MYOTONE DYSTROFIE EXPERTISECENTRUM

ENTRY DM

Funded by the European Union

Focusing on foci: Expansion Microscopy for high-resolution characterization of repeat RNA-protein aggregates in DM

Sofia Gaspari[1], Marieke Willemse[1,2], Thomas D. Hoekman[1], Hans van Bokhoven[2], Derick G. Wansink[1]

[1]Department of Medical BioSciences, Radboudumc, Nijmegen, 6525 GA, The Netherlands

[2]Department of Human Genetics, Radboudumc, Nijmegen, 6500 HB, The Netherlands

Introduction

A key feature of myotonic dystrophy type 1 (DM1) and 2 (DM2), is the accumulation of RNA-protein aggregates, primarly in cell nuclei. However, unravelling their structure, dynamics, and function remains a challenge. To resolve RNA foci ultrastructure, we introduced Expansion Microscopy (ExM) as an innovative approach to DM research. The combination of ExM with other techniques, such as single-molecule inexpensive FISH (smiFISH), provides another approach to high-resolution visualization of MBNL-C(C)UG repeat transcript complexes in cell types relevant to DM pathology.

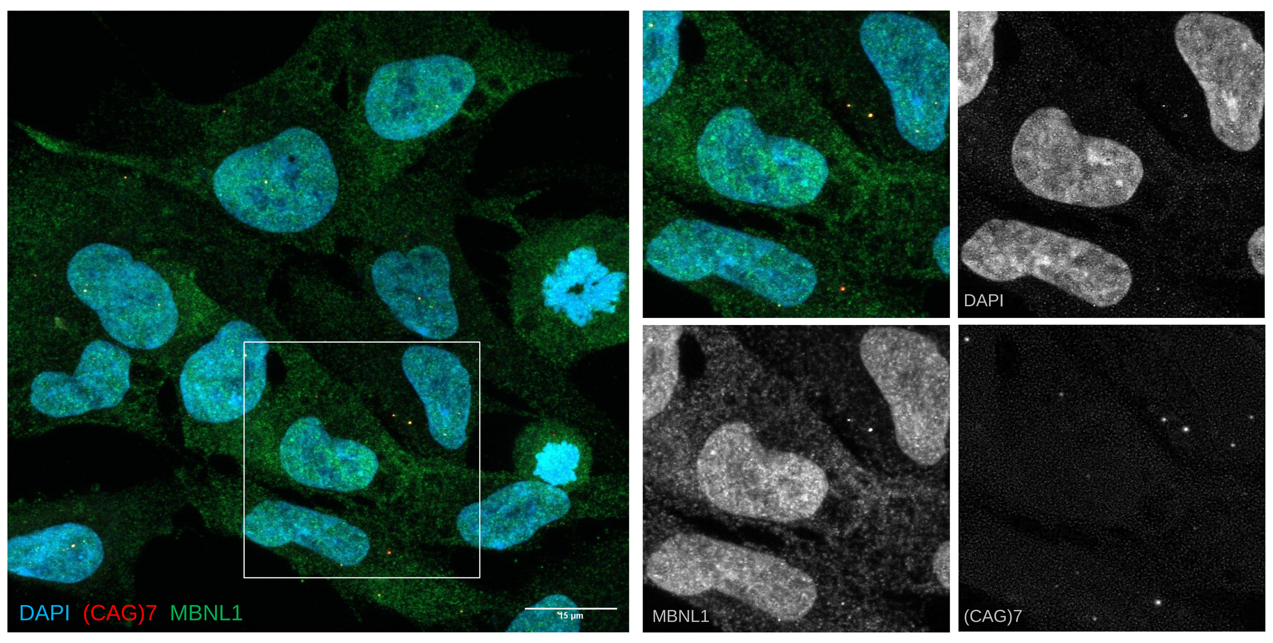

***Figure 1.* Visualization of (CUG)n RNA foci by confocal microscopy.** iPSc-derived muscle progenitor cells (MPCs, CTG ≈4000) were stained with a $(CAG)_7$ smiFISH probe and antibody against MBNL1. Images were acquired on a Zeiss LSM900 confocal microscope. Scalebar is 15 μm.

AIM:
Characterization of the nanoscale structure of RNA foci through smiFISH and Expansion Microscopy

Methods and Results

For analysis of cultures of DM patient-derived cells, we employed small-molecule inexpensive fluorescence *in situ* hybridization (smiFISH). This technique allows RNA localization by imaging individual mRNAs in single cells, by exploiting the presence of two fluorophores on each probe, ideally allowing a stronger and more sensitive signal detection. In combination with immunofluorescence assays, this enabled us to study DM hallmarks such as nuclear and cytoplasmic RNA foci, and free and foci-sequestered MBNL1/2 protein levels. To perform expansion microscopy, immunolabelled samples were uniformly enlarged via hydrogel expansion, enabling super-resolution imaging of foci using confocal fluorescence microscopy.

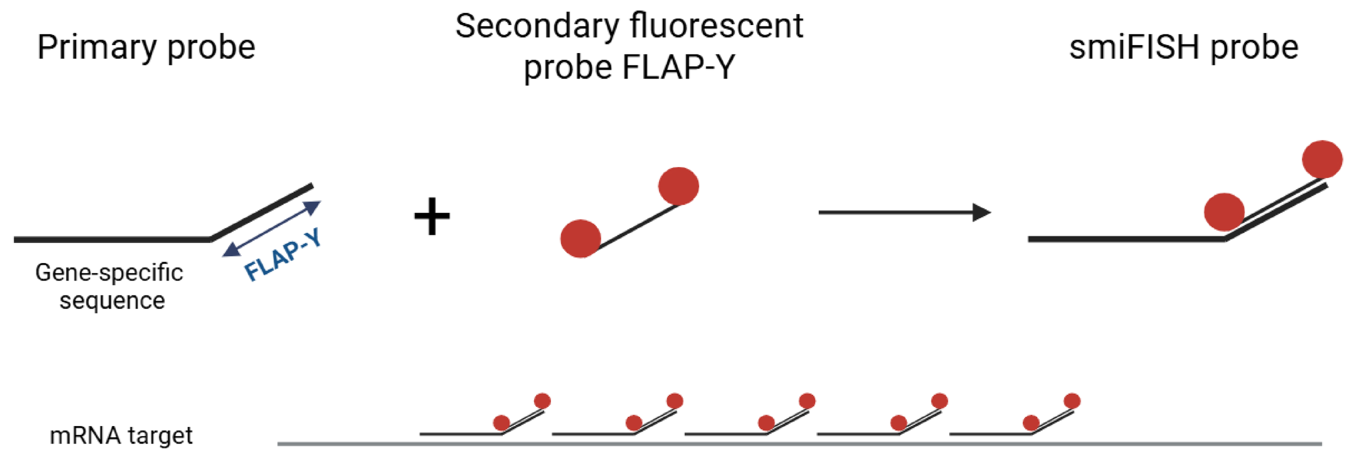

***Figure 2.* smiFISH probe structure.** The primary probe consists of the gene-specific sequence, followed by a FLAP-Y sequence, which will be recognized by the secondary probe. The secondary probe has fluorophores attached to both ends. This techniques allows to design secondary probes with different fluorophores, leading to various combinations of colours for the detection. Image adapted from *Tsanov et al. (2016).*

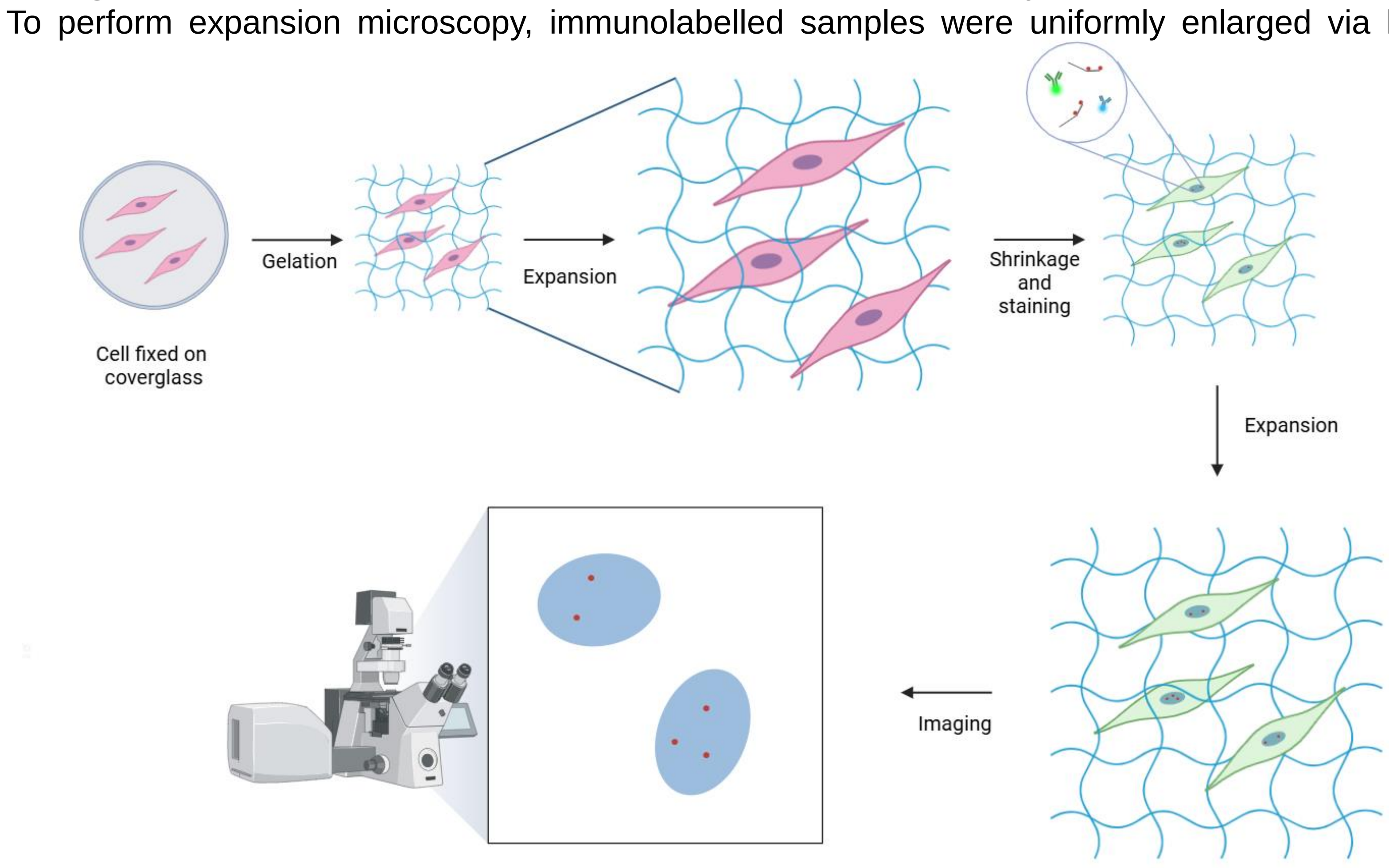

***Figure 3.* Schematic representation of Expansion Microscopy workflow.** After seeding, cells are fixed on coverslips. Then, they are embedded into a hydrogel, which is expanded by immersion in water. FISH and immunofluorescence are performed on the samples and, after a second expansion, imaged through confocal microscopy (Airyscan mode).

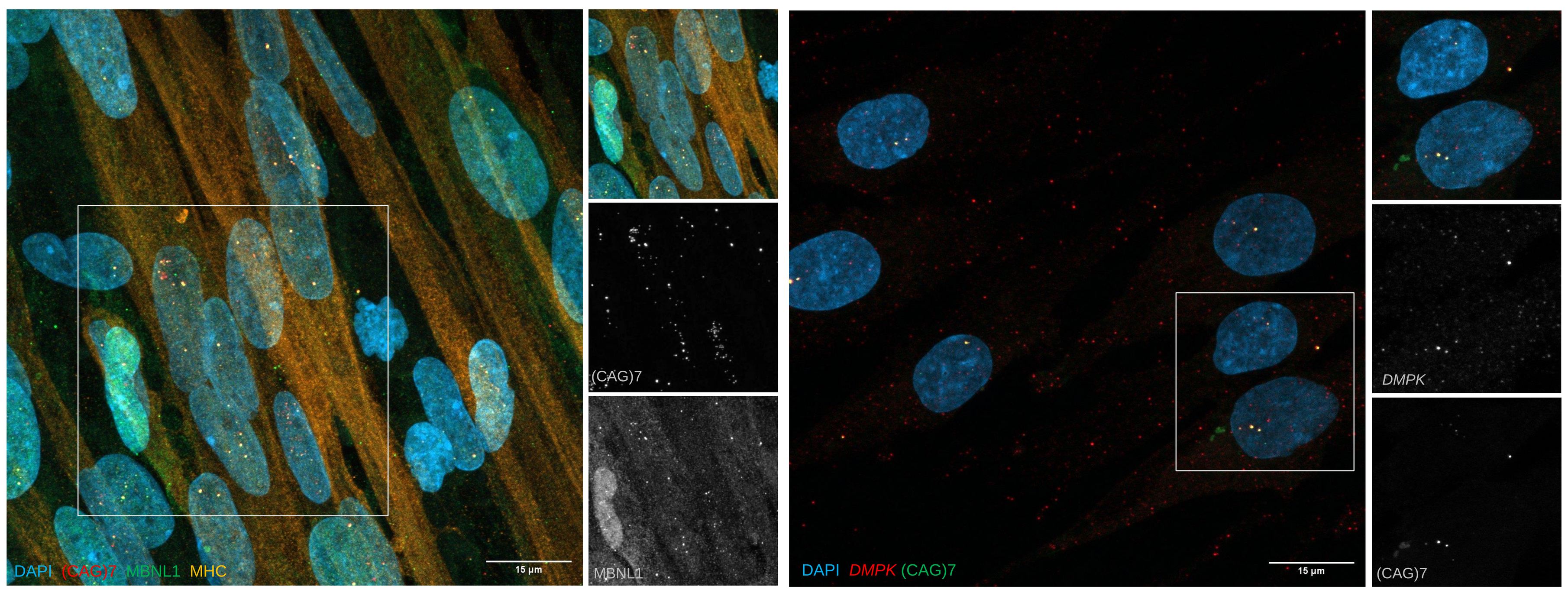

***Figure 4.* Application of smiFISH in combination with immunofluorescence.** *On the left:* iPSC-derived muscle progenitor cells after 3 days of differentiation. (CUG)n RNA foci were identified with a $(CAG)_7$ -ATTO 647N probe as reported above. *On the right*: (CUG)n repeats and *DMPK* transcripts were detected in immortalized DM1 myoblasts (DM11, CTG ≈2600), respectively with $(CAG)_7$ -ATTO 488 and *DMPK*-ATTO 647N probes. A significant number of both nuclear and cytoplasmic foci was detected. Images were acquired on a Zeiss LSM900 confocal microscope. Scalebar is 15 μm.

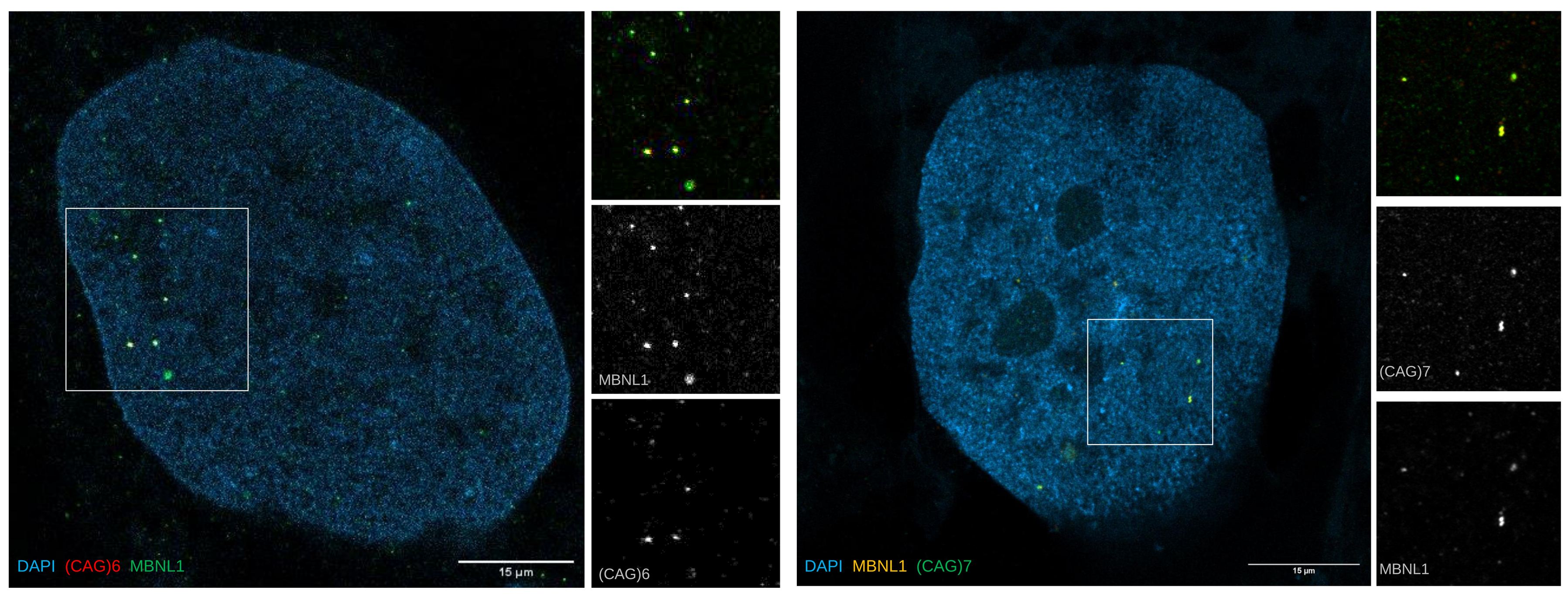

***Figure 5.* Visualization of (CUG)n RNA foci by Expansion Microscopy.** *On the left*: immortalized DM1 myotubes (DM11) were stained with a $(CAG)_6$-ALEXA 647 FISH probe and antibody against MBNL1. *On the right*: MPCs after 1 day of differentiation, stained with $(CAG)_7$ -ATTO 488 probe and antibody against MBNL1. Images were acquired on a Zeiss LSM900 microscope with Airyscan mode. Scalebar is 15 μm.

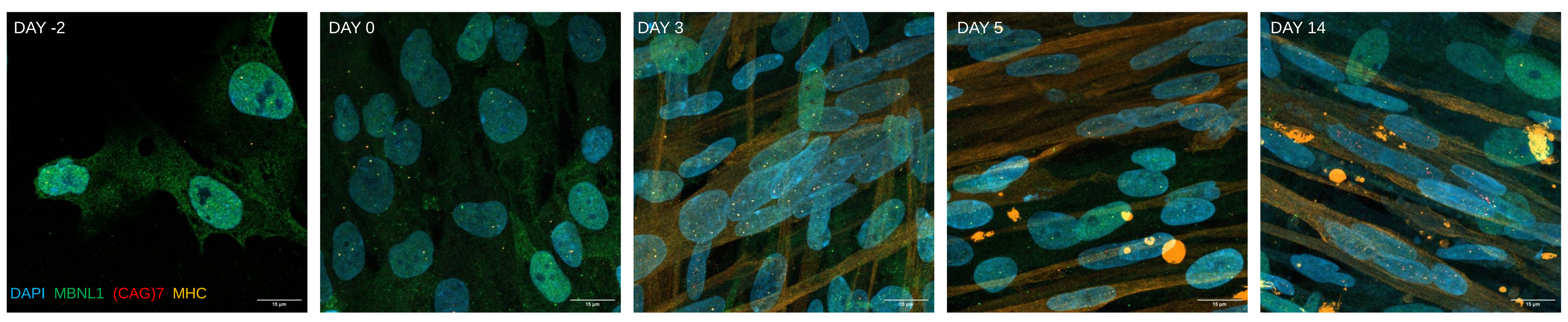

***Figure 6.* MPCs at different timepoints of differentiation.** MPCs were cultured for 14 days after inducing differentiation, then smiFISH was performed using the $(CAG)_7$ -ATTO 647N probe; antibodies against myosin heavy chain (MHC) and MBNL1 were used for immunofluorescence. Images were acquired on a Zeiss LSM900 confocal microscope. Scalebar is 15 μm.

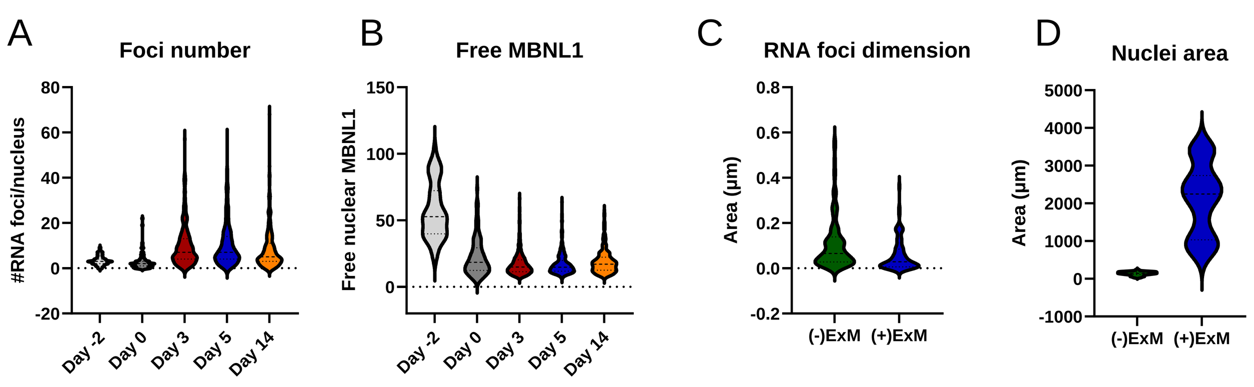

***Figure 7.* Analysis of RNA foci in MPCs.** (A-B) Number of nuclear RNA foci and MBNL1 sequestration in MPCs at different point of differentiation**.** (C-D) Differences in area of the nuclei and RNA foci with or without expansion microscopy. The expansion factor of cell nuclei is ≈4 times.

Conclusion

The combination of established techniques, such as smiFISH, with more innovative ones like expansion microscopy, is a successful approach to the characterization of RNA foci in DM. The analysis of ribonuclear aggregates through ExM indicates that they are much smaller than was previously thought, and structurally diverse. This finding highlights the potential of this technique as a nanoscale tool to study DM1 pathogenesis.

Want to know more about our expertise center?

Correspondence to:

Sofia.Gaspari@radboudumc.nl

Radboud University · Maastricht University · DONDERS INSTITUTE · MHENS · Radboudumc · Maastricht UMC+ · EURODMCRN · EURO-NMD Registry Hub, European Reference Network for neuromuscular diseases