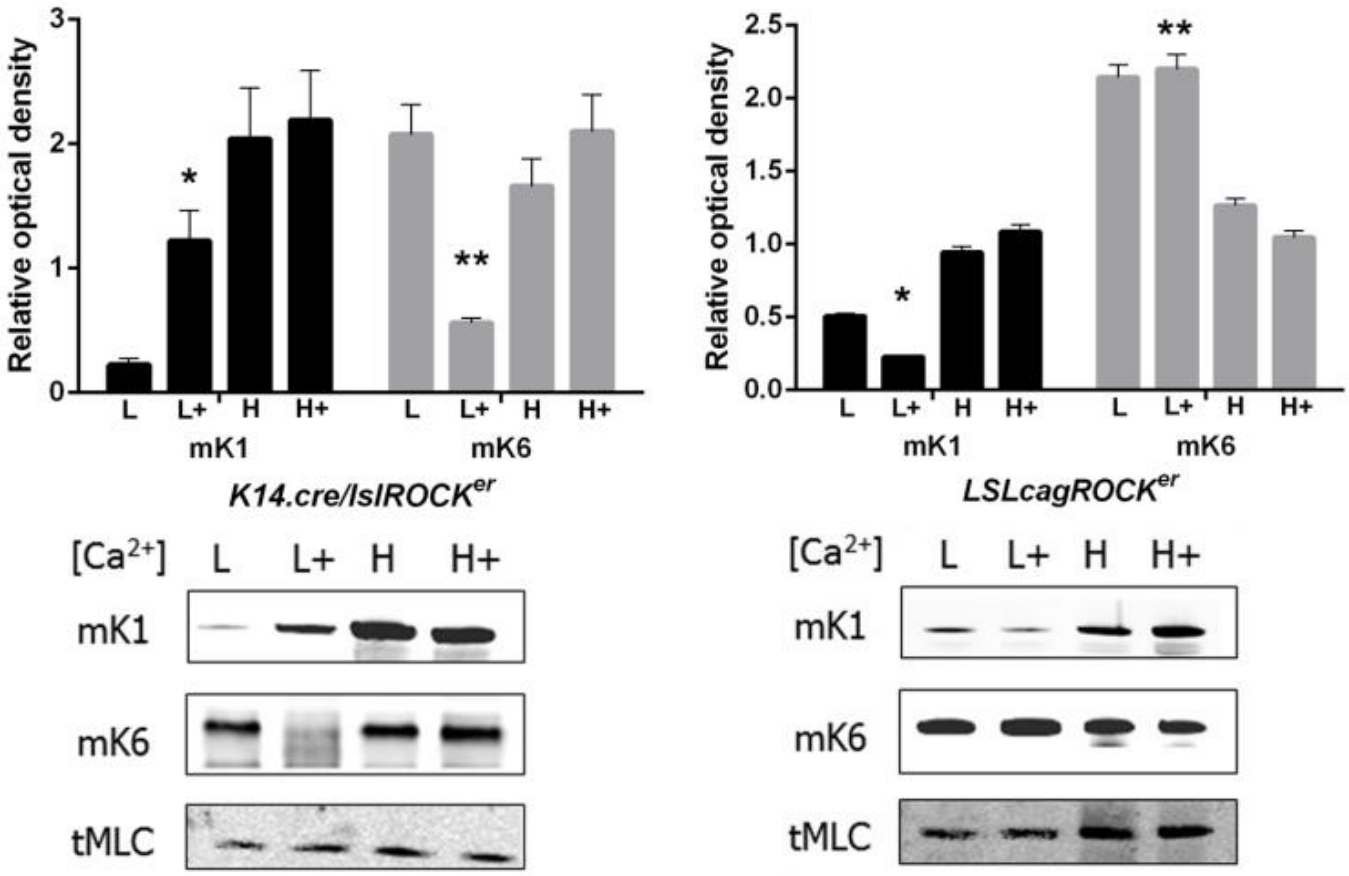


Figure S1: Quantitation of differentiation marker expression.

Quantitation of mK1 and mK6α expression levels as relative optical density ratio compared to MLC loading control. Each column represents mean±SEM. Statistical analysis (one-way anova) of K1 (L+*) or K6 (L+**) expression in *K14.cre/lslROCK*^{er} vs l*slcagROCK*^{er} keratinocytes gives significance p<0.001. Primary *K14creP.lslROCK*^{er} or control *lslcagROCK*^{er} keratinocytes were cultured in low or high calcium media ((L= 0.05mM; H= 0.12mM) containing RU486 (5nM) with/without 4HT (1nM; L+/H+). ROCK^{er} activation induced anomalous K1 in low calcium medium absent in untreated or 4HT–treated *lslROCK*^{er} controls (L+ versus L); with normal K1 expression in high calcium. ROCK^{er} activation also down-regulated K6α in hyperproliferative, low calcium *K14creP.lslROCK*^{er} keratinocytes (L+); to date a result unique to these keratinocytes. Total MLC served as a loading control.

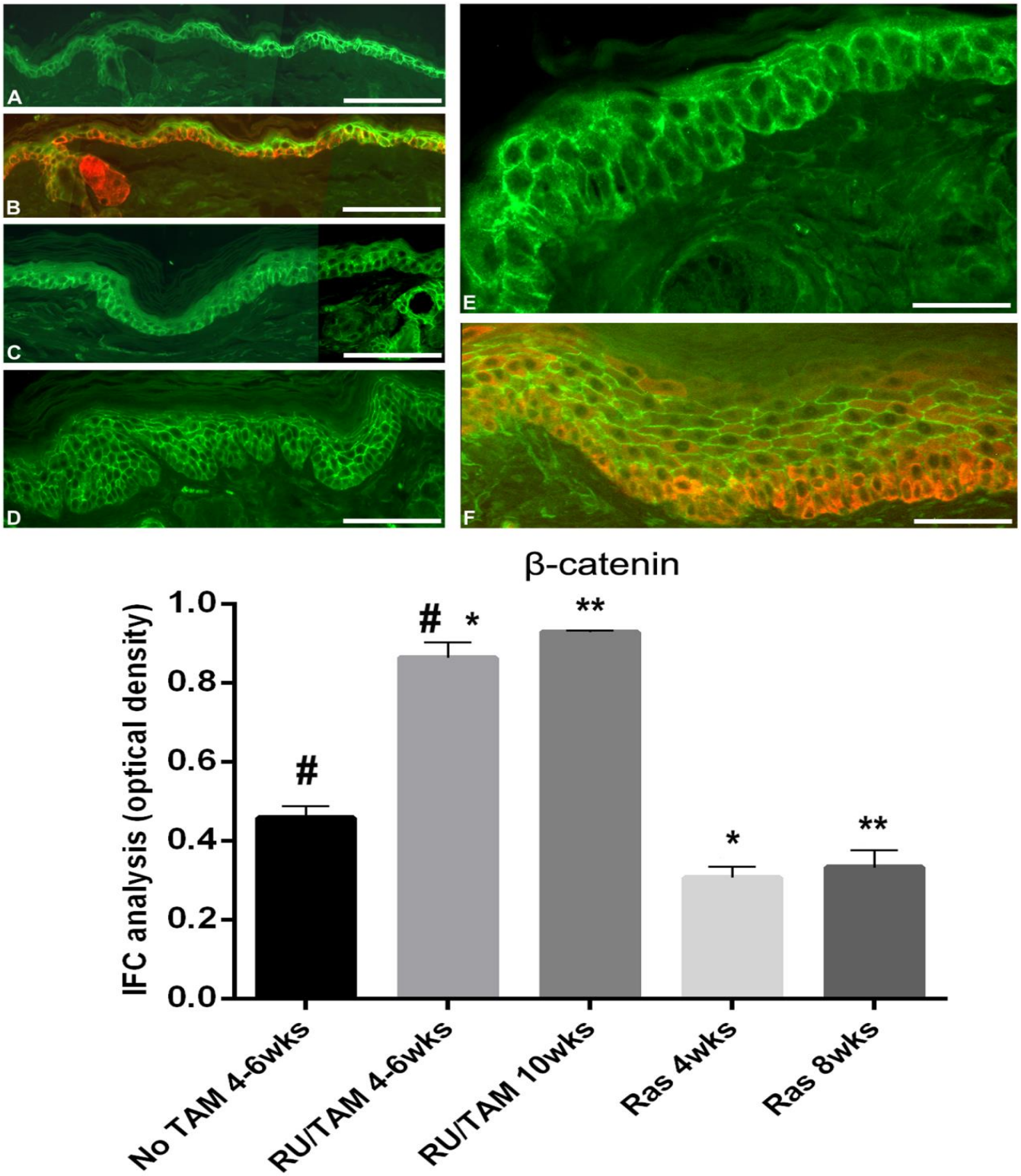


Figure S2: Quantitation of β-catenin immunefluorescence analysis [modified Figure 4] (A and B) β-catenin expression in *K14.creP/lslROCK^{er}* epidermis (4-6wks) at junction between RU486 alone (left) and 4HT/RU486-treatment (right) shows expression increases in 4HT-treated areas (B: double-label K1 (green) vs K14 (red) counterstain) (C) Older 4HT/RU486-treated *K14.creP/lslROCK^{er}* hyperplasia (10wks) shows elevated β-catenin in basal and supra-basal layer keratinocytes compared to (D) *HK1.ras* hyperplasia (4wks) which displays membranous, supra-basal expression (*p<0.001). (E) At higher magnification, *K14.creP/lslROCK^{er}* epidermis (10wks) shows detectable cytoplasmic/nuclear expression in basal layer keratinocytes compared to (F) *HK1.ras* hyperplasia (8wks) with less expression in basal-layer membranes and infrequent nuclear expression. Quantitation of [#] treated *K14.creP/lslROCK^{er}* epidermis vs untreated skin shows approx. 50 % increase over normal (p<0.0001); similarly [*] treated *K14.creP/lslROCK^{er}* epidermis vs HK1.ras skin at 4-6 wks or [**] 10 wks also increased by approx. 60 % (p<0.001). (*bars: A and E approx.100μm; C: approx.75μm; B,D,F approx.50-60μm)*.

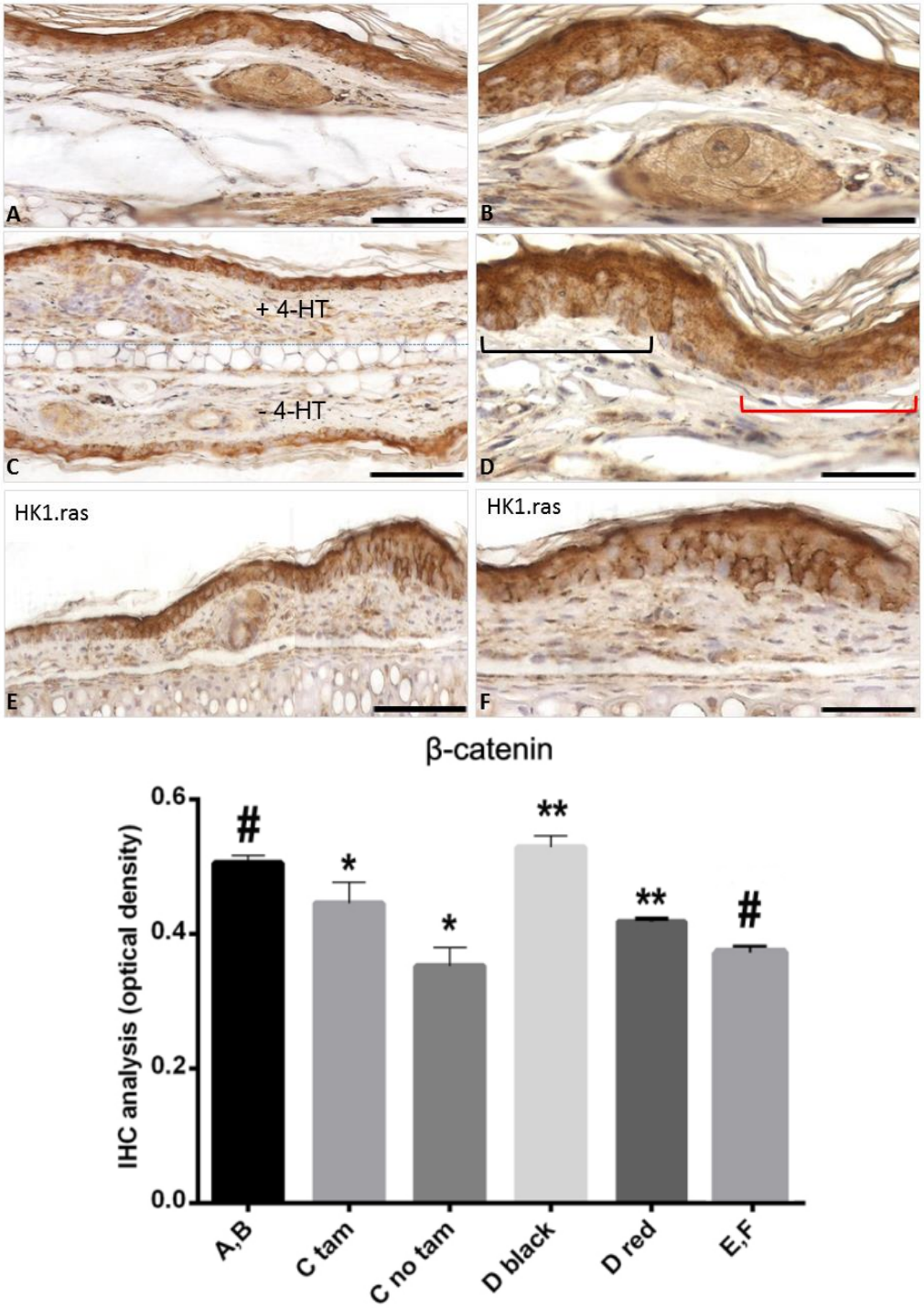


Figure S3: Quantitation of *β-catenin expression* via *Immunohistochemical analysis* [A and B] 4HT/RU486-treated *K14.creP/lslROCK^{er}* hyperplasia [at 4wks] show uniform, elevated β-catenin expression; with membranous and cytoplasmic/nuclear β-catenin expression in basal layer keratinocytes. [C] 4HT/RU486-treated *K14.creP/lslROCK^{er}* vs. untreated epidermis [serial sections of p-GSK3β; Fig.S4] show ROCK^{er}-mediated increased β-catenin expression. [D] At 6wks magnification of 4HT/RU486-treated *K14.creP/lslROCK^{er}* epidermis [left/black bar] shows a subtle β-catenin increase in basal layer keratinocytes vs. RU486 treatment alone [right/red bar]; which exhibits supra-basal expression and predominantly blue basal layer nuclei. [E] 4HT/RU486-treated *HK1.ras* hyperplasia [4wks] displays membranous β-catenin expression in basal- and supra-basal layers, with infrequent detectable nuclear expression as basal keratinocytes possessed predominantly blue nuclei. [F] With time, less β-catenin expression was detectable in basal-layer membranes. Quantitation of [*] treated dorsal ear skin vs untreated ventral skin (Fig.S3C) shows increased βcatenin expression ($p<0.05$) parallels p-GSK3β [below]; [**] comparison of junctional expression shows elevated basal layer β-catenin in treated [brown nuclei] vs untreated [blue nuclei] areas [$p<0.05$; Fig.S2D, black vs red bar]. [#] 4HT/RU486-treated *K14.creP/lslROCKer* epidermis expressed higher β-catenin levels vs. *HK1.ras* hyperplasia [$p<0.05$; A and B vs E and F] (*bars: C approx. 125μm; A approx. 75μm; B, D and F approx. 25-30μm).*

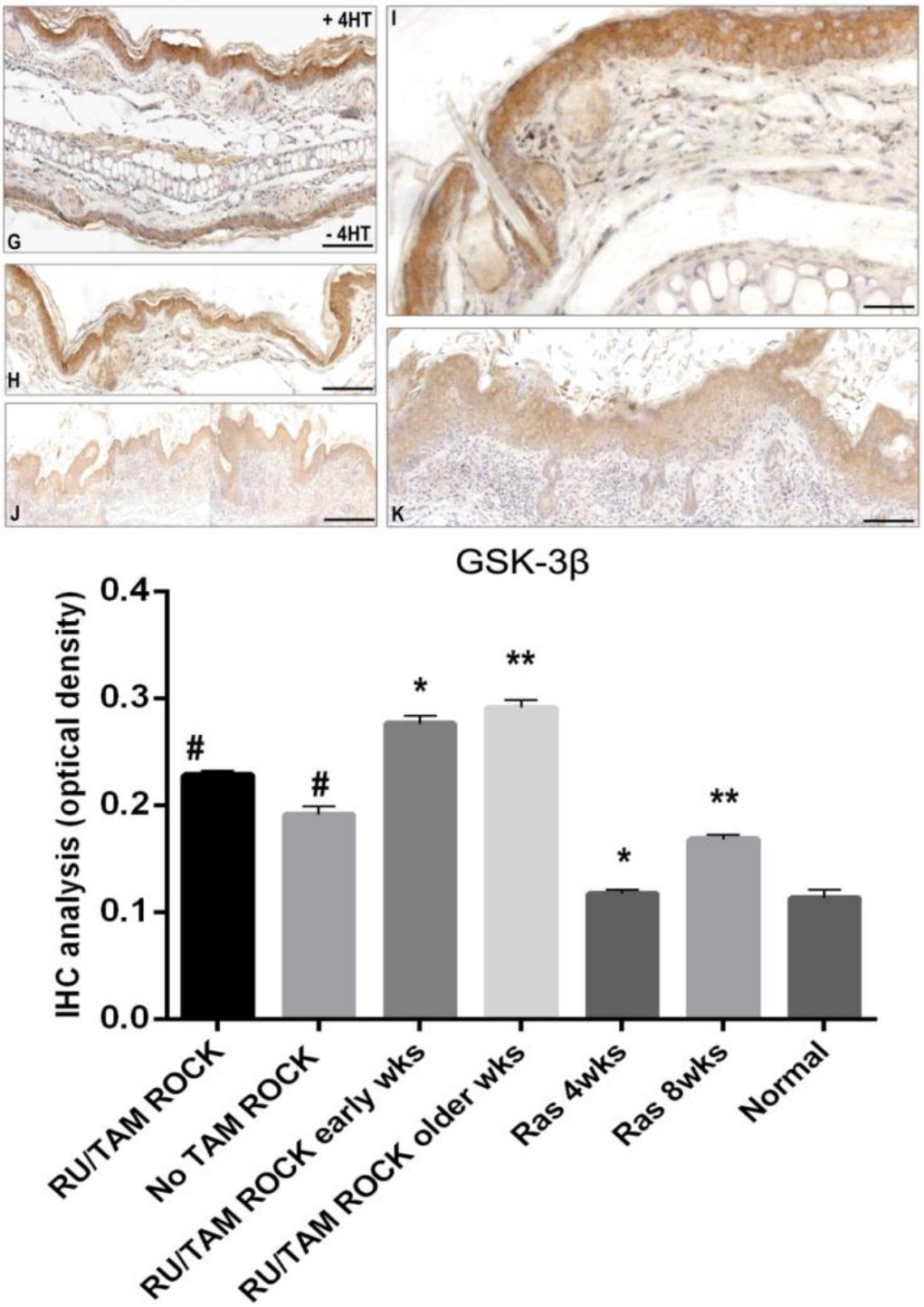
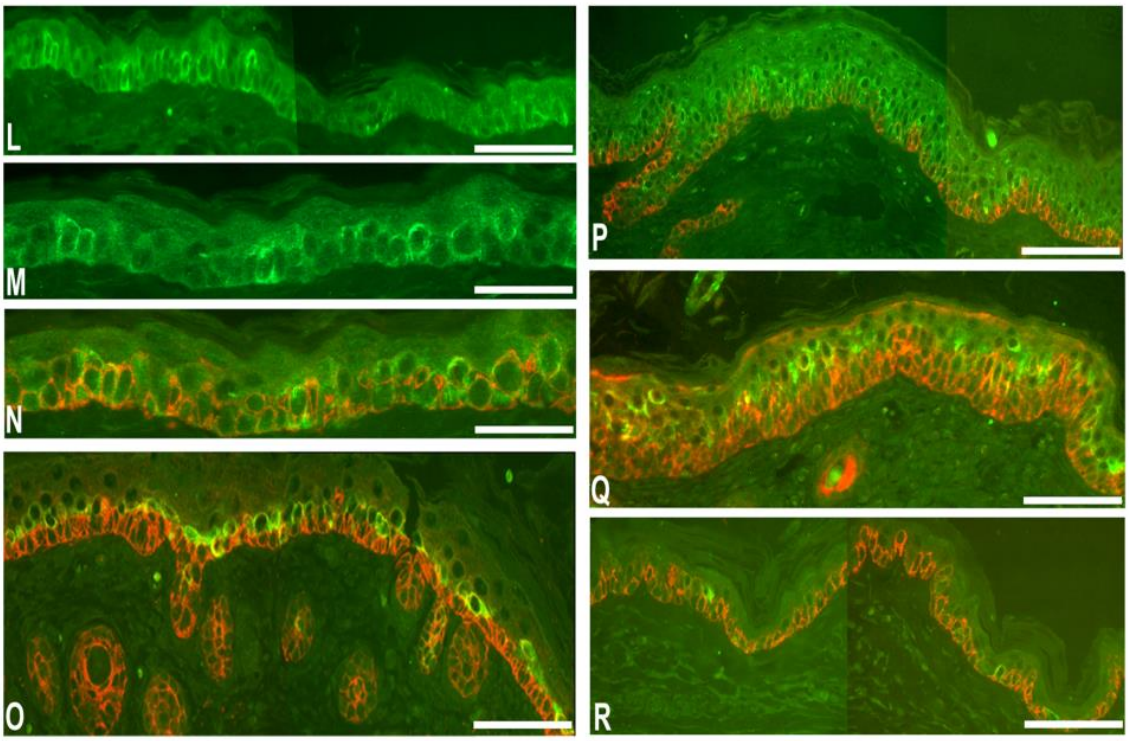


Figure S4: Quantitation of p-GSK3β immunohistochemical expression analysis. (G) p-GSK3β analysis of 4HT-treated (upper dorsal) vs untreated (lower ventral) *K14.creP/lslROCK*^{er} ear skin shows elevated expression in 4HT-treated epidermis became (H) stronger/uniform with time in 4HT/RU486-treated *K14.creP/lslROCK*^{er} hyperplasia (12wks). (I) Higher magnification of 4HT/RU486-treated *K14.creP/lslROCK*^{er} hyperplasia shows elevated p-GSK3β expression in both basal and suprabasal layers; whereas (J) *HK1.ras* hyperplasia displays less basal layer p-GSK3β at 4wks (that (K) weakens by 8wks, becoming increasingly supra-basal in parallel to β-catenin [above]. Quantitation of [#] treated dorsal ear skin vs untreated ventral skin (Fig.S4C) shows moderate, increased p-GSK3β expression ($p<0.05$) became stronger with time. 4HT/RU486-treated *K14.creP/lslROCKer* epidermis expressed significantly higher p-GSK3β levels vs. *HK1.ras* hyperplasia at both [*] 4wks ($p< 0.001$) and [**] 8wks ($p<0.001$) (*bars: H and J approx.100μm; G and K approx.75μm; I approx. 50μm)*.



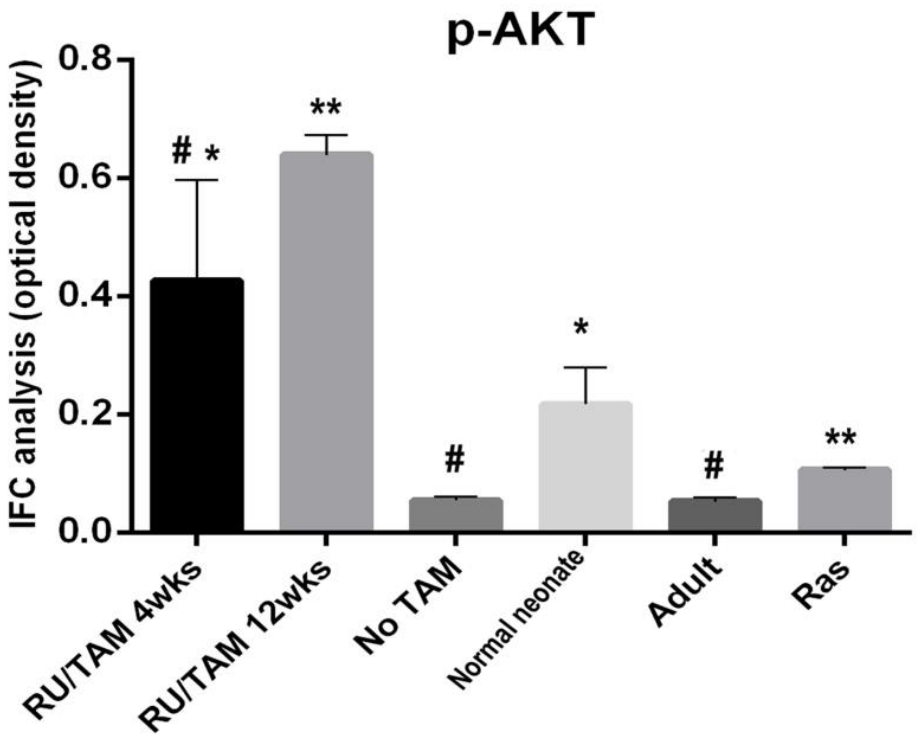


Figure S5: Quantitation of p-AKT1 immunefluorescence expression analysis. (L-N) p-AKT1 in 4HT/RU486-treated *K14.creP/lslROCK*^{er} epidermis (4wks) shows elevated, basal-layer expression at levels similar to (O) normal neonatal skin (24hrs) that exhibits supra-basal p-AKT1. (P) Older (12wks) 4HT/RU486-treated *K14.creP/lslROCK*^{er} epidermis retains elevated p-AKT1 but with a trend towards supra-basal expression similar to neonates. (Q) *HK1.ras* hyperplasia (4-5wks) displays less, supra-basal p-AKT1; whilst (R) wild type adult keratinocytes display sporadic, supra-basal p-AKT. Image quantitation of [#] treated skin (4wks) vs untreated areas; (Fig. S5L-N (4wks) vs R (12wks) and 4wks (not shown)] expressed a significant increase in p-AKT1 ($p<0.0001$) as observed on comparison to [*] neonatal epidermis ($p<0.001$; Fig. S5L-N vs S5O) or [**] equivalent *HK1.ras* hyperplasia ($p<0.0001$; Fig.S5P vs S5Q). (*bars: P approx.80µm; L and O, 60µm; Q and R approx.50µm; M and N: approx.25-30µm*).

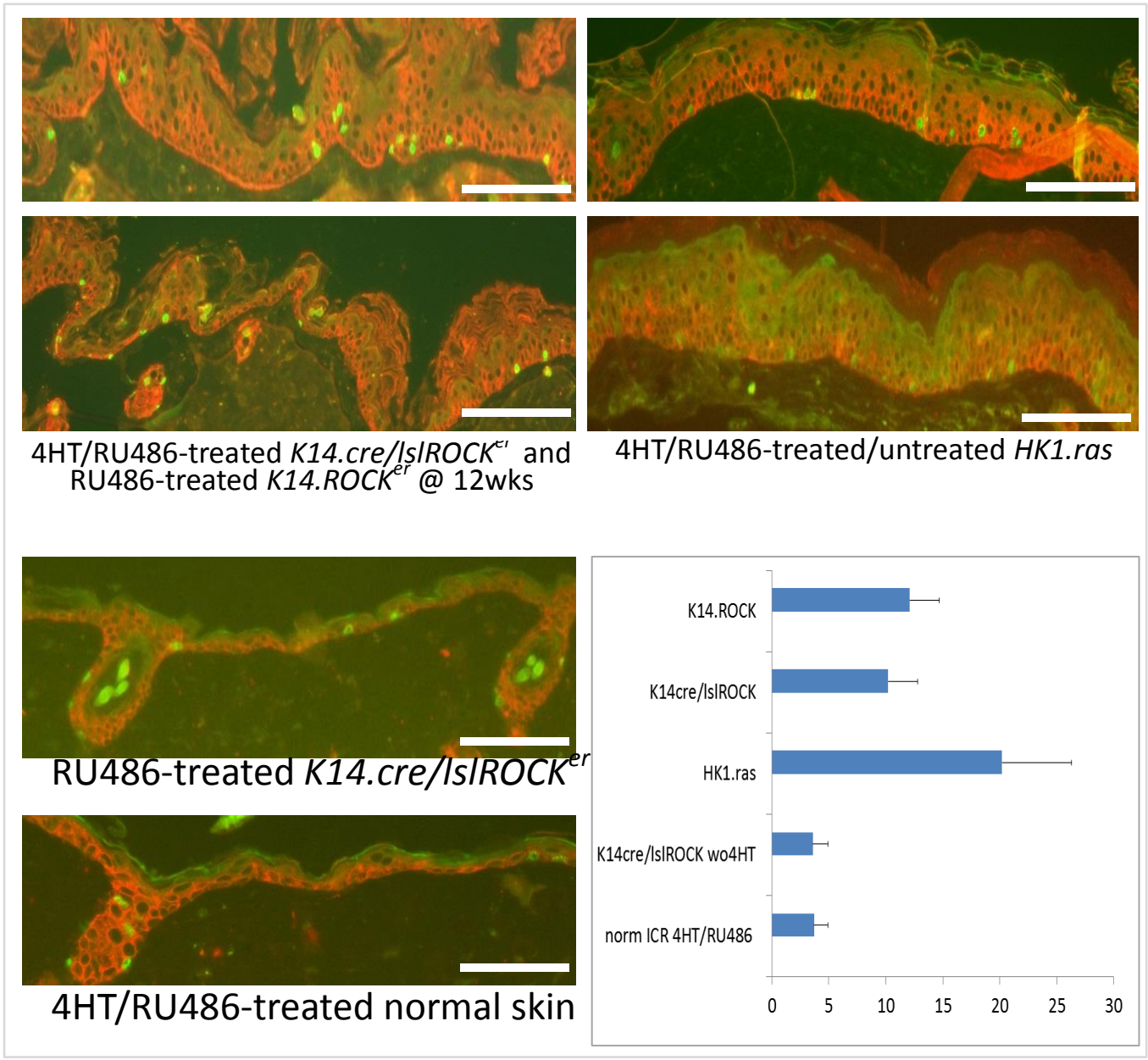


Figure S6: Mitotic indices via BrdU labelling. Mitotic index [number of BrdU-labelled cells per mm of basement membrane] in RU486/4HT-treated *K14.creP/lslROCK*^{er} or 4HT-treated *K14.ROCK*^{er} hyperplasia gave a mitotic index [12.1^{+}/- 2.6; 10.1^{+}/- 2.6] approximately triple that of normal epidermis [3.7^{+}/-1.2] but approximately half that of equivalent *HK1.ras* mice [20.2 $^{+}$/- 6.1]; hence the milder hyperplasia. Numbers of positive BrdU-labelled cells were counted in 3 areas of 3 separate sections from 3 different mice per cohort, taken at 12 weeks *[Scale bars: approx. 75 µm].*