AAN - April 23 2023

ProMIS™ Neurosciences

RACK1 Knockdown is a Potential Therapeutic Target in ALS and FTLD-TDP

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Disclosure

Drs. NRC, JK and BZ are employees of ProMIS Neurosciences

NRC is a Professor Emeritus at the University of British Columbia

Introduction

- The Receptor of Activated C-Kinase 1 (RACK1) is a ribosomal protein that co-aggregates with pathogenic TDP-43 cytoplasmic inclusions characteristic of ALS and FTLD-TDP, participating in consolidation of TDP-43 aggregation and impaired global protein translation.

- The objective of the current study was to assess the potential of RACK1 as a therapeutic target in models of TDP-43 proteinopathy.

Design/Methods

TDP-43 proteinopathy was modeled *in vitro* by transfection of an HA-tagged nuclear localization signal defective mutant of TDP-43, "dNLS-TDP-43", in HEK293T cells. Morphology and localization of dNLS-TDP-43 aggregates were detected by an HA antibody, and global protein translation was measured by Surface Sensing of Translation (SUnSET) assay. *In vivo*, the *Drosophila melanogaster* expression system (UAS-Gal4) was used to direct expression of hTDP-43^{WT} or hTDP-43^{Q331K} to retinal or motor neurons, with and without RACK1 knockdown*.

- ***Retinal degeneration*** in flies was measured according to the scoring system published[1]: 0= normal; 1= <25% ommatidia loss; 2= 25-50% ommatidia loss; 3= 50-75% ommatidia loss with small regions of necrosis (black patches); 4= >75% ommatidia loss with massive regions of necrosis.

- ***Motor function*** was scored using a modification of a previously published system[2] wherein the height climbed by each fly in 10 seconds was measured every week for 28 days.

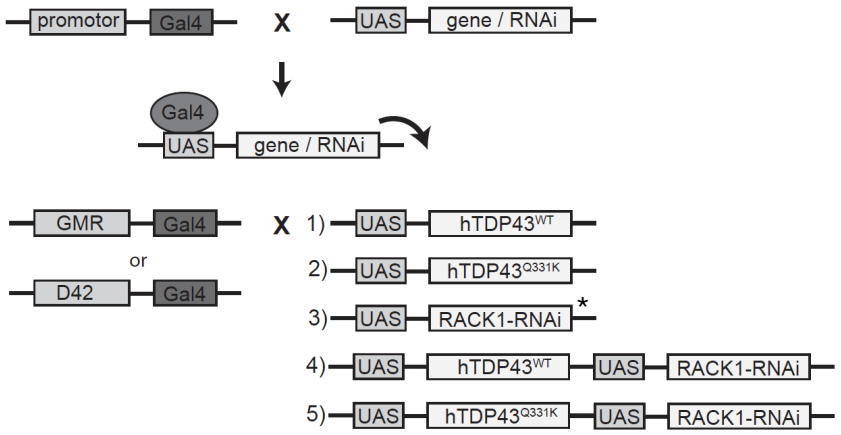


Fig. 1: Schematic illustration for the construction of transgenic *D. melanogaster* lines specifically targeting retinal (GMR-driven) or motor (D42-driven) neurons.

[1]Li Y, Ray P, Rao E et al, PNAS 2010;

[2]Elden A, Kim H-J, Hart M et al, Nature 2010

**The RACK1-RNAi line used in the current study may also affect another gene, "wds".*

RACK1 co-aggregates with TDP-43 in ALS spinal cords

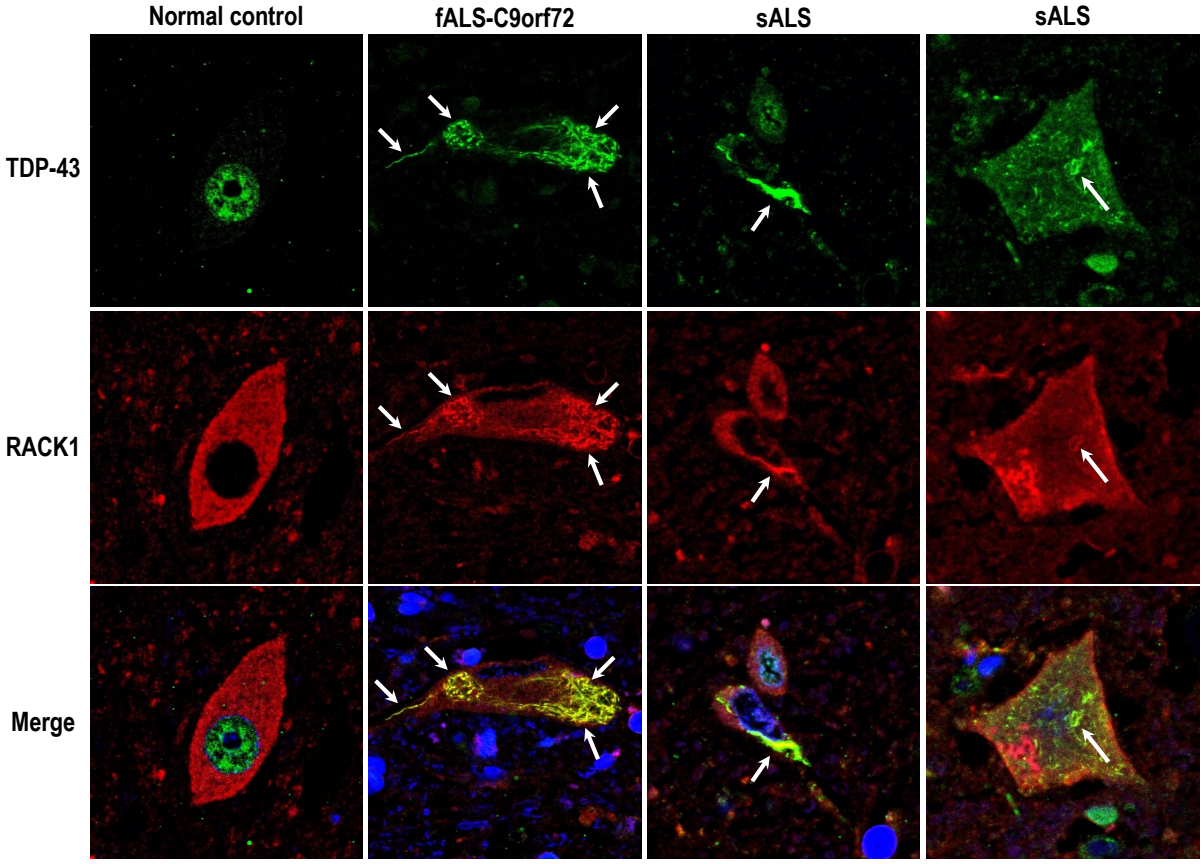


Fig. 2: Immunohistochemical analysis with of post-mortem spinal cord sections with pan-RACK1 antibody showing that RACK1 co-aggregates with TDP-43 cytoplasmic inclusions in C9orf72-linked familial ALS (fALS-C9orf72) and sporadic ALS (sALS) (*arrows*).

Misfolded RACK1 is present in sALS spinal cords and FTLD-TDP brains

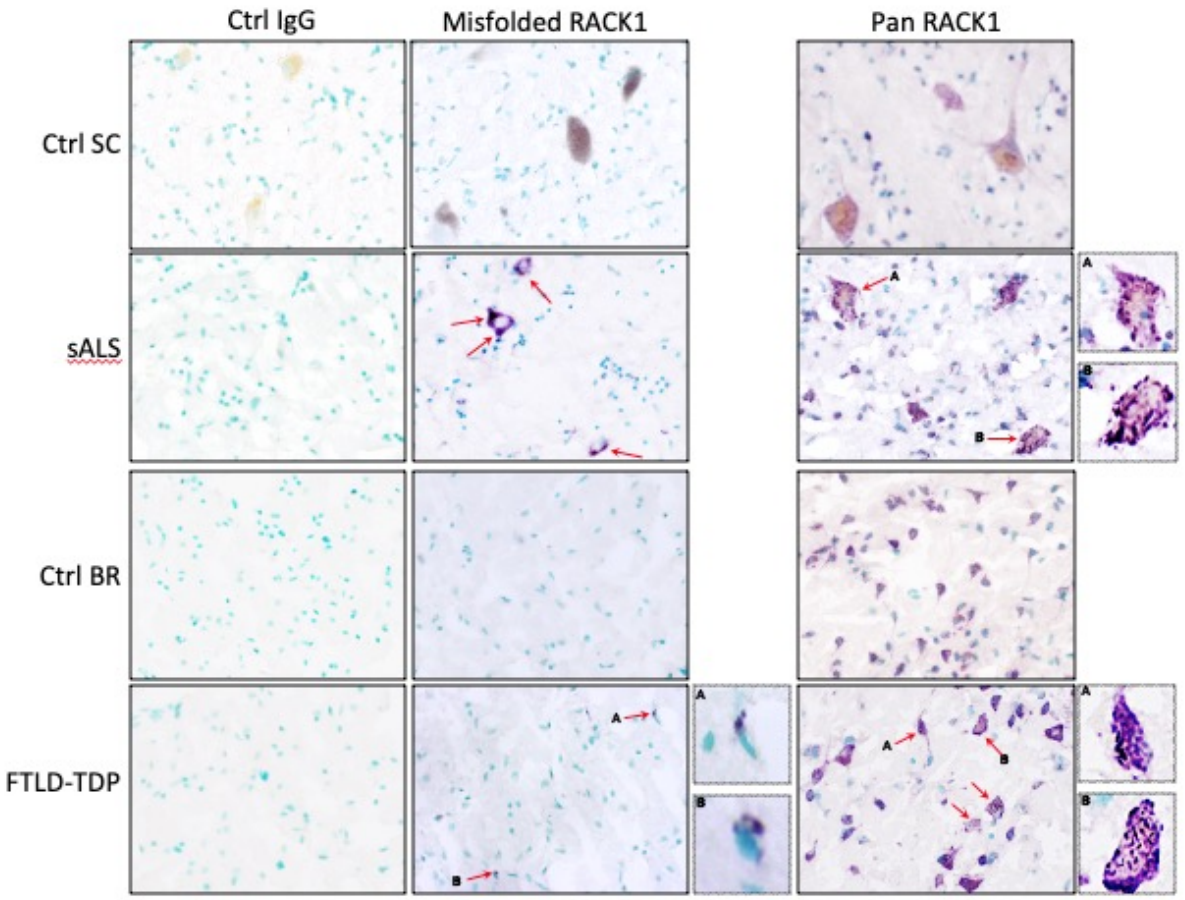


Fig 4. Immunohistochemical analysis demonstrates the immunoreactivity of RACK1 misfolding-specific monoclonal antibody (RACK1^{mis} mAb) developed by ProMIS Neurosciences, showing cytoplasmic aggregates in sporadic ALS spinal cord (sALS SC) and FTLD-TDP brain (BR) (*arrows*), but not in corresponding control (Ctrl) tissue. Pan RACK1 antibody reacts with both aggregated and normal RACK1 in all tissues.

Misfolded RACK1 is specifically present in dNLS-TDP-43/RACK1 co-aggregates *in vitro*

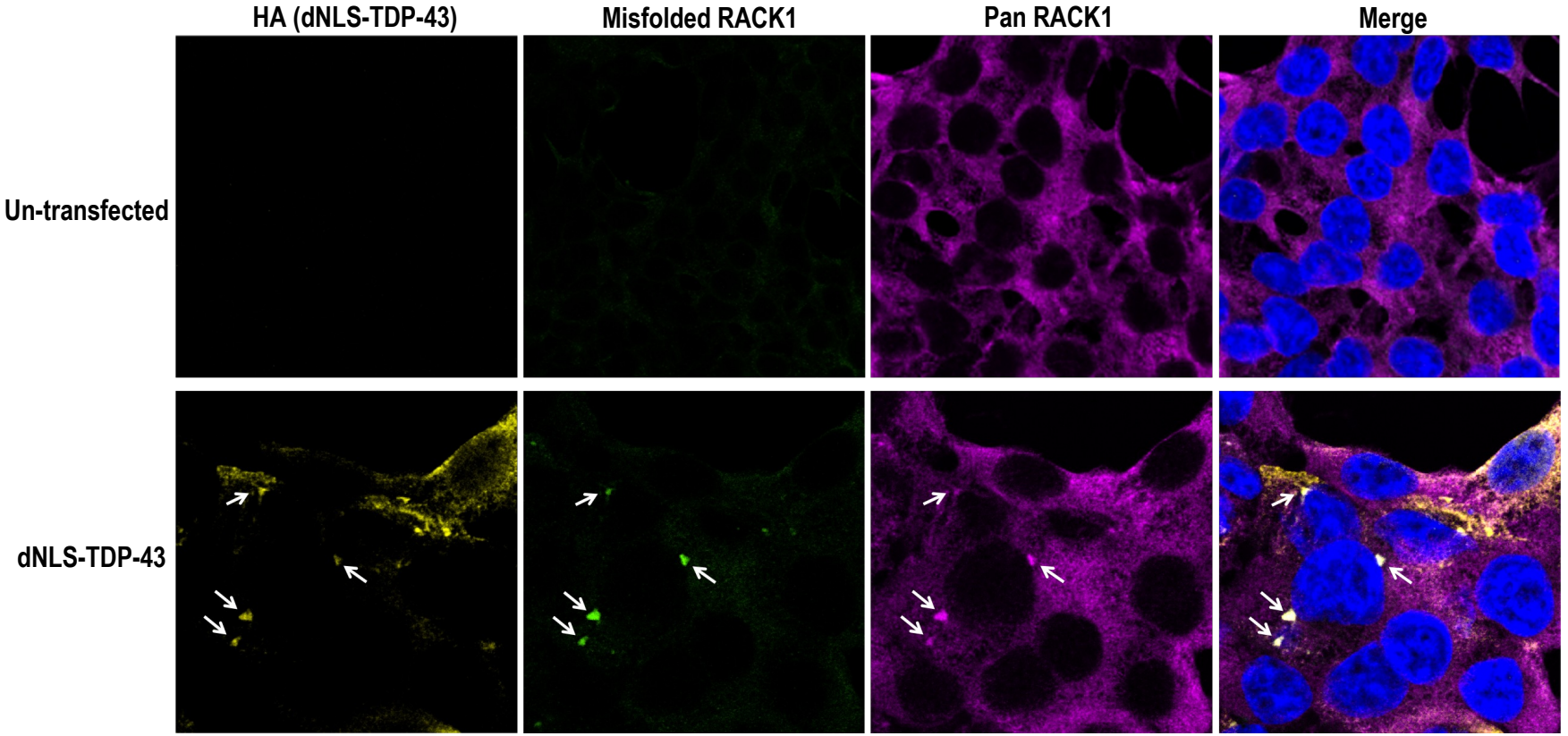
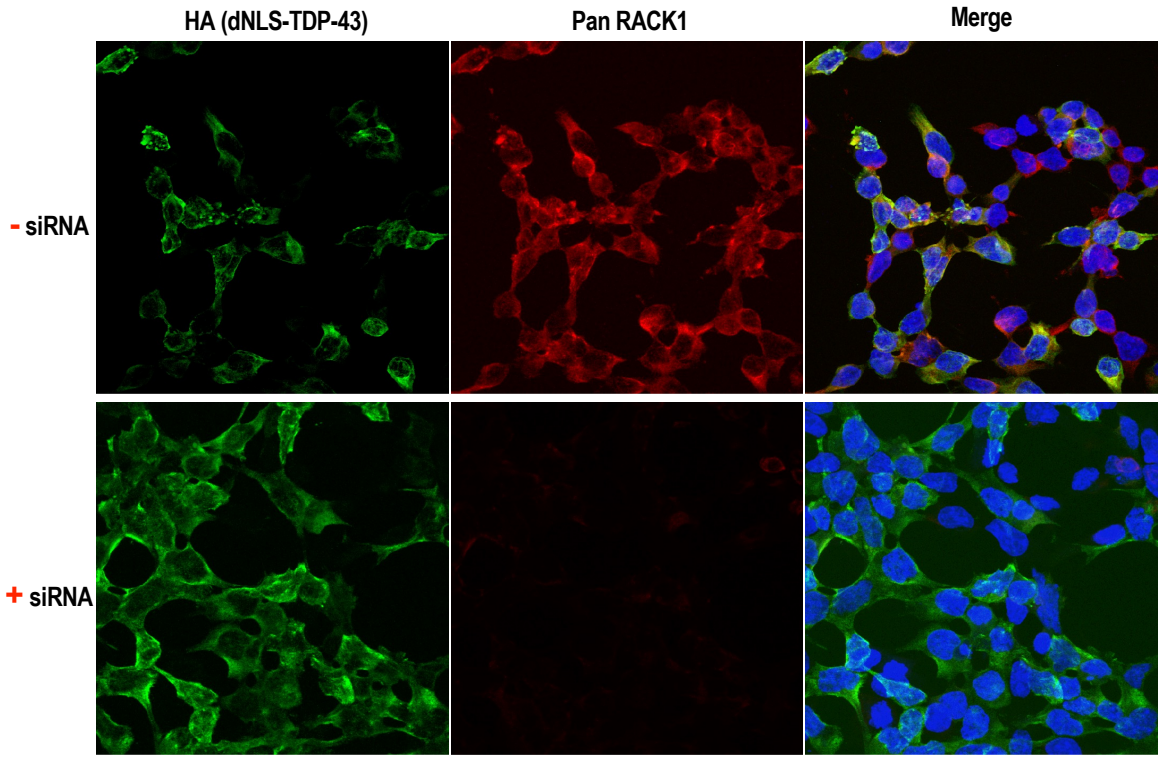


Fig 3. Immunocytochemical analysis performed using a commercial pan RACK1 antibody and ProMIS RACK1^{mis} mAb. The RACK1^{mis} mAb does not react with normal diffuse RACK1 in the cytoplasm but only with aggregates formed in HEK293T cells transfected with dNLS-TDP-43, demonstrating co-aggregation of misfolded RACK1 and dNLS-TDP-43.

RACK1 knockdown reduces dNLS-TDP-43 aggregation



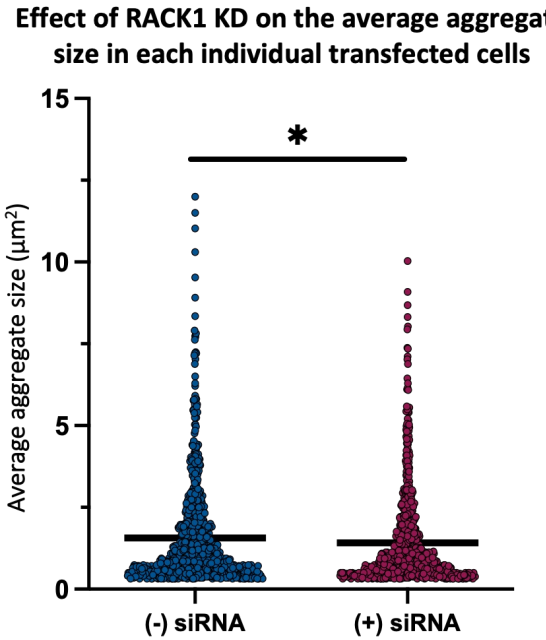
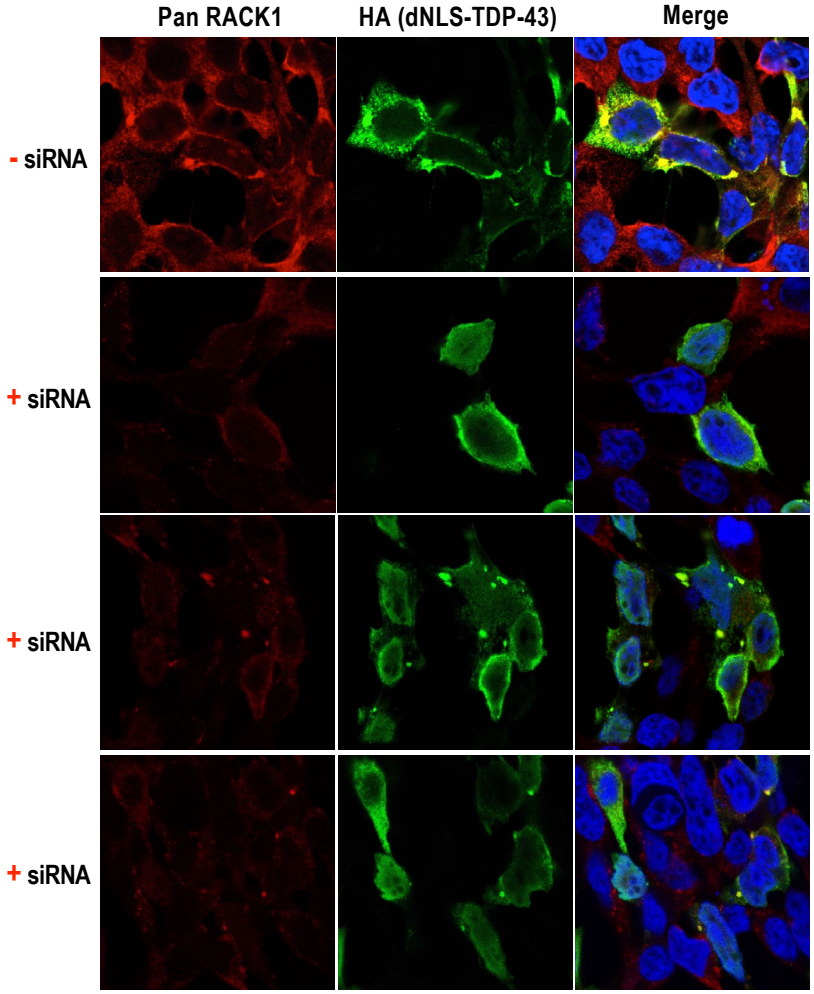


Fig. 5 RACK1 knock-down (KD) alleviates dNLS-TDP-43 aggregation in the cytoplasm of HEK293T cells, resulting in more diffuse staining and a reduction in the average aggregate size. Each dot in the graph represents the average aggregate size in a particular HA-dNLS-TDP-43 expressing cell. Z-stack images were acquired on a Leica TCS SP8 MP confocal microscope using a 63x oil objective. *Statistics*: Student *t-test* two-tailed unpaired. n=6 *p<0.05.

RACK1 knockdown surprisingly promotes nuclear localization of dNLS-TDP-43



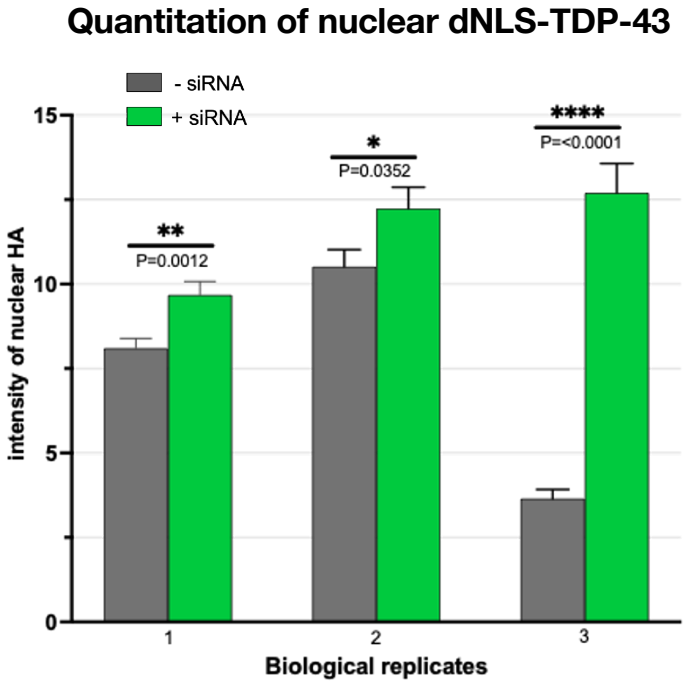


Fig. 6 RACK1 KD induces unexpected nuclear localization of dNLS-TDP-43 (despite its lack of a functional nuclear localization signal) in a sub-population of transfected HEK293T cells. Z-stack images were acquired on a Leica TCS SP8 MP confocal microscope using a 63x oil objective. *Statistics*: Student *t-test* two-tailed unpaired. 5-6 z-stacked images were acquired for each biological repeat. Error bars: SEM.

RACK1 knockdown restores dNLS-TDP-43 induced global translational suppression

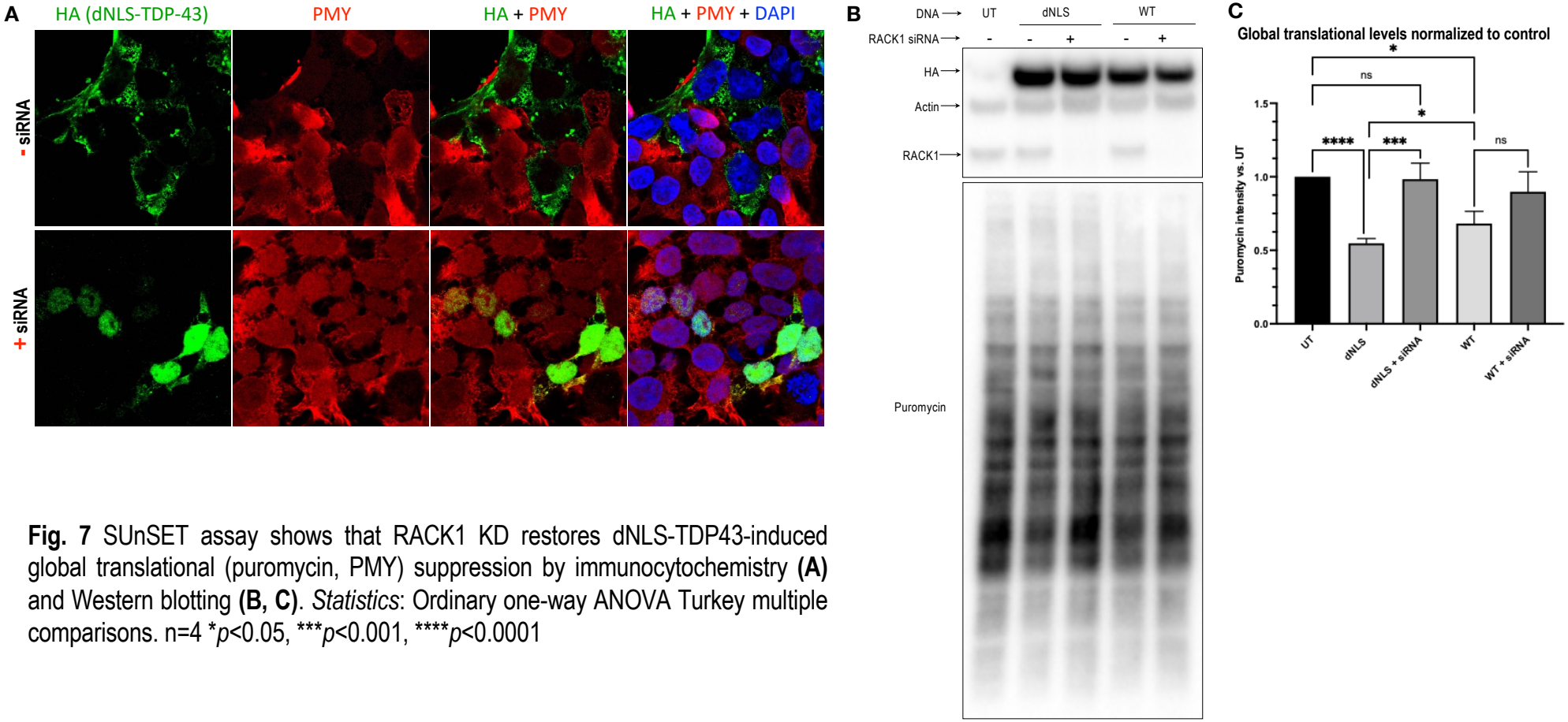


Fig. 7 SUnSET assay shows that RACK1 KD restores dNLS-TDP43-induced global translational (puromycin, PMY) suppression by immunocytochemistry **(A)** and Western blotting **(B, C)**. *Statistics*: Ordinary one-way ANOVA Turkey multiple comparisons. n=4 *p<0.05, ***p<0.001, ****p<0.0001

1) RACK1 knockdown ameliorates retinal neurodegeneration *in vivo*

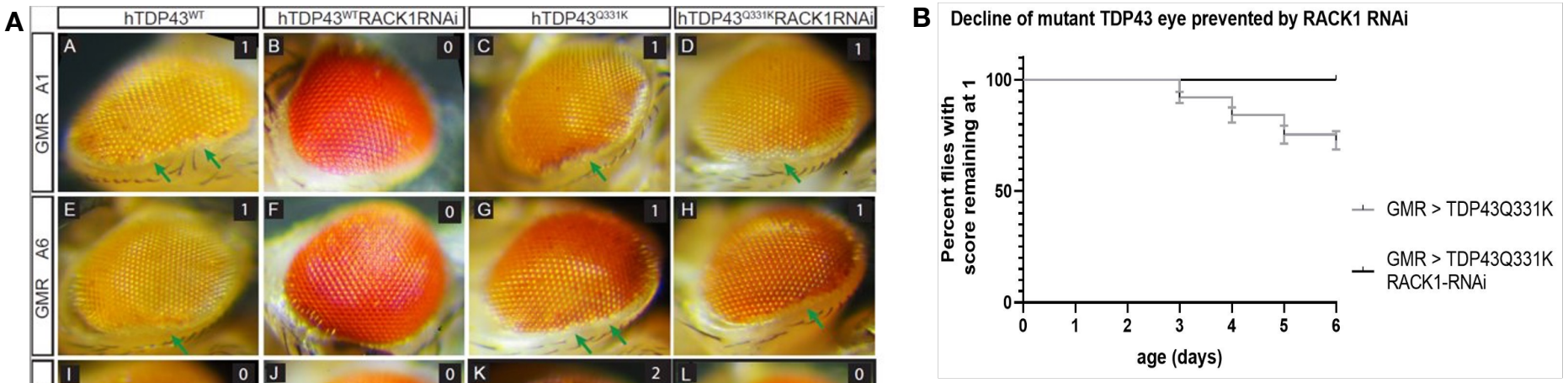


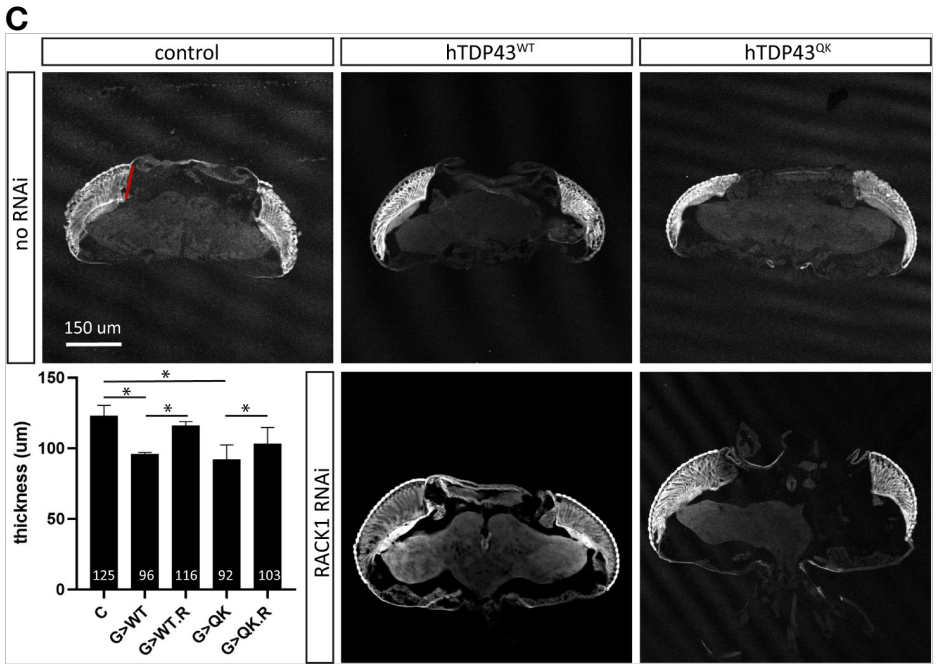
Fig. 8 (A) *D. melanogaster* eyes at adult day 1 or 6 (A1, A6) were scored (top right corner of each panel) for retinal neurodegeneration*. Phenotypes of retinal neurodegeneration: 1) ommatidia are often missing from the ventral margin (*arrows*); and 2) darker dots of dying ommatidia. *Statistics:* Chi^2 test.

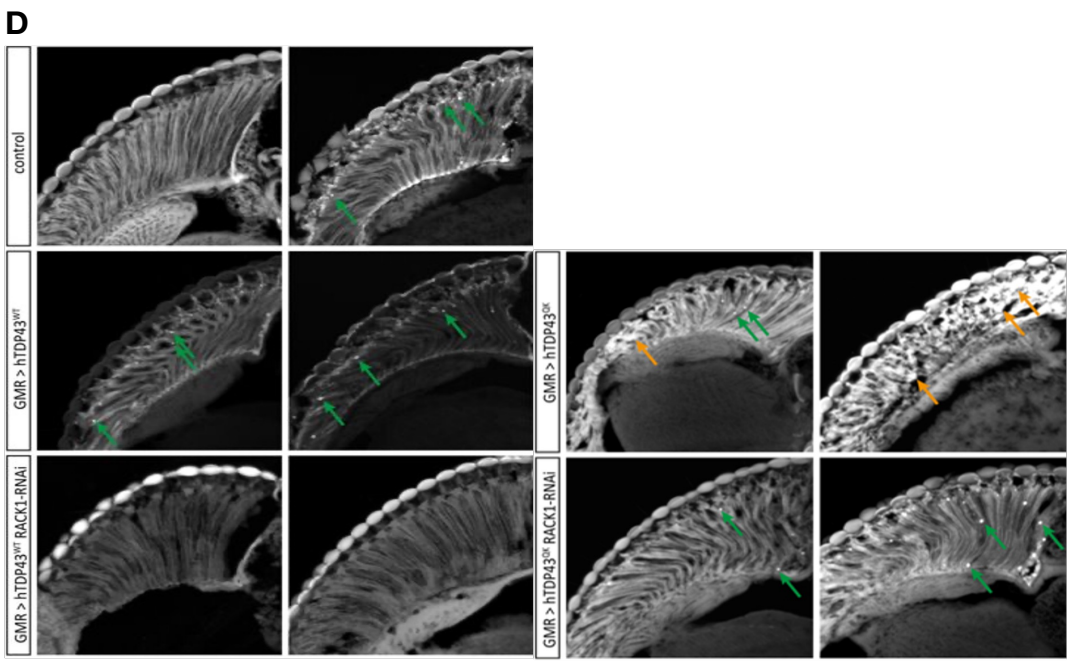
- hTDP-43^{WT}: mild neurodegeneration from A1 to A6 (score = 1, $p<0.0001$) (*panel A & E*)
- hTDP-43^{WT}/RACK1-RNAi: normal (score = 0, $p<0.0001$) (*panel B & F*)
- hTDP-43^{Q331K}: more severe neurodegeneration than hTDP-43^{WT} at A1, worsening over time (score = 1 → 2, *panel C, G, & K*, $p<0.0001$)
- hTDP-43^{Q331K}/RACK1-RNAi: score remains "1" from A1-A6 (panel *D & H*, $p<0.01$)
- RACK1-RNAi alone: comparable to control (score = 0, *panel I, J, & L*)

(B) hTDP-43^{Q331K}/RACK1-RNAi: score remains "1" from A1-A6 (Log-rank test, $p<0.01$). Error bars: 95% confidence intervals.

**Described in "Design/Methods".*

2) RACK1 knockdown ameliorates retinal neurodegeneration *in vivo*





(C) Histological analysis of retinal thickness at A6. Retina thickness significantly reduced by hTDP-43^{WT} and hTDP-43^{Q331K}, both of which are improved by RACK1-RNAi (One-way ANOVA, n=4, *p<0.05);

(D) Structural organization and pigmentation of the retina are disrupted by hTDP-43^{WT}, and markedly further by hTDP-43^{Q331K}, both of which are improved by RACK1-RNAi. Small puncta of retinal pigment (*green arrows*) are sometimes observed in control and often in hTDP-43^{WT}. Large puncta (*orange arrows*) are seen on expression of hTDP-43^{Q331K}. RACK1-RNAi reduces both types of puncta in both hTDP-43 transgenic fly eyes.

RACK1 knockdown ameliorates TDP-43 motor neuron impairment *in vivo*

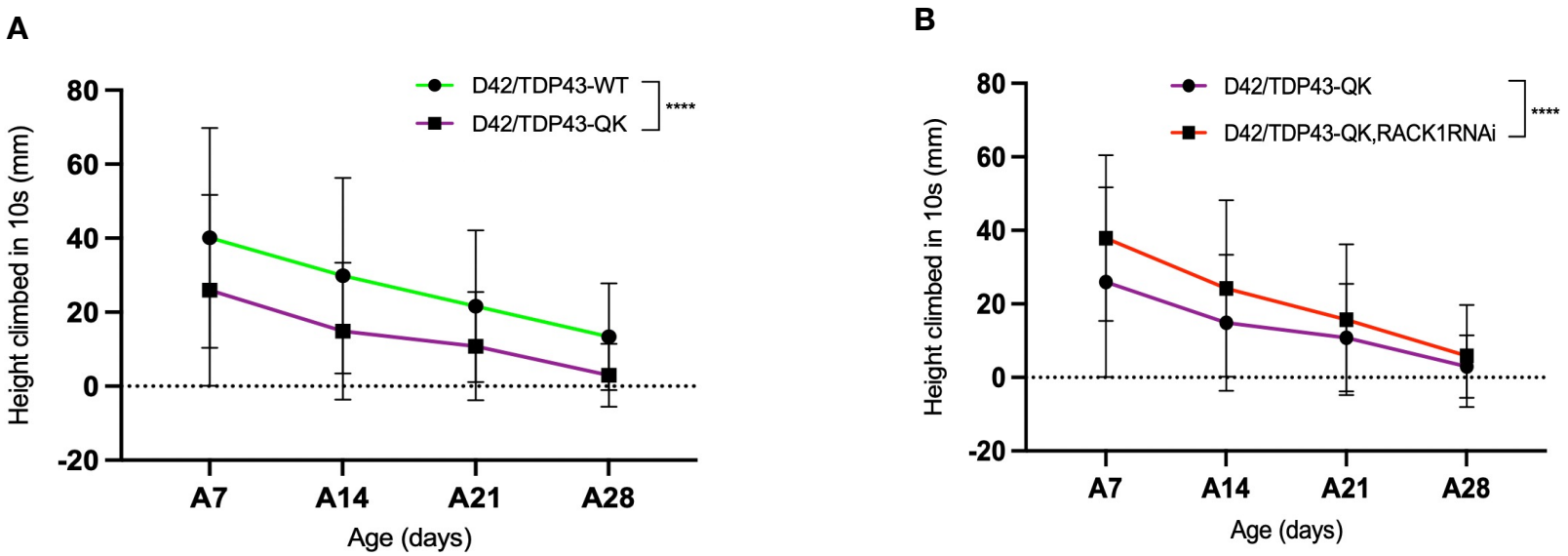


Fig. 9 (A) hTDP-43^{Q331K} causes more severe motor defect than hTDP-43^{WT}. 83-140 flies were scored; **(B)** RACK1 RNAi KD improves the motor defect caused by hTDP-43^{Q331K}. 79-95 flies were scored. *Statistics*: two-way ANOVA, ****p<0.0001. Error bars: SD.

Summary

- ProMIS monoclonal antibodies selective for misfolded RACK1 (RACK1^{mis} mAb) showed coaggregation of RACK1 and TDP-43 in ALS and FTLD-TDP, and in cell culture;
- RACK1 knockdown alleviated TDP-43 aggregation as well as associated global translational suppression in cultured cells;
- RACK1 knockdown was well-tolerated in cultured cells in vitro and D. melanogaster neurons in vivo;
- RACK1 knockdown ameliorated retinal and motor neuron neurodegeneration in D. melanogaster in vivo;
- These findings support RACK1 knockdown as a potential therapeutic approach for TDP-43 proteinopathy in non-SOD1 and non-FUS ALS, and FTLD-TDP.

Acknowledgments

University of British Columbia

Neil Cashman
Catherine Cowan
Ananya Saraph
Juliane Coutts
Darren Christy
Ian Mackenzie

ProMIS Neurosciences

Neil Cashman
Johanne Kaplan
Beibei Zhao

University of Toronto

Janice Robertson