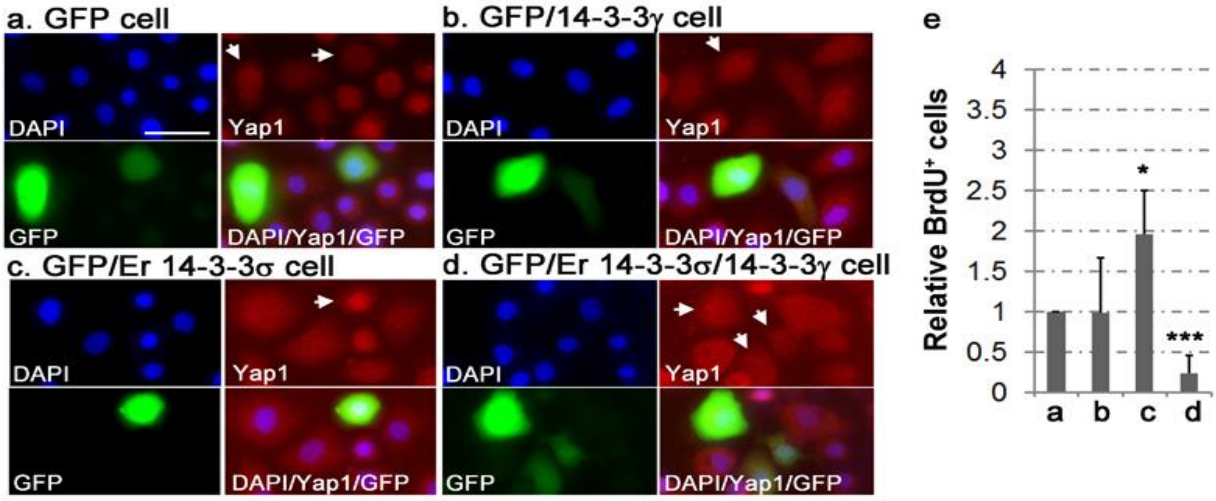

Figure S1. 14-3-3γ over-expression suppressed the *Er* 14-3-3σ induced Yap1 nuclear localization and HaCat cell proliferation. (**a-d**) Immunostaining of DAPI (purple), Yap1 (red), GFP (green) and the merged images. HaCat cells were transfected with plasmid DNA expressing GFP, or GFP/HA14-3-3γ (1:4 ratio), GFP/flag-*Er* 14-3-3σ (1:4 ratio) or GFP/HA-14-3-3γ/flag-*Er* 14-3-3σ (1:2:2 ratio). The arrows indicate yap1 staining of the transfected cells. (**e**) Co-expression of 14-3-3γ suppressed cell proliferation induced by over-expression of *Er* 14-3-3σ. The values represents the number of $BrdU^+$ cells in the *Er* 14-3-3σ transfected HaCat cells relative to the number of $BrdU^+$ cells from those transfected with control GFP plasmid. Data are expressed as mean±SD, n=3. Two-tailed Student's *t*-test: $*p < 0.05$, $***p < 0.005$. The scale bar in (**a**) represents 50 μm and all pictures (**a-d**) are in same magnification.

Table S1. List of primers used for qPCR	
Gene	**Primer sequence**
Mouse *Gapdh*	F-5'- TTCACCACCATGGAGAAGGC -3' R-5'- GGCATGGACTGTGGTCATGA -3'
Mouse *Actb*	F-5'- CCAGTTGGTAACAATGCCATGT -3' R-5'- TGTATGCTATACGAAGTTAT -3'
Mouse *Cyr61*	F-5'- CTGCGCTAAACAACTCAACGA -3' R-5'- GCAGATCCCTTTCAGAGCGG -3'
Mouse *Ctgf*	F-5'- GGGCCTCTTCTGCGATTTC -3' R-5'- ATCCAGGCAAGTGCATTGGTA -3'
Mouse *Yap1*	F-5'- ACCCTCGTTTTGCCATGAAC -3' R-5'- TGTGCTGGGATTGATATTCCGTA -3'
Mouse *Zeb1*	F-5'- TGGCAAGACAACGTGAAAGA -3' R-5'- AACTGGGAAAATGCATCTGG -3'
Mouse *Snai2*	F-5'- TGATGCCCAGTCTAGGAAAT -3' R-5'- AGTGAGGGCAAGAGAAAGG -3'
Mouse *14-3-3σ*	F-5'- GTGTGTGCGACACCGTACT -3' R-5'- CTCGGCTAGGTAGCGGTAG -3'
Mouse *14-3-3β*	F-5'- TGGATAAGAGTGAGCTGGTACA -3' R-5'- CGTGTCCCTGCTCTGTTACG -3'
Mouse *14-3-3γ*	F-5'- GTGACCGAGCTGAACGAAC -3' R-5'- GATGCTGCTGATGACCCTCC -3'
Mouse *14-3-3η*	F-5'- ACGAAGATCGAAATCTCCTCTCT -3' R-5'- CCGGTAGGCTTTAACTTTCTCCA -3'
Mouse *14-3-3ε*	F-5'- ACTGGCGAGTCCAAGGTTTTC -3' R-5'- TGTCATCGCAATGTCACTAGC -3'
Mouse *14-3-3ζ*	F-5'- GAAAAGTTCTTGATCCCCAATGC -3' R-5'- TGTGACTGGTCCACAATTCCTT -3'
Mouse *14-3-3θ*	F-5'- ATTGAGCAGAAGACCGACACC -3' R-5'- TGTTTTCGATCATCGCCACAA -3'

Supplementary Materials and Methods.

Animals. *Er/+* mutant mice in a mixed C57BL/6J and CBA/CaGnLeJ genetic background (Stock #000515) were purchased from the Jackson Laboratory. Experimental animals were housed under pathogen-free conditions and handled in accordance with guidelines approved by the Institutional Animal Care and Use Committee of the University of Louisville. *Er/Er* homozygous mutants were generated from *Er/+* heterozygous mice by intercrossing. To label cell proliferation in embryos, 5-bromo-2'-deoxyuridine (BrdU) was injected intraperitoneally into pregnant mice at a dose of 150 mg/kg body weight 2 hours prior to collection of the embryos on embryonic day 18.5 (E18.5). The embryo tails were saved and processed for protein preparation for western analysis of the 14-3-3σ genotypes, and the rest of the embryos were fixed in 4% paraformaldehyde (PFA) at 4^{o}C overnight.

Histology and immunostaining. Tumors induced with 7,12-dimethylbenzanthracene/12-O-tetradecanoyl-phorbol-13-acetate (DMBA/TPA) in *Er/+* mice were described in our previous study. Briefly, *Er/+* mice at 6 weeks old received 100 nmol of DMBA in 0.2 ml acetone topically on the shaved dorsal skin. One week after a single DMBA treatment, TPA (17 nmol in 0.2 ml acetone) was applied topically to the shaved dorsal skin three times per week for 19 weeks. Eight weeks after stopping TPA treatment, the tumors were collected. E18.5 embryo tissues were fixed immediately in 4% PFA at 4 ^{o}C overnight and then subjected to paraffin embedding and sectioning for histological studies. The paraffin-embedded sections (7 μm thick) were prepared for hematoxylin and eosin (H&E) staining. For most immunostaining, the tissue sections or cultured cell preparations were subjected to an antigen-retrieval procedure by heating the slides at 95^{o}C for 30 min in 10 mM Tris-ethylendiaminetetraacetic acid (EDTA) buffer (pH 9.0). The primary antibodies used in this study were goat anti-C terminus for 14-3-3σ (1:200, cat # sc-7683, Santa Cruz Biotechnology), goat anti-ΔNp63 (1:200, cat #sc-8609, Santa Cruz Biotechnology), rabbit anti-filaggrin (1:500, PRB-417P, Covance Research Products, Denver, PA), rat anti-BrdU (1:800, MAS 250c, Harlan-Sera Lab, Belton Loughborough, Leicestershire, England), and mouse anti-Yap1 (1:100, cat # sc-101199, Santa Cruz Biotechnology). The secondary antibodies, conjugated with either carbocyanine 3 (Cy3) or fluorescein isothiocyanate (FITC), were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). The HRP-conjugated secondary antibody was visualized with DAB substrate following the manufacturer's instructions (Cat # SK-4600, Vector Labs, Burlingame, CA).

Keratinocyte culture. Preparation and culture of keratinocytes followed a published procedure (Xin *et al.*). Keratinocytes plated in collagen-coated 6-well plates or 8-well chambers were cultured in 0.02 mM calcium containing Keratinocyte Serum-free Media (SFM, Invitrogen, Carlsbad, CA). For lentiviral transduction, cells were infected with lentivirus collected in SFM overnight (2nd day), incubated in fresh SFM (3rd day), and then incubated in SFM with 0.02 or 0.5 mM calcium for an additional 48 hours (days 5–7). Puromycin (2.5 μg/ml) was added to the medium to select for cells that stably express shRNA. Cells were collected for protein or RNA isolation or fixed for immunostaining. For BrdU incorporation, the cells were incubated in medium containing 10 μM BrdU for 90 min and then fixed with 4% PFA for 15 min at room temperature before immunostaining with anti-BrdU antibody.

RNA isolation and quantitative (q) PCR. Primary cultured keratinocytes at 70–90% confluence were prepared at the indicated time points for RNA extraction using TRIzol reagent (Invitrogen). The A260/A280 ratio of all RNA samples was >2.0, as measured by Nanodrop. Double-stranded cDNA was reverse-transcribed using random primers and the SuperScript VILO cDNA synthesis kit (Invitrogen).

Real-time qPCR was performed in a SYBR green-based PCR reaction mixture on a MX3005p system (Agilent Technologies, Inc., Santa Clara, CA), programmed with a 10-minute initial hot-start activation of *Taq* polymerase at 95°C, followed by 40 cycles of amplification (95°C for 10 seconds, 56°C for 5 seconds, and 72°C for 10 seconds). The comparative threshold cycle (CT) method normalized to *Actb* or *Gapdh* was used to analyze relative changes in gene expression. The qPCR primer sequences for *Zeb1*, *Snai2* and *Gapdh* have been published elsewhere (Liu *et al.*, 2009; Wu *et al.*, 2010). The primers for mouse *Cyr61* (ID 6753594a1), *Ctgf* (ID 6753878a1), *Yap1* (ID 15928514a1), 14-3-3σ (ID 134023661c1), 14-3-3β (ID 31543974a1), 14-3-3γ (ID 31543976a1), 14-3-3ζ (ID 6756041a1), 14-3-3ε (ID 31981925a1), 14-3-3θ (ID 6756039a1), and 14-3-3η (ID 6756037a1) were designed based on the online PrimerBank database (Harvard Medical School, Boston, MA; *http://pga.mgh.harvard.edu/primerbank*). The primer sequences are listed in **Table S1**.

293T culture, transfection, and co-immunoprecipitation. Wild type keratinocytes were infected with lentivirus expressing Flag-tagged mouse 14-3-3σ and lysed in cold RIPA buffer (20 mM Tris-HCl, 100 mM NaCl, 0.2% deoxycholic acid, 0.2% Triton X-100, 0.2% NP-40, and protease inhibitor cocktail [Roche]) three days after transduction. 293T human embryonic kidney cells (Invitrogen, Carlsbad, California) in 10-cm plates were maintained in DMEM medium containing 10% FBS and were transfected by the calcium phosphate method. Two days after transfection, the cells were lysed in cold RIPA buffer. After clearance by incubation for 30 min with normal IgG (1 μg/ml) from the same species as the primary antibody used to immunoprecitate the target proteins and 100 μl of a protein G-coupled agarose bead slurry (Amesham Biosciences), the lysates were incubated for 2 h at 4ºC with 30 μl of a protein G-coupled agarose bead slurry with mouse anti-Flag (M2, cat #F1804, Sigma) or rat anti-HA (3F10, cat # 11867423001, Roche Applied Science) antibody. The beads were then washed five times before the antibody–antigen complexes were released from the beads with SDS lysis buffer and loaded on 10% SDS-polyacrylamide gels and subjected to western analysis with antibodies recognizing the different 14-3-3 isoforms: rabbit anti-Flag (1:500, cat #600-401-383, Rockland), mouse anti-HA (1:500, cat #9658, Sigma), or protein A-HRP (1:1500, from Amersham for rabbit antibody).

HaCat cell culture and transfection. HaCaT cells were cultured in 0.05 mM calcium containing Keratinocyte Serum-free Media (SFM, Invitrogen, Carlsbad, CA). Transfections with GenCarrier-1™ were performed according to the manufacturer's protocol. 8-chamber slides were used for cell culture and cells in each well were transfected with 0.5 μg DNA containing GFP, or GFP/HA14-3-3γ (1:4 ratio), GFP/flag-*Er* 14-3-3σ (1:4 ratio) or GFP/HA-14-3-3γ/flag-*Er* 14-3-3σ (1:2:2 ratio). Fresh medium was added at 12 hrs post-transfection. For BrdU incorporation, the cells were incubated in medium containing 10 μM BrdU for 90 min at 36 hrs post-transfection and then fixed with 4% PFA for 15 min at room temperature before immunostaining with anti-BrdU and anti-Yap1 antibodies. Transfected cells were visualized by GFP fluorescence.

Plasmid information. Expression vectors encoding the Yap1 shRNA and scrambled control shRNA were ordered from Open Biosystems, Inc.. pCSC-SP-PW-flag-14-3-3σ is the lentivirus vector expressing flag-tagged wild type mouse 14-3-3σ under CNV promoter. The construct was made by generating insert cDNA from mouse genomic DNA of WT embryos using primers including 5'-cgcggatcccatATGGATTACAAGGACGACGACGATAAGATCatggagagagccagtctgatc-3' and 5'-cttgtaggagtcctcactga-3', then inserting it into BamHI site in pBOB lentiviral vector (kindly provided by Drs. Robert Marr and Inder Verma). Similarly, pCSC-SP-PW-flag-*Er* 14-3-3σ was constructed with cDNA fragment amplified from mouse genomic DNA of *Er/Er* embryos using primers as 5'-

cgcggatcccatATGGATTACAAGGACGACGACGATAAGATCatggagagagccagtctgatc-3' and 5'-cttgtaggagtcctcactgaa-3'. pcDNA3-HA-14-3-3γ (Plasmid #132274) and pcDNA3-HA-14-3-3σ (Plasmid #11946) and pCSC-SP-PW-GFP (Plasmid #12337) were obtained from Addgene Inc..

Western blot analysis. Keratinocytes were lysed in cold radioimmunoprecipitation assay buffer (20 mM Tris-HCl, pH 7.4, 100 mM NaCl, and 0.2% each of deoxycholate, Triton X-100, and Nonidet P-40) containing 1x complete protease-inhibitor mixture (Roche Diagnostics, Indianapolis, IN). Equal amounts of whole-cell lysates were separated on a 10% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and transferred to a nitrocellulose membrane. For immunoblotting, rabbit anti-14-3-3β (1:1000, Cat. #9636), rabbit anti-14-3-3γ (1:1000, Cat. #5522), rabbit anti-14-3-3ζ (1:1000, Cat. #7413), rabbit anti-14-3-3ε (1:1000, Cat. #9635), rabbit anti-14-3-3θ (1:1000, Cat. #9638), rabbit anti-14-3-3η (1:1000, Cat. #5521), and rabbit anti- Yap1^{Ser127} (1:500, Cat. #4911) antibodies were purchased from Cell Signaling Technology. The specificity of the antibodies could be found from the company website: http://www.cellsignal.com.
Goat anti-N terminus of 14-3-3σ (1:200, cat. # sc-7683) and mouse anti-Yap1 (1:100, cat. # sc-101199) antibodies were purchased from Santa Cruz Biotechnology, and mouse anti-β-actin antibody (1:1000, cat, #A2228) was purchased from Sigma. Horseradish peroxidase-conjugated secondary antibody and an enhanced chemiluminescence (ECL) system (Amersham Pharmacia, Piscataway, NJ) were used to visualize the signals.