secreted by HEK cells. The percentage of ApoE+ beads decreases proportionately to the concentration of ApoE. Number of independent experiments: E2: 6; E3: 6; E4: 6.
H. Plot indicating the percentage of Dynabeads that are positive for the goat anti-ApoE antibody that was conjugated to 594Dylight. Approximately 97% of dynabeads were positive for antibody binding. Number of independent experiments: E2: 7; E3: 7; E4: 7.
I. 7PA2 Aβ was attached to dynabeads using the 4G8 antibody overnight, following by detection of the Aβ with the 6e10-650 antibody the following day. When a serial dilution of Aβ and a stable concentration of 6E10-650 antibody were used, the 650MFI was proportionate to the decrease in concentration of Aβ. When a stable concentration of Aβ and a concentration gradient of 6E10-650 were used, the 650MFI was proportionate to the amount of the antibody. Number of independent experiments: 7PA2 gradient: 6; 6E10 gradient: 5.
J. HEK-secreted ApoE-GFP was attached to dynabeads using an anti-GFP antibody. 2h later, 7PA2 Aβ was added to the sample for overnight incubation. The following day, the Aβ was detected by using the 6e10-650 antibody. When a concentration gradient of Aβ and a stable concentration of 6E10 antibody were used, the results differed from the equivalent experiment without ApoE. When the concentration of Aβ was stable and the concentration of 6E10-650 antibody followed a gradient, the results were identical to the experiment where there was no ApoE present. Number of independent experiments: 7PA2 gradient: 6; 6E10 gradient: 5.
K. Plots indicating the 650MFI in ApoE+ and ApoE- populations when recombinant E3 was incubated with 7PA2 or 7w Aβ. Both plots indicate the presence of a mild interaction between recombinant E3 and Aβ. Number of independent experiments: 7w: 5; 7PA2: 5.
L. Antibody titration assays were performed in order to determine the minimum concentration of the 6E10 antibody that is suitable for the assay.

In these plots, each dot represents the mean value from one independent experiment. The data was normalized to E3 or to ApoE- before metaanalysis (apart from panel G). Statistical analysis was done with 1 way ANOVA with Tukey's post-hoc correction. Mean+/-SD are shown on the plots. The asterisks on the graph indicate *P* values as follows: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$;



Supplementary figure 3: Illustration summarizing the workflow of the experiments. Conditioned media from cells transfected with ApoE-GFP was concentrated 15-fold, and ApoE-GFP was attached to Dynabeads with a GFP antibody. Aβ from a variety of sources was used: synthetic tagged with a far red fluorophore, 15-fold concentrated conditioned media of 7w or 7PA2 CHO cells or HEK cells transfected with Aβ42, or conditioned media from Tg2576 primary neurons expressing the APP Swedish mutation. 2 hours after addition of ApoE to Dynabeads, physiological Aβ was added and the sample was incubated at 4°C overnight with rotation. The next morning, the 6e10 or 4G8 anti-amyloid antibodies were added to the mix and incubated for 5.5 hours before flow cytometry. In the case of synthetic Aβ, this was added to the ApoE-GFP+Dynabeads for 4h after overnight incubation, followed by analysis through flow cytometry.