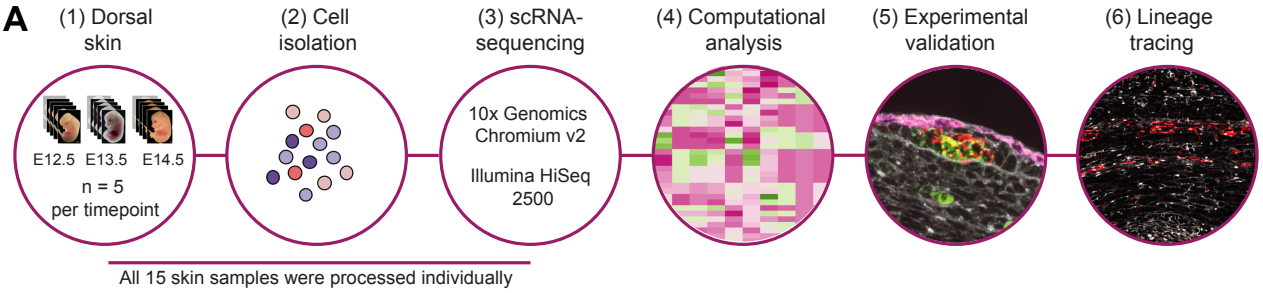
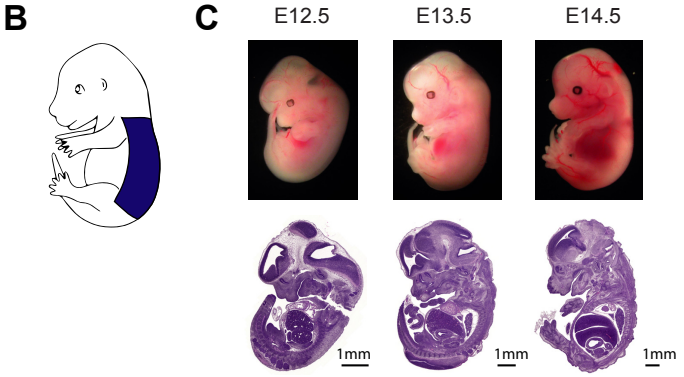
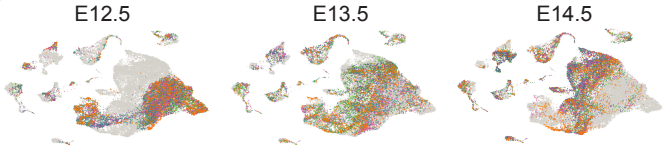
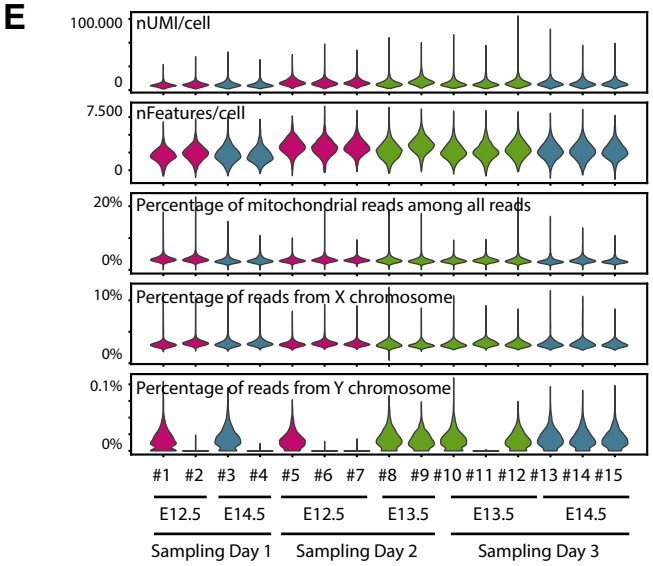
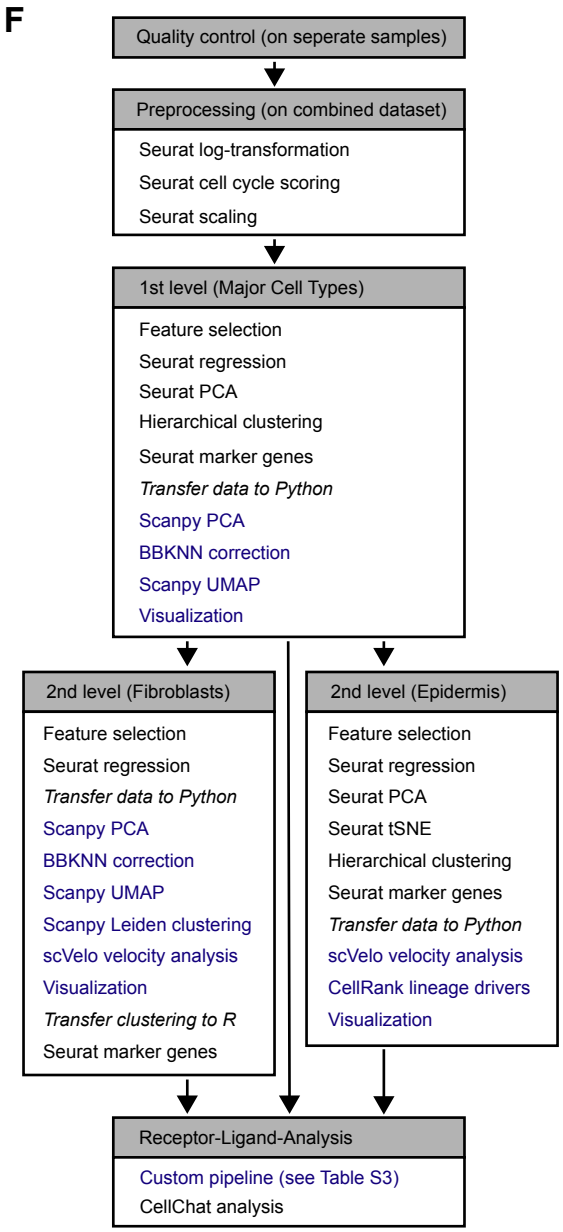
Figure S1


A
(1) Dorsal skin
(2) Cell isolation
(3) scRNA-sequencing
(4) Computational analysis
(5) Experimental validation
(6) Lineage tracing
E12.5 E13.5 E14.5
n = 5 per timepoint
10x Genomics Chromium v2
Illumina HiSeq 2500
All 15 skin samples were processed individually


B
C
E12.5
E13.5
E14.5
1mm
1mm
1mm


D
E12.5
E13.5
E14.5


E
100.000
0
nUMI/cell
7.500
0
nFeatures/cell
20%
0%
Percentage of mitochondrial reads among all reads
10%
0%
Percentage of reads from X chromosome
0.1%
0%
Percentage of reads from Y chromosome
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14 #15
E12.5 E14.5 E12.5 E13.5 E13.5 E14.5
Sampling Day 1
Sampling Day 2
Sampling Day 3


F
Quality control (on seperate samples)
Preprocessing (on combined dataset)
Seurat log-transformation
Seurat cell cycle scoring
Seurat scaling
1st level (Major Cell Types)
Feature selection
Seurat regression
Seurat PCA
Hierarchical clustering
Seurat marker genes
Transfer data to Python
Scanpy PCA
BBKNN correction
Scanpy UMAP
Visualization
2nd level (Fibroblasts)
Feature selection
Seurat regression
Transfer data to Python
Scanpy PCA
BBKNN correction
Scanpy UMAP
Scanpy Leiden clustering
scVelo velocity analysis
Visualization
Transfer clustering to R
Seurat marker genes
2nd level (Epidermis)
Feature selection
Seurat regression
Seurat PCA
Seurat tSNE
Hierarchical clustering
Seurat marker genes
Transfer data to Python
scVelo velocity analysis
CellRank lineage drivers
Visualization
Receptor-Ligand-Analysis
Custom pipeline (see Table S3)
CellChat analysis

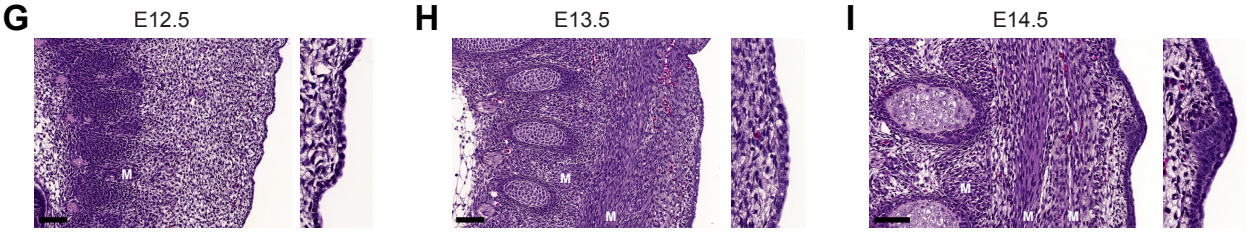

G
E12.5
H
E13.5
I
E14.5

Figure S1. Details of experimental approach and quality control, Related to Figure 1

(A) Overview of the experimental workflow.
(B) Sampling area highlighted in blue.
(C) Representative photos and H&E sagital sections of embryos at the analysed embryonic time points.
(D) Sample contributions (n = 5 biological replicates) per timepoint overlayed on combined UMAP.
(E) Violin plots showing a uniform distribution of QC measures (reads/cell, genes/cell and percentage of mitochondrial reads among all reads) among all 15 biological replicates (upper 3 panels). Violin plots showcasing the approach of retrospective sex determination (lower 2 panels). Only male embryos show reads from Y chromosome.
(F) Flow diagram summarizing the analysis workflow. Black font marks steps that were performed in R and blue font marks steps that were performed in Python.
(G-I) H&E stained tissue sections of dorsal skin. Zoom in on epidermis (includind a developing hair follicle in (I)). M marks developing muscle layers (for orientation in the tissue). Scale bars, 100µm.

Figure S2

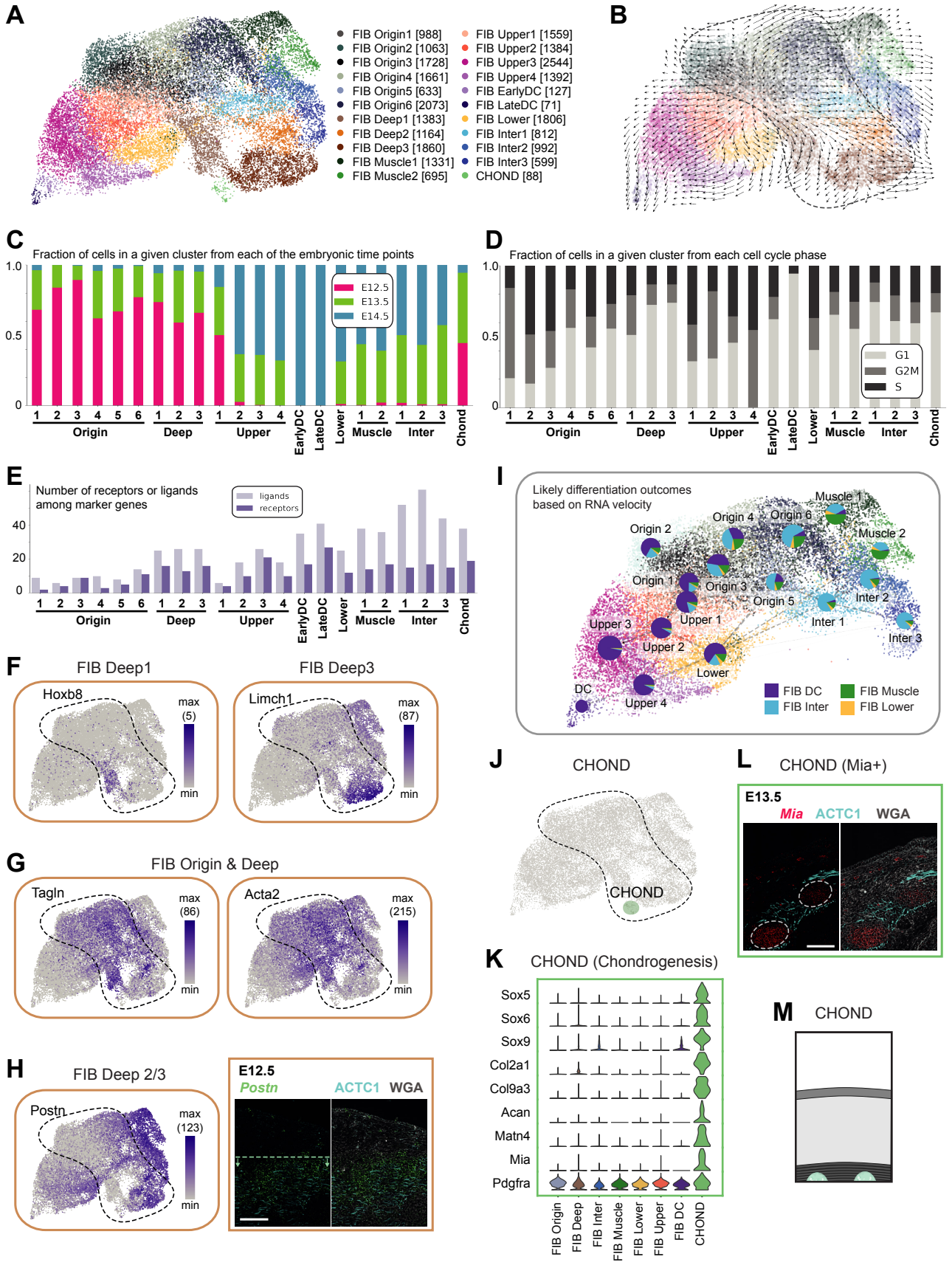

A
FIB Origin1 [988]
FIB Origin2 [1063]
FIB Origin3 [1728]
FIB Origin4 [1661]
FIB Origin5 [633]
FIB Origin6 [2073]
FIB Deep1 [1383]
FIB Deep2 [1164]
FIB Deep3 [1860]
FIB Muscle1 [1331]
FIB Muscle2 [695]
FIB Upper1 [1559]
FIB Upper2 [1384]
FIB Upper3 [2544]
FIB Upper4 [1392]
FIB EarlyDC [127]
FIB LateDC [71]
FIB Lower [1806]
FIB Inter1 [812]
FIB Inter2 [992]
FIB Inter3 [599]
CHOND [88]
B
C
Fraction of cells in a given cluster from each of the embryonic time points
E12.5
E13.5
E14.5
Origin
Deep
Upper
EarlyDC
LateDC
Lower
Muscle
Inter
Chond
D
Fraction of cells in a given cluster from each cell cycle phase
G1
G2M
S
E
Number of receptors or ligands among marker genes
ligands
receptors
I
Likely differentiation outcomes based on RNA velocity
Origin 1
Origin 2
Origin 3
Origin 4
Origin 5
Origin 6
Muscle 1
Muscle 2
Inter 1
Inter 2
Inter 3
Upper 1
Upper 2
Upper 3
Upper 4
Lower
DC
FIB DC
FIB Muscle
FIB Inter
FIB Lower
F
FIB Deep1
Hoxb8
max (5)
min
FIB Deep3
Limch1
max (87)
min
G
FIB Origin & Deep
Tagln
max (86)
min
Acta2
max (215)
min
H
FIB Deep 2/3
Postn
max (123)
min
E12.5
Postn
ACTC1
WGA
J
CHOND
CHOND
L
CHOND (Mia+)
E13.5
Mia
ACTC1
WGA
K
CHOND (Chondrogenesis)
Sox5
Sox6
Sox9
Col2a1
Col9a3
Acan
Matn4
Mia
Pdgfra
FIB Origin
FIB Deep
FIB Inter
FIB Muscle
FIB Lower
FIB Upper
FIB DC
CHOND
M
CHOND

Figure S2. Deconstruction of fibroblast heterogeneity at E12.5 (expression and location), Related to Figure 2

(A) UMAP with subclustering of all fibroblasts. Cell numbers per subcluster are displayed in square brackets.
(B) UMAP, colored according to fibroblast subclustering and overlayed with RNA velocity vectors that predict developmental trajectories.
(C-D) Bar plot visualizing the contribution of each embryonic time point (C) or each (predicted) cell cycle phase (D) to each fibroblast subcluster.
(E) Bar plot showing the number of ligands and receptors among the marker genes of each fibroblast subcluster.
(F-G) UMAP with expression pattern of additional *FIB Deep* marker genes (F) and additional marker genes shared between *FIB Origin* and *FIB Deep* (G). Number in brackets shows max number of RNA copies detected per cell (absolute abundance).
(H) UMAP with expression pattern of *Postn* (left panel). *Postn* mRNA staining plus ACTC1 protein staining (right panel). Dashed lines and arrows highlight the region with highest expression. Counterstained with WGA (membraneous stain). Microscope images originate from larger tile scan (n = 3 mice). Scale bars, 100µm.
(I) Directed PAGA plot showing the most likely differentiation outcomes of each fibroblast subpopulation based on RNA velocity. Cells are colored according to fibroblast subclustering and pie chart segments are colored according to the differentiation outcomes.
(J) *FIB CHOND* cells highlighted on the UMAP.
(K) Violin plots of *FIB CHOND* marker gene expression.
(L) *Mia* mRNA and *ACTC1* protein staining reveals location of *FIB CHOND* cells. Microscope image originates from larger tile scan (n = 3 mice). Scale bar, 100µm.
(M) Scheme summarizing the location of *FIB CHOND* cells within the tissue at E12.5.

Figure S3

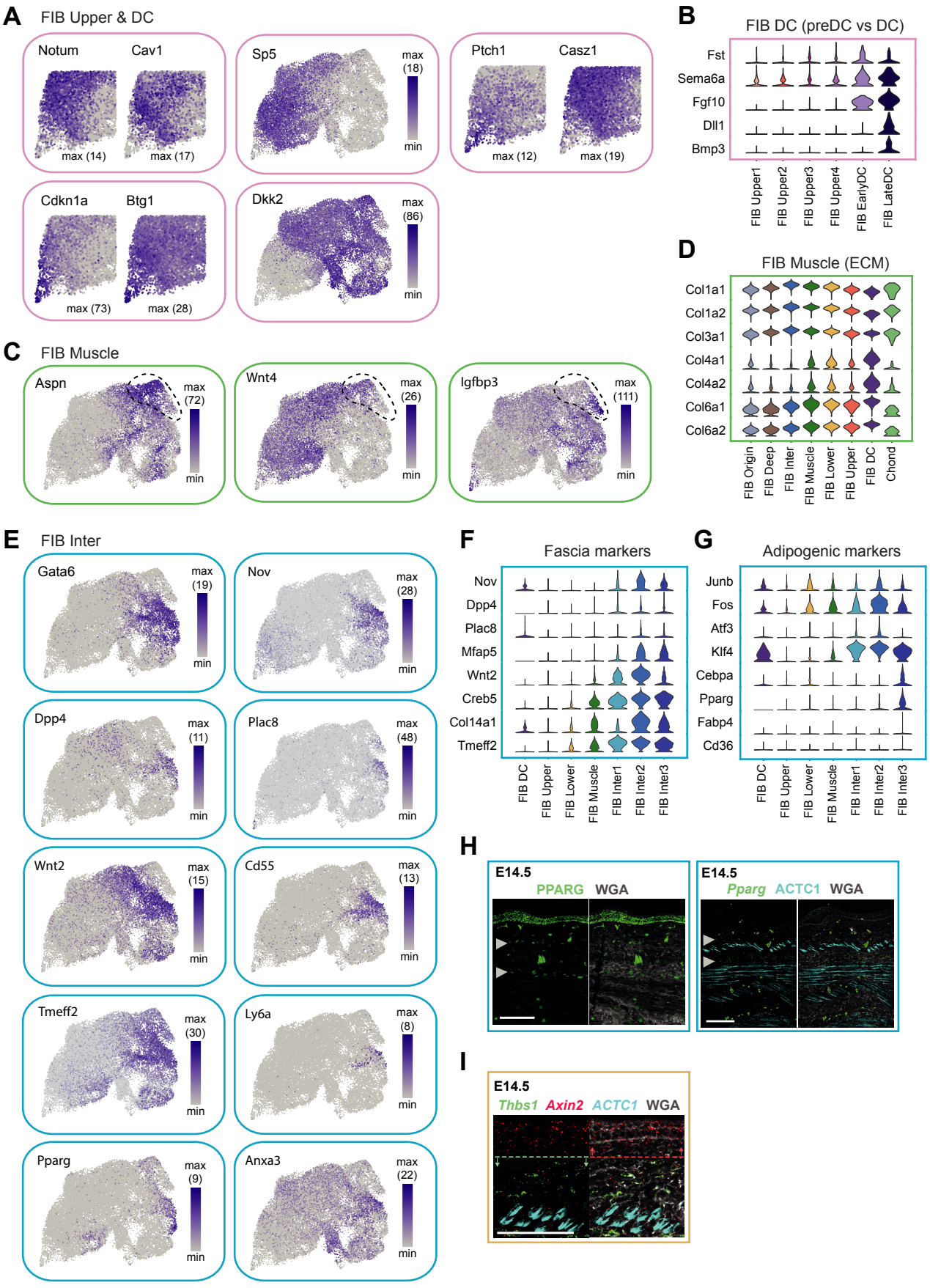
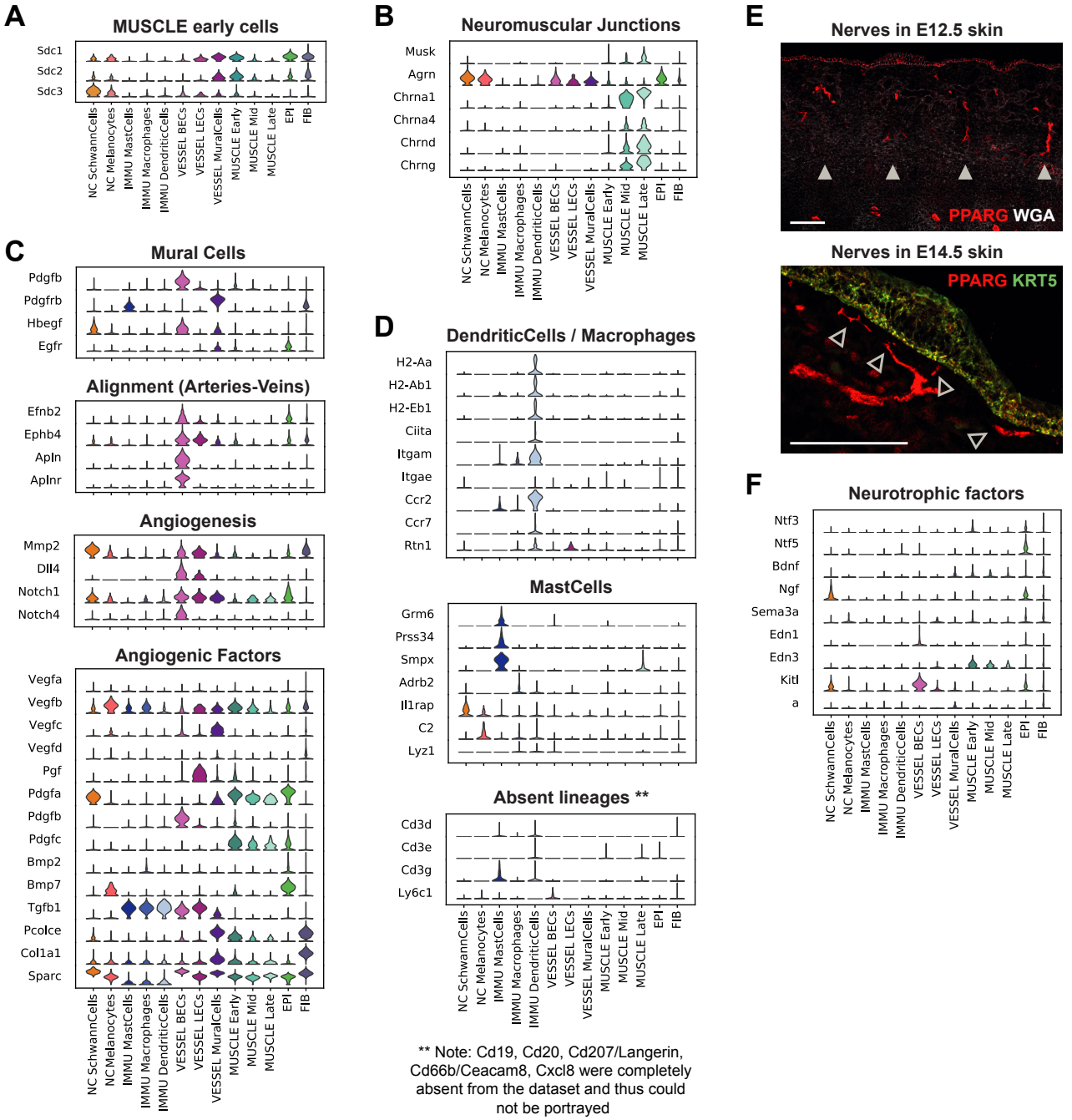

A
FIB Upper & DC
Notum
Cav1
max (14)
max (17)
Sp5
max (18)
min
Ptch1
Casz1
max (12)
max (19)
Cdkn1a
Btg1
max (73)
max (28)
Dkk2
max (86)
min
B
FIB DC (preDC vs DC)
Fst
Sema6a
Fgf10
Dll1
Bmp3
FIB Upper1
FIB Upper2
FIB Upper3
FIB Upper4
FIB EarlyDC
FIB LateDC
C
FIB Muscle
Aspn
max (72)
min
Wnt4
max (26)
min
Igfbp3
max (111)
min
D
FIB Muscle (ECM)
Col1a1
Col1a2
Col3a1
Col4a1
Col4a2
Col6a1
Col6a2
FIB Origin
FIB Deep
FIB Inter
FIB Muscle
FIB Lower
FIB Upper
FIB DC
Chond
E
FIB Inter
Gata6
max (19)
min
Nov
max (28)
min
Dpp4
max (11)
min
Plac8
max (48)
min
Wnt2
max (15)
min
Cd55
max (13)
min
Tmeff2
max (30)
min
Ly6a
max (8)
min
Pparg
max (9)
min
Anxa3
max (22)
min
F
Fascia markers
Nov
Dpp4
Plac8
Mfap5
Wnt2
Creb5
Col14a1
Tmeff2
FIB DC
FIB Upper
FIB Lower
FIB Muscle
FIB Inter1
FIB Inter2
FIB Inter3
G
Adipogenic markers
Junb
Fos
Atf3
Klf4
Cebpa
Pparg
Fabp4
Cd36
FIB DC
FIB Upper
FIB Lower
FIB Muscle
FIB Inter1
FIB Inter2
FIB Inter3
H
E14.5
PPARG WGA
E14.5
Pparg ACTC1 WGA
I
E14.5
Thbs1 Axin2 ACTC1 WGA

Figure S3. Deconstruction of fibroblast heterogeneity at E13.5 and E14.5 (expression and location), Related to Figure 3

(A,C,E) UMAP with expression pattern of additional *FIB Upper / DC* marker genes (A), *FIB Muscle* marker genes (C), and *FIB Inter* marker genes (F).
(B,D,F,G) Violin plots of DC marker gene expression (B), ECM-related gene expression (D), fascia-related marker gene expression (F), and adipogenesis-related marker gene expression (G).
(H) PPARG protein staining (left panel) and *Pparg* mRNA staining plus ACTC1 protein staining (right panel). Arrowheads mark layers with maximal staining.
(I) *Thbs1* and *Axin2* mRNA staining plus ACTC1 protein staining. Dashed lines with arrows highlight the region with highest expression.
(H-I) Microscope images originate from larger tile scan (n = 3 mice). Scale bars, 100μm.

Figure S4

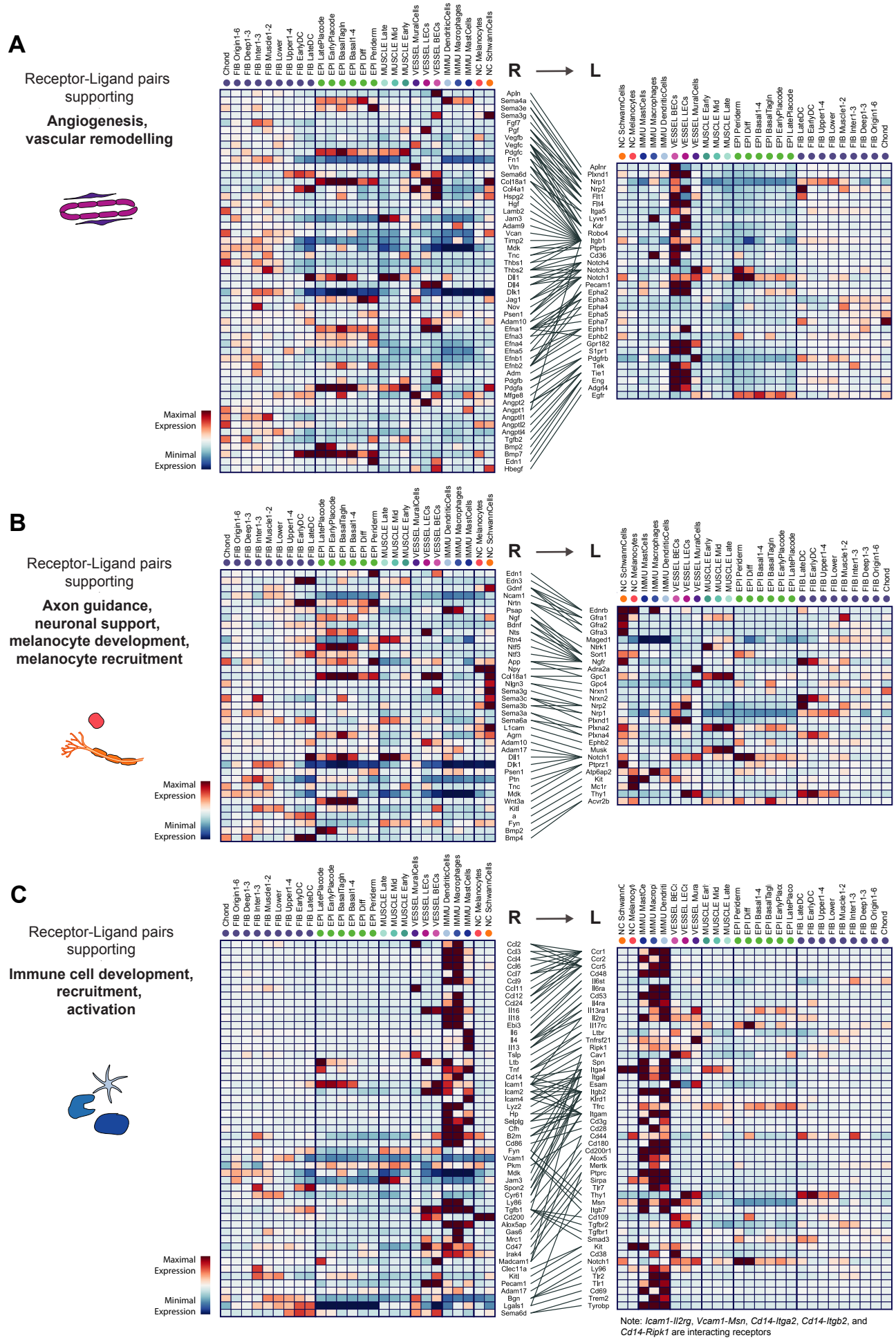


** Note: Cd19, Cd20, Cd207/Langerin, Cd66b/Ceacam8, Cxcl8 were completely absent from the dataset and thus could not be portrayed

Figure S4. Cell types contributing to embryonic skin besides fibroblasts and keratinocytes, Related to Figure 5 and 6

(A-D,F) Violin Plots showing expression of markers used to further characterize the captured muscle cells (A,B), vessel-associated cells (C), immune cells (D), and neural crest-derived cells (F).
(E) PPARG and KRT5 protein staining at E12.5 and E14.5, respectively. Filled arrowheads mark thick nerve bundles traversing dermis at the height of each vertebrae (asterisks). Empty arrowheads mark nerve endings extending towards epidermis. Microscope images originate from larger tile scans (n = 3 mice). Scale bars, 100µm.

Figure S5



Note: *Icam1-Il2rg*, *Vcam1-Msn*, *Cd14-Itga2*, *Cd14-Itgb2*, and *Cd14-Ripk1* are interacting receptors

Figure S5. Receptor-ligand interactions supporting recruitment/maintenane of vessel-associated, immune and neural crest-derived cells, Related to Figure 6.

(A-C) Receptor-ligand pairs with probable involvement in angiogenesis and/or vascular remodelling (A), axon guidance, neuronal support, melanocyte development, and/or melanocyte recrtuitment (B), or immune cell development, recruitment and/or activation (C). Heatmaps show the expression pattern of interaction partners among all first-level clusters as well as fibroblast and keratinocyte subclusters. Central lines mark significant receptor-ligand pairs observed in our dataset.

Figure S6

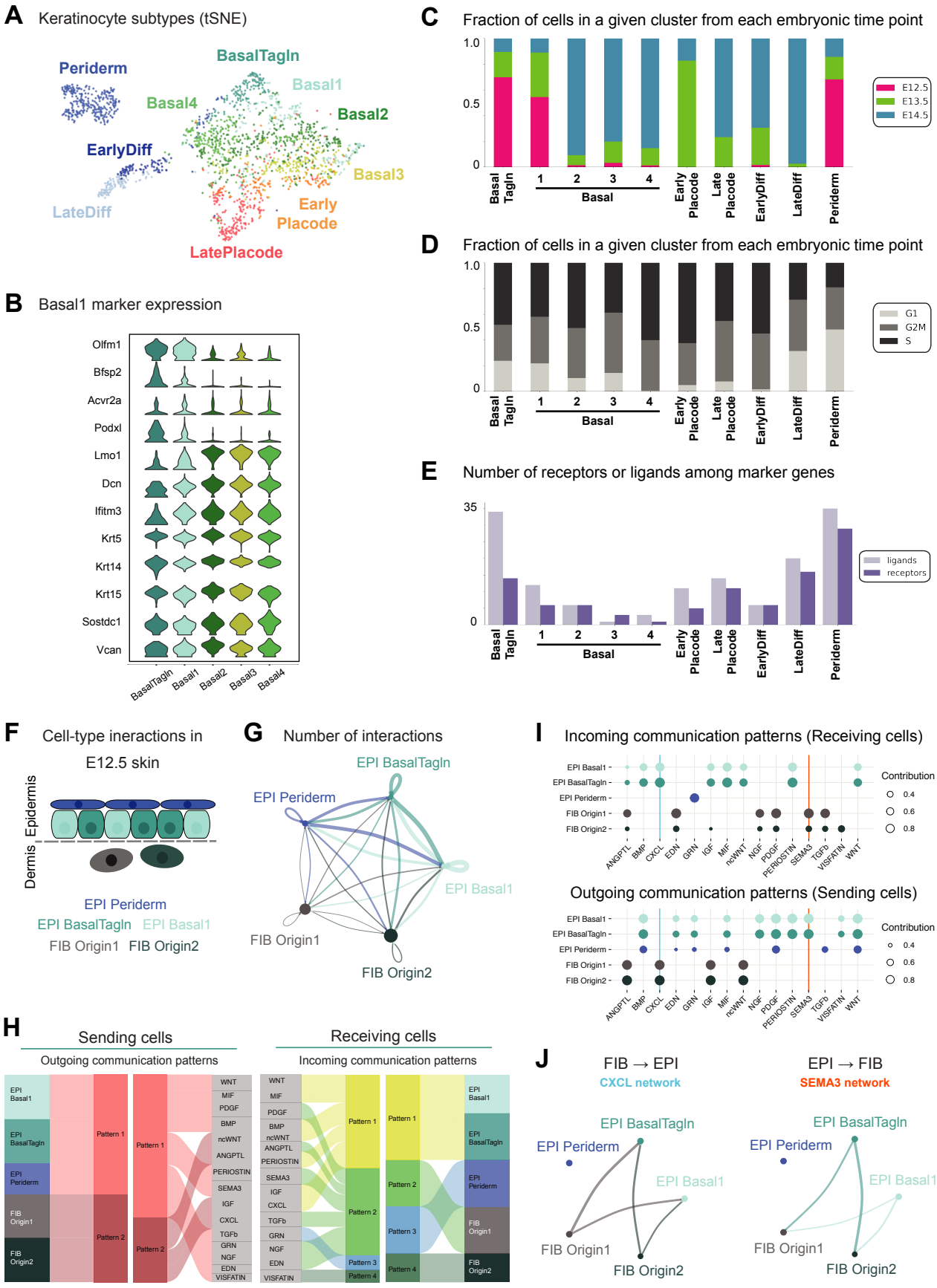

A
Keratinocyte subtypes (tSNE)
Periderm
BasalTagIn
Basal1
Basal4
Basal2
EarlyDiff
Basal3
LateDiff
Early Placode
LatePlacode
B
Basal1 marker expression
Olfm1
Bfsp2
Acvr2a
Podxl
Lmo1
Dcn
Ifitm3
Krt5
Krt14
Krt15
Sostdc1
Vcan
BasalTagIn
Basal1
Basal2
Basal3
Basal4
C
Fraction of cells in a given cluster from each embryonic time point
1.0
0.5
0
E12.5
E13.5
E14.5
Basal TagIn
1
2
3
4
Basal
Early Placode
Late Placode
EarlyDiff
LateDiff
Periderm
D
Fraction of cells in a given cluster from each embryonic time point
1.0
0.5
0
G1
G2M
S
E
Number of receptors or ligands among marker genes
35
0
ligands
receptors
F
Cell-type ineractions in E12.5 skin
Epidermis
Dermis
EPI Periderm
EPI BasalTagIn
EPI Basal1
FIB Origin1
FIB Origin2
G
Number of interactions
EPI BasalTagIn
EPI Periderm
EPI Basal1
FIB Origin1
FIB Origin2
I
Incoming communication patterns (Receiving cells)
EPI Basal1
EPI BasalTagIn
EPI Periderm
FIB Origin1
FIB Origin2
ANGPTL
BMP
CXCL
EDN
GRN
IGF
MIF
ncWNT
NGF
PDGF
PERIOSTIN
SEMA3
TGFb
VISFATIN
WNT
Contribution
0.4
0.6
0.8
Outgoing communication patterns (Sending cells)
H
Sending cells
Outgoing communication patterns
Receiving cells
Incoming communication patterns
Pattern 1
Pattern 2
Pattern 3
Pattern 4
J
FIB → EPI
CXCL network
EPI → FIB
SEMA3 network
EPI BasalTagIn
EPI Periderm
EPI Basal1
FIB Origin1
FIB Origin2

Figure S6. Epidermal development – Cellular heterogeneity at E12.5, Related to Figure 7

(A) tSNE visualization of all keratinocytes, colored according to keratinocyte subclustering.
(B) Violin plot showing overlapping signatures in *EPI Basal1* population.
(C-D) Bar plot visualizing the contribution of each embryonic time point (C) or each (predicted) cell cycle phase (D) to each keratinocyte subcluster.
(E) Bar plot showing the number of ligands and receptors among the marker genes of each keratinocyte subcluster.
(F) Scheme of E12.5 keratinocyte and fibroblast subclusters included in the interaction analysis; only FIB Origin clusters located in close proximity to epidermis were included.
(G-J) CellChat generated cell communication analysis.
(G) Circle plot visualizing number of interactions between E12.5 keratinocyte and fibroblast subclusters. Edge width proportional to the number of interactions. Edges colored according to sending cell population.
(H) Sankey plot with inferred outgoing and incoming latent communication patterns. The thickness of the flow indicates the contribution of the cell group or signaling pathway, respectively, to each latent pattern.
(I) Dot plot showing outgoing and incoming signaling patterns between E12.5 keratinocyte and fibroblast subclusters. Dot size proportional to enrichment of signaling pathway in the cell population.
(J) Circle plots for selected signaling pathways with significant interactions.

Figure S7

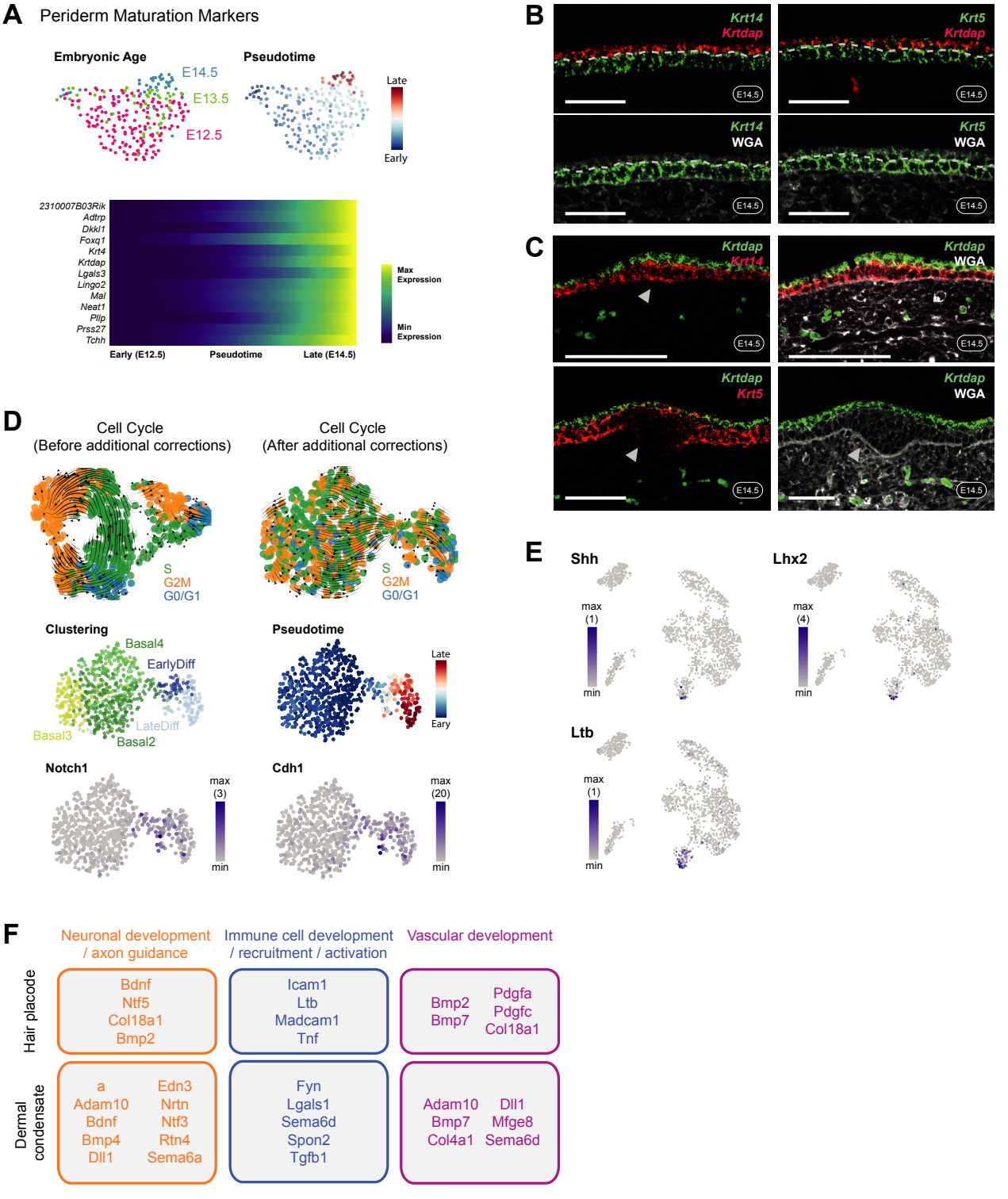

A
Periderm Maturation Markers
Embryonic Age
E14.5
E13.5
E12.5
Pseudotime
Late
Early
2310007B03Rik
Adtrp
Dkkl1
Foxq1
Krt4
Krtdap
Lgals3
Lingo2
Mal
Neat1
Pllp
Prss27
Tchh
Early (E12.5)
Pseudotime
Late (E14.5)
Max Expression
Min Expression
B
Krt14 Krtdap
Krt5 Krtdap
Krt14 WGA
Krt5 WGA
E14.5
C
Krtdap Krt14
Krtdap WGA
Krtdap Krt5
Krtdap WGA
D
Cell Cycle (Before additional corrections)
Cell Cycle (After additional corrections)
S
G2M
G0/G1
Clustering
Basal4
EarlyDiff
LateDiff
Basal2
Basal3
Pseudotime
Late
Eary
Notch1
Cdh1
max (3)
max (20)
min
E
Shh
Lhx2
Ltb
max (1)
max (4)
min
F
Neuronal development / axon guidance
Immune cell development / recruitment / activation
Vascular development
Hair placode
Dermal condensate
Bdnf
Ntf5
Col18a1
Bmp2
Icam1
Ltb
Madcam1
Tnf
Bmp2
Bmp7
Pdgfa
Pdgfc
Col18a1
a
Adam10
Bdnf
Bmp4
Dll1
Edn3
Nrtn
Ntf3
Rtn4
Sema6a
Fyn
Lgals1
Sema6d
Spon2
Tgfb1
Adam10
Bmp7
Col4a1
Dll1
Mfge8
Sema6d

Figure S7. Epidermal development - From a single basal layer towards a HF-inducing and stratified epithelium, Related to Figure 7

(A) UMAP of periderm cells, colored according to embryonic age (upper left panel) or pseudotime (upper right panel), respectively. Heatmap of genes related to periderm maturation (upper panel). A pseudotime was modeled based on *EPI Periderm* cells from all embryonic time points and pseudotime-dependent genes were determined. Genes peaking latest in pseudotime were chosen for display on heatmap.
(B) *Krt14* and *Krtdap* mRNA staining (left panels) and *Krt5* and *Krtdap* mRNA staining (right panels); revealing differing expression pattern of Krt5 and Krt14 in suprabasal layer. Dashed line marks basal-suprabasal border.
(C) *Krt14*, *Krt5* and *Krtdap* mRNA staining; confirming downregulation of *Krt5* and maintenance of *Krt14* in the developing hair placode (arrowhead) and showing a continous layer of suprabasal *Krtdap*$^{+}$ cells covering the hair placode.
(B-C) Microscope image originates from larger tile scan (n = 3 mice). Scale bars, 50µm.
(D) UMAP of keratinocyte subset (*EPI Basal1-4*, *EPI EarlyDiff*, and *EPI LateDiff* cells from E14.5) used to delineate epidermal stratification. Row one: UMAP before and after additional corrections for cell cycle (see **Methods**), colored according to (predicted) cell cycle phase and overlayed with velocity vectors predicting developmental trajectories. Row two: UMAP (from upper right panel), colored according to keratinocyte subclustering (left panel) or pseudotime (right panel), respectively. Row three: UMAP (from upper right panel) with expression of *Notch1* and *Cdh1*.
(E) UMAP with expression pattern of placode markers.
(F) Summary scheme with selected hair placode- and dermal condensate-derived factors promoting neuronal, immune cell, and vascular development.

Table S4. Oligonucleotides, Related to STAR Methods and Key Resources Table.

REAGNET or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
3-plex Positive Control Probe	ACDBio/Bio-Techne	Cat# 320881
3-plex Negative Control Probe	ACDBio/Bio-Techne	Cat# 320871
Mm-Axin2	ACDBio/Bio-Techne	Cat# 400331-C3
Mm-Creb5	ACDBio/Bio-Techne	Cat# 572891-C1
Mm-Dkk1	ACDBio/Bio-Techne	Cat# 402521-C3
Mm-Dkk4	ACDBio/Bio-Techne	Cat# 404851
Mm-Ebf2	ACDBio/Bio-Techne	Cat# 550841-C3
Mm-Gal	ACDBio/Bio-Techne	Cat# 400961-C3
Mm-Hapln1	ACDBio/Bio-Techne	Cat# 448201-C2
Mm-Krt5	ACDBio/Bio-Techne	Cat# 415041-C2
Mm-Krt8	ACDBio/Bio-Techne	Cat# 424521-C3
Mm-Krt10	ACDBio/Bio-Techne	Cat# 457901
Mm-Krt14	ACDBio/Bio-Techne	Cat# 422521-C3
Mm-Krtdap	ACDBio/Bio-Techne	Cat# 500671
Mm-Lef1	ACDBio/Bio-Techne	Cat# 441861
Mm-Ltb	ACDBio/Bio-Techne	Cat# 315681-C3
Mm-Mfap5	ACDBio/Bio-Techne	Cat# 490211-C2
Mm-Mia	ACDBio/Bio-Techne	Cat# 498011
Mm-Notum	ACDBio/Bio-Techne	Cat# 428981
Mm-Nppc	ACDBio/Bio-Techne	Cat# 493291
Mm-Pax7	ACDBio/Bio-Techne	Cat# 314181-C3
Mm-Pdgfc	ACDBio/Bio-Techne	Cat# 441551
Mm-Pecam1	ACDBio/Bio-Techne	Cat# 316721-C2
Mm-Pmel	ACDBio/Bio-Techne	Cat# 422481-C2
Mm-Postn	ACDBio/Bio-Techne	Cat# 418581
Mm-Pparg	ACDBio/Bio-Techne	Cat# 418821
Mm-Ptch1	ACDBio/Bio-Techne	Cat# 402811
Mm-Rgs5	ACDBio/Bio-Techne	Cat# 430181
Mm-Shh	ACDBio/Bio-Techne	Cat# 314361-C2
Mm-Sox9	ACDBio/Bio-Techne	Cat# 401051-C2
Mm-Sox10	ACDBio/Bio-Techne	Cat# 435931-C3
Mm-Thbs1	ACDBio/Bio-Techne	Cat# 457891