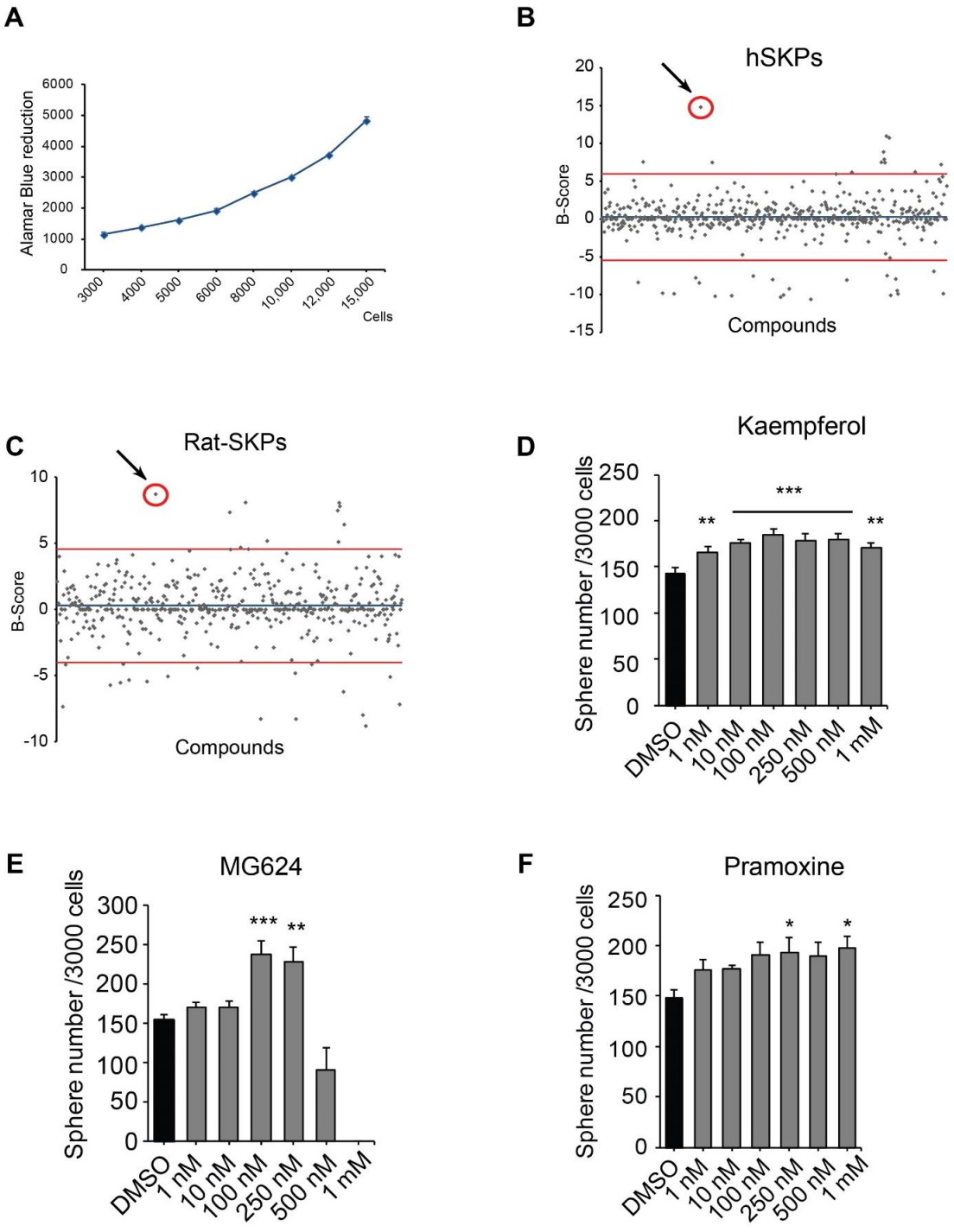
Stem Cell Reports, Volume *6*

Supplemental Information

Identification of Drugs that Regulate Dermal Stem Cells and Enhance Skin Repair

Sibel Naska, Scott A. Yuzwa, Adam P.W. Johnston, Smitha Paul, Kristen M. Smith, Maryline Paris, Michael V. Sefton, Alessandro Datti, Freda D. Miller, and David R. Kaplan

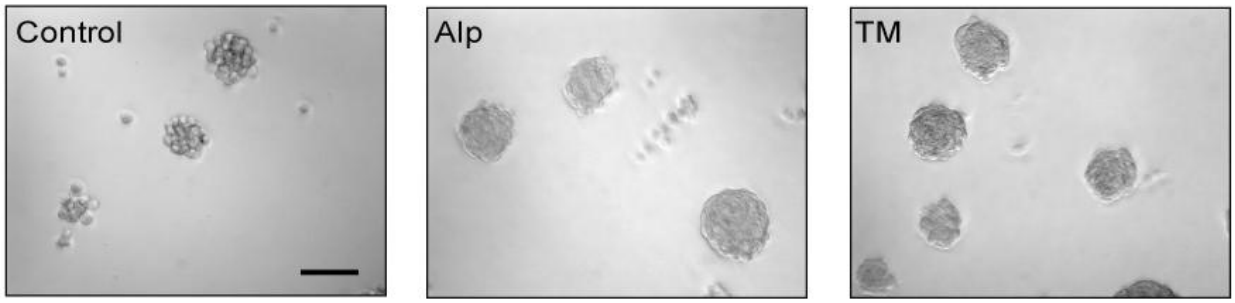
Supplemental Information



Supplemental Figure S1: ***Identification of compounds that enhance self-renewal and proliferation of cultured SKPs, Related to Figure 1.*** (A) Dissociated secondary neonatal rat

SKPs were robotically plated at 3,000 to 15,000 cells/well in 96 well plates, alamarBlue® was added after 30 hours and the plates were analyzed after an additional 24 hours. The graph shows the fluorescence signal caused by reduction of alamarBlue® (with the medium value subtracted) in wells with increasing numbers of cells. (B,C) Representative B-Score plots from the NIH collection library screened against human (B) or rat (C) SKPs. The red lines indicate three standard deviations from the mean. Compounds with B-scores greater than three standard deviations were considered primary hits. Arrows indicate the hit compound TM that was identified in both human (B) and rat (C) SKP screens. (D-F) Number of SKP spheres generated from secondary human SKPs grown for 7 days in varying concentrations of kaempferol (D), MG-624 (E) or pramoxine (F). Controls were treated with DMSO alone. Results are pooled from at least 3 independent experiments with 3 different human SKP lines ($n \geq 3$ wells each). Error bars indicate SEM, and in all cases *$p<0.05$, **$p<0.01$, ***$p<0.001$, one-way ANOVA with multiple comparison post-hoc tests.

A



B

Overlapping Genes:

uck2; slc44a1; 9430024e24rik; trim34a; gm20257; krtap9-1; cox1; tuba1b; gm10517; vstm2l; oxct1; gm10684; 2310079g19rik; 4930590j08rik; mdh1b; tmed11; olfr898; htatsf1; foxj1; gm8579; paqr9

Supplemental Figure S2: ***In vivo aprostadil and TM treatment enhance SKP sphere formation and induce overlapping differentially expressed genes, Related to Figure 3.*** (A) Representative images of cultured secondary SKP spheres generated from injured skin treated with alprostadil (Alp), TM or vehicle (Control) for 7 days. Scale bar = 100 μm. (B) mRNA from secondary SKP spheres generated as in (A) were analyzed on Affymetrix GeneChip Mouse Gene 2.0 ST arrays. The list indicates overlapping genes that differ by $p < 0.01$ (unadjusted) in pairwise comparisons of vehicle (Control) versus TM or alprostadil-treated injured skin.

Supplemental Table S1: ***Differentially expressed genes in secondary SKP spheres generated from injured skin treated with vehicle versus alprostadil, Related to Figure 3.*** Secondary SKP spheres were generated from the skin of 9 month old mice that had received punch wounds and were treated topically with vehicle or alprostadil for 7 days. mRNA from these SKP spheres was analyzed on Affymetrix GeneChip Mouse Gene 2.0 ST arrays, and differentially expressed genes were determined using the limma bioconductor package at the probeset level to generate $\log_2$ fold-change (shown here as inverse $\log_2$ fold-change) and moderated t-statistics and comparing vehicle versus alprostadil. Differentially expressed genes were annotated using the mogene20sttranscriptcluster.db bioconductor annotation package to annotate the gene symbol and description shown here. Differentially expressed genes with known annotations and p-values < 0.01 were included.

Supplemental Table S2*: Differentially expressed genes in secondary SKP spheres generated from injured skin treated with vehicle versus TM, Related to Figure 3.* Secondary SKP spheres were generated from the skin of 9 month old mice that had received punch wounds and were treated topically with vehicle or TM for 7 days. mRNA from these SKP spheres was analyzed on Affymetrix GeneChip Mouse Gene 2.0 ST arrays, and differentially expressed genes were determined using the limma bioconductor package at the probeset level to generate $\log_2$ fold-change (shown here as inverse $\log_2$ fold-change) and moderated t-statistics and comparing vehicle versus TM. Differentially expressed genes were annotated using the mogene20sttranscriptcluster.db bioconductor annotation package to annotate the gene symbol and description shown here. Differentially expressed genes with known annotations and p-values < 0.01 were included.

Supplemental Table S3*: Differentially expressed genes in neonatal rat SKPs treated for 24 hours with alprostadil, Related to Figure 3:* Three independent preparations of primary neonatal rat SKPs were dissociated and cultured for 24 hours in alprostadil or vehicle, and mRNA was isolated and analyzed on Affymetrix GeneChip Rat Gene 2.0 ST arrays. Differentially expressed genes were determined for the pairwise comparisons between vehicle and alprostadil-treated SKPs, computed using the limma bioconductor package at the probeset level to generate $\log_2$ fold-change (shown here as inverse $\log_2$ fold-change) and moderated t-statistics. Differentially expressed genes were annotated using the ragene20sttranscriptcluster.db bioconductor annotation package to annotate the gene symbol and description shown here. Differentially expressed genes with known annotations and p-values < 0.01 are included.

Supplemental Table S4*: Differentially expressed genes in neonatal rat SKPs treated for 24 hours with TM, Related to Figure 3:* Three independent preparations of primary neonatal rat SKPs were dissociated and cultured for 24 hours in TM, or vehicle, and mRNA was isolated and analyzed on Affymetrix GeneChip Rat Gene 2.0 ST arrays. Differentially expressed genes were determined for the pairwise comparisons between vehicle and TM treated SKPs, computed using the limma bioconductor package at the probeset level to generate $\log_2$ fold-change (shown here as inverse $\log_2$ fold-change) and moderated t-statistics. Differentially expressed genes were annotated using the ragene20sttranscriptcluster.db bioconductor annotation package to annotate the gene symbol and description shown here. Differentially expressed genes with known annotations and p-values < 0.01 are included.

Supplemental Table S5*: Overlapping differentially expressed genes (p < 0.01) comparing neonatal rat SKPs treated for 24 hours with alprostadil or TM, Related to Figure 3.*

Gene symbols: *loc100910839, lyz2, ccl6, klra17, siglec5, myo1f, lcp1, scimp, rgs1, ptprc, smpdl3a, loc24906, loc681325, ptk2b, prg4, rgd1561143, rspo3, bmp3, rgd1561730, cidec, adipoq, gpd1, csf1r, gldn, cpa3, cd37, car3, cfh, ccl9, bcl2a1, fcgr2a, spi1, parvg, lcp2, fam105a, loc681182, igsf6, slc25a43, tbxas1, ncf1, cd53, clec4a3, mmp11, lipg, gpr116, itgb2, sirpa, cttnbp2, kcnj2, lrrc15, sema4a, tnfsf13, cd74, lilrb4, selplg, trpv2, ptpn6, tmem176a, rbp4, fgr, bmp7, acsl1, lyn, itgal, tfpi2, alox5, cfp, sla, nfam1, ucp2, npy1r, sort1, il18, dock8, was, aqp7, fbn2, prelp, emr4, gmfg, tfec, fabp4, nnat, cd180, fcer1g, rgs18, des, gpr65, pla2g7, retn, nckap1l, kcnn4, fcgr1a, siglec10, s100b, tmem2, myo1g, ass1, sfrp1, tusc5, lipa, siglec8, tlr7, prss23, rpp25, dock10, inhba, syk, slc6a6, hpse2, ar, clec4a1, chrna5, cidea, frmpd4, plekha6, gpnmb, c3, ccl22, qprt, hist1h3a, sh2d1b, sertm1, bmp5, lrfn5, prdm6, cdc6, serpina3n, tacr3, akap6, col14a1, tyrobp, scd1, serpinb1a, gpr133, pck1, nefl, ccl3, atp1b1, ces1a, tll1, lrrn3, arhgdib, ptprn, dgat2, slc6a12, fcgr3a, gabarapl1, aspn, vav1, tmem176b, dpep1, ifi30, creg1, loc688459, stxbp2, itga8, c1qb, naaa, scn7a, ceacam1, gm2a, myo1d, epha4, ptpro, sult1a1, megf10, gpx3, rbm47, epha7, ptgs2, tnfrsf26, cfd, aspa, kcnn3, tmem100, prl3d4, sdc3, itgam, ddah1, clec5a, clec4e, pla2g2d, npr3, has1, msr1, epha3, cpxm1, lcn2, elmo1, mfap5, cyp4b1, sox4, pik3r5, fermt3, mgst1, march1, nos1, mir323, dapp1, gas6, clec4d, gpc3, b3galt1, slitrk5, mir223, cst6, fam111a, gramd1b, ptn, mir493, prdx5, chaf1b, il1rn, mctp2, rtp3, tnfaip8l2, sox2, unc93b1, bin2, akap5, abi3bp, cytip, dhrs3, n4bp2, coro1a, mirlet7c-1, abhd2, il1b, il33, cmklr1, treml4, nlrc4, drp2, pdia5, abcd2, apobec1, capn6, ptger3, rgd1566085, aoah, abcg1, mcemp1, cdh13, ebpl, tf, myo1e, crabp1, plbd1, foxp1, ms4a7, jam2, atp8b1, mdga2, h19, crip2, rgd1305089, dgkb, tmem35, lonrf2, cd302, fst, b3gnt7, sp100, zdhhc14, shc4, nqo2, parm1, hpgds, cyp26b1, olr1051, napsa, rac2, plin1, il20rb, diras2, krt31, slc7a1, fli1, dtl, sez6l, fxyd1, ms4a4a, slco2a1, steap1, inpp5d, ablim3, vldlr, cdo1, cdc42ep3, lrrc25, cyp4f39, rgd1309362, shc3*

Overlapping differentially expressed genes from the pairwise comparisons of neonatal rat SKPs treated with vehicle versus alprostadil or TM, as shown in Suppl. Tables S3 and S4, and as computed using the limma bioconductor package at the probeset level to generate $\log_2$ fold-change (shown here as inverse $\log_2$ fold-change) and moderated t-statistics. Differentially expressed genes were annotated using the ragene20sttranscriptcluster.db bioconductor annotation package.

Supplemental Table S6*: Top 50 differentially expressed genes in neonatal rat SKPs treated for 24 hours with alprostadil, Related to Figure 3.*

Gene Symbol	Description	Fold-Change	P-value (moderated t-statistic)
LOC100910839	leucine zipper protein 2-like	1.001921	3.18E-05
Lyz2	lysozyme 2	1.041509	5.20E-05
Il6	interleukin 6	6.07487	6.07E-05
Ccl6	chemokine (C-C motif) ligand 6	1.794098	6.35E-05
Klra17	killer cell lectin-like receptor, subfamily A, member 17	0.961245	6.87E-05
Siglec5	sialic acid binding Ig-like lectin 5	1.08744	8.78E-05
Myo1f	myosin IF	0.71582	8.79E-05
Lcp1	lymphocyte cytosolic protein 1	0.835355	8.99E-05
Scimp	SLP adaptor and CSK interacting membrane protein	1.306032	0.000104
Rgs1	regulator of G-protein signaling 1	1.169478	0.000113
Ptprc	protein tyrosine phosphatase, receptor type, C	0.93395	0.000132
Cxcl13	chemokine (C-X-C motif) ligand 13	4.189169	0.000139
Smpdl3a	sphingomyelin phosphodiesterase, acid-like 3A	1.173385	0.000144
LOC24906	RoBo-1	1.835212	0.000145
LOC681325	hypothetical protein LOC681325	1.154336	0.000156
Ptk2b	protein tyrosine kinase 2 beta	1.013942	0.000173
Prg4	proteoglycan 4	1.167444	0.000178
Ccl12	chemokine (C-C motif) ligand 12	0.326009	0.000181
RGD1561143	similar to cell surface receptor FDFACT	1.009887	0.000185
Rspo3	R-spondin 3	1.649091	0.000185
Bmp3	bone morphogenetic protein 3	0.927272	0.000198
RGD1561730	similar to cell surface receptor FDFACT	1.184263	0.000207
Cidec	cell death-inducing DFFA-like effector c	0.826168	0.000221
Adipoq	adiponectin, C1Q and collagen domain containing	1.014716	0.000228
Gpd1	glycerol-3-phosphate dehydrogenase 1 (soluble)	0.908651	0.000256
Csf1r	colony stimulating factor 1 receptor	1.088615	0.000259
Gldn	gliomedin	0.896379	0.000262
Cpa3	carboxypeptidase A3, mast cell	0.925526	0.000265
Cd37	CD37 molecule	1.019646	0.00027
Car3	carbonic anhydrase 3	0.924688	0.00027
Cfh	complement factor H	0.713577	0.00029
Phlda1	pleckstrin homology-like domain, family A, member 1	2.12465	0.000292
Ccl9	chemokine (C-C motif) ligand 9	0.907055	0.000309
Bcl2a1	BCL2-related protein A1	0.965589	0.000313
Fcgr2a	Fc fragment of IgG, low affinity IIa, receptor	1.194083	0.000323
Spi1	Spi-1 proto-oncogene	0.952852	0.000349
Parvg	parvin, gamma	0.997287	0.000382
Lcp2	lymphocyte cytosolic protein 2	0.955288	0.000406
Fam105a	family with sequence similarity 105, member A	1.002798	0.000411
LOC681182	similar to paired immunoglobin-like type 2 receptor beta	1.129033	0.000411
Igsf6	immunoglobulin superfamily, member 6	1.34594	0.000411
Slc25a43	solute carrier family 25, member 43	0.954538	0.000421
Tbxas1	thromboxane A synthase 1, platelet	1.070738	0.000431
Ncf1	neutrophil cytosolic factor 1	0.903086	0.000433
Cd53	Cd53 molecule	1.342827	0.000435
Hs3st1	heparan sulfate (glucosamine) 3-O-sulfotransferase 1	1.997368	0.000456
Clec4a3	C-type lectin domain family 4, member A3	0.596755	0.000458
Mmp11	matrix metallopeptidase 11	0.855951	0.00046
Slpi	secretory leukocyte peptidase inhibitor	1.494137	0.000472
Lipg	lipase, endothelial	0.972027	0.000486

Three independent preparations of primary neonatal rat SKPs were dissociated and cultured for 24 hours in alprostadil, or vehicle, and mRNA was isolated and analyzed on Affymetrix GeneChip Rat Gene 2.0 ST array. Shown here are the top 50 differentially expressed genes

(highest significance and known annotations) for the pairwise comparisons between vehicle and alprostadil treated SKPs, as computed using the limma bioconductor package at the probeset level to generate $\log_2$ fold-change (shown here as inverse $\log_2$ fold-change) and moderated t-statistics. Differentially expressed genes were annotated using the ragene20sttranscriptcluster.db bioconductor annotation package to annotate the gene symbol and description shown here.

Supplemental Table S7*: Top 50 differentially expressed genes in neonatal rat SKPs treated for 24 hours with TM, Related to Figure 3.*

Gene Symbol	Description	Fold-Change	P-value (moderated t-statistic)
Scimp	SLP adaptor and CSK interacting membrane protein	1.233397	4.00E-05
Ptprc	protein tyrosine phosphatase, receptor type, C	1.145283	4.63E-05
Siglec5	sialic acid binding Ig-like lectin 5	1.15008	5.18E-05
Fabp4	fatty acid binding protein 4, adipocyte	0.964711	8.74E-05
Clec4a3	C-type lectin domain family 4, member A3	0.916971	0.000114
Cidec	cell death-inducing DFFA-like effector c	1.024594	0.000122
Fam105a	family with sequence similarity 105, member A	1.071321	0.000126
Cpa3	carboxypeptidase A3, mast cell	0.779702	0.000173
LOC100910839	leucine zipper protein 2-like	1.109524	0.000179
Adipoq	adiponectin, C1Q and collagen domain containing	0.962054	0.000181
LOC681182	similar to paired immunoglobin-like type 2 receptor beta	1.049692	0.000206
Gpr133	G protein-coupled receptor 133	1.043488	0.000242
Acsl1	acyl-CoA synthetase long-chain family member 1	1.006802	0.000253
Selplg	selectin P ligand	1.089982	0.000254
Fcgr3a	Fc fragment of IgG, low affinity IIIa, receptor	1.053318	0.000266
Il1rn	interleukin 1 receptor antagonist	1.123454	0.00031
Naaa	N-acylethanolamine acid amidase	1.068007	0.000316
Bcl2a1	BCL2-related protein A1	0.943153	0.000333
Sema4a	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A	1.068099	0.000356
Smpdl3a	sphingomyelin phosphodiesterase, acid-like 3A	1.146783	0.000357
Ccl6	chemokine (C-C motif) ligand 6	1.187186	0.000366
Lrfn5	leucine rich repeat and fibronectin type III domain containing 5	0.991541	0.000387
Tbxas1	thromboxane A synthase 1, platelet	1.13564	0.000392
Bmp3	bone morphogenetic protein 3	1.02108	0.000413
Csf1r	colony stimulating factor 1 receptor	0.987028	0.000415
Hist1h3a	histone cluster 1, H3a	0.680929	0.000421
Il18	interleukin 18	0.865269	0.000426
Npy1r	neuropeptide Y receptor Y1	0.914961	0.000448
Fcgr1a	Fc fragment of IgG, high affinity Ia, receptor (CD64)	1.0427	0.000459
Cxcr1	chemokine (C-X-C motif) receptor 1	0.938585	0.000461
Abcd2	ATP-binding cassette, subfamily D (ALD), member 2	1.020682	0.000487
Gpd1	glycerol-3-phosphate dehydrogenase 1 (soluble)	0.914691	0.00049
Aoah	acyloxyacyl hydrolase (neutrophil)	1.077756	0.000492
Sp100	SP100 nuclear antigen	0.919426	0.000529
Rspo3	R-spondin 3	1.104077	0.000537
LOC24906	RoBo-1	0.953287	0.000543
Lyz2	lysozyme 2	1.075892	0.000555
Gpc3	glypican 3	0.941928	0.000557
Spi1	Spi-1 proto-oncogene	1.03597	0.000566
Olr1726	olfactory receptor 1726	0.64766	0.000581
Ccl3	chemokine (C-C motif) ligand 3	0.906386	0.000581
Mmp11	matrix metallopeptidase 11	1.015164	0.00059
Prg4	proteoglycan 4	0.97482	0.000591
Gpr116	G protein-coupled receptor 116	1.004721	0.000619
Pla2g7	phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	1.000346	0.000627
Ptpn6	protein tyrosine phosphatase, non-receptor type 6	0.995589	0.000648
Mir145	microRNA 145	0.956693	0.000659
Fbn2	fibrillin 2	0.984266	0.000719
Mirlet7c-1	microRNA let-7c-1	0.846102	0.000731
Olr819	olfactory receptor 819	1.208596	0.000733

Three independent preparations of primary neonatal rat SKPs were dissociated and cultured for 24 hours in TM or vehicle, and mRNA was isolated and analyzed on Affymetrix GeneChip Rat

Gene 2.0 ST array. Shown here are the top 50 differentially expressed genes (highest significance and known annotations) for the pairwise comparisons between vehicle and TM-treated SKPs, as computed using the limma bioconductor package at the probeset level to generate $\log_2$ fold-change (shown here as inverse $\log_2$ fold-change) and moderated t-statistics. Differentially expressed genes were annotated using the ragene20sttranscriptcluster.db bioconductor annotation package to annotate the gene symbol and description shown here.

Supplemental Table S8*: Differentially expressed genes associated with GO terms for proliferation from neonatal rat SKPs treated for 24 hours with TM or alprostadil, Related to Figure 3.* Three independent preparations of primary neonatal rat SKPs were dissociated and cultured for 24 hours in alprostadil (Alp), TM or vehicle (Ctrl), and mRNA was isolated and analyzed on Affymetrix GeneChip Rat Gene 2.0 ST array. Gene-ontology (GO) enrichment analysis identified genes that were significantly different in the comparisons of vehicle versus TM or alprostadil-treated SKPs and that were associated with GO categories for cell proliferation (as shown in Fig. 3J). These genes are listed here. Some listed genes are associated with more than one category.

Supplemental Table S9: *Differentially expressed genes associated with GO terms for MAPK/ERK from neonatal rat SKPs treated for 24 hours with TM or alprostadil, Related to Figure 3.* Three independent preparations of primary neonatal rat SKPs were dissociated and cultured for 24 hours in alprostadil (Alp), TM or vehicle (Ctrl), and mRNA was isolated and analyzed on Affymetrix GeneChip Rat Gene 2.0 ST array. Gene-ontology (GO) enrichment analysis identified genes that were significantly different in the comparisons of vehicle versus TM or alprostadil-treated SKPs and that were associated with GO categories for MAPK/ERK (as shown in Fig. 3K). These genes are listed here. Some listed genes are associated with more than one category.

Supplemental Experimental Procedures

Preparation of SKP cultures. SKPs were isolated from the back skin of newborn Sprague-Dawley rat pups or from the back skin of 9 month old CD57/Bl6 mice. Skin was dissected and cut into 1-2 mm pieces and incubated in collagenase type XI (Sigma) for 30 min at 37°C. Samples were centrifuged, the supernatant was removed, and tissue pieces were resuspended in medium (DMEM/F12, 3:1 [Invitrogen] containing 1% penicillin/streptomycin [P/S]) and manually dissociated by pipetting. Cells were cultured at 50,000 cells/ml in SKPs basal growth medium (DMEM-F12, 3:1 and 40 ng/ml FGF2 [Peprotech], 20 ng/ml EGF [BD Biosciences], 2% B27 [Invitrogen], and 1 μg/ml fungizone [Invitrogen]) and allowed to form spheres. For human SKPs, anonymized foreskin tissue from voluntary circumcisions was cut into 4-6-mm pieces, and incubated in Liberase DH Research grade Dispase High (0.60 Wunsch U/ml; Roche Molecular Biochemicals) overnight at 4°C. Epidermis was manually removed and dermis was further cut into 1 mm pieces and incubated in Liberase for 30-40 min at 37°C. DNase I was added for 1 min, and 10% fetal bovine serum (FBS; Fisher Scientific) was added to inhibit the enzymes. The supernatant was removed, and tissue pieces were resuspended in medium (DMEM/F12, 3:1 [Invitrogen] containing1% penicillin/streptomycin [P/S]) and manually dissociated by pipetting. Once tissue was dissociated, the cell suspension was filtered through a 70-μm-cell strainer (BD Biosciences). The strained cell suspension was centrifuged at 1,000 rpm for 5 min, supernatant was aspirated, and cells were plated at a density of 50-100,000 cells/ml in SKPs basal growth medium. To generate secondary spheres, SKPs were digested in collagenase (1 mg/ml; type XI; Sigma) for 15-30 min at 37°C and then mechanically dissociated to single cells by pipetting. Medium (DMEM/F12) was added to inhibit the enzyme. Cells were centrifuged at 1,000 rpm for 5 min. Supernatant was removed and cells were passaged into 50% new growth medium and 50% of the same medium pre-conditioned by SKPs to generate secondary spheres. All experiments were done using secondary or tertiary spheres. For SKPs drug-screening assays, secondary or tertiary passage human or rat SKP spheres were dissociated and 3000 human or 5000 rat SKP cells were robotically seeded in each well of 96-well uncoated plates (Costar, Corning Life Sciences). Compounds (1-5 μM) were added in singlet and plates were incubated in SKPs basal growth medium plus 50% of the same medium pre-conditioned by

SKPs. AlamarBlue dye (Life Technologies) was added after 30 hrs, and its reduction to a fluorescent compound was measured after another 24 hrs.

NIH 3T3 cultures. NIH 3T3 cells were grown in DMEM containing 10% FBS and 1% penicillin/streptomycin and plated at a density of 25,000 cells / cm^2. The following day, alprostadil and TM were added to the NIH 3T3 cultures at 100 nM and 24 hours later cells were fixed and immunostained for Ki67.

Skin wounding and morphometric analysis. To induce skin wounds 9 month old mice were anesthetized, the dorsal hair was shaved and the exposed skin was cleaned with ethanol. Two 6 mm diameter full thickness excisional wounds were performed on each side of the dorsal midline using a biopsy punch (Miltex). For consistency, only one wound on the right dorsal side of each mouse was used for analysis (punch from outside to inside skin). Wounds were left uncovered and mice were given Anafen for analgesia and housed individually. To quantify wound size, digital photographs (Canon) were taken at day 0, 3, 7 and 9 post wounding. A ruler was used to calibrate the images and the wound margins were manually outlined using Northern Eclipse software (Empix). The wound closure rate was calculated as a percentage of the wound size on the day of surgery, using the following formula: % wound closure = $(area_i\text{-}area_f) \times 100/area_i$ where $area_i$ is the initial wound area at day 0 and $area_f$ is the final area (Martin et al., 2010). At 9 days post-injury, mice were sacrificed, and the wound bed was excised and fixed in 10% formalin and embedded in paraffin. Wounds were bisected in a caudocranial direction and serial sections from the central portion of the wound were stained with hematoxylin and eosin and used for histology. Sections were scanned using a 20x/0.75 lens (Zeiss Mirax Scan) and were stitched together using the Mirax Scan software. Morphometric analysis were performed as described previously (Johnston et al., 2013; Martin et al., 2010), using Northern Eclipse software (Empix). Epithelial gap was measured as the distance between the new epithelial tongues. Wound width was measured as the distance between the wound margins which were defined by the last hair follicles. Finally, dermal tissue thickness was given a score of 1, 2 or 3 for very thin, moderate or thick dermis, respectively.

Antibodies. For immunohistochemistry of skin sections primary antibodies were rabbit anti-Ki67 (1:100; LabVision) or rabbit anti-CD31 (1:100; Abcam) followed by a biotin-conjugated goat anti-rabbit secondary, and the ABC-DAB detection kit (Vector Laboratory). For western blotting, primary antibodies were rabbit anti-pERK1/2 T202/Y204 (1:2000, Cell Signaling), rabbit anti-ERK1/2 (1:10,0000, Santa Cruz), rabbit anti-pSTAT3 Tyr705 (1:1000, Cell Signaling) and mouse anti-STAT3 (1:1000, Cell Signaling) and secondary antibodies were HRP-conjugated goat anti-mouse (1:10,000; Millipore) or goat anti-rabbit (1:10,000). Protein was detected using the ECL kit (GE Healthcare)

Supplemental References

Martin, D.C., Semple, J.L., and Sefton, M.V. (2010). Poly(methacrylic acid-co-methyl methacrylate) beads promote vascularization and wound repair in diabetic mice. J. Biomed. Mater. Res. A *93*, 484–492.