SUPPLEMENTARY MATERIAL

The APP C-terminal fragment C100 occurs in monomeric and dimeric stable conformations and binds gamma-secretase modulators

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Supp. Fig. 1: CD and ESR spectra of F19C in DOC buffer resemble those in the presence of SDS.

A - B: C100 F19C was purified from inclusion bodies with the urea buffer containing 2% DOC (instead of Triton x-100 and SDS), labeled with 10-fold molar excess of MTSL in the presence of 0.02 mM TCEP and 0.1% DOC and concentrated on a centrifugal filter device using washing buffer containing 0.1% DOC. Both CD (A) and ESR (B) spectra for DOC resemble those measured in the presence of SDS.

Supp. Fig. 2: Optimization of spin labeling conditions.

A - B: C100 F19C labeled with 10-fold molar excess of MTSL spin label in the presence of varying concentrations of TCEP (A) or S59C labeled with 10-, 20- or 80-fold molar excess of spin label in the presence of 0.02 to 0.05 mM TCEP (B), separated on an SDS-PA gel under non-reducing conditions and stained with Coomassie Brilliant Blue. To differentiate between disulfide-bridged and SDS-resistant dimers, a labeled control was monomerized by addition of 2 mM TCEP (ctr). C100 was labeled with equal efficiency (as indicated by equal ratios of monomer:dimer) in the presence of 0.02 to 0.2 mM TCEP and a molar MTSL excess of 10-fold to 20-fold.

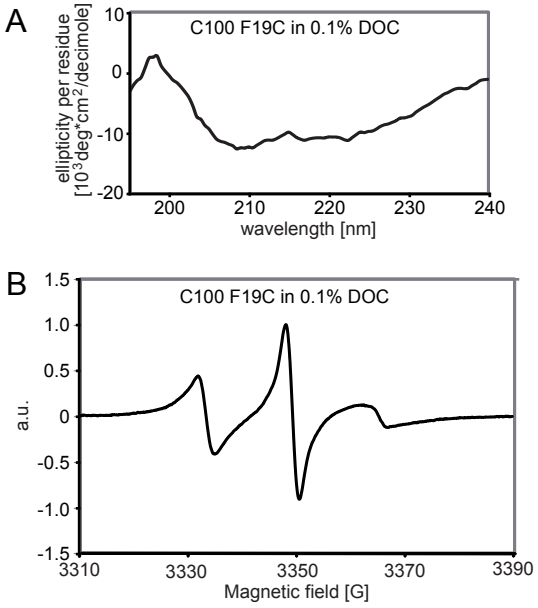
C: Continuous wave electron paramagnetic resonance (cw-ESR) spectra of C100 S59C. C100 was labeled with 10- to 20-fold MTSL excess in the presence of 0.02 mM TCEP or 80-fold MTSL excess in the presence of 0.05 mM TCEP. After elution, free label and TCEP were removed by washing on a centrifugal filter device. Arrows indicate peaks of free spin label at 20x and 80x MTSL (a.u.: arbitrary units).

Supp. Fig. 3: Equal mobility of spin label coupled to C100 mutants L17C and F19C indicates identical oligomerization states.

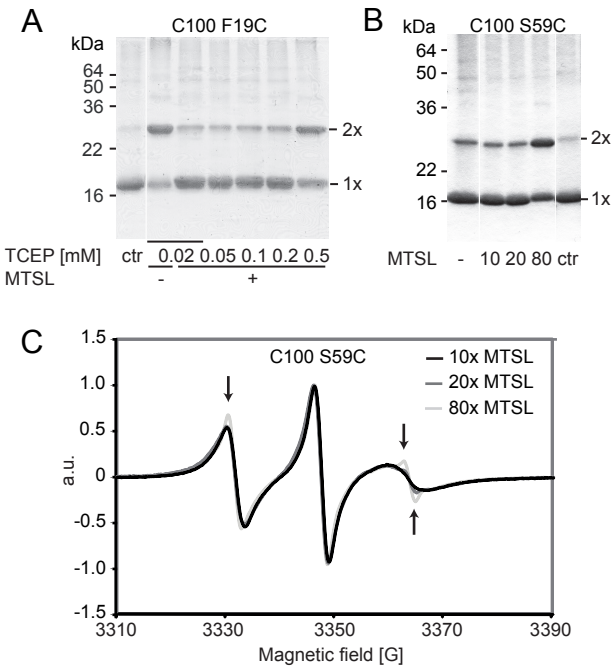
Continuous wave electron paramagnetic resonance (cw-ESR) spectra of C100 L17C and F19C. C100 was labeled with a 10-fold MTSL excess in the presence of 0.02 to 0.2 mM TCEP. Spectra shown are the mean of 5 (L17C) and 7 (F19C) independent measurements (a.u.: arbitrary units).

Supp. Fig. 4: SPR analysis of sulindac sulfide binding to C100 G33I and L17C.

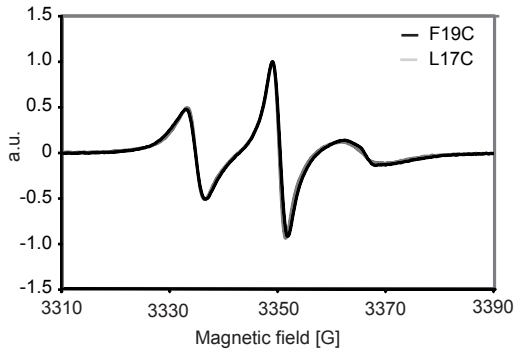
Interaction of sulindac sulfide with C100 mutants determined by SPR analysis. Overlays of representative SPR sensorgrams obtained from injections of sulindac sulfide after coupling of C100 mutants L17C or G33I to the NTA sensor chip. Sulindac sulfide was injected for 60 s at a flow rate of 30 µl/min at indicated concentrations. All binding curves were double-reference subtracted from DMSO buffer blank and the reference flow cell. Sensorgrams shown are the mean of 3 independent measurements. Binding of 50 µM sulindac sulfide to each C100 mutant is set as 100%.



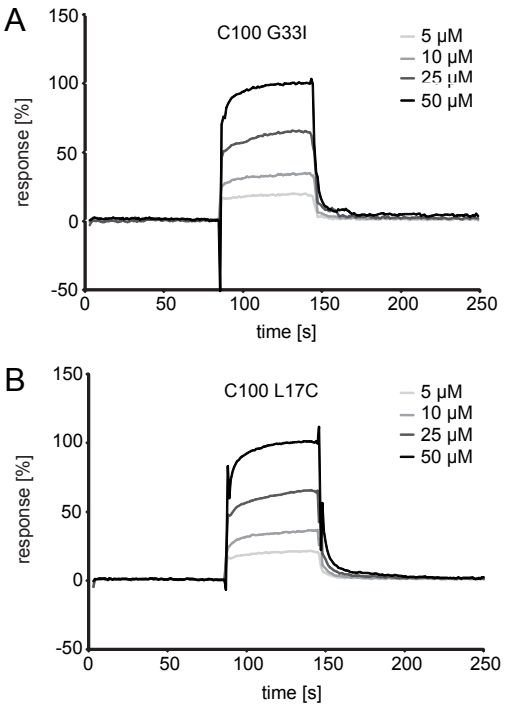
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4