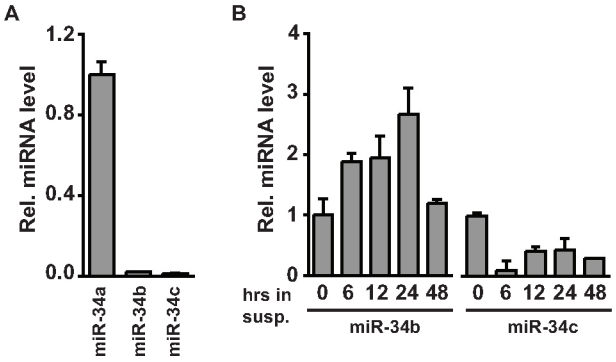
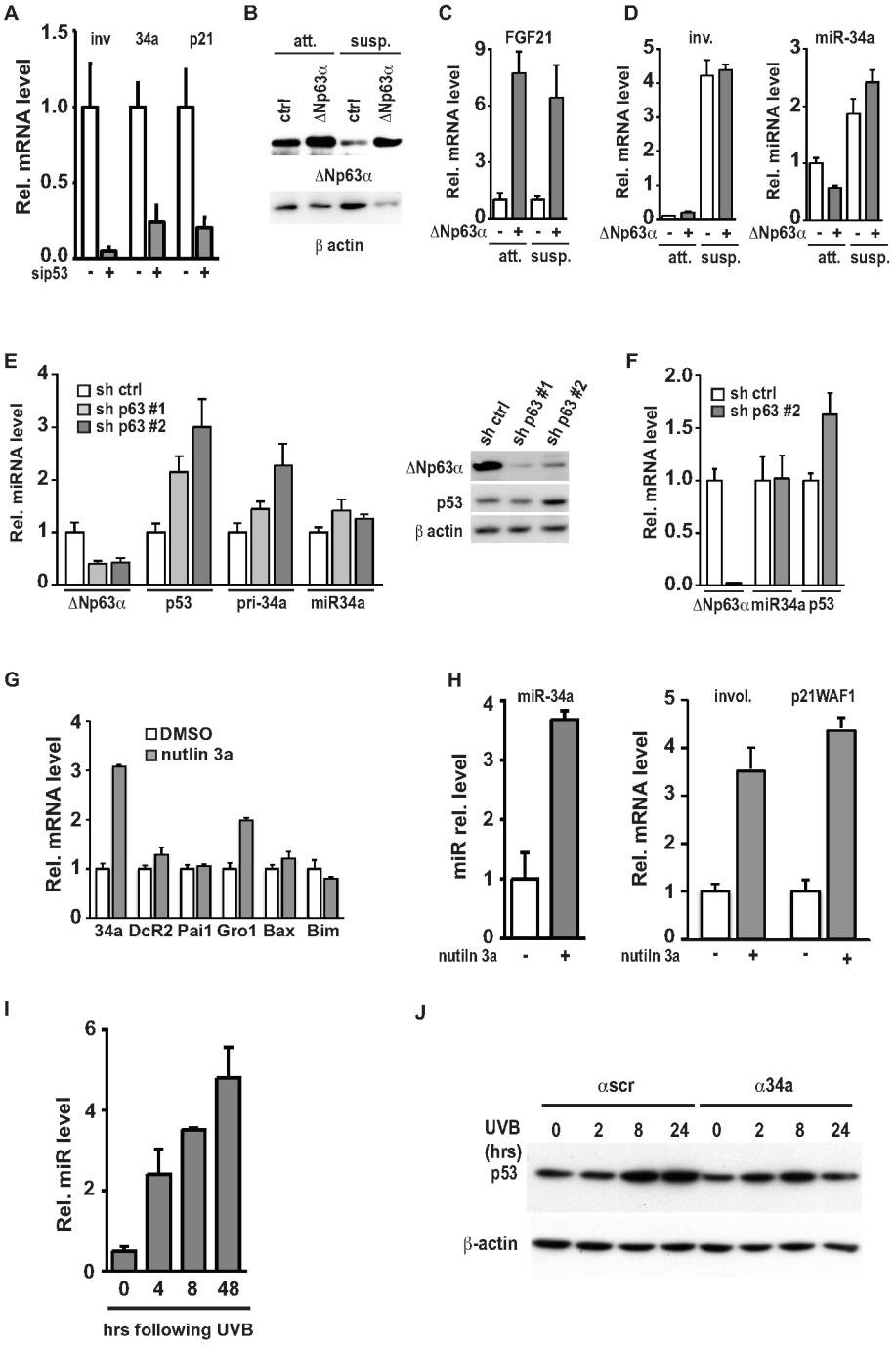
Supplementary Figure S1



Supplementary Figure S1. Pattern of miR-34 expression in HKCs. (A) Levels of mature miR-34a, b and c family members were measured in growing HKCs by qRT-PCR using specific Taqman probes with the small non coding RNA reference Z30 for normalization. (B) Levels of mature miR-34b and c in HKCs induced to differentiate by culture in suspension for the indicated times. Samples were the same as those analyzed for miR-34a expression in Figure 1B.

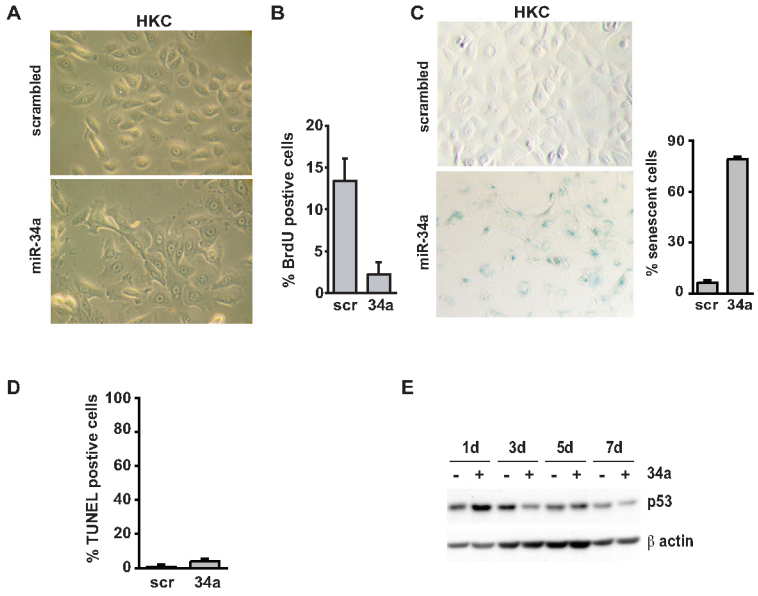
Supplementary Figure S2



Supplementary Figure S2. Control of miR-34a expression and differentiation by p53 versus p63 proteins. (A) HKCs were transfected with siRNAs against p53 versus scrambled controls followed, 72 hours later, by qRT-PCR analysis of expression of precursor miR-34a (34a) in parallel with the involucrin and p21 genes, using 36β4 for normalization. (B) HKCs were stably infected with a ΔNp63α expressing retrovirus (ΔNp63α) in parallel with empty vector control (ctrl). Cells were kept under attached growing conditions (att.) or induced to differentiate by culture in suspension (susp.) for 24 hours followed by immunoblot analysis of p63 protein expression. (C) Parallel cultures of cells treated as in the previous panel were analyzed by qRT-PCR for expression of FGF21, a known p63 targets, with 36β4 for normalization. Values are expressed as relative FGF21 levels in ΔN-p63α over-expressing HKCs versus controls, under either growing or differentiating conditions. (D) HKCs plus/minus ΔN-p63α over-expression and under growing versus differentiating conditions as in the previous panels were analyzed by qRT-PCR for expression of the involucrin differentiation marker and mature miR-34a. (E) HKCs stably infected with shRNA lentiviral vectors against p63 in parallel with empty vector control were analyzed for levels of mature (miR-34a) and primary (pri-34a) miR-34a transcripts as well as p53 by qRT-PCR (Left panel). Immunoblot analysis of the same cells for p63 and p53 protein expression (Right panel). (F) SCC13 cells infected with an shRNA lentiviral vector against p63 in parallel with empty vector control were analyzed for p63 and p53 expression as well as mature miR-34a by qRT-PCR with 36β4 and Z30, respectively, for normalization. (G and H) HKCs were treated

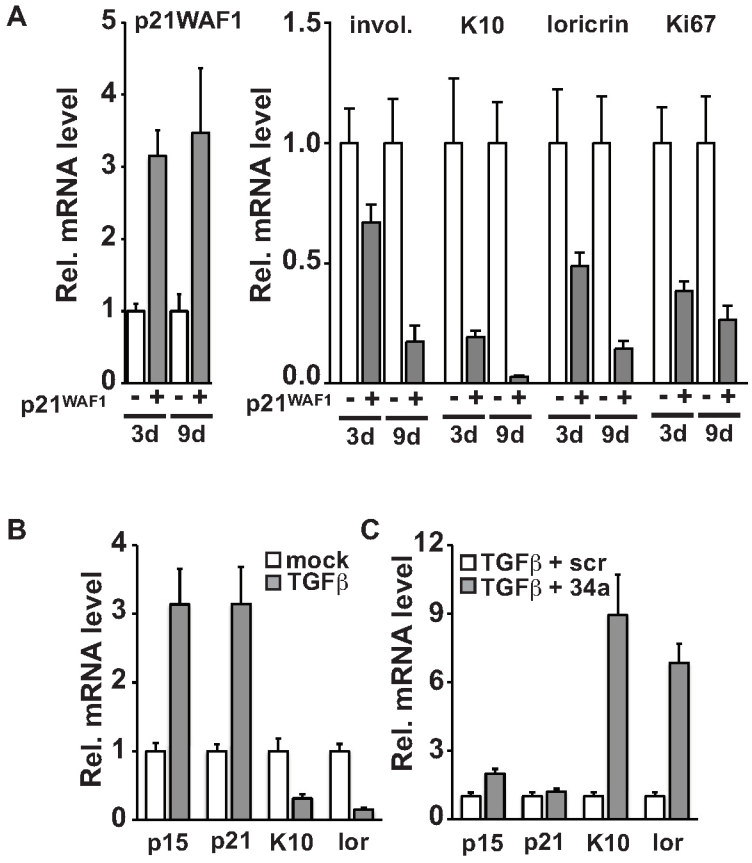
with nutlin-3a (10 μM) or DMSO vehicle alone for 48 hours (left panel) or for 30 hours (right panel) followed by qRT-PCR analysis of mature miR-34a as well as oher indicated gene expression with Z30 and 36β4, respectively, for normalization. (I) HKCs at the indicated times after UVB irradiation (30 mJ/cm^2) were analyzed for levels of mature miR-34a expression, using Z30 for normalization. (K) HKCs transfected with antagomiR against miR-34a (α34a) or control (αscr) for a total of 3 days were UVB irradiated (50 mJ/cm^2) at the indicated times from the end of the experiment, followed by immunoblot analysis for p53 expression. Samples were the same as those analyzed for involucrin expression in Figure 3C.

Supplementary Figure S3



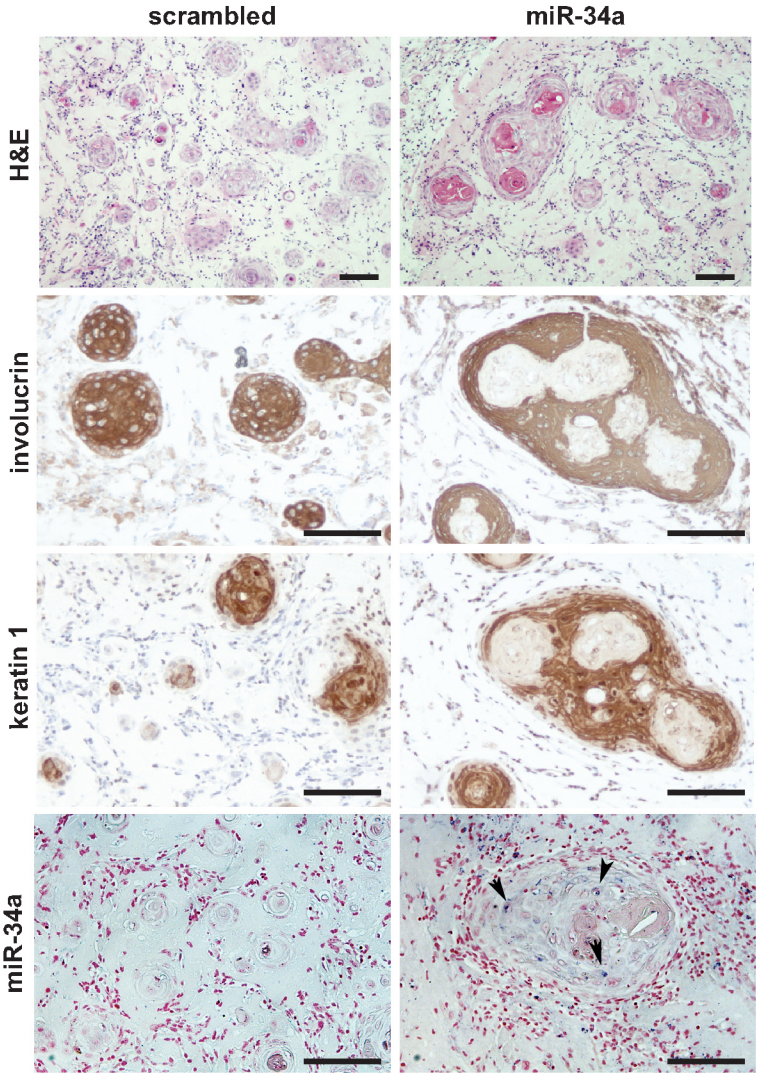
Supplementary Figure S3. Induction of keratinocyte growth arrest and senescence by elevated miR34a levels. (A) Phase contrast images of HKCs 72 hours after transfection with 25 nM miR-34a precursors (miR-34a) or scrambled control oligonucleotides (scrambled). (B) Proliferation of HKCs at 72 hours after transfection as in the previous panel was assessed by a 2 hours 5-bromo-2`-deoxyuridine (BrdU) incorporation assay. Percentage of BrdU-positive cells was evaluated in triplicate wells by digitally captured images and Imaris software analysis, using DAPI staining for total cells identification. (C) HKCs treated as in the previous panels were assayed for senescence-associated (SA)-β−Gal staining. Shown are representative cell images as well as quantification of SA-β−Gal positive cells (left and right panels, respectively). (D) The apoptotic response was evaluated by TUNEL assay in the same cells as in the previous panels. Percentages of apoptotic TUNEL positive cells are shown. (E) HKCs were analyzed by immunoblotting for p53 protein expression at various days after transfection with miR-34a precursors (+) or scrambled control oligonucleotides (-) as in the previous panels.

Supplementary Figure S4



Supplementary Figure S4. Impact of increased $p21^{WAF1/Cip1}$ expression, TGFβ1 treatment and/or miR-34a on keratinocyte differentiation. (A) HKCs were stably infected with a lentivirus for doxycycline inducible expression of $p21^{WAF1/Cip1}$. Cells were either untreated (-) or treated (+) with doxycycline (500 ng/ml) for 3 or 9 days followed by qRT-PCR analysis of expression of the indicated genes. (B) HKCs were either untreated (-) or treated with recombinant TGFβ1 (4 ng/ml) for 4 days, followed by qRT-PCR analysis of expression of the indicated genes. lor = loricrin. (C) HKCs treated with recombinant TGFβ1 (4 ng/ml) for 4 days were transfected with miR-34a mimics (25 nM) or scrambled controls for the last 3 days of the experiment. Expression of the indicated genes was assessed by qRT-PCR with 36β4 for normalization.

Supplementary Figure S5



Supplementary Figure S5. Impact of miR-34a on keratinocyte differentiation *in vivo*. HKCs transfected with miR-34a precursor or scrambled oligonucleotides were injected, 48 hours later, at the dermal–epidermal junction of immuno-compromised NOD/SCID mice. Nodules formed 8 days after injection were processed for haematoxylin and eosin (H&E) staining and immuno-histochemistry for involucrin and keratin 1 expression as in Figure 5. Parallel tissue sections were analyzed by *in situ* hybridization with double digoxigenin (DIG)-miR-34a probes. Arrows point to the miR-34a positive signal in the well differentiated cysts formed by the miR-34a transfected keratinocytes. No such signal was found in the nodules formed by control HKCs. Bars = 180 μm.