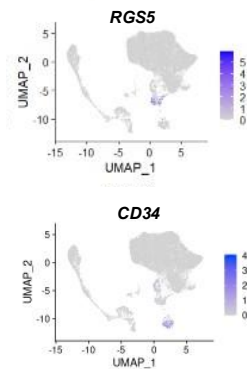


1 **Supplementary materials**

2 **Supplementary Figure S1. Signature gene expression for disc pericytes (*RGS5*) and endothelial**

3 **cells (*CD34*).**



4

5

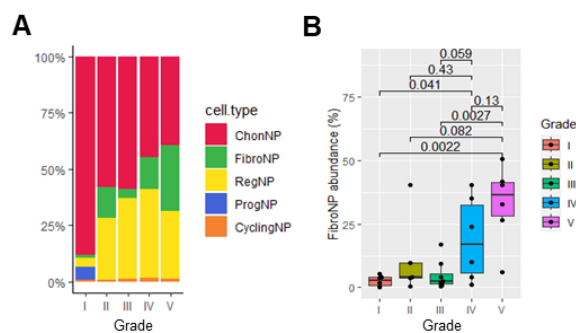
6

7

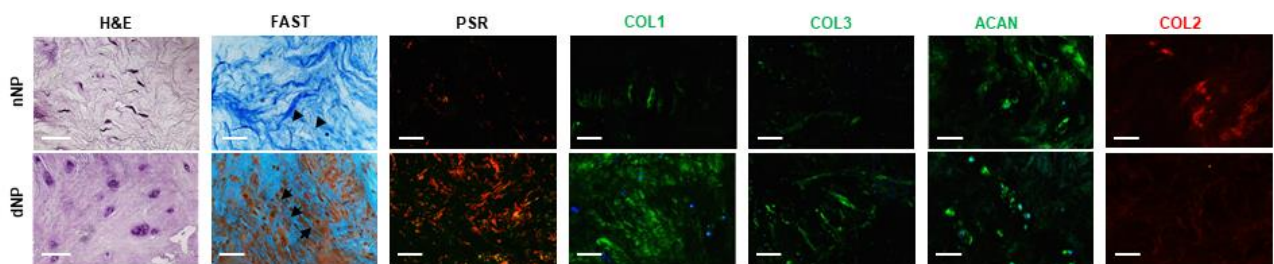
8 **Supplementary Figure S2. Association of NPC clusters with disc degeneration severity. (A)**

9 **Distribution of the five NPC clusters in different disc degeneration grades. (B) Abundance of the**

10 **FibroNP population in different grades. P values were calculated based on unpaired t test.**



17 **Supplementary Figure S3. Histological evaluation of nucleus pulposus fibrosis in human disc**
 18 **degeneration.** Various staining were performed on NP sections representing non-degenerative (nNP)
 19 or degenerative (dNP) condition: Hematoxylin and eosin staining (H&E); Multichromatic staining of
 20 Fast green/Alcian blue/Safranin O/Tartrazine (FAST); Polarized microscopy of Sirius red (PSR);
 21 immunofluorescence staining of fibrotic collagen I (COL1, green) and collagen III (COL3, green),
 22 and hyaline matrix components of aggrecan (ACAN, green) and collagen II (COL2, red). Arrow
 23 head: round-shaped cells; Arrow: spindle-shaped cells.



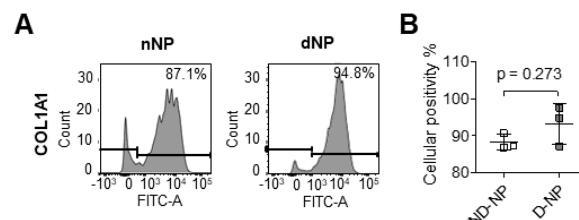
24

25

26

27

28 **Supplementary Figure S4. Flow cytometry analysis of COL1⁺ NP cells.** (A) Representative COL1
 29 flow cytometry of freshly isolated NP cells from non-degenerative scoliotic (nNP) and degenerative
 30 and herniated (dNP) discs. (B) Quantification of COL1⁺ cells (n=3). Two-tailed unpaired *t*-tests.

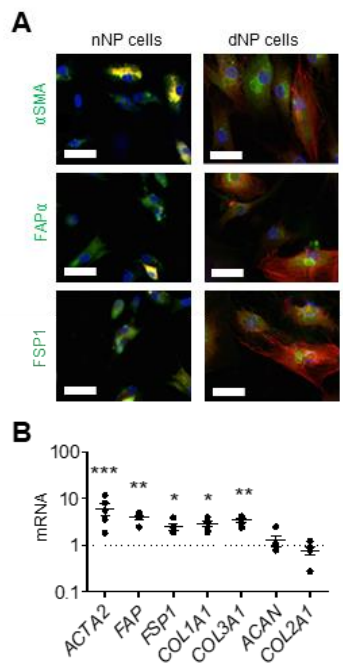


31

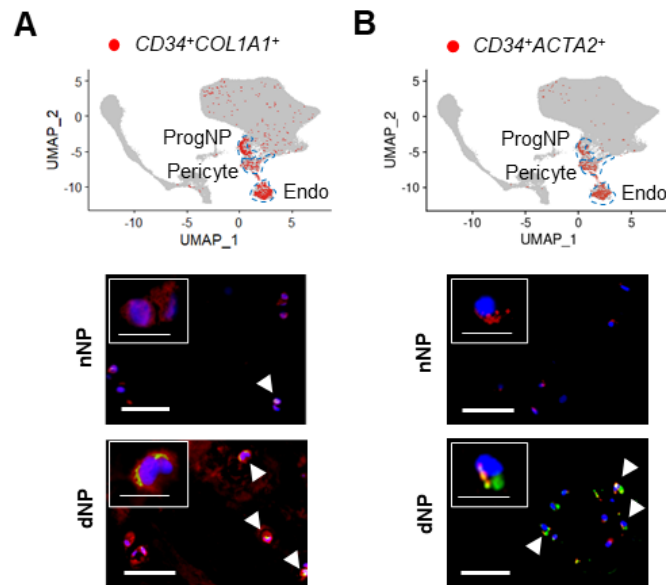
32

33

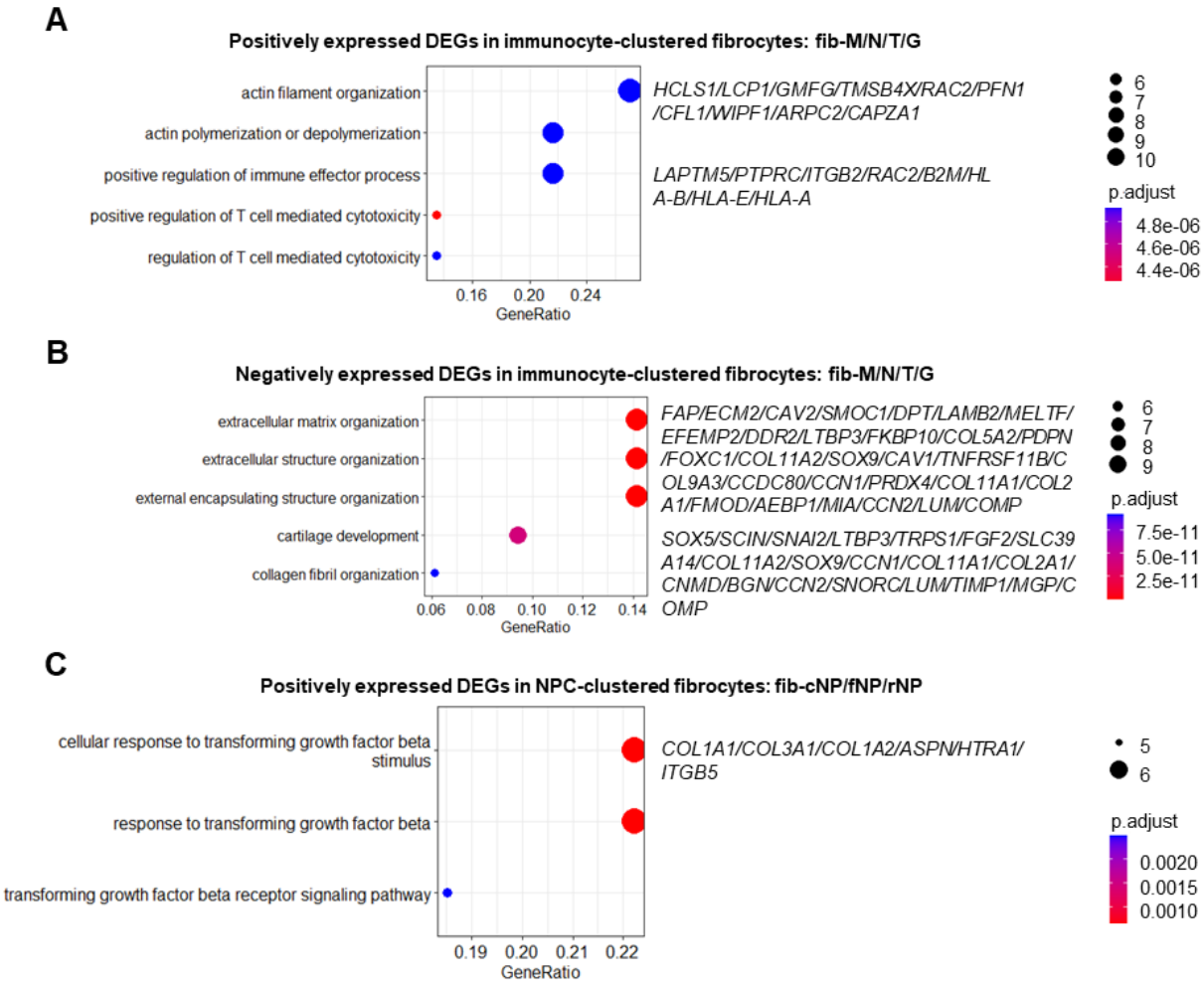
34 **Supplementary Figure S5. Characterization of myofibroblastic phenotype in NP cells culture.**
35 **(A)** Representative immunofluorescence of cultivated primary cells from scoliotic (nNP) and
36 degenerative (dNP) human NP tissues. Scale bar: 50 μ m. **(B)** RT-qPCR of gene markers of
37 myofibroblast and NPCs. * $p < 0.05$, ** $p < 0.01$ by one sample t -tests.



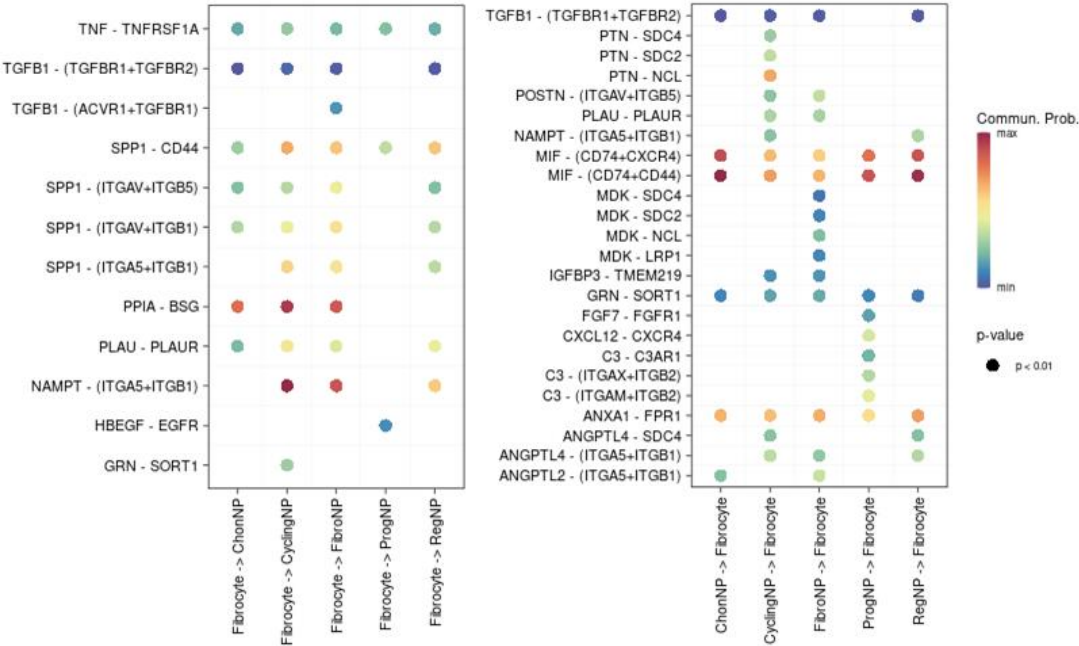
49 **Supplementary Figure S6. Analysis of putative fibrocyte marker CD34.** UMAP distribution and
50 immunodetection of co-expression for *CD34* with *COL1A1* (A) and *ACTA2* (B) in disc cells.
51 Immunostaining was conducted on human NP tissue sections from non-degenerative scoliotic (nNP)
52 or degenerated and herniated IVDs (dNP). CD34 was in green, and COL1/ α SMA in red. Scale bar:
53 50 μ m. Insert: representative cells micrograph showing positive signals; scale bar: 12 μ m.



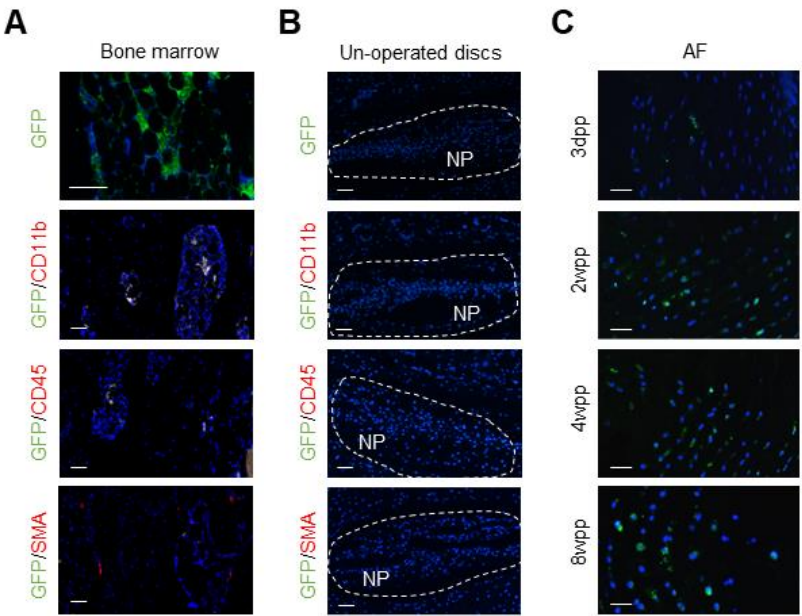
66 **Supplementary Figure S7. GO (gene ontology) functions of immunocyte- and NPC-clustered**
67 **fibrocytes.** (A) from 47 positively expressed DEGs and (B) from 236 negatively expressed DEGs of
68 immunocyte-clustered fibrocytes; (C) from 43 positively expressed DEGs of NPC-clustered
69 fibrocytes. Fib-M/N/T/G: fibrocyte distributed in macrophage, neutrophil, T cell and G-MDSC
70 clusters; fib-cNP/fNP/rNP: fibrocyte distributed in ChonNP, FibroNP and RegNP clusters.



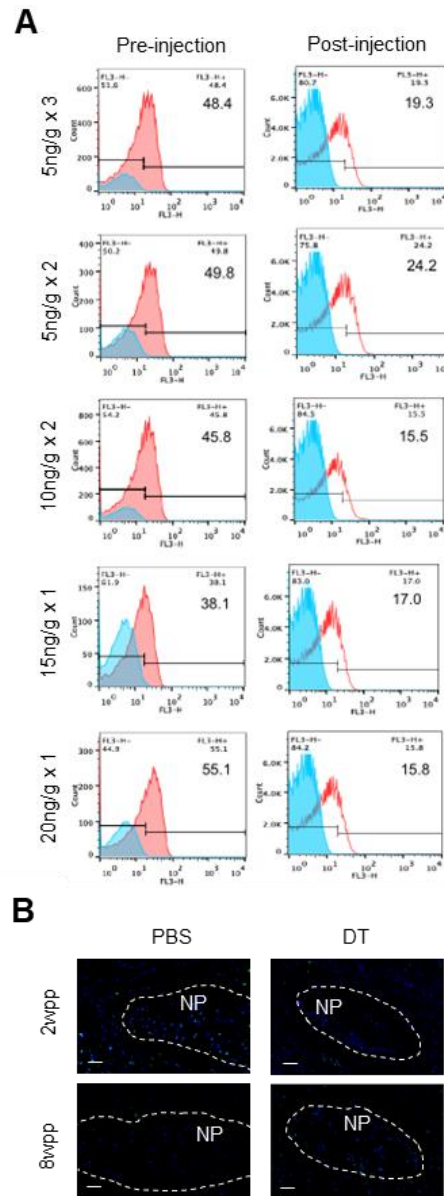
78 **Supplementary Figure S8. Bubble plot from CellChat analysis showing the molecular pattern**
 79 **of interaction between disc fibrocytes and the NPC clusters.**



84 **Supplementary Figure S9. Immunodetection of monocytic cells by GFP.** (A) Bone marrow stain;
85 (B) Healthy discs of CD11b-DTR mice. Non-punctured discs serve as sham control and negative to
86 GFP staining; (C) Monocytic fibrocyte presence in annulus fibrosus (AF). Scale bar: 50µm. dpp:
87 days post-puncture; wpp: weeks post-puncture.



101 **Supplementary Figure S10. Monocyte depletion in CD11b-DTR mice. (A)** Representative flow
 102 cytometry of CD11b⁺ monocyte in peripheral blood. Isotype control was shown in blue. (B)
 103 Representative micrographs for GFP stain in punctured discs. DT injection was conducted on
 104 CD11b-DTR mice 3 days prior to disc puncture and 4 weeks later at dosage of 10ng/g. Scale bar:
 105 50μm. wpp: weeks post-puncture.



109 **Supplementary Table S1. Summarization of disc cells atlas and conventional cellular markers.**

110 Four published single cell RNA sequencing analysis using human disc/NP tissues were included.

Ref	Annotation	Defined markers	Additional information
7	C1 chonNPC	<i>MMP3, PDE4B, CXCL8, TNFRSF11B, MGP, ADPRHL1, FGF2, BMP2, C11orf96, SOD2, CXCL2, GPX3, CLU</i>	Inflammatory process
	C2 chonNPC	<i>PRG4, SPARC, MSMO1, CYTL1, COL2A1, ABI3BP, S100A2, VCAN, TIMP1, HAPLN1</i>	Cholesterol biosynthetic process and maintenance of the structure
	C3 chonNPC	<i>COL12A1, SAA1, MMP13, TNC, CXCL1, CHI3L2, MMP2, AEBP1, GREM1, IFITM1</i>	Inflammatory responses and matrix disassembly
	C4 chonNPC	<i>POSTN, COL1A1, COL3A1, COL1A2, SPP1, TMSB4X, LGALS1, MMP13, COL6A3, MMP14</i>	Fibrous characteristics
	Cartilage Progenitor	<i>STMN1, HIST1H4C, LGALS1, NUSAP1, TOP2A, HIST1H1B, TUBB, CKAP2, PTTG1, POSTN, CENPF, BIRC5</i>	NP-derived progenitor
	Fibrochondrocyte Progenitor	<i>MYLK, TRIB3, TCEA1, ATF3, ATF5, SYNE1, GOT1, GDF15, CYR61, DEPP1, CANX</i>	Protein folding quality control and ER stress
	Homeostatic chondrocytes	<i>FOS, JUN, ZFP36, RGS16, IER2, ID3, JUNB, ATF3, EGR1, IRF1</i>	Responsive to stress
	Endothelial cells	<i>HES1, CALCRL, COL4A1, SPARCL1, PECAM1, IFI27, PLVAP, AQP1, COL15A1, LAMA4, CD34</i>	
	Macrophage	<i>CCL3L1, CCL4L2, CCL4, IL1B, CD74, CXCL3, CXCL8, HLA-DRA, CCL3, TNF, LAPTM, TYROBP</i>	
14	NP progenitor	<i>PLA2G2A, FBLN1, SERPINF1, CFD, PTGDS, WISP2, IGFBP6, GSN, MMP2, C1R, PDGFRA, PAX1, ANGPT1, PRG4, PRRX1</i>	Bone morphogenesis, connective tissue development

	Stromal cells	<i>ACTA2, MYL9, RGS5, MYH11, SYNPO2, STEAP4, NOTCH3, MCAM, ADIRF, ADIRF, FOXC2, GJA1, HES4</i>	
	Stromal cells- Fibroblast	<i>CEMIP, AKR1C1, MGP, COMP, DNER, MELTF</i>	
	Stromal cells- Neurogenic	<i>SOX2, NGFR, NCMAF, CLDN19</i>	
	Stromal cells- Osteogenic	<i>RUNX2, DLX5, SP7, BGLAP, MMP11</i>	
	Pericyte	<i>ITGB1, CARMN, ID3, C1QTNF1, MYL6, MAP3K20, TNS1, LGI4, ABCC9, ACTA2, TAGLN, MCAM</i>	
	Chon1 (C1/2)	<i>CYTL1, IBSP, CRYAB, APOD, IL17B, RBP4, C2orf40, CHAD, SPP1, HSPA6</i>	Regulatory chondrocytes secreting growth factors, AF and CEP chondrocytes
	Chon2 (C3/4)	<i>NEAT1, MALAT1, DST, COL11A1, COL2A1, FN1, ACAN, FMOD, COMP, SLC5A3, CCNL1, WSB1</i>	Homeostatic chondrocytes, ECM homeostasis and circadian rhythm
	Chon3 (C5/6)	<i>CHRD12, CAPS, NDUFA4L2, ABI3BP, CNMD, CHI3L1, MT1E, MT1X, VCAN, CRISPLD1, PRG4, KLF2, COL5A1, EPYC</i>	Effector chondrocytes, metabolic active
	Notochord NPC	<i>KRT19, CA3, EPYC, ENPP2, CD24, CTHRC1, ACTC1, PCSK2, RAPGEF5, RAB3B, KRT8, TBXT</i>	
	Endothelial cells	<i>CD74, IFI27, PECAM1, RAMP2, GNG11, CALCRL, VWF, EGFL7, STC1, HLA-E, CD34, CDH5, ERG</i>	
	Blood cells	<i>LYZ, S100A9, S100A8, CXCL8, SRGN, AC020656.1, MPO, CCL3, TYROBP, AZU1</i>	

12	Effector NPC	<i>MSMO1, HMGCS1, INSIG1</i>	Metabolic process, Positive regulation of ECM assembly
	Hypertrophic chonNPC	<i>FRZB, DKK1, EGR1</i>	Programmed cell death; ECM disassembly
	Adhesion NPC	<i>FN1, CRTAC1, FMOD</i>	Cell migration, cell– matrix adhesion.
	FibroNPC	<i>COL1A1, COL3A1, MMP2, COL6A1, TGFB</i>	Fibrosis related
	Homeostatic NPC	<i>RPS29, RPS21, RPL31</i>	Cellular homeostasis
	Regulatory NPC	<i>CHI3L1, NFKB, CXCL2, CXCL3, IL6</i>	Cellular responses to inflammation and endogenous stimuli
	NK cells	<i>CD94</i>	
	Macrophage	<i>CD163</i>	
	T cell	<i>TRAC</i>	
	G-GMP	<i>MS4A3, MPO, ELANE</i>	
	Neutrophil	<i>FCGR3B, HLADR</i>	
	G-MDSC	<i>ITGAM, OLR1, ARG1, CD45</i>	Immunopression, ROS production
11	Endothelial progenitor	<i>PECAM1, CDH5, CD34, KDR</i>	
	Erythrocytes	<i>HBA1, HBB</i>	
	Effector NP cells, EffectorNP	<i>COL2A1, SPARC, CLEC3A, COL3A1, FMOD, CYTL1</i>	ECM organization, cartilage development
	Homeostasis NP cells, HomNP	<i>MT1G, EMP1, HSPB1, S100A2, RGCC, ANXA1</i>	Responsive to stress, detoxification of inorganic compound
	Hypertrophic NP cells, HTNP	<i>MMP3, CXCL2, SOD2, C11orf96, BMP2, SERPINE2</i>	Cellular response to external stimulus, inflammatory reaction
	NP progenitor cells, NPPC	<i>TMSB4X, NEAT1, XIST, MALAT1</i>	Chondrocyte differentiation,

			extracellular structure organization, ossification
	CD24+ progenitor	<i>CD24, KRT19, KRT8, GJA1, LGALS3, APOE, CA3</i>	Protein synthesis, cellular pluripotency
	MK167+ progenitor	<i>MK167, CENPF, STMN1, POSTN, TUBB, TUBA1B, COL1A1</i>	Epithelial-mesenchymal transition (EMT), inflammatory response
	AF cells	<i>COL1A1, CRTAC1, ASPN, MMP2</i>	

111

112

113

114

115

116

117

118

119

120

121

122

123

124

125

126

127

128

129

130 **Supplementary Table S2. Fibrocyte count in NP cells clusters.** Either *ITGAM*⁺*COL1A1*⁺ or
 131 *PTPRC*⁺*COL1A1*⁺ cells are regarded as disc fibrocytes, and their number in NP cells clusters (as
 132 described in Fig. 1) were count.

NP cells clusters	<i>ITGAM</i> ⁺ <i>COL1A1</i> ⁺		<i>PTPRC</i> ⁺ <i>COL1A1</i> ⁺		<i>Disc fibrocytes</i>	
	Count	%	Count	%	Count	%
ChonNP	38	3.47	54	3.11	89	4.16
FibroNP	97	8.87	69	3.97	160	7.48
RegNP	36	3.29	30	1.73	64	2.99
ProgNP	0	0.00	3	0.17	3	0.14
CyclingNP	4	0.37	6	0.35	9	0.42
Neutrophil	411	37.57	647	37.23	732	34.24
G-MDSC	21	1.92	52	2.99	63	2.95
Macrophage	462	42.23	629	36.19	756	35.36
T cell	16	1.46	238	13.69	243	11.37
Endothelial cell	4	0.37	3	0.17	7	0.33
Pericyte	4	0.37	6	0.35	10	0.47
Erythrocyte	1	0.09	1	0.06	2	0.09

133
 134
 135
 136
 137
 138
 139
 140
 141
 142

143 **Supplementary Table S3. Safety evaluation of DT injection in CD11b-DTR mice.** Depletion
 144 efficiency was calculated based on number of CD11b+ cells in peripheral blood. Three animals were
 145 included for each condition. A: >1 living mice at week 8; D: all three mice dead at week 8.

Dosage	Frequency	Time frame/ weeks					Depletion efficiency
		1 st	3 rd	5 th	6 th	8 th	
5ng/g	3	√	√	√		A	55.80%
	2	√		√		A	45.78%
10ng/g	3	√	√	√		D	N/A
	2	√		√		A	65%
15ng/g	2	√		√		D	N/A
	1	√				A	55.46%
20ng/g	2	√		√		D	N/A
	1	√				A	68.50%
25ng/g	2	√		√		D	N/A
	1	√				D	N/A

146
 147
 148
 149
 150
 151
 152
 153
 154
 155
 156
 157

158 **Supplementary Table S4. Demographics of IVD donors.** Grade of disc degeneration was
 159 determined by MRI according to Pfirrmann Scale. F, female; M, male; AIS, adolescent idiopathic
 160 scoliosis; DDD, disc degeneration disease; ND, not determined.

Sample ID	Gender	Age (Years)	Level	Pathology	Disc grade
1	F	13	L2-3	AIS	ND
2	F	13	L2-3	AIS	ND
3	M	15	L2-3	AIS	ND
4	F	15	L2-3	AIS	ND
5	F	16	L1-2	AIS	ND
6	F	15	L2-3	AIS	ND
7	M	28	L5-S1	DDD	IV
8	M	40	L3-L4	DDD	III
9	F	42	L4-L5	DDD	IV
10	F	47	L3-L4	DDD	V
11	F	56	L4-L5	DDD	III
12	M	58	L3-L4	DDD	V
13	M	59	L4-L5	DDD	IV
14	F	45	L4-L5	DDD	IV
15	M	71	L4-L5	DDD	IV

161
 162
 163
 164
 165
 166
 167
 168
 169
 170

171 **Supplementary Table S5. Antibodies used for immunofluorescence and flow cytometry (FC)**
172 **analysis.**

Product Cat#	Antigen	Host	Conjugation	Application
EPR14664	Aggrecan	Rabbit	N/A	ACAN in human NP (Supplementary Fig. S1)
Ab34710	Collagen I	Rabbit	N/A	COL1 in human NP (Supplementary Fig. S1); FC for COL1 (Supplementary Fig. S4); Co-stain with CD34 in Cd11b-DTR mice IVDs
Ab88147	Collagen I	Mouse	N/A	FC for COL1 (Fig. 2); Co-stain with CD45, α SMA in human NP (Fig. 2)
EPR12268	Collagen II	Rabbit	N/A	COL2 in human NP (Supplementary Fig. A1)
Ab7778	Collagen III	Rabbit	N/A	COL3 in human NP (Supplementary Fig. S1)
EPR5368	α SMA	Rabbit	N/A	FC for α SMA (Fig. 1); α SMA in human NP (Fig. 1); co-stain with GFP in CD11b-DTR mice IVDs (Fig. 3)
Ab7817	α SMA	Mouse	N/A	Co-stain with CD45 in human NP (Fig. 2)
EPR20021	Fap- α	Rabbit	N/A	FC for FAP α (Fig. 1); FAP α in human NP (Fig. 1);
EPR2761-2	Fsp1	Rabbit	N/A	FC for FSP1 (Fig. 1); FSP1 in human NP (Fig. 1);
Ab23910	CD45	Rat	N/A	Co-stain with COL1 and α SMA in CD11b-DTR mice IVDs (Fig. 4)
Ab10558	CD45	Rabbit	N/A	Co-stain with COL1 in human NP (Fig. 2), and GFP in CD11b-DTR mice IVDs (Fig. 3)
9B10D4	CD34	Mouse	N/A	Co-stain with COL1 and α SMA in human NP (Supplementary Fig. S6)
Ab184308	CD11b	Rabbit	N/A	Co-stain of CD11b with GFP in CD11b-DTR mice IVDs (Fig. 3); FC for CD11b in CD11b-DTR mice IVDs (Supplementary Fig. S9)
Ab5450	GFP	Goat	N/A	GFP in CD11b-DTR mice IVDs (Fig. 3)

Ab150077	Rabbit IgG H&L	Goat	Alexa Fluor® 488	Immunostaining of COL1/3, ACAN, α SMA, FAP α (Supplementary Fig. S1 and Fig. 1); FC for α SMA, FAP α , FSP1, COL1 and CD45 in human NP cells (Fig. 1 & 2, Supplementary Fig. S4); co-stain of CD45 with COL1 and α SMA in human NP (Fig. 2); co-stain of COL1 and α SMA with CD45 in CD11b-DTR mice (Fig. 4)
Ab150080	Rabbit IgG H&L	Goat	Alexa Fluor® 594	Immunostaining of COL2, FSP1 in human NP (Supplementary Fig. S1 & Fig. 1) and COL1 in CD11b-DTR mice IVDs (Fig. 4); FC for α SMA, Fap- α and Fsp1 (Fig. 1); Co-stain of CD45 with COL1 (Fig. 2), COL1 and α SMA with CD34 in human NP (Supplementary Fig. S6)
Ab150116	Mouse IgG H&L	Goat	Alexa Fluor® 594	FC for COL1 (Fig. 2); Co-stain of COL1 and α SMA with CD45 in human NP (Fig. 2)
Ab150160	Rat IgG H&L	Goat	Alexa Fluor® 594	Co-stain of CD45 with COL1 and α SMA in CD11b-DTR mice IVDs (Fig. 4)
Ab150113	Mouse IgG H&L	Goat	Alexa Fluor® 488	Co-stain of COL1 and α SMA with CD34 in human NP (Supplementary Fig. S6)
Ab150129	Goat IgG H&L	Donkey	Alexa Fluor® 488	GFP and co-stain with CD11b, CD45, α SMA in CD11b-DTR mice IVDs (Fig. 3)
Ab175470	Rabbit IgG H&L	Donkey	Alexa Fluor® 568	Co-stain of CD11b, CD45, α SMA with GFP in CD11b-DTR mice IVDs (Fig. 3)

173

174

175

176

177 **Supplementary Table S6.** Primers for real-time PCR.

Gene name	Forward	Reverse
<i>ACTA2</i>	CTATGAGGGCTATGCCTTGCC	GCTCAGCAGTAGTAACGAAGGA
<i>FAP</i>	ATGAGCTTCCTCGTCCAATTCA	AGACCACCAGAGAGCATATTTTG
<i>SI00A4</i>	GATGAGCAACTTGGACAGCAA	CTGGGCTGCTTATCTGGGAAG
<i>COL1A1</i>	ATCAACCGGAGGAATTTCCGT	CACCAGGACGACCAGGTTTTC
<i>COL3A1</i>	GCCAAATATGTGTCTGTGACTCA	GGGCGAGTAGGAGCAGTTG
<i>COL2A1</i>	TGGACGATCAGGCGAAACC	GCTGCGGATGCTCTCAATCT
<i>ACAN</i>	ACTCTGGGTTTTTCGTGACTCT	ACACTCAGCGAGTTGTCATGG
<i>GAPDH</i>	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

178

179

180 **Supplementary methods**

181 *Disc height index calculation*

182 Based on the X-rays for coccygeal IVDs of Cd11b-DTR mice, the lengths of the discs and adjacent
183 vertebral bodies were measured with Image J (Version 1.42, National Institutes of Health). Disc
184 height index (DHI) was calculated according to the formula: DHI = the disc height (averaging three
185 measurements of the heights of lateral and middle portions of the disc)/ the vertebral body height
186 (averaging three measurements of the heights of the lateral and middle adjacent caudal vertebral
187 body), and. Level C6/7 of tail disc was un-operated and further used as reference for DHI
188 calculation.

189