Supplemental Data

Cell-derived hexameric β-amyloid: a novel insight into composition, self-assembly and nucleating properties

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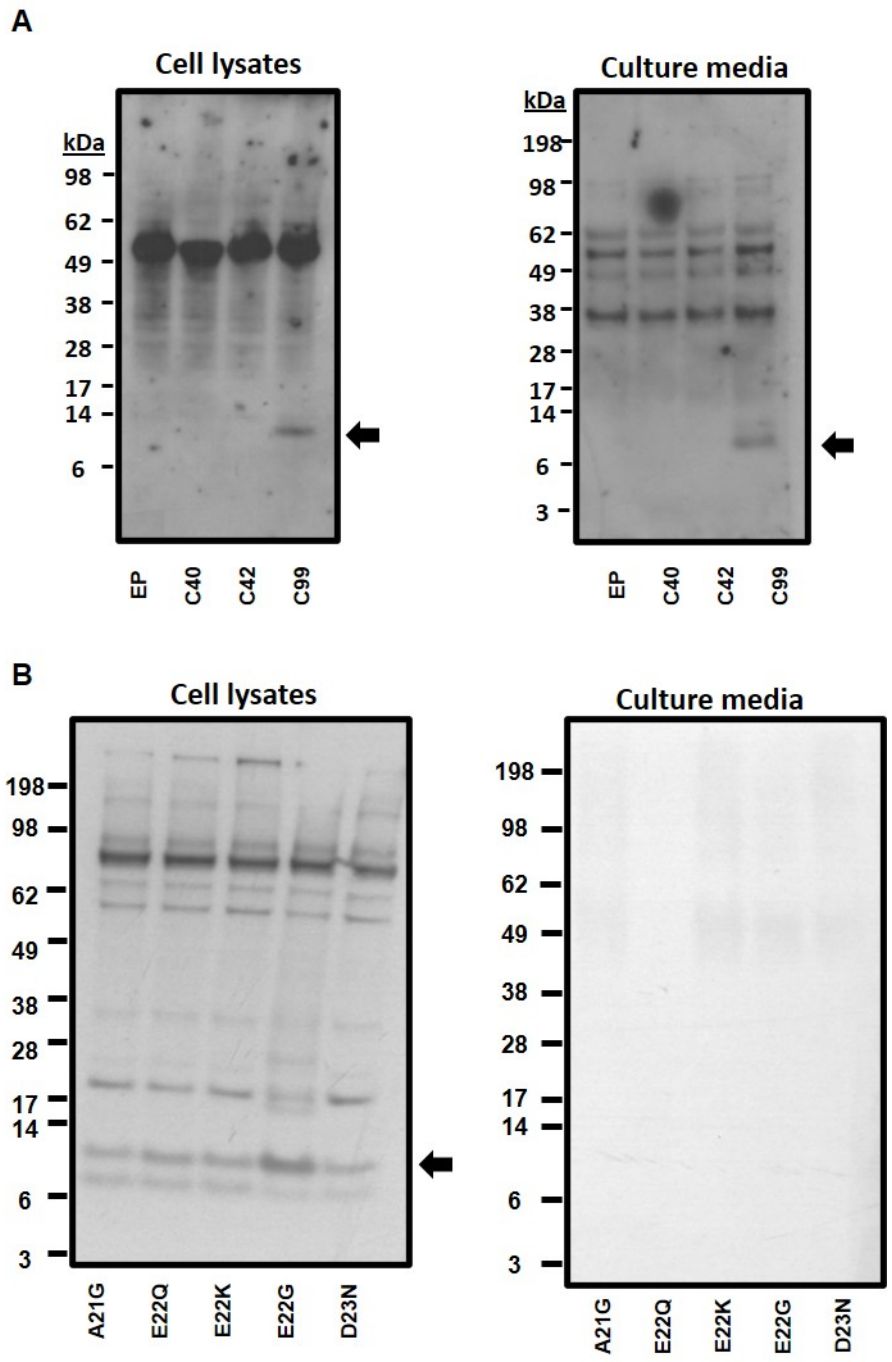
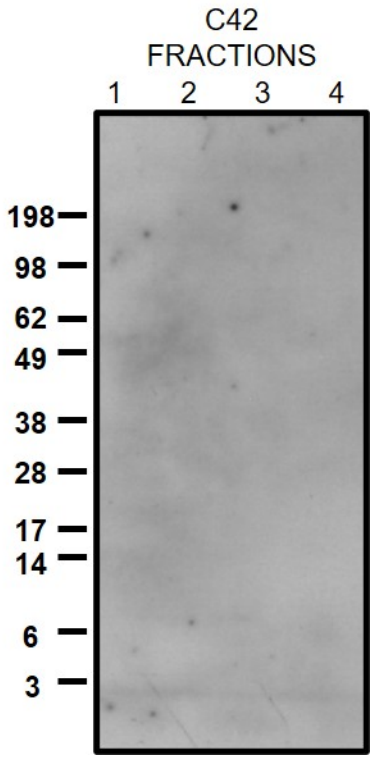
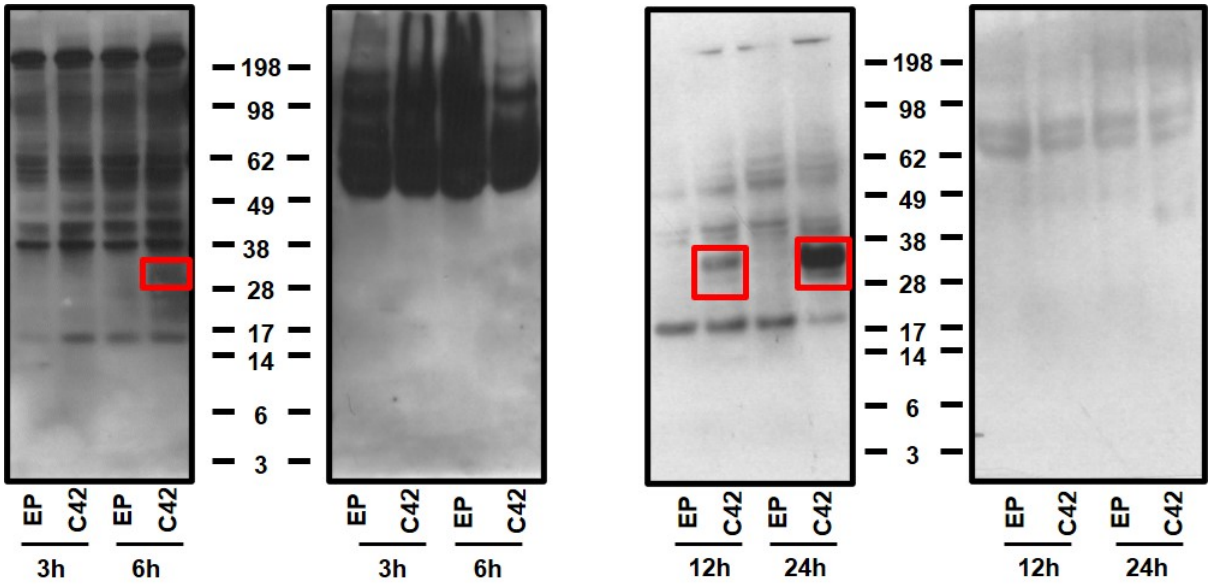


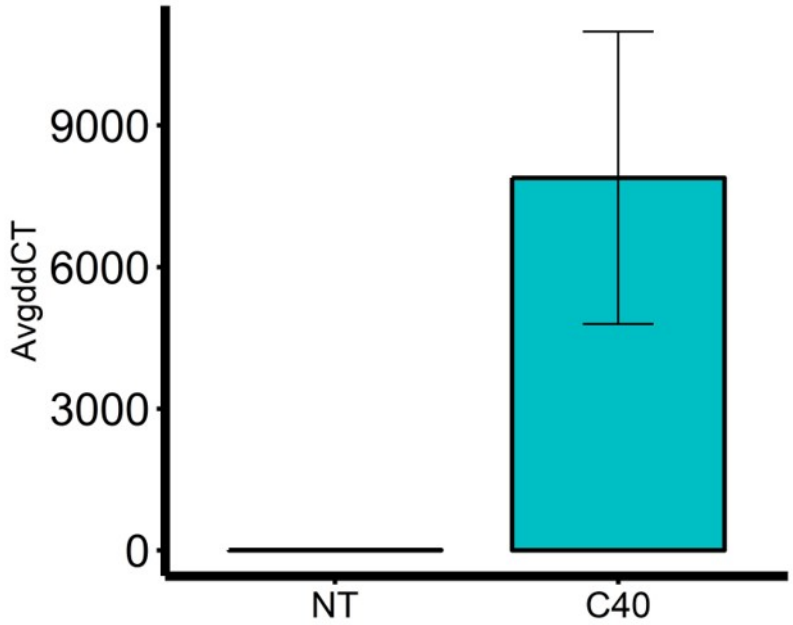
Supplemental Figure 1. APP C-terminal detection with C-ter antibody confirms assemblies to be Aβ. (A) Hexameric assemblies are not detected in the cell lysates or culture media of CHO cells transfected with EP, C40, C42 or C99 **(B)** FAD mutants. The C-terminal fragment is seen in the cell lysates of CHO cell transfected with C99 sequences (black arrows).



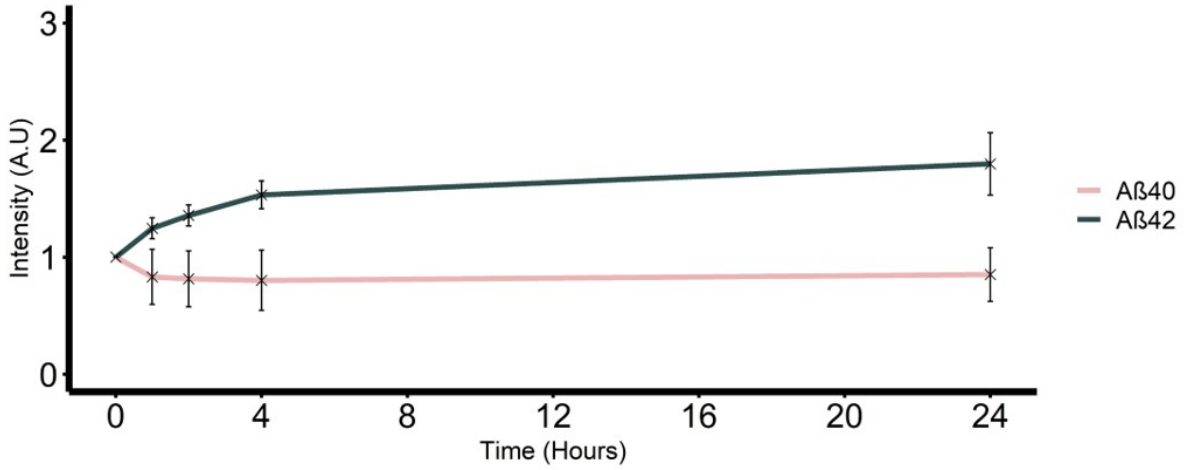
Supplemental Figure 2. Isolation of C42 derived hexamers: Fractions 1-4. No Aβ assemblies were isolated in fraction 1-4 collected after separation by the GELFrEE 8100 system. Western blot detected with the W02 antibody.



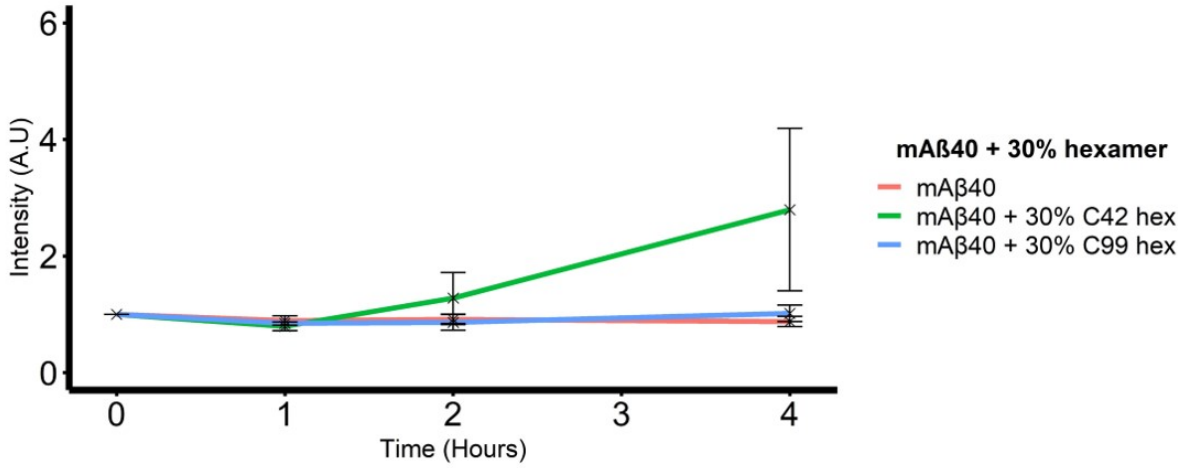
Supplemental Figure 3. Lower molecular weight Aβ assemblies were not detected in C42 transfected CHO cells. CHO cells were transfected with C42 for 3,6,12 and 24hours. Formation of hexameric assemblies were seen in cell lysates by 6 hours with no lower weight assemblies seen at the earlier time point of 3 hours. Aβ assemblies were not detected in the media of these cells even after 24 hours. Western blot detection with the W02 antibody.



Supplemental Figure 4. C40 transfection efficiency. qPCR confirms that the lack of hexameric assemblies is not due to low transfection efficiency (N=5)



Supplemental Figure 5. ThT fluorescence of Aβ40 and Aβ42 over 24 hours. 50μM of each peptide was incubated with 20μM ThT and fluorescence intensity was measured over 24hours. Aβ40 does not display an increase in fluorescence however Aβ42 displays a sharp increase in fluorescence intensity from 0-4hours which then plateaus over 24 hours.



Supplemental Figure 6. monomeric Aβ40 does show accelerated aggregation after 30% hexamer addition. 30% addition both hexamers leads to increased ThT fluorescence over 24 hours.

	Sequence
C42	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
vC42	DAEFRHDSGYEVHHQKLV**S**FAEDVGSNKGAIIGLMV**D**GVVIA

Supplemental Table S1. **Primary sequence of the wild type (C42) and variant C42 (cvC42) sequences**

	Forward	Reverse
A21G	5'-ggtgttctttggagaagatgtgggttcaaac-3'	5'-cacatcttctccaaagaacaccaatttttgatg-3'
E22Q	5'-ggtgttctttgcacaagatgtgggttcaaac-3'	5'-cccacatcttgtgcaaagaacaccaatttttg-3'
E22K	5'-ggtgttctttgcaaaagatgtgggttcaaac-3'	5'-cccacatcttttgcaaagaacaccaatttttg-3'
E22G	5'-gtgttctttgcaggagatgtgggttcaaac-3'	5'-cccacatctcctgcaaagaacaccaatttttg-3'
D23N	5'-ggtgttctttgcagaaaatgtgggttcaaac-3'	5'-cccacattttctgcaaagaacaccaatttttg-3'

Supplemental Table S2. **Primer sequences for site directed mutagenesis of C99 to introduce FAD mutations**

	Forward	Reverse
GAPDH	5'-acccagaagactgtggatgg-3'	5'acacattgggggtaggaaca-3'
C40	5' gctggaggatgcagaattccgac 3'	5' cgcccaccatgagtccaatgattg 3'

Supplemental Table S3. **Primer sequences for qPCR to probe for C40 transfection efficiency in CHO cells.**