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Mammalian Merkel cells are descended from the epidermal lineage

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Abstract

Merkel cells are specialized cells in the skin that are important for proper neural encoding of light touch stimuli. Conflicting evidence suggests that these cells are lineally descended from either the skin or the neural crest. To address this question, we used epidermal (*Krt14*^{Cre}) and neural crest (*Wnt1*^{Cre}) *Cre*-driver lines to conditionally delete *Atoh1* specifically from the skin or neural crest lineages, respectively, of mice. Deletion of *Atoh1* from the skin lineage resulted in loss of Merkel cells from all regions of the skin, while deletion from the neural crest lineage had no effect on this cell population. Thus, mammalian Merkel cells are derived from the skin lineage.

Keywords

skin; development; touch; sensation

Introduction

Four main classes of sensory receptors in mammalian skin mediate different aspects of the sense of touch (Johnson, 2001; Johnson et al., 2000). One of these specialized structures, the Merkel cell-neurite complex, is thought to be important for two-point discrimination and the detection of texture, shape and curvature. These receptors consist of Merkel cells, a distinct cell population found at the epidermal/dermal border, and the afferent somatosensory fibers that innervate them (Merkel, 1875). Merkel cell-neurite complexes are found in touch-sensitive areas of the skin including whisker follicles, glabrous (hair-less) skin surfaces such as the hands and feet, and specialized epithelial structures in the hairy skin called touch domes (Halata et al., 2003). Although the precise function of Merkel cells is unknown, it is clear that they are required for the characteristic neurophysiological response of Merkel cell-neurite complexes to tactile stimuli (Maricich et al., 2009).

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The developmental origins of Merkel cells have been debated for 135 years. Contradictory evidence suggests that these cells are lineally derived from either the neural crest (Grim and Halata, 2000; Szeder et al., 2003) or the skin (Compton et al., 1990; Moll et al., 1990; Moll et al., 1986; Tweedle, 1978). Definitive assignment of the origins of this cell population could provide key insights into the pathogenesis of human diseases such as Merkel cell carcinoma (MCC), a devastating and deadly form of human skin cancer for which no effective treatments are available (Sidhu et al., 2005).

Atoh1 is a basic helix-loop-helix transcription factor that is a mammalian homolog of the *Drosophila atonal* gene (Akazawa et al., 1995; Ben-Arie et al., 1996). Neurons in the hindbrain and spinal cord, mechanosensory hair cells of the inner ear, and secretory cells of the gut are all derived from the *Atoh1*-lineage (Ben-Arie et al., 1997; Bermingham et al., 1999; Bermingham et al., 2001; Wang et al., 2005; Yang et al., 2001). In each of these cell lineages, *Atoh1* expression begins in precursor cells and continues for varying lengths of time in the terminally differentiated progeny. *Atoh1* is also expressed by and necessary for the production of Merkel cells (Ben-Arie et al., 2000; Maricich et al., 2009).

We used genetic lineage tracing and conditional knockout techniques to determine the lineal origin of murine Merkel cells. Selective deletion of *Atoh1* from the neural crest and its derivatives had no effect on the Merkel cell population, while deletion in the skin lineage resulted in an absence of Merkel cells from all areas of the body skin. These data provide conclusive evidence for an epidermal origin of mammalian Merkel cells.

Materials and Methods

Mice

The generation of $Atoh1^{flox}$, $Atoh1^{LacZ}$, $Krt14^{Cre}$, $ROSA^{R26R}$ and $Wnt1^{Cre}$ mice were described previously (Ben-Arie et al., 1996; Danielian et al., 1998; Dassule et al., 2000; Shroyer et al., 2007; Soriano, 1999). All animals were maintained on mixed genetic backgrounds except for $Atoh1^{LacZ}$ and $ROSA^{R26R}$ mice, which are congenic on the C57Bl/6J strain background. All animal work was conducted in accordance with institutional IACUC guidelines.

Atoh1 conditional knockout ($Atoh1^{CKO}$) mice were generated by first crossing $Krt14^{Cre}$ or $Wnt1^{Cre}$ mice with $Atoh1^{LacZ}$ mice to generate $Krt14^{Cre/+}$; $Atoh1^{LacZ/+}$ and $Wnt1^{Cre/+}$; $Atoh1^{LacZ/+}$ animals. These animals were mated with $Atoh1^{flox/flox}$ mice to generate mice of four genotypes: [$Cre^{+/+}$; $Atoh1^{+/flox}$], [$Cre^{Cre/+}$; $Atoh1^{+/flox}$], [$Cre^{+/+}$; $Atoh1^{LacZ/flox}$] and [$Cre^{Cre/+}$; $Atoh1^{LacZ/flox}$]. Only animals with $Krt14^{Cre}$ and $Atoh1^{LacZ}$ or $Wnt1^{Cre}$ and $Atoh1^{LacZ}$ alleles lack *Atoh1* expression in the $Krt14^{Cre}$ (*Krt14; Atoh1*^{CKO}) or $Wnt1^{Cre}$ (*Wnt1; Atoh1*^{CKO}) distributions. Mice of the other three genotypes ([$Cre^{+/+}$*; Atoh1*$^{+/flox}$], [$Cre^{Cre/+}$; $Atoh1^{+/flox}$], and [$Cre^{+/+}$*; Atoh1*$^{LacZ/flox}$]) are collectively referred to as “control” because they displayed no abnormal phenotypes and were indistinguishable based on the testing reported here.

Tissue harvesting

For embryos, the day of conception was designated as E0. Pregnant dams were euthanized and embryos dissected from the uterus. Embryonic tails were collected for genotyping.

Adult animals were euthanized, and the skin was shaved and depilated with Surgicream. Back and belly skin, foot pads, and whisker pads were dissected.

All samples were immersion fixed for 15–30 minutes (X-gal) or overnight (immunostaining) in ice-cold 10% neutral buffered formalin (NBF).

Wholemount X-gal staining

Tissue was X-gal stained overnight at 37°C, fixed overnight in 10% NBF at 4°C, then photographed. Tissue was equilibrated in 30% sucrose/1× PBS, embedded in OCT, serially sectioned on a cryostat (25 μm), then counterstained with nuclear fast red solution.

Immunostaining

Tissue was equilibrated in sucrose, embedded and cryosectioned. Antibodies: rat anti-keratin 8 (TROMA1, DSHB) 1:20; chicken anti-β-galactosidase (AbCam, cat #ab9361) 1:1000; rabbit anti-Rab3c (Genetex, cat #13298) 1:1000; and rabbit anti-VGLUT2 (Synaptic Systems, cat #135402) 1:3000. Secondary antibodies (Jackson Immunochemicals) were all used at a 1:500 dilution. DAPI was used for counterstaining.

Results

Atoh1 expression in the skin during embryonic development

Atoh1^{LacZ} is an *Atoh1*-null allele that drives β-galactosidase expression under the control of the endogenous *Atoh1* locus, recapitulating *Atoh1* expression in all areas of the developing embryo (Ben-Arie et al., 2000; Bermingham et al., 1999; Yang et al., 2001). We compared X-gal staining patterns in the skin of heterozygous *Atoh1*$^{LacZ/+}$ (control) and *Atoh1*$^{LacZ/LacZ}$ (null) embryos. No X-gal staining was detectable in whisker follicles or body skin of E13.5 embryos of either genotype (data not shown). X-gal staining was first seen in whisker follicles of both genotypes at E14.5 (Fig. 1A, G). Developing touch domes of the body skin were first labeled with X-gal product at E15.5 in both genotypes (Fig. 1E, F, K, L).

Given the nearly identical appearance of the wholemount preparations, we were surprised to find clear differences between the X-gal staining patterns in skin tissue sections from *Atoh1*$^{LacZ/+}$ and *Atoh1*$^{LacZ/LacZ}$ embryos (Fig. 1B, D, F, H, J, L). Strong cellular staining was present in *Atoh1*$^{LacZ/+}$ embryos in the upper portions of whisker follicles and in multiple cells within developing touch domes of the body skin (Fig. 1B, D, F). As in the wholemount preparations, the overall distribution of X-gal staining was the same in *Atoh1*$^{LacZ/LacZ}$ embryos. However, complete labeling of single cells was replaced by punctate staining of lesser intensity (Fig. 1H, J, L). This quantitative decrease in X-gal staining of *Atoh1*$^{LacZ/LacZ}$ embryos is likely secondary to the loss of autoregulation of *Atoh1* expression in these animals (Helms et al., 2000). The maintenance of low levels of -galactosidase expression in epidermal cells of whisker follicles and touch domes of *Atoh1*$^{LacZ/LacZ}$ embryos and the presence of similar punctate staining in some epidermal cells of *Atoh1*$^{LacZ/+}$ embryos suggested that these cells might serve as Merkel cell precursors.

Atoh1$^{LacZ/LacZ}$ animals lack Merkel cells

We next compared the distribution of β-galactosidase protein with that of keratin 8 (Fig. 2), an early marker of Merkel cells (Moll et al., 1984;Vielkind et al., 1995). We first detected keratin 8 expression in whisker follicles of *Atoh1*$^{LacZ/+}$ embryos at E14.5. We counted the number of β-galactosidase-positive, keratin 8-positive and double-positive cells in the whisker follicles of E14.5 (n=3) and E15.5 (n=2) *Atoh1*$^{LacZ/+}$ embryos. At E14.5, we found 6 (13%) β-galactosidase-positive cells, 0 (0%) keratin 8-positive cells and 39 (87%) double-positive cells in seven whisker follicles. By E15.5 a large expansion of the Merkel cell population had occurred: we found 91 (27%) β-galactosidase-positive cells, 14 (4%) keratin 8-positive cells and 230 (69%) double-positive cells in seven whisker follicles. Keratin 8 expression in touch domes of body skin was first detected at E16.5 (data not shown), well after the onset of *Atoh1* expression (Fig. 1F, L). These data demonstrate that *Atoh1* expression precedes the onset of keratin 8 expression.

By contrast, we never found keratin 8-positive cells in E14.5 (n=3) or E15.5 (n=3) *Atoh1*$^{LacZ/LacZ}$ embryos (Fig. 2B-B‴), suggesting that *Atoh1* function is required to initiate keratin 8 expression during Merkel cell development. This agrees with our previous data demonstrating the absence of Merkel cells in adult animals harboring a conditional deletion of *Atoh1* (Maricich et al., 2009), but extends those data by showing that Merkel cells are missing from the earliest developmental times in *Atoh1*-null mice.

Merkel cells are not descended from the neural crest lineage

A previous report suggested that murine Merkel cells were descended from the neural crest (Szeder et al., 2003). We attempted to reproduce these findings by conditionally deleting *Atoh1* from neural crest cells using the *Wnt1*^{Cre} line, which drives *Cre* expression in neural crest and dorsal domains of the developing hindbrain and spinal cord beginning at E8 (Danielian et al., 1998). Conditional knockout (*Wnt1; Atoh1*^{CKO}; see Materials and Methods for mating strategy) animals died within 24–36 hours of birth, likely secondary to effects of *Atoh1* loss on the respiratory centers of the brainstem (SMM, unpublished).

The X-gal staining patterns in the skin of E16.5 *Wnt1; Atoh1*^{CKO} and control embryos were identical (Fig. 3A,B), and robust staining was present in whisker follicles and touch domes of both genotypes (Fig. 3A′, A″, B′, B″, C, E). In addition, all β-galactosidase-positive cells in the skin of P0 *Wnt1; Atoh1*^{CKO} newborns were also keratin 8-positive (Fig. 3D-D‴, F-F‴). Thus, the *Wnt1*^{Cre} driver is incapable of deleting *Atoh1* from the skin.

These data could be explained by inefficient recombination of the floxed *Atoh1* sequence in *Wnt1; Atoh1*^{CKO} animals. We performed two types of experiments to rule out this possibility. First, we examined wholemount X-gal staining in hindbrains of *Wnt1; Atoh1*^{CKO} embryos (Fig. S1). *Wnt1* is expressed throughout the rhombic lip, and it encompasses the expression domain of *Atoh1* both spatially and temporally. *Atoh1*$^{LacZ/LacZ}$ animals lose neurons and β-galactosidase expression from multiple nuclei in the hindbrain, including the external cuneate nuclei, cochlear nuclei, and external granular layer of the cerebellum (Wang et al., 2005). P0 *Wnt1; Atoh1*^{CKO} animals also lose *Atoh1*-lineal neurons in these hindbrain regions (Fig. S1B), demonstrating that efficient recombination occurs where *Cre* is expressed. Second, we crossed the *Wnt1*^{Cre} line to the *ROSA*^{R26R} reporter line (Soriano, 1999) to fate map neural crest cells in the skin. At E16.5, strong X-gal labeling was present in the head and peripheral nerves of the body but was absent from touch domes and foot pads (Fig. S2A-A‴). In the skin of adult *Wnt1*^{Cre}; *ROSA*^{R26R} mice, X-gal labeling was present in buccal pads, cutaneous nerves, foot pads and touch domes (Fig. S2B, D, F). We therefore examined the distribution of β-galactosidase and keratin 8 in over 100 whisker pad, hairy skin and foot pad sections from two *Wnt1*^{Cre}; *ROSA*^{R26R} mice to determine whether any cells co-expressed these proteins (Fig. S2C-C‴, E-E‴, G-G‴). The β-galactosidase protein distribution was identical to the distribution of X-gal staining in all skin regions (Fig. S2B and C′, D and E′, F and G′). β-galactosidase-positive and keratin 8-positive cells were found in close association in all three regions of the epidermis, but no double-labeled cells were present in any region of the skin in adult *Wnt1*^{Cre}; *ROSA*^{R26R} mice. These data verify that Merkel cells are not derived from the *Wnt1*-expressing neural crest lineage.

Merkel cells are derived from the skin lineage

The *Krt14*^{Cre} line drives high levels of *Cre* expression in the basal layer of the epidermis (Dassule et al., 2000; Vassar et al., 1989; Wang et al., 1997). We verified this expression pattern in *Krt14*^{Cre}; *ROSA*^{R26R} embryos and adult animals (Fig. S3). X-gal staining was first detected at E13.5 in limited regions of the body skin and face (Fig. S3A-A″). Expression increased throughout the skin by E14.5, especially in developing hair follicles (Fig. S3B-B″). By E15.5, the entire skin surface expressed the reporter (Fig. S3C-C″). This level and distribution of

expression was maintained through adulthood (Fig. S3D-D‴). Thus, *Krt14*^{Cre} drives *Cre* expression in all skin regions prior to the onset of *Atoh1* expression. We proceeded to generate *Krt14; Atoh1*^{CKO} animals, which are born in the expected Mendelian ratio, survive to adulthood, and display no overt phenotypes.

Wholemount X-gal stained skin specimens from control and *Krt14; Atoh1*^{CKO} embryos and adults displayed only minor differences in β-galactosidase expression driven by the *Atoh1*^{LacZ} locus (Fig. 4A, D, G, J, M, P). However, tissue sections from adult *Krt14; Atoh1*^{CKO} animals revealed a distribution of X-gal staining reminiscent of that seen in *Atoh1*$^{LacZ/LacZ}$ embryos (Fig. 1H, J, L). Specifically, punctate X-gal staining was present in touch domes and rete ridges (epithelial invaginations) of the feet in *Krt14; Atoh1*^{CKO} animals, in contrast to the heavy labeling of individual cells present in control animals (Fig. 4B, E, N, Q). Strong labeling of epidermal cells in the whisker follicles was also present in control and *Krt14; Atoh1*^{CKO} animals, but individual heavily-labeled cells were present only in control animals (Fig. 4H, K).

We next used immunocytochemistry to compare the distribution of Merkel cell markers with that of β-galactosidase in the skin of *Krt14; Atoh1*^{CKO} animals. In addition to keratin 8, we also examined Rab3c and VGLUT2, two synaptic vesicle proteins that robustly label Merkel cells (Haeberle et al., 2004; Hitchcock et al., 2004; Nunzi et al., 2004). Punctate staining for β-galactosidase was found in all touch domes, whisker follicles and foot rete ridges of *Krt14; Atoh1*^{CKO} animals, consistent with the X-gal staining pattern in these regions (Fig. 4F′, L′, R′ and Fig. 5B′, D′). However, expression of all three Merkel cell marker proteins was absent from all skin areas of *Krt14; Atoh1*^{CKO} animals (Fig. 4F″, L″, R″ and Fig. 5B″, D″). These data demonstrate that Merkel cells are absent from the skin of *Krt14; Atoh1*^{CKO} animals.

Discussion

Our data provide the first direct evidence that mammalian Merkel cells are descended from the epidermal lineage. These findings are consistent with indirect evidence for an epidermal origin of Merkel cells in humans (Compton et al., 1990; Moll et al., 1990; Moll et al., 1986) and amphibians (Tweedle, 1978). Interestingly, data from chick/quail chimera studies suggest that avian Merkel cells are derived from the neural crest lineage (Grim and Halata, 2000). Whether mammals and birds truly demonstrate a divergence in the origins of these cells remains unclear.

Our data are somewhat surprising given that a previous study that examined whisker pads from E16.5 *Wnt1*^{Cre}; *ROSA*^{R26R} mice reached the conclusion that Merkel cells were derived from the neural crest (Szeder et al., 2003). Our analysis was much more comprehensive, and included examination of the foot pads, hairy skin, and whisker pads from mice of multiple ages in both lineage tracing and conditional knockout experiments. In addition, our data show that the neural crest and Merkel cell lineages are in close contact in all regions of the skin (Fig. S2B, D, F). This raises the possibility that the punctate β-galactosidase and keratin 8 co-labeling seen in the previous study represents contacts between these cells rather than true overlap of these markers within Merkel cells themselves.

Our data establish *Atoh1* as the earliest known marker of the Merkel cell population. Simple keratins such as keratins 8 and 18 have been reported to be expressed by murine Merkel cells in the whisker pad as early as E12 and in the body skin at E14 or E15 (Pasche et al., 1990; Vielkind et al., 1995). We first detected keratin 8 expression in Merkel cells of the whisker pads at E14.5, but we did not detect significant keratin 8 expression in touch domes at the embryonic ages that we examined. *Atoh1* expression preceded the onset of keratin 8 expression in both regions, and keratin 8 expressing cells always formed a subset of the *Atoh1*-positive cell population. The early expression of *Atoh1*, the loss of Merkel cells in animals with

constitutive (*Atoh1*$^{LacZ/LacZ}$) and conditional (*Krt14; Atoh1*^{CKO}) deletions of *Atoh1*, and the importance of *Atoh1* for the specification of other cell populations (Ben-Arie et al., 1997; Bermingham et al., 1999; Bermingham et al., 2001; Yang et al., 2001) all suggest that the gene plays an important role in the early specification of Merkel cells.

The Merkel cell population is morphologically heterogeneous (Halata et al., 2003; Nakafusa et al., 2006). This heterogeneity extends to the expression of different neural (neurofilament proteins, nerve growth factor receptor, synaptophysin) and epithelial (villin) proteins by Merkel cells located in different body regions (Eispert et al., 2009). These findings led to the suggestion that some Merkel cells might function in mechanoreception of tactile stimuli, while other "Merkel-like" cells form part of the diffuse neuroendocrine system involved with modulation of peripheral neural responses (Halata et al., 2003). Our data suggest that the morphological and molecular heterogeneity of Merkel cells arise after the cells are specified, as all Merkel cells in the skin are derived from the epidermis and are dependent upon *Atoh1* function. The mechanisms involved in subspecialization of this cell population remain unknown, but could be secondary to local environmental factors.

One intriguing finding of our study is the presence of β-galactosidase expression driven from the *Atoh1* locus in whisker pads, touch domes and foot pad rete ridges of embryonic *Atoh1*$^{LacZ/LacZ}$ and adult *Krt14; Atoh1*^{CKO} animals. This diffuse expression is reminiscent of *atonal* expression during the formation of *Drosophila* chordotonal organs. Chordotonal organs are cuticular mechanoreceptors that arise from sensory organ precursor cells, which are specified by *atonal* (Jarman et al., 1993). Initially, all of the cells in the presumptive receptor field express *atonal* at low levels. Stochastic events cause up-regulation of *atonal* in a single cell, which then becomes the sensory organ precursor, while lateral inhibition mediated by the Notch signaling pathway insures that the remaining cells adopt support cell fates (Bertrand et al., 2002). Notch signaling also plays a role in the development of mammalian skin and hair follicles (Fuchs and Raghavan, 2002), and it is critically important for the development of *Atoh1*-dependent cell populations such as inner ear hair cells and granule cells of the cerebellum (Gazit et al., 2004; Lanford et al., 1999; Woods et al., 2004).

We propose that the β-galactosidase expression seen in the skin of *Atoh1*$^{LacZ/LacZ}$ embryos and adult *Krt14; Atoh1*^{CKO} animals identifies developmental fields equivalent to those that give rise to chordotonal organs of the fly. *Atoh1*-null epidermal cells of these fields are unable to produce Merkel cells because they lack *Atoh1*, and they are therefore arrested in a primordial developmental field state where all of the cells attempt to express the gene. This interpretation is supported by the fact that the observed intensity of epidermal β-galactosidase expression (high levels in the whisker pads and low levels in other areas) in adult *Krt14; Atoh1*^{CKO} animals is positively correlated with the normal number of Merkel cells found in those regions. The location and maintenance of these proposed fields are consistent with the observation that Merkel cell numbers are dynamic in adult mammals during the hair cycle and in response to denervation and reinnervation (English et al., 1983; Nakafusa et al., 2006; Nurse et al., 1984). In addition, new Merkel cells must arise from a progenitor population because adult Merkel cells are non-mitotic (Merot and Saurat, 1988; Moll et al., 1996; Vaigot et al., 1987). Finally, dysregulation of gene expression in this proposed developmental field secondary to genetic and environmental insults could lead to the generation of Merkel cell carcinoma. Further studies are needed to determine what roles the Notch signaling pathway may play in the establishment and/or maintenance of this presumptive developmental field, and if these cells remain competent to produce Merkel cells in adult *Atoh1* conditional knockout animals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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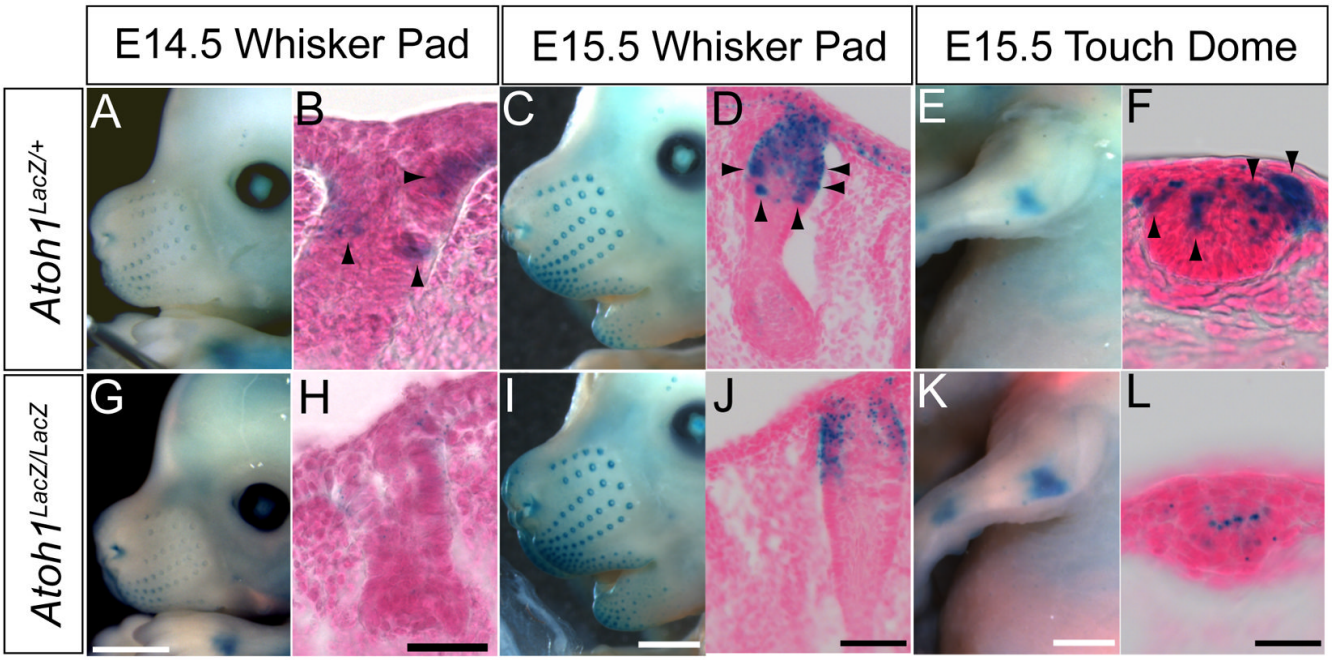

Fig. 1.

β-galactosidase expression driven from the *Atoh1*^{LacZ} locus is altered in the skin of *Atoh1*$^{LacZ/LacZ}$ embryos. (A, B, G, H) Whisker pads from E14.5 *Atoh1*$^{LacZ/+}$ (A, B) and *Atoh1*$^{LacZ/LacZ}$ (G, H) embryos. (C, D, I, J) Whisker pads from E15.5 *Atoh1*$^{LacZ/+}$ (C, D) and *Atoh1*$^{LacZ/LacZ}$ (I, J) embryos. Note the increased amount of X-gal staining in both genotypes compared to E14.5. (E, F, K, L) Forelimb, body skin and touch domes of E15.5 *Atoh1*$^{LacZ/+}$ (E, F) and *Atoh1*$^{LacZ/LacZ}$ (K, L) embryos. Head is at the top of (E, K), and dorsal is to the right. Arrowheads in (B, D, F) denote single cells that are completely labeled by X-gal reaction product. Only punctate X-gal staining is present in whisker follicles and touch domes of *Atoh1*$^{LacZ/LacZ}$ embryos. Scale bars: 1mm (A, C, G, I), 2.5 μm (E, K), 25 m (B, H), 100 μm (D, J), 30 μm (F, L).

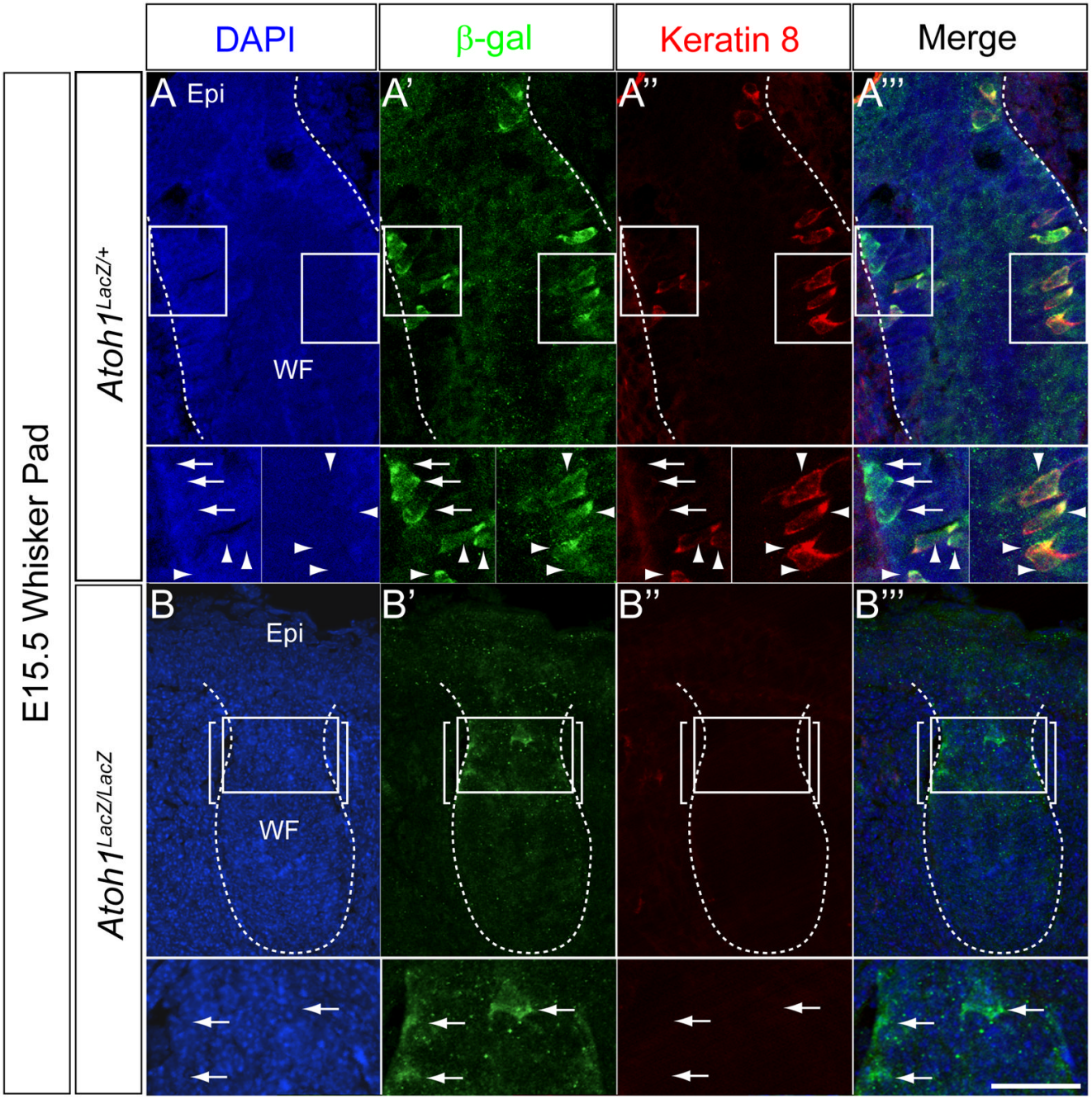

Fig. 2.

Merkel cells are absent from the skin of *Atoh1*$^{LacZ/LacZ}$ embryos. Tissue sections from whisker pads of E15.5 *Atoh1*$^{LacZ/+}$ and *Atoh*$^{LacZ/LacZ}$ embryos immunostained for β-galactosidase (A′, B′) and keratin 8 (A″, B″). Arrowheads denote double-labeled cells; arrows denote β-galactosidase-positive, keratin 8-negative cells; brackets in (B′-B‴) denote the region of punctate β-galactosidase immunoreactivity; dotted lines identify the epidermal/dermal boundary; boxes denote regions shown in subpanels. Epi – epidermis, WF – whisker follicle. Scale bar: 30 μm (panels), 22.5 μm (subpanels).

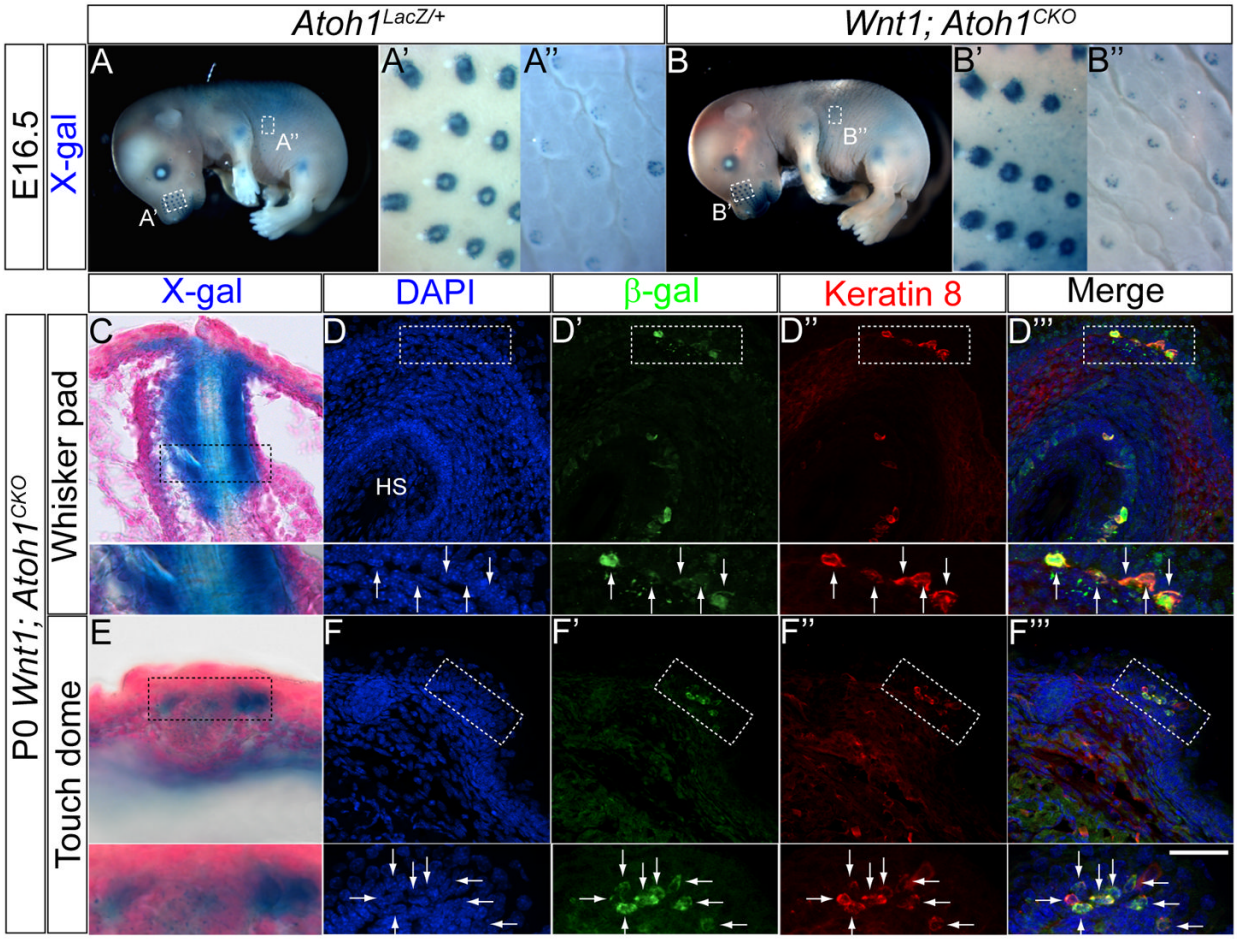

Fig. 3.

Merkel cells are present in the skin of *Wnt1; Atoh1*^{CKO} animals. (A-B″) Wholemount X-gal staining of E16.5 *Wnt1*$^{+/+}$*; Atoh1*$^{LacZ/flox}$ (*Atoh1*$^{LacZ/+}$; A-A″) and *Wnt1; Atoh1*^{CKO} (B-B″) embryos. Boxed areas in (A) and (B) are shown in (A′, A″) and (B′, B″), respectively. (C-F‴) Whisker pad (C-D‴) and touch dome (E-F‴) sections from P0 *Wnt1; Atoh1*^{CKO} animals stained with X-gal (C, E) or immunostained with β-galactosidase and keratin 8 (D-D‴, F-F‴). Dotted lines demarcate the regions shown in subpanels. Merkel cells are present in both tissues. HS – hair shaft. Scale bar: 50 μm C-F‴ main panels, 25 μm subpanels.

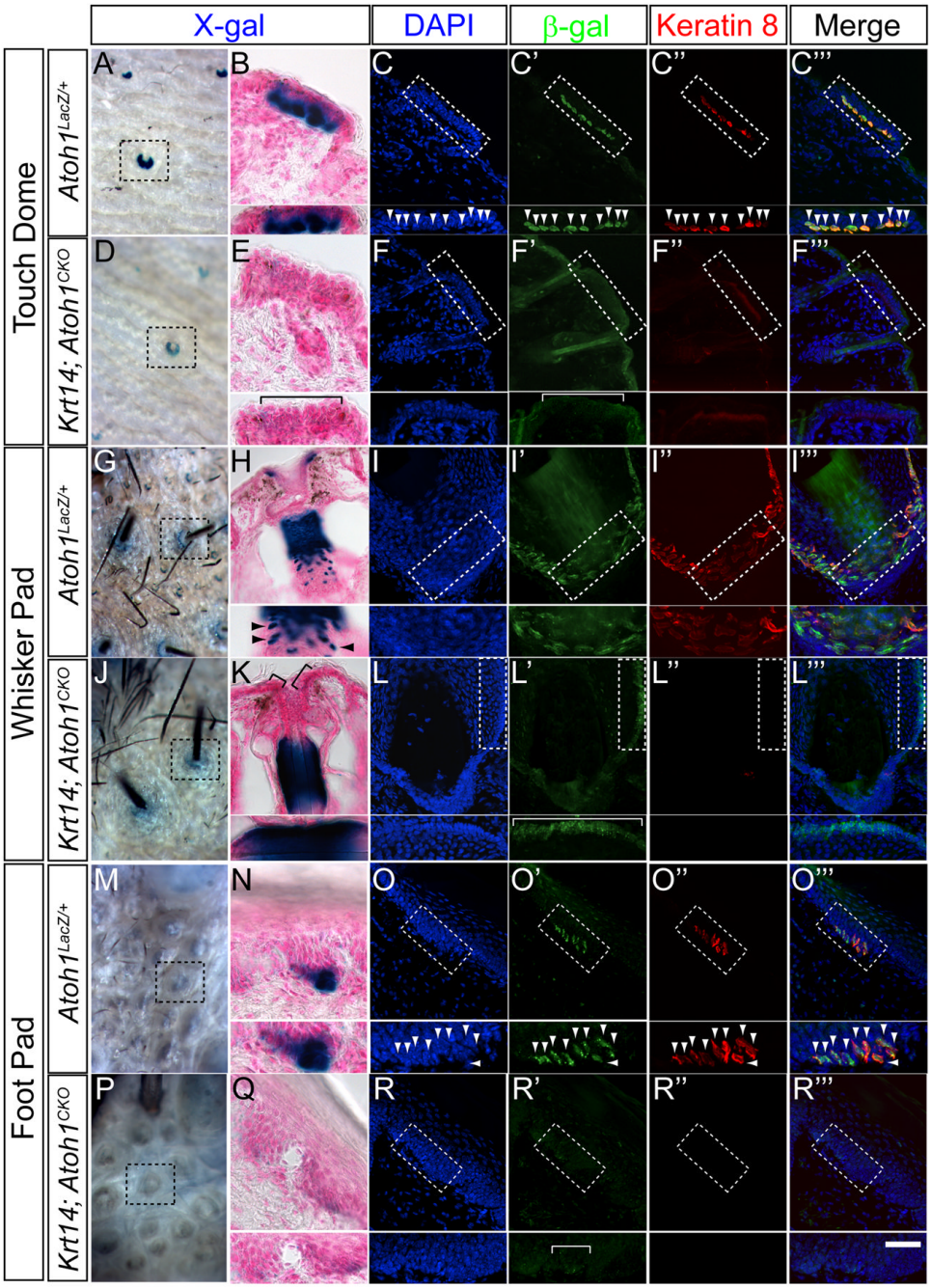

Fig. 4.

Merkel cells are absent from the skin of adult *Krt14; Atoh1*^{CKO} animals. All tissue was obtained from 22–25 day old mice. Tissue was prepared for wholemount X-gal staining (A, B, D, E, G, H, J, K, M, N, P, Q) or immunostaining (C-C‴, F-F‴, I-I‴, L-L‴, O-O‴, R-R‴). (A-F‴) Back skin from *Krt14*$^{+/+}$*; Atoh1*$^{LacZ/flox}$ (*Atoh1*$^{LacZ/+}$; A-C‴) and *Krt14; Atoh1*^{CKO} (D-F‴) animals. (H-L‴) Whisker pad from *Krt14*$^{+/+}$*; Atoh1*$^{LacZ/flox}$ (G-I‴) and *Krt14; Atoh1*^{CKO} (J-L‴) animals. (M-R‴) Foot pad from *Krt14*$^{+/+}$*; Atoh1*$^{LacZ/flox}$ (M-O‴) and *Krt14; Atoh1*^{CKO} (P-R‴) animals. Boxes in (A, D, G, J, M, P) denote areas shown in (B, E, H, K, N, Q), respectively; boxes in immunostaining panels denote regions shown in subpanels.

Arrowheads in subpanels denote double-labeled cells. No keratin 8-positive cells were found in *Krt14; Atoh1*^{CKO} animals in any skin region. Punctate X-gal and β-galactosidase staining (brackets) is present in epidermal cells of touch domes, whisker follicles and foot pads in *Krt14; Atoh1*^{CKO} animals. Scale bars: 300 μm (A, D, G, J, M, P), 50 μm all other panels, 25 μm subpanels.

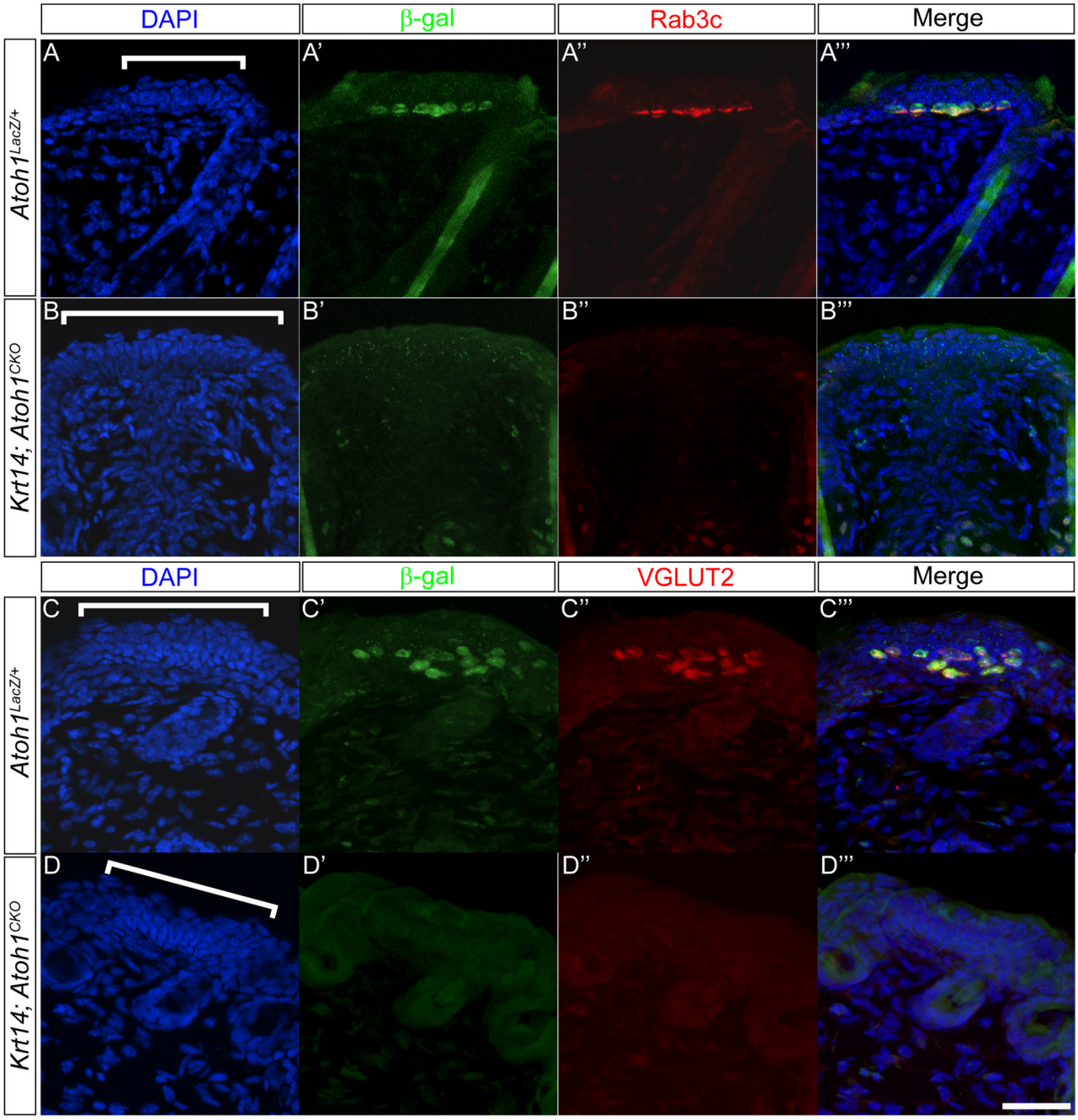

Fig. 5.

Rab3c and VGLUT2 are not expressed in touch domes of *Krt14; Atoh1*^{CKO} animals. Hairy skin from P25 *Krt14*$^{+/+}$*; Atoh1*$^{LacZ/+}$ (*Atoh1*$^{LacZ/+}$) (A-A‴, C-C‴) and *Krt14; Atoh1*^{CKO} (B-B‴, D-D‴) mice was immunostained for β-galactosidase, Rab3c, and/or VGLUT2. Punctate β-galactosidase staining is present in epidermal cells overlying touch domes in both genotypes, but Rab3c and VGLUT2 labeled cells are absent in *Krt14; Atoh1*^{CKO} animals. Brackets denote touch domes. Scale bar: 50 μm.