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Supplementary Materials for

Selective targeting of primary and secondary nucleation pathways in Aβ42 aggregation using a rational antibody scanning method

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/3/6/e1700488/DC1)

- movie S1 (.avi format). Representative video clip of the Aβ42 *C. elegans* worms GMC101 at day 7 upon treatment with empty vesicles at days 1 and 3 (AP1, early treatment).
- movie S2 (.avi format). Representative video clip of the Aβ42 *C. elegans* worms GMC101 at day 7 upon treatment with empty vesicles at day 6 (AP2, late treatment).
- movie S3 (.avi format). Representative video clip of the control *C. elegans* worms N2 at day 7 upon treatment with empty vesicles at day 6 (AP2, late treatment).
- movie S4 (.avi format). Representative video clip of the Aβ42 *C. elegans* worms GMC101 at day 7 upon treatment with $DesAb_{18–25}$ at days 1 and 3 (AP1, early treatment).
- movie S5 (.avi format). Representative video clip of the Aβ42 *C. elegans* worms GMC101 at day 7 upon treatment with $DesAb_{29–36}$ at days 1 and 3 (AP1, early treatment).
- movie S6 (.avi format). Representative video clip of the Aβ42 *C. elegans* worms GMC101 at day 7 upon treatment with $DesAb_{18–25}$ at day 6 (AP2, late treatment).
- movie S7 (.avi format). Representative video clip of the Aβ42 *C. elegans* worms GMC101 at day 7 upon treatment with $DesAb_{29–36}$ at day 6 (AP2, late treatment).

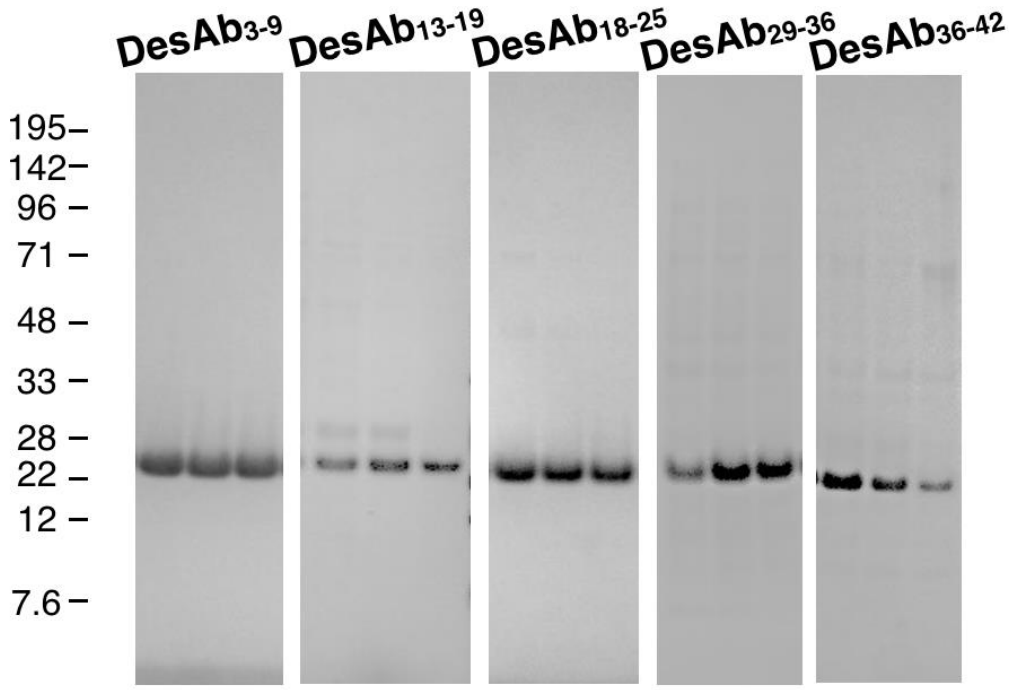

fig. S1. Purified DesAbs used in this study. SDS-PAGE analysis of the protein fractions obtained from an exemplary purification of the different DesAbs. Only the purest fractions were used in other experiments.

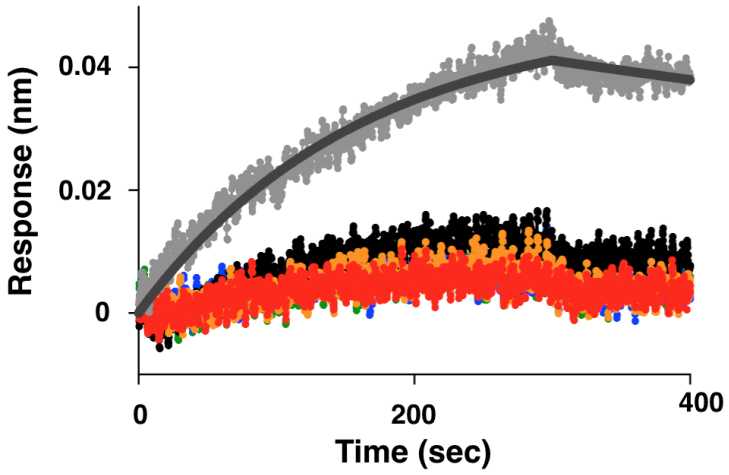

fig. S2. BLI analysis of the interaction of different DesAbs with monomeric α-synuclein. As expected no binding is detected. Color code: $DesAb_{3-9}$ (black), $DesAb_{13-19}$ (orange), $DesAb_{18-25}$ (blue), $DesAb_{29-36}$ (green), $DesAb_{36-42}$ (red) and DesAb-F, a DesAb that targets α-synuclein (grey).

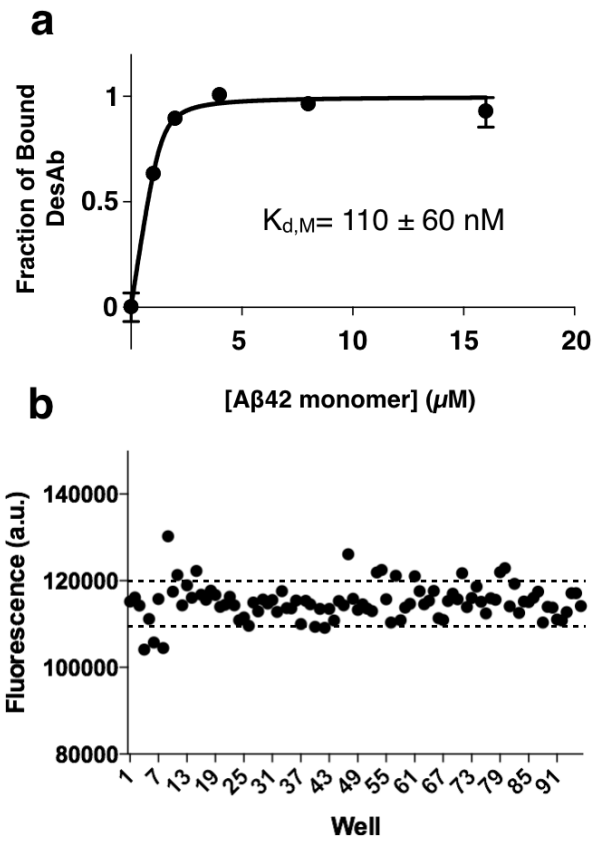

fig. S3. Biotin-mediated affinity measurement of DesAb$_{3-9}$ binding to monomeric Aβ42 and setup of the experimental conditions. (**a**) Binding curve of DesAb$_{3-9}$ to monomeric N-terminal biotinylated Aβ42. The fit resulted in a K_d value of 110±60 nM. (**b**) Quantification by fluorescence measurements of the level of Alexa488-streptavidin coating the wells of a representative ELISA plate used for the biotin-mediated affinity measurements. Dashed lines represent standard deviation of the average value of fluorescence.

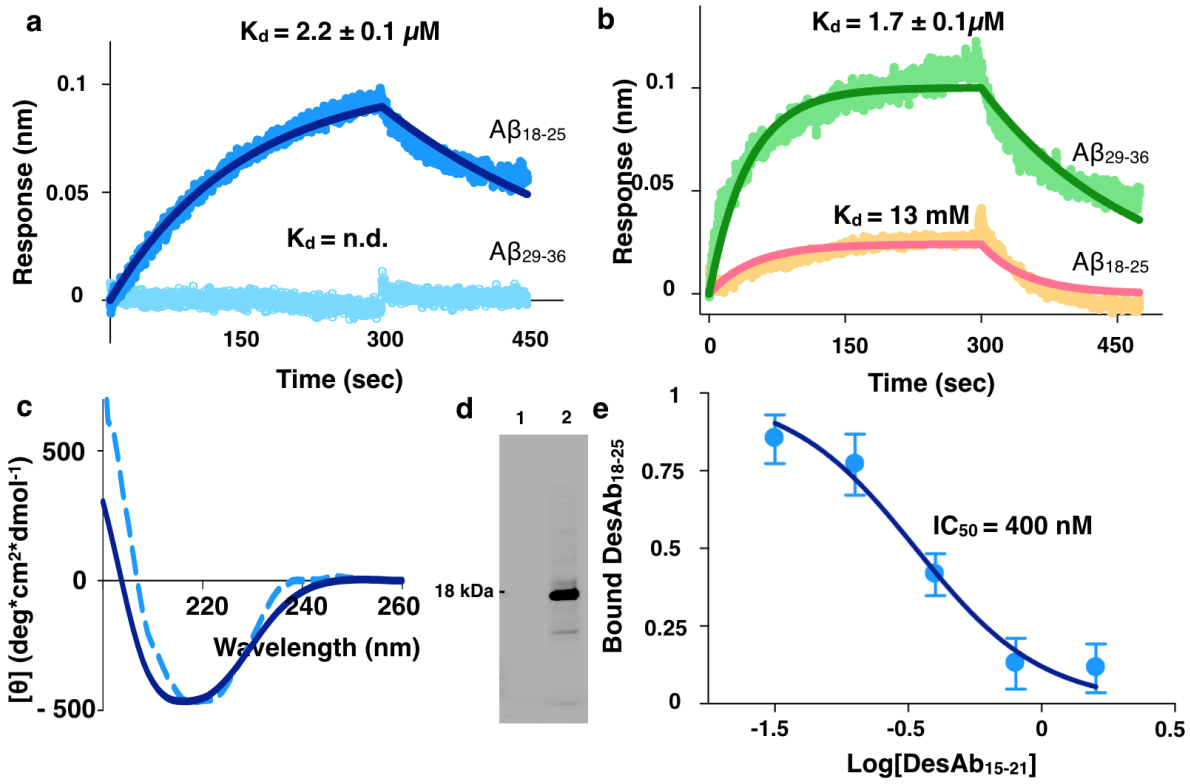

fig. S4. DesAb binding specificity assessment and interaction of DesAb$_{18-25}$ and DesAb$_{29-36}$ with the respective target peptides. (**a** and **b**) BLI measurements at 6 μM of (a) target peptide Aβ$_{18-25}$ with DesAb$_{18-25}$ and (b) target peptide Aβ$_{29-36}$ with DesAb$_{29-36}$. Color code: binding of DesAb$_{18-25}$ with Aβ$_{18-25}$ (blue), binding of DesAb$_{29-36}$ with Aβ$_{29-36}$ (cyan), binding of DesAb$_{29-36}$ with Aβ$_{29-36}$ (green) and binding of DesAb$_{29-36}$ with Aβ$_{18-25}$ (gold). Solid lines represent the fitting of the data; K_d values are reported when data allowed a reliable fitting. (**c**) CD spectrum of Alexa647-labeled DesAb$_{18-25}$ (solid blue line) compare to unlabeled DesAb$_{18-25}$ (dashed cyan line). (**d**) SDS-PAGE of DesAb$_{18-25}$ before and after labeling. The gel was imaged using excitation and emission set up for Alexa647 using a Typhoon Trio. (**e**) Alexa647-DesAb$_{18-25}$/DesAb$_{15-21}$ competition assay.

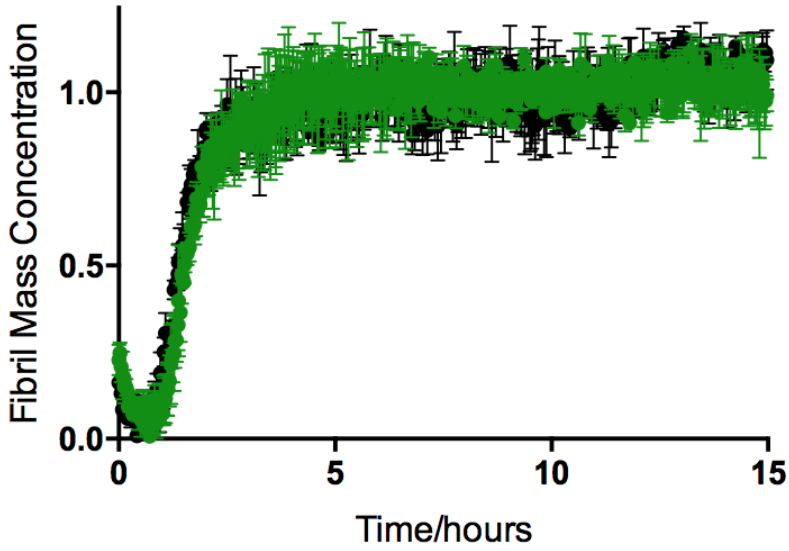

fig. S5. A DesAb designed to target α-synuclein does not inhibit Aβ42 aggregation. ThT-aggregations experiments of 2 μM Aβ42 peptide alone (black line) and in the presence of DesAb-F, an antibody designed to target α-synuclein (green line, Table 1). The overlap of the two ThT traces indicates that DesAb-F does not have any effect on Aβ42 aggregation.

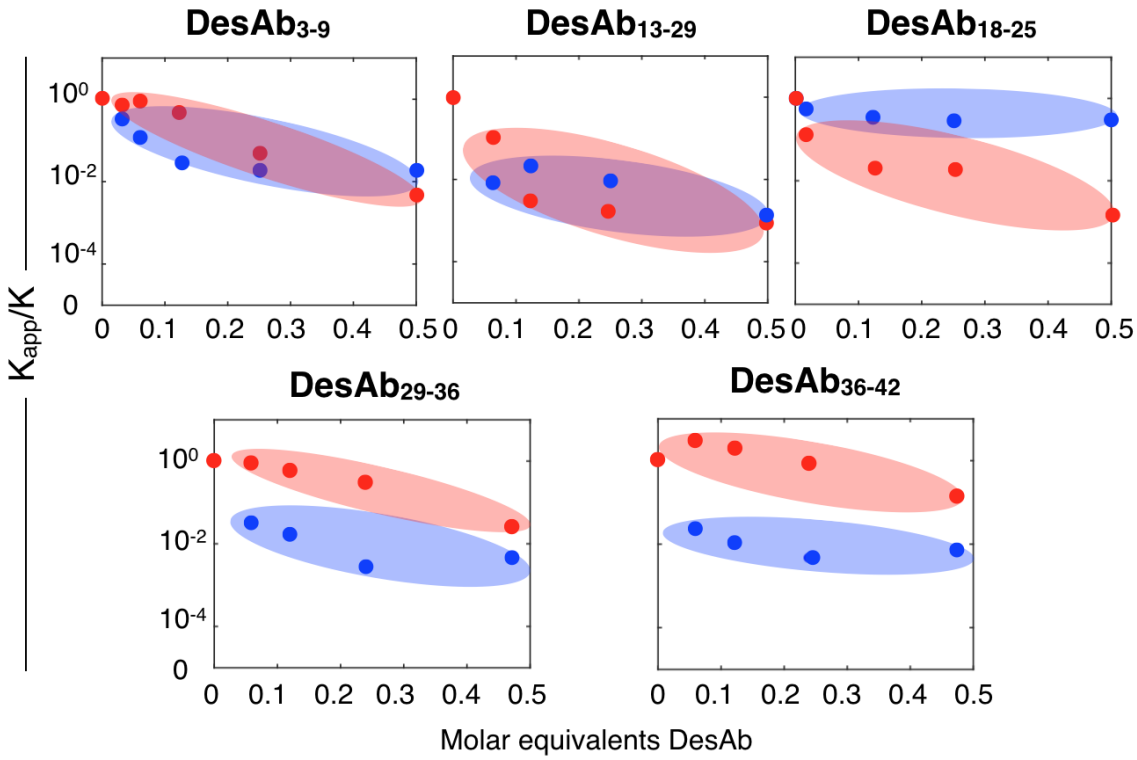

fig. S6. Effect of the DesAbs on the global parameters k_+k_n and k_+k_2 of Aβ42 aggregation. Decrease of the global parameters k_+k_n (red) and k_+k_2 (blue) evaluated from the fit as a function of the relative antibody concentration shown in Fig. 3a.

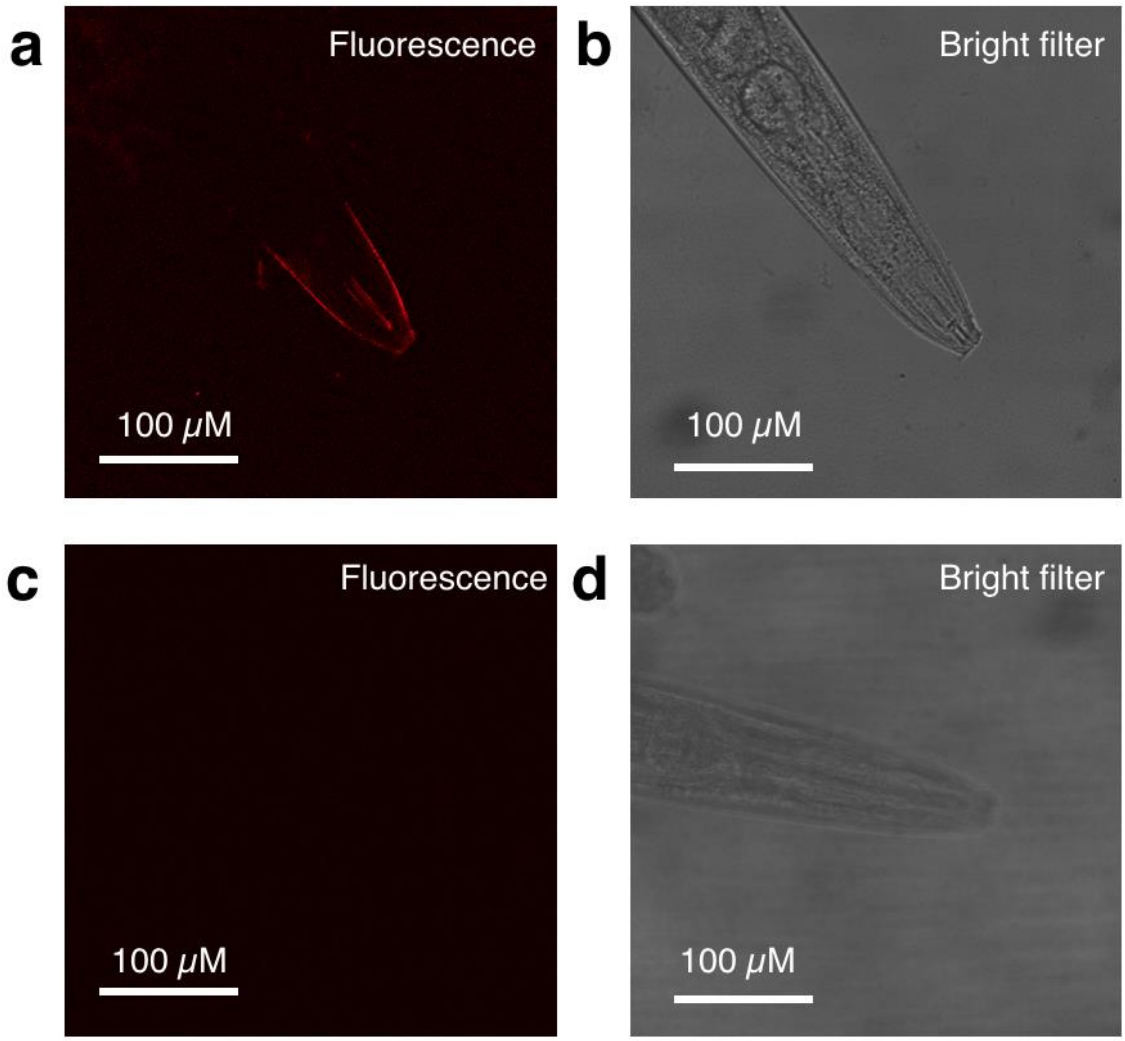

fig. S7. Transduction of the fluorescent protein mCherry into wild-type worms. *Wild type* worms were transducted overnight with 20 μM of mCherry protein encapsulated into lipid vesicles and then imaged after washing using confocal microscopy. (**a** and **b**) Fluorescence and bright filter images of the magnified head of a worm transducted with the protein mCherry. (**c** and **d**) Same images of a wild type worm subjected to the same treatment in the absence of mCherry.

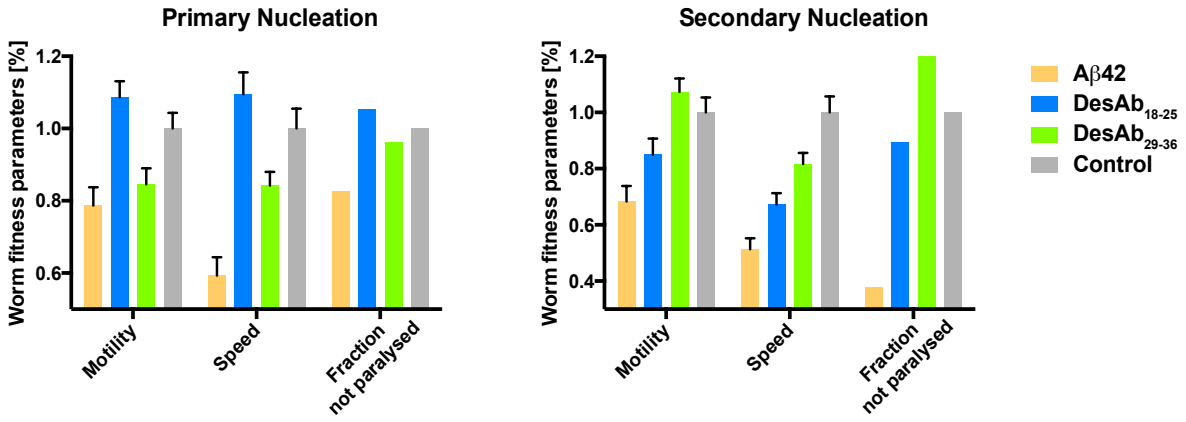

fig. S8. Effects of DesAb$_{18-25}$ and DesAb$_{29-36}$ treatments on the *C. elegans* worms. Bar plots showing the changes of the phenotypic parameters (motility, speed and fraction not paralysed) used for the fingerprint analysis and the total fitness estimation shown in Fig. 4.

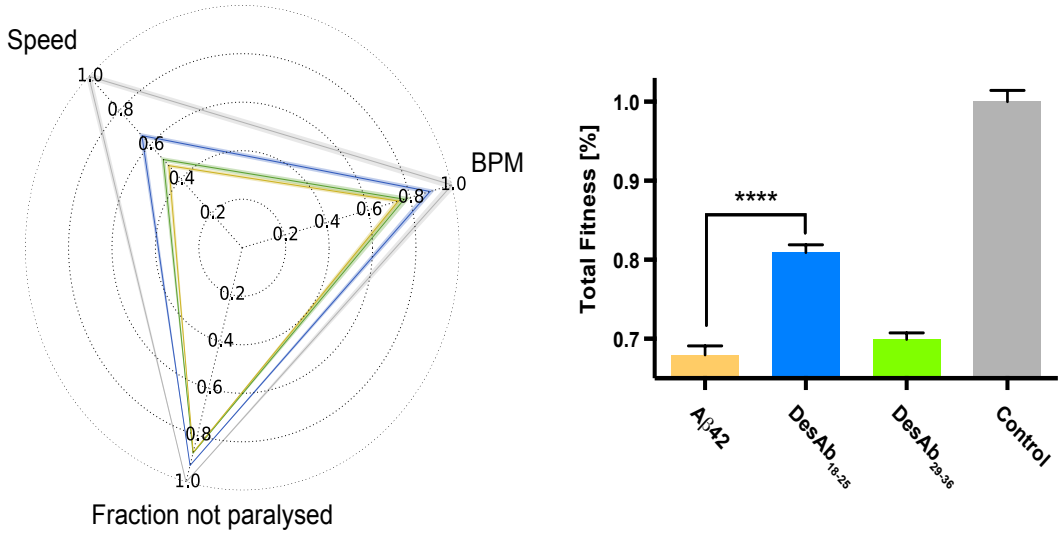

fig. S9. Fingerprints of the Aβ42 worms screened at day 4 of adulthood. Phenotypic fingerprints of *C. elegans* N2 (grey) and *C. elegans* GMC101 treated with empty lipid vesicles (yellow), *C. elegans* GMC101 treated with DesAb$_{29-36}$ (orange) and DesAb$_{18-25}$ (green). Worms are screened at day 4 of adulthood after administration of the antibodies at days 1 and 3.

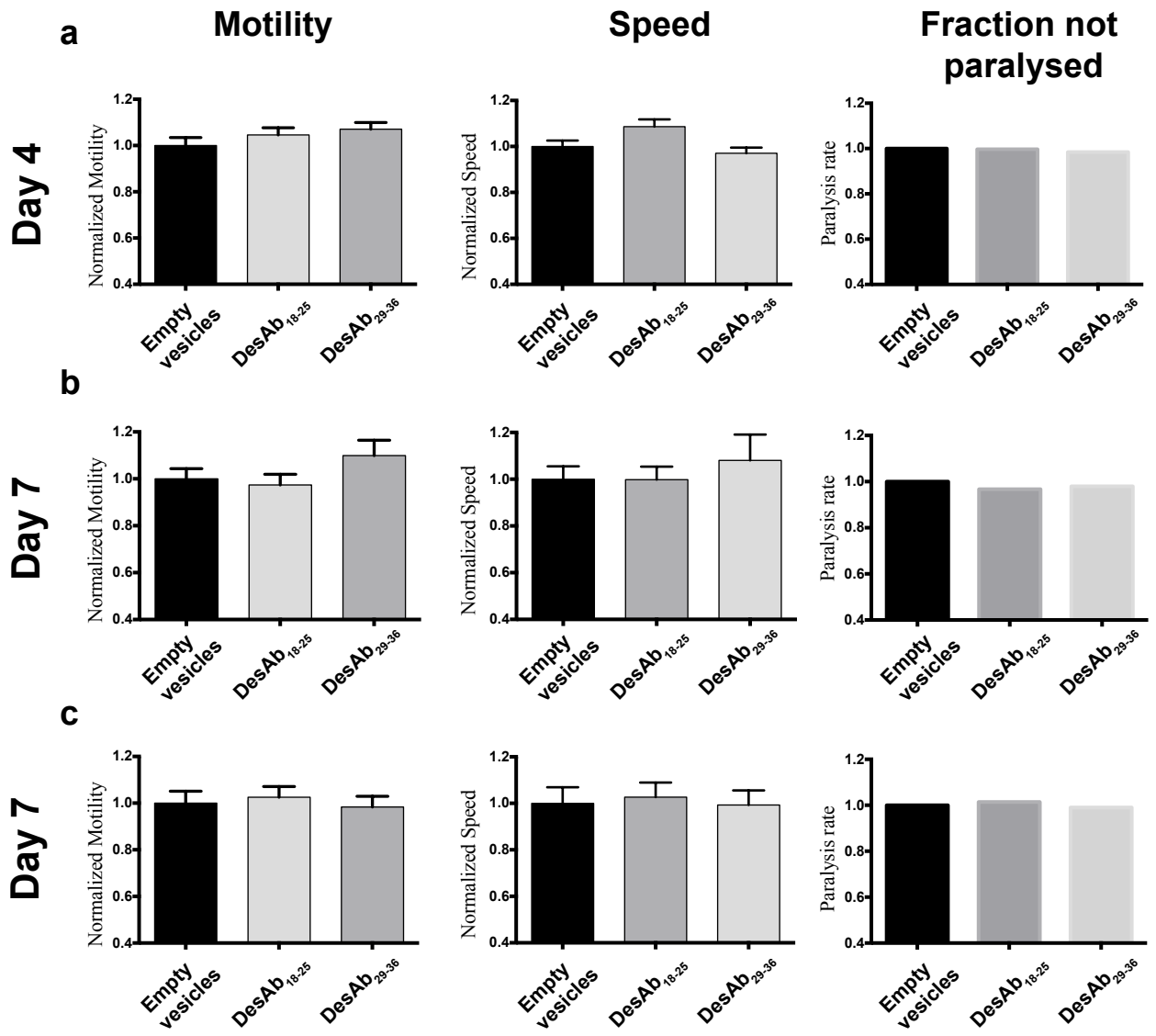

fig. S10. Effects of DesAb$_{18-25}$ and DesAb$_{29-36}$ treatments on wild-type control worms. Bar plots of the motility, speed and fraction of worms that are not paralysed (survival fraction) of *wild type* N2 worms treated with empty vesicles or with vesicles containing DesAb$_{29-36}$ or DesAb$_{18-25}$. Bar plots of measurements at day 4 (**a**) and day 7 (**b**) after double administration at days 1 and 3. (**c**) Bar plot of measurements at day 7 after administration of the antibodies at day 6. The plots are representative of three replicates, which show similar results and errors represent standard error on the mean (SEM).

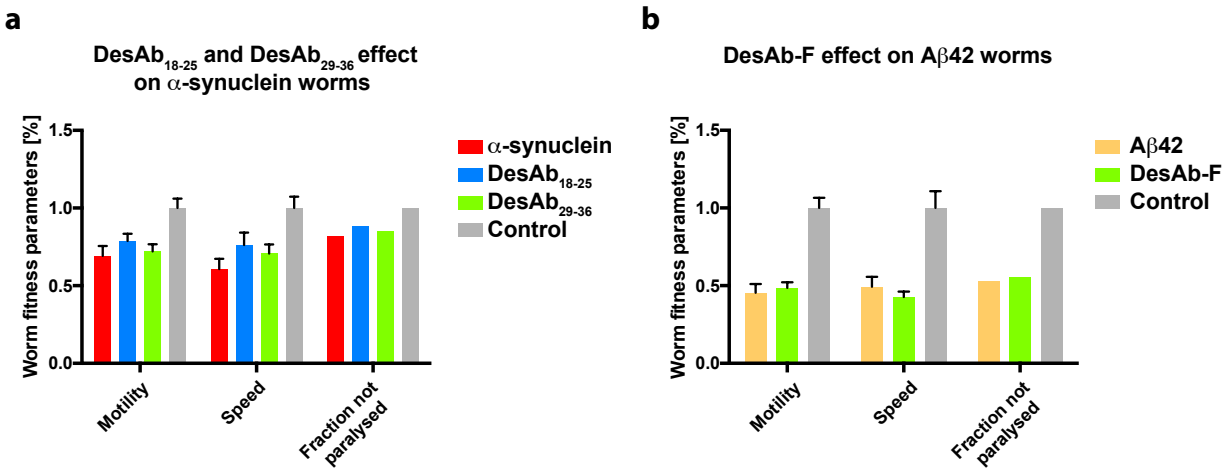

fig. S11. Analysis on the specificity of the treatment with the DesAbs in *C. elegans*. (**a**) Effect of the administration of DesAb18-25 and DesAb29-36 in α-synuclein *C. elegans* worms (OW40). (**b**) Effect of the administration of DesAb-F (designed to bind α-synuclein) in Aβ42 *C. elegans* worms (GMC101). In both panels phenotypic measurements are carried out at day 7 of adulthood following administration at day 6. No significant effect is present in both experiments. The plots are representative of three replicates, which show similar results and errors represent standard error on the mean (SEM).

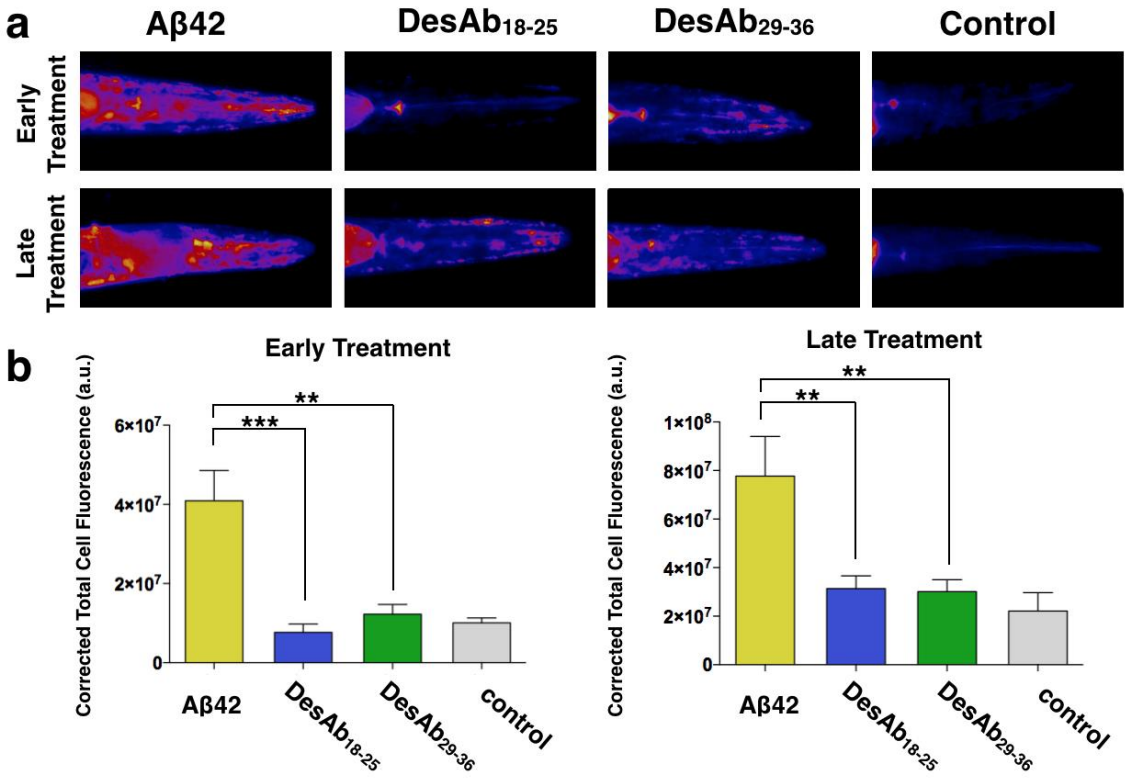

fig. S12. Effects of DesAb$_{18-25}$ and DesAb$_{29-36}$ treatments on the aggregation of Aβ42 in *C. elegans* models. (**a**) Fluorescence microscopy images of the Aβ42 aggregates in Aβ42 *C. elegans* worms (GMC101) at day 10 of adulthood, obtained by using the amyloid-specific fluorescent probe NIAD-4. The effect of the AP1 (early treatment) and the AP2 (late treatment) of DesAb$_{18-25}$ and DesAb$_{29-36}$ is shown. Untreated Aβ42 worms and control worms (N2) are shown for comparison. (**b**) Bar plot representing the fluorescence associated to the different quantification of the aggregates in the different treatment conditions (early and late treatment) for the control *C. elegans* worms N2 (grey) and the Aβ42 *C. elegans* model GMC101 (yellow) treated in the absence of antibodies, the Aβ42 *C. elegans* model GMC101 after the administration of DesAb$_{18-25}$ (blue) and DesAb$_{29-36}$ (green). The errors represent standard error on the mean (SEM).

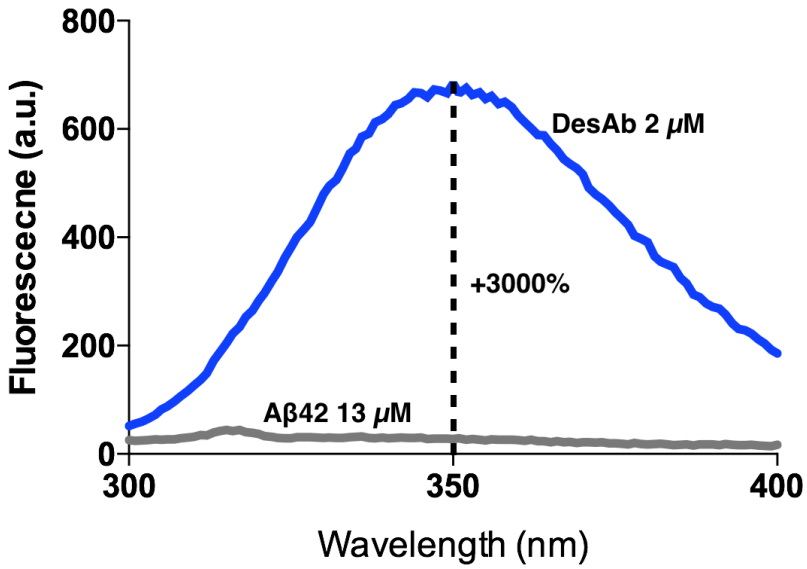

fig. S13. Difference between the spectrum of DesAb$_{18-25}$ and the background. The spectra of 2 µM DesAb$_{18-25}$ and of 13 µM Aβ42 are shown in blue and in grey, respectively. Both protein samples were incubated in streptavidin-coated wells blocked with BSA as described in materials and methods.