Why are only basketless nuclear pore complexes found in the nucleolar territory?

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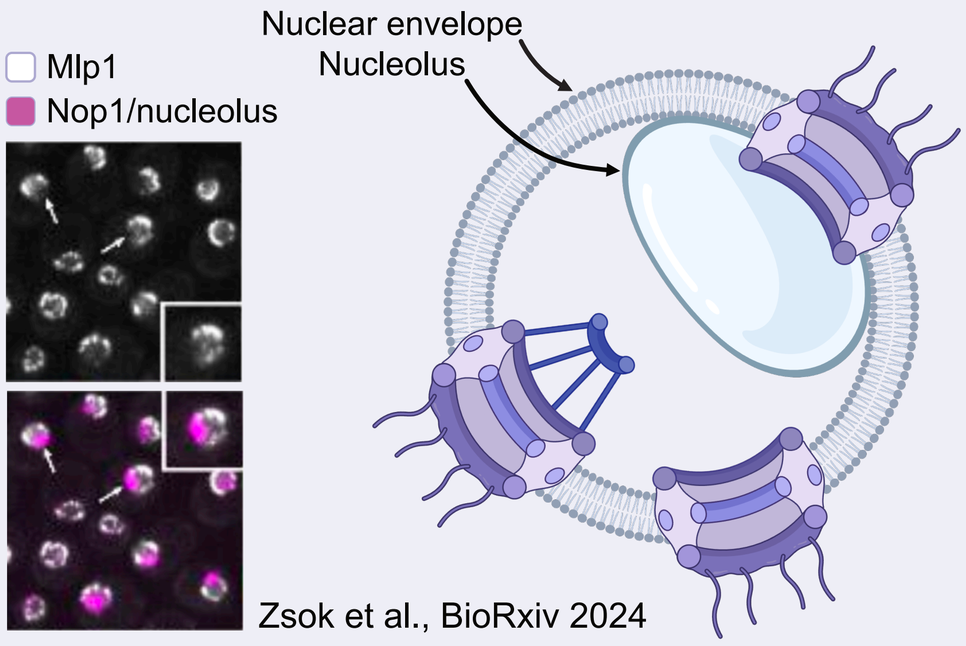
Background

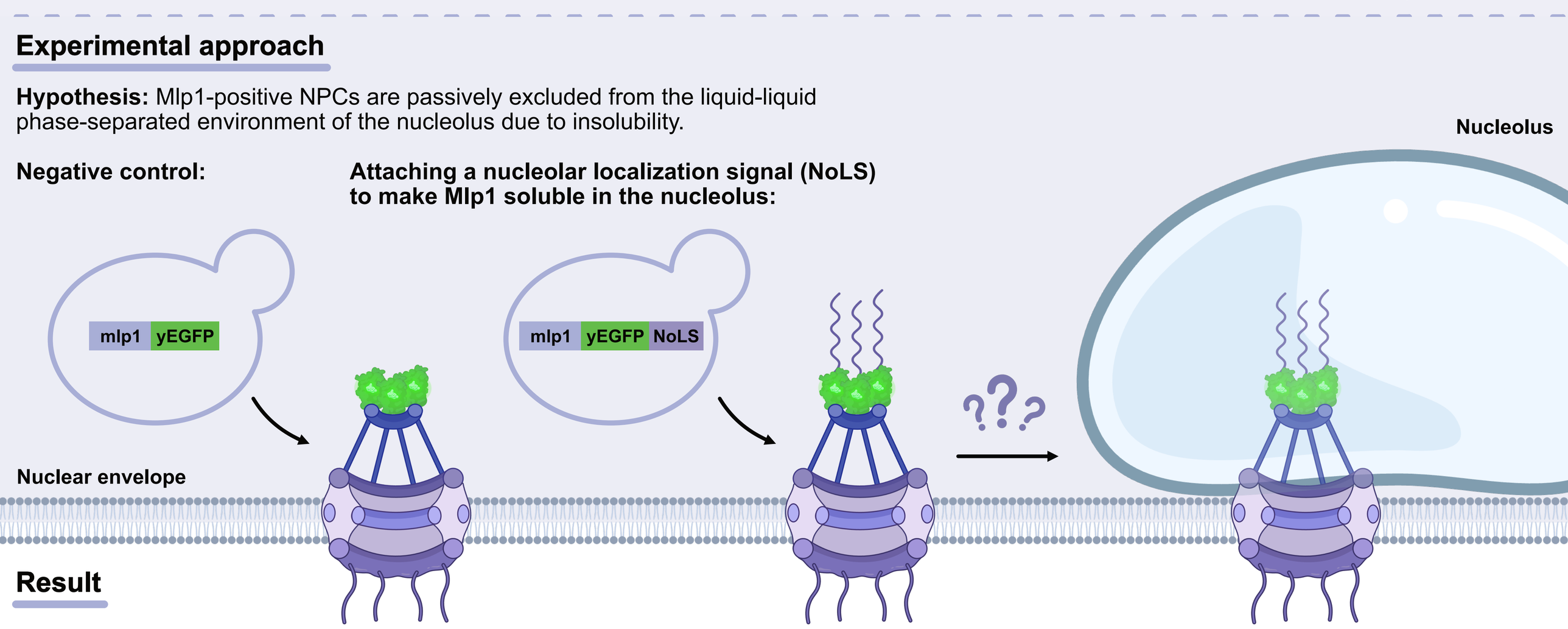
Nuclear pore complexes (NPCs) are big protein complexes in the nuclear envelope that mediate nucleocytoplasmic transport[1]. On the nucleoplasmic side, NPCs have a feature called the nuclear basket which is important for chromatin organization and mRNA export[1]. In budding yeast (S. cerevisiae), there are two variants of NPCs that differ in the presence or absence of the key structural basket component Mlp1[2]. Curiously, Mlp1-positive NPCs are confined to the extranucleolar territory of the nuclear envelope, while the basketless NPCs can be found in both regions[2]. The nucleolus of budding yeast is a membraneless organelle localized adjacent to the nuclear envelope[3]. It forms through the condensation of nucleolar material into a distinct phase, separated from the rest of the nuclear environment, akin to an immiscible liquid[3,4]. The aim of this project was to probe the liquid-liquid phase separation mechanism in Mlp1-positive NPCs exclusion from the nucleolar territory by endogenously fusing Mlp1 with previously characterized nucleolar localization signal (NoLS) sequences[5,6].



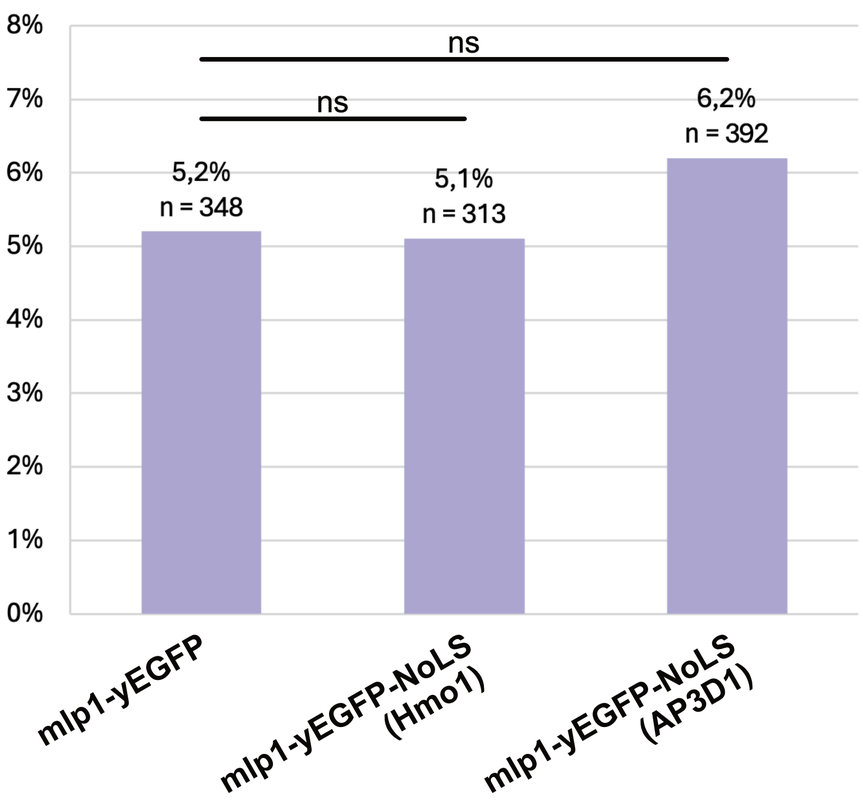
Zsok et al., BioRxiv 2024

Experimental approach

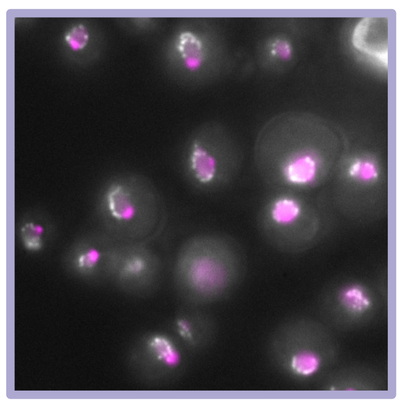
Hypothesis: Mlp1-positive NPCs are passively excluded from the liquid-liquid phase-separated environment of the nucleolus due to insolubility.



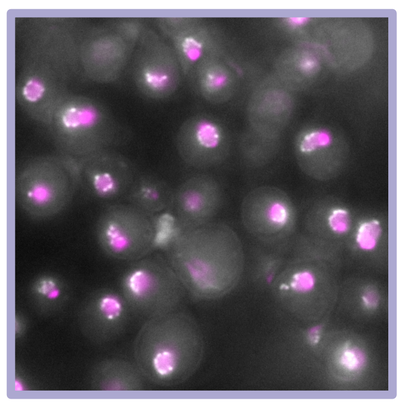
Result



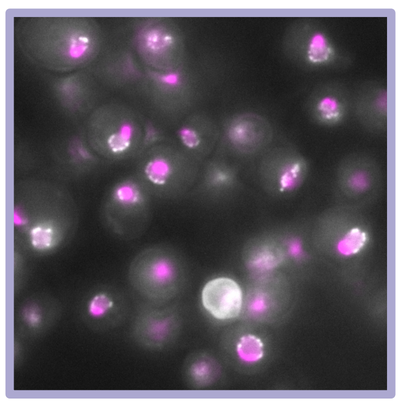
Statistical significance: Fisher's exact test.



mlp1-yEGFP-NoLS (Hmo1)

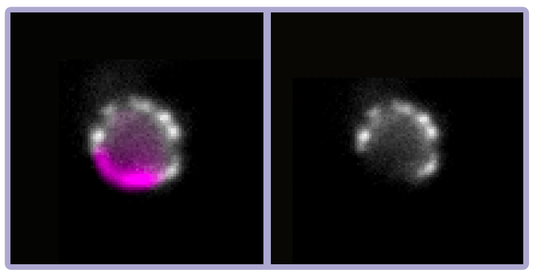


mlp1-yEGFP-NoLS (AP3D1)

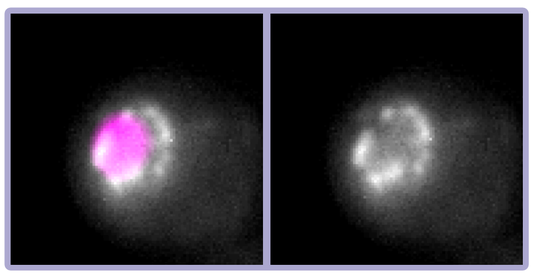


mlp1-yEGFP

Nucleolar exclusion pattern:



Nucleolar inclusion pattern:



Mlp1::yEGFP or Mlp1::yEGFP-NoLS

Nop1::mCherry

Nop1 is a nucleolar marker[7].

Conclusions and future perspectives

- Fusion of the tested NoLSs to Mlp1 did not significantly affect nucleolar exclusion of Mlp1-positive NPCs.
- Future perspectives: Test the nucleolar localization functionality of NoLSs; use bona fide nucleolar proteins as targeting means for the NPCs; investigate the effects of NoLSs on Mlp1-negative NPCs.

Illustrations created with BioRender.

1. Beck, Martin, and Ed Hurt. 2017. "The nuclear pore complex: understanding its function through structural insight." Nature Reviews Molecular Cell Biology 18: 73–89. doi: 10.1038/nrm.2016.147
2. Zsok, Janka, et al. 2024. "Nuclear basket proteins regulate the distribution and mobility of nuclear pore complexes in budding yeast." BioRxiv, preprint version. doi:10.1101/2023.09.28.558499
3. Miné-Hattab, Judith, and Angela Taddei. 2019. "Physical principles and functional consequences of nuclear compartmentalization in budding yeast." Current Opinion in Cell Biology 58: 105–113. doi: 10.1016/j.ceb.2019.02.005
4. Lafontaine, Denis L. J., et al. 2021. "The nucleolus as a multiphase liquid condensate." Nature Reviews Molecular Cell Biology 22: 165–182. doi: 10.1038/s41580-020-0272-6
5. Scott, Michelle S., et al. 2010. "Characterization and prediction of protein nucleolar localization sequences." Nucleic Acids Research 38, no. 21: 7388–7399. doi: 10.1093/nar/gkq653
6. Girke, Philipp, et al. 2023. "Targeting of Hmo1 to subcompartments of the budding yeast nucleolus." Molecular Biology of the Cell 34, no. 3. doi: 10.1091/mbc.E22-07-0261
7. Schimmang, Thomas, et al. 1989. "A yeast nucleolar protein related to mammalian fibrillarin is associated with small nucleolar RNA and is essential for viability." The EMBO Journal 8, no. 13: 4015–4024. doi: 10.1002/j.1460-2075.1989.tb08584.x

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