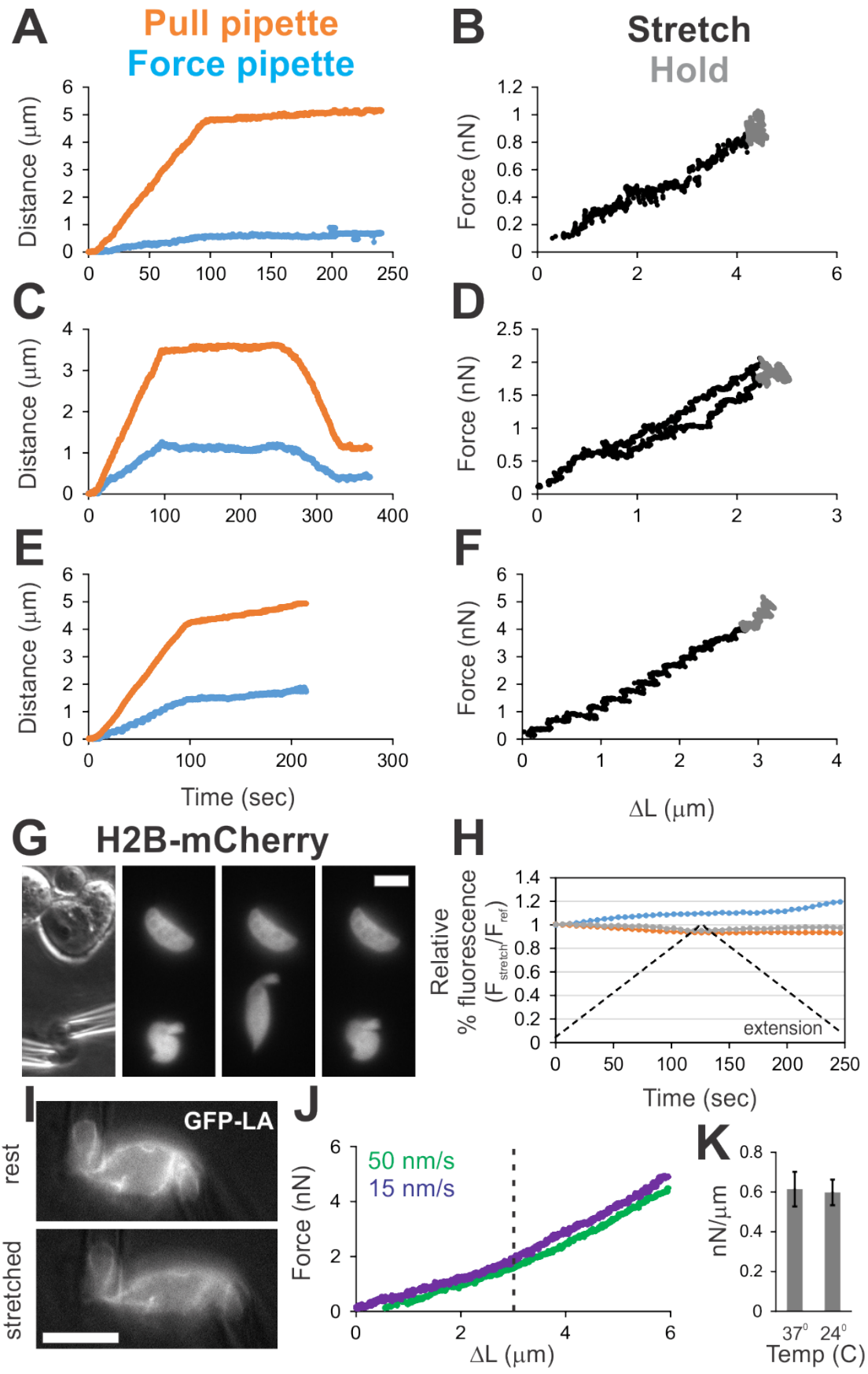
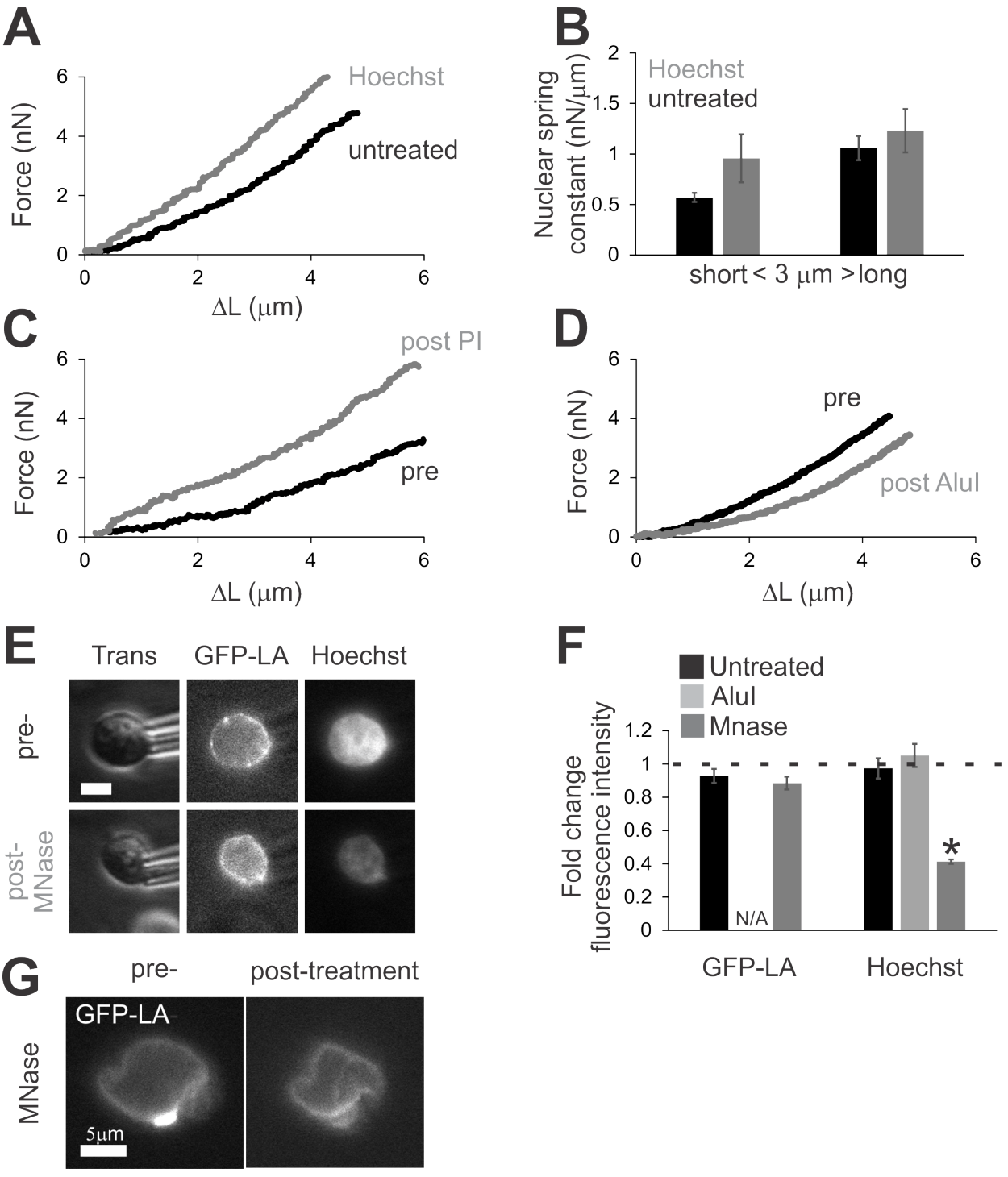
Supplemental Materials

Molecular Biology of the Cell

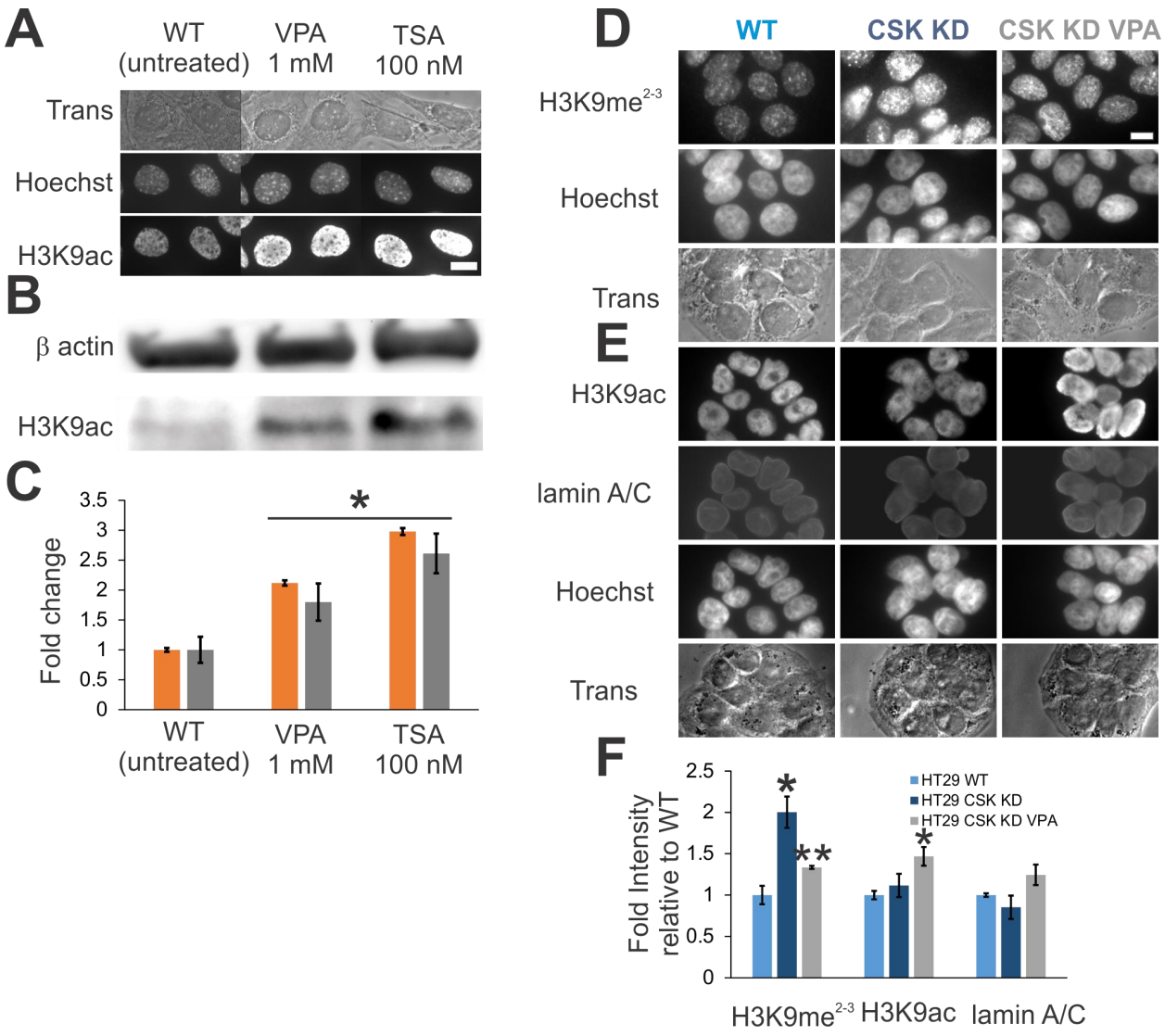
Stephens et al.


A
Pull pipette
Force pipette
Distance (μm)
B
Stretch
Hold
Force (nN)
C
Distance (μm)
D
Force (nN)
E
Distance (μm)
Time (sec)
F
Force (nN)
ΔL (μm)
G
H2B-mCherry
H
Relative % fluorescence (F_stretch/F_ref)
extension
Time (sec)
I
GFP-LA
rest
stretched
J
50 nm/s
15 nm/s
Force (nN)
ΔL (μm)
K
nN/μm
37° 24°
Temp (C)

Supplemental Figure S1. Extending a whole nucleus slowly at 50 nm/s probes elastic responses and maintains nuclear integrity. (A, C, E) Raw distance versus time data for pull (orange) and force (blue) pipettes during experiments stretching a nucleus extracted from a (A) MEF V-/-, (C) MEF WT, or (E) HeLa cell. Nuclei are extended at 50 nm/s and then held to watch for length relaxation. Significant relaxation does not occur during the >2 minute holding time in which the viscous properties of the nucleus relax (Guilak et al., 2000; Dahl et al., 2005). (B, D, F) Corresponding force-extension plots show that during holding (gray), the measured force across the nucleus does not relax from the force measured when maximal stretch is attained (black). (G) Example bright field and histone H2B-mCherry images of an isolated nucleus (bottom) and a nucleus still inside a HeLa cell (top). (H) Relative fluorescence intensity, $F_{stretch}/F_{ref}$, of H2B-mCherry in an isolated nucleus undergoing extension ($F_{stretch}$) compared to a nucleus still inside a cell (F_{ref}) over the course of a stretching experiment. The different colors represent measurements from three different nuclei. (I) Example images of HeLa nuclei expressing GFP-LA before and during extension. (J) Mechanical response of the nucleus remains similar for the an individual nucleus stretched at different rates (green: 50 nm/s pulling, purple: 15 nm/s; for n = 4 nuclei the relative change in nuclear spring constant for short 0.90 and long 0.88 extensions). (K) Nuclear spring constant remains similar for MEF V-/- nuclei isolated and measured at 37 and 24°C ($p > 0.05$, n = 12 and 10 respectively). Scale bar = 10 μm.

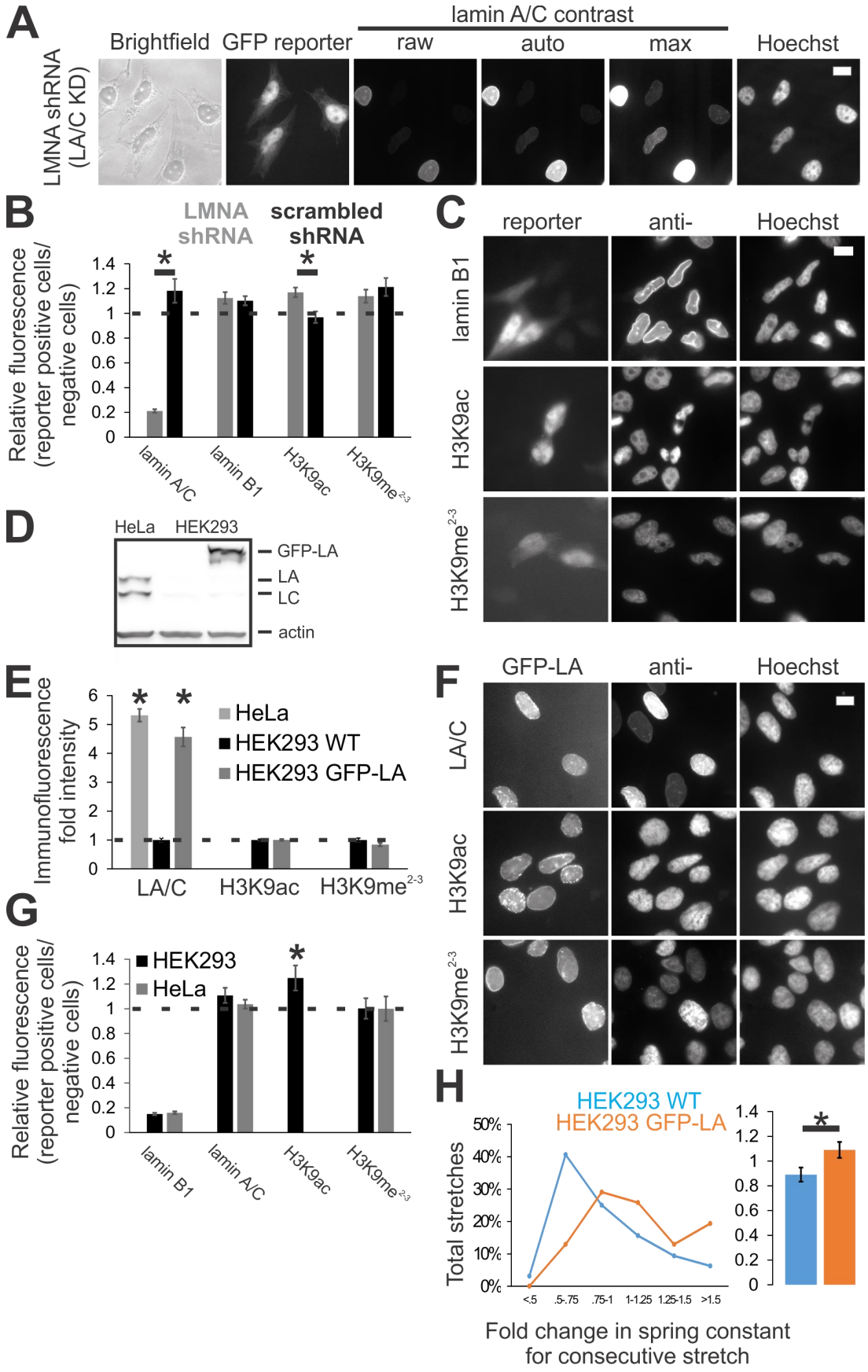

A
Force (nN)
ΔL (μm)
Hoechst
untreated
B
Nuclear spring constant (nN/μm)
Hoechst
untreated
short < 3 μm > long
C
Force (nN)
ΔL (μm)
post PI
pre
D
Force (nN)
ΔL (μm)
pre
post AluI
E
Trans
GFP-LA
Hoechst
pre-
post-MNase
F
Untreated
AluI
Mnase
Fold change fluorescence intensity
N/A
*
GFP-LA
Hoechst
G
pre-
post-treatment
MNase
GFP-LA
5μm

Supplemental Figure S2. Longer extensions reveal that strain stiffening and lamin A remains after biochemical treatment of chromatin. (A) Representative force-extension relation for a nucleus pretreated with the DNA intercalator Hoechst (gray) compared to the force-extension relation for an untreated nucleus. (B) Nuclear spring constant averages for MEF V-/- nuclei untreated (black, n= 15) and Hoechst-treated (gray, n = 4). Error bars denote SEM and the lack of * indicates that the data are not statistically significantly different. (C, D) Representative force-extension plots for a nucleus pre- and post-treatment with (C) the strong DNA intercalator propidium iodide (PI) or (D) blunt restriction endonuclease AluI. (E) HEK293 GFP-LA nuclei stained with Hoechst for DNA were isolated from the cell and imaged pre- and post-treatment with MNase. (F) Graph of fold change in nucleus fluorescence intensity of GFP-LA (for lamin A/C) and Hoechst (for DNA) above background pre- and post-treatment with MNase or AluI (fluorescence shown for DNA only). Fluorescence is also shown for untreated nuclei, which are nuclei that remained in cells in the same field of view. Isolated nuclei were imaged before and after spray treatments with treatment times of 5 min for AluI or 1 min for MNase (AluI n = 2 MEF V-/- and MNase n = 3 MEF V-/- and n = 4 HEK293 GFP-LA, * $p < 0.05$). (G) Representative image of HeLa GFP-LA nucleus pre- and post-treatment with MNase. Scale bar = 5 μm. Error bars denote SEM.



Supplemental Figure S3. HDAC inhibitors VPA and TSA increase the amount of acetylated histones/euchromatin. (A) Representative images of WT (untreated), VPA-treated, and TSA-treated MEF V-/- cells fixed and labeled with H3K9ac primary and Alexa 594 secondary antibody. (B) Representative image of western blot for untreated, VPA-treated, and TSA-treated nuclei probed for β-actin (loading control) and H3K9ac. (C) Graph of fold change in intensity for immunofluorescence (orange, n = 100 cells each) and western blot (gray, n = 5 each). (D,E) Representative images of HT-29 WT, CSK KD, and CSK KD cell nuclei treated with HDAC inhibitor VPA (CSK KD VPA) stained for (D) heterochromatin marker H3K9me2-3, (E)

euchromatin marker H3K9ac and lamin A along with Hoechst to label DNA. (F) Summary graph of fold intensity relative to HT-29 WT (n = 50 each). * and ** denote $p < 0.05$ relative to WT and to each other. Scale bar = 10 μm. Error bars denote SEM.


A
lamin A/C contrast
Brightfield
GFP reporter
raw
auto
max
Hoechst
LMNA shRNA (LA/C KD)
B
LMNA shRNA
scrambled shRNA
Relative fluorescence (reporter positive cells/ negative cells)
lamin A/C
lamin B1
H3K9ac
H3K9me2-3
C
reporter
anti-
Hoechst
lamin B1
H3K9ac
H3K9me2-3
D
HeLa
HEK293
GFP-LA
LA
LC
actin
E
Immunofluorescence fold intensity
HeLa
HEK293 WT
HEK293 GFP-LA
LA/C
H3K9ac
H3K9me2-3
F
GFP-LA
anti-
Hoechst
LA/C
H3K9ac
H3K9me2-3
G
Relative fluorescence (reporter positive cells/ negative cells)
HEK293
HeLa
lamin B1
lamin A/C
H3K9ac
H3K9me2-3
H
HEK293 WT
HEK293 GFP-LA
Total stretches
<.5
.5-.75
.75-1
1-1.25
1.25-1.5
>1.5
Fold change in spring constant for consecutive stretch

Supplemental Figure S4. Modulation of lamin levels via shRNA knockdown and ectopic overexpression. HeLa or HEK293 cells were transfected with either lamin A/C, lamin B1, or scrambled shRNA on a plasmid with a GFP reporter and allowed to incubate for 5 days. (A) Images of fixed HeLa cells transfected with lamin A/C (LMNA) shRNA. (B) Graph of relative fluorescence for GFP-reporter-positive cells (>1.25-fold greater than background) compared to reporter-negative cells for LMNA (gray) and scrambled (black) shRNA. (C) Representative images from HeLa lamin A/C knockdown cells for immunofluorescence of lamin B1, H3K9ac (euchromatin marker), and H3K9me$^{2-3}$ (heterochromatin marker, (n = 20 – 100). (D) Western blot of lamin A/C and actin in HeLa WT, HEK293WT, and HEK293 GFP-LA. (E) Graph of fold immunofluorescence intensity relative to WT for HeLa WT (light gray) and HEK293 WT (black) and GFP-LA (gray) expressing nuclei (n = 50 nuclei for each condition). (F) Representative image of mixed populations of WT and ectopic GFP-LA-expressing HEK293 cells with immunofluorescence staining for lamin A/C (LA/C), H3K9ac, and H3K9me$^{2-3}$ visualized by Alexa 594 secondary antibody. GFP was used to differentiate GFP-LA-expressing HEK293 nuclei from WT. (G) Graph of immunofluorescence intensity for GFP positive lamin B1 (LMNB1) knockdown relative to GFP negative WT nuclei for HeLa and HEK293. (H) Histogram and bar graph of average fold change in nuclear spring constant for short extensions for a consecutive stretch as compared to the previous stretch. HEK293 with lamin A (GFP-LA ectopic expression, orange) display slight strengthening upon the next stretch. Conversely, HEK293 (blue), which have low levels of lamin A, are skewed toward weaker spring constants upon the next stretch, suggesting that nuclei with low levels of lamin A deform plastically. Scale bar = 10 μm. Error bars denote SEM and * indicates $p < 0.05$.

Supplemental Table S1. Dimension measurements of nuclei used for force measurement experiments.

	Length				Diameter			
MEF V-/-	WT	VPA	TSA		WT	VPA	TSA	
avg	12.3	12.2	12.9		8.6	8.7	9.1	
st err	0.5	0.5	0.4		0.3	0.5	0.6	
n	19	10	10		19	10	10	
HT29	WT	CSK KD	CSK KD VPA		WT	CSK KD	CSK KD VPA	
avg	8.9	9.2	9.4		6.1	6.3	6.7	
st err	0.2	0.3	0.3		0.2	0.3	0.4	
n	22	23	14		22	23	14	
Hela	WT	VPA	LA/C KD VPA		WT	VPA	LA/C KD VPA	
avg	11.1	10.9	10.2		7.8	7.4	7.3	
st err	0.3	0.3	0.3		0.3	0.4	0.4	
n	19	9	10		19	9	10	
Hela	scramble	LA/C KD	LB1 KD		scramble	LA/C KD	LB1 KD	
avg	11.1	10.8	9.9*		8.2	7.5	7.6	
st err	0.4	0.3	0.7		0.3	0.2	0.2	
n	13	24	10		13	24	10	
HEK293	WT	GFP-LA	scramble	LB1 KD	WT	GFP-LA	scramble	LB1 KD
avg	10.0	10.9	11.0	9.9	6.2*	8.0	7.6	7.4
st err	0.4	0.4	0.4	0.7	0.4	0.4	0.3	0.3
n	15	16	8	8	15	16	8	8

*Denotes statistically significantly different ($p < 0.05$)