RACK1 Knockdown Alleviates TDP-43 Associated Global Translational Suppression *in vitro*, and Neurodegeneration *in vivo*.

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INTRODUCTION

The receptor for activated C kinase 1 (RACK1) is a well-conserved scaffold protein with >100 recognized activities (1). Co-aggregation of RACK1 with TDP-43 inclusions has been observed in sporadic ALS (2), suggesting that it may be part of a pathogenic interactome involving the 2 proteins. While RACK1 knock-out is embryonic lethal in mice (3), we hypothesized that modulation of RACK1 through knockdown in mature cells and *D. melanogaster* (fruit flies) expressing pathogenic TDP-43 may provide a functional benefit.

DESIGN/METHODS

- ***Cell culture and transfection.*** RACK1 knockdown was achieved by transfecting a pool of 3 target-specific siRNA plasmids against human RACK1 using Lipofectamine RNAiMAX. HA-tagged cDNA plasmids encoding wild-type or dNLS-TDP43 (K82A/R83A/K84A) were transfected using Lipofectamine LTX.
- ***Surface Sensing of Translation (SUnSET)*** was performed as previously described (2), followed by immunocytochemical or biochemical detection of newly synthesized proteins using a puromycin-specific antibody.
- ***Construction of RACK1 RNAi hTDP43 transgenic D. melanogaster lines.*** *UAS-Gal4* system was used to target expression of previously published alleles hTDP-43^{WT} and hTDP-43^{Q331K} (3) to retinal (GMR-driven) and motor (D42-driven) neurons (**Fig. 1**), cell populations widely used for their read-out of neuronal degeneration.
- ***Retinal degeneration*** in flies was scored according to the system published (4): 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 (3) wherein the height climbed by each fly in 10 seconds was measured every week for 28 days.

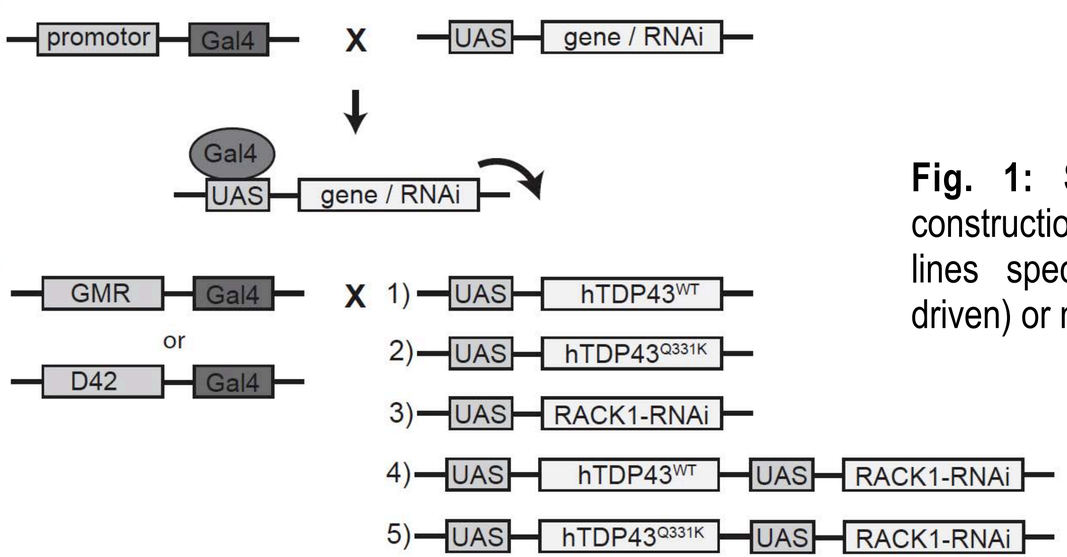


Fig. 1: Schematic illustration for the construction of transgenic *D. melanogaster* lines specifically targeting retinal (GMR-driven) or motor (D42-driven) neurons.

RESULTS

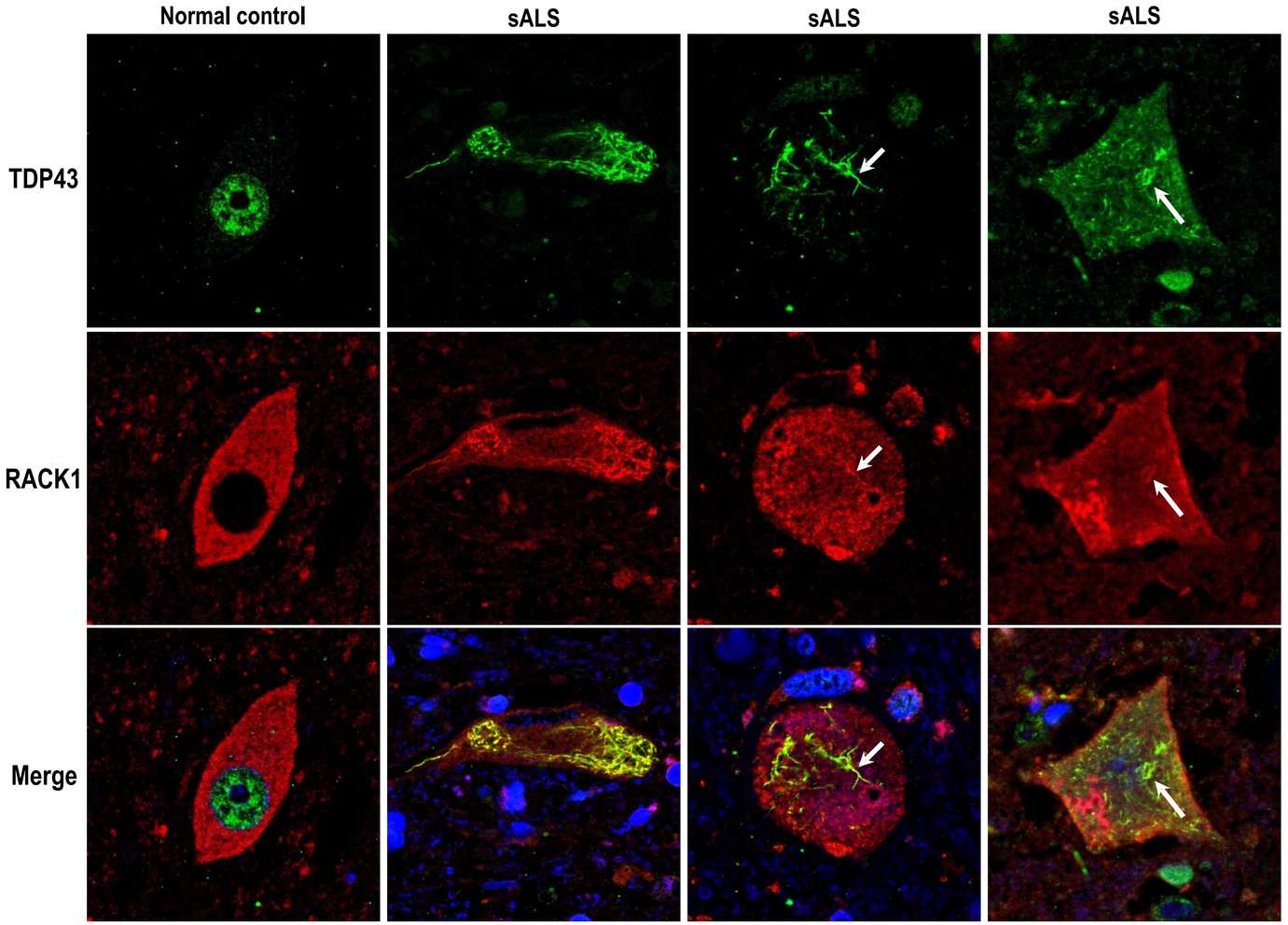
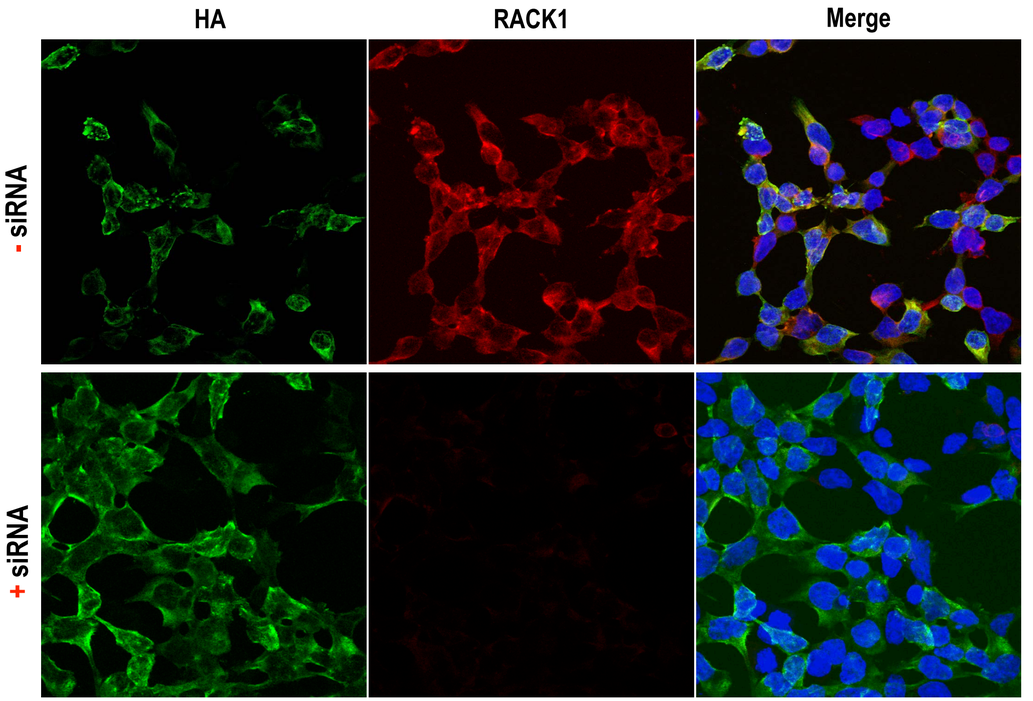


Fig. 2: Immunohistochemical analysis of post-mortem spinal cord sections confirms that RACK1 co-aggregates with TDP-43 cytoplasmic inclusions (*arrows*) in sporadic ALS, as previously published (2).



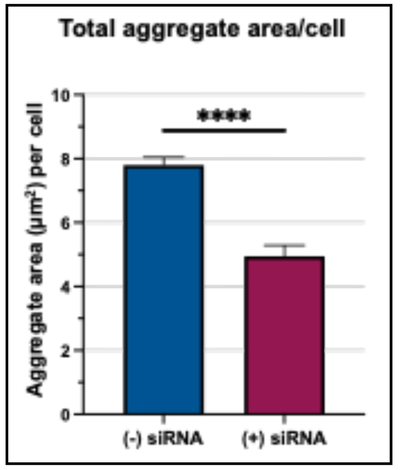


Fig. 3 RACK1 KD alleviates dNLS-TDP43 (HA) aggregation. Z-stack images were acquired on a Leica TCS SP8 MP confocal microscope using a 63x oil objective to capture the full volume of cells. Statistics: Student *t-test* two-tailed unpaired. N=6 *p*-value ****: < 0.0001 Error bars: SEM.

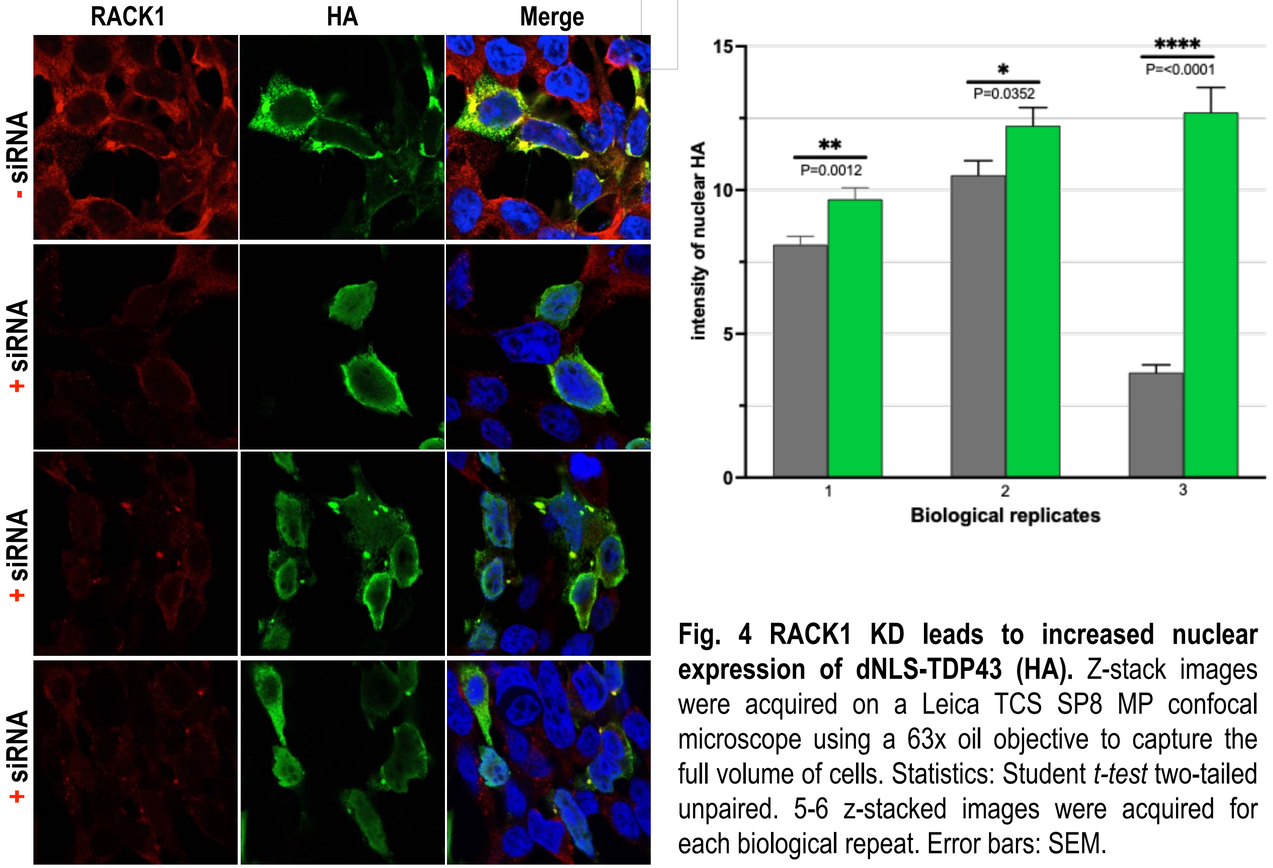


Fig. 4 RACK1 KD leads to increased nuclear expression of dNLS-TDP43 (HA). Z-stack images were acquired on a Leica TCS SP8 MP confocal microscope using a 63x oil objective to capture the full volume of cells. Statistics: Student *t-test* two-tailed unpaired. 5-6 z-stacked images were acquired for each biological repeat. Error bars: SEM.

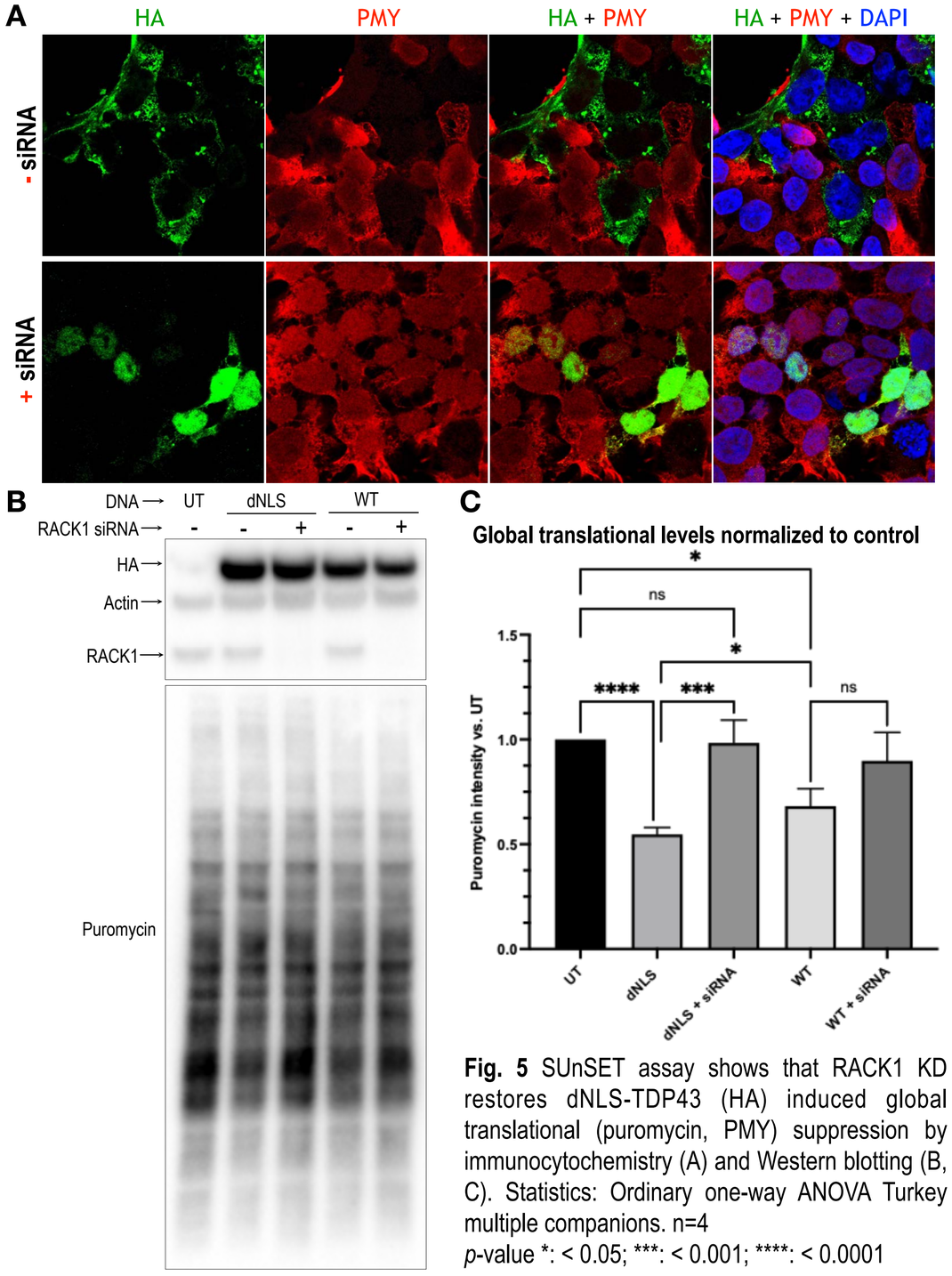


Fig. 5 SUnSET assay shows that RACK1 KD restores dNLS-TDP43 (HA) induced global translational (puromycin, PMY) suppression by immunocytochemistry (A) and Western blotting (B, C). Statistics: Ordinary one-way ANOVA Turkey multiple companions. n=4
p-value *: < 0.05; ***: < 0.001; ****: < 0.0001

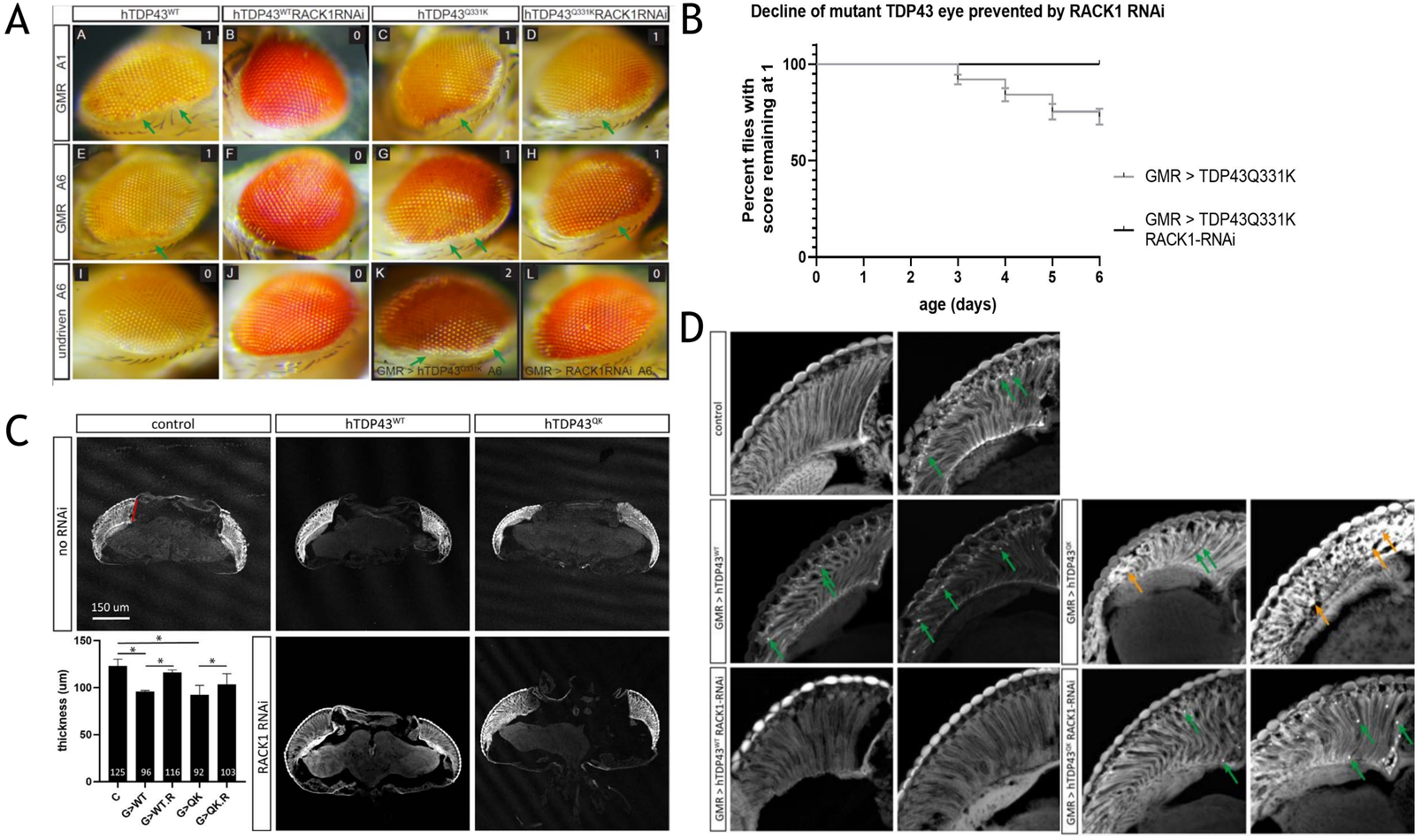


Fig. 6 RACK1 KD ameliorates retinal neurodegeneration in GMR-driven hTDP43^{WT} and hTDP43^{Q331K} transgenic *D. melanogaster.* **(A)** Representative photos of fly eyes, transgene expression shown at adult day 1 (A1, A-D) or A6 (E-H,K,L) and un-driven controls shown at A6 (I,J). In eyes displaying mild degeneration, ommatidia are often missing from the ventral margin (*arrows*). Additionally, darker dots of dying ommatidia can be observed. hTDP43^{WT} causes mild neurodegeneration at A1 and persists to A6 (Chi^2 test, p<0.0001). This is fully rescued by RACK1-RNAi (p<0.0001). hTDP43^{Q331K} causes more severe neurodgeneration than hTDP43^{WT} at A1, worsening over time (p<0.0001). In contrast, in eyes co-expressing RACK1-RNAi and hTDP43^{Q331K}, degeneration remains mild from A1-A6 (p<0.01). RACK1-RNAi alone (L) is comparable to control. Neurodegeneration scores are shown in the top right corner of each panel. **(B)** Kaplan-Meier curve shows the percentage of flies whose score remains at 1 on any given day. RACK1-RNAi significantly improves neurodegeneration caused by hTDP43^{Q331K} (Log-rank test: p=0.002. Error bars: 95% confidence intervals). **(C)** Histological analysis of retinal thickness of transgenic flies at A6 shows that retina thickness is significantly reduced by hTDP43^{WT} and hTDP43^{Q331K}, both of which are improved by RACK1-RNAi (ANOVA, $p<0.05$, n=4). **(D)** Structural organization and pigmentation of the retina are disrupted by hTDP43^{WT}, and markedly further by hTDP43^{Q331K}, both of which are improved by RACK1-RNAi. Small puncta of retinal pigment (*green arrows*) are sometimes observed in control and often in hTDP43^{WT}. Additionally, large puncta (*orange arrows*) are seen on expression of hTDP43^{Q331K}. RACK1-RNAi reduces both types of puncta in both hTDP43 transgenic fly eyes.

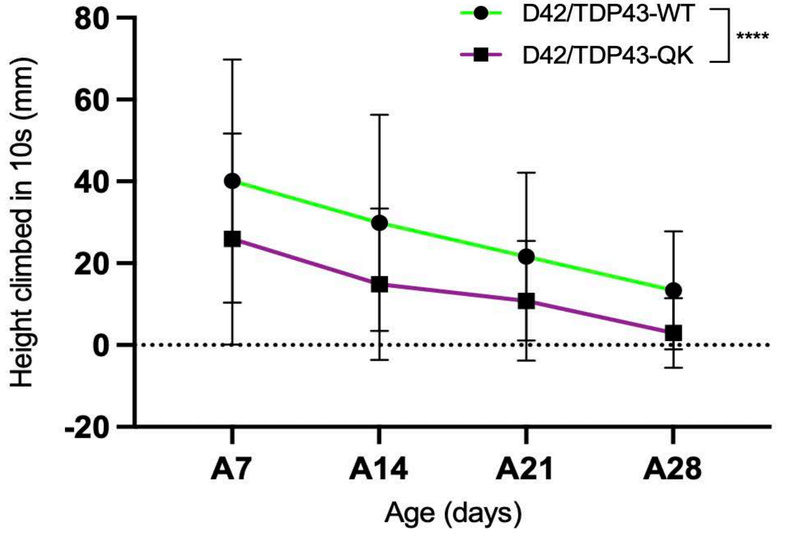


Fig 7. hTDP-43^{Q331K} expression causes more severe motor defect than hTDP-43^{WT} in flies. Statistics: two-way ANOVA, p<0.0001. 83-140 flies were scored. Error bars: SD.

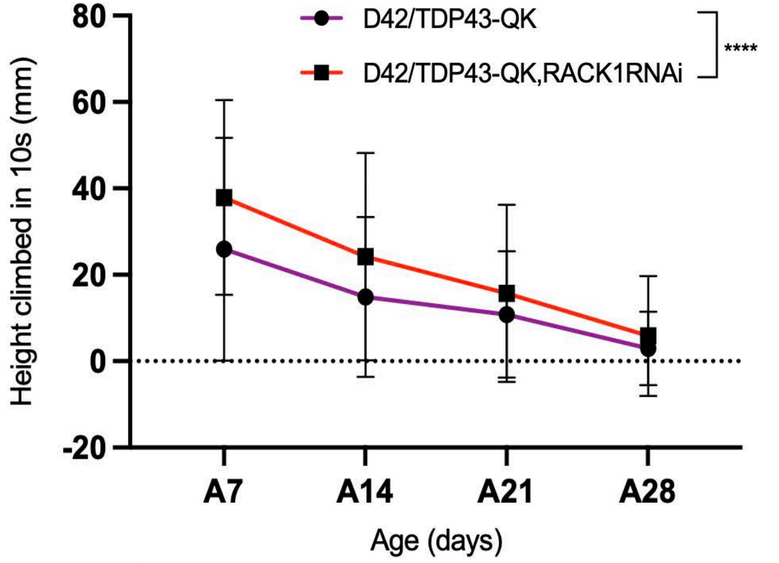


Fig 8. RACK1 RNAi KD improves the motor defect caused by hTDP-43^{Q331K} expression in flies. Statistics: two-way ANOVA, p< 0.0001. 79-95 flies were scored. Error bars: SD.

DISCUSSION

Our results are consistent with the existence of a pathogenic interaction between TDP-43 and RACK1 in misfolded aggregates and support targeting of RACK1 to alleviate TDP-43 proteinopathy.

REFERENCES

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