

Supplementary Materials for
Subthalamic nucleus deep brain stimulation alleviates oxidative
stress via mitophagy in Parkinson's disease

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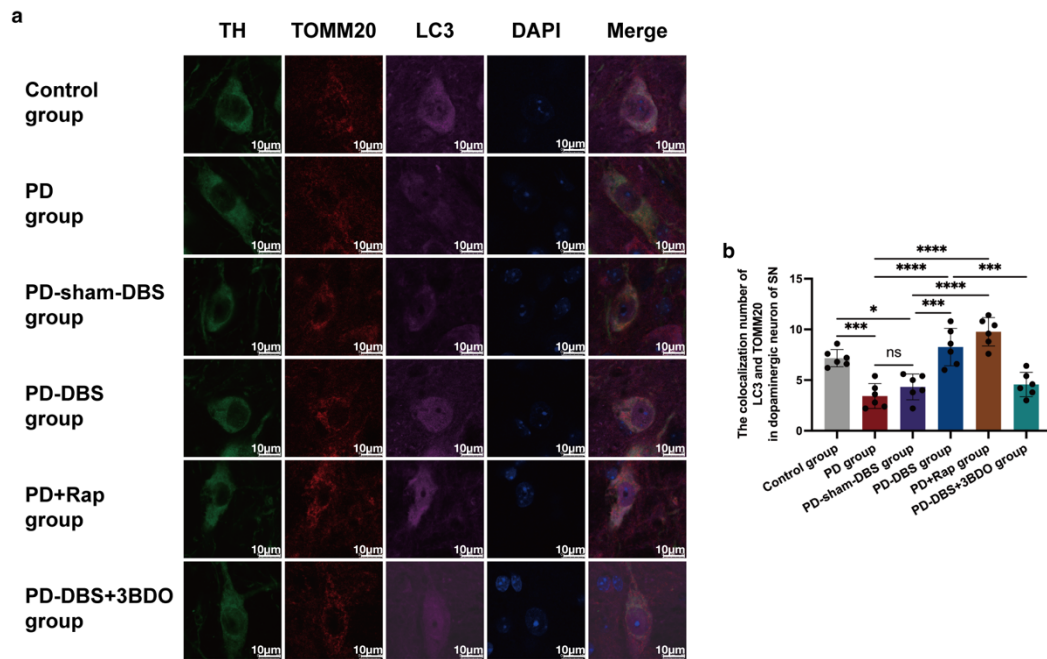
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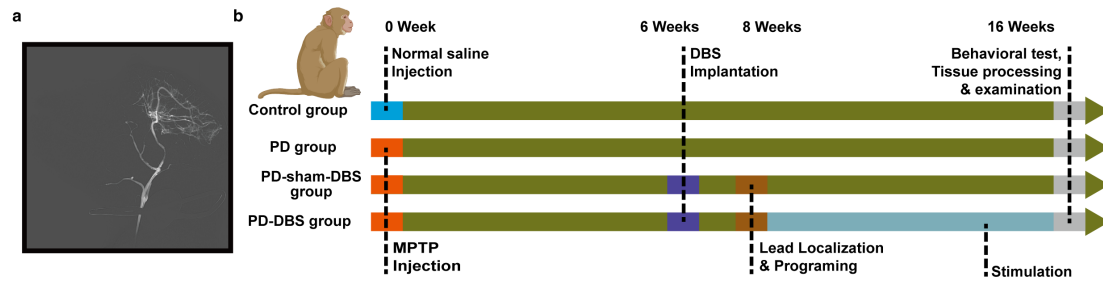
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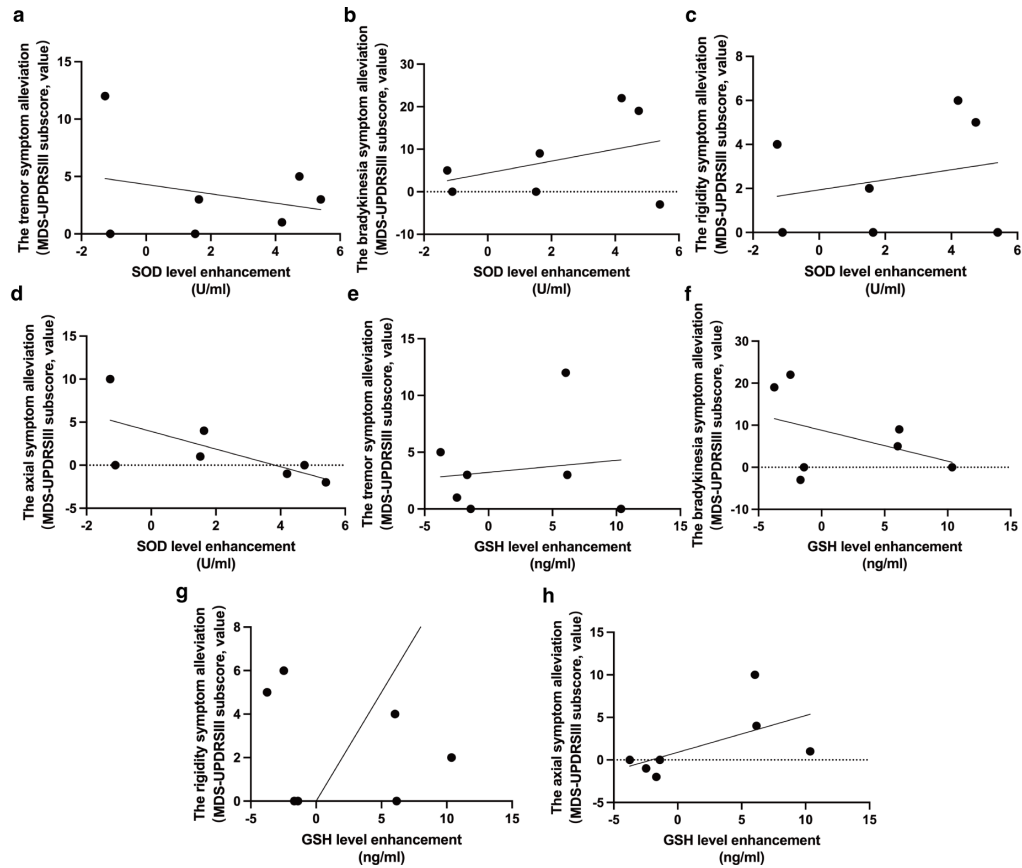
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Supplementary Fig. 1. The mTOR-dependent mitophagy contributed to the neuroprotective effects of STN-DBS in PD mice. **(a).** Co-localization of TH, TOMM20, and LC3 in SN via IF staining (shared with the same mice in Fig. 3h). **(b).** The STN stimulation and rapamycin increased the co-expression of TOMM20 (mitochondria marker) and LC3 in the TH⁺ cell in the SN of the PD model, nevertheless, this could be interrupted by treatment with 3BDO ($n = 6$ per group; $F_{(5,30)} = 21.4$, $P < 0.0001$; one-way ANOVA followed by a Tukey post-hoc correction). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. STN-DBS: subthalamic nuclei deep brain stimulation; PD: Parkinson's disease; SN: substantia nigra; IF: immunofluorescence; DAPI: 4',6-diamidino-2-phenylindole; TH: tyrosine hydroxylase; ns: not significant.



Supplementary Fig. 2. Chronic STN-DBS exerts an antioxidative role and stabilizes mitochondrial homeostasis in a PD monkey model. **(a).** MPTP internal carotid artery injection via DSA. **(b).** Experimental design of monkey STN-DBS. STN-DBS: subthalamic nuclei deep brain stimulation; PD: Parkinson's disease; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NS: normal saline; DSA: digital subtraction angiography.



Supplementary Fig. 3. STN-DBS suppresses oxidative stress in PD patients. **(a–h).** no obvious correlation between changes in levels of SOD (tremor: $P=0.5685$; rigidity: $P=0.6012$; bradykinesia: $P=0.3863$; axial symptoms: $P=0.0939$) or GSH (tremor: $P=0.7668$; rigidity: $P=0.5942$; bradykinesia: $P=0.3561$; axial symptoms: $P=0.1740$), and alteration in tremor, rigidity, bradykinesia, and axial symptoms ($(Preoperative\ MDS - UPDRS)_{med-off} - (Postoperative\ MDS\ UPDRS)_{stm-on,med-off}$) ($n = 7$; Pearson correlation test). STN-DBS: subthalamic nuclei deep brain stimulation; PD: Parkinson's disease; MDS-UPDRS: Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale; SOD: superoxide dismutase; GSH: glutathione.

Supplementary Table. 1. Monkey hemiparkinsonism rating scale.

Tremor	Dyskinesia
0=none	0=absent
1=some episodes	1=mild and intermittently present
2=frequent episodes	2=moderate and consistently present
3=persistent	3=severe and persistent, large amplitude
Bradykinesia	Localization (if present)
0=normal speed	0=contralateral
1=mild slowing of overall movements	1=ipsilateral
2=severe slowing of movements	2=both sides
3=akinesia (no movement)	
Posture	Vomiting
0=normal rising position	0=absent
1=flexed posture (0 ° - 45 °)	1=present
2=severely flexed posture (>90 °)	
3=dystonic posture	
Rigidity*	Overall level of activity
0=none	+2=severe hyperkinesia; persistent excessive involuntary movement
1=mild	+1=moderate hyperkinesia; noticeable increase in involuntary movement
2=moderate	0=normal amount of movement
3=severe	-1=moderate hypokinesia; sparse movement
	-2=severe hypokinesia
Arm movements (reaching for food outside of task) *	
3=no movements	
2=sometimes	
1=frequent	
0=no differences with normal side	

*Only the contralateral side is quantified.

Supplementary Table. 2. Mitochondrial injury scale.

Score	Observation
0	Normal mitochondria (mitochondria appeared highly dense with well-organized cristae)
1	Early swelling as manifested by early clearing of matrix density and separation of cristae (large amorphous matrix density and linear density are present)
2	More marked swelling as manifested by further clearing of matrix density and separation of cristae
3	More extensive mitochondrial swelling with disruption of cristae
4	Severe mitochondrial swelling with disruption of cristae and rupture of inner and outer mitochondrial membranes

P values in “STN-DBS relieved motor impairment and loss of dopaminergic neurons in the PD model”

Rotarod test Adjusted P Value

Control group vs. PD group <0.0001
Control group vs. PD-sham-DBS group <0.0001
Control group vs. PD-DBS group 0.0246
Control group vs. PD+Rap group 0.0045
Control group vs. PD-DBS+3BDO group <0.0001
PD group vs. PD-sham-DBS group 0.9899
PD group vs. PD-DBS group 0.0038
PD group vs. PD+Rap group 0.021
PD group vs. PD-DBS+3BDO group 0.9695
PD-sham-DBS group vs. PD-DBS group 0.0204
PD-sham-DBS group vs. PD+Rap group 0.0918
PD-sham-DBS group vs. PD-DBS+3BDO group >0.9999
PD-DBS group vs. PD+Rap group 0.9891
PD-DBS group vs. PD-DBS+3BDO group 0.0318
PD+Rap group vs. PD-DBS+3BDO group 0.1328

TH-WB-SN Adjusted P Value

Control group vs. PD group <0.0001
Control group vs. PD-sham-DBS group <0.0001
Control group vs. PD-DBS group 0.0021
Control group vs. PD+Rap group 0.0421
Control group vs. PD-DBS+3BDO group <0.0001
PD group vs. PD-sham-DBS group 0.9994
PD group vs. PD-DBS group 0.0008
PD group vs. PD+Rap group <0.0001
PD group vs. PD-DBS+3BDO group 0.8639
PD-sham-DBS group vs. PD-DBS group 0.0003
PD-sham-DBS group vs. PD+Rap group <0.0001
PD-sham-DBS group vs. PD-DBS+3BDO group 0.6852
PD-DBS group vs. PD+Rap group 0.8385
PD-DBS group vs. PD-DBS+3BDO group 0.0159
PD+Rap group vs. PD-DBS+3BDO group 0.0007

TH-DAB-striatum Adjusted P Value

Control group vs. PD group <0.0001
Control group vs. PD-sham-DBS group <0.0001
Control group vs. PD-DBS group <0.0001
Control group vs. PD+Rap group <0.0001
Control group vs. PD-DBS+3BDO group <0.0001
PD group vs. PD-sham-DBS group >0.9999
PD group vs. PD-DBS group 0.143
PD group vs. PD+Rap group 0.0364
PD group vs. PD-DBS+3BDO group >0.9999
PD-sham-DBS group vs. PD-DBS group 0.1263
PD-sham-DBS group vs. PD+Rap group 0.0314
PD-sham-DBS group vs. PD-DBS+3BDO group >0.9999
PD-DBS group vs. PD+Rap group 0.9875
PD-DBS group vs. PD-DBS+3BDO group 0.0971
PD+Rap group vs. PD-DBS+3BDO group 0.023

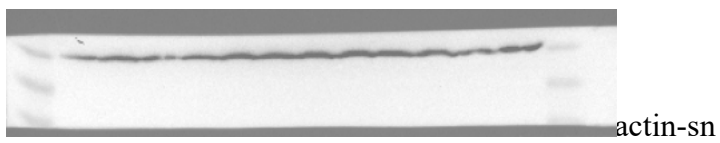
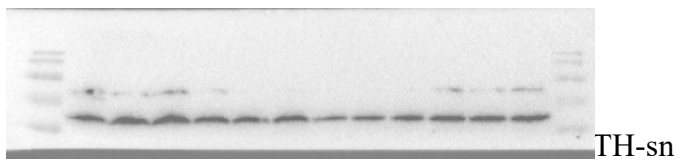
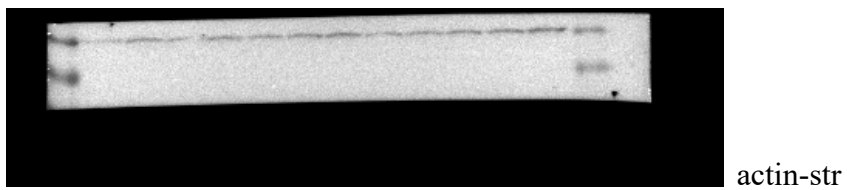
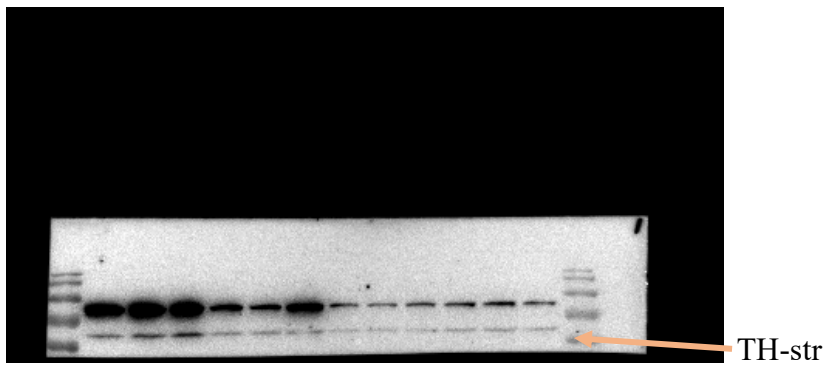
TH-DAB-SN Adjusted P Value

Control group vs. PD group <0.0001
Control group vs. PD-sham-DBS group <0.0001
Control group vs. PD-DBS group <0.0001
Control group vs. PD+Rap group <0.0001
Control group vs. PD-DBS+3BDO group <0.0001
PD group vs. PD-sham-DBS group >0.9999
PD group vs. PD-DBS group 0.0074
PD group vs. PD+Rap group <0.0001
PD group vs. PD-DBS+3BDO group >0.9999
PD-sham-DBS group vs. PD-DBS group 0.0062
PD-sham-DBS group vs. PD+Rap group <0.0001
PD-sham-DBS group vs. PD-DBS+3BDO group 0.9998
PD-DBS group vs. PD+Rap group 0.5557
PD-DBS group vs. PD-DBS+3BDO group 0.0122
PD+Rap group vs. PD-DBS+3BDO group 0.0001

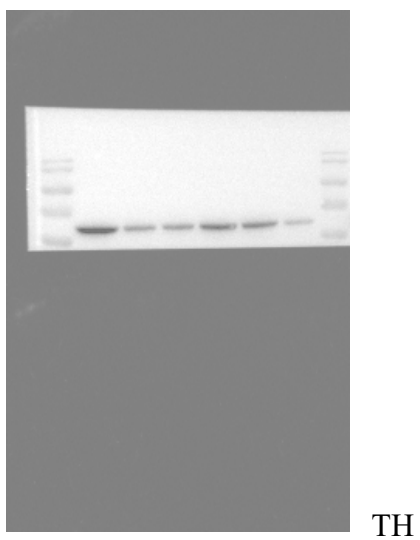
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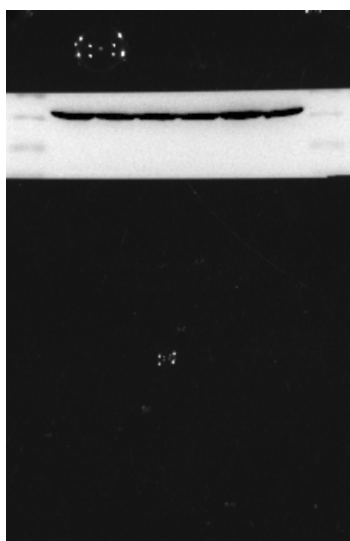
Mice

Full unedited gel for Figure 1



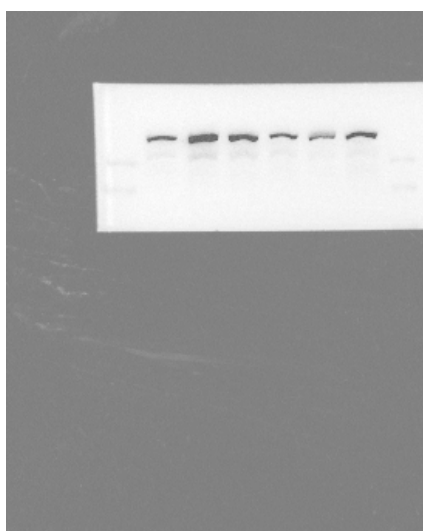
Full unedited gel for Figure 2



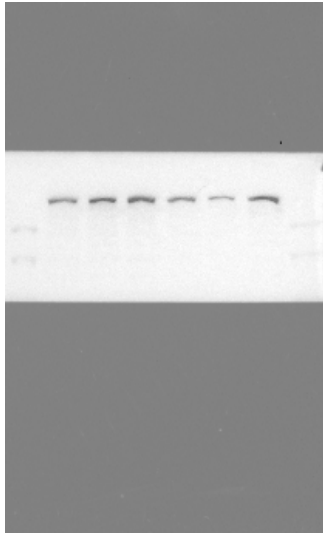


actin

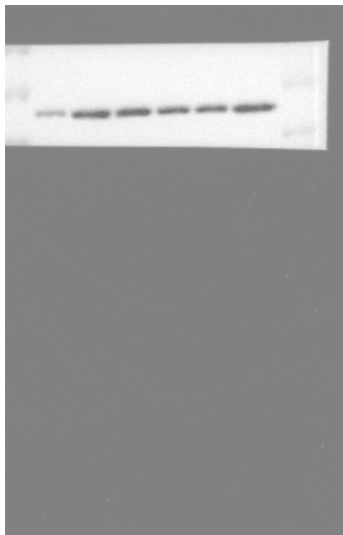
Full unedited gel for Figure 3



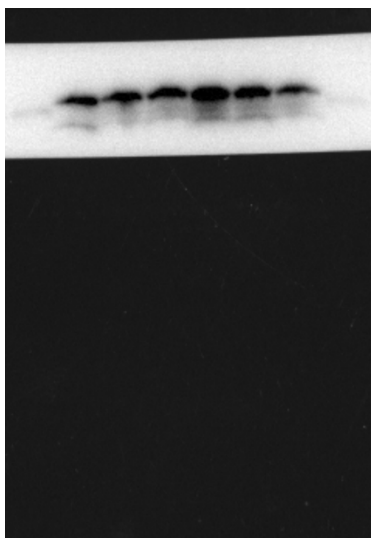
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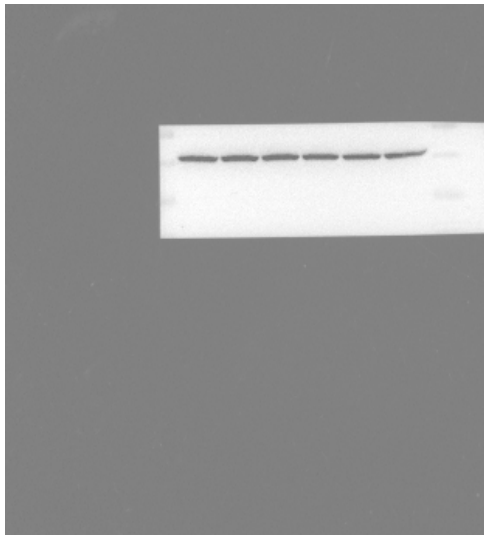
mTOR



P62

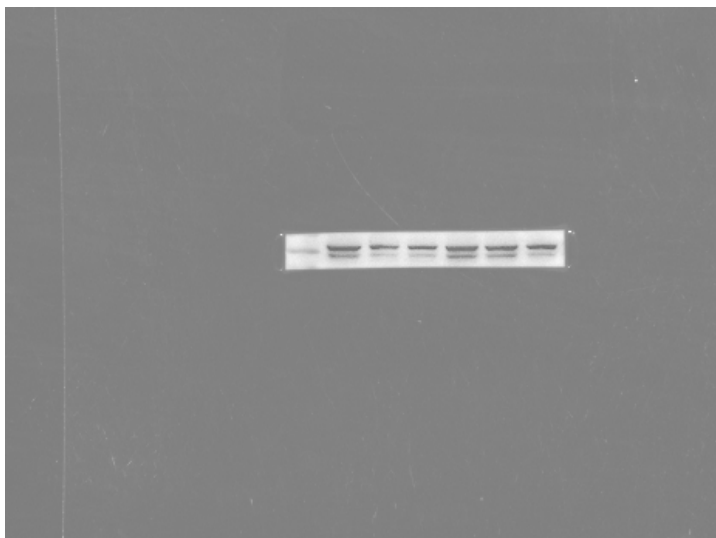


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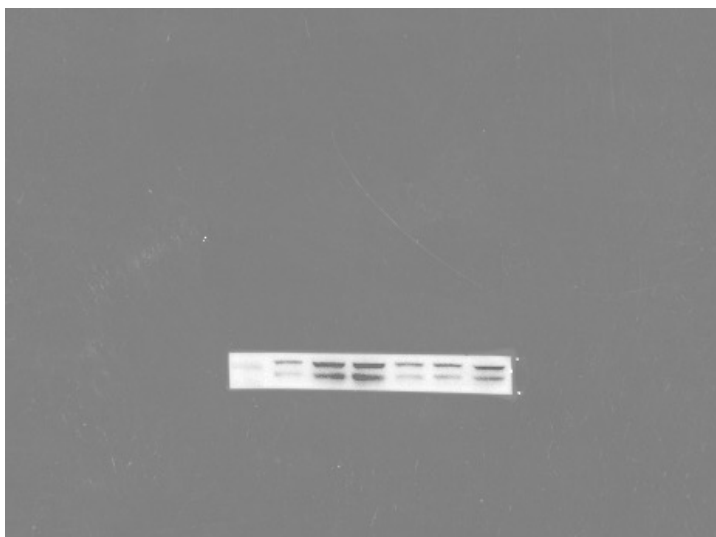


β -actin

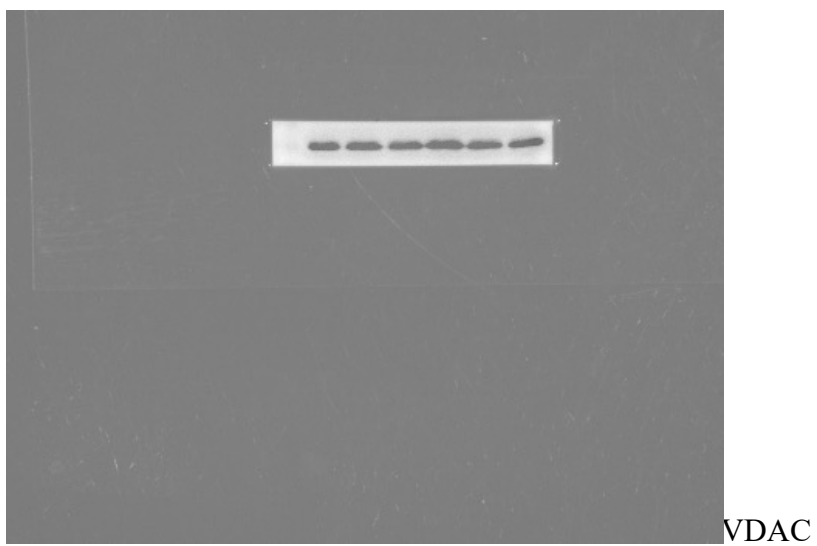
Full unedited gel for Figure 4



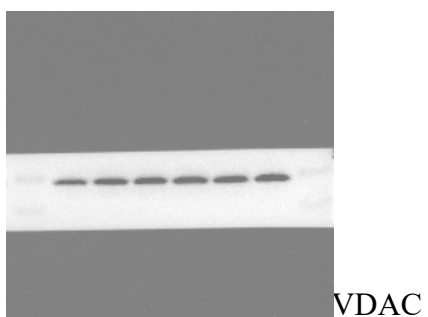
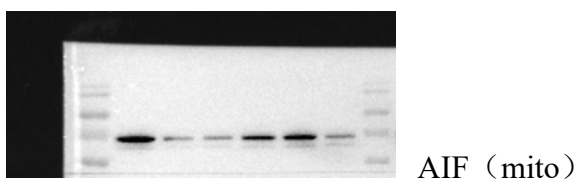
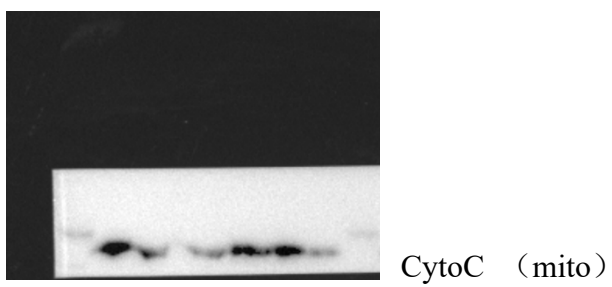
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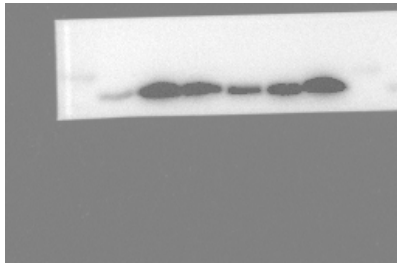


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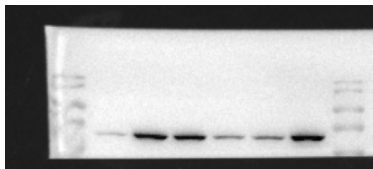


Full unedited gel for Figure 5

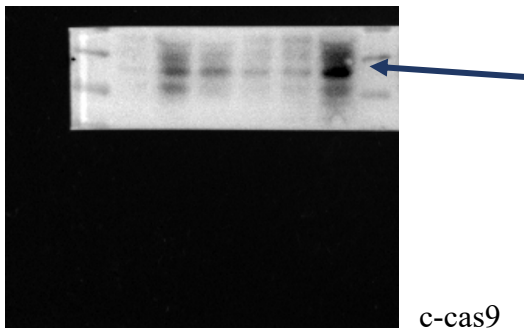




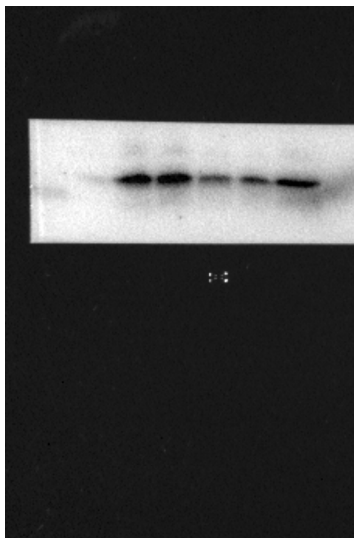
CytoC (cyto)



AIF (cyto)



c-cas9

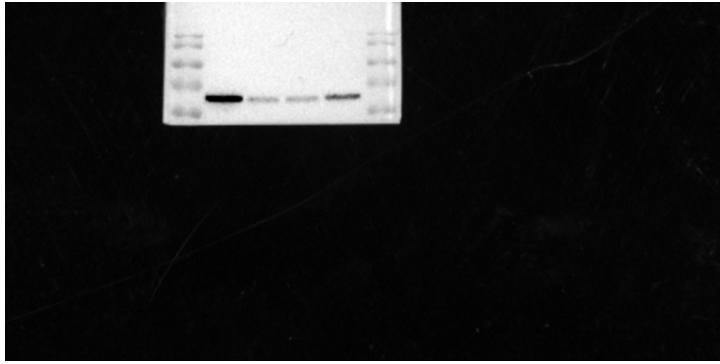


c-cas3

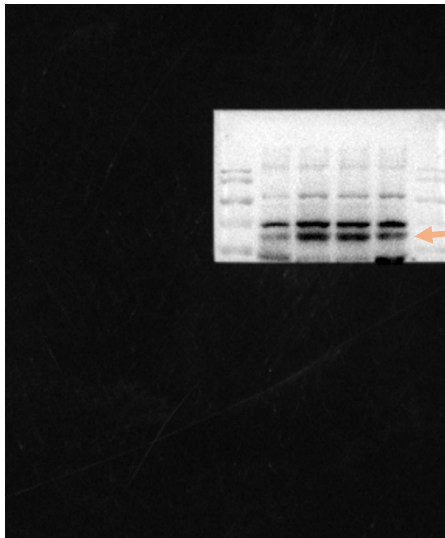


β -actin

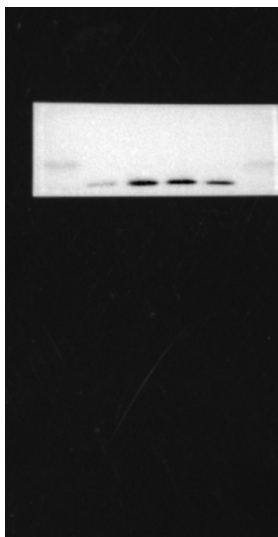
Monkey western blot
Full unedited gel for Figure 6



TH



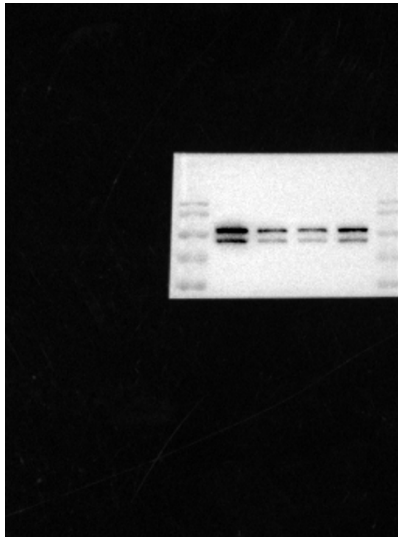
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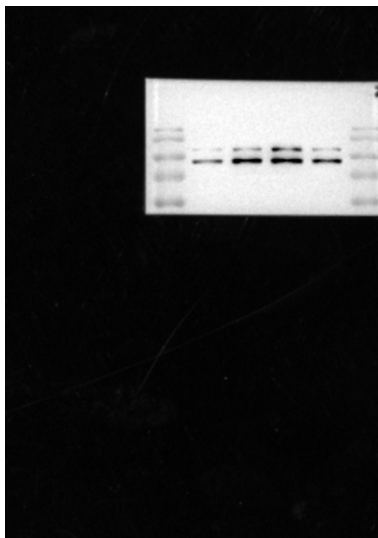
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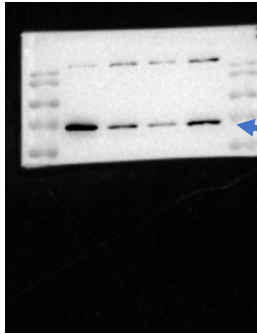
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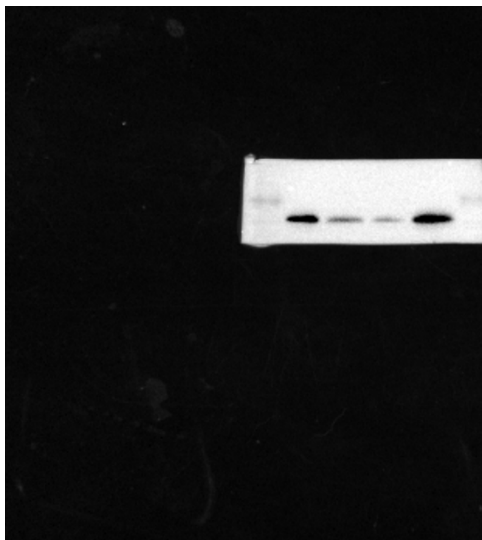
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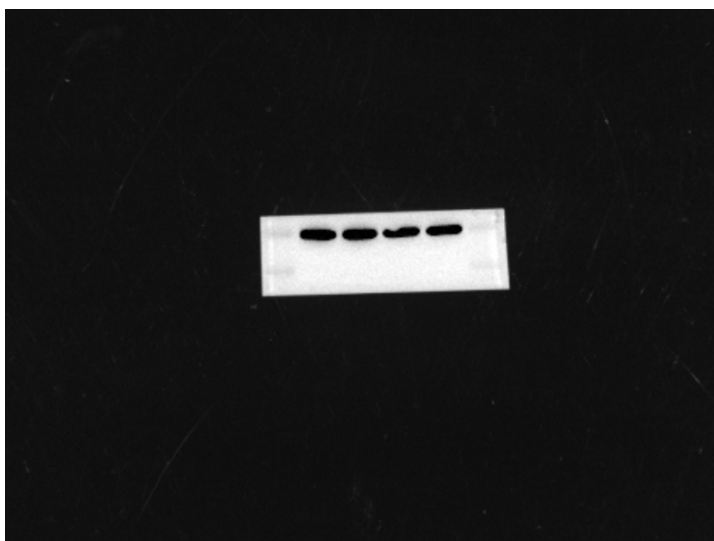
Drp1 (mito)



AIF (mito)



CytoC (mito)



VDAC (mito)