

Substrate Stiffness Enhances Human Regulatory T Cell Induction and Metabolism

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Supplementary Figures

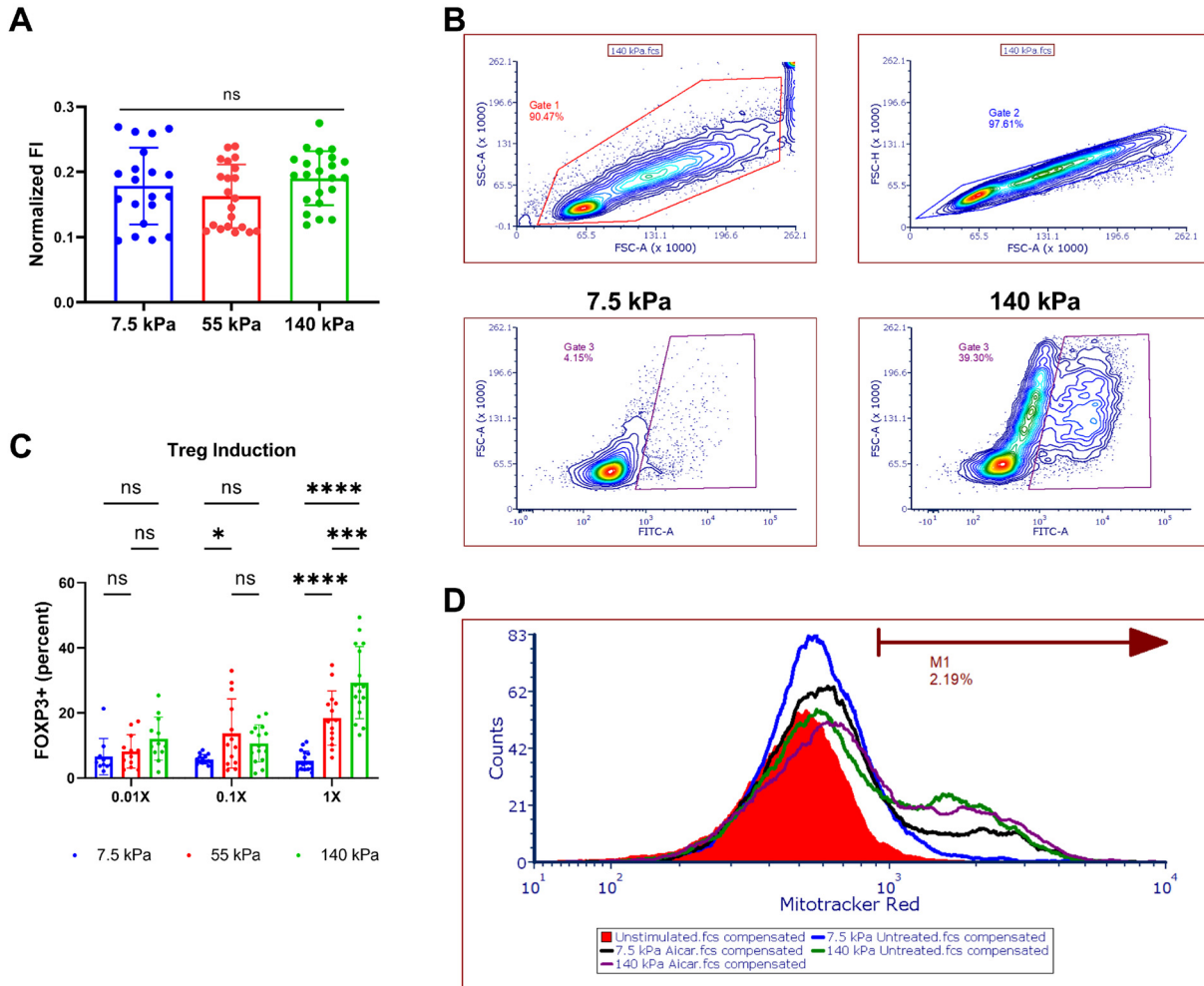


Figure S1. **(A)** Streptavidin-acrylamide concentrations were varied to obtain a similar coating of antibodies on PA-gels, and confirmed by fluorescence intensities on the surface of gels ($n = 4$ gels for each formulation, Kruskal-Wallis test with Dunn's multiple comparisons). **(B)** Representative gating strategies for FXP3 expression from PA-gels. **(C)** Treg induction with various concentrations of streptavidin-acrylamide (4 independent experiments and 2 replicates each for 0.1X and 0.01X, two-way ANOVA with Tukey's multiple comparisons * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$). **(D)** A representative gating strategy for percent mitochondrial high cells for Fig. 4.

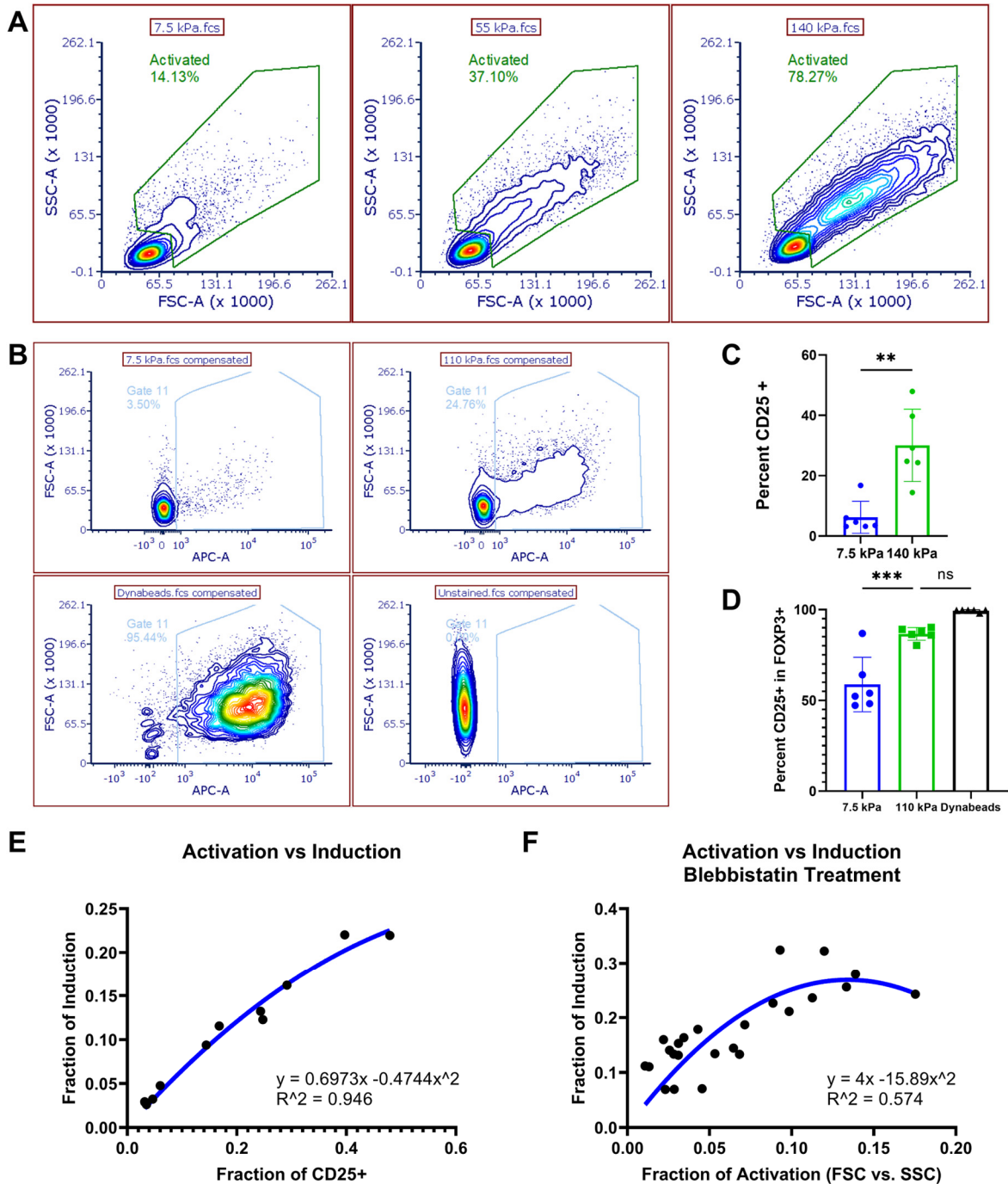


Figure S2. **(A)** Gating of activated T cells on the forward scattering and side scattering of flow cytometry data. **(B)** Representative flow gating strategy for CD25⁺ population (APC-A, CD25). **(C)** Percent CD25⁺ on both 7.5 kPa and 140 kPa (3 independent experiments and 2 replicates each, Mann-Whitney test, ** $p < 0.01$). **(D)** Percent of CD25⁺ population in FOXP3⁺ population (3 independent experiments and 2 replicates each, One-way ANOVA, *** $p < 0.001$). **(E)** Fraction of CD25⁺ versus fraction of induction, measured as fraction of cells that are FOXP3⁺, (Extra sum-of-squares F test ($\alpha = 0.05$), 3 independent experiments and 2 replicates each). **(F)** Activation vs induction of all the data from blebbistatin treatment and the second-order polynomial fit was identified with the Extra sum-of-squares F test ($\alpha = 0.05$).

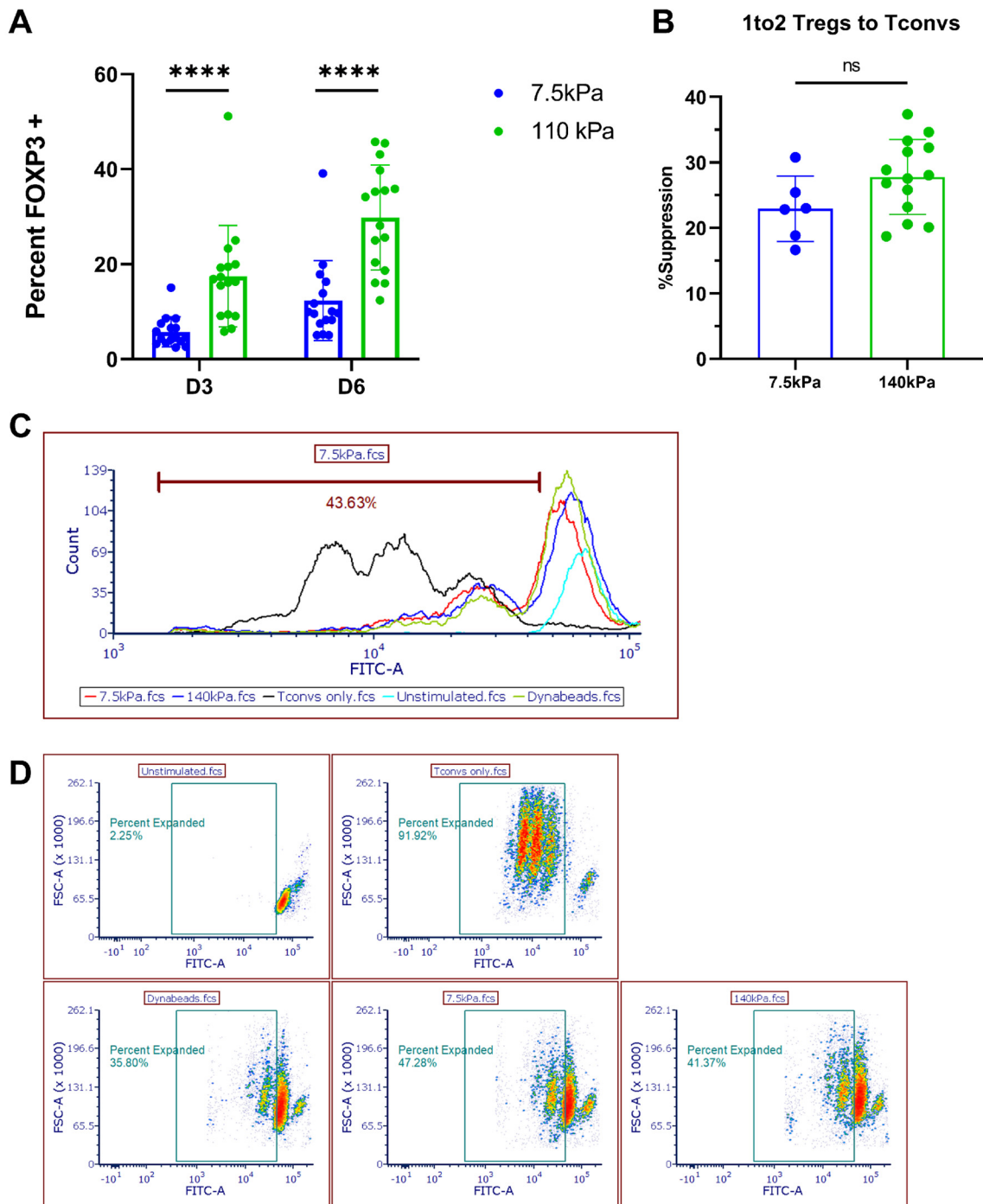


Figure S3. **(A)** Percent FOXP3⁺ on day 6 (Mann-Whitney test was used to compare gels on day 3 and day 6 individually, **** $p < 0.0001$). **(B)** Treg suppressive capacities with 1to2 ratio of Tregs to Tconvs ($n = 5$ independent experiments, and the number of replicates depends on the number of cells obtained after sorting, unpaired t -test). **(C)** Representative gating strategies for calculation of percent suppression with a 1to1 ratio of Tregs to Tconvs samples. Tconvs only is the positive control without Tregs with Dynabeads. **(D)** Representative CFSE(FITC-A) vs FSC-A density plots for 1to1 ratio of Tregs to Tconvs samples.

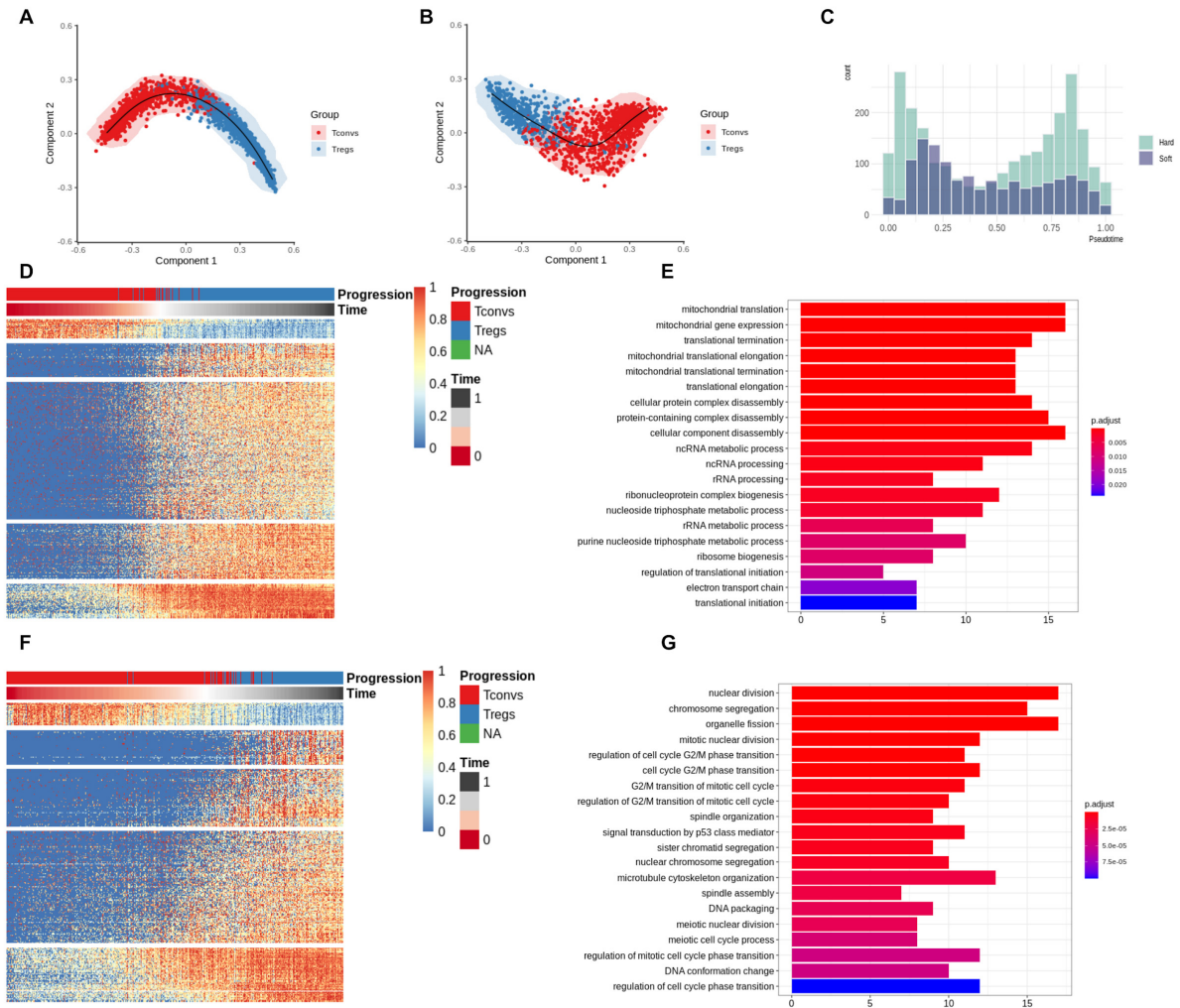


Figure S5. Trajectory analyses on either the hard or the soft samples. **(A, B)** Trajectory analyses were performed with SCORPIUS with T cells from either the hard **(A)** or the soft **(B)** sample separately. **(C)** Histogram of cells along the trajectory on either the hard or the soft sample. **(D, F)** Important genes that contribute to Treg induction were organized into modules for either the hard **(D)** or the soft **(F)** sample. **(E, G)** Over-representation analysis of genes uniquely identified in either the hard **(E)** or the soft **(G)** sample.

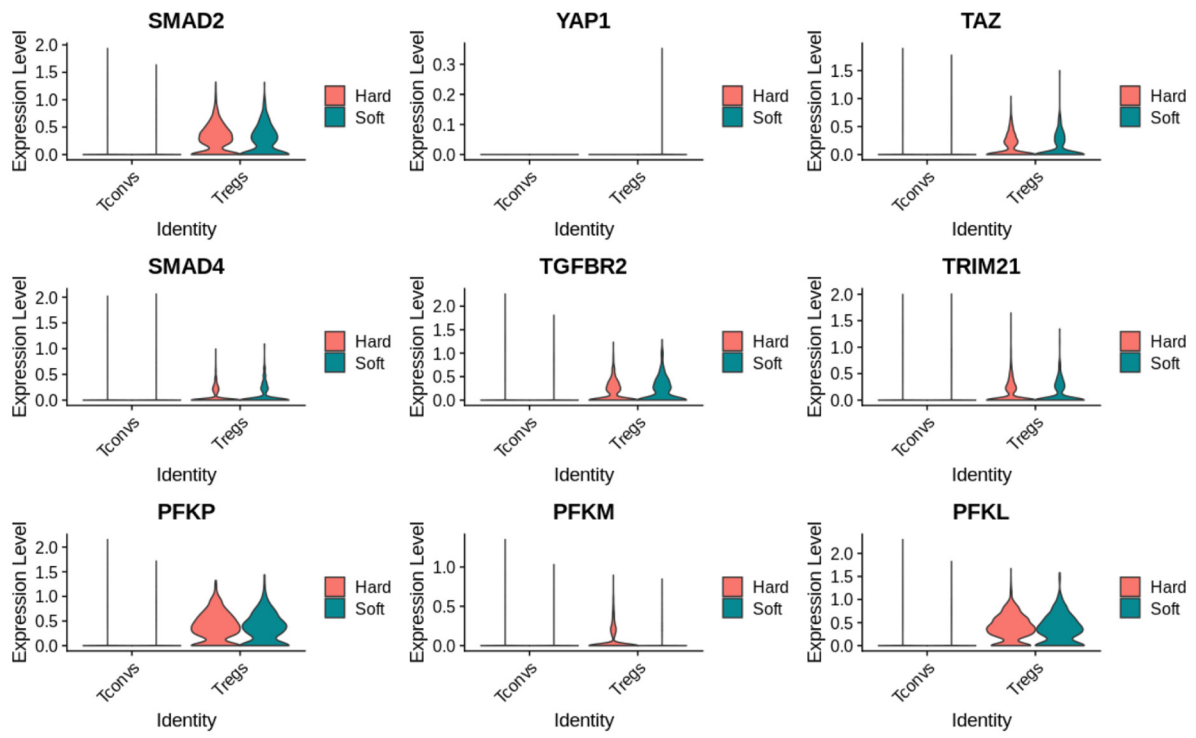
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Figure S6. (A) Violin plots of SMAD2, YAP1, TAZ, SMAD4, TGFBR2, TRIM21, PFKP, PFKM, and PFKL.