Supplemental Materials

Molecular Biology of the Cell

Zhang et al.

Supplemental Figures:

Figure S1. A. Histograms of EGFP-NLS intensity normalized to initial intensity in unstressed (blue, Ref) and stressed nuclei (orange) at the indicated pressures, at 120 s after the stress pulse. N≥20 for each stress level. P_R shows the probability of rupture calculated as the fraction of samples that exhibited NLS intensity less than the mean reference intensity (i.e. of the unstressed nucleus) less the SEM. B. Histograms of EGFP-NLS intensity normalized to initial intensity in unstressed (blue, Ref), 10 kPa stressed lamin A/C depleted, lamin B2 depleted (orange) and scrambled siRNA transfected nuclei (black) 120 s after the stress pulse. N≥22 for each condition.

Figure S2. A. Plot shows the pooled normalized mean EGFP-NLS intensity at 10 kPa in MEF WT cells at 10 kPa and 30 kPa. Error bars are S.E.M (N≥17). Statistical differences were detected with two-way ANOVA (* represents $p < 0.05$). B. Plot shows area strain of nuclear deformation in MEF WT cells. Error bars are S.E.M (N≥17). Statistical differences were detected with Student's T-test (* represents $p < 0.05$).

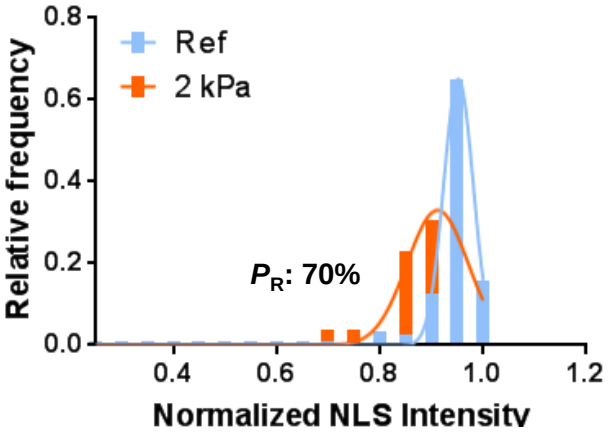
Figure S3. Validation of siRNA transfection. Fluorescent images show the MCF-10A cells transfected with scrambled (non-targeting) siRNA (top) and siRNA targeting *LMNA* (A) or *LMNB2* (B). C. Fluorescence intensity of indicated proteins in nuclei relative to control (scrambled siRNA transfected cells). siRNA transfection results in nearly 60% decrease in fluorescence intensity of the targeted protein. Student's t-test showed no statistically significant difference. D. Relative levels of mRNA transcripts measured by RT-qPCR showing a more than 80% decrease upon siRNA transfection for both lamin A/C and lamin B2. Student's t-test showed no statistically significant difference.

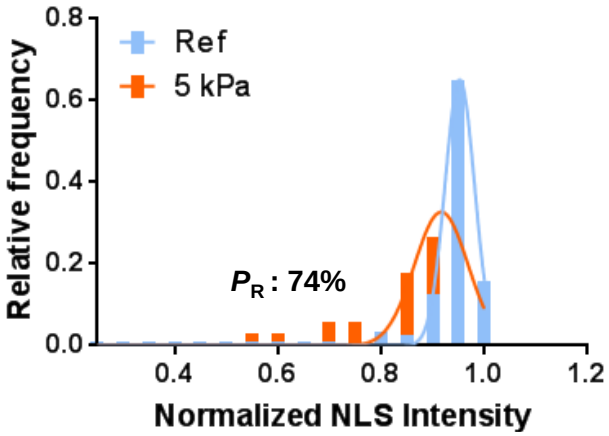
Figure S4. Plot shows maximum nuclear deformation (quantified as area strain) under a 10 kPa stress pulse in MCF-10A cells transfected with scrambled siRNA or siRNA targeting lamin A/C and lamin B2. Error bars are S.E.M (N≥21). Area strain is higher in lamin A/C depleted cells but not lamin B2 depleted cells. Statistical differences were detected with Student's T-test (* represents $p < 0.05$).

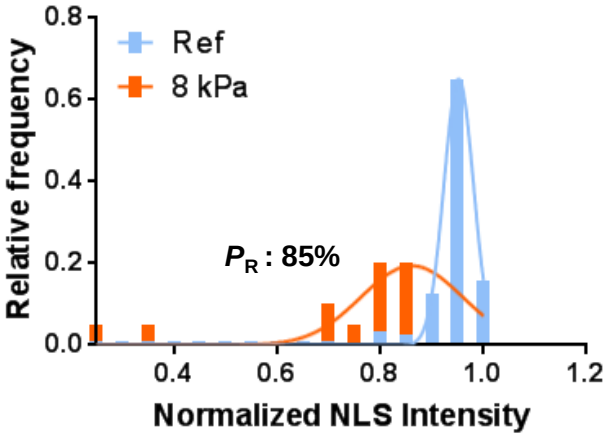
Figure S5. A. Computation of membrane geometry for donut-shaped holes in the nuclear bilayers corresponding to a membrane tension of ~0.08 mN/m (top) and 0.002 mN/m (bottom). Lower tension corresponds to a larger hole diameter. B. Schematic of membrane geometry. r is the radial distance, z is the height, and ψ is the angle of the tangent vector with the radial direction.

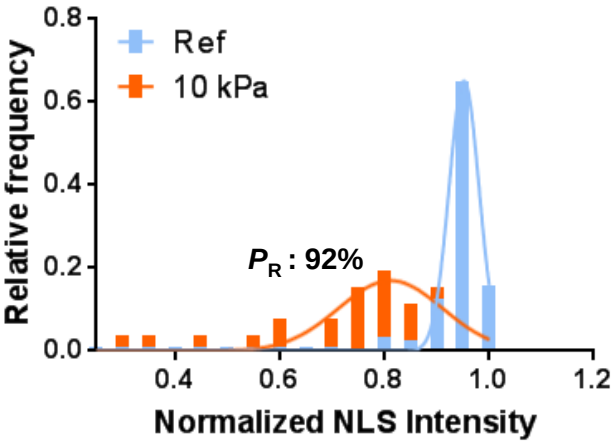
Figure S6. Applying suction pressure to the nuclear envelope for several seconds does not cause leakage of EGFP-NLS into the micropipette. A. DIC image shows the micropipette tip attached to the nuclear surface. Suction was applied to the nucleus for 10.9 s without any measurable change in the nuclear EGFP-NLS intensity. At 10.9 s, the pipette was moved away, at which point the nucleus deformed. The deformation demonstrates that the micropipette tip in this experiment was indeed suction-sealed to the nuclear surface. The plot in B shows the corresponding quantification of fluorescence intensity (grey curve is intensity in the nucleus in A after suction was applied), and quantification of intensity in another nucleus (orange) in the field of view (not shown in A) that was not subjected to micromanipulation. Comparison of the orange and grey curves indicates that the small decrease in EGFP-NLS intensity in the experiment in A is caused by photobleaching due to image capture, and not due to leakage from the nucleus. C. Pooled average of normalized fluorescence intensity (suction/unstressed) for 12 different nuclei. Suction was applied for ~ 5 s, which is a much longer time than the <1s time for which the nucleus was deformed in the rupture experiments. In each experiment, suction-sealing of the pipette to the nuclear surface was confirmed by moving the pipette away after 5 s and checking that the nucleus deformed. Error bar is standard deviation.

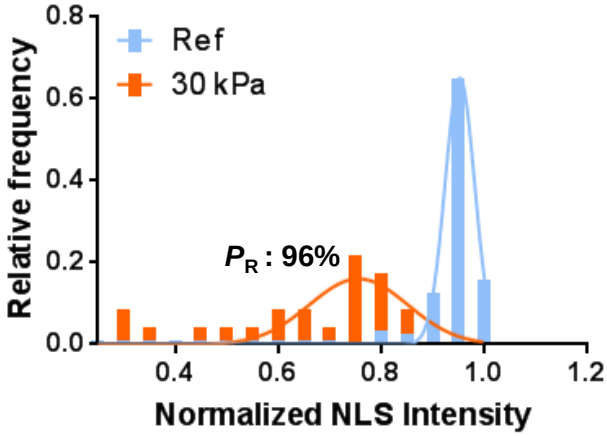
A


Ref
2 kPa
P_R: 70%
Relative frequency
Normalized NLS Intensity

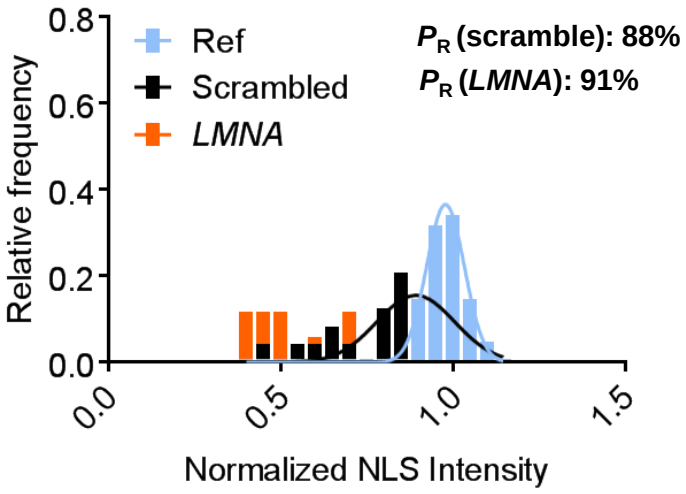

Ref
5 kPa
P_R: 74%
Relative frequency
Normalized NLS Intensity

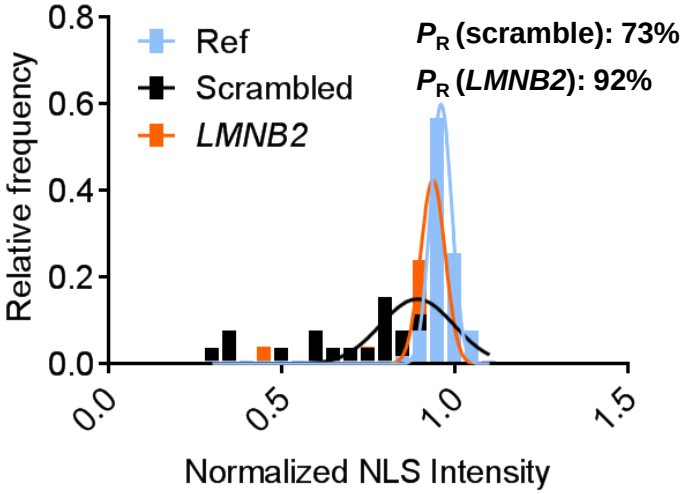

Ref
8 kPa
P_R: 85%
Relative frequency
Normalized NLS Intensity


Ref
10 kPa
P_R: 92%
Relative frequency
Normalized NLS Intensity

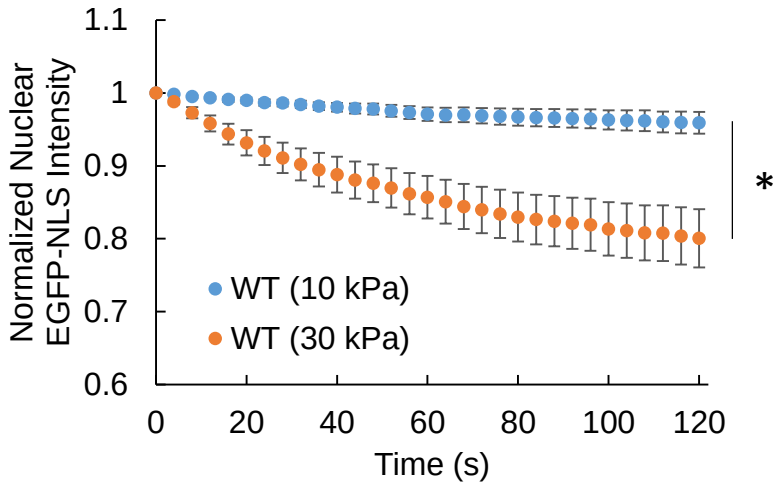

Ref
30 kPa
P_R: 96%
Relative frequency
Normalized NLS Intensity

B

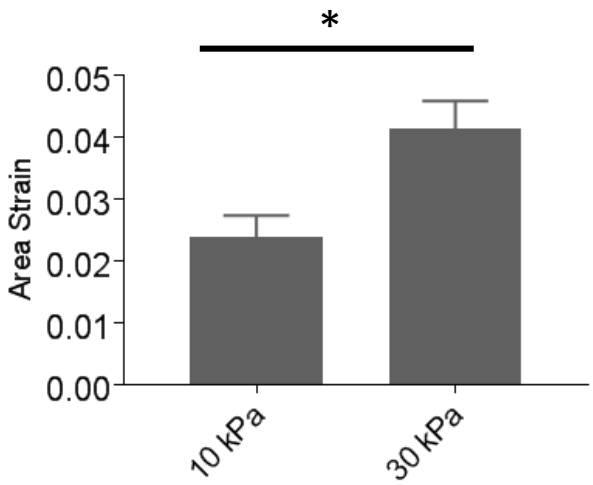

Ref
Scrambled
LMNA
P_R (scramble): 88%
P_R (LMNA): 91%
Relative frequency
Normalized NLS Intensity

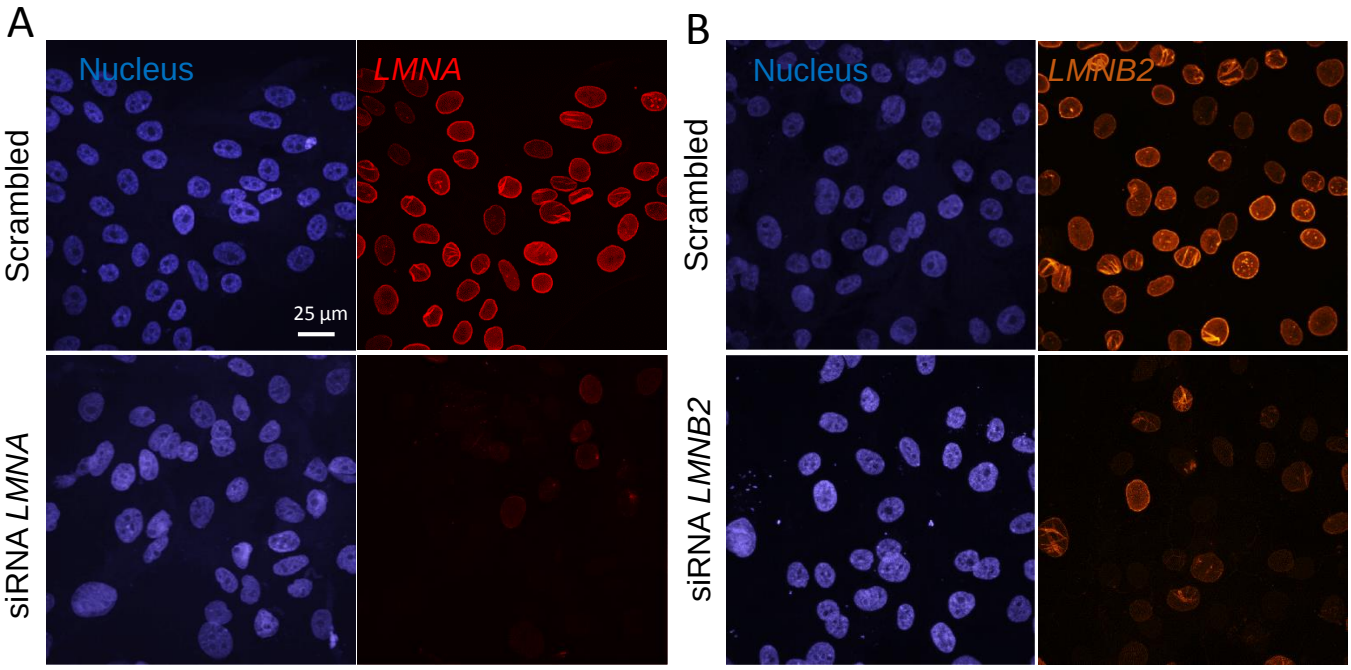

Ref
Scrambled
LMNB2
P_R (scramble): 73%
P_R (LMNB2): 92%
Relative frequency
Normalized NLS Intensity

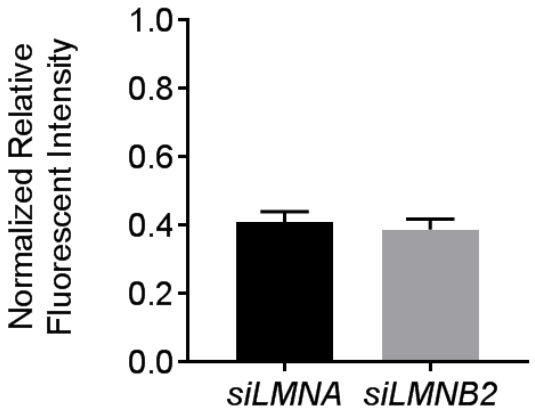
A

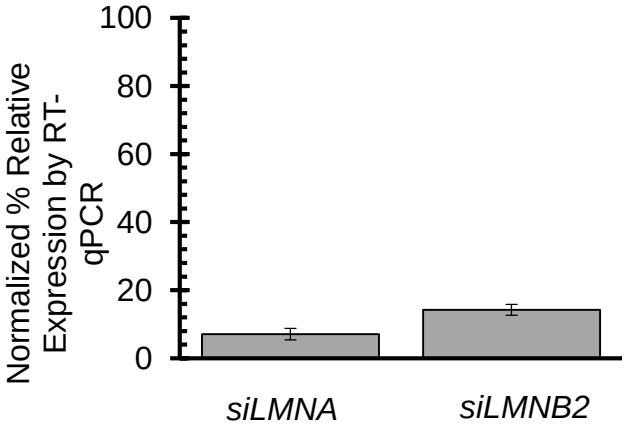

Normalized Nuclear EGFP-NLS Intensity
1.1
1
0.9
0.8
0.7
0.6
0
20
40
60
80
100
120
Time (s)
WT (10 kPa)
WT (30 kPa)
*

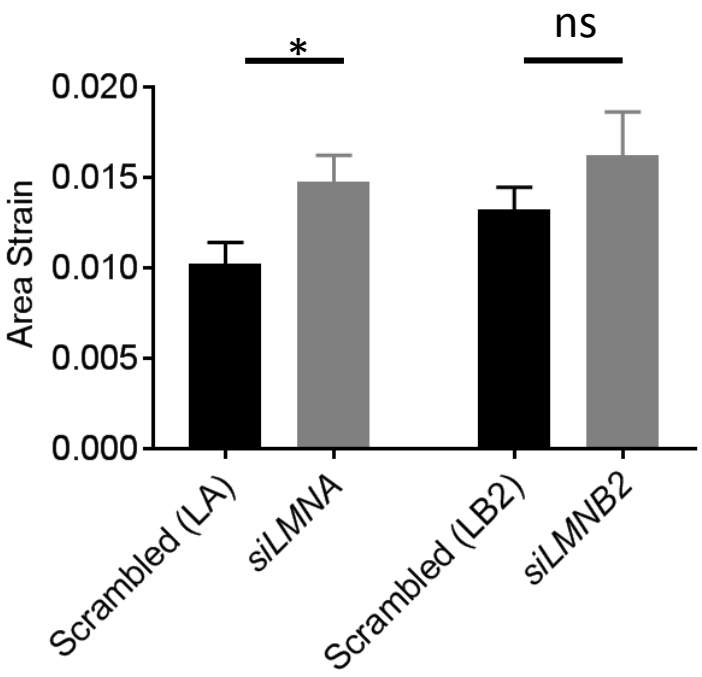
B


*
Area Strain
0.05
0.04
0.03
0.02
0.01
0.00
10 kPa
30 kPa

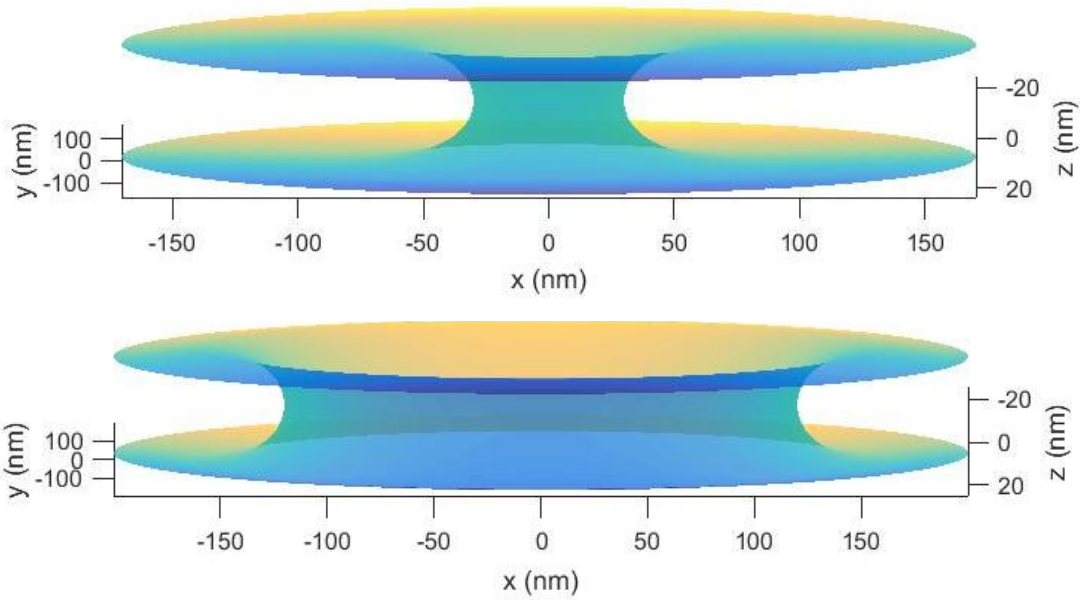

A
Nucleus
LMNA
Scrambled
25 µm
siRNA LMNA
B
Nucleus
LMNB2
Scrambled
siRNA LMNB2


C
Normalized Relative Fluorescent Intensity
1.0
0.8
0.6
0.4
0.2
0.0
siLMNA
siLMNB2

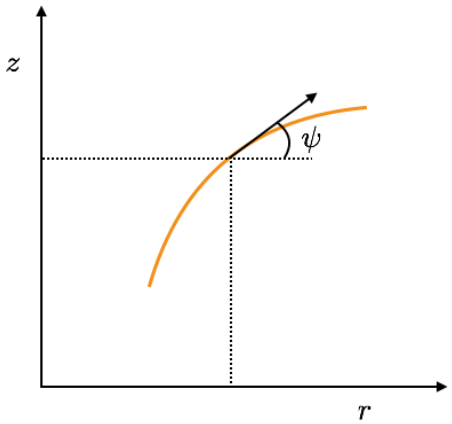

D
Normalized % Relative Expression by RT-qPCR
100
80
60
40
20
0
siLMNA
siLMNB2

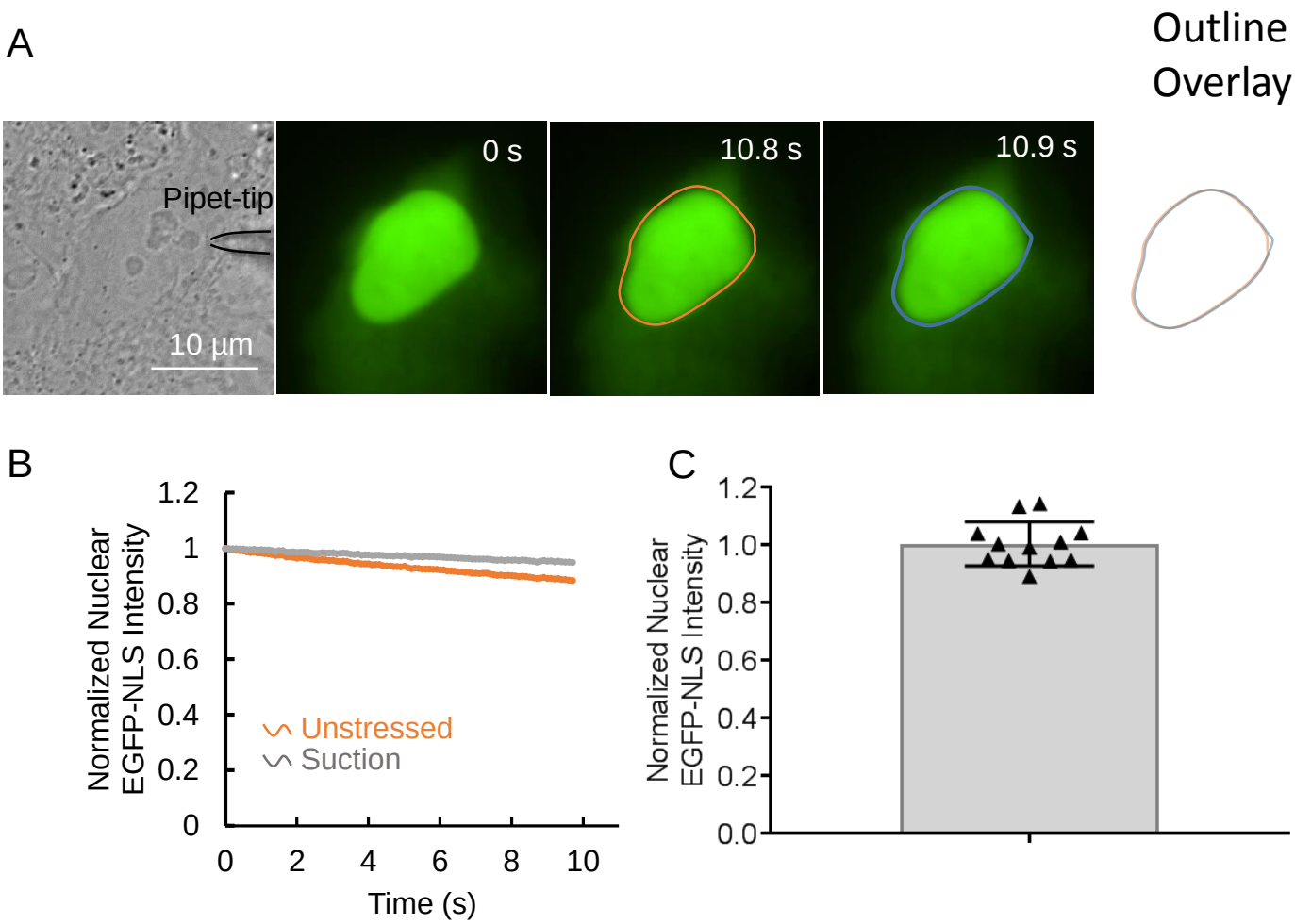

*
ns
0.020
0.015
0.010
0.005
0.000
Area Strain
Scrambled (LA)
siLMNA
Scrambled (LB2)
siLMNB2

A


y (nm)
100
0
-100
-150
-100
-50
0
50
100
150
x (nm)
-20
0
20
z (nm)
y (nm)
100
0
-100
-150
-100
-50
0
50
100
150
x (nm)
-20
0
20
z (nm)

B


z
ψ
r


A
Outline
Overlay
Pipet-tip
10 µm
0 s
10.8 s
10.9 s
B
Normalized Nuclear
EGFP-NLS Intensity
1.2
1
0.8
0.6
0.4
0.2
0
Unstressed
Suction
0
2
4
6
8
10
Time (s)
C
Normalized Nuclear
EGFP-NLS Intensity
1.2
1.0
0.8
0.6
0.4
0.2
0.0