

Expanded View Figures

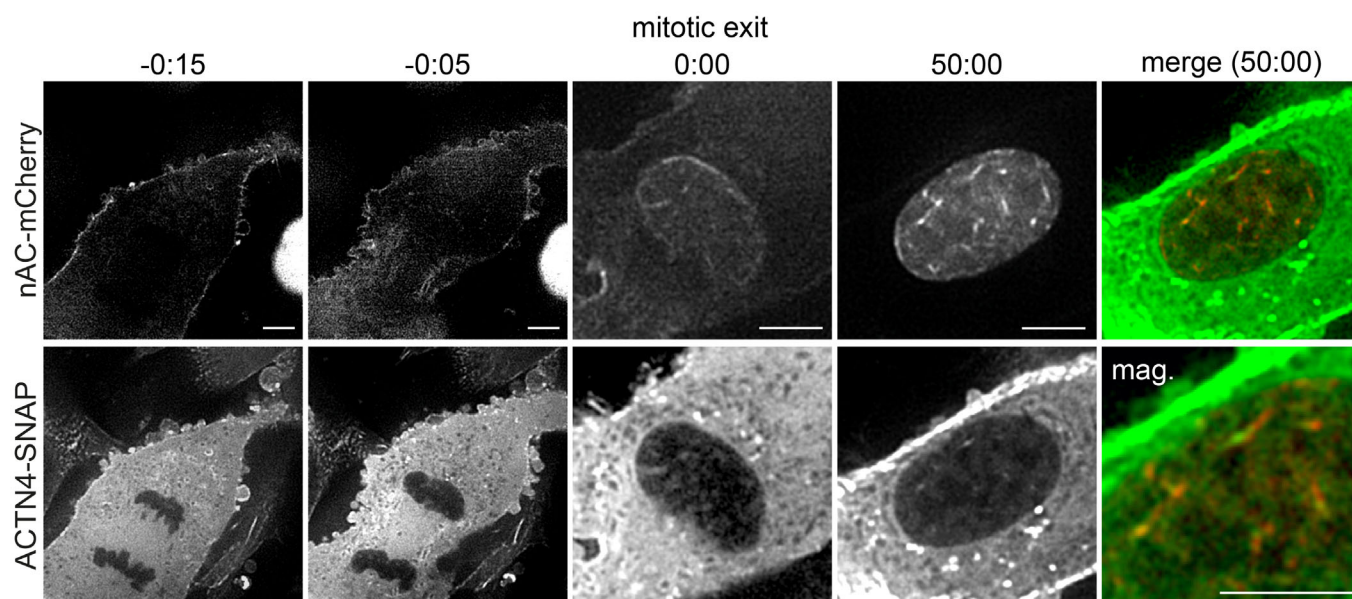


Figure EV1. ACTN4 associates with postmitotic nuclear actin filaments.

Cells stably expressing nAC-mCherry and doxycycline-inducible ACTN4-SNAP were analyzed by time-lapse microscopy. The cell is an alternative example of Fig 2A that additionally covers the mitotic phase. ACTN4-SNAP was labeled by SNAP-Cell 647SiR (green). The magnification changes at time point 0:00. Scale bar 5 μ m.

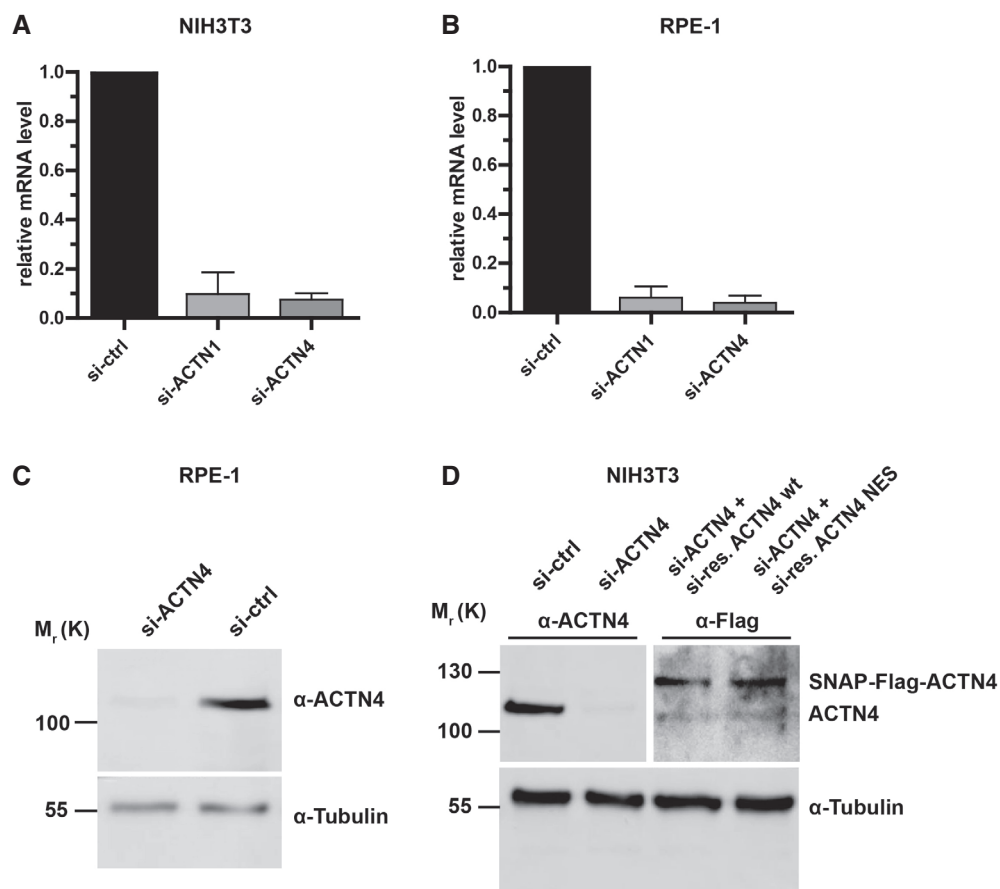


Figure EV2. Representative Western blots and qPCR data for knockdown and reconstitution experiments.

A qPCR results for knockdown efficiency of ACTN1 (si-ACTN1) and ACTN4 (si-ACTN4) mRNA levels in NIH3T3 cells. Data are mean \pm SD of three biological replicates.
 B qPCR results for knockdown efficiencies of ACTN1 (si-ACTN1) and ACTN4 (si-ACTN4) mRNA levels in RPE-1 cells. Data are mean \pm SD of three biological replicates.
 C Immunoblot displaying knockdown efficiency of ACTN4 (si-ACTN4) in RPE-1 cells (α -ACTN4).
 D Immunoblots displaying ACTN4-knockdown efficiency (si-ACTN4) in NIH3T3 cells (α -ACTN4) and re-expression of siRNA-resistant (si-res.) SNAP-Flag-ACTN4-wt and SNAP-Flag-ACTN4-NES, detected by anti-Flag antibodies (α -Flag).

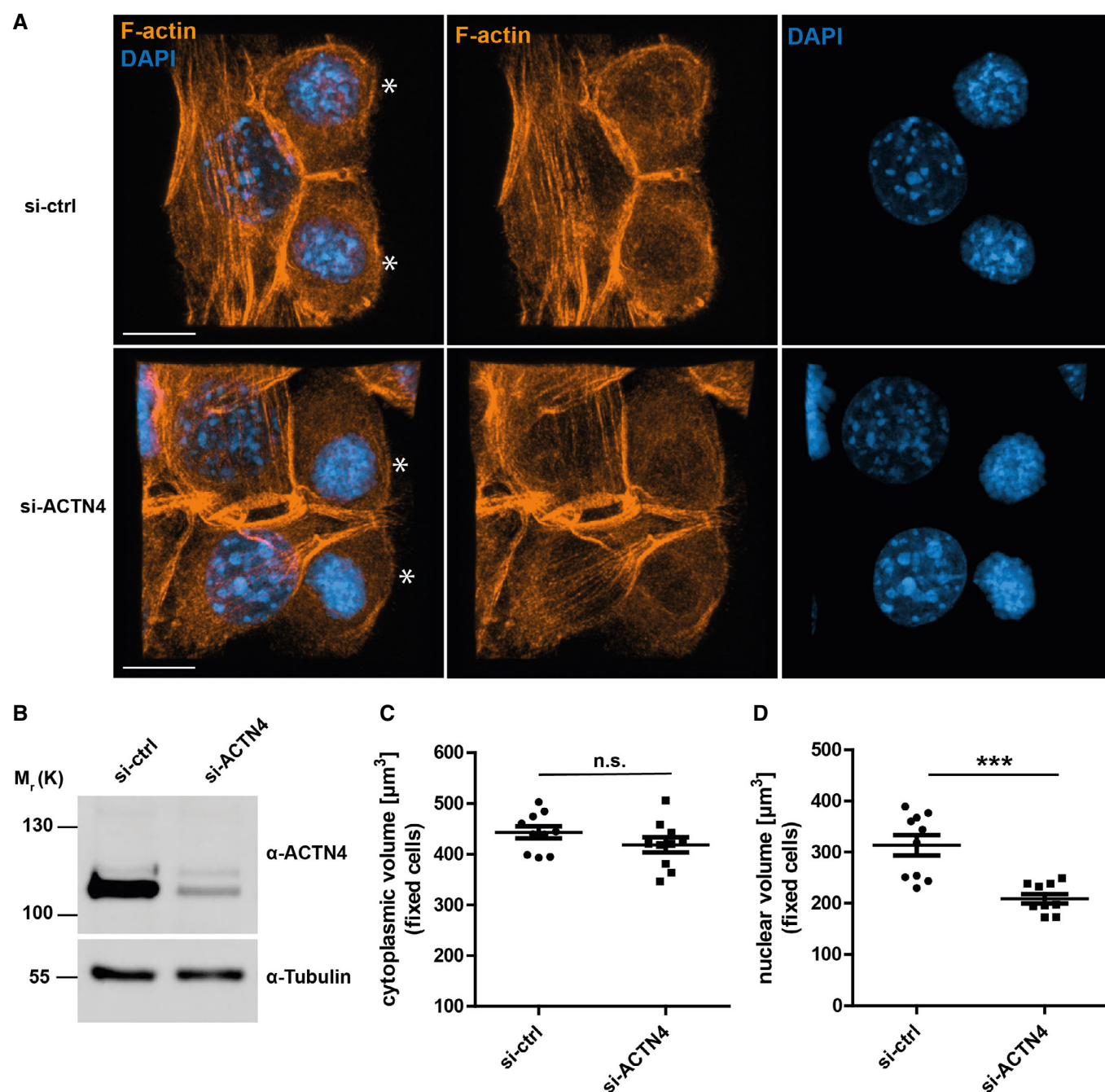


Figure EV3. Nuclear and cytoplasmic volume measurements of fixed NIH-3T3 cells silenced for ACTN4.

- A** Fluorescence image example of fixed NIH3T3 cells in early-G1 (mitotic cells are indicated by asterisks), stained with Phalloidin (orange) for F-actin and DAPI (blue) for nuclei. Shown is a maximum intensity projection that was used for further analysis to determine volume parameters using Imaris (z-distance: 0,14 μm), scale bar: 10 μm . Note the smaller nuclei and more condensed DNA in si-ACTN4 postmitotic cells.
- B** Immunoblot showing ACTN4-knockdown (si-ACTN4) in NIH3T3 cells.
- C** Dot plots of cytoplasmic volumes from fixed NIH3T3 cells in early-G1 for control (siCtrl) and ACTN4-knockdown (si-ACTN4) conditions. Data are mean \pm SEM from 2 biological replicates with 5 cells each, two-tailed *t*-test.
- D** Dot plots of nuclear volumes from fixed NIH3T3 cells in early-G1 for control (siCtrl) and ACTN4-knockdown (si-ACTN4) conditions. Data are mean \pm SEM from 2 biological replicates with 5 cells each analyzed as two-tailed *t*-test; ****P* < 0.001.

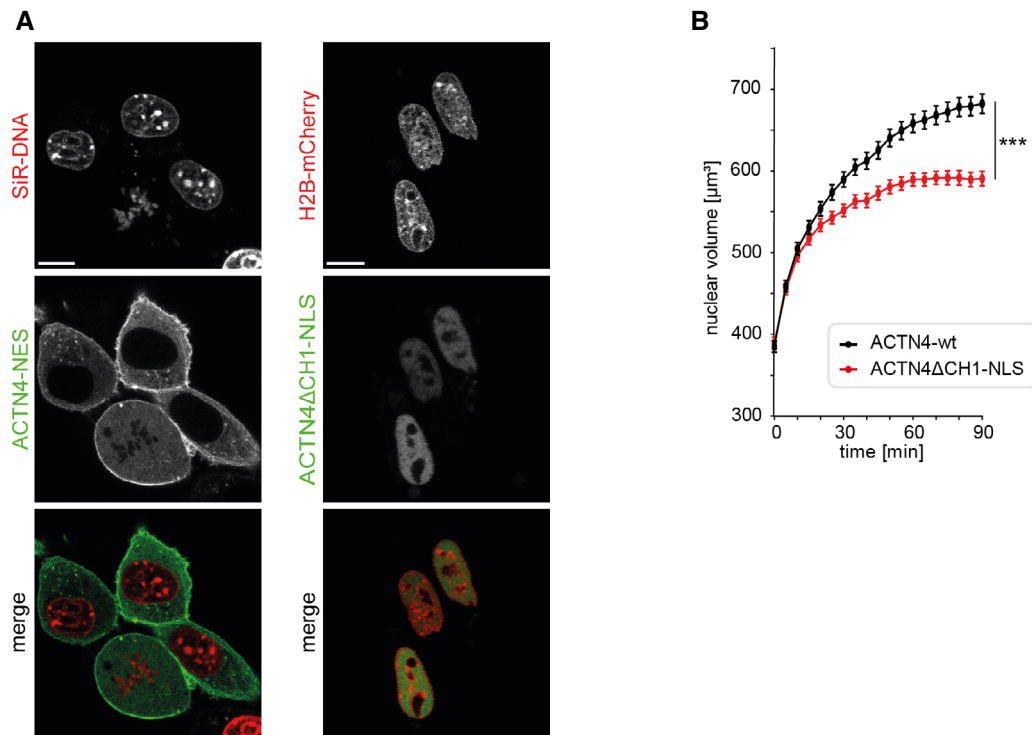


Figure EV4. Localization of ACTN4 Δ CH1-NLS and ACTN4-NES. Effects of ACTN4 Δ CH1-NLS on nuclear volume expansion after mitotic exit.

A NIH3T3 cells expressing H2B-mCherry and the indicated ACTN4 constructs were stained by SNAP488; scale bars 10 μm .

B NIH3T3 cells stably expressing H2B-mCherry were transfected with ACTN4-wt and ACTN4 Δ CH1-NLS. After mitotic exit, z-stacks (30 planes) were acquired, every 5 min. Nuclei were 3D-reconstructed, and volume was measured by Imaris software. Data are shown as mean \pm SEM from 4 biological replicates with 56–62 nuclei (ACTN4-wt) and 51–57 nuclei (ACTN4 Δ CH1-NLS); *** P < 0.001 by Wilcoxon test and **** P < 0.0001 by t -test at 90 min.