SUPPLEMENTARY MATERIAL

Supplementary Materials and Methods

Histology, Microscopy, and Morphometric Analysis

Unless otherwise indicated, all reagents were from Sigma (St. Louis, MO) or Fisher (Waltham, MA). Tissues were fixed overnight in 10% formalin, paraffin embedded, and processed for histology (H&E). A Zeiss Axioshop microscope (Carl Zeiss Microscopy, Thornwood, NY) with SPOT Insight camera and software (HiTech Instruments, Pennsburg, PA) was used to acquire brightfield images. For epidermal thickness measurements, 5 images were taken for each sample and 4-5 measurements of the IFE were taken at standard intervals from each image using image J. Analysis was completed in xcel. Areas of cell crowding/palisading were defined when 5 or more consecutive elongated and more tightly packed basal cells that are not associated with a hair follicle or obvious processing issue with the tissue happened. They are presented as percentage of the length of total epidermis per mouse that they encompass.

Immunohistochemistry (IHC)

IHC was completed with EnVision HRP antibody system (Dako, Carpinteria, CA). Antibodies: FLAG pAb (1:1000; Sigma)(Brennan *et al.*, 2007), Anti-Dsg2 Ab10 antibody (1:200)(Brennan and Mahoney, 2009), CK17 (Gift from Pierre Coloumbe, Johns Hopkins University); Phospho-Stat3, and Phospho-Erk1/2 1:400 (Cell Signaling). Immunostaining was completed following manufacturer protocols or as previously reported.

Cell culture experiments

ASZ001 cells (kind gift from Ervin H. Epstein, Children's Hospital Oakland Research Institute, Oakland, CA) were maintained in M154 medium with 2% calcium-chelated, heat inactivated FBS; antibiotics/antimycotics; and 0.05 mM Ca^{2+} as previously reported.(So *et al.*, 2006) Cells were transfected with mDsg2.Flag in pcDNA3 or vector alone using Fugene HD (Promega, Madison, WI) according to the manufacturer's protocols. Cells were serum starved starting 24 hours post transfection and sub-confluent cells were collected 48hrs post-transfection for Western and RT-qPCR analysis. ASZ001 cells were treated with 1 µM Stattic or 20 µM ruxolitinib (Cayman Chemical, Ann Arbor, MI), 3 µM C188-9 (SelleckChem, Ely, UK) and/or 50-100 nM Vismodegib (Adooq Bioscience, Irvine, CA) in plain M154 medium for 48 hours. Sub-confluent cells were collected for Western, RT-qPCR and WST-1. For conditioned medium experiments, cells were transfected as indicated and fresh serum-free medium was added 12 hrs post transfection. Conditioned serum-free medium was collected ~48 hrs post transfection, and added to untreated (naïve) cells for 30 mins for signaling Westerns or 24 hours for RT-qPCR analysis.

RNA-extraction and qPCR

RNA was extracted from cultured using RNAeasy kit (Qiagen; Valencia, CA). DNA was removed using TURBO DNase I (Life Technologies) and cDNA was generated using High-Capacity cDNA Reverse Transcription Kit (Life Technologies). qPCR was performed on BioRad MiniOpticon with Sso Eva Green (Bio-Rad, Hercules, CA) using primers previously reported.(Brennan-Crispi *et al.*, 2015a)

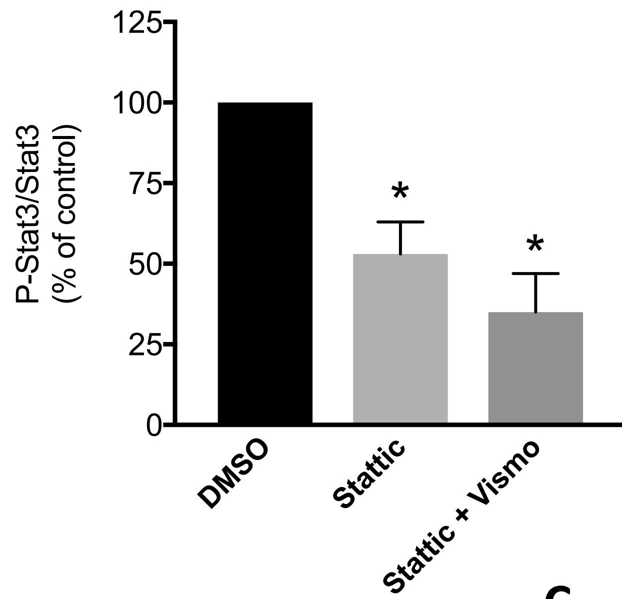
Western blotting

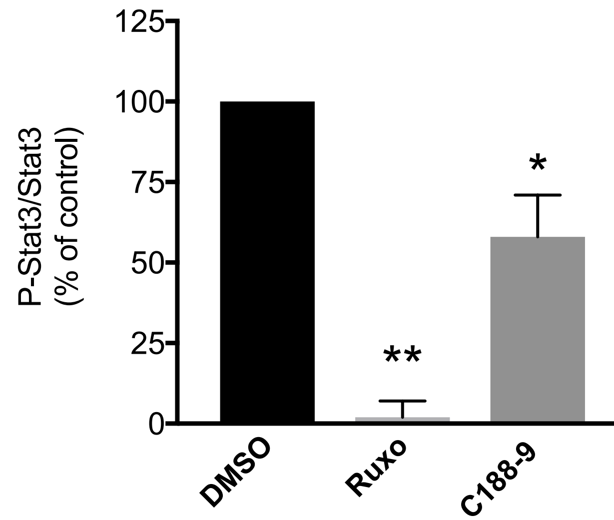
Proteins were resolved over 5-10% SDS-PAGE (Bio-Rad Laboratories), transferred to PVDF membrane, non-specific sites blocked in 5% milk in TBS/Tween-20 and incubated in primary antibodies in 5% BSA in PBS/T-20 overnight at 4°C. Membranes were washed and incubated with HRP-conjugated secondary antibodies from Jackson Immunoresearch (West Grove, PA) and signal was detected with chemiluminescence (SuperSignal West Pico). Antibodies: anti-FLAG pAb (1:1000; Sigma), GAPDH (1:300; Fitzgerald, Acton, MA), Stat3, Phospho-Stat3, and Gli1 (1:1000; Cell Signaling Technology, Danvers, MA).

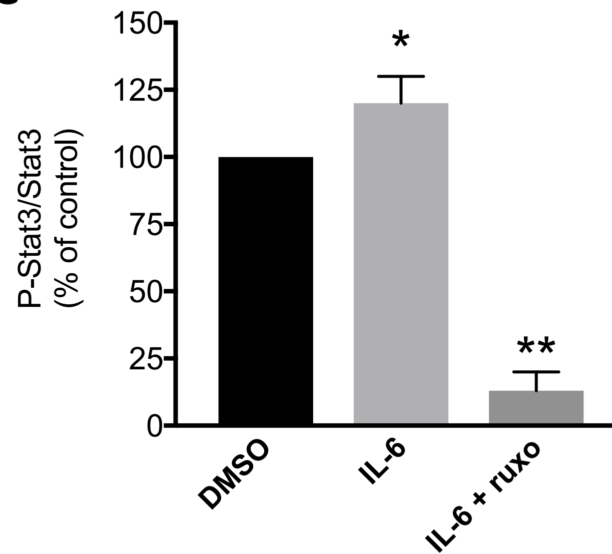
Cell viability assay

WST-1 reagent (Roche Applied Science, Indianapolis, IN) was added directly to cell medium as per manufacturer protocols. Cells were then incubated for 4.5 hours at 37°C. After 1 min shaking, absorbance at 450 nm was determined using a BioRad spectrophotometer. Readings were normalized to cell-free medium blanks.

Supplementary Figure 1. (a) Densitometric analysis of P-Stat3 levels normalized to total Stat3 in ASZ001 cells treated with vehicle (DMSO), 1 μM Stattic, or 1 μM Stattic plus 100 nM vismodegib for 48 h. (n=3; *$p<0.01$), Student's *t*-test. **(b)** Densitometric analysis of P-Stat3 levels normalized to total Stat3 in ASZ001 cells treated with 20 μM ruxolitinib (Ruxo) or 1 μM C188-9 for 48 h. (n=3; *$p<0.05$, **$P<0.0001$) **(c)** Quantification of Stat3 phosphorylation in ASZ001 cells stimulated with 10 ng/ml IL-6 or IL-6 plus 20 μM ruxolitinib (Ruxo) (n=3; *$p<0.05$, **$P<0.001$).







Supplementary Figure 1
Brennan-Crispi et al.