Supplemental Materials

Molecular Biology of the Cell

Stephens et al.

Supplemental Figure 1. Nuclear blebbing induced by altered histone modificationsoccurs in many cell lines, occurs during interphase, and requires the actin cytoskeleton. (A) Graph of the amount of blebbed nuclei in the population of MEF WT untreated (UT) or MEF V-/- cells untreated (UT) or treated with VPA, TSA, or DZNep (n = 428, 439, 181, 151, and 201, respectively) after 16-24 hours.MEF WT and V-/- untreated show similar blebbing percentages (2%, p =0.33 χ^2, n = 4 sets of >100 nuclei each).(B) Representative example of HDACi-induced nuclear blebbing in U2OS and HeLa Kyoto nuclei. (C) Time course of nuclear blebbing frequency upon treatment with 100 nM TSA (n > 80 per time point). (D) Example of bleb formation in nuclei coincident with nuclear rupture, similar to reported bleb formation in lamin perturbations(Vargas et al., 2012; Robijns et al., 2016; Hatch and Hetzer, 2016). (E) Graph of nuclear blebbing percentage of MEF wild-type untreated, VPA-treated for 4-6 hours, and joint treatment with VPA and cytochalasin D for 4-6 hours where the last hour is washed free of cytochalasin D (n = 4 data sets each, 597-605 nuclei). In the same graph, nuclear blebbing percentage for MEF VPA-treated 24 hours were compared to those treated with cytochalasin D for 1 hour with 1 hour and 3 hour post-washout recovery times. (F) Graph for increase in nuclear spring constant upon strain-stiffening in the long-extension regime (> 3 μm) for MEF VPA, DZNep, WT (untreated) and methylstat (MS; n = 5, 6, 8 , and 9 respectively; p > 0.05). Long-extension additional spring constant, from lamin-based strainstiffening, is calculated as long-extension spring constant (3-6 μm) minus short-extension spring constant (0-3 μm). (G) Graph of relative change in 2D nuclear area upon treatment with VPA, TSA, and DZNep in MEF cells and VPA treatment in HT1080 (n = 50 – 300 each). (H) Nucleus height measured by Hoechst half-max intensity of MEF WT untreated (UT) and VPA treated (n = 23 and 26, p = 0.28 t-test). Scale bar = 10 μm.

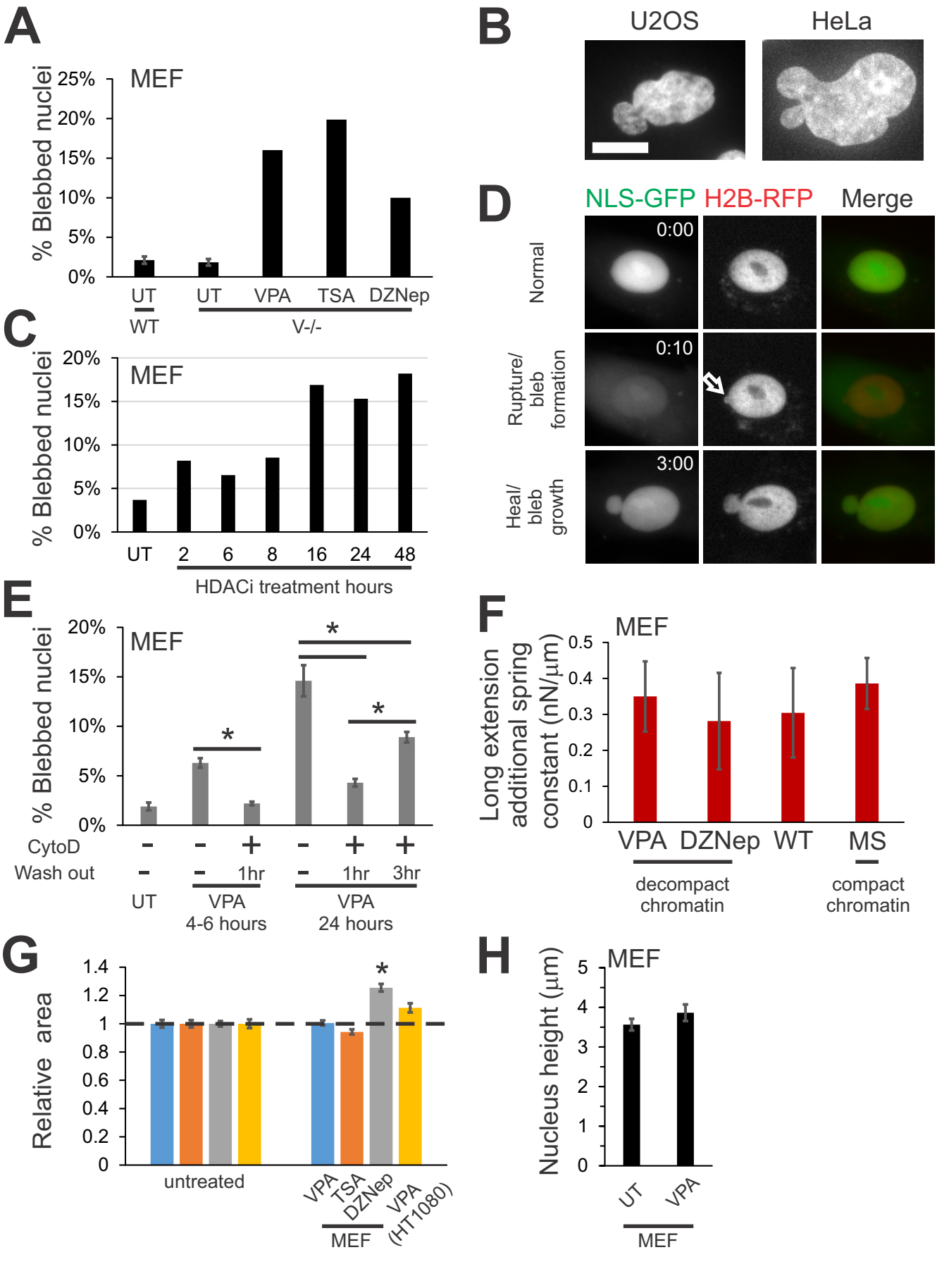
Supplemental Figure 2. HT1080 VPA induced blebs also display bleb position preference on the major axis with no strong correlation to nuclear body aspect ratio. (A) HT1080 and (B) MEF representative images of untreated and VPA-treated (16-24 hours) nuclei stained for DNA (Hoechst, Lamin A (LA) and euchromatin (H3K9ac) or DNA and euchromatin (H4K5ac), respectively. White arrows denote bleb. (C) Graphs of percentage of nuclear blebs forming proximal to the major axis, and histogram of nuclear bleb location relative to the major axis of

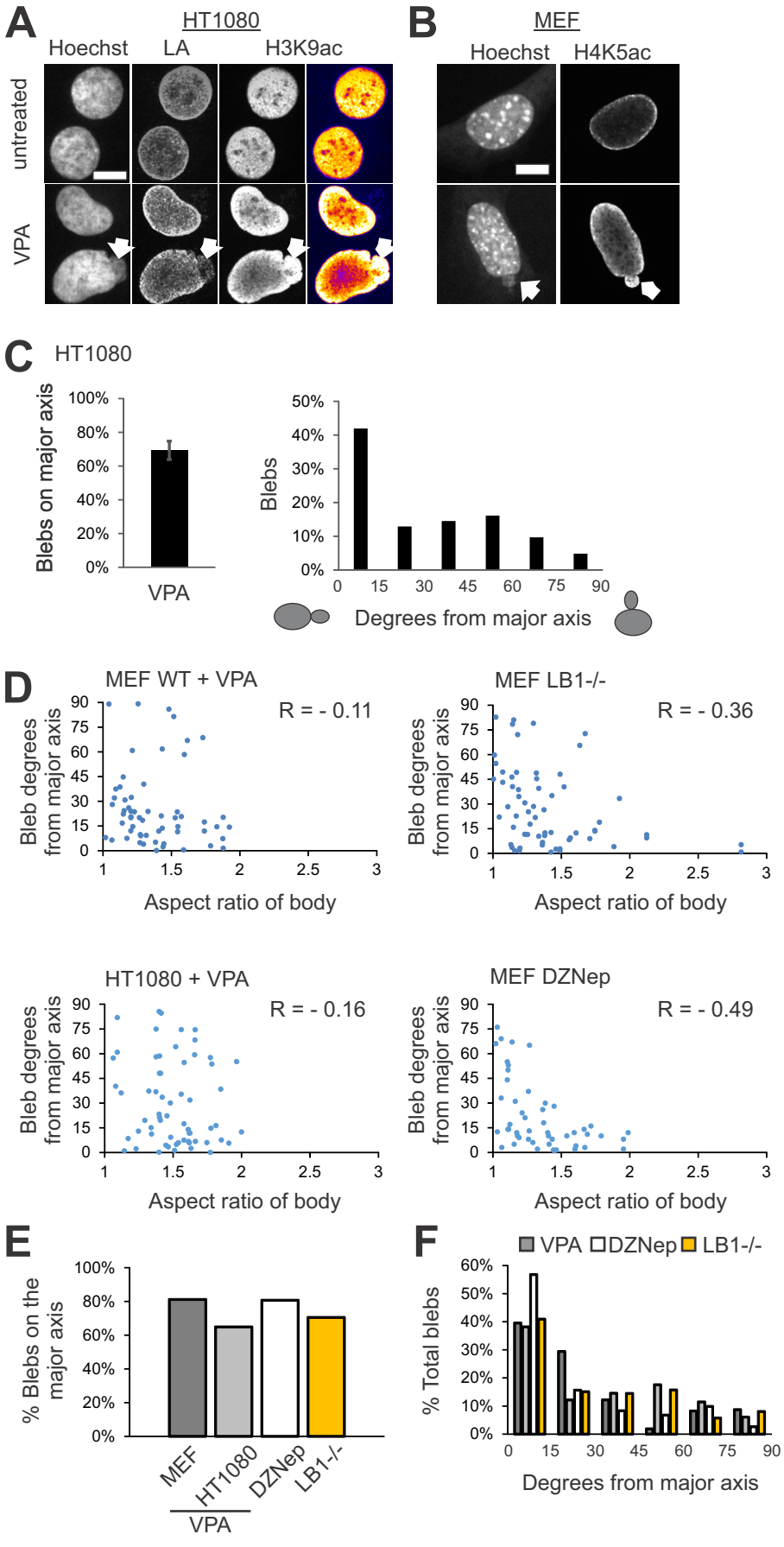
the nuclear body. (D) Aspect ratio of nuclear body vs. the location of the nuclear bleb relative to the major axis reveals no consistent anti-correlation.Graphs of (E) bleb percentage on axis and (F) histogram of bleb position relative to the major axis of the nucleus scaled by the perimeter in each angle bin, which is non-uniform for an ellipse. Darker gray represents MEF VPA-treated and light gray represents HT1080 VPA-treated.Scale bar = 10 μm. Error bars represent standard error.

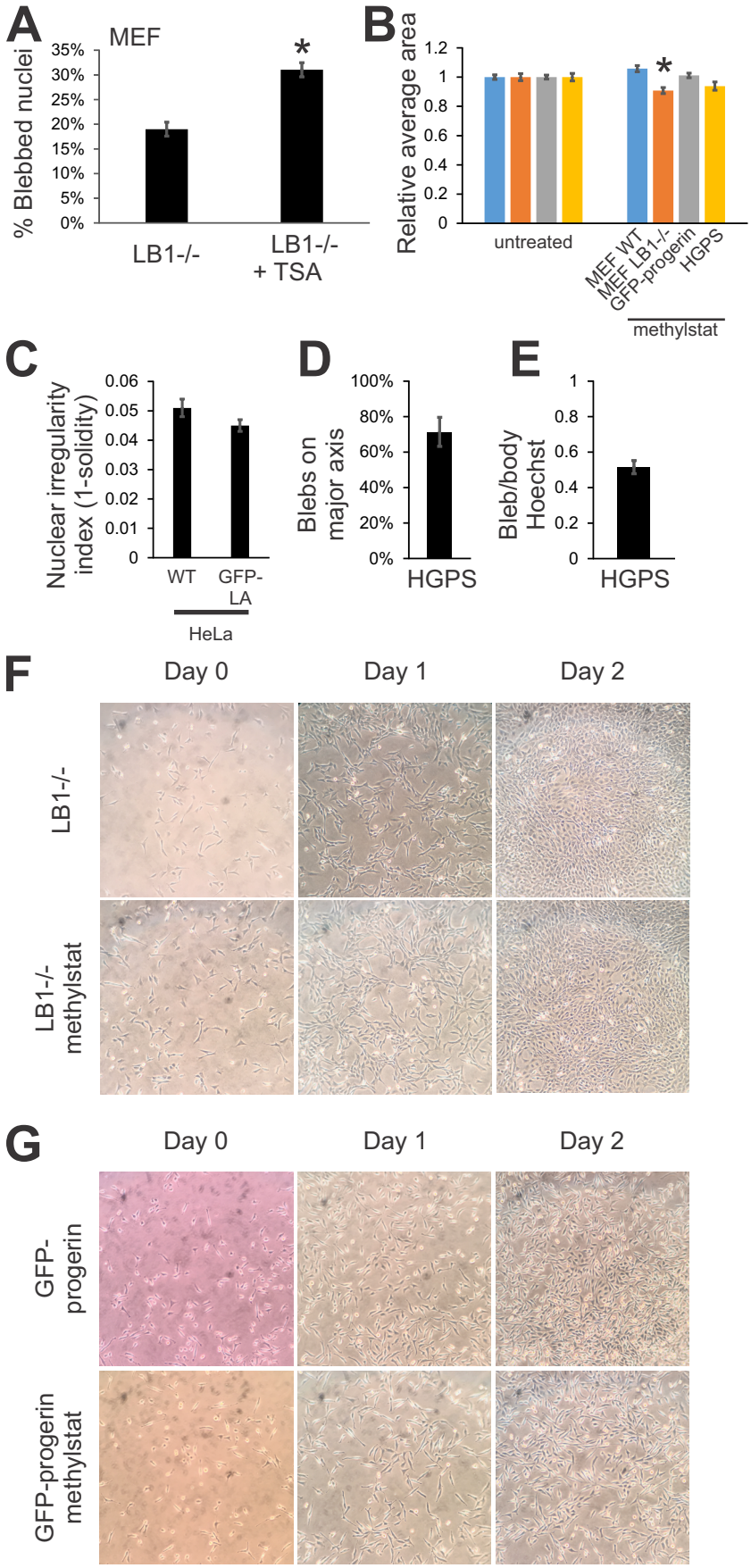
Supplemental Figure 3.Altered histone modification state can also increase nuclear blebbing in LB1-/- MEFs, does not affect increase of spring constant in long regime, and does not affect cell growth. (A) Graph of percentage of blebbed nuclei reveals coupling MEF LB1-/- and HDACi (TSA) for 24 hours results in increased blebbing ($p < 0.001$, total $n = 631$ and 834, respectively two experiments each).(B) Graph of 2D nuclear area relative to untreated for MEF WT, MEF LB1-/- and HeLa GFP-progerin treated with methylstat for 48 hours ($n = 130 – 300$). (C) Nuclear irregularity index is similar for HeLa WT and GFP-lamin A expressing cells (0.051 vs. 0.045, $n > 100$, $p > 0.05$ t-test).Western blots reveal increased lamin A but similar heterochromatin levels for HeLa WT and GFP-lamin A expressing cells (Supplemental Figure 4E). (D)Percentage of HGPS patient cells' nuclear blebs that are proximal (< 45 degrees) to the nuclear major axis. (E) Ratio of bleb vs. body Hoechst average signal intensity. Images of cell growth over 2 days for (F) MEF LB1-/- untreated and methylstat treated and (G) HeLa cells expressing GFP-progerin untreated or treated with methylstat. Error bars represent standard error.

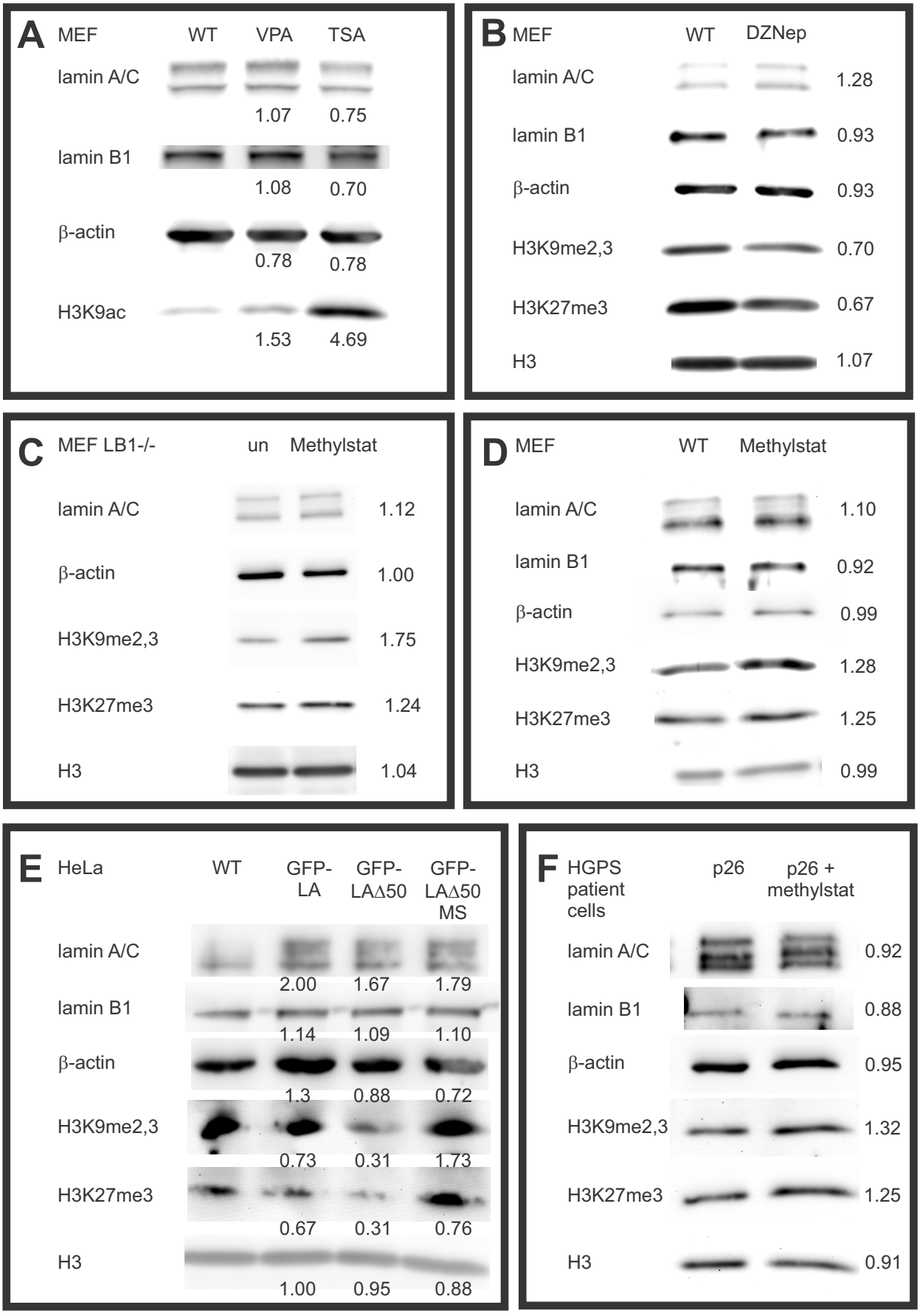
Supplemental Figure 4. Western blots confirm immunofluorescence measurements of alterations in histone modification states. (A) MEF WT untreated (WT) and treated with HDACi VPA or TSA for 24 hours confirms increase in euchromatin marker H3K9ac. (B) MEF WT untreated (WT) and treated with HMTi DZNep for 24 hours confirms decrease in constitutive ($H3K9me^{2,3}$) and facultative ($H3K27me^{3}$) heterochromatin markers. Treatment of (C) MEF LB1-/- (un, untreated), (D) MEF WT, (E) HeLa progerin (GFP-LAΔ50), and (F) human Hutchinson-Gilford progeria syndrome patient cells (HGPS p26) with HDMi methylstat

for 48 hours confirms increase in heterochromatin markers. Quantifications via ImageJ are posted to the right or underneath the blot and are relative to the first lane.


A
MEF
% Blebbed nuclei
25%
20%
15%
10%
5%
0%
UT
UT
VPA
TSA
DZNep
WT
V-/-
B
U2OS
HeLa
C
MEF
% Blebbed nuclei
20%
15%
10%
5%
0%
UT
2
6
8
16
24
48
HDACi treatment hours
D
NLS-GFP
H2B-RFP
Merge
Normal
Rupture/ bleb formation
Heal/ bleb growth
0:00
0:10
3:00
E
MEF
% Blebbed nuclei
20%
15%
10%
5%
0%
*
*
*
CytoD
Wash out
UT
VPA 4-6 hours
VPA 24 hours
1hr
1hr
3hr
F
MEF
Long extension additional spring constant (nN/μm)
0.5
0.4
0.3
0.2
0.1
0
VPA
DZNep
WT
MS
decompact chromatin
compact chromatin
G
Relative area
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
untreated
VPA
TSA
DZNep
VPA (HT1080)
MEF
H
MEF
Nucleus height (μm)
5
4
3
2
1
0
UT
VPA
MEF


A
HT1080
Hoechst
LA
H3K9ac
untreated
VPA
B
MEF
Hoechst
H4K5ac
C
HT1080
Blebs on major axis
100%
80%
60%
40%
20%
0%
VPA
Blebs
50%
40%
30%
20%
10%
0%
0
15
30
45
60
75
90
Degrees from major axis
D
MEF WT + VPA
R = - 0.11
MEF LB1-/-
R = - 0.36
HT1080 + VPA
R = - 0.16
MEF DZNep
R = - 0.49
Bleb degrees from major axis
Aspect ratio of body
E
% Blebs on the major axis
MEF
HT1080
DZNep
LB1-/-
VPA
F
VPA
DZNep
LB1-/-
% Total blebs
Degrees from major axis


A
MEF
% Blebbed nuclei
35%
30%
25%
20%
15%
10%
5%
0%
*
LB1-/-
LB1-/-
+ TSA
B
Relative average area
1.2
1
0.8
0.6
0.4
0.2
0
*
untreated
MEF WT
MEF LB1-/-
GFP-progerin
HGPS
methylstat
C
Nuclear irregularity
index (1-solidity)
0.06
0.05
0.04
0.03
0.02
0.01
0
WT
GFP-
LA
HeLa
D
Blebs on
major axis
100%
80%
60%
40%
20%
0%
HGPS
E
Bleb/body
Hoechst
1
0.8
0.6
0.4
0.2
0
HGPS
F
Day 0
Day 1
Day 2
LB1-/-
LB1-/-
methylstat
G
Day 0
Day 1
Day 2
GFP-
progerin
GFP-progerin
methylstat


A
MEF
WT
VPA
TSA
lamin A/C
1.07
0.75
lamin B1
1.08
0.70
β-actin
0.78
0.78
H3K9ac
1.53
4.69
B
MEF
WT
DZNep
lamin A/C
1.28
lamin B1
0.93
β-actin
0.93
H3K9me2,3
0.70
H3K27me3
0.67
H3
1.07
C
MEF LB1-/-
un
Methylstat
lamin A/C
1.12
β-actin
1.00
H3K9me2,3
1.75
H3K27me3
1.24
H3
1.04
D
MEF
WT
Methylstat
lamin A/C
1.10
lamin B1
0.92
β-actin
0.99
H3K9me2,3
1.28
H3K27me3
1.25
H3
0.99
E
HeLa
WT
GFP-LA
GFP-LAΔ50
GFP-LAΔ50 MS
lamin A/C
2.00
1.67
1.79
lamin B1
1.14
1.09
1.10
β-actin
1.3
0.88
0.72
H3K9me2,3
0.73
0.31
1.73
H3K27me3
0.67
0.31
0.76
H3
1.00
0.95
0.88
F
HGPS patient cells
p26
p26 + methylstat
lamin A/C
0.92
lamin B1
0.88
β-actin
0.95
H3K9me2,3
1.32
H3K27me3
1.25
H3
0.91