

1 **Supplemental Materials**

Marker Types	Antibody	Isotope
Chondrocyte	SOX9	147
	CD24	160
Cell cycle	IDU	127
	Ki67	154
Inflammation	HIF2A	142
	pNF-κB(S29)	149
	iNOS	158
	CD126	163
	CD121B	143
	CD121A	156
	CD120B	165
Signaling	pJNK (T183/Y185)	175
	pSMAD1/5(S463/465)	176
	pSTAT3(pY705)	145
	TET1	148
	SOD2	144
	RUNX1	166
	RUNX2	167
CPC	CD106	159
	CD73	151
	NOTCH1	155
	STRO1	164
	CD171	170
	CD105	173
	CD33	168
	CD49E	169
	CD146	153
TLRs and Senescence	TLR4	162
	TLR2	171
	p16 ^{ink4a}	174

2

3 **Supplemental Table 1. cyTOF marker panel.** List of antibody markers categorized into
 4 chondrocyte, cell cycle, inflammation, signaling, chondroprogenitor (CPC) or TLRs, and
 5 senescence-related marker types with corresponding metal isotope conjugates identified by
 6 unique mass numbers.

7

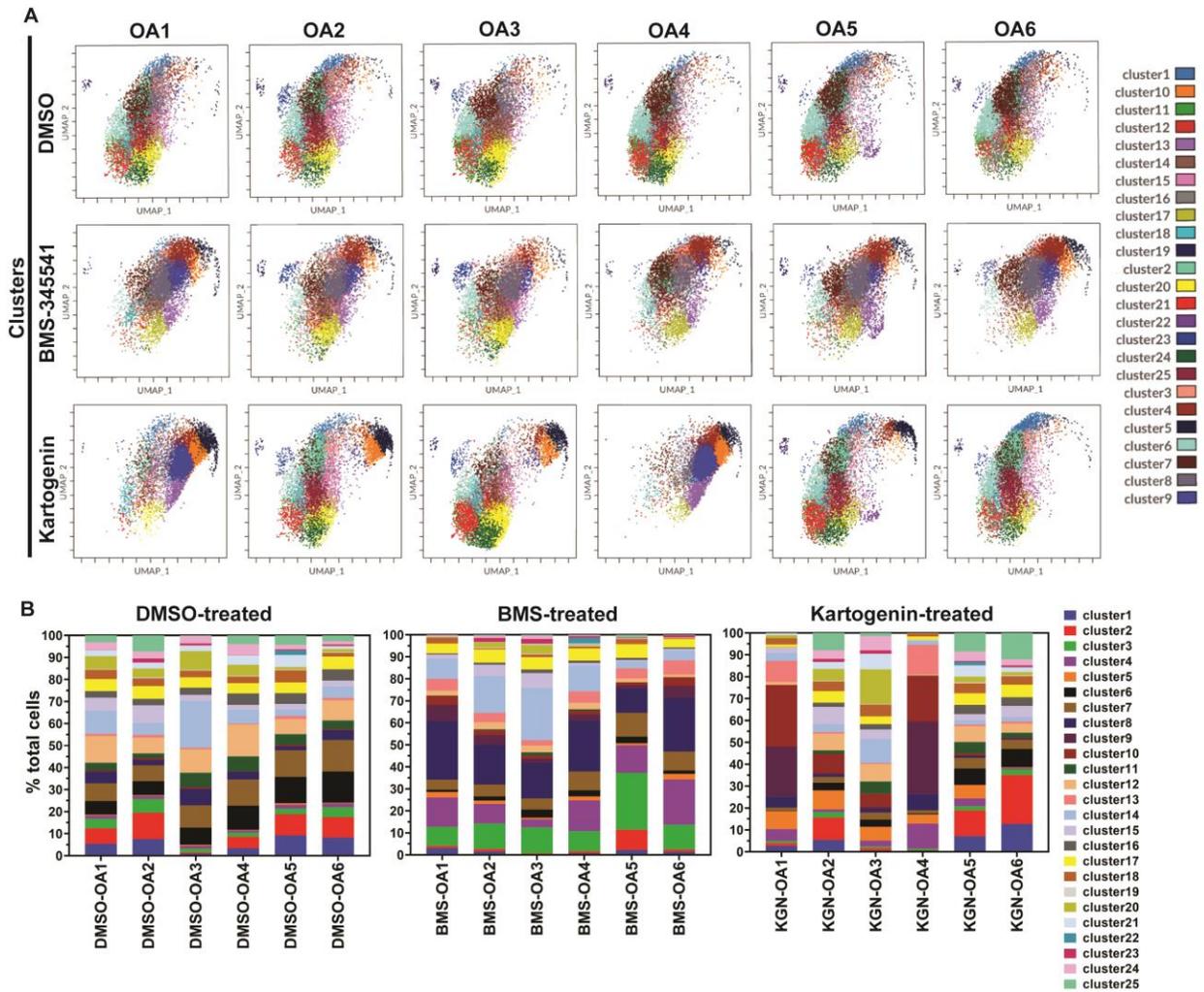
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Cluster Number	Group	Cluster Identity	Annotation
4,5,9,10	SOX9 ^{low}		
1,3,8,13,22	SOX9 ^{low-medium}		
2	SOX9 ^{medium}		
6		CD33 ⁺ TLR2 ⁺ p16 ^{ink4a+}	SnC I
7			
12			
14			
15			
16		CD105 ⁺ NOTCH1 ⁺ STRO1 ⁺ CD33 ⁺ TLR2 ⁺ p16 ^{ink4a+}	SnC CPC III
17		CD105 ⁺ p16 ^{ink4a+}	SnC III
18			
19		CD121A ⁺ CD120B ⁺	Inf-A
23		3CD24 ⁺	Inf-D
25			
11	SOX9 ^{high}	NOTCH1 ⁺ CD33 ⁺ TLR2 ⁺ p16 ^{ink4a+}	SnC II
20			
21			
24		CD105 ⁺ p16 ^{ink4a+}	SnC IV

9

10 **Supplemental Table 2. Table of high confidence cluster identities.** Categorization of clusters
11 based on their median SOX9 expression, and empirical identities, and annotation of select
12 clusters using the markers included in the cyTOF panel are shown.

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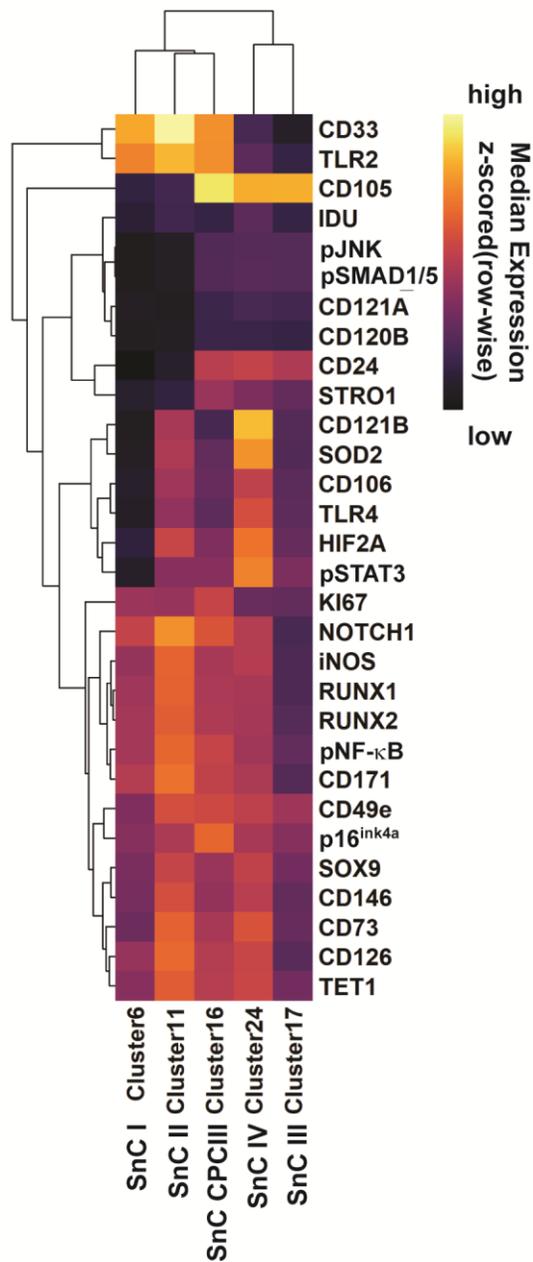


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15 **Supplemental figure 1. FlowSOM identified the OA chondrocyte landscape.**

16 (A) UMAP of all 25 clusters identified by FlowSOM in individual DMSO, BMS-345541 and
 17 Kartogenin treated samples (n=6 per treatment). Each UMAP is a representation of 8043 cells.
 18 (B) Proportional representation of all clusters as a percent of total cells in each sample across
 19 treatment groups.

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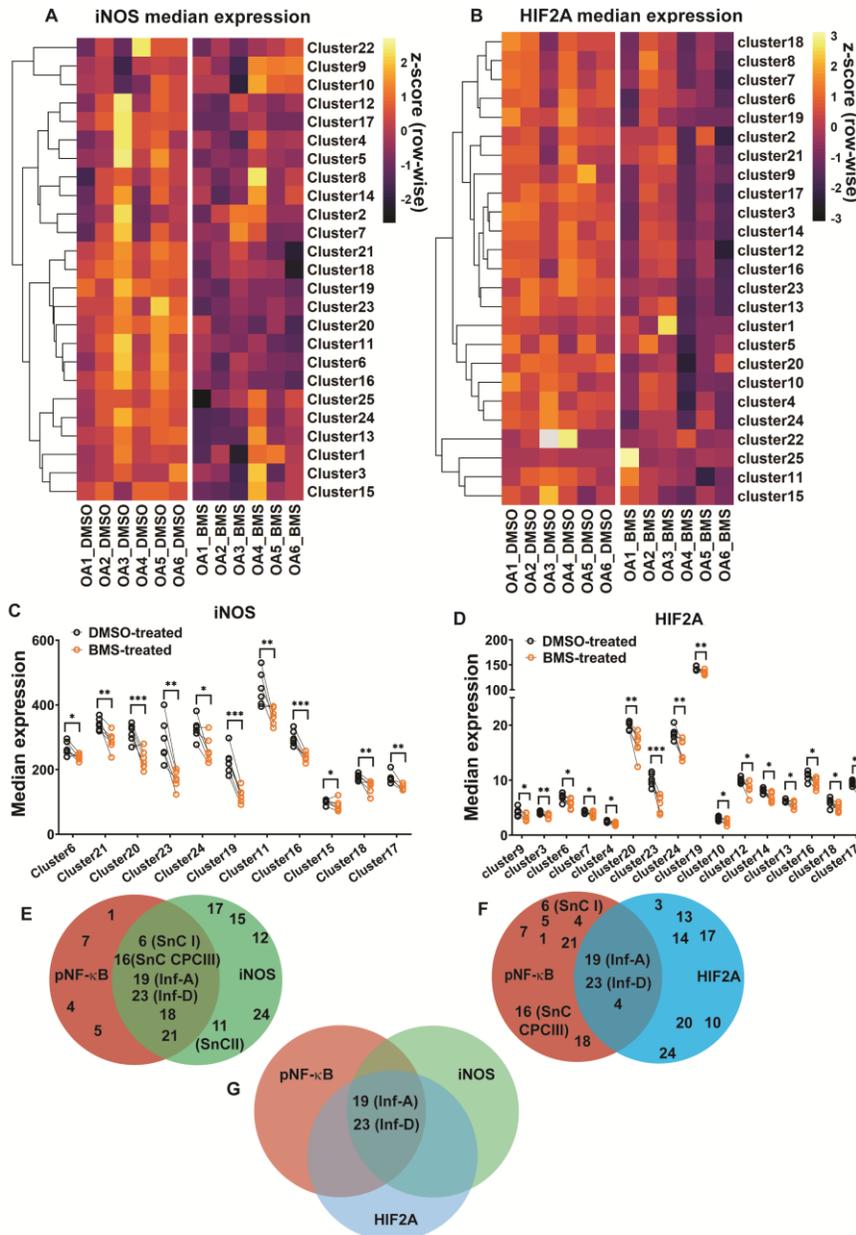


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22 **Supplemental figure 2. Median expression profile of p16^{ink4a} clusters.**

23 Heatmap of the median expression of markers in the four p16^{ink4a} clusters, namely SnC I, SnC II,
24 SnC III, SnC CPC III, and Snc IV in combined DMSO-treated samples. The median intensities
25 were z-scored row-wise (per marker) for standardization.

26

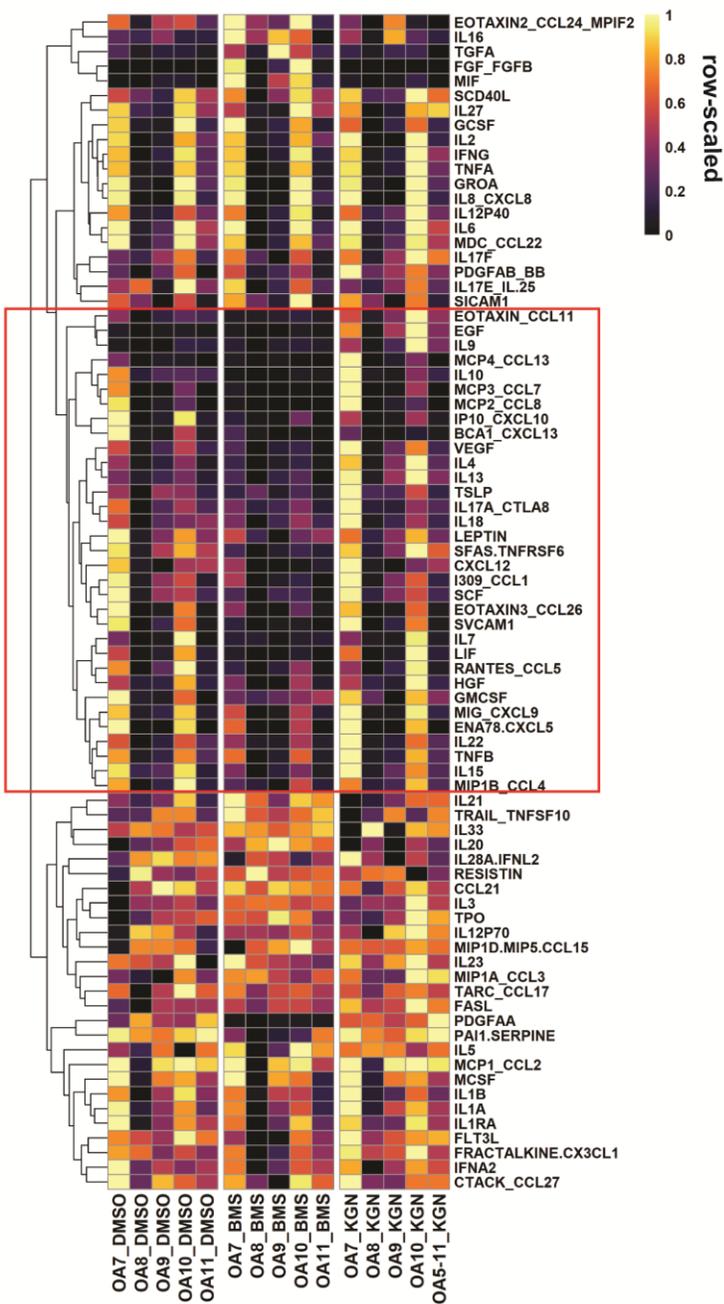


27

28 **Supplemental figure 3. Effect of NF-κB pathway inhibition on iNOS and HIF2A**
 29 **expression.**

30 Heatmap of the median expression of (A) iNOS and (B) HIF2A in all clusters between DMSO-
 31 treated controls and BMS-345541 -treated samples. The significant difference in the mean
 32 expression of iNOS or HIF2A was calculated by paired t-test at 95% confidence level. Venn
 33 diagram representing clusters where the expression of (C) pNF-κB and iNOS, (D) pNF-κB and
 34 HIF2A, and (E) pNF-κB, iNOS and HIF2A were commonly downregulated in BMS-345541 -
 35 treated samples when compared with DMSO-treated controls.

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49

50 **Supplemental figure 5. Drug treatment effects on the secretory profile of OA chondrocytes.**

51 Heatmap represents the raw MFI of 80 analytes (scaled row-wise) measured by multiplex
 52 autoantibody assay by Luminex in all DMSO, BMS-345541 and Kartogenin treated samples.
 53 Hierarchical clustering of rows as a measure of Euclidean distance was performed to visualize
 54 treatment effects on analytes. Analytes that are dampened in BMS-345541 treatment group
 55 compared to DMSO or Kartogenin treatment groups cluster together and are indicated by
 56 rectangle box in red.