

Supplementary data (Ula V. Jurkunas et al.)

Table 1. Antibodies Used for Immunofluorescence Staining (IF)

Antibodies	Source	Dilution
Cytokeratin 12 (L-20)	Santa Cruz (Santa Cruz, CA)	1:500
Cytokeratin 3 (AE5)	Millipore (Billerica, MA)	1:100
Cytokeratin 8 (E432)	Millipore (Billerica, MA)	1:100
Cytokratin 14 (C-14)	Santa Cruz (Santa Cruz, CA)	1:200
Cytokeratin 15 (LHK15)	Santa Cruz (Santa Cruz, CA)	1:250
Pan-cytokeratins (PCK) Recognizes cytokeratins 4, 5, 6, 8, 10, 13 & 18	Millipore (Billerica, MA)	1:200
E-cadherin (36/-E-cadherin)	BD Biosciences	1:2000
Oct4 (Octamer-binding Protein 4)	Abcam (Cambridge, MA)	1:100
TRA-1-60	Abcam (Cambridge, MA)	1:100
SSEA4 (Stage Specific Embryonic Antigen) (for immunofluorescence)	Abcam (Cambridge, MA)	1:100
SSEA4 (for separation and sorting)	R&D Systems Inc., Minneapolis	1:100
N-cadherin	Santa Cruz (Santa Cruz, CA)	1:50
Mouse monoclonal IgG	Millipore (Billerica, MA)	1:50
Vimentin (5G3F10)	Santa Cruz (Santa Cruz, CA)	1:500
Alexa Fluor 488 anti-mouse IgG	Invitrogen (Carlsbad, CA)	1:1000
Alexa Fluor 647 anti-Rabbit IgG	Invitrogen (Carlsbad, CA)	1:1000

Table 2. Assay ID and Probe Sequences Used for Real-time PCR

Gene Name	Assay ID (Taqman Expression Assay)
GAPDH	Hs99999905_m1
p63	Hs00978340_m1
K12	Hs00165015_m1
ABCG2 (ATP-binding cassette sub-family G member 2)	Hs01053790_m1
K8	Hs01670053_m1
DSC1 (Desmoclin-1)	Hs00245189_m1
DSG1 (Desmoglein-1)	Hs00170047_m1
Integrin, beta 1	Hs00559595_m1
Oct4 (Octamer-binding Protein 4)	Hs00999634_gH
Nanog (Transcription Factors)	Hs04260366_g1
Sox2 (POU family binder Transcription Factors)	Hs00415716_m1
Rex1 (Zinc-finger Transcription factor)	Hs00381890_m1
Vim	Hs00185584_m1

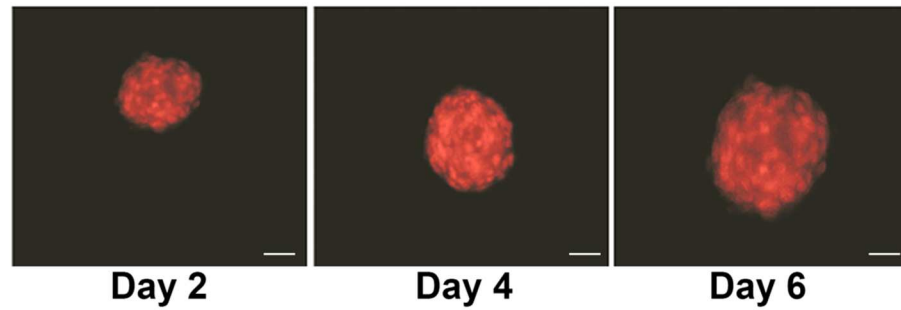
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Table 3: Surface antigen profile of Unsorted LFs, LF SSEA4+, SSEA4-, and Unsorted BM MSCs by FACS Analysis.

Marker	Unsorted LFs	LF SSEA4+	LF SSEA4-	Unsorted BM MSCs	Predominant expression
CD106	59.24 ± 0.25%	58.54 ± 0.55%	60.64 ± 0.45%	59.44 ± 0.85%	Mesenchymal stem cells
CD166	88.24 ± 0.15%	86.24 ± 0.45%	88.74 ± 0.55%	84.34 ± 0.55%	Mesenchymal stem cells
CD71	69.07 ± 6.57%	64.34 ± 0.57%	69.83 ± 0.77%	49.73 ± 0.83%	Mesenchymal stem cells
CD90	95 ± 0.8 %	96 ± 0.2%	96 ± 0.5%	94.7 ± 3.00 %	Mesenchymal stem cells
CD29	85.6 ± 1.2 %	85.2 ± 1.2%	84.6±1.3%	84.0 ± 1.65 %	Mesenchymal stem cells
CD34	0.10 ± 0.05 %	0.9 ± 0.02 %	0.15 ± 0.04%	0.13 ± 0.02 %	Endothelial cells
CD45	Negative	Negative	Negative	0.8 ± 0.2 %	Hematopoietic cells
CD11b	0.51 %	0.5 ± 0.2%	0.51 ± 0.42%	0.35 ± 0.3 %	Microglial cells
CK3, CK12, CK10 and CK15	Negative	Negative	Negative	Negative	Epithelial cells

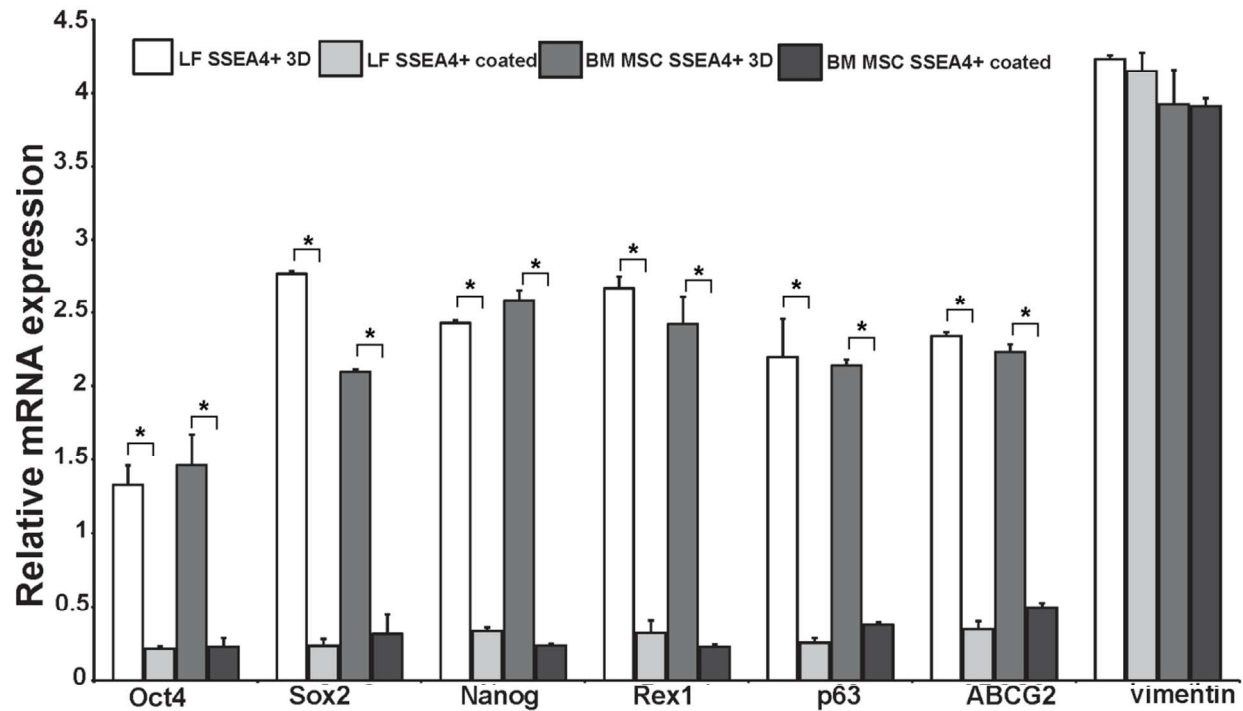
* Results represent percentage of expression of a marker (average values of n=3, ± standard deviation).

Supplementary data (Ula V. Jurkunas et al.)



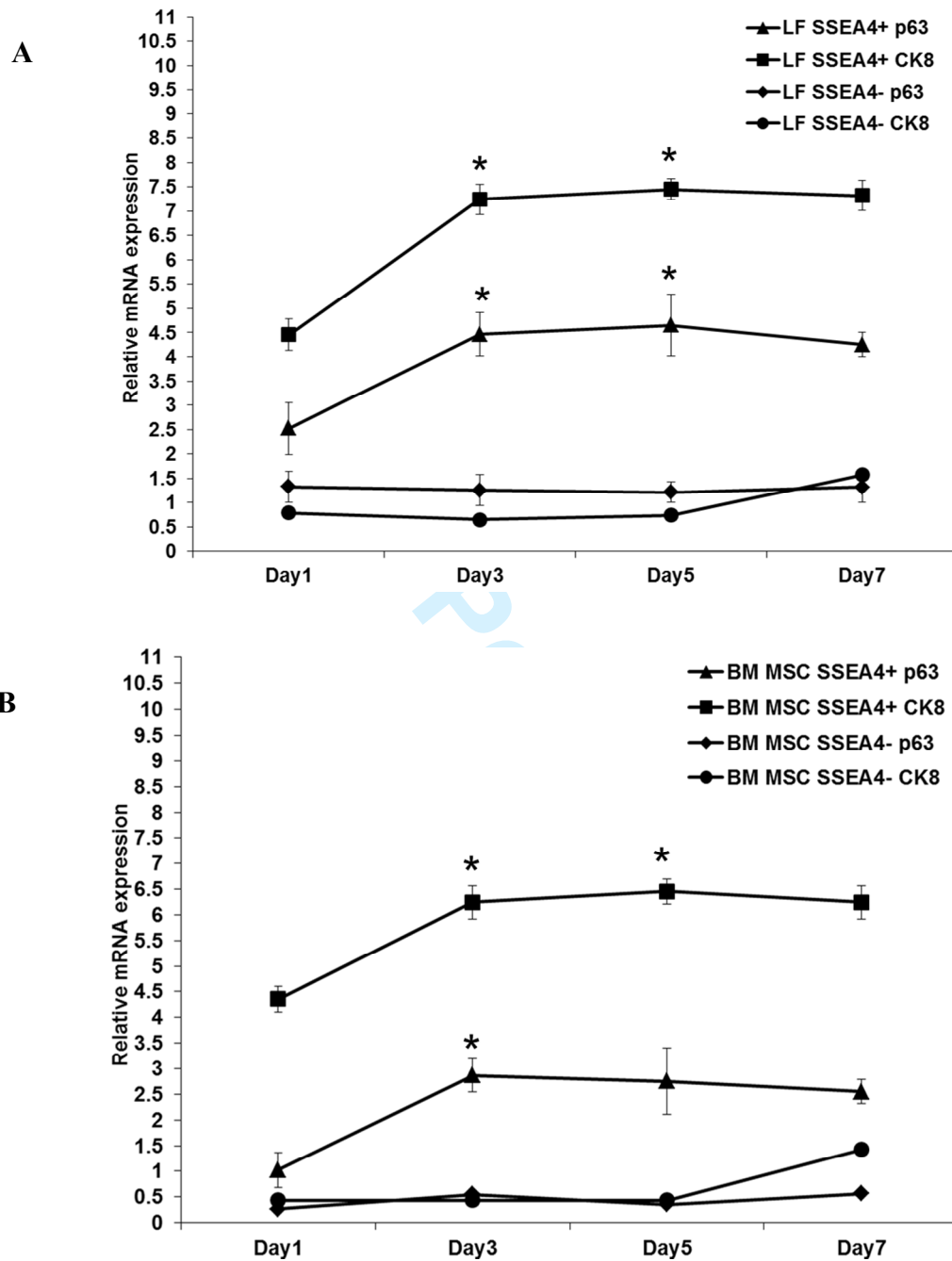
Supplementary Figure S1: LF SSEA4⁺ cell sphere formation assay. Spheres were labeled with BrdU 24 hours before fixation at days 2, 4, and 6 to detect dividing cells (10 x magnification).

Supplementary data (Ula V. Jurkunas et al.)



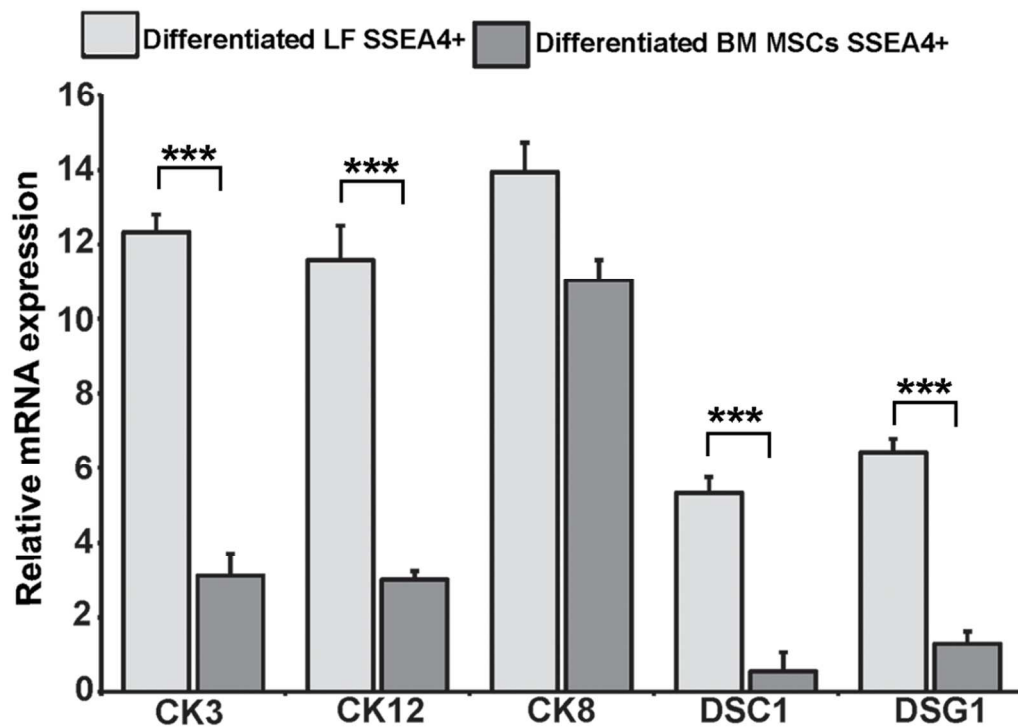
Supplementary Figure S2: Comparison of stem cell marker expression between 3D Matrigel and thin-coated Matrigel culture systems. The data represent the mean \pm SEM from 6 experiments. Statistical analysis was performed using Two tailed Student's *t* test ($*p < 0.05$).

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Supplementary Figure S3: Ectodermal induction of LFs and BM MSCs. **(A):** LFs and **(B):** BM MSCs; mRNA levels of p63 and CK8, determined at 1, 3, 5, and 7 days post induction. The marker levels increased to their highest levels between days 3 and 5 as compared to day 1 of induction. The data represent the mean \pm SEM from 3 experiments. Statistical analysis was performed using Two tailed Student's *t* test ($*p < 0.05$).

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Supplementary Figure S4: Differentiation of LF SSEA4+ and BM MSC SSEA4+ cells into corneal epithelial cells. LF SSEA4+ cells show a higher significant differentiation potential into corneal epithelial cells than do BM MSC SSEA4+ cells. The data represent the mean \pm SEM from 6 experiments. Statistical analysis was performed using Two tailed Student's *t* test. (***) $p < 0.001$.