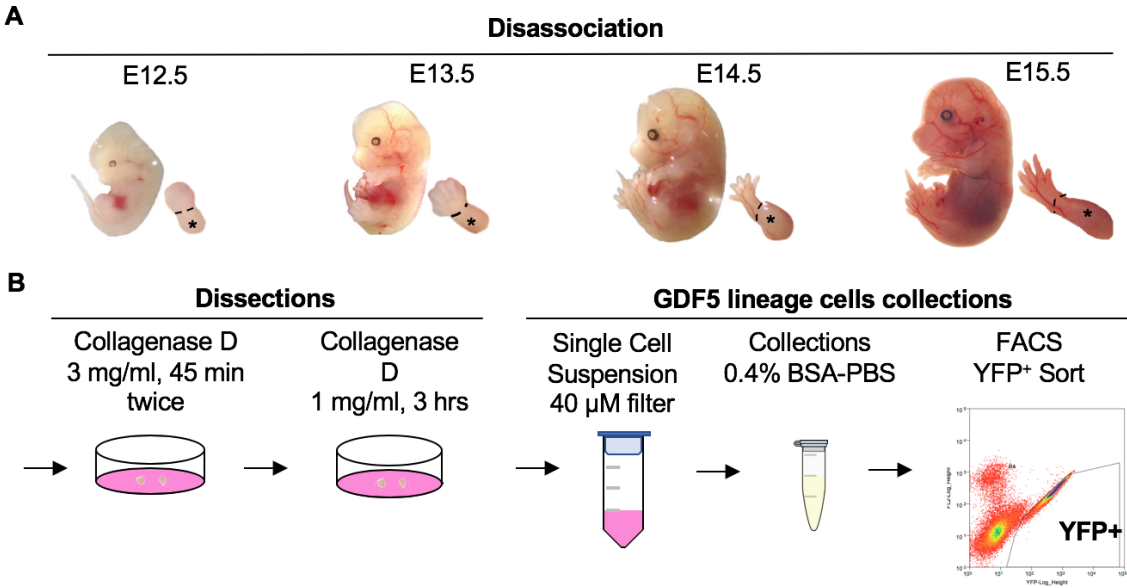


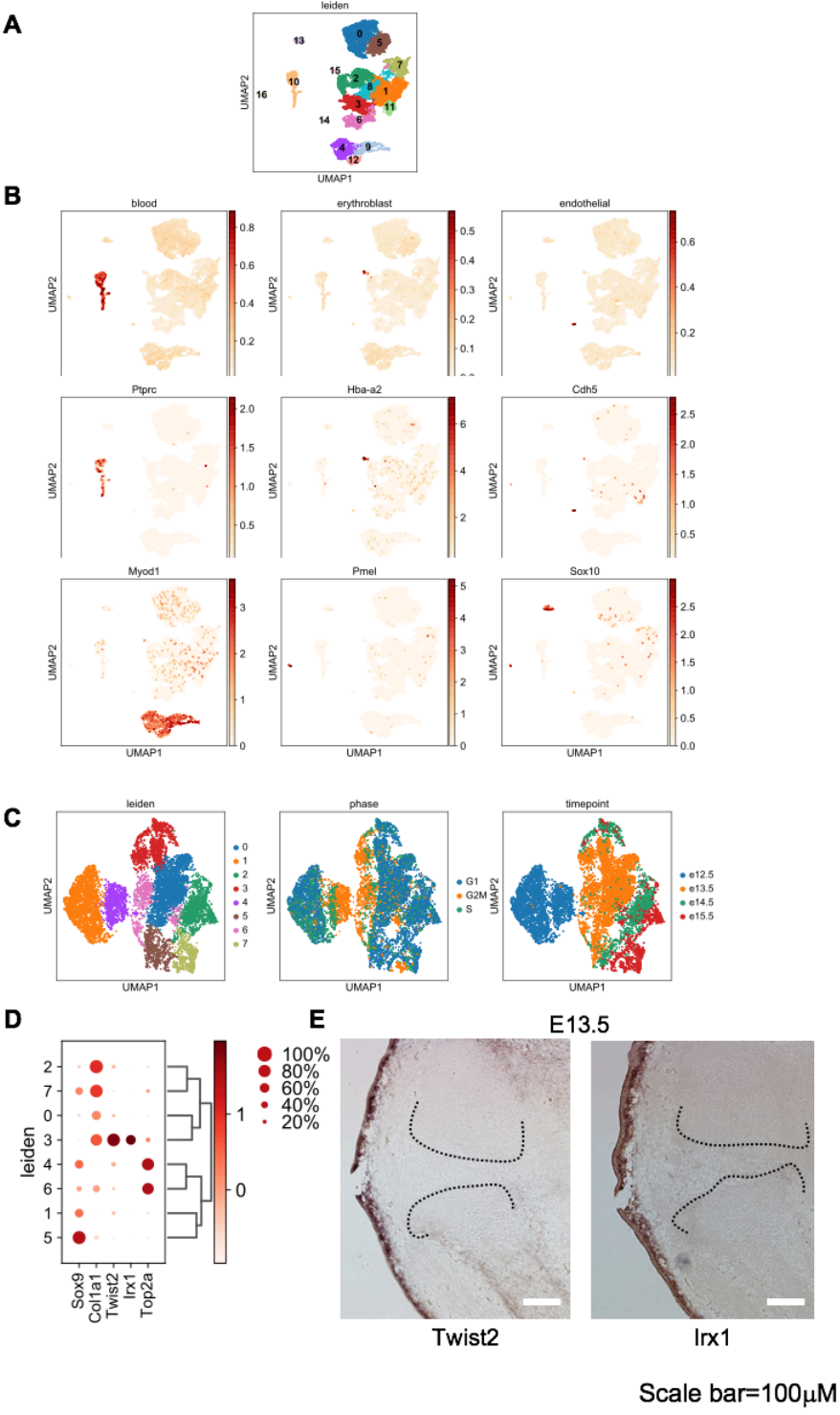
Supplemental Information

Supplemental Figure 1



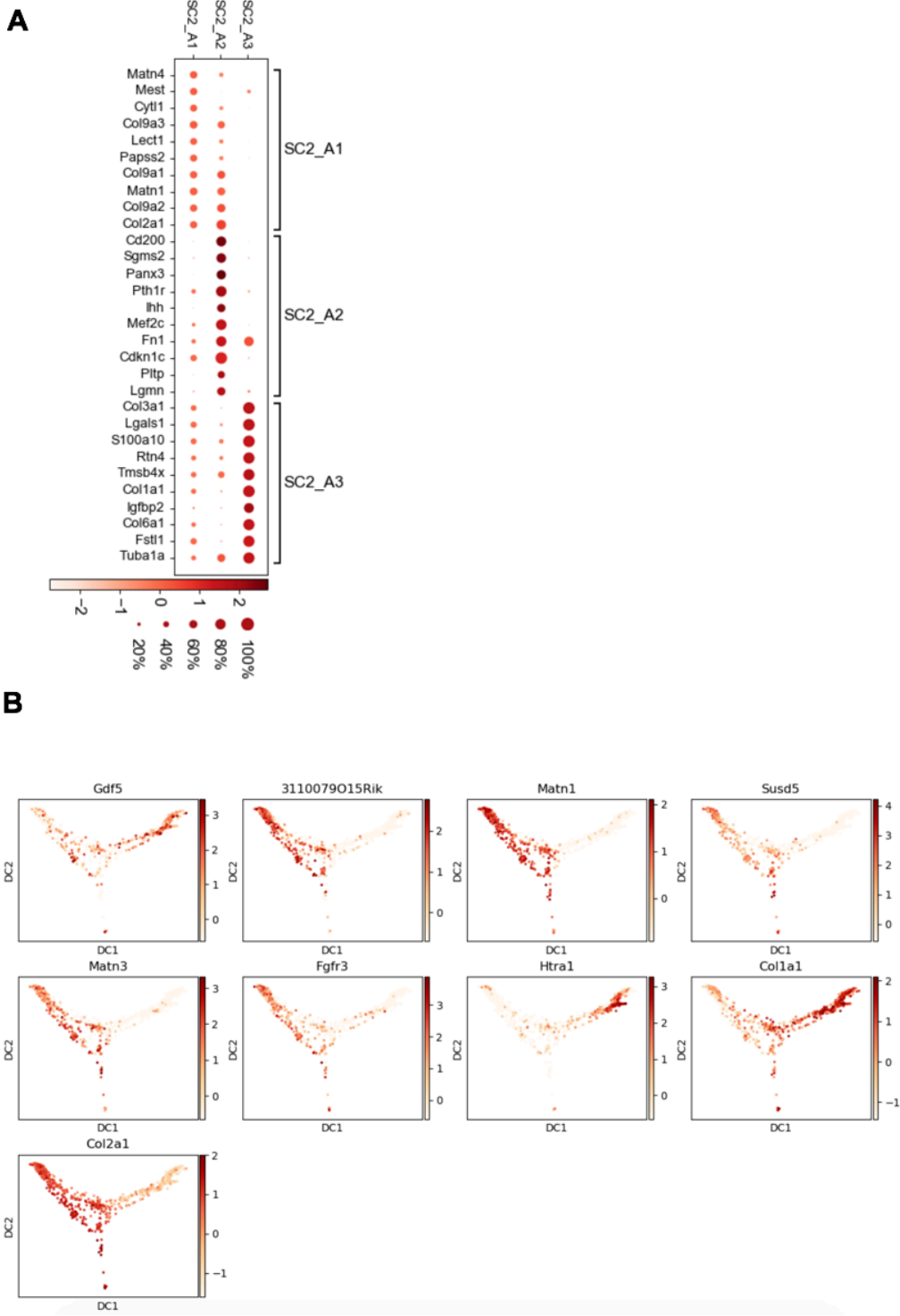
**Supplemental Figure 1:** (A) Developmental stage and region of hind limb dissected for cell isolation. (B) Schematic of single cell dissociation and YFP<sup>+</sup> cell isolation.

**Supplemental Figure 2**



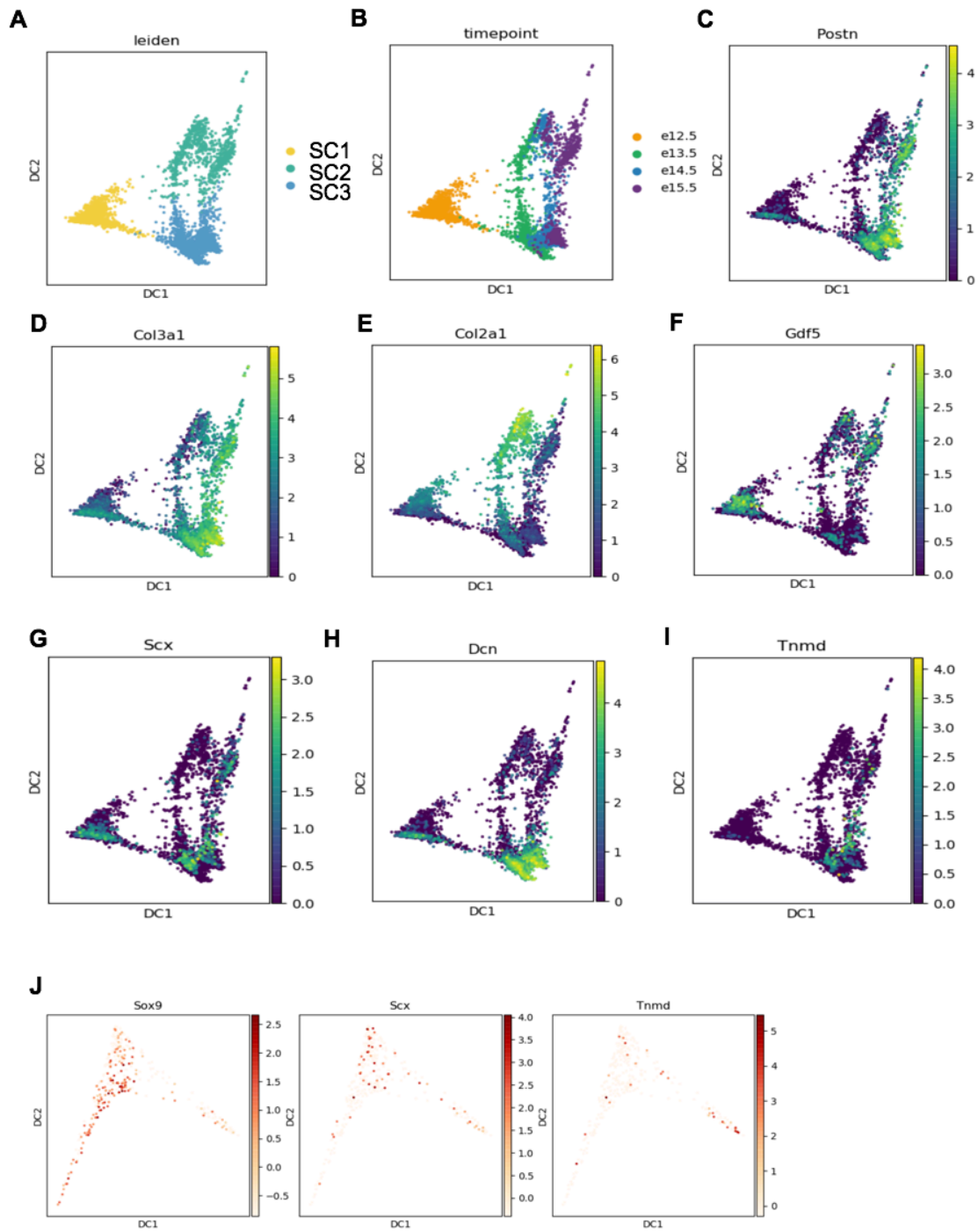
**Supplemental Figure 2:** Initial clustering and identification of non-joint cells and clusters of cells defined by stage of cell cycle.

**Supplementary Figure 3**



**Supplemental Figure 3:** Expression distribution of outer IZ and intermediate IZ representative genes of SC2.

### Supplementary Figure 4



**Supplemental Figure 4:** (A, B) Leiden clustering of SCs, colored by groups (A) and timepoints (B). (C-I) Expression distribution of representative genes on 3 of SCs. (J) Typical tendon/ligament developmental marker genes expression on SC3\_A3 and SC3\_B3 arranged by pseudotime.

Supplemental Table 5

## Reagent Source

REAGENT	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Anit-GFP antibody (Rabbit polyclonal to GFP)	Abcam	Ab6556
Anti-TNMD antibody (Rabbit polyclonal to TNMD)	Abcam	Ab203676
Anti-SOX9 antibody (Rabbit monoclal to SOX9)	Abcam	Ab185966
Anti-THY1 antibody (Rabbit monoclal to THY1)	Abcam	Ab3105
Goat anti-rabbit IgG H&L(Alexa Fluor 488)	Abcam	Ab150077
Goat anti-rabbit IgG secondary antibody, Cy3 conjugate	Boster	BA1032
CD140a (PDGFRA) monoclonal antibody, APC	eBioscience	17-1401-81
CD9 monoclonal antibody, eFluor 450	eBioscience	48-0091-82
<b>Chemicals, enzymes, recombinant proteins</b>		
Antibody Diluent	Agilent Dako	S080981-2
Vectashield Antifade Mounting Medium with DAPI	Vector Laboratories	H-1200
Trichrome Stain (Connective Tissue Stain) kit	Abcam	ab150686
Propidium Iodide	Sigma	P4864
GenElute™ Mammalian Total RNA Miniprep Kit	Sigma Aldrich	RTN70
autoMACS™ Rinsing Solution	Miltenyi Biotec	130-091-222
SuperScript™ III Reverse Transcriptase	Invitrogen	18080044
Collagenase D	Roche	11088858001
Extracta™DNA Prep for PCR	QuantaBio	95091-025
DreamTaq Green PCR Master Mix (2X)	Thermo Scientific™	K1081
iTaq Universal SYBR Green Supermix	BIO-RAD Laboratories	1725121
RNase A	Roche	10109142001

<b>Proteinase K</b>	Sigma-Aldrich	P2308-25MG
<b>DIG RNA Labeling Mix</b>	Roche	11277073910
<b>SP6 RNA Polymerase</b>	Millipore Sigma	10810274001
<b>BCIP 5-bromo-4-chloro-3-indolyl-phosphate, 4-toluidine salt</b>	Millipore Sigma	11585002001
<b>NBT Substrate powder(nitro-blue tetrazolium chloride)</b>	Thermo Scientific™	34035
<b>DNA, MB-grade from fish sperm</b>	Millipore Sigma	11467140001
<b>tRNA from brewer's yeast</b>	Millipore Sigma	10109525001
<b>MEM <math>\alpha</math>, no nucleosides</b>	Gibco™	12561056
<b>Smooth Muscle Cell Differentiation Medium</b>	Sigma-Aldrich	311D-250
<b>Synoviocyte Growth Medium</b>	Sigma-Aldrich	415F-500
<b>Horse Serum</b>	Gibco™	16050114
<b>Recombinant Mouse BMP-6 Protein</b>	R&D	6325-BM-020
<b>Chicken Embryo Extract Powder</b>	Gemini	100-163P
<b>TGF Beta 3</b>	Lonza	PT-4124

### Supplemental Table 6

#### Oligonucleotides for PCR amplification of templates for antisense RNA probes

Gene Symbol	Forward Primer	Reverse Primer	Probe size (bp)
<i>Osr1</i>	AAGCGTCAGAAGTCTAGTTCG	GCTTCTTTTCTGGGGATAGCTT	634
<i>Col2a1</i>	GTCCTACTGGAGTGACTGGTCC	CCAGATTCTCCTTTGTCACCTC	738
<i>Irx1</i>	ATGTCCTTCCCGCAG	TCAGGCAGACGGGAG	1444
<i>Sfrp2</i>	AGCAACTGCAAGCCCATC	ATGGAGAGAAGCCACCCC	803
<i>Twist2</i>	CGCCAGGTACATAGACTTCCTC	GTAAGAAGCAGGAGTATGCGGG	675
<i>Col9a1</i>	AGAGGCCAGATTGATGCG	CATCAAATCCCCGAGCAC	843
<i>Postn</i>	TTTAGAGCAGCCGCCATC	CTGCAGCTTCAAGGAGGC	811
<i>Col3a1</i>	CTCAGGGTATCAAGGGTGAAAG	AGACTTTTCACCTCCAACCTCCA	739
<i>Sox9</i>	ATGAATCTCCTGGACCCC	TCAGGGTCTGGTGAGCTGTG	1532
<i>Gdf5</i>	GCCTTGTTCTAGTGTTTGGTC	CAGCCCCTGTAATGAACATCTC	899