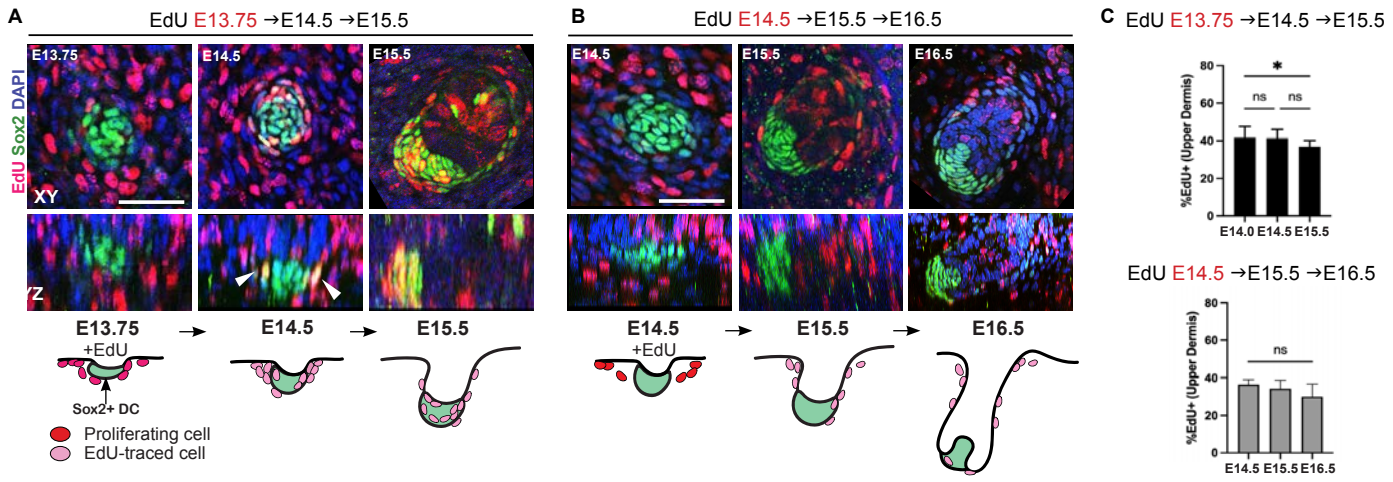
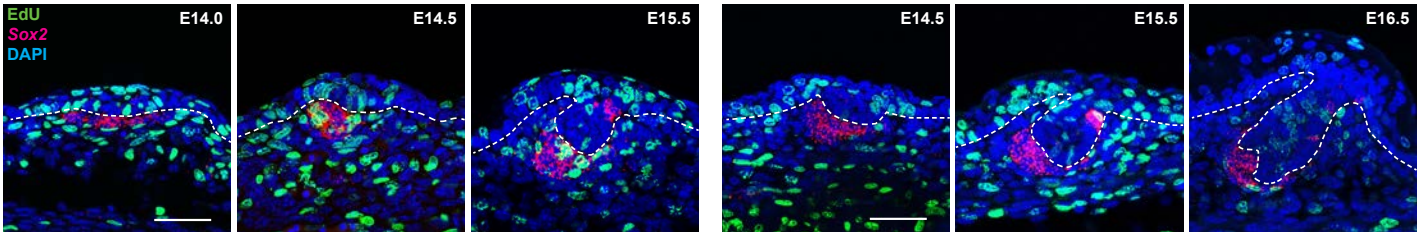
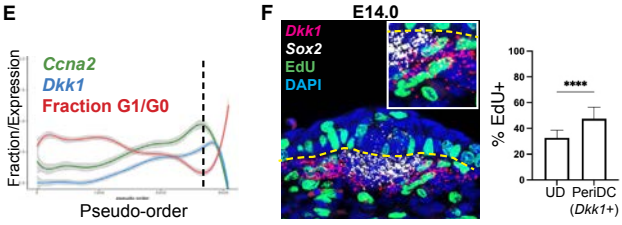

A
EdU E13.75 →E14.5 →E15.5
EdU Sox2 DAPI
E13.75
E14.5
E15.5
XY
YZ
E13.75 +EdU
E14.5
E15.5
Sox2+ DC
Proliferating cell
EdU-traced cell
B
EdU E14.5 →E15.5 →E16.5
E14.5
E15.5
E16.5
E14.5 +EdU
E15.5
E16.5
C
EdU E13.75 →E14.5 →E15.5
%EdU+ (Upper Dermis)
ns
ns
*
E14.0 E14.5 E15.5
EdU E14.5 →E15.5 →E16.5
%EdU+ (Upper Dermis)
ns
E14.5 E15.5 E16.5


D
EdU E13.75 →E14.5 →E15.5
EdU
Sox2
DAPI
E14.0
E14.5
E15.5
EdU E14.5 →E15.5 →E16.5
E14.5
E15.5
E16.5


E
Ccna2
Dkk1
Fraction G1/G0
Fraction/Expression
Pseudo-order
F
E14.0
Dkk1
Sox2
EdU
DAPI
% EdU+
****
UD
PeriDC
(Dkk1+)
G
% EdU+
****
Dkk1+ p21-
Dkk1+ p21+

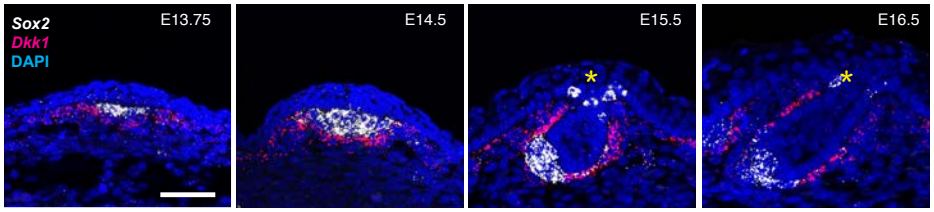

H
Sox2
Dkk1
DAPI
E13.75
E14.5
E15.5
E16.5

Figure S1. Related to Figure 1. Selectively proliferative DC progenitors express Dkk1 and reside in the peri-DC.

(A) 3D view of skin explants after pulsing with EdU at E13.75. Cartoons beneath whole-mount views depict the location of EdU-traced dermal cells. **(B)** 3D view of skin explants after pulsing with EdU at E14.5. Cartoons beneath whole-mount views depict the location of EdU-traced dermal cells. **(C)** %EdU+ of upper dermal (UD) cells at indicated time points after either E13.75 EdU pulse (top) or E14.5 EdU pulse (bottom). **(D)** FISH: Sox2, EdU with EdU pulsed at E13.75 and harvested at indicated times (left); FISH: Sox2, EdU with EdU pulsed at E14.5 and harvested at indicated times (right); n=3 per time point. **(E)** Pseudo-order showing *Dkk1* and *Ccna2* expression and the fraction of G0/G1 cells; dashed line indicates highly proliferative state. **(F)** FISH showing *Dkk1*+ peri-DC cells are highly proliferative. **(G)** %EdU+ of Dkk1+/p21- or Dkk1+/p21+ (corner) peri-DC cells. **(H)** FISH showing *Dkk1* expression in the peri-DC/perifollicular region over time (asterik=Merkel cells); n=5 per time point. Data as mean ± SEM, **P*<0.05, ***P*<0.01, ****P*<0.001 and *****P*<0.0001, Student's *t*-test or one-way ANOVA; n=3; ns, not significant. Scale bars, 50 μm.

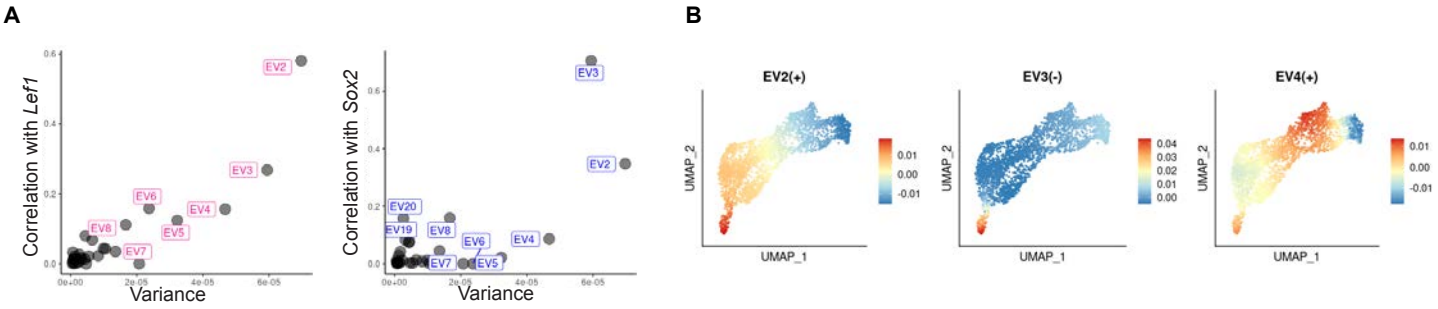

A
Correlation with Lef1
Variance
EV2
EV3
EV6
EV4
EV5
EV8
EV7
Correlation with Sox2
Variance
EV3
EV2
EV20
EV19
EV8
EV6
EV4
EV7
EV5
B
EV2(+)
EV3(-)
EV4(+)
UMAP_2
UMAP_1
0.01
0.00
-0.01
0.04
0.03
0.02
0.01
0.00

Figure S2. Related to Figure 2. Defining components of a non-linear DC differentiation process by eigenvectors

(A) Calculating correlation between leading 30 eigenvectors (EV) and expression level of *Lef1* or *Sox2*, as well as variance of eigenvectors that indicate relative informativeness. **(B)** UMAP of E14.5 dermal populations overlaid by values of EV2, EV3 and EV4. Different gradient patterns reflect their association with different biological processes.

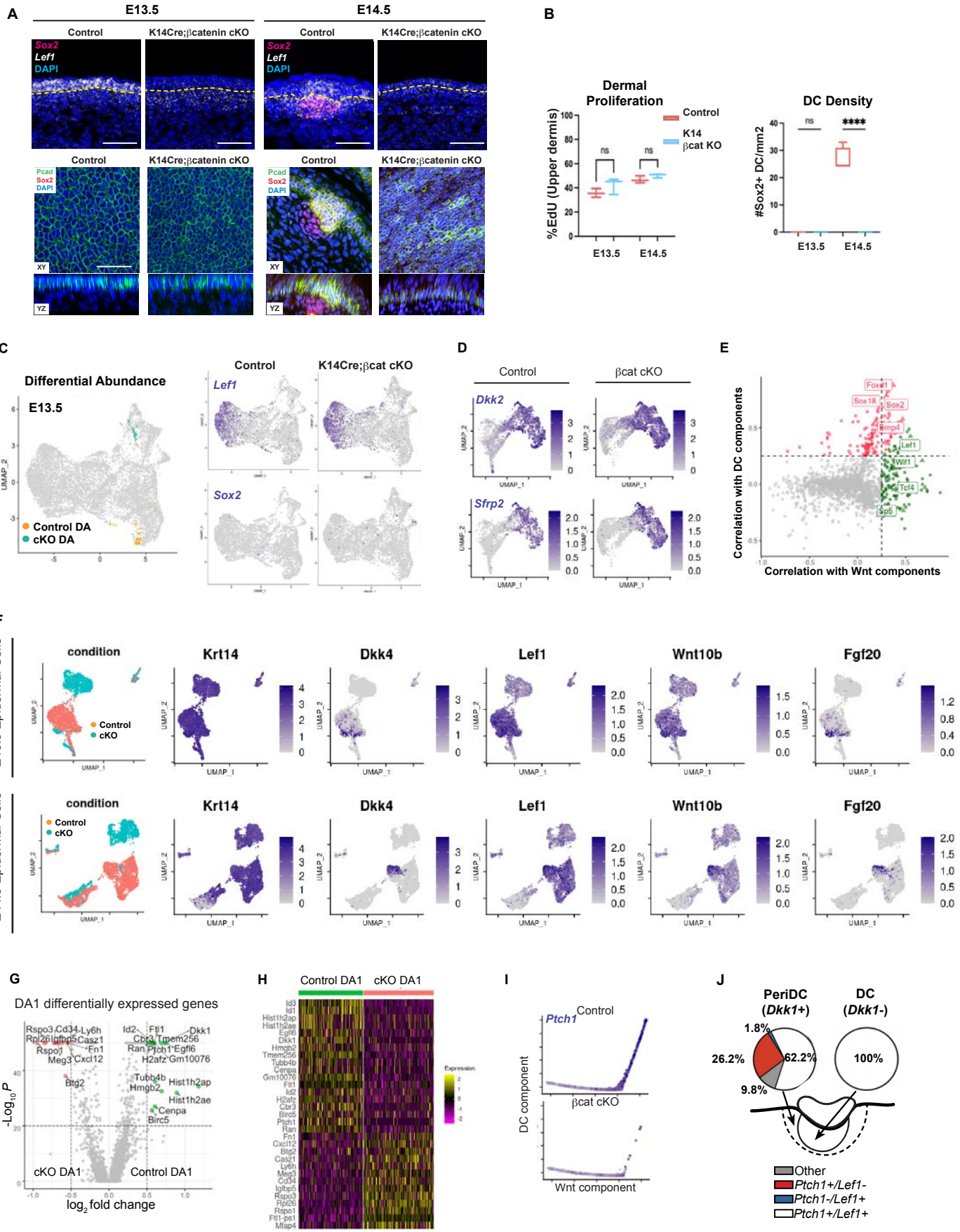

A
E13.5
E14.5
Control
K14Cre;βcatenin cKO
Sox2
Lef1
DAPI
Pcad
XY
YZ
B
Dermal Proliferation
%EdU (Upper dermis)
Control
K14 βcat KO
ns
DC Density
#Sox2+ DC/mm2
****
C
Differential Abundance
UMAP2
Control DA
cKO DA
K14Cre;βcat cKO
D
βcat cKO
Dkk2
Sfrp2
E
Correlation with DC components
Correlation with Wnt components
Foxd1
Sox18
Sox2
Bmp4
Lef1
Wif1
Tcf4
Sp5
F
E13.5 Epidermal Cells
E14.5 Epidermal Cells
condition
Krt14
Dkk4
Lef1
Wnt10b
Fgf20
G
DA1 differentially expressed genes
-Log10 P
log2 fold change
cKO DA1
Control DA1
H
Control DA1
cKO DA1
Expression
I
Ptch1
Control
βcat cKO
DC component
Wnt component
J
PeriDC (Dkk1+)
DC (Dkk1-)
1.8%
26.2%
62.2%
100%
9.8%
Other
Ptch1+/Lef1-
Ptch1-/Lef1+
Ptch1+/Lef1+

Figure S3. Related to Figure 3. Using a genetic placode-deficient mutant to identify the placode-dependent factor required for DC differentiation.

(A) FISH: *Sox2*, *Lef1* at E13.5 and E14.5 by condition (top, n=3); skin whole mounts at E13.5 and E14.5 by condition showing a lack of Sox2+ DCs in the mutant at both times (bottom). **(B)** EdU% of control and *K14Cre*;β*cat*$^{fl//fl}$ UD cells at E13.5 and E14.5 (left); DC density by condition at E13.5 and E14.5. **(C)** UMAP of E13.5 dermal populations overlaid by differentially abundant (DA) cell populations ($P < 0.01$) and UMAPs of E13.5 dermal populations parsed by condition with *Lef1* and *Sox2* genes. **(D)** UMAPs of E14.5 dermal populations parsed by condition with LD markers, *Dkk2* and *Sfrp2*. **(E)** Correlation plot of top 2000 variable genes by Wnt and DC components (threshold 0.25), determined based on the diffusion maps of combined E14.5 control and *K14Cre*;β*cat*$^{fl//fl}$ dermal cells**. (F)** UMAPs of E13.5 (Top) and E14.5 (bottom) epidermal populations overlaid by condition or expression level of canonical placode markers. **(G)** Volcano plot of DEGs between control and *K14Cre*;β*cat*$^{fl//fl}$ DA1 populations. **(H)** Heatmap showing top significant DEGs between control and *K14Cre*;β*cat*$^{fl//fl}$ DA1 populations. **(I)** Diffusion maps of E14.5 *K14Cre*;β*cat*$^{fl//fl}$ and control dermal cells showing sparse DA2 outliers of mutant express *Ptch1*. **(J)** Distribution of *Lef1*+/*Ptch1*+ copositive vs. other cells in peri-DC and DC cells. Data as mean ± SEM, **P*<0.05, ***P*<0.01, ****P*<0.001 and *****P*<0.0001, Student's *t*-test; n=3; ns, not significant. Scale bars, 50 μm.

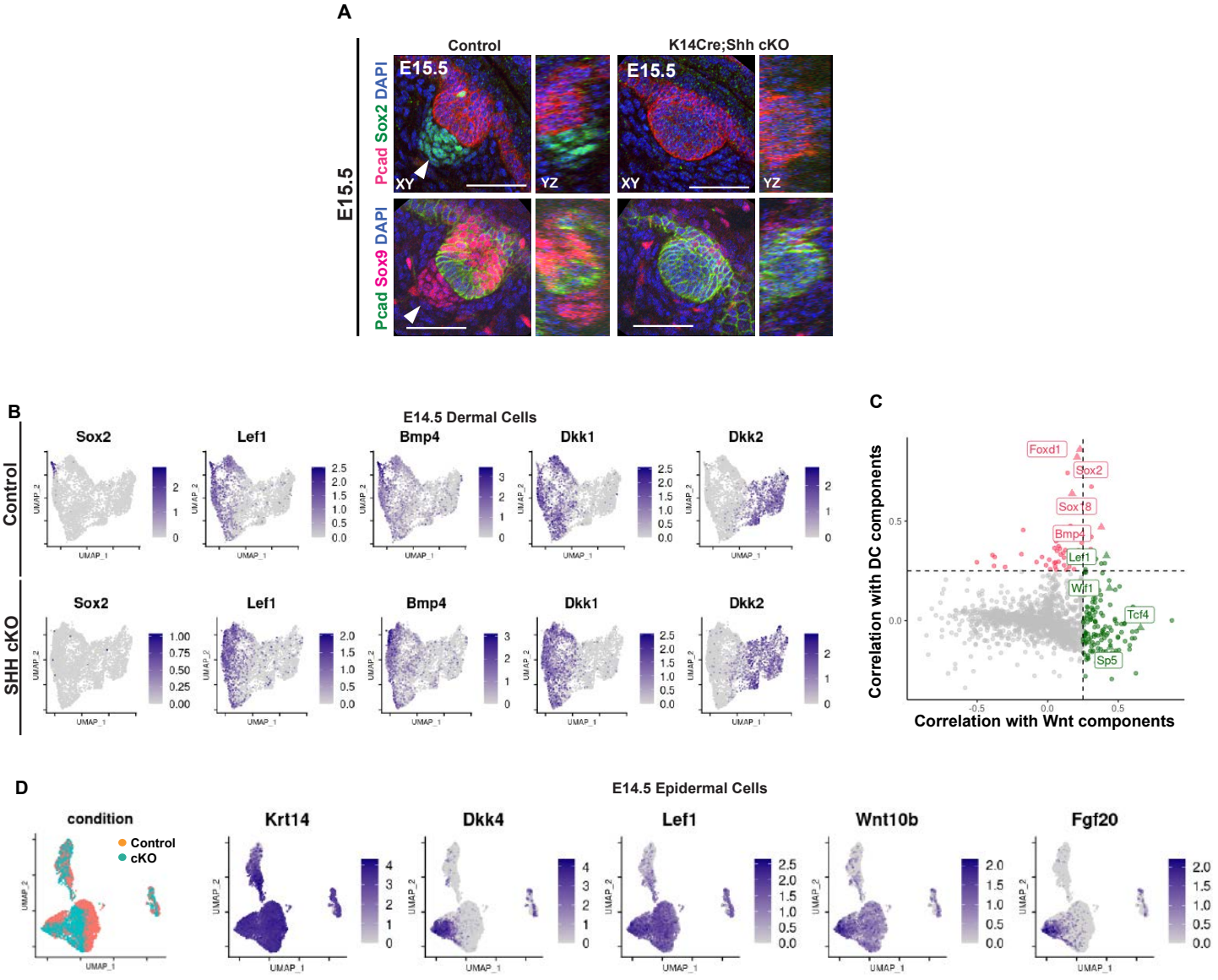

A
Control
K14Cre;Shh cKO
E15.5
Pcad Sox2 DAPI
Pcad Sox9 DAPI
XY
YZ
B
E14.5 Dermal Cells
Control
SHH cKO
Sox2
Lef1
Bmp4
Dkk1
Dkk2
UMAP_1
UMAP_2
C
Correlation with DC components
Correlation with Wnt components
Foxd1
Sox2
Sox18
Bmp4
Lef1
Wif1
Tcf4
Sp5
D
E14.5 Epidermal Cells
condition
Control
cKO
Krt14
Dkk4
Lef1
Wnt10b
Fgf20

Figure S4. Related to Figure 4. Characterization of SHH cKO scRNA-seq populations

(A) E15.5 control and SHH cKO skin whole mount views stained for Sox2 or Sox9 DC markers (arrowhead) and epithelial marker, P-cadherin (n=6). **(B)** UMAPs of E14.5 dermal populations parsed by condition showing expression of canonical dermal gene markers. **(C)** Correlation plot of top 2000 variable genes by Wnt and DC components (threshold 0.25), based on the diffusion maps of combined E14.5 control and SHH cKO dermal cells**. (D)** UMAPs of E14.5 epidermal populations overlaid by condition or with placode gene markers. Scale bars, 50 μm.

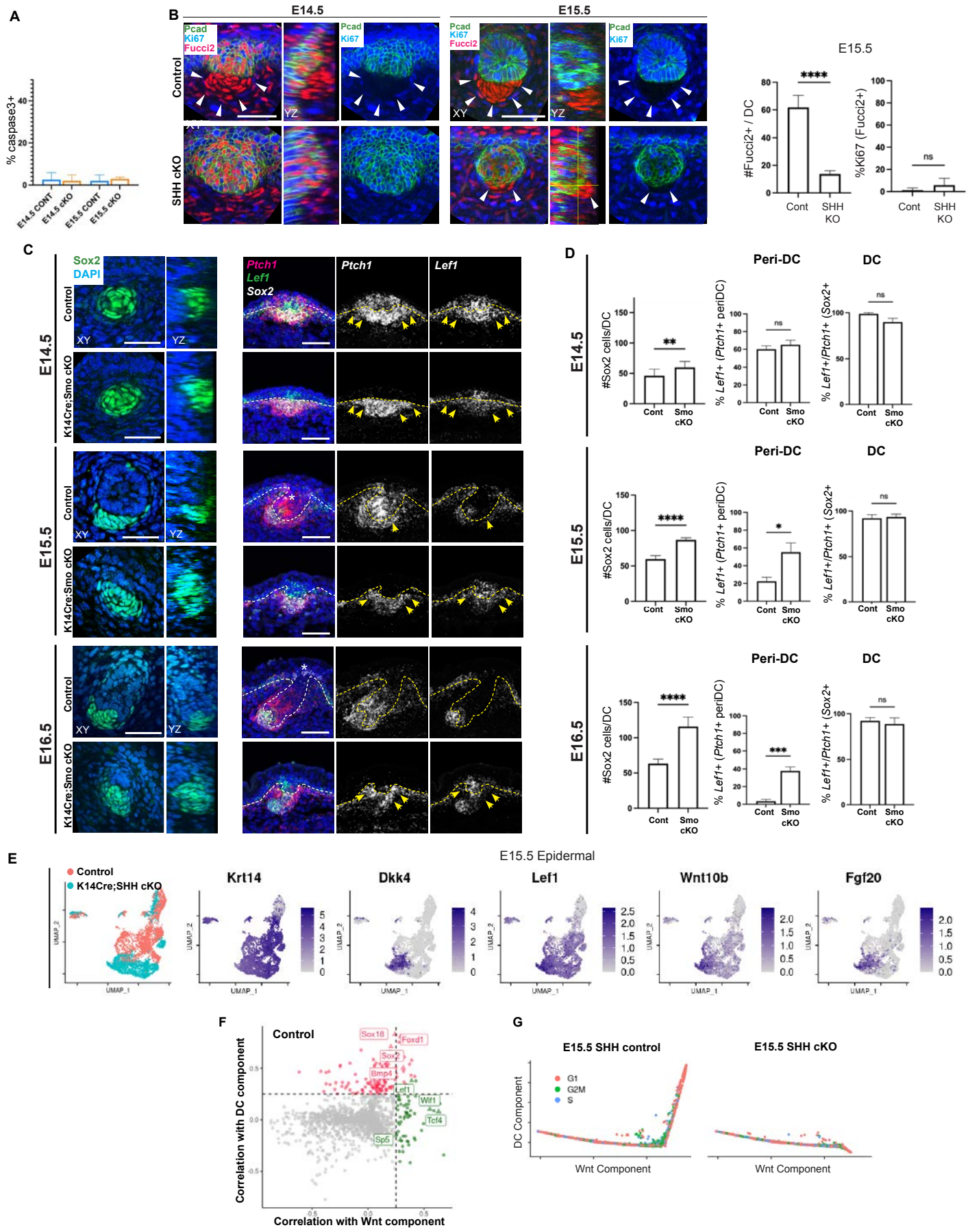

A
% caspase3+
E14.5 CONT
E14.5 cKO
E15.5 CONT
E15.5 cKO
B
E14.5
E15.5
Pcad
Ki67
Fucci2
Control
SHH cKO
XY
YZ
E15.5
#Fucci2+ / DC
%Ki67 (Fucci2+)
****
ns
Cont
SHH KO
C
Sox2
DAPI
Ptch1
Lef1
Sox2
E14.5
E15.5
E16.5
Control
K14Cre;Smo cKO
D
Peri-DC
DC
#Sox2 cells/DC
% Lef1+ (Ptch1+ periDC)
% Lef1+/Ptch1+ (Sox2+
**
*
***
Cont
Smo cKO
E
E15.5 Epidermal
Control
K14Cre;SHH cKO
Krt14
Dkk4
Lef1
Wnt10b
Fgf20
UMAP_1
UMAP_2
F
Control
Correlation with DC component
Correlation with Wnt component
Sox18
Foxd1
Sox9
Bmp4
Lef1
Wif1
Tcf4
Sp5
G
E15.5 SHH control
E15.5 SHH cKO
G1
G2M
S
DC Component
Wnt Component

Figure S5. Related to Figure 5. Dermal SHH activation is required for transition to quiescence and is due to a cell-autonomous requirement for dermal SHH signaling

(A) %Caspase3+ apoptotic dermal cells in control and SHH cKO at E14.5 and E15.5 (n=3). **(B)**, Skin whole mounts of E14.5 and E15.5 Fucci2+ control and *K14Cre;Shh*$^{fl/fl}$ stained for RFP, Ki-67, Pcad (left). White arrowheads, Ki67-/MCherry+ cluster. Quantification of MCherry+ (G0/G1) cells/DC and %Ki67+ cells per MCherry+ cluster at E15.5 (right, n=3). **(C)** Skin whole mounts of control and *K14Cre;Smo*$^{fl/fl}$ skin explants at E14.5, E15.5 and E16.5 and corresponding FISH showing where *Ptch1*+ and *Lef1*+ are located over time in control and Smo cKO mutants (n=3 per time point; yellow arrowheads, peri-DC *Ptch1*/*Lef1* co-positive cells; white asterisk, Merkel cell). **(D)** Quantification of number of Sox2+ DC cells in control and *K14Cre;Smo*$^{fl/fl}$ embryos or proportion of peri-DC cells and Sox2+ DC cells copositive for *Lef1* and *Ptch1* in control and *K14Cre;Smo*$^{fl/fl}$ embryos at each time point (n=4). **(E)** UMAPs of E15.5 epidermal populations overlaid by condition or with canonical epidermal placode markers. **(F)** Correlation plot of top 2000 variable genes by Wnt and DC components (threshold 0.25), based on the diffusion maps of combined E14.5 control and *K14Cre;Shh*$^{fl/fl}$ dermal cells. **(G)** Diffusion maps of E15.5 dermal cells parsed by condition showing cell cycle phase**.** Data as mean ± SEM, **P*<0.05, ***P*<0.01, ****P*<0.001 and *****P*<0.0001, Student's *t*-test, n=3; ns, not significant. Scale bars, 50 μm.

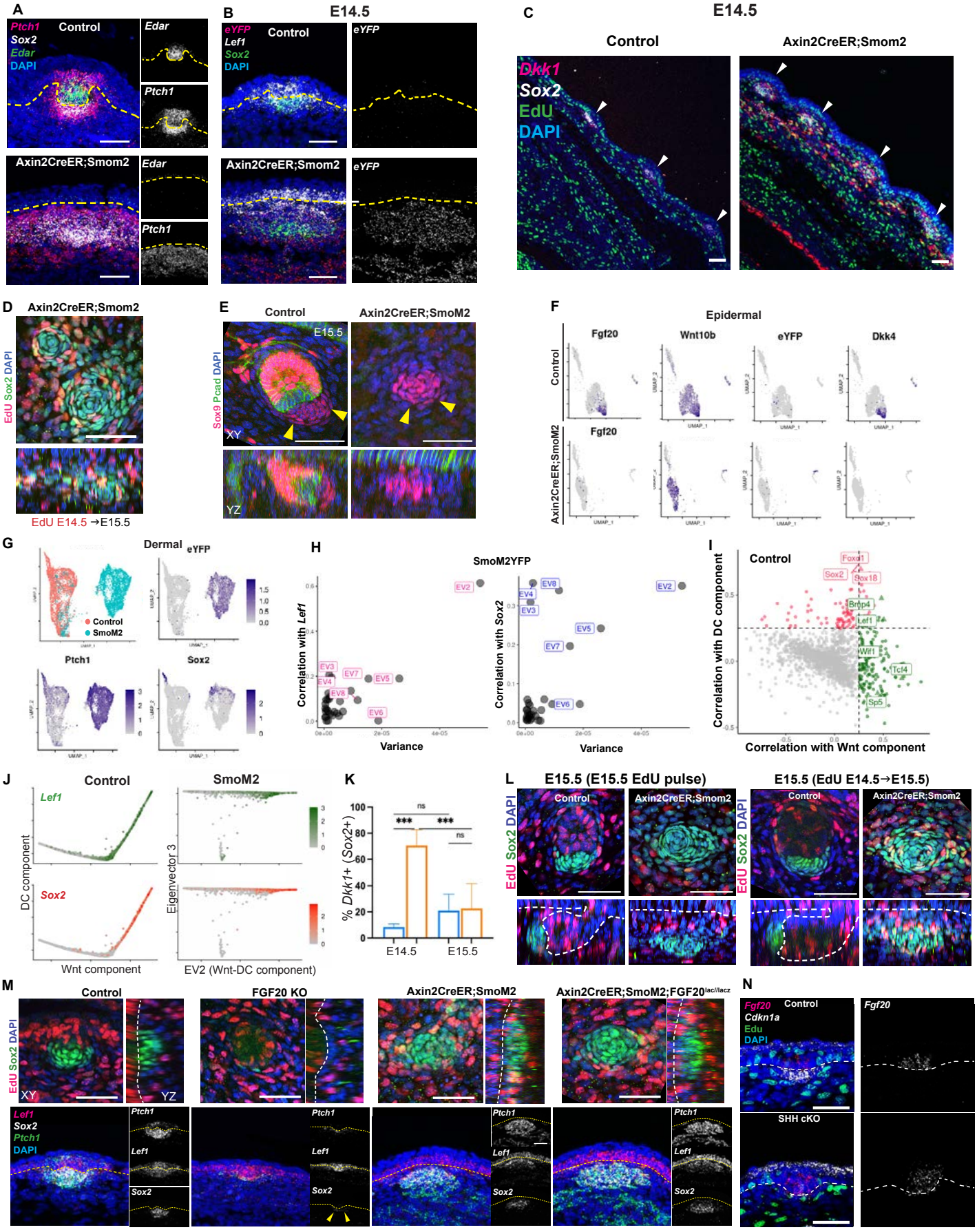

A
Ptch1
Sox2
Edar
DAPI
Control
Edar
Ptch1
Axin2CreER;Smom2
Edar
Ptch1
B
E14.5
eYFP
Lef1
Sox2
DAPI
Control
eYFP
Axin2CreER;Smom2
eYFP
C
E14.5
Control
Axin2CreER;Smom2
Dkk1
Sox2
EdU
DAPI
D
Axin2CreER;Smom2
EdU Sox2 DAPI
EdU E14.5 →E15.5
E
Control
Axin2CreER;SmoM2
Sox9 Pcad DAPI
E15.5
XY
YZ
F
Epidermal
Fgf20
Wnt10b
eYFP
Dkk4
Control
Fgf20
Axin2CreER;SmoM2
G
Dermal
eYFP
Control
SmoM2
Ptch1
Sox2
H
SmoM2YFP
Correlation with Lef1
Correlation with Sox2
Variance
EV2
EV3
EV4
EV5
EV6
EV7
EV8
I
Control
Correlation with DC component
Correlation with Wnt component
Foxa1
Sox2
Stox18
Bmp4
Lef1
Wif1
Tcf4
Sp5
J
Control
SmoM2
Lef1
Sox2
DC component
Wnt component
Eigenvector 3
EV2 (Wnt-DC component)
K
% Dkk1+ (Sox2+)
ns
***
***
ns
E14.5
E15.5
L
E15.5 (E15.5 EdU pulse)
E15.5 (EdU E14.5→E15.5)
Control
Axin2CreER;Smom2
EdU Sox2 DAPI
M
Control
FGF20 KO
Axin2CreER;SmoM2
Axin2CreER;SmoM2;FGF20lac/lacz
EdU Sox2 DAPI
XY
YZ
Lef1
Sox2
Ptch1
DAPI
N
Fgf20
Cdkn1a
Edu
DAPI
Control
SHH cKO
Fgf20

Figure S6. Related to Figure 6.Dermal SHH activation induces DC genesis in early Wnt-active cells independent of placodes

(A) FISH: E14.5 control and *SmoM2YFP* stained for *Ptch1*, *Sox2*, *Edar*. **(B)** FISH: control and *SmoM2YFP* stained for *eYFP*, *Lef1*, *Sox2*. **(C)** Low power axial view of E14.5 dorsolateral flank skin stained for *Dkk1, Sox2*, EdU. **(D)** Skin whole mount of E15.5 *SmoM2YFP* mutant showing abutting DCs (EdU pulsed at E14.5). **(E)** Skin whole mount of control and mutant stained with placode and DC marker, Sox9. (**F)** UMAPs of E14.5 epidermal populations parsed by condition with placode gene markers and *eYFP*. **(G)** UMAPs of E14.5 dermal populations showing *eYFP*, *Ptch1* and *Sox2*. **(H)** Calculating correlation between leading 30 eigenvectors (EV) of *SmoM2YFP* diffusion maps and expression level of *Lef1* or *Sox2*, as well as variance of eigenvectors that indicate relative informativeness. **(I)** Correlation plot of top 2000 variable genes by Wnt and DC components (threshold 0.25), based on the diffusion maps of E14.5 wildtype dermal cells. **(J)** Dermal diffusion maps of E14.5 control and *SmoM2YFP* biological replicate scRNA-seq data with *Sox2* and *Lef1* expression shown. **(K)** Proportion of Sox2+ cells co-expressing *Dkk1* at E14.5 in control and mutant (n=3). **(L)** Skin whole mount views of E15.5 control and mutant pulsed with EdU and harvested 1.5 hours or 24 hours later (n=3). **(M)** Whole mount 3D images of E14.5 control, $FGF20^{lacZ/lacZ}$, *SmoM2YFP* and *SmoM2YFP;*$FGF20^{lacZ/lacZ}$ embryos after EdU pulse (top). E14.5 FISH: *Lef1*, *Sox2*, *Ptch1* across conditions (bottom) (n=5). **(N)** Representative FISH: E14.5 control and SHH cKO stained for *Fgf20*, *Cdkn1a*, EdU (n=3). Data as mean ± SEM, **P*<0.05, ***P*<0.01, ****P*<0.001 and *****P*<0.0001, one-way ANOVA; n=3; ns, not significant. Scale bars, 50 μm.

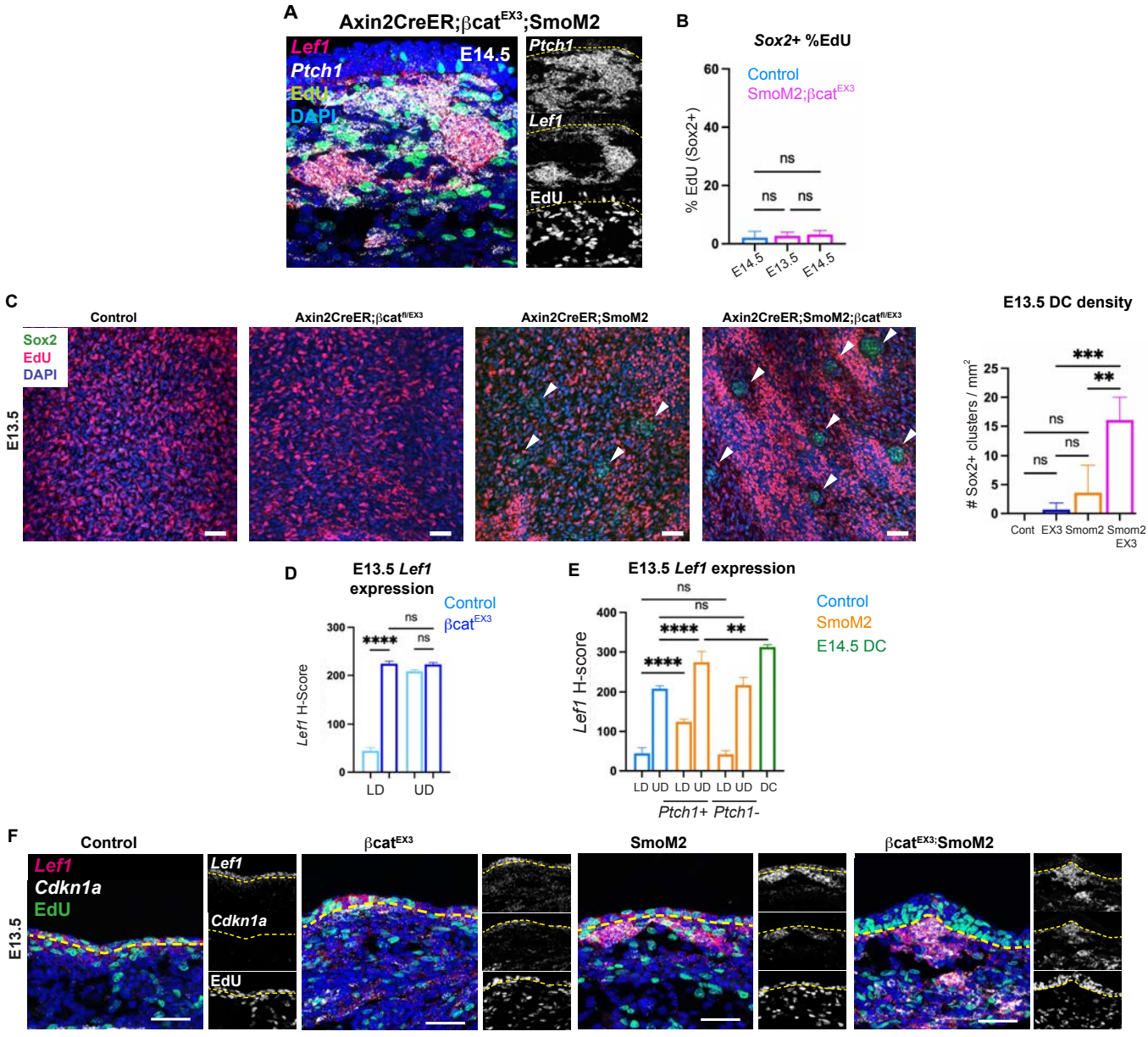

A
Axin2CreER;βcat^EX3;SmoM2
Lef1
Ptch1
EdU
DAPI
E14.5
Ptch1
Lef1
EdU
B
Sox2+ %EdU
Control
SmoM2;βcat^EX3
% EdU (Sox2+)
ns
E14.5
E13.5
E14.5
C
Control
Axin2CreER;βcat^fl/EX3
Axin2CreER;SmoM2
Axin2CreER;SmoM2;βcat^fl/EX3
Sox2
EdU
DAPI
E13.5
E13.5 DC density
Sox2+ clusters / mm^2
***
**
Cont
EX3
Smom2
Smom2 EX3
D
E13.5 Lef1 expression
Control
βcat^EX3
Lef1 H-Score
****
LD
UD
E
E13.5 Lef1 expression
Control
SmoM2
E14.5 DC
Lef1 H-score
LD UD LD UD LD UD DC
Ptch1+ Ptch1-
F
Control
βcat^EX3
SmoM2
βcat^EX3;SmoM2
E13.5
Lef1
Cdkn1a
EdU

Figure S7. Related to Figure 6 and 7. Co-activation of sufficient levels of dermal SHH and Wnt signaling induces DCs prior to HF morphogenesis

(A) FISH of E14.5 control and *SmoM2YFP;βcat*$^{fl/EX3}$ embryos showing quiescent *Sox2*+ clusters in the UD and LD that co-express *Lef1* and *Ptch1*. **(B)** %EdU+ of *Sox2*+ cells in E14.5 control and *SmoM2YFP;βcat*$^{fl/EX3}$ DCs and E13.5 *SmoM2YFP;βcat*$^{fl/EX3}$ DCs. **(C)** Widefield view (500 x 500 μm) of skin whole mounts of E13.5 control and indicated mutants (white arrowheads, DCs) and quantification of DC density by condition (per mm^2). **(D)** *Lef1* levels (H-score) of E13.5 control and *βcat*$^{fl/EX3}$ in the LD or UD. **(E)** *Lef1* levels (H-score) of E13.5 control, *Ptch1*+ (*Smom2YFP* mutant) or *Ptch1*- (WT, un-recombined cells) in the LD or UD; *Lef1* levels of E14.5 WT DC cells are shown as green bar. **(F)** FISH showing expression of *Cdkn1a*, *Lef1*, and EdU across conditions at E13.5. Data as mean ± SEM, **P*<0.05, ***P*<0.01, ****P*<0.001 and *****P*<0.0001, one-way ANOVA; n=3; ns, not significant. Scale bars, 50 μm