Figure S1

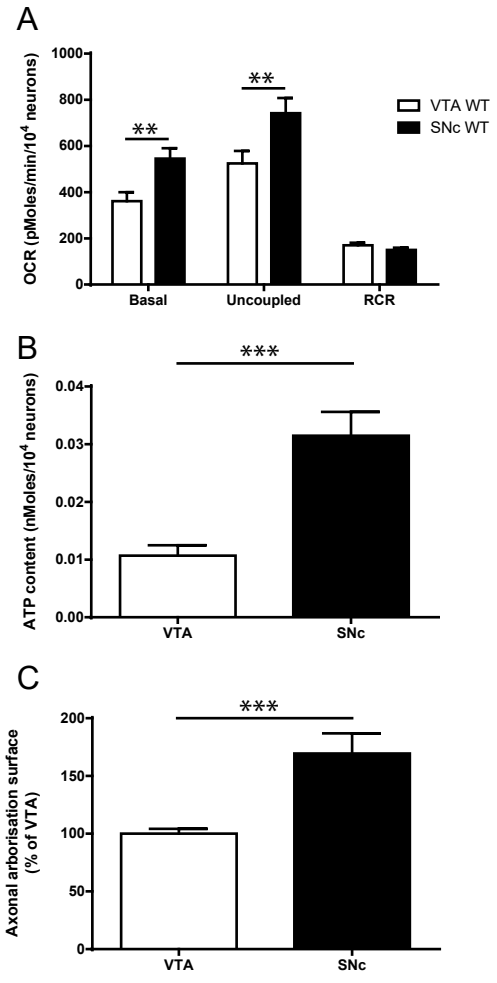
A
OCR (pMoles/min/10^4 neurons)
1000
800
600
400
200
0
**
**
VTA WT
SNc WT
Basal
Uncoupled
RCR
B
ATP content (nMoles/10^4 neurons)
0.04
0.03
0.02
0.01
0.00
***
VTA
SNc
C
Axonal arborisation surface (% of VTA)
200
150
100
50
0
***
VTA
SNc

Figure S2

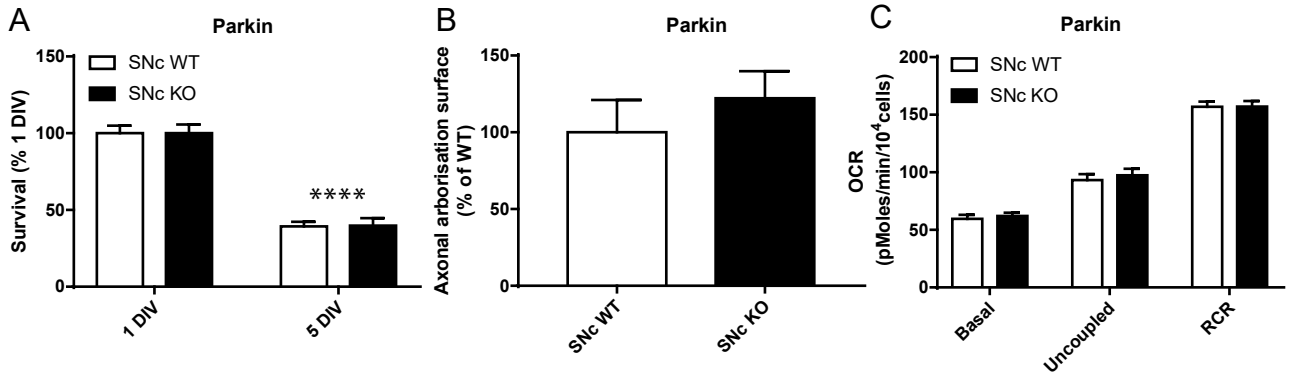
A
Parkin
SNc WT
SNc KO
Survival (% 1 DIV)
150
100
50
0
****
1 DIV
5 DIV
B
Parkin
Axonal arborisation surface (% of WT)
150
100
50
0
SNc WT
SNc KO
C
Parkin
SNc WT
SNc KO
OCR (pMoles/min/10^4 cells)
200
150
100
50
0
Basal
Uncoupled
RCR

Figure S3

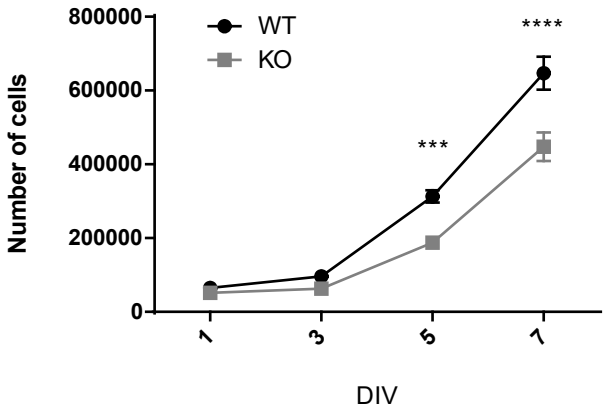

Figure S4

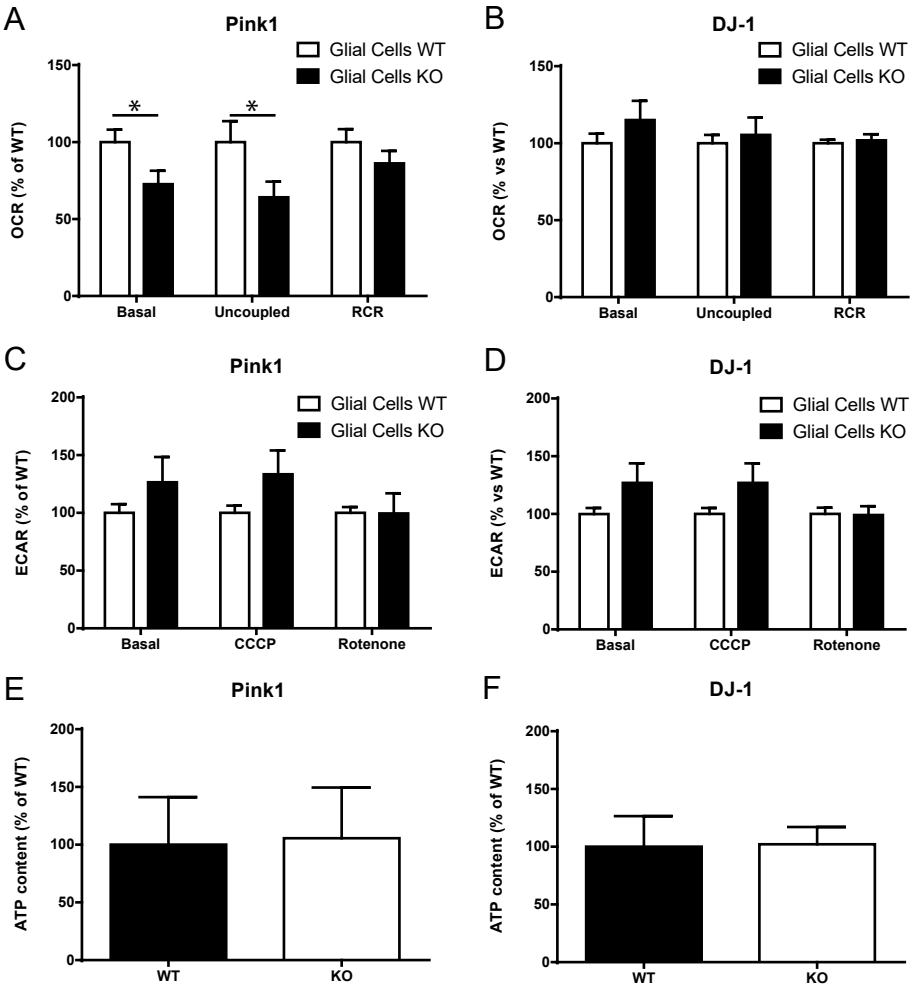
A
Pink1
Glial Cells WT
Glial Cells KO
OCR (% of WT)
150
100
50
0
*
*
Basal
Uncoupled
RCR
B
DJ-1
Glial Cells WT
Glial Cells KO
OCR (% vs WT)
150
100
50
0
Basal
Uncoupled
RCR
C
Pink1
Glial Cells WT
Glial Cells KO
ECAR (% of WT)
200
150
100
50
0
Basal
CCCP
Rotenone
D
DJ-1
Glial Cells WT
Glial Cells KO
ECAR (% vs WT)
200
150
100
50
0
Basal
CCCP
Rotenone
E
Pink1
ATP content (% of WT)
200
150
100
50
0
WT
KO
F
DJ-1
ATP content (% of WT)
200
150
100
50
0
WT
KO

Figure S5

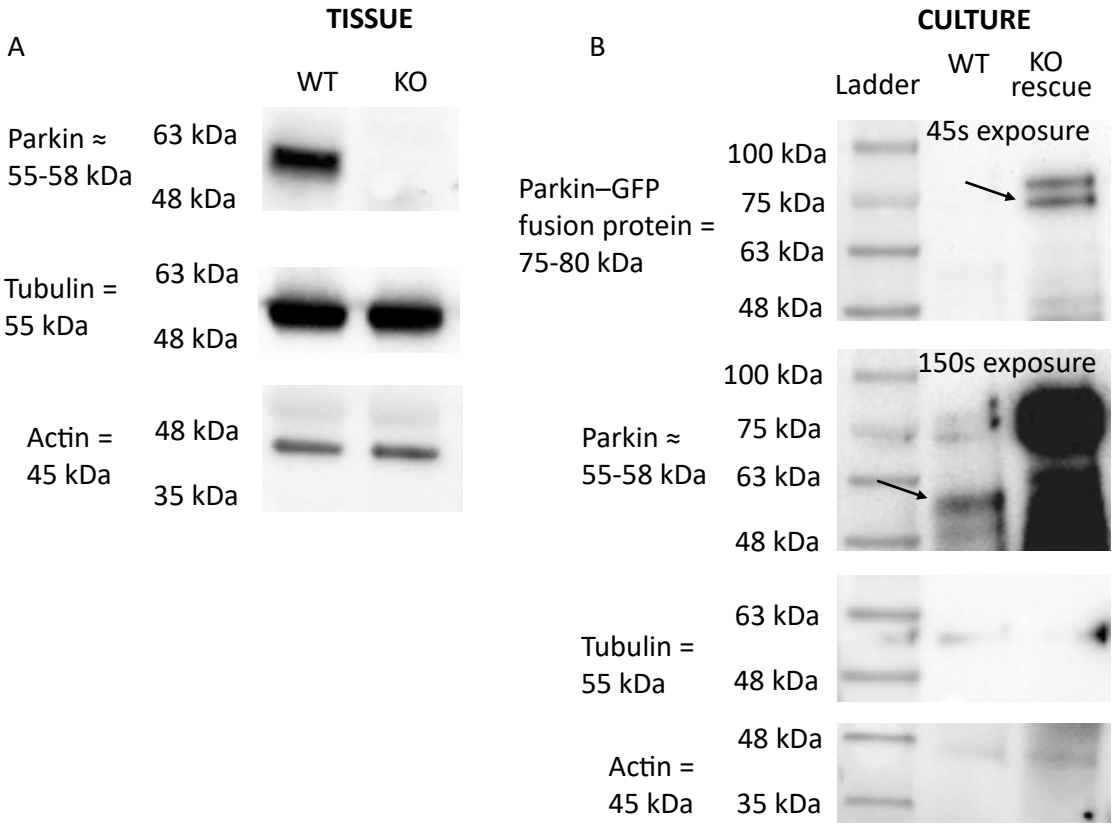
A
TISSUE
WT
KO
Parkin ≈ 55-58 kDa
63 kDa
48 kDa
Tubulin = 55 kDa
63 kDa
48 kDa
Actin = 45 kDa
48 kDa
35 kDa
B
CULTURE
Ladder
WT
KO rescue
45s exposure
150s exposure
Parkin–GFP fusion protein = 75-80 kDa
Parkin ≈ 55-58 kDa
Tubulin = 55 kDa
Actin = 45 kDa
100 kDa
75 kDa
63 kDa
48 kDa
35 kDa

FIGURE S1. OCR (A), ATP content (B) and axonal arborization surface (C) of SNc and VTA DA neurons pooled from the WT non-normalized data from Parkin, Pink1 and DJ-1 cultures. OCR (A) was measured using a XF24 Analyzer from all WT SNc and VTA cultures. The values represent the mean ± SEM, n = 39-78 wells from at least 12 different cultures. **$p < 0.01$. ATP content under basal conditions was quantified in all WT SNc and VTA cultures. The values represent the mean ± SEM, n = 19-20 coverslips from at least 9 different cultures ***$p < 0.001$. (C) Axonal arborization size was measured removing MAP2 signal surface from TH signal surface in random fields. The values obtained were normalized to the number of TH-positive SNc or VTA DA neurons in all WT cultures. The values represent the mean ± SEM, n = 46-47 coverslips from at least 9 different cultures. ***$p < 0.001$.

FIGURE S2. Parkin WT and KO SNc cultures at 5 DIV (A) Basal survival rate was measured by counting the proportion of DA neurons with a clear round nucleus at 1 DIV that survived until 5 DIV. (B) Axonal arborization size was measured by removing somatodendritic (MAP2 signal) surface from TH surface in random fields. Data were normalized by the control condition. The values represent the mean ± SEM, n =20-24 coverslips from at least 3 different cultures. *$p < 0.05$, ***$p < 0.001$. (C) Oxygen consumption rates (OCR) were measured using a XF24 Analyzer. Basal OCR, uncoupled OCR in the presence of 0.5 mM CCCP and the respiratory control ratio (RCR), calculated by dividing uncoupled by basal OCR, were measured. Data were normalized to the control condition. The values represent the mean ± SEM, n =18-21 wells from at least 3 different cultures. ****$p < 0.0001$.

FIGURE S3. Mesencephalic Parkin KO glial cells were grown for 1, 3, 5 and 7 DIV. The number of glial cells was measured by DAPI staining. The values represent the mean ± SEM, n = 12 coverslips from at least 3 different cultures. ***$p < 0.001$, ****$p < 0.0001$.

FIGURE S4. OCR (A-B) and ECAR (C-D) were measured using a XF24 Analyzer from Pink1 (A, C) and DJ-1 (B, D) glial cell cultures. The values represent the mean ± SEM, n = 16–21 wells from at least 4 different cultures. *$p < 0.05$. ATP content under basal conditions was quantified in Pink1 (E) and DJ-1 (F) glial cell cultures. The values represent the mean ± SEM, n = 7 coverslips from at least 3 different cultures.

FIGURE S5. Parkin expression was measured by western blot in Parkin WT and KO P0 mouse brain tissue (A) and in Parkin WT and KO (with Parkin-GFP AAV rescue) SNc cultures. (B). Parkin overexpression in cultures was strong (B top, arrow), and the presence of the second band is likely due to parkin's ability to self-ubiquitinate. It was necessary to increase blot exposure to reveal the WT band in the WT culture condition (B middle, arrow). The bands for reference genes are weak for cultures because the amount of material obtained from culture coverslips is limited (B bottom). Antibodies used were anti-Parkin mouse 1:200 (sc-32282, Santa Cruz Biotechnology), anti-α/β-Tubulin rabbit 1: 1000 (2148S, Cell Signaling Technology) and anti-β-Actin mouse 1:2500 (ab6276, Abcam).