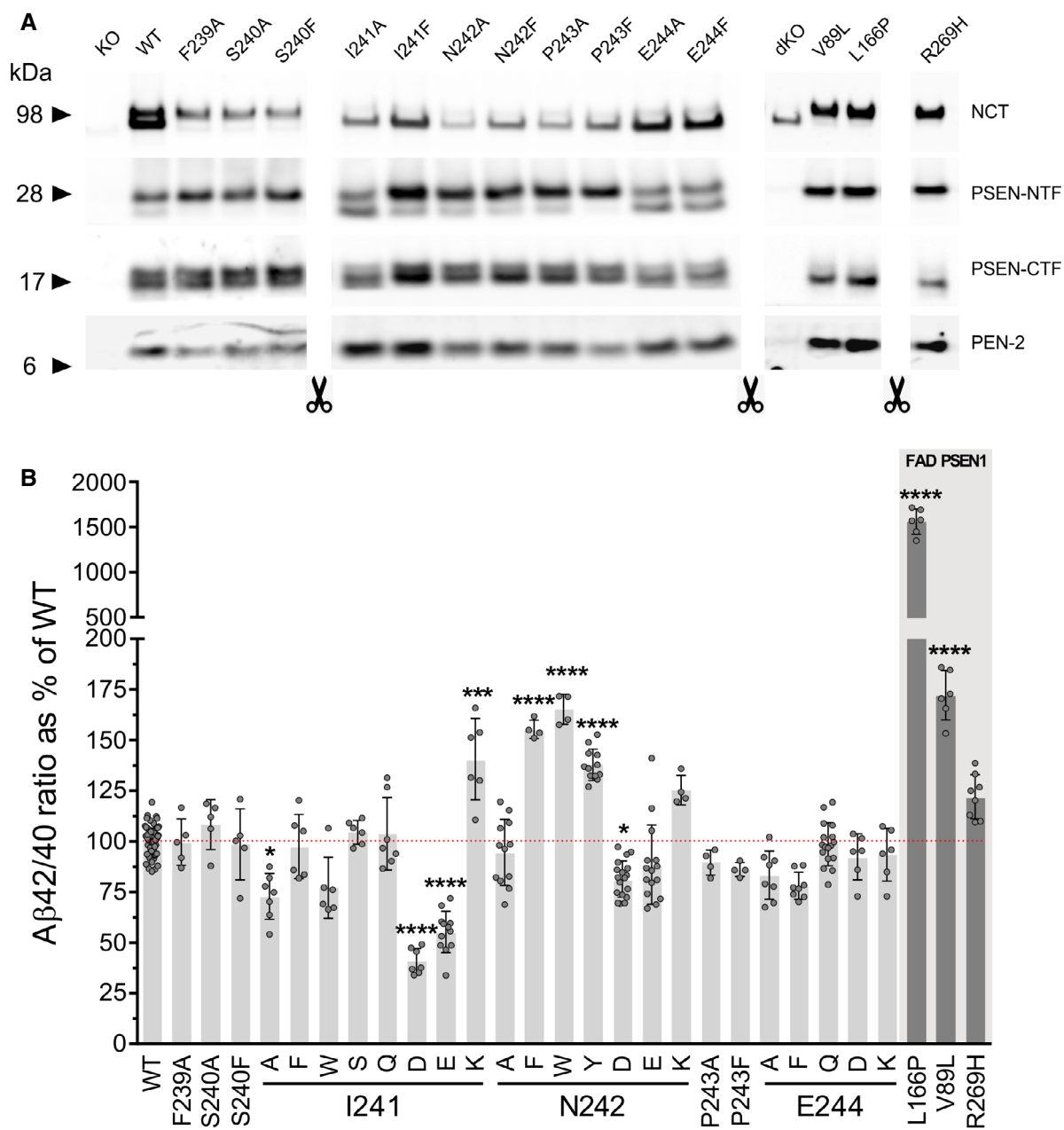


## Expanded View Figures

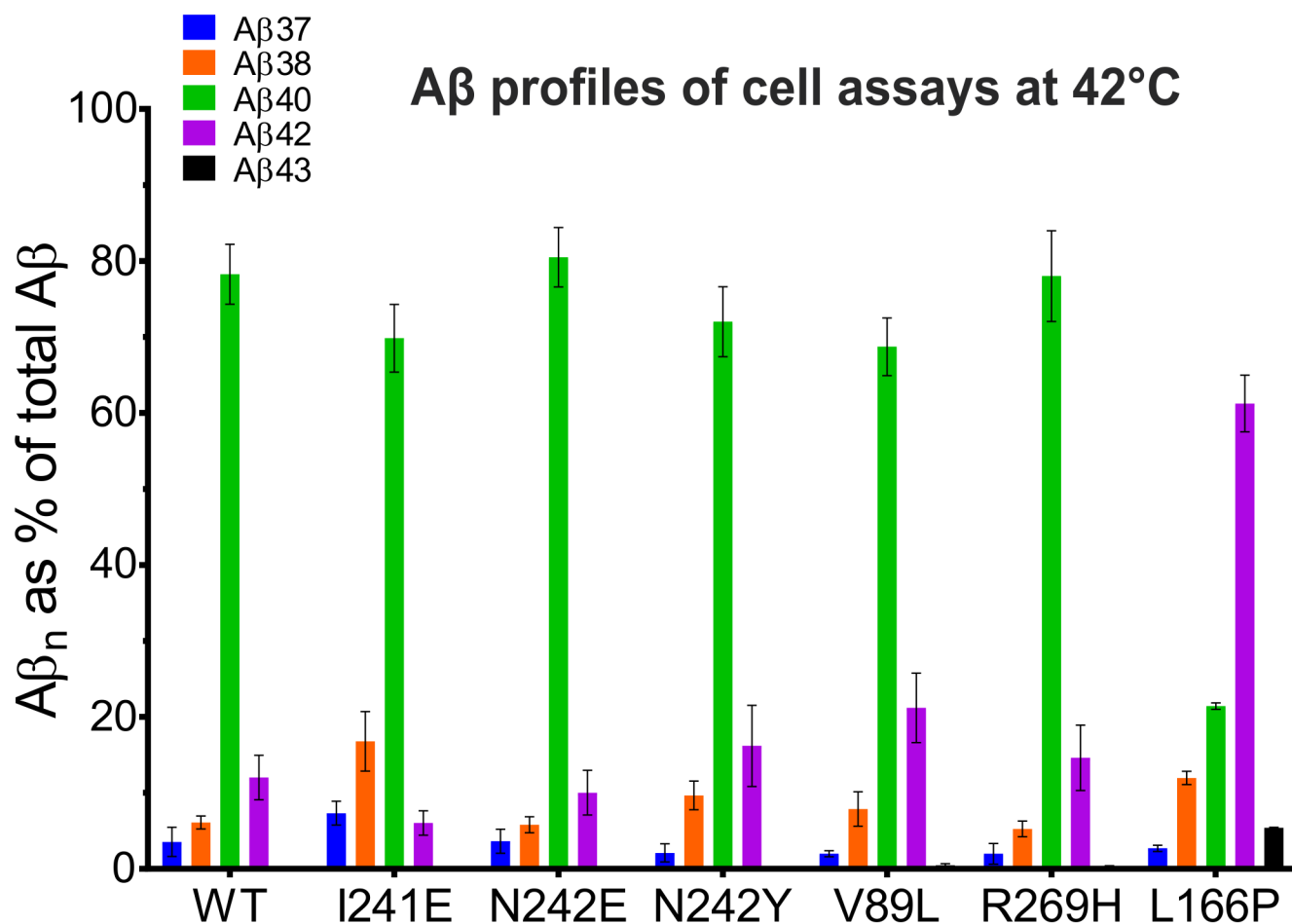


**Figure EV1. NCT ectodomain regulates the Aβ 42/40 ratio.**

**A** Representative SDS-PAGE/Western blot analysis of CHAPSO-solubilized membrane proteins from *Ncstn*<sup>-/-</sup> or *Psen1*<sup>-/-</sup>/*Psen2*<sup>-/-</sup> MEF cell lines stably expressing WT or mutant mouse NCT/human PSEN1 subunits (shown in Fig 2A). The presence of mature, glycosylated NCT, N-terminal, and C-terminal fragments of the endoproteolyzed PSEN1 and PEN-2, compared to NCT knock-out (KO) or PSEN1/2 double KO (dKO) cells, indicates that WT and mutants reconstitute GSEC complexes. Arrowheads indicate the position of molecular weight markers.

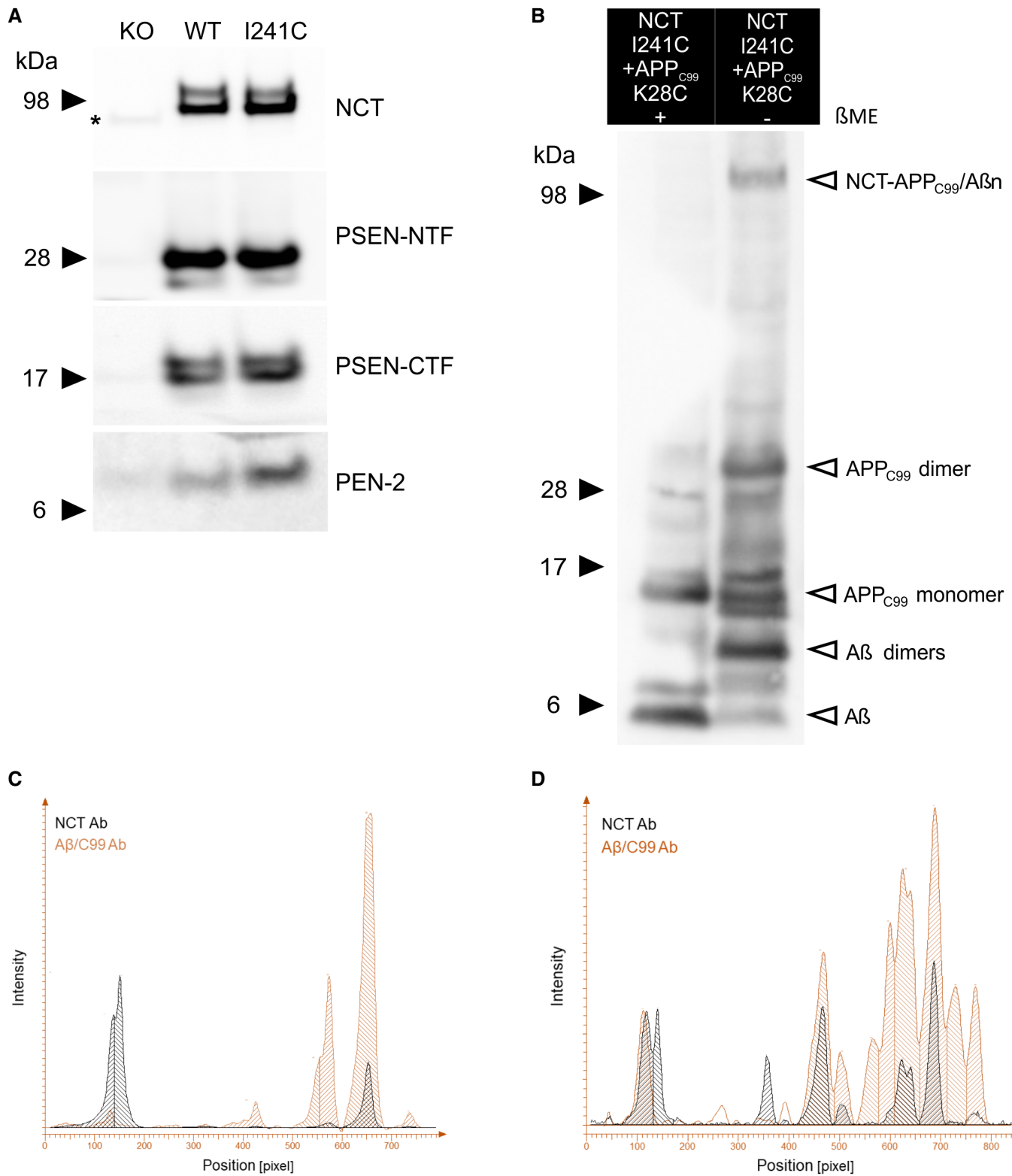
**B** Aβ peptides present in the extracellular media of cultured WT or mutant mNCT/hPSEN1 MEF cell lines, transiently expressing APP<sub>C99</sub> substrate, were quantified by ELISA, and the Aβ 42/40 ratio were determined and plotted as % of the WT condition. The data are represented as mean ± SD, *N* ≥ 4 independent experiments. One-way ANOVA and Dunnett's post hoc test were used to determine the statistical significance; \*\*\**P* > 0.001 and \*\*\*\**P* > 0.0001 (compared to WT condition). *F*(DFn, DFd): *F*(31, 290) = 873.6.

Source data are available online for this figure.



**Figure EV2. Secreted A $\beta$  profiles in cell-based thermoactivity assays.**

A $\beta$  profiles (% contribution of individual A $\beta$ 37, A $\beta$ 38, A $\beta$ 40, A $\beta$ 42, and A $\beta$ 43 peptides to the total A $\beta$  levels (A $\beta$ 37 + 38 + 40 + 42 + 43)) in the conditioned medium collected from WT or mutant (NCT) MEF cells lines co-expressing WT or mutant APP<sub>C99</sub> substrates, subjected to temperature challenge, were assayed by ELISA. The contribution of the A $\beta$ 43 peptides to the secreted A $\beta$  pool is < 1% for the WT or mildly destabilizing FAD mutants (V89L and R269H). Data are represented as mean  $\pm$  SD,  $N \geq 3$ .



**Figure EV3. Cross-linking data demonstrate a short-distance interaction between NCT 241 and APP<sub>C99</sub> K28.**

- A Representative SDS–PAGE/Western blot analysis of CHAPSO-solubilized membrane proteins extracted from *Ncstn*<sup>-/-</sup> MEF cell lines stably expressing WT or mutant mNCT-I241C subunits (shown in Fig 5). The presence of mature NCT, N-terminal, and C-terminal fragments of the endoproteolyzed PSEN1 as well as PEN-2, compared to NCT knock-out (KO) cells, indicates that WT and the I241C mutant reconstitute GSEC complexes. \*Non-specific band. Arrowheads indicate the position of molecular weight markers.
- B Representative SDS–PAGE/Western blot presenting NCT-APP<sub>C99</sub>/A $\beta$  and APP<sub>C99</sub> expression (as detected by 82E1 anti-A $\beta$  antibody) in detergent-extracted membranes prepared from MEF cell lines co-expressing respective NCT-I241C mutant GSEC complex with APP<sub>C99</sub>-K28C substrate. In the non-reduced condition (-  $\beta$ ME) bands corresponding to APP<sub>C99</sub> and/or A $\beta$  dimers are appearing, as indicated with arrowheads. To estimate the amount of substrate cross-linked to GSEC (i.e., spontaneous disulfide bond formation between APP<sub>C99</sub>/A $\beta$  and NCT), the NCT-APP<sub>C99</sub>/A $\beta$  band was quantified and normalized to “total APP/A $\beta$  expression” (defined as the sum of the bands indicated with arrowheads). Mean efficiency  $\pm$  SD = 15.4  $\pm$  5.7%, *N* = 3 independent experiments. Arrowheads indicate the position of molecular weight markers.
- C, D Analysis of the integrated density profiles on the full blots shown in Fig 5B (C) and 5E (D).

Source data are available online for this figure.

**Figure EV4. NCT ectodomain (N242) modulates the response toward imidazole-based GSEC modulators.**

- A A $\beta$  profiles generated by 0.3 and 1  $\mu$ M GSM (I-II-III) or vehicle control (0.1% DMSO)-treated mNCT mutant MEF cell lines (shown in Fig 6).
- B, C A $\beta$  37/A $\beta$ 40 and A $\beta$  38/A $\beta$ 42 ratios were quantified to evaluate the effect of GSM 0.3  $\mu$ M (B) or 1  $\mu$ M (C) treatment on the processivity of different mNCT mutant GSECs (shown in Fig 6). The A $\beta$  ratios are normalized to the response of the wild-type GSEC toward vehicle (0.1% DMSO condition) or GSM treatment. The arrows indicate the lack of response to GSM II of the N242F/Y substitutions.

Data information: Data are presented as mean  $\pm$  SD, *N*  $\geq$  3 independent experiments.

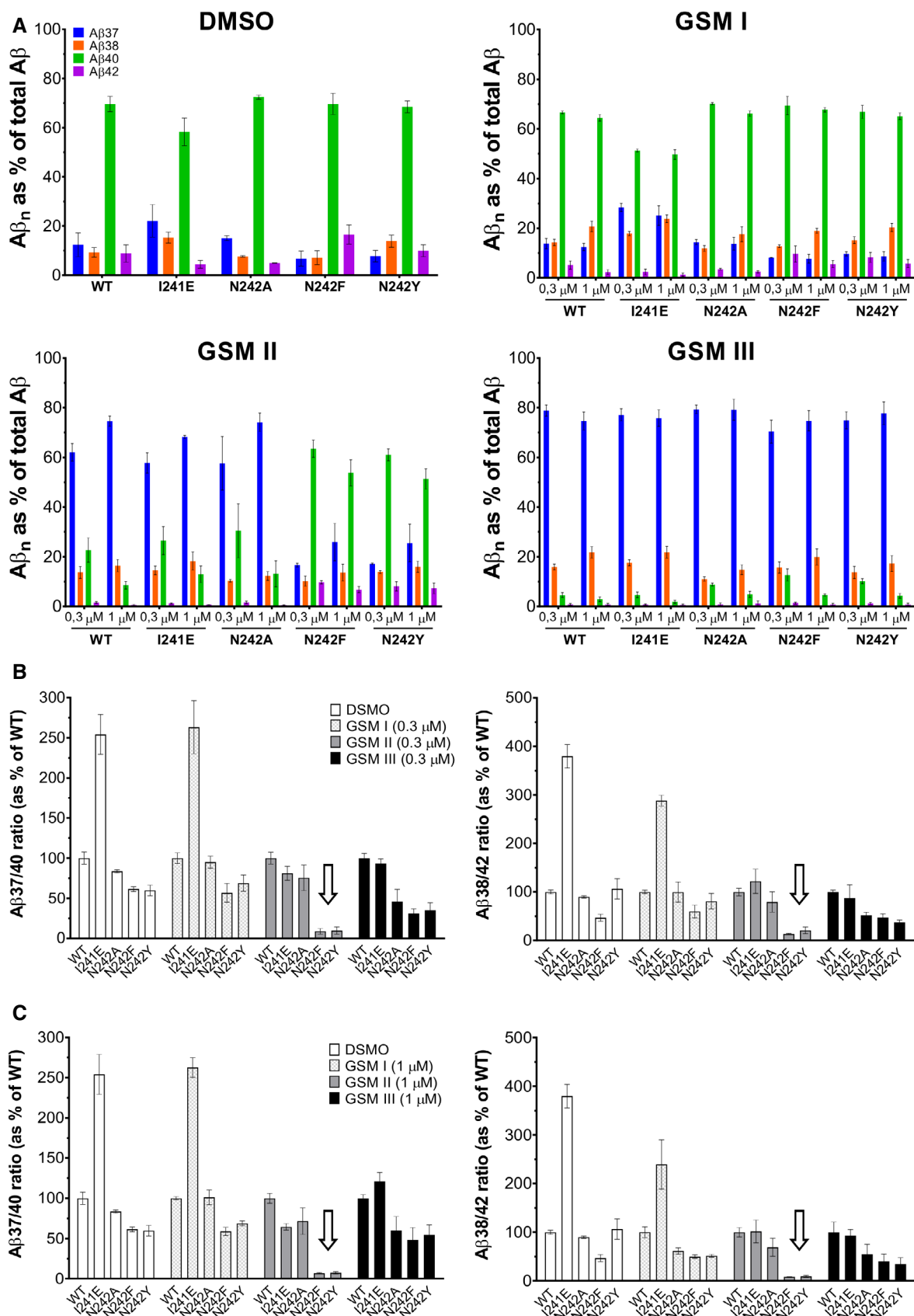
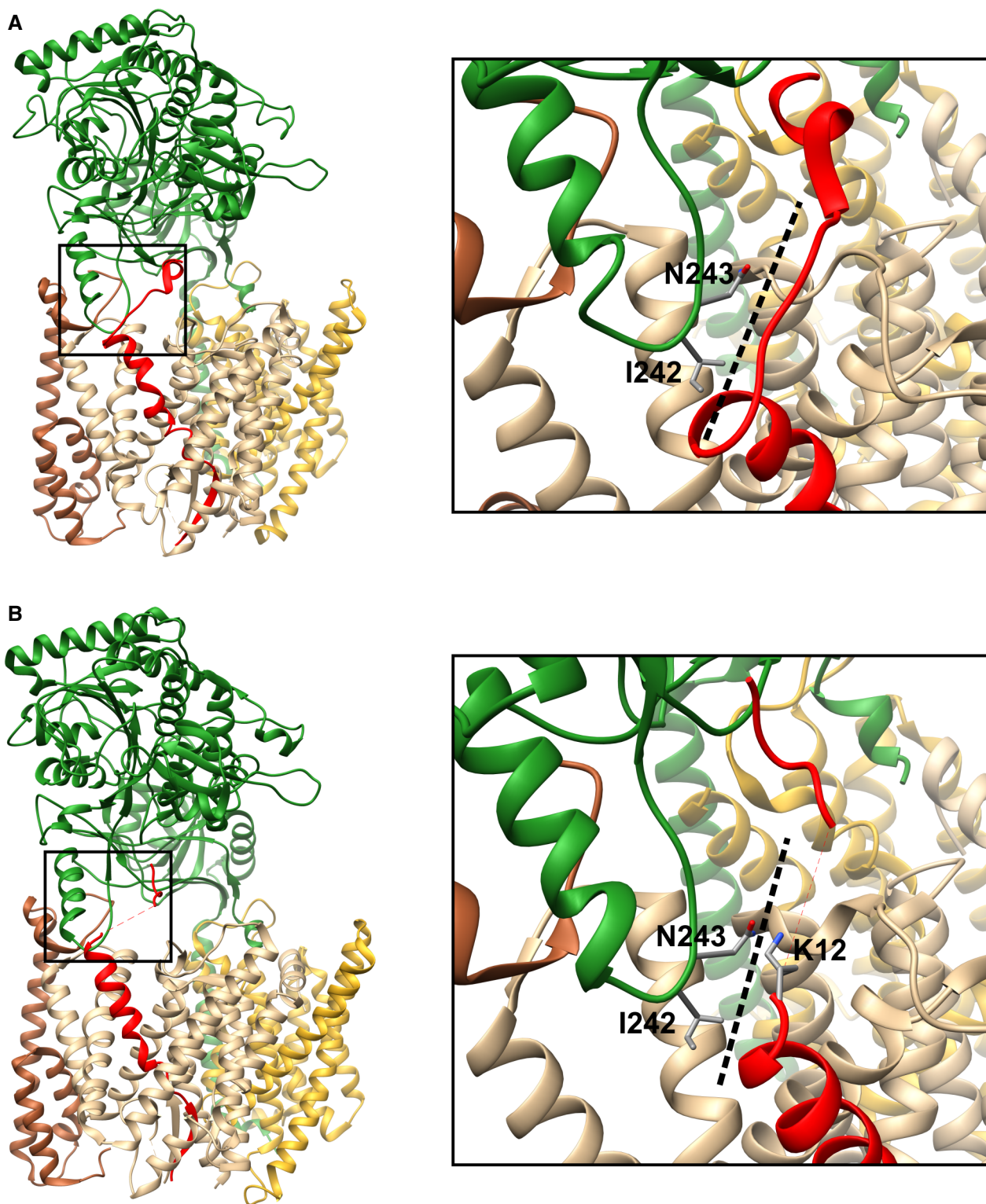


Figure EV4.



**Figure EV5. NCT-APP interface highlighted in the 6IDF and 6IYC GSEC-substrate co-structures.**

A, B GSEC co-structure with Notch (PDB:6IDF) (A) or APP<sub>C83</sub> (PDB:6IYC) (B) substrate is presented. PSEN1 is shown in light brown, PEN-2 in dark brown, APH1A in gold, NCT in green, and Notch/APP<sub>C83</sub> in red. The side chains of the NCT-I241 and NCT-N242 (together with K12 (corresponding to K28 in APP<sub>C99</sub>) in the APP<sub>C83</sub> structure) are shown, and the interface between Notch/APP<sub>C83</sub>, NCT ectodomain, and PSEN1 is magnified and marked with a dashed line.