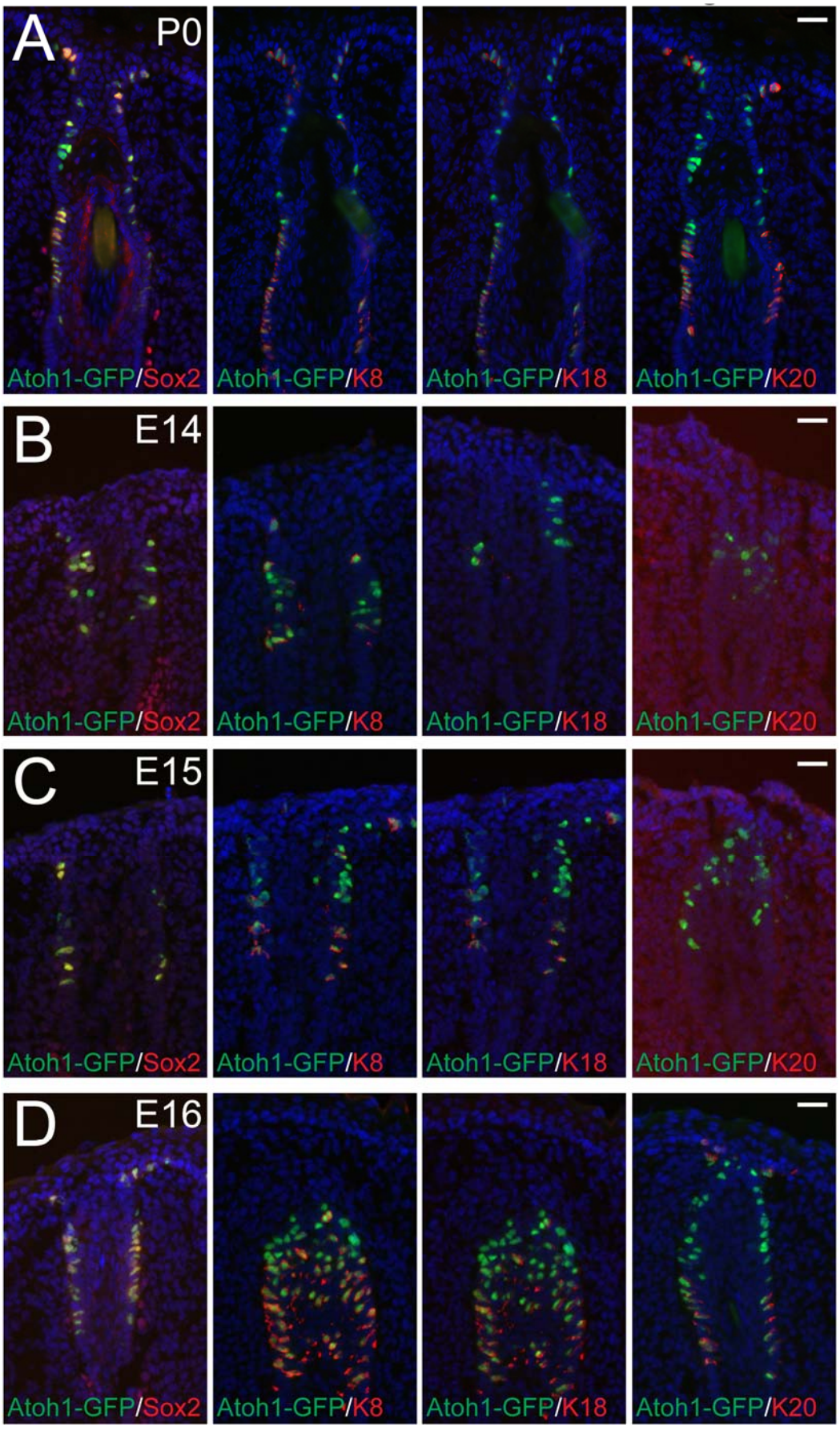
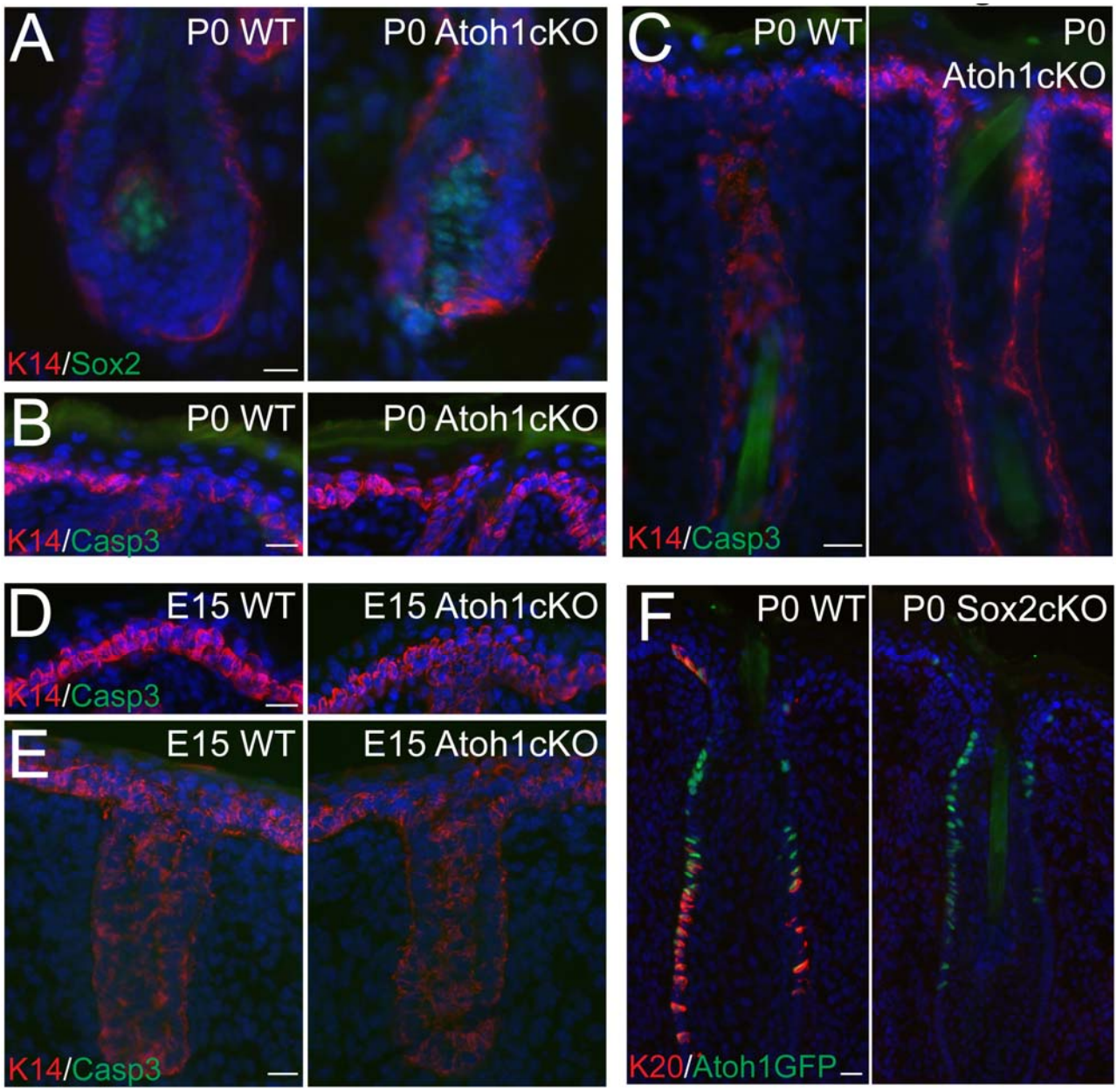
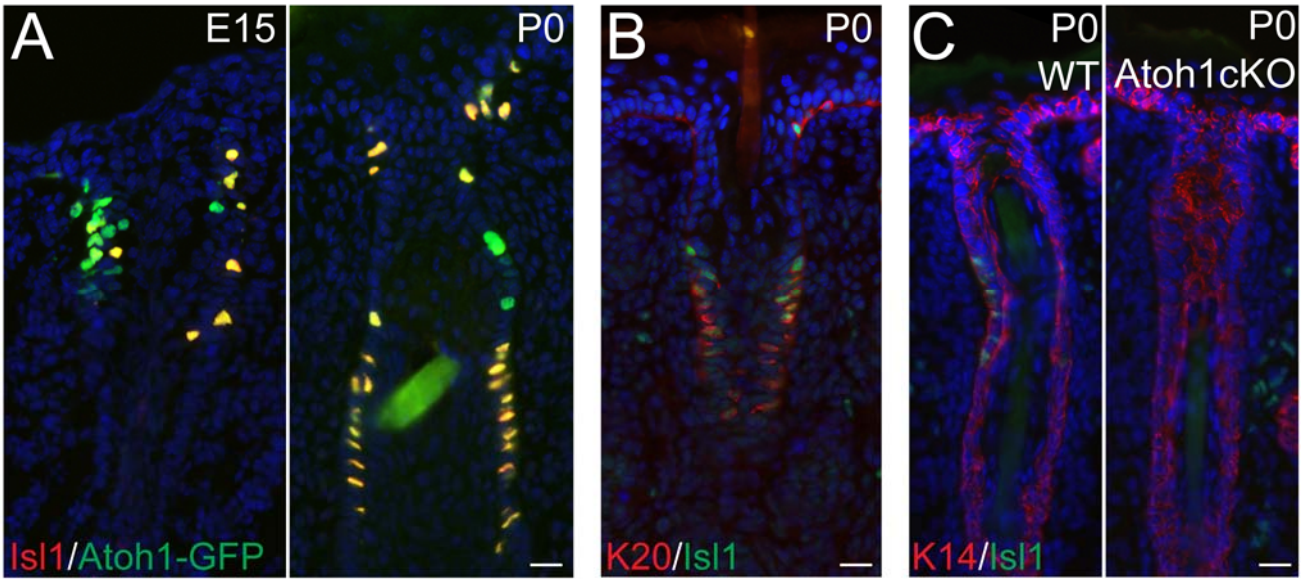

A
P0
Atoh1-GFP/Sox2
Atoh1-GFP/K8
Atoh1-GFP/K18
Atoh1-GFP/K20
B
E14
Atoh1-GFP/Sox2
Atoh1-GFP/K8
Atoh1-GFP/K18
Atoh1-GFP/K20
C
E15
Atoh1-GFP/Sox2
Atoh1-GFP/K8
Atoh1-GFP/K18
Atoh1-GFP/K20
D
E16
Atoh1-GFP/Sox2
Atoh1-GFP/K8
Atoh1-GFP/K18
Atoh1-GFP/K20

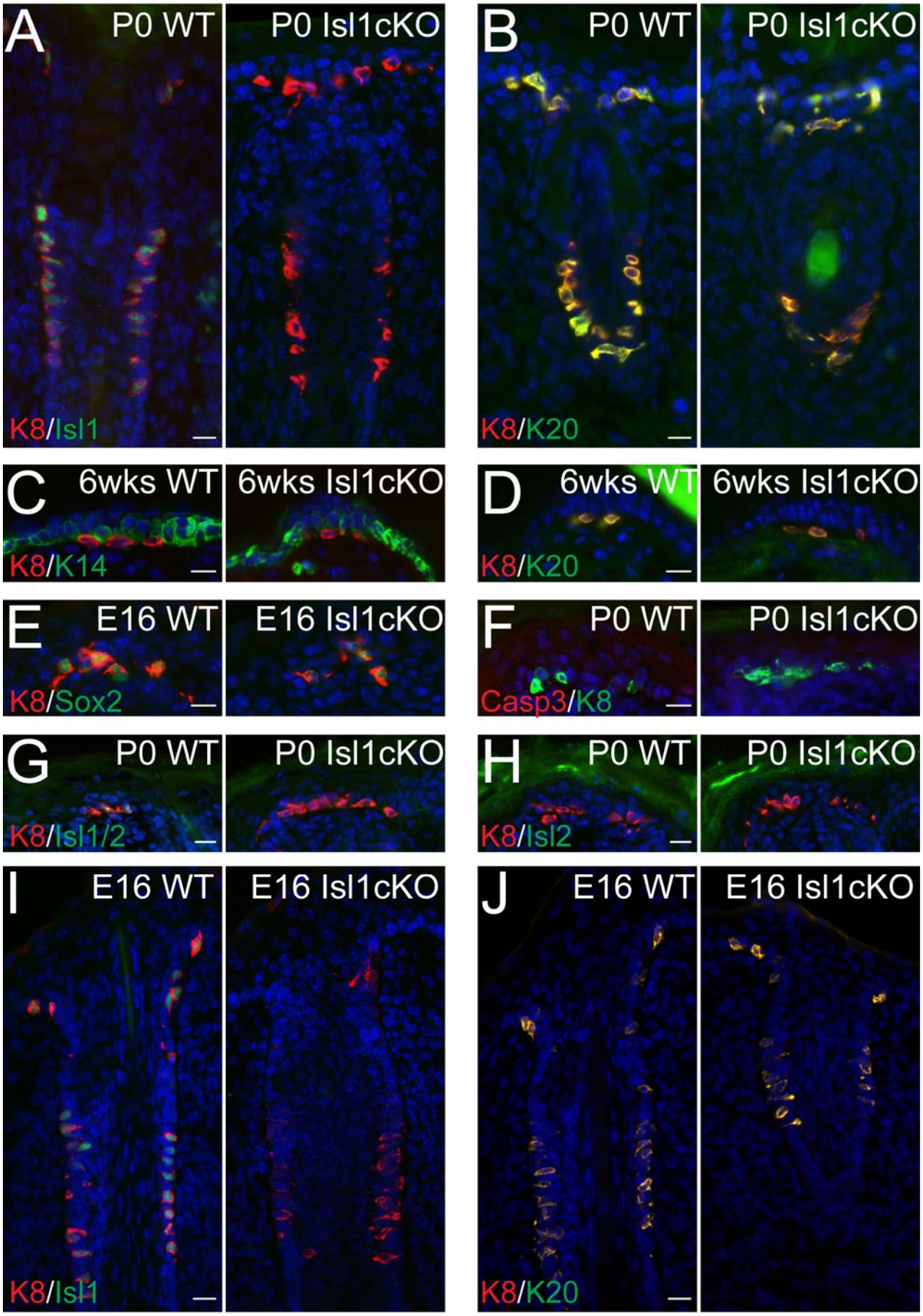
Supplementary Figure 1 Merkel cell differentiation is a temporal maturation process. (A-D) Immunofluorescence (IF) of whisker follicles with antibodies against Atoh1-GFP and Sox2 (left), K8 (center-left), K18 (center-right), and K20 (right) at P0 (A) E14 (B), E15 (C), and E16 (D) shows progressive accumulation of markers through development. Note that this process is advanced by one day compared to back skin. Scale bars are 50 µm.



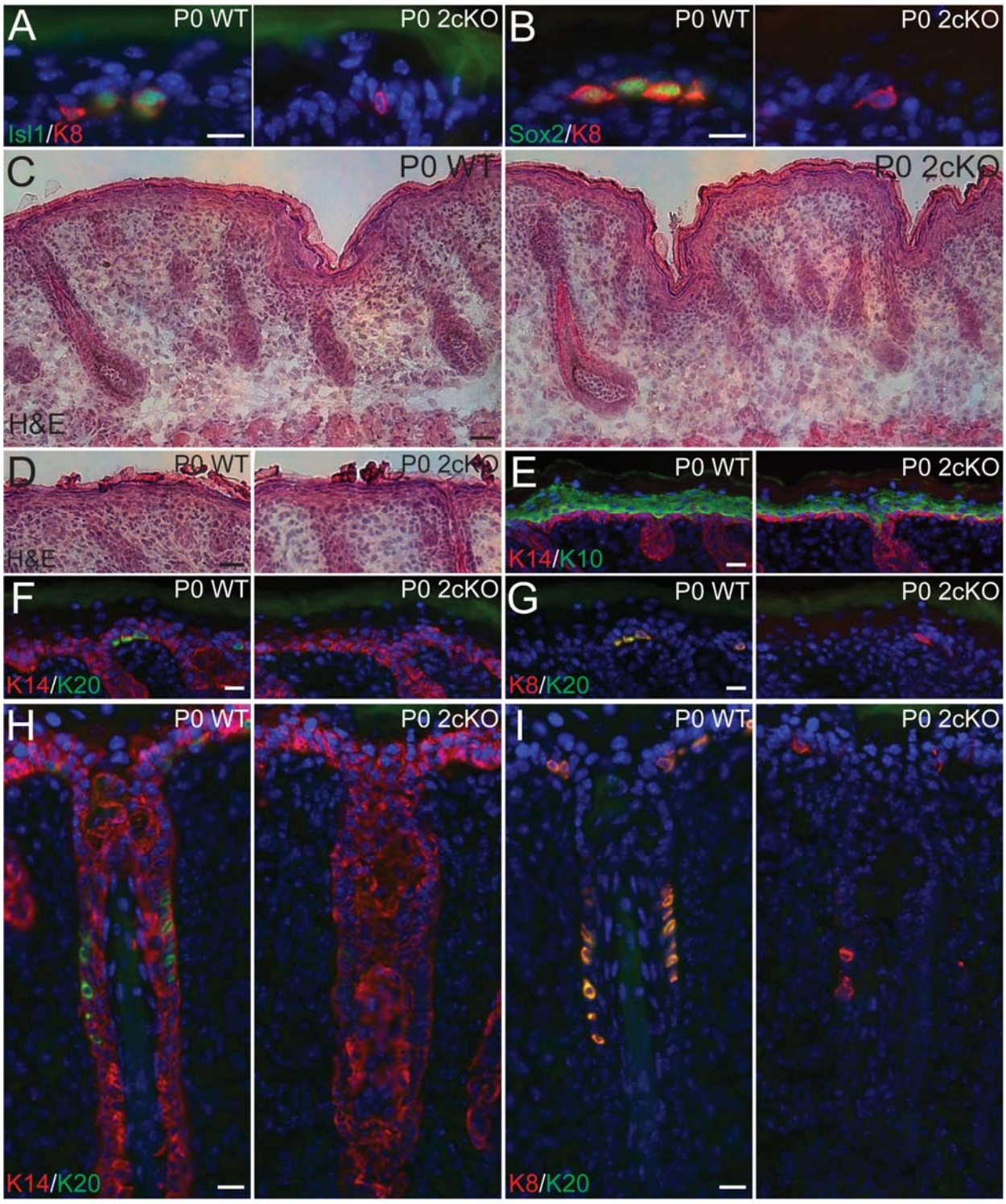
Supplementary Figure 2 Atoh1 is essential for Merkel cell specification, while Sox2 is required for late phase of Merkel cell differentiation. (A) IF with antibodies against Sox2 shows that mesenchymal Dermal Papilla (DP) cells are unaffected in the Atoh1cKO mice. (B-E) Analysis of P0 (B-C) and E15 (D-E) WT and Atoh1cKO mice showed no differences in apoptosis, as measured by IF against Activated Caspase3, in either back skin (B, D) or whisker follicles (C, E). (F) IF analysis of P0 WT and Sox2cKO whisker follicles shows a complete lack of K20+ cells, but only a partial loss of Atoh1-GFP+ cells. Scale bars are 25 μm.



Supplementary Figure 3 Transcription factor Isl1 is expressed during the middle stages of Merkel cell differentiation. (A) IF analysis of whisker follicles showing the onset of Isl1 expression in Atoh1-GFP+ Merkel cells at E15 and a complete overlap by P0. (B) Further analysis shows that Isl1 is expressed in fully differentiated K20+ Merkel cells of the whisker follicle at P0. (C) Analysis of WT and Atoh1cKO whisker follicles at P0 revealed a complete absence of Isl1 in the Atoh1cKO mice. Scale bars are 25 µm.

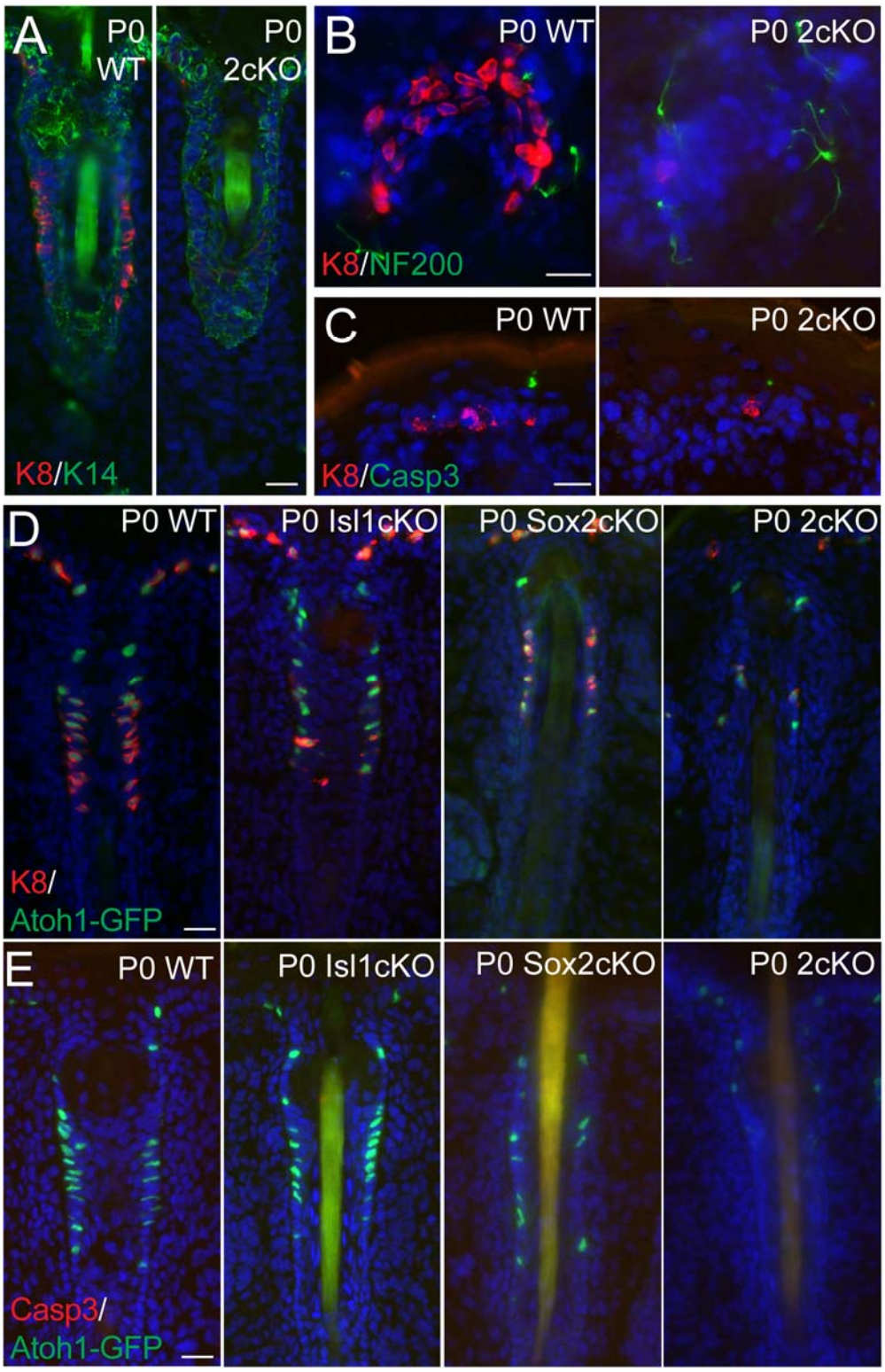

A
P0 WT
P0 Isl1cKO
K8/Isl1
B
P0 WT
P0 Isl1cKO
K8/K20
C
6wks WT
6wks Isl1cKO
K8/K14
D
6wks WT
6wks Isl1cKO
K8/K20
E
E16 WT
E16 Isl1cKO
K8/Sox2
F
P0 WT
P0 Isl1cKO
Casp3/K8
G
P0 WT
P0 Isl1cKO
K8/Isl1/2
H
P0 WT
P0 Isl1cKO
K8/Isl2
I
E16 WT
E16 Isl1cKO
K8/Isl1
J
E16 WT
E16 Isl1cKO
K8/K20

Supplementary Figure 4 Isl1 is not required for Merkel cell development. (A) IF analysis of P0 WT and Isl1cKO whisker follicles confirmed the loss of Isl1 from K8+ Merkel cells. (B) Compared to WT, Isl1cKO whisker follicles showed no difference in the number of K8+ or K20+ Merkel cells. (C-D) IF analysis of Merkel cell markers K8 (C) and K20 (D) in epidermis of 6-week-old mice shows that Merkel cells are maintained through adulthood in Isl1cKO mice. (E) IF with antibodies against Sox2 and K8 shows that Sox2 expression is unaffected in Isl1cKO mice at E16. (F) No differences in apoptosis were seen, as detected by IF against Active Caspase3, between WT and Isl1cKO mice. (G-H) IF using antibodies against both Isl1 and Isl2 (Isl1/2) (G) or Isl2 alone (H) showed that there was no expression of the Isl1 homolog in Isl1cKO epidermis. (I) IF analysis of E16 whisker follicles confirmed that Isl1 was absent from K8+ Merkel cells in the Isl1cKO mice. (J) At E16, Isl1cKO whisker follicles contain K8+ or K20+ Merkel cells. Scale bars are 25 μm.



Supplementary Figure 5 Sox2 and Isl1 cooperate to orchestrate Merkel cell differentiation. (A-B) IF with antibodies against Isl1 (A) or Sox2 (B) confirmed the loss of these proteins from the epidermal Merkel cells in Sox2 Isl1 2cKO mice. (C-D) Histological analysis with hematoxylin and eosin showed no defects in hair follicle (C)

or suprabasal layer (D) formation in the 2cKO mice. (E) Additional analysis with antibodies against the suprabasal marker K10 showed no defect in spinous layer formation. (F-I) IF staining of WT and 2cKO back skin (F-G) and whisker follicles (H-I) at P0 shows a complete loss of K20+ Merkel cells (F, H) and a drastic reduction in the number of K8+ cells (G, I). Scale bars are 50 µm for panels C-D and 25 µm for all others.



Supplementary Figure 6 Sox2 and Isl1 cooperate to orchestrate Merkel cell differentiation. (A) Removal of Isl1 and Sox2 in mice results in dramatic loss of K8 + cells in the whisker follicles. IF analysis of P0 WT and 2cKO whisker follicles

showing a reduction in the number of K8+ cells present. (B) WMIF with antibodies against K8 and NF200 show that neuronal innervation remains intact in the absence of K8+ Merkel cells in 2cKO epidermis. (C) No differences were seen in apoptosis, as measured by IF for active Caspase3. (D) IF analysis of WT, Isl1cKO, Sox2cKO, and Isl1+Sox2 2cKO whisker follicles showing a reduction in the number of Atoh1GFP+ K8+ cells in the 2cKO whiskers. (E) WT, Isl1cKO, Sox2cKO, and Isl1+Sox2 2cKO whisker follicles showed no differences in apoptosis, as measured by IF against Activated Caspase3. Scale bars are 25 µm.