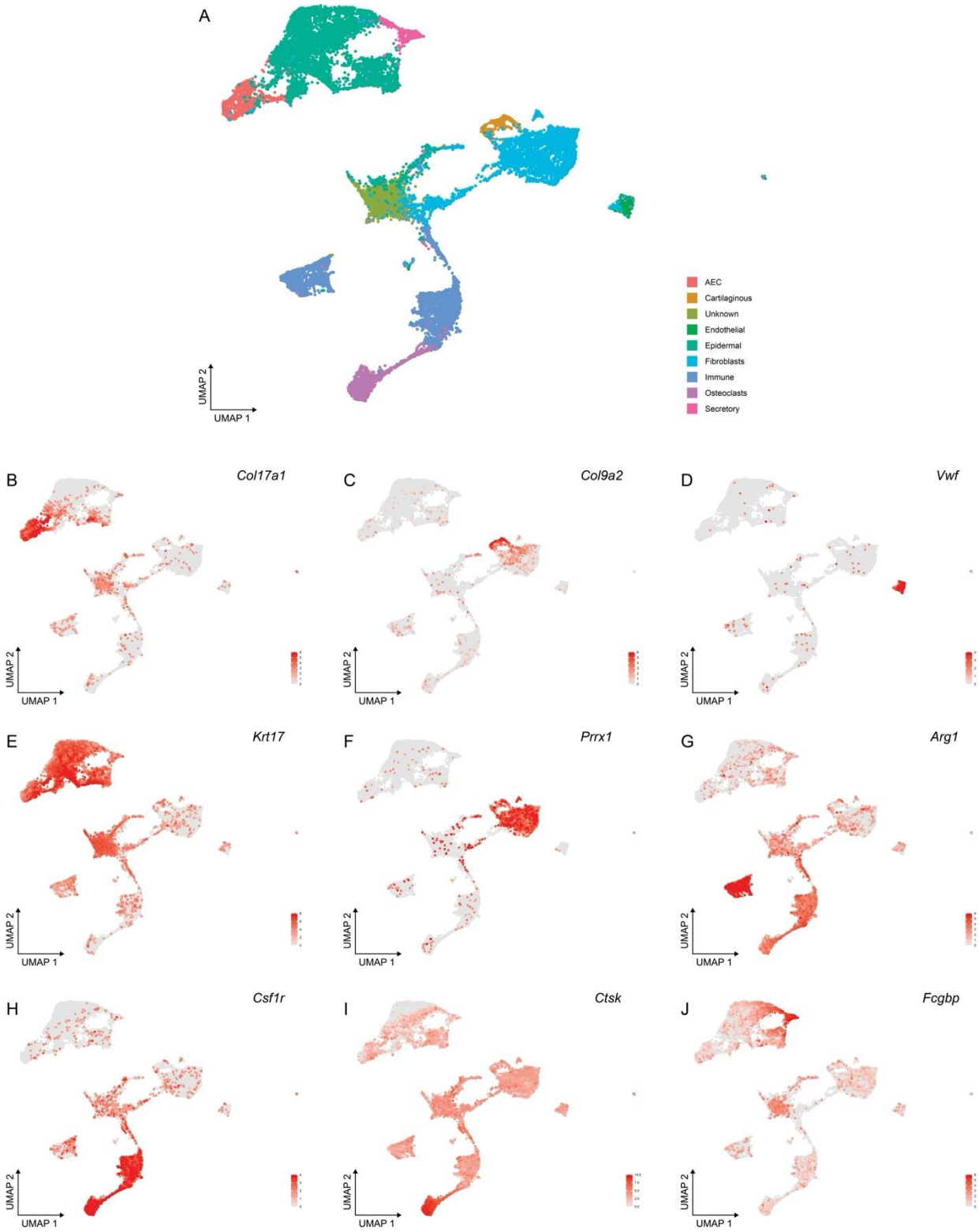
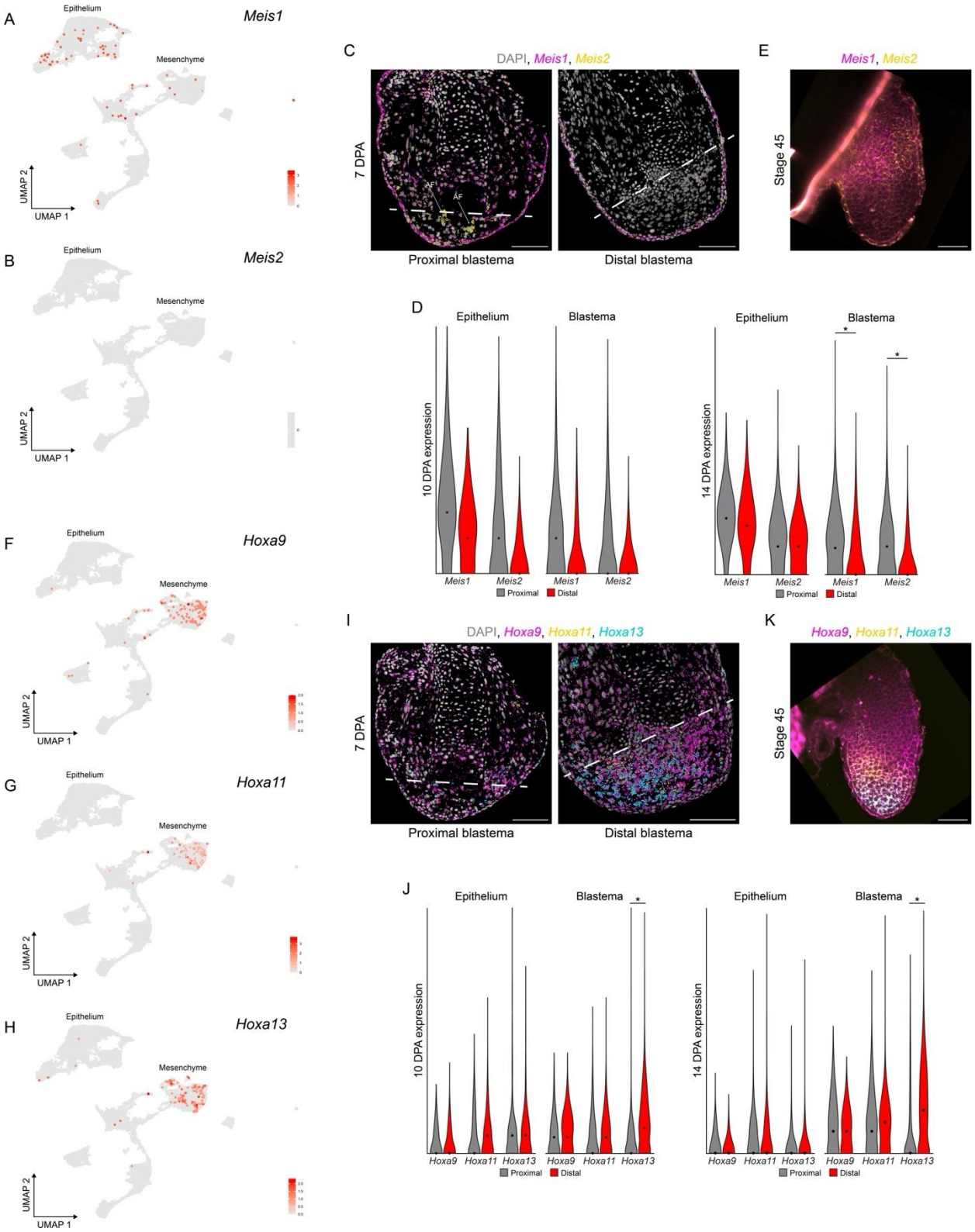


Figure S1: **Cluster identification in reanalysis of blastemas from Li et al. 2021**

(A) Clustering of 7, 14, and 22 DPA blastemas from Li et al. 2021 with 9 clusters marked by designated genes. (B) *Col17a1* marks the apical epithelial cap (AEC). (C)

Col9a2 marks chondrocytes. (D) *Vwf* marks endothelial cells. (E) *Krt17* marks an unknown cell population. (F) *Prrx1* marks dedifferentiated limb CT cells. (G-H) *Arg1* and *Csf1r* mark immune cells. (I) *Ctsk* marks osteoclasts. (J) *Fcgbp* marks secretory cells.



7 **Figure S2: Additional characterization of PD patterning gene expression**

(A) UMAP showing *Meis1* in blastemas at 7, 14, and 22 DPA. (B) UMAP showing *Meis2* expression was undetected in blastemas at 7, 14, or 22 DPA. (C) HCR-FISH for *Meis1* and *Meis2* in PBs and DBs at 7 DPA. Dashed lines indicate the amputation plane. AF = autofluorescence. Scale bars = 200 μm. (D) Quantification in the epithelium and whole blastema for *Meis1* and *Meis2* in PBs and DBs at 10 and 14 DPA (3.5 cm (HT) animals aged 2.5 months). Axes and analyses as in Fig. 1E. * = $p < 0.05$. (E) HCR-FISH for *Meis1* and *Meis2* expression in whole mount developing limb buds at stage 45. The image represents a single, 2D z-plane within a 3D image stack. Scale bar = 50 μm. (F-H) UMAP showing *Hoxa9* (F), *Hoxa11* (G), and *Hoxa13* (H) in blastemas at 7, 14, and 22 DPA. (I) HCR-FISH for *Hoxa9*, *Hoxa11*, and *Hoxa13* in PBs and DBs at 7 DPA. Dashed lines indicate the amputation plane. Scale bars = 200 μm. (J) Quantification in the epithelium and whole blastema for *Hoxa9*, *Hoxa11*, and *Hoxa13* in PBs and DBs at 10 and 14 DPA (3.5 cm (HT) animals aged 2.5 months). Axes and analyses as in Fig. 1E. * = $p < 0.05$. (K) HCR-FISH for *Hoxa9*, *Hoxa11*, and *Hoxa13* in whole mount developing limb buds at stage 45. The image represents a single, 2D z-plane within a 3D image stack. Scale bar = 50 μm. ScRNA-seq data were reanalyzed from a previously published dataset (Li et al., 2021). Sample size and exact p-values are located within the source data file. Source data are provided as a source data file.

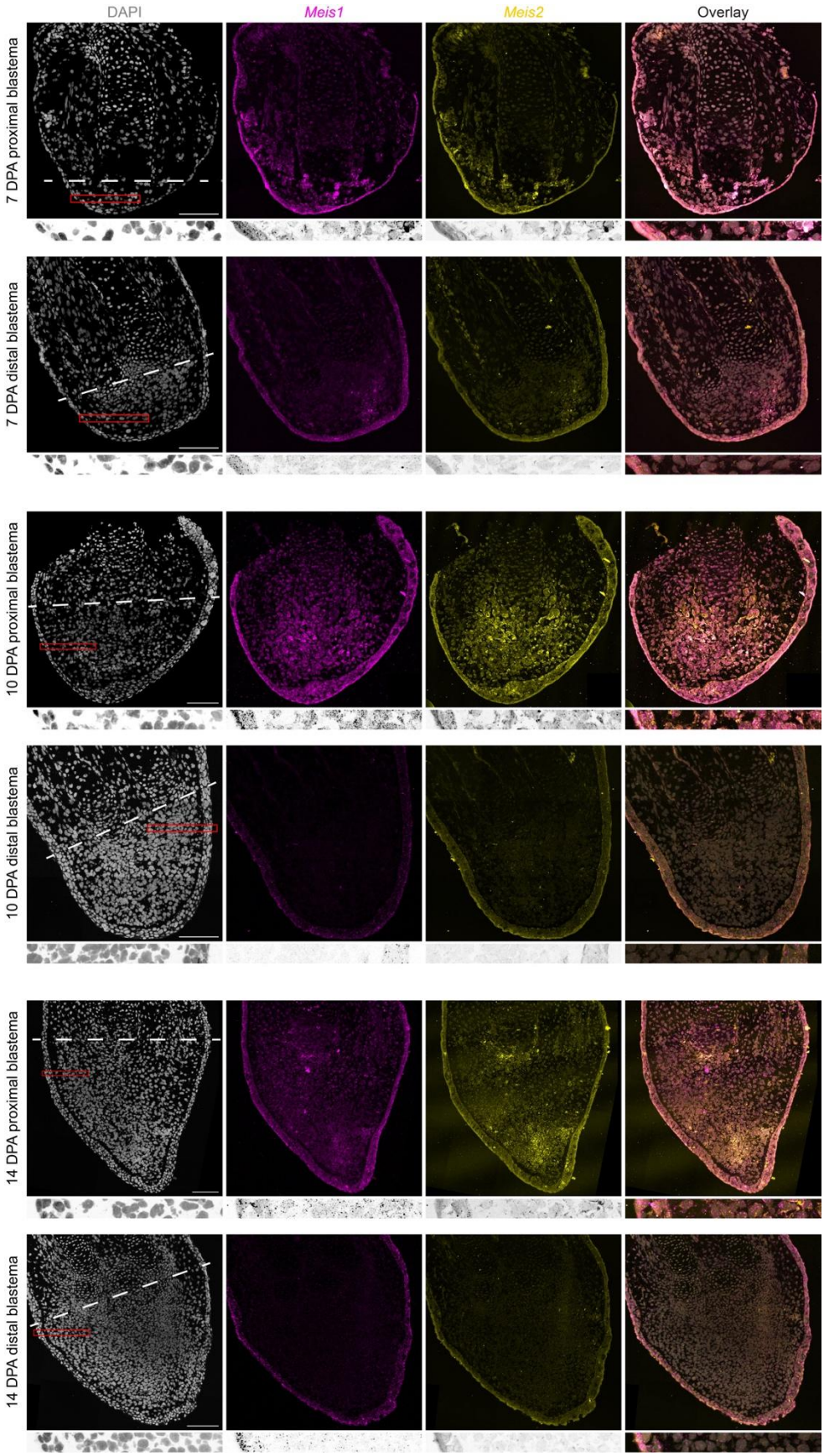

DAPI
Meis1
Meis2
Overlay
7 DPA proximal blastema
7 DPA distal blastema
10 DPA proximal blastema
10 DPA distal blastema
14 DPA proximal blastema
14 DPA distal blastema

Figure S3: Individual FISH channels for *Meis1* and *Meis2* in PBs and DBs from Fig. 1 and Fig. S2

Individual fluorescent channels for *Meis1* and *Meis2* in PBs and DBs at 7, 10, and 14 DPA. Boxes in the DAPI channel represent the location of the inset images. Dashed lines indicate the amputation plane. Scale bars = 200 µm.

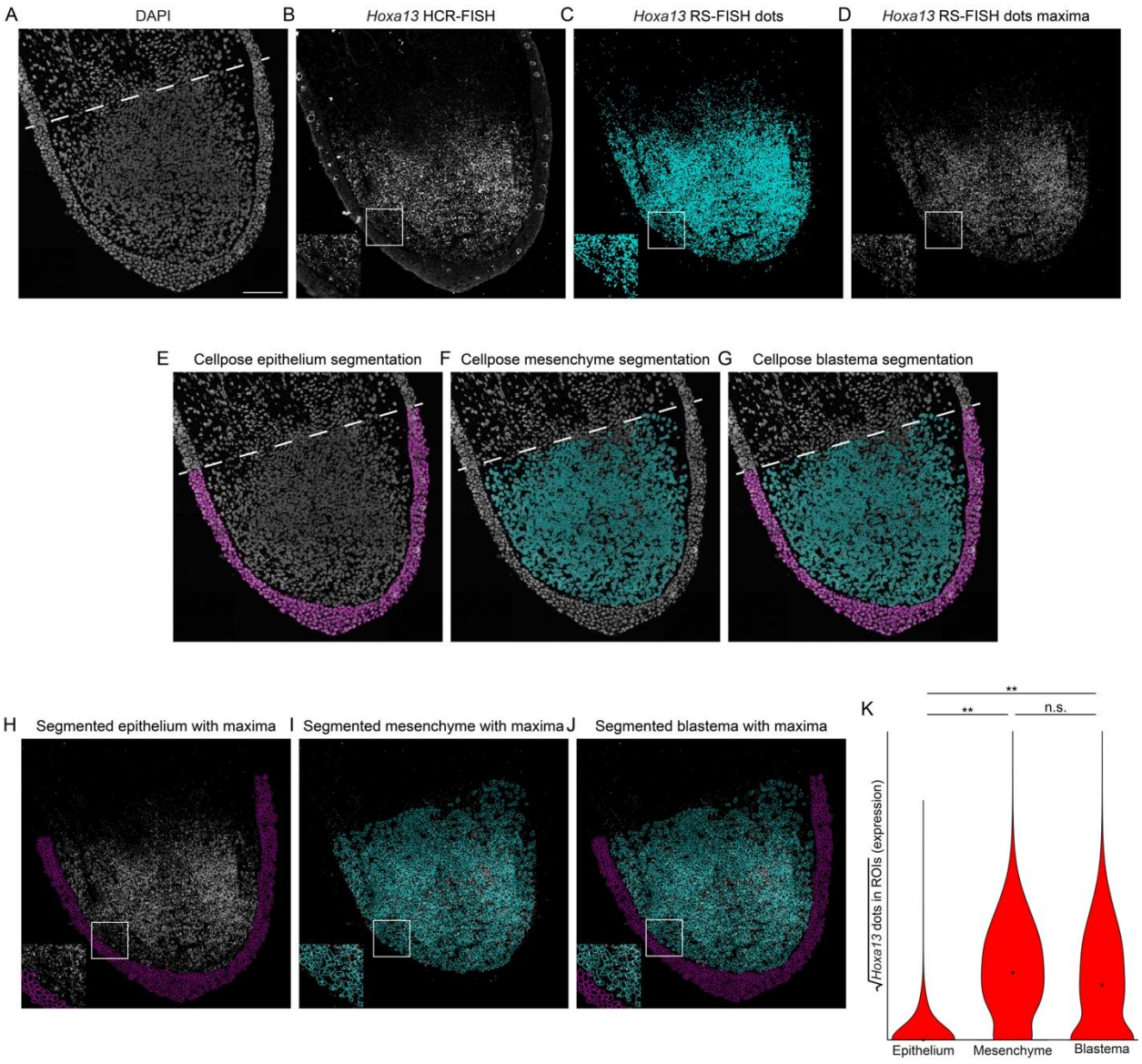


Figure S4: HCR-FISH dot quantification workflow

(A) DAPI channel for a representative DB replicate at 14 DPA. Scale bar = 200 µm. (B) Raw imaging data for *Hoxa13* expression. Inset scale bar = 20 µm. (C) Pseudodots obtained by RS-FISH (Bahry et al., 2022). (D) Maxima-converted pseudodots, each containing a pixel value of 255. (E-G) Cellpose-generated segmentation of epithelium (E), mesenchyme (F), and whole blastema (G) from DAPI channel in panel A (Stringer et al., 2021). (H-J) Segmentation for epithelium (H), mesenchyme (I), and whole blastema (J) overlayed on atop maxima from panel D. (K) Quantification in each tissue type for *Hoxa13* expression in DBs at 14 DPA (3.5 cm (HT) animals aged 2.5 months). The sum of the pixel values within each cell was divided by 255 to obtain the total

number of HCR-FISH dots within a cell. These values were then square root normalized for visualization in violin plots. Groups were analyzed using a clustered Wilcoxon rank sum test according to the Datta-Satten method. n.s. = no statistical difference, ** = $p < 0.01$. Sample size and exact p-values are located within the source data file. Source data are provided as a source data file.

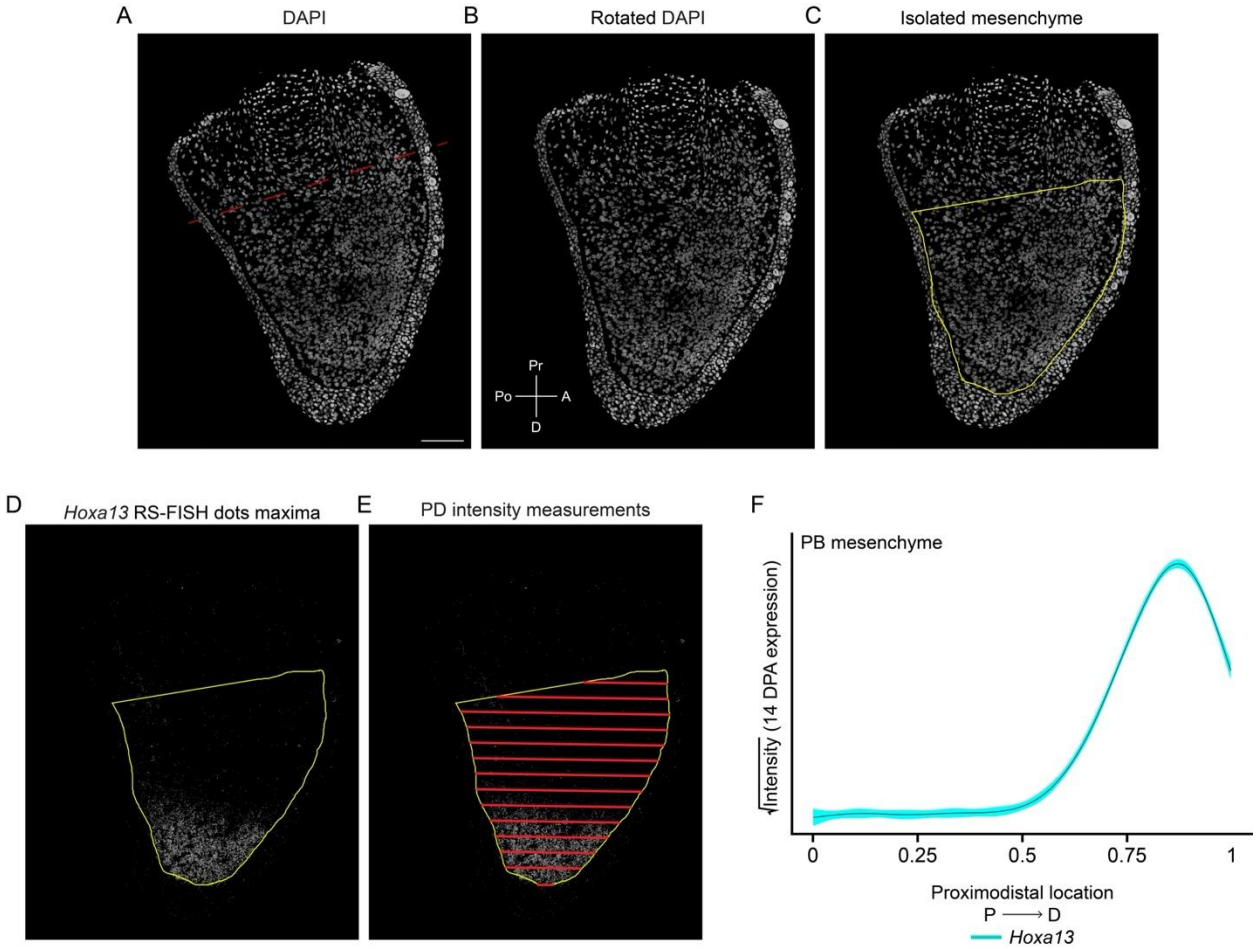


Figure S5: Workflow for generating HCR-FISH PD intensity plots

(A) DAPI channel for a representative PB replicate at 14 DPA. Scale bar = 200 µm. (B) Rotated DAPI channel from panel A. Pr = proximal, A = anterior, D = distal, Po = posterior. (C) Rotated DAPI image with mesenchyme outlined. (D) Mesenchyme outline with maxima-converted pseudodots. Pseudodots obtained via workflow outlined in Figure S3. (E) Intensity measurements obtained continuously along the proximodistal axis. (F) Intensity plots for *Hoxa13* expression along the PD axis within the mesenchyme of PBs at 14 DPA. Lines represent average signal intensity (expression) along a normalized PD axis across each sample. Shaded region represents 99% confidence interval. Source data are provided as a source data file.

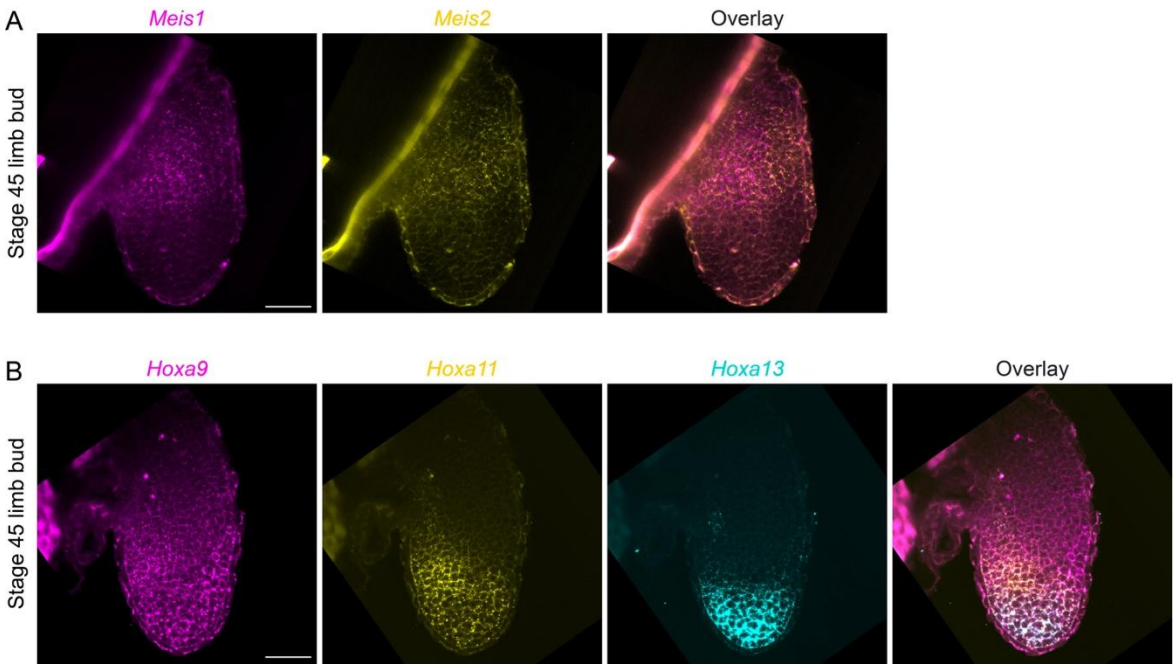


Figure S6: Individual FISH channels in stage 45 developing limbs from Fig. S2

(A) Individual fluorescent channels for *Meis1* and *Meis2* in a stage 45 developing limb bud. The images represent a single, 2D z-plane within a 3D image stack. (B) Individual fluorescent channels for *Hoxa9*, *Hoxa11*, and *Hoxa13* in a stage 45 developing limb bud. The images represent a single, 2D z-plane within a 3D image stack. Scale bars = 50 µm.

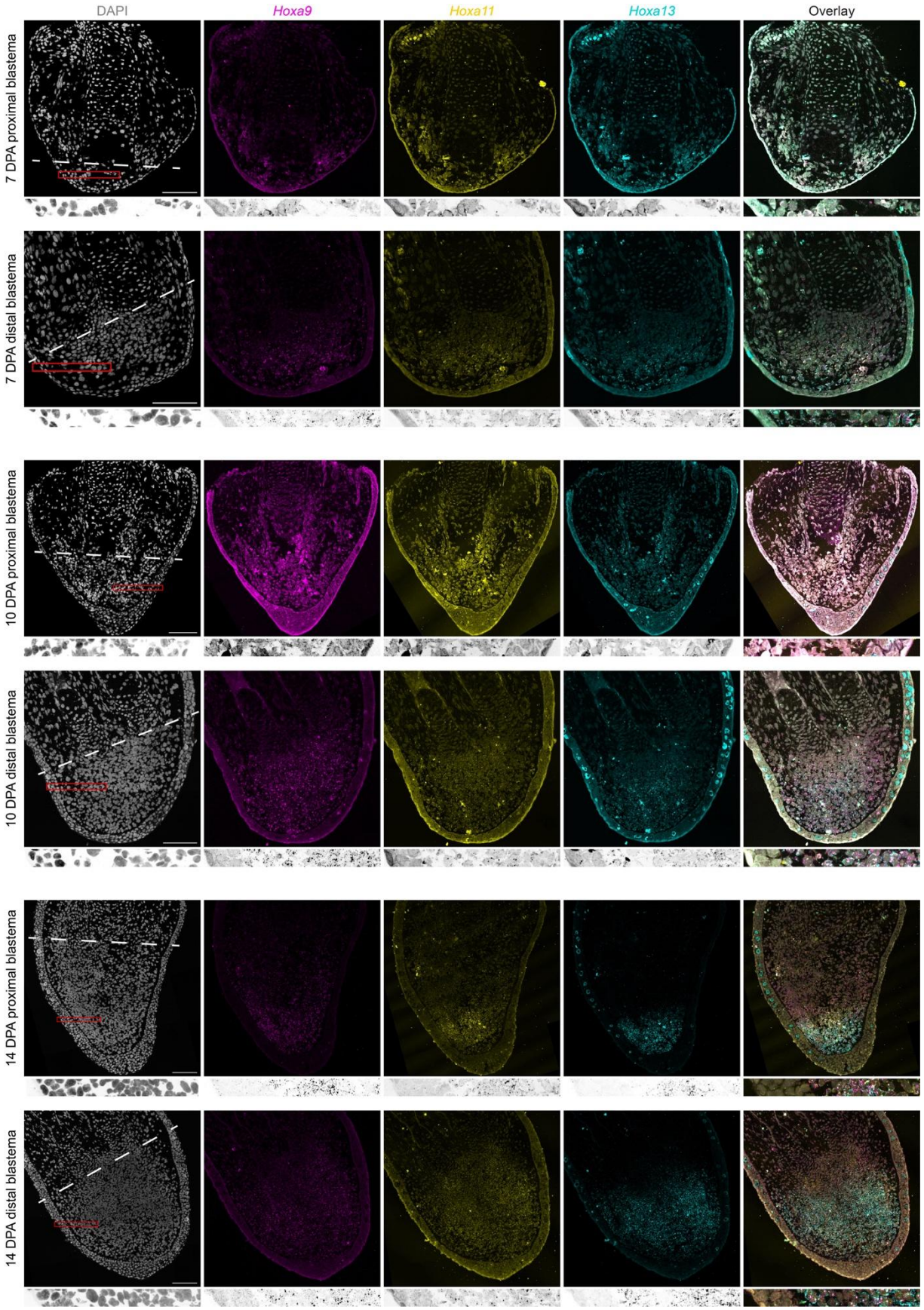
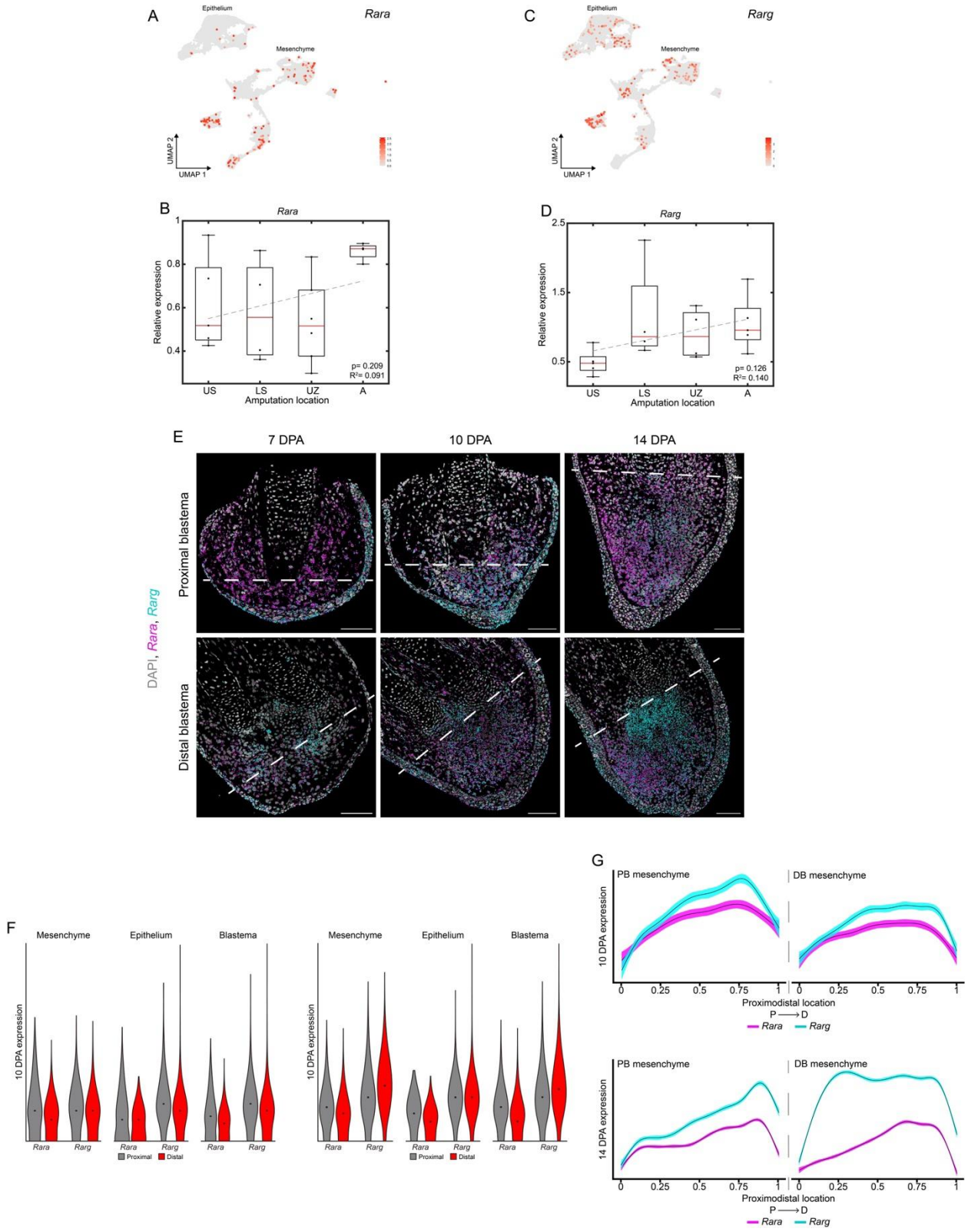

DAPI
Hoxa9
Hoxa11
Hoxa13
Overlay
7 DPA proximal blastema
7 DPA distal blastema
10 DPA proximal blastema
10 DPA distal blastema
14 DPA proximal blastema
14 DPA distal blastema

Figure S7: Individual FISH channels for *Hoxa9*, *Hoxa11*, and *Hoxa13* in PBs and DBs from Fig. 1 and Fig. S2

Individual fluorescent channels for *Hoxa9*, *Hoxa11*, and *Hoxa13* in PBs and DBs at 7, 10, and 14 DPA. Boxes in the DAPI channel represent the location of the inset images. Dashed lines indicate the amputation plane. Scale bars = 200 µm.



67 **Figure S8: *Rar* expression along the regenerating PD axis**

(A) UMAP showing *Rara* in blastemas at 7, 14, and 22 DPA. (B) qRT-PCR quantification of *Rara* at different amputation locations along the PD axis (.5 cm (HT) animals aged 2.5 months, blastemas collected at 10 DPA). Boxplots and analyses as in Fig. 1B-C. (C) UMAP showing *Rarg* in blastemas at 7, 14, and 22 DPA. (D) qRT-PCR quantification of *Rarg* at different amputation locations along the PD axis (3.5 cm (HT) animals aged 2.5 months, blastemas collected at 10 DPA). Boxplots and analyses as in Fig. 1B-C**.** (E) HCR-FISH for *Rara* and *Rarg* in PBs and DBs at 7, 10, and 14 DPA. Dashed lines indicate the amputation plane. Scale bars = 200 µm. (F) Quantification for *Rara* and *Rarg* expression in the mesenchyme, epithelium, and whole blastema of PBs and DBs at 10 DPA (3.5 cm (HT) animals aged 2.5 months). Axes and analyses as in Fig. 1E. (L) PD Intensity plots for mesenchymal *Rara* and *Rarg* in PBs and DBs at 10 and 14 DPA (3.5 cm (HT) animals aged 2.5 months). Axes and analyses as in Fig. 1F. Sample size and exact p-values are located within the source data file. Source data are provided as a source data file.

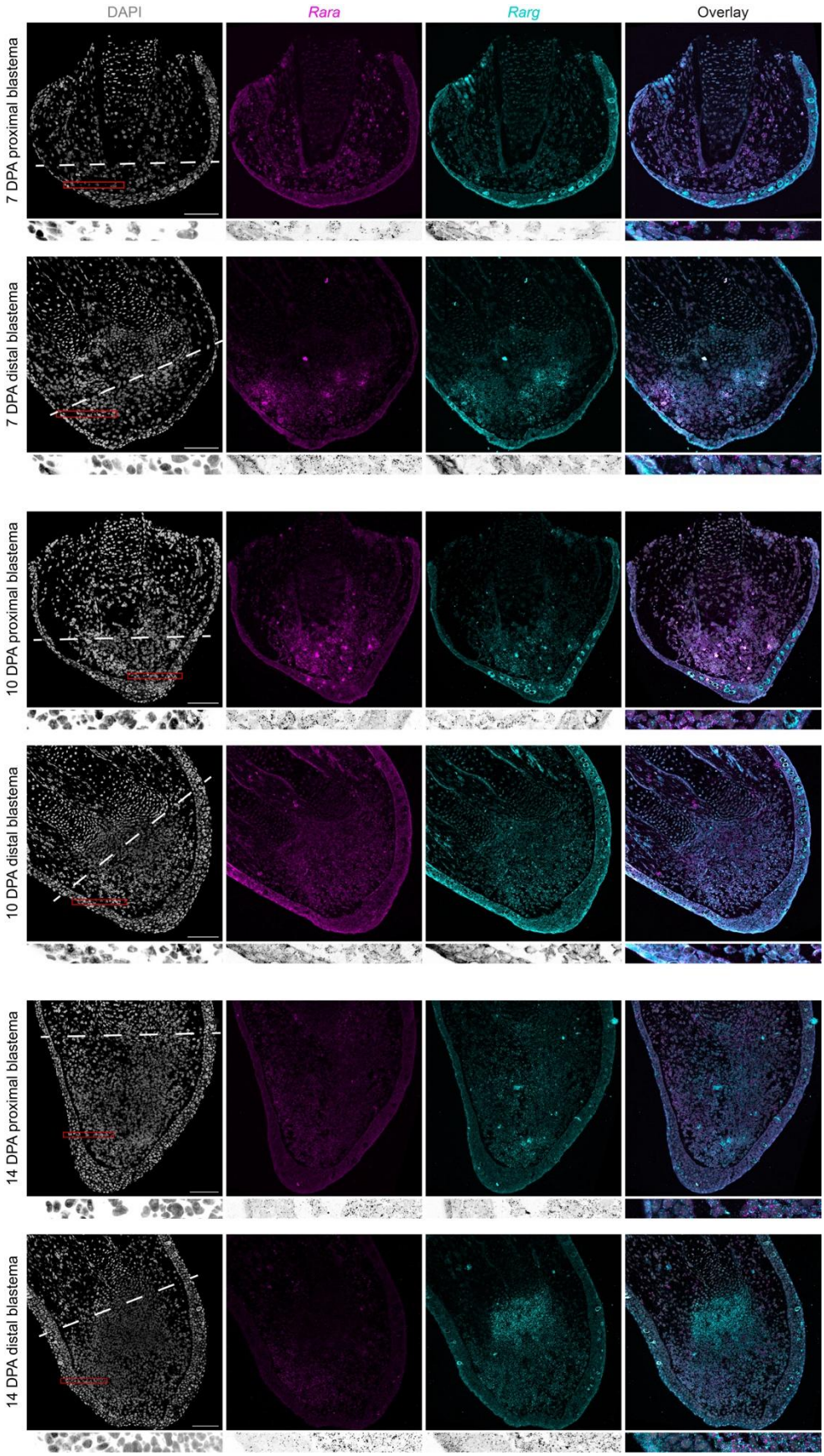
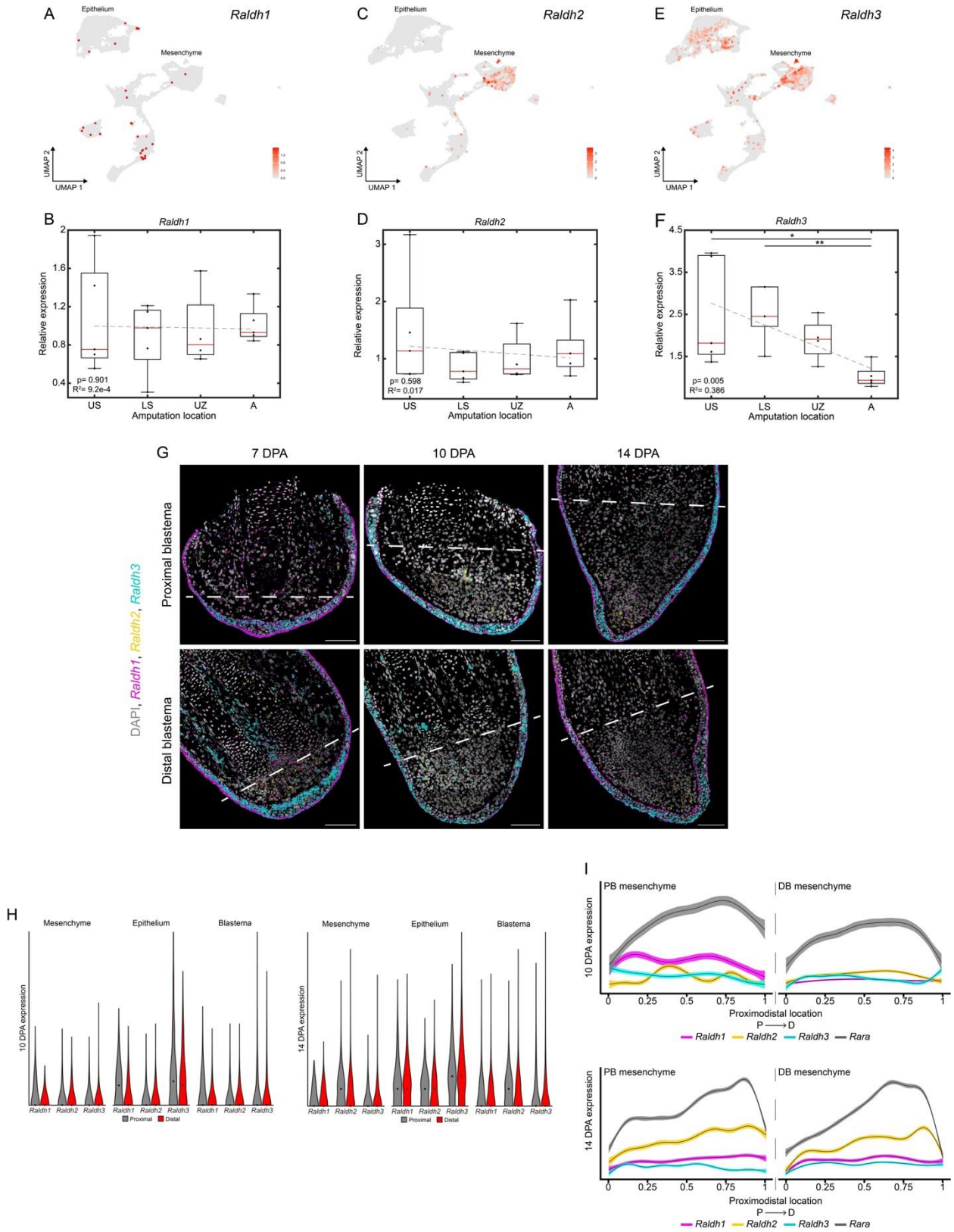

DAPI
Rara
Rarg
Overlay
7 DPA proximal blastema
7 DPA distal blastema
10 DPA proximal blastema
10 DPA distal blastema
14 DPA proximal blastema
14 DPA distal blastema

Figure S9: Individual FISH channels for *Rara* and *Rarg* in PBs and DBs from Fig. S8

Individual fluorescent channels for *Rary* and *Rarg* in PBs and DBs at 7, 10, and 14 DPA. Boxes in the DAPI channel represent the location of the inset images. Dashed lines indicate the amputation plane. Scale bars = 200 µm.



87 **Figure S10: *Raldh* expression along the regenerating PD axis**

(A) UMAP showing *Raldh1* in blastemas at 7, 14, and 22 DPA. (B) qRT-PCR quantification of *Raldh1* at different amputation locations along the PD axis (3.5 cm (HT) animals aged 2.5 months, blastemas collected at 10 DPA). Boxplots and analyses as in Fig. 1B-C. (C) UMAP showing *Raldh2* in blastemas at 7, 14, and 22 DPA. (D) qRT-PCR quantification of *Raldh2* at different amputation locations along the PD axis (3.5 cm (HT) animals aged 2.5 months, blastemas collected at 10 DPA). Boxplots analyses as in Fig. 1B-C**.** * = $p < 0.05$, ** = $p < 0.01$. (E) UMAP showing *Raldh3* in blastemas at 7, 14, and 22 DPA. (F) qRT-PCR quantification of *Raldh3* at different amputation locations along the PD axis (3.5 cm (HT) animals aged 2.5 months, blastemas collected at 10 DPA). Boxplots and analyses as in Fig. 1B-C. (G) HCR-FISH for *Raldh1*, *Raldh2*, and *Raldh3* in PBs and DBs at 7, 10, and 14 DPA. Dashed lines indicate the amputation plane. Scale bars = 200 µm. (H) Quantification for *Raldh1*, *Raldh2*, and *Raldh*3 in the mesenchyme, epithelium, and whole blastema of PBs and DBs at 10 and 14 DPA (3.5 cm (HT) animals aged 2.5 months). Axes and analyses as in Fig. 1E. (I) PD intensity plots for mesenchymal *Raldh1*, *Raldh2*, *Raldh3*, and *Rara* in PBs and DBs at 10 and 14 DPA (3.5 cm (HT) animals aged 2.5 months). *Rara* was included as a reference for a more highly expressed gene in the mesenchyme. Axes and analyses as in Fig. 1F. Sample size and exact p-values are located within the source data file. Source data are provided as a source data file.

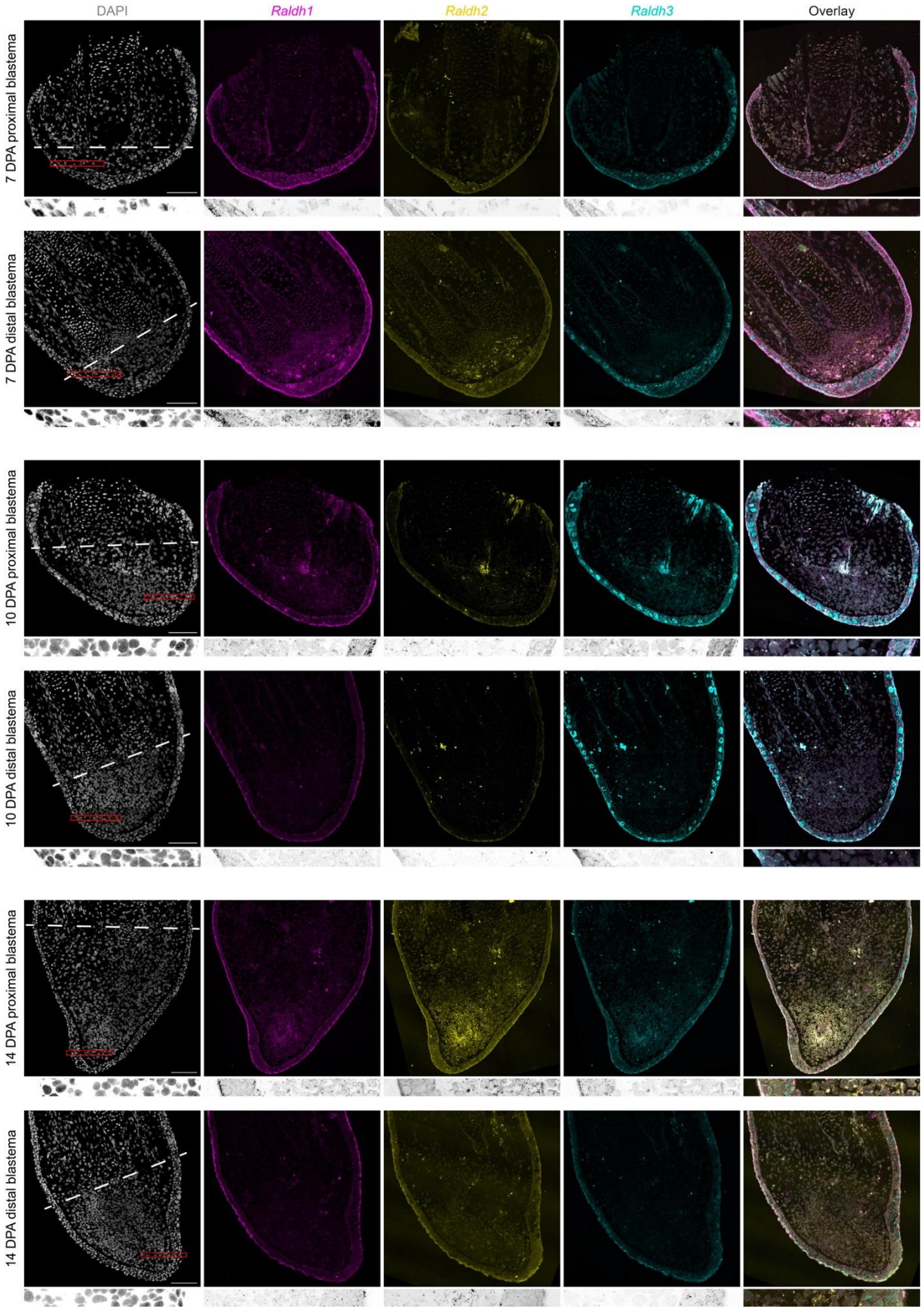

DAPI
Raldh1
Raldh2
Raldh3
Overlay
7 DPA proximal blastema
7 DPA distal blastema
10 DPA proximal blastema
10 DPA distal blastema
14 DPA proximal blastema
14 DPA distal blastema

Figure S11: Individual FISH channels for *Raldh1*, *Raldh2,* and *Raldh3* in PBs and DBs from Fig. S10

Individual fluorescent channels for *Raldh1*, *Raldh2*, and *Raldh3* in PBs and DBs at 7, 10, and 14 DPA. Boxes in the DAPI channel represent the location of the inset images. Dashed lines indicate the amputation plane. Scale bars = 200 µm.

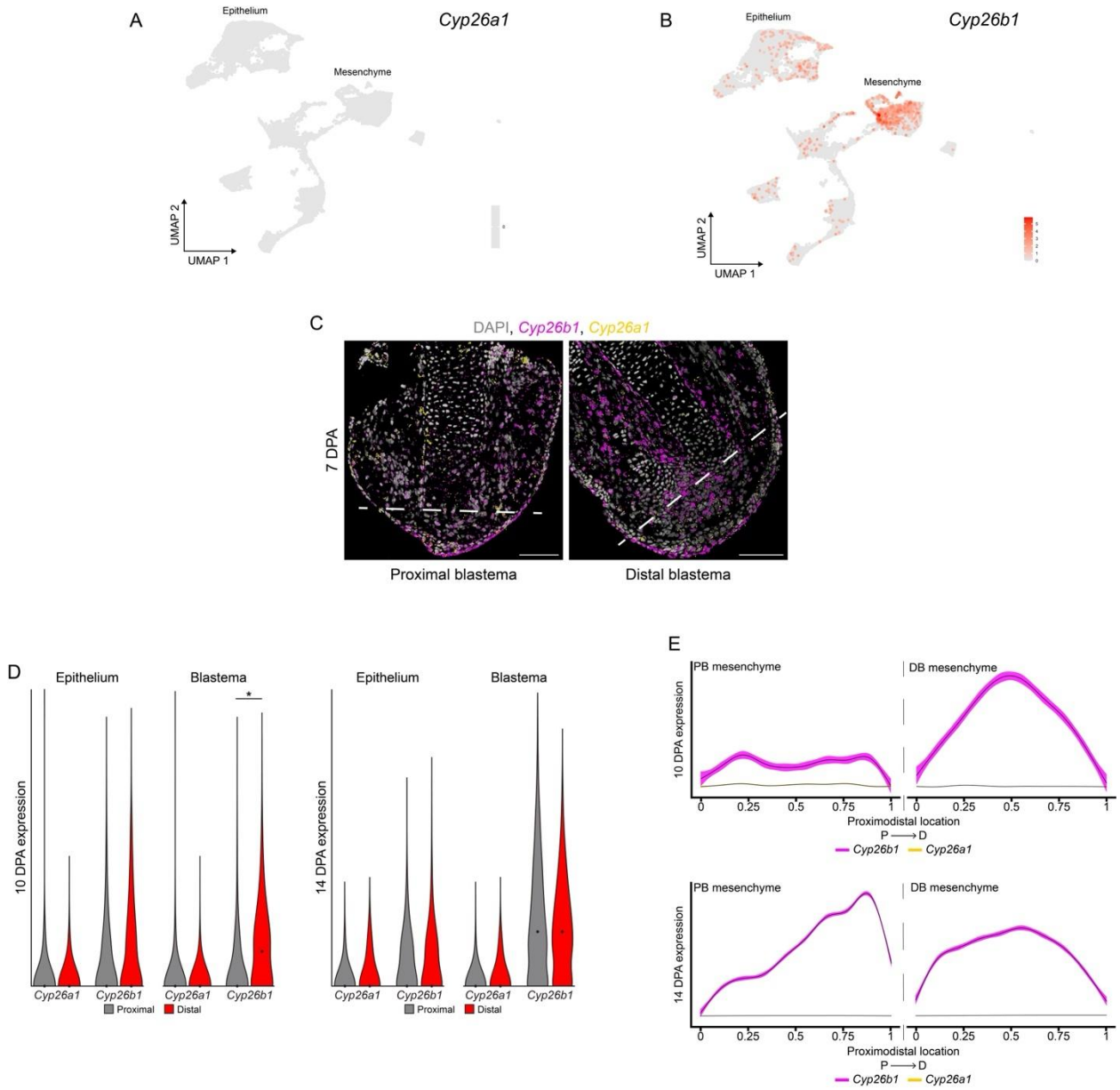


Figure S12: Additional characterization of *Cyp26a1* and *Cyp26b1* expression

(A) UMAP showing *Cyp26a1* expression was undetected in blastemas at 7, 14, or 22 DPA. (B) UMAP showing *Cyp26b1* in blastemas at 7, 14, and 22 DPA. (C) HCR-FISH for *Cyp26a1* and *Cyp26b1* in PBs and DBs at 7 DPA. Dashed lines indicate the amputation plane. Scale bars = 200 µm. (D) Quantification in the epithelium and whole blastema for *Cyp26a1* and *Cyp26b1* expression in PBs and DBs at 10 and 14 DPA (3.5 cm (HT) animals aged 2.5 months). Axes and analyses as in Fig. 1E. * = $p < 0.05$. (E) PD intensity plots for *Cyp26a1* and *Cyp26b1* in PBs and DBs at 10 and 14 DPA (3.5 cm (HT) animals aged 2.5 months). Axes and analyses as in Fig. 1F. Sample size and

exact p-values are located within the source data file. Source data are provided as a source data file.

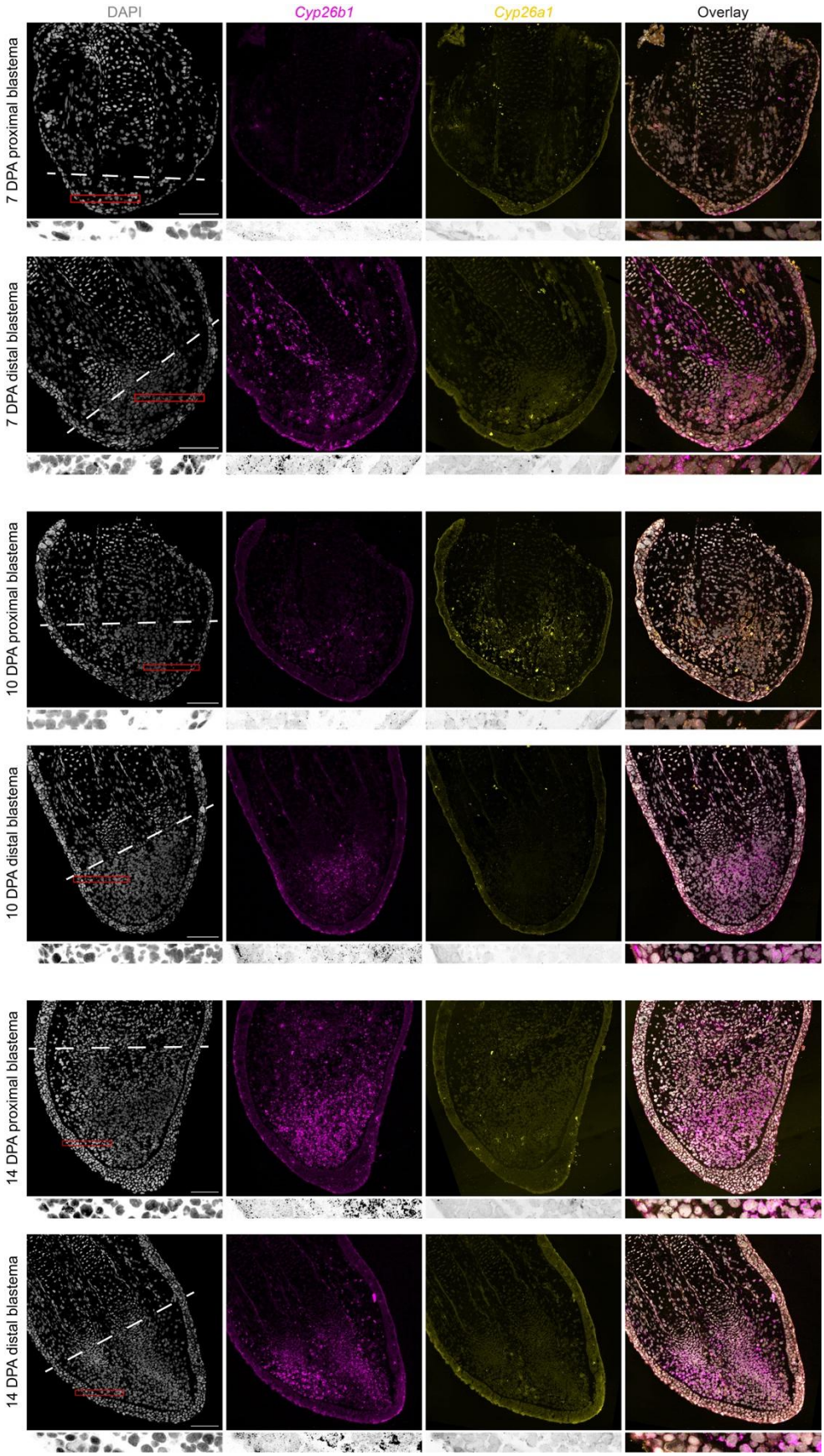

DAPI
Cyp26b1
Cyp26a1
Overlay
7 DPA proximal blastema
7 DPA distal blastema
10 DPA proximal blastema
10 DPA distal blastema
14 DPA proximal blastema
14 DPA distal blastema

Figure S13: Individual FISH channels for *Cyp26a1* and *Cyp26b1* in PBs and DBs from Fig. 2 and Fig. S12

Individual fluorescent channels for *Cyp26a1* and *Cyp26b1* in PBs and DBs at 7, 10, and 14 DPA. Boxes in the DAPI channel represent the location of the inset images. Dashed lines indicate the amputation plane. Scale bars = 200 µm.

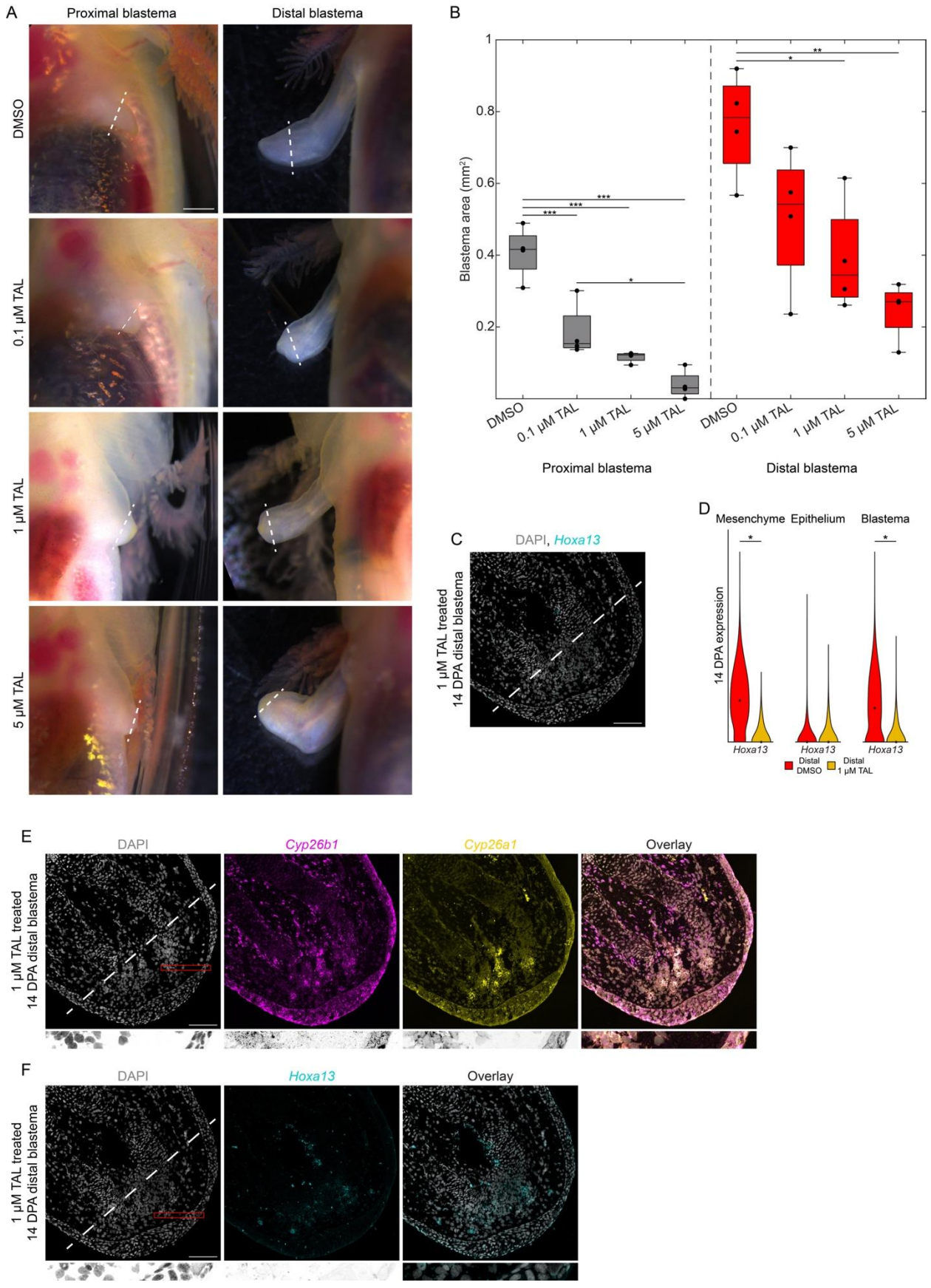

A
Proximal blastema
Distal blastema
DMSO
0.1 µM TAL
1 µM TAL
5 µM TAL
B
Blastema area (mm²)
1
0.8
0.6
0.4
0.2
***
***
***
*
**
*
DMSO
0.1 µM TAL
1 µM TAL
5 µM TAL
DMSO
0.1 µM TAL
1 µM TAL
5 µM TAL
Proximal blastema
Distal blastema
C
DAPI, Hoxa13
1 µM TAL treated
14 DPA distal blastema
D
Mesenchyme
Epithelium
Blastema
14 DPA expression
*
*
Hoxa13
Hoxa13
Hoxa13
Distal DMSO
Distal 1 µM TAL
E
DAPI
Cyp26b1
Cyp26a1
Overlay
1 µM TAL treated
14 DPA distal blastema
F
DAPI
Hoxa13
Overlay
1 µM TAL treated
14 DPA distal blastema

Figure S14: Additional characterization of the impact of TAL during limb regeneration

(A) Brightfield images of PBs and DBs treated with DMSO or 0.1, 1, or 5 µM TAL at 14 DPA. Dashed lines indicate the amputation plane. Scale bar = 1 mm. (B) Quantification of blastema area for PBs and DBs treated with DMSO or 0.1, 1, or 5 µM TAL at 14 DPA (4.5 cm (HT) animals aged 4 months). Boxplot top and bottom represent the 75th and 25th percentiles, the red line indicates median, and whisker ends show minima and maxima. Groups were analyzed using a one-way ANOVA using a Tukey-Kramer multiple comparison test. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. (C) HCR-FISH for *Hoxa13* in DBs treated with 1 µM TAL at 14 DPA. Dashed line indicates amputation plane. Scale bar = 200 µm. (D) Quantification in the epithelium and whole blastema for *Cyp26a1* and *Cyp26b1* in PBs and DBs at 10 and 14 DPA (3.5 cm (HT) animals aged 2.5 months). Axes and analyses as in Fig. 1E. * = $p < 0.05$. (E-F) Individual fluorescent channels for *Cyp26a1* and *Cyp26b1* (E) or *Hoxa13* (F) in TAL treated DBs at 14 DPA. Boxes in the DAPI channel represent the location of the inset images. Dashed lines indicate the amputation plane. Scale bars = 200 µm. Sample size and exact p-values are located within the source data file. Source data are provided as a source data file.

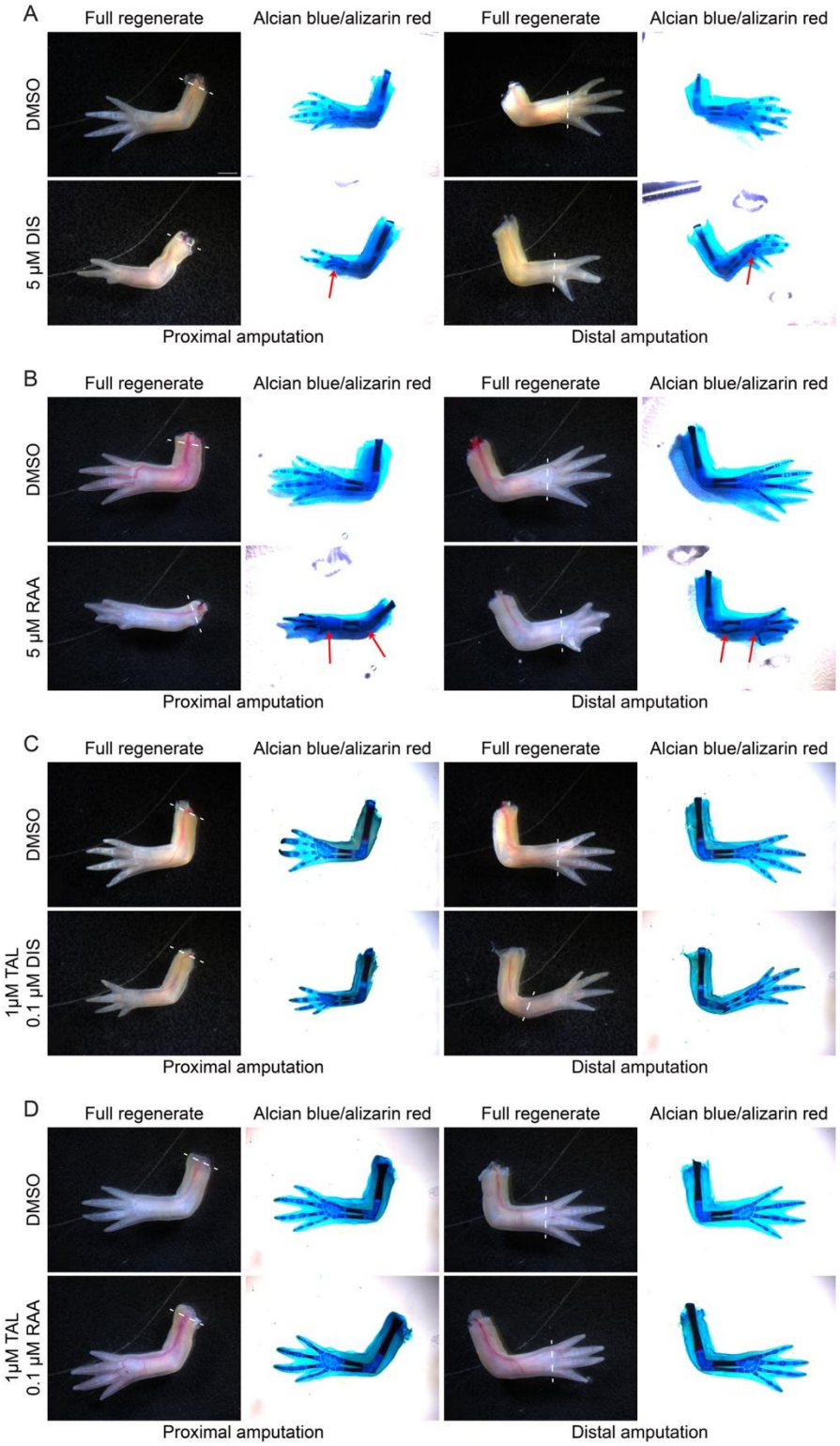
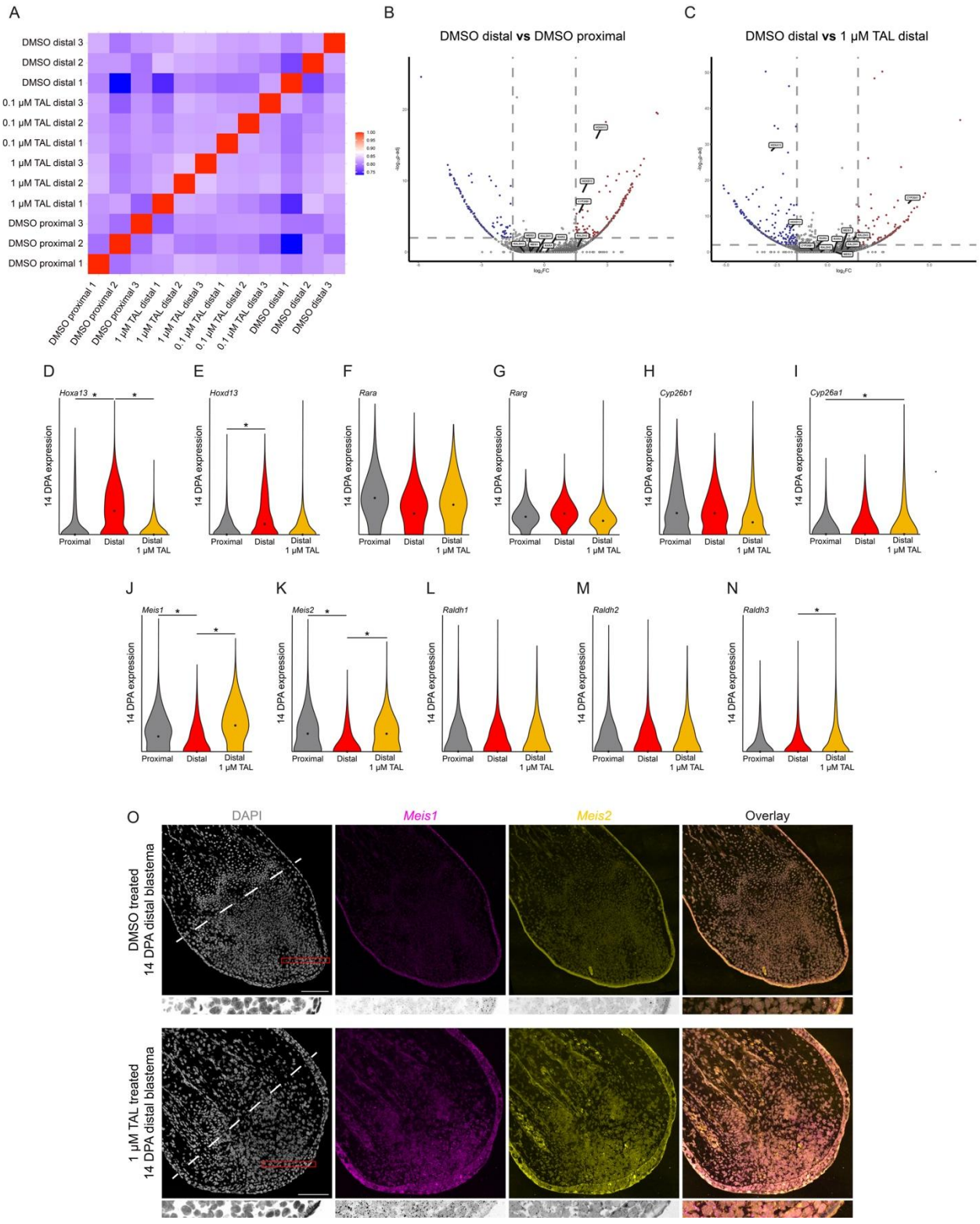


Figure S15: Cotreating TAL with DIS or RAA prevents proximalization

(A) Brightfield images of regenerates and skeletal structures of PBs and DBs treated with DMSO or 5 µM DIS. (B) Brightfield images of regenerates and skeletal structures

of PBs and DBs treated with DMSO or 5 µM RAA. (C) Brightfield images of regenerates and skeletal structures of PBs and DBs treated with DMSO or 1 µM TAL/0.1 µM DIS. (D) Brightfield images of regenerates and skeletal structures of PBs and DBs treated with DMSO or 1 µM TAL/0.1 µM RAA. Arrows point to skeletal abnormalities. Dashed lines indicate the amputation plane. Scale bar = 2 mm.



153 **Figure S16: Additional bulk RNAseq characterization and validation**

154 (A) Sample correlation matrix showing relatedness between each sample. (B-C)

155 Volcano plots showing expression of genes quantified in D-M. (D-N) Quantification in

the whole blastema for *Hoxa13*, *Hoxd13*, *Rara*, *Rarg*, *Cyp26b1*, *Cyp26a1*, *Meis1*, *Meis2*, *Raldh1*, *Raldh2*, and *Raldh3* expression in DMSO treated PBs, DMSO treated DBs, and 1 µM TAL treated DBs at 14 DPA (3.5 cm (T) animals aged 2.5 months). Groups were analyzed using a clustered Wilcoxon rank sum test. * = $p < 0.05$. (O) Individual fluorescent channels for *Meis1* and *Meis2* in control or TAL treated DBs at 14 DPA. Boxes in the DAPI channel represent the location of the inset images. Dashed lines indicate the amputation plane. Scale bars = 200 µm. Sample size and exact p-values are located within the source data file. Source data are provided as a source data file.

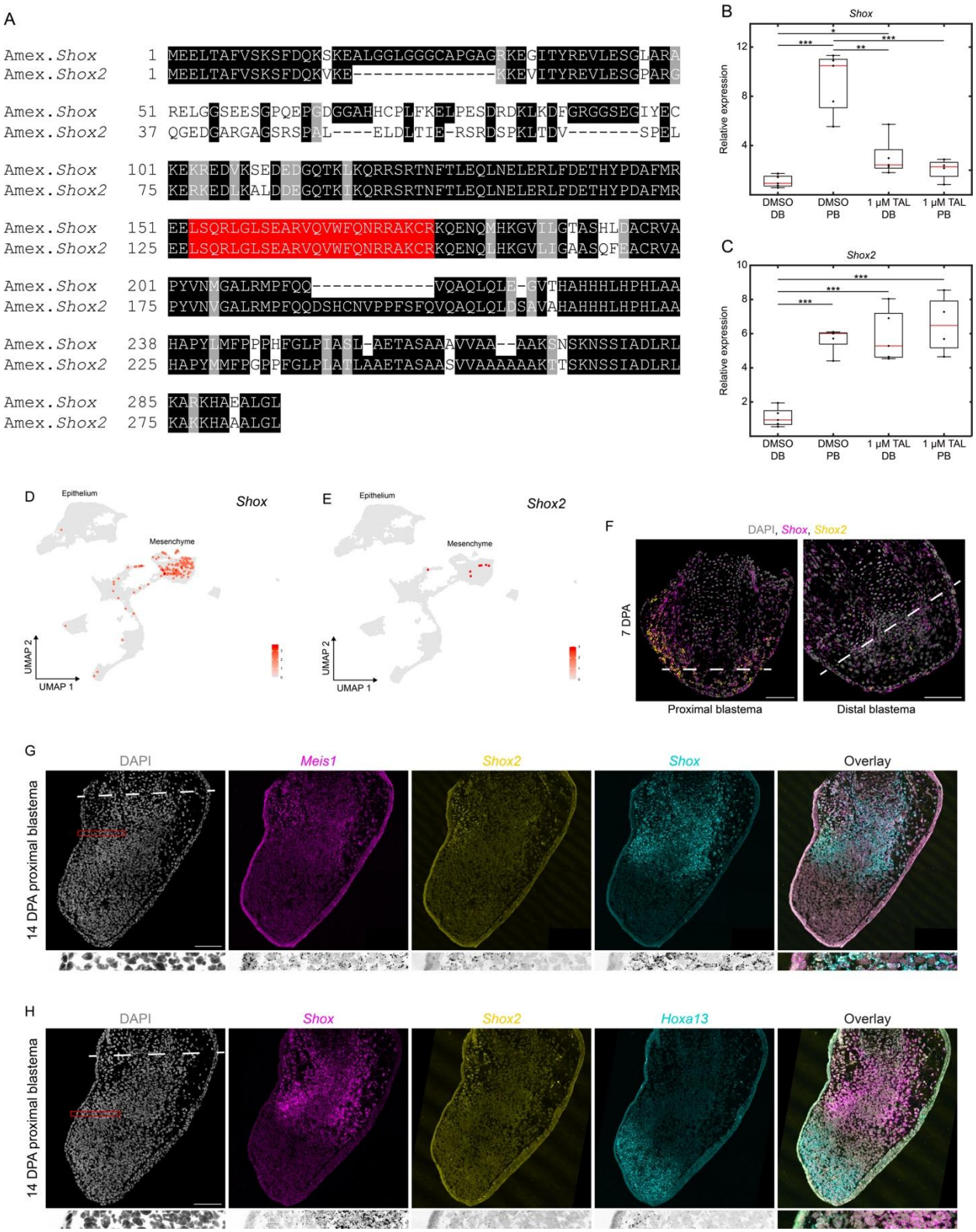

A
Amex.Shox 1 MEELTAFVSKSFDQKSKEALGGLGGGCAPGAGRKEGITYREVLESGLARA
Amex.Shox2 1 MEELTAFVSKSFDQKVKE----------KKEVITYREVLESGPARG
Amex.Shox 51 RELGGSEESGPQEPGDGGAHHCPLFKELPESDRDKLKDFGRGGSEGIYEC
Amex.Shox2 37 QGEDGARGAGSRSPAL----ELDLTIE-RSRDSPKLTDV-------SPEL
Amex.Shox 101 KEKREDVKSEDEDGQTKLKQRRSRTNFTLEQLNELERLFDETHYPDAFMR
Amex.Shox2 75 KERKEDLKALDDEGQTKIKQRRSRTNFTLEQLNELERLFDETHYPDAFMR
Amex.Shox 151 EELSQRLGLSEARVQVWFQNRRAKCRKQENQMHKGVIIGTASHLDACRVA
Amex.Shox2 125 EELSQRLGLSEARVQVWFQNRRAKCRKQENQLHKGVLIGAASQFEACRVA
Amex.Shox 201 PYVNMGALRMPFQQ----------VQAQLQLE-GVTHAHHHLHPHLAA
Amex.Shox2 175 PYVNVGALRMPFQQDSHCNVPPFSFQVQAQLQLDSAVAHAHHHLHPHLAA
Amex.Shox 238 HAPYLMFPPPHFGLPIASL-AETASAAAVVAA--AAKSNSKNSSIADLRL
Amex.Shox2 225 HAPYMMFPGPPFGLPLATLAAETASAASVVAAAAAAKITSKNSSIADLRL
Amex.Shox 285 KARKHAEALGL
Amex.Shox2 275 KAKKHAAALGL
B
Shox
Relative expression
DMSO DB
DMSO PB
1 µM TAL DB
1 µM TAL PB
C
Shox2
Relative expression
DMSO DB
DMSO PB
1 µM TAL DB
1 µM TAL PB
D
Epithelium
Mesenchyme
Shox
UMAP 2
UMAP 1
E
Epithelium
Mesenchyme
Shox2
UMAP 2
UMAP 1
F
DAPI, Shox, Shox2
7 DPA
Proximal blastema
Distal blastema
G
DAPI
Meis1
Shox2
Shox
Overlay
14 DPA proximal blastema
H
DAPI
Shox
Shox2
Hoxa13
Overlay
14 DPA proximal blastema

Figure S17: Additional characterization of *Shox* and *Shox2*

(A) Amino acid alignment of SHOX and SHOX2. Highlighted in red is the 100% conserved homeobox domain. (B-C) qRT-PCR of *Shox* (A) and *Shox2* (B) in DMSO treated DBs, DMSO treated PBs, 1 µM TAL treated DBs, and 1 µM TAL treated PBs (3.5 cm (HT) animals aged 2.5 months, 14 DPA). Box plot and analyses as in Fig. 1B-C. (D) UMAP showing *Shox* in blastemas at 7, 14, and 22 DPA. (E) UMAP showing *Shox2* in blastemas at 7, 14, and 22 DPA. (F) HCR-FISH for *Shox* and *Shox2* in PBs and DBs at 7 DPA. (G) Individual FISH channels for *Meis1*, *Shox2*, and *Shox* in a PB at 14 DPA. (H) Individual FISH channels for *Meis1*, *Shox2*, and *Shox* in a PB at 14 DPA. Dashed lines indicate the amputation plane. Scale bars = 200 µm. Sample size and exact p-values are located within the source data file. Source data are provided as a source data file.

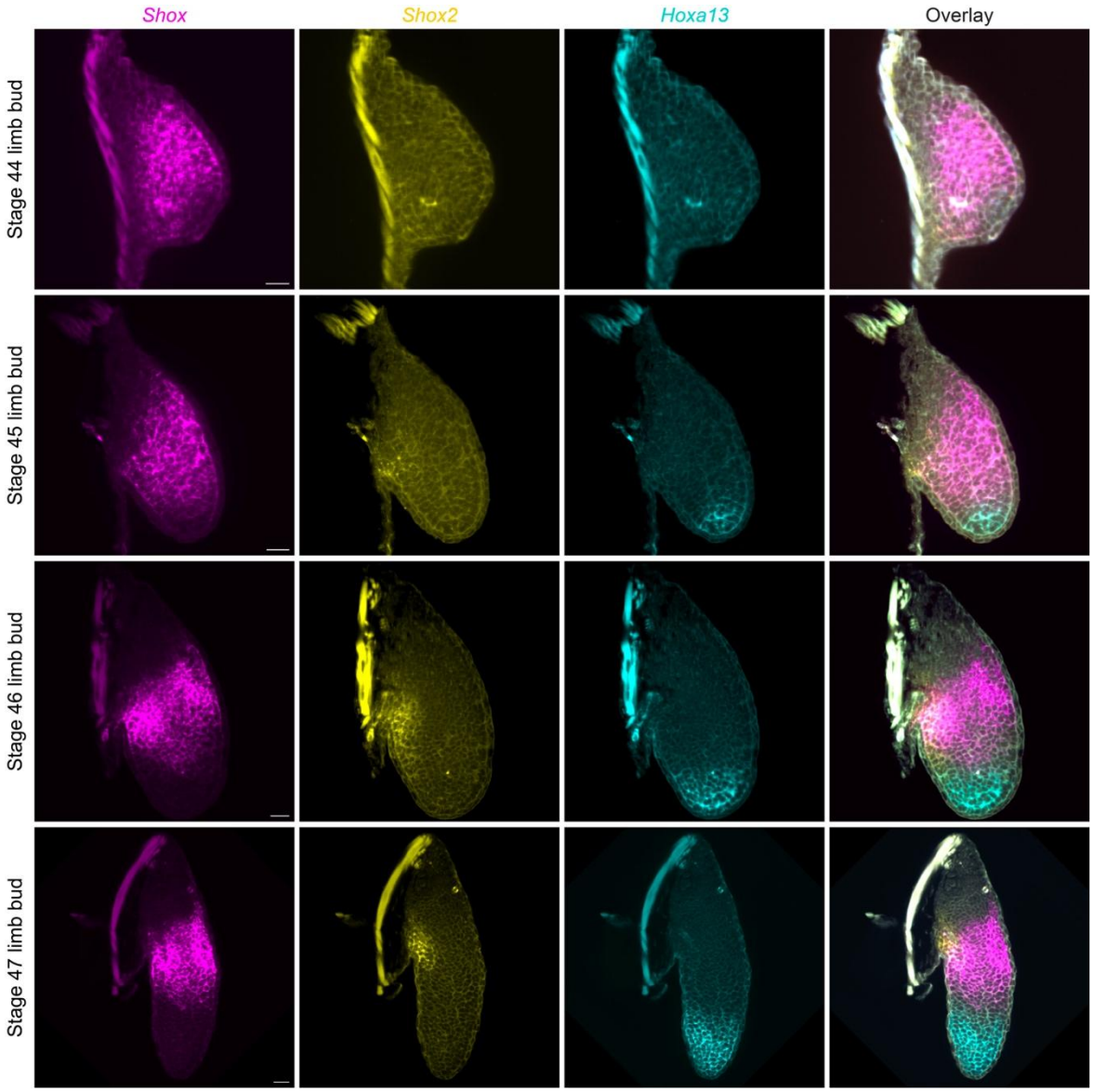


Figure S18: Individual FISH channels for *Shox*, *Shox2*, and *Hoxa13* in stage 44-47 developing limb buds from Fig. 5

Individual fluorescent channels for *Shox*, *Shox2*, and *Hoxa13* in stage 44-47 limb buds. The images represent a single, 2D z-plane within a 3D image stack. Scale bars = 50 µm.

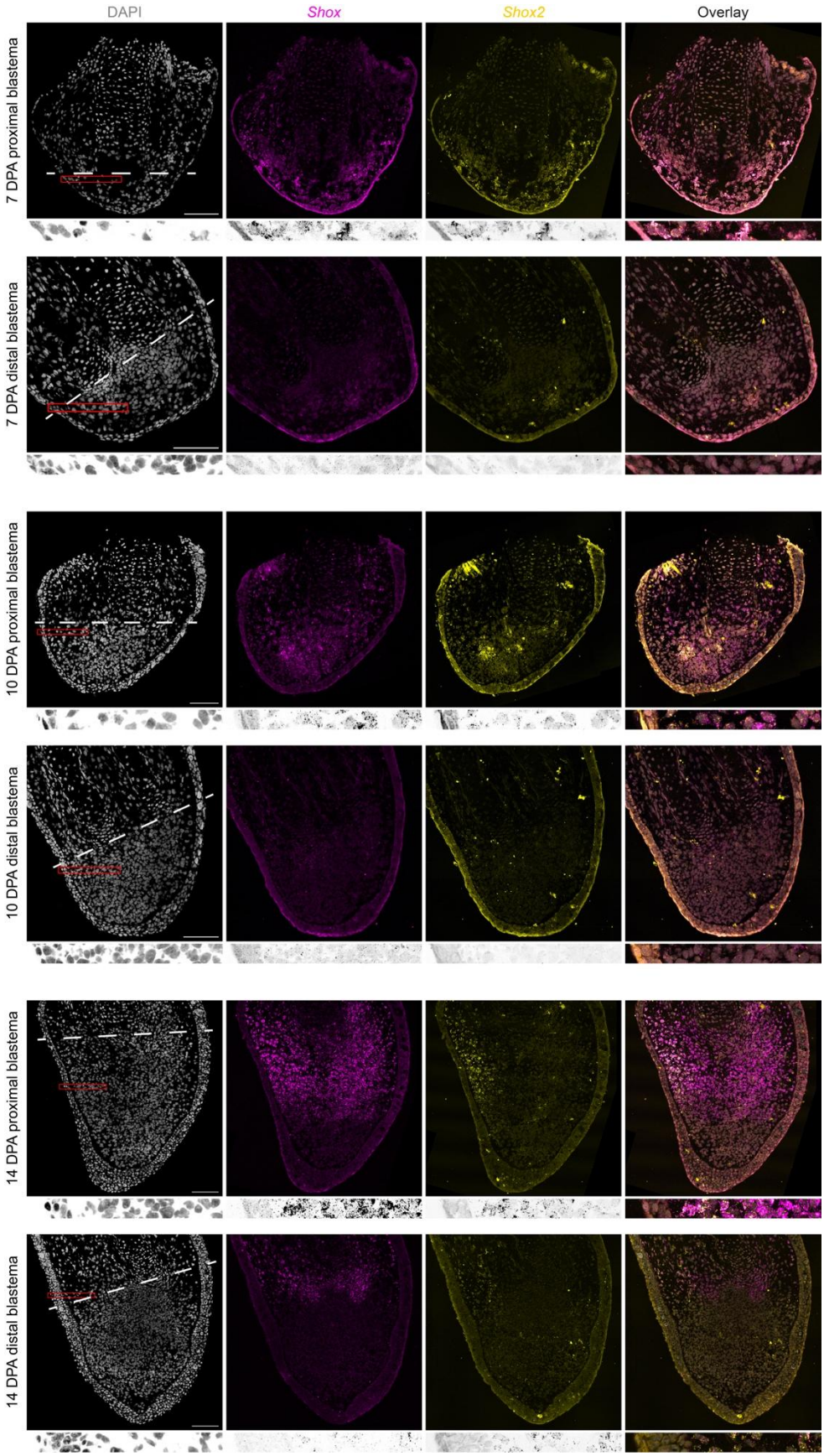
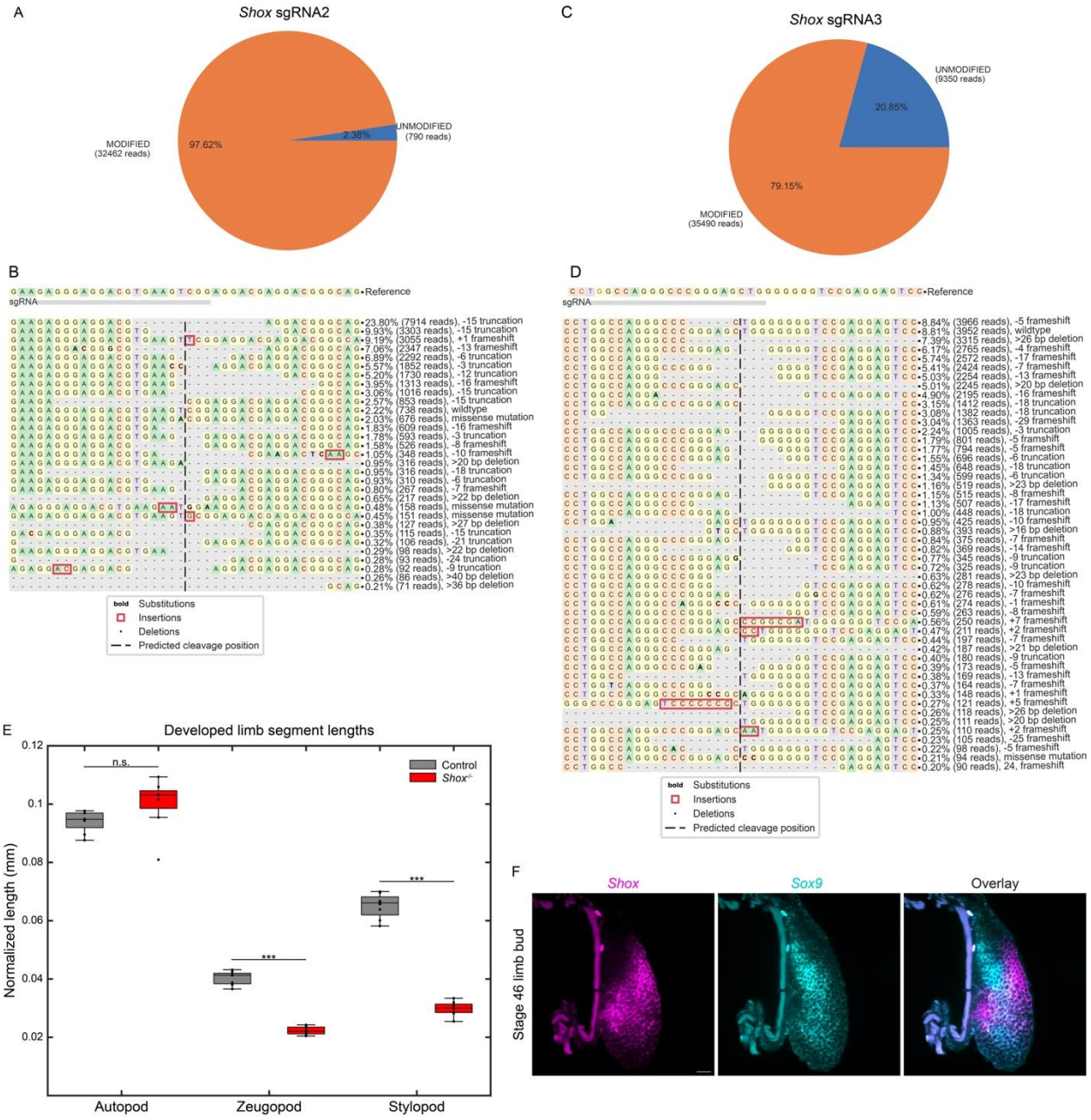

DAPI
Shox
Shox2
Overlay
7 DPA proximal blastema
7 DPA distal blastema
10 DPA proximal blastema
10 DPA distal blastema
14 DPA proximal blastema
14 DPA distal blastema

Figure S19: Individual FISH channels for *Shox* and *Shox2* in PBs and DBs from Fig. 5 and Fig. S17

Individual fluorescent channels for *Shox* and *Shox2* in PBs and DBs at 7, 10, and 14 DPA. Boxes in the DAPI channel represent the location of the inset images. Dashed lines indicate the amputation plane. Scale bars = 200 µm.



187 **Figure S20: Additional characterization of *Shox* crispants and *Shox*$^{-/-}$ animals, and**
188 **individual FISH channels for *Shox* and *Sox9* in a stage 46 developing limb bud**
189 **from Fig. 6**

190 (A) Pie cart for *Shox* sgRNA2 showing that 97.62% of alleles sequenced from 10 pooled
191 tail tips were modified. (B) Sequence alignment for *Shox* sgRNA2 depicting the
192 frequency of mutation type in each *Shox* crispant. (C) Pie chart for *Shox* sgRNA3
193 showing that 79.15% of alleles sequenced from 10 pooled tail tips were modified. (D)
194 Sequence alignment for *Shox* sgRNA3 depicting the frequency of mutation type in each

Shox crispant. Genotyping data generated from CRISPResso2 (Clement et al., 2019). (E) Skeletal element quantification in control and *Shox*$^{-/-}$ limbs (3.2 cm and 2.2 cm (HT) in control and *Shox*$^{-/-}$ animals, respectively aged 2 months). Segment size was normalized to HT length to account for differences in size. Box plot and analyses as in Fig. 6C. n.s. = no statistical difference, *** = $p < 0.001$. (F) Individual fluorescent channels for *Shox* and *Sox9* in a stage 46 limb bud from Fig. The images represent a single, 2D z-plane within a 3D image stack. Scale bar = 50 µm. Sample size and exact p-values are located within the source data file. Source data are provided as a source data file.

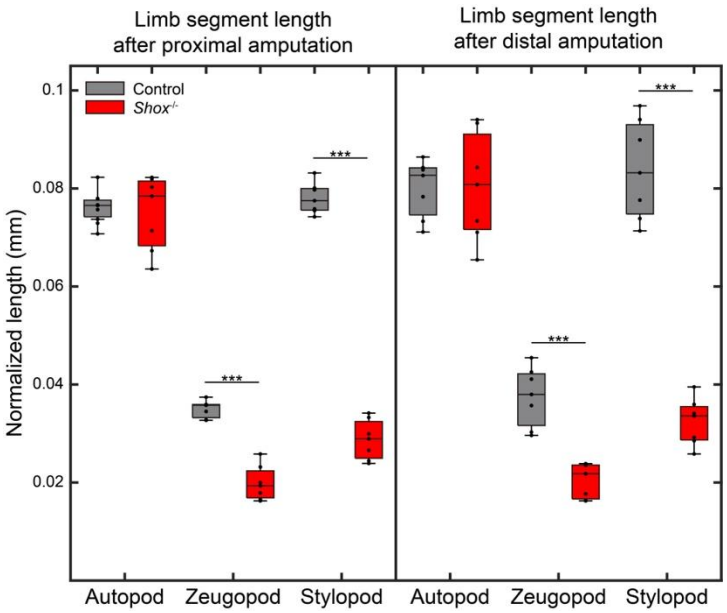


Figure S21: Skeletal element quantification in regenerating control and *Shox*$^{-/-}$ limbs

Skeletal element quantification in regenerating control and *Shox*$^{-/-}$ limbs amputated proximally or distally (4.0 cm and 3.3 cm (HT) in control and *Shox*$^{-/-}$ animals, respectively aged 2 months). Segment size was normalized to HT length to account for differences in size. Box plot and analyses as in Fig. 6C. n.s. = no statistical difference, *** = $p < 0.001$. Sample size and exact p-values are located within the source data file. Source data are provided as a source data file.

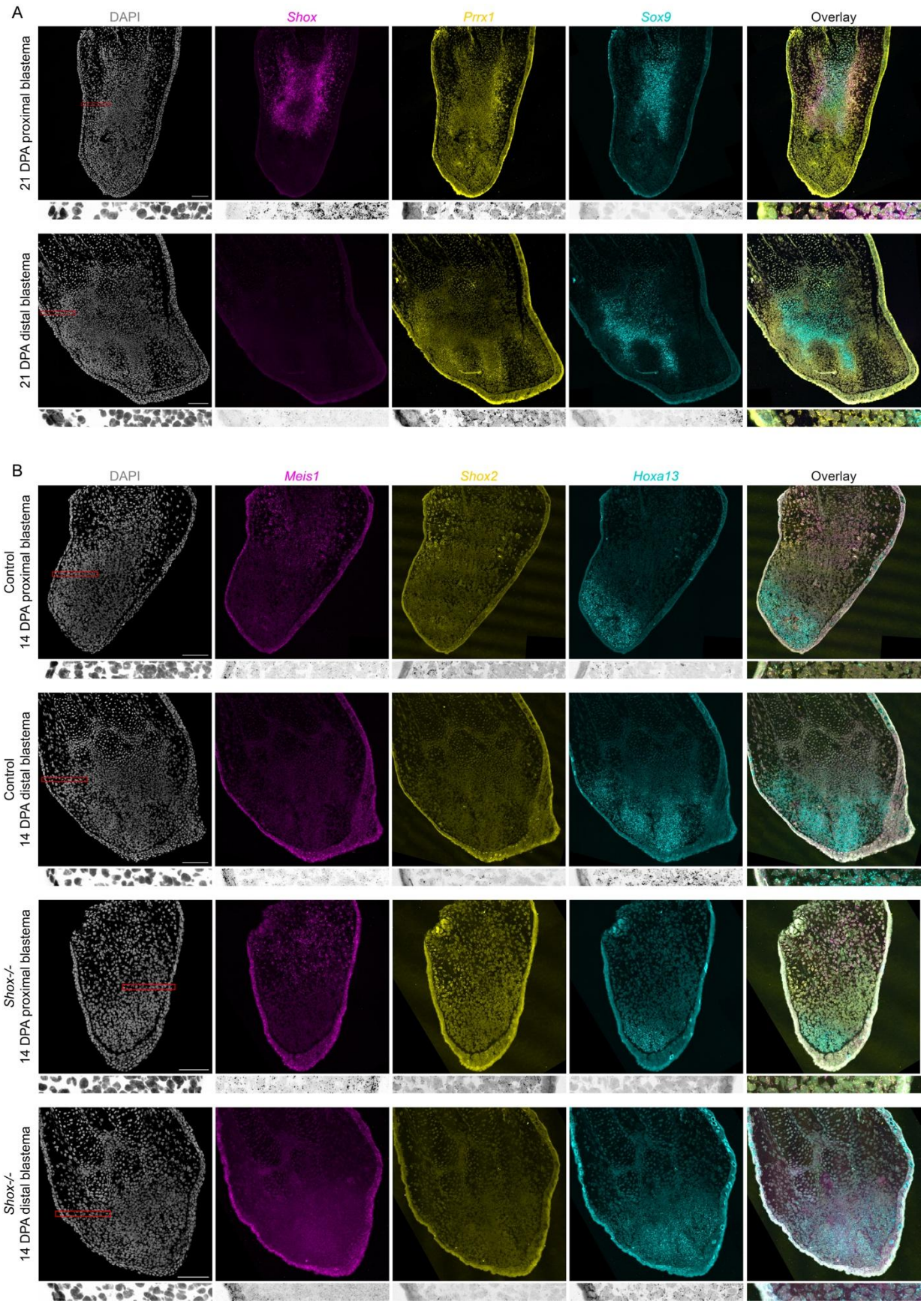

A
DAPI
Shox
Prrx1
Sox9
Overlay
21 DPA proximal blastema
21 DPA distal blastema
B
DAPI
Meis1
Shox2
Hoxa13
Overlay
Control
14 DPA proximal blastema
Control
14 DPA distal blastema
Shox-/-
14 DPA proximal blastema
Shox-/-
14 DPA distal blastema

Figure S22: Individual FISH channels for *Shox*, *Prrx1*, and *Sox9* in PBs and DBs and individual FISH channels for *Shox*, *Shox2*, and *Hoxa13* in control and *Shox*$^{-/-}$ PBs and DBs

(A) Individual fluorescent channels for *Shox*, *Prrx1*, and *Sox9* in PBs and DBs at 21 DPA. (B) Individual fluorescent channels for *Shox*, *Shox2*, and *Hoxa13* in control and *Shox*$^{-/-}$ PBs and DBs at 14 DPA. Boxes in the DAPI channel represent the location of the inset images. Dashed lines indicate the amputation plane. Scale bars = 200 µm.

Table S1: Primers used for qRT-PCR

Primer name	5' --> 3' sequence
Meis1_qPCR_F	TCCCCCTGAACGTGTTGATG
Meis1_qPCR_R	AGTCGGACAGCGAAGACATG
Meis2_qPCR_F	TGTCATGCCCAGCAGTATGG
Meis2_qPCR_R	ACTTCTCAAAGACCAGGGCG
Hoxa9_qPCR_F	GGAGACAAGCCTGCCATTGA
Hoxa9_qPCR_R	TGTAAGGGCAGCGCTTCTTG
Hoxa11_qPCR_F	GAGTCCTCTTCCGGCAACAA
Hoxa11_qPCR_R	TTCGCGGATCTGGTACTTGG
Hoxa13_qPCR_F	ACTGTTCCAAGGAGCAAGGG
Hoxa13_qPCR_R	GTACGAGTTCGCATCCGAGG
Rara_qPCR_F	CATTATGGTGTCAGCGCGTG
Rara_qPCR_R	GATGATGCACGTCTTGTCGC
Rarg_qPCR_F	CAAAGGTCAGCAAGGCACAC
Rarg_qPCR_R	TCGCCAGCTCGCTGAATTTA
Raldh1_qPCR_F	ATCGACAAGGCACTGACGTT
Raldh1_qPCR_R	TTTCTCTCCCGTTGCCTGAC
Raldh2_qPCR_F	ATGTCCGGTAATGGGAGGGA
Raldh2_qPCR_R	CTGCAGCGCTCTTAGGAGTT
Raldh3_qPCR_F	GAACATAGTCCCCGGCTACG
Raldh3_qPCR_R	TGGAGGCAGCTTCCTTGATG
Cyp26a1_qPCR_F	CATCGATGTGCCTTTCTGCG
Cyp26a1_qPCR_R	GAGTCCTTGGCCATTTTCGC
Cyp26b1_qPCR_F	AAAGAGCATGGCAAGGAGCT
Cyp26b1_qPCR_R	TTGTGGAGGATACCGTTGCC
GFP_qPCR_F	AGCTGAAGGGCATCGACTTC
GFP_qPCR_R	TTCTGCTTGTCGGCCATGAT
Shox_qPCR_F	TCAGTTGCAGCTGGAAGGAG
Shox_qPCR_R	GTCCAAAGTGAGGAGGTGGG
Shox2_qPCR_F	GAAGTGCCGGAAACAGGAGA
Shox2_qPCR_R	CGTTGACATAGGGTGCGACT
Ef1a_qPCR_F	AACATCGTGGTCATCGGCCAT
Ef1a_qPCR_R	GGAGGTGCCAGTGATCATGTT

Table S2: Quantification of PD limb duplications after TAL treatment

					PD duplication				
Dose	Amputation plane	Total animals	No duplication	No regeneration	Half radius/ulna	Full radius/ulna	Half humerus	Full humerus	Shoulder
DMSO	Proximal	14	14	—	—	—	—	—	—
DMSO	Distal	14	14	—	—	—	—	—	—
0.1 µM TAL	Proximal	13	13	—	—	—	—	—	—
0.1 µM TAL	Distal	13	1	—	4	8	—	—	—
1 µM TAL	Proximal	12	12	—	—	—	—	—	—
1 µM TAL	Distal	12	—	—	—	—	4	8	—
5 µM TAL	Proximal	13	2	11	—	—	—	—	—
5 µM TAL	Distal	13	—	12	—	—	—	1	—

221 **Table S3: Quantification of PD limb duplications after DIS or TAL/DIS treatment**

					PD duplication				
Dose	Amputation plane	Total animals	No duplication	No regeneration	Half radius/ulna	Full radius/ulna	Half humerus	Full humerus	Shoulder
DMSO	Proximal	6	6	—	—	—	—	—	—
DMSO	Distal	6	6	—	—	—	—	—	—
0.1uM DIS	Proximal	7	7	—	—	—	—	—	—
0.1uM DIS	Distal	7	7	—	—	—	—	—	—
1uM DIS	Proximal	6	5	1	—	—	—	—	—
1uM DIS	Distal	6	6	—	—	—	—	—	—
5uM DIS	Proximal	5	5	—	—	—	—	—	—
5uM DIS	Distal	5	5	—	—	—	—	—	—
0.1uM DIS/1 uM TAL	Proximal	4	4	—	—	—	—	—	—
0.1uM DIS/1 uM TAL	Distal	4	—	—	—	4	—	—	—
1uM DIS/1 uM TAL	Proximal	5	5	—	—	—	—	—	—
1uM DIS/1 uM TAL	Distal	5	2	1	2	—	—	—	—

Table S4: Quantification of PD limb duplications after RAA or TAL/RAA treatment

					PD duplication				
Dose	Amputation plane	Total animals	No duplication	No regeneration	Half radius/ulna	Full radius/ulna	Half humerus	Full humerus	Shoulder
DMSO	Proximal	4	4	—	—	—	—	—	—
DMSO	Distal	4	4	—	—	—	—	—	—
0.1 µM RAA	Proximal	5	5	—	—	—	—	—	—
0.1 µM RAA	Distal	5	5	—	—	—	—	—	—
1 µM RAA	Proximal	7	5	2	—	—	—	—	—
1 µM RAA	Distal	7	5	2	—	—	—	—	—
5 µM RAA	Proximal	7	7	—	—	—	—	—	—
5 µM RAA	Distal	7	7	—	—	—	—	—	—
0.1 µM RAA/1 µM TAL	Proximal	6	6	—	—	—	—	—	—
0.1 µM RAA/1 µM TAL	Distal	6	6	—	—	—	—	—	—
1 µM RAA/1 µM TAL	Proximal	6	6	—	—	—	—	—	—
1 µM RAA/1 µM TAL	Distal	6	5	1	—	—	—	—	—

Table S5: Putative *Meis1* binding sites within 20 Kb *Shox* promoter region

P-values determined using default setting from https://molotool.autosome.org**.**

Human gene	Sequence	Start (relative to TSS)	End (relative to TSS)	Strand	P-value	-log10(P-value)
MEIS1	atgatgtatgggg	1608	1620	-	3.090e-5	4.51
MEIS1	atgagttatgggt	4189	4201	-	8.054e-6	5.094
MEIS1	ttgatttacgtgc	19128	19140	+	2.972e-5	4.527
MEIS1	gtgatggatgtgc	19877	19889	+	7.638e-5	4.117