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Regulation of mitophagy by the NSL complex underlies genetic risk for Parkinson's disease: Drosophila Husbandry, Assays and Immunohistochemistry

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ABSTRACT

This protocol describes *Drosophila* stocks and husbandry, Locomotor and lifespan assays and, Immunohistochemistry and sample preparation in order to assess the role of KAT8/KANSL1 in neuronal survival *in vivo*.

MATERIALS TEXT

Drosophila Strains

Bloomington *Drosophila* Stock Center (RRID:SCR_006457): *mof* RNAi lines, P{TRiP.JF01701} (RRID:BDSC_31401); and P{TRiP.HMS00537} (RRID:BDSC_58281); *ns1* RNAi lines, P{TRiP.HMJ22458} (RRID:BDSC_58328); the pan-neuronal *nSyb-GAL4* driver (RRID:BDSC_51941); and dopaminergic neuron driver (TH-GAL4; RRID:BDSC_8848); and control (*lacZ*) RNAi P{GD936}v51446 from the Vienna *Drosophila* Resource Center (RRID:SCR_013805).

Medium

Agar, cornmeal, molasses, propionic acid and yeast

OPEN ACCESS

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Drosophila stocks and husbandry

- 1 Raise flies under standard conditions in a humidified, temperature-controlled incubator with a 12h:12h light:dark cycle at  25 °C , on food consisting of agar, cornmeal, molasses, propionic acid and yeast.

Note

The following strains were obtained from the Bloomington *Drosophila* Stock Center (RRID:SCR_006457): *mof*RNAi lines, P{TRiP.JF01701} (RRID:BDSC_31401); and P{TRiP.HMS00537} (RRID:BDSC_58281); *ns1* RNAi lines, P{TRiP.HMJ22458} (RRID:BDSC_58328); the pan-neuronal *nSyb-GAL4* driver (RRID:BDSC_51941); and dopaminergic neuron driver (TH-GAL4; RRID:BDSC_8848); and control (*lacZ*) RNAi P{GD936}v51446 from the Vienna *Drosophila* Resource Center (RRID:SCR_013805).

All experiments are conducted using male flies.

Locomotor Assay

- 2 Perform the startle induced negative geotaxis (climbing) assay using a counter-current apparatus.
- 3 Place 20-23 males into the first chamber, and tap to the bottom.
Give 10 s to climb a 10 cm distance.
Repeat this procedure five times (five chambers), and count the number of flies that remain in each chamber.
- 4 Normalize the weighted performance of several group of flies for each genotype to the maximum possible score and expressed as *Climbing index* as described previously in the manuscript by [Greene et al., 2003](#).

Note

Reference:

Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ. Mitochondrial pathology and

apoptotic muscle degeneration in *Drosophila parkin* mutants. *Proc Natl Acad Sci*. 2003;100(7):4078 LP - 4083. doi:10.1073/pnas.0737556100

Lifespan Assay

- 5 For lifespan experiments, grow flies at low-density under identical conditions as described in Step 1
[⇒ go to step #1](#) .
- 6 Collect progeny under very light anaesthesia and keep in tubes of approximately 20 males each, around 50-100 in total.
- 7 Transfer flies every 2-3 days to fresh medium and record the number of dead flies.
- 8 Calculate percent survival at the end of the experiment after correcting for any accidental loss.

3d 5h 30m

Sample Preparation and Immunohistochemistry

- 9 Dissect *drosophila* brains from aged flies and immunostain as previously described in the manuscript by [Whitworth et al., 2005](#).

Note

Reference:

Whitworth AJ, Theodore DA, Greene JC, Beneš H, Wes PD, Pallanck LJ. Increased glutathione *S*-transferase activity rescues dopaminergic neuron loss in a *Drosophila* model of Parkinson's disease. *Proc Natl Acad Sci*. 2005;102(22):8024 LP - 8029. doi:10.1073/pnas.0501078102

- 10 Dissect adult brains in PBS and fix in **[TM] 4 % (w/v)** formaldehyde for **[🕒] 00:30:00** **[🧊] On ice** .

30m

11 Permeabilize in [M] 0.3 % (v/v) Triton X-100 for 3 times ⌚ 00:20:00 . 20m

12 Block with [M] 0.3 % (v/v) Triton X-100 plus [M] 4 % (v/v) goat serum in PBS for ⌚ 04:00:00 at 🌡 Room temperature . 4h

13 Incubate tissues with anti-tyrosine hydroxylase (Immunostar Inc. #22491), diluted in [M] 0.3 % (v/v) Triton X-100 plus [M] 4 % (v/v) goat serum in PBS for ⌚ 72:00:00 at 🌡 4 °C , then rinse 3 times ⌚ 00:20:00 with [M] 0.3 % (v/v) Triton X-100 in PBS, and incubate with the appropriate fluorescent secondary antibodies ⌚ Overnight at 🌡 4 °C . 3d 0h 40m

14 Wash tissues 2 times in PBS and mount on slides using Prolong Diamond Antifade mounting medium (Thermo Fisher Scientific). 🧪

15 Image brains with a Zeiss LMS 880 confocal. Count tyrosine hydroxylase-positive neurons under blinded conditions. 🔬