ENDOTHELIAL NUCLEAR PROTEINS SUN1 AND SUN2 REGULATE MICROTUBULE ORGANIZATION IN ENDOTHELIAL CELLS

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Abstract

Malfunctions in the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex machinery lead to varying cases of muscular dystrophy, cardiac and skeletal muscle pathologies, and progeria. Progeria is a premature aging syndrome characterized by the development of atherosclerosis in young patients. Although endothelial cell (EC) dysfunction contributes to cardiovascular impairments in progeria patients, the role of the LINC complex in EC is still poorly understood. Our study examines how the depletion of LINC complex proteins affects microtubule organization in endothelial cells. Specifically, we investigate the unique and shared functions of SUN1, SUN2, and SYNE1 (nesprin-1) proteins. Through siRNA-mediated depletion, re-nucleation assays, and orbital flow experiments, the research identifies how SUN1, SUN2, and SYNE1 influence centrosome positioning and microtubule nucleation in cultured endothelial cells. Results indicate that the depletion of *SUN1*, *SUN2*, or *SYNE1*, individually or in combination, impacts centrosome distance from the nucleus, induces Golgi apparatus dispersal, and delays microtubule re-nucleation. The findings suggest that the LINC complex components are critical for the communication between the nucleus and the centrosome, which has implications in endothelial cell polarization and signal transduction, contributing to vascular biology and pathology.

Introduction

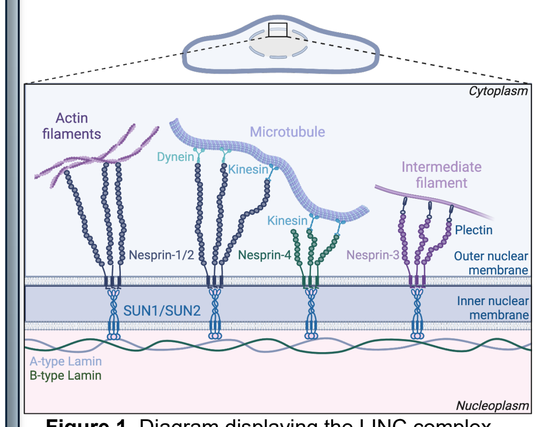
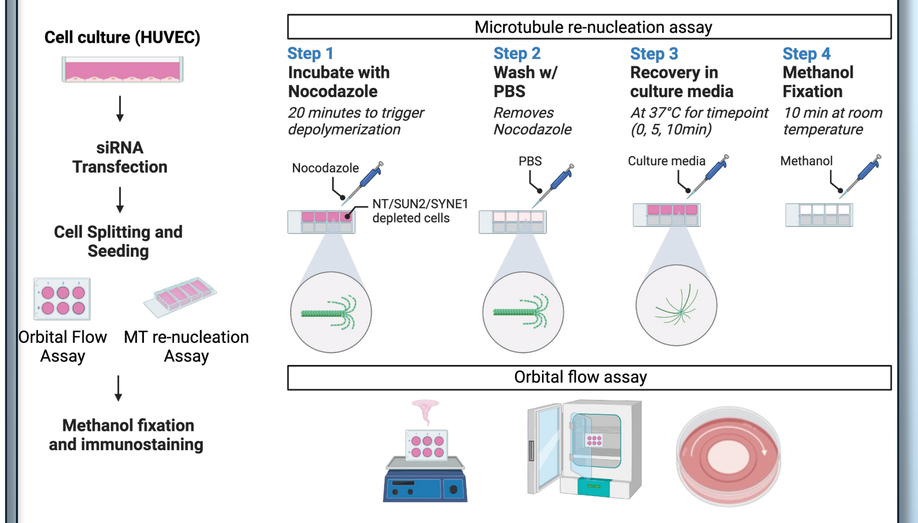


Figure 1. Diagram displaying the LINC complex.

- The LINC complex (Linker of Nucleoskeleton and Cytoskeleton) bridges the nucleus, and the cytoskeleton is composed of the SUN proteins and nesprins located in the inner and outer nuclear membrane, respectively **(Figure 1).**
- SUN2 is hypothesized to play a role in regulating the behavior of endothelial cells by transmitting forces between the nucleus and cytoskeleton.[1]
- Our lab showed that SUN1 regulates microtubule dynamic and proposed that it acts as a decoy preventing the interaction between SYNE1 (Nesprin-1) and other nuclear envelope proteins, such as SUN2. [2]
- Preliminary results from our lab showed that unlike SUN1, SUN2 regulates flow-induced endothelial cell polarization, a process regulated by microtubule organization in other cell types.[3]
- How do the LINC complex proteins SUN1, SUN2, and SYNE1 regulate microtubule organization and dynamics in endothelial cells?

Materials and Methods



Results

siRNA transfection successfully decreased SUN1, SUN2 and SYNE1 protein expression

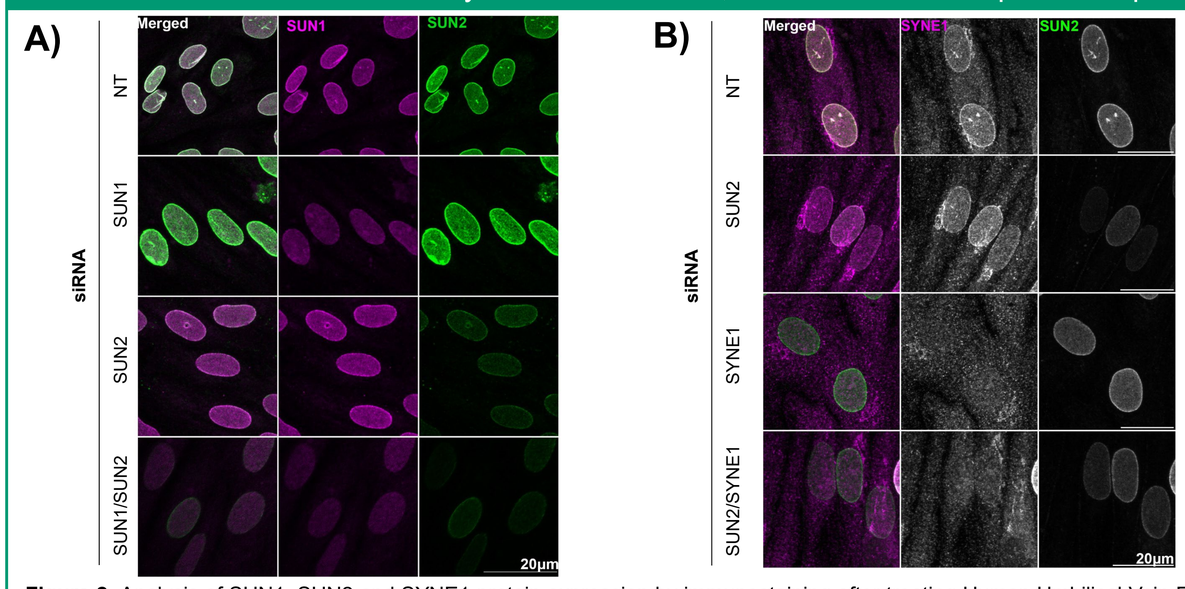


Figure 2. Analysis of SUN1, SUN2 and SYNE1 protein expression by immunostaining after treating Human Umbilical Vein Endothelial Cells (HUVEC) with *NT*, *SUN1*, *SUN2* or *SYNE1* siRNA and staining with SUN2 (in green), SUN1 (in pink) antibodies **(A)** or with SUN2 (in green) or SYNE1 (in pink) antibodies **(B)**.

SUN1 and SUN2 regulate centrosome distance from the nucleus under flow

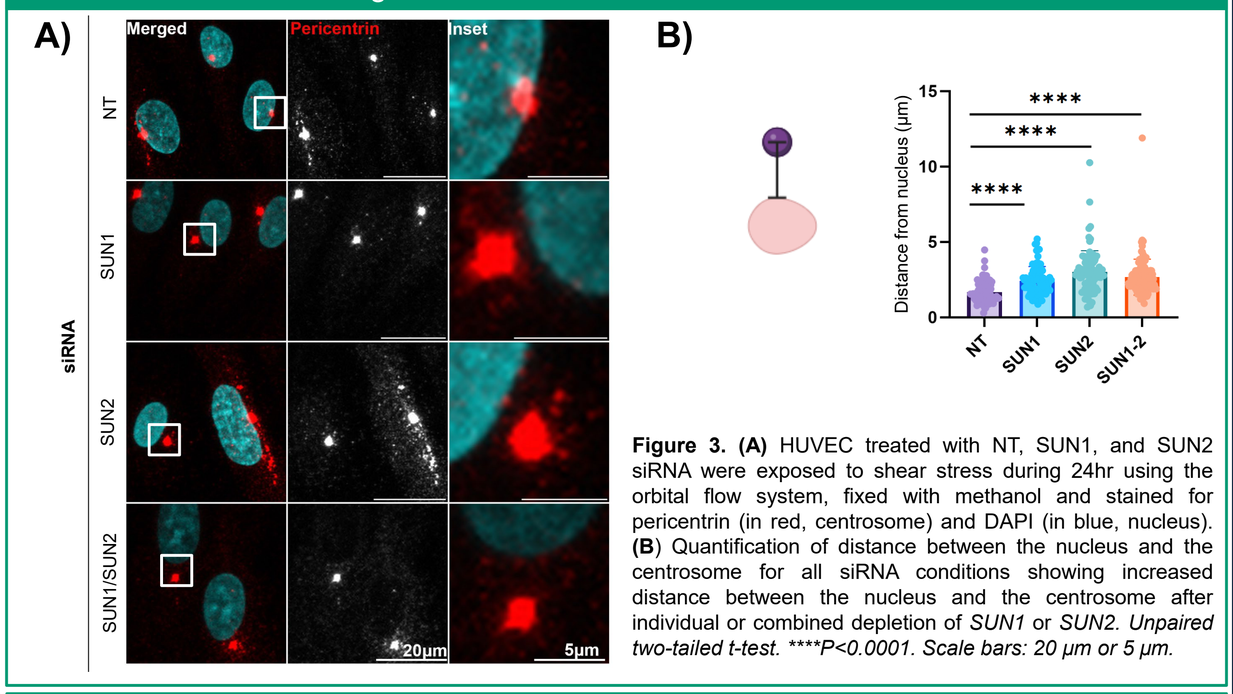


Figure 3. (A) HUVEC treated with NT, SUN1, and SUN2 siRNA were exposed to shear stress during 24hr using the orbital flow system, fixed with methanol and stained for pericentrin (in red, centrosome) and DAPI (in blue, nucleus). **(B)** Quantification of distance between the nucleus and the centrosome for all siRNA conditions showing increased distance between the nucleus and the centrosome after individual or combined depletion of *SUN1* or *SUN2*. *Unpaired two-tailed t-test. ****P<0.0001. Scale bars: 20 µm or 5 µm.*

SUN1 and SUN2 regulate microtubule dynamics during re-nucleation assay

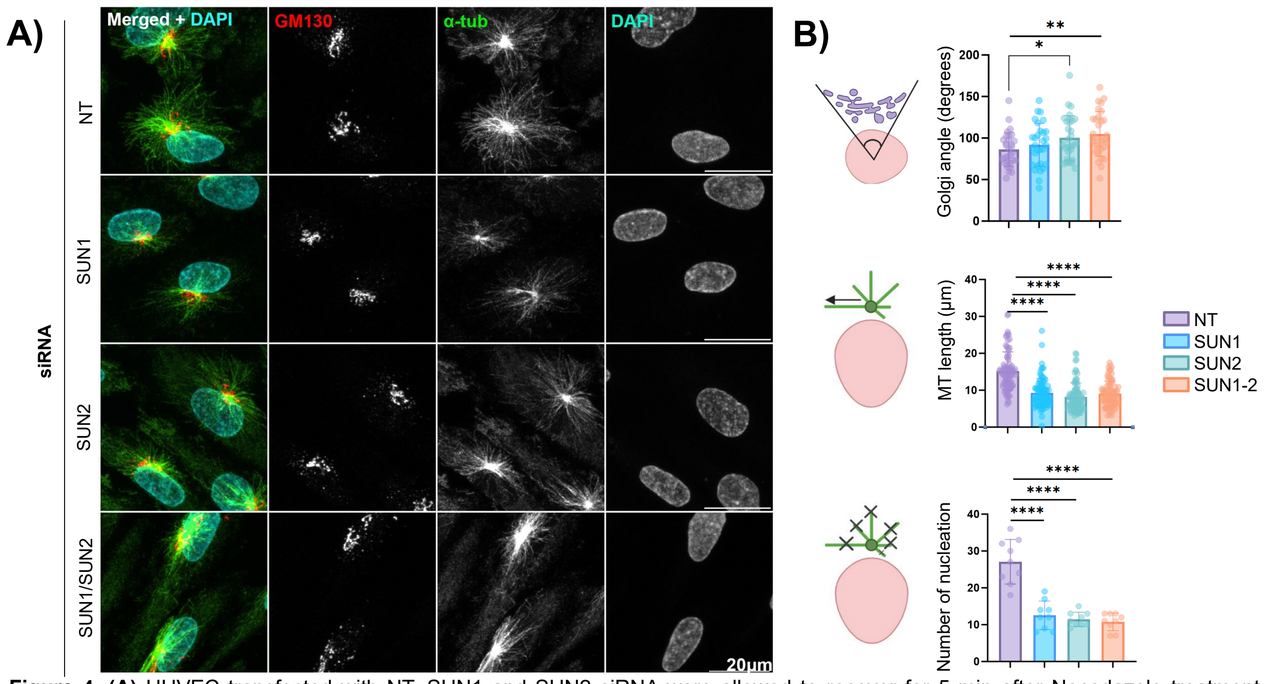


Figure 4. **(A)** HUVEC transfected with NT, SUN1 and SUN2 siRNA were allowed to recover for 5 min after Nocodazole treatment. Cells were then fixed and stained for α-tubulin (in green), GM130 (in red, Golgi apparatus) and DAPI (in blue, nucleus). **(B)** Quantification of Golgi apparatus angle, microtubule length and nucleation for all siRNA conditions showing increased Golgi dispersal in *SUN2*-depleted cells and delayed microtubule re-nucleation after individual depletion of *SUN1* or *SUN2*. *SUN1*/*SUN2* co-depletion showed a similar effect to the individual depletion of *SUN2*. *Unpaired two-tailed t-test. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; Scale bars: 20 µm.*

SUN2 and SYNE1 show synergistic effects on microtubule dynamics

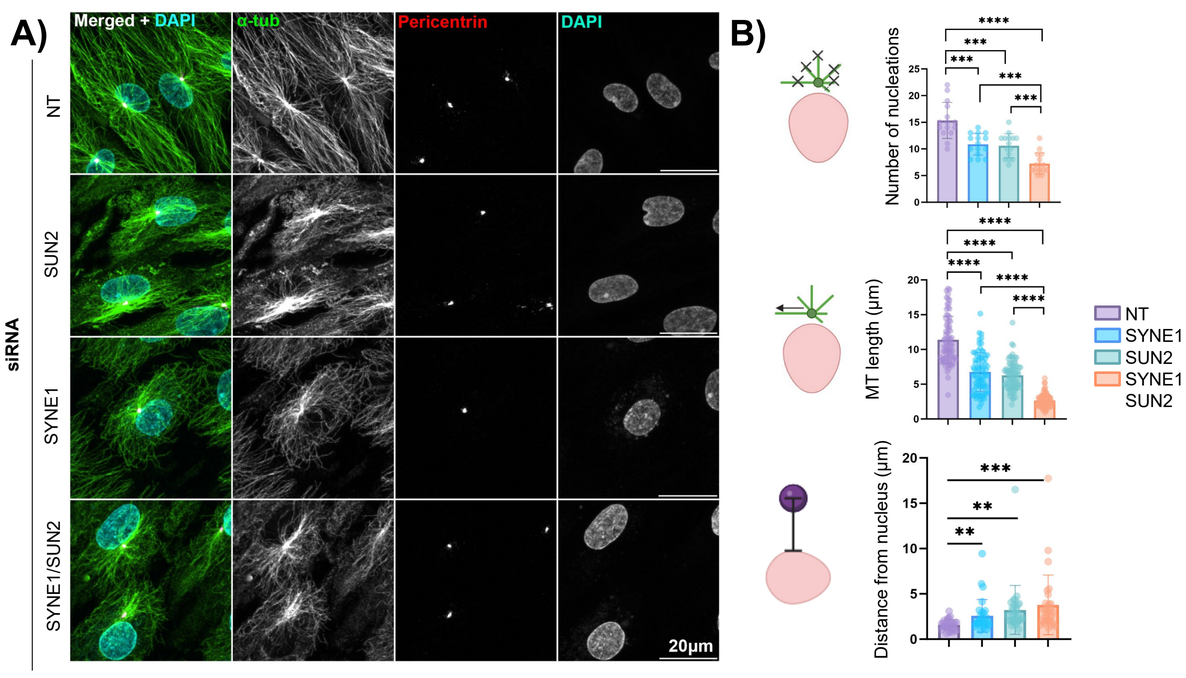
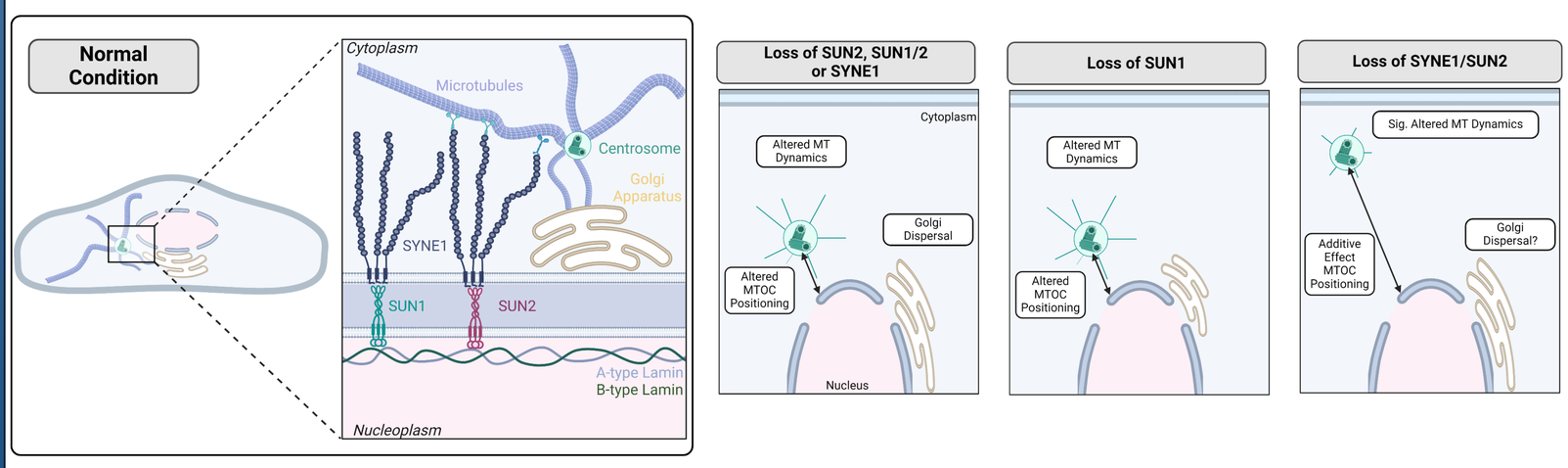


Figure 5. **(A)** HUVEC transfected with NT, SUN2 and SYNE1 siRNA were allowed to recover for 10 min after Nocodazole treatment. Cells were then fixed and stained for α-tubulin (in green), pericentrin (in red, centrosome) and DAPI (in blue, nucleus). **(B)** Quantification of number of renucleations, microtubule length, and distance between nucleus and centrosome for all siRNA conditions showing delayed microtubule re-nucleation and increased distance between the nucleus and the centrosome after individual depletion of *SUN2* or *SYNE1*. *SUN2*/*SYNE1* co-depletion showed additive effect. *Unpaired two-tailed t-test. **P<0.01; ***P<0.001; ****P<0.0001; Scale bars: 20 µm.*

Conclusions



- Taken together, these findings demonstrate impaired signal transduction between the nucleus and the cytoplasm with depletion of LINC complex proteins. They also suggest alterations in the microtubule dynamics, potentially decreasing the ability of endothelial cells to polarize adequately, highlighting a potential perspective on the nuanced interplay within the LINC complex and its implications for cardiovascular health and progeria.

Future Directions

- Further investigation of the role of SYNE1 in nucleocytoplasmic communication, specifically in interactions with the Golgi Apparatus.
- Exploring the role of SUN1/2 in nucleocytoplasmic communication via non-centrosomal microtubules
- Investigation of motor proteins adaptors and their relationship to LINC Complex
- Investigation of impacts of SUN2/SYNE1 on actin cytoskeleton

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References

1) King, M. C. Dynamic Regulation of LINC Complex Composition and Function across Tissues and Contexts. FEBS Letters 2023, 597 (22), 2823–2832. https://doi.org/10.1002/1873-3468.14757.
2) Buglak, D. B., Kulikauskas, M. R., Liu, Z., Gold, A. L., Marvin, A. P., Burciu, A., Tanke, N. T., Ricketts, S. N., Kinghorn, K., Oatley, M., Johnson, B. N., Bougaran, P., Shiau, C. E., Rogers, S. L., & **Bautch, V. L.** (2021). Nuclear SUN1 stabilizes endothelial cell junctions via microtubules to regulate blood vessel formation. https://doi.org/10.1101/2021.08.11.455980
3) Martin, M.; Veloso, A.; Wu, J.; Katrukha, E. A.; Akhmanova, A. Control of Endothelial Cell Polarity and Sprouting Angiogenesis by Non-Centrosomal Microtubules. eLife 2018, 7, e33864. https://doi.org/10.7554/eLife.33864.