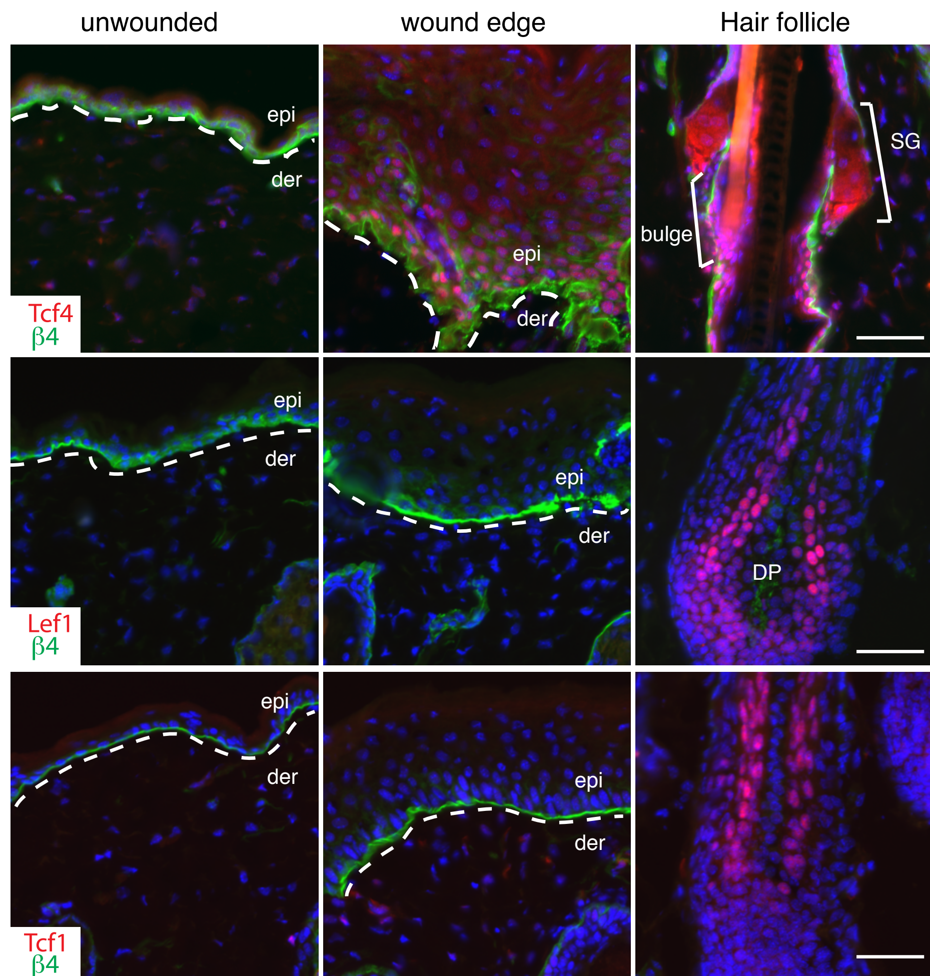
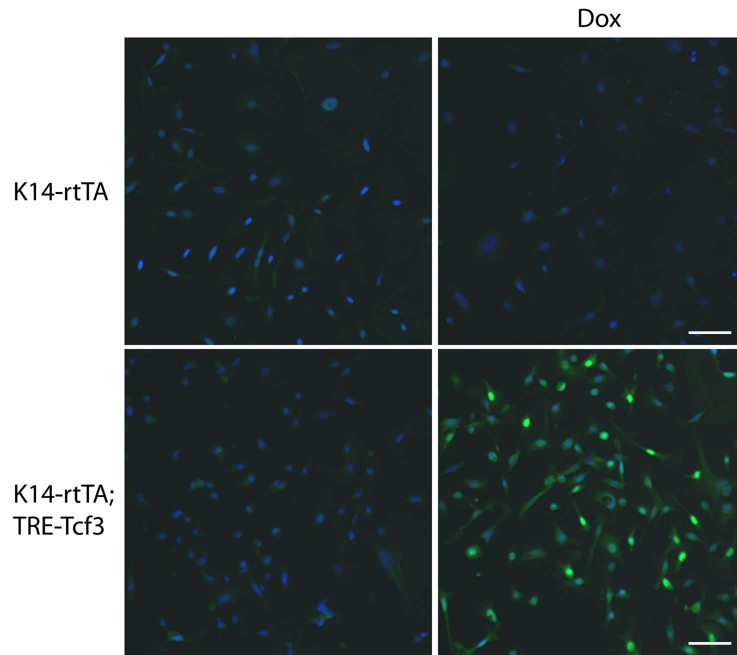
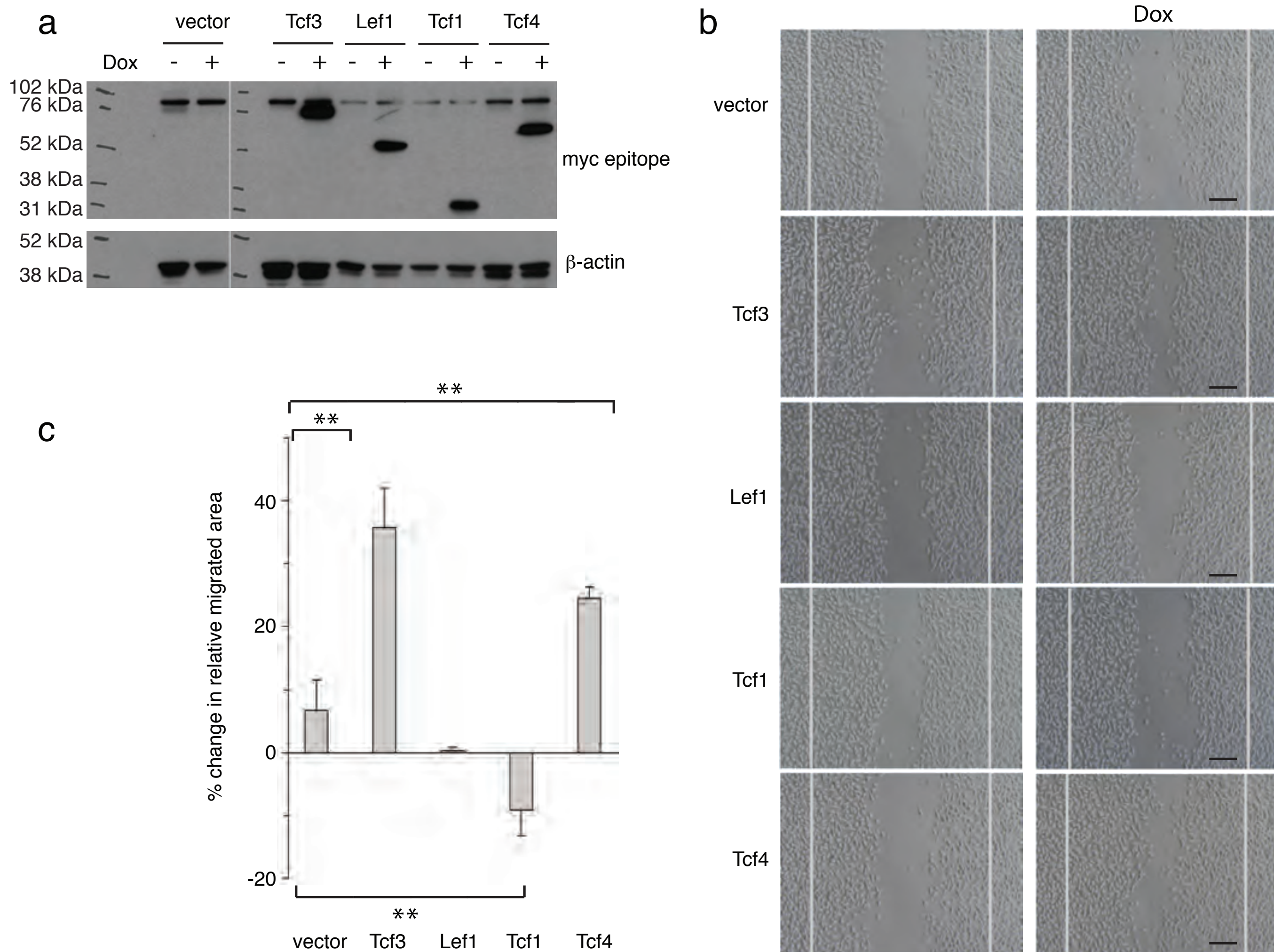


Supplementary Figure 1. Tcf4 is also induced at the skin wound edge.
Images of immunofluorescence analysis of wounded and unwounded skins with hair follicles as positive control. Full thickness wounds were created on dorsal skins of 10-week old mice and isolated 5 days post wounding. Skins containing the wound sites were embedded in OCT, sectioned and analyzed by immunofluorescence with antibodies against Tcf4, Lef1, Tcf1 and integrin β4 (color coding according to the secondary abs). Unwounded skins from the same mice were used as unwounded controls and hair follicles were used as positive controls. Bar denotes 50µm.



Supplementary Figure 2. Keratinocytes isolated from *K14rtTA;TRE-Tcf3* skin express tet-inducible Tcf3.
Immunofluorescence images of keratinocytes expressing tet-inducible myc-tagged Tcf3. Keratinocytes were isolated from tet-inducible Tcf3 (*K14rtTA;TRE-mycTcf3)* or control (*K14-rtTA)* mice. Cells were grown in media containing doxycycline (Dox) or vehicle control for 24 hours prior to being fixed and immunostained for myc-tagged Tcf3 (green). Bar denotes 20μm.

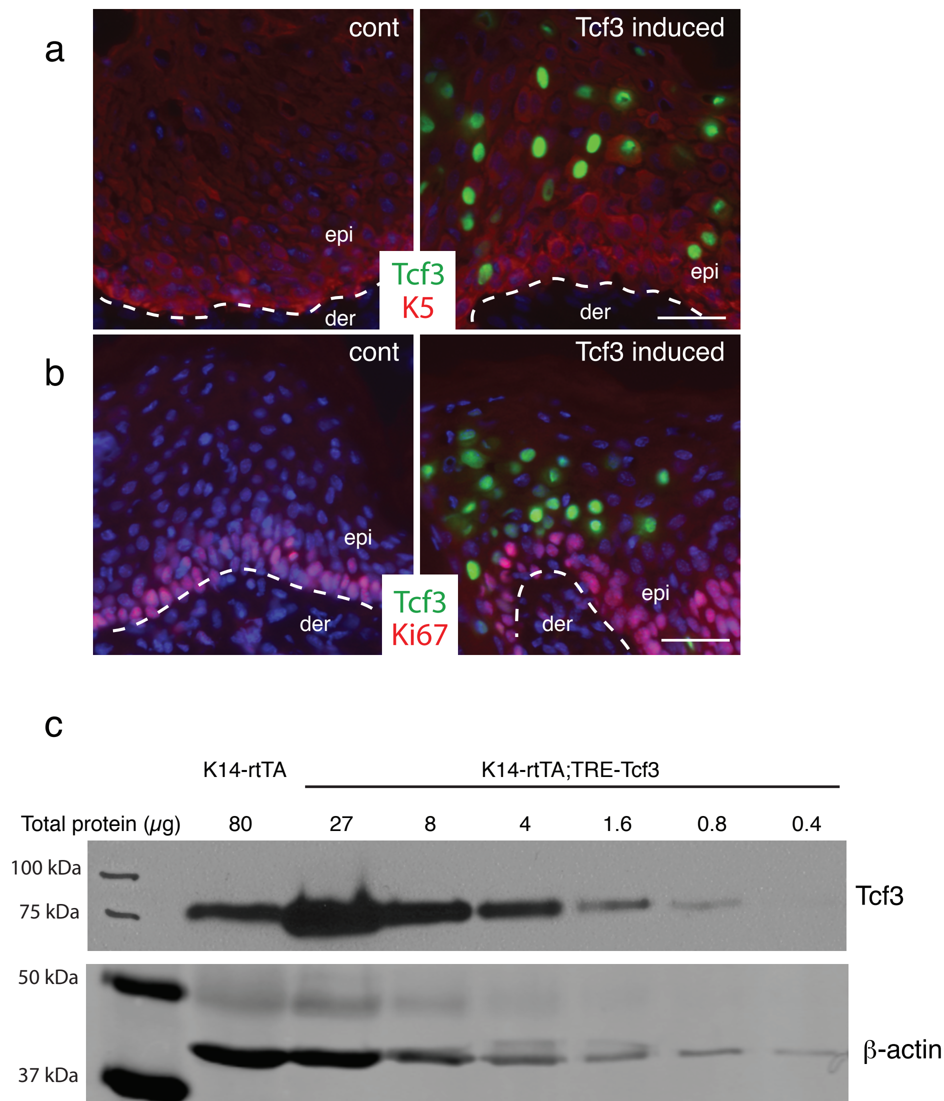


Supplementary Figure 3. Tcf3 and Tcf4 are the only Lef/Tcf members that promote cell migration.

(a) Keratinocytes were transduced with GFP-tagged lentiviral vectors expressing tet-inducible myc-tagged Lef/Tcfs. After the transduced cells were enriched by fluorescent cell sorting, they were treated with Dox or vehicle control for 24 hours and harvested at the initiation of the migration assay. Western blot analysis was performed with antibodies against myc-epitope tag and β-actin.

(b) Keratinocytes were transduced to express tet-inducible Tcf3, Tcf4, Lef1, or Tcf1 and then subjected to the migration assay with or without Dox. Images were taken 16 hrs after the initiation of the migration assay. Black bar denotes 200μm.

c) Graph quantifying the area migrated by transduced cells treated with Dox relative to the area migrated by cells treated with vehicle control. Data are mean ± s.d. **$p<0.001$ (Student's *t* test).



Supplementary Figure 4. Tcf3 is overexpressed in skins of tet-induced Tcf3 mice. (a-b) Immunofluorescence images of skins of tet-inducible Tcf3 (*K14-rtTA;TRE-mycTcf3*) or control (*K14-rtTA or TRE-mycTcf3*) mice that had been maintained on a doxycycline containing diet. 6 days post wounding, skins were embedded in OCT and immunostained with antibodies specific to Tcf3 (green) and integrin β4 (red) or Ki67 (red). Bar denotes 20μm.

(c) Western analysis of total proteins isolated at the wound edge of control (*K14-rtTA*) or Tcf3 induced (*K14-rtTA;TRE-Tcf3*) skins 5 days post wounding. Tcf3 and β-actin were probed with HRP- or fluorescence-labeled secondary antibody, respectively.

```
Human            -------TCCCTCTCAATGAACCAA----ATCCAAATTCTTTTGGAACCCAAGACCGCGA -952
Mouse            TGATAATTTCTTCTTAA--AATCAATACAGTTAAAATCCCGTCTGAATCCAAGACAGTGA -943
Rat              -----ATTTCCTCTTGA--AATCA-----GTTAAAATCCGGCCTGAACCCAAGACAGTGA -953
                        * * ***  *  ** **      *  **** *     *** ******* * **

Human            TTTTTCGATTTAGGACCTAGTCCCAAACAATGA--AGAAAAGGTGATCTTCAAGATTTCA -894
Mouse            GTATTGAATTTCACACCTCTTCTCCAGCTTTAGAGAGAAAGCGTGATCTTCAAGGTTGCA -883
Rat              GTATTGGATTTCACACCTTGTCTCCGGCTTTA---AGAAAGAGTGATCTTTAAGGTTTCA -896
                  * **  ****   ****  ** *   *  *    *****  ******** *** ** **

Human            GCAGGGAAATCTGTA-TATCTGTACAAGGTTGAAAACCTGGGCCGGGGGT-----CGCGT -840
Mouse            GTAGT----TCCTTACTAAGAGGAAGTGATCAAAAACACAGTTTGGGGACAGGAATGCGT -827
Rat              GTGGT----TCTTTACTGAGAGGAAGCAATCAAAAACACAGTTTGGGGACAGGAATGCCT -840
                 *  *     **  ** *    * *     *  *****   *   ****        ** *

Human            TGGAACCCCACAGGAAAAAGGCGCGGAAAGCCGCCGGGCAT-TTTCCGGGGTTCCATAGA -781
Mouse            TGGAATACCATAGGAAAGGGA-ATGTAAGACCGCTCAACTTGTTTC-AAAGTGTCACAAA -769
Rat              TGAAACAGCATAGGAAAGGGA-AGGGAAGGCCGCTCAGCTTGTTTCCGAAGTGTCACAAA -781
                 ** **   ** ******  *    * **  ****    * * ****    **  ** * *

Human            TGTCCCCAGTGTCCTAGTCCG-----TGCATCAGCTCGCGCACTCGGAGGGACTCTAGGC -726
Mouse            -ATC-----TGGCTGAGTAGAAGGGCTG-ATGGGATCCTGTACTCGG--------CAGGC -724
Rat              -ATC-----TAGCTGAGTAGGA---CTG-ATGGGATCCTGCACTCTGAGGAACCGCGGGC -731
                   **     *  *  ***        ** **  * **  * **** *          ***

Human            AGGGGGAGGGCCCCGCGGCCAGTATGTGCGTCCGAGGCTTTCCCGCAGGGGG-CAGTGCC -667
Mouse            TGAGTGGTTGTGCT-CGGCGGATCTGCGAGTCCTCGGCTGTCCCGCAGGGGG-CAGTGCT -665
Rat              TGAGTGGTTGTGCT-CAGCGAATCTGCGACTCCTCAGCTTTCCCGCAGGGGGGCAGTGCT -672
                  * * *   *  *  * **   * ** *  ***   *** ************ ******

Human            GCCCGCCCGCGCGCCGATACGGTGGGAG-GGGTGGGAACCTGCGCGGAGTTCTGGAGGT -608
Mouse            GCCTGCC--------AGTGCGGTGGGAGCGGAGTGGGAACCCGCGCGGAGTTCTGGGGCT -614
Rat              GCCTGCC--------ACTGCGGTGGGAGCTGAGTGGGAACCCGCGCTGAGTTCTGGGGGT -620
                 *** ***          * *********  * ********* **** ********* * *

Human            TCTTTG-GGAGAAAGTTAGGGGATGCGGAGGGGTGGGCGCAAGACTTCCAGGACTCCAGG -519
Mouse            TGTTTGCAAAGAAAGTTAGGGGATGTGAAGGAGTGCGGGCCAGACTCT------TAGAGG -560
Rat              TGTTTGCCAAGAAAGTTAGGGGATGTGAAGGAGTGCAGGCCAGACTCTCGGGCTTAGCGG -560
                 * ****   **************** * *** ***   ** *****        *   **

Human            GAGGCCGTGGGGA-GGGCCGCCGAGGGT-GCAGTGTGAGGCGCAGGAGGGGGT-TGG--- -495
Mouse            GAGGGTGCGAGAAAGGACTTCCCAGGGTAGCCATGGAGTTCACTGGAGGGGATCTGGATA -500
Rat              GAGGGGGCAAGAA-GGACTTCCCAGGGTAGCCATGGAGTTCAGTGGATGGGATCTGGATA -501
                 ****  *   * * ** *  ** ***** **  **     *   *** *** * ***

Human            -------------GGGCGGT-GCACGTTGCAGGGAGACGCAGCCCCTGGAAGATGCGAGT -449
Mouse            AGTTCGAGGGGTAGGGTGGTCGCACGAGGAGGGTAGTTGTAGGCCCCGGAAGGTGCAAGC -440
Rat              AGTACGAGGAGTAGGGTGGTCGCACGCGGAGGGGAGTTGCAGTCCCCGGAAGGTGCAAGC -441
                              *** *** *****  *  ** **  * ** *** ***** *** **

Human            GTGAACGTGTGAGTGTGAGTGCGTGTGTATGTGTGTGTGTGCGCGCGCACCGCAGCTCTC -389
Mouse            GAGCA-----GGACGCGAGTG----TGAATACGAGTGTGCGTGCGCGCGCTGCAGTTCGC -389
Rat              GAGCA-----GGACGCGAGTG----TGAATGCGAGTGTGTGTGCGCGCGCTGCAGTTCGC -389
                 * * *     *   * *****    ** **  * ***** * ****** * **** ** *

Human            CGGGTTCCGCGAGGCGCGCGGGTGTCAGCTTGCAGCCGGGGCTCCTCCCTCCGGCCCCCC -345
Mouse            CTGGTTCCGAGAGGCGCG--GGTGTCAGCTTGCAGCCGGGGCTCCTCCCTCCGGCCCCCC -331
Rat              CTGGTTCCGCGAGGCGCG--GGTGTCAGCTTGCAGCCGGGGCTCCTCCCTCCGGCCCCCC -332
                 * ******* ********  ****************************************

Human            TGCCCAGCCCGGCGGTCCCTCCTCCCTCCCTCCCCGCTCGCCCCTCCCCGGCGGGCCAGG -269
Mouse            TGCCCCGCCGGGCGGTCCCTCC---CTCCTTCCCCA-TCGCCCCTCCCCGGCGGGCCAGG -275
Rat              TGCCCCGCCGGGCGGTCCCTCC---CTCCTTCCCCA-TCGCCCCTCCCCGGCGGGCCAGG -276
                 ***** *** ************   **** *****  ***********************

Human            GGCTGGGACGCCCCGGCGGAGCAGGCGGCGGCGGTGGCG------AGTTGGGGAGCCCTA -215
Mouse            CGCTGGGACGCCCGGGCCGAGAAGGCAGCGGCGGTGGCGGCGGCTAGTTGGG-AGTCCTA -216
Rat              CGCTGGGACGCCCCGGCCGAGCAGGCAACGGCGGTGGCGGCGGCTAGTTGGGGAGCCCTA -216
                  ************ *** *** ****  ***********      ******* ** ****

Human            GGCTCGGCGCTGCCGGAGGGGCCCGAGCCGAGCCGCCTGCGCCCCGGCCGGGCAGCGCCG -155
Mouse            GGTTCCGTGCTGCGGGACGATTCGGAGCCGAGTGTCTCTTGCCCCGGCTGGGCGGTGCTG -156
Rat              GGTTCCGTACTGCGGGACGATCCGGAGCCGAGCGTTTCTTGCCCCGGCTGGGCGGTGCTG -156
                 ** ** *  **** *** *   * ********        ******** **** * ** *

Human            GGCCCGCTTCCCGCGGGGCCACGCCCTGTCAAA-CTTTGT---TGCGGCGGCTAG----- -105
Mouse            GGCCCGCTTCCCACAGAGCCACGCCCTGTCAAAACTTTGTCGCCGCAGCGGCCAAACGAT -97
Rat              GGCCCGCTTCCCACGGAGCCACGCCCTGTCAAAACTTTGTCGCTGCAGCGGCCAAACGAT -97
                 ************ * * **************** ******    ** ***** *

Human            CGCAGC-GGGCCCGCAAGCGGGCGGGAGGGGCGCCGGGCCGGGCCGGGCAGGGCGCGGGC -45
Mouse            CGGAGCCGGGCCAGCGAGAGGGAGGGAGGGGCGCCGGGCCGGGCCAGGCAGGGCGCGGG- -37
Rat              CGGAGCCGGGCCAGCGAGAAGGAGGGAGGGGCGCCGGGCCGGGCCAGGCAGGGCGCGGG- -37
                 ** *** ***** ** **  ** ********************** *************

Human            GGCTAGGGGCTCCGAGAGCGGCGGCCCCGGCCCGCGGCCCCACC -1
Mouse            --------GCTCCGAGCGCAGCGGCCCCGGCCCGTGGCCCCACC -1
Rat              --------GCTCTGAGCGCAGCGGCCCCGGCCCGTGGCCCCACC -1
                         **** *** ** ************** *********
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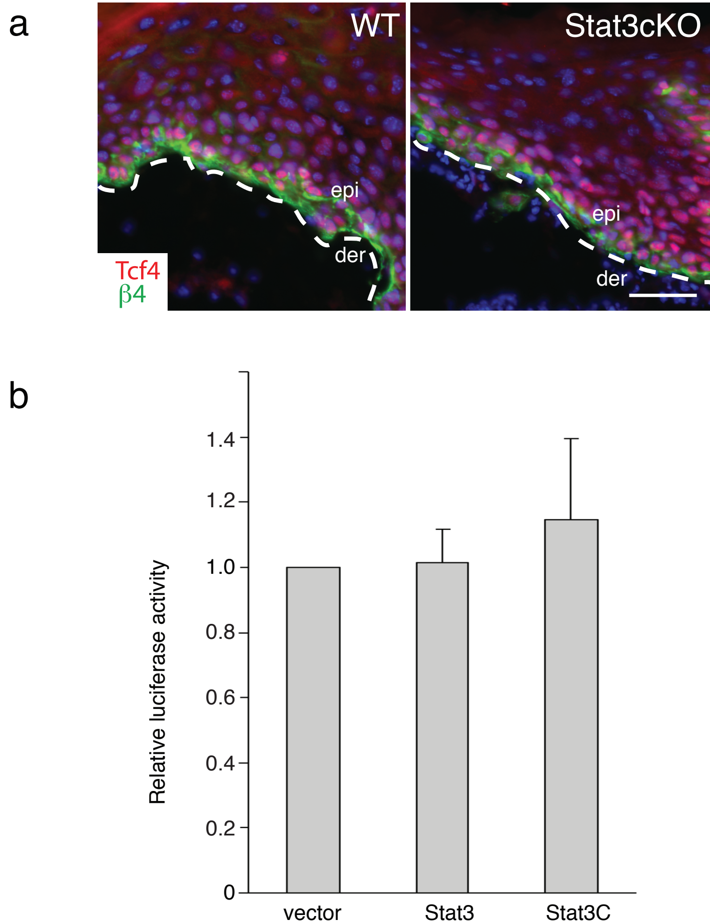
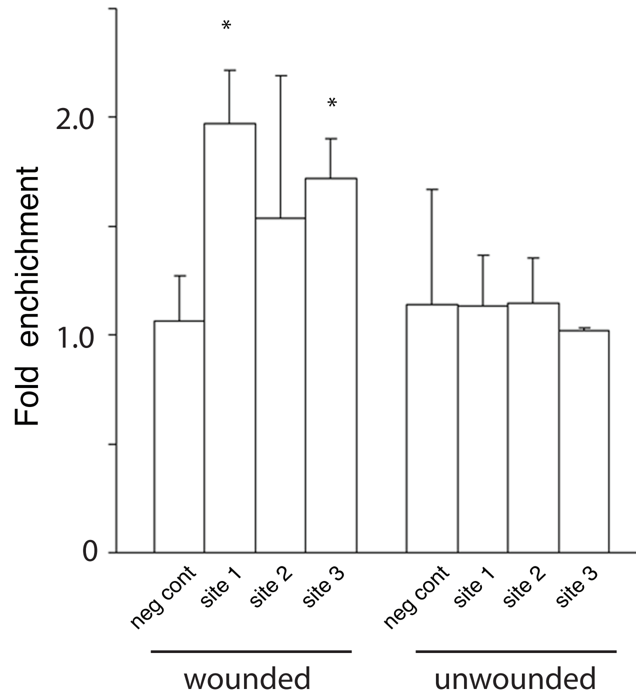
Supplementary Figure 5. Alignment of Tcf3 promoters 1 kb upstream of transcription start site.
Putative Stat3 transcription factor binding sites were predicted using the rVista function within the ECR browser with standard settings. The Stat3 binding sites conserved between human, mouse, and rat were indicated in bold.



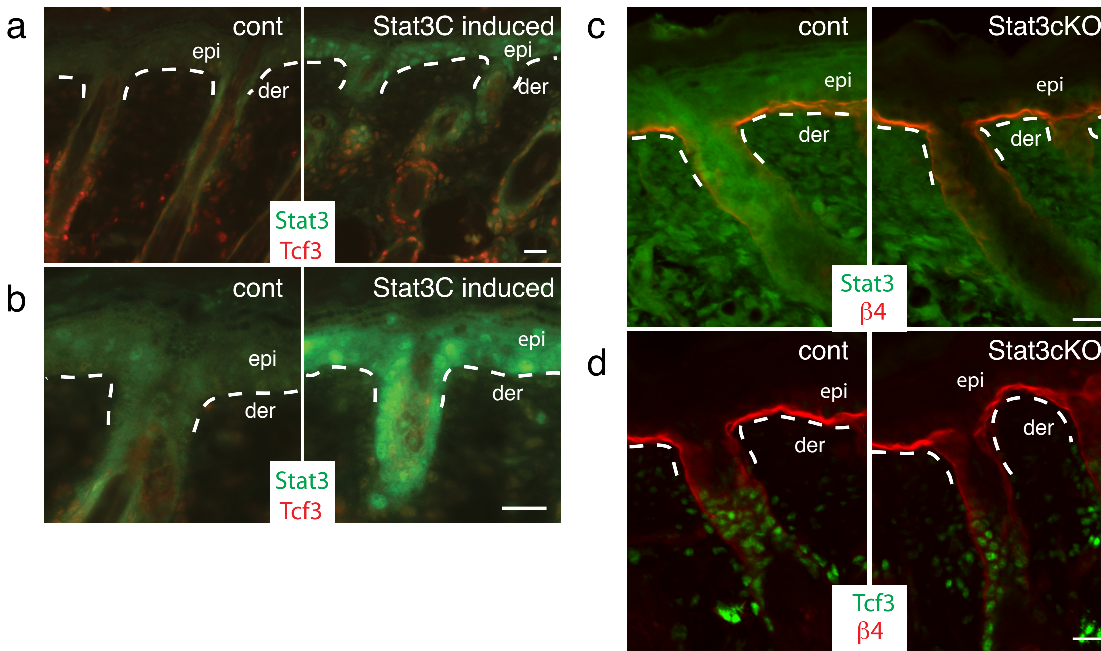
Supplementary Figure 6. Tcf4 induction at the wound edge is independent of Stat3.

(a) Ablation of Stat3 does not affect Tcf4 induction at the wound edge. Images of immunofluorescence analysis of the wound edge of skin from *Stat3$^{fl/fl}$;K14-Cre (Stat3* cKO*)* and *Stat3$^{fl/fl}$* (WT) mice. Full-thickness wounds were created on dorsal skins of 10-week old mice and isolated 5 days post wounding. Skins containing the wound sites were embedded in OCT, sectioned and analyzed by immunofluorescence with antibodies against phospho-Tcf4 (red) and integrin β4 (Green). Bar denotes 50μm.

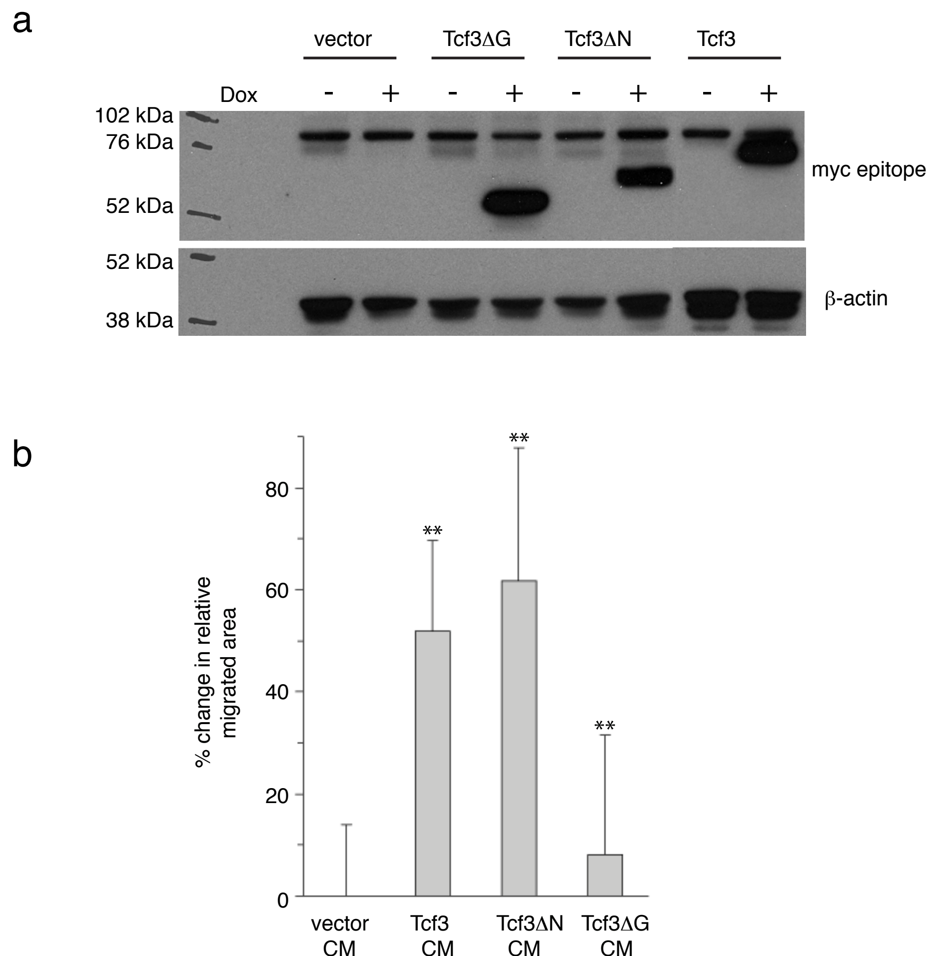
(b) Keratinocytes were transfected with the 5kb *Tcf4* promoter-Firefly luciferase and *Renilla* luciferase constructs, together with constructs expressing either Stat3, constitutively active Stat3 (Stat3C) or control vector. Luciferase activity was measured and Firefly luciferase activity was normalized over *Renilla* luciferase activity. Graph shows normalized luciferase activity relative to vector control. Experiments were repeated three times. Data are mean ± s.d.



Supplementary Figure 7. Endogenous Stat3 binds to the *Tcf3* promoter in wounded skins.
Chromatin immunoprecipitation (ChIP) was performed with anti-Stat3 or isotype control antibodies on crosslinked chromatin lysates from wounded skins or adjacent unwounded skins. Amount of chromatin precipitated by Stat3 or IgG was measured by qPCR using primers spanning regions containing Stat3 binding sites (sites 1-3) or regions without Stat3 conserved binding sites (neg cont). The graph shows the amount of fold enrichment of Stat3-immunoprecipitated DNA relative to IgG-immunoprecipitated DNA. Experiments were repeated three times. Data are mean ± s.d. *$p<0.05$ (Student's *t* test).



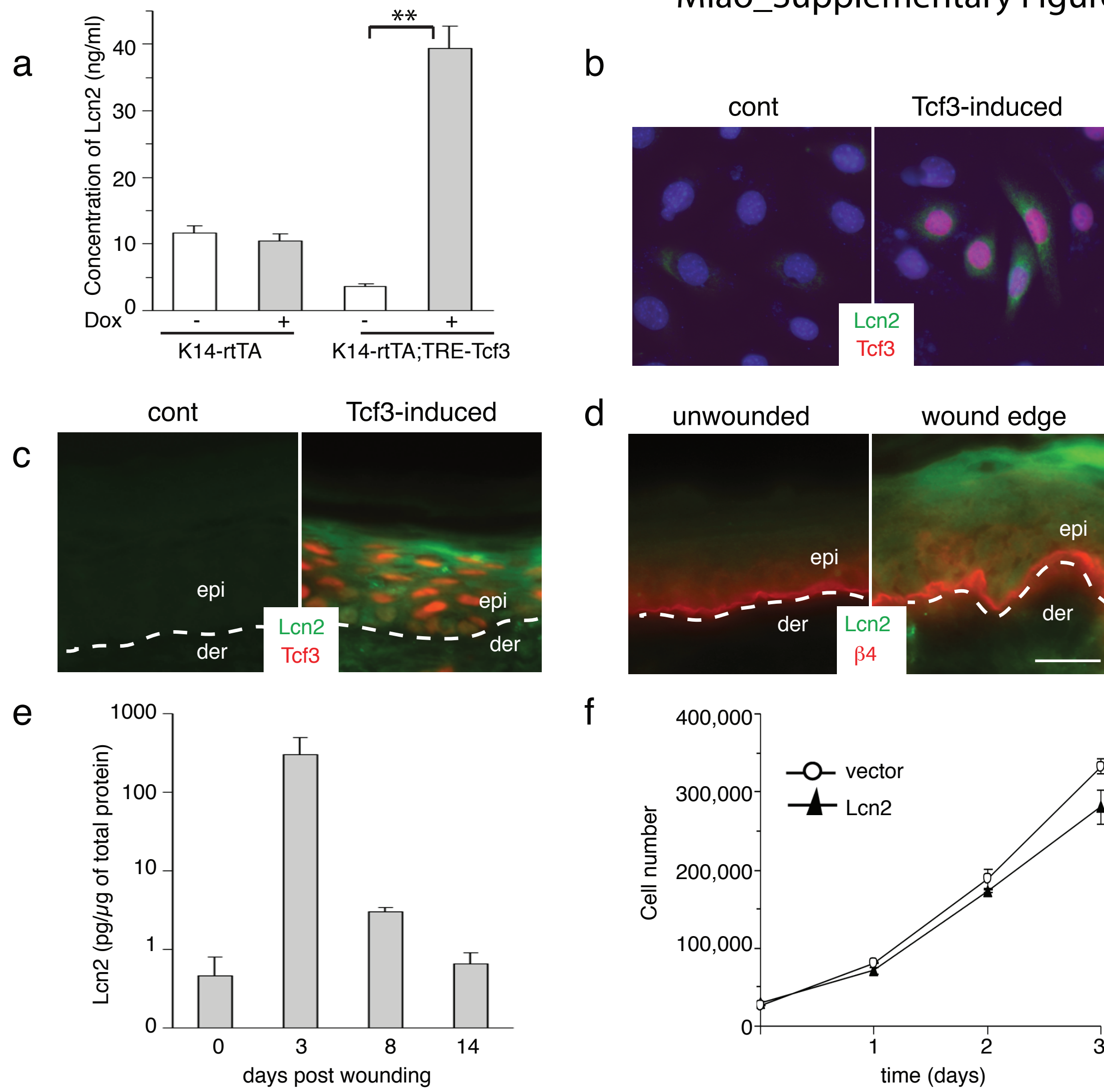
Supplementary Figure 8. Stat3 overexpression or ablation does not affect Tcf3 expression during normal development.
(a-b) Immunofluorescence images of P4 skin sections from *K14-rtTA* (cont) or *K14-rtTA;TRE-Stat3C* (*Stat3*C induced) mice that had been on Dox for 4 days. Skins were immunostained with antibodies specific to Stat3 (green) and Tcf3 (red).
(c-d) Immunofluorescence images of new born skin sections from *Stat3*$^{fl/fl}$ (cont) or *Stat3*$^{fl/fl}$*;K14-Cre* (*Stat3* cKO) mice that were immunostained with antibodies specific to (c) Stat3 (green) (D)Tcf3 (green) and integrin β4 (red). Bar denotes 20μm.



Supplementary Figure 9. Tcf3 promotes cell migration non-cell autonomously and independently of β-catenin.

(a) Keratinocytes were transduced with GFP-tagged lentiviral vectors expressing tet-inducible myc-tagged Tcf3 and its mutant versions Tcf3ΔN and Tcf3ΔG. After the transduced cells were enriched by fluorescent cell sorting, they were treated with Dox or vehicle control for 24 hours and harvested at the initiation of the migration assay. Western blot analysis detected Tcf3 and its mutant versions with antibodies against myc epitope only in cells treated with Dox.

(b) Migration assay was performed on wild-type keratinocytes that were incubated with conditioned media from transduced cells expressing tet-inducible Tcf3 and its mutant versions Tcf3ΔN, Tcf3ΔG. Graph quantifying the migrated area of wild-type cells that were incubated with Dox treated conditioned media during the migration assay relative to the area migrated by cells treated with vehicle treated conditioned media. Experiments were repeated twice. Data are mean ± s.d. $^{**}p<0.001$ (Student's *t* test).



Supplementary Figure 10. Lcn2 is induced in response to Tcf3 overexpression and wounding.

(a) Keratinocytes from tet-inducible Tcf3 (*K14-rtTA;TRE-Tcf3)* and control *(K14-rtTA)* mice were cultured in the presence or absence of Dox for 48 hours and their CM were collected. Lcn2 levels in the CM were determined by ELISA with the Lcn2 quantikine kit (R&D Systems) Experiments were repeated twice. Data are mean ± s.d. **$p<0.001$ (Student's *t* test).

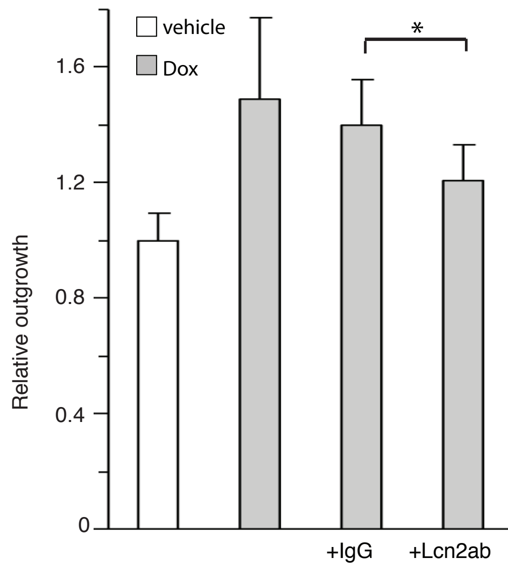
(b) Immunofluorescence images of keratinocytes expressing tet-inducible myc-tagged Tcf3. Keratinocytes from tet-inducible Tcf3 (*K14-rtTA;TRE-Tcf3*) or control (*K14-rtTA*) mice and were grown in media containing Dox for 24 hours prior to being fixed and immunostained for myc-tagged Tcf3 (red) and Lcn2 (green).

(c) Immunofluorescence images of skins of tet-inducible Tcf3 (*K14-rtTA;TRE-Tcf3*) or control (*K14-rtTA*) P5 mice that had been injected with Dox (100 μl, 1 mg/ml in PBS) for 48 hours. Skins were embedded in OCT and immunostained with antibodies specific to Lcn2 (green) and Tcf3 (red).

(d) Immunofluorescence analysis of the skin wound edge of wild-type mice using antibodies against integrin β4 (red) and Lcn2 (green). Full thickness wounds were created on dorsal skins of 10-week old mice and isolated 5 days post wounding. Unwounded skins from the same mice were used as controls. Bar denotes 20μm.

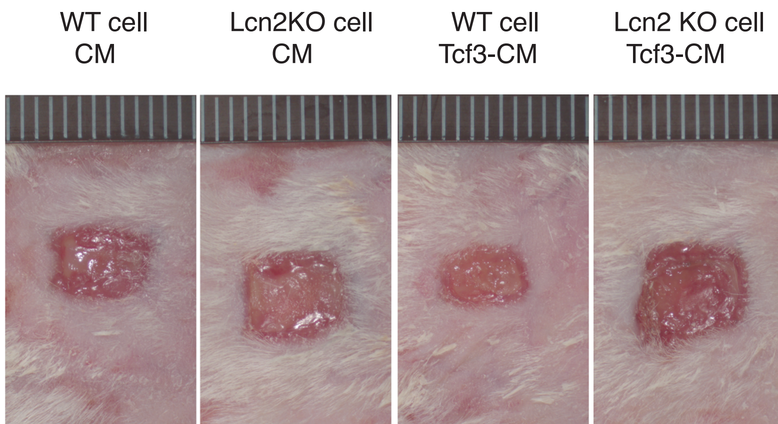
(e) Lcn2 levels in the total protein lysates isolated from the wound edge were determined by ELISA with the Lcn2 quantikine kit (R&D Systems). Experiments were repeated twice. Data are mean ± s.d.

(f) Keratinocytes were transduced with retroviral vectors *pBabe or pBabe-Lcn2* and the transduced cells were selected with puromycin before being plated for proliferation assay. Experiments were repeated three times. Data are mean ± s.d.

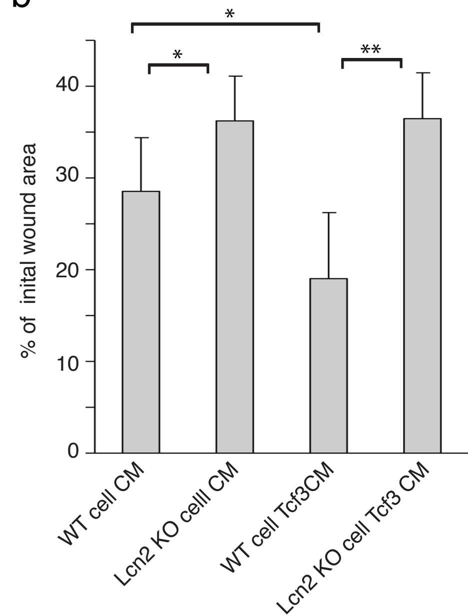


Supplementary Figure 11. Inhibition of Lcn2 decreases the ability of epithelial cells from Tcf3-induced skin to migrate.
Graph quantifying the relative distance of outgrowth of epithelial cells from skin explants from tet-inducible Tcf3 mice (*K14-rtTA;TRE-Tcf3)* that were treated with vehicle or Dox with the addition of anti- Lcn2 antibody or IgG isotype control. 4-mm dorsal skin punches were cultured in the presence or absence of Dox. Explants were treated with Mitomycin C treatment on day 3, followed by the incubation with anti- Lcn2 antibody or IgG isotype control. Explants from a minimum of 4 mice were analyzed for each condition 8 days after plating. Data are mean ± s.d. *$p<0.05$ (Student's *t* test).

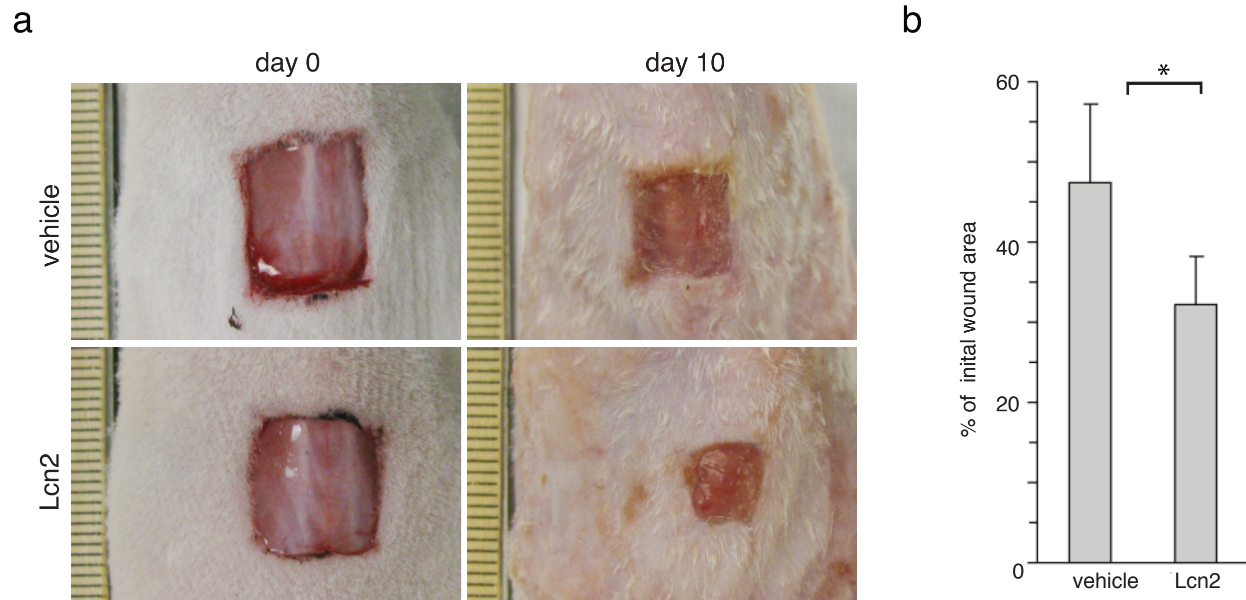
a



b



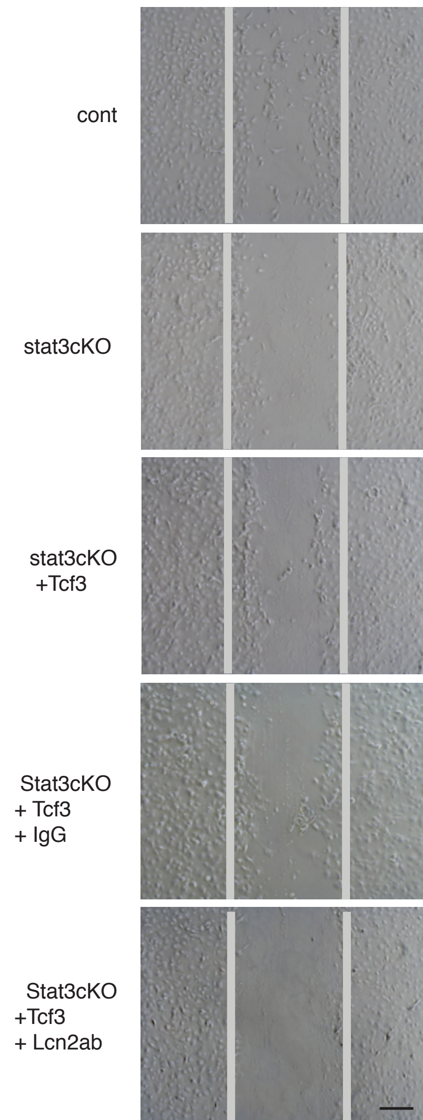
Supplementary Figure 12. Ablation of Lcn2 decreases the ability of Tcf3-CM to promote wound healing.
(a) Images of the wound sites were taken 10 days post wounding. 1cm^2 full-thickness wounds were created on dorsal skins of *ICR* mice and were treated every other day for 10 days with topical application of conditioned media collected from WT cells overexpressing Tcf3 (WT cell Tcf3-CM) or empty vector (WT cell CM), or Lcn2 KO cells overexpressing Tcf3 (Lcn2 KO cell Tcf3-CM) or empty vector (Lcn2 KO cell CM. Surface areas of the wounds were measured at the initial time point and 10 days post wounding.
(b) Graph quantifying the surface areas of the wounds as a percentage of the original wounds 10 days post wounding, WT cells overexpressing Tcf3 (WT cell Tcf3-CM) (n=6) or empty vector (WT cell CM) (n=7), or Lcn2 KO cells overexpressing Tcf3 (Lcn2 KO cell Tcf3-CM) (n=8) or empty vector (Lcn2 KO cell CM) (n=7). Data are mean ± s.d. *p<0.05, **p<0.001 (Student's *t* test).



Supplementary Figure 13. Lcn2 accelerates wound closure.

(a) Images of skin wound sites taken immediately after wounding and 10 days post wounding show that topical application of recombinant Lcn2 accelerates wound healing. 1cm^2 full-thickness wounds were created on dorsal skins of wild-type mice and were treated daily with topical application of recombinant Lcn2 (200ng) or vehicle control. Surface areas of the wounds were measured at the initial time point and 10 days post wounding.

(b) Graph quantifying the effect of topical application of recombinant Lcn2 protein on wound closure (n=6 for each condition). Average surface areas of the wounds 10 days post wounding is quantified as a percentage of the initial wound areas. Areas of the wounds were measured with ImageJ software. Data are mean ± s.d. *p<0.05 (Student's *t* test).



Supplementary Figure 14. Inhibition of Lcn2 decreases the ability of Tcf3 to rescue defective migration in Stat3-deficient keratinocytes.
Images of keratinocytes 48hrs after initiation of the migration assay. Migration assays were performed on *Stat3$^{+/fl}$;K14-cre* (cont) or *Stat3$^{fl/fl}$;K14-cre* (*Stat3* cKO) keratinocytes that were transduced with control or tet-inducible Tcf3 treated with Dox together with anti-Lcn2 antibody or IgG isotype control. Black bar denotes 200μm.

Miao Supplementary Table 1

Primers for real-time PCR

	Forward	Reverse
Mrpl19	ACCCCTATGCCAGTGGAAA	TCAAAGCAAATCTCCACACCT
Tcf3	CGGGACAACTATGGGAAGAAG	CCTCTTGGATTTGCTGCTGA
Tcf4	CACTCCACAGCTCAAAGCATC	CACCACCTTCGCTCTCATCT
Lef1	GCACGTGAAGCCTCAACAC	TAGCGTGCACTCAGCTACGA
Tcf1	CGCGGGATAACTACGGAAAG	AGAGCACTGTCATCGGAAGG
Lcn2	CCCTGTATGGAAGAACCAAGGA	CACACTCACCACCCATTCAGT

Primers for ChIP-qPCR

		Spanning regions, relative to TSS
mTcf3-1U	GAGTCCTAGGTTCCGTGCTG	-223 to -112
mTcf3-1L	GGCGACAAAGTTTTGACAGG	
mTcf3-2U	GCGGGCCAGACTCTTAGA	-579 to -468
mTcf3-2L	CCTCCTCGTGCGACCA	
mTcf3-3U	TGTGCTCGGCGGATCT	-715 to -575
mTcf3-3L	CCCGCACTCCTTCACAT	
mTcf3NC-U	GGGACCATACTCCTGTAGGTT	-6035 to -5930
mTcf3NC-L	CCGAGGGGTCTTTCATATAC	

Primers for Cloning Lef/Tcf genes

myctag_EcoRI_fw	CTAGAATTCGCCACCATGGCATCAATGCAGAAGCTGATCTC
Tcf3_XhoI_rv	CCTCTCGAGTTAGTGGGCAGACTTGGTGACCAAG
Tcf3dC_XhoI_rv2	CTTCTCGAGTTA CGCTTGCTCGGAGTGGGTAGC
mTcf1_myc_Eco_fw	ATCCAGGAATTCGCCACCATGGAGCAGAAGCTGATCAGCGAGGAGGACCTGATGTACAAAGAGACTGTCTACTCTG
mTcf1_Not_rv	ACGGATGCGGCCGCCTAGAGCACTGTCATCGGAAG

Primers for cloning promoters

mTcf3_ MluI_fw	CTCACGCGTATCACAGCAATCAAGACGCTA
mTcf3_ XhoI_rv	TAGCTCGAGGGTGGGGCCACGGGCCGGGGC
mTcf4_SacI_fw	CCGAGCTCCCAAGCCCAGGTGTCTATTCT
mTcf4_HindIII_rv	GCGAAGCTTTTCACCCACCAGCAGCAGCAATTTTG

Primers for mutating Stat3 binding sites in Tcf3 promoter

Tcf3mStat3-1_s	GGTGCTGGGCCCGCAATCCACAGAGCCACGC
Tcf3mStat3-1_as	GCGTGGCTCTGTGGATTGCGGGCCCAGCACC
Tcf3mStat3-2_s	AGGGTGCGAGAAAGGACCCACCAGGGTAGCCATGGAG
Tcf3mStat3-2_as	CTCCATGGCTACCCTGGTGGGTCCTTTCTCGCACCCT
Tcf3mStat3-3_s	GGTGGGAGCGGAGTGGTCCACCGCGCGGAGTTCTGG
Tcf3mStat3-3_as	CCAGAACTCCGCGCGGTGGACCACTCCGCTCCCACC

Primers for cloning Lcn2

Lcn2_XhoI_fw	att ctcgag ACCATGGCCCTGAGTGTCATGT
Lcn2_SfuI_rv	atc ttcgaa GTTGTCAATGCATTGGTCGG