

Podocalyxin and ciliary neurotrophic factor receptor are novel components of the surfaceome of chondrogenic cells

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Figure S1. Volcano plots showing differentially expressed proteins (DEPs) between consecutive time points, and between undifferentiated (day 1) and mature/hypertrophic (day 15) chondrogenic micromass cultures (total lysates). Cutoff values: $\log_2$ fold change > 1 (x-axis) and adjusted p -value < 0.05 (y-axis). Red dots signify proteins that were upregulated in later time points, whereas blue signifies proteins downregulated in later time points.

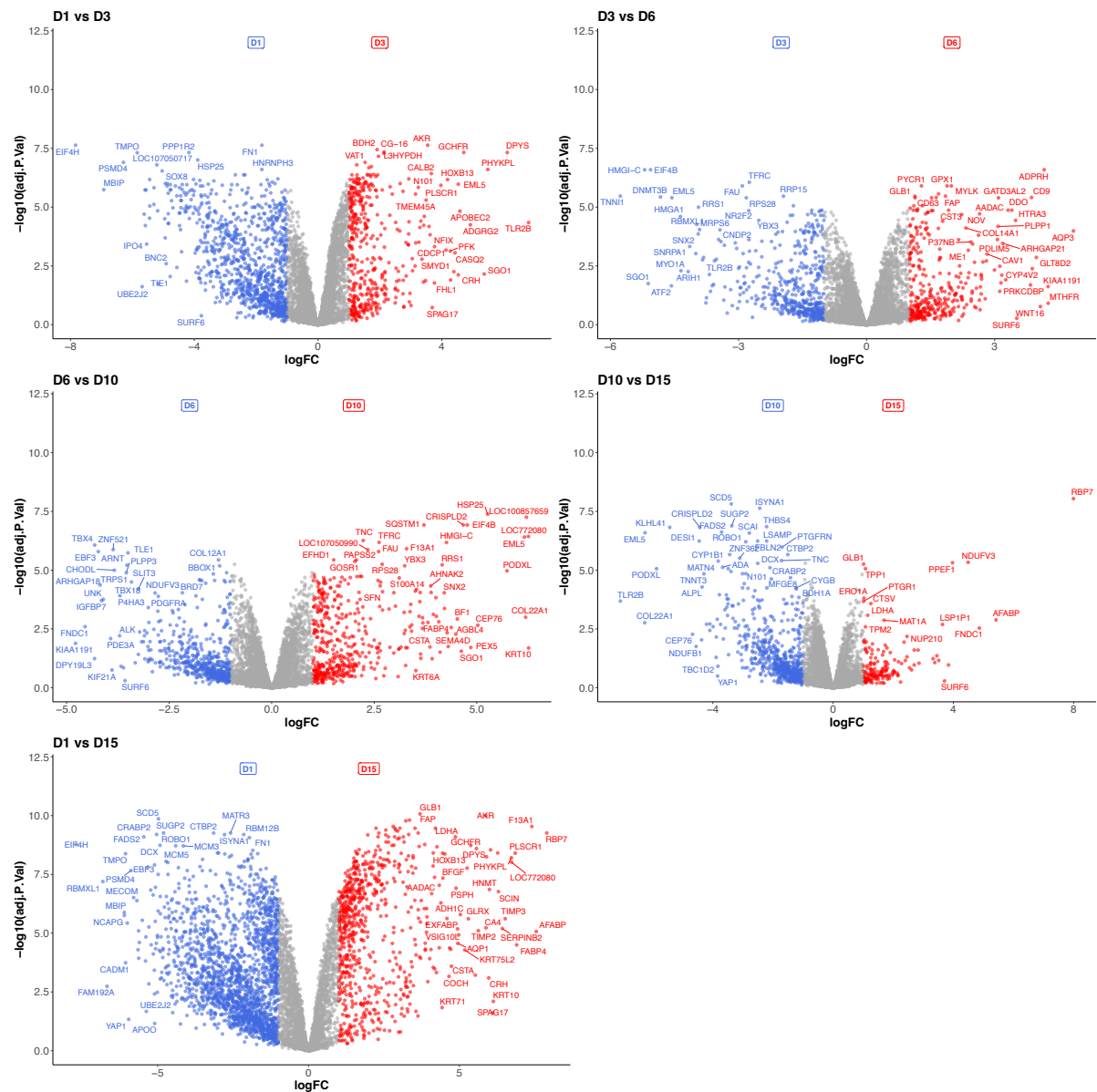


Figure S2. Protein-protein interaction (PPI) analysis of the 6 clusters in total cell lysates using STRING based on the top 20 (hub) proteins. In each subnetwork, the size of the circles represented the degree of value: larger circles represent proteins with more connections (higher degree), meaning they interact with more other proteins in the network.

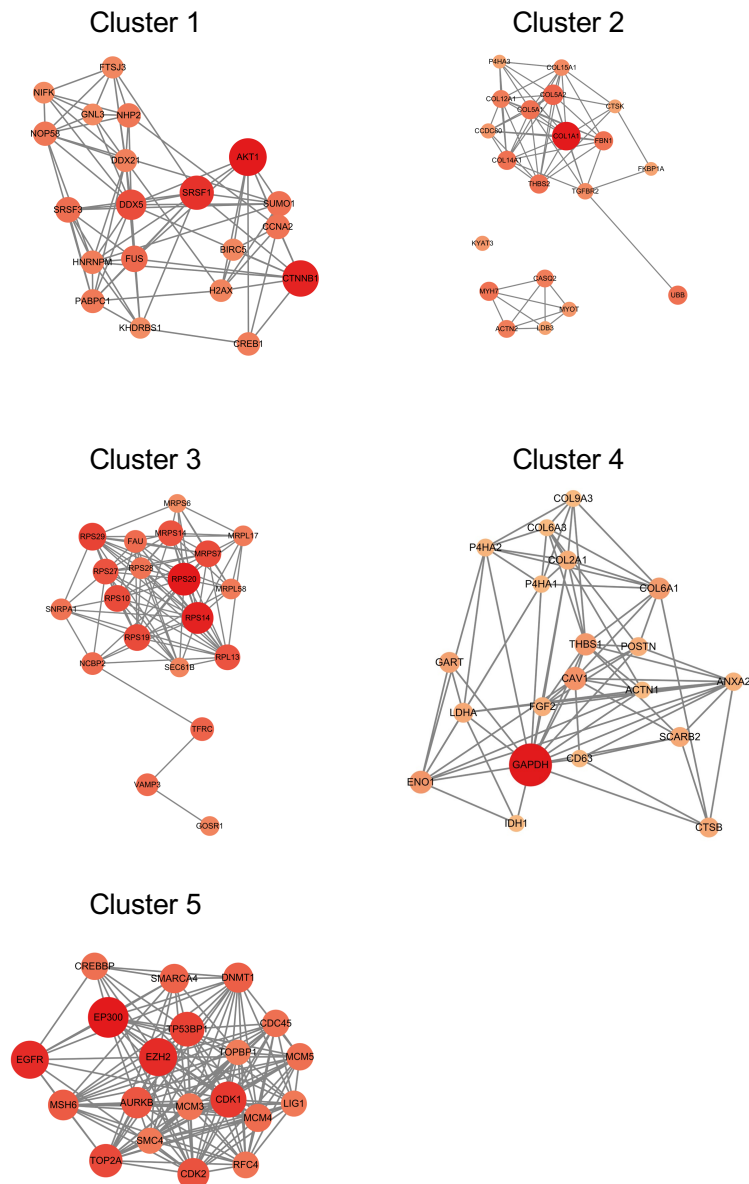


Figure S3. Volcano plot highlighting differentially expressed proteins between consecutive time points, and between Day 1 (undifferentiated) and Day 15 (mature/hypertrophic) chondrogenic micromass cultures after AOB-enrichment. Cutoff values: $\log_2$ fold change > 1 (horizontal axis) and adjusted p -value < 0.05 (vertical axis). Red dots signify proteins that were upregulated in later time points, whereas blue signifies proteins downregulated in later time points.

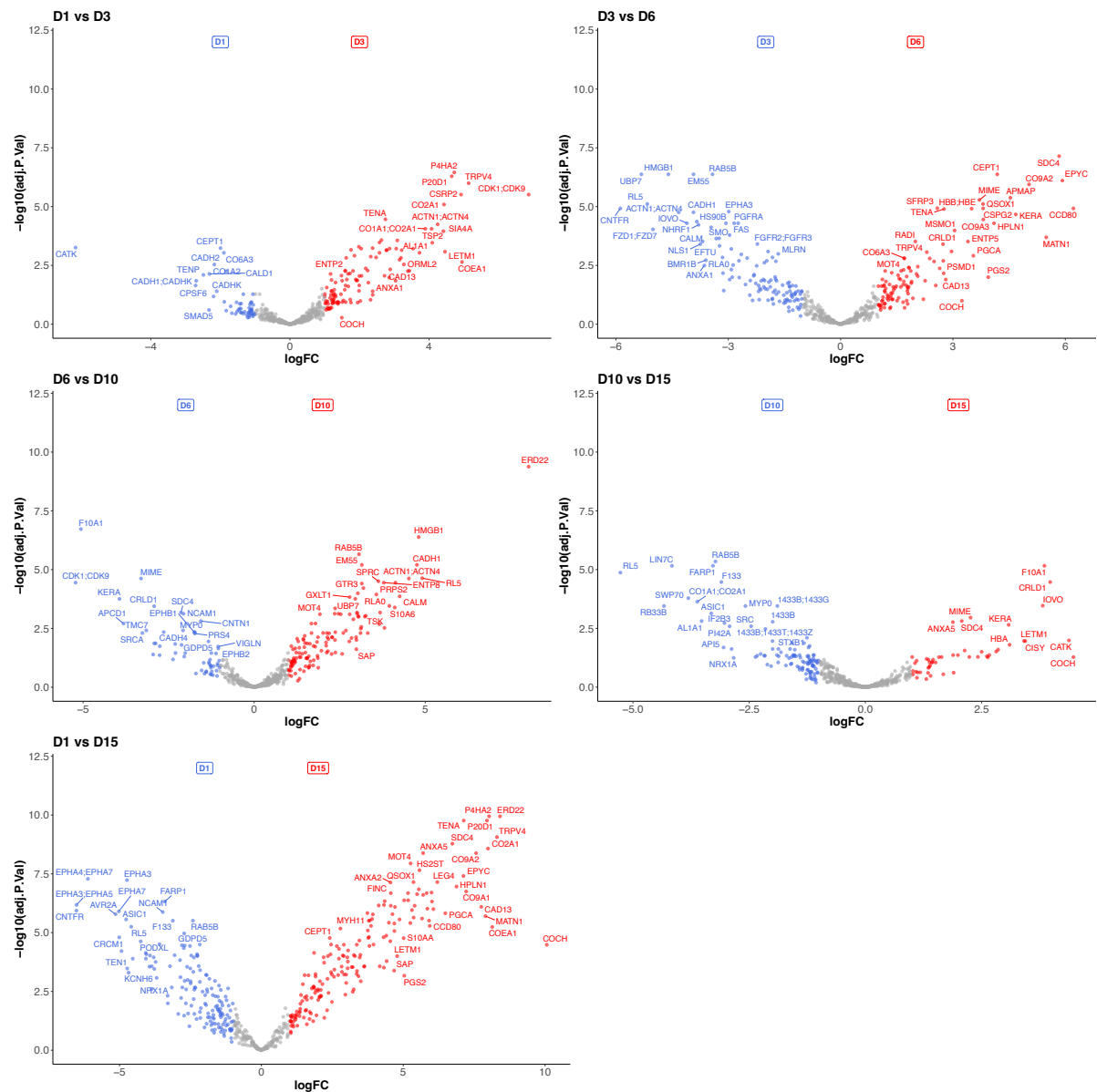


Figure S4. Protein-protein interaction (PPI) analysis of the 4 clusters in surfaceome samples using STRING. In each network/subnetwork, the size of the circles represented the degree of value: larger circles represent proteins with more connections (higher degree), meaning they interact with more other proteins in the network.

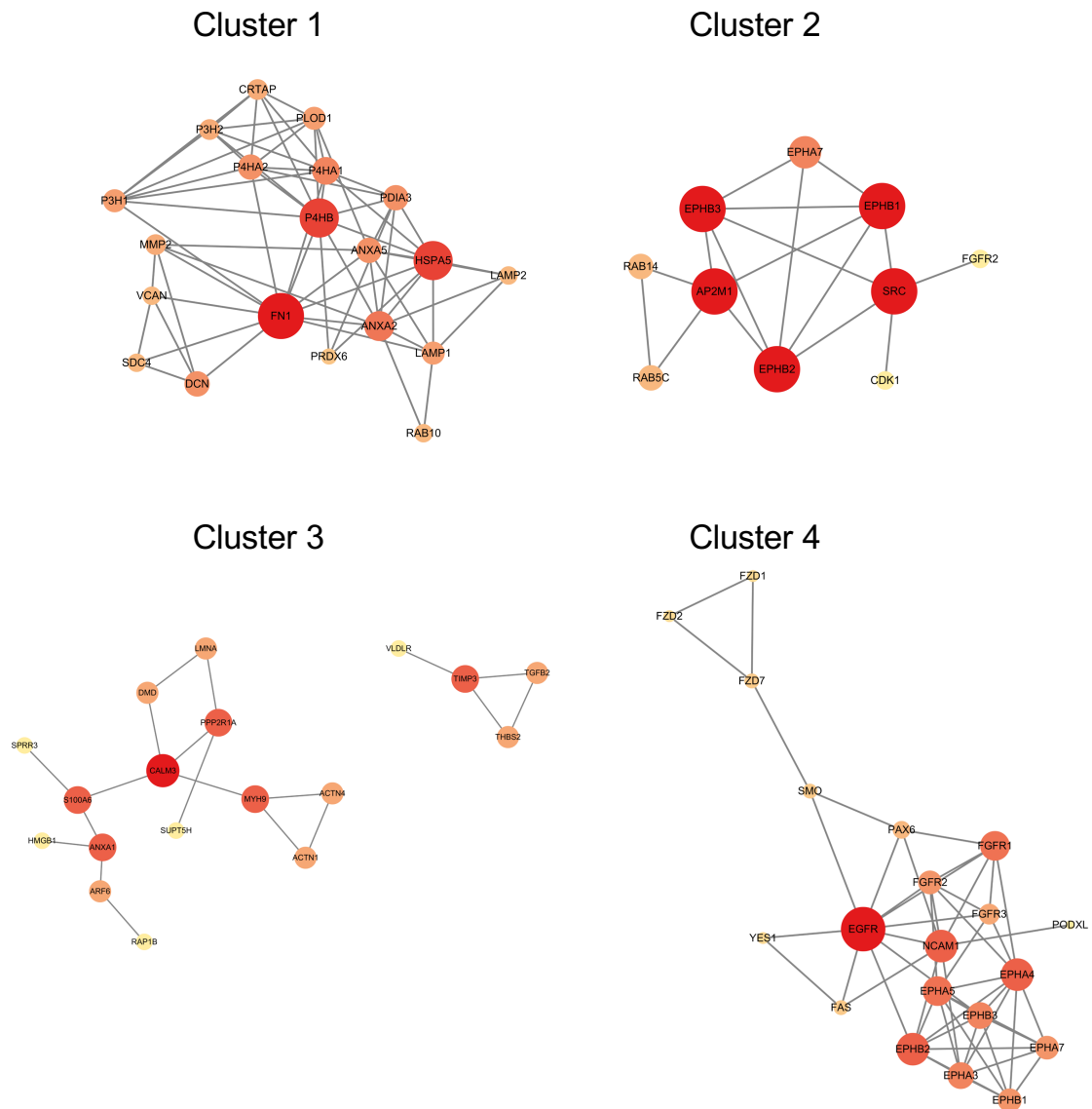


Figure S5. Full-length uncropped blots for the western blot images presented in Figure 7.

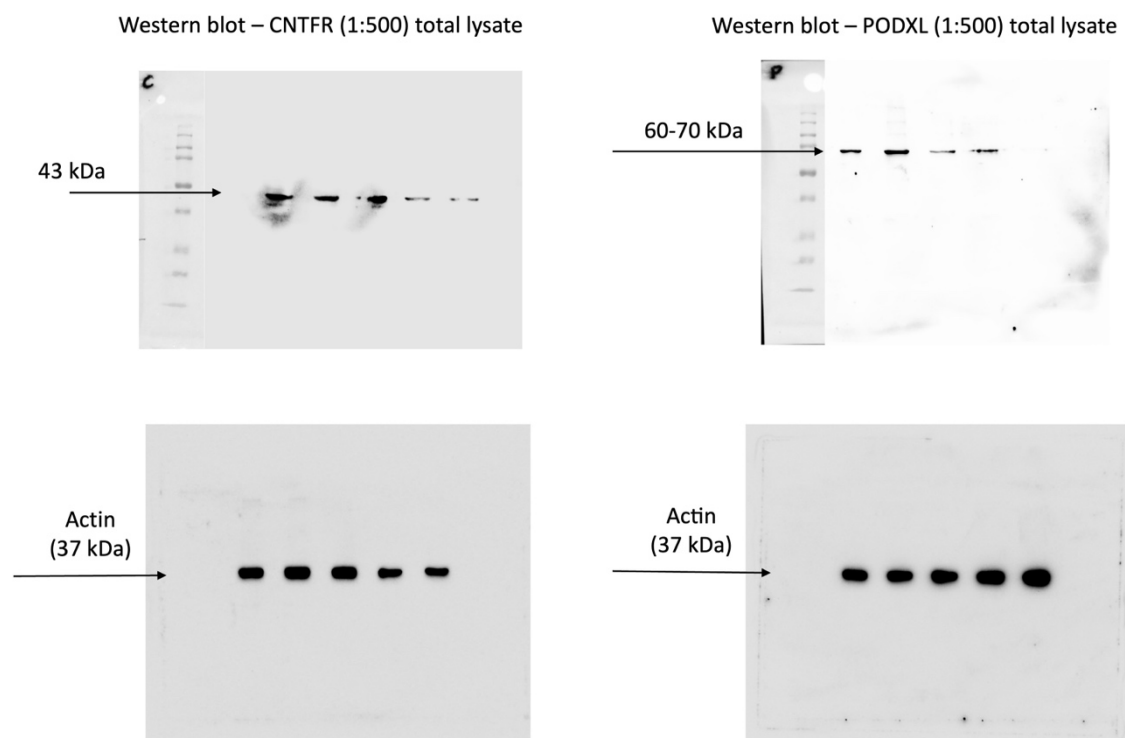


Figure S6. Weighted gene co-expression network analysis (WGCNA) results performed on the NGS dataset to identify clusters of highly correlated genes to CNTFR or PODXL, using culture age (time) as a trait. For CNTFR, the turquoise module eigengene (ME) was showing the highest degree of correlation; for PODXL, the black ME showed the highest correlation to the trait.

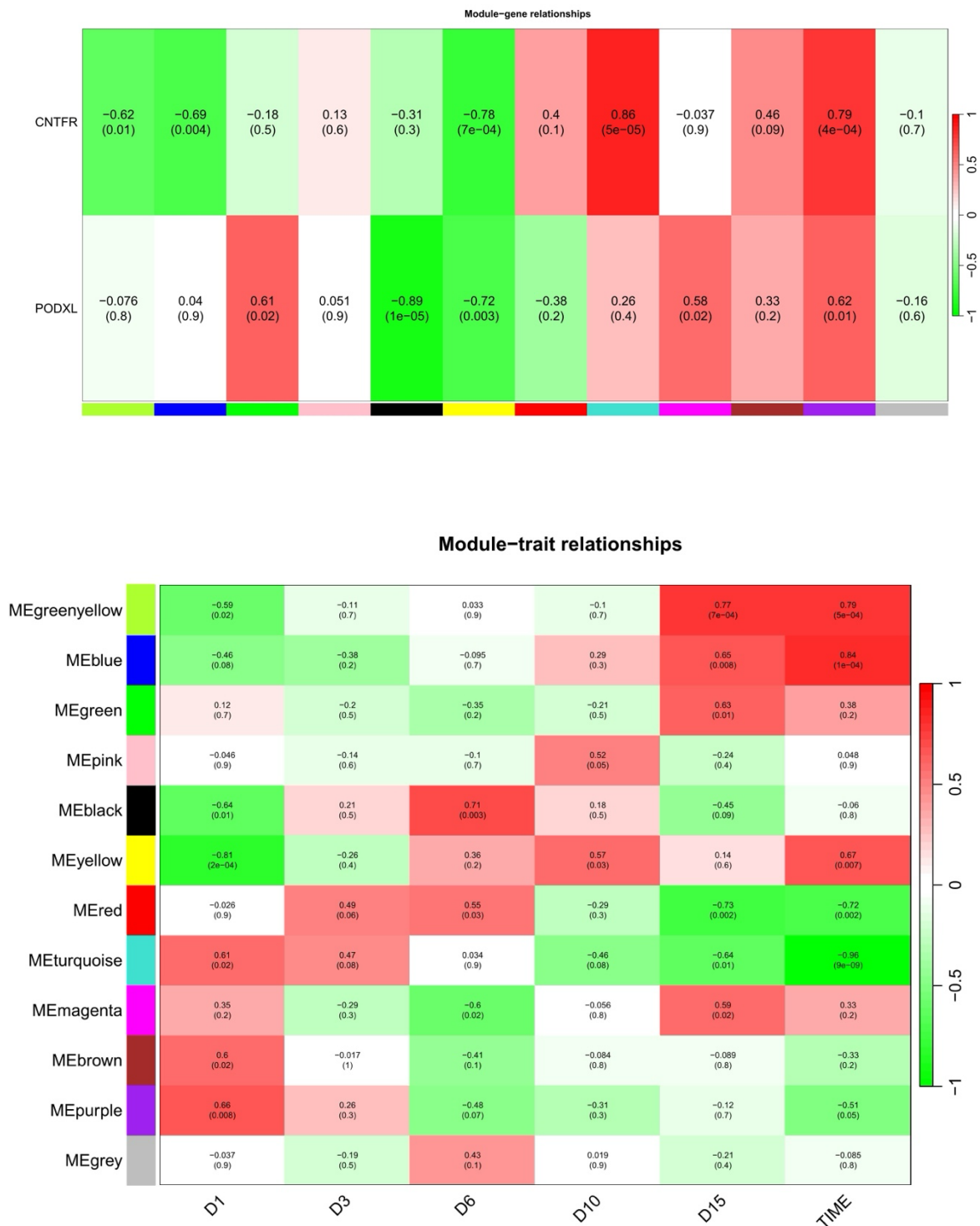
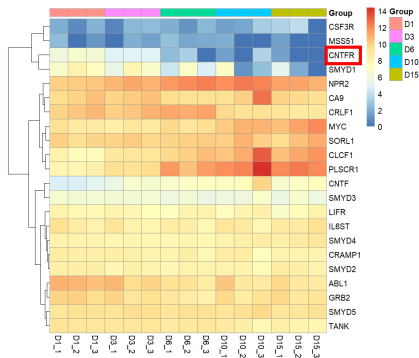


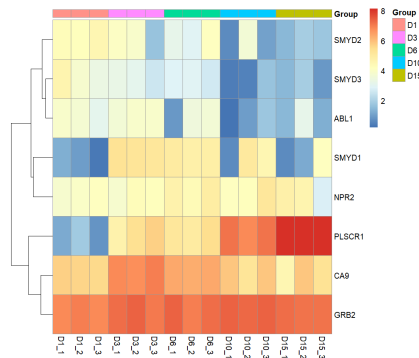
Figure S7. Transcript and protein expression patterns for the closest 25 interactants, as well as WGCNA co-expression networks, and enriched gene ontology (GO) terms in the MEs for CNTFR (A) and PODXL (B).

A. CNTFR

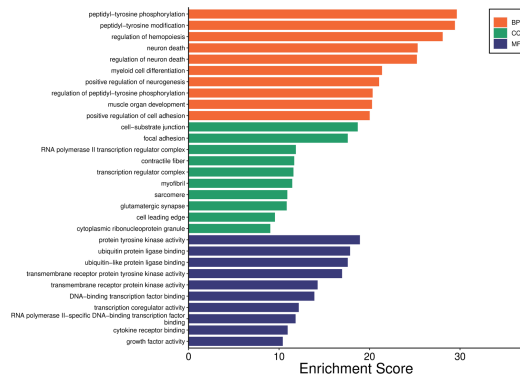
Transcript expression pattern



Protein expression pattern

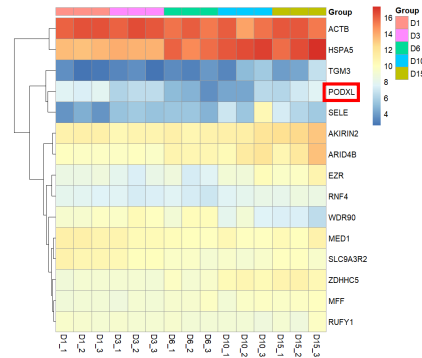


Gene ontology enrichment

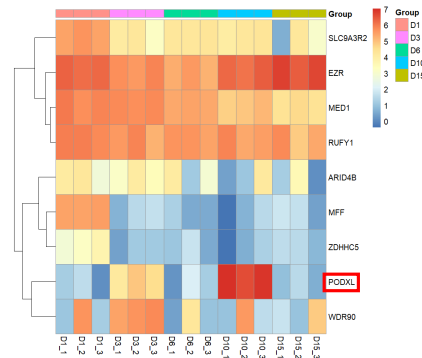


B. PODXL

Transcript expression pattern



Protein expression pattern



Gene ontology enrichment

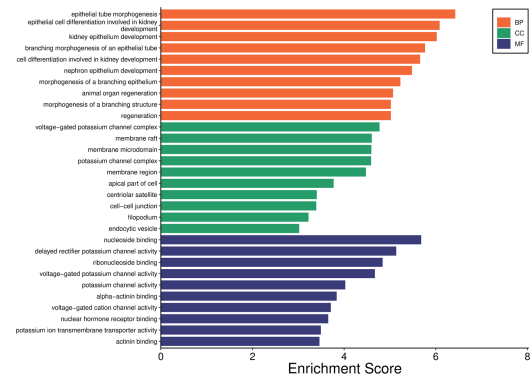
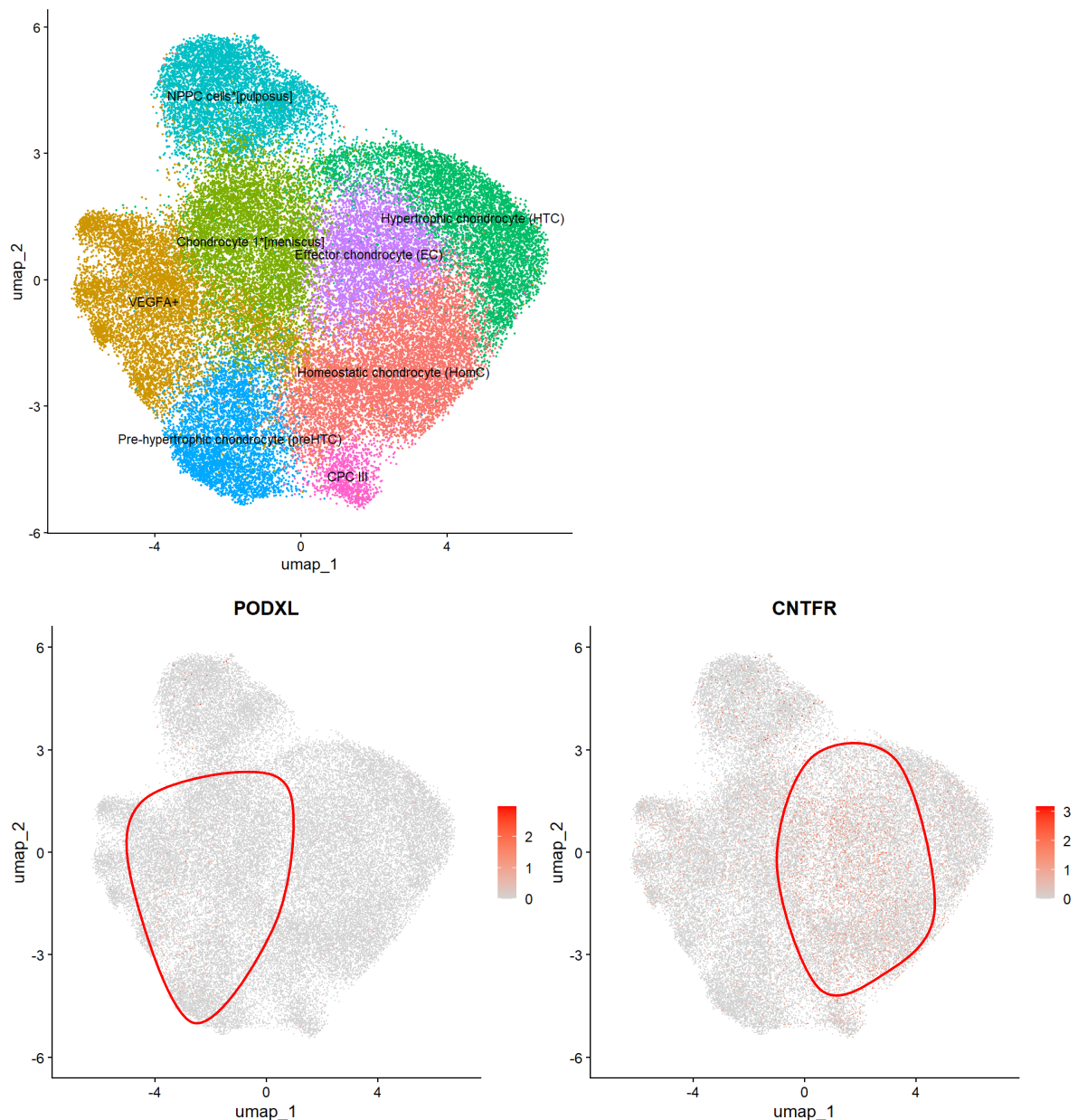


Figure S8. The single-cell atlas of healthy cartilage tissue integrates data from two public datasets: cartilage samples derived from the ankle joints of five healthy individuals (GSE216578), and healthy knee joint cartilage specimens obtained from three individuals in dataset GSE255460. Data integration and analytical processing were performed using the Seurat R package. In the single-cell atlas of healthy cartilage tissue, we identified eight chondrocyte clusters and systematically annotated their cellular identities (upper panel). Subsequent analysis revealed the expression patterns of CNTFR and PODXL across these chondrocyte subpopulations (lower panel). Notably, CNTFR demonstrated predominant expression enrichment in both effector chondrocytes (EC) and homeostatic chondrocytes (HomC). This expression signature suggests potential functional regulation of CNTFR during chondrocyte differentiation stages. PODXL demonstrated much weaker expression profile throughout chondrocyte populations, perhaps with somewhat higher transcript levels in pre-hypertrophic chondrocyte (Pre-HTC) subpopulations.



The dot plot below shows that CNTFR is highly expressed mainly in Homeostatic chondrocytes (HomC) and Effector chondrocytes (EC), while PODXL is predominantly expressed in Regulatory chondrocytes (RegC) and Pre-hypertrophic chondrocytes (preHTC).

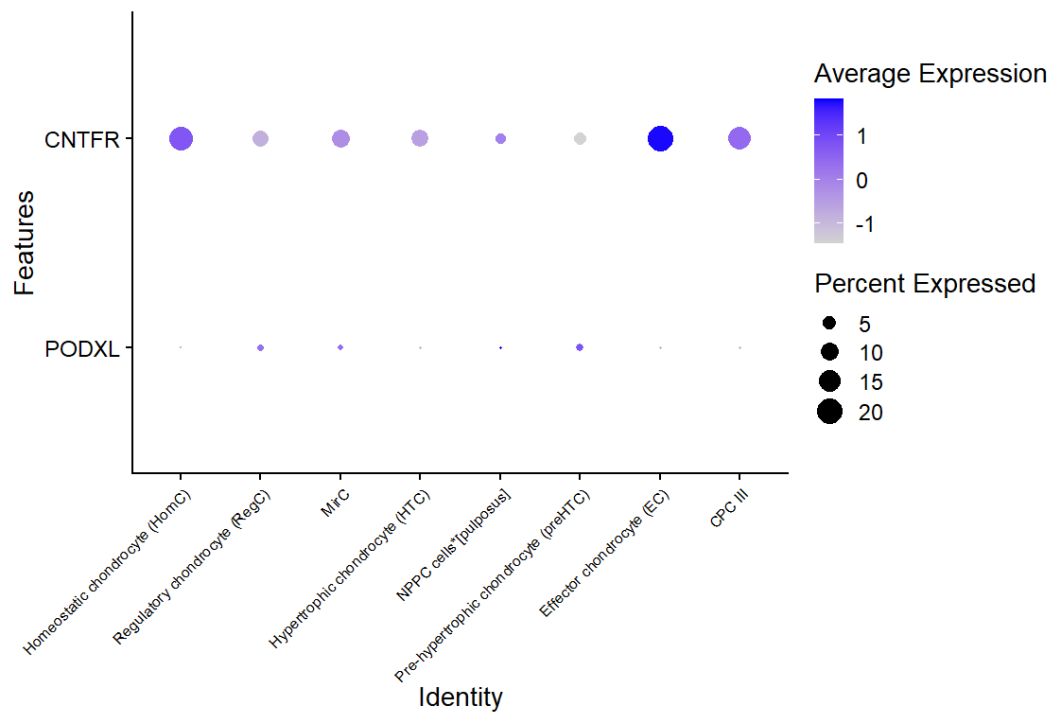


Table S1. Forward and reverse primer sequences, gene accession numbers and amplicon length for each primer pair employed in this study.

Gene symbol, species	Accession number	Primer sequence	Product length (bp)
RPS7 (<i>Gallus gallus</i>)	XM_004940515.4	FW: CGGAGTGCCGCGAAAGG REV: TGTGATGTTCAACTCCCGCA	157
YWHAZ (<i>Gallus gallus</i>)	NM_001031343	FW: GTTCCCTTGCAAAAACGGCT REV: GAGGCAGACGGAAGTTGGAA	199
PPIA (<i>Gallus gallus</i>)	NM_001166326.1	FW: GAGCTCTTCGCTGACAAGGT REV: GCGTAAAGTCACCACCCTGA	139
HPRT1 (<i>Gallus gallus</i>)	NM_204848.1	FW: TGGTGGGGATGACCTCTCAA REV: TCCAACAAAGTCTGGCCGAT	190
RPL4 (<i>Gallus gallus</i>)	NM_001007479	FW: TGTTTGCCCCAACCAAGACT REV: CTCCTCAATGCGGTGACCTT	137
RPL13 (<i>Gallus gallus</i>)	NM_204999.1	FW: GGCCCGTGTTATCTCAGAGG REV: CCGCTTCTTTGGCACGTTTT	113
SOX9 (<i>Gallus gallus</i>)	NM_204281.1	FW: TTTCCGAGACGTGGACATCG REV: GTACCGCTGTAGGTGGTGAC	150
ACAN (<i>Gallus gallus</i>)	NM_204955	FW: AGCAGTAGATGCACTGGGAC REV: GCCAGGTCGATCTCACACAG	153
COL2A1 (<i>Gallus gallus</i>)	NM_204426.1	FW: GGGACCTCAAGGCAAAGTCG REV: TTCCAGGCTCACCATTAGCG	140
COL1A1 (<i>Gallus gallus</i>)	NM_001396622.1	FW: TACTGCAACATGGAGACGGG REV: CCGCCGTACTCAAAGTGGAA	152
RUNX2 (<i>Gallus gallus</i>)	NM_204128	FW: GAGTCAGATTACAGACCCCAGG REV: AAATGGGCCCAGCTCGGAA	199
CNTFR (<i>Gallus gallus</i>)	XM_046934809.1	FW: ATCACGGATGCCTATGCTGG REV: GTGCTCGTCGTCTCTGTGAT	164
PODXL (<i>Gallus gallus</i>)	XM_046906716.1	FW: CATGTCATTGTGCATCGCGT REV: TAGGTGACGTTGGTGATGCC	101

Table S2. Comparative analysis of all total lysate and surfaceome-enriched samples, which revealed limited overlap, with only approx. 30% of proteins identified in both fractions.

Sample ID	Surfaceome only	Total lysate only	Overlap	Overlap %
D1_1	289	5016	84	22.52%
D1_2	305	5010	87	22.19%
D1_3	312	5013	86	21.61%
D3_1	317	4944	93	22.68%
D3_2	335	4995	100	22.99%
D3_3	328	4998	98	23.00%
D6_1	305	5004	94	23.56%
D6_2	326	5000	100	23.47%
D6_3	303	4980	89	22.70%
D10_1	338	4900	100	22.83%
D10_2	309	4986	95	23.51%
D10_3	336	4997	101	23.11%
D15_1	326	5001	99	23.29%
D15_2	306	5008	92	23.12%
D15_3	329	4950	98	22.95%

Table S3. Relative distribution of GO functional classification of the identified surface proteins during chondrogenic differentiation in micromass cultures

	Day 1	Day 3	Day 6	Day 10	Day 15
Receptors	28%	29%	27%	28%	28%
Enzymes	33%	31%	32%	32%	31%
Transporters	21%	21%	19%	19%	20%
Adhesion proteins	24%	23%	23%	23%	23%
Miscellaneous	20%	24%	25%	25%	26%