SUPPLEMENTARY MATERIALS

Table 1-Supp. *SNCA*-Rep1 transgenic mice lines

LINE	Rep1 Allele	Copy no.	Integration chromosome
259-A	Rep1-259bp	4	3
259-B	Rep1-259bp	1	19
261-A	Rep1-261bp	7	1
261-B	Rep1-261bp	5	17
Delta-A	Rep1-deletion	2	3
Delta-B	Rep1-deletion	3	10

Figure 1-Suppl.

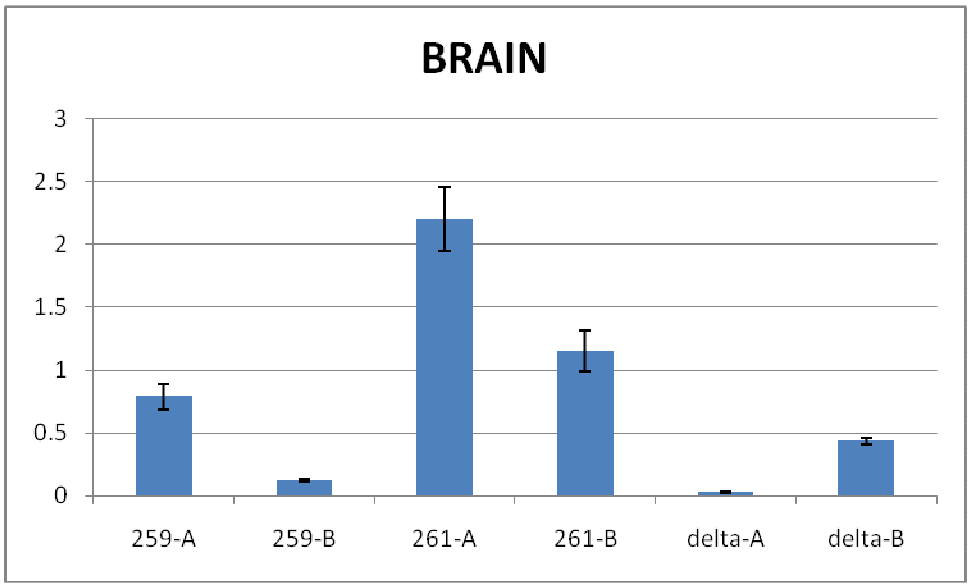


Figure 2-Suppl.

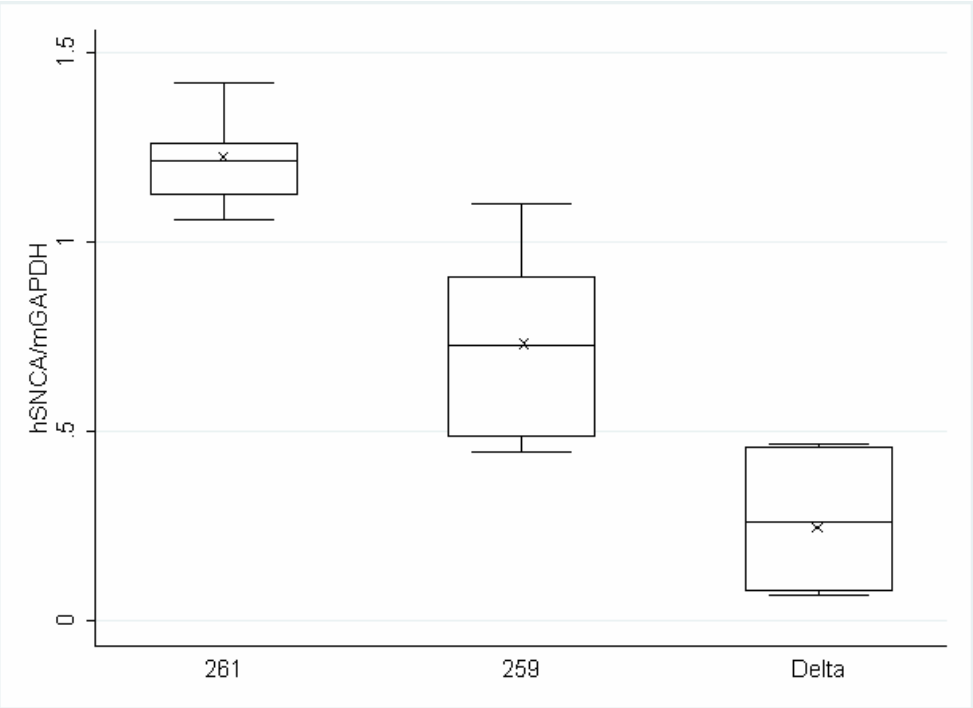
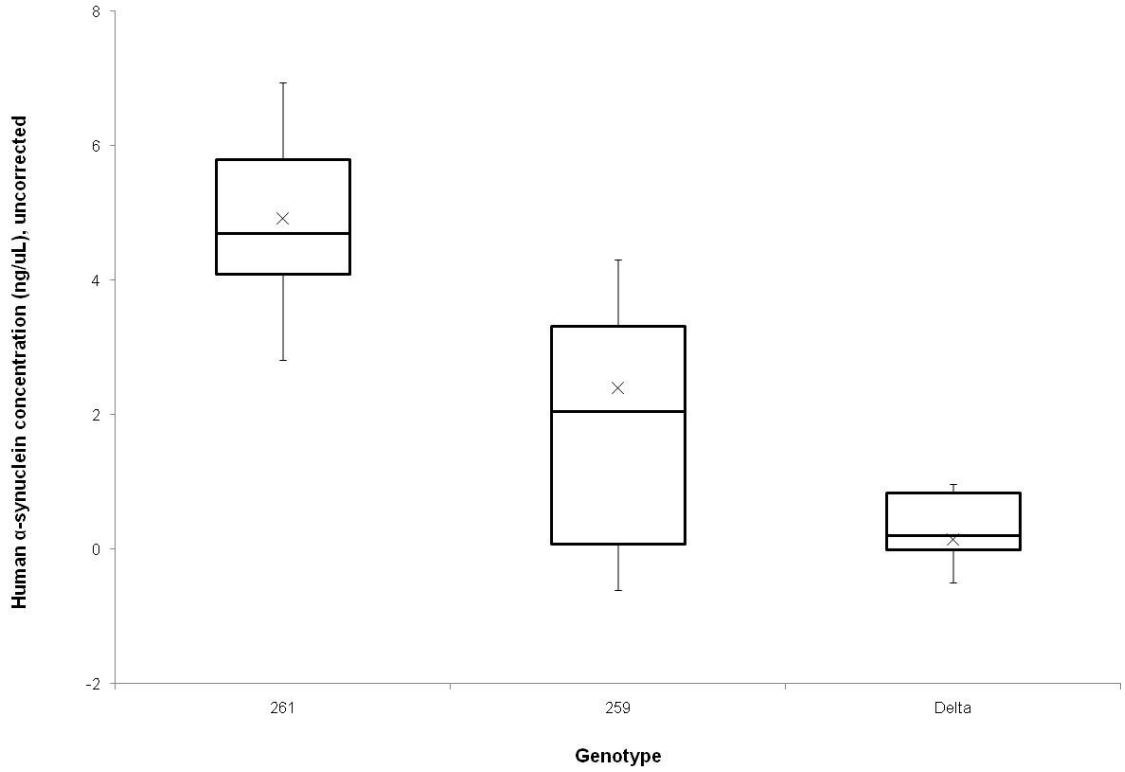
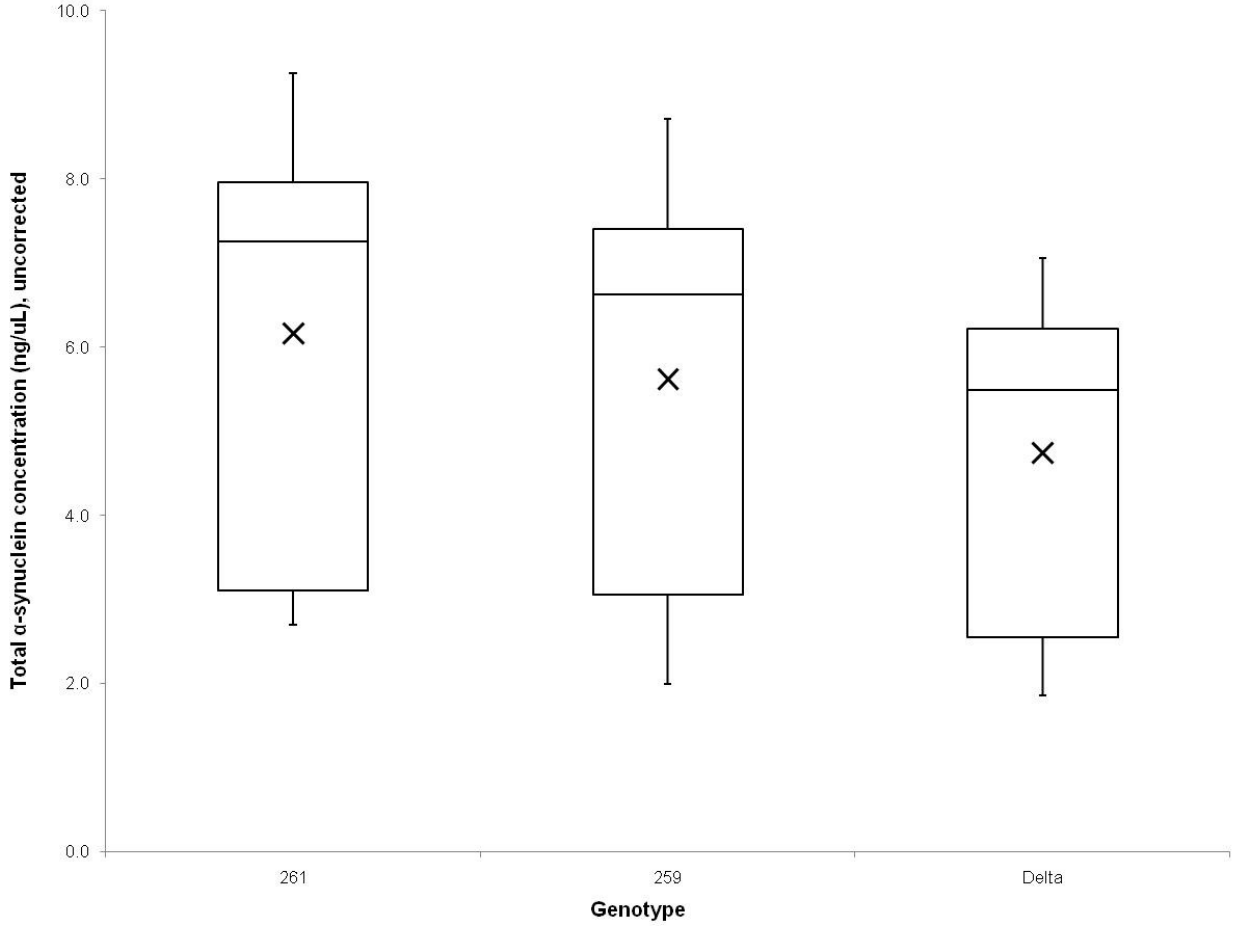


Figure 3-Suppl.

A



B



C

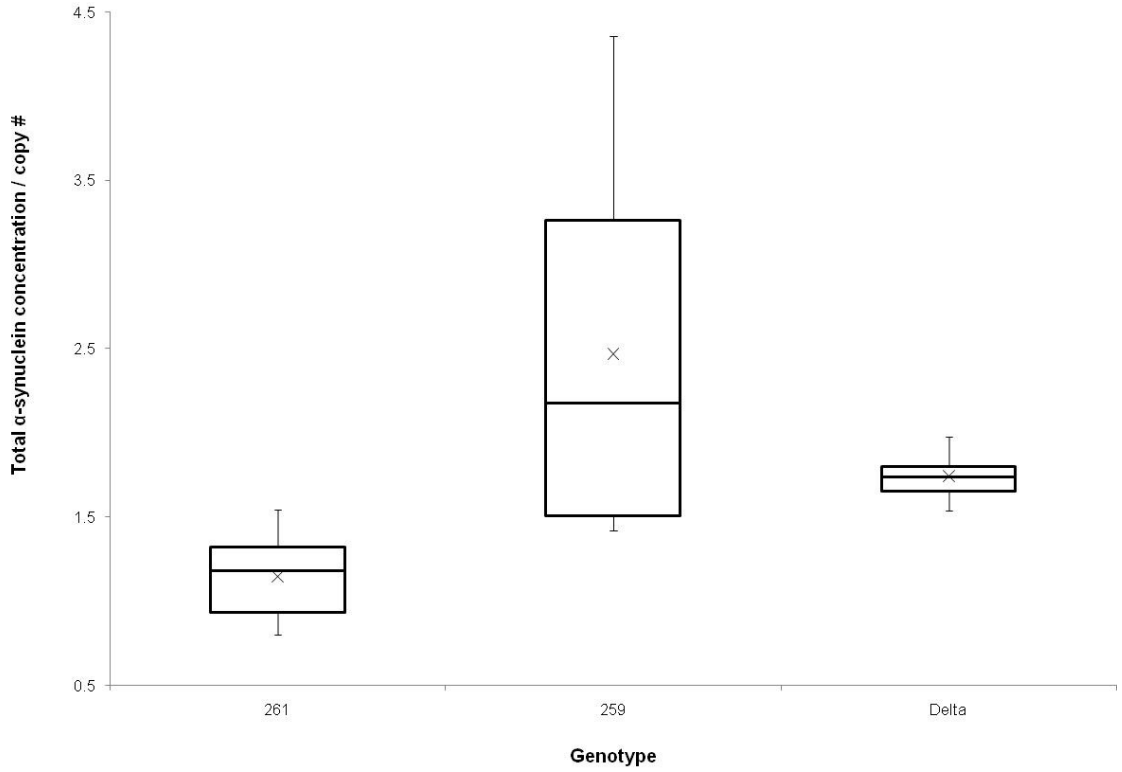
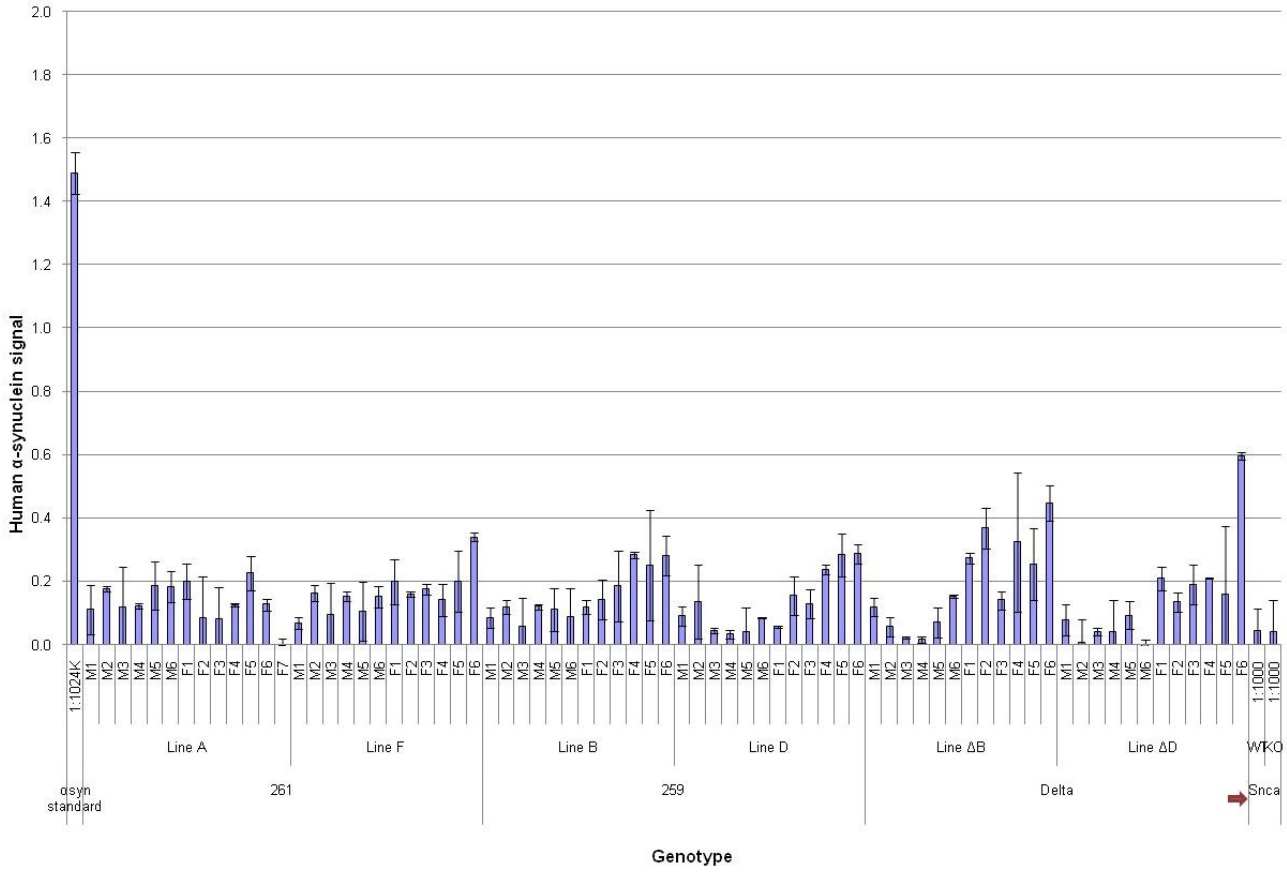

4.5
3.5
2.5
1.5
0.5
Total α-synuclein concentration / copy #
261
259
Delta
Genotype

Figure 4-Suppl.

A



B

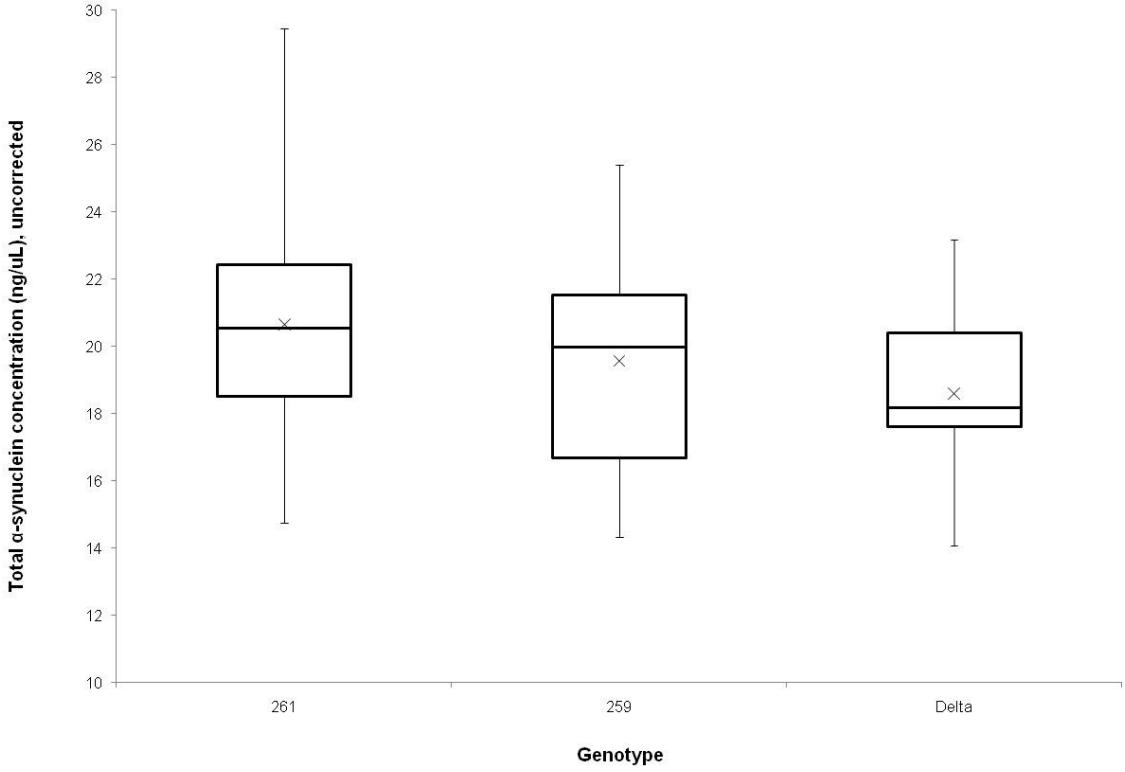
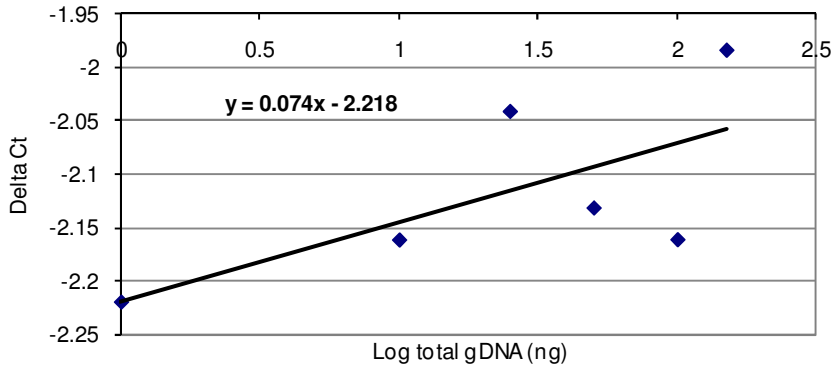


Figure 5-Suppl.

A



B

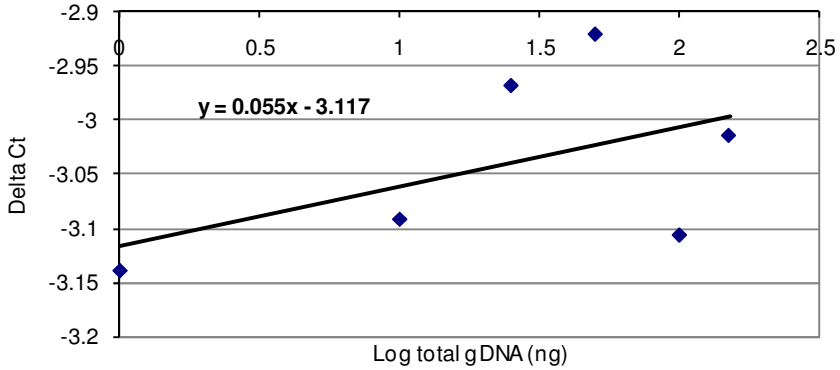


Figure 1 Suppl.

Human *SNCA*-mRNA expression levels in mouse brains of each transgenic line.

Fold levels of human *SNCA*-mRNA were assayed by real-time RT-PCR and calculated relative to mouse *Syp*-mRNA reference control using the $2^{-\Delta Ct}$ method. For each mouse line the bar graph represents the average values (mean ± standard error of the mean) of the analysis performed using twelve animals from each line (six males and six females), each of which was analyzed twice independently.

Figure 2 Suppl.

Effect of the *SNCA*-Rep1 promoter genotypes on human *SNCA*-mRNA expression levels relative to mouse *Gapdh*-mRNA in transgenic mouse brains. Fold levels of human *SNCA*-mRNA were assayed by real-time RT-PCR and calculated relative to mouse *Gapdh*-mRNA reference control using the $2^{-\Delta Ct}$ method. No amplification of the human *SNCA*-mRNA was detected with the control brain of a wild type mouse. The risk genotype 261/261 correlates with higher *SNCA*-mRNA levels than the protective genotype 259/259. The deleted *SNCA*-Rep1 genotype correlates with the lowest *SNCA*-mRNA levels compared with the Rep1 genotypes 261/261 and 259/259 ($P<0.0001$). A subset of 24 mice were analyzed; for each genotype: 261/261, 259/259, and Δ/Δ the box plot represents the analysis performed using two transgenic lines, four animals from each line (two males and two females), comprising 8 total brain samples per genotype. The average values are presented in 'X'. The box plot shows the median (horizontal line inside the box) and the 25th and 75th percentiles (horizontal borders of the box). The range between the 25th and 75th percentiles is the interquartile-range (IQR). The whiskers show the minimal and maximal values inside the main data body.

We conclude from these data that the differences observed in brain tissue did not depend on the use of neuronal reference genes (mouse *Syp* and *Eno2)* for normalization controls for the entire brain set.

Figure 3 Suppl.

(A) Quantification of human SNCA protein in transgenic mouse brain homogenates using sandwich ELISA (hSA-3 / 211-B) and a 384-well plate format (triplicates; 50 ul / well). Concentration values (in ng / uL) were interpolated from the standard curve. Values shown are not yet corrected for the calculated gene copy number in each mouse line.

(B) Quantification of total SNCA protein in transgenic mouse brain. The latter was measured using sandwich ELISA [hSA3/Syn1-B] on 384-well plates quantifying both mouse snca and human SNCA; graph shows values without correction for gene copy number.

(C) Graph shows values from (B) after correction for calculated gene copy number.

Figure 4 Suppl.

(A) Quantification of human SNCA protein in transgenic mouse blood homogenates using sandwich ELISA (hSA-3 / 211-B) and 384-well plate format (triplicates; 50 ul / well). Signals were specific for human SNCA protein because whole blood from wild-type (WT) and *snca* knock-out (KO) mice did not generate ELISA signals above background (see red arrow at bottom right). Values are shown in OD 405 nm signals given their overall low levels (M, male; F, female).

(B) Quantification of total SNCA protein concentration levels in transgenic mouse blood was carried out with sandwich ELISA [hSA3/Syn1-B] and 384-well plates. This ELISA measures both mouse snca and human SNCA proteins; values (in ng / uL) are shown without correction for gene copy numbers.

Figure 5 Suppl.

Validation curve of the Δ real time assay for relative quantization of PAC transgene copy number relative to mouse β-actin measured by real time qPCR targeted to gDNA sequences of the human-*SNCA* **(A)** exon 2 and **(B)** exon 6. Relative efficiency plots of (A) human-*SNCA* exon 2 and mouse-*β actin* (B) human-*SNCA* exon 6 and mouse-*β actin* were formed by plotting the log input amount (ng of total gDNA) versus the ΔCt=[Ct(*SNCA* exon 2 or exon 6)-Ct(*β-actin*)].

The slops are (A) 0.074 and (B) 0.055, which indicated the validation of the ΔCt calculation in the range between 1-150 ng gDNA.