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Supplemental information

Single-cell transcriptomics reveals

heterogeneity and intercellular crosstalk

in human intervertebral disc degeneration

Dandan Wang, ZiZhang Li, Weimin Huang, Shengnan Cao, Liangyu Xie, Yuanzhen Chen, Huazhong Li, Lei Wang, Xiaoshu Chen, and Jian-Rong Yang



Supplementary Fig. 1 Principal component analysis (PCA) of single-cell transcriptomes of human NP cells, Related to Figure 2.(A) PCA plot showing Part 1-4 NP clusters and colored according to the IVD degeneration grades. D1-D5, Pfirrmann grade I-V. (B) Distribution of four parts of NP clusters (defined in A) in human D1-D5 IVDs. (C) Heatmap revealing the scaled expression of differentially expressed genes along PC1 and PC2.



Supplementary Fig. 2 The expression and heatmap of marker genes of NP subclusters, Related to Figure 2. (A) The average expression of curated feature genes for cell clusters defined in Figure 2 A on the UMAP map. (B) Heatmap showing the scaled expression of differentially expressed genes of NP subclusters defined in Figure 2 A.

2 1 0 -1 -2

А В NPPC MKI67+ progenitor Cell cycle HTNP phase G1 G2M S HomNP IMAP EffectorNP UMA Epithelial mesenchymal transition D CD24+ progenitor 0.5 Running Enrichment Score 0.4 50% 0% 25% 75% 100% 0.3 0.2 0. С CD24+ progenitor MKI67+ progenitor 0.0 KRT19 KRT8 LGALS3 MKI67+ progenito RAB3B Rank in Ordered Dataset CD24 HOPX G2M checkpoint PDE1A



Cell cycle

phase

• G1 • G2M

• S

CD24-



Supplementary Fig. 3 The functional comparation and DEGs of CD24+ progenitor and MKI67+ progenitor, Related to Figure 2and Figure 3.

2

1

0

-1

-2

(A) The distribution of NP cell clusters in different cell cycle phase. MKI67+ progenitor showing more percentage in G2/M phase.

- (B) UMAP plot showing the distribution of NP cell clusters and colored according to the different cell cycle phase.
- (C) Heatmap showing the scaled expression of differentially expressed genes of CD24+ progenitor and MKI67+ progenitor.
- (D) Gene Set Enrichment Analysis (GSEA) dataset showing the main functional difference between CD24+ progenitor and

MKI67+ progenitor.

RAPGEF5

APOE RARRES2 ID4 KRT18

> GJA1 LYST

ACAN ADIRF

SNORC

PCDH9 CA3

CHI3L2 TYMS

PCLAF

STMN1 CENPF

TOP2A NUSAP1 PTTG1

MKI67 TUBA1B DPT NUCB2 UBE2C TNFAIP6 CLEC11A LGALS1

PRG4 HMGB2 POSTN HIST1H4C

COL1A1



Supplementary Fig. 4 GO functions and heatmap of the expression of differentially expressed genes of NPPC_1-3 and EffectorNP_1-3, Related to Figure 3.

(A) GO functions of NPPC_1-3 clusters. (B) Heatmap showing the scaled expression of differentially expressed genes of NPPC_1-3 clusters. (C) GO functions of EffectorNP_1-3 clusters.(D) Heatmap showing the scaled expression of differentially expressed genes of EffectorNP_1-3 clusters.



Supplementary Fig. 5 Intercellular crosstalk analysis among NP cells, AF cells, progenitors, and immune cells in the degeneration process of human IVD, Related to Figure 7.

(A) Heatmap showing the intercellular crosstalk among NP cells, AF cells, progenitors, and immune cells in normal (D1), mild (D2-3) and severe (D4-5) degenerated IVDs. Height of column represent differential interaction strength between corresponding two cell clusters compared with other groups. (B) Outgoing and incoming interaction strength of NP cells, AF cells, progenitors, and immune cells in normal, mild and severe degenerated IVDs. Dots indicate the ligand amount. (C) Histogram showing the number of inferred interactions between NP cells, AF cells, progenitors, and immune cells in normal, mild and severe degenerated IVDs.



Supplementary Fig. 6 The GO functions of NP (A), AF (B), CD24+ progenitor (C), MKI67+ progenitor (D), MCAM+ progenitor (E), and M φ -SPP1 clusters (F) regulated by ligands of other cell clusters in normal (D1), mild (D2-3) and severe (D4-5) degenerated grades of IVDs, Related to Figure 7.

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