

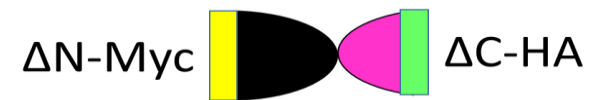


Advanced Cell Biology and Biotechnology

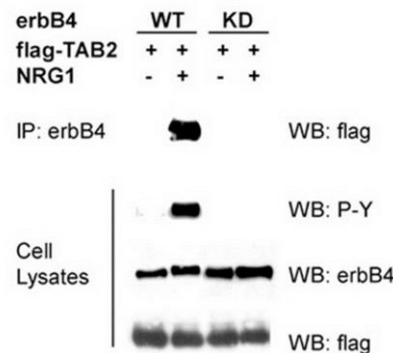
ACBB 2021/22

Summary of the previous lesson

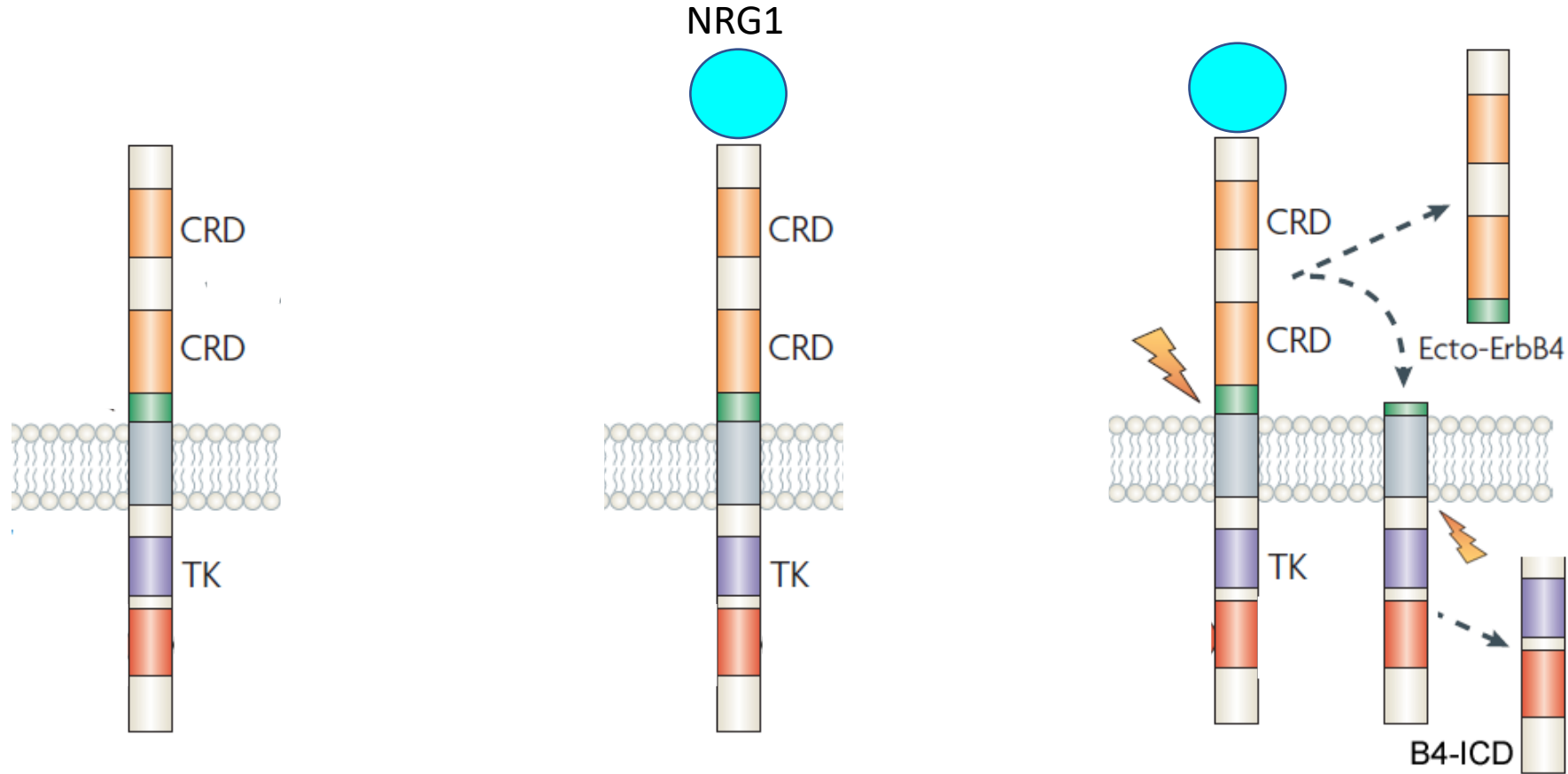
- protein expression analysis (western blot on total cell lysate or on IP)
- protein-protein interactions (co-immunoprecipitation)
- TAG proteins
 - Myc tag – (EQKLISEEDL)
 - HA tag – (YPYDVPDYA)
 - FLAG tag – (DYKDDDDK)
 - to discriminate between endogenous and exogenous recombinant proteins
 - when the antibody for the protein of interest is not available
- domain deletions (ΔN , ΔC , Δ internal domains) to identify domains involved in protein-protein interactions or in specific activities



- example of co-IP of a tagged protein



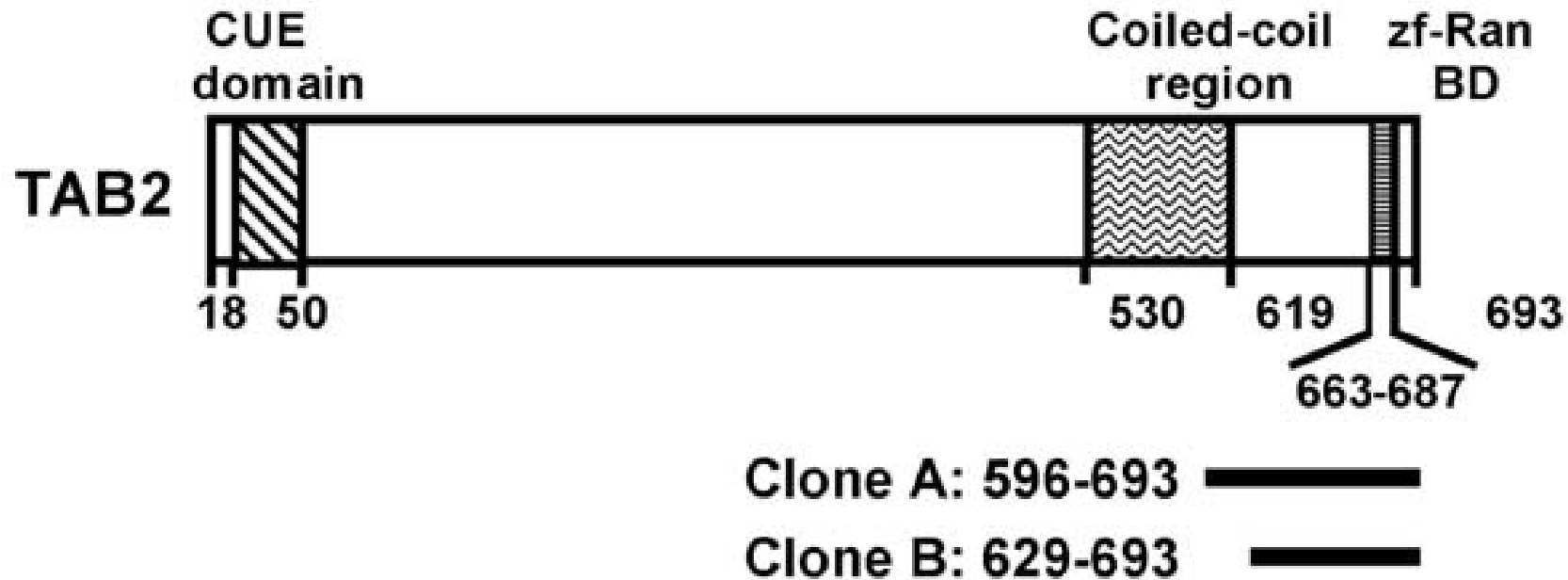
ErbB4



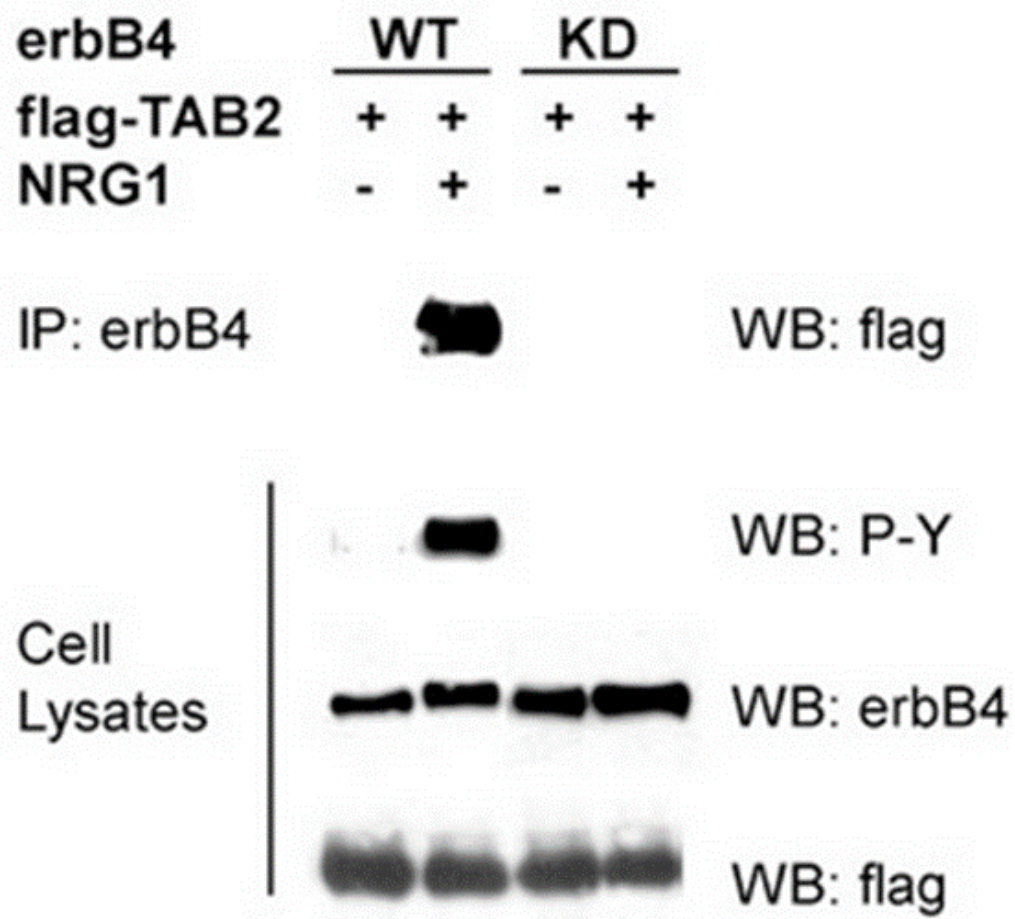
To identify ErbB4-binding proteins that are involved in transcriptional regulation they used a **yeast two-hybrid system** that facilitates the isolation of proteins that interact with activated RTKs

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)



Regions of TAB2 included in two clones identified in the screen are indicated.



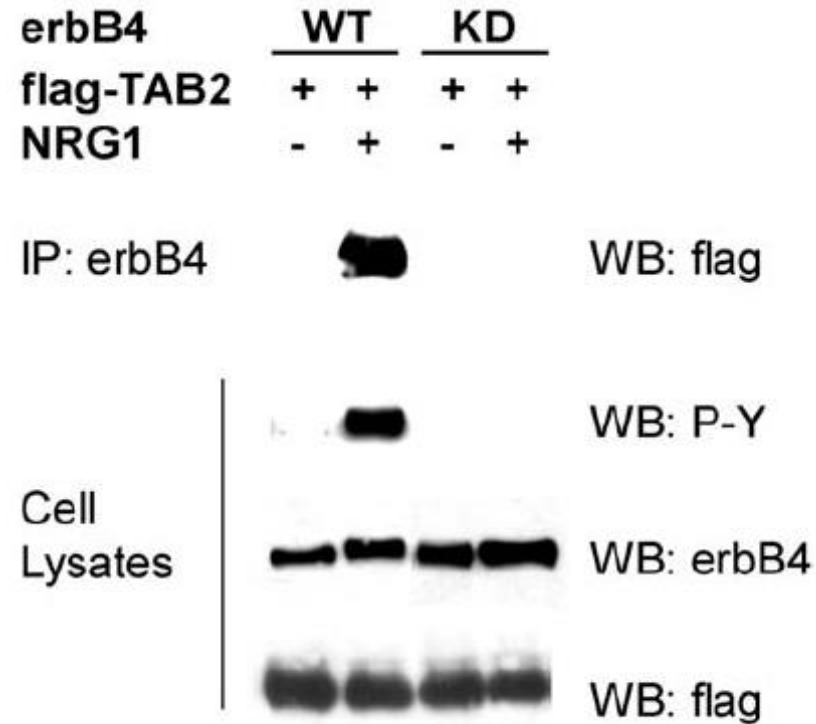
Panel 5

- Cells were transiently transfected with an expression vector for ErbB4 wild type (WT) or kinase dead (KD) and with flag-TAB2.
- 24 hours later cells were treated or mock treated with NRG1 for 15 minutes.
- Proteins were extracted, immunoprecipitated for ErbB4 and analysed by WB with an antibody recognizing the flag epitope.
- Total cell lysate (input) was also run and analysed with antibodies recognizing phosphorylated tyrosines (P-Y), ErbB4, Flag.

Does TAB2 binds to ErbB4?

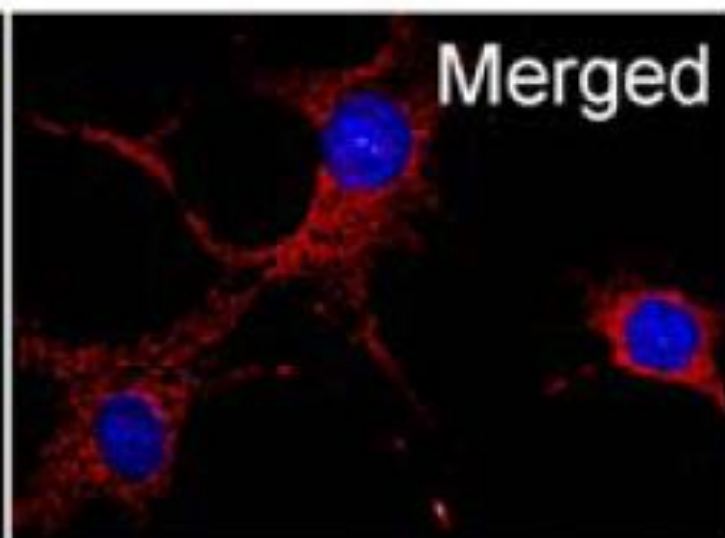
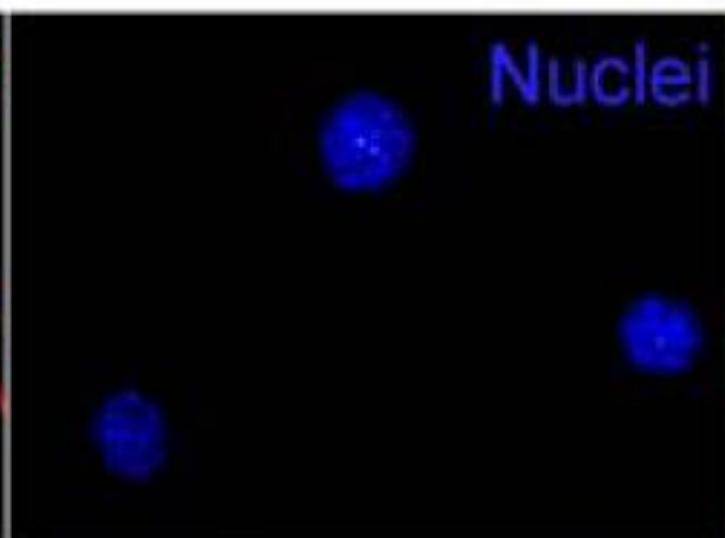
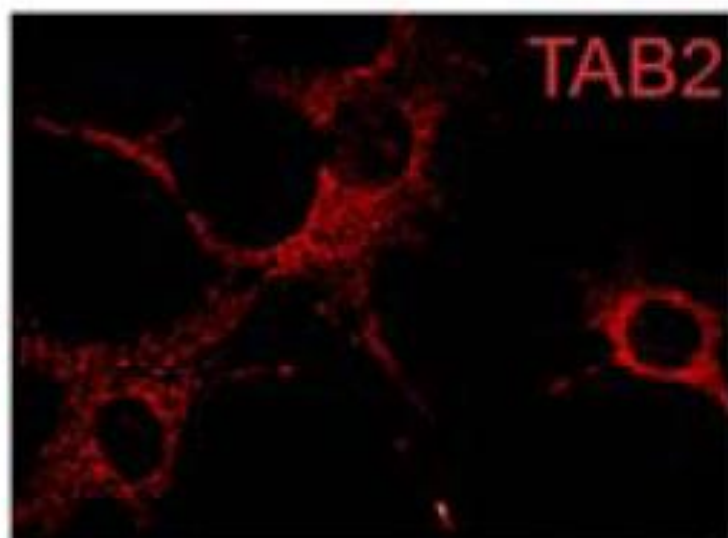
Does this interaction depend on receptor activation by NRG1?

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

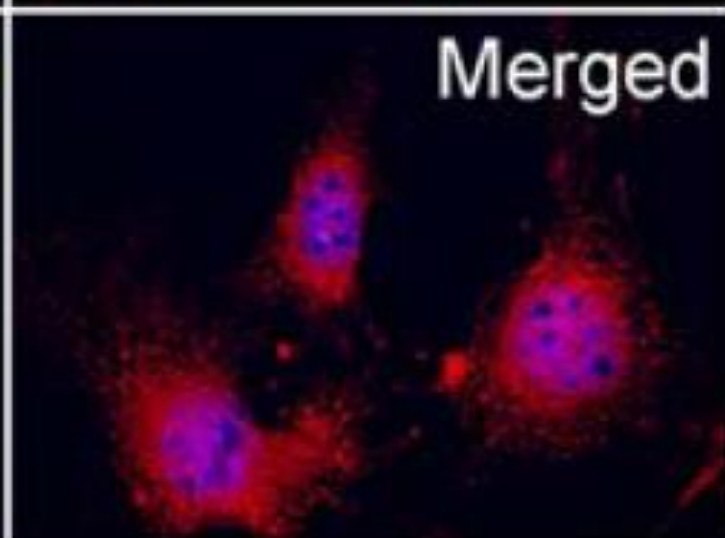
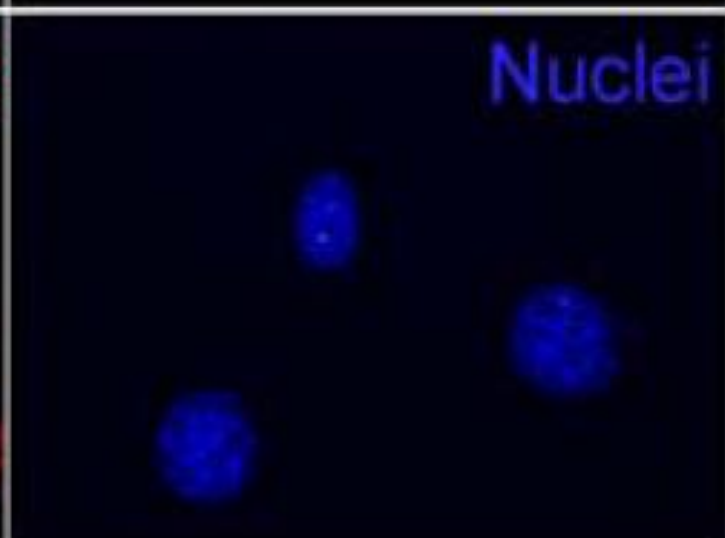
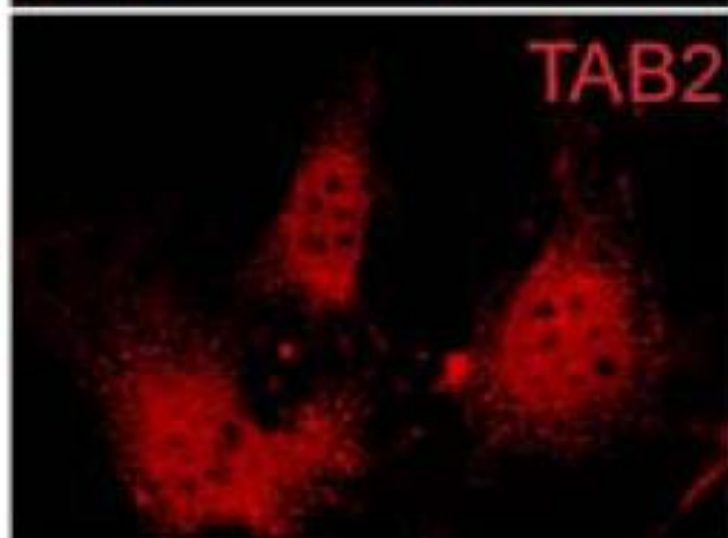


→ TAB2 coprecipitated with ErbB4 only after NRG1 treatment and only if ErbB4 kinase is active

control

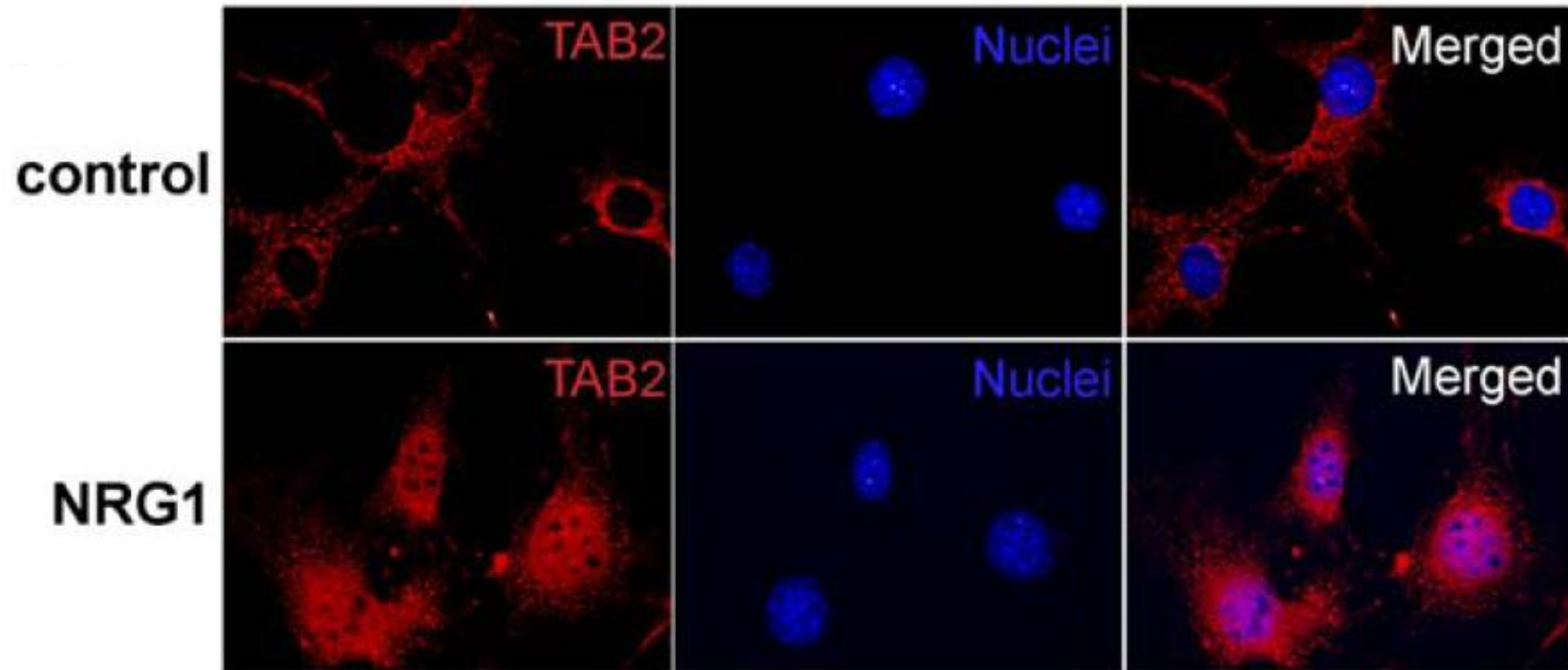


NRG1



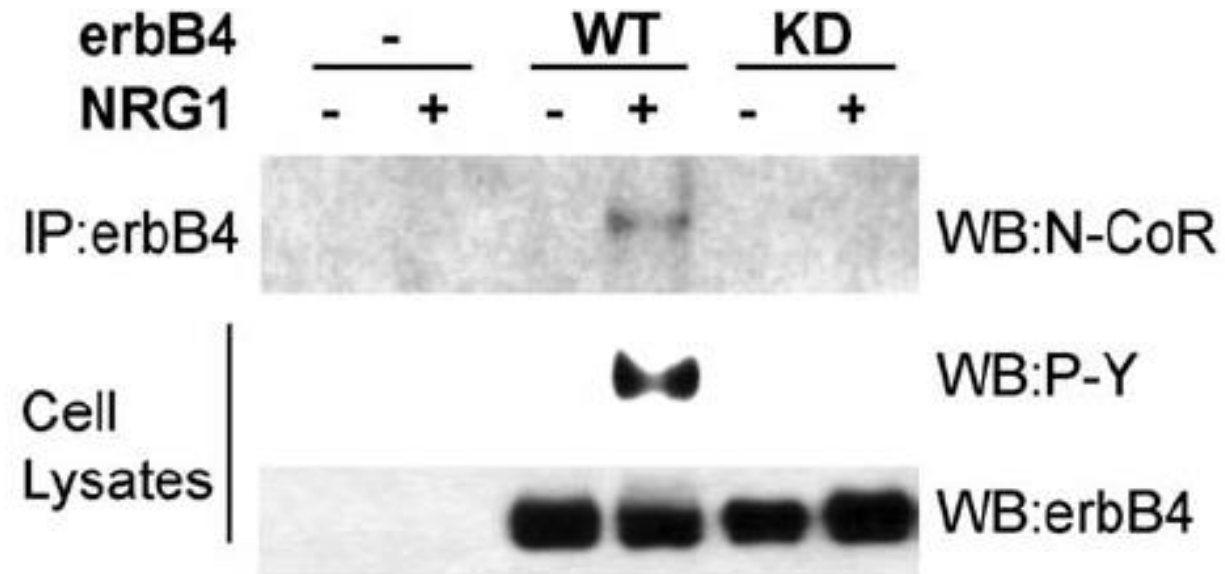
Cleavage of ErbB4 induced by NRG1 promotes TAB2 Nuclear Translocation

NRG1-ErbB4 signaling had dramatic effects on the cellular distribution of TAB2:



- in quiescent NIH 3T3 cells, TAB2 was excluded from the nuclei
- upon treatment with NRG1 for 2 hours, TAB2 translocates to the nucleus

Cells were transfected with full-length ErbB4 or ErbB4KD, treated with NRG1, and immunoprecipitated with ErbB4 antibodies.

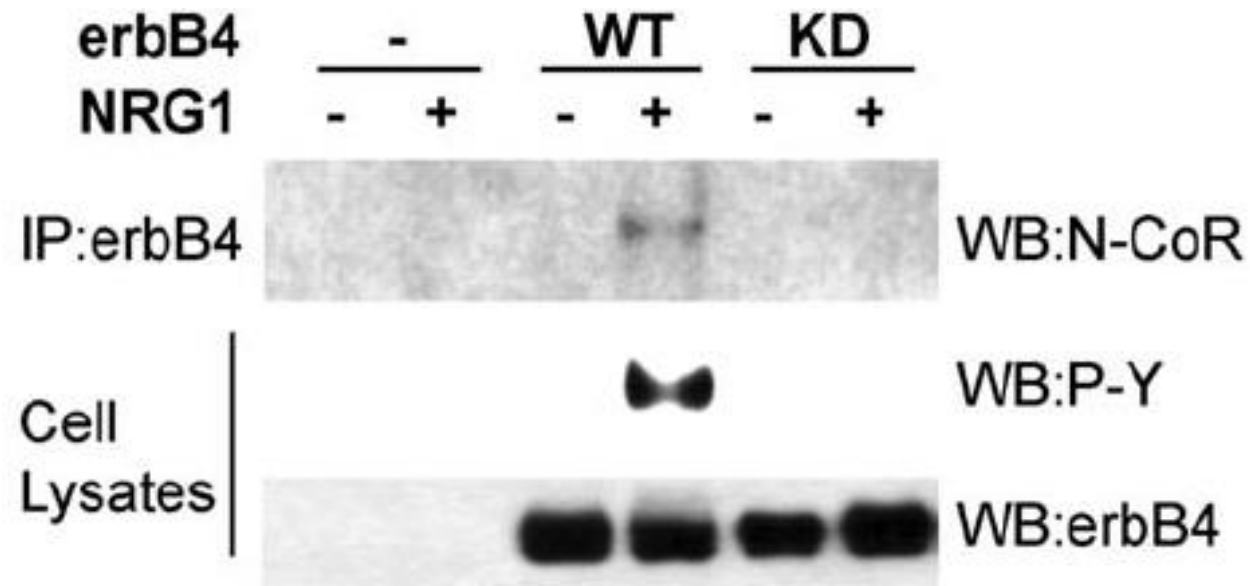


Panel 6

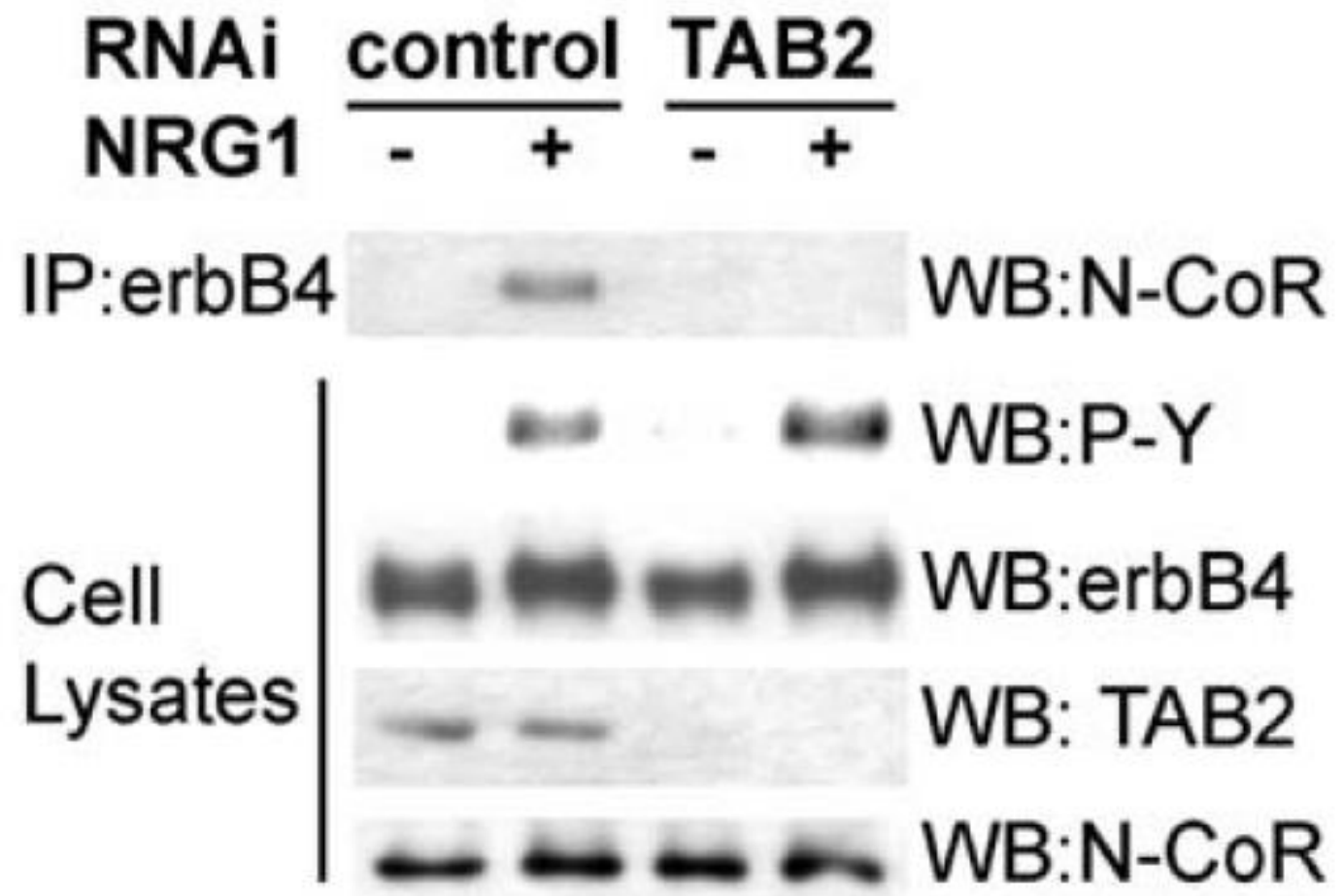
TAB2 can form a complex with the transcriptional corepressor N-CoR

Does N-CoR interact with ErbB4?

Cells were transfected with full-length ErbB4 or ErbB4KD, treated with NRG1, and immunoprecipitated with ErbB4 antibodies.

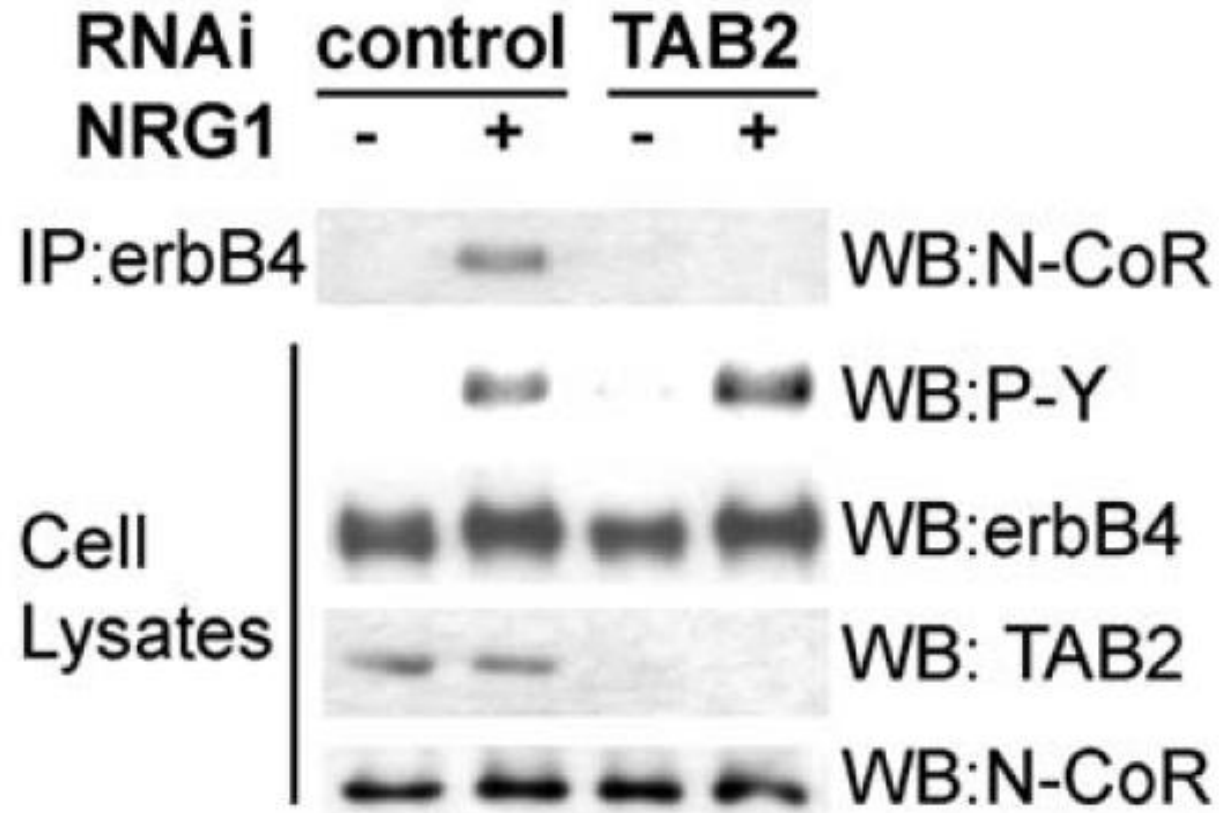


→ Only wild-type ErbB4 coimmunoprecipitated with endogenous N-CoR, and this occurred only when the cells were stimulated with NRG1



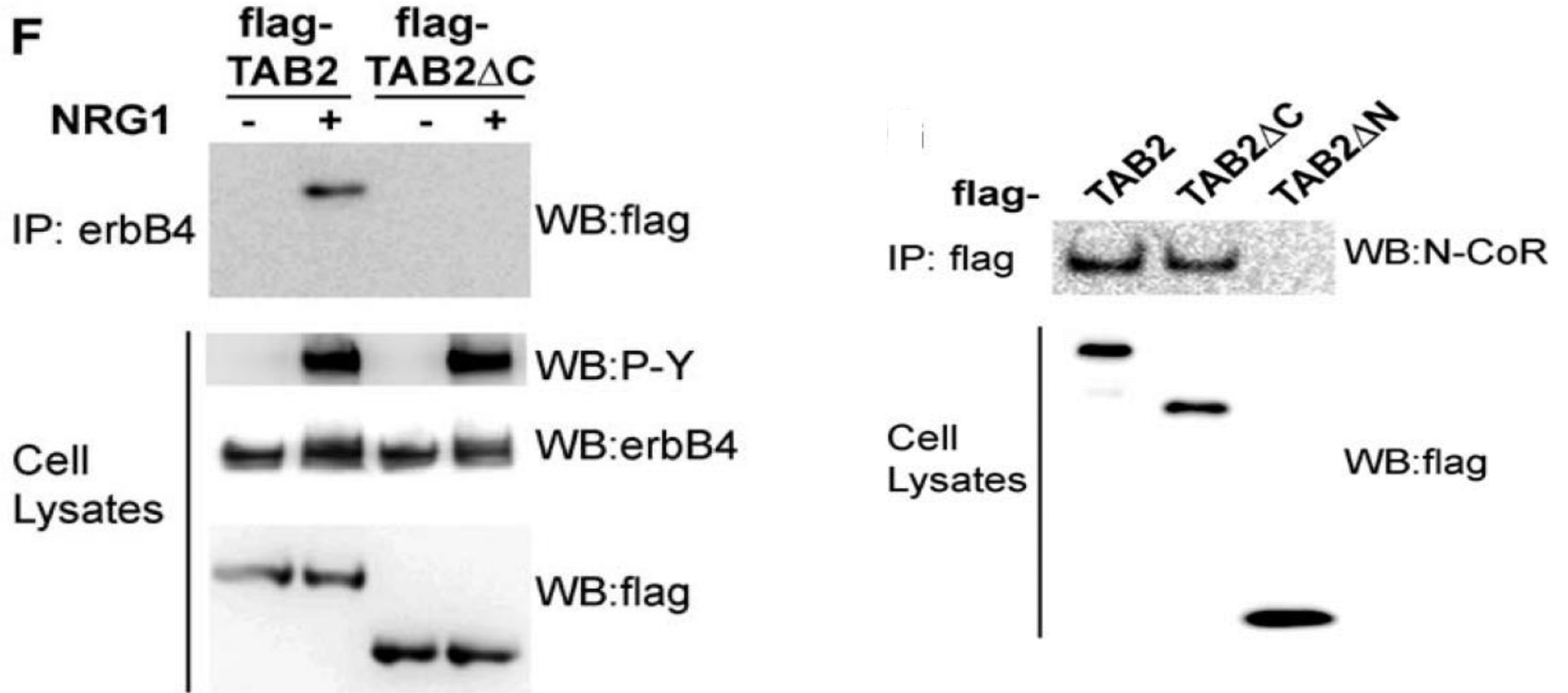
Panel 7

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?



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

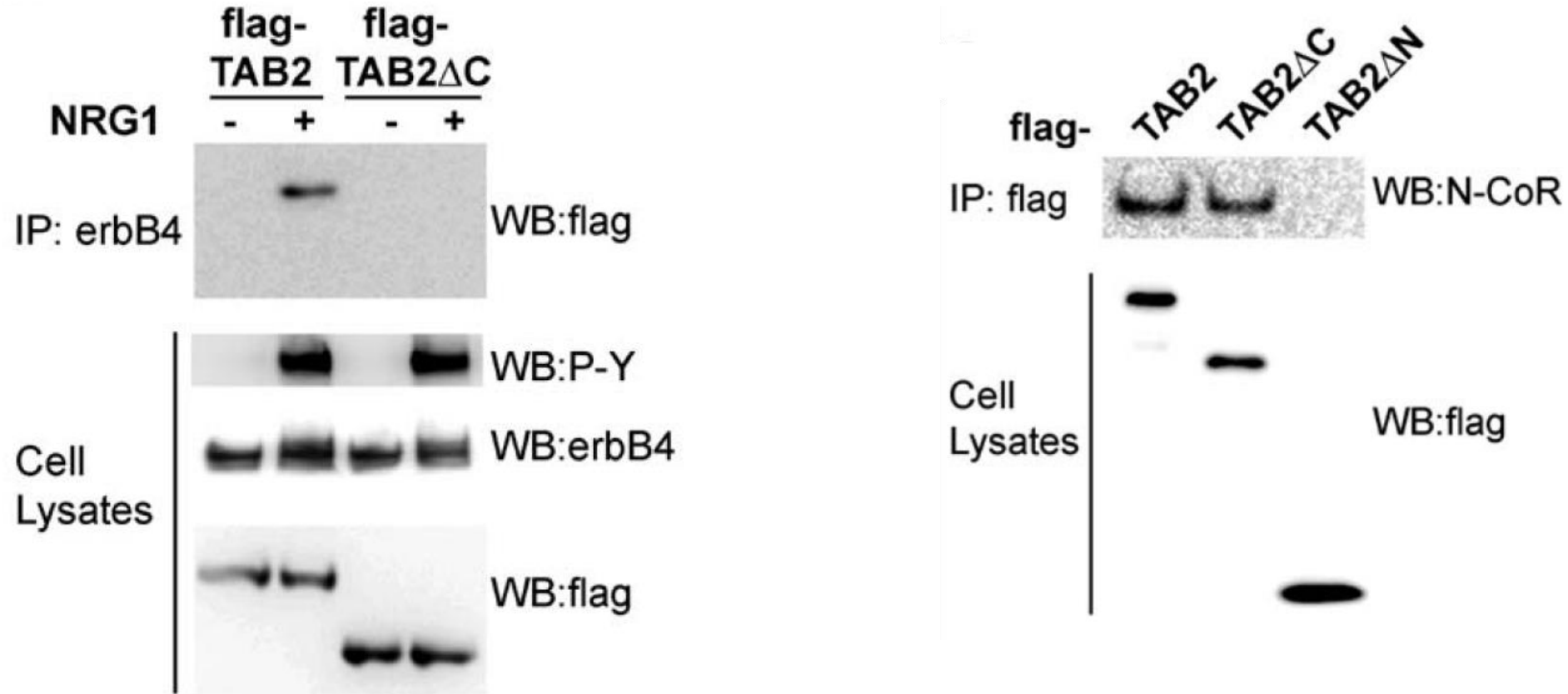
Which domain of TAB2 is involved in the interaction with E4ICD & N-CoR?



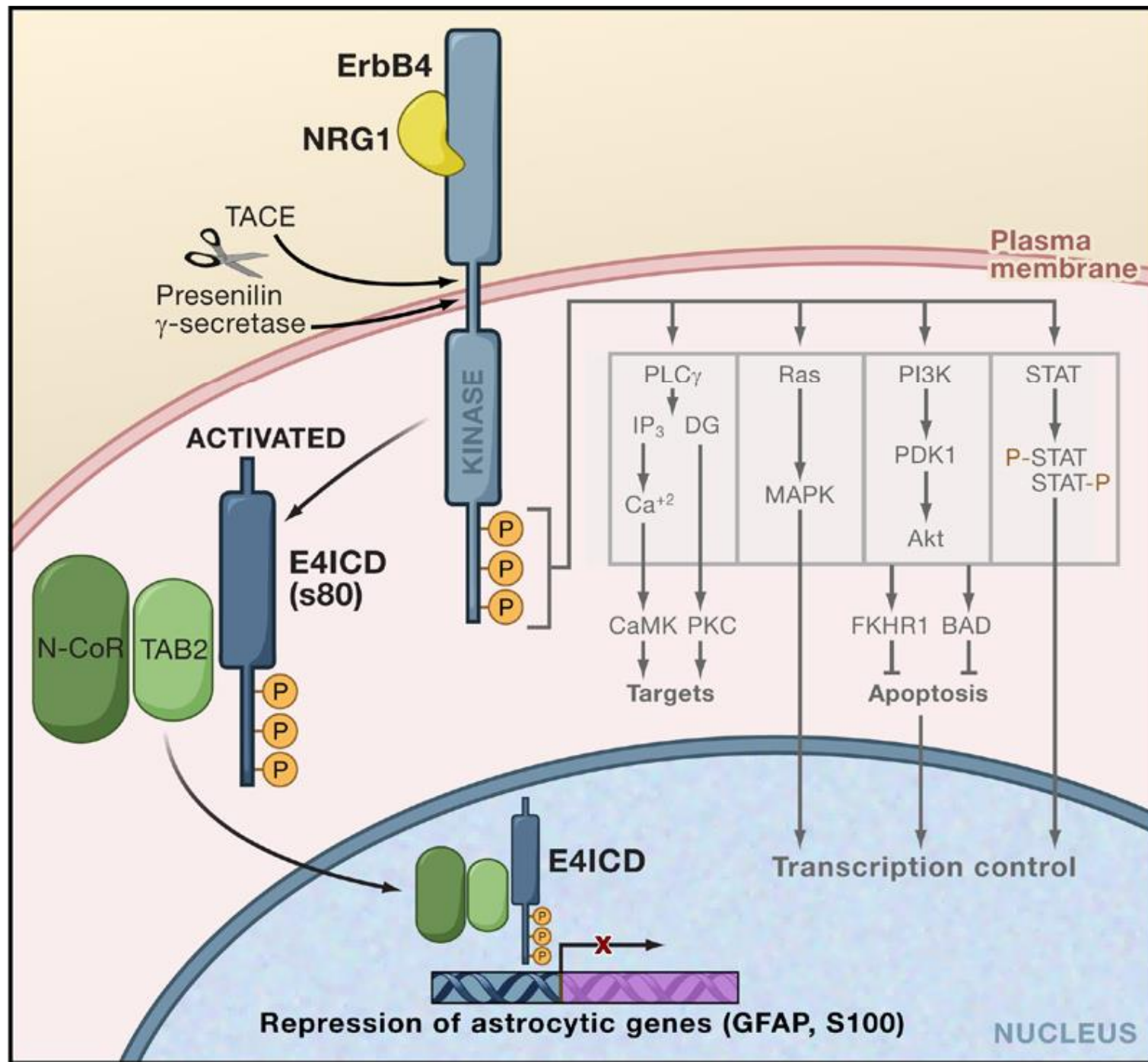
Panel 8

Which domain of TAB2 is involved in the interaction with E4ICD & which in the interaction with N-CoR?

Truncated versions of TAB2 were used to define the specific domains within this protein responsible for its ability to link E4ICD to N-CoR.



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.



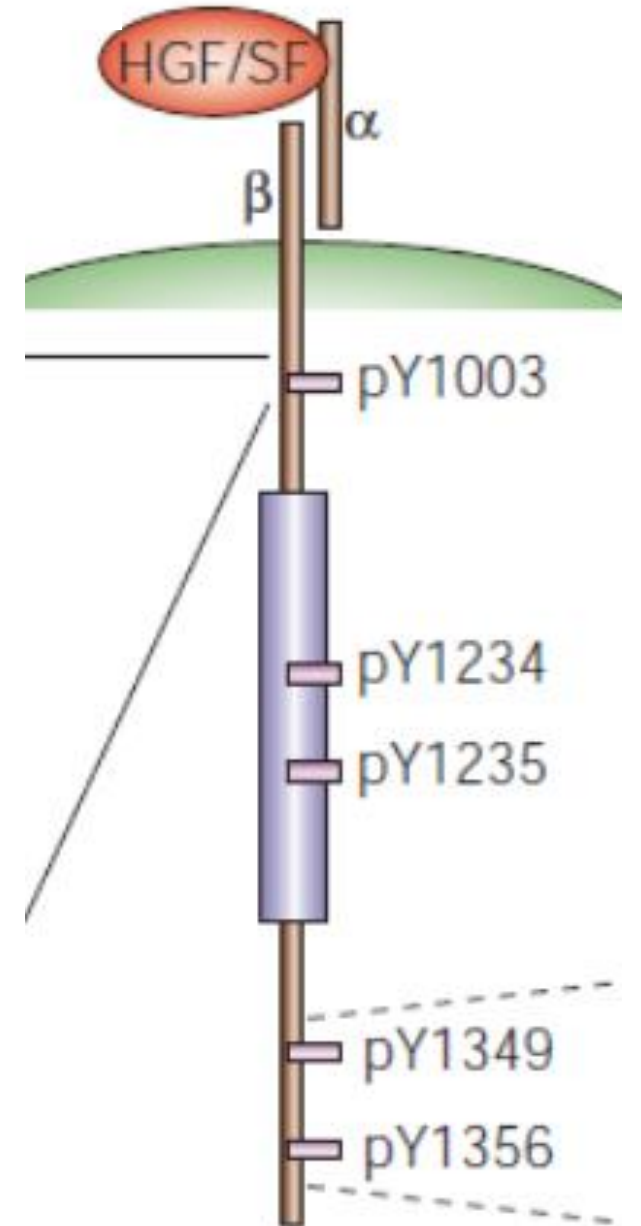


Protein phosphorylation

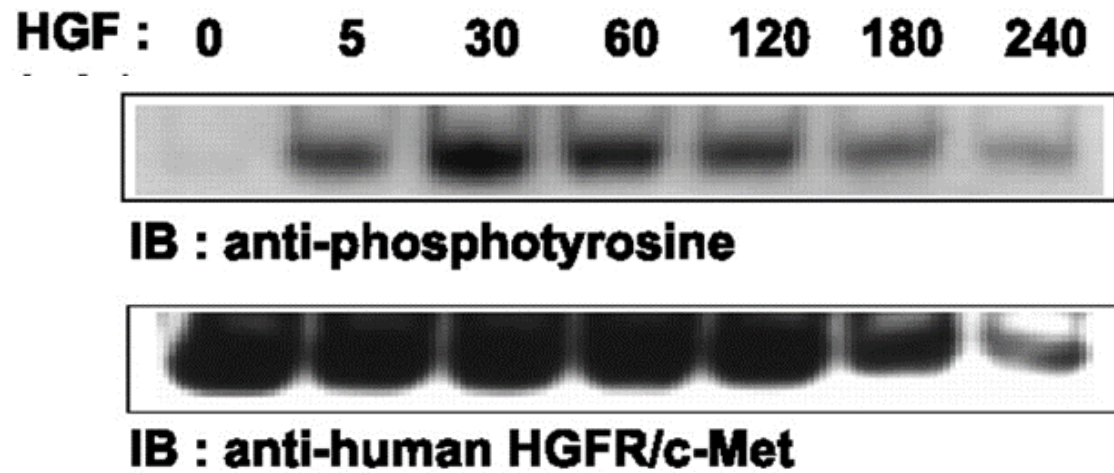
- pTyr antibody
- Tyrosine specific antibodies

Phosphorylation is a common mode of activating or deactivating a protein as a form of regulation. Within cells, proteins are commonly modified at serine, tyrosine and threonine amino acids by adding a phosphate group.

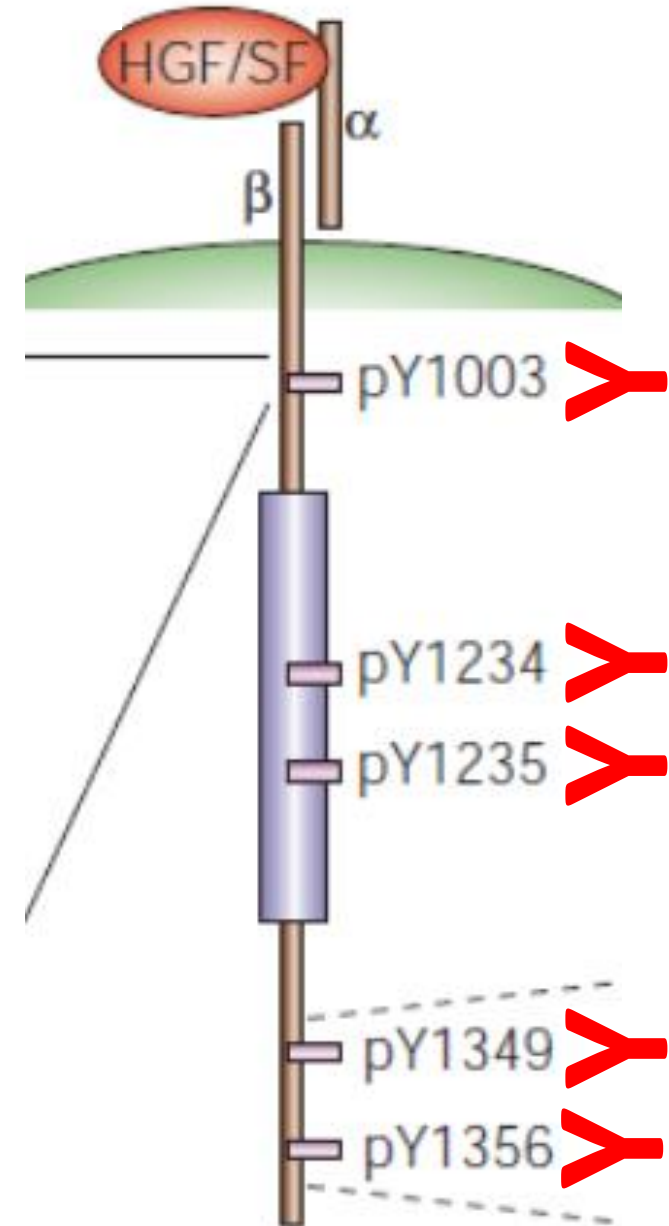
Is Met phosphorylated following stimulation with HGF/SF?



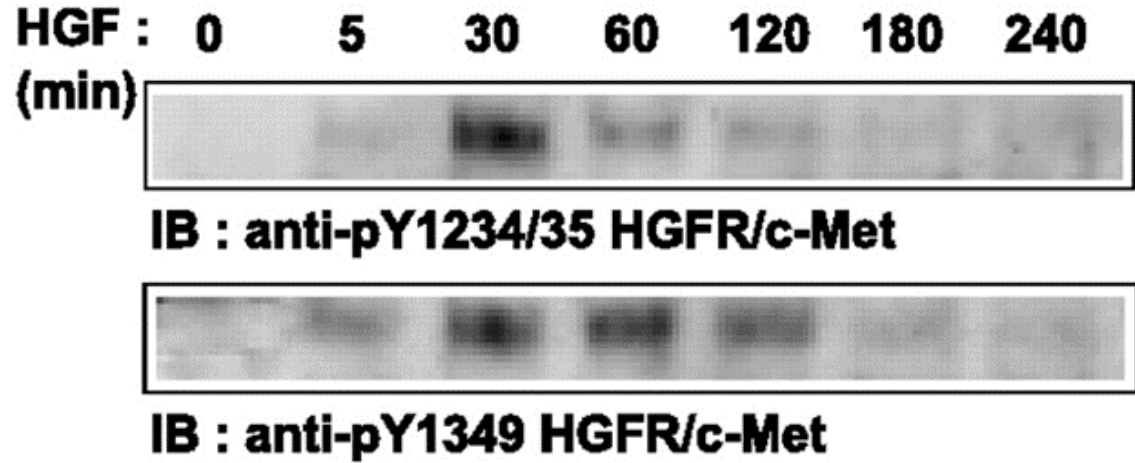
Is Met phosphorylated following stimulation with HGF/SF?



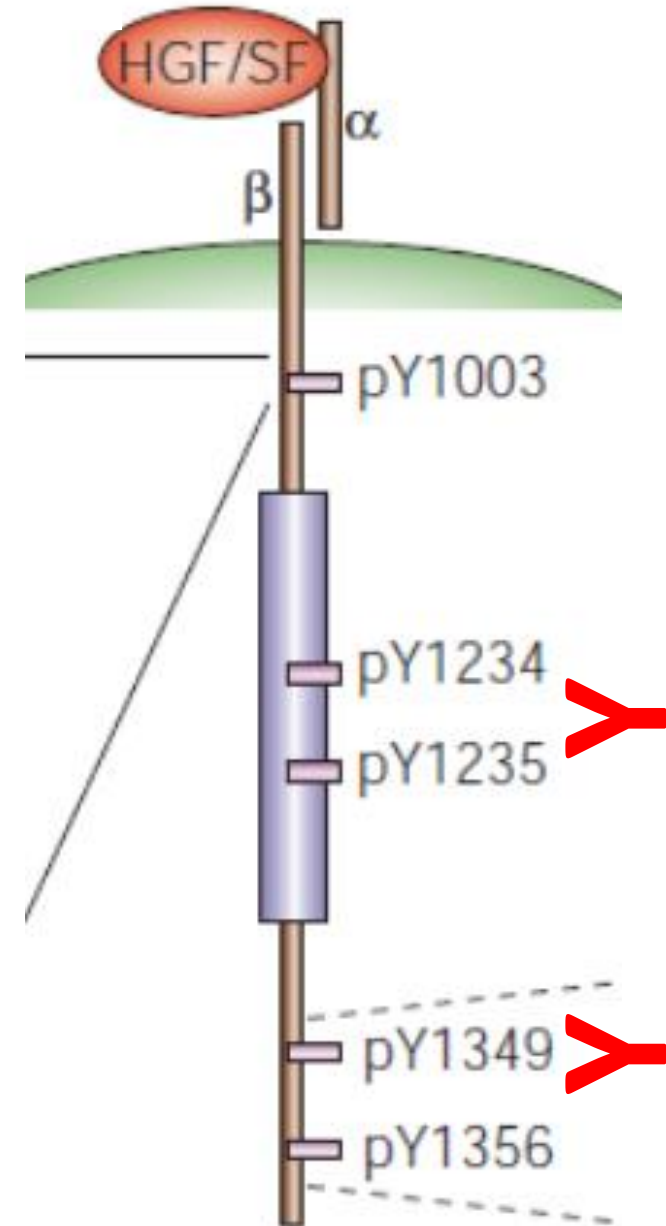
IP α HGFR/c-Met



Is Met phosphorylated following stimulation with HGF/SF?

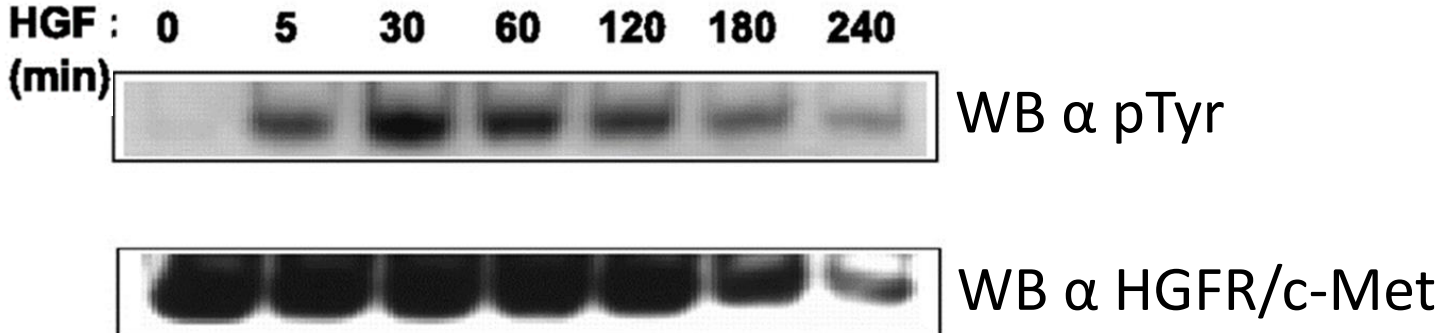


Total cell lysate



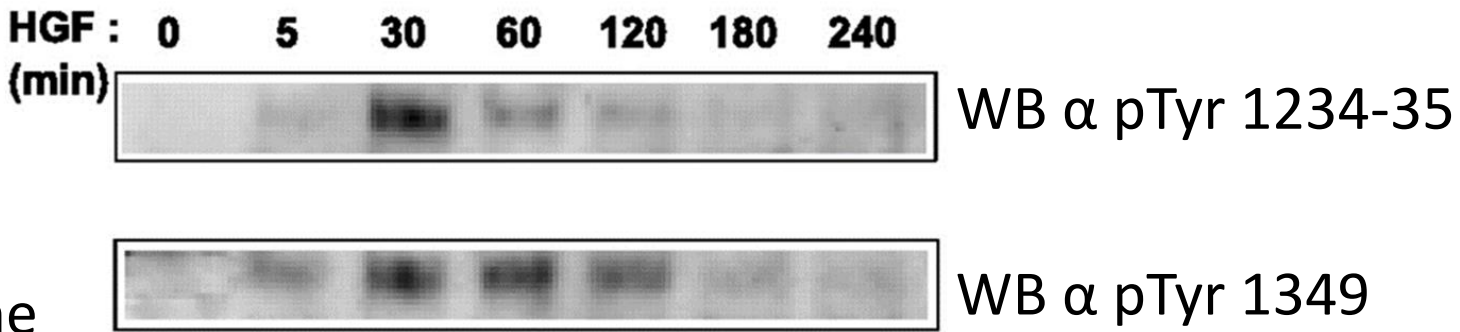
Ab α generic phospho-tyrosine:

- 1-total extract
- 2-IP α protein of interest
- 3-SDS-PAGE
- 4-WB α phospho-tyrosine

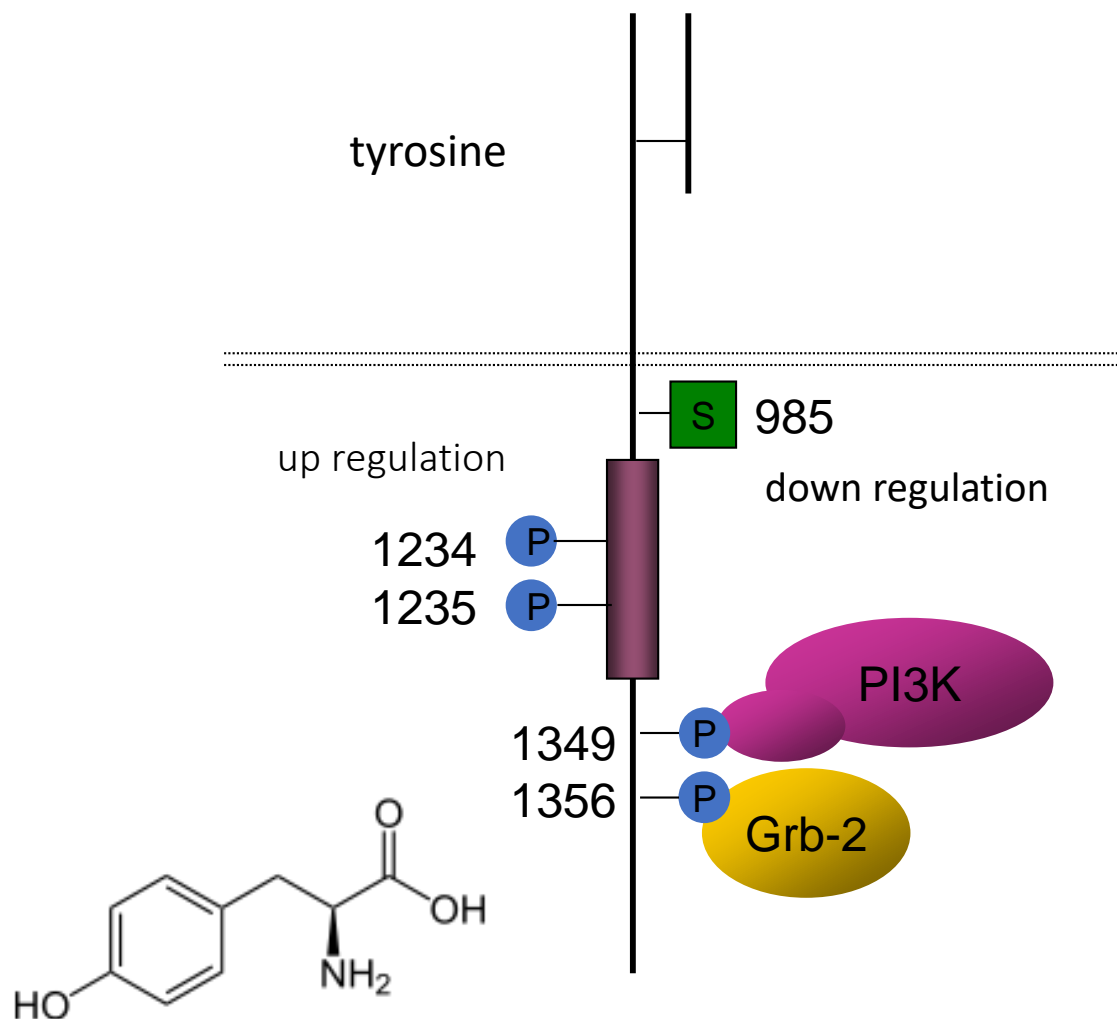


Ab α specific tyrosine:

- 1-total extract
- 2-SDS-PAGE
- 3-WB α specific phospho-tyrosine



THE HGF/SF RECEPTOR (*MET*)



Anti-Met (c-Met) (phospho Y1230 + Y1234 + Y1235) antibody (ab5662)

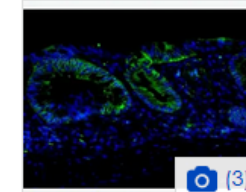
★★★★☆ Reviews (7) Specific References (17)

Description: Rabbit polyclonal to Met (c-Met) (phospho Y1230 + Y1234 + Y1235)

Application: ICC/IF, IHC-P, WB

Reactivity: Mouse, Human (predicted: Rat)

Conjugate: Unconjugated



(3)

Compare (max 4)

Recombinant RabMAb 10 µl Trial Size

Anti-Met (c-Met) (phospho Y1349) antibody [EP2367Y] (ab68141)

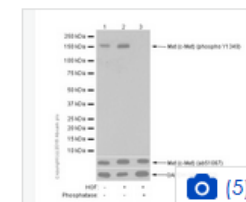
★★★★☆ Reviews (4) Specific References (8)

Description: Rabbit monoclonal [EP2367Y] to Met (c-Met) (phospho Y1349)

Application: Dot, IHC-P, IP, WB

Reactivity: Human

Conjugate: Unconjugated



(5)

Compare (max 4)

Anti-Met (c-Met) (phospho Y1356) antibody (ab73992)

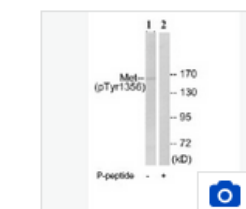
Specific References (3)

Description: Rabbit polyclonal to Met (c-Met) (phospho Y1356)

Application: ELISA, WB

Reactivity: Mouse, Human (predicted: Rat)

Conjugate: Unconjugated



(1)

Compare (max 4)

Anti-Met (c-Met) (phospho Y1003) antibody (ab193270)

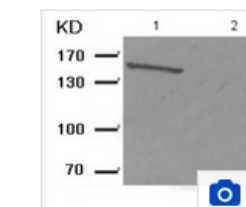
Specific References (1)

Description: Rabbit polyclonal to Met (c-Met) (phospho Y1003)

Application: WB

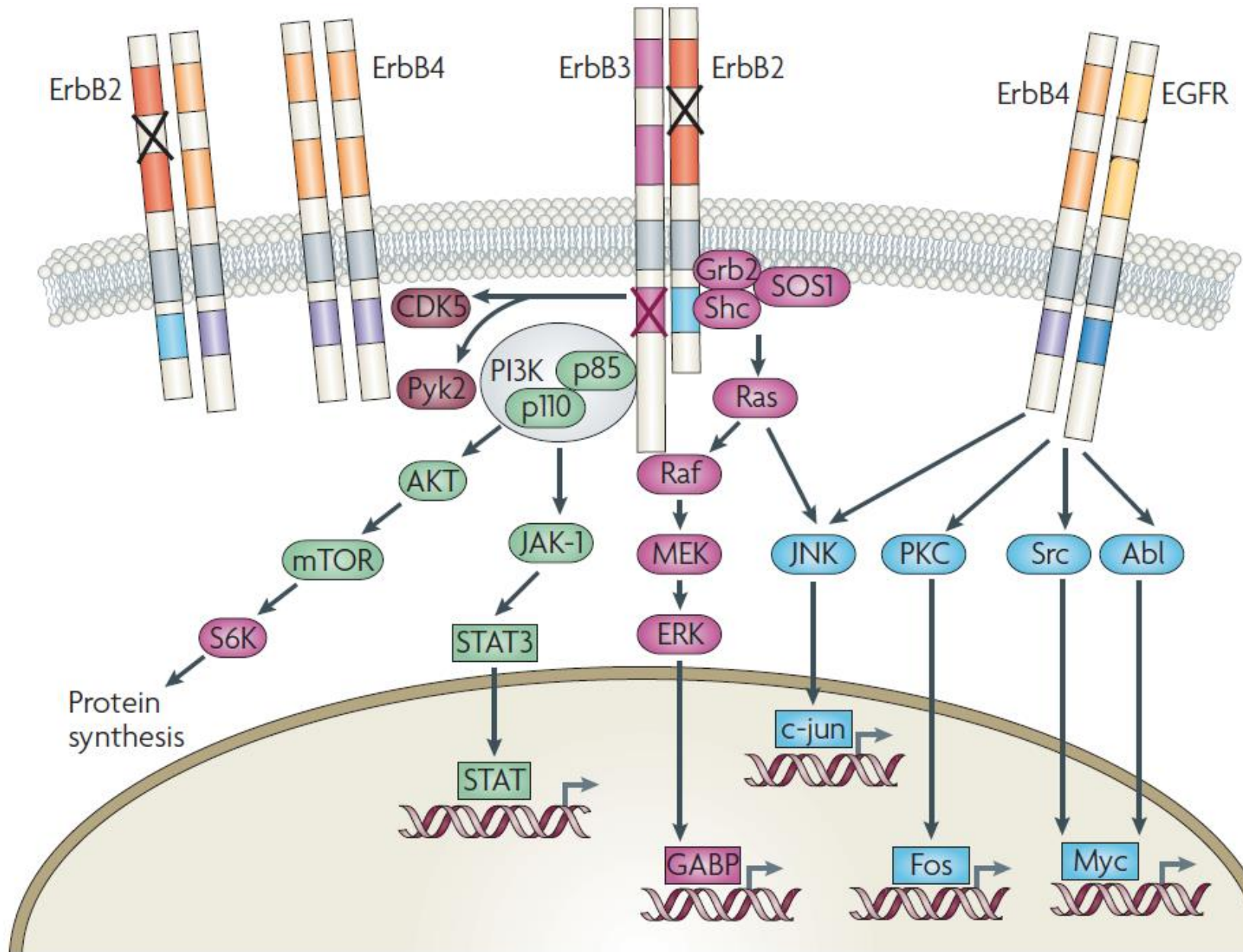
Reactivity: Mouse, Rat, Human

Conjugate: Unconjugated



(1)

Compare (max 4)



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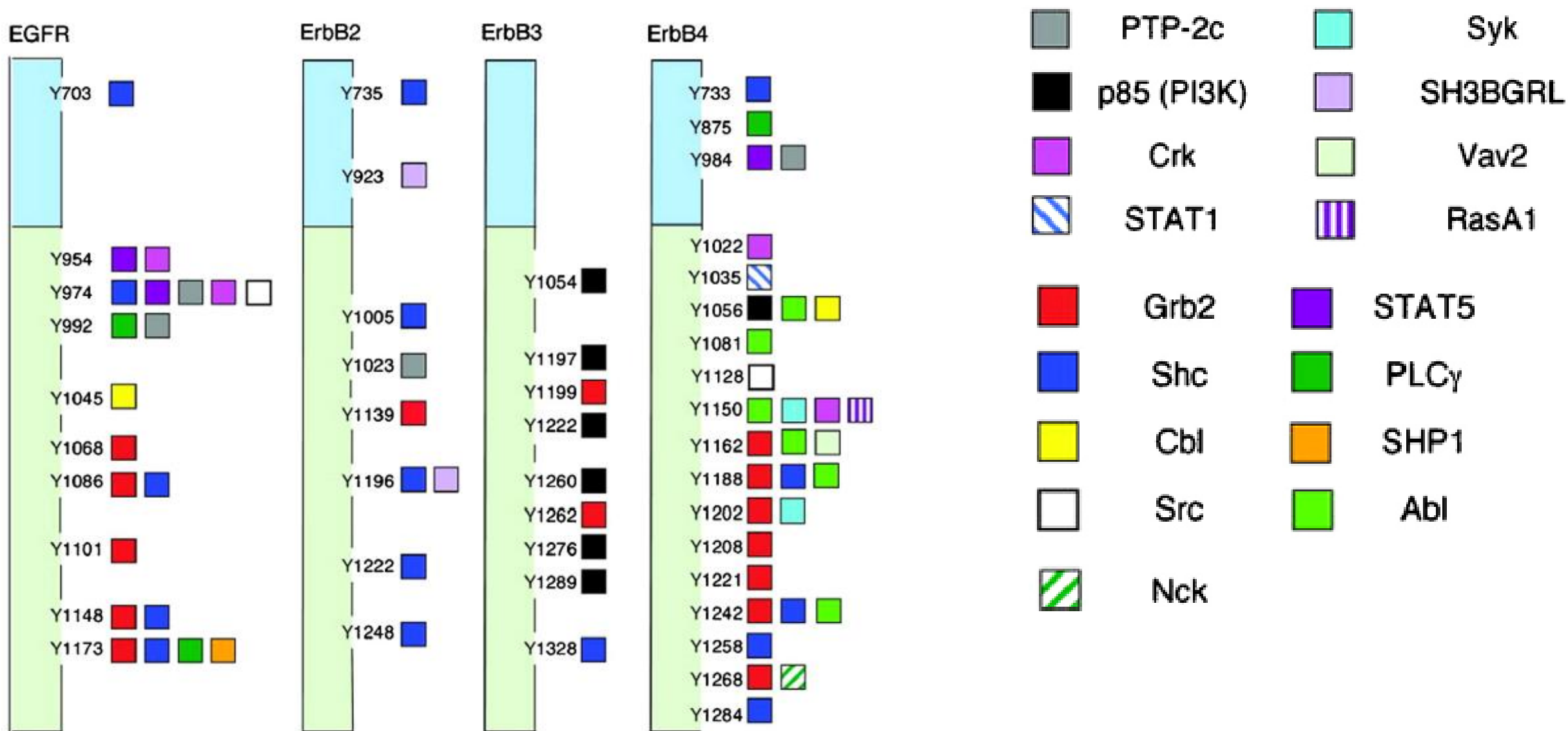
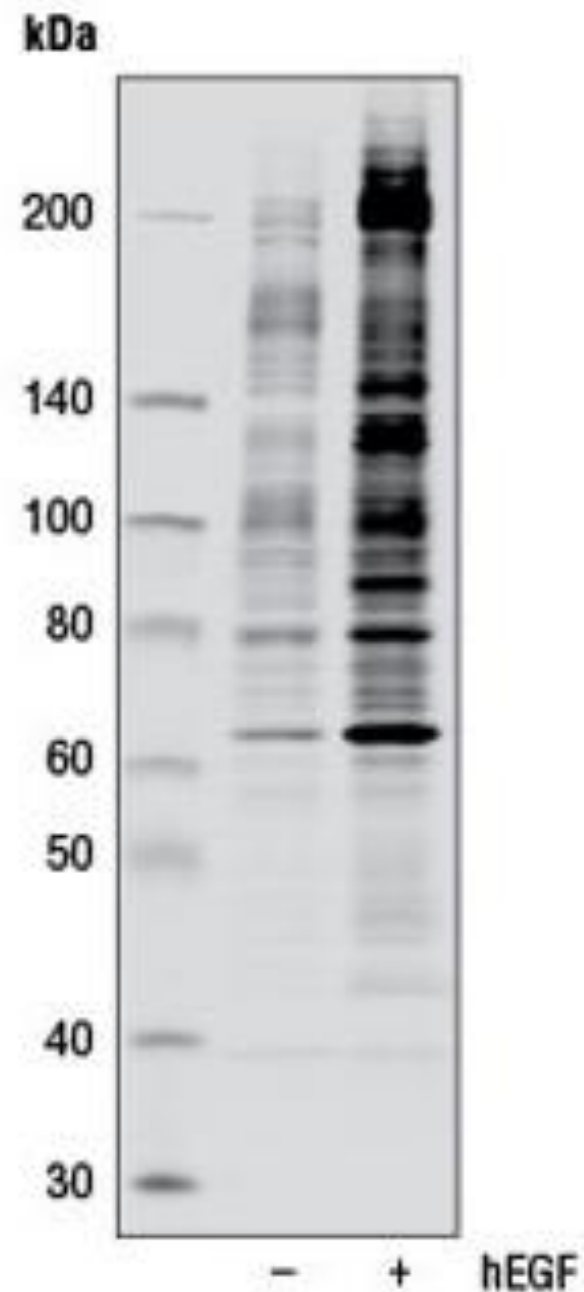


Fig. 8. Signaling by the ErbB/HER protein tyrosine kinases. The tyrosyl phosphorylation sites and their binding partners are depicted. The receptors are not drawn to scale. The residue numbers on EGFR correspond to the mature protein while those of ErbB2/3/4 correspond to the nascent protein including the signal peptides. The figure is reproduced from Ref. [83] with copyright permission from Elsevier.

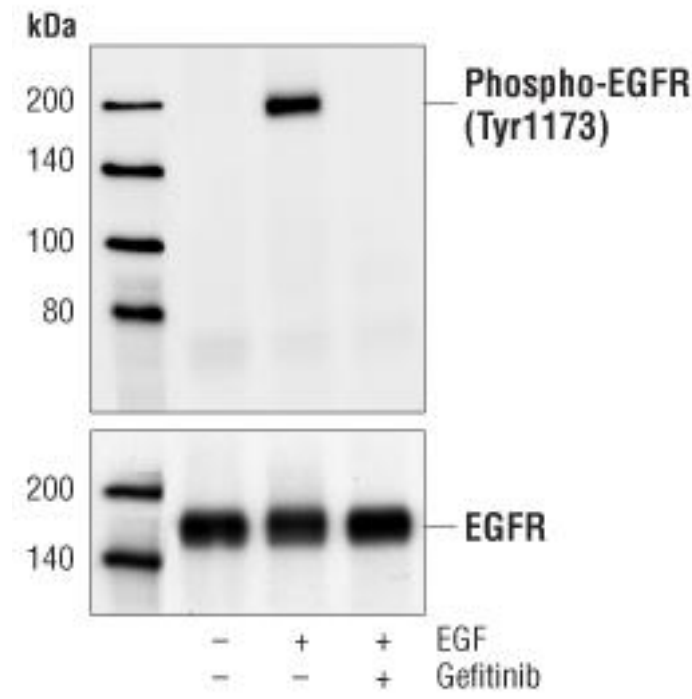
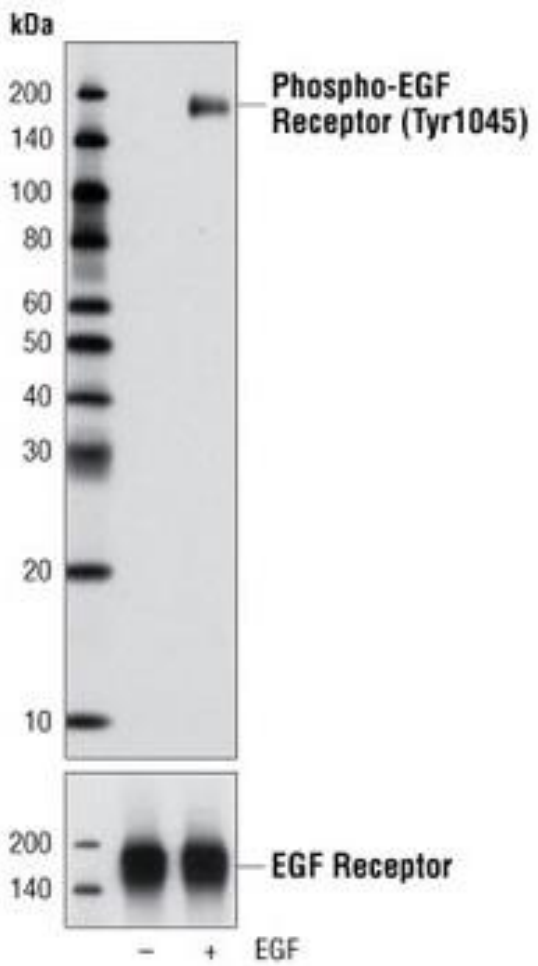
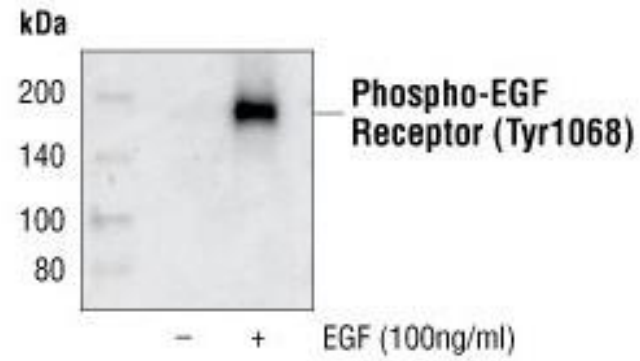
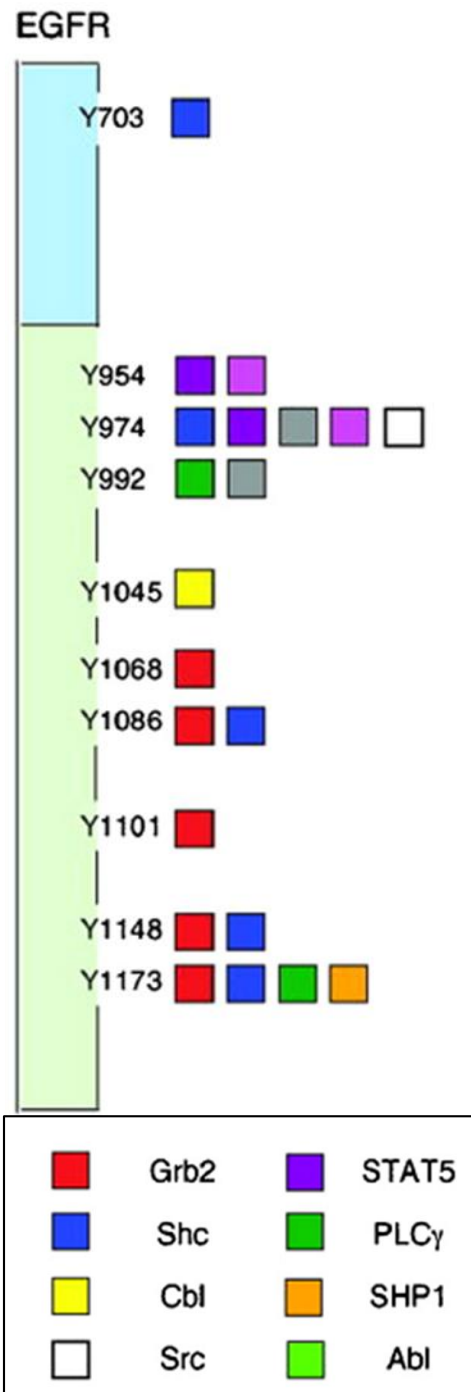
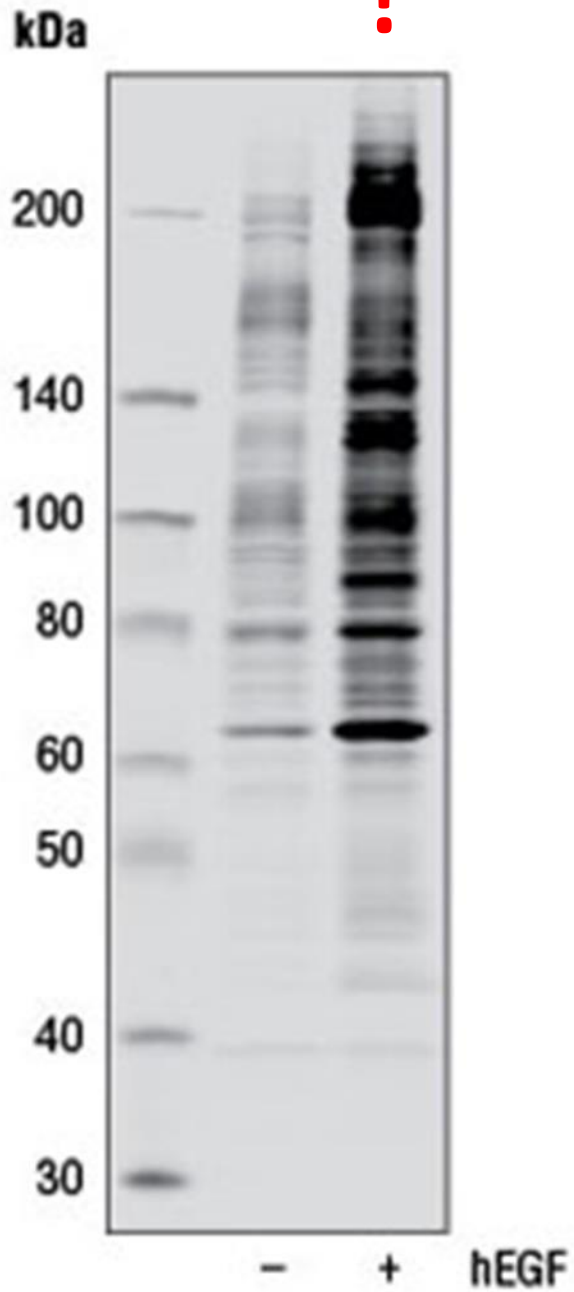
Anti-Phosphotyrosine antibody

Product name	Anti-Phosphotyrosine antibody [PY20]
Description	Mouse monoclonal [PY20] to Phosphotyrosine
Host species	Mouse
Specificity	This antibody recognizes phospho-tyrosine and phosphotyrosine- containing proteins.
Tested applications	Suitable for: WB, ICC/IF, IHC-P, IHC-FoFr
Species reactivity	Reacts with: Species independent
Immunogen	Chemical/ Small Molecule corresponding to Phosphotyrosine conjugated to keyhole limpet haemocyanin.

- Which proteins are phosphorylated in tyrosine?



?



Anti-ErbB 3 (phospho Y1289) antibody 1

Overview

Product name	Anti-ErbB 3 (phospho Y1289) antibody
Description	Rabbit polyclonal to ErbB 3 (phospho Y1289)
Host species	Rabbit
Tested applications	Suitable for: ICC/IF, ELISA, WB
Species reactivity	Reacts with: Human Predicted to work with: Mouse, Rat, Cow, Macaque monkey, Chinese hamster, Orangutan ▲
Immunogen	Synthetic peptide corresponding to Human ErbB 3 aa 1250 to the C-terminus conjugated to keyhole limpet haemocyanin.

Anti-ErbB 3 (phospho Y1328) antibody 2

Overview

Product name	Anti-ErbB 3 (phospho Y1328) antibody
Description	Rabbit polyclonal to ErbB 3 (phospho Y1328)
Host species	Rabbit
Specificity	ab131444 detects endogenous levels of ErbB 3 only when phosphorylated at Tyrosine 1328.
Tested applications	Suitable for: WB, IHC-P, ICC/IF
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic phosphopeptide conjugated to KLH, surrounding the phosphorylation site of Tyrosine 1328 (P-D-Y(p)-W-H) of Human ErbB3 (NP_001005915.1).

Anti-ErbB 3 (phospho Y1222) antibody 3

Overview

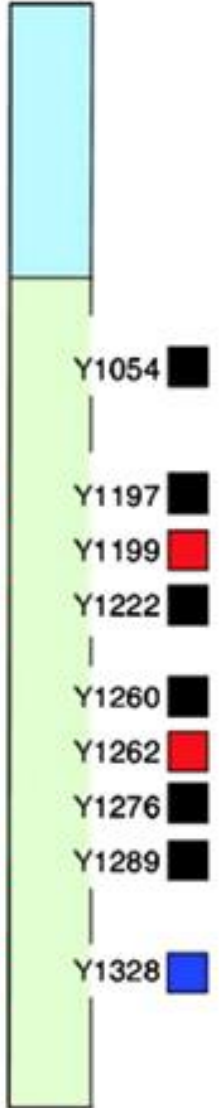
Product name	Anti-ErbB 3 (phospho Y1222) antibody [EPR5807]
Description	Rabbit monoclonal [EPR5807] to ErbB 3 (phospho Y1222)
Host species	Rabbit
Specificity	ab133445 only detects ErbB 3 phosphorylated at Tyrosine 1222.
Tested applications	Suitable for: WB, ICC/IF, IP Unsuitable for: Flow Cyt or IHC-P
Species reactivity	Reacts with: Human
Immunogen	Synthetic phosphopeptide corresponding to a region surrounding Tyrosine 1222 of Human ErbB 3.
Positive control	Lysate of SKBR3 cells treated with neuregulin.
General notes	A trial size is available to purchase for this antibody. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

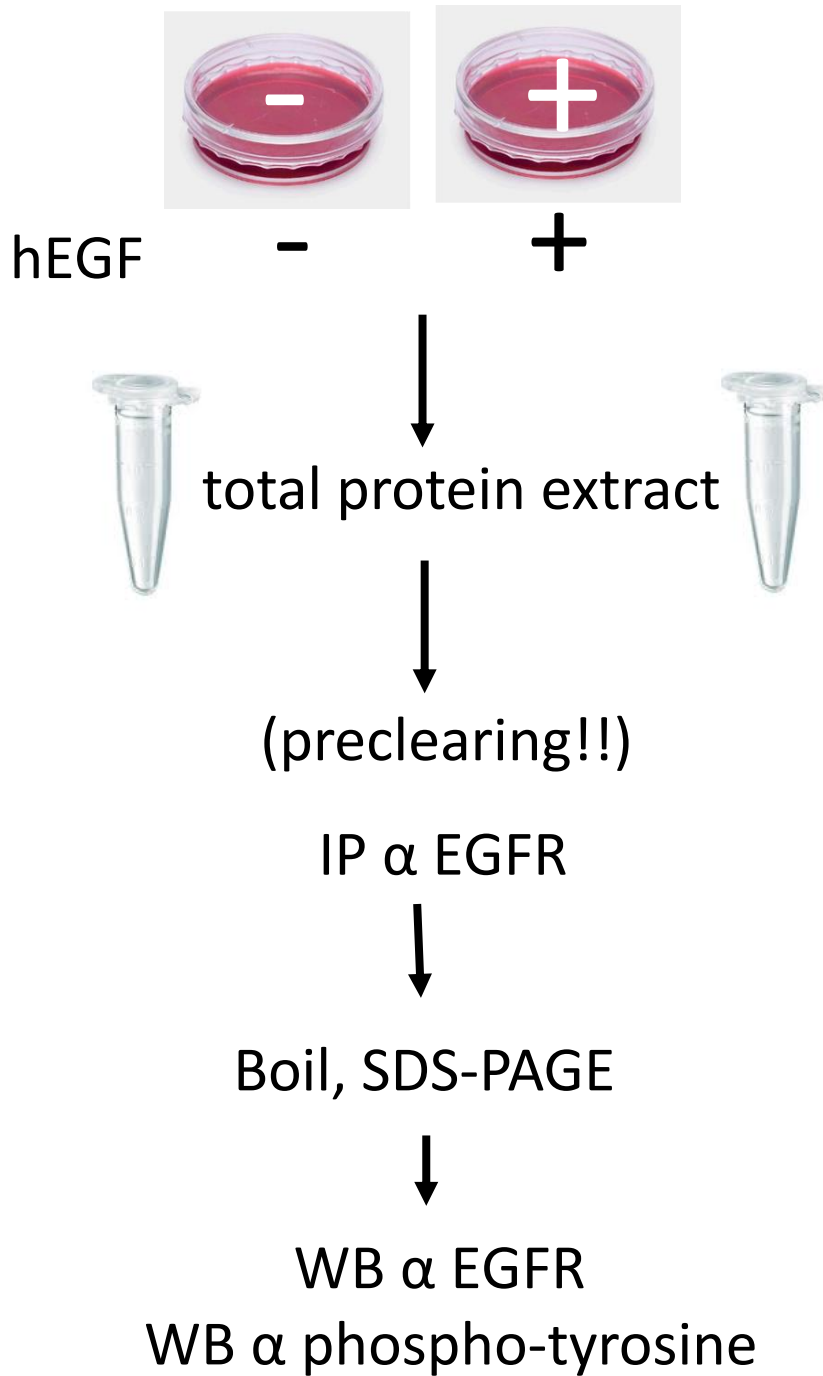
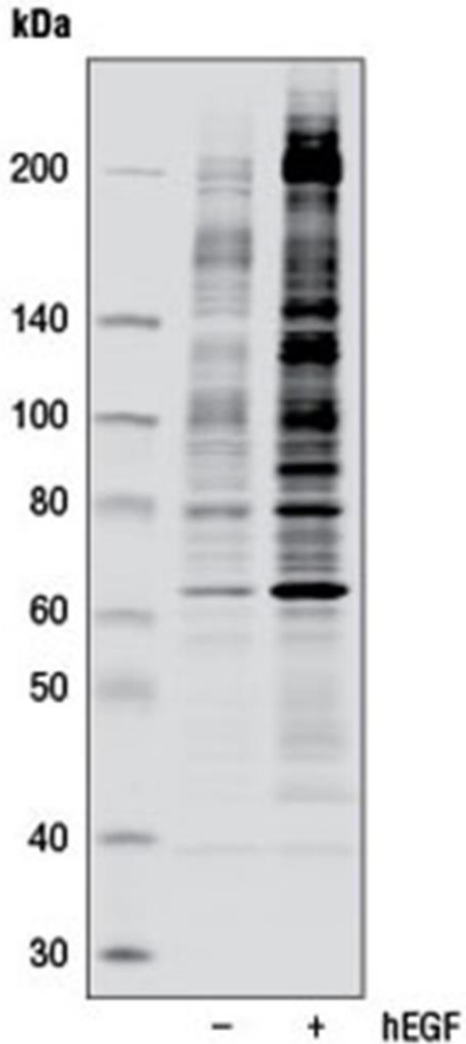
Anti-ErbB 3 (phospho Y1197) antibody [EPR5806] 4

Overview

Product name	Anti-ErbB 3 (phospho Y1197) antibody [EPR5806]
Description	Rabbit monoclonal [EPR5806] to ErbB 3 (phospho Y1197)
Host species	Rabbit
Specificity	ab133444 only detects ErbB 3 phosphorylated at Tyrosine 1197.
Tested applications	Suitable for: WB, IP Unsuitable for: Flow Cyt, ICC/IF or IHC-P
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide within Human ErbB 3 (phospho Y1197). The exact sequence is proprietary.
Positive control	Lysate of SKBR3 cells treated with neuregulin.
General notes	A trial size is available to purchase for this antibody. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

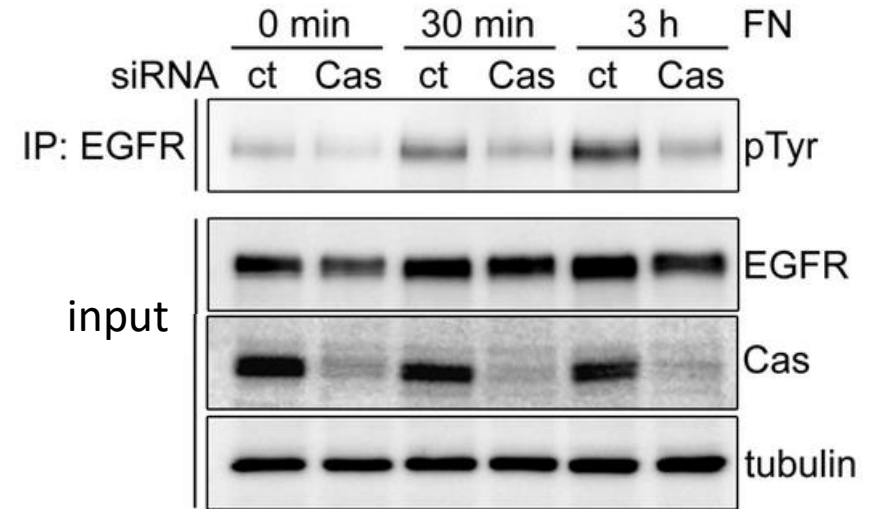
ErbB3





If you have only an anti-phosphotyrosine antibody, how can you verify if EGFR is phosphorylated?

IP α EGFR

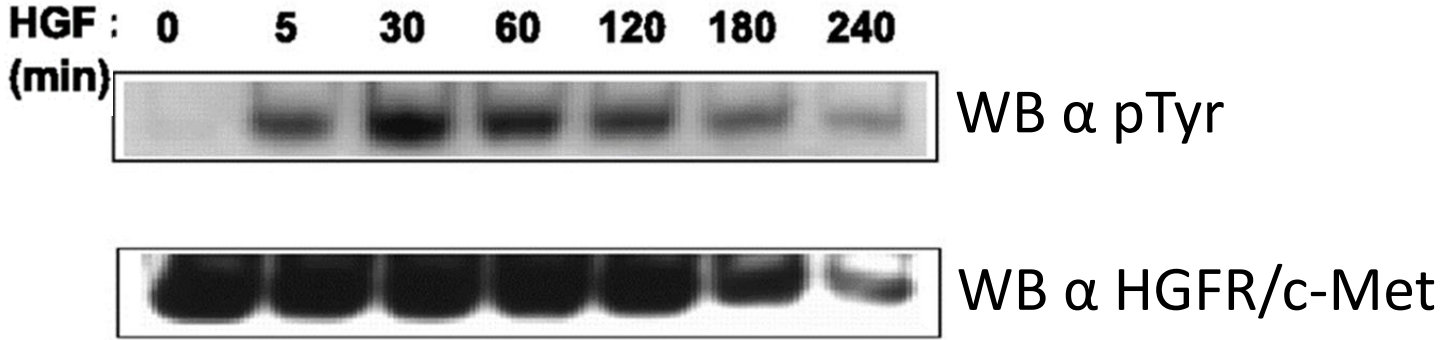


Cells were transfected with non-targeting (ct) or p130Cas-specific siRNA (Cas) duplexes and cultured for 60 h. The cells were then serum starved for 12 h, incubated in suspension for 1 h, and plated on fibronectin (FN) for 0 min, 30 min or 3 h. Tyrosine phosphorylation of EGFR was analyzed by immunoblotting EGFR immunoprecipitates with an anti-phosphotyrosine antibody (pTyr).

total protein extract

Ab α generic phospho-tyrosine:

- 1-total extract
- 2-IP α protein of interest
- 3-SDS-PAGE
- 4-WB α phospho-tyrosine



Ab α specific tyrosine:

- 1-total extract
- 2-SDS-PAGE
- 3-WB α specific phospho-tyrosine

