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SELECTIVE TARGETING OF INTRACELLULAR MISFOLDED, PATHOGENIC TDP-43 WITH RATIONALLY DESIGNED INTRABODIES. ABSTRACT #55269.

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Selective targeting of pathogenic TDP-43 for optimal safety and efficacy

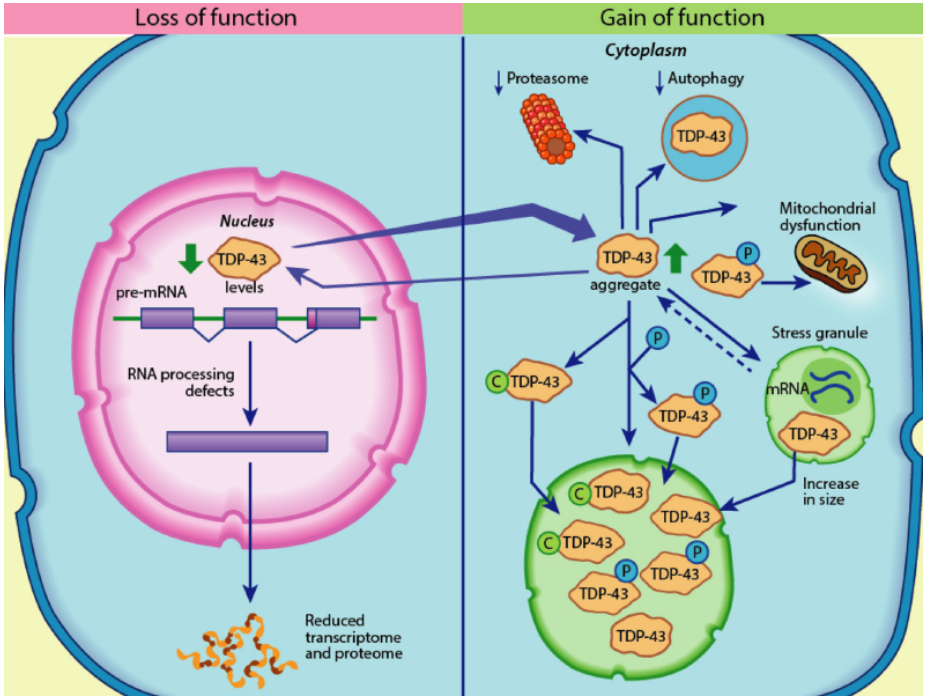


Figure from de Boer et al[1]

TDP-43 is essential to neuronal cell survival[1]

- TDP-43 is normally present in the nucleus of all cells and performs an essential role in RNA splicing and transport
- Under stress conditions (e.g. oxidative stress) normal TDP-43 also forms protective stress granules in the cytoplasm

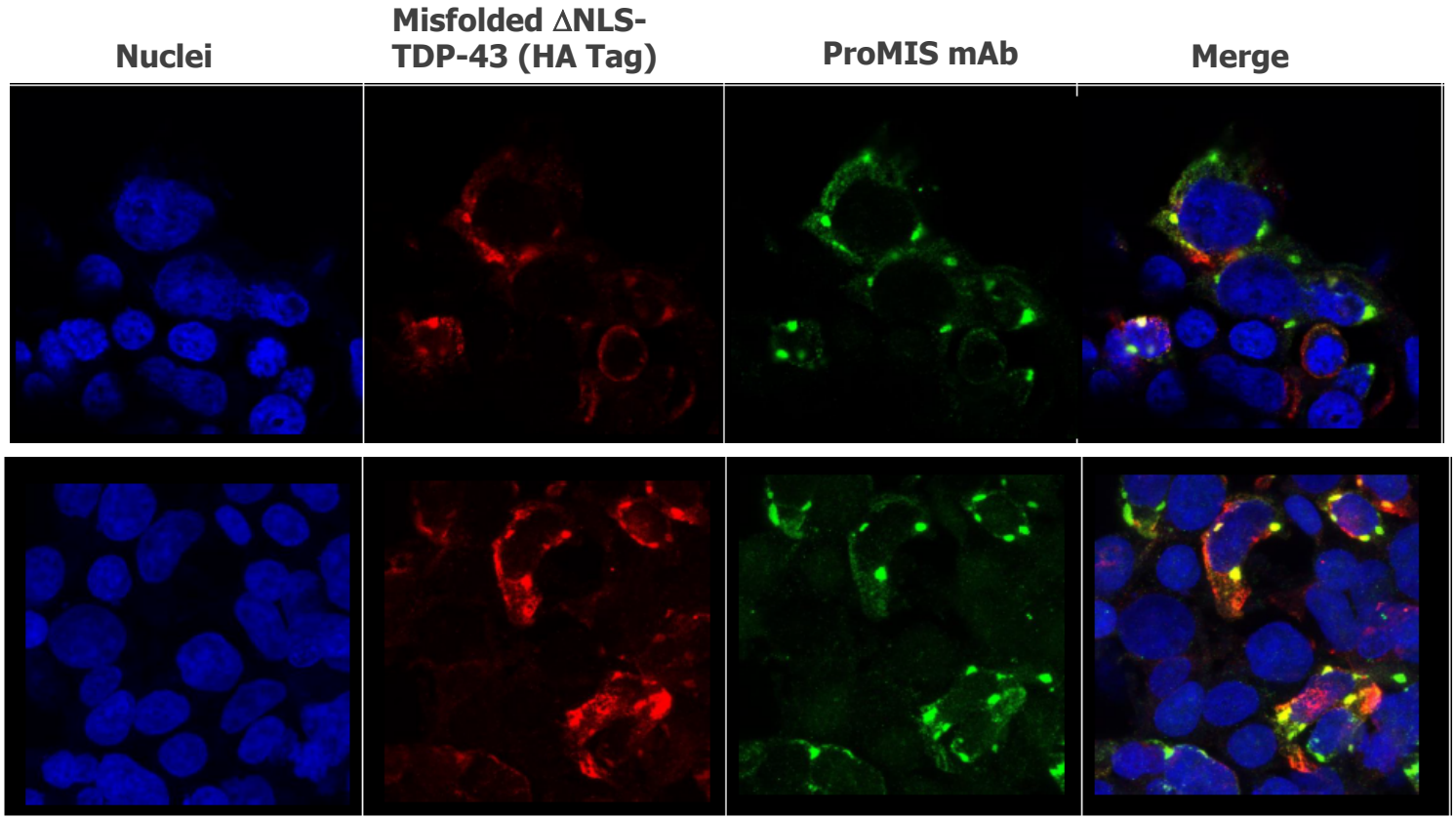
Misfolded TDP-43 gives rise to both loss of function and toxic gain of function[1]

- Loss of function: Under disease conditions, misfolding of TDP-43 causes formation of mislocalized cytoplasmic aggregates. Nuclear depletion leads to defective splicing and transport of mRNA.
- Toxic gain of function: Cytoplasmic aggregates of misfolded TDP-43 template the misfolding of healthy TDP-43, and are toxic to mitochondria, ER and physiologic stress granule function. They also induce misfolding of other proteins into pathogenic aggregates[2-4] - "TDP-43 Pathological Interactome"

Targeting of pathogenic TDP-43 requires stringent selectivity for the misfolded form of the protein to avoid safety concerns

[1]de Boer, EMJ et al, 2020, J Neurol Neurosurg Psychiatry; [2]Pokrishevsky et al, 2016, Scientific Reports; [3]Chou et al, 2018, Nat Neurosci; [4]Endo et al, 2018, Biological Psych

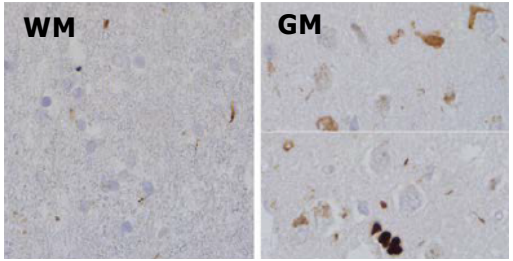
ProMIS mAbs to misfolded TDP-43 recognize mislocalized, aggregated ΔNLS-TDP-43, but not nuclear wild-type TDP-43 physiological oligomers



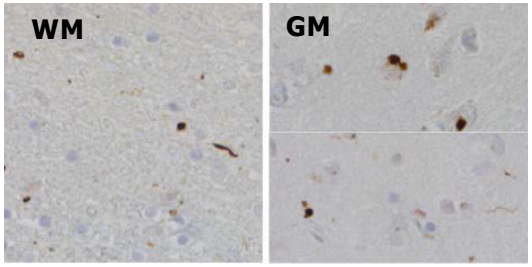
- HEK-293 cells transfected with ΔNLS TDP-43 lacking a functional nuclear localization signal
- Cells stained for HA tag (red) of overexpressed ΔNLS TDP-43 or with rabbit mAb to misfolded TDP-43 epitope at 2 μg/ml (green).
- Nuclei stained with DAPI (blue)
- Images analyzed by confocal microscopy (Z-stacks)

Misfolded TDP-43 is recognized by ProMIS antibody in brain tissue from FTLD patients with pathology subtypes A,B,C,E and in spinal cord from ALS patients

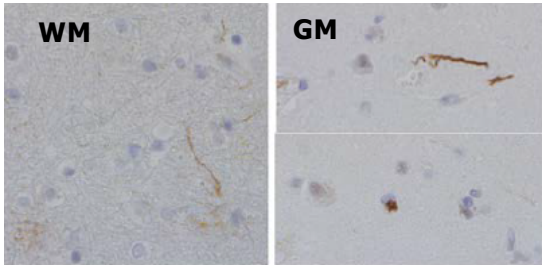
FTD brain, TDP-43 pathology type A



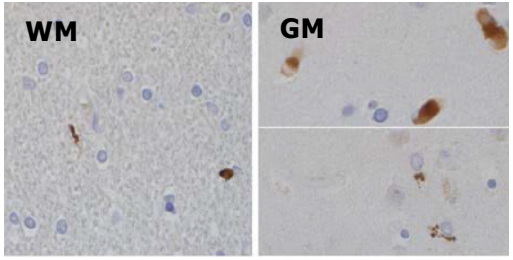
FTD brain - TDP-43 pathology type B



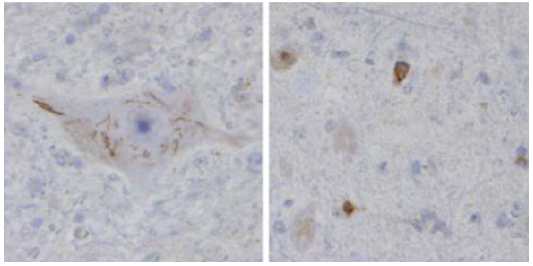
FTD brain - TDP-43 pathology type C



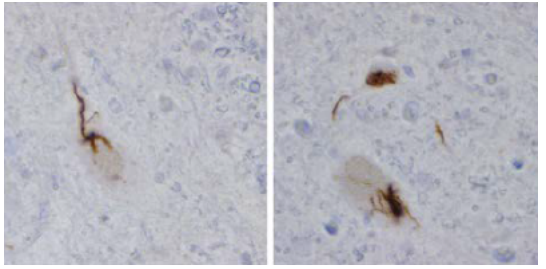
FTD brain - TDP-43 pathology type E



ALS patient 1 – Spinal cord



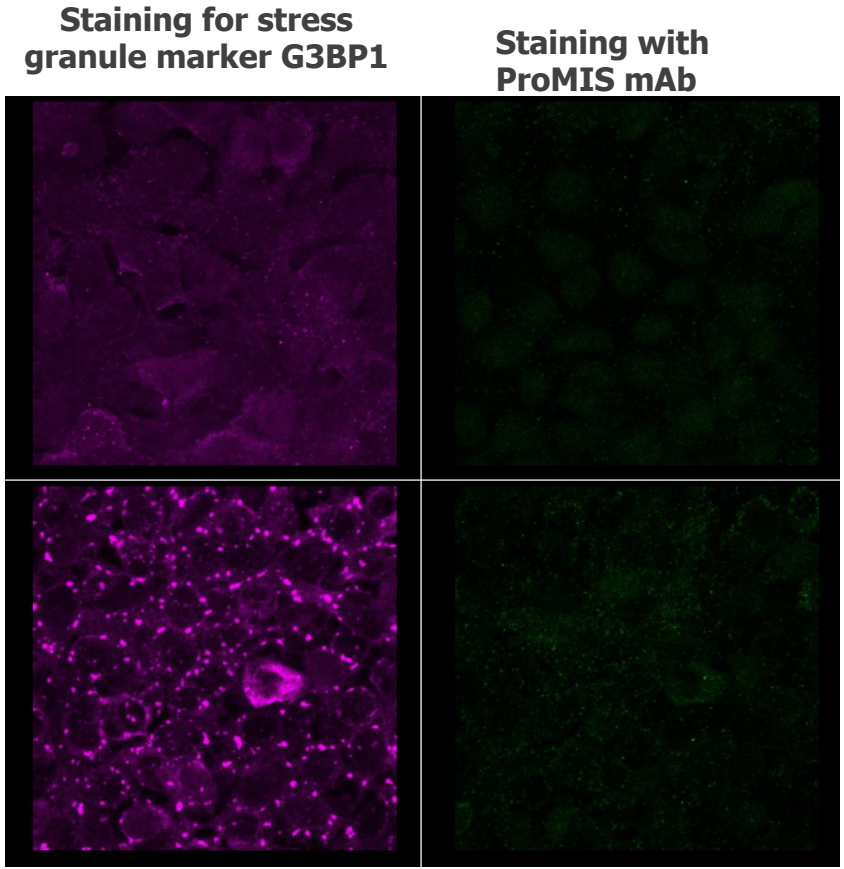
ALS patient 2 – Spinal cord



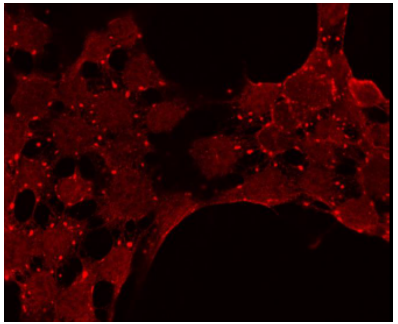
WM – White matter
GM = Grey matter

Staining with ProMIS rabbit pAb to misfolded TDP-43
Performed by Dept. of Pathology, Amsterdam Neuroscience, VU University Medical Center, Amsterdam, The Netherlands

ProMIS mAbs to misfolded TDP-43 do not recognize physiological oligomers of TDP-43 in stress granules



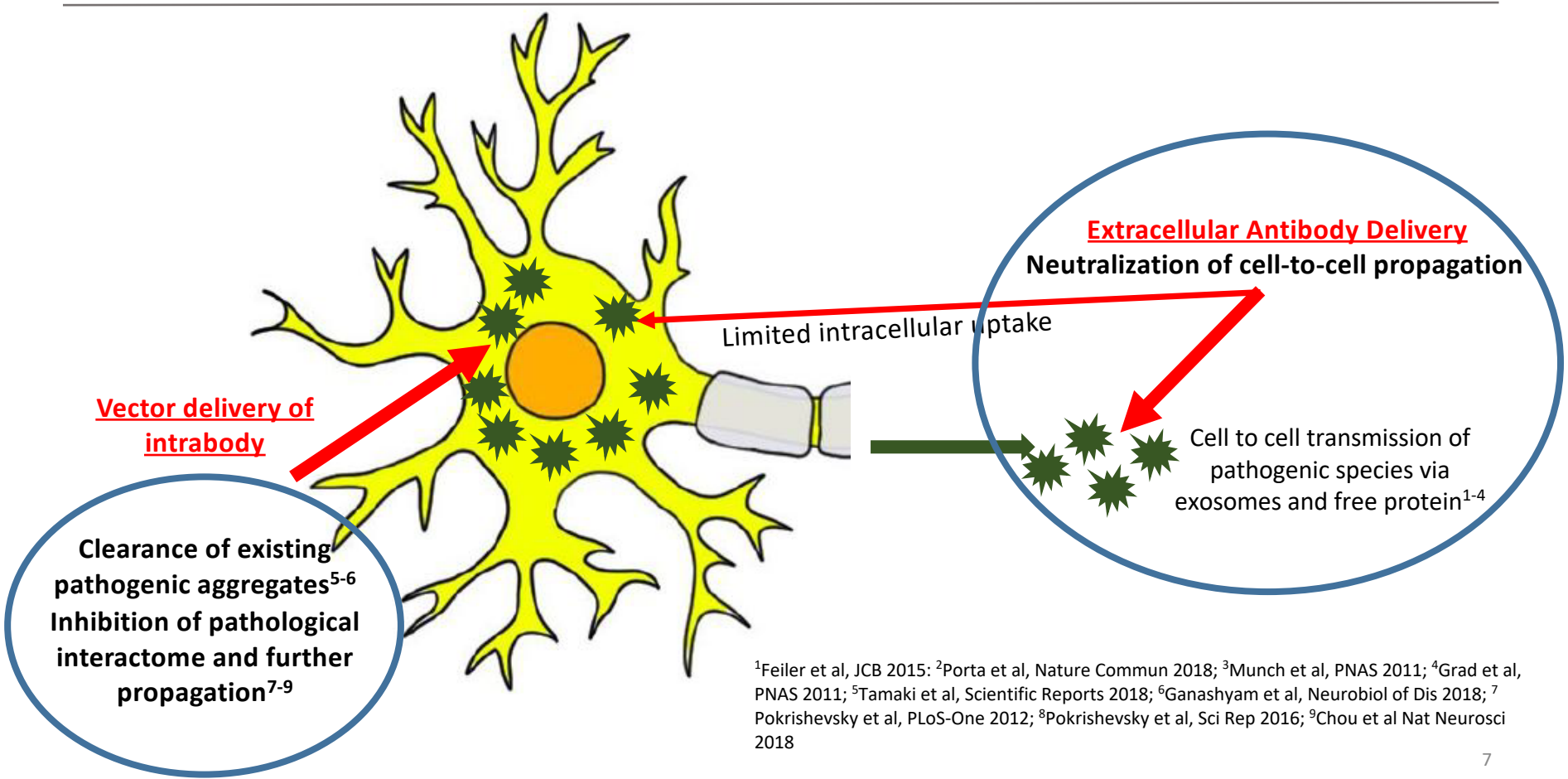
Staining with pan-TDP-43



Staining with a pan-reactive TDP-43 antibody confirms that normal TDP-43 is present in physiologic stress granules -> not recognized by misfolded-specific ProMIS mAb

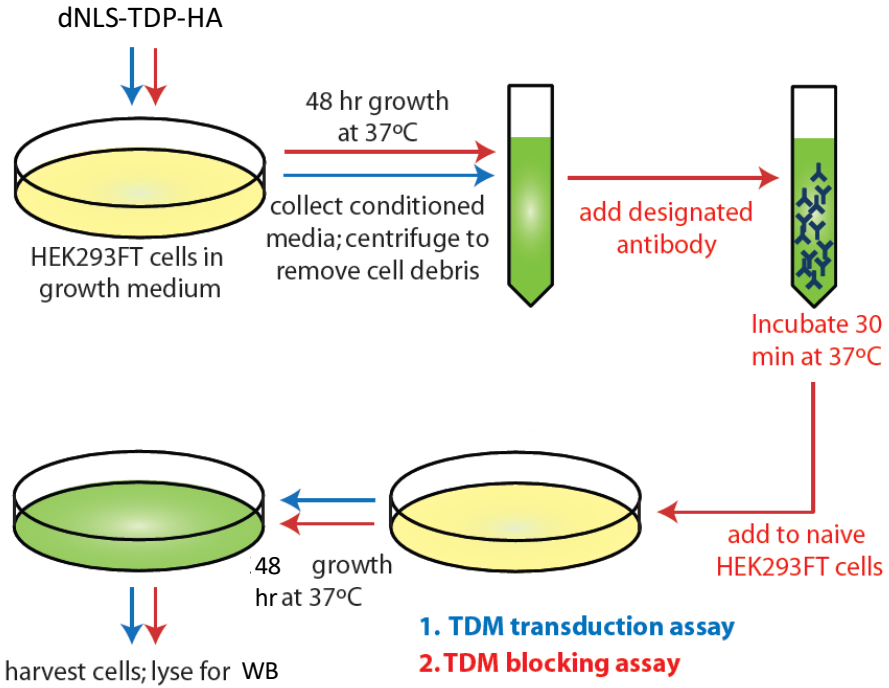
- HEK293 cells stressed by 60min exposure to 1mM sodium arsenite for 60 min
- Cells stained with ProMIS rabbit mAb at 10 μg/ml (green) or with antibody to G3BP1 (magenta)
- Images analyzed by confocal microscopy

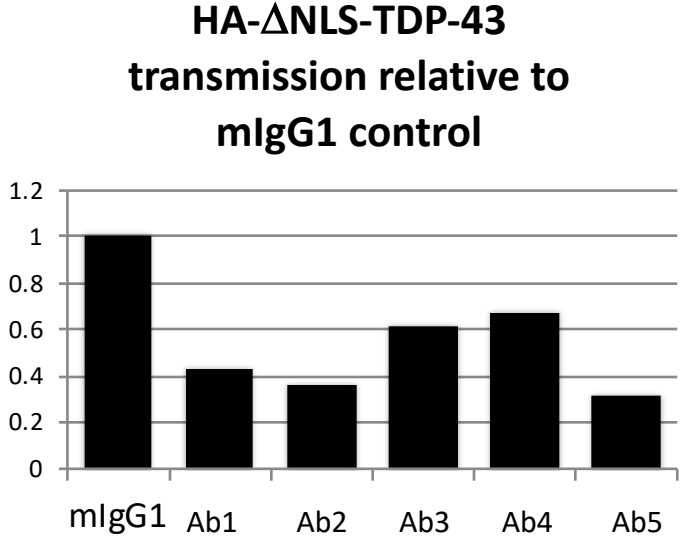
Antibody-Based Targeting of Pathogenic TDP-43



[1]Feiler et al, JCB 2015: [2]Porta et al, Nature Commun 2018; [3]Munch et al, PNAS 2011; [4]Grad et al, PNAS 2011; [5]Tamaki et al, Scientific Reports 2018; [6]Ganashyam et al, Neurobiol of Dis 2018; [7] Pokrishevsky et al, PLoS-One 2012; [8]Pokrishevsky et al, Sci Rep 2016; [9]Chou et al Nat Neurosci 2018

Functional Assay: ProMIS mAbs inhibit cell-to-cell transmission of misfolding ΔNLS-TDP43

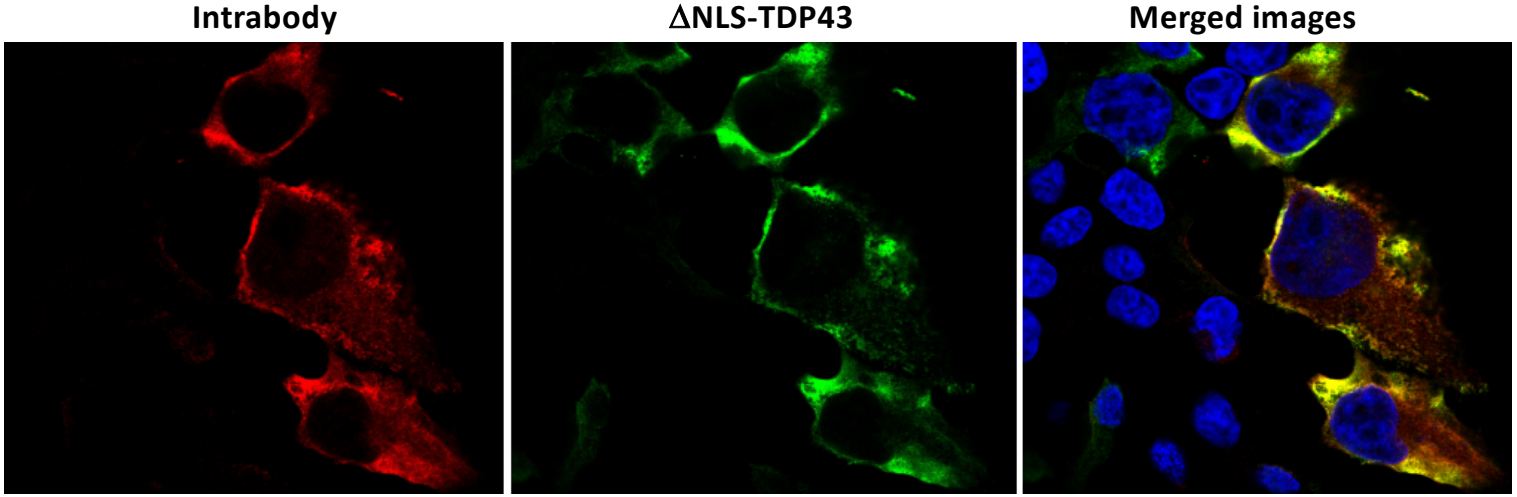




ProMIS mAbs inhibit transmission of misfolding TDP-43 from the conditioned medium of donor HEK293 cells transfected with ΔNLS-TDP-43 to naïve recipient cells

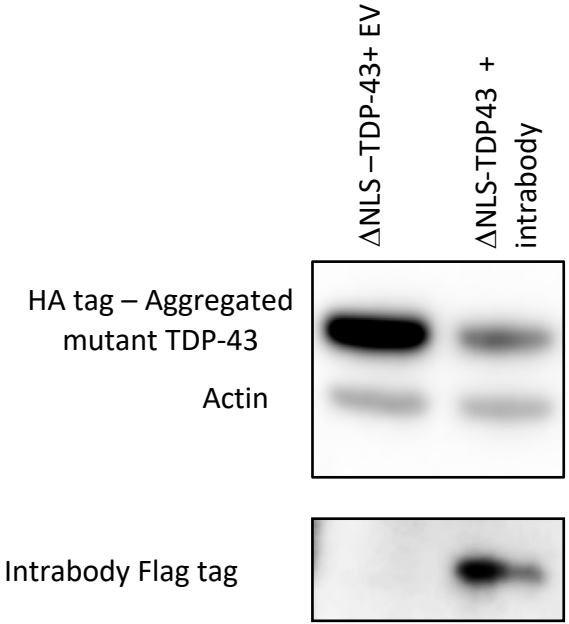
ProMIS intrabodies only interact with cytoplasmic ΔNLS-TDP43 aggregates and not normal nuclear TDP43

HEK293 cells transfected with ΔNLS-TDP43 and single chain ProMIS intrabody

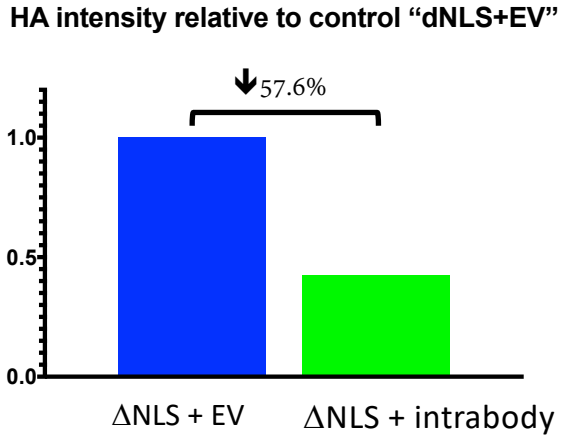


- Expression of ProMIS TDP43 intrabody is not toxic to cells
- Intrabody co-localizes with mislocalized, aggregated cytoplasmic ΔNLS-TDP43
- Intrabody does not interact with endogenous normal TDP43 in the nucleus

TDP-43 intrabody promotes clearance of misfolded TDP-43 aggregates



Misfolded TDP43-selective intrabody with lysosomal targeting signal promotes degradation of TDP-43 aggregates without cellular toxicity



EV = empty vector

ProMIS selectivity for the toxic form of TDP-43 is critical for gene therapy vectorization of intrabodies in order to preserve normal cell function

- **ProMIS antibodies are highly selective for misfolded TDP-43**

  - ✓Epitope binding affinity in the sub-nanomolar range
  - ✓Reactive and specific for aberrant cytoplasmic TDP-43 aggregates with no reactivity with wild-type nuclear TDP-43 → preserves normal, essential TDP-43 function
  - ✓No binding to physiological stress granules → preserves stress-protective function
  - ✓Reactive with native pathological TDP-43 from human brain and spinal cord samples