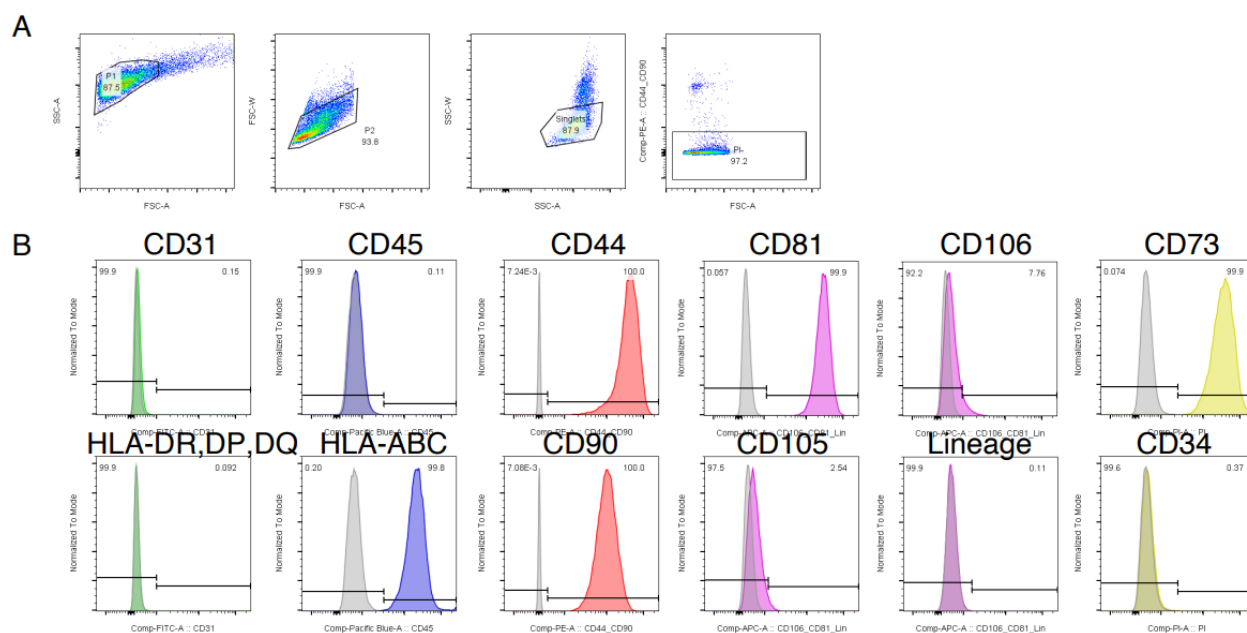


## SUPPLEMENTARY MATERIALS

### Supplementary methods

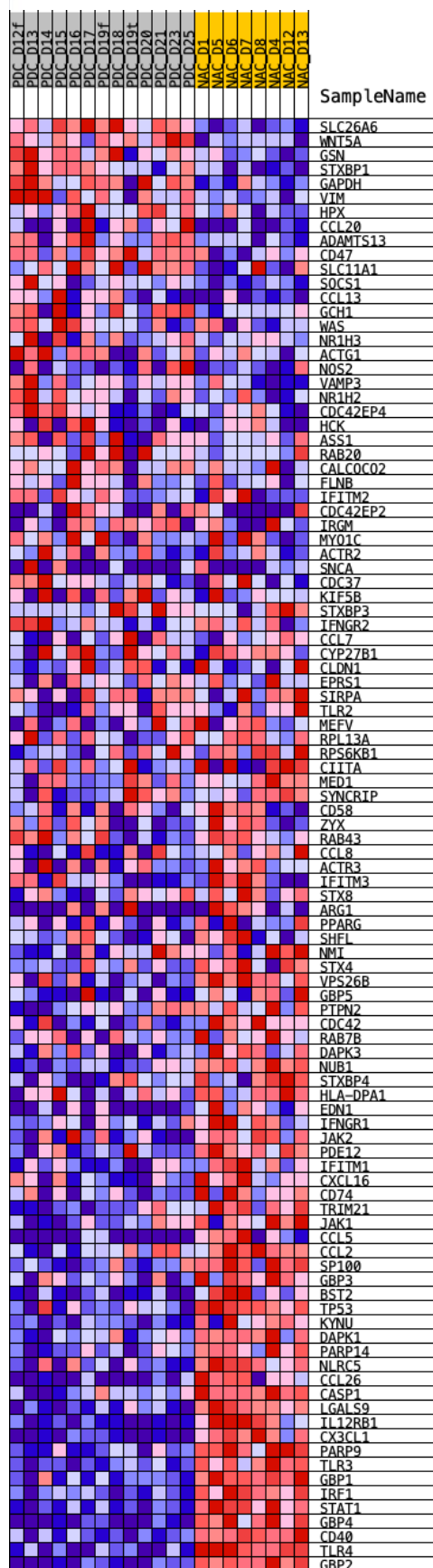
#### Flow Cytometry

Isolated cell suspensions from chondrocyte sheets (dissociated as described in manuscript) were aliquoted and incubated in 1  $\mu\text{g/mL}$  Fc block solution (564220, BD, Franklin Lakes, USA), resuspended in 10% FBS-containing PBS for 5-10 min, then labeled with fluorescent-conjugated antibodies (**Supplementary Table 4**) for 15 minutes. Cells were washed with 10% FBS-containing PBS, centrifuged, resuspended with 1:1000 propidium iodide (PI) (556463, BD) in 10% FBS-containing PBS. Samples were analyzed with a cell analyzer (Canto, BD). Doublets were excluded with FSC-W and SSC-W gating, then the PI-negative population was analyzed. Gating strategy is shown in **Supplementary Fig. 1A**.



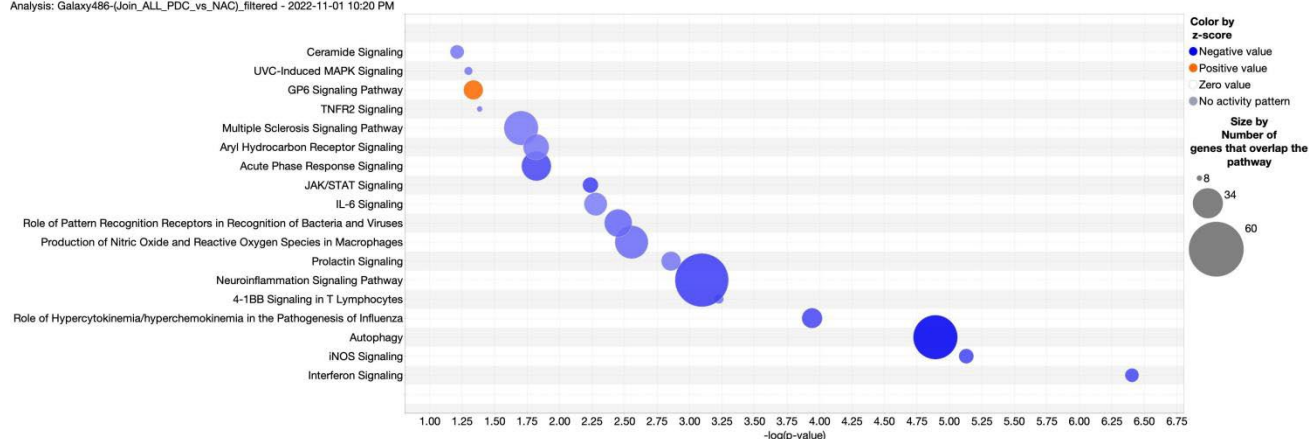
**Supplementary Fig. 1.** Flow cytometry analysis for chondrocyte cell purity and surface marker characterization. (A) Gating strategy for analysis. (B) Representative histograms for CD45, lineage cocktail (mixture of CD3, CD14, CD16, CD19, CD20, CD56), CD31, HLA-ABC and HLA-DR, -DP, -DQ, CD44, CD90, CD81, CD106, CD73, and CD34. Column colors represent fluorophores (blue: Pacific blue, green: FITC or Alx488, red: PE, magenta: APC or Alx647, yellow: PerCP-Cy5.5). Antibody information is provided in Supplementary Table 3. This figure was created with FlowJo and Microsoft Power Point.

**Supplementary Fig. 2.** Heatmap of the most significantly different genes between JCC sheets and ACC sheets. Red/blue indicates high/low expression in RNAseq. The heatmap was created with GSEA.

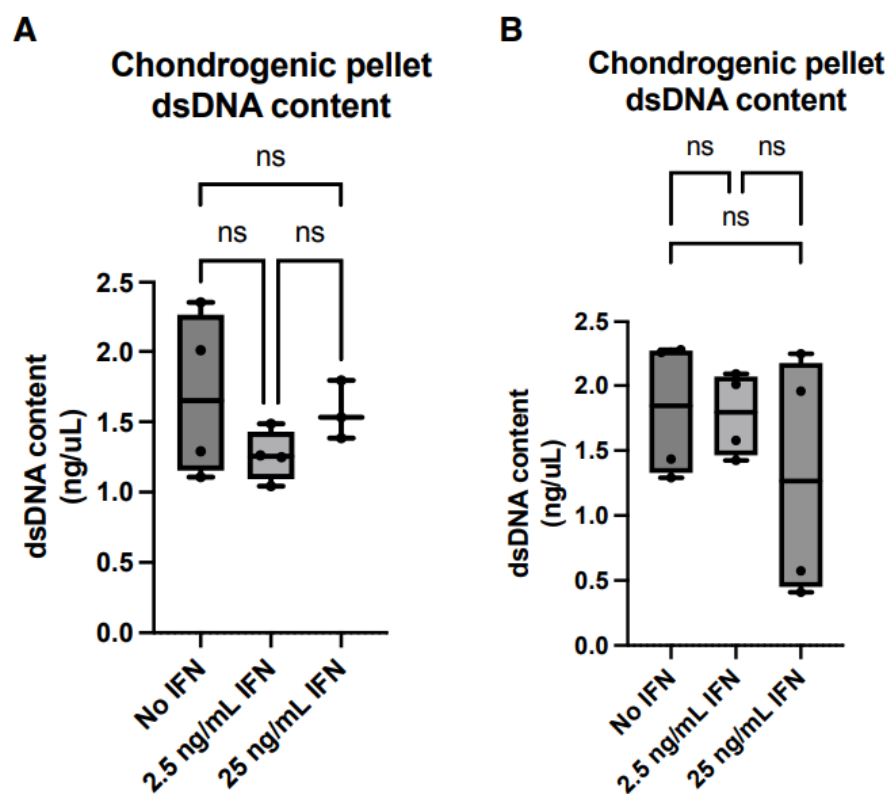


**Supplementary Fig. 3.** An unsupervised total gene list of GOBP\_RESPONSE\_TO\_INTERFERON\_GAMMA\_210 created by GSEA software. Red/blue indicates high/low expression in RNAseq. The heatmap was created with GSEA.

Analysis: Galaxy486-(Join\_ALL\_PDC\_vs\_NAC)\_filtered - 2022-11-01 10:20 PM



**Supplementary Fig. 4.** Significantly different pathways obtained from Ingenuity Pathway Analysis. Circle sizes indicate number of genes that overlap the pathway. Orange/blue indicates positive/negative activation. The heatmap was created with Ingenuity Pathway Analysis.



5

**Supplementary Fig. 5.** Interferon  $\gamma$  treatments in (A) passage culture and (B) cell sheet culture showed no significant differences in double-stranded DNA (dsDNA) contents. No statistical significance was found with ANOVA analysis and indicated as ns. The graphs were created with GraphPad Prism.

**Supplementary Table 1.** Primary antibodies used in immunohistochemistry.

Target	Host	Clone/ID	Dilution factors	Original conc.	Company/pr oducer	Cat #
<b>COL II</b>	Mouse	2B1.5 (IgG2a)	1:200	200 $\mu$ g/mL	Invitrogen (Thermo)	MA5-12789
<b>COL I</b>	Goat	poly	1:200	400 $\mu$ g/mL	Southern Biotech	1310-01
<b>Aggrecan</b>	Goat	poly	1:100	200 $\mu$ g/mL	R&D	AF1220
<b>Human Vimentin</b>	Rabbit	SP20	1:200	10-50 $\mu$ g/mL	Abcam	ab16700
<b>Isotype</b>	Mouse	IgG2a	1:100	100 $\mu$ g/mL	Dako/Agilent	X0943
<b>Isotype</b>	Mouse	IgG1 $\kappa$ DAK-GO1	1:10	100 $\mu$ g/mL	Dako/Agilent	X0931
<b>Isotype</b>	Goat	-	1:50	100 $\mu$ g/mL	Chalbiochem /Merck	NI02-100UG
<b>Isotype</b>	Rabbit	-	1:200,000	15 mg/mL	Dako	X0936

**Supplementary Table 2.** Secondary antibodies used in immunohistochemistry.

Target	Host	Conjugate	Dilution factors	Original conc.	Company	Cat #
<b>Mouse IgG</b>	Goat	HRP	1:1000	0.8 mg/mL	Jackson	115-035-166
<b>Goat IgG</b>	Donkey	HRP	1:1000	0.8 mg/mL	Jackson	705-035-147
<b>Rabbit IgG</b>	Goat	HRP	1:1000	0.8 mg/mL	Jackson	111-035-144

**Supplementary Table 3.** Differentially expressed gene list of GO terms of interest.

Description	LogP	Term/List	Symbols
cartilage development	-16.84	37/157	BMP5,BMPR1B,RUNX3,CHI3L1,COL2A1,COL11A1,COL11A2,COMP,EPYC,EDN1,FGF9,FGFR3,GHR,HOXA11,HOXC4,HYAL1,MGP,MMP13,MSX1,MSX2,ROR2,PITX1,SHOX2,STC1,WNT7B,WNT2B,FGF18,NOG,SULF1,SOX6,CSGALNACT1,SULF2,ADAMTS12,OSR1,RSPO2,SNORC,SCX
cell-cell adhesion	-16.39	71/537	ASTN1,BCL2,BMP5,CDH2,CDH4,CDH15,CDH18,COMP,CSTA,CXADR,CYP1B1,DCC,DSC3,DSCAM,DSG2,DSG3,FAT2,GPC4,FOXF1,GRID2,HBB,ICAM2,ITGA6,ITGA3,ITGA9,KIT,EPCAM,NINJ2,NRCAM,PCDH1,PCDH7,RAC2,CCL5,CX3CL1,SELL,TGFB2,THBS4,ICAM5,TNXB,COL14A1,WNT7B,ITGA8,NRXN2,NTN1,IL1RAPL1,NLGN1,CD93,ADGRL3,SLC7A11,FLRT3,TENM4,CDH19,LEF1,PCDHB18P,DCHS2,TENM3,PCDHB7,PCDHB4,TENM2,NLGN4X,PCDH10,LRRC4,CADM3,LRRC4,TLN2,MYPN,IGFN1,FAT3,SDK1,GLDN,AMIGO2
chondrocyte development	-5.05	8/26	BMPR1B,COL11A1,COMP,MSX2,SHOX2,FGF18,SULF1,SULF2
chondrocyte differentiation	-8.12	17/72	BMPR1B,RUNX3,COL2A1,COL11A1,COMP,FGF9,FGFR3,MSX2,SHOX2,WNT2B,FGF18,SULF1,SOX6,SULF2,ADAMTS12,OSR1,SCX
NABA CORE MATRISOME	-21.07	55/275	COL2A1,COL4A3,COL4A4,COL4A5,COL4A6,COL7A1,COL9A2,COL9A3,COL10A1,COL11A1,COL11A2,COMP,DPT,EPYC,ECM2,EFEMP1,MATN2,MGP,OMD,OGN,THBS4,TNXB,COL14A1,MFAP5,MATN4,CCN5,CCN4,SLIT2,NTN1,PRG4,FBLN5,FGL2,ESM1,MXRA5,PCOLCE2,SMOC2,SMOC2,VWA1,THSD4,FRAS1,COL21A1,PAPLN,PODN,FBLN7,EGFLAM,COL26A1,VWA3A,BMPER,VWCE,HMCN2,IGSF10,RSPO2,VWA2,GLDN,EYS
NABA ECM GLYCOPROTEINS	-11.43	34/196	COMP,DPT,ECM2,EFEMP1,MATN2,MGP,THBS4,TNXB,MFAP5,MATN4,CCN5,CCN4,SLIT2,NTN1,FBLN5,FGL2,MXRA5,PCOLCE2,SMOC2,VWA1,THSD4,FRAS1,PAPLN,FBLN7,EGFLAM,VWA3A,BMPER,VWCE,HMCN2,IGSF10,RSPO2,VWA2,GLDN,EYS
regulation of cell growth	-11.62	53/419	AGT,APOE,ARHGAP4,BCL2,BDKRB1,BDNF,BST2,CDH4,CRABP2,DCC,DSCAM,HBEGF,EDN1,SFN,HPN,HYAL1,MSX1,MT3,NKX6-1,NRCAM,ENPP1,SERPINE2,RGS2,CXCL12,SFRP1,TGFB2,VEGFA,WNT11,SOC2,SLIT2,NTN1,AKAP6,RIMS2,SEMA3E,MUC12,SEMA6B,FBLN5,FAM107A,RIMS1,EPB41L3,NEDD4L,LPAR3,OSGIN1,TRPV2,BCL11A,SEMA6A,CPNE5,SEMA6D,SLC44A4,DCSTAMP,RERG,PI16,NANOS1
skeletal system development	-22.76	79/505	BMP5,BMPR1B,RUNX3,CHI3L1,COL2A1,COL9A2,COL10A1,COL11A1,COL11A2,COMP,EPYC,EDN1,EN1,ENG,EXTL1,FGF9,FGFR3,GDF10,GHR,HAS2,HOXA1,HOXA11,HOXA13,HOXB7,HOXC4,HOXC5,HOXC6,HOXC8,HOXC9,HOXC10,HOXC11,HOXD9,HOXD10,HOXD12,HOXD13,HYAL1,KIT,MEIS1,MGP,MMP13,MSX1,MSX2,ROR2,TNFRSF11B,PHEX,PITX1,PTGER4,SFRP1,SHOX2,SOX11,STC1,TFAP2A,TGFB2,VDR,WNT7B,WNT11,WNT2B,WNT9B,TP63,FGF18,CCN4,NOG,RASSF2,LRRC17,IRX5,SULF1,TTC9,SOX6,CSGALNACT1,SULF2,SPEF2,ADAMTS12,OSR1,RFLNA,GSC,RANBP3L,RSPO2,SNORC,SCX



**Supplementary Table 4.** Antibodies used in flow cytometry.

Targets	Conjug.	Company	Cat #	Clone	Lot	Conc.	Dilution factors
CD45	PB	Biologend	304021	HI30 (IgG1κ)	B225702	500 µg/mL	5/100
HLA-ABC	PB	Biologend	311417	W6/32 (IgG2a)	B191432	500 µg/mL	3/100
CD31	AF488	Biologend	303109	WM59 (IgG1κ)	B190516	400 µg/mL	5/100
HLA -DR, -DP, -DQ	FITC	Biologend	361705	Tu39 (IgG2a)	B228758	200 µg/mL	5/100
CD44	PE	Biologend	338807	BJ18 (IgG1κ)	B222834	50 µg/mL	1/100
CD90	PE	Biologend	328109	5E10 (IgG1κ)	B206721	50 µg/mL	1/100
CD106	APC	Biologend	305809	STA (IgG1κ)	B208208	100 µg/mL	5/100
CD81	APC	BD	551112	JS-81 (IgG1κ)	8005529	12.5 µg/mL	20/100
Lineage (CD3, CD14, CD16, CD19, CD20, CD56)	APC	Biologend	348803	Mix of 6 ab (CD3, CD14, CD16, CD19, CD56: IgG1κ, CD20: IgG2b)	B199913	IgG1: 99.25 µg/mL IgG2b 6.25 µg/mL	20/100
CD73	PerCp/Cy5.5	Biologend	344013	AD2	B272402	200 µg/mL	5/100
CD34	PerCp/Cy5.5	Biologend	343521	581	B267999	200 µg/mL	5/100
Normal IgG1κ	PB	Biologend	400131	MOPC-21 (IgG1κ)	B229538	500 µg/mL	Set as same conc. to sample
Normal IgG2a	PB	Biologend	400235	MOPC-173 (IgG2a)	B243657	500 µg/mL	Set as same conc. to sample
Normal IgG1κ	AF488	Biologend	400129	MOPC-21 (IgG1κ)	B220820	200 µg/mL	Set as same conc. to sample
Normal IgG2a	FITC	Biologend	400207	MOPC-173 (IgG2a)	B235551	500 µg/mL	Set as same conc. to sample
Normal IgG1κ	PE	Biologend	400111	MOPC-21 (IgG1κ)	B244596	200 µg/mL	Set as same conc. to sample
Normal IgG1κ	APC	Biologend	400120	MOPC-21 (IgG1κ)	B257952	200 µg/mL	Set as same conc. to sample
Normal IgG2b	APC	Biologend	400319	MPC-11 (IgG2b)	B202284	200 µg/mL	Set as same conc. to sample