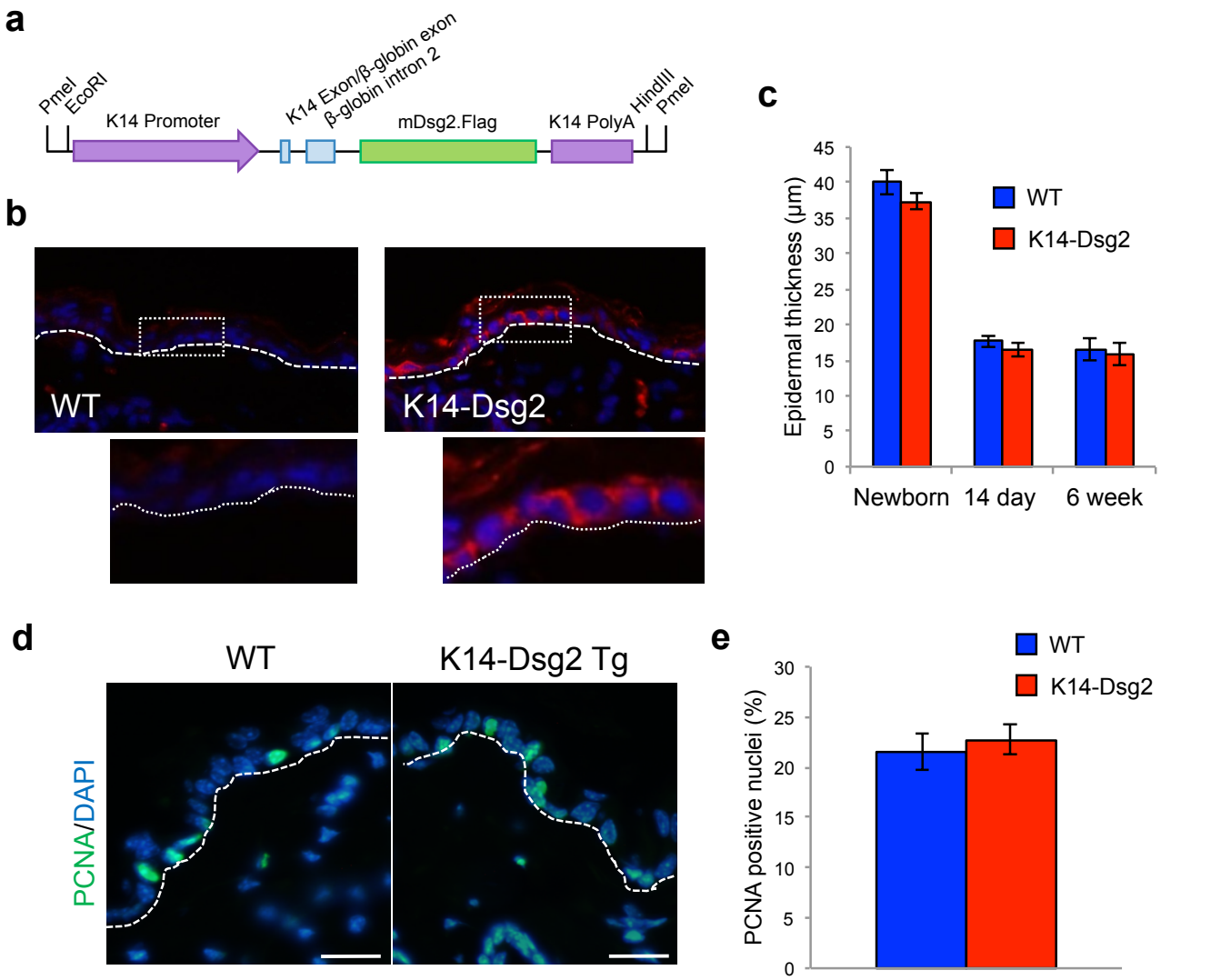
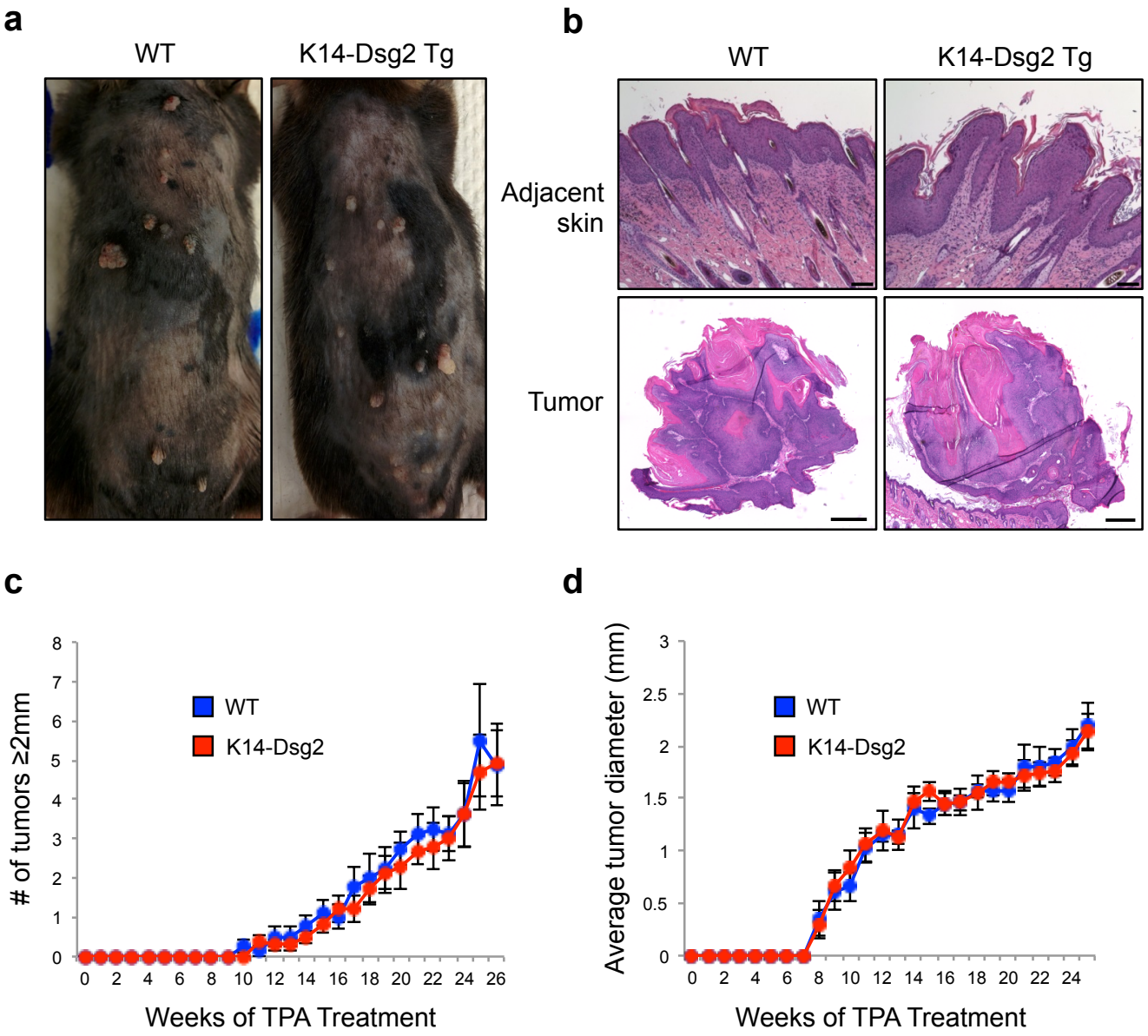


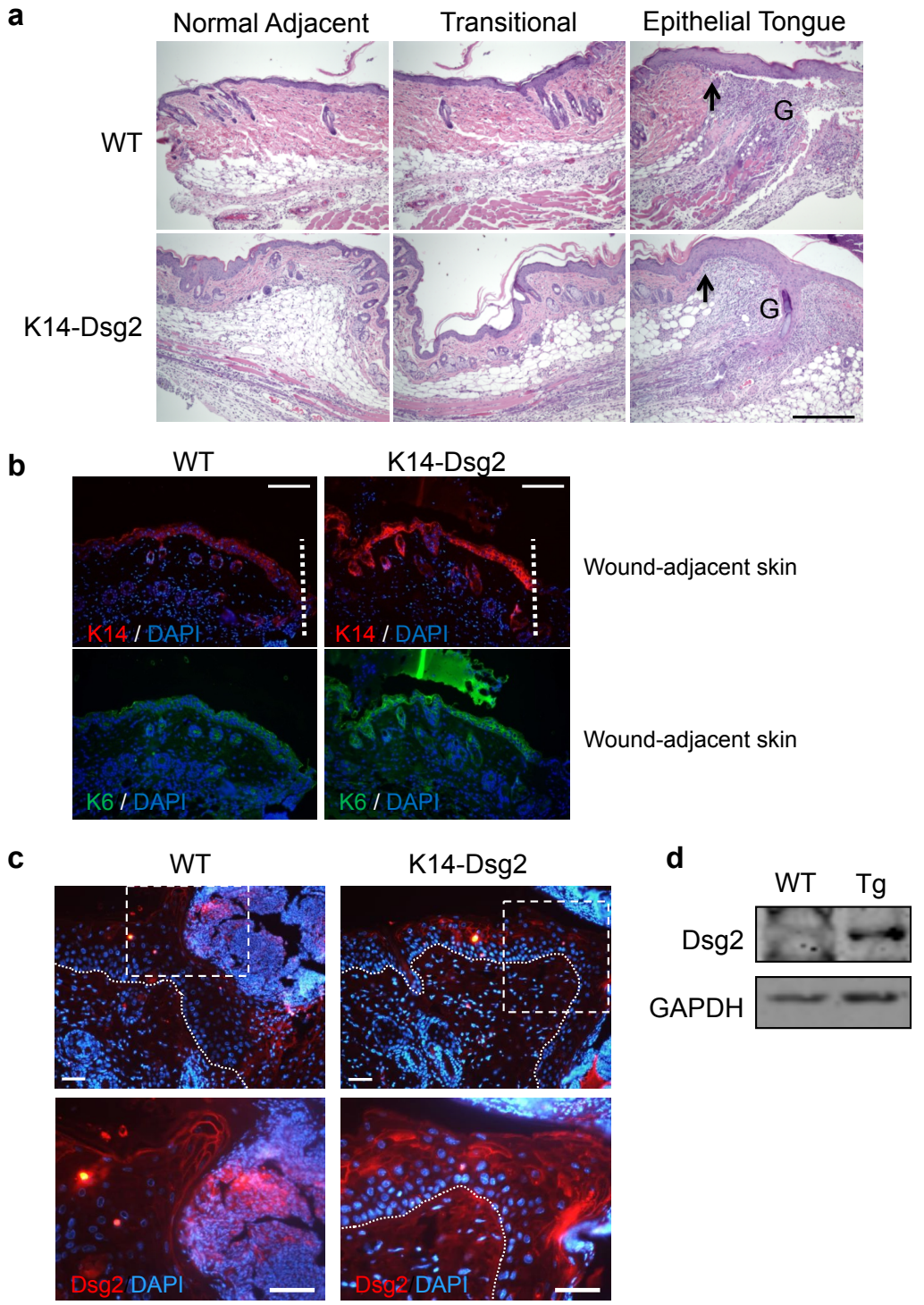
Cooper *et al.* Supplemental Figure S1

Supplemental Figure S1. Generation and characterization of the K14-Dsg2 transgenic mice. (**a**) Schematic of K14-mDsg2.Flag construct. (**b**) Dorsal skin of adult wild-type and K14-Dsg2 transgenic mice was frozen in OCT, sectioned, and immunostained with anti-Flag antibodies. (**c**) Epidermal thickness of newborn, 14-day, and 6-week old wild-type and K14-Dsg2 mice was measured and presented as average thickness of epidermis (μm) ± SEM. N=3. (**d**) Representative images of 6-week wild-type and K14-Dsg2 dorsal mouse skin immunostained for PCNA (green) and DAPI (blue). Dashed line indicates basement membrane zone. (**e**) Quantification of PCNA-positive nuclei in wild-type and K14-Dsg2 mice. Data is presented as average number of PCNA-positive nuclei in epidermis ± SEM. N=3 mice (three sections per mouse). Scale bars=50 μm



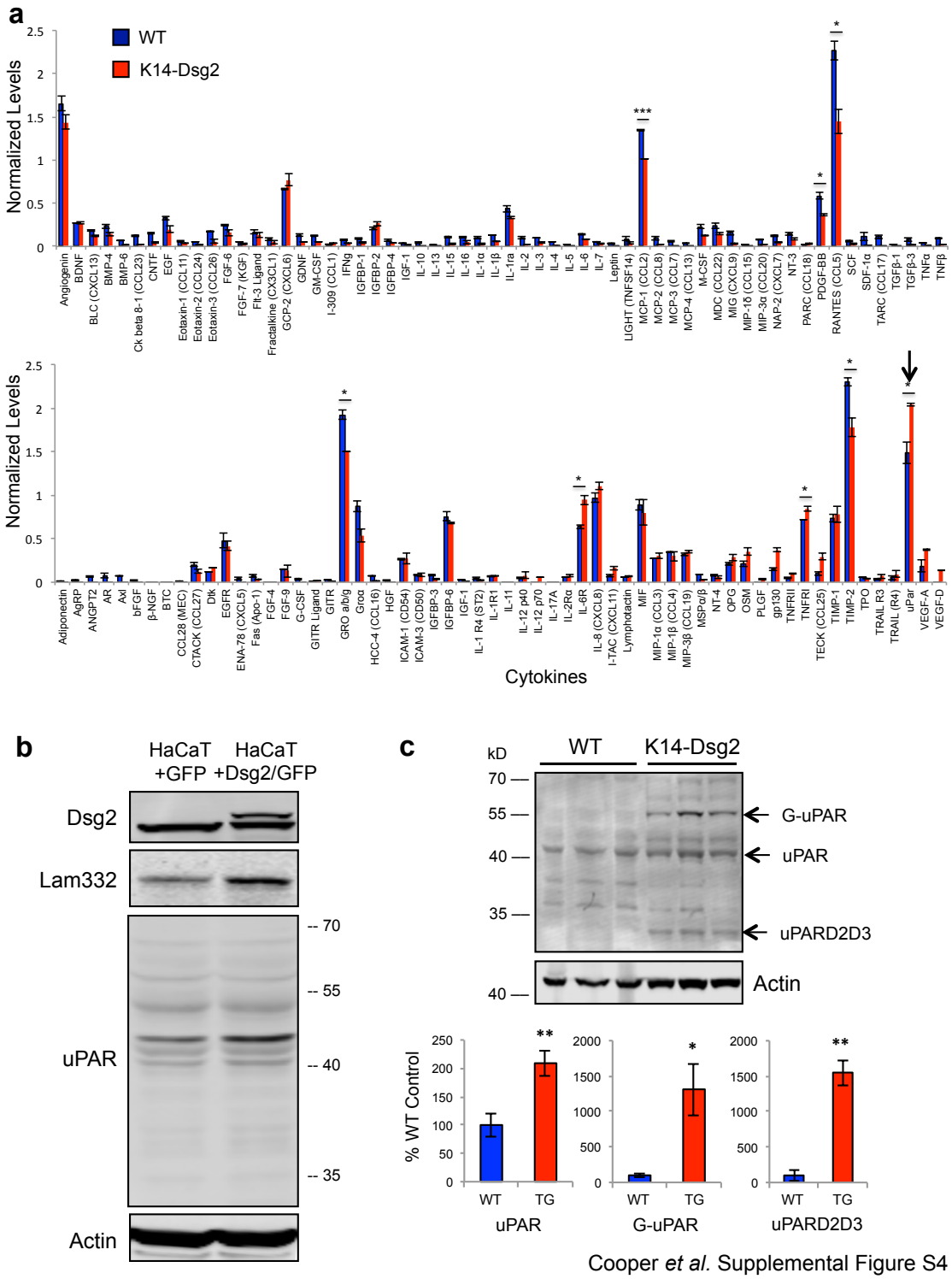
Cooper *et al.* Supplemental Figure S2

Supplemental Figure S2. K14-Dsg2 mice are not susceptible to chemical-induced carcinogenesis. (**a**) Wild-type and K14-Dsg2 transgenic mice were treated once with DMBA, followed by twice weekly of TPA in the dorsal skin for 25 weeks to induce squamous lesions. (**b**) Representative H&E of formalin-fixed, paraffin-embedded sections of wild-type and transgenic mice demonstrating epidermal hyperplasia (top row) and well-differentiated papillomas (bottom row) after 25 weeks. (**c-d**) The number of papillomas greater ≥2 mm in diameter and the average diameter of the papillomas were determined. Data expressed as average number of ≥2 mm papillomas per mouse ± SEM. (**c**) or average tumor diameter ± SEM. (**d**). N=11 wild-type; 9 transgenic.

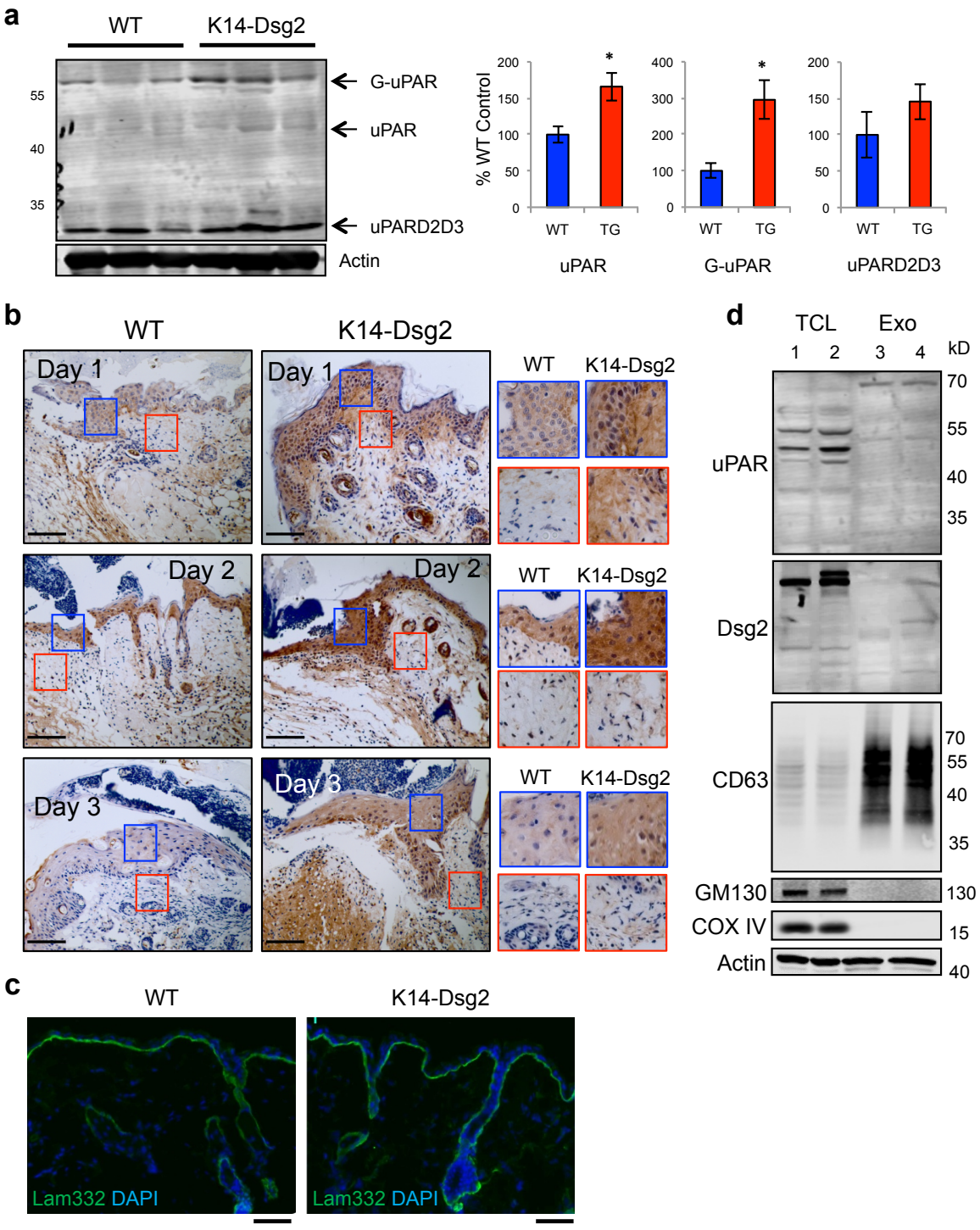


Cooper *et al.* Supplemental Figure S3

Supplemental Figure S3. Activation of the K14 promoter at the wound edge. (**a**) H&E staining of wild-type and K14-Dsg2 wounds and adjacent skin 24 h post-wounding. Scale bar = 300 µm. (**b**) FFPE tissues from 1-day old wounds were immunostained for K14 (red), K6 (green), and DAPI (blue). Vertical white dashed line demarcates the boundary between the wound and undamaged tissue. Scale bar=100 µm. (**c**) Wild-type and K14-Dsg2 wounds were immunostained for Dsg2. Dashed line demarcates regenerating basement membrane, and dashed box on top panels highlight area of bottom panels. (**d**) Skin from wild-type and transgenic mice 24 h after wounding was excised, lysed and proteins immunoblotted for Dsg2 and GAPDH. Scale bar=50 µm.



Supplemental Figure S4. Dsg2 enhances uPAR expression. (**a**) Complete array of cytokines, chemokines, and growth factors assayed in conditioned media of HaCaT+GFP and +Dsg2/GFP cells. Data are normalized to on-membrane positive control and depicted as relative abundancy in samples ± SEM. Arrow delineates abundance of uPAR secretion from cells. N=2. (**b**) Total cell lysates from HaCaT+GFP and +Dsg2/GFP cells immunoblotted for Dsg2, Laminin-332, uPAR, and actin. (**c**) Wild-type and K14-Dsg2 mouse dermis (N=3) was immunoblotted for uPAR and actin. Signals of various uPAR species were quantified, normalized to actin, and expressed as % of wild-type (bottom). N=3.



Cooper *et al.* Supplemental Figure S5

Supplemental Figure S5. uPAR expression and secretion potentiated in K14-Dsg2 wounds. (**a**) Wounds from wild-type and K14-Dsg2 mice were lysed and immunoblotted for uPAR and GAPDH. Signals of various uPAR species were quantified, normalized to GAPDH, and expressed as % of wild-type (right). N=3. (**b**) Wild-type and K14-Dsg2 Day 1-3 wounds were immunostained for uPAR. Right panels: Enlarged representative images of the epidermis (blue) and dermis (red) immunostained for uPAR. (**c**) Skin of wild-type and K14-Dsg2 mice was immunostained for Laminin-332. (**d**) Total cell lysates (TCL) and exosomes (Exo) from HaCaT+GFP and HaCaT+Dsg2/GFP immunoblotted for uPAR, Dsg2, CD63, GM130, COX IV, and Actin. Scale bar=50 µm.

MATERIALS AND METHODS

Ethics statement on animal research

Animal experiments were facilitated within the guidelines provided within the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and in accordance with Laboratory Animal Services at Thomas Jefferson University. The animal usage protocol was approved by the Institutional Animal Care and Use Committee at Thomas Jefferson University.

Antibodies

Antibodies against Flag (F7425) was from Sigma (St. Louis, MO); Laminin 332 (ab14509) was from Abcam (Cambridge, MA); Actin (CP01) was from EMD Millipore (Billerica, MA); activated β-catenin (#8814), PCNA (#13110), p42/44 MAPK (#9102), phospho-p42/44 MAPK (#9101), Akt (#9272), phospho-Akt S473 (#4060), phospho-PTEN S380/T382/T383 (#9554), GAPDH (#5174), COXIV (#4850) and phospho-PDK1 (#3438) were from Cell Signaling (Danvers, MA); Cyclin D1 (#RM9104), CD63 (10628), phospho-EGFR Y845 (44-784G), and uPAR (PA5-15478) were from ThermoFisher (Waltham, MA); cytokeratin 6 and 14 was a kind gift from Dr. Dennis Roop (University of Colorado) (Roop et al., 1984); and Dsg2, β-catenin, plakoglobin, plakophillin-1, plakophillin-3, and Dsg1 were previously described (Roberts et al., 2016, Wahl et al., 1996, Wahl, 2002). AlexaFluor-488 or -594-conjugated mouse, rabbit, and sheep antibodies were from Life Technologies (Grand Island, NY); and IRDye-680 and -800-conjugated mouse and rabbit were from Li-Cor Biosciences (Lincoln, NE).

Generating transgenic mice and genotyping

The Flag-tagged Dsg2 cDNA previously described (Brennan et al., 2007) was subcloned into a targeting construct containing ~2,300 bp of the human cytokeratin KRT14 promoter and a

polyadenylation tail (kind gift from Dr. Elaine Fuchs, Rockefeller University). All sequences were confirmed by automated nucleotide sequencing (ABI, Foster City, CA). The K14-Dsg2/Flag transgenic construct was purified and injected into the male pronuclei of FVB/NJ fertilized eggs. Embryos were then implanted into the oviducts of pseudo-pregnant FVB/N foster mothers. K14-Dsg2 Tg mice were genotyped to confirm the presence of the transgene by PCR on DNA extracted from a tail snip using 50 mM sodium hydroxide. PCR primers (Fwd: $^{5'}$ATGAGGCGGATGAGAGGAGG$^{3'}$; Rev: $^{5'}$GAAGACCTCTAAGTGAAGTCC$^{3'}$) were designed to span exons 1 and 2 of the mouse Dsg2 gene to generate a 1500 bp product for the endogenous gene and a 243 bp product for the transgene. PCR reactions were performed in a 20 µL volume containing 1 µl of crude genomic DNA, 0.20 µM primers, 1x Buffer Q, 1.5 mM $MgCl_2$, 200 µM dNTPs, and 1.25 units of Taq polymerase (Qiagen Taq PCR Core Kit). PCR cycles were as follows: 4 minutes at 95°C, followed by 30 cycles of 30 seconds at 94°C, 1 minute at 58°C, and 1 minute at 72°C. The K14-Dsg2 construct was used to generate 3 Tg founder males that were backcrossed to C57BL/6J females; progeny were backcrossed at least 5 generations to the C57BL/6J inbred strain.

Histological analysis of skin

WT and K14-Dsg2 Tg dorsal skin was evaluated at 1-2 days, 14 days, 6 weeks, and 9 months after birth for morphological changes. Skin was fixed in 10% neutral buffered formalin. Tissues were paraffin embedded and cut at 4 µm, mounted onto positively charged slides, and stained with Hematoxylin and Eosin (ThermoFisher).

Tissue extraction

Mice were sacrificed and punch biopsies (4 mm) from dorsal skin were excised and flash frozen in liquid nitrogen for protein extraction. Tissue was ground in mortar and pestle and lysed in

Urea Lysis buffer (9M Urea, 63mM Tris (pH 6.8), 1% SDS, 10% glycerol, 0.01% pryonin, 0.5% β-mercaptoethanol) with a protease inhibitor (PI) cocktail containing pancreas-extract, pronase, thermolysin, chymotrypsin, trypsin and papain (#11836170001; Sigma), 1 mM PMSF, and phosphatase inhibitors (PhI, #P5726; Sigma). Epidermal/dermal separation for lysis and immunoblotting was achieved by proteolytic digestion of the basement membrane as adapted from (Li et al., 2017). Briefly, skin from juvenile (P3) WT and K14-Dsg2 was harvested, washed in PBS with 1X penicillin-streptomycin and 1µg/mL amphotericin B, and digested in supplemented EpiLife medium (ThermoFisher, 0.06mM Ca^{2+}) with 1X penicillin-streptomycin, 1µg/mL amphotericin B, and 2.4U/mL Dispase II (Sigma) overnight at 4°C while rocking vigorously. The following day, the digested skin was gently rinsed in PBS and moved to a sterile, dry petri dish—epidermis face down—to tease away the loosened dermis with forceps. Both the dermis and epidermis were flash frozen in liquid N_2, pulverized in mortar and pestle, lysed in Urea lysis buffer+PI, PhI, and PMSF, and very briefly sonicated while on ice.

Immunoblotting

Proteins were separated SDS-PAGE and immunoblotted as previously described (Overmiller et al., 2016). Membranes were blocked 1 h in blocking buffer (0.5% fish gelatin (Sigma) in Tris-buffered saline (0.5M Tris and 0.15M sodium chloride in deionized water) with 0.1% Tween-20) followed by overnight incubation in primary antibody diluted in blocking buffer. Signal was detected by IRDye-680 or 800 secondary antibodies and visualized by using the Odyssey infrared imaging system (Li-Cor). Protein bands were quantified using the Image Studio Lite v5.2 (Li-Cor). Protein signals were normalized to corresponding loading control bands (Actin or GAPDH).

Immunofluorescence and immunohistochemistry

Harvested skin was either formalin fixed in 10% neutral buffered formalin and paraffin embedded (FFPE) or flash-frozen in Tissue-Tek OCT Compound (Sakura Finetek USA, Inc., Torrance, CA) and sectioned at 4 μm. Frozen tissues were immunostained with Flag, Dsg2, pEGFR (Tyr845), pSrc (Tyr416), or Laminin 332 antibodies, and FFPE sections were immunostained for the proliferating cell nuclear antigen (PCNA), K14, K6, and uPAR as previously described (Brennan et al. 2007). Frozen sections were fixed with 100% methanol at -20°C for 20 min (Flag, Dsg2) or 4% PFA for 15 min (pSrc, pEGFR, Laminin 332). FFPE antigen retrieval was performed with sodium citrate (10 mM, pH 6.0) at 95˚C for 20 minutes.

Keratinocyte proliferation

For analysis of proliferation, the total number of PCNA+ nuclei in the epidermis were counted and divided by the total number of DAPI stained nuclei. A minimum of three random epidermal high-powered fields (40x) per animal was used to calculate and average %PCNA+ nuclei. For proliferation calculation in the wounded epidermis, a single medium powered field (20x) often encompassed the entire wound area and was used to quantify %PCNA+ nuclei.

DMBA-TPA-induced skin carcinogenesis

Adult male and female mice (6-8 weeks) were treated with DMBA (7,12-dimethylbenz[a]antracene) and biweekly applications of TPA (12-*O*-tetradecanoylphorbol 13-acetate) to promote skin tumor growth as previously described (Filler et al., 2007). Briefly, mice had their dorsal back skin shaved, painted with DMBA (400 nmol in 20 μL acetone), and maintained under yellow light for 24 hr. TPA (17 nmol in 200 μL acetone) was then applied twice-weekly for 24 weeks, starting 1 week post DMBA treatment. Papillomas were measured weekly by digital calipers, and mice were sacrificed and tumors harvested at week 26 of the

carcinogenesis protocol. Tissues were formalin fixed and paraffin-embedded for histological analysis by H&E. Significance measured by repeated-measures ANOVA.

Full-thickness skin wounding

Adult male and female mice (6-8 weeks) were given 1-2 mg/ml dose of acetaminophen in drinking water 24 hours prior to the beginning of the wounding protocol and for the entire duration of protocol. Mice were anesthetized using 4% isofluorane and injected intramuscularly with buprenorphine (0.6 mg/kg). Dorsal skin was then shaved and disinfected using 4% chlorhexidine surgical scrub. Two uniform full-thickness excisional wounds were created with a 4 mm punch biopsy without penetrating the parietal wall. Wounds were treated with 500 units Bacitracin topical antibiotic immediately following wounding and once every 24 h until the end of the experiment. Wounds were photographed daily using a hand-held digital microscope (Plugable USB 2.0 Digital Microscope) for up to 4 days post-wounding. The initial area of non-epithelized tissue for each wound was calculated immediately after punch biopsy, and the % area that closed in each wound was calculated by measuring the non-epithelized tissue each day using ImageJ. Animals were sacrificed and wounds were excised and processed for histological analysis, immunostaining, and immunoblotting. Significance measured by repeated-measures ANOVA.

Cytokine antibody array

A panel of 120 cytokines and chemokines from HaCaT-GFP and HaCaT-Dsg2/GFP was detected from the 48 h conditioned media with the C-series Human Cytokine Antibody Array C1000 (AAH-CYT-1000-2; Raybiotech, Inc., Norcross, GA) according to manufacturer's protocol. Briefly, HaCaT-GFP and HaCaT-Dsg2/GFP cells were grown to confluency in 10 cm^2 tissue culture dishes and then incubated in serum-free medium for 48 hr. Conditioned medium

was centrifuged to remove dead and live cells and further concentrated using Vivaspin centrifugal concentrators (VS2001; Fisher Scientific). The Quantification of dot intensity was performed in ImageJ following background subtraction, and the raw data normalized to the array-specific positive control dots.

Scratch assay

$3x10^5$ HaCaT+GFP and +Dsg2/GFP were seeded into individual wells of a 24-well plate and cultured overnight, coming to complete confluence the following day. A single scratch down the middle of each well was made with a fresh P200 pipet tip. Wells were rocked, washed 1X with DMEM, and incubated in DMEM ± 1μg/mL anti-uPAR antibody for 24 hr. Photos at the same location in each well (3x spots) were imaged immediately following scratch and at 24 hr. The distance between cell sheets was measured 3x within each spot (9 measurements total for each well; 3 wells each condition), and the % of the initial wound distance remaining open was calculated. Significance measured by ANOVA.

Exosome isolation

Exosomes were purified from conditioned medium of HaCaT keratinocytes by sequential ultracentrifugation as previously described (Greening et al., 2015, Overmiller et al., 2017). Briefly, medium was centrifuged at 300 x g (10 min), 2,000 x g (10 min) and 10,000 x g (30 min) to remove live and dead cells, and microvesicles. Supernatant was centrifuged at 110,000 x g (Beckman 45Ti) for 70 min followed by a PBS wash. Pellets containing exosomes were suspended in PBS.

REFERENCES

Brennan D, Hu Y, Joubeh S, Choi YW, Whitaker-Menezes D, O'Brien T, et al. Suprabasal Dsg2 expression in transgenic mouse skin confers a hyperproliferative and apoptosis-resistant phenotype to keratinocytes. J Cell Science 2007;120(5):758-71.

Filler RB, Roberts SJ, Girardi M. Cutaneous two-stage chemical carcinogenesis. CSH Protoc 2007;2007:pdb prot4837.

Greening DW, Xu R, Ji H, Tauro BJ, Simpson RJ. A protocol for exosome isolation and characterization: evaluation of ultracentrifugation, density-gradient separation, and immunoaffinity capture methods. Methods Mol Biol 2015;1295:179-209.

Li F, Adase CA, Zhang LJ. Isolation and Culture of Primary Mouse Keratinocytes from Neonatal and Adult Mouse Skin. J Vis Exp 2017(125).

Overmiller AM, McGuinn KP, Roberts BJ, Cooper F, Brennan-Crispi DM, Deguchi T, et al. c-Src/Cav1-dependent activation of the EGFR by Dsg2. Oncotarget 2016;7(25):37536-55.

Overmiller AM, Pierluissi JA, Wermuth PJ, Sauma S, Martinez-Outschoorn U, Tuluc M, et al. Desmoglein 2 modulates extracellular vesicle release from squamous cell carcinoma keratinocytes. FASEB J 2017;31(8):3412-24.

Roberts BJ, Svoboda RA, Overmiller AM, Lewis JD, Kowalczyk AP, Mahoney MG, et al. Palmitoylation of Desmoglein 2 Is a Regulator of Assembly Dynamics and Protein Turnover. J Biol Chem 2016;291(48):24857-65.

Roop DR, Cheng CK, Titterington L, Meyers CA, Stanley JR, Steinert PM, et al. Synthetic peptides corresponding to keratin subunits elicit highly specific antibodies. J Biol Chem 1984;259(13):8037-40.

Wahl JK, Sacco PA, MaGranahan-Sadler TM, Sauppe LM, Wheelock MJ, Johnson KR. Plakoglobin domains that define its association with the desmosomal cadherins and the classical cadherins: identification of unique and shared domains. J Cell Sci 1996;109:1143-54.

Wahl JKr. Generation of monoclonal antibodies specific for desmoglein family members. Hybrid Hybridomics 2002;21(1):37-44.