Cytoskeletal tension induces the polarized architecture of the nucleus

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THIS DOCUMENT CONTAINS THE FOLLOWING SUPPLEMENTARY INFORMATION

Figure S1

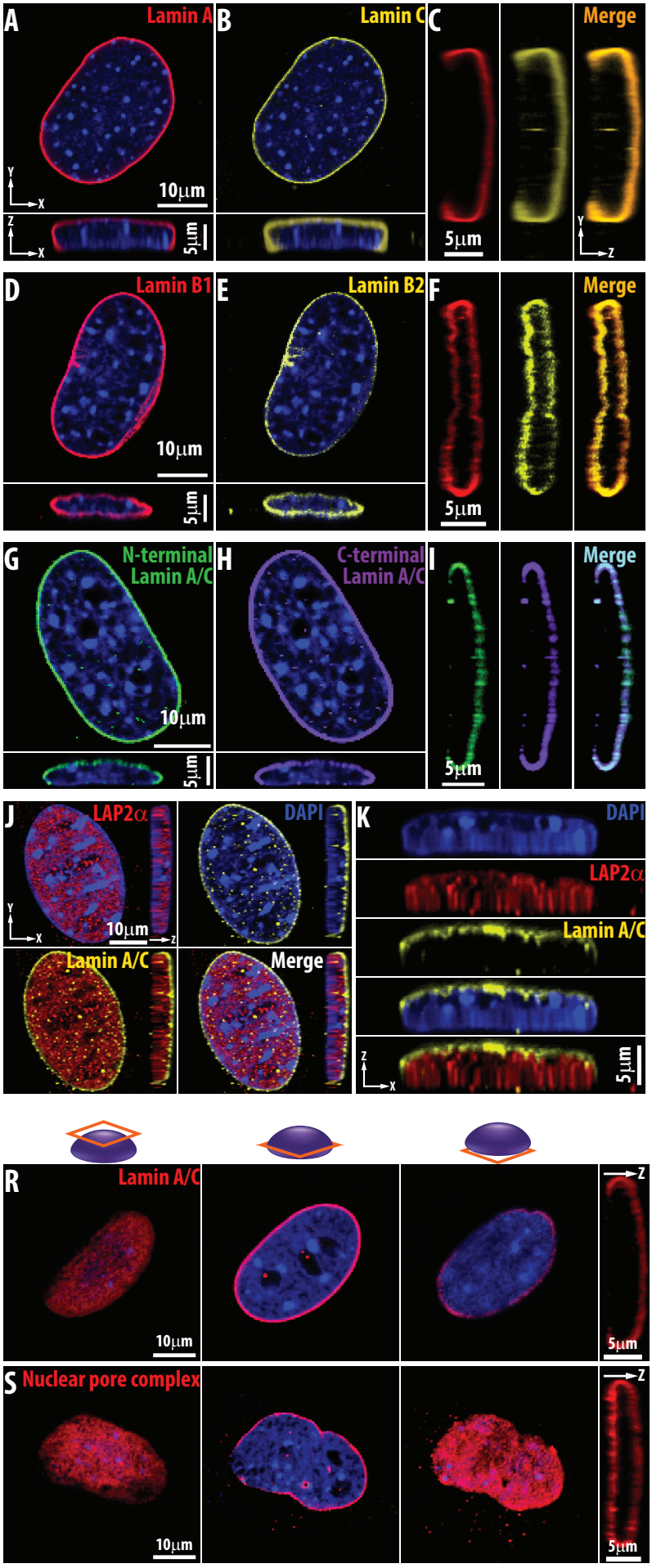

A
Lamin A
B
Lamin C
C
Merge
10μm
5μm
D
Lamin B1
E
Lamin B2
F
Merge
10μm
5μm
G
N-terminal Lamin A/C
H
C-terminal Lamin A/C
I
Merge
10μm
5μm
J
LAP2α
DAPI
Lamin A/C
Merge
10μm
K
DAPI
LAP2α
Lamin A/C
5μm
L
Emerin
M
Merge
Nesprin2giant
10μm
5μm
N
Emerin
O
Merge
Nesprin3
10μm
5μm
P
Emerin
Q
Merge
SUN1
5μm
R
Lamin A/C
10μm
5μm
S
Nuclear pore complex
10μm
5μm

Figure S2

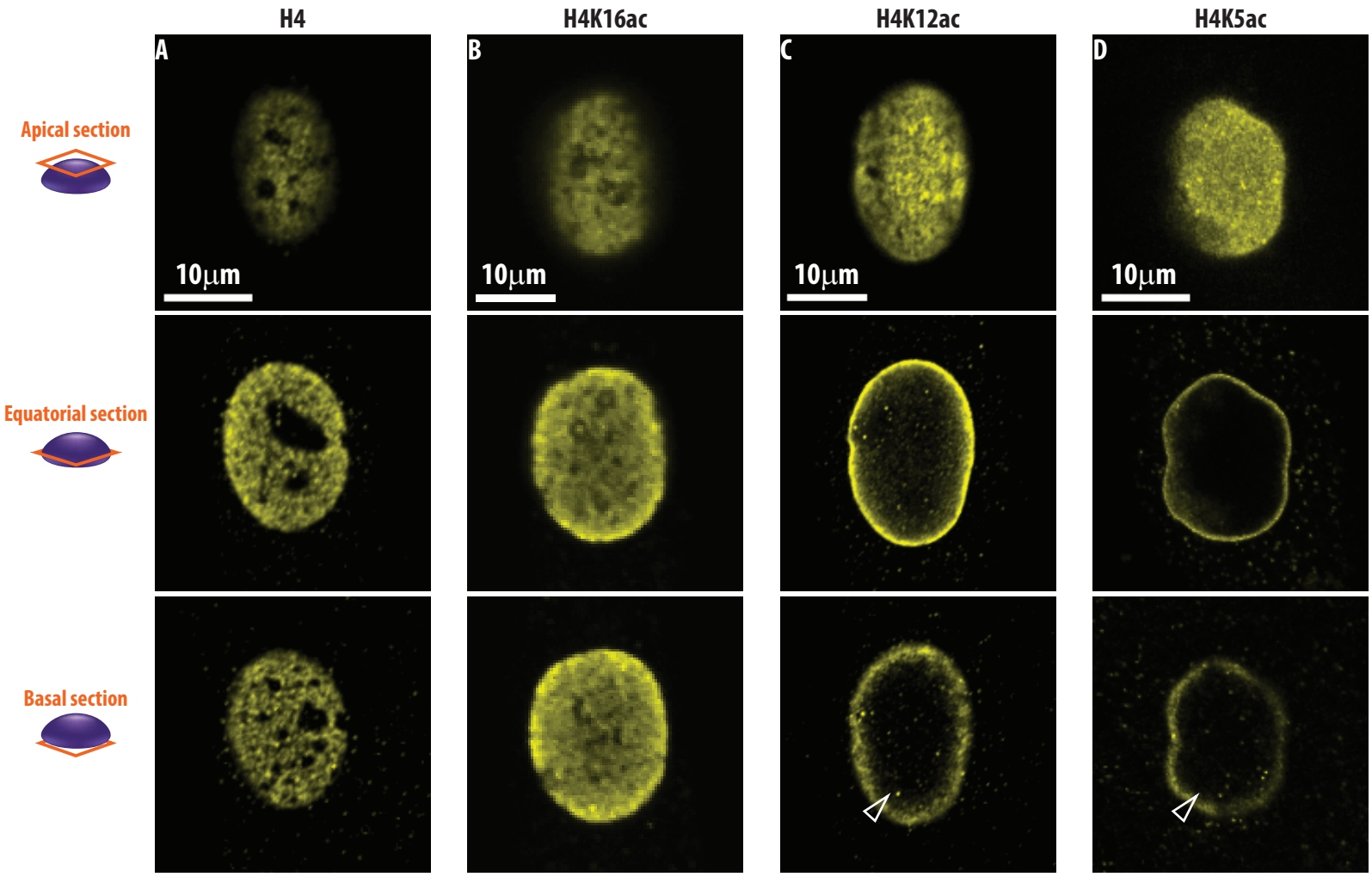
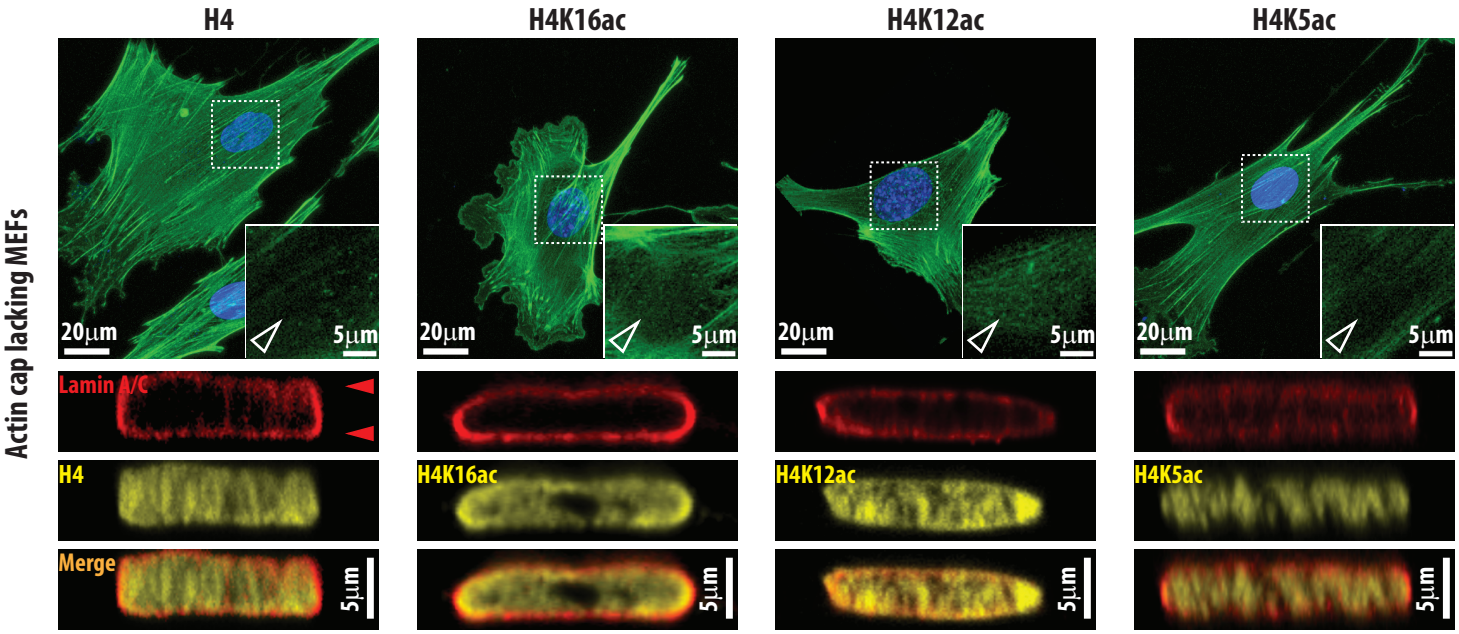

H4
H4K16ac
H4K12ac
H4K5ac
A
B
C
D
Apical section
Equatorial section
Basal section
10μm
10μm
10μm
10μm

Figure S3


H4
H4K16ac
H4K12ac
H4K5ac
Actin cap lacking MEFs
20μm
5μm
Lamin A/C
H4
H4K16ac
H4K12ac
H4K5ac
Merge
5μm

Supplemental Figure S1. Molecular composition of the lamin dome. A-F. A-type lamins *vs*. B-type lamins. Immunofluorescence confocal microscopy of A-type lamins, lamin A (A,C) and lamin C (B,C), and B-type lamins, lamin B1 (D,F) and lamin B2 (E,F) shows A-type lamins are apically polarized as stained by an anti-lamin A/C antibody (Figure 1A,B) but B-type lamins are organized as conventional symmetric thin shells. **G-I.** N-terminal lamin A/C *vs*. C-terminal lamin A/C. Antibodies binding specifically to the N-terminal (G) or C-terminal domains (H) of lamin A/C depict the identical asymmetric dome structures (I). **J-K.** Lamin A/C *vs*. lamin A/C binding partner. Immunofluorescence confocal microscopy shows that lamin A/C forms the apically polarized dome structure (yellow) but lamina-associated polypeptide 2alpha (LAP2α) that specifically binds to A-type lamins are uniformly distributed in the intranuclear space without vertical localization (red). **L-Q.** Spatial organization of nuclear membrane associated proteins. Outer-nuclear-membrane associated protein nesprin isoforms nesprin2giant and nesprin3 (yellow, L-O) and inner-nuclear-membrane-associated proteins emerin (red, L-Q) and SUN1 (yellow, P-Q) are evenly distributed along the periphery of the DAPI-stained nuclei. **R-S.** Structural difference between lamin A/C and nuclear pore complex protein. Apical polarization of lamin A/C (R) and isotropic distribution of nuclear pore complex (S) are visualized along the vertical direction; apical, equatorial, and basal cross-sectional planes, respectively. Nuclear DNA was stained by DAPI (blue). See also Supplemental Movie S1.

Supplemental Figure S2. Spatial distribution of epigenetic marks. A-D. Cross-sectional confocal images of the nuclei in MEFs stained with antibodies against unmodified histone H4 (H4, A), histone H4 acetylated at lysine 16 (H4K16ac, B), histone H4 acetylated at lysine 12 (H4K12ac, C), and histone H4 acetylated at lysine 5 (H4K5ac, D) along the apical, equatorial, and basal surfaces (i.e., XY planes). Each column corresponds to Figure 4 D-G. Empty arrow heads indicate the absent or faint staining of epigenetic marks (C, D).

Supplemental Figure S3. Representative organization of lamin A/C and epigenetic marks in the actin-cap-lacking MEFs. While actin-cap-bearing control MEFs reveal

actin-cap-mediated spatial re-organization of lamin A/C and highly acetylated histone marks, H4K12ac and H4K5ac (Fig. 4D-G *vs*. Fig. 4H-K), a small population of control MEFs transiently lacking an actin cap (<30%) forms an unpolarized lamin A/C (red) and acetylated histones (yellow) in the nucleus. See also Fig. 4H-K.

Supplemental Movie S1. 3D reconstruction of lamin A/C and nuclear pore complex. Mouse embryonic fibroblasts were fixed and stained with anti-lamin A/C or anti-nuclear pore complexes, respectively and nuclear DNA was stained by DAPI (blue). Gradual movement of the XZ cross sectional plane and rotation along the X and Z axis clearly show the distinct Frisbee-like apical dome structure of lamin A/C, while nuclear pore complexes isotropically surround DAPI stained nucleus. See also Supplemental Figure S1.