Supplementary Materials for

Insulin-like growth factor II prevents oxidative and neuronal damage in cellular and mice models of Parkinson's disease

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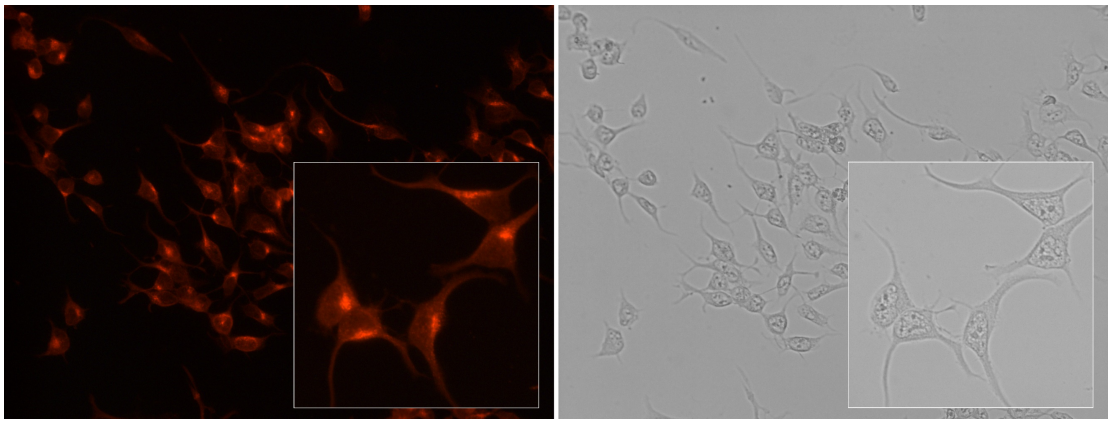
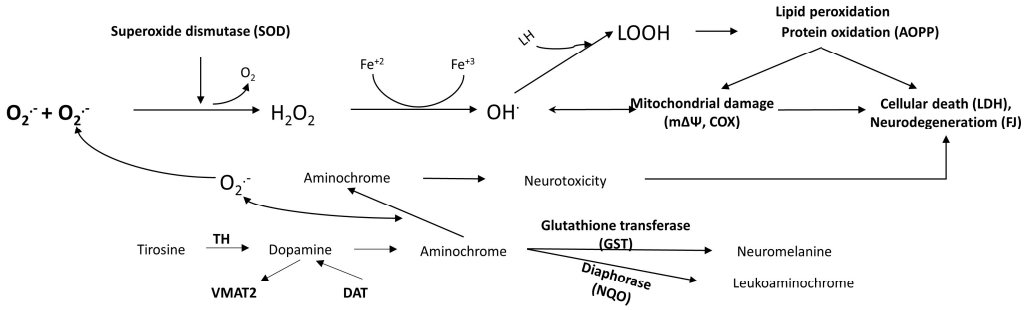
This word file includes:

Supplementary Figure 1.



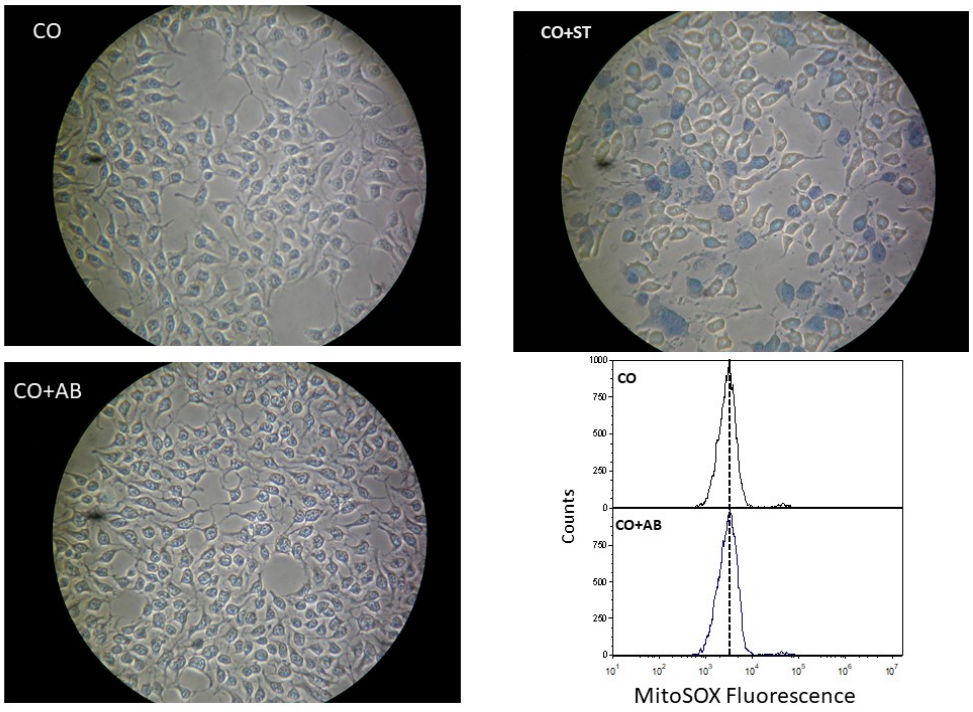
Supplementary Fig. 1: Representative immunocytochemistry images and bright field and of IGF-IIr in SN4741 dopaminergic neurons. Images were acquired using an Olympus BX51 epifluorescence microscope at 40x magnification.

Supplementary Fig. 2:



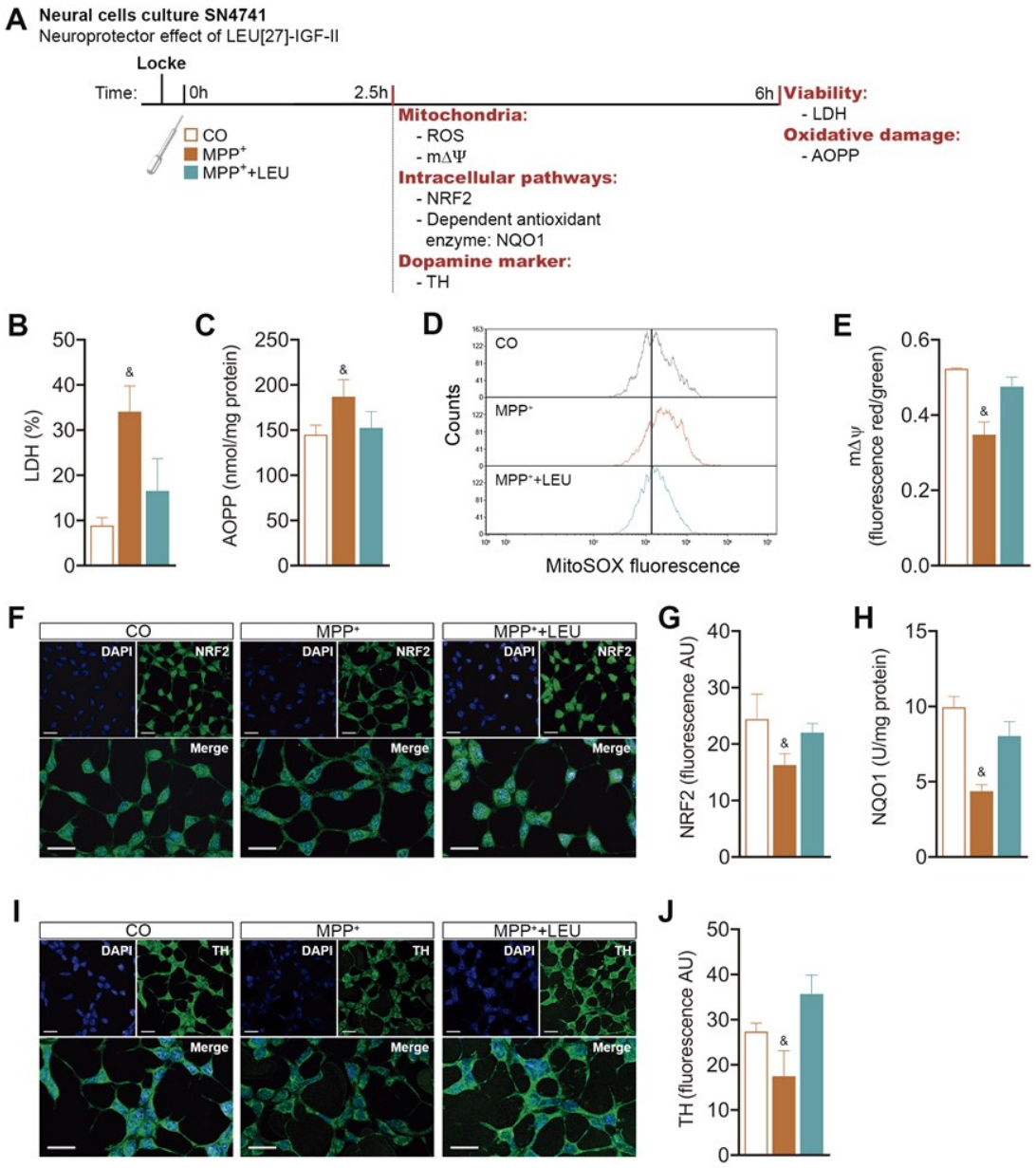
Supplementary Fig. 2: Major pathways of oxidative homeostasis, survival, and neuronal dopaminergic markers, with the parameters studied in bold.

Supplementary Fig. 3:



Supplementary Fig. 3: Effects of AB on SN4741 dopaminergic cells. Survival was measured using Trypan Blue stain after 6h of incubation of cells, at 37 ºC, with Locke or AB (20ng/mL) or staurosporine (ST) 500nM. Images were acquired in an inverted microscope at 4X magnification. Redox production was assessed by MitoSOX probes and flow cytometry after incubation of cells for 2.5 h at 37°C with Locke or AB (20ng/mL).

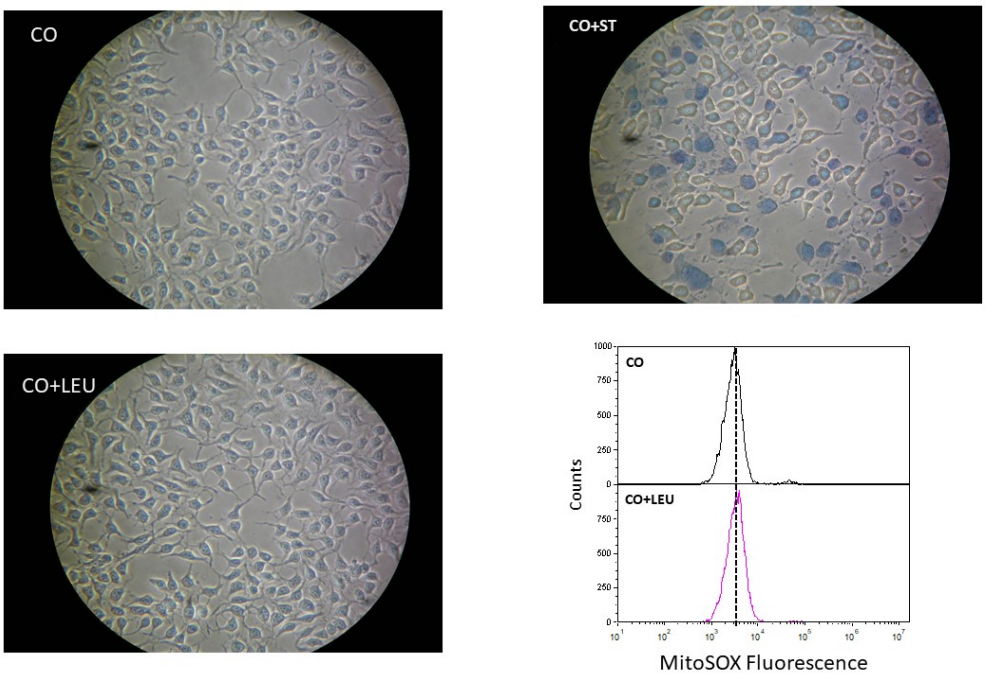
Supplementary figure 4



Supplementary Fig. 4: Effects of LEU[27]-IGF-II on SN4741 dopaminergic cells against MPP$^+$-induced toxicity. (A) Experimental design to assess the relevance of IGF-IIr in the main action of IGF-II. The study was taken in SN4741 cells after 2.5 h or 6h, at 37°C, of incubation with MPP$^+$, in the presence or absence of LEU (10nM), an IGF-II analogue with high affinity for

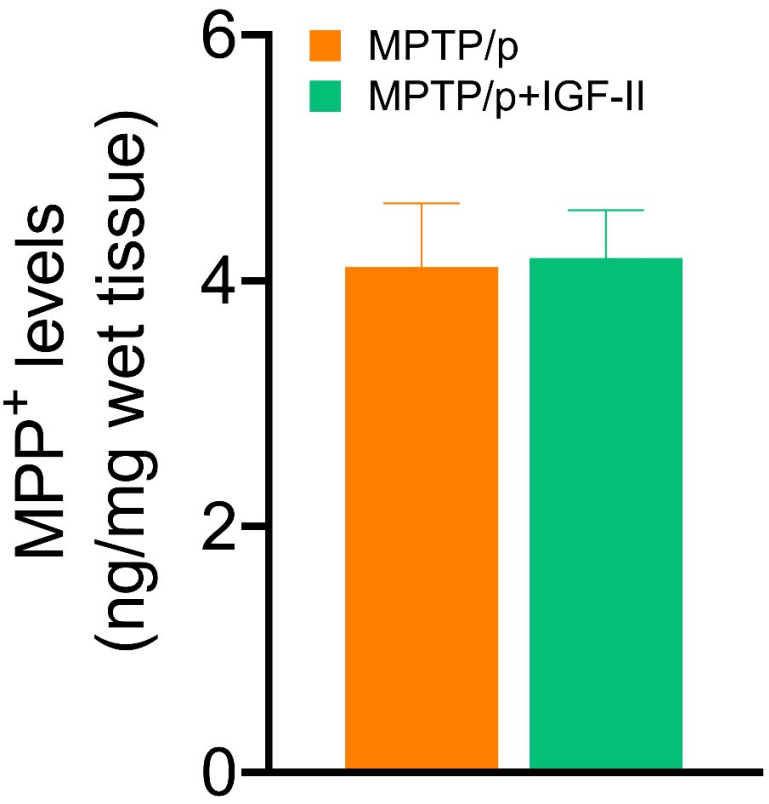
IGF-IIr. **(B)** Cytotoxicity, measured by quantifying LDH release and expressed as % of control. **(C)** Oxidative damage was evaluated as Protein oxidation (AOPP). **(D)** Mitochondrial function was estimated using MitoSOX fluorescence as mitochondrial ROS production and **(E)** Mitochondrial mΔΨ measured as JC1 fluorescence aggregates. **(F)** Intracellular signalling pathway was evaluated as immunocytochemistry stain for NRF2 and nuclear DAPI (Representative images), **(G)** Quantification of NRF2 immunofluorescence and **(H)** NRF2 target gene NQO1 activity. **(I)** Dopamine marker was assessed as immunocytochemistry stain for nuclear DAPI and TH (Representative images). **(J)** Quantification of TH immunofluorescence. Data are expressed as mean ± SEM. n = 6 each group (3 independent experiment). & $P< 0.05$, versus all other groups. Data were analysed by one-way ANOVA followed by Tukey´s multiple comparison test.

Supplementary figure 5



Supplementary Fig. 5: Effect of LEU[27]-IGF-II on SN4741 dopaminergic cells. Survival was measured using Trypan Blue stain after 6h at 37 ºC of incubation with Locke or Leu (100 ng/mL) or staurosporine (ST) 500nM. Images were acquired in an inverted microscope at 4X magnification. Redox production was assessed by MitoSOX probes and flow cytometry, after incubation of cells for 2.5 h at 37°C with Locke or Leu (10 nM).

Supplementary figure 6



Supplementary Fig. 6: Measurement of the striatal MPP^+ concentration. Quantification was performed 2 h after the administration of MPTP/p, in the presence and absence of IGF-II. Data are expressed as mean ± SEM and analysed by unpaired Student's t-test ($P > 0.05$).