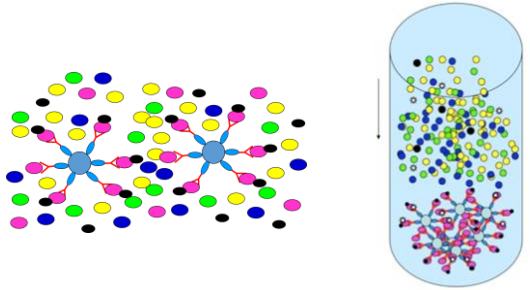
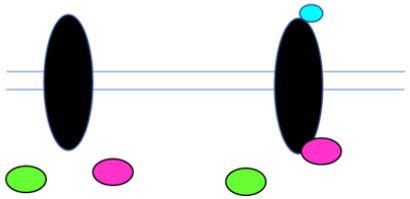
Advanced Cell Biology and Biotechnology

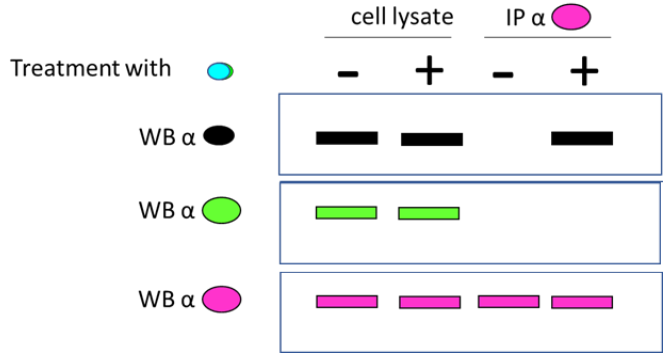
ACBB 2021/22

Summary of the previous lesson

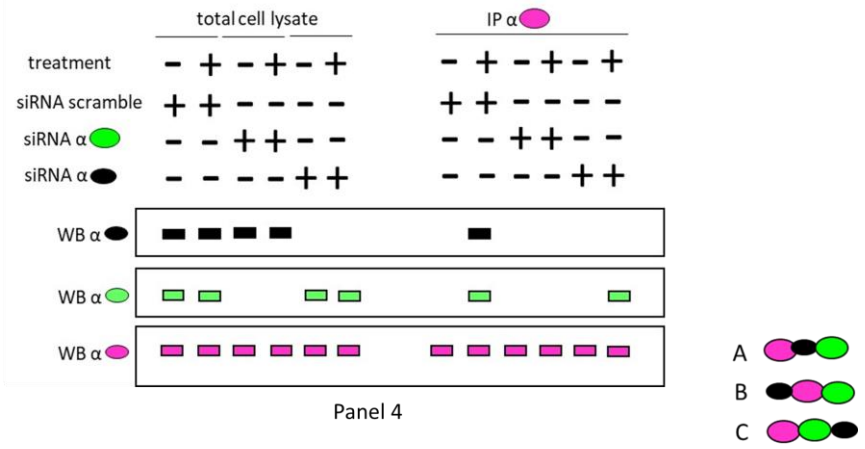
- co-immunoprecipitation



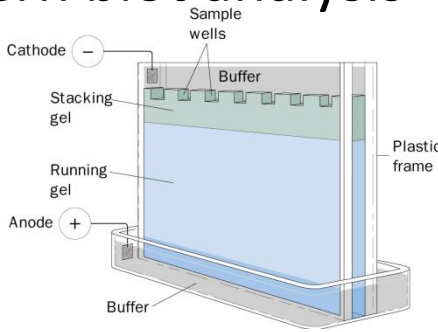




- use of siRNAs to investigate the protein interactions within a complex



Gel run: Western blot analysis



cell lysate/input

cell lysate

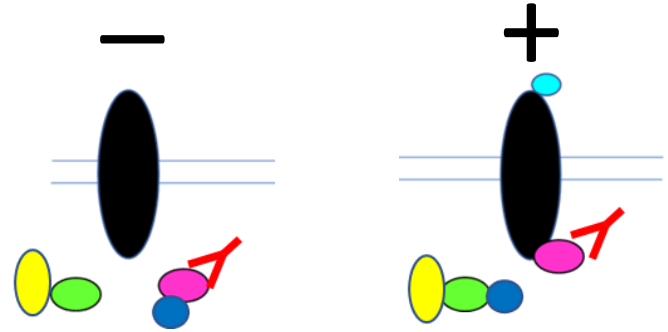
immunoprecipitation

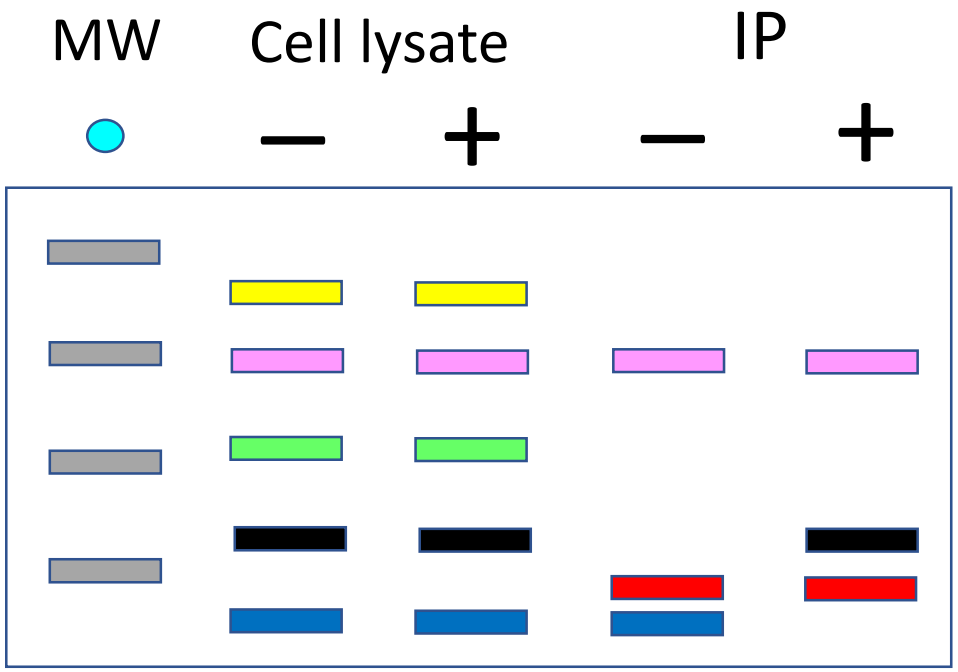
IP

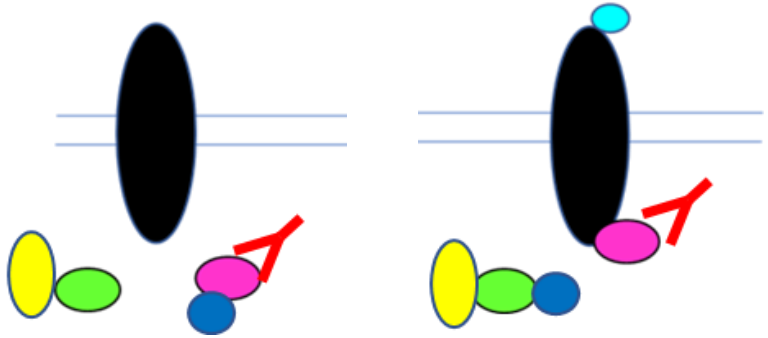
Cell lysate

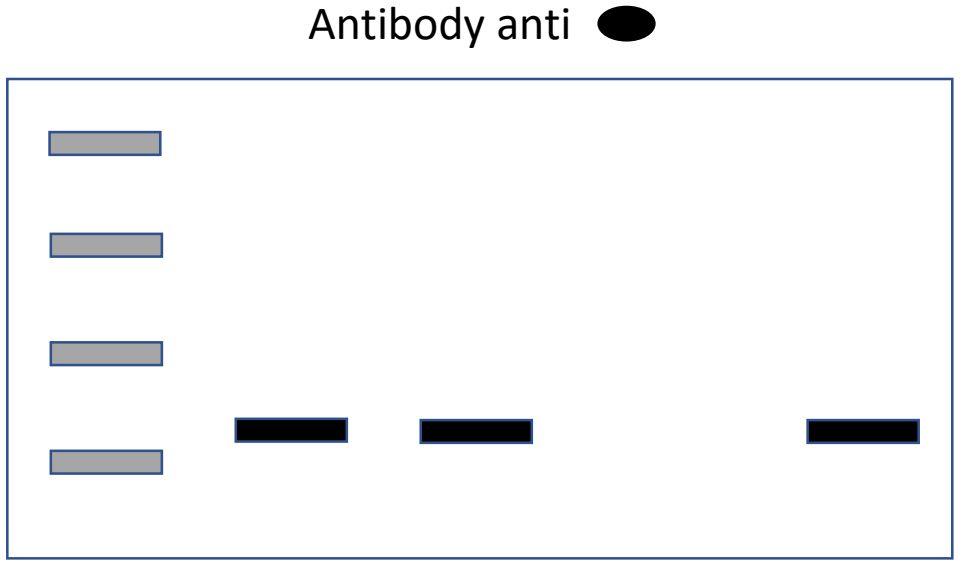
IP

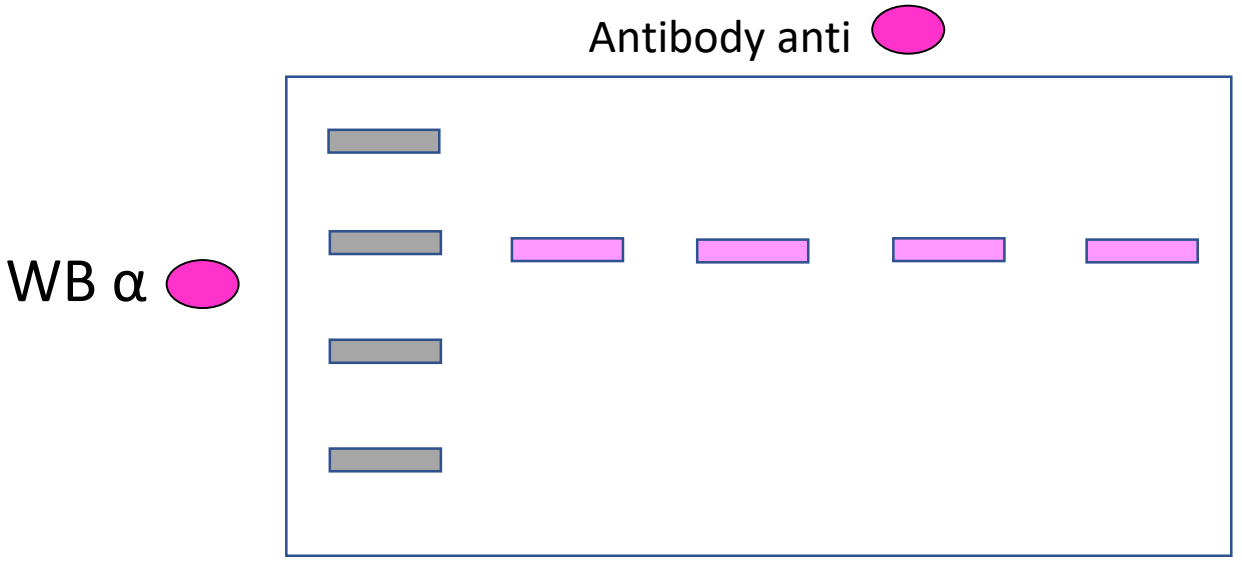
MW

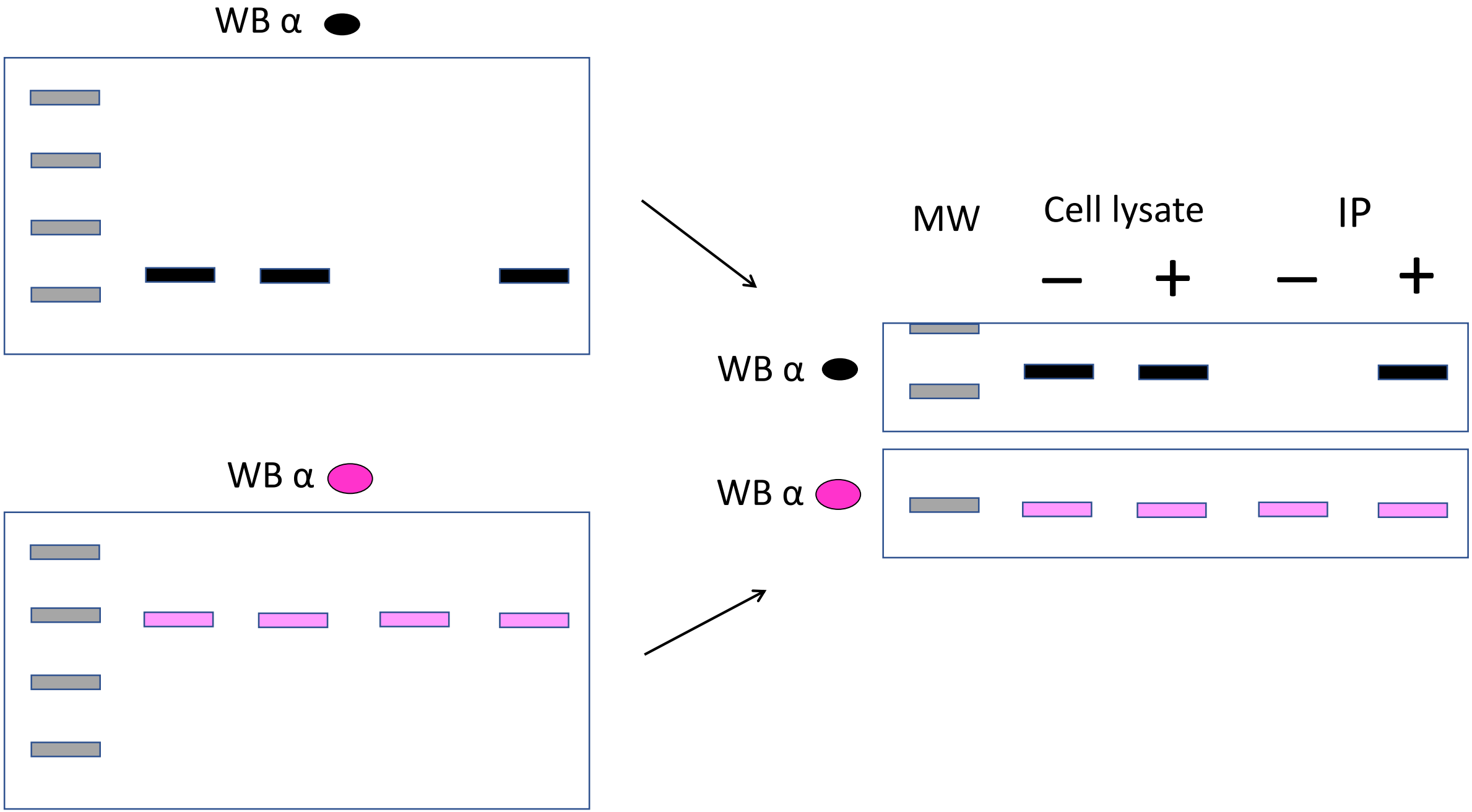


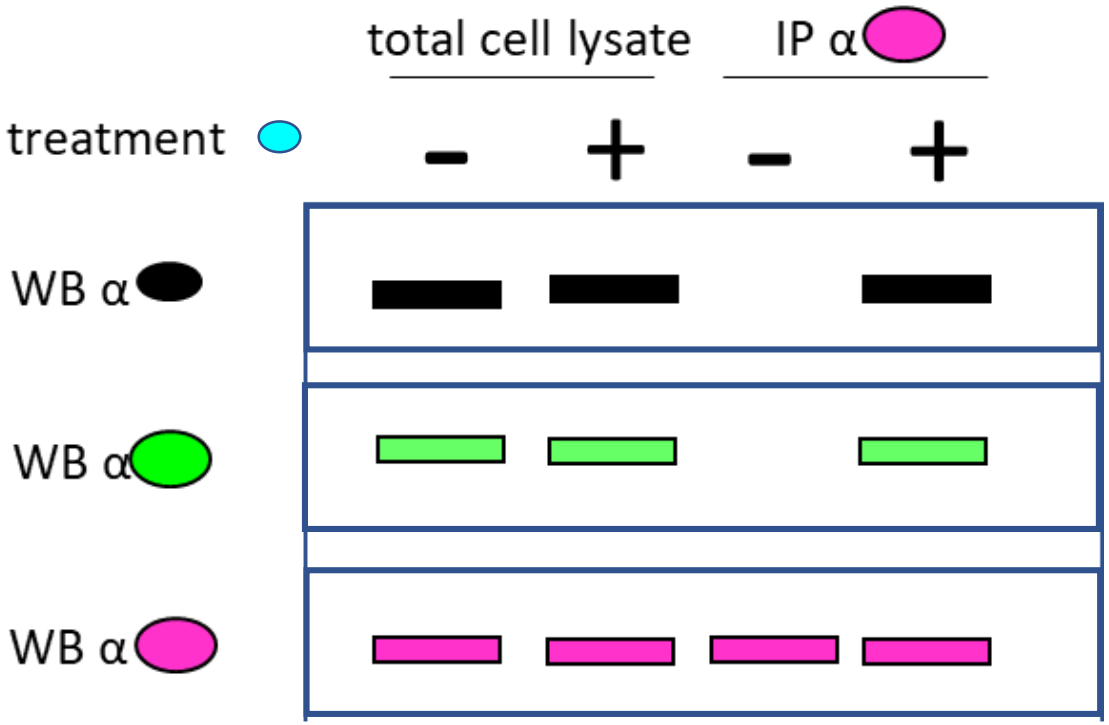

MW
Cell lysate
IP
–
+
–
+



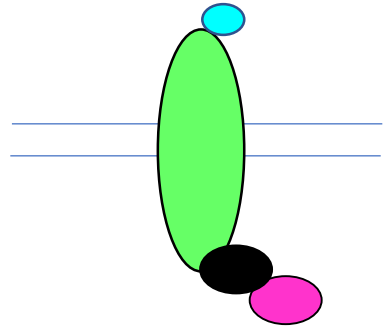

Antibody anti
WB α

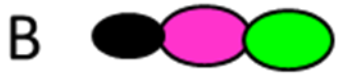

Antibody anti
WB α

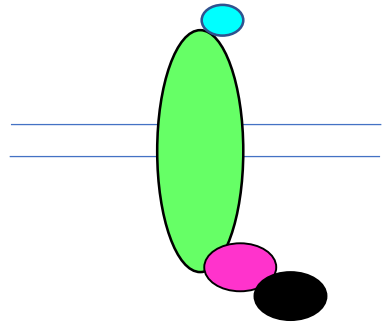

WB α
WB α
MW
Cell lysate
IP
–
+
–
+
WB α
WB α

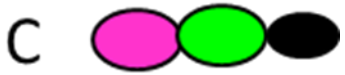


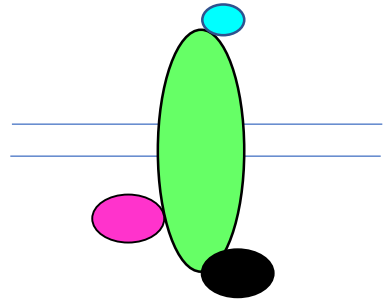
A strategy to understand
if and how these proteins interact.

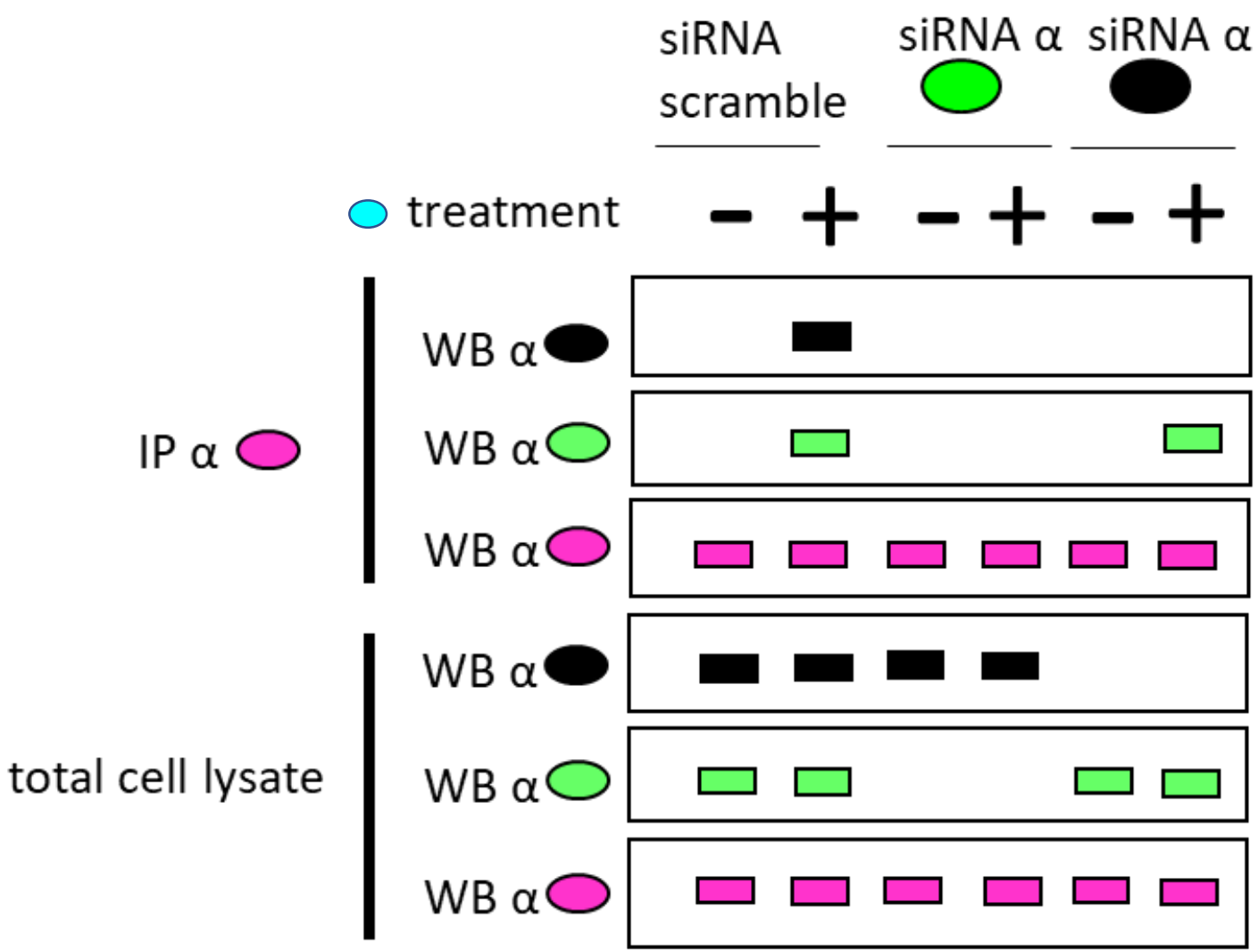


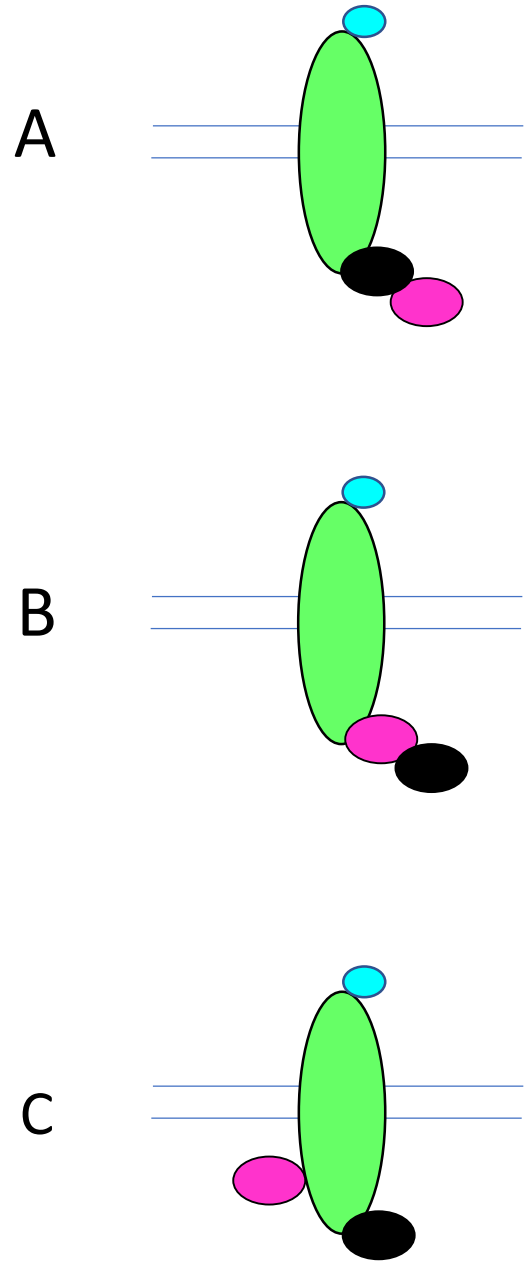






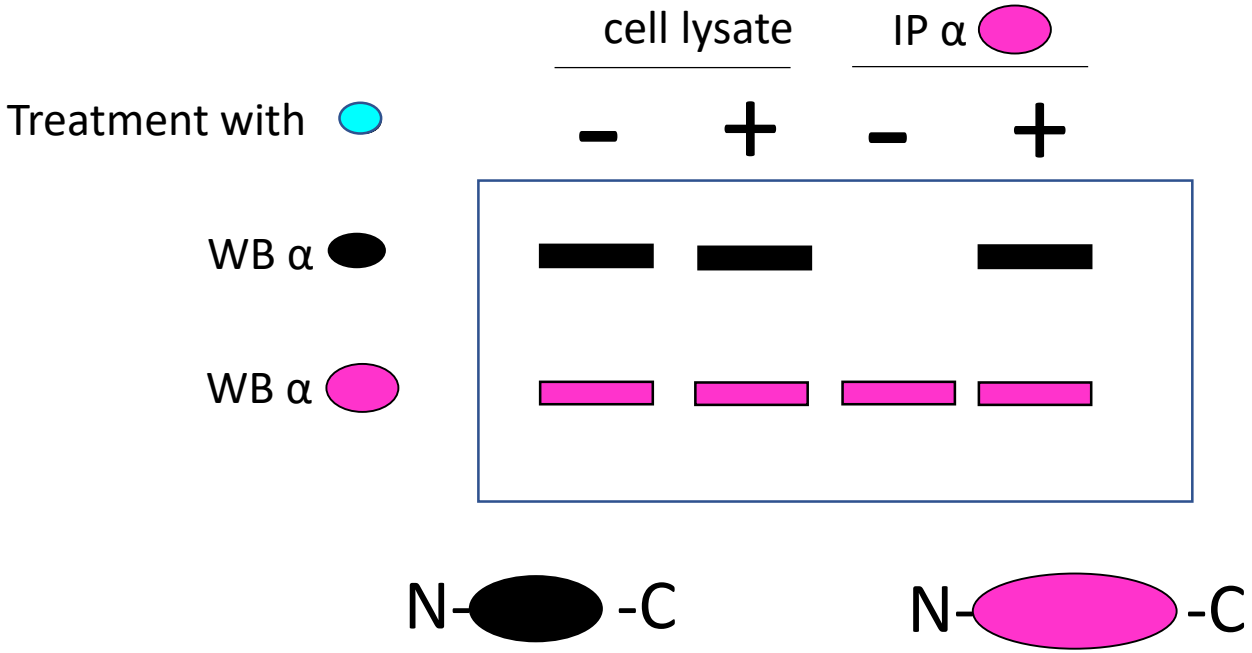






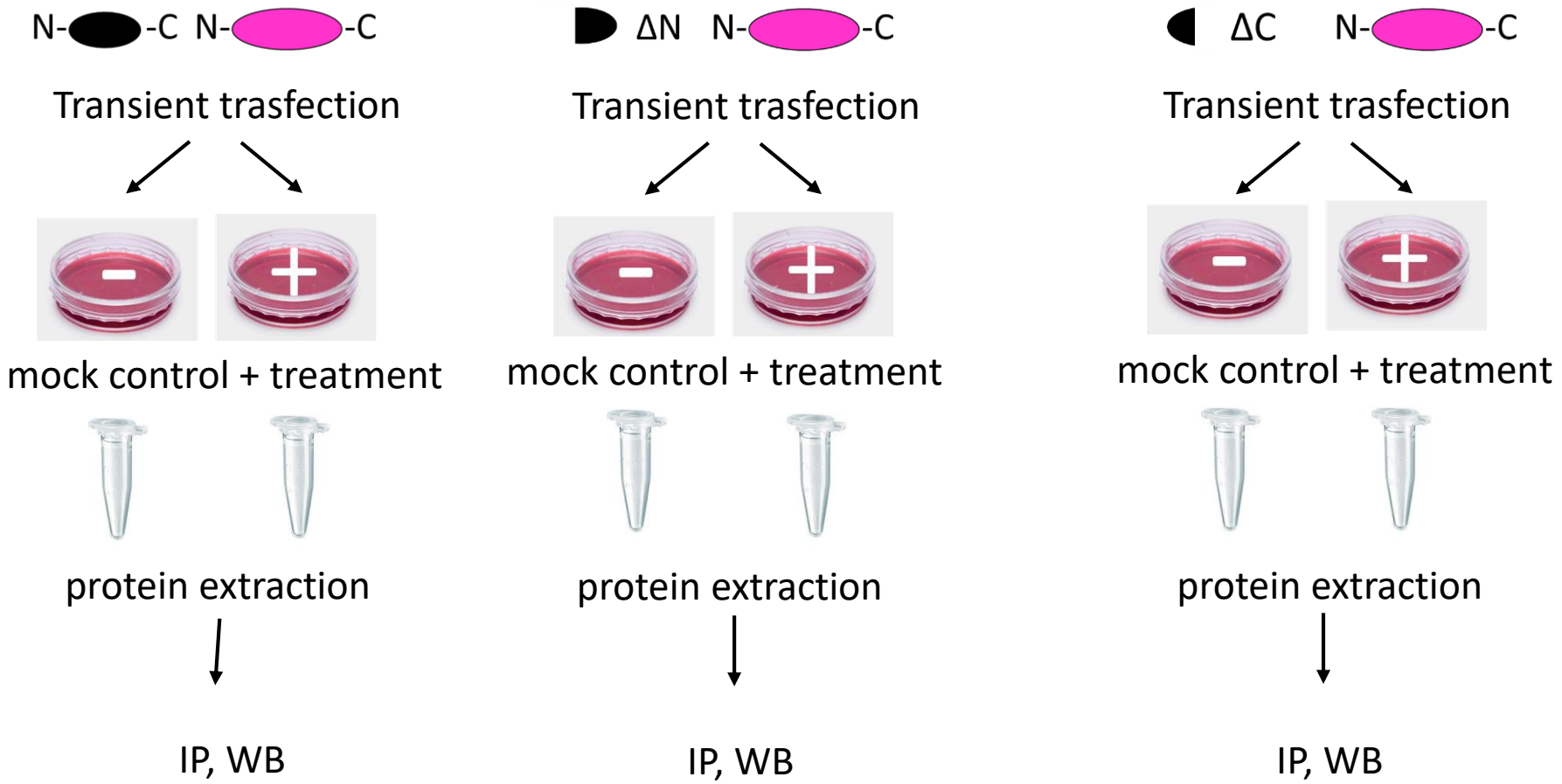
These results suggest that the **green** protein forms a bridge between **pink** and **black** proteins, because when the **green** protein is missing, **pink** and **black** proteins do not co-precipitate.

Which domain of the **PINK** protein is necessary to interact with the **BLACK** protein?
Which domain of the **BLACK** protein is necessary to interact with the **PINK** protein?

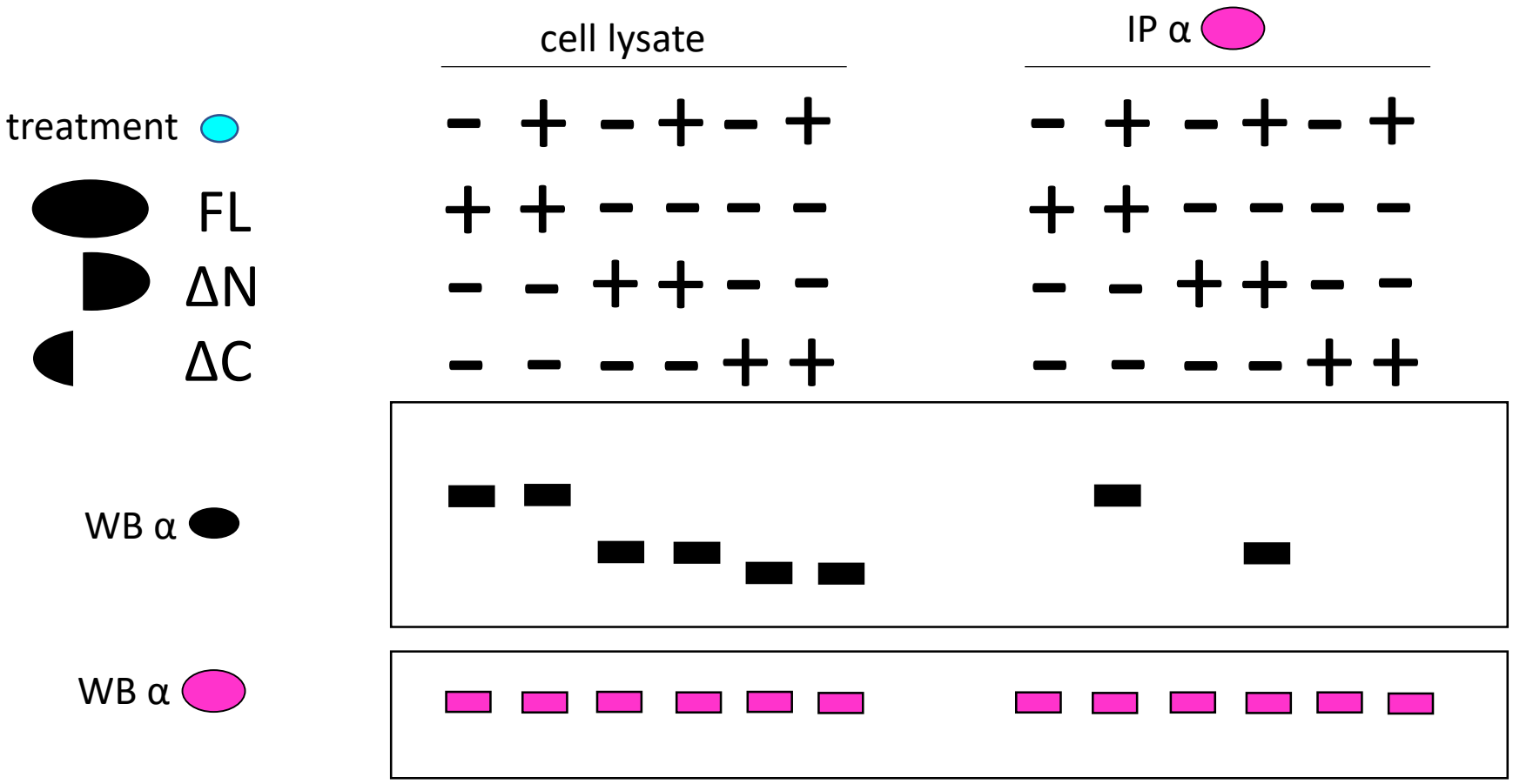


? Which strategy would you use to answer this question ?

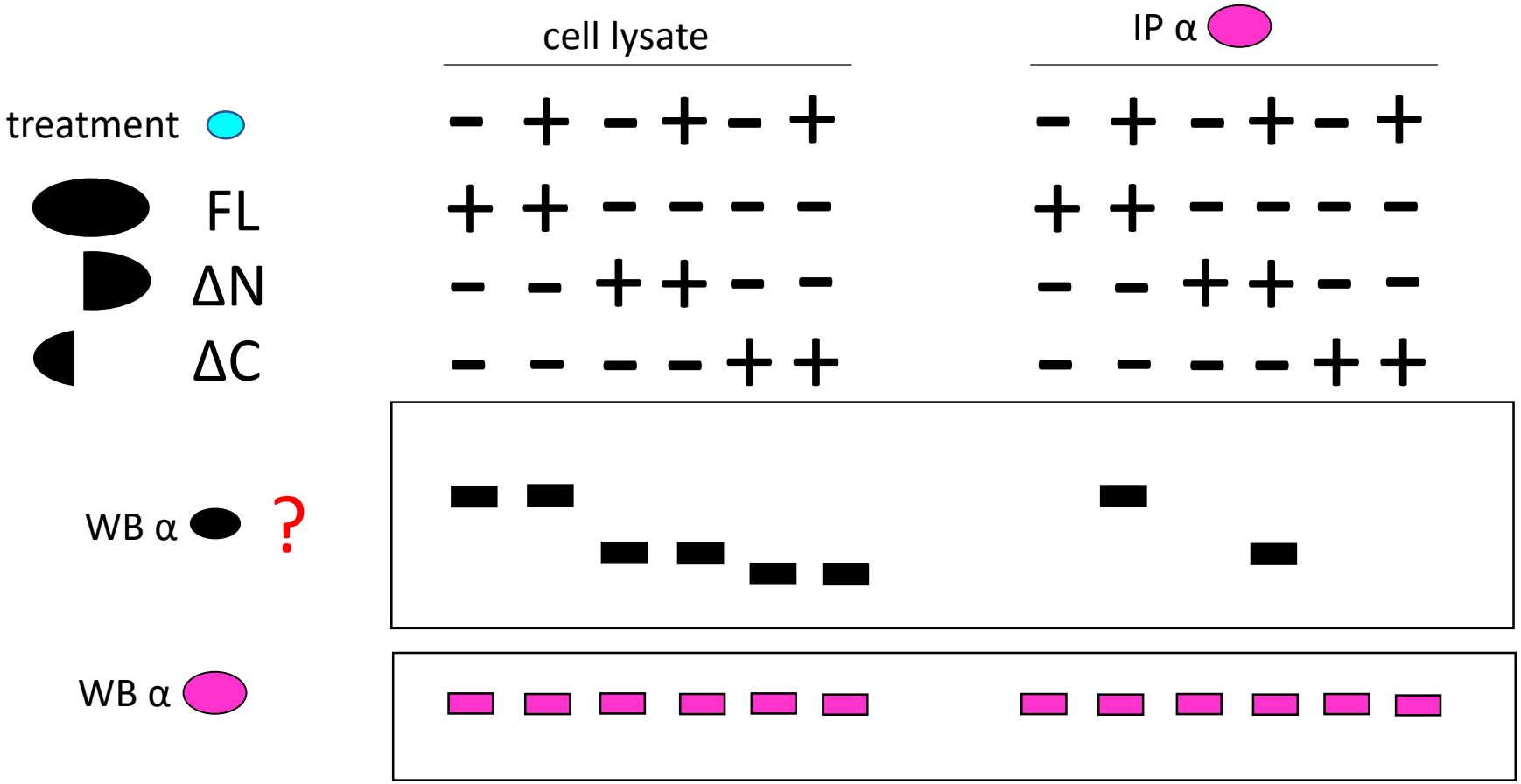
Which domain of the **BLACK** protein is necessary to interact with the **PINK** protein?



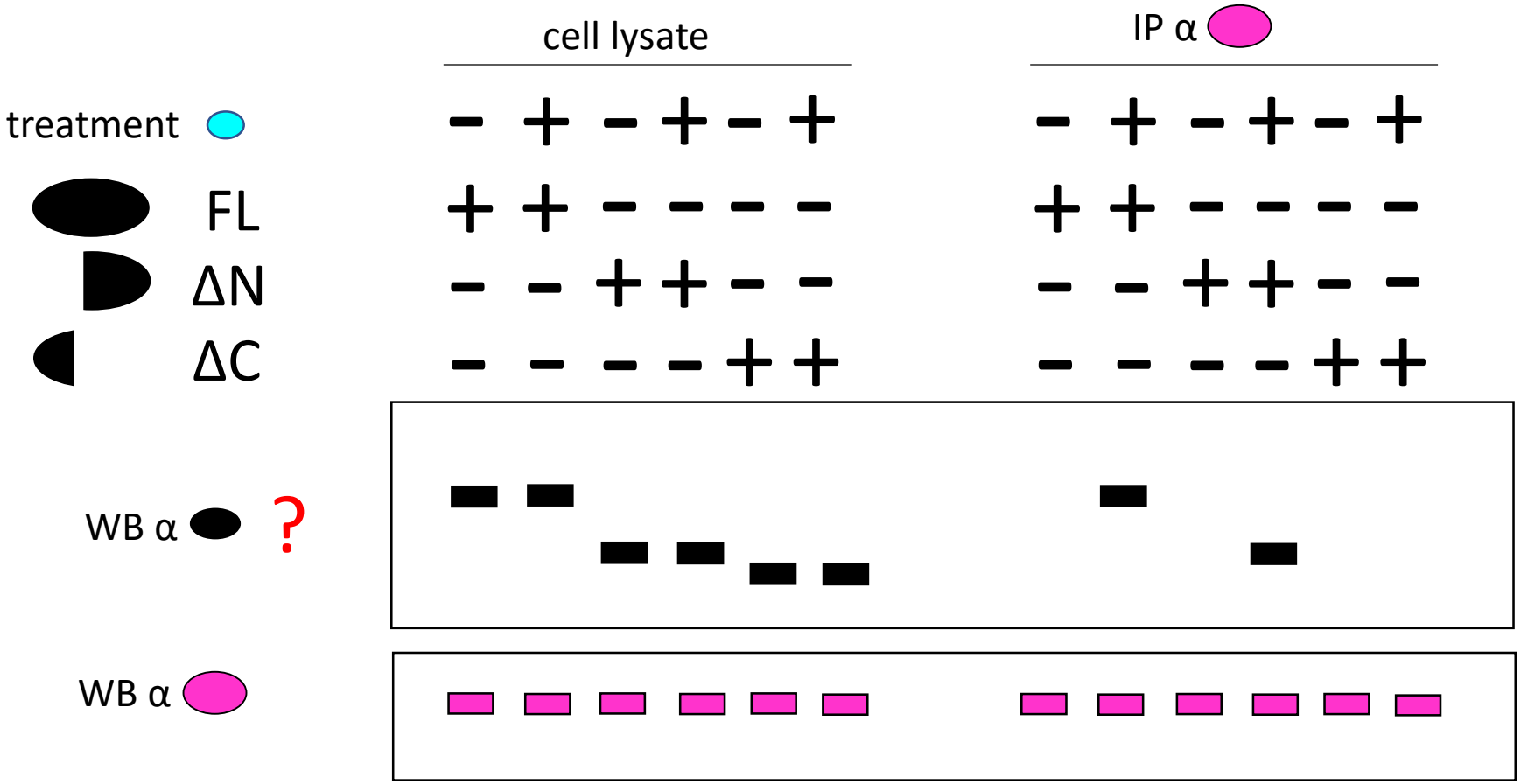
Which domain of the **BLACK** protein is necessary to interact with the **PINK** protein?



Which domain of the **BLACK** protein is necessary to interact with the **PINK** protein?



Which domain of the **BLACK** protein is necessary to interact with the **PINK** protein?



- How can I discriminate between endogenous and exogenous proteins?
- How can I identify the protein if the antigen recognized by the primary antibody is lost?

TAG proteins

- to discriminate between endogenous and exogenous recombinant proteins, if the endogenous protein is expressed.
- to identify the protein if the antibody for my protein of interest is not available.
- to identify the recombinant protein if the antigen recognized by the primary antibody is lost (deletions, mutations).

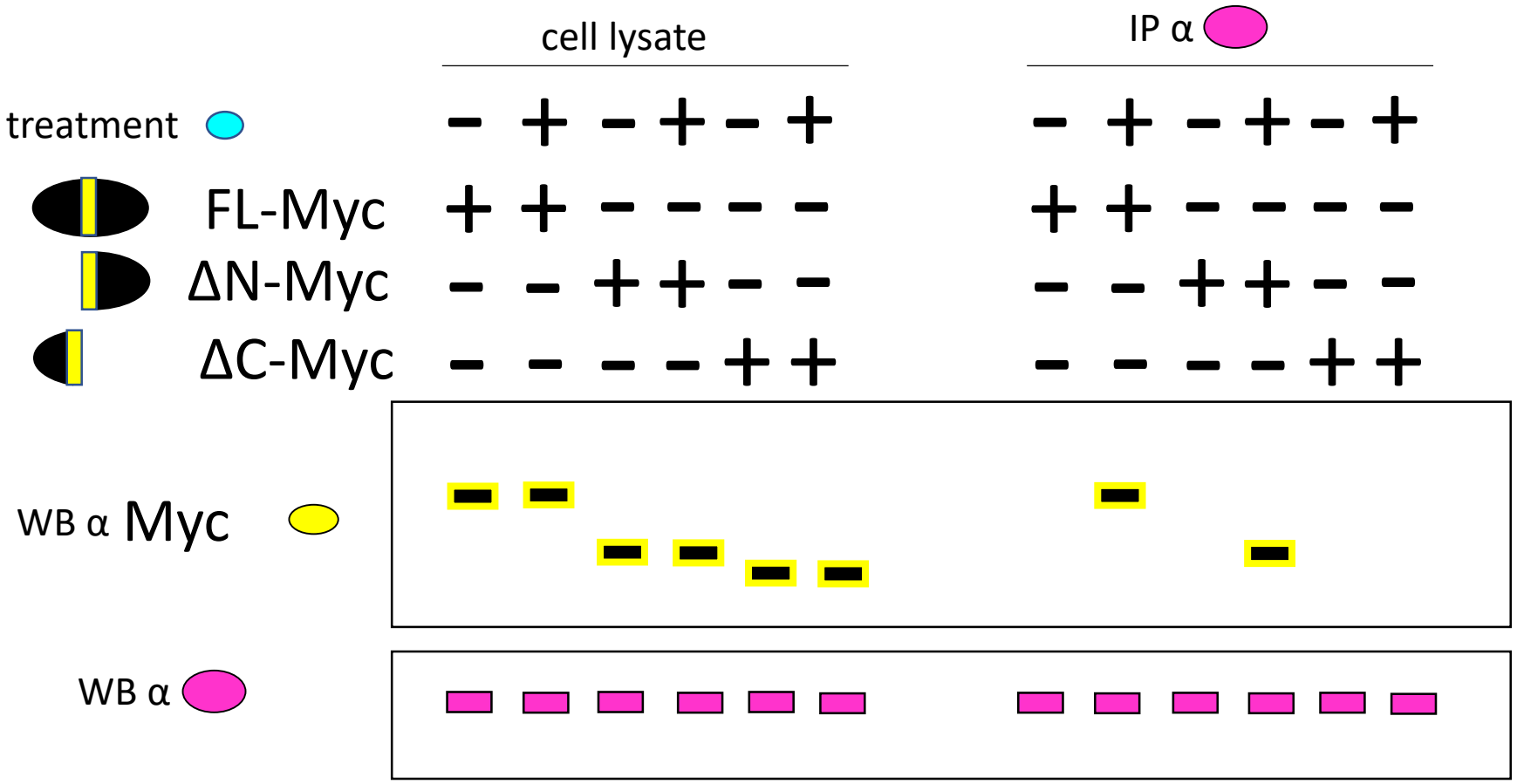
TAG proteins

• **Myc tag** –short peptide sequence (**EQKLISEEDL**) derived from the c-myc gene product and recognized by numerous commercial antibodies. It can be added to a protein using recombinant DNA technology and may be used for affinity chromatography and for isolating protein complexes with multiple subunits.

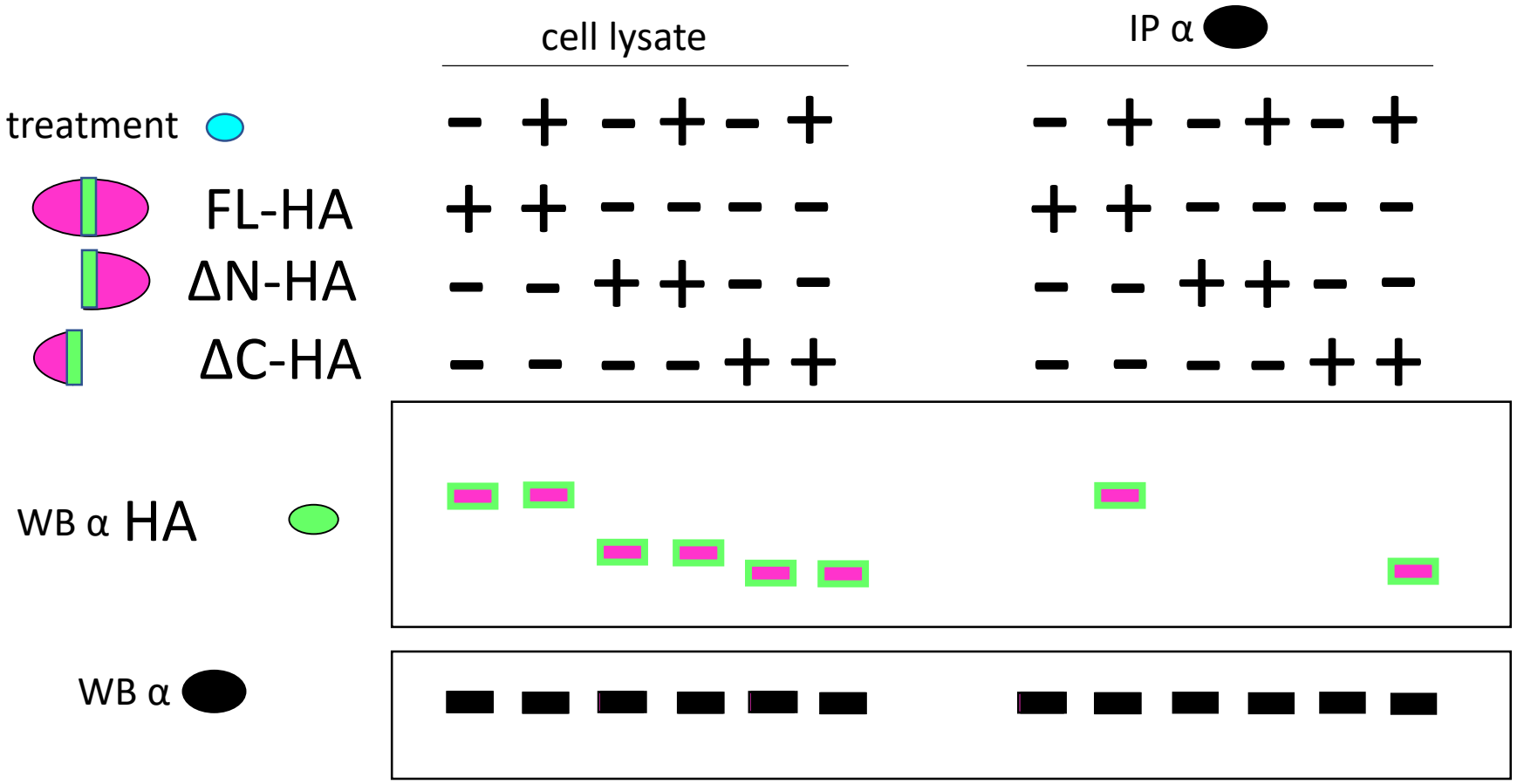
• **Human influenza hemagglutinin (HA) tag** – The HA tag is a peptide sequence (**YPYDVPDYA**) derived from the surface glycoprotein that facilitates the ability of the influenza virus to infect its host and is recognized by numerous commercial antibodies. It is used as a general epitope tag in expression vectors and is useful for the detection, isolation, and purification of proteins.

• **FLAG tag** – The FLAG tag is a popular short peptide tag (**DYKDDDDK**) used in recombinant DNA technology and can be used for affinity chromatography and for isolating protein complexes with multiple subunits. It is recognized by numerous commercial antibodies, can be fused to the C-terminus or the N-terminus of a protein and can also be used with other affinity tags. The FLAG tag is more hydrophilic as compared to other tags in its class so they do not denature or inactivate the proteins to which they are attached.

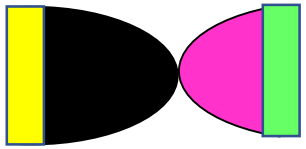
Which domain of the **BLACK** protein is necessary to interact with the **PINK** protein?



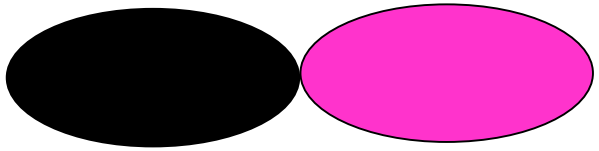
Which domain of the **PINK** protein is necessary to interact with the **BLACK** protein?



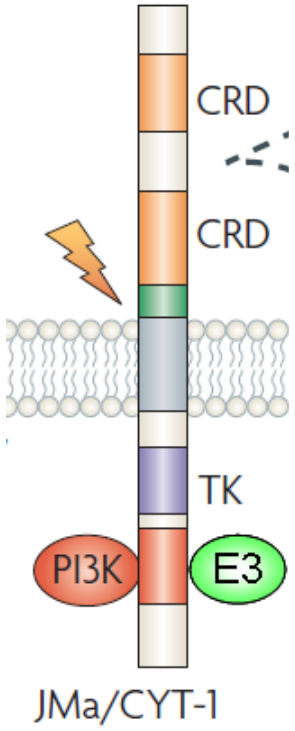
Conclusions

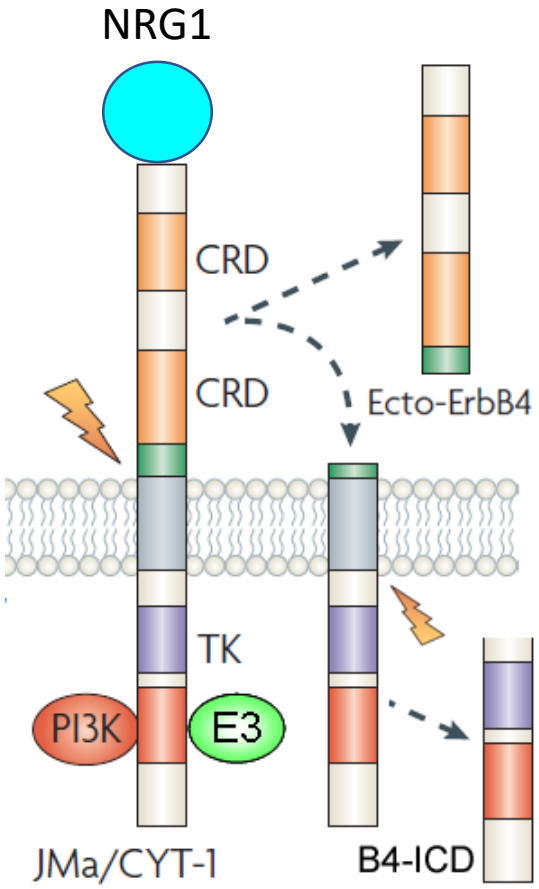
ΔN-Myc  ΔC-HA

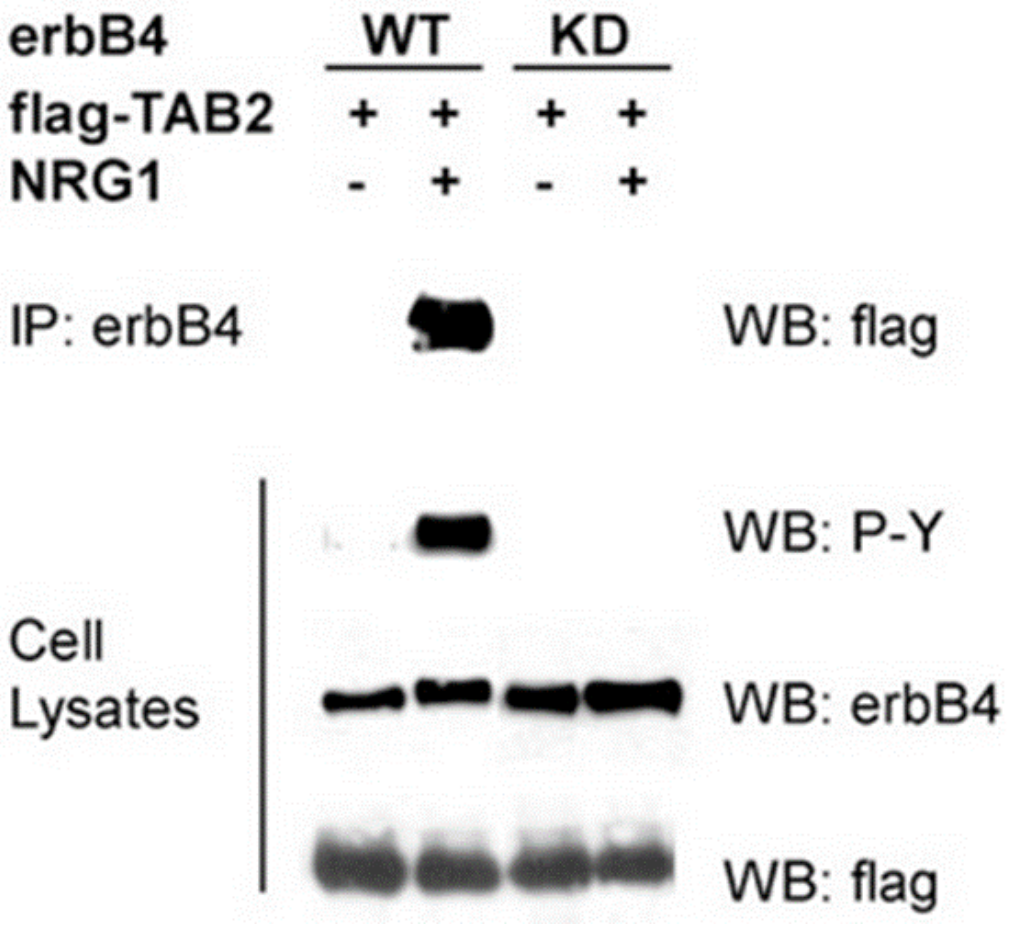
Following the treatment, the C terminus domain of the **BLACK** protein interacts with the N terminus domani of the **PINK** protein.



ErbB4


CRD
CRD
TK
PI3K
E3
JMa/CYT-1


NRG1
CRD
CRD
Ecto-ErbB4
TK
PI3K
E3
JMa/CYT-1
B4-ICD



- 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, immunoprecipitaed 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.

wooclap

Panel 5