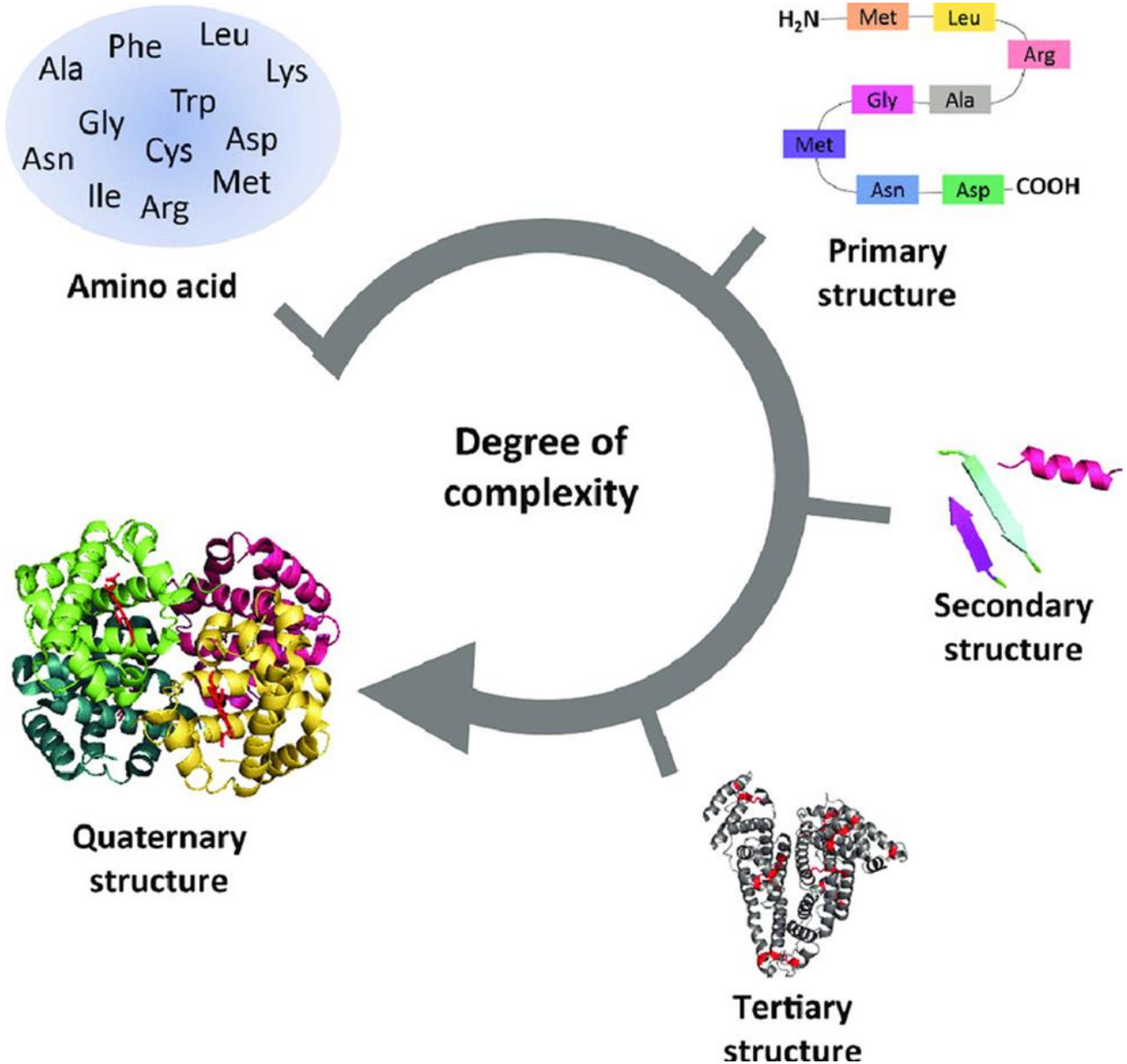
Biological Networks

Maggie Chrostowski and
Brooke Fuerstenau

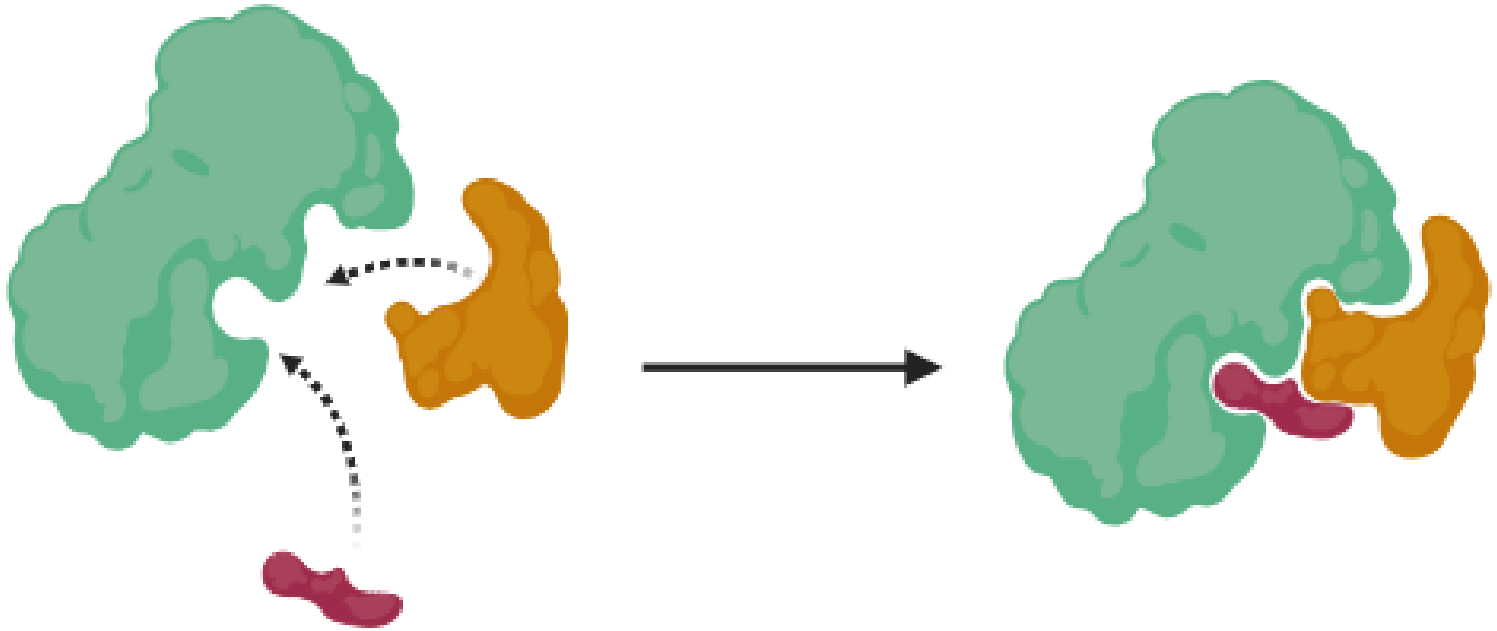
cupcake art by Ahna Skop!

What is a protein?



(Facci, 2019)

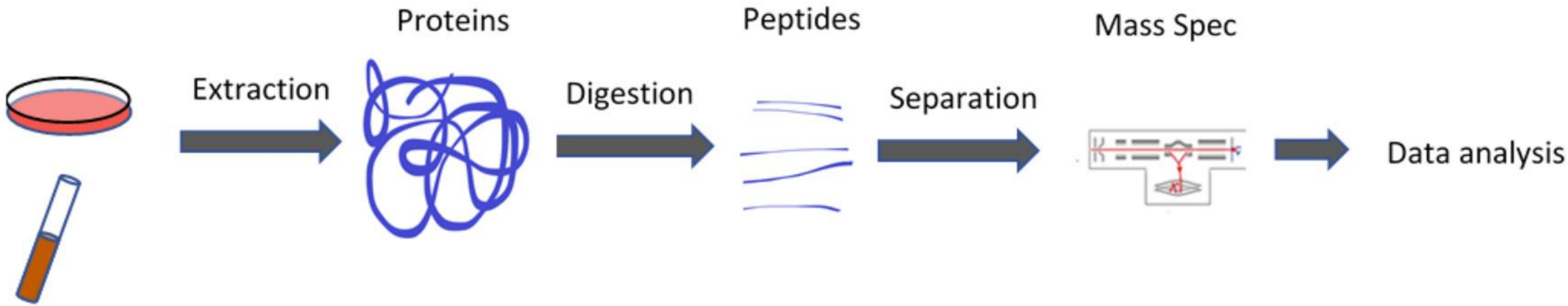
What are protein-protein interactions (PPIs)?



Proteins bind directly or indirectly to one another in many cellular processes.

(De Las Rivas & Fontanillo, 2010)

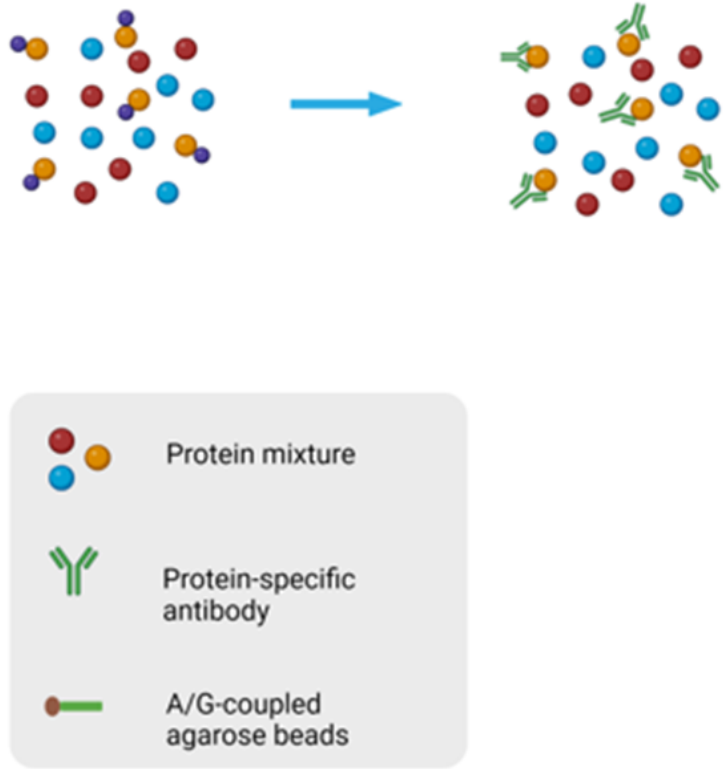
What is proteomics?



Study of isolated peptides through mass spectroscopy.

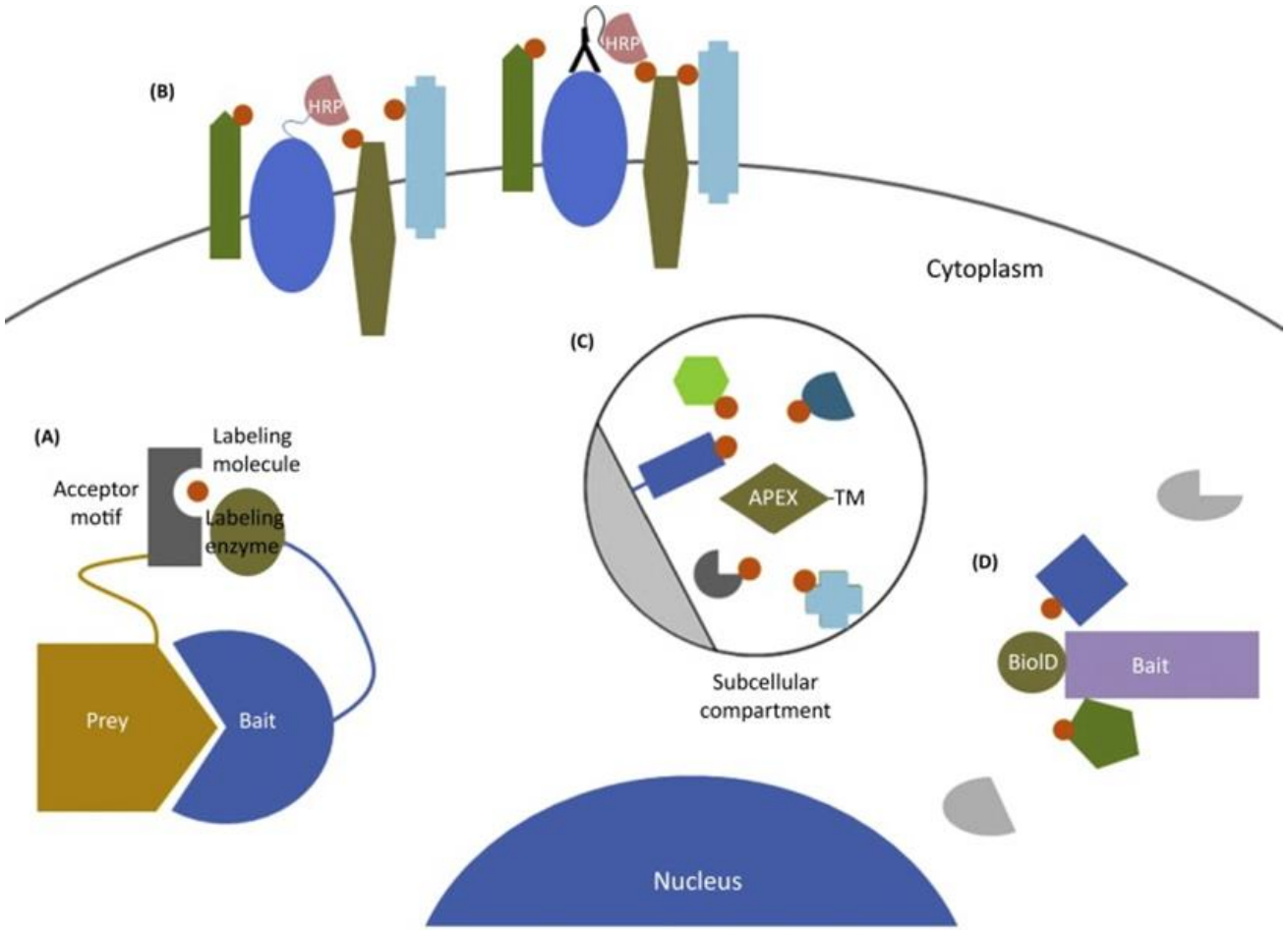
(University of South Florida, n.d.)

1) What are the ways to isolate proteins?



Immunoprecipitation
[Affinity Purification]

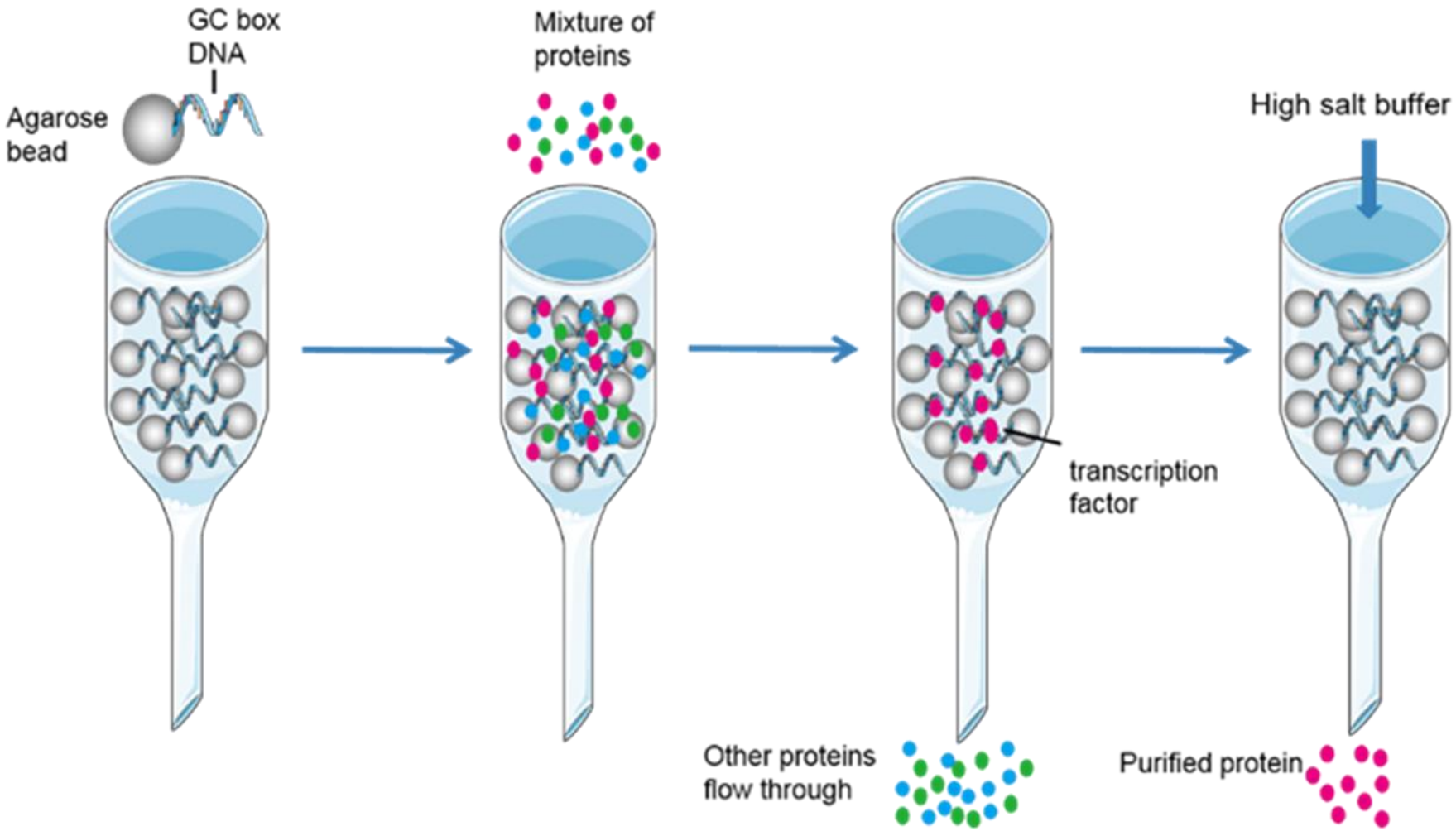
(Fhearraigh, 2021)



Proximity Dependent Labeling

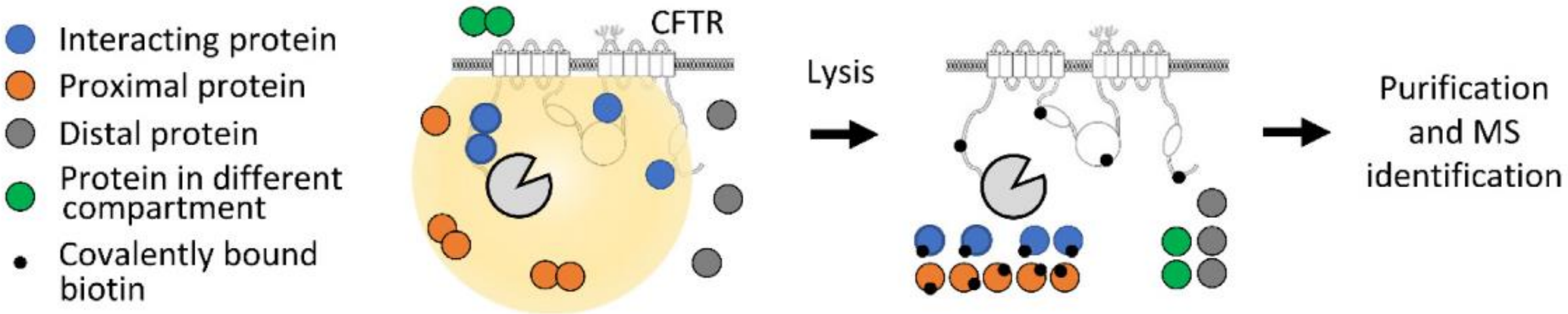
(In Kim & Roux, 2016)

1) What is Affinity Purification (AP)?



(Creative BioMart, n. d.)

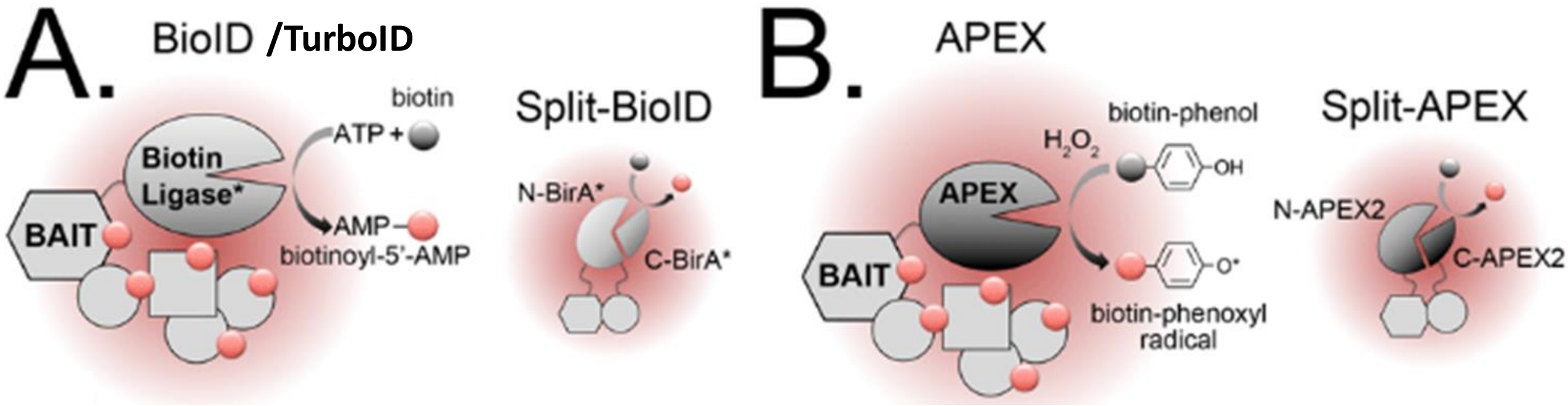
1) What is Proximity Dependent Labeling?



It uses enzymes that construct reactive molecules to bind and label neighboring proteins.

(Chevalier et al., 2022)

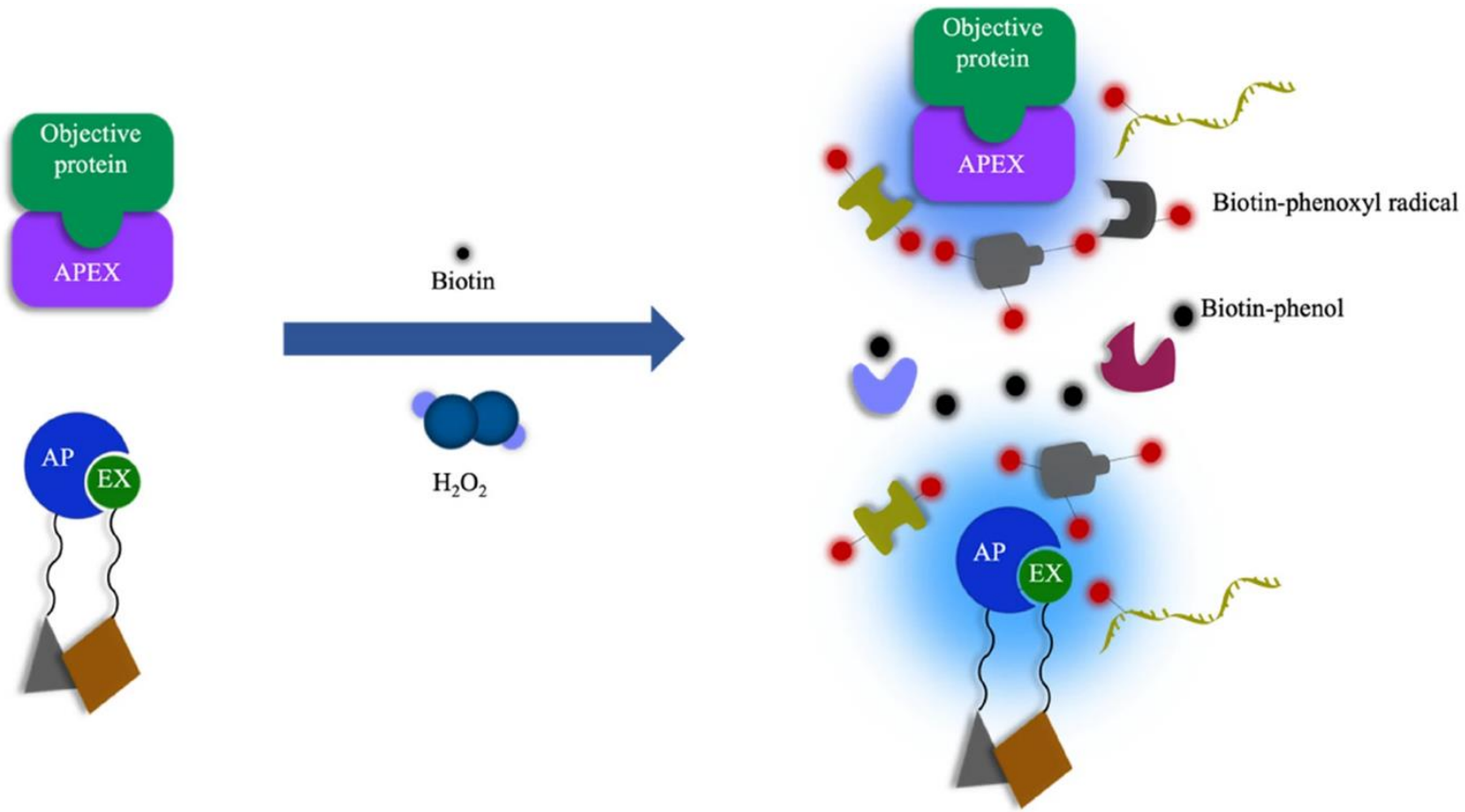
1) What are the standard proximity-dependent labeling methods?



(Cecchetelli, 2019)

1) How does APEX work?

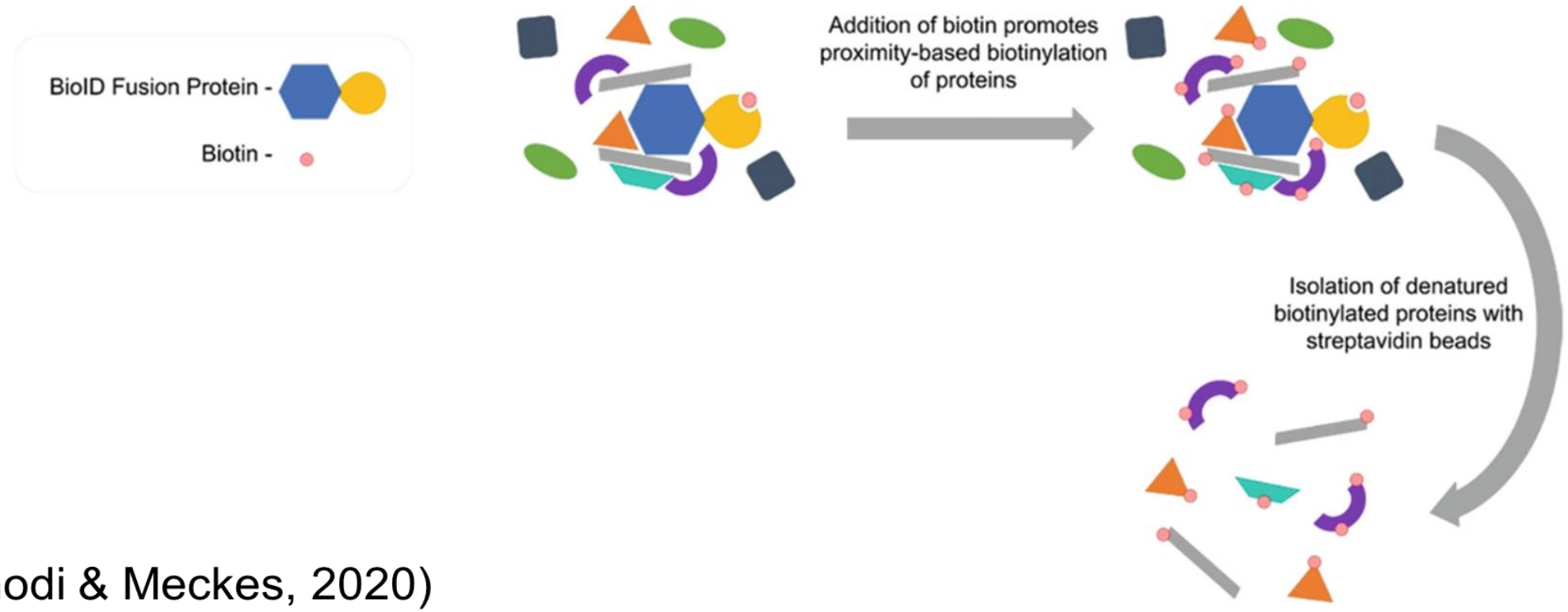
Enzyme	Type	Size (kDa)	Labelling time	Substrate	Target
APEX2	Peroxidase	28	1 min	Biotin-phenol + H_2O_2	Tyr, Trp, Cys, His



(Gou et al., 2023)

1) How does BioID work?

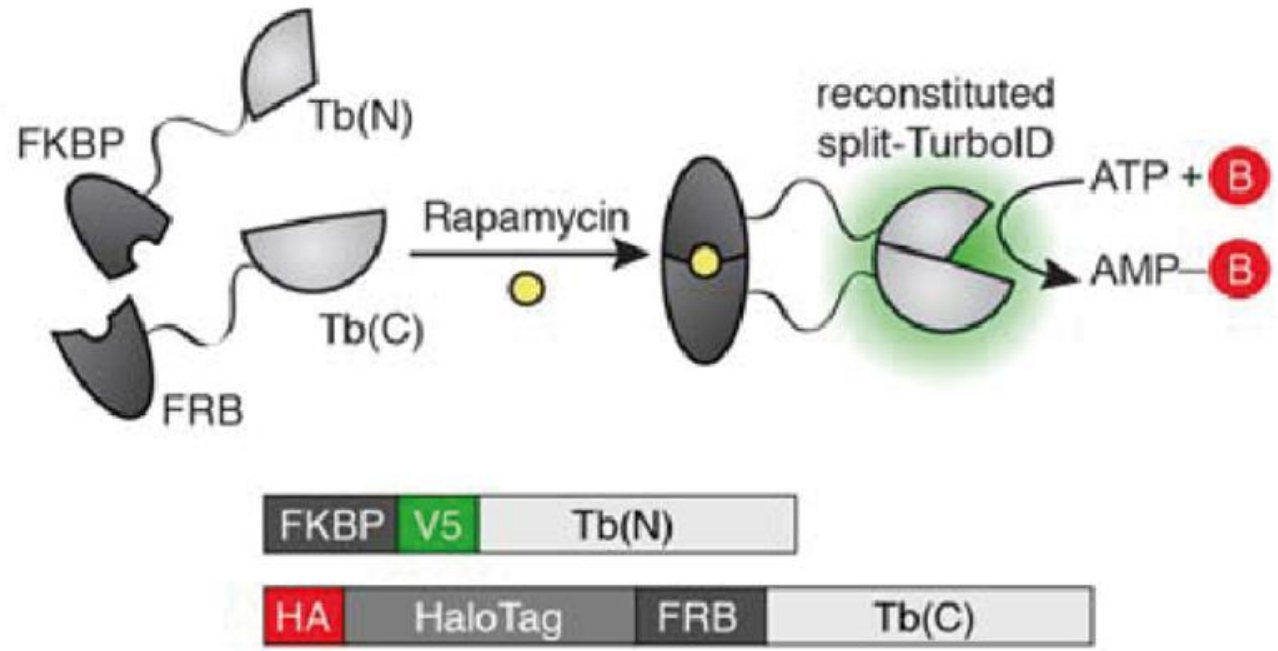
Enzyme	Type	Size (kDa)	Labelling time	Substrate	Target
BioID	Biotin ligase	35	18 h	Biotin	Lys



(Cherrathodi & Meckes, 2020)

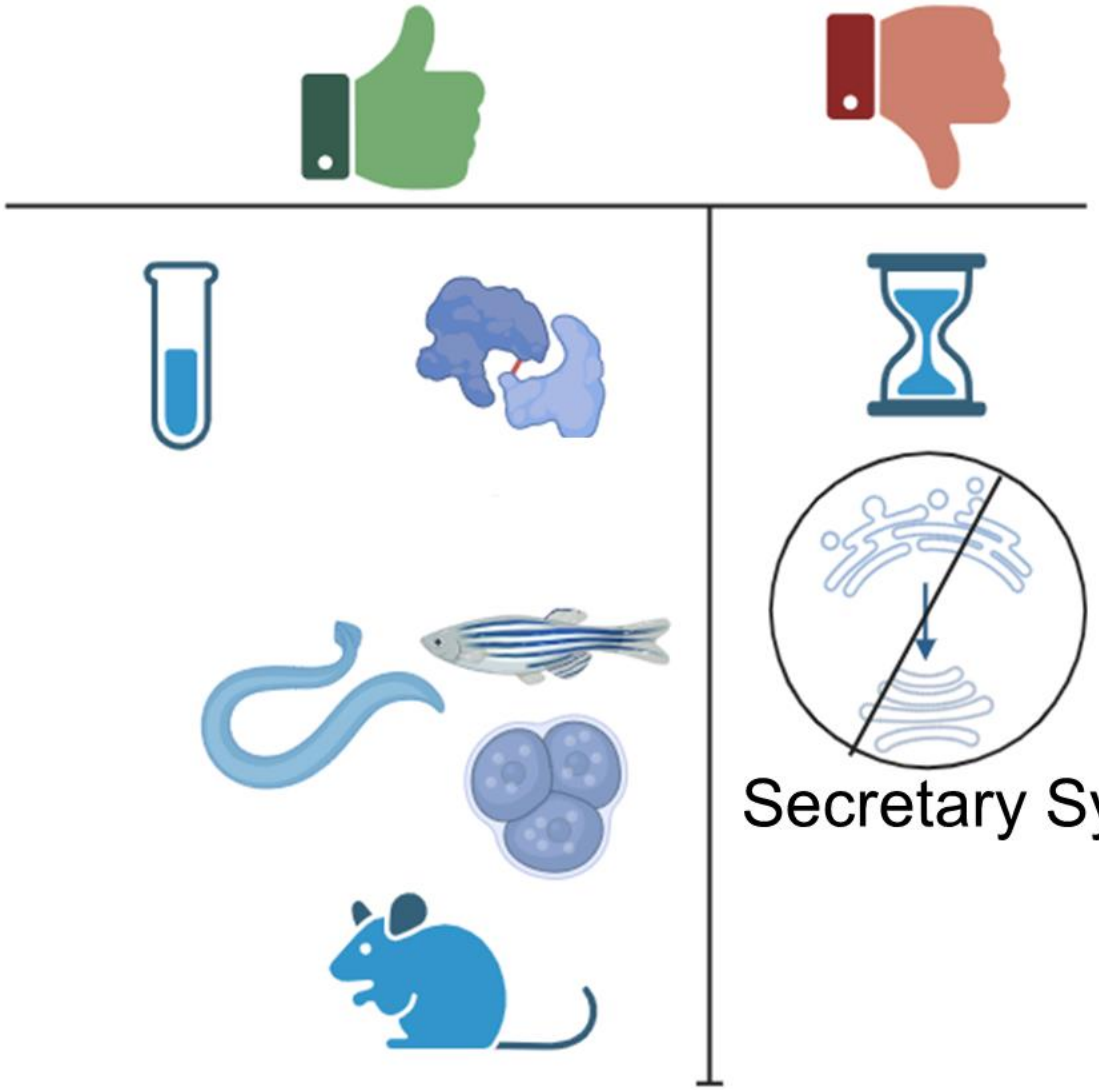
1) How does TurboID work?

Enzyme	Type	Size (kDa)	Labelling time	Substrate	Target
TurboID	Biotin ligase	35	10 min	Biotin	Lys



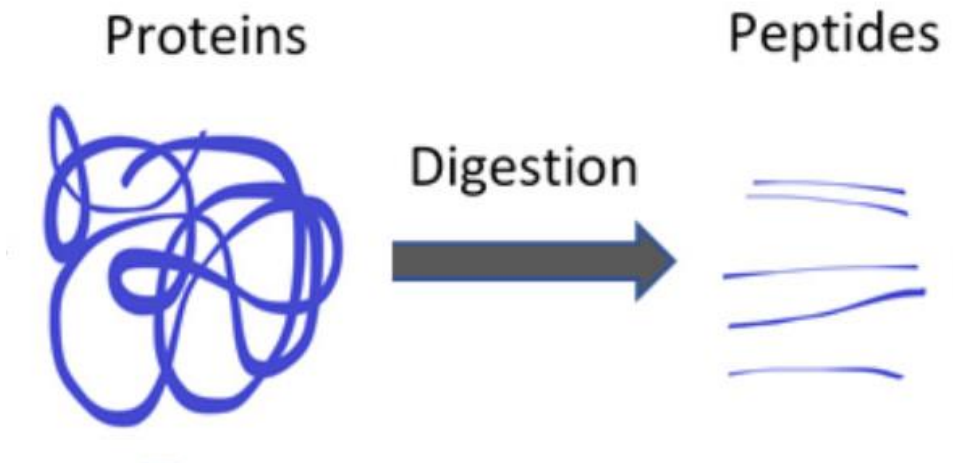
(Cho et al., 2020)

1) What are the pros and cons of using BioID and TurboID?



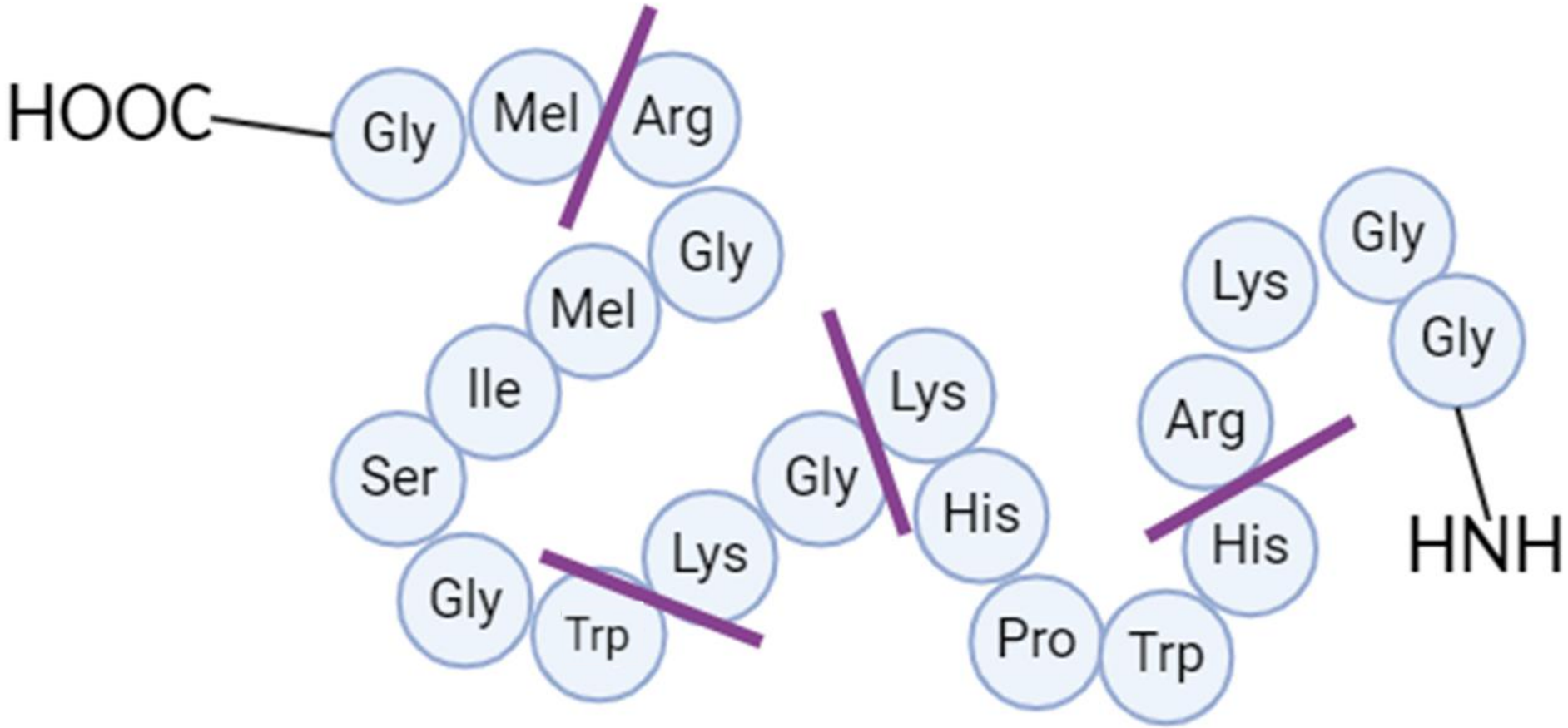
(Varnaitė & MacNeill, 2016)

Step 2: Digestion

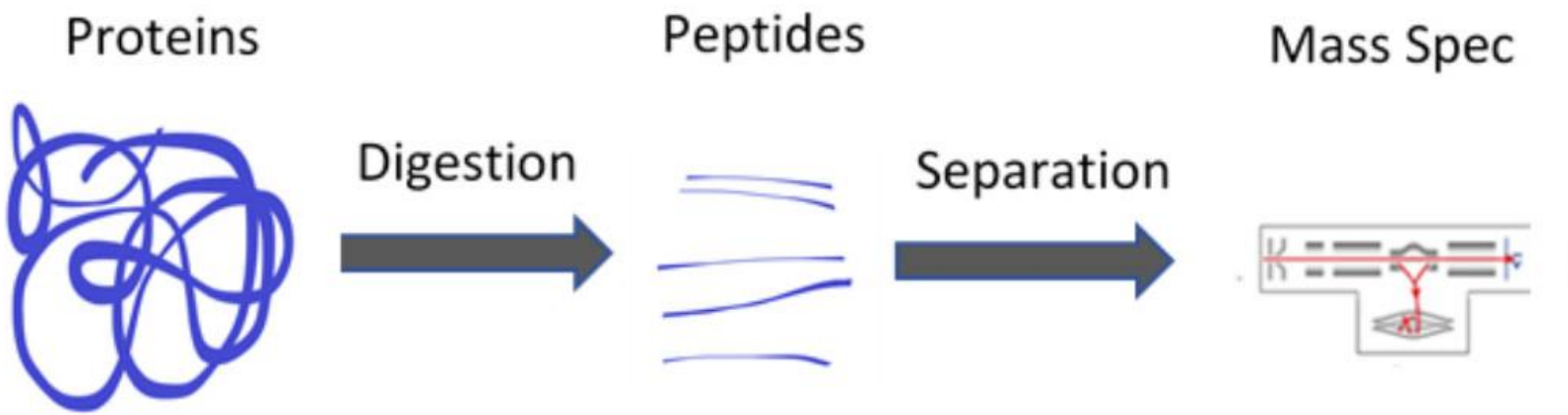


(University of South Florida, n.d.)

2) How does Trypsin Cut proteins?

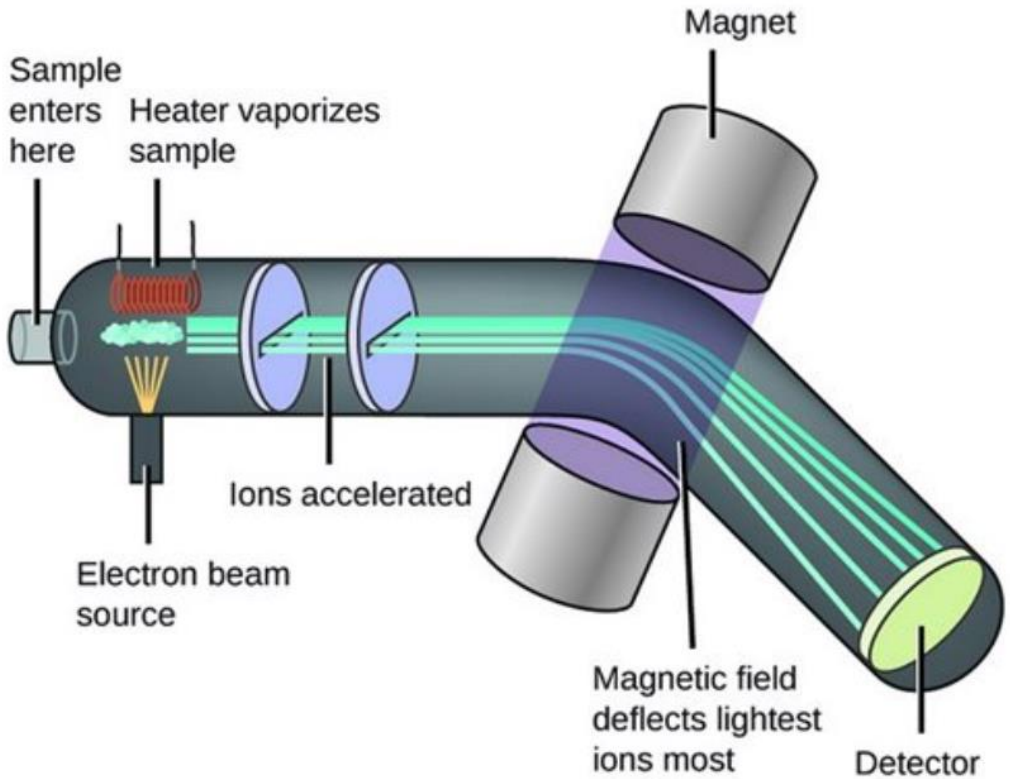


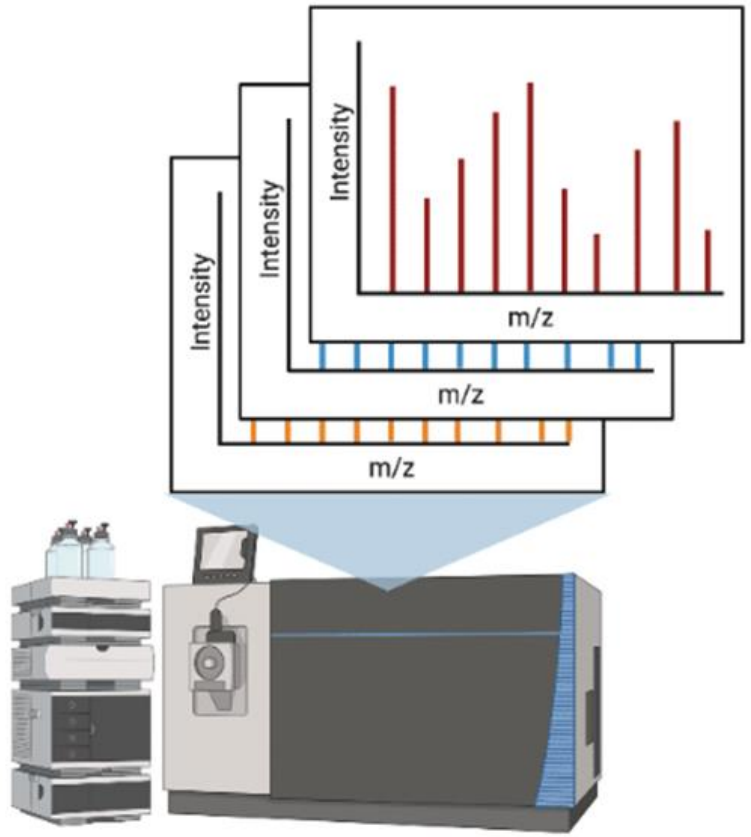
Step 3: Mass Spec



(University of South Florida, n.d.)

3) How do you perform a simple mass spectroscopy experiment?

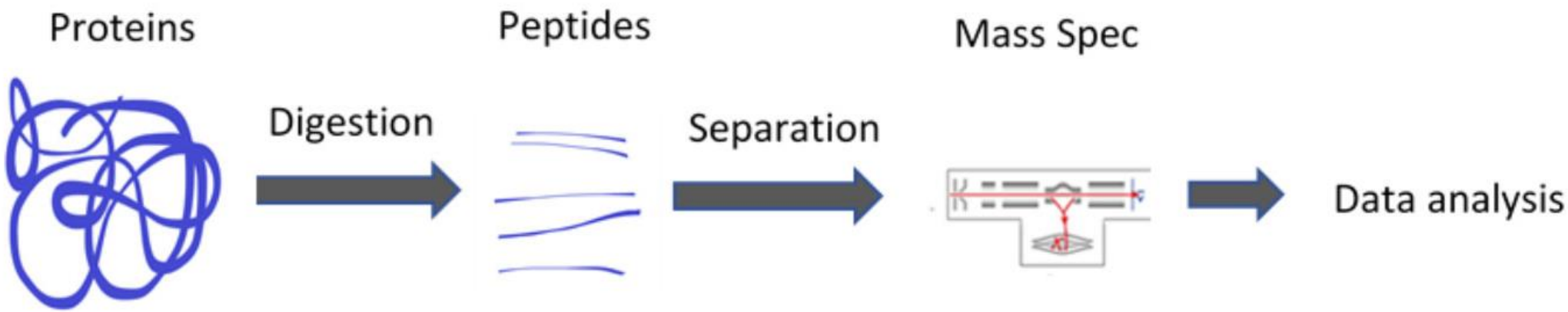




After preparing the sample, a mass spectrometer collects data to obtain separate mass/charge ratios for isolated peptides.

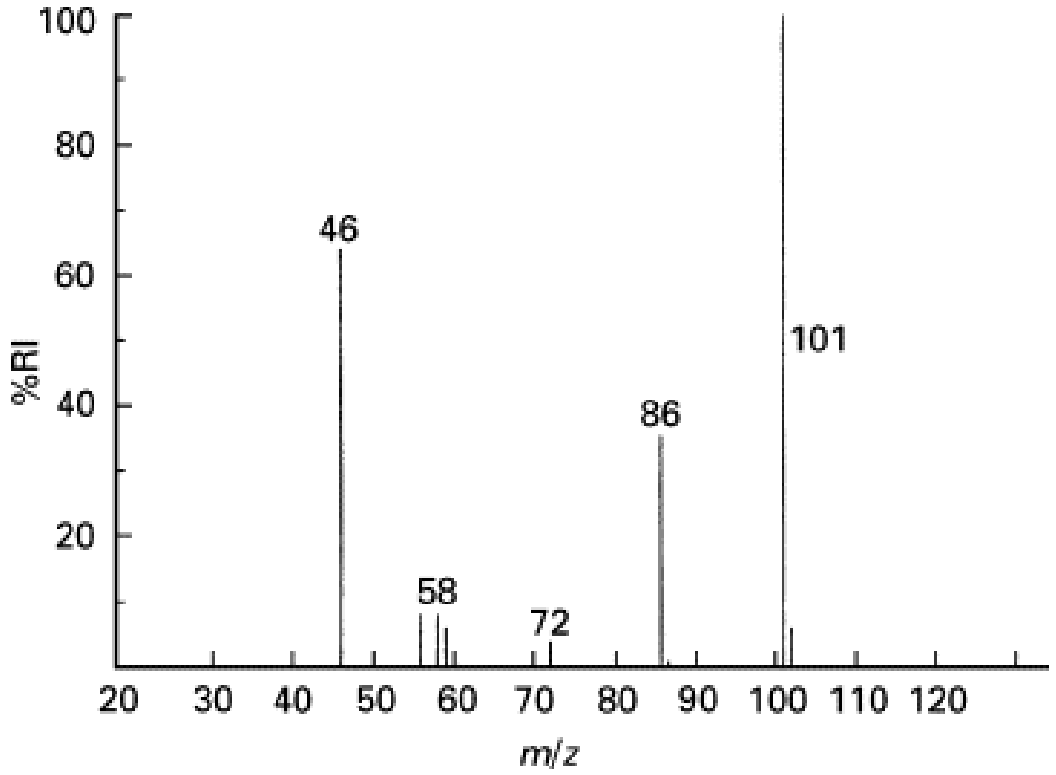
(Science Ready, 2021)

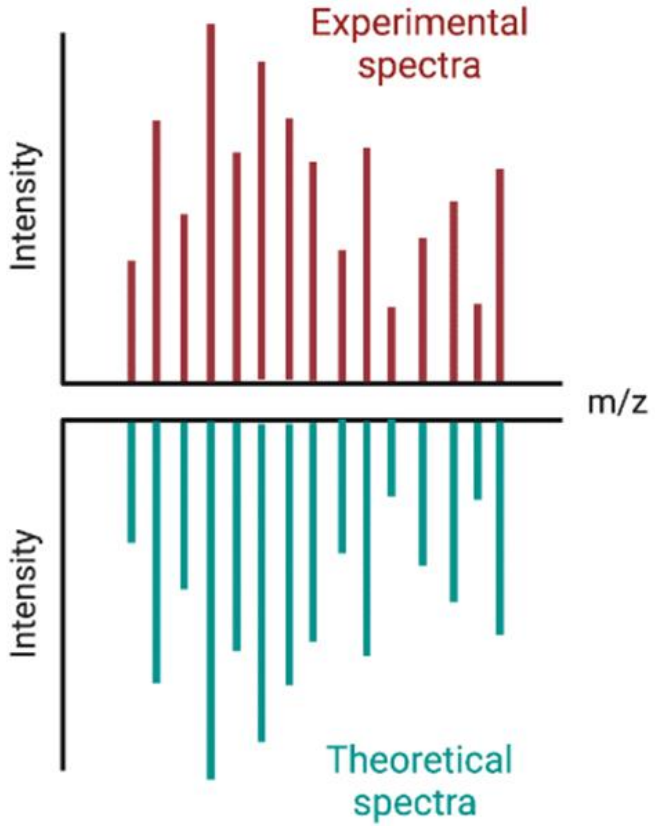
Step 4: Data Analysis



(University of South Florida, n.d.)

4) What does the mass\charge ratio mean in MS?



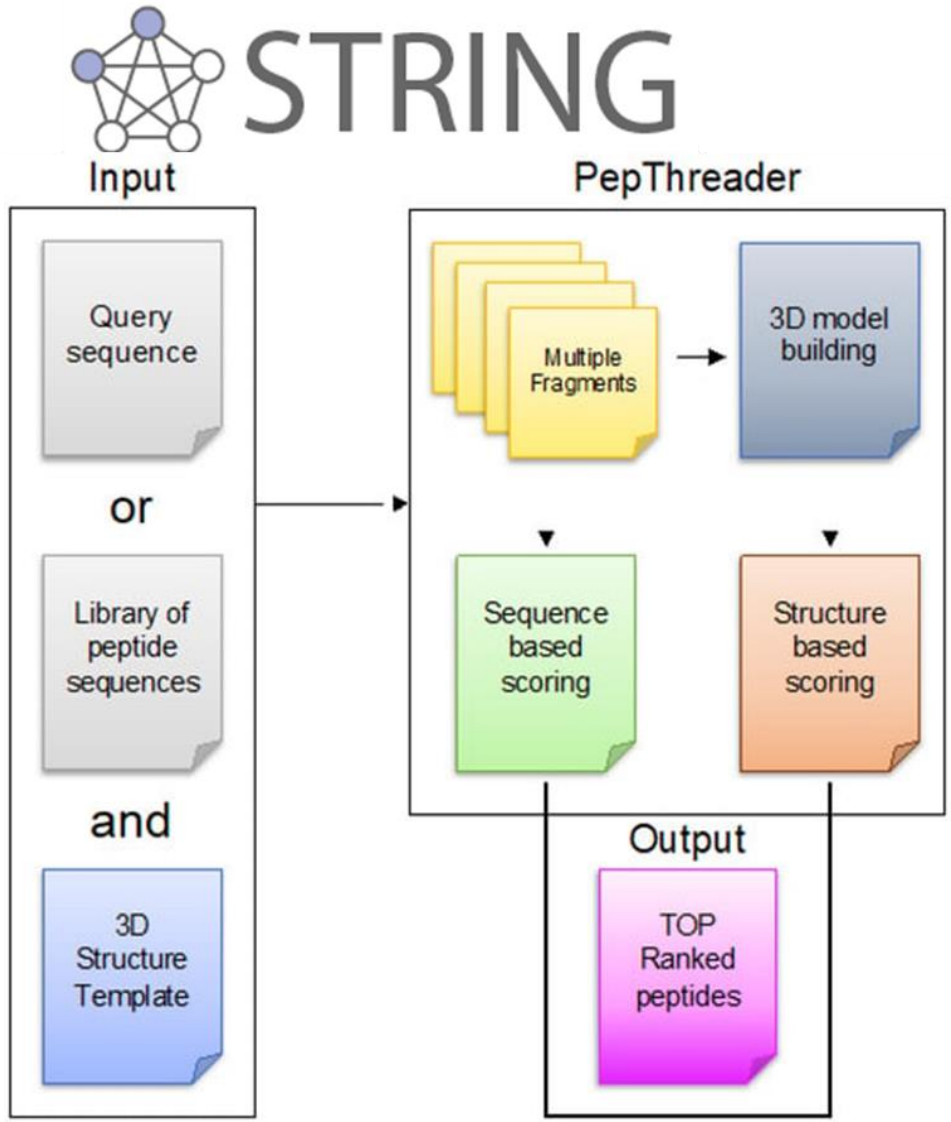


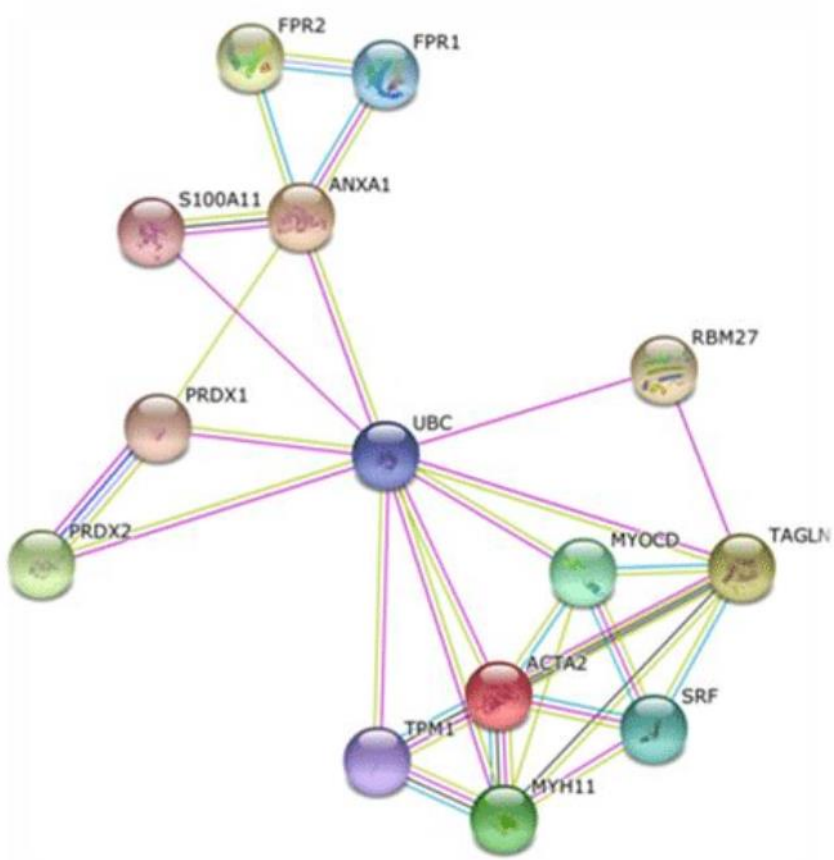
The peptide mass is divided by the charge of the protein, allowing us to identify any peptides present and cross reference with databases.

(Babele & Yadav, 2023)

4) What databases do you use to analyze data?

How do you build interaction models?

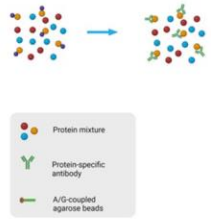




(Gasbarri, 2022)

Summary

1) What are the ways to isolate proteins?



Immunoprecipitation
[Affinity Purification]

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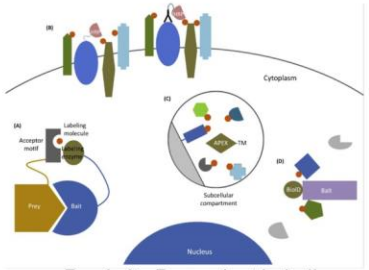
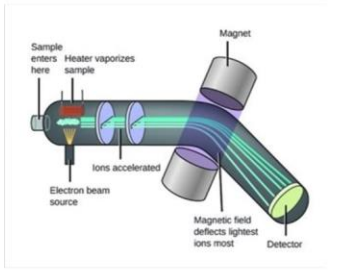


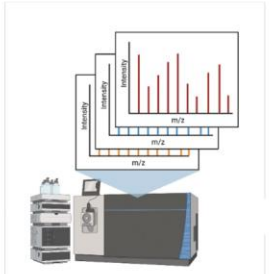
Proximity Dependent Labeling

(In Kim & Roux, 2016)

Immunoprecipitation and Proximity Dependent Labeling are two ways to isolate protein. TurboID is a biotin ligase that uses ATP to target Lysine.

3) How do you perform a simple mass spectroscopy experiment?



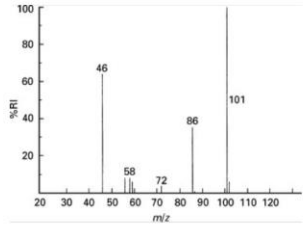


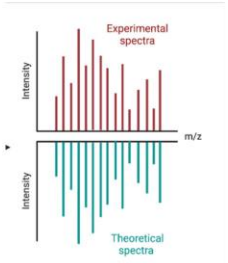
After preparing the sample, a mass spectrometer collects data to obtain separate mass/charge ratios for isolated peptides.

(Science Ready, 2021)

Mass spectroscopy takes the tagged peptides (cut-up proteins) and analyzes them through a magnetic field to get mass/charge ratio.

4) What does the mass\charge ratio mean in MS?





The peptide mass is divided by the charge of the protein, allowing us to identify any peptides present and cross reference with databases.

(Babele & Yadav, 2023)

The mass/charge ratio allows you to determine the proteins present by comparing the experimental data to the theoretical data found in a database. To do this, you can use UniProt, NCBI, nextprot, or EMBL.


Questions?

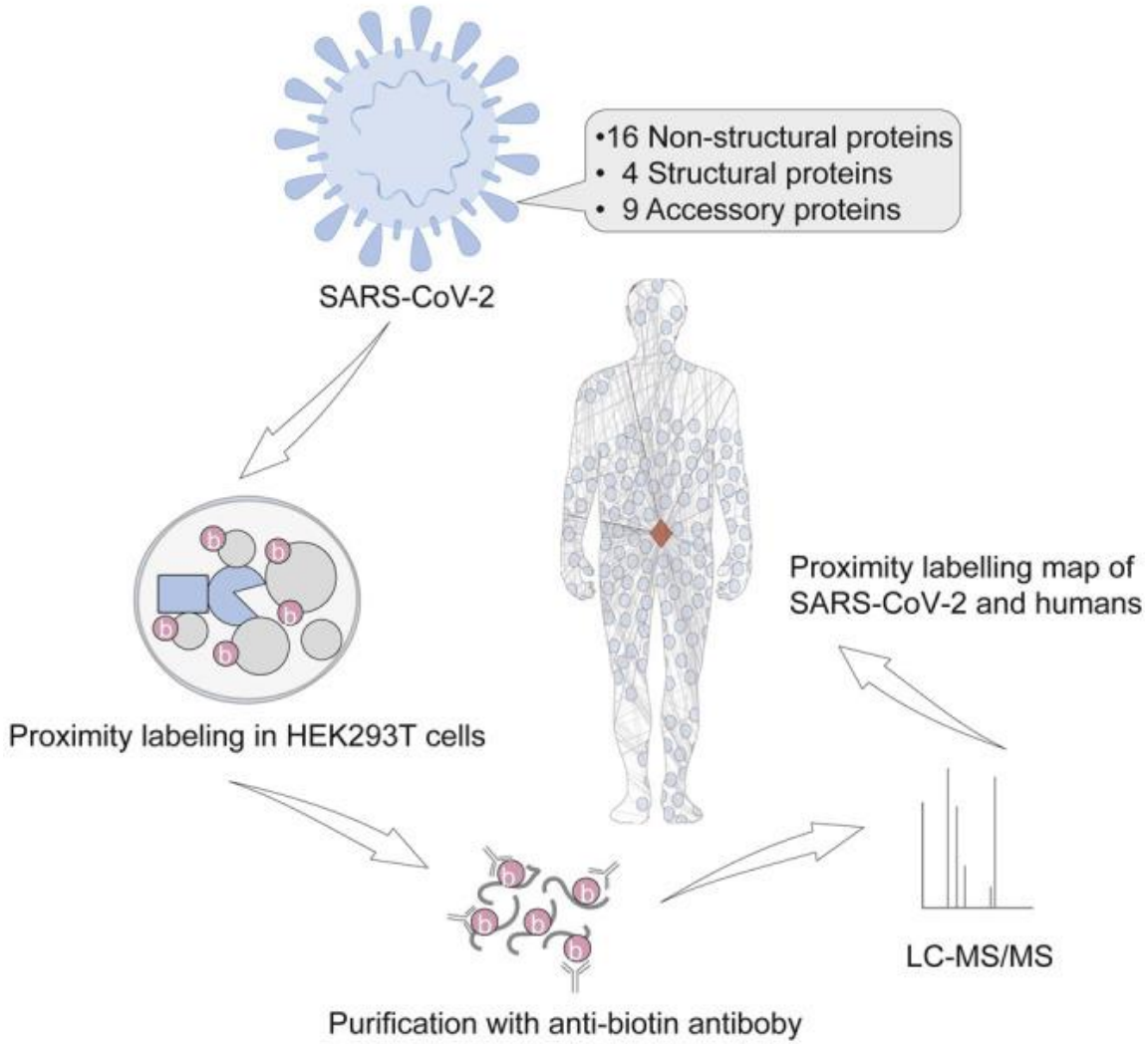
“An antibody-based proximity labeling map reveals mechanisms of SARS-CoV-2 inhibition of antiviral immunity”

Authors :

Yuehui Zhang

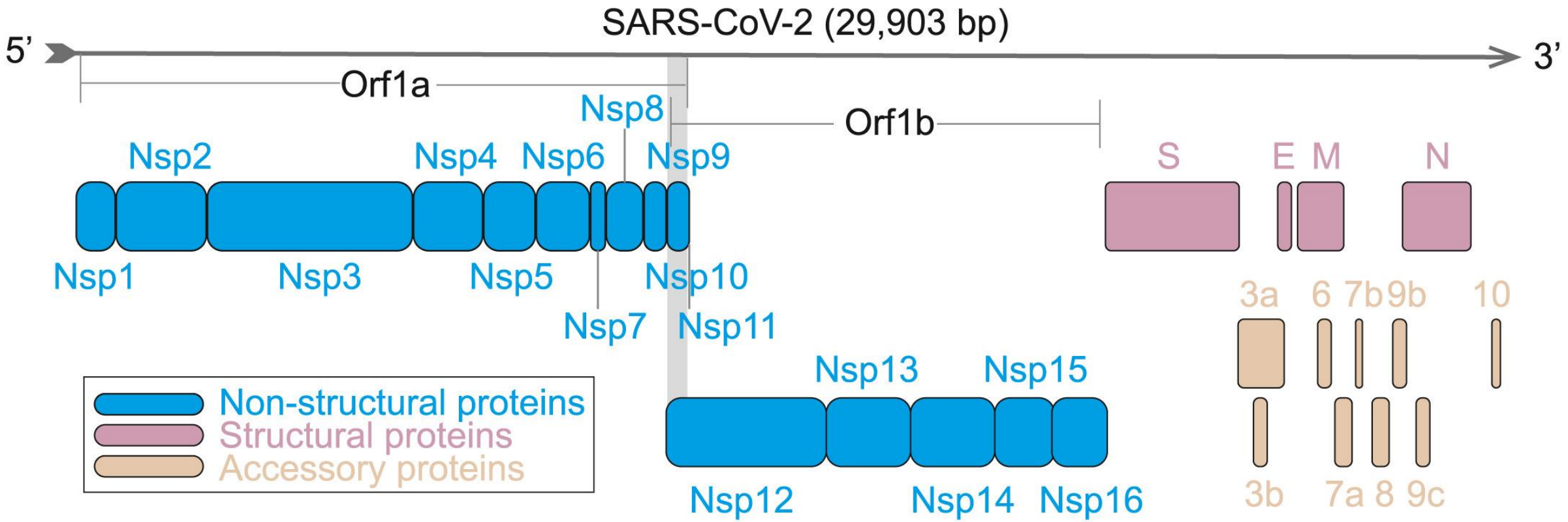
Limin Shang

How can TurboID be used in SARS-CoV-2 research?



(Zhang et al., 2021)

What proteins are encoded by SARS-CoV-2?



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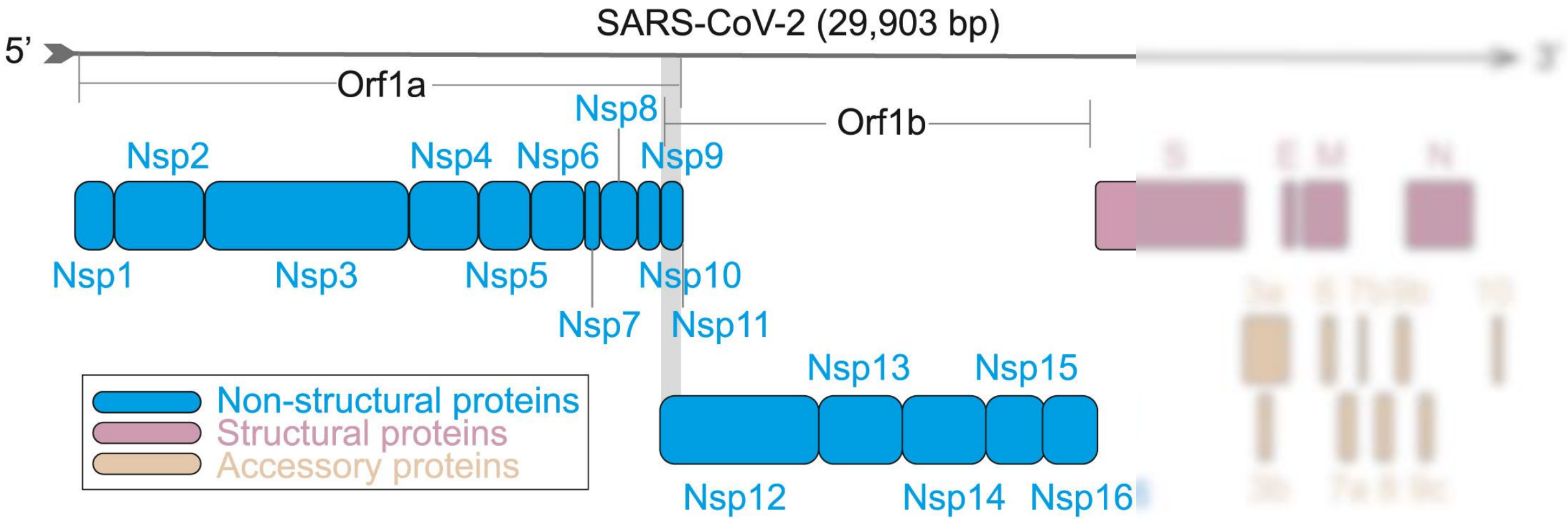

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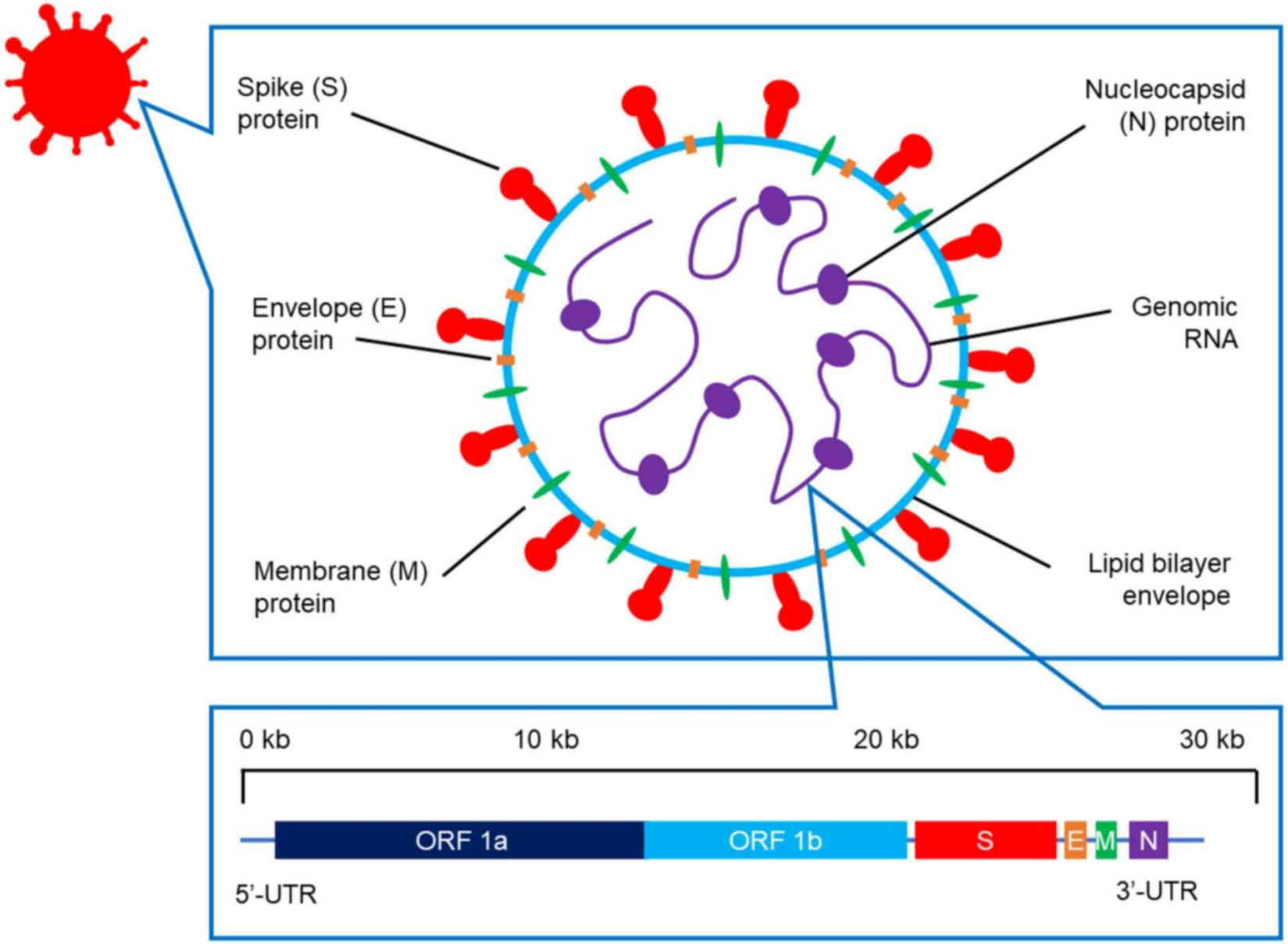
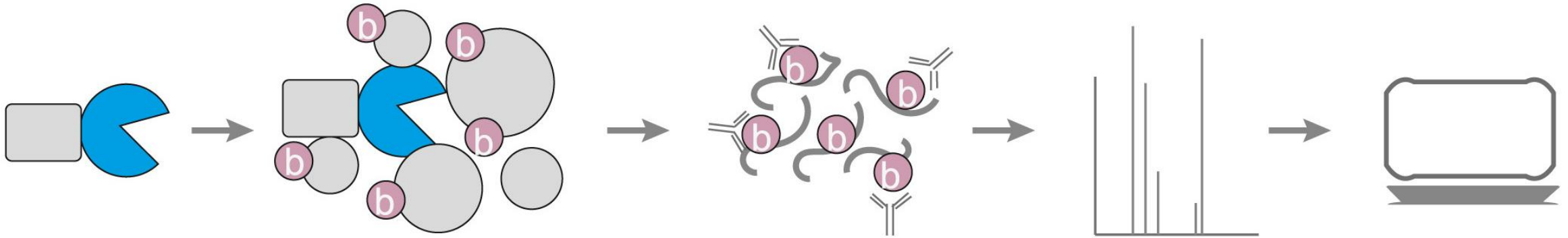
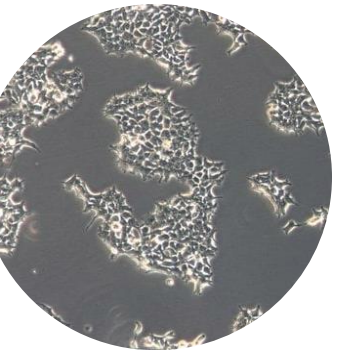


(Mammas et al., 2022)

How was TurboID used to identify proximal proteins?

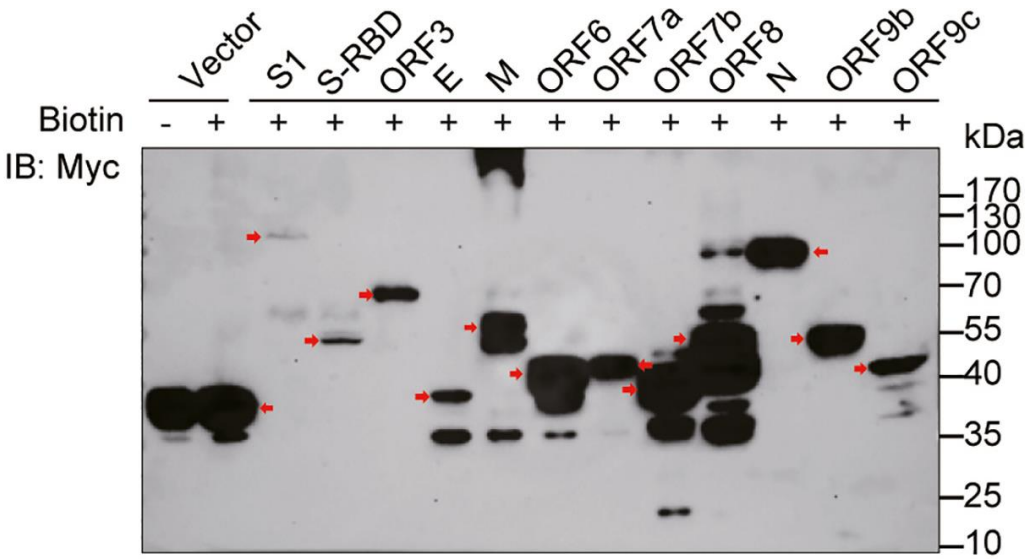


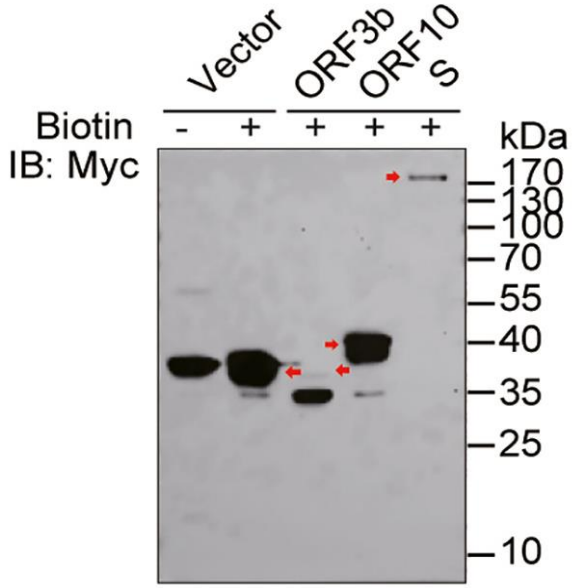


HEK293T cells

(Zhang et al., 2021)

What protein expressions were detected by western blot assays?





Indicates expression of target proteins

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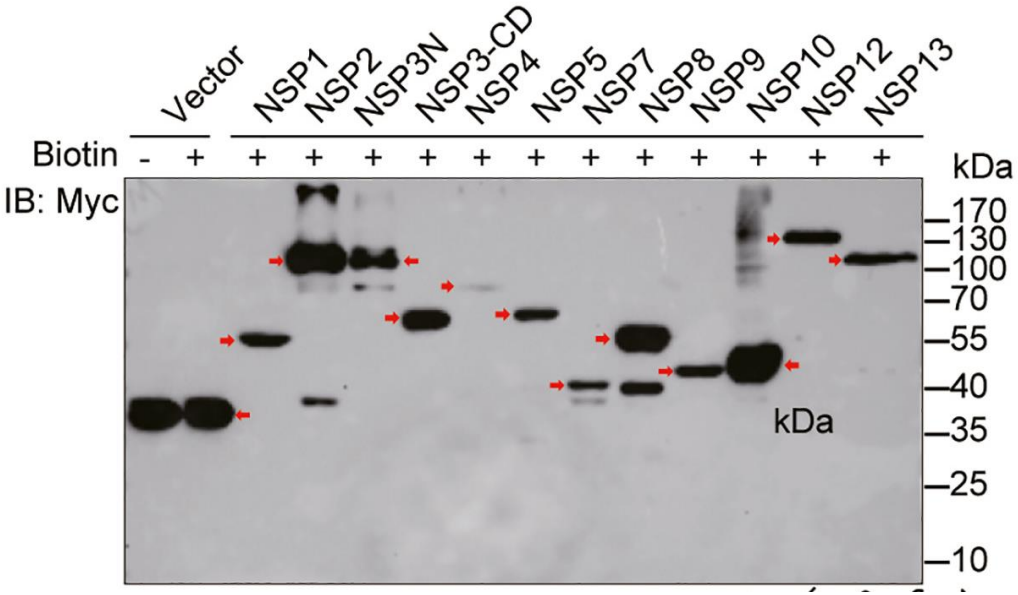

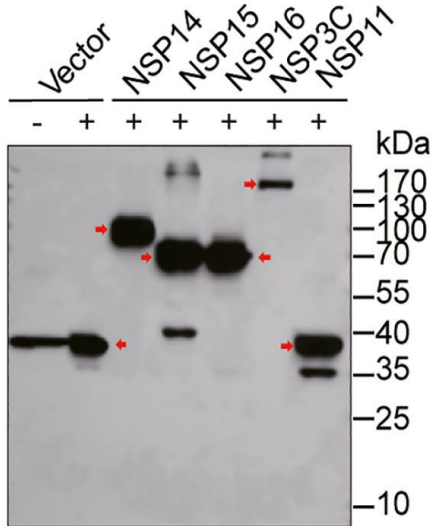

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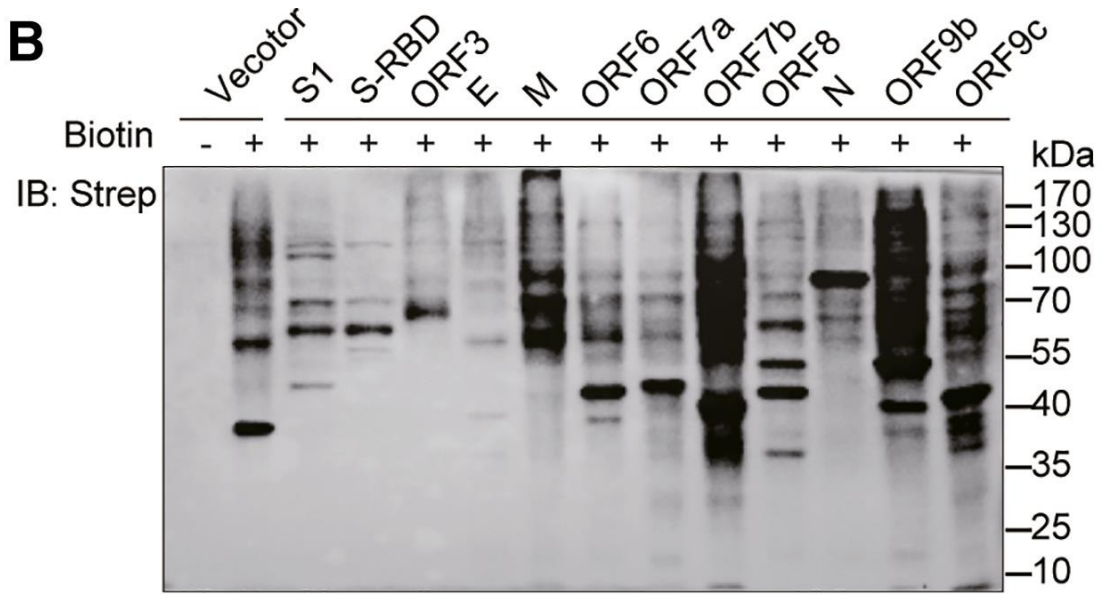

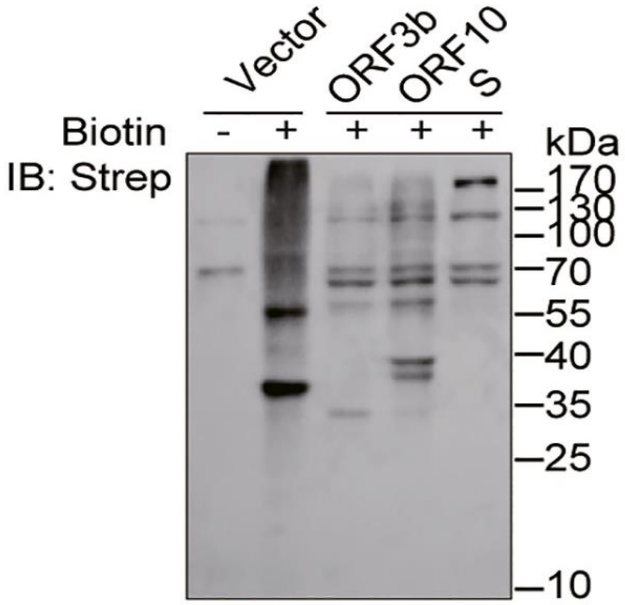

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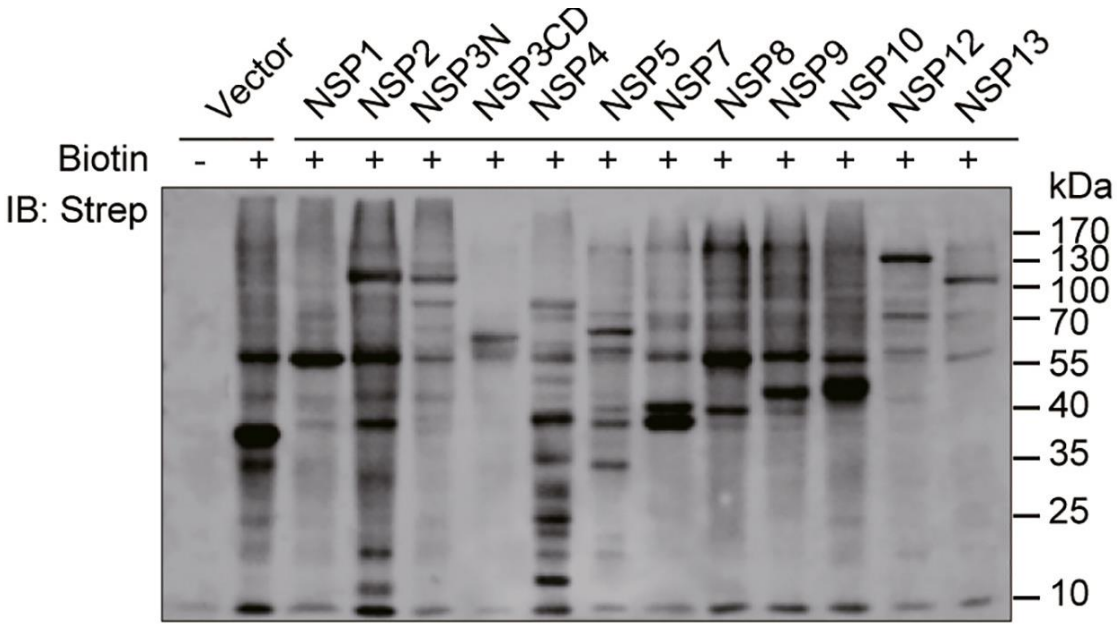
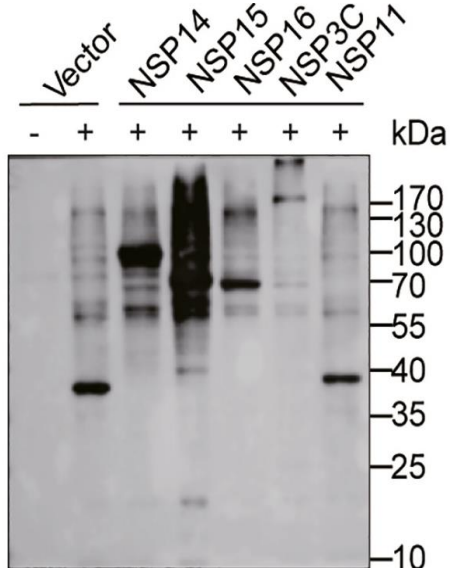
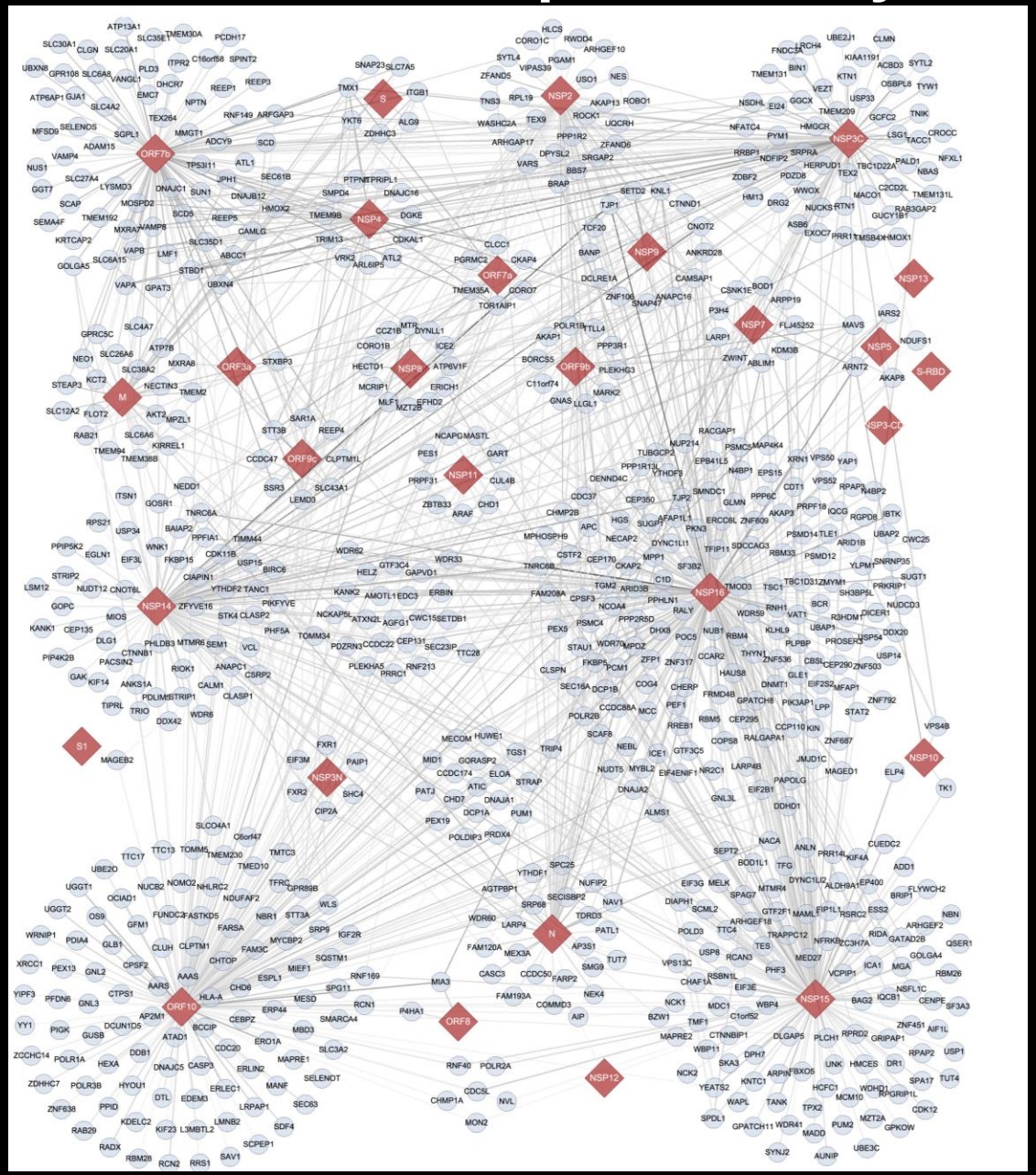




How does mass spectrometry confirm these identified protein interactions?



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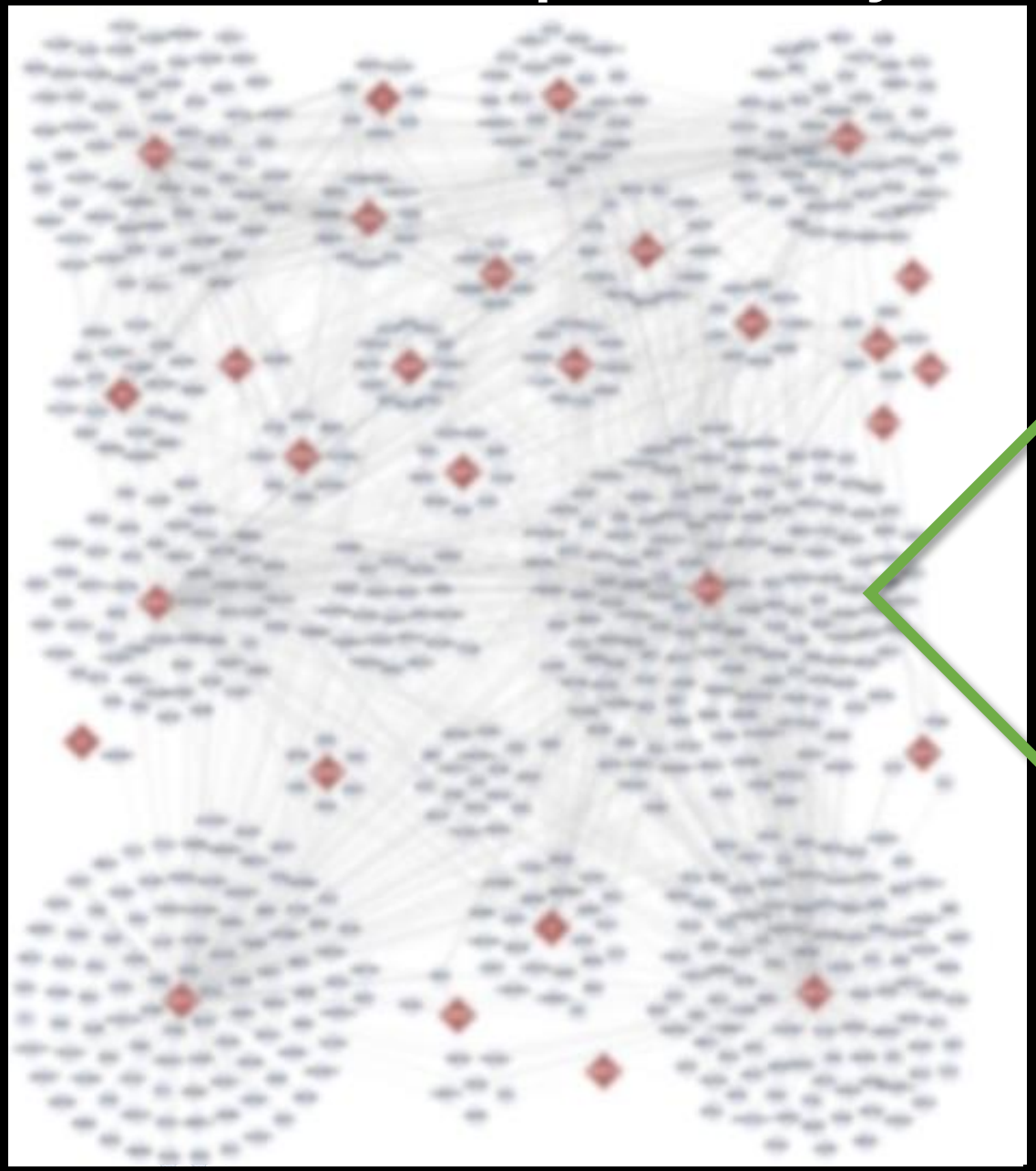
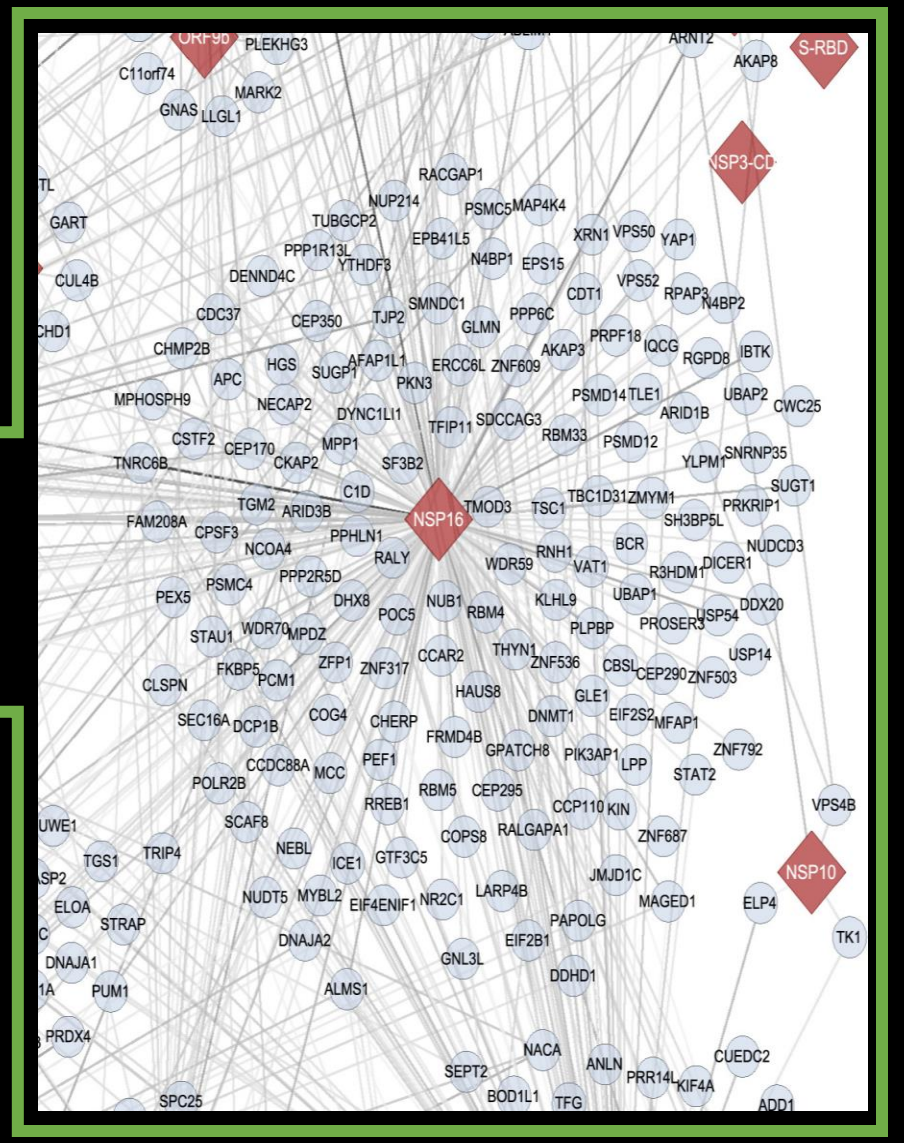
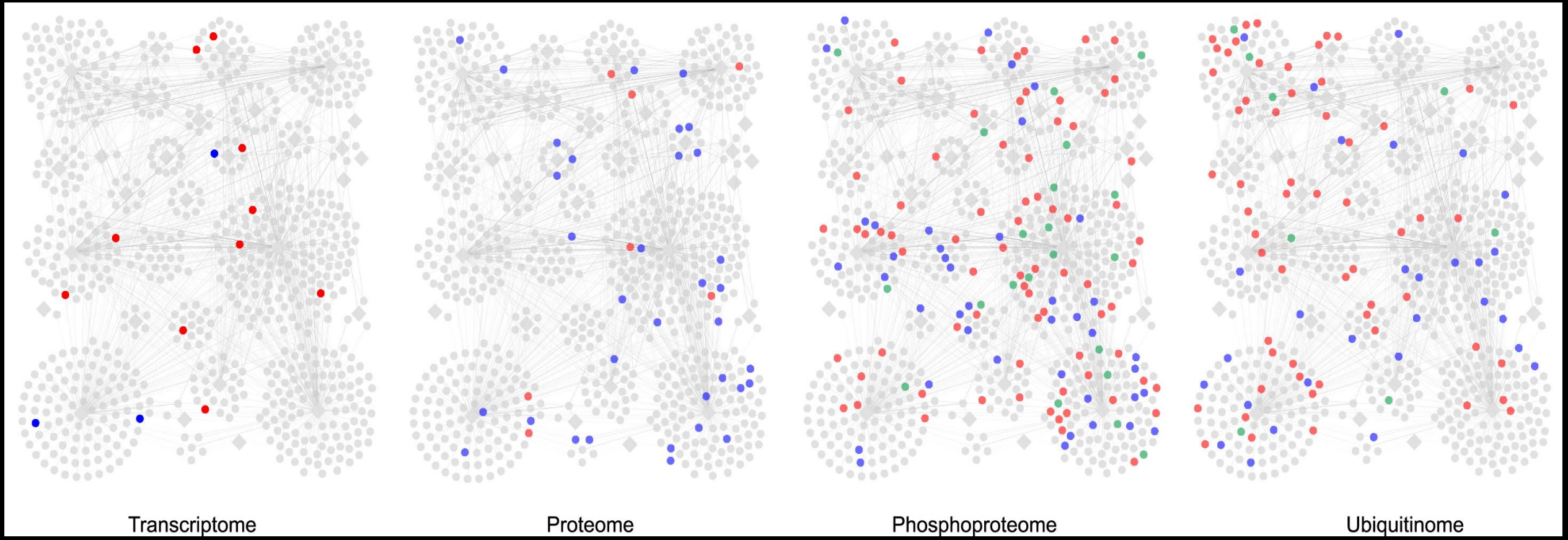




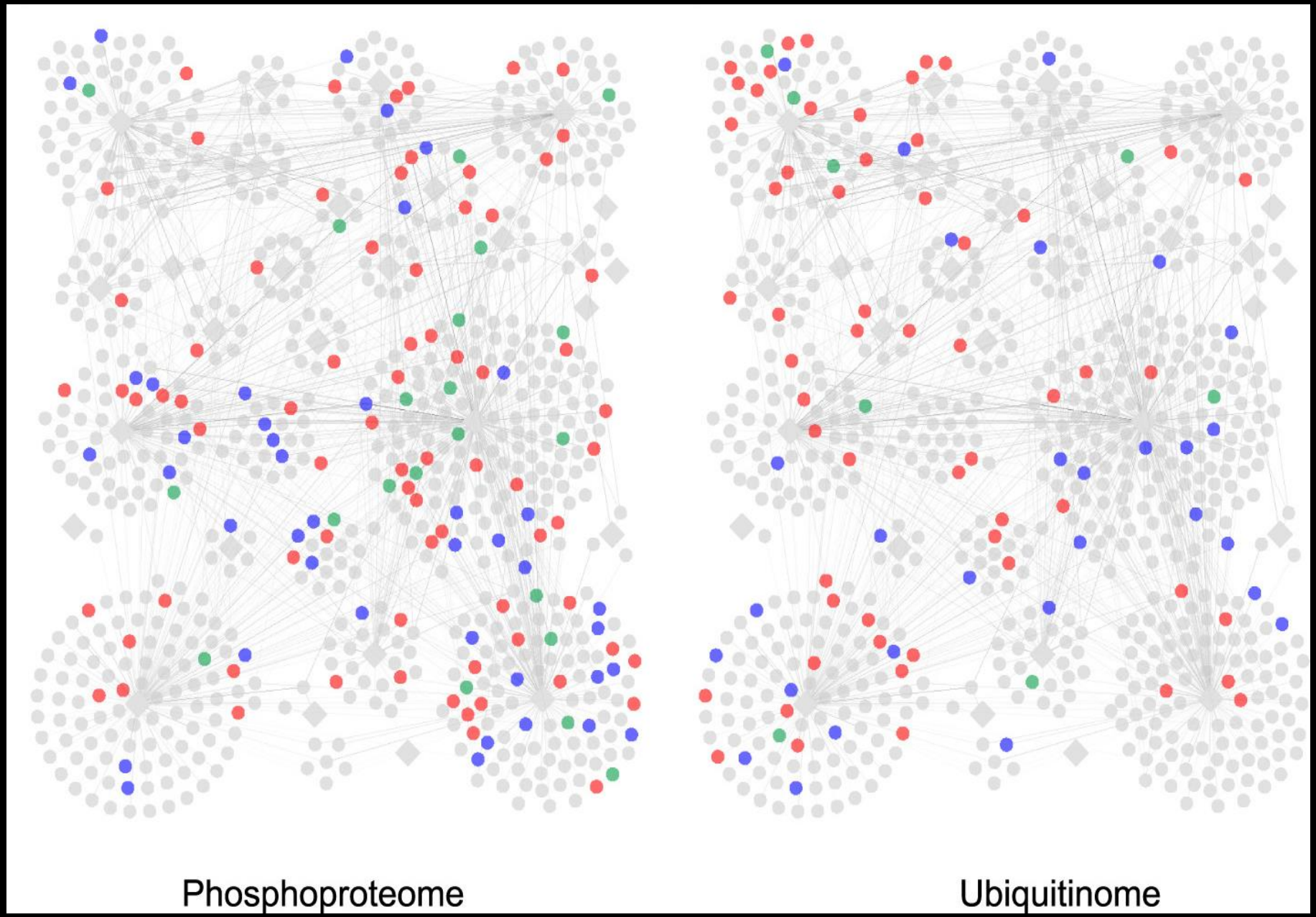
How do these proximal proteins compare to altered proteins of our genome?



downregulated proteins

upregulated proteins

dynamically regulated proteins



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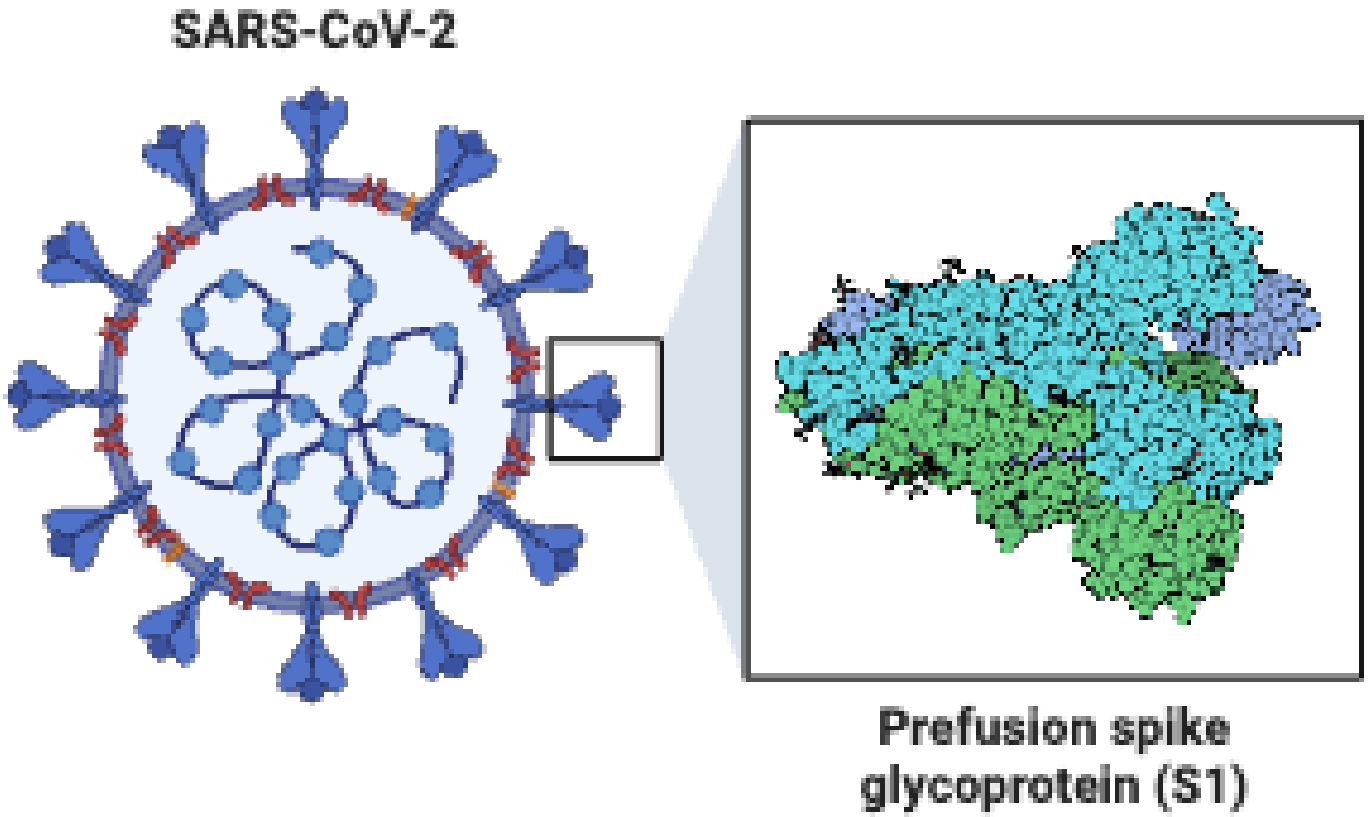
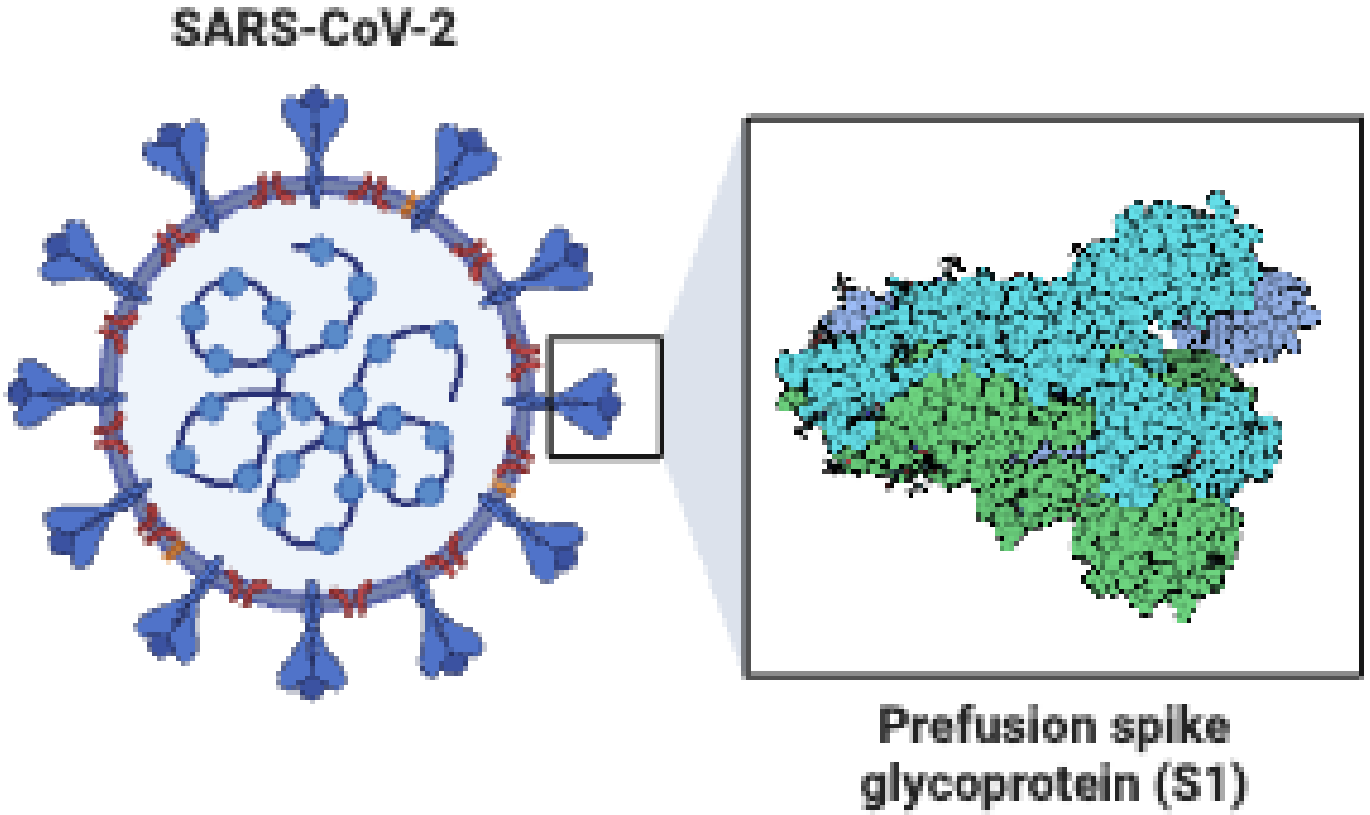
Interactions with S protein



BioRender

Interactions with S protein

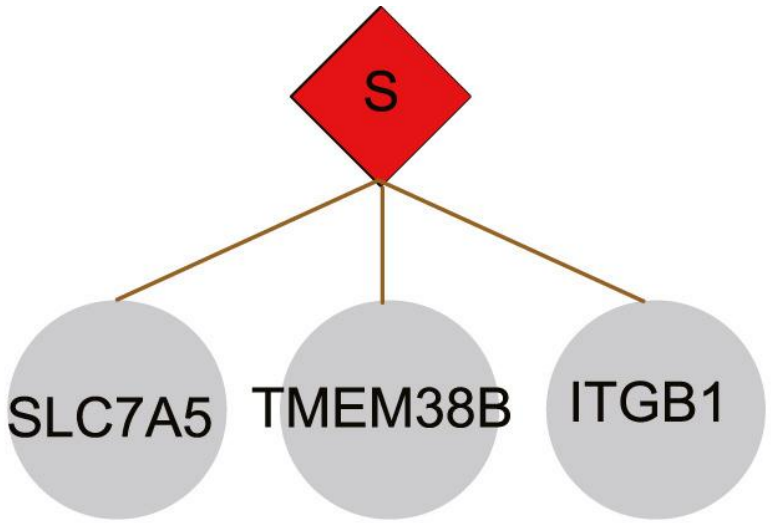


Integrin subunit beta 1 **(ITGB1)**
Transmembrane protein 38B **(TMEM38B)**
Solute carrier family 7 member 5 **(SLC7A5)**

BioRender

How do these membrane proteins interact with the S protein of SARS-CoV-2?

A



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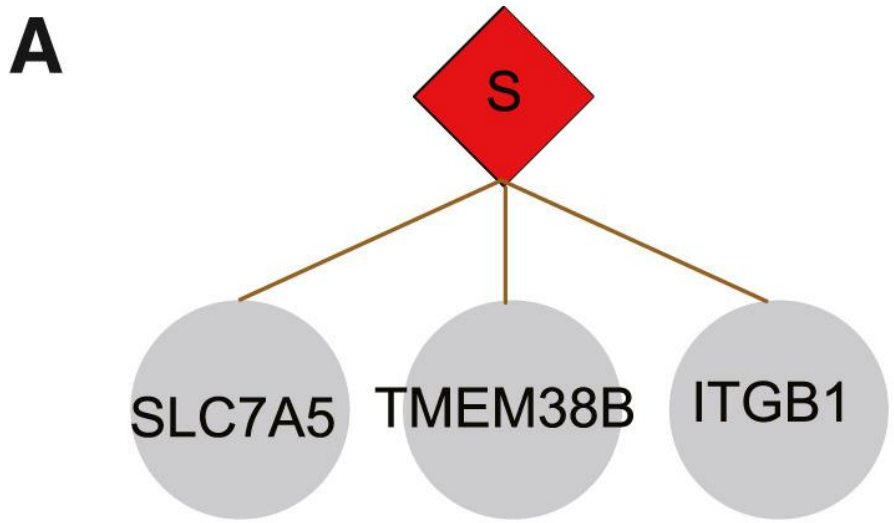

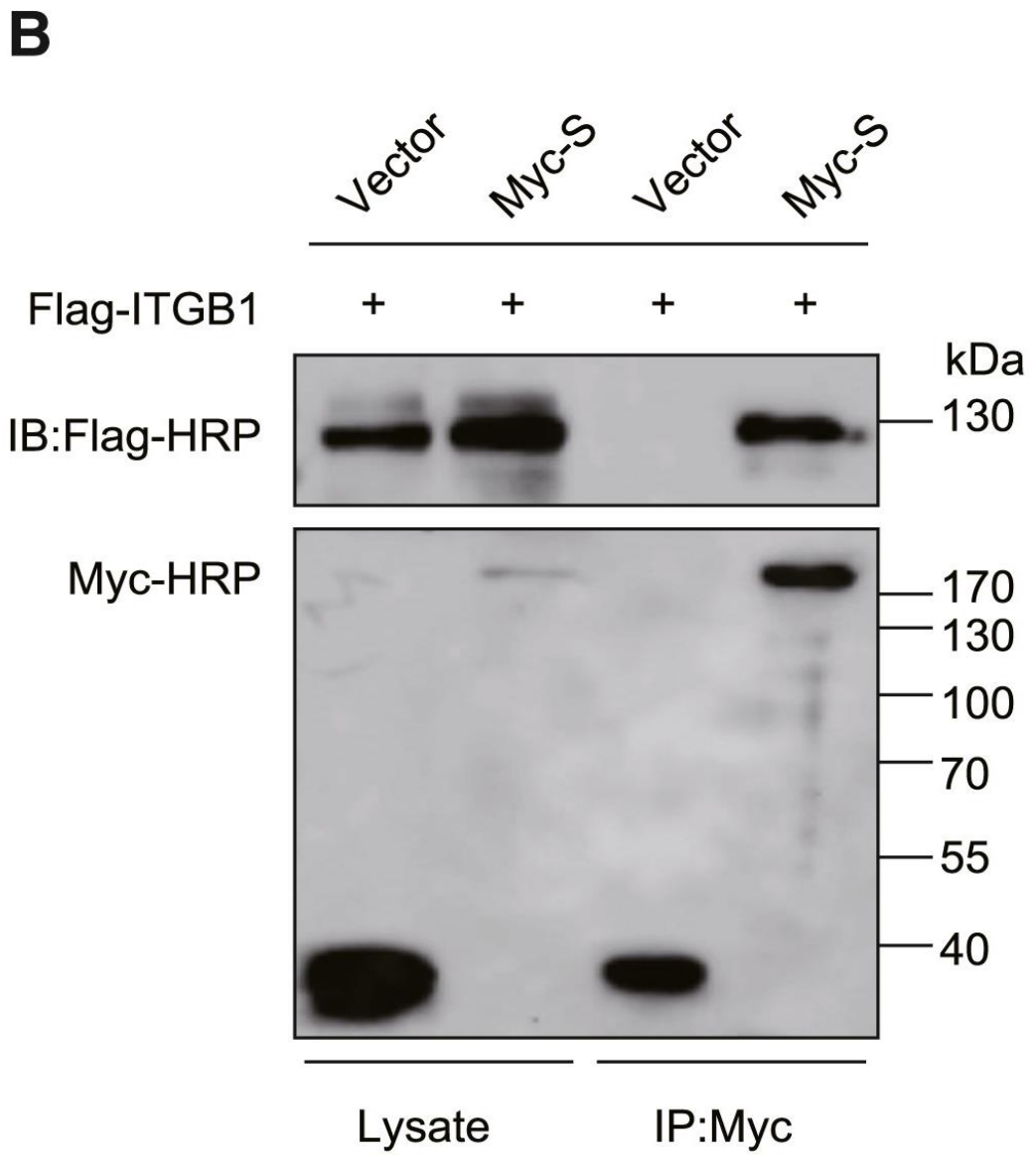

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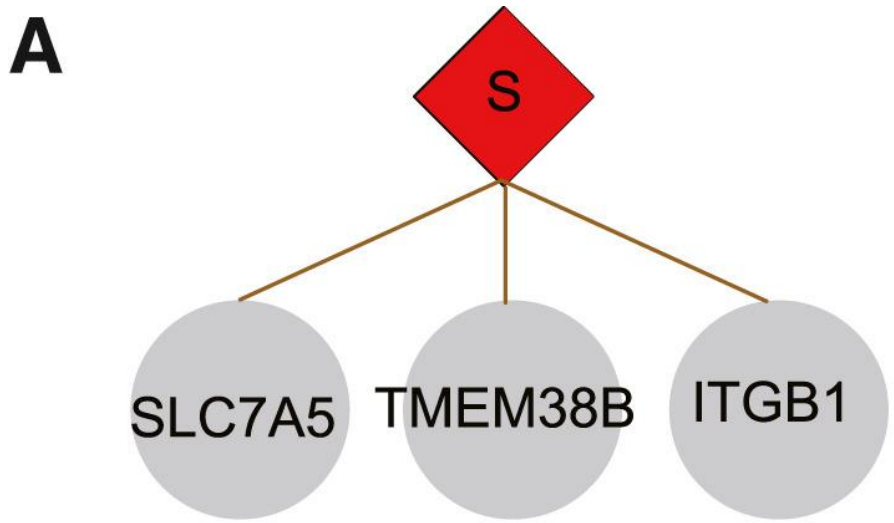
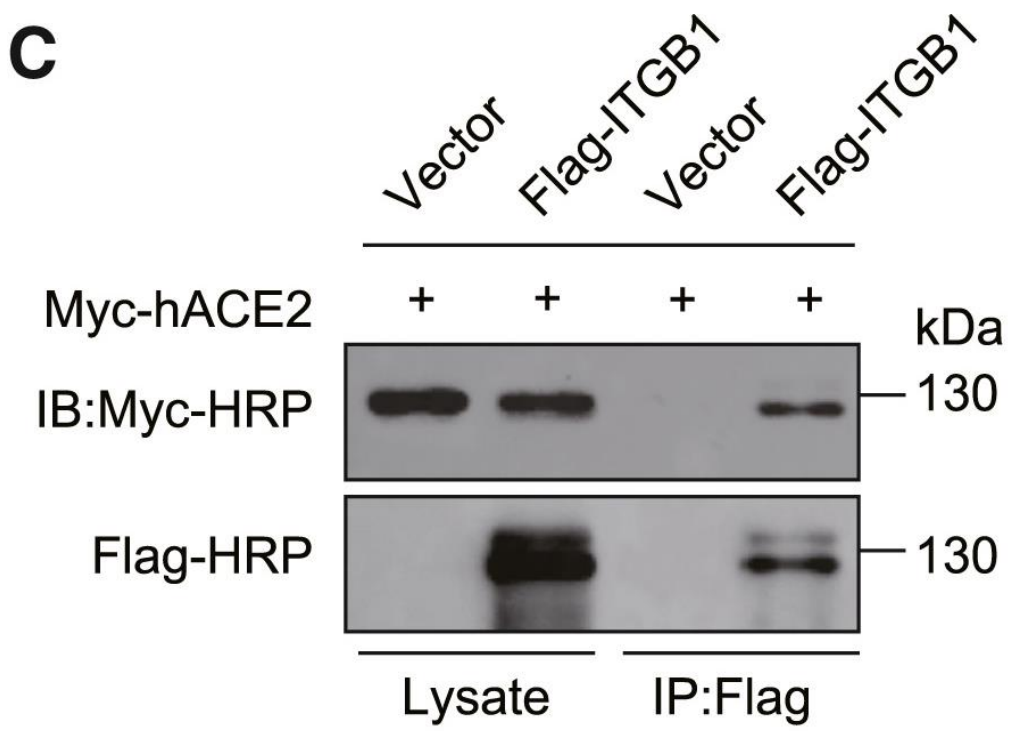
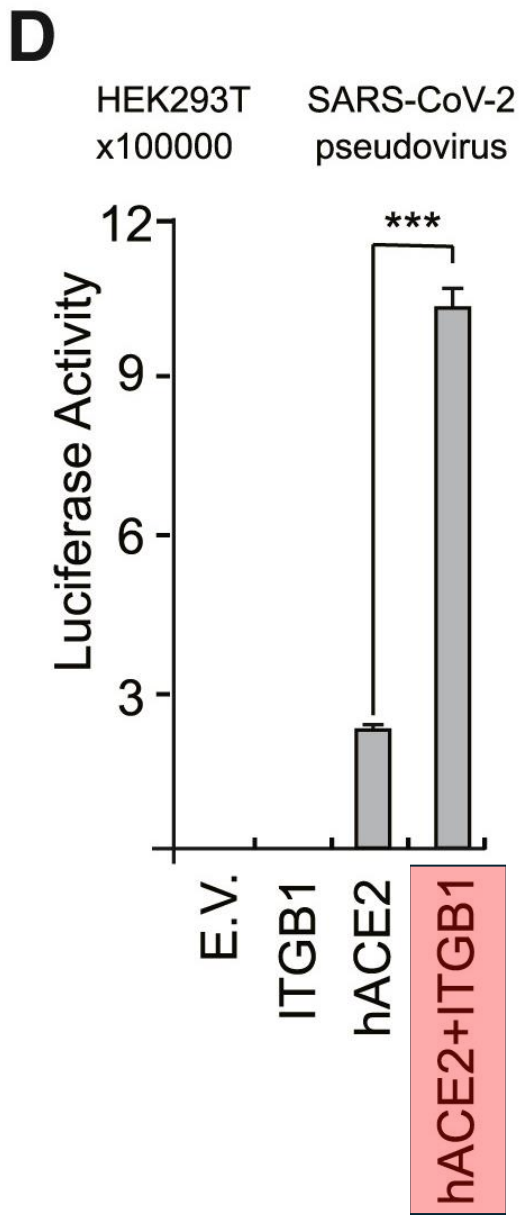




How does ITGB1 mediate the entry of SARS-CoV-2 into HEK293T cells?



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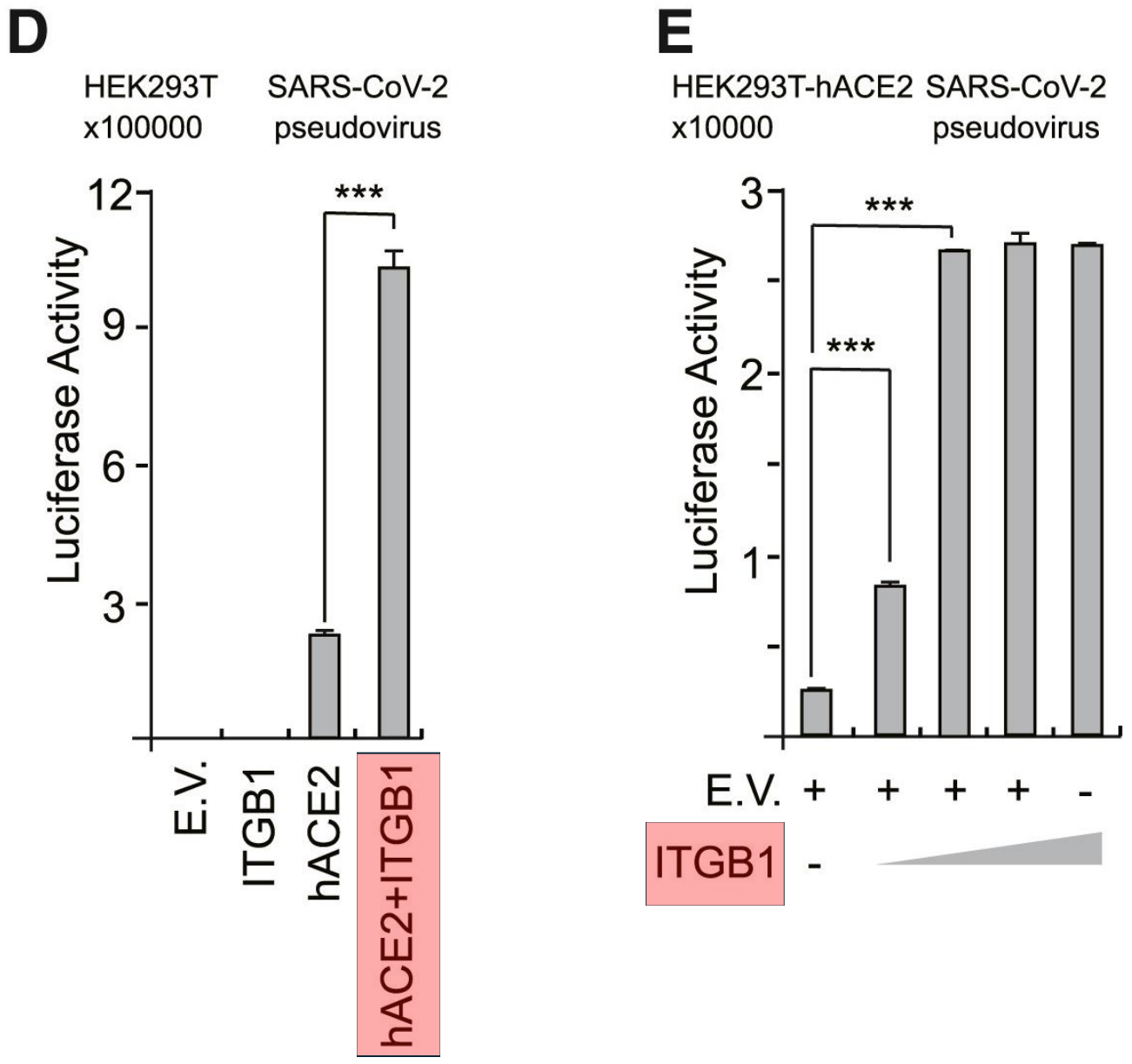
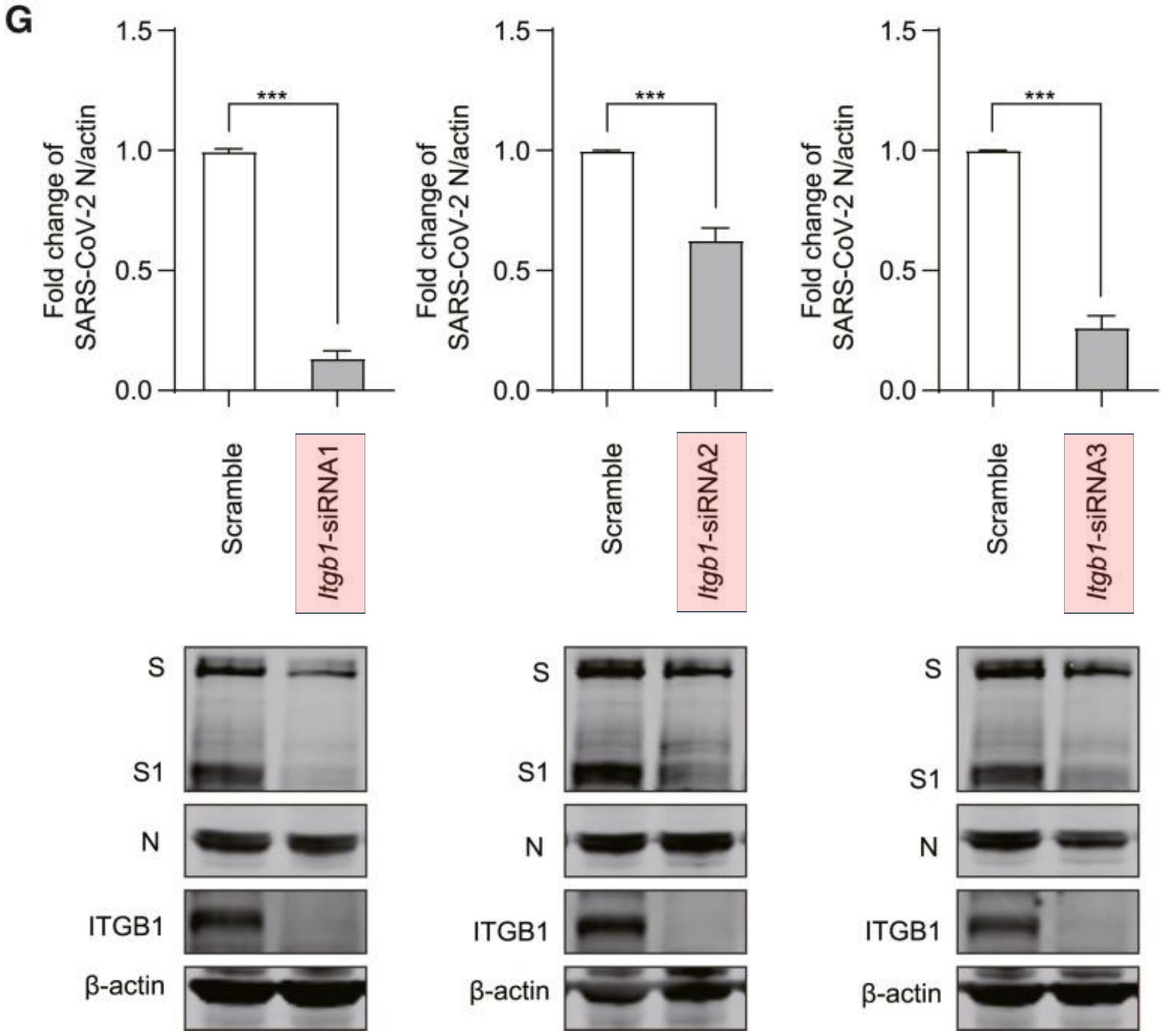
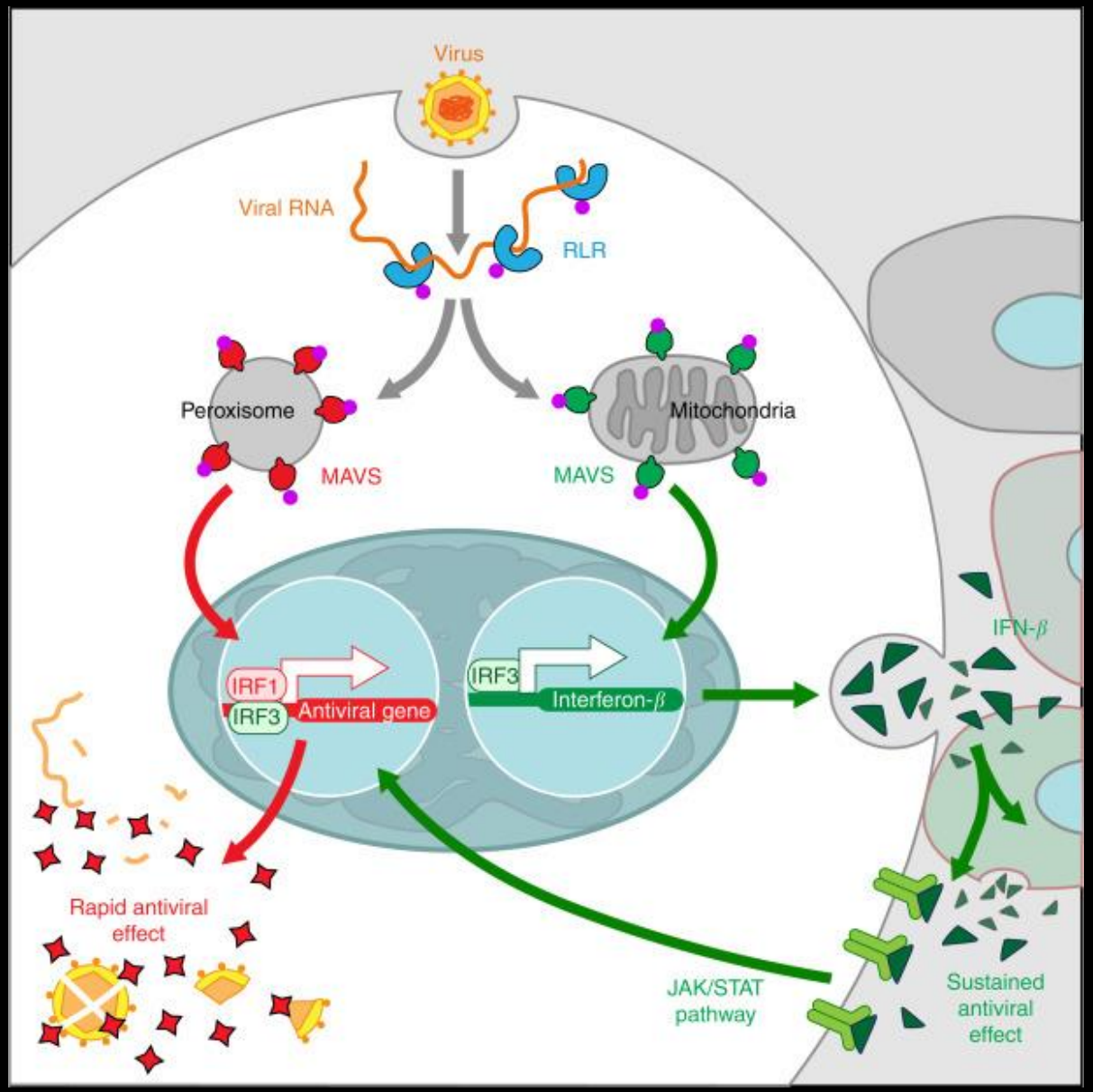


These results indicate that ITGB1 is a co-factor for SARS-CoV-2 entry.

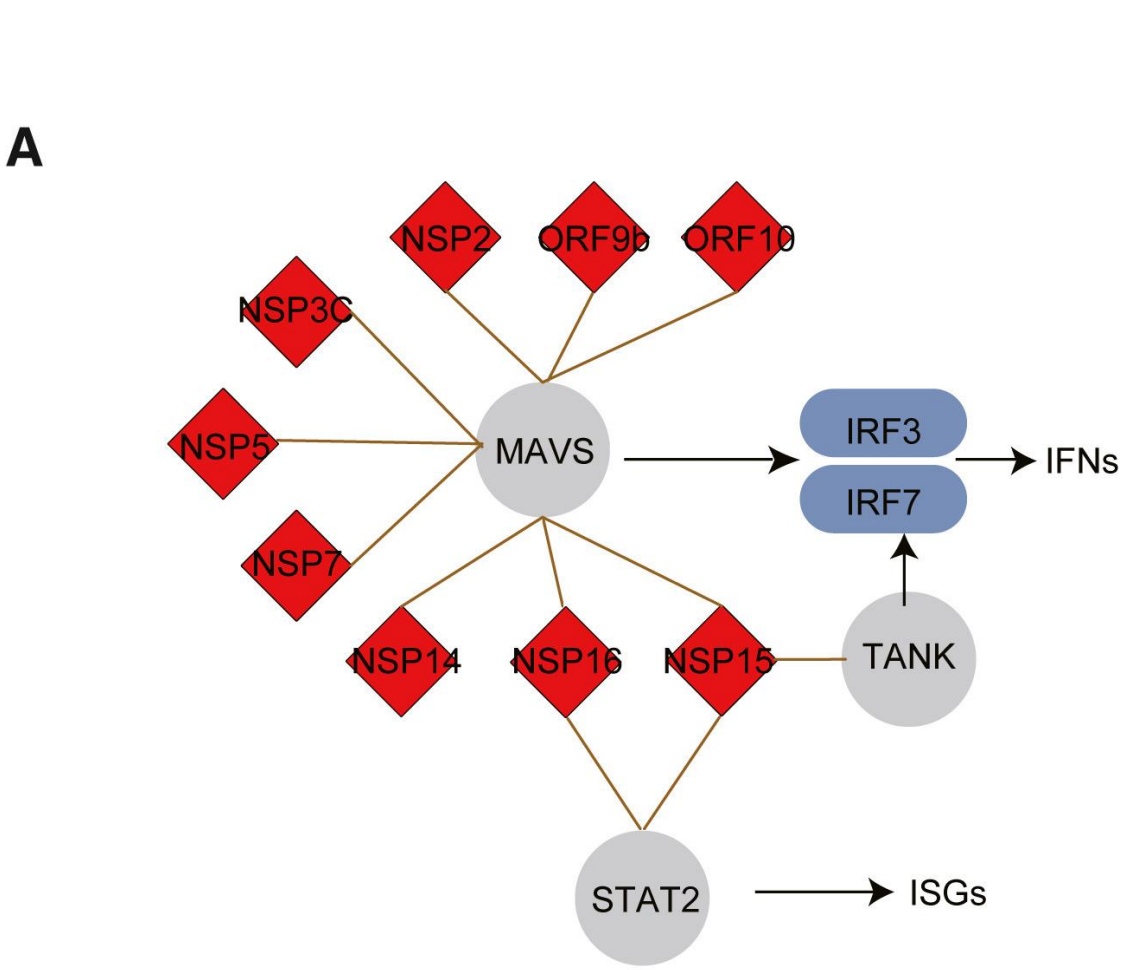
How is this proof that ITGB1 is a co-factor for SARS-CoV-2?

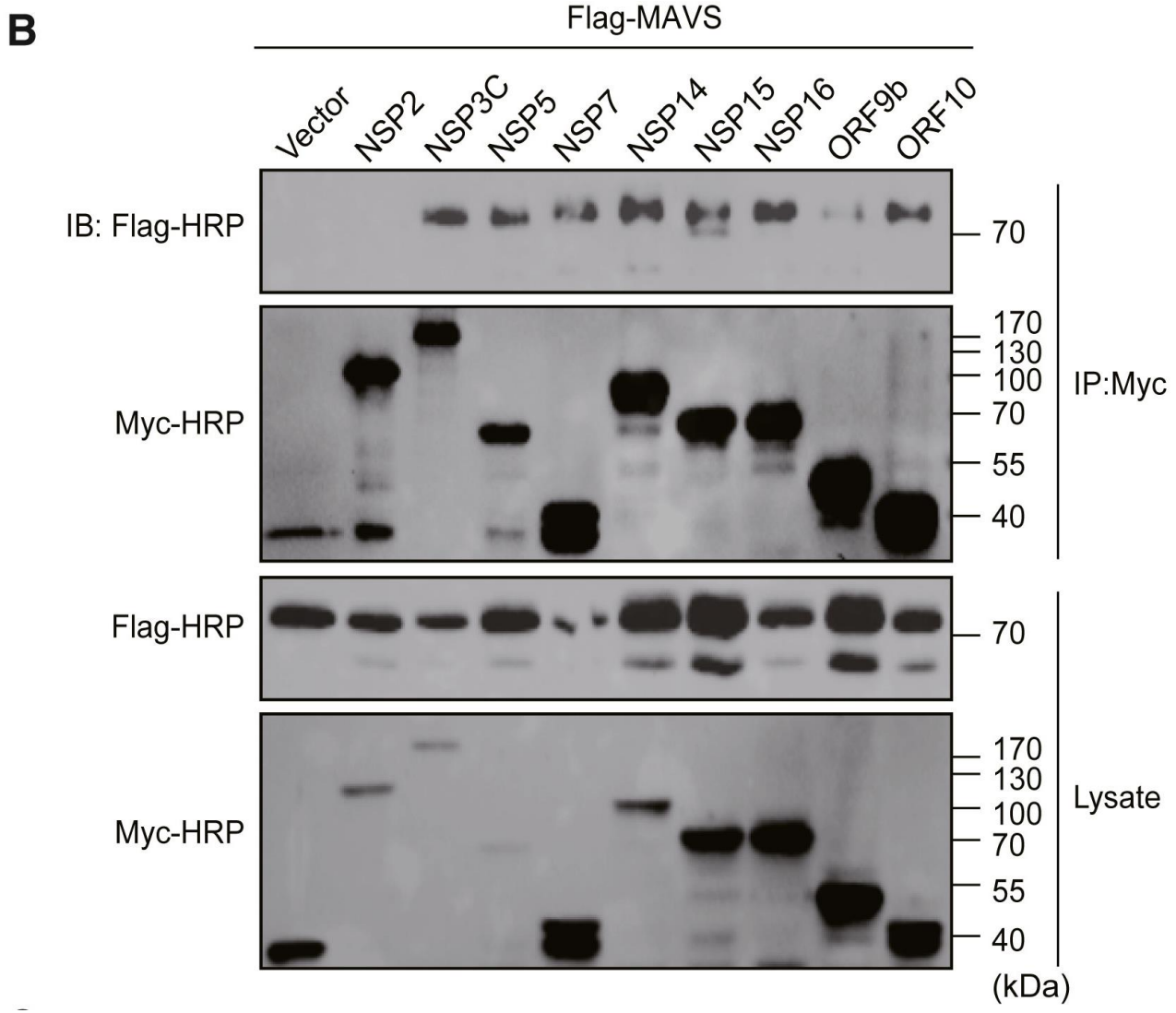


What is the MAVS signaling pathway?

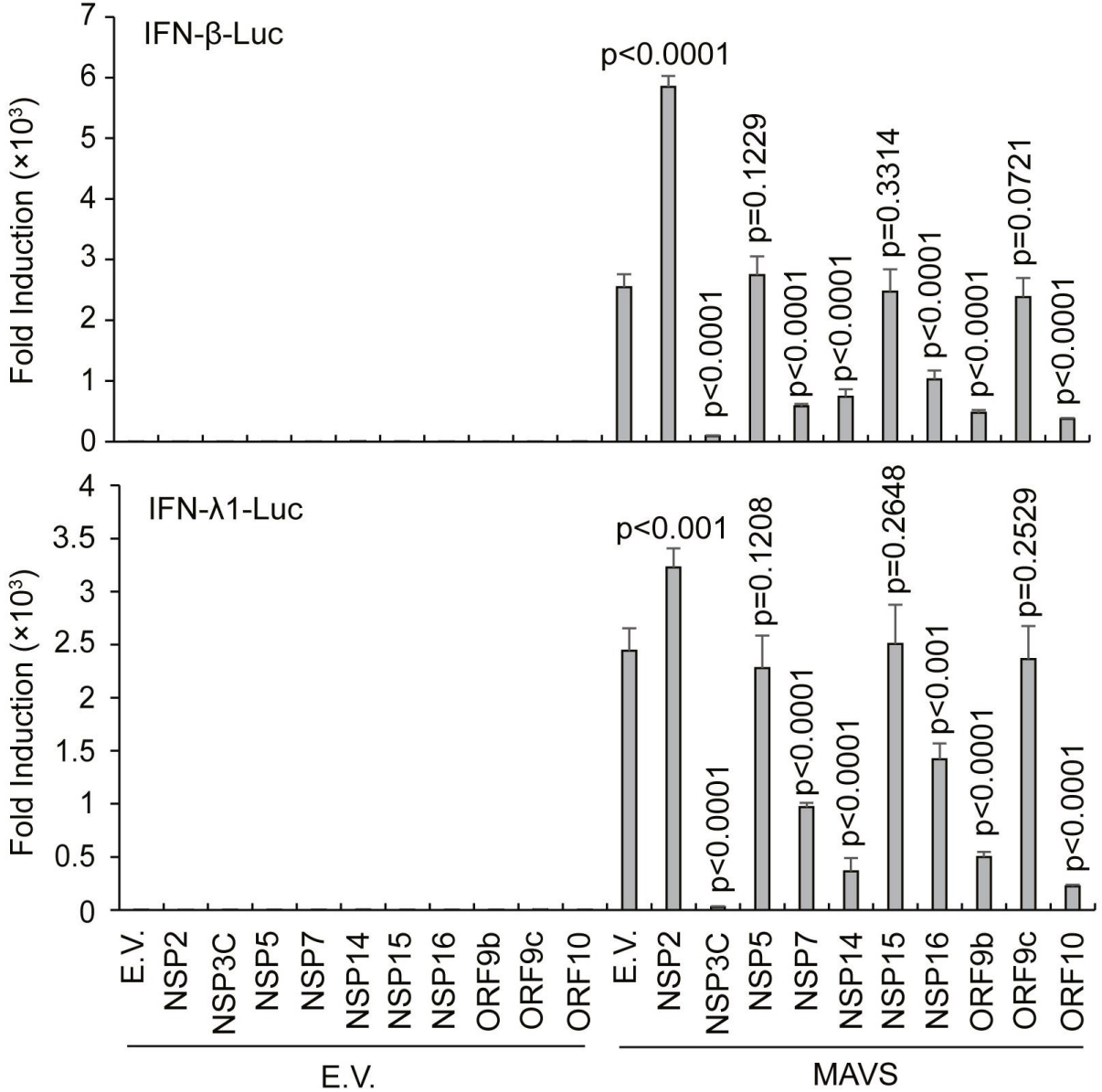


What proteins involved in immune response are targeted by SARS-CoV-2?

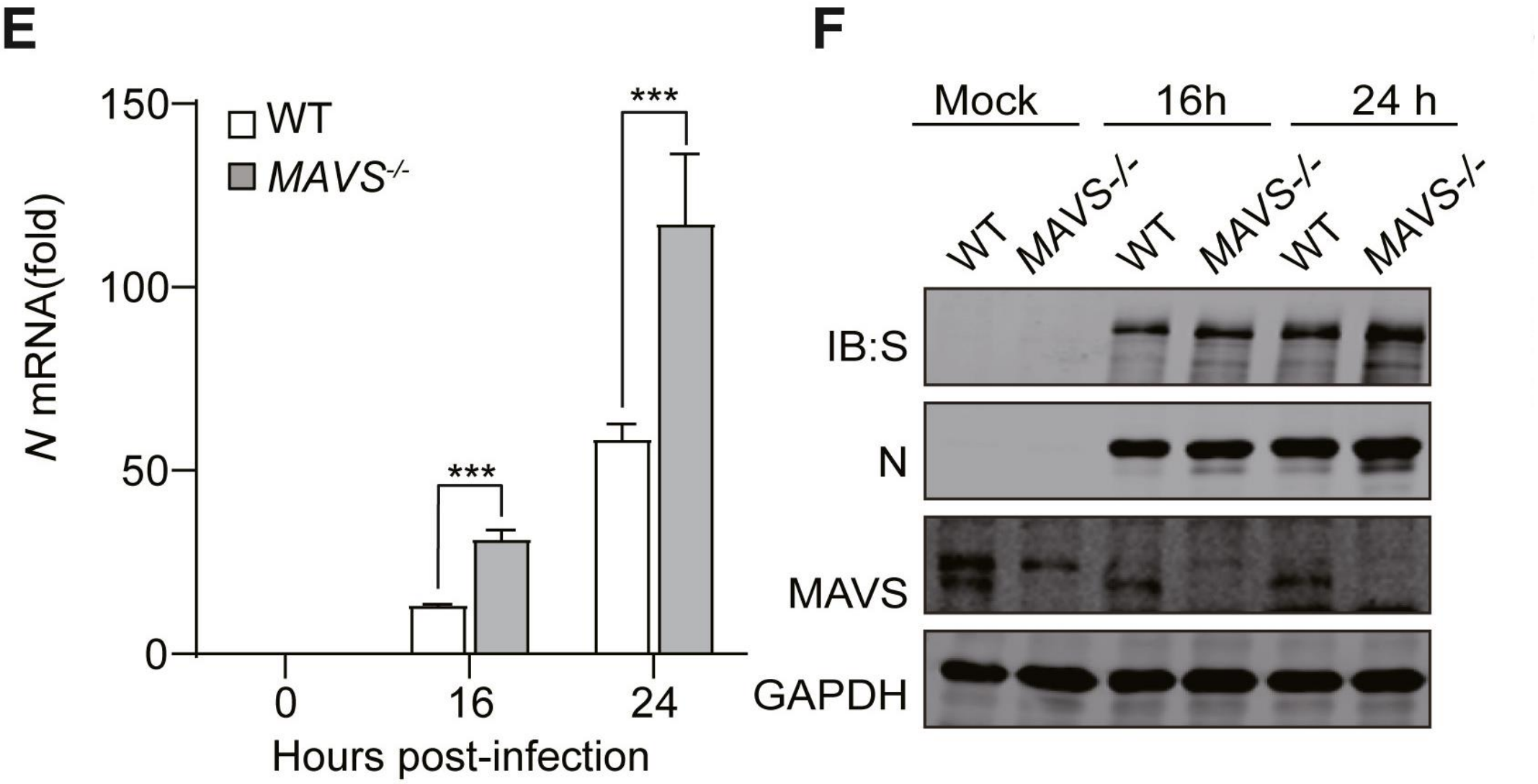




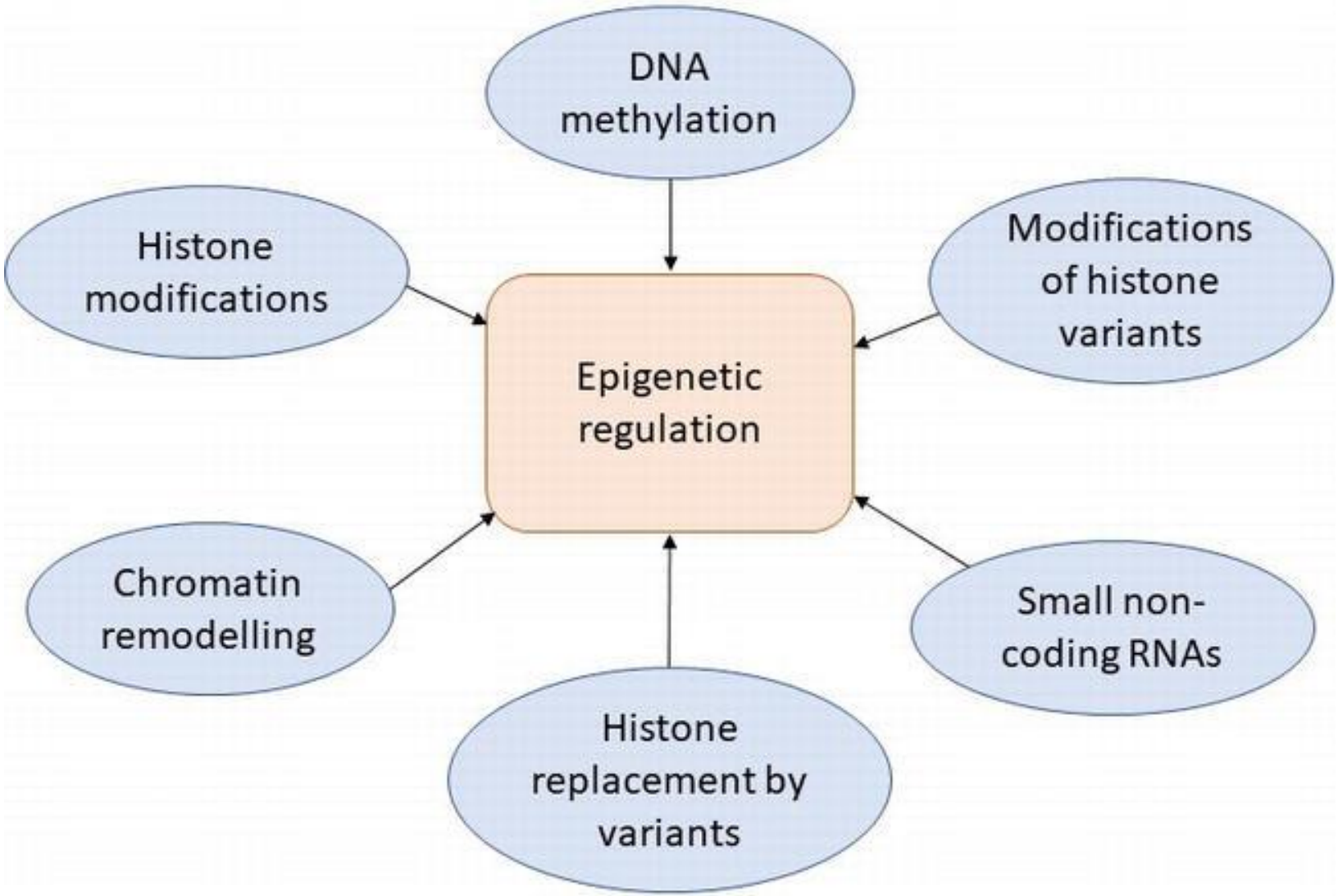
What effect do the proteins of SARS-CoV-2 have on MAVS-induced activation of IFN reporters?



How was the role of MAVS during infection confirmed?

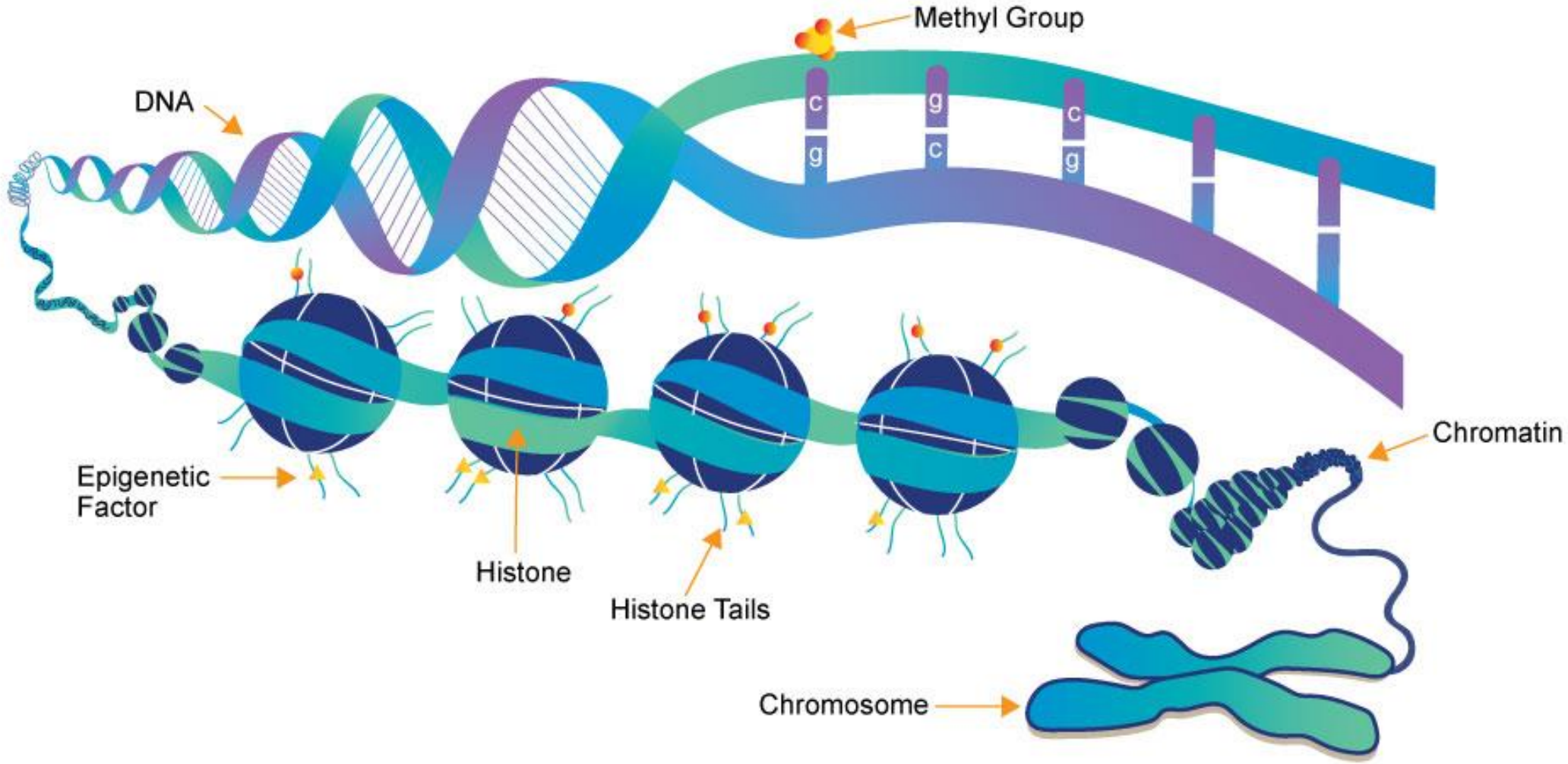


What are epigenetic regulators?



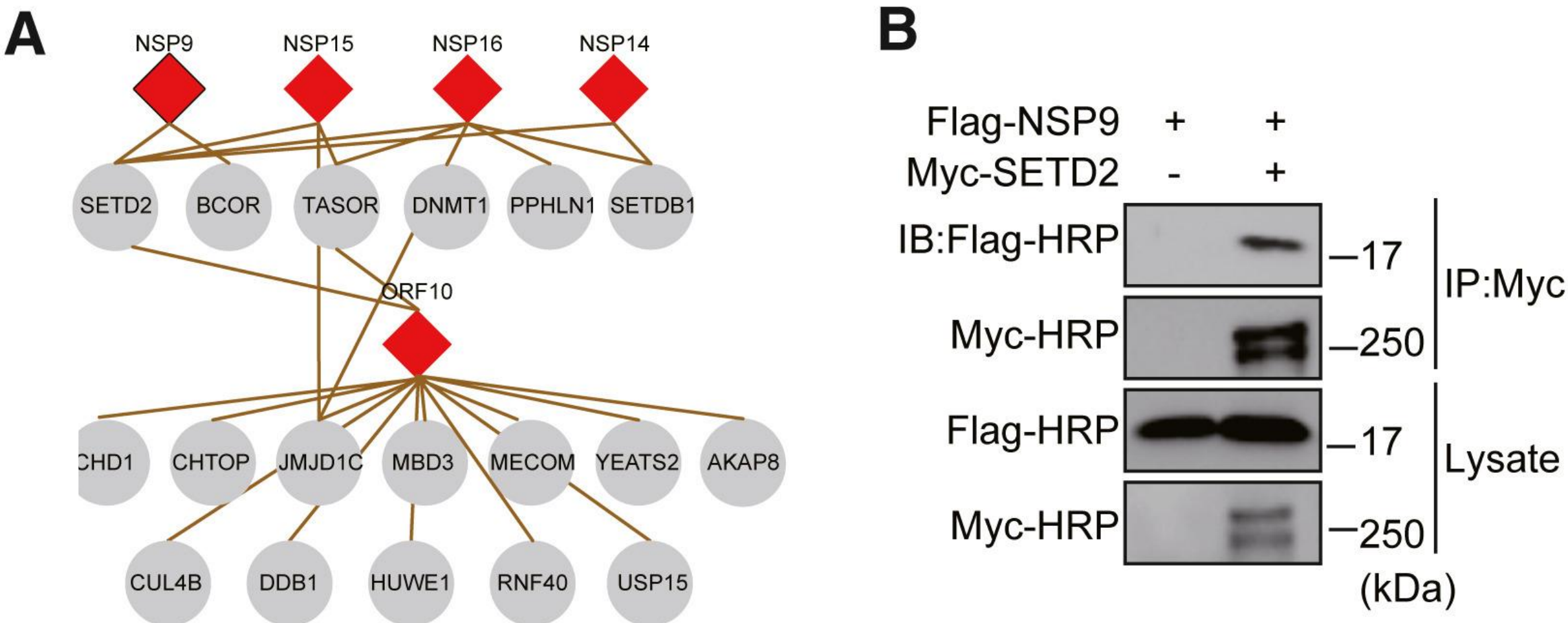
(Shah, Joseph 2022)

What are epigenetic regulators?

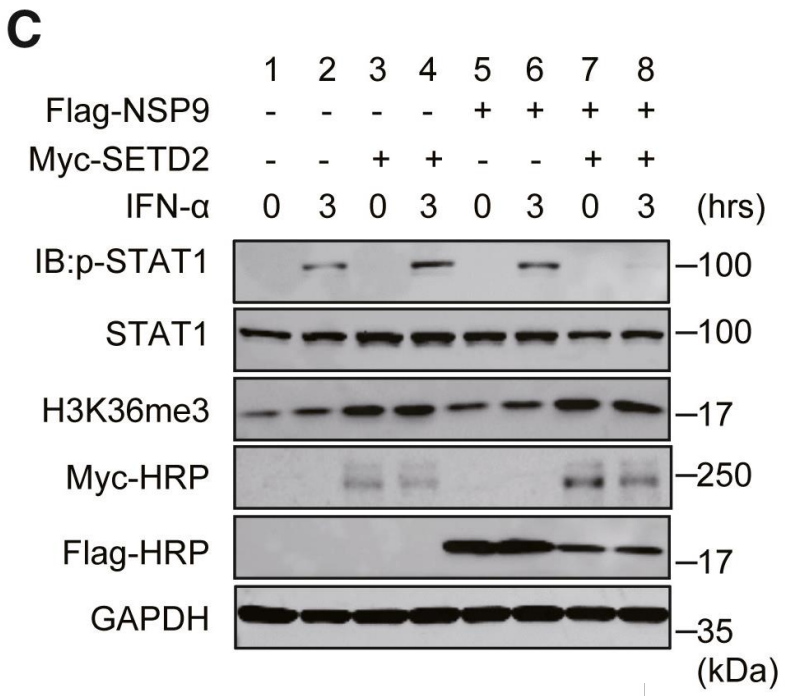


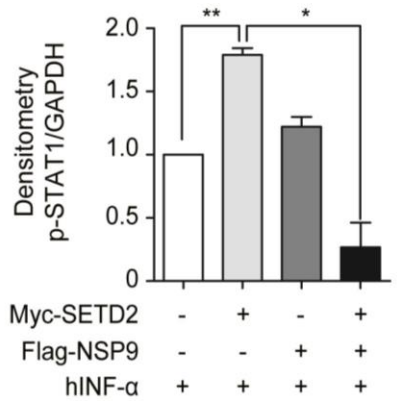
(Mammas et al., 2022)

What proteins target epigenetic regulators for viral infection?



How were epigenetic regulators tested to confirm blocking of IFN signaling?





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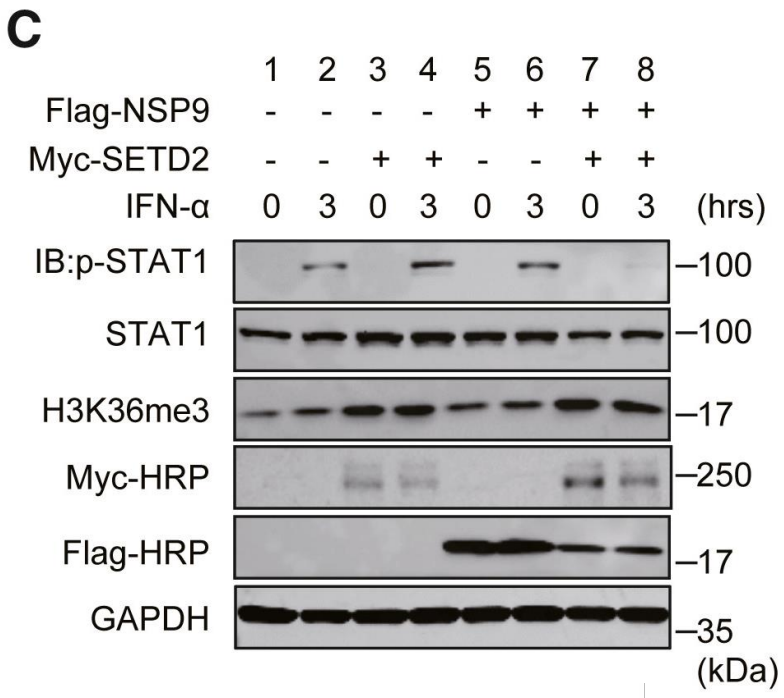
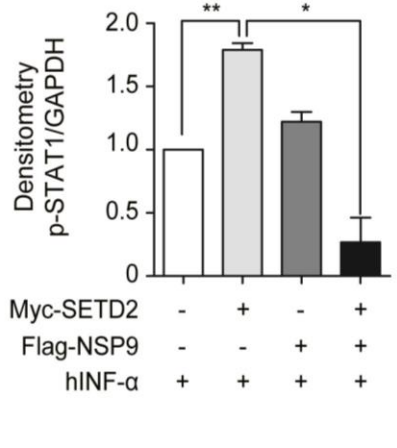
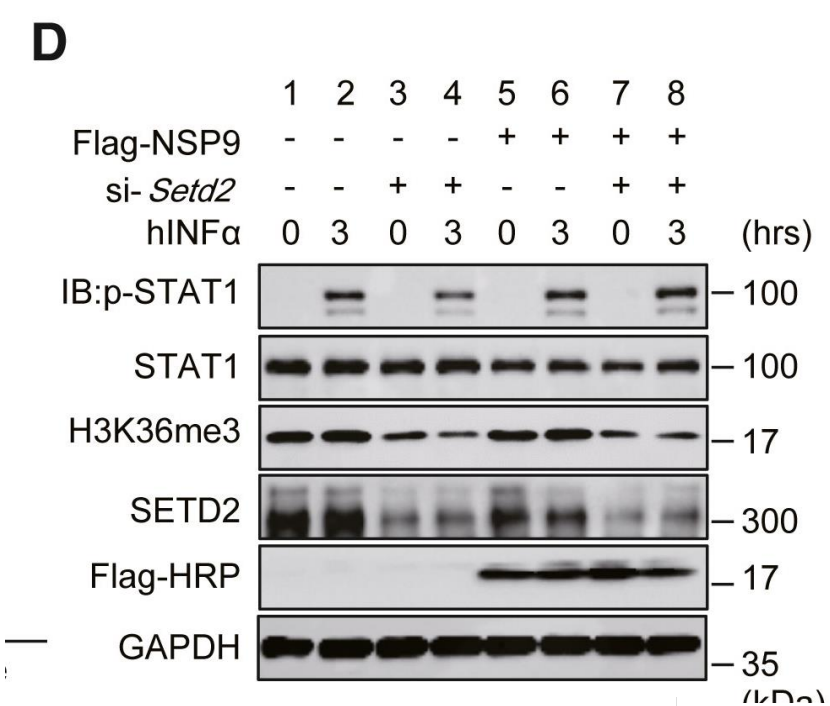
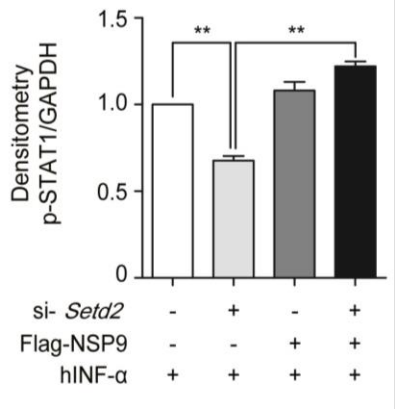
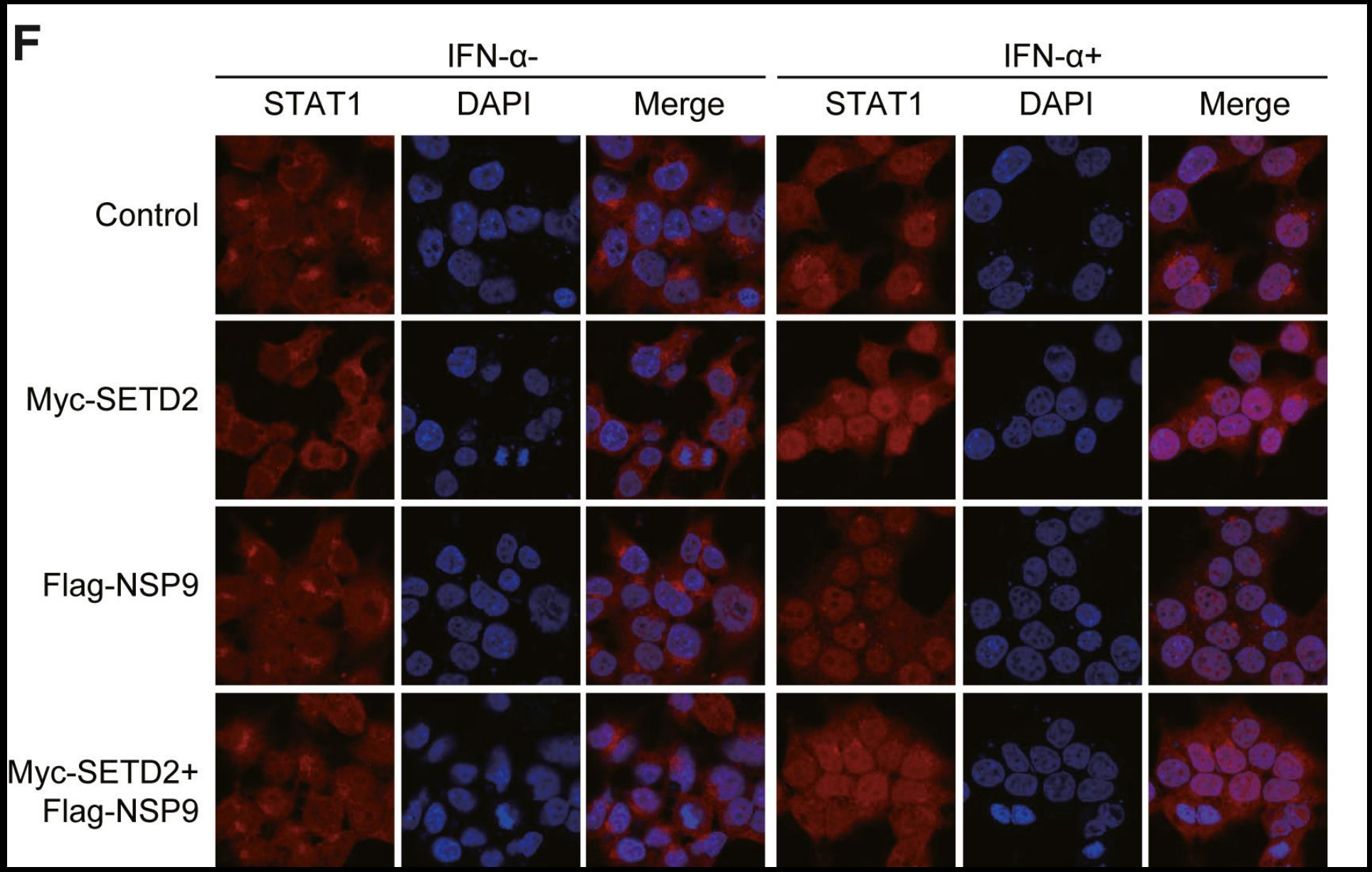








How was microscopy used to show how NSP9-SETD2 cells inhibit translocation of STAT1?



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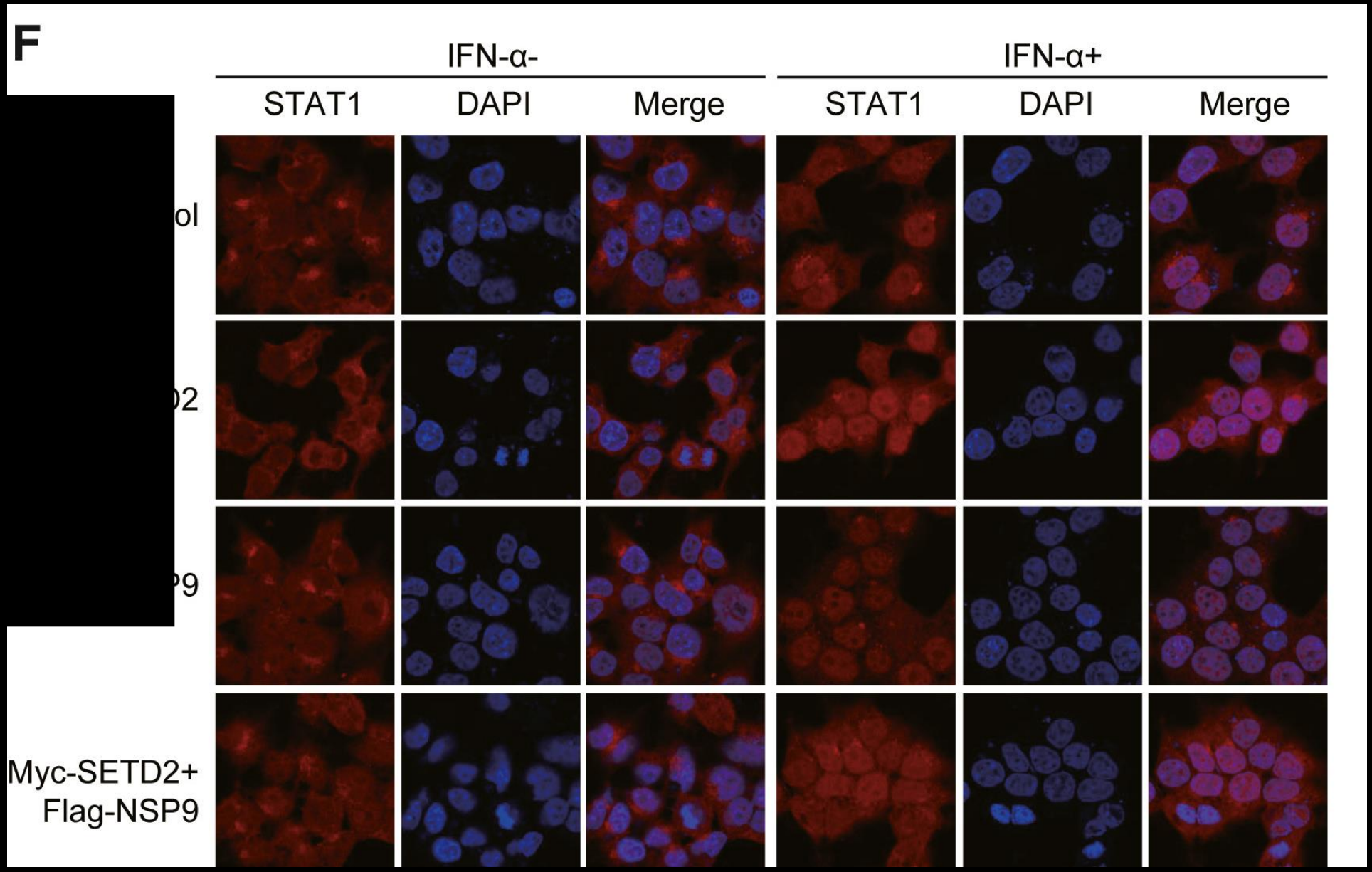

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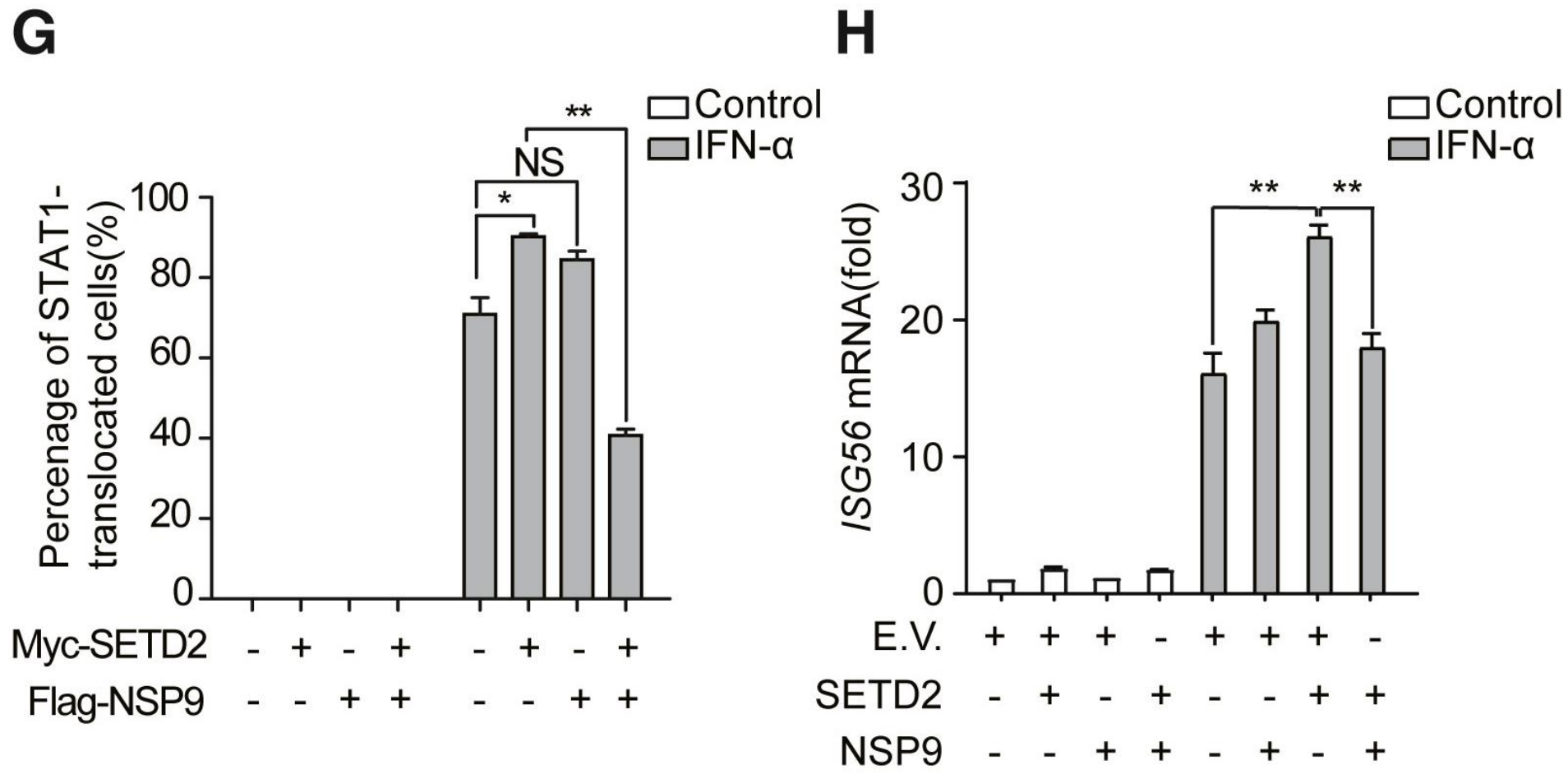
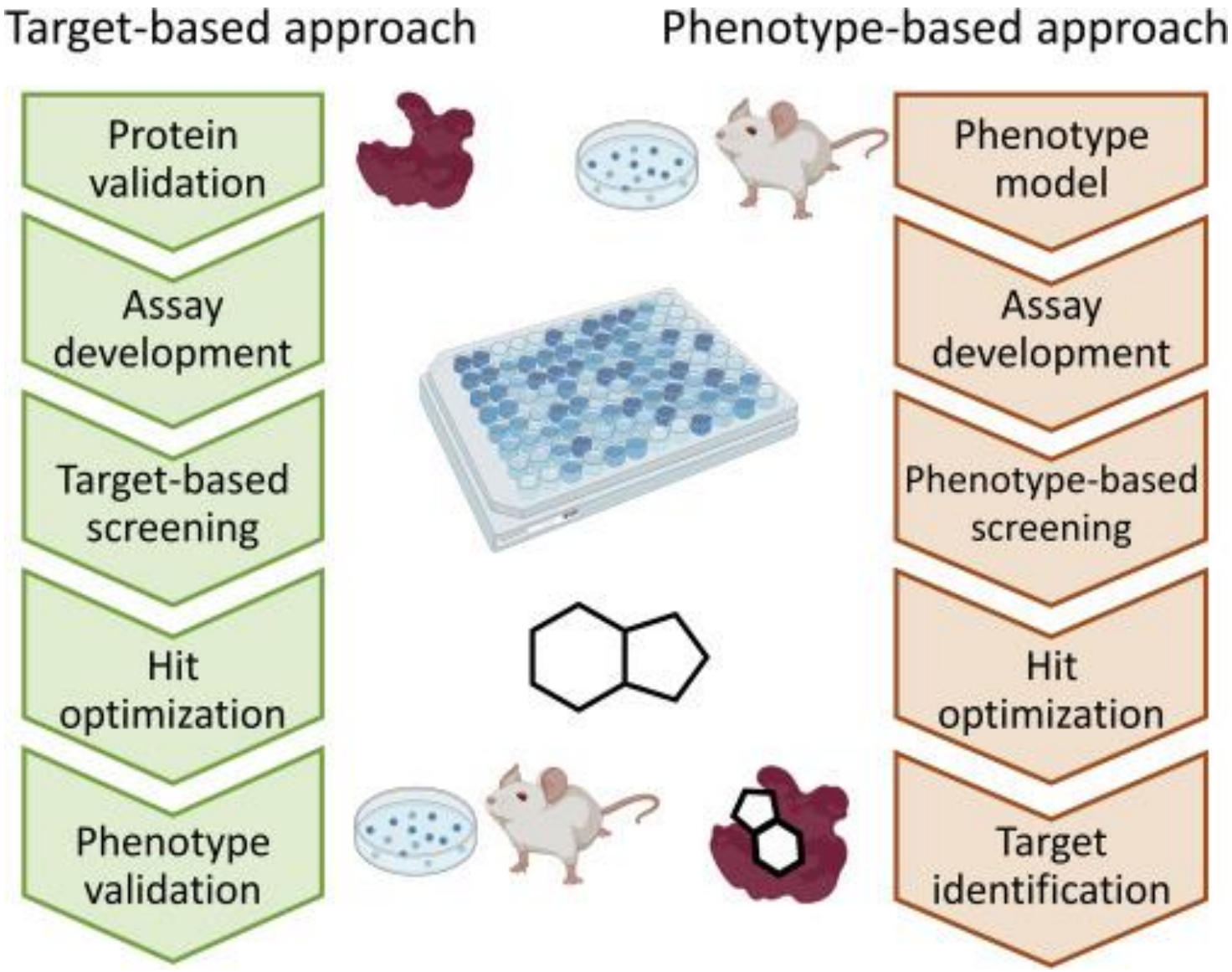
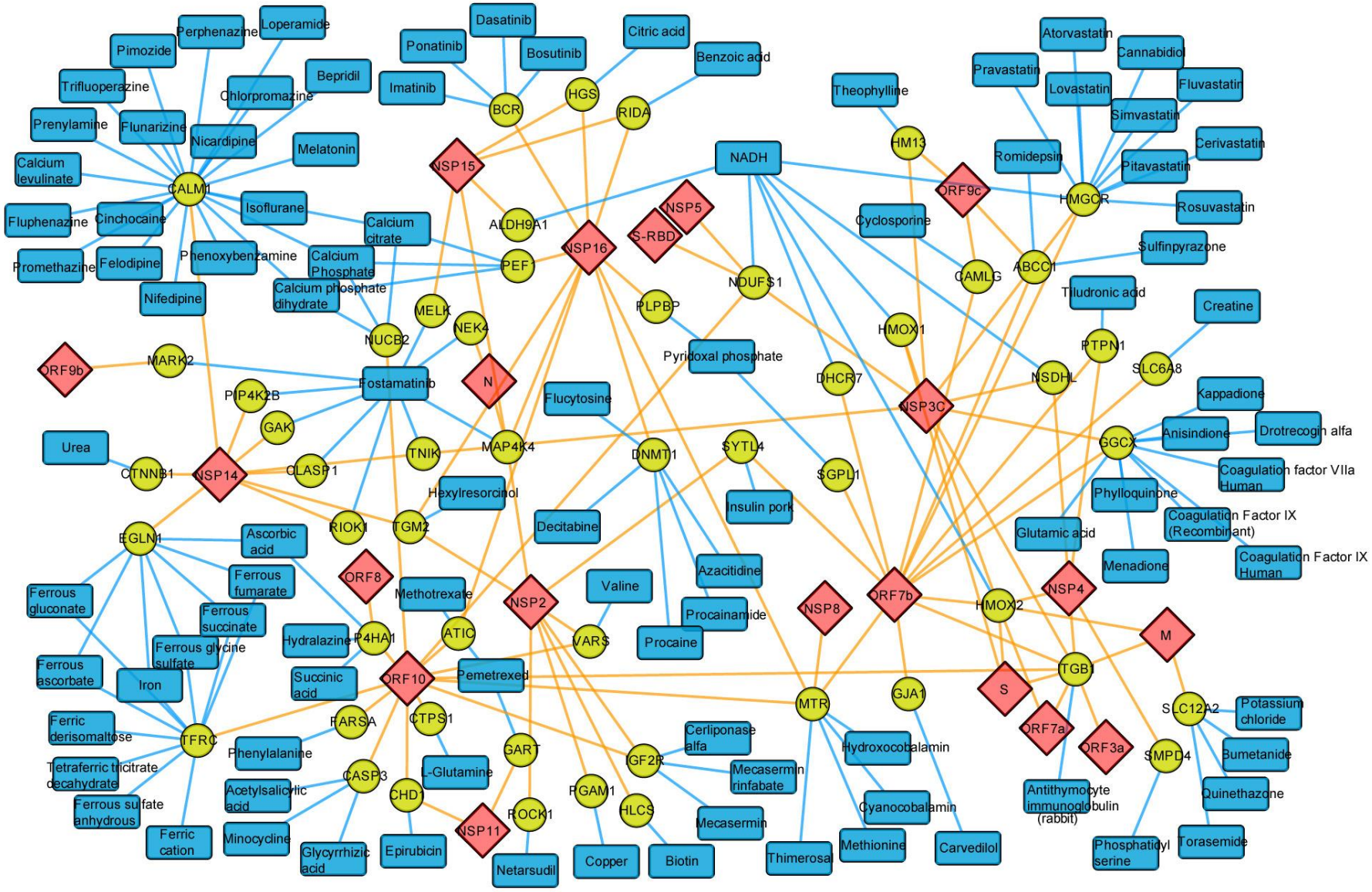


How are drugs used to target proximal proteins?



How were potential drug targets revealed by proximity labeling map?



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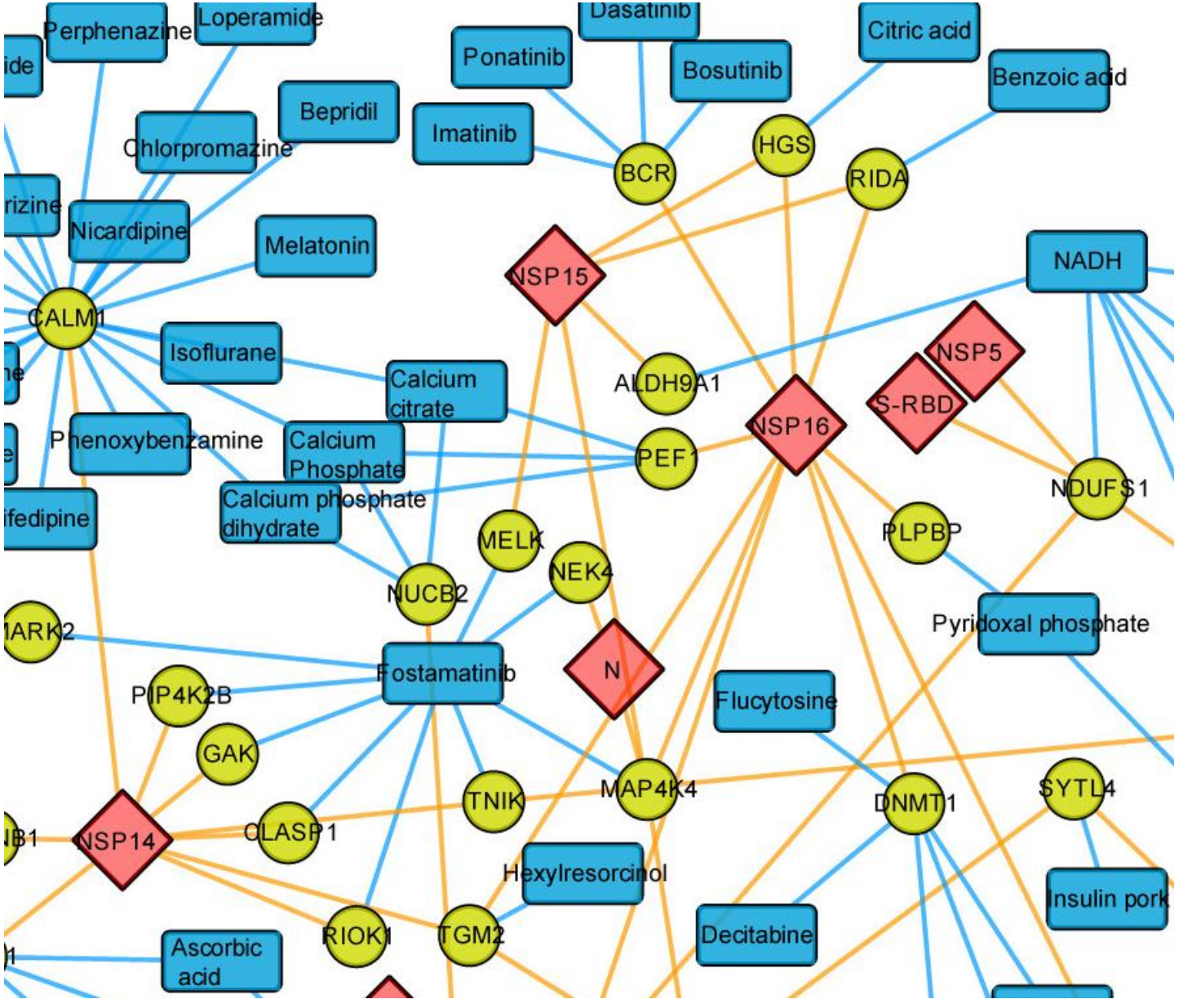
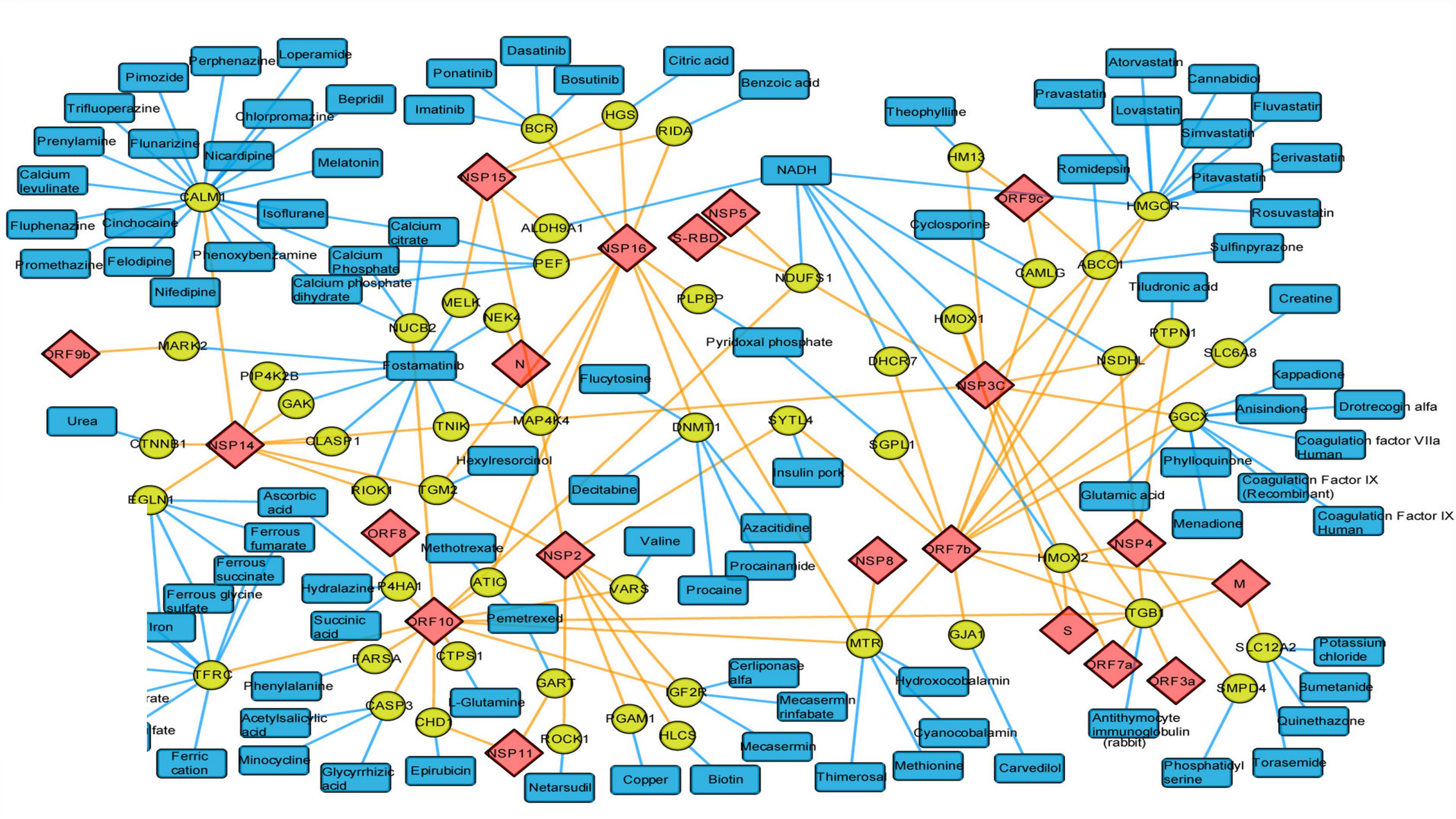
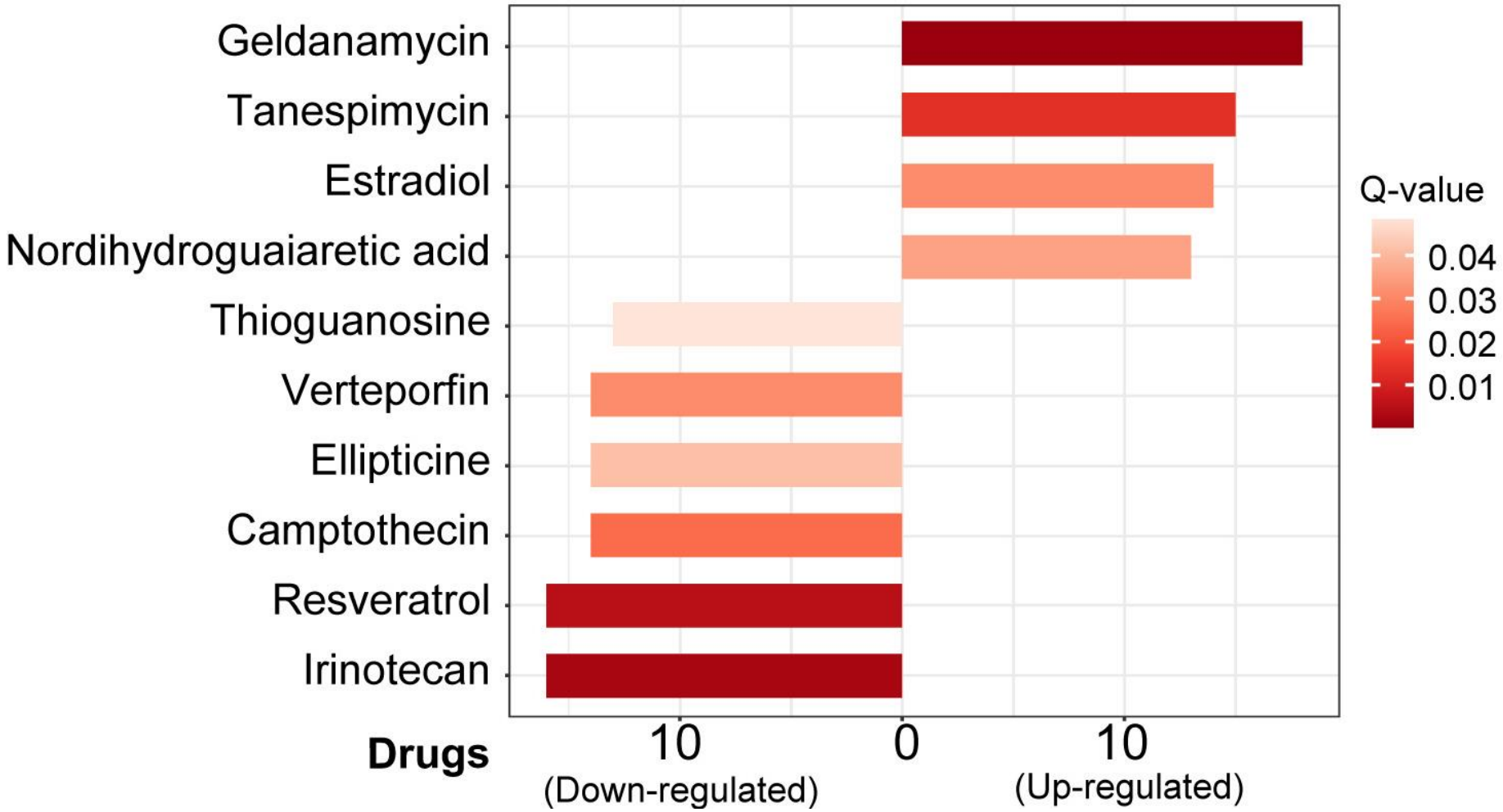
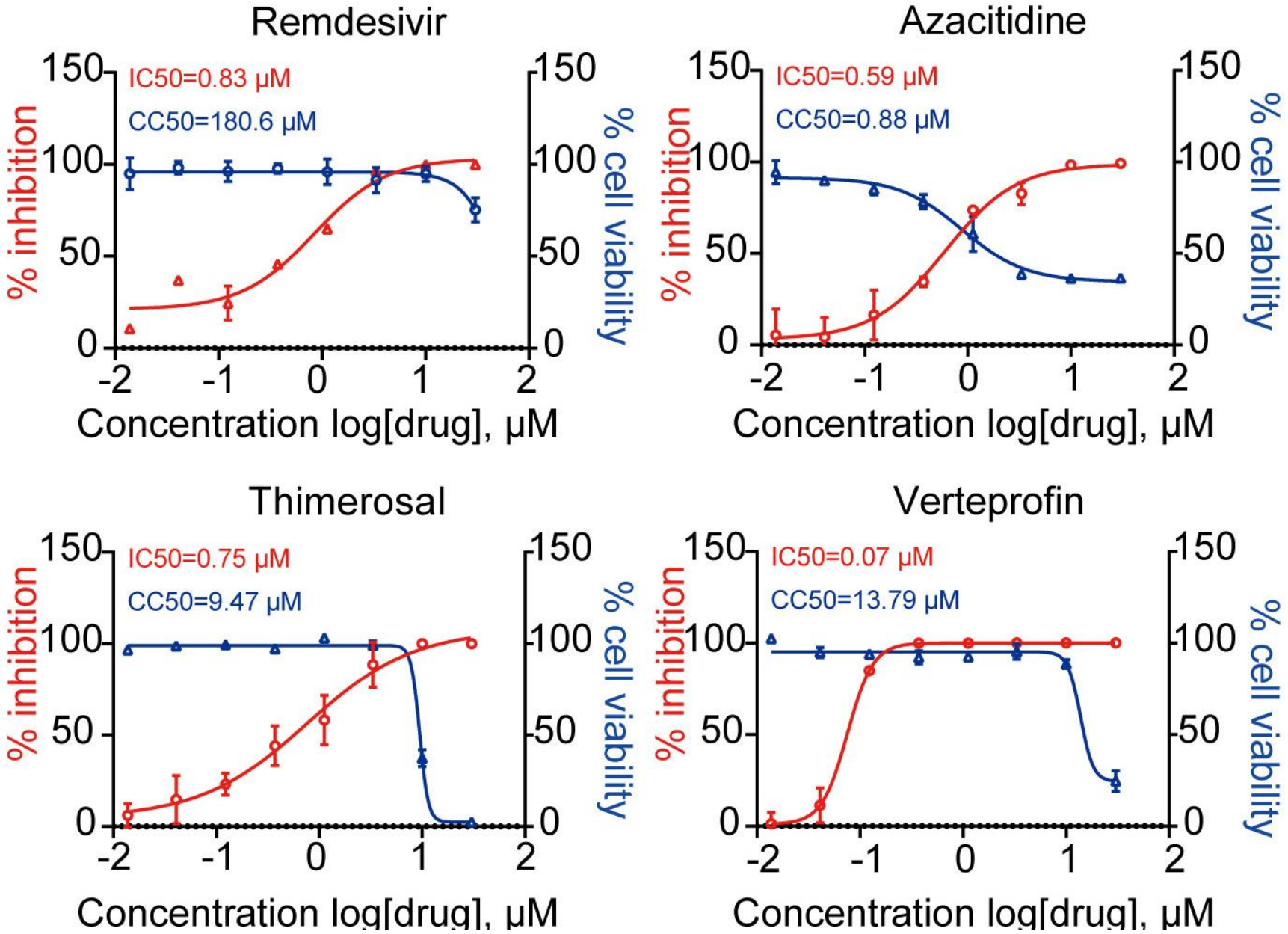



Perphenazine
Loperamide
Pimozide
Trifluoperazine
Chlorpromazine
Bepridil
Prenylamine
Flunarizine
Nicardipine
Melatonin
Calcium levulinate
CALM1
Fluphenazine
Cinchocaine
Isoflurane
Promethazine
Felodipine
Phenoxybenzamine
Nifedipine
Calcium citrate
Calcium Phosphate
Calcium phosphate dihydrate
Dasatinib
Ponatinib
Bosutinib
Imatinib
BCR
Citric acid
Benzoic acid
HGS
RIDA
NSP15
ALDH9A1
PEF1
NSP16
NSP5
S-RBD
NADH
NDUFS1
PLPBP
Pyridoxal phosphate
MELK
NEK4
NUCB2
ORF9b
MARK2
Fostamatinib
PIP4K2B
GAK
N
Flucytosine
DNMT1
Urea
CTNNB1
NSP14
CLASP1
TNIK
MAP4K4
Hexylresorcinol
RIOK1
TGM2
Decitabine
Azacitidine
Procainamide
Procaine
Valine
VARS
SYTL4
Insulin pork
SGPL1
EGLN1
Ascorbic acid
Ferrous fumarate
Ferrous succinate
Ferrous glycine sulfate
Iron
TFRC
ORF8
Methotrexate
ATIC
NSP2
Hydralazine
P4HA1
Succinic acid
ORF10
Pemetrexed
RARSA
CTPS1
Phenylalanine
CASP3
L-Glutamine
CHD1
GART
NSP11
ROCK1
Acetylsalicylic acid
Minocycline
Glycyrrhizic acid
Epirubicin
Netarsudil
PGAM1
HLCS
Copper
Biotin
IGF2R
Cerliponase alfa
Mecasermin rinfabate
Mecasermin
Theophylline
HM13
ORF9c
Cyclosporine
CAMLG
HMOX1
DHCR7
NSP3C
NSDHL
Atorvastatin
Pravastatin
Lovastatin
Cannabidiol
Fluvastatin
Simvastatin
Cerivastatin
Romidepsin
Pitavastatin
HMGCR
Rosuvastatin
Sulfinpyrazone
ABCC1
Tiludronic acid
PTPN1
Creatine
SLC6A8
Kappadione
Anisindione
Drotrecogin alfa
GGCX
Coagulation factor VIIa Human
Phylloquinone
Coagulation Factor IX (Recombinant)
Coagulation Factor IX Human
Menadione
Glutamic acid
NSP8
ORF7b
HMOX2
NSP4
M
TGB1
S
ORF7a
ORF3a
Antithymocyte immunoglobulin (rabbit)
MTR
GJA1
Hydroxocobalamin
Cyanocobalamin
Methionine
Thimerosal
Carvedilol
SLC12A2
Potassium chloride
Bumetanide
Quinethazone
Torasemide
SMPD4
Phosphatidyl serine

What drugs target these identified proteins?



How do different drugs interact differently within our genome?



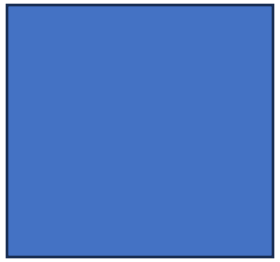
Cell viability

Percent inhibition

Future research??

Summary

AP-MS has been used to further look into human proximal protein reactions with SARS-CoV-2, however integrating this with other data, such as TurboID, allows for more information to be determined regarding protein interactions



Approximately 1,388 interactions were found between proximal proteins and SARS-CoV-2, notably finding manipulations in key cellular processes regarding antiviral and immune response

Three compounds were identified that inhibit replication of SARS-CoV-2 : azacitidine, thimerosal, and verteporfin


Questions?

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