

Supplemental Table and Figure Legend

Table S1. List of genes altered in Notch1-deficient proximal hair bulbs in GO categories of cell growth, cycle, proliferation, and programmed cell death.

622 genes differentially regulated in Notch1-deficient proximal bulbs were classified by GO term. “emN1 vs anN1 (1)” and “emN1 vs. anN1 (2)” indicate two different microarray experiments. Validated genes by qRT-PCR were marked as “√” and the genes with no significant change as “NC”.

Table S2. Altered expression of melanocyte signature genes in Notch1-deficient hair bulbs.

Figure S1. p53 does not contribute to the Notch1-deficient phenotype in skin.

(A) Histological (H&E staining) and stereoscopic view of the genotypes specified. Note that p53 loss did not rescue Notch1-deficient follicular phenotype. Skin stripes for stereoscopic views were prepared as described in Materials and Methods. Scale Bars 200 μ M.

Figure S2. mRNA expression patterns of TGF β and IGF signaling components.

TGF β 1 is expressed in hair matrix while TGF β 2 only in proximal hair matrix. TGF β receptors are found in matrix as well as dermal papilla (Arrows). IGFBP2 and 3 are found in dermal fibroblasts and epidermal

basal keratinocytes, respectively. Scale Bars 50 μ M

Figure S3. TGF β signaling in epithelial cells do not participate in Notch1-deficient phenotype.

(A) Histological (H&E staining) and stereoscopic view of the genotypes specified. Note that no observable difference between the presence and absence of TGF β R2. Scale Bars 200 μ M. (B) Semi-quantitative genomic DNA PCR of the plucked hairs from wild-type and embryo-deleted *Msx2-Cre;Notch1^{flox/flox};Tgfr2^{flox/flox}* (emN1;Tgfr2^{-/-}). *Nrarp* primer sets were used as a positive control. Note the absence of Tgfr2 floxed allele in emN1;Tgfr2^{-/-} hairs.

Figure S4. Cell non-autonomous role of Notch1 in maintaining melanocyte population in hair follicles.

Immunohistochemical staining of wild-type, *Msx2Cre;Rosa26R/+* (albino), *Msx2Cre;Notch1^{flox/flox};Rosa26R/+* with Dct-1 (green) and LacZ (red) . Nuclei were stained with DAPI. Note that melanocytes (Dct-1-positive) do not show LacZ staining and that the number of melanocytes in Notch1-deficient hair follicles is reduced. Scale bar 50 μ M.

Figure S5. Igfbp3 mRNA expression in the follicles of P9 emN1 and P16 wild-type

Non-radioactive mRNA in situ hybridization with Igfbp3 probe on P9 Notch1-deficient mouse skin and

on P16 wild-type. (c) and (d) are magnified view of the follicles in (a) and (b), respectively. Note that P9 emN1 sections ((a) and (c)) were developed for 2 days while P16 wild-types for 3 hours. Scale bars, (a) and (b), 200uM; (c) and (d), 50uM.

Figure S6. Confirmation of Notch1 deficiency in IGF1 rescued hair follicles.

Immunohistochemical analyses with full-length Notch1 (1:50, rabbit, Abcam ab27526) and cleaved Notch1 (V1744, 1:100, rabbit, Cell Signaling, #2421) antibodies. Note the absence of Notch1 and V1744 immunoreactivity in emN1 and emN1;Ivl-IGF1 follicles. Scale bars, 50uM.

Table S1

Probe Set ID	Gene Symbol	Gene Title	emN1 vs anN1 (1)	emN1 vs anN1 (2)	qRT-PCR validation
Cell Cycle / Growth / Proliferation					
1456022_at	B230339E18Rik	RIKEN cDNA B230339E18 gene	-2.32	-1.74	
1418971_x_at	Bcl10	B-cell leukemia/lymphoma 10	1.56	1.62	
1418970_a_at	Bcl10	B-cell leukemia/lymphoma 10	1.62	1.62	
1422477_at	Cables1	Cdk5 and Abl enzyme substrate 1	2.5	1.63	
1441938_x_at	Cables1	Cdk5 and Abl enzyme substrate 1	2.01	1.92	
1455869_at	Camk2b	Calcium/calmodulin-dependent kinase II, beta	2.56	2.3	
1449145_a_at	Cav1	caveolin, caveolae protein 1	2.98	3.23	
1417327_at	Cav2	caveolin 2	3.31	2.29	
1417419_at	Cend1	cyclin D1	-2.37	-2.32	NC
1448698_at	Cend1	cyclin D1	-1.5	-1.79	NC
1417649_at	Cdkn1c	cyclin-dependent kinase inhibitor 1C (P57)	2.43	2.22	√
1449152_at	Cdkn2b	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	1.72	1.83	√
1450944_at	Cspg4	chondroitin sulfate proteoglycan 4	3.53	1.94	
1423341_at	Cspg4	chondroitin sulfate proteoglycan 4	4.9	3.12	
1448823_at	Cxcl12	chemokine (C-X-C motif) ligand 12	6.22	4.19	
1421117_at	Dst	dystonin	2.96	2.39	
1419431_at	Ereg	epiregulin	2.87	2.88	
1416268_at	Ets2	E26 avian leukemia oncogene 2, 3' domain	1.78	1.78	
1438883_at	Fgf5	fibroblast growth factor 5	-1.56	-2.57	
1416855_at	Gas1	growth arrest specific 1	2.3	2.78	
1417399_at	Gas6	growth arrest specific 6	2.52	2.1	
1459211_at	Gli2	GLI-Kruppel family member GLI2	1.58	1.6	
1446086_s_at	Gli2	GLI-Kruppel family member GLI2	2.08	1.61	
1419292_at	Htra3	serine protease HTRA3	2.33	4.63	
1454159_a_at	Igfbp2	insulin-like growth factor binding protein 2	5.8	7.05	√
1458268_s_at	Igfbp3	insulin-like growth factor binding protein 3	8.03	4.05	√
1423062_at	Igfbp3	insulin-like growth factor binding protein 3	6.4	5.98	√
1437406_x_at	Igfbp4	insulin-like growth factor binding protein 4	1.51	1.75	√

1423757_x_at	Igfbp4	insulin-like growth factor binding protein 4	1.74	1.85	√
1423756_s_at	Igfbp4	insulin-like growth factor binding protein 4	1.89	1.89	√
1423584_at	Igfbp7	insulin-like growth factor binding protein 7	2.58	1.96	
1421066_at	Jak2	Janus kinase 2	1.63	1.51	
1421317_x_at	Myb	myeloblastosis oncogene	1.86	2.37	
1450194_a_at	Myb	myeloblastosis oncogene	2.22	2.56	
1422818_at	Nedd9	neural precursor cell expressed, developmentally down-regulated gene 9	2.09	1.53	
1438157_s_at	Nfkbia	nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	1.5	1.8	
1448306_at	Nfkbia	nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	1.89	2.25	
1421917_at	Pdgfra	platelet derived growth factor receptor, alpha polypeptide	1.56	1.84	
1417133_at	Pmp22	peripheral myelin protein	2.11	1.99	
1438251_x_at	Prss11	protease, serine, 11 (Igf binding)	2.29	2.35	
1416749_at	Prss11	protease, serine, 11 (Igf binding)	2.47	2.55	
1447830_s_at	Rgs2	regulator of G-protein signaling 2	5.19	3.54	
1419248_at	Rgs2	regulator of G-protein signaling 2	6.32	3.63	
1419247_at	Rgs2	regulator of G-protein signaling 2	5.96	3.84	
1418448_at	Rras	Harvey rat sarcoma oncogene, subgroup R	2.1	1.95	
1456212_x_at	Socs3	suppressor of cytokine signaling 3	6.63	2.13	
1455899_x_at	Socs3	suppressor of cytokine signaling 3	2.73	2.21	
1421943_at	Tgfa	transforming growth factor alpha	4.31	3.28	
1423250_a_at	Tgfb2	transforming growth factor, beta 2	3.45	1.83	√
1450923_at	Tgfb2	transforming growth factor, beta 2	3.36	2.23	√
1450922_a_at	Tgfb2	transforming growth factor, beta 2	3.15	2.86	√
1426397_at	Tgfbr2	transforming growth factor, beta receptor II	2.23	2.16	√
1460302_at	Thbs1	Thrombospondin 1	3.46	3.06	
Programmed Cell Death					
1456022_at	B230339E18Rik	RIKEN cDNA B230339E18 gene	-2.32	-1.74	
1418971_x_at	Bcl10	B-cell leukemia/lymphoma 10	1.56	1.62	
1418970_a_at	Bcl10	B-cell leukemia/lymphoma 10	1.62	1.62	
1421392_a_at	Birc3	baculoviral IAP repeat-containing 3	2.17	1.77	
1454880_s_at	Bmf	Bcl2 modifying factor	1.9	1.69	

1417040_a_at	Bok	Bcl-2-related ovarian killer protein	1.57	1.71	
1416855_at	Gas1	growth arrest specific 1	2.3	2.78	
1435040_at	Irak3	interleukin-1 receptor-associated kinase 3	2.56	2.79	
1421066_at	Jak2	Janus kinase 2	1.63	1.51	
1420640_at	Jmy	junction-mediating and regulatory protein	1.53	1.66	
1454974_at	Ntn1	netrin 1	4.22	2.89	
1423986_a_at	Scotin	scotin gene	1.91	1.73	√
1450036_at	Sgk3	serum/glucocorticoid regulated kinase 3	2.11	1.62	
1418011_a_at	Sh3glb1	SH3-domain GRB2-like B1 (endophilin)	1.66	1.56	
1433699_at	Tnfaip3	tumor necrosis factor, alpha-induced protein 3	1.71	1.59	
1416926_at	Trp53inp1	transformation related protein 53 inducible nuclear protein 1	1.81	1.72	
1452325_at	Trp73	transformation related protein 73	4.19	3.28	√
Others					
1460302_at	Thbs1	thrombospondin 1	4.2	3.65	
1421811_at	Thbs1	thrombospondin 1	3.64	5.04	
1415855_at	Kitl	Kit ligand	-3.24	-2.63	√

Table S2

ProbeSet ID	Gene Symbol	Gene Title	emN1 vs anN1 (1)	emN1_vs anN1 (2)
1423989_at	2210010N04Rik	RIKEN cDNA 2210010N04 gene	-1.78	-3.8
1434175_s_at	2210010N04Rik	RIKEN cDNA 2210010N04 gene	-2.12	-2.25
1416607_at	4931406C07Rik	RIKEN cDNA 4931406C07 gene	-2.52	-1.99
1433671_at	A130022J15Rik	RIKEN cDNA A130022J15 gene	-1.7	-1.89
1454678_s_at	A130022J15Rik	RIKEN cDNA A130022J15 gene	-1.58	-1.82
1450380_at	AU040950	ependymin 2	-3.54	-3.49
1435758_at	B4galt6	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide6	-1.68	-1.75
1416673_at	Bace2	beta-site APP-cleaving enzyme 2	-2.19	-3.34
1424383_at	BC003277	cDNA sequence BC003277	-1.87	-1.71
1418476_at	Crlf1	cytokine receptor-like factor 1	-3.46	-3.63

1418365_at	Ctsh	cathepsin H	3.08	2.63
1450779_at	Fabp7	fatty acid binding protein 7, brain	-4.48	-3.55
1420581_at	Gpr143	mouse homolog of human ocular albinism 1 (Nettleship-Falls)	-5.11	-4.18
1421040_a_at	Gsta2	glutathione S-transferase, alpha 2 (Yc2)	-7.07	-5.76
1451055_at	Matp	membrane associated transporter protein	-3.71	-4
1437430_at	Matp	membrane associated transporter protein	-2.82	-3.27
1437540_at	Mcoln3	mucolipin 3	-2.99	-2.97
1450391_a_at	Mgll	monoglyceride lipase	-1.73	-1.82
1449896_at	Mlph	melanophilin	-2.1	-2.02
1419127_at	Npy	neuropeptide Y	-2.21	-2.51
1426588_at	Ocsp	oculospanin	-3.09	-3.11
1418211_at	p	pink-eyed dilution	-3.52	-4.09
1418835_at	Phlda1	pleckstrin homology-like domain, family A, member 1	-1.5	-1.53
1423213_at	Plxnc1	plexin C1	-3.17	-3.42
1450905_at	Plxnc1	plexin C1	-3.19	-2.52
1423860_at	Ptgds	prostaglandin D2 synthase (brain)	-2.63	-2.83
1423859_a_at	Ptgds	prostaglandin D2 synthase (brain)	-2.65	-2.07
1416527_at	Rab32	RAB32, member RAS oncogene family	-1.85	-1.75
1417700_at	Rab38	Rab38, member of RAS oncogene family	-2.94	-3.35
1448595_a_at	Rex3	reduced expression 3	3.79	4.5
1449078_at	Siat10	sialyltransferase 10 (alpha-2,3-sialyltransferase VI)	-3.32	-3.18
1418326_at	Slc7a5	solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	-1.61	-2.85
1451689_a_at	Sox10	SRY-box containing gene 10	-3.12	-3.26
1420928_at	St6gal1	sialyltransferase 1 (beta-galactoside alpha-2,6- sialyltransferase)	-2	-1.69
1415845_at	Syt4	synaptotagmin 4	-4.61	-3.07
1450881_s_at	Tm7sf1	transmembrane 7 superfamily member 1	-2.83	-2.33
1439255_s_at	Tm7sf1	transmembrane 7 superfamily member 1	-2.14	-2.06
1422754_at	Tmod1	tropomodulin 1	2.79	26.42
1418935_at	Trpm1	transient receptor potential cation channel, subfamily M, member 1	-4.26	-4.51

1437531_at	Trpm1	transient receptor potential cation channel, subfamily M, member1	-3.01	-3.76
1448361_at	Ttc3	tetratricopeptide repeat domain 3	-1.51	-1.75
1448821_at	Tyr	tyrosinase	-3.58	-4.39
1456095_at	Tyr	tyrosinase	-3.75	-3.17
1417717_a_at	Tyr	tyrosinase	-2.95	-3.09
1415862_at	Tyrp1	tyrosinase-related protein 1	-3.76	-3.19
1415861_at	Tyrp1	tyrosinase-related protein 1	-2.34	-2.56

Fig.S1

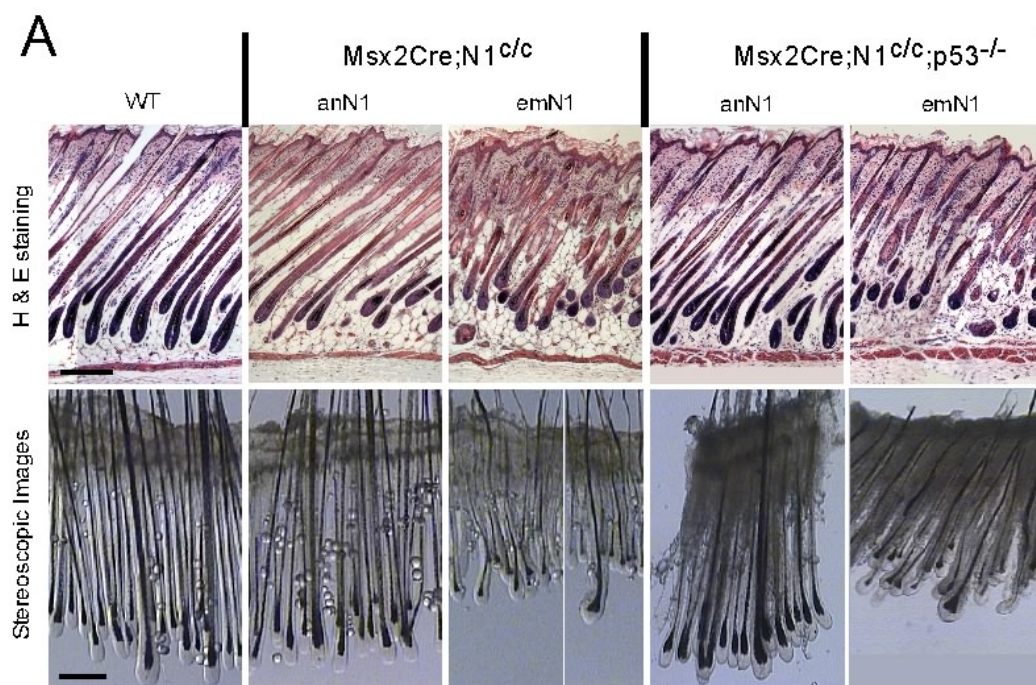


Fig S2

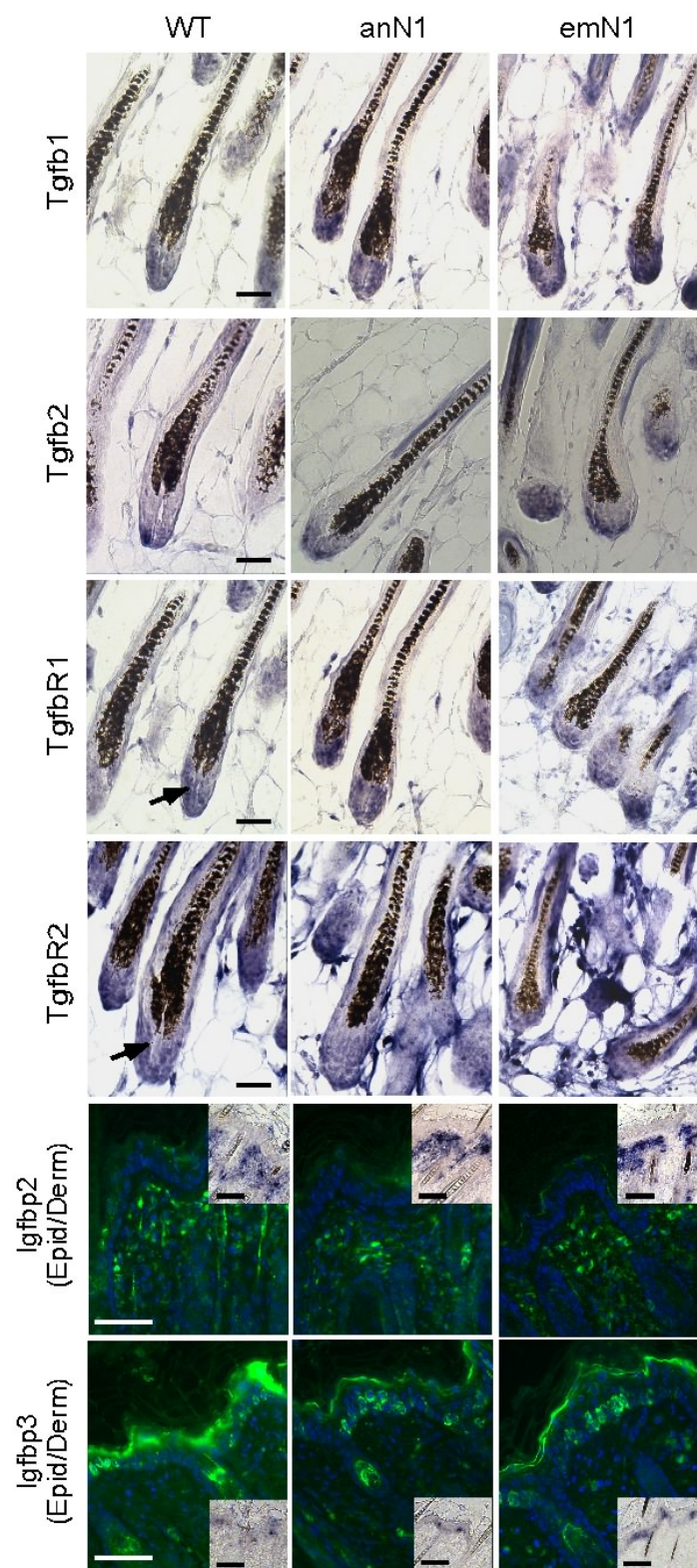


Fig. S3

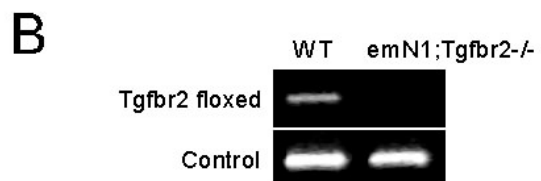
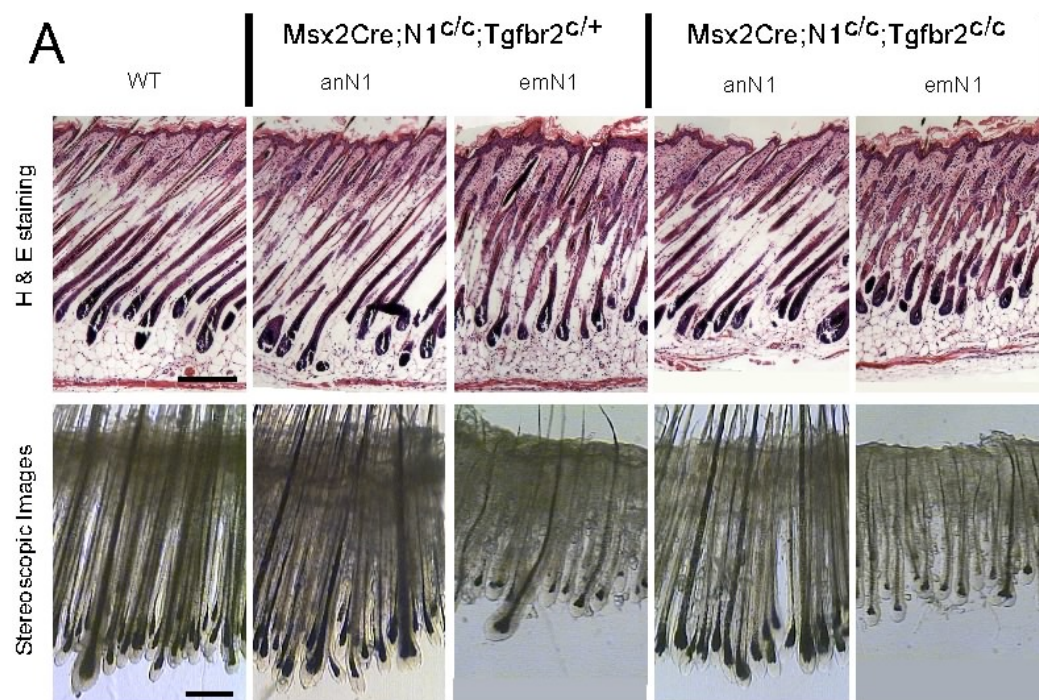


Fig S4

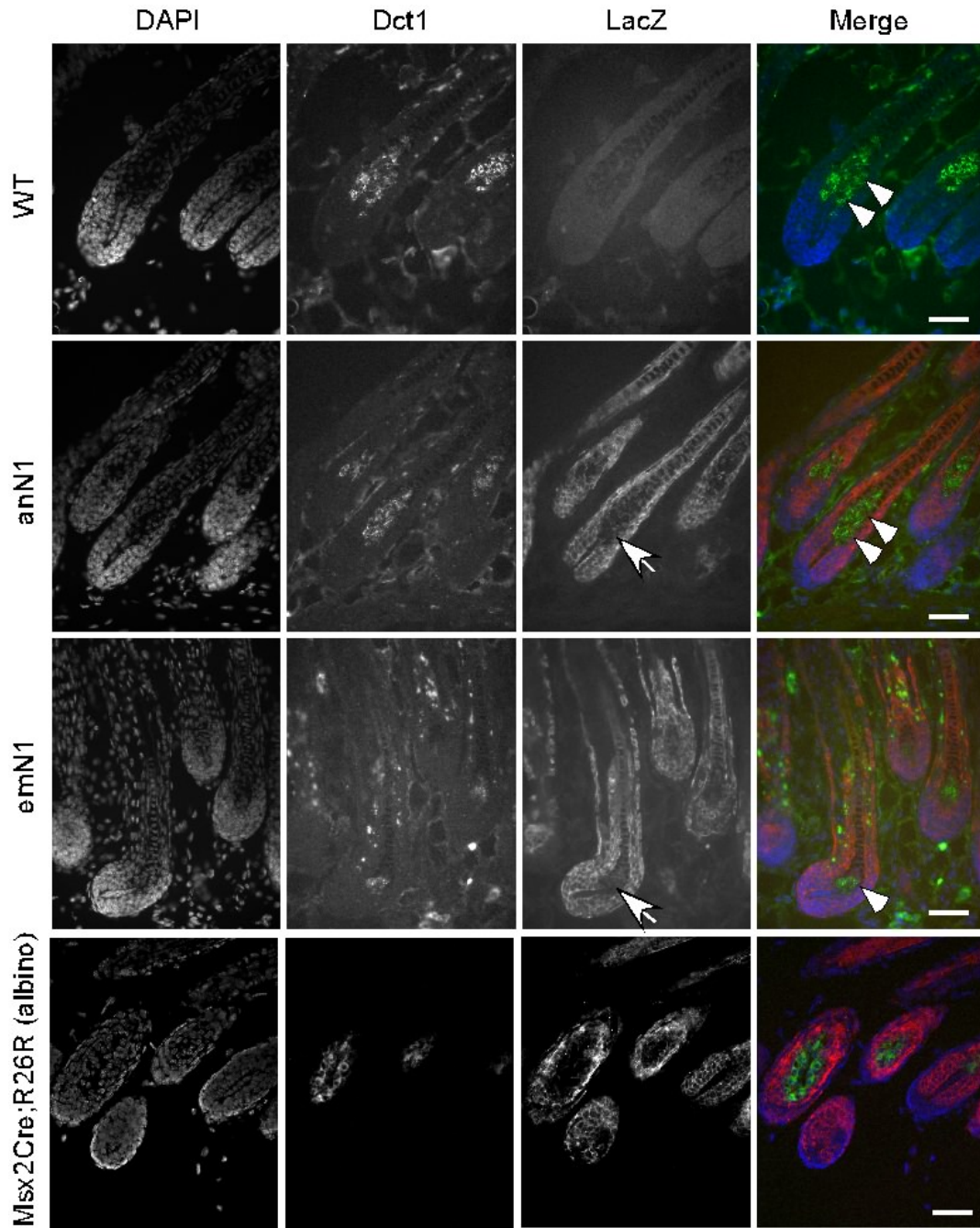


Fig. S5

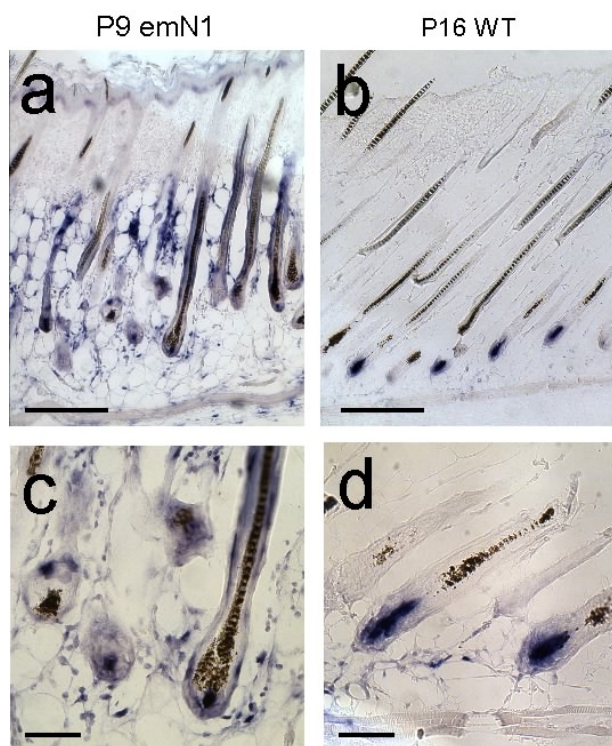


Fig. S6

