**Parkinson disease-linked *GBA* mutation effects reversed by molecular chaperones in human cell and fly models**

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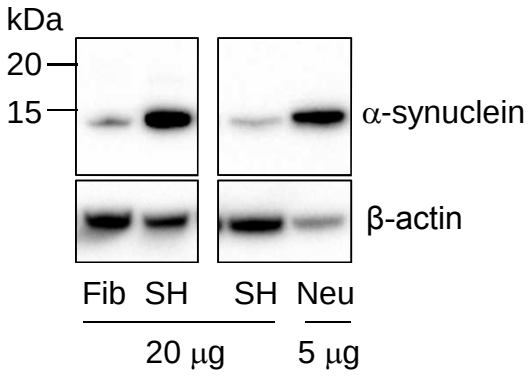
†The authors wish it to be known that, in their opinion, the first three authors should be regarded as joint First Authors

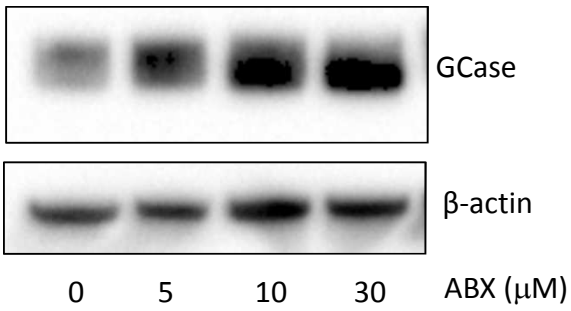



**Supplementary Figure 1. Expression of α-synuclein in fibroblasts is very low.** Human dermal fibroblasts (Fib), human neuroblastoma SH-SY5Y cell line (SH) and primary cortical mouse neurons (neu) were lysed in buffer containing 0.1% (w/v) SDS, 150 mM NaCl, 10 mM Tris (pH 7.5) and separated by SDS-PAGE. Proteins were transfered to Hybond P membrane (GE Healthcare), fixed for 30 minutes with 4% (v/v) paraformaldehyde, 0.01% (v/v) glutaraldehyde in PBS (pH 7.4)[1] and total α-synuclein levels measured by western blot using a mouse-monoclonal antibody that detects both human and mouse α-synuclein (abcam, clone 4D6). Human fibroblasts have barely detectable α-synuclein levels compared to SH-SY5Y cells, which in turn contain much less α-synuclein than primary mouse neurons. Note that only 5 μg of lysate was loaded for neurons compared to 20 μg for fibroblasts and SH-SY5Y cells. Fibroblasts had to be run on a separate blot to neurons to allow for the very weak signal in fibroblasts to be detected by ECL reagent.

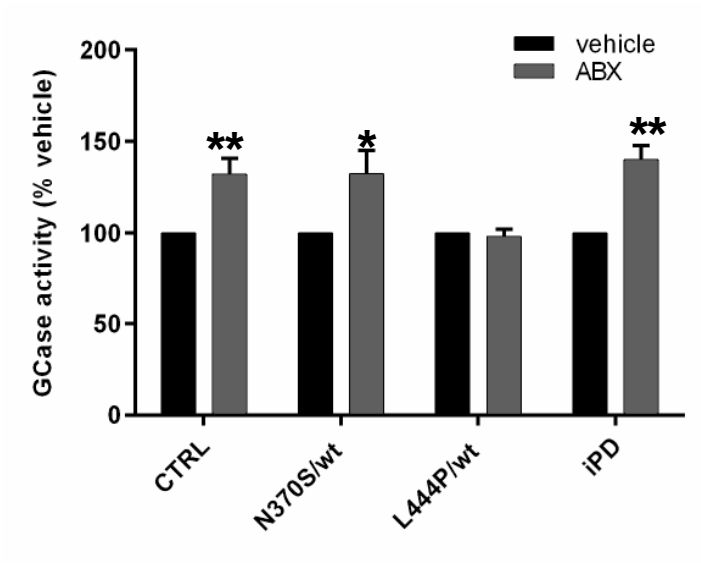
[1] Sasaki A, Arawaka S, Sato H, Kato T. Sensitive western blotting for detection of endogenous Ser129-phosphorylated α-synuclein in intracellular and extracellular spaces. Sci Rep. 2015. 5:14211. doi: 10.1038/srep14211.

A

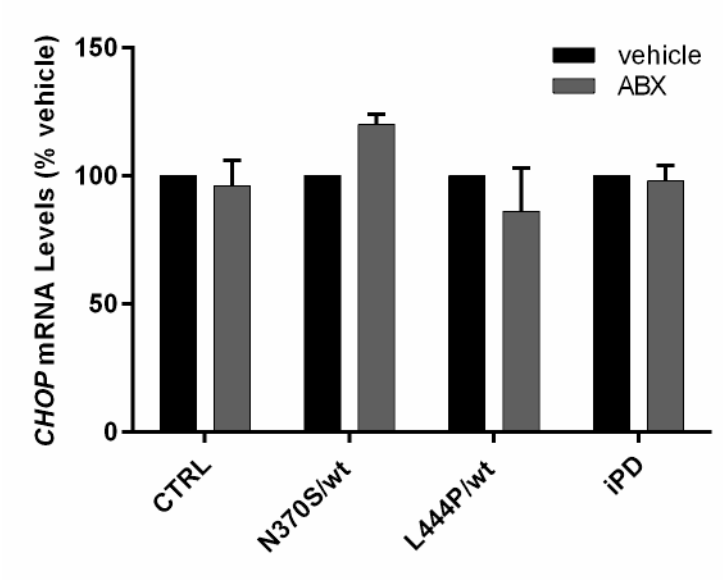
| ABX (μM) | GCase Activity | GBA mRNA |
|---|---|---|
| vehicle | 100 | 100 |
| 5 | 145 | 152 |
| 10 | 207 | 201 |
| 30 | 245 | 271 |



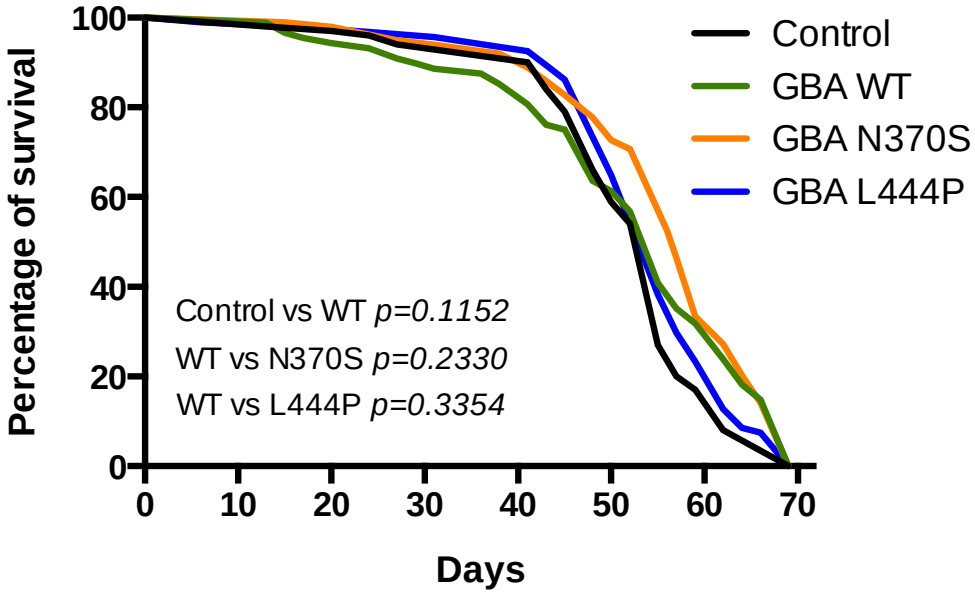

B



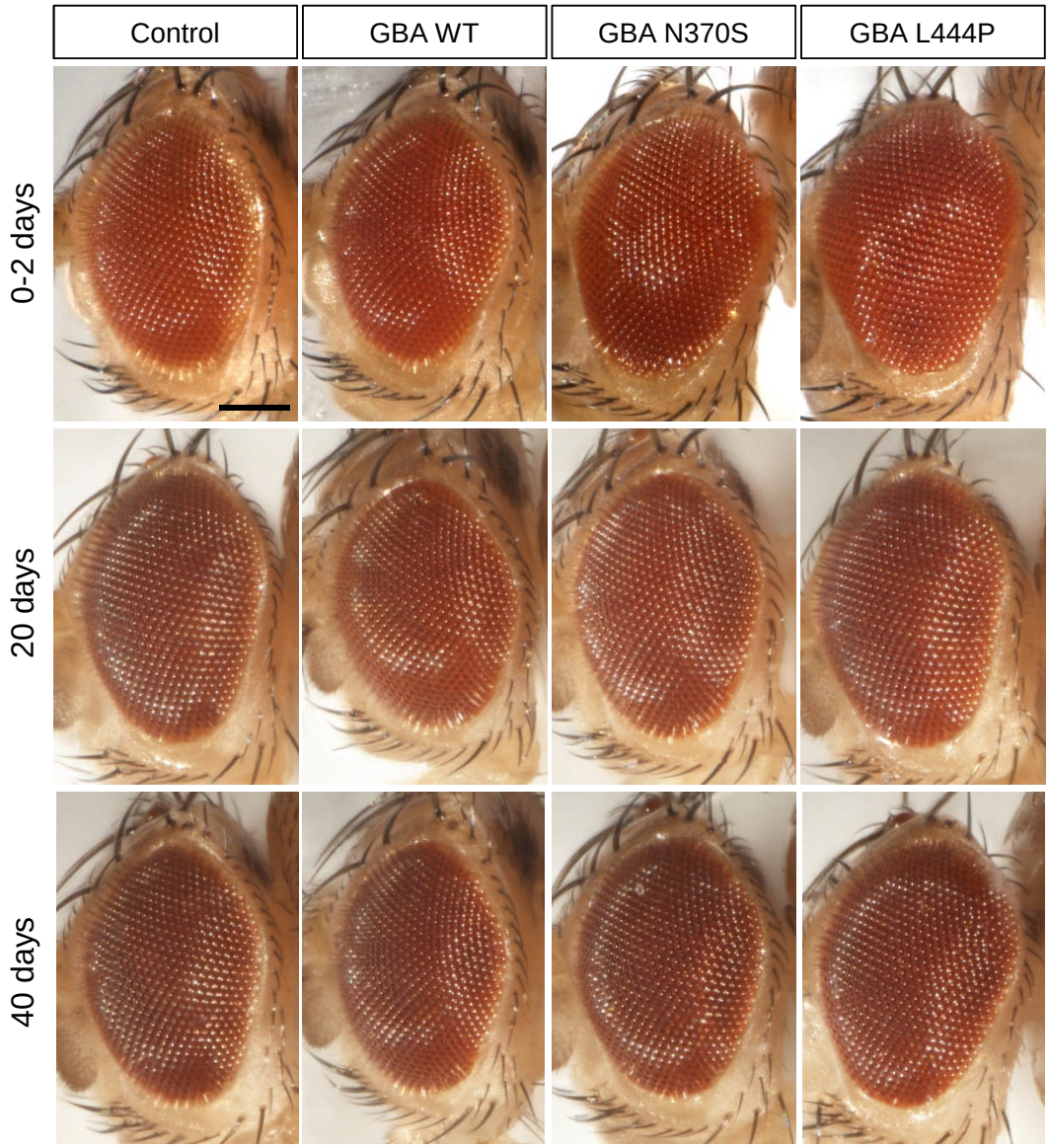

C




**Supplementary Figure 2. The effect of lower ambroxol concentrations on GCase expression and *CHOP* mRNA.** (A) Control fibroblasts (wt/wt) were treated with 0 – 30 μM ambroxol (ABX) for 4 days (treatment on days 0 and 2) and GCase activity and *GBA* mRNA levels measured. Data normalised against protein concentration and *β-actin* mRNA levels for activity and qRT-PCR analysis, respectively. Data expressed as % of vehicle treated. Representative immunoblot for GCase showing increased protein expression with ambroxol treatment. (B) Treatment of CTRL, N370S/wt, L444P/wt or iPD fibroblasts with vehicle (veh) or 5 μM ambroxol (ABX) for 4 days. GCase activity normalised against protein concentration and expressed as % of vehicle treated cells for each cell line. * $P < 0.05$ vs. respective vehicle control; ** $P < 0.05$ vs. respective vehicle control using one-tailed t-test. (C) qRT-PCR analysis of *CHOP* mRNA levels in fibroblasts treated with veh or 5 μM ABX. Data normalized against β-actin mRNA levels and data expressed as % of vehicle treatment for respective cell line (n=3).




**Supplementary Figure 3. Expression of *GBA* variants with a pan-neural driver *elav-GAL4* does not alter life span.**




**Supplementary Figure 4. Overexpression of *GBA* variants in adult eye using *GMR-GAL4* does not show an overt phenotype neither in young nor old flies.**