

p53 activity contributes to defective interfollicular epidermal differentiation in hyperproliferative murine skin

DOI: 10.1111/bjd.14048

DEAR EDITOR, Skin diseases affect a significant percentage of the population and are often the result of a complex interplay between autoimmune dysregulation, and abnormal epidermal differentiation and proliferation. Origins may be genetic and/or environmental, and while no complete cure exists for conditions such as psoriasis, a range of treatments, including retinoids and antibodies against tumour necrosis factor- α and interleukin-17, have shown therapeutic efficacy, although relapses can occur.¹ While there is no definitive mouse model for psoriasis, some do present with abnormal skin phenotypes that have revealed interesting molecular drivers.¹ For example, agonist-treated peroxisome proliferator-activated receptor (PPAR) β/δ transgenic mice develop psoriasis-like conditions with failed compaction of the granular layer, which can be countered by PPAR β/δ antagonists.² PPAR β/δ is also upregulated in human psoriasis and murine ichthyosis,^{2,3} but has proinflammatory, anti-inflammatory and prodifferentiation properties in various contexts.^{4,5} PPAR γ , although predominantly expressed in the sebaceous gland, has similar prodifferentiation effects, and can reduce inflammation and promote barrier formation in mice with induced parakeratosis (Fig. S1; see Supporting Information).⁶

The tumour suppressor p53 is upregulated in the pathogenesis of human chronic plaque-type psoriasis (Fig. 1a), and its

role in skin disease has long been questioned.⁷ In additional animal studies, p53 has been found to be largely dispensable to epidermal homeostasis, with gene loss only causing minor alterations in murine catagen,⁸ and paradoxically, p53 deletion reduces oncogenesis in transgenic mouse skin carcinogenesis studies.⁹ p53 knockdown also promotes squamous differentiation in human keratinocytes cultured *in vitro*,¹⁰ which suggests p53 activation may impair keratinocyte differentiation in the interfollicular epidermis; however, this has not been tested *in vivo*.

A parakeratotic differentiation programme can be invoked by high MYC activity in keratinocytes; thus, K14MycER mice form a useful model of hyperproliferative skin. They overexpress MYC fused with the tamoxifen-responsive mutant oestrogen receptor ligand binding domain in the keratin 14 (K14)-positive basal layer of the epidermis and, upon activation with high-dose 4-hydroxytamoxifen (4OHT), exhibit parakeratotic lesions of acanthosis, hyperkeratosis and dermal inflammatory infiltration (see Fig. S1; see Supporting Information).^{11,12} Our K14MycER mice also show dose-dependent activation of the tumour suppressor p53 (Fig. 1b).¹³

We previously crossed K14MycER mice with p53 knockout animals and demonstrated that aberrant p53 activity interferes with sebaceous gland differentiation by impairing androgen receptor function.¹³ In this study we investigated if p53 activity also contributes to defective interfollicular epidermal differentiation in the same cohort of animals. Full materials and methods are available in Appendix S1 (see Supporting Information). Our results show K14MycER p53null mice exhibited persistent hyperproliferation (Fig. S1; see Supporting

Fig 1. Characterization of K14MycER p53null mouse epidermis. (a) Human scalp and psoriasis lesions immunostained for p53 ($n = 6$). (b) Mouse back skin from wild-type (WT:), K14MycER (Myc:) and K14MycER p53 knockout (Myc: p53null) mice 4 days after treatment with 0.1 mg or 1.5 mg 4-hydroxytamoxifen as indicated, immunostained for p53 and counterstained with haematoxylin. (c) Mice treated as in (b), including p53 knockout (p53null) controls stained with haematoxylin and eosin (H&E). The granular layer was not visible in $21.39 \pm 0.01\%$ of K14MycER p53 wild-type (WT; p53wt) skin (as a proportion of length) and $22.64 \pm 0.03\%$ of K14MycER p53 heterozygous (p53het) skin. In contrast, the granular layer was only absent in $10.85 \pm 0.03\%$ of K14MycER p53null skin (* $P < 0.05$). (d) Mouse skin immunostained for keratin 6 (K6) and keratin 14 (K14), and counterstained with nuclear dye 4',6-diamidino-2-phenylindole (DAPI); (e) mouse skin immunostained for K14, fatty acid binding protein 5 (FABP5) and DAPI; and (f) mouse skin immunostained for K14, loricrin (LOR) and DAPI. (g–n) Quantitative reverse transcription polymerase chain reaction for indicated mRNAs relative to *Gapdh*, standardized to WT mice (defined as 1). K14MycER (Myc:) mice shown by grey bars of p53wt, p53het and p53null status. (j) MYC activity alone induced downregulation of *Krt10* mRNA, although (k) the *K10* protein persists in this time frame and there is upregulation of *Flg* mRNA (Fig. S1; see Supporting Information). (l, m) Genes that showed MYC and p53-dependent regulation included *Ivl* and *Ppl*, such that deletion of even one functional p53 allele resulted in a significant upregulation of mRNA. (g, n) Most significantly, hyperproliferative *Krt6a* and *Ppar β/δ* expression, which was upregulated in K14MycER p53wt/het mice, was reduced in K14MycER p53null mice. We have shown previously that K14MycER p53null mice have increased *Pparg* mRNA expression,¹³ and here demonstrate the increased peroxisome proliferator-activated receptor (PPAR) γ protein expression is predominantly in the sebaceous gland (Fig. S1; see Supporting Information). $n = 3$ –5. Error bars represent SEM. # $P < 0.06$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$. Scale bars 50 μ m. Rel. Exp., relative expression.

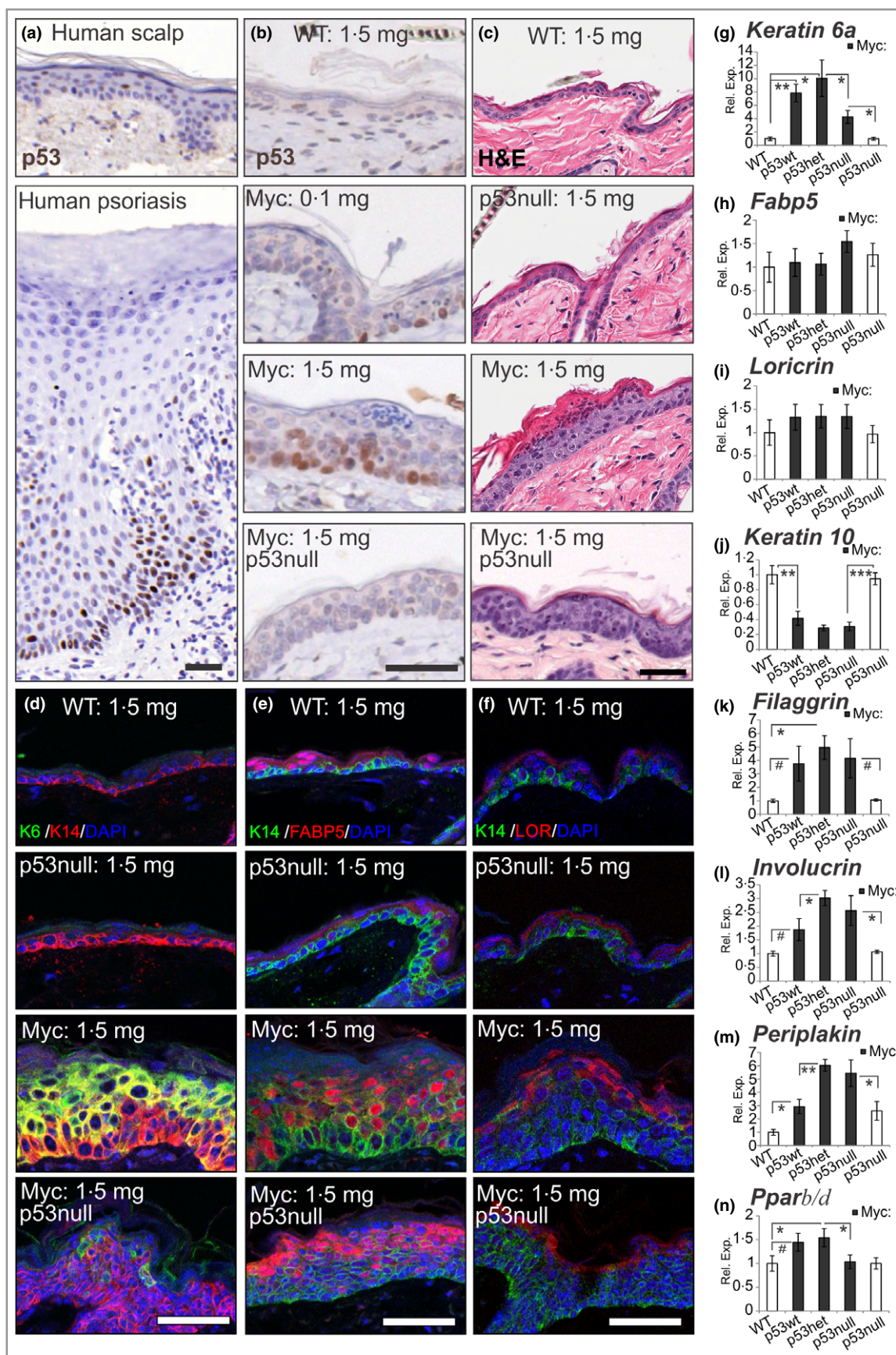


Fig 2. Retinoic acid signalling and p53. (a) Mouse telogen back skin [wild-type (WT:) and K14MycER (Myc:) mice] treated once with acetone or 1.5 mg 4-hydroxytamoxifen (4OHT) and daily with 0.016 mg BMS493 for 4 days, stained with haematoxylin and eosin (H&E) and (b) immunostained for p53 with haematoxylin counterstain. (c) Haematoxylin and eosin analysis of K14MycAER (MycA:) mice treated with 1.5 mg 4OHT after 4 days. (d) p53 immunostaining of mice in (c) with haematoxylin counterstain. (e) Quantitative reverse transcription polymerase chain reaction (qRT-PCR) for p53 mRNA relative to *Gapdh*, standardized to wild-type (WT) mice (defined as 1). Mice (including transgenics and WT siblings) from the K14MycER (Myc:) strain are shown by white bars and mice from the K14MycAER (MycA:) strains are shown by the grey bars. (f) Immunostained for keratin 6 (K6), keratin 14 (K14) and 4',6-diamidino-2-phenylindole (DAPI), (g) immunostained for K14, fatty acid binding protein 5 (FABP5) and DAPI, and (h) immunostained for K14, loricrin (LOR) and DAPI. (i–l) qRT-PCR for indicated mRNAs relative to *Gapdh*, standardized to WT mice (defined as 1). Mice (including transgenics and WT siblings) from the K14MycER (Myc:) strain are shown by white bars and mice from the K14MycAER (MycA:) strains are shown by the grey bars. (m) Summary of gene changes in transgenic mouse strains. (+) indicates gain, (–) indicates reduction, (o) indicates unchanged. Magnitude indicated by number of $\pm$ symbols. (n) Summary model of proposed signalling pathway (note that BMS493 and MYCA can inhibit retinoic acid receptor activity and prevent p53 activity). $n = 3$ –9. Error bars represent SEM. $^{\#}P < 0.06$, $^*P < 0.05$, $^{***}P < 0.005$, $^{**}P < 0.01$. Scale bars = 50 μ m. N/A, not applicable; Rel. Exp., relative expression; PPAR, peroxisome proliferator-activated receptor.

Information),¹³ skin thickening and K14-positive basal layer expansion (Fig. 1c, d); however, deletion of p53 causes a number of positive changes, with reduced keratin 6 (K6) expression (Fig. 1d, g), partial redistribution of the keratinocyte differentiation marker fatty acid binding protein 5 (FABP5) towards terminal differentiating layers (Fig. 1e, h), and improved granular layer compaction (Fig. 1). Additional quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analysis showed that MYC activity reduced *Krt10* mRNA expression (Fig. 1j), although keratin 10 (K10) protein persisted in the uppermost keratinocyte layers (Fig. S1; see Supporting Information). MYC activity also promoted the expression of *Flg* and *Ivl* but these (and K10) did not change further upon deletion of p53 (Fig. 1j–m). However, loss of p53 reduced *Krt6a* (Fig. 1g), upregulated *Pparg* and normalized mRNA expression of *Pparb/d* [Fig. 1n; Fig. S1 (see Supporting Information)].¹³

Retinoic acid (RA) signalling is important to skin biology yet there is no previous evidence for cross-talk between p53 and RA signalling in skin. A recent study has demonstrated that p21(RAC)-activated kinase 2 (PAK2)-phosphorylated MYC binds and co-activates RA receptor (RAR) α , while unphosphorylated MYC acts as a co-repressor.¹⁴ As we observed non-apoptotic p53 activation in response to MYC, we set out to examine if this was related to RAR signalling by using the reverse agonist BMS493 to promote stabilization of RAR/retinoid X receptor repressive complexes.¹⁵ This drug promoted granular formation and prevented the induction of p53 (Fig. 2a, b). We had also previously generated K14MycAER mice, possessing three MYC point mutations to prevent PAK2 phosphorylation, and predicted 'MYCA' would therefore mimic BMS493 to function as a RAR co-repressor. Our prediction proved true, as K14MycAER mice did, indeed, retain granular formation (Fig. 2c), and showed markedly reduced p53 expression and p53 activation (Fig. 2d, e). As in K14MycER mice, hyperproliferation, skin thickening and basal layer expansion still occurred [Fig. 2c, f; Fig. S1 (see Supporting Information)], and *Krt10* mRNA expression was also reduced, while the K10 protein persisted [Fig. 2i; Fig. S1 (see Supporting Information)]. However, points of difference included that K6 was not as greatly upregulated and FABP5 was predomi-

nantly observed in upper differentiating keratinocytes in K14MycAER mice (Fig. 2f, g). Loricrin was also more compacted (Fig. 2h). Further qRT-PCR analysis of K14MycAER mice confirmed reduced *Krt6a* expression (Fig. 2j), along with upregulation of *Pparg* and normalized *Pparb/d* mRNA expression (Fig. 2k, l). Thus, K14MycAER mice phenocopy K14MycER p53null mice (Fig. 2m). Of further interest, in contrast to K14MycER mice, K14MycAER mice also maintained their granular layer when challenged with the strongly p53-activating compound, camptothecin (Fig. S1; see Supporting Information), suggesting PAK2 unphosphorylated MYC is the form of MYC that counteracts p53.

A question that arises is how p53 activity predominantly detected in basal cells can trigger such changes in differentiating keratinocytes. One possibility is that p53 promotes basal keratinocyte-secreted inflammatory mediators that influence differentiating cells. In line with this general idea, we recently showed how chemokine upregulation increases disease severity by impairing keratinocyte terminal differentiation in a mouse model of harlequin ichthyosis.³

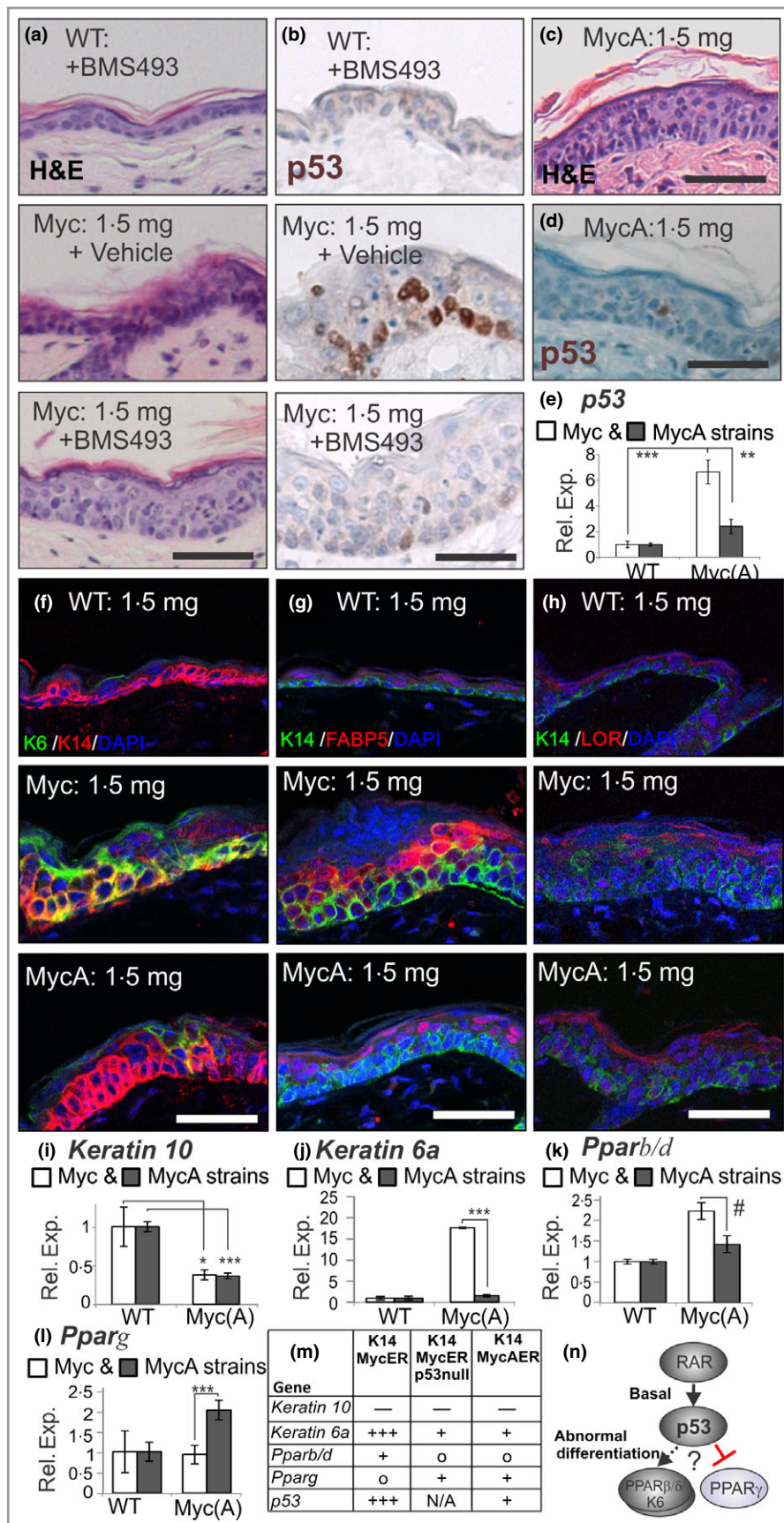
In conclusion, we identified that p53 signalling reduces *Pparg* and promotes *Pparb/d* and *Krt6a* expression. This has physiological significance as it controls granular layer formation (Fig. 2n). We also highlight for the first time, the significant convergence of the p53 and RAR signalling pathways via MYC and demonstrate that repression of RARs can inhibit p53 activity to restore granular layer formation, which is presumably good for barrier function. Given p53 is an integrator of multiple stress responses, these findings may provide mechanistic insight into the pathogenesis of human skin diseases where granular formation is disrupted.

¹Wellcome Trust–Medical Research Council
Cambridge Stem Cell Institute, University of
Cambridge, Cambridge CB2 1QR, U.K.

²Department of Biochemistry and Molecular
Biology, Monash University, Clayton 3800,
VIC Australia

³Centre for Stem Cell and Regenerative
Medicine Research, King's College London,
London SE1 9RT, U.K.

D.L. COTTLE^{1,2}
K. KRETZSCHMAR^{1,3,6}
H.P. GOLLNICK⁴
S.R. QUIST^{4,5}



⁴Clinic of Dermatology and Venereology,
Otto-von-Guericke University, DE-39120,
Magdeburg, Germany

⁵CR-UK Cambridge Research Institute, Li Ka
Shing Centre, Cambridge CB2 0RE, U.K.

⁶Present address: Hubrecht Institute –
KNAW and University Medical Centre,
Utrecht Uppsalalaan 8 3584 CT Utrecht,
the Netherlands
E-mail: denny.cottle@monash.edu

References

- Wagner EF, Schonthaler HB, Guinea-Viniegra J et al. Psoriasis: what we have learned from mouse models. *Nat Rev Rheumatol* 2010; **6**:704–14.
- Hack K, Reilly L, Palmer C et al. Skin-targeted inhibition of PPAR β/δ by selective antagonists to treat PPAR β/δ -mediated psoriasis-like skin disease in vivo. *PLoS ONE* 2012; **7**:e37097.
- Cottle DL, Ursino GM, Ip SC et al. Fetal inhibition of inflammation improves disease phenotypes in harlequin ichthyosis. *Hum Mol Genet* 2015; **24**:436–49.
- Schmuth M, Haqq CM, Cairns WJ et al. Peroxisome proliferator-activated receptor (PPAR)-beta/delta stimulates differentiation and lipid accumulation in keratinocytes. *J Invest Dermatol* 2004; **122**:971–83.
- Tan NS, Michalik L, Noy N et al. Critical roles of PPAR beta/delta in keratinocyte response to inflammation. *Genes Dev* 2001; **15**:3263–77.
- Mao-Qiang M, Fowler AJ, Schmuth M et al. Peroxisome-proliferator-activated receptor (PPAR)-gamma activation stimulates keratinocyte differentiation. *J Invest Dermatol* 2004; **123**:305–12.
- Baran W, Szepletowski JC, Szybejko-Machaj G. Expression of p53 protein in psoriasis. *Acta Dermatovenol Alp Panonica Adriat* 2005; **14**:79–83.
- Botchkarev VA, Komarova EA, Siebenhaar F et al. p53 Involvement in the control of murine hair follicle regression. *Am J Pathol* 2001; **158**:1913–9.
- Greenhalgh DA, Wang XJ, Donehower LA et al. Paradoxical tumor inhibitory effect of p53 loss in transgenic mice expressing epidermal-targeted v-rasHa, v-fos, or human transforming growth factor alpha. *Cancer Res* 1996; **56**:4413–23.
- Freije A, Molinuevo R, Ceballos L et al. Inactivation of p53 in human keratinocytes leads to squamous differentiation and shedding via replication stress and mitotic slippage. *Cell Rep* 2014; **9**:1349–60.
- Berta MA, Baker CM, Cottle DL et al. Dose and context dependent effects of Myc on epidermal stem cell proliferation and differentiation. *EMBO Mol Med* 2010; **2**:16–25.
- Arnold I, Watt FM. c-Myc activation in transgenic mouse epidermis results in mobilization of stem cells and differentiation of their progeny. *Curr Biol* 2001; **11**:558–68.
- Cottle DL, Kretschmar K, Schweiger PJ et al. c-MYC-induced sebaceous gland differentiation is controlled by an androgen receptor/p53 axis. *Cell Rep* 2013; **3**:427–41.
- Uribealago I, Buschbeck M, Gutierrez A et al. E-box-independent regulation of transcription and differentiation by MYC. *Nat Cell Biol* 2011; **13**:1443–9.
- Chapellier B, Mark M, Messaddeq N et al. Physiological and retinoid-induced proliferations of epidermis basal keratinocytes are differently controlled. *EMBO J* 2002; **21**:3402–13.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Appendix S1. Materials and methods.

Fig S1. Further characterization of K14MycER, K14MycER p53null and K14MycAER mouse epidermis.

Funding sources: This work was supported by Professor Fiona M. Watt via the Medical Research Council, Wellcome Trust, CRUK, EU FP7 programme, the University of Cambridge, and Hutchison Whampoa Ltd. This work was also supported by Associate Professor Ian M. Smyth and Monash University.

Conflicts of interest: none declared.