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Supplemental Information

Cellular Stiffness as a Novel Stemness Marker in the Corneal Limbus

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FIGURES

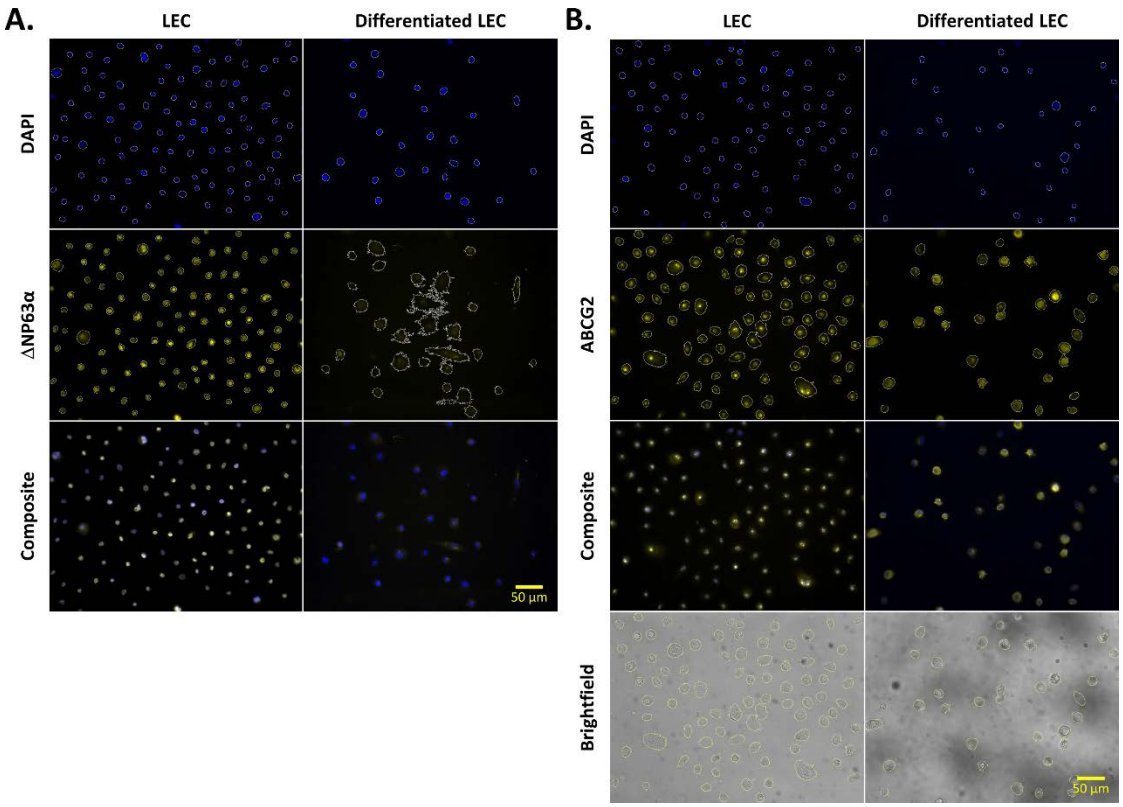

Figure S1. Image Quantification. The nuclei of limbal epithelial cells (LECs) and differentiated LECs were first stained with DAPI to serve as a seed for the identification of individual cells. The seeds were used to identify either the ΔNP63α-stained area (panel A, second row, white outlines), as identified by ΔNP63α intensity (yellow), or the cytoplasmic area (panel B, second row, white outlines), as identified by ABCG2 (yellow). The cellular boundaries are similar whether defined by the brightfield images or the automatically identified cytoplasmic areas (panel B, fourth row, yellow outlines). Scale bars, 50 µm.

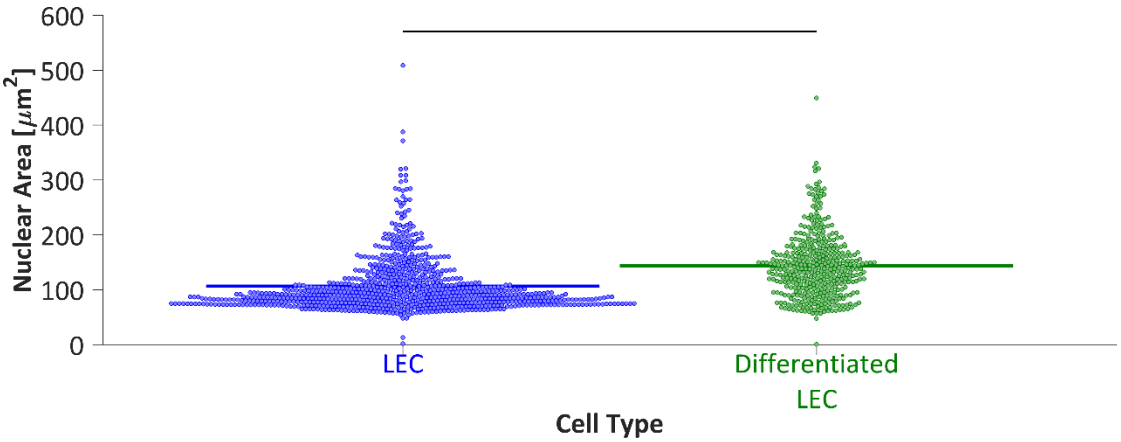

Figure S2. Nuclear Area. The nuclear area, as measured from DAPI channel images taken during ΔNP63α and ABCG2 staining, was significantly higher for differentiated LECs than LECs ($p<10^{-6}$).

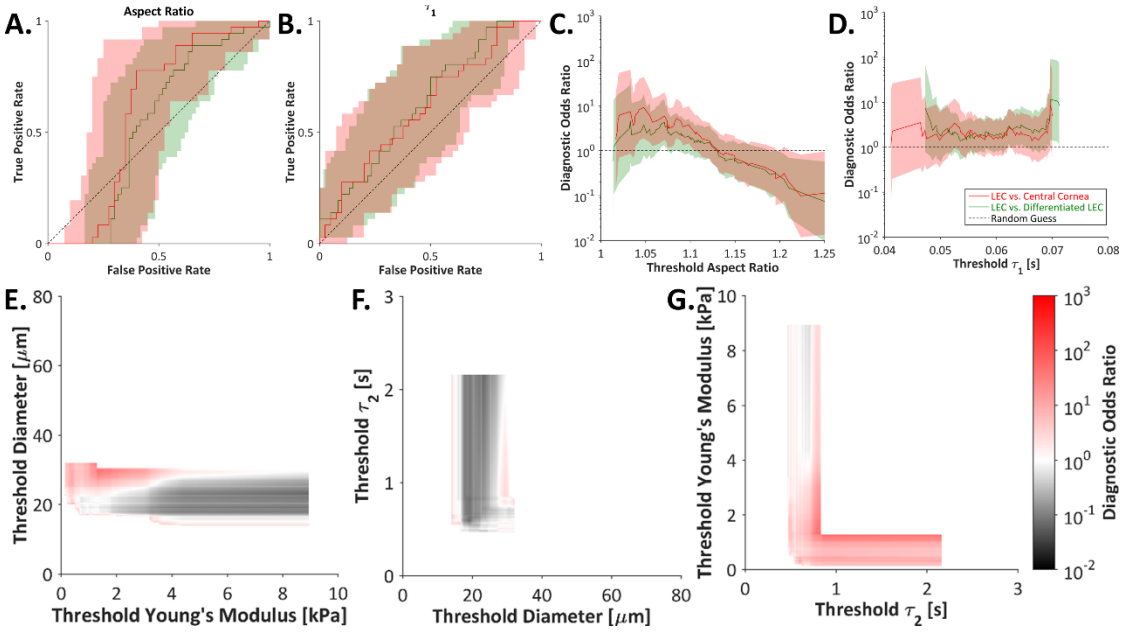

Figure S3. Receiver Operating Characteristic (ROC) Curves. A-B) Selection for limbal epithelial cells (LECs) against central cornea cells (red) or differentiated LECs (green) on the basis of A) aspect ratio and B) the fast viscoelastic time constant (τ_1) was poor, as indicated by areas under the curve of only marginally greater than 0.5, which is the area under the curve for a random classifier. **C-D)** The low diagnostic odds ratios (DORs) for classifiers based on C) the aspect ratio and D) the fast viscoelastic time constant further indicate the poor quality of classification. **E-G)** For all possible combinations of E) threshold diameter and threshold Young's modulus, F) threshold slow viscoelastic time constant (τ_2) and threshold diameter, and G) threshold Young's modulus and threshold slow viscoelastic time constant, the DOR was calculated for selection of LECs against central cornea cells. Overall, the DORs were lower for selection of LECs against central cornea cells than against differentiated LECs (Figs. 7H-J). The DORs ranged from 0.01 (black) to 1 (white) to 1000 (bright red).

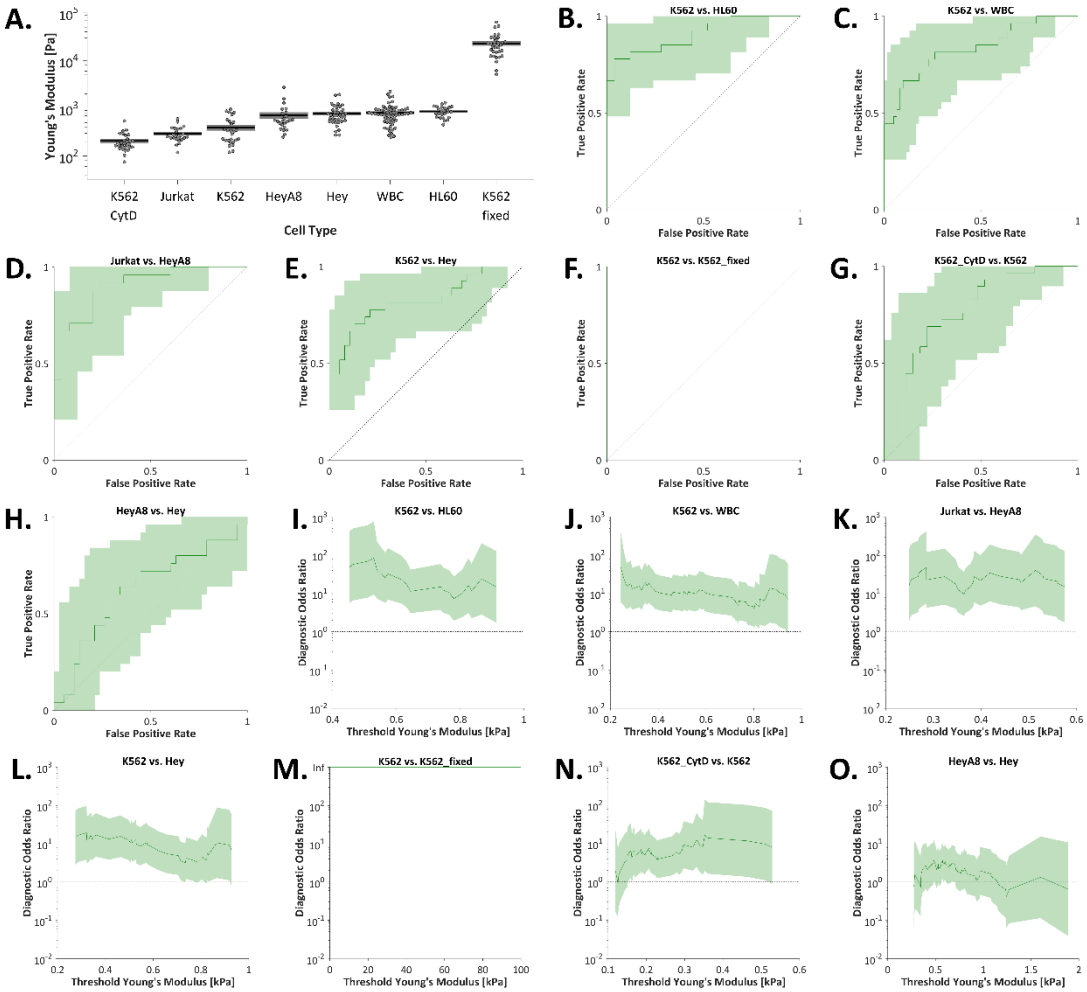

Figure S4. Differences in Adherent Cell Young's Moduli are Correlated with the Microfluidic Sorting Diagnostic Odds Ratio. A. Cellular Young's moduli ranged from 0.1-100 kPa for cell lines previously sorted using microfluidic technology. **B-H.** For various combinations of cell types, the receiver operating curves based on adherent-cell Young's modulus yielded areas under the curve ranging from 0.626 (H, HeyA8 vs. Hey) to 1 (F, K562 vs. K562_fixed), where the soft cell type was taken as condition positive. **I-O.** The maximum diagnostic odds ratio (DOR) based on adherent-cell Young's modulus ranged from 3.713 (O, HeyA8 vs. Hey) to infinity (M, K562 vs. K562_fixed). Shaded regions indicate 95% confidence interval. WBC, white blood cell; fixed, treatment with 4% paraformaldehyde; CytD, treatment with 2 μM cytochalasin-D. Pre-sort Young's moduli of various cell types (Panel B) replotted from (33-36). Young's modulus ROC and DOR curves (C-J) calculated from previously published data (33-36).

TABLES

Table S1. Diagnostic Odds Ratios for Single Parameters. For each parameter, the maximum and mean diagnostic odds ratios (DORs) were calculated for classifying limbal epithelial cells (LECs) versus differentiated LECs or central cornea cells. The DOR of a perfect classifier is infinite for any threshold value, whereas the DOR of a random classifier is approximately 1, and a test classifier will have a finite DOR>1. τ_1, fast viscoelastic time constant; τ_2, slow viscoelastic time constant.

	Maximum DOR		**Mean DOR**	
	LEC vs. Differentiated LEC	**LEC vs. Central Cornea**	**LEC vs. Differentiated LEC**	**LEC vs. Central Cornea**
Mean ΔNP63α Intensity	89.92		16.82	
Nucleus-to-Cytoplasm Ratio	30.44		7.80	
Young's Modulus	28.64	38.68	8.30	7.11
Diameter	28.64	3.55	11.31	0.28
Aspect Ratio	4.62	9.15	1.94	2.96
τ_1	11.67	8.75	2.75	2.31
τ_2	14.24	2.84	6.37	1.03

Table S2. Area under the Receiver Operating Curves. For each parameter, the area under the receiver operating curve was calculated for classifying limbal epithelial cells (LECs) versus differentiated LECs or central cornea cells. The area under the curve of a useful test ranges from 0.5 (random classification) to 1 (perfect classification). τ_1, fast viscoelastic time constant; τ_2, slow viscoelastic time constant; CI, confidence interval.

	LEC vs. Differentiated LEC			**LEC vs. Central Cornea**		
	Area Under Curve	**Lower 95% CI**	**Upper 95% CI**	**Area Under Curve**	**Lower 95% CI**	**Upper 95% CI**
Mean ΔNP63α Intensity	0.860	0.800	0.913			
Nucleus-to-Cytoplasm Ratio	0.757	0.654	0.847			
Young's Modulus	0.801	0.626	0.928	0.802	0.607	0.943
Diameter	0.827	0.654	0.947	0.216	0.051	0.422
Aspect Ratio	0.531	0.344	0.725	0.597	0.381	0.805
τ_1	0.637	0.422	0.823	0.615	0.370	0.827
τ_2	0.777	0.578	0.931	0.500	0.263	0.743

Table S3. Diagnostic Odds Ratios for Two-Parameter Combinations. For each pair of parameters, the maximum and mean diagnostic odds ratios (DORs) were calculated for classifying limbal epithelial cells (LECs) versus differentiated LECs or central cornea cells. The DOR of a perfect classifier is infinite for any threshold value, whereas the DOR of a random classifier is approximately 1, and a test classifier will have a finite DOR>1. τ_2, slow viscoelastic time constant.

	Maximum DOR		**Mean DOR**	
	LEC vs. Differentiated LEC	**LEC vs. Central Cornea**	**LEC vs. Differentiated LEC**	**LEC vs. Central Cornea**
Diameter + Young's Modulus	65.00	42.78	11.89	1.33
τ_2 + Diameter	73.75	3.55	12.23	0.39
Young's Modulus + τ_2	37.55	47.35	8.67	2.74