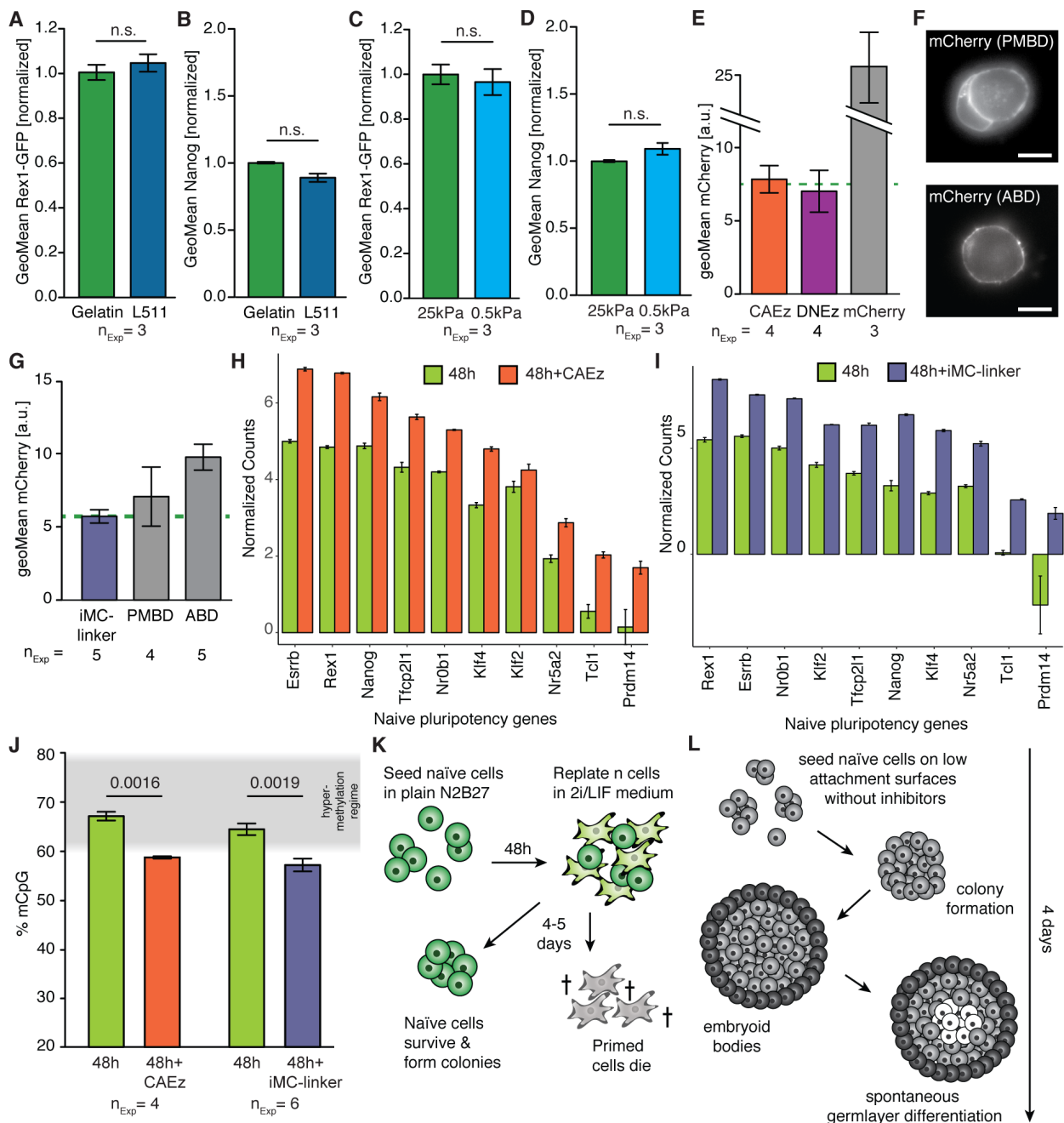# Supplemental Information

Cell Surface Mechanics Gate

Embryonic Stem Cell Differentiation

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Supplemental Figure 1. Quantifications of naïve pluripotency features and expression levels. Related to Figure 1 and 2. (**A**) Normalized GFP geometric mean intensities for mESCs Rex1-GFPd2 in 2i/LIF medium plated for 48h on Gelatin or on Laminin 511 (L511). (**B**) Normalized geometric mean intensities of Nanog immunofluorecence levels for mESCs Rex1-GFPd2 in 2i/LIF medium plated for 48h on Gelatin or on Laminin 511. n_{Exp}: number of independent experiments. Error bars: SEM. p-Values: Welch's *t*-test. (**C**) Normalized GFP geometric mean intensities for mESC Rex1-GFPd2 cells in 2i/LIF medium after plating for 48 h on Laminin 511-coated hydrogels of 25 kPa or 0.5 kPa. (**D**) Normalized geometric mean intensities of Nanog immunofluorescence levels for mESC Rex1-GFPd2 cells in 2i/LIF medium after plating for 48 h on Laminin 511-coated hydrogels of 25 kPa or 0.5 kPa. n_{Exp}: number of independent experiments. Error bars: SEM. p-Values: Welch's *t*-test. (**E**) mCherry geometric mean intensities for mESC Rex1-GFPd2 *ind*-CAEz, *ind*-DNEz and *ind*-mCherry cells during exit from pluripotency in N2B27±Dox medium at 48 h. n_{Exp}: number of independent experiments. Error bars: SEM. (**F**) Representative fluorescent images of naïve mESC Rex1-GFPd2 *ind*-PMBD (plasma membrane-binding domain) and mESC Rex1-GFPd2 *ind*-ABD (actin-binding domain) cells in 2i/LIF+Dox medium. Scale bar: 10 *µ*m. (**G**) mCherry geometric mean intensities for mESC Rex1-GFPd2 *ind*-iMC, *ind*-PMBD and *ind*-ABD cells during exit from pluripotency in N2B27±Dox medium at 48 h. n_{Exp}: number of independent experiments. Error bars: SEM. (**H-I**) Bar plots of naïve pluripotency markers (Kalkan et al., 2017) upregulated upon CAEzrin or iMC-linker expression (RNAseq-derived, data from 3 independent experiments). All pairs (48 h *vs.* 48 h+Dox) are significant (p<0.01). (**J**) Global DNA methylation levels of mESC Rex1-GFPd2 *ind*-CAEz and *ind*-iMC cells plated for 48 h in N2B27 media or in N2B27+Dox media. Gray region depicts typical hypermethylation regime (60-80 %mCpG) acquired upon 2i/LIF withdrawal (Hackett et al., 2018). n_{Exp}: number of independent experiments. Error bars: SEM. p-Values: Welch's *t*-test. (**K**) Schematic of the re-plating assay used to assess the pluripotency state (Betschinger et al., 2013; Cirera-Salinas and Ciaudo, 2017). (**L**) Schematic of embryoid body formation.

A GeoMean Rex1-GFP [normalized]

n.s.

Gelatin L511
$n_{Exp}= 3$

B GeoMean Nanog [normalized]

n.s.

Gelatin L511
$n_{Exp}= 3$

C GeoMean Rex1-GFP [normalized]

n.s.

25kPa 0.5kPa
$n_{Exp}= 3$

D GeoMean Nanog [normalized]

n.s.

25kPa 0.5kPa
$n_{Exp}= 3$

E geoMean mCherry [a.u.]

CAEz DNEz mCherry
$n_{Exp}=$ 4 4 3

F mCherry (PMBD)

mCherry (ABD)

G geoMean mCherry [a.u.]

iMC- PMBD ABD
linker
$n_{Exp}=$ 5 4 5

H Normalized Counts

48h 48h+CAEz

Esrrb Rex1 Nanog Tfcp2l1 Nr0b1 Klf4 Klf2 Nr5a2 Tcl1 Prdm14

Naïve pluripotency genes

I Normalized Counts

48h 48h+iMC-linker

Rex1 Esrrb Nr0b1 Klf2 Tfcp2l1 Nanog Klf4 Nr5a2 Tcl1 Prdm14

Naïve pluripotency genes

J % mCpG

hyper-methylation regime

0.0016   0.0019

48h 48h+ 48h 48h+
CAEz   iMC-linker
$n_{Exp}= 4$ $n_{Exp}= 6$

K Seed naïve cells in plain N2B27

Replate n cells in 2i/LIF medium

48h

4-5 days

Naïve cells survive & form colonies

Primed cells die

L seed naïve cells on low attachment surfaces without inhibitors

colony formation

embryoid bodies

spontaneous germlayer differentiation

4 days

Supplemental Figure 2. DNEzrin decreases MCA but does not speed up exit from naïve pluripotency. Related to Figure 2. (**A**) Representative brightfield (DIC) and fluorescent images of mESC Rex1-GFPd2 *ind*-DNEz cells expressing DNEz-mCherry in 2i/LIF+Dox medium. **(B)** Force-velocity curve from dynamic tether pulling on mESC Rex1-GFPd2 *ind*-DNEz cells in 2i/LIF medium and during exit from pluripotency in N2B27±Dox medium at 48 h. Data points are mean tether force f±SEM at 2, 5, 10 and 30 μm/sec pulling velocity. n: number of cells analysed in 3 independent experiments. Insert shows mean and standard deviation of the MCA parameter a obtained from Monte-Carlo-based fitting (see STAR Methods for details). p-Value: *Z*-test. **C**) Normalized GFP geometric mean intensities for mESC Rex1-GFPd2 *ind*-DNEz cells plated for 48 h in 2i/LIF or in 2i/LIF+Dox medium. n_{Exp}: number of independent experiments. Error bars: SEM. p-Values: Welch's *t*-test. (**D**) Normalized geometric mean intensities of Nanog immunofluorecence levels for mESC Rex1-GFPd2 *ind*-DNEz cells plated for 48h in 2i/LIF or in 2i/LIF+Dox medium. n_{Exp}: number of independent experiments. Error bars: SEM. p-Values: Welch's *t*-test. **(E)** Normalized GFP geometric mean intensities for mESC Rex1-GFPd2 *ind*-DNEz and *ind*-mCherry cells in 2i/LIF medium and during exit from naïve pluripotency in N2B27+/-Dox medium at 48 h. n_{Exp}: number of independent experiments. Error bars: SEM. p-Value: Welch's *t*-test. **(F)** Normalized colony number (Dox/Ctrl) mESC Rex1-GFPd2 *ind*-DNEz and *ind*-mCherry cells re-plated after a 48 h exit in N2B27+/-Dox medium. n_{Exp}: number of independent experiments. Error bars: SEM. p-Value: Welch's *t*-test.

A

mCherry (DNEz)

B

Naïve (2i/LIF)
n=13

Naïve (2i/LIF) + DNEz
n=16

1.12e-13

MCA α
[10⁴ pN³s/μm]

Mean tether force f [pN]

Pulling velocity [μm/sec]

C

n.s.

GeoMean Rex1-GFP [normalized]

2i/LIF   2i/LIF
          +DNEz

n_{Exp}= 3

D

n.s.

GeoMean Nanog [normalized]

2i/LIF   2i/LIF
          +DNEz

n_{Exp}= 3

E

n.s.   n.s.

GeoMean Rex1-GFP [normalized]

2i/LIF 48h 48h+   2i/LIF 48h 48h+
             DNEz   mCherry

n_{Exp} = 4 n_{Exp} = 3

F

n.s.

Colony number (Dox/Ctrl)

DNEz   mCherry
n_{Exp}= 3 n_{Exp}= 3