SUPPLEMENTAL INFORMATION

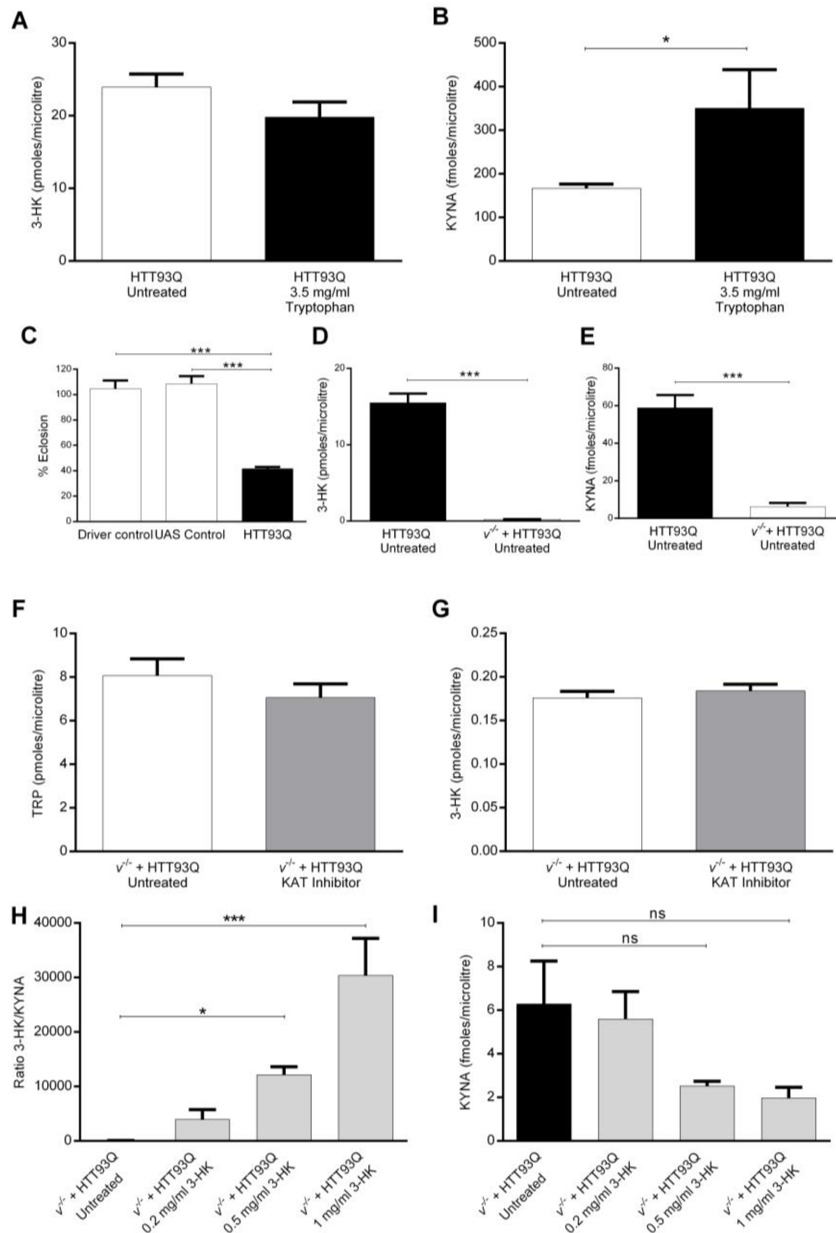

Figure S1. Levels of KP metabolites in HTT93Q and $v^{-/-}$ HTT93Q flies treated with TRP or 3-HK. (A) No significant difference in 3-HK levels is observed between untreated and TRP-treated Htt93Q flies. *n* = 5 - 6 per condition. (B) TRP fed Htt93Q flies display a higher level of KYNA compared to untreated controls. *n* = 5 - 6 per condition. * P < 0.05. (C) The frequency of eclosion in HD flies is significantly reduced compared to controls. Driver control flies: *n* = 1437; UAS control flies: *n* = 1066; HD flies; *n* = 1216. *** P < 0.001. $v^{-/-}$ HTT93Q flies show a decreased level of 3-HK (D) and KYNA (E). *n* = 5 per condition. *** P < 0.001. TRP (F) and 3-HK (G) levels are not affected by the administration of the KAT inhibitor AOAA (100 µM in the food). *n* = 5 per condition. (H) The 3-HK/KYNA ratio is increased in $v^{-/-}$ HTT93Q flies fed with 3-HK. *n* = 5 – 6 per condition, * P < 0.05 and *** P < 0.001. (I) No significant difference in KYNA levels is observed in $v^{-/-}$ HTT93Q flies treated with increasing concentrations of 3-HK. *n* = 5 - 6 per condition. Data are the mean ± SEM (one-way ANOVA with Newman-Keuls *post hoc* test).

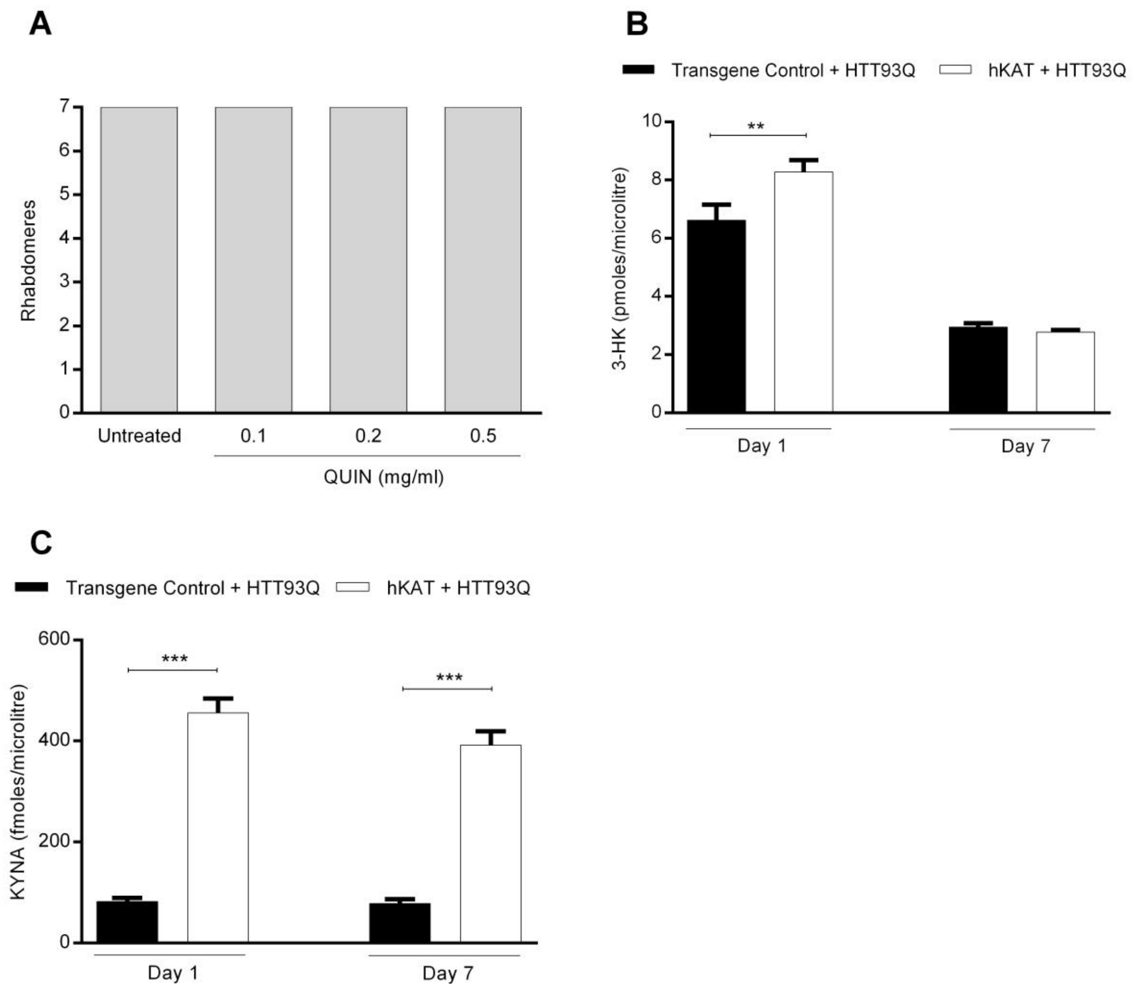

Figure S2. (A) QUIN feeding does not lead to neurodegeneration in wild-type flies. Wild-type flies fed with increasing concentrations of QUIN do not exhibit rhabdomere degeneration compared to untreated flies. n = 6 – 7 per condition. **(B, C) Levels of 3-HK and KYNA in flies overexpressing hKAT.** Days 1 and 7. n = 4 - 5 per condition. ** $P < 0.01$ and *** $P < 0.001$. Data are the mean ± SEM (one-way ANOVA with Newman-Keuls *post hoc* test).

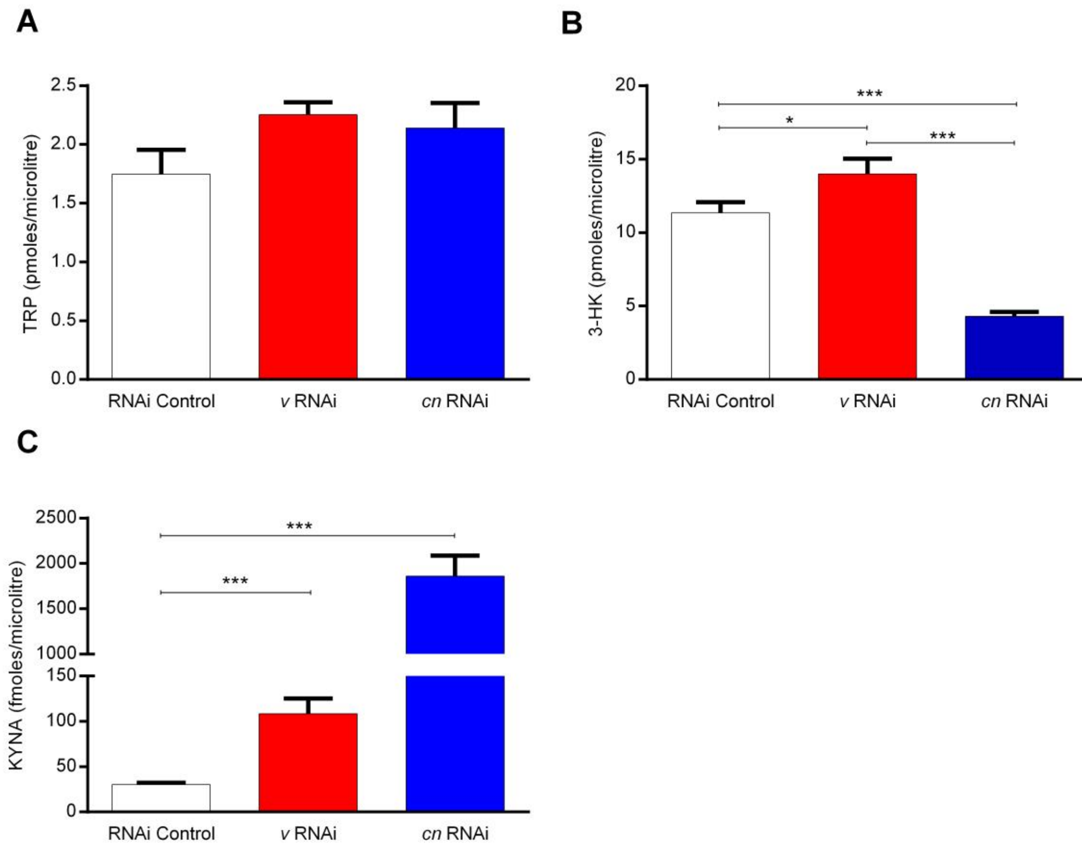

Figure S3. *v* and *cn* silencing alters levels of KP metabolites. (A) TRP levels are unaltered in flies down-regulating *v* and *cn* compared to the RNAi control. *n* = 5 per genotype. (B) The level of 3-HK is significantly increased in *v* RNAi and decreased in *cn* RNAi flies. *n* = 5 per genotype. * P < 0.05 and *** P < 0.001. (C) KYNA levels are increased in both *v* and *cn* RNAi lines compared to the control. *n* = 5 per genotype. *** P < 0.001. Data are the mean ± SEM (one-way ANOVA with Newman-Keuls *post hoc* test).

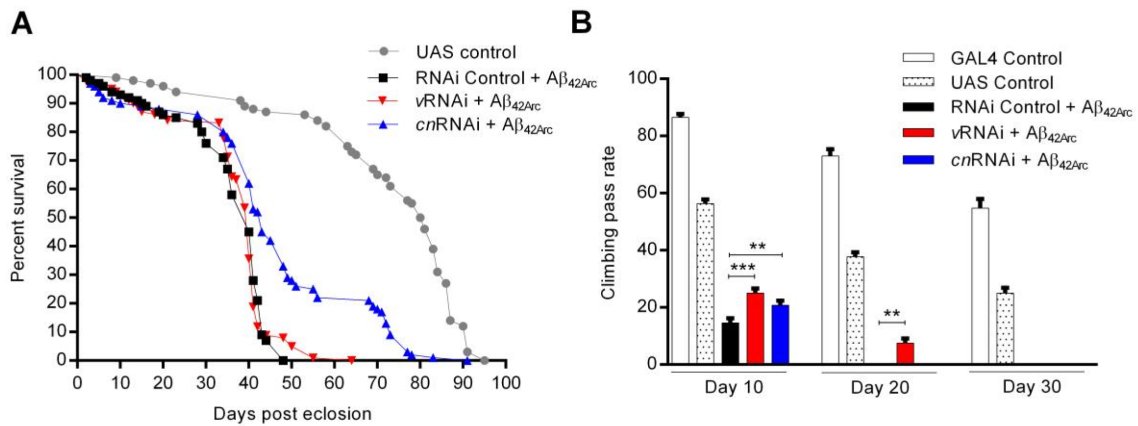

Figure S4. *v* and *cn* silencing in aβ_{42Arc}-expressing flies improves diseases-related phenotypes. (A) Survival curve of flies expressing Aβ42Arc pan-neuronally and down-regulating *v* and *cn*. The silencing of *cn* ameliorates decreased lifespan observed in flies. *n* = 100 per genotype. (B) Mean climbing pass rate at different post-eclosion ages of flies expressing Aβ_{42Arc} pan-neuronally. Aβ_{42} reduces climbing, which is significantly rescued by the downregulation of *v* or *cn* at day 10 post-eclosion. *n* = 50 - 60 per genotype and condition. ** P < 0.01 and *** P < 0.001. Data are the mean ± SEM (one-way ANOVA with Newman-Keuls *post hoc* test).

Materials and Methods

Pseudopupil analysis

Visible rhabdomeres were scored from 50-150 ommatidia per fly at day 0 or 7 post-eclosion using the scoring method published previously (1). Aged fly heads were fixed to slides using fingernail polish, and rhabdomeres were examined at 500 X magnification using a Nikon Optiphot-2 microscope (1).

Eclosion analysis

Eclosion in the fly lines was assessed as described previously (2). Briefly, males carrying the sex-linked *elav*GAL4 driver were crossed to virgin females homozygous for the *UAS-HTT93Q* transgene generating experimental females and control males in the F1 generation. Flies were allowed to lay eggs on vials with or without treatment, and the number of adult females and males emerging from the pupal case in each vial was counted every day for 9 days. Eclosion percent was determined by the following calculation: (number of female flies/number of male flies)*100.

Feeding experiments

Tryptophan (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in water and subsequently added to the standard food at several concentrations (0.4, 0.8, 1.7, 3.5, 7 and 10 mg/ml). 3-HK (Sigma-Aldrich) and QUIN (Sigma-Aldrich) were dissolved in water and then mixed with standard maize food at the concentrations of 1 mg/ml (3-HK) or 0.1, 0.2 and 0.5 mg/ml (QUIN). Crosses were set up on the supplemented media, and rhabdomeres were scored at day 0 (newly emerged adult flies). The non-specific KAT inhibitor aminooxyacetic acid (AOAA) - also referred to as O-(Carboxymethyl)hydroxylamine hemihydrochloride – from Sigma-Aldrich was dissolved in water whereas the TDO inhibitor 680C91 (Tocris Bioscience, Bristol, UK) was dissolved in dimethyl sulfoxide (DMSO; 0.001% final). Compounds were added to maize media at 100 µM (final concentration). Flies were introduced to the media upon eclosion, supplemented media were changed every day, and rhabdomeres were scored after 7 days of treatment.

Measurement of KP metabolites

Ten fly heads were homogenized by sonication in 100 µl of ultrapure water, and the homogenate was further diluted (1:10, v/v) in ultrapure water. One hundred µl of the preparation were then thoroughly mixed with 25 µl of 6% perchloric acid, and the precipitated proteins were removed by centrifugation (16,000 x g, 15 min). For the determination of KYNA, the resulting supernatant was further diluted 1:10 (v/v) with ultrapure water, and 20 µl were subjected to HPLC analysis. KYNA was isocratically eluted from a 3 µm C_{18} reverse-phase column (HR-80; 80 mm x 4.6 mm, ESA, Chelmsford, MA, USA), using a mobile phase containing 0.25 M zinc acetate, 50 mM sodium acetate and 3% acetonitrile (pH adjusted to 6.2 with glacial acetic acid) at a flow rate of 1 ml/min and was then quantitated fluorimetrically (excitation: 344 nm, emission: 398 nm; Perkin Elmer Series 200, Waltham, MA, USA). The retention time of KYNA was ~7 min. For the measurement of 3-HK, the deproteinized supernatant was either used directly or diluted (1:10 or 1:20, v/v) with ultrapure water, and 20 µl of the solution were applied to a 3 µm C_{18} reverse-phase column (HR-80; 80 mm x 4.6 mm, ESA), using a mobile phase containing 1.5% acetonitrile, 0.9% triethylamine, 0.59% phosphoric acid, 0.27 mM EDTA and 8.9 mM sodium heptane sulfonic acid. 3-HK was isocratically eluted at a flow rate of 0.5 ml/min and detected electrochemically using a HTEC 500 detector (Eicom Corp., San Diego, CA; oxidation potential: +0.5 V). The retention time of 3-HK was ~11 min.

For the measurement of TRP, the deproteinized supernatant was further diluted 1:100 (v/v) with ultrapure water, and 20 µL were subjected to HPLC analysis. TRP was isocratically eluted from a 3 µm C_{18} reverse phase HPLC column (150 mm × 4 mm; Dr. Maisch GmbH, Ammerbuch, Germany), using a mobile phase containing 50 mM sodium acetate and 5% acetonitrile (pH adjusted to 6.2 with glacial acetic acid) at a flow rate of 0.5 ml/min. Zinc acetate, 0.5 M (not pH adjusted), was delivered post column by a peristaltic pump (Dionex AXP, Thermo Fisher, Waltham, MA, USA) at a flow rate of 0.1 ml/min. In the eluate, TRP was detected fluorimetrically (excitation: 365 nm, emission: 385 nm; S200a fluorescence detector; Perkin Elmer). The retention time of TRP was ~7 min.

QUIN levels were quantified by GC/MS/MS. To this end, 10 fly heads were homogenized by sonication in 100 µl of ultrapure water, and the homogenate was further diluted (1:10, v/v)

in 0.1 % ascorbic acid. 50 µl of a solution containing internal standard ([$^2H_3$]QUIN) were added to 50 µl of the respective sample, and proteins were precipitated with 50 µL of acetone. After centrifugation (13,700 x g, 5 min), 50 µl of methanol:chloroform (20:50) were added to the supernatant, and the samples were centrifuged (13,700 x g, 10 min). The upper layer was added to a glass tube and evaporated to dryness (90 min). The samples were then derivatized with 120 µl of 2,2,3,3,3-pentafluoro-1-propanol and 130 µl of pentafluoropropionic anhydride at 75°C for 30 min, dried down again and reconstituted in 50 µl of ethyl acetate. One µl was injected into the gas chromatograph. GC/MS analysis was carried out with a 7890A GC coupled to a 7000B MS/MS (Agilent Technologies, Santa Clara, CA, USA), using electron capture negative chemical ionization (3).

Behavioral assays

For larval crawling experiments, crosses were set up on standard maize fruit fly food mixed with 0.05% Bromophenol Blue (FisherBiotech, Loughborough, UK) as described previously (4). Briefly, deeply blue colored third instar wandering larvae were washed in distilled water, and the distance covered by larvae in 2 min was manually tracked on a transparent paper placed on the top of the petri dish lid. The tracks were scanned, and the distance calculated using ImageJ (5).

Negative geotaxis assays were performed as previously described (4). Briefly, flies of the desired genotype were placed in a cylinder with a diameter of 2.3 cm and a total length of 18.4 cm. Before the experiment, flies were left to acclimatize for 1 min. For each trial, the tubes were tapped gently in order to gather the flies at the bottom. The flies were then allowed to fly or scale the sides of the tube for 10 sec, and the number of flies which passed an 8 cm height threshold was recorded. The same cohort of flies was re-tested 10 times. In between climbing trials, the flies were allowed to rest for 1 min. This process was repeated on days 10, 20 and 30 post-eclosion.

Longevity analysis

Virgin females of the desired genotype were collected and kept in groups of 10 in separate vials. Vials were inspected and changed every 2-3 days, and the number of flies remaining alive was scored.

Statistical analyses

Statistical analyses were performed using Prism 6 (GraphPad Software). Analyses were carried out using ANOVA with the Newman–Keuls *a posteriori* test. For longevity, survival curves were generated and data were analysed using the Kaplan–Meier method and log-rank statistics.

References

1. Campesan S*, et al.* (2011) The kynurenine pathway modulates neurodegeneration in a Drosophila model of Huntington's disease. *Curr Biol* 21(11):961-966.
2. Varadarajan S*, et al.* (2015) The transrepression arm of glucocorticoid receptor signaling is protective in mutant huntingtin-mediated neurodegeneration. *Cell Death Differ* 22(8):1388-1396.
3. Notarangelo FM, Wu HQ, Macherone A, Graham DR, & Schwarcz R (2012) Gas chromatography/tandem mass spectrometry detection of extracellular kynurenine and related metabolites in normal and lesioned rat brain. *Anal Biochem* 421(2):573-581.
4. Breda C*, et al.* (2015) Rab11 modulates alpha-synuclein-mediated defects in synaptic transmission and behaviour. *Hum Mol Genet* 24(4):1077-1091.
5. Schneider CA, Rasband WS, & Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9(7):671-675.