Tissue-specific downregulation of *EDTP* removes polyglutamine protein aggregates and extends lifespan in *Drosophila*

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ABSTRACT

Drosophila egg-derived tyrosine phosphatase (EDTP, also called JUMPY) is a lipid phosphatase essential in the oogenesis and muscle function in the adult stage. Although mammalian JUMPY negatively regulates autophagy, loss-of-JUMPY causes muscle dysfunction and is associated with a rare genetic disorder centronuclear myopathy. Here we show that tissue-specific downregulation of EDTP in *Drosophila* non-muscle tissues, particularly glial cells, completely removes polyglutamine (polyQ) protein aggregates and improves survivor. Additionally, tissue-specific downregulation of EDTP in glial cells or motoneurons extends lifespan. We demonstrate an approach to fine-tune the expression of a disease-associated gene *EDTP* for the removal of polyQ protein aggregate and lifespan extension in *Drosophila*.

Introduction

Egg-derived tyrosine phosphatase (EDTP, also called JUMPY) is a lipid phosphatase that removes 3-position phosphate at the inositol ring of phosphatidylinositol 3-phosphate (PtdIns3P) and phosphatidylinositol (3, 5)-bi-phosphate (PtdIns(3,5)P_2)[1]. EDTP has opposite function to Vps34, a sole class III phosphoinositide 3-kinase[2,3], in the regulation of PtdIns3P pool.

The most interesting characteristics of EDTP expression in *Drosophila* is that there are two peaks, one at the stages of oogenesis[4,5], and the other at adult stage[6]. The transcription of *mJUMPY*, a mouse homolog to *EDTP*, also shows a peak at day five of differentiation, followed by a decline in C2C12 myoblasts[1]. In addition, human JUMPY is detectable in all tested tissues at the age of 19 - 69[1]. Another expression characteristic is that, human *JUMPY* is ubiquitously expressed and abundant in skeletal muscle[1]. Such an expression pattern is highly coincident with its opposite functioning gene *Vps34*[7]. The decline of EDTP between early embryogenesis and young adult stage is accompanied with *Drosophila* metamorphosis, a process requiring extensive autophagy and apoptosis for histolysis[8]. These observations suggest a role for EDTP in the regulation of autophagy. This is indeed supported by the findings that PtdIns3P stimulates autophagy in human HT-29 cells[9], that mJUMPY negatively controls autophagosome formation and maturation in mammalian cells[10], and that an EDTP/JUMPY inhibitor, AUTEN-99, activates autophagy in human cell lines and mouse tissues[11].

Despite the negative regulation of autophagy, *Drosophila* null *EDTP* mutant is lethal at stages of embryo or first instar, and germline clones with null *EDTP* allele fail to produce mature oocytes[5]. Homozygous flies carrying a hypomorphic allele of *EDTP* are short-lived with impaired motor functions and reduced fecundity[12]. Additionally, the muscles of mJUMPY-deficiency mouse have decreased force production, prolonged relaxation and exacerbated fatigue[13]. Human JUMPY missense variant (R336Q) has a link to the centronuclear myopathy, a rare genetic disorder with muscle weakness and wasting[1]. Therefore, the function of autophagy initiation is likely overwhelmed by the lethality or disease-causing effects of loss-of-JUMPY ubiquitously or in the skeletal muscles.

There are advantages of autophagy in degrading and recycling disrupted organelles, long-lived proteins and denatured protein aggregates[9,14]. A strategy to maximize the potentially beneficial effects of EDTP is to manipulate the expression in the tissues favored for improved survivor while remaining EDTP unaffected in the muscles. This seems to be feasible by using *Drosophila* Gal4/UAS expression system[15]. We therefore hypothesized that selective downregulation of EDTP in the central nervous system removes protein aggregates and extends lifespan in *Drosophila*.

In the current study, we performed several sets of experiments to examine the effects of selective downregulation of EDTP on the removal of protein aggregates and lifespan extension in *Drosophila*. We first demonstrate that heterozygous EDTP mutant improves survivor to prolonged anoxia exposure, a stress condition inducing autophagy[16]. We next show that RNAi knockdown of EDTP in glial cells removes polyglutamine (polyQ) protein aggregates. Finally, RNAi knockdown of EDTP in glial cells or motoneurons extends lifespan in *Drosophila*.

Results

Improved survival to prolonged anoxia in *EDTP* mutant

Heterozygous flies of DJ694, an *EDTP* mutant[6,12], were exposed to a prolonged anoxia of six hours at the ages of 7-8 days. DJ694/+ males showed overall improved survival (median 43 days, n = 167) compared with their sibling controls w^{1118} flies (median 8 days, n = 163) ($P = 0.0033$, Mantel-Cox test) (Fig. 1a). Female DJ694/+ flies also showed better survival (median 70.5 days, n = 150) than controls (median 18 days, n = 148) ($P = 0.0001$, Mantel-Cox test) (Fig. 1b). Without anoxia exposure, the survivorship were the same between DJ694/+ males (median 76 days, n = 102) and w^{1118} males (median 76 days, n = 96) ($P = 0.9452$, Mantel-Cox test) (Fig. 1c). The survivorship were also the same between DJ694/+ females (median 81 days, n = 107) and w^{1118} females (median 81 days, n = 97) ($P = 0.8688$, Mantel-Cox test) (Fig. 1d). Heterozygous *EDTP* mutant displayed improved survivor to a 6-h anoxia.

Polyglutamine protein aggregates expressed in glial cells

Extreme hypoxia induces autophagy in mammalian and human cells[16]. *Drosophila* EDTP might have functions similar to mammalian counterpart in the negative regulation of autophagy. To support this proposal, a *Drosophila* model with protein aggregates expressed in the central nervous system is desired. We directed the expression of a genetic construct, UAS-Httex1-Q72-eGFP[17], in the glial cells with repo-Gal4[18,19]. This UAS line has been successfully used in the expression of polyglutamine (polyQ) protein aggregates, each of which contains 72 tandem repeats of glutamine (Q72) in an abnormal huntingtin protein, in the compound eyes[17].

PolyQ protein aggregates were clearly observed in the brain of UAS-Httex1-Q72-eGFP/+;;repo-Gal4/+ fly (Fig. S1). There was no expression of protein aggregate in the brains of control flies (UAS-Httex1-Q72-eGFP/+ and repo-Gal4/+). Expression of heat shock protein 70[20], a soluble molecular chaperone, did not form protein aggregates in glial cells in repo-Gal4/UAS-hsp70-myc fly. Also, expression of mCD8-GFP, a membrane-fused protein, resulted in no protein aggregates in glial cells in repo-Gal4/20×UAS-IVS-mCD8::GFP fly. The polyQ protein aggregates expressed in glial cells were used as an indicator to examine the effects of *EDTP* downregulation.

RNAi knockdown of *EDTP* in glial cells removed polyQ protein aggregates

PolyQ protein aggregates were observed in the brains of UAS-Httex1-Q72-eGFP/+;;repo-Gal4/+ flies. At day 1, 10 and 19 the protein aggregates were seen all over the brain. The large protein plaques were observed to be located in the surface, median septum and grooves between middle brain and optic lobes. The relatively small aggregates were diffused throughout the brain. Few flies survived more than 19 days (Fig. 2a).

RNAi knockdown of *EDTP* was performed by using an RANi line carrying a dsRNA construct on the second chromosome (BSC #41633). PolyQ protein aggregates were seen in the brains at day 1, 10 and 19 but markedly reduced in flies UAS-Httex1-Q72-eGFP/+; UAS-EDTP-RNAi/+; repo-Gal4/+ compared with the controls without RNAi. Furthermore, UAS-Httex1-Q72-eGFP/+; UAS-EDTP-RNAi/+; repo-Gal4/+ flies lived up to 38 days, which were a 2-fold survivor of UAS-Httex1-Q72-eGFP/+;;repo-Gal4/+ (Fig. 2b).

Most strikingly, polyQ protein aggregates were completely removed at day 30 and 38 with the presence of EDTP-RNAi in UAS-Httex1-Q72-eGFP/+; UAS-EDTP-RNAi/+; repo-Gal4/+ flies (Fig. 2b).

In order to support the effects of RNAi knockdown of *EDTP*, another independent RNAi line (on the third chromosome, BSC #36917) was used. Flies UAS-Httex1-Q72-eGFP/+;;repo-Gal4/UAS-EDTP-RNAi lived up to 38 days. PolyQ protein aggregates were observed at day 1, 10, 19 and 38 (Fig. S2). Therefore, the improved survivor was observed in UAS-Httex1-Q72-eGFP/+;;repo-Gal4/UAS-EDTP-RNAi compared with flies without RNAi, although polyQ protein aggregates were present throughout the life. These data supported the observation that *EDTP* downregulation improved the survivor to polyQ protein aggregates.

Two RNAi were used together with an expectation to achieve a better removal of polyQ protein aggregates. Flies UAS-Httex1-Q72-eGFP/+;UAS-EDTP-RNAi/+;repo-Gal4/UAS-EDTP-RNAi lived up to 38 days. PolyQ protein aggregates were seen at day 1, 10, 19 and 38 (Fig. S2). There were few flies lived greater than 38 days, and polyQ protein aggregates in the brains were observed at all tested ages. These findings were similar to the observations using a single RNAi on the third chromosome, and did not reproduce the effect of complete removal of protein aggregates by the RNAi on the second chromosome. Therefore, double RNAi to *EDTP* was unable to confer a better removal of polyQ protein aggregates than single RNAi.

RNAi knockdown of *EDTP* in glial cells improved the survivor to polyQ protein aggregates

We examined the survivor of flies to polyQ protein aggregates expressed in glial cells. The survivor of UAS-Httex1-Q72-eGFP/+;;repo-Gal4/+ flies (median 15 days, n = 44) was remarkably shorter than repo-Gal4/+ flies (median 76 days, n = 37) ($P < 0.0001$, Mantel-Cox test) or UAS-Httex1-Q72-eGFP/+ flies (median 84 days, n = 62) ($P < 0.0001$, Mantel-Cox test)

(Fig. 3a). All UAS-Httex1-Q72-eGFP/+;;repo-Gal4/+ flies died out around 20 days. This was consistent with the imaging observations that few flies of UAS-Httex1-Q72-eGFP/+;;repo-Gal4/+ were available with ages greater than 19 days.

Expression of polyQ protein aggregates and simultaneous RNAi knockdown of *EDTP* in glial cells were performed. The survivor of UAS-Httex1-Q72-eGFP/+;; repo-Gal4/UAS-EDTP-RNAi (median 25 days, n = 50) was longer than UAS-Httex1-Q72-eGFP/+;;repo-Gal4/+ flies (median 18 days, n = 84) ($P < 0.0001$, Mantel-Cox test). The survivor of UAS-Httex1-Q72-eGFP/+; UAS-EDTP-RNAi/+; repo-Gal4/+ (median 27 days, n = 61) was also longer than UAS-Httex1-Q72-eGFP/+;;repo-Gal4/+ flies ($P < 0.0001$, Mantel-Cox test) (Fig. 3b). There was no statistical difference of survivor between UAS-Httex1-Q72-eGFP/+;; repo-Gal4/UAS-EDTP-RNAi and UAS-Httex1-Q72-eGFP/+; UAS-EDTP-RNAi/+; repo-Gal4/+. Therefore, RNAi knockdown of *EDTP* in glial cells improved the survivor to polyQ protein aggregates.

RNAi knockdown of *EDTP* in glial cells or motoneurons extended lifespan

A critical question is whether tissue-specific downregulation of *EDTP* extends lifespan.

The lifespan of repo-Gal4/UAS-EDTP-RNAi flies (median 100 days, n = 106) was longer than that of repo-Gal4/+ flies (median 59 days, n = 51) ($P < 0.0001$, Mantel-Cox test) or that of UAS-EDTP-RNAi/+ flies (median 83 days, n = 78) (Fig. 4a). Therefore, RNAi knockdown of *EDTP* in glial cells resulted in extended lifespan in *Drosophila*.

Two additional Gal4 drivers, motoneuron-specific D42-Gal4[21] and pan-neuronal driver elav-Gal4[22], were used for tissue-specific RNAi knockdown of *EDTP*. The lifespan of D42-Gal4/UAS-EDTP-RNAi flies (median 113 days, n = 132) was extended compared with D42-Gal4/+ flies (median 73 days, n = 137) ($P < 0.0001$, Mantel-Cox test) or UAS-EDTP-RNAi/+ flies (median 89 days, n = 65) ($P < 0.0001$, Mantel-Cox test) (Fig. 4b). There was no statistical difference of lifespan between elav-Gal4/+; UAS-EDTP-RNAi/+ flies (median 83 days, n = 93) and elav-Gal4/+ flies (median 88 days, n = 57) or UAS-EDTP-RNAi/+ flies (median 83 days, n = 78) (Fig. 4c). Thus, tissue-specific downregulation of *EDTP* in glial cells or motoneurons extended lifespan in *Drosophila*.

Discussion

Drosophila EDTP is essential in the regulation of oogenesis[5] as well as muscle performance at adult stage[12]. The mammalian homolog *mJUMPY* negatively regulates the initiation of autophagy[10]. However, loss-of-JUMPY is also associated with muscle dysfunction[13,23] and a rare genetic disease centronuclear myopathy[1]. Here we show that tissue-specific downregulation of *EDTP* in non-muscle cells, particularly glial cells, removes polyQ protein aggregate, and extends lifespan in *Drosophila*.

It is striking that polyQ protein aggregates are completely removed by downregulation of EDTP in glial cells. Translation of *repo* and repo-Gal4-driven mCD8-GFP expression in glial cells persist to at least 56 - 63 days[24]. The effects of EDTP downregulation must be strong enough to clear accumulated polyQ protein aggregates during young ages (i.e. < 30 days), while at the same time to sufficiently remove persistently synthesized protein aggregates. It has been shown that several proteins, including histone deacetylase (HDAC)[25], dHDJ1 (homolog o human HSP40)[26], dTPR2 (homolog to human tetratricopeptide repeat protein 2)[26], molecular chaperones HDJ1 and HDJ2[27–29], human Hsp70[30], yeast Hsp104[31], baculoviral antiapoptotic protein P35[32], and several modifiers for Spinocerebellar ataxia type 1 (SCA1)[33], are the molecular targets for suppressing the expression of polyQ protein aggregates. However, there is previously no evidence of complete removal of polyQ protein aggregates by manipulating the expression of these proteins. Therefore, we have identified a novel molecular target sufficient for the complete removal of persistent expression of polyQ protein aggregates in glial cells.

In addition to the removal of polyQ protein aggregates, lifespan extension by downregulation of *EDTP* in glial cells firmly supports the hypothesis that tissue-specific manipulation of EDTP expression circumvents the mutation-related muscle phenotypes, shifting the overall effect to lifespan extension. *Drosophila* heterozygous *EDTP* mutant appears to have unaffected muscle function and lifespan[12]. This suggests that one genomic copy of *EDTP* is sufficient to produce normal level of EDTP in muscles. However, heterozygous *EDTP* mutant might have reduced EDTP in non-muscle tissues, which would affect the balance of PtdIns3P and promote autophagy. This is supported by the findings that heterozygous *EDTP* flies have improved survivor to prolonged anoxia, a condition inducing autophagy in mammalian and human cells[16]. Through tissue-specific downregulation of EDTP, we also find that motoneurons are another target tissue favored for lifespan extension. A study has shown that motoneuronal overexpression of a human CuZn superoxide dismutase (SOD1) extends *Drosophila* lifespan[21]. By targeting all neurons in the central nervous system for EDTP downregulation, we observe no beneficial effect in lifespan extension. This would be because of possibly low expression of EDTP in neurons, or these cells have developed processes independent of EDTP for self clearance/disposal of protein aggregates.

Two UAS-RNAi lines have different effects in the removal of polyQ protein aggregates. A combination of two RNAi is unable to produce better effect than a single RNAi. Each RNAi line carries a short hairpin RNA (shRNA) targeting a unique exon of *EDTP*. The shRNAs carried in RNAi lines have respectively potential off-targets. The shRNA in RNAi line giving complete removal of polyQ protein aggregates has a potential target gene *Traf-like* (CG4394), encoding TNF-receptor-associated factor-like. Traf-like is inferred to have ubiquitin-protein transferase activity (http://flybase.org/reports/FBgn0030748.html),

which might be harnessed by downregulation to assist in the removal of polyQ protein aggregates. The shRNA in second RNAi line has two potential off targets, *CG5142* and *ORCT*. The product of *CG5142* has a tetratricopeptide repeat domain (http://flybase.org/reports/FBgn0032470.html). A *Drosophila* tetratricopeptide repeat protein dTPR2 has been shown to suppress polyQ protein aggregates in the compound eyes[26]. *ORCT* encodes an organic cation transporter (http://flybase.org/reports/FBgn0019952.html). How ORCT affects the removal of polyQ protein aggregates is unclear. Potential off-targets might modify the effects of EDTP downregulation, giving complete or incomplete removal of polyQ protein aggregates. Both RNAi lines have the effects of survivor improvement to polyQ protein aggregates to a similar level, indicating that there is little relation between complete removal of polyQ protein aggregates and survivor improvement.

EDTP/JUMPY represents a novel phosphoinositide phosphatase that belongs to myotubularian (MTM) family[1]. EDTP/JUMPY hydrolyzes 3-position phosphate from PtdIns3P and PtdIns(3,5)P_2. Mouse JUMPY negatively controls PtdIns3P levels[10]. PtdInd3P regulates many aspects of autophagy. In the early events of autophagy, PtdInd3P recruits effectors and initiates the formation of autophagosome[34]. Later, PtdInd3P attracts MTM3 for its turnover and promotes autophagosome maturation into autolysosome[35]. Thus, JUMPY is actively involved in the regulation of autophagy. In addition, *JUMPY* mutation causes the accumulation of PtdIns(3,5)P_2 in skeletal muscles. Accumulated PtdIns(3,5)P_2 binds directly to ryanodine receptors, RyR1 and RyR2, in sarcoplasmic reticulum and increases intracellular Ca^{2+} in both skeletal and cardiac muscle[13,23], resulting in muscle dysfunction[13] and altered cardiac contractility[23].

Abundant expression of EDTP/JUMPY in muscles could be a barrier for utilizing the effect of negative regulation of autophagy through ubiquitous downregulation. Tissue-specific manipulation of EDTP expression by targeting non-muscle tissues would circumvent this problem. The advantage of tissue-specific downregulation of EDTP is to avoid or minimize the *EDTP*/*JUMPY*-mutation-associated muscle phenotypes. Targeting glial cells provides an additional advantage. Restricted downregulation of EDTP in glial cells causes no/minor disturbance to maternally derived EDTP expression in the development of oocyte. Transcription of *repo*, which is trapped for Gal4 expression in repo-Gal4 fly, first appears to be highly restricted in glioblasts in stage 9 embryos[18]. *EDTP* mRNA, however, is detected uniformly in the cytoplasm of eggs at stage 1, 5 and 11 but mostly disappears at stage 15 and after[5]. There is little spatial and temporal overlap of transcription between *repo* and *EDTP* during oogenesis. Therefore, glial cell-specific downregulation of EDTP occurs mostly at adult stage, during which EDTP reappears around day 7, peaks at day 20-30 and decreases after in whole-fly preparations[6].

In conclusion, we demonstrate an approach to fine-tune the expression of a disease-associate gene *EDTP*/*JUMPY* for the removal of polyQ protein aggregates and lifespan extension in *Drosophila*.

Methods

Flies

Fly strains and their sources are: DJ694[6], repo-Gal4 (Bloomington Stock Center (BSC) #7415); D42-Gal4 (BSC #8816); elav-Gal4 (BSC #8765); UAS-EDTP-RNAi (with RNAi construct on the second chromosome, BSC #41633); UAS-EDTP-RNAi (with RNAi construct on the third chromosome, BSC #36917); UAS-Httex1-Q72-eGFP[17]; UAS-hsp70-myc[20]; 20×UAS-IVS-mCD8::GFP (BSC #32914) and w1118. We recombined several transgenes and generated these flies: UAS-EDTP-RNAi (on II); UAS-EDTP-RNAi (on III) and UAS-Httex1-Q72-eGFP;;repo-Gal4. Flies were maintained with standard medium (cornmeal, agar, molasses and yeast) at 21-23 °C in a 12/12 h light/dark condition. Male flies were used for the experiments, unless otherwise stated.

Heterozygous DJ694 flies and their sibling controls were prepared by two consecutive crosses between homozygous DJ694 and w1118. We chose virgin female progenies from first mating, and crossed them to w1118 males. Flies carrying EDTP mutation (red eyed) and their siblings (white eyed) were collected for the tests of survivor to prolonged anoxia.

Survivor to prolonged anoxia

Flies were exposed to a 6-h anoxia (generated by pure nitrogen gas) at the ages of 7-8 days. Dead flies were scored daily during the first week of recovery. After then dead flies were counted twice a week until all the flies were counted. Throughout the survivor experiments flies were transferred to fresh food vials twice a week.

Immunohistochemistry

Immunohistochemistry was performed by following two similar protocols[36,37]. Briefly, dissected brains were fixed in freshly prepared 4% paraformaldehyde for 1 h. After three washes with PAT (PBS with 0.5 % BSA and 0.5 % Triton X-100), tissues were incubated with saturation buffer (10 % goat serum in PAT) for 1 h at room temperature. Brain tissues were then incubated with primary antibody at 4 °C for 1-2 days with gentle rotation. Following three washes, tissues were incubated with appropriate secondary antibody. Incubation with secondary antibody was performed at 4 °C in a dark room for 1-2 days with gentle rotation. Flies and their specific antibodies were: (1) repo-Gal4/UAS-hsp70-myc flies, primary antibody: rabbit anti-cmyc (A00173, GenScript) at 1:50, secondary antibody: DyLight goat anti-rabbit IgG (111-485-144, Jackson ImmunoResearch) at 1:500. (2)

repo-Gal4/20×UAS-IVS-mCD8::GFP flies, primary antibody: mouse anti-GFP supernatant (12A6, DSHB) at 1:20, secondary antibody: Alexa Fluor 488 conjugated goat anti-mouse IgG (115-545-003, Jackson ImmunoResearch) at 1:500. After three washes tissues were suspended in 200 μl SlowFade Gold antifade reagent (S36938, Life Technologies) and mounted on slides for microscopy. Image stacks were taken using a Carl Zeiss LSM 710NLO laser scanning confocal/multiphoton microscope (Carl Zeiss), and processed with ImageJ (NIH).

PolyQ-expressing flies were imaged without immunohistochemistry. Briefly, fly brains with polyQ expression were dissected in Schneider's insect medium (S0146, Sigma-Aldrich) containing 0.5 % Triton-X-100, mounted on a slide immediately with a small drop of SlowFade Gold antifade reagent (S36938, Life Technologies), and imaged within an hour. The iamges of Z stacks spaced by 5-7 μm throughout the thickness of tissue were taken.

Lifespan experiments

Flies were collected at 0-2 days since emergence at a density of 20-25 flies per vial. They were transferred into fresh food vials twice a week. Dead flies were scored during transfer until all the flies were scored.

Statistics

Mantel-Cox test was performed for survivor analysis. Statistics were performed using GraphPad Prism5 software. A $P < 0.05$ was considered significant difference.

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Acknowledgements

This work was funded by Natural Sciences and Engineering Research Council of Canada (NSERC) grant (RGPIN 40930-09) to R.M.R. The authors declare no conflict of interest.

Author contributions statement

C.X. contributed to experimental design; C.X. and S.Q. contributed to data collection, analysis and manuscript preparation; R.M.R. contributed to funding support and manuscript editing; L.S contributed to research materials, comments and discussion. All authors reviewed the manuscript.

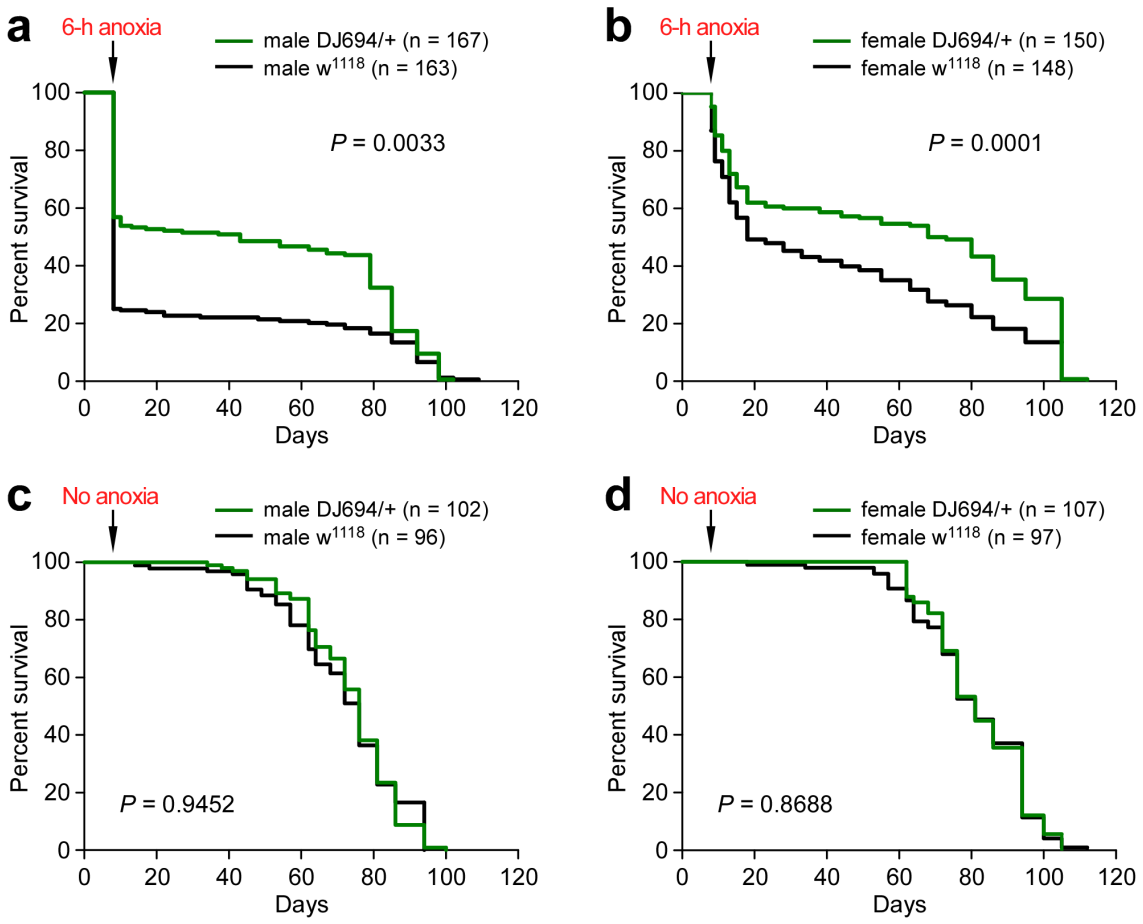

Figure 1. Improved survival to prolonged anoxia in *EDTP* mutant. (**a**) Survival of male flies with an exposure to 6-h anoxia. Anoxia was applied to flies at the ages of 7-8 days. DJ694/+ and w^{1118} flies were siblings prepared by two consecutive crosses. Numbers of flies (n) were indicated. DJ694 is an *EDTP* mutant line[6]. (**b**) Survival of female flies with an exposure to 6-h anoxia. Flies were prepared in a procedure similar to males. (**c**) Survival of male flies without anoxia exposure. (**d**) Survival of female flies without anoxia exposure.

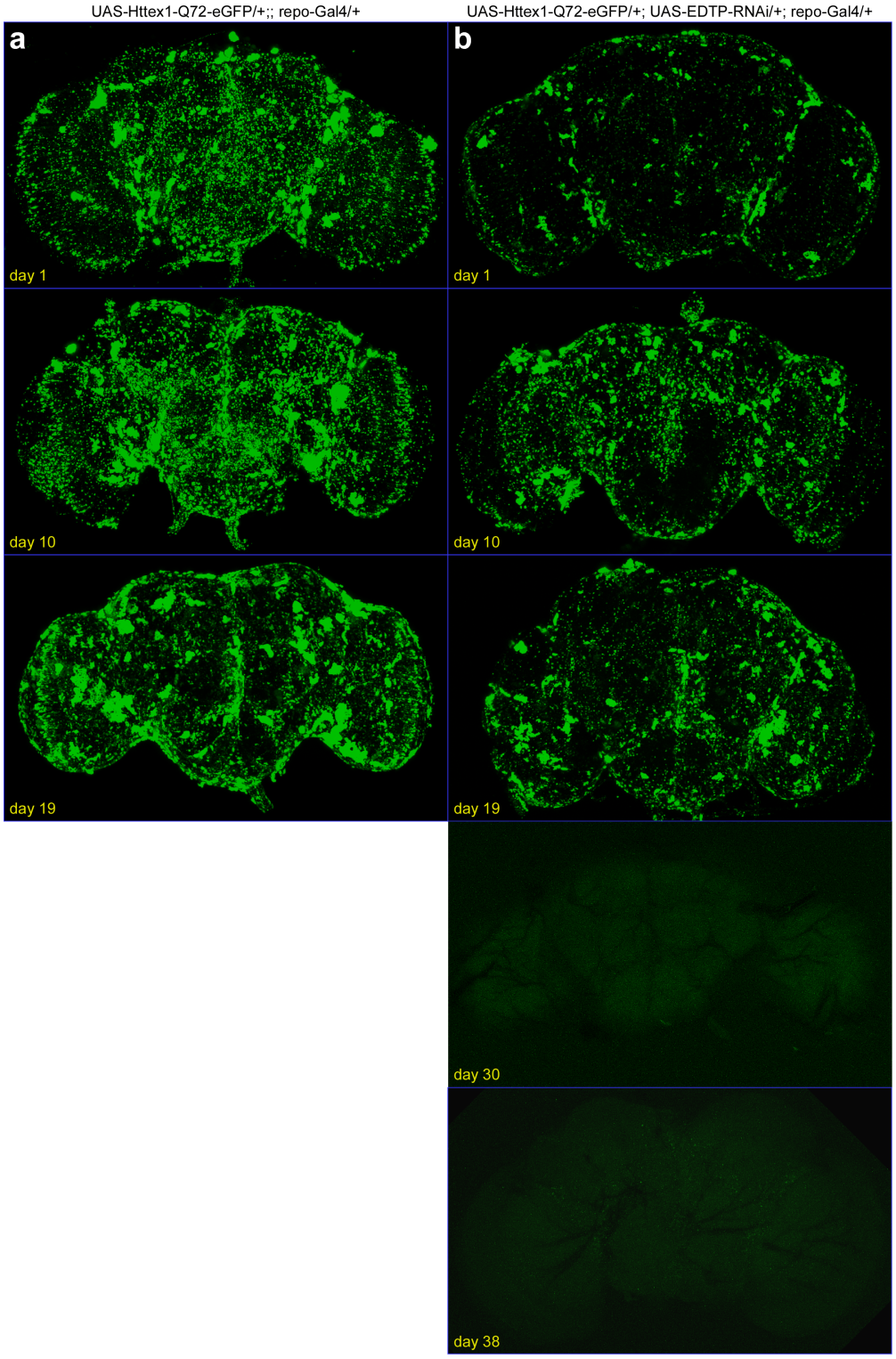

Figure 2. Removal of polyQ protein aggregates through *EDTP* downregulation. (**a**) Expression of polyQ protein aggregates in glial cells in UAS-Httex1-Q72-eGFP/+;;repo-Gal4/+ male flies. Shown are the brains at the ages of 1, 10 and 19 days. Few flies survive greater than 19 days. (**b**) Expression of polyQ protein aggregates in glial cells with RNAi knockdown of *EDTP*. Brains of UAS-Httex1-Q72-eGFP/+;UAS-EDTP-RNAi/+;repo-Gal4/+ male flies at day 1, 10, 19 and 38 are shown. At day 38, polyQ protein aggregates in the brain are completely removed. UAS-EDTP-RNAi line: BSC# 41633.

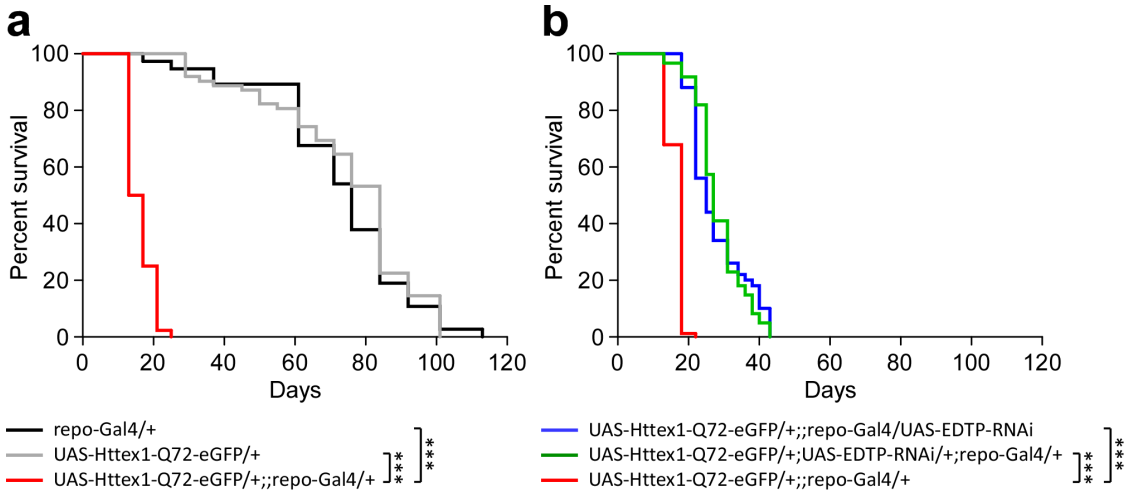

Figure 3. Improved survivor to polyQ protein aggregates by *EDTP* downregulation. (**a**) Expression of polyQ protein aggregates in glial cells shortened the lifespan. Survivor of UAS-Httex1-Q72-eGFP/+;;repo-Gal4/+ flies (red) was markedly decreased compared with repo-Gal4/+ (black) or UAS-Httex1-Q72-eGFP/+ (grey). ***, $P < 0.0001$, Mantel-Cox test. Male flies were examined. (**b**) *EDTP* downregulation in glial cells improved survivor to polyQ protein aggregates. Survivor of UAS-Httex1-Q72-eGFP/+;; repo-Gal4/UAS-EDTP-RNAi flies (blue) or UAS-Httex1-Q72-eGFP/+; UAS-EDTP-RNAi/+; repo-Gal4/+ flies (green) was improved compared with UAS-Httex1-Q72-eGFP/+;; repo-Gal4/+ flies (red). Experiments with two independent RNAi lines showed similar results. ***, $P < 0.0001$, Mantel-Cox test.

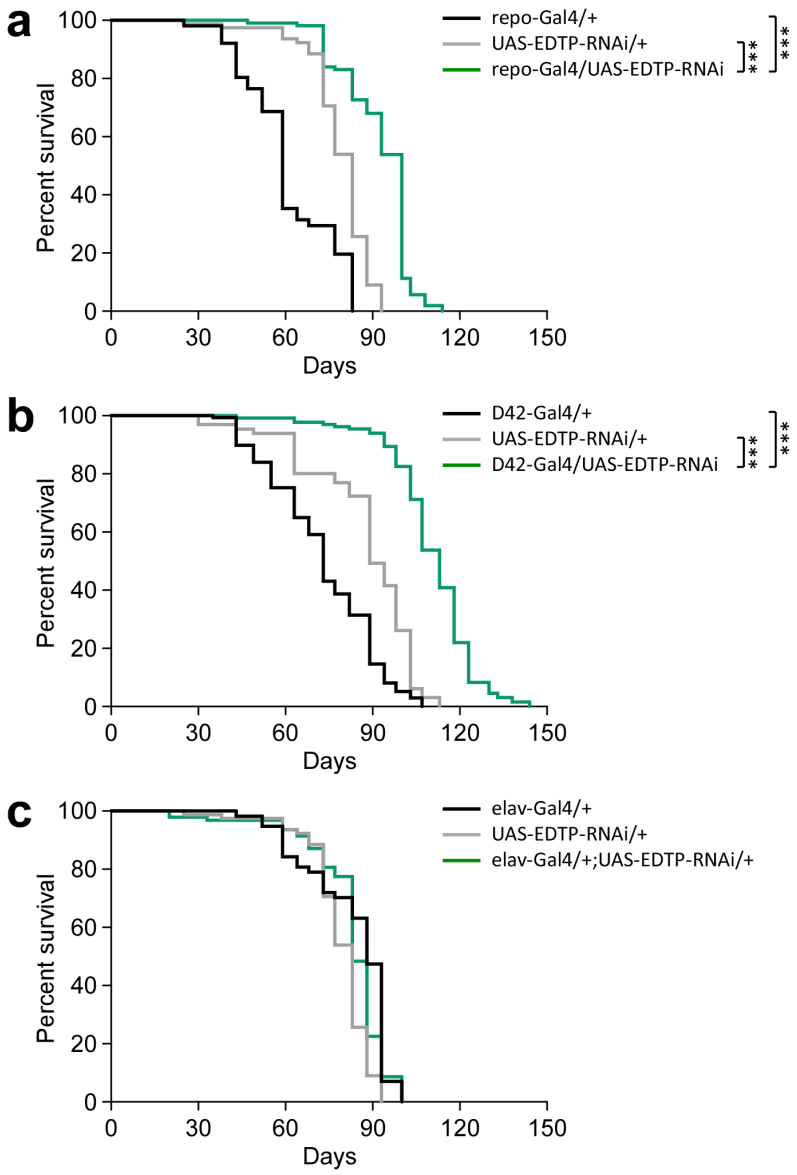

Figure 4. Tissue-specific downregulation of *EDTP* in glial cells or motoneurons extends lifespan. (**a**) Lifespan extension by *EDTP* downregulation in glial cells. Survivorship of repo-Gal4/UAS-EDTP-RNAi (green), repo-Gal4/+ (black) and UAS-EDTP-RNAi/+ (grey) were compared. An ENTP-RNAi line (on the third chromosome) was used. ***, $P < 0.0001$, Mantel-Cox test. (**b**) Lifespan extension by *EDTP* downregulation in motoneurons. Survivorship of D42-Gal4/UAS-EDTP-RNAi (green), D42-Gal4/+ (black) and UAS-EDTP-RNAi/+ (grey) were compared. D42-Gal4 is a motoneuronal driver[21]. ***, $P < 0.0001$, Mantel-Cox test. (**c**) Pan-neuronal downregulation of *EDTP* had no effect on lifespan. elav-Gal4 is a pan-neuronal driver[22].

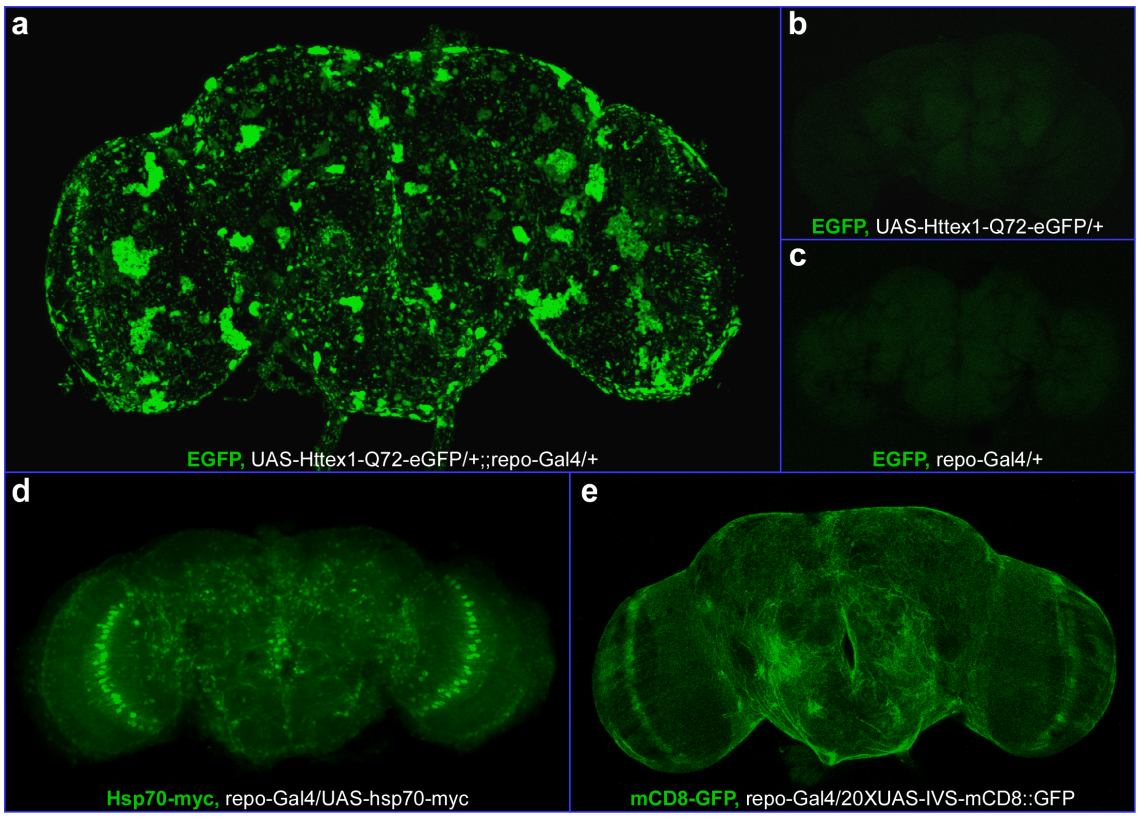

Figure S1. PolyQ protein aggregates expressed in glial cells. (**a**) PolyQ protein aggregates expressed in glial cells in UAS-Httex1-Q72-eGFP/+;;repo-Gal4/+ fly. Immunostaining of the target proteins (in green) and fly genotype (in white) are indicated. Large protein aggregates are seen located around the surface of the brain. (**b**) No detectable protein aggregates in UAS-Httex1-Q72-eGFP/+ fly. (**c**) No detectable protein aggregates in repo-Gal4/+ fly. (**d**) Expression of a soluble molecular chaperone (Hsp70-myc) in glial cells in repo-Gal4/UAS-hsp70-myc fly. Experiment was repeated here from a previous report[38] as a comparison for the expression of polyQ protein aggregates. (**e**) Expression of a membrane protein (mCD8-GFP) in glial cells in repo-Gal4/20$\times$UAS-IVS-mCD8::GFP fly. Male flies at 1-7 days were used for imaging.

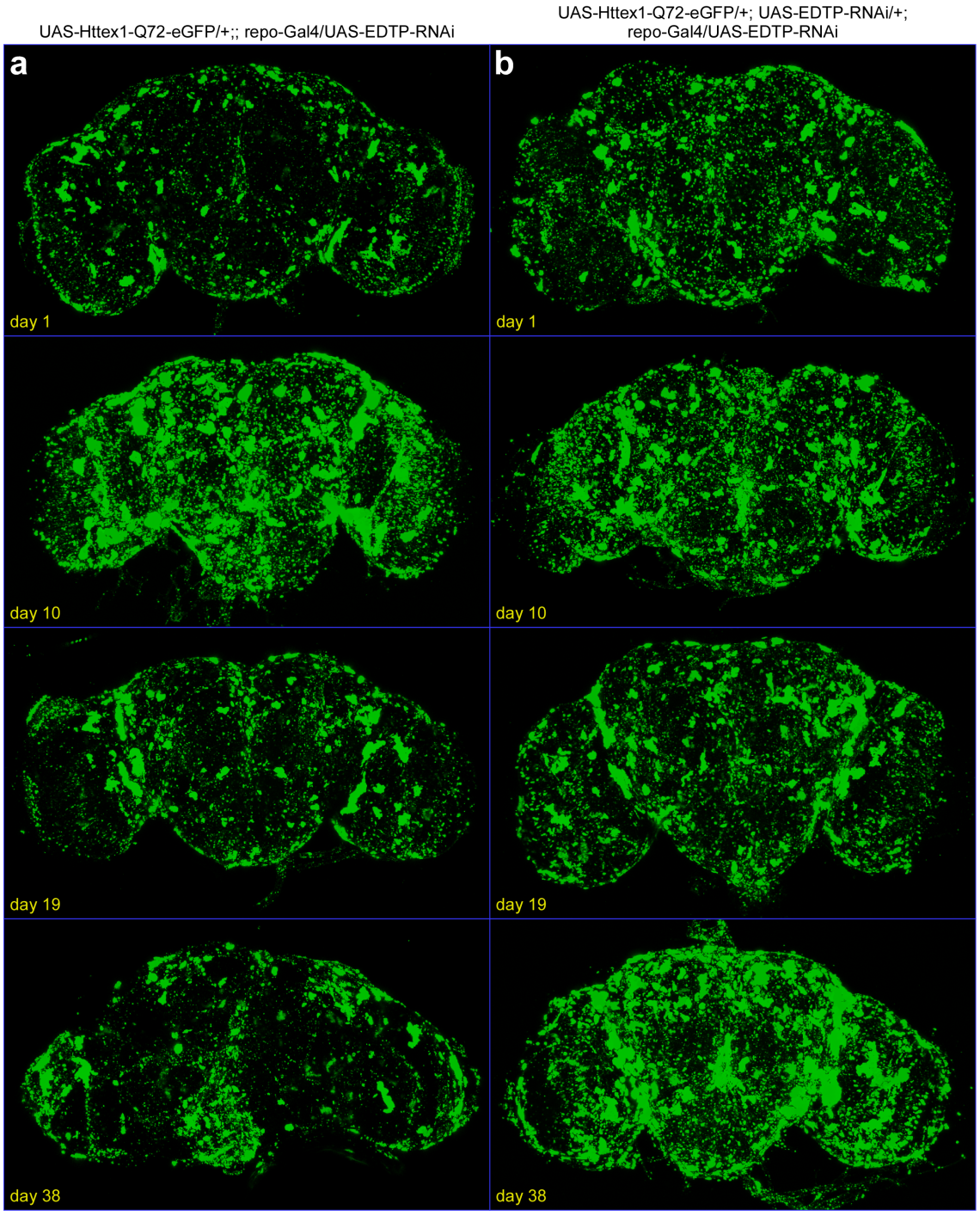

Figure S2. Expression of polyQ protein aggregates in glial cells with *EDTP* downregulation. (**a**) Expression of polyQ protein aggregates and simultaneous RNAi knockdown of *EDTP* in glial cells in UAS-Httex1-Q72-eGFP/+;; repo-Gal4/UAS-EDTP-RNAi flies. Shown are the brains of males at 1, 10, 19 and 38 days. Another UAS-EDTP-RNAi line (BSC# 36917) was used for these experiments. At day 38, polyQ protein aggregates are still present in the brain. (**b**) Expression of polyQ protein aggregates and *EDTP* knockdown by two RNAi in glial cells. Fly genotype: UAS-Httex1-Q72-eGFP/+; UAS-EDTP-RNAi/+; repo-Gal4/UAS-EDTP-RNAi. At day 38, polyQ protein aggregates are present in the brain. Male flies are used for imaging.