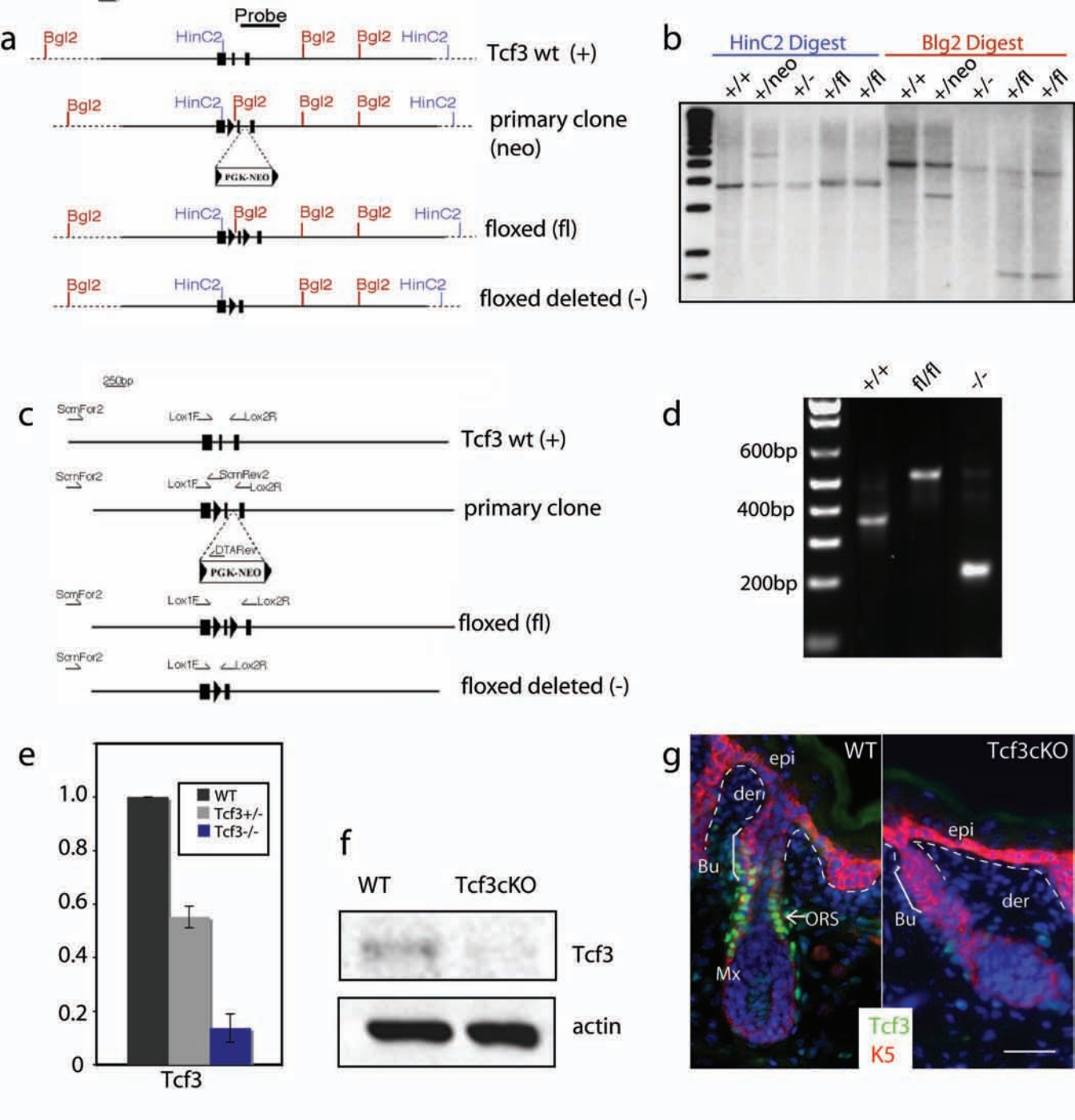
Tcf3 and Tcf4 are essential for long-term homeostasis of skin epithelia

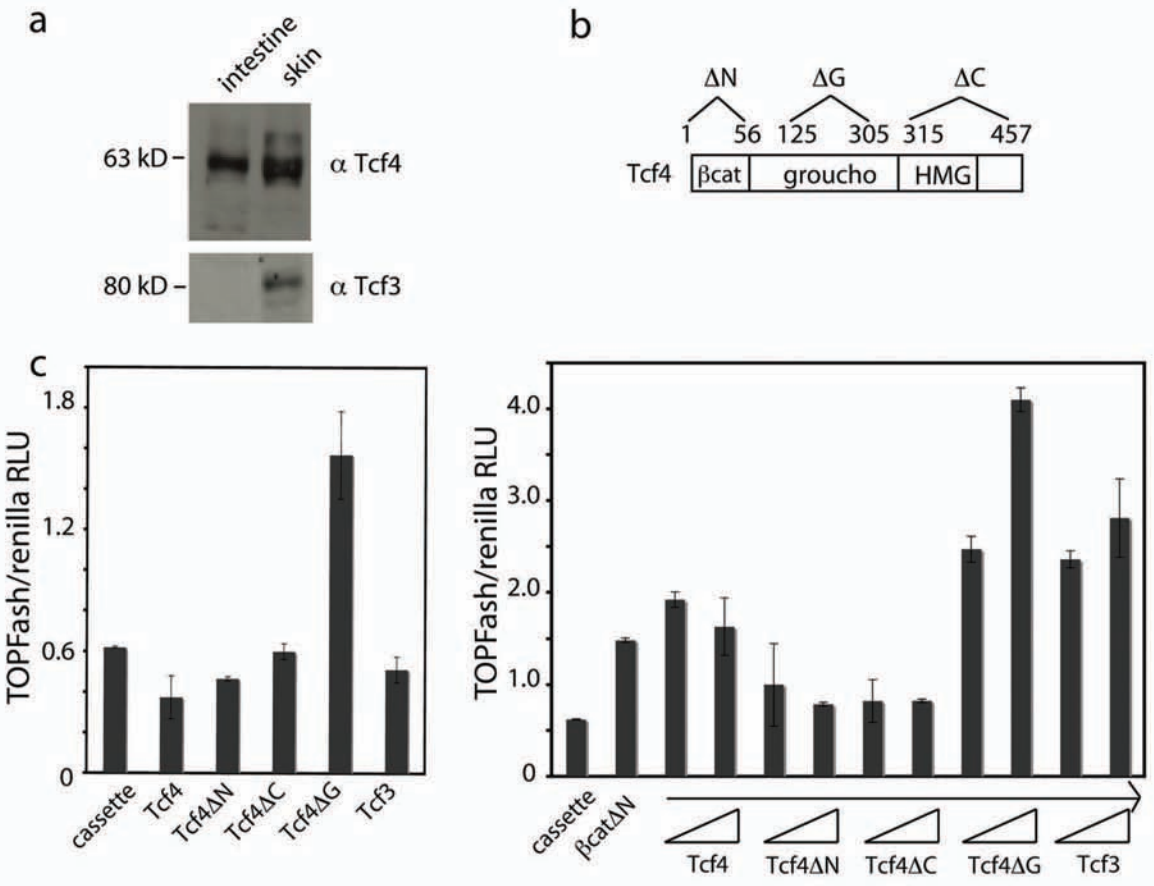
Hoang Nguyen[1,3], Bradley J. Merrill[1,3],
Lisa Polak[1], Maria Nikolova[1], Michael Rendl[1,3], Timothy M. Shaver[2],
H. Amalia Pasolli[1] and Elaine Fuchs[1]

Supplemental Material

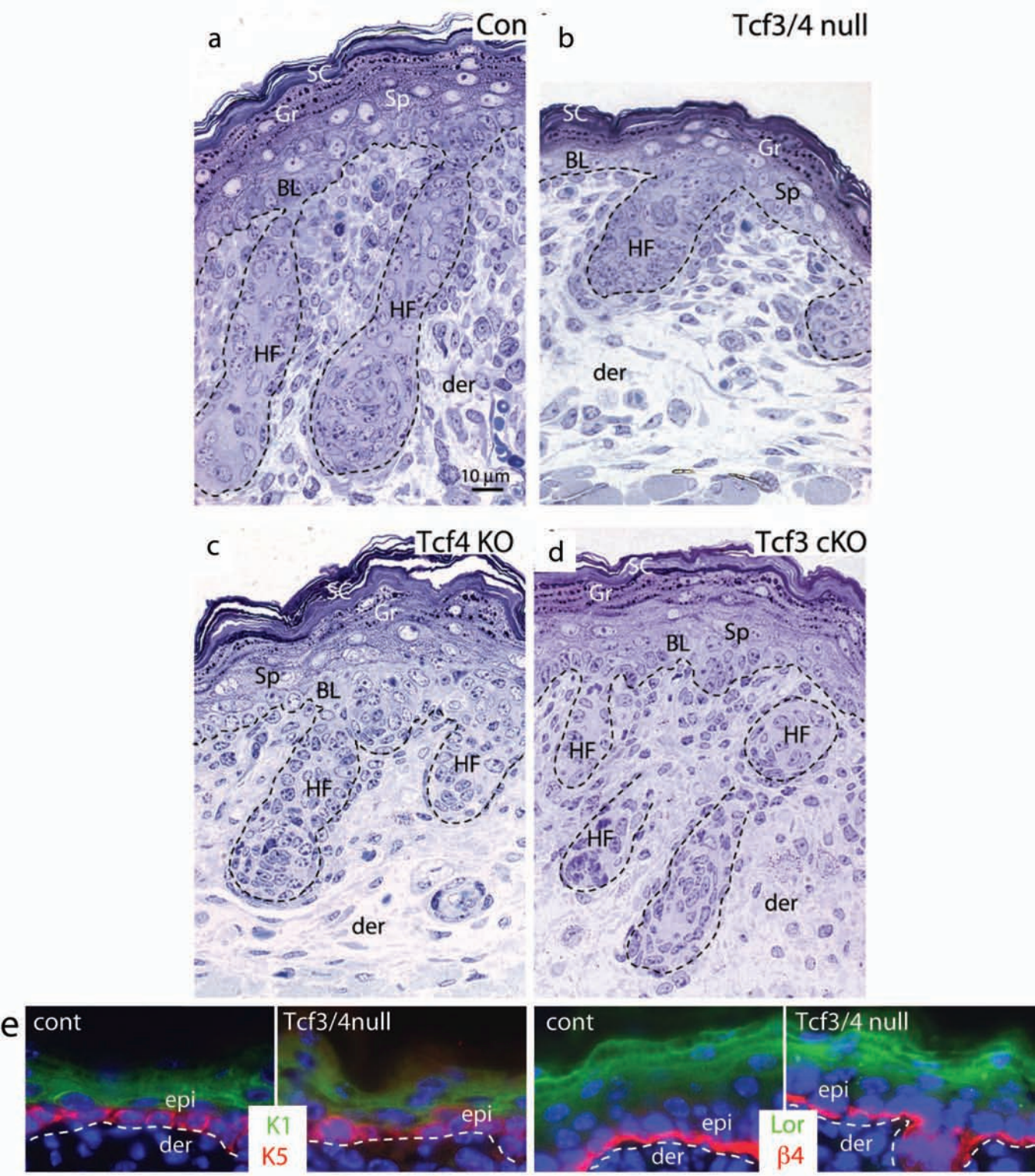
Nature Genetics: doi:10.1038/ng.431



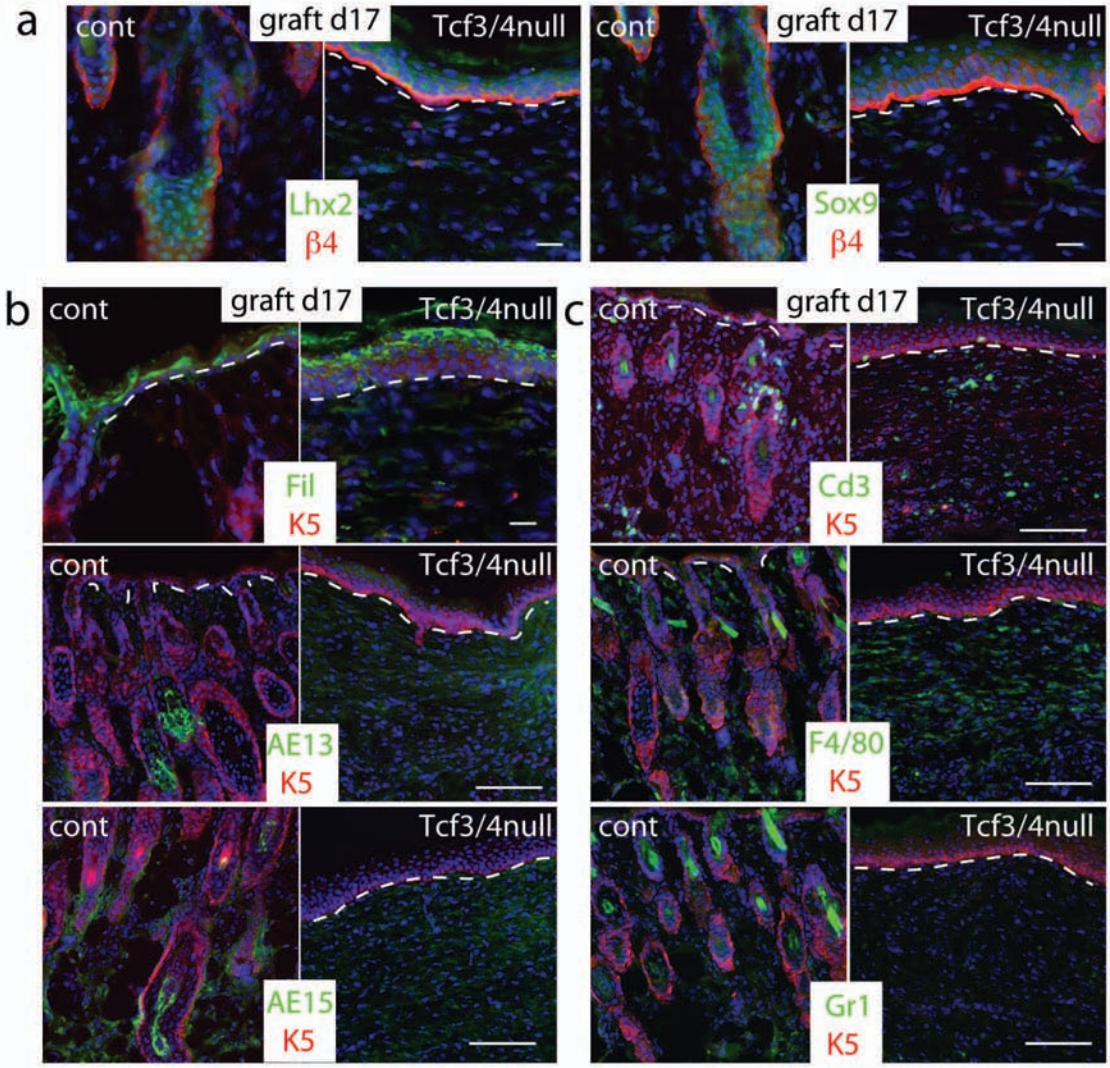
Supplementary Figure 1. Conditional ablation of *Tcf3* in the skin. (a) Targeting scheme to introduce two loxP sites flanking exon 2 of *Tcf3,* thereby generating a putative frame shift and premature termination of the protein. Shown are maps of the targeting vector and the targeted *Tcf3* exon 2 before and after Cre recombination; triangles represent *lox*P sites. (b) Southern blot analysis verified the correct targeting event and the proper Cre-mediated excision of *Neo* gene to yield the *Tcf3* floxed allele. (c) Scheme of the design of genotyping primers that yield PCR products that distinguish WT, floxed, and floxed deleted allele. (d) PCR analysis confirmed the deletion of exon 2 upon Cre recombination. (e) Real Time PCR analysis of mRNAs from FACS-purified basal epidermal cells of WT, *Tcf3*$^{+/-}$ and *Tcf3* cKO E17.5 skins. (f-g) Immunoblot and immunofluorescence analyses, respectively, confirmed the efficient loss of Tcf3 in *Tcf3* cKO E17.5 skin epidermis. β-actin served as the loading control in the immunoblot and K5 serves as the control for the immunofluorescence (DAPI in blue marks the nuclei). Bar represents 20μm.



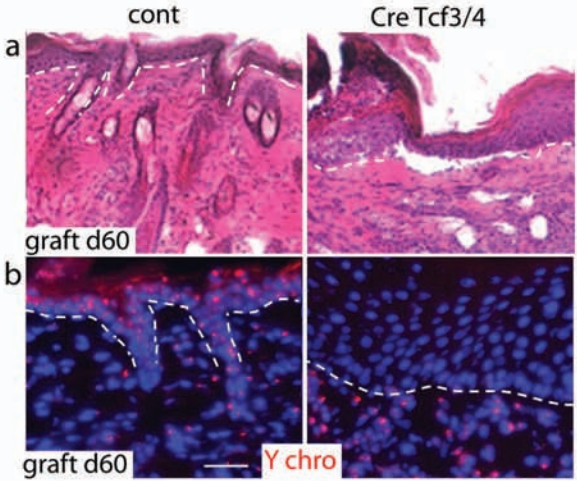
Supplementary Figure 2. Tcf4 has repressive activity on WNT reporter TOPFlash *in vitro* (a) Western analysis confirmed that the predominant isoform of Tcf4 in the skin to be the Tcf4B isoform that is expressed in the intestine. (b) Scheme of Tcf4 constructs expressing mutant forms which either lacks the β-catenin binding domain (ΔN), or Groucho binding domain (ΔG), or the DNA binding domain (ΔC). (c) Tcf4 has repressive activity on WNT reporter TOPFlash in keratinocytes and its repressive function requires its binding domain to corepressor Groucho. Note that by lacking the Groucho binding domain, Tcf4ΔG has higher basal activating activity on TOPFlash.



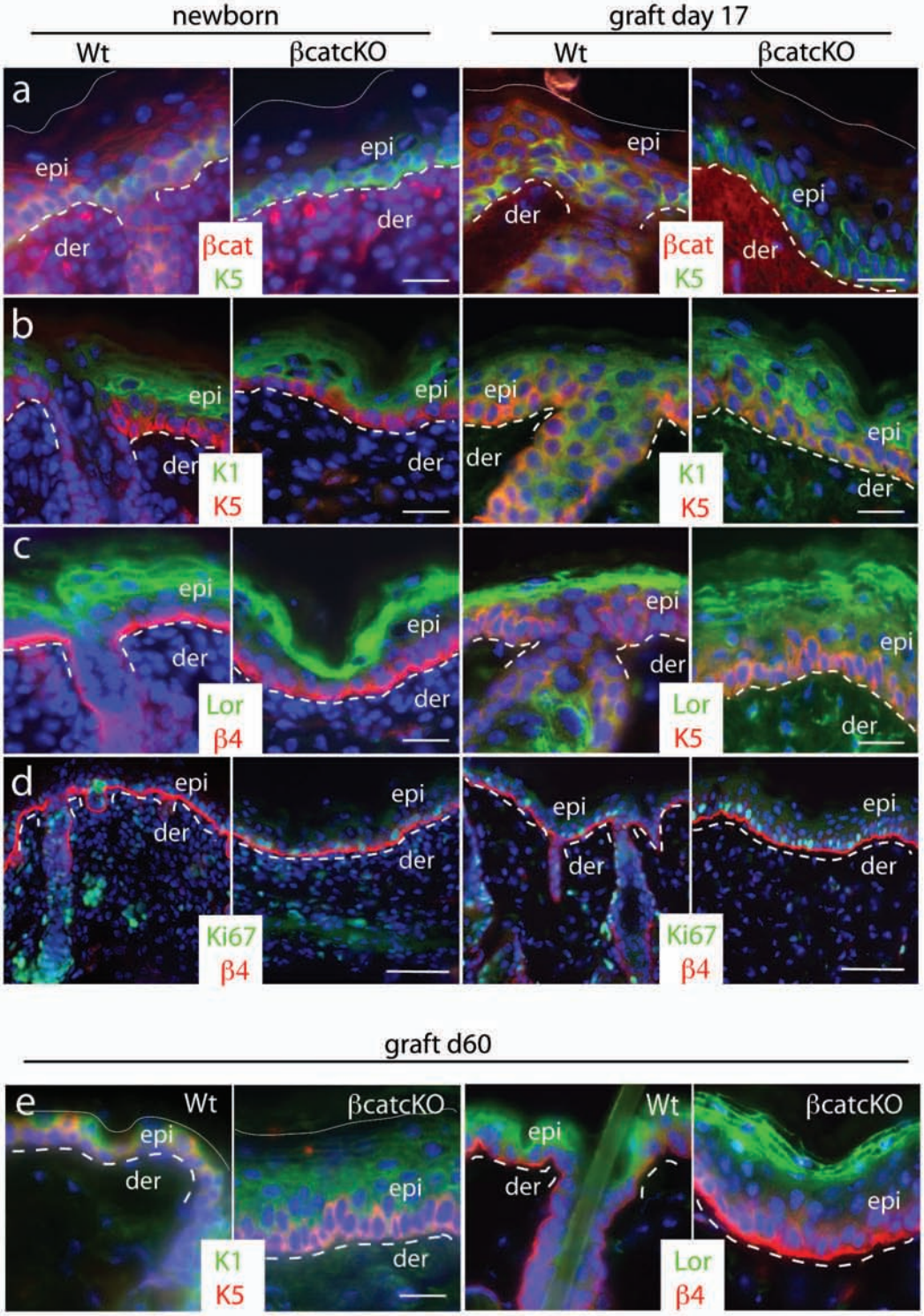
Supplementary Figure 3. Histological and immunofluorescence analyses of *Tcf3* and *Tc4* null mutants. (a-d) Newborn backskins from control, *Tcf4*KO and *Tcf3*cKO, and *Tcf3/4* null were fixed, embedded in Epon, and sectioned (1um). Sections were stained with toluidine blue and subjected to light microscopic analysis. *Tcf3*cKO and *Tcf4*KO exhibit similar phenotype as the control while the *Tcf3/4* null showed less developed hair follicles and thinner epidermis. SC, stratum corneum; Gr, granular layer; Sp, spinous layer; BL, basal layer; HF, hair follicle; der, dermis. Dotted line delineates the b asal lamina. Bar 10µm applies to all p anels. (e) B asal (K5), spinous (K1) and granular (Loricrin, Lor) markers are properly expressed in *Tcf3/4* null epidermis.



Supplementary Figure 4. Hair follicle and immune cell analyses of *Tcf3/4* null vs Wt skins at d17 after engraftment. *Tcf3/4* null grafted skin does not maintain hair follicles and does not express follicle stem cell markers Lhx2 and Sox9 (a) or hair differentiation markers AE13 and AE15 (b). At d17 the double null skin still expresses normal epidermal terminal differentiation marker Filaggrin and does not have abnormal inflammation. Expression level of lymphocyte (Cd3), granulocyte (Gr1) and macrophage (F4/80) is comparable in the double null and *Wt* skin. Bar denotes 20μm and 100μm in a and b, respectively.



Supplemetary Figure 5. Skin grafting permits evaluation of the long-term consequences of *Tcf3* and *Tcf4* ablation. (a-b) Skins from P0 male *Tcf3/4* null and control littermates were grafted onto female *Nude* mice and analyzed after 2 months. (a) H&E analysis of grafted skins. Note that after 2 months, the epidermis is drastically altered in architecture, resembling a chronic wound state. (b) Y chromosome FISH shows that in contrast to the control, bordering Y-chromosome negative, female *Nude* epidermal cells have replaced the diminishing *Tcf3/4* null (male) epidermal cells, while the underlying dermis of the graft remains *Tcf3/4* null.



Supplementary Figure 6. Analyses of *K14-Cre/β-catenin*$^{fl/fl}$ skin at P0 and at d17 and d60 after engraftment. (a) β-catenin immunostaining verified the absence of β-catenin in newborn cKO and engrafted cKO skins (b-c) *β-catenin* cKO skin lacks hair follicle but still exhibits a seemingly healthy epidermis as previously reported[15]. At newborn or d17 and d60 after grafting, *β-catenin* cKO epidermis still expresses normal terminal differentiation markers K1 and Loricrin. The numbers of epidermal cells displaying Ki67 immunostaining are even higher in *β-catenin* cKO than in Wt d17 grafts. This contrasts strikingly with *Tcf3/4* cKO skin grafts at d17, which show little or no Ki67 immunostaining (see main text). Bar denotes 100μ m in d, and 20μ m the rest of the panels.

Supplementary Table 1. List of sequences of primers used in cloning and Real Time PCR

Real Time Primers	Sequences
Actc1 F	TCCCCCTGAGCGTAAATACTCTG
Actc1 R	GGGCCTGCCTCATCATACTCTT
Adam19F	CCTGGGCTCAATTCACTTCCTTAT
Adam19R	ACGGGGTACCTTTCCAGTTGG
axin2F	TAGTCCCAGAGCCCGTCACAG
axin2R	GAACGCTGGCAGACAGGACATA
Basp1F	GGGGAGGGAGGCGTTTGA
Basp1R	CTAAGTGGGCTCCGTCTGAAAGTT
bcat F exon4	GAGCTGCCATGTTCCCTGAGA
bcat R exon 4	CAAGTTCCGCGTCATCCTGATA
BdnfF	TGGCGGGACGGTCACAGT
BdnfR	TAGTTCGGCATTGCGAGTTCC
Cldn4F	CAGCGCTACTCTTGCCATTACG
Cldn4R	AGAGGCCCAGGGTCCTTCTG
Csrp1 F	CCGGGAAGTCCTGGCATAAGT
Csrp1 R	CTGAGCCACAAAAGCCAGATACC
Ctgf F	GCAGTGGGAATTGTGACCTGAGT
Ctgf R	TACCCTGAGCCAGCCATTTCTTA
Cxxc5 F	ATTCCCCCTACCCCAACAGTG
Cxxc5 R	GCTGCGAGCAAGGCTGAGA
cyclin D2 F	CCGTCGCATACTCTTGCTGACTA
cyclin D2 R	GTCTCCATCTGCCCCCATTTAG
Dkkl1F	GGAGCACAGAATGGGCAACC
Dkkl1R	GTGCAAGCTGTCGGTGACCTT
Dlc1F	TCCCCAGACCAAGGTCAGAAAG
Dlc1R	AAATCGTGGCCACAGTACAAGATG
Etv1 F	AAGGGGGCTTTTCCTGTTGC
Etv1 R	ATCCGCCATCTTCCTGTTACAAA
Frat2 F	GGGCGCCTCTCCTGCTAAA
Frat2 R	AGGATGACCGAGCCATTGAATC
gadd45gF	GAGCCGCAGCTTCAACGACT
gadd45gR	CCGCGCTCCTCGCTCTC
Grrp1 F	GGACGCCCCACGGTGTC
Grrp1 R	TGGCCCCTGCCTGTGGT
Hexa F	AGGCCCAGCCCATCAGTGT
Hexa R	GGCCGGGAGGCAGTGAA
Hhip F	CGGCTGGGAGGGAGACTTCT
Hhip R	AGGATACCTGCCCTGGTCACTCT
Klf12F	TGCCCAGGCAAAGTCACTGA
Klf12R	CCGGGTGGCTGTAACTAAGACC
Klf2F	GCGCGACTGTGGCAGGTT
Klf2R	GGGGACCCGAGGGAAATAAGT
Klf9F	TCCGCGTACTCGGCTGATG
Klf9R	CGTGGCGGTCGCAAGTTTA

Lamb1-1F	CAAGTGCGGCCTTTCAAGAAC
Lamb1-1R	CCCAAGCCTTCCCAAAGTCA
McamF	ACCTGGGCACATGGTCACATTAT
McamR	CGGGAAGCTTGGGCTCAGTA
MdkF	GGAGCCGACTGCAAATACAAGTT
MdkR	TTAGTCACGCGGATGGTCTCC
Mef2cF	GTTTGCCGTATCGTTTTTCTTCCT
Mef2cR	TATGCCGCTGTGAGCCTCTATTT
Mfge8F	CTGGGGGAGTTGGACAGGTCT
Mfge8R	TTGGGTAGCAAGCCAGCAGAG
MycF	AAGCCACCGCCTACATCCTG
MycR	AAAGCCCCAGCCAAGGTTG
Nav1F	TGCTGCCCAGCCAGTGC
Nav1R	AGTATGCGAGGCCTCCAGAATC
Ncam1F	GAAGGGCAGATGGGAGAGGAC
Ncam1R	CACCGCAGAGAAAAGCAATGAG
notch4F	GCTCACTTGTCTCCCCCATAGAGT
notch4R	ACACCCGGCACATCGTAGGT
Npr3F	TATCGCCGGGCAGGTGTC
Npr3R	TTCCCGATGTTGCTTTCCTCTT
Nr2f2F	CGCCGAGTATAGCTGCCTCAA
Nr2f2R	TCGATGGGGGTTTTACCTACCA
Nr4a2F	GGGGCATCCTGGATTTAGAAAAC
Nr4a2R	CATGCCACCCACGCAACA
PdgfrlF	TCTTGGCCTCCTCTAACAAAGTGA
PdgfrlR	ATATGTAGTAGCCCGCATCAATGG
Phf17F	ATGGGGCCTGCCACCAG
Phf17R	CAGGGGCGTACCCATCATTC
Pim1F	ATCGGCCCTCCTTTGAAGAAAT
Pim1R	AGAGGGGCCCAGGACCAGT
Plekhg2F	CTGATGCCGCTCTCCGTATGT
Plekhg2R	GGAGGGCCATCTGTGGACAC
PodxlF	CTGGGGAGGGAGAATGGACTC
PodxlR	CTGGGCTCAGGCACAAGTAGG
rtn4F	AACCCCTAGCAACTGTGTTTA
Rtn4R	CTCAATACATTACAATGGAGATACTG
Socs2F	GCCGATTGCTTTAACCCAAGTT
Socs2R	TGGCGAGAAATTTCCCAGATG
SostdcF	ACGCGCACCTACAAAATCACC
SostdcR	GGGGGAGGGGATGGAAACTA
Sox11F	GCTGCCCACAGTGGAATAAGC
Sox11R	AGCAACTGCCCCTGAATAATCC
Sox2F	AGGGTTCTTGCTGGGTTTTGATT
Sox2R	CGGTCTTGCCAGTACTTGCTCTC
Tcf3F_exon2	GGAGCCGGGGCAACCAGTG
Tcf3R_exon2	CATCCTGGGGCCTTCTCACTTC
Tcf4F	CACCCGGCCATCGTCACAC
Tcf4R	GCCACCTGCGCCCGAGAAT
Tubb2bF	GACCAGTGCGGCAACCAGA

Tubb2bR	GGATGGCCCCTAGGCACATA

Cloning Primers	**Sequences**
Tcf4bR-BamH1R	CCGGATCCTCAAAGACTGCAGGGGCCG CACCA
Tcf4F166 –BamH1	CCGGATCCATGCAAGACAGCTCCTCCGATTCC
Tcf4bR-BamH1	CCGGATCCTCAAAGACTGCAGGGGCCGCACCA
Tcf4R948-stopXba1	CGCTCTAGATCAGGAGTCCGTATGCTTTG AGC
Tcf4R385	GATAGGT CCGGGCGACGTCGGTGGGCGAGAGCG
Tcf4F359 –aattII	CGCTCTCGCCCACCGACGTCGCCCGAACCTATC
BglII-Tcf4F1	GGTAGATCTCTATGCCGCAGCTGAACGGC
BlgII –stop-Tcf4 R12889	CGCAGATCTTCAGGTTTCCCCCGGCTGCTTG
mycBamH1F824	CGCGGATCCCCACCATCGCATCAATGC
mycBamH1R939	GTCTGGATCCCCGCGGCCGCGG