Supporting Information

Glutathione S-Transferase Omega 1 Activity is Sufficient to Suppress Neurodegeneration in a *Drosophila* Model of Parkinson's Disease

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FIGURE S1. **Generation of *DmGSTO1* mutants in *Drosophila*.** *A*, Genomic structures of *DmGSTO1* (*CG6673*). Transposon insertion sites are indicated above the map by an inverted triangle. The *GE26508 P*-element was imprecisely excised to generated $DmGSTO1^{null}$. $DmGSTO1^{null}$ was a 593 bp deletion, which removed the *DmGSTO1*, and *CG6662* coding regions. *DmGSTO1* codes for two transcripts, A and B, which share the first exon. *B*, Quantitative RT-PCR analysis on extracts from mutant and control flies. $DmGSTO1^{null}$ mutants showed loss of the two transcripts (*DmGSTO1A* and *DmGSTO1B*), and *CG6662*. *GAPDH* was used as a control. *C*, GSH-dependent DHA reductase activity in wild type and $DmGSTO1^{null}$. Error bars indicate standard deviation. The experimental significance was determined by one-way ANOVA (* is $P < 0.0001$). Experiments were performed in triplicate. *D*, *CG6776* and *CG6662* mRNA levels in $park^{1}$ mutants are the same as in *WT*. *GAPDH* was used as a control.

FIGURE S2**. DmGSTO1 suppresses the defective thorax, and downturned wing phenotypes of $park^{1}$ mutants.** *A* and *B*, Upregulation of DmGSTO1A suppressed the collapsed thorax (white arrows), and downturned wing phenotypes of *parkin* mutant flies.

FIGURE S3. **$Park^{1}$ mutants display accumulation of tubulin in DA neurons.** Immunostaining with anti-α-tubulin, and anti-TH antibodies in DA neurons in *Drosophila* brains. Accumulation of α-tubulin was observed in DA neurons from $park^{1}$ mutants and $park^{1}/DmGSTO1^{null}$ double mutants (white arrowheads). Immunostaining with anti-TH antibody was performed to identify DA neurons.

FIGURE S4. **CG6662, another GSTO in *Drosophila*, was unable to rescue glutathionylation of the ATP synthase β subunit in $park^{1}$ mutants.** Glutathionylated proteins were immunoprecipitated from thorax extracts with an anti-GSH antibody, and were immunoblotted with an anti-ATPsyn β antibody. CG6662 was unable to glutathionylate the endogenous ATP synthase β subunit in $park^{1}$ mutants. Experiments were performed in triplicate.

FIGURE S5. **Mitochondrial F1F0-ATP synthase (Complex V) assembly in the ATP synthase β subunit RNAi mutants.** Mitochondrial protein extracts from the thorax were subjected to BlueNative-PAGE, followed by western blot analysis with anti-ATPsyn α subunit antibody. All bands were decreased in the ATP synthase β subunit RNAi mutants. Prohibitin was used as a mitochondrial loading control.

FIGURE S6. **DmGSTO1A is not important for the suppression of mitochondrial morphological defects in *park*^{1} mutants.** Mitochondria in flight muscle were stained with Alexa 488-conjugated streptavidin. Compared with *WT*, *park*^{1} mutants displayed large clumps of intense signal. Upregulation of DmGSTO1A in *park*^{1} mutants did not suppress either the *park*^{1} mutant phenotype or the mitochondrial morphological defects (first panel). *ATP synthase β subunit* RNAi mutants also displayed normal mitochondrial morphology (second panel). Mitochondria in IFM tissues of *Drosophila* thorax were labeled by *mito-GFP*. Compared with *park*^{1} mutant, DmGSTO1A or DmGSTO1A^{C31A} expressing lines in a *park*^{1} mutant background also showed mitochondrial morphological defects (third panel).

FIGURE S7. **The endogenous levels of the glutathionylated form of the ATP synthase β subunit in thorax extracts were decreased in *PINK1*^{B9} mutants.** *A*, *parkin* and *DmGSTO1* mRNA levels were also reduced in the *PINK1*^{B9} mutants. Error bars represent standard deviation. Experimental significance was determined by one-way ANOVA (* is $P < 0.05$). Experiments were performed in triplicate. *B*, The levels of the glutathionylated ATP synthase β subunit in thorax extracts were decreased in *PINK1*^{B9} mutants. The total level of the ATP synthase β subunit was unchanged in *PINK1*^{B9} mutants. β-actin was used as a loading control.

FIGURE S1.

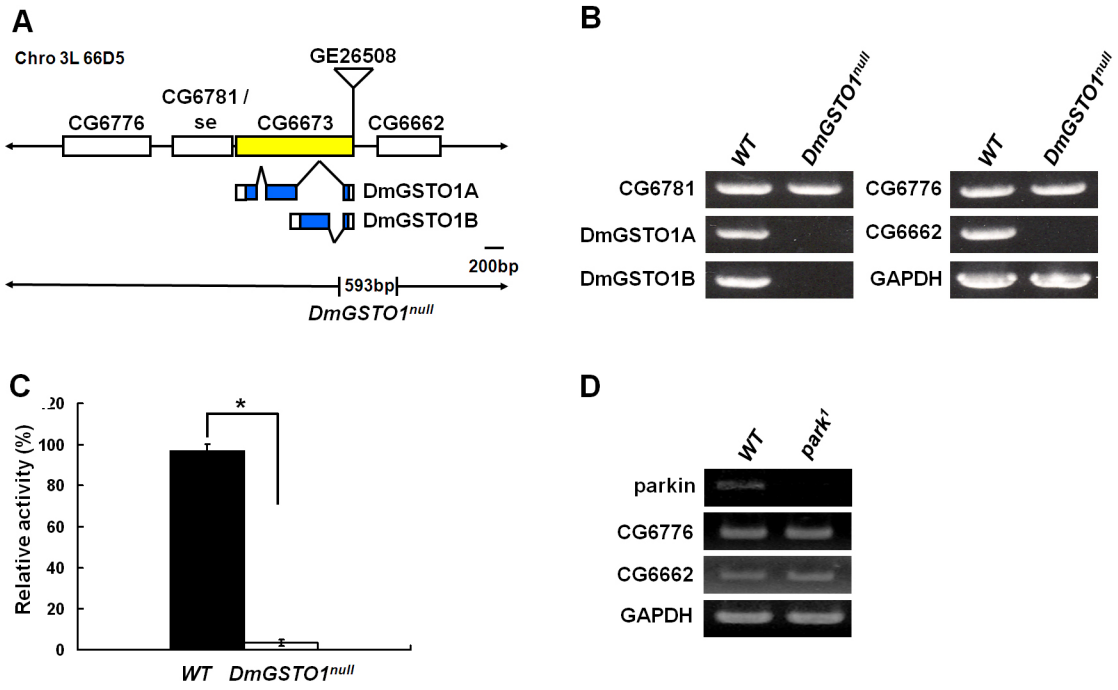

A
Chro 3L 66D5
GE26508
CG6781 /
se
CG6776
CG6673
CG6662
DmGSTO1A
DmGSTO1B
200bp
593bp
DmGSTO1null
B
WT
DmGSTO1null
CG6781
DmGSTO1A
DmGSTO1B
CG6776
CG6662
GAPDH
C
Relative activity (%)
120
100
80
60
40
20
0
*
WT
DmGSTO1null
D
WT
park1
parkin
CG6776
CG6662
GAPDH

FIGURE S2.

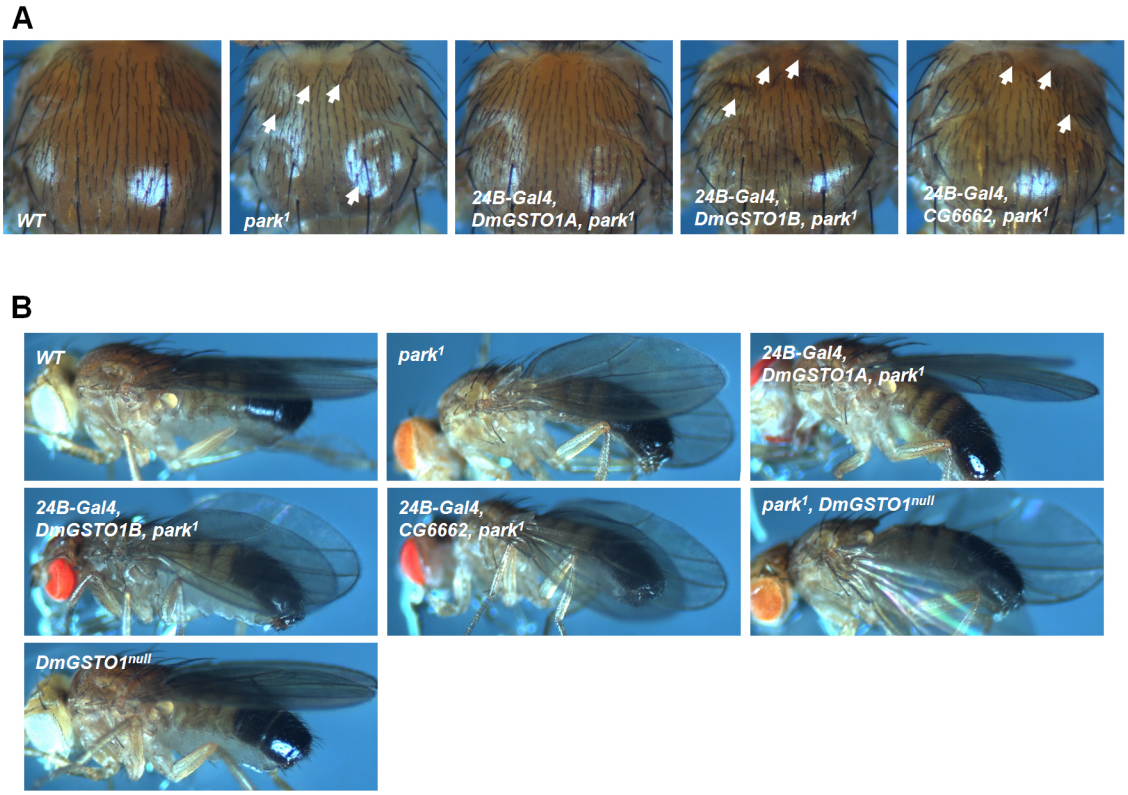

A
WT
park1
24B-Gal4, DmGSTO1A, park1
24B-Gal4, DmGSTO1B, park1
24B-Gal4, CG6662, park1
B
WT
park1
24B-Gal4, DmGSTO1A, park1
24B-Gal4, DmGSTO1B, park1
24B-Gal4, CG6662, park1
park1, DmGSTO1null
DmGSTO1null

FIGURE S3.

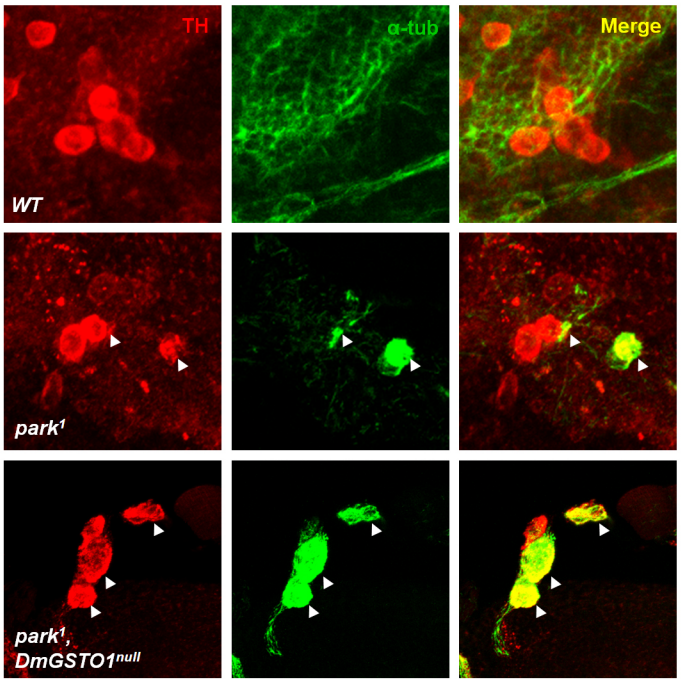


FIGURE S4.

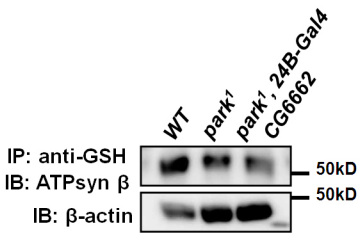

WT
park1
park1; 24B-Gal4
CG6662
IP: anti-GSH
IB: ATPsyn β
IB: β-actin
50kD
50kD

FIGURE S5.

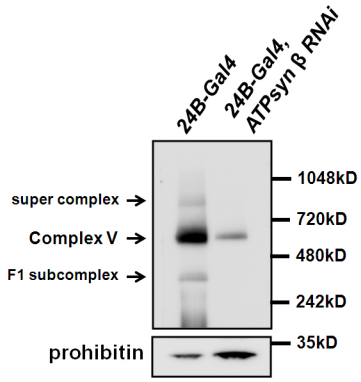

24B-Gal4
24B-Gal4, ATPsyn β RNAi
super complex
Complex V
F1 subcomplex
prohibitin
1048kD
720kD
480kD
242kD
35kD

FIGURE S6.

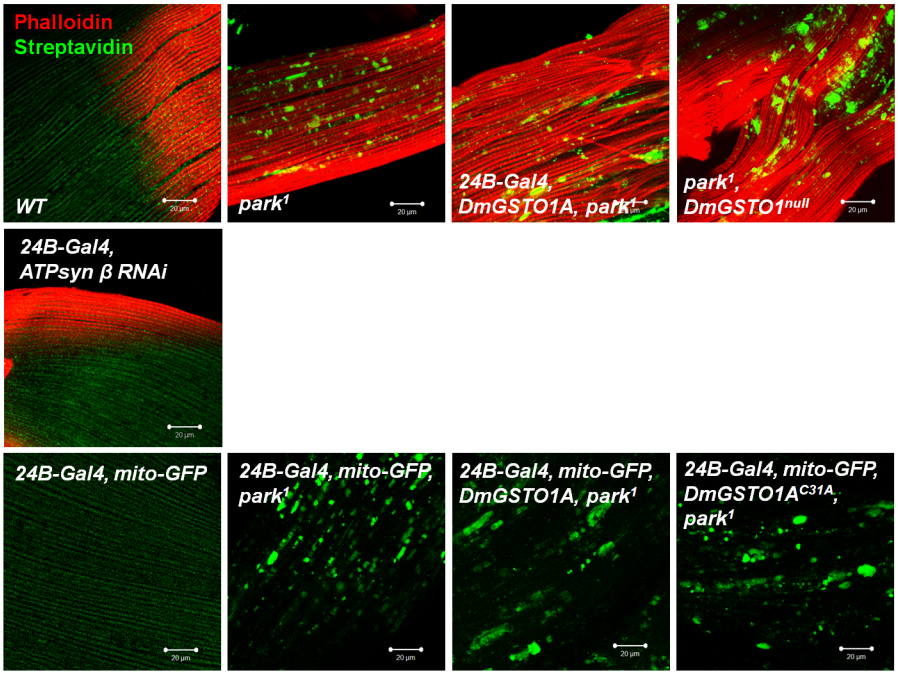
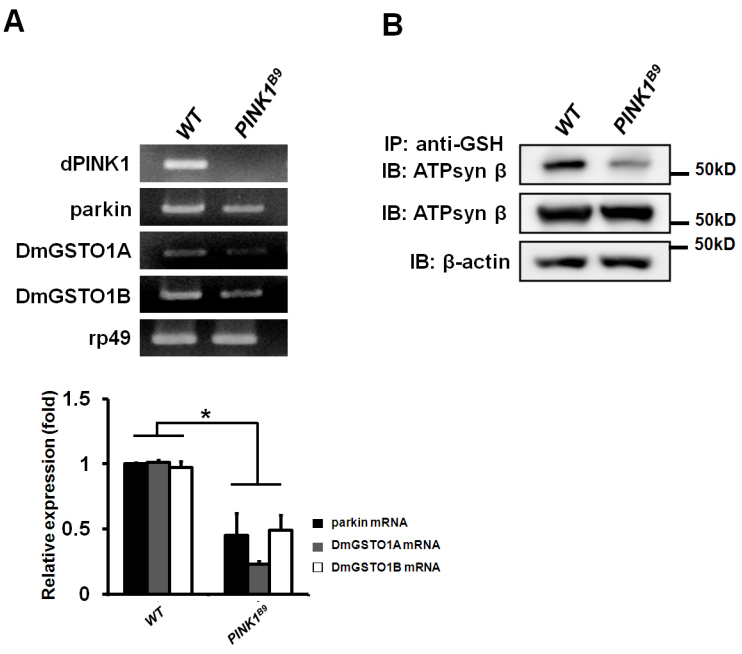

Phalloidin
Streptavidin
WT
park1
24B-Gal4,
DmGSTO1A, park1
park1,
DmGSTO1null
24B-Gal4,
ATPsyn β RNAi
24B-Gal4, mito-GFP
24B-Gal4, mito-GFP,
park1
24B-Gal4, mito-GFP,
DmGSTO1A, park1
24B-Gal4, mito-GFP,
DmGSTO1AC31A,
park1

FIGURE S7.


A
WT
PINK1B9
dPINK1
parkin
DmGSTO1A
DmGSTO1B
rp49
Relative expression (fold)
1.5
1
0.5
0
*
parkin mRNA
DmGSTO1A mRNA
DmGSTO1B mRNA
WT
PINK1B9
B
WT
PINK1B9
IP: anti-GSH
IB: ATPsyn β
50kD
IB: ATPsyn β
50kD
IB: β-actin
50kD