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Supplemental Information

***Lgr5* and *Col22a1* Mark Progenitor Cells in the Lineage toward Juvenile
Articular Chondrocytes**

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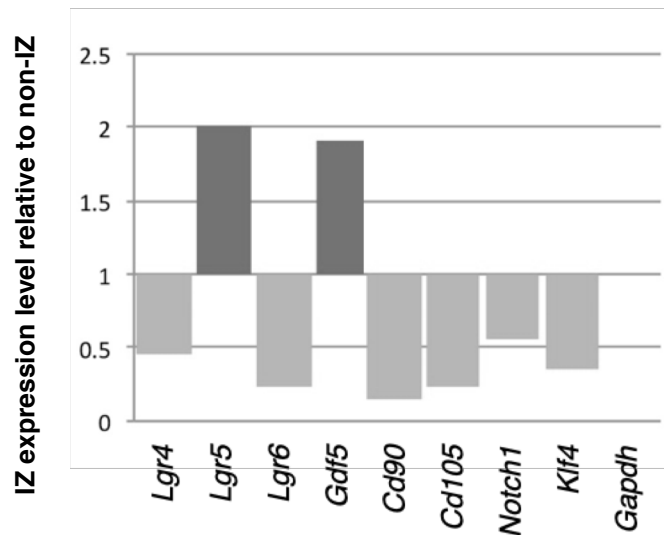


Figure S1. Quantitative PCR showed expression of *Gdf5* and *Lgr5* in digit interzone mRNA was extracted from the digit interzone (IZ) and surrounding non-interzone regions of a E14.5 embryo to generate cDNA for QPCR. Expression of *Gapdh* was normalized to 1.0, values higher than 1.0 indicates these genes are upregulated, vice versa. Among all the selected stem cell/progenitor markers, known marker of joint interzone *Gdf5* and novel marker identified in this paper *Lgr5* were upregulated in the interzone region.

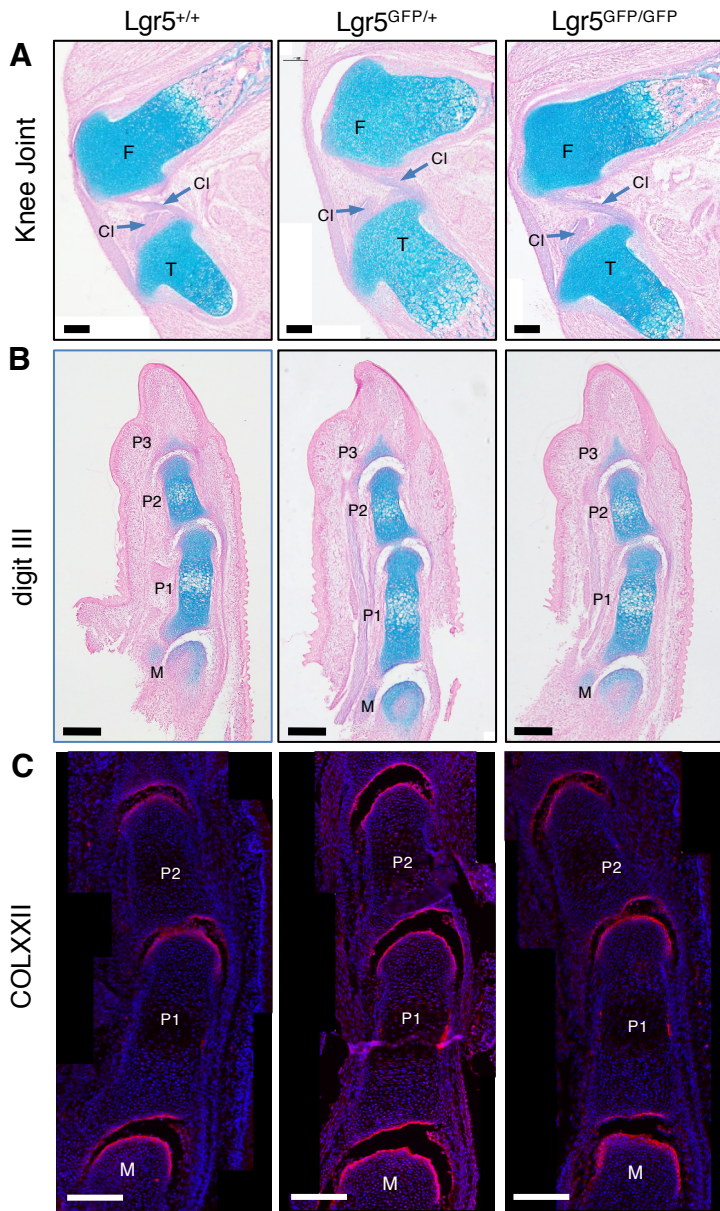


Figure S2. *Lgr5-GFP* mutant developed normal knee and digit joints

Representative histology sections stained with Alcian blue and nuclear fast red, showing no observable difference between the developing knee (A) and digit joints (B) of *Lgr5*^{+/+}, *Lgr5*^{GFP/+} and *Lgr5*^{GFP/GFP} E18.5 embryos (n=3 per genotype). (C) The articular cartilages appeared to have formed normally with the expected expression and localization of COLXXII in the superficial layer. F=femur, T=tibia, CI=cruciate ligament, M=metacarpal, P1= proximal phalange, P2=middle phalange, P3=distal phalange. Scale bar, 200µm

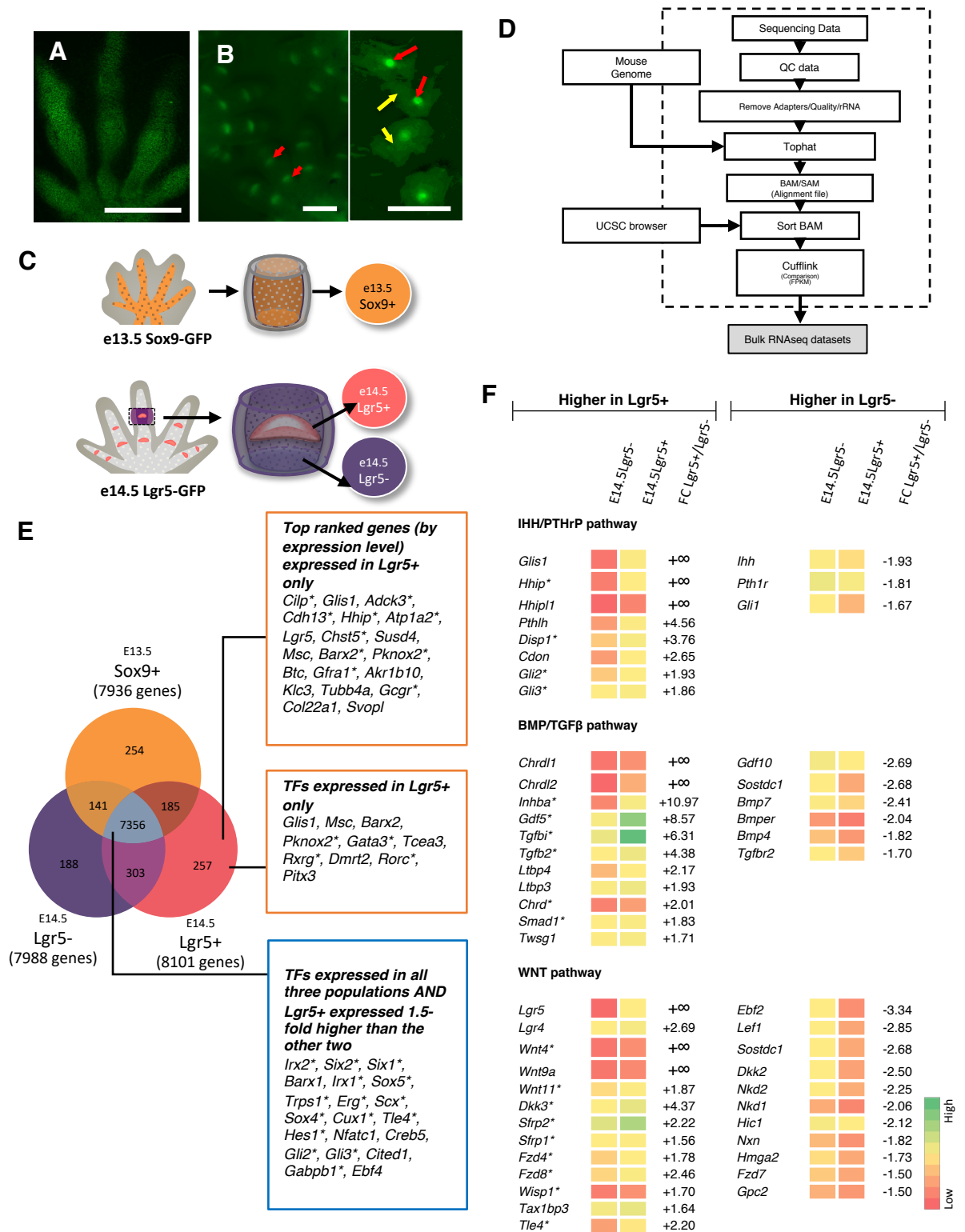


Figure S3. Bulk transcriptomic analysis of cell populations in joint development.

(A) Three population of cells isolated. One from Sox9 expressing cells in E13.5 digit of Sox9-eGFP mice, and two populations from the E14.5 interzone region of the developing digits that contains the Lgr5+ interzone (red arrows) and surrounding non-GFP region (yellow arrows) from Lgr5-GFP mice (B). Scalebars, 1mm. (C) Diagrammatic illustration of the protocol with all cell isolated were sorted for the appropriate population of cells for subsequent for RNA-seq for the generating the three datasets. (D) Workflow for bulk RNAseq data processing. (E) Venn diagram showing the number of genes identified, and overlaps between datasets. Genes with higher expression in Lgr5+ population are shown on the right. (F) Heat maps depicting comparative gene expression levels of the top genes involved in specified molecular pathways. + Fold change (FC) indicates an increase and -FC for decrease in Lgr5+ cells. "+∞" are given to indicate genes that are expressed only in Lgr5+ cells. Genes with expression data available in the Euxpress database are marked with an asterisk.

Related to figure 3.

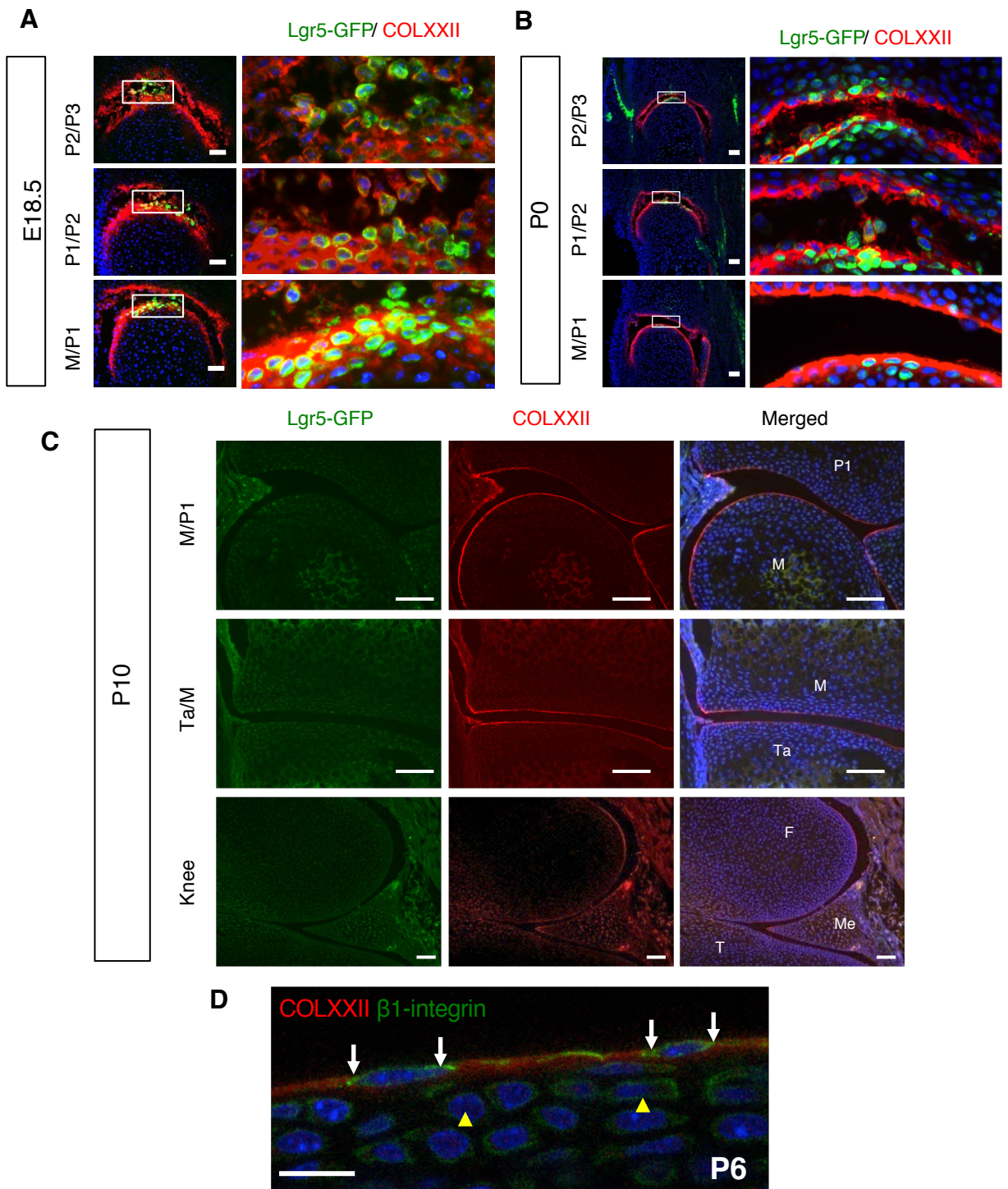


Figure S4. Expression of Lgr5 and COLXXII in phalangeal and knee joints.

Co-localization of Lgr5 (Green) and COLXXII (Red) in E18.5 **(A)** and newborn (P0) **(B)** digit joints. **(C)** Expression in M/P1, Ta/M and Knee joints at P10. M, metatarsal. P1, proximal phalange. P2, middle phalange. P3, distal phalange. Ta/M, tarsometatarsal joint. **(D)** Cells embedded in the COLXXII layer are flat compare to the round chondrocytes in the deeper zones (yellow arrow) of P6 mouse. Focal adhesions (β 1-integrin) are found at both sides of the cells where they are in direct contact with the COLXXII matrix (white arrows). Scale bars in (A-C), 100 μ m and in (D), 10 μ m. **Related to figure 3.**

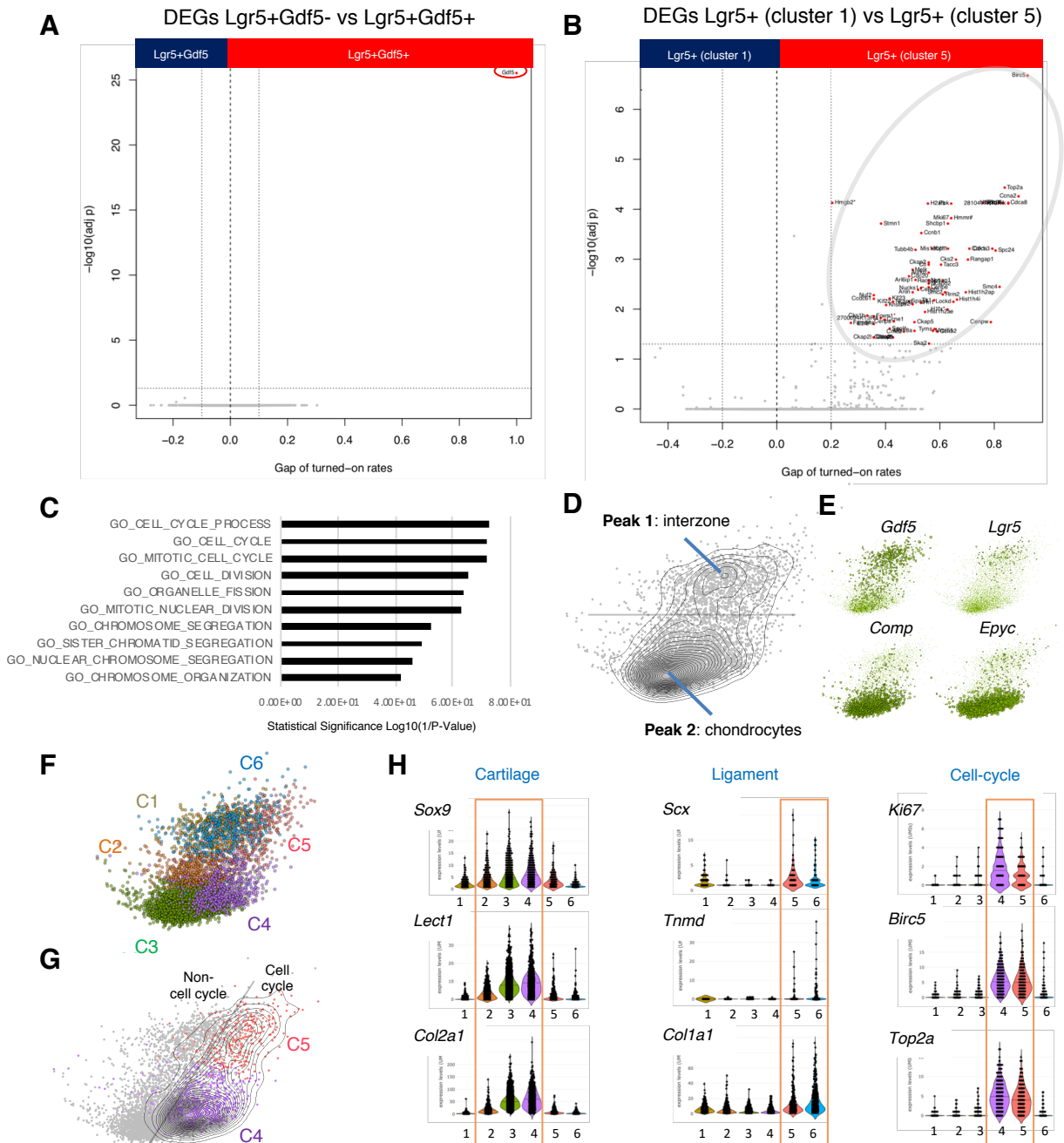


Figure S5 Additional information for single cell analysis

(A) Volcano plot showing *Gdf5* (red circle) is the only differential expressing gene (DEG) between *Lgr5*⁺/*Gdf5*⁻ and *Lgr5*⁺/*Gdf5*⁺ subpopulations, and these cells are essentially identical. (B) Volcano plot shows DEGs between *Lgr5*⁺ cells from cluster 1 and cluster 5, also show they are the same cells, differ only as proliferating cells in cluster 5. (C) GO term analysis of the DEGs from panel (B) (circled). (d-g) Principle component analysis (PCA) for the 5,460 E14.5 knee joint cells. (D) A contour plot showing the density of cells, two major peaks are found. (E) Peak 1 corresponds to interzone cells expressing *Gdf5* and *Lgr5*, and peak 2 as non-interzone expressing mature chondrocyte markers, *Comp* and *Epyc*. (F) t-SNE clusters 1, 2, 5, and 6 are located in peak 1, whereas clusters 3 and 4 are in peak 2, consistent with the 2D t-SNE finding. (G) Contour plot of the cell cycle genes showing the existence of a proliferating subpopulation of cells in the interzone (cluster 5) and non-interzone (cluster 4) clusters. (H) Violin plots showing the expression profiles of representative cartilage, ligament and cell cycle genes in each cluster supporting their identities/signatures. **Related to figure 4.**

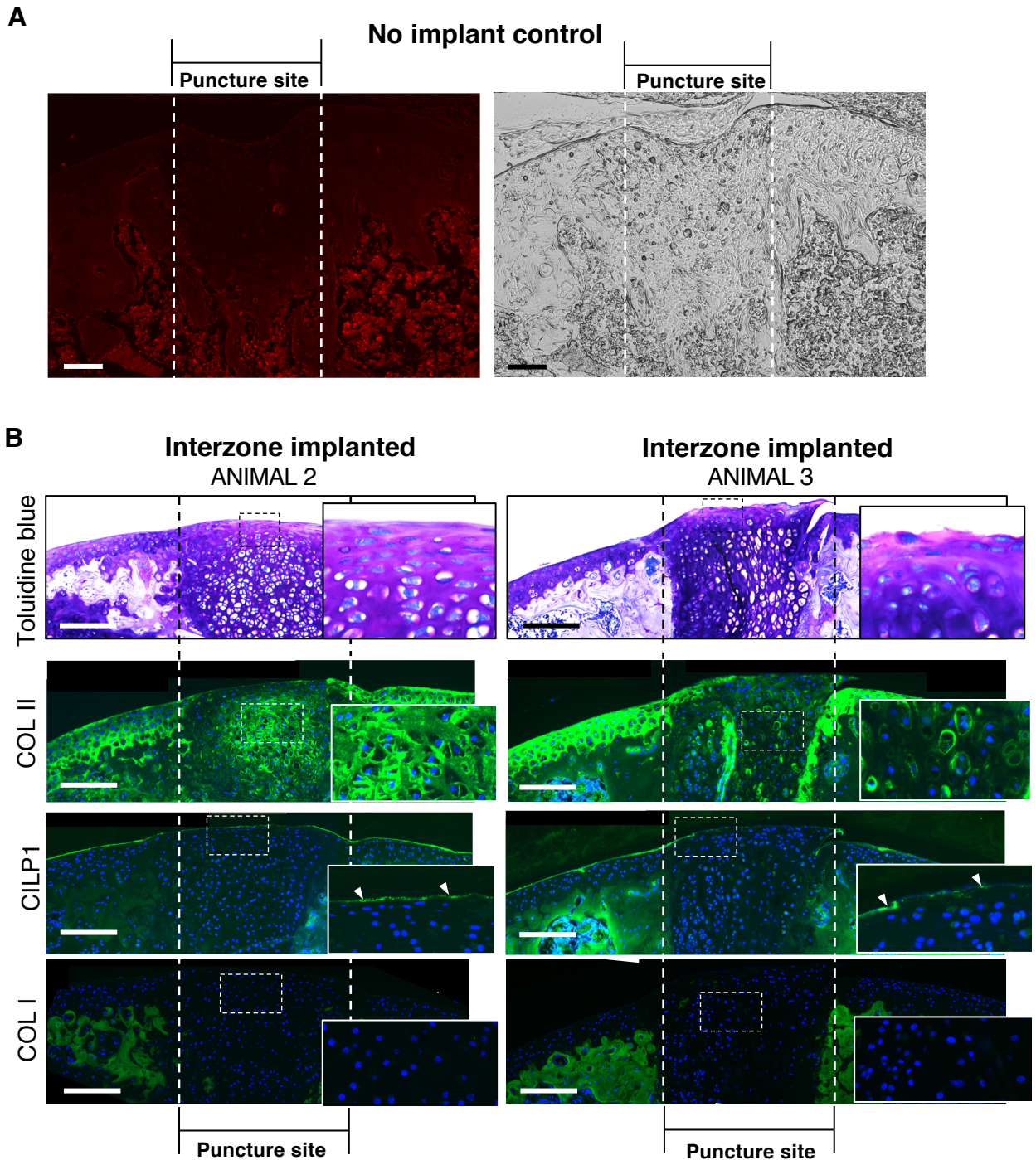


Figure S6. *Lgr5+* interzone tissue transplantation for repairing of a cartilage lesion

(A) No tdTomato+ cells was detected in the puncture site without interzone tissue insert. Bright-field image of the same region was shown on the right. (B) Analysis of two additional animals receiving interzone tissue transplantation. Similar positive outcomes among all three operated animals, with differentiation of the interzones to chondrocytes expressing COL II, reestablishment of the superficial layer (CILP1 expressing), and suppressing COL I expression and fibrotic tissue. Scalebars, 50 μ m. **Related to figure 6.**

Table S1. Genes expressed by only one population (top 100 ranked by level) (Digit bulk)

	Expressed in Sox9+ only (FPKM>5)				Expressed in Lgr5- only (FPKM>5)				Expressed in Lgr5+ only (FPKM>5)			
	Gene	e13.5 Sox9+	e14.5 Lgr5-	e14.5 Lgr5+	Gene	e13.5 Sox9+	e14.5 Lgr5-	e14.5 Lgr5+	Gene	e13.5 Sox9+	e14.5 Lgr5-	e14.5 Lgr5+
1	<i>Epyc</i>	48.82	0.63	1.2	<i>Alas2</i>	0.86	25.41	1.16	<i>Cilp</i>	0.2	0.75	58.03
2	<i>Smpd3</i>	30.66	0.29	0.19	<i>Foxf2</i>	2.52	20.2	2.85	<i>Glis1</i>	4.33	3.54	52.57
3	<i>Panx3</i>	27.67	1.16	1.14	<i>Stra6</i>	4.58	18.26	4.13	<i>Adck3</i>	0.99	2.62	35.46
4	<i>Epha2</i>	20.86	4.24	4.68	<i>Cpa3</i>	0.81	15.64	2.79	<i>Cdh13</i>	2.91	2.97	35.39
5	<i>Frzb</i>	20.82	1.77	2.12	<i>Sst</i>	0.23	15.56	4.07	<i>Hhip</i>	2.97	4.94	30.22
6	<i>Foxd1</i>	19.47	3.43	0.71	<i>Cd74</i>	1.82	12.69	4.52	<i>Atp1a2</i>	3.4	1.89	27.54
7	<i>Cxcl10</i>	18.67	1.73	3.35	<i>Zfhx4</i>	4.26	12.46	4.66	<i>Lgr5</i>	3.48	1.36	26.75
8	<i>Pltp</i>	17.52	3.89	2.93	<i>Cma1</i>	0.39	12.41	2.26	<i>Chst5</i>	4.06	3.01	26.54
9	<i>Hoxc10</i>	17.02	0.21	0.37	<i>Ablim1</i>	3.74	12.18	4.9	<i>Susd4</i>	3.6	4.89	22.94
10	<i>Tnfaip3</i>	16.98	4.13	4.71	<i>S100a8</i>	0	11.5	0.86	<i>Msc</i>	2.18	1.28	22.79
11	<i>Peg10</i>	14.87	3.71	2.4	<i>Grp</i>	1.24	11.19	4.11	<i>Barx2</i>	1.27	0.25	20.41
12	<i>Spp1</i>	14.72	2.5	2.13	<i>Snca</i>	0.69	9.95	1.52	<i>Pknx2</i>	3.74	1.42	19.37
13	<i>Sgms2</i>	14.54	3.67	3.36	<i>Fgf2</i>	4.52	9.61	4.19	<i>Btc</i>	0.46	0.56	19.22
14	<i>Chac1</i>	14.27	0.64	1.38	<i>Angpt2</i>	0.78	9.45	1.97	<i>Gfra1</i>	2.9	3.67	18.25
15	<i>Ndufa4l2</i>	14.12	2.13	3.35	<i>Kcp</i>	4.12	9.03	4.84	<i>Akr1b10</i>	3.87	1.78	18.06
16	<i>Chst11</i>	14.06	4.4	4.75	<i>Arhgdig</i>	4.34	9.01	4.21	<i>Klc3</i>	3.59	4.05	17.07
17	<i>Ptgs2</i>	13.6	1.04	1.41	<i>Axin2</i>	2.12	8.94	3.13	<i>Tubb4a</i>	4.29	4.63	16.12
18	<i>Mpzl2</i>	13.06	3.79	4.67	<i>Cd52</i>	0	8.86	2.87	<i>Gegr</i>	3.2	1.77	15.97
19	<i>Edil3</i>	12.86	3.73	4.66	<i>Kcnk2</i>	3.13	8.79	4.11	<i>Col22a1</i>	1.5	1.93	15.82
20	<i>S100b</i>	12.1	2.56	2.62	<i>Stmn2</i>	2.71	8.7	1.51	<i>Svopl</i>	1.11	1	15.72
21	<i>Foxo6</i>	12.07	4.31	3.36	<i>Dbx2</i>	1.4	8.68	0.74	<i>Vaultrc5</i>	4.63	2.57	15.48
22	<i>Sytl2</i>	12.03	2.32	4.18	<i>Alox5ap</i>	1.35	8.64	2.61	<i>Pde2a</i>	2.11	2.81	15.23
23	<i>C1galt1</i>	11.68	3.47	4.69	<i>Nxn12</i>	2.17	8.62	3.73	<i>Ppp1r1b</i>	2	2.62	14.44
24	<i>Cgref1</i>	11.62	4.64	4.25	<i>Srgn</i>	0.45	8.58	2.88	<i>Sntb1</i>	1.77	2.9	13.36
25	<i>Pmaip1</i>	11.61	2.98	2.64	<i>Cmpk2</i>	3.86	8.56	4.93	<i>Thbs4</i>	2.09	2.94	12.9
26	<i>Tefl5</i>	11.41	4.13	3.25	<i>F11r</i>	3.94	8.27	3.13	<i>Krt5</i>	4.61	1.24	12.62
27	<i>Saa1</i>	11.33	2.08	4.06	<i>Kif26a</i>	3.06	8.07	1.13	<i>Lrrn3</i>	2.5	1.52	12.38
28	<i>Hck</i>	10.72	4.42	1.37	<i>Gstm6</i>	2.7	8.06	3.74	<i>Tnnc1</i>	1.83	1.07	12.27
29	<i>Xylt1</i>	10.7	4.44	3.26	<i>Heph</i>	2.99	7.92	3.35	<i>Ephx1</i>	1.74	2.72	12.15
30	<i>Entpd6</i>	10.53	4.55	4.95	<i>H2-T22</i>	2.59	7.91	4.31	<i>Stc1</i>	3.61	4.5	11.55
31	<i>Col15a1</i>	10.4	3.93	2.15	<i>Il17rd</i>	2.63	7.89	2.76	<i>Cer1</i>	2.53	0.03	11.04
32	<i>Magel2</i>	10.39	4.97	3.5	<i>Gpr124</i>	4.32	7.8	2.74	<i>Ptpro</i>	0.68	0.76	10.97
33	<i>Ifitm5</i>	10.38	2.22	1.74	<i>Fbxl19</i>	4.86	7.78	4.63	<i>Matk</i>	2.46	2.32	10.56
34	<i>Bmp5</i>	10.25	4.5	2.69	<i>Tmtc2</i>	2.81	7.77	2.37	<i>Acta2</i>	2.64	3.18	10.21
35	<i>E2f7</i>	9.81	4.32	3.38	<i>Lgals7</i>	2.52	7.75	3.95	<i>Ppp1r1a</i>	3.75	2.33	9.98
36	<i>Foxa3</i>	9.6	2.47	3.36	<i>Raver1-fdx11</i>	3.61	7.36	3.59	<i>Kcnma1</i>	4.52	2.85	9.71
37	<i>Ssfa2</i>	9.58	4.32	4.96	<i>Adamts9</i>	3.79	7.33	3.19	<i>Sh2b2</i>	3.44	4.96	9.6
38	<i>Clec11a</i>	9.43	2.23	1.37	<i>Nek8</i>	4.51	7.31	4.84	<i>Glr3</i>	2.27	4.98	9.5
39	<i>Eif3j</i>	9.34	0.33	0.66	<i>Hdc</i>	0.85	7.31	0.71	<i>Des</i>	1.17	1.61	9.33
40	<i>Serinc5</i>	9.13	2.57	2.94	<i>Ngfr</i>	0.67	7.28	3.72	<i>Camk1g</i>	3.22	4.51	9.32
41	<i>Ncoa7</i>	9.03	2.27	2.52	<i>Scara5</i>	1.44	7.15	3.18	<i>Pitpnm1</i>	4.26	4.74	9.26
42	<i>Hoxa5</i>	8.96	2.36	1.97	<i>Pdxk</i>	4.82	7.12	4.5	<i>Gdf1</i>	3.37	3.67	9.1
43	<i>Dlc1</i>	8.89	3.62	3.41	<i>Kctd1</i>	4.39	7.12	3.9	<i>Krtdap</i>	0.62	0.19	9.02
44	<i>E2f8</i>	8.67	2.56	3.61	<i>Cav1</i>	2.19	6.92	3.6	<i>Kcnj15</i>	0.4	0.47	9.02
45	<i>Otor</i>	8.65	3.95	2.44	<i>Tubb3</i>	2.06	6.9	1.24	<i>Ccl4</i>	1.41	4.75	8.88
46	<i>Stil</i>	8.53	3.47	3.27	<i>Arhgap39</i>	4.5	6.89	3.82	<i>Sfn</i>	3.54	1.38	8.86
47	<i>Avpr1a</i>	8.45	1.25	0.8	<i>Lypd6</i>	2.57	6.71	3.31	<i>Sema3e</i>	3.21	3.2	8.8
48	<i>Fndc3c1</i>	8.39	2.26	2.93	<i>Lbh</i>	4.96	6.7	2.52	<i>Myod1</i>	0.46	1.69	8.77

49	<i>Frat1</i>	8.29	2.48	4.36	<i>Slc25a14</i>	3.08	6.7	4.78	<i>Smtnl2</i>	3.1	4.07	8.64
50	<i>Armcx5</i>	8.25	3.75	4.53	<i>Ophn1</i>	4.37	6.68	4.33	<i>Pf4</i>	1.69	3.34	8.57
51	<i>Nol12</i>	8.23	4.59	4.21	<i>Adamts17</i>	3.42	6.67	3.66	<i>Snap91</i>	2.29	1.93	8.43
52	<i>Ptger4</i>	8.16	2.88	2.04	<i>Mterfd3</i>	3.9	6.62	4.78	<i>Dcx</i>	2.93	2.18	8.41
53	<i>Till3</i>	8.13	4.77	4.21	<i>Rev3l</i>	4.38	6.58	4.67	<i>Fam159a</i>	3.68	3.42	8.39
54	<i>Dclre1c</i>	8.02	2.77	4.02	<i>Acsf3</i>	3.84	6.54	3.97	<i>Accs</i>	2.66	3.37	8.32
55	<i>Stkl7b</i>	8	2.3	1.7	<i>Stom</i>	2.8	6.54	4.95	<i>Gata3</i>	3.49	0.84	8.3
56	<i>Hist1h2bn</i>	8	3.32	3.49	<i>Ank2</i>	3.39	6.48	4.86	<i>Myl1</i>	1.83	2.24	8.28
57	<i>Omd</i>	7.96	3.38	3.72	<i>Bcl2l11</i>	3.94	6.42	4.94	<i>Slc25a23</i>	2.77	4.17	8.13
58	<i>Pcdh1</i>	7.9	0.67	2.57	<i>Ankrd44</i>	2.78	6.42	3.49	<i>Tcea3</i>	1.68	4.53	8.08
59	<i>Npat</i>	7.88	4.84	4.74	<i>Nkain1</i>	3.17	6.4	3.93	<i>Doc2g</i>	4.83	4.62	7.9
60	<i>Cbr2</i>	7.86	4.12	3.25	<i>Ptges</i>	3.93	6.39	3.44	<i>Spon2</i>	0.21	2.12	7.89
61	<i>Hspg2</i>	7.84	3.74	2.28	<i>Kit</i>	2.35	6.38	2.5	<i>Abcg4</i>	3.87	4.93	7.88
62	<i>Pygl</i>	7.81	3.93	3.2	<i>Mgat3</i>	3.77	6.35	3.81	<i>Adamts14</i>	3.42	2.32	7.81
63	<i>Krtcap3</i>	7.75	2.34	4.88	<i>Fzd10</i>	1.6	6.33	1.17	<i>Krt15</i>	2.78	1.78	7.81
64	<i>Shroom3</i>	7.72	4.68	4.46	<i>Slitrk5</i>	3.45	6.31	3.4	<i>Dleu2</i>	1.11	3.16	7.77
65	<i>Hist1h3g</i>	7.67	4.3	2.68	<i>Ddx23</i>	4.78	6.29	4.63	<i>Cntfr</i>	2.19	1.1	7.71
66	<i>Scd1</i>	7.62	3.42	1.66	<i>Camk2g</i>	4.35	6.26	4.27	<i>Phactr1</i>	1.35	1.55	7.69
67	<i>Ung</i>	7.59	3.32	2.78	<i>Fap</i>	1.5	6.26	3.85	<i>Fbp2</i>	4.11	1.92	7.65
68	<i>Nppc</i>	7.59	3.69	3.88	<i>Rhobtb2</i>	4.82	6.25	4.68	<i>Ano1</i>	2.91	2.32	7.62
69	<i>Baiap2l1</i>	7.58	2.65	2.78	<i>Tgfb3</i>	4.87	6.22	3.44	<i>Ehbp1l1</i>	3.16	3.34	7.59
70	<i>Tgfa</i>	7.57	2.66	1.99	<i>Atxn7l1</i>	4.59	6.19	3.89	<i>Uap1l1</i>	3.42	4.25	7.53
71	<i>Adamts3</i>	7.53	4.01	2.9	<i>Wbp7</i>	4.39	6.12	4.97	<i>Wdr67</i>	4.62	4.69	7.41
72	<i>Prkg2</i>	7.48	1.73	1.22	<i>Iqgap2</i>	4.99	6.1	4.34	<i>Tmem117</i>	1.95	1.4	7.39
73	<i>Orc1</i>	7.4	3.33	3.57	<i>Sdpr</i>	2.35	6.09	3.2	<i>Dynl13</i>	4.2	4.23	7.38
74	<i>Aldoc</i>	7.38	3.34	4.4	<i>Zfp322a</i>	4.84	6.06	4.9	<i>Tle6</i>	3.26	4.42	7.36
75	<i>Foxf1a</i>	7.37	0.58	1.26	<i>Slc2a4</i>	3.46	6.06	2.19	<i>Ramp1</i>	0.56	0.62	7.33
76	<i>Cenpk</i>	7.36	4.04	4.36	<i>Pear1</i>	4.79	6.04	3.57	<i>Rxrg</i>	4.78	2.31	7.22
77	<i>Fam180a</i>	7.35	1.15	2.41	<i>Mtss1</i>	3.04	6.03	4.92	<i>St7l</i>	4.32	4.72	7.18
78	<i>Tgm2</i>	7.34	3.85	3.84	<i>Traf5</i>	4.46	6.01	4.74	<i>Wnt9a</i>	3.13	3.77	7.17
79	<i>Esco2</i>	7.34	4.01	4.35	<i>Fgf10</i>	2.45	6	2.21	<i>Tesk2</i>	4.7	4.25	7.16
80	<i>Hip1r</i>	7.33	3.6	4.68	<i>Gdf11</i>	3.4	5.98	4.88	<i>Coq10a</i>	4.14	4.78	7.16
81	<i>Slc19a2</i>	7.32	4.28	4.71	<i>Txnrd2</i>	3.51	5.95	3.92	<i>Fam20c</i>	3.93	3.24	7.16
82	<i>Cenpf</i>	7.32	3.48	4.93	<i>Tspan11</i>	1.58	5.95	3.57	<i>Rarres2</i>	3.09	3.66	7.16
83	<i>Pdlim1</i>	7.29	3.41	2.27	<i>Kpna6</i>	4.89	5.94	4.26	<i>Pip4k2a</i>	3.77	3.38	7.12
84	<i>Per2</i>	7.28	2.93	4.73	<i>Fert2</i>	4.55	5.9	4.66	<i>Dmrt2</i>	1.31	2.73	7.06
85	<i>Ascc3</i>	7.27	4.03	4.96	<i>Zfp397</i>	4.26	5.9	4.14	<i>Nt5e</i>	2.7	1.32	7.02
86	<i>St6galnac2</i>	7.25	4.83	3.19	<i>Smcr8</i>	4.69	5.89	3.4	<i>Tagln</i>	3.33	2.95	7.01
87	<i>Scn1b</i>	7.22	2.19	2.08	<i>Gng8</i>	3.13	5.88	3.46	<i>Coll3a1</i>	0.75	4.35	6.98
88	<i>Grem1</i>	7.16	1.11	2.71	<i>Sall1</i>	2.8	5.88	2.77	<i>Aard</i>	3.16	3.38	6.97
89	<i>Nptx2</i>	7.12	1.28	0.73	<i>Nlrx1</i>	2.62	5.87	4.8	<i>Tesc</i>	1.57	0.78	6.95
90	<i>Usp2</i>	6.93	4.18	3.64	<i>Prmt10</i>	4.21	5.86	4.73	<i>Shq1</i>	4.9	4.87	6.91
91	<i>Gpd2</i>	6.93	4.65	4.22	<i>Tcf7</i>	2.91	5.86	3.27	<i>Ell3</i>	3.03	2.34	6.91
92	<i>Snhg4</i>	6.91	3.3	2.85	<i>Slc22a5</i>	3.73	5.84	4.4	<i>Clqa</i>	0.31	0.86	6.91
93	<i>Isl1</i>	6.86	0.14	0.02	<i>Ptch2</i>	2.46	5.83	1.86	<i>Tmem175</i>	4.76	4.96	6.87
94	<i>Lingo1</i>	6.78	3.68	2.82	<i>Prr5l</i>	1.38	5.83	1.75	<i>Dner</i>	0.57	1.67	6.83
95	<i>Pcp4</i>	6.75	2.28	1.82	<i>Tyk2</i>	4.38	5.79	4.23	<i>Apba2</i>	3.22	3.57	6.81
96	<i>Fam84b</i>	6.75	2.9	3.26	<i>Cdc42bpa</i>	4.25	5.75	4.38	<i>Ppp1r3g</i>	3.54	1.43	6.79
97	<i>Dlx3</i>	6.69	3.11	1.26	<i>Srd5a1</i>	4.44	5.73	4.28	<i>Dcaf6</i>	3.38	3.82	6.78
98	<i>Rfx1</i>	6.68	4.95	3.69	<i>Ccdc28a</i>	3.3	5.73	3.59	<i>Igsf11</i>	2.7	1.46	6.77
99	<i>Arl4c</i>	6.67	2.97	3.03	<i>Penx</i>	4.42	5.72	4.81	<i>Krt14</i>	4.43	0.54	6.76
100	<i>Foxn2</i>	6.64	3.72	4.97	<i>Hnrnpul2</i>	4.27	5.69	3.96	<i>Fbp1</i>	1.5	1.46	6.76

Table S2. Transcription factors expressed in specific cell population (Bulk transcriptome of digit joint)

Gene	e13.5 Sox9+	e14.5 Lgr5- (FPKM)	e14.5 Lgr5+
Transcription factors expressed in Lgr5+ only			
<i>Glis1</i>	4.33	3.54	52.57
<i>Msc</i>	2.18	1.28	22.79
<i>Barx2</i>	1.27	0.25	20.41
<i>Pknox2</i>	3.74	1.42	19.37
<i>Gata3</i>	3.49	0.84	8.3
<i>Tcea3</i>	1.68	4.53	8.08
<i>Rxrg</i>	4.78	2.31	7.22
<i>Dmrt2</i>	1.31	2.73	7.06
<i>Rorc</i>	1.03	0.62	6.02
<i>Pitx3</i>	0.79	1.01	5.71
Transcription factors expressed in Lgr5- only			
<i>Foxf2</i>	2.52	20.2	2.85
<i>Dbx2</i>	1.4	8.68	0.74
<i>Lbh</i>	4.96	6.7	2.52
<i>Ptges</i>	3.93	6.39	3.44
<i>Wbp7</i>	4.39	6.12	4.97
<i>Rfx5</i>	4.76	5.54	4
<i>Tcf20</i>	3.21	5.48	3.03
Transcription factors expressed in Sox9+ only			
<i>Foxd1</i>	19.47	3.43	0.71
<i>Hoxc10</i>	17.02	0.21	0.37
<i>Tnfaip3</i>	16.98	4.13	4.71
<i>Foxo6</i>	12.07	4.31	3.36
<i>Tcf15</i>	11.41	4.13	3.25
<i>Foxa3</i>	9.6	2.47	3.36
<i>Hoxa5</i>	8.96	2.36	1.97
<i>Foxf1a</i>	7.37	0.58	1.26
<i>Pdlim1</i>	7.29	3.41	2.27
<i>Per2</i>	7.28	2.93	4.73
<i>Rfx1</i>	6.68	4.95	3.69
<i>Foxn2</i>	6.64	3.72	4.97
<i>Pou3f3</i>	6.58	1.83	2.45
<i>Zim1</i>	6.44	1.13	1.16
<i>Per1</i>	6.41	3.16	2.64
<i>Hoxc9</i>	6.32	0.06	0.34
<i>Meox1</i>	6.03	1.63	3.26
<i>Nkx6-2</i>	5.41	4.07	4

Table S3. Transcription factors expressed in all three populations AND Lgr5+ expressed 1.5-fold higher than the other two populations (Bulk transcriptome of digit joint)

Gene	e13.5 Sox9+	e14.5 Lgr5- (FPKM)	e14.5 Lgr5+	Fold change Lgr5+/Lgr5-	Fold change Lgr5+/Sox9+
<i>Irx2</i>	9.97	5.84	57.21	+9.80	+5.74
<i>Six2</i>	50.34	53.86	277.21	+5.15	+5.51
<i>Six1</i>	16.24	9.49	37.33	+3.93	+2.30
<i>Barx1</i>	55.41	23.81	92.04	+3.87	+1.66
<i>Irx1</i>	15.6	11.76	43.98	+3.74	+2.82
<i>Sox5</i>	21.32	10.02	35.08	+3.50	+1.65
<i>Trps1</i>	15.71	12.68	33.33	+2.63	+2.12
<i>Erg</i>	10.34	9.99	25.21	+2.52	+2.44
<i>Scx</i>	42.52	80.56	194.54	+2.41	+4.58
<i>Sox4</i>	416.76	318.43	701.26	+2.20	+1.68
<i>Cux1</i>	47.5	33.43	73.42	+2.20	+1.55
<i>Tle4</i>	14.27	11.65	25.58	+2.20	+1.79
<i>Hes1</i>	298.41	284.69	604.86	+2.12	+2.03
<i>Nfatc1</i>	52.48	39.2	80.79	+2.06	+1.54
<i>Creb5</i>	19.1	25.8	49.93	+1.94	+2.61
<i>Gli2</i>	13	19.73	38.16	+1.93	+2.94
<i>Gli3</i>	22.69	32.62	60.75	+1.86	+2.68
<i>Cited1</i>	10.79	11.28	19.14	+1.70	+1.77
<i>Gabpb1</i>	30.91	29.95	50.67	+1.69	+1.64
<i>Ebf4</i>	7.67	11.14	18.09	+1.62	+2.36

Table S4. Genes involved in molecular signaling pathways, cell-matrix contact, ECM degradation and organisation (Bulk transcriptome of E14.5 digit joint)

Gene	E14.5	E14.5	Fold change relative to Lgr5-	Gene	E14.5	E14.5	Fold change relative to Lgr5-
	Lgr5-	Lgr5+			Lgr5-	Lgr5+	
	(FPKM)				(FPKM)		
IHH/PTHrP pathway				Cell surface markers and receptors			
<i>Glis1</i>	3.54	52.57	+∞	<i>Cd9</i>	105	296.73	+2.83
<i>Hhip</i>	4.94	30.22	+∞	<i>Cd200</i>	6.66	14.9	+2.24
<i>Hhipl1</i>	0.92	6.36	+∞	<i>Cd83</i>	22.25	45.54	+2.05
<i>Pthlh</i>	11	50.11	+4.56	<i>Cd47</i>	22.63	37.49	+1.66
<i>Disp1</i>	19.78	74.28	+3.76	<i>Cd52</i>	8.86	2.87	-∞
<i>Cdon</i>	10.93	28.96	+2.65	<i>Cd74</i>	12.69	4.52	-∞
<i>Gli2</i>	19.73	38.16	+1.93	<i>Cd248</i>	46.41	21.55	-2.15
<i>Gli3</i>	32.62	60.75	+1.86	<i>Cd82</i>	10.93	6.35	-1.72
<i>Ihh</i>	45.36	23.54	-1.93	<i>Cdh13</i>	2.97	35.39	+∞
<i>Pth1r</i>	131.09	72.3	-1.81	<i>Igla11</i>	16.04	81.87	+5.10
<i>Gli1</i>	26.77	16.03	-1.67	<i>Iga5</i>	29.83	52.72	+1.77
BMP/TGFβ pathway				<i>Igav</i>	9.39	5.39	+1.37
<i>Chrd11</i>	3.52	8.64	+∞	<i>Iga9</i>	8.32	11.38	+1.05
<i>Chrd12</i>	0.85	14.32	+∞	<i>Igb1</i>	92.38	97.13	+1.05
<i>Inhba</i>	6.76	74.18	+10.97	<i>Igb5</i>	70.15	73.66	-2.02
<i>Gdf5</i>	71.72	614.83	+8.57	<i>Cdh3</i>	33.83	16.78	-1.62
<i>Tgfb1</i>	136.76	863.5	+6.31	<i>Cdh2</i>	21.39	13.23	-1.76
<i>Tgfb2</i>	27.44	120.3	+4.38	<i>Ddr2</i>	15.06	8.55	-1.74
<i>Ltbp4</i>	16.72	36.21	+2.17	<i>Iga6</i>	5.78	5.44	-1.06
<i>Ltbp3</i>	61.32	118.37	+1.93	Enzymes for ECM degradation			
<i>Chrd</i>	5.69	11.43	+2.01	<i>Adamts14</i>	2.32	7.81	+∞
<i>Smad1</i>	32.44	59.33	+1.83	<i>Adamtsl2</i>	6.99	14.82	+2.12
<i>Twsg1</i>	43.31	73.92	+1.71	<i>Timp3</i>	49.55	85.52	+1.73
<i>Gdf10</i>	86.28	32.02	-2.69	<i>Ctsf</i>	24.52	36.84	+1.50
<i>Sostdc1</i>	36.56	13.65	-2.68	<i>Chst10</i>	10.7	6.78	-1.58
<i>Bmp7</i>	52.52	21.81	-2.41	<i>Chst15</i>	7.46	3.17	-∞
<i>Bmper</i>	10.35	5.08	-2.04	<i>Mmp11</i>	124.76	77.61	-1.61
<i>Bmp4</i>	18.37	10.07	-1.82	ECM components			
<i>Tgfb2</i>	33.63	19.82	-1.70	<i>Cilp</i>	0.75	58.03	+∞
WNT pathway				<i>Col22a1</i>	1.93	15.82	+∞
<i>Lgr5</i>	1.36	26.75	+∞	<i>Thbs4</i>	2.94	12.9	+∞
<i>Lgr4</i>	28.27	76.06	+2.69	<i>Spon2</i>	2.12	7.89	+∞
<i>Wnt4</i>	3.52	8.09	+∞	<i>Tgfb1</i>	136.76	863.5	+6.31
<i>Wnt9a</i>	3.77	7.17	+∞	<i>Fmod</i>	24.45	134.56	+5.50
<i>Wnt11</i>	23.2	43.28	+1.87	<i>Col8a1</i>	6.86	22.27	+3.25
<i>Dkk3</i>	37.72	164.84	+4.37	<i>Dcn</i>	35.98	115.28	+3.20
<i>Sfrp2</i>	203.06	450.71	+2.22	<i>Efemp1</i>	12.14	34.69	+2.86
<i>Sfrp1</i>	28.5	44.32	+1.56	<i>Col9a2</i>	216.81	479.99	+2.21
<i>Fzd4</i>	20.58	36.73	+1.78	<i>Ltbp4</i>	16.72	36.21	+2.17
<i>Fzd8</i>	21.71	53.42	+2.46	<i>Col9a3</i>	206.32	440.71	+2.14
<i>Wisp1</i>	5.11	8.68	+1.70	<i>Col6a1</i>	82.63	167.27	+2.02
<i>Tax1bp3</i>	92.74	151.9	+1.64	<i>Ltbp3</i>	61.32	118.37	+1.93
<i>Tle4</i>	11.65	25.58	+2.20	<i>Vcan</i>	31.28	56.73	+1.81
<i>Ebf2</i>	27.59	8.27	-3.34	<i>Wisp1</i>	5.11	8.68	+1.70
<i>Lef1</i>	36.27	12.73	-2.85	<i>Col9a1</i>	184.09	302.35	+1.64
<i>Sostdc1</i>	36.56	13.65	-2.68	<i>Col11a1</i>	104.96	169.86	+1.62
<i>Dkk2</i>	30.03	11.99	-2.50	<i>Col2a1</i>	1400.84	2253.09	+1.61
<i>Nkd2</i>	32.47	14.43	-2.25	<i>Postn</i>	52.87	81.99	+1.55
<i>Nkd1</i>	13.91	6.74	-2.06	<i>Emilin1</i>	159.14	244.21	+1.53
<i>Hic1</i>	78.08	36.77	-2.12	<i>Fbln2</i>	159.5	243.39	+1.53
<i>Nxn</i>	16.76	9.19	-1.82	<i>Prg4</i>	2.36	1.14	not exp. in both
<i>Hmga2</i>	23.57	13.6	-1.73	<i>Comp</i>	15.75	3.9	-∞
<i>Fzd7</i>	19.6	13.08	-1.50	<i>Emilin2</i>	18.61	6.65	-2.80
<i>Gpc2</i>	15.06	10.06	-1.50	<i>Col23a1</i>	25.86	10.96	-2.36
				<i>Matn1</i>	93.79	41.46	-2.26
				<i>Fbn1</i>	26.53	12.34	-2.15
				<i>Col4a1</i>	50.96	28.98	-1.76
				<i>Fbln5</i>	21.76	13.65	-1.59
				<i>Lox</i>	47.87	31.36	-1.53

+∞ and -∞ indicate genes that are not expressed in Lgr5- and Lgr5+ respectively, fold change is not applicable.

Table S5. Details and parameters for single cell transcriptome sequencing and analysis. Related to Figures 4 and 5.

Sequencing and quality check	
Sequencing depth/read length	50Gbp/101bp pair end
Total read-pairs	304 million
Percentage achieving a quality score of Q30 (equivalent to 1 mistake in 1,000 bps)	72%
Mapping rate (to mouse reference mm10)	93.0%
Percentage of valid barcodes	97.7%
Sequencing saturation	77.2%
Fraction of reads in cells and beads	100%
<i>Cells</i>	90.9%
<i>Background beads</i>	9.1%
Transcriptome mapping	
Total number of genes detected in aggregate	18,247
Total number of UMIs detected	46,239,500
Number of reads (R2) per UMI detected	6.6 (=304million/46million)
Total number of cells captured and sequenced	5,649
Average number of gene detected	2,058 (s.d. 637 genes)
Number of UMIs per cell	6,601
Blood cell captured	189
Average number of genes detected in blood cell	216
Non-blood cells	5,460 (=5649-189)
Total number of Lgr5+ cell detected	207
<i>Express normal Lgr5 allele only</i>	94
<i>Express Lgr5-CRE-ERT2 allele only</i>	87
<i>Express both alleles</i>	26
Data analysis	
DEG stringency	<ul style="list-style-type: none"> • FDR cutoff of <0.05. • expression difference at least 20% (defined as $\% \{ \text{turned on in cluster}_i \} - \% \{ \text{turned on in all_other_clusters} \}$)
Clustering of dispersed genes (Heatmap)	<ul style="list-style-type: none"> • Top 5% dispersed gene (genome-wide)
Pseudotime trajectory	<ul style="list-style-type: none"> • at least 50 cells expressed • Negative binomial distribution • based on the genome-wide UMI count data for genes expressing in 10 cells or more

Table S6. DEGs “Cluster 1 vs non-cluster 1” and “Lgr5+ vs Lgr5-” (Single cell transcriptome of E14.5 knee joint). Related to Figure 4

<p>Genelist B (n=27) DEGs (Lgr5+ (cluster 1) vs Lgr5-)</p> <p>Genelist C (n=48) Common</p> <p>Genelist A (n=62) DEGs (Cluster 1 vs non-cluster 1)</p>	<p>Genelist A (n=62) <i>Osr2, Man1a, Nbl1, Angptl2, Dusp1, Egfl6, Gas2, Rbms3, Creb5, Rnd3, Zfp503, Dclk1, F2r, Zfp3611, Igfbp4, 3632451o06rik, Epb41l3, Svl, Tpm1, Palld, Tuba1a, Hmcn1, Cdh11, Hspal1a, Pdgfra, Cpq, Epha7, Foxp1, Loxl2, Csrp1, Dbn1, Col6a1, Col8a2, Vcan, Ddah2, Col6a2, Cdon, Prrx1, Tagln2, Sulf2, Col5a2, Tax1bp3, Zyx, Nrep, Ltbp3, Nfia, Rcan1, Fbln1, Igfbp7, Arhgap31, Marcks11, Kctd12, Hoxa10, Abracl, Marcks, Hmgb3, Timp2, Map1lc3a, Ifitm3, Aes, Trps1, Barx1</i></p>
	<p>Genelist B (n=27) <i>F2r11, Fhod3, Adam33, Tagln, Fst, Khdrbs2, Hspb8, Chst5, Inhba, Tnni2, Etl4, Meox2, Sorcs2, Tlr2, Bmp4, Gfra4, Syt11, Mafb, Ppm11, Pdgfrl, Gpm6b, Fjx1, Tmem119, Pcdh18, Enpp1, Rgcc, Pdgfc</i></p>
	<p>Genelist C (n=48) <i>Lrrc17, Pamr1, Htra1, Cdh13, Phactr1, Aqp1, Tm4sf1, Nupr1, Tcea3, Lrrn1, Sfrp2, Ccdc80, Cntfr, Tgfbi, Ssc5d, Gas1, Pax9, Mkx, Pid1, Map1b, Gdf5, Dkk3, Sulf1, Gm26771, Dusp8, Mylk, Shisa2, Adamts11, Prss23, Krt17, Cited2, Wisp1, Loxl1, Sox4, Lmo1, Akr3, Bcl11a, Scx, Phldb2, Myo1b, Spint2, Fbn2, Postn, Mfap4, Col12a1, Tgfb2, Fn1, Ier3</i></p>

Bold = transcription factors

Table S7. Primers used in this paper

	Forward primer	Reverse primer
Primers for genotyping Lgr5-eGFP-IRES-CreER^{T2} mice		
wildtype	5'-CTGCTCTCTGCTCCCAGTCT-3'	5'-ATACCCCATCCCTTTTGAGC-3'
mutant		5'-GAACTTCAGGGTCAGCTTGC-3'
Primers for genotyping R26R-lacZ mice		
wildtype	5'-AAAGTCGCTCTGAGTTGTTAT-3'	5'-GGAGCGGGAGAAATGGATATG-3'
mutant		5'-GCGAAGAGTTTGTCCCTCAACC-3'
Primers for Quantitative PCR		
<i>Lgr4</i>	5'-CACAACCTGTCTGATCTGCAT-3'	5'-TGGTGCTTATTTTTGTCCCTGT-3'
<i>Lgr5</i>	5'-CACAGCCACTGCGGCGACTT-3'	5'-CAATGGGCGTCTGCCGGGTC-3'
<i>Lgr6</i>	5'-CTCCTTGCTGGACAAGCTGA-3'	5'-TCTCAAAGAGGTGCTCGCAG-3'
<i>Gdf5</i>	5'-AAAGGGAGGTAACAGCAGCG-3'	5'-CCCATCCTTCTCCAAGGCAC-3'
<i>Cd90</i>	5'-TCTCCTGCTCTCAGTCTTGC-3'	5'-TATTCTCATGGCGGCAGTCC-3'
<i>Cd105</i>	5'-TAGCACCTTGTCAGGAAG-3'	5'-CAGTACAGAGGGCAGGACAA-3'
<i>Notch1</i>	5'-TGTGAGTCCAACCCTTGTGT-3'	5'-CGTTGATGTTGGTCTGGCAA-3'
<i>Klf4</i>	5'-AAAAGAACAGCCACCCACACTT-3'	5'-CCCAGTCACAGTGGTAAGGTTTC-3'
<i>Gapdh</i>	5'-TGCACCACCAACTGCTTAG-3'	5'-GATGCAGGGATGATGTTC-3'