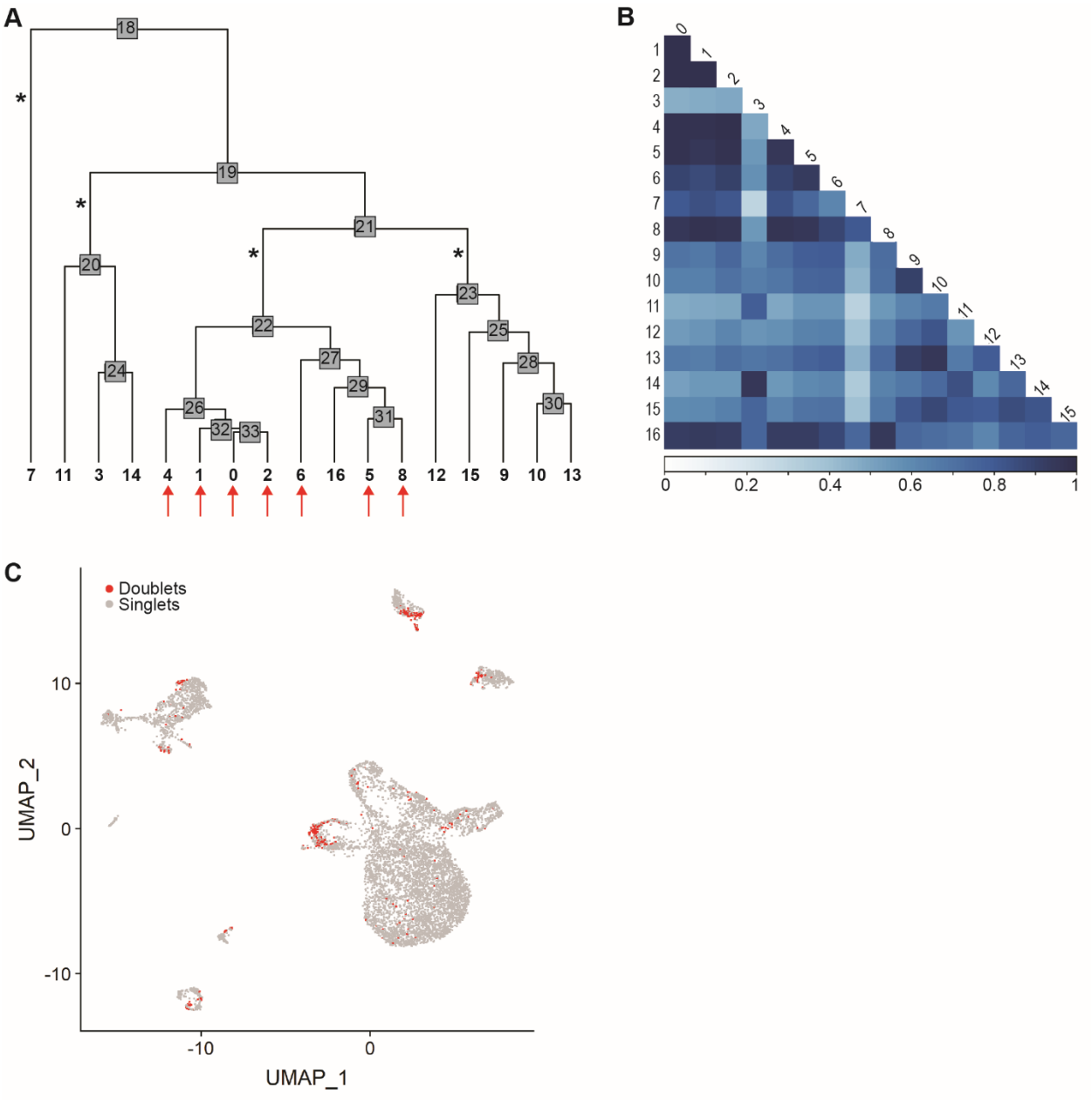
A
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
7 11 3 14 4 1 0 2 6 16 5 8 12 15 9 10 13
B
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
0 0.2 0.4 0.6 0.8 1
C
Doublets
Singlets
UMAP_2
UMAP_1
10
0
-10
-10
0

Figure S1

11dpa cluster relationships and doublet detection (Relates to Figures 1 and 2)

(A) Dendrogram showing the relationships between clusters; cell cluster numbers correlate with cluster numbers shown on UMAP plot in Figure 1B. Asterisks denote four main branches of the dendrogram, and red arrows denote fibroblast clusters. (B) Heatmap showing the Pearson correlation between each cell cluster, where dark blue represents highly correlated (r nears 1) and light blue represents lowly correlated (r nears 0). (C) 419 cells classified as doublets were excluded from all subsequent analyses and are shown in red; all singlet cells are in gray.

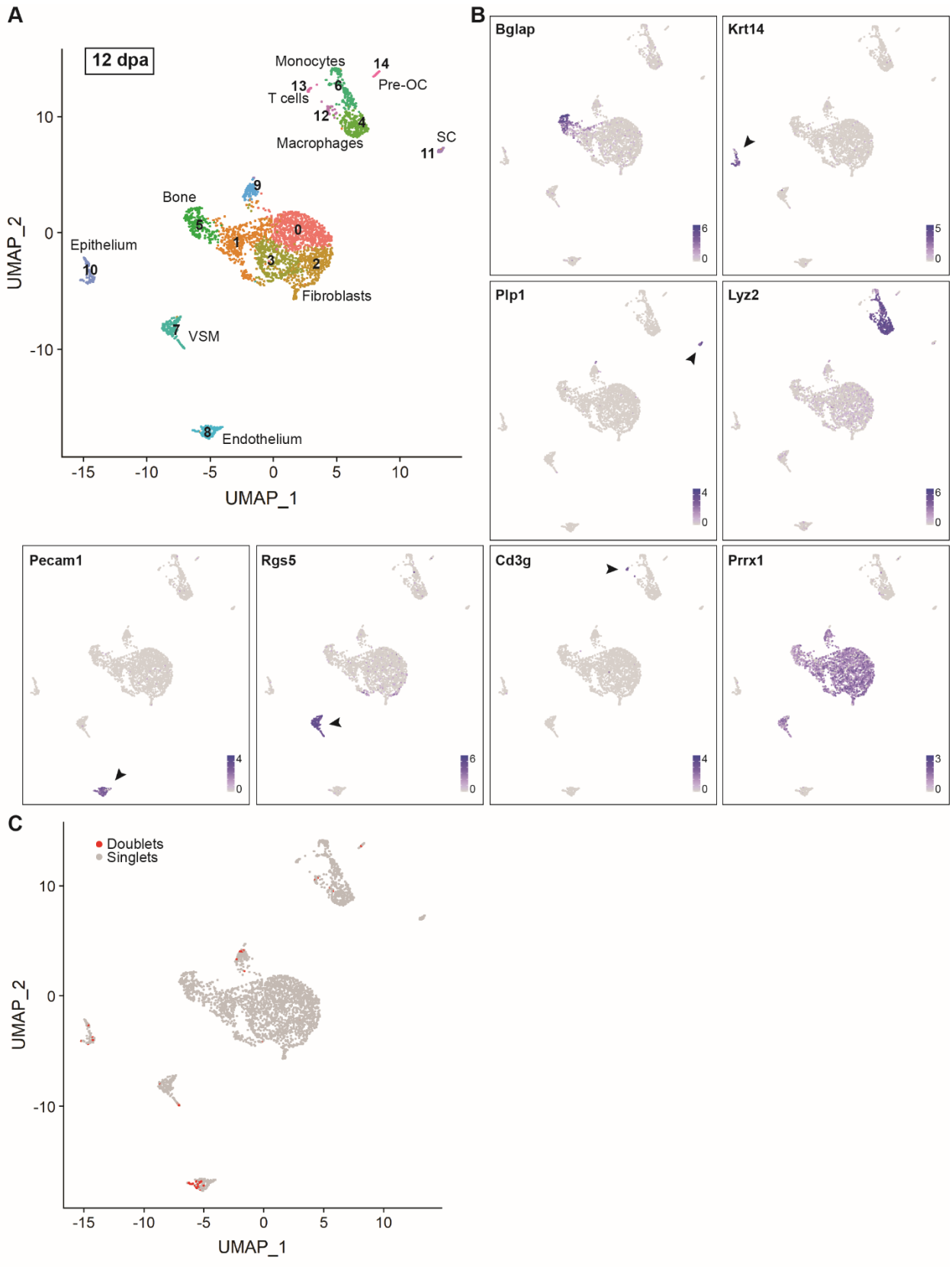
A
12 dpa
Monocytes
14
Pre-OC
13
6
T cells
12
4
Macrophages
SC
11
9
Bone
5
1
0
Epithelium
10
3
2
Fibroblasts
7
VSM
8
Endothelium
UMAP_2
UMAP_1
B
Bglap
Krt14
Plp1
Lyz2
Pecam1
Rgs5
Cd3g
Prrx1
C
Doublets
Singlets
UMAP_2
UMAP_1

Figure S2

Single cell RNAseq of 12dpa blastema (Relates to Figure 3 and Table S1)

(A) Unbiased single cell clustering of 3,309 high quality cells visualized by UMAP. Each dot represents a single cell and cells assigned to the same cluster are similarly colored. Cell type identities are assigned as follows: fibroblasts (clusters 0-3, and 9), macrophages (clusters 4 and 12), bone (cluster 5), monocytes (cluster 6), vascular smooth muscle cells (VSM) (cluster 7), endothelial cells (cluster 8), epithelial cells (cluster 10), Schwann cells (SC) (cluster 11), T cells (cluster 13), and pre-osteoclasts (Pre-OC) (cluster 14). (B) Gene expression UMAP overlay with examples of highly expressed, cell type specific markers used to assign cluster cell identities: *Bglap* (bone), *Krt14* (epithelial cells), *Plp1* (SCs), *Lyz2* (monocytes/macrophages), *Pecam1* (endothelial cells), *Rgs5* (vascular smooth muscle cells), *Cd3g* (T cells), *Prrx1* (fibroblasts). Gray depicts low expression and purple depicts high expression as specified on the scale for each gene. Black arrowheads denote the population of cells expressing a particular marker. (C) 83 cells were classified as doublets (red) and excluded from all subsequent analyses.

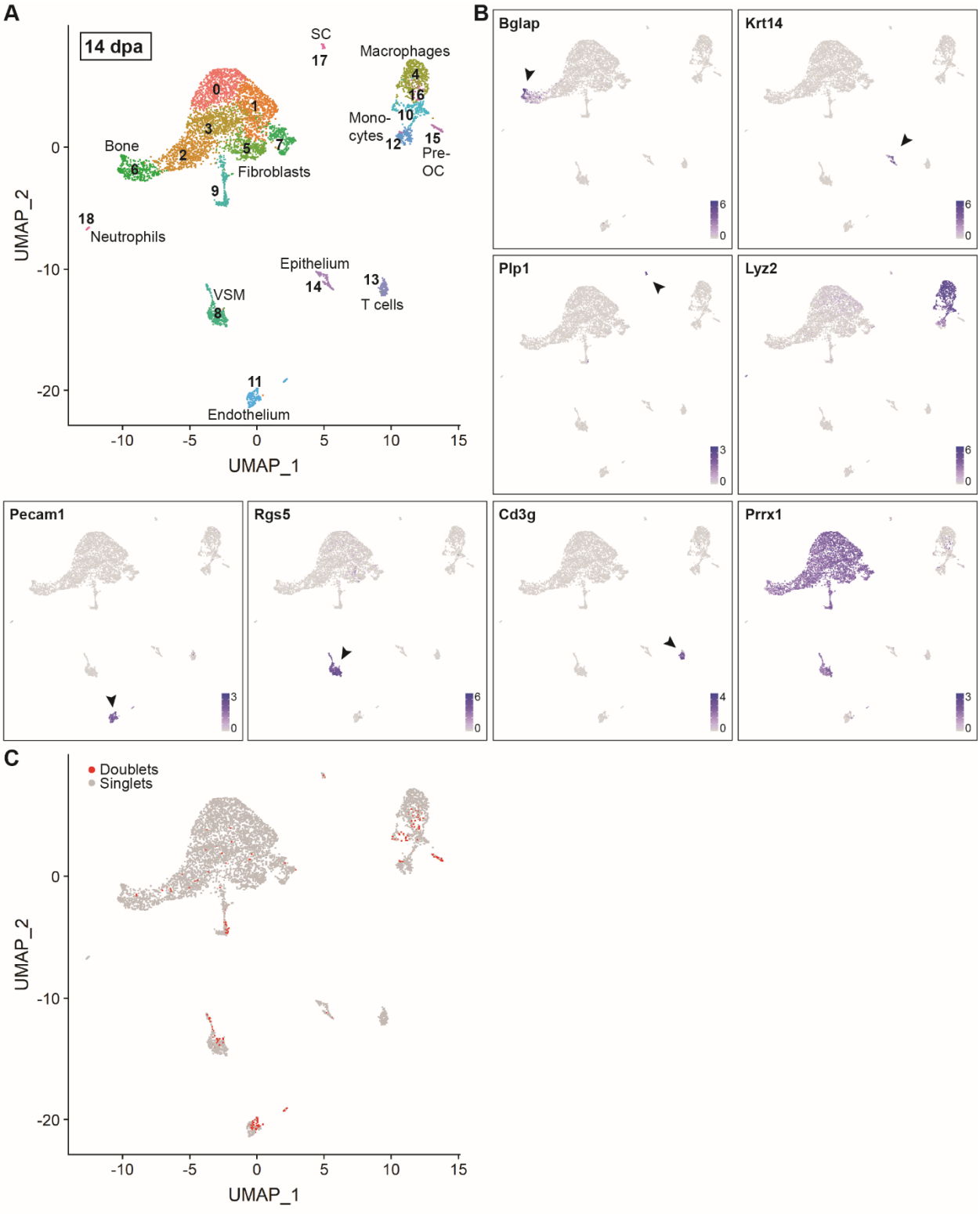
A
14 dpa
SC
17
Macrophages
4
16
10
Mono-
cytes
12
15
Pre-
OC
0
1
3
Bone
2
5
7
6
Fibroblasts
9
18
Neutrophils
Epithelium
14
13
T cells
VSM
8
11
Endothelium
UMAP_2
UMAP_1
B
Bglap
Krt14
Plp1
Lyz2
Pecam1
Rgs5
Cd3g
Prrx1
C
Doublets
Singlets

Figure S3

Single cell RNAseq of 14dpa blastema (Relates to Figure 3 and Table S1)

(A) Unbiased single cell clustering of 5,896 high quality cells visualized by UMAP. Each dot represents a single cell and cells assigned to the same cluster are similarly colored. Cell type identities are assigned as follows: fibroblasts (clusters 0-3, 5, 7, and 9), macrophages (clusters 4 and 16), bone (cluster 6), vascular smooth muscle cells (VSM) (cluster 8), monocytes (clusters 10 and 12), endothelial cells (cluster 11), T cells (cluster 13), epithelial cells (cluster 14), pre-osteoclast (Pre-OC) (cluster 15), Schwann cells (SC) (cluster 17), neutrophils (cluster 18). (B) Gene expression UMAP overlay with examples of highly expressed, cell type specific markers used to assign cluster cell identities: *Bglap* (bone), *Krt14* (epithelial cells), *Plp1* (SCs), *Lyz2* (monocytes/macrophages), *Pecam1* (endothelial cells), *Rgs5* (vascular smooth muscle cells), *Cd3g* (T cells), *Prrx1* (fibroblasts). Gray depicts low expression and purple depicts high expression as specified on the scale for each gene. Black arrowheads denote the population of cells expressing a particular marker. (C) 271 cells were classified as doublets (red) and excluded from all subsequent analyses.

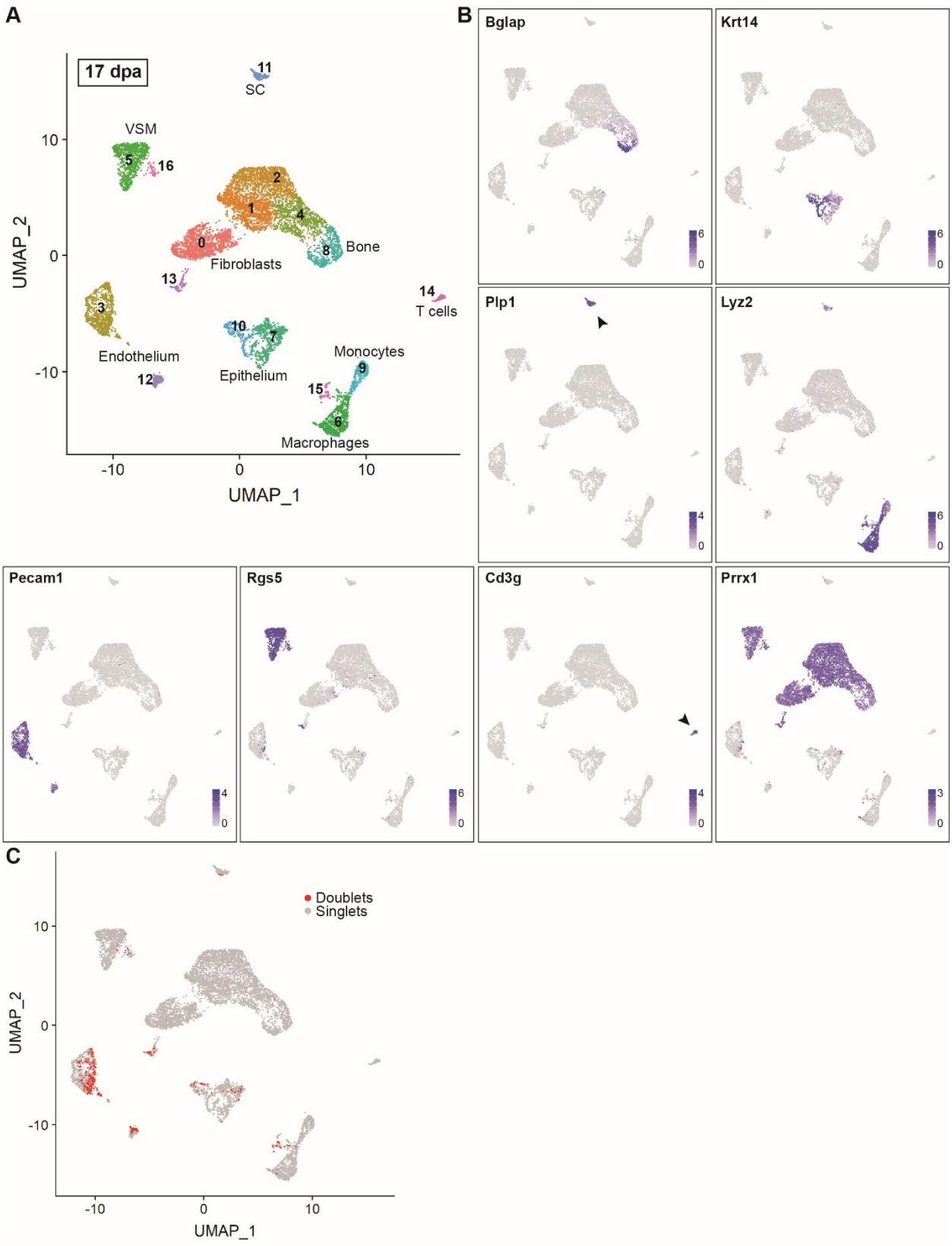
A
17 dpa
SC
VSM
Fibroblasts
Bone
Endothelium
Epithelium
Monocytes
Macrophages
T cells
UMAP_1
UMAP_2
B
Bglap
Krt14
Plp1
Lyz2
Pecam1
Rgs5
Cd3g
Prrx1
C
Doublets
Singlets

Figure S4

Single cell RNAseq of 17dpa blastema (Relates to Figure 1 and Table S1)

(A) Unbiased single cell clustering of 8,778 high quality cells visualized by UMAP. Each dot represents a single cell and cells assigned to the same cluster are similarly colored. Cell type identities are assigned as follows: fibroblasts (clusters 0-2, and 4), endothelial cells (clusters 3 and 12), vascular smooth muscle cells (VSM) (clusters 5 and 16), macrophages (cluster 6), epithelial cells (clusters 7 and 10), bone (cluster 8), monocytes (clusters 9 and 15), Schwann cells (SC) (cluster 11), T cells (cluster 14). (B) Gene expression UMAP overlay with examples of highly expressed, cell type specific markers used to assign cluster cell identities: *Bglap* (bone), *Krt14* (epithelial cells), *Plp1* (SCs), *Lyz2* (monocytes/macrophages), *Pecam1* (endothelial cells), *Rgs5* (vascular smooth muscle cells), *Cd3g* (T cells), *Prrx1* (fibroblasts). Gray depicts low expression and purple depicts high expression as specified on the scale for each gene. Black arrowheads denote the population of cells expressing a particular marker. (C) 606 cells were classified as doublets (red) and excluded from all subsequent analyses.


A
Unamputated
Bone
Epithelium
Endothelium
VSM
T cells
Mono-
cytes
Fibroblasts
SC
Macrophages
UMAP_1
UMAP_2
B
Bglap
Krt14
Plp1
Lyz2
Pecam1
Rgs5
Cd3g
Prrx1
C
Doublets
Singlets
UMAP_1
UMAP_2

Figure S5

Single cell RNAseq of the unamputated digit tip (Relates to Figure 1 and Table S1)

(A) Unbiased single cell clustering of 12,871 high quality cells visualized by UMAP. Each dot represents a single cell and cells assigned to the same cluster are similarly colored. Cell type identities are assigned as follows: fibroblasts (clusters 0-3, 5, 6, 9, and 21), macrophages (clusters 4 and 16), vascular smooth muscle cells (VSM) (cluster 7), epithelial cells (clusters 8, 12, and 13), endothelial cells (cluster 10, 18, and 20), Schwann cells (SC) (clusters 11 and 19), monocytes (cluster 14), bone (cluster 15), T cells (cluster 17). (B) Gene expression UMAP overlay with examples of highly expressed, cell type specific markers used to assign cluster cell identities: *Bglap* (bone), *Krt14* (epithelial cells), *Plp1* (SCs), *Lyz2* (monocytes/macrophages), *Pecam1* (endothelial cells), *Rgs5* (vascular smooth muscle cells), *Cd3g* (T cells), *Prrx1* (fibroblasts). Gray depicts low expression and purple depicts high expression as specified on the scale for each gene. Black arrowheads denote the population of cells expressing a particular marker. (C) 978 cells were classified as doublets (red) and excluded from all subsequent analyses.

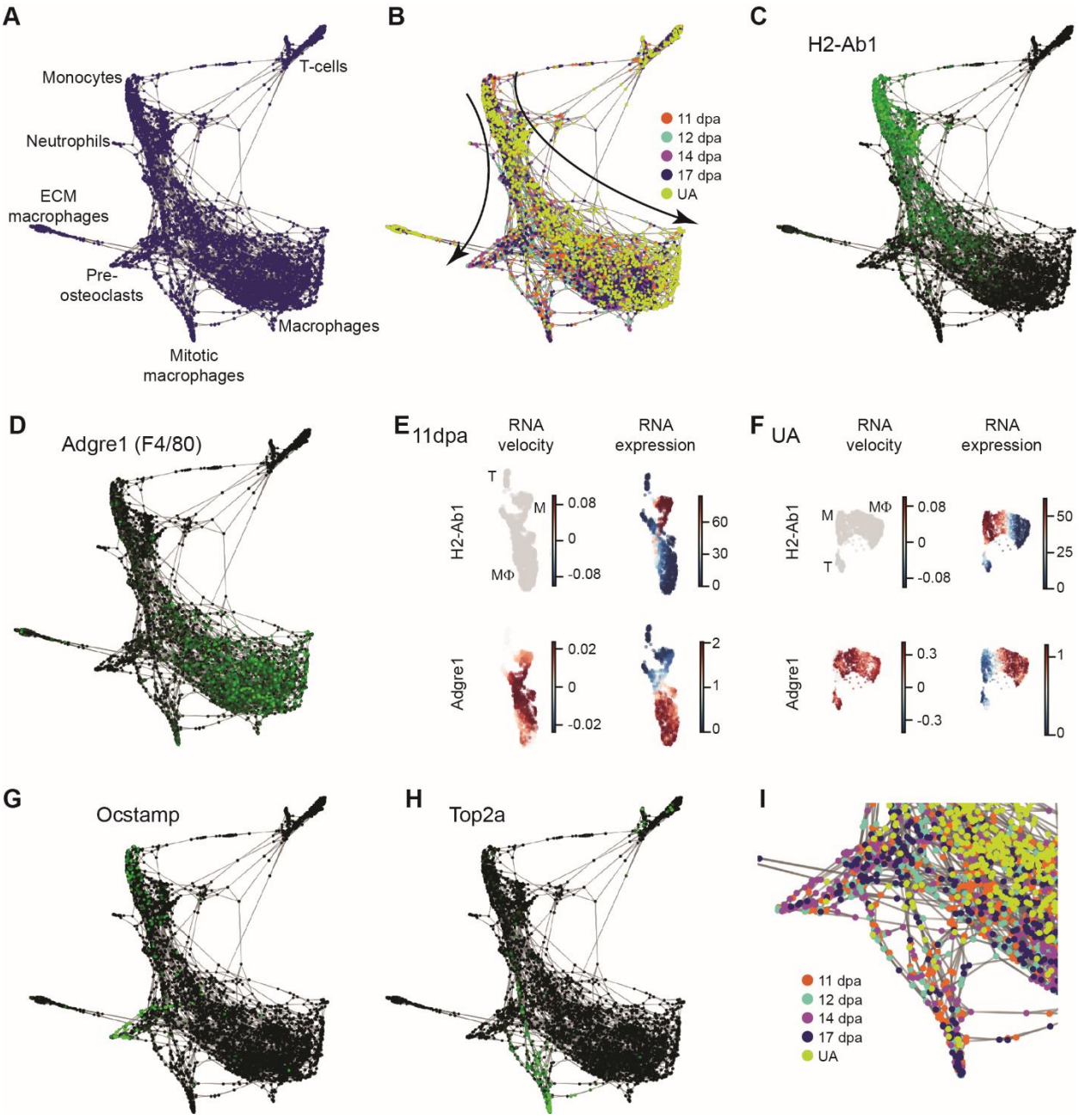
A
Monocytes
T-cells
Neutrophils
ECM
macrophages
Pre-
osteoclasts
Macrophages
Mitotic
macrophages
B
11 dpa
12 dpa
14 dpa
17 dpa
UA
C
H2-Ab1
D
Adgre1 (F4/80)
E
11dpa
RNA
velocity
RNA
expression
H2-Ab1
T
M
MΦ
0.08
0
-0.08
60
30
0
Adgre1
0.02
0
-0.02
2
1
0
F
UA
RNA
velocity
RNA
expression
H2-Ab1
M
MΦ
T
0.08
0
-0.08
50
25
0
Adgre1
0.3
0
-0.3
1
0
G
Ocstamp
H
Top2a
I
11 dpa
12 dpa
14 dpa
17 dpa
UA

Figure S6

Differentiation trajectory analysis of immune-related cells (Relates to Figure 3)

Computationally derived SPRING lineage trajectory analysis of cells from the integrated data set immune-related clusters 7, 11, 12, 17, 19, 21, and 22. (A) Force-directed SPRING plot of cells showing monocytes, macrophages, ECM macrophages (express ECM related genes; population of unknown relevance), mitotic macrophages, pre-osteoclasts, T cells. (B) SPRING plot as in (A) with regenerative stages of each cell colored coded: 11dpa (orange), 12dpa (light blue), 14dpa (purple), 17dpa (dark blue), unamputated (yellow). Known differentiation trajectories from monocytes to macrophages, and monocytes to pre-osteoclasts are depicted with curved arrows. (C and D) Marker gene expression overlay supports computationally defined monocyte to macrophage differentiation. *H2-Ab1* is expressed in monocytes and *Adgre1*(F4/80) is expressed in macrophages. High expression is in green and low expression is black. (E and F) UMAP plots of RNA velocity and expression data for individual monocyte and macrophage genes *H2-Ab1* and *Adgre1* in 11dpa and UA samples; M = monocytes, T = T cells, and MΦ = macrophages. (G and H) Gene expression overlay showing *Ocstamp* pre-osteoclast expression and *Top2a* mitotic macrophage expression. (I) Close-up of pre-osteoclasts and mitotic macrophages in (B); qualitative evaluation shows enrichment for blastema stages.

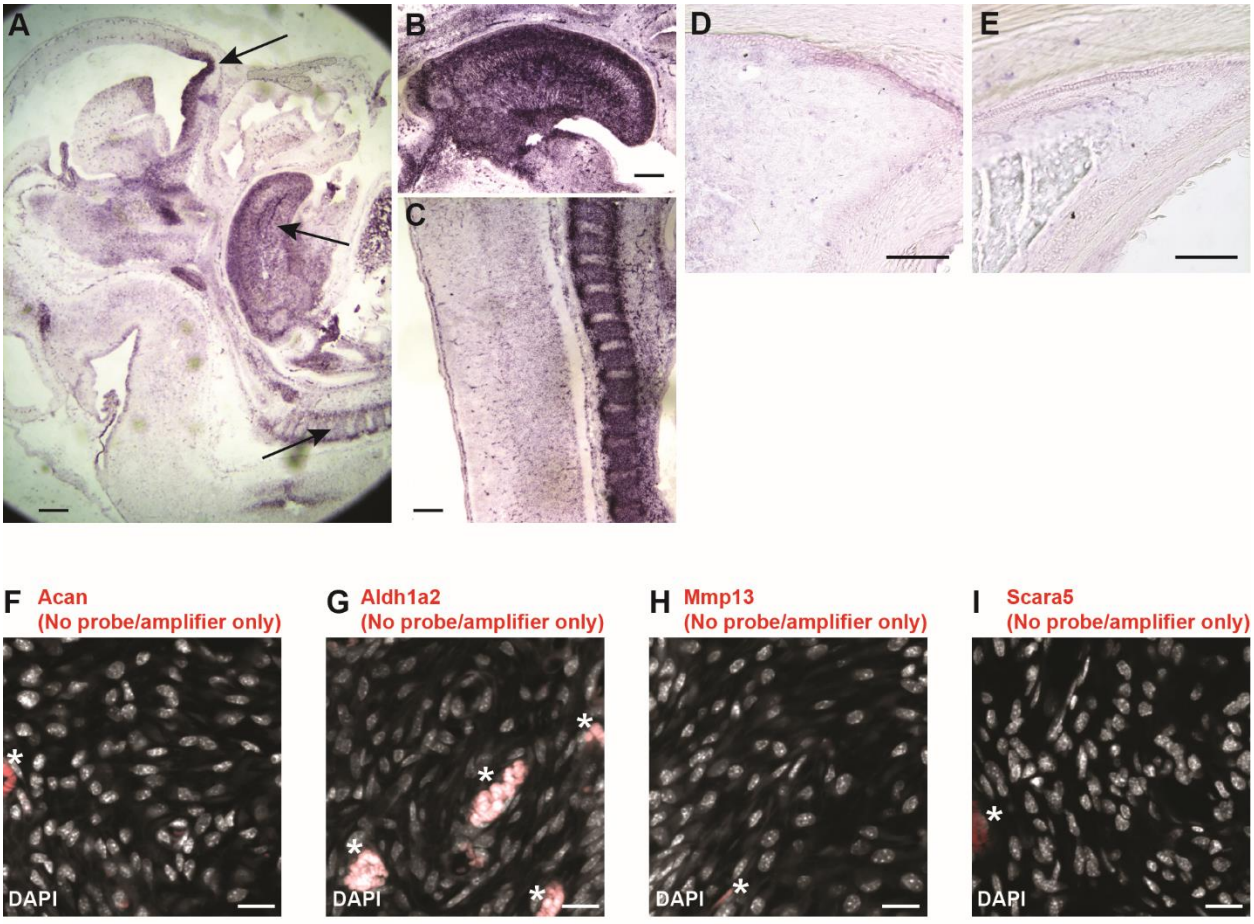
A
B
C
D
E
F Acan
(No probe/amplifier only)
G Aldh1a2
(No probe/amplifier only)
H Mmp13
(No probe/amplifier only)
I Scara5
(No probe/amplifier only)
DAPI
DAPI
DAPI
DAPI

Figure S7

RNA in situ control expression (Relates to Figures 2 and 7)

(A-E) DIG labeled section RNA in situ controls. (A-C) *Mest* antisense probe positive control on E12.5 mouse embryo sections. (A) Transverse section through head and neck region with positive expression (purple) in the developing forebrain, tongue, and vertebrae (arrows). (B and C) Magnified view of panel (A) of (B) tongue and (C) vertebrae. (D and E) *Mest* sense probe negative control on adult mouse digit tip sections. No appreciable expression from sense probe is found on (D) 12dpa or (E) unamputated tissues. Scale bars = 400μm for (A), 200μm for (B and C), and 100μm for (D and E). (F-I) Mid-blastema fields of no probe/amplifier only HCR RNA FISH negative controls. (F) Amplifier for *Acan* probe, (G) amplifier for *Aldh1a2* probe, (H) amplifier for *Mmp13* probe, (I) amplifier for *Scara5* probe. Scale bars = 20μm.

11dpa fibroblast cluster #	GO significant categories
0	granulocyte chemotaxis, inflammatory response, Wnt signaling pathway, response to cytokine, regulation of signal transduction
1	antimicrobial humoral immune response, response to lipopolysaccharide, response to cytokine
2	none significant
4	skeletal system development, extracellular matrix organization
5	extracellular matrix organization, cell development, regulation of signal transduction
6	iron ion import, transmembrane receptor protein tyrosine kinase signaling pathway
8	DNA recombination, chromosome segregation, chromosome condensation, regulation of cyclin-dependent protein serine/threonine kinase activity, positive regulation of cell cycle, mitotic nuclear division, chromatin organization, microtubule cytoskeleton organization, nucleotide biosynthetic process

Table S2

GO terms associated with 11dpa fibroblast cluster gene expression (Relates to Figure 2 and S1)

Significant GO slim biological process categories for 11dpa fibroblast clusters with adjusted p-value ≤ 0.05 and average log fold-change ≥ 0.05.

	ua vs. 11	ua vs. 12	ua vs. 14	ua vs. 17	11 vs. 12	11 vs. 14	11 vs. 17	12 vs. 14	12 vs. 17	14 vs. 17
cluster_0	0.0000	0.0031	0.0009	0.1675	0.1033	0.0484	0.0000	0.4417	0.0234	0.0132
cluster_1	0.0004	0.0335	0.0182	0.4171	0.2297	0.1847	0.0009	0.4767	0.0577	0.0335
cluster_2	0.3133	0.4055	0.4055	0.4055	0.4055	0.4055	0.4055	0.4944	0.4944	0.4944
cluster_3	0.4083	0.4100	0.4083	0.4083	0.4083	0.4083	0.4083	0.4083	0.4933	0.4083
cluster_4	0.2039	0.2039	0.2568	0.2039	0.4547	0.4547	0.4547	0.4547	0.4547	0.4547
cluster_5	0.0080	0.1525	0.1630	0.1630	0.3354	0.1630	0.1525	0.3598	0.3354	0.4094
cluster_6	0.2034	0.3882	0.3825	0.0206	0.3882	0.3882	0.0034	0.4327	0.0328	0.0117
cluster_7	0.4374	0.4374	0.4374	0.4477	0.4477	0.4374	0.4374	0.4477	0.4374	0.4374
cluster_8	0.0004	0.0008	0.0000	0.4231	0.4231	0.3606	0.0016	0.4231	0.0020	0.0003
cluster_9	0.2619	0.2619	0.1057	0.2165	0.4217	0.2523	0.1057	0.2931	0.1110	0.0233
cluster_10	0.2558	0.1640	0.1640	0.2134	0.3480	0.3480	0.4327	0.4223	0.3480	0.3634
cluster_11	0.4826	0.4826	0.4826	0.4826	0.4826	0.4905	0.4826	0.4826	0.4826	0.4826
cluster_12	0.4776	0.4776	0.4776	0.4776	0.4776	0.4776	0.4776	0.4776	0.4776	0.4776
cluster_13	0.0002	0.0047	0.0039	0.2585	0.4124	0.2585	0.0016	0.3426	0.0169	0.0169
cluster_14	0.0347	0.1077	0.0095	0.1550	0.4658	0.3193	0.2792	0.3193	0.3193	0.1550
cluster_15	0.0107	0.1713	0.0107	0.4448	0.3003	0.4544	0.0287	0.3003	0.2297	0.0287
cluster_16	0.0001	0.0022	0.0001	0.0264	0.4547	0.4609	0.0768	0.4547	0.1741	0.0927
cluster_17	0.4741	0.4741	0.1840	0.4741	0.4741	0.1840	0.4741	0.1840	0.4741	0.1840
cluster_18	0.1061	0.2972	0.1182	0.4041	0.3917	0.4041	0.1182	0.4041	0.3501	0.1941
cluster_19	0.3950	0.3950	0.3950	0.3950	0.3950	0.3950	0.3950	0.3950	0.3950	0.3950
cluster_20	0.0438	0.0438	0.0438	0.0438	0.4732	0.4732	0.4732	0.4732	0.4732	0.4732
cluster_21	0.3208	0.3208	0.3208	0.3283	0.3854	0.4668	0.3854	0.3854	0.3854	0.3854
cluster_22	0.3524	0.3060	0.2741	0.3524	0.3054	0.2741	0.3081	0.3054	0.3081	0.2741

Table S4

P-values for differential population analysis of all stage integrated dataset (Relates to Figure 3)

All resultant p-values for regenerative stage pairwise differential proportion analyses testing for significant changes in proportion of cells within clusters. Reported values have been corrected for multiple hypothesis testing. Column headers indicate regenerative stages being compared: 11 = 11dpa, 12 = 12dpa, 14 = 14dpa, 17 = 17dpa, and ua = unamputated. Cluster numbers in each row refer to UMAP cluster classification in Figure 3B. All table cells in gray are noted as significant with $p \leq 0.05$.

	ua vs. 11	ua vs. 12	ua vs. 14	ua vs. 17	11 vs. 12	11 vs. 14	11 vs. 17	12 vs. 14	12 vs. 17	14 vs. 17
cluster_0	0.0000	0.0022	0.0022	0.0743	0.1598	0.0464	0.0005	0.2858	0.0597	0.0871
cluster_1	0.0716	0.4114	0.4114	0.4516	0.2965	0.1889	0.0716	0.4516	0.4114	0.4114
cluster_2	0.0368	0.1355	0.0837	0.4148	0.4148	0.4148	0.0837	0.4896	0.1763	0.1362
cluster_3	0.0004	0.0597	0.0730	0.2955	0.2656	0.0960	0.0053	0.2955	0.0960	0.1489
cluster_4	0.0005	0.0031	0.0031	0.0281	0.4892	0.4150	0.1646	0.4162	0.2219	0.2686
cluster_5	0.4785	0.4785	0.4785	0.4785	0.4785	0.4785	0.4785	0.4785	0.4785	0.4785
cluster_6	0.1910	0.0331	0.1002	0.2719	0.1584	0.2854	0.3283	0.2598	0.1002	0.2404
cluster_7	0.4539	0.4539	0.4539	0.4539	0.4539	0.4539	0.4539	0.4539	0.4539	0.4539
cluster_8	0.3678	0.4254	0.4254	0.4254	0.4254	0.4254	0.4254	0.4254	0.4599	0.4254
cluster_9	0.3265	0.2042	0.2042	0.2042	0.3265	0.3723	0.3265	0.4309	0.4309	0.4309
cluster_10	0.0027	0.0238	0.0184	0.2907	0.4103	0.2907	0.0182	0.3887	0.0668	0.0668
cluster_11	0.4710	0.0952	0.0294	0.0294	0.0952	0.0294	0.0340	0.4227	0.4710	0.4227
cluster_12	0.0000	0.0000	0.0000	0.0008	0.4960	0.4960	0.2256	0.4960	0.2256	0.2256
cluster_13	0.0966	0.0978	0.0966	0.0966	0.4440	0.4440	0.4440	0.4440	0.4440	0.4440

Table S6

P-values for differential population analysis of re-clustered fibroblast and bone populations (Relates to Figures 5 and 6)

All resultant p-values for regenerative stage pairwise differential proportion analyses testing for significant changes in proportion of cells within clusters. Reported values have been corrected for multiple hypothesis testing. Column headers indicate regenerative stages being compared: 11 = 11dpa, 12 = 12dpa, 14 = 14dpa, 17 = 17dpa, and ua = unamputated. Cluster numbers in each row refer to UMAP cluster classification in Figure 5A. All table cells in gray are noted as significant with $p \leq 0.05$.