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Supplemental Information

A Neuron-Glial Trans-Signaling Cascade Mediates LRRK2-Induced Neurodegeneration

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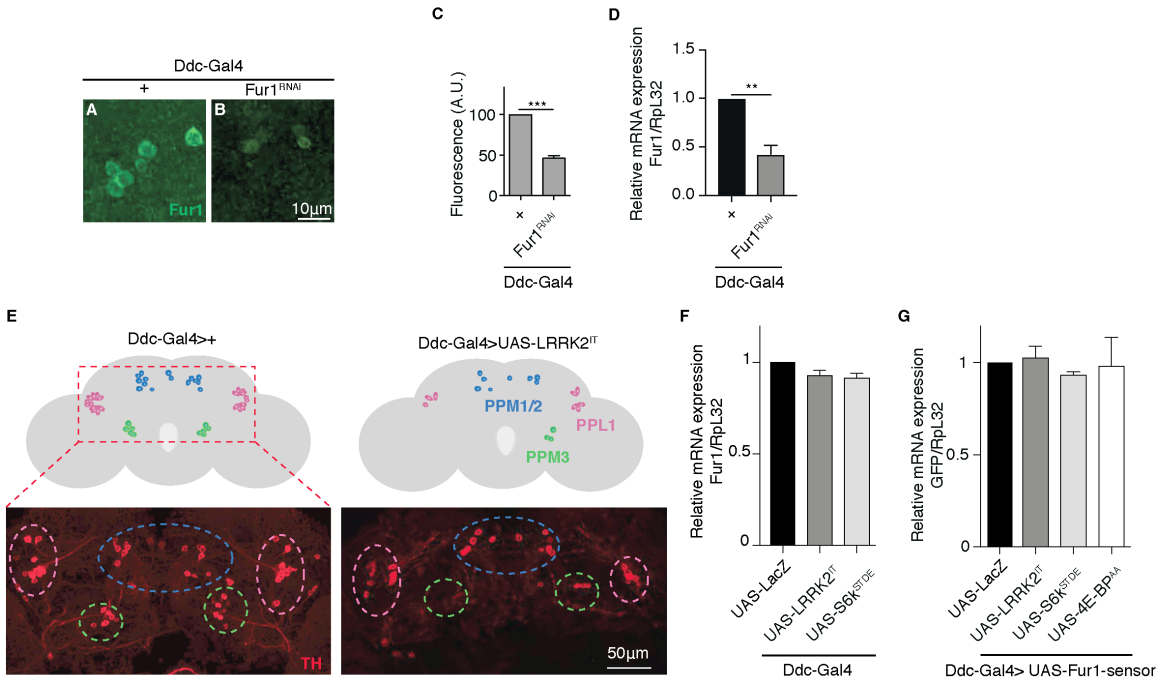

Figure S1, Related to Figure 1: LRRK2^{IT} overexpression in DA neurons doesn't affect Fur1 or Fur1 sensor transcription levels.

(A-B) Representative images of PPL1 clusters from 10-day-old female brains stained with anti-Fur1 (green) in (A) + (Ddc-Gal4/W1118) and (B) Fur1^{RNAi} (Ddc-Gal4/+; UAS-Fur1-RNAi/+).

(C) Quantification of the fluorescence associated with Fur1 antibody from genotypes in A and B in 10-day-old female flies. n=50 neurons from 5 PPL1 clusters for each genotype. Data are represented as mean ± SEM. Student's t-test.

(D) Quantification of Fur1 mRNA expression relative to RpL32 from female fly heads by qPCR for genotypes in A and B. n=3. Data are represented as mean ± SEM. Student's t-test.

(E) Top: Schematic representation of the adult *Drosophila* brain DA neurons clusters PPL1, PPM1/2 and PPM3. Bottom: Dorsal view of the whole mount *Drosophila* brain for the following genotypes: Ddc-Gal4>+ (Ddc-Gal4/UAS-LacZ) and Ddc-Gal4> UAS-LRRK2^{IT} (DDC-Gal4/+; UAS-LRRK2^{IT}/+). DA neurons are stained with an anti-TH antibody in red.

(F) Quantification of Fur1 mRNA expression relative to RpL32 by qPCR from female fly heads for the following genotypes: UAS-LacZ (Ddc-Gal4/UAS-LacZ), UAS-LRRK2^{IT} (Ddc-Gal4/+; UAS-LRRK2^{IT}/+) and UAS-S6K^{STDE} (Ddc-Gal4/+; UAS-S6K^{STDE}/+). n=3 for each genotype. Data are represented as mean ± SEM. One-way ANOVA with Bonferroni post-test.

(G) Quantification of GFP mRNA expression relative to RpL32 by qPCR from female fly heads for the following genotypes: UAS-LacZ (UAS-Fur1 sensor/UAS-LacZ; Ddc-Gal4/+), UAS-LRRK2^{IT} (UAS-Fur1 sensor/+; Ddc-Gal4/UAS-LRRK2^{IT}), UAS-S6K^{STDE} (UAS-Fur1 sensor/+; Ddc-Gal4/UAS-S6K^{STDE}) and

UAS-4E-BP^{AA} (UAS-Fur1 sensor/+; Ddc-Gal4/UAS-4E-BP^{AA}). n=3 for each genotype. Data are represented as mean ± SEM. One-way ANOVA with Bonferroni post-test.
** $P<0.01$; *** $P<0.001$.

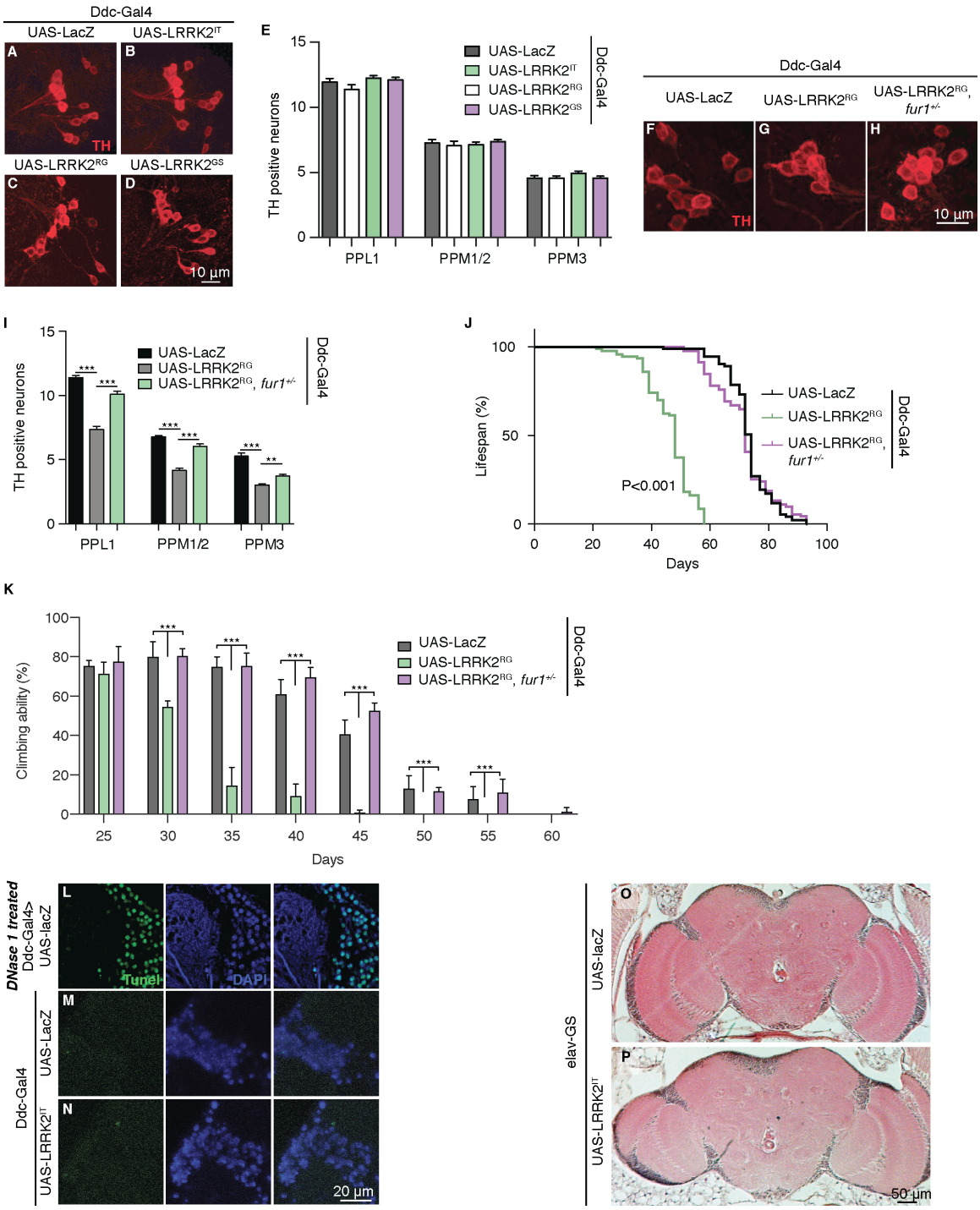

Figure S2, Related to Figure 2: Fur1 heterozygosity is protective in LRRK2^{RG} over-expressing neurons.

(A-D) Representative images of DA neurons stained with anti-TH antibody (red) from PPL1 clusters in 10-day-old female flies for the following genotypes: (A) UAS-LacZ (Ddc-Gal4/UAS-LacZ), (B) UAS-

LRRK2^{IT} (Ddc-Gal4/+; UAS-LRRK2^{IT}/+), (C) UAS-LRRK2^{RG} (Ddc-Gal4/UAS- LRRK2^{RG}) and (D) UAS-LRRK2^{GS} (Ddc-Gal4/+; UAS-LRRK2^{GS}/+).

(E) Quantification of the number of TH positive DA neurons in the PPL1, PPM1/2 and PPM3 clusters in 10-day-old females for genotypes in A to D. n=22 hemispheres for each genotype. Data are represented as mean ± SEM. One-way ANOVA with Bonferroni post-test.

(F-H) Representative images of DA neurons stained with anti-TH antibody (red) from PPL1 clusters in 60-day-old female flies for the following genotypes: (F) UAS-LacZ (Ddc-Gal4/UAS-LacZ), (G) UAS-LRRK2^{RG} (Ddc-Gal4/UAS-LRRK2^{RG}) and (H) UAS-LRRK2^{RG}, *fur1*$^{+/-}$ (Ddc-Gal4/UAS- LRRK^{RG}; *fur1*^{rl205}/+).

(I) Quantification of the number of TH positive DA neurons in the PPL1, PPM1/2 and PPM3 clusters in 60-day-old females for genotypes in F to H. n=22 hemispheres for each genotype. Data are represented as mean ± SEM. One-way ANOVA with Bonferroni post-test.

(J-K) Representative survival curves (J) and climbing activity (K) for genotypes in F to H. Survival: n=100 flies for each genotype (See Table S1). Log-rank and Wilcoxon tests. Climbing n=60 flies for each genotype. For climbing activity, data are represented as mean ± SEM. Two-way ANOVA with Dunnett post-test.

(L-N) Representative images of TUNEL (green) and DAPI (blue) staining in 5 um paraffin sections of 50 day-old *Drosophila* heads: (L) Upper right quadrant at approximately mid-brain of a DNase1 treated section of the following genotype: Ddc-Gal4/UAS-LacZ; (M-N) Upper right quadrant at approximately mid-brain in *Drosophila* of the following genotypes: (M) UAS-LacZ (Ddc-Gal4/UAS-LacZ) and (N) UAS-LRRK2^{IT} (Ddc-Gal4/+; UAS-LRRK2^{IT}/+).

(O-P) Representative images of 5 μm paraffin sections at approximately midbrain of 50-day-old female fly brains of the following genotypes: (O) UAS-lacZ (elav-GS/UAS-lacZ) and (P) UAS-LRRK2^{IT} (elav-GS/+; UAS-LRRK2^{IT}/+). ** P<0.01; *** P<0.001.

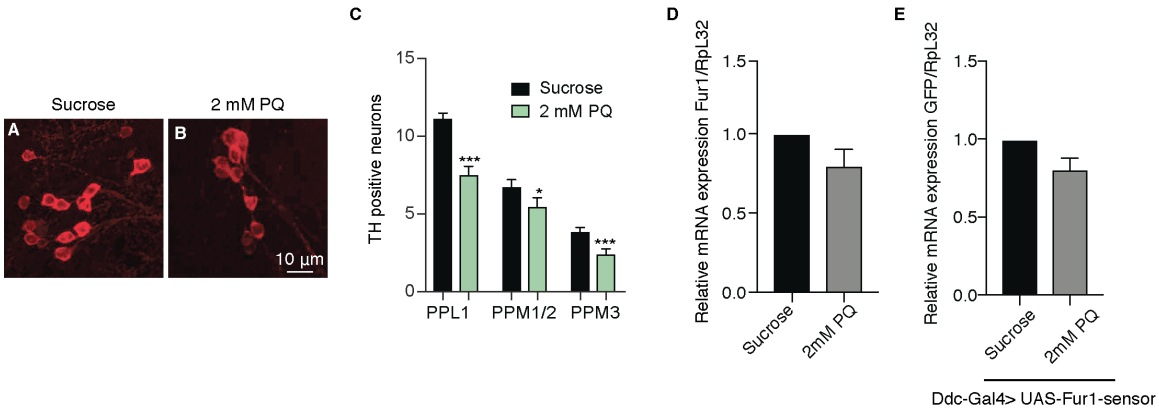

Figure S3, Related to Figure 3: Paraquat exposure is toxic to DA neurons.

(A-B) Representative images of DA neurons stained with anti-TH antibody (red) from PPL1 cluster in (*yw*) female flies raised on (A) sucrose or (B) 2 mM paraquat containing media for 5 days.

(C) Quantification of the number of TH positive DA neurons in the PPL1, PPM1/2 and PPM3 clusters for conditions A and B. n=22 hemispheres for each genotype. Student's t-test.

(D) Quantification of Fur1 mRNA expression relative to RpL32 by qPCR from (*yw*) female fly heads raised on sucrose or 2 mM paraquat containing media for 5 days. n=3 for each genotype. Student's t-test.

(E) Quantification of GFP mRNA expression relative to RpL32 by qPCR from female fly heads of (UAS-Fur1 sensor/+; Ddc-Gal4/+) flies raised on sucrose or 2 mM paraquat containing media for 5 days. n=3 for each genotype. Student's t-test

Error bars represent SEM. * $P<0.05$, *** $P<0.001$.

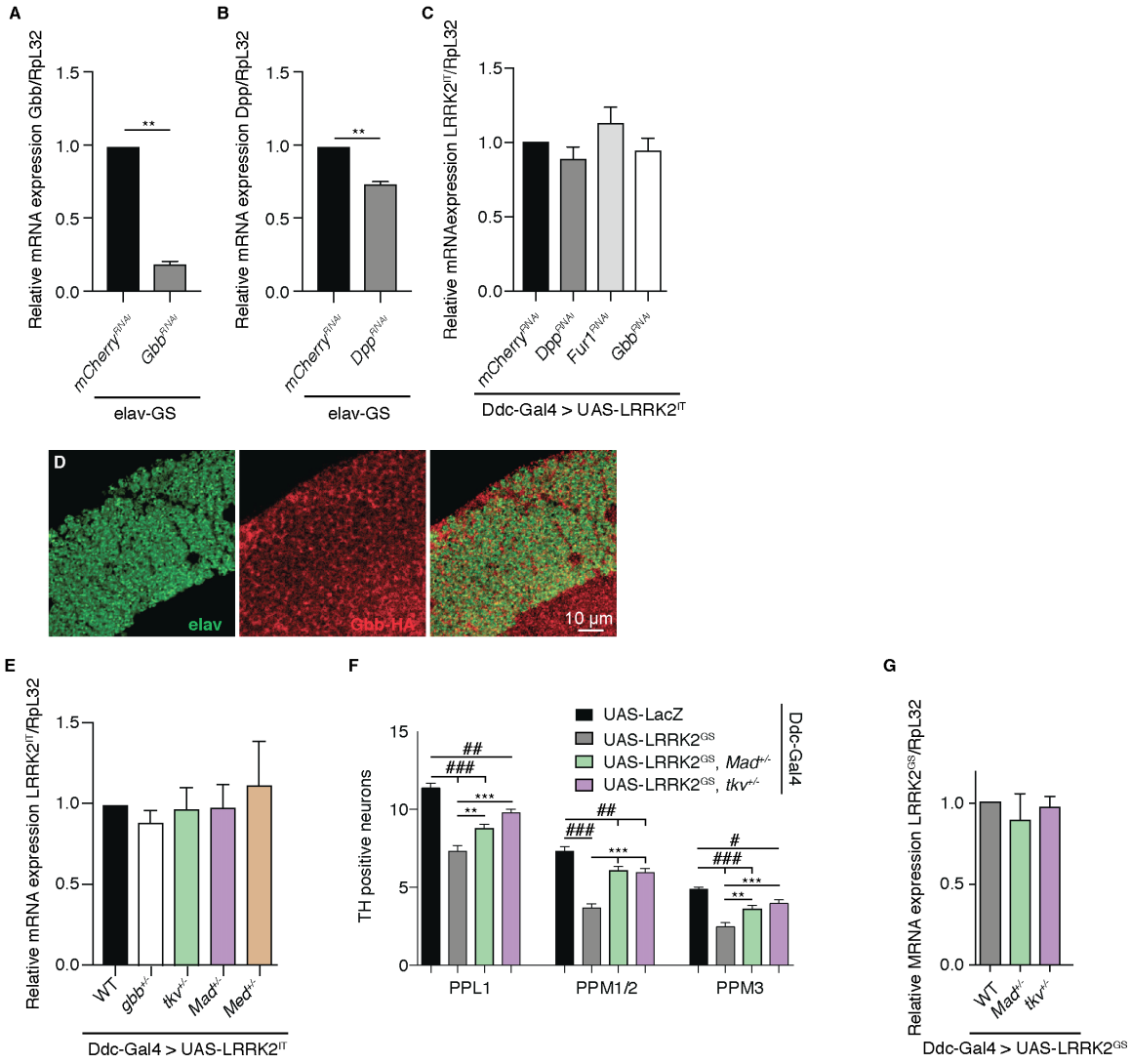

Figure S4, Related to Figure 4: LRRK2^{GS} toxicity is mediated by BMP signaling.

(A) Quantification of Gbb mRNA expression relative to RpL32 by qPCR from female fly heads of the following genotypes: mCherry^{RNAi} (elav-GS/+; UAS-mCherry-RNAi/+) and Gbb^{RNAi} (elav-GS/+; UAS-Gbb-RNAi/+). n=3 for each genotype. Student's t-test.

(B) Quantification of Dpp mRNA expression relative to RpL32 by qPCR from female fly heads of genotypes: mCherry^{RNAi} (elav-GS/+; UAS-mcherry-RNAi /+) and Dpp^{RNAi} (elav-GS/+; UAS-Dpp-RNAi /+). n=3. Student's t-test.

(C) Quantification of LRRK2^{IT} mRNA expression relative to RpL32 by qPCR from female fly heads of the following genotypes: mCherry^{RNAi} (Ddc-Gal4/+; UAS- LRRK2^{IT}/UAS-mCherry-RNAi), Dpp^{RNAi} (Ddc-Gal4/+; UAS- LRRK2^{IT}/UAS-Dpp-RNAi), Fur1^{RNAi} (Ddc-Gal4/+; UAS- LRRK2^{IT}/UAS-Fur1-RNAi) and

Gbb^{RNAi} (Ddc-Gal4/+; UAS- $LRRK2^{IT}$/UAS-Gbb-RNAi). n=3 for each genotype. One-way ANOVA with Bonferroni post-test.

(D) Representative images of the upper left quadrant of the adult *Drosophila* brain (Gbb-HA) double stained with anti-elav (green) and anti-HA (red).

(E) Quantification of $LRRK2^{IT}$ mRNA expression relative to RpL32 by qPCR from female fly heads of the following genotypes: WT (Ddc-Gal4/+; UAS- $LRRK2^{IT}$/+), $gbb^{+/-}$ (Ddc-Gal4/gbb^{1}; UAS- $LRRK2^{IT}$/+), $tkv^{+/-}$ (Ddc-Gal4/tkv^{7}; UAS- $LRRK2^{IT}$/+), $Mad^{+/-}$ (Ddc-Gal4/ Mad^{2371}; UAS- $LRRK2^{IT}$/+) and $Med^{+/-}$ (Ddc-Gal4/+; UAS- $LRRK2^{IT}$/Med^{C246}). n=3 for each genotype. One-way ANOVA with Bonferroni post-test.

(F) Quantification of the number of TH positive DA neurons in the PPL1, PPM1/2 and PPM3 clusters of 60-day-old female flies of the following genotypes: UAS-LacZ (Ddc-Gal4/UAS-LacZ), UAS-$LRRK2^{GS}$ (Ddc-Gal4/+; UAS-$LRRK2^{GS}$/+), UAS-$LRRK2^{GS}$; $Mad^{+/-}$ (Ddc-Gal4/Mad^{K00237}; UAS-$LRRK2^{GS}$/+) and UAS-$LRRK2^{GS}$; $tkv^{+/-}$ (Ddc-Gal4/tkv^{7}; UAS- $LRRK2^{GS}$/+). One-way ANOVA with Bonferroni post-test.

(G) Quantification of $LRRK2^{GS}$ mRNA expression relative to RpL32 by qPCR from female fly heads of the following genotypes: WT (Ddc-Gal4/+; UAS-$LRRK2^{GS}$/+), UAS-$LRRK2^{GS}$; $Mad^{+/-}$ (Ddc-Gal4/Mad^{K00237}; UAS- $LRRK2^{GS}$/+) and UAS-$LRRK2^{GS}$; $tkv^{+/-}$ (Ddc-Gal4/tkv^{7}; UAS- $LRRK2^{GS}$/+) . One-way ANOVA with Bonferroni post-test.

Error bars represent SEM. ** $P<0.01$*** $P<0.001$; # $P<0.05$; ## $P<0.01$; ### $P<0.001$

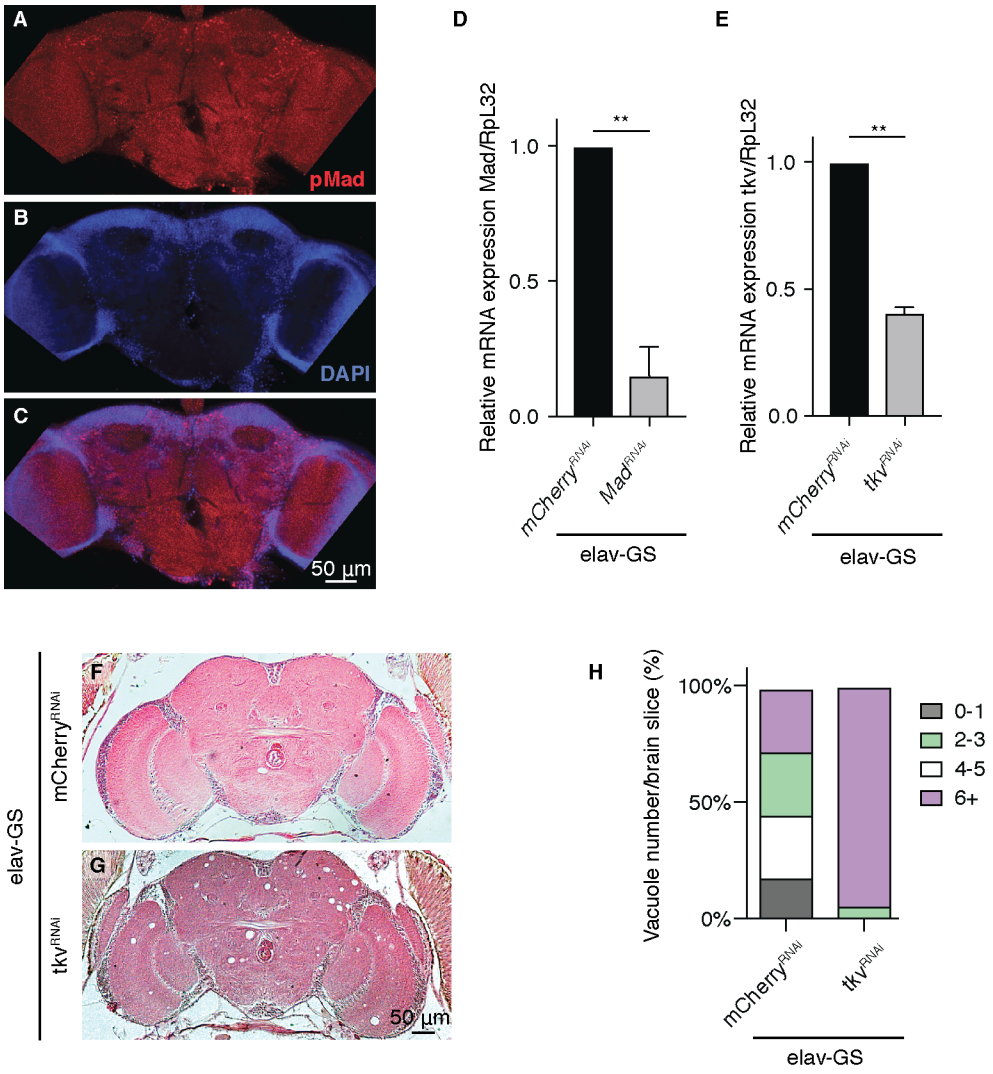

Figure S5, Related to Figure 5: Loss of BMP signaling in neurons causes neurodegeneration.

(A-C) Adult *Drosophila* brain double stained for (A) anti-pMad (red) and (B) DAPI (blue) in 10-day-old (*yw*) flies, (C) merged image.

(D) Quantification of Mad mRNA expression relative to RpL32 by qPCR from female fly heads of genotypes: mCherry^{RNAi} (elav-GS/+; UAS-mCherry-RNAi/+) and Mad^{RNAi} (elav-GS/+; UAS-Mad-RNAi/+). n=3 for each genotype. Student's t-test.

(E) Quantification of tkv mRNA expression relative to RpL32 by qPCR from female fly heads of genotypes: mCherry^{RNAi} (elav-GS/+; UAS-mCherry-RNAi/+) and tkv^{RNAi} (elav-GS/+; UAS-tkv-RNAi/+). n=3 for each genotype. Student's t-test.

(F-G) Representative images of 5 µm paraffin sections at approximately midbrain of 40-day-old female fly brains of the following genotypes: (F) mCherry^{RNAi} (elav-GS/+; mCherry-RNAi/+) and (G) tkv^{RNAi} (elav-GS/+; tkv-RNAi/+). White vacuoles indicate neurodegeneration.

(H) Percentage of brain sections from independent brains for genotypes S5F and S5G with (0-1), (2-3), (4-5) and (6+) vacuoles per section. n=20 sections from 20 brains for each genotype.
Error bars represent SEM. ** $P<0.01$

		Median survival (days)		
Figure:	Genotypes	N1	N2	N3
2F	Ddc-Gal4/UAS-LacZ	57	63	65
	Ddc-Gal4/+; *fur1*^{rl205}/+	60	65	62
	Ddc-Gal4/+; UAS-LRRK2^{IT}/+	34	31	38
	Ddc-Gal4/+; UAS- LRRK2^{IT}/*fur1*^{rl205})	55	57	57
3D	Ddc-Gal4/+; UAS-eGFP/+	60	58	62
	Ddc-Gal4/UAS-eGPF::Fur1	39	35	44
3K	Ddc-Gal4/+ (w^{1118})	5.5	6	5
	Ddc-Gal4/+; UAS-Fur1-RNAi	8	9	8.5
3J	Ddc-Gal4/+; UAS-eGFP/+	5.5	6	5
	Ddc-Gal4/UAS-eGPF::Fur1	8	9	8.5
5E	elavGS/+; UAS-mCherry-RNAi/+	8.5	9	8
	elavGS/+; UAS-tkv-RNAi/+	4.5	5	4.5
	elavGS/+; UAS-Mad-RNAi/+	4	5.5	4.5
5F	tub-Gal80^{ts}/+; repo-Gal4/UAS-mCherry-RNAi	9	8.5	8
	tub-Gal80^{ts}/+; repo-Gal4/UAS-tkv-RNAi	10	9.5	10
	tub-Gal80^{ts}/+; repo-Gal4/UAS-Mad-RNAi	10	10	9.5
S2J	Ddc-Gal4/UAS-LacZ	74	67	72
	Ddc-Gal4/UAS-LRRK^{RG}	48	53	50
	Ddc-Gal4/UAS- LRRK^{RG}; *fur1*^{rl205}/+	72	65	68

Table S1, Related to Figures 2, 3, 5 and S2: Median lifespan of three repeats (N) for each experiment. Summary of median lifespan in days of three repeats for experiments from Fig 2F, 3D, 3K, 3L, 5E, 5F and S2E. N refers to a single experiment based on an independent set of genetic crosses. n= 100 female flies for each genotype.

Primers	
Furin1 forward	AGGAATATGCAGCAGGTGGG
Furin1 reverse	TGCACTCTAAGCACTTGCGA
GFP forward	GGGCTTGGATGTTTTAATCTTG
GFP reverse	AGAAGAAGCCCCGCTTGTA
tkv forward	ATGGAACCTGCGAGACCAGAC
tkv reverse	CTCCTCGTACATCCCGGT
Mad forward	GCACATTTGCGTGTCGAA
Mad reverse	GCGGATAGTGCGTGGATTTAG
Gbb forward	GAGTGGCTGGTCAAGTCGAA
Gbb reverse	GAAGCCGATCATGAAGGGCT
Dpp forward	TGGCGACTTTTCAAACGATTGT
Dpp reverse	CAGCGGAATATGAGCGGCAA
RpL32 forward	AAGCGGCGACGCACTCTGTT
RpL32 reverse	GCCCAGCATACAGGCCCAAG
LRRK2 forward	CGATCCATGGCTAGTGGCAGCTGT
LRRK2 reverse	CCTCTGAGACTCTCTCAAACAGC
OED461	GCACCGGTATATGAAAAACGACGTCGTGCGAT
OED462	GAGCGGCCGCTTATCTAATGCATTTGATAATGTTGTTTTT

Table S2, Related to figures S1, S3, S4 and S5 and Key Resources Table: Primers' list.