APPENDIX FOR

Spatial control of nucleoporin condensation by Fragile X-related proteins

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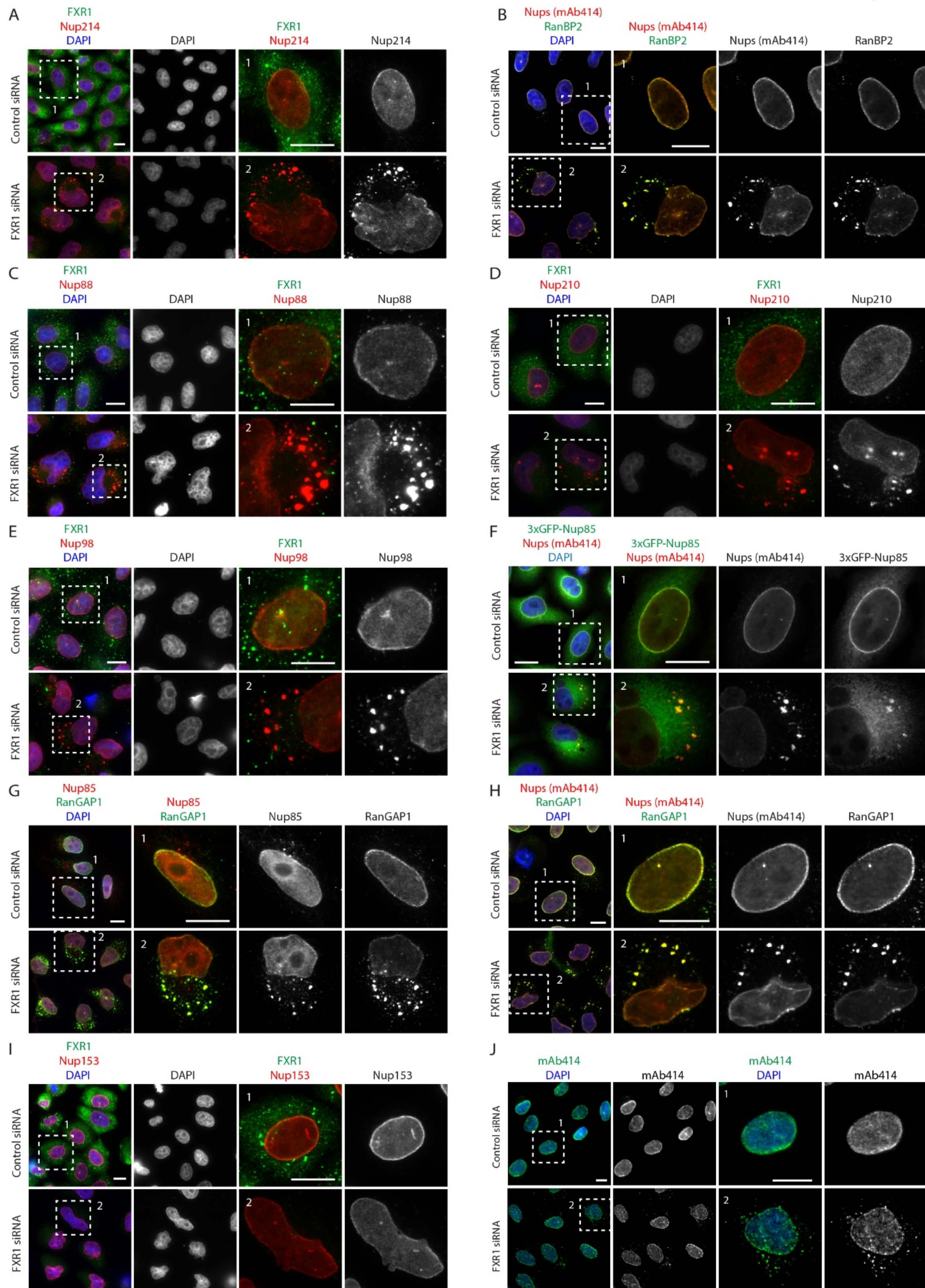
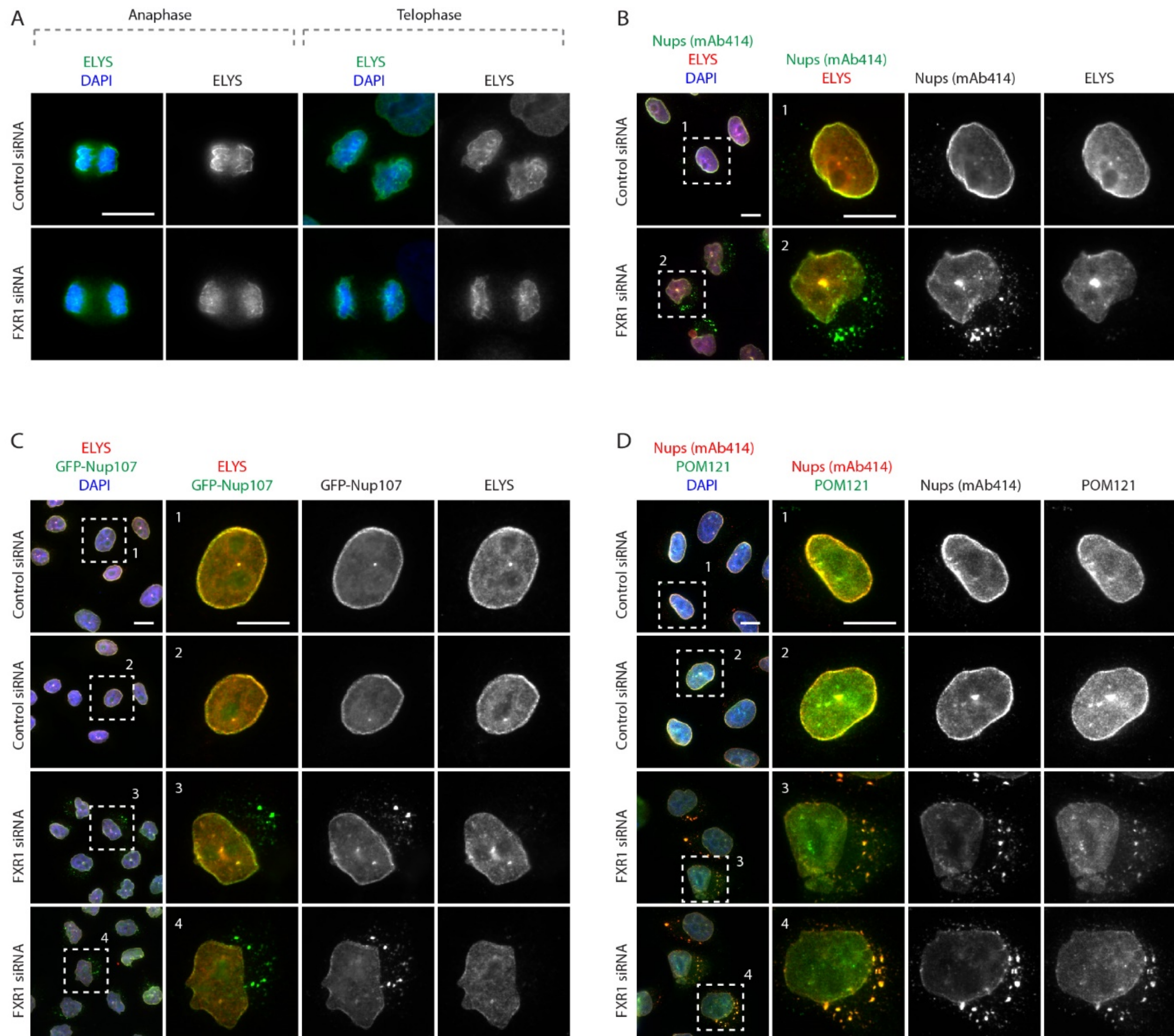


Appendix Figure S1 - FXR1 inhibits aberrant assembly of cytoplasmic Nups.

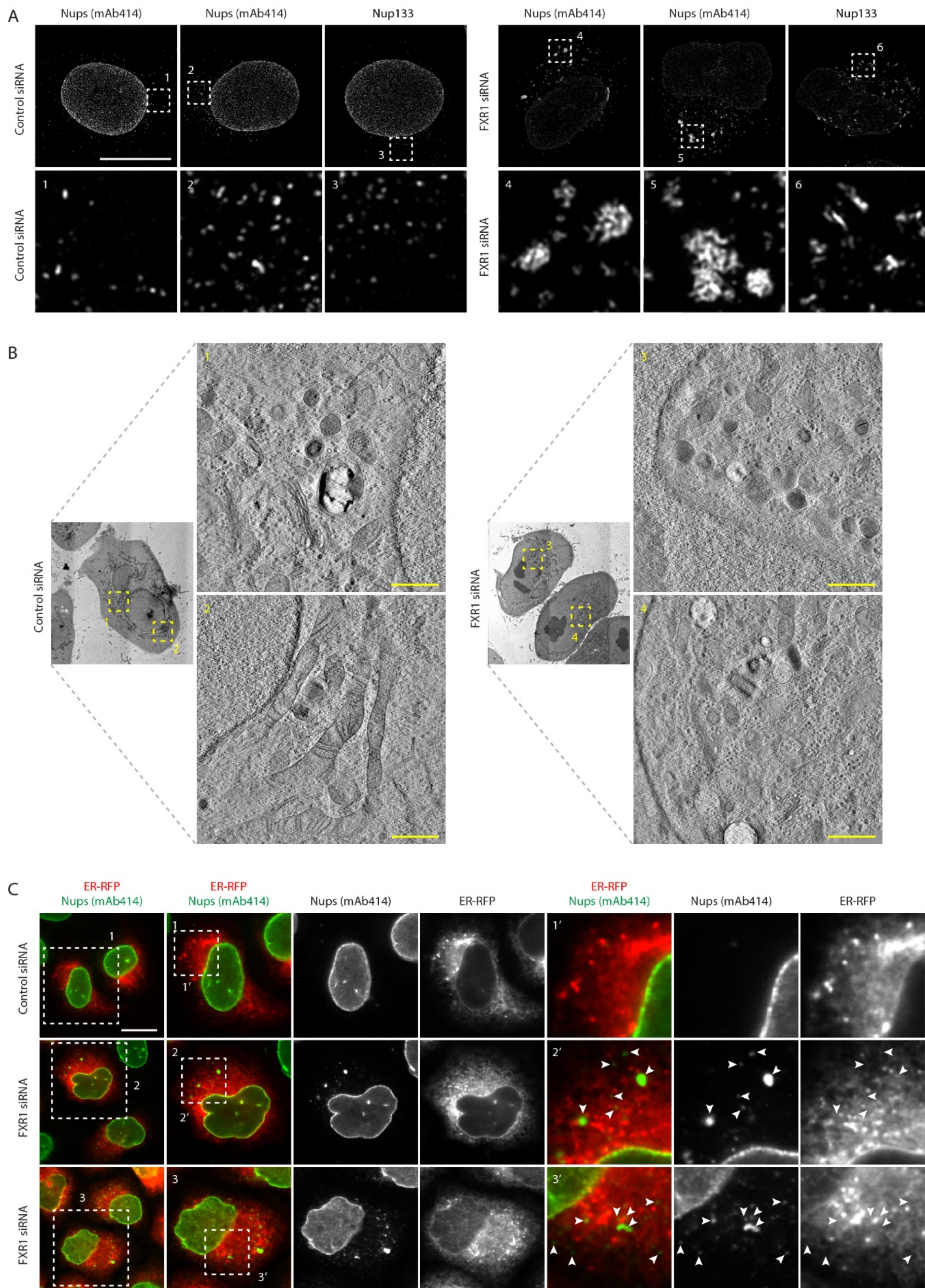
A-I HeLa cells were treated with the indicated siRNAs, synchronized by double thymidine block and released for 12 hours and analysed by immunofluorescence microscopy. The magnified framed regions are shown in the corresponding numbered panels (**related to Figure 2C**). Scale bars are 5 μm.

J U2OS cells were treated with the indicated siRNAs and analysed by immunofluorescence microscopy for FG-Nups using mAb414 antibody. The magnified framed regions are shown in the corresponding numbered panels (**related to Figure 2G, H**). Scale bars are 5 μm.



Appendix Figure S2 - FXR1 inhibits aberrant assembly of cytoplasmic POM121 but not ELYS.

A-D HeLa cells (**A, B, D**) or HeLa cells stably expressing GFP-Nup107 (**C**) were treated with the indicated siRNAs, synchronized by double thymidine block and released for 9 (telophase) (**A**) and 12 (early G1 phase) (**B-D**) hours and analysed by immunofluorescence microscopy. The magnified framed regions are shown in the corresponding numbered panels (**related to Figure 2C**). Scale bars are 5 µm.



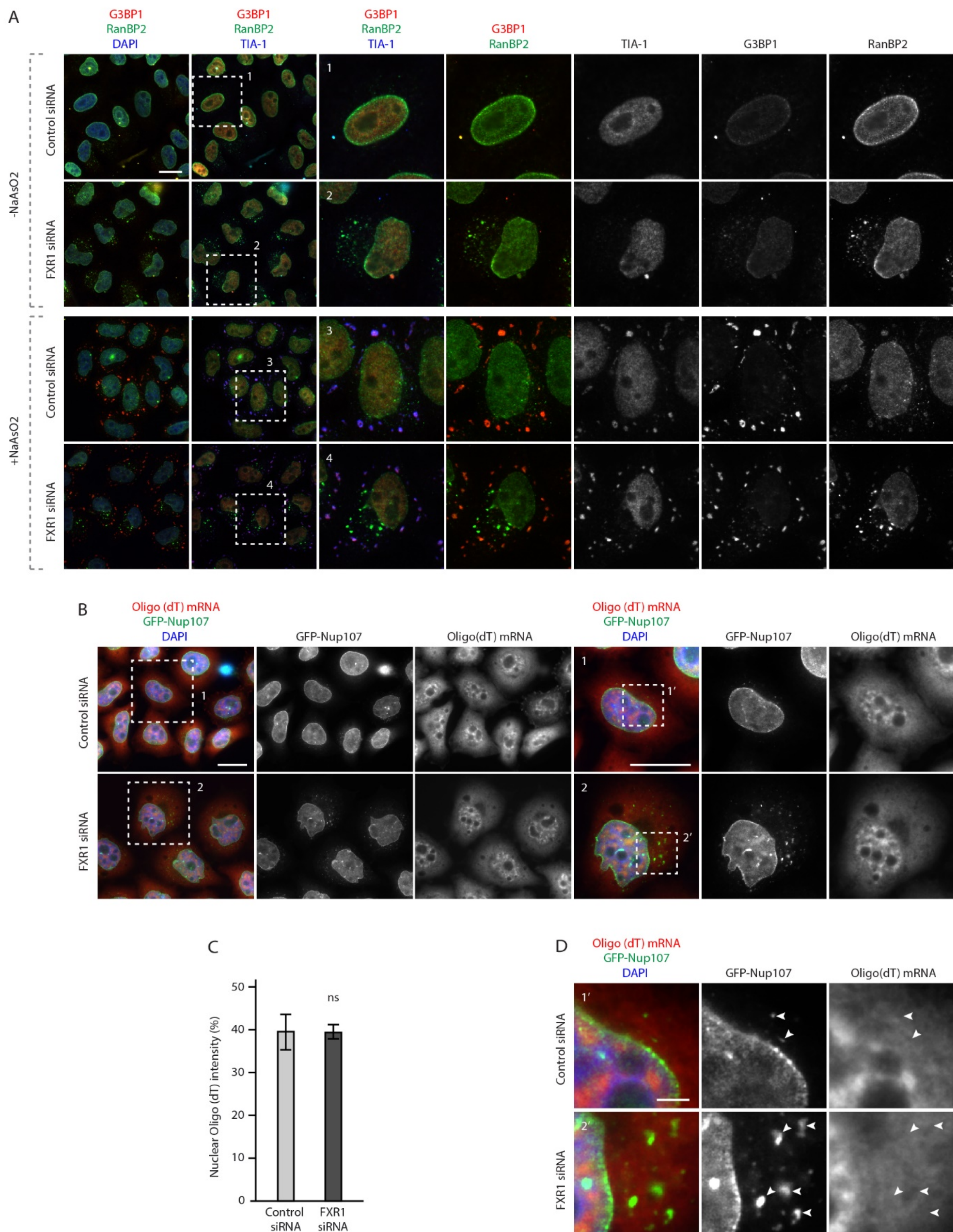
Appendix Figure S3 - The cytoplasmic nucleoporin granules are Nup assemblies distinct

from ALs.

A HeLa cells were treated with the indicated siRNAs, synchronized by double thymidine block and released for 12 hours and analysed by superresolution confocal microscopy. Scale bar is 5 µm.

B HeLa cells were treated with the indicated siRNAs, synchronized by double thymidine block and released for 12 hours and analysed by electron microscopy. The magnified framed regions are shown in the corresponding numbered panels. Scale bar is 1 µm.

C HeLa cells were treated with indicated siRNAs, transfected with ER-RFP reporter for 24h, synchronized by double thymidine block and released for 12 hours and analysed by immunofluorescence microscopy. The magnified framed regions are shown in the corresponding numbered panels. Arrowheads point to the FG-Nup-positive cytoplasmic granules. Scale bar is 5 µm.

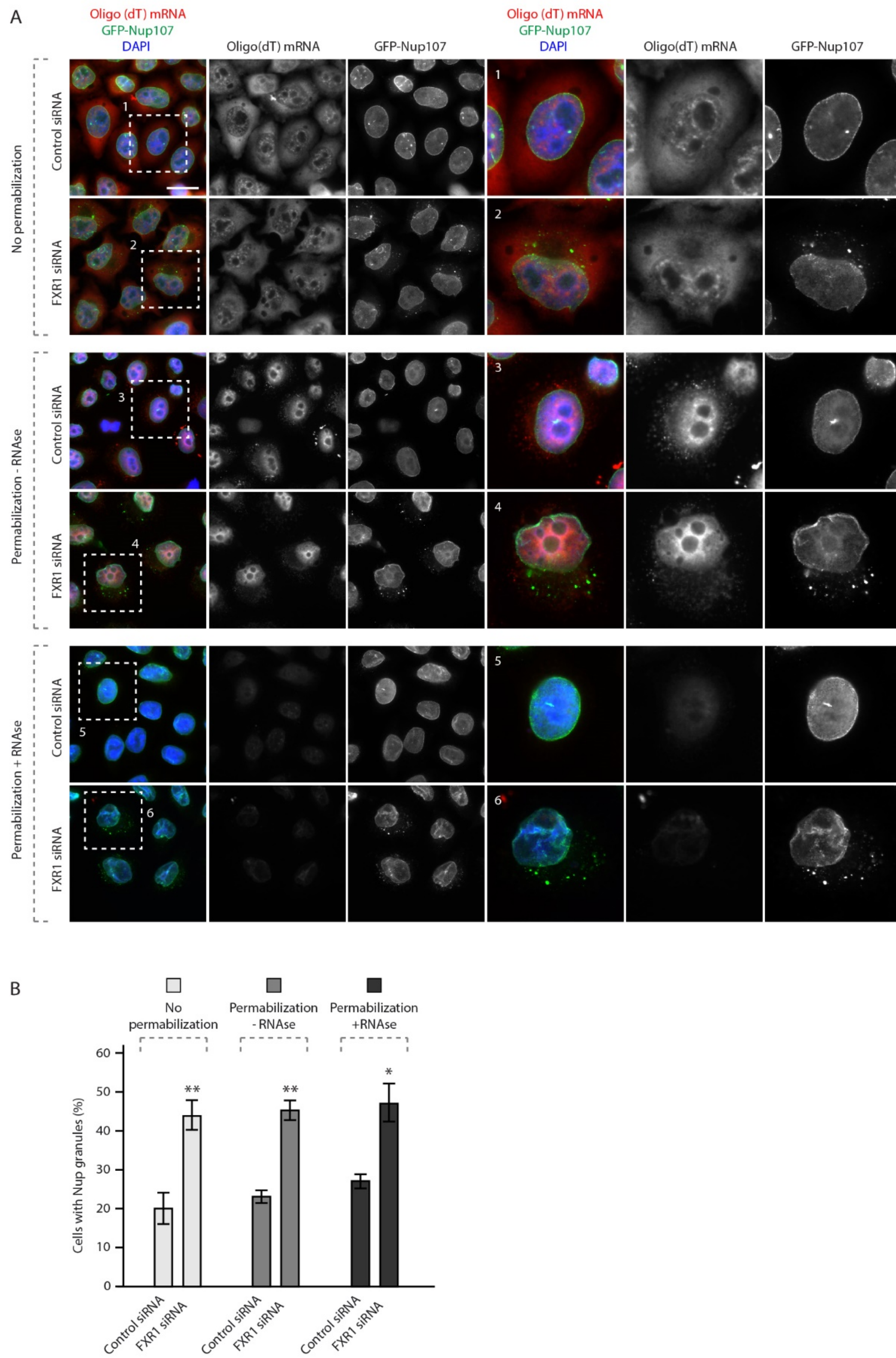


Appendix Figure S4 - The cytoplasmic nucleoporin granules are distinct from SGs and do not contain mRNAs.

A HeLa cells were treated with the indicated siRNAs, synchronized by double thymidine block and released for 12 hours, treated with or without the stress inducing factor NaAsO2 0,5 mM for 1 hour and analysed by immunofluorescence microscopy for the stress granule markers G3BP1 and TIA-1 and the nucleoporin RanBP2. The magnified framed regions are shown in the corresponding numbered panels.

B-D HeLa cells stably expressing GFP-Nup107 were treated with the indicated siRNAs, synchronized by double thymidine block and released for 12 hours, hybridized with the oligo d(T) mRNA FISH probe and analysed by immunofluorescence microscopy. The magnified framed regions are shown in the corresponding numbered panels in (**B**) and higher magnifications are shown in (**D**). Arrowheads point to GFP-Nup107-positive cytoplasmic granules. The percentage of nuclear mRNA intensity was quantified in (**C**), 600 cells were analysed (mean ±SD, ns = non-significant; N = 3).

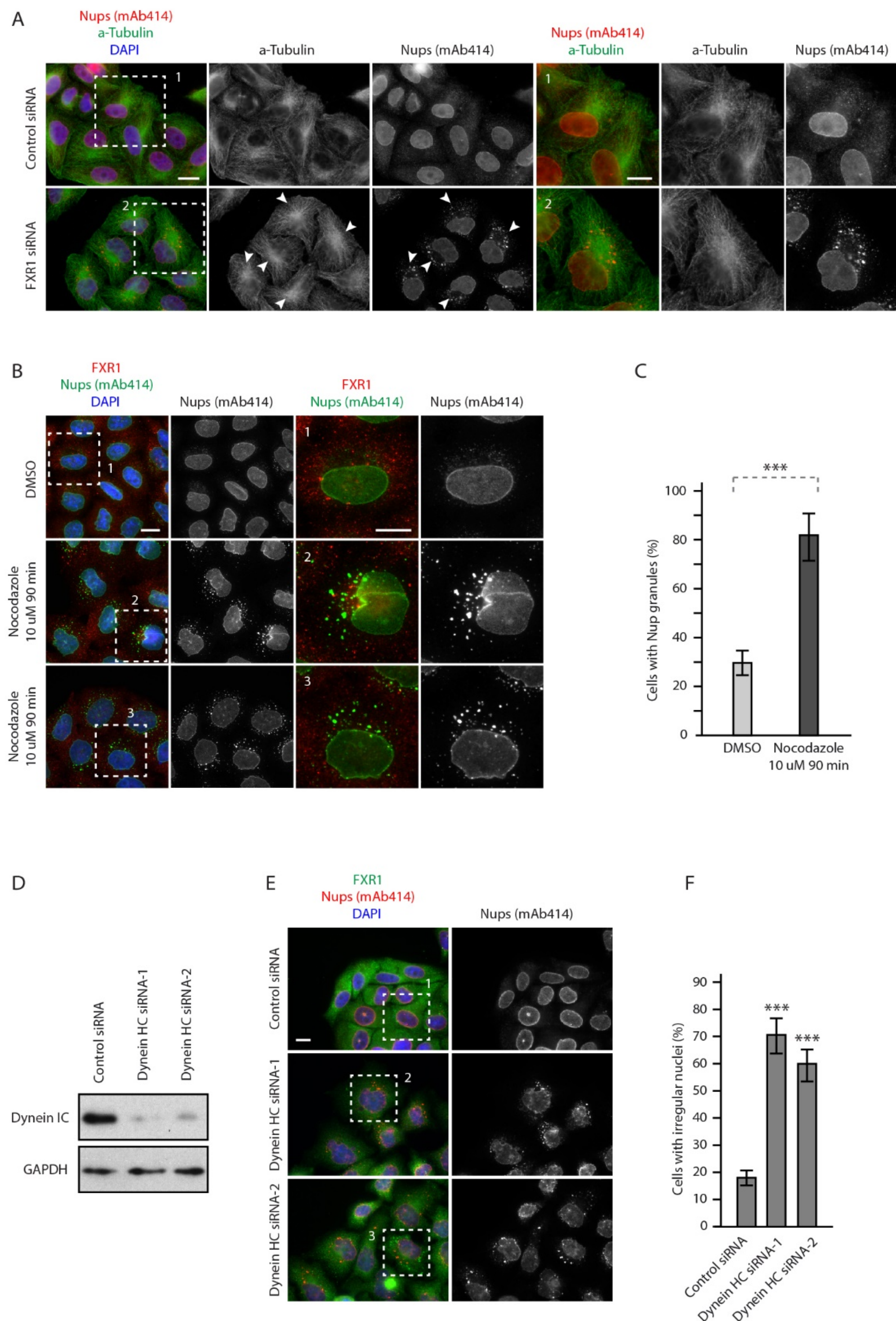
Data information: Scale bars are 5 µm (**A, B**) and 1 µm (**D**). Statistical significance was assessed by two-tailed unpaired Student’s T-test.



Appendix Figure S5 - RNAs are dispensable for maintenance and dynamics of the cytoplasmic nucleoporin granules.

A, B HeLa cells stably expressing GFP-Nup107 were treated with the indicated siRNAs, synchronized by double thymidine block and released for 12 hours, hybridized with the oligo d(T) mRNA FISH probe, treated with or without RNaseA/T1 and/or digitonin permabilization for 5 min and analysed by fluorescence microscopy. The magnified framed regions are shown in the corresponding numbered panels. The percentage of cells with GFP-Nup107-positive cytoplasmic granules was quantified in (**B**), 8900 cells were analysed (mean ±SD, $*P < 0.05$; $**P < 0.01$; $N = 4$). Scale bar is 5 μm.

Data information: Statistical significance was assessed by one-way ANOVA test with Sidak's correction.



Appendix Figure S6 - FXR1 inhibits formation of the cytoplasmic nucleoporin granules by dynein microtubule-dependent transport.

A HeLa cells were treated with the indicated siRNAs, synchronized by double thymidine block and released for 12 hours and analysed by immunofluorescence microscopy. Arrowheads point to the positions of the cytoplasmic nucleoporin granules relative to MTOC.

B, C HeLa cells were synchronized by double thymidine block and released for 12 hours, treated with nocodazole 10 uM or solvent (DMSO) for 90 min and analysed by immunofluorescence microscopy. The magnified framed regions are shown in the corresponding numbered panels in (**B**). The percentage of cells with cytoplasmic nucleoporin granules was quantified in (**C**), 2500 cells were analysed (mean ±SD, ***$P < 0.001$; $N = 3$).

D-F HeLa cells were treated with the indicated siRNAs, synchronized by double thymidine block and released for 12 hours and analysed by Western blot (**D**) and immunofluorescence microscopy (**E**). The percentage of cells with irregular nuclei was quantified in (**F**), 900 cells were analysed (mean ±SD, ***$P < 0.001$; $N = 3$).

Data information: Scale bars are 5 µm. Statistical significance was assessed by two-tailed unpaired Student's T-test (**C**) and one-way ANOVA test with Dunnett's correction (**F**).