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Supplementary Information for

Probing the Mechanism of Inhibition of Amyloid β(1-42) Induced Neurotoxicity by the Chaperonin GroEL

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This PDF file includes:

Figs. S1 to S12

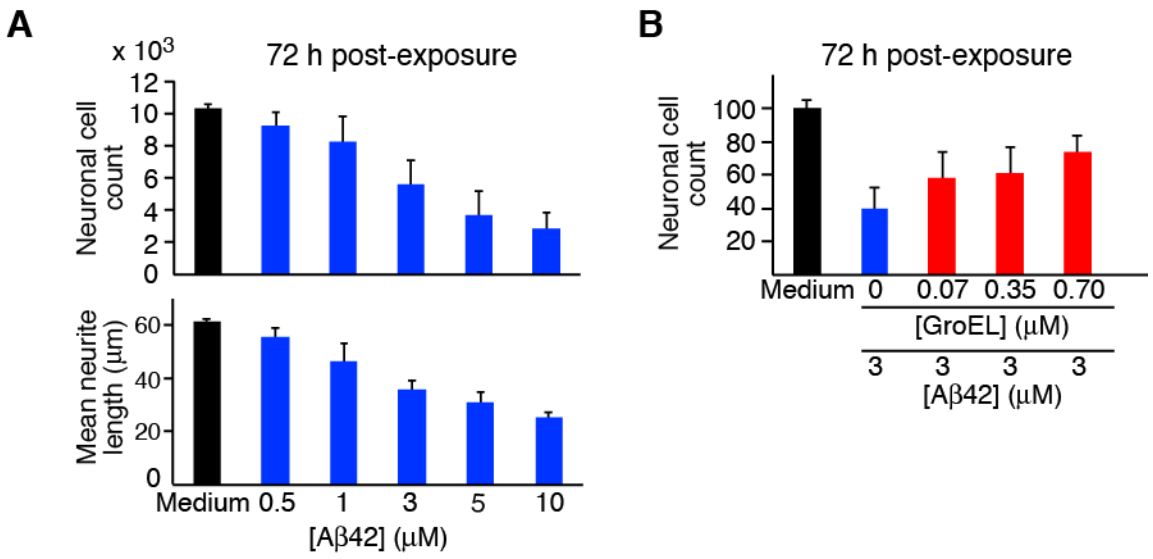

Figure S1. Aβ42 induced neuronal cell damage and protection by GroEL. (A) Neuronal cell count and mean neurite length 72 hours after exposure to varying concentrations of Aβ42 (from 0.5 to 10 µM; blue bars); the control is medium only (black bar). Neuronal cell count decreased from 10319 ± 240 for medium alone to 9209 ± 883, 8259 ± 1591, 5596 ±1458, 3696 ± 1518 and 2848 ± 1021 upon the addition of 0.5, 1, 3 and 5 µM Aβ42, respectively. Neurite cell length decreased from 61 ± 1 µm for medium alone to 56 ±3, 47 ± 7, 36 ± 3, 31 ± 4, and 26 ± 2 µm upon the addition of 0.5, 1, 3 and 5 µM Aβ42, respectively. (B) Protective effect of varying concentrations of GroEL (from 0 to 0.7 µM) on Aβ42 (3 µM) induced neuronal cell toxicity, measured by neuronal cell count 72 h after exposure to Aβ42. The control is medium only (black bar). In absence of GroEL only ~40% of the neurons survive at 72 h; upon addition of 0.07, 0.35 and 0.7 µM GroEL, 58, 61 and 74% neuronal survival, respectively, is observed. Aβ42 was dissolved in 20 mM sodium phosphate buffer (pH 7.4) at a concentration of 300 µM and left for 3 days at 4 °C, prior to addition to the neuronal cell cultures (37 °C) at the indicated final concentrations.

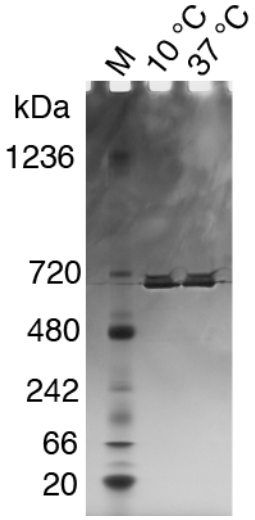

Figure S2. BN-PAGE analysis of the stability of GroEL. 0.7 µM GroEL was stored at 10 and 37 °C for 6 days in 20 mM sodium phosphate (pH 7.4) and then subjected to BN-PAGE. GroEL remains as a stable tetradecamer at both temperatures. Lane M is the molecular weight standard with masses indicated in kDa.

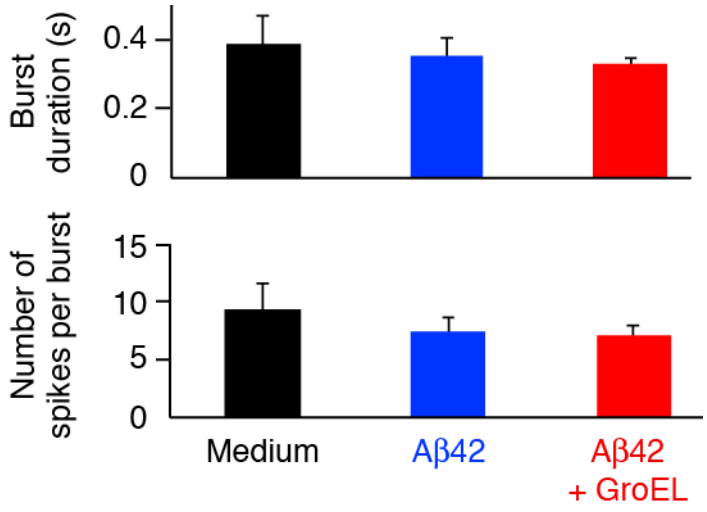

Figure S3. Effect of GroEL on neuronal cell activity after Aβ42 treatment for 24 hours. Neuronal cell activity measured by burst duration (top) and number of spikes per burst (bottom) in medium only (black), and in the presence of either 3 μM Aβ42 (blue) or 3 μM Aβ42 plus 0.7 μM GroEL (red). Aβ42 was dissolved in 20 mM sodium phosphate buffer (pH 7.4) at 300 μM and left for 3 days at 4 °C. The peptide was then added to the neuronal cell cultures to a final concentration of 3 μM.

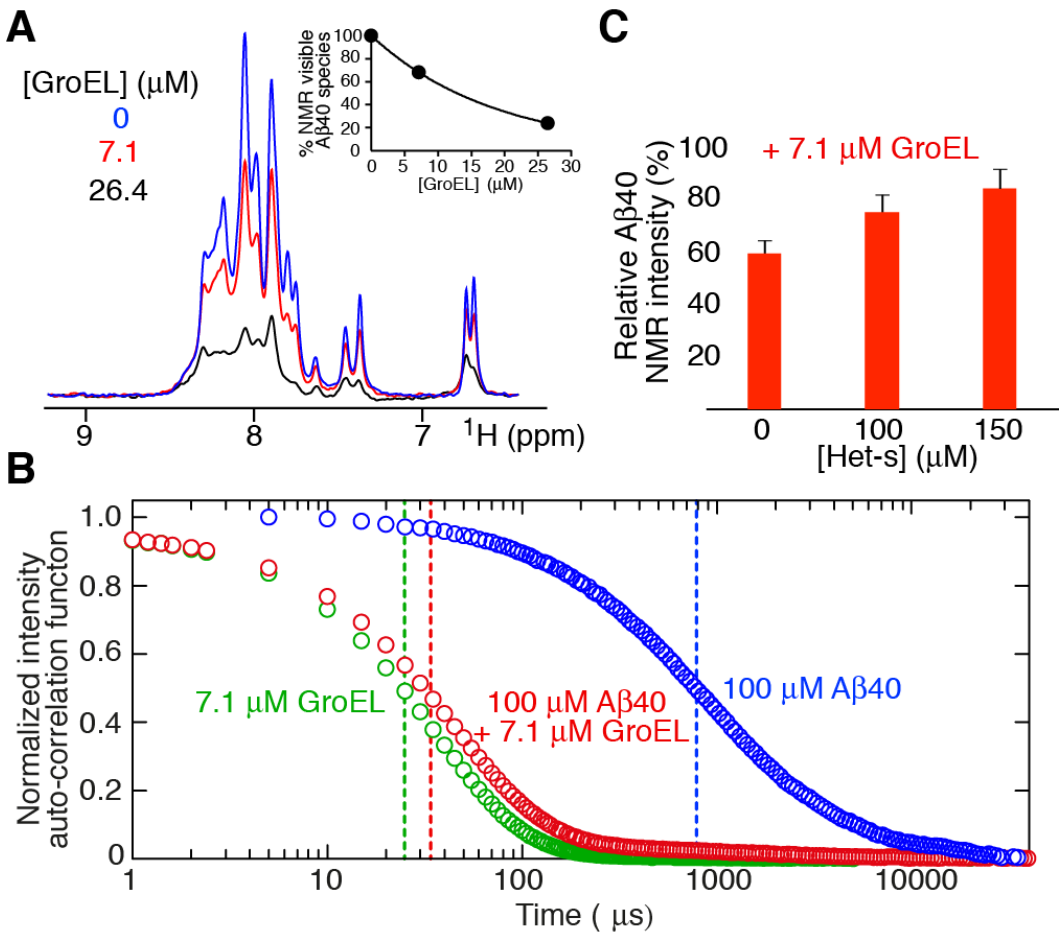

Figure S4. Interaction of Aβ40 with GroEL. (A) Fourier transform of the FID of the first t_1 increment of a ^{1}H-^{15}N correlation experiment of 100 μM ^{15}N-labeled Aβ40 recorded immediately (time point zero) upon adding 0 to 26.4 μM GroEL. The inset shows the overall decrease in the intensity of the amide proton envelope of the Aβ40 NMR spectrum as a function of GroEL concentration. (B) Dynamic light scattering normalized intensity autocorrelation functions ($g^{(2)}$-1) obtained immediately after dilution at room temperature of 7.1 μM GroEL (green), 100 μM Aβ40 (blue), and 100 μM Aβ40 plus 7.1 μM GroEL (red). The vertical dashed lines indicate the apparent decay times ($t_{1/2}$). (C) Overall relative intensity of the first t_1 increment of a ^{1}H-^{15}N correlation experiment of 100 μM ^{15}N-labeled Aβ40 immediately after dissolving the peptide (i.e. time point zero) in the absence (blue) and presence (red) of GroEL (7.1 μM); addition of Het-s displaces GroEL-bound Aβ40 resulting in restoration in the intensity of the amide proton envelope of the Aβ40 spectrum.

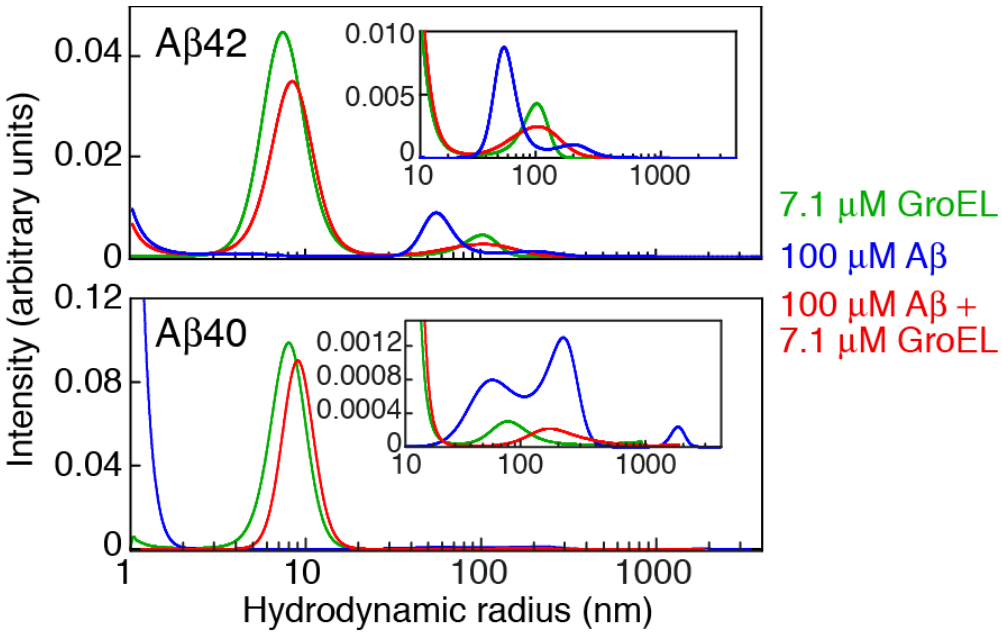

Figure S5. Dynamic light scattering $I(R_h)$ profiles. The hydrodynamic radii (R_h) of Aβ42 (top) and Aβ40 (bottom) in the absence of GroEL (blue lines) are indicative of polydisperse high molecular weight species, which decrease to 10.0 ± 0.8 nm and 9.8 ± 0.1 nm for Aβ42 and Aβ40, respectively, upon addition of GroEL (red lines). These radii are very close to that of GroEL alone (R_h = 8.74 ± 0.06 nm; green lines). The measurements were performed at room temperature immediately after dissolving the Aβ peptides in 20 mM sodium phosphate (pH 7.4).

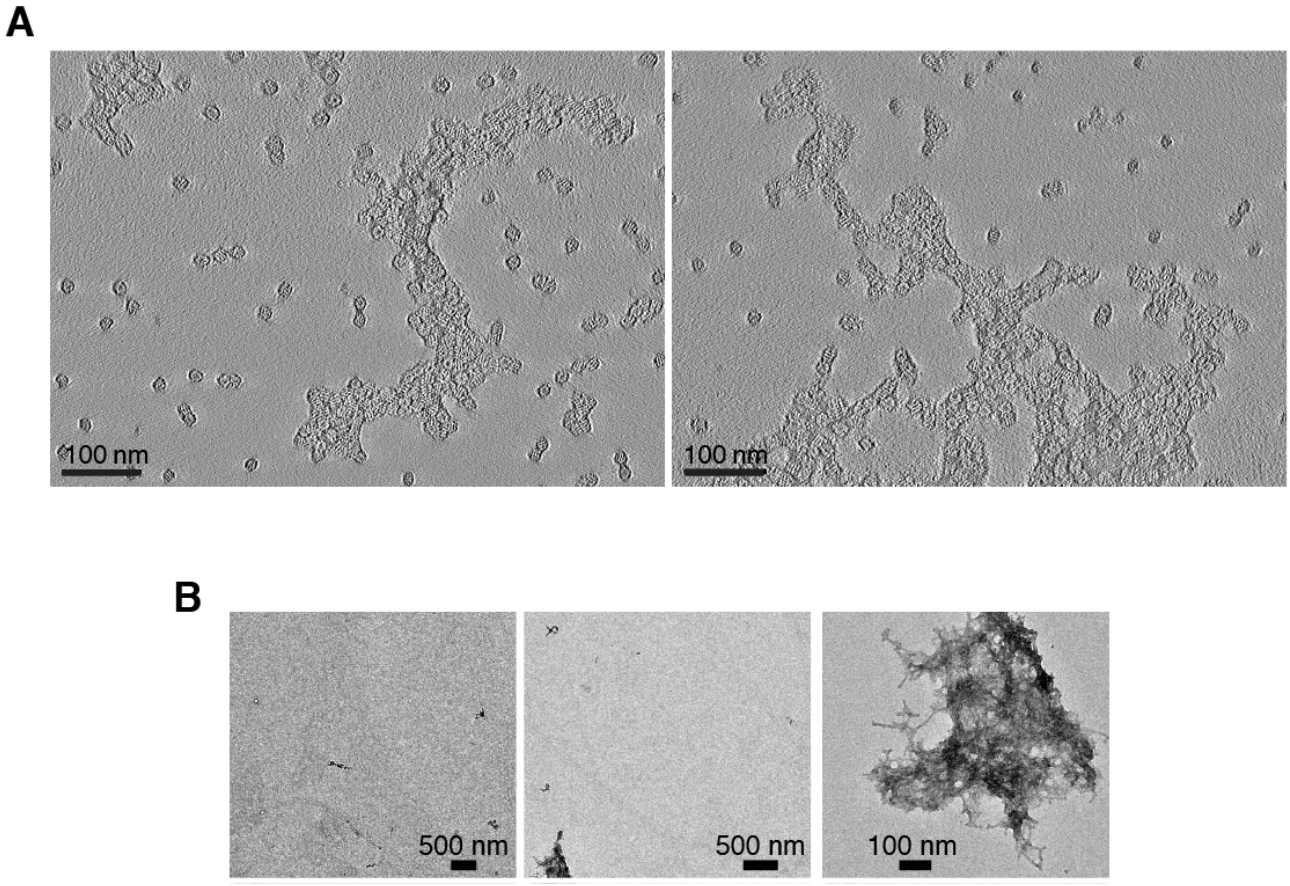

Figure S6. Electron tomography of a GroEL/Aβ42 mixture. (A) Negative stain electron tomogram images at two different slices of 100 μM Aβ42 in the presence of 7.1 μM GroEL immediately after dissolving Aβ42. No aggregates, protofibrils or fibrils of Aβ42 are found between the GroEL molecules in any of the tomogram slices, indicating that Aβ42 binds to GroEL as monomeric or small oligomeric species. Note that some clusters of GroEL are found both in the absence and presence of Aβ42 and are possibly an artefact of blotting GroEL on the hydrophobic surface of the EM grids. (B) Electron micrographs of GroEL alone showing that GroEL can form small clusters on the EM grid. GroEL, stored at a concentration of 36 μM at 4 °C, was diluted to 0.36 μM and blotted on the grid for analysis.

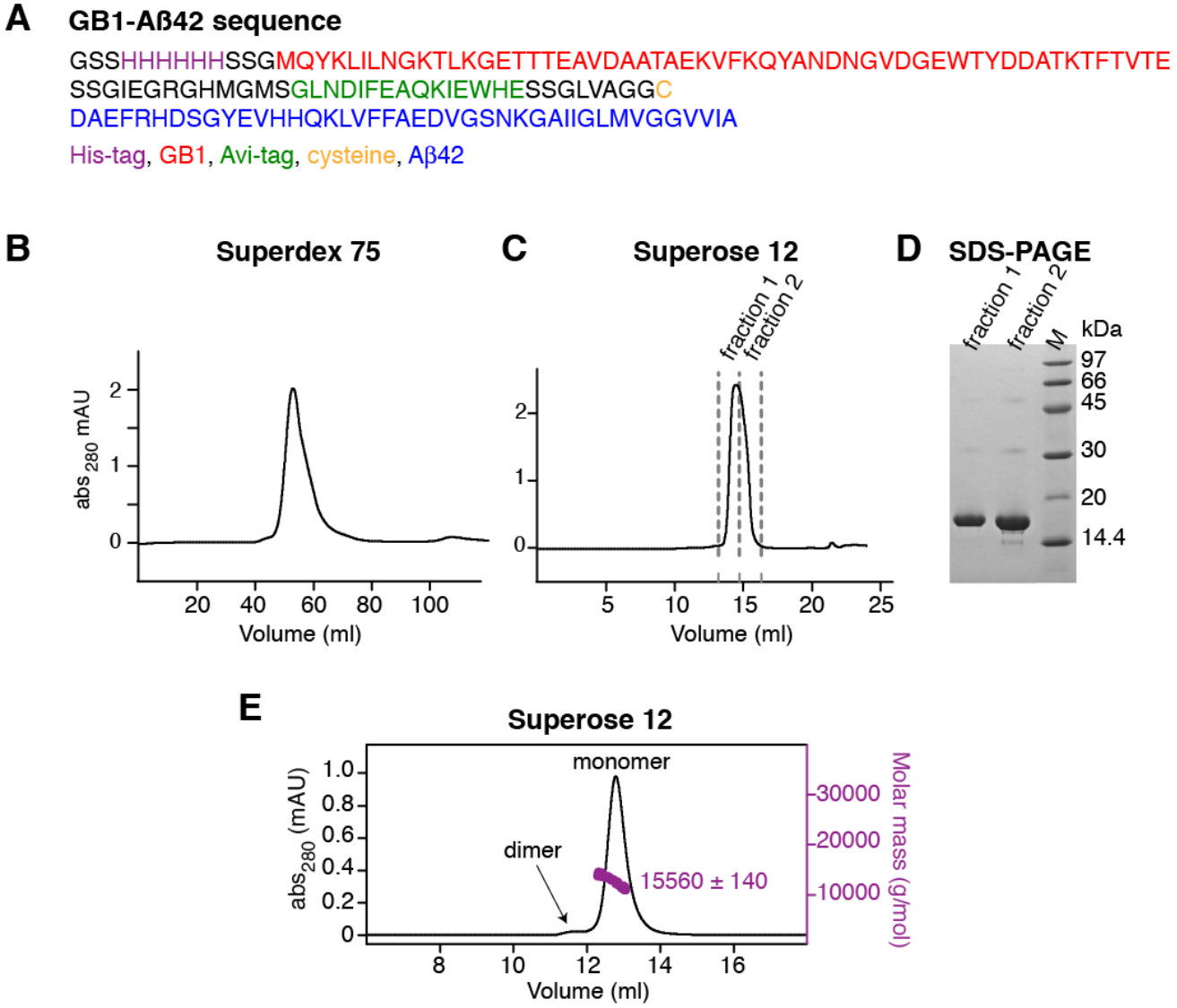

Figure S7. GB1-Aβ42 construct. (A) Sequence of GB1-Aβ42. (B) Elution profile of the last purification step of the GB1-Aβ42 purification obtained by size exclusion chromatography (HiLoad 16/60 Superdex 75) equilibrated in 8 M urea, 50 mM Tris (pH 8.0) and 20 mM imidazole and run at a flow rate of 1.5 ml/min. (C) Elution profile of GB1-Aβ42 on a Superose 12 column, used for buffer exchange into 20 mM sodium phosphate (pH 7.4) and 25 mM NaCl. (D) SDS-PAGE gel electrophoresis of the two fractions shown in panel C. M is the molecular weight standard (GE Healthcare catalog number 17-0446-01) with masses indicated in kDa. (E) SEC-MALS analysis of purified GB1-Aβ42. 250 µg GB1-Aβ42 was injected onto a Superose 12 column at a flow rate of 0.5 ml/min. The sample elutes primarily (95%) as a monomer with a mass of 15560±140 Da with a small fraction of dimer (5%) which is readily separated by gel filtration. The running buffer was 20 mM sodium phosphate (pH 7.4) and 25 mM NaCl.

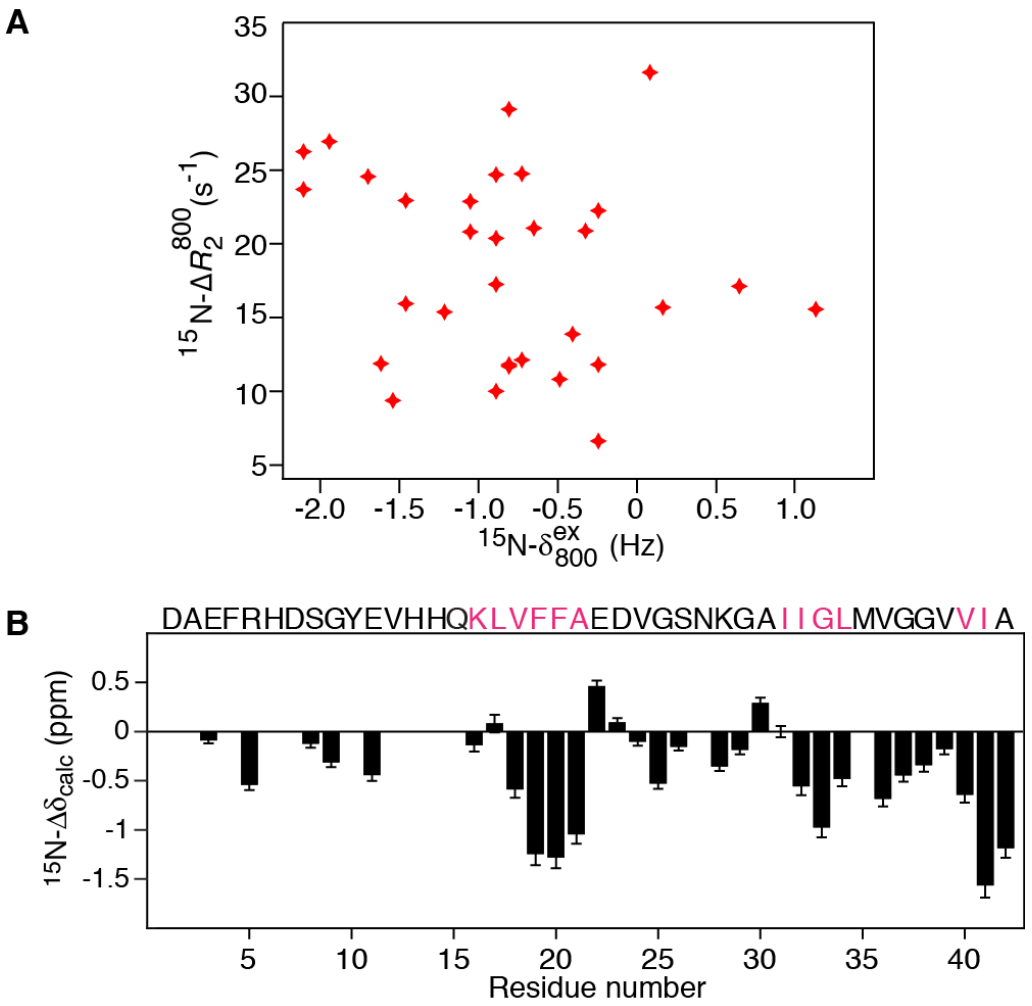

Figure S8. Backbone ^{15}N exchange-induced shifts. (A) Correlation plot showing that ^{15}N-lifetime line-broadening (ΔR_2) and exchange-induced shifts (δ_{ex}) measured at 800 MHz upon addition of 8.6 μM GroEL to 50 μM Aβ42 are not correlated to one another. (B) Optimized values of the ^{15}N chemical shift differences between GroEL-bound and free states of Aβ42 obtained from the global fits of a two-site exchange model to the NMR data (see Figures 5 and 6 of main text). The sequence is shown above the plot and the residues in magenta are those exhibiting the largest ^{15}N-R_2 values in the bound state (see Fig. 6B, main text).

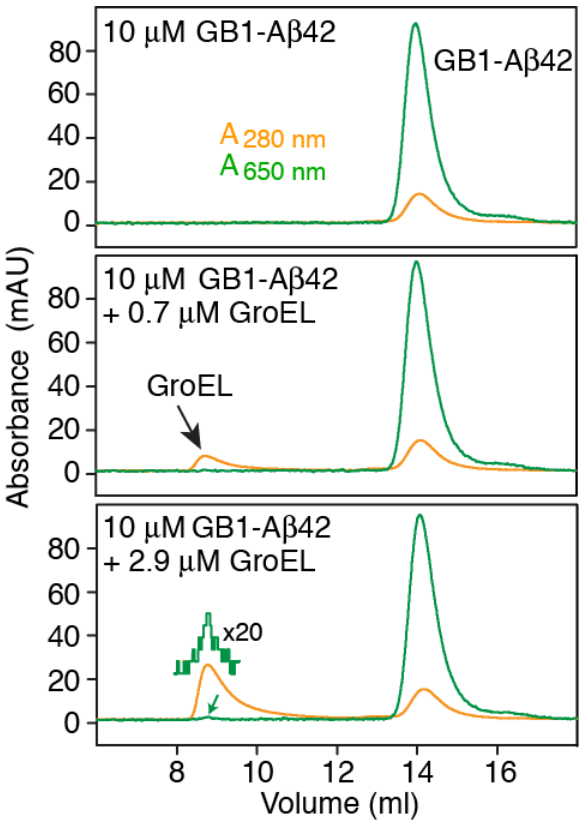

Figure S9. Size exclusion chromatography of AL647-GB1-Aβ42 in the absence and presence of GroEL. 100 µl of a 10 µM solution AL647-GB1-Aβ42 alone (top) and with 0.7 (middle) and 2.9 µM (bottom) GroEL was loaded onto an analytical Superose 12 column (1 x 30 cm) at a flow rate of 0.7 ml/min in 25 mM sodium phosphate (pH 7.4) and 25 mM NaCl. Eluted proteins were detected by absorbance at 280 and 650 nm. The latter wavelength is specific to the AL647 fluorescent tag and hence only detects AL647-GB1-Aβ42. A very small (~1%) fraction of AL647-GB1-Aβ42 co-migrates with GroEL (green arrow, bottom panel), consistent with the K_D value of ~0.3 mM determined from analysis of the NMR data (see Figures 5 and 6 of main text). (Note that the use of higher concentrations of GroEL on an analytical column is undesirable if column resolution is to be maintained, given that 2.9 µM GroEL corresponds to ~2.4 mg/ml of protein.)

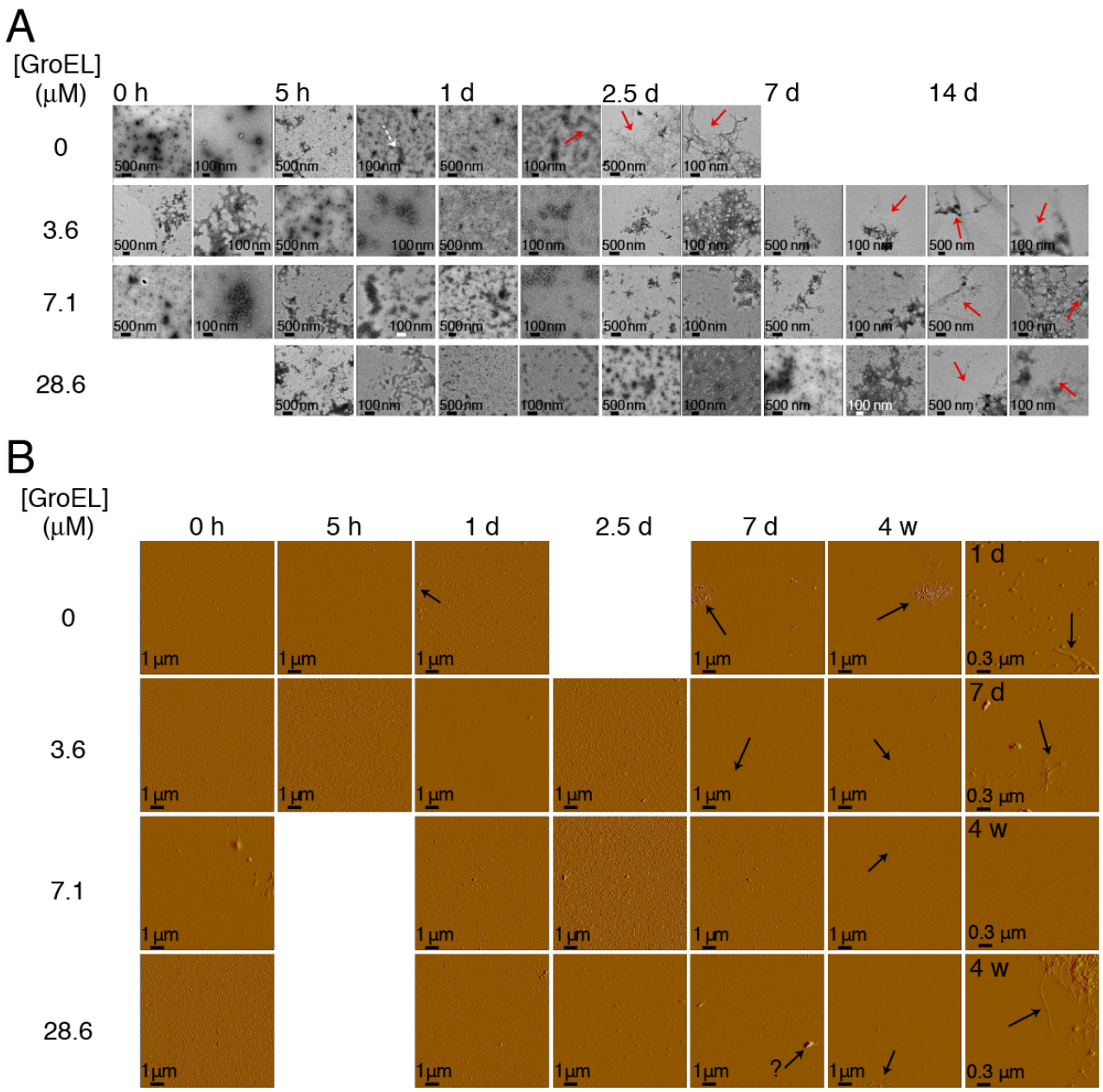

Figure S10. (A) EM and (B) AFM images of Aβ42 aggregation over time in the presence of varying amounts of GroEL. 100 μM Aβ42 was allowed to aggregate at room temperature under quiescent (unshaken) conditions in the presence of varying amounts of GroEL. Samples were taken at different time points and subject to EM and AFM analysis. For the EM the samples were diluted to individual concentrations ranging from 1 to 100 μM, optimized for each sample, and blotted on the grid. For AFM samples were diluted to 5 μM Aβ42 and blotted on mica. In the EM images (panel A) in the absence of GroEL, Aβ42 is seen to form protofibrils after 5 hours (white dashed arrow), and some fibrils are apparent at 1 day (red arrow). Protofibrils or fibrils of Aβ42 are only seen at 7 days (red arrows) in the presence of 3.6 μM GroEL and at 14 days in the presence of 7.1 or 28.6 μM GroEL. In the AFM images (panel B) fibrils (black arrows) are apparent after 1 day in the absence of GroEL, at 7 days in the presence of 3.6 μM GroEL, and at 4 weeks in the presence of 7.1 or 28.6 μM GroEL.

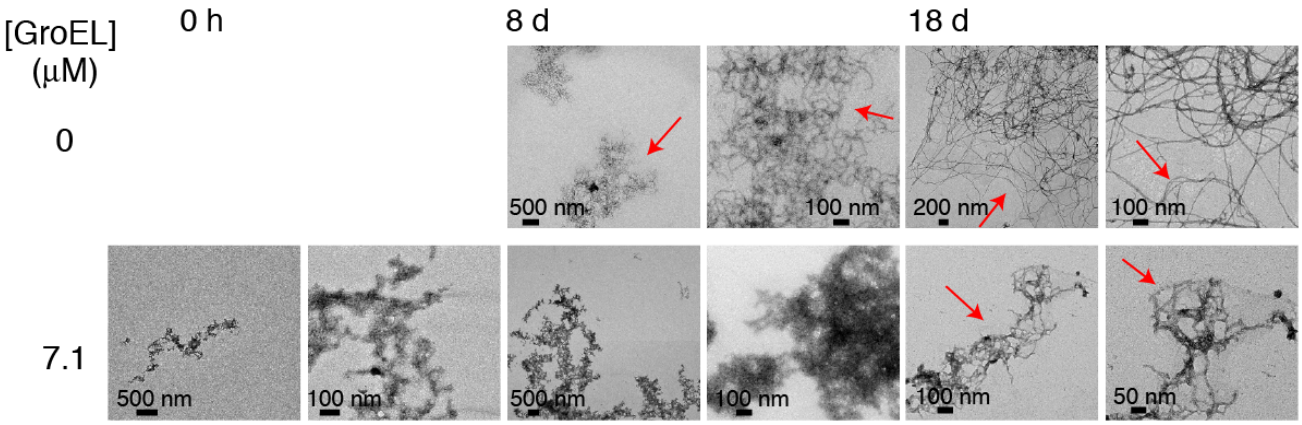

Figure S11. Electron micrographs of Aβ40 aggregation over time in the absence and presence of GroEL. 100 μM Aβ40 was allowed to aggregate at room temperature under quiescent conditions (i.e. not shaken) in the absence (top row) and presence (bottom row) of 7.1 μM GroEL. Samples taken at different times were diluted to a concentration of 5 μM and blotted on EM grids. Fibrils of Aβ40 (indicated by the red arrows) are formed after 8 days in the absence of GroEL (top), but are only visible after 18 days with GroEL present (bottom).

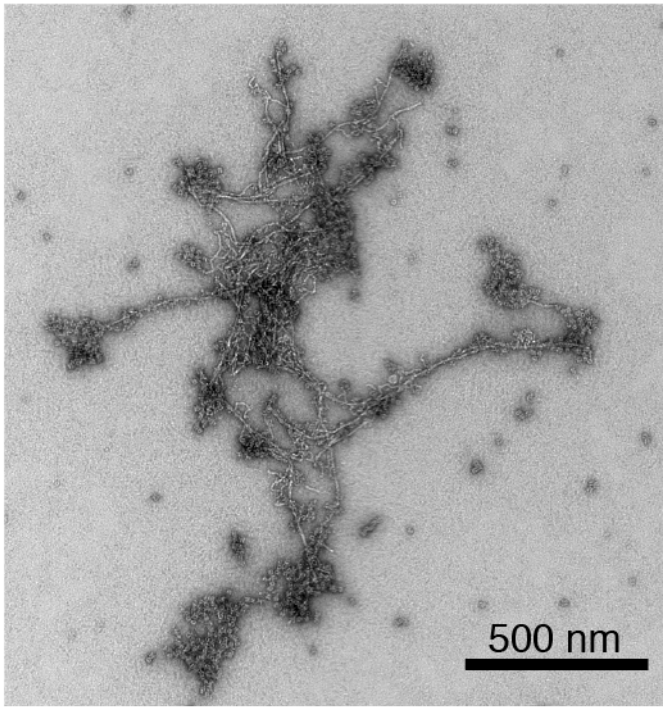

Figure S12. Electron micrograph of 100 μM Aβ42 fibrils incubated with 7.1 μM GroEL. The sample was washed three times by spinning down at a maximum speed with a table top centrifuge and dissolved in water before blotting on the grid.