Supporting information for

Eda-activated RelB recruits a SWI/SNF (BAF) chromatin remodeling complex and initiates gene transcription in skin appendage formation

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SI Materials and Methods

Plasmids and antibodies. Mouse Edar cDNA (BC068315) was purchased from ATCC and cloned into pEGFP-N1 plasmid (Clontech). Tfg promoter luciferase reporter plasmid (Tfg-luc) was constructed by cloning Tfg promoter (500bp) into pGL4.14 luc plasmid (Promega). Eda-A1-Myc expression plasmid was previously constructed by our lab. Briefly, mouse Eda-A1 cDNA (AF016628) was cloned into pFlag-CMV20 plasmid with an additional c-terminal Myc tag. Flag-tagged NF-kB subunits expression plasmids and BAF250a-V5 expression plasmid were from Addgene.

www.pnas.org/cgi/doi/10.1073/pnas.1800930115

BRG1WT and BRG1Mu (K798R) expression plasmids were provided by Dr. Weidong Wang. Antibodies used in this study are listed in Table S3.

Conditioned medium collection and promoter luciferase reporter assay. Eda-A1 CM was produced as previously described (1). Briefly, HEK293 cells (2×10^8) were transfected with 100 μg Eda-A1 plasmids for 16 h, and then changed to culture in DMEM medium with 1% FBS. After 24 h, medium was concentrated 10-fold using Centricon Plus-10 filters (Millipore) and stored at -80°C until use. We performed the luciferase reporter assays using a dual-luciferase kit (Promega). HaCaT or Kera308 cells (1×10^6/500 μl DMEM) were transiently transfected with 100 ng of *Renilla* luciferase vector (Promega) and 1 μg of pNFkB-luc (Stratagene) or Tfg-luc, using the Nucleofector system (Lonza). After transfection for 24 h, cells were incubated with or without Eda-A1 CM for 16 h, then firefly and *Renilla* luciferase activity was measured according to the manufacturer's instructions. The firefly luciferase activity was normalized to *Renilla* luciferase activity.

Gel filtration, mass spectral analysis, immunoprecipitation (IP), gel silver staining and Western blotting. BAF complex was directly immunopurified with a BAF170 antibody from HaCaT nuclear extract by using an IP protocol as described (2). The superpose 6 gel-filtration analysis, western blotting, immunoprecipitation and Mass spectral analysis has been described (3). Gel silver staining was performed using the SilverQuestTM silver staining kit (Invitrogen).

***In vitro* protein synthesis and protein-protein binding assay.** Proteins were synthesized using the T7 TNT quick coupled transcription/translation kit based on rabbit reticulocyte lysates system (Promega), according to the

manufacturer's instructions. Briefly, five NF-kBs were synthesized using cFlag-tagged expression plasmids (Addgene) as templates. BAF components and Tfg protein were synthesized using cDNA (Addgene or Dharmacon) as templates. T7 promoter and Flag/HA tag were fused into each BAF or Tfg cDNA by PCR, according to the manufacturer's instructions. The PCR primers used were listed in Table S4 and PCR products were purified using a DNA purification kit (Qiagen). The synthesized proteins were purified with a Flag or HA affinity gel (Sigma Aldrich). Protein-protein incubation (1:1) were set up in tubes and rotated at 4°C for overnight and followed by further IP-western blotting experiments.

NF-kB oligonucleotide immunoprecipitation. 200ul Nuclear extracts were diluted into 1ml IP buffer and incubated with 20ul NF-kB mutant or consensus oligonucleotide (oligo) agarose beads (Santa Cruz) at 4°C for overnight rotation. The oligo beads were washed and eluted by following the supplier's protocol.

Mice. All research was conducted according to the guidelines of the Office of Animal Care and Use in the NIH Intramural Program, and all animal study protocols were approved by the NIA Animal Care and Use Committee (ACUC). Skin-specific *BAF250a* knockout mice (BAF250a cKO) were generated by crossing the *BAF250a*$^{loxP/loxP}$ mice (provided by Dr. Zhong Wang) with the *K14-Cre* mice. Tabby mice were purchased from The Jackson Laboratory. Two sets of timed mating were set up. WT C57BL/6J male mice were crossed with Tabby females to get Tabby homozygote and WT progeny. Skin tissues were excised under dissection microscopy and were fixed, cultured or stored at -80°C until use. Livers were used to provide

DNA for genotyping. Genotyping for *BAF250a*, *K14-Cre*, *Tabby* and WT mice was carried out by PCR.

Histology and immunohistochemistry. As a described protocol before (1, 4), eyelids or back skin from mice at each time point were fixed in 10% formaldehyde and embedded in paraffin and 5 μm sections were then cut for H&E staining or immunofluorescence. Images were then collected by DeltaVision microscopy.

Eyelid organotypic culture and MG length measurement. Eyelid skin from embryos at E15.5 was separated and cultured as previously described (1). Briefly, eyelids were dissected on ice under microscopy, after two washes with cold PBS, then were placed on a 0.4 μm Millicell culture insert (Millipore) and cultured with 10% FBS in full DMEM. In cultures, lentiviral particles (1×10^7 GC/ml) expressing LentiCon or shRNAs (Santa Cruz) were added to medium. After 2 days (d) or 4 d, eyelids were fixed in 10% formaldehyde and followed our immunohistochemistry protocol for paraffin-embedded tissue sections. The longest MG germs from serial sections were measured, and the distance between epidermal basal cell edge and the outer most edge of germs was calculated.

Gene expression profiling and qRT-PCR. HaCaT cells (ATCC) were cultured in DMEM plus 10% FBS and transfected with scrambled siRNA or SMARTpooled siRNAs (Dharmacon) aginst hRelB, hBRG1, hTfg or hBAF45d for 48 h. Then total RNA from HaCaT cells were isolated using a RNeasy extraction kit (Qiagen) for microarray gene expression profiling or Quantitative RT-PCR (qPCR). For microarray assays, total RNA quantity and quality was tested using the Agilent Bioanalyzer RNA 6000 Chip (Agilent).

500 ng total RNA was labeled according to the manufacturer's instructions using the Illumina® TotalPrepTM RNA amplification kit (Illumina). A total of 750 ng biotinylated aRNA was hybridized to Illumina HumanHT-12 v4 BeadChips overnight. Following posthybridization rinses, arrays were incubated with streptavidin-conjugated Cy3, and scanned at a resolution of 0.53 μm using an Illumina iScan scanner. Hybridization intensity data was extracted from the scanned images using Illumina BeadStudio GenomeStudio software, V2011.1. Each group of samples were set as triplicates. Hybridization data have been deposited in the Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) with accession number GSE97783. Microarray data were analyzed using DIANE 6.0, a spreadsheet-based microarray analysis program based on the JMP7.0 system. Raw microarray data were subjected to filtering by Z normalization and p-value. Individual genes with *P* value <0.05, *Z* ratio >1.5, and FDR <0.03 were considered significantly changed. qPCR was performed using SYBR Green PCR master mix (Applied Biosystems) and all primers were listed in Table S4.

Promoter analysis and chromatin immunoprecipitation. Core promoter analysis was carried out using a computational program ConTra v2 (http://bioit.dmbr.ugent.be/contrav2/index.php). Chromatin was extracted from cells or skin tissues and immunoprecipitated with a Chromatin-IP kit (Cell Signaling Technology) following the supplier's protocol. Treated DNA was amplified by qPCR. Antibodies used in this study were listed in Table S3, and primers used are listed in Table S4.

DNase sensitivity assay. HaCaT, T47D or SW13 cells were cultured in DMEM plus 10% FBS. The cells were extracted 48 h after transfection with

siRNA, empty vector, or expressing vectors coding BAF250a, BRG1WT, or BRG1Mu. Nuclei isolation and DNase digestion were performed as previously described, with modifications. Nuclei were isolated by incubation for 10 min on ice with 5 ml lysis buffer (20 mM Tris–HCl pH 7.5, 3 mM CaCl2, 2 mM MgCl2, 0.5% Nonidet P-40, 1x protease inhibitor cocktail) followed by Dounce homogenization. Nuclei were collected by centrifugation at 1000*g* for 10 min. Nuclei were washed twice in resuspension buffer (10 mM Tris–HCl pH 7.5, 10 mM NaCl, 3 mM MgCl2) and pelleted by centrifugation at 1000*g* for 10 min. Nuclei were then resuspended in 1 ml resuspension buffer and nuclei were counted. Additional resuspension buffer was used to generate equal concentrations of nuclei between samples. Nuclei were aliquoted into microcentrifuge tubes and incubated at 37°C for 5 min. Different concentration of DNase (1U, 2U and 5U) was added to each of the aliquoted samples with the exception of the undigest control and the samples were incubated at 37°C. Digestion was stopped by addition of an equal volume of stop buffer (20 mM Tris pH 7.5, 200 mM NaCl, 2 mM EDTA, 1% SDS, 200 µg/ml proteinase K). Reaction mixtures were incubated overnight at 37°C, followed by DNA purification using a kit (Qiagen). DNA was measured on a Nanodrop 3000 and equal amounts of DNA were analyzed by qPCR using the appropriate primers (Table S4).

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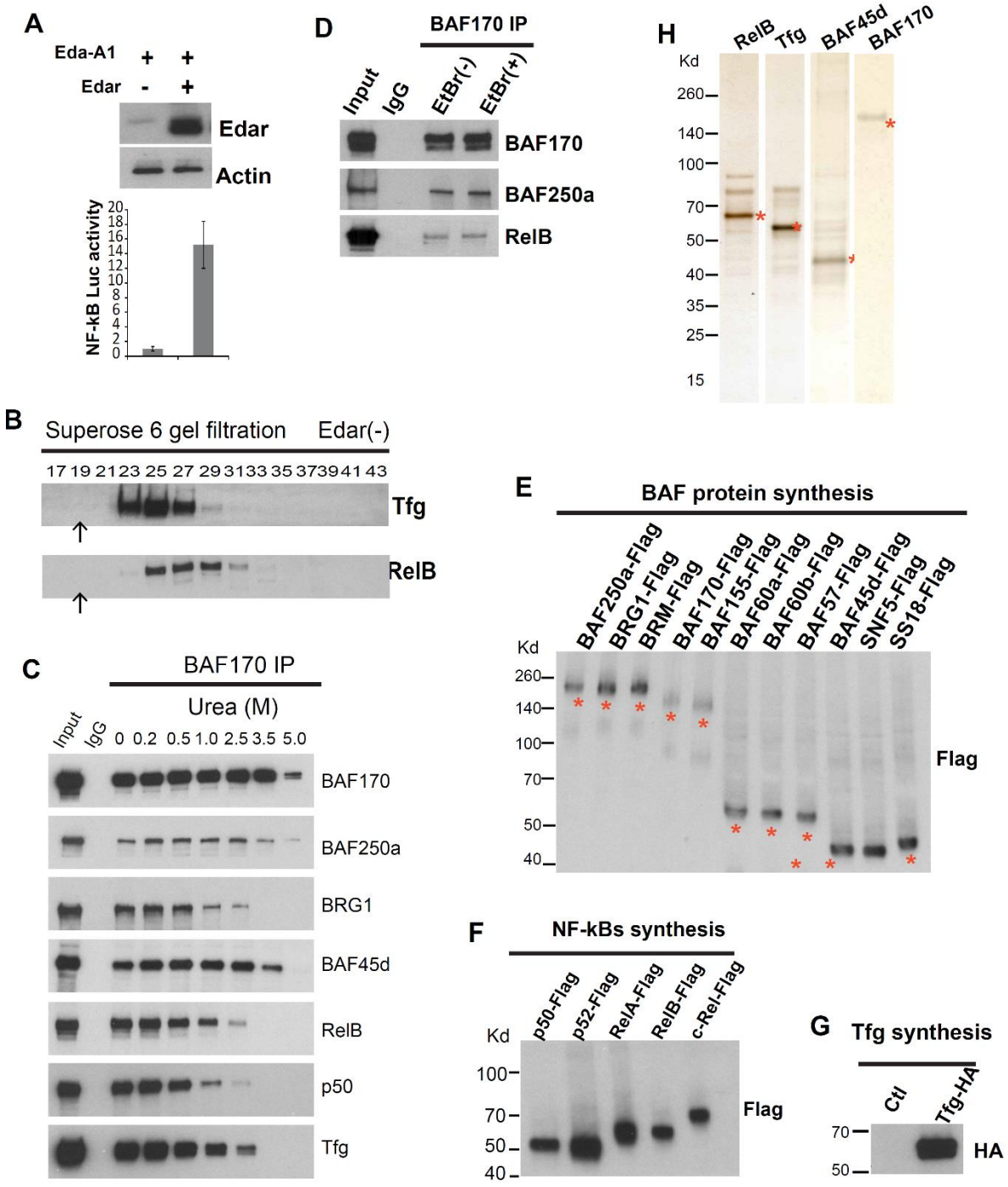

Fig. S1. Identification of the "linker" components in Eda-induced BAF complex. (**A**) Immunoblotting shows the expression of Edar in Hacat cells and indicated treatment. The bar graph shows the parallel NF-kB luciferase activity. NF-kB activity in Edar- cells was normalized to 1. Error bars, mean ± SEM. (**B**) Immunoblotting shows the fully ECL-exposed gel filtration profiles of Tfg and RelB in nuclear extract (NE) from Edar- cells.

Arrows indicate the absence of Tfg and RelB in high molecular weight position. (**C**) Urea denaturation analyses using BAF170 IP shows existence of BAF, RelB/p50 and Tfg in NE (from Edar+ cells) treated with 0-5M urea. (**D**) Immunoblotting shows the presence of BAF250a and RelB in BAF170 IP from the NE of Edar+ cells. The presence (+) or absence (−) of ethidium bromide (EtBr) (100 μg/ml) are indicated. (**E**) Immunoblotting shows the purified BAF proteins from a cell-free transcription /translation system (TNT). Flag-tagged BAF subunits were immunoprecipitated (IP) with anti-Flag affinity gel. Red stars indicate BAF proteins with predicted molecular weight. (**F**) Immunoblotting shows each Flag-tagged NF-kB protein synthesized and purified as described in (**E**). (**G**) Immunoblotting shows HA-tagged Tfg synthesized as described in (**E**) and purified with anti-HA affinity gel. Protein synthesis buffer without cDNA template was loaded as control (Ctl). (**H**) Silver stained gels show immuno-purified RelB, Tfg, BAF45d and BAF170 with expected molecular weight (red star) *in vitro*.

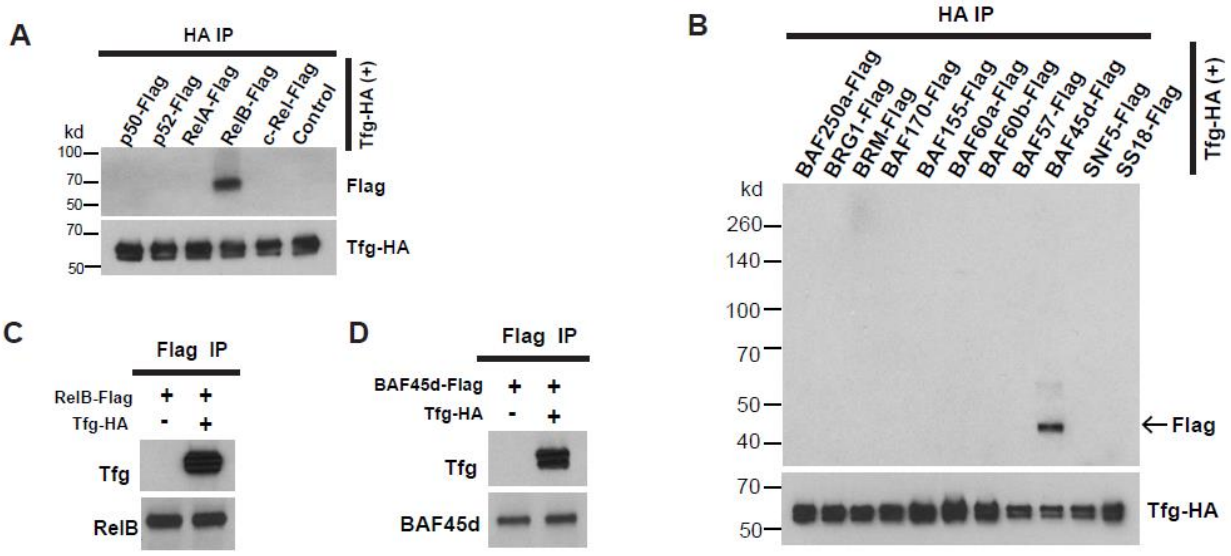

Fig. S2. Tfg directly binds to RelB and BAF45d. (**A**) Immunoblotting shows direct protein binding of Tfg-HA to RelB-Flag in vitro in HA IP. (**B**) Immunoblotting shows that Tfg-HA binds to BAF45d-Flag but not to other BAF proteins in HA IP. Reverse Flag IP confirms the direct Tfg-RelB binding in (**C**) and Tfg-BAF45d binding in (**D**).

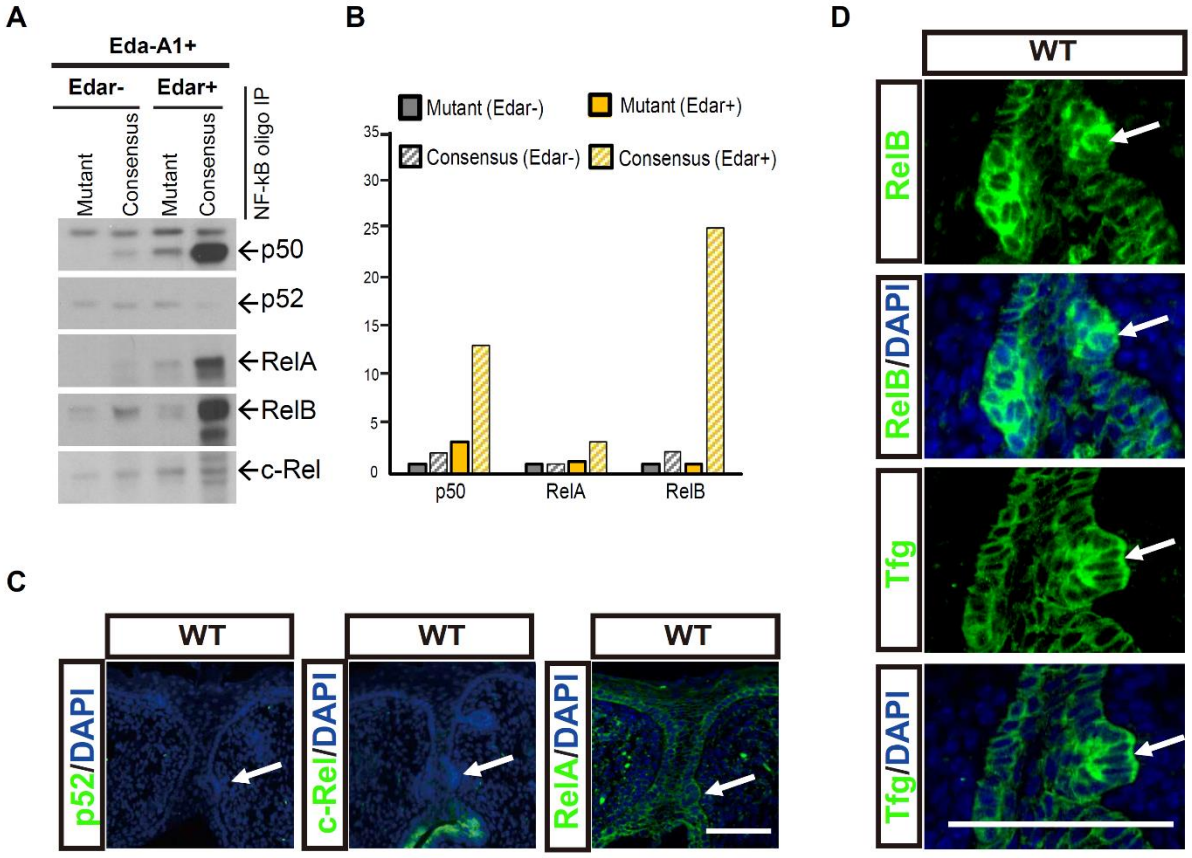

Fig. S3. Activation pattern of NF-kB subunits upon Eda signaling. (**A**) Immunoblotting shows the presence of NF-kB subunits in the NF-kB oligonucleotides (oligo) IP contents of mutant or consensus of NF-kB binding oligos. NE from Edar- or Edar+ cells were used for oligo IP. (**B**) The measured density of protein bands in (**A**). The band density in each first lane was normalized to 1. (**C**) IHC staining (green) of p52, c-Rel and RelA of wild type (WT) eyelids at E16.5 are shown. (**D**) IHC staining (green) of RelB and Tfg of WT eyelids at E16.5. Arrows indicate MG germs. Tissue counterstained with DAPI (blue). Scale bar, 50 μm.

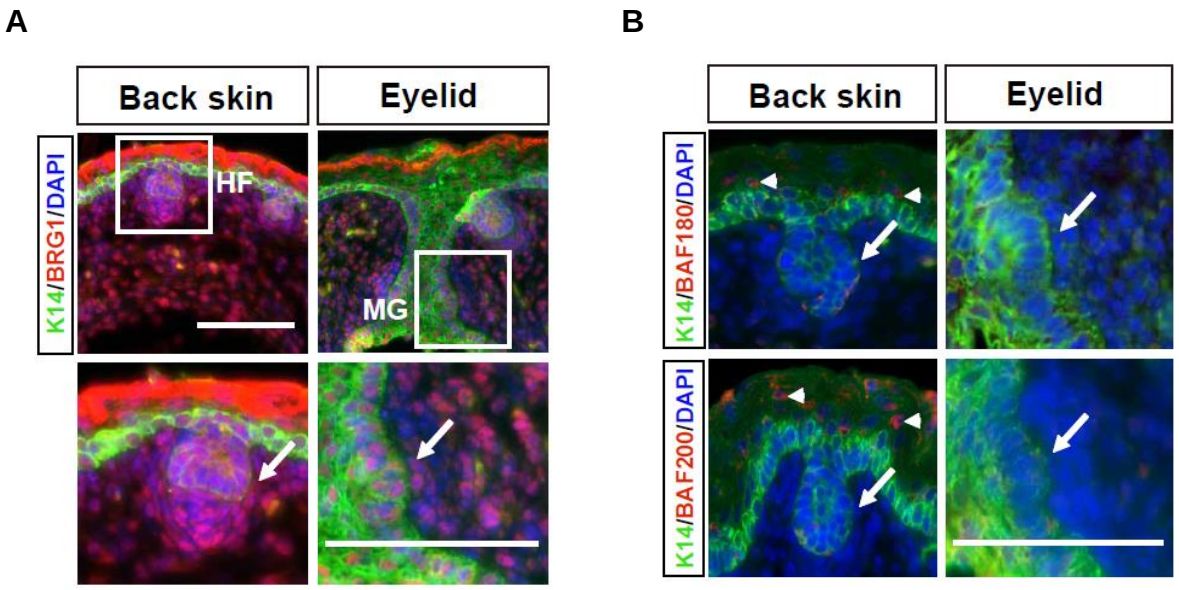

Fig. S4. The expression pattern of BRG1, BAF180 and BAF200 in skin appendages. (**A**) Immunohistochemistry (IHC) of K14 (green) and BRG1 (red) in back skin (left) and eyelid (right) from wild type (WT) mice at E16.5. Enlarged images of hair follicle (HF) and Meibomian gland (MG) are shown in lower panels. (**B**) As described in lower panels of (**A**), IHC images of PBAF components BAF180 (upper) and BAF200 (lower) are shown. Arrows indicate HFs (left) or MGs (right). Arrowheads indicate cells with positive staining of red signal. Scale bar, 50 μm.

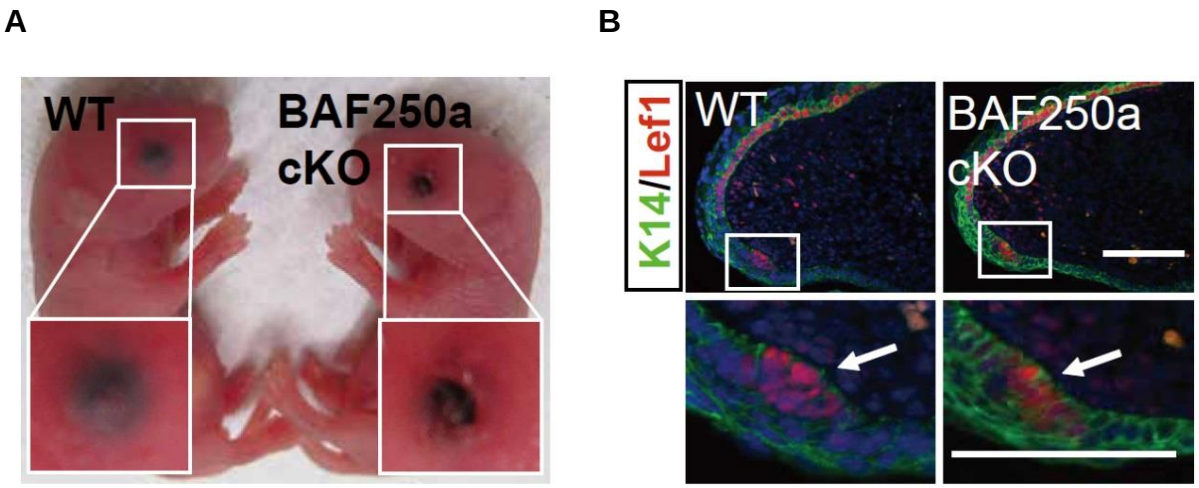

Fig. S5. Eye-open at birth phenotype in BAF250a cKO does not affect MG induction. (**A**) Photograph of P0 mouse of WT and BAF250a cKO. Images of eyelids in boxes are amplified. (**B**) K14 (green) and Lef1 (red) IHC staining of WT and BAF250a cKO eyelids at E15.5.

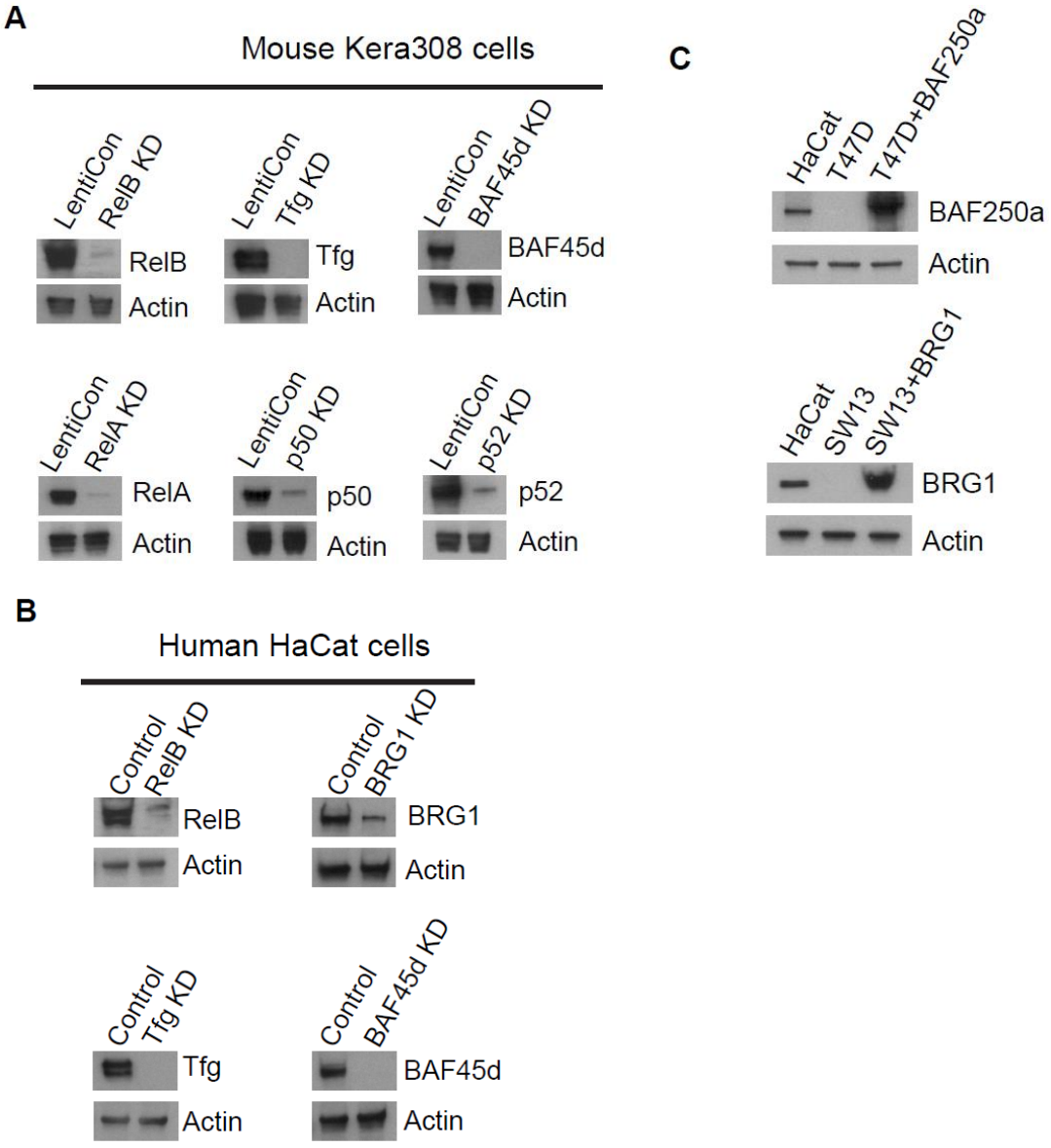

Fig. S6. Knockdown and overexpression of NF-kBs and BAF components. (**A**) Immunoblotting shows the efficacy of shRNA against NF-kBs, Tfg or BAF45d in mouse kera308 cells. LentiCon indicates lentivirus coding a scrambled shRNA. Actin as a loading control. (**B**) Immunoblotting shows the efficacy of siRNA against human RelB, BRG1, Tfg and BAF45d respectively. (**C**) Immunoblotting shows the expression of BAF250a or BRG1 in each indicated cell line with or without plasmid transfection.

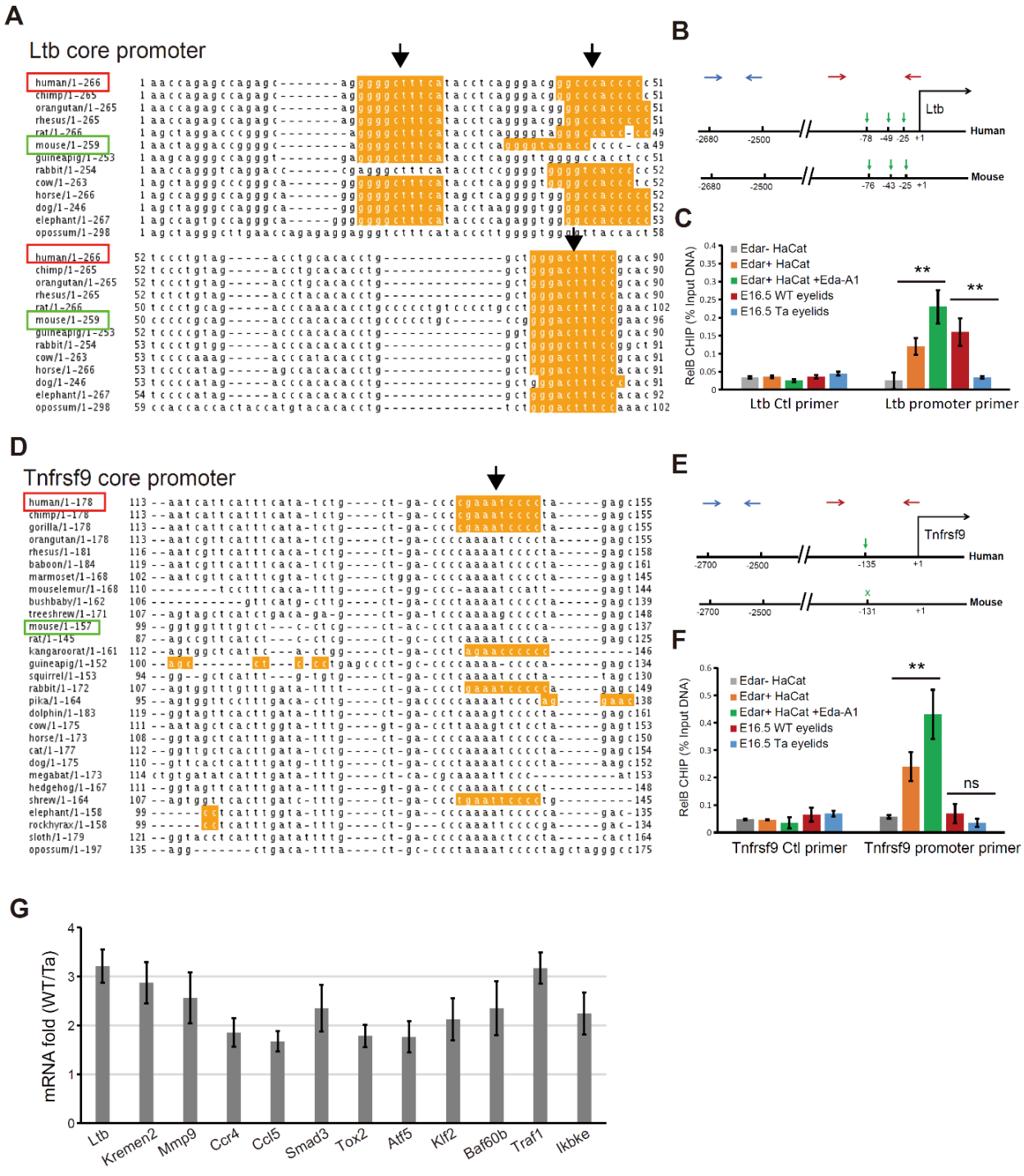

Fig. S7. Differential species binding capacity of NF-kB in the promoters of Eda targets. (**A**) Sequence alignment of NF-kB binding sites in the core promoter of Ltb across species**.** Yellow marks predicted high-scoring NF-kB binding sites. Black arrows indicate the positions of NF-kB binding sites. Red rectangle, human; green rectangle, mouse. (**B**)

Schematic diagram of Ltb promoter in human and mouse. Green arrows indicate the position of NF-kB binding site. Red arrows indicate the position of promoter primers designed for Chromatin IP (CHIP)-qPCR. Blue arrows indicate the position of control primers for CHIP-qPCR. (**C**) CHIP-qPCR assays show Ltb promoter binding activity of RelB in empty, Edar, Edar+Eda-A1 treated human HaCaT cells or WT/Ta mouse eyelids. A RelB antibody was used for CHIP. (**D**) Sequence alignment of Tnfrsf9 core promoter as described in (**A**). (**E**) Schematic diagram of Tnfrsf9 promoter as described in (**b**), except green cross indicates loss of NF-kB binding site in the promoter of mouse Tnfrsf9. (**F**) CHIP-qPCR assays show Tnfrsf9 promoter binding activity of RelB in empty, Edar, Edar+Eda-A1 treated human HaCaT cells or WT/Ta mouse eyelids. (**G**) qPCR assays show the mRNA fold (WT/Ta) of indicated genes in eyelids at E16.5. 2% chromatin DNA was used for qPCR as input. Error bars, mean ± SEM for triplicates. $^{**}P \leq 0.01$ by Student's *t*-test. ns, no significant change.

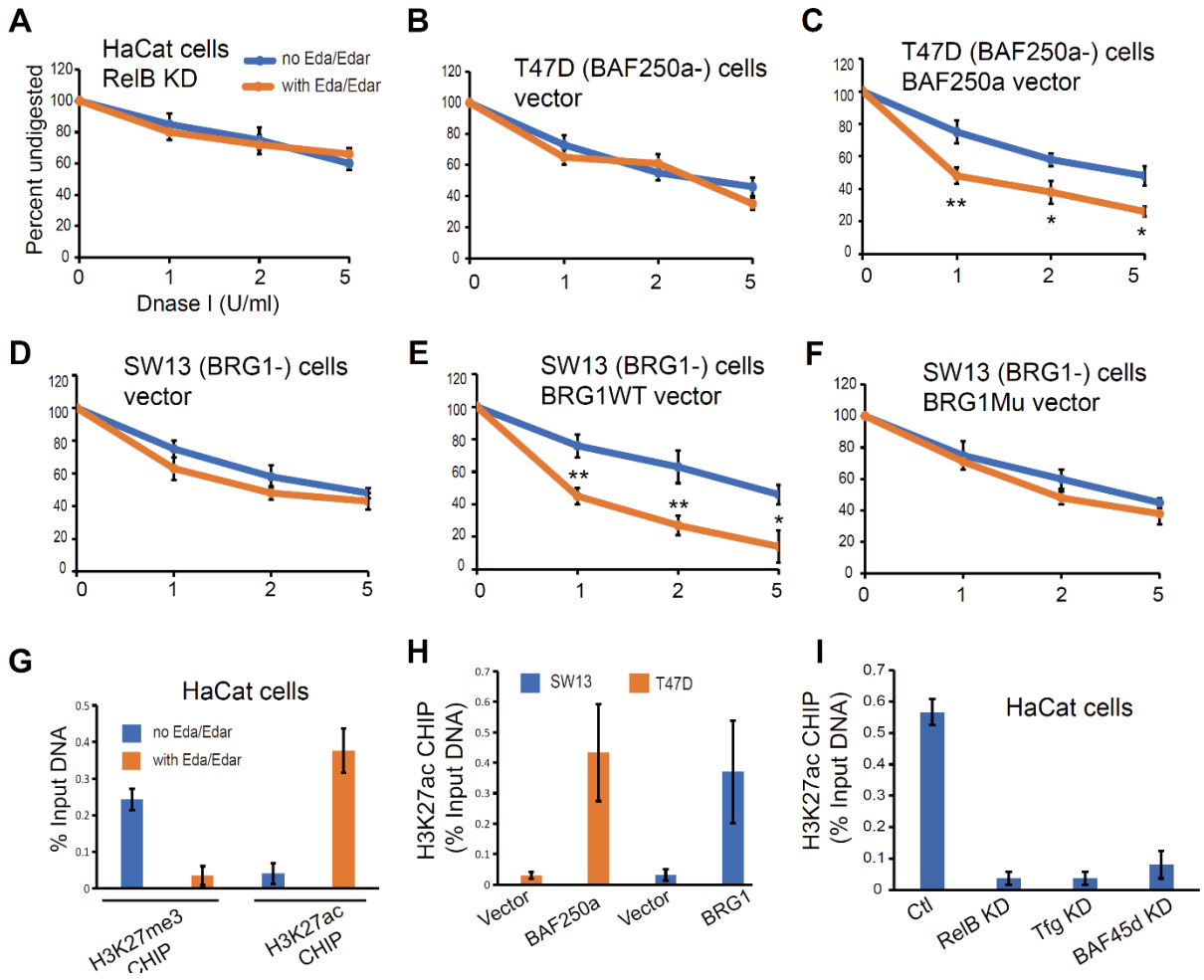

Fig. S8. Chromatin remodeling of Ltb gene requires both Eda signaling and BAF complex. (**A**) Chromatin were extracted from Edar- or Edar+ cells in addition to Eda CM. Extracted chromatin was treated with DNase I (from 0 to 5 U/ml). Undigested DNA was amplified by q-PCR using primers targeting Ltb loci. Signals for DNase-untreated chromatin are normalized to 100%.Subsequent panels show data generated following the same experiment procedure but starting with different cell types or treatments: (**B**) T47D cells pre-transfected with an empty vector; (**C**) T47D cells pre-transfected with a BAF250a expression vector; (**D**) SW13 cells pre-transfected with an empty vector; (**E**) SW13 cells pre-transfected with a BRG1-WT expression vector; (**F**) SW13 cells pre-transfected with a BRG1-Mu expression vector; (**G**) CHIP-qPCR assays show Ltb promoter binding activity of H3K27me3 and H3K27ac. Chromatin from HaCat cells were used for each CHIP by antibodies against H3K27me3 or H3K27ac. (**H**) CHIP-qPCR assays show Ltb

promoter binding activity of H3K27ac in T47D or SW13 cells treated with Eda/Edar. Cells were pre-transfected with indicated vector respectively. (**I**) CHIP-qPCR assays show Ltb promoter binding activity of H3K27ac in HaCat cells treated with Eda/Edar, pre-transfected with scrambled siRNA (Ctl), or KD of RelB, Tfg or BAF45d respectively. Error bars, mean ± SEM for triplicates. *$P \leq 0.05$, **$P \leq 0.01$ by Student's *t*-test.

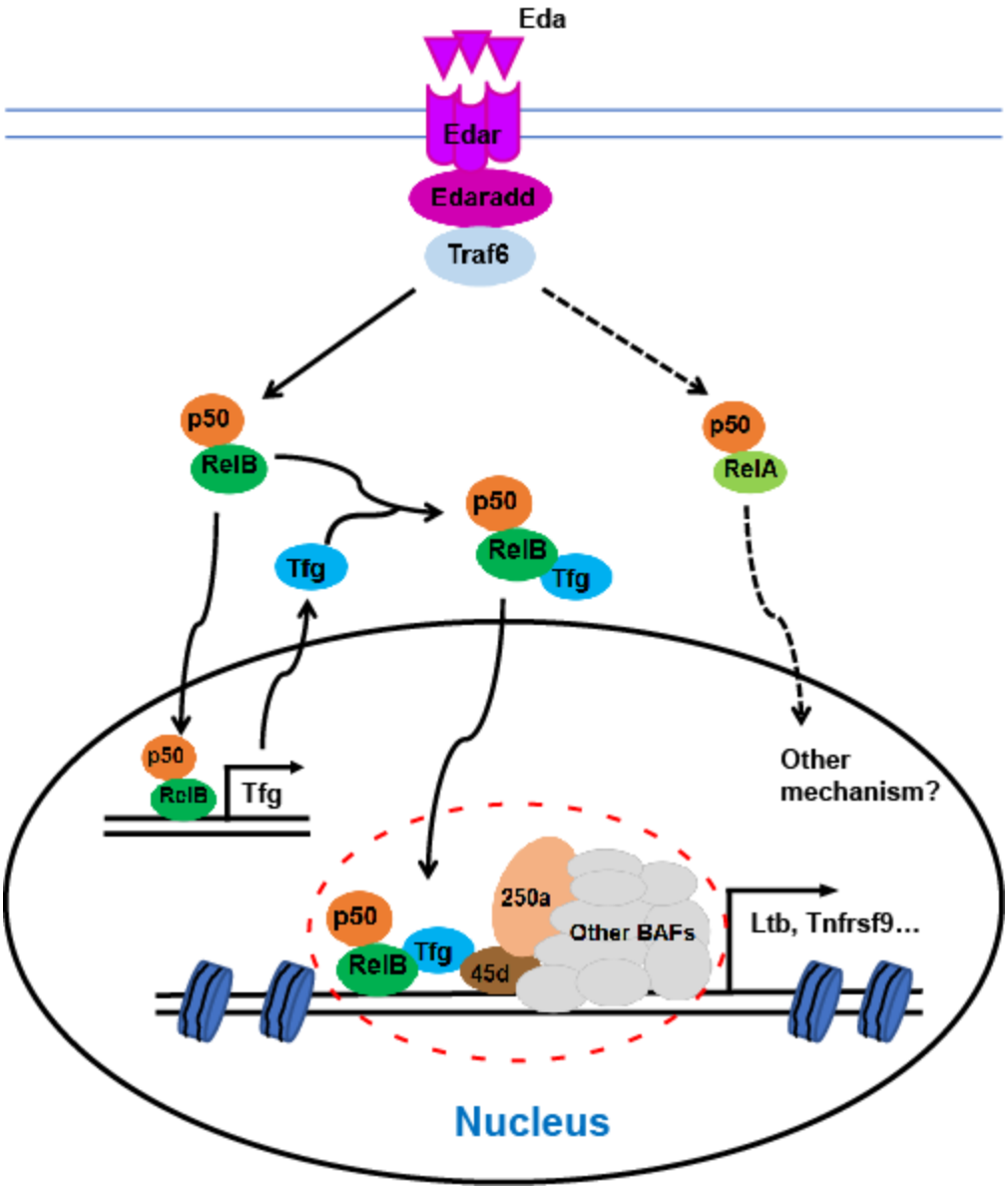

Fig. S9. Schematic model of gene regulation coordinated by Eda signaling and BAF complex during skin appendage development.

Table S1: Components of BAF complexes purified from HaCat cells as determined by mass spectrometric analysis

BAF protein	BAF170 immunoprecipitate				RelB immunoprecipitate			
	Eda- cells		Edar+ cells		Eda- cells		Edar+ cells	
	#pept total(unique)	%cov	#pept	%cov	#pept	%cov	#pept	%cov
BAF250a (Arid1a)	109(54)	22.4	230(97)	45.1	0	0	0	0
BRG1 (Smarca4)	42(30)	27.9	111(45)	38.1	0	0	4(2)	3.6
BRM (Smarca2)	67(42)	27.3	136(71)	44.3	0	0	3(3)	1.9
BAF170 (Smarcc2)	229(64)	38.6	213(61)	49.0	0	0	12(12)	11.4
BAF155 (Smarcc1)	65(26)	21.6	150(35)	31.3	0	0	1(1)	0.8
BAF60a (Smarcd1)	34(23)	43.7	51(32)	55.1	0	0	1(1)	2.5
BAF60b (Smarcd2)	46(21)	39.7	61(28)	51.9	0	0	1(1)	3.8
BAF57 (Smarce1)	70(28)	74.9	61(31)	75.4	0	0	2(2)	5.6
BAF45d (Dpf2)	19(12)	34.8	20(11)	30.1	0	0	1(1)	3.8
SNF5 (Smarcb1)	20(13)	30.9	91(21)	46.7	0	0	1(1)	3.4
SS18 (SS18)	2(2)	2.8	4(2)	2.8	0	0	1(1)	2.9
BAF180 (PBRM1)	30(26)	16.8	117(67)	38.7	0	0	0	0
BAF200 (Arid2)	20(18)	15.1	96(49)	32.8	0	0	0	0
NF-kBs								
P50	0	0	6(6)	8.9	7(6)	7.8	6(6)	7.6
P52	0	0	3(2)	5.8	57(32)	38.6	41(28)	36.5
RelA	0	0	2(2)	5.4	4(4)	8.2	2(2)	4.1
RelB	0	0	23(18)	40.2	16(15)	25.6	20(16)	33.5
c-Rel	0	0	0	0	0	0	0	0
Tfg protein								
Tfg	0	0	7(6)	26.0	0	0	7(7)	19.5

Table S2: List of selected genes regulated by Eda, RelB and BRG1 from the microarrays

Selected genes from total 314 genes regulated by Eda, RelB and BRG1 (Fig. 5A) are listed. Separated classes of genes are shown according to gene function.

	Gene	Z-score
Known Eda target	Ltb	3.87
	Kremen2	2.56
	Mmp9	5.21
Chemokine and Cell migration	Ccr4	8.67
	Ccl5	4.87
	Smad3	6.09
	Elmo1	5.73
TFs and chromatin binding	Tox2	8.12
	Atf5	7.00
	Klf2	6.24
	BAF60b	3.01
TNF signaling	Traf1	3.46
	Ikbke	2.28
	Tnfrsf9	2.03
	Tnfrsf14	1.62

Table S3: Antibodies used in this study

Antibody	Vendor
Anti-GFP (1:1000)	Invitrogen (# A11122)
Anti-Actin (1:1000)	Bethyl Lab (#A300-491A)
Anti-BAF250a (WB: 1:1000; IHC: 1:500; CHIP: 1:500)	Cell signaling Tech (#12354)
Anti-BRG1 (WB: 1:1000; IHC: 1:500)	Bethyl Lab (A303-877A)

Anti-BAF170 (WB: 1:3000; IP: 1:1000)	Cell signaling Tech (#12760)
Anti-BAF180 (WB: 1:1000; IHC: 1:250)	Bethyl Lab (A301-590A)
Anti-BAF200 (WB: 1:1000; IHC: 1:250)	Novus Biologicals (NBP1-615)
Anti-RelB (WB: 1:500; IHC: 1:250; IP: 1:250)	Santa cruz (sc-226)
Anti-Tfg (WB: 1:3000; IHC: 1:500; CHIP: 1:500)	Bethyl Lab (A302-341A)
Anti-Flag (1:1000)	Sigma-Aldrich (F1084)
Anti-HA (1:1000)	Cell signaling Tech (3724)
Anti-Flag affinity gel	Sigma-Aldrich (A2220)
Anti-HA affinity gel	Cell signaling Tech (3956)
Anti-Lef1 (1:200)	Cell signaling Tech (2230)
Anti-K14 (1:2000)	Progen biotechnik (GP-K14)
Anti-p50 (WB: 1:1000; IHC: 1:500; CHIP: 1:500)	Cell signaling Tech (13586)
Anti-p52 (WB: 1:500; IHC: 1:250)	Cell signaling Tech (4882)
Anti-RelA (WB: 1:1000; IHC: 1:500)	Cell signaling Tech (8242)
Anti-c-Rel (WB: 1:500; IHC: 1:250)	Santa cruz (sc-70)
Anti-Histone H3 (1:3000)	Cell signaling Tech (4499)
Anti-BAF45d (WB: 1:1000; IHC: 1:500; CHIP: 1:500)	Abcam (ab134942)
Anti-H3K27me3 (CHIP: 1:500)	Cell signaling Tech (#9733)
Anti-H3K27ac (CHIP: 1:500)	Cell signaling Tech (#8173)
Anti-Ring1b (CHIP: 1:500)	Cell signaling Tech (#5694)
Anti-Ezh2 (CHIP: 1:500)	Active Motif (39875)

Table S4: PCR primers used in this study

Primer name	DNA sequence (5'-3')
Edar-cloning F	aactctcgagatggcccacgtcggggactgc aa
Edar-cloning R	aattaagcttggacgcagctgggggtgggga
BAF250a-TNT F	ccgtcatagttaatacgactcactatagggga ctaaccaccatggccgcgcaggtcgcccccg ccgccgccag
BAF250a-TNT R	ttttttttttttttttttttttttttttttcacttgtcgtcatcgt ctttgtagtctgactggccaatcaaaaacagta catcacaaat
BRG1-TNT F	ccgtcatagttaatacgactcactatagggga ctaaccaccatgtccactccagacccaccct gggc
BRG1-TNT R	ttttttttttttttttttttttttttttttcacttgtcgtcatcgt ctttgtagtcgtcttcttcgctgccacttcctgagc gg
BRM-TNT F	ccgtcatagttaatacgactcactatagggg ctaaccaccatgtccacgcccacagaccctg gtgcga
BRM-TNT R	Ttttttttttttttttttttttttttttttcacttgtcgtcatcgt ctttgtagtcctcatcatccgtcccacttccttctg
BAF170-TNT F	ccgtcatagttaatacgactcactatagggga ctaaccaccatggcggtgcggaagaaggac ggcggcccca
BAF170-TNT R	ttttttttttttttttttttttttttttttcacttgtcgtcatcgt ctttgtagtcctgtggaggtggcacaggggtga
BAF155-TNT F	ccgtcatagttaatacgactcactatagggg ctaaccaccatggccgcagcggcgggcggc ggcg
BAF155-TNT R	ttttttttttttttttttttttttttttttcacttgtcgtcatcgt ctttgtagtcaggagcagctgaggctggcggg c
BAF60a-TNT F	ccgtcatagttaatacgactcactatagggga ctaaccaccatggcggcccgggcgggtttcc agtc
BAF60a-TNT R	ttttttttttttttttttttttttttttttctacttgtcgtcatcgt ctttgtagtctgtattccggattcccagggcttgc tc
BAF60b-TNT F	ccgtcatagttaatacgactcactatagggga ctaaccaccatgtcgggccgaggcgcgggc gg

BAF60b-TNT R	ttttttttttttttttttttttttttttttttacttgtcgtcatcgt ctttgtagtcggtcaggcgaattcccagcacct gt
BAF57-TNT F	ccgtcatagttaatacgactcactatagggggg ctaaccaccatgtcaaaaagaccatcttatgc ccc
BAF57-TNT R	ttttttttttttttttttttttttttttttttacttgtcgtcatcgt ctttgtagtcttctttttctcatcttctggtatggg
BAF45d-TNT F	ccgtcatagttaatacgactcactatagggggg ctaaccaccatggcggctgtggtggagaatgt agtg
BAF45d-TNT R	ttttttttttttttttttttttttttttttttacttgtcgtcatcgt ctttgtagtcagaggagttctggttctggtagat gg
SNF5-TNT F	ccgtcatagttaatacgactcactatagggggg ctaaccaccatgatgatgatggcgctgagca agacc
SNF5-TNT R	Tttttttttttttttttttttttttttttttacttgtcgtcatcgt ctttgtagtcccaggccggggccgtgttggcaag
SS18-TNT F	ccgtcatagttaatacgactcactatagggggg ctaaccaccatgtctgtggctttcgcggccccg aggca
SS18-TNT R	ttttttttttttttttttttttttttttttttacttgtcgtcatcgt ctttgtagtcctgctggtaatttccatactgtccct ggtcat
Tfg-TNT F	ccgtcatagttaatacgactcactatagggggg ctaaccaccatgaacggacagttggatctaa gtgggaagcta
Tfg-TNT R	ttttttttttttttttttttttttttttttttaagcgtagtctgg gacgtcgtatgggtatcgataaccaggtccag gttgggt
hLtb-qPCR F	cctatcactgtcctggctg
hLtb-qPCR R	ctgagatctgtttctggctcc
hKremen2-qPCR F	acaacttctgccgtaaccc
hKremen2-qPCR R	agtccacaaagcatcccag
hMmp9-qPCR F	cgaactttgacagcgacaag
hMmp9-qPCR R	cactgaggaatgatctaagccc
hCcr4-qPCR F	tgtgctctgccaatactgtg
hCcr4-qPCR R	gtggaaagttcattgactctgc
hCcl5-qPCR F	tgcccacatcaaggagtatttc
hCcl5-qPCR R	ccatcctagctcatctccaaag

hSmad3-qPCR F	tccatccccgaaaacactaac
hSmad3-qPCR R	catcttcactcaggtagccag
hElmo1-qPCR F	tgggatacattttcggtggc
hElmo1-qPCR R	ctggtagaggtcatggctattg
hTox2-qPCR F	caagtttgatggtgacagtgc
hTox2-qPCR R	gaggttgggaggtgttatcg
hAtf5-qPCR F	gctcgtagactatgggaaactc
hAtf5-qPCR R	aatcaactcgctcagtcatcc
hKlf2-qPCR F	cctacaccaagagttcgcatc
hKlf2-qPCR R	tgtgctttcggtagtggc
hBAF60b-qPCR F	tccattccgaaaacgcctg
hBAF60b-qPCR R	ccatgtatgcctgagactctg
hTraf1-qPCR F	aagatcaccaatgtcaccagg
hTraf1-qPCR R	gccatctccattcaggtacag
hIkbke-qPCR F	aaaatatcacggagacccagg
hIkbke-qPCR R	catcttgtccaaacagcactg
hTnfrsf9-qPCR F	actgcgttgctcttcctg
hTnfrsf9-qPCR R	cagccatcttcctcttgagtag
hTnfrsf14-qPCR F	catcgtcattgtttgctccac
hTnfrsf14-qPCR R	ctcctgtcttttccgctgg
mLtb-qPCR F	ctgcggattctacaccagatc
mLtb-qPCR R	ttgcccactcatccaagc
mTnfrsf9-qPCR F	cctgtgataactgtcagcctg
mTnfrsf9-qPCR R	tcttgaacctgaaatagcctgc
hLtb-CHIP F	aaccagagccagagc
hLtb-CHIP R	gtaaacaggaagctgggtga
mLtb-CHIP F	aactaggaccggggc
mLtb-CHIP R	ggggttgggtggctggatg
hLtb control-CHIP F	taaatgaaagaagcaagttgc
hLtb control-CHIP R	agttgaaattttcttttaaaga
mLtb control-CHIP F	tgggtcccaaccccaccacac
mLtb control-CHIP R	agtccctcatgttgtgctgac
hTnfrsf9-CHIP F	tagagagctgcttccaagaa

hTnfrsf9-CHIP R	ggagtgcggtgactcagcca
hTnfrsf9 control-CHIP F	tggccctccatatccatgttacat
hTnfrsf9 control-CHIP R	tttatcatctcacttctccataa
mTnfrsf9-CHIP F	tgataagcgaccaagtctgt
mTnfrsf9-CHIP R	acaggacctgtgactcaactga
mTnfrsf9 control-CHIP F	tcctgattgtcttgttctttatt
mTnfrsf9 control-CHIP R	ttaccactgcacgggggtggg