Figures and figure supplements

TCF7L1 promotes skin tumorigenesis independently of β-catenin through induction of LCN2

Amy T Ku et al
Figure 1. TCF7L1 is overexpressed in papillomas and skin SCC. (A) Immunofluorescence analyses of frozen sections of normal skin, papilloma and SCC induced by DMBA/TPA using antibodies against TCF7L1 (green) and β4-integrin (red). (B) Immunohistochemistry of untreated abdomen epidermis and UV-induced papilloma and SCC with antibody against TCF7L1. Bar denotes 50 μm. DOI: 10.7554/eLife.23242.002
Figure 2. TCF7L1 overexpression increases tumor incidence and multiplicity and accelerates malignancy progression in the mouse model of skin SCC. (A) Experimental scheme testing the role of TCF7L1 in tumorigenesis. Eight-week-old tet-inducible Tcf7l1 (KRT14-rtTA;TRE-Tcf7l1) and control (KRT14-

Figure 2 continued on next page
rtTA or TRE-Tcf7l1) mice were put on a doxycycline-containing diet one week prior to DMBA treatment and for the remainder of the experiment. Shaved backskins were topically treated once with DMBA and then twice weekly with TPA for 25 weeks. (B–D) Effect of TCF7L1 overexpression under a high dose of DMBA (100 nmol) and TPA (10 nmol) on skin tumorigenesis. n = 17 (KRT14-rtTA;TRE-Tcf7l1), n = 16 (KRT14-rtTA or TRE-Tcf7l1). (B) Tumor incidence. **p<0.01. (C) Average number of tumors per mouse. (D) Percentage of mice with SCC. p=0.08. (E–G) Effect of TCF7L1 overexpression under a low dose of DMBA (25 nmol) and TPA (1 nmol) on skin tumorigenesis. n = 16 (KRT14-rtTA;TRE-Tcf7l1), n = 21 (KRT14-rtTA or TRE-Tcf7l1). (E) Tumor incidence. ***p<0.001. (F) Average number of tumors per mouse. (G) Percentage of mice with SCC. ***p<0.001. (H–J) Effect of TCF7L1 overexpression on skin tumorigenesis following a low dose of DMBA without TPA treatment. 8-week-old tet-inducible Tcf7l1 (KRT14-rtTA;TRE-Tcf7l1) and control (KRT14-rtTA or TRE-Tcf7l1) mice were put on a doxycycline-containing diet one week prior to DMBA treatment and for the remainder of the experiment. Shaved backskins were topically treated once with low dose DMBA (25 nmol) and then twice weekly with an acetone vehicle control in place of TPA. n = 22 (KRT14-rtTA;TRE-Tcf7l1), n = 26 (KRT14-rtTA or TRE-Tcf7l1). (H) Tumor incidence. TCF7L1-induced versus control: *p<0.05. (I) Average number of tumors per mouse. (J) Percentage of mice with SCC. TCF7L1-induced versus control: p=0.1198.

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Control TCF7L1-induced

Ki67

MYC-TCF7L1

β4

epidermis

HF

epidermis

HF

epidermis

HF

1 month

1 month

1 month

1 month

5 days

5 days

5 days

5 days

Figure 2—figure supplement 1. Prolonged induction of TCF7L1 in adult skin results in hyperproliferation. (A–B) Immunofluorescence image of Ki-67 (green) and myc-TCF7L1 (red) expression in adult skins from control (KRT14-rtTA) or tet-inducible Tcf7l1 (KRT14-rtTA;TRE-Tcf7l1) mice that were placed on a doxycycline-containing diet for 1 month. (C) Immunofluorescence image of KRT6 (green) and myc-TCF7L1 (red) expression in adult skins from control (KRT14-rtTA) or tet-inducible Tcf7l1 (KRT14-rtTA;TRE-Tcf7l1) mice that were placed on a doxycycline-containing diet for 1 month. (D) Histology image of epidermis (epi) and dermis (der) in control (left) and TCF7L1-induced (right) adult skin.
Figure 2—figure supplement 1 continued

Five days (A) or one month (B) β-4 integrin (magenta) marks the basement membrane, which marks the border separating the epidermis (epi) and hair follicle (HF) from the dermis (der). (C) Immunofluorescence image of Keratin 5 (K5) (red) and Keratin 6 (K6) (green) expression and H and E image (D) of skin sections from mice that were on the doxycycline-containing diet for one month. Bar denotes 50 μm.

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Figure 2—figure supplement 2. Myc epitope-tagged TCF7L1 is induced in skins and tumors of tet-inducible mice on a doxycycline-containing diet. Normal skins from control (KRT14-rtTA) or TCF7L1-induced (KRT14-rtTA;TRE-Tcf7l1) mice along with papilloma and SCC samples from TCF7L1-induced mice were analyzed by immunofluorescence with antibodies against myc-epitope to detect myc-TCF7L1 (green). β4-integrin (red) marks the basement membrane. Bar denotes 50 μm. DOI: 10.7554/eLife.23242.005
Figure 2—figure supplement 3. Tet-inducible TCF7L1 is tightly regulated and not sufficient to promote tumor formation. (A–B) Eight-week-old tet-inducible TCF7L1 (KRT14-rtTA; TRE-Tcf7l1) and control (KRT14-rtTA or TRE-Tcf7l1) mice were maintained on a doxycycline-free diet and their backs were treated with a high dose of DMBA (100 nmol) and TPA (10 nmol). n = 17 (KRT14-rtTA; TRE-Tcf7l1), n = 16 (KRT14-rtTA or TRE-Tcf7l1). (A) Tumor incidence. TCF7L1-uninduced versus control: *p=0.92. (B) Average number of tumors per mouse. (C–D) 8-week-old tet-inducible Tcf7l1 (KRT14-rtTA; TRE-Tcf7l1) and control (KRT14-rtTA or TRE-Tcf7l1) mice were placed on doxycycline-containing diet and their backs were treated with an acetone vehicle control in place of DMBA/TPA. n = 20 (KRT14-rtTA; TRE-Tcf7l1), n = 29 (KRT14-rtTA or TRE-Tcf7l1). (C) Tumor incidence. (D) Average number of tumors per mouse.

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Figure 3. Downregulation of TCF7L1 and TCF7L2 reduces human skin SCC growth. (A) Expression of TCF7L1 and TCF7L2 in adult human keratinocytes (KER) and different human SCC cell lines, including SCC12, SCC13, SRB1 and SRB12. The human skin SCC cell line SCC13 was transduced to express tet-inducible control shRNA (shNS) or shRNAs against TCF7L1 and/or TCF7L2. After drug selection, transduced cells were treated with doxycycline for five days prior to mRNA and protein isolation for (B) Real time PCR analysis or (C) Western analysis. The transduced cells with effective shRNAs were xenografted into flanks of immunodeficient mice, which were then put on a doxycycline-containing diet. Tumors were isolated and analyzed at the end of eight weeks. (D) Mass of tumors derived from SCC cells expressing control shRNAs (shNS), shRNAs against TCF7L1 (shTCF7L1#1), shRNAs against TCF7L2 (shTCF7L2#1), or both (shTCF7L1#1+shTCF7L2#1). (E) Immunohistochemical analysis of TCF7L1 and TCF7L2 expression in xenografted tumors derived from SCC cells expressing both TCF7L1 and TCF7L2 (shTCF7L1#1+shTCF7L2#1). Bar denotes 100 μm. DOI: 10.7554/eLife.23242.007
**Figure A**

MTT Absorbance (570nm) over time (days) for shNS, shTCF7L1#1, and shTCF7L2#1. The graph shows a significant increase in absorbance over time for shTCF7L1#1 and shTCF7L2#1 compared to shNS.

**Figure B**

Images of shNS, shTCF7L1, and shTCF7L1 + shTCF7L2 treatments stained for Ki67 and KRT5. The images illustrate cell proliferation and differentiation markers.

**Figure C**

Bar graph showing relative mRNA expression of TCF7L1 and TCF7L2 in shNS, shTCF7L1, and shTCF7L1 + shTCF7L2 treatments. TCF7L1 expression is significantly upregulated in shTCF7L1 and shTCF7L1 + shTCF7L2 compared to shNS.

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**Figure 3—figure supplement 1.** Silencing TCF7L1 and TCF7L2 in SCC cells reduces proliferation in vitro and in vivo. (A) Silencing TCF7L1 and TCF7L2 reduces SCC cell proliferation in vitro. Human SCC (SCC13) cells expressing inducible shRNA against TCF7L1 and TCF7L2 (shTCF7L1#1+shTCF7L2#1) or nonspecific control (shNS) were treated with doxycycline the following morning after plating, indicated as day 0. Cell growth curve was recorded for seven days using MTT assay. For each time point, 10 μL MTT at 5 mg/mL was added to 90 μL of DMEM media and incubated for 4 hr before DMSO lysis for 15 min in room temperature, followed by absorbance measurement at 570 nm. Data are presented as mean ± s.d. **p<0.01, ***p<0.001 (Student's T test). (B) Xenografted tumors expressing shRNAs against TCF7L1 and TCF7L2 have fewer proliferative cells. Immunofluorescence images of xenografted tumors expressing control shRNA or shRNAs against both TCF7L1 and TCF7L2 that were immunostained with antibodies against the proliferative marker Ki67 (green) and epithelial marker Keratin 5 (red). Bar denotes 100 μm. (C) Real Time PCR quantifying the relative mRNA level of TCF7L1 and TCF7L2 in xenografted tumors expressing control shRNA (n = 4) or shRNAs against TCF7L1 and TCF7L2 (n = 2).

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Figure 4. TCF7L1 stimulates tumor growth and increases tumorigenic capacity. (A) TCF7L1 promotes xenografted tumor growth. Human skin SCC cell line SRB12 transduced with control vector or vector expressing tet-inducible TCF7L1 was injected into nude mice. (B) Summary of tumor growth in mice injected with vector or Tcf7l1. (C) Ki67 and BrdU staining of control and TCF7L1-induced tumors. (D) Ki67+ cell density in control and TCF7L1-induced tumors.
Figure 4 continued

Tcf7l1 were drug selected and expanded. 1 million cells were xenografted onto NSG mice. Grafted mice were put on doxycycline-containing chow the next day. Tumor weight was measured at the end of 10 weeks. The grafted tumor mass data are presented as box and whisker plots where boxes span first and third quartiles, bars as the median values, and whiskers as minimum and maximum of all data. n = 5. ***p<0.001 (Student T test). (B) Limiting-dilution transplantation assay from SRB12 cells with or without TCF7L1 overexpression. Transduced SRB12 cells were grafted onto NSG mice at serial diluted cell numbers as indicated. Mice were put on doxycycline-containing chow the next day and were given a pulse of BrdU 3 hr prior to tumor isolation at the end of 10 weeks. A presence of a tumor is scored when it is over 10 mg. (C) Immunofluorescence analysis of expression of induced TCF7L1 and proliferative markers BrdU (top panel) and Ki67 (bottom panel) with Keratin 5 marking epithelial cells. Bar denotes 100 μm. (D) Quantification of Ki67 positive cells in a minimum of 5 fields per tumor and three tumors/group. *p<0.05 (Student T test).

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Figure 5. TCF7L1 promotes tumor growth independently of β-catenin binding. The human skin SCC cell line SCC13 was transduced with pINDUCER21 lentiviral vectors expressing GFP alone or GFP with tet-inducible, myc-tagged murine Tcf7l1 or Tcf7l1 deletion mutants. After enrichment by FACS, the transduced cells were xenografted into flanks of immunodeficient mice, which were then put on a doxycycline-containing diet. (A) TCF7L1 and its mutant versions are schematized with amino acid deletion and point mutation annotated. (B) Immunofluorescence analysis of myc-TCF7L1 expression in cells overexpressing TCF7L1 or its mutants. Bar denotes 50 μm. (C) Western analysis of protein expression against myc epitope in transduced cells. (D) Graph quantifying the mass of tumors derived from SCC cells with vector or the overexpression of Tcf7l1 or its mutants. n = 12 (vector), n = 19 (Tcf7l1), n = 15 (Tcf7l1ΔN), n = 7 (Tcf7l1ΔG), n = 4 (Tcf7l1*). The grafted tumor mass data is presented as box and whisker plots where boxes span first and third quartiles, bars as the median values, and whiskers as minimum and maximum of all data **p<0.01 (One-way ANOVA with Dunnett’s post-hoc test).

Figure 5 continued on next page
**Figure 5—figure supplement 1.** Activity of TCF7L1, TCF7L1 deletion mutants and TCF7L2 on TCF/β-catenin responsive promoter (TOPFlash). Mouse keratinocytes that were transduced and drug selected to express tet-inducible Tcf7l1, Tcf7l1 deletion mutants, or Tcf7l2 were transiently co-transfected with TOPFlash reporter plasmids and TK-Renilla as internal control. They were also co-transfected with KRT14 empty vector or KRT14-ΔNβcat construct. Cells were treated with doxycycline treatment the following day and luminescence was recorded 72 hr post transfection.

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**Figure 6.** TCF7L1 suppresses HRAS$^{G12V}$-induced senescence independently of β-catenin interaction. Mouse primary keratinocytes were transduced to express tet-inducible Tcf7l1 or Tcf7l1 deletion mutants. After cell line establishment by G418 selection, cells were re-infected to express oncogenic HRAS$^{G12V}$ vector or HRAS$^{G12V}$ + Tcf7l1. 

A) Bright field SA-β-gal activity.

B) Side scatter SSC-A (x1000) and fluorescence (488 nm) for vector, HRAS$^{G12V}$, HRAS$^{G12V}$ + Tcf7l1, and HRAS$^{G12V}$ + Tcf7l1ΔNβ.

C) MFI (median fluorescent intensity) for fluorescence (488 nm).

D) MFI (median fluorescent intensity) for Side scatter SSC-A (x1000).

E) MFI (median fluorescent intensity) for fluorescence (488 nm) for HRAS$^{G12V}$, Tcf7l1, ΔNβcat, Tcf7l1ΔG, and Tcf7l1ΔNβ.
HRAS<sup>G12V</sup> or pBabe-puro vector as control. Cells were selected with puromycin, treated with doxycycline two days post-infection, and analyzed 5–6 days post-infection as indicated. (A) Fluorescent detection of SA-β-gal activity five days post HRAS<sup>G12V</sup> infection. Transduced cells were incubated with β-gal substrate C<sub>12</sub>FDG (5-Dodecanoylaminofluorescein Di-β-D-Galactopyranoside), which is cleaved by β-gal to release a fluorescent product that is retained by the cells. Bar denotes 200 μm. (B) Flow cytometry analysis of SA-β-gal activity in mouse keratinocytes with or without Tcf7l1 overexpression six days post HRAS<sup>G12V</sup> infection. pBabe-transduced cells with C<sub>12</sub>FDG incubation serve as control. Cells were pretreated with 300 μM chloroquine for lysosomal neutralization followed by C<sub>12</sub>FDG substrate incubation for another 2 hr. Samples were then analyzed with Arial II flow cytometer using a 488 nm filter. Results were represented in dot plot (x axis, C<sub>12</sub>FDG fluorescent intensity; y axis, side scatter in area) after size exclusion. The threshold to define SA-β-gal positive cells is set by 5% gate in the control sample as presented in green population. (C) SA-β-gal activity of each sample in B is indicated by median fluorescent intensity (MFI) after size exclusion. (D) Flow cytometry analysis of SA-β-gal activity in HRAS<sup>G12V</sup>-transduced mouse keratinocytes with the overexpression of Tcf7l1 or Tcf7l1 mutants. (E) Flow cytometry analysis of SA-β-gal activity in HRAS<sup>G12V</sup>-transduced mouse keratinocytes with the overexpression of ΔN-βcat, Tcf7l1, or both. Data is presented as mean ± s.d. *p<0.05, **p<0.01, ***p<0.001 (One way ANOVA with Dunnett’s post-hoc test). DOI: 10.7554/eLife.23242.012
Figure 6—figure supplement 1. TCF7L1 overrides HRAS$^{G12V}$-induced senescence in fibroblasts. (A) Measurement of SA-β-gal activity by X-gal staining at pH 6.0 in fibroblasts transduced to express oncogenic HRAS$^{G12V}$ with or without TCF7L1 overexpression. Bar denotes 200 μm. (B) Quantification of SA-β-gal positive cells. Data are presented as mean ± s.d. ***p<0.001 (One-way ANOVA).

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Figure 6—figure supplement 2. ΔNβcat has no effect on HRAS<sub>G12V</sub>-induced senescence in keratinocytes. Tcf7l1-inducible primary keratinocytes were transduced to express oncogenic HRAS<sub>G12V</sub> and then re-transduced with pLenti-RFP or pLenti-RFP-ΔNβcat after two days. After drug selection and/or doxycycline treatment for additional four days, the transduced cells were sorted on the basis of RFP fluorescence. (A) Measurement of SA-β-gal activity by flow cytometry analysis in control or HRAS<sub>G12V</sub>-expressing mouse keratinocytes with overexpression of ΔNβcat, TCF7L1, or both. Cells were pretreated with 300 μM chloroquine for lysosomal neutralization followed by C<sub>12</sub>FDG substrate incubation for another 2 hr. Samples were then analyzed with Aria II flow cytometer using a 488 nm filter. pBabe-transduced cells with C<sub>12</sub>FDG incubation serve as control. Results were represented in dot plot (x axis, C<sub>12</sub>FDG fluorescent intensity; y axis, side scatter in area). The percentage of positive cells over 5% background threshold in control is indicated. (B) Immunofluorescence image of keratinocytes transduced to express pLenti-RFP or pLenti-RFP-ΔNβcat that were immunostained for β-catenin expression (green). Arrowheads point to nuclei of RFP-positive transduced cells. Bar denotes 100 μm.

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Figure 7. TCF7L1 promotes cell migration in SCC cells independently of β-catenin binding. The human skin SCC cell lines SCC13 and SRB12 were transduced with tet-inducible Tcf7l1 or Tcf7l1 deletion mutants. After drug selection, the transduced cells were treated with doxycycline for 48 hr and their migratory ability was measured using the Boyden chamber-based cell migration assay. Bar denotes 200 μm. (A) Representative images of migrated SCC13 cells stained with crystal violet. (B–C) Quantification of migrated SCC cells, SCC13 (B) or SRB12 (C), per 5X field. Data are presented as mean ± s.d. *p<0.05, **p<0.01, ***p<0.001 (One-way ANOVA with Dunnett’s post-hoc test).

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Figure 8. Overexpression of TCF7L2 promotes tumor growth, increases cell migration, and represses HRAS$^{G12V}$-induced senescence similarly to TCF7L1. The human skin SCC cell line SCC13 was transduced to express tet-inducible, myc-tagged TCF7L1 or TCF7L2. After G418 drug selection, transduced cells were grafted into flanks of immunodeficient nude mice, which were then put on a doxycycline containing diet for eight weeks. (A) Graph quantifying the mass of tumors derived from SCC cells with vector or the overexpression of Tcf7l1 or Tcf7l2. n = 11 (vector), n = 18 (Tcf7l1), n = 4 (Tcf7l2). The grafted tumor mass data is presented as box and whisker plots where boxes span first and third quartiles, bars as the median values, and whiskers as minimum and maximum of all data. **p<0.01, ***p<0.001 (One-way ANOVA with Dunnett’s post-hoc test). (B–C) Boyden chamber-based cell migration assay. SCC cells were treated with doxycycline for 48 hr to overexpress TCF7L1 or TCF7L2. (B) Representative image of migrated SCC13 cells stained with crystal violet. Bar denotes 200 μm. (C) Quantification of migrated SCC13 or SRB12 SCC cells per 5X field. Data are presented as mean ± s.d. ***p<0.001 (One-way ANOVA with Dunnett’s post-hoc test). (D) Flow cytometry analysis of SA-β-gal activity in HRAS$^{G12V}$-transduced mouse keratinocytes with the overexpression of TCF7L1 or TCF7L2. The SA-β-gal activity of each sample is indicated by median fluorescent intensity (MFI) with 5000 cells (after size exclusion) per sample in duplicates. Data are presented as mean ± s.d. **p<0.01 (One-way ANOVA). Note that the overexpression of TCF7L2 experiments were performed together with the overexpression of TCF7L1 experiments, whose data were shown in Figures 5–7.

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Figure 9. TCF7L1 overexpression alters transcriptional landscape in SCC cells. The human skin SCC cell line SCC13 was transduced with lentiviral vectors expressing tet-inducible Tcf7l1 or Tcf7l1 deletion mutants. After drug selection, the transduced cells were treated with doxycycline for 48 hr and Figure 9 continued on next page.
RNA was harvested for gene expression profiling by RNAseq. **(A)** Heat map of genes differentially expressed in SCC13 cells with vector, overexpression of Tcf7l1 or Tcf7l1 deletion mutants. Gene list is ordered by clustering. Induced gene sets are shown in red, and repressed gene sets are shown in green. **(B)** Venn diagram of genes induced (as indicated in red) or repressed (as indicated in green) by both TCF7L1 and TCF7L1ΔN but not TCF7L1*. Numbers of overlaid genes were annotated across groups. Differentially expressed genes from each group were those with significant difference (q < 0.05) above the threshold, log₂(Fold Change)>1 or <-1. **(C)** Biological process and KEGG pathway analyses of differentially expressed genes sets in B. Gene set analysis (GSA) was performed using Enrichr tool (Chen et al., 2013). The list includes up to 10 significantly involved terminologies (p<0.05) for GO ontology (biological process) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. List of top 20 genes significantly induced or repressed by TCF7L1 and TCF7L1ΔN but not TCF7L1* in **(D)** Volcano plot and in **(E)** Table with corresponding fold change. Outliers were excluded from the list.

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Figure 10. TCF7L1 promotes tumor growth through induction of LCN2. (A) Real time PCR analysis of expression of LCN2 in SCC13 and SRB12 cells that were transduced to express Tcf7l1 and its various mutants. (B) Real time PCR analysis of Lcn2 in enriched epithelial cells from mouse normal skin, Figure 10 continued on next page
Figure 10 continued

DMBA/TPA-induced papilloma and SCC. (C) Real-time PCR analysis of LCN2 in SRB12 cells that were transduced to express shRNA against nonspecific sequence (shNS) or two different sets of shRNAs against LCN2. (D) Western analysis of LCN2 in SRB12 cells that were transduced to express empty vector control or Tcf7l1 with shRNA against nonspecific sequence or two different sets of shRNAs against LCN2. Drug selected transduced cells expressing shRNAs were grafted onto NSG mice and tumors were isolated at the end of eight weeks. (E) Quantification of tumor mass. Data are presented as box and whisker plots where boxes span first and third quartiles, bars as the median values, and whiskers as minimum and maximum of all data. *p<0.05, **p<0.01, ***p<0.001 (One-way ANOVA with Dunnett’s post-hoc test). n = 5 (shNS+vector), n = 4 (shLCN2#1+vector), n = 2 (shLCN2#2+vector), n = 3 (shNS+Tcf7l1), n = 3 (shLCN2#1+Tcf7l1), n = 3 (shLCN2#2+Tcf7l1). (F) Representative images of tumors. (G) Quantification of cell migration in transwell assay. Human SCC (SRB12) cells with tet-inducible Tcf7l1 were treated with doxycycline for 48 hr and their migratory ability was measured using the Boyden chamber-based cell migration assay. Cells were allowed to migrate toward the lower compartment containing media with 10% FBS as chemoattractant, supplemented with neutralizing antibody against LCN2 or mouse IgG isotype control. After 30 hr, migrated cells were fixed by 4%PFA, counterstained with crystal violet, and quantified. Data are presented as mean ± s.d. **p<0.01 (One-way ANOVA with Dunnett’s post-hoc test).

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**Figure 10—figure supplement 1.** LCN2 is induced by TCF7L1 and is overexpressed in murine skin papilloma and SCC. (A) Immunofluorescence images of LCN2 staining in control and TCF7L1-induced skin. Control or tet-inducible Tcf7l1 mice were on a doxycycline containing diet for five days and their skins were immunostained for LCN2 (green) and basement membrane marker β4-integrin (red). (B) Images of immunohistochemical staining of LCN2 in normal mouse skin, and DMBA/TPA-induced papilloma and skin SCC. Bar denotes 100 μm.

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Figure 10—figure supplement 2. TCF7L1 and LCN2 are expressed in human skin SCC. (A) Representative images of negative and positive immunohistochemical staining of TCF7L1 and LCN2 in human skin SCC (middle and bottom panels) and adjacent normal skin (top). Bar denotes 100 μm.
Figure 10—figure supplement 2 continued

µm. (B) Graph summarizing the expression of TCF7L1 and LCN2 in human skin SCC samples. (C) Graph summarizing the expression of LCN2 in TCF7L1-subgrouped human skin SCC samples (n = 16).

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Overexpression of TCF7L1 increases neutrophil infiltration independently of LCN2. Xenografted tumors (n = 3) expressing empty vector or vector expressing tet-inducible Tcf7l1 with or without shLCN2#1 were dissociated and analyzed by flow cytometry for CD11b+Ly6G+Ly6C+ (neutrophils) and CD11b +F4/80+ (macrophages). Representative plots showing the gating of (A) CD11b+Ly6G+Ly6C+ and (B) CD11b +F4/80+. Graph quantifying the percentage of total cells that express (C) CD11+Ly6G+Ly6C+ and (D) CD11b +F4/80+. Data are presented as mean ± s.d. **p<0.05 (One-way ANOVA with Dunnett’s post-hoc test).

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