DMM000653 Supplementary Material
Files in this Data Supplement:

Fig. S1. Sequence and structural comparison between human and Drosophila Huntingtin proteins. (A) Schematics of homologous regions between the human and Drosophila Huntingtin proteins adopted from Li et al., (1999). Four regions with high sequence homology are colored, with their sequence similarity labeled according to Li et al. (B) Putative HEAT repeats and their localizations in human and Drosophila Huntingtin proteins. Notice that the putative HEAT repeats are clustered similarly in the N-, central-, and two C-terminal regions in both proteins, as highlighted underneath by the light blue lines.

Fig. S2. In situ hybridization against the *tartan* gene (positive control for RNA in situ hybridization). As the positive control for the RNA in situ hybridization experiment, a DIG-labeled antisense probe against the *tartan* gene was hybridized in parallel against third instar larval tissues. Specific signals were observed in the eye (A), wing (B) and leg imaginal discs (C), confirming the robustness of the in situ assay and suggesting that the weak in situ signals observed for *dhtt*were not the result of failed experiments.

Fig. S3. Normal synaptic organization and axonal transport in *CG9990* transgenic flies. Confocal images of glutamatergic NMJs (A-C) and neighboring axons (D-F) from *CG9990* transgenic animals. (A-C) NMJs in abdominal segment A3 of third instar muscles 6 and 7, double labeled with anti-HRP (red) and anti-Dlg (green) antibodies, which reveal the well-defined pre- and post-synaptic NMJ structures in *CG9990* transgenic animals. (C) Overlayed image of anti-Dlg and anti-HRP double stainings. Bars, 23.8 Âµm. (D-F) Confocal images of NMJs and neighboring axons (white arrows) of the larval peripheral nervous system (anti-HRP, red) in abdominal segment A2 of third instar muscles 6 and 7. Synaptic vesicles (green, anti-Syt) are properly delivered to NMJs and show no obvious accumulation in the axons of *CG9990* transgenes, resembling that observed in wild-type controls (Fig 4A-C). (F) Overlayed image of anti-HRP and anti-Syt double stainings. Bars, 10 Âµm.

Fig. S4. Axonal terminal morphology of A307-positive neurons in young fly brains. Representative images of the axonal termini morphology of the A307-positive neurons in young (3-day-old) wild-type (A-C) and *dhtt-ko* mutant (D-F) adult brains. The termini of A307-positive neurons are labeled by a membrane-bound mCD8-eGFP reporter (C,F) (green). The white dashed lines delineate the region magnified in (C1) and (C2) and in (F1) and (F2). Notice that there are hardly any prominent synaptic boutons in these axonal termini (compare with Figs 7F and 7I in 40-day-old flies). (B,E) Brains are co-stained with anti-FasII antibody (red) for mushroom bodies (MBs), which show that the axonal termini are located at similar positions in both brains. Also, notice that there is a much higher level of background signal from the A307>mCD8-eGFP reporter in young brains than in older brains (compare Figs S4C1-2 and S4F1-2 in 3-day-old flies with Figs 7F and 7I in 40-day-old flies). For clear visualization of the axonal termini, the images in S4 are presented as montage images of confocal sections that cover only the depth of axonal termini (which correspond to only the very anterior part of the MB) and not the whole brain. The top of each image is the dorsal end of the brain. WT=w^{1118} wild-type control. Bars, 75 Âµm (A-C,D-F); 10 Âµm (C1-2,F1-2).

Fig. S5. Normal axonal terminal complexity in aged wild-type fly brains. Anterior view of an 83-day-old wild-type brain. Axon termini of A307-positive neurons in the brain were revealed by using the membrane-bound mCD8-eGFP reporter (green) (A), which showed similar complexity to that in younger brains (Fig. 7). The same brain was also co-stained with DAPI (blue) (B) and anti-FasII (red) (C) to label cell nuclei and MBs, respectively. (D) Overlay of images (A-C) showing the relative positions of MBs and axonal termini of A307-positive neurons in the brain. The top of each image is the dorsal end of the brain. Bars, 75 Âµm.

Fig. S6. Axonal termini morphology of the giant fiber (GF) neurons. (A) Schematic of the GF system in the adult Drosophila central nervous system adopted from Allen et al. (1998). *J. Comp. Neurol.***397**, 519-531. The pair of GF neurons, which are located in the central brain, each projects a prominent unbranched axon to the mesothoracic neuromere (T2), where it forms the characteristic terminal bend and synapses with other interneurons and motor neurons. The red dashed lines delineate the T2 neuromere region imaged in (B-G). (B-F) Montage images of projected confocal sections of the axonal termini of GF neurons, which are labeled by the membrane-bound mCD8-eGFP reporter, driven by the GF-specific A307-Gal4 line. (B,C) Representative images of 8-day-old wild-type (WT) (B) and *dhtt-ko* (C) flies. Notice the similar morphology and comparable

signal intensity of the GF axonal termini in WT and *dhtt-ko* mutants. Bar, 47.62 Âµm. (D-F) Representative images of 40-day-old wild-type (WT) (D) and *dhtt-ko* (F) flies, which are co-stained with anti-Elav antibody (red) to reveal the nuclei of neuronal cells that are located in the same confocal plain. Notice that the overall morphology of the GF axonal termini are similar, but their signal intensity (i.e. the mCD8-eGFP reporter) is much weaker in the *dhtt-ko* mutant than in the age-matched WT control. (E,G) High-magnification views of the terminal bends highlighted in (D) and (F), respectively. Bar, 15Âµm (D,F).

Fig. S7 and S8. Loss of endogenous *dhtt* affects the pathogenesis of HD flies. Confocal images of five sets of 5-day-old HD-Q93 (Fig. S7) and HD-Q93; *dhtt-ko* (Fig. S8) brains, showing the distribution of neuronal cells (anti-Elav, green) and MB neurons (anti-FasII, white) in brains. Notice the enlarged regions devoid of neuronal cells in the HD-Q93; *dhtt-ko* brains (Fig. S8) as compared with the corresponding regions in the HD-Q93 brains (Fig. S7, highlighted with white dashed lines). The MB in HD-Q93; *dhtt-ko* brains (B1-B5) in Figs S7 and S8 is less well organized and shows weaker signal intensity. As cells in the fly brain are mainly localized at its surface, for better visualization of the distribution pattern of the cells, the anterior (A1-A5) and posterior (C1-C5) halves of the brains are projected separately. The arrows in (C) in Fig. S8 indicate the area lacking neuronal cells at the posterior of the brain. Note that for the bottom two brains in Fig. S7, part of the optic lobe region was removed during sample preparation. Bars, 100 Âµm.

Movie S1. *dhtt* mutants have normal mobility at a young age. Climbing assay of wild-type (WT) and *dhtt* mutant flies at 7 days of age. *dhtt* mutants at this age showed similar climbing ability to wild-type controls. The wild-type vial is labeled as â€˜WTâ€™ on the tape (and as â€˜1â€™ on the vial).The *dhtt* mutant vial is labeled as â€˜dhtt-â€˜ on the tape (and as â€˜4â€™ on the vial).

Movie S2. *dhtt* mutants have significantly reduced mobility as they age. Climbing assay of wild-type (WT) and *dhtt* mutant flies at 30 or 36 days of age. The ages and genotypes of these flies are labeled on the vials. Compared with the 36-day-old wild-type controls (WT, left vial), *dhtt* mutants displayed an obvious decreased climbing ability at 30 days of age (*dhtt-ko*, middle vial). This mobility defect became even more pronounced in 36-day-old *dhtt* mutants (right vial).

Movie S3. The mobility defect of *dhtt-ko* mutants can be rescued by ectopically expressed *dhtt* from a mini-*dhtt* transgene. Climbing assays involving 36-day-old flies with the genotypes of: wild type (WT, left vial), *dhtt-ko* mutants (*dhtt-ko*, middle vial), and *dhtt-ko* mutants with the *dhtt* genomic minigene rescue construct (Rescued, right vial). In the presence of a wild-type mini *dhtt*-transgene, the mobility defect of *dhtt-ko* mutants was rescued, demonstrating that the observed mobility defect in *dhtt-ko* mutants is indeed because of the loss of normal *dhtt* function. The age and abbreviated genotypes of these flies were also marked on the tape at the bottom of the vials. Genotypes (from left to right): (1) WT:w^{1118}; (2) *dhtt-ko*: w^{1118}*; dhtt-ko*/*dhtt-ko*; and (3) Rescued: w^{1118}*; Pmini-dhtt*/+*; dhtt-ko*/*dhtt-ko.*

Movies S4-S8. Loss of endogenous *dhtt* accelerates the mobility phenotypes associated with the fly model of HD. In Movies S4-S8, flies of the same age were tested at different time points to examine their age-dependent mobility. Abbreviated genotypes of these flies were labeled on the tape at the bottom of the vials. The detailed genotypes of these flies are: (1) WT: wild-type control flies. Genotype: w^{1118}/ w^{1118}; +/+; +/+; (2) dhtt^{-}/dhtt^{-}: homozygous *dhtt-ko* mutants alone. Genotype: w^{1118}/ w^{1118}*; dhtt-ko*/*dhtt-ko*; (3) Elav-Gal4/uas-HD93: HD-Q93 flies, an established fly HD model in which the human huntingtin exon 1 containing a 93 glutamine repeat (Httexon1-Q93) was expressed specifically in all neurons under the control of the *elav*-Gal4 driver Steffan et al. (2001). *Nature***413**, 739-743. Genotype: *elav-Gal4*/w^{1118}; *UAS-Httexon1-Q93*/+; +/+; and (4) Elav-Gal4/uas-HD93; dhtt^{-}/dhtt^{-}: HD-Q93; *dhtt-ko* flies (HD-Q93 in a *dhtt-ko* mutant background). Genotype: *elav-Gal4*/w^{1118}; *UAS-Httexon1-Q93*/+; *dhtt-ko*/*dhtt-ko.*

Movie S4. Climbing assay for 5-day-old flies. Notice that at day 5, the HD-Q93; *dhtt-ko* flies (in the second vial from the left) already displayed detectable mobility phenotypes, showing a slightly delayed response at the beginning of the climbing assay, a high frequency of falling while climbing and, at the end of the assay, more flies remained at the bottom of the vial.

Movie S5. Climbing assay for the 7-day-old flies. Climbing assay for the same batch of flies at day 7. Notice that by day 7, the same group of HD-Q93; *dhtt-ko* flies (vial 2) showed a more pronounced mobility defect, because they climbed at a slower speed than HD-Q93 flies (vial 3) and other age-matched controls. They were

also slower than at 5 days of age, showing more frequent falling during climbing. In addition, several HD-Q93; *dhtt-ko* flies failed to climb to the top of the vial at all and remained at the bottom during the assay. It is also noticeable that, at this age, the HD-Q93 flies (vial 3) also showed a slightly increased frequency of falling during climbing.

Movie S6. Close-up view of a climbing assay for 7-day-old flies. Close-up view of 7-day-old flies in vial 2 (left vial, HD-Q93; *dhtt-ko* flies) and vial 3 (right vial, HD-Q93 flies) during a climbing assay. Notice that more flies in the left vial (HD-Q93; *dhtt-ko* flies) displayed obvious mobility defects at this age: some flies could barely move or climb, several flies constantly fell while trying to climb, and more flies remained at the bottom of the vial at the end of the assay. Note that, by this time, some HD-Q93 flies (right vial) also started to show a similar, although less severe, mobility defect.

Movie S7. Climbing assay for 11-day-old flies. Climbing assay for the same batch of flies at day 11. By this time, most of the HD-Q93; *dhtt-ko* flies (vial 2) were already dead and had been removed from the vial. The few remaining flies showed a more reduced mobility, had difficulty moving or climbing, and fell during attempted climbs. It is also noticeable that by this age, the mobility defect of the HD-Q93 flies (vial 3) also became more pronounced (compared with in Movies S4-S6), as more flies had difficulty in successfully climbing to the top, and some became hyperactive.

Movie S8. Close-up view of the 11-day-old flies. Close-up view of the 11-day-old flies in vial 2 (left vial, HD-Q93; *dhtt-ko* flies) and vial 3 (right vial, HD-Q93 flies) during a climbing assay. Notice that most of the remaining HD-Q93; *dhtt-ko* flies (left vial) had difficulty walking, whereas those that could move showed uncoordinated walking. At this time, the HD-Q93 flies also displayed a high frequency of falling during climbing. Some HD-Q93 flies appeared to become hyperactive, showing uncontrolled jumping and wing beating. Notice that during the latter part of the movie, two flies at the right corner of the vial showed uncontrollable, excessive wing beating after they fell to the bottom of the vial.