SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1 Number of Merkel cells is increased in Ezh1/2 2KO epidermis, paws, whisker IFEs and ventral skin. **(A-F)** Immunofluorescence (IF) analysis of 90-day old grafted back skin (A), P0 ventral skin (B), P0 paw (C), P0 whiskers IFE (D), and P0 whisker follicles (E) using antibodies against K18 or K20 shows an increase in the number of Merkel cells in Ezh1/2 2KO mice in all areas except for whisker follicles. Quantifications are presented in (F) (WT/2KO Whisker follicle p=0.6128, total length analysed: 4.4/2.1mm; Whisker IFE p<0.0001, total length analysed: 7.2/5.4mm; Paw p<0.0001, total length analysed: 13.3/13.3mm. Statistics for Epidermis are in main figure 1. **(G)** H&E analysis of E15 WT and Ezh1/2 2KO skin shows normal development of guard hairs. The number of guard hairs in WT and Ezh1/2 2KO skin is comparable, as shown by quantifications at right (WT/2KO p=0.2119, total length analysed: 86/87mm. Scale bars are 50um.

Supplementary Figure 2 Accelerated formation of Merkel cells in Ezh1/2 2KO skin. **(A)** IF using antibodies against H3K27me3 and the melanocyte marker CD117 shows that H3K27Me3 is not lost from melanocytes in Ezh1/2 2KO skin. **(B)** IF studies of WT and Ezh1/2 2KO mice expressing K14-GFP-H2B using antibodies against CD117 show that the non-K14 derived melanocytes do not express GFP. (**C**) Greyscale images showing GFP channel from Figure 2B shows the presence of GFP signal in all K18(+) Merkel cells in 2KO skin. Intensity analysis in Figure 2B incorporated GFP intensity from all K18+ cells and is described in the Methods. **(D-H)** IF analysis of WT and Ezh1/2 2KO mice shows that K18(+) and K20(+) Merkel cells are postmitotic throughout stages of Merkel cell lineage specification. The analysis was performed on back skin (D-F), paw (G) or whisker (H) regions at E16 (D), P0 (E, G, H) and P14 (F) using antibodies against BrdU (D-G) or Ki67 (H). **(I)** Analysis of Merkel cell organization in WT and Ezh1/2 2KO skin reveals that there are no changes in the number of Merkel cells per cluster (E, WT/2KO p=0.5571, total length analysed: 81/84mm). However, the number of clusters is

increased in Ezh1/2 2KO skin (F, WT/2KO $p<0.0001$, total length analysed: 133/110mm), suggesting that these clusters are developing normally but specified excessively. Corresponding IF images are presented in Figure 1A and 1B. Scale bars are 50um.

Supplementary Figure 3 Sox2 is expressed in Merkel cells and epidermal progenitors in Ezh1/2 2KO skin. (A) Table shows raw numbers of hybridization signals of total cDNAs isolated from WT and Ezh1/2 2KO basal cells for top three genes expressed in Merkel cells. Please note that none of the genes are upregulated in 2KO epidermis suggesting the purity of FACS-isolated populations and confirming the selective activation of a few Merkel genes in 2KO basal cells. A: absent, M: marginal, P: present. (B) In vitro ChIP-QPCR shows the presence of Ezh1 (left) and Ezh2 (right) at the Sox2 gene. Cultured Ezh1KO and Ezh2cKO keratinocytes were used as negative controls for antibody specificity. **(C)** IF analysis using antibodies against K18 and Sox2 shows that K18(+) Merkel cells coexpress Sox2, and that Sox2 is also expressed in epidermal progenitors (arrows) in Ezh1/2 2KO skin confirming RT-QPCR data. Scale bar is 50um.

Supplementary Figure 4 Sox2 is required for proper Merkel cell development. **(A-E)** IF analysis of P0 whisker IFE (J-K), P0 paw (L), and P0 whisker follicles (M) using antibodies against K20 or Rab3c shows a decrease in the number of fully differentiated Merkel cells in Sox2cKO mice in all analysed regions. Quantifications are presented in (N) (WT/Sox2cKO Whisker follicle $p<0.0001$, total length analysed: 4.4/1.3mm; Whisker IFE $p=0.0371$, total length analysed: 7.2/2.5mm; Paw $p=0.0064$, total length analysed: 13.3/3.3mm. Statistics for Epidermis are in main figure 4. **(F)** Sox2cKO mice show no gross developmental defects. **(G)** Normal differentiation of hair types was observed in both WT and Sox2cKO skin. **(H-I)** Epidermal differentiated layers develop normally in Sox2cKO skin, as shown by IF using antibodies against Keratin10 and Loricrin. **(J)** IF studies show the presence of NF200 neurons in WT and Sox2cKO skin. **(N-S)** IF studies of E15, E16, P0 and P22 WT and Sox2cKO mice with

antibodies against activated caspase3 (Ac-Casp3) confirm that there is no increase in apoptosis in Sox2cKO skin throughout stages of Merkel cell development. Body regions analyzed include back skin (A-C), paws (D, F), whisker interfollicular epidermis (E, G), and whisker follicles (H-I). A rare apoptotic cell present in E16 WT skin serves as a positive control for antibody staining. GH: Guard Hair; AU: Auchene; ZZ: Zigzag.

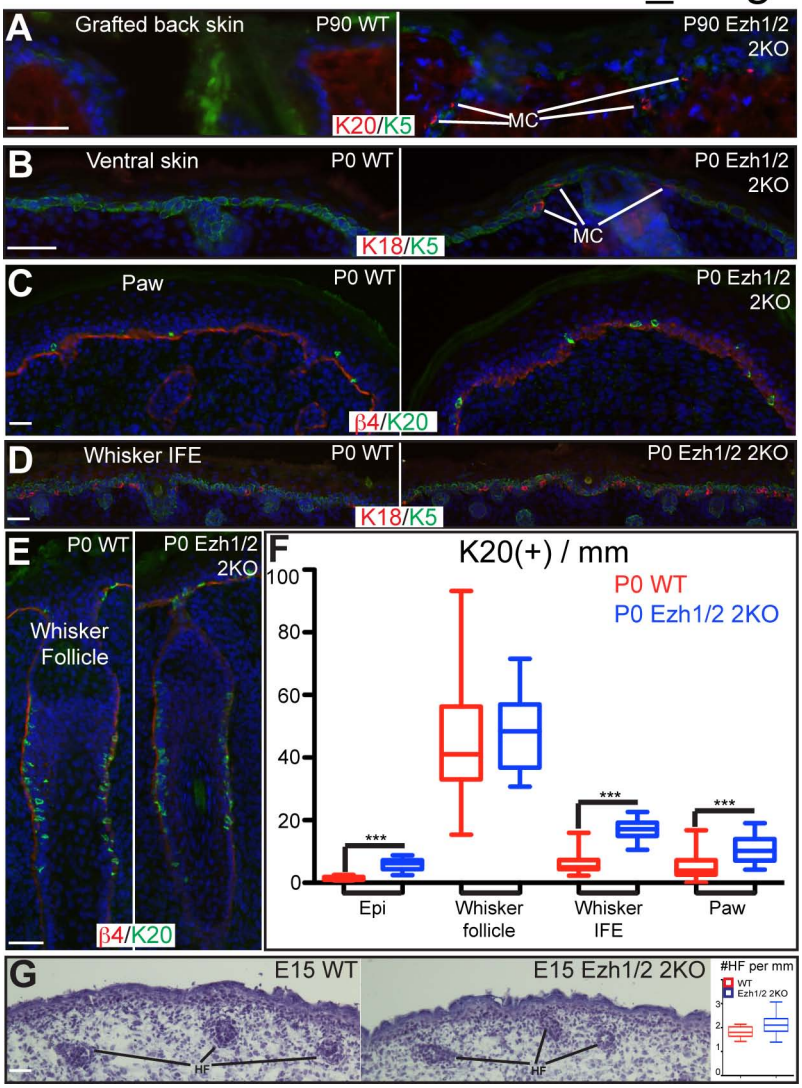
Supplementary Figure 5 Sox2 regulates expression of both Atoh1 and Isl1. **(A)** IF using antibodies against K20 shows dramatic decrease in the number of Merkel cells in Atoh1-GFP Sox2cKO skin. Quantifications are shown at right. WT/Sox2cKO p=0.0014, total length analysed: 67/44mm. **(B)** FACS analysis of P2 WT and Sox2cKO mouse skin expressing an Atoh1-GFP fusion protein shows a reduction in the number of Atoh1-GFP(+) cells. Graph at right also shows reduction in Atoh1-GFP mean fluorescence intensity, normalized to WT levels (p=0.0028). (**C**) *In vivo* ChIP-qPCR analysis shows the presence of Ezh1/2-dependent H3K27me3 histone mark at the *Atoh1* gene confirming published ChIP-seq data showing that *Atoh1* is a Polycomb target genes in basal epidermal cells. The H3K27me3 ChIP signal is drastically decreased in total skin cells isolated from P0 Ezh1/2 2KO mice. Please note that total skin contained both epithelial and a small fraction of non-epithelial cells (melanocytes, immune cells, etc) that were not targeted by the K14-Cre mediated ablation of Ezh1/2. Thus small residual level of H3K27me3 at *Sox2* is observed in 2KO cells. **(D)** Sox2 is necessary for Isl1 expression. (left) mRNA analysis of total Sox2cKO skin by RT-qPCR shows a reduction in the expression of Isl1, a Merkel signature gene found to be highly upregulated in Ezh1/2 2KO epidermis. (right) Quantification of Isl1 fluorescence intensity (arbitrary units) in P1 Sox2cKO Merkel cells confirms the decrease in Isl1 protein level (WT/Sox2cKO p<0.0001, 15/18 cells). **(E)** IF analysis of WT and Sox2cKO skin shows a reduction in the number of Isl1(+) cells. Quantifications are shown at right (WT/Sox2cKO p=0.0006, total length analysed: 70/106mm). Scale bars are 50um.

Supplementary Figure 6 Loss of Sox2 attenuates the Ezh1/2 2KO Merkel cell phenotype. **(A-B)** IF analysis using antibodies against Sox2 shows loss of Sox2 from the epidermis but not from the dermal papilla in Ezh1/2 Sox2 3KO mice. **(C)** IF using antibodies against H3K27me3 confirms loss of Ezh1/2 activity in Ezh1/2 Sox2 3KO epithelium. **(D)** IF using antibodies against NF200 shows the presence of neurons in both WT and Ezh1/2 Sox2 3KO skin. **(E)** Epidermal differentiated layers develop normally in Ezh1/2 Sox2 3KO skin, as shown by IF using antibodies against Keratin10. **(F-I)** IF analysis of the apoptosis marker Act-Casp3 shows no difference in cell death between WT and Ezh1/2 Sox2 3KO back skin (F), paws (G), whisker interfollicular epidermis (H), or whisker follicles (I). **(J)** Confirmation of the reduction in Merkel cell numbers in Ezh1/2 Sox2 3KO mice compared to Ezh1/2 2KO mice as shown by IF with antibodies against K18. Quantifications are show at right (WT/2KO $p=0.0006$, total length analysed: 38/76mm). **(K-N)** IF studies of whisker IFE (**K-L**), paws (**M**) and whisker follicles (**N**) of WT and Ezh1/2 Sox2 3KO mice using antibodies against K20 or Rab3c show the reduction of Merkel cells in 3KO skin. Quantifications are presented in (**O**) (WT/3KO Whisker follicle $p<0.0001$, total length analysed: 4.4/3.1mm; Whisker IFE $p=0.0371$, total length analysed: 7.2/2.7mm; Paw $p=0.0002$, total length analysed: 13.3/4.8mm. Statistics for Epidermis are in main figure). Scale bars are 50um.

Supplementary Table 1 List of H3K27Me3 targeted Merkel-signature genes enriched in Ezh1/2 2KO skin

Supplementary Table 2 Details of Merkel cell quantifications, including number of sections, total cells, and total length of skin analyzed.

Bardot_SFig1



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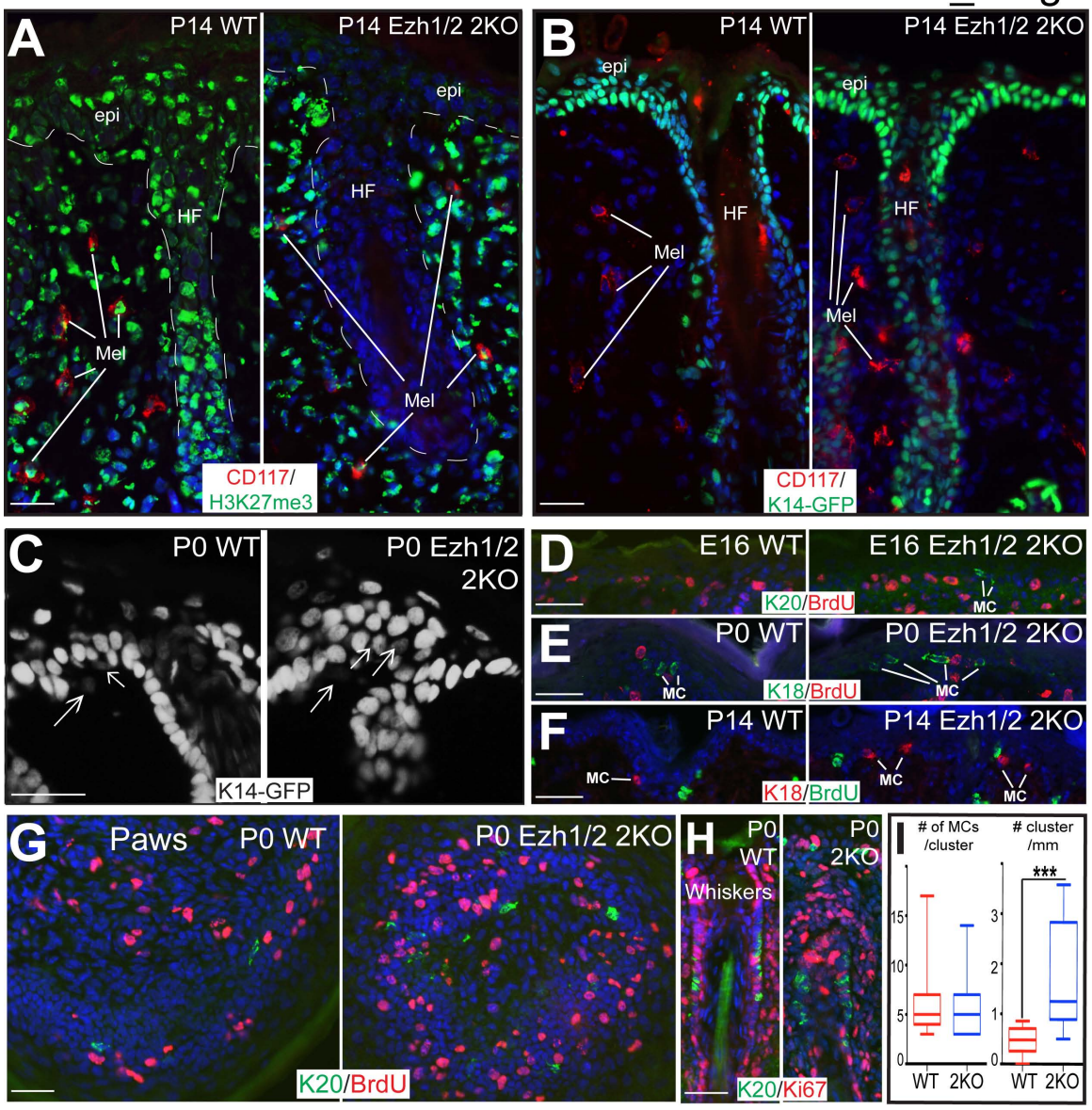

Bardot_SFig3

A

Gene name	Probe accession #	WT_Epi		2KO_Epi		MC fold enrichment
Lhx3	1421753_a_at	22.58110733	A	21.03636918	A	3729
Lhx3	1425041_at	41.22429161	A	44.06327017	A	3729
Krt20	1426284_at	32.21694725	A	71.42694451	M	3184
Insm1	1421399_at	27.78621391	M	37.29018703	P	1393
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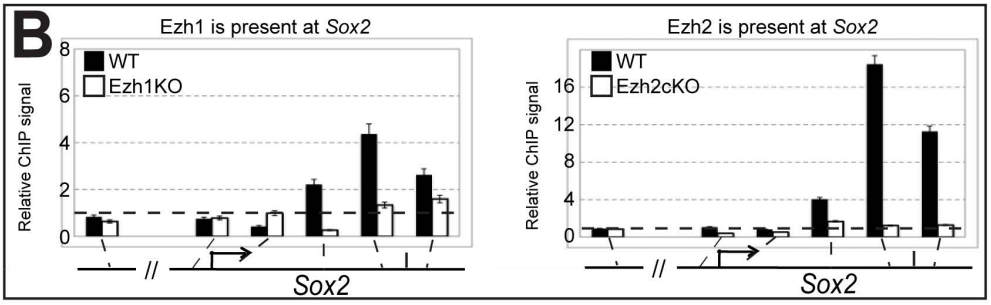

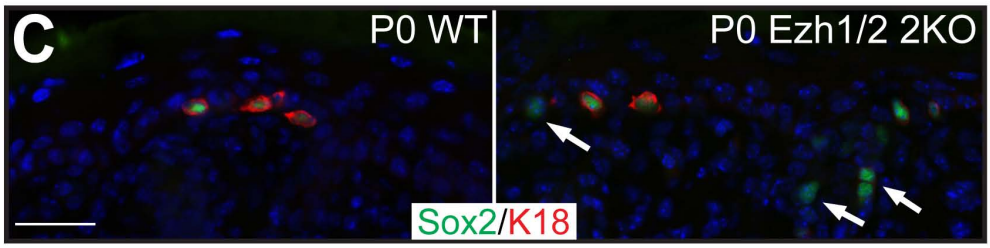

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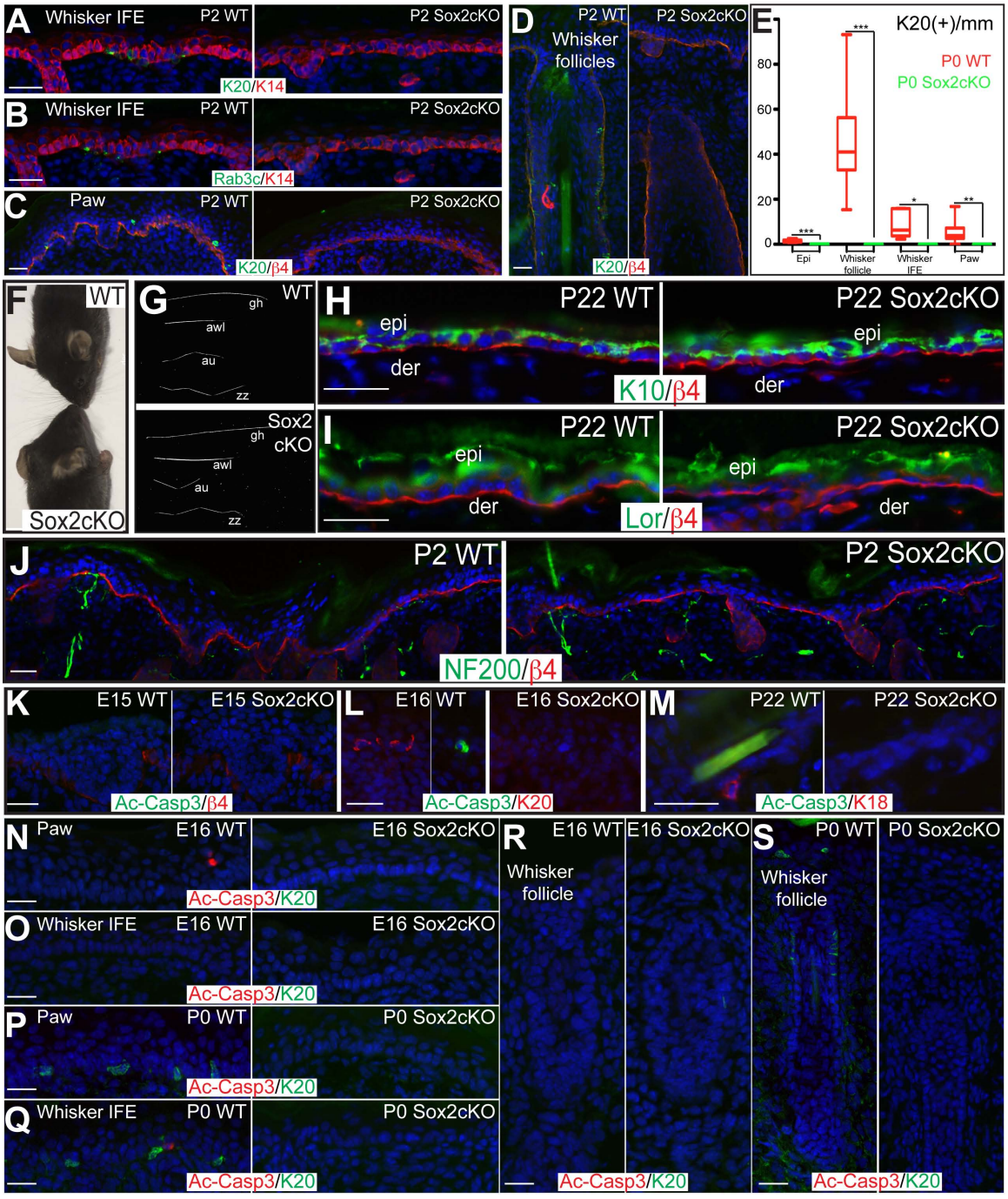

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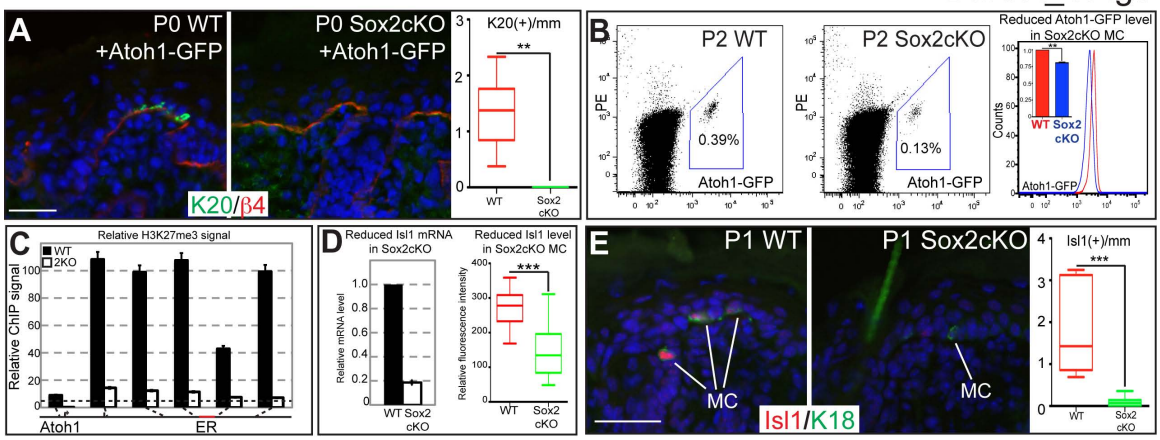

Bardot_SFig6