Supplemental Material:

Supplemental Materials and Methods:

Media conditions for cell culture

KCs were propagated in M154 medium supplemented with human KC growth supplement (Life Technologies/Thermo Fisher Scientific, Waltham, MA), 1,000 x gentamycin/amphotericin B solution (Life Technologies/Thermo Fisher), and 0.07 mM $CaCl_2$ (low calcium). Confluent KC monolayers were induced to differentiate by the addition of 1.2 mM $CaCl_2$ (high calcium) in M154 in the presence of human KC growth supplement and gentamycin/amphotericin B solution. MCs were cultured in OptiMEM (Life Technologies/Thermo Fisher) containing 1% penicillin/streptomycin (Corning, Corning, NY), 5% fetal bovine serum (Millipore Sigma, St. Louis, MO), 10 ng/ml bFGF (ConnStem Inc., Cheshire, CT), 1 ng/ml heparin (Millipore Sigma), 0.1 mM N^6, 2'-O-dibutyryladenosine 3:5-cyclic monophosphate (dbcAMP; Millipore Sigma), and 0.1 mM 3-isobutyl-1-methyl xanthine (IBMX; Millipore Sigma).

Fixation and processing of organotypic cultures for imaging

Organotypic cultures fixed in 10% neutral buffered formalin were embedded in paraffin blocks and cut into 4 μm sections. For indirect immunofluorescence microscopy, slides were baked at 60°C, de-paraffinized using xylene, dehydrated with ethanol, rehydrated in PBS and permeabilized by 0.5% Triton X-100 in PBS. Antigen retrieval was performed by incubation in 0.01 M Citrate buffer (pH 6.0) at 95°C for 15 minutes. Sections were blocked in 1% BSA/2% Normal Goat Serum/PBS for 30 minutes at 37°C. Primary antibody incubation was carried out overnight at 4°C in 1% BSA/2% Normal Goat Serum/PBS followed by washing in PBS. Secondary antibody incubation was carried out at 37°C for 45 minutes followed by washing in PBS. Sections were stained with 4′,6-Diamidino-2-phenylindole (DAPI – Millipore Sigma) at a final concentration of 5 ng/μl at room temperature for 2 minutes followed by washing in PBS. Cover slips were mounted on the sections with ProLong Gold Antifade Reagent (Life Technologies/Thermo Fisher).

For whole mount imaging: Six days after lifting to the air-liquid interface, the epidermal equivalent layer was removed from the collagen plug and fixed in 4% paraformaldehyde in PBS for 15 min on ice. Samples were then washed three times in PBS for 5 min each at room temperature.

Subsequently, samples were incubated in blocking buffer (1% Triton-X 100 with 5% goat serum in PBS) for 1 hr at 37°C followed by incubation overnight at 37°C with S100 (ab52642, anti-S100 beta; Abcam, Cambridge, UK) diluted 1:100 in blocking buffer. Samples were washed 3 times for 10 min each with PBS at room temperature and then incubated overnight at 37°C with Alexa Fluor-

conjugated secondary antibodies (1:250) in blocking buffer that included DAPI (2 μg/ml). Samples were washed 3 times for 10 min each with PBS at room temperature and mounted onto glass slides with Prolong Gold Antifade Reagent.

Quantitative real-time PCR

RNA was isolated from KCs and MCs using the RNeasy Mini Kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. MCs were grown in monoculture prior to being incubated for 7 days with either MC media alone or with a 1:1 mixture of MC media and conditioned media. Total RNA concentrations were equalized between samples and cDNA was prepared using the Superscript III First Strand Synthesis Kit (Life Technologies/Thermo Fisher). Quantitative PCR was performed using SYBR Green PCR master mix (Life Technologies/Thermo Fisher) and gene-specific primers (Supplemental Table 1) in a StepOnePlus instrument (Thermo Fisher Scientific Applied Biosystems). Calculations for relative mRNA levels were performed using the ΔΔCt method, normalized to GAPDH.

MTT assay and cell counting

MCs were plated at 5000 cells/well in 96 well plates and the next day conditioned media diluted 1:1 in MC media was added. The MTT assay protocol (Abcam), which was followed according to the manufacturer's instructions, is based on the conversion of water soluble MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) compound to an insoluble formazan product by viable cells. MC viability was assayed 1 day and 7 days after initiating incubation in conditioned media diluted 1:1 in MC media. On days 1 and 7, MCs were incubated for 3 hours with MTT reagent and 15 minutes with MTT solvent and absorbance at 590nm was measured with an ELx800 microplate reader (Bio-Tek Instruments, Inc). Cells were also counted on days 0, 1, and 3 after initiating incubation in conditioned media using a hemocytometer.

Immunoblot analysis of proteins

Whole cell lysates were collected from confluent monolayers of KCs or 80% confluent MCs in urea-SDS buffer (8 M urea/1% SDS/60 mM Tris (pH 6.8)/5% ß-mercaptoethanol/10% glycerol) and sonicated. Samples separated by SDS-PAGE were transferred to nitrocellulose, blocked in 5% milk/PBS, and incubated with primary antibodies in milk/PBS for 1 hour at room temperature or overnight at 4°C. After PBS washes, secondary antibodies diluted in milk/PBS were added to blots. Protein bands were visualized using exposure to X-ray film. Densitometric analyses were performed of scanned immunoblots using ImageJ software.

Antibodies

Mouse monoclonal antibodies: P124 (anti-Dsg1 extracellular domain; Progen, Heidelberg, Germany) and 27B2 (anti-Dsg1 cytodomain; Life Technologies/Thermo Fisher). Rabbit monoclonal antibody EP1576Y (ab52642, anti-S100 beta; Abcam, Cambridge, UK). Rabbit polyclonal antibodies: Flag (Cell Signaling Technologies), TYRP1 (ab83774; Abcam), and GAPDH (G9545, glyceraldehyde-3-phosphate dehydrogenase; Millipore Sigma). Secondary antibodies for immunoblotting were goat anti-mouse and goat anti-rabbit peroxidase (SeraCare Life Sciences, Milford, MA). Secondary antibodies for immunofluorescence were goat anti-mouse and goat-anti-rabbit linked to fluorophores of 488 and 568 nm (Alexa Fluor; Life Technologies/Thermo Fisher).

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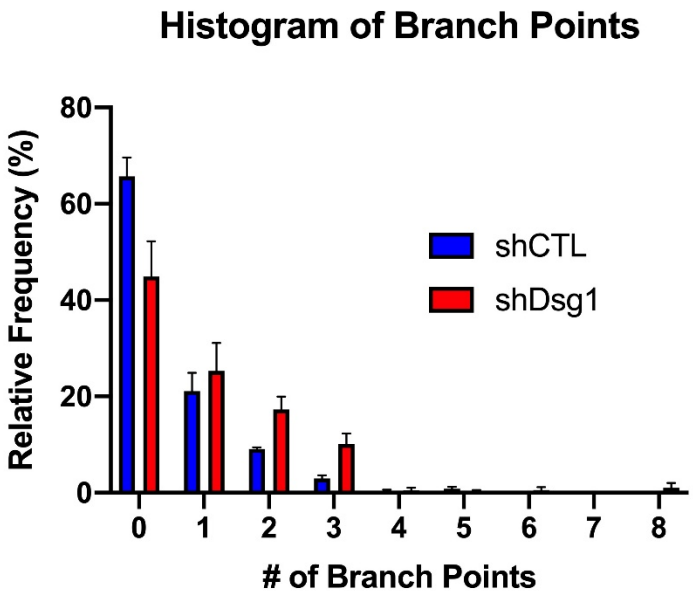
78 **Supplemental Table 1. Primers used in this study.**

Target	Primers
Dsg1	F: 5'-TCCATAGTTGATCGAGAGGTCAC-3' R: 5'-CTGCGTCAGTAGCATTGAGTATC-3'
Dsg3	F: 5'-ATCAATGCAACAGATGCAGATGA-3' R: 5'-TGTCAAAGTGTAGCTGCTGTGT-3'
IL1α	F: 5'-AGTAGCAACCAACGGGAAGG-3' R: 5'-TGGTTGGTCTTCATCTTGGG-3'
IL1β	F: 5'-GCAAGGGCTTCAGGCAGGCCGCG-3' R: 5'-GGTCATTCTCCTGGAAGGTCTGTG-3'
IL2	F: 5'-GTCACAAACAGTGCACCTAC-3' R: 5'-CCCTGGGTCTTAAGTGAAAG-3'
IL4	F: 5'-ACTTTGAACAGCCTCACAGAG-3' R: 5'-TTGGAGGCAGCAAAGATGTC-3'
IL6	F: 5'- ACAGCCACTCACCTCTTCAG -3' R: 5'- CCATCTTTTTCAGCCATCTTT -3'
IL8	F: 5'- ATGACTTCCAAGCTGGCCGT -3' R: 5'- TCCTTGGCAAAACTGCACCT -3'
IL10	F: 5'- GGTTGCCAAGCCTTGTCTGA -3' R: 5'- AGGGAGTTCACATGCGCCT -3'
IL19	F: 5'- GAGCCATCCAAGCTAAGGACA -3' R: 5'- CTTGGTCACGCAGCACACAT -3'
IL23	F: 5'- GCTTCAAAATCCTTCGCAG -3' R: 5'- TATCTGAGTGCCATCCTTGAG -3'
CXCL1	F: 5'- AACCGAAGTCATAGCCACAC -3' R: 5'- GTTGGATTTGTCACTGTTCAGC -3'
TNFα	F: 5'- ATGAGCACTGAAAGCATGATCC -3' R: 5'- GAGGGCTGATTAGAGAGAGGTC -3'
IFNγ	F: 5'- CTAATTATTCGGTAACTGACTTGA -3' R: 5'- ACAGTTCAGCCATCACTTGGA -3'
POMC	F: 5'- AGGCACTTGCTGGATTCTCC -3'

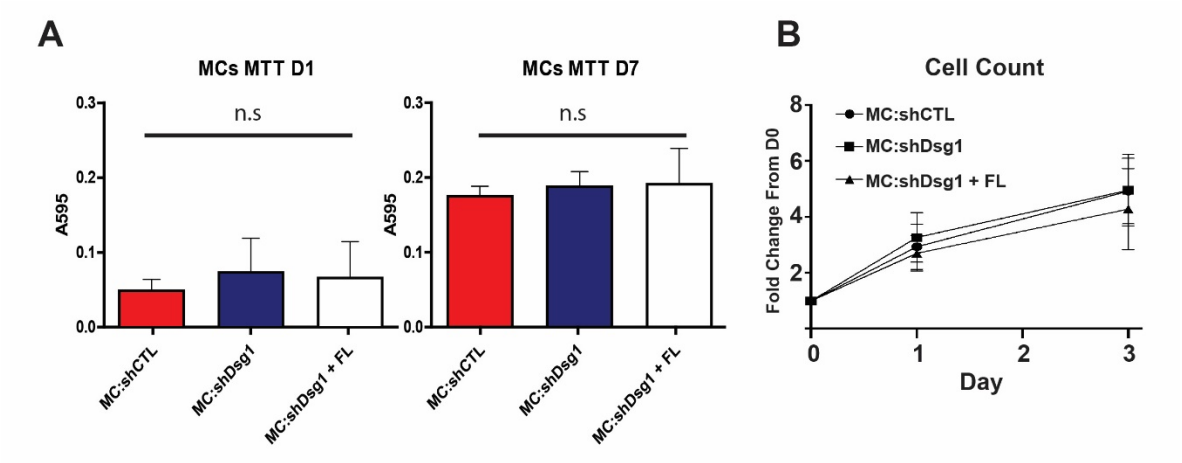
	R: 5’- GCCCTTCTTGTAGGCGTTCT -3’
KITL	F: 5’- TCGATGACCTTGTGGAGTGC -3’ R: 5’- TGCTGTCATTCCTAAGGGAGC -3’
END-1	F: 5’- GACATCATTTGGGTCAACACTC -3’ R: 5’- GGCATCTATTTTCACGGTCTGT-3’
MITF	F: 5’- TTATAGTACCTTCTCTTTGCCAGTCC -3’ R: 5’- CTTATAAAATCCCTCTTTTTCACAGTTGGA -3’
MC1R	F: 5’- ACTTCTCACCAGCAGTCGTG -3’ R: 5’- CATTGGAGCAGACGGAGTGT -3’
TYRP1	F: 5’- GTGCCACTGTTGAGGCTTTG -3’ R: 5’- ATGGGGATACTGAGGGCTGT -3’
GAPDH	F: 5’- ACCACAGTCCATGCCATCAC -3’ R: 5’- TCCACCACCCTGTTGCTGTA -3’

Dsg1, Desmoglein 1; Dsg3, Desmoglein 3; IL, Interleukin, CXCL1, Chemokine ligand 1; TNF, Tumor Necrosis Factor; IFN, Interferon; POMC, Pro-opiomelanocortin; MITF, Melanogenesis associated transcription factor; MC1R, Melanocortin 1 receptor; TYRP1, Tyrosinase related protein 1; KITL, Kit ligand; END-1, Endothelin-1; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase.

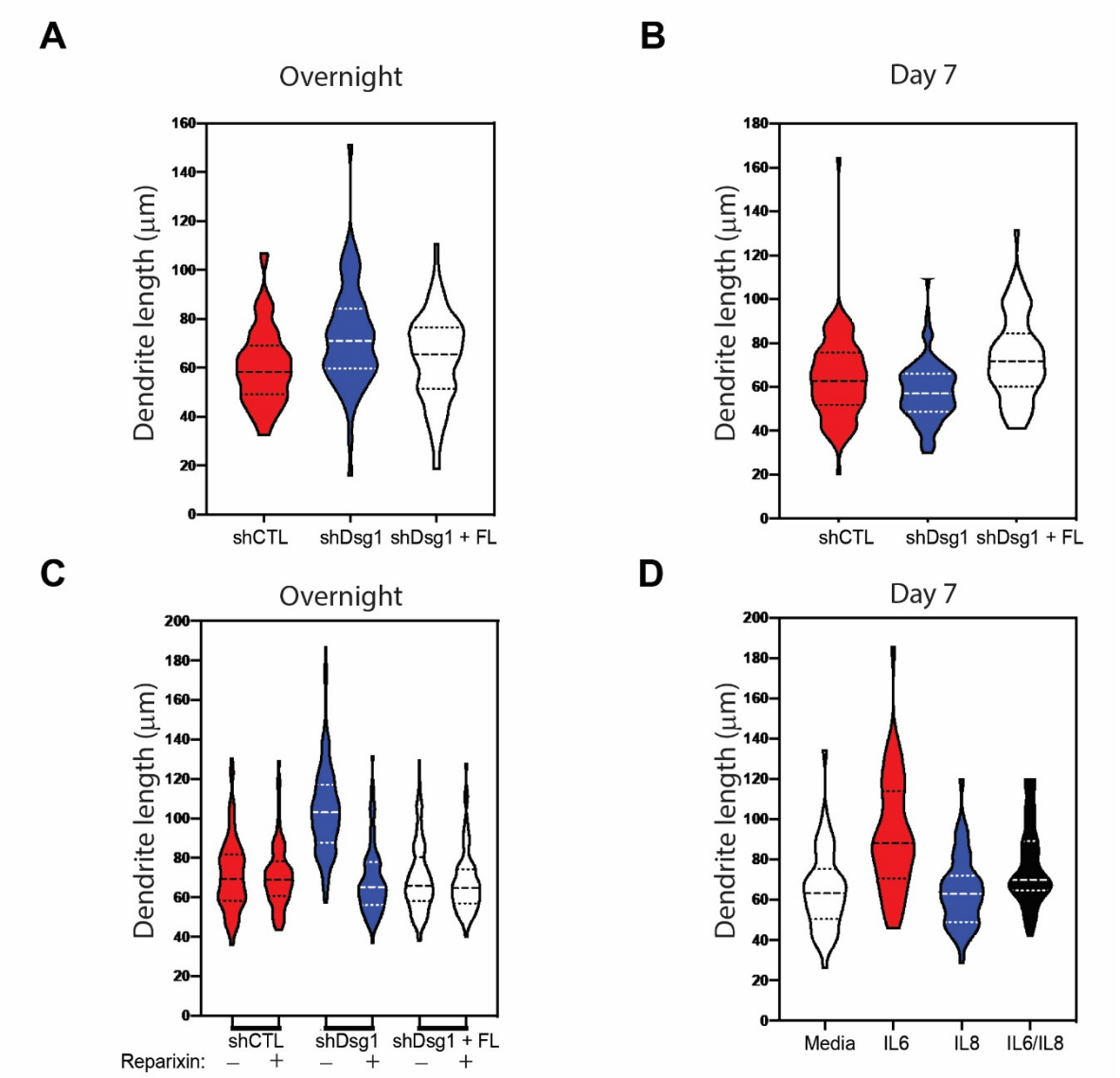
Supplemental Figure Legends:



Supplemental Figure 1. ***MCs in Dsg1-deficient organotypic cultures exhibit increased dendrite branching compared to controls.*** Histogram of the frequency of occurrence of 0, 1, 2, 3, 4, 5, 6, 7, and 8 branch points per cell. The average number of branch points per cell are presented graphically in Figure 1F. There were fewer occurrences of 0 MC dendrite branch points and more occurrences of 2 and 3 branch points in the Dsg1-depleted organotypic cultures compared to the controls. A total of 239 MCs were analyzed for shCTL samples and 204 MCs for shDsg1 samples across all images (around 50 cells per organotypic culture). Data are presented as mean (N=4 independent experiments) and SEM.



Supplemental Figure 2. ***MCs are viable and have similar growth curves when grown in conditioned media from control, Dsg1-deficient, and Dsg1-deficient rescued with full length Dsg1 KCs.*** **(A)** The MTT assay was conducted on MCs 1 day or 7 days after incubation in 1:1 mixture of MC media: KC conditioned media (MC:shCTL, MC:shDsg1, MCLshDsg1 + FL). No significant changes were observed in cell viability at either time point regardless of type of conditioned media (One Way ANOVA, repeated measures, Tukey's post-hoc test, N=3 MC isolates, 1 KC conditioned media, bar graph represents mean and SEM). **(B)** MCs (3 MC isolates) were counted using a hemocytometer on days 1, 2, and 3 after incubation in 1:1 MC:KC conditioned media. Fold changes from day 0 were calculated and represented as the mean and SEM of the 3 MC isolates treated with 1 set of KC conditioned media (MC:shCTL, MC:shDsg1, MC:shDsg1 + FL).



Supplemental Figure 3. ***Dsg1-deficient KCs change MC dendricity, partially dependent upon cytokine/chemokine signaling.*** **A)** Violin plots of the spread of dendrite lengths of all cells after overnight incubation in media conditioned by KCs infected with shCTL, shDsg1, or shDsg1 + FL-expressing viruses. Incubation with media from KCs depleted of Dsg1 resulted in dendrite lengthening compared to shCTL, as shown in Figure 4B. N = 5 independent MC:KC pairs. **B)** Violin plots as in A except after 7 days of incubation in media conditioned by KCs expressing shCTL, shDsg1, or shDsg1 + FL. Incubation with media from KCs depleted of Dsg1 resulted in dendrite shortening compared to the other two treatment groups, as shown in Figure 4C. N = 5 independent MC:KC pairs. **C)** Violin plots of dendrite lengths after overnight incubation in media conditioned by

KCs expressing shCTL or shDsg1 with and without treatment with the CXCL1/IL8 receptor inhibitor, reparixin. While media from Dsg1-depleted KCs resulted in lengthened MC dendrites as in A, treatment with reparixin blocked the effect, as shown in Figure 4D. N = 3 independent experiments. **D)** Violin plots of dendrite lengths from MCs treated for 7 days with media spiked with recombinant IL6, IL8, or the combination of IL6/IL8. Exposure to IL6 resulted in increased dendrite length as shown in Figure 4E. N = 3 independent experiments. Dotted lines inside violin plots represent means and SEM of all data points.