Appendix

In silico modelling of GSM III interactions with GSEC-APP\textsubscript{C99}

To choose an appropriate target conformation for docking, we tracked the size of the putative modulator binding cavity (c.f. Figure 1C) in a 2 µs long, unrestrained simulation of the enzyme substrate complex using the MD-Pocket software (Schmidtke et al., 2010). To optimize the success of the GSM docking runs, we chose a structural snapshot with a maximum-sized binding cavity as ‘receptor’ for compound docking analysis using the Autodock Vina software (Trott and Olson, 2010). However, even the most spacious pocket conformation was too narrow to accommodate GSM III. In consequence, initial rigid sidechain/flexible ligand docking and subsequent 400ns simulations of the top two docking poses did not yield binding modes that could explain the mutagenesis data. Notably no interactions with Y106 were recorded.

Therefore, we applied the flexible ligand/flexible side chain docking technique to the problem, defining the side chains of the outlining residues mentioned above, as flexible. This produced a number of more promising docking poses for GSM III (higher degree of ligand burial). A set of promising and diverse docking poses were then chosen for 400ns of MD simulations. The resulting binding poses could, however, still not satisfyingly explain the extreme lowering of GSM response for the Y106F mutation.

Subsequently, we re-docked GSM III (rigid sidechain docking), choosing a snapshot of the simulation
with the maximum number of interactions between GSM III and PSEN1 residues outlining the putative pocket, as target receptor.

We again selected a diverse set from the resulting poses for 400ns unrestraint MD simulations. The GSM III simulations yielded two binding modes exhibiting pronounced interactions with all implied PSEN1 residues. Above all, these poses also exhibited stable hydrogen bonds with Y106. The only difference between the two models was the orientation of the methyl group on the central bicyclic ring of GSM III. In one case this methyl group pointed towards the substrate APP$_{C99}$ (GSM III_M1), while in the second binding mode, it was oriented in the direction of the receptor (GSM III_M2).

Analysis of the side-chain rotamers in these binding modes revealed that PSEN1-Y240 was the only residue exhibiting a flipped side chain, relative to the experimental PDB structure 6IYC, and oriented towards TMD2 (“flipped-out”). This flipping was caused by the flexible side chain docking approach and very likely enabled initial ligand binding by increasing the size of the putative binding cavity. Of note, MD simulations of the apo-GSEC + APP$_{C99}$ complex strongly suggested that PSEN1-Y240 exists entirely in the “flipped-in” position. In this “natural” conformation, the hydroxyl group forms polar contacts with water molecules or polar GSEC residues. Besides that, mutagenesis studies suggest strong hydrophobic interactions between the studied GSMs and residue PSEN1 Y240, which is not very well reflected by the binding mode in the flipped-out” conformation (GSM III_M1).

In order to assess if ligand binding remains stable within the “flipped-in” model, we performed 400ns of MD simulations. The simulations showed that once the GSM is bound to PSEN1, inducing the formation of a fitting binding pocket, it remains stable and established pronounced π-π sandwich stacking interactions with the aromatic Y240 ring. The binding mode with π-π stacking and the methyl group directed towards APP (GSMIII_M1a) fits very well to the experimental finding that the Y240A mutation leads to a drastic decrease of GSEC modulation by imidazole-based GSMs.

We hence strongly favoured the binding mode presented in GSMIII_M1a over the other model generated in the course of this study and discussed only this model in the manuscript.

**Dose-response analysis for GSM III** (data presented in Figure S3 A-C).

HEK cells overexpressing APP.KM670/671NL (Swedish) were plated into poly-L-lysine coated 96-well plates at the density of 50000 cells per well in OPTIMEM (Thermofisher Scientific) supplemented with 5% foetal bovine serum (Merck). 24h after plating the cells were treated with respective compound or vehicle control diluted in serum-free OPTIMEM. After 24h incubation, conditioned medium was collected, cleared by centrifugation and Aβ37, Aβ38, Aβ40 and Aβ42 levels determined by custom-made multipot ELISA, as indicated in main method section.
**Appendix supplementary tables and legends:**

| Mutation | Forward primer | Reverse primer |
|----------|----------------|----------------|
| F105A    | GTCACTGAGCCGAAGG | TTATGGTTAGGAACG |
| Y106A    | AGTCAGCTTTGGACCCGGAAAGG | GACTTTAATGGTAGCCAG |
| Y106F    | AGTCAGCTTTGGACCCGGAAAGG | GACTTTAATGGTAGCCAG |
| Y106Q    | AGTCAGCTTTGGACCCGGAAAGG | GACTTTAATGGTAGCCAG |
| Y106W    | AGTCAGCTTTGGACCCGGAAAGG | GACTTTAATGGTAGCCAG |
| K109A    | TTATCCCCGGGAGGTGAGAATTC | AAGCTGACTGCTTAAATGG |
| K109F    | TTATCCCCGGGAGGTGAGAATTC | AAGCTGACTGCTTAAATGG |
| D110A    | TACCCGGGACTGTAATGAGAATTC | TAAAGCTGACTGCTTAAATGG |
| D110F    | TACCCGGGACTGTAATGAGAATTC | TAAAGCTGACTGCTTAAATGG |
| G111A    | CCGGAAGGATGCTGCTTAAATGG | TAAAGCTGACTGCTTAAATGG |
| G111F    | CCGGAAGGATGCTGCTTAAATGG | TAAAGCTGACTGCTTAAATGG |
| Q112A    | GAAGGTACCCGGATATGAGAATTC | CCGGTATTAAAGCTGACTG |
| Q112F    | GAAGGTACCCGGATATGAGAATTC | CCGGTATTAAAGCTGACTG |
| L113A    | GCAGTGGGCAGCTTAAATGGTATGC | TTCCGGGCTAATGGCAG |
| L113F    | GCAGTGGGCAGCTTAAATGGTATGC | TTCCGGGCTAATGGCAG |
| F177A    | GCTGTTCTTTGGACTGCTTAAATGG | AAACAGTATATAAAGCT |
| I180A    | TTTTTCATTCGAGTCTGCTTAAATGG | AAAGAAGCAGCAACATAG |
| I180F    | TTTTTCATTCGAGTCTGCTTAAATGG | AAAGAAGCAGCAACATAG |
| Y181A    | TTTTTCATTCGAGTCTGCTTAAATGG | AAAGAAGCAGCAACATAG |
| Y181F    | TTTTTCATTCGAGTCTGCTTAAATGG | AAAGAAGCAGCAACATAG |
| L232A    | GATTAGGTCCGAGTCTGCTTAAATGG | ATATGAGATTGCTTAAATGG |
| L232F    | GATTAGGTCCGAGTCTGCTTAAATGG | ATATGAGATTGCTTAAATGG |
| L235A    | CCTGATCCGCTGAGTCTGCTTAAATGG | GCAGCTAATTAGAAGTAGAT |
| L235F    | CCTGATCCGCTGAGTCTGCTTAAATGG | GCAGCTAATTAGAAGTAGAT |
| V236A    | CATGGGCCTGAGTCTGCTTAAATGG | AGAGGACATATTAGAG |
| V236F    | CATGGGCCTGAGTCTGCTTAAATGG | AGAGGACATATTAGAG |
| F237A    | GCCCGTGACTGTGCTGCTTAAATGG | AAGGAGCATATTAGAG |
| K239A    | GGCCTATTGCTGCTTAAATGGTATGC | AGGAGCATATTAGAG |
| K239F    | GGCCTATTGCTGCTTAAATGGTATGC | AGGAGCATATTAGAG |
| Y240A    | GTTTTATCTAAGTCGCTGCTTAAATGGTATGC | AGGAGCATATTAGAG |
| Y240F    | GTTTTATCTAAGTCGCTGCTTAAATGGTATGC | AGGAGCATATTAGAG |
| Y240W    | GTTTTATCTAAGTCGCTGCTTAAATGGTATGC | AGGAGCATATTAGAG |
| Ins113A  | gTTATGCTGCTTAAATGGTATGC | TAACTGCCCATCTTCCG |
| Ins113F  | gTTATGCTGCTTAAATGGTATGC | TAACTGCCCATCTTCCG |
| Ins113T  | gTTATGCTGCTTAAATGGTATGC | TAACTGCCCATCTTCCG |

**Table S1: Primers used in the generation of GSEC or APP mutants.** List of all mutagenic primers used in the generation of mutant PSEN1/GSECs or mutant APP substrates. *The Intron4 mutant cell line was generated and reported before (Szaruga et al., 2015). The q5 (New England Biolabs) and QuikChange II
Petit D., Hitzenberger M., et al

(agilent) Site-Directed Mutagenesis kits were used in the generation of mutant PSEN1 and APP expression vectors, respectively.

Table S2: Pocket filling mutation PSEN1-V236W, mimicking GMS-GSEC interactions stabilizes GSEC-\(\beta\) complexes. Data derived from thermo-activity assays using WT or mutant PSEN1-V236W GSECs presented in Figure 7. Temperatures (Tm) at which half of the production of \(\alpha\)-s is reached and related 95% CIs are presented.

| System                  | \(\Delta G\) (kcal/mol) | Std. Dev. (kcal/mol) | Std. Error (kcal/mol) |
|-------------------------|--------------------------|----------------------|-----------------------|
| GSEC + APP\(_{C99}\) + GSMIII | -227.8                   | 9.9                  | 0.8                   |
| GSEC + APP\(_{C99}\) - GSMIII   | -219.0                   | 10.8                 | 0.9                   |

Table S3: Calculated affinities of APP\(_{C99}\) to GSEC in presence and absence of GSM III. Free energies have been calculated using the MMGBBSA method on 150 simulation snapshots.

| Steps | Time Step | K Protein | K Lipid | K Dihed. | Temp. | Pressure |
|-------|-----------|-----------|---------|----------|-------|----------|
| min   | 2000      | 10.0      | 2.5     | 250      | ---   | ---      |
| Eq1   | 125000    | 10.0      | 2.5     | 100      | 303.15K | NA       |
| Eq2   | 125000    | 5.0       | 2.5     | 50       | 303.15K | NA       |
| Eq3   | 125000    | 10.0      | 1.0     | 50       | 303.15K | 1 bar    |
| Eq4   | 250000    | 1.0       | 0.5     | 25       | 303.15K | 1 bar    |
| Eq5   | 250000    | 0.5       | 0.1     | 0        | 303.15K | 1 bar    |
| Eq6   | 250000    | 0.1       | 0.0     | 0        | 303.15K | 1 bar    |

Table S4.1: Overview of the 7 equilibration steps performed for all simulations. The initial minimization is followed by six equilibration simulations with decreasing force constants (K) on positional restraints of amino acids (Protein, all atoms), positional restraints on lipid headgroups (Lipid, phosphorus atom) as well as restraints on the lipid dihedrals (Dihed.). Temp. = temperature, NA = not applicable.
Petit D., Hitzenberger M., *et al*

| Name | Sampling Time |
|------|---------------|
| GSEC+ APP$_{C99}$, hPSEN1 WT | 2000ns |
| GSEC+APP$_{C99}$, hPSEN1 V236W | 1000ns |
| GSEC, hPSEN1 V236W | 400ns |
| GSEC+ APP$_{C99}$ (RMSF) | 10 x 30ns |
| GSEC+ APP$_{C99}$ +GSM III, hPSEN1 Y240W | 3x400ns |
| GSEC+ APP$_{C99}$ + GSM III | 3x400ns |
| GSEC+GSM III | 3x400ns |
| GSEC+GSM III (RMSF) | 10x30ns |
| GSEC+ APP$_{C99}$ (RMSF) | 10x30ns |
| GSEC+ APP$_{C99}$ +GSM III, hPSEN1 Y106Q | 400ns |
| GSEC+ APP$_{C99}$ +GSM III, hPSEN1 Y106W | 400ns |
| GSEC+ APP$_{C99}$ +GSM III, hPSEN1 Y240W | 3x400ns |
| GSEC+ APP$_{C99}$ +GSM III, hPSEN1 V236F | 400ns |
| GSEC + short substrate, PSEN1 V236W | 280ns |

Table S4.2: Sampling times of simulations discussed in this study.
Appendix supplementary figures and legends:

**Figure S1. Wild type (WT) and mutant GSEC reconstitution in Psen1<sup>−/−</sup>/Psen2<sup>−/−</sup> MEFs**
Representative western blot analysis of CHAPSO solubilized membranes from double knock-out (dKO) MEF Psen1<sup>−/−</sup>/Psen2<sup>−/−</sup> cell lines stably expressing WT or respective mutant human PSEN1s. The rescue of mature and active GSEC complexes in these stable lines is evidenced by the presence of mature, glycosylated Nicastrin (NCSTN); the enrichment of N-terminal and C-terminal fragments (NTF/CTF) of the endoproteolysed PSEN1 over full-length PSEN1 (FL PSEN1) and the presence of PEN2. These features are absent in the Psen dKO cells. In panel D: the YW-VW legend refers to Y106W-236W.

**Figure S2. Effects of mutations on the global GSEC activity and product line preference for the cleavage of APP<sub>C99</sub> in WT and mutant PSEN1/GSEC cell lines.** Secreted Aβ peptides generated by WT and mutant PSEN1 MEF lines, overexpressing APP<sub>C99</sub>, were measured by ELISA. (A) Total secreted Aβ, defined as the sum of Aβ peptides (lengths: 37+38+40+42) was used to estimate the global endopeptidase GSEC activity levels. Mean ± SD, N ≥ 3. One-way ANOVA followed by Dunnett’s post-hoc test with comparison to WT; F (DFn, DFd): F (36, 168) = 6, statistical significance is depicted by **P < 0.01, ***P < 0.001, ****P < 0.0001. (B) Aβ(37+40)/(38+42) ratios were calculated to estimate the significance of the mutation-driven changes in GSEC product line. Mean ± SD, N ≥ 3. One-way ANOVA followed by Dunnett’s post-hoc test with comparison to WT. In both panels, statistical significance (P < 0.05) is depicted by **P < 0.01, ***P < 0.001, ****P < 0.0001; F (DFn, DFd): F (34, 85) = 178.5.

**Figure S3. GSEC processing of APP<sub>C99</sub> in WT and mutant GSEC cell lines upon treatment with GSM III or vehicle.** (A-C) HEK cells overexpressing APP.KM670/671NL (Swedish) were treated for 24h with GSM III at increasing concentrations. The amounts of Aβ37, Aβ38, Aβ40 and Aβ42 secreted into the medium were determined by ELISA. GSM III activates GSEC processivity as determined by the Aβ(37+38)/(40+42) ratio (A); enhances the global (endopeptidase) activity, as shown by the total Aβ levels (37+38+40+42) (B); and shifts its proteolytic activity towards Aβ38 and Aβ42 production line, as indicated by the Aβ (37+40)/(38+42) ratio (C). Statistics were calculated using one-way ANOVA followed by Dunnett’s multiple comparison test with DMSO set as reference. The data are presented as mean ± 95% CI, N>18, *** P<0.0005, **** P<0.0001. Paneld: WT or mutant MEFs, over expressing APP<sub>C99</sub>, were treated with DMSO vehicle or 1 µM GSM III. The Aβ (37+38)/(40+42) ratios were calculated and normalized to the WT ratio in DMSO (set to 1). The significance of the responses (vehicle- vs. GSM- treatment) were evaluated by t-test. GSM III treatment induced significant changes (P < 0.05) in all mutant cell lines, excepting the PSEN1-Y106A line, marked with the symbol “#”. Mutant cell lines significantly responding to GSM III treatment,
Petit D., Hitzenberger M., et al

according to the Aβ (37+38)/(40+42) ratio, were analysed by one-way ANOVA followed by Dunnett’s post-hoc test with comparison to WT. Data shown in Figure 3B.

**Figure S4. Generation of the initial in silico GSEC-APPC99-GSM III system.**

Residues missing in the PDB structure 7IYC were added for in-silico modelling. Added residues are highlighted in purple. Colour-code: PSEN1 in tan, PEN-2 in sienna brown, APH1A in gold, NCSTN in green. (B) The volume of the investigated pocket was followed during MD simulations and a snapshot with maximum volume taken for compound docking. The figure shows the pocket volume vs simulation snapshot, calculated on the simulation of the GSEC-APP complex, using MDPocket. (C) This panel shows the initial configuration of the GSEC-APPC99-GSM III system. The merged subpockets shown in Figure 1C-D form the GSM-binding pocket depicted in this panel.

**Figure S5. The Aβ (37+38)/(40+42) ratio shows mutation- or GSM- driven effects on GSEC processivity of APPC99.** WT and mutant PSEN1 MEFs, overexpressing APPC99, were treated with 1 µM GSM III, or vehicle, and the secreted Aβ peptide levels were determined by ELISA. (A) Tested mutations in PSEN1 drastically impair the responses of GSEC to GSM III. The detrimental effects of PSEN1- Y106Q, Y106W or Y240W substitutions were determined by the Aβ (37+38)/(40+42) ratio. Panel shows the fold-change between DMSO- and GSM III- treatments for WT or indicated cell line. One-way ANOVA followed by Dunnett’s post-hoc test with comparison to WT show statistical significance (P < 0.05) **P < 0.01, ***P < 0.001, ****P < 0.0001. (B) The Aβ (37+38)/(40+42) ratio shows concentration-dependent activation profiles of WT GSEC by GSM II (black) and GSM III (blue). Dose response experiments using the WT GSEC cell line and testing GSM III (blue) or GSM II (black) modulator demonstrated a higher potency for GSMIII, relative to GSM II, in the modulation of GSEC activity. Data shown mean ± SD, N=2. GSM III and GSM II activated wild type GSEC with EC_{50} values = 633 nM and 682 nM, respectively. (C) Table shows the 95%CI for the indicated Aβ peptide levels generated by the WT and PSEN1- Y240W mutant cell lines in presence of DMSO, 1 µM GSM III or 1 µM GSM II. The overlap in the 95%CI peptide data determined in DMSO and GSMII for the PSEN1- Y240W mutant cell line demonstrates the lack of response towards this modulator; data relate to Figures 5A and 5C.

**Figure S6. ELISA and Mass Spectrometry (MS) analyses of secreted Aβ peptides generated from WT cells overexpressing WT or mutant APPC99 substrates.** Aβ peptides produced in HEK293T cells transfected with WT or mutant APPC99 treated with DMSO or 0.3 µM GSM III were analysed. (A) Aβ (37+38)/(40+42) ratios, derived from ELISA measurements, were calculated and normalized to the WT ratio in DMSO (set to 1). The responses (vehicle- vs. GSM- treatment) and statistical significance (t-test) are shown. GSM III treatment induced significant changes in all cases, statistical significance is depicted by **P < 0.01, ***P < 0.001, ****P < 0.0001. The mutations in APP however drastically
Petit D., Hitzenberger M., et al

diminished the magnitude of the responses. The analysis of Aβ (37+38)/(40+42) ratios in vehicle vs GSM III treatment (fold-response) are presented in Figure 5E. (B) Representative MS spectra of immunoprecipitated Aβ peptides produced in HEK293T cells transfected with WT or mutant APP 

c9 treated with DMSO (blue profile) or 0.3 µM GSM III (magenta profile). We note that very low levels of short Aβ1-34 and/or Aβ 1-33 were detected in the WT and M35A APP profiles.

Figure S7. Acidic GSM I-mediated activation of WT and selected GSEC mutants. WT and mutant PSEN1 MEFs, overexpressing APPC99, were treated with 1 µM (acidic) GSM I or vehicle. The secreted Aβ peptide levels were determined by ELISA. (A) Aβ profiles secreted by WT and indicated PSEN1 mutants in presence of 1 µM acidic GSM I. The GSM I structure is depicted. Data are presented as mean ± SD, N ≥ 3 independent experiments. (B) GSM I selectively modulates the cleavage of Aβ42 into Aβ38. This panel shows the relative proportions of Aβ42 and Aβ38 peptides generated by the cell lines analysed in panel A.

Figure S8. Effects of pocket filling mutations PSEN1-V236W and Y102W-V236W on GSEC product line preference and corresponding responses towards GSM III treatment. WT, PSEN1-V236W or Y102W-V236W MEF lines, overexpressing APPC99, were treated with 1 µM GSM III or vehicle. (A) Aβ 37+40 (blue) vs. Aβ 38+42 (orange) relationships for the WT and mutant GSEC cell lines. Data presented as mean ± 95% CI, N ≥ 3. One-way ANOVA followed by Dunnett’s post-hoc test with comparison to WT show statistical significance: ****P < 0.0001. (B) Dose-response analysis in WT and mutant PSEN1 V236W MEFs overexpressing APPC99 demonstrated that GSM II fails to modulate Aβ profiles generated by the mutant GSEC / PSEN1-V236W mutation. Aβ37, Aβ38, Aβ40 and Aβ42 peptide levels, normalized to total Aβ (Aβ37+Aβ38+Aβ40+Aβ42), are presented as mean ± SD, N ≥ 3 independent experiments. Dotted lines indicate the production of Aβn by the PSEN1-V236W cell line in vehicle (DMSO).

Figure S9. Fluorogenic peptide reporter on GSM- or mutation-driven allosteric modulation of GSEC activity. (A-B) In vitro activity assays using purified WT GSEC and 20 µM fluorogenic peptide as a substrate in presence of vehicle (DMSO), inhibitor X or GSMs (GSM III or GSM II). Note that this short peptide substrate lacks an ectodomain. Fluorescence (emission at λ = 440 nm), indicative of the amount of processed fluorogenic peptide substrate by purified GSEC, is plotted over reaction time. GSEC specific activities- derived from the slopes in A, upper panel- are presented in the lower panel. Data are shown as mean ± 95% CIs, N = 3 independent experiments (2 technical replicates). (C) In silico modelling of the GSEC bearing the PSEN1-V236W mutation and a small peptide substrate (sequence: W-G-G-V-V-I-A-T-V-K) revealed no direct interactions between the substrate and the PSEN-1 W236 position. The center of mass – center of mass distances between the side chains of W236 and the N-
terminal Trp of the peptide during the MD are shown. The average distance is highlighted as a red, dashed line. Colour-code: PSEN1 in tan, PEN-2 in sienna brown, APH1A in gold, NCSTN in green.

**Figure S10. Impact of GSM III on N-terminal APP helix fluctuations.** RMSF of APP\textsubscript{C99} residues derived from MD simulations with GSEC-APP\textsubscript{C99} in the presence (black profile) or absence of GSM III (red profile). We performed simulations on structurally closely coupled pairs: GSEC-APP\textsubscript{C99} +/- GSM III; i.e. modulator-free simulations were started from the same initial structure as the respective GSM-bound simulations. Error bars indicate standard errors calculated from 10 x 30 ns long trajectories.

**Appendix References:**
Schmidtke, P., Le Guilloux, V., Maupetit, J., and Tuffery, P. (2010). fpocket: Online tools for protein ensemble pocket detection and tracking. Nucleic Acids Res. 38, 582–589.
Szaruga, M., Veugelen, S., Benurwar, M., Lismont, S., Sepulveda-Falla, D., Lleo, A., Ryan, N.S., Lashley, T., Fox, N.C., Murayama, S., et al. (2015). Qualitative changes in human gamma-secretase underlie familial Alzheimer’s disease. J. Exp. Med. 212, 2003–2013.
Trott, O., and Olson, A.J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 31, 455–461.
FIGURE S1

Panel A shows a gel with protein bands labeled NCSTN, FL PSEN, PSEN-NTF, PSEN-CTF, and PEN-2 at various molecular weights. The bands are labeled with kDa values of 98, 38, 28, 17, and 6.

Panel B is a replicate of Panel A with a different set of samples.

Panel C mirrors Panel A with a different set of samples.

Panel D is a replicate of Panel C with a different set of samples.
**FIGURE S2**

**A**  
Global GSEC activity levels

**B**  
GSEC product-line preference
FIGURE S3

A

B

C

D

DMSO treatment as 100%

GSM III

DMSO

Aβ(37+38)/(40+42)

WT, DMSO normalized to 1

GSM III

Aβ(37+38)/(40+42)

DMSO treatment as 100%

GSM III

Aβ(37+38)/(40+42)

DMSO treatment as 100%

GSM III
FIGURE S5

A

B

C

| 95% CI | WT | Y240W |
|-------|----|-------|
|       | DMSO | GSM II | GSM III | DMSO | GSM II | GSM III |
| Aβ 37 | 5.1 - 5.3 | 29.7 - 31.6 | 53.7 - 55.4 | 5.7 - 6.7 | 5.6 - 7.1 | 52.3 - 56.1 |
| Aβ 38 | 13.2 - 13.4 | 26.2 - 28.2 | 40.1 - 41.9 | 13.0 - 17.4 | 13.9 - 16.3 | 24.5 - 27.5 |
| Aβ 40 | 73.48 - 73.9 | 37.8 - 40.64 | 3.9 - 4.0 | 69.4 - 75.6 | 68.4 - 74.0 | 18.0 - 19.2 |
| Aβ 42 | 7.6 - 7.9 | 2.8 - 3.1 | 0.4 - 0.6 | 5.0 - 7.4 | 6.3 - 8.5 | 0.6 - 1.3 |
FIGURE S6

A

WT, DMSO is normalized to 1

GSMIII : -     + -     + -     + -     + -     +
✱✱✱✱ ✱✱✱✱
✱ ✱✱✱✱ ✱✱

B

C99 WT (DMSO)•
C99 WT (GSM III)•

C99 I32A (DMSO)•
C99 I32A (GSM III)•

C99 M35A (DMSO)•
C99 M35A (GSM III)•

C99 V36A (DMSO)•
C99 V36A (GSM III)•

15 | Appendix
FIGURE S7

A

WT, DMSO
WT, GSM I
Y106A, DMSO
Y106A, GSM I
L113A, DMSO
L113A, GSM I
V236W, DMSO
V236W, GSM I
Y240W, DMSO
Y240W, GSM I
Ins113T, DMSO
Ins113T, GSM I

Aβₙ as % of total Aβ

B

WT, DMSO
WT, GSM I
Y106A, DMSO
Y106A, GSM I
L113A, DMSO
L113A, GSM I
V236W, DMSO
V236W, GSM I
Y240W, DMSO
Y240W, GSM I
Ins113T, DMSO
Ins113T, GSM I

Aβ (38+42) as 100 %
FIGURE S8

A

B

C

Aβn as % of total Aβ

WT V236W Y106W-V236W

Aβ(37+40) Aβ(38+42)

✱✱✱✱✱✱✱✱
FIGURE S9

A

B

C
FIGURE S10

A

GSM III
no GSMIII