Ankersmit Laboratory

for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Regulation of axon regeneration by the RNA repair and splicing pathway.

Nature neuroscience. 2015;18(6):817-25.

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Introduction

Regeneration associated genes

- limited potential of regeneration of injured neurons in the CNS
- hundreds of regeneration associated genes (RAGs) and transcription factors (TF) in peripheral nerves
- very limited/no RAG response in CNS
- Small effects on forced RAG-expression in the CNS

van Kesteren RE, Mason MRJ, MacGillavry HD, Smit AB, Verhaagen J. A Gene Network Perspective on Axonal Regeneration. Frontiers in Molecular Neuroscience. 2011;4:46.

Introduction

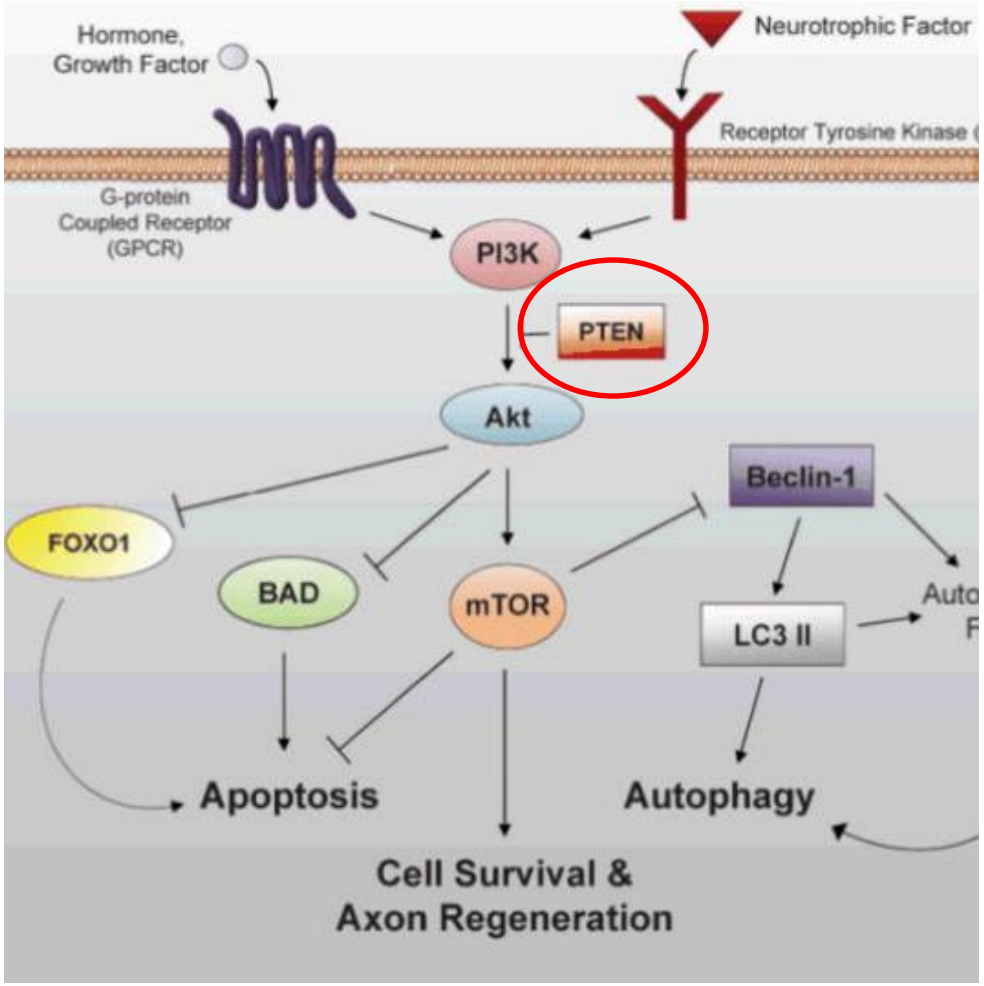
Regeneration associated genes

- RAGs: GAP-43, Sprr1a, Itga7
- Manipulation of RAGs does not enhance CNS regeneration → identification of TF hub-genes
- TFs: C-JUN, ATF3, SOX11, CREB, p53, STAT3, KLF4, SMAD1

Introduction

Regeneration regulators

- **Pten** = Phosphatase and tensin homolog
- A negative regulator of mTOR pathway
- Deletion has shown increase in CNS axon regeneration

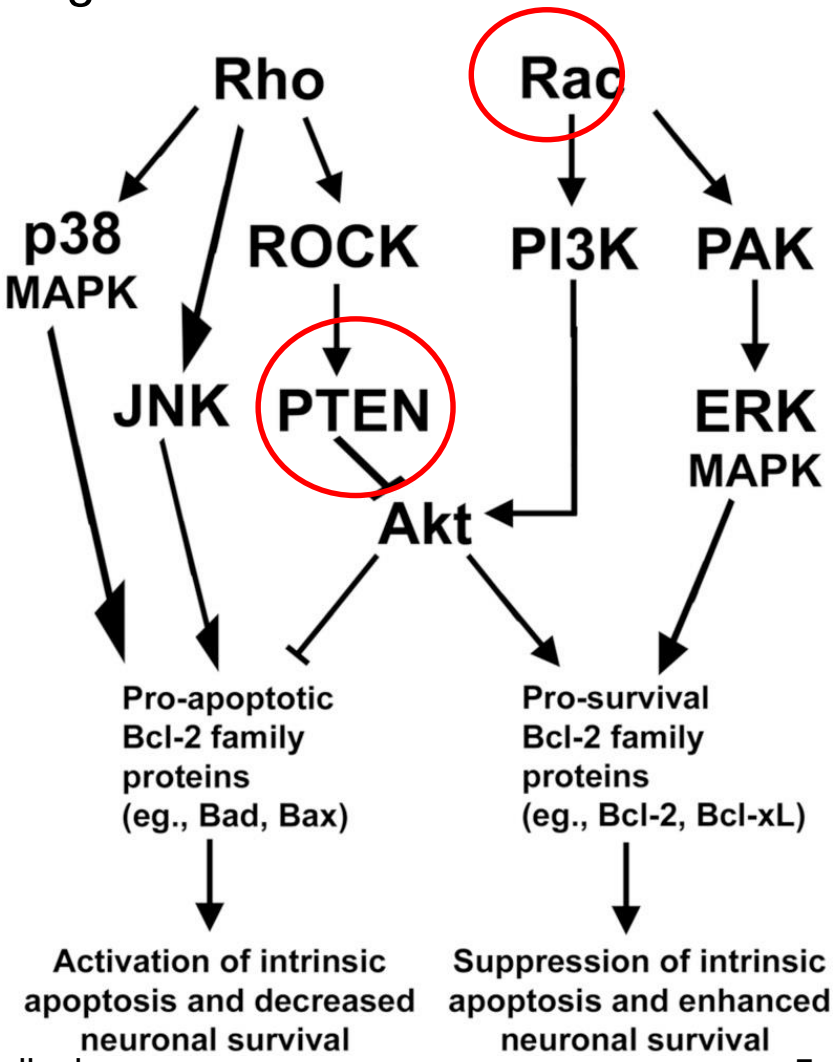


Walker CL, Liu NK, Xu XM. PTEN/PI3K and MAPK signaling in protection and pathology following CNS injuries. Frontiers in biology. 2013;8(4).

Introduction

Regeneration regulators

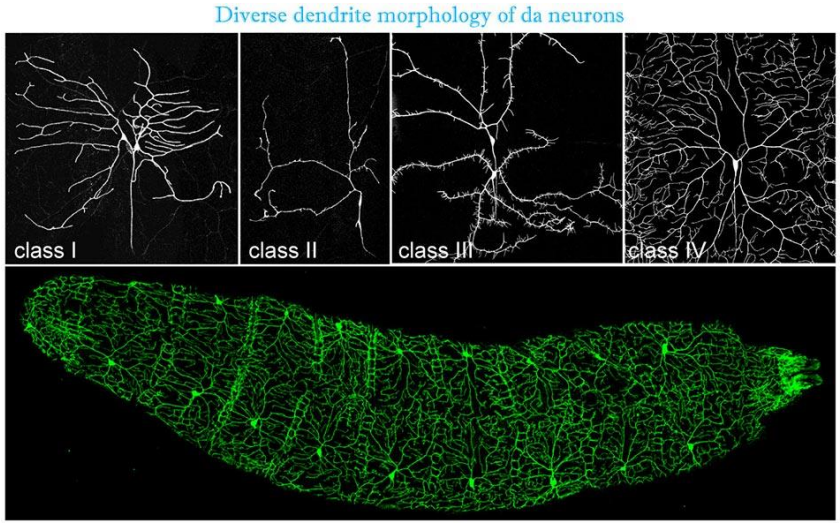
- Rac1 GTPase
- Cytosceletal regulator



Stankiewicz TR, Linseman DA. Rho family GTPases: key players in neuronal development, neuronal survival, and neurodegeneration. Frontiers in cellular neuroscience. 2014;8:314.

Methods

- Drosophila sensory neuron injury model
- VNC, Class III + IV dendritic arborization (da) neuron in Drosophila larvae



The Weill institute research summary
http://han.wicmb.cornell.edu/research/

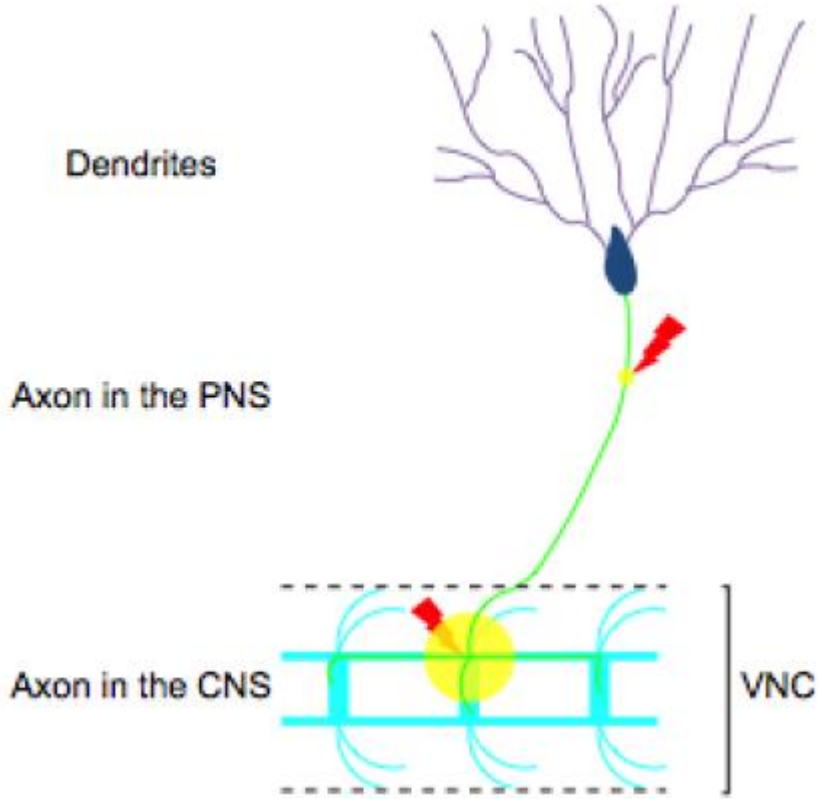
Methods

- Class IV da neurons regenerates its axon in periphery but shows limited regrowth in CNS
- Similar phenotype and molecular levels

  → resembles mammalian neurons

Methods

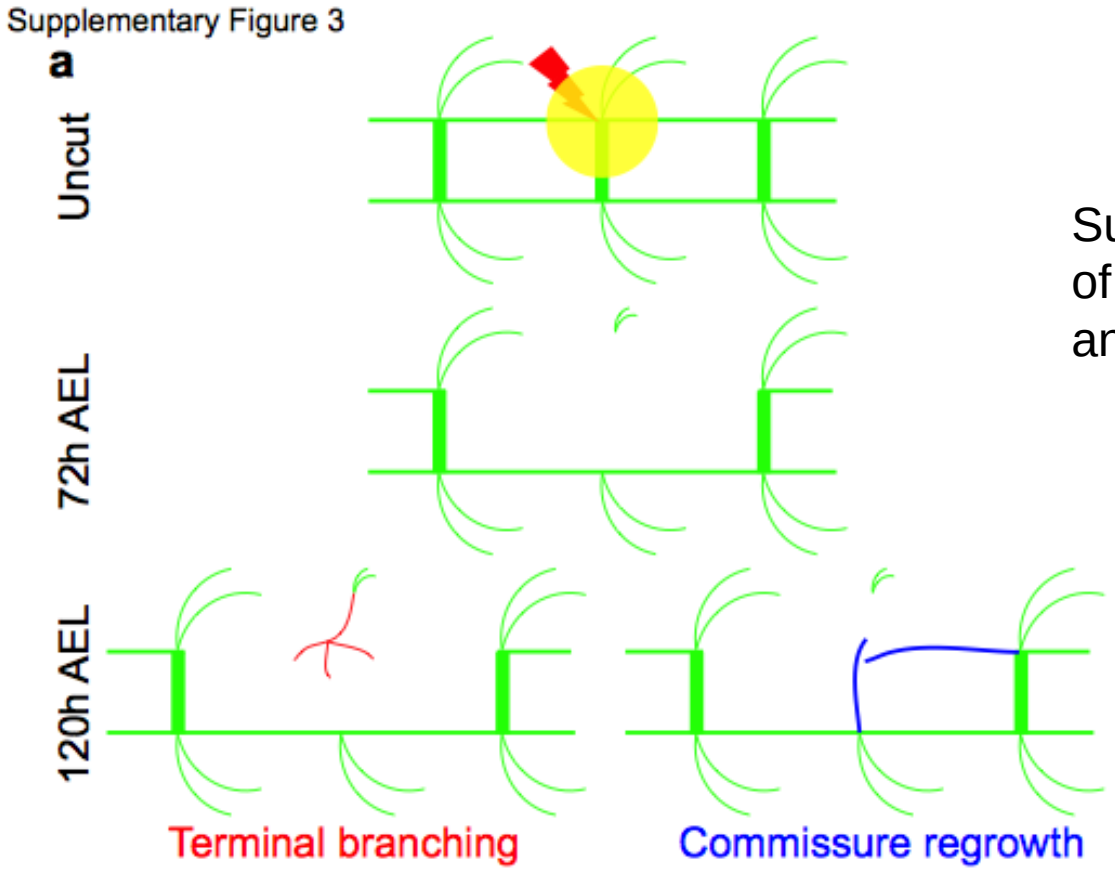
Axotomy



Suppl. Fig 1: axotomy protocol of neurons in the PNS and CNS of Drosophila

Methods

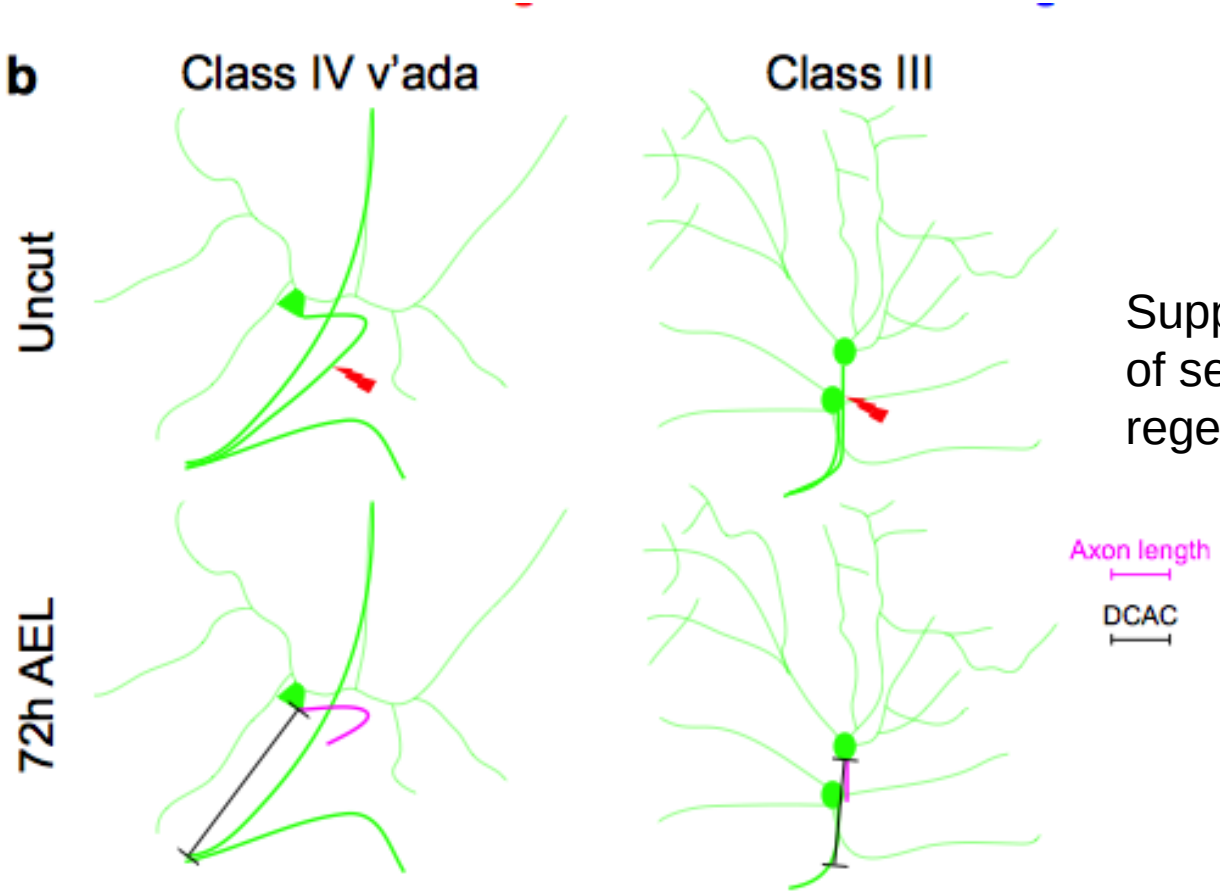
Axotomy



Suppl Fig 3a: axotomy protocol of neurons in the PNS and CNS

Methods

Axotomy



Suppl Fig 3b: Quantification of sensory axon regeneration

Methods

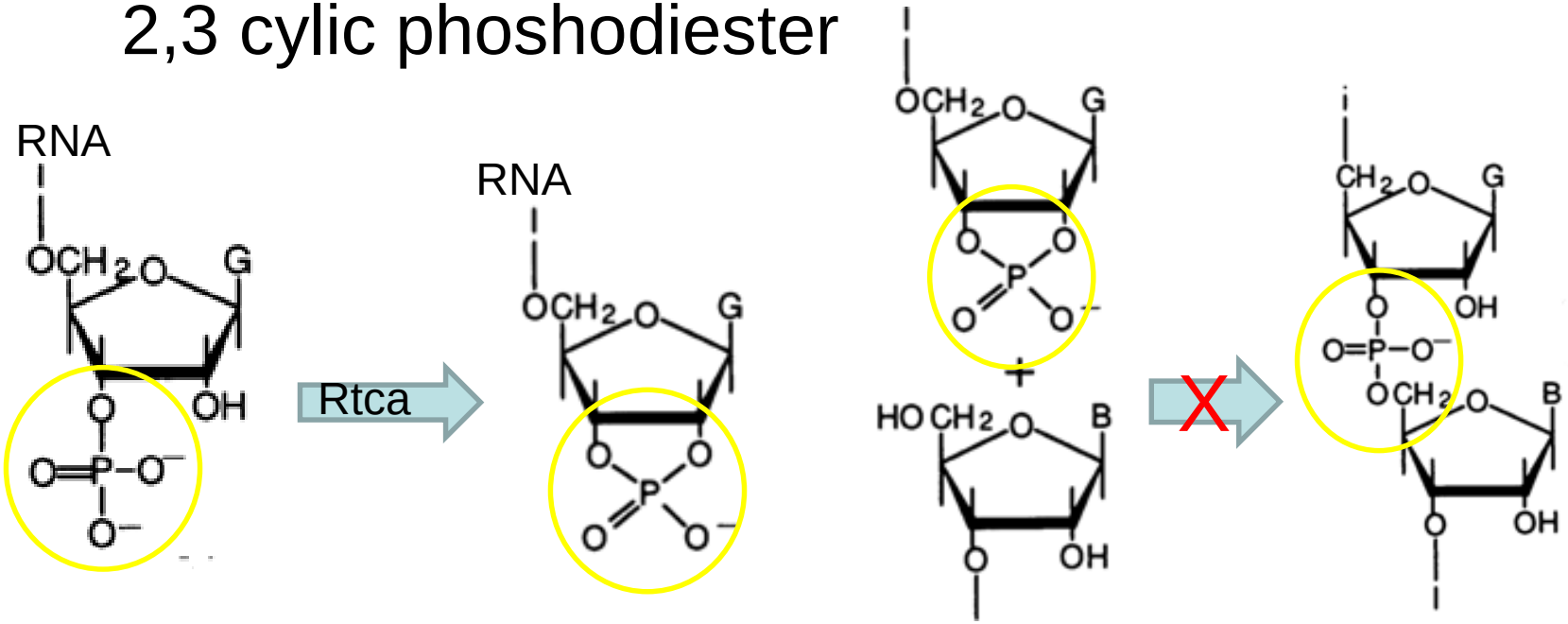
- Identification of axotomy-regulated genes in Drosophila via candidate-based genetic screening

- Drosophila Rtca (CG4061)
- Drosophila Archease
- Xbp1

Methods

Rtca

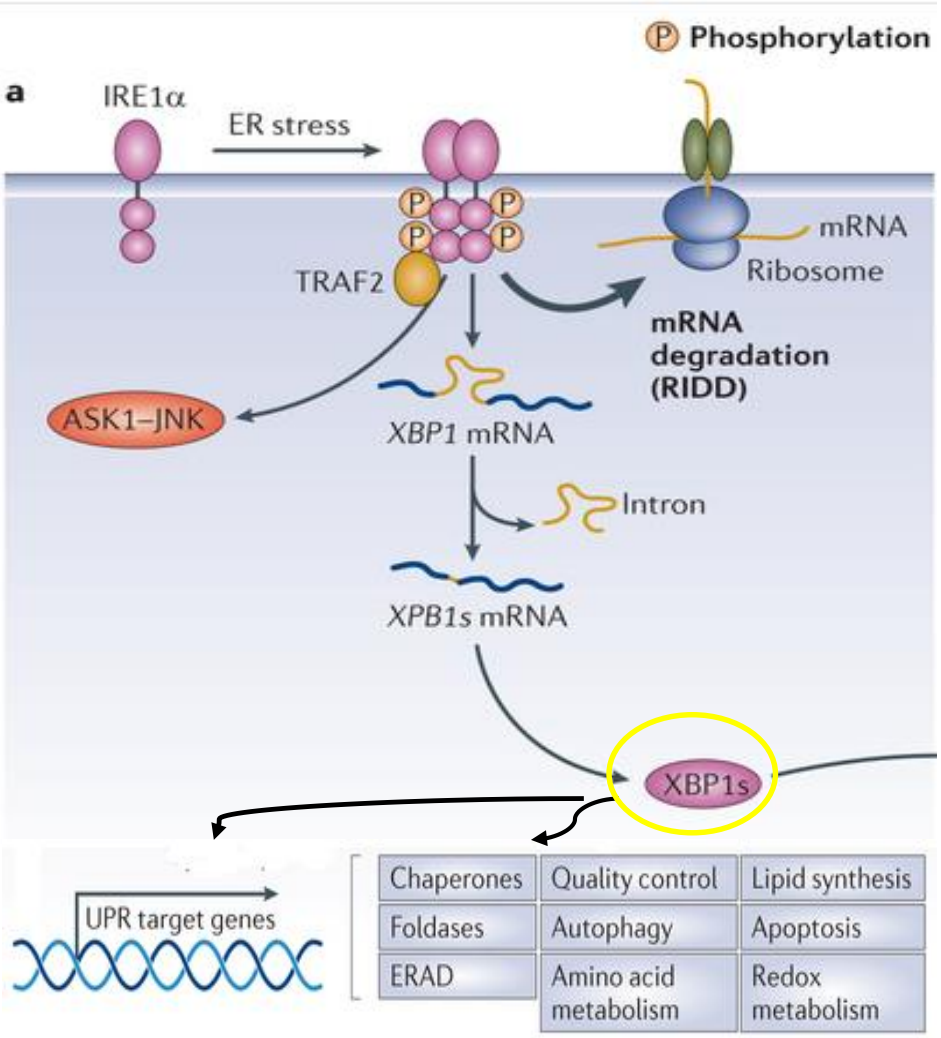
- RNA 3´-terminal phosphate cyclase
- Enzyme:
  catalyzes conversion of RNA-3´phosphate to 2,3 cylic phoshodiester



Methods

Xbp1

- X-box-binding protein 1
- =Transcription factor
- Part of UPR (unfolded protein response)
- Cellular stress reaction



UPR-Response

Hetz C, Chevet E, Harding HP.
Targeting the unfolded protein response in disease.
Nature reviews Drug discovery. 2013;12(9):703-19.

Methods

Archease & Rtcb

- **Rtcb** = part of RNA-ligase complex
  - → tRNA-ligation after intron-removal
  - → Xbp1-ligation in UPR

- **Archease** = Rtcb-ligase-cofactor

Kosmaczewski SG, Han SM, Han B, Irving Meyer B, Baig HS, Athar W, et al. RNA ligation in neurons by RtcB inhibits axon regeneration. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(27):8451-6.

Methods

Drosophila mutants

- $Rtca^{NP5057}$: LOF allele of Rtca
- Df(1)BSC718: Rtca deficiency line
- $Rtca^{delta}$: Rtca zygotic deletion allele
- $Rtca^{deltaMat}$: Rtca zygotic and maternal deletion allele
- ppkGal4>Rtca: Rtca overexpression
- 19-12-Gal4>RtcaRNAi: RNA interference knockout in Class III da

Results

Drosophila Rtca loss of function enhances axon regeneration

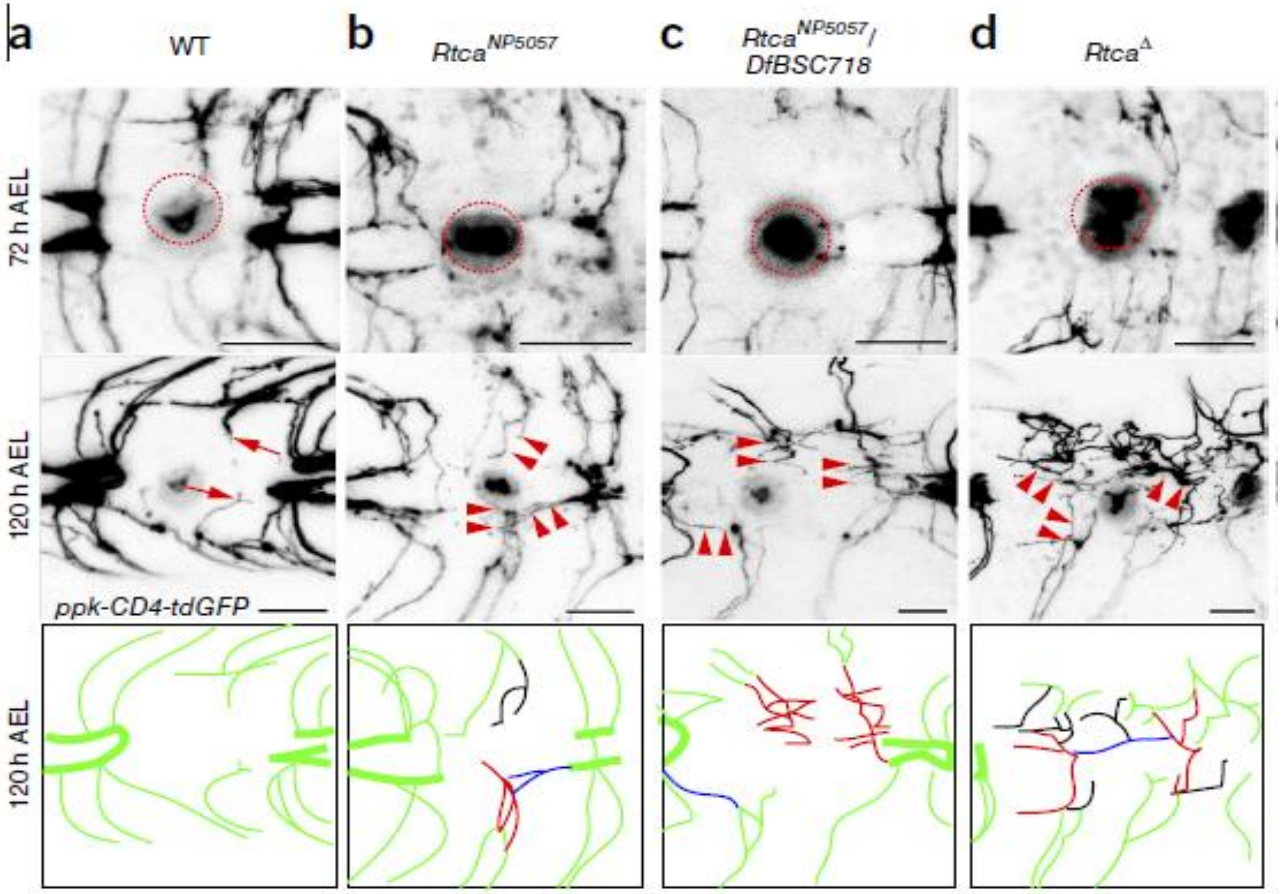


Figure 1 a-d: Rtca-removal increases class IV neuron axon regrowth

Results

Drosophila Rtca loss of function enhances axon regeneration

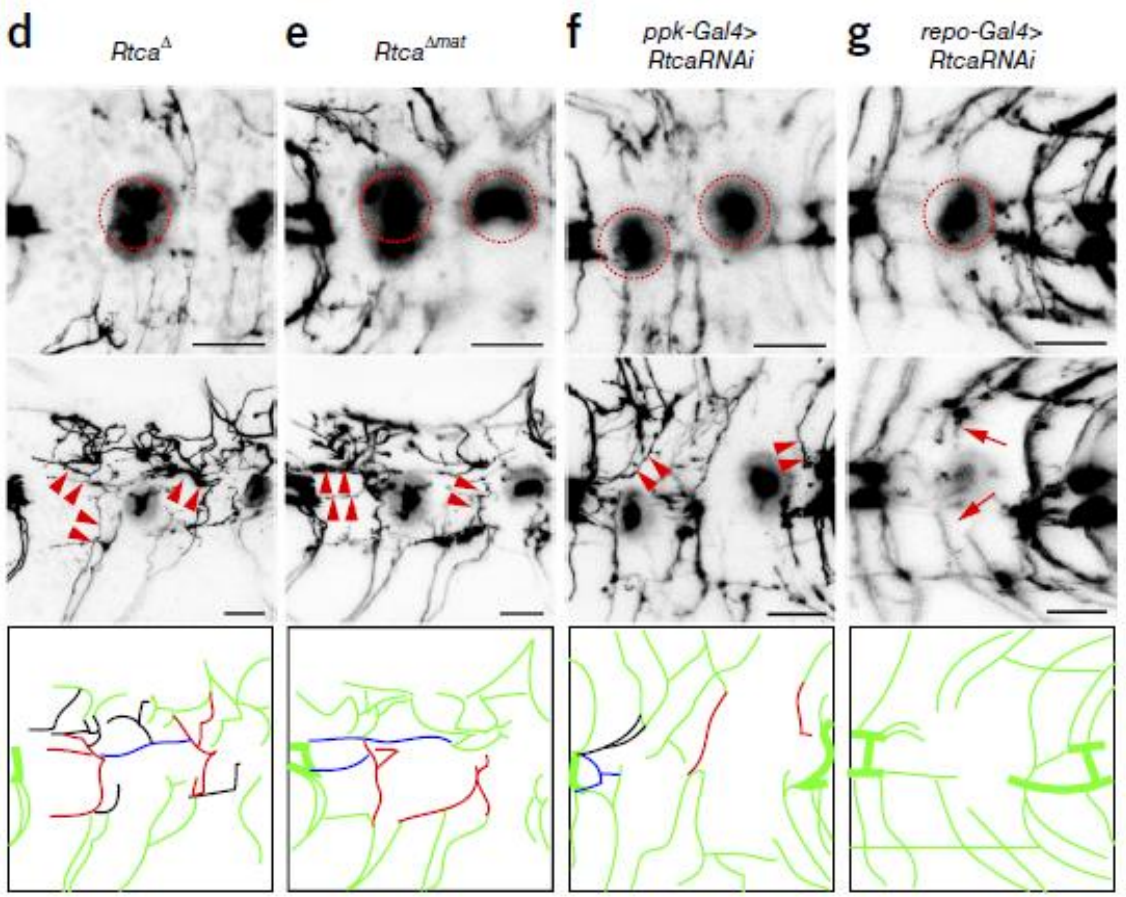


Figure 1 d-g: Rtca removal increases class IV neuron axon regrowth

Results

Drosophila Rtca loss of function enhances axon regeneration

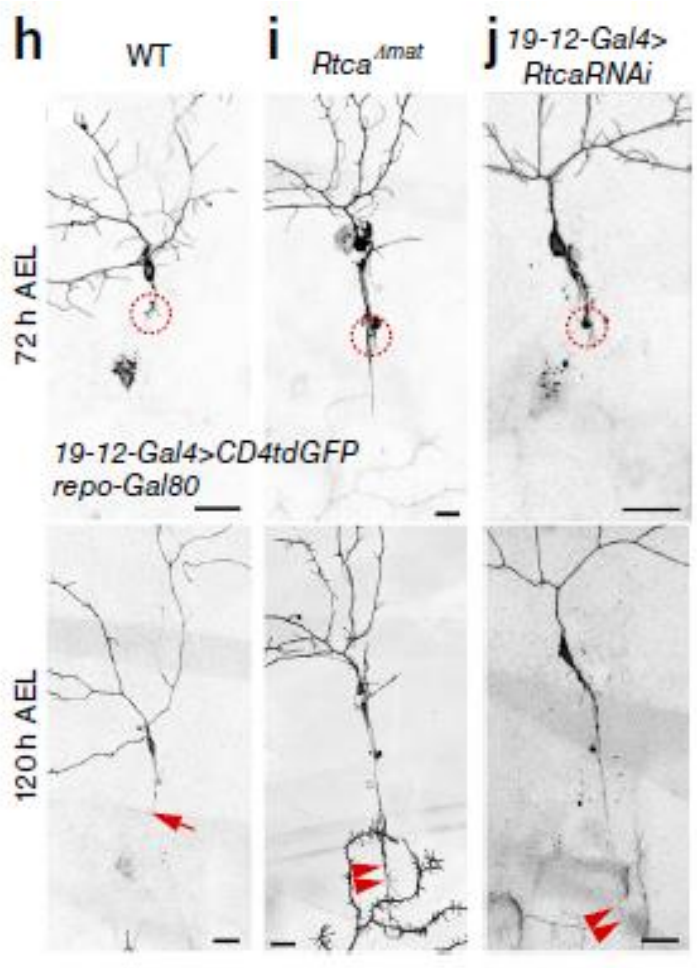


Figure 1 h-j: Rtca removal promotes class III da axon regeneration in the periphery

Results

Drosophila Rtca loss of function enhances axon regeneration

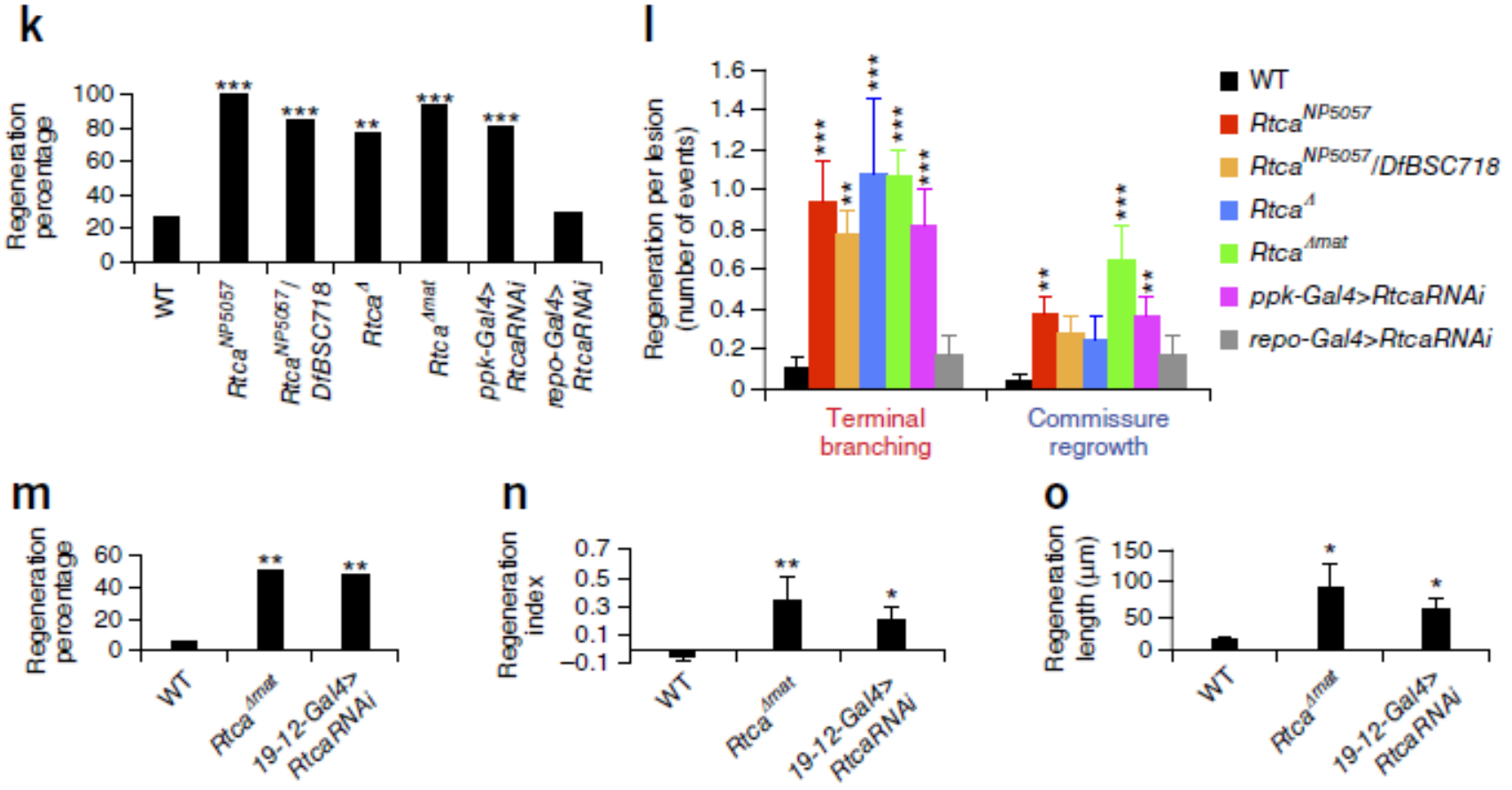


Figure 1 k-o: Rtca LOF increases the regeneration percentage, regeneration index and regeneration length

Results

Drosophila Rtca gain of function reduces axon regeneration

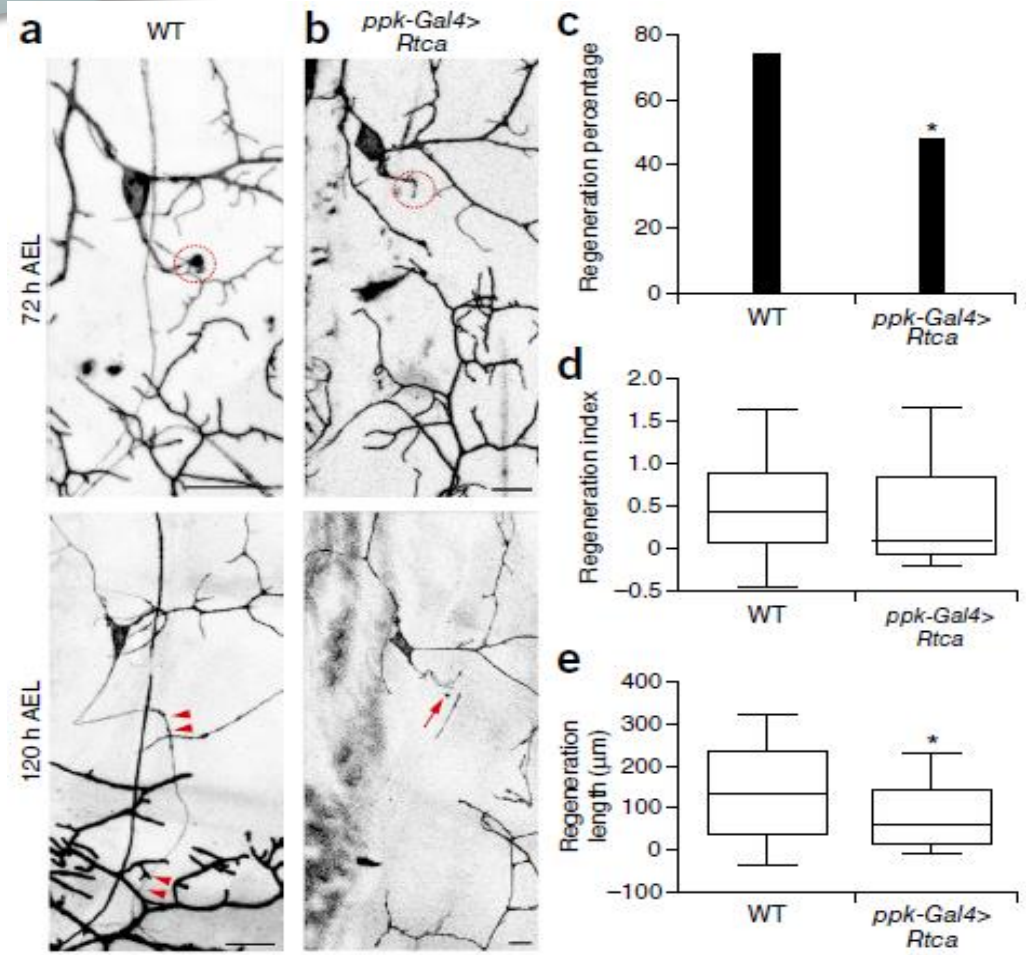


Fig 2: Drosophila overexpression reduces axon regeneration in PNS

Results

Drosophila Rtca gain of function reduces axon regeneration

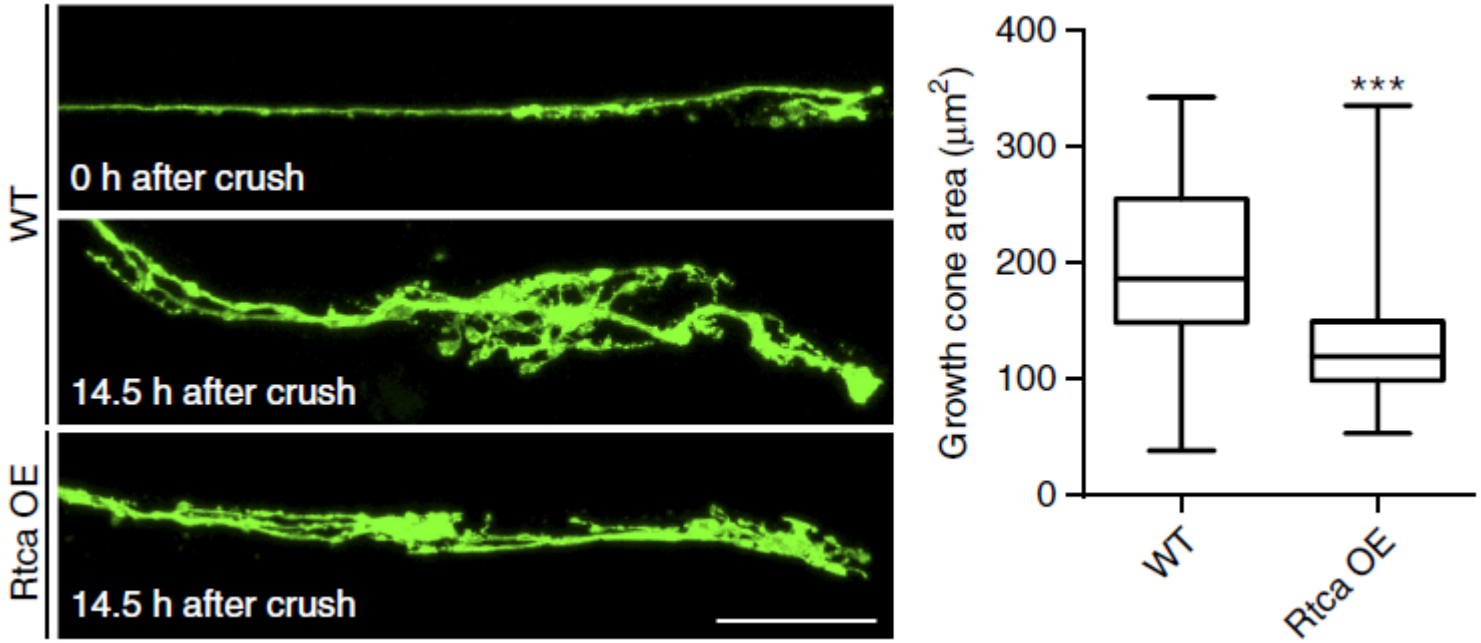


Fig 3: Drosophila overexpression reduces motor axon regeneration

Results

The expression pattern of Drosophila Rtca

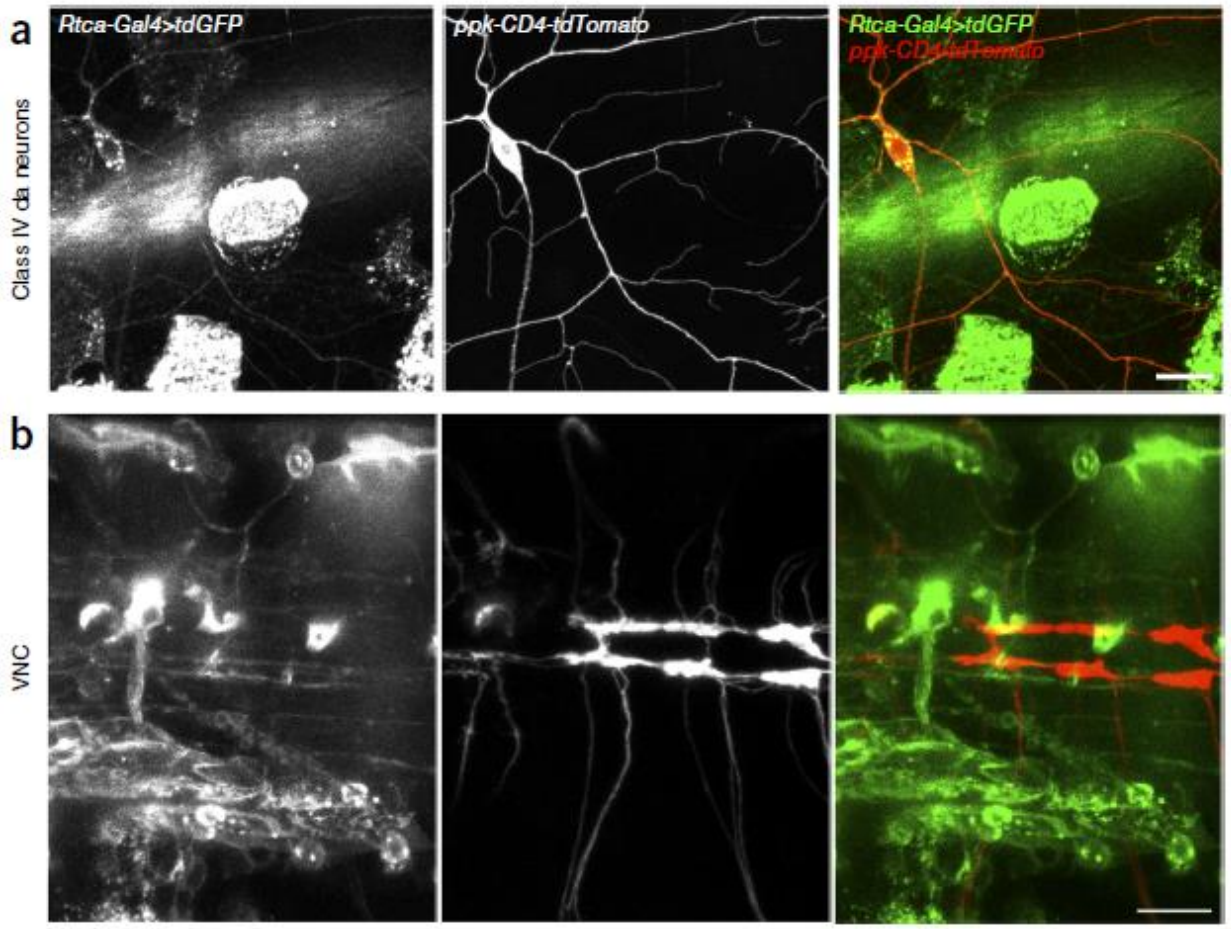


Figure 4 a,b: The expression pattern in of Rtca in Drosophila

Results

The expression pattern of Drosophila Rtca

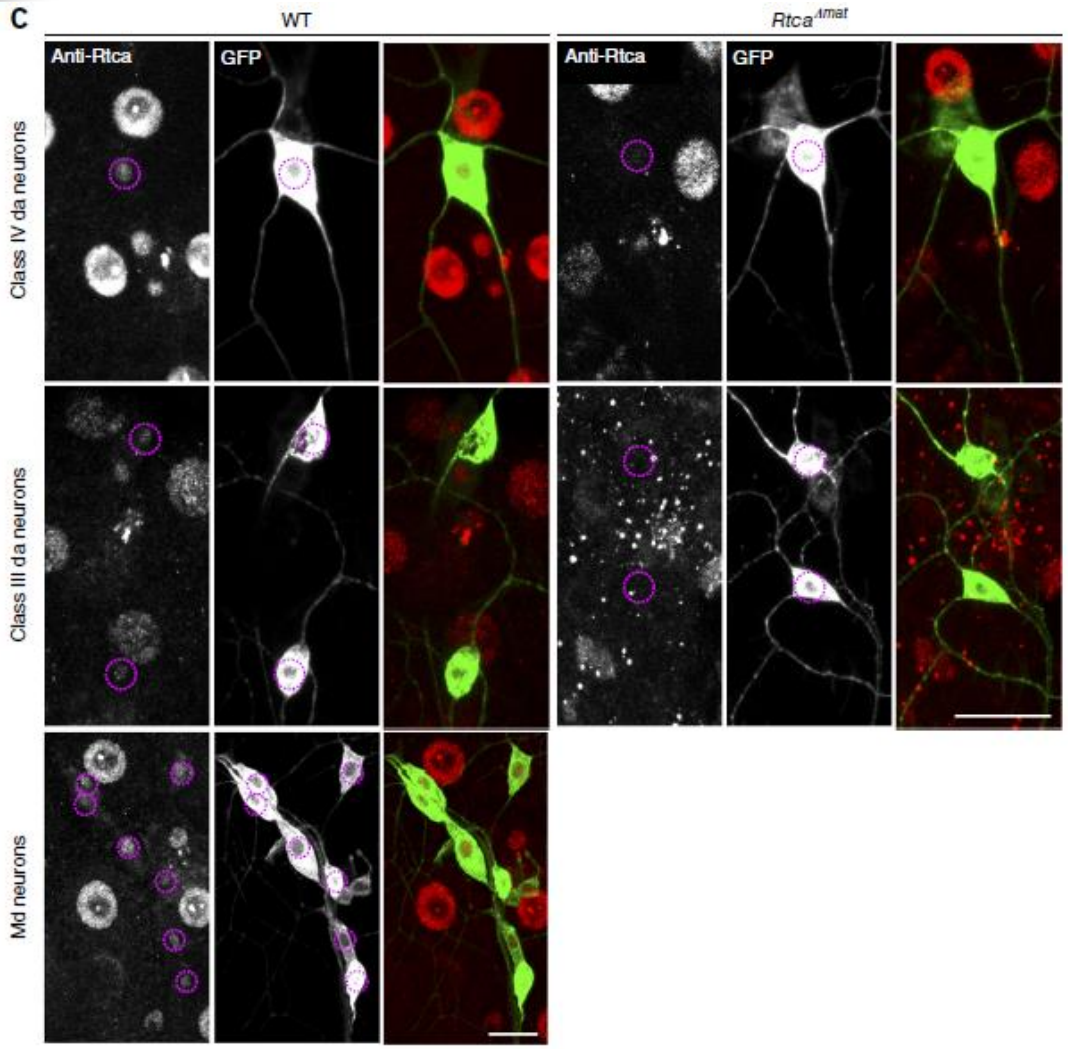


Figure 4c: The expression pattern in of Rtca in Drosophila

Results

Interaction of Rtca with known regeneration regulators

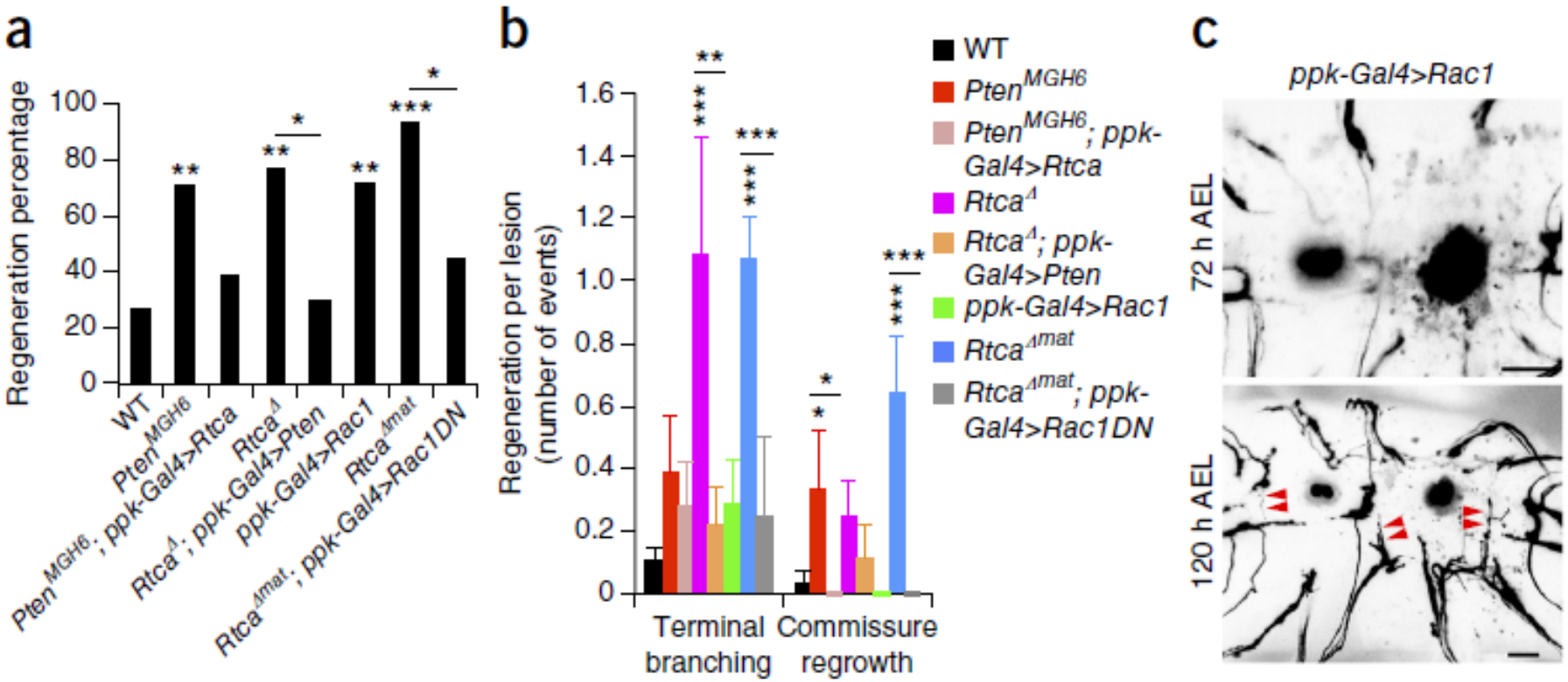
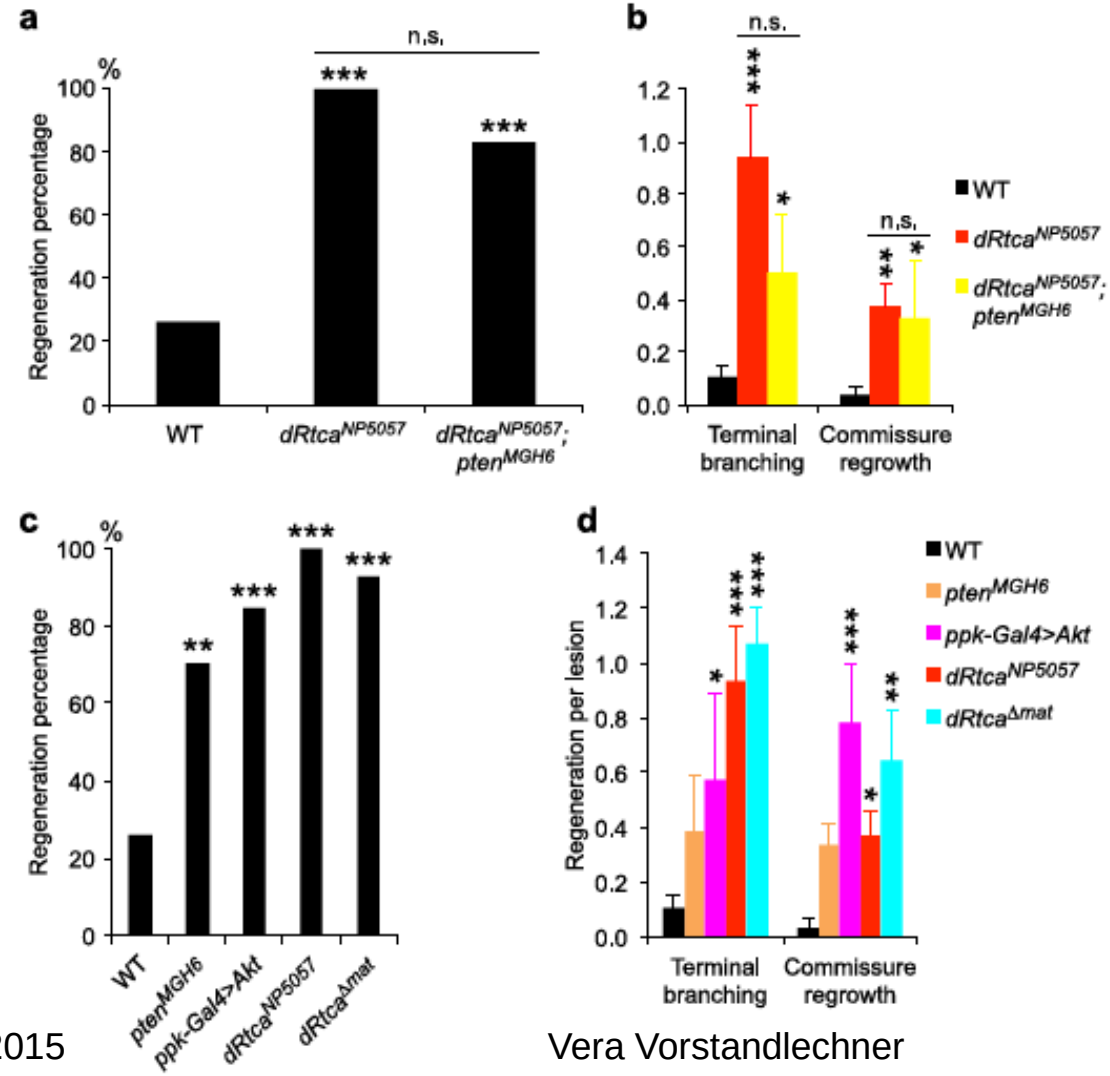


Figure 5 a-c: The interaction of Drosophila with known regeneration regulators

Results

Interaction of Rtca with known

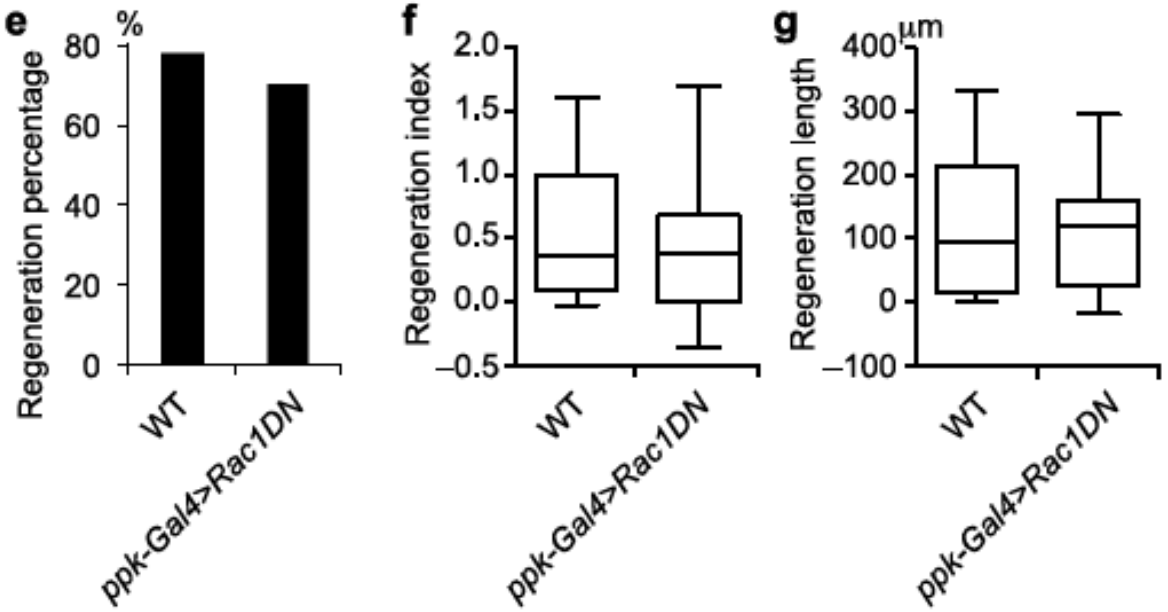
Supplementary Figure 4



Suppl. Figure 4a-d: double LOF mutants Rtca + Pten do not further improve regeneration

Results

Interaction of Rtca with known regeneration regulators



Suppl. Figure 4 e-g: Rac1DN overexpression does not affect regeneration in the periphery

Results

RNA repair and splicing pathway regulates axon regeneration

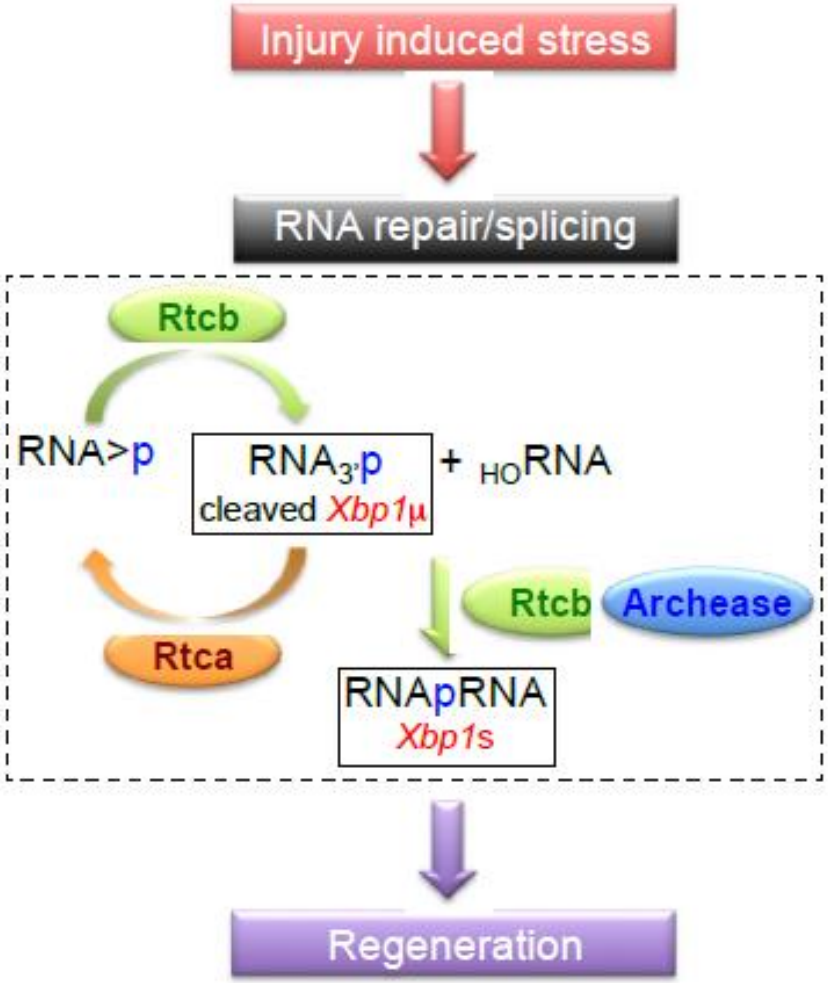
Hypothesis:

- Rtca catalyzes conversion of phosphodiesters at the end of RNA
- Neuron injury triggers cell stress leading to RNA damage and splicing → RNA 3´phosphates need to be rejoined by ligase (*Rtcb-ligase*)
- *Rtcb* is catalyzed by *Archease*
- RNA-phosphodiesters converted by Rtca slow ligation →
- Silencing Rtca promote axon regeneration

→ Role of Archease in regeneration?

Results

RNA repair and splicing pathway regulates axon regeneration



Suppl. Figure 6:
proposed model of Rtca-Arche-ase-Xbp1-pathway in regulation of regeneration

Results

RNA repair and splicing pathway regulates axon regeneration

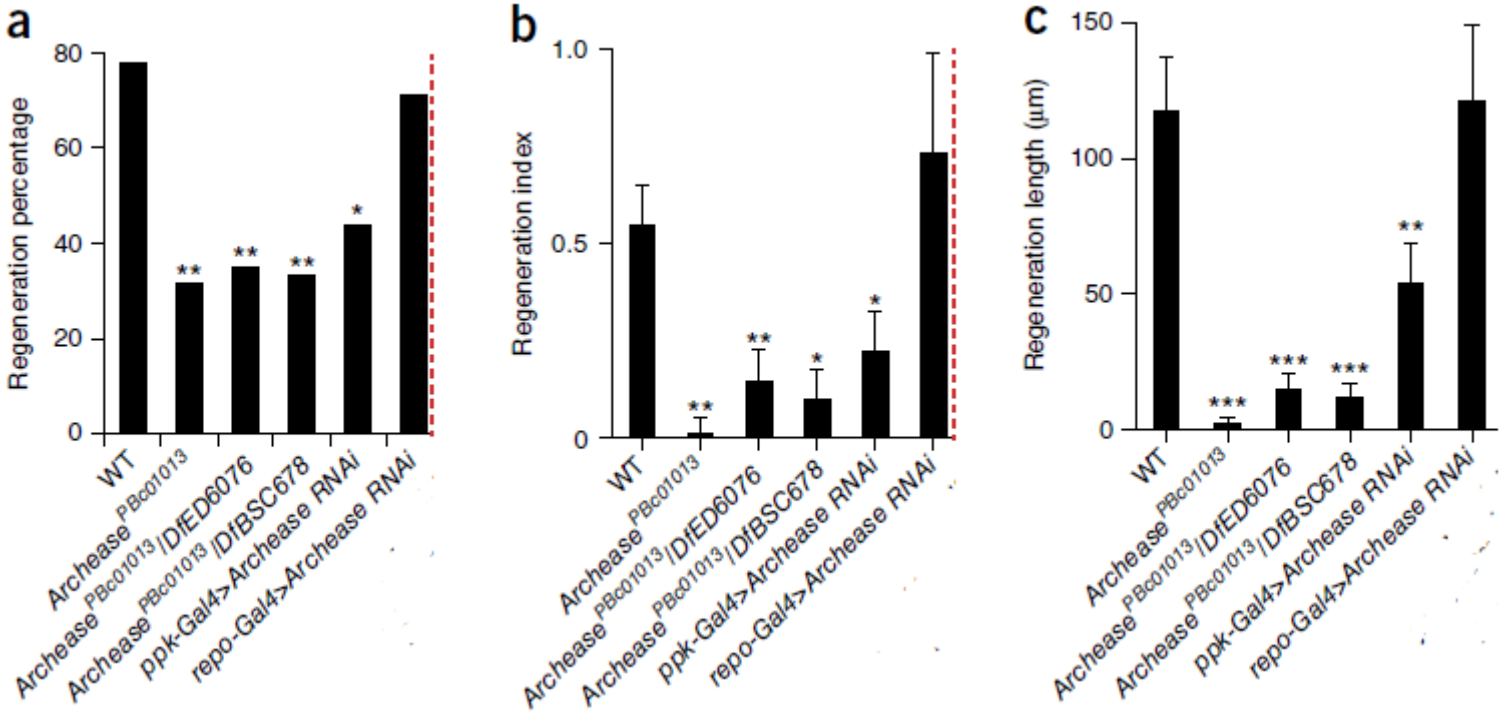


Figure 6 a-c: Archease and Xbp1 are required for class IV da neuron regeneration

Results

RNA repair and splicing pathway regulates axon regeneration

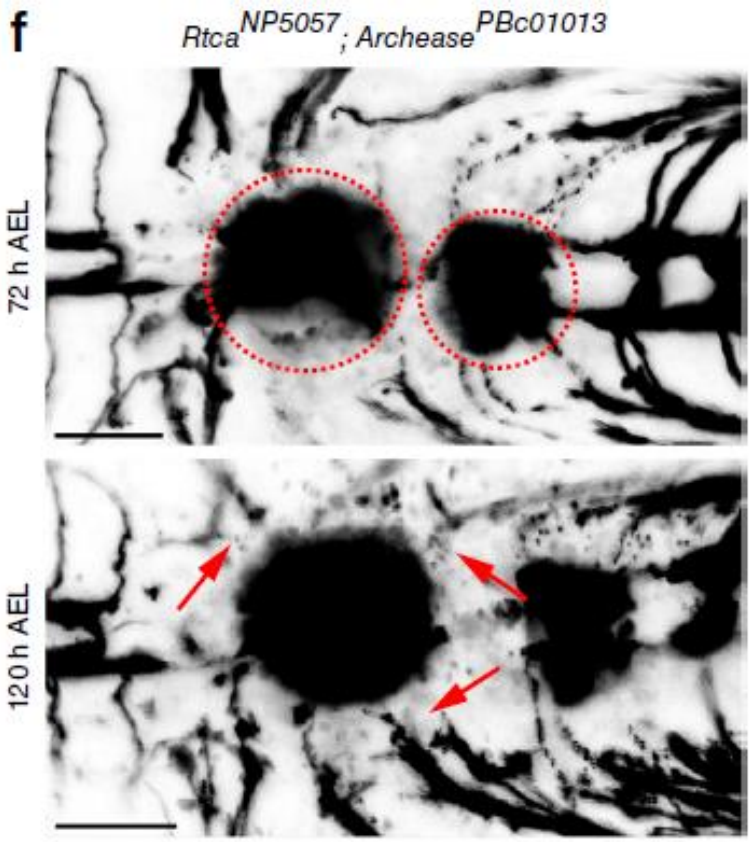
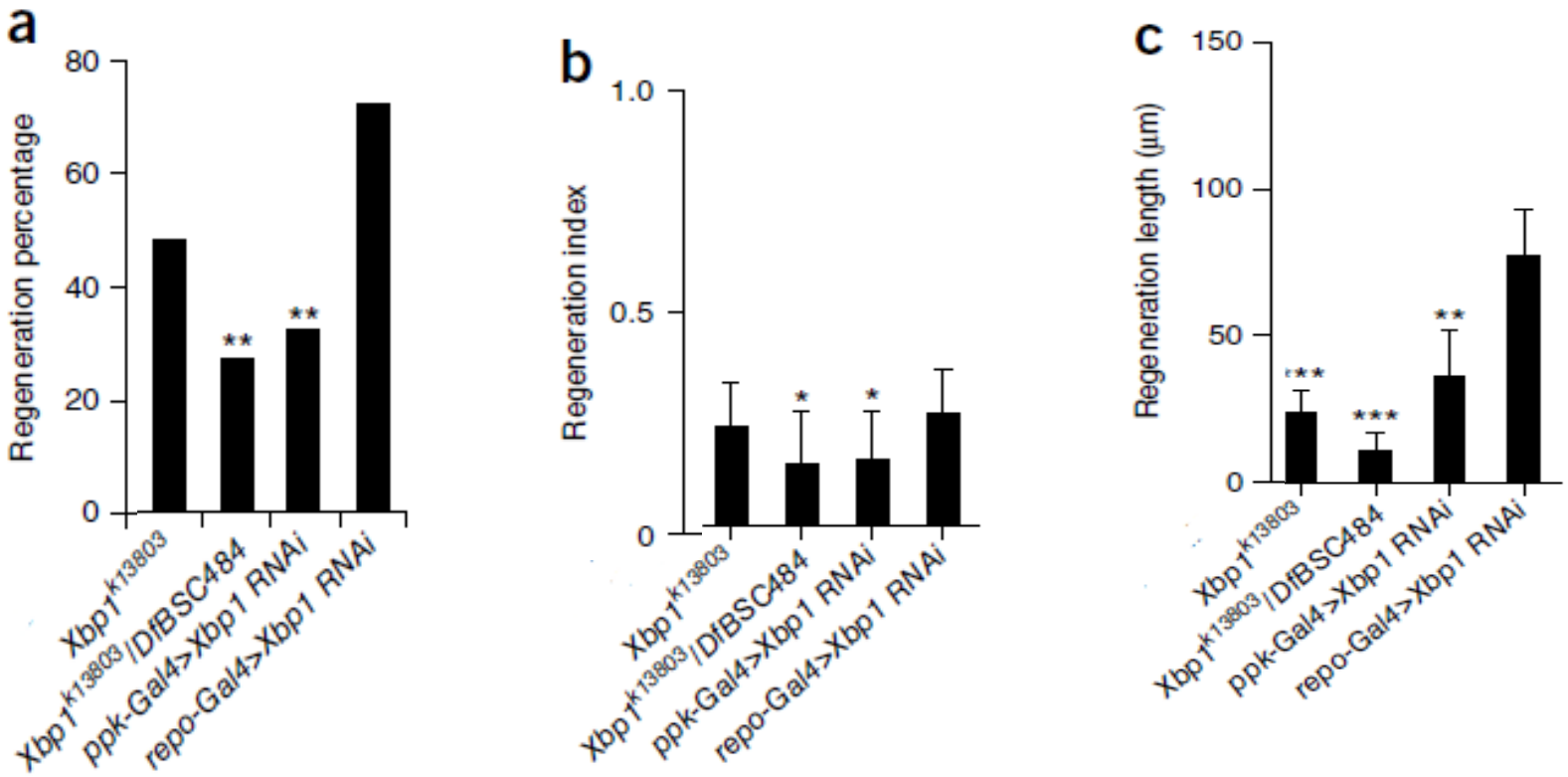


Figure 6f: LOF of Rtca and Archease double mutants produce stalled and retracted axons

Results

Xbp1 is a substrate of Rtca-Archease in regeneration

Figure 6 a-c: Xpb1 is required for class IV da neuron regeneration



Results

Xbp1 is a substrate of Rtca-Archease in regeneration

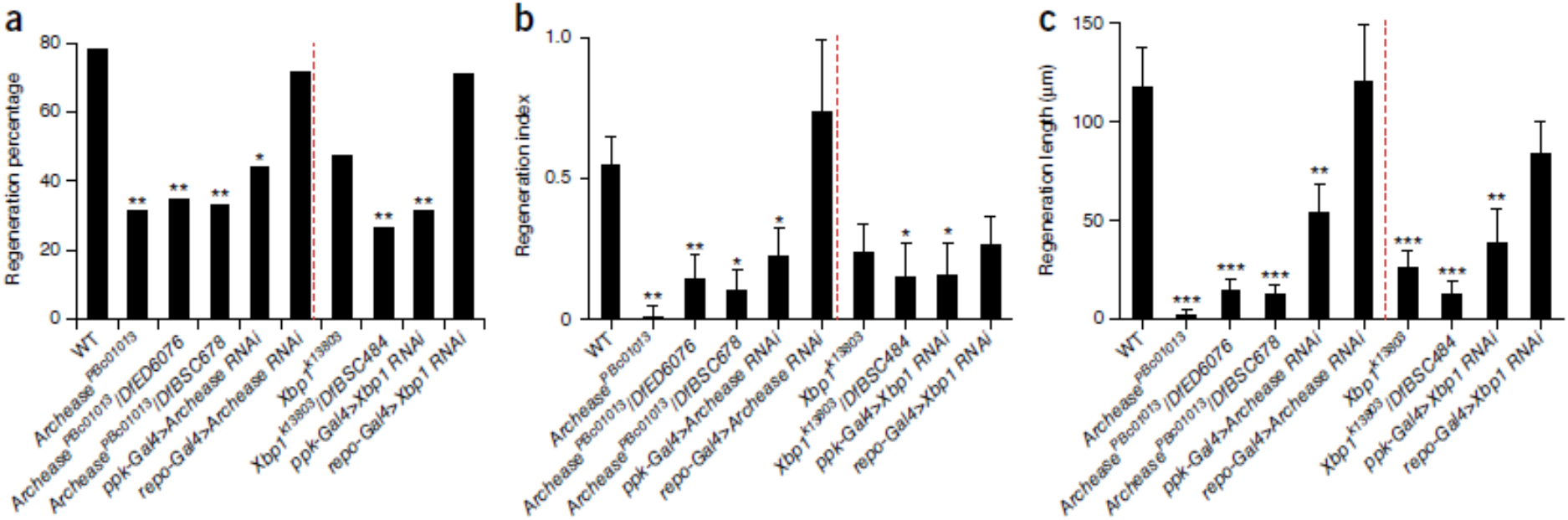


Fig 6 a-c: Archease and Xbo1 are required in neuronal regeneration

Results

Xbp1 is a substrate of Rtca-Archease in regeneration

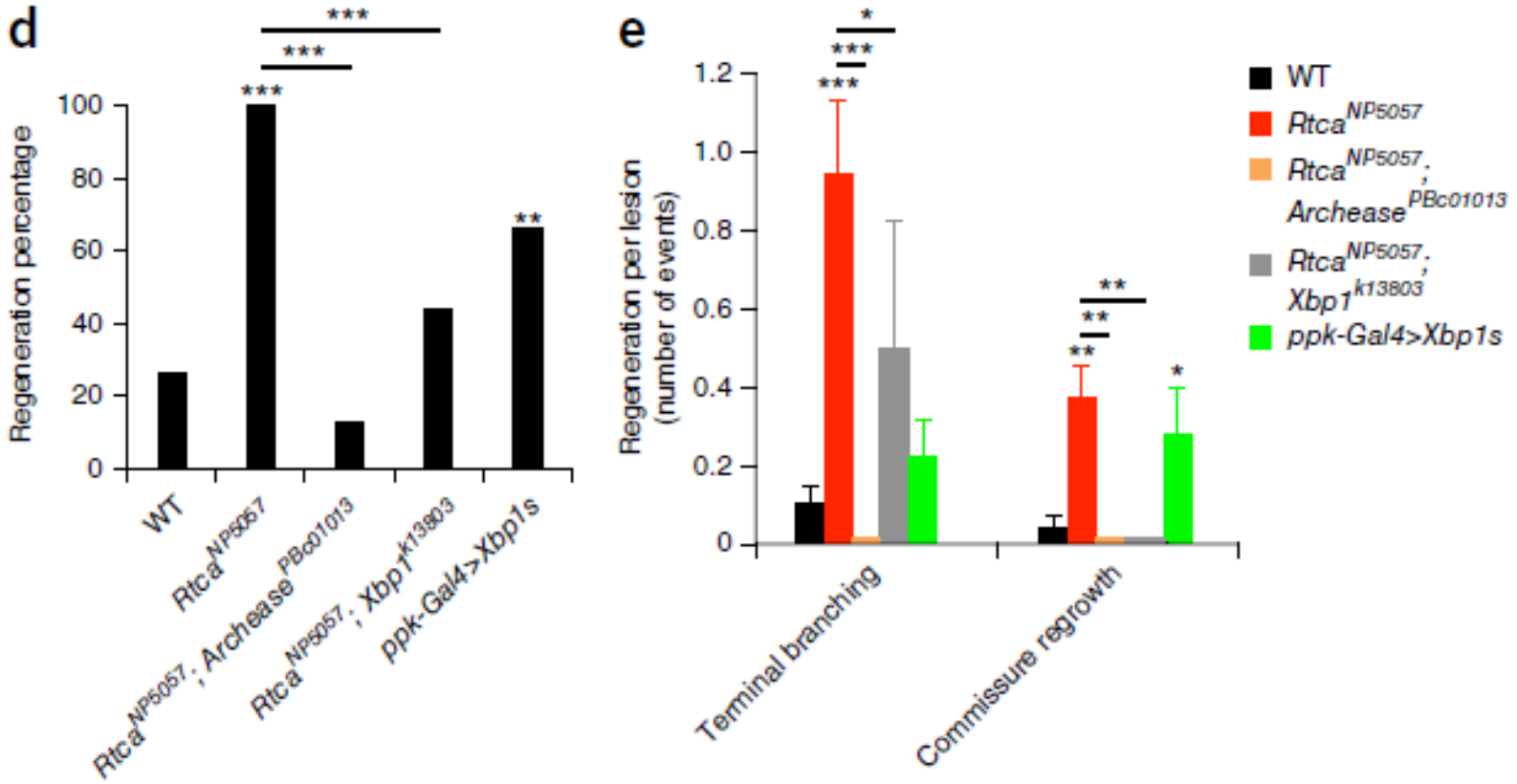
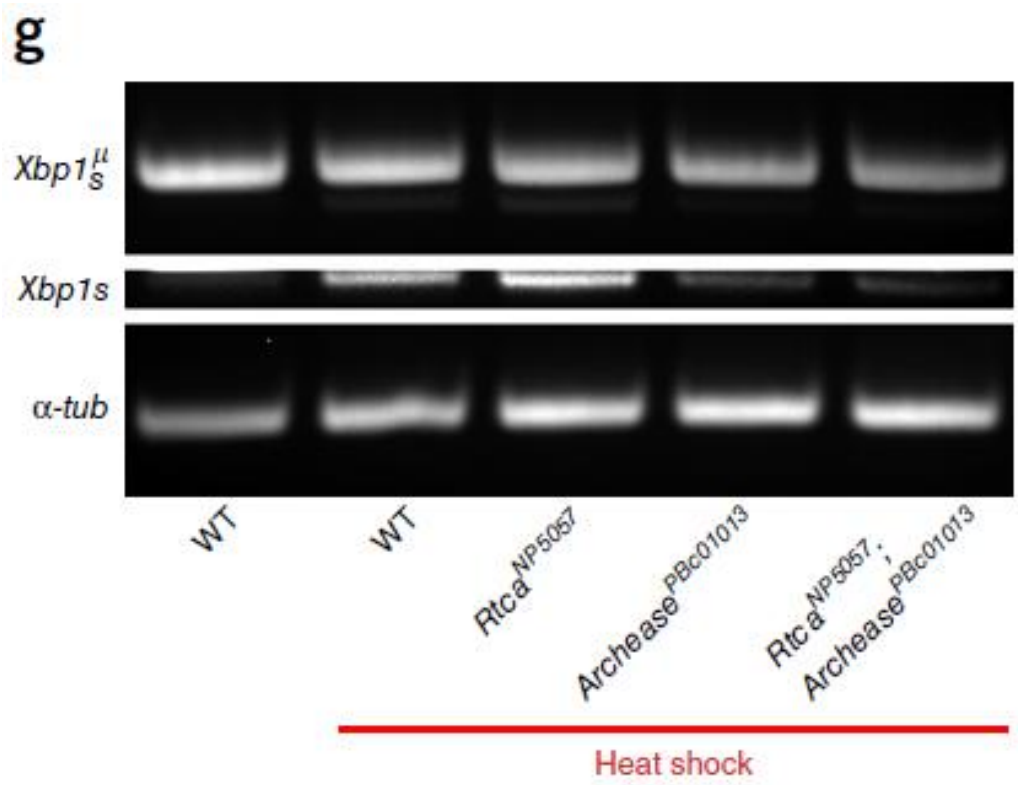


Figure 6 d,e: Analysis indicates that Archease and Xbp1 function downstream of Rtca

Results

Xbp1 is a substrate of Rtca-Archease in regeneration



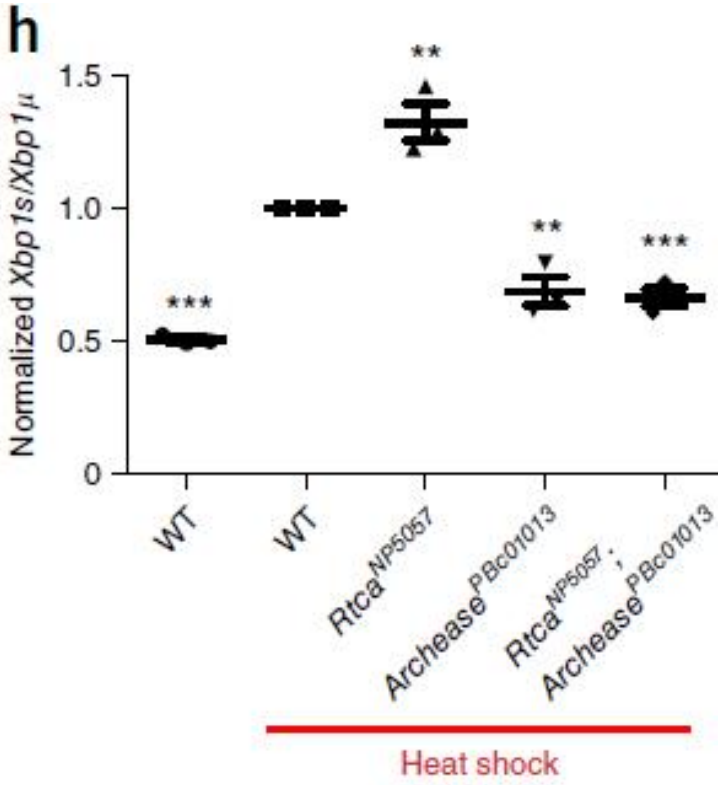


Figure 6g: Semiquantative RT-PCR of Xbp1sμ and Xbp1s after heat shock in WT and in mutants

Figure 6h: Quantification of Xbp1s/Xbp1sμ-ratio

Results

Mammalia Rtca inhibits CNS axon regeneration

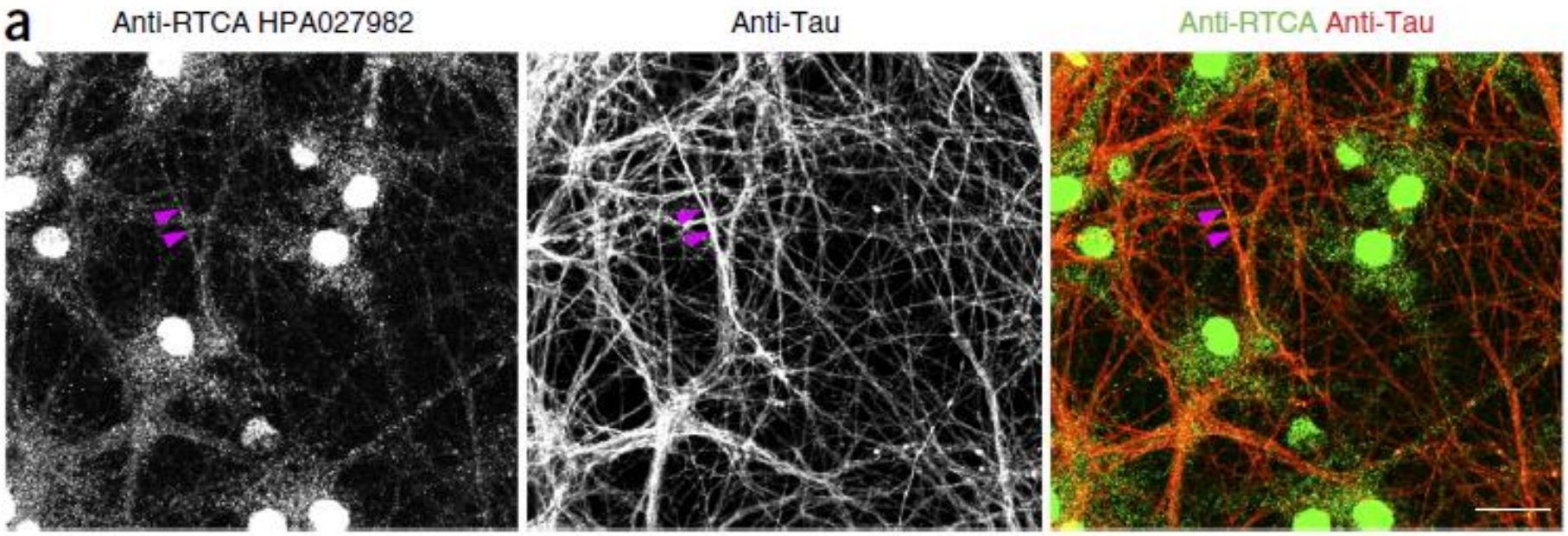


Figure 7a: Rtca-Expression in vitro in rats via human Anti-Rtca-Antibodies

Results

Mammalia Rtca inhibits CNS axon regeneration

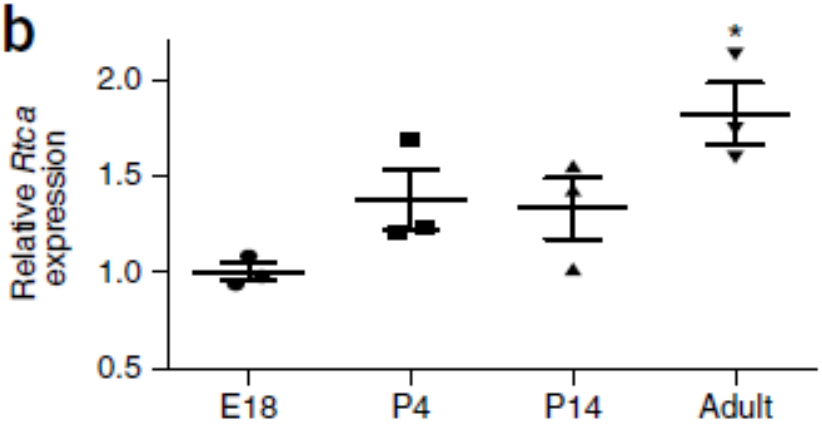


Figure 7b: expression of *Rtca* transcripts in DRG is upregulated throughout development

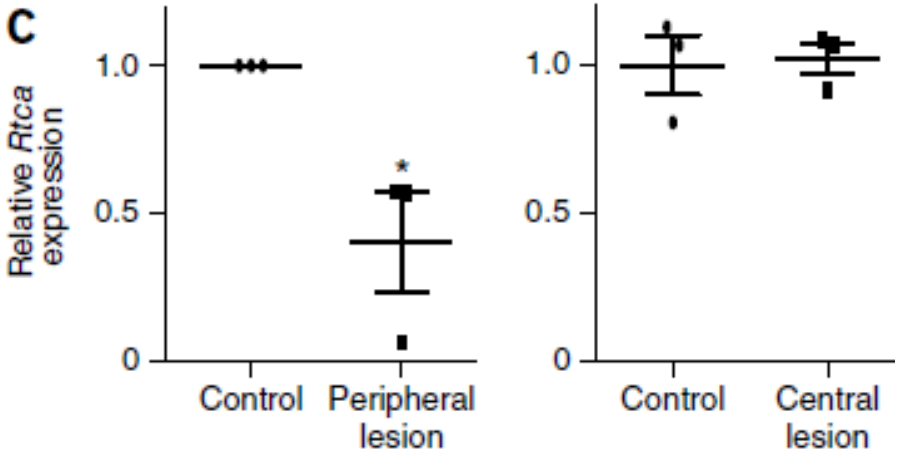


Figure 7c: laesion in sciatic nerve reduces Rtca transcript levels, but not in DRG after spinal cord laesion

Results

Mammalia Rtca inhibits CNS axon regeneration

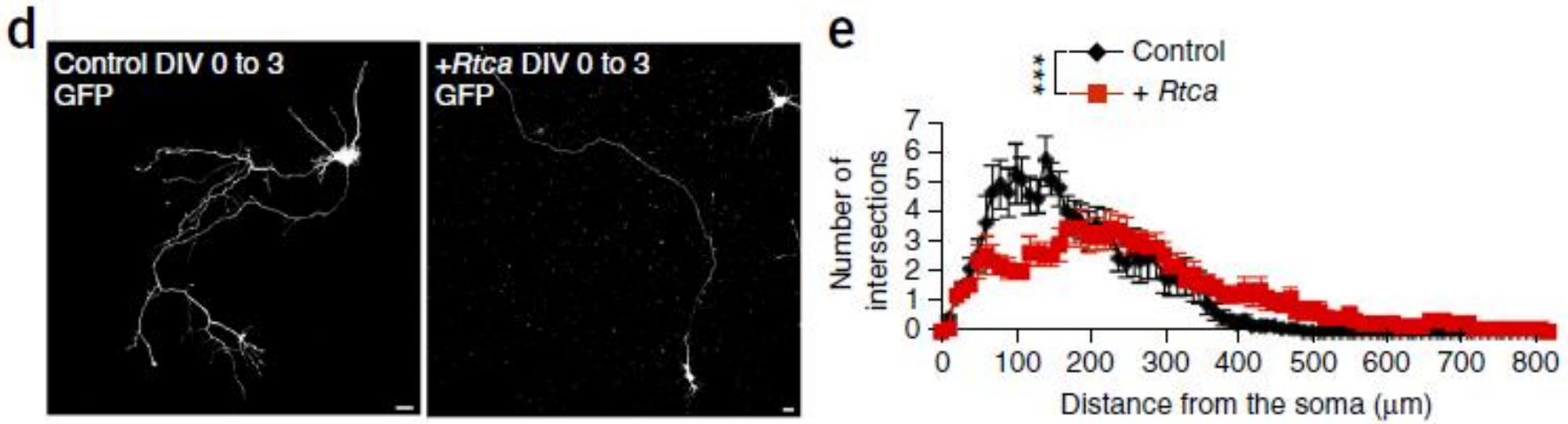


Figure 7 d,e : overexpression of Rtca in cultured hippocampal neurons reduced axon complexity and reduced proximal axonal branching

Results

Does knockout of Rtca enhance axon regeneration in adult mice?

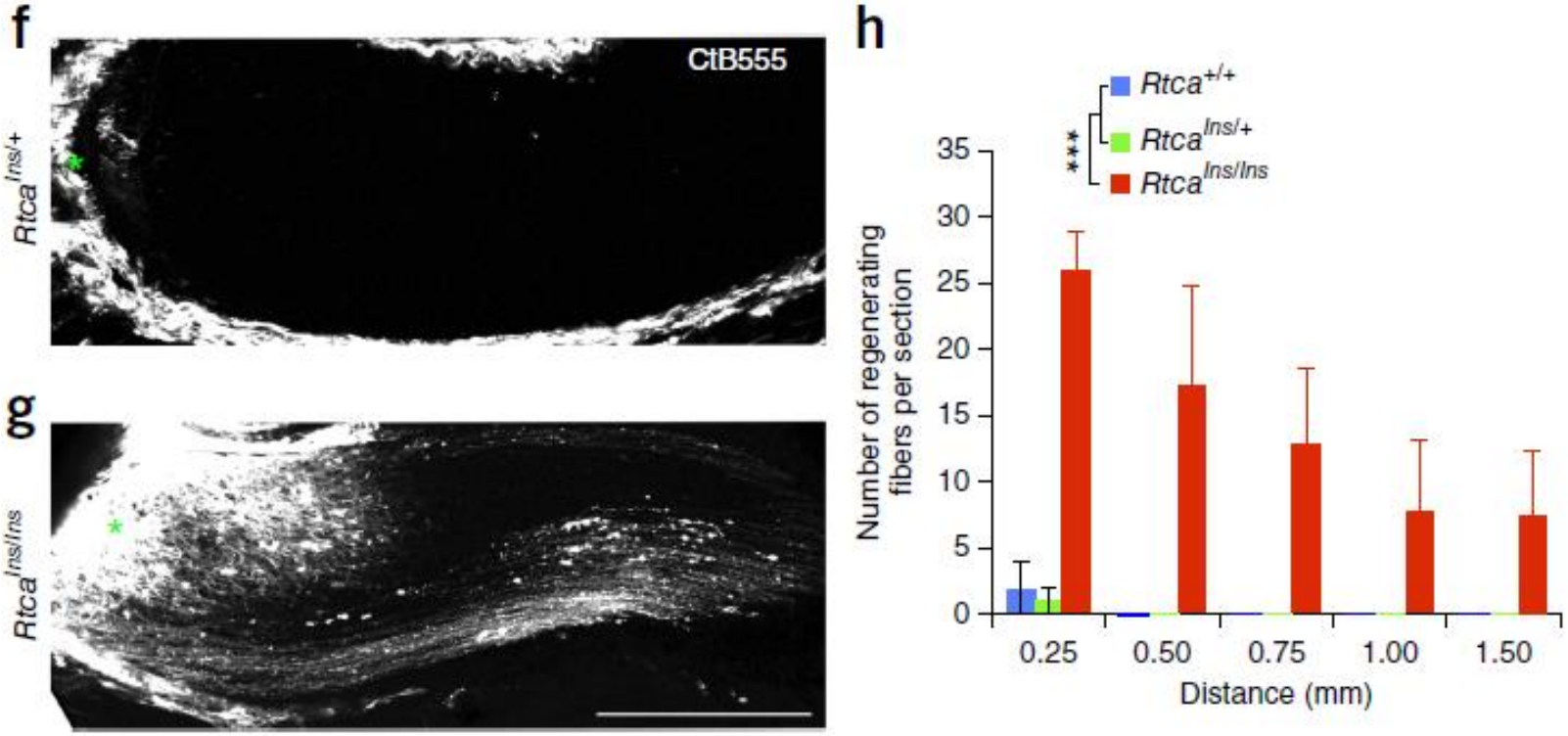
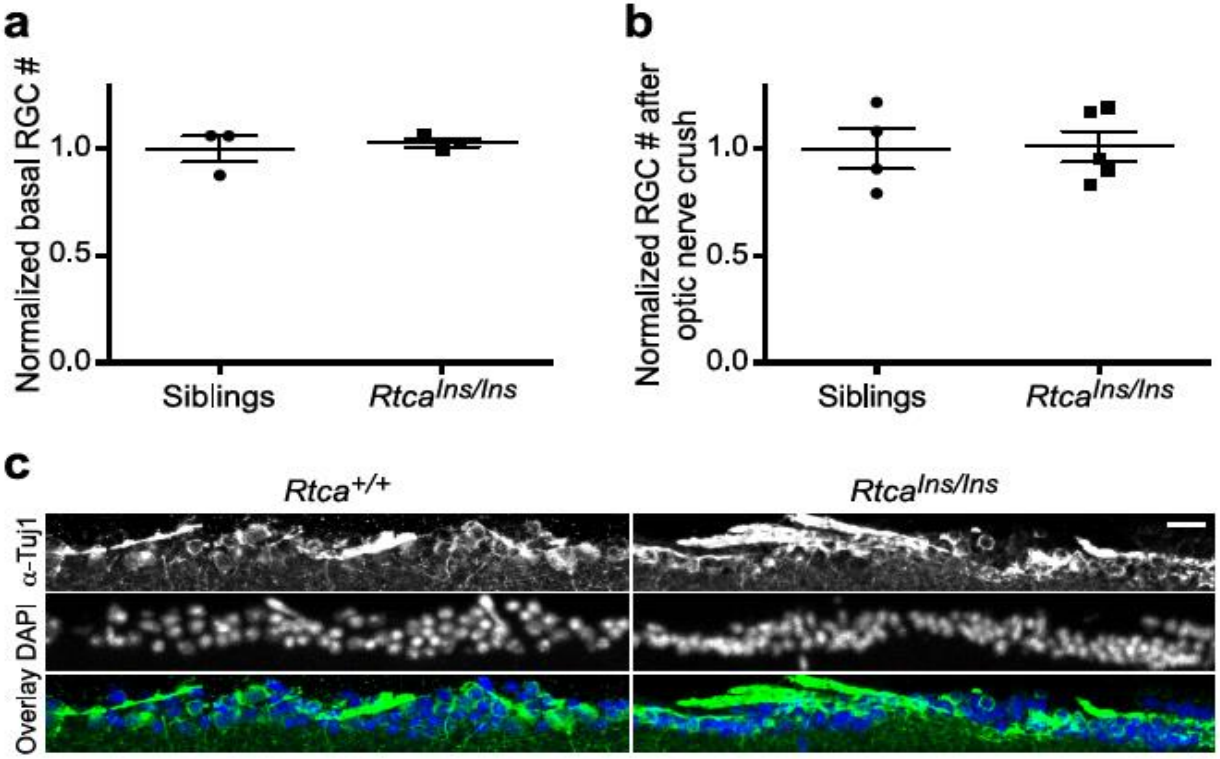


Figure 7 f-h: regeneration after optic nerve crush in Rtca +/+, Rtca +/ins and Rtca ins/ins mice

Results

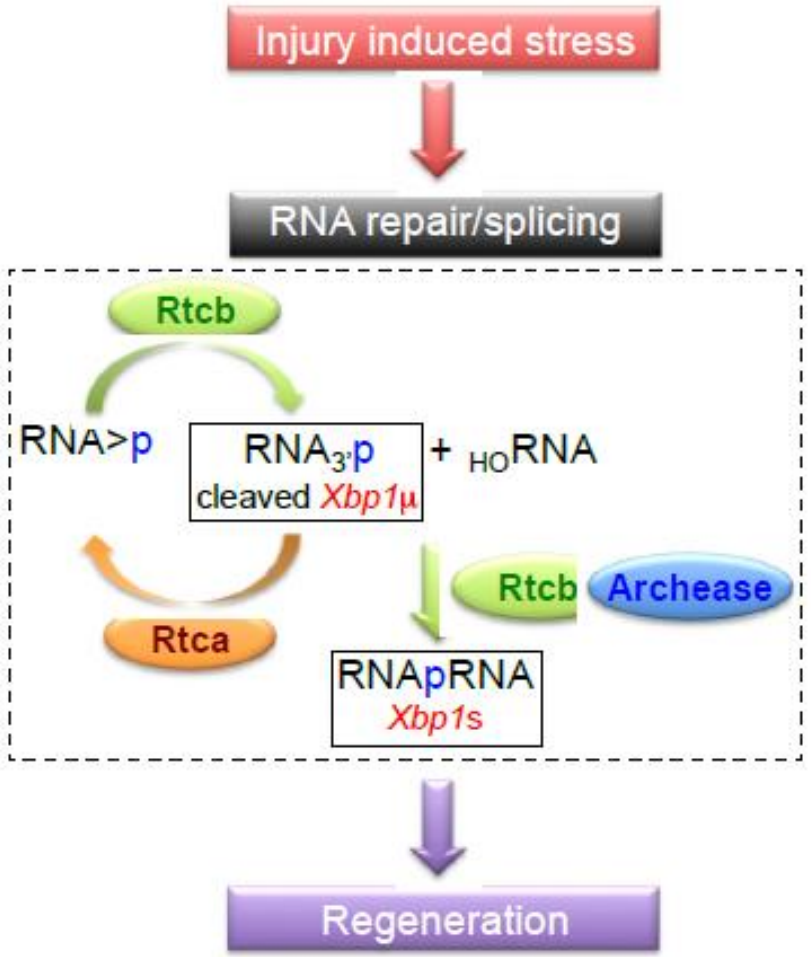
Does knockout of Rtca enhance axon regeneration in adult mice?



Suppl. Figure 9: Rtca LOF during development does not affect adult RGC number or survival after injury.

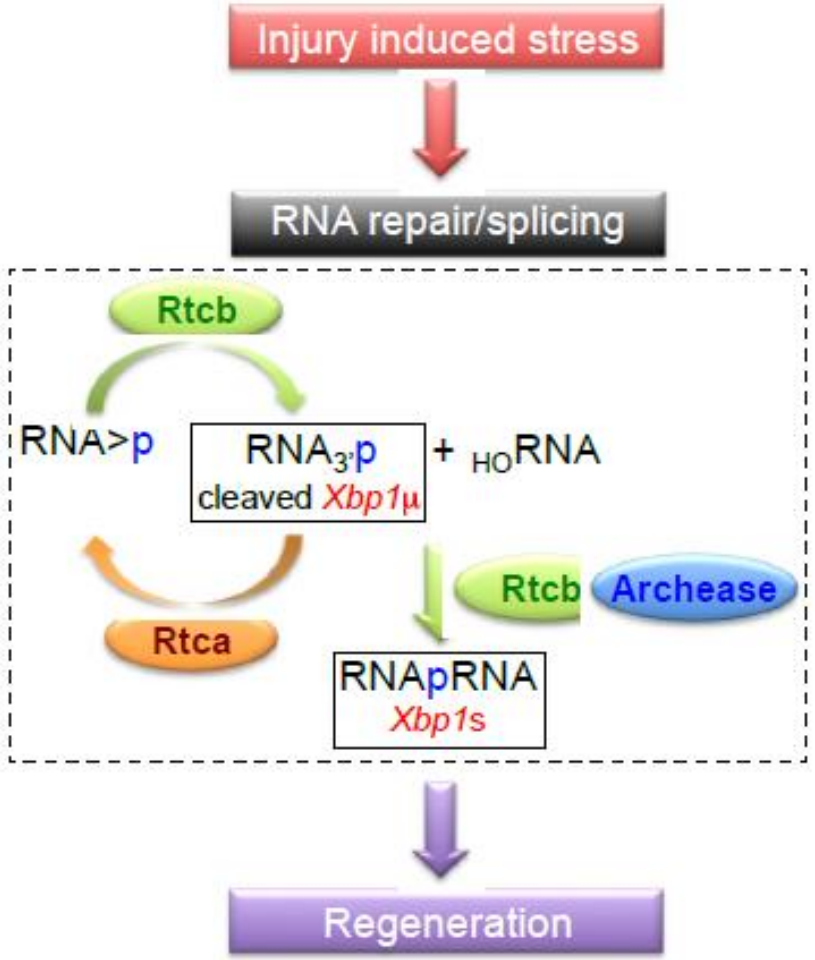
Discussion

- RNA repair and splicing plays an important role in axon regeneration
- Rtca and Archease integrate injury signals



Discussion

- Stress triggered by axotomy leads to response cascade, e.g. Xbp1-splicing
- Splicing of Xbp1 is important for axon regeneration



Discussion

Stress pathways

- Missing link: how is injury signal relayed to stress response?
- → Does axonal injury signal to stress pathway, which recruit Rtca-Archease?
- → Do Rtca-Archease work as upstream elements of stress pathways?

Discussion

Xpb1

- Xpb1 works as an effector downstream of Rtca →
- Does it impinge other stress pathways?

Discussion

Rtca, Archease and Xpb1

as possible targets for therpeutic intervention in nervous system injury?

Discussion

- Rtca-knockout phenotype is modest compared to Pten, Klf4 or Socs3-knockout

→Residual Rtca-function?

→Developmental compensation

→Future mammalian models with Rtca-null mutants needed

Comments

- Thoroughly researched paper
- very conclusively written and illustrated
- Inconsistent knowledge of exact Rtca / Rtcb / Xpb1 / Archease-function
- Only hypothesized model of UPR-response / Rtca /Archease /neuron regeneration
- Rtca-function in cells other than neurons?
- Any possibility of therapeutic drug-interaction with Rtca?

Fragen?

Danke!

Sources

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- van Kesteren RE, Mason MRJ, MacGillavry HD, Smit AB, Verhaagen J. A Gene Network Perspective on Axonal Regeneration. Frontiers in Molecular Neuroscience. 2011;4:46.
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