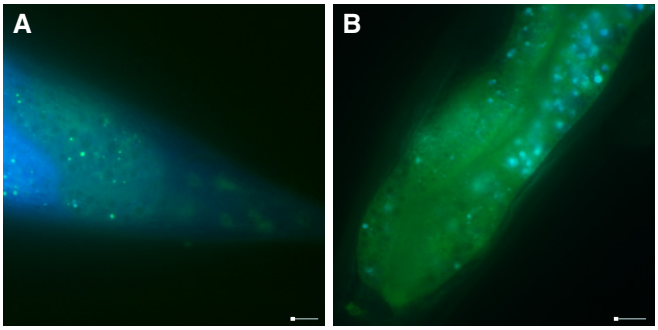
Expanded View Figures



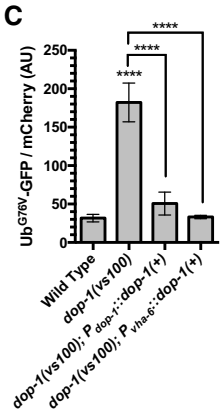


Figure EV1. DOP-1 receptors act in epithelial cells.

A Fluorescence image of the tail region of transgenic *dop-1::gfp* animals, with GFP in green and endogenous autofluorescence (captured with DAPI filters) in blue. Scale bar, 10 μm.

B Fluorescence image of the intestine of transgenic *dop-1::gfp* animals, with GFP in green and endogenous autofluorescence (captured with DAPI filters) in blue. Scale bar, 10 μm.

C Quantified fluorescence of Ub^{G76V}-GFP normalized to mCherry in the intestine from L4 + 48 h animals of the indicated genotypes. ****$P < 0.0001$, ANOVA with Dunnett's *post hoc* comparison to the wild-type control (for *dop-1* mutants) or with Sidak *post hoc* comparison between indicated genotypes (brackets). $N = 20$ animals per genotype and time point. Error bars indicate SEM.

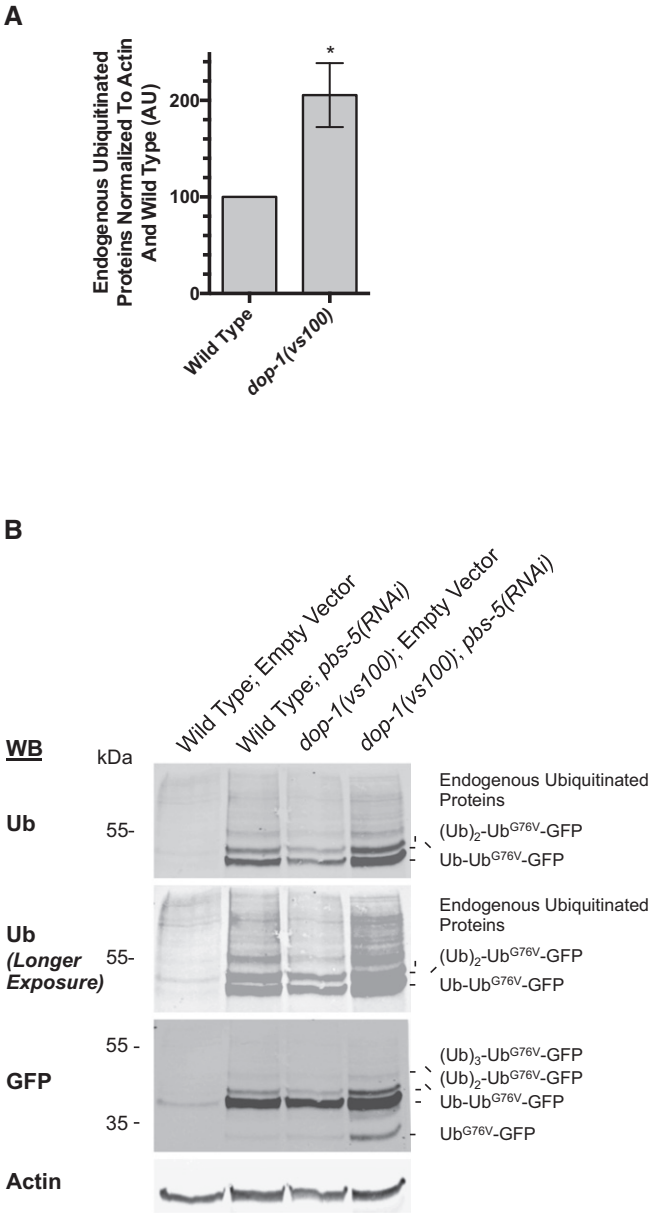


Figure EV2. Loss of proteasome activity and DOP-1 results in an additive effect on protein turnover.

A Quantification of endogenous ubiquitinated proteins in the 50–80 kDa range detected from whole nematode lysates by Western blot using the anti-ubiquitin antibody. Lysates are from animals of the indicated genotype, and quantification of each replicate blot (N = 4 biological replicates) was normalized to an anti-actin probing of the same blot followed by normalization to the wild-type ubiquitin/actin ratio for that blot. Averages are shown along with SEM for error bars. *$P < 0.02$ using a paired Student's t-test.

B Western blot of lysed nematodes at the L4 + 48 h stage, probed with antibodies recognizing ubiquitin, GFP, or actin as a loading control. Animals express Ub^{G76V}-GFP protein in their hypodermis. The position of molecular weight markers is shown on the left of each blot. The position of Ub^{G76V}-GFP protein, as well as Ub^{G76V}-GFP with the indicated number of additional ubiquitin moieties, based on molecular weight, is indicated on the right of each blot. Twenty animals were loaded per lane for each indicated genotype and RNAi treatment. For the anti-ubiquitin by Western blot, a longer exposure is shown to make poly-ubiquitinated species more visible.

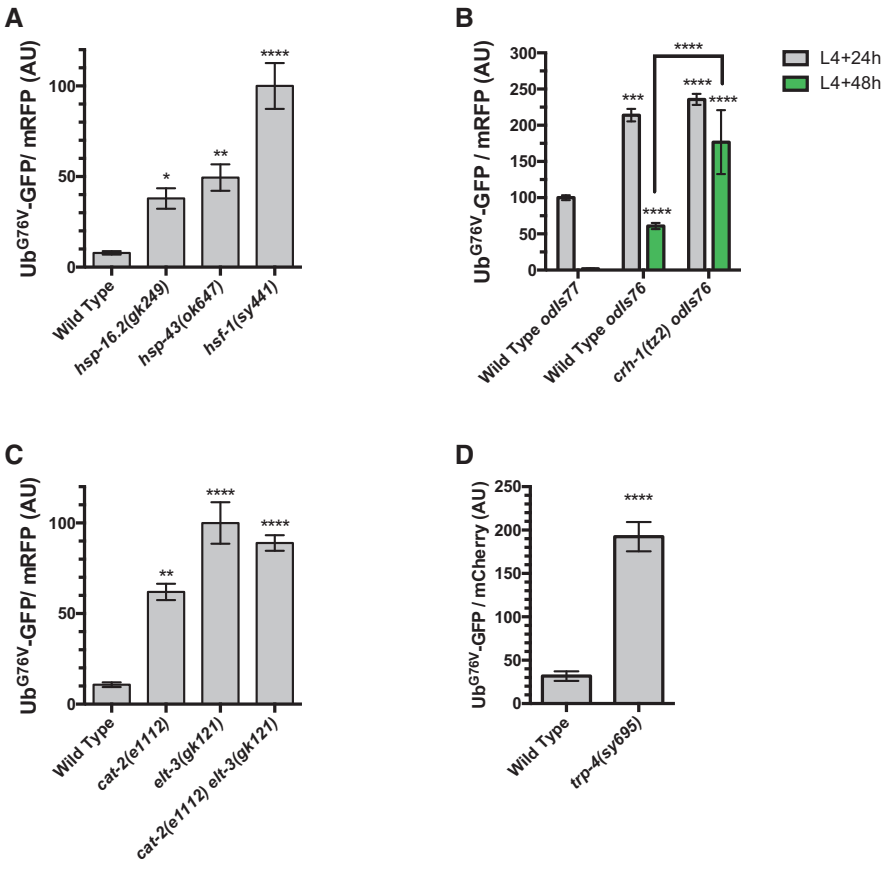


Figure EV3. Additional mutations that slow reporter protein turnover.

A Quantified fluorescence of Ub^{G76V}-GFP normalized to mRFP in the hypodermis from L4 + 48 h animals of the indicated genotype. ****$P < 0.0001$, **$P < 0.01$, *$P < 0.05$, ANOVA with Dunnett's *post hoc* comparison to the wild-type control for the respective time point. $N = 20$ animals per genotype and time point. Error bars indicate SEM.

B Whereas the *odIs77[$P_{col\text{-}19}$::Ub^{G76V}-GFP]*-integrated transgene is used for all other experiments, it is closely linked to the *crh-1* gene. Therefore, we examined Ub^{G76V}-GFP levels in *crh-1* mutants using the *odIs76[$P_{col\text{-}19}$::Ub^{G76V}-GFP]*-integrated transgene, which expresses Ub^{G76V}-GFP at higher levels than does the *odIs77* transgene, but with the same pattern of increased turnover at L4 + 48 h (some residual Ub^{G76V}-GFP remains at L4 + 48 h in *odIs76* transgenics, but all of the protein is turned over by L4 + 72 h). Mutations in *crh-1* block Ub^{G76V}-GFP turnover in *odIs76* transgenics. Quantified fluorescence of Ub^{G76V}-GFP normalized to mRFP in the hypodermis from animals of the indicated stage (gray for L4 + 24 h, green for L4 + 48 h) and the indicated mutant. ****$P < 0.0001$, ***$P < 0.001$, ANOVA with Dunnett's *post hoc* comparison to the wild-type *odIs77* transgenics at L4 + 24 h. Bracket indicates a Bonferroni *post hoc* comparison, ****$P < 0.0001$. $N = 20$ animals per genotype and time point. Error bars indicate SEM.

C Quantified fluorescence of Ub^{G76V}-GFP normalized to mRFP in the hypodermis from L4 + 48 h animals of the indicated genotype. ****$P < 0.0001$, **$P < 0.01$, ANOVA with Dunnett's *post hoc* comparison to the wild-type control for the respective time point. $N = 20$ animals per genotype and time point. Error bars indicate SEM.

D Quantified fluorescence of Ub^{G76V}-GFP normalized to mCherry in the intestine from L4 + 48 h animals of the indicated genotypes. ****$P < 0.0001$, Student's *t*-test. $N = 20$ animals per genotype and time point. Error bars indicate SEM.

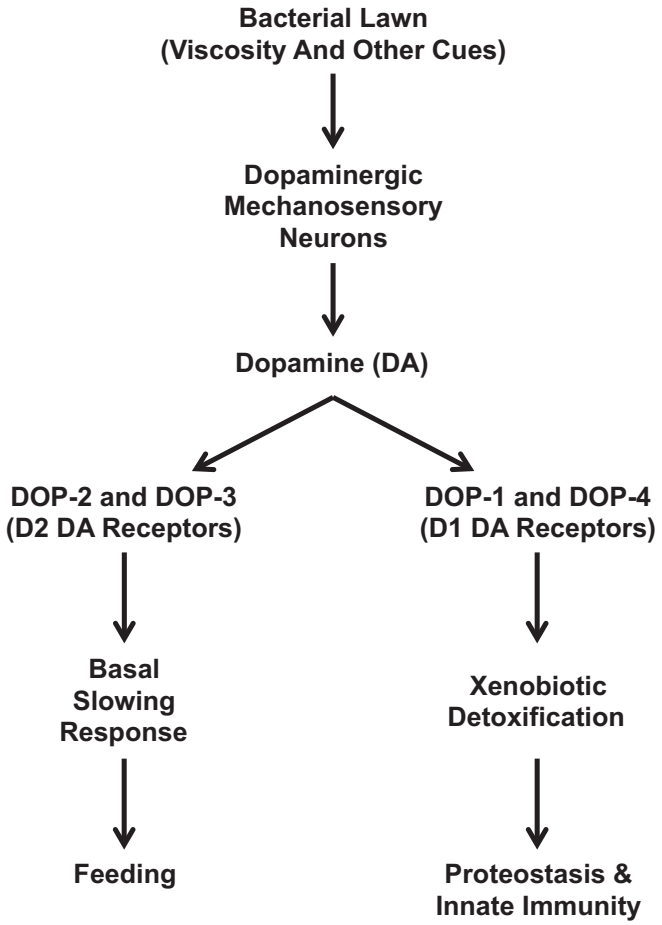


Figure EV4. Dopamine regulates behavior and proteostasis through separate receptor pathways.

When nematodes encounter a bacterial lawn, the lawn's viscosity is sensed by dopaminergic mechanosensory neurons, which then release the neurohormone dopamine (DA) into the pseudocoelomic body cavity in response. DA binds to the D2-like DA receptors DOP-2 and DOP-3 on motoneurons, resulting in reduced activity of the locomotion circuit and a basal slowing response behavior of the animal, allowing it to feed. DA also binds to the D1-like DA receptors DOP-1 and DOP-4, and we propose that these receptors activate the expression of xenobiotic detoxification genes. The resulting detoxification enzymes maintain protein homeostasis in the barrier epithelia of the hypodermis and the intestine, as well as promote innate immunity.