Abnormal expression of TIP30 and arrested nucleocytoplasmic transport within oligodendrocyte precursor cells in multiple sclerosis

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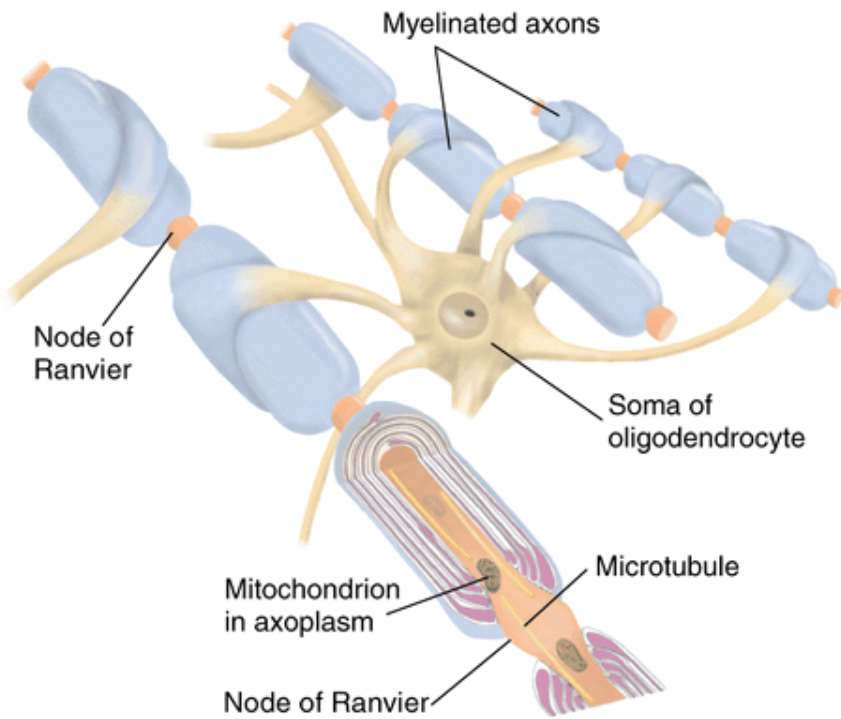
Commentator: Thy-Sheng Lin & Shun-Fen Tzeng

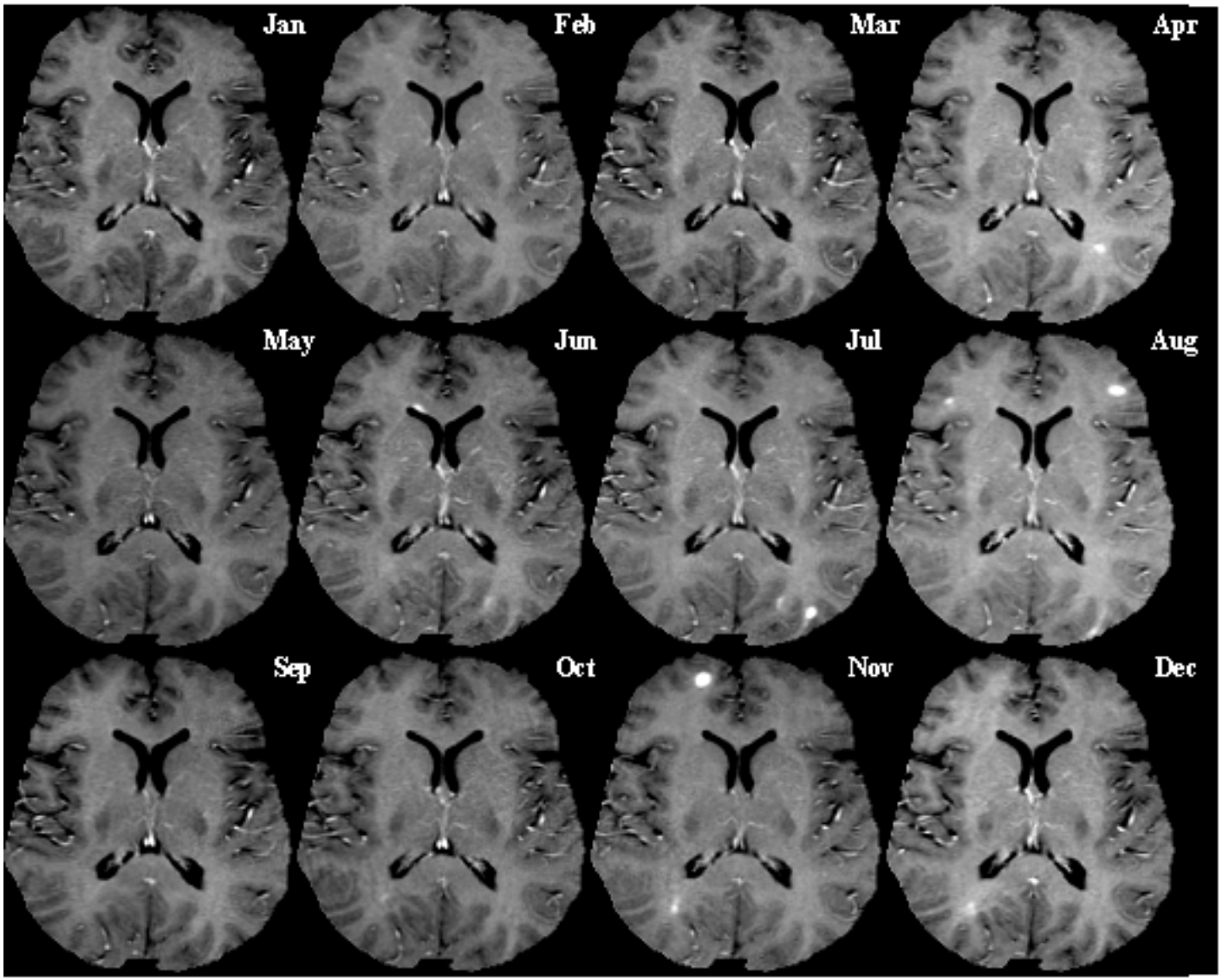
Multiple sclerosis (MS)

- **Multiple sclerosis** is an autoimmune condition
- attacks the central nervous system, leading to demyelination.

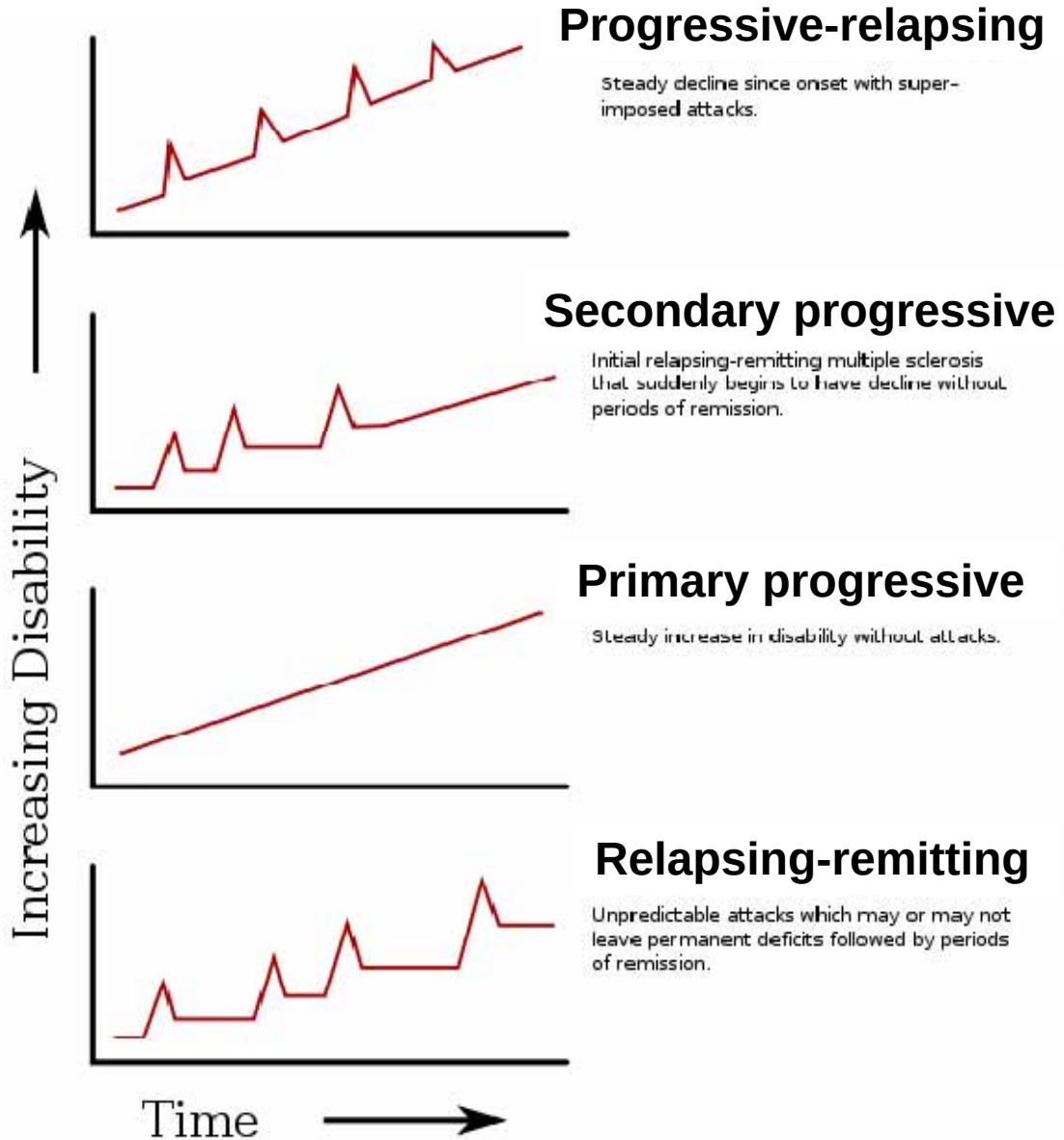


http://en.wikipedia.org/wiki/Multiple_sclerosis

Multiple sclerosis



Multiple sclerosis



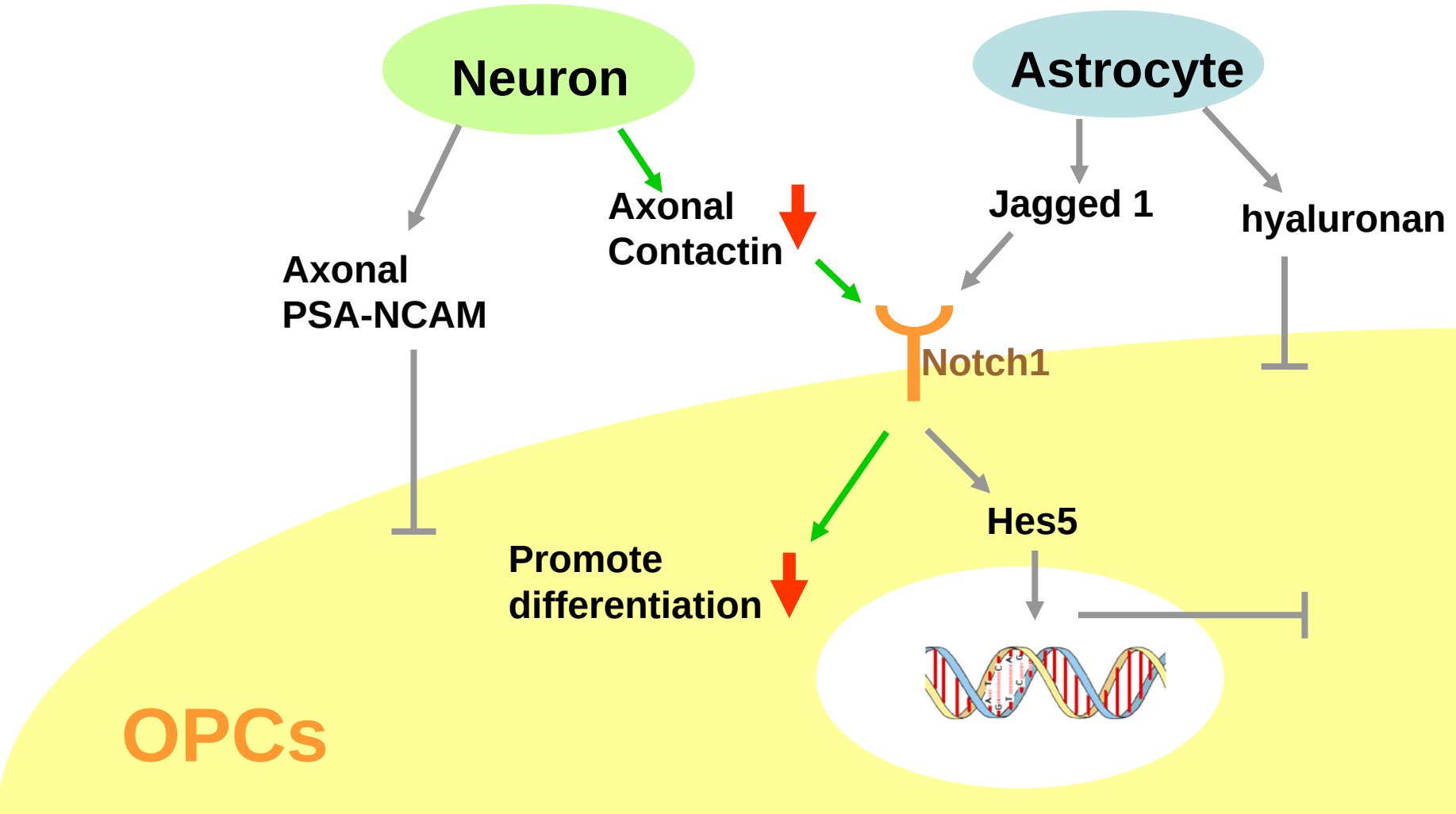
Chronic MS

- Persistent demyelination even in the absence of active inflammation

- Oligodrndrocyte precursor cells (OPCs) are preserved in demyelinated lesion but are failure to differentiate into mature oligodendrocytes.

Why?

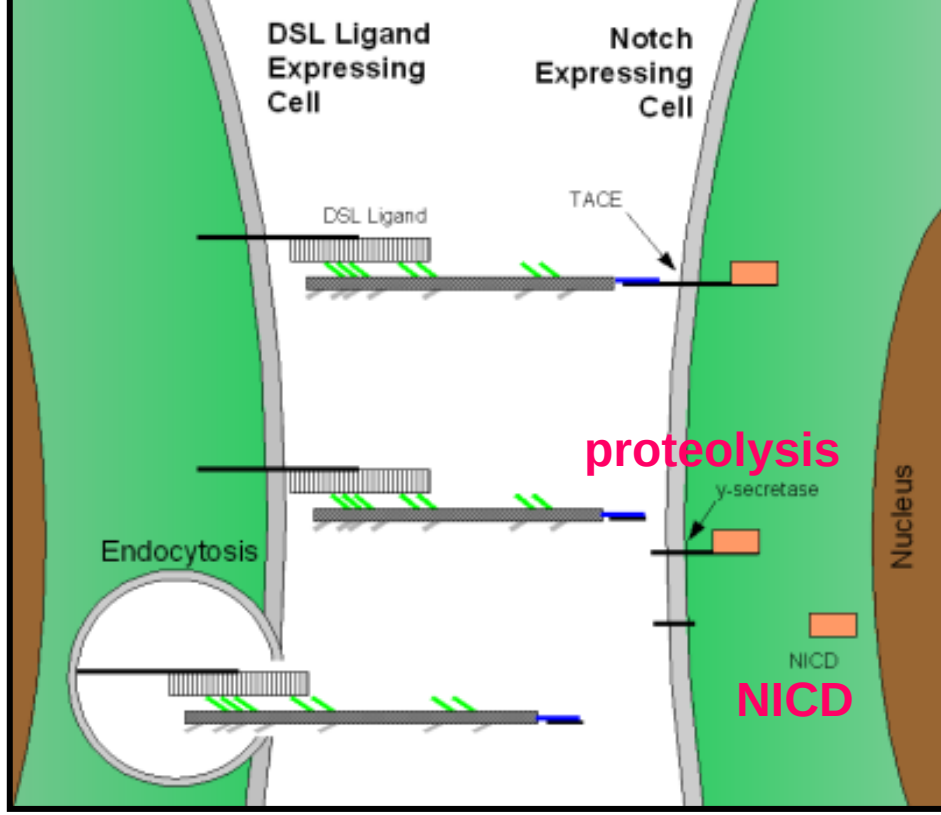
Possible mechanisms that inhibit differentiation of oligodendrocytes

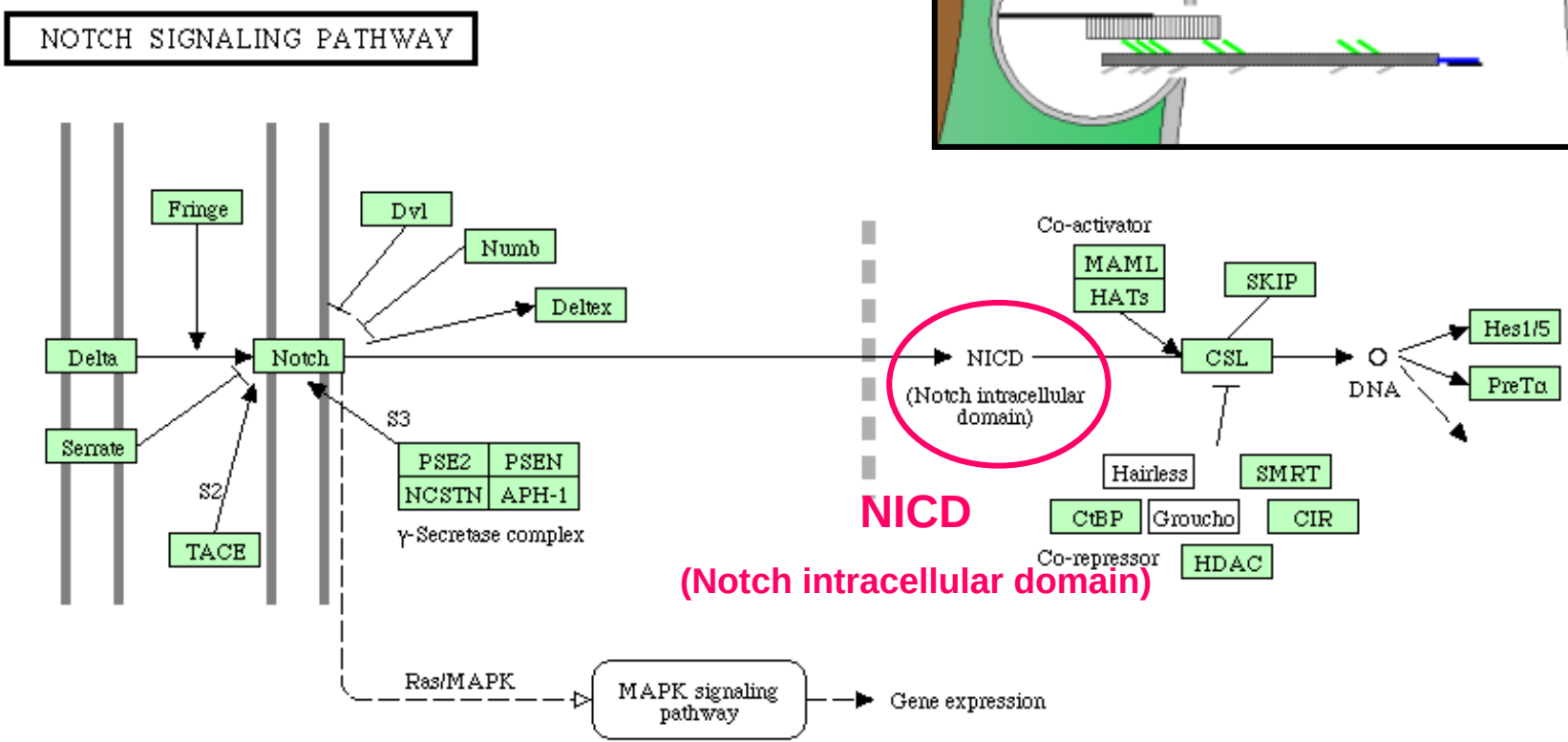


Contactin

- a glycosylphosphatidylinositol-anchored neuronal cell adhesion molecule
- promotes neurite outgrowth
- induction of oligodendroglial differentiation
- expression is dependent on neuronal electrical activity
- could be detectable in the CSF
  - developing brain
  - some but not all MS patients

Notch 1 signaling





Hypothesis

- The upregulation of Contactin may be altered in the preserved axons, possible due to impaired activity, and that this reduction contributes to the failure of remyelination in MS

Specific aims

- To check the expression pattern of Contactin on axons in demyelinated MS
- To detect Contactin/Notich1 signaling within OPCs in MS lesion
- To clarify the cytoplasmic aggregation of NICD
- To exam the expression of TIP30, an importin β inhibitor, in MS lesion.
- To determine the relationship between TIP30 and remyelination
- To test the effect of TIP30 on nuclear translocalization and OPCs differentiation.

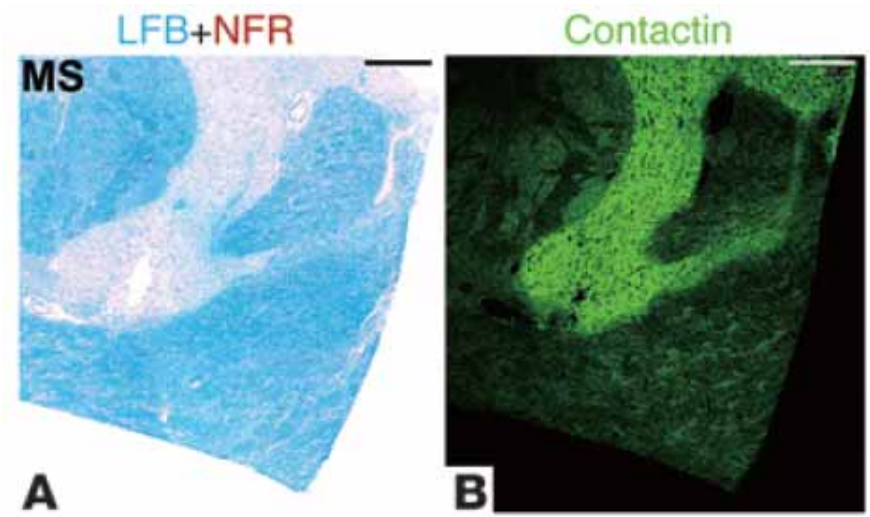
Brain tissue

Cell

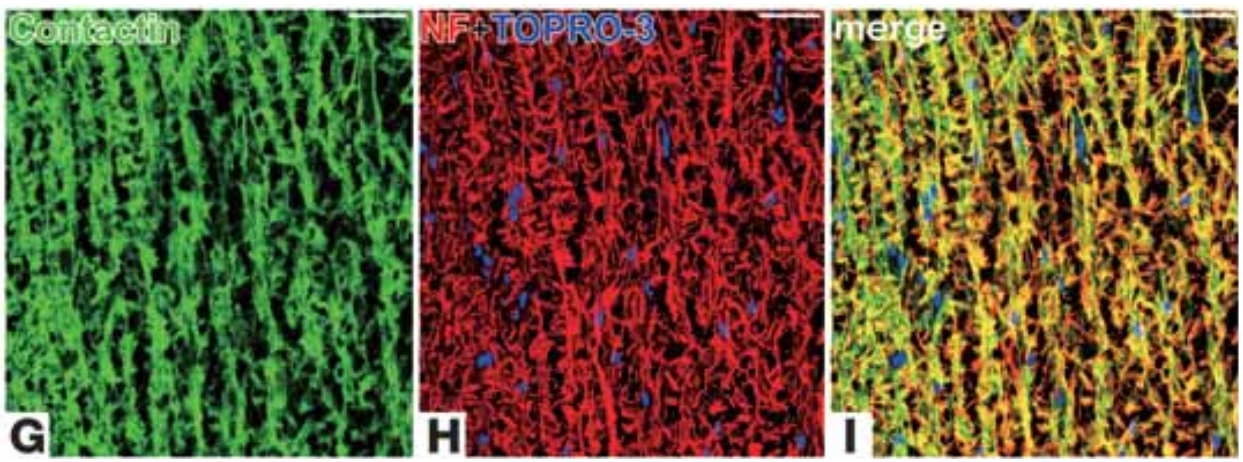
Results

Demyelinated axons in MS express Contactin

Contactin expressed in demyelinated lesion

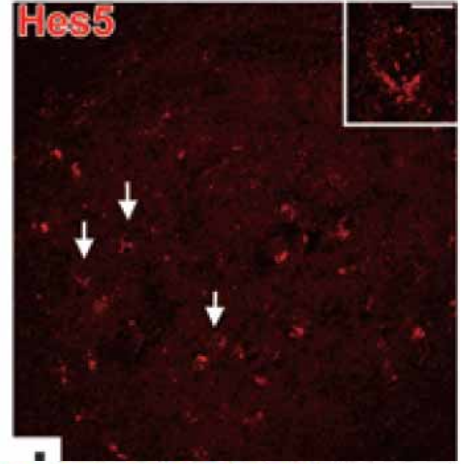


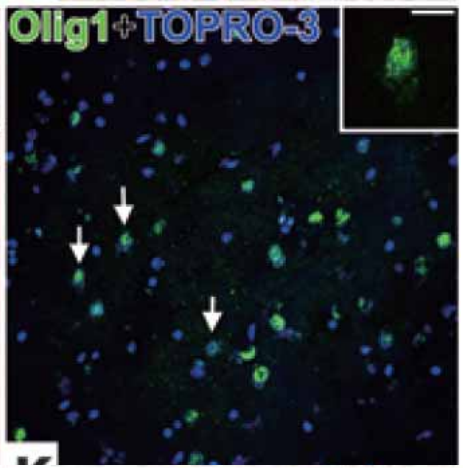
Contactin expressed on axonal surface

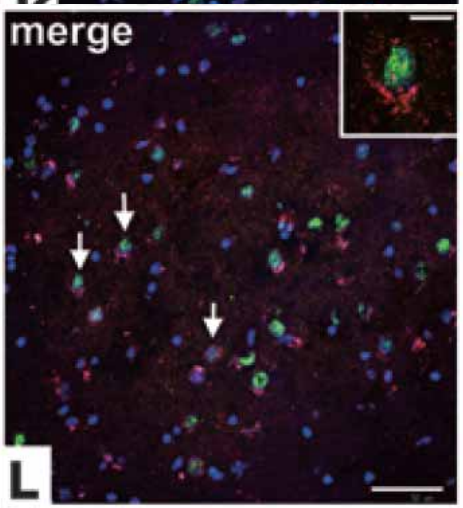


Contactin Neurofilament Merge

Autopsied brain sample from 10 chronic MS patients and 5 normal subjects

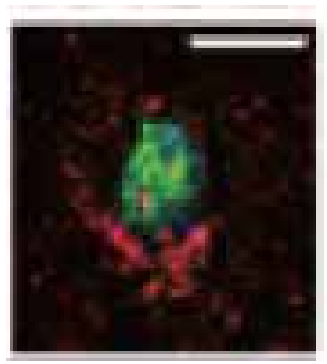


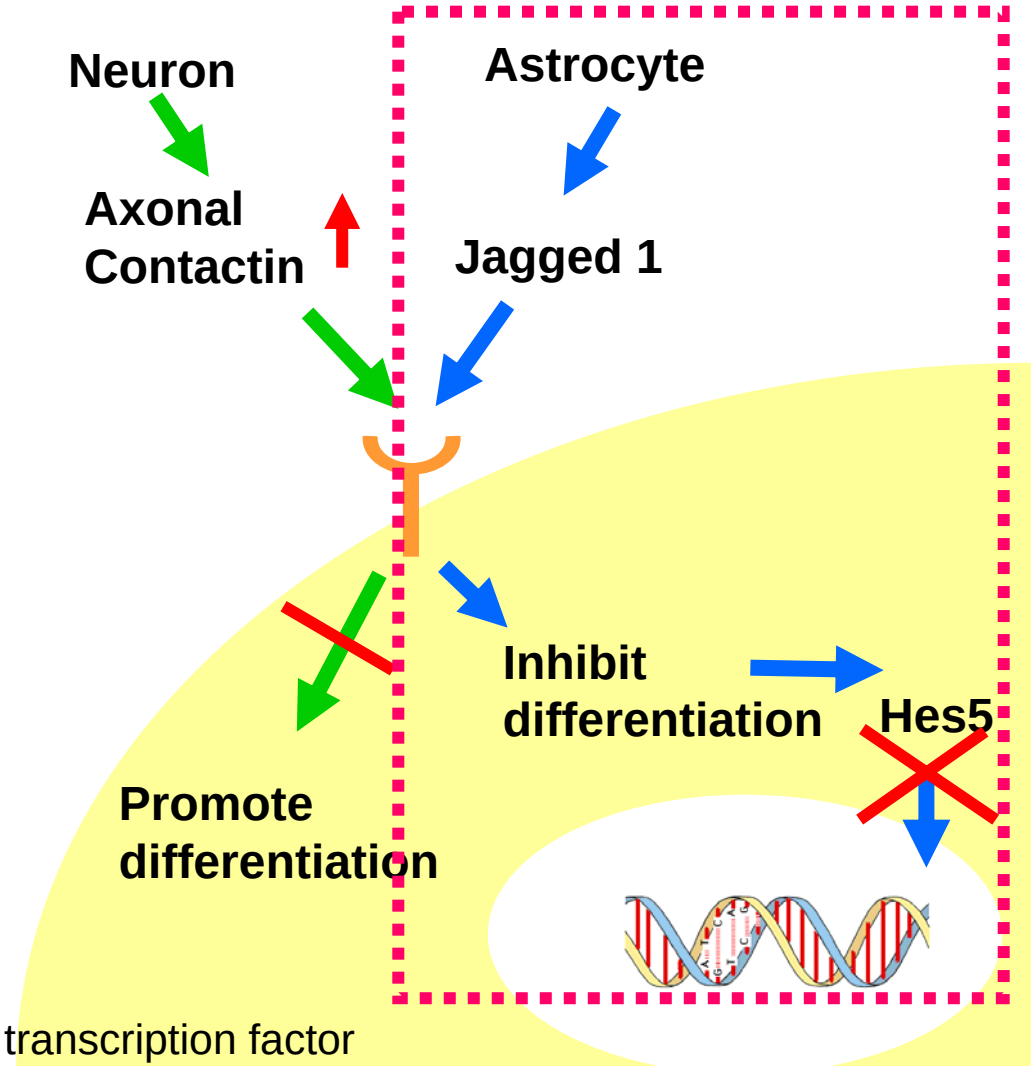




Hes5 was detected weakly in OPCs recruited for remyelination

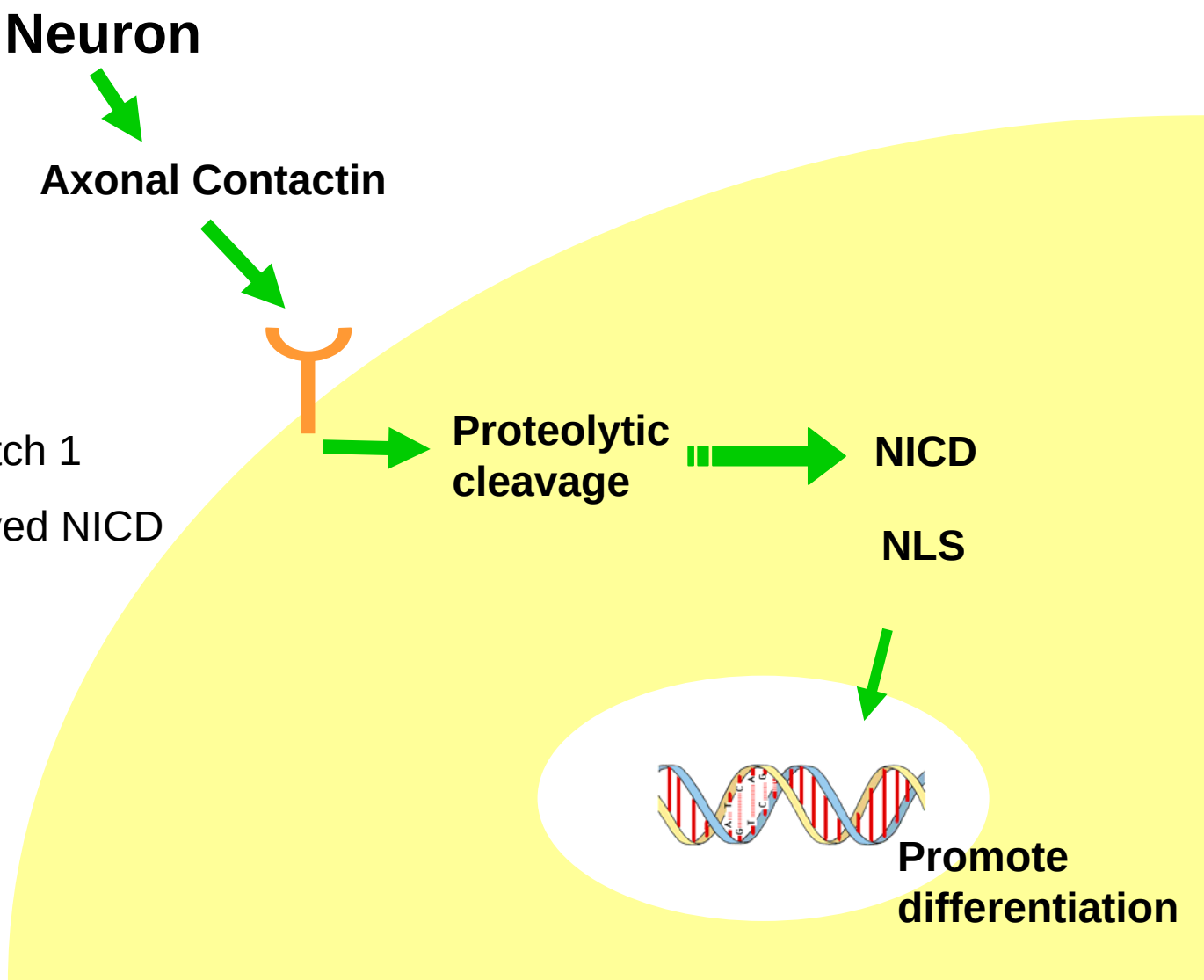
The remyalination failure was unlikely to be due to the lack of axonal signals.





Olig1: nuclear Olig1 transcription factor

Contactin/Notch1 signaling

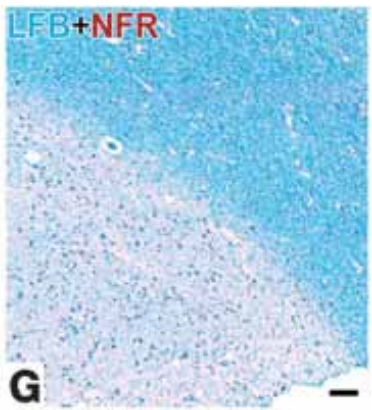


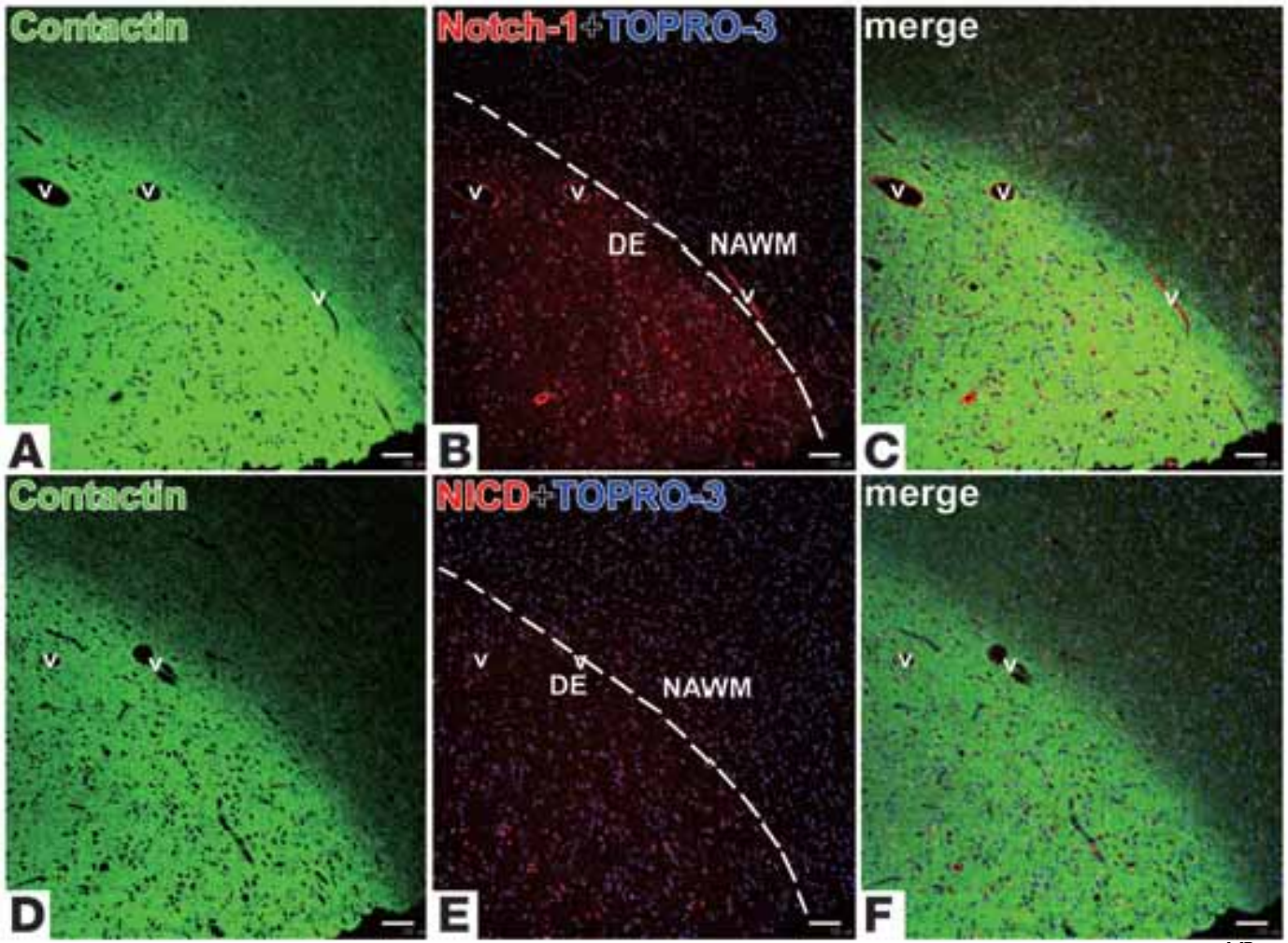
Two Abs:

Against full-length Notch 1

Against val1744-cleaved NICD

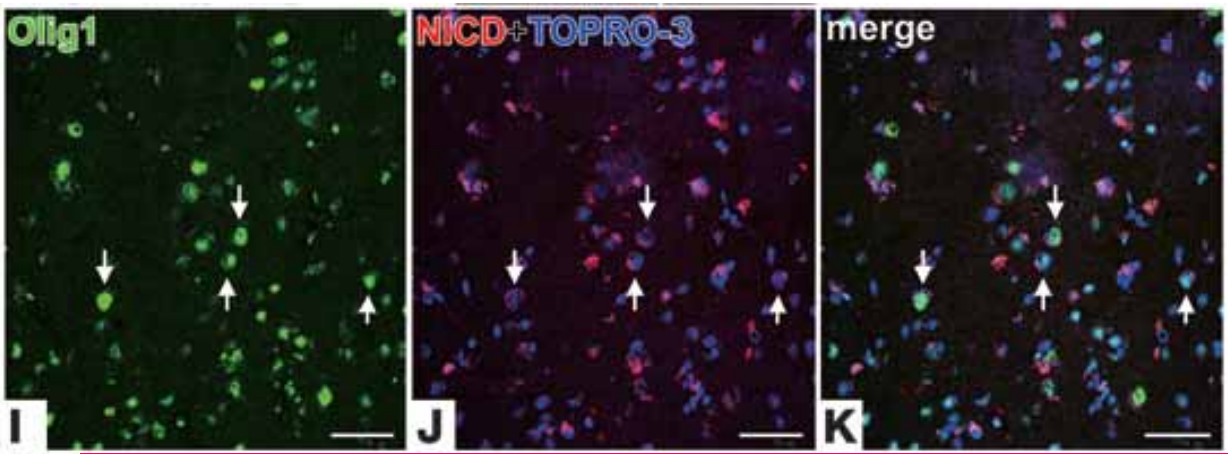
Detection of axoglial Contactin/Notch1 signaling within OPCs in MS lesions

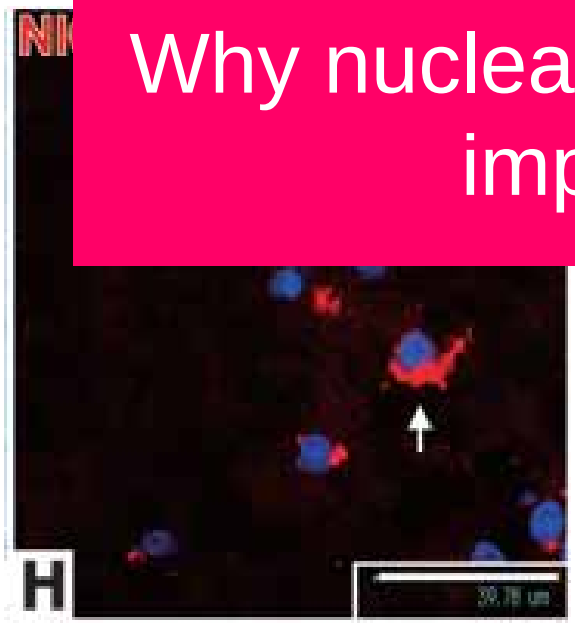




The receptor engagement and sequential cleavages of Notch1 did take place in demyelinated region

NICD was aggregated in the cytoplasm





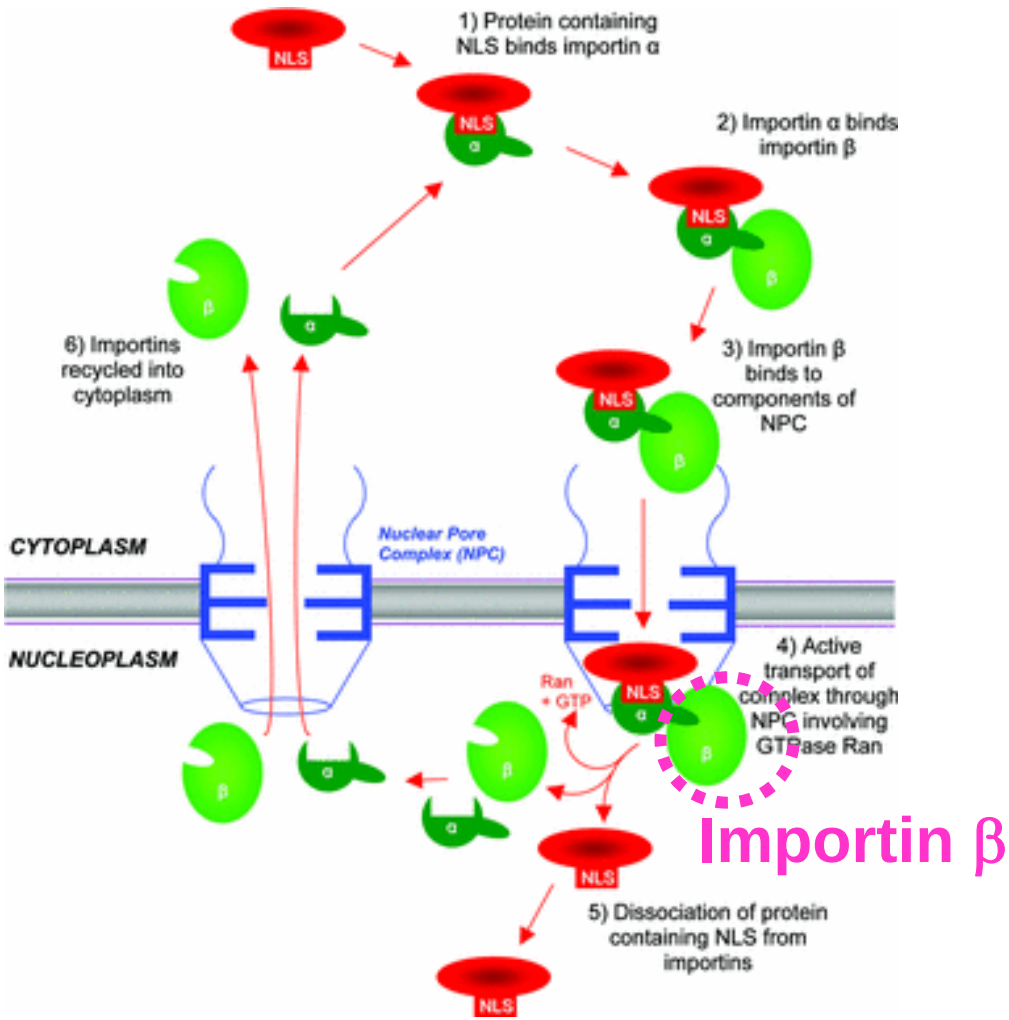
Axonal Contactin

Proteolytic cleavage

Why nuclear translocalization of NICD is impaired in the OPCs?

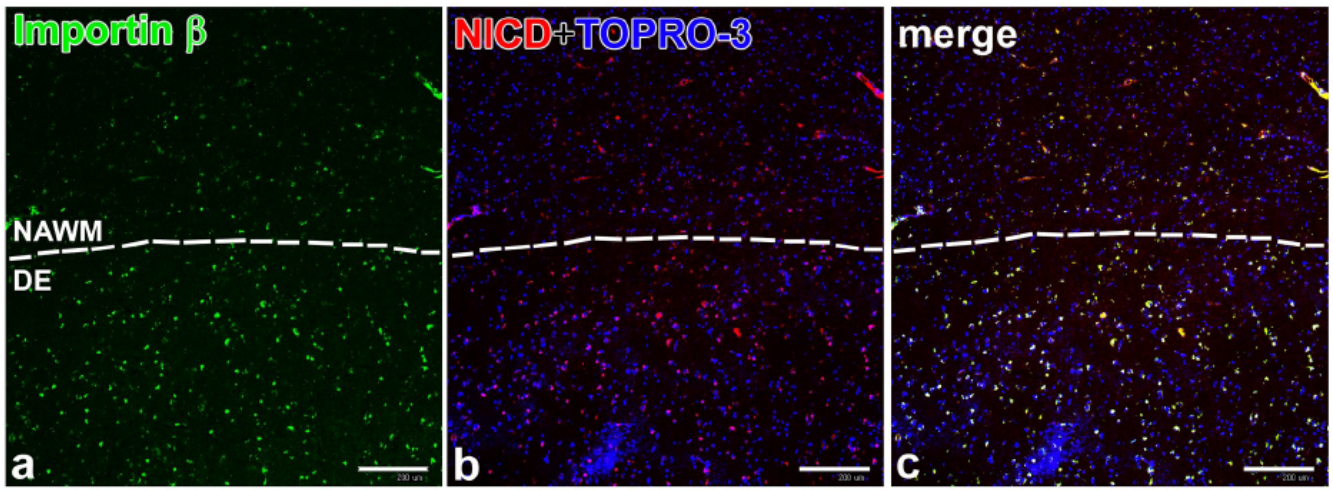
Promote differentiation

Nuclear translocation

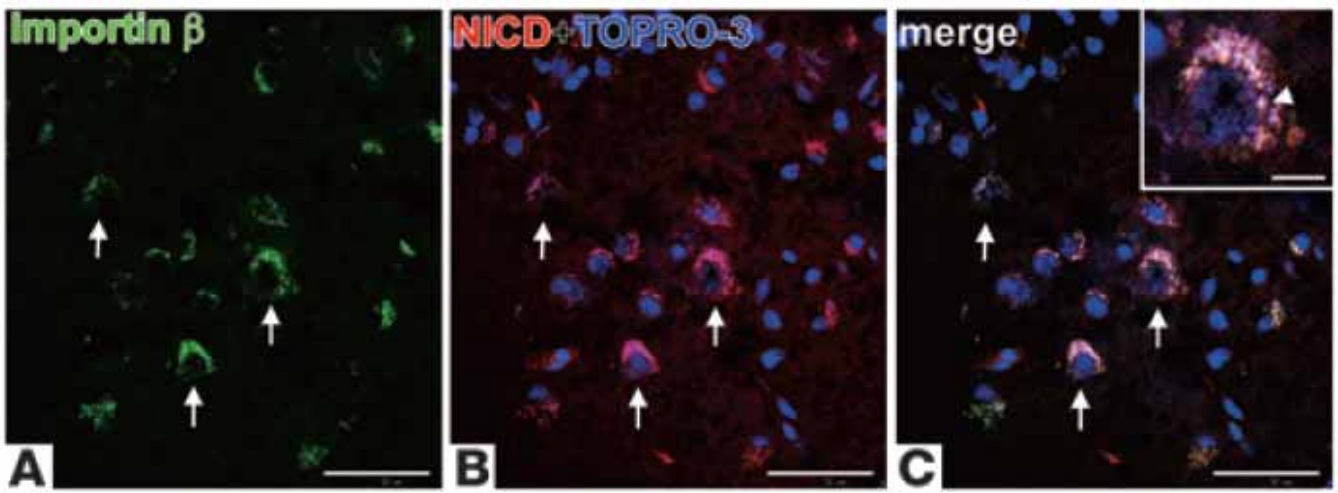


- determine nuclear import velocity
- Previous microarray analysis: reduced Importin β mRNA levels in MS lesion

Importin β was detected in the demyelinated lesions

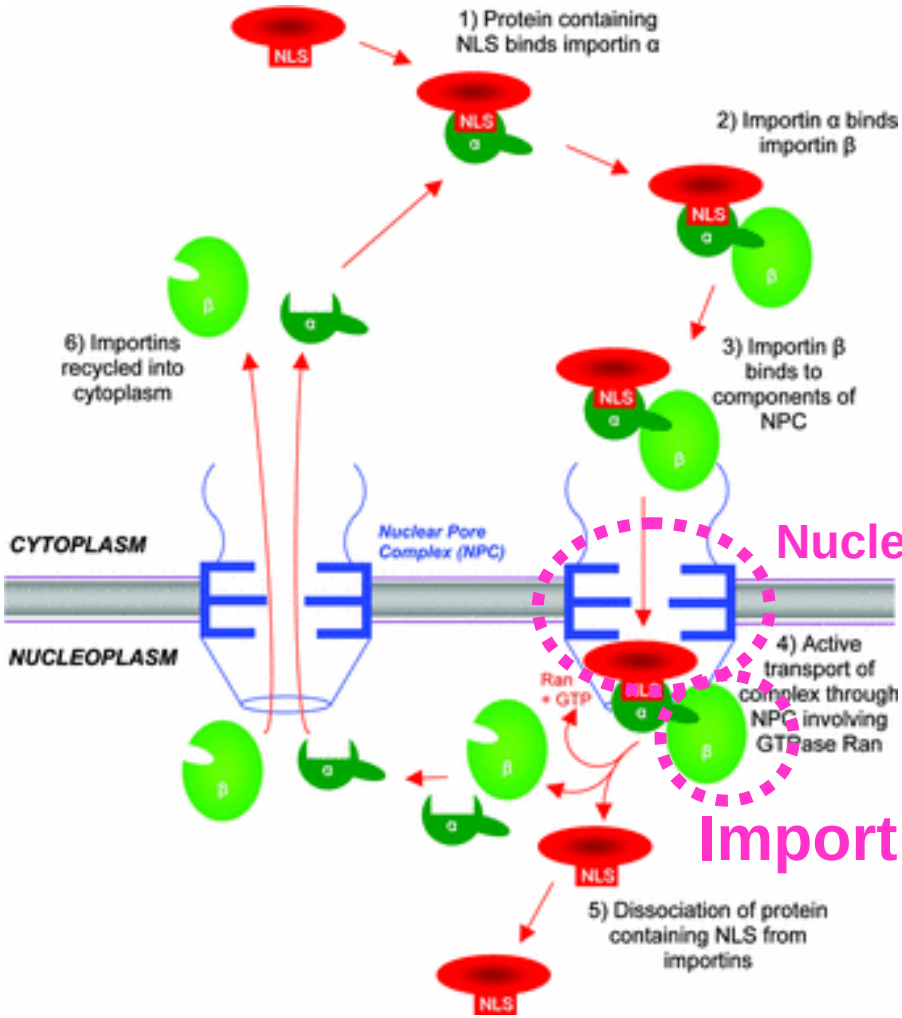


Contrary to the results of microarray



Colocalize with cytoplasmic NICD aggregates

Nuclear translocation



Nup153:

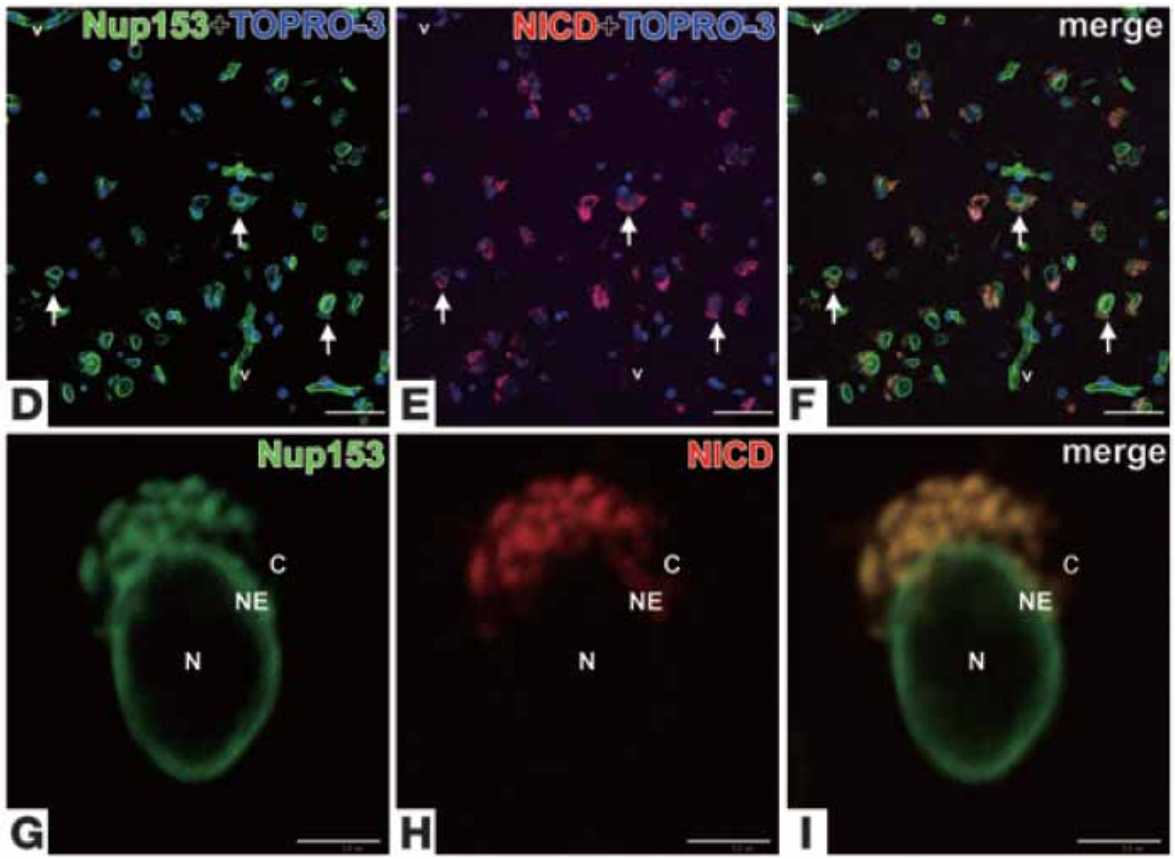
key protein involved in the formation of NPC

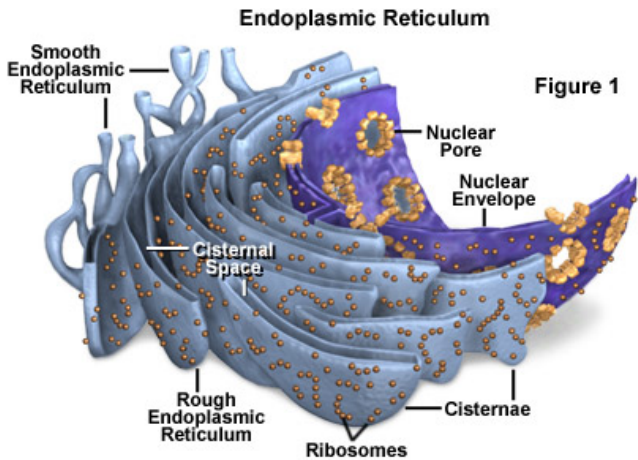
Required for the Importin β-mediated nuclear transport

Nuclear pore complex (NPC)

Importin β

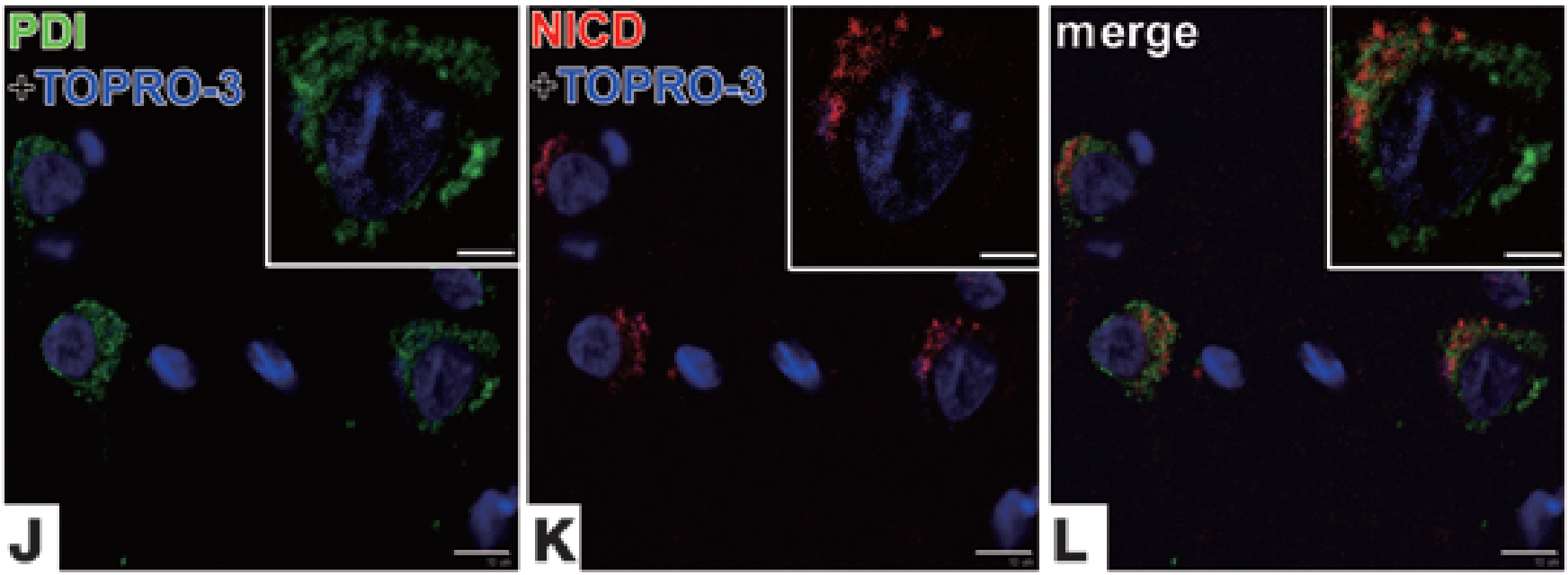
Nup153 localized outside the envelope was associated with the NICD aggregates





- Annulate lamellae?

The NICD aggregates and associated Nup153 are cytoplasmic



- PDI (protein disulfide isomerase): an ER marker

Why cytoplasmic?

- The stability of Nup153 is largely determined by its interactions with lamins

The EMBO Journal Vol. 19 No. 15 pp. 3918–3931, 2000

Incorporation of the nuclear pore basket protein Nup153 into nuclear pore structures is dependent upon lamina assembly: evidence from cell-free extracts of *Xenopus* eggs

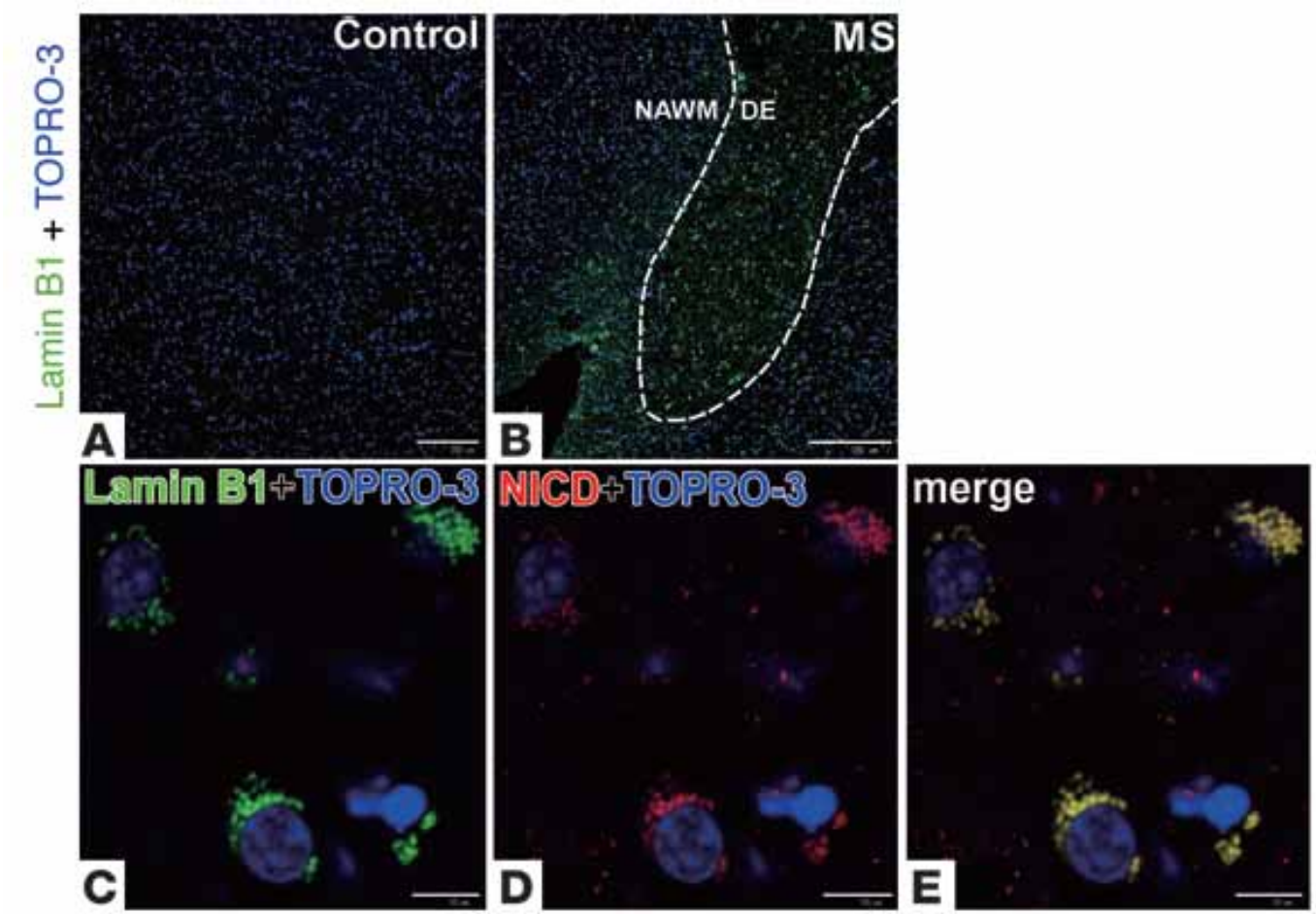
nature genetics

Lamin B1 duplications cause autosomal dominant leukodystrophy

Hereditary demyelinating disease without remyelination

Quasar S Padiath[1], Kazumasa Saigoh[1,8], Raphael Schiffmann[2], Hideaki Asahara[3], Takeshi Yamada[4], Anulf Koeppen[5], Kirk Hogan[6], Louis J Ptáček[1,7] & Ying-Hui Fu[1]

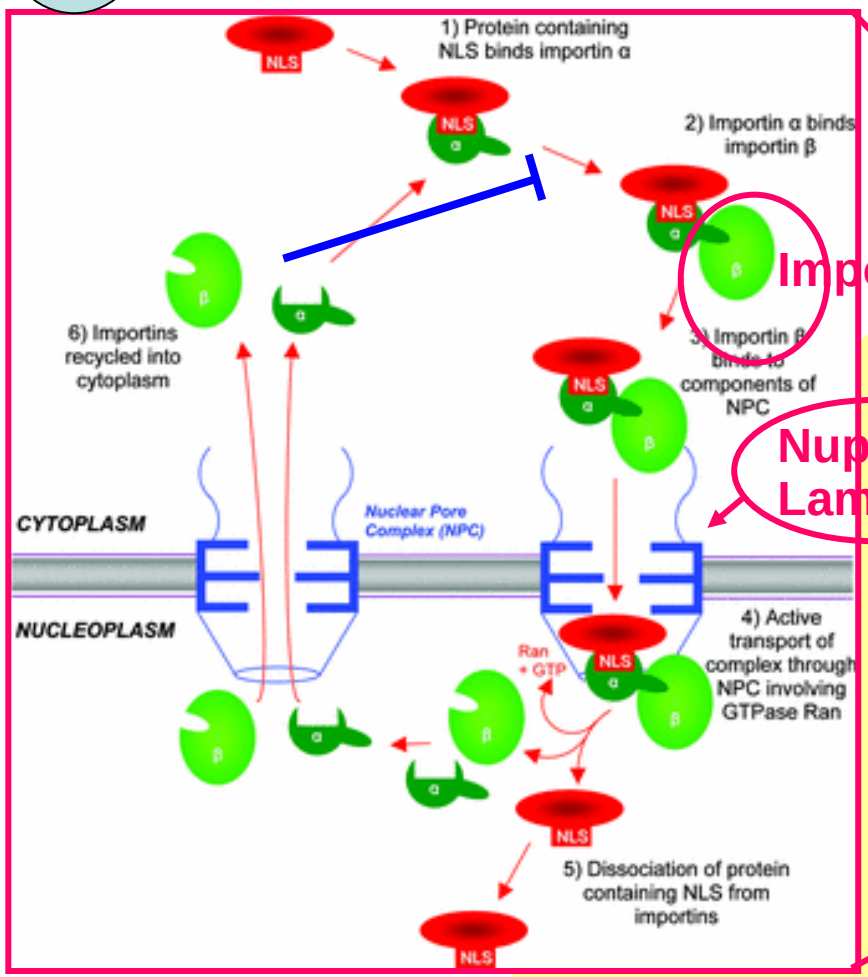
The subcellular localization of Lamin B1 was associated with NICD



Cytoplasmic aggregation of NICD with Importin β, Nup 153 and Lamin B1

General impairment in Importin β-mediated nucleocytoplasmic transport

TIP30



Importin β

Nup153
Lamin B1

Neuron → **Axonal Contactin**

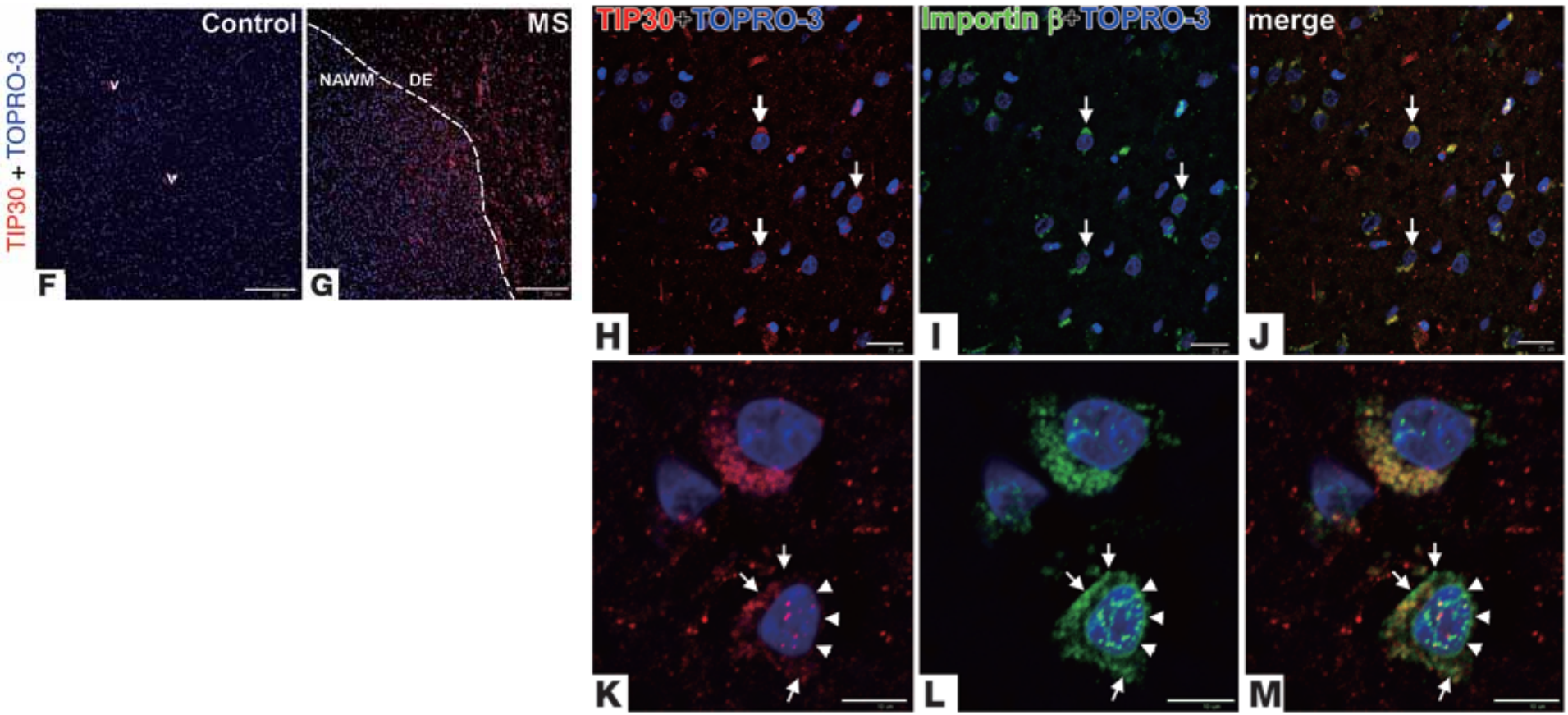
Proteolytic cleavage

NICD

NLS

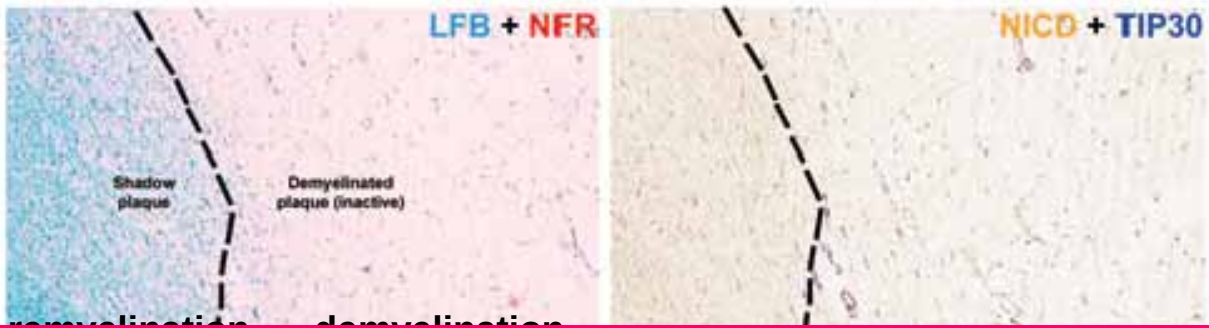
Promote differentiation

Upregulated TIP30 interferes nuclear translocalization

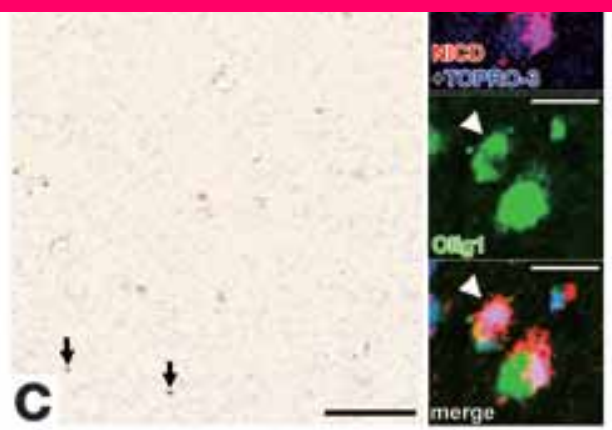


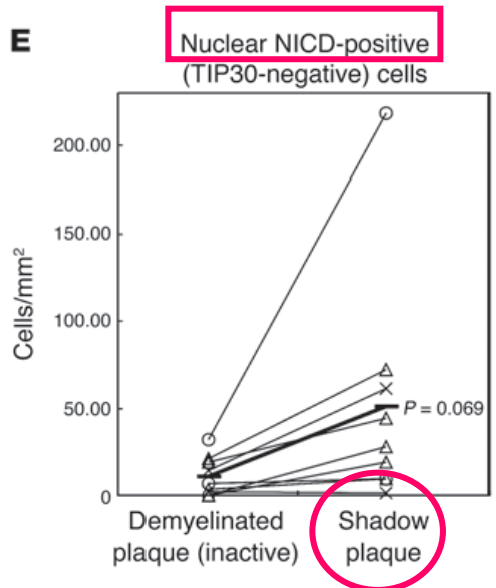
TIP30: TAT-interacting protein 30kDa

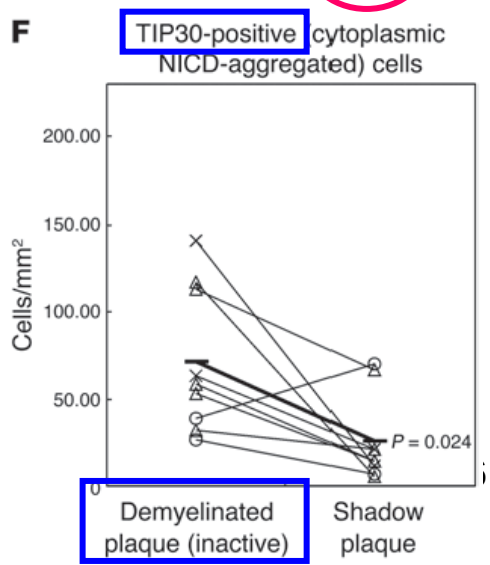
TIP30-positive cells are less frequent in remyelinating shadow plaques



Upregulated TIP30 is associated with remyelination failure in MS







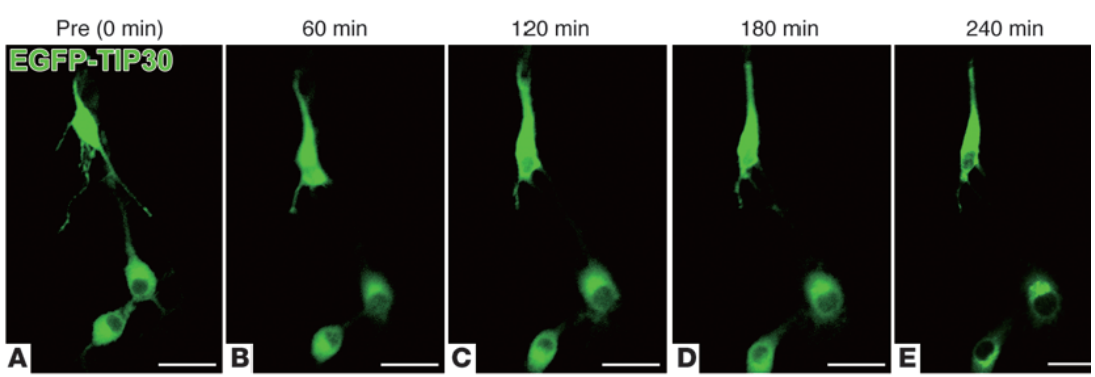
Specific aims

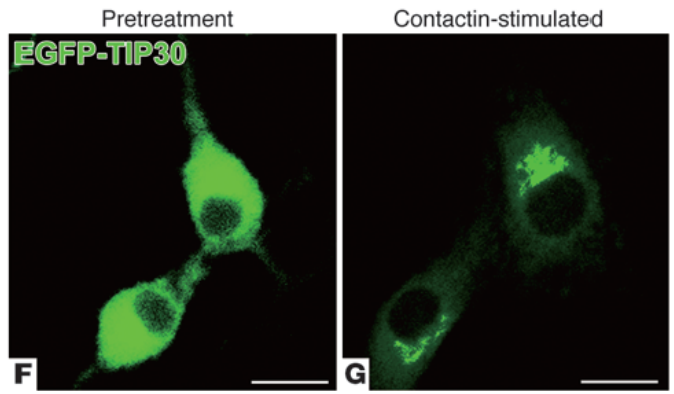
• Demyelinated axons in MS express Contactin. • Detection of axoglial contactin/Notch1 signaling within OPCs in MS lesion • Cytoplasmic aggregation of NICD with impotin β, Nup153, and lamin B1 • OPCs express TIP30, an importin β inhibitor, in MS lesion. • TIP30-positive cells are less frequent in remyelinating shadow plaques	Brain tissue

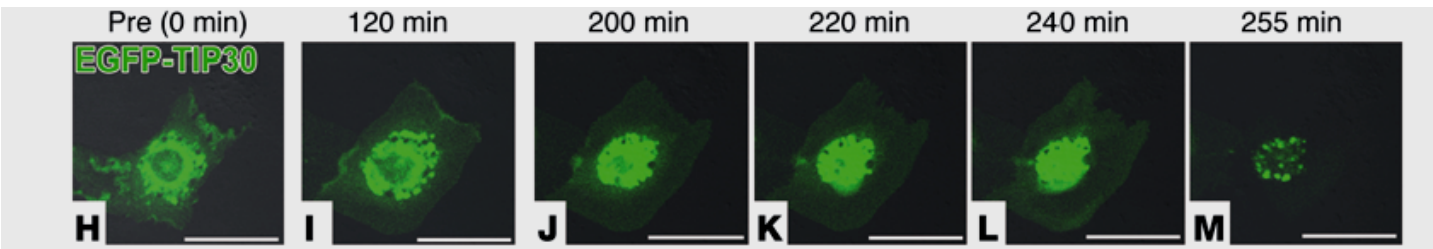
• TIP30 blocks the ① nuclear translocalozation of NICD and ② OPCs differentiation.
  • **Biochemical**
  • **Morphological**
	Cell line

TIP30 blocks the nuclear translocalization of NICD and OPCs differentiation

- Transfect EGFP tagged TIP30 into OLN-93 cells → induce differentiation with soluble Contactin-Fc

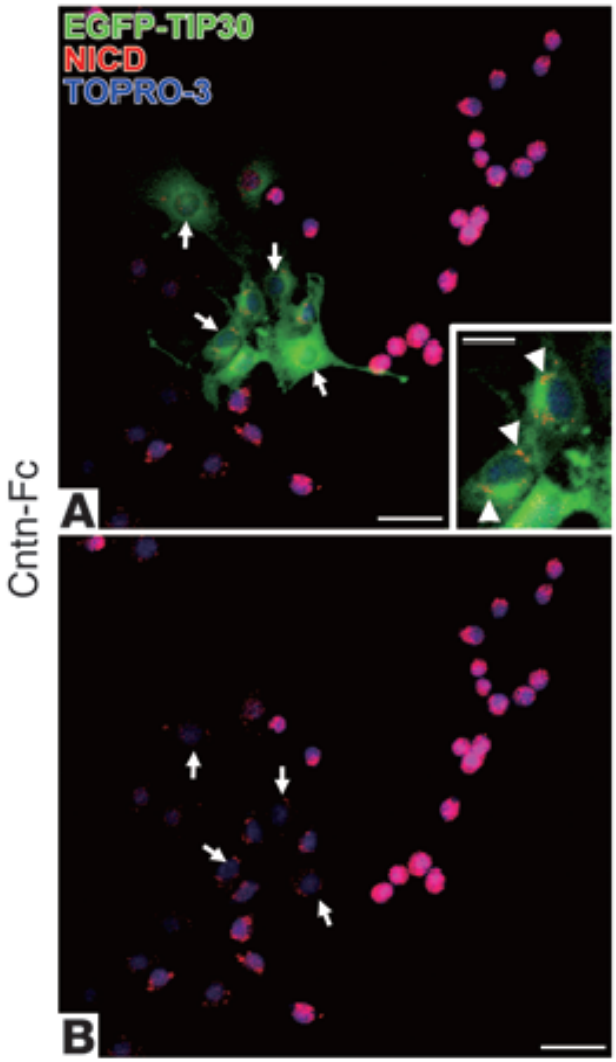


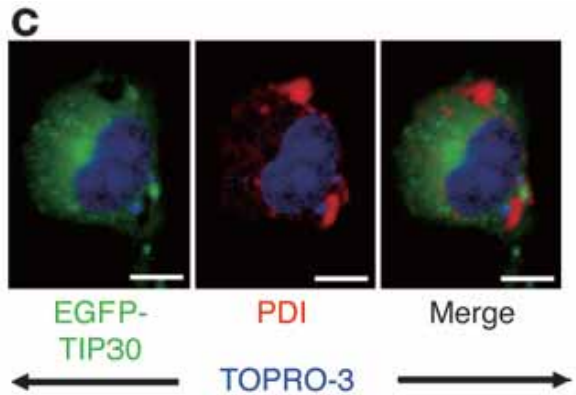


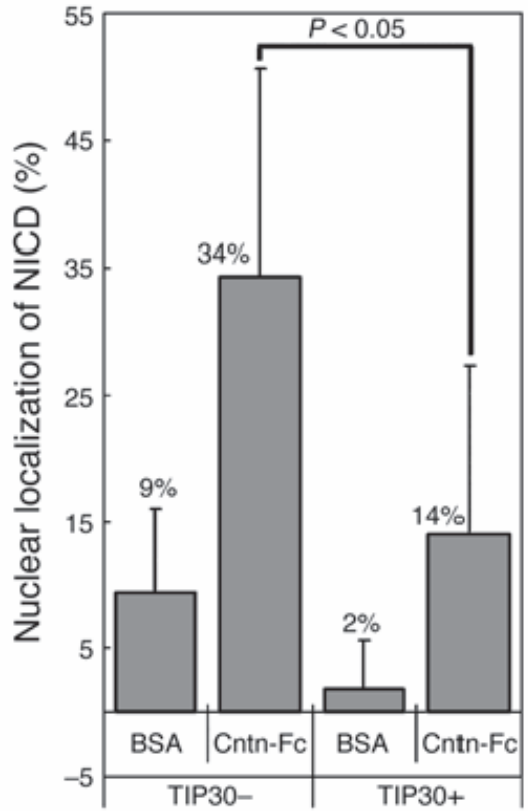


Breakdown of nuclear membrane

Nuclear translocalization of NICD was inhibited in TIP30-positive cells

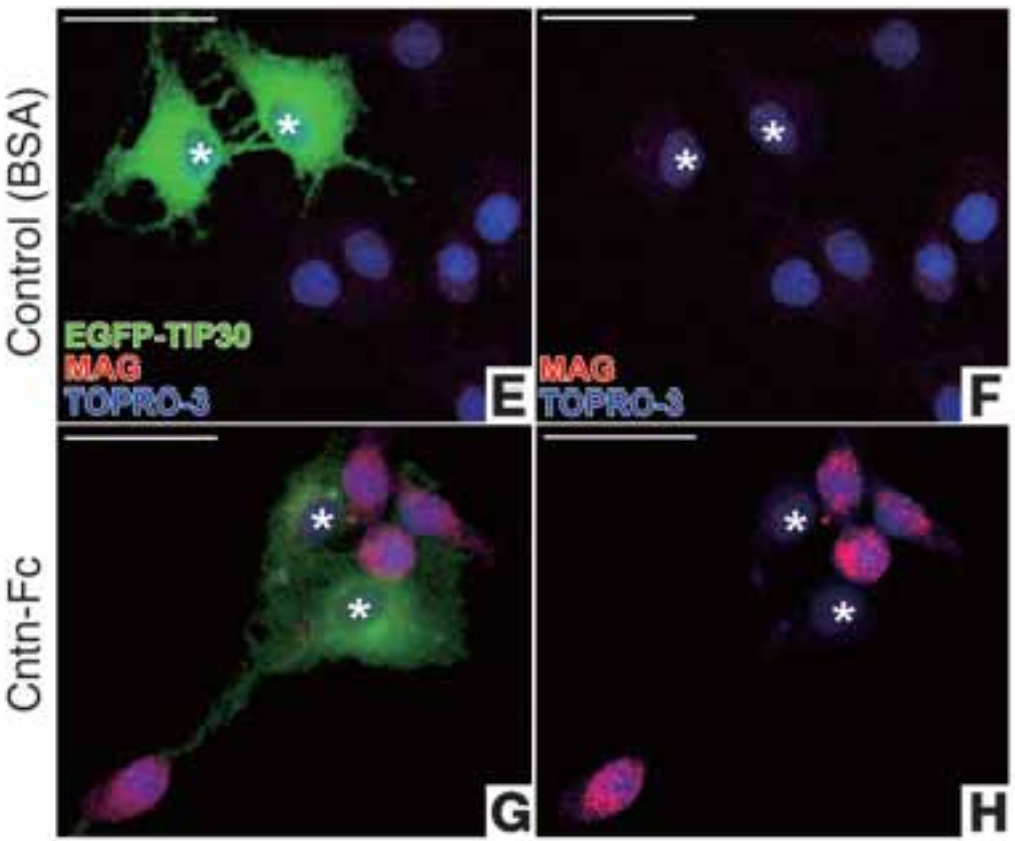


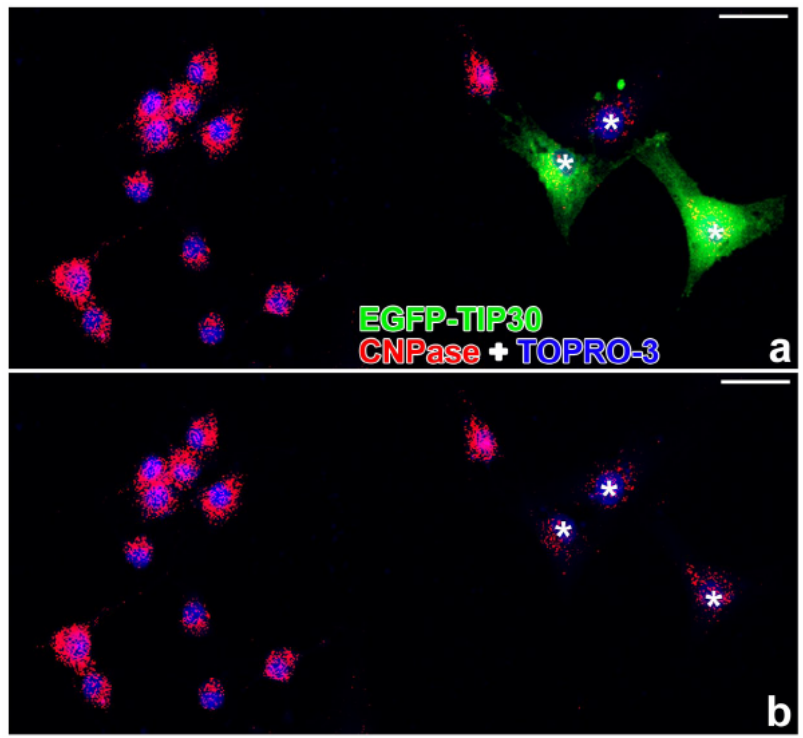




TIP30 blocks the nuclear translocalization of NICD and OPCs differentiation

Biochemical: TIP30 expresses in less differentiated OPCs

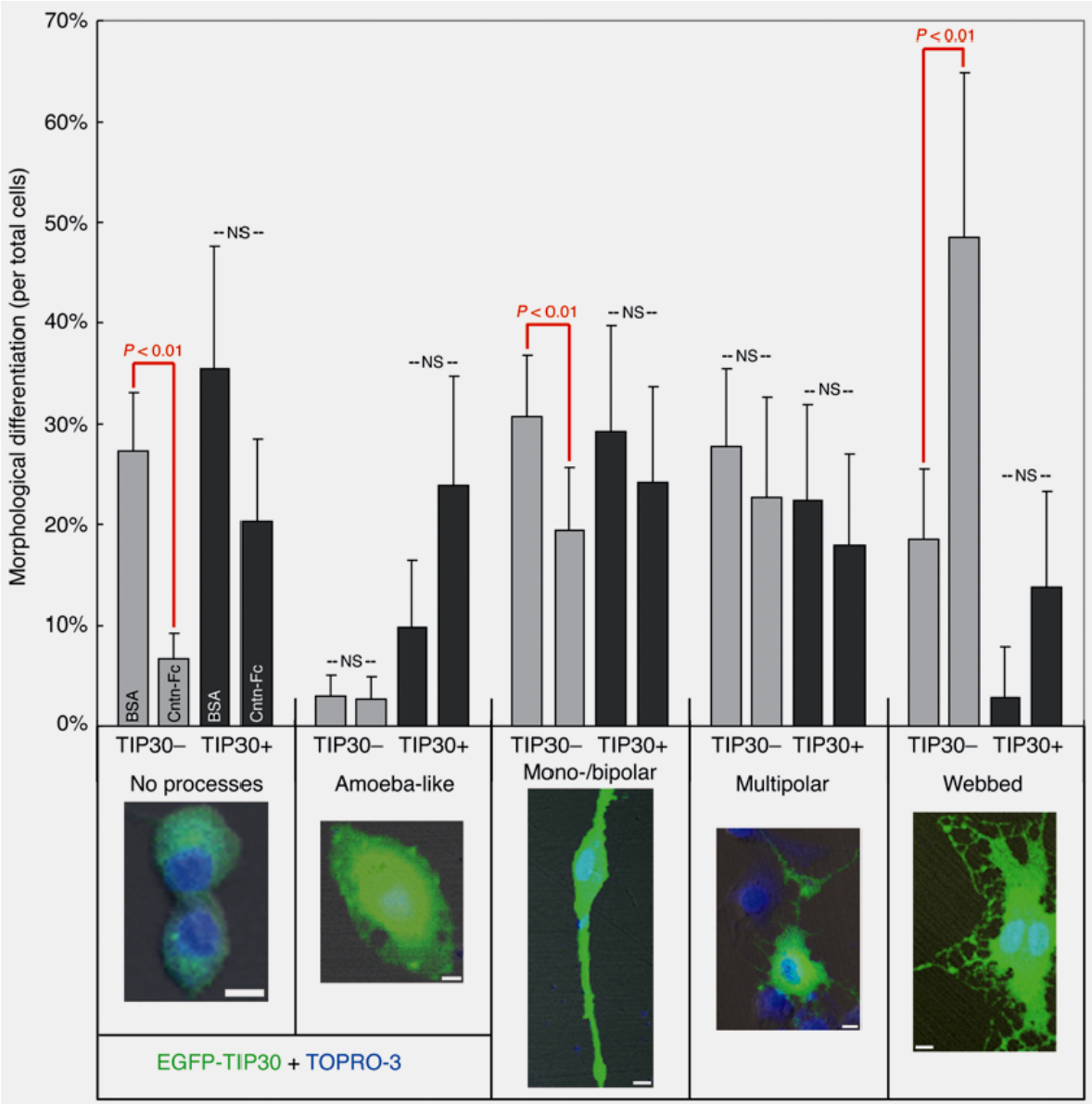




Myelin-associated glycoprotein (MAG), CNPase : markers of mature oligodendrocytes

TIP30 blocks the nuclear translocalization of NICD and OPCs differentiation

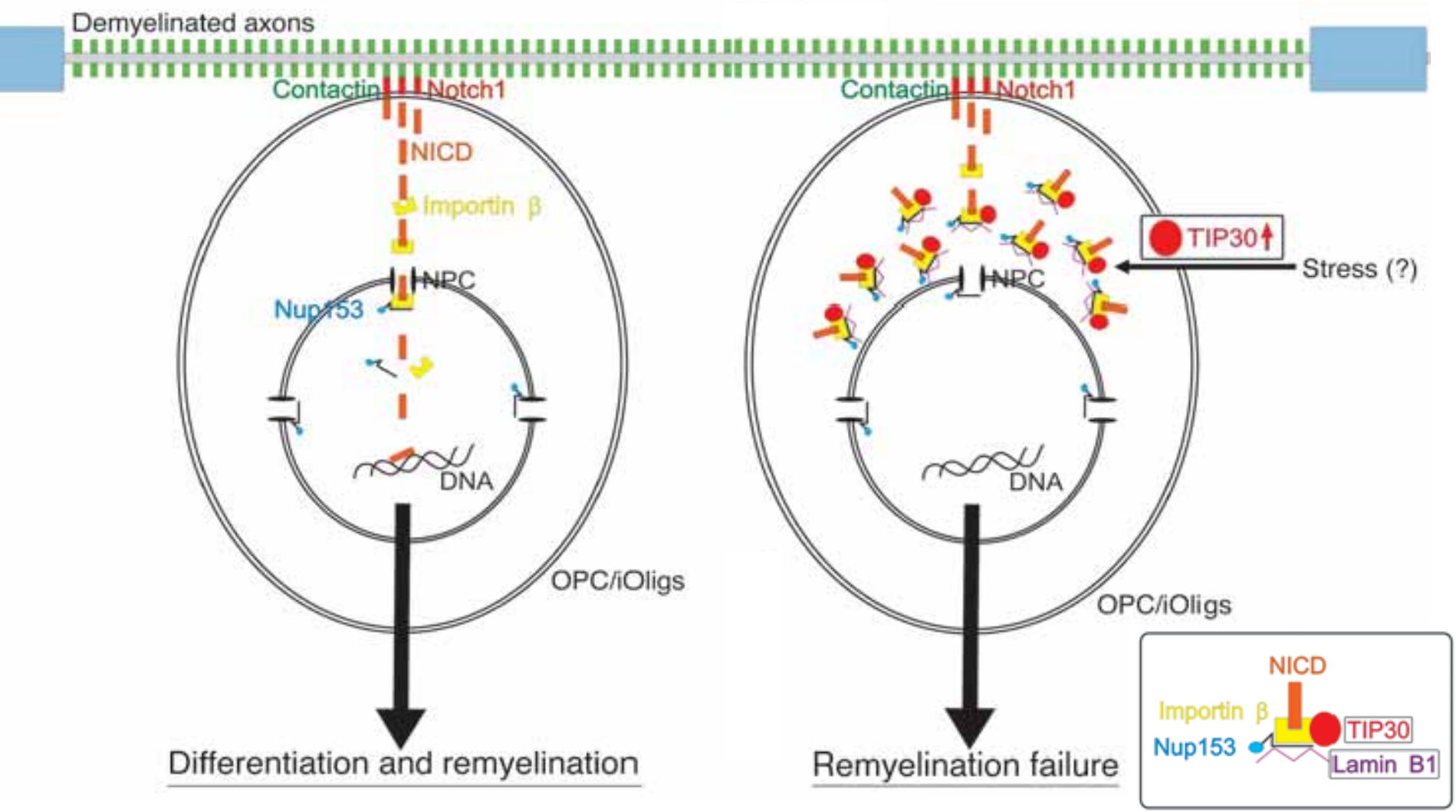
Morphological:



Conclusion

- Axons in demyelinated MS express Contactin.
- Detection of axoglial contactin/Notich1 signaling within OPCs in MS lesion
- Cytoplasmic aggregation of NICD with importin β, Nup153, and lamin B1
- OPCs express TIP30, an importin β inhibitor, in MS lesion.
- TIP30-positive cells are less frequent in remyelinating shadow plaques
- TIP30 blocks the nuclear translocalization of NICD and differentiation.

Summary


Demyelinated axons
Contactin
Notch1
NICD
Importin β
NPC
Nup153
DNA
OPC/iOligs
Differentiation and remyelination
Contactin
Notch1
TIP30
Stress (?)
NPC
DNA
OPC/iOligs
Remyelination failure
NICD
Importin β
TIP30
Nup153
Lamin B1

Discussion

Implication

- Remyelination failure may not be entirely lack of axonal signals
- Abnormal upregulation of TIP30 results in impaired nucleocytoplasmic transport.
  - TIP30 is induced by specific environmental factor in MS lesion. **?**
- Systemic blockade of TIP30: potential tumorigenesis

Implication

- Nucleocytoplsmic transport of NICD seems intact in EAE animal model
- TIP30 is not induced in the demyelinated lesion of EAE animal

Thanks for your attention!

TIP30

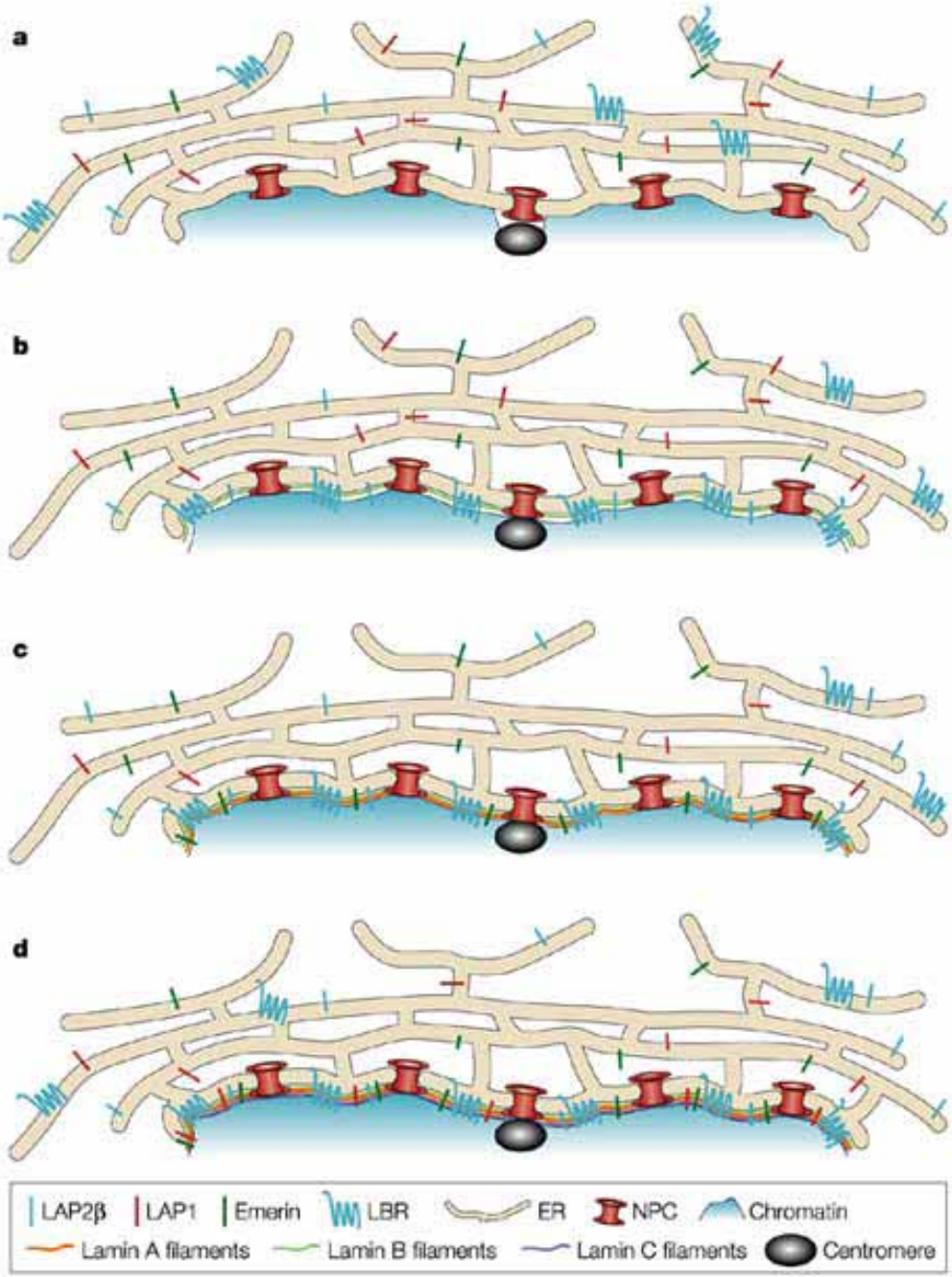
TAT-interacting protein 30kDa

- Tumor suppressor
  - Associated with metastasis of small lung CA and breast CA
- Proapoptotic protein
  - Induced by heat shock, irradiation, aging or TGF-β1
- Stimulates HIV-1 Tat-activated transcription by interacting with both Tat and RNA polymerase II
- Serine/threonine kinase
- Low expression in brain

EMBO J. 2000 Mar 1;19(5):956-63.

Clinical information of the ten MS patients

- Patients:
  - Subtypes:
    - Relapsing/remitting MS: 2
    - Primary progressive MS: 2
    - Secondary progressive MS: 5
    - Without clinical information: 1
  - Average age: 54.9 years (range: 47-72 years)
  - Disease duration: 12-59 years
  - Lesion sites: 9 inactive lesion, 1 active lesion
- Normal control:
  - Average age: 76.2 years (range 54-91 years)


a
b
c
d
LAP2β
LAP1
Emerin
LBR
ER
NPC
Chromatin
Lamin A filaments
Lamin B filaments
Lamin C filaments
Centromere
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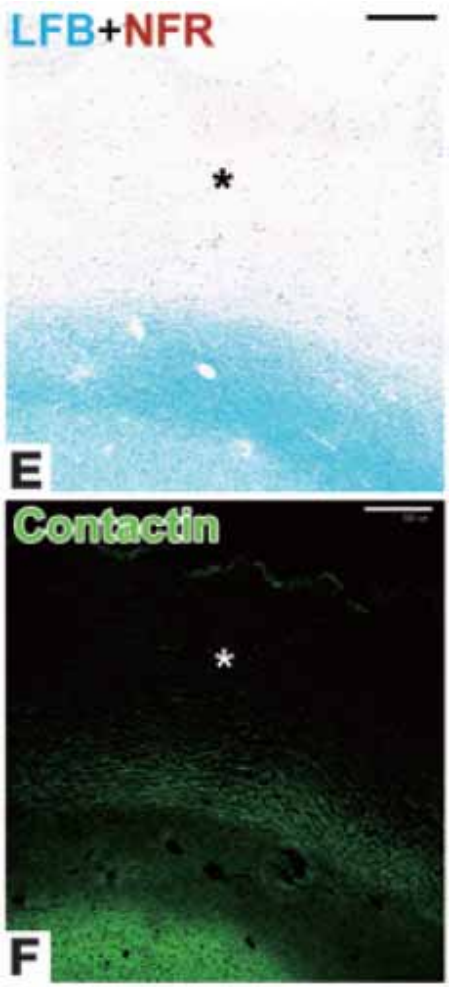
Contactin immunoreactivity was lost in demyelinated area with severe tissue damage



Increased expression of Contactin within demyelinated lesions in all MS samples