

Figure S1. Nesprin1 α forms filamentous structures in photoreceptors. Related to Figure 1

A. Maximum intensity projection of seven single planes (500 nm apart) from adult *Rho-CreNes1^{Δ/WT}* (left panel) and *Rho-CreNes1^{Δ/Δ}* (middle panel) littermate retinas immunostained with Nesprin1. Note the absence of Nesprin1 immunoreactivity in the IS and ONL of *Rho-CreNes1^{Δ/Δ}* rods (middle panel). Right Panel: Maximum intensity projection of a whole *Rho-CreNes1^{Δ/Δ}* retina immunostained with cone arrestin (CAR) and Nesprin1 showing that depletion of Nesprin1 in rods does not obviously affect retinal organization. Related to Figure 1B. **B.** Isolated photoreceptor IS/OS compartment immunostained with Nesprin1 and rod transducin (*Gat1* that label rods OS) antibodies. Note the restriction of Nesprin1 immunoreactivity to the IS. **C.** Nesprin1 immunofluorescence of 3 months-old wild-type (*Rho^{+/+}*, top) and Rhodopsin knockout (*Rho^{-/-}*, bottom) littermate retinas. Note the loss of the entire ONL and IS/OS interface in *Rho^{-/-}* retinas. Scale bars: 20 μ m. Right: Nesprin1 immunoblotting of corresponding retinal lysates showing the sharp decrease of a ~120 kDa Nesprin1 immunoreactive band (arrowhead). Note that a ~55kDa immunoreactive is also absent from *Rho^{-/-}* retinal lysates (arrow). Related to Figure 1C. **D.** Nesprin1 immunoblot of pelleted wild-type retinas and IS/OS supernatant after brief vortexing (see STAR Methods for more details). Note the enrichment of the ~120 kDa (arrowhead) and ~55kDa (arrow) Nesprin1-immunoreactive bands in the IS/OS-enriched fraction. Related to Figure 1C. **E.** Exonic organization of Nesprin1 α transcripts relative to transcripts encoding longer isoforms of Nesprin1 (see text for details). Grey shaded areas correspond to UTRs of Nesprin1 α transcripts. Red: denotes the *XbaI* restriction strategy used to discern Nesprin1 transcripts that harbor exon-2 (535 bp) from transcript in which exon-2 is alternatively spliced (476 bp). Related to Figure 1E. **F.** Upper panels: RT-PCR amplification (30 cycles) of Nesprin1 α transcripts with the p1532/p1535 primer pair or of transcripts encoding longer Nesprin1 isoforms (Long) with the p1350/p1535 primer pair from total RNA from various C57/Bl6 mouse tissues and NIH3T3 cells. Lower panels: *XbaI* digestion of these amplicons. *: 476 bp *XbaI* restriction fragment indicative of the alternative splicing of exon-2 of transcripts encoding long isoforms of Nesprin1 in retina, cerebrum and cerebellum. Related to Figure 1E.

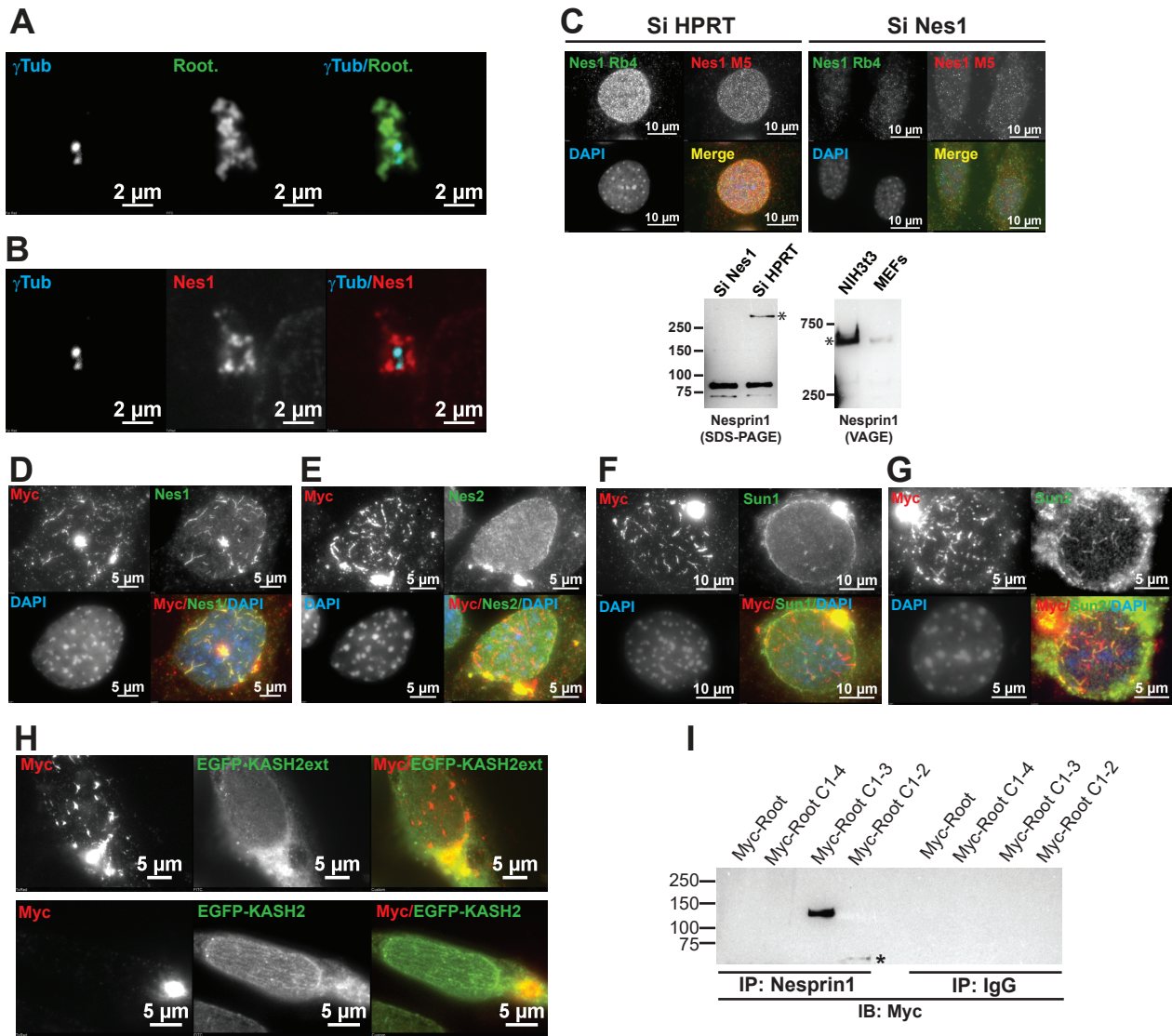


Figure S2: Recombinant rootletin filaments induce the aggregation of Nesprin1. Related to Figure 3.

A, B. Relative localization of γ -tubulin and Myc-root (labeled with rootletin) (A) and of γ -tubulin and Nesprin1 (B) within the same single focal plane. Note that Myc-root and endogenous Nesprin1 form distinct structures that wrap the centrosome. Related to Figure 3A. **C.** NIH3t3 cells express a ~600 kDa isoform of Nesprin1. Top: NIH3t3 cells transfected for 48h either with control (SiHPRT, left) or Nesprin1 (SiNes1, right) SiRNA targeting exon-13 and colabeled with mouse (M5) or rabbit (Rb4) antibodies that are both directed against the same C-terminal epitope of Nesprin1. Note the efficient downregulation of endogenous Nesprin1 in SiNes1-treated cells. Bottom left: immunoblotting of corresponding cell lysates. *: ~600kDa Nesprin1 isoform endogenous to NIH3t3. Bottom right: Nesprin1 immunoblotting of NIH3t3 and primary mouse embryonic fibroblasts (MEFs) lysates processed for vertical agarose gel electrophoresis (VAGE). Related to Figure 3A. **D-G.** Maximum intensity projections of Myc-root-transfected NIH3t3 cells coimmunolabeled with Myc and Nesprin1 (D), Nesprin2 (E), Sun1 (F) or Sun2 (G). Related to Fig.3A-D. **H:** Apical views of NIH3t3 cells transfected either with dominant negative EGFP-KASH2 that disrupts LINC complexes or with EGFP-KASH2ext that does not [S1]. Note the absence of perinuclear rootletin filaments in cells transfected with EGFP-KASH2. **I:** Nesprin1 and rabbit immunoglobulins (IgG, used as a negative control) immunoprecipitations (IP) of RIPA lysates from NIH3t3 cells transfected with Myc-Roof deletion constructs. Immunoprecipitates were immunoblotted (IB) with Myc to detect the coimmunoprecipitation of Myc-Roof deletion mutants with endogenous Nes1600kDa. The asterisk denotes the weak immunoreactivity of Myc-Roof C 1-2 in Nesprin1 immunoprecipitates. Related to Figure 3K.

Supplemental references

- S1. Stewart-Hutchinson, P.J., Hale, C.M., Wirtz, D., and Hodzic, D. (2008). Structural requirements for the assembly of LINC complexes and their function in cellular mechanical stiffness. *Exp Cell Res* 314, 1892-1905.