Supporting Information

Disease-specific phenotypes in dopamine neurons from human iPS-based models of genetic and sporadic Parkinson's disease

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Fig. S1

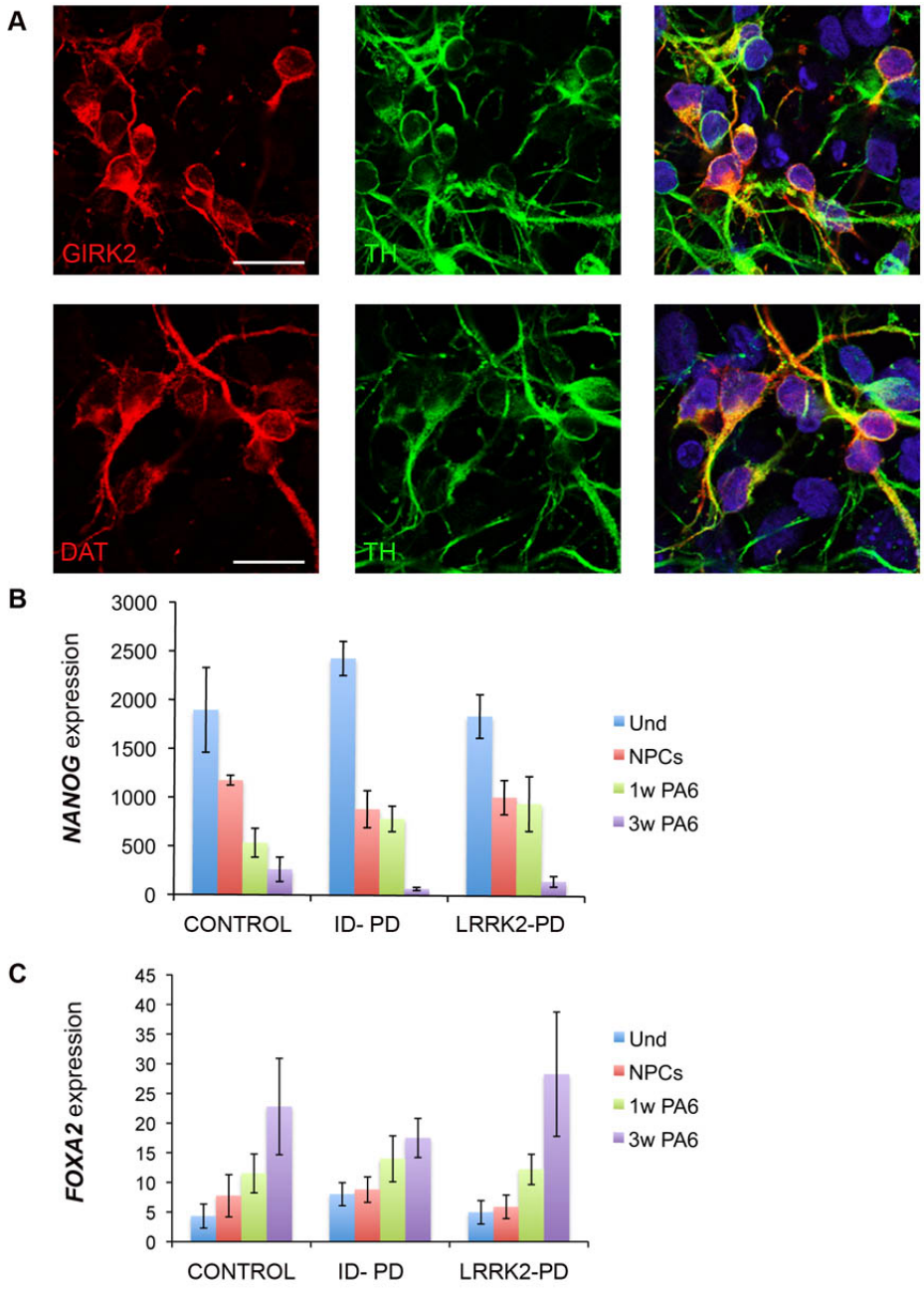

Fig. S1. Directed differentiation of iPSC towards vmDA neurons. (**A**) DA neurons (TH labelled cells) generated from iPSC transduced with a lentivirus that forced the expression of LMX1A in neural progenitors express the vmDA markers DAT and GIRK2 at the end of the differentiation protocol. Cell nuclei counterstained with DAPI (blue). (**B**) Quantitative PCR with reverse transcription (RT-qPCR) for *NANOG*, a pluripotency-associated transcription factor. (**C**) RT-qPCR for *FOXA2*, a dopaminergic progenitor marker. Data for CONTROL is the average of three different Ctrl-iPSC clones, for ID-PD is the average of five different ID-PD iPSC clones, and for LRRK2-PD group is the average of three LRRK2-PD iPSC. *HPRT* and *B2M* were used for normalization. Und refers to iPSC in undifferentiated state, NPCS to neural precursor cells, 1w PA6 to NPCs co-cultured one week with PA6 and 3w PA6 refers to the end of the differentiation protocol when vmDA neurons have been generated. Representative pictures shown in **A** correspond to the SP11.1 cell line. Scale bar, 12.5 μm.

Fig. S2

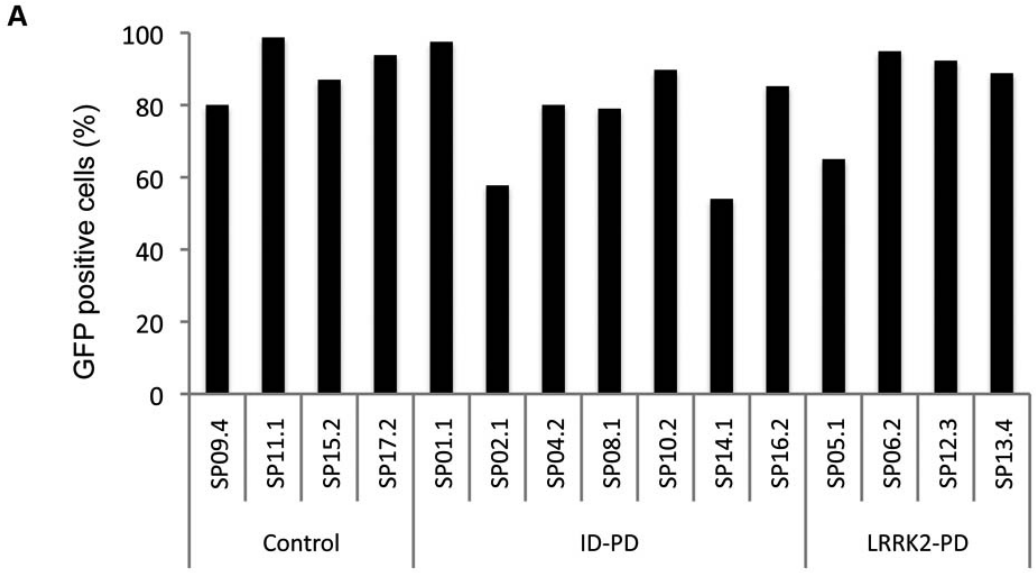

Fig. S2.: Efficiency of lentiviral transduction. (**A**) Analysis of GFP expression in neural precursor cells (NPCs) transduced with the lentivirus LV.NESE.LMX1A.IRES.GFP. Note that LMX1A and GFP are expressed when the *NESTIN* neural enhancer is active, corresponding to neural precursor cells. Neural precursors cells cultured in suspension were trypsinized to single cells and analysed by flow cytometry for GFP expression. An average of 50,000 cells per each condition were analyzed.

Fig. S3

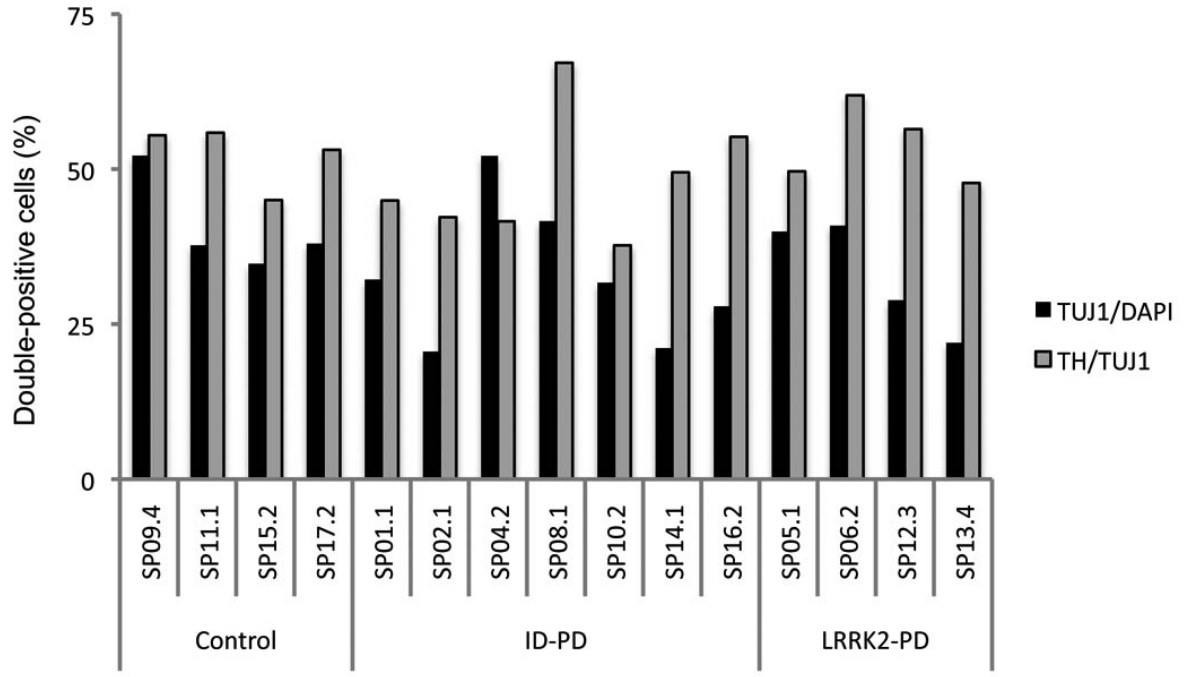

Fig. S3. Neuronal and dopaminergic yield varies within iPSC clones. (**A**) iPSC clones transduced with LV.NESE.LMX1A.IRES.GFP were differentiated to DA neurons and, at the end of the protocol, stained for the DA marker TH, the neuronal marker TUJ1, and DAPI. For quantification of stained cells, 300 cells were randomly scored per differentiated aggregate, with an average of 6 differentiated aggregates per each iPSC clone.

Fig. S4

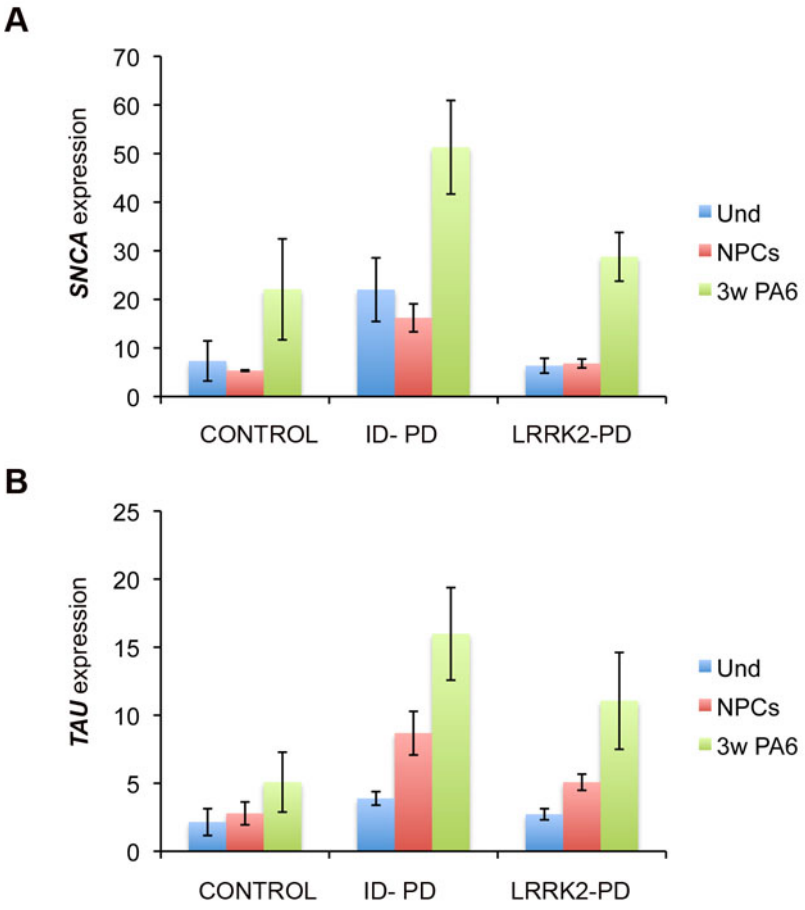

Fig. S4. Upregulation of the expression of PD-related genes during DA neuron differentiation. (**A**) Quantitative PCR with reverse transcription (RT-qPCR) for *SNCA* and (**B**) *TAU*. Data for CONTROL is the average of three different Ctrl-iPSC clones, for ID-PD is the average of four different ID-PD iPSC clones, and for LRRK2-PD group is the average of three LRRK2-PD iPSC. *HPRT* and *B2M* were used for normalization. Und corresponds to iPSC in undifferentiated state, NPCs to neural precursor cells, and 3w PA6 refers to the end of the differentiation protocol, when vmDA neurons have been generated.

Fig. S5

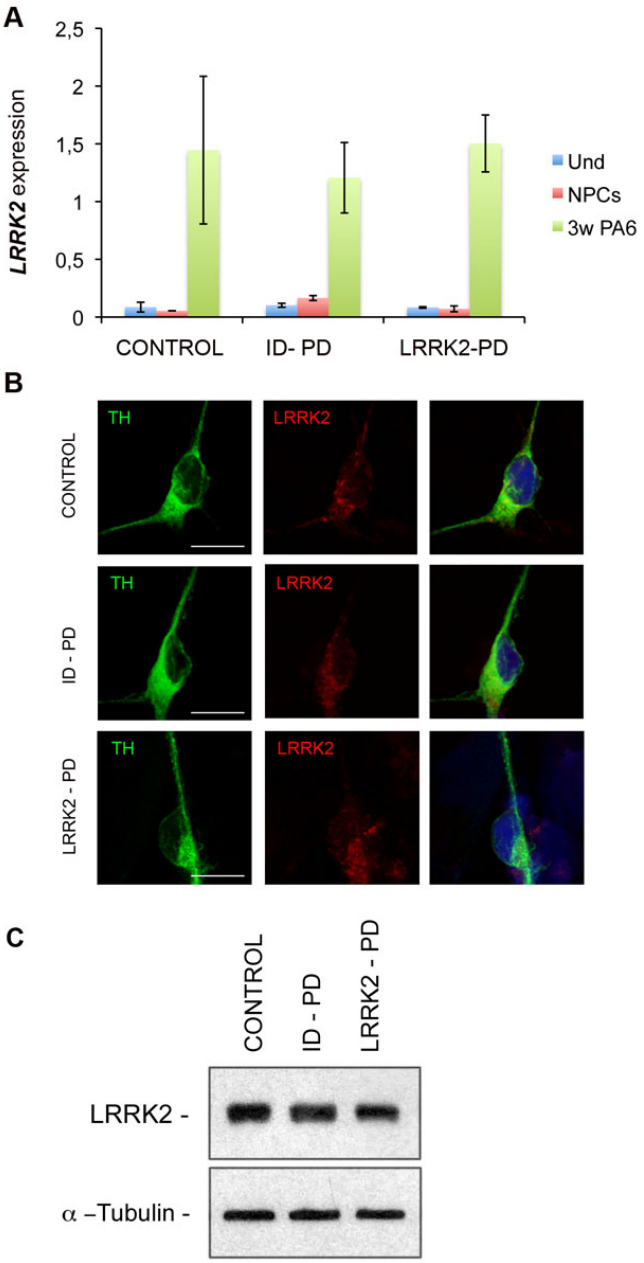

Fig. S5. LRRK2 is expressed in dopaminergic neurons at the end of the 30-day differentiation protocol. (A) Quantitative PCR with reverse transcription (RT-qPCR) for *LRRK2*. Data for CONTROL is the average of three different Ctrl-iPSC clones, for ID-PD is the average of four different ID-PD iPSC clones, and for LRRK2-PD group is the average of three LRRK2-PD iPSC. *HPRT* and *B2M* were used for normalization. Und refers to iPSC in undifferentiated state, NPCs to neural precursor cells, and 3w PA6 refers to the end of the differentiation protocol, when vmDA neurons have been obtained. (**B**) Dopaminergic neurons (TH positive cells, green) generated on the top of PA6 for three weeks and cultured on matrigel for three days stained for LRRK2 (red). Representative images are shown from CONTROL (SP11.1), ID-PD (SP08.1) and LRRK2-PD (SP12.3). (**C**) Western blot shows LRRK2 expression in dopaminergic neuron cultures at the end of 30-day dopaminergic differentiation protocol for CONTROL (SP15.2), ID-PD (SP16.2) and LRRK2-PD (SP12.3). Alpha-tubulin is used as a loading control. In **B** nuclei are counterstained with DAPI, shown in blue. Scale bars, 10μm.

Fig. S6

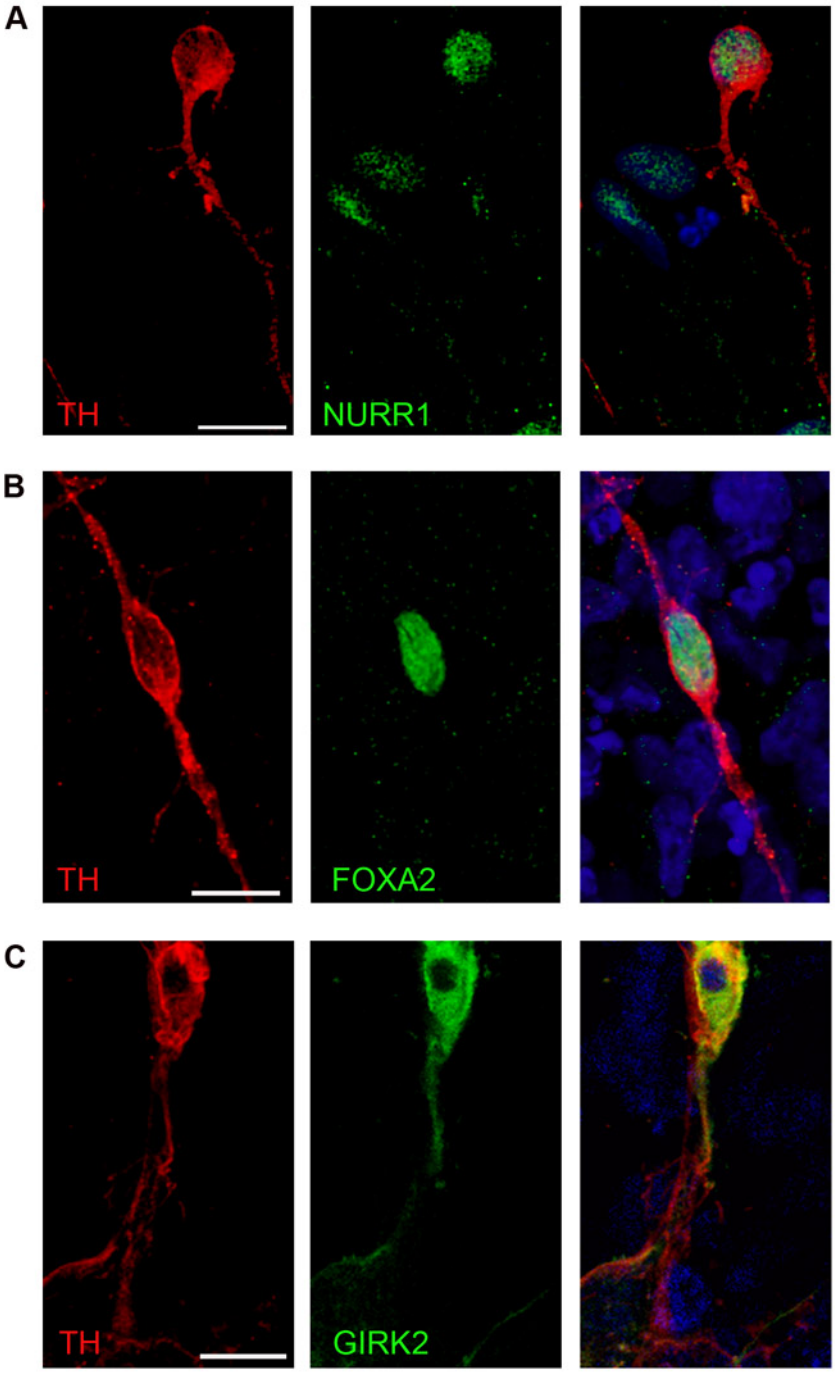

Fig. S6. DA neurons in long-term cultures show a vmDA phenotype. (**A**) The differentiation of NPCs on the top of cortical astrocytes gave rise to neurons that presented a vmDA phenotype, indicated by the presence of cells double positive for TH/NURR1 (**A**), TH /FOXA2 (**B**), and TH/GIRK2 (**C**), which could be maintained for an extended culture time span (75 days). Cell nuclei are counterstained with DAPI, shown in blue. Shown are representative pictures from the SP11.1 cell line. Scale bar, 10 μm.

Fig. S7

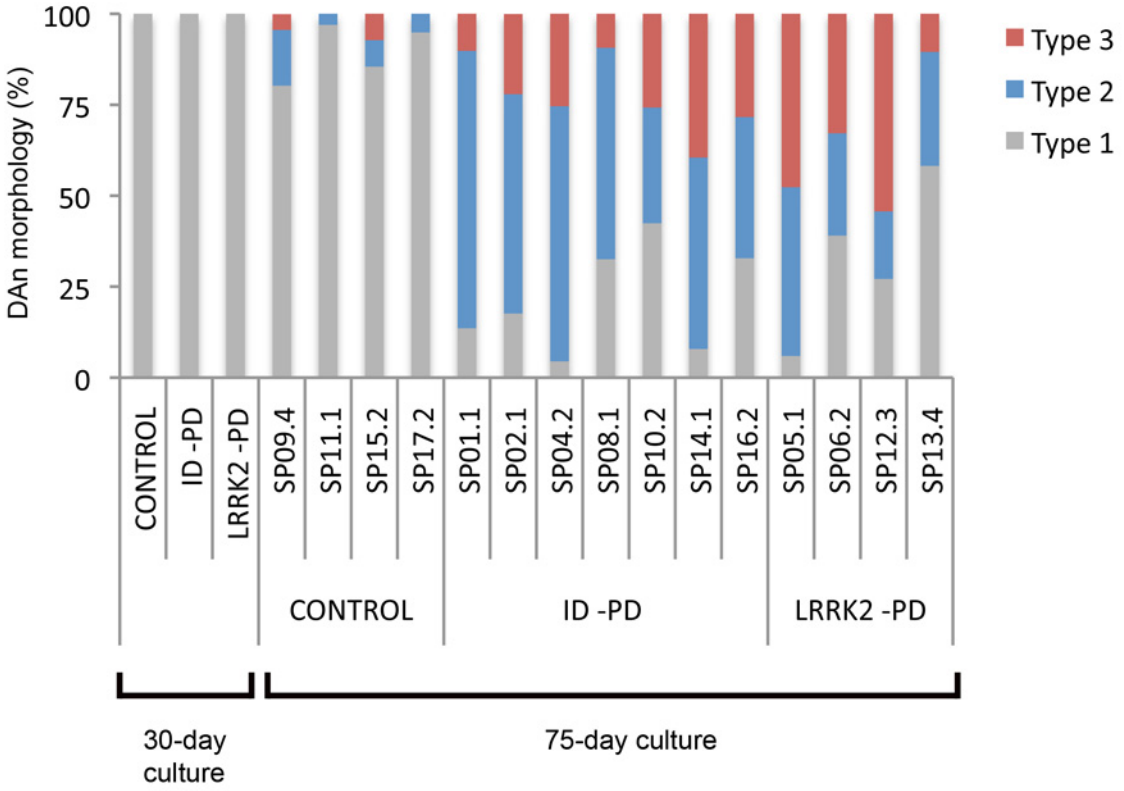

Fig. S7. Morphological alterations of DAn from PD-iPSC upon long-term culture. Quantitative analyses of the percentage of DAn showing the three types of morphology after 30 or 75 days of culture, in the indicated cell lines. Data for 30-day cultures are average of 4 Ctrl-iPSC lines, 7 ID-PD iPSC lines, and 4 LRRK2-PD iPSC lines. For 75-day cultures, individual data for each iPSC line are shown.

Fig. S8

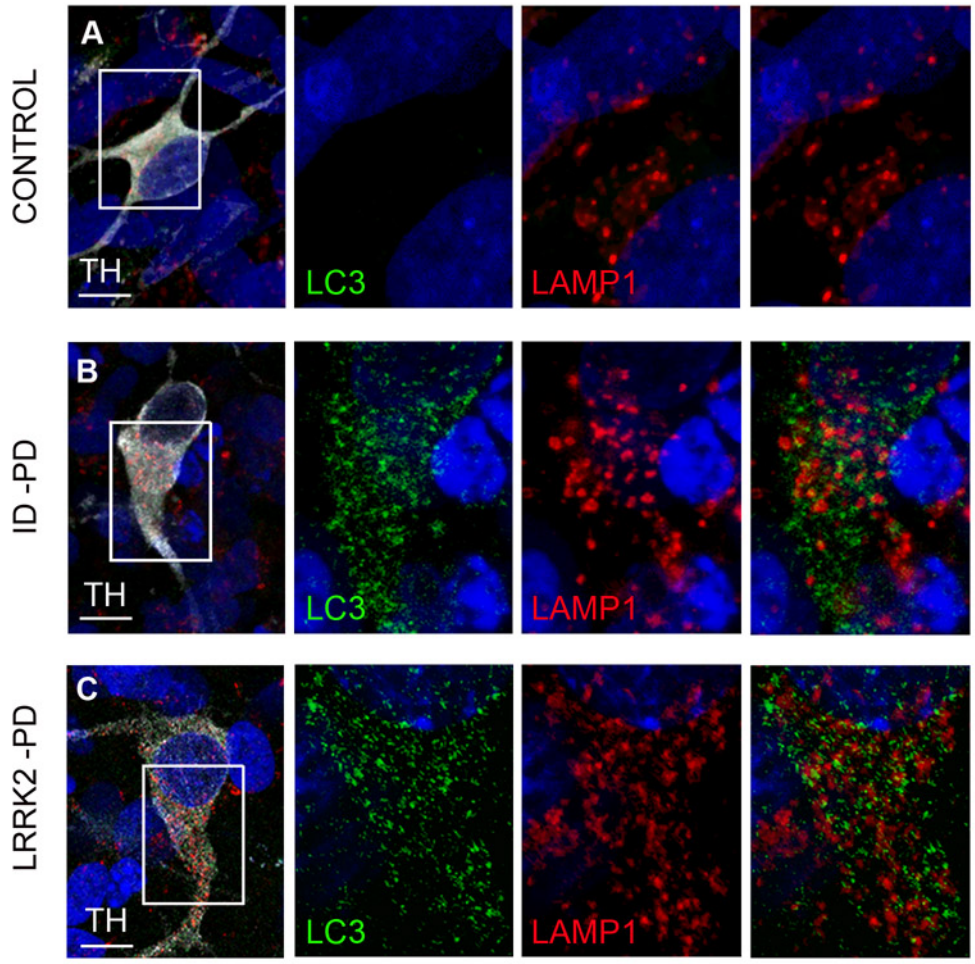

Fig. S8. Expression of autophagic markers in iPSC-derived dopaminergic neurons. (A-C) Dopaminergic neurons (TH positive, white) from (**A**) CONTROL (SP11.1), (**B**) ID-PD (SP10.2) and (**C**) LRRK2-PD (SP05.1) after 75 days of culture analysed for LC3 (green) and LAMP1(red) showing basal levels of autophagy. Cell nuclei are counterstained with DAPI, shown in blue. Scale bar, 5μm.

Fig. S9

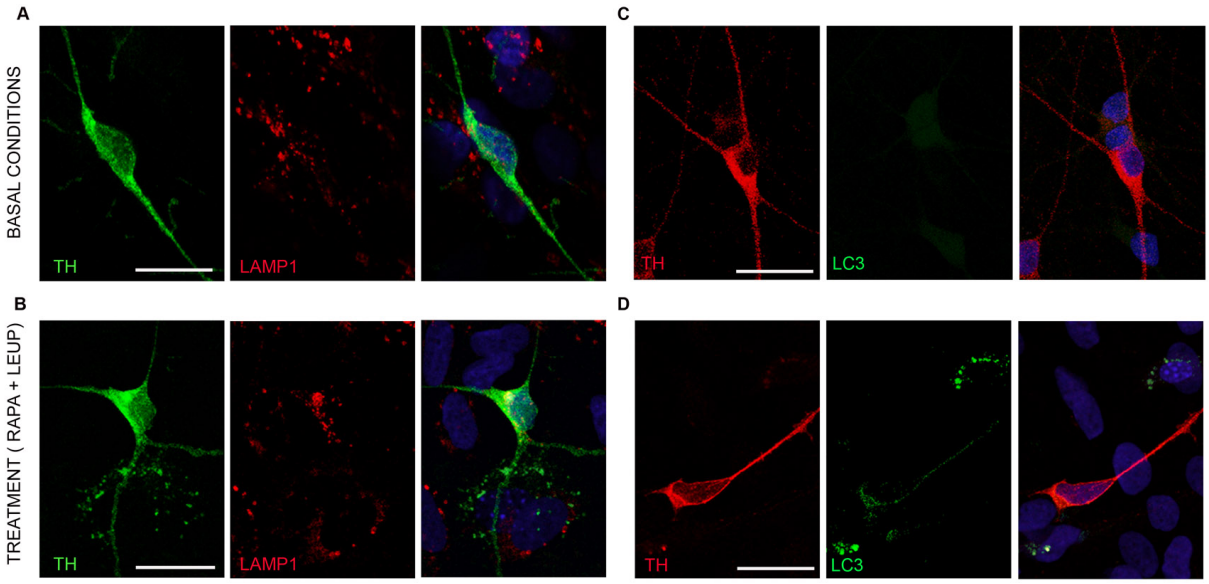

Fig. S9. Treatment with rapamycin and leupeptin causes an increase in the expression of autophagic markers in iPSC-derived DA neurons. (**A** and **C**) DA neurons from Ctrl-iPSC (SP11.1 cell line) after 75 days of culture were analyzed for the autophagic markers LAMP1 (**A**) and LC3 (**C**), showing basal levels of autophagy. (**B** and **D**) DA neurons after 6h of treatment with rapamycin and leupeptin were stained for LAMP1 (**B**) and LC3 (**D**). Cell nuclei are counterstained with DAPI, shown in blue. Scale bar, 15 μm.

Fig. S10

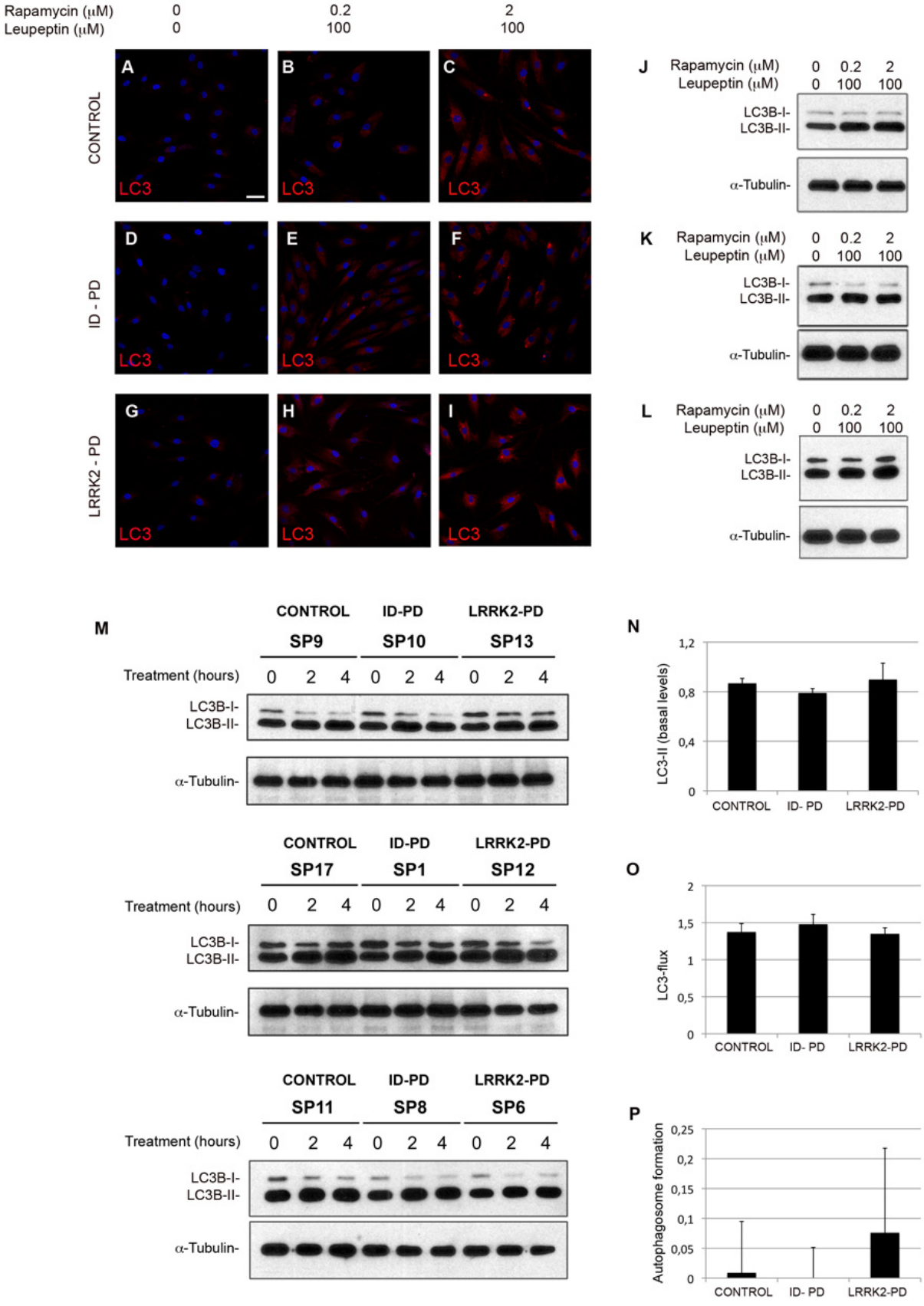

Fig. S10. Normal autophagy in fibroblasts from PD patients. (**A-I**) Immunofluorescence analyses of dermal fibroblasts stained for LC3 in **(A-C)** CONTROL, (**D-F**) ID-PD and (**G-I**) LRRK2-PD fibroblasts, after treatment with the indicated concentrations of rapamycin and

leupeptin. (**J-L**) Western blot analysis for LC3 expression in dermal fibroblasts, after different concentrations of rapamycin and leupeptin treatment in (**J**) CONTROL, (**K**) ID-PD and (**L**) LRRK2-PD. α-tubulin is used as a loading control. (**A-L**) For immunocytochemistry and western blot analysis representative images are shown, CONTROL refers to SP17 cell line, ID-PD to SP16 and LRRK2-PD to SP12. (**M**) Western blot analysis for LC3 in fibroblasts after treatment with leupeptin and NH_4Cl during the indicated periods of time. α-tubulin is used as a loading control. (**N**) LC3B-II basal level quantification normalized to α-tubulin. Values calculated using the no-treatment condition (0 hours). (**O**) Quantification of LC3-flux normalized to α-tubulin. (**P**) Quantification of the autophagosome formation normalized to α-tubulin. (**N-P**) Data per each group is the average of three cell lines (shown in **M**). Note that, as previously described, the autophagic flux in fibroblasts is markedly slower than in neurons (compare with Fig 5K-N). In **A**-**I** nuclei are counterstained with DAPI, shown in blue. Scale bar, 50μm.

Fig. S11

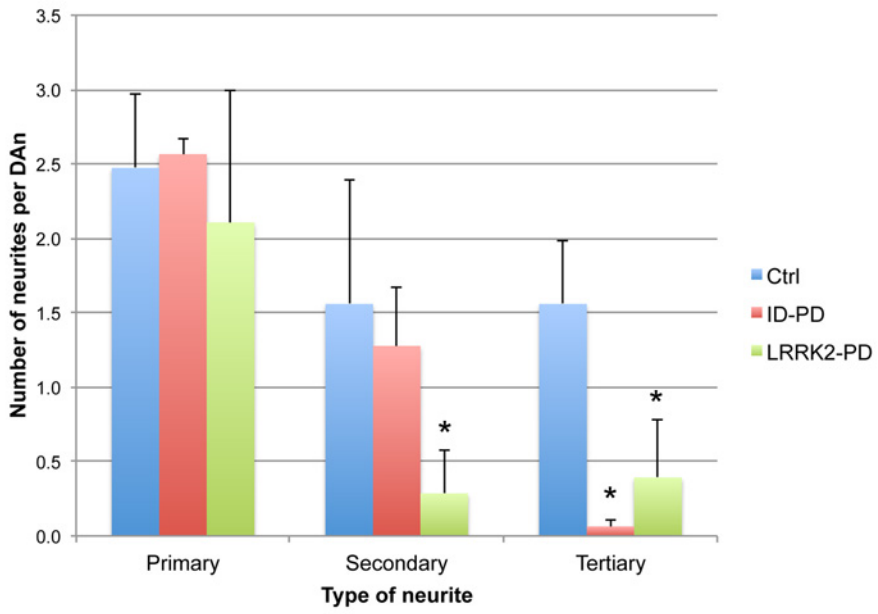

Fig. S11. Increased susceptibility of PD-iPSC-derived DA neurons to MPP+ treatment. Quantitative analyses of the number of the indicated types of neurites in DA neurons differentiated from Ctrl-iPSC (blue bars), ID-PD iPSC (red bars), and LRRK2-PD iPSC (green bars), after 30 days of differentiation and treated with sub-lethal concentrations of MPP+. Data shown are average and SEM of three Ctrl-iPSC lines, three ID-PD iPSC lines, and three LRRK2-PD iPSC lines. Statistically significant differences compared with Ctrl-iPSC-derived DA neurons are indicated with asterisks.