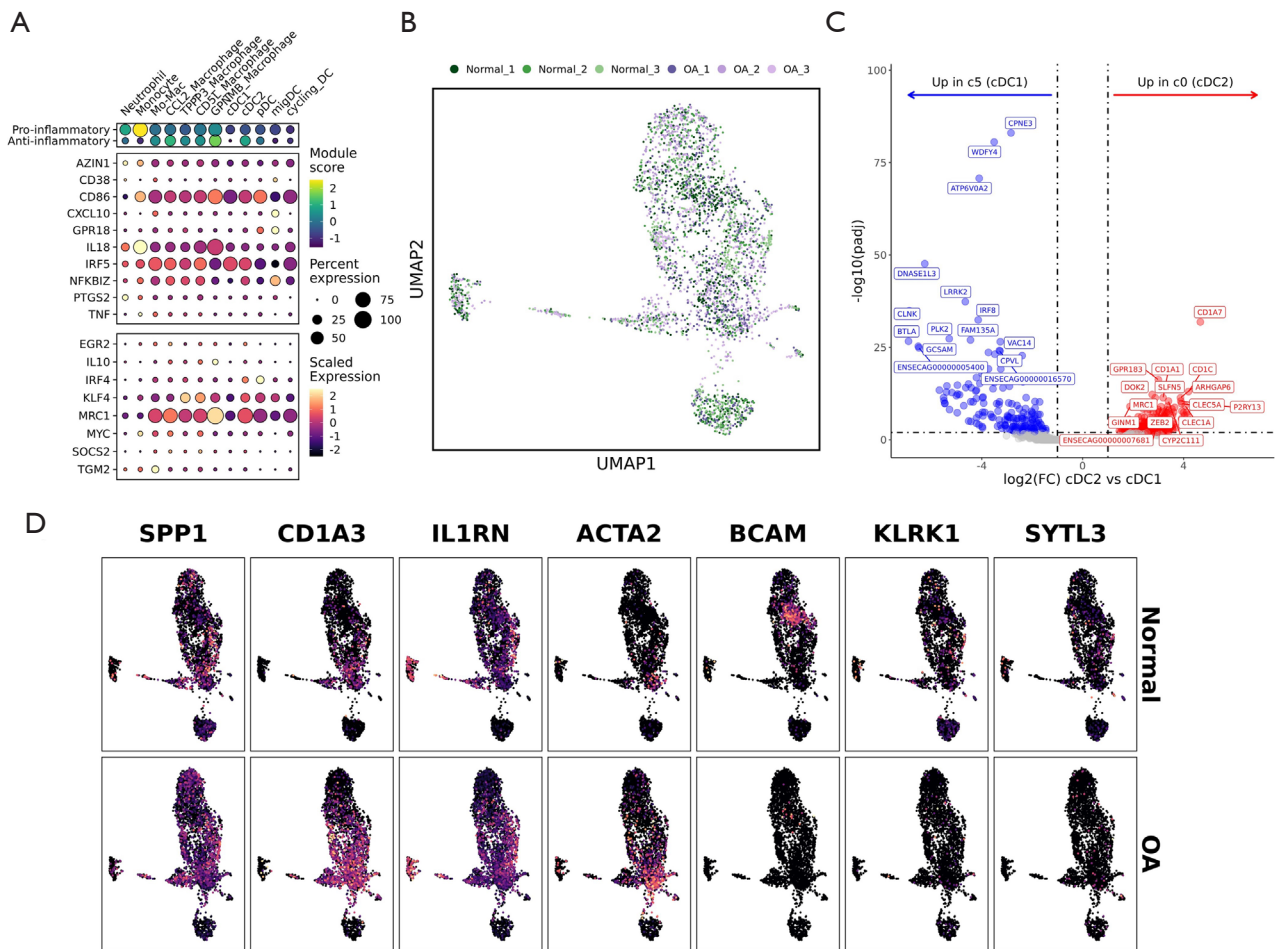
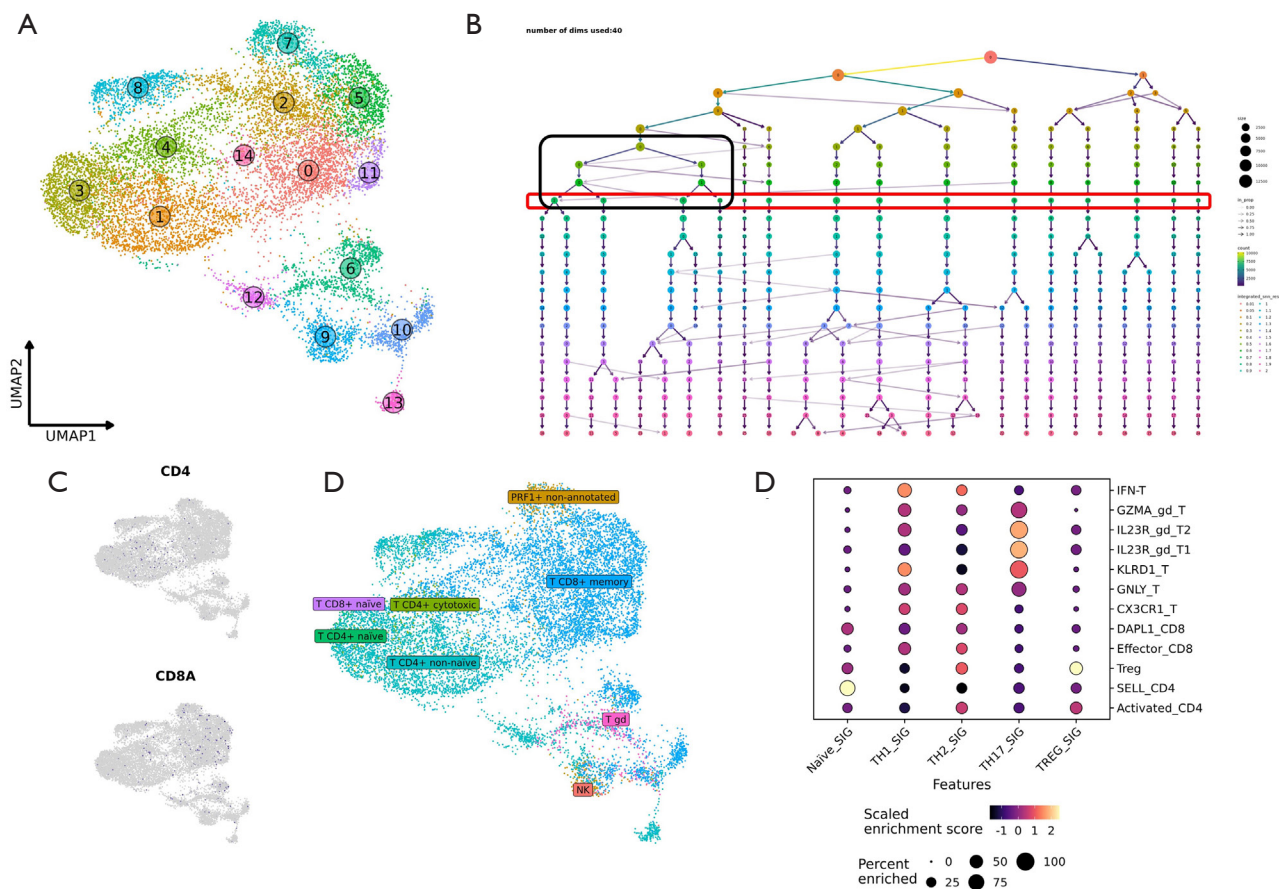


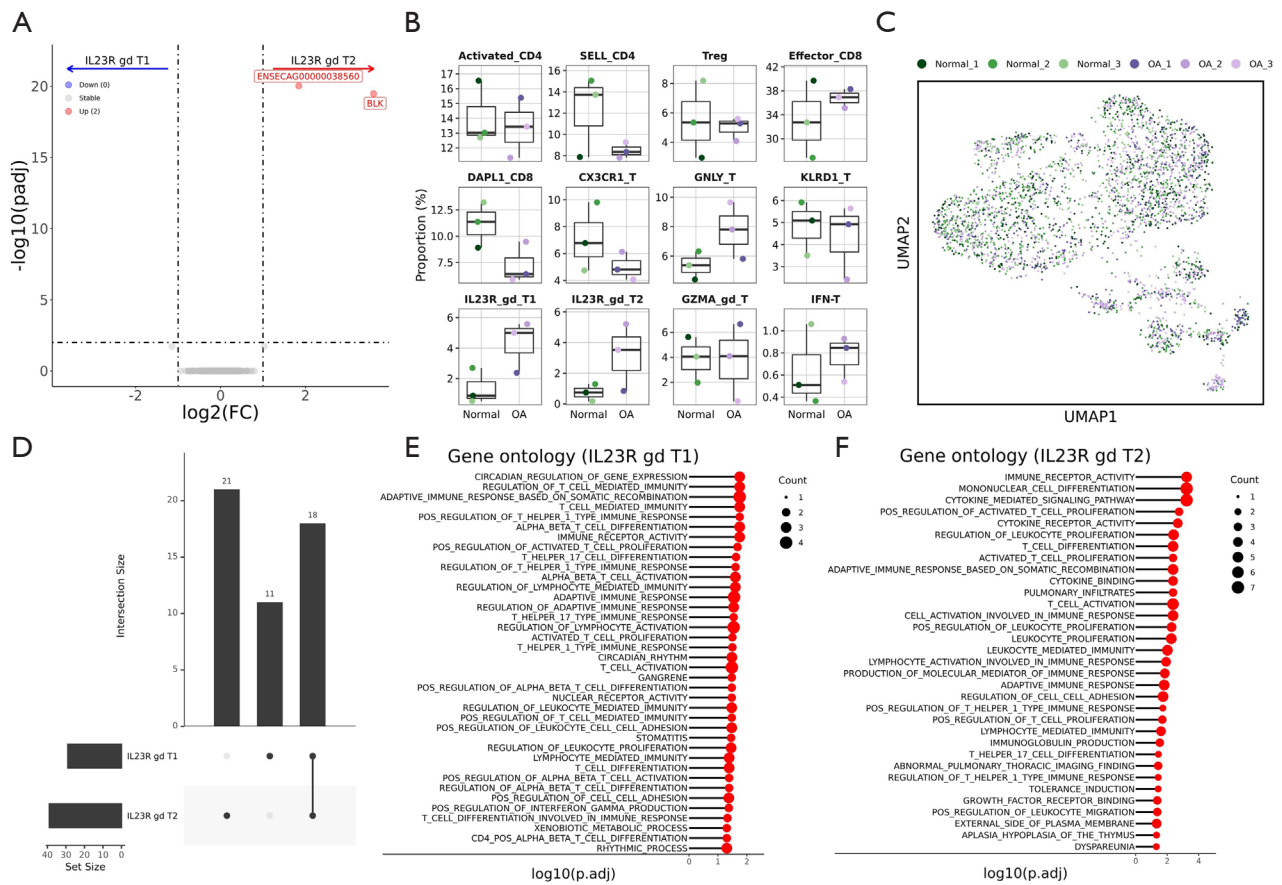
**Figure S1** Additional plotting associated with differential abundance analysis of the full synovial fluid dataset presented in primary *Figure 2*. (A) Stacked bar graph depicting the composition of each cluster. Purple bars indicate cells came from osteoarthritis-affected synovial fluid (n=3 horses) while green bars indicate cells came from normal synovial fluid (n=3 horses). (B) Differential abundance analysis of major cell types in OA relative to normal synovial fluid (y-axis indicates the log-fold change in cell type abundance, significance determined using Monte Carlo permutation test with adjusted P value threshold set to 0.01). The lower heatmap depicts the natural log transformed odds ratio with significantly under or overrepresented cell types (relative to a random distribution across all samples) indicated with an \*. Statistical significance was classified based on an adjusted P value <0.01 and  $|\log OR| > 0.69$  (the equivalent of OR >2 or OR <0.5). (C) Box plots depicting cell type proportions in normal and OA affected synovial fluid. Each data point represents a biological replicate. OA, osteoarthritis; FC, fold change; OR, odds ratio.



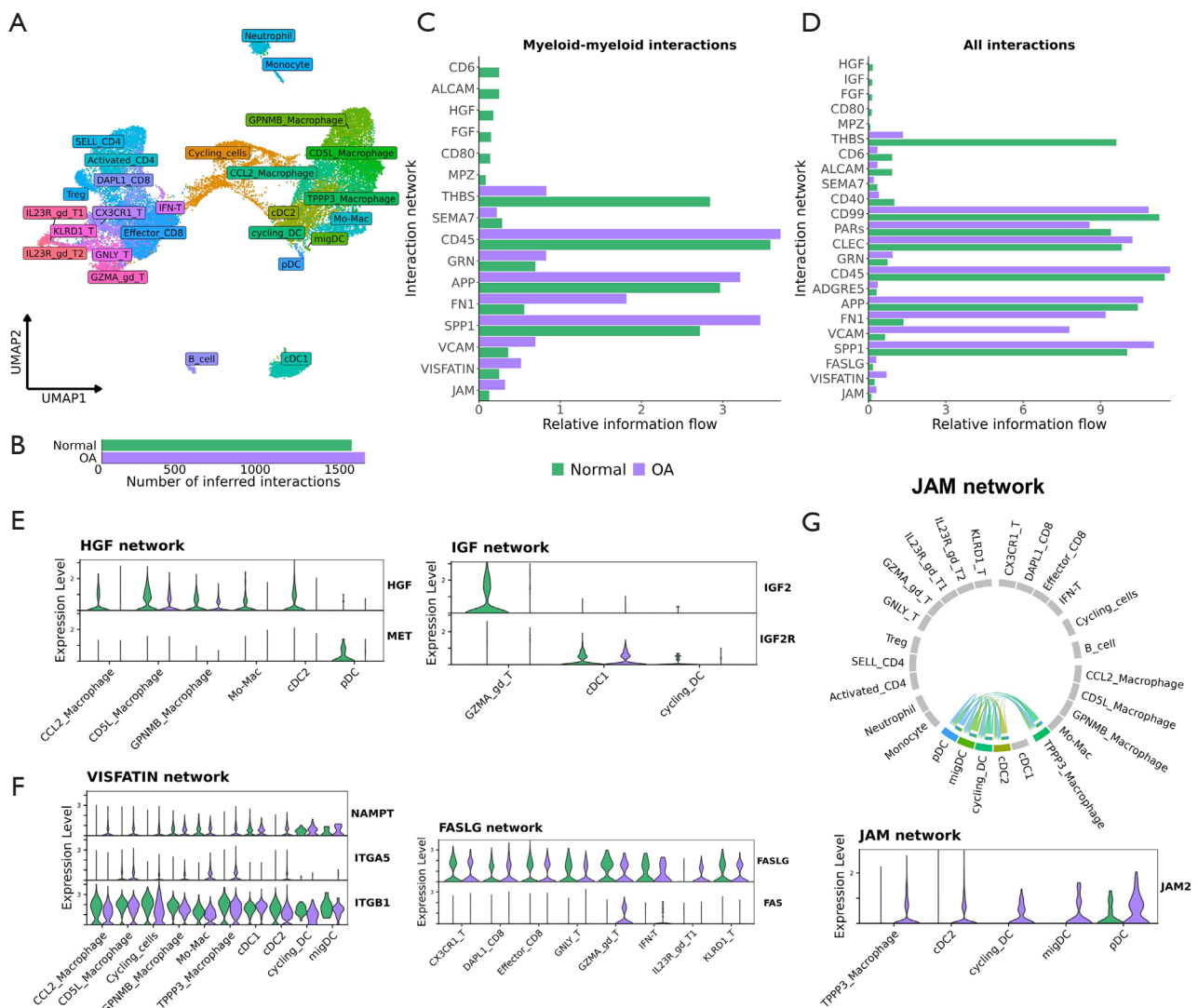
**Figure S2** Additional plotting associated with analysis of myeloid cells in primary Figure 3. (A) Dot plot depicting module scoring of “Pro-inflammatory” and “anti-inflammatory” gene signatures (top facet) as well as scaled gene expression (bottom two facets). (B) UMAP representation of all myeloid cells colored by sample. (C) Volcano plot depicting results of pseudobulk differential gene expression analysis between cDC2s and cDC1s using DESeq2. Top 20 genes (weighted by adjusted P value) are labeled in red (enriched in cDC2s) and blue (enriched in cDC1s). (D) UMAP split by cell source (normal = top row, osteoarthritis-affected = bottom row) that depicts the expression of differentially expressed features identified in primary Figure 3D. OA, osteoarthritis; FC, fold change; UMAP, Uniform Manifold Approximation and Projection.



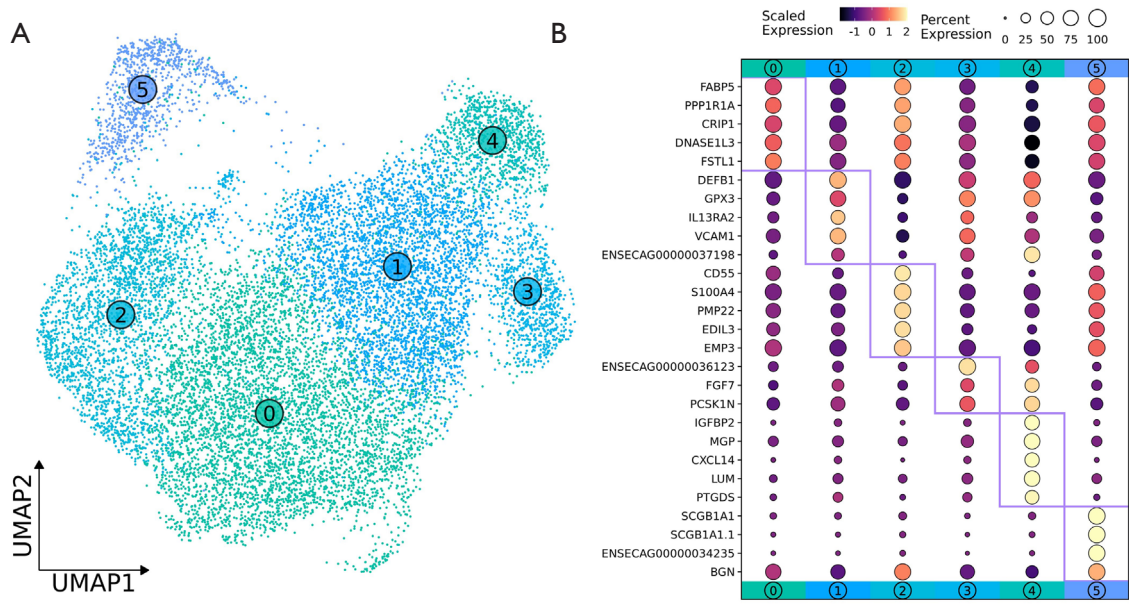
**Figure S3** Additional plotting associated with annotation of T cells in primary *Figure 4*. (A) UMAP embedding of T cells colored by unsupervised clustering results. (B) Clustering tree diagram demonstrating cluster stability as resolution is increased. The row boxed in red indicates the resolution used to generate (A), while the black box indicates that Clusters 0, 2, 5, and 11 were collapsed into one cluster to generate primary *Figure 4A*. (C) Feature plots depicting expression of CD4 and CD8A. (D) UMAP embedding colored by cell type as determined by reference mapping to equine PBMC reference. (E) Dot plot depicting enrichment scores for annotated T cell subsets (y-axis) using published human T cell gene signatures (x-axis) (Bukhari, 2023). Bukhari *et al.* Single-cell RNA sequencing reveals distinct T cell populations in immune-related adverse events of checkpoint inhibitors. *Cell Reports Medicine* 2023;4:100868. UMAP, Uniform Manifold Approximation and Projection; PBMC, peripheral blood mononuclear cell.



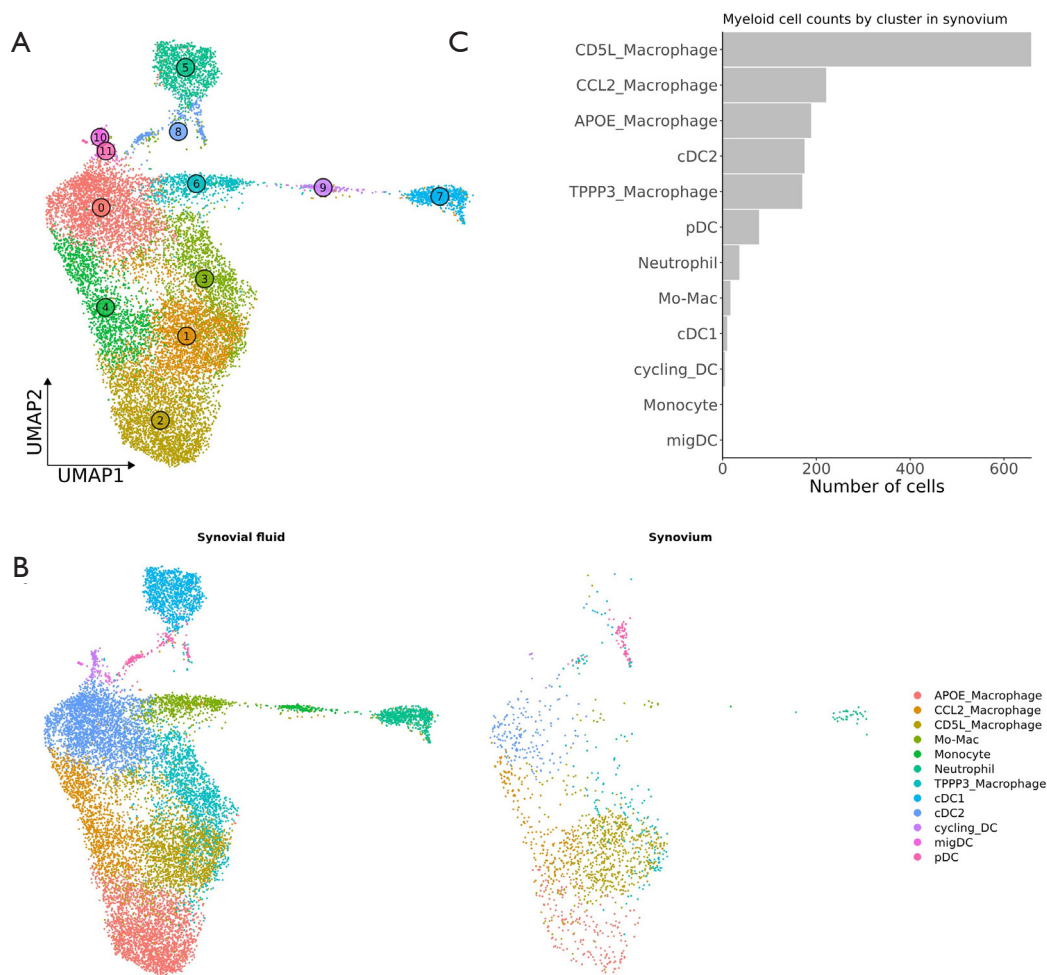
**Figure S4** Additional plotting associated with analysis of T cells in primary *Figure 4*. (A) Volcano plot depicting results of pseudobulk differential gene expression analysis between IL23R gd T2 and IL23R gd T1. Top 20 genes (weighted by adjusted P value) are labeled in red (enriched in IL23R gd T2) and blue (enriched in IL23R gd T1). Only 2 features met significance thresholds. (B) Box plots depicting cell type proportions in normal and osteoarthritis-affected synovial fluid. Each dot represents a biological replicate. Legend shared with (C). (C) UMAP representation of the T cell subset colored by sample. (D) Upset plot depicting overlap of gene signatures that define IL23R gd T1 and IL23R gd T2 (gene signatures are upregulated features identified in primary *Figure 4E,4F*, respectively). The plot indicates 21 features unique to IL23R gd T1, 11 unique to IL23R gd T2, and 18 shared features. Gene set enrichment analysis of (E) IL23R gd T1 and (F) IL23R gd T2 gene signatures using gene ontology database. Count (size of dot) indicates the number of features mapping to the gene set. FC, fold change; OA, osteoarthritis; UMAP, Uniform Manifold Approximation and Projection.



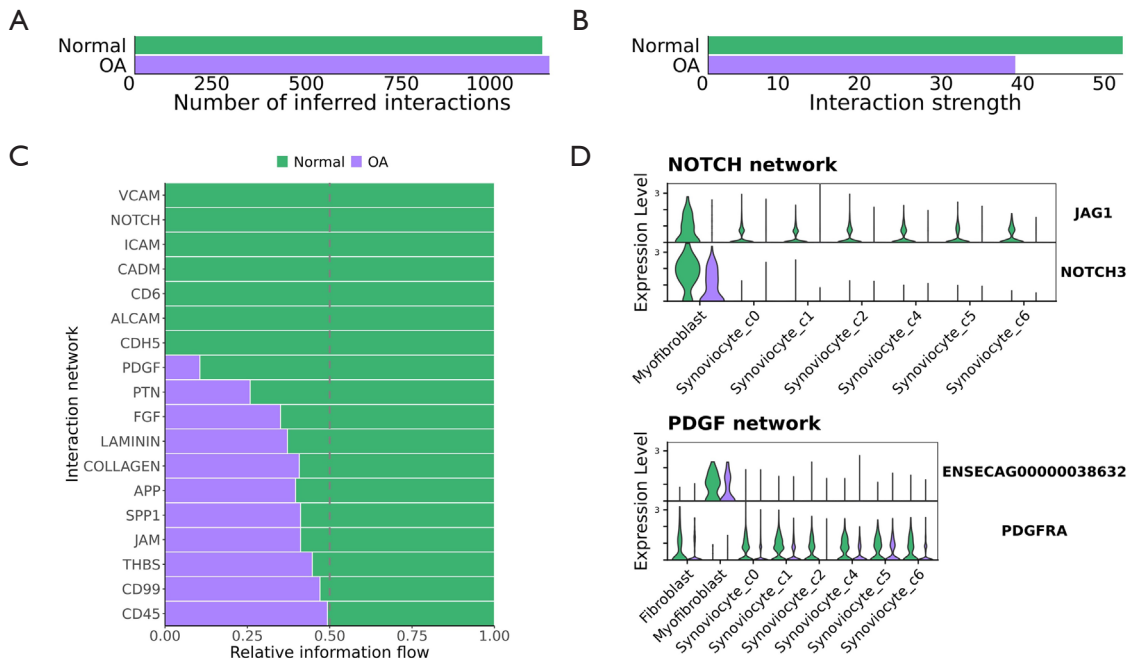
**Figure S5** Additional plotting associated with CellChat analysis of all synovial fluid cells in primary *Figure 5*. (A) UMAP embedding of all synovial fluid cells (corresponds to primary *Figure 2A*) colored by cell type as annotated through subcluster analysis. Bar charts of (B) number of inferred interaction, (C) inferred information transfer between myeloid cells, and (D) inferred information transfer between all cell types. Violin plots of features involved in enriched CellChat networks in cells from (E) normal and (F) osteoarthritic joints. (G) Circos plot depicting the inferred interactions between cell types within the JAM network. For (C-F) green bars/violins indicate normal and purple bars/violins indicate OA. UMAP, Uniform Manifold Approximation and Projection; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; VISFATIN, nicotinamide phosphoribosyltransferase (aka NAMPT); FASLG, FAS ligand; JAM, junctional adhesion molecule; OA, osteoarthritis.



**Figure S6** Subcluster analysis of synoviocytes (Clusters 0, 1, 2, 4, 5, and 6 in primary *Figure 6A*). (A) UMAP of synoviocyte subcluster analysis colored by the results of unsupervised clustering. (B) Dot plot depicting scaled expression of the top 3–5 (weighted by adjusted P value) features that define each of the 6 synoviocyte subclusters. Clusters 0, 1, 2, 3, 4, and 5 correspond to Clusters 0, 1, 2, 4, 5, and 6 from primary *Figure 6A*, respectively. UMAP, Uniform Manifold Approximation and Projection.



**Figure S7** Integrated analysis of synovial fluid and tissue myeloid cells. (A) UMAP embedding of myeloid cells integrated from synovial fluid and synovium colored by the results of unsupervised clustering. (B) Split UMAP embedding depicting cell distribution of myeloid cells in synovial fluid (left) and synovium (right). Plots were not down sampled, so 13,173 cells plotted in synovial fluid (left) and 1,570 cells in synovium (right). (C) Bar chart depicting number of cells within each annotated cell type of myeloid cells using nomenclature established in the synovial fluid. UMAP, Uniform Manifold Approximation and Projection.



**Figure S8** Additional plotting associated with CellChat analysis of all synovium cells in primary *Figure 6*. Bar charts of (A) number of inferred interactions, (B) strength of inferred interactions, and (C) inferred information transfer between all cell types present in equine synovium. (D) Violin plots of features involved in enriched CellChat networks in cells from normal joints. For all plots green bars/violins indicate cells are from normal synovium and purple bars/violins indicate cells are from osteoarthritis-affected synovium. OA, osteoarthritis; NOTCH, Notch; PDGF, platelet-derived growth factor.



**Table S1** Summary metrics from Cell Ranger alignment

Source	Sample name	Estimated number of cells	Mean reads per cell	Median genes per cell	Number of reads	Valid barcodes	Sequencing saturation	Q30 bases in barcode	Q30 bases in RNA read	Q30 bases in UMI	Reads mapped to genome	Reads mapped confidently to genome	Reads mapped confidently to intergenic regions	Reads mapped confidently to intronic regions	Reads mapped confidently to exonic regions	Reads mapped confidently to transcriptome	Reads mapped antisense to gene	Fraction reads in cells	Total genes detected	Median UMI counts per cell
Synovium	Normal_1	9,409	48,760	1,777	458,778,175	97.60%	55.40%	95.20%	91.80%	92.60%	97.00%	91.90%	24.20%	18.80%	49.00%	41.30%	0.60%	92.30%	15,784	8,032
Synovium	OA_1	13,093	38,095	1,563	498,782,613	97.60%	49.50%	95.00%	91.30%	92.40%	96.90%	91.50%	24.20%	16.60%	50.70%	42.40%	0.60%	88.40%	15,643	7,100
Synovial fluid	Normal_1	8,621	146,561	1,289	1,263,499,959	94.00%	79.50%	95.60%	89.20%	95.30%	88.40%	81.50%	24.20%	22.90%	34.40%	25.90%	1.30%	75.60%	15,269	3,629
Synovial fluid	Normal_2	4,034	94,601	1,232	381,622,256	97.40%	77.00%	95.50%	91.00%	92.60%	95.50%	90.40%	22.90%	22.00%	45.60%	38.20%	0.60%	80.00%	14,820	3,976
Synovial fluid	Normal_3	8,025	33,204	833	266,463,057	97.80%	52.80%	97.30%	93.20%	96.90%	95.20%	89.80%	25.20%	21.40%	43.30%	34.50%	0.70%	58.50%	13,641	2,467
Synovial fluid	OA_1	8,386	105,110	1,840	881,448,973	94.30%	68.40%	95.70%	89.80%	95.40%	89.70%	85.20%	23.00%	22.20%	40.00%	30.90%	1.30%	86.00%	15,124	7,158
Synovial fluid	OA_2	6,267	68,859	2,107	431,539,173	97.70%	58.50%	95.50%	91.10%	92.60%	95.70%	91.40%	23.10%	18.00%	50.40%	42.80%	0.50%	90.00%	14,745	10,674
Synovial fluid	OA_3	8,982	36,540	755	328,201,488	97.80%	42.80%	96.50%	90.50%	96.20%	95.80%	88.80%	24.20%	14.30%	50.30%	41.70%	0.70%	41.10%	14,461	1,969

UMI, unique molecular identifier; OA, osteoarthritis.

**Table S2** Cell counts for every cluster split out for each sample in synovial fluid

Cell type	Normal_1	Normal_2	Normal_3	OA_1	OA_2	OA_3
Activated_CD4	488	251	420	291	61	274
B_cell	64	10	57	25	29	32
CCL2_Macrophage	216	54	97	340	247	317
CD5L_Macrophage	680	93	63	529	346	265
CX3CR1_T	200	189	157	91	33	83
Cycling_cells	430	222	383	660	361	540
DAPL1_CD8	263	219	437	121	51	120
Effector_CD8	1171	517	1082	724	189	753
GNLY_T	130	122	178	110	42	197
GPNMB_Macrophage	364	184	24	413	1300	156
GZMA_gd_T	166	38	134	126	22	10
IFN-T	15	7	35	16	5	11
IL23R_gd_T1	26	52	17	45	30	102
IL23R_gd_T2	22	25	6	16	28	72
KLRD1_T	150	114	116	93	13	115
Mo-Mac	62	47	38	179	390	174
Monocyte	9	13	17	13	73	39
Neutrophil	223	80	19	94	111	125
SELL_CD4	232	290	454	158	42	189
TPPP3_Macrophage	245	91	163	392	289	221
Treg	87	103	271	100	22	114
cDC1	231	171	107	567	50	44
cDC2	639	111	38	958	862	374
cycling_DC	4	7	9	13	24	30
migDC	5	2	2	17	6	4
pDC	8	6	7	50	15	17

OA, osteoarthritis.

**Table S3** Cell counts for every cluster split out for each sample in synovial tissue

Cell type	Normal	OA
Endothelial	249	586
Fibroblast	244	202
Macrophage/DC	1050	520
Myofibroblast	44	97
Synoviocyte_c0	2065	3369
Synoviocyte_c1	1675	2060
Synoviocyte_c2	814	1578
Synoviocyte_c4	599	363
Synoviocyte_c5	391	557
Synoviocyte_c6	341	544
T cell	83	110

OA, osteoarthritis.

**Table S4** Clustering resolutions and dimension reduction parameters used for analysis of each cell subset

Tissue	Subset	Associated UMAP	# of integration anchors	Cluster resolution	Dimensions	Minimum distance	n.neighbors
Synovial fluid	Myeloid	<i>Figure 2A</i>	2500	1	40	0.3	30
Synovial fluid	T cells	<i>Figure 3A</i>	2500	0.7	40	0.3	30
Synovial fluid	All cells	<i>Figure 1A</i>	2500	0.7	40	0.3	30
Synovial fluid + synovium	Myeloid	<i>Figure S6A</i>	2500	0.6	40	0.3	30
Synovium	Synoviocytes	<i>Figure S5A</i>	2500	0.3	40	0.3	30
Synovium	All cells	<i>Figure 5A</i>	2500	0.7	50	0.2	25

UMAP, Uniform Manifold Approximation and Projection.

**Table S5** Summary statistics and the results of statistical testing using Monte Carlo permutation testing for *Figure S1B*

Cell type	Mean_Normal	SD_Normal	Mean_OA	SD_OA	obs_log2FD	P value	FDR	boot_mean_log2FD	boot_CI_2.5	boot_CI_97.5	Significance
Macrophage	16.66	8.39	37.16	15.96	1.138780433	0.000999001	0.001332001	1.137514898	1.061136019	1.216490583	Up
DC	9.36	5.37	19.16	7.82	1.00503559	0.000999001	0.001332001	1.006040465	0.899568981	1.120117512	Up
Neutrophil	2.56	1.54	3.15	1.23	0.328383117	0.006993007	0.007992008	0.334269222	0.107356825	0.591859347	n.s.
CD4	20.31	6.7	8.27	5.28	-1.3242784	0.000999001	0.001332001	-1.329460593	-1.439024513	-1.218227673	Down
gdT	10.03	1.81	6.86	4.23	-0.536776036	0.000999001	0.001332001	-0.536855319	-0.69154565	-0.397442273	n.s.
CD8	32.43	6.45	14.53	8.09	-1.132354926	0.000999001	0.001332001	-1.130430364	-1.217716921	-1.050476592	Down
Cycling_cells	7.74	0.97	10.29	2.31	0.39329572	0.000999001	0.001332001	0.390805136	0.26037188	0.530372738	n.s.
B_cell	0.9	0.51	0.59	0.16	-0.506959989	0.022977023	0.022977023	-0.510157479	-1.000366169	0	n.s.

SD, standard deviation; OA, osteoarthritis; FD, fold difference (aka fold change); FDR, false discovery rate; CI, confidence interval; n.s., not significant.

**Table S6** Summary statistics and the results of statistical testing using Monte Carlo permutation testing for primary *Figure 3C*

Cell type	Mean_Normal	SD_Normal	Mean_OA	SD_OA	obs_log2FD	P value	FDR	boot_mean_log2FD	boot_CI_2.5	boot_CI_97.5	Significance
Neutrophil	6.95	3.25	4.24	2.47	-0.432315491	0.015984016	0.02740117	-0.433987013	-0.853314688	-0.058242025	n.s.
Monocyte	1.59	1.29	1.51	1.01	0.096215315	0.444555445	0.444555445	0.115148175	-0.560065786	0.807820314	n.s.
Mo-Mac	4.76	2.19	8.46	2.99	0.558873272	0.002997003	0.005994006	0.567822606	0.224333286	0.920955155	n.s.
CCL2_Macrophage	10.31	5.52	11.38	5.87	0.254572827	0.030969031	0.046453546	0.253028567	-0.022492913	0.540599038	n.s.
TPPP3_Macrophage	15.87	10.45	10.43	2.42	-0.577226852	0.000999001	0.002397602	-0.581307162	-0.83209245	-0.333931869	n.s.
CD5L_Macrophage	15.65	8.38	13.06	3.24	-0.401740677	0.000999001	0.002397602	-0.401692688	-0.640862121	-0.156607443	n.s.
GPNMB_Macrophage	13.03	8.67	18.47	14.39	0.442274084	0.000999001	0.002397602	0.436481685	0.196272506	0.666970813	n.s.
cDC1	15.61	6.12	6.58	8.09	-1.337581359	0.000999001	0.002397602	-1.341217219	-1.652128431	-1.028123601	Down
cDC2	14.41	8.74	23.76	2.88	0.828974964	0.000999001	0.002397602	0.834466363	0.62893849	1.038241433	Up
pDC	0.73	0.45	0.92	0.5	0.415037499	0.254745255	0.33966034	0.428624698	-0.515522369	1.415037499	n.s.
migDC	0.25	0.08	0.29	0.17	0.736965594	0.351648352	0.421978022	0.771069575	-1.321928095	2.874780699	n.s.
cycling_DC	0.83	0.7	0.9	0.71	0.180572246	0.434565435	0.444555445	0.169793245	-0.907368316	1.241181085	n.s.

SD, standard deviation; OA, osteoarthritis; FD, fold difference (aka fold change); FDR, false discovery rate; CI, confidence interval; n.s., not significant.

**Table S7** Summary statistics and the results of statistical testing using Monte Carlo permutation testing for primary *Figure 4D*

Cell type	Mean_Normal	SD_Normal	Mean_OA	SD_OA	obs_log2FD	P value	FDR	boot_mean_log2FD	boot_CI_2.5	boot_CI_97.5	Significance
Activated_CD4	14.09	2.13	13.39	2.03	-0.060541542	0.33966034	0.407592408	-0.058871834	-0.300555402	0.203817803	n.s.
SELL_CD4	12.21	3.83	8.48	0.73	-0.66862043	0.000999001	0.002997003	-0.672245466	-0.961344322	-0.397460193	Down
Treg	5.5	2.62	4.99	0.79	-0.016119665	0.504495504	0.504495504	-0.01373887	-0.415037499	0.391637584	n.s.
Effector_CD8	33.08	6.44	36.78	1.58	0.221993722	0.001998002	0.004795205	0.224680349	0.082111184	0.355147755	n.s.
DAPL1_CD8	11.16	2.15	7.25	1.95	-0.859822342	0.000999001	0.002997003	-0.862043833	-1.219939713	-0.529504087	Down
CX3CR1_T	7.11	2.55	5	1.04	-0.38502493	0.038961039	0.066790353	-0.388251527	-0.787481378	0	n.s.
GNLY_T	5.37	0.96	7.76	1.92	0.349007629	0.037962038	0.066790353	0.348128883	-0.04396612	0.713296828	n.s.
KLRD1_T	4.84	1.22	4.33	1.69	-0.167456746	0.27972028	0.407592408	-0.176404224	-0.642950306	0.275891303	n.s.
IL23R_gd_T1	1.36	1.17	4.32	1.7	1.807354922	0.000999001	0.002997003	1.82140039	1.186413124	2.606907917	Up
IL23R_gd_T2	0.74	0.56	3.19	2.19	2.155278225	0.000999001	0.002997003	2.211722715	1.338801913	3.322642324	Up
GZMA_gd_T	3.88	1.84	3.75	3.1	0.071553261	0.424575425	0.46317319	0.079570965	-0.415037499	0.585188629	n.s.
IFN-T	0.64	0.37	0.77	0.21	0.459431619	0.314685315	0.407592408	0.528026518	-0.679190296	2	n.s.

SD, standard deviation; OA, osteoarthritis; FD, fold difference (aka fold change); FDR, false discovery rate; CI, confidence interval; n.s., not significant.

**Table S8** Short gene lists that define cell types in synovial tissue

Cell type	Markers
Synoviocyte	DEFB1, ENSECAG00000020648, FN1, DNASE1L3, HHIPL2, ITGEBL1, CLU, ERFF1
Synoviocyte_c0	PPP1R1A, FSTL1, FABP5, DNASE1L3, EEF1B2, IQGAP2, ENSECAG00000030892
Synoviocyte_c1	DEFB1, IL13RA2, GPX3, ENSECAG00000037198, HHIPL2, RCAN2
Synoviocyte_c2	CD55, S100A4, PMP22, TIMP2, CRIP1, EDIL3, SBSPO
Synoviocyte_c4	ENSECAG00000036123, GPX3, FGF7, COL28A1, ENAH
Synoviocyte_c5	IGFBP2, PTGDS, CXCL14, MGP, LUM, CP, COL14A1, VCAN
Synoviocyte_c6	SCGB1A1, SCGB1A1.1, ENSECAG00000034235, EDIL3, CD55, STRA6
Endothelial	APOE, PLTP, C1QC, CD68, ENSECAG00000024790, LGMN
Fibroblast	SFRP4, LUM, MMP2, COL14A1, FNDC1, FBLN1, TFPI2, ASPN
Myeloid cells	C1QB, C1QC, FCER1G, C4BPA, CXCL8, CST3, CD68, AIF1
Myofibroblast	SIDT2, APOE, ACTA2, HIGD1B, PPP1R14A, CPE, NOTCH3, DMP1
T cell	CCL5, PTPRC, CD3E, CTSW, LAPTM5, GIMAP7, CD7, CD2

**Table S9** Summary statistics and the results of statistical testing using Monte Carlo permutation testing for *Figure 6C*

Cell type	Mean_ Normal	SD_ Normal	Mean_ OA	SD_ OA	obs_ log2FD	P value	FDR	boot_mean_ log2FD	boot_ CI_2.5	boot_ CI_97.5	Significance
Endothelial	3.3	NA	5.87	NA	0.821358	0.000999	0.001832	0.816675	0.604725	1.036785	Up
Fibroblast	3.23	NA	2.02	NA	-0.64534	0.000999	0.001832	-0.64677	-0.93148	-0.3546	Down
Macrophage/DC	13.9	NA	5.21	NA	-1.41779	0.000999	0.001832	-1.41933	-1.57832	-1.27067	Down
Myofibroblast	0.58	NA	0.97	NA	0.730393	0.005994	0.008242	0.727392	0.192343	1.243167	Up
Synoviocyte_c0	27.33	NA	33.74	NA	0.299822	0.000999	0.001832	0.29685	0.229689	0.363407	n.s.
Synoviocyte_c1	22.17	NA	20.63	NA	-0.1091	0.007992	0.009768	-0.10673	-0.19187	-0.02103	n.s.
Synoviocyte_c2	10.77	NA	15.8	NA	0.545434	0.000999	0.001832	0.549266	0.433689	0.662288	n.s.
Synoviocyte_c4	7.93	NA	3.64	NA	-1.04154	0.000999	0.001832	-1.04407	-1.25221	-0.83113	Down
Synoviocyte_c5	5.18	NA	5.58	NA	0.120294	0.121878	0.134066	0.124592	-0.06989	0.304331	n.s.
Synoviocyte_c6	4.51	NA	5.45	NA	0.255257	0.005994	0.008242	0.256587	0.06073	0.453666	n.s.
T cell	1.1	NA	1.1	NA	0.067904	0.414585	0.414585	0.065574	-0.35517	0.495996	n.s.

SD, standard deviation; OA, osteoarthritis; FD, fold difference (aka fold change); FDR, false discovery rate; CI, confidence interval; NA, not applicable; n.s., not significant.