#### **Title**

Rapid Restoration of Cell Phenotype and Matrix Forming Capacity Following Transient Nuclear Softening

## **Author names**

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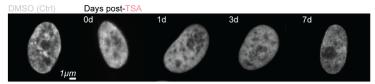
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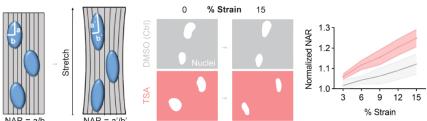
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# Supplemental data

a. Representative images for chromatin condensation parameter (CCP)



b. Nuclear deformability increased following TSA treatment



c. No differences in 2D wound closure following TSA treatment

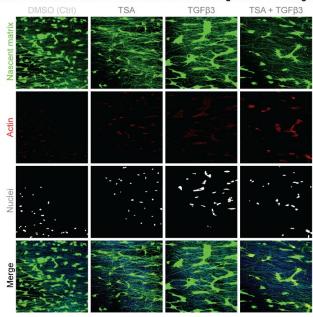


**Supplemental Figure 1.** (a) Representative images of high magnification DAPI-stained nuclei used for quantification of chromatin condensation parameter edge density. (b) Schematic of nuclear deformability assay and representative binarized images of DAPI-stained nuclei at 0 and 15% strain for DMSO-treated controls and TSA-treated cells. Quantification of the nuclear aspect ratio (NAR) at incremental strain steps for DMSO-treated controls and TSA-treated cells, showing increased nuclear deformability at each strain step following TSA treatment. (c) Representative images of 2-dimensional scratch assay over time for DMSO-treated controls and TSA-treated cells, and quantification of percent wound closure over time. N=5-6.

### a. Study design for nascent matrix formation to 2-weeks



#### b. Maintence of functional nascent matrix formation following nuclear softening at 2-weeks



Supplemental Figure 2. Representative confocal images of nascent matrix at 14 days following DMSO, TSA, TGF $\beta$ 3, or TSA + TGF $\beta$ 3 treatment. Green: deposited nascent matrix over the culture period, yellow: overlay of nascent matrix and actin (red), blue: nanofibrous scaffold autofluorescence, magenta: nuclei.

a. Complete recovery of bulk transcriptional phenotype and normal respose to pro-matrix stimuli differentially expressed genes between low- and high-dose TSA treatments at day 0 following washout of low-dose TSA Low (150nM) TSA dose TSA 0d vs TSA + TGFR3 7d vs 125 none Low-dose TSA 0d <u>High-dose</u> 60 100 0/14a1 200 0261055 -Log<sub>10</sub> (p<sub>adj</sub>) 75 oc10062099 Cpt1 4a Inhb 40 1472 83 826 Tnfrsf2 34 14 107 818 50 100 Chst1 20 Stmr Ncami **Amp13** 25 Down Both compared to Ctrl 0d 10 -5 -5 5 -10 -5 5 Log<sub>2</sub> (Fold Change) Log<sub>2</sub> (Fold Change) Log<sub>2</sub> (Fold Change) d. Minimal differences in response to subsequent TGF $\beta 3$  following prior exposure to c. Minimal differences in differential expression between low- and high-dose TSA low- or high-dose TSA compared to TGFβ3-control 7d: High-dose vs. Low-dose TSA Low-dose 7d: TGFβ3 vs. TSA + TGFβ3. High-dose 7d: TGFβ3 vs. TSA + TGFβ3. 80 100 none c10216812 none 60 60 75 4xin2 Mki6 -Log<sub>10</sub> (p<sub>adj</sub>) Aldh1a3 eg10 40 40 40 50 elsr2 Csf 20 20 25 20 Krt18 Mmp13 0 -10 0 -5

b. Significant overlap and differences in

0

Log<sub>2</sub> (Fold Change)

5

Supplemental Figure 3. (a) Volcano plots for low-dose TSA treatment groups. (b) Venn diagram comparing differentially expressed genes at day 0 following low- and high-dose TSA. Numbers indicate upregulated (top number) genes and downregulated (bottom number) genes. Non-overlapping quadrants represent unique genet sets for each group. (c) Volcano plots for high-dose compared to low-dose TSA treatment at day 0 and 7. (d) Volcano plots for TGF $\beta$ 3 only compared to low- or high-dose TSA + TGF $\beta$ 3 at day 7.

-5

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Log<sub>2</sub> (Fold Change)

5

Log<sub>2</sub> (Fold Change)

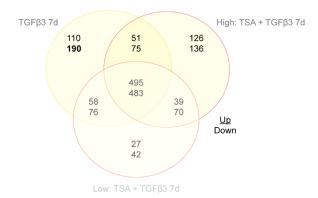
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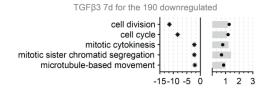
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-5

#### a. High similarity of differentially expressed genes for TGF-treated groups



#### b. Minimal pathway differences between unique genes for TGF-treated groups



Supplemental Figure 4. (a) Venn diagram of differentially expressed genes between groups treated with TGF $\beta$ 3, including TGF $\beta$ 3 only, high-dose TSA + TGF $\beta$ 3, and low-dose TSA + TGF $\beta$ 3. Numbers indicate upregulated (top number) genes and downregulated (bottom number) genes. Non-overlapping quadrants represent unique gene sets for each group. (b) Gene ontologies for unique upregulated genes following high-dose TSA + TGF $\beta$ 3. Note: The unique downregulated genes following high-dose TSA + TGF $\beta$ 3 and both the up- and down-regulated genes following low-dose TSA + TGF $\beta$ 3 were not significantly enriched into specific gene ontologies.