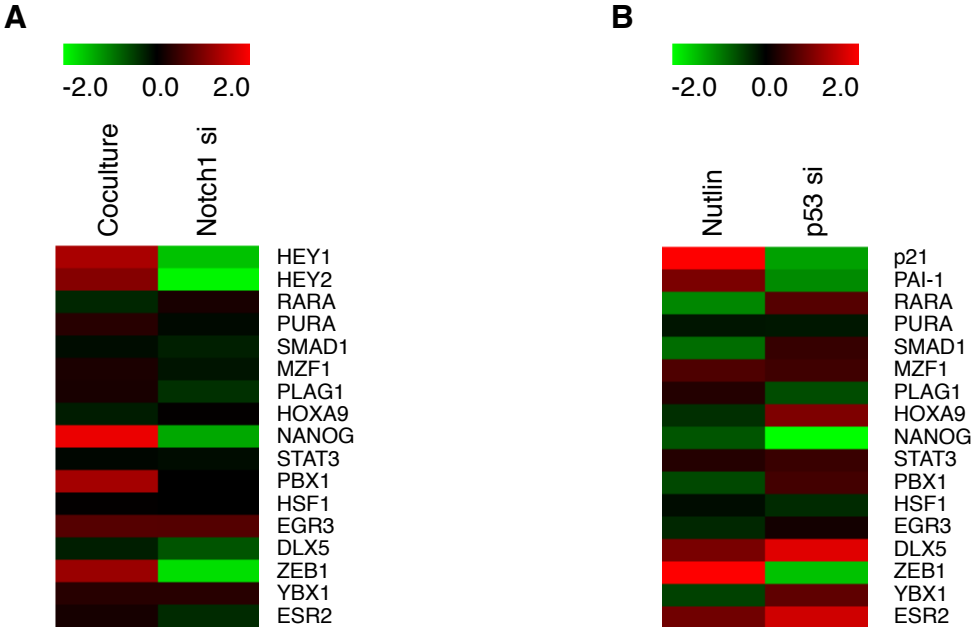
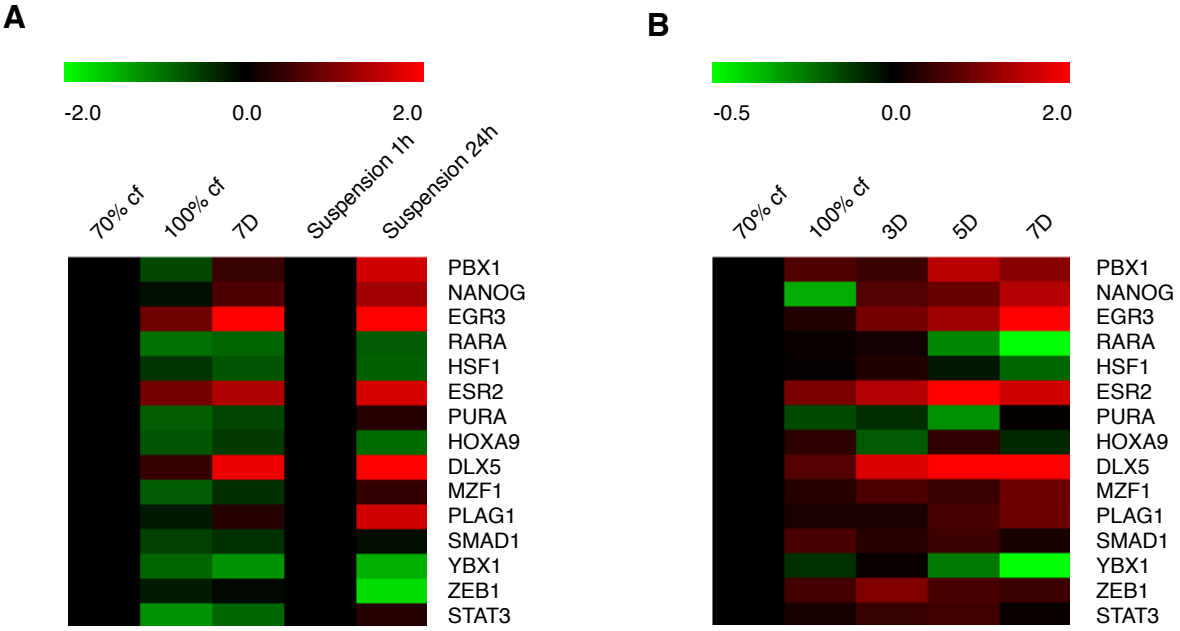


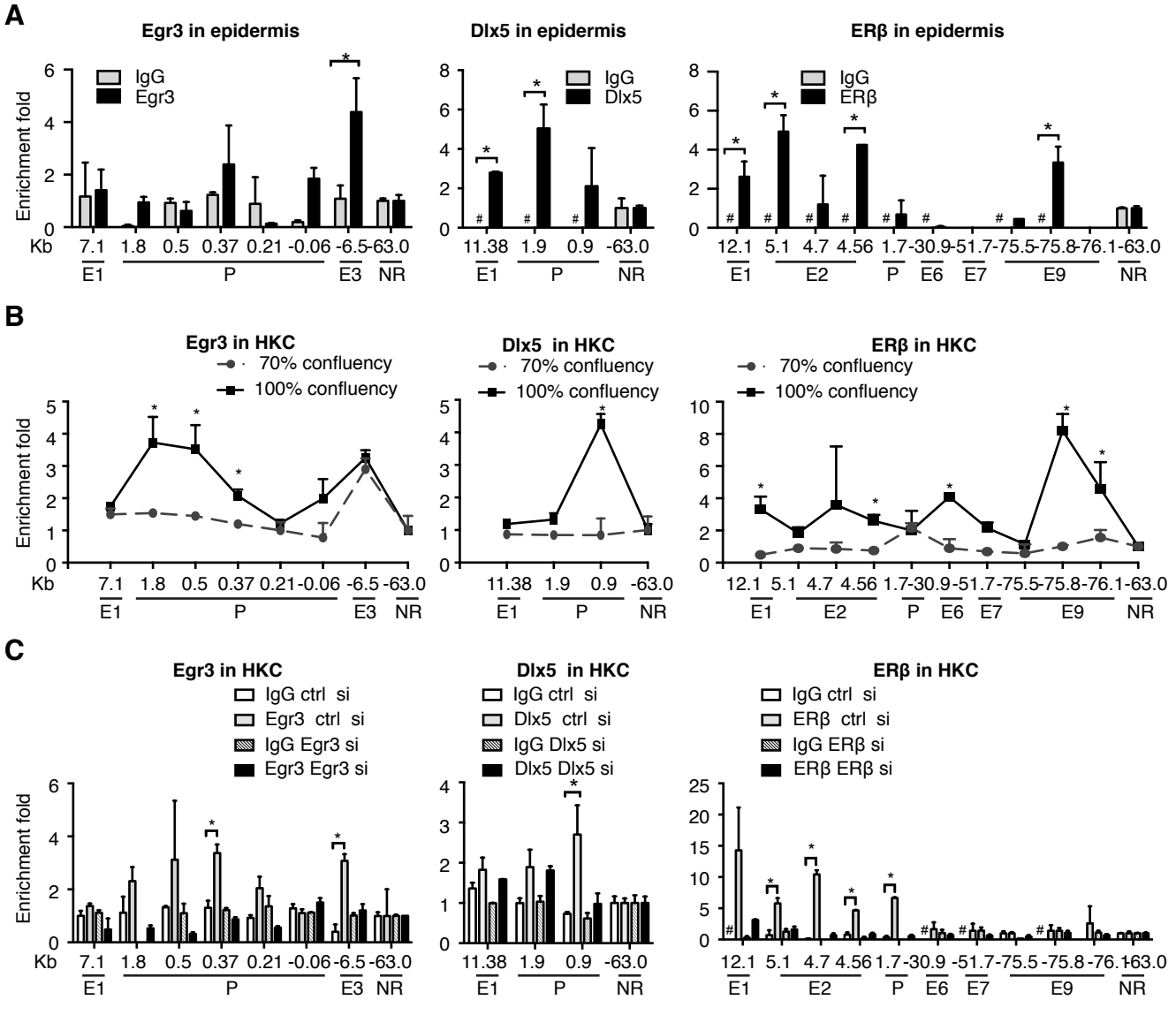
Supplemental Figure 1

Transcriptional control of Notch1 regulators by Notch and p53 signaling. (A) HKCs were co-cultured with NIH3T3 cells expressing full length Jagged2 or empty vector control (for 24 hours) as a way to activate endogenous Notch signaling, or were transfected with siRNA against Notch1 (30 nM) or scrambled siRNA control (30 nM) (for 1 week) for Notch signaling attenuation. Expression of the indicated genes was assessed by RT-qPCR analysis with 36ß4 for normalization and Hey1 and Hey2 as control of "canonical" Notch responsive genes. Results are expressed as heat maps of log2 ratios in cells with modulation of Notch signaling versus controls. (B) HKCs were treated with Nutlin-3A (10 µm; for 24 hours) to stabilize the endogenous p53 protein, or transfected with siRNA against p53 (30 nM) or scrambled siRNA control (30 nM; for 1 week) to suppress p53 activity. Expression of the indicated genes was assessed by RT-qPCR analysis with 36ß4 for normalization and $p21^{CIP1/WAF1}$ and PAI-1 as control of "canonical" p53 responsive genes. Results are expressed as heat maps of log2 ratios in cells with modulation of p53 signaling versus controls.



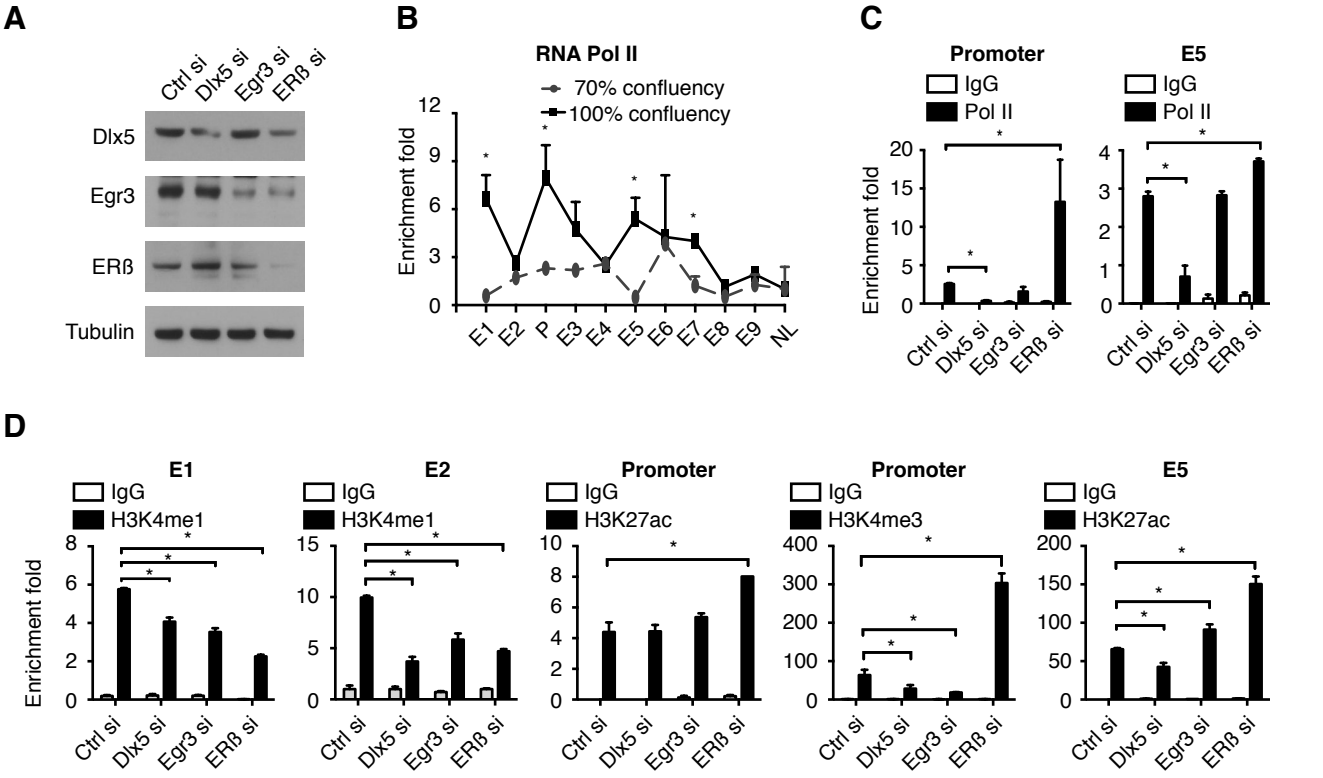
Supplemental Figure 2

Expression of the identified *Notch1* regulators in growing versus differentiating keratinocytes. Two different strains of HKCs (A and B) under growing conditions (70% confluence, cf) or induced to differentiate by high density (100 % confluence, cf) for the indicated number of days (D) or by suspension culture conditions for the indicated times (days and hours, respectively) were analyzed for expression of the indicated genes by RT-qPCR with 36ß4 for normalization. Results are expressed as heat map of log2 ratios in cells under differentiating versus growing conditions.



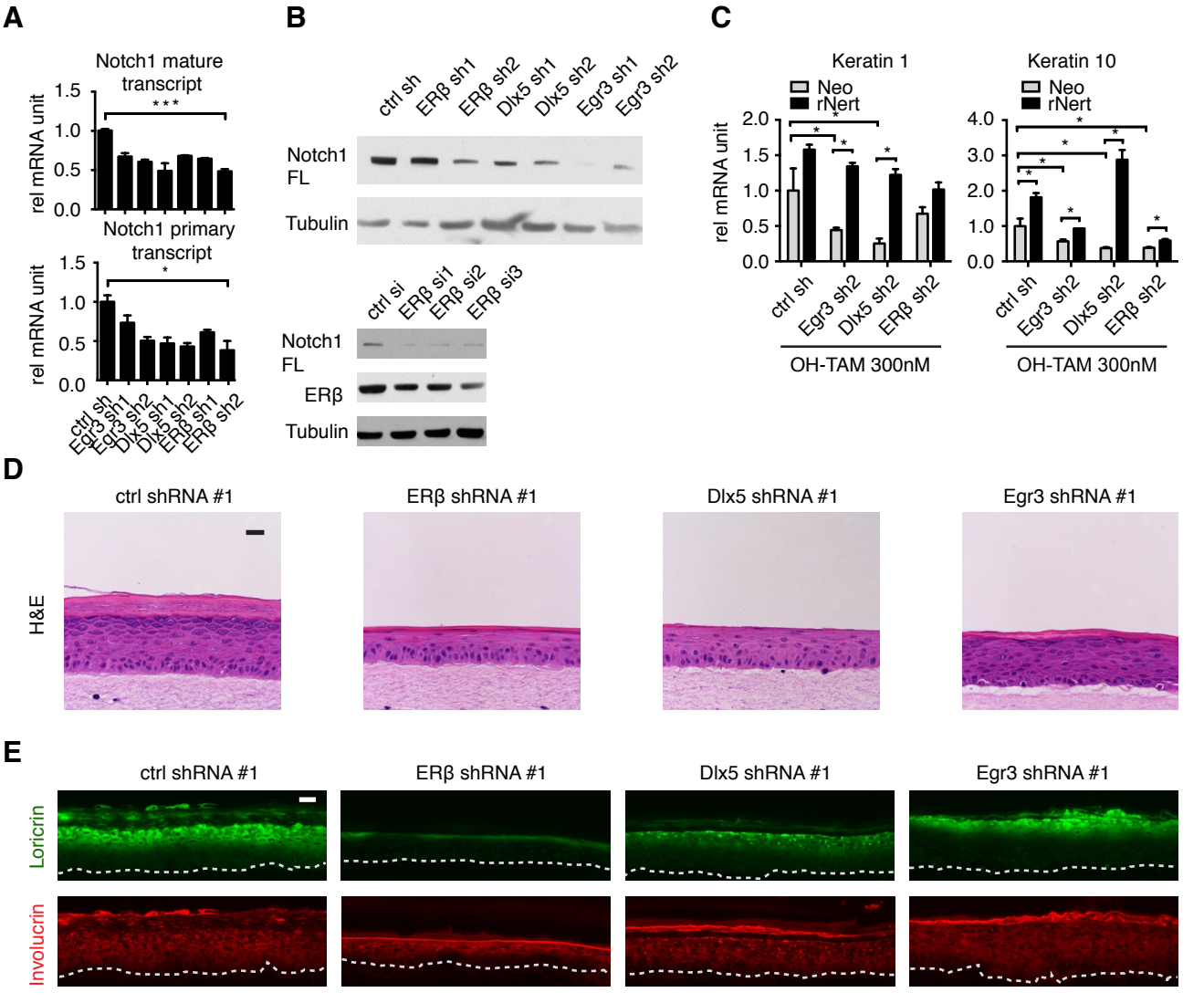
Supplemental Figure 3

Binding of endogenous Egr3, Dlx5 and ERß to the *Notch1* gene locus in human epidermis and HKCs. (A) Similar ChIP assays as those described for Figure 2B were undertaken utilizing an epidermal sample of different origin. # refers to undetectable signal in some ChIP assays with non immune IgG (*p<0.05). (B and C) Similar ChIP assays as those described for Figure 2, C and D, utilizing HKCs of a different orgin under growing versus differentiating conditions (B) or plus/minus knock-down of the Egr3, Dlx5 and ERß genes (C). Data were similarly analyzed and plotted (*p<0.05).



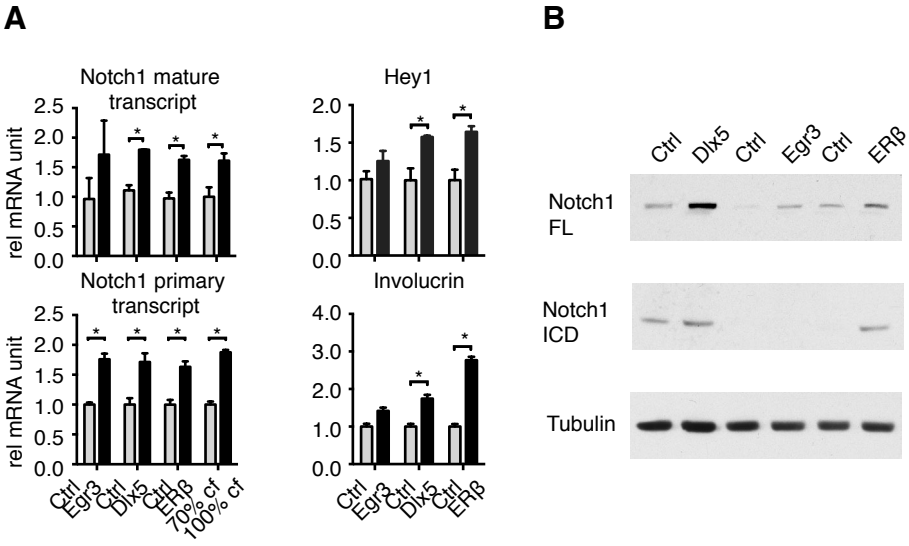
Supplemental Figure 4

Essential role of Egr3, Dlx5 and ERß in RNA Pol II recruitment to the Notch1 locus and/or pause release. (A) HKCs transfected with siRNAs against Dlx5, Egr3, ERß versus scrambled controls utilized for the ChIP assays shown in Figure 3B were analyzed 96h later by immunoblot of the indicated proteins. (B and C) ChIP analysis of HKCs under growing versus differentiating conditions (B) and in differentiating HKCs plus/minus Egr3, Dlx5 and ERß (C) for levels of polymerase II occupancy of the Notch1 locus. Conditions were the same as in Figure 3, A and B, utilizing HKCs of a different origin (*$p<0.05$). (D) ChIP analysis of activated histone marks at the indicated regions of the Notch1 locus in HKCs of different origin from those in Figure 3C, plus/minus Egr3, Dlx5 and ERß knock-down. Data were similarly analyzed and plotted (*$p<0.05$).



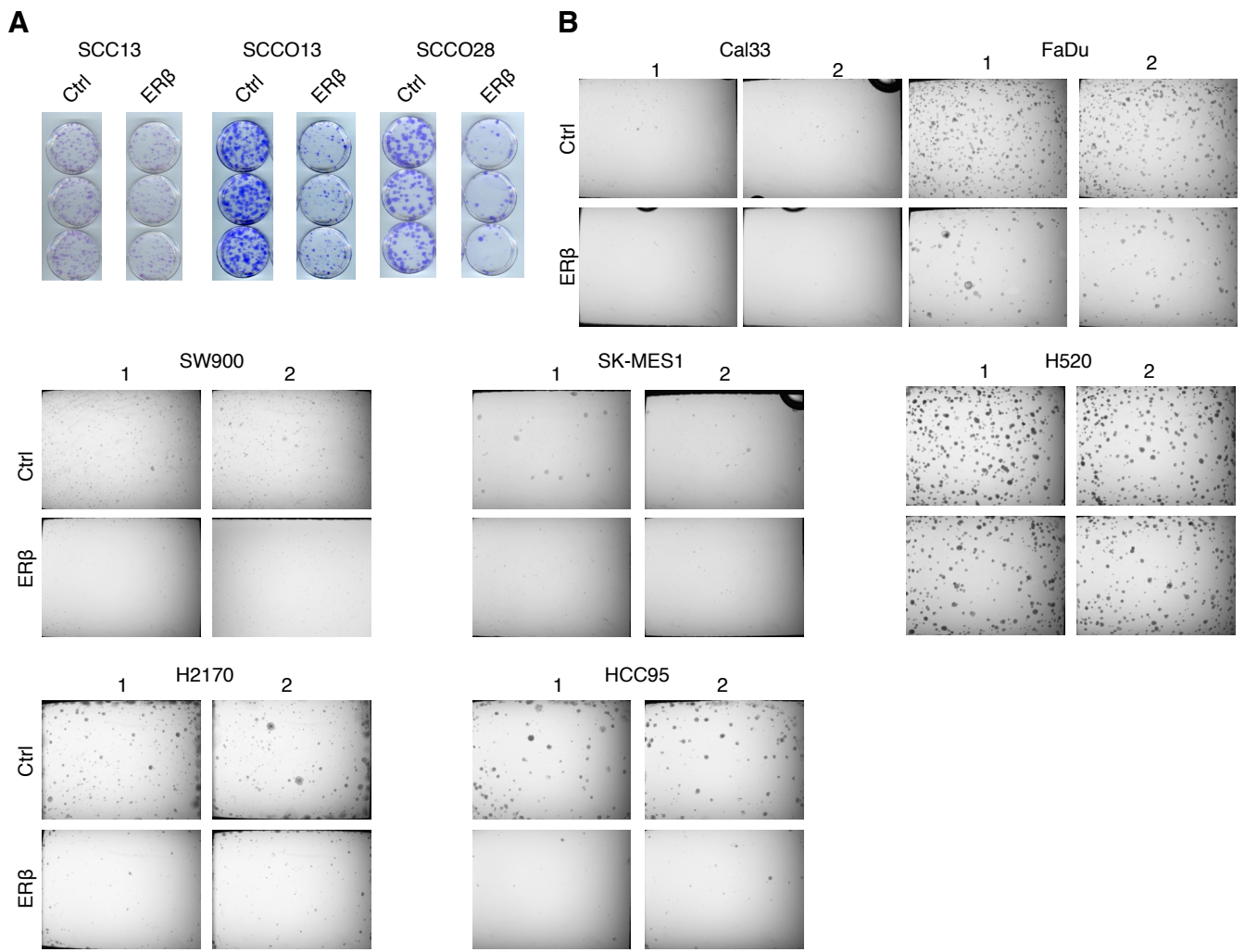
Supplemental Figure 5

Egr3, Dlx5 and ERß as regulators of Notch1 gene expression and function. (A) Parallel HKC cultures plus/minus shRNA-mediated knock down of the indicated genes as in Figure 4A were analyzed by RT-qPCR for expression of the primary and mature Notch1 transcripts (*p<0.001). (B-C) Similar experiments as the ones shown in Figure 4, A and B, with HKCs of an independent origin. In B, lower panel, a similar immunoblot analysis of Notch 1 expression is shown for HKCs 3 days after transfection with 3 different siRNAs against ERß versus scrambled controls. (D-E) HKCs stably infected with shRNA expressing lentiviruses against Dlx5, Egr3, ERß versus luciferase control were induced to differentiate in a 3D organotypic culture system as in Figure 4, D and E. Reconstituted epidermis were collected at day 12 after lifting for H&E (D) and immunofluorescence analysis of the involucrin and loricrin differentiation markers (E). For each marker, identical image capture conditions were used. Images are representative of 3 independent fields, two epidermis per genotype. Bar=100 µm.



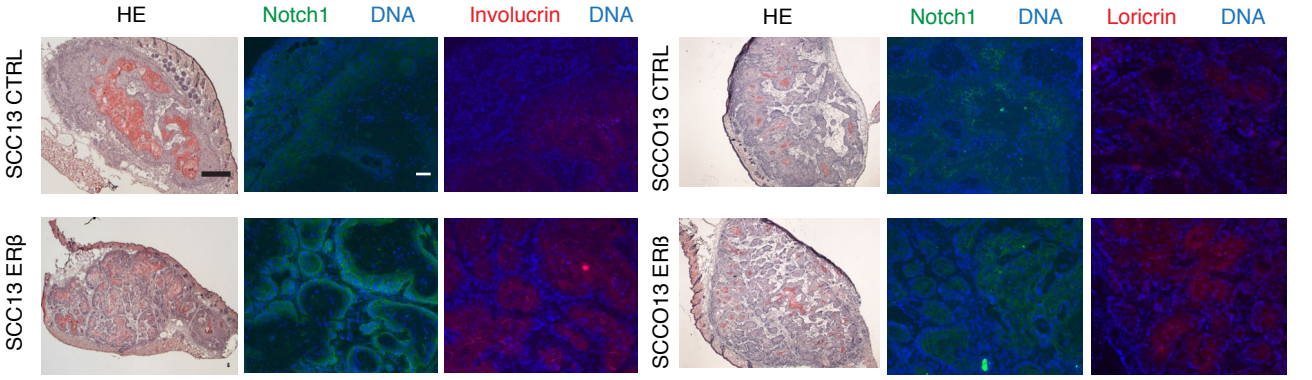
Supplemental Figure 6

Egr3, Dlx5 and ERß as regulators of Notch1 gene expression and function. (A) Parallel HKC cultures plus/minus retroviral-mediated expression of the indicated genes as in Figure 5, A and B were analyzed by RT-qPCR for expression of the primary and mature Notch1 transcripts using primers targeting the third intron/exon junction and junction between exon 32 and 33, respectively. The expression levels for canonical Notch target Hey1 and differentiation marker involucrin were also determined. mRNA levels were normalized for 36ß4, and presented as fold-changes over control ± S.D. (*p<0.001). (B) HKCs of an independent origin plus/minus retroviral-mediated expression of genes as in Figure 5A were similarly analyzed by immunoblotting.



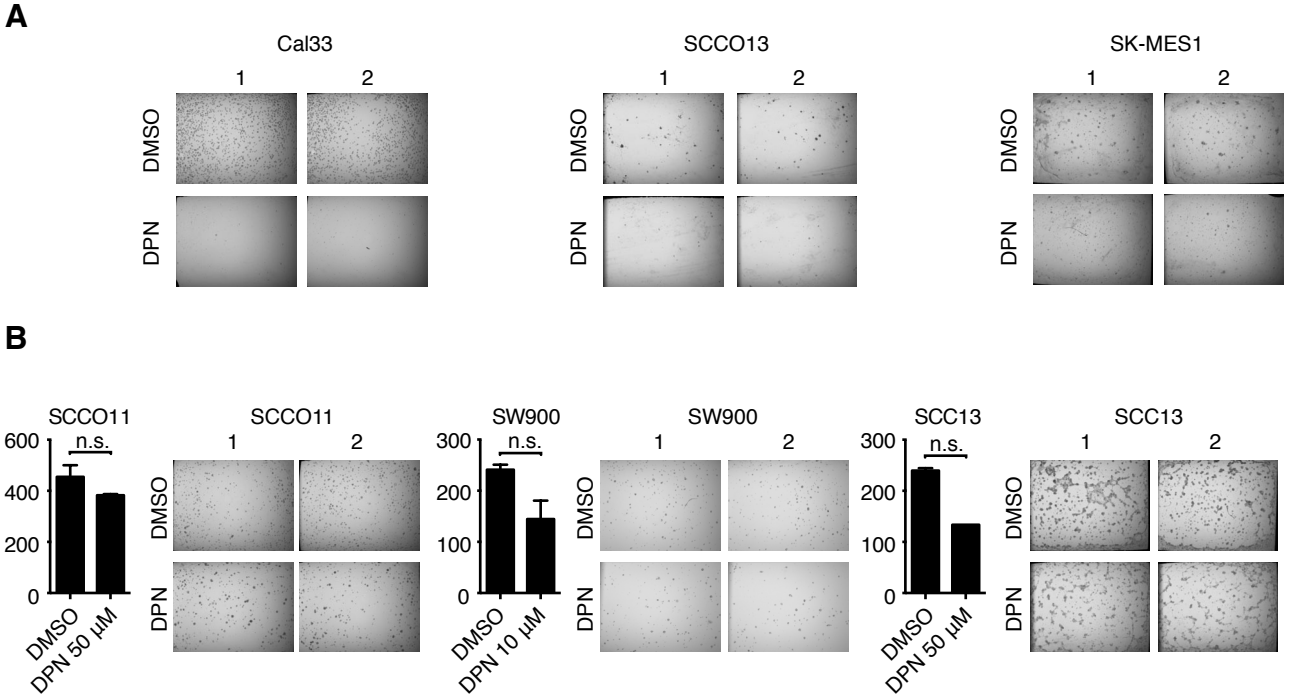
Supplemental Figure 7

Increased ERβ level leads to reduced proliferation of SCC cells. (A) Whole dish images of colonies formed by the indicated SCC cells infected with an ERβ expressing lentivirus versus empty vector control. SCC13, SCCO13 and SCCO28 cells were plated at limited density on triplicate dishes (10^3 cells/60 mm dish) 96 hours after infection of lentiviruses and stained for colonies10 days later.Images correspond to the experiment and colony quantification data shown in Figure 8B. (B) Whole well images of spheroids formed by the indicated SCC cells infected with ERβ expressing versus control viruses. Stably infected cells were trypsinized and plated in 8-well chambers coated with matrigel at a density of 2000 cells/well in duplicates.Images correspond to the experiment and sphere quantification data shown in Figure 8, C and D. Some SCC cell lines formed small size spheres that can be seen after magnification of the digital images.



Supplemental Figure 8

Increased ERß level up-regulates Notch1 expression and induces differentiation of SCC cells in tumor xenografts. SCC13 and SCCO13 cells infected with ERß expressing versus control vectors were tested by parallel intradermal injections into mice as in Figure 9. Animals were sacrificed 3 weeks later and tumor samples were processed for H&E and immunofluorescence analysis of Notch1 and differentiation marker expression as indicated. Black bar=250µm, white bar=100µm.



Supplemental Figure 9

ERß agonist treatment suppresses proliferation of SCC cells. (A) Whole well images of spheroids formed by the indicated SCCs treated with DPN versus DMSO control in Figure 10. (B)Whole well images and quantification of spheroids formed by the indicated SCC cells treated with DPN versus DMSO control. SCC cells were trypsinized and plated in 8-well chambers coated with matrigel at a density of 2000 cells/well in duplicates. Data quantification is presented as mean of duplicates ± S.D. n.s. stands for not significant.

Supplemental Table 1. List of genes included in the siRNA library and the location of their putative binding sites in the regulatory regions of the *Notch1* gene locus.

RefSeq Accession Number	Gene Symbol	Full Gene Name	Gene ID	Predicted binding sites in the regulatory regions
NM_002585	PBX1	pre-B-cell leukemia homeobox 1	5087	E1, E2, E4, E5,
NM_001042539	MAZ	MYC-associated zinc finger protein (purine-binding transcription factor)	4150	E1, E2, P, E3, E5, E6, E7, E8, E9
NM_003998	NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	4790	E2, P, E3, E4, E5, E6, E7, E8, E9
NM_005269	GLI1	glioma-associated oncogene homolog 1 (zinc finger protein)	2735	E1, E2, P, E3, E6, E8, E9
NM_002908	REL	v-rel reticuloendotheliosis viral oncogene homolog (avian)	5966	E2, P, E3, E4, E5, E6, E7, E8, E9
NM_001530	HIF1A	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	3091	E1, E2, P, E5, E7, E8, E9
NM_003593	FOXN1	forkhead box N1	8456	E1, E2, P, E3, E7, E9
NM_024865	NANOG	Nanog homeobox	79923	E2, P, E3, E4, E5, E6, E7, E8, E9
NM_000926	PGR	progesterone receptor	5241	E1, P, E3, E4, E5
NM_005859	PURA	purine-rich element binding protein A	5813	P, E3, E4, E5, E7, E9
NM_006599	NFAT5	nuclear factor of activated T-cells 5, tonicity-responsive	10725	E1, E2, P, E3, E4, E6, E7, E9
NM_005270	GLI2	GLI-Kruppel family member GLI2	2736	E1, E2, P, E3, E6, E8, E9
NM_006286	TFDP2	transcription factor Dp-2 (E2F dimerization partner 2)	7029	E1, P, E6, E7
NM_021813	BACH2	BTB and CNC homology 1, basic leucine zipper transcription factor 2	60468	E1, E2, E3, E4, E5, E6, E7, E9
NM_021632	ZNF350	zinc finger protein 350	59348	E1, P, E3, E5, E6, E7, E8, E9
NM_004430	EGR3	early growth response 3	1960	P, E3
NM_182907	PRDM1	PR domain containing 1, with ZNF domain	639	E1, E2, E3, E4, E5, E7, E9
NM_003131	SRF	serum response factor (c-fos serum response element-binding transcription factor)	6722	E1, E2, P, E3, E4, E5, E7, E8, E9
NM_001621	AHR	aryl hydrocarbon receptor	196	E2, E3, E4, E5,
NM_002943	RORA	RAR-related orphan receptor A	6095	E2, E7, E9

NM_003200	TCF3	transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)	6929	E1, E2, E3, E4, E5, E7, E8, E9
NM_014323	PATZ1	POZ (BTB) and AT hook containing zinc finger 1	23598	E1, E2, P, E3, E4, E5, E6, E7, E8, E9
NM_000125	ESR1	estrogen receptor 1	2099	E1, E2, P E6, E7, E9
NM_003153	STAT6	signal transducer and activator of transcription 6, interleukin-4 induced	6778	E1, E2, P, E3, E4, E5, E8, E9
NM_021964	ZNF148	zinc finger protein 148	7707	E1, E2, P, E3, E4, E5, E6, E7, E8, E9
NM_014352	POU2F3	POU class 2 homeobox 3	25833	P, E5, E7
NM_016269	LEF1	lymphoid enhancer-binding factor 1	51176	E2, P, E3, E4, E5, E6, E7, E8, E9
NM_001040110	NRF1	nuclear respiratory factor 1	4899	E2, P, E9
NM_001040275	ESR2	estrogen receptor 2 (ER beta)	2100	E1, E2, P E6, E7, E9
NM_000964	RARA	retinoic acid receptor, alpha	5914	E1, E2, P, E3, E4, E5, E6, E7, E8, E9
NM_152739	HOXA9	homeobox A9	3205	E4, E5
NM_004559	YBX1	Y box binding protein 1	4904	E1, E2, P, E3, E4, E5, E7, E9
NM_002653	PITX1	paired-like homeodomain 1	5307	P, E9
NM_005526	HSF1	heat shock transcription factor 1	3297	E1, E2, P, E3, E4, E5, E6, E7, E8, E9
NM_002460	IRF4	interferon regulatory factor 4	3662	E2, P, E3, E4, E5, E7, E8, E9
NM_001032280	TFAP2A	transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)	7020	E2, P, E3, E4, E5, E7, E8, E9
NM_002655	PLAG1	pleiomorphic adenoma gene 1	5324	E1, E2, P, E3, E4, E5, E6, E7, E8, E9
NM_030751	ZEB1	zinc finger E-box binding homeobox 1	6935	E1, E2, P, E3, E4, E5, E6, E7, E9
NM_001003688	SMAD1	SMAD family member 1	4086	E2, P, E5, E7, E9
NM_003422	MZF1	myeloid zinc finger 1	7593	E1, E2, P, E3, E4, E5, E6, E7, E8, E9
NM_005221	DLX5	distal-less homeobox 5	1749	E1, P
NM_005524	HES1	hairy and enhancer of split 1, (Drosophila)	3280	E1, P, E3, E5, E7, E8, E9
NM_000966	RARG	retinoic acid receptor, gamma	5916	E1, E2, P, E3,

				E4, E5, E6, E7, E8, E9
NM_002198	IRF1	interferon regulatory factor 1	3659	E2, P, E3, E4, E5, E6, E7, E8, E9
NM_001964	EGR1	early growth response 1	1958	E1, E2, P, E3, E5, E7, E8, E9
NM_002196	INSM1	insulinoma-associated 1	3642	E1, E2, P, E3, E4, E5, E6, E7, E8, E9
NM_006186	NR4A2	nuclear receptor subfamily 4, group A, member 2	4929	E1, P, E7, E9
NM_005225	E2F1	E2F transcription factor 1	1869	E1, E2, P, E3, E4, E5, E6, E8, E9
NM_000346	SOX9	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	6662	E1, E2, P, E3, E5, E7,
NM_001668	ARNT	aryl hydrocarbon receptor nuclear translocator	405	E3, E4, E5, E8
NM_000044	AR	androgen receptor	367	E1, E2, P, E3, E4, E5, E6, E7, E8, E9
NM_002382	MAX	MYC associated factor X	4149	E6, E9
NM_001913	CUX1	cut-like homeobox 1	1523	E1, E2, P, E4, E5, E7, E9
NM_030762	BHLHB3	basic helix-loop-helix domain containing, class B, 3	79365	E1, E2, P, E3, E4, E5, E6, E7, E9
NM_000168	GLI3	GLI-Kruppel family member GLI3 (Greig cephalopolysyndactyly syndrome)	2737	E1, E2, P, E3, E5, E6, E7, E8, E9
NM_015069	ZNF423	zinc finger protein 423	23090	E1, E2, P, E3, E5, E6, E7, E9
NM_003150	STAT3	signal transducer and activator of transcription 3 (acute-phase response factor)	6774	E1, E2, P, E3, E4, E5, E6, E7, E9
NM_002701	POU5F1	POU class 5 homeobox 1	5460	E1, E2, P, E3, E5, E7, E9
NM_021953	FOXM1	forkhead box M1	2305	P, E3, E4, E5, E6, E9
NM_000376	VDR	vitamin D (1,25- dihydroxyvitamin D3) receptor	7421	E1, E2, P, E3, E4, E5, E6, E7, E8, E9

Supplemental Table 2. Summary of gene information and protein functions of 15 identified *Notch1* regulators.

Gene Symbol	Gene Name	TF family	General Function	Epidermal Function	Known genetic alteration
PBX1	pre B cell leukemia homeobox 1	PBX homeobox family	1. At transcription regulation level, PBX1 protein interacts with other homedomain-containing proteins, including HOX and MEIS to form transcription complexes. It also acts as a pioneer factor to facilitate the recruitment of ERalpha in breast cancer. 2. PBX1 regulates the development of spleen, pancreas, kidney, adrenal gland, and skeleton and is involved in tumorigenesis.	1.PBX1 competes with sp1/3 factors to bind to the promoters of Late cornified envelope protein genes and activates their expression in differentiated keratinocytes. 2. Epidermal-specific Pbx1-null mice are viable and have epidermal barrier abnormalities.	TCF3-PBX1 and E2A-PBX1 translocations in pre-B-cell acute lymphoblastic leukemia (ALL)
RARA	retinoic acid receptor alpha	Nuclear hormone receptor family	1. RARA plays an important role in regulation of development, differentiation, apoptosis, granulopoeisis, and transcription of clock genes. 2. RARA regulates transcription by dimerizing with RXR and in a ligand-dependent manner. 3 RARA is a critical factor in ER transcription complex by maintaining cofactor interaction.	1. Both RARG and RARA are expressed in epidermal keratinocytes with RARG being the predominant isoform. RAR-beta was induced by RA in dermal fibroblasts, but not in keratinocytes. 2. Terminal differentiation of epidermal keratinocytes is inhibited by retinoic acid in parallel with the inhibition of the synthesis of suprabasal keratins, filaggrin, and transglutaminase.	BCOR-RARA, PML-RARA, NPM-RARA, NuMA-RARA, STAT5b-RARA and PLZF-RARα translocations in acute promyelocytic leukemia (APL)
PURA	purine-rich element binding protein A	PUR DNA-binding protein family	1. PURA is a sequence-specific single-stranded DNA-binding protein which binds purine rich elements present at the	No study has been reported.	Deletion of PURA has been reported in myelodysplastic syndrome and

			origins of replication and in gene flanking regions. It is implicated in the control of both DNA replication and transcription. 2. PURA is a component of the transcription repressor complex on the promoter of adrogen receptor gene. The loss of PURA leads to AR overexpression and androgen-independent prostate cancer.		acute myelogenous leukemia.
MZF1	myeloid zinc finger 1	Krüppel family of zinc finger proteins	1.MZF1 is critical for blood cell development and its disruption leads to a block of granulopoiesis. 2. Mzf1 can act as a tumor/growth suppressor in the hemopoietic compartment.	In normal human keratinocytes, MZF1 activates the transcription of peptidylarginine deiminase type I gene (PADI1) which is involved in keratinocyte differentiation.	No study has been reported.
YBX1	Y box binding protein 1	cold-shock protein superfamily	1. YBX1 binds to both DNA and RNA and can exert its function in cytoplasm, nucleus as well as cell surface. 2. YBX1 participates in various cellular processes, including apoptosis, cell proliferation, development and differentiation. The YB_1 gene knockout in mice results in serious distortions of embryonic development and in early (prenatal) death. 3. YBX1 is involved in tumorigensis by interacting with E2F, PI3K/Akt/mTOR and	No study has been reported.	No study has been reported.

			Ras/Raf/MEK/ERK pathways pathways.		
PLAG1	pleiomorphic adenoma gene 1	PLAG gene family	1. The expression of PLAG1 is developmentally regulated. 2. PLAG1 promotes cell proliferation by inducing cell growth pathways , such as IGF- II pathway. 3. PLAG1 transgenic mice develop a variety of tumor types.	No study has been reported.	CTNNB1-PLAG1. LIFR-PLAG1, SII/TCEA1-PLAG1, CHCHD7-PLAG1 Pleomorphic adenomas of the salivary gland. **(HAS2-PLAG1,** COL1A2-PLAG1 in lipoblastomas. Gene amplification in hepatoblastoma
HOXA9	homeobox A9	HOX gene family	1. HOXA9 promoters ovarian cancer progression by creating permissive microenvironment for tumor growth through TGFB2 action in fibroblasts. 2. HOXA9 restrains breast cancer progression by BRCA1 upregulation.	Mice with conditional deletion of the HoxA cluster, combined with deletions within the HoxD cluster exhibit a striking skin phenotype: largely edematous skin, without epidermal stratification and differentiation of epithelial appendages.	NUP98-HOXA9 in myeloid leukemogenesis
HSF1	heat shock transcription factor 1	HSF gene family	1. HSF1 is an evolutionarily conserved gene which can be activated by various stressors. 2. HSF1 mediates adaptive responses in an array of physiological processes, such as cell cycle control, ribosome biogenesis, protein translation, and glucose metabolism. 4. In mouse models and cell cultures, HSF1 enables oncogenic transformation and maintains malignant	It regulates heat shock protein-72 expression in human keratinocytes exposed to ultraviolet B light to protect against UV induced damage. HSF1 is required for normal growth and survival of melanoma cells under heat shock conditions.	No study has been reported.

			phenotype.		
NANOG	Nanog homeobox	NANOG homeobox family	1. NANOG is involved in the maintenance of stemness (self-renewal, undifferentiated state) and immunomodulation in ESC and adult stem cells. 2. Nanog's expression in cancer cells promotes cancer stem cell characteristics and is positively correlated with tumor maliganancy.	No study has been reported.	No study has been reported.
STAT3	signal transducer and activator of transcription 3	STAT protein family	1. STAT3 is activated by IL-6, IL-11, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M and cardiotropin. 2. Constitutively activated STAT3 is found in a number of epithelial cancers, including including prostate, breast, lung, head and neck, and pancreas, as well as hematopoietic malignancies, such as, lymphomas, leukemias, mucosis fungoides, and multiple myelomas. 3. Ablation of STAT3 leads to apoptosis and inhibition of cell proliferation.	1. In mouse skin, activated STAT3 is found at the wounding edge and is involved in re-epithelization process. 2. Transgenic mice with keratinocyte specific-deletion of STAT3 display markedly reduced wound healing capability and enhanced resistance to chemical-induce carcinogenesis. 3. Various skin tumor promoters can activate STAT3 in mouse epidermis trhough EGFR activation. 4. In human psoriatic patients, activated STAT3 is found in the lesional keratinocytes.	No study has been reported
ZEB1	zinc finger E-box binding homeobox 1	ZEB family	1. ZEB1 is cruicial for neural crest cell migration and formation of derivative structures during embryonic development. 2. ZEB1 is an inducer of epithelial-	1. Estrogen increases ZEB1 expression in a human foreskin fibroblast cell line in vitro. 2. ZEB1 is significantly over expressed in the penile	No study has been reported

			mesenchymal transition and its elevated expression is observed in cancerous tissues 3. ZEB1 is involved in the regulatory loop with mir-200 microRNA family to mediate EMT.	skin of subjects with severe hypospadias.	
SMAD1	SMAD family member 1	SMAD family	1. SMAD1 is a signal transducer of BMP signaling which is involved in cellular processes, such as cell growth, apoptosis, morphogenesis, development and immune responses.	1. SMAD1 mediated BMP signaling plays pivotal roles in the control of cutaneous development and also possesses a potent antitumor activity in postnatal skin. 2. Overexpression of BMP4/6 in murine epidermis is accompanied by increased resistance to chemically induced carcinogenesis. 3.In chemically induced murine epidermal tumors and in human basal cell carcinoma cells, Smad1/5 are strongly down-regulated. 4. Total and phosphorylated Smad1 levels are significantly elevated in systemic sclerosis (SSc) skin biopsy samples and in cultured SSc fibroblasts.	No study has been reported
DLX5	distal-less homeobox 5	homeobox transcription factor **family**	1. Dlx5;Dlx6 double-knockout mice exhibit Split Hand-Foot Malformation phenotype which is observed in patients with p63 mutation. 2. Dlx5;Dlx6 are under positive transcriptional control of	No study has been reported	1. DLX5 mutation is associated with split-hand/split-foot malformation. 2. The chromosomal region containing DLX5/DLX6

			ΔNp63 during embryonic limb development (E10.5 to E12.5).		undergoes a recurrent inversion in T-cell lymphoma from Lck-Akt2 transgenic mice.
EGR3	early growth response 3	EGR family of C2H2-type zinc-finger proteins	1. EGR3 is an immediate-early growth response gene which is induced by mitogenic stimulation. 2. Egr3-deficient animals had gait ataxia (lack muscle spindles), increased frequency of perinatal mortality, scoliosis, resting tremors and ptosis.	No study has been reported	No study has been reported
ESR2	estrogen receptor 2 (ER beta)	Nuclear hormone receptor family	Estrogen receptor mediated transcription plays an important role in human physiology and pathology.	1. ESR2 is the ER subtype in the epidermis. 2. ESR2 is a tumor suppressor in skin and oral epithelium.	No study has been reported

Supplemental Table 7. DNA Oligonucleotide primers used in the study.

GENE	Forward	Reverse
Primers for cloning		
PMx-ERβ	GATTCCGGATCCGCCACCATGGAC TACAAGGACGACGATGACAAGGAT ATAAAAAACTCACCA	GCTGCTGCGGCCGCCTACTG AGACTGTGGGTTCTG
CSII-Dlx5	CACCATGGACTACAAGGACGACGA TGACAAGACAGGAGTGTTTGACAGA	CTAATAGAGTGTCCCGGAGGC
CSII-ERβ	CACCACCATGGACTACAAGGACGAC GATGACAAGGATATAAAAAACTCAC CA	CTACTGAGACTGTGGGTTCTG
Primers for RT-PCR		
NOTCH1	TTGGGAGGAGCAGATTTTTG	CACTGGCATGACACACAACA
NOTCH1 PRIMARY	TTGTCTCCAGGGAAATCGTG	GGCAGTGGCAGATGTAGGAG
HEY1	TCATTTGGAGTGTTGGTGGA	CTCGCACACCATGATCACTT
NOTCH2	GTTTCCAGTGCCTGTGTCCT	CGATACACTTTGCCCCATTC
JAG2	GGAGGTTCTGCGATGAGTGT	GCTGCCACAGTAGTTCAGGT
CSL	CAAAAGTTGCACAGAAGTCATA	TGCTGCATTTCTTGGTCAC
STAT3	AGTTTCTGGCCCCTTGGA	CTTCGTAGATTGTGCTGATAGAGA
HOXA9	AGAACCGCAGGATGAAAATG	GGGTGAGAGAAGGGAGAAGG
PBX1	GAAGCAGGACATTGGAGACA	GGCTCCTCGGATACTCAAAAC
RARA	GAAGAAGGAGGTGCCCAAG	TCTGAGCTGTTGTTCGTAGTGT
EGR3	GATGGCTACAGAGAATGTAATGGA	AGTTGGAAGGGGAGTCGAAG
HSF1	GCAACAGAAAGTCGTCAACAAG	GGCTATACTTGGGCATGGAA
PLAG1	CATCCCTCTCACCACCTTTC	CGCCACCTTGTAACTCCATC
SMAD1	TGCCCTCAGAAATCAACAGA	TGAAACCATCCACCAACACA
NFKB1	ACCAGCCTCTGTGTTTGTCC	TGACGTTTCCTCTGCACTTCT
DLX5	TGAGCGATGACAGGAGTGTT	CTGAGACGGATGGTGCATAG
PURA	CCTATCGCAACTCCATCACC	CCTCTGCTTCTCTTGAATCTTCTT
MZF1	GTGTAAGCCCTCACCTCCAC	TGGGGTCCTGTTCACTCCT
NANOG	GCAAGAACTCTCCAACATCCT	GCGTCACACCATTGCTATTC
ZEB1	TGCACAAGAAGAGCCACAAG	GCGCAAGACAAGTTCAAGG
ESR2	CAGCTAGTGCTCACCCTCCT	ACACCTCCATCCAACAGCTC
YBX1	AAGGAACGGATATGGTTTCA	CCACAGTCTCTCCATCTCCT
IVL	GGCCCTCAGATCGTCTCATA	CACCCTCACCCCATTAAAGA
KRT1	GTTCCAGCGTGAGGTTTGTT	TAAGGCTGGGACAAATCGAC
KRT10	GAAAAGCATGGGCAACTCACA	TGTCGATCTGAAGCAGGATG
36B4	GCAATGTTGCCAGTGTCTGT	GCCTTGACCTTTTCAGCAAG
Primers for ChIP in Fig 4		
DLX5 11.38kb	GGGGCATTTACCCAGCTTT	TCGTGTTGTTCCTTCTGCTC
DLX5 1.9KB	AGCTCCACACGCAGCATAA	CGCAGGGGACAGAACACT
DLX5 0.9KB	GCCGACACCCAATACCTG	TCTCAGCCCCGGTAAGATG
EGR3 7.1KB	GCTGCTGGTATTTCCTCCTC	GTGCCAGTGCAGTTTTCATC
EGR3 1.8KB	CTGCCCTCGCAAAGCAAC	CGGTGAGACCTGCCTGAA

EGR3 0.5KB	CCGCAGAGCCCACACTCC	TTTGGTTTCCTGTTGCTTCTC
EGR3 0.37KB	AGATAAATGGCCCGGAGAAG	GAGTTAGGAGGCCGGTGTG
EGR3 0.21KB	CACACCGGCCTCCTAACTC	CCAGCATGGAGAGGGAAAA
EGR3 -0.06KB	CGCCAAAGTTTCCAAAGG	CCAGCCGGGGAAGAGAGG
EGR3 -6.5KB	AGAGGCGCTGCTGAGTGT	AGGCCAAGAGAAAAGGCAAG
ESR2 12.1KB	GTCGAGGGCAGGACACTT	GTAGGAGGCTGGAGCTTTTG
ESR2 5.1KB	CCTCACACACCACCAAGAGA	CGCTTTAGCTTTGGACAACC
ESR2 4.7KB	GCCCTCTTCAGTACCCCTTG	CGGAGGATGGTTGGTCTCT
ESR2 4.56KB	CCCAGAGACCAACCATCCT	TGGTGCAAAATGCCTTCC
ESR2 1.7KB	TCACACTTCCCGCCATTC	CCCCAGCAACCCATGATAC
ESR2 -30.9KB	TGAGGTGAGGGCAGGAGTAG	TGAGGAGTGGATGAGGGTGT
ESR2 -51.7KB	CACTAGGAGCAGGGCGAGT	AGATGCAGATGGCCTCAGTT
ESR2 -75.5KB	ACCCCGTGGAGACCTGCT	CTGTTCAGAGGCGGGAAA
ESR2 -75.8KB	GCTGTGAACAACCACGTCTC	TGAGGGCAAGACTCCACAC
ESR2 -76.1KB	GCAGCAGATGGTGAAGGAG	TCCTGGGGTAGAGAGGAGGT
Negative Region	TCCTGACTGGGTCTCTCTCC	TTGGCATTTGTCCCTCAAC

Primers for ChIP in Fig 5

E1	GCCTCCTGTGCTACCTGTG	CTCTGAAGGGCTTGAATTGG
E2	TGAAAACTGCACTGGCACAC	GCGTCTAGCTTGCCTTCCT
P	ATCTTACCGGGGCTGAGAAA	GTCTCTGGGGAATCGAGTGA
E3	GCTGCACTCTCTCTTCCCTTT	AACGCTCAGACTTTTCTTGCT
E4	ACTTTCCCCTTCGCTGTCTC	AATCAGCTCACAGTCCCACA
E5	GCGCCTCAGTCTTCTCTCCT	GTTCCTGGTCGTTCCCATTC
E6	AGGGCAGGAGTAGCGAGAAG	GAGGAGTGGATGAGGGTGTG
E7	CAGGGGATGTCGGTGTGT	ACAGTGAAGACTGAAACCAGGAG
E8	TTGACAGCATCTTGGCATC	TCCAGACATGACCTGCATC
E9	GCTGTGAACAACCACGTCTC	GAGGGCAAGACTCCACACC
Negative Region	TCCCACCAGCGTACACTAAA	TCCCTGGTGTCTGAGTGTGA

Supplemental Table 8. Information on antibodies used in the study.

Name	Manufacturer	Cat. No.	Application and Dilution
Notch1	Santa Cruz	sc-6014R	WB: 1/1000, IF: 1/200
Notch1 ICD	Cell Signaling	2421	WB: 1/1000
Egr3	Santa Cruz	sc-191x	WB: 1/1000, IF: 1/100, ChIP: 5ug per million cells
Dlx5	Santa Cruz	sc-18152x	WB: 1/1000, IF: 1/100, ChIP: 2ug per million cells
ERβ	Santa Cruz	sc-8974x	WB: 1/1000, IF: 1/100, ChIP: 5ug per million cells
Keratin 1	Covance	PRB-149P	WB: 1/2000
Keratin 10	Covance	PRB-159P	WB: 1/500
Involucrin	Sigma	Mob270	WB: 1/2000, IF: 1/500
Loricrin	Covance	PRB-145P	IF: 1/500
γ -Tubulin	Sigma	GTU-88	WB: 1/2000
RNA Polymerase II	Santa Cruz	sc-899x	ChIP: 5ug per million cells
H3K4me1	Abcam	ab8895	ChIP: 2ug per million cells
H3K4me3	Abcam	ab8580	ChIP: 2ug per million cells
H3K27ac	Abcam	ab4729	ChIP: 2ug per million cells
H3K9ac	Abcam	Ab4441	ChIP: 2ug per million cells
H3K9me3	Abcam	ab6001	ChIP: 2ug per million cells
H3K27me3	Upstate	07-449	ChIP: 2ug per million cells

Figure 1 original blots scan

E

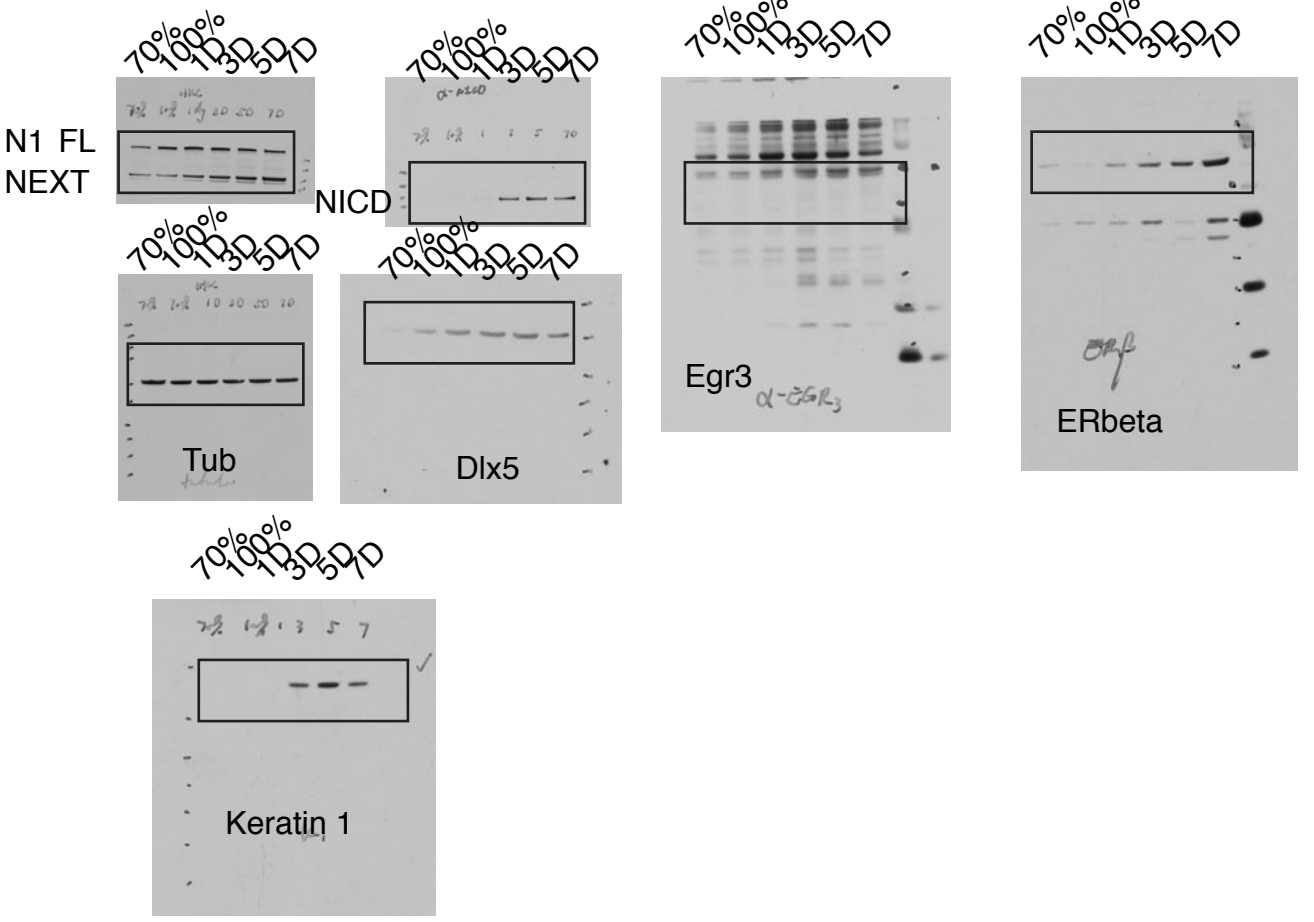


Figure 1 (E) Same amounts of protein lysates were loaded as two parallel sets on a 15-well gel. After transfer, the membrane was cut in half and probed with different antibodies without stripping. One half of the membrane was sequentially blotted for the Notch1 full length (FL) protein, Keratin 1 and Egr3 and the other half for Dlx5, ERß and γ-tubulin.

Figure 4 original blots scan

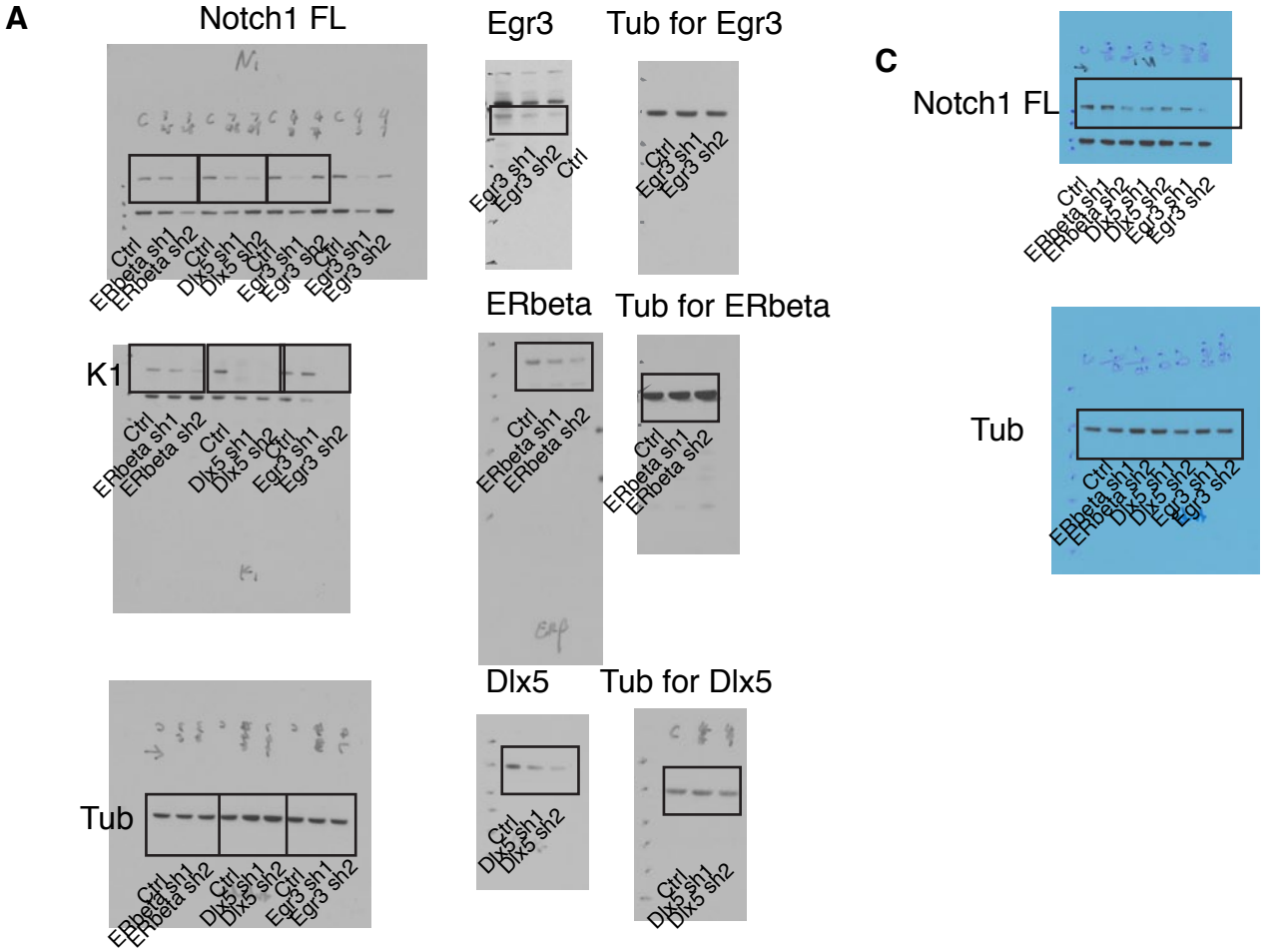


Figure 4 (A) Same amounts of protein lysates were loaded as three parallel sets on a 15-well gel. After transfer, the membrane was cut in three and probed with different antibodies without stripping. First set was probed with Notch ICD, Notch1 FL, Egr3 and Tubulin sequentially. The second set was probed with Notch ICD, Notch1 FL, Dlx5 and Tubulin sequentially. The third set was probed with Notch ICD, Notch1 FL, ERß and Tubulin sequentially.

Figure 5 original blots scan

A

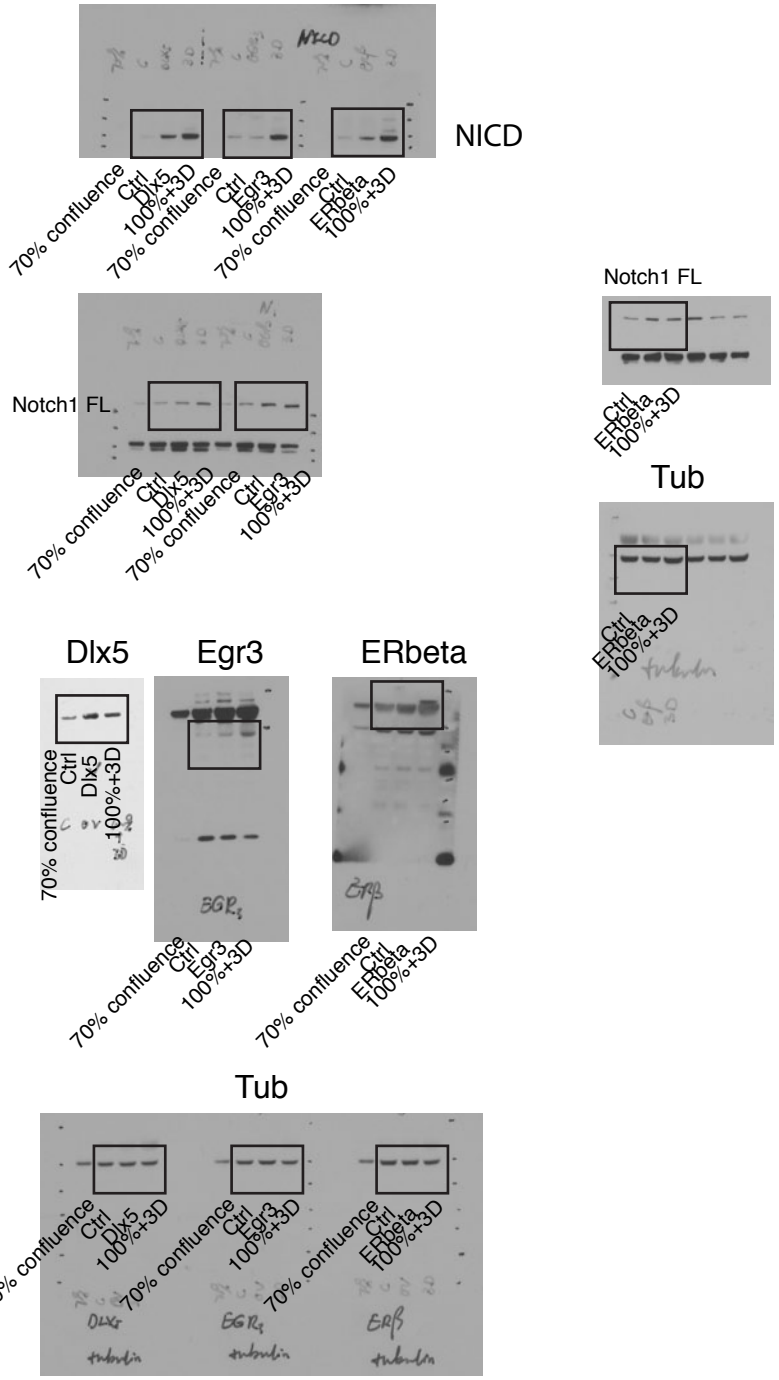


Figure 5 (A) Protein lysates were loaded in one 15 well gel. After transfer, the membrane was cut at 75KD horizontally. The membrane below 75KD was cut in three parts corresponding to Dlx5, Egr3 and ERβ sets, respectively. The upper blot was probed with antibodies for NICD and subsequently Notch1. The lower blots were probed with Dlx5, Egr3 and ERβ antibodies in parallel then reprobed with γ-tubulin for loading control.

Figure 6 original blots scan

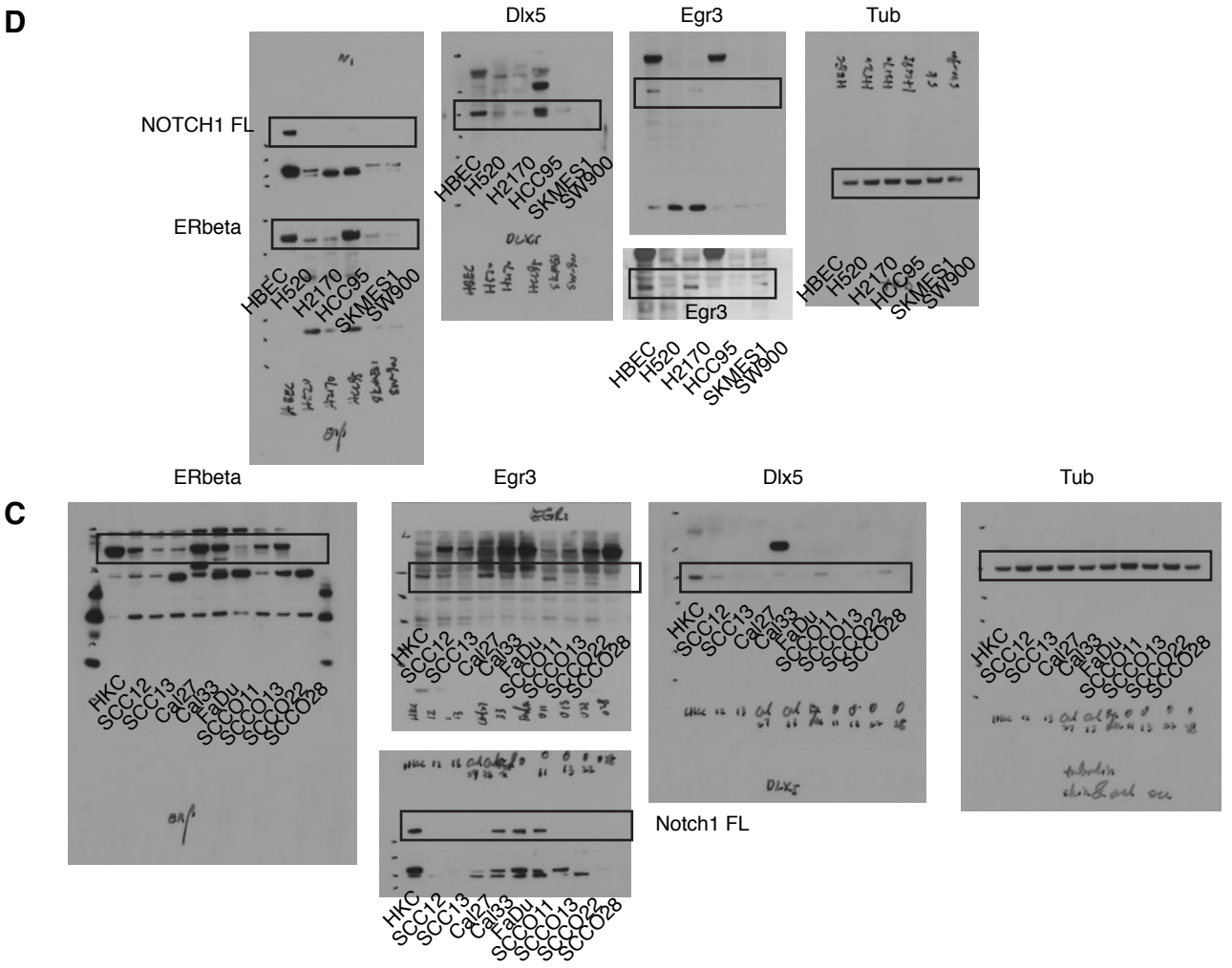


Figure 6 (C) Skin (SCC12 and 13) and H/N (Cal27, Cal33, FaDu, SCCO11, SCCO13, SCCO22 and SCCO28) SCC cell lines were analyzed, in parallel with HKCs under growing conditions (70% confluence) for Notch1, Egr3, Dlx5 and ERß expression by immunoblotting. Same amounts of protein lysates were loaded as parallel sets on two gels. One membrane was blotted for Notch1, Dlx5 and tubulin and the other for ERß and Egr3, in the order. (D) The indicated lung SCC cell lines were analyzed, in parallel with human bronchial epithelial cells (HBEC) for Notch1, Egr3, Dlx5 and ERß expression by immunoblotting. Same amounts of protein lysates were loaded as two parallel sets on a 15-well gel. After transfer, the membrane was cut in half and probed with different antibodies without stripping. One half of the membrane was sequentially blotted for the Notch1 full length (FL) protein and ERß and the other half for Dlx5 and γ-tubulin.

Figure 9 original blots scan

A

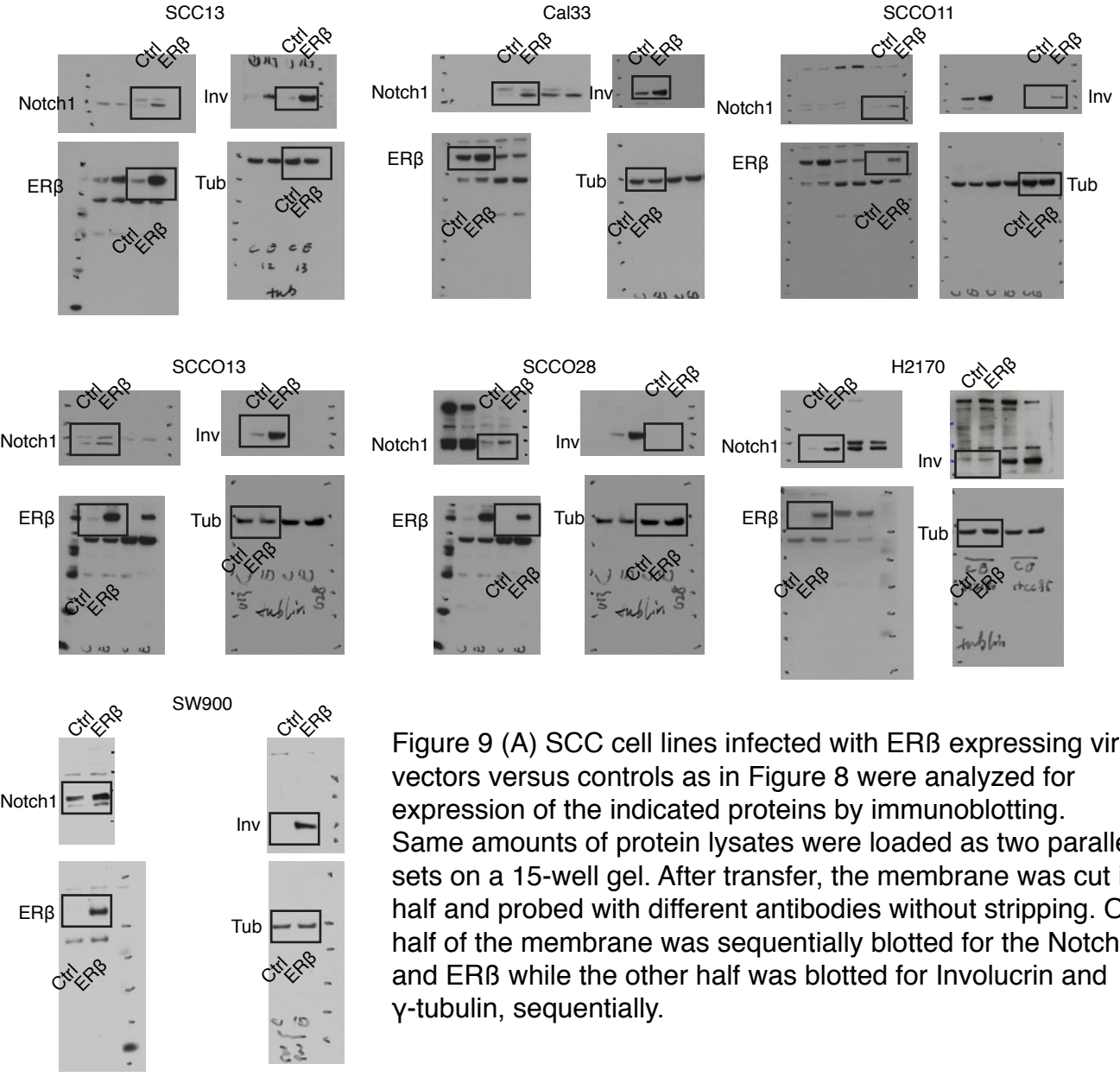
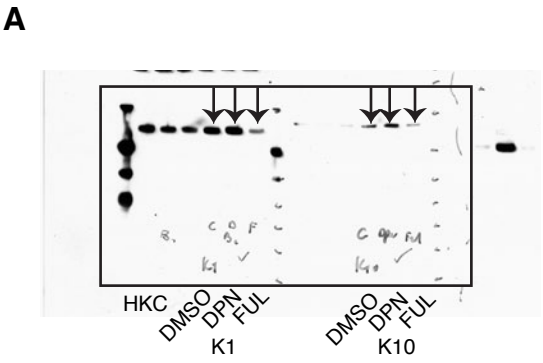


Figure 9 (A) SCC cell lines infected with ERß expressing viral vectors versus controls as in Figure 8 were analyzed for expression of the indicated proteins by immunoblotting. Same amounts of protein lysates were loaded as two parallel sets on a 15-well gel. After transfer, the membrane was cut in half and probed with different antibodies without stripping. One half of the membrane was sequentially blotted for the Notch1 and ERß while the other half was blotted for Involucrin and γ-tubulin, sequentially.

Figure 10 original blots scan



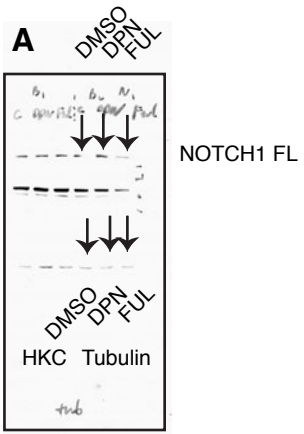


Figure 10 (A) Differentiating HKCs (100% confluence) were treated with 10 nM estradiol (E2), 100 nM ERß specific agonist (DPN), 100 nM ERα specific agonist (PPT), 10 nM estrogen receptor pan-antagonist (fulvestrant) or DMSO control followed, 72 hours later, by RT-qPCR and immunoblot analysis of the indicated genes/proteins (* $p<0.05$). Same amounts of protein lysates were loaded as two parallel sets on a 15-well gel. After transfer, the membrane was cut in half and probed with different antibodies without stripping. One half of the membrane was sequentially blotted for the Notch1 full length (FL) protein and γ-tubulin, while the other half was blotted for keratin 10.(E) The indicated SCC cells were treated with the ERß-specific agonist DPN at the indicated doses for 10 days followed by immunoblot analysis of the indicated proteins. Same amounts of protein lysates were loaded as two parallel sets on a 15-well gel. After transfer, the membrane was cut in half and probed with different antibodies without stripping. One half of the membrane was sequentially blotted for Notch1 full length (FL) and tubulin while the other half was blotted for keratin 10.

Figure 10 Original blots scan

E