To further define cell types in the ‘coarse grained’ mesenchyme cloud, we did sub-clustering of these clusters (FIGXXX). By taking a closer look at the sample origin, we see that head mesenchyme and trunk mesenchyme cells are naturally divided.

For trunk mesenchyme cells, we trimmed off all well isolated clusters, then pooled all remaining cells and ran different clustering algorithms. Using Latent Class Analysis (REF) on these clustering as our final clusters (FIGXXX), then we performed DGE and GSEA using our regular pipeline to annotate these clusters. Of the 16 clusters we annotated, we clearly see different cell types, and related cell types show trajectories on plot (FIGXXX).

On the other hand, we analysed Hox family gene expression across trunk mesenchyme clusters. It is known that certain Hox genes follow a nested expression pattern in limb (REF), thus we hypothesize that the expression of these genes in related clusters on tSNE plot would follow the same nested pattern. To test the hypothesis, we set the centroid of ZPA cluster as reference point, and calculated distance of specific Hox gene and Shh expression cells(FIGXXX). ~~By plotting these cells in tSNE space, they largely expand along tSNE 2 coordinate (MAYBE CALCULATE DISTANCE NOT ONLY TSNE2)~~. We averaged all cells for each Hox gene tested, and the results matched our hypothesis (FIGXXX).

Head mesenchyme cells show a simpler structure, and we sub-clustered these cells into 3 clusters by Ward Hierarchical clustering. By analysing these clustering using same work flow as trunk mesenchyme, we found that they all show strong cranial neural crest signature. Besides that, one cluster which is connected to trunk mesenchyme shows significant signature of cell adhesion related biological processes, while the other two clusters show stronger proliferation signature (FIGXXX).