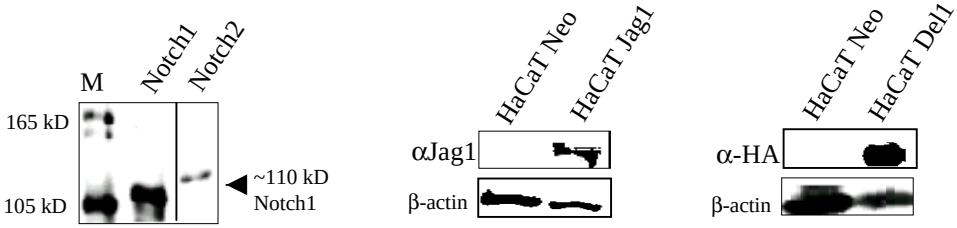
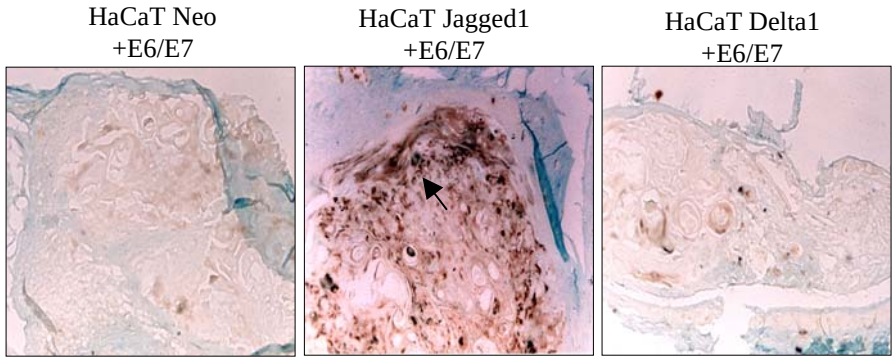
Supplementary Fig. 1

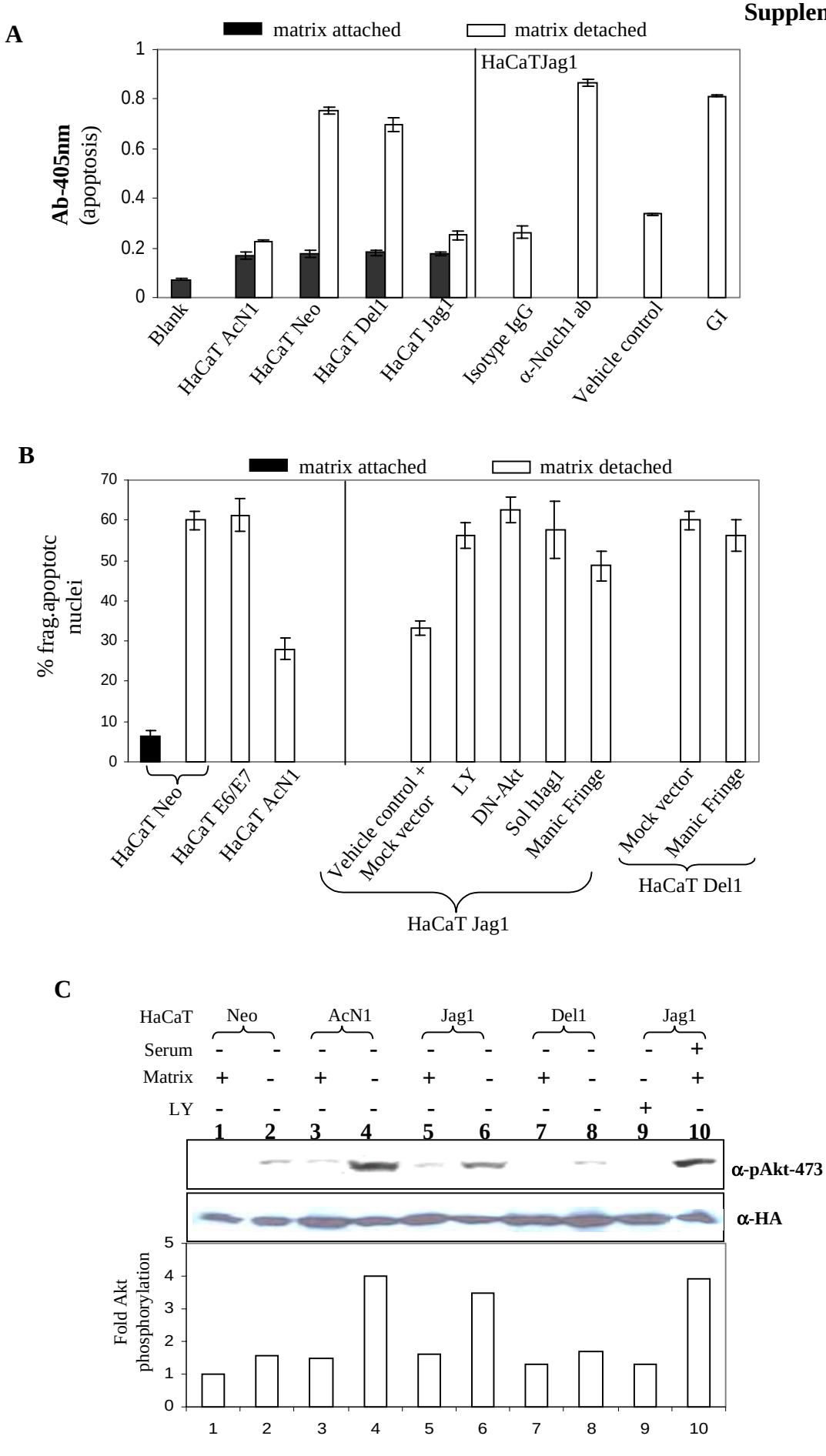
A



B

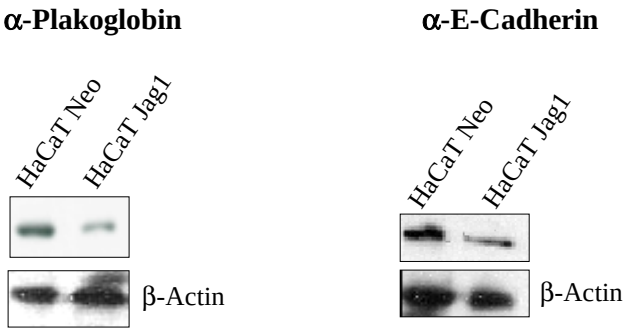


Supplementary Fig. 2



Supplementary Fig. 3

A



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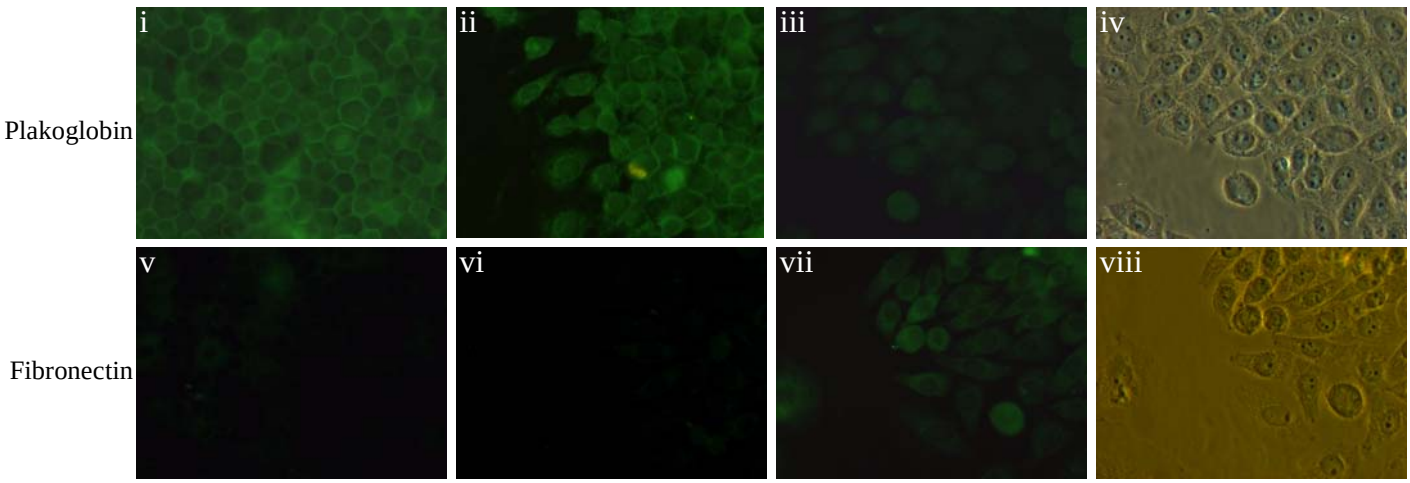
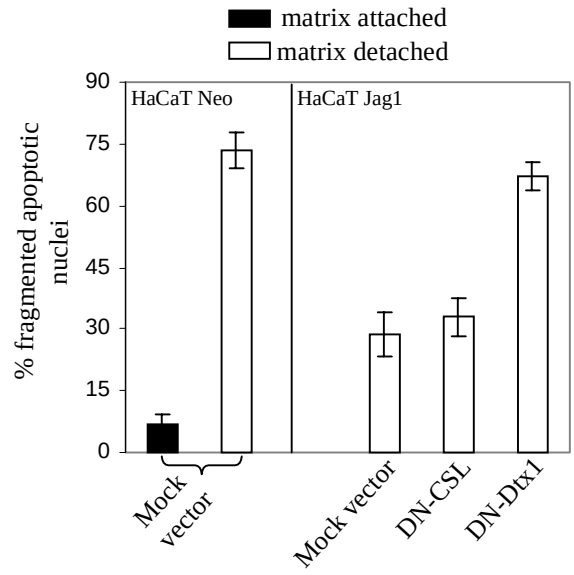
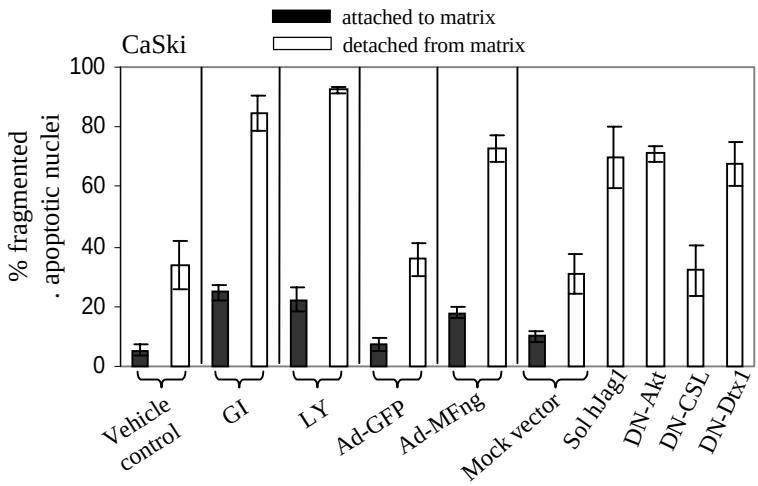
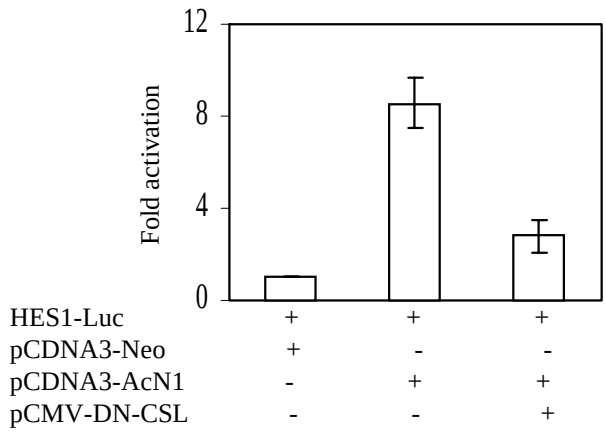


A


matrix attached
matrix detached
HaCaT Neo
HaCaT Jag1
% fragmented apoptotic nuclei
90
75
60
45
30
15
0
Mock vector
Mock vector
DN-CSL
DN-Dtx1

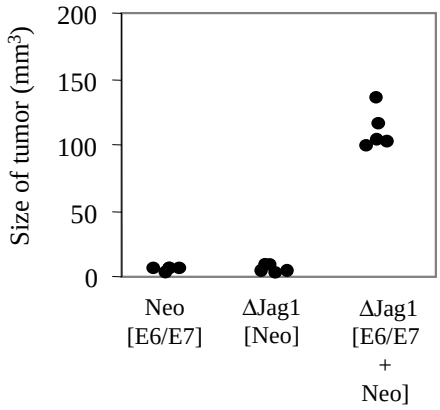

attached to matrix
detached from matrix
CaSki
% fragmented apoptotic nuclei
100
80
60
40
20
0
Vehicle control
GI
LY
Ad-GFP
Ad-MFng
Mock vector
Sol hJag1
DN-Akt
DN-CSL
DN-Dtx1

B

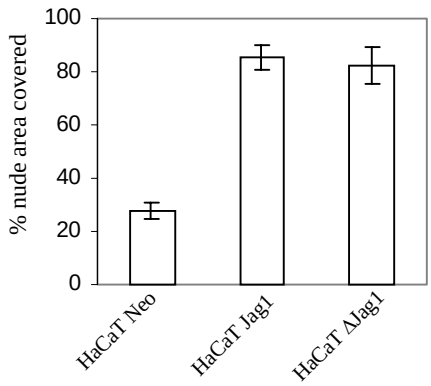

Fold activation
12
8
4
0
HES1-Luc + + +
pCDNA3-Neo + - -
pCDNA3-AcN1 - + +
pCMV-DN-CSL - - +

Supplementary Fig. 5

A



B



Supplementary Figure Legends

Supplementary Fig. 1. (A) Immunoblot on the left shows expression levels of endogenous Notch1 and Notch2 determined by immunoblotting total lysates of HaCaT cells using anti-Notch1 [1:10; bTAN20; DSHB] and anti-Notch2 [1:10; DSHB] antibodies. Immunoblots in the middle and right show stable expression of Jagged1 and HA-tagged Delta1 in mentioned cell lines, respectively. Expression was determined by immunoblotting total lysates from mentioned cells using anti-Jagged1 [1:300; sc-6011; Santa Cruz Biotech] (α-Jag1) and anti-HA antibodies [1:1000; Cell Signaling Technology] (α-HA). Appropriate HRP-conjugated secondary antibodies were used for chemiluminescent detection. As a gel-loading control, same blots were re-probed with β-actin antibody [1:4000; Sigma] and M refers to molecular weight marker lane.

(B) Representative microphotographs show levels of phospho-PKB/Akt (ser-473) in subcutaneous tumors generated by HaCaT cells expressing the mentioned combination of genes. Tumor sections derived from E6 and E7 expressing HaCaT-Neo, -Jagged1 and -Delta1 cells were immunostained with anti-phospho-PKB/Akt (ser-473) [1:1000; Cell Signaling Technology] antibody as described earlier (8). Arrow indicates areas of positive DAB staining. The counter stain is fast green. Microphotographs were taken under 20X magnification.

Supplementary Fig. 2. Expression of Jagged1 correlates with PI3K-mediated generation of anoikis resistance. (A) Histogram shows quantity of cytoplasmic histone-associated DNA fragments under matrix attached and detached conditions from HaCaT cells stably expressing mock vector (Neo), AcN1, Jagged1 (Jag1) or Delta1 (Del1) as measured by photometric enzyme-immunoassay at 405 nm. A well with no lysates was included as negative control (Blank). In a similar assay, the detached single suspension of HaCaT-Jagged1 cells were treated with either neutralizing α-Notch1 antibody targeted against the ligand-binding domain of Notch1 [11-12 EGF repeats; 1:300; Neomarkers] or 20μM of the pharmacological inhibitor of presenilin-dependent γ-secretase (GI; Calbiochem) that blocks ligand-induced proteolytic processing of Notch1. Treatments with either mouse isotype antibody (Isotype IgG) or DMSO (Vehicle control) were included as controls.

(B) Graph shows % of cells with fragmented apoptotic nuclei as measured by microscopic examination after hoechst staining in matrix attached or detached HaCaT-Neo, -E6/E7, -AcN1, -Jagged1 or -Delta1 cells expressing the mentioned gene combinations or treated with 20μM of PI3K inhibitor, LY 294002 (LY). The plasmids encoding dominant negative PKB/Akt (DN-Akt; 4μg), dominant negative human soluble Jagged1 (Sol hJag1 {pcDNA-sol Jag1}; 4μg) or Manic Fringe {pcDNA3-MFng; 4μg} transfected cells were identified using a tracer GFP expressing vector (1μg) by cotransfection microscopic assay as described earlier (33). 100 GFP positive cells were scored for apoptotic features. Each bar in both (A) and (B) histograms represent the mean ± standard error of three independent experiments.

(C) Immunoblot shows the levels of phosphorylated-PKB/Akt (pAkt-ser-473; Cell Signaling Technology) in total lysates prepared from HaCaT cells stably expressing mentioned genes that were transiently transfected with HA-tagged PKB/Akt (33) and subjected to 2 hours of matrix withdrawal under reduced serum conditions (0.2 % serum). Blot was reprobed with anti-HA antibody to normalize for HA-Akt (α-HA) expression. The graph below shows the normalized fold Akt phosphorylation. As a positive control 10 % serum was added 5 minutes prior to lysis in HaCaT-Jagged1 cells (lane 10). In lane 9, HaCaT-Jagged1 cells were treated with 20μM LY following detachment from matrix.

Supplementary Fig. 3. (A) immunoblots reveal the analysis of protein lysates prepared from HaCaT cells stably expressing mock vector (HaCaT-Neo) or Jagged1 (HaCaT-Jag1) 20 hours post wounding for the expression of plakoglobin [1:200; Sigma](left panel) and E-cadherin [1:100; Calbiochem](right panel). As a gel-loading control, same blots were re-probed with β-actin antibody. (B) Representative photomicrographs (40X magnification) show alterations in the expression of EMT markers in SiHa cells at 40 hours. Immunofluorescence staining of plakoglobin [1:300; Sigma] and fibronectin [1:300; Sigma] is shown in SiHa cells.Immunofluorescence staining of plakoglobin (B, i) and fibronectin (B, v) in confluent monlolayer culture of SiHa cells are shown. Expression levels of plakoglobin (B, ii) and fibronectin (B, vii) by 24 hours are shown. Downregulation of plakoglobin (B, iii) and upregulation of fibronectin (B, vii) in SiHa cells at 40 hours near the wound healing edge are shown. (B, iv, viii) represent the bright field images of the wound healing edge.

Supplementary Fig. 4. Jagged1-induced PI3K activation and anoikis resistance is independent of CSL-mediated Notch signaling. (A) Graph on the top shows percentage (%) of cells with fragmented apoptotic nuclei as measured by microscopic examination after hoechst staining in matrix attached or detached HaCaT-Neo or -Jagged1 cells transiently transfected with 4μg of mentioned plasmids [pcDNA 3.0-Neo (mock vector) or dominant negative CSL (DN-CSL) {pcDNA3-DN CSL} or dominant negative Dtx1 (DN-Dtx1) {pEF-BOS-HA-hDxI-II}]. Graph below shows % of cells with fragmented apoptotic nuclei as measured by microscopic examination after hoechst staining in matrix attached or detached CaSki cells either; treated with DMSO (vehicle control), 20μM GI, 20μM LY; or infected with equal titre of recombinant adenovirus encoding for GFP (Ad-GFP), Manic Fringe (Ad-MFng) (41); or transiently transfected with 4μg of mentioned plasmids [pCDNA 3.0-Neo (mock vector), dominant negative human soluble Jagged1 (Sol hJag1 {pcDNA-sol Jag1}; 4μg), dominant negative PKB/Akt (DN-Akt; 4μg), dominant negative CSL (DN-CSL), dominant negative Dtx1 (DN-Dtx1)]. Transfected cells were identified using a tracer GFP expressing vector (1μg) by cotransfection microscopic assay as described earlier (33). 100 GFP positive cells were scored for apoptotic features. Each bar in both graphs represents the mean ± standard error of three independent experiments.

(B) CSL-dependent Notch signaling is inhibited by DN-CSL contstruct. HaCaT cells were transfected with Notch/CSL-responsive promoter reporter (HES1-Luc reporter; 1μg). This reporter contains a luciferase gene driven by the HES1 promoter that harbors two CSL-binding sites (gift of A. Israel). Activation of Notch signaling results in transcriptional upregulation of this HES1-Luc reporter. In addition to the reporter construct, cells were cotransfected with 3μg of mentioned plasmids [mock vector (pCDNA3-Neo), pCDNA3-AcN1, pCMV-DN-CSL]. The graph shows that expression of AcN1 resulted in 8-fold increase in the reporter activity compared to mock vector. Coexpression of DN-CSL along with AcN1 strongly inhibited the reporter activity from 8 to 2-fold. Each bar in the graph represents the mean ± standard error of three independent experiments. Transfection efficiency was normalized using the dual luciferase assay system (Promega) as described earlier (41).

Supplementary Fig. 5. (A) HaCaT cells stably expressing mock vector (Neo) or c-terminus PDZ-binding motif mutant Jagged1 (ΔJag1) {pcDNA-J1$^{\Delta PL}$; gift of A.Capobianco} (3) were transiently transfected with the plasmid combinations (total of 10μg) encoding for either bicistronic HPV-16 E6 and E7 (E6/E7) {pMSIIref-E6/E7} (gift of M. Conrad-Stoppler and H. Stoppler) or neomycin (Neo) {pcDNA3.0-Neo}, and injected subcutaneously into nude mice. After 3 weeks, the mice were sacrificed, and tumor sizes were measured as described previously (8). Graph shows tumor volume after 3 weeks and each dot represents a tumor from one mouse.

(B) Graph represents the percentage wounded nude area covered at 24 hours by HaCaT cells stably expressing mock vector (Neo) or Jagged1 (Jag1) or c-terminus PDZ-binding motif mutant Jagged1 (ΔJag1) {pcDNA-J1$^{\Delta PL}$} (3) in *in vitro* wound-healing assay. The % wound area covered was measured using a Nikon inverted microscope and the associated Image-pro Plus software. Graph shows that HaCaT-ΔJagged1 covers 80% of the wounded nude area by 24 hours. The results shown represent the means ± standard deviation of the results from three independent experiments.