Supplemental data to the manuscript:

Aberrant APP processing in hereditary forms of Alzheimer disease caused by APP-FAD mutations can be rescued by mutations in the APP GxxxG motif

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Fig. S1: ELISA data comparing the APP-FAD-G33A constructs to APP-wt in Aβ production.
A: sAPPα levels were quantified by ELISA, APP-wt set as 100% (mean ± s.e.m, n= 5-16). Only the sw mutation reduced sAPPα levels significantly, as expected. Addition of G33A to the FAD variants had no influence on sAPPα levels (compare black bars, G33A, with grey bars, G33-wt).
B: Total secreted Aβ levels, APP-wt set as 100% (mean ± s.e.m, n= 6-14). The sw mutation significantly increased total Aβ approximately 2-fold. G33A had no influence on total Aβ levels compared to the respective APP-FAD variant. Note that A692G and E693G could not be analyzed by total Aβ ELISA as the FAD mutations alter the epitope of the antibody 4G8 (see Fig. 2C).
C: Aβ38 levels, G33A set as 100% (mean ± s.e.m, n= 4-12). All FAD mutants except I716V, V717G, and L723P generated more Aβ38 compared to APP-wt. Addition of the G33A mutation resulted in increased Aβ38 levels compared to the respective G33-wt constructs.
D: Aβ40 levels, APP-wt set as 100% (mean ± s.e.m, n= 7-16). Whereas sw, A692G, and E693G increased Aβ40 levels compared to APP-wt, FAD mutations at the C-terminal TMS end caused a decrease in Aβ40 levels. The addition of G33A had no influence on Aβ40 levels.
A-E: Asterisks indicate significant differences to APP-wt (*P<0.01, **P<0.001, one-way Anova type Dunnett). Horizontal lines mark the level of APP-wt for better comparison of the mutational effects.

Fig. S2: MALDI-MS analysis of APP-FAD and APP-FAD-G33A.
Aβ was immunoprecipitated from conditioned cell culture media with W0-2. The mass shifts in G33A peptides are attributable to the amino-acid exchange glycine to alanine causing an increase by 14 Da. For better resolution, the Aβ42 peaks are shown enlarged on the right. No aberrant Aβ species are generated by the APP-FAD-G33A constructs, no unusual amounts of Aβ37 or Aβ39 are observed. A: Artefact peak, peaks result from Protein-G used for the immunoprecipitation.

Fig. S3: Expression controls of SH-SY5Y cell lines by Western blot analysis.
A: Western blot analysis of PS1 using the antibody NT1 against the N-terminus of PS1. PS1 holoprotein is detected only in PS1 overexpressing lines, not in the mock control. The N-terminal fragment (NTF) of PS1 runs higher compared to the endogenous PS1-NTF (mock) attributable to the N-terminal HA-tag. This indicates suppression of endogenous PS1 upon overexpression of exogenous PS1 as described previously (1, 2).
B: Comparable SPA4CT expression levels were observed for all cell lines, detection of SPA4CT with antibody W0-2.
A, B: As a loading control the lower panels show actin levels.

Fig. S4: ELISA data comparing the SPA4CT-G33A cell lines with SPA4CT-wt cell lines. Aβ levels of PS1-wt/SPA4CT-wt cells were set as 100%.
A: total Aβ levels (mean ± s.e.m, n= 3-10). All cell lines secrete similar amounts of total Aβ indicating that all PS1 mutants are overall working efficiently on both substrates, SPA4CT-wt (grey bars) and SPA4CT-G33A (black bars).
B: Aβ40 levels (mean ± s.e.m, n= 3-10). Aβ40 levels were only significantly decreased by PS1-G384A, while no difference between wt or G33A substrate was observed.
C: Aβ38 levels (mean ± s.e.m, n=3-9). All PS1-FAD mutants generated less Aβ38 compared to PS1-wt. The G33A substrate increased Aβ38 levels produced by PS1-G378V and -L381V, although not significantly.

D: Aβ42 levels (mean ± s.e.m, n= 3-8). All PS1-FAD mutants generated more Aβ42 than PS1-wt (grey bars). Co-expression of SPA4CT-G33A with PS1-FAD mutants decreased Aβ42 levels although not significantly.
B-D: Asterisks indicate significant differences to PS1-wt/SPA4CT-wt (*P<0.01, **P<0.001, one-way Anova type Dunnett).

Fig. S5: MALDI-MS analysis of PS1-FAD/SPA4CT-wt and PS1-FAD/SPA4CT-G33A.
As Fig. S2. All Aβ peptides derived from SPA4CT carry two extra amino acids at their N terminus (LE) leading to a mass shift of 242 Da. No aberrant Aβ species are generated, no unusually intense peaks of Aβ37 or Aβ39 were observed.

1. Thinakaran, G., Harris, C. L., Ratovitski, T., Davenport, F., Slunt, H. H., Price, D. L., Borchelt, D. R. and Sisodia, S. S. (1997) *J Biol Chem* **272**, 28415-28422
2. Uemura, K., Kitagawa, N., Kohno, R., Kuzuya, A., Kageyama, T., Shibasaki, H. and Shimohama, S. (2003) *J Neurosci Res* **73**, 166-175

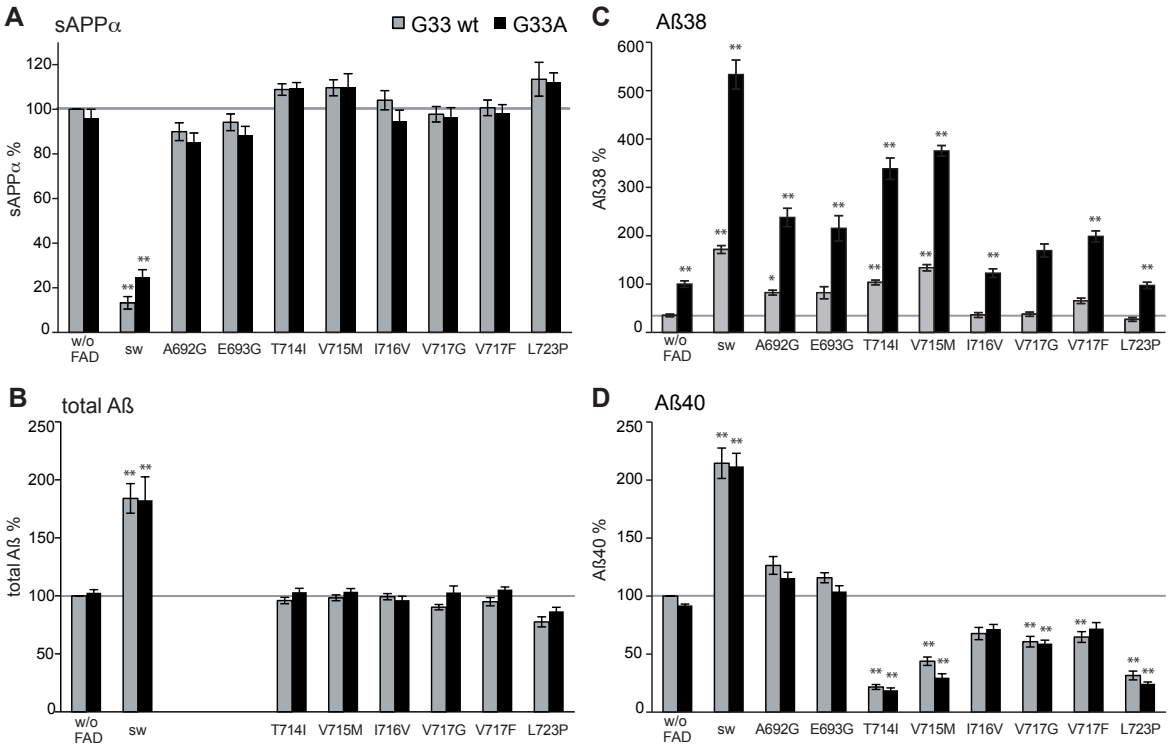
A
sAPPα
G33 wt
G33A
sAPPα %
w/o FAD
sw
A692G
E693G
T714I
V715M
I716V
V717G
V717F
L723P
B
total Aß
total Aß %
C
Aß38
Aß38 %
D
Aß40
Aß40 %

Figure S1

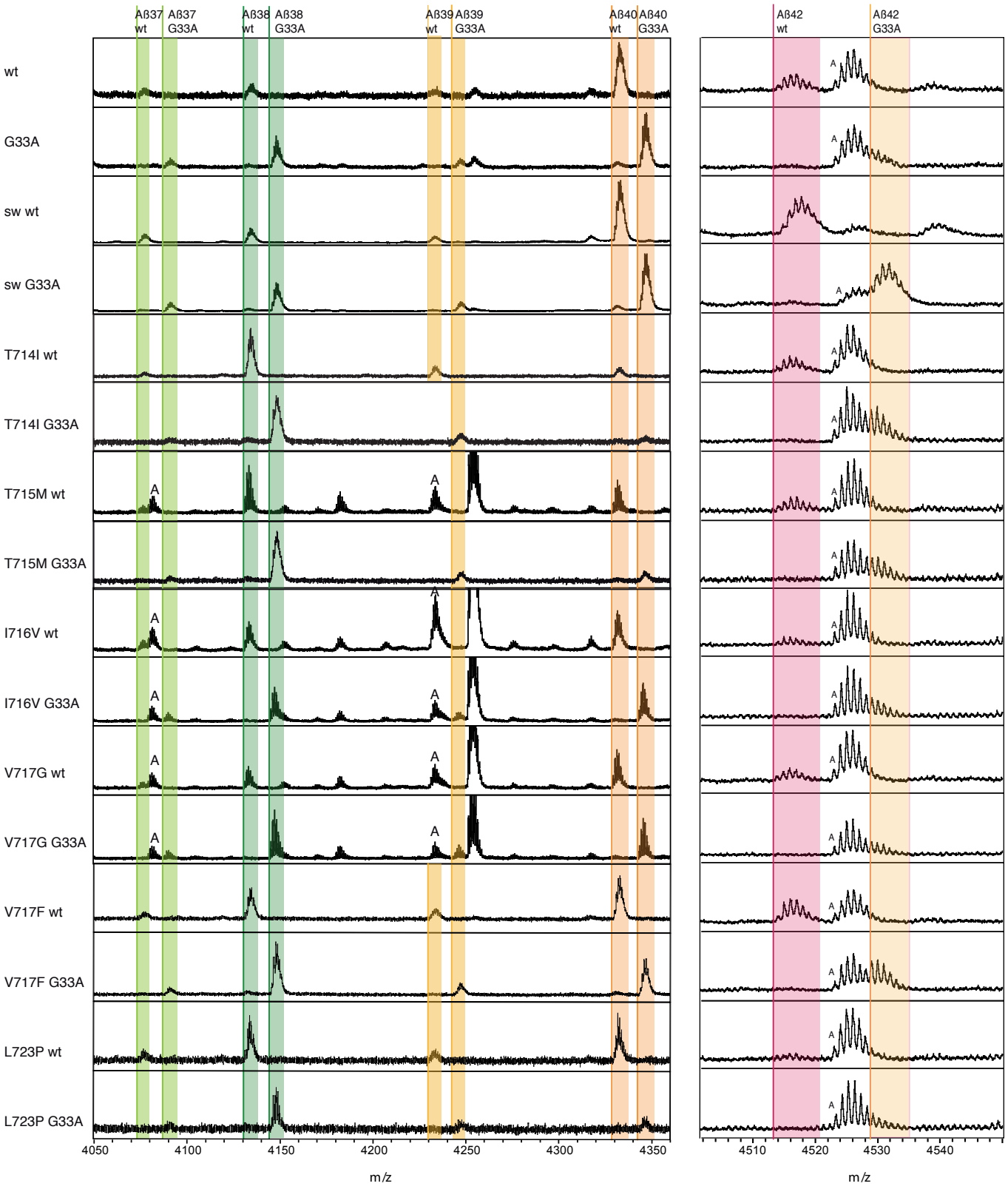
Aß37 wt
Aß37 G33A
Aß38 wt
Aß38 G33A
Aß39 wt
Aß39 G33A
Aß40 wt
Aß40 G33A
Aß42 wt
Aß42 G33A
wt
G33A
sw wt
sw G33A
T714I wt
T714I G33A
T715M wt
T715M G33A
I716V wt
I716V G33A
V717G wt
V717G G33A
V717F wt
V717F G33A
L723P wt
L723P G33A
A
4050
4100
4150
4200
4250
4300
4350
4510
4520
4530
4540
m/z
m/z

Figure S2

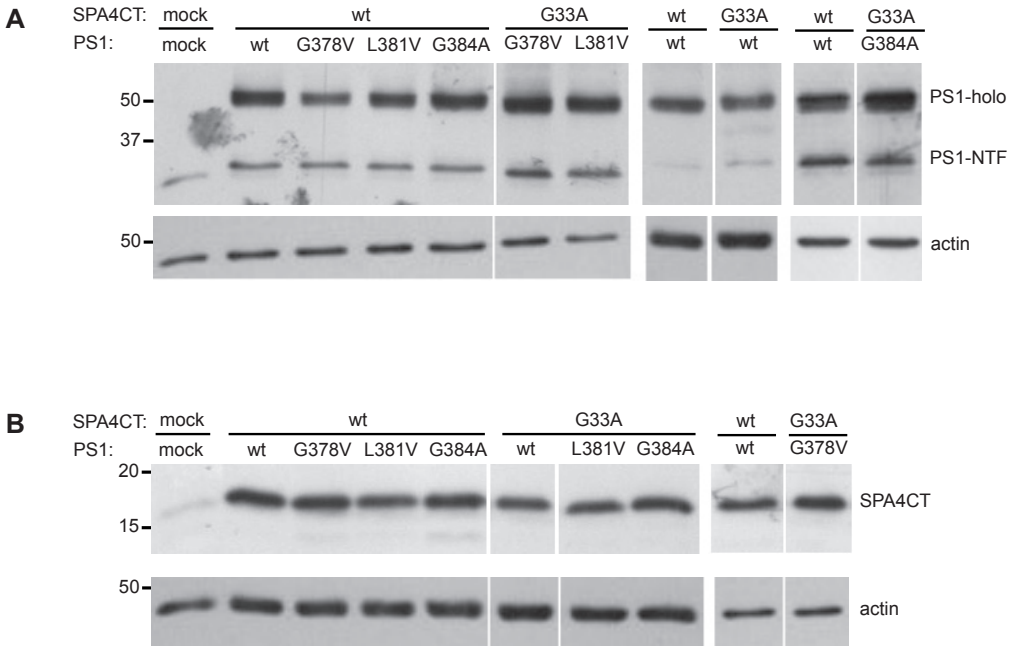
A
SPA4CT:
PS1:
mock
mock
wt
wt
G378V
L381V
G384A
G33A
G378V
L381V
wt
wt
G33A
wt
wt
G33A
G384A
50
37
50
PS1-holo
PS1-NTF
actin
B
SPA4CT:
PS1:
mock
mock
wt
wt
G378V
L381V
G384A
G33A
wt
L381V
G384A
wt
wt
G33A
G378V
20
15
50
SPA4CT
actin

Figure S3

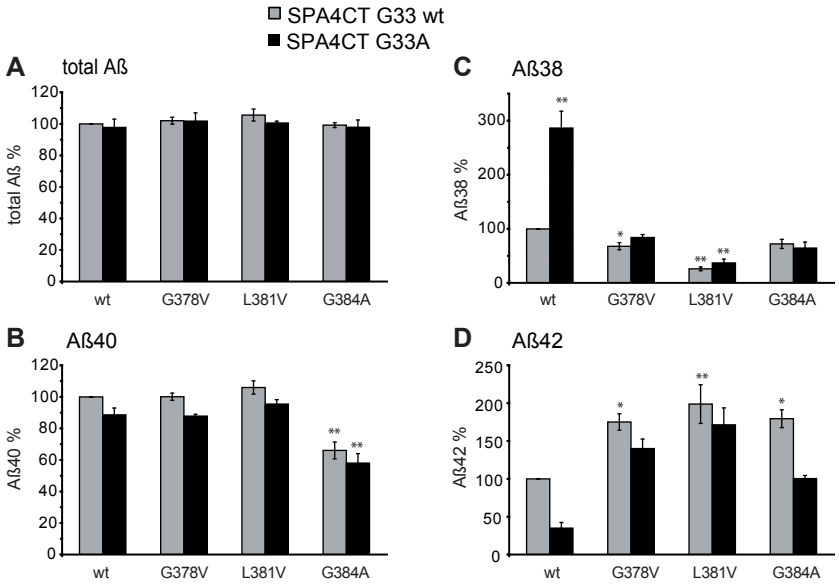
SPA4CT G33 wt
SPA4CT G33A
A
total Aß
total Aß %
120
100
80
60
40
20
0
wt
G378V
L381V
G384A
C
Aß38
Aß38 %
300
200
100
0
B
Aß40
Aß40 %
D
Aß42
Aß42 %
250
200
150
100
50

Figure S4

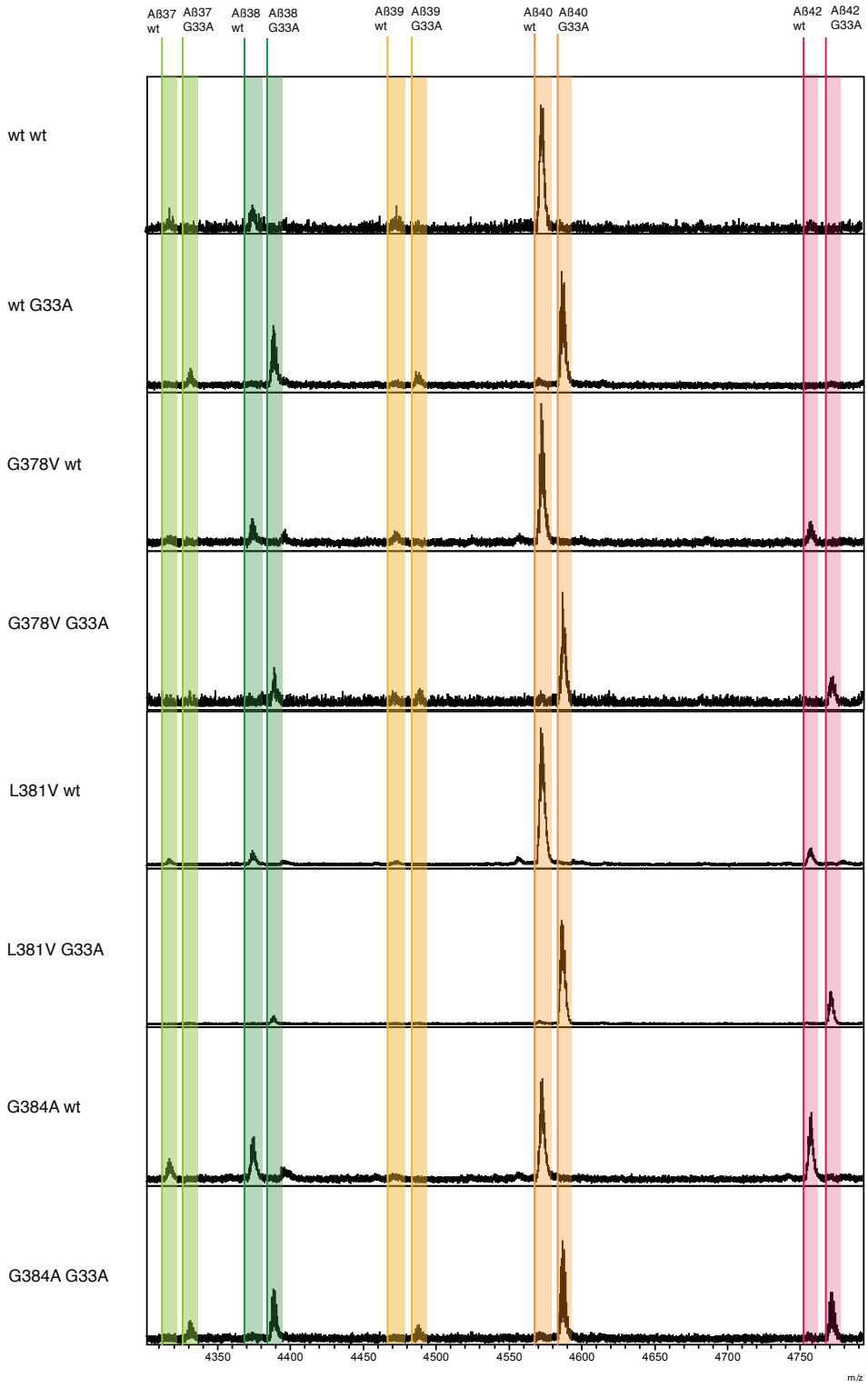
Aß37 wt
Aß37 G33A
Aß38 wt
Aß38 G33A
Aß39 wt
Aß39 G33A
Aß40 wt
Aß40 G33A
Aß42 wt
Aß42 G33A
wt wt
wt G33A
G378V wt
G378V G33A
L381V wt
L381V G33A
G384A wt
G384A G33A
4350
4400
4450
4500
4550
4600
4650
4700
4750
m/z

Figure S5