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Interplay between TDP-43 and FUS in protein assemblies

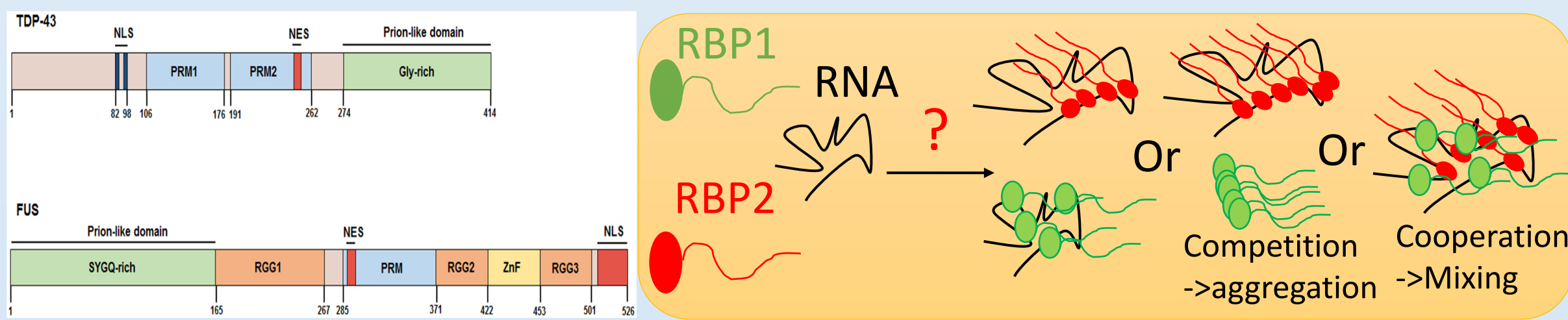
Influence of mRNA on the aggregation process

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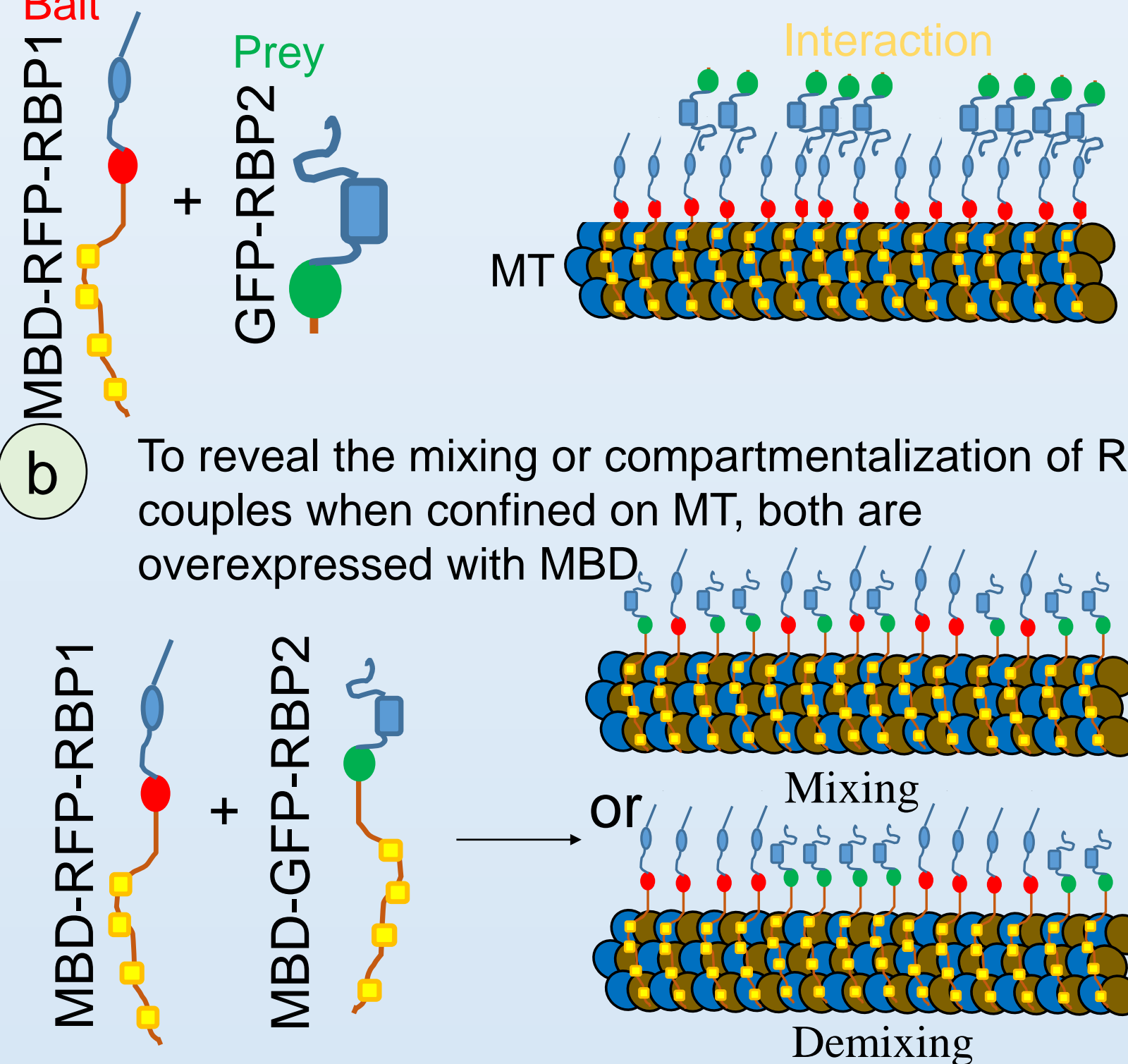
1 Introduction

TDP43 and FUS are 2 RNA Binding Proteins (RBP) involved in neurodegenerative diseases. These 2 nuclear proteins are very similar in structure and function; they both harbor RRM (RNA Recognition Motif) to bind to RNA, and a domain of low complexity promoting protein-protein interaction and potentially, aggregation. They are involved in several mRNA-related processes as RNA biogenesis, transport, and splicing. TDP-43 targets more than 6000 mRNA thanks to its RRMs with specificity towards GU motif while FUS interact with broad varieties of mRNA through its ZnF, RGG motifs and RRM without any specific nucleotide sequence. Despite this difference in RNA interaction mode, these proteins promote the formation of mRNA rich compartments like stress granules, drosha complexes, splicing complexes... via the process of liquid-liquid phase separation (LLPS), and within these structures, they accumulate on common RNA targets. Such proximity in functions and localization point toward an interplay between FUS and TDP-43. Here, we analyze their ability to mix or phase separate in cellular context using the microtubule bench technology. Then we reveal the ultrastructure of pure and mixed TDP43 and FUS assemblies as well as the influence of RNA.



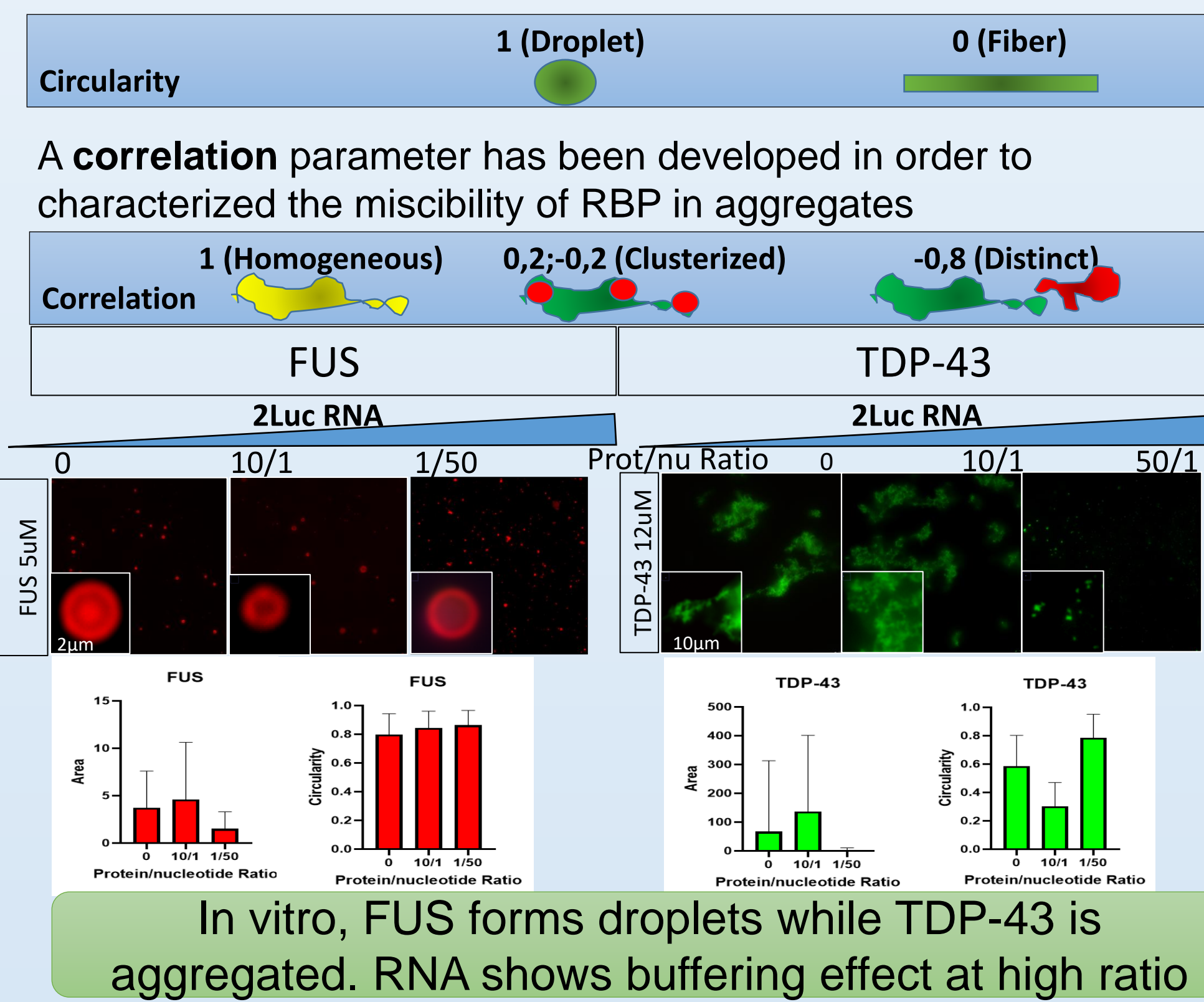
2,1 The Microtubule Bench

To detect the interaction between proteins: one RBP (the "bait"), fused to an MBD (Microtubule Binding Domain) and a fluorescent tag is confined on the microtubule network and the second RBP (the "prey") with another fluorescent tag is overexpressed [1]



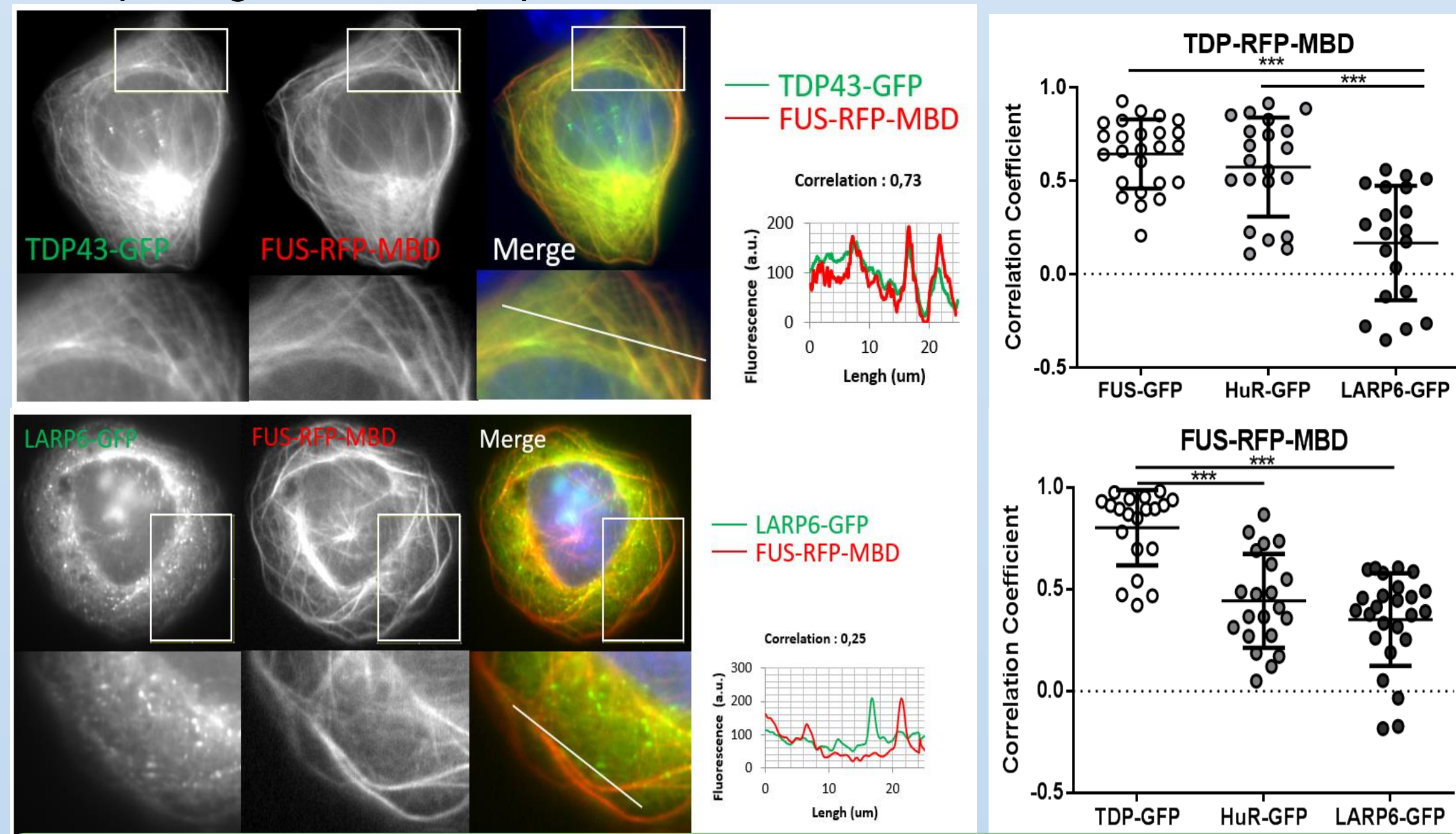
2,2 Structural analysis of RBP assemblies in vitro

Mixed protein samples are incubated then fixed and glass slides before being marked with antibodies. **Circularity** and **Area** have been used to describe shape of the aggregates



3 TDP-43 and FUS interaction with various RBPs

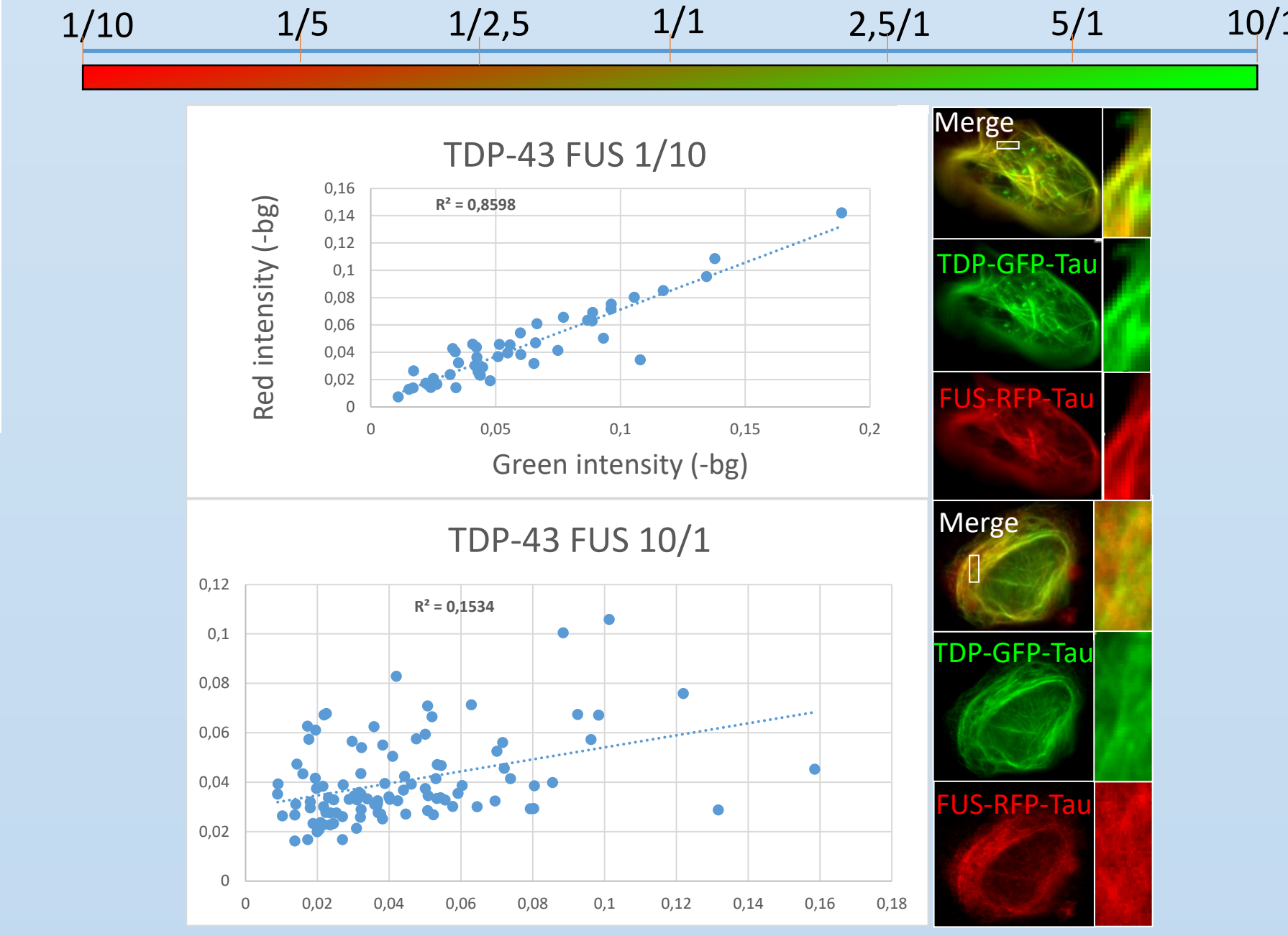
Bait/prey system has been used in order to detect protein-protein interaction in cellular context. Several RBPs have been tested comprising HuR and Larp6



TDP-43 and FUS interaction is higher than other RBPs

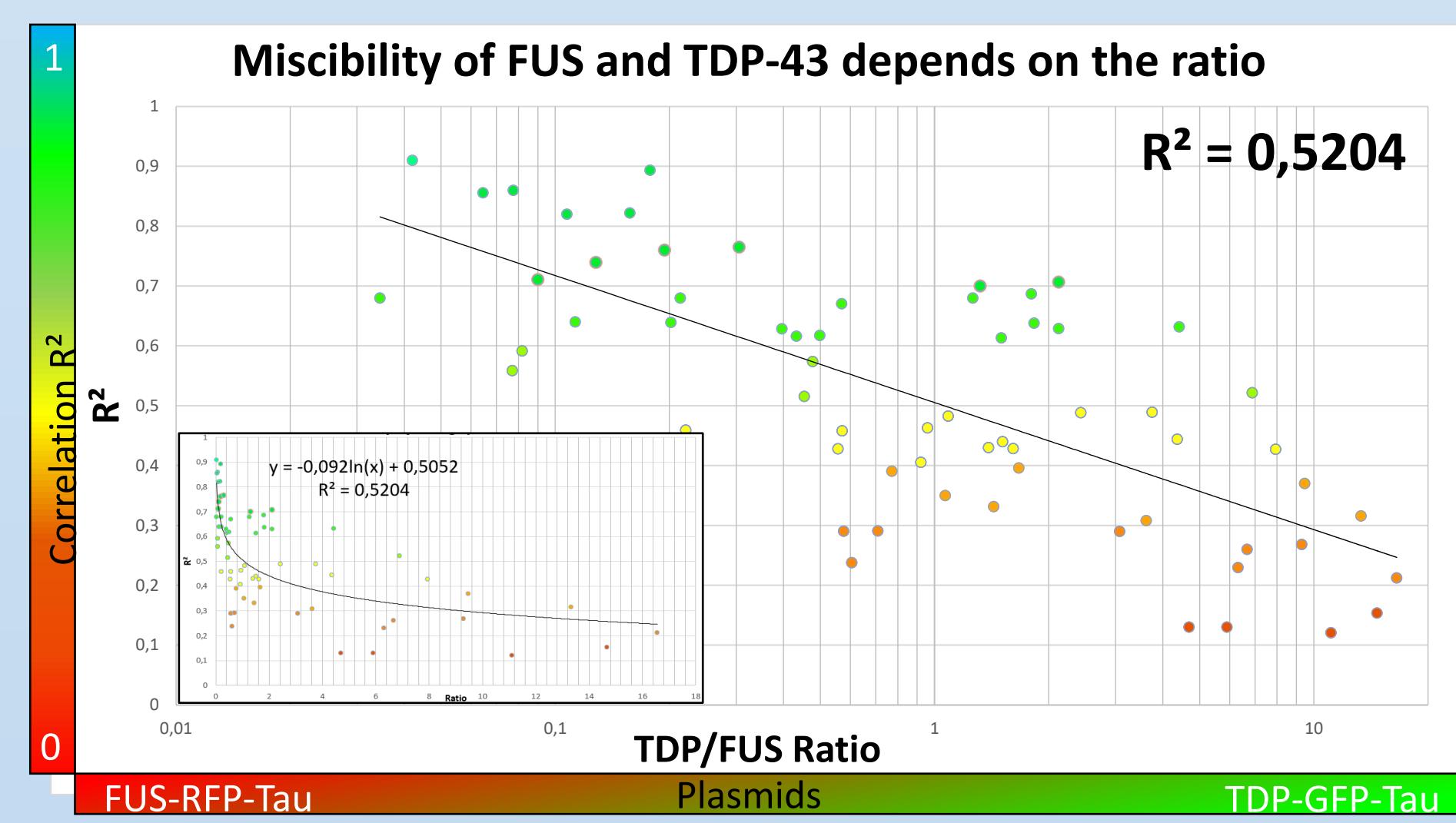
4 Mixing of TDP-43 and FUS in cellular context

U2OS cells have been transfected with several ratios of plasmid of FUS-RFP-Tau/TDP43-GFP-Tau RBP2-GFP-Tau



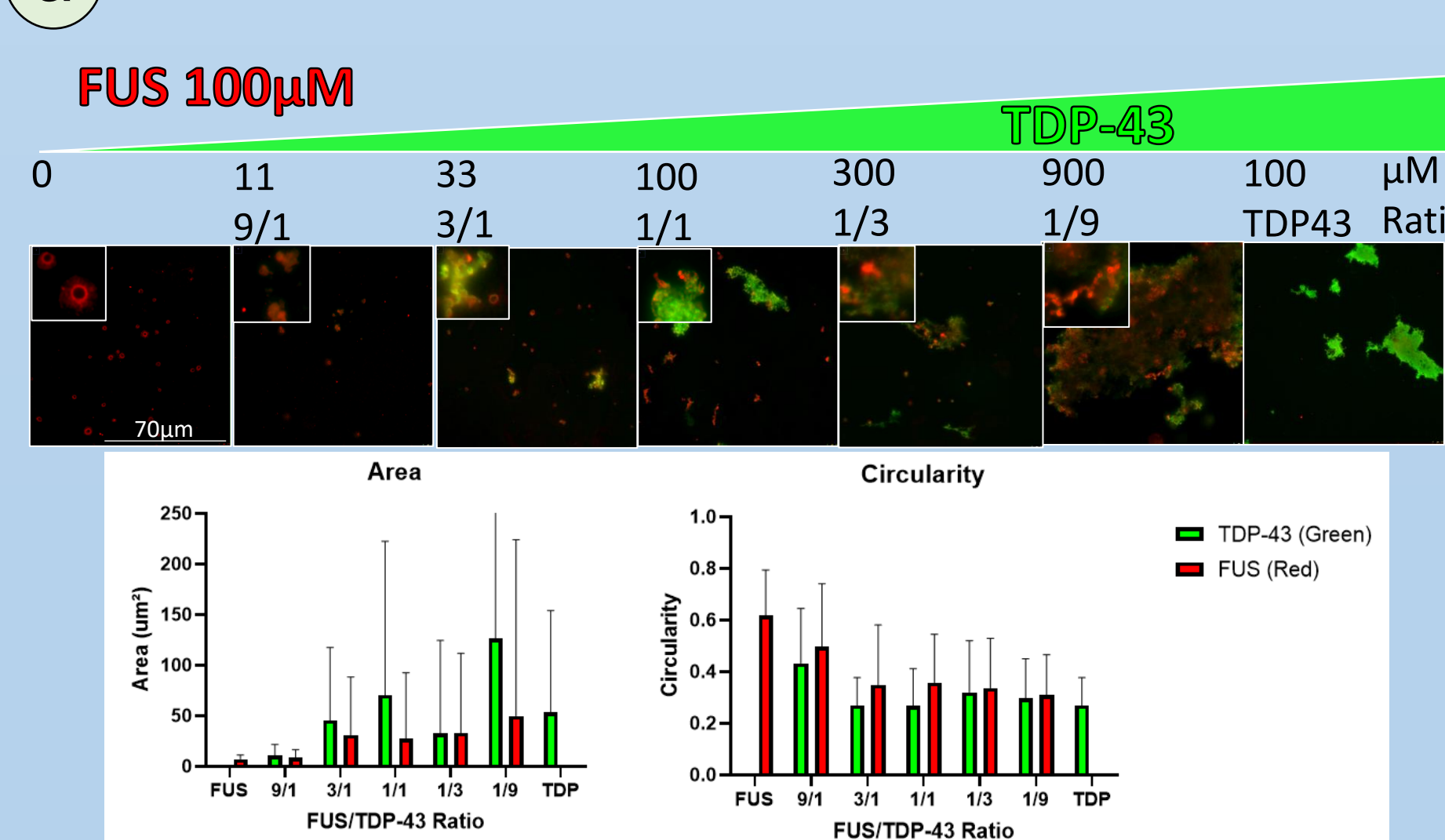
Miscibility of TDP-43 and FUS depends on the expression level, FUS solubilizes TDP-43, while TDP-43 prefers to interact with itself

Determination of the correlation coefficient according to the expression level of TDP-43 and FUS. Correlation has been measured automatically (Cell profiler) by analyzing repartition of fluorescence intensity along microtubule



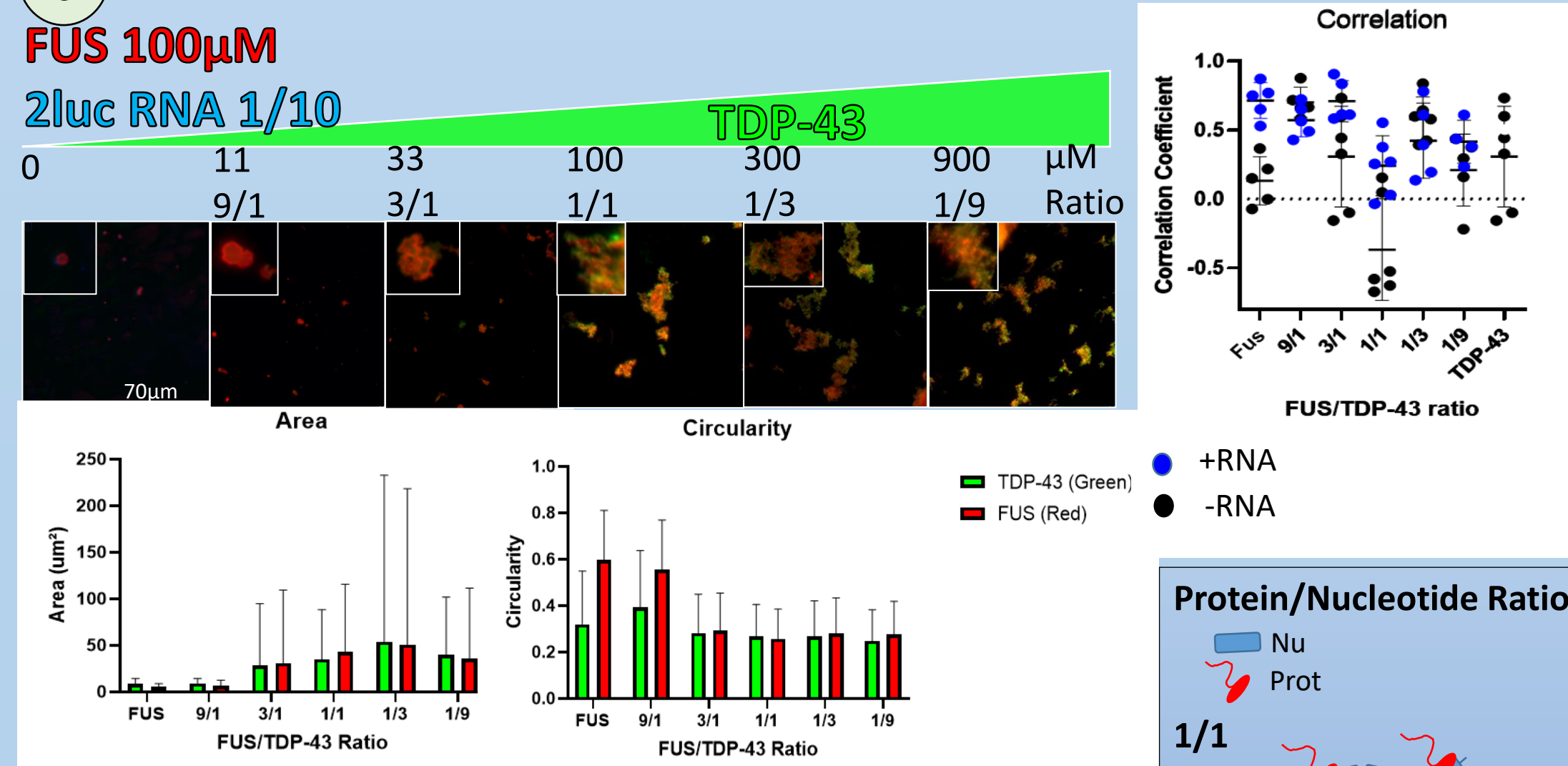
5 Ultrastructural analysis of FUS and TDP-43 assemblies

Ratio of TDP-43 and FUS without RNA



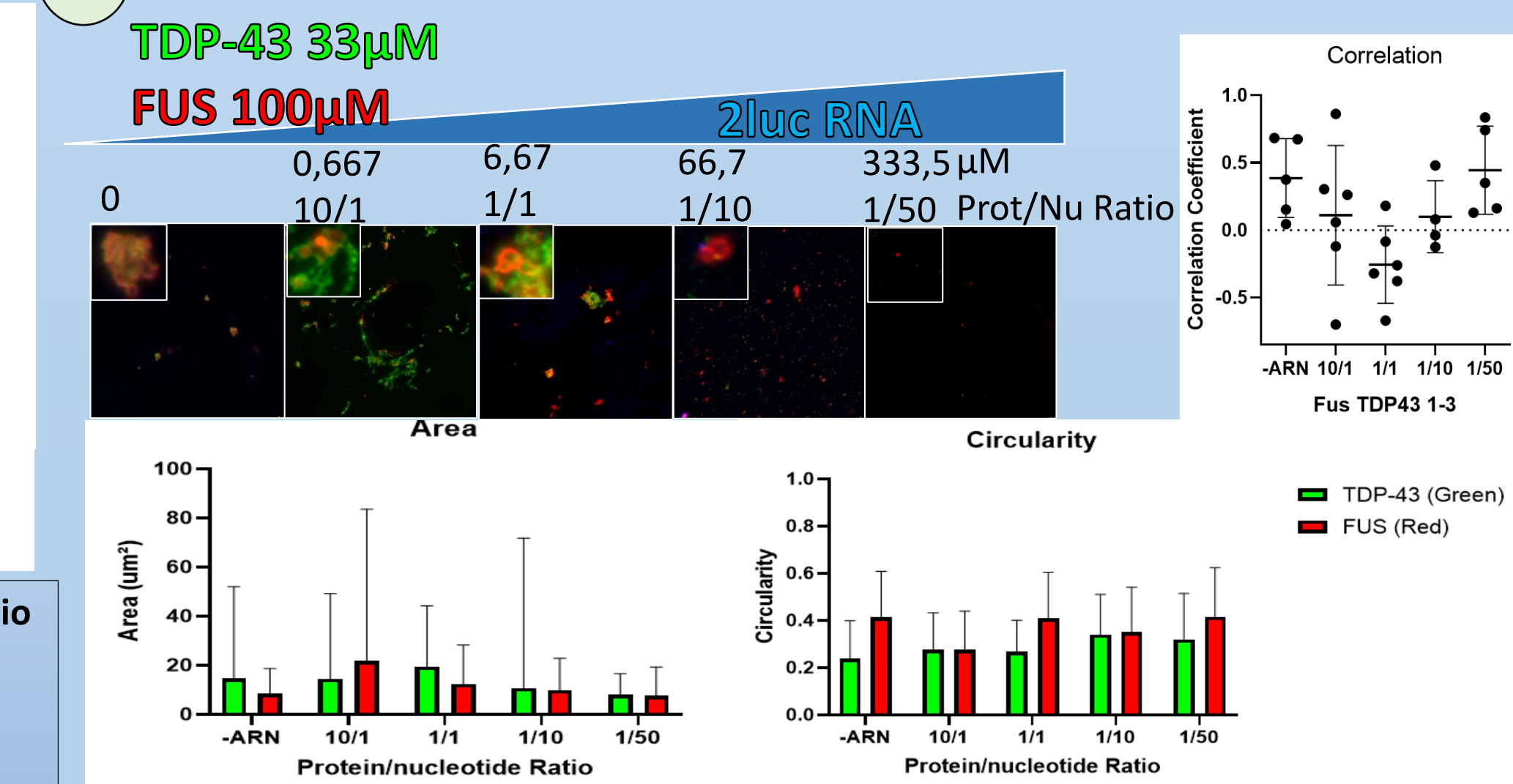
TDP-43 and FUS interact without RNA: FUS solubilizes TDP-43 in its phase at a high ratio, while FUS forms clusters in TDP-43 aggregates at a low ratio

Ratio of TDP-43 and FUS with RNA



Miscibility of TDP-43 and FUS is improved by RNA but still depends on the protein ratio

Influence of RNA ratio on RBPs solubility and mixing [2]



RNA decreased miscibility at a low ratio (Prot/Nu) and improved miscibility at a high ratio while preventing aggregation

Conclusion Using an original method, we demonstrated that TDP-43 and FUS interact in cellular context and In vitro. However, this interplay depends on the expression level of proteins, it appears that FUS is able to accept TDP43 in its environment while TDP43 forms a dense and rich phase where FUS is hardly inserted. Such differences could be related to the structure of the RBP assemblies. FUS forms droplet that could solubilize TDP43 upon quite elevated ratio while TDP43 has the propensity to produce higher order assemblies with a filamentous structure in which FUS clustered. Moreover, RNA, through its buffering effect, is important for protein solubility and seems to increase the interplay between these 2 RBPs. Regarding their involvement in neurodegenerative diseases as Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Lobar Degeneration (FTLD) [3] and the more recently described Limbic-predominant age-related TDP-43 Encephalopathy (LATE), it seems important to better characterized the compartment of these RBPs. Indeed, these proteins are aggregated in neurons of patients under different forms (ubiquitinated, mutated, phosphorylated...) and could coexist in the same assemblies. The overlap of these neurodegenerative diseases suggests a common mechanism of degeneration, where loss of function in RNA metabolism is suspected. Better understanding the aggregation process and how to prevent it remains essential.

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