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Supplementary Materials for

Single-cell mass cytometry reveals cross-talk between inflammation-dampening and inflammation-amplifying cells in osteoarthritic cartilage

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/11/eaay5352/DC1)

Supplemental Figures

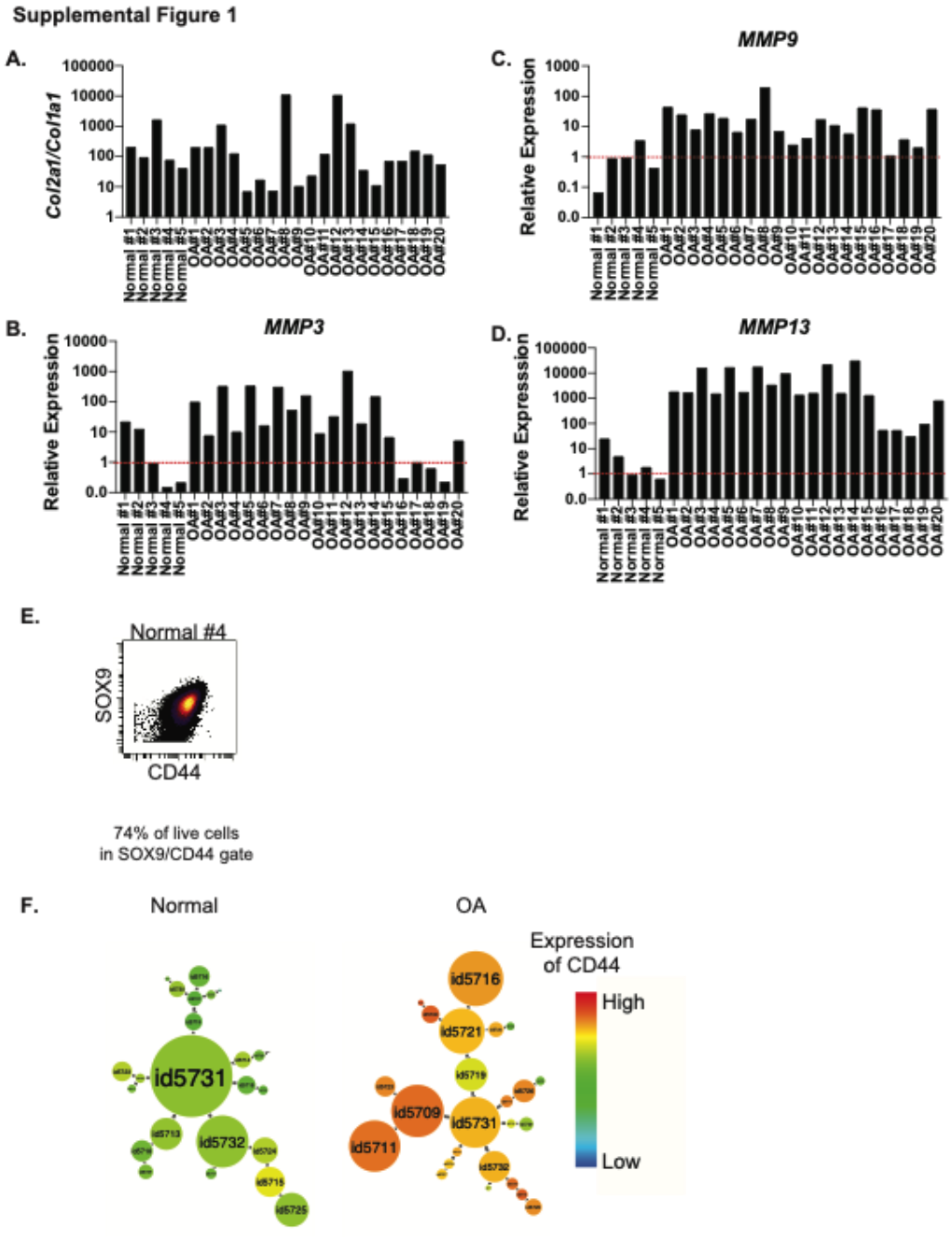

Fig. S1. Quality control of normal and OA chondrocyte samples used for cyTOF analysis. A. Ratio between the ΔΔ Ct of *Col2a1* and *Col1a1* as measured by RT-qPCR. **B.** RT-qCPR of *MMP3* gene expression normalized to Normal #3. **C.** RT-qCPR of *MMP9* gene expression normalized to Normal #3. **D.** RT-qCPR of *MMP13* gene expression normalized to Normal #3. **E.** Example gating of live cells on SOX9 and CD44

expression. **F.** Minimum spanning tree (MST) for the 24 clusters found by the X-shift algorithm. The size of each circle corresponds to the relative size of this cluster (cell-population) with respect to the rest. Size can be compared between normal and OA samples. Each circle is colored by the expression of CD44.

Supplemental Figure 2

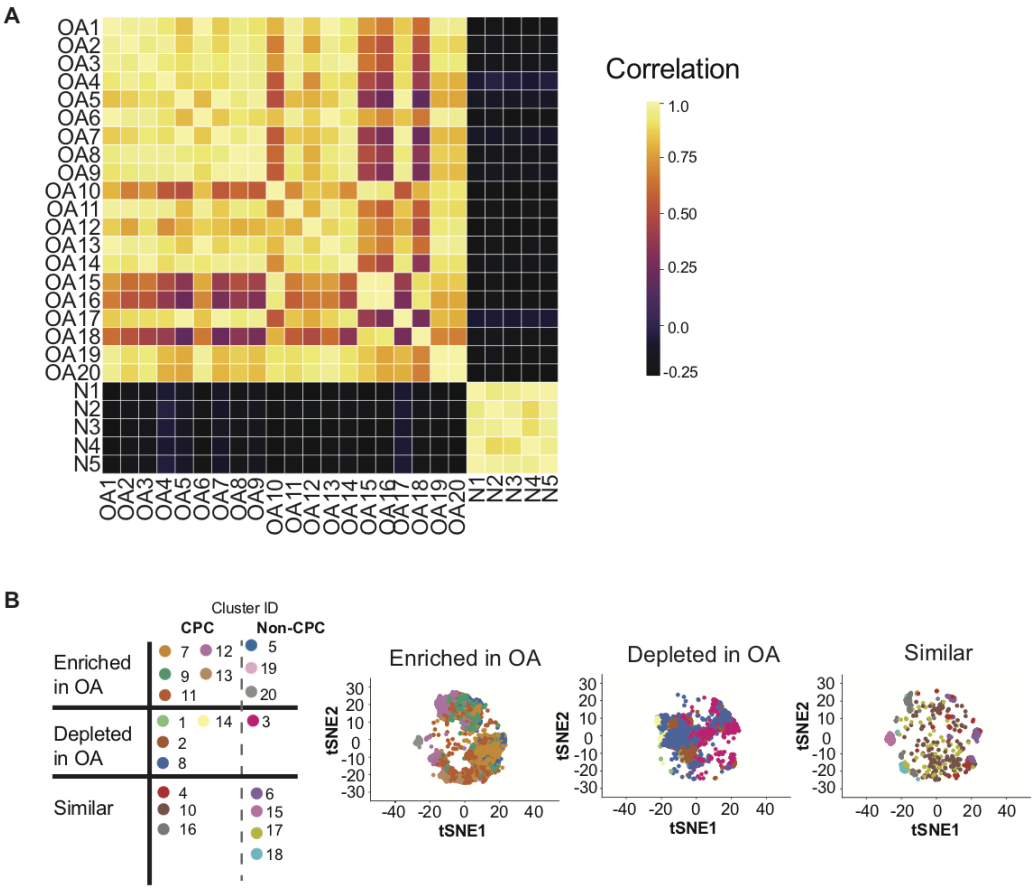

Fig. S2. OA and normal samples are characterized by different populations of cells. A. Correlation map between 20 OA samples and 5 normal samples. R value is represented by color-scale. **B.** Table of the cluster IDs, partially reproduced from Figure 2C, which are enriched, depleted or similar between OA and normal samples. Colors in the enriched section correspond to the tSNE projections on the right. The tSNE projections contain cells from clusters that are enriched, depleted or similar in OA compared to normal, sampled to 9000 cells. Enrichment, depletion or similarity between the ranked means of normal (n=5) and OA (n=20) cluster abundance was tested using an unpaired, two-tailed Mann-Whitney test with Bonferroni correction (alpha = 0.0025). Adjusted p-values for all enriched or depleted clusters are 0.002 (**).

Supplemental Figure 3

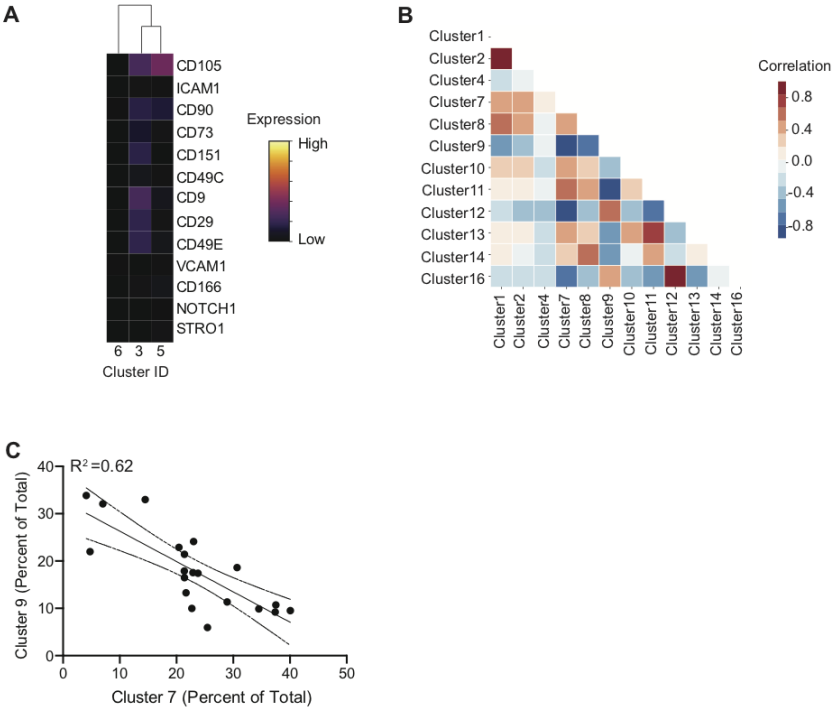

Fig. S3. CPC clusters differentiate OA patient subtypes. A. Heatmap for three clusters (3,5,6) which show low expression for the CPC markers. Expression is scaled to 1, and is comparable to the heatmaps in Figure 3A. **B.** Correlation matrix between CPC clusters. Colormap gives correlation. **C.** Correlation between abundance of Cluster 7 and 9. Each point represents a single OA patient. Collectively, these points give the R^2 value between Cluster 7 and 9 from panel A.

Supplemental Figure 4

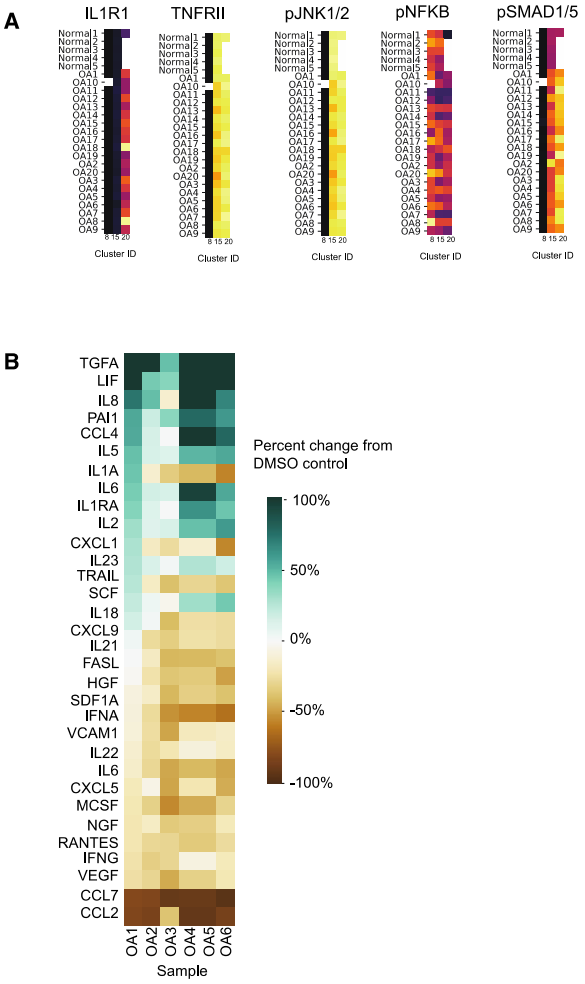

Fig. S4. Inf-A population is marked by TNFRII and IL1R1. A. Average expression of all the cells in a given cluster (8, 15 or 20) for each sample. Squares that are white represent that there were no cells to plot for that given sample. Expression is given for IL1R1, TNFRII, pJNK, pNFKB and pSMAD1/5. Color scale is normalized to highest value of each marker and is comparable within each heatmap. **B.** Heatmap for percent change from DMSO of the secretion of each of the cytokines that was found to change after JNK inhibitor treatment.

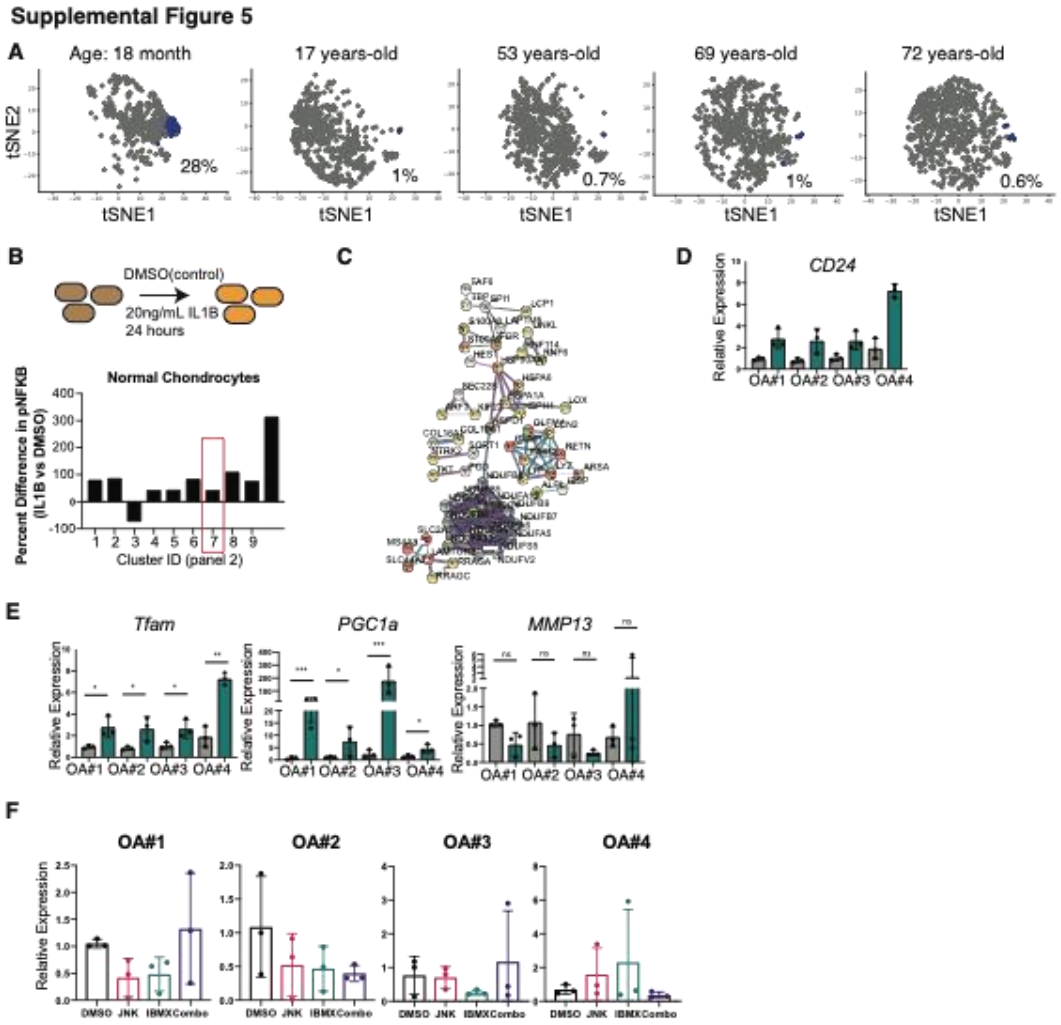

Fig. S5. Inf-D population is marked by CD24 expression and low reactivity to inflammatory cues. A. A limited cyTOF panel was used to test the age dependence of CD24 expression. CD24 positive cells are highlighted in blue in each sample, and the percent of all cells measured is given. **B.** With the same limited panel as in (A), normal chondrocytes were treated with either DMSO or IL1B for 24 hours to stimulate a NFK-B response. FlowSOM analysis was performed on samples before and after treatment. The percent change in pNFKB in each cluster is plotted. CD24 positive cells are in cluster 7. **C.** STRING network analysis for CD24 positive cells from the scRNA-sequencing dataset of OA patients. Red and yellow nodes are related to the immune

system and immune signaling, while blue and green nodes are related to oxidative phosphorylation and mitochondrial homeostasis **D.** RT-qPCR of OA patients treated with IBMX for 48 hours, for *CD24*. Each point represents an independent replicate of the experiment with cells from a given patient (n=3); significance is tested with Student's t-test. p-value < 0.05 (*), 0.001(**). **E.** RT-qPCR of OA patients treated with IBMX for 48 hours, for *TFAM, PGC1a,* and *MMP13.* Each point represents an independent replicate of the experiment with cells from a given patient (n=3); significance is tested with Student's t-test. p-value < 0.05 (*), 0.001(**), 0.0001(***). **F.** RT-qPCR of OA patients treated with IBMX + JNK inhibitor for 48 hours for *MMP13.* Each point represents an independent replicate of the experiment with cells from a given patient (n=3); significance is tested with Welch's T-test with multiple hypothesis testing (alpha = 0.016) to account for multiple drug treatments. No significant differences were found between groups.