**Supplementary materials and methods.**

**Human samples, laser capture microdissection, cell culture and treatment.** Oral SCC tumors from 7 patients in parallel with corresponding normal tissue from a distant region were obtained at time of surgery at the Ear, Nose and Throat Department of the University Hospital Zurich after approval of the regional board of the Medical Ethics Commission. To rule out gene expression alterations due to stromal cell contamination, we confirmed that each tumour specimen and corresponding normal tissue used in this analysis contained > 80% epithelial cells by analysis of corresponding H&E-stained sections. Tissue samples were fresh frozen in liquid nitrogen and stored at –80°C before RNA extraction.

Normal skin and matched cutaneous actinic keratosis or SCC samples were obtained at the Department of Dermatology of the Medical University of Tübingen, Germany, from clinical biopsies. Parts not needed for histological diagnosis were further processed with institutional review board approval. Tissues were processed for laser capture microdissection (LCM) or *in situ* hybridization and/or immunofluorescence analysis, as described further below. LCM was performed as previously described (Hu et al., 2012).

Culture of primary human keratinocytes (Nguyen et al., 2006) and suspension-induced differentiation were previously reported (Watt et al., 1988). SCC O11, O12, O22 and O28 cells were provided by Dr. J. Rocco (Mass. Gen. Hosp., Boston, MA), and SCC12 and SCC13 cells by Dr. J. Rheinwald (Brigham and Women's Hospital, Boston, MA), while other cells were from ATCC. Where

specified, cells were treated with nutlin-3a (Sigma) at 10 μM (stock solution prepared in DMSO), doxycycline (Sigma) at 750 ng/ml (or lower when specified) (stock solution prepared in $ddH_2O$) or 5-aza-2'-deoxycytidine (Sigma) at the indicated concentrations and times (stock solution prepared in DMSO) adding the 5-aza-dC containing medium every day. TGFβ1 was purchased from PeproTech and used at a final concentration of 4 ng/ml.

For UVB treatment, medium was removed, cells were rinsed twice with PBS, and were irradiated with a spectrolinker XL 1000 (Spectronics Corporation) consisting of 6 tubes whose emission peak is 312 nm. A Kodacel filter was used to eliminate wavelengths below 290 nm and the delivered UVB dose was monitored each time by using a photometer (model IL 1400A, International Light, Inc., Newburyport, MA). Depending on the experiments, cells were exposed to UVB (30 $mJ/cm^2$ or 50 $mJ/cm^2$), and the medium put back onto cells.

For clonogenicity assay, cells were counted and plated at low density (500 cells per 35 mm) in triplicate. One week after, cells were fixed and stained with crystal violet. Cells were manually counted.

**miR-34a promoter methylation analysis.** Genomic DNA was isolated by incubating cells overnight at 55°C in the DNA extraction Buffer (Tris pH 8 100 mM, EDTA 1 mM, NaCl 100 mM, SDS 1%, proteinase K 50 μg/ml). After RNase treatment, DNA was extracted with phenol/chloroform precipitated with isopropanol using standard protocol. 2 μg of purified DNA were subjected to bisulfite treatment using the EZ-methylation kit according to the manufacturer's

instructions (Zymo Research). After column purification, DNA was eluted with 16 μl of elution buffer and 2 μl were used as template for methylation specific PCR (MSP) using specific primers differentiating between the unmethylated versus the methylated miR-34a promoter sequence, as already described (Lodygin et al., 2008) (primer sequences in Supplementary Table 3). PCR products were run on a 8% polyacrylamide gel and visualized by staining with SYBR Green on a blue light transilluminator.

**mRNA and miR expression analysis.** Total RNAs were prepared with Tri-Reagent (Sigma) according to the manufacturer's protocol and DNase I treated. For conventional qRT-PCR, one microgram was used for cDNA preparation with random hexamers and Superscript II (Invitrogen) according to the manufacturer's protocol, and qRT-PCR analysis was performed with gene-specific primers (Supplementary Table 3) using 36β4 for normalization. The expression levels of mature miRNAs were quantified using TaqMan MicroRNA Assays (Applied Biosystems, Foster City, CA; miR-34a: no. 000426; miR-34b: no. 002102, and miR-34c: no. 000428). The expression of each miRNA was normalized to the expression of Z30 snRNA (Applied Biosystems, no. 001092). When specified, miR-34a was detected by qRT-PCR according an alternative protocol previously described (Raymond et al., 2005) using 5s RNA for normalization (Primer sequences in Supplementary Table 3). Each sample was tested in triplicate on a Lightcycler 480 Real-Time detection System (Roche) according to the manufacturer's recommendation, with SYBR Green (Roche) for detection.

**Microarray hybridization.** For conventional microarray hybridization of HKCs total RNA (5-8 μg) was used as template for double stranded cDNA preparations with T7-$(dT)_{24}$ oligonucleotide primers for the first strand reaction. The resulting cDNAs were used for preparation of biotin-labeled cRNA probes preparation and hybridization to the Affymetrix HG-U133A 2.0 gene expression platform containing 22000 probe sets according to the manufacturer's recommendation (two chips per sample were generated, following the manufacturer's instructions). For miRNA microarray, total RNA was processed for array hybridization using two different platforms: LC Sciences (Houston, TX) and Agilent Technologies (performed at Center for Integrative de Genomics, University of Lausanne, Switzerland), and data acquisition was performed by respective service providers as described previously (Pradervand et al., 2009; Tay et al., 2008).

**Microarray data analysis.** For gene expression analysis, Affymetrix .CEL files were loaded into the Resolver SE System (Rosetta Biosoftware) for data processing and normalization, using platform-specific error models. Intensity profiles were then compared to form ratio experiments where each gene is associated to an expression fold-change and a p-value that assesses the statistical significance of modulation in the treated versus control sample.

Genes significantly down-regulated (fold change < -2 and p-value < 0.01) by the over-expression of miR-34a in HKCs and in differentiated primary keratinocytes were extracted and analyzed for the presence of recognition sequences for miR-

34a itself in their 3' UTR regions. For this purpose, we exploited three different miRNA prediction algorithms (PicTar, TargetScan and miRBase) and we considered as reliable targets those predicted by 2 out of 3 algorithms.

For assignment of modulated genes to Biological Processes-level 5 (BP5) of Gene Ontology (GO), we used the functional annotation tool available within the DAVID website (http://david.abcc.ncifcrf.gov/). The Affymetrix HG-U133A.2 platform was used as reference list.

For miRNA expression analysis from Agilent platform, raw data were processed in R statistical environment through the method of invariant selection and normalization (Pradervand et al., 2009). Differential expression was then tested applying the LIMMA package available at www.bioconductor.org (Gentleman et al., 2004). For miRNA microarrays from LC Sciences, normalized data were provided by the LC Sciences service (www.lcsciences.com). In both cases, differentially expressed miRNAs with fold change > 2 or <-2 and p-value < 0.01 were considered as differentially expressed.

***In situ* hybridization (ISH).** Skin and SCC specimens were fixed in 4% paraformaldehyde (PFA), washed in PBS and incubated in 30% sucrose/PBS before embedding in OCT. 7 µm skin sections were fixed in 4% PFA, washed in phosphate buffer saline (PBS) and then treated with Proteinase K (10 mg/ml; Sigma) for 5 min, washed, and PFA fixed again. Sections were then incubated for 10 min in triethanolamine pH 8, and then for 10 min in triethanolamine/acetic anhydride. Hybridization was performed at 55°C overnight, with 5'- and 3'-

digoxigenin (DIG)-coupled miRCURY LNA™ Detection antisense probes specific for miR-34a (Exiqon) in hybridization buffer (formamide 50%, SCC 5X, Denhardt's solution 5X, yeast tRNA (Ambion) 250 mg/ml, salmon sperm DNA (Sigma), 500 mg/ml. After washing, sections were incubated with a mouse anti-digoxigenin antibody coupled to alkaline phosphatase (AP). Sections were stained with the AP substrate NBT/BCIP (Promega) and development of the precipitate was monitored under a Leica (DM2000) microscope. The ISH on miR-34a done on the paraffin embedded cyst formed *in vivo* was performed using the miRCURY LNA$^{TM}$ microRNA ISH kit (FFPE) from Exiqon according to the manufacturer's instructions (Exiqon).

**Immunoblotting, immunofluoresence, SA-βGal assay and BrdU labeling.** For immunoblotting, total proteins were extracted as previously described (Lefort et al., 2001). Unless otherwise specified, for equal loading control, the same blots were reprobed, without stripping the membrane, with either antibody against γ-tubulin or β-actin. For immunofluoresence, frozen sections were fixed with paraformaldehyde 3%, washed 3 times in PBS-1X-triton 1%, incubated for 1 hour in the blocking buffer (PBS-1X, triton 1%. BSA 5%) before overnight incubation with corresponding antibodies. Tissue sections were washes 3 times in PBS-1X-triton 1% and incubated for 1 hour with the appropriate secondary antibodies (Invitrogen, Molecular probes). Finally sections were washed 3 times in PBS-1X-triton 1% with the second wash including DAPI (Sigma). After a final wash in PBS-1X, tissue sections were mounted with fluorescence mounting medium

(DAKO) and fluorescence was monitored on the Upright microscope Axio Imager.Z1 (Zeiss). Antibodies against the following proteins were used: $p21^{WAF1}$, cyclin E2, p53, Axl, Notch1, c-Met, FosL1 (i.e. Fra-1), p63, β-actin (Santa Cruz), HES-1, HERP1 (i.e. HEY2) (Chemicon), involucrin (Abcam), keratin 1, 10 and 14 (Covance), SIRT1 and SIRT6 (Cell Signalling) and γ-tubulin (Sigma). Proliferative cells were assessed by incorporation of BrdU (Amersham) and revealed by anti-BrdU antibody (BD Biosciences) according to the manufacturer's instructions. Senescent cells were visualized using the SA-β-gal staining kit (Cell Signaling) according to the manufacturer's instructions (Roche). Positive blue cells versus total cells (phase contrast) were counted under a Leica (DM2000) microscope.

**siRNA, miRNA and antagomiR transfection.** For siRNA experiments, cells were transfected as described (Nguyen et al., 2006) with a total 20 nM of Ambion Silencer Select siRNAs (Invitrogen) against *Axl* (si n°1 and n°2), *FosL1* (si n° 1, 2 and 3 pooled together) or *c-Met* (si n°1 and si n°2) or the Stealth siRNAs against *SIRT6* or *p53* (duplex 2) (Invitrogen) in parallel with corresponding siRNA controls (Supplementary Table 3). miR-34a precursor oligonucleotides (as well as scrambled control) and miRCURY LNA™ microRNA Power inhibitor, antagomiR-34a) (as well as antagomiR scrambled) were purchased from Ambion or Exiqon respectively. Cells were transfected with mimics or antagomiRs using Interferin (Polyplus, France) overnight (final concentration of 25 nM or less where specified for mimics and 50 nM for antagomiRs).

**Luciferase activity assays.** For cloning the Notch1-3'UTR-luc WT and mutant as well as the SIRT6-3'UTR-luc WT or mutant, see Supplementary materials and methods. HeLa cells or HKCs, respectively, were transfected with the Notch1-3'UTR-luc WT or mutant as well as the SIRT6-3'UTR-luc WT or mutant reporter vectors together with miR-34a oligonucleotides or scrambled control. Cells were harvested 40 hours or 48 hours, respectively, after transfection and assayed for luciferase activity using total protein concentration for normalization. All experiments were performed in triplicate.

**In vivo experiments.** For in vivo cyst assays, HKCs were transfected with miR-34a precursor oligonucleotides or scrambled control at a final concentration of 25 nM. Two days later, cells were brought into suspension, admixed with Matrigel (BD Biosciences) and injected ($2x10^6$ cells per injection) intra-dermally into NOD/SCID mice. Cysts formed were dissected 8 days later. Chemically induced papillomas in $p53^{KI/KI}$ mice control mice and tamoxifen treatment were as described in (Guinea-Viniegra et al., 2012).

**Luciferase activity assays.** Human Notch1 WT-3′-UTR was amplified by PCR from the first nucleotide after the stop codon to the last nucleotide before the polyadenylation signal from human genomic DNA using the following primers: Notch1UTR-XbaIF 5′-tgctctagacggcgcgccccacgag-3′; Notch1UTR-SpeIR 5′-ggccactagtgcaaatcagttaacaaaaaagatg-3′. The 1600-bp fragment, after XbaI/SpeI

restriction, was ligated to a compatible XbaI-linearized pGL3Control vector (Promega). The construct Notch1-mutant-3'UTR-luc was obtained by deletion of the 2 miR-34a-predicted target sites (7 bp, CACTGCC) by PCR using a series of 3 successive sub-clonings using the overlapping primers: 1) Notch1UTR-XbaIF2 5′-tgctctagagggctgaccccggtggc-3' and Notch1UTR-SpeIR, 2) Notch1UTR-XbaIF1 5′-tgctctagatttttatttatatgtactgttttatct-3' and Notch1UTR-SpeIR2 5′-ggccactagtctttccccagaaaagggtag-3' and 3) Notch1UTR-XbaIF and Notch1UTR-SpeIR1 5′-ggccactagtttttctgtgtaaaataaaagtacataa-3'. The human SIRT6 WT-3′-UTR was amplified by PCR from the first nucleotide after the stop codon to the last nucleotide before the polyadenylation signal from human genomic DNA using the following primers: SIRT6-UTR-SpeIF 5′- ggccactagtccagggtgcttggggag-3′; SIRT6-UTR-SpeIR 5′- ggccactagtgaggacttttccagaagcct-3′. The 479-bp fragment, after SpeI restriction, was ligated to a compatible XbaI-linearized pGL3Control vector (Promega). The construct SIRT6-mutant-3'UTR-luc was obtained by deleting the last 57 bp of the WT construct containing the miR-34a-predicted target site (7 bp, CACTGCC) by PCR using the previous SIRT6-UTR-SpeIF and the following primer: SIRT6-UTR-XbaIR1 5'-tgctctagacaagcctctactgatcccc-3'.

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