Supplemental Materials

Molecular Biology of the Cell

Stephens et al.

Supplemental Figure Legends

Supplemental Figure 1. Increased extracellular divalent cations do not alter normal cell growth or characteristics. (A) Viability measured by the absence of propidium iodide staining, which marks dead cells (n = 200 – 330). MEF cells treated with VPA or null for lamin B1 (LB1-/-) in normal or $MgCl_2$ supplemented media display similar (B-D) nuclear sizedistribution (Pearson correlation coefficient r > 0.9), (C) cell confluency and growth, and (D) actin cables running over the nucleus.(E) Western blot of MEF cells with and without $MgCl_2$ supplemented mediaand with or without mechanosensitive ion channel inhibitors GsMTx4 (GMT) and $GdCl_3$. Scale bar = 10 μm.

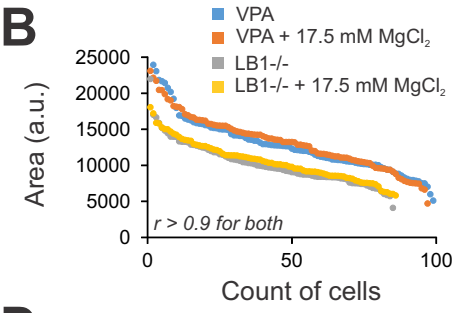
Supplemental Figure 2. The extracellular amount of divalent or polyamine cations alters heterochromatin levels and nuclear blebbing over hours, but does not alter euchromatin levels. (A) Western blot of MEF VPA plus increased extracellular $CaCl_2$. (B) Western blot and (C) Immunofluorescence of euchromatin marker H3K9ac inMEF VPA-treated cells with added $MgCl_2$ (n > 50 cells).(D) MEF nuclei treated with VPA for 24 hours, and then with continued VPA treatment and additional extracellular $MgCl_2$ 17.5 mM for 0, 2, and 8 hours were assayed for $H3K27me^3$ immunofluorescence signal and nuclear blebbing percentage (n > 50 cells). (E) Relative immunofluorescence signal of heterochromatin markers in MEF LB1-/- with and without added extracellular $MgCl_2$(n = 2 experiments, > 20 cells). (F) HT1080 cells treated with VPA and extracellular $MgCl_2$ or (G) MEF cells treated with VPA and spermidine (n > 50 cells). (H) Relative nuclear blebbing in MEF TSA-treated or MEF V-/- VPA-treated without or with increased extracellular divalent cations (n = 2-4 experiments each > 100 cells). (I) MEF cells treated with DZNep and additional extracellular $MgCl_2$ and labeled for heterochromatin markers via immunofluorescence (n = 3 experiments, > 50 cells each). (J) Graph of percentage of nuclei that exhibit nuclear rupture over 3 hours on average for MEF VPA, MEF LB1-/-, and HT1080 VPA with and without additional extracellular $MgCl_2$(n = 3 experiments, >50 cells each). (K) Relative nuclear size for MEF cells treated with either VPA or DZNep with increasing extracellular $MgCl_2$for 24 hours ($p > 0.05$, n = 3 experiments, >50 cells each).Error bars represent standard error. Asterisks denote statistically significant differences ($p < 0.05$ or $\chi = 0.05$).

Supplemental Figure 3. HeLa GFP-progerin expressing cells display extracellular $MgCl_2$-induced heterochromatin increase and rescued nuclear morphology dependent on mechanosensitive ion channels. (A) Western blot of $H3K27me^3$decrease upon GFP-Progerin and increase/rescue upon increased extracellular $MgCl_2$ in HeLa cells. (B) Graph of relative nuclear area for HeLa cells (GFP-Progerin and wild-type) and patient HGPS cells with and without additional extracellular $MgCl_2$($n \geq 3$ sets of > 50 cells, $p > 0.05$). (C) Relative nuclear irregularity index in HeLa GFP-progerin treated with just 17.5 mM extracellular $MgCl_2$ and co-treated with GsMTx4 mechanosensitive channel inhibitor (n = 3 sets of >50 cells each).Error bars represent standard error. Asterisks denote statistically significant differences*** $p < 0.001$).

A

	MEF WT	MEF VPA	MEF LB1-/-
-	97.3%	100.0%	99.5%
7.5 mM	100.0%	99.5%	100.0%
17.5 mM	99.1%	99.7%	99.7%

B



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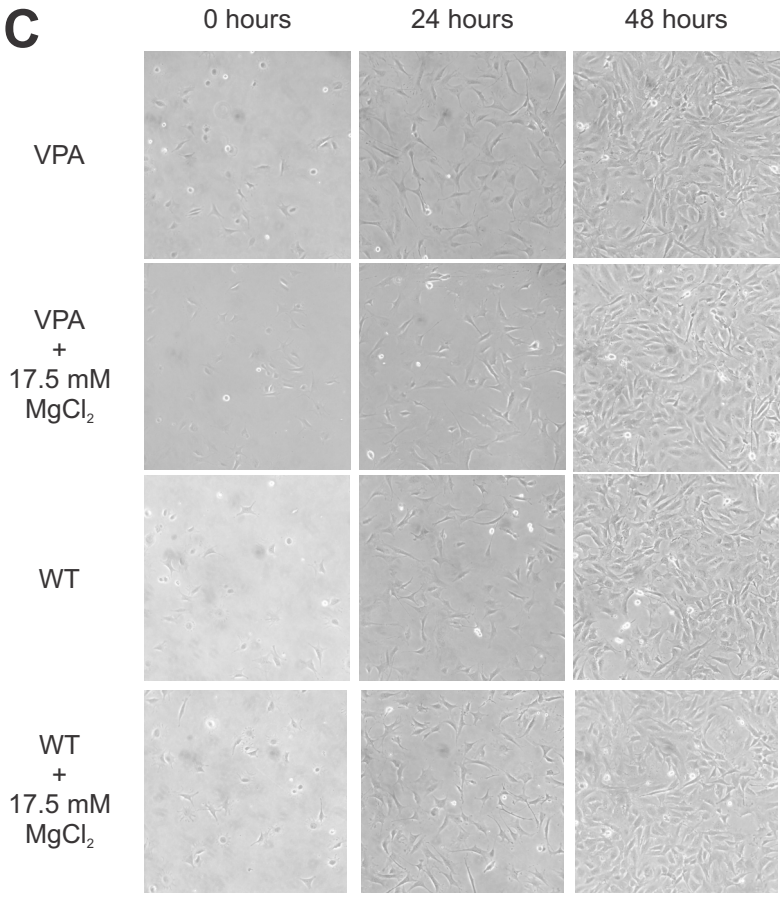
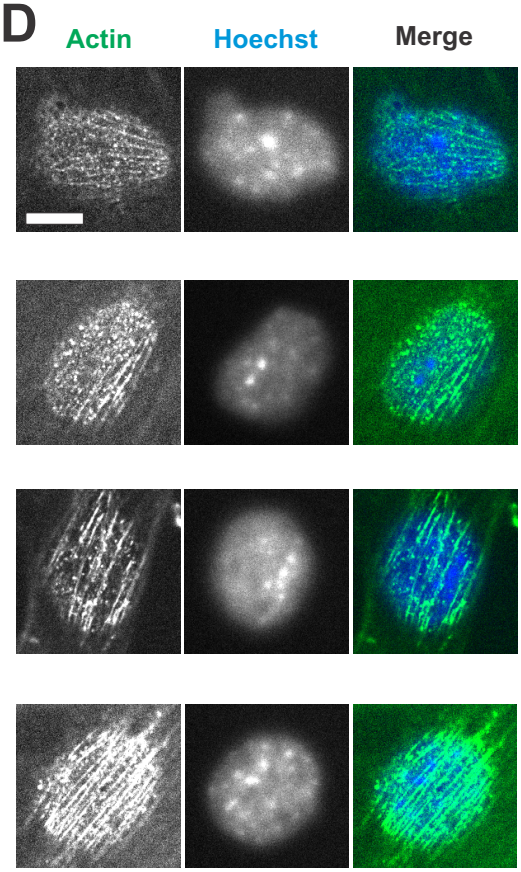


D



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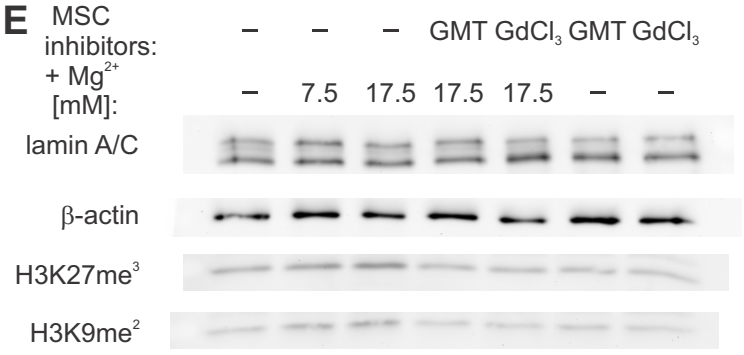
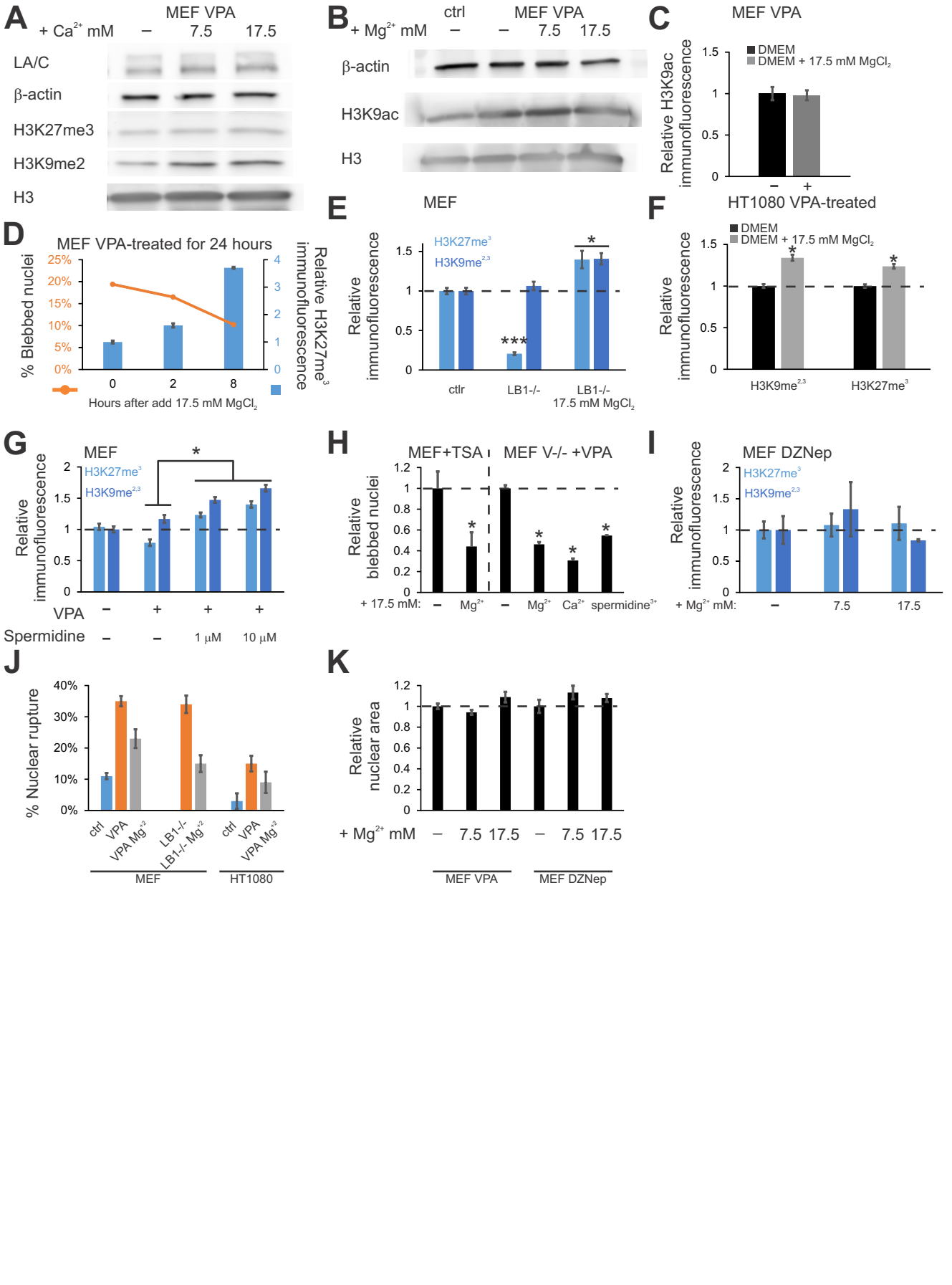
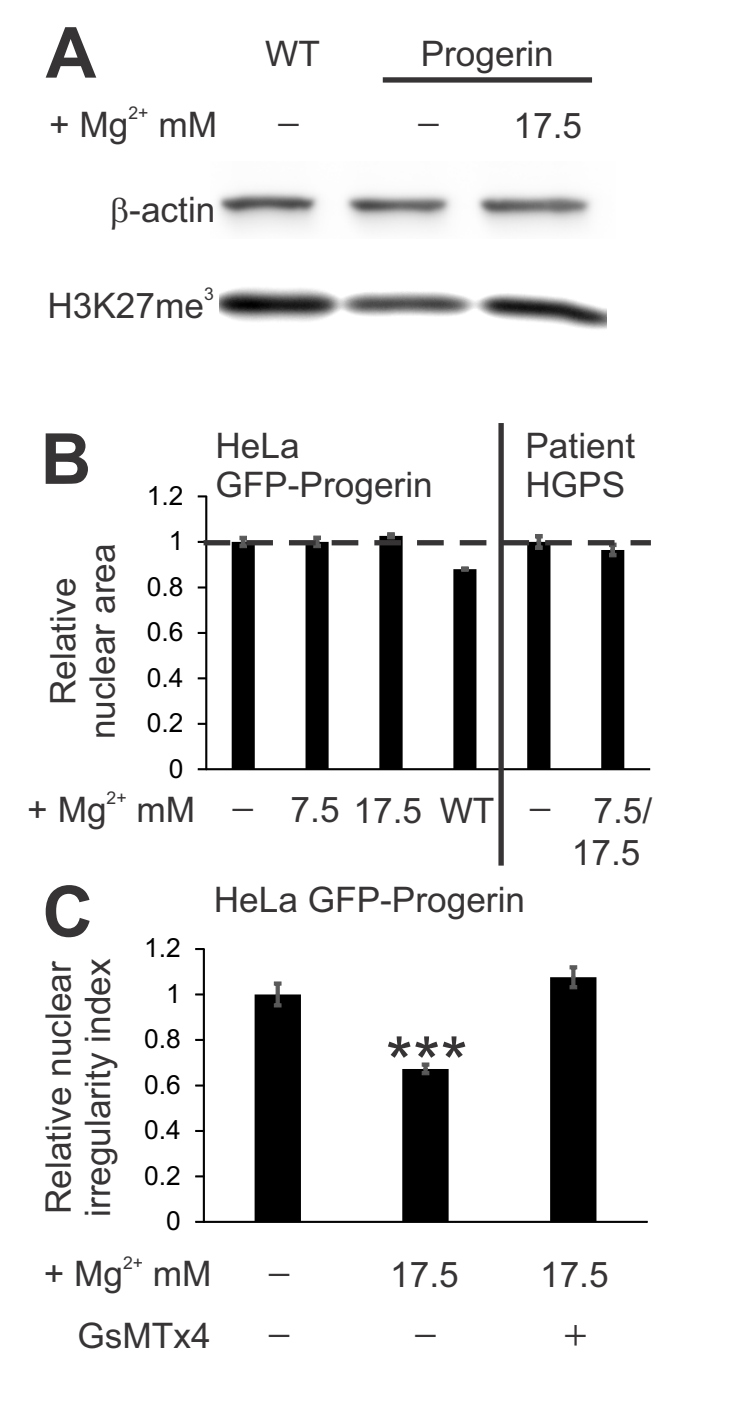



A
MEF VPA
+ Ca2+ mM – 7.5 17.5
LA/C
β-actin
H3K27me3
H3K9me2
H3
B
ctrl MEF VPA
+ Mg2+ mM – – 7.5 17.5
β-actin
H3K9ac
H3
C
MEF VPA
Relative H3K9ac immunofluorescence
DMEM
DMEM + 17.5 mM MgCl2
D
MEF VPA-treated for 24 hours
% Blebbed nuclei
Relative H3K27me3 immunofluorescence
Hours after add 17.5 mM MgCl2
E
MEF
Relative immunofluorescence
H3K27me3
H3K9me2,3
ctlr
LB1-/-
LB1-/- 17.5 mM MgCl2
F
HT1080 VPA-treated
DMEM
DMEM + 17.5 mM MgCl2
Relative immunofluorescence
H3K9me2,3
H3K27me3
G
MEF
Relative immunofluorescence
H3K27me3
H3K9me2,3
VPA
Spermidine
1 μM
10 μM
H
MEF+TSA
MEF V-/- +VPA
Relative blebbed nuclei
+ 17.5 mM:
Mg2+
Ca2+
spermidine3+
I
MEF DZNep
Relative immunofluorescence
H3K27me3
H3K9me2,3
+ Mg2+ mM:
7.5
17.5
J
% Nuclear rupture
ctrl
VPA
VPA Mg+2
LB1-/-
LB1-/- Mg+2
MEF
HT1080
K
Relative nuclear area
+ Mg2+ mM – 7.5 17.5 – 7.5 17.5
MEF VPA
MEF DZNep


A
WT
Progerin
+ Mg2+ mM
–
–
17.5
β-actin
H3K27me3
B
HeLa
GFP-Progerin
Patient
HGPS
Relative
nuclear area
1.2
1
0.8
0.6
0.4
0.2
0
+ Mg2+ mM
–
7.5
17.5
WT
–
7.5/
17.5
C
HeLa GFP-Progerin
Relative nuclear
irregularity index
1.2
1
0.8
0.6
0.4
0.2
0
***
+ Mg2+ mM
–
17.5
17.5
GsMTx4
–
–
+