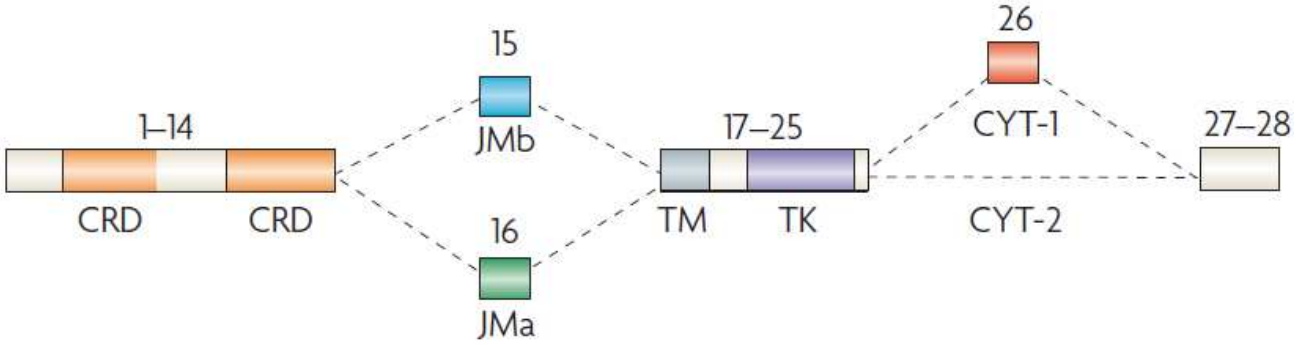
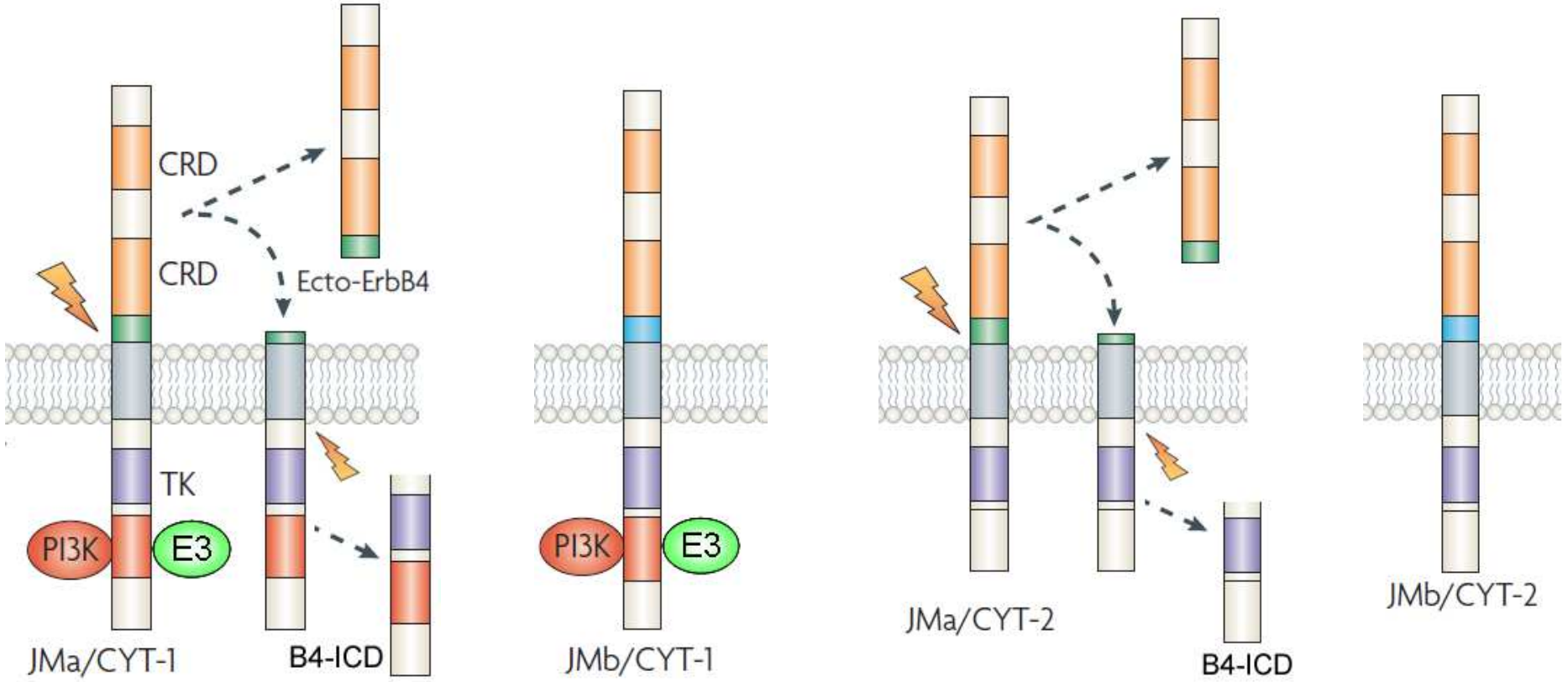


ErbB4 isoforms



(Mei and Xiong, 2008)

Presenilin-Dependent ErbB4 Nuclear Signaling Regulates the Timing of Astrogenesis in the Developing Brain

S. Pablo Sardi,[1] Joshua Murtie,[1] Samir Koirala,[1] Brooke A. Patten,[1] and Gabriel Corfas[1,*]
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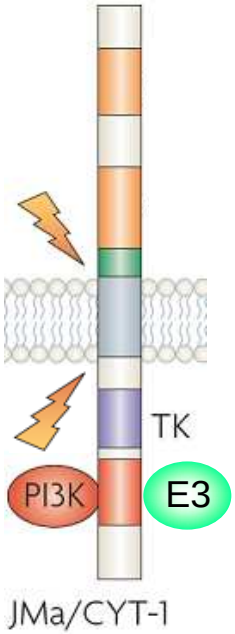
DOI 10.1016/j.cell.2006.07.037

Cell *127*, 185–197, October 6, 2006

Identification of Proteins that Interact with Activated E4ICD

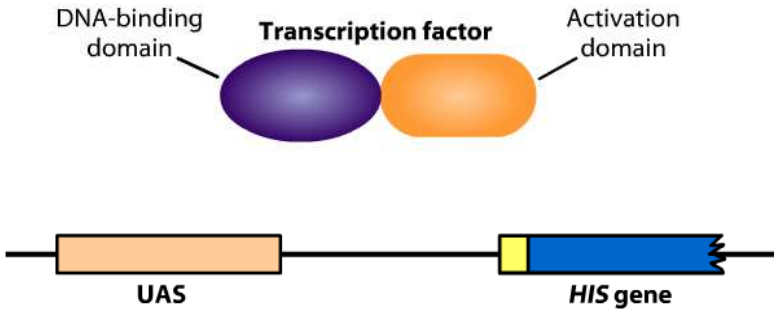


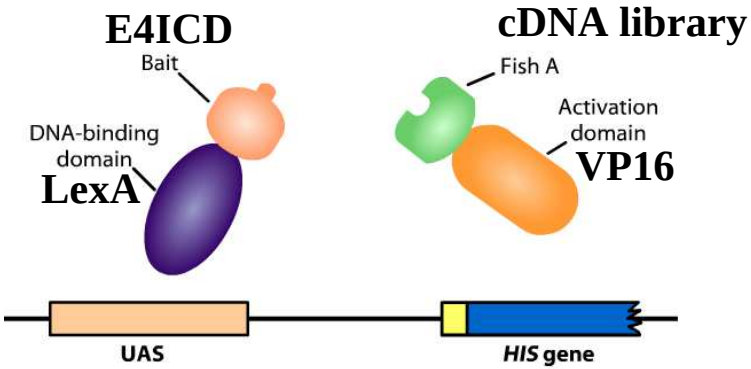
E4ICD

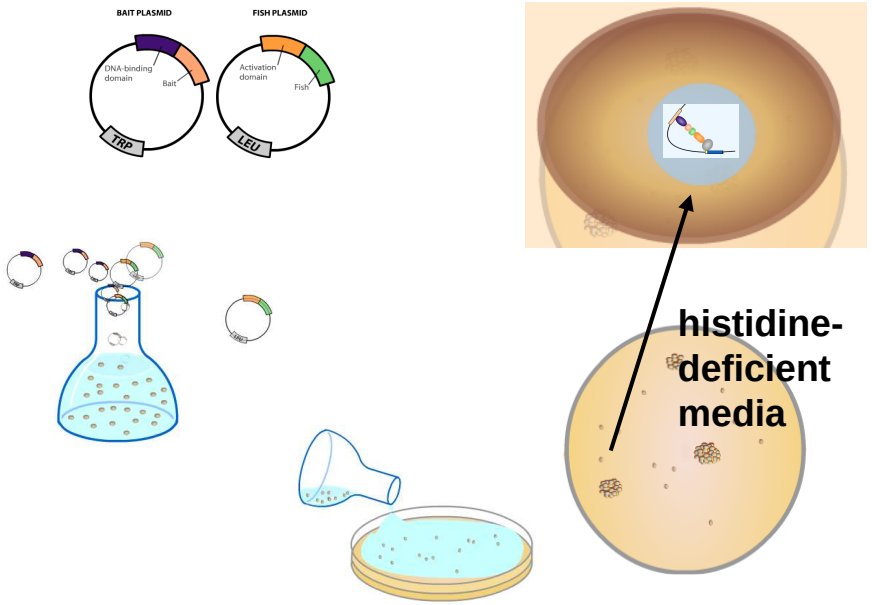
Which assay would you suggest to identify proteins interacting with the ErbB4 intracellular domain (E4ICD)?

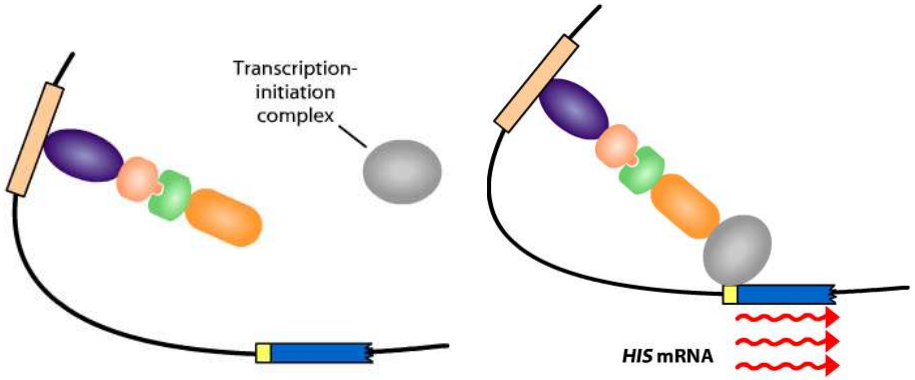
- screening of a cDNA expression library from rat embryonic day 14 (E14) spinal cord and dorsal root ganglia with a bait containing the entire E4ICD in an activated state

E4ICD



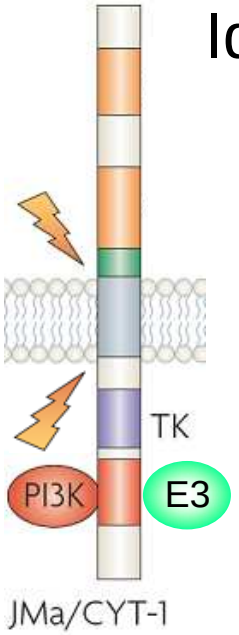






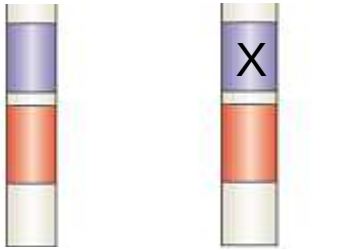
What is a cDNA library? How do you prepare it?

Identification of Proteins that Interact with Activated E4ICD

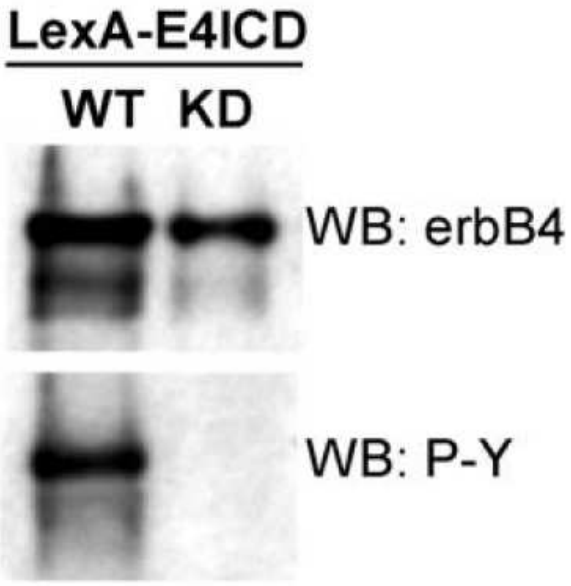


- yeast two-hybrid system to identify ErbB4-binding proteins that are involved in transcriptional regulation

- screening of a cDNA expression library from rat embryonic day 14 (E14) spinal cord and dorsal root ganglia with a bait containing the entire E4ICD in an activated state led to isolation of several putative E4ICD-interacting proteins



$E4ICD^{WT}$ $E4ICD^{KD}$



LexA-E4ICD fusion, when expressed in mammalian cells, dimerizes and becomes autophosphorylated

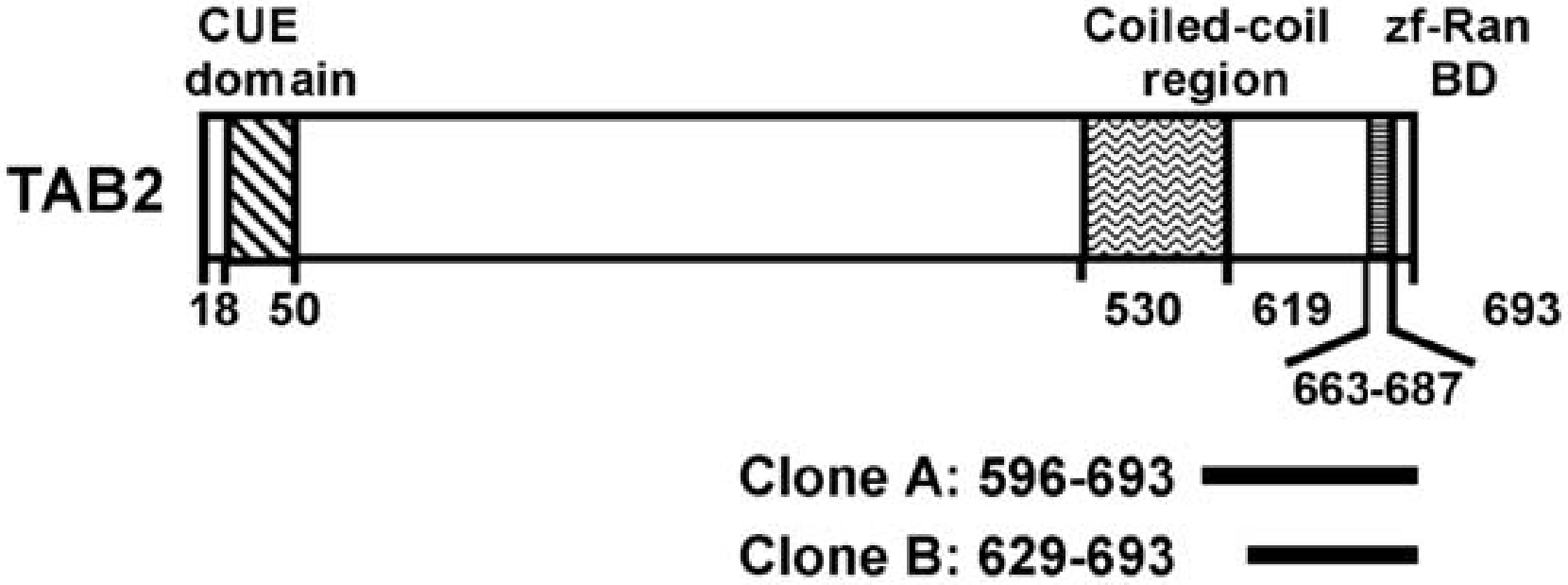
Yeast expressing wild-type (WT) and kinase-dead (KD) LexA-E4ICD fusion proteins were lysed and immunoblotted with ErbB4 or phosphotyrosine (P-Y) antibodies, showing that both proteins are expressed (top panel) but only WT is tyrosine phosphorylated

The two hybrid screening led to isolation of several putative E4ICD-interacting proteins

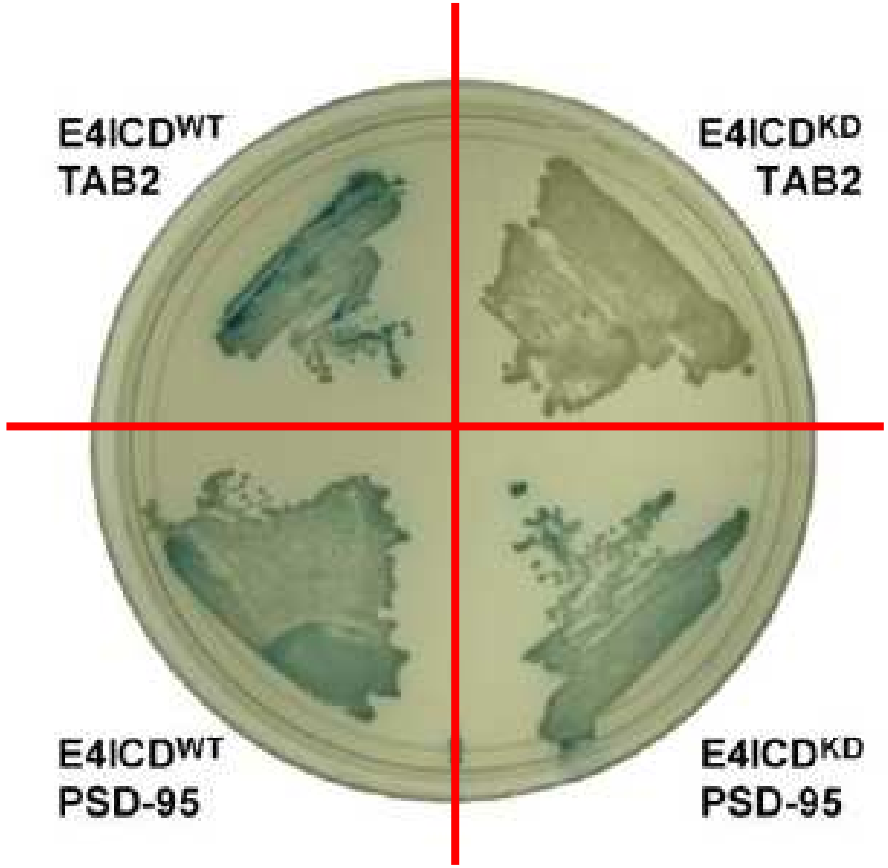
Two clones contained cDNAs encoding the C-terminal region of
TAB2 (TAK1 binding protein 2), a protein first identified as an adaptor for
TAK1 (transforming growth factor β-activated kinase 1)

Schematic diagram of TAB2

Regions of TAB2 included in two clones identified in the screen are indicated.
zf-Ran BD: zinc finger Ran-binding domain.



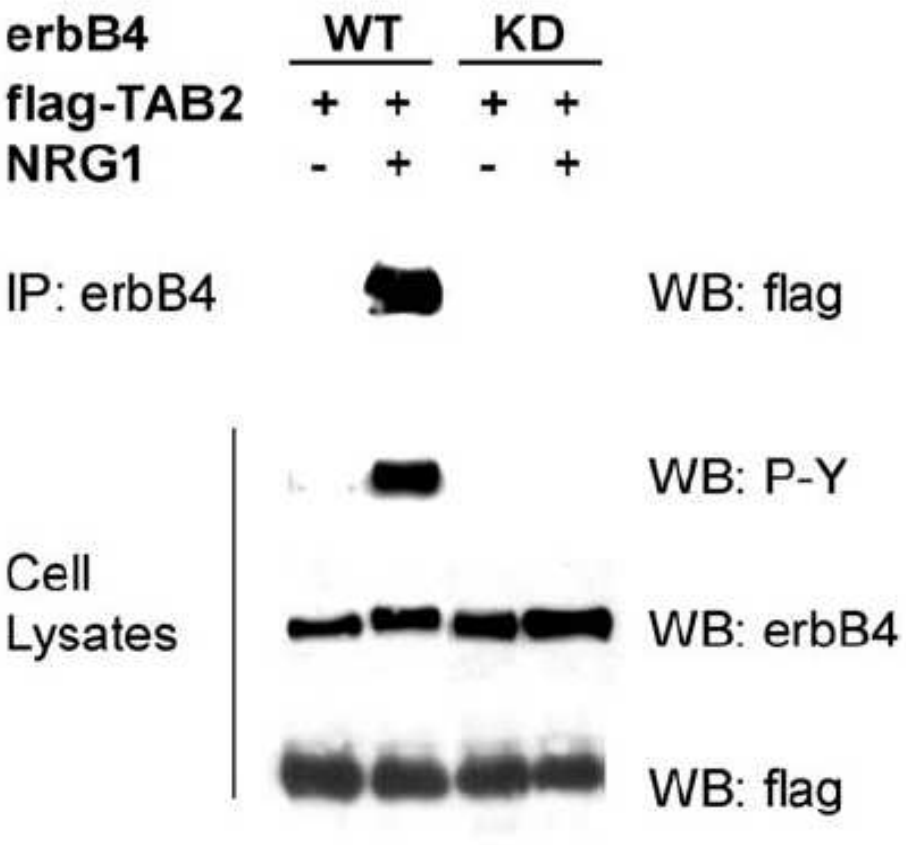
Wild-type E4ICD also interacted with full-length TAB2 in yeast, and this association was abolished when the tyrosine kinase activity of E4ICD was eliminated by a mutation in the ATP-binding site ($E4ICD^{KD}$).

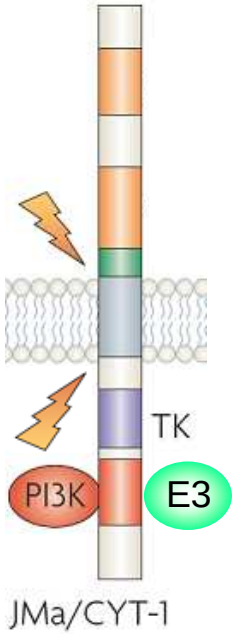


Tyrosine kinase activity of E4ICD is necessary for interaction with TAB2 in yeast. WT or KD LexA-E4ICD was coexpressed in yeast with full-length TAB2 or PDZ domains 1 and 2 of PSD-95 as fusion proteins with the activation domain.
TAB2 only interacts with phosphorylated E4ICD (blue color), whereas PSD-95 interacts with both baits.

→ binding of TAB2 to ErbB4 in yeast appears to occur only when the tyrosine kinase receptor is activated.

Does TAB2 binds to ErbB4 in mammalian cells?
Does this interaction depend on receptor activation by NRG1?





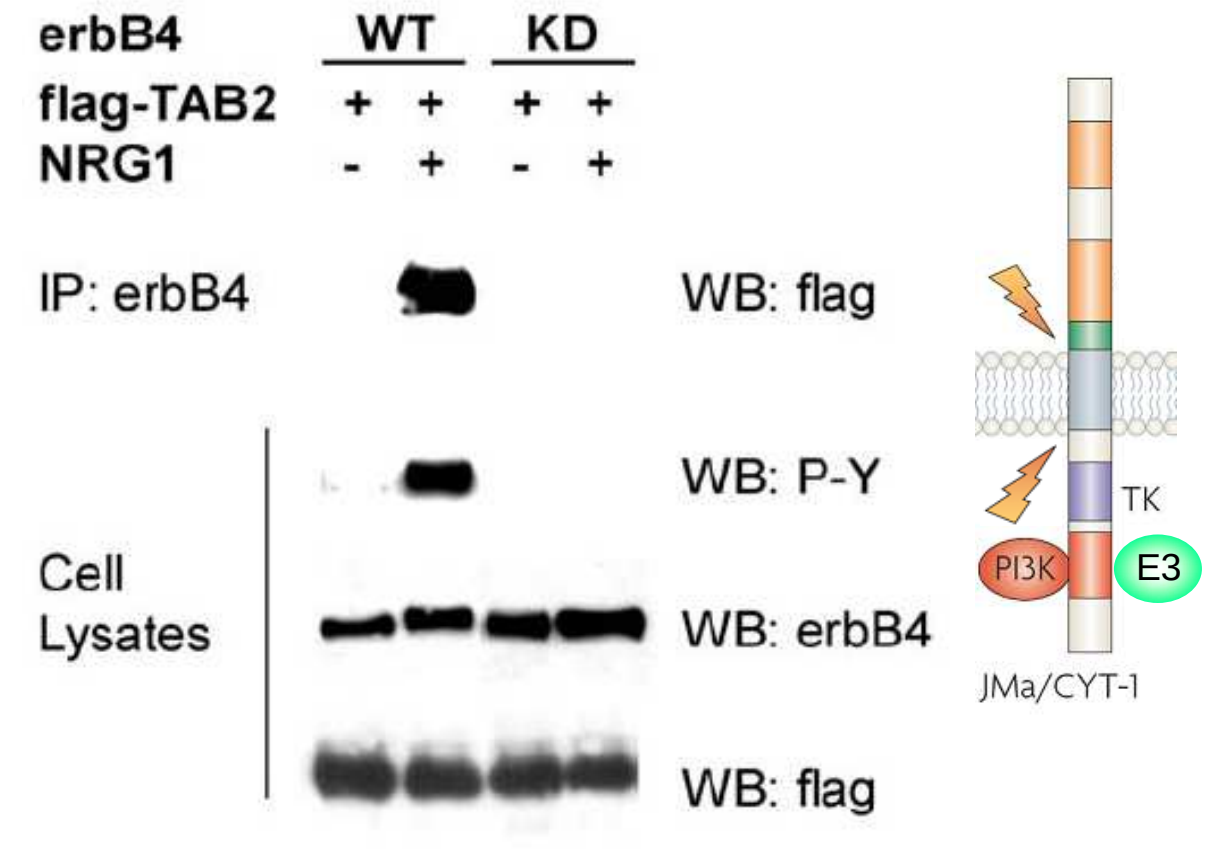
1- Why do they use full length ErbB4 and not LexA-E4ICD ?

2- Which control is missing in this IP?

3- What can you conclude from this IP-western blot?

Does TAB2 binds to ErbB4 in mammalian cells?
Does this interaction depend on receptor activation by NRG1?

Cells were cotransfected with FLAG-TAB2 and full-length ErbB4 expression constructs and then subjected to immunoprecipitation with ErbB4 antibodies.



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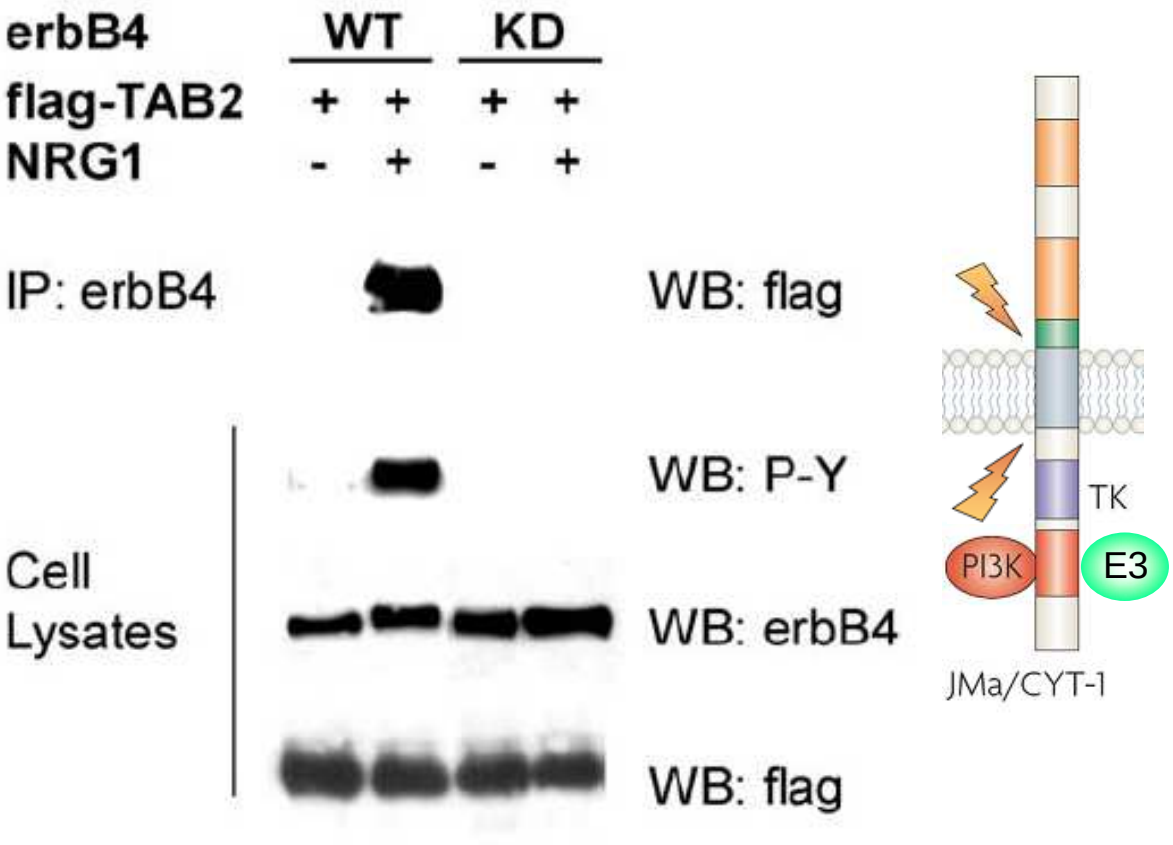


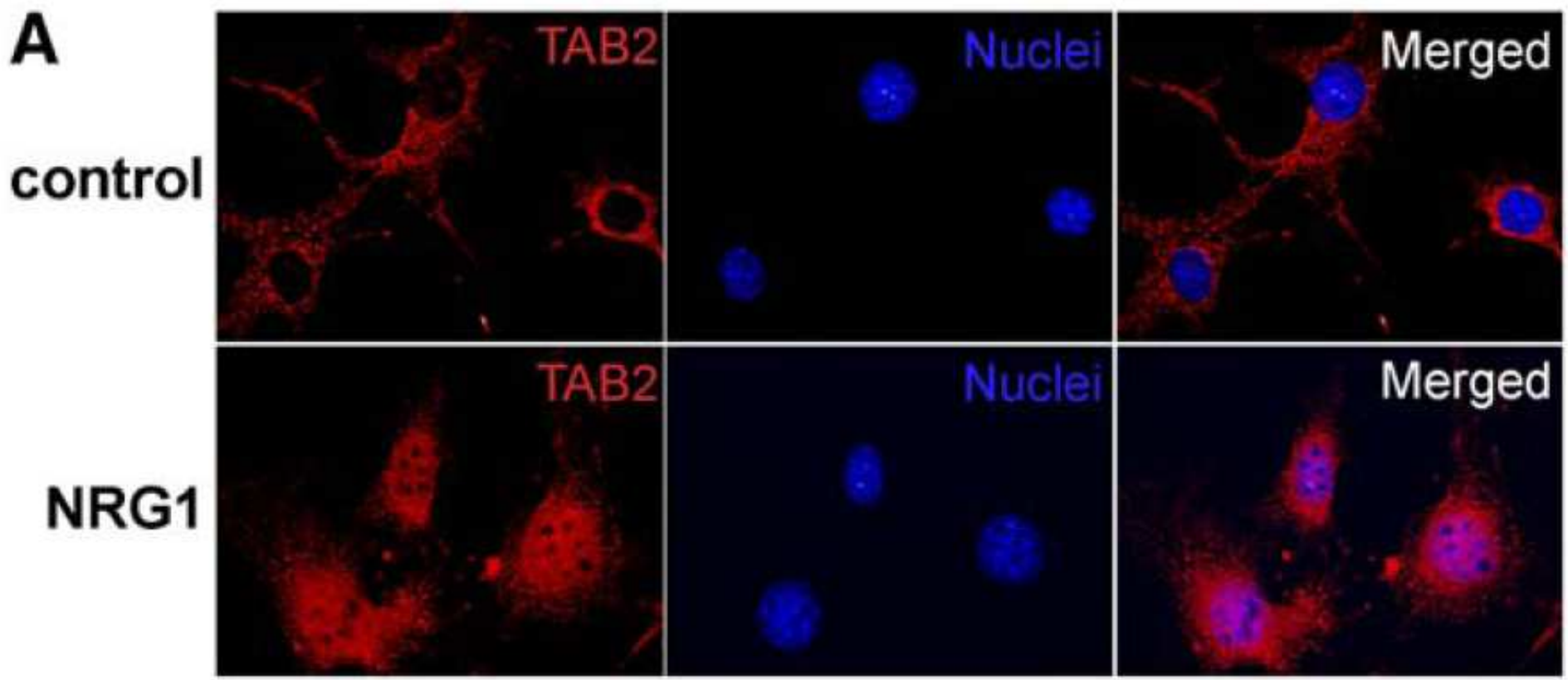
→ TAB2 coprecipitated with ErbB4 only after NRG1 treatment

Presenilin-Dependent Cleavage of ErbB4 does not induce TAB2 phosphorylation

The dependence of ErbB4/TAB2 interaction on NRG1 suggested that ErbB4 activation could alter TAB2, particularly its state of phosphorylation or its cellular localization.

Phosphotyrosine western blot and ^{32}P-ATP incorporation assays in cells expressing ErbB4 did not demonstrate induction of TAB2 phosphorylation by NRG1 (data not shown).

→ It appears that ErbB4 activation does not induce TAB2 phosphorylation.



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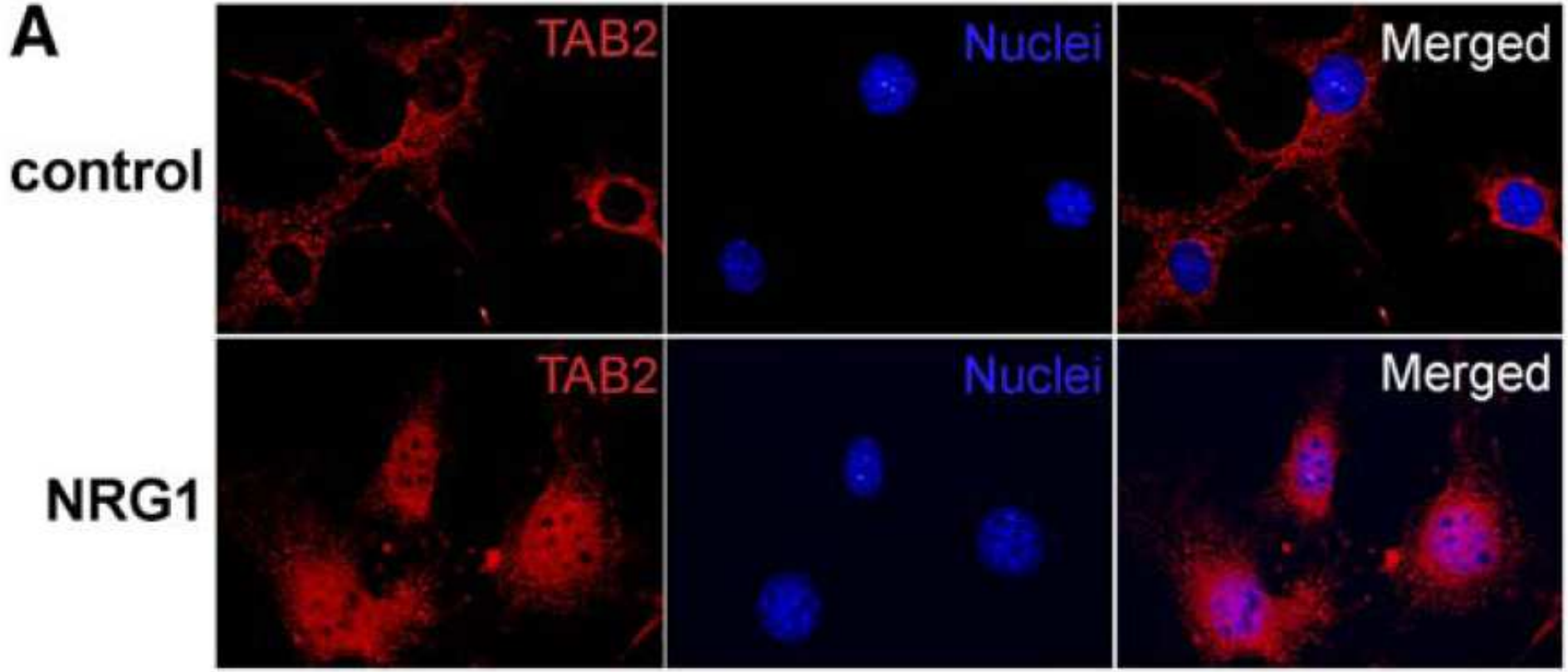
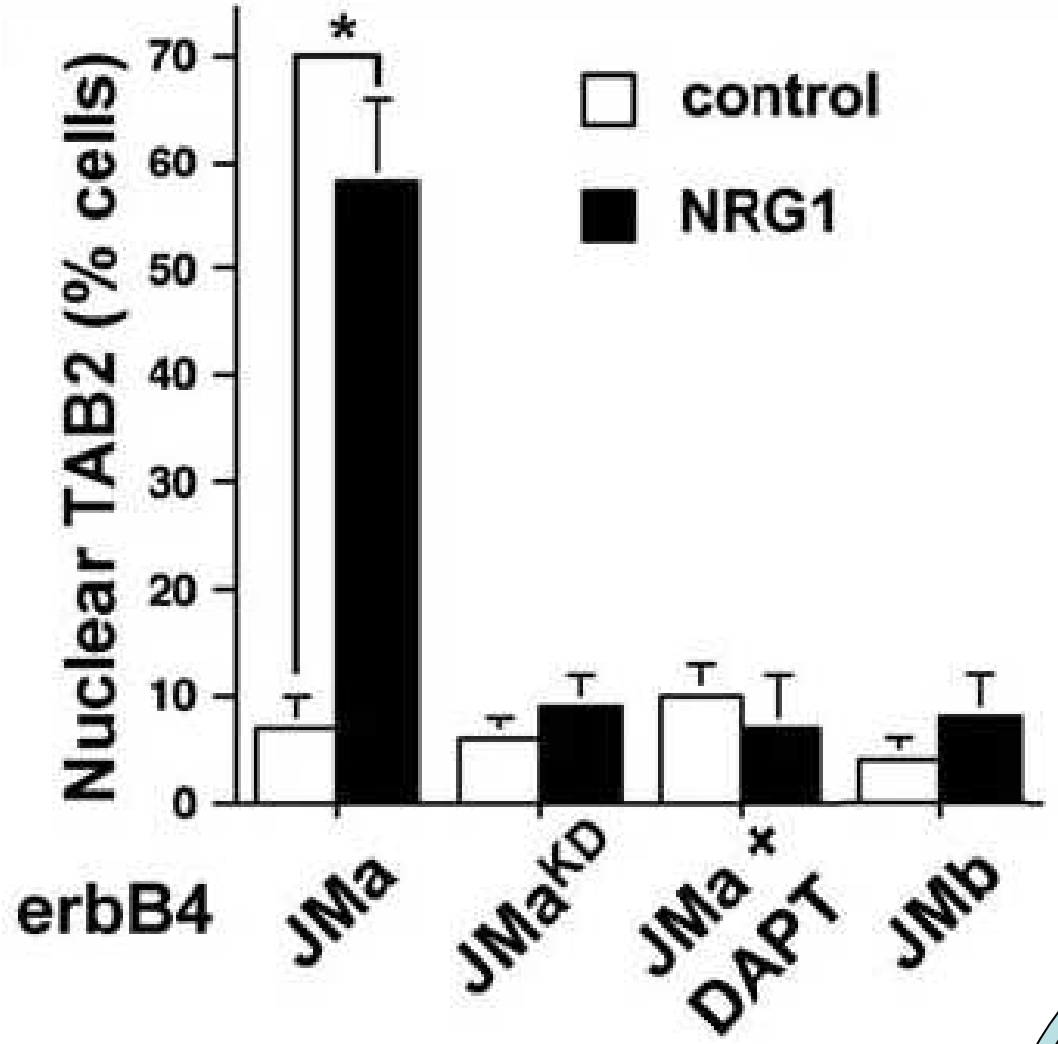
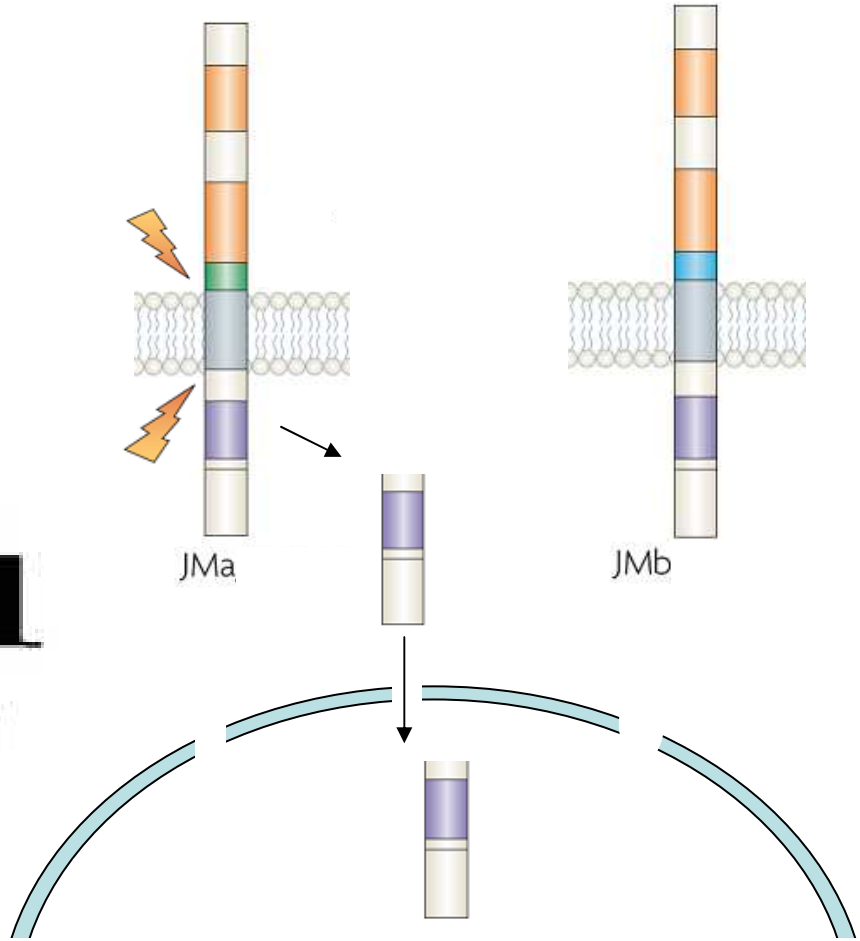
Presenilin-Dependent Cleavage of ErbB4 promotes TAB2 Nuclear Translocation



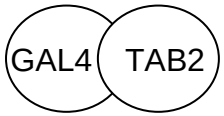
NRG1-ErbB4 signaling had dramatic effects on the cellular distribution of TAB2:
- in quiescent NIH 3T3 cells, TAB2 was excluded from the nuclei independently of whether the cells expressed ErbB4 or not
- upon treatment with NRG1 for 2 hours, TAB2 translocated to the nucleus in cells expressing ErbB4-JMa

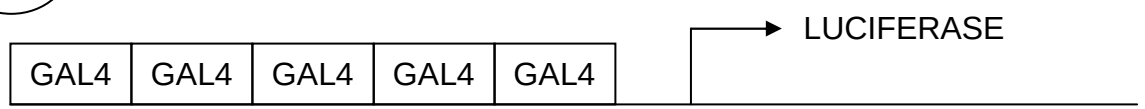
Does NRG1-induced TAB2 nuclear translocation depend on ErbB4 activation and cleavage?

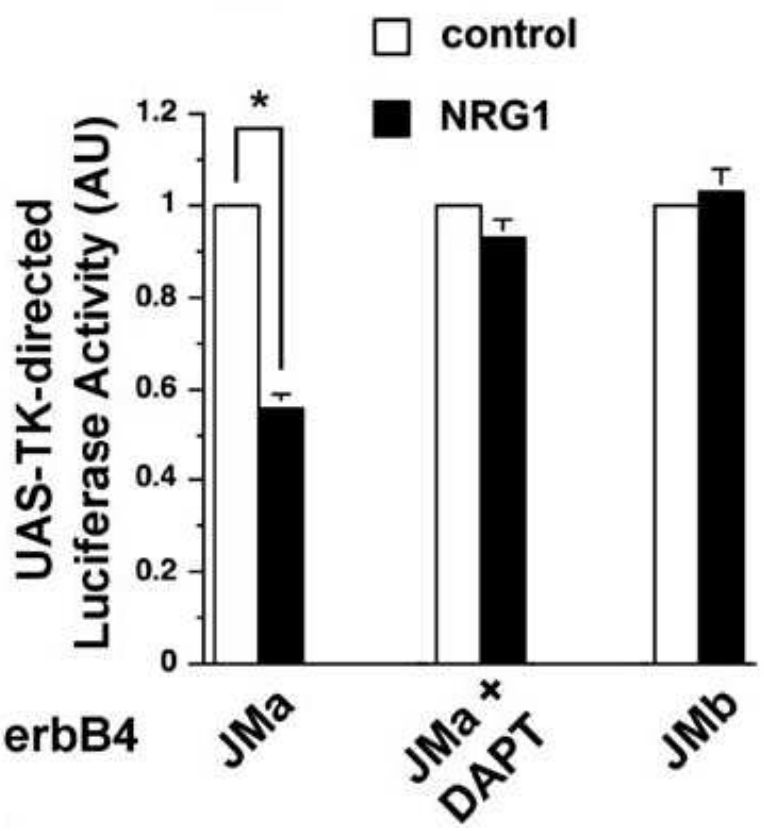


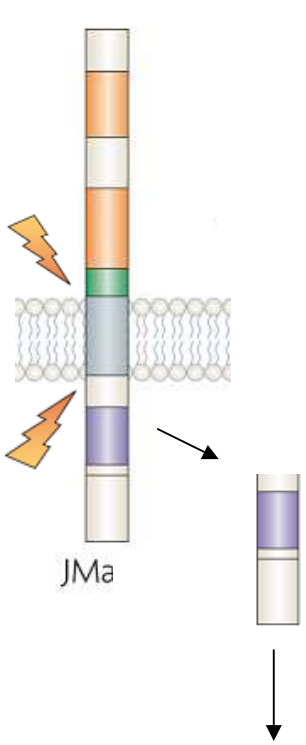


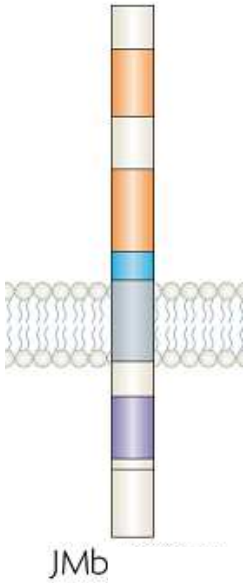
Could TAB2 be mediating potential effects of E4ICD on gene expression?











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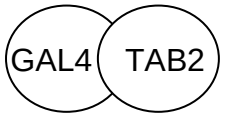
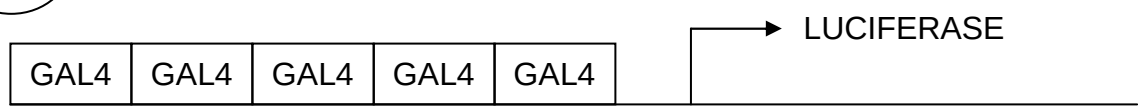
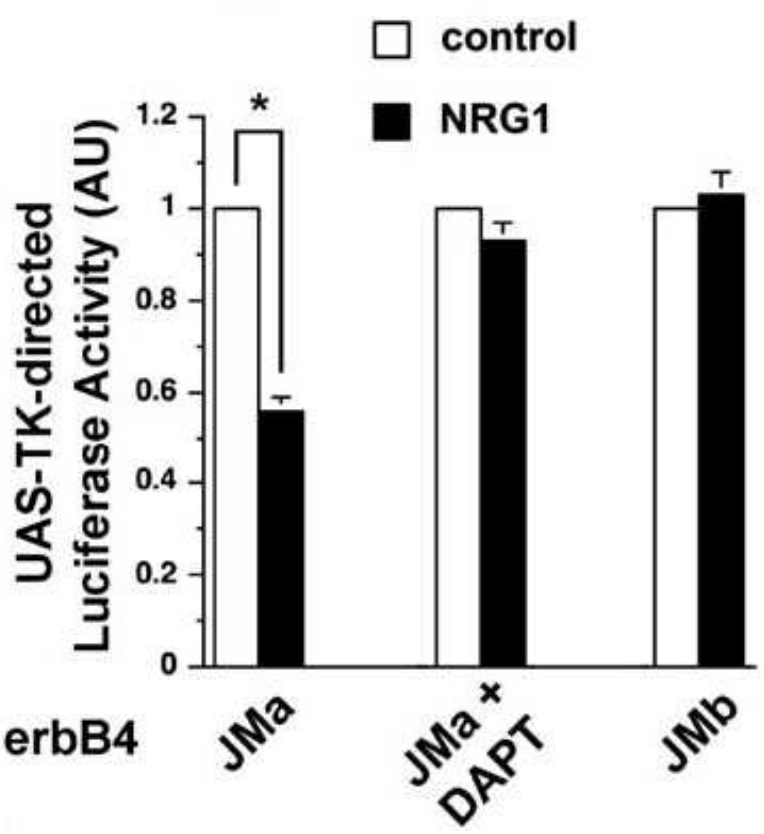
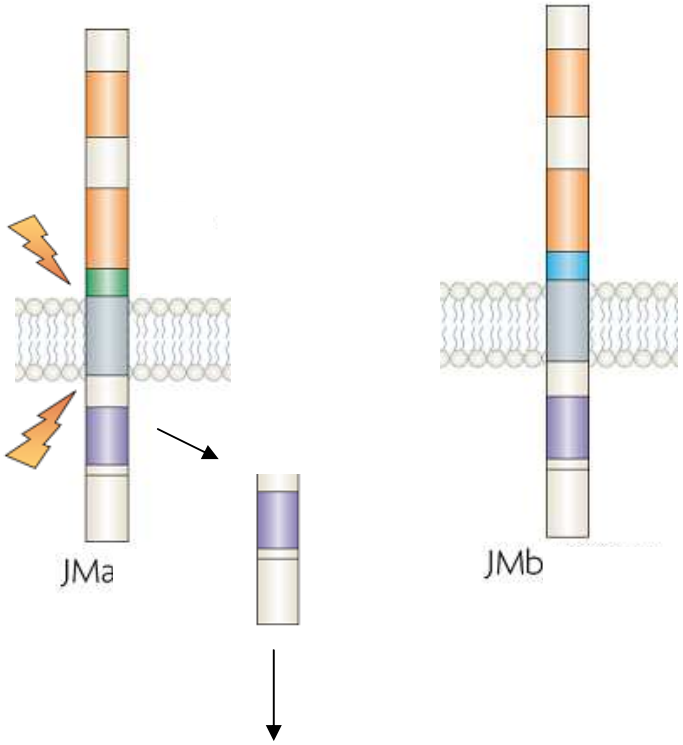




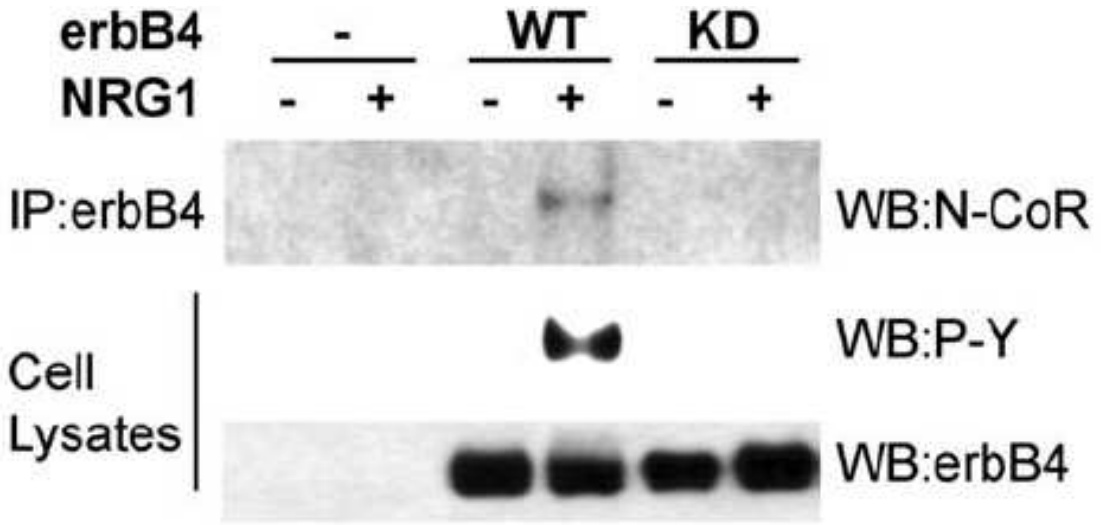




→ NRG1-mediated ErbB4 activation and cleavage induces nuclear translocation of TAB2 and raised the possibility that an E4ICD/TAB2 complex represses transcription.

TAB2 can form a complex with the transcriptional corepressor N-CoR

Does N-CoR also interacts with ErbB4?



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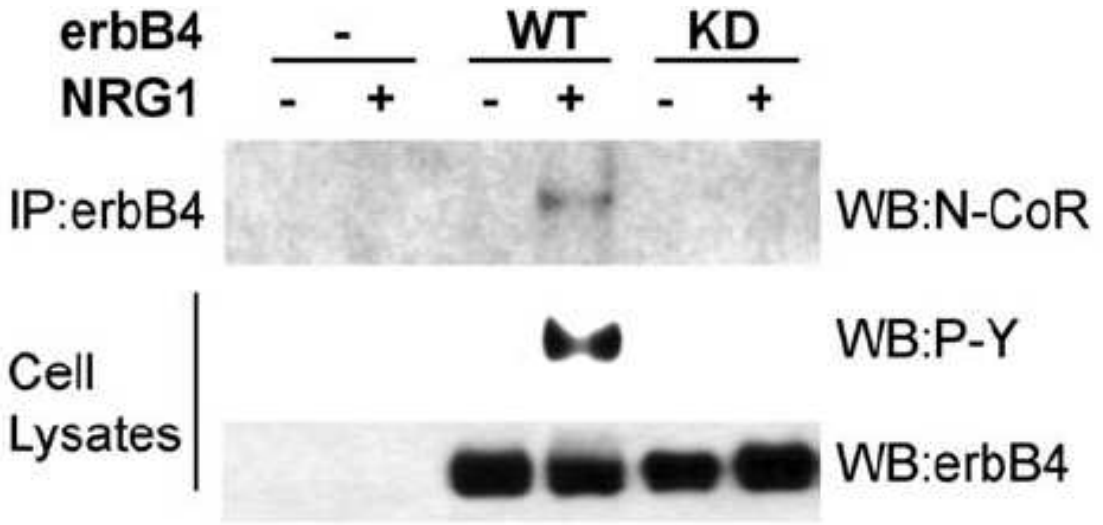
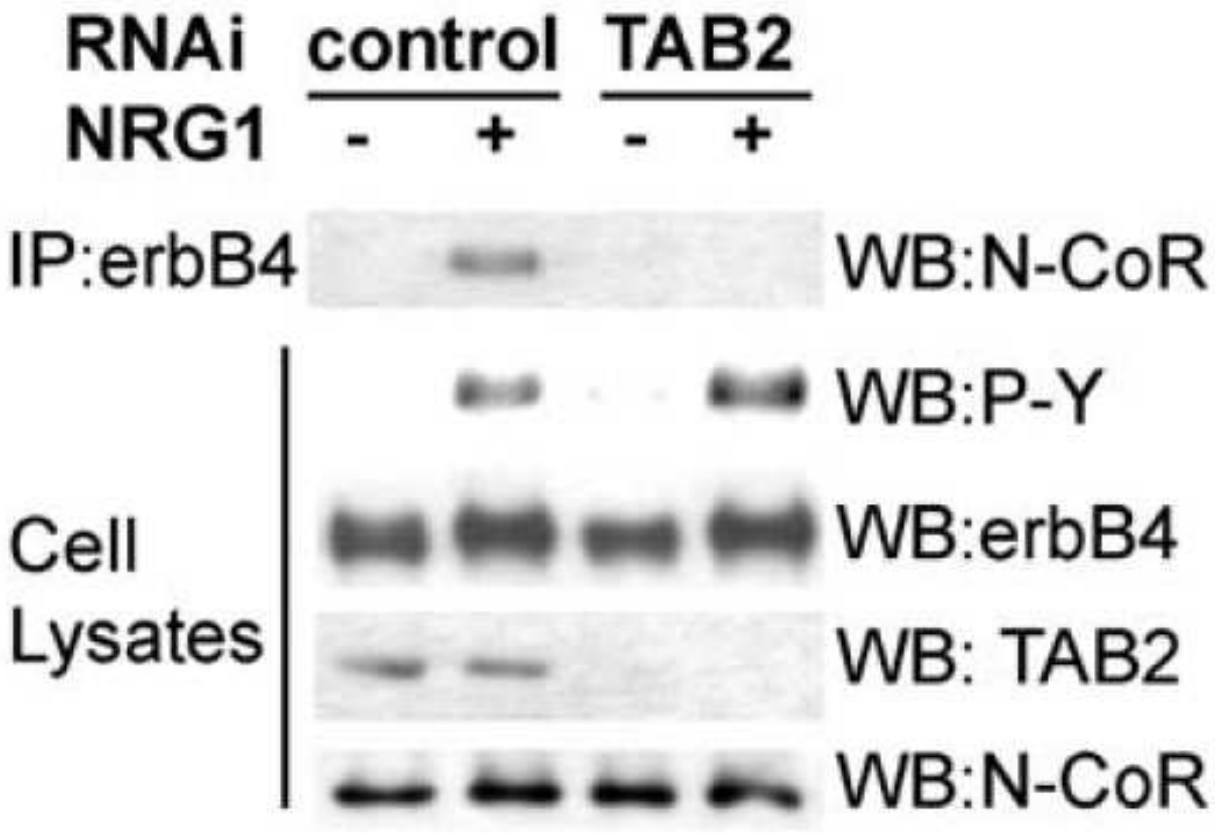
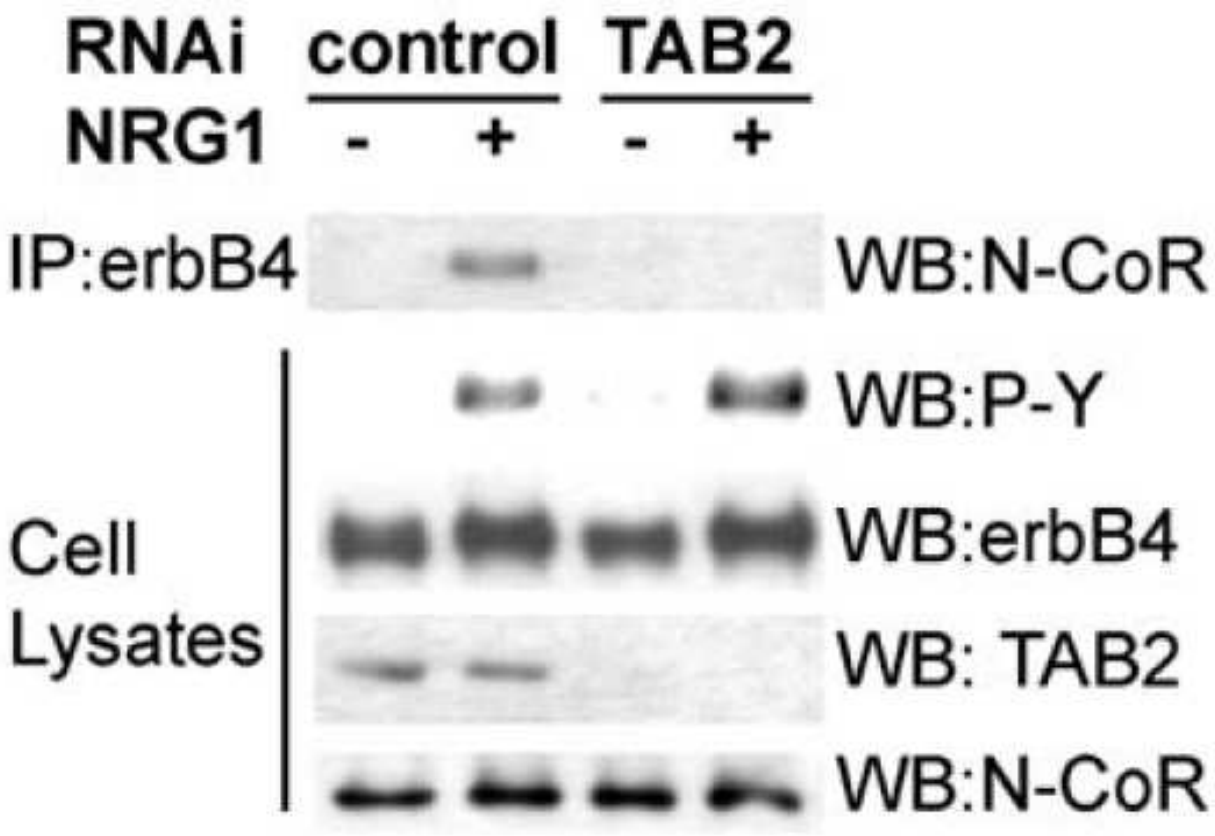


No tyrosine phosphorylation of N-CoR was detected when ErbB4 was activated (data not shown).

→ NRG1 induces the formation of an E4ICD/TAB2/N-CoR complex



Which is the role of TAB2 in the interaction between E4ICD and N-CoR? Could ErbB4 bind to N-CoR in the absence of TAB2?



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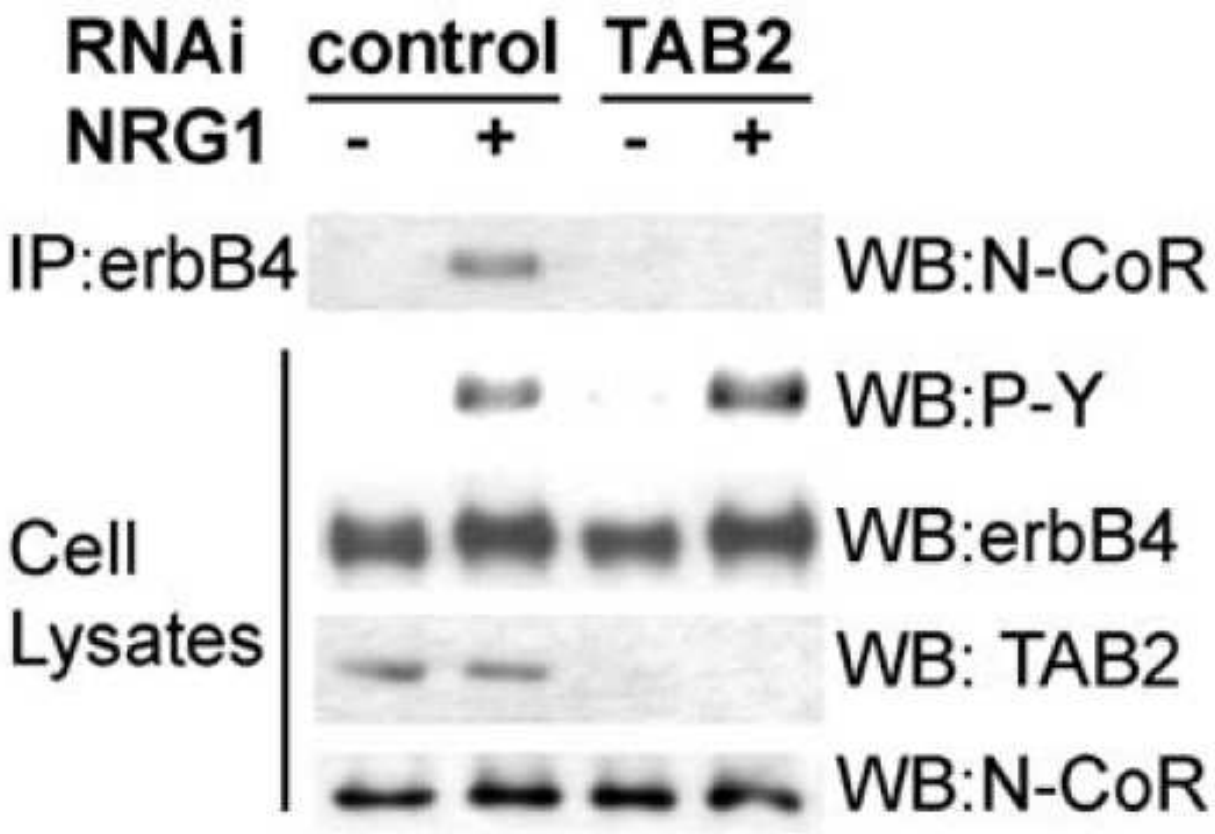
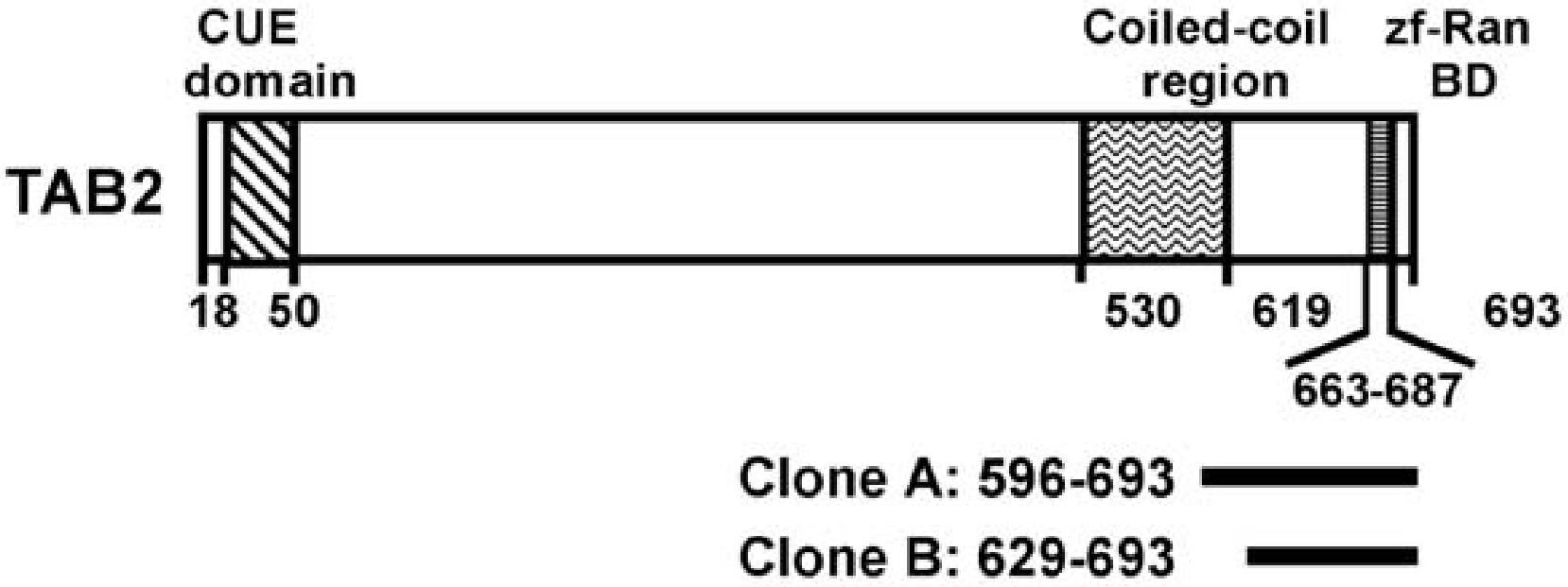


Lack of TAB2 expression (using RNAi) abolished the NRG1-dependent E4ICD/N-CoR association

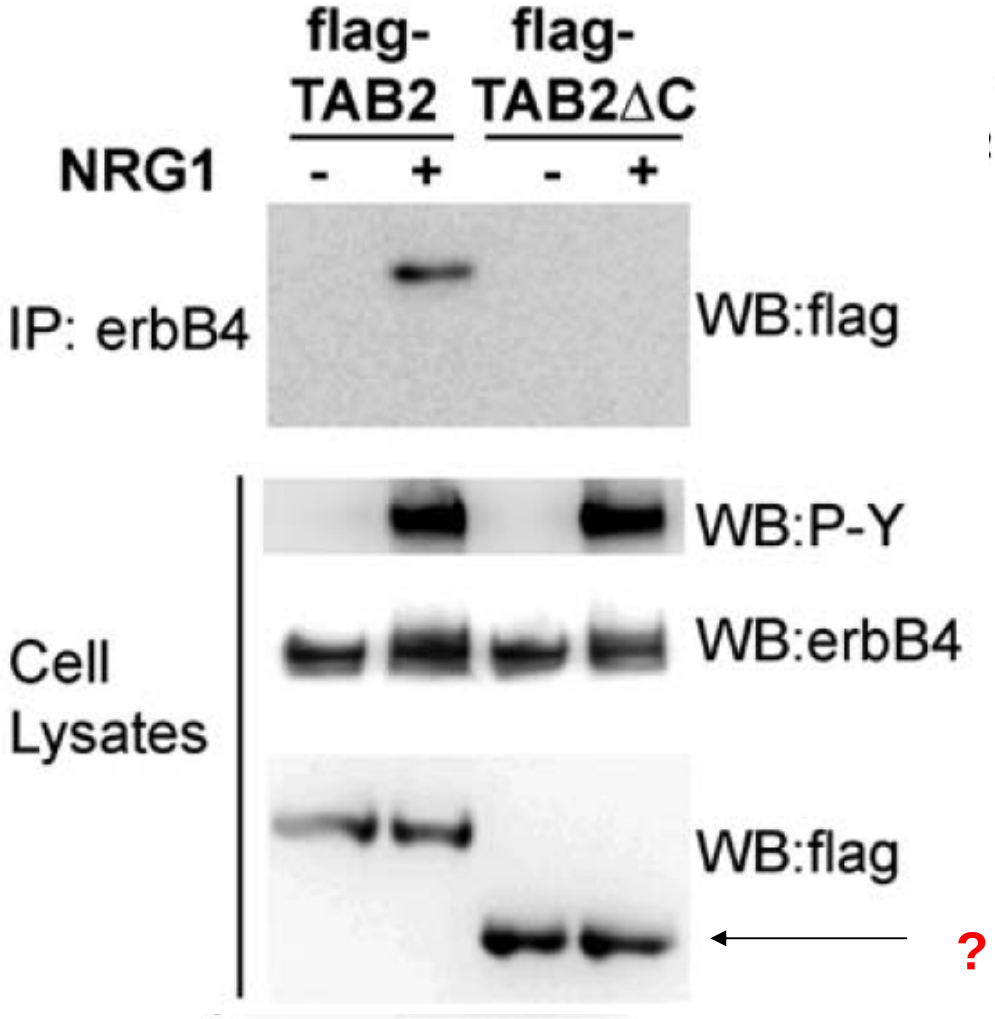
→ suggesting that TAB2 forms the bridge between E4ICD and N-CoR

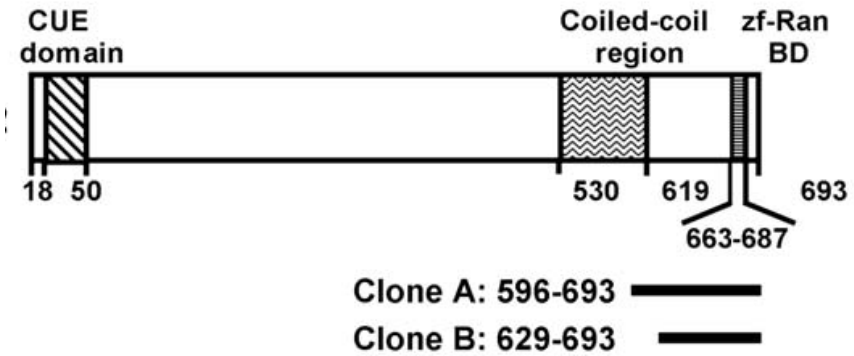
Which domain of TAB2 is involved in the interaction with E4ICD? Why?



Which methods could you use to obtain deleted constructs (ΔN or ΔC)?

Which domain of TAB2 is involved in the interaction with E4ICD?





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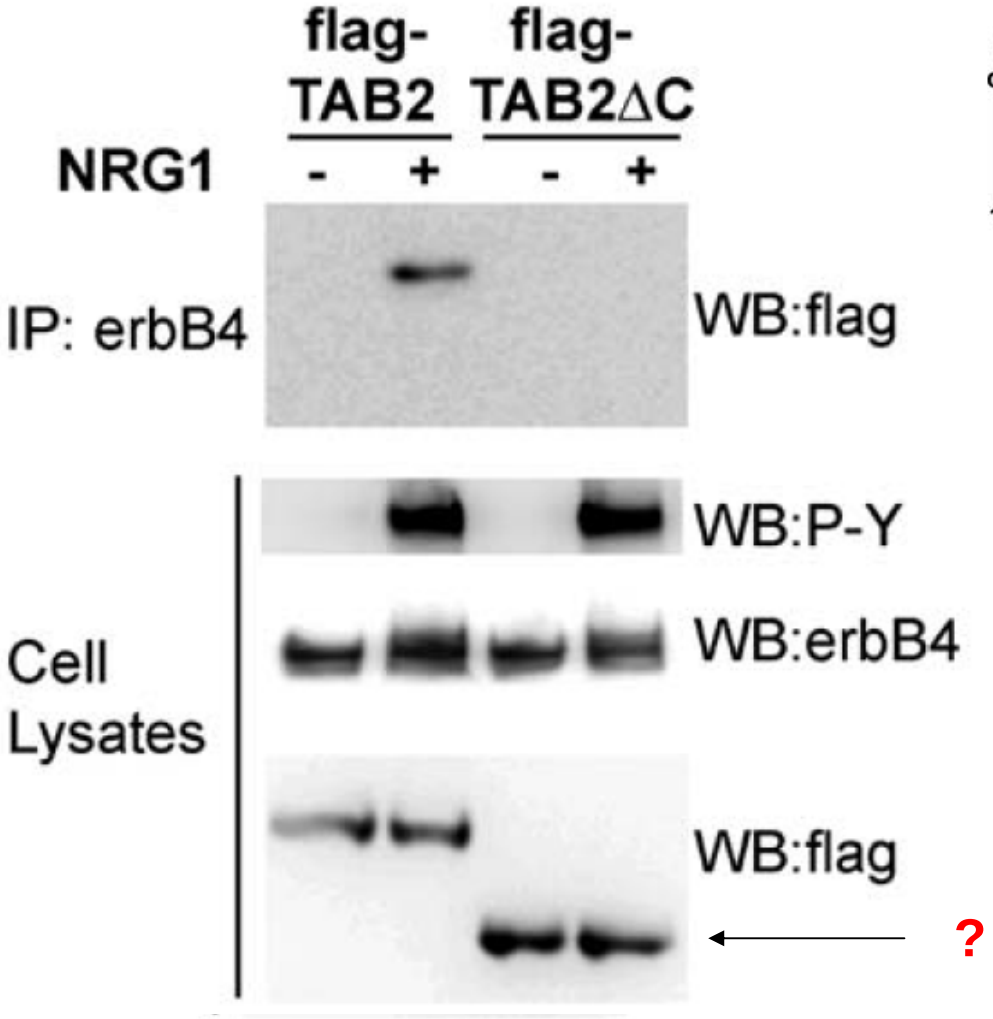
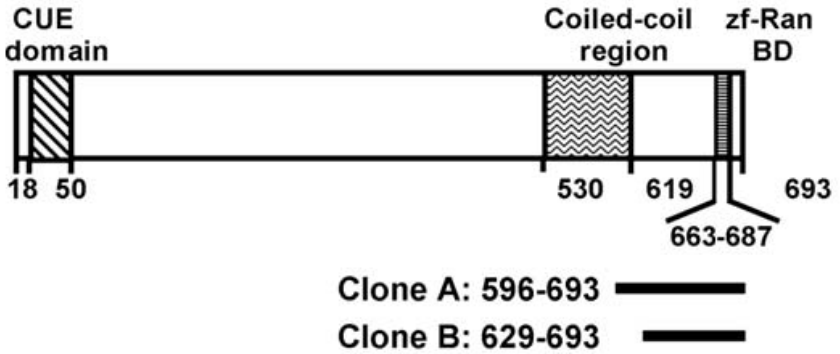
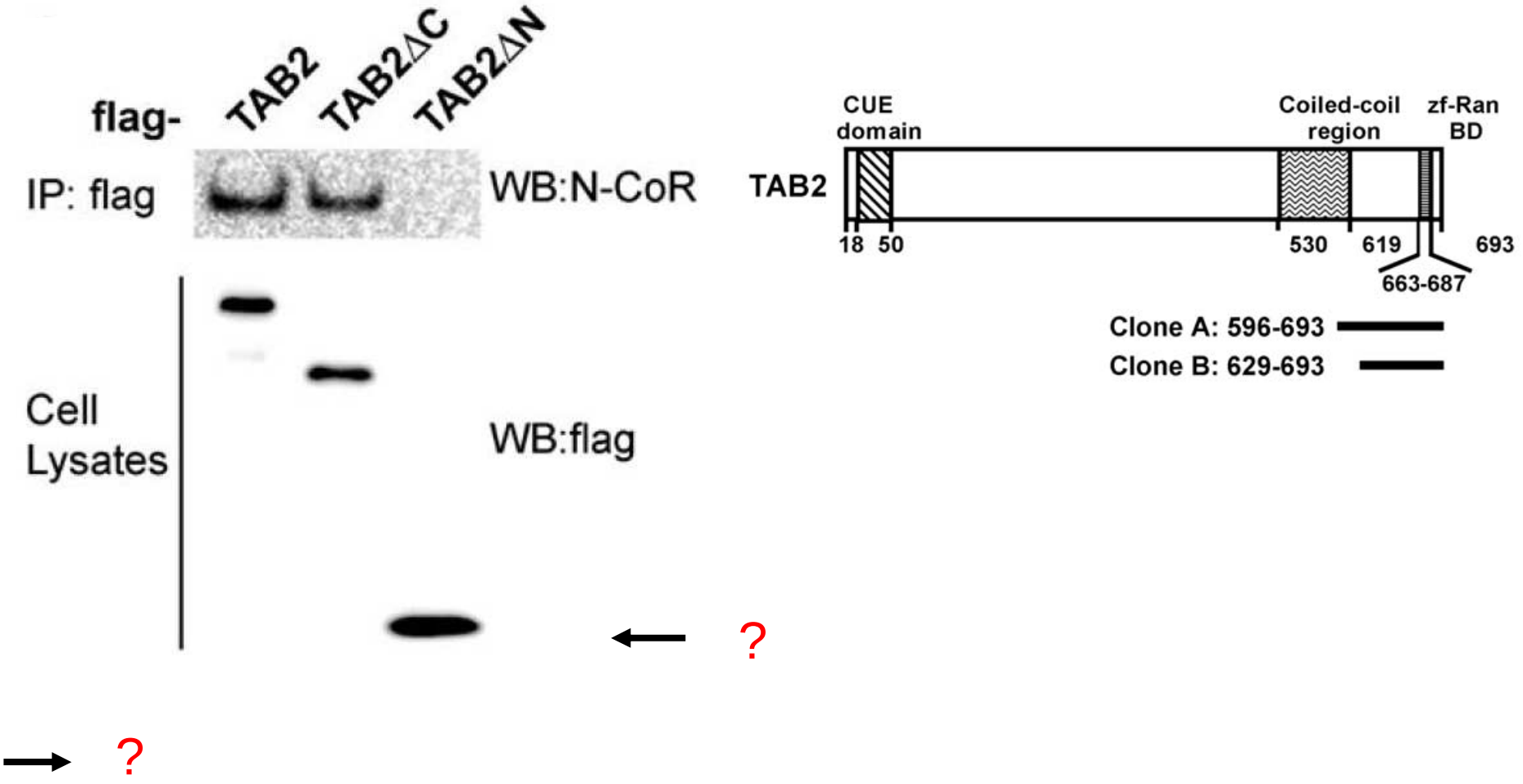




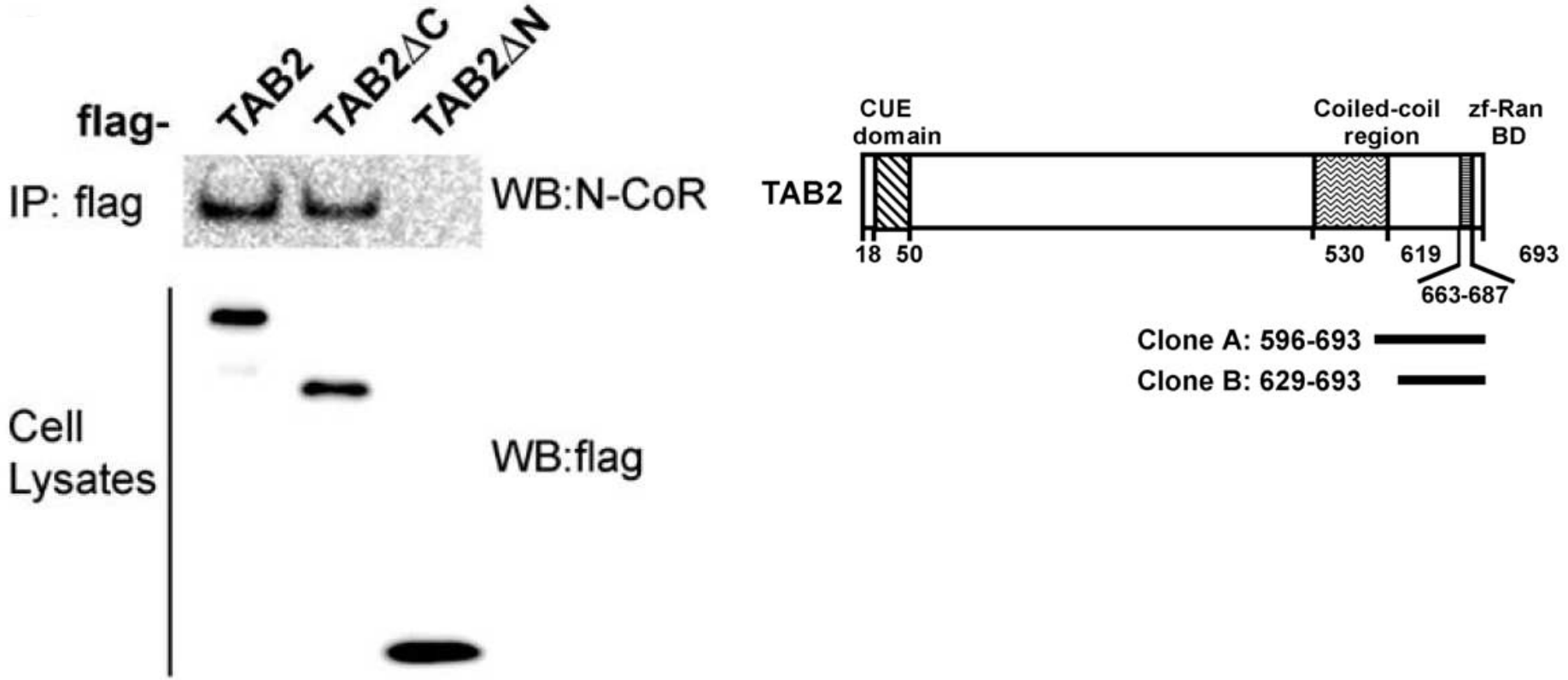
- the C-terminal 64 amino acids of TAB2 are not only sufficient but also necessary for ErbB4/TAB2 interaction

Which domain of TAB2 is involved in the interaction with N-CoR?



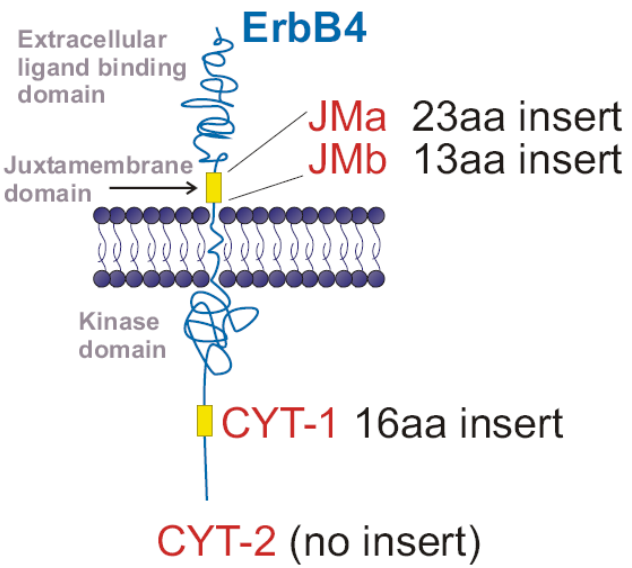
Which domain of TAB2 is involved in the interaction with N-CoR?

TAB2 binds constitutively to N-CoR.
N-CoR physically interacts with TAB2ΔC but not with TAB2ΔN, the C-terminal E4ICD-interaction domain of TAB2.



→ TAB2 is a bifunctional molecule with non-overlapping binding sites for ErbB4 and N-CoR and it provides the critical link for the formation of the ErbB4/TAB2/N-CoR complex.

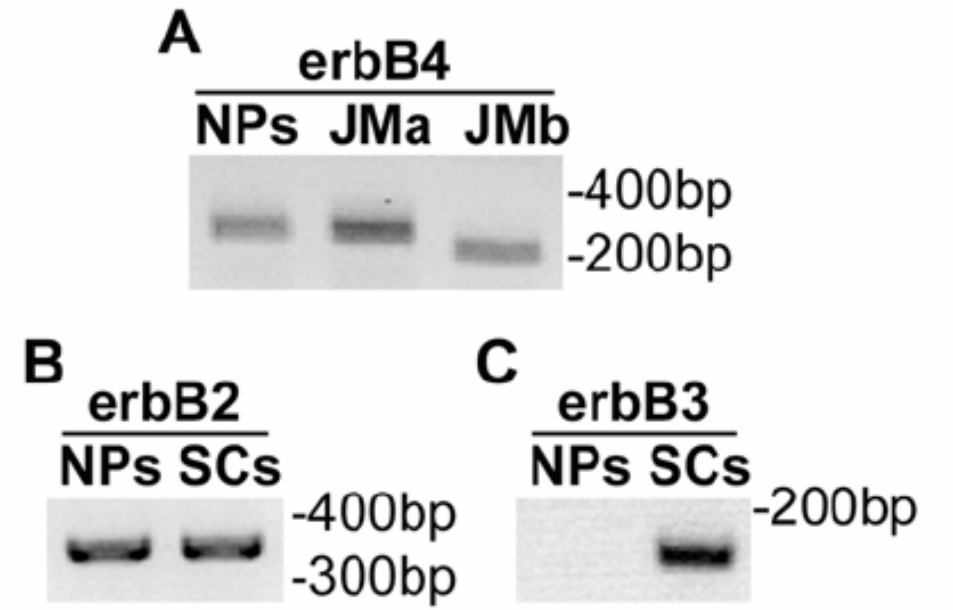
Could E4ICD/TAB2/N-CoR complex be of biological significance for Primary Neuronal precursors in the central nervous system?



How can they identify the ErbB4 isoforms expressed by primary neuronal precursors?

SC=Schwann cells (positive control)

Which assay did they use to test ErbB expression and identify ErbB4 isoforms?
Which results did they obtain?



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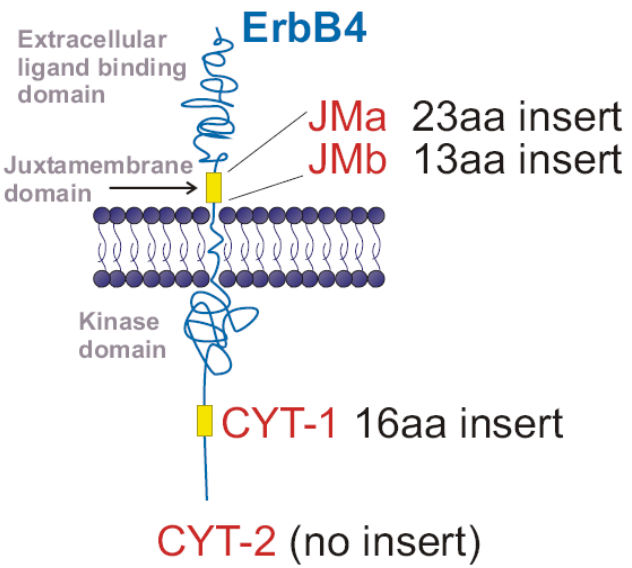
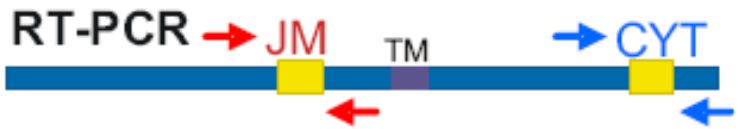
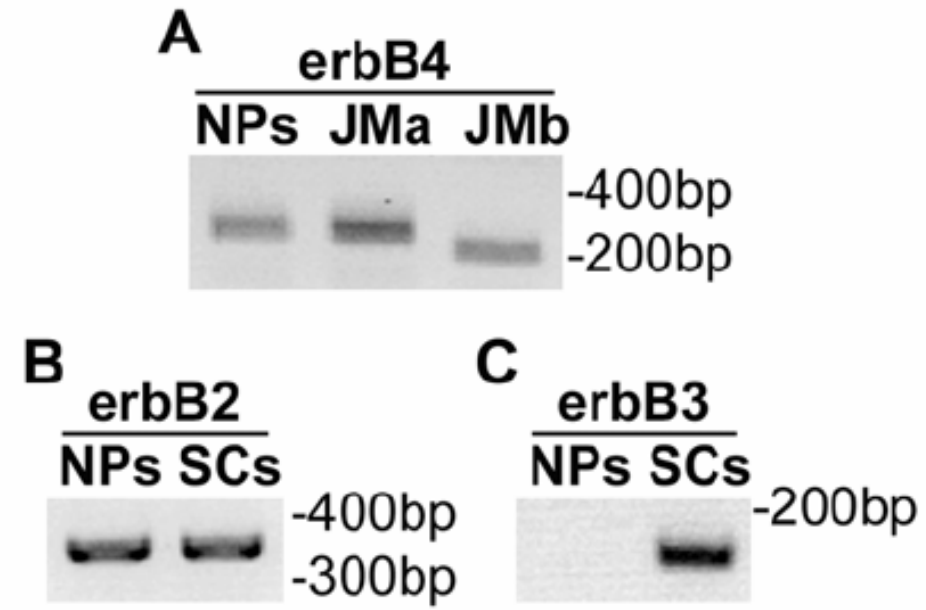


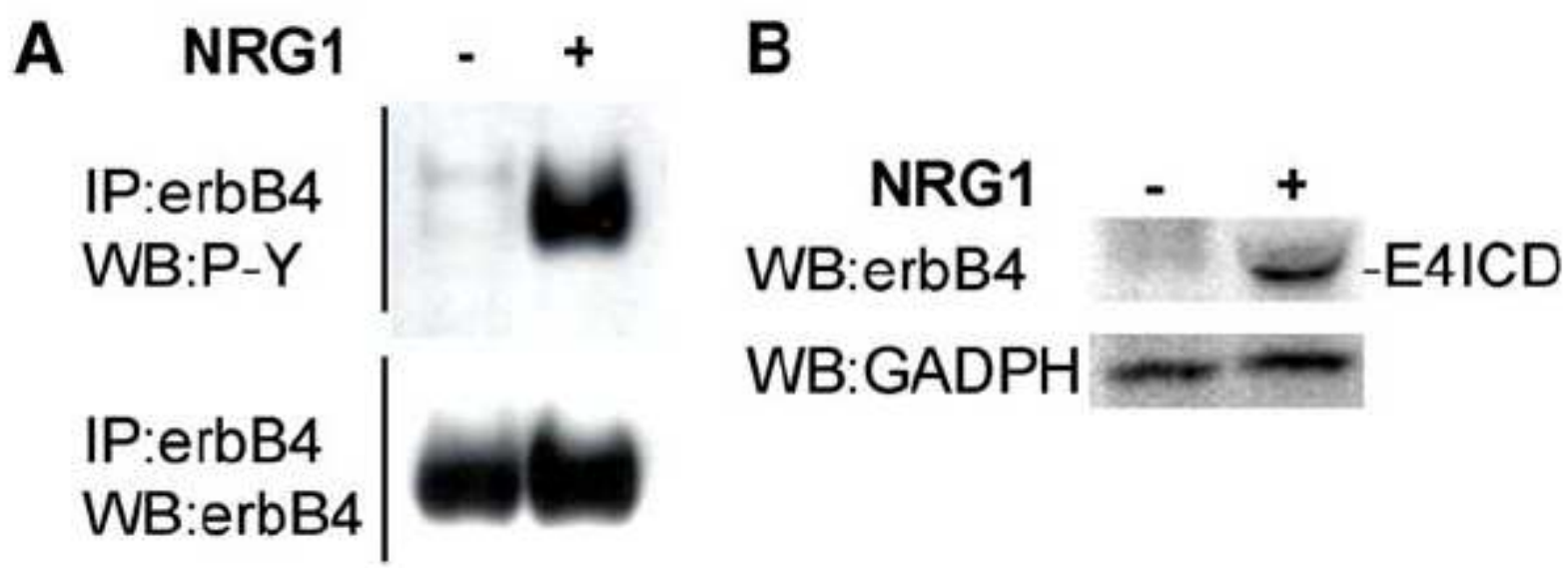


Primary Neuronal precursors (NPs) obtained from E14.5 rat cortices express only the ErbB4 JMa isoform (and ErbB2).

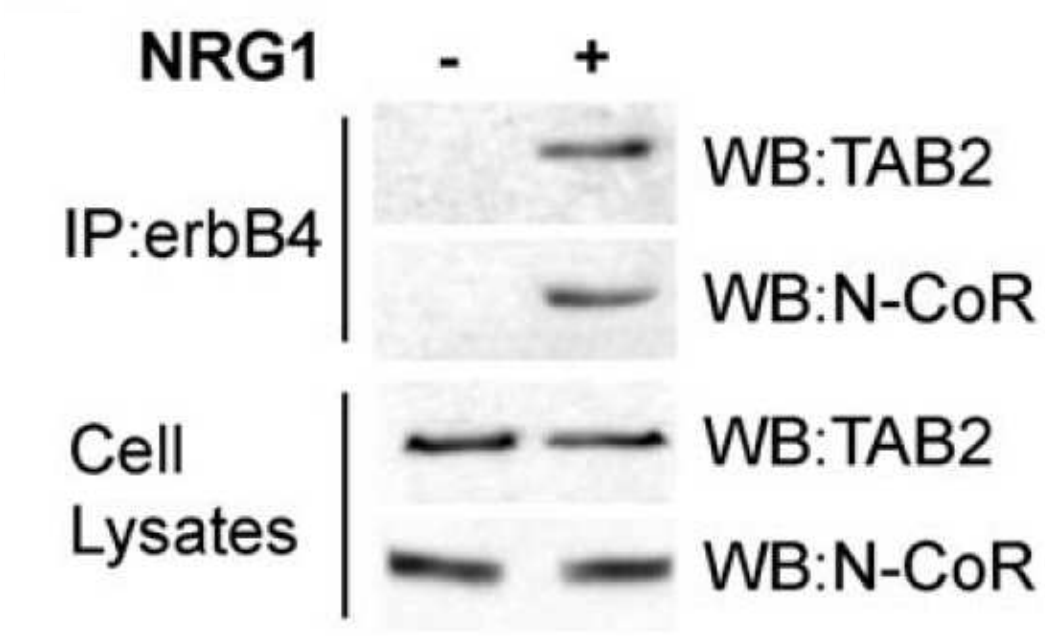
Since ErbB2 does not bind to NRG1, any response of NPs to NRG1 would require ErbB4, acting as either a homodimer or an ErbB4/2 heterodimer.



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.