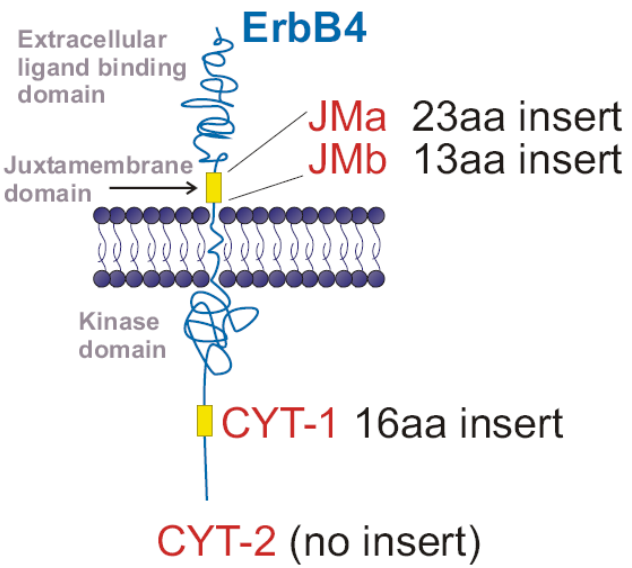
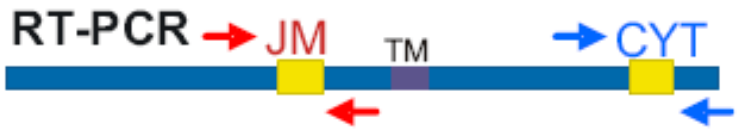
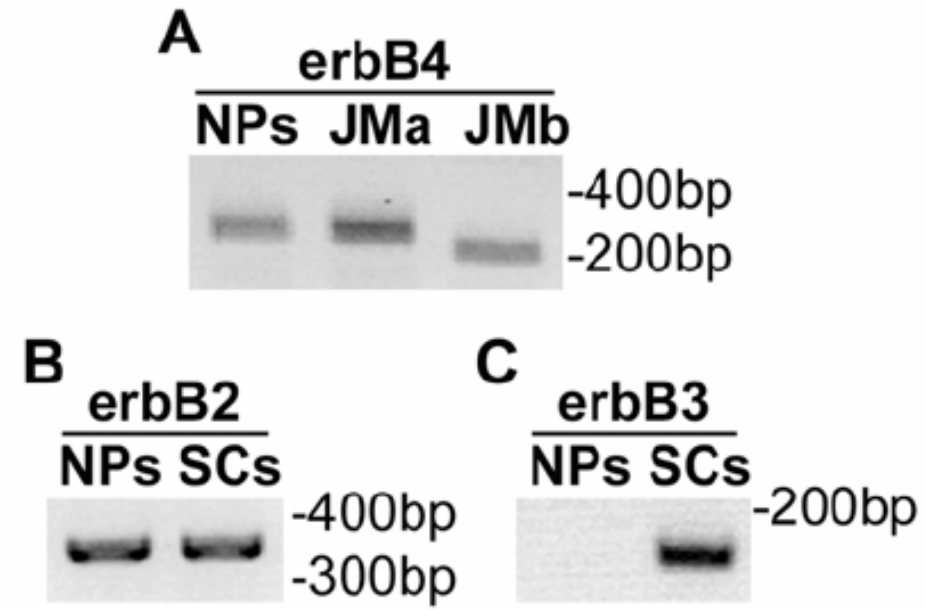
Could E4ICD/TAB2/N-CoR complex be of biological significance for Primary Neuronal precursors in the central nervous system?



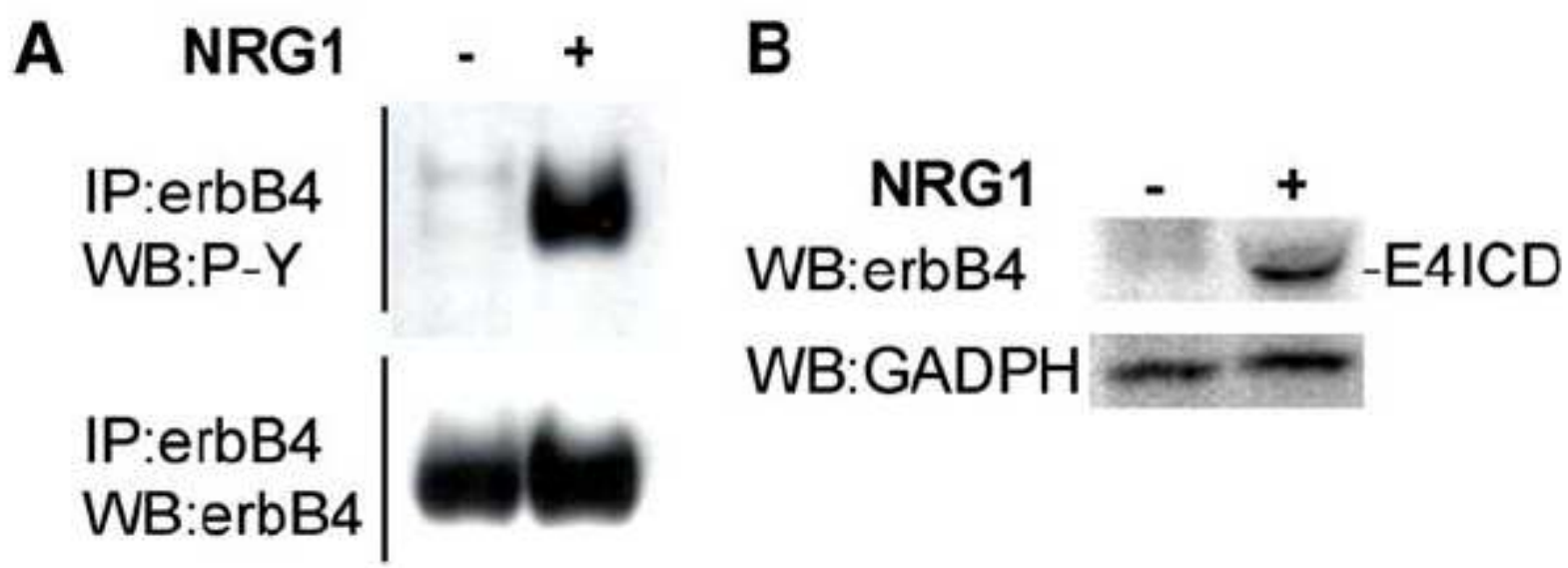


Primary Neuronal precursors (NPs) obtained from E14.5 rat cortices express only the ErbB4 JMa isoform (and ErbB2).

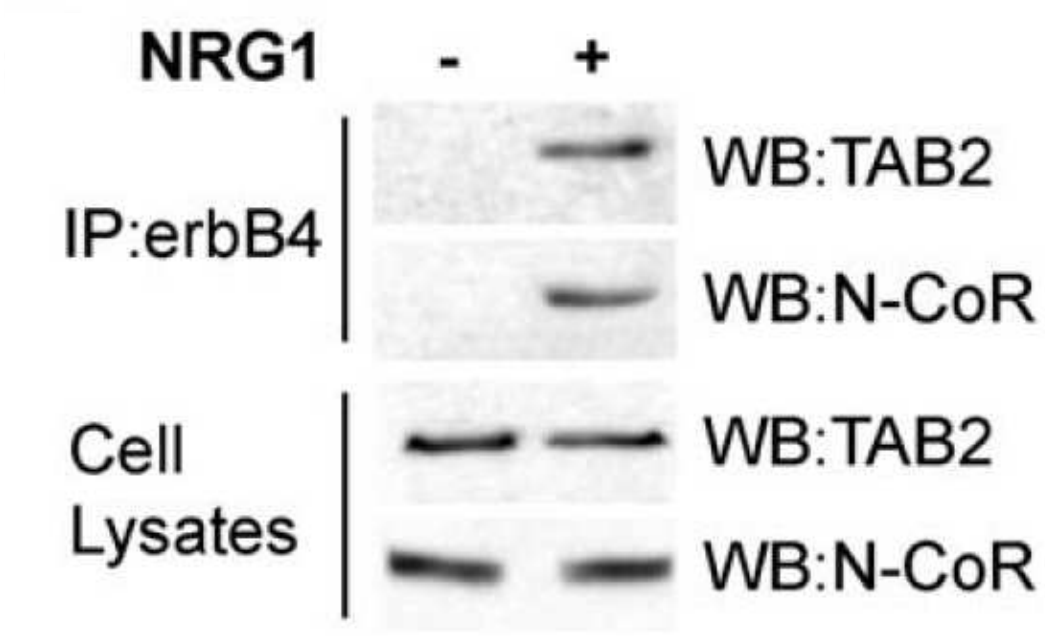
Since ErbB2 does not bind to NRG1, any response of NPs to NRG1 would require ErbB4, acting as either a homodimer or an ErbB4/2 heterodimer.



In Primary Neuronal precursors ErbB4 is readily activated by NRG1, leading to its cleavage and release of the 80 kDa E4ICD.

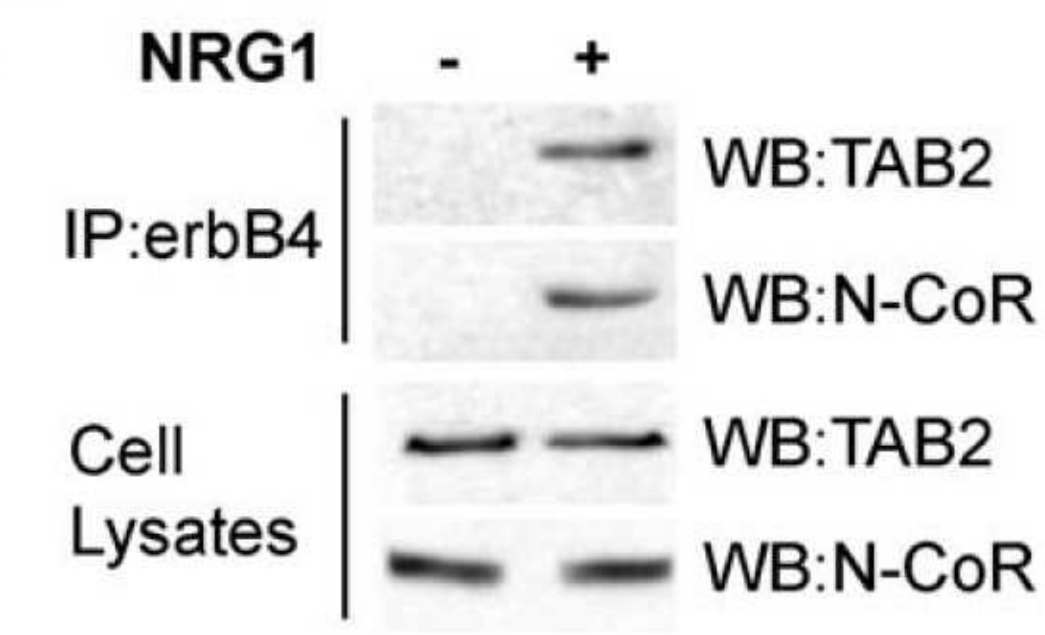


Does ErbB4 endogenous to Primary Neuronal precursors interact with TAB2 and N-CoR?

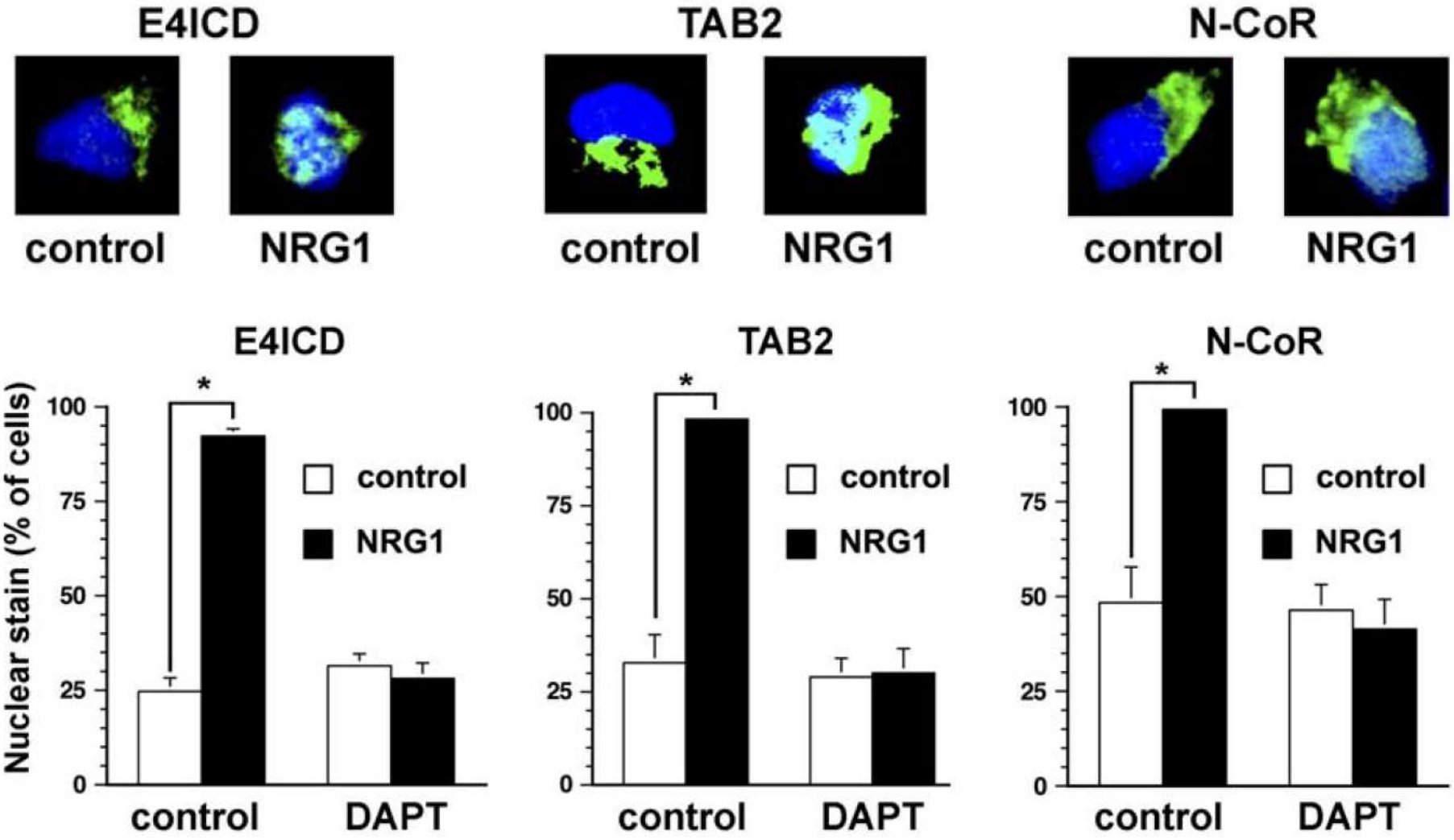


Does ErbB4 endogenous to Primary Neuronal precursors interact with TAB2 and N-CoR?

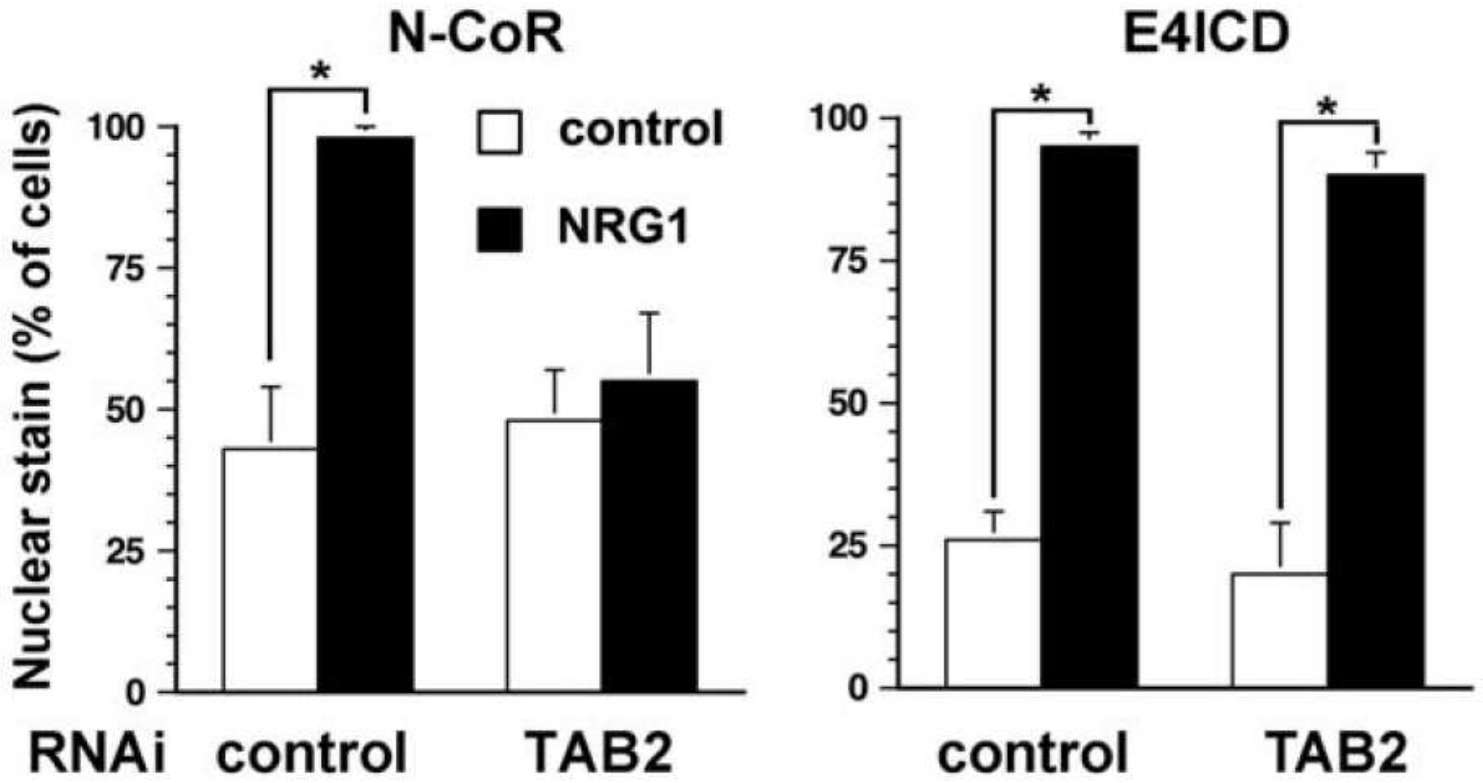
Immunoprecipitation assays show that NRG1 induces ErbB4 JMa association with both endogenous TAB2 and N-CoR.



Immunostaining showed that NRG1 promotes nuclear translocation of E4ICD, TAB2, and NCoR in virtually all Primary Neuronal precursors and that this depends on presenilin activity

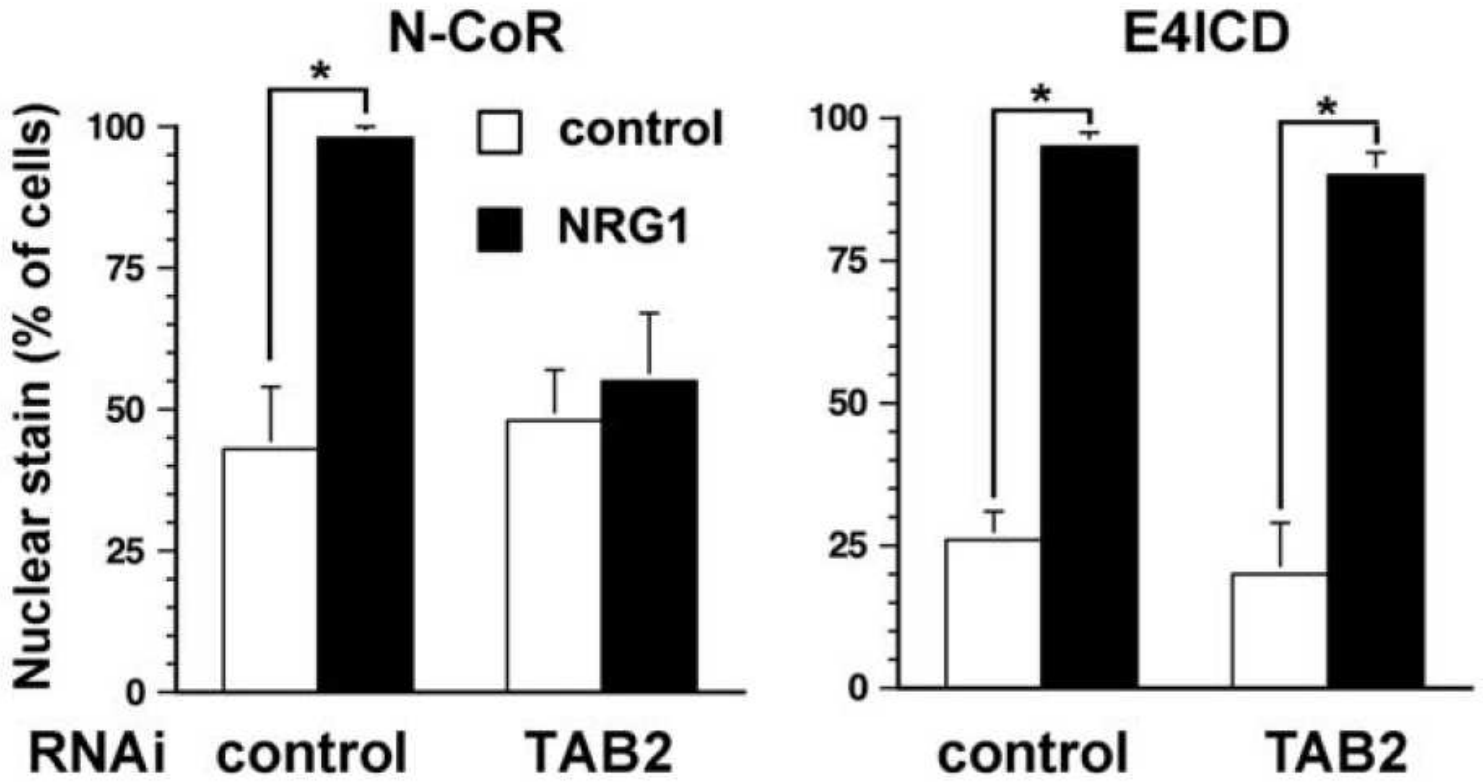


Do you observe differences among E4ICD, TAB2 and N-CoR?



→ ?

Lentivirus-mediated RNAi knockdown of TAB2 abolished the NRG1-induced nuclear translocation of N-CoR, whereas E4ICD nuclear translocation was not affected.



→ E4ICD might be responsible for nuclear shuttling of the E4ICD/TAB2/N-CoR complex in NPs.

Could NRG1 stimulation of ErbB4 JMa nuclear signaling regulate aspects of Primary Neuronal precursors biology?

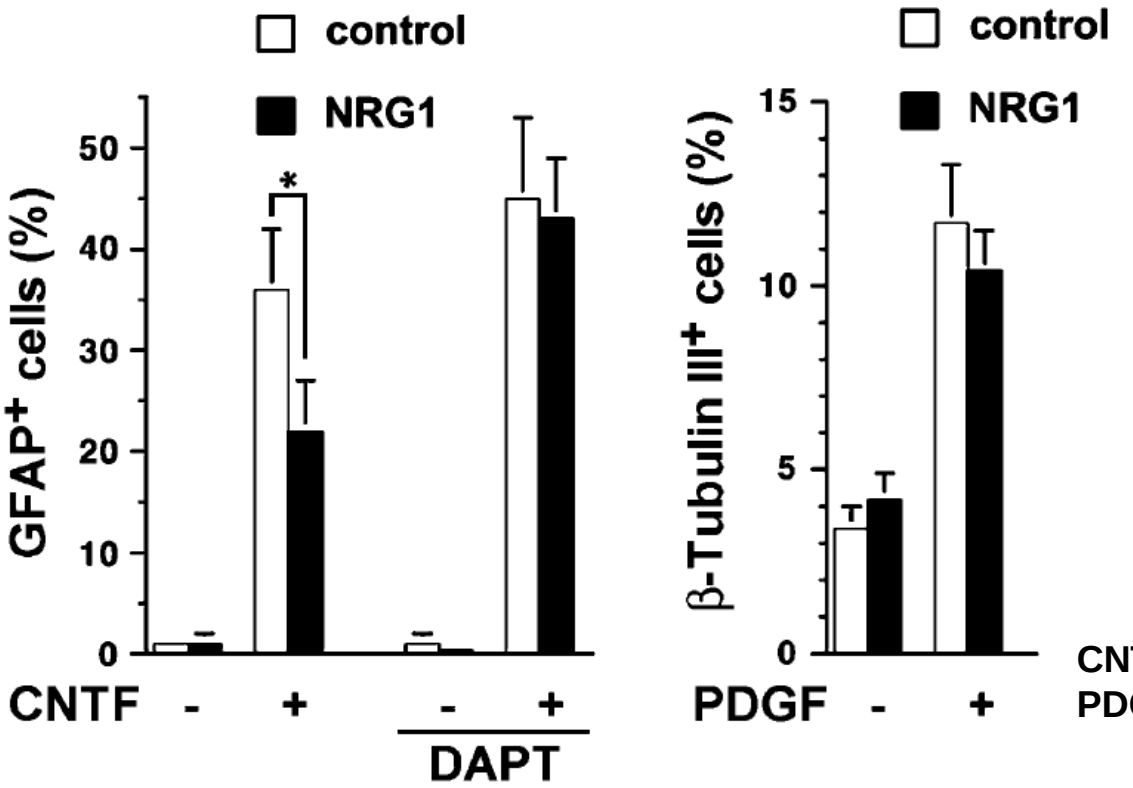
Multipotent Primary Neuronal precursors can be isolated from embryonic brains and maintained in culture in a proliferative undifferentiated state or can be induced to adopt astrocytic or neuronal fates by extracellular signaling molecules.

ciliary neurotrophic factor (CNTF) -> astrocytic differentiation (GFAP+)

platelet-derived growth factor (PDGF) -> neuronal differentiation (β-tubulin III+)

NRG1 stimulation of Primary Neuronal precursors did not induce the acquisition of either neuronal or astrocytic fates, and did not modify survival or proliferation.

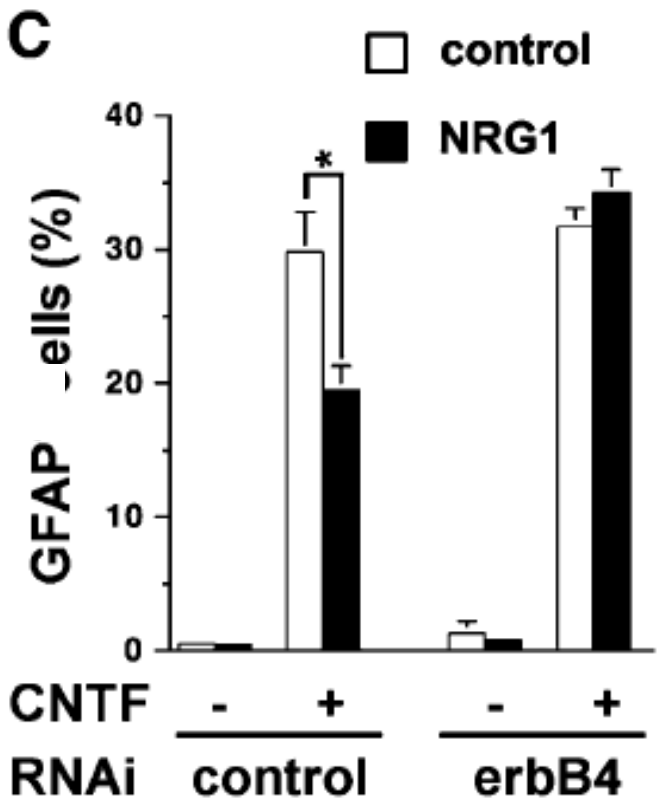
NRG1 antagonized the effects of CNTF on astrogenesis without altering the ability of PDGF to induce Primary Neuronal precursors to adopt a neuronal fate.



CNTF -> astrocytic differentiation (GFAP+)
PDGF -> neuronal differentiation (β-tubulin III+)

Activation and cleavage of ErbB4 JMa after NRG1 stimulation might contribute to maintenance of the Primary Neuronal precursors pool in a neurogenic state by preventing their differentiation into astrocytes.

Is ErbB4 the NRG1 receptor implicated in this differentiation effect?



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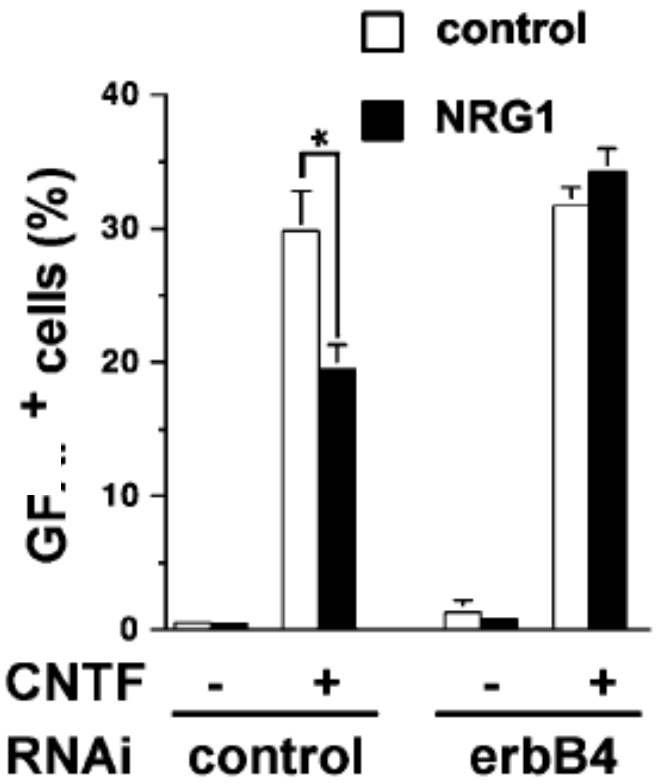
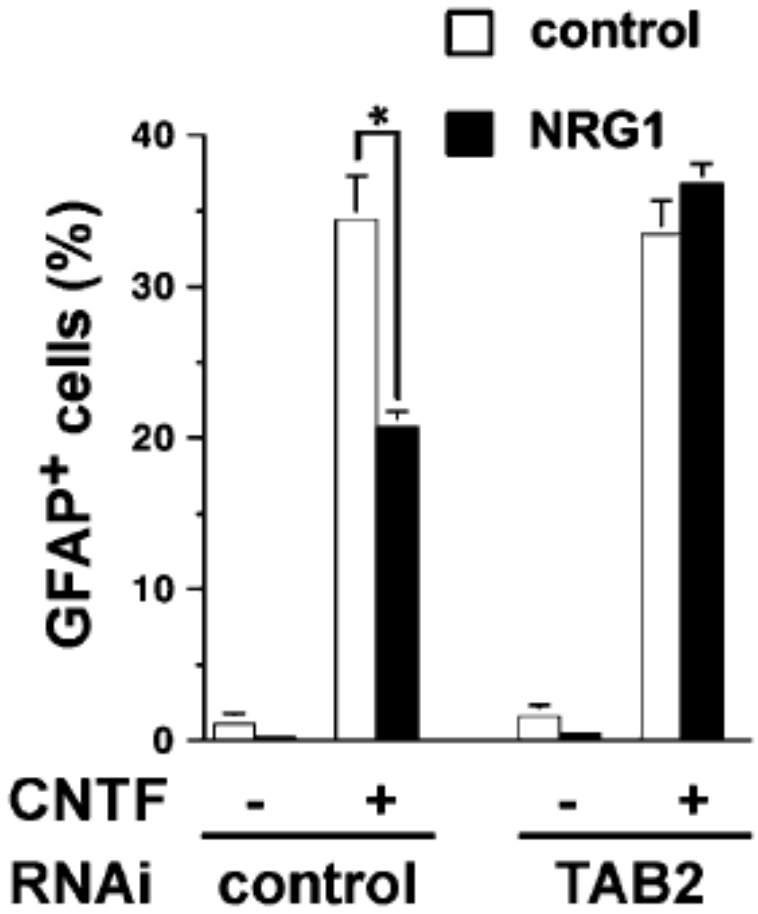
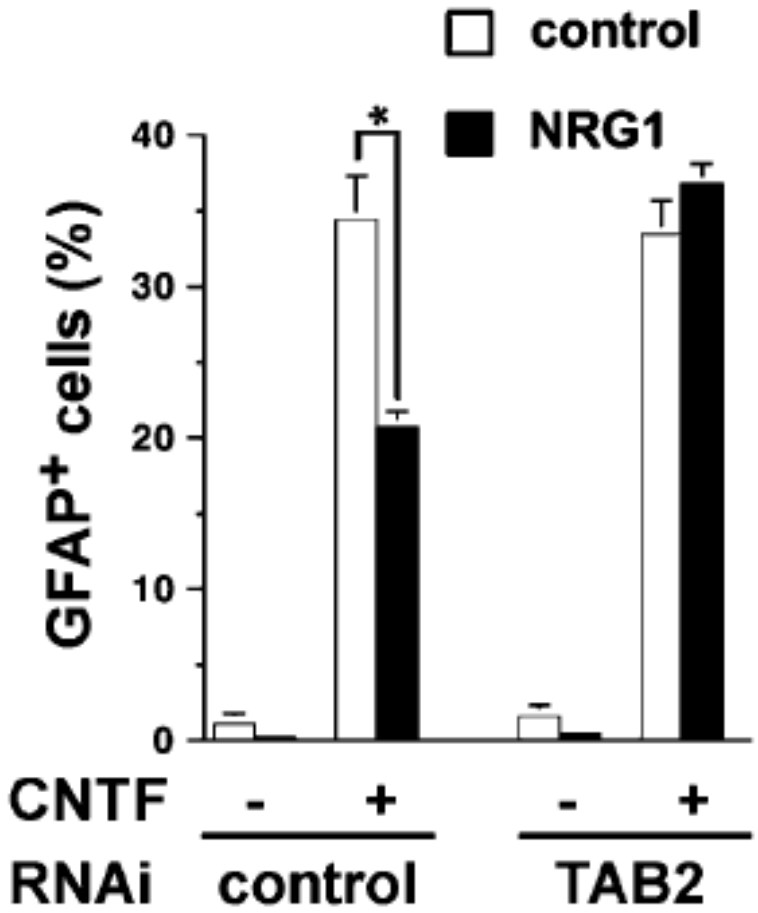


ErbB4 expression in Primary Neuronal precursors was eliminated using lentivirus-mediated RNAi knockdown. Infection with control lentivirus did not alter the number of GFAP-positive astrocytes found in untreated, NRG1-treated, and/or CNTF-treated cultures.
Knockdown of ErbB4 completely abolished the ability of NRG1 to antagonize the CNTF-induced astrogenesis, indicating that this receptor is essential for the NRG1-mediated effect.

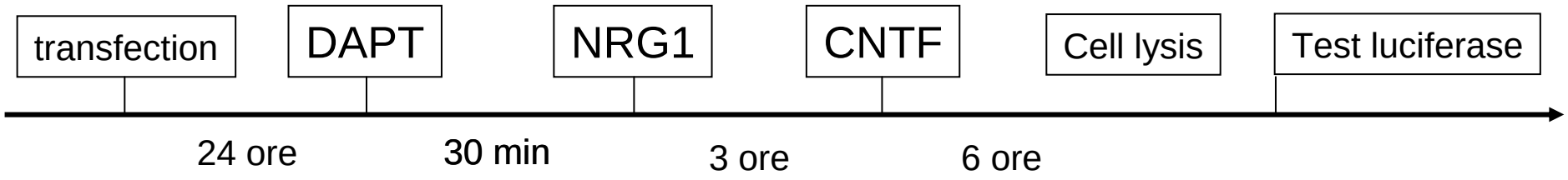


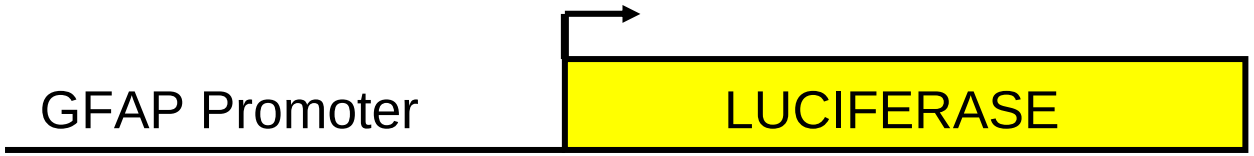
Similar experiments carried out with TAB2 RNAi produced identical results showing that TAB2 is required for the inhibition of the NRG1-dependent inhibition of astrocyte differentiation.

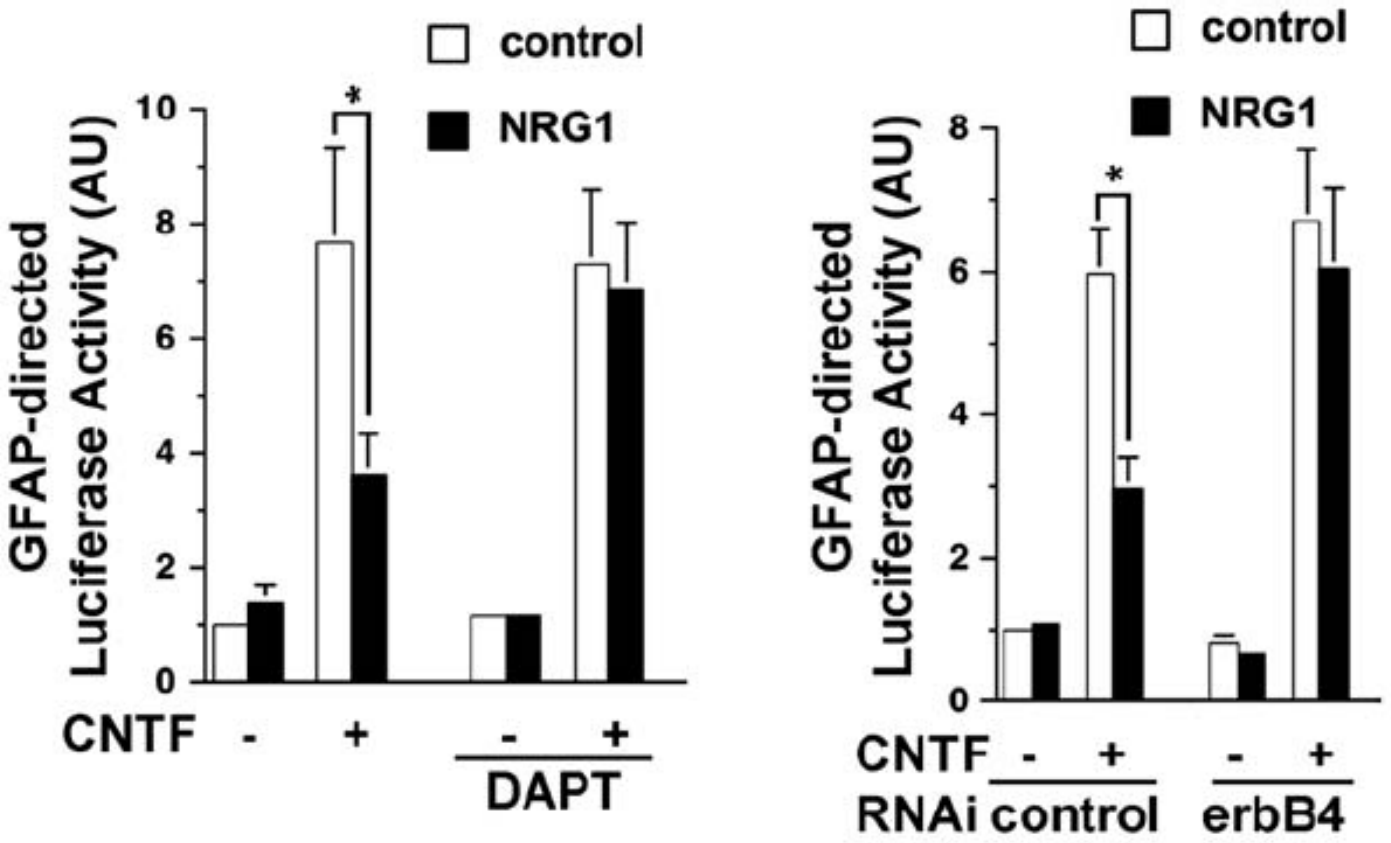


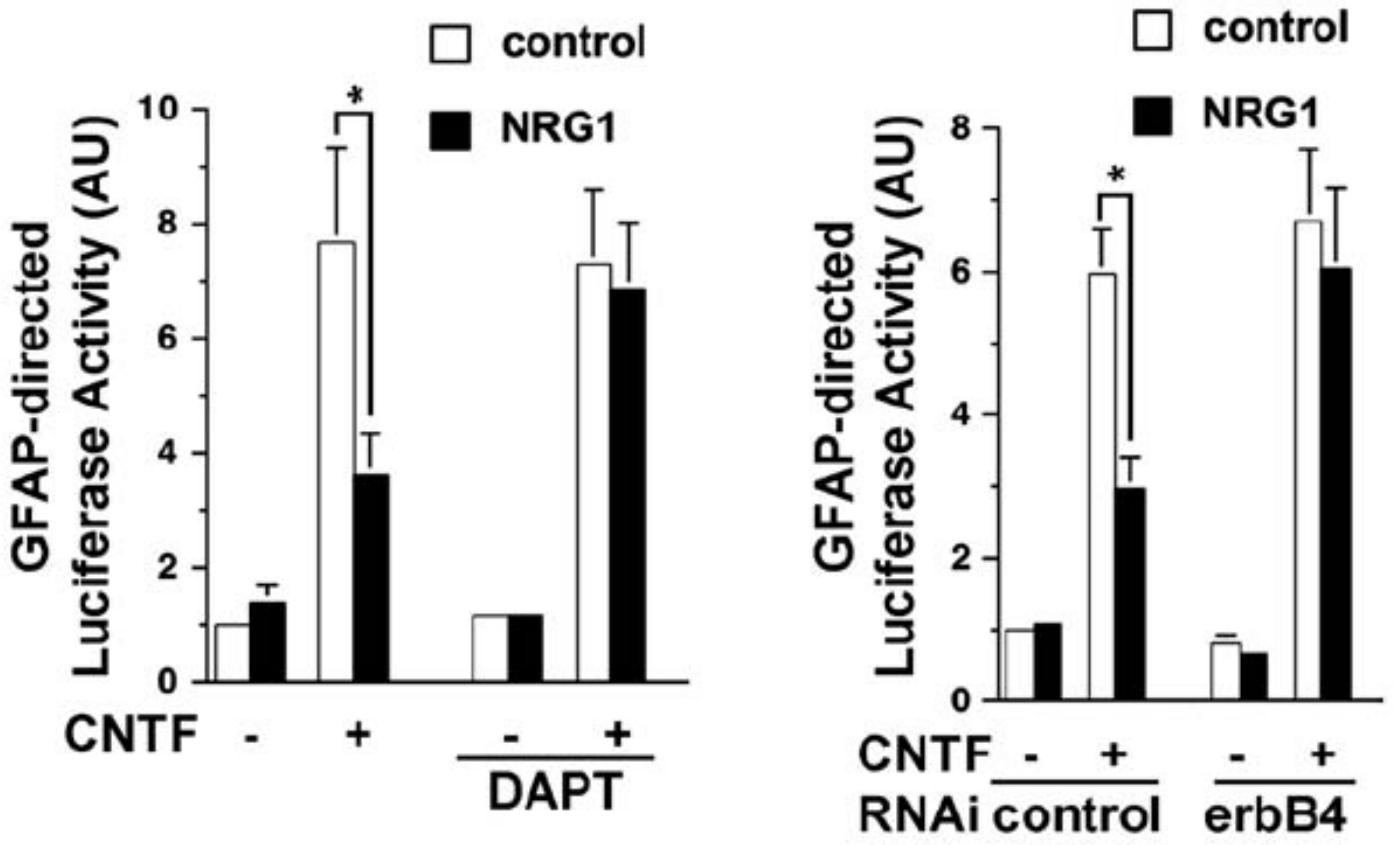
Does E4ICD Nuclear Signaling Inhibit Astrogenesis through Transcriptional Repression of Astrocytic Genes?

1. Primary Neuronal precursors were cotransfected with GFAP-luciferase
The next day, cells were treated with NRG1, CNTF, and/or DAPT:
2. DAPT was added 30 min prior to NRG1
3. NRG1 was added 3 hr prior to CNTF
4. Six hours after CNTF addition, Primary Neuronal precursors were lysed and luciferase activity was measured





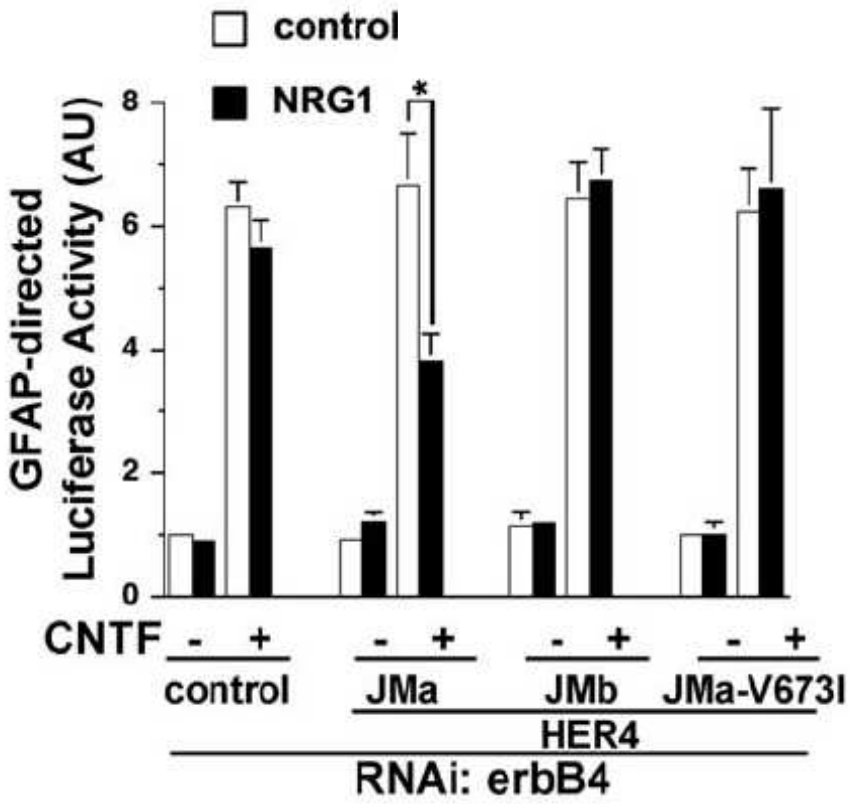




NRG1 had no effect on GFAP promoter basal activity in a luciferase reporter assay in Primary Neuronal precursors, but it significantly reduced the effects of CNTF on this promoter activity.
This antagonistic effect of NRG1 was blocked by the presenilin inhibitor DAPT, and knockdown of ErbB4.

Importantly, NRG1 had similar effects on S100β, another astrocyte protein.

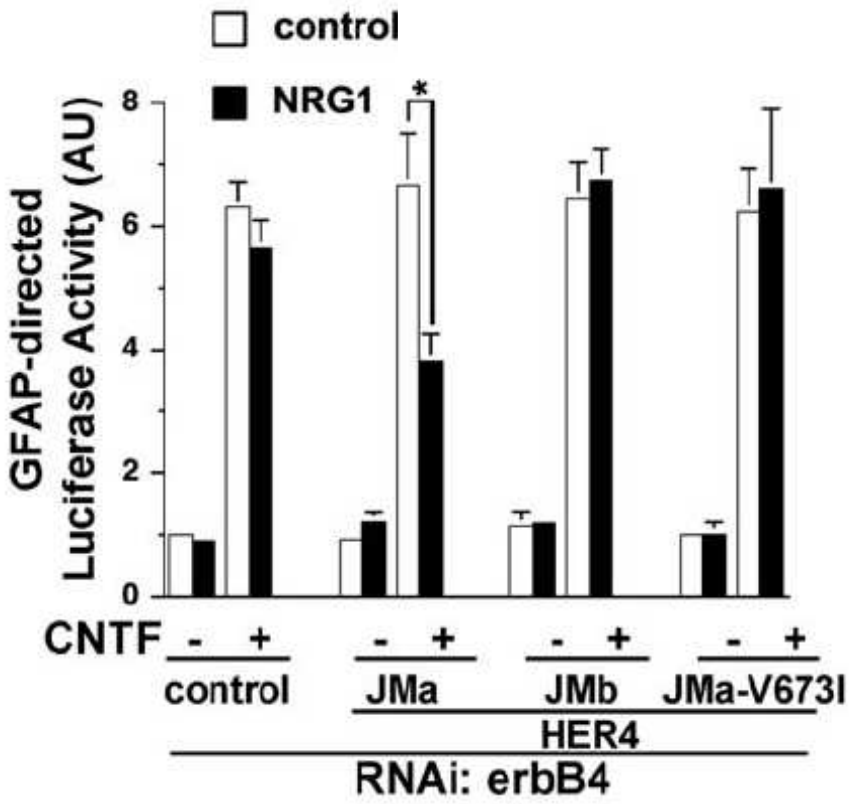
Can ErbB4 variants with diverse sensitivities to proteases **rescue** the effects of NRG1 after RNAi knockdown of endogenous ErbB4?



What is “rescue”?

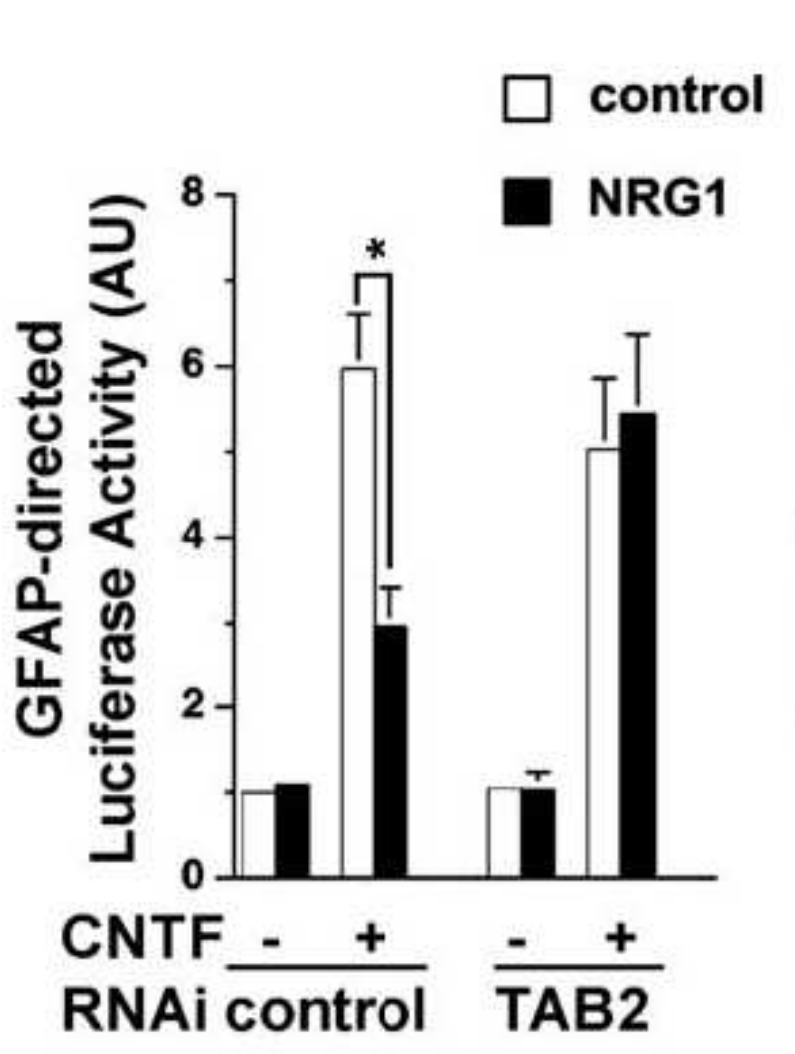
Why exogenous ErbB4 JMa and JMb is resistant to siRNA?

Can ErbB4 variants with diverse sensitivities to proteases rescue the effects of NRG1 after RNAi knockdown of endogenous ErbB4?

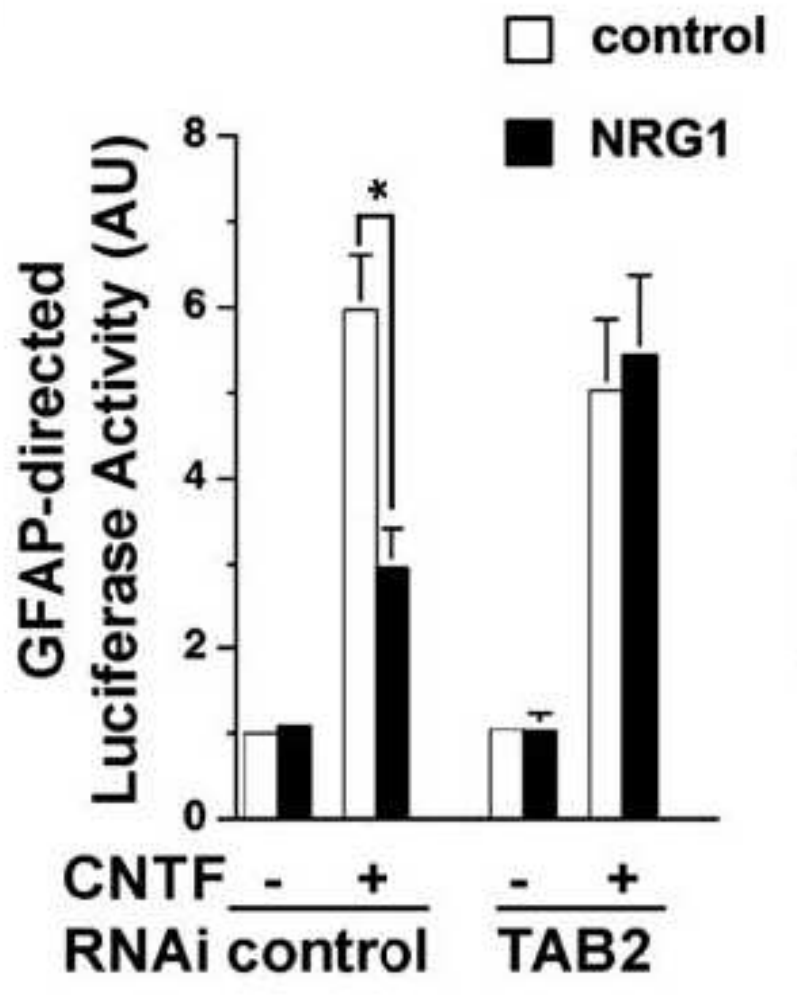


ErbB4 endogenous to NPs was knocked down by RNAi. After 3 days, the human ErbB4 juxtamembrane isoforms (HER4 JMa or JMb) or the presenilin-resistant HER4 JMa V673I, which are resistant to the RNAi, were transfected along with the reporters.

Only the cleavable isoform HER4 JMa rescues the ErbB4 knockdown phenotype.



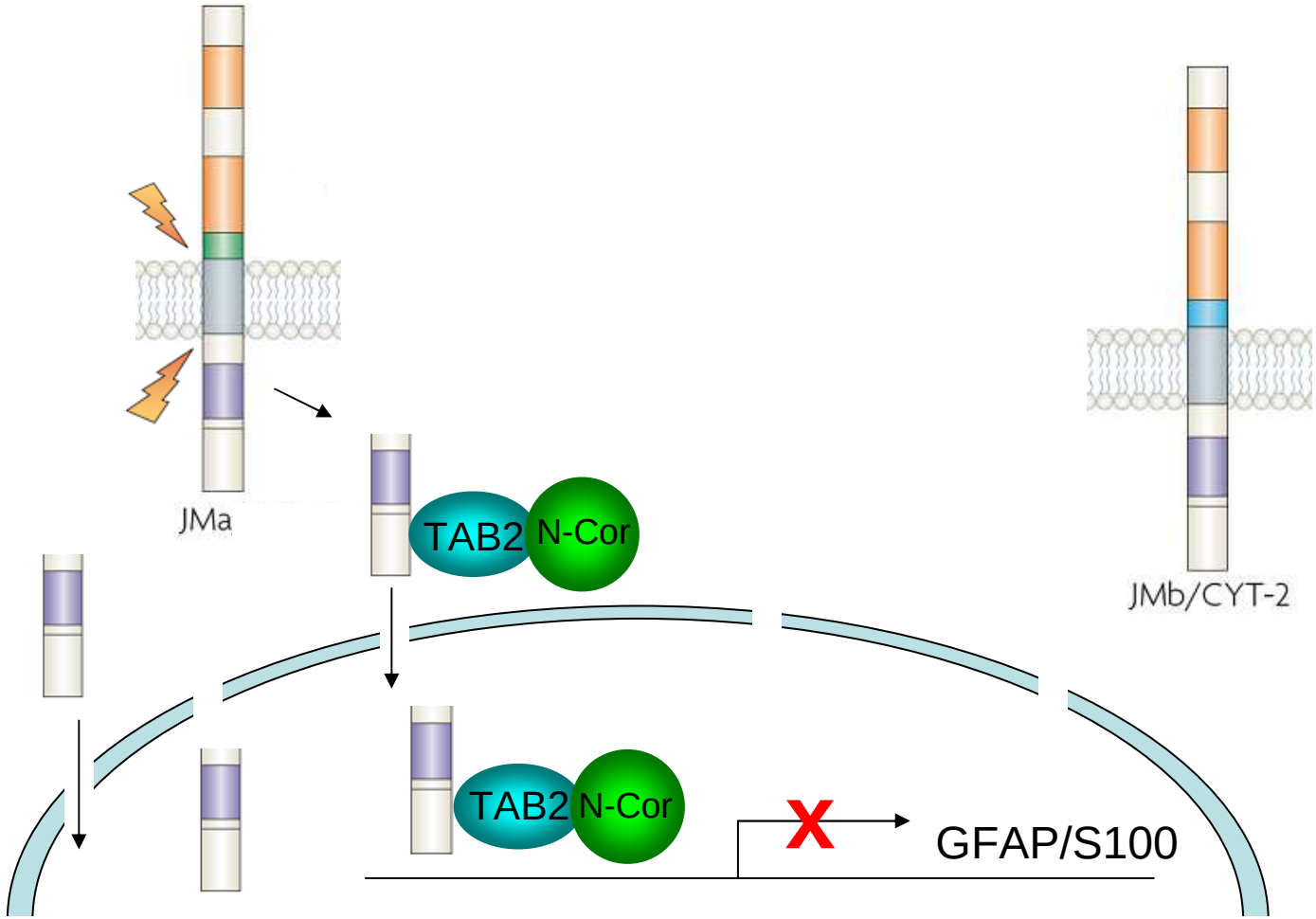
Similar to ErbB4, knockdown of TAB2 eliminated the antagonistic effect of NRG1 on GFAP expression.



These results

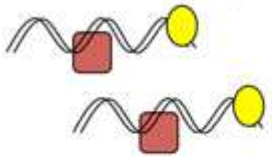
❇ indicate that repression of the GFAP promoter by ErbB4 nuclear signaling requires the presence of TAB2, which brings together E4ICD and N-CoR.

❇ suggested that E4ICD could be part of the transcriptional repressor complex that mediates the NRG1 inhibition of GFAP and S100β expression.

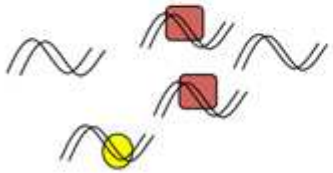


To test this possibility, they used a Chromatin Immunoprecipitation (ChIP) assay.

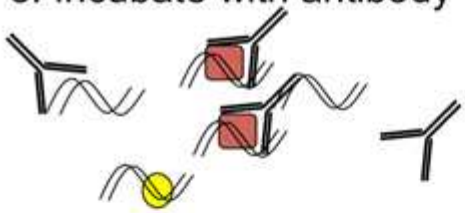
1. Crosslink proteins to DNA



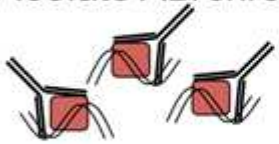
2. Isolate chromatin and sonicate



3. Incubate with antibody



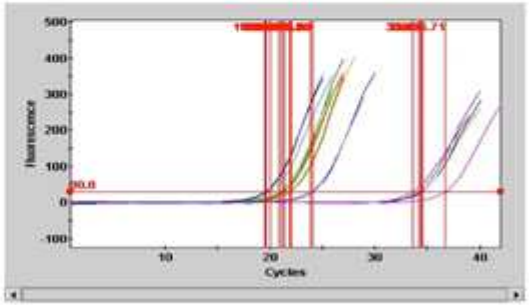
4. Isolate AB/chromatin complexes



5. Isolate DNA from complexes

Chromatin Immuno-precipitation - ChIP

qPCR

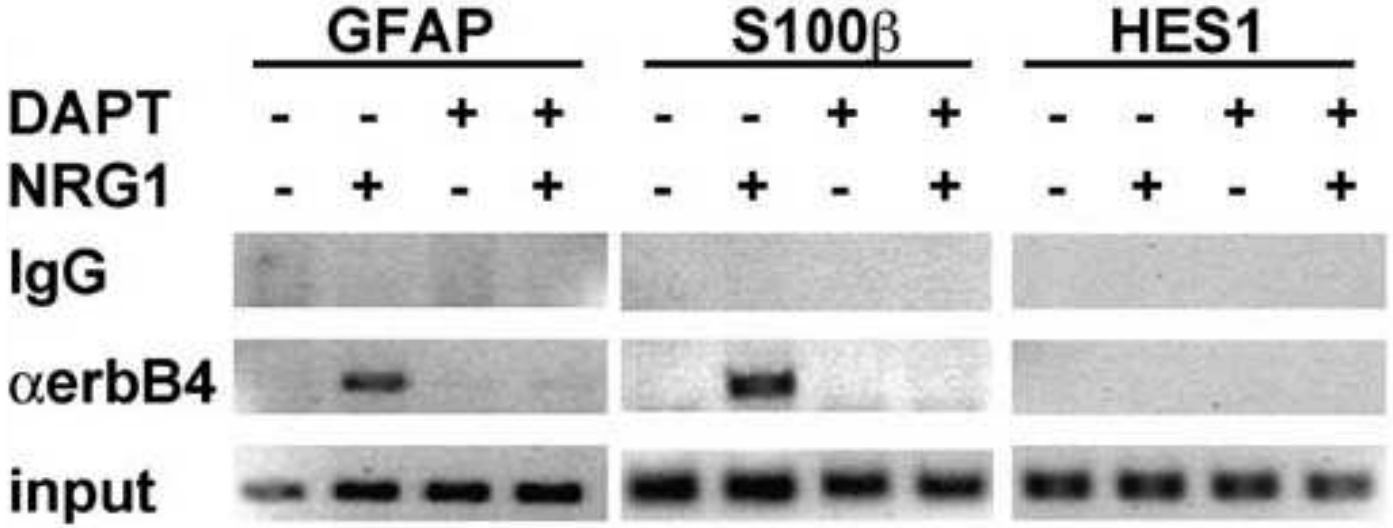


ChIP-chip

ChIP-seq

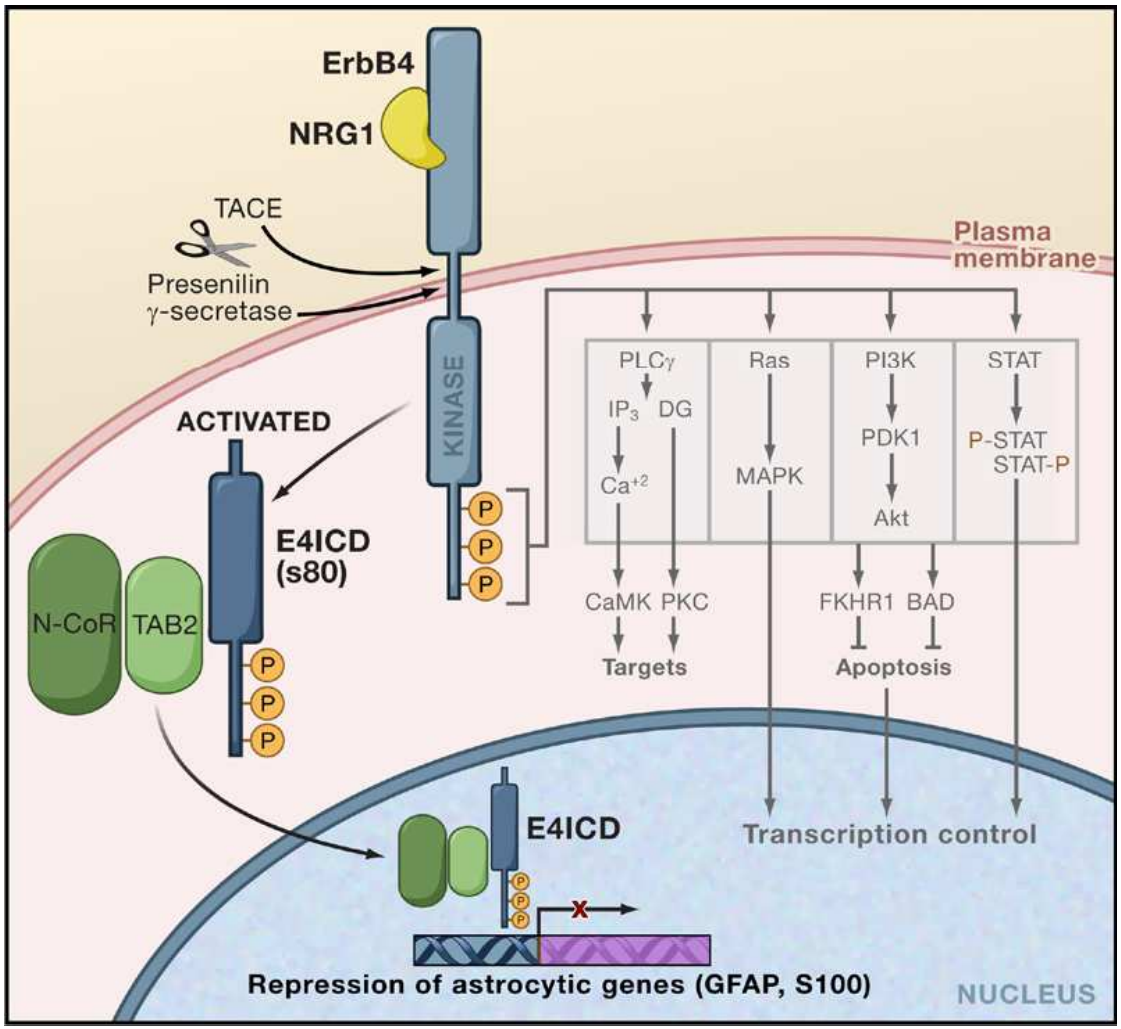
N Riddle

Immunoprecipitation with ErbB4 antibodies showed that E4ICD associates with the GFAP and S100β promoters in NPs, but only after treatment with NRG1. Moreover, these associations were blocked by presenilin inhibition.



The association of E4ICD with the glial promoters was specific since normal rabbit immunoglobulin G (IgG) failed to immunoprecipitate these promoters and ErbB4 antibodies did not precipitate a control promoter, HES1.

In this paper authors show that NRG1-induced presenilin-dependent ErbB4 nuclear signaling regulates the timing of astrogenesis in the developing brain. Upon activation and presenilin-dependent cleavage of ErbB4, E4ICD forms a complex with the signaling protein TAB2 and the corepressor N-CoR.



This complex translocates to the nucleus of undifferentiated neural precursors and inhibits their differentiation into astrocytes by repressing the transcription of glial genes.

Consistent with this observation, cortical astrogenesis occurs precociously in ErbB4 knockout embryos, a phenotype that is rescued by re-expression of human ErbB4 JMa but not by the uncleavable ErbB4 JMb.