Supplement Figure 1. Multiple epitope-tagged parkin transgenes confirm the neurotoxicity of mutant parkin. ***A***, Quantification of DA neurons from independent parkin transgenic lines at five weeks post eclosion in DM (upper panel) and DL (lower panel) clusters show significant loss of TH-immunoreactive neurons in brains expressing mutant parkin^{Q311X} (blue bars) and parkin^{T240R} (red bars) as compared to *ddc*-GAL4 control (black bars); brains expressing FLAG-tagged parkin^{wt} were indistinguishable from controls(green bars). Values represent the mean $\pm$ SEM, n = 10. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ relative to the *ddc* control (one-way ANOVA with Bonferroni's post test). ***B***, Representative confocal images of *ddc*-GAL4 (***a***-***c***) and FLAG-tagged parkin^{wt} (***d***-***f***) brains aged five weeks post eclosion co-stained with anti-TH (green) and anti-parkin (red). The number of anti-TH labeled neurons for parkin^{wt} is comparable to the control (compare ***b*** and ***e***). Anti-parkin also colocalized with all DA neurons in parkin^{wt} brain (***d***,***f***); arrows, somata of non-DA neurons that are negative for TH staining but labeled with anti-parkin (***d***,***f***). The branch-like staining in the lower part of ***a*** and ***d*** is due to non-specific secondary antibody background staining. Circles, DM; rectangles, DL.

Supplement Figure 2. Immunoblots and immunohistochemistry demonstrating expression of human parkin transgenes. ***A***, Immunoblot using polyclonal antibody directed against residues surrounding amino acid 400 of human parkin (Cell Signaling) detects ~45 kDa bands in *ddc*::parkin^{wt} and *ddc*::parkin^{T240R}; the truncated mutant parkin^{Q311X} protein cannot be detected by this antibody. The same blot was stripped and then probed with anti-β-tubulin to serve as a loading control. Lane 1, *ddc*-GAL4/+.Lane 2, *ddc*-GAL4/UAS-parkin^{Q311X}-29. Lane 3, *ddc*-GAL4/+; UAS-parkin^{wt}-45/+. Lane 4, *ddc*-GAL4/UAS-parkin^{T240R}-2.130. ***B***, Immunoblot using monoclonal antibody PRK8 directed against the second RING finger domain of human parkin (Pawlyck, et al., 2003). The antibody recognizes wild type and T240R mutant parkin. Lane 5, *ddc*-GAL4/+. Lane 6, *ddc*-GAL4/UAS-parkin^{wt}-45 (Myc). Lane 7, *ddc*-GAL4/UAS-parkin^{wt}-41 (FLAG). Lane 8, *ddc*-GAL4/UASparkin^{wt}-47 (FLAG). Lane 9, *ddc*-GAL4/UAS-parkin^{T240R}-2.130 (FLAG). Lane 10, *ddc*-GAL4/UAS-parkin^{T240R}-2.147 (FLAG). Lane 11, *ddc*-GAL4/UAS-parkin^{T240R}-2.164 (FLAG). Parallel

blots were used for the β-tubulin antibody. ***C***, Immunoblot demonstrating expression of the Q311X transgene using the Sigma M2 anti-FLAG monoclonal antibody. Lane 12, *ddc*-GAL4/+. Lane 13, *ddc*-GAL4/UAS-parkin^{Q311X}-21 (FLAG). ***D-F***, immunohistochemistry demonstrating expression of the parkinQ311X transgenes in a subset of DA neurons. Images were taken of cells in the peripheral brain using whole mount preps microwaved for 1 min for antigen retrieval. ***D***, polyclonal anti-TH + FITC-anti-rabbit. ***E***, anti-FLAG monoclonal M5 + Cy3-anti-mouse. ***F***, merged image.

Supplement Figure 3. Expression of TNT using the *ddc*-GAL4 driver produces markedly abnormal motor function in *Drosophila*. ***A***, climbing assay; ***B***, righting reflex; and ***C***, *Drosophila* rotarod assay show that disruption of neurotransmission in *ddc*-expressing cells using TNT (*ddc*::TNT) causes significant motor deficit at two weeks as compared to age-matched controls. Sample size: n = 32 (***A***), 10 (***B***), and 12 (***C***). Values shown represent the mean $\pm$ SEM; ***, $p < 0.001$ relative to control *ddc*-GAL4 using unpaired t-test (***A***,***B***) and two-way ANOVA with Bonferroni post test (***C***).

Supplement Figure 4. Schematic of the *Drosophila* rotarod and sample readout. Normal flies break beams at all five positions, whereas flies with postural impairment break beams at 60 and 120 degrees more frequently (due to falls) but fail to break beams at 180, 240, or 300 degrees. Flies positioned at the peripheral edges of cylinders fail to break any beams.

Supplement Figure 5. Expression of human parkin^{T240R} in DA neurons causes age-dependent neurodegeneration. ***A***, Representative confocal images of *ddc*::parkin^{T240R} brains stained with anti-TH reveal progressive, age-dependent decreases in DA neurons. ***B***, Confocal images of brains aged five weeks after eclosion with expression of parkin transgenes using the DA-specific TH-GAL4 driver (***Ba***-***d***). Anti-TH staining showed significant reductions of DA neurons in parkin^{T240R} brain (***Bb***) as compared to control (***Ba***), while brains expressing wild type parkin (***Bd***) resembled controls (***Bc***). Confocal images were re-oriented from adjacent brains imaged

simultaneously for paired comparison (a and b, c and d). Quantitation of TH-positive neurons showed significant decreases in both DM and DL clusters in parkin^{T240R} brains (e, mean ± SEM, one-way ANOVA with Bonferroni post test; n = 6; *, $p < 0.05$; ***, $p < 0.001$). DM (circles) and DL clusters (rectangles) are indicated.

Supplement Figure 6. Selective vulnerability of aminergic neurons to mutant parkin is not due to higher expression. ***A***, Immunoblot using monoclonal PRK8 comparing expression levels of UAS-parkin^{T240R}-2.130 in head extracts under control of *ddc*-, GMR-, and *chat*-GAL4. Parallel blots were probed with the β-tubulin antibody. ***B***, Quantitation derived from three sets of extracts for each genotype. Values shown represent the mean ± SEM. Failure to obtain toxicity with *chat*-GAL4 or GMR-GAL4 as compared to *ddc*-GAL4 was not due to lower expression, as the former two drivers generated more robust expression (more than twofold higher for *chat* and more than sixfold higher for GMR as compared to *ddc*). *, $p < 0.05$; one way ANOVA with Holm-Sidak comparison. For *chat*-GAL4 as compared to *ddc*-GAL4, there was a trend toward significance ($p = 0.05$).

Supplement Figure 7. Expression of DVMAT RNAi construct reduces endogenous DVMAT immunoreactivity. ***A***, Confocal image of *ddc*-GAL4 control brain. ***B***, *ddc*::DVMATi brain. Adjacent brains were stained with anti-DVMAT and images was acquired simultaneously on the same slide. DVMAT immunoreactivity was reduced in DVMATi-expressing cells (circles). ***C***, Quantitation of DVMAT staining showed significantly reduced pixel density in *ddc*::DVMATi brains as compared to *ddc* control (unpaired t-test, *, $p < 0.05$).

D, Immunoblot demonstrating that DVMAT RNAi decreases DVMAT level expression. Protein extracts from freshly eclosed fly heads were separated by SDS-PAGE. Upper blot, anti-DVMAT antibody demonstrates decreased immunoreactivity in ddc::DVMATi extracts (lane 2), whereas there was increased signal in flies overexpressing DVMAT (lane 3) as compared to the *ddc*-GAL4 control (lane 1). Asterisk, nonspecific band. The same blot was stripped and then subsequently blotted with anti-β-tubulin (middle blot) and anti-VGLUT (*Drosophila* vesicular glutamate

transporter, lower blot) to demonstrate equal loading of protein and DVMATi specificity, respectively.

Supplement Figure 8. Modulation of DVMAT affects DA concentration in fly heads. ***A***, HPLC analysis indicates that DA concentrations are lower in head extracts of flies homozygous for both *ddc*-GAL4 and UAS-DVMAT (mean $\pm$ SEM; n = 4 samples, 3 heads/sample) relative to *ddc*-GAL4 (*, $p < 0.05$, two-tailed t-test). ***B*,** In a separate set of experiments, HPLC analysis of flies homozygous for both *ddc*-GAL and UAS-DVMAT-RNAi shows higher DA concentrations (mean $\pm$ SEM, n = 6 samples, 3 heads/sample) as compared to *ddc*-GAL4 (** $p < 0.001$).