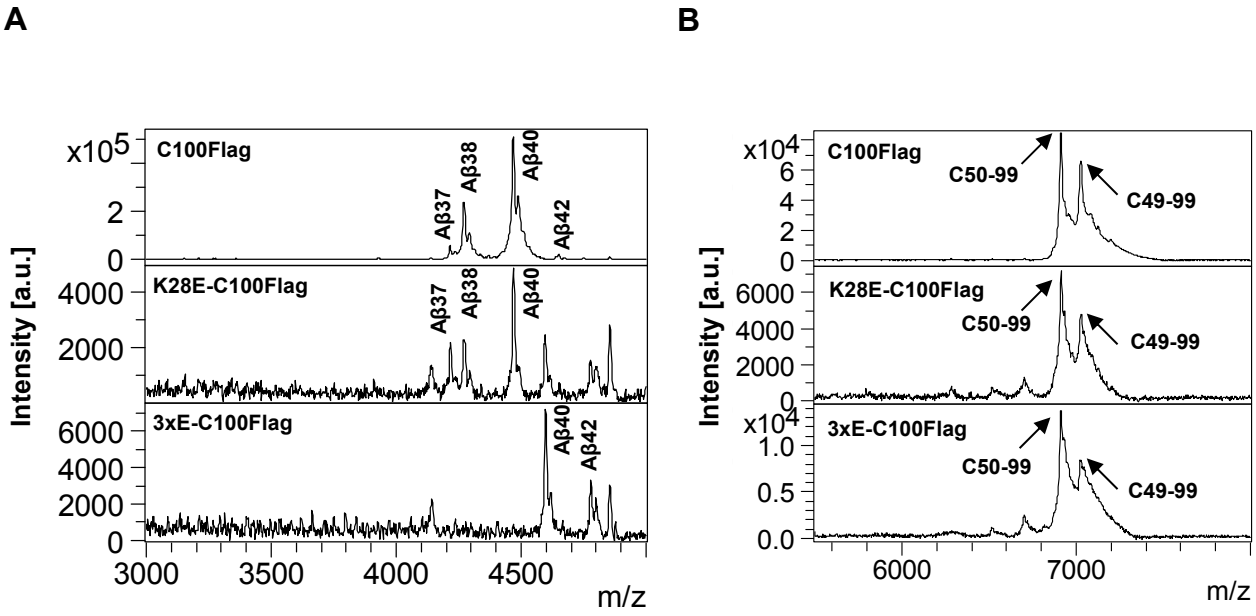
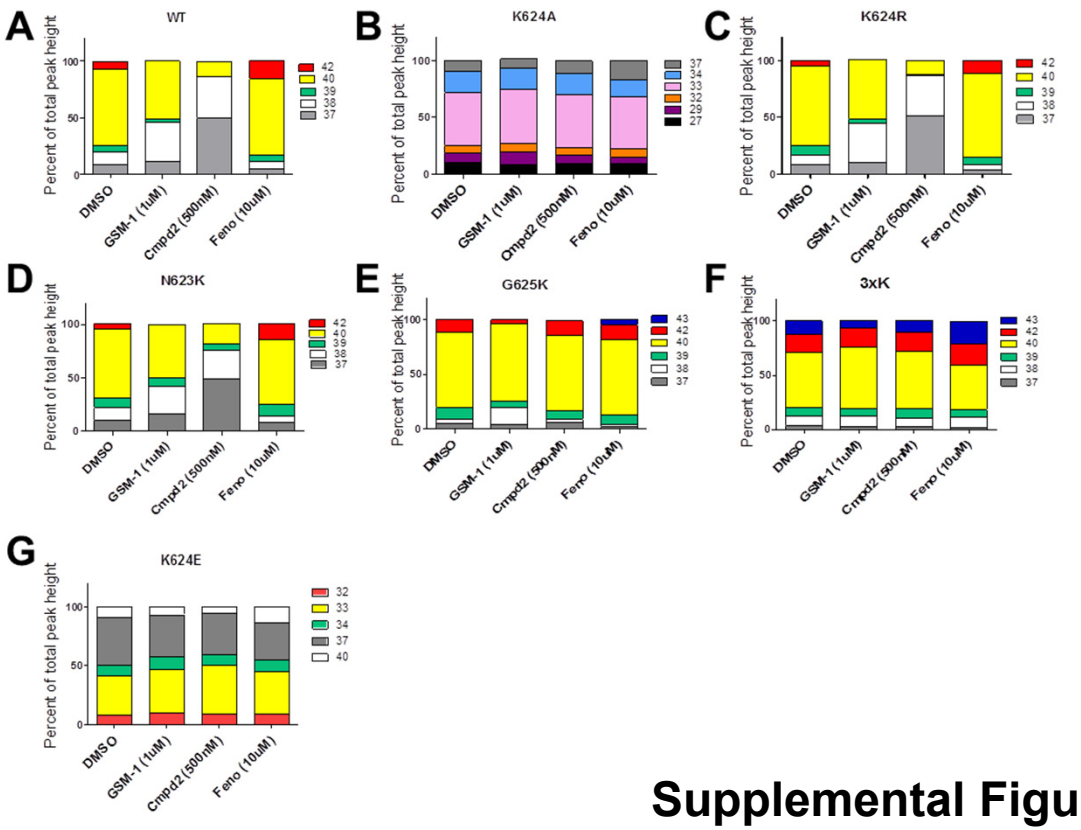
Supplemental Figure 1. AICD production is unchanged upon introduction of mutations with negative charge. (A) Recombinant C100Flag produced Aβ37, Aβ38, Aβ40 and Aβ42 as the major constituents. In the K28E mutant, Aβ37, Aβ38, and Aβ40 were identified. 3xE C100Flag showed decreased processivity by showing increased longer Aβ peptides, i.e., Aβ42. (B) No major alterations were detected in AICD fragments produced from the C100Flag and the K28E and 3xE mutants. In all cases, the major peaks were C50-99 and C49-99. (Unmarked peaks are non-specific).

Supplemental Figure 2. Quantitation of GSM and iGSM effect on the APP mutants. (A-F) In order to compare the major changes in each Aβ isoform within each construct after drug treatment, The Aβ profiles from Figure 2B-2G were quantified as described (*39, 54*). Each stack in the graph represents the ratio of each peak height to the sum of the all the peaks. (A) GSM-1 dramatically lowered Aβ42 and raised Aβ38 without any major changes in other Aβ isoforms. Cmpd2 lowered both Aβ42 and Aβ40, and raised both Aβ38 and Aβ37. Fenofibrate raised Aβ42 and lowered Aβ38. (B) The isoforms identified in the K624A mutant did not demonstrate any major changes after GSM or iGSM treatment. (C) The K624R showed a response similar to the WT. (D) The N623K showed slightly diminished effects after treatment with either GSM, whereas fenofibrate was still active. For example, the effect for raising Aβ38 was reduced by ca. 9% for GSM-1 and 11% for Cmpd2, respectively relative to the wt. (E) The G625K and (F) the 3xK mutants illustrated a more pronounced decrease in responsiveness to GSM treatment. Fenofibrate treatment raised not only Aβ42 but also Aβ43 in both mutants. For example, the capacity to raise Aβ38 reduced by ca. 20% for GSM-1 and 34% for Cmpd2 for G625K, and reduced by 26% and 29% for GSM-1 and Cmpd2 for 3K, respectively. (G) The K624E demonstrated reduced activity for both GSMs and iGSM. These analyses were based on 2-3 experiments with 2 replicates in each experiment. (Maximal S.E.M=±5.5)



Supplemental Figure1



Supplemental Figure2

Supplemental table1. Molecular weight of Aβ and AICD detected

Construct	Aβ	MW Calculated (Da)	MW Observed (Da)
WT	Aβ37	4074.5	4074.625
	Aβ38	4131.5	4132.999
	Aβ39	4230.7	4226.845
	Aβ40	4329.8	4329.133
	Aβ42	4514.0	4513.029
K624A	Aβ27	3134.3	3131.529
	Aβ29	3262.4	3260.147
	Aβ32	3559.8	3554.966
	Aβ33	3616.8	3613.253
	Aβ34	3730.0	3727.300
	Aβ37	4017.4	4014.180
K624R	Aβ37	4102.5	4101.014
	Aβ38	4159.5	4159.255
	Aβ39	4258.7	4261.102
	Aβ40	4357.8	4363.323
	Aβ42	4542.0	4554.425
N623K	Aβ37	4088.6	4082.701
	Aβ38	4145.6	4140.781
	Aβ39	4244.8	4239.172
	Aβ40	4343.9	4340.822
	Aβ42	4528.1	4527.033
G625K	Aβ37	4145.5	4157.145
	Aβ38	4202.5	4210.454
	Aβ39	4301.7	4314.058
	Aβ40	4400.8	4415.871
	Aβ42	4585.0	4608.463
G625K/A626K (3xK)	Aβ37	4202.5	4193.461
	Aβ38	4259.5	4235.670
	Aβ39	4358.7	4356.073
	Aβ40	4457.8	4458.888
	Aβ42	4642.0	4649.134
	Aβ43	4743.1	4754.816
K624E	Aβ32	3617.9	3613.898
	Aβ33	3675.0	3672.439
	Aβ34	3788.1	3785.239
	Aβ37	4075.5	4071.524
	Aβ40	4330.8	4327.860
3xE-C100Flag	Aβ40	4598.0	4592.0
	Aβ42	4782.1	4776.3
C100Flag	C50-99	6905.66	6903.964
	C49-99	7018.82	7018.86
3xK-C100Flag	C50-99	6905.66	6905.473
	C49-99	7018.82	7017.932
K28E-C100Flag	C50-99	6905.66	6913.932
	C49-99	7018.82	7028.528
3xE-C100Flag	C50-99	6905.66	6911.40
	C49-99	7018.82	7028.379

Supplemental table2. EC50 values for Aβ42 lowering effect of GSMs and iGSM

Mutants	Baseline Aβ42 (pM)	GSM-1 (nM)	Cmpd 2 (nM)
APP695wt	174.5±7.5	183.4±1.5	43.6±1.2
K624A	Under detection limit	NC	NC
K624R	68.1±4.1	232.5±1.5	83.5±1.2
N623K	52.8±3.6	896.7±1.5	149.1±1.3
G625K	120.2±3.5	1055.0±1.4	NC
3xK	215.7±6.4	NC	NC
K624E	Under detection limit	NC	NC

Supplemental table3. EC50 values for Aβ40 altering effects of GSMs and iGSM

Mutants	Baseline Aβ40 (pM)	GSM-1 (nM)	Cmpd 2 (nM)
APP695wt	997.8±66.0	NC	126.2±1.2
K624A	Under detection limit	NC	NC
K624R	476.3±23.4	NC	437.5±1.2
N623K	682.0±51.0	NC	455.2±1.3
G625K	312.7±11.7	NC	NC
3xK	264.0±13.2	NC	NC
K624E	Under detection limit	NC	NC