Supplementary Material

Regulation of DJ-1 by glutaredoxin 1 *in vivo – implications for Parkinson's disease*

William M. Johnson[1], Marcin Golczak[1], Kyonghwan Choe[2], Pierce L. Curran[2], Olga Gorelenkova Miller[1] Chen Yao[2], Wenzhang Wang[2], Jiusheng Lin[3], Nicole M. Milkovic[3], Ajit Ray[5], Vijayalakshmi Ravindranath[5], Xiongwei Zhu[2], Mark A. Wilson[3], Amy L. Wilson-Delfosse[1], Shu G. Chen[2,*] John J. Mieyal[1,4,*]

[1]Department of Pharmacology, Case Western Reserve University, Cleveland, OH 44106, USA.

[2]Department of Pathology, Case Western Reserve University, Cleveland, OH 44106, USA.

[3]Department of Biochemistry and the Redox Biology Center, University of Nebraska-Lincoln, NE, 68588, USA.

[4]Louis B. Stokes Veterans Affairs Medical Research Center, Cleveland, Ohio 44106, USA.

[5] Centre for Neuroscience, Indian Institute of Science, C.V. Raman Avenue, Bangalore 560012, India

*To whom correspondence should be addressed. E-mail: jjm5@case.edu (J.J.M.); shu.chen@case.edu (S.G.C.)

Supplemental Figure Legends

S1. QPCR and Western blot data for transgenic Grx1 and WT mice. (A) QPCR analysis of DJ-1 mRNA from WT and *Grx1*$^{-/-}$ mice in the C57BL/6 background. N=3 mice per group, no statistical difference *via* Student's T-Test. (B) Western blot analysis of transgenic mice over-expressing hGrx1; tissue probed with anti-Grx1, anti-DJ-1, and anti-actin antibodies, respectively. (C) Western blot analysis of WT and *Grx1*$^{-/-}$ mice in the BALB/c background; tissue probed with anti-Grx1, anti-DJ-1, and anti-actin antibodies, respectively. N=3 mice per group. (D) QPCR analysis of DJ-1 mRNA from WT and *Grx1*$^{-/-}$ mice in the BALB/c background. N=3 mice per group, no statistical difference *via* Student's T-Test.

S2. Western blot data for human samples. (A) Western blot analysis of samples extracted from human PBMCs probed with anti-Grx1, anti-DJ-1, and anti-actin antibodies, respectively; n= 14 independent biological samples. (B) Western blot analysis of samples extracted from human midbrain tissue probed with anti-Grx1, anti-DJ-1, and anti-actin antibodies, respectively; n = 14 independent biological samples.

S3. Validation of the DJ-1 antibody. (A) Western blot analysis of immunopreciptated DJ-1. Left lane, reduced (+ DTT); right lane, non-reduced (- DTT). (B) Western blot analysis of brain tissue homogenates from DJ-1$^{-/-}$ and WT mice treated with DTT and probed with anti-DJ-1, and anti-actin antibodies, respectively. N=2 mice per genotype.

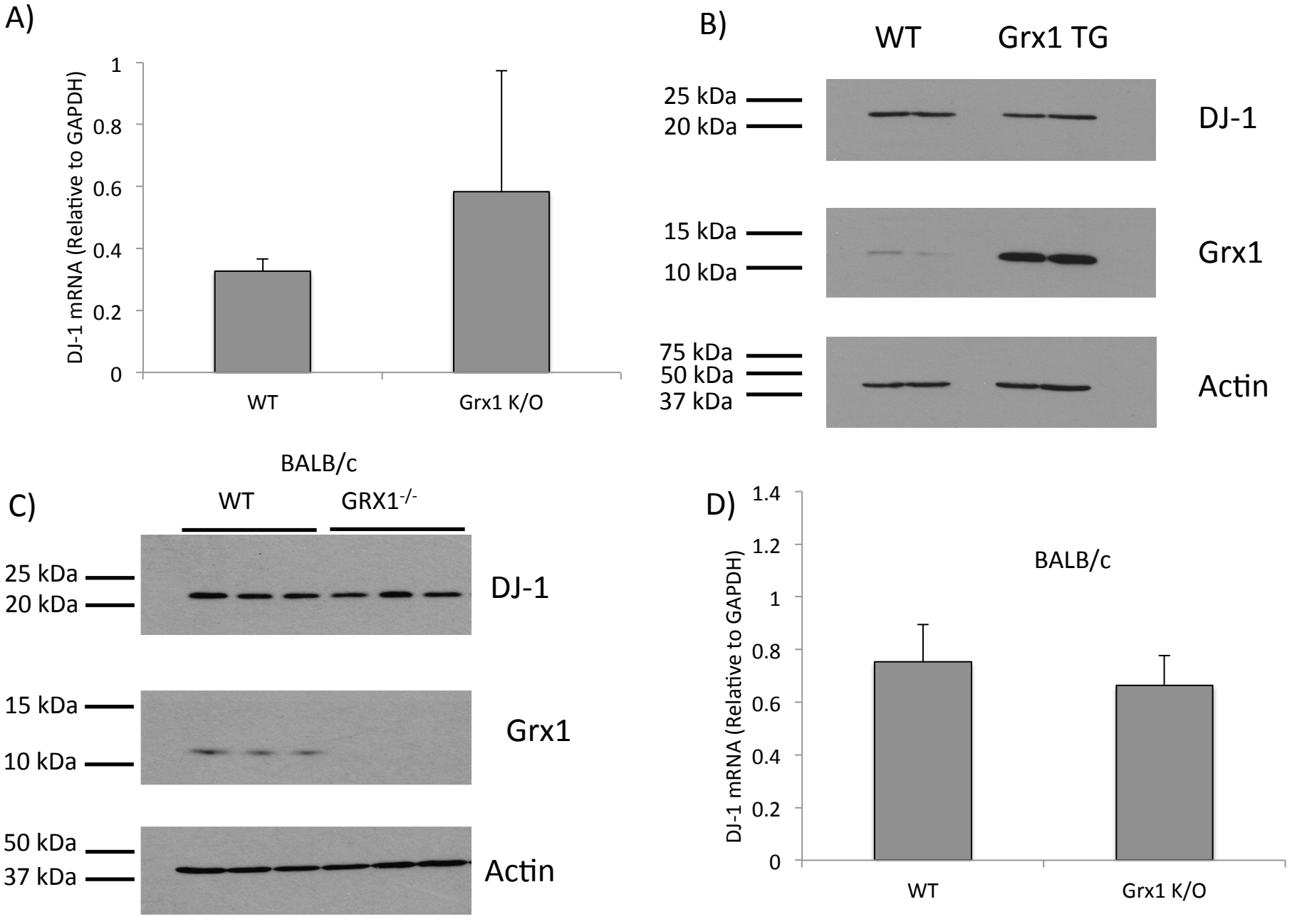
S4. Validation of the DJ-1 glutathionylation. DJ-1 was immunoprecipitated from mouse brain lysate, run under non-reducing conditions and transferred to PVDF membrane. Half of the blot was soaked in TBST buffer without DTT, and the other half was soaked in TBST buffer containing 100 mM DTT. The blots then were probed with the anti-GSH antibody, stripped, and re-probed with the anti-DJ-1 antibody for confirmation of DJ-1 protein. Loss of anti-GSH signal in the presence of DTT indicates that the disulfide reducing agent ablates the anti-GSH signal consistent with loss of the glutathionyl mixed disulfide adduct of DJ-1 (A) Western blot analysis of the membrane incubated with TBST (B) Western blot analysis of membrane incubated in TBST and DTT. Arrows indicate the ~45 kDa immunoreactive band.

S5. Quantification of glutathionylated DJ-1 peptides. (A) and (B) represent selective ion chromatograms for two cysteine-containing DJ-1 peptides unmodified by glutathionylation; (C) and (D) correspond to the same peptides obtained from ^{15}N-labeled DJ-1, serving as internal standards. Areas under the ion intensity peaks, colored in gray were used to calculate ratios between ^{15}N-enriched and natural abundance peptides to determine the fraction of each peptide covalently modified by glutathione. (E) and (F) represent MS spectra averaged at the peak of ion intensities for DVVIC^{53}PDASLEDAK and GLIAAIC^{106}AGPTALLAHEIGFGSK peptides, respectively. Ions and their isotopic distribution for both 15N-enriched (blue) and natural abundance (black) peptides are shown.

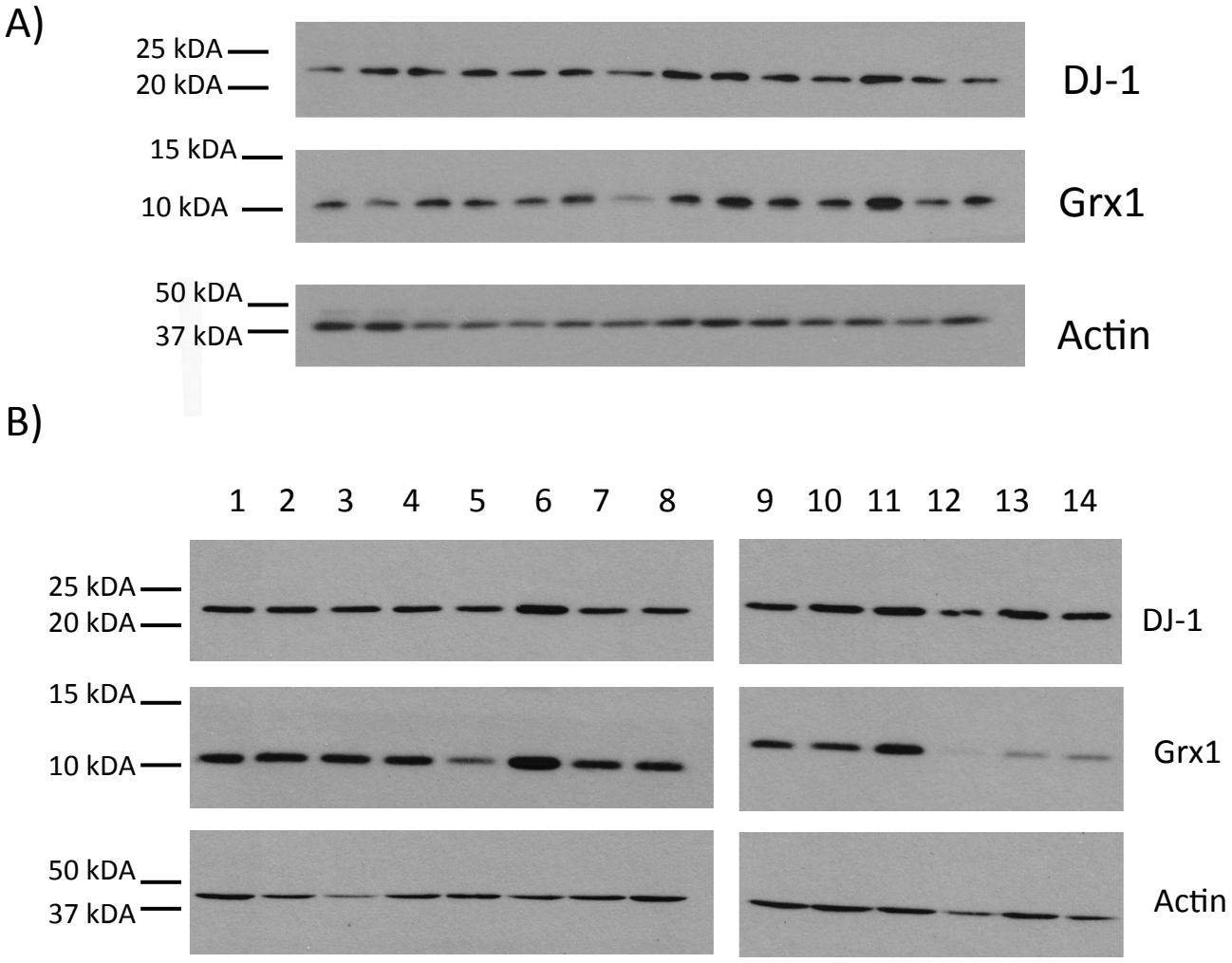
S6. Quantification of mCherry and DJR-1.2:mCherry protein content in the dopaminergic neurons of *C. elegans* by fluorescence microscopy. Quantification indicates that content of mCherry and DJ-1:mCherry protein (relative to GFP expression specifically in the dopaminergic neurons) are equivalent and do not change over time.

S7. Quantification of relative DJ-1 protein content in Neuro-2A cells transfected with scRNA/Grx1 shRNA and treated with individual protease inhibitors from the protease inhibitor cocktail. Neuro-2A cells transfected with scRNA/Grx1 shRNA were treated with individual protease inhibitors or with DMSO (**D**; vehicle) for 48 hr with fresh medium containing inhibitor or vehicle replaced every 12 hr. Panels show representative Western blots of DJ-1 along with respective loading control β-tubulin (Tub) and densitometric analyses. The inhibitors added with scRNA/shRNA are: (A) 52 μM 4-(2-aminoethyl) benzenesulfonyl fluoride, (**Ae**), (B) 40 nM aprotinin, (**Ap**), (C) 2 μM bestatin (**Be**), (D) 700 nM E-64 (**E64**), (E) 1 μM leupeptin (**Le**) and (F) 750 nM pepstatin A (**Pe**). All data were analyzed using one-way ANOVA followed by Student-Newman-Keuls test to determine significant differences with * representing $p < 0.05$. n = 3-6 independent samples in each experimental group. None of the individual protease inhibitors tested alone afforded protection of DJ-1 from degradation, unlike the complete cocktail.

Supplemental Figure S1

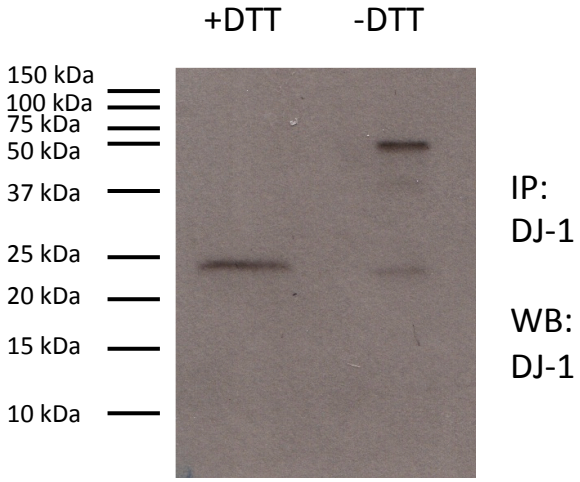


Supplemental Figure S2

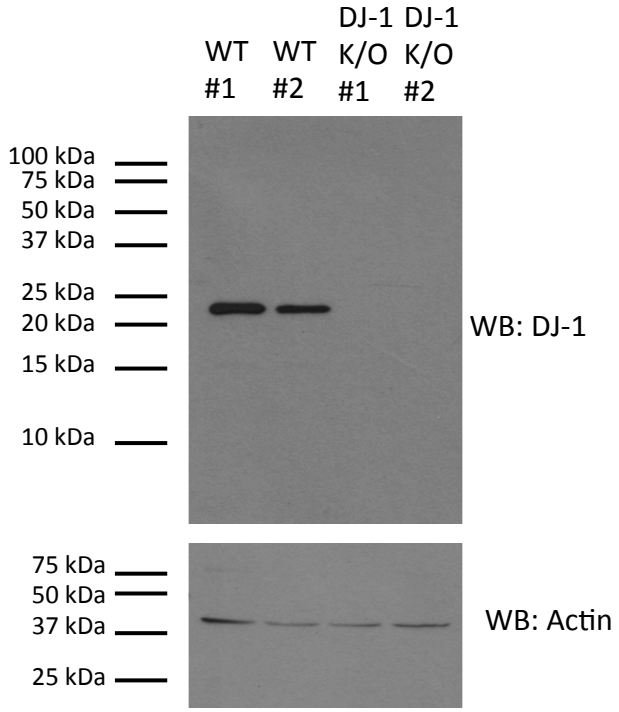


Supplemental Figure S3

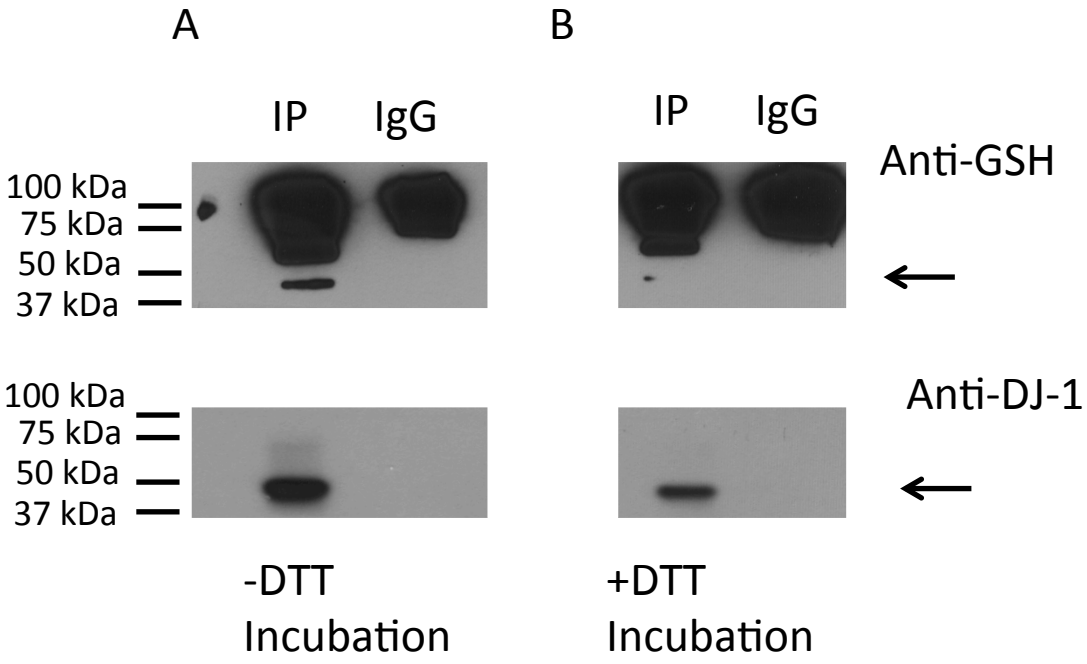
A)



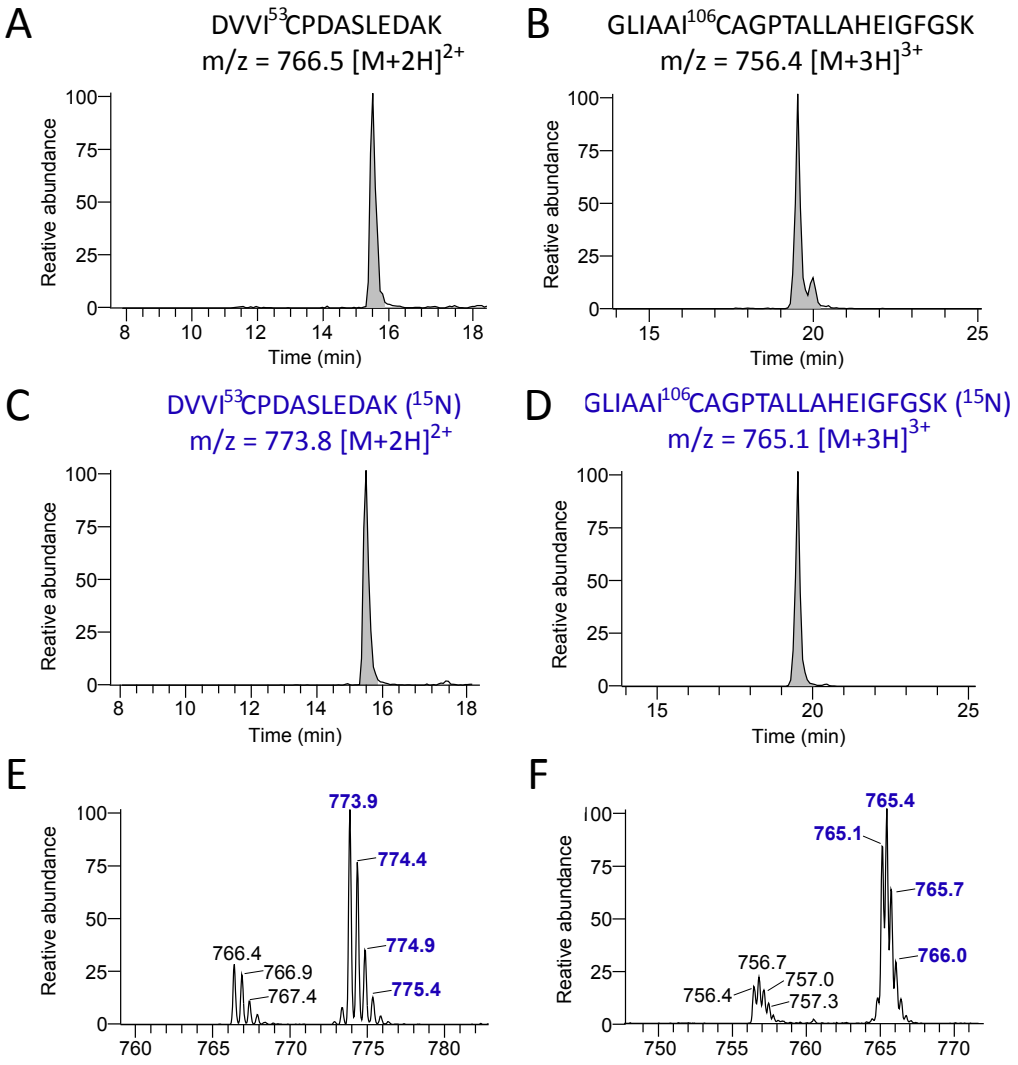
B)



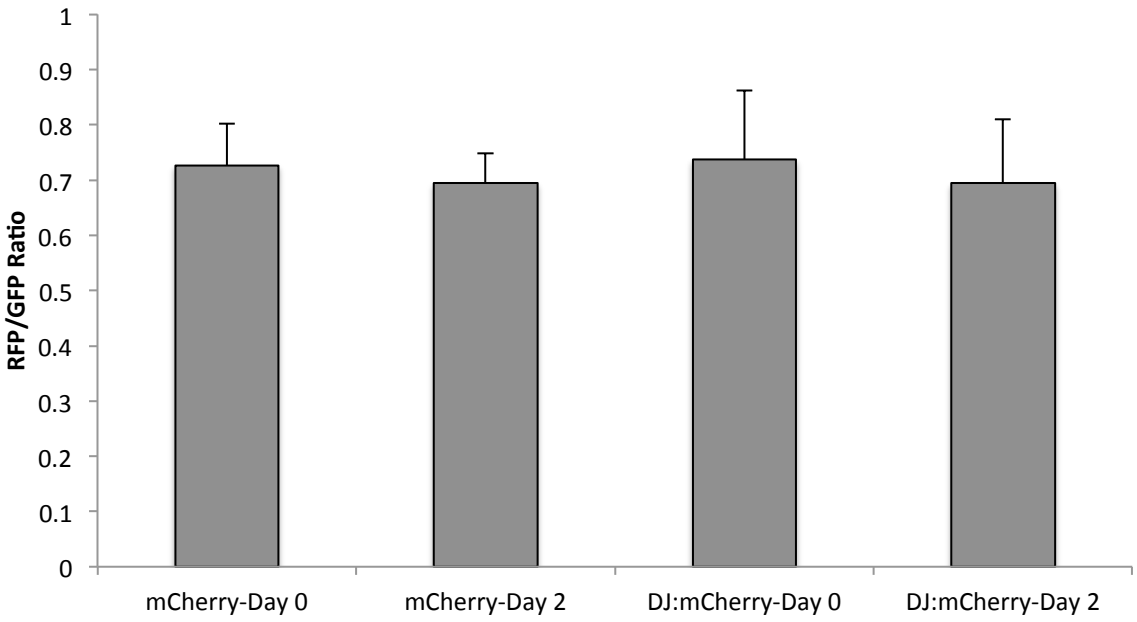
Supplemental Figure S4



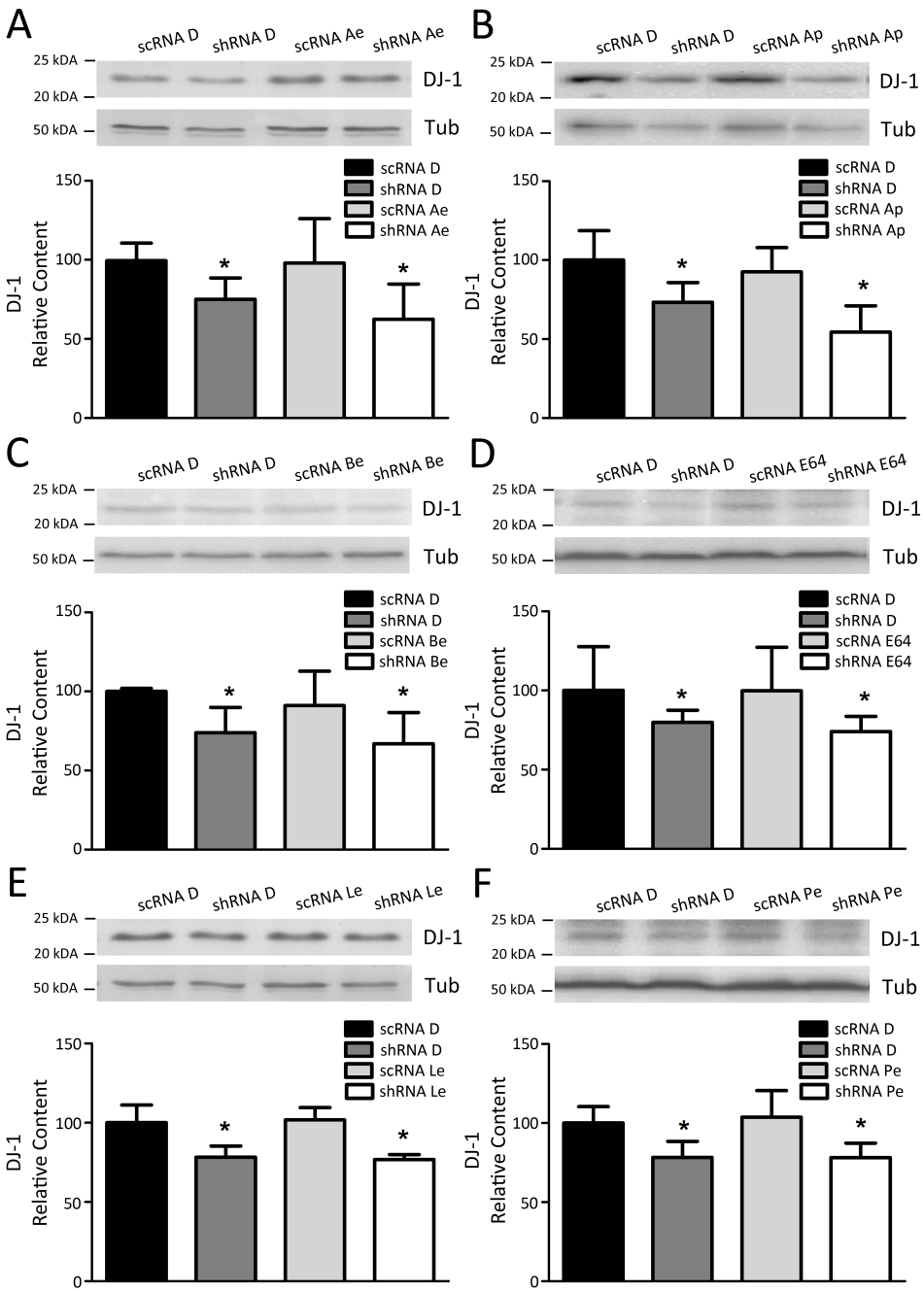
Supplemental Figure S5



Supplemental Figure S6



Supplemental Figure S7



Supplemental Tables

A Patient information for PBMC analysis

Patient Refernce #	Age	Sex	Diagnosis
137	62	F	PD
219	67	M	PD
228	77	M	PD
236	67	M	PD
254	65	M	PD
256	74	M	PD
257	80	M	PD
260	46	M	PD
262	73	M	PD
264	57	M	PD
266	70	M	PD
268	64	M	PD
270	75	M	PD
271	58	F	PD

Cases used for Western blot analysis from PBMC lysate.

B Patient information for brain lysate analysis

Patient Refernce #	Sex	Age	Diagnosis
P08-40	F	86	PD
P08-41	F	83	PD
P08-42	M	75	PD
P08-43	M	71	PD
P08-34	M	51	Control
P08-35	M	89	Control
P08-36	F	86	Control
P08-37	M	73	Control
P08-38	M	69	Control
PD1	M	77	PD
PD2	F	49	PD
PD3	F	61	PD
PD4	M	73	PD

Cases used for Western blot analysis from post mortem midbrain tissue.

C

Designation	Genotype	Strain	Reference
Control (GFP)	*lin-15(n765ts)X; cwrIs730[Pdat-1::GFP, lin-15(+)]*	SGC730	[6]
R1441C	*lin-15(n765ts)X; cwrIs851[Pdat-1::GFP, Pdat-1::LRRK2(R1441C), lin-15(+)]*	SGC851	[6]
G2019S	*lin-15(n765ts)X; cwrIs856[Pdat-1::GFP, Pdat-1::LRRK2(G2019S), lin-15(+)]*	SGC856	[6]
R1441C; *glrx-10*$^{-/-}$	*glrx-10(tm4634)I; cwrIs851*	SGC302	[20]
djr-1.2$^{-/-}$	*djr-1.2(tm1348)V*	tm1346	NBP
R1441C; *djr-1.2*$^{-/-}$	*djr-1.2(tm1348)V; cwrIs851*	SGC10	This study
G2019S; *djr-1.2*$^{-/-}$	*djr-1.2(tm1348)V; cwrIs856*	SGC11	This study
R1441C; *glrx-10*$^{-/-}$; DJR-1.2	*glrx-10(tm4634)I; cwrIs851; cwrEx20[Pdat-1::DJR-1.2::mCherry]*	SGC20	This study
R1441C; *glrx-10*$^{-/-}$; Vector	*glrx-10(tm4634)I; cwrIs851; cwrEx22[Pdat-1::mCherry]*	SGC22	This Study

NBP: National Bioresource Project (Japan)

Caenorhabditis elegans strains used in this study