

**Maintenance of mitochondrial integrity in midbrain dopaminergic neurons
governed by a conserved developmental transcription factor**

SUPPLEMENTARY INFORMATION

Supplementary Figures

Supplementary Figure 1

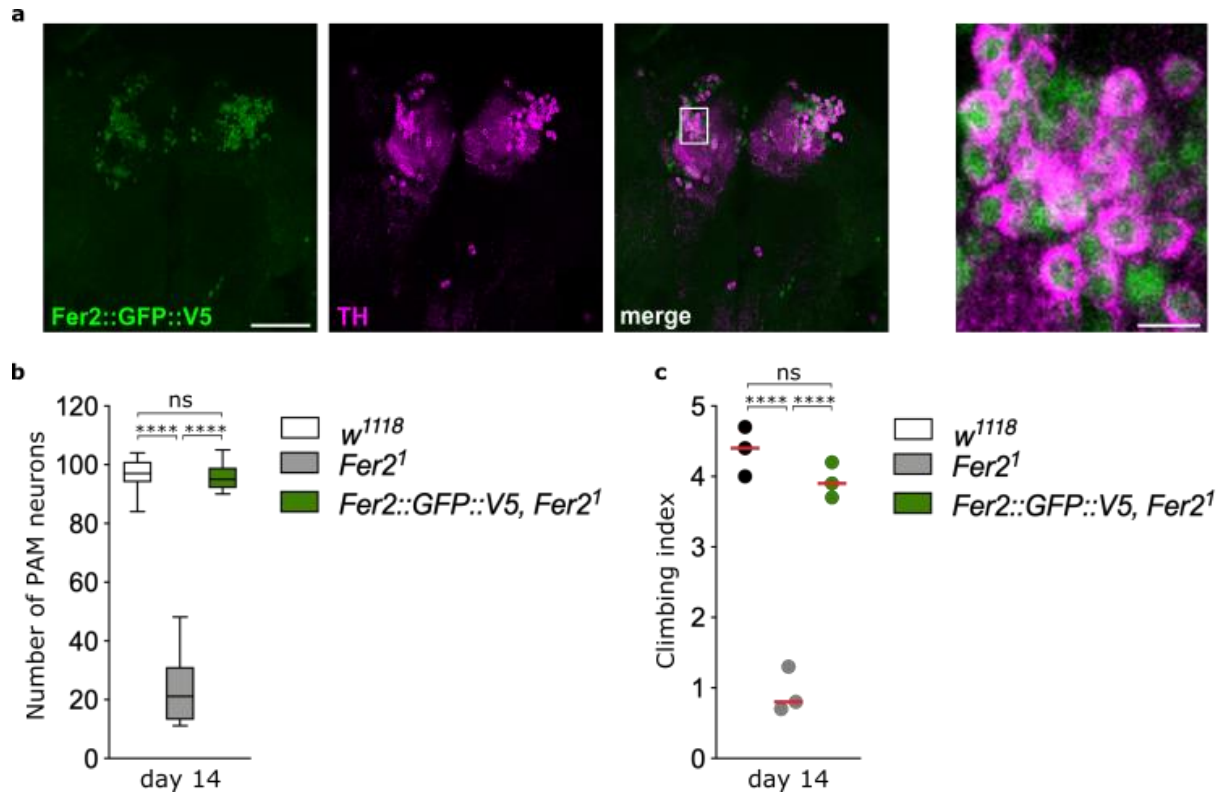
Supplementary Figure 2

Supplementary Figure 3

Supplementary Figure 4

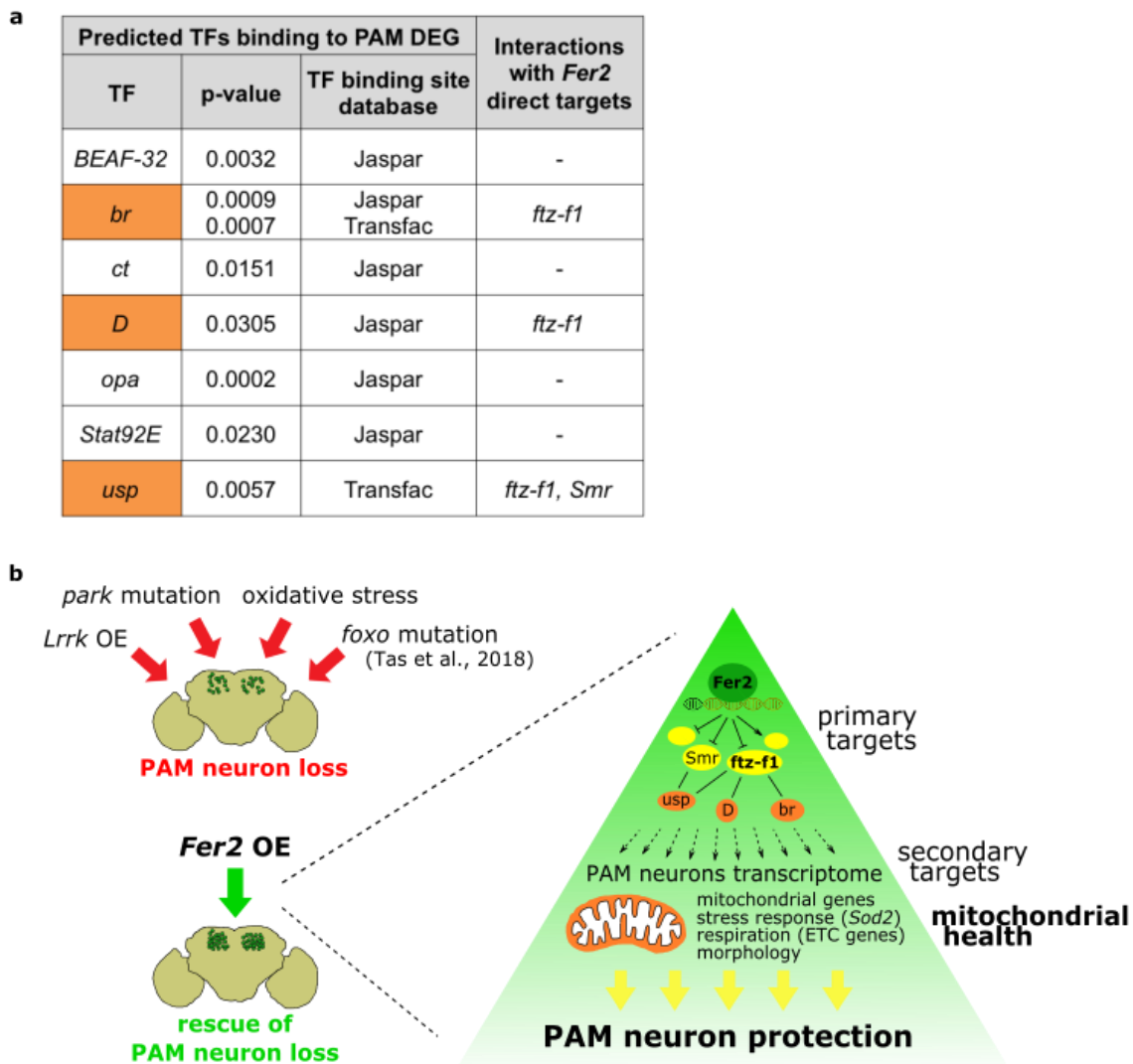
Supplementary Figure 5

One-way ANOVA followed by a Turkey's test for multiple group comparison. **(d)** Quantification of the number of PAM neurons labeled with RedStinger in *park¹* mutants at day 14. The elements of the box plots are as in (a-c) and defined in the Method section. n=16 hemispheres per group. Box boundaries in **(a)**, **(b)**, **(c)** and **(d)** are the 25th and 75th percentiles, the horizontal line across the box is the median, and the whiskers indicate the minimum and maximum values. Two-tailed Mann-Whitney test found no difference between two genotypes.



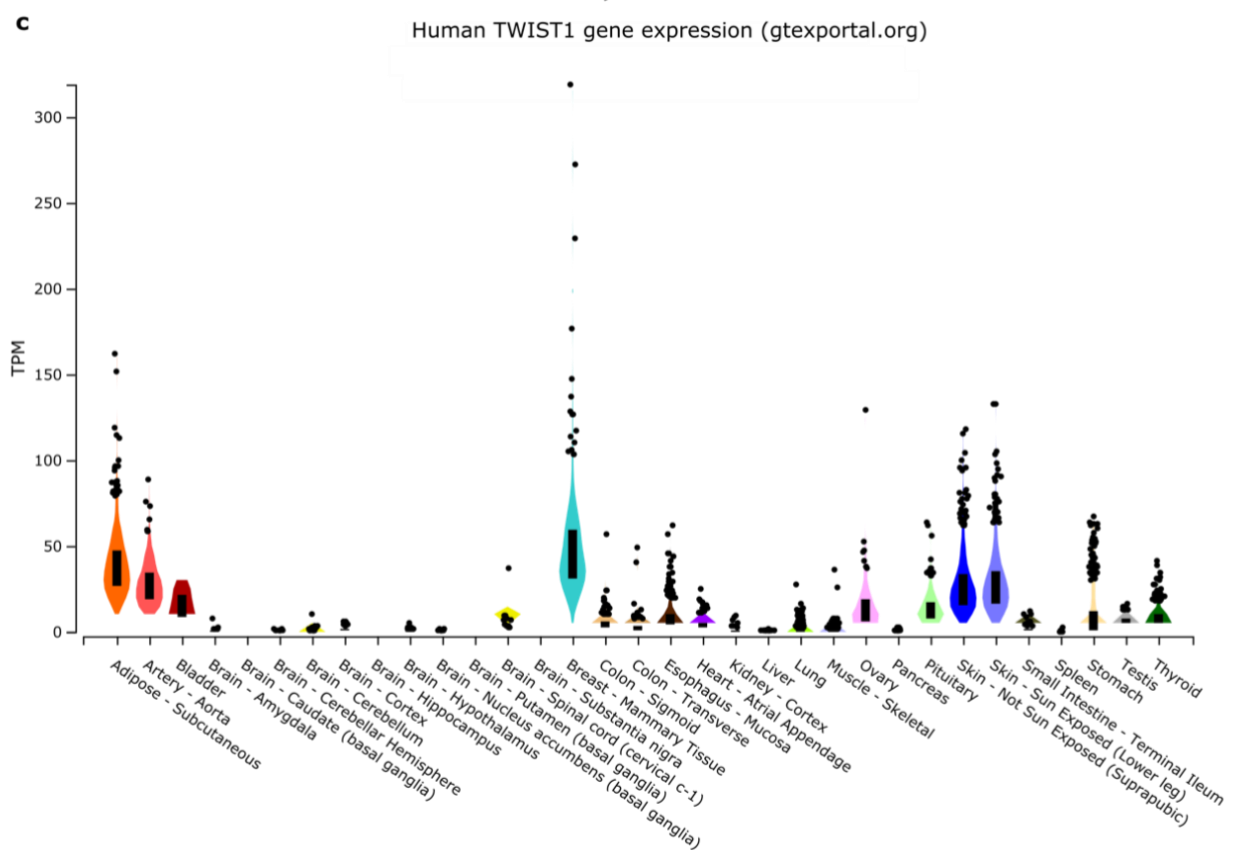
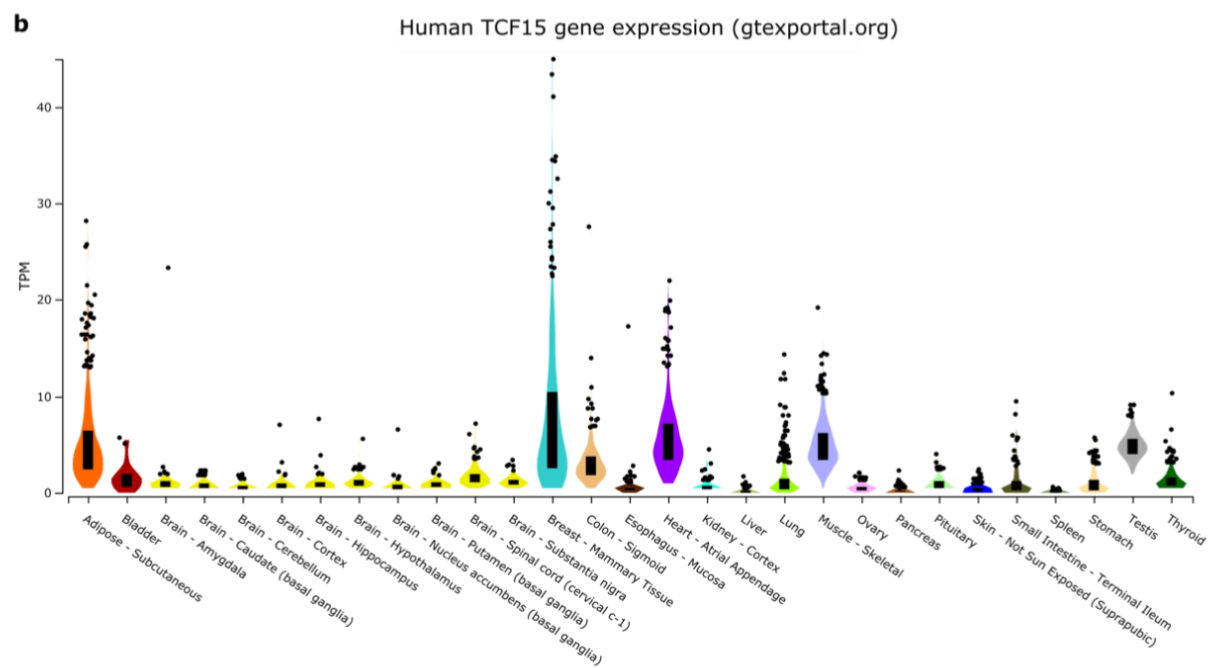
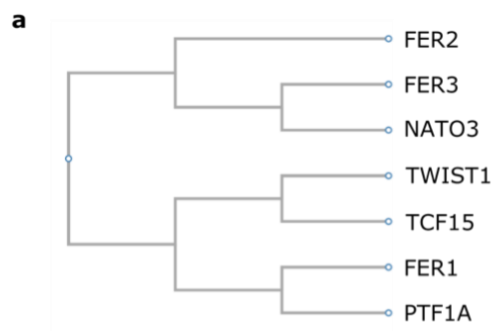
Supplementary Figure 2. *Fer2::GFP::V5* transgene rescues PAM neuron loss and locomotor deficits in *Fer2¹* mutants.

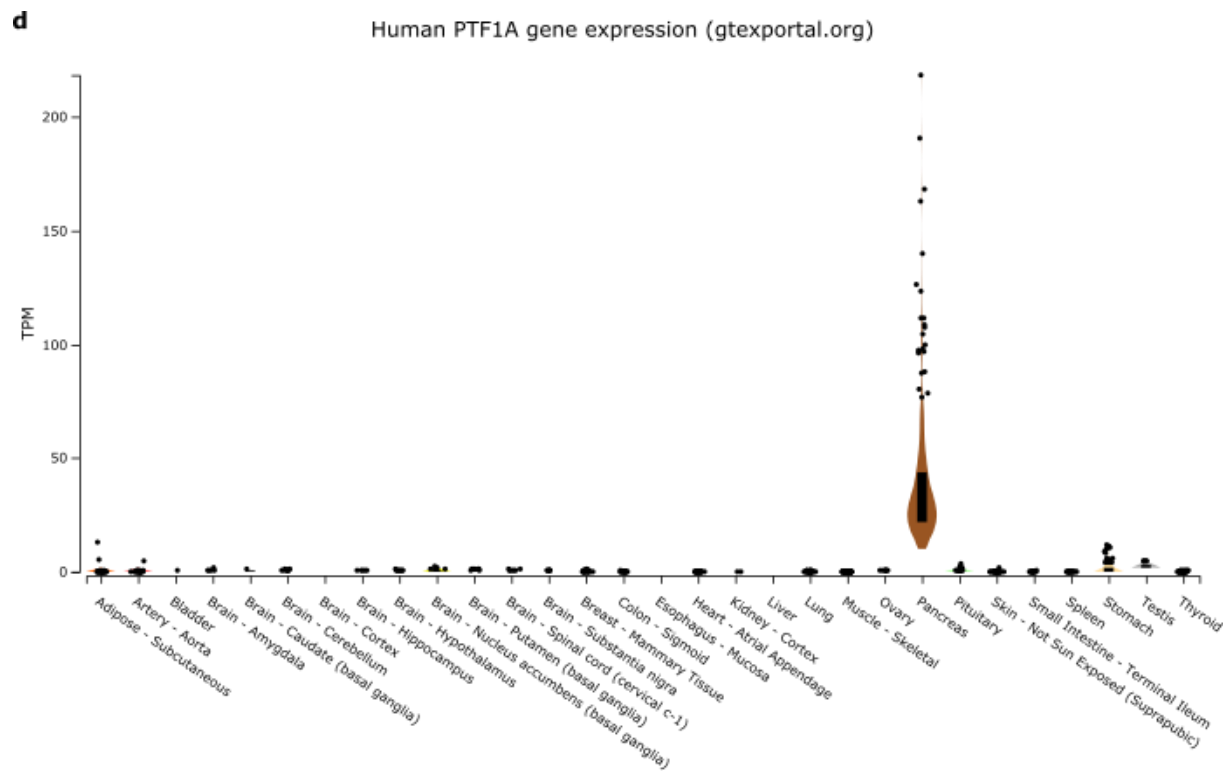
(a) Brains of *Fer2::GFP::V5, Fer2¹* flies at day 14 were stained with anti-GFP (green) and anti-TH (magenta) antibodies. The right panel presents a high-magnification image of the PAM neurons in the square from the left panel. Left panel scale bar, 50 μ m. Right panel scale bar, 5 μ m. **(b)** Quantification of the number of PAM neurons per hemisphere, as detected by anti-TH immunostaining in *w¹¹¹⁸*, *Fer2¹* and *Fer2::GFP::V5, Fer2¹* flies at day 14. n=14 hemispheres per group. Box boundaries are the 25th and 75th percentiles, the horizontal line across the box is the median, and the whiskers indicate the minimum and maximum values. One-way ANOVA followed by a Turkey's test for multiple group comparison, **** p <0.0001. ns, not significant. **(c)** Climbing index of *w¹¹¹⁸*, *Fer2¹* and *Fer2::GFP::V5, Fer2¹* flies at day 14. Three independent experiments. One-way ANOVA followed by a Turkey's test for multiple group comparison, **** p <0.0001. ns, not significant.



Supplementary Figure 3. Genetic pathways downstream of *Fer2*.

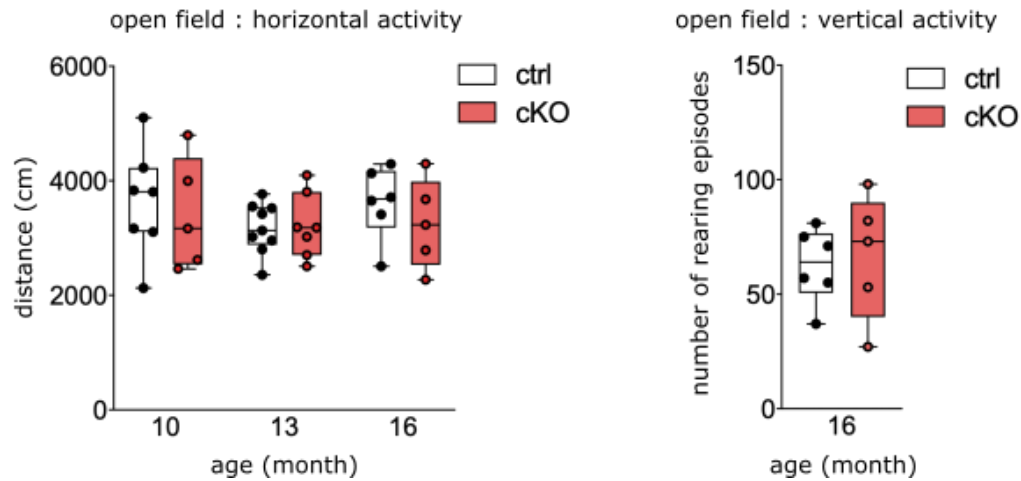
(a) List of transcription factors that are expressed in PAM neurons and whose binding sites are significantly overrepresented in the promoters of PAM RNA-seq DEGs. Enrichment *p*-value (z-test) and the databases used for the analysis are indicated. Transcription factors known or predicted to interact with one or more *Fer2* direct targets are highlighted in orange. **(b)** A model for the role of *Fer2* in dopaminergic neuroprotection. *Fer2* binds to and regulates the expression of a set of direct target genes, including multiple transcription factors and chromatin regulators. *Fer2* direct targets FTZ-F1 and SMR interact with D, Br and USP and regulate a large set of genes within PAM neurons. In this manner, *Fer2* controls multiple pathways leading to regulation of mitochondrial gene expression, improved mitochondrial health, and PAM neuron survival against genetic and oxidative insults.





Supplementary Figure 4. Phylogenetic and expression analysis of mammalian genes with significant similarity to FER2 protein sequence.

(a) Phylogenetic tree generated by multiple protein sequence alignment of bHLH domains of *Drosophila* FER1, FER2, FER2 and mouse TCF15, NATO3, PTF1A and TWIST1, using ClustalW. bHLH domains were identified with PROSITE Expasy tool. **(b-d)** TCF15 **(b)**, TWIST1 **(c)** and PTF1A **(d)** expression levels in human tissues measured by RNA-seq as reported in GTEx portal (gtexportal.org).



Supplementary Figure 5. Locomotor activity measured in the open field test is not affected in *Nato3* cKO mice.

Levels of horizontal activity (left), measured as the total distance traveled in the arena, and vertical activity (right), measured as the number of rearing events, were not different between *Nato3* cKO and control mice. Horizontal activity: n=7 (ctrl, 10 months; cKO, 13 months), n=5 (cKO, 10 months), n=9 (ctrl, 13 months), n=6 (ctrl, 16 months), n=5 (cKO, 16 months) mice per group. Vertical activity: n=6 (ctrl), n=5 (cKO) mice per group. Box boundaries are the 25th and 75th percentiles, the horizontal line across the box is the median, and the whiskers indicate the minimum and maximum values. No statistically significant difference between ctrl and cKO by two-tailed Mann-Whitney test.