Supplementary Material

Nucleocytoplasmic shuttling of the Usher syndrome 1G protein SANS differs from its paralogue ANKS4B

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**1. Supplementary Figures and Tables**

Supplementary\_Figure\_File.docx: all Figures S1-S5 referred to in this work;

Table\_S1.xlsx: GO-term analysis of SANS nuclear interactome from (Yildirim et al. 2021)

CellProfiler workflow available on GitHub:

<https://github.com/LabWolfrum/Fritze_et_al_2024_SANS_Nuclear_localization>

* 1. **Supplementary Figures**

**Figure S1:**

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Automatisch generierte Beschreibung

**Figure S1.** **Subcellular localization of SANS NLS mutants in HEK293T cells.** **(A)** Confocal microscopy of HEK293T cells transfected with eYFP-SANS (red) or SANS NLS mutants, counterstained with DAPI. **(B)** Quantification of (A)by CellProfiler. eYFP-SANSK213E and eYFP-SANSR447W differed significantly from eYFP-SANS. White arrows in merge images: regions of interests (ROI) of fluorescence intensity plots; blue dashed lines: DAPI positive nuclear extension; black arrows: position of Z-projections. Scale bars: horizontal = 10 µm; vertical = 2 µm. Students t-test was performed for 3 independent experiments with a minimum of 75 cells.

**Figure S2:**

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Automatisch generierte Beschreibung

**Figure S2. Nuclear localization of eYFP-SANS treated with Importazole.** **(A)** Confocal microscopy of HeLa cells transfected with eYFP-SANS (red) and treated with DMSO or 40 µM Importin-β inhibitor Importazole (IPZ). **(B)** Quantification of (A)by CellProfiler. eYFP-SANS did not differ in its localization after IPZ treatment. White arrows in merge images: regions of interests (ROI) of fluorescence intensity plots; blue dashed lines: DAPI positive nuclear extension; black arrows: position of Z-projections. Scale bars: horizontal = 10 µm; vertical = 2 µm. Students t-test was performed for three independent experiments with a minimum of 75 cells.

**Figure S3:**

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**Figure S3.** **Localization of SANS NES mutants in HEK293T cells.** **(A)** Confocal microscopy of HEK293T cells transfected with eYFP-SANS (red) or NES mutants, counterstained with DAPI. **(B)** Quantification of (A)by CellProfiler. eYFP-SANSL195E was significantly enriched in the nucleus compared to eYFP-SANS. White arrows in merge images: regions of interests (ROI) of fluorescence intensity plots; blue dashed lines: DAPI positive nuclear extension; black arrows: position of Z-projections. Scale bars: horizontal = 10 µm; vertical = 2 µm. Students t-test was performed for 3 independent experiments with a minimum of 75 cells.

**Figure S4:**

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**Figure S4.** **Localization of pathogenic variant of SANS in HEK293T cells.** **(A)** Confocal microscopy of HEK293T cells transfected with eYFP-SANS (red), eYFP-SANSS278Pfs\*71, eYFP-SANSS243\* and eYFP-SANSV132Gfs\*3, counterstained with DAPI. **(B)** Quantification of (A)with CellProfiler. All pathogenic variants were significantly enriched in the nucleus. White arrows in merge images: regions of interests (ROI) of fluorescence intensity plots; blue dashed lines: DAPI positive nuclear extension; black arrows: position of Z-projections. Scale bars: horizontal = 10 µm; vertical = 2 µm. Students t-test was performed for 3 independent experiments with a minimum of 75 cells.

**Figure S5:**

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Automatisch generierte Beschreibung

**Figure S5.** **Localization of eYFP-SANS under siRNA-based PRPF31 knock down in HeLa cells.** Quantification with CellProfiler. eYFP-SANS was not significantly enriched in the cytoplasm after siRNA-based knockdown of PRPF31. Students t-test was performed for 3 independent experiments with a minimum of 75 cells.