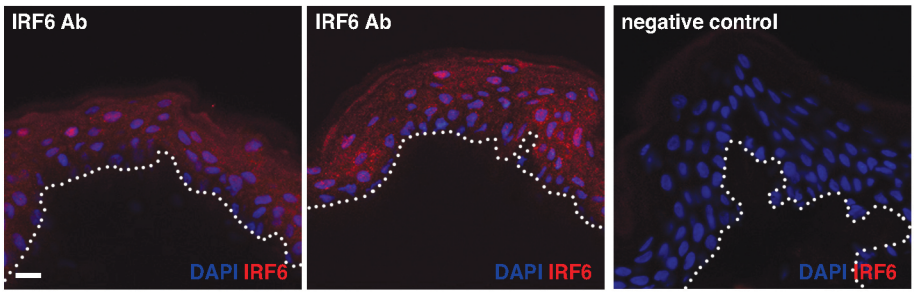
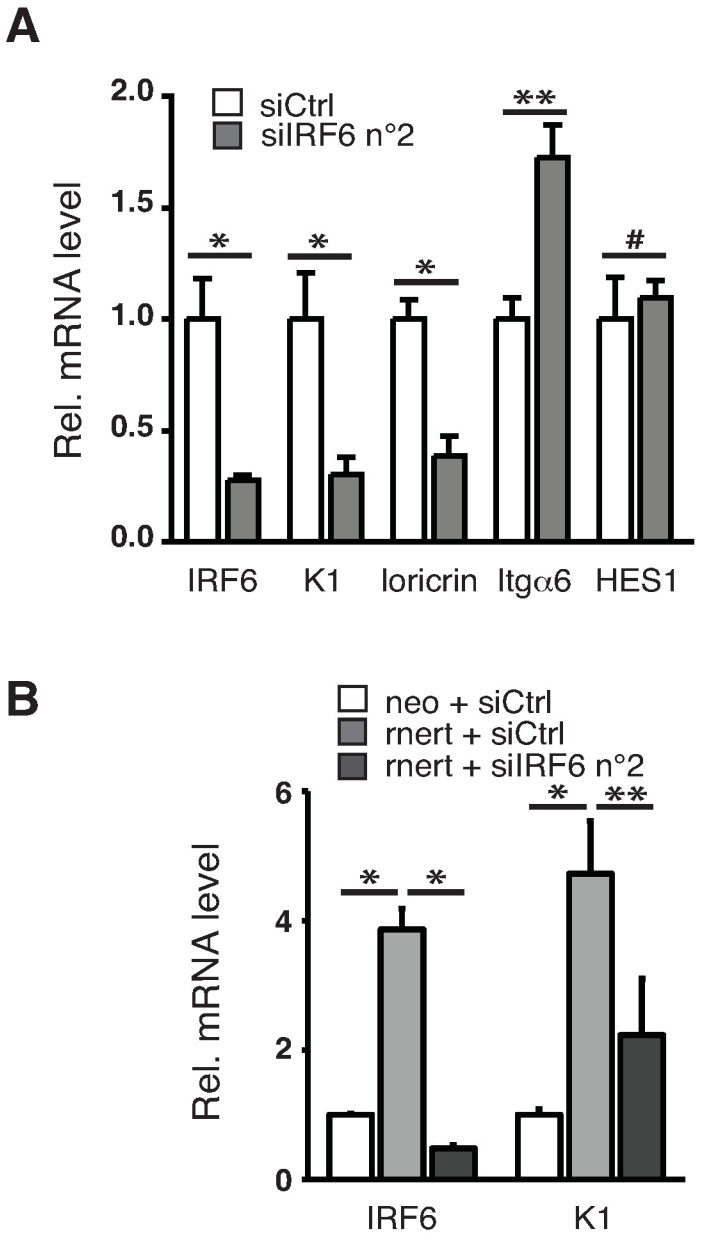
Supplementary Figure S1



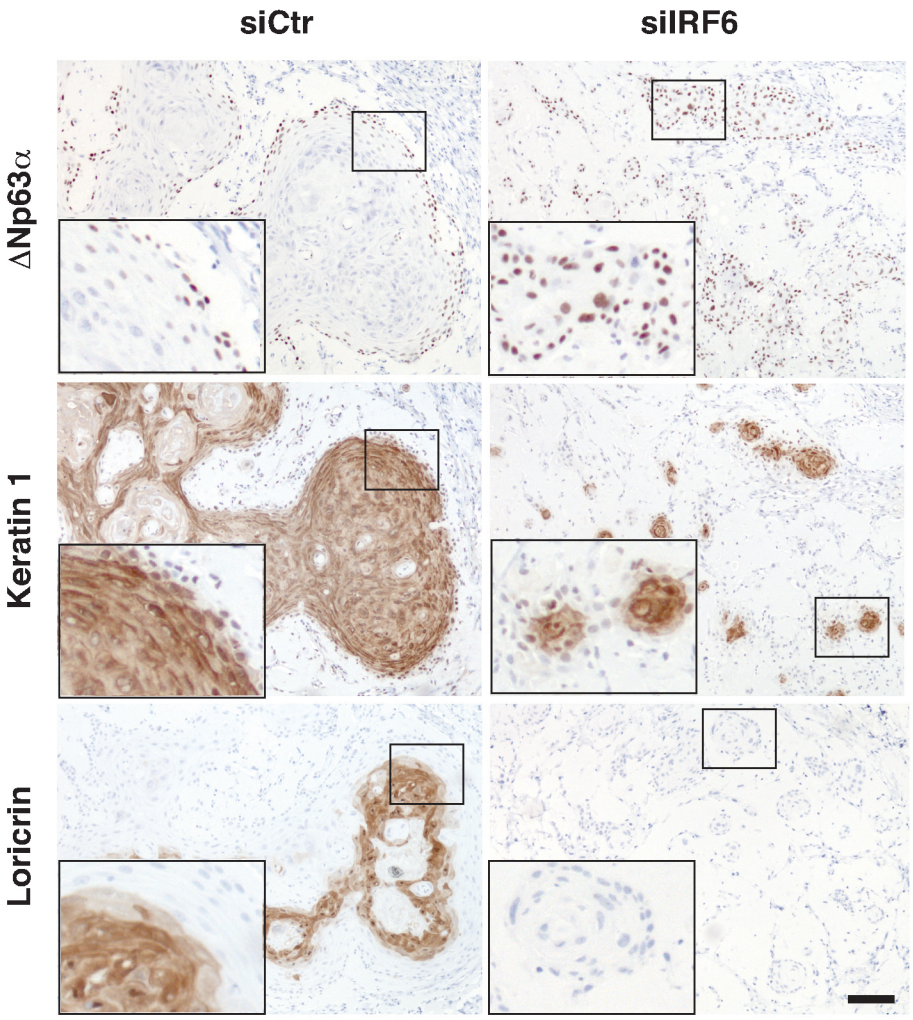
Supplementary Figure S1. IRF6 protein is mostly localized in the suprabasal layers of human epidermis. Confocal immunofluorescence analysis of IRF6 expression in human epidermis, showing representative images of two independent fields (left panels) in parallel with immune-negative control (right panel). Staining conditions were similar to those used for Fig. 1A, except for the use of frozen rather than formalin-fixed skin sections. Note the prevalent cytoplasmic distribution of IRF6, with sporadic nuclear localization limited to cells of the outermost layers. Images are representative of several independent fields. Bar : 15 μm.

Supplementary Figure S2



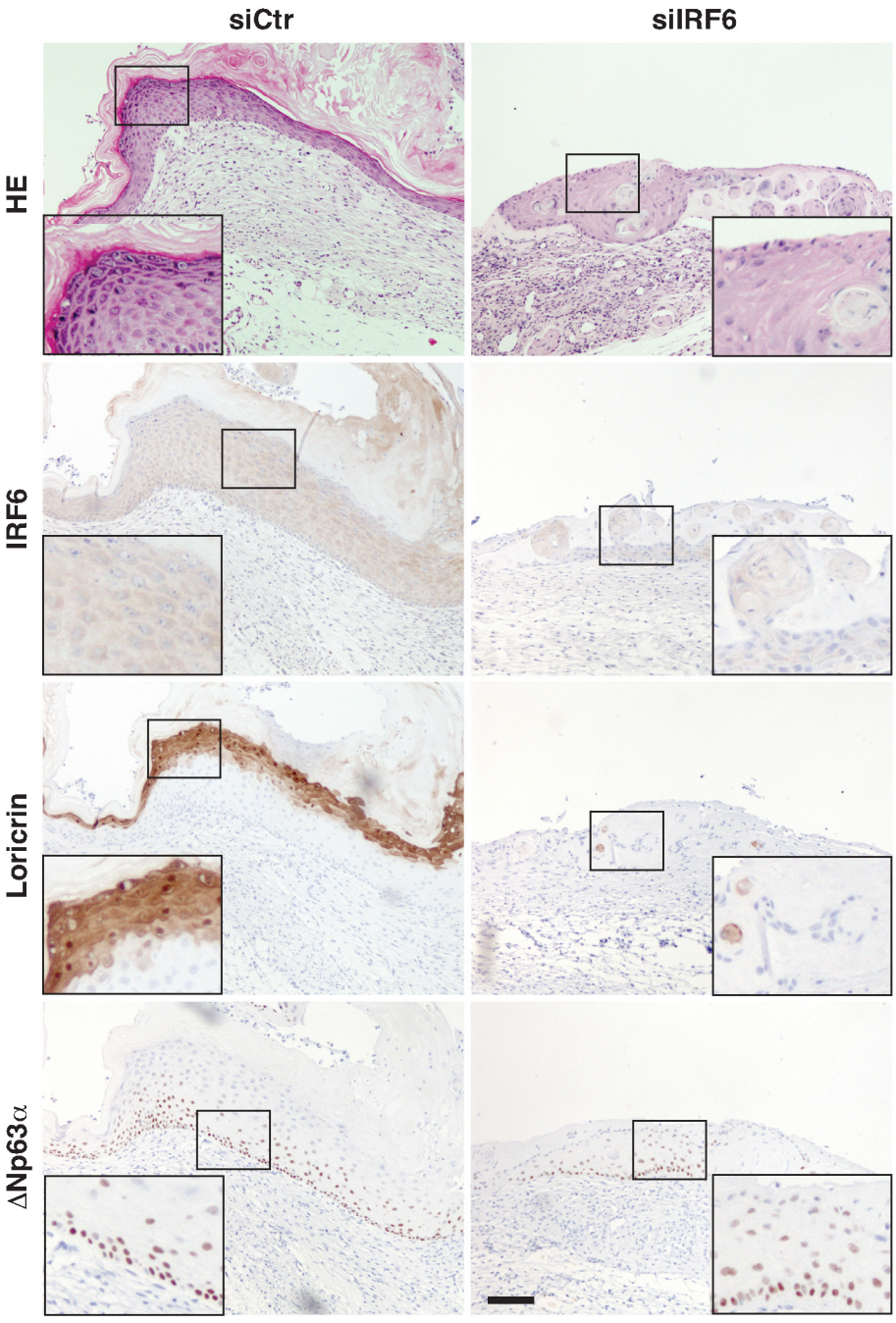
Supplementary Figure S2. (**A**) HKCs were transfected with the siRNAs against IRF6 (siIRF6 n°2) in parallel with scrambled siRNA control (siCtrl) for 72 hours followed by real time RT-PCR analysis for the indicated genes. *$P < 0.0001$, ** $P < 0.0002$, # not significant. **(B)** SCC13 cells expressing the Notch1 protein fused to the human estrogen receptor (rNERT), or empty vector control (Neo) were transfected with siRNAs against IRF6 (siIRF6 n°2) in parallel with scrambled siRNA control (siCtrl) for 48 hours and treated with 4-hydroxytamoxifen (OH-TAM) at a concentration of 1 μM for additional 24 hours. Expression of the indicated genes was analyzed by real time RT-PCR. *$P < 0.0001$, ** $P < 0.005$.

Supplementary Figure S3



Supplementary Figure S3. Silencing of IRF6 expression alters the *in vivo* differentiation process of HKCs as assessed by intradermal injection assays. HKCs transfected with siRNA against IRF6 or scrambled siRNA control for 3 days were collected, admixed with Matrigel and injected intra-dermally into the skin of NOD/SCID mice. The two types of cells were injected in parallel in the right and left flank of mice, to avoid the risk of individual animal variations. A week later, nodules formed at the sites of injection were excised and tissues were processed for H&E staining and immunohistochemical analysis with antibodies against the indicated proteins. Bars = 50 μm. Inserts : high magnification images of the indicated low magnification areas.

Supplementary Figure S4



Supplementary Figure S4. **Silencing of IRF6 expression alters the *in vivo* differentiation process of HKCs as assessed by grafting assays.** Histological and immunohistochemical analysis of the indicated proteins in reconstituted skin formed by grafted human primary keratinocytes transfected with siRNA against IRF6 (siIRF6) or scrambled siRNA control (siCtrl), 11 days after grafting. Bars = 50 μm. Inserts : high magnification images of the indicated low magnification areas.

Supplementary Table 1: Primers used for real time RT-PCR reactions

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
human		
IRF6	GCTCTCTCCCAATGACTGACCTGGA	CCATGACGTCCAGCAGCTTGCTA
INTEGRINα6	ATAAATTTTGCACCCGAGAAGGAA	GTTGGAAGGGCTGTTTGTCACTGT
INTEGRINβ4	CCCAACCACTCCTACGTGTT	GGGAGTGCTCAAAGTGAAGG
INVOLUCRIN	GGCCCTCAGATCGTCTCATA	CACCCTCACCCCATTAAAGA
IRF3	TCAGGGCCTTGGTAGAAATG	GCAGGTAGGCCTTGTACTGG
IRF7	GGCTGGAAAACCAACTTCC	GAGCGCGTACACCTTGTGC
36B4	GCAATGTTGCCAGTGTCTGT	GCCTTGACCTTTTCAGCAAG
ΔNP63α	ATTGCATCACTGTATCATTTTCT	TGCTCTGTGGGGACCTTTCA
KERATIN1	GTTCCAGCGTGAGGTTTGTT	TAAGGCTGGGACAAATCGAC
NOTCH1	GAACCAATACAACCCTCTGC	AGCTCATCATCTGGGACAGG
HES1	GGTGCTGATAACAGCGGAAT	TGAGCAAGTGCTGAGGGTTT
P21WAF1/CIP1	CCCAAGCTCTACCTTCCCAC	ACAGGTCCACATGGTCTTCC
LORICRIN	ATGATGCTACCCGAGGTTTG	ACTGGGGTTGGGAGGTAGTT
Nascent INVOL.	AGGGAAGAGGGGATGCTAAA	GTGTGTGTTGCTGGGACATC
Nascent IRF6	GGCATAGCCCTCAACAAGAA	CACCCCCATCATAAGCATTC
Ki67	CTGCTTGTTTGGAAGGGGTA	AGCCGTACAGGCTCATCAAT
mouse		
GAPDH	ATCACTGCCACCCAGAAGAC	CAGTGAGCTTCCCGTTCAG
P21	AAGGGTGCCGTTGTCTCTTC	GTCAAAGTTCCACCGTTCTC
IRF6	TGGACAGTGGCCTCTACCCTGGTCT	TGTTGAGAGCACAGCGGAGCTGAG
KERATIN 1	TCGTGACCATCAAGAAGGAT	ACAACATTGGTTTCGCTGAT
INVOLUCRIN	GGG ACA GAA ACA GAA GCAGA	CAGTTCTGGCTCAGGTGACT
P63	TGCCCAGACTCAATTTAGTG	TCACGCTATTCTGTGCGTGG
HES1	CTACCCCAGCCAGTGTCAAC	ATGCCGGGAGCTATCTTTCT
HEY1	GAAGGAGTTGCAGGTGAAGC	AACTTGGGGTTGCTGAGTTG
HEY2	CCAATTCACCGACAACTACC	TTGGCAGATCCTTGTTTTTC
INTEGRINα6	CGGGAACTTCCTGAAAAACA	GGCACCTGATGTTCACACAC
INTEGRINβ4	GAAGGAGTTGCAGGTGAAGC	AACTTGGGGTTGCTGAGTTG