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Supplemental Data

Ezh2 Orchestrates Gene Expression for the Stepwise Differentiation of Tissue-Specific Stem Cells

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Supplemental Figures

Supplementary Figure 1 **Ezhkova et al**

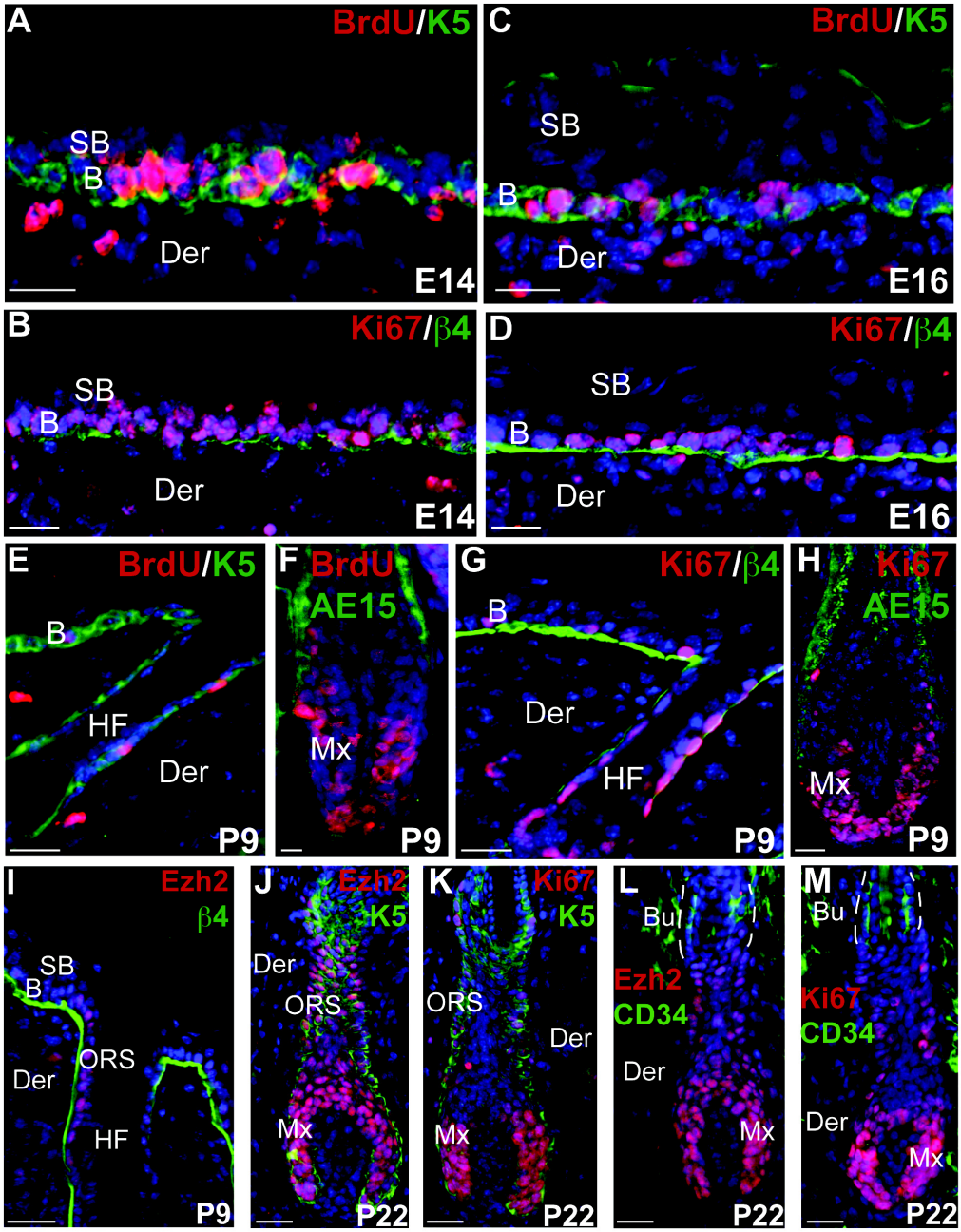

Figure S1. Ezh2 Colocalizes with Highly Proliferative Cells in the Epidermis

(**A** and **B**) Both basal (B) and suprabasal (SB) cells are proliferative at E14 as shown by immunofluorescent microscopy using BRDU and Ki67 antibodies. (**A** to **H**) Proliferation of basal cells wanes as epidermis matures as shown by immunofluorescent staining

with BRDU and Ki67 antibodies at indicated ages. Matrix (Mx) and outer root sheath (ORS) of hair follicle (HF) contain proliferative cells. (**I** to **M**) Ezh2 is maintained postnatally in the proliferative (Ki67+) cells of the hair follicle ORS and Mx, but not the quiescent follicle stem cells within the CD34(+) bulge. Thus, in both the developing epidermis and hair follicle, Ezh2 correlated with an undifferentiated, proliferative state. Abbreviations are as follows: Der, dermis; K5, keratin 5; β4, β4 integrin. Scale bar is 30μm.

Supplementary Figure 2 **Ezhkova et al**

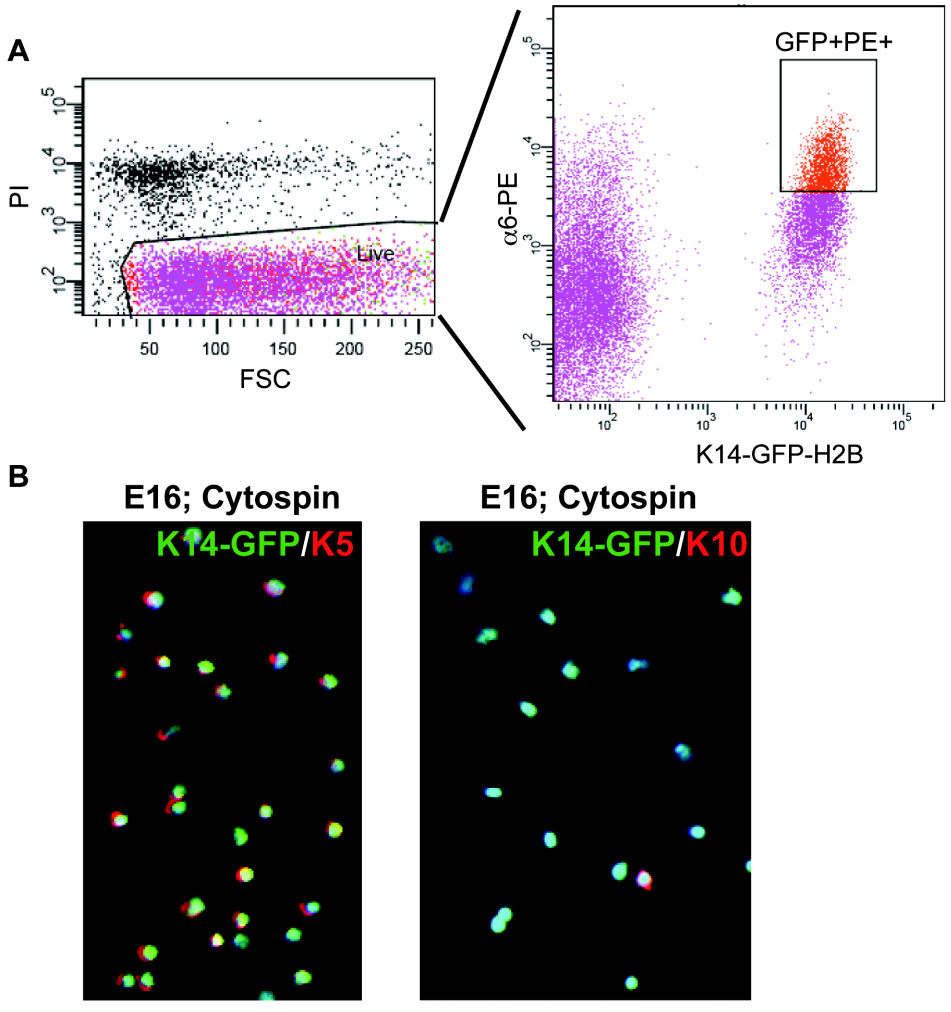

Figure S2. Scheme of Purification of Basal Cells for *In Vivo* ChIP Analysis

(**A**) Scheme of FACS purification of basal layer (BL) cells from E16 embryos that express GFP-tagged histone H2B under promoter of basal layer *keratin 14* gene. Cells were stained with propidium iodide (PI) to exclude dead cells (left) and with antibodies against α6 integrin (α6) conjugated to fluorescent dye PE (right). Gating was done on α6 integrin positive and K14-GFP positive basal cells. (**B**) Immunofluorescent stainings on cytospins of FACS isolated basal cells with basal Keratin 5 (K5) and spinous Keratin 10 (K10) layer markers confirms purity of a sorted population. A few basal cells that initiated program of terminal differentiation, but did not downregulate basal markers are K14-GFP and K10 positive.

Supplementary Figure 3 **Ezhkova et al**

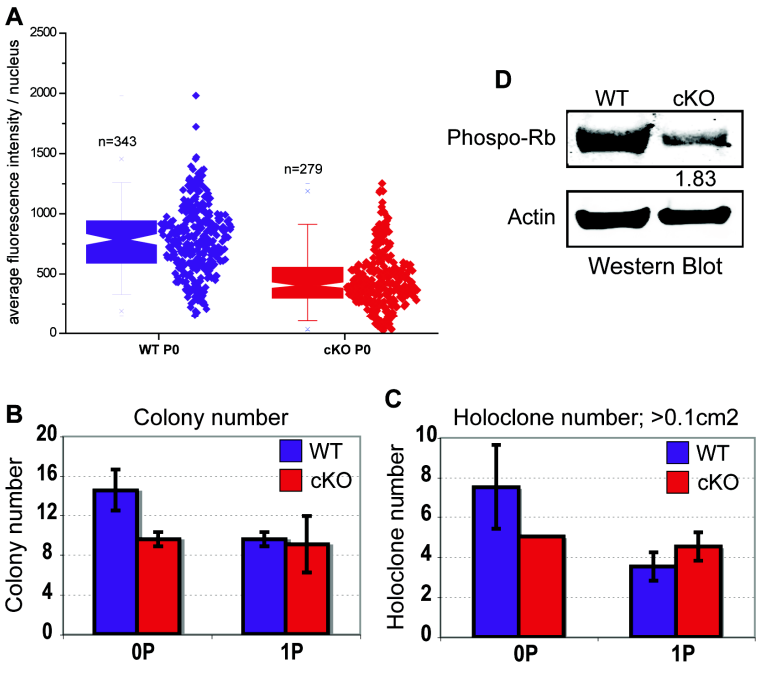

Figure S3. Loss of Ezh2 in Basal Cells Leads to Proliferation Defect

(**A**) Average fluorescent intensity for triMeK27-H3 in nuclei of P0 Ezh2cKO and WT basal cells shows reduction of this histone mark in Ezh2cKO basal cells. (**B** and **C**) Colony in **B** and holoclone in **C** forming efficiency are unchanged in Ezh2cKO vs WT cells analyzed at different passages. (**D**) Level of phosphorylated Retinoblastoma (phospho-Rb) protein is reduced more than two fold in Ezh2cKO cells compared to WT.

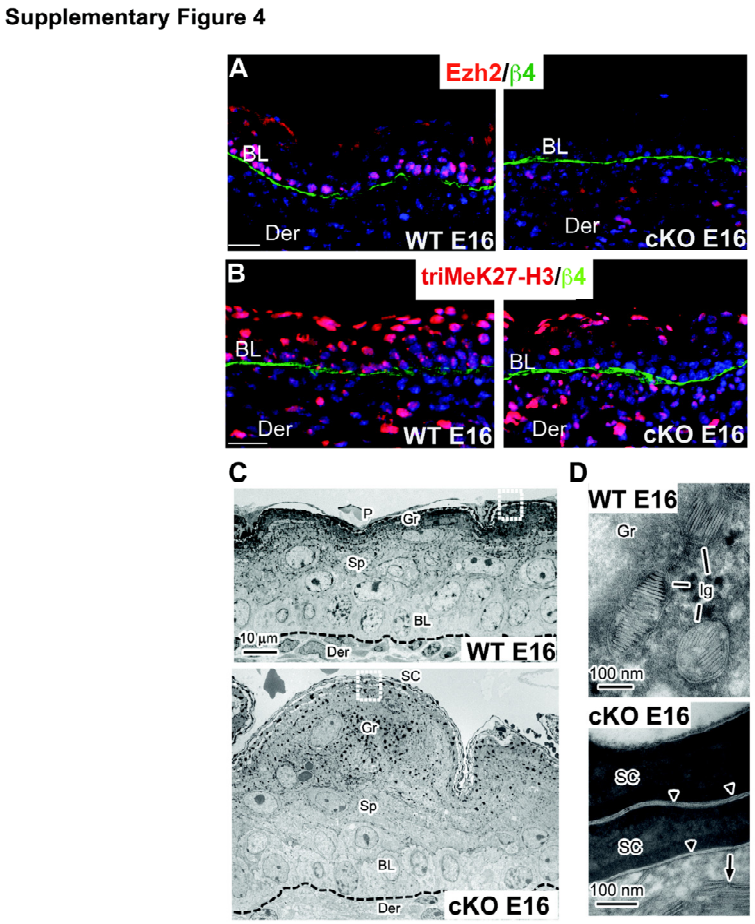

Figure S4. Epidermal Differentiation Is Accelerated in Ezh2cKO E16 Mice

(**A** and **B**) Immunofluorescence confirms the absence of Ezh2 and triMeK27-H3 in E16 Ezh2cKO basal cells. (**C**) Electron microscopy analysis reveals acceleration in epidermal development in Ezh2cKO mice as shown by the presence of several layers of

granular cells and stratum corneum, whereas WT had thinner granular layer and lacked stratum corneum. (**D**) Fixation of skin with ruthenium tetroxide to preserve lipid structure reveals that lamellar granules (lg) are observed in both WT and Ezh2cKO epidermis while the highly ordered tracks of alternating electron-dense and electron-lucent lipid lamellae interconnecting stratum corneum cells are present only in the Ezh2cKO. Scale bar is 30μm.

Supplementary Figure 5 **Ezhkova et al**

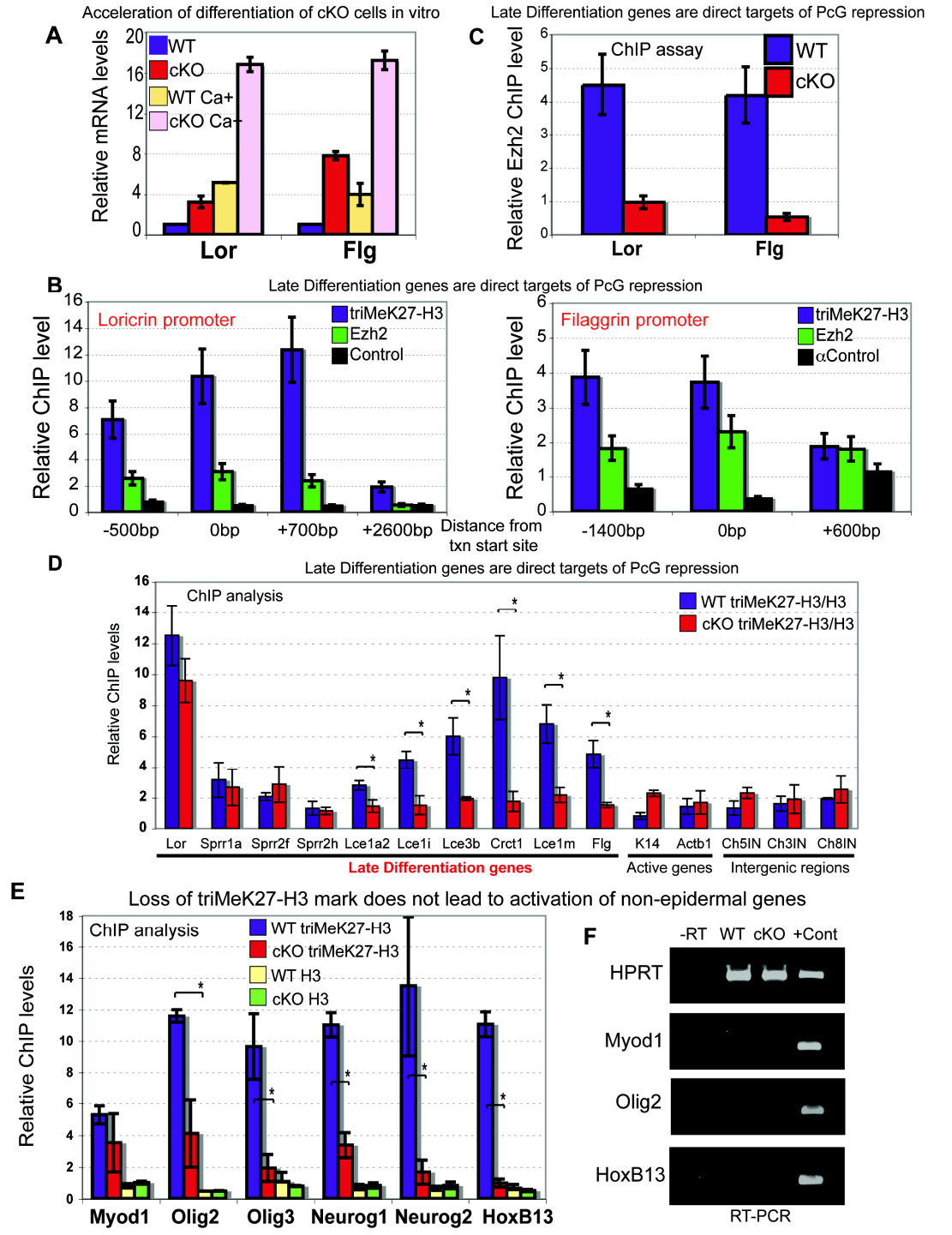

Figure S5. Late Differentiation Genes Are Direct Targets of Ezh2 Repression

(**A**) Semi-quantitative RT-PCR of mRNAs from WT and Ezh2cKO cultures ± Ca+ (24hr) shows induced terminal differentiation. Semi-quantitative RT-PCR with primers against the *HPRT* gene is used for normalization. (**B**) ChIP analysis of association of Ezh2 and triMeK27-H3 with *loricrin* (left) and *filaggrin* (right) promoters shows their enrichment around transcriptional (txn) start sites (0bp). Immunoprecipitation with no antibodies (Control) serves as a negative control. (**C**) ChIP analysis revealed reduction of Ezh2 at promoters of *loricrin* and *filaggrin* genes in Ezh2cKO cells. (**D**) ChIP analysis of triMeK27-H3 signal normalized to total histone H3 level at late differentiation genes shows reduction of this mark at most EDC genes in Ezh2cKO cells compared to WT. Data are mean ±st. dev. N=2. Asterisks indicate significance, $p < 0.05$. Normalization of triMeK27-H3 signal over bulk H3 showed no statistically significant reduction at *Loricrin* gene due to reduction in the level of total histone H3 at this gene. (**E**) ChIP analysis revealed reduction of the triMeK27-H3 mark at non-epidermal genes in Ezh2cKO cells. Data are mean ±st. dev. N=2. Asterisks indicate significance, $p < 0.05$. Semi-quantitative PCR with primers against the promoter of basal *keratin 5* gene was used for normalization in **C-E**. (**F**) RT-PCR analysis revealed that loss of triMeK27-H3 at promoters of non-epidermal genes did not result in their activation. PCR with RNA as a template served as a negative control (-RT). RT-PCR with RNA isolated from skeletal muscle or brain served as a positive control (+Control). Control is HPRT (unchanged).

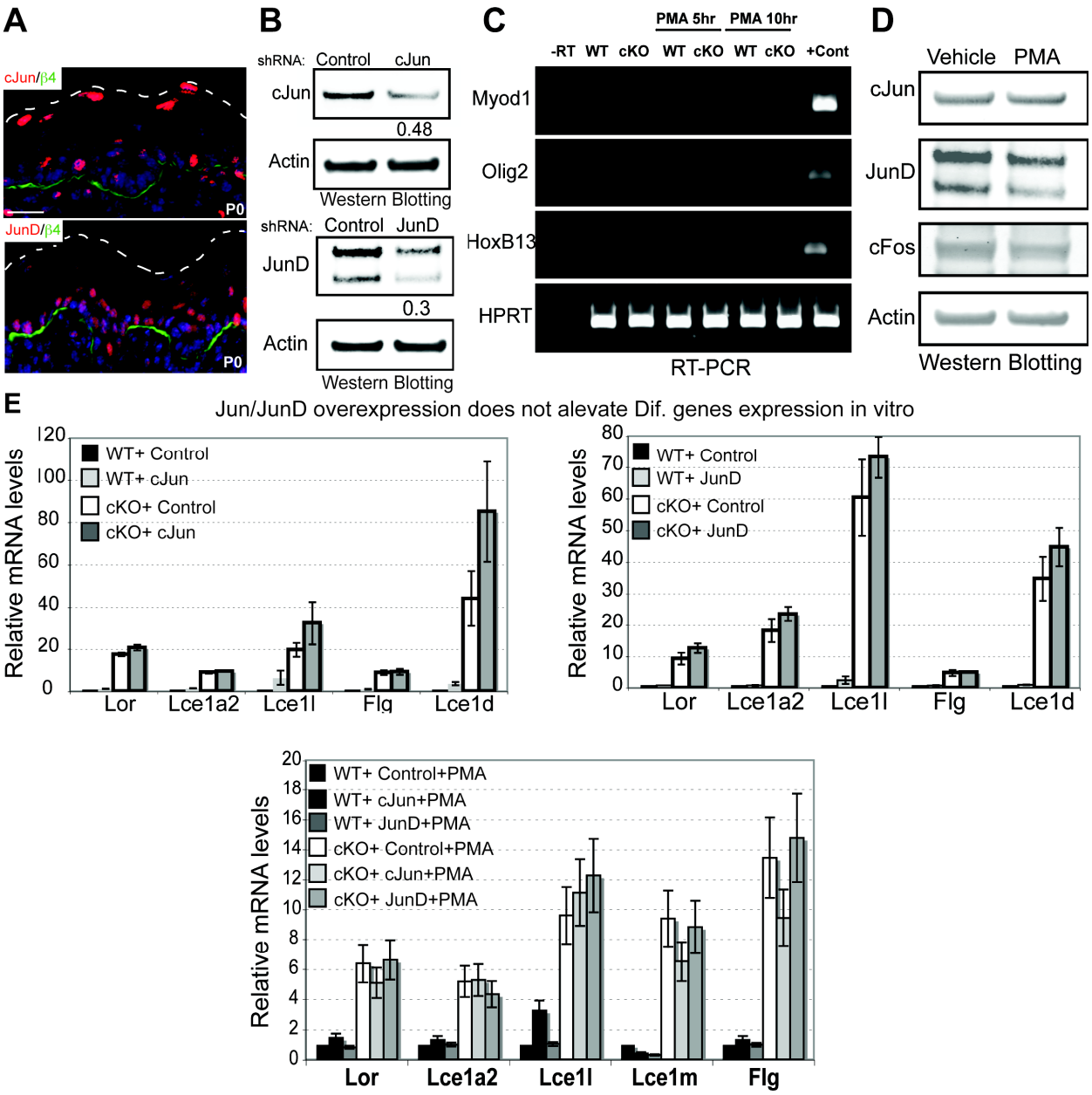

Figure S6. AP1 Family Members Control Late Differentiation Gene Expression in Ezh2cKO Cells

(**A**) Immunofluorescent studies revealed expression of AP1 family members, cJun and JunD, in both basal and suprabasal cells of WT P0 epidermis. Note upregulation of cJun expression in late differentiation layers of the epidermis. (**B**) Efficiency of RNAi

knockdown of cJun and JunD proteins analyzed by Western Blotting. (**C**) RT-PCR analysis revealed that PMA treatment of either WT or Ezh2cKO cells did not result in upregulation of *Myod1*, *Olig2*, or *HoxB13* genes. PCR with RNA as a template served as a negative control (-RT). RT-PCR with RNA isolated from skeletal muscle or brain served as a positive control (+Control). Control is HPRT (unchanged). (**D**) Levels of Ap1 proteins are unchanged upon PMA treatment as revealed by Western Blotting. Control is actin (unchanged) in A and D. (**E**) Overexpression of either cJun or JunD ±PMA treatment does not lead to changes in expression of late differentiation genes as revealed by semi-quantitative RT-PCR. Semi-quantitative RT-PCR with primers against the *HPRT* gene was used for normalization.

Supplementary Figure 7 **Ezhkova et al**

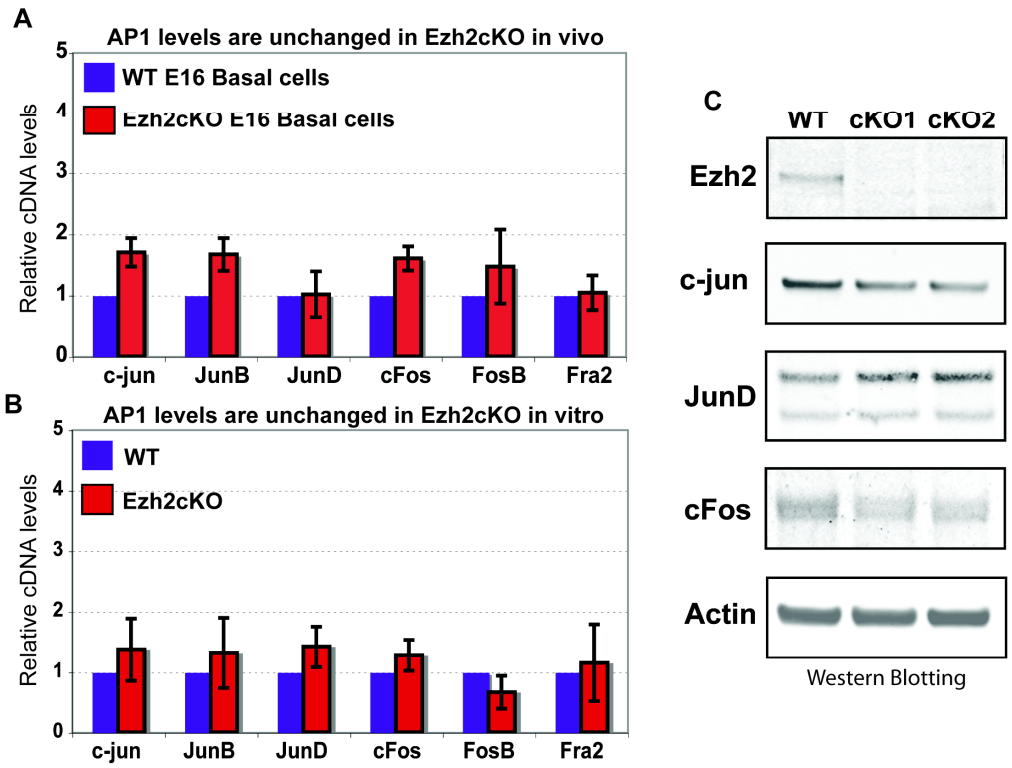

Figure S7. AP1 Levels Are Unchanged in Ezh2cKO Compared to WT Cells *In Vivo* and *In Vitro*

(**A-C**) Semi-quantitative RT-PCR (**A** and **B**) and Western Blotting (**C**) analyses showed no significant changes in expression of Ap1 family members between WT and Ezh2cKO cells *in vivo* and *in vitro*. Semi-quantitative RT-PCR with primers against the *HPRT* gene was used for normalization.

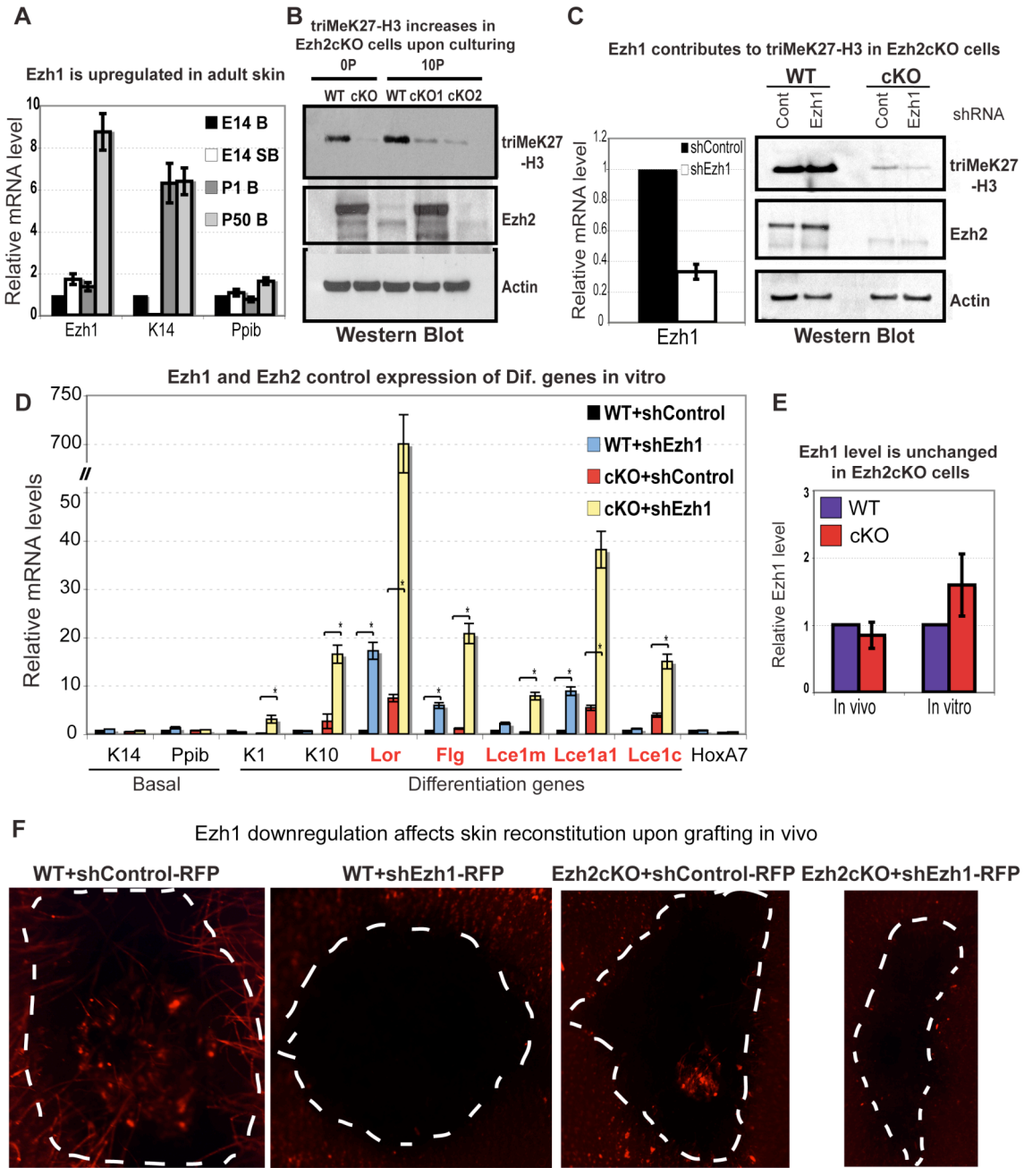

Figure S8. Ezh2 paralogue, Ezh1, Is a New Histone H3 Lysine 27 Methyltransferase that Contributes to Repression of Late Differentiation Genes.

(**A**) Semi-quantitative RT-PCR analysis of expression of *Ezh1*, basal *keratin 14* and housekeeping *Ppib* genes in basal and suprabasal cells at indicated times. (**B**) Western

blotting analysis revealed upregulation of triMeK27-H3 in passaged Ezh2cKO keratinocytes. (**C**) RNAi studies revealed that Ezh1 contributes to triMeK27-H3 in Ezh2cKO cells. Semi-quantitative RT-PCR analysis showed downregulation of Ezh1 expression in keratinocytes infected with lentiviral RNAi construct against Ezh1 (shEzh1) and Western blotting revealed reduction of triMeK27-H3 in Ezh2cKOshEzh1 keratinocytes. Control is actin (unchanged) in B and C. (**D**) Semi-quantitative RT-PCR analysis revealed synergistic role for Ezh1 and Ezh2 in activation of late differentiation genes. Data are mean ±st. dev. N=2. Asterisks indicate significance, $p < 0.05$. Semi-quantitative RT-PCR with primers against silenced *HoxA7* gene served as a control. (**E**) Ezh1 level is unchanged in Ezh2cKO cells *in vivo* and *in vitro* as shown by semi-quantitative RT-PCR. Semi-quantitative RT-PCR with primers against the *HPRT* gene was used for normalization in **A**, **C, D** and **E**. (**F**) Grafting of primary WT and Ezh2cKO cells that express sh-Ezh1-RFP failed to reconstitute skin whereas WT and cKO cells that express sh-Control-RFP led to reconstitution of skin. Abbreviations are B, basal cells; SB, suprabasal cells.

Supplemental Experimental Procedures

Antibodies

The following antibodies and dilutions used were α6-integrin, β4-integrin (rat, 1:100; BDPharmingen), Ezh2 (rabbit, 1:400; Lake Placid Biologicals); trimethylated K27 histone H3 (rabbit, 1:100; Upstate); CD34 (rat, 1:100; Pharmingen); K5 (rabbit, 1:500; Fuchs Laboratory), AE15 (mouse, 1:50; T.T. Sun, New York University, New York), BrdU (rat, 1:100; Abcam), Filaggrin (rabbit, 1:100; Fuchs Laboratory), Loricrin (rabbit,

1:400; Fuchs Laboratory), K10 (rabbit, 1:250; Covance); Ki67 (rabbit, 1:100; Castro-nova), active capase-3 (rabbit, 1:500; R&D Systems), cJun (rabbit, 1:250, Santa Cruz), JunD (rabbit, 1:250, Santa Cruz), cFos (rabbit, 1:250, Santa Cruz), Actin (mouse, 1:1000, Sigma), H3 (rabbit, 1:1000, Abcam). Secondary Abs coupled to FITC, Alexa488, or Texas-Red were from Jackson Laboratories.

Immunostaining and Western Blots

Tissues were embedded in OCT, frozen, sectioned (10 μm) and fixed with formaldehyde for immunofluorescence microscopy. To prevent nonspecific binding with mouse monoclonal Abs, the MOM Basic Kit (Vector Laboratories) was used. Antibodies and dilutions are included in Supplementary Data. Nuclei were labeled by 4'v6'-diamidino-2-phenylindole (DAPI) in conjunction with immunofluorescence microscopy. To visualize actin, FITC-coupled Phalloidin was used. Images were taken by a mot plus microscope (Axioskop 2; Carl Zeiss MicroImaging, Inc.). The objectives used were 20x NA 0.5 plan Neofluar /0.17 and 40x NA 1.3 oil plan Neofluar /0.17 (Carl Zeiss MicroImaging, Inc.).

Cell fractionation was performed using NE-PER Nuclear and Cytoplasmic Extraction Kit (Pierce). Total cell lyses were prepared by boiling for 10 minutes in EZ Lysis Buffer (60 mM Tris-HCl (pH 6.8); 10% glycerol; 2% SDS; 2% β-mercaptoethanol freshly added). Samples were run on 4%–12% gradient gels, transferred to nitrocellulose, and blotted overnight with the indicated Abs.

Histology and Electron Microscopy

For transmission electron microscopy (TEM), samples were fixed in 2% glutaraldehyde, 4% PFA, and 2 mM $CaCl_2$ in 0.05M sodium cacodylate buffer, pH 7.2, at RT for >1 h. Samples were postfixed in either 1% osmium tetroxide or 0.2% ruthenium tetroxide and processed for Epon embedding; semi-thin sections (1 μm) were stained with toluidine blue and examined by light microscopy. For TEM, ultrathin sections (60–70 nm) were counterstained with uranyl acetate and lead citrate. EM images were taken with a transmission electron microscope (Tecnai G2-12; FEI) equipped with a digital camera (Model XR60; Advanced Microscopy Techniques, Corp.)

RNA Isolation and Semi-Quantitative RT-PCR

RNAs were isolated from cells using Absolutely RNA kit (Stratagen), and DNase treatment was performed to remove genomic DNA (Absolutely RNA, Stratagene). Equal RNA amounts were added to reverse-transcriptase reaction mix (Invitrogen) with oligo-dT(12) as a primer. Semi-Quantitative PCR was conducted with a LightCycler system (Roche Diagnostics). RT–PCRs of RNA (i.e., not reverse-transcribed) were used as negative controls. Reactions were performed using the indicated primers and template mixed with the LightCycle DNA master SYBR Green kit and run for 45 cycles. Specificity of the reactions was determined by subsequent melting curve analysis. LightCycle analysis software was used to remove background fluorescence (noise band). The number of cycles needed to reach the crossing point for each sample was used to calculate the amount of each product using the 2^{-CP} method. Levels of PCR product were expressed as a function of GAPDH and/or HPRT. Sequences of primers are

available upon request.

RNAi Constructs

RNAi lentiviral constructs targeting Ezh1 mRNA (TRCN0000095696 and TRCN0000095698) were obtained from Open Biosystems. Selection with puromyocin (2μg/ml) was done two days upon infection with lentiviruses and keratinocytes were collected for analysis a week after infection.

In Vivo Grafting Experiments

Grafting of primary cells infected with lentiviruses that express either shControl or sh-Ezh1 vectors was done as described (Guasch et al., 2007). Briefly two days upon infection 80% infected cells were RFP positive. 1-1.5*10^6 cells of keratinocytes and 3-5*10^6 dermal fibroblast cells were grafted onto nude mouse in which piece of back skin was removed. Reconstitution of skin with grafted cells was analyzed 4-8 weeks later.

Reference

Guasch, G., Schober, M., Pasolli, H. A., Conn, E. B., Polak, L., and Fuchs, E. (2007). Loss of TGFbeta signaling destabilizes homeostasis and promotes squamous cell carcinomas in stratified epithelia. Cancer Cell *12*, 313-327.

Table S1. Summary of Microarray Analysis of Changes in Gene Expression in E16 Ezh2cKO vs WT Basal Cells

Probe Set ID	UniGene Title	UniGene Symbol	Mean Fold Change Ezh2cKOvs WT
1453218_at	late cornified envelope 1C // RIKEN cDNA 1110014K05 gene	Lce1c // 1110014K05Rik	10.5
1457945_at	Eukaryotic translation initiation factor 2, subunit 3	Eif2s3y	7.8
1427268_at	filaggrin	Flg	6.7
1420332_x_at	late cornified envelope 1D // small proline rich-like 7	Lce1d // Sprrl7	6.61
1429565_s_at	late cornified envelope 1M // late cornified envelope 5A	Lce1m // Lce5a	5.42
1451612_at	metallothionein 1	Mt1	5.07
1436277_at	ring finger protein 207 // RIKEN cDNA 330010C22 gene	Rnf207 // D330010C22Rik	4.95
1457396_at	---	---	4.9
1453092_at	Cysteine-rich C-terminal 1 // RIKEN cDNA 2300002G24 gene	Crct1 // 2300002G24Rik	4.87
1456787_at	---	---	4.64
1419231_s_at	keratin complex 1, acidic, gene 12	Krt1-12	4.39
1419409_at	Late cornified envelope 1B // small proline rich-like 5	Lce1b // Sprrl5	4.2
1420350_at	Late cornified envelope 1A2 // small proline rich-like 2	Lce1a2 // Sprrl2	4.1
1420676_at	Late cornified envelope 1A1 // small proline rich-like 3	Lce1a1 // Sprrl3	3.95
1457823_at	cysteine rich protein 61	Cyr61	3.54
1420517_at	Chromatin modifying protein 4C // RIKEN cDNA 2310010I16 gene	Chmp4c // 2310010I16Rik	3.25
1420677_x_at	Late cornified envelope 1A1 // small proline rich-like 3	Lce1a1 // Sprrl3	3.15
1449984_at	chemokine (C-X-C motif) ligand 2	Cxcl2	3.06
1434353_at	Scm-like with four mbt domains 2	Sfmbt2	3.05
1437777_at	activating transcription factor 2	Atf2	3.05
1442340_x_at	cysteine rich protein 61	Cyr61	2.99
1451613_at	hornerin	Hrnr	2.98
1420741_x_at	Late cornified envelope 1I // RIKEN cDNA 2310069N01 gene	Lce1i // 2310069N01Rik	2.97
1422860_at	neurotensin	Nts	2.97
1453599_at	Tripartite motif-containing 71 // RIKEN cDNA 2610206G21 gene	Trim71 // 2610206G21Rik	2.85
1451854_a_at	shroom	Shrm	2.82
1422134_at	FBJ osteosarcoma oncogene B	Fosb	2.79
1428980_at	Keratinocyte expressed, proline-rich // RIKEN cDNA 1110001M24 gene	Kprp // 1110001M24Rik	2.79
1458980_at	---	---	2.78
1455865_at	insulinoma-associated 1	Insm1	2.78
1438841_s_at	arginase type II	Arg2	2.75
1441793_at	ring finger protein 39	Rnf39	2.72
1421882_a_at	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B)	Elavl2	2.7

1434526_at	abhydrolase domain containing 7	Abhd7	2.7
1453202_at	RIKEN cDNA E330016A19 gene	E330016A19Rik	2.65
1455626_at	homeo box A9	Hoxa9	2.6
1458088_at	Ubiquitin-associated protein 2	Ubap2	2.6
1457404_at	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	Nfkbiz	2.6
1440681_at	cholinergic receptor, nicotinic, alpha polypeptide 7	Chrna7	2.59
1437695_at	prokineticin receptor 2	Prokr2	2.58
1420183_at	Loricrin	Lor	2.58
1428706_at	proline-rich polypeptide 6	Prr6	2.53
1449799_s_at	plakophilin 2	Pkp2	2.52
1437460_x_at	Ras and Rab interactor 1	Rin1	2.46
1440490_at	Membrane protein, palmitoylated 6 (MAGUK p55 subfamily member 6)	Mpp6	2.45
1449499_at	homeo box A7	Hoxa7	2.39
1417414_at	septin 3	Sepetin3	2.37
1433699_at	tumor necrosis factor, alpha-induced protein 3	Tnfaip3	2.35
1447882_x_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 54	Ddx54	2.35
1434092_at	nitric oxide synthase 3 antisense	Nos3as	2.33
1456326_at	gene model 784, (NCBI)	Gm784	2.33
1440299_at	RIKEN cDNA E330016A19 gene	E330016A19Rik	2.33
1441044_at	RIKEN cDNA C130002M15 gene	C130002M15Rik	2.33
1449290_at	dihydropyrimidinase-like 5	Dpysl5	2.32
1453486_a_at	signal peptide, CUB domain, EGF-like 2	Scube2	2.32
1447932_at	---	---	2.32
1418536_at	similar to H-2 class I histocompatibility antigen, Q7 alpha chain precursor (QA-2 antigen) /// region containing histocompatibility 2, Q region locus 9; histocompatibility 2, Q region locus 7	LOC630509 /// LOC674192	2.3
1448745_s_at	loricrin	Lor	2.29
1418655_at	beta-1,4-N-acetyl-galactosaminyl transferase 1	B4galnt1	2.29
1457644_s_at	chemokine (C-X-C motif) ligand 1	Cxcl1	2.28
1446805_at	Gamma-aminobutyric acid (GABA-A) receptor, subunit alpha 2	Gabra2	2.28
1435193_at	RIKEN cDNA A230050P20 gene	A230050P20Rik	2.28
1439878_at	involucrin	Ivl	2.26
1451846_at	nebulette	Nebl	2.22
1433579_at	transmembrane protein 30B	Tmem30b	2.19
1419810_x_at	Rho GTPase activating protein 9	Arhgap9	2.19
1418203_at	phorbol-12-myristate-13-acetate-induced protein 1	Pmaip1	2.18
1448459_at	Kv channel-interacting protein 1	Kcnip1	2.17
1417932_at	interleukin 18	Il18	2.1
1434848_at	Transcribed locus	---	2.1
1449612_x_at	M-phase phosphoprotein 1	Mphosph1	2.09
1428209_at	brain expressed gene 4	Bex4	2.09
1449319_at	R-spondin homolog (Xenopus	Rspo1	2.09

	laevis)		
1458367_at	gene model 94, (NCBI)	Gm94	2.08
1443772_at	---	---	2.08
1420437_at	indoleamine-pyrrole 2,3 dioxygenase	Indo	2.07
1417946_at	abhydrolase domain containing 3	Abhd3	2.07
1459672_at	Topoisomerase (DNA) I	Top1	2.07
1426620_at	carbohydrate sulfotransferase 10	Chst10	2.07
1448733_at	B lymphoma Mo-MLV insertion region 1	Bmi1	2.07
1423952_a_at	keratin complex 2, basic, gene 7	Krt2-7	2.06
1449451_at	serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 11	Serpinb11	2.05
1427683_at	early growth response 2	Egr2	2.05
1448830_at	dual specificity phosphatase 1	Dusp1	2.04
1437667_a_at	BTB and CNC homology 2	Bach2	2.03
1424948_x_at	histocompatibility 2, D region locus 1 /// histocompatibility 2, K1, K region /// MHC (A.CA/J(H-2K-f) class I antigen	H2-D1 /// H2-K1 /// LOC56628	2.03
1448932_at	keratin complex 1, acidic, gene 16	Krt1-16	2.02
1429230_at	kallikrein 5	Klk5	2.02
1446290_at	RIKEN cDNA 2410129H14 gene	2410129H14Rik	2.02
1422865_at	runt related transcription factor 1	Runx1	2.01
1455898_x_at	solute carrier family 2 (facilitated glucose transporter), member 3	Slc2a3	2.01
1447339_at	Transcribed locus	---	2.01
1417312_at	dickkopf homolog 3 (Xenopus laevis)	Dkk3	2.01
1419700_a_at	prominin 1	Prom1	2.01
1424984_at	RIKEN cDNA 2700078E11 gene	2700078E11Rik	0.49
1419171_at	RIKEN cDNA 2310044D20 gene	2310044D20Rik	0.49
1427262_at	inactive X specific transcripts	Xist	0.48
1439620_at	---	---	0.48
1438462_x_at	---	---	0.48
1460039_at	C-type lectin domain family 1, member a	Clec1a	0.48
1434186_at	G protein-coupled receptor 23	Gpr23	0.48
1443541_at	Hypothetical gene supported by AK039231; AK039519; AK039710; AK045832	LOC381438	0.48
1449347_a_at	X-linked lymphocyte-regulated 4B /// X-linked lymphocyte-regulated 4A /// X-linked lymphocyte-regulated 4E	Xlr4b /// Xlr4a /// Xlr4e	0.48
1453319_at	cell division cycle and apoptosis regulator 1	Ccar1	0.47
1425890_at	lymphocyte antigen 6 complex, locus I	Ly6i	0.47
1451602_at	sorting nexin 6	Snx6	0.46
1444121_at	Transcribed locus	---	0.46
1452406_x_at	erythroid differentiation regulator 1	Erdr1	0.46
1426558_x_at	RIKEN cDNA 3100002L24 gene /// similar to zinc finger protein 14 ///	3100002L24Rik /// LOC627901 ///	0.46

	RIKEN cDNA 0610010B08 gene /// similar to zinc finger protein 91 /// similar to zinc finger protein 709 /// similar to gonadotropin inducible ovarian transcription factor 1 /// similar to zinc finger protein 97 /// similar to gonadotropin inducible ovarian transcription factor 1	0610010B08Rik /// LOC628084 /// LOC628147 /// LOC668030 /// LOC668039 /// LOC668066	
1458438_at	RIKEN cDNA 4933415L06 gene	4933415L06Rik	0.45
1434340_at	---	---	0.44
1439396_x_at	glycerol-3-phosphate dehydrogenase 1 (soluble)	Gpd1	0.43
1441388_at	Adult male hippocampus cDNA, RIKEN full-length enriched library, clone:C630020O05 product:unclassifiable, full insert sequence	---	0.42
1431503_at	RIKEN cDNA 4930513O06 gene	4930513O06Rik	0.42
1418094_s_at	carbonic anhydrase 4	Car4	0.4
1439200_x_at	---	---	0.39
1460256_at	carbonic anhydrase 3	Car3	0.39
1447815_x_at	RIKEN cDNA 6430527G18 gene	6430527G18Rik	0.36
1422651_at	adiponectin, C1Q and collagen domain containing	Adipoq	0.34
1458219_at	breast carcinoma amplified sequence 2	Bcas2	0.34
1416544_at	enhancer of zeste homolog 2 (Drosophila)	Ezh2	0.28
1449434_at	carbonic anhydrase 3	Car3	0.27
1439716_at	piwi-like homolog 4 (Drosophila)	Piwil4	0.27

Table S2. Primers

Below are sequences of the primers that were used for Semi-Quantitative PCR. Please note that for ChIP assay (if not indicated otherwise) primers span the transcriptional start site of a gene.

	Assay	Forward primer	Reverse Primer
Ezh1	RT-QPCR	TCAGTGGCAACATGCCTAAGC	GCCTAGATTAGGCCCAGCTACC
Ezh2	RT-QPCR	CATTTCATACGCTCTTCTGTCGAC	CCCTCCAGATGCTGGTAACACT
Eed	RT-QPCR	CCCTCTGGTGTTTGCAACTGT	CATCTGCATCAGCATCTACATAGGA
Bmi1	RT-QPCR	GCAATGACTGTGATGCACTTGA	AAGGTAGTGGGCCATTTCTTCTC
Cbx2	RT-QPCR	AACACGTCTTTGTCACGGATGTC	CAGGTCAGGCATCAAAGCAA
Pcgf2	RT-QPCR	CAACGGCTCCAACGAGGA	GTCCCTGACGCCTTCGTAGA
Ring1a	RT-QPCR	GCCGATGCCTGGATCTGAT	GGGCGGAGTCAGAGCTTACAT
HPRT	RT-QPCR	GATCACTCAACGGGGGACATAAA	CTTGCGCTCATCTTAGGCTTTGT
Ppib	RT-QPCR	GTGAGCGCTTCCCAGATGAGA	TGCCGGAGTCGACAATGATG
Myod1	RT-QPCR	GCTCAAACCCCAATGCGAT	AACGGCTTCGAAAGGACAGTT
Olig2	RT-QPCR	AAAATAGCACTTGTGGAATGGAATAATC	AAGAAACCAATTGATGTGTGCTTAGA
HoxB13	RT-QPCR	CCCAAAGGCCAAATGACATAA	CCCTTCAATACACCTCAGTCAA
K14	RT-QPCR	CGCCGCCCCTGGTGTGG	ATCTGGCGGTTGGTGGAGGTCA
K5	RT-QPCR	AACATTTTGGGGTCTGGGTCAC	GGCCCACAGAGACTGCTTCTTT
α6	RT-QPCR	CTGGACACCCGCGAGGACAAC	TCAACCGGCCATCGCAGAAACT
β4	RT-QPCR	GGGGCCGAGAGCGAGAGGGTGTC	CGTGGTGCTGTGCGTGCTGGTCA
K1	RT-QPCR	GACACCACAACCCGGACCCAAAACTTAG	ATACTGGGCCTTGACTTCCGAGATGATG
K10	RT-QPCR	GGAGGGTAAAATCAAGGAGTGGTA	TCAATCTGCAGCAGCACGTT
Lor	RT-QPCR	TCACTCATCTTCCCTGGTGCTT	GTCTTTCCACAACCCACAGGA
Inv	RT-QPCR	GTCCGGTTCTCCAATTCGTGTTT	GCAATTGGAAGAGAAGCAGCATCAG
Lce1a1	RT-QPCR	CACTTTAGACAAACCATTCAGGAGAA	CCAAGAAGACAAACCCAGCAA
Lce1a2	RT-QPCR	GAGGTGCCCAAGGATCTTGTAC	CCAGGCTACAGCAGGAAGACAC
Lce1c	RT-QPCR	AGATCTCAAACATTCTATGCAGAGGAA	TCACAAAATACTGAAGAAGAAAGGGATT
Lce1d	RT-QPCR	GACTGCTGCTGATCTCAATGAGAA	TGCAGAAACTTTCCCTGAAGATTTC
Lce1l	RT-QPCR	CATGAAGGCTTCAGACAAGCAAT	TTGGAATCACAGAAGGAGATGAGAC
Lce1m	RT-QPCR	AGCATTGACTGAAGACCTGCAA	GCAAAGCCAATGCATCTCAGA
Crct1	RT-QPCR	ACTGCACTTTGATGTTCAGAAACTTC	CAGGAGGCCTGTTTTGAACACT
Flg	RT-QPCR	GGAGGCATGGTGGAACTGA	TGTTTATCTTTTCCCTCACTTCTACATC
HoxA7	RT-QPCR	AAAATTCCAGGAGATGGCAGTGT	CAGGGAAAATATACGACTGCTGCT
cJun	RT-QPCR	AAGCGCAAAACTCCGAGCT	CATGAGTTGGCACCCACTGT
JunB	RT-QPCR	GGAGGACAAGGTGAAGACACTCA	GGCAAGGGAGGCTCTCAGA
JunD	RT-QPCR	GACACGCAAGAACGCATCAA	TTGACGTGGCTGAGGACTTTC
cFos	RT-QPCR	TGGCCCTGTGAGCAGTCA	AGCCTGGTGTGTTTCACGAAC
FosB	RT-QPCR	TGACGGCTTCTCTCTTTACACACA	GGAGGGAGGGCGAGTTCA
Fra2	RT-QPCR	GCCCTAGGAGGGTCGCTCTA	TCCATAGCAGCAAGCTCCTACC
Ink4A	RT-QPCR	GTGTGCATGACGTGCGGG	GCAGTTCGAATCTGCACCGTAG
Ink4B	RT-QPCR	CCCTGTGAACTGAAAATGCAGA	TGTCGAGCTGGAGGTGACTTC
Myod1	ChIP-QPCR	ACTCTCACGGCTTGGGTTGA	GAGTCGAAACACGGGTCATCA
Olig2	ChIP-QPCR	TGCTTATTACAGACCGAGCCAAC	CTAAATCCTAGCCACTTTGGAGAAGT
Olig3	ChIP-QPCR	ACCATCAGGAGAGTCGTCTGAACT	GAAGATCCTGCTCCGACAGCT
Neurog1	ChIP-QPCR	CCGGGTCGTACGGACAGTAA	TCGGTGAGGAAGCTGCACA
Neurog2	ChIP-QPCR	CAGATCTGATTGTTTTCTTGGTGGTATA	GCGTGGTTGTCTGTCAGTCAGT
HoxB13	ChIP-QPCR	TCTGGAAAGCAGCGTTTGC	CTTAGTGATAAACTTGTTGGCTGCA
HoxA11	ChIP-QPCR	CTGCAGTGGAGAATCATGTTAAGC	AGAAGGGAGGCTGGAGAAATCT
HoxD10	ChIP-QPCR	TTTTCCTAGCGATGTCAGCCTAC	TGCTCGAATAAAAACTGTCACTCCT

K14	ChIP-QPCR	AGGAGGGATCTGATCGGGAGT	AGAGCTGCTCAGGTGTGTTAGAAAA
K5	ChIP-QPCR	GTTGAACGCCGCTGACCT	CTTCGGAAGGACACACTGGAC
Actb1	ChIP-QPCR	CCGTAAAGACCTCTATGCCAACAC	GCTAGGAGCCAGAGCAGTAATCTC
K1	ChIP-QPCR	CTCAGTATATAAGGGCACGGCACT	GACTCATGATGCCTTAGAGAGAGGT
K10	ChIP-QPCR	AGCAAAGCCTAGCACCTGTGA	GCTGCTGGAGCTGTATAGAACAGAC
Lor (TSS, AP1site)	ChIP-QPCR	CAGAGCAGGACAAGAGTATAAAACACA	GCCCACACTTACCTGAAGCAC
Lor(-500)	ChIP-QPCR	GGGACTCATGAACTCTCACAACC	CCATCTCTCTTATGCCACAATTTCT
Lor(+700)	ChIP-QPCR	AGGACCACAGTAGAGCTGTCTTGTCT	GCATTTCCTGCATAAAGTCCTTAGTT
Lor(+2000)	ChIP-QPCR	GCGCCGATGGGCTTAACT	CTTGAGCGACTCAATGGCTTC
Flg	ChIP-QPCR	TCCCTTTTACAGGTGCATACACAC	CCTCCTTATCACTGGTTGAGTATTGTT
Flg(-1400)	ChIP-QPCR	CCCACTTGCTGAAAAGGTCAA	CAGGTTTGGCAGGTGCTGTA
Flg(+600)	ChIP-QPCR	GTGATGCCCAGGGAAGACA	TGTCTGCTCCCACAATGAAGC
Lce1a2	ChIP-QPCR	GAGGTGCCCAAGGATCTTGTAC	CCTTCCCCCAACACTTCCAT
Lce1m	ChIP-QPCR	AGCATTGACTGAAGACCTGCAA	GCAAAGCCAATGCATCTCAGA
Lce1i	ChIP-QPCR	TCCTGGAGGTAAGGGCAGAGA	CAAGGCTGTGGCTAGTTATTGTCA
Lce3b	ChIP-QPCR	AAAGCATCCTCAGACACGGACTT	CCTATTGCACTTATGTCTGGATTTCTGT
Crct1	ChIP-QPCR	TCTGCCTAGCAGGTGTCAAGTTC	GCTACATTCTGGCTGCATCCTACT
Ch5IN	ChIP-QPCR	CCCTCATCACAGACCCACTTCT	GTGGGAGTGGATGTATCTCTGACTT
Ch3IN	ChIP-QPCR	TGTCTGGAATGTGGTGGTTTGA	GCCCACTGCTATAATTAGGAAGGA
Ch8IN	ChIP-QPCR	AAGGGGCCTCTGCTTAAAAA	AGAGCTCCATGGCAGGTAGA
Sprr1a	ChIP-QPCR	GGTGCTGCCTACAGTTAGTGAACAGTA	TGAATATTTGAATGGAGGTTTCCTTT
Sprrr2f	ChIP-QPCR	TCTTTGAAAGGCCATATACCTCAGC	GTTCTGGTGCCCTGAGAAACC
Sprr2h	ChIP-QPCR	TGGTTCCAAACTCTGAGCAAGTGTA	CCTGTATATCAAGAGAGGGCATCAGAT
K5 (AP1site)	ChIP-QPCR	GGAGGCTGGCAGAGCAGTC	AGCCCTTCACAGTCCCTCCTA
Lce1c (AP1 site)	ChIP-QPCR	CCACAGATCTTGTGCTACCCTTC	TGCCTTAACCTCACATTTGAACAT
Lce1a2 (AP1 site)	ChIP-QPCR	ACAAGAGCATGGCCTGTACCA	TACCCAGCTCCTTCTACTGCTTTC
Crct1(AP1 site)	ChIP-QPCR	GAAGGACAGGCAAGAAAGAAGGT	CGCAGCTCTTTCAAGTGGTAATCT
Lce1m (AP1 site)	ChIP-QPCR	GAGGGAGGAATTAGATCTTTGGGAT	AGTAATGGATTGAGGTGCTATGAGAA