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Supplementary Information for

Cellular Clearance of Circulating Transthyretin Decreases Cell-nonautonomous Proteotoxicity in *Caenorhabditis elegans*

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SUPPLEMENTAL INFORMATION
Materials and Methods

Strains and transgenes

N2 Bristol was used as the wild-type strain and standard nematode culture methods and genetics were followed as previously described (1). Nematodes were grown on NGM plates seeded with the *E. coli* strain OP50 at 15˚C or 20°C, unless otherwise specified. The strain CX11480 *kyEx3017[des-2p::myr::GFP + unc-122p::DsRed]* (2) was provided by C. Bargmann (Rockefeller University). Integration of *kyEx3017* was done by gamma irradiation following standard methods (3). Integrated strains were outcrossed at least 4-8 times into N2 wild-type background. The strain NP717 *unc-119(ed3); arIs37[myo-3p::ssGFP; dpy-20(+)]; cdIs32[pcc-1p::DT-A(E148D); unc-119(+) myo-2p::GFP]* (4) was provided by H. Fares (University of Arizona). The *cdIs32* transgene was isolated by outcrossing the NP717 strain at least 4 times into N2 wild-type background and selecting only for *cdIs32[pcc-1p::DT-A(E148D); unc-119(+) myo-2p::GFP]* transgene expression. The strain LG398 *geIs101[rol-6(su1006)]* was obtained from the *Caenorhabditis* Gene Center (CGC) and outcrossed 8 times before use.

Generation of TTR variant constructs

The following constructs were generated using standard molecular biology techniques, including quick change site directed mutagenesis using KOD-polymerase (EMD Novagen) of construct pCL10 *unc-54p::hTTR(WT*) that was generated and provided by Christopher Link (5)(University of Colorado Boulder): pSEE054 *unc-54p::hTTR(V30M*), pSEE055 *unc-54p::hTTR(D18G*), pSEE057 *unc-54p::hTTR(T119M*), pSEE106 *unc-54p::hTTR(V30M ΔSS*).

The following primers were used for site directed mutagenesis of pCL10 *unc-54p::hTTR(WT*) construct:
V30M Forward 5'-CCTGCCATCAATGTGGCCATGCATGTGTTCAGAAAGGCT-3´
V30M Reverse 5'-CAGCCTTTCTGAACACATGCATGGCCACATTGATGGCAGG-3´
D18G Forward 5'-GGTCAAAGTTCTAGGTGCTGTCCGAGGCAGTCC-3'
D18G Reverse 5'-GGACTGCCTCGGACAGCACCTAGAACTTTGACC-3'
T119M Forward 5'-CTACTCCTATTCCACCATGGCTGTCGTCACCAATC-3'
T119M Reverse 5'-GATTGGTCACGACAGCCATGGTGGAATAGGAGTAG-3'
ΔSS Forward 5'-GGCTAGCGTCGACGGTACCATGGGCCCTACGGGCACCGGTGAATC-3'
ΔSS Reverse 5'-GATTCACCGGTGCCCGTAGGGCCCATGGTACCGTCGACGCTAGCC-3'

Quick change site directed mutagenesis reactions were performed in final concentrations of reaction buffer #2 (0.12 M Tris-HCl, 10 mM KCl, 6 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 0.001% BSA, pH 8.8), $MgCl_2$ 1.5 mM, dNTPs 0.2 mM (each), KOD DNA Polymerase (0.05 U μl^{-1}), forward and reverse primers 0.4 µM, and template 5 ng μl^{-1}. Amplification was performed by initial denaturation at 95°C for 10 minutes followed by 34 cycles consisting of a 45 second 95°C denaturation, annealing at 61°C for 45 seconds and elongation at 72°C for 90 seconds. Addition of 0.4 U μl^{-1} DPN1 restriction enzyme (New England Biolabs), digested the methylated template DNA during a 1-hour incubation at 37°C followed by a 20-minute heat activation at 80°C. The PCR products were purified with a QIAquick PCR Purification Kit (QIAGEN) and competent DH5α bacteria were transformed with 2 μg DNA by 30-minute incubation on ice followed by a heat shock for 90 seconds at 42°C. Positive clones were selected on agar plates containing ampicillin (100 μg ml^{-1}) and larger cultures were grown in LB media, and vectors were purified with QIAprep Spin Miniprep Kit a (QIAGEN) and sequenced to verify the single site mutations.

Generation of transgenic *C. elegans* strains

Germline transformation was performed as described (3) to generate two sets of TTR strains, using either the pRF4 *rol-6(su1006)* plasmid (100 ng μl^{-1}), or *odr-1p*::RFP (100 ng μl^{-1}) as co-injection markers (see below). To enable the characterization of thermal avoidance and of forward locomotion, as well as for the ELISA assay in Fig. 6, we used control and TTR strains carrying an *odr-1p*::RFP co-injection marker. For all other experiments the TTR strains carrying *rol-6(su1006)* co-injection marker were used. All strains were tested by western blot, and showed comparable TTR protein levels to each other (Fig. S5 and S10A). Briefly, constructs containing human WT TTR, V30M TTR, D18G TTR, or T119M TTR sequences were co-injected (100 ng μl^{-1}) with either plasmid pRF4 *rol-6(su1006)* or plasmid *odr-1p*::RFP at a concentration of 100 ng μl^{-1} for each plasmid, plus 50 ng μl^{-1} of empty vector (E.V.) (total 250 ng μl^{-1} injected). Injections were performed using a Zeiss Axio Observer A1 inverted microscope (Carl Zeiss MicroImaging) connected to an Eppendorf femto jet express microinjection system (Eppendorf). Microinjection needles were made from borosilicate glass capillaries (World Precision Instruments) using a P-30 needle puller (Sutter Instruments). Injected animals were kept at 20°C and progeny were screened for either the roller phenotype or red chemosensory neurons. Since the roller phenotype was found to be temperature sensitive, the animals were always maintained at 15°C. The extrachromosomal arrays were integrated by a dose of 3800 rad γ-irradiation(3) and outcrossed at least 4 times before use.
The following strains were generated and/or used in this study:

STRAIN NAME ABBREVIATION	STRAIN NAME	GENOTYPE	REFERENCE
control	SEE047	*geIs101[rol-6(su1006)]*	Strain first generated in Viswanathan and Guarente (6). Outcrossed 8X.
WT TTR	SEE037	*scrIs008[unc-54p::hTTR(WT) + rol-6]*	This study
V30M TTR	SEE034	*uthIs378[unc-54p::hTTR(V30M) + rol-6]*	This study
D18G TTR	SEE046	*uthIs352[unc-54p::hTTR(D18G) + rol-6]*	This study
T119M TTR	SEE158	*scrIs038[unc-54p::hTTR(T119M) + rol-6]*	This study
Red coelomocyte marker	SEE064	*scrIs010[des-2p::myr::GFP + unc-122p::DsRed]*	Strain first generated in Maniar*, et al.* (2). Integrated and outcrossed 4X.
Control; Red coelomocytes marker	SEE106	*geIs101[rol-6(su1006)]*; *scrIs010[des-2p::myr::GFP + unc-122p::DsRed]*	This study
WT TTR; Red coelomocyte marker	SEE143	*scrIs006[unc-54p::hTTR(WT) + rol-6]; scrIs010[des-2p::myr::GFP + unc-122::DsRed]*	This study
WT TTR; Red coelomocyte marker	SEE151	*scrIs008[unc-54p::hTTR(WT) + rol-6]; scrIs010[des-2p::myr::GFP + unc-122p::DsRed]*	This study

V30M TTR; Red coelomocytes	SEE145	*uthIs378[unc-54p::hTTR(V30M) + rol-6]; scrIs010[des-2p::myr::gfp + unc-122p::DsRed]*	This study
D18G TTR; Red coelomocytes	SEE141	*uthIs353[unc-54p::hTTR(D18G) + rol-6]; scrIs010[des-2p::myr::GFP + unc-122::DsRed]*	This study
D18G TTR; Red coelomocytes	SEE222	*uthIs352[unc-54p::hTTR(D18G) + rol-6]; scrIs010[des-2p::myr::GFP + unc-122p::DsRed]*	This study
T119M TTR; Red coelomocytes	SEE223	*scrIs038[unc-54p::hTTR(T119M)+rol-6]; scrIs010[des-2p::myr::gfp + unc-122::DsRed] Heterozygous*	This study
DT-A	SEE061	*cdIs32[pcc1p::DT-A(E148D)]; unc-119(+) myo-2p::GFP*	Strain first generated in Schwartz*, et al.* (4). Outcrossed 8X.
control; DT-A	SEE079	*geIs101[rol-6(su1006)]*; *cdIs32[pcc-1p::DT-A(E148D); unc-119(+) myo-2p::GFP]*	This study
Control; DT-A; red coelomocytes	SEE106	*geIs101[rol-6(su1006)]; cdIs32[pcc1p::DT-A(E148D)]; unc-119(+) myo-2p::GFP; scrIs010[des-2p::myr::GFP + unc-122p::DsRed]*	This study
WT TTR; DT-A	SEE080	*scrIs008[unc-54p::hTTR(WT) + rol-6]; cdIs32[pcc-1p::DT-A(E148D); unc-119(+) myo-2p::GFP]*	This study
WT TTR; DT-A	SEE120	*scrIs006[unc-54p::hTTR(WT)+rol-6]; cdIs32[pcc1p::DT-A(E148D)]; unc-119(+) myo-2p::GFP*	This study
V30M TTR; DT-A	SEE128	*uthIs378[unc-54p::hTTR(V30M) + rol-6]; cdIs32[pcc-1p::DT-A(E148D); unc-119(+) myo-2p::GFP]*	This study
T119M TTR; DT-A	SEE168	*scrIs038[unc-54p::hTTR(T119M) + rol-6]; cdIs32[pcc-1p::DT-A(E148D); unc-119(+) myo-2p::GFP]*	This study
D18G TTR; DT-A	SEE152	*uthIs353[unc-54p::hTTR(D18G) + rol-6]; cdIs32[pcc1p::DT-A(E148D)]; unc-119(+) myo-2p::GFP*	This study
D18G TTR; DT-A	SEE221	*uthIs352[unc-54p::hTTR(D18G) + rol-6]; cdIs32[pcc-1p::DT-A(E148D); unc-119(+) myo-2p::GFP]*	This study
control	SEE109	*scrIs020[odr-1p::RFP]; scrIs010[des-2p::myr::GFP + unc-122p::DsRed]*	This study

V30M TTR	SEE130	*scrIs024[unc-54p::hTTR(V30M) + odr-1p::RFP]; scrIs010[des-2p::myr::GFP + unc-122p::DsRed]*	This study
D18G TTR	SEE115	*scrIs032[unc-54p::hTTR(D18G) + odr-1p::RFP]*	This study
control; DT-A	SEE169	*scrIs020[odr-1p::RFP]; scrIs010[des-2p::myr::GFP + unc-122::DsRed]; [cdIs32[pcc-1p::DT-A(E148D); unc-119p::myo-2p::GFP]*	This study
V30M TTR; DT-A	SEE170	*scrIs024[unc-54p::hTTR(V30M)+odr-1p::RFP]; scrIs010[des-2p::myr::GFP+unc-122::DsRed]; cdIs32[pcc-1p::DT-A(E148D); unc-119p::myo-2p::GFP]*	This study
V30M TTR ΔSS	SEE299	*scrEx033[unc-54p::hTTR(V30MΔSS) + odr-1p::RFP]*	This study
mtGFP	SEE045	*jsIs609[mec-7p::mtGFP + pJM23 [lin-15]]*	
ctrl; mtGFP	SEE110	*scrIs020[odr-1p::RFP]; jsIs609[mec-7p::mtGFP]*	This study
V30M; mtGFP	SEE148	*scrIs024[unc-54p::hTTR(V30M) + odr-1p::RFP]; jsIs609[mec-7p::mtGFP]*	This study
T119M; mtGFP	SEE329	*scrIs040[unc-54p::hTTR(T119M) + odr-1p::RFP]; jsIs609[mec-7p::mtGFP]*	This study
V30M TTR ΔSS	SEE313	*scrEx047[des-2p::hTTR(V30MΔSS) + odr-1p::RFP] + scrIs010[des-2p::myr::GFP + unc-122p::Ds-red]*	This study

mRNA extraction and quantitative reverse transcriptase (RT)-PCR

Bleached synchronized animals (~1000 to 10,000 nematodes) were grown in 50 ml S-complete medium supplemented with 50 μg ml^{-1} carbenicillin, 0.1 μg ml^{-1} fungizone and 6 mg ml^{-1} (< 5000 animals) or 12 mg ml^{-1} (> 5000 animals) freshly prepared *E. coli* OP50. Animals were cultured in 15 cm petri dishes at 20°C. To arrest embryos prior to hatching, 5-fluoro- 2´-deoxyuridine (FUDR, Sigma) was added to a final concentration of 0.12 mM to L4 larvae animals. Animals were aged until day 1, day 5, and day 8 of adulthood, with new OP50 added (1.2 ml of 100 mg ml^{-1} solution) on day 5 to prevent starvation. At desired time point, animals were thoroughly washed three times in 1x M9 buffer and remove as much buffer as possible and flash freeze the samples in liquid nitrogen. RNA was extracted using the QIAzol lysis reagent (QIAGEN), followed by DNase I treatment (Sigma). mRNA was reverse transcribed using the iScript cDNA Synthesis kit (Bio-Rad). cDNA (20 ng) was used for real-time PCR amplification using the FastStart Universal SYBR Green Mastermix (Roche) and the ABS 7900HT Fast Real-Time PCR System. The relative TTR gene expression levels were determined using the Comparative C_T Method. TTR gene expression levels were normalized

relative to those of the following housekeeping genes: the 60S ribosomal protein L6 (*rpl-6*), and of the plasma membrane protein 3 (*pmp-3*) for each sample (internal controls).

The following primer sequences were used:

TTR Forward 5'-ATTTGCCTCTGGGAAAACCAG-3'
TTR Reverse 5'-GGCTGTGAATACCACCTCTGC-3'
rpl-6 Forward 5'-TTCACCAAGGACACTAGCG-3'
rpl-6 Reverse 5'-GACAGTCTTGGAATGTCCGA-3'
pmp-3 Forward 5'-TGGCCGGATGATGGTGTCGC-3'
pmp-3 Reverse 5'-ACGAACAATGCCAAAGGCCAGC-3'

Expression and purification of recombinant TTR and preparation of non-native TTR forms

Recombinant WT TTR, T119M TTR, D18G TTR and V30M TTR were expressed in and purified from *Escherichia coli* as described previously (7). Protein was eluted in a standard phosphate buffer (10 mM sodium phosphate (pH 7.6), 100 mM KCl, 1 mM EDTA). The molar absorptivities (ε) of TTR (73800 M^{-1} cm^{-1}) tetramers in standard phosphate buffer were used to calculate TTR concentrations using the nanodrop. Non-native (NN) TTR forms were prepared using 0.2 mg/ml monomeric (M) TTR or by incubating the same protein concentration of recombinantly-made normally folded tetrameric TTR proteins at 37˚C in 100mM pH 4.3 acetate buffer for 16 hours (8).

Antibodies against oligomeric TTR

The polyclonal MDX114 antibody, and monoclonal MDX102 and MDX108 antibodies were generated by Misfolding Diagnostics, and have been optimized for use in an ELISA assay to specifically detect non-native (NN) TTR forms that include partially unfolded or misfolded TTR monomers, and oligomeric TTR (patent WO2014/124334A2). The specificity toward NN TTR of MDX102 and MDX108 antibodies was first tested by SDS-PAGE by incubating recombinant (rec) native WT tetrameric TTR in SDS sample buffer in the absence of boiling. Rec tetrameric TTR is kinetically stable and resisted denaturation in the absence of boiling in SDS-PAGE, and was unrecognized by MDX antibodies (rec WT TTR shown in Fig. S3A, left panels). In contrast, NN monomeric (M)-TTR oligomers produced as stated above, by incubation of an engineered monomeric variant of TTR (M-TTR) at 37°C, were denatured at 25°C by unboiling SDS treatment (rec WT TTR shown in Fig. S3A, left panels), and were recognized by MDX102 in a native gel (Fig. S3A, right panel)(9). MDX114 is a rabbit polyclonal antibody that was used as the capture antibody in the ELISA assay (see below), while the MDX102 was used as the detection antibody.

Western blot analysis for quantification of total TTR protein levels

Bleached synchronized animals (~1000 to 10,000 nematodes) were grown in 50 ml S-complete medium supplemented with 50 μg ml^{-1} carbenicillin, 0.1 μg ml^{-1} fungizone and 6 mg ml^{-1} (< 5000 animals) or 12 mg ml^{-1} (> 5000 animals) freshly prepared *E. coli* OP50. Animals were cultured in 15 cm petri dishes at 20°C. To arrest embryos prior to hatching, 5-fluoro- 2´-deoxyuridine (FUDR) was added to a final concentration of 0.12 mM to L4 larvae animals (Sigma). Animals were aged until day 1, day 5, and day 8 of adulthood, with new OP50 added (1.2 ml of 100 mg ml^{-1} solution) on day 5 to prevent starvation. At desired time point, animals were thoroughly washed three times in 1x PBS buffer and worm pellet was re-suspended in 300 μl cold 1x PBS supplemented with complete protease inhibitors (Roche). The approximate concentration of animals for each sample was determined by counting the number of animals in ten 10 μl drops using a dissecting scope. The animal suspension was added to a hard tissue homogenizing CK28 Precellys tube (Bertin Technologies) and subjected to Precellys-24 homogenization at 6500 x *g* for 3 x 10 sec bursts (Bertin Technologies). Sample homogenates

were spun at 3000 x *g* for 15 minutes at 4°C, where the supernatant was collected as the soluble fraction and the pellet was collected as the insoluble fraction. The insoluble pellet was re-suspended in a 10% SDS solution and boiled for 10 minutes at 95°C. Both soluble and insoluble protein fraction concentrations were determined using the Bio-Rad DC Protein Assay kit (Bio-Rad).

Samples (5-10 μg total protein) were mixed with 6x SDS loading sample buffer (20% glycerol, 12% SDS, 125 mM Tris pH 6.8) and with 100 mM DTT (Sigma), boiled for 10 minutes and loaded into the wells of a 15% SDS-Page Gel. Protein separation was performed at 150V in SDS running buffer and blotted onto a nitrocellulose membrane in transfer buffer (20 mM Tris, 150 mM glycine, 20% methanol) for 2 hours at 0.25A. Membranes were incubated in blocking solution (1X TBS with 5% non-fat milk) for 1 hour followed by an overnight incubation at 4°C with primary monoclonal antibody MDX102 against TTR (Misfolding Diagnostics, Inc.) at a 1:1000 dilution, and with a monoclonal antibody against actin (α-actinC4; MAB1501, Millipore) at a 1:4000 dilution. After three rinses in 1X TBS-T, the membrane was incubated with secondary donkey anti-mouse IRDye 800 nm conjugated antibody (LI-COR) for 1 hour at room temperature diluted 1:10,000 in blocking solution, rinsed 3 times in TBS-T, and scanned with an Odyssey infrared imager (LI-COR). Protein band intensities were measured using Image J.

Quantification of *in vitro* TTR tetramer levels

TTR tetramer levels were measured as previously described (10). Briefly, soluble protein lysates (10 μg) were incubated with the fluorogenic small molecule **A2** (10 μM final concentration, prepared in DMSO) in 1x PBS buffer. To generate a TTR tetramer standard curve, recombinant WT TTR was added to day 1 soluble non-TTR control lysate at various concentrations (serial dilution: 2 μM, 1 μM, 0.5 μM, 0.25 μM, 0.125 μM, 0.0625 μM, 0.03125 μM, 0.0156 μM, 0.00781 μM) and incubated with **A2** (10 μM) for 10-12 hours at 4°C to allow for complete covalent modification by **A2** of the two thyroxine binding sites within the TTR tetramer. A 50 μL sample was injected onto a Waters Acquity H-Class Bio-UPLC (Ultra Performance Liquid Chromatography) instrument fitted with a strong anion exchange column (Waters Corp). TTR was eluted from the column using a nonlinear gradient at a flow rate of 0.6 ml min^{-1} over 33 min. **A2**-modified TTR conjugate fluorescence was monitored using excitation at 328 nm with emission at 430 nm, at a sampling rate of one point per second. The TTR peak was extracted by plotting the data in excel.

Native PAGE gel analysis for detection of TTR oligomers

Soluble lysate and insoluble pellet were obtained from synchronized *C. elegans* as mentioned above in the section 'Western blot analysis for quantification of total TTR protein levels'. The insoluble pellet was re-suspended in 50mM Tris pH8, 0.5M NaCl, 1% v/v Igepal CA630+ Protease Inhibitor to maintain the structure of the TTR proteins (11). The samples were resolved on NativePAGE™ Novex™3-12% Bis-Tris protein gels by loading 10 μg total protein and running at 150V for 90 min at room temperature. After transferring on nitrocellulose membrane, the membrane was probed with the MDX102 antibody against oligomeric TTR. Validation of antibodies used in this assay is reported above in the section 'Antibodies against oligomeric TTR' (Fig. S3A and B).

Quantification of non-native TTR levels *in vitro* by ELISA

Non-native (NN) TTR levels were quantitated using a sandwich ELISA developed by Misfolding Diagnostics, Inc. (patent WO2014/124334A2). Validation of antibodies used in this assay is reported in SI Materials and Methods under 'Antibodies against oligomeric TTR' (Fig. S3A and B). The lysates were obtained as mentioned above in the section 'Native PAGE gel analysis for detection of TTR oligomers'. Each sample containing 2.4 μg of the total protein was

used in the assay. A recombinant form of misfolded TTR protein was used to generate the standard curve.

***In vivo* TTR tetramer localization with compound 5 (CMPD 5)**

Ten L4 larvae were transferred into a well of a 96-well plate containing 150 μL liquid culture media (S-complete media with 50 μg mL^{-1} carbenicillin, 0.1 μg mL^{-1} fungizone, OP50 6 mg mL^{-1}, FUDR 0.12 mM). Animals were incubated on a nutator and overnight at 20°C. On day 1 of adulthood, **CMPD5** (0.5 μL of a 3 mM solution in DMSO, for a final concentration of 10 μM) was added to animals in culture (12). Animals were again incubated on a nutator overnight at 20 °C. On day 2 of adulthood, animals were collected and washed three times in 1 mL M9 buffer and transferred to a fresh 6 cm NGM plate prior to image analysis. For light and fluorescence microscopy, animals were mounted on 3% agar pads in 2% sodium azide buffer (prepared in 1x PBS), and covered with a coverslip. Imaging of coelomocytes was done on live animals expressing Ds-Red under the coelomocyte-specific *unc-122* promoter (13). To image the animals, we used simultaneous differential interference contrast microscopy (DIC) and epifluorescence modalities using a Nikon Ti-E Perfect Focus inverted microscope equipped with a iXon+ DU897 EM Camera, a 100X/1.49 NA oil objective and an Intensilight System M lamp. We used 340-380 nm/500-550 nm excitation and emission filters, respectively, to detect **CMPD5** fluorescence, and 545-570 nm/578-625 nm emission and excitation filters, respectively, to detect DsRed fluorescence.

Immunofluorescence

The *in vivo* localization of TTR was visualized by immunofluorescence (IF) microscopy and staining was performed following a modified protocol developed by the Loer laboratory (http://home.sandiego.edu/~cloer/loerlab/anti5htshort.html). The primary antibodies used were either the polyclonal antibody against TTR (DAKO) at a 1:100 dilution or monoclonal anti-mouse MDX102 (Misfolding Diagnostics, Inc) at a 1:100 dilution. Secondary antibodies used were Alexa647-conjugated Donkey anti-rabbit (Life Technologies) at a 1:100 dilution, or Alexa647-conjugated Donkey anti-mouse (Life Technologies) at a 1:100 dilution. To visualize the body-wall muscle, animals were incubated with Rhodamine Phalloidin (Invitrogen) at 1:10 dilution for 30 mins at RT before mounting. Whole animals were imaged using a Nikon A1R+ laser scanning confocal microscope system and a 60X/1.4 NA oil objective. The 561 nm laser was used to image Phalloidin and the 647 nm laser for imaging TTR.

Thermal avoidance assay for nociception

The thermal avoidance assay was done on day 1 adult animals and their response to noxious heat was scored in one of the 4 categories, as published before: class I: rapid withdrawal reflex, backing, and change in direction; class II: rapid withdrawal reflex with little backing; class III: slow backing; class IV: no response (14). Because the dominant roller phenotype induced by the *rol-6(su1006)* co-injection transgene interfered with this behavioral assay, we used control, TTR transgenic animals with an *odr-1p*::RFP co-injection marker. The heat source was delivered by heating a blunt $30^{1/2}$-gauge needle for ~ 10 seconds in a burner and immediately placing it 1-3 mm in front of the animal when in forward motion. The following guidelines were strictly followed: The heated tip was placed in front of a single animal at a time; each animal was removed after testing to prevent re-testing; and only the initial response of the worm was recorded, i.e., animals were not re-tested. Class IV animals were moved into separate 6 cm NGM plates and were each tested with a soft touch to the nose with an eyelash to score for backing.

Analysis of FLP dendritic branching

C. elegans were picked as L4 larvae, placed in fresh NGM (+OP50) plates, and incubated at 20°C for 15 hours to allow growth into day 1 adult animals. Visualization of the branching of FLP neurons was done using a myristoyl::GFP signal under the *des-2* promoter (2). Animals were mounted in 2% sodium azide buffer (prepared in 1x PBS) on 3% agarose pads and covered with a coverslip. FLP dendritic branching was imaged using a Nikon A1R+ laser scanning confocal microscope system, a 60X/1.4 NA oil objective, and a 488 nm laser for excitation of GFP. The number of quaternary dendritic branches per 100 μm (from the tip of the nose) were counted manually for each animal image of each strain.

Coelomocyte uptake assay of dextran

Injections into the pseudocoelom (body cavity) were performed as previously described (15). Briefly, approximately 10-15 L4 animals were transferred onto NGM (+OP50) plates and incubated overnight at 20°C. Alexa Fluor 488 3,000 MW Dextran [1 mg $ml^{-}1$] (prepared in ddH_2O, Invitrogen) was injected into the pseudocoelom near the pharynx, of day 1 adult animals. Injected animals were kept at 20°C on NGM (+OP50) plates overnight and Dextran-488 fluorescence was imaged after 24 hours using epifluorescence in a Nikon Ti-E Perfect Focus inverted microscope equipped with a iXon+ DU897 EM Camera, a 100X/1.49 NA oil objective and an Intensilight System M lamp.

Quantitation of mitochondrial size in ALM neurons

L4 larvae were picked and placed in NGM (+OP50) plates at 20˚C overnight. Day 1 adults were immobilized with Polybead® Polystyrene 0.10 micron Microspheres (2.6% Solids-Latex) (Polysciences, Inc., Illinois) on ~1mm 8% agarose pads covered with a coverslip. Proximal regions of ALM neurons expressing a mitochondrial localization signal fused to GFP (mtGFP) were imaged using a Nikon Ti-E A1 confocal laser microscope system equipped with a scanning stage and Piezo-Z control, and a 60X/1.4 NA oil objective. A 488 nm laser was used for excitation of GFP. The mitochondrial length was measured using the ImageJ software and normalized by the total axonal length for each mitochondrion, expressing the values in "parts per mille" (‰) of the total axon length. Non-parametric Kruskal-Wallis with Dunn's multiple comparisons test was performed for statistical analysis.

RNA interference

Reduction of TTR transgene activity was accomplished by feeding *C. elegans* with RNAi bacterial clones expressing double-stranded RNA (dsRNA) targeting TTR. The bacterial clones were generated by amplification of a 460 bp sequence by PCR that includes the complete human TTR sequence. The primers used for the amplification were the following: forward primer 5´-AATGAGCTCATGGCTTCTCATCGTCTGCTCCTC-3´and reverse primer 5´-ATTAGGTTACCTCATTCCTTGGGATTGGTGACG-3´. The sequence was purified and ligated between Sac1 and Kpn1 sites in the multiple cloning site region of the L4440 vector (plasmid 1654, Addgene) and transformed into *E. coli* HT115 with tetracycline resistance. *E. coli* HT115 bacteria expressing RNAi were cultured in Luria-Bertani (LB) media containing 10 mg ml^{-1} ampicillin and spotted on NGM plates containing 100 μg ml^{-1} carbenicillin. RNAi production was induced with addition of 100 mM IPTG placed directly on the bacterial lawn of each plate. Animals were grown on new plates seeded with RNAi expressing bacteria for two generations prior to analysis. Lysates were made by picking animals into 300 μl cold PBS and lysed as described above (”Western blot analysis for quantification of total TTR protein levels”). Day 2 adult animals were recorded as mentioned below (”Locomotion assay and worm tracking analysis”).

Locomotion assay and worm tracking analysis

Animals were maintained at 22°C for multiple generations on NGM + OP50 seeded plates. Because the dominant roller phenotype induced by the rol-6(su1006) co-injection transgene interfered with this behavioral assay, we examined control, TTR transgenic animals with an *odr-1p*::RFP co-injection marker. L3 larvae were picked onto 150 μl liquid culture medium (S-complete media with 50 μg ml^{-1} carbenicillin, 0.1 μg ml^{-1} fungizone and 6 mg ml^{-1} OP50) in a 96-well plate and incubated at 22°C. To arrest embryos prior to hatching, 0.12 mM FUDR was added to each well when larvae reached the L4 stage. Individual day 2 adult animals were placed in an unseeded 35 mm plate for 40 minutes, then transferred to a new unseeded 35 mm plate and their movement trajectories recorded for a duration of 40 seconds using the Stemi 508 microscope (Zeiss) with SwiftCam2 camera and imaging software (Swift). All videos were analyzed using wrMTrck plugin for ImageJ to obtain average speed and representative tracks of each of the trajectories (16). All locomotion assays including the tracking analysis were performed blind.

Compound 5 (CMPD5) treatment for locomotion assay

Thirty L3 larvae were picked into 150 μl liquid culture medium (S-complete media with 50 μg ml^{-1} carbenicillin, 0.1 μg ml^{-1} fungizone and 6 mg ml^{-1} OP50) in a 96-well plate and incubated at 22°C. Animals were fed **CMPD5** (10 μM) or DMSO (concentration) starting at the L3 stage through to day 1 of adulthood. At day 1, animals were transferred onto NGM (+OP50) plates (containing 10 μM **CMPD5** or 0.3% DMSO). Day 2 adult animals were assessed for locomotion as mentioned above.

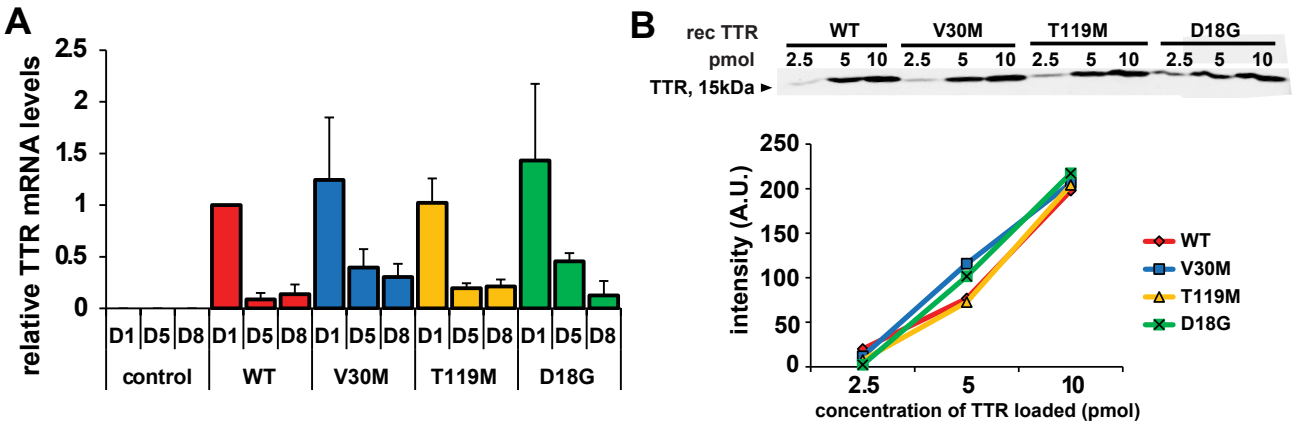

Fig. S1. Messenger RNA levels and TTR antibodies in transgenic *C. elegans* strains, Related to Fig. 1. (A) Quantitative real-time PCR analysis of synchronized adult day 1 (D1), 5 (D5), and 8 (D8) mRNA levels. TTR gene expression levels were compared to that of 60S ribosomal protein L6 (*rpl-6*) mRNA, and to the plasma membrane protein 3 (*pmp-3*) mRNA, and normalized to D1 WT TTR. Data is from two biological samples for each condition (n = 3 technical replicates, mean ± s.e.m.). The percentage difference of relative TTR mRNA levels between the strains with respect to the WT TTR D1 are 24%, 2% and 43% for V30M, T119M and D18G, respectively. (B) Top panel: Western blot of recombinant TTR protein probed with MDX102 antibody against TTR. Bottom panel: TTR protein levels are plotted as fluorescence intensity, against loaded protein amount to show linear antibody detection of TTR protein amount. Blot is representative of 2 biological replicates.

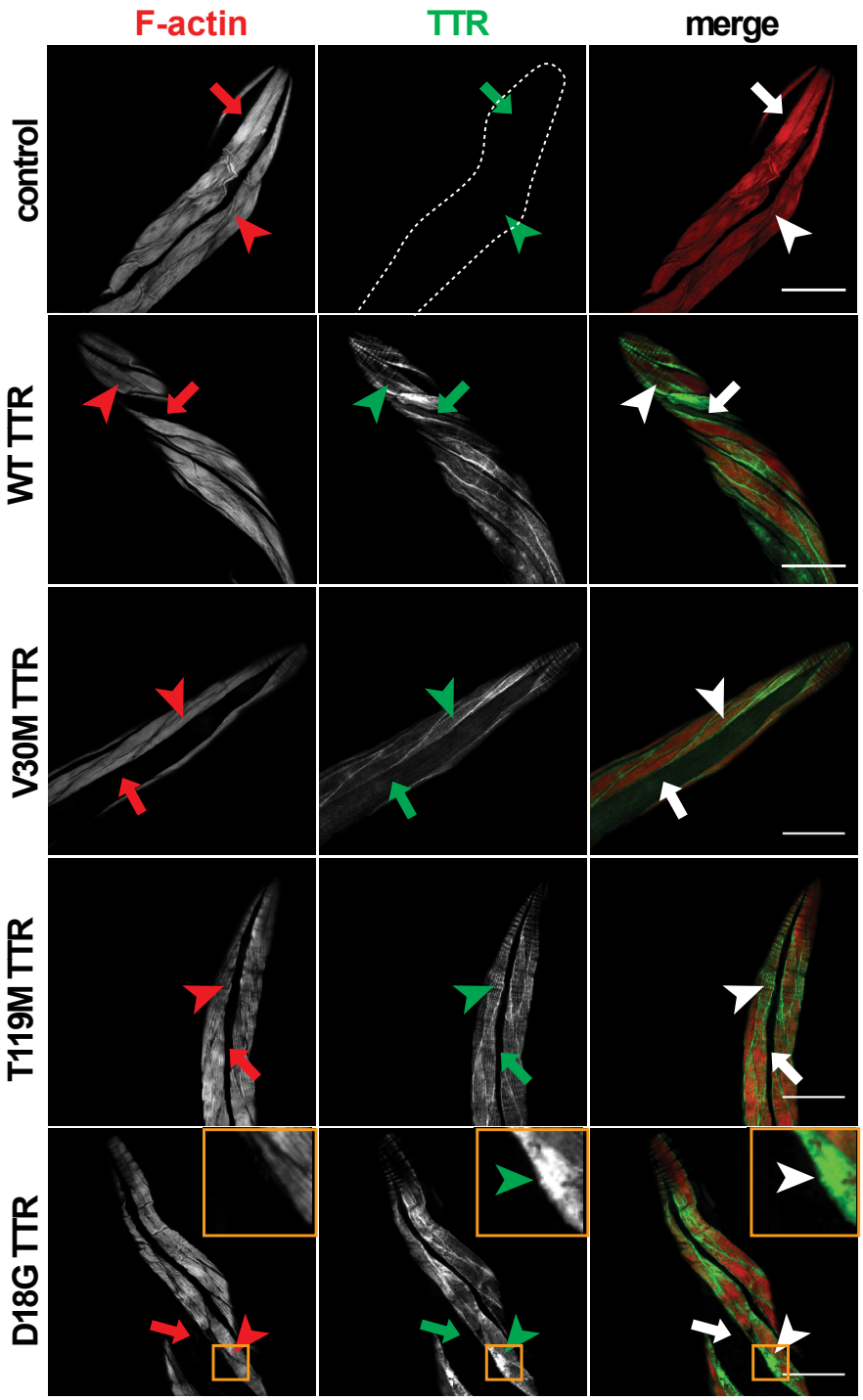

Fig. S2. Immunofluorescence (IF) of TTR transgenic animals with a non-native TTR specific antibody, Related to Fig. 2. Representative confocal images of the head region of animals stained with antibody MDX102 against oligomeric TTR (green), and with phalloidin (F-actin; red). Control n=6, WT TTR n=11, V30M TTR n=10, T119M TTR n=10, D18G TTR n=6. Arrowheads point to muscle cells; arrows point to the body cavity. Insets inside D18G TTR panel shows enlargement of aggregates. Scale bars = 50μm.

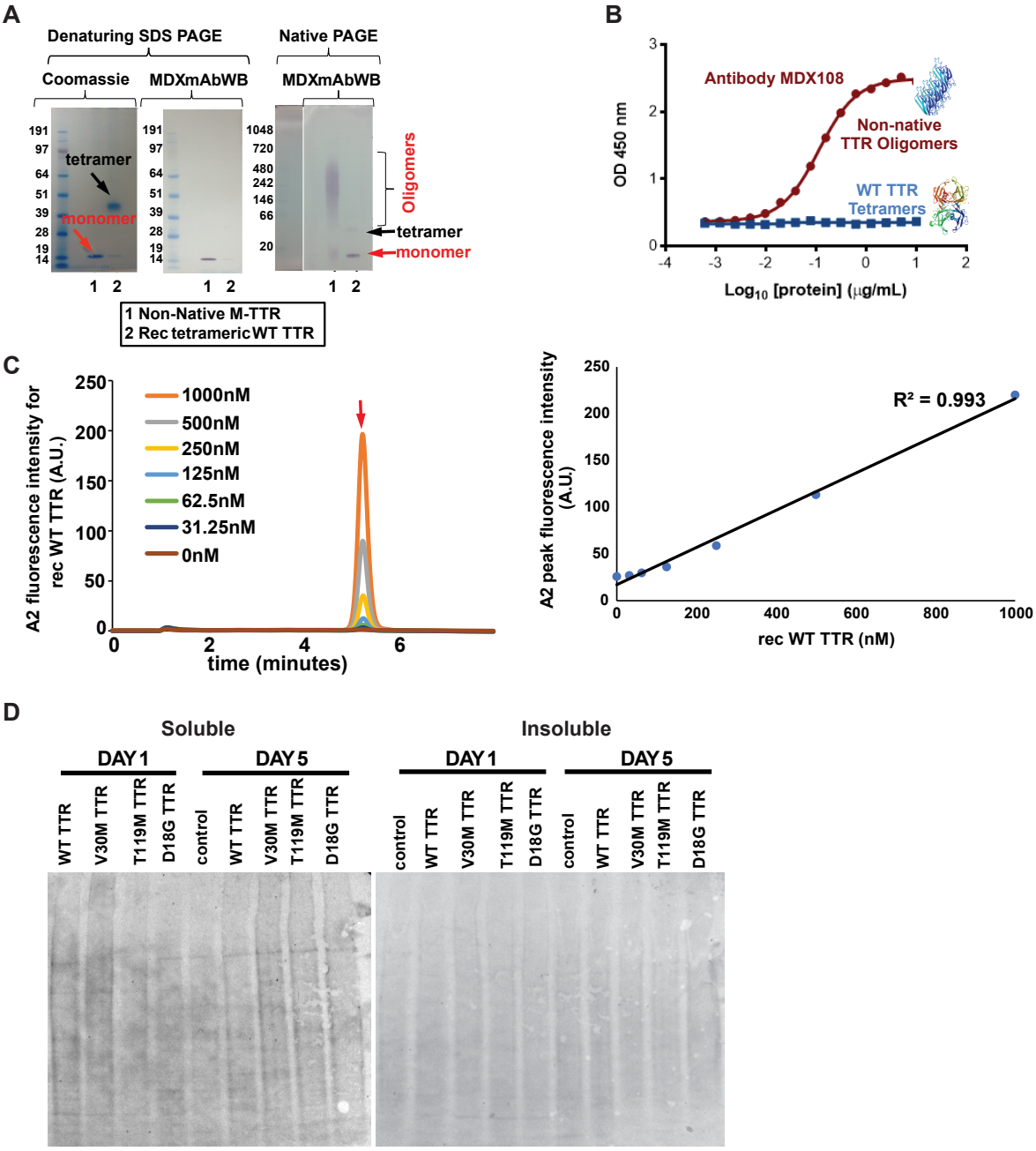

Fig. S3. Generation of soluble and insoluble TTR and oligomeric aggregates in transgenic *C. elegans* strains, Related to Fig. 3. (A) SDS-and Native-PAGE gels showing specificity of antibody MDX108 for recombinant (rec) non-native TTR monomer and oligomers of M-TTR, but not for rec WT TTR tetramer that is kinetically stable in a non-boiled SDS buffer. WB = western blot. (B) ELISA binding affinities of the MDX108 antibody to native WT TTR and to non-native TTR. (C) Left panel: UPLC chromatogram of the TTR tetramer-(**A2**)$_2$ conjugate peak for various amounts of recombinant WT TTR tetramer. Red arrow points to eluted recombinant TTR tetramers. Right panel: standard linear regression curve for quantification of the TTR tetramer-(**A2**)$_2$ conjugate peak heights in top panel. This standard curve was used to determine the amount of TTR tetramer in lysates. (D) Ponceau S staining of soluble and insoluble samples run on the native gels showing total equivalent protein loading.

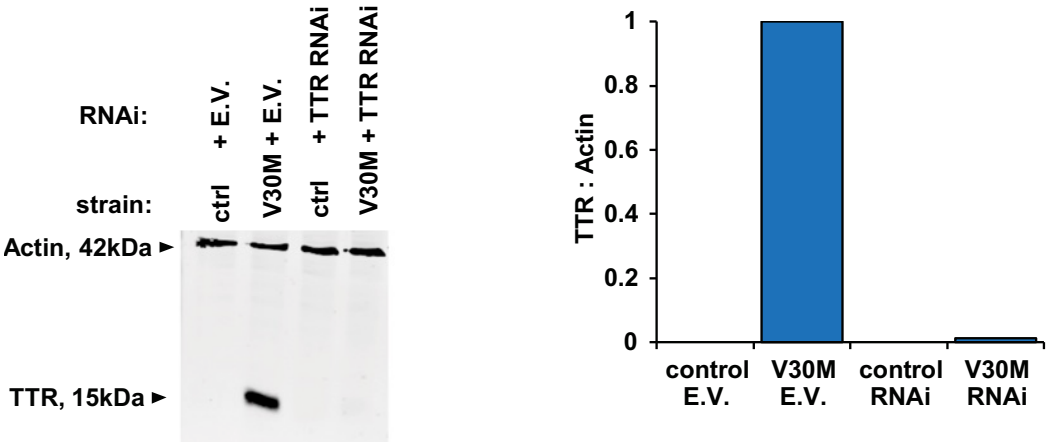

Fig. S4. RNAi treatment of V30M TTR transgenic animals, Related to Fig. 4. Western blot (left) and quantification of Western blot (right) of lysates of control and V30M TTR transgenic animals fed either with control bacteria (E.V. – empty vector) or with bacteria expressing dsRNA against TTR (TTR RNAi). Membrane was probed with the monoclonal MDX102 antibody against TTR.

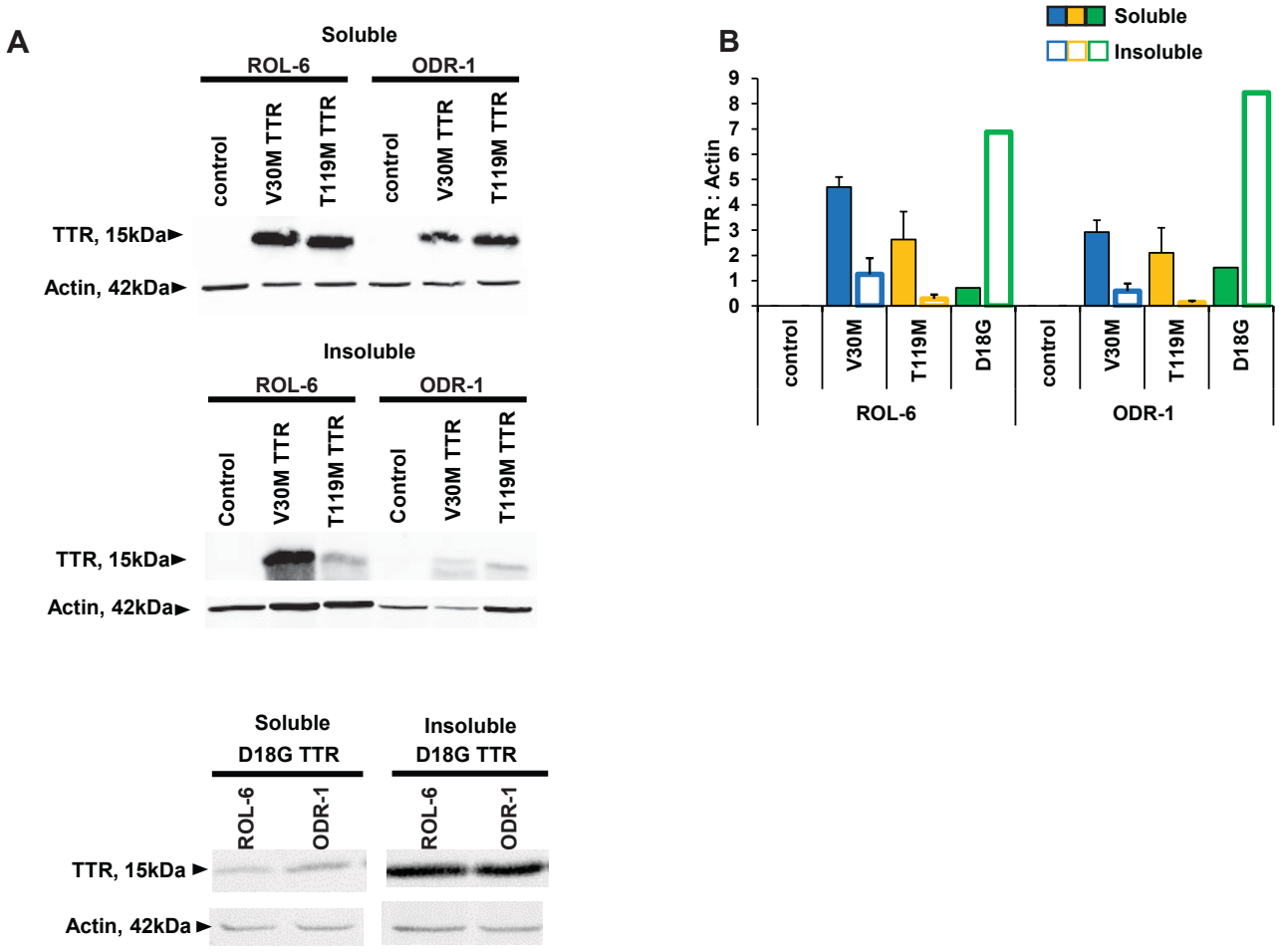

Fig. S5. Newly generated *C. elegans* strains for the characterization of nociception, locomotion, and neuronal mitochondrial morphology phenotypes, Related to Fig. 4, and Fig. 7. Analyses of nociception and neuronal mitochondrial morphology were performed on strains that had *odr-1p*::RFP instead of ROL-6 co-injection markers to avoid the rolling phenotype of *rol-6(su1006)* animals. (A) Western blot comparing TTR levels in soluble and insoluble fractions in TTR strains that were generated with *rol-6(su1006)* versus *odr-1p*::RFP as co-injection markers. Membranes were probed with the monoclonal MDX102 antibody against TTR. Images are representative of 2 independent experiments. (B) Quantification of the TTR : Actin ratio of the samples shown in (A) (mean ± s.e.m.).

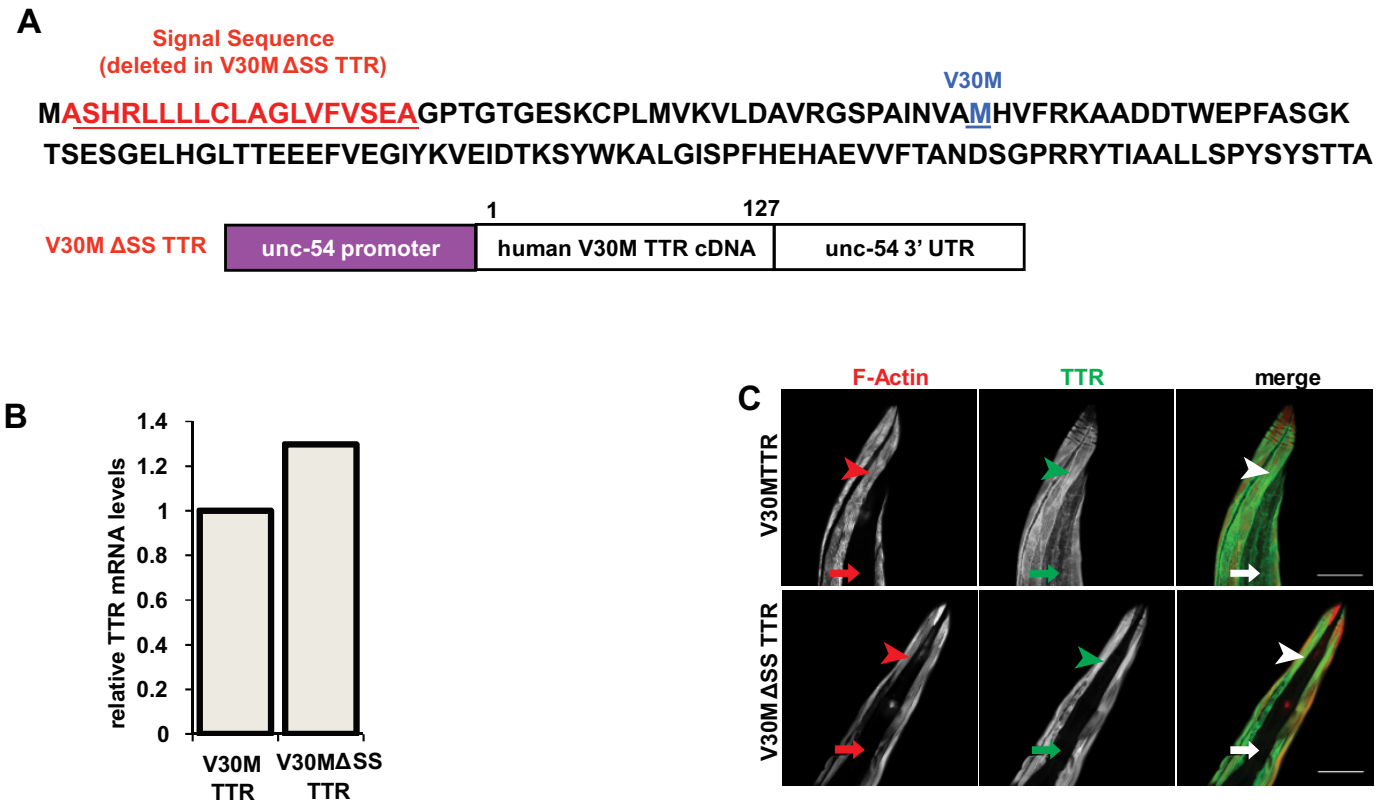

Fig. S6. Characterization of V30M ΔSS TTR animals lacking the TTR signal sequence (SS), Related to Fig. 4. (A) Top panel: TTR protein sequence showing the signal sequence (red) that was deleted in the V30M ΔSS TTR transgenic strain. Bottom panel: Schematic of the construct expressed in the V30M ΔSS TTR transgenic animal. (B) Messenger RNA levels as measured by quantitative real-time PCR analysis of synchronized day 1 V30M TTR; *odr-1p*::RFP, and of V30M ΔSS TTR; *odr-1p*::RFP transgenic animals. TTR gene expression levels were normalized relative to the 60S ribosomal protein L6 (*rpl-6*) mRNA, or to the plasma membrane protein 3 (*pmp-3*) mRNA, and normalized to day 1 V30M TTR levels. (C) Representative confocal immunofluorescence (IF) images of the head region of day 1 TTR transgenic animals stained with polyclonal antibody against TTR (green) and phalloidin (F-actin; red). n $\geq$ 5 per strain. Arrowheads point to muscle cells; arrows point to the body cavity. Scale bar = 50μm.

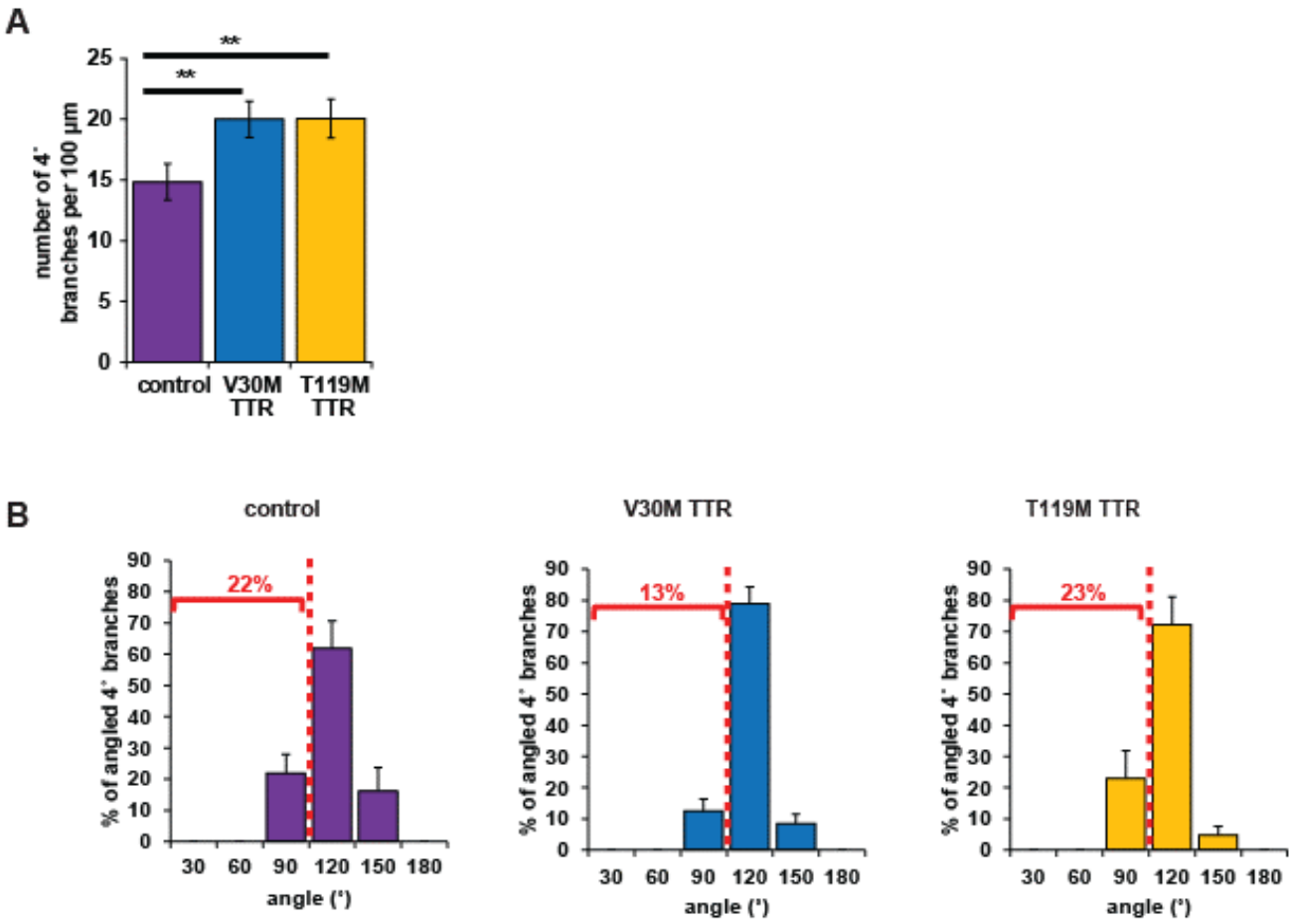

Fig. S7. FLP dendritic branching defects in L4 larval TTR transgenic animals, Related to Fig. 5. (A) Quantification of number of FLP 4˚ dendritic branches per animal in 100 µm starting from the tip of the head (mean ± s.e.m.). n=20 animals per strain. **p<0.01 by permutation t-test. (B) Histograms showing the proportion of angular branches with a deviation angle greater than 0˚ in L4 larvae, as measured in Fig. 5D. The two-sample Kolmogorov-Smirnov test showed no significant differences between the strains.

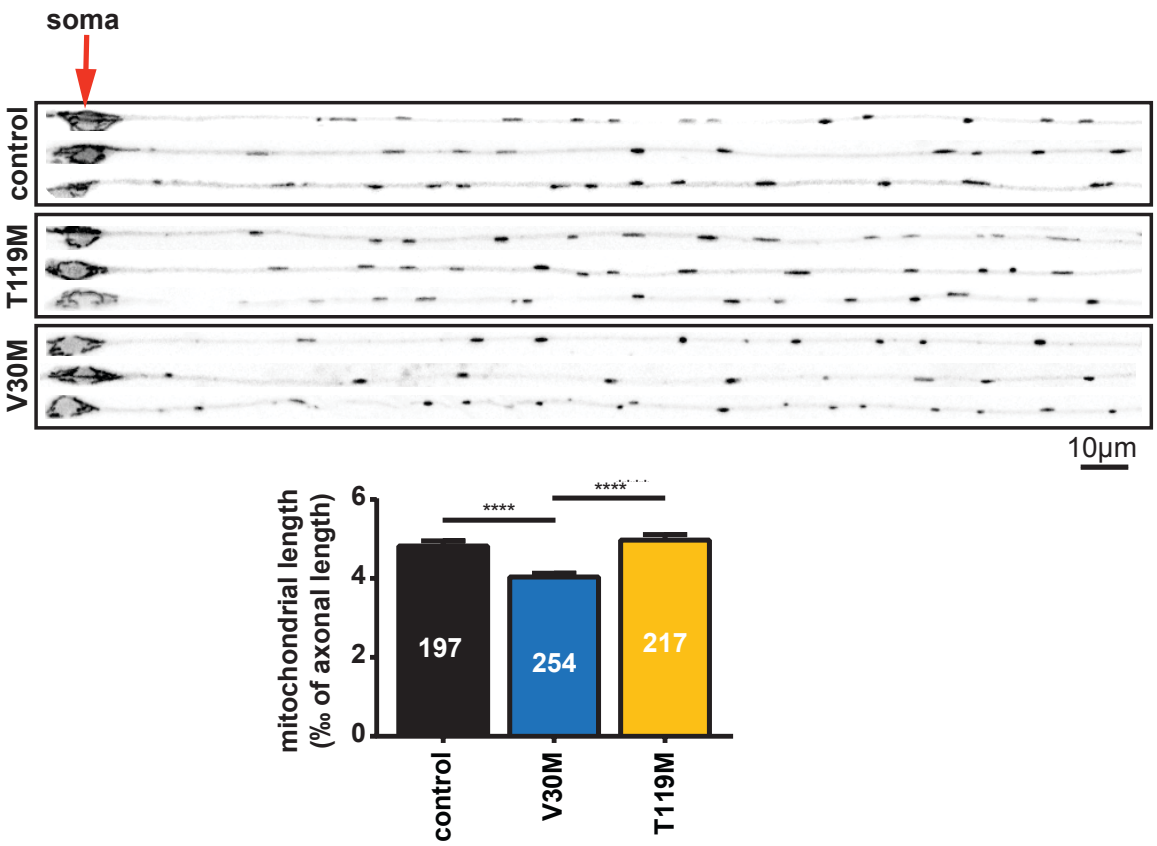

Fig. S8. Neuronal mitochondria morphology phenotypes, Related to Fig. 5. Top panels: confocal fluorescence images of the proximal region of three straightened and aligned ALM axons, expressing a mitochondrial localization sequence::GFP (MLS::GFP) fusion. Position of cell bodies is indicated by red arrow. Bottom panel: mean ± SEM of mitochondrial length in ALM axons of day 1 adult animals. The length is expressed in "parts per mille" (‰) of the total axon length. The numbers of mitochondria analyzed for each strain are indicated inside each bar. The number of axons analyzed are the following: $n_{control}$ = 9, n_{T119M} = 11, n_{V30M} = 11. Non-parametric Kruskal-Wallis with Dunn's multiple comparisons tests were performed for statistical analyses. ****$p<0.0001$.

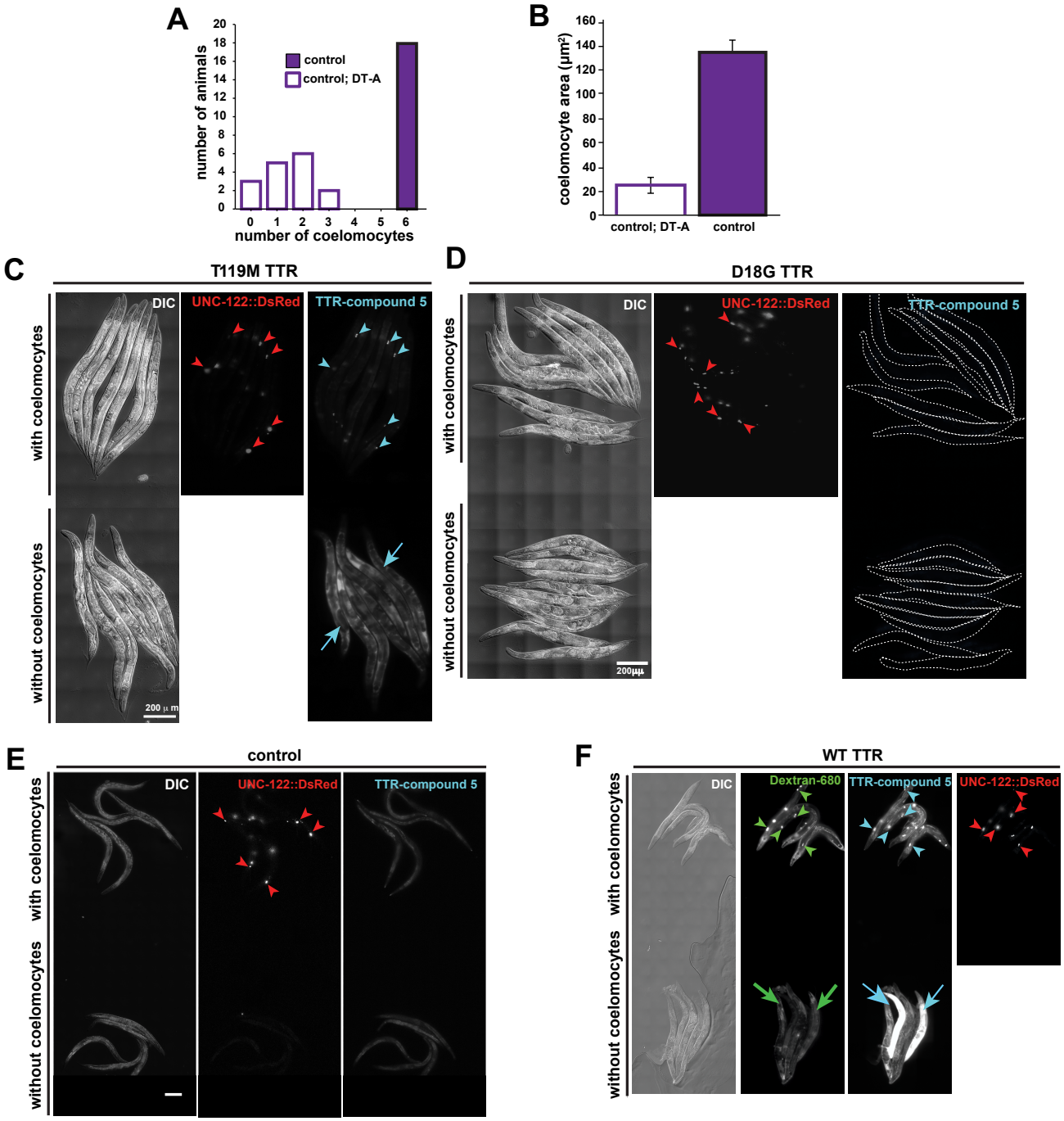

Fig. S9. Characterization of the formation and activity of coelomocytes after genetic ablation by a diphtheria-toxin (DT-A) mutation in TTR strains, Related to Fig. 6. (A) Coelomocytes counts scored with UNC-122::DsRed marker. (B) Area of coelomocytes (control $n_{coelomocytes}$ = 32; for DT-A $n_{coelomocytes}$ = 23). (C-F) Representative image of day 2 adult animals. Control n= 11, control; DT-A n= 5, WT n= 12, WT; DT-A n= 13, T119M = 21, T119M; DT-A n = 19, D18G = 23, D18G; DT-A n= 24. Animals were injected in the body cavity with Dextran-680 (if indicated) and fed with fluorogenic **CMPD5**. Coelomocytes were visualized with UNC-122::Ds-Red. Arrowheads indicate position of representative coelomocytes in focus. Arrows point to body cavity fluorescence of corresponding molecule in DT-A animals. Animals without coelomocytes were not crossed to the UNC-122::Ds-Red marker strain (except control; DT-A animals).

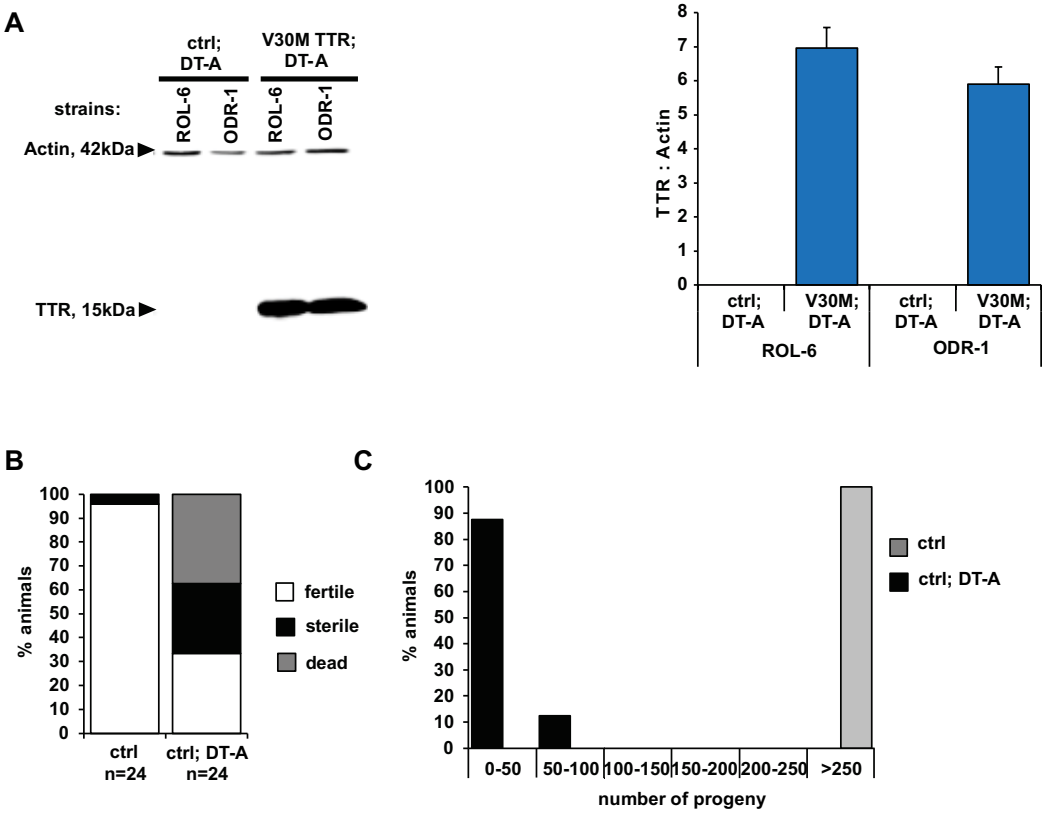

Fig. S10. Characterization of TTR protein expression levels, and viability of transgenic animals with and without coelomocytes, Related to Fig. 6. (A) Western blot (left) and quantification (right) (mean ± s.e.m.) of TTR protein levels in the soluble lysates of control and V30M TTR strains without coelomocytes (DT-A) that were generated with *rol-6(su1006)* versus *odr-1p*::RFP as co-injection markers. Membranes were probed with the monoclonal MDX102 antibody against TTR. Images are representative of 2 independent experiments. (B) Viability and fecundity analyses of animals singled out as L1s and scored as young adults (day 1 adulthood) for fertility, sterility, and viability. (C) Percentage (brood size) of animals that hatched and developed at least until the L3 larval stage from fertile parents in (B); ctrl n = 8; ctrl; DT-A n = 23, ctrl = control. The observed reduction in nociception in the control; DT-A strains in Fig. 6C could be due to the generalized toxicity exhibited as a reduction in brood size and the number of viable and fertile animals compared to control animals.

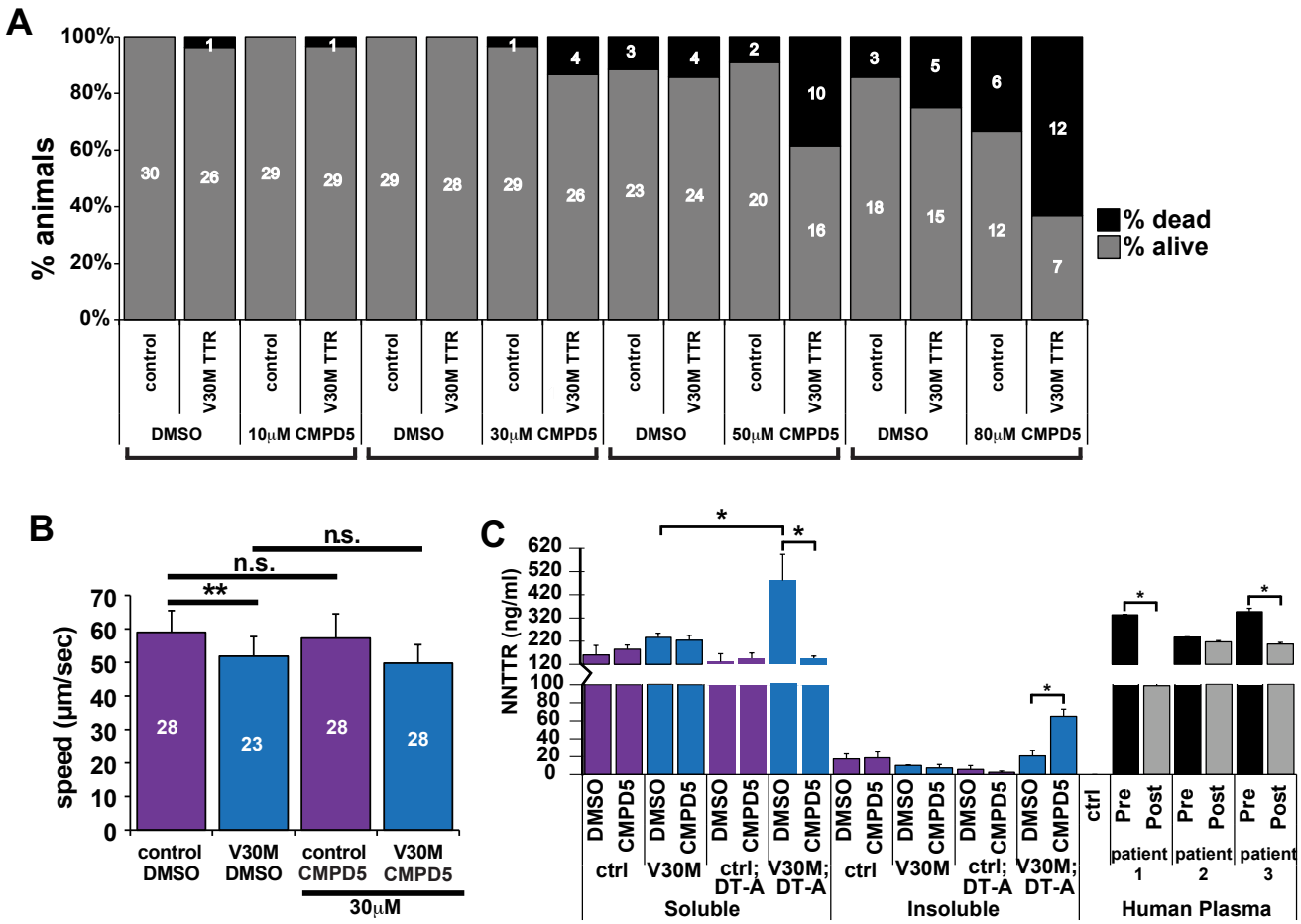

Fig. S11. Toxicity assay of V30M TTR animals treated with CMPD5, Related to Fig. 7. (A) Percentage of animals dead or alive after treatment with the same volume of vehicle (DMSO) and/or increasing concentrations of **CMPD5.** Animals were treated in liquid culture from L3 to adult day 1 and were scored on day 2 of adulthood. Brackets indicate experimental conditions with the same concentration of DMSO. (B) Locomotion rates for adult day 2 strains treated with 30 μM **CMPD5** and DMSO. Numbers of animals analyzed for each strain are shown inside bars. Plot is representative of 1 experiment (mean ± s.e.m., * $p < 0.005$, by Student's t-test). (C) Quantification of non-native (NN) TTR oligomer levels by a sandwich ELISA assay from lysates of day 2 TTR animals, and treated or not with **CMPD5** at the L3 larval stage. Data plotted shows mean ± s.e.m., n=3, * $p < 0.005$, by Student's t-test. Three human patient samples pre- and post-tafamidis treatment were included as positive controls for this assay. ctrl = control.

Table S1: Quantification of nociception defect in day 1 adult TTR transgenic animals.
[a]Student t-test.

		% animals displaying no avoidance response (Class IV)	Total # animals	# replicates	Difference from control[a]	Difference from V30M TTR[a]
eat-4(n2474)		47 ± 8.6	100	2	*** p<0.0001	-
unc-86(n846)		7 ± 2.2	85	2	*** p<0.0001	-
control		0	486	6	-	*** p<0.0001
unc-54p	**V30M TTR**	10 ± 0.6	577	6	*** p<0.0001	-
	T119M TTR	0	188	3	n.s.	*** p<0.0001
	D18G TTR	1.3 ± 1.3	148	2	*** p<0.0001	*** p<0.0003
	V30M ΔSS TTR	2.6 ± 0.5	310	3	*** p<0.0001	*** p<0.0003
des-2p	**V30M ΔSS TTR**	0	90	2	-	*** p<0.0001

Table S2: Quantification of nociception defect in day 1 adult TTR transgenic animals treated with RNAi against TTR.
[a]Student t-test.

	% animals displaying no avoidance response (Class IV)	Total # animals	# replicates	Difference from control[a]	Difference from V30M TTR[a]
control + E.V.	0	106	2	-	*** p<0.0001
control + TTR RNAi	0	111	2	n.s.	*** p<0.0001
V30M TTR + E.V.	9.5 ± 0.4	148	2	*** p<0.0001	-
V30M TTR + TTR RNAi	0	158	2	n.s.	*** p<0.0001

Table S3: Quantification of 4° FLP dendritic branches in day 1 adult animals.

Angle	# branches in control animals (n=19)	# branches in V30M animals (n=18)	# branches in T119M animals (n=18)
0°-30°	0.4	1.2	0.0
31°-60°	0.0	7.8	0.7
61°-90°	14.7	20.0	16.7
91°-120°	63.2	54.7	66.7
121°-150°	19.7	14.9	16.0
151°-180°	2.0	1.4	0.0

Table S4: Quantification of % 4° FLP dendritic branches in L4 animals.

Angle	# branches in control animals (n=19)	# branches in V30M animals (n=19)	# branches in T119M animals (n=15)
0°-30°	0	0	0
31°-60°	0	0	0
61°-90°	21.9	12.5	22.9
91°-120°	61.8	78.9	72.2
121°-150°	16.2	8.5	4.8
151°-180°	0	0	0

Table S5: Number of coelomocytes in day 2 adult animals as quantitated using dextran uptake assays.
[a] See List of Strains above for full strain genotypes.

Strain Name	Abbreviated Strain Name[a]	# animals Injected with dextran-A488	# animals with dextran-A488 in coelomocytes after 24 hrs
SEE064	control	9	9
SEE061	DT-A	11	0
SEE079	control; DT-A	8	0
SEE080	WT TTR; DT-A	12	0
SEE128	V30M TTR; DT-A	9	0
SEE168	T119M TTR; DT-A	9	0
SEE221	D18G TTR; DT-A	18	0

Table S6: Quantification of nociception defect in day 1 adult TTR DT-A transgenic animals.
[a]Student t-test.

	% animals displaying no avoidance response (Class IV)	Total # animals	# replicates	Difference from control[a]	Difference from V30M TTR[a]
control	0	486	6	-	*** p<0.0001
V30M TTR	10 ± 0.6	577	6	*** p<0.0001	-
control; DT-A	9.1 ± 2.5	143	2	*** p<0.0001	n.s.
V30M TTR; DT-A	22 ± 2	196	2	*** p<0.0001	*** p<0.0001

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