## METHODS

**Generation of mice and tumour analysis.** *TAp63*$^{-/-}$ mice[19] were intercrossed with *p53*$^{-/-}$ mice[16] to generate compound mutant *TAp63/p53* mice on an enriched C57BL/6 background ((C57BL/6 = 95%) and (129/SvJae = 5%)). For analysis of tumour formation and metastatic disease, 30 mice of each genotype were aged for 2.5 years. Moribund mice were killed, following the approved guidelines of the IACUC at the University of Texas M. D. Anderson Cancer Center and analysed histologically by haematoxylin/eosin (H&E) staining as described previously[8]. Tumour-free survival of mice was plotted with PRISM5 software (GraphPad).

**Migration and invasion assay.** MEFs or mouse and human tumour cell lines were resuspended in 500 µl of DMEM with 10% FBS and 1 × penicillin/streptomycin ($5 \times 10^4$ cells) and added to the top of each chamber containing BD BioCoat cell culture inserts (354578; BD Biosciences) or Matrigel Invasion Chamber (354480; BD Biosciences)[19,24]. Non-invasive cells were removed from the upper chamber. Cells remaining on the membrane were fixed, stained with Diff-Quik (Dade Behring, Inc.), and counted with a Zeiss AxioObserver A1 inverted microscope.

**Mouse and human tumour cell lines.** Tumours were excised from *p53* and *TAp63* mutant mice at the time of necropsy. Half of the tumour was fixed in 10% formalin and analysed histologically by H&E staining. The other half of the tumour was minced and treated for 20 min with 0.25% trypsin-EDTA solution at 37 °C. Cells were suspended in DMEM medium containing 10% fetal bovine serum (FBS) and 1 × penicillin/streptomycin. Human primary and metastatic squamous cell carcinomas (cell lines 10A, 10B, 17A, 17B, 22A and 22B) were cultured similarly[20].

**Immunocytochemistry.** Cells were fixed on chamber slides for 30 min at room temperature (25 °C) with 4% paraformaldehyde in PBS and incubated in blocking buffer (8% FBS, 0.3% Triton X-100 in PBS) for 30 min at room temperature as described previously[19]. Samples were incubated overnight in anti-γH2AX (dilution 1:100; Upstate Biotechnology, Inc.) at 4 °C under humidified conditions, followed by incubation for 1 h with secondary antibodies (goat anti-mouse Texas Red, dilution 1:500; Jackson ImmunoResearch Laboratories) at room temperature. 4′,6-Diamidino-2-phenylindole (DAPI; Pierce Biotechnology) was used as a nuclear counterstain. Photomicrographs were taken with a Zeiss AxioObserver A1 inverted fluorescence microscope.

**Immunofluorescence and immunohistochemistry.** Paraffin-embedded sections of tumours were prepared as described previously[8]. Tissue microarrays containing primary and metastatic HNSCCa (HN242), lung adenocarcinomas and squamous cell carcinomas (LC1005) and mammary adenocarcinomas (BR480) were obtained from US Biomax. For detection of Dicer, sections were incubated overnight with anti-Dicer (dilution 1:100; Abcam) at 4 °C in a humidified chamber followed by incubation for 1 h with goat anti-mouse-fluorescein isothiocyanate (dilution 1:500; Jackson ImmunoResearch Laboratories) at room temperature for immunofluorescence or using the Vectastain universal ABC Kit (Vector Labs), followed by the DAB kit (Vector Labs) for immunohistochemistry. For detection of TAp63, sections were incubated with anti-TAp63 (dilution 1:100; gift from C. Prives) followed by detection for immunohistochemistry as described above. For detection of γH2AX in frozen tumour sections, tumours were fixed in 4% paraformaldehyde, frozen in OCT medium (Tissue Tek) and detected by incubation in anti-γH2AX (dilution 1:100; Upstate Biotechnology, Inc.) followed by incubation for 1 h with goat anti-mouse Texas Red (dilution 1:500; Jackson ImmunoResearch Laboratories) at room temperature. DAPI (Pierce Biotechnology) was used as a nuclear counterstain for immunofluorescence or haematoxylin (Vector) for immunohistochemistry. Photomicrographs were taken with a Ziess Axioplan2 imaging fluorescence microscope.

**Human HNSCCs and lung adenocarcinomas.** Total RNA was isolated from 25 human HNSCCs and 19 lung adenocarcinomas of various grades (well differentiated (grade I), moderately differentiated (grade II) and poorly differentiated and invasive (grade III)) for qRT-PCR using primers for *TAp63*, *Dicer* and *miR-130b* as described below.

**SA-β-gal staining.** SA-β-gal staining was performed on frozen sections of tumours as described previously[19].

**qRT-PCR.** Total RNA was isolated from mouse MEFs, human squamous cell carcinomas and cell lines, or mouse tumours and tumour cell lines by using TRIzol LS Reagent (Invitrogen). qRT-PCR was performed with a StepOnePlus real-time PCR system (Applied Biosystems), a SuperScript First-Strand Synthesis System (Invitrogen) and Power SYBR Green PCR Master Mix (Applied Biosystems) in accordance with the manufacturers' protocols. The following primers were used: human TAp63 (ref. 25), human ΔNp63 (ref. 25) and murine or human Dicer (ref. 26). Murine or human glyceraldehyde-3-phosphate dehydrogenase was used as an internal control[19,25]. Average cycle threshold ($C_t$) values were calculated from triplicate reactions of three biological replicates.

**Cytogenetics.** Tumour cells at 70% confluence were treated for 3 h with 0.02 µg ml$^{-1}$ colcemid and prepared as described previously[19]. Images were captured and processed with MetaMorph Premier (Molecular Devices) using a Nikon Eclipse E400 microscope.

**Northern blot analysis.** Total RNA was fractionated on a denaturing 15% polyacrylamide gel containing 8 M urea, and transferred to Hybond-N$^+$ membrane (Amersham Biosciences) by a semi-dry gel transfer method. Membranes were hybridized with end-labelled [γ-$^{32}$P]ATP miRCURY LNA detection probes (Exiqon) in ULTRA hyb-Oligo Hybridization Buffer (Ambion) at 37 °C. Membranes were washed twice for 5 min in 2 × SSC, 0.1% SDS at 37 °C followed by a prolonged wash for 15 min at 25 °C, and then exposed to X-ray film overnight.

**miRNA TaqMan assays.** Applied Biosystems TaqMan miRNA assays were used to detect and quantify mature miRNAs using looped-primer real-time PCR. Total RNA samples were prepared from tumour cell lines and MEFs with TRIzol LS Reagent (Invitrogen). Total RNA (1 µg) was used to synthesize complementary DNA with the Two-Step TaqMan MicroRNA Assay kit (Applied Biosystems) in accordance with the protocol. qRT-PCR was performed with the Step One Real Time PCR System, TaqMan PCR master mix, and TaqMan primers for the specific miRNAs (Applied Biosystems). Each sample was run in triplicate. $C_t$ values for miRNAs were calculated and normalized to $C_t$ values for *RNU6B*.

**ChIP assay.** ChIP was performed with nuclear extracts from wild-type and *p63*$^{-/-}$;*Arf*$^{G/G}$ keratinocytes as described previously[19,24,27]. qRT-PCR was performed with the following primer sequences specific for p63-binding sites: murine Dicer, −1433, 5′-AGGCTGGCCTTGATCTGTGA-3′ (forward) and −1333, 5′-CACATCCTCGGCTGTCTTTCA-3′ (reverse); mmu-miR-34a, +367, 5′-TAGCCAAACAGCCACCATCTT-3′ (forward) and +457, 5′-CCCCAGCCCTCCACAAG-3′ (reverse); and mmu-miR-130b, −850, 5′-CACGTGAGTAACTGGTCTGGGATA-3′ (forward) and −763, 5′-TCCTAACAGATTCTCCTGCCTAGAA-3′ (reverse). Primer sequences for non-specific binding sites were as follows: murine Dicer promoter, −2560, 5′-CGAACCCAGAGAGTCCACAAG-3′ (forward) and −2499, 5′-CCCCATCCCCGACACTTAC-3′ (reverse); mmu-miR-34a promoter, +732, 5′-AAGCGGGTTTCAAGTGCATCTCAG-3′ (forward) and +796, 5′-TCAGGCTACTAAACCAGTTGCCCT-3′ (reverse); and mmu-miR-130b promoter, −1431, 5′-AAATGTCCCATCCTGGAGGAGCAA-3′ (forward) and −1323, 5′-TCACCAAATTAGCGAGGGCTCTGA-3′ (reverse). Primers for the p53-binding site for *miR-34a* were used as described previously[23].

**Lentiviral infection.** Lentivirus-based vectors (2 µg) containing shRNAs (SBI System Biosciences) for mmu-miR-34a, mmu-miR-126, mmu-miR-130b or scramble sequence (as control) and tagged with green fluorescent protein (GFP) or pDESTmycDICER[28] were transfected into 293T cells along with 2 µg of each vector required for lentivirus packaging (pCMV-VSVG, pRSV-REV or pMDLg/pRRE) using Fugene HD (Roche) in accordance with the manufacturer's protocol. After transfection of 293T cells, supernatants containing the lentivirus were collected, filtered and added to target MEF or tumour cells for 24 h in the presence of 8 µg ml$^{-1}$ Polybrene. At 24 h after infection, puromycin was added to the media at 3 µg ml$^{-1}$ for 7 days. Infection efficiency of the cells was calculated by dividing the number of GFP-expressing cells by the total number of cells, with the use of a Zeiss AxioObserver A1 inverted fluorescence microscope. The infection efficiency was calculated to be 100% in all experiments. Infected cells were analysed by TaqMan assay to determine the level of miRNA silencing. These cells were further analysed by migration and invasion assays. All experiments were performed in triplicate.

**Retroviral infection (pSuper mouse Dicer1).** Wild-type and *TAp63*$^{-/-}$ MEFs ($10^6$) were infected with pSuper mouse Dicer1 (Addgene) as described previously[22].

**Adenovirus-Cre infection.** *TAp63*$^{fl/fl}$ MEFs ($10^6$) were plated on 10-cm dishes and infected with $5 \times 10^3$ Ad-Cre–GFP (Vector Development Laboratory) particles per cell. Infection efficiency was calculated on the basis of expression of GFP and found to be 98%. To verify recombination, qRT-PCR was performed for *TAp63* mRNA.

**Dual luciferase reporter assay.** Luciferase assays were performed with *p53*$^{-/-}$;*p63*$^{-/-}$ MEFs as described previously[29]. To generate the luciferase reporter genes *pGL3-Dicer, pGL3-miR-130b* and *pGL3-miR-34a*, the DNA fragment containing the p63-binding site identified by ChIP was amplified from C57BL/6 genomic DNA by PCR with the following primers containing 5′ *Nhe*I and 3′ *Bgl*II cloning restriction enzyme sites: DICER1, 5′-GCTAGCATGTGCCAGGGCTTTGGCATGTA-3′ (forward) and 5′-AGATCTTCCTGGAACTTGCTCTGTACACCA-3′ (reverse); mmu-miR-34a, 5′-GCTAGCTGGAGTGTGAGCACTTCTGGCTAA-3′ (forward) and 5′-AGATCTTGGACATTCAGGTGAGGGTCTTGT-3′ (reverse); and mmu-miR-130b, 5′-GCTAGCATGGTTAAAGATGGAGCCGAGGGA-3′ (forward) and 5′-AGATCTTCTCCTGCCTAGAAGAGCAGAACT-3′ (reverse).

**Statistics.** Data are represented as means and s.e.m. All experiments were performed in triplicate. Student's *t*-test was used for comparison between two groups or one-way analysis of variance test. $P = 0.05$ was considered significant.


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