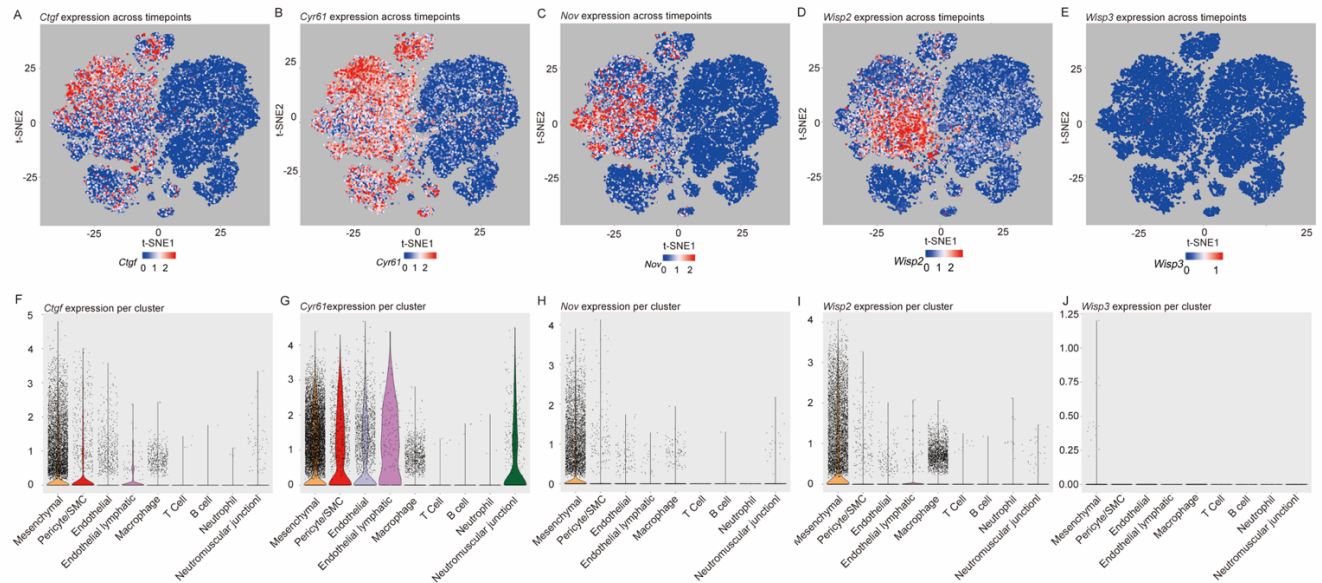
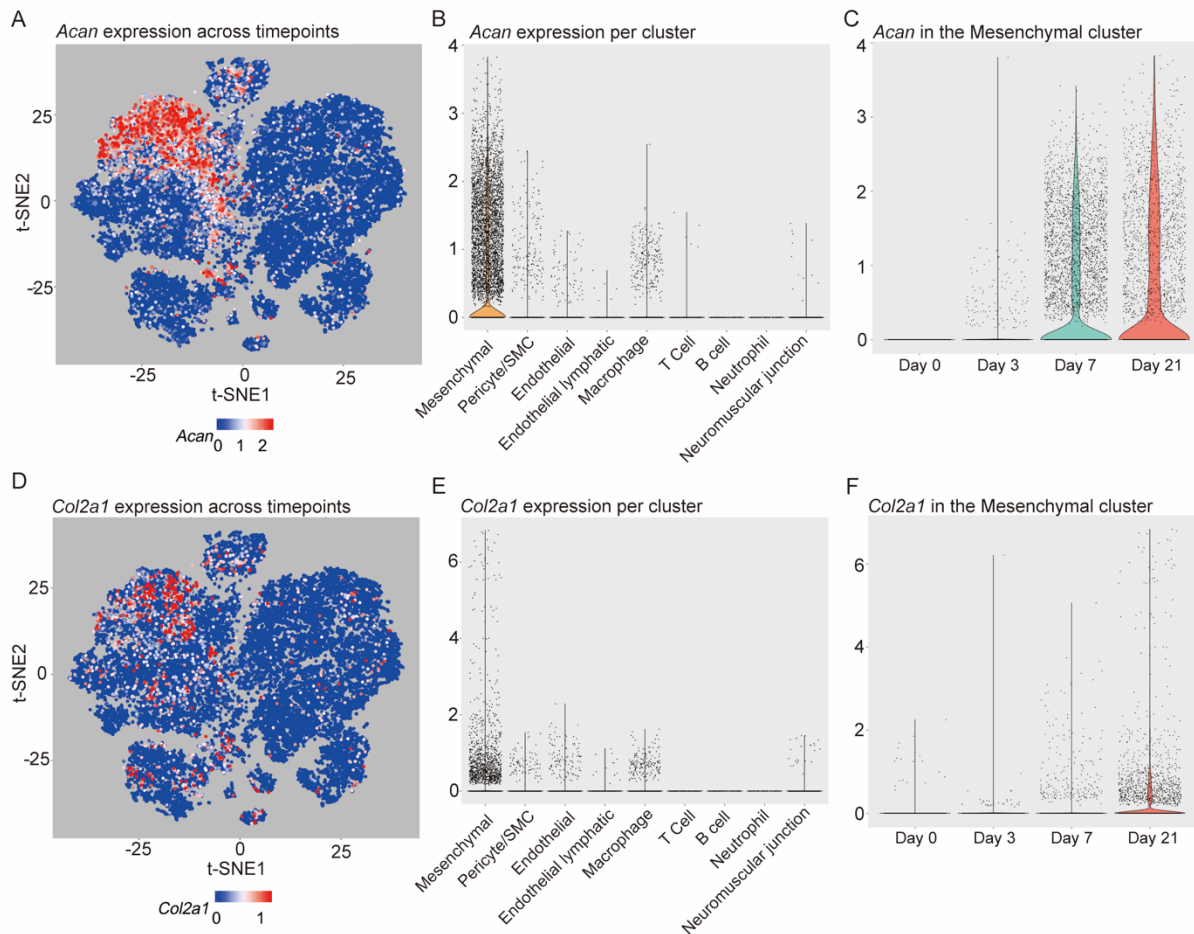


## Supplemental Figure 1.



**Supplemental Figure 1: Single cell RNA sequencing analysis demonstrates CCN family member expression during HO formation.** C57BL/6J mice were subjected to HO induction, and the injury site was microdissected and examined by single cell RNA sequencing up to 21 d thereafter (days 0, 3, 7, and 21 after induction). **(A-E)** t-SNE plots of CCN family members from pooled cells across all time points after HO induction, including *Ctgf*, *Cyr61*, *Nov*, *Wisp2* and *Wisp3*. **(F-J)** Violin plots of CCN family members across each cell cluster. Data represents pooled cells across all timepoints after HO induction. N=3-4 animals per timepoint.

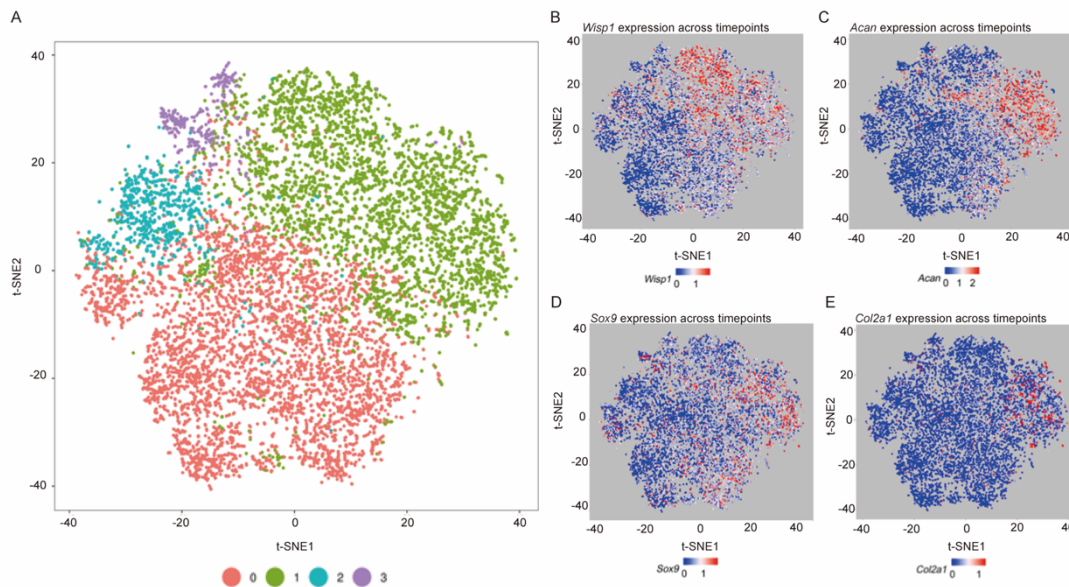
## Supplemental Figure 2



**Supplemental Figure 2: Single cell RNA sequencing analysis demonstrates select chondrocyte associated gene enrichment in mesenchymal cells overtime during HO formation.** C57BL/6J mice were subjected to HO induction, and the injury site was microdissected and examined by single cell RNA sequencing up to 21 d thereafter (days 0, 3, 7, and 21 after induction). **(A-C)** Aggrecan (*Acan*) expression shown as **(A)** t-SNE plot of pooled cells from all timepoints, **(B)** violin plots to demonstrate enrichment in the mesenchymal cluster, pooled cells from all timepoints, and **(C)** violin plots to demonstrate increased gene expression within the mesenchymal cluster across time

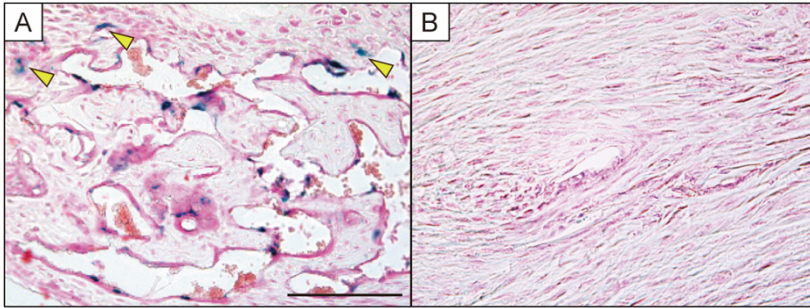
after HO induction. **(D-F)** Type II Collagen (*Col2a1*) expression shown as **(D)** t-SNE plot of pooled cells from all timepoints, **(E)** violin plots to demonstrate enrichment in the mesenchymal cluster, pooled cells from all timepoints, and **(F)** violin plots to demonstrate increased gene expression within the mesenchymal cluster across time after HO induction. N=3-4 animals per timepoint.

### Supplemental Figure 3



**Supplemental Figure 3: Single cell RNA sequencing analysis demonstrates select chondrocyte associated gene enrichment in mesenchymal cell sub-clusters.** Single cell RNA sequencing of mesenchymal cell sub-clusters in HO induction site up to 21 d thereafter (days 0, 3, 7, and 21 after induction) was examined (A) 4 sub-clusters were identified within mesenchymal cell cluster in HO. (B) *Wisp1* expression, (C) Aggrecan (*Acan*), (D) SRY-Box Transcription Factor 9 (*Sox9*), and (E) Type II Collagen (*Col2a1*) expression shown as t-SNE plot of pooled mesenchymal cells alone.

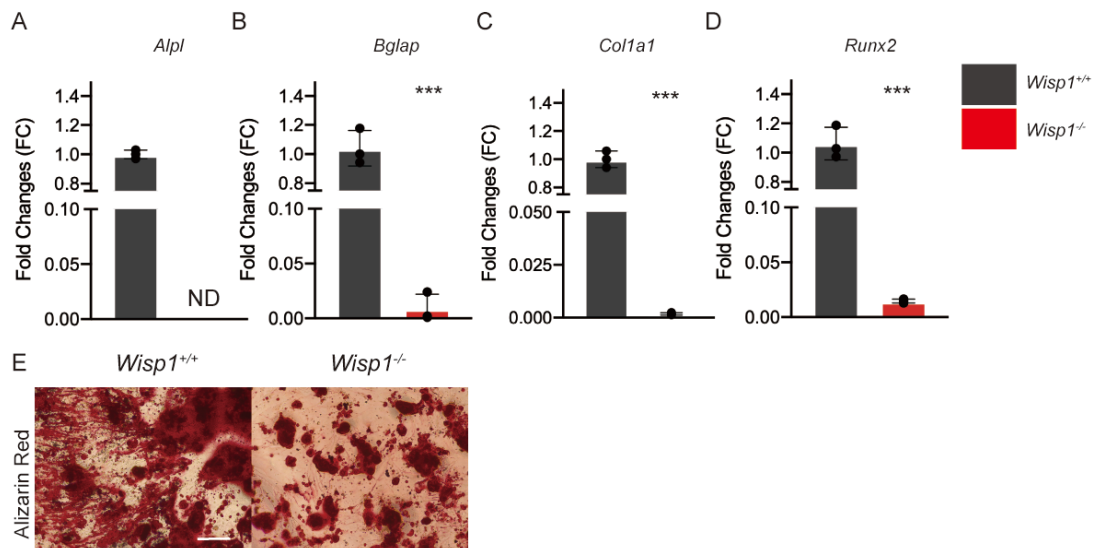
#### Supplemental Figure 4.



#### Supplemental Figure 4: Reporter activity in *Wisp1-LacZ* (*Wisp1*<sup>LacZ/LacZ</sup>)

**heterotopic ossification.** HO induction was performed in *Wisp1*<sup>LacZ/LacZ</sup>, and Xgal staining performed at 9 weeks post-injury. **(A)** *Wisp1* reporter activity in bone lining cells of the HO site. **(B)** As a negative control, injury sites from *Wisp1*<sup>+/+</sup> littermates were also used. No Xgal staining was confirmed. Fast red counterstain appears pink, reporter activity appears blue. Scale bar: 100  $\mu$ m.

## Supplemental Figure 5.



**Supplemental Figure 5: Osteogenic differentiation among *Wisp1*<sup>+/+</sup> and *Wisp1*<sup>-/-</sup> adipose stromal cells.** Gene expression for markers of osteogenic differentiation at d 7. **(A)** Alkaline phosphatase (*Alpl*) **(B)** Osteocalcin (*Bglap*), **(C)** Type 1 Collagen (*Col1a1*) and **(D)** Runt related transcription factor 2 (*Runx2*). \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Differences were calculated between groups by Student's t test. Data represent the mean  $\pm$ S.D. and triplicate experimental replicates in biological duplicate. **(E)** Alizarin red staining of *Wisp1*<sup>+/+</sup> and *Wisp1*<sup>-/-</sup> adipose derived-stromal cells in osteogenic differentiation medium on d 14. Scale bar: 100 $\mu$ m.