SUPPLEMENTARY MATERIAL

SUPPLEMENTARY MATERIAL AND METHODS

Generation of K14 V12Rac1 transgenic mice

Rac1 c-DNA containing a point mutation C17 to A and a C-terminal Myc-tag (1) was inserted via *Sac*I-*Xba*I sites into a keratin 14 expression cassette harboring an SV40 intron and an SV40 polyA signal sequence at the N- terminus. Correct insertion was confirmed by direct DNA sequencing. After digestion of the plasmid with *Bss*H2 the transgene was separated by agarose gel electrophoresis and isolated from the gel using the MinElute gel extraction kit (Qiagen, Hilden, Germany). Purification was performed using an Elutip mini column (Schleicher & Schüll, Dassel, Germany) and subsequent precipitation with ethanol. For pronucleus injection, the DNA was dissolved in microinjection buffer and adjusted to a final concentration of 10 ng/ml. Transgenic mice were generated by injection of the DNA construct into the pronucleus of fertilized oocytes. For screening of transgene insertion, genomic DNA was isolated from mouse tails and analyzed by means of polymerase chain reaction (PCR) using the primers SF3-25 5'-TTGGTTGTGTAACTGATCAGTAGGC-3' and SF5-23 5'-TGGAGAGCTAGCAGGAAACTAGG-3'. Insertion was confirmed by Southern blot analysis using a 600-bp fragment as probe.

RNA isolation and primer sequences for RT-qPCR

RNA was extracted from cell lysates; or mouse skin from day 1 or day 7 Rac1 or wild type pup skin using a Qiagen RNA plus miniprep kit. RNA concentration was determined with spectrophotometric analysis; purity analyzed with 260:280 absorbance ratios. One ug of RNA was reverse-transcribed using iSCRIPT cDNA synthesis kit (Bio-Rad) or High Capacity RNA-to-cDNA Kit (Invitrogen).

Human qPCR primer sequences

18S F: GCAATTATTCCCCATGAACG; 18S R: GGCCTCACTAAACCATCCAA; ZNF750 F:

AGCTCGCCTGAGTGTGAC; ZNF750 R: TGCAGACTCTGGCCTGTA; LCE3D F: GCTGCTTCCTGAACCAC; LCE3D R: GGGAACTCATGCATCAAG; SPRR2G F: GGACTCTCCACCACACTGATG; SPRR2G R: CTGCTGCTGCTGGTAAGACAT; SPRR3 F: CCAGGCTACACAAAGCTAC; SPRR3 R: GCTTAATTCAGGGGCTTAC; IVL F: AAAGCACCTAGAGCACCC; IVL R: GGTTGAATGTCTTGGACCT; LOR F: CTCTGTCTGCGGCTACTCTG; LOR R: CACGAGGTCTGAGTGACCTG.

Mouse qPCR primer sequences

CCL2: Mm00441242_m1 (Thermo Scientific); CCL5 F: GCAAGTGCTCCAATCTTGCA; CCL5 R: CTTCTCTGGGTTGGCACACA; CCL5 Probe: TGTTTGTCACTCGAAGGAACCGCCA; CCL20: Mm00444228_m1 (Thermo Scientific); CXCL1: Mm00433859_m1 (Thermo Scientific); CXCL2: Mm00436450_m1 (Thermo Scientific); CXCL10: Mm00445235_m1 (Thermo Scientific); Cxcl11: Mm00444662_m1 (Thermo Scientific); β4 Defensin F: TGGTGCTGCTGTCTCCACTTGC; β4 Defensin R: CGAAAAGCGGTAGGGCACGGA. CCL17 F: GCCTCTCGTACATACAGACGC; CCL17 R: CCAGTTCTGCTTTGGATCAGC; CCL20 F: TACCATGAGGTCACTTCAGATGC; CCL20 R: GCACTCTCGGCCTACATTGG; IL17RE F: CAGTCCCAGTGACGCTAGAC; IL17RE R: ACCCACTAGAGCGGTGAGAG; TSLP F: ACGGATGGGGCTAACTTACAA; TSLP R: AGTCCTCGATTTGCTCGAACT; OSMR F: GCATCCCGAAGCGAAGTCTT; OSMR R: GGGCTGGGACAGTCCATTCTA; CCL5 F: GCTGCTTTGCCTACCTCTCC; CCL5 R: TCGAGTGACAAACACGACTGC; CCR6 F: TGGGCCATGCTCCCTAGAA; CCR6 R: GGTGAGGACAAAGAGTATGTCTG; IL23P19 F: CAGCAGCTCTCTCGGAATCTC; IL23P19 R: TGGATACGGGGCACATTATTTTT; IL1β F: GAAATGCCACCTTTTGAC AGT G; IL1β R: TGGATGCTCTCATCAGGACAG; IL1F6 F: GCAGCATCACCTTCGCTTAGA; IL1F6 R: CAGATATTGGCATGGGAGCAAG

Immunoblot assays

Cells or tissue was lysed in 1x cell lysis solution (Thermo Scientific) with 1% Halt proteinase-phosphatase inhibitor (Thermo Scientific), incubated at 4° C under rotation for 1 hour and centrifuged 15 minutes for 13200 rpm at 4° C. Lysates were quantified based on absorbance with a Bradford assay, using standard conditions.

Lysates were denatured in 100°C for 5 minutes with 4x NUPAGE sample loading buffer (Invitrogen), 10x NUPAGE sample reducing agent (Invitrogen), and 5% β mercaptoethanol. Subsequently, lysates were loaded on a 4-12% bis-tris gel with 1X MOPS running buffer and run for 90 min at 150V. Gels were transferred with 1x transfer buffer in 10% methanol for 2.5 hours at 25V. Membranes were stained with Ponceau red, prior to being blocked (5% milk or 5% or 3% BSA), washed and incubated with primary antibody in 3% BSA overnight, washed and incubated with a HRP-tagged secondary antibody for 1 hour at RT in 2% BSA or 5% milk, washed and developed.

Tissue and cells for confocal microscopy

Tissue sections were embedded in OCT, snap frozen, and 7 µm sections cut on a cryostat (Leica), and sections or cells on coverslips were fixed for 10 minutes with cold methanol, washed with TBS, then blocked for one hour at room-temp with 10% normal goat, donkey or human serum, and incubated with primary antibody in PBS or TBS overnight. 12 hours later, washing was repeated 3 times 5 minutes, followed by incubation with secondary antibodies 1:400 together with Hoescht 1:5000 for 1 hour at room temperature washed and mounted with fluoromount (Southern Biotech). For mouse antibodies on mouse tissue, sections were treated with MOM igG blocking kit (Vector laboratories), according to manufacturer's recommendations.

Antibodies

Confocal microscopy

Rac1GTP (#26903, NewEast Biosciences), RhoAGTP (#26904, NewEast Biosciences), phospho-STAT3 (tyr705, d3a7xp, #9145, Cell Signaling), phospho-nfκb p65 (ser536,93h1,

#3033, Cell Signaling), total nf-κb p65 (d14e12xp,#8242, Cell Signaling), STAT3α (d1a5xp, #8768, Cell Signaling), anti-Traf3ip2 (#hpa036352, Atlas antibodies), IL23p19 (#ab45420, Abcam), MDA5(#ab79055, Abcam), anti-CD49f (#555736, Fisher Scientific), CD3 (#ab5690-100, Abcam), CD11c (ep1347y,#ab52632, Abcam and cd11c alexa fluor 488, Biolegend), CD68 (#ab125212, Abcam), desmoglein 3 (#32-6300, Cell Tech). Type VII collagen polyclonal rabbit antibody used in the study has been previously described (2).

Western blot

Total Rac1 (#1862341, Thermo Scientific), acetyl NFKB p65 (Lys310, #3045P, Cell Signaling), phospho-STAT3 (Tyr705, D3A7xp,#9145, Cell Signaling), phospho-NF-κB p65 (Ser536,93H1, #3033, Cell Signaling), total NF-κB p65 (D14E12xp, #8242, Cell Signaling), STAT3α (D1A5xp,#8768, Cell Signaling), ZNF750 (#HPA023012, Sigma), Il17RC (#ab69673, Abcam), ARHGEF6 (#ab184569, Abcam), RACGAP1 (#ab2270, Abcam), TIAM1 (#sc-872, Santa Cruz), Rac1/2/3 (#2465, Cell Signaling) RhoA (#2117, Cell Signaling), CDC42 (#2462, Cell Signaling), GAPDH (#sc-25778, Santa Cruz), βactin (#A5441, Sigma Aldrich).

Flow cytometry

Anti-CD3 Pacific Blue (clone 17A2, #100214, Biolegend), anti-CD4 PerCPCy5.5 (clone GK1.5, #100434, Biolegend), anti-CD8 Alexa 700 (clone 53-6.7, #100730, Biolegend), anti-CD19 PE/Cy7 (clone 6D, #115520, Biolegend), anti-Ly6g APC (clone 1A8, #127614, Biolegend), anti-CD11b Pacific Blue (clone M1/70, #101224, Biolegend, anti-CD11c APC Cy7 (clone N418, #117324, Biolegend), anti-Ly6C Fitc (clone HK1.4, #128006, Biolegend).

Cell culture

Neonatal foreskin, adult human control or adult human psoriatic non-lesional skin was incubated overnight at 4°C in HBSS with 25 U/ml dispase. Only non-lesional skin was harvested for in vitro analysis and xenograft production. Epidermis-dermis was separated

with forceps, epidermal sheets trypsinized for 15 min, neutralized with DMEM (Mediatech Inc) containing 10% FBS, 1%antibiotic-antimycotic (30-004-CI, Mediatech Inc), centrifuged for 5 min at 1000 rpm, and re-suspended in a 50-50 mixture of supplemented (Human Keratinocytes Growth Supplement, S-001-5, Invitrogen) medium 154 (M16 254-500, Invitrogen) and K-SFM (Defined Keratinocyte SFM, 10744-019, Invitrogen) and 1 % antibiotic-antimycotic solution (0-004-CI, Mediatech Inc). Adult cells were at passage 3 prior to in vitro analysis or xenografted.

SRPG-1 peptidoglycan stimulation

Primary human keratinocytes (n=3) from non-lesional psoriatic (n=3) or healthy control (n=3) skin were isolated from skin biopsies through dispase treatment as previously described, and cultured on collagen-coated coverslips in 6-well plates. Cells were attached for 4 hours, growth factor starved for 24 hours and stimulated with 1ug/ml-1 SRPG-1 (#SRPG-1, lot 112503SRPG, Toxin Technologies, Saratoga, FL, US) for 10 or 90 minutes.

Cytokine stimulation

Primary human adult psoriatic or adult normal control keratinocytes were growth factor starved for 24 hours then stimulated using 5 or 50ng/ml EGF (PHG0311, Life Technologies), 100ng/ml TNFα, (PHC3016, Gibco), 25ng/ml IL22 (NBP1-99226, Novus) or 100ng/ml IL17A/F (P4799, Novus Biologicals) and harvested after 0, 10 and 90 minutes; or for Il17A/F experiments GF starved or stimulated for 24 hours.

MTT assay

5000 first-passage neonatal keratinocytes per well were seeded on a collagen-coated 96 well plate, and incubated with 50/50 (Medium 154 and Keratinocyte –SFM medium, Invitrogen with Human Keratinocytes Growth Supplement, S-001-5, Invitrogen, and medium 154 supplement M-154-

500, Invitrogen) with 1 % antibiotic-antimycotic (30-004-CI, Mediatech Inc) for 48 hours. 10 ul MTT reagent (MTT proliferation assay, ATCC, Manassas,US) was added for 4 hours, 100 ul detergent was added to each well for 2 hours, and absorbance read at 570 nm (Spectramax M5, Molecular Devices, US), normalized to cell-free control absorbance.

Keratinocyte differentiation assay

36 hours after transduction, keratinocytes were plated at 40k in 6-well plates for the undifferentiated condition or 400k in 12-well plates for the differentiated condition. Each condition was in triplicate. After 16 hours, 1.2mM calcium was added to the differentiated condition. After 3 days, RNA was extracted using a RNeasy plus kit (Qiagen) or cells lysed using 1X cell lysis solution (Thermo Scientific) with 1% halt proteinase-phosphatase inhibitor (Thermo Scientific).

cDNA and siRNA constructs and vector information

V12 Rac1,V14 RhoA, dominant negative N17 Rac1 or LacZ control constructs were generated and cloned as previously described (3). Human V12 Rac1, N17Rac1 and LacZ constructs were a kind gift of Dr John Collard, Nethelands Cancer Institute, Amsterdam, The Netherlands. Human V14 RhoA was a kind gift from Dr Alan Hall, University College London, UK. ZNF750 was cloned into pLEX (Open Biosystems) with C-terminal FLAG, HA, and 6XHIS tags with the following primers: ZNF750 F: ACGCAGGATCCGCCACCATGAGTCTCCTCAAAGAGCGGAAGCCAAAAA; ZNF750 R: ACGCAGCGGCCGCGGGGACACCCGGGCCCTCCTTCGTAGTGTG.

Lentiviral gene transfer

293T cells were transfected with 8 ug of lentiviral expression construct, 6 ug of pCMVD8.91, and 2 ug of pUCMD.G. Transfections were done in 10-cm plates using Lipofectamine 2000 (Life Technologies). Viral supernatant was collected 72 h after transfection and concentrated

using a Lenti-X concentrator (Clontech). For ZNF750 experiments cells were after 48 hours transduced with pLEX control or pLEX ZNF750 lentivirus overnight.

Retroviral gene transfer

Phoenix cells were transfected with V12Rac1, V14 RhoA, N17 Rac1 or LacZ in 10-cm plates using Lipofectamine 2000 (Life Technologies). Cells were grown to 80% confluency, transferred to 32°C incubation, and viral supernatant was collected after 24, 48 and 72 h. Cell cultures were incubated with polybrene for 10 minutes at 37°C, (5ug/mL), media replaced by viral media with polybrene (5ug/mL), centrifuged 1 hour at 1000 rpm, followed by incubation for 4 hours at 37°C, prior to media change.

SiRNA transduction and sequences

1 million keratinocytes were electroporated with 1 nmol control or ZNF750 siRNA using Amaxa nucleofection reagents with siRNA sequences (control) GUAGAUUCAUAUUGUAAGGUU; (ZNF750): CCACCAGAGUUUCCACAUA'.

Luminex assays

Luminex assays were performed in the Human Immune Monitoring Center at Stanford University.

Polystyrene bead kits

Human 51-plex kits were purchased from Affymetrix and used according to the manufacturer's recommendations with modifications as described below. All samples were mixed with antibody-linked polystyrene beads on 96-well filter-bottom plates and incubated at room temperature for 2 h followed by overnight incubation at 4°C. Room temperature incubation steps were performed on an orbital shaker at 500-600 rpm. Plates were vacuum filtered and washed twice with wash buffer, then incubated with biotinylated detection antibody for 2 h at room temperature. Samples were then filtered and washed twice as above

and resuspended in streptavidin-PE. After incubation for 40 minutes at room temperature, two additional vacuum washes were performed, and the samples resuspended in Reading Buffer. Plates were read using a Luminex 200 instrument with a lower bound of 100 beads per sample per cytokine. Custom assay Control beads by Radix Biosolutions were added to all wells. All sample groups were run in duplicates and normalized to the PBMC only condition.

eBioscience/Affymetrix Magnetic bead Kits

Mouse 38 plex kits were purchased from eBiosciences/Affymetrix and used according to the manufacturer's recommendations with modifications as described below. Beads were added to a 96 well plate and washed in a Biotek ELx405 washer. Samples were added to the plate containing the mixed antibody-linked beads and incubated at room temperature for 1 hour followed by overnight incubation at 4°C with shaking. Cold and Room temperature incubation steps were performed on an orbital shaker at 500-600 rpm. Following the overnight incubation plates were washed in a Biotek ELx405 washer and then biotinylated detection antibody added for 75 minutes at room temperature with shaking. Plate was washed as above and streptavidin-PE was added. After incubation for 30 minutes at room temperature wash was performed as above and reading buffer was added to the wells. Each sample was measured in duplicate. Plates were read using a Luminex 200 instrument with a lower bound of 50 beads per sample per cytokine. Custom assay Control beads by Radix Biosolutions were added to all wells. All samples were run in duplicates and fold changes calculated between Rac1 and WT control mice.

Flow cytometry

Live cells (Dapi -) from Rac1 or WT littermate control skin were gated on total T-cells (CD3+), CD4+ cells, CD8+ cells, B-cells (CD19+), granulocytes (Lin-, Ly6g high), dendritic cells (Lin-, CD11b+, CD11c+), and monocytes (Lin-, CD11b+, Ly6c+). Cells were stained in FACS buffer (2mM EDTA with BSA in PBS), FC blocked using an-anti CD16/CD32 antibody

(#101320 Biolegend) and stained in 100ul with 1ul of each antibody added for 20 min at 4C prior to washing and DAPI staining.

Transcriptional expressional analysis

Mouse MEEBO microarray

RNA was extracted using a Qiagen RNA miniprep kit. RNA concentration was determined with spectrophotometric analysis; purity analyzed with 260:280 absorbance ratios; RNA quality and integrity were assessed and ensured using Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) and RNA 6000 NanoAssay. MEEBO microarrays covering 24886 mRNA probe-sets were hybridized and scanned at the Stanford Microarray Facility. Each RNA sample was amplified using the Ambion Illumina RNA amplification kit with biotin UTP labeling. T7 oligo (dT) primer was used to generate single stranded cDNA, followed by second strand synthesis to generate double-stranded cDNA, which is then column purified. *In vitro* transcription was performed to synthesize biotin-labeled cRNA using T7 RNA polymerase. The cRNA was then column purified and measured. A total of 1500 ng of cRNA was hybridized for each array using standard Illumina protocols with streptavidin-Cy3 for detection. Slides were scanned on an Illumina Beadstation and analyzed using BeadStudio (Illumina, Inc). Microarray data have been deposited in NCBI's Gene Expression Omnibus according to MIAME guidelines and is accessible through GEO Series accession number GSE71683.

Human psoriasis gene expression data

An analysis of microarray data was conducted from publicly available datasets found on the NCBI Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo): GSE13355, GSE14905, GSE30999 and GSE41664. All samples were hybridized on Affymetrix Human Genome U133 Plus 2.0 Array chips.

Mouse models of psoriasis gene expression data

Additionally microarray data deposited on the NCBI Gene Expression Omnibus was obtained for microarray data from K5-Tie2, K14-AREG (tail dataset), K5-STAT3, K5-TGFβ1 and IMQ mouse models of psoriasis (GSE27628). Transcripts were merged into unique genes, and only genes annotated across all experimental conditions were analyzed.

Normalization, gene set enrichment and pathway analysis

Partek Genomics Suite 6.6 (Partek Inc., St. Louis, MO, USA, www.partek.com) was used for quantile normalization and $\log^2$ transformation of datasets, removing batch effects, quality control and statistical analysis. GSE27628 were normalized as previously described (4). For human data-sets, samples in GSE13355 were quartile normalized and a reference file was generated. The reference file was used as a reference for GSE14905, GSE30999, and GSE41664. Differential expression detected by ANOVA were filtered (FC >1.5 or <1.5, unadjusted p=0.05, FDR<0.25, or significance-ranked) and exported to Ingenuity Pathway Analysis (IPA; Ingenuity Systems, Redwood City, CA, http://www.ingenuity.com/), DAVID and KEGG pathway analysis, or the Protein ANalysis THrough Evolutionary Relationships database. Mouse and human homologs were compared using IPA, Biomart (Wellcome Trust Sanger Institute and the European Bioinformatics Institute), Mammalhom (http://depts.washington.edu/l2l/mammalhom.html) and Homologene ((http://www.ncbi.nlm.nih.gov/HomoloGene/) resources and non-homologous genes excluded. For IPA upstream regulation and pathway analysis, findings were ranked according to activation z score, ratio or number of annotated genes. (http://pages.ingenuity.com/rs/ingenuity/images/0812%20upstream_regulator_analysis_whitepaper.pdf).

Human xenografts

Keratinocytes were grown in supplemented 50/50 medium-154 and defined keratinocyte SFM as described, and fibroblasts in DMEM (Mediatech Inc), with 10% FBS. $0.5x10^6$ fibroblasts were centrifuged (2x20 min, 1000 rpm) onto reticular side of a 10x10 mm^2

devitalized dermis (New York Firefighters Biobank, NY, US), and cultured in DMEM with 10% FBS for 3 days. Primary keratinocytes of early passage (P1) were retro-virally transduced with LZRS LacZ or N17 Rac1 in the presence of polybrene 5ug/ml^{-1}. After 3 days, skin equivalents were transferred to an annular dermal support (ADS) tissue culture insert, as previously described (5), with minor modifications. 35 mm plastic inserts were prepared with an 8mm^2 square central orifice resting on anchored 3mm glass beads inside a 60mm plastic tissue culture dish. Reticular side of skin equivalent was covered in matrigel (BD Biosciences), dried for 5 minutes and flipped onto 35 mm insert, covering an 8mm^2 orifice, exposing papillary side of skin equivalent to the air-fluid interphase. 1x10^6 LacZ or N17 Rac1 keratinocytes were seeded in 100 ul 50/50 M-154/defined keratinocyte SFM media into orifice on papillary side, settled for 10 minutes, and 5 ml of KGM pipetted into lower chamber comprising of a 60 mm tissue culture dish. Media was changed in lower chamber daily for 7 days. On day 8, skin equivalents containing psoriatic keratinocytes and autologous fibroblasts (LacZ n=4 and N17 n=2) or control keratinocytes (LacZ n=4) and autologous fibroblasts were grafted onto 8 week old NOD/SCID male mice (Jackson Labs), sutured and bandaged. After ten days, bandages and sutures were removed. The following day, blood samples were obtained from each subject, and PBMCs were isolated using Ficoll-paque plus per manufacturer's recommendations (GE Healthcare).150 ul of RPMI or RPMI with PBMCs (1x10^6) were subsequently injected intradermally into LacZ-psoriasis (PBMCs n=2, RPMI n=2), N17 psoriasis (PBMCs n=2) or LacZ-control xenografts (PBMCs n=2, RPMI n=2). Samples were harvested after 14 days.

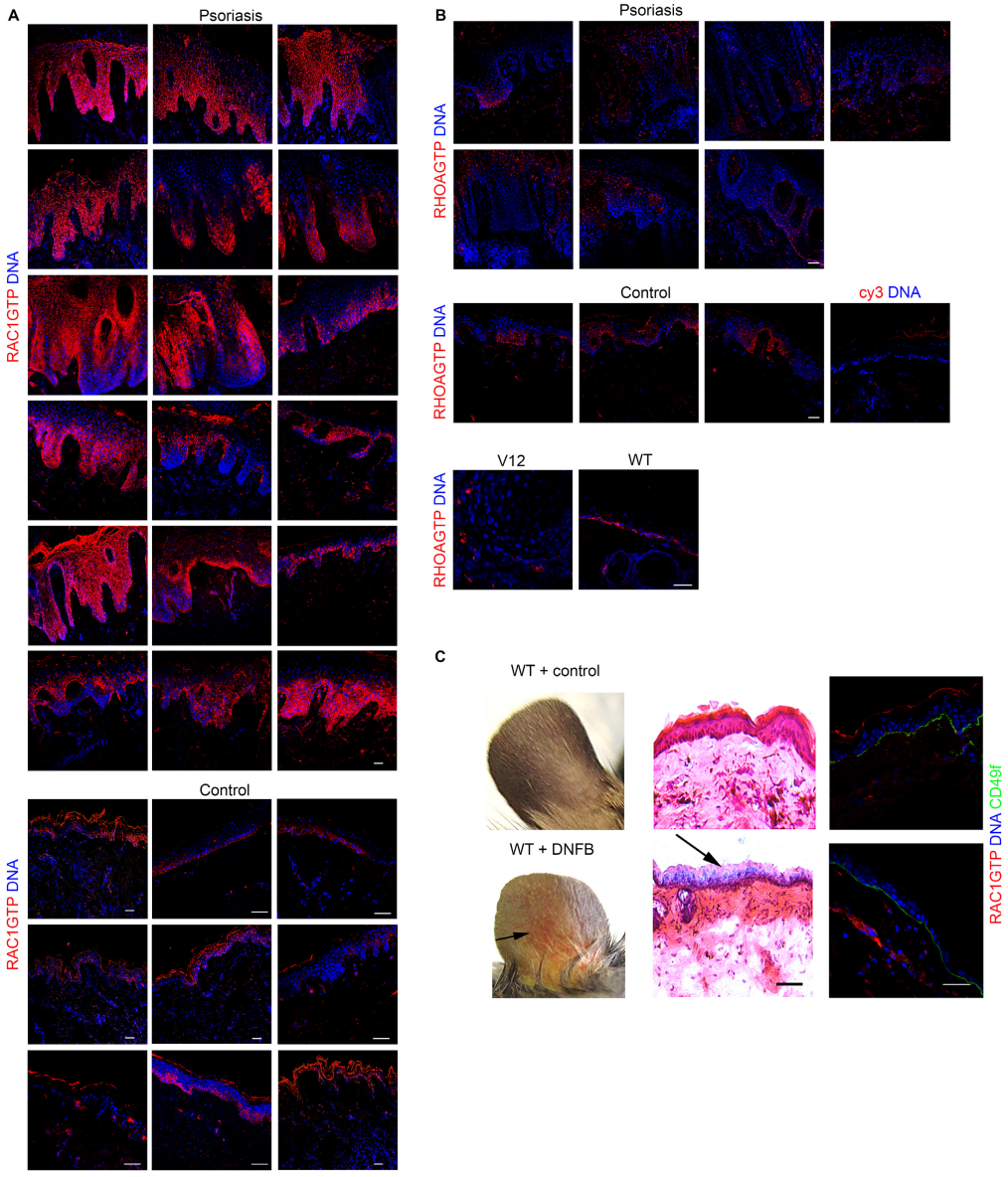
Organotypic 3D skin equivalents

Keratinocytes were grown in supplemented 50/50 medium-154 and defined keratinocyte SFM as described, and fibroblasts in DMEM (Mediatech Inc), with 10% FBS. 0.5x10^6 fibroblasts were centrifuged (2x20 min, 1000 rpm) onto reticular side of a 10x10 mm^2 devitalized dermis (New York Firefighters Biobank, NY, US), and cultured in DMEM with 10% FBS for 3 days. Primary keratinocytes of early passage (P1) were retro-virally transduced

with LZRS V12 Rac1, V14 RhoA or LacZ in the presence of polybrene 5ug/ml^{-1}. After 3 days, skin equivalents were transferred to an annular dermal support (ADS) tissue culture insert, as previously described (5), with minor modifications. 35 mm plastic inserts were prepared with an 8mm^2 square central orifice resting on anchored 3mm glass beads inside a 60mm plastic tissue culture dish. Reticular side of skin equivalent was covered in matrigel (BD Biosciences), dried for 5 minutes and flipped onto 35 mm insert, covering an 8mm^2 orifice, exposing papillary side of skin equivalent to the air-fluid interphase. 1x10^6 keratinocytes were seeded in 100 ul 50/50 M-154/defined keratinocyte SFM media into orifice on papillary side, settled for 10 minutes, and 5 ml of KGM pipetted into lower chamber comprising of a 60 mm tissue culture dish. Media was changed in lower chamber daily for 7 days, then harvested, embedded in OCT, and snap-frozen.

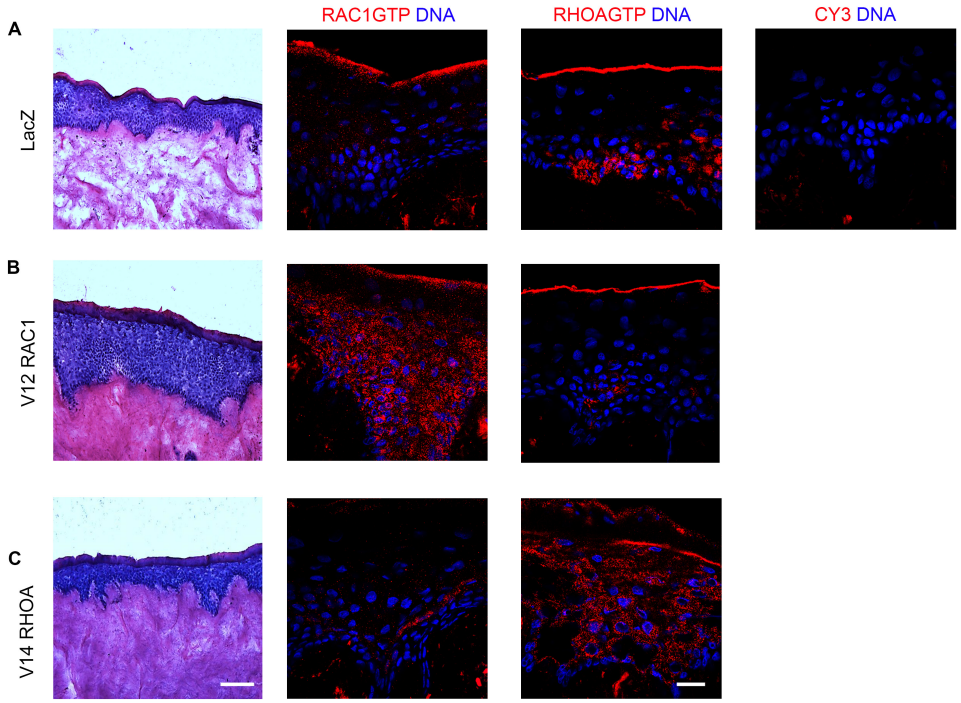
SUPPLEMENTARY FIGURES AND TABLES

FIGURE S1. Rac1GTP and RhoAGTP expression in psoriasis lesional and control skin, and Rac1GTP in a DNFB-induced model of contact dermatitis.



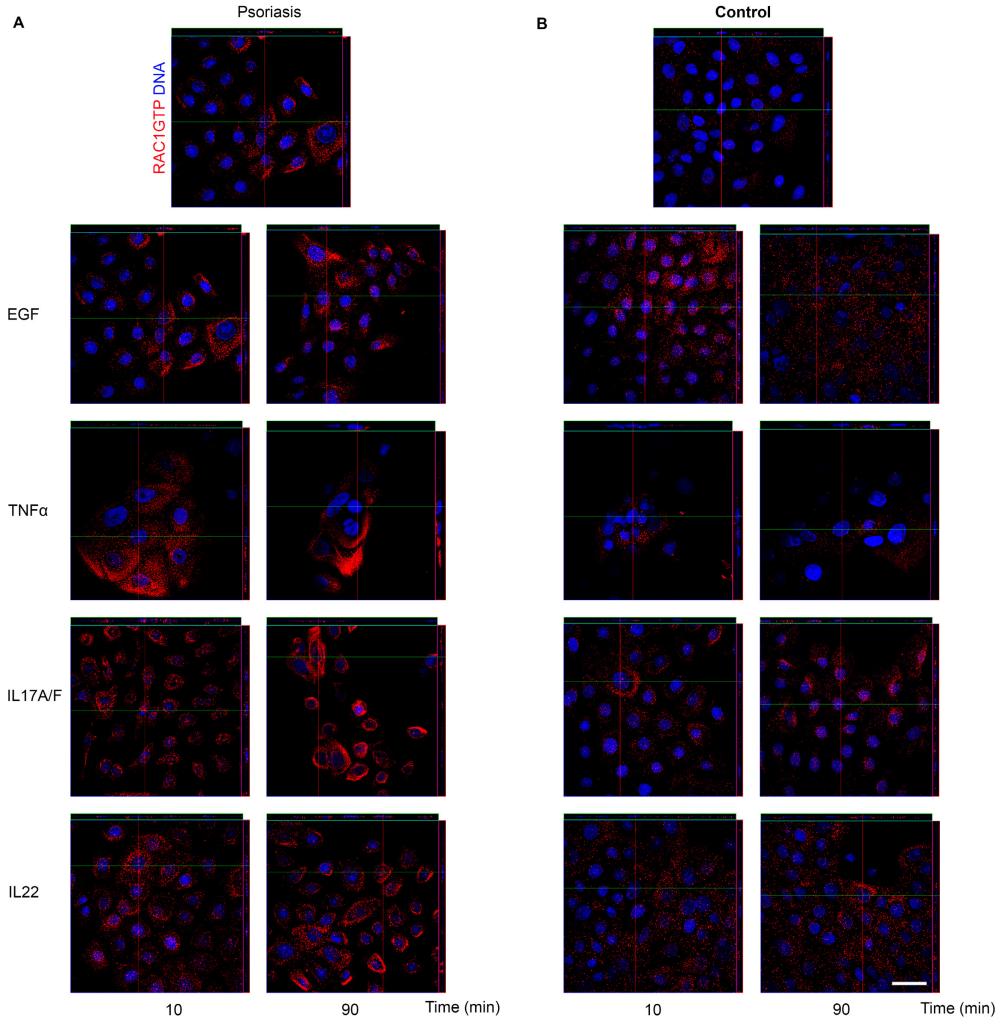
(A) IDIF using a Rac1-GTP specific mAb showed marked upregulation in basal and suprabasal layers of human psoriatic lesional skin compared to control skin. Rac1GTP-red, DNA-blue. (B) IDIF using a RhoA-GTP specific mAb did not show substantial differences of RhoAGTP in psoriatic lesional (n=7) skin compared to control skin (n=3), whereas RhoAGTP was reduced in V12 Rac1 skin compared to wildtype. RhoAGTP-red, DNA-blue. (C) Wild-type mice were painted with DNFB, and sensitized 10 days later by ear application. No increased epidermal Rac1GTP was noted in spongiotic epidermis of CAD mice compared to controls (n=3 per condition). Rac1GTP-red, DNA-blue CD49f-green. Arrow in C (middle) depicts epidermis. A psoriasis n=18, control n=9. B psoriasis n=7, control n=3, V12 representative of n=2, WT representative of n=2. C representative of n=3 per condition. Scalebar 50 µm (A-C).

FIGURE S2 Positive and negative control staining for immunofluorescence specificity of Rac1- and RhoA GTP antibodies



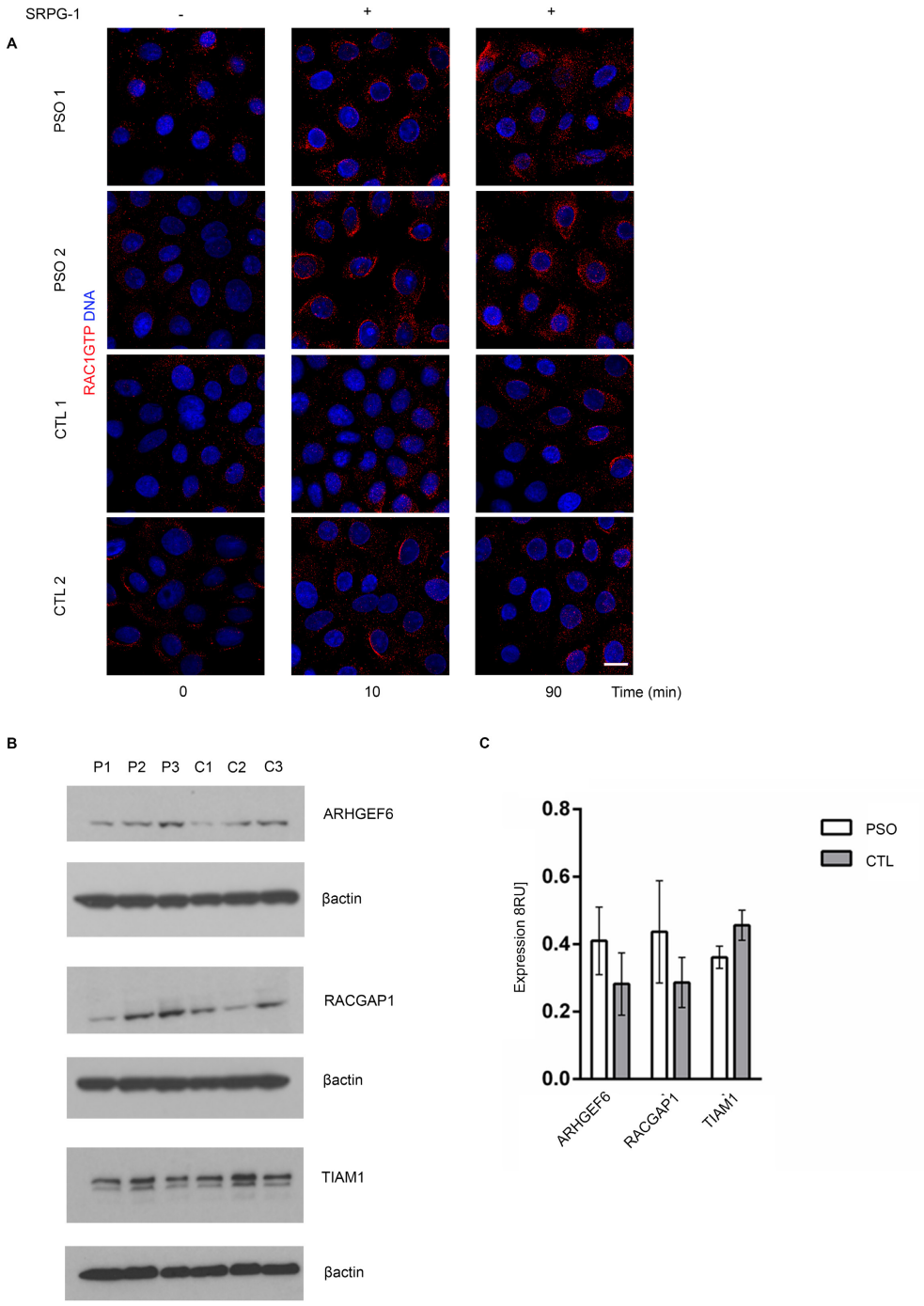
Organotypic 3D skin equivalents grown at air/fluid interphase containing keratinocytes retrovirally overexpressing (A) LacZ control, (B) V12 Rac1 or (C) V14 RhoA assayed with IDIF using confocal microscopy. Rac1GTP/RhoAGTP-red, DNA-blue. Representative of n=2 per condition. Scalebar 50µm (A-C left) / 25µm (A-C middle, right).

FIGURE S3. Stimulated psoriatic keratinocytes exhibit Rac1GTP hyperresponsiveness.



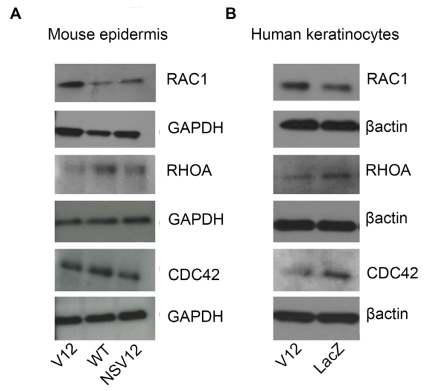
Following growth factor starvation, (A) psoriasis patient or (B) control keratinocytes were assayed for Rac1GTP by IDIF using confocal microscopy. Orthogonal view of z stacks of each condition shown at baseline or 10 and 90 minutes after addition of EGF, TNFα, IL17A/F or IL22. A-B Rac1GTP-red, DNA-blue. Representative of n=2 per condition. Scalebar 25μm

FIGURE S4. Upregulation of Rac1GTP by GAS capsular extract, and Rac1 GEF and GAP expression in psoriatic and control keratinocytes.



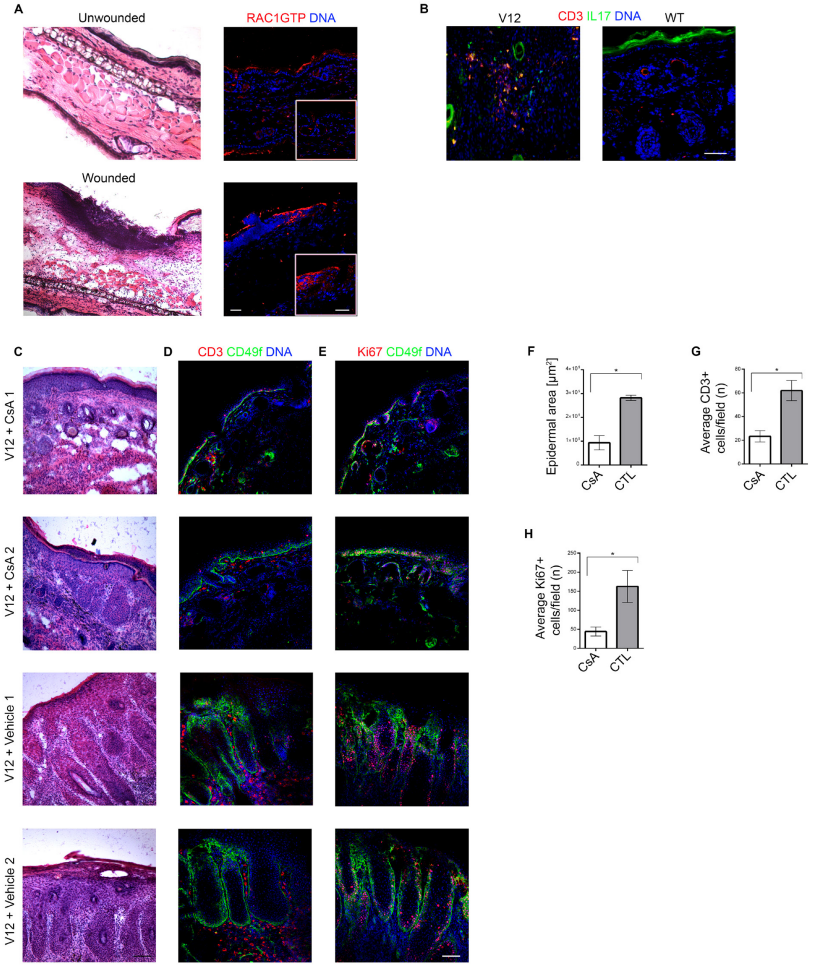
(A) GAS capsular extract exhibited pronounced induction of Rac1GTP in psoriatic keratinocytes compared to controls. Rac1GTP-red, DNA-blue. (B) ARHGEF6, TIAM1 and RACGAP1 were assayed by immunoblot in psoriatic patient or control keratinocytes. (C) Quantification of (B) showed a non-significant trend of induced ARHGEF6 and RACGAP1 but not TIAM1 in psoriatic compared to control keratinocytes. A representative of n=2 per condition. B-C n=3 per condition. Unpaired t-test (C). Scalebar 10μm (A).

FIGURE S5. Expression of RhoGTPases in V12 Rac1 mouse epidermis and V12 Rac1 keratinocytes



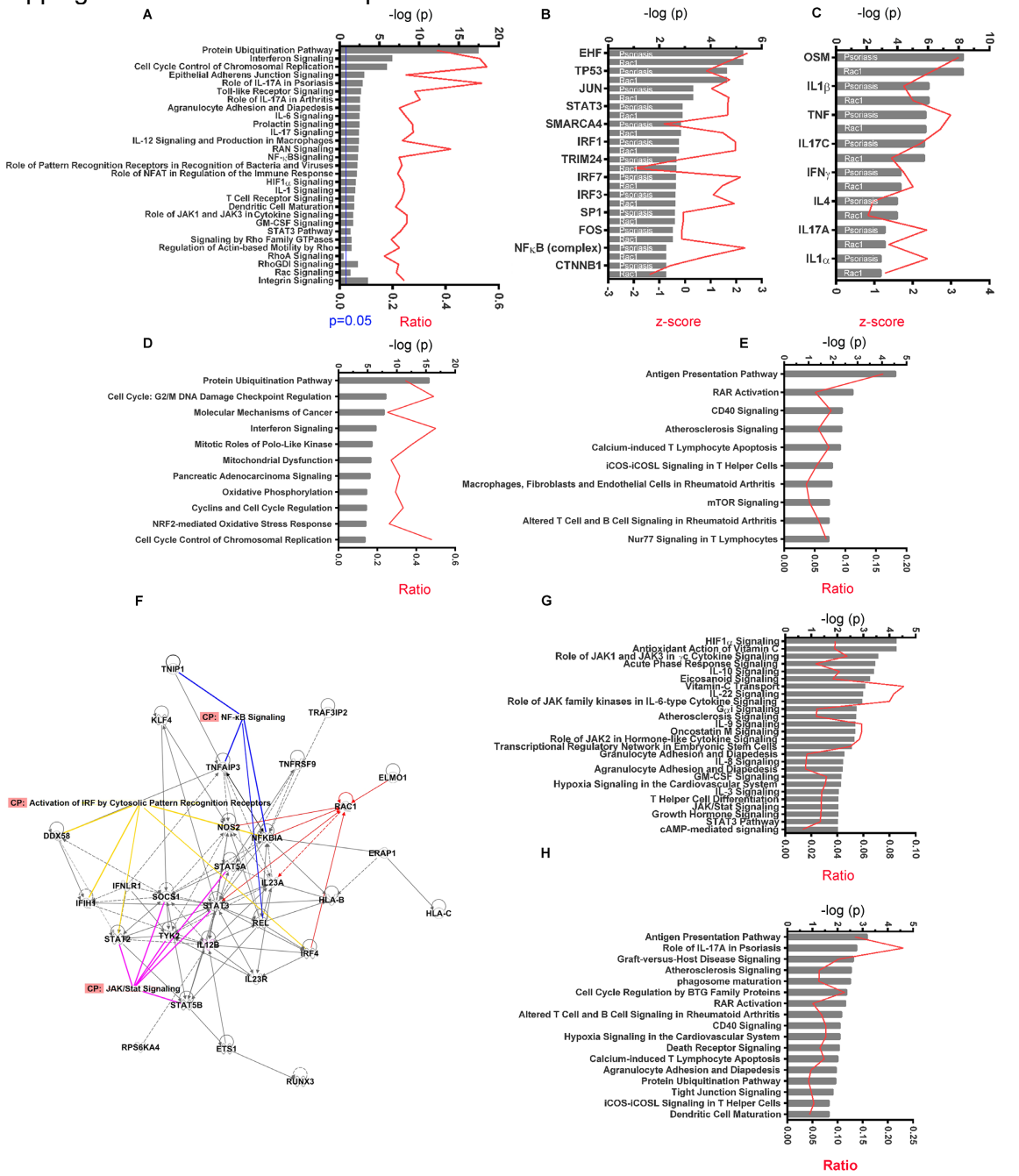
(A) Cell lysates from dispase separated epidermis of V12 Rac1, WT or Nod-SCID V12 Rac1 mice were assayed for total Rac1, RhoA and Cdc42. (B) Rac1, RhoA and Cdc42 levels were compared in lysates from V12 or LacZ transduced primary human keratinocytes. n=2 per condition.

FIGURE S6. Rac1GTP induced in healing epidermal wound edges, increased CD3+ T cell infiltration and IL17 production in Rac1 lesional skin, and effect of Cyclosporin A on psoriasiform hyperplasia in Rac1 mice.



(A) Rac1GTP was assayed by IDIF in healing skin after 4mm punch biopises, and detected at healing epidermal wound edges 24 hours after wounding, but not detected in healed skin. Rac1GTP-red, DNA-blue. Inserts 63x magnification. B) Pronounced infiltration of Il17 producing CD3+ T cells into upper dermis and epidermis in lesional Rac1 skin compared to littermate controls. CD3-red, IL17-green, DNA-blue. (C) H&E of tail sections from seven day old Rac1-pups treated daily (IP) for 21 days with cyclosporine A (15mg/kg) or vehicle, quantified in (F). (D) CD3+ cells, quantified in (G) and (E) epidermal Ki67 positive cells, quantified in (H). A representative of n=2 per condition. B-E representative of n=3 per condition. F-H n=3 per condition. G-H unpaired t test. F: one-tailed unpaired t-test. *p<0.05. Ki67/CD3–red, cd49f -green, DNA-blue. Error bars SEM. Scalebars 50µm (A-E).

FIGURE S7. Signature of Rac1-signaling in psoriatic lesional skin on signaling pathways, in silico-GWAS susceptibility gene association, transcription factor enrichment and pathways overlapping other mouse models of psoriasis.



(A) Canonical pathway analysis of a human dataset including 3642 unique transcripts, depicting selected pathways highly enriched as well as signatures for annotated RhoGTP family signaling. (B)Transcription factors and (C) cytokines enriched in signature overlapping Rac1 mouse and human psoriatic lesional skin. (D) Canonical pathways of DEGs in psoriatic lesional skin non-overlapping Rac1 mouse DEGs. (E) Canonical pathways of DEGS in Rac1 mouse skin not overlapping human psoriatic lesional skin. (F) In silico mapping of published psoriasis susceptibility genes to interactions (across multiple celltypes) with each other and Rac1 using IPA pathway analysis. (G) The shared signature between K14V12Rac1-K14AREG and K5STAT3C included enrichment for STAT3-, immune cell-, JAK- and IL22 signaling. (H) Non-overlapping transcripts in K14V12Rac1 skin included enrichment for antigen presentation pathway, role of IL17 in psoriasis as well as arthritis signaling. (A-E, G, H ANOVA and Fisher Exact test.

TABLE S1. Most significant differentially expressed genes in Rac1 mouse skin.

Gene symbol	p-value (Rac1 vs. WT)[1]	Fold-Change (Rac1 vs. WT)[2]
Sprr2d	1,6E-05	27,53
Tspan8	1,7E-04	5,20
Krt16	2,2E-04	25,75
Slc34a2	2,6E-04	2,41
BC117090	4,5E-04	2,18
Sbsn	4,8E-04	2,66
Stfa2	5,5E-04	5,85
4930583H14Rik	6,0E-04	2,21
Slc25a17	6,4E-04	2,75
Krt6b	6,7E-04	9,48
Oas1f	7,9E-04	3,17
Tomm22	8,5E-04	2,51
Sprr2e	9,4E-04	3,03
2310042E22Rik	1,2E-03	2,86
Spink4	1,3E-03	4,04
Cyp2b10	1,6E-03	3,17
Sdcbp2	1,6E-03	2,07
Ccl21a	1,7E-03	2,59
S100a9	1,7E-03	9,88
Krt7	1,8E-03	2,94
Cyp2f2	1,8E-03	-2,95
Chi3l1	1,9E-03	6,05
Slpi	1,9E-03	4,53
Uchl3	2,0E-03	-2,28
Serpinb3a	2,0E-03	4,26
Ptges	2,1E-03	2,07
Gsdmc1	2,1E-03	3,31
Pdzk1ip1	2,2E-03	2,62
Chit1	2,9E-03	2,88
Cxcl16	2,9E-03	2,18
Serpina3h	3,0E-03	3,03
Ccl21c	3,0E-03	2,45
Clca3	3,2E-03	2,59
Sprr2g	3,2E-03	3,15
Dnase1l3	3,3E-03	2,19
EG408196	3,4E-03	3,14
Egln3	3,6E-03	2,20
C79267	4,0E-03	2,55
Map3k14	4,1E-03	2,08

[1]filtered by FDR <0.25; ANOVA. [2]Log^2 fold change.

TABLE S2. Biological functions of significance-ranked differentially expressed genes in Rac1 mouse skin

Category	**p-value**[1, 2]
Dermatological Diseases and Conditions	8,76E-15-2,52E-03
Inflammatory Response	8,76E-15-3,83E-03
Cellular Movement	4,85E-11-3,73E-03
Cellular Growth and Proliferation	6,96E-11-3,74E-03
Organismal Survival	8,01E-11-3,37E-03
Embryonic Development	2,13E-10-3,9E-03
Hair and Skin Development and Function	2,13E-10-3,73E-03
Organ Development	2,13E-10-3,9E-03
Organismal Development	2,13E-10-3,9E-03
Tissue Development	2,13E-10-3,9E-03
Organ Morphology	6,14E-09-3,74E-03
Cellular Function and Maintenance	1,12E-08-3,71E-03
Cell Death and Survival	1,38E-08-3,8E-03
Hematological System Development and Function	3,35E-08-3,73E-03
Tissue Morphology	3,35E-08-3,74E-03
Cell-To-Cell Signaling and Interaction	1,21E-07-3,38E-03
Organismal Injury and Abnormalities	1,47E-07-3,83E-03
Cellular Development	3,44E-07-3,74E-03
Immune Cell Trafficking	5,14E-07-3,73E-03
Cancer	1,41E-06-3,81E-03
Cardiovascular System Development and Function	2,16E-06-4,06E-03
Skeletal and Muscular Disorders	4,09E-06-3,31E-03

[1]Cutoff of ranked list $p<0.05$, ANOVA. Log^2 FC 1.5. [2]p-values of biological functions by Fisher Exact test.

TABLE S3. Overlap of significance-ranked differentially expressed transcripts in Rac1 and human psoriasis lesional skin

Homolog	p-value[1]	FC[2]	Hu probeset	p-value[1]	FC[2]
IL36A	1,63E-04	1,81	221404_at	1,20E-52	1,99
KRT16	2,16E-04	25,75	209800_at	1,08E-80	4,49
LTBR	3,82E-04	1,51	203005_at	2,03E-39	1,22
KRT6A	6,70E-04	9,48	209125_at	3,26E-66	2,66
OAS1	7,94E-04	3,17	205552_s_at	1,68E-53	2,24
PRSS27	1,08E-03	1,91	232074_at	3,92E-62	2,44
TAP1	1,30E-03	1,84	202307_s_at	4,16E-51	1,47
S100A9	1,75E-03	9,88	203535_at	1,24E-114	10,68
SERPINB4	1,99E-03	4,26	210413_x_at	6,49E-89	7,82
PTGES	2,12E-03	2,07	207388_s_at	2,70E-35	1,22
WWP1	3,56E-03	-1,50	212638_s_at	2,16E-51	-1,25
ALOX12B	4,23E-03	1,77	207381_at	9,96E-74	2,35
LTB4R	5,53E-03	2,23	236172_at	1,84E-44	1,56
STAT3	7,46E-03	1,77	208992_s_at	6,10E-45	1,50
SLC2A13	8,61E-03	-1,61	227176_at	8,69E-35	-1,43
HLA-G	9,57E-03	1,52	210514_x_at	2,12E-33	1,17
TMPRSS4	1,02E-02	2,12	218960_at	9,45E-66	1,83
KLK6	1,08E-02	2,82	204733_at	4,11E-39	2,73
STK3	1,14E-02	-1,81	204068_at	5,94E-29	-1,16
SCNN1A	1,43E-02	1,92	203453_at	5,05E-29	1,26
CALML5	1,65E-02	2,82	220414_at	1,11E-46	1,45
CGNL1	1,66E-02	-1,62	225817_at	3,05E-62	-1,66
IL36RN	1,79E-02	1,71	222223_s_at	2,50E-91	3,03
S100A8	1,99E-02	2,28	202917_s_at	1,05E-114	6,61

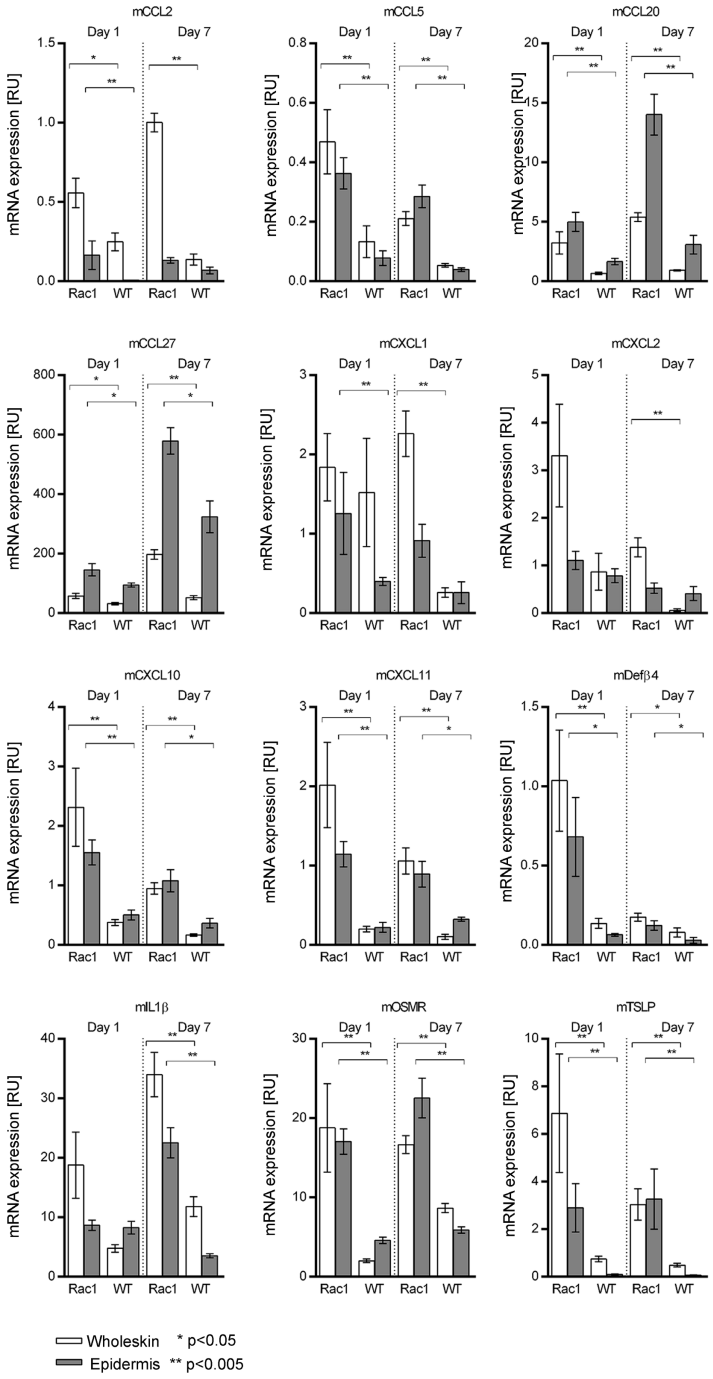
[1] ANOVA. [2] Log^2 FC

Table S4. Overlapping signature in Rac1 mouse skin and five mouse models of psoriasis

Model	Overlap (%)[1]	p-value[2]
AREG	8.7	1,75E-13
STAT3C	10.2	1,20E-10
IMQ	4.5	2,00E-06
TGFβ	3.4	1,90E-04
TIE2	1.4	0,052

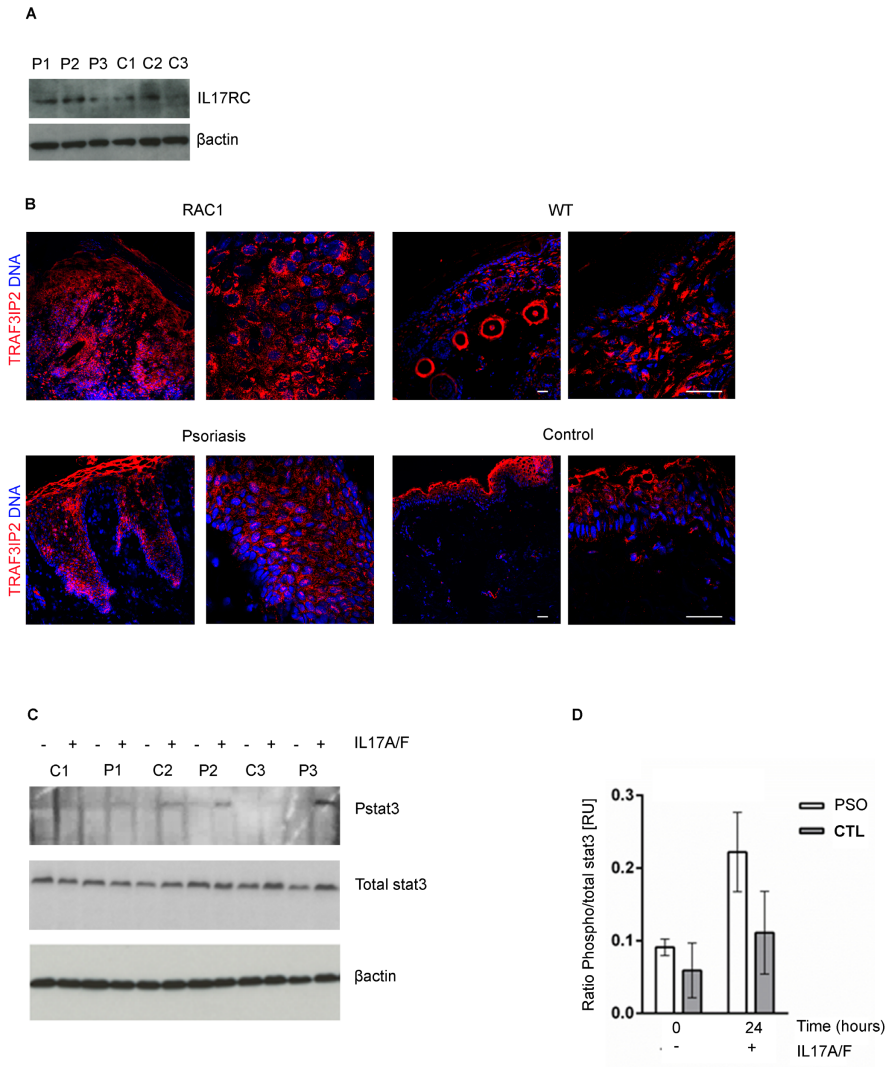
[1]Cutoff p<0.05, ANOVA, FC 1.5. [2]Hypergeometric mean

FIGURE S8. mRNA expression of cytokine and chemokines involved in immune recruitment and activation in pre-lesional and lesional Rac1 mouse or WT control skin.



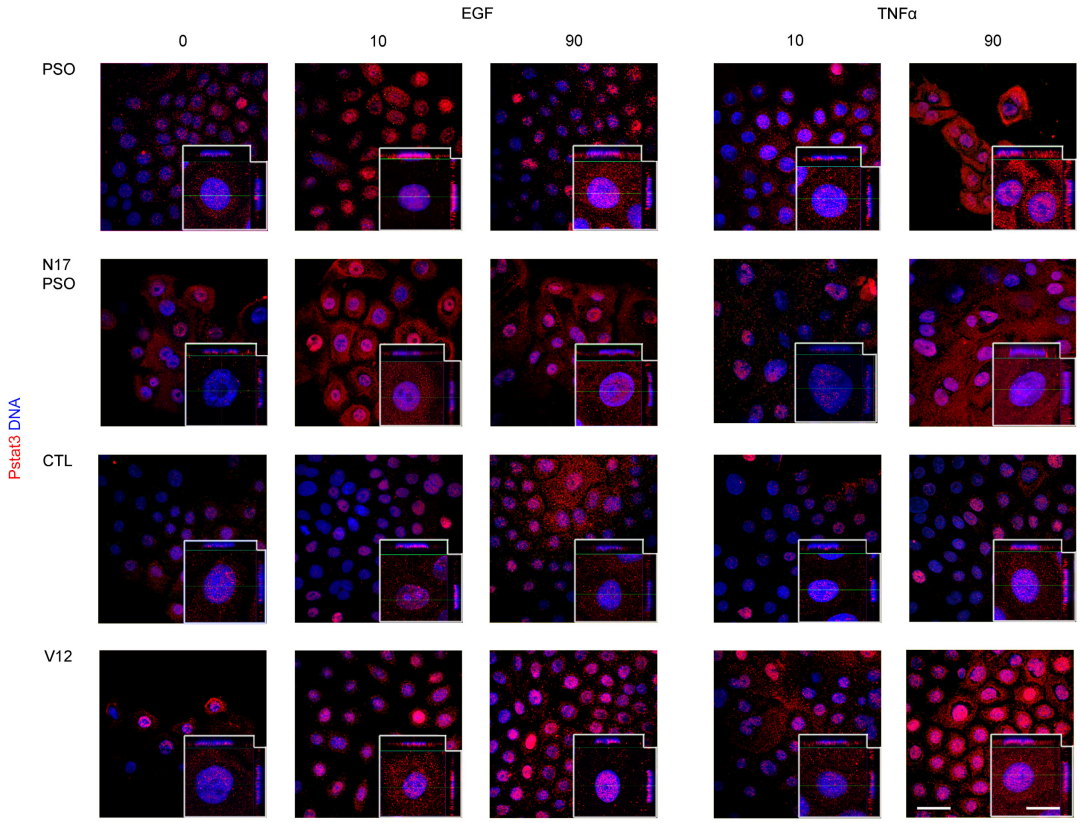
RT-qPCR of CCL2, CCL5, CCL20, CCL27, CXCL1, CXCL2, CXCL10, CXCL11, β4-Defensin, IL1β, OSMR and TSLP in dispase-treated epidermis or wholeskin from Rac1 or WT control skin. Rac1 n= 6-12 WT n=3-7. Error bars SEM. Mann-Whitney ranked test. *p<0.05, **<0.005. White bars – wholeskin, grey bars – epidermis.

Figure S9. Components of IL17 signaling axis expressed by human keratinocytes



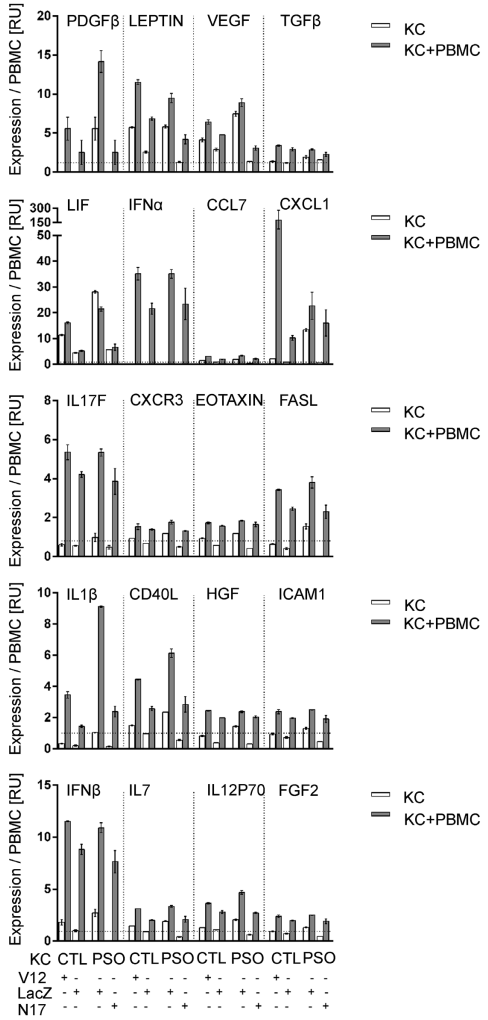
(A) Expression of Il17RC was detected and was modestly increased in a panel of primary psoriasis patient keratinocytes compared to controls. (B) Expression of the IL17R adaptor TRAF3IP2 was detected in skin of V12 Rac1 mice and in human psoriatic lesional skin. (C) Control (C1-C3) or psoriatic (P1-P3) keratinocytes were growth factor starved for 24 hours, then incubated with vehicle or 100ng/ml Il17A/F for 24 hours, and lysates assayed by immunoblot. (D) Quantification of densitometry of (C) showing increased PSTAT3 in psoriatic keratinocytes after Il17 stimulation. A, C-D n=3 per condition. B representative of n=2 per condition. Scalebars 50um (B).

FIGURE S10. Rac1-dependent nuclear translocation of phosphorylated STAT3 in psoriatic and normal keratinocytes.



IDIF of PSTAT3 after addition of EGF or TNFα in primary psoriatic keratinocytes transduced with LacZ control or dominant negative (N17) Rac1, compared to LacZ control or V12Rac1 overexpressing primary control keratinocytes. Inserts showing orthogonal view of z-stacks. PSTAT3-red, DNA-blue. Scalebars 20/10 μm (inserts). Representative images of 3 experiments per condition.

FIGURE S11. Expression of pro-inflammatory molecules in human keratinocyte/PBMC *in vitro* co-cultures.



Luminex panel of selected cytokine expression (PDGFβ, LEPTIN, VEGF,TGFβ, LIF, IFNα, CCL7, CXCL1, Il17F, CXCR3, EOTAXIN, FASL, IL1β, CD40L, HGF, ICAM1, IFNβ, IL7, IL12P70 and FGF2) in supernatants of LacZ, N17 PSOKCs or LacZ or V12 PHKCs grown alone or in co-cultures with PBMCs. Relative expression values normalized to a PBMC alone condition (dotted line). All conditions in duplicates. Error bars SEM.

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