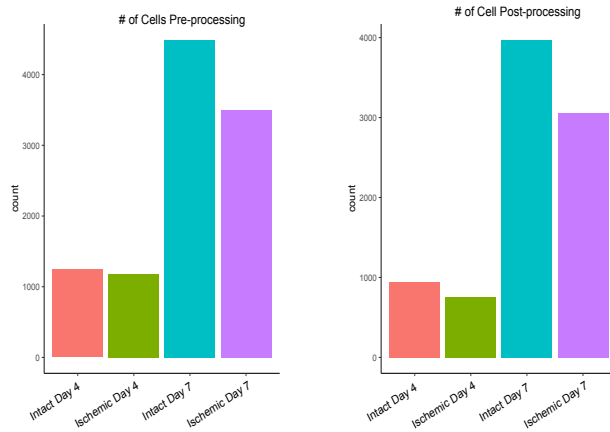
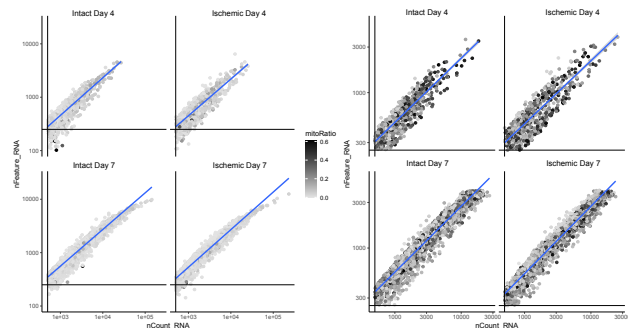


SUPPLEMENTAL FIGURES

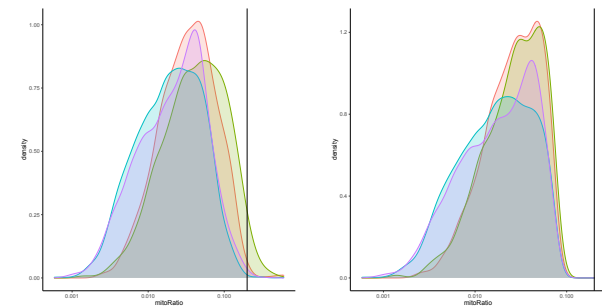
A.



B.



C.

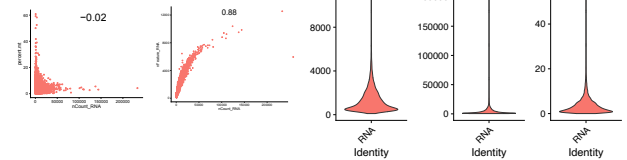
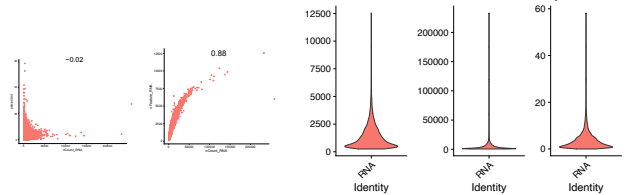
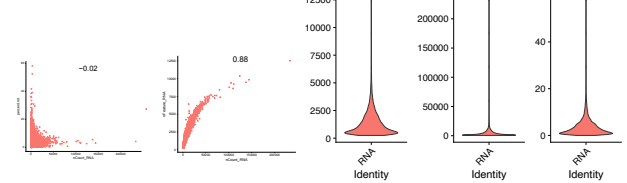
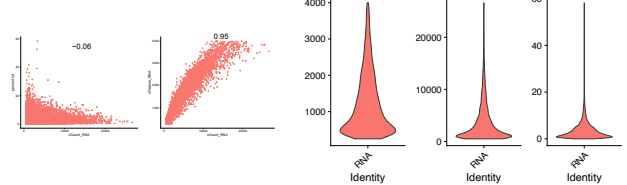
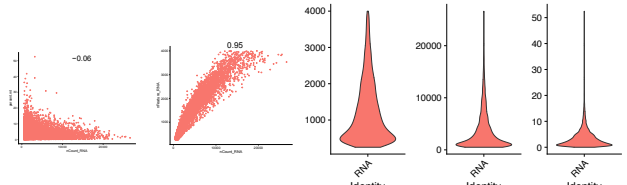
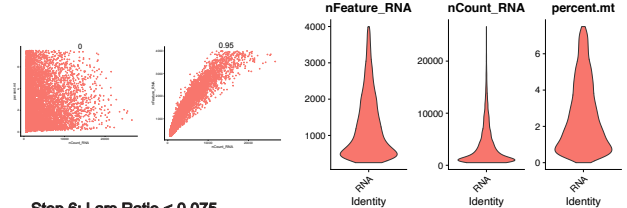
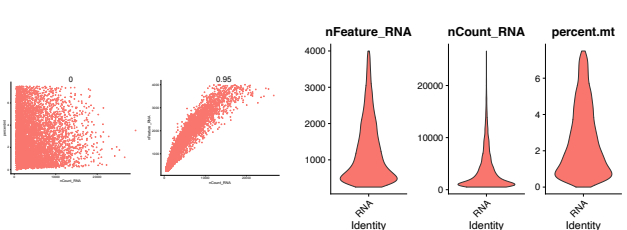


D.

	Pre-processing Step	Sample			
		Intact Day 4	Ischemic Day 4	Intact Day 7	Ischemic Day 7
Step 1	$nFeature \geq 250$	62	50	17	24
Step 2	$nCount \geq 500$	0	0	0	0
Step 3	$nFeature \leq 4000$	4	3	219	91
Step 4	$\log_{10}GenesPerUMI > 0.8$	27	39	6	3
Step 5	$mitoRatio < 7.5\%$	210	328	275	282
Step 6	$larsRatio < 7.5\%$	1	0	3	46
Original Cell #		1244	1178	4491	3501
Total # Cells lost		304	420	520	446
Total % Cells lost		24.4	35.7	11.6	12.7
Total Cells at the end		941	758	3971	3055

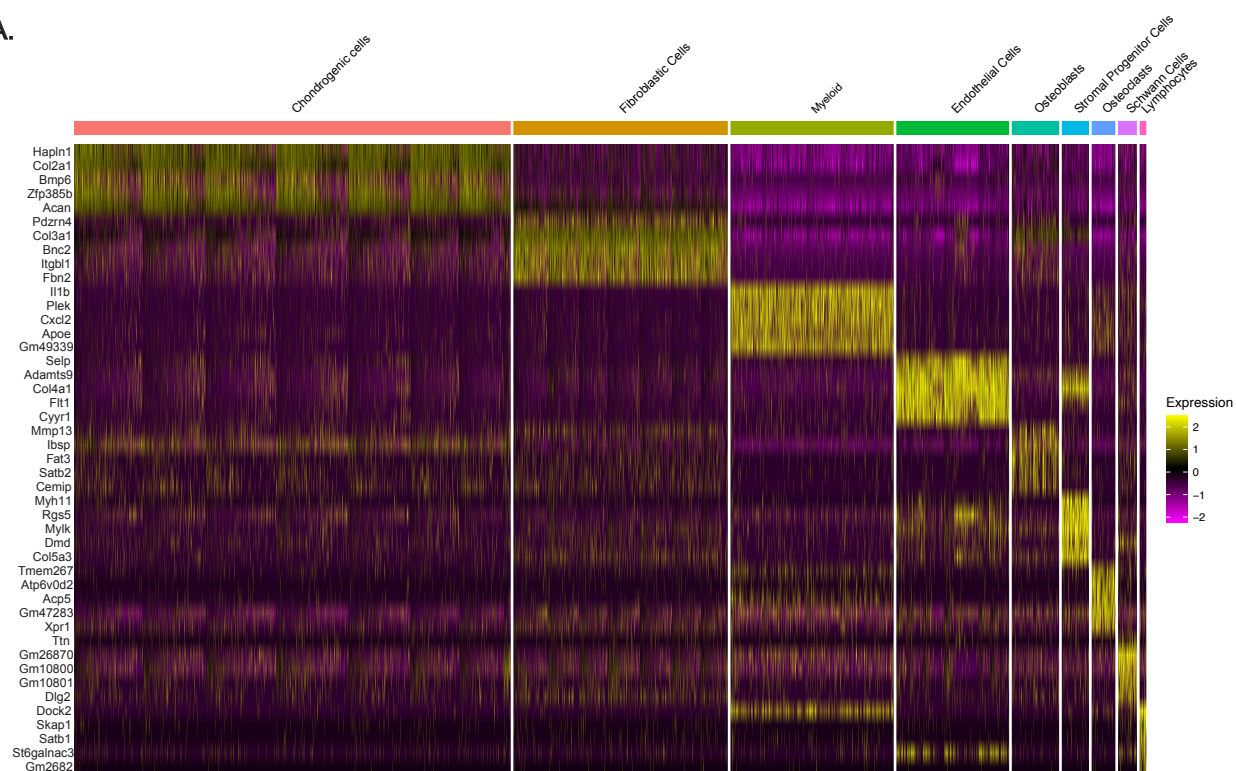
E.

Pre-Processing

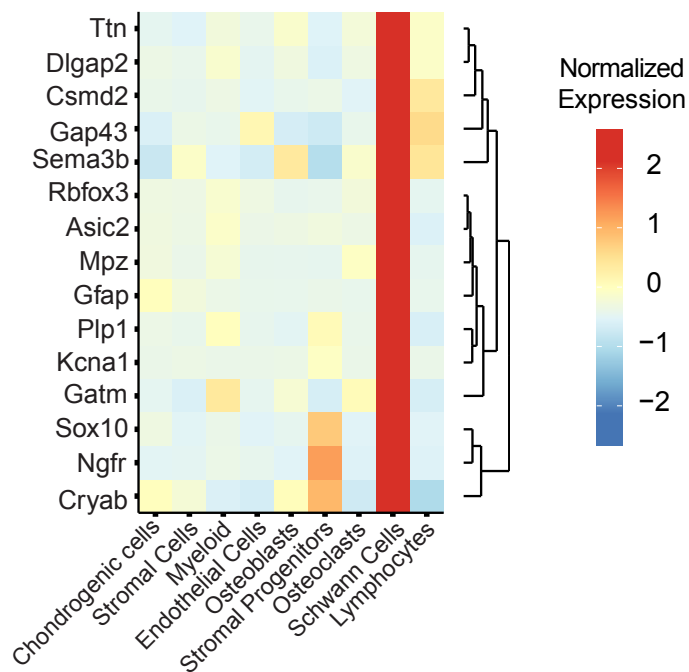
Step 1: $nFeature \geq 250$ Step 2: $nCount \geq 500$ Step 3: $nFeature \leq 4000$ Step 4: $\log_{10}GenesPerUMI > 0.80$ Step 5: $Mito.Ratio < 0.075$ Step 6: $Lars.Ratio < 0.075$ 

Supplemental Figure 1: Quality control and preprocess filtering of scRNAseq dataset. (A) Bar graph of cell numbers before and after quality control pre-process filtering for each condition: Intact Day 4, Ischemic Day 4, Intact Day 7, Ischemic Day 7. (B) Slope of the line and scatter of cells according to nFeature. Cells with comparatively high mitoRatio are darker in color. Line plots demonstrate before and after filtering. (C) Mitochondrial counts ratio of contamination from dead or dying cells before and after removal of cells containing >7.5% mitochondrial genes. (D) Preprocessing workflow and the number of cells lost at each step. (E) Scatterplot and violin plot of nFeature RNA, nCount RNA, and percent mitochondria (percent.mt) at each preprocessing step.

A.

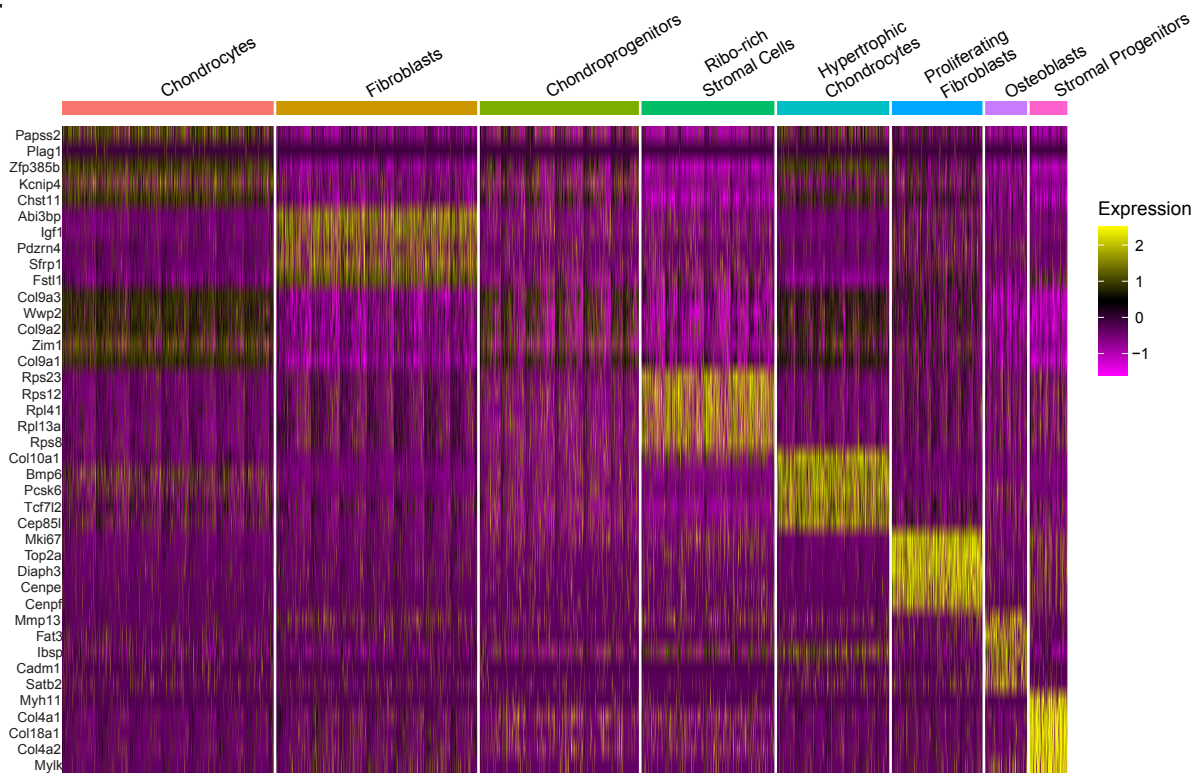


B.

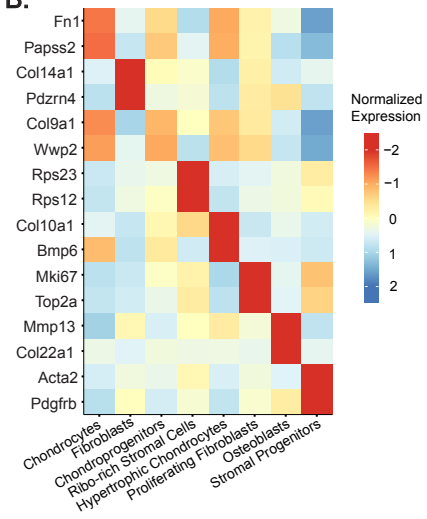


Supplemental Figure 2: Annotation of cell populations and condition-wise breakdown **(A)** Heatmap of the top 5 genes from *FindAllMarkers* output according to log2FC for each cell population from all fracture conditions (Figure 1A). **(B)** Heatmap of known marker genes for Schwann cells. Normalized expression is averaged, log transformed and then plotted.

A.



B.



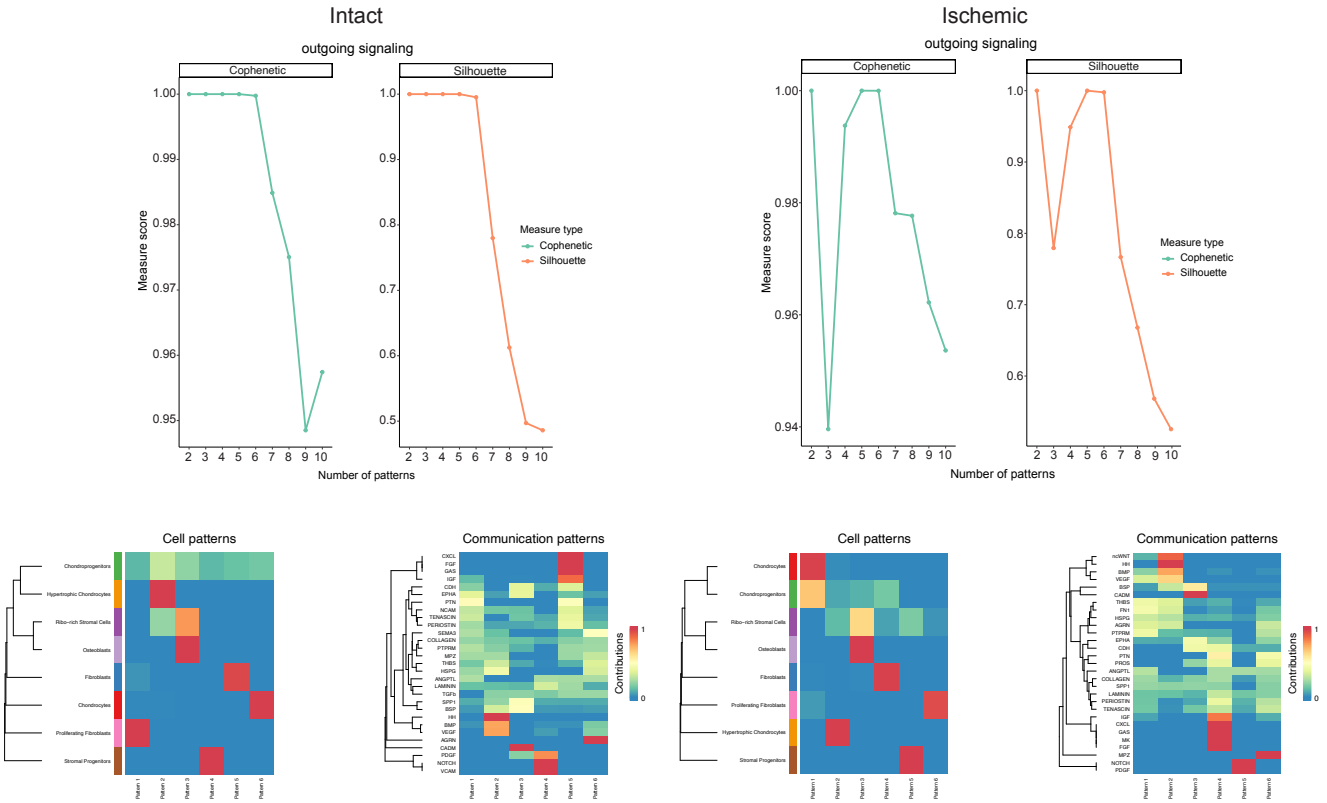
C.



Supplemental Figure 3: Annotation of stromal cell populations (A) Heatmap of top 5 genes from *FindAllMarkers* output according to log2FC for each population in the stromal subset (Figure 4A). (B) Heatmap of normalized expression of thematically selected marker genes for each cluster. Normalized expression is averaged, log transformed and then plotted. (C) Bubble plot depicting gene ontology terms relevant to stromal function (thematically selected) and corresponding log(FDR) and rich ratio for each stromal subset according to PantherDB.

A.

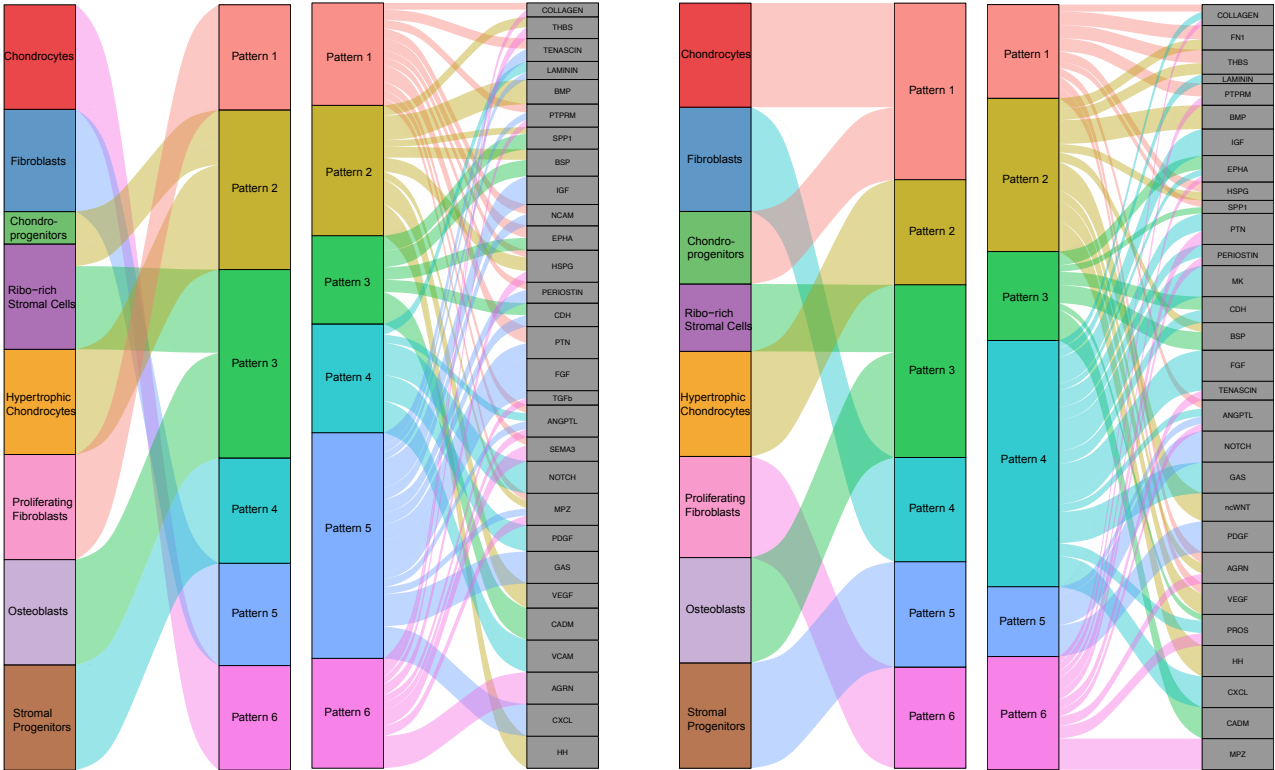
Stromal Outgoing Day 7



B.

Stromal Intact Day 7 Outgoing

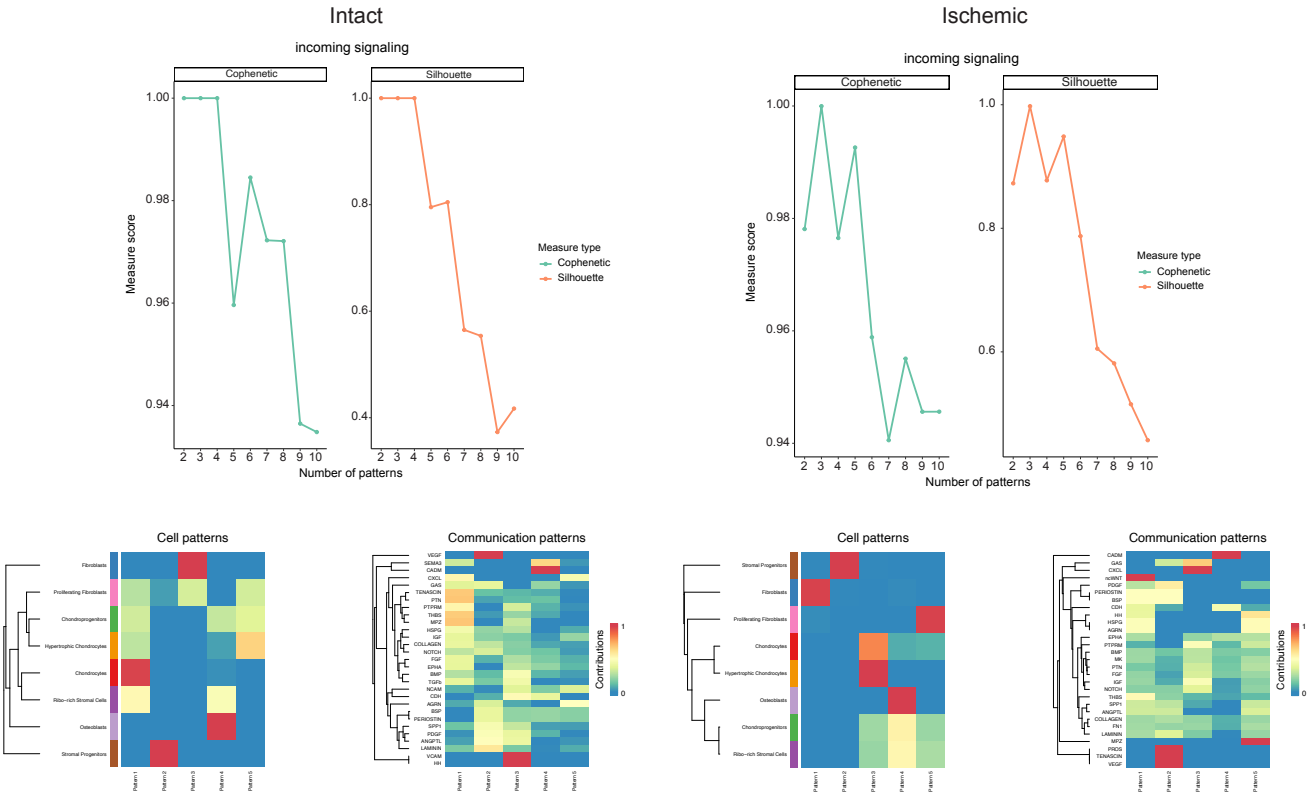
Stromal Ischemic Day 7 Outgoing



Supplemental Figure 4: Outgoing and incoming signaling patterns in the stromal population under intact and ischemic fracture healing conditions at 7 days post fracture. Related to Figure 1. **(A)** Line plots to determine the number of outgoing signaling patterns according to CellChat for intact and ischemic fracture at day 7 post fracture. Corresponding heatmap of signaling pattern for each population. **(B)** River plot from CellChat depicting outgoing signaling patterns by stromal clusters (Figure 1) at Day 7 post fracture.

A.

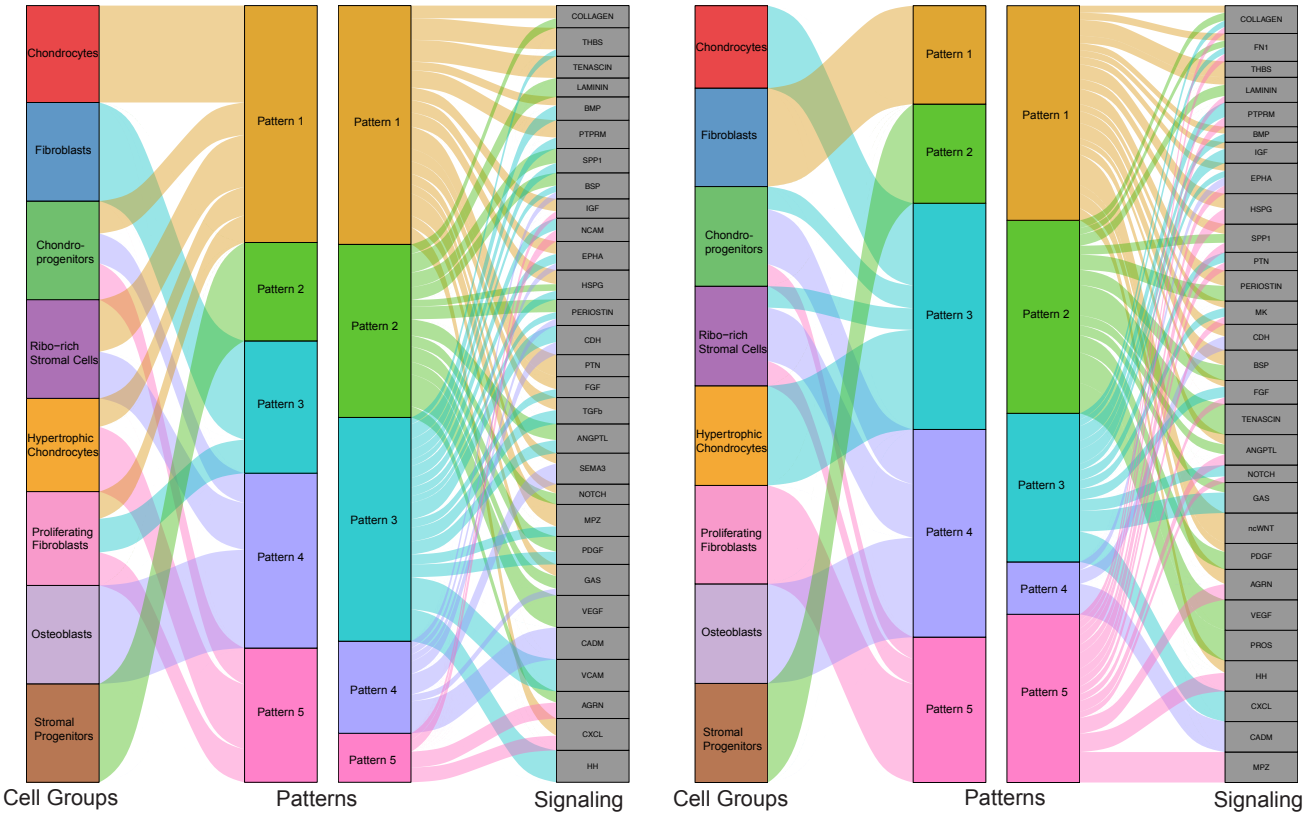
Stromal Incoming Day 7



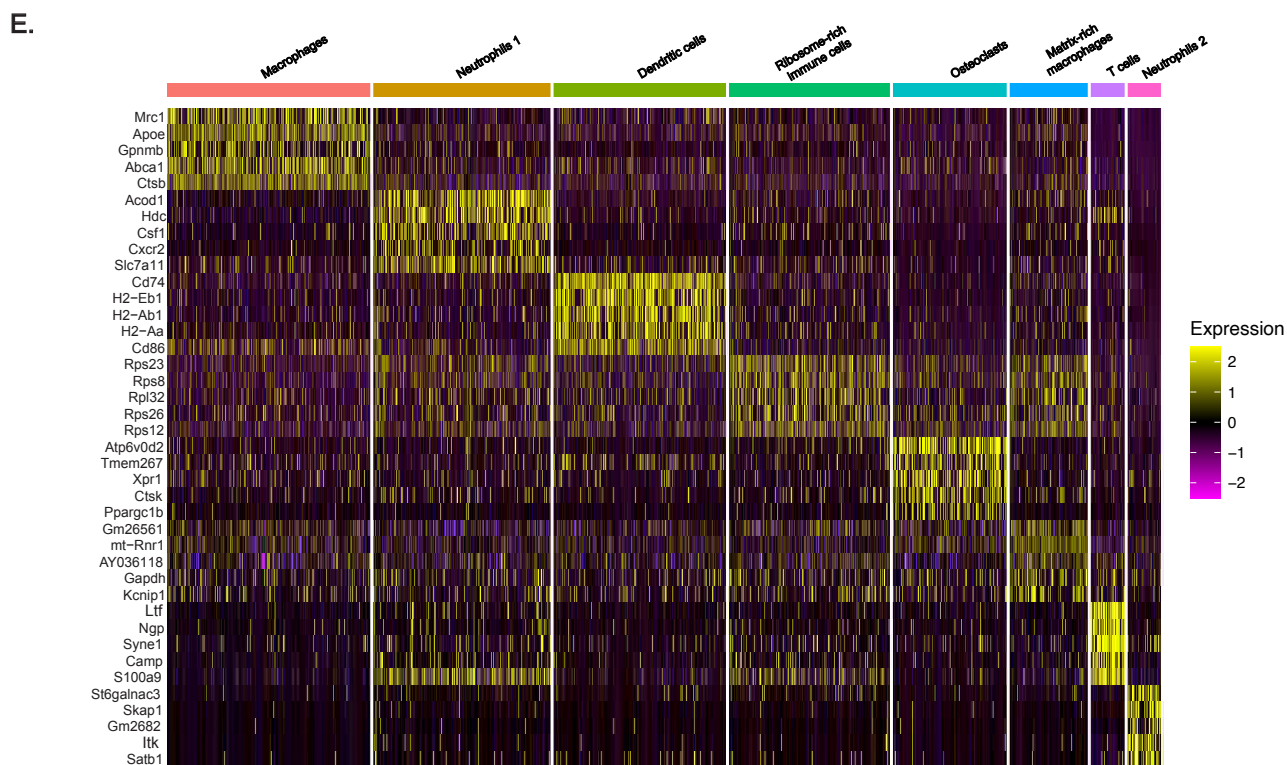
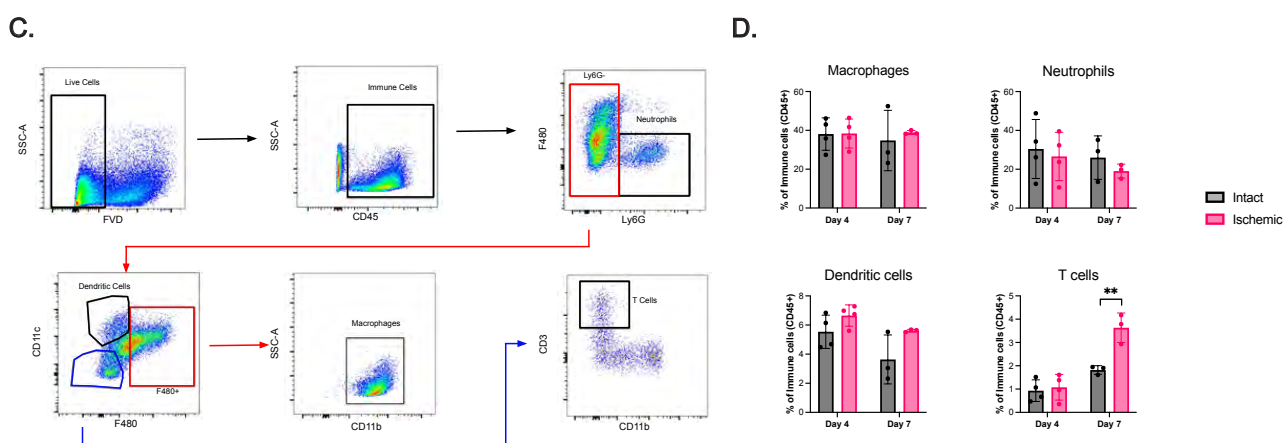
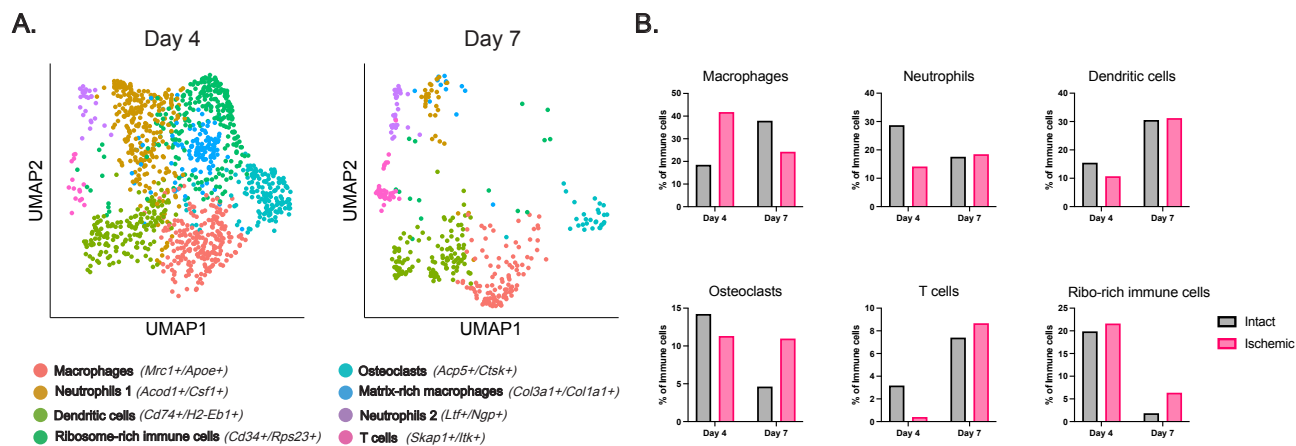
B.

Stromal Intact Day 7 Incoming

Stromal Ischemic Day 7 Incoming

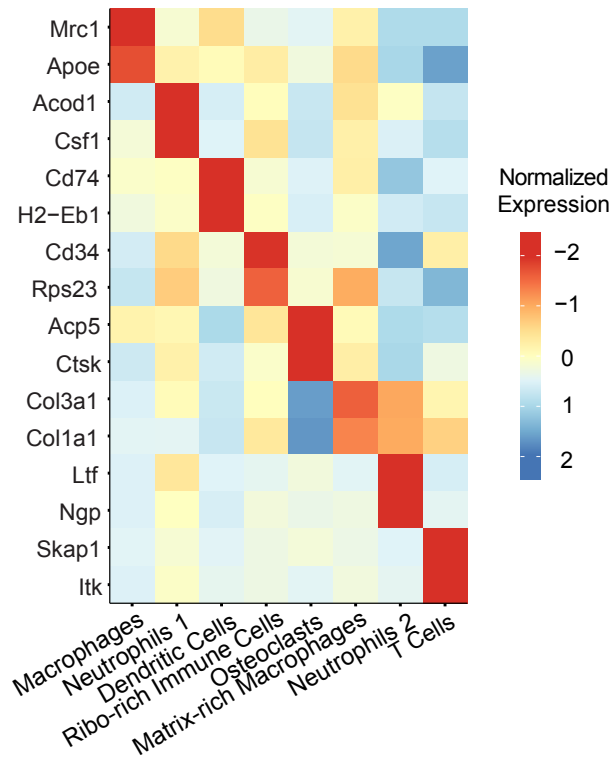


Supplemental Figure 5: Incoming signaling patterns in the stromal population under intact and ischemic fracture healing conditions at 7 days post fracture. Related to Figure 1. **(A)** Line plots to determine the number of incoming signaling patterns according to CellChat for Intact fracture conditions and heatmap of corresponding pattern for each population. **(C)** River plot from CellChat depicting incoming signaling patterns by stromal clusters (Figure 1) at Day 7 post fracture.

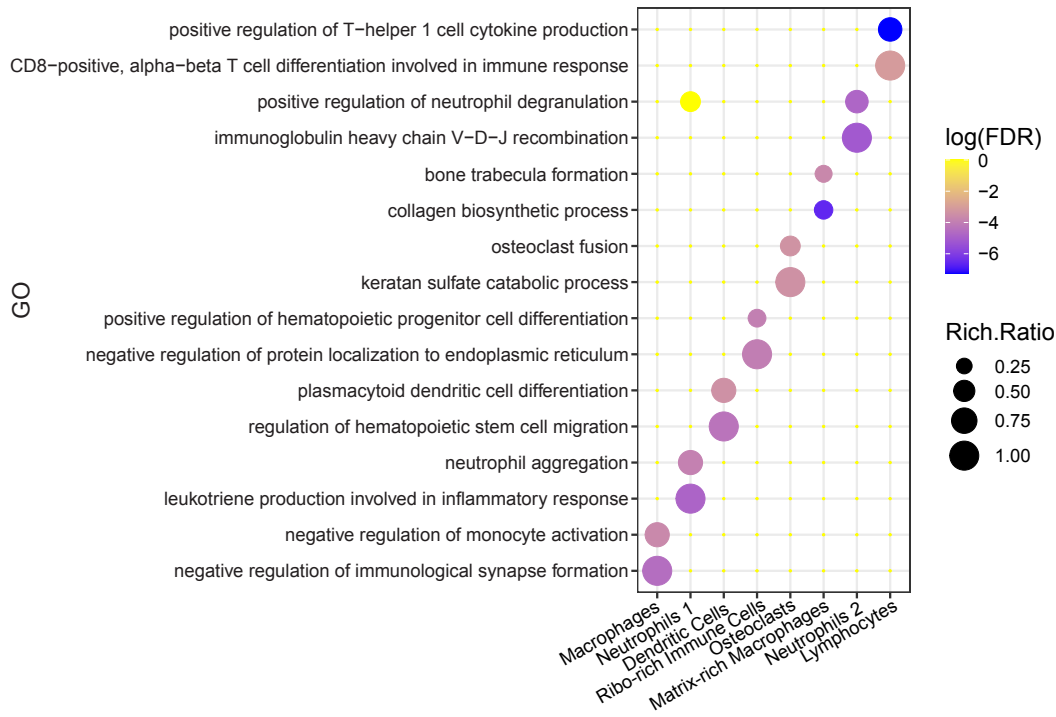


Supplemental Figure 6: Assessment of broad immune populations and discrete distribution across fracture conditions (A) UMAP plots of the immune population at day 4 and 7 post fracture in the intact and ischemic conditions combined. (B) Proportion plots of broad populations of macrophages, neutrophils, dendritic cells, hematopoietic progenitors, and T cells in intact and ischemic conditions at day 4 and 7 post fracture. (C) Overview of flow cytometry gating strategy for immune panel. Gating strategy depicts subsetting live cells, followed by gating on CD45⁺ cells. Ly6G⁺ identified granulocytes; Ly6G⁻/CD11c⁺ identified dendritic cells; Ly6G⁻/F480⁺/CD11b⁺ identified macrophages; Ly6G⁻/CD11c⁻/F480⁻/CD11b⁻/CD3⁺ identified T cells. *P<0.05, multiple two-sided t tests. (D) Bar graphs depicting scRNAseq % breakdown of the immune population for intact and ischemic conditions at day 4 and 7 post fracture. (E) Heatmap of top 5 genes from *FindAllMarkers* output according to log2FC for each population in the immune subset.

A.

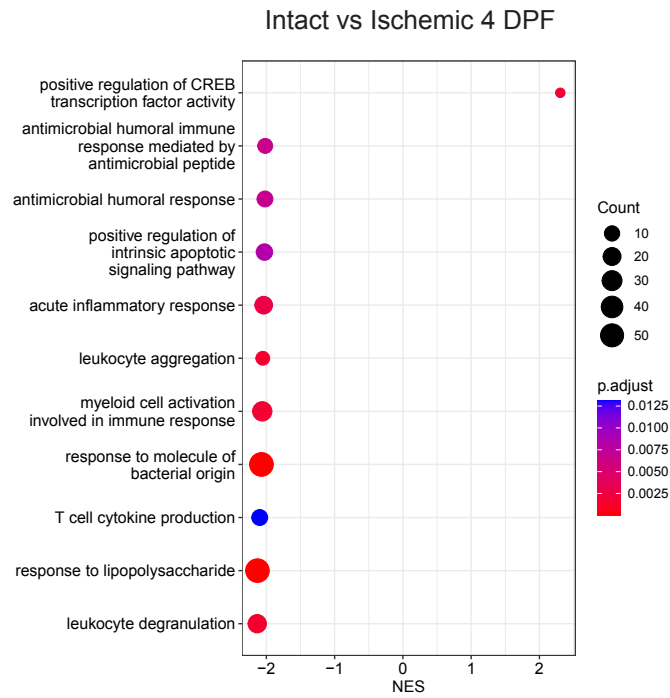


B.

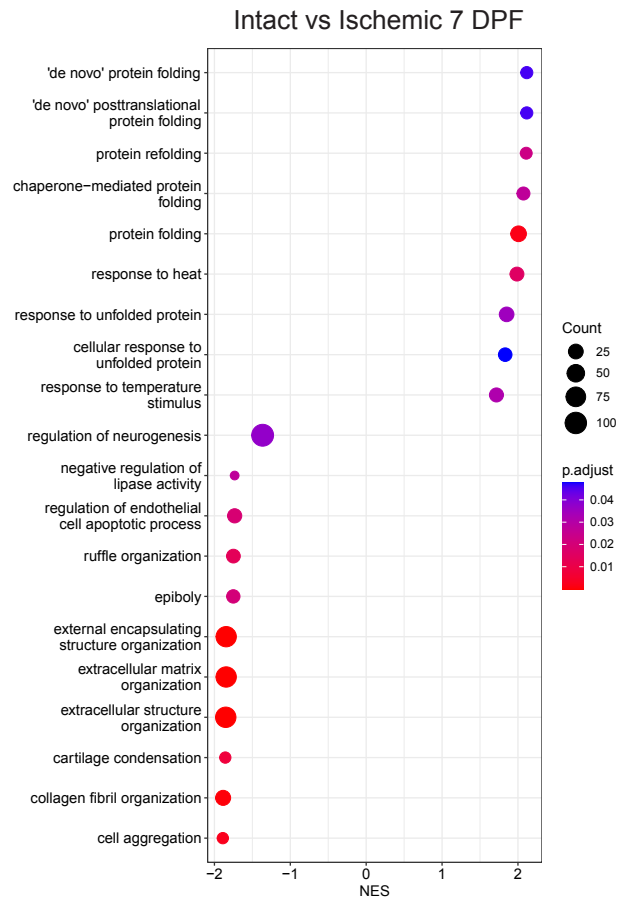


Supplemental Figure 7: Annotation of immune cell populations. (A) Heatmap of normalized expression of marker genes for each cluster. Normalized expression is averaged, log transformed and then plotted. (B) Bubble plot depicting gene ontology terms and corresponding log(FDR) and rich ratio for each stromal subset according to PantherDB.

A.

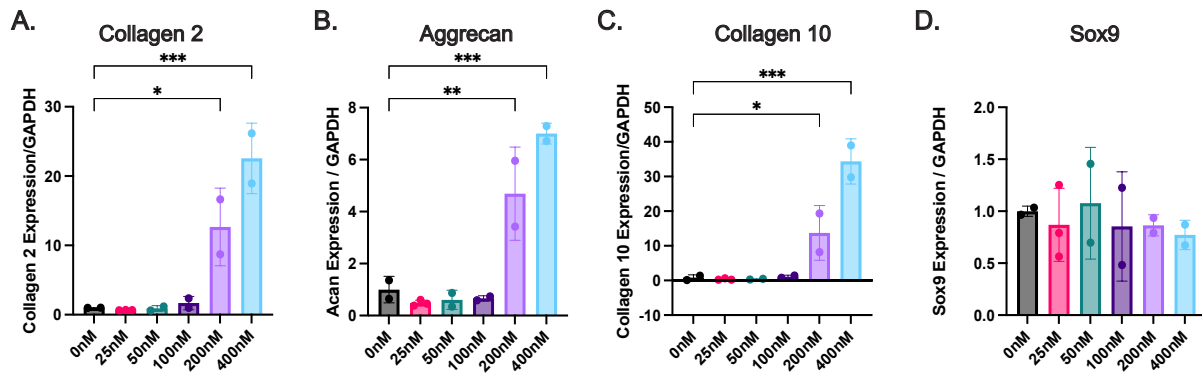


B.



+ terms are upregulated under ischemic conditions

Supplemental Figure 8: Unbiased GSEA of immune population in the Intact and Ischemic callus at 4 and 7 dpf. Unbiased gene set enrichment analysis using the entire gene and output the top gene ontology terms upregulated under Ischemic fracture conditions (+) and the top 10 gene ontology terms for control Intact fracture conditions (-) according to normalized expression scores at **(A)** 4 dpf and **(B)** 7 dpf.



Supplemental Figure 9: SNX631-6 induces increased chondrogenic gene expression in ATDC5 cells.

ATDC5 cells were treated with TGF β +/- SNX631-6 for 10 days. Chondrogenic gene expression of ATDC5 cells treated with 0nM, 25nM, 50nM, 100nM, 200nM, and 400nM of SNX631-6 via RT-qPCR of (A) Collagen 2, (B) Aggrecan, (C) Collagen 10, and (F) Sox9.

SUPPLEMENTAL TABLES

Supplemental Table 1. Area Under the Curve (AUC) Differences in genes along pseudotime (progenitor to hypertrophic chondrocytes, i.e. low to high pseudotime) in Intact and Ischemic conditions

Gene names	Intact	Ischemic	Difference	% Difference
Epas1	8.8	5.5	-3.4	-38.0
Zeb2	16.5	15.3	-1.2	-7.0
Pde5a	3.1	1.9	-1.2	-38.5
Gm14005	6.0	6.2	0.2	3.0
Arhgap6	3.7	1.9	-1.8	-49.6
Pdgfrb	4.9	5.9	1.1	22.0
Abcc9	2.5	1.8	-0.7	-26.7
Dmd	11.4	6.8	-4.6	-40.5
Ebf2	6.3	3.8	-2.5	-40.2
Akap12	4.7	3.4	-1.3	-28.5
Nhs12	4.1	3.1	-1.0	-24.9
Ctnna3	4.7	3.2	-1.5	-31.6
Pde1a	4.5	2.1	-2.5	-54.1
Adamts2	5.9	7.0	1.1	17.9
Gucyl1a2	3.8	2.2	-1.6	-42.4
Mylk	11.6	6.6	-4.9	-42.8
Lama5	0.8	1.0	0.2	32.6
Synpo2	4.7	2.0	-2.6	-56.9
Mcam	3.8	3.3	-0.5	-12.6
Notch3	5.1	2.9	-2.3	-44.4
Kcnab1	3.3	2.3	-0.9	-28.6
Trpc6	1.6	0.8	-0.8	-48.6
Col4a1	29.6	20.3	-9.3	-31.5
Syne2	6.5	5.6	-0.9	-13.3
Acta2	5.7	5.0	-0.7	-12.2
Gm38048	3.6	1.7	-1.9	-52.3
Myocd	1.8	1.2	-0.6	-30.7
Col18a1	14.9	10.2	-4.7	-31.5
Adamts4	5.4	2.1	-3.3	-61.3
Filip1	2.7	1.2	-1.5	-55.1
Col4a2	21.9	14.6	-7.4	-33.6

Sparcl1	11.9	6.8	-5.0	-42.5
Tpm2	4.3	4.9	0.5	12.4
Col5a3	9.7	11.2	1.4	14.8
Ednra	2.1	1.6	-0.5	-24.7
Rgs5	35.3	22.2	-13.1	-37.1
Thy1	2.3	1.5	-0.8	-36.1
Sgip1	2.4	1.0	-1.4	-57.9
Cap2	1.1	0.5	-0.6	-53.1
Dennd2a	1.5	2.4	0.8	54.5
Tagln	7.6	2.9	-4.7	-62.3
Pi15	1.2	1.0	-0.2	-18.7
Lmod1	3.3	0.9	-2.5	-73.9
Ccdc3	1.0	0.5	-0.6	-54.7
Myl9	3.2	1.9	-1.3	-40.4
Myh11	18.1	6.8	-11.3	-62.2
Wwp2	103.7	71.2	-32.5	-31.4
Col9a2	101.3	82.1	-19.2	-19.0
Kcnma1	161.2	122.9	-38.3	-23.8
Col11a1	1167.5	779.4	-388.1	-33.2
Peg3	161.5	151.9	-9.6	-5.9
Edil3	63.7	41.0	-22.6	-35.5
Fn1	1552.6	875.5	-677.1	-43.6
Matn3	93.1	49.8	-43.3	-46.5
Col9a1	186.8	125.1	-61.6	-33.0
Sobp	78.9	47.2	-31.7	-40.2
Chst11	173.0	109.5	-63.5	-36.7
Gpc6	136.3	89.4	-47.0	-34.5
Plagl1	126.0	89.7	-36.3	-28.8
Xylt1	214.5	158.2	-56.4	-26.3
Mir99ahg	199.3	128.5	-70.8	-35.5
Col11a2	161.8	152.7	-9.1	-5.6
Adk	175.6	94.2	-81.3	-46.3
Kif21a	28.5	21.7	-6.7	-23.7
Gramd2	33.2	14.8	-18.4	-55.3
Col6a2	89.8	86.1	-3.7	-4.1
Raph1	121.7	63.7	-58.0	-47.7
Mbnl1	82.2	47.8	-34.4	-41.9
Clint1	65.9	41.7	-24.2	-36.7
Zfp385b	187.8	93.6	-94.1	-50.1
Sox9	40.1	39.3	-0.9	-2.2

Fndc3b	193.7	148.0	-45.7	-23.6
Col2a1	2265.0	1593.0	-672.0	-29.7
Col9a3	138.0	119.9	-18.1	-13.1
Plod2	126.4	71.7	-54.6	-43.2
Papss2	96.3	42.9	-53.4	-55.5
Slc26a2	40.0	43.1	3.2	7.9
Sgms2	49.6	32.9	-16.6	-33.6
Plekhh2	25.0	16.3	-8.7	-34.7
Tgm2	36.4	13.2	-23.2	-63.8
Stk38l	38.3	20.8	-17.4	-45.5
Wnt5b	28.7	17.9	-10.8	-37.6
Vav3	35.8	15.4	-20.4	-57.0
Ppfibp1	42.1	37.2	-5.0	-11.8
Rapgef2	78.6	66.6	-12.1	-15.4
Phtf2	29.1	18.3	-10.9	-37.3
Mef2c	84.8	44.0	-40.8	-48.1
Prkg1	397.4	199.0	-198.4	-49.9
Fam129b	26.4	18.3	-8.1	-30.7
Zbtb20	382.7	179.8	-202.9	-53.0
Nfatc2	197.9	110.5	-87.4	-44.1
Ldlrad3	69.1	51.8	-17.3	-25.1
Sox6	197.0	139.7	-57.2	-29.1
Col27a1	322.2	217.9	-104.2	-32.4
Hspg2	112.5	96.4	-16.1	-14.3
Hapln1	391.1	215.3	-175.8	-44.9
Hip1r	12.8	10.0	-2.8	-21.9
4930523C07Rik	47.4	21.2	-26.1	-55.2
Sdk2	75.0	49.6	-25.4	-33.8
Cpe	49.5	31.9	-17.7	-35.7
Trpv4	33.3	20.3	-13.0	-39.1
Comp	237.3	157.1	-80.2	-33.8
Msi2	209.5	146.6	-62.9	-30.0
Atp10a	57.1	40.9	-16.2	-28.4
Ywhaq	38.2	23.2	-15.0	-39.3
Prkg2	46.9	25.1	-21.8	-46.4
Ugp2	40.0	27.4	-12.7	-31.6
Nfat5	83.2	44.1	-39.1	-47.0
Acan	659.8	471.3	-188.5	-28.6
Serinc5	51.0	31.4	-19.6	-38.4
Tiam2	224.2	156.5	-67.7	-30.2

Pacsin2	33.4	22.0	-11.3	-34.0
Scube1	38.3	19.3	-19.0	-49.5
Smpd3	37.1	24.0	-13.2	-35.5
Gm26917	737.7	807.9	70.2	9.5
Trps1	215.7	123.3	-92.4	-42.8
C430049B03Rik	40.9	26.6	-14.4	-35.1
Usp53	11.5	6.2	-5.3	-46.0
Wnk4	4.6	3.6	-1.1	-23.3
Tcf7l2	50.2	44.8	-5.4	-10.7
Col10a1	62.1	43.2	-19.0	-30.5
Pde11a	6.1	3.8	-2.3	-38.3
Cds1	11.7	7.3	-4.4	-37.4
Slc25a36	9.3	5.5	-3.8	-40.8
Gm4258	18.1	8.6	-9.5	-52.6
Gsk3b	40.9	32.6	-8.3	-20.4
Runx2	75.3	57.2	-18.1	-24.0
Loxl4	25.7	14.2	-11.5	-44.7
Col6a3	92.0	94.1	2.1	2.3
Pcsk6	28.5	22.0	-6.5	-22.9
Crybg3	26.5	20.6	-5.9	-22.3
Alpl	31.4	23.5	-8.0	-25.4
Bmp6	93.8	64.3	-29.5	-31.4
Cep85l	32.8	27.4	-5.4	-16.4
Stk39	48.2	46.8	-1.4	-2.9
Enah	71.9	54.5	-17.4	-24.2
Gm42418	8123.4	8467.5	344.2	4.2
mt-Rnr2	932.4	1030.3	97.9	10.5

Supplemental Table 2. Reagents and Resources

REAGENT or RESOURCE	SOURCE	Catalog ID
Antibodies		
Brilliant Violet 650™ anti-mouse CD45	Biolegend	103151
Brilliant Violet 605™ anti-mouse/human CD11b Antibody	Biolegend	101257
Brilliant Violet 421™ anti-mouse Ly-6G Antibody	Biolegend	127627
PE/Dazzle™ 594 anti-mouse CD11c Antibody	Biolegend	117347
Fixable Viability Dye eFluor™ 660	Fisher	50-112-8848
F4/80 Rat anti-Mouse, APC-R700	Fisher	BDB565787
APC/Fire™ 750 anti-mouse CD3 Antibody	Biolegend	100247
Chemicals, peptides, and recombinant proteins		
Ethiqa XR (Buprenorphine extended-release injectable suspension)	MWI Veterinary Supply	0099114
Bupivacaine Hydrochloride Injection	MWI Veterinary Supply	121952
Collagenase P	Sigma	11249002001
Hyaluronidase	Sigma	H3506
DNase	Sigma	DN25
TRIzol Reagent	Invitrogen	15596018
POWER SYBR Green	Applied Biosystems	4368708
Recombinant TGFb (carrier-free)	Biolegend	580702 781802
Recombinant BMP-2 Protein (carrier-free)	R&D Systems	355-BM-050/CF
Human Fibroblast Growth Factor basic 154	Shenandoah (Fujifilm)	100-146
Gibco Fetal Bovine Serum	Fisher	26140079
Low glucose DMEM	Invitrogen	11995065
High glucose DMEM	Invitrogen	11965092
High glucose DMEM, pyruvate	Invitrogen	11965095
MEM Alpha (1X)	Gibco	12571063
Gibco L-glutamine	Fisher	25030081
L-proline	Sigma	P0380
L-ascorbic acid 2-phosphate sequimagnesium salt hydrate	Sigma	A8960
B-glycerophosphate disodium salt hydrate	Sigma	154804510
ITS+ 1 Liquid Media Supplement (100X)	Sigma	SLCF9930
Antibiotic-antimycotic (100X)	Fisher	15240096
Dexamethasone	Sigma	D4902
Gibco Nonessential Amino acids	Fisher	11140050
Senexin B	Fisher	50-213-4492
SNX631-6	University of South Carolina, Senex Biotech	N/A
Propylene Glycol	Sigma	P4347-4L
Lab Alley PEG 400 Food Grade FCC	Fisher	NC2160118
Alcian Blue 1% in 3% Acetic Acid	Poly Scientific R&D	NC9303153

Electron Microscopy Sciences Nuclear Fast Red Solution	Fisher	26078-05
Alizarin Red S	Sigma	A5533
Wiegert's Iron Hematoxylin A	Electron Microscopy Sciences	50-297-13
Wiegert's Iron Hematoxylin B	Electron Microscopy Sciences	50-297-14
Safranin O	Fisher	LC223408
Fast Green	Fisher	AAA1652006
1 % Acetic Acid	Fisher	A38-500
Papain	Sigma	P4762
L-Cysteine Hydrochloride	Sigma	30120
EDTA	Sigma	ED2SS
Sodium phosphate (disodium anhydrous)	Sigma	S7907
1,9 Dimethylene Blue	Sigma	341088
Ethanol 200 proof	Fisher	04355223
Chondroitin-6-sulfate	Sigma	C4384
Glycine	Sigma	G8898
NaCl	Sigma	S7653
Quant-iT PicoGreen dsDNA Assay Kit	Invitrogen	P7589
10% Neutral Buffered Formalin	Sigma	HT501128
Critical commercial assays		
Single Cell Whole Transcriptome Kit	Parse	20105
High-Capacity cDNA Reverse Transcription Kit	Applied Biosystems	4368814
Biological samples		
Human donor cells	Case Western University; Wagley et al. ⁷⁶ ; Kaur et al. ⁶⁷	N/A
Human donor cells	Texas A & M; Wagley et al. ⁷⁶ ; Kaur et al. ⁶⁷	N/A
Experimental models: Cell lines		
ATDC5 mouse cell line	Sigma	99072806
Experimental models: Organisms/strains		
Mouse: C57 Bl/6J	The Jackson Laboratory	RRID: IMSR_JAX:000664
Oligonucleotides		
Primers, Supplemental Table 2	This paper, ordered from IDT	N/A
Software and algorithms		
ImageJ (FIJI)	Schindelin et al. ⁷⁷	https://imagej.net/software/fiji/downloads
R Studio	R Studio Team	https://posit.co/
R Console v. 4.1.3	R Core Team	

Seurat	Satija et al. ⁶⁹
CellChat	Jin et al. ²⁴
Monocle3	Trapnell et al. ²⁶
PantherDB	Mi et al. ^{71,72}

Supplemental Table 3. Monocle Plotting Parameters

Condition	Intact	Ischemic
num_dim	12	13
k	12	13
resolution	4e-4	2e-3
minimal_branch_len	12	12
euclidean_distance_ratio	1	1
geodesic_distance_ratio	1/3	1/3
rann.k	NULL	NULL
ncenter	350	320

Supplemental Table 4. Exclusion Criteria for μ CT and Histologic Analysis Studies

Intact vs Ischemic Day 7 Analysis				
	Intact		Ischemic	
Starting Sample Size	8		8	
Comminuted Fracture	0		0	
Partial Fracture	0		0	
Improper Pin Removal	0		0	
Morbidity Requiring Euthanasia	0		0	
Ending Sample Size	8		8	
Single Cell RNA Sequencing Analysis				
	Intact Day 4	Intact Day 7	Ischemic Day 4	Ischemic Day 7
Starting Sample Size	8	8	8	8
Comminuted Fracture	0	0	0	0
Partial Fracture	0	0	0	0
Improper Pin Removal	0	0	0	0
Morbidity Requiring Euthanasia	0	0	0	0
Ending Sample Size	8	8	8	8
SNX631-6 vs Vehicle Day 15 Analysis				
	Cohort 1 Vehicle	Cohort 1 SNX631-6	Cohort 2 Vehicle	Cohort 2 SNX631-6
Starting Sample Size	10	10	10	10
Comminuted Fracture	0	0	1	0
Partial Fracture	0	0	0	1
Improper Pin Removal	0	0	0	1
Morbidity Requiring Euthanasia	1	2	1	0
Ending Sample Size	9	8	8	8

Supplemental Table 5. Primer Sequences

Gene Name	5'-3' sequence	Organism
Gapdh Fwd	GCCTCTCTTGCTCAGTGTCC	<i>Mus musculus</i>
Gapdh Rev	CTCCCACTCTTCCACCTTCG	<i>Mus musculus</i>
Col2a1 Fwd	ACTTGCCAAGACCTGAAACTCTG	<i>Mus musculus</i>
Col2a1 Rev	AAACTTTCATGGCGTCCAAGG	<i>Mus musculus</i>
Col10a1 Fwd	TCTCCCAGCACCAGAATCTATC	<i>Mus musculus</i>
Col10a1 Rev	CTTTATGCCTGTGGGCGTTT	<i>Mus musculus</i>
Acan Fwd	CCTGCTACTTCATCGACCCC	<i>Mus musculus</i>
Acan Rev	AGATGCTGTTGACTCGAACCT	<i>Mus musculus</i>
Sox9 Fwd	GTGCAAGCTGGCAAAGTTGA	<i>Mus musculus</i>
Sox9 Rev	TGCTCAGTTCACCGATGTCC	<i>Mus musculus</i>
Runx2 Fwd	CCCAGCCACCTTTACCTACA	<i>Mus musculus</i>
Runx2 Rev	TATGGAGTGCTGCTGGTCTG	<i>Mus musculus</i>
Tgfb1 Fwd	ACGTCACTGGAGTTGTACGG	<i>Mus musculus</i>
Tgfb1 Rev	GGGGCTGATCCCGTTGAT	<i>Mus musculus</i>
GAPDH Fwd	TGGTATCGTGGAAGGACTCATGAC	<i>Homo sapiens</i>
GAPDH Rev	ATGCCAGTGAGCTTCCCGTTCAGC	<i>Homo sapiens</i>
COL2A1 Fwd	CTCCAATGGCAACCCTGGAC	<i>Homo sapiens</i>
COL2A1 Rev	CAGAGGGACCGTCATCTCCA	<i>Homo sapiens</i>

COL10A1 Fwd	ATGCTGCCACAAATACCCTTT	<i>Homo sapiens</i>
COL10A1 Rev	GGTAGTGGGCCTTTTATGCCT	<i>Homo sapiens</i>
ACAN Fwd	ACTCTGGGTTTTTCGTGACTCT	<i>Homo sapiens</i>
ACAN Rev	ACACTCAGCGAGTTGTCATGG	<i>Homo sapiens</i>
SOX9 Fwd	AGCGAACGCACATCAAGAC	<i>Homo sapiens</i>
SOX9 Rev	CTGTAGGCGATCTGTTGGGG	<i>Homo sapiens</i>
CDK8 Fwd	CCAAGAGGAAAGATGGAAGG	<i>Homo sapiens</i>
CDK8 Rev	ATGCCAGAGGTCATGTTCAG	<i>Homo sapiens</i>

Supplemental Table 6: ARRIVE Essential 10: Compliance Questionnaire

	Item	Question(s)	Answers
1	Study Design	Are all experimental and control groups clearly identified?	☒ Yes, for at least one experiment ☐ No
		Is the experimental unit (e.g. an animal, litter or cage of animals) clearly identified?	☒ Yes, for at least one experiment ☐ No
2	Sample Size	Is the exact number of experimental units in each group at the start of the study provided (e.g. in the format 'n=')?	☒ Yes, for at least one experiment ☐ No
		Is the method by which the sample size was chosen explained?	☒ Yes, for at least one experiment ☐ No
3	Inclusion & Exclusion Criteria	Are the criteria used for including and excluding animals, experimental units, or data points provided?	☒ Yes, for at least one experiment ☐ No
		Are any exclusions of animals, experimental units, or data points reported, or is there a statement indicating that there were no exclusions?	☒ Yes, for at least one analysis ☐ No
4	Randomization	Is the method by which experimental units were allocated to control and treatment groups described?	☒ Yes, for at least one experiment ☐ No
5	Blinding	Is it clear whether researchers were aware of, or blinded to, the group allocation at any stage of the experiment or data analysis?	☒ Yes, for at least one experiment ☐ No
6	Outcome Measures	For all experimental outcomes presented, are details provided of exactly what parameter was measured?	☒ Yes, for at least one experiment ☐ No
7	Statistical Methods	Is the statistical approach used to analyze each outcome detailed?	☒ Yes, for at least one analysis ☐ No
		Is there a description of any methods used to assess whether data met statistical assumptions?	☒ Yes, for at least one analysis ☐ No ☐ Not applicable
8	Experimental Animals	Are all species of animal used specified?	☒ Yes, for at least one experiment ☐ No

		Is the sex of the animals specified?	☒ Yes, for at least one experiment ☐ No ☐ Not applicable to the species
		Is at least one of age, weight or developmental stage of the animals specified?	☒ Yes, for at least one experiment ☐ No
9	Experimental Procedures	Are both the timing and frequency with which procedures took place specified?	☒ Yes, for at least one experiment ☐ No
		Are details of acclimatization periods to experimental locations provided?	☒ Yes, for at least one experiment ☐ No
10	Results	Are descriptive statistics for each experimental group provided, with a measure of variability (e.g. mean and SD, or median and range)?	☒ Yes, for at least one experiment ☐ No ☐ Not applicable to the type of analysis used
		Is the effect size and confidence interval provided?	☐ Yes, for at least one experiment ☐ No ☒ Not applicable to the type of analysis used