

# Characterization of *In Vivo* and *In Vitro* Drug Screening Models for Gaucher Disease Based on GBA-D409V-KI MICE

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## BACKGROUND

It is well-described that mutations in the human GBA (glucosylceramidase- $\beta$ ) gene and associated lowered glucosylceramidase- $\beta$  (GCase) activity, can cause Gaucher disease (GD). Next to the significance of GCase for GD, the enzyme is highly discussed as therapeutic target in Parkinson's disease (PD) research. To study both diseases and test possible therapeutic agents *in vivo*, specific mouse models were generated. Here we characterize GBA-D409V-KI mice, that express the mutant D427V GBA protein which corresponds to the D409V mutation in the mature human GBA protein, for expression of typical GD and PD biomarkers. Additionally, a corresponding *in vitro* model, embryonic fibroblasts generated from GBA-D409V-KI mice, is validated by evaluation of GCase activity after treatment with the  $\beta$ -glucosidase inhibitor isofagomine.

## MATERIALS and METHODS

Liver samples and brains hemispheres of 4 to 12 months old homo- (tm/tm) and heterozygous (tm/wt) GBA-D409V-KI mice as well as age-matched wild type littermates (wt/wt) were assessed for GCase activity using the 4-MUG assay. Each sample was analyzed in duplicates and a third replicate including 1 mM CBE was used to subtract the GBA1 unspecific signal.

An aliquot of the hemisphere homogenate from 12 months old animals was used for extraction of soluble and Triton X-100 insoluble proteins and subsequently analyzed for murine  $\alpha$ -synuclein level with a self-established immunosorbent assay based on the Mesoscale Discovery (MSD) platform.

The corresponding *in vitro* model, mouse embryonic fibroblasts (MEFs) generated from homo- or heterozygous GBA-D409V-KI E14 embryos or the wild type littermates, were validated by testing the effects of isofagomine on GCase activity normalized to viability.

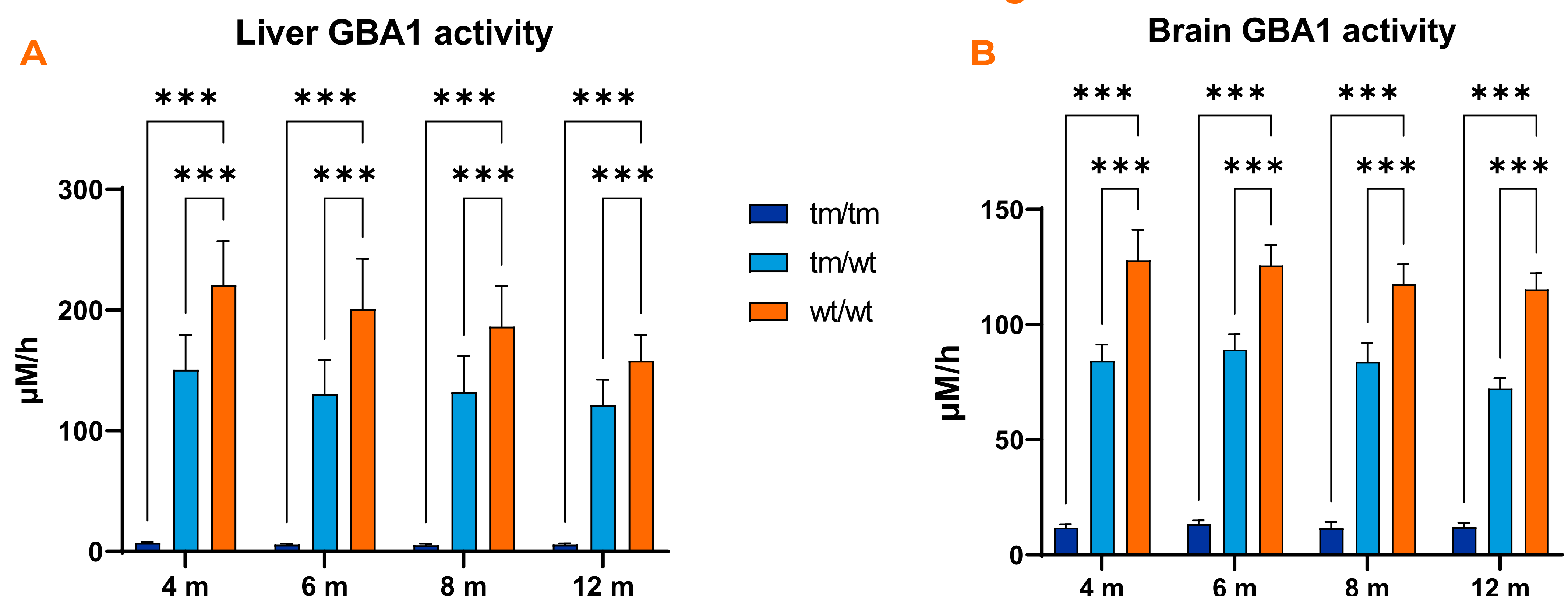
GCase activity was determined with an on-cell 4-MUG assay, viability was assessed using the crystal violet assay. Data are given as relative fluorescent units (RFU) of 4-MUG assay normalized to optical density (OD) values derived from the crystal violet assay.

For more information about the models please visit:

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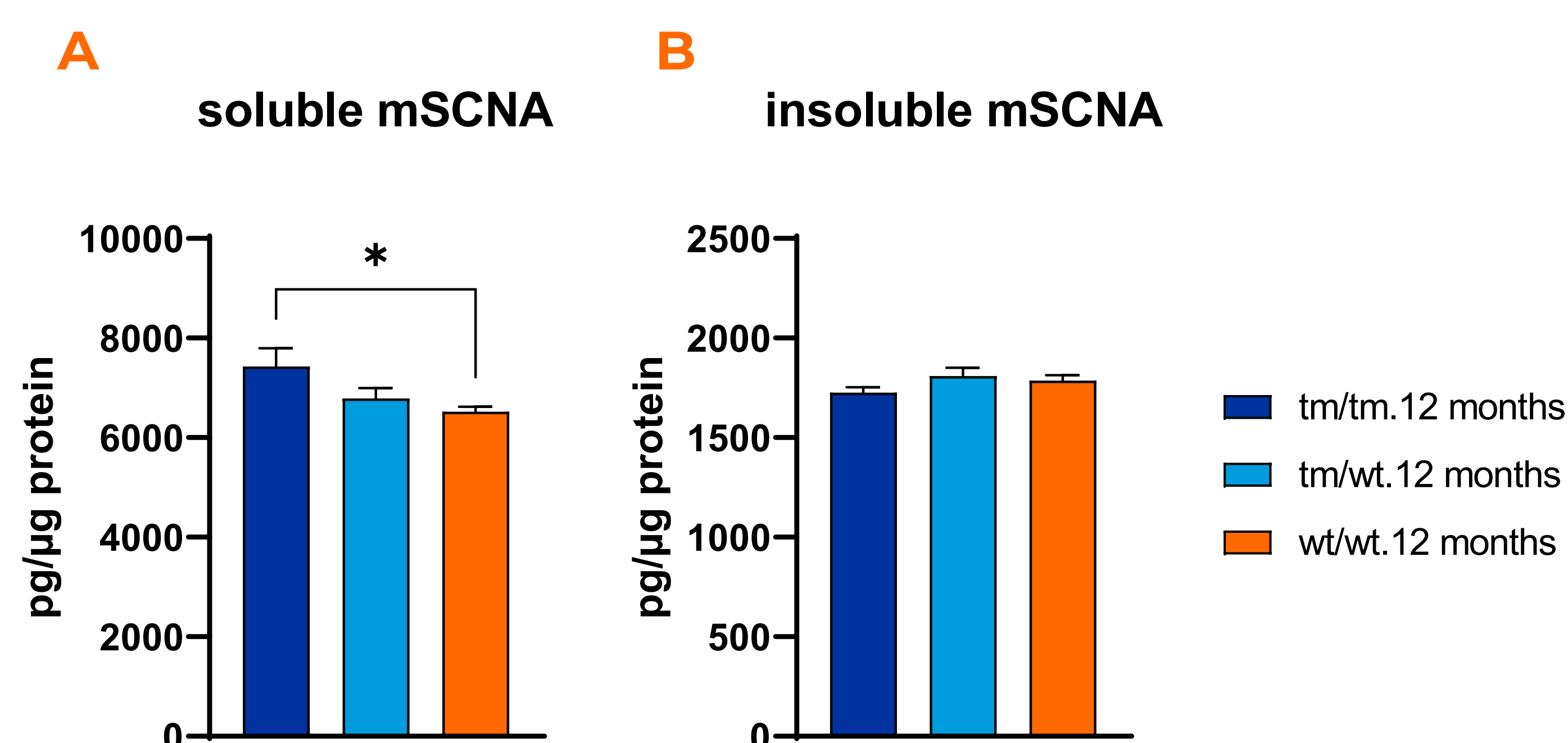
## RESULTS

### GBA1 Activity



**Figure 1. GBA1 activity in liver and brain samples of GBA-D409V-KI mice with age.** GBA1 activity as  $\mu\text{M/h}$  in liver (A) and brain samples (B) of homo- (tm/tm) and heterozygous (tm/wt) GBA-D409V-KI mice as well as age-matched wild type littermates (wt/wt) at 4, 6, 8 and 12 months of age; n=12 per group. Two-way ANOVA with Bonferroni's *post hoc* test; mean + SEM; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

### Soluble and Insoluble Cerebral $\alpha$ -synuclein

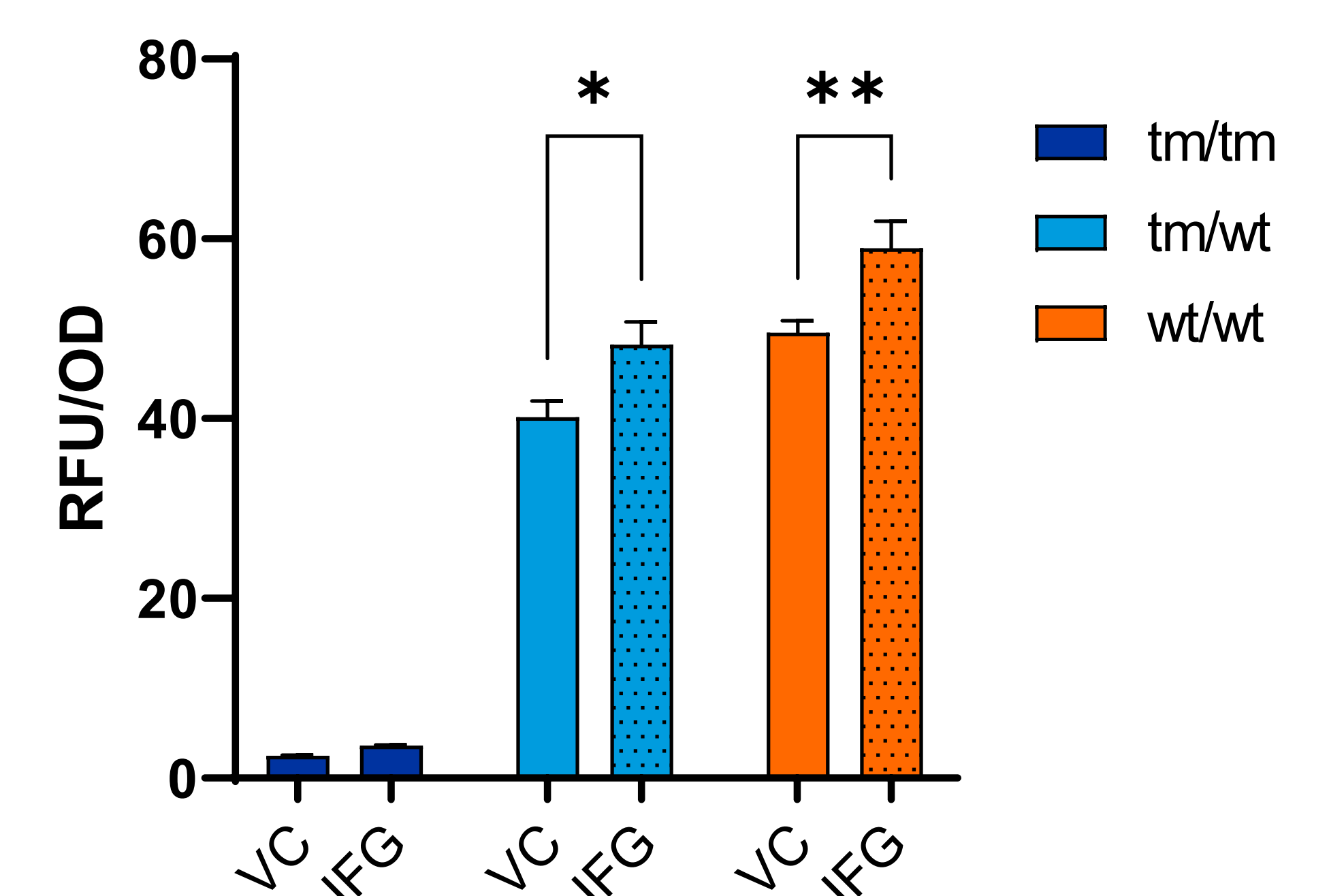


**Figure 2. Soluble and insoluble  $\alpha$ -synuclein in brain samples of 12 months old animals.** Murine  $\alpha$ -synuclein (mSCNA) in soluble (A) and insoluble (B) brain fractions of homo- (tm/tm) and heterozygous (tm/wt) GBA-D409V-KI mice as well as age-matched wild type littermates (wt/wt) at 12 months of age; n=12 per group. Two-way ANOVA with Bonferroni's *post hoc* test; mean + SEM; \* $p < 0.05$ .

### Mouse Embryonic Fibroblasts

**Figure 3. GCase activity in mouse embryonic fibroblasts (MEFs) of different genotypes treated with vehicle (VC) or Isofagomine (IFG).** Mouse embryonic fibroblasts were isolated from homo- (tm/tm) and heterozygous (tm/wt) GBA-D409V-KI E14 embryos as well as age-matched wild type littermates (wt/wt). Cells were cultivated in 96-well plates and treated with either vehicle (0.1 % DMSO) or 20  $\mu\text{M}$  isofagomine for 7 days. Thereafter, cells were subjected to either an adapted on-cell 4-MUG or crystal violet assay. Data are given as relative fluorescent units (RFU) of 4-MUG assay normalized to optical density (OD) values derived from crystal violet assay; n=5 per group. Two-way ANOVA with Bonferroni's *post hoc* test; mean + SEM; \* $p < 0.05$ ; \*\* $p < 0.01$ .

### MEF on-cell GCase assay



## SUMMARY and CONCLUSION

In summary, we provide a baseline characterization of GD and PD biomarkers in GBA-D409V-KI mice and corresponding MEFs. Genotype-specific reduction of GCase activity is reliably present in both models. MEFs are a suitable *in vitro* screening tool before selected compounds are tested in the corresponding *in vivo* mouse model.