Developmental Cell, Volume *56*

Supplemental information

Fibroblast dedifferentiation as a determinant of successful regeneration

Tzi-Yang Lin, Tobias Gerber, Yuka Taniguchi-Sugiura, Prayag Murawala, Sarah Hermann, Lidia Grosser, Eri Shibata, Barbara Treutlein, and Elly M. Tanaka

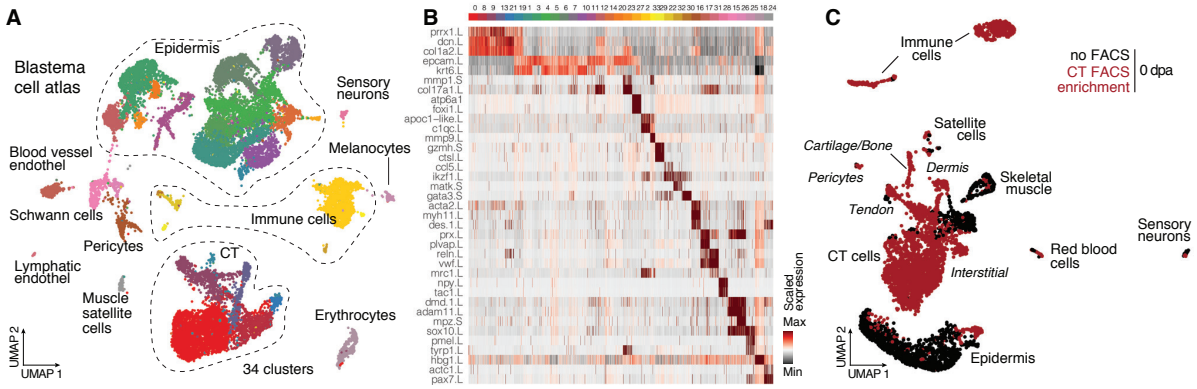

Figure S1. Frog Limb Blastema Cell Atlases. Related to Figure 2.

(A) UMAP embedding of scRNA-seq data of 20,139 froglet blastema cells over a time course of 7, 10, 14, 20 and 52 dpa. Cells are colored by cluster. Cell type annotations based on canonical markers are shown next to corresponding cells.

(B) Heatmap visualization of canonical marker gene expression used to assign cell types in (A) for cells. Colors in top bar correspond to cluster colors in (A). Only 50 cells are visualized per cluster.

(C) UMAP embedding of scRNA-seq data of uninjured mature frog CT cells with one set of cells being enriched through FACS (dark red) and one without FACS (black). Data integration using Harmony (Korsunsky et al., 2019) was performed.

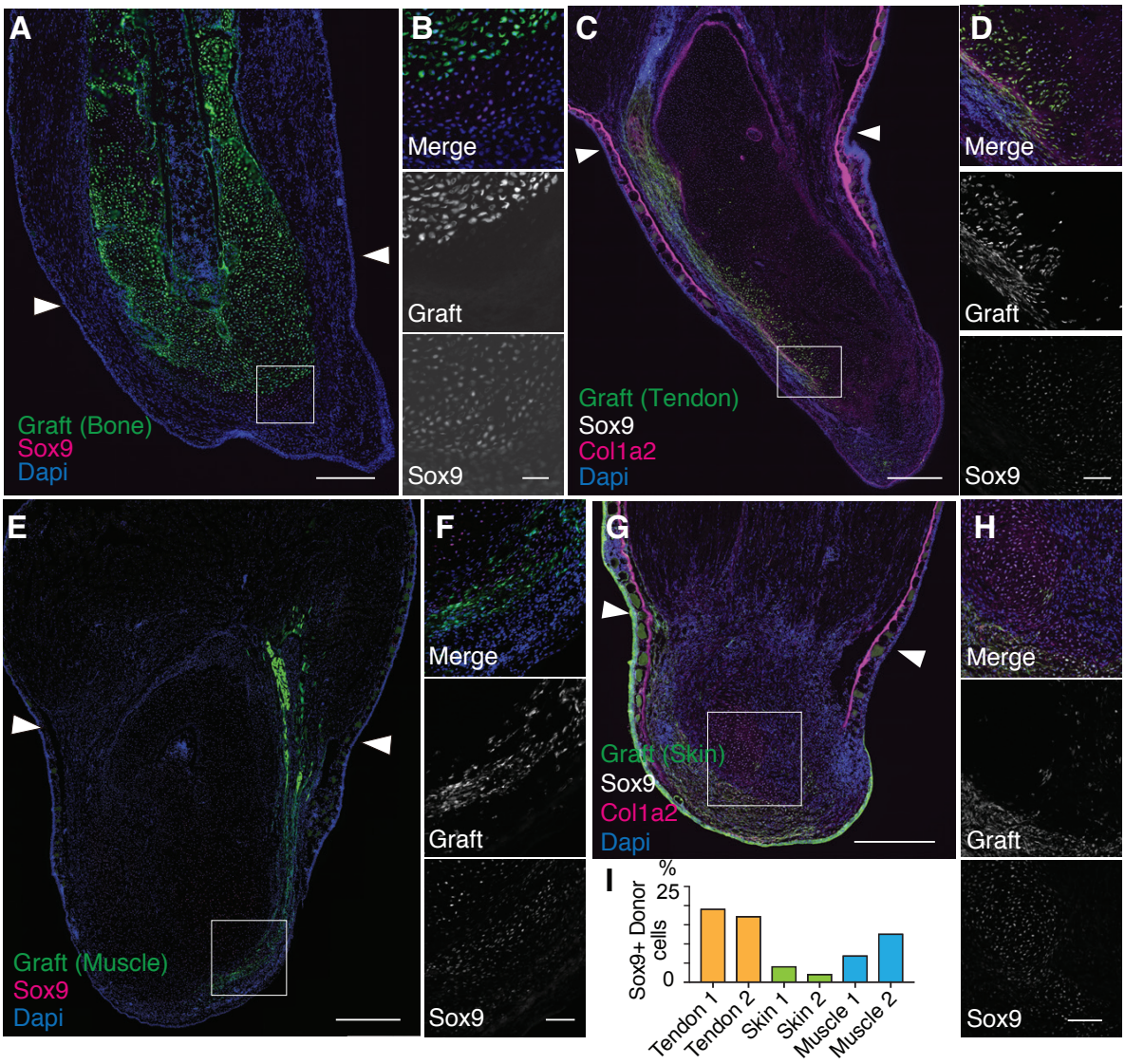

Figure S2. Tissue Scale Transplantation Experiments in *Xenopus laevis* Froglets. Related to Figure 2.

(A, C, E and G) Example image of longitudinal sections from froglets transplanted with GFP+ bone (A), tendon (C), muscle tissue (E), and skin (G). Blue: DAPI-stained nuclei. Magenta/white: Sox9 immunostaining (chondrocytes). Green: GFP immunostaining (donor cells). Magenta: Col1a2 immunostaining. White boxes indicate the respective field of view in (B), (D), (F) and (H). Arrowheads indicate amputation plane. Scale bars represent 500 µm.

(B, D, F and H) Insets from (A), (C), (E) and (G), respectively, showing overlay of immunofluorescence signals (top), only GFP (donor cells from the grafted tissue, center) or Sox9 (chondrocytes, bottom). Scale bars represent 100 µm.

(I) A bar plot showing the percentage of Sox9-expressing GFP+ donor cells in tendon (orange), skin (green), and muscle (blue) transplants. Two samples of each transplantation type were analyzed in this study.

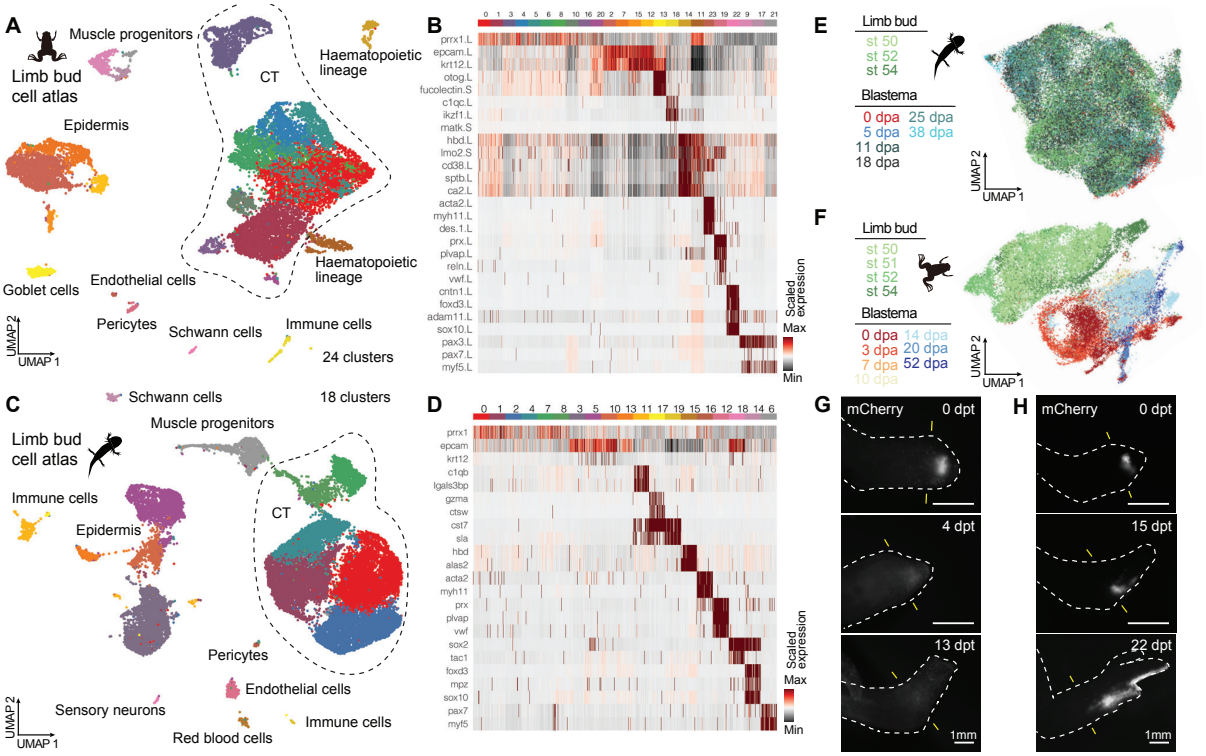

Figure S3. Frog and Axolotl Limb Bud Cell Atlases. Related to Figure 2 and Figure 3.

(A) UMAP embedding of scRNA-seq data of 16,120 tadpole cells originating from embryonic stage 50, 51 and 52. Cells are colored by cluster. Cell type annotations based on canonical markers are shown next to corresponding cells.

(B) Heatmap visualization of canonical marker gene expression used to assign cell types in (A). Only 50 cells are visualized per cluster.

(C) UMAP embedding of scRNA-seq data of 29768 larval axolotl limb bud cells originating from embryonic stage 50, 52 and 54. Cells are colored by cluster. Cell type annotations based on canonical markers are shown next to corresponding cells.

(D) Heatmap visualization of canonical marker gene expression used to assign cell types in (C). Only 50 cells are visualized per cluster.

(E) UMAP embedding of scRNA-seq data of 43,915 axolotl CT cells originating from different limb bud stages, blastema states and mature uninjured CT cells. Cellular transcriptomes were integrated using Harmony (Korsunsky et al., 2019).

(F) UMAP embedding of scRNA-seq data of 26,152 frog CT cells originating from different limb bud stages, blastema states and mature uninjured CT cells. Cellular transcriptomes were integrated using Harmony (Korsunsky et al., 2019) with exactly the same parameters as used for axolotl data integration shown in (E).

(G) Fluorescence images of cross-species transplantation. Unsorted frog mCherry^{+} blastema cells were transplanted to non-fluorescent axolotl blastemas and the mCherry signal were traced for 13 days. Fluorescence signal rapidly disappeared at day 4 (n=4). White dashed lines outline the samples. Yellow lines indicate the amputation planes. Scale bars represent 1 mm.

(H) Fluorescence images of control transplantation experiments of (G). Unsorted axolotl mCherry^{+} blastema cells were transplanted to non-fluorescent axolotl blastemas and the mCherry signal were traced for 22 days. Donor mCherry^{+} cells were consistently observed in the patterning limb (n=4). White dashed lines outline the samples. Yellow lines indicate the amputation planes. Scale bars represent 1 mm.

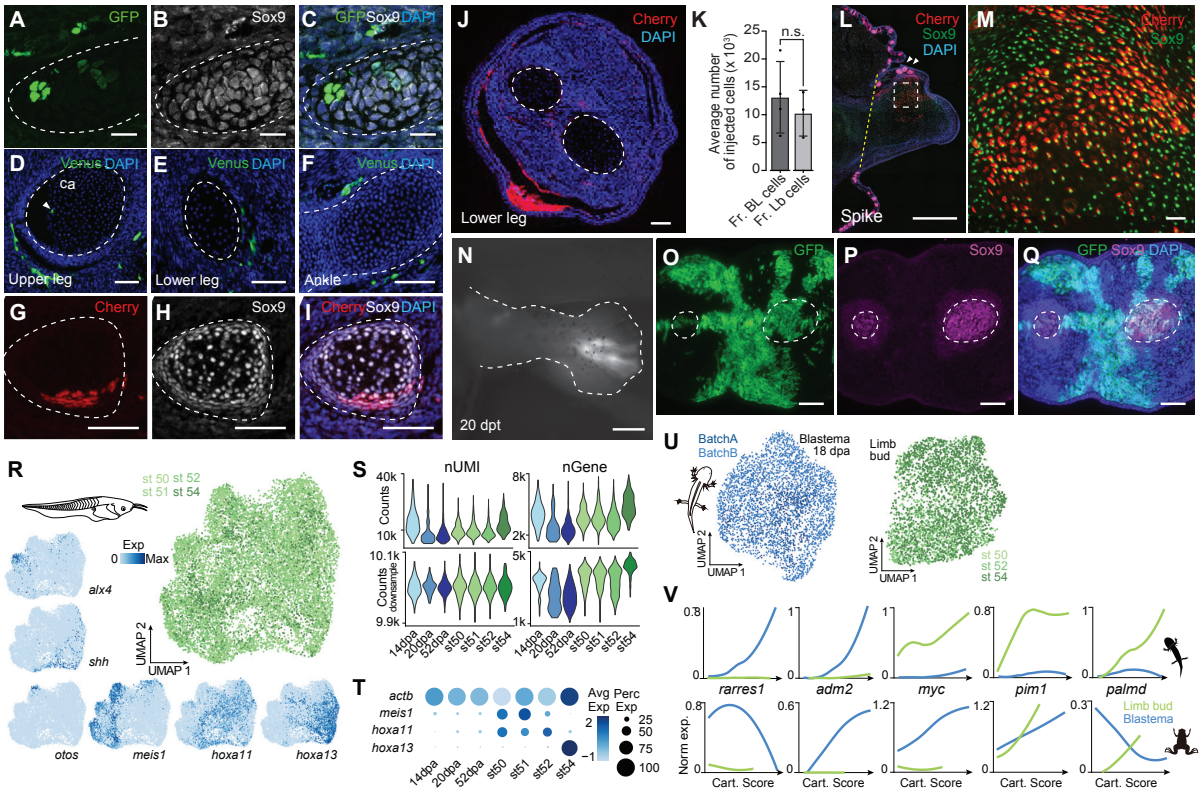

Figure S4. Heterochronic Transplantation Assay and Limb Bud Single-Cell RNA-seq Analysis of Frog and Axolotls. Related to Figure 3 and Figure 4.

(A–C) A transverse section at the foot level from a developing axolotl limb transplanted with unsorted axolotl early embryonic limb bud cells (GFP$^+$) 22 days earlier. Sections were immunostained for GFP (donor cells, green) (A), Sox9 (Chondrocytes, white) (B) and the combined overlay with DAPI (nuclei, blue) (C). Dashed circles indicate the cartilage region. Scale bars represent 50 µm.

(D–F) Transverse sections at the levels of upper leg (D), lower leg (E), and foot (F) regions from a developing tadpole limb transplanted with unsorted frog 14 dpa blastema cells (Venus$^+$) 21 days earlier. Sections were immunostained for GFP (donor cells, green) and DAPI (nuclei, blue). Dashed circles indicate the cartilage regions. The white arrowhead indicates autofluorescence. Scale bars represent 50 µm.

(G–I) A transverse section at the foot level from a developing tadpole limb transplanted with unsorted stage 50 tadpole limb bud cells (mCherry$^+$) 21 days earlier. Sections were immunostained for Cherry (donor cells, red) (G), Sox9 (Chondrocytes, white) (H) and the combined overlay with DAPI (nuclei, blue) (I). Dashed circle outlines the cartilage regions. Scale bars represent 50 µm.

(J) A transverse section at the lower leg level of a developing tadpole limb transplanted with FAC-sorted Prrx1-converted cells (mCherry$^+$) from uninjured frog upper legs 21 days earlier. Sections were immunostained for Cherry (donor cells, red) and DAPI (nuclei, blue). Dashed circles indicate the cartilage regions. Scale bar represents 100 µm.

(K) Bar plot showing the average number of injected frog donor cells per transplant sample. The number of cells transplanted per sample is comparable for both frog blastema-to-limb bud (Fr. BL cells, N=4 experiments) and frog limb bud-to-limb bud (Fr. Lb cells, N=3 experiments). Statistical significance is calculated using unpaired Student's t test. n.s.: p>0.05.

(L) A transverse section from a froglet spike transplanted with unsorted frog 14 dpa blastema cells (mCherry$^+$) 30 days earlier. The section was immunostained for Cherry (donor cells, red), Sox9 (Chondrocytes, green), and DAPI (nuclei, blue). White arrowheads indicate autofluorescence. Yellow dashed line indicates the amputation plane. White dashed box indicated the field of view in (M). Scale bar represents 1mm.

(M) Inset of (L). Only the overlay of Cherry (red) and Sox9 (green) signals is shown. Scale bar represents 50 μm.

(N) Fluorescent images of a developing tadpole limb 20 days post-transplantation of unsorted Venus-expressing 10 dpa blastema cells of stage 53 frog limb buds (regenerative stage). White dashed lines outline the host limb buds. Scale bar represents 500 μm.

(O–Q) Transverse sections at the digit level of (N) immunostained for GFP (donor cells, green) (O), Sox9 (chondrocytes, magenta) (P), and the combined overlay with DAPI (nuclei, blue) (Q). Dashed circles indicate the cartilage regions. Scale bars represent 50 μm.

(R) UMAP embedding of scRNA-seq data of 13,028 CT cells from a tadpole limb bud time course after data integration using Harmony (Korsunsky et al., 2019). Cells are colored by embryonic stage. Feature plots show expression of proximal-distal (*meis1*, *hoxa11*, *hoxa13*) and anterior-posterior (*alx4*, *shh*) patterning genes as well as *otos* as mature cartilage marker.

(S) Violin plots show the detected number of transcripts (UMI) and genes per sample across 3 frog blastema time points and 4 frog limb bud stages. Top: Raw transcript counts for the gene-cell-matrix that was used to generate the dotplot in Figure 4D. Bottom: Transcript counts and corresponding gene counts for the same but downsampled gene-cell-matrix that was used to generate the dotplot in (T).

(T) Dotplot showing gene expression intensity and frequency differences between developing frog tadpole limb bud cartilage and frog blastema cartilage cells after downsampling transcripts to similar values (see Figure S4S). Avg Exp: Average expression values, Perc. Exp: Percent of cells expressing each gene.

(U) UMAP embeddings of scRNA-seq data of 3,720 cartilage cells from an 18 dpa axolotl blastema (left) and 3643 cartilage cells from 3 different axolotl limb bud stages (right). Cells are colored by batch or stage, respectively.

(V) Gene expression visualization for differentially expressed genes between axolotl limb bud and blastema cartilage differentiation across the cartilage scores in frog (bottom) and axolotl (top) limb bud and blastema samples. A loess fitted curve through the data points is shown. Green: limb bud. Blue: blastema.

Tissue type	Fractions of *Prrx1*$^+$ cells in the uninjured upper leg[1]	Expand ratio[2]	Contribution to blastema[3]	Fraction of Sox9$^+$ cells in the expanded population[4]	Contribution to Sox9$^+$ cartilage in blastema[5]
Interstitial CT	86.4%	3.15	82.9%	10%	85.6%
Tendon	4.1%	4.62	5.8%	19%	10.6%
Dermis	4.9%	7.59	11.3%	3%	3.8%
Bone	3.6%	ND	ND	ND	ND
Others	1%	ND	ND	ND	ND

Table S1. Contribution of CT Cell Populations to Frog Blastema and Developing Cartilage. Related to Figure 2.

[1]based on scRNA-seq analysis of uninjured upper leg (0 dpa);

[2]calculated by dividing the GFP$^+$ cells in the blastema region over GFP$^+$ cells within the 500 μm zone proximal to the amputation plane;

[3]calculated by multiplying (1) and (2) and then divided by the three populations (interstitial CT, tendon, and dermis) combined;

[4]calculated by dividing the GFP$^+$/Sox9$^+$ cells over total GFP$^+$ cells in the blastema region;

[5]calculated by multiplying (1), (2), and (3) and then divided by the three populations (interstitial CT, tendon, and dermis) combined; ND: not determined.

Feature	0 dpa	3 dpa	Early BL	Late BL	14 dpa	Trans-plant	Stage 50 Lb	Stage 51 Lb	Stage 52 Lb	Stage 54 Lb/ 0 dpa
nUMI high	30,000	60,000	40,000	40,000	40,000	60,000	40,000	40,000	40,000	40,000
nUMI low	2,000	2,000	3,000	2,000	3,000	2,000	2,000	2,000	2,000	3,000
mt	0.1	0.1	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.1
Sample mix	Single	Single	Pool	Pool	Single	Single	Single	Single	Single	Pool
Genotype	Prrx1	Prrx1	Venus (7dpa); Cherry (10dpa); TFP (14dpa)	TFP (14dpa); Cherry (20dpa); Venus (52dpa)	Venus	Cherry (Host); Venus (Donor)	GFP	GFP	GFP	TFP (st54); tdTom (0dpa)
FACS	Yes	Yes	No	No	No	No	No	No	No	No

Table S2. Metadata for Xenopus scRNAseq Analysis. Related to STAR methods.
Number of transcripts (nUMI) and Fraction of mitochondrial genes (mt) were used to remove low quality cells as well as cell multiplets from the data sets. Some experiments were run as cell pools with cells expressing different fluorescent labels (see STAR Methods for details). CT enrichment by FACS was performed for some experiments. Prrx1: *Prrx1:CreER;CAGGs:lp-Cherry*. Venus: *CAGGs:Venus*. Cherry: *CAGGs:mCherry*. TFP: *CAGGs:nucTFP*, tdTom: *CMV:tdTomato*.

Feature	0 dpa	5 dpa	11 dpa	18 dpa	25 dpa	38 dpa	Stage 50	Stage 52	Stage 54
nUMI high	15,000	30,000	30,000	10,000	20,000	15,000	25,000	25,000	25,000
nUMI low	1,000	500	500	1,000	1,000	1,000	1,000	1,000	1,000
mt	0.1	0.1	0.1	0.05	0.05	0.05	0.2	0.2	0.2
FACS	Yes	No	No	Yes	Yes	Yes	No	No	No

Table S3. Metadata for Axolotl scRNAseq Analysis. Related to STAR methods.
Number of transcripts (nUMI) and Fraction of mitochondrial genes (mt) were used to remove low quality cells as well as cell multiplets from the data sets. CT enrichment by FACS was performed for some experiments. Samples 0, 18, 25 and 38 dpa are published data sets and were reanalyzed from Gerber et al., 2018. (see STAR Methods for details).