Modifications of FUS and implications in ALS and FTD

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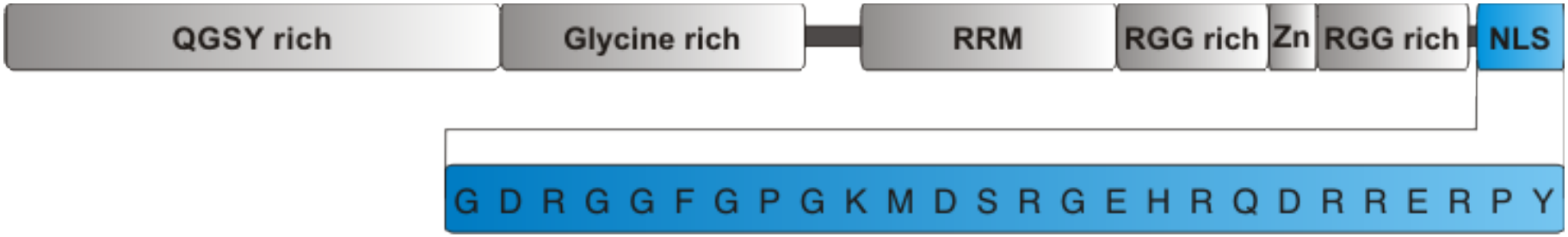
Fused in sarcoma



Implication in cancer and neurodegeneration.

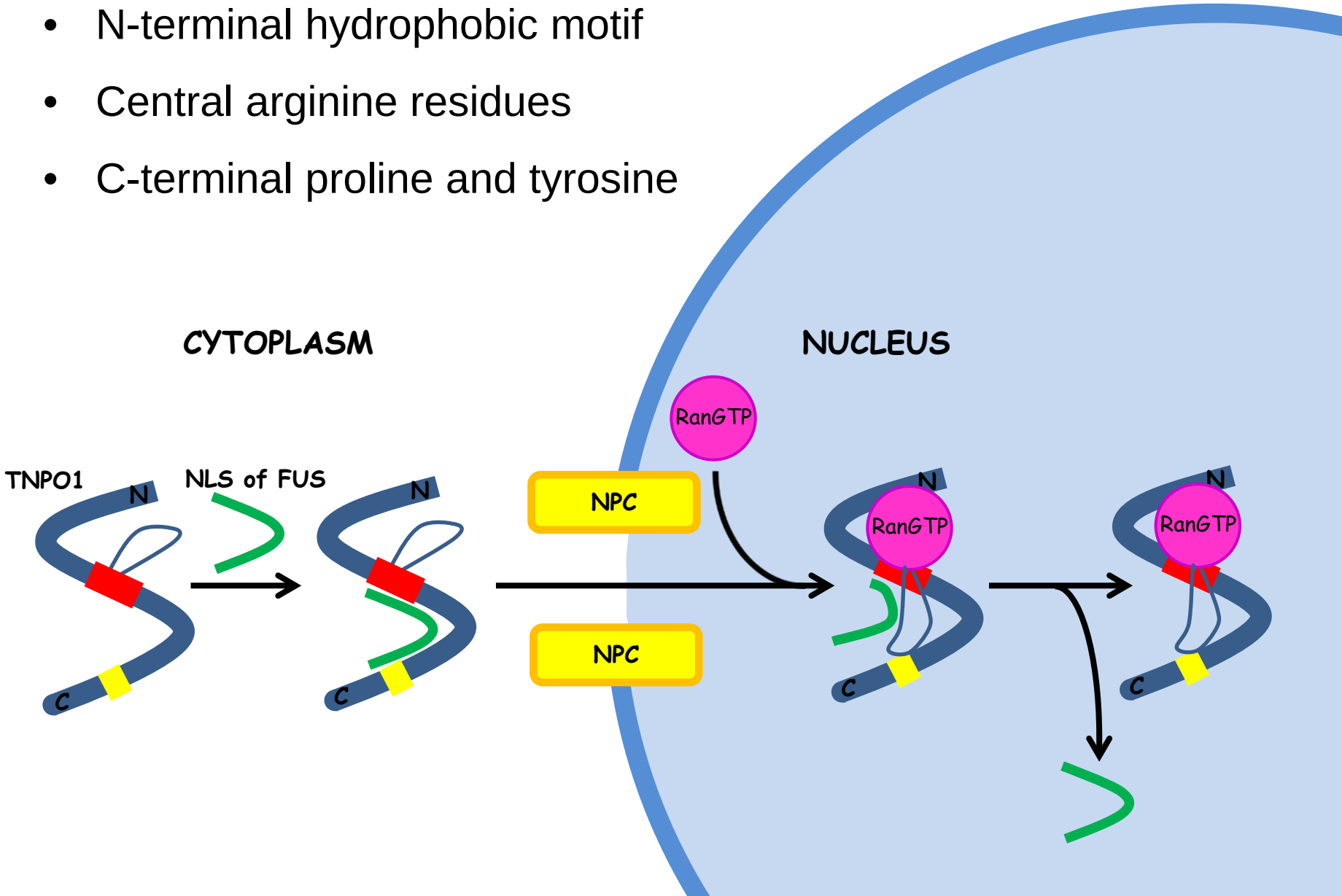
FET family of multifunctional RNA/DNA binding proteins (FUS, EWS, TAF15).

Nuclear protein with non-classical PY type NLS at its extreme C-terminus.

NLS mediates interaction with Transportin-1 and enables transport of FUS through nuclear membrane.

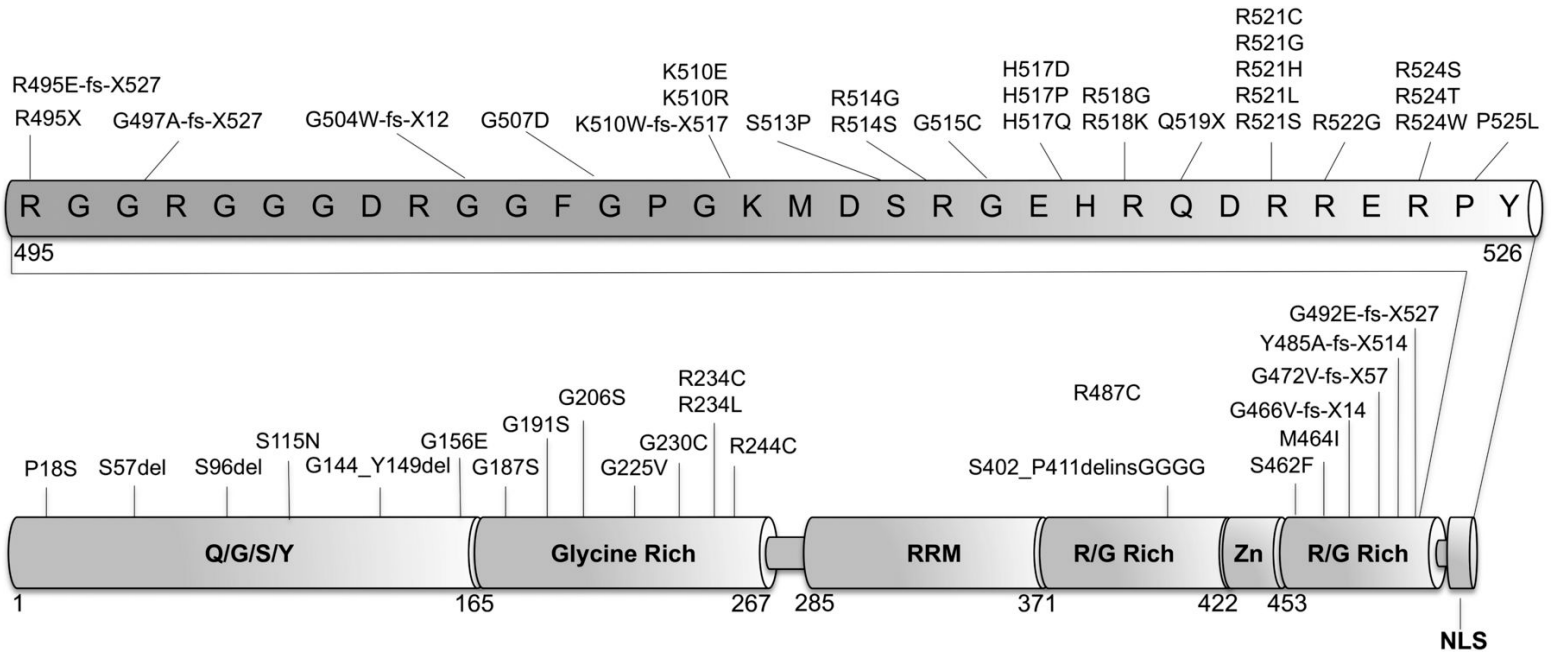
Rules of PY type NLS:

- N-terminal hydrophobic motif
- Central arginine residues
- C-terminal proline and tyrosine



Mutations in FUS are responsible for 3 % of familial ALS and rare sporadic cases.

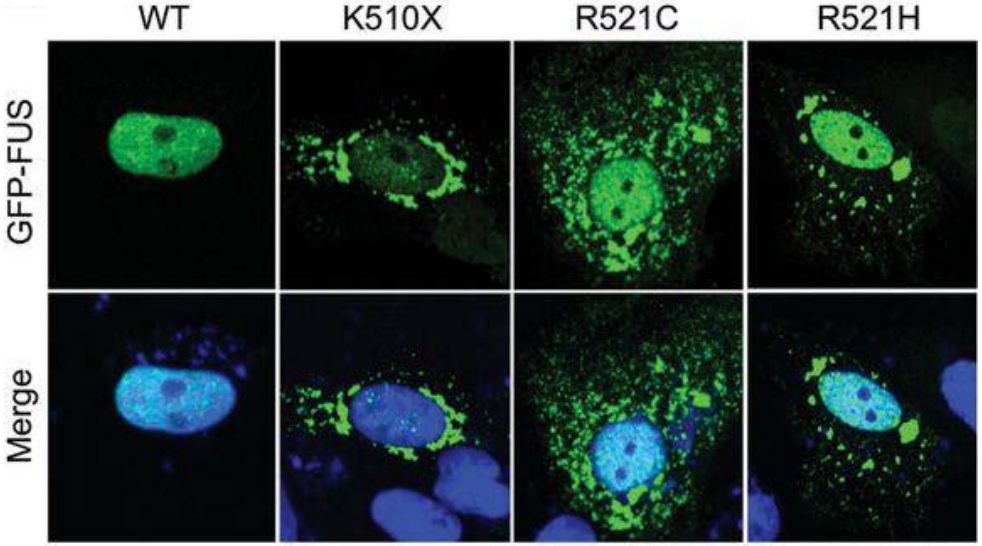
Majority of pathogenic mutations identified so far are located at the very C-terminus of FUS.



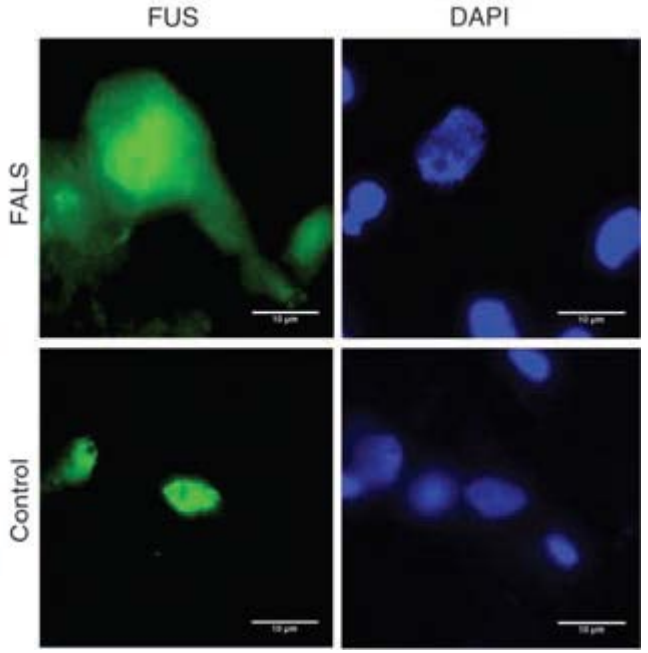
Vance et al, 2013

Mutations impair nuclear transport of FUS, which leads to abnormal cytoplasmic aggregation of protein.

ALS patients with FUS mutations have FUS immunoreactive cytoplasmic inclusions in neuronal and glial cells.



Vance et al, 2013

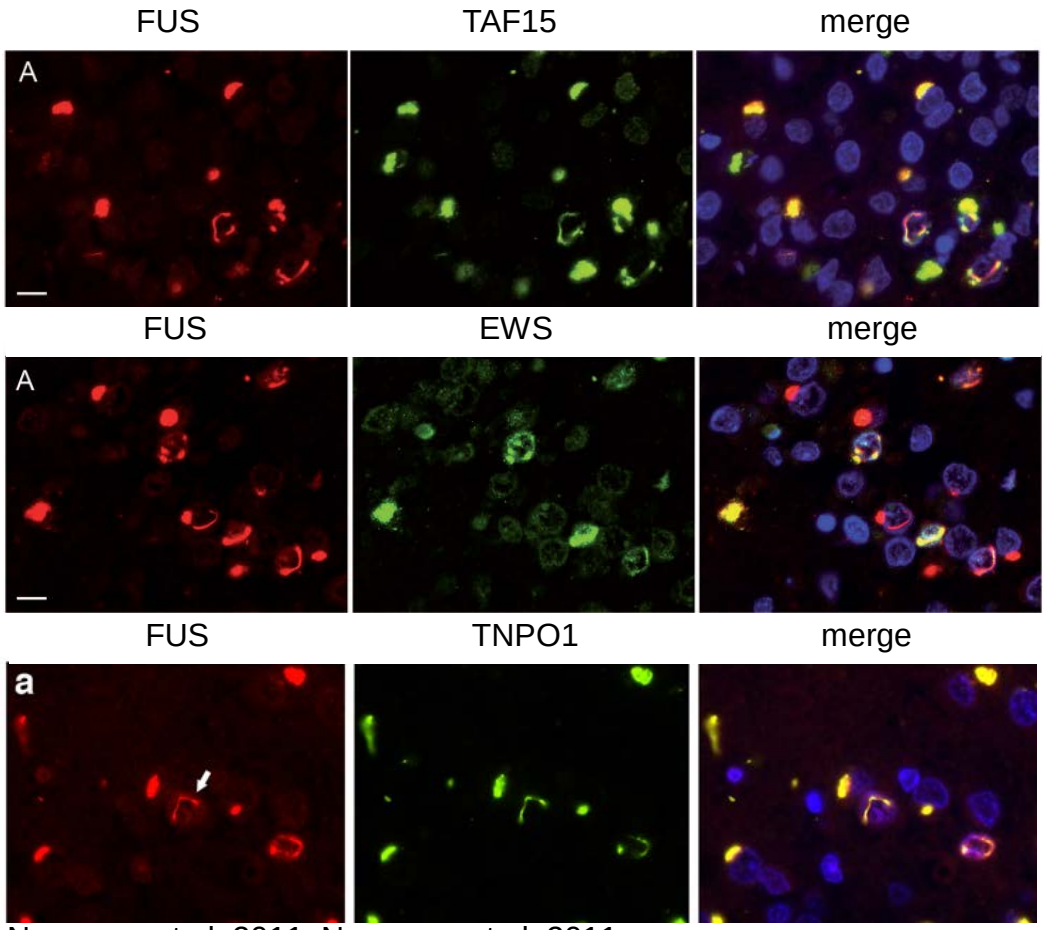


Kwiatkowski et al, 2009

Cytoplasmic aggregates of FUS are present in 10 -15 % of FTLD patients.

FUS is not mutated in FTLD-FUS patients.

FUS in aggregates colocalizes with TAF15, EWS and Transportin-1.

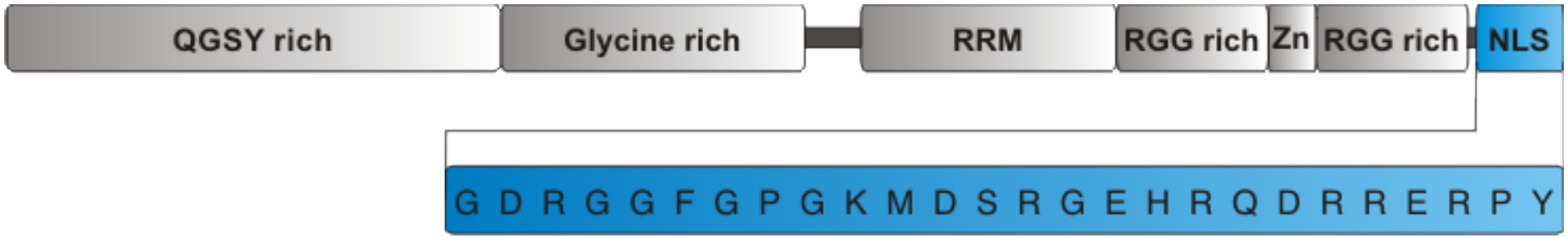


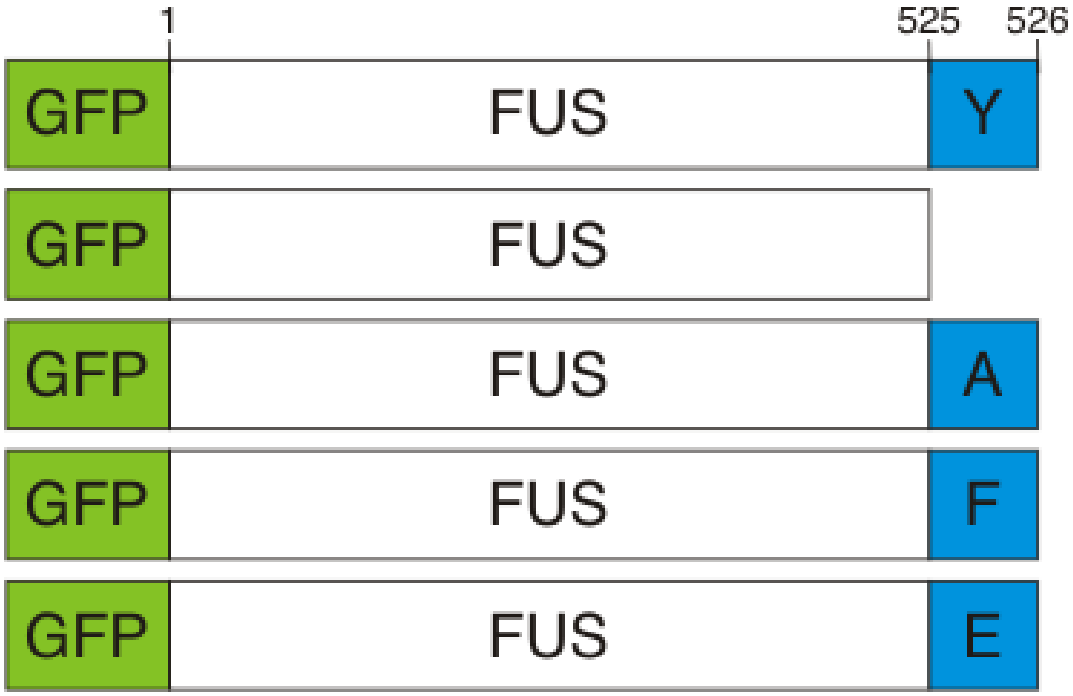
Neumann et al, 2011, Neumann et al, 2011

It is still unknown why FUS forms pathological aggregates in neurons of FTLD-FUS patients.

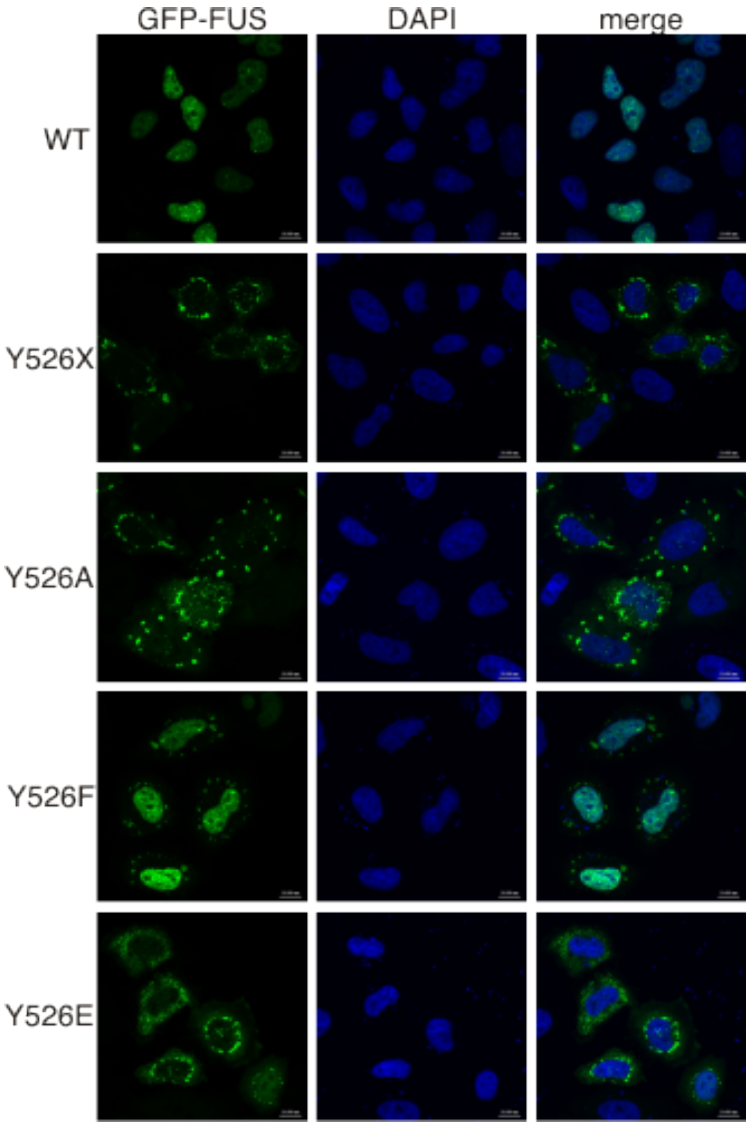
Nucleo-cytoplasmic transport can be regulated at multiple levels, including post-translational modifications of transport cargo, such as phosphorylation.

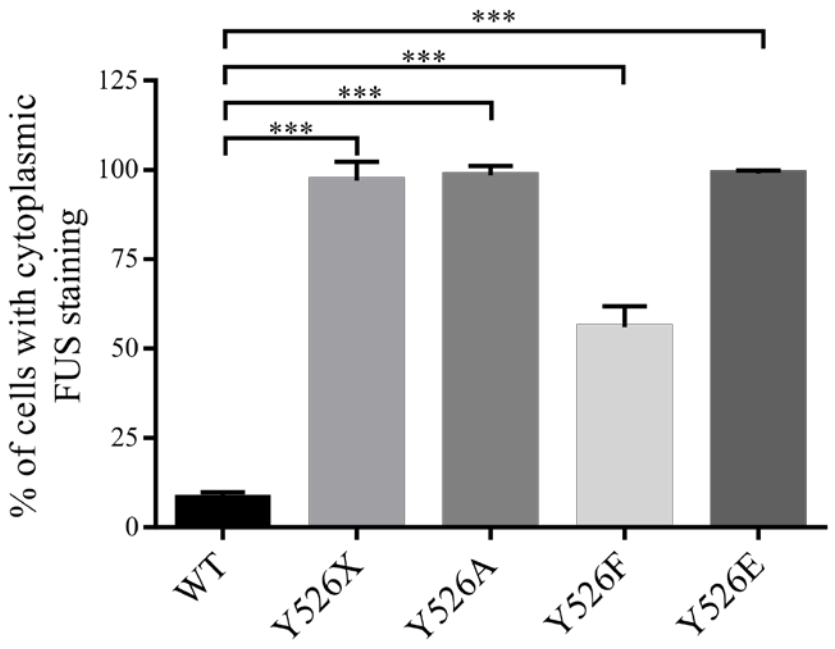
Posttranslational modification of nuclear localization signal could impair binding of Transportin-1 and lead to cytoplasmic aggregation of FUS.


QGSY rich
Glycine rich
RRM
RGG rich
Zn
RGG rich
NLS
G D R G G F G P G K M D S R G E H R Q D R R E R P Y


1
525
526
GFP
FUS
Y
GFP
FUS
GFP
FUS
A
GFP
FUS
F
GFP
FUS
E

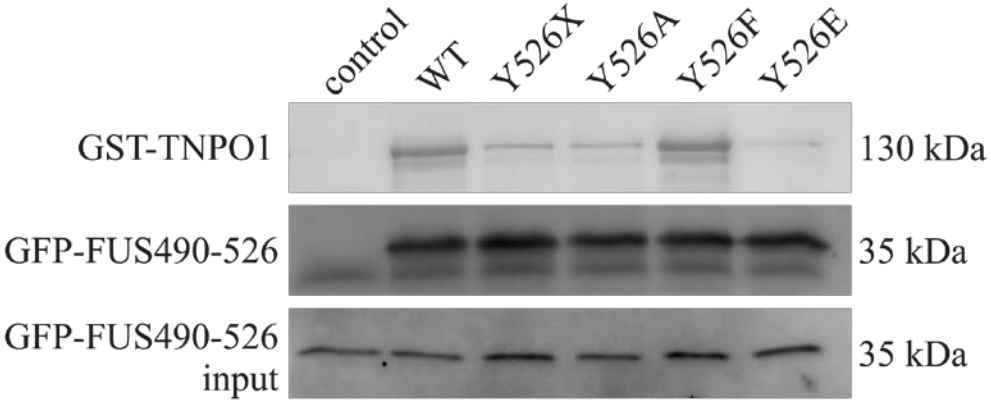
Effect of Y526 mutations on FUS localization

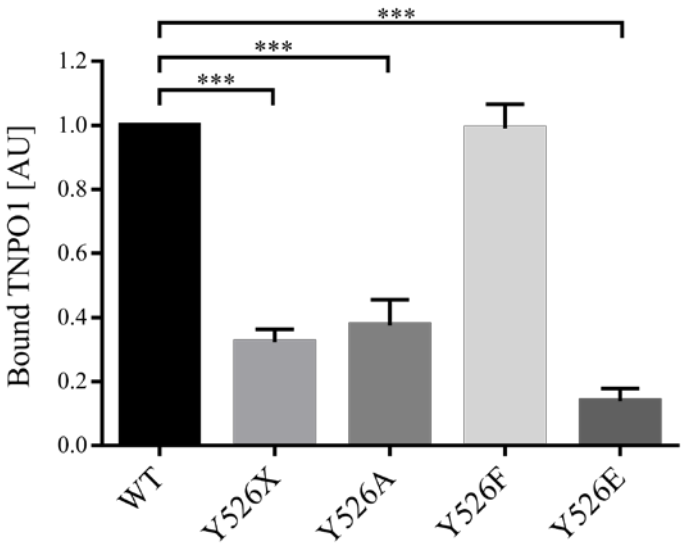




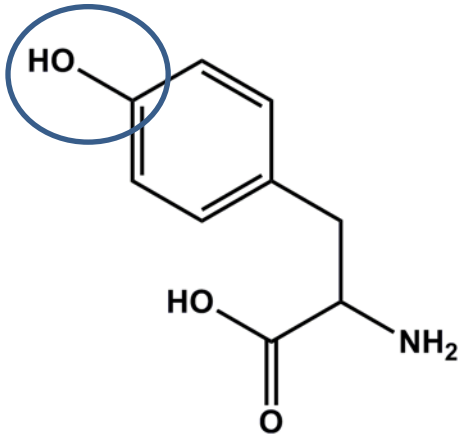
Neither alanine, phenylalanine nor glutamate can fulfil function of tyrosine 526 in nuclear import of FUS.

Effect of Y526 mutations on interaction with TNPO1

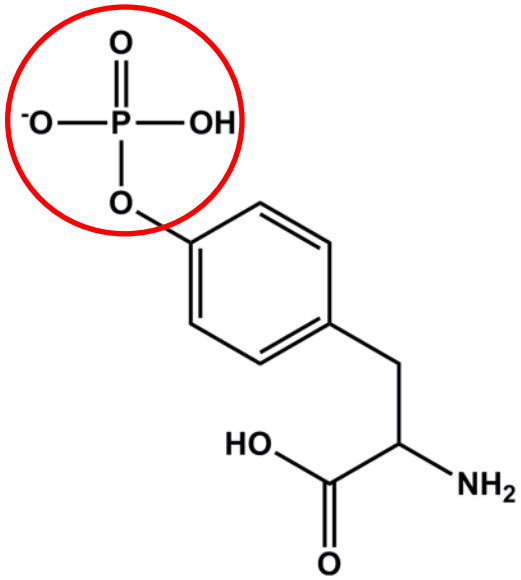




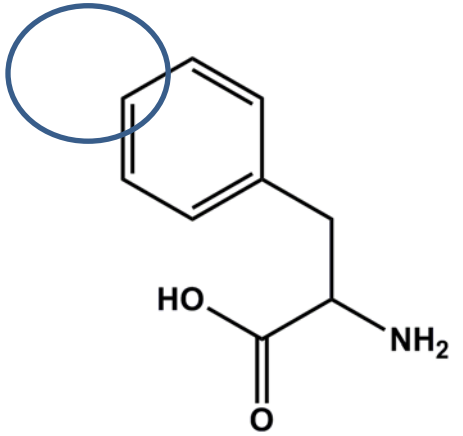
Mutants Y526X, Y526A and Y526E bound less GST-TNPO1 compared to wild type fragment, while mutant Y526F did not significantly affect binding of GST-TNPO1.



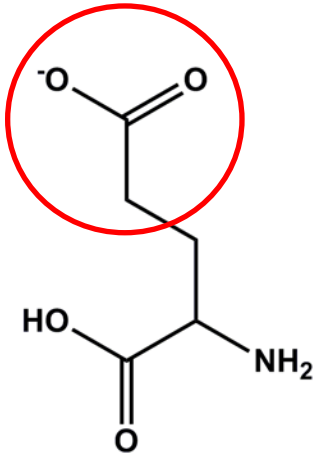
Tyrosine



Phosphotyrosine

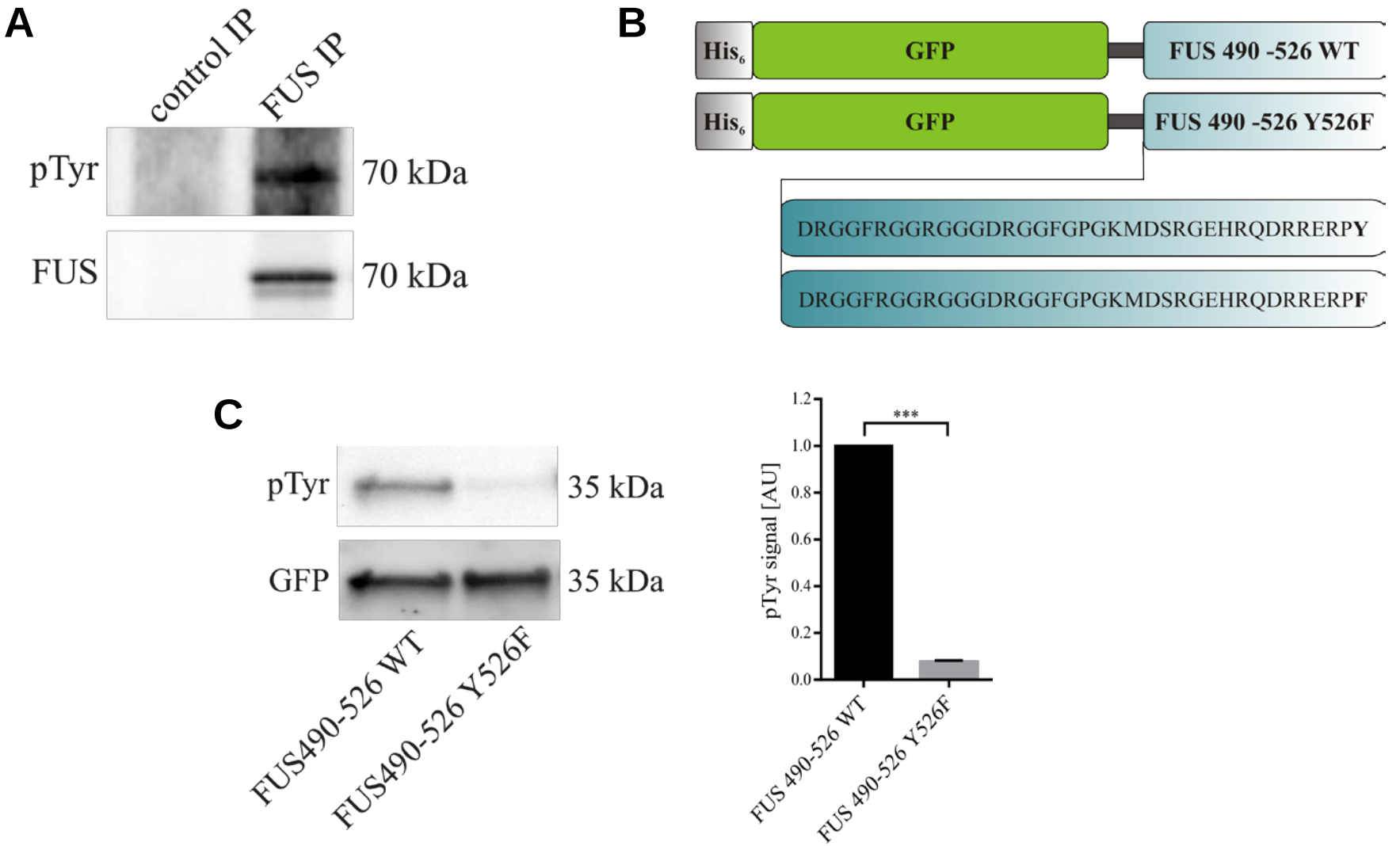


Phenylalanine

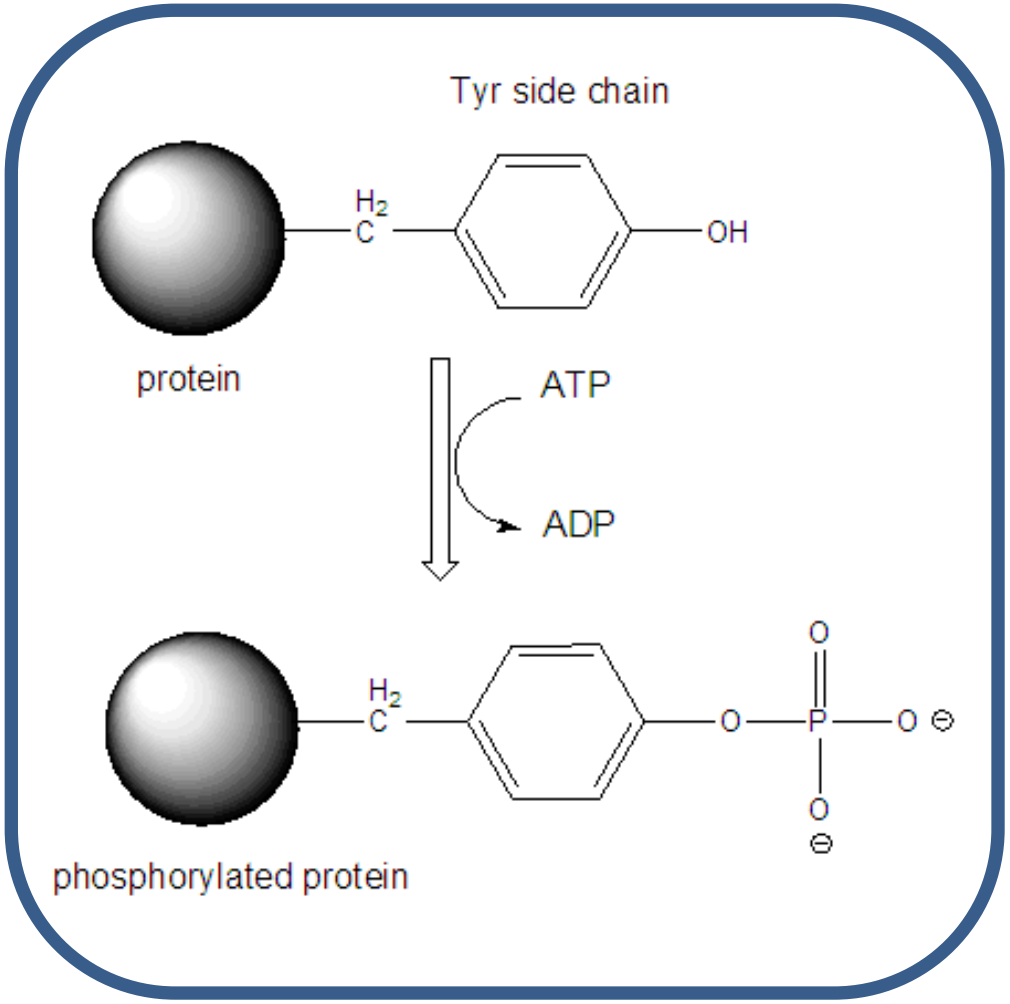


Glutamate

C-terminal tyrosine in FUS is phosphorylated



FUS undergoes phosphorylation on C-terminal Y526.

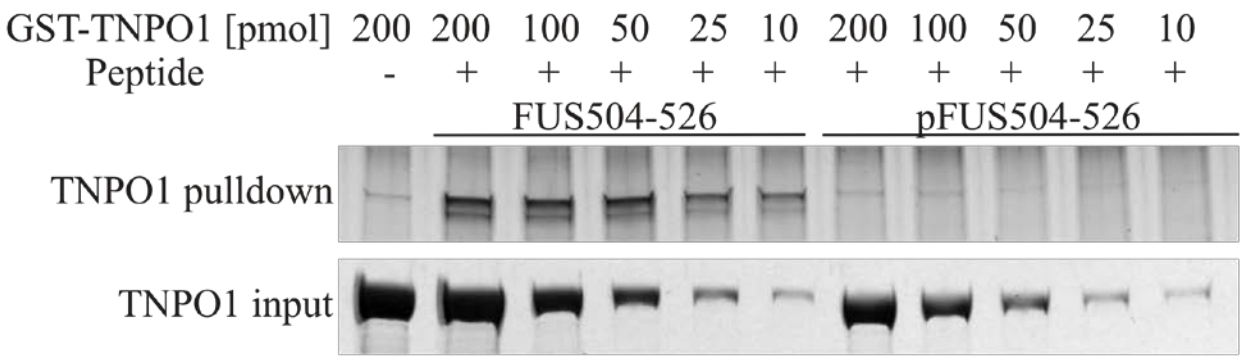


Negative charge

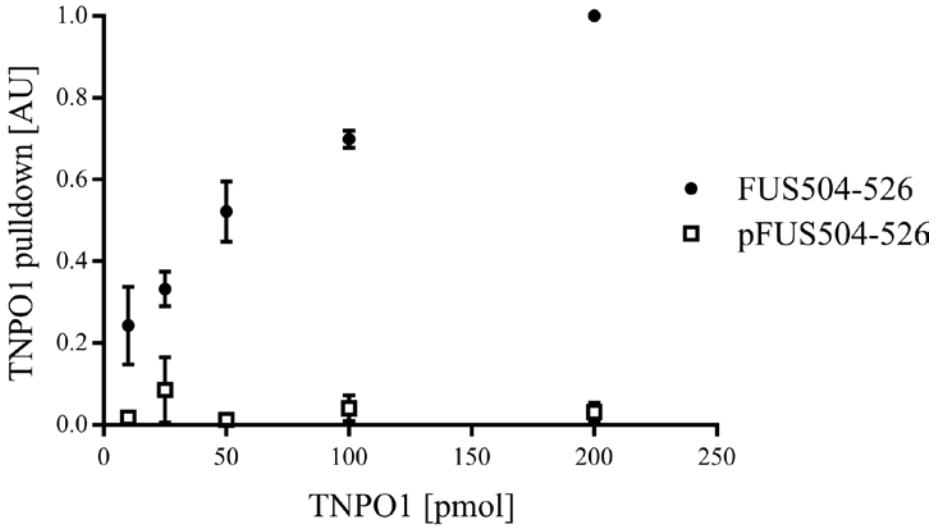
Enlargement

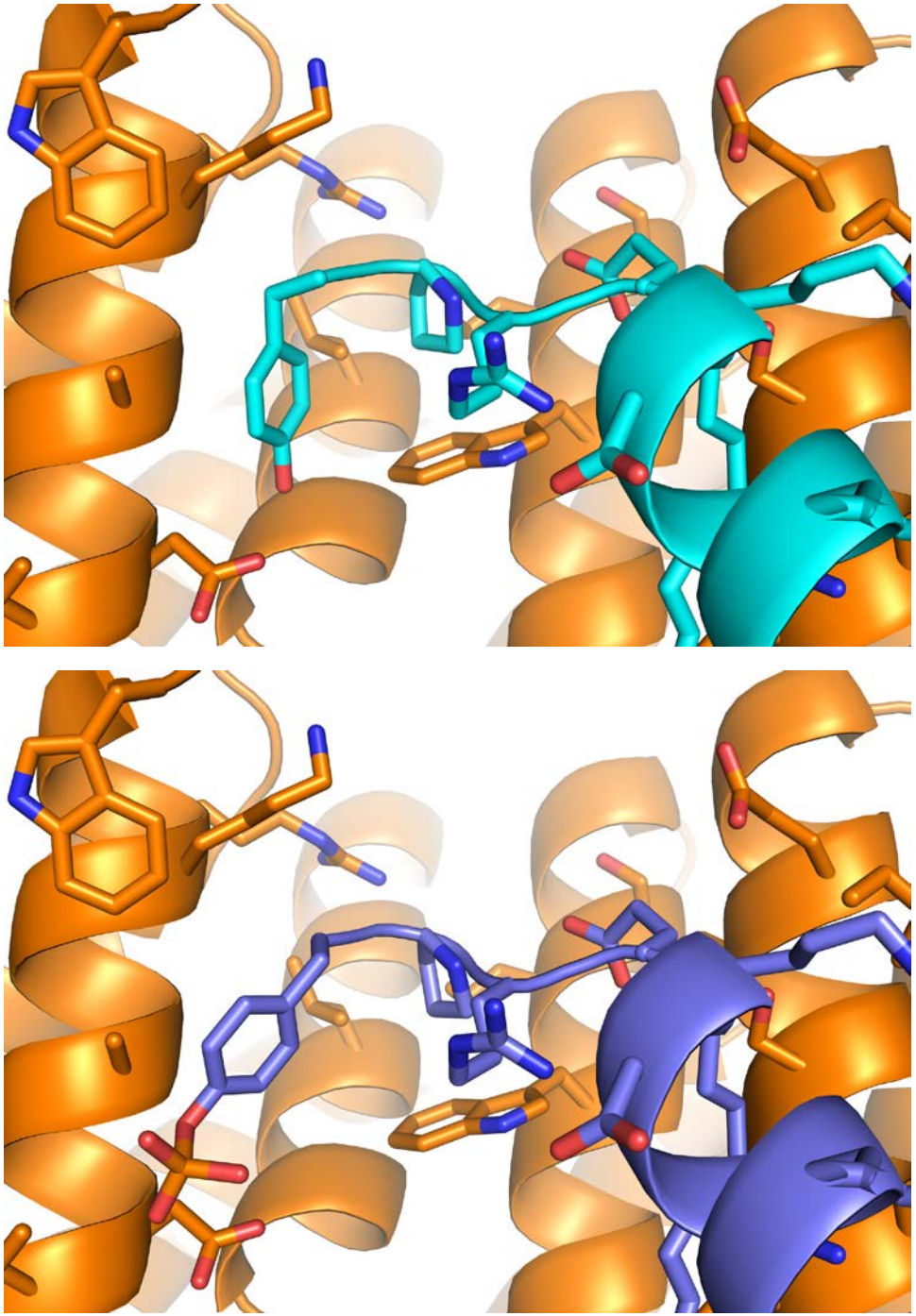
Impact on interaction with Transportin-1

Effect of Y526 phosphorylation on interaction with Transportin-1



Phosphorylation of Y526 impairs interaction with Transportin-1.





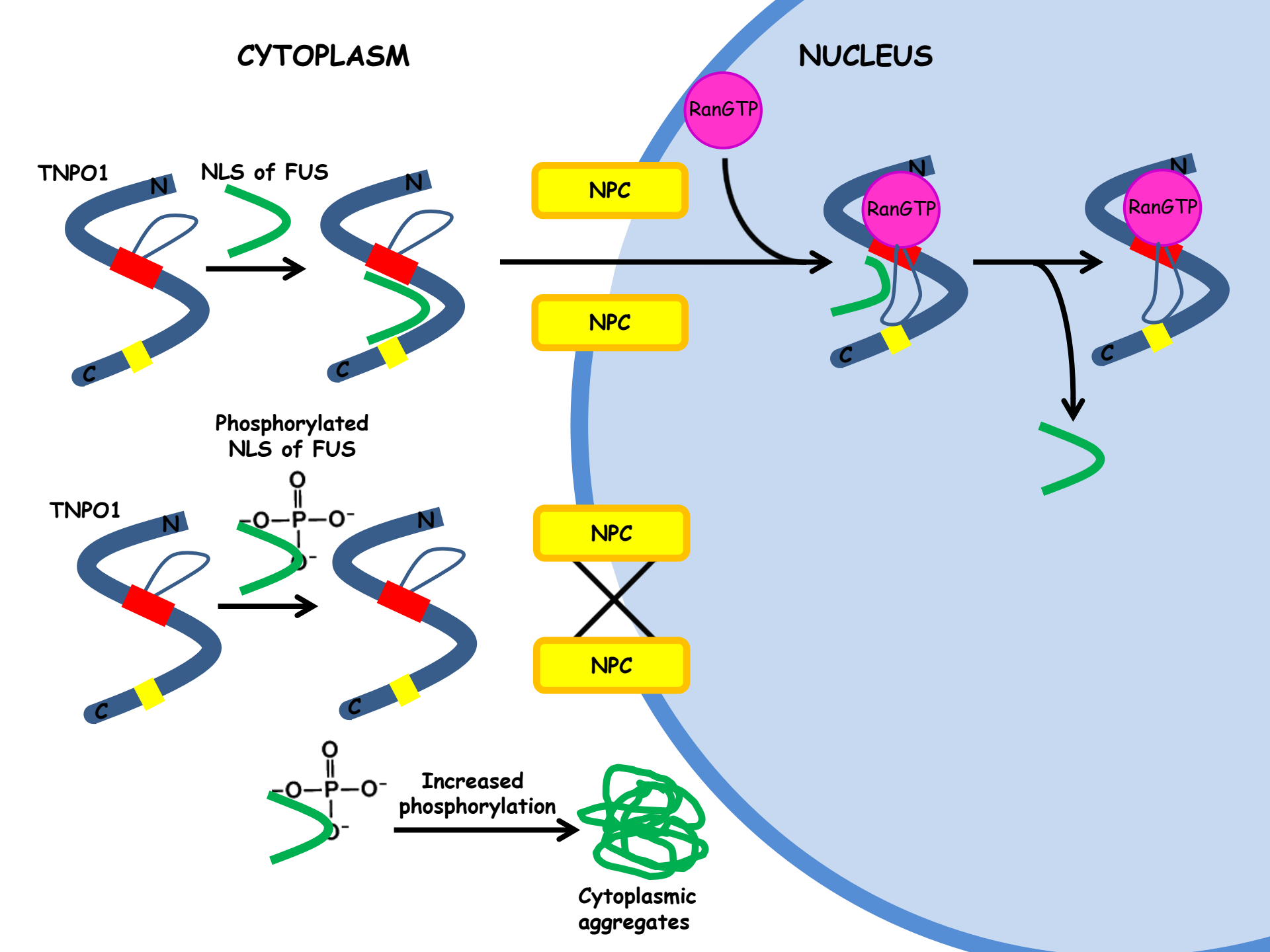
Y526 is important for interaction with TNPO1 and normal nuclear import.

Y526 undergoes phosphorylation.

Phosphorylation of Y526 abolishes interaction with TNPO1.

Does phosphorylation serve for dissociation of FUS from TNPO1 in nucleus?

Can increased phosphorylation of FUS in cytoplasm prevent its transport into the nucleus?


CYTOPLASM
NUCLEUS
TNPO1
NLS of FUS
N
C
NPC
NPC
RanGTP
RanGTP
RanGTP
Phosphorylated
NLS of FUS
TNPO1
NPC
NPC
Increased
phosphorylation
Cytoplasmic
aggregates

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Thank you for your attention