Supplemental Data for:

Integrative metabolic pathway analysis reveals novel therapeutic targets in osteoarthritis

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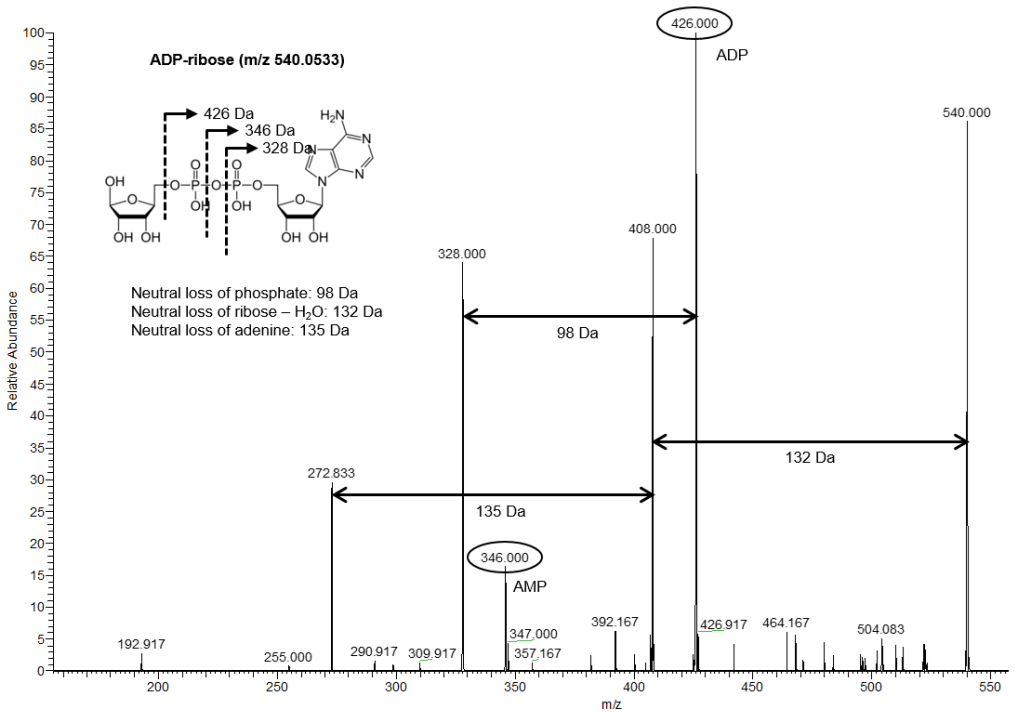

Figure S1. MALDI-Orbitrap-MS/MS spectrum of the *m/z* 540.0533 ([ADP-ribose – H_2O-H]$^-$) precursor ion. The mass peak of *m/z* 426 confirms the presence of ADP, whereas the *m/z* 132 fragment ion corresponds to the neutral loss of the ribose sugar minus water. Neutral loss of phosphate and adenine nitrogenous base was confirmed by the mass differences of 98 Da (between *m/z* 426 and *m/z* 328) and 135 Da (between *m/z* 408 and *m/z* 273), respectively. Thus, we can confidently assign the signal *m/z* 540.0533 as adenine diphosphate-ribose (ADP-ribose).

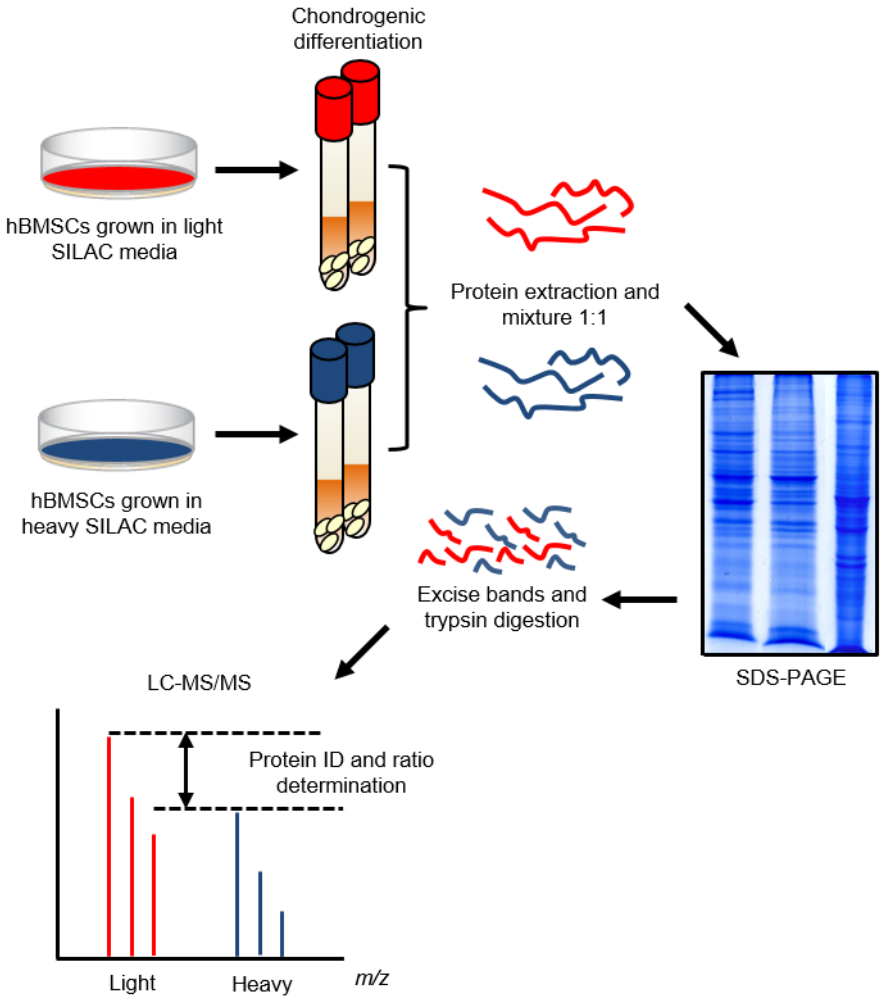

Figure S2. SILAC-based quantitative proteomics. Workflow of SILAC coupled with nanoLC-MS/MS for the comparative analysis of protein expression in OA hBMSCs after 2 and 14 days of chondrogenic differentiation.

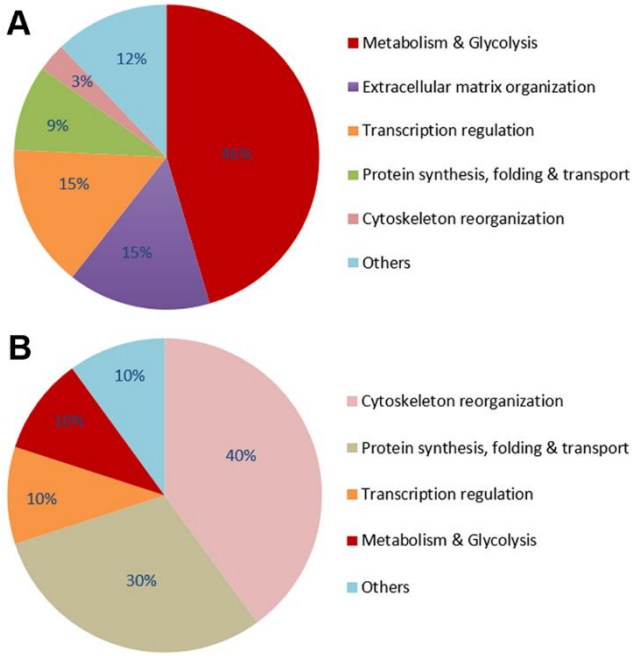

Figure S3. Predicted biological process of the differentially abundant proteins identified in the proteomic analysis. A, B) Predicted biological process of the set of proteins increased (A) or decreased (B) at day 14, according to GO annotation.

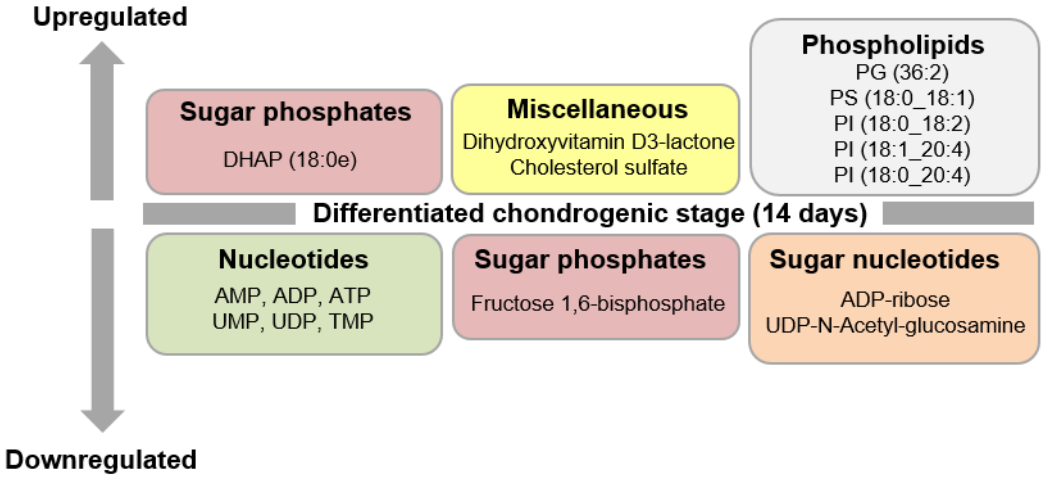

Figure S4. Schematic representation of metabolites presented a significant alteration of their levels in control hBMSCs due to chondrogenic differentiation. Levels of 17 metabolites, including phospholipids, were significantly altered between day 2 and 14 of chondrogenic differentiation. AMP, adenine monophosphate; ADP, adenine diphosphate; ATP, adenine triphosphate; UMP, uridine monophosphate; UDP, uridine diphosphate; TMP, thymidine monophosphate; DHAP, dyhidroxyacetone phosphate; P, phosphate; PG, phosphatidylglycerol; PS, phosphatidylserine; PI, phosphatidylinositol.

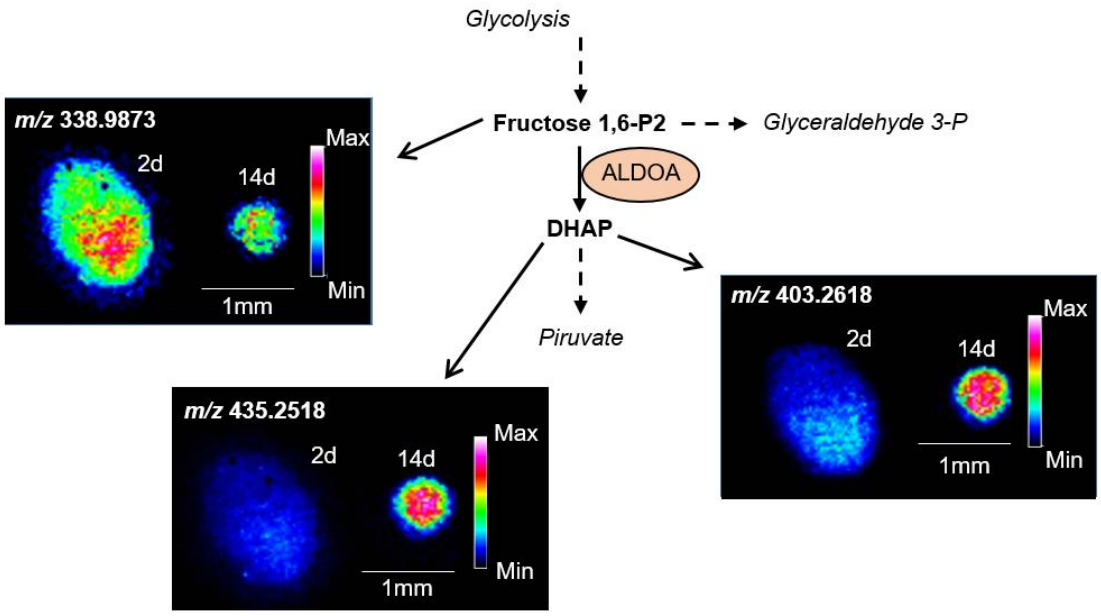

Figure S5. Spatial distribution and abundance of intermediate metabolites of glycolytic pathway in OA micromasses. Fructose 1,6-bisphosphate (Fru-1,6-P2) and dihydroxyacetone phosphate (DHAP) are two important intermediates in the glycolysis metabolic pathway. Fru-1,6-P2 can be converted to DHAP by the action of the enzyme fructose-bisphosphate aldolase (ALDOA). The abundance of Fru-1,6-P_2 (*m/z* 338.9874) was relatively higher in the micromasses collected at day 2, whereas DHAP (*m/z* 403.2618 and *m/z* 435.2518) were increased at day 14 and specifically localized in the core of the pellet.

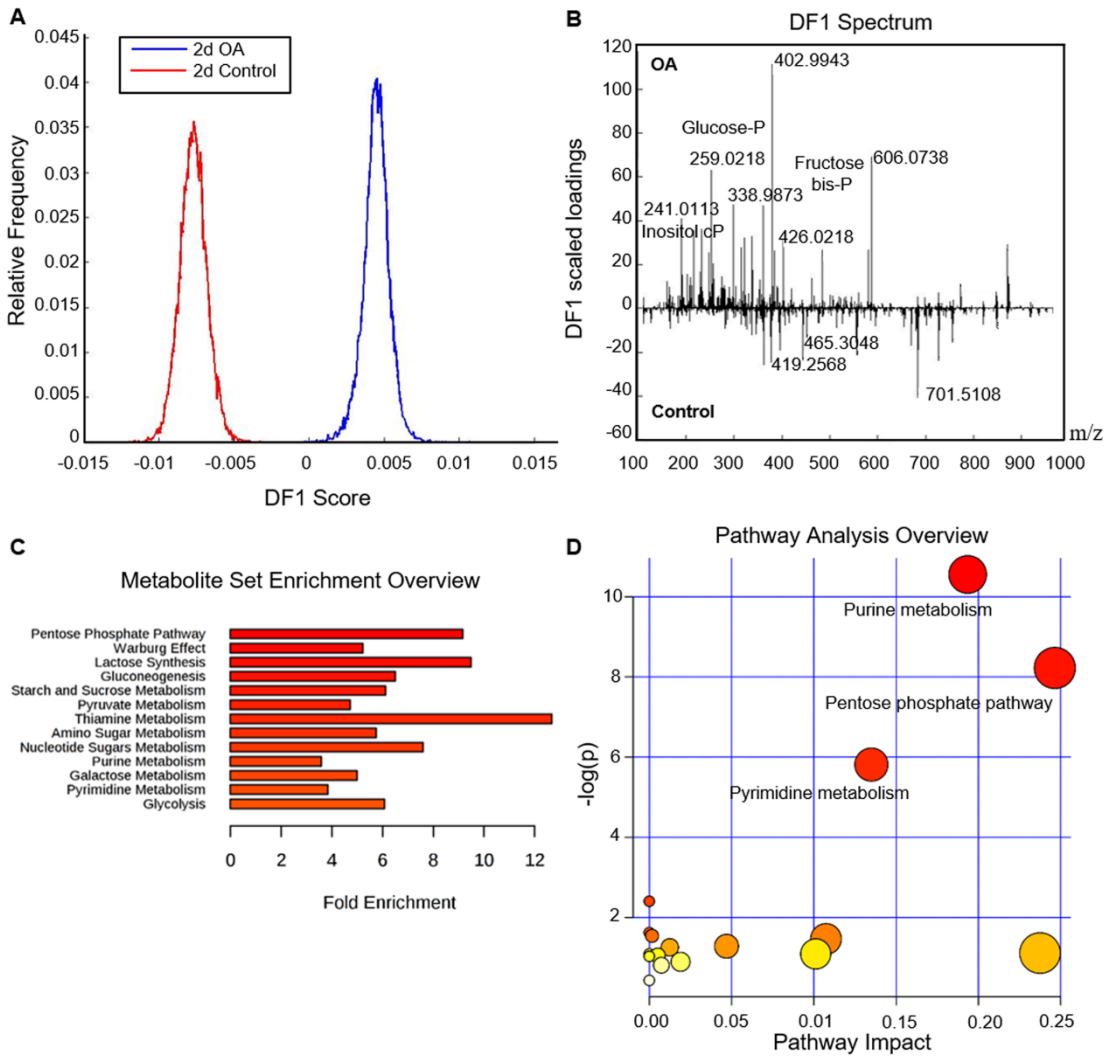

Figure S6. PCA-DA analysis and altered metabolic pathways observed in OA and control hBMSCs at the undifferentiated stage (day 2). A) Statistical analysis showing metabolic differences between undifferentiated OA vs. control hBMSCs. **B**, Discriminant fuction 1 (DF1) spectra loading plot showing the discriminating masses between control and OA hBMSCs at day 2 of chondrogenic differentiation. **C**, The top 13 significantly altered pathways (*p* value <0.05) determined by metabolite set enrichment analysis (MSEA), ranked by raw *p* value calculated from the enrichment analysis. **D**, Metabolic pathway analysis overview. In D, all the matched pathways are represented by circles, with the circle size indicating pathway impact (arbitrary scale). Pathways in the top right

diagonal region indicates that metabolites involved in those pathways are significantly changed and these are more likely to have significant impacts on the pathways. The color of the circles indicates unadjusted *p* values (y axis) from pathway enrichment analysis, with red < 0.03; orange < 0.29, and yellow < 0.36. The pathways are identified based on overrepresentation analysis (ORA), using the significantly altered metabolites. The x axis represents increasing metabolic pathway impact according to the degree of centrality from pathway topology analysis.

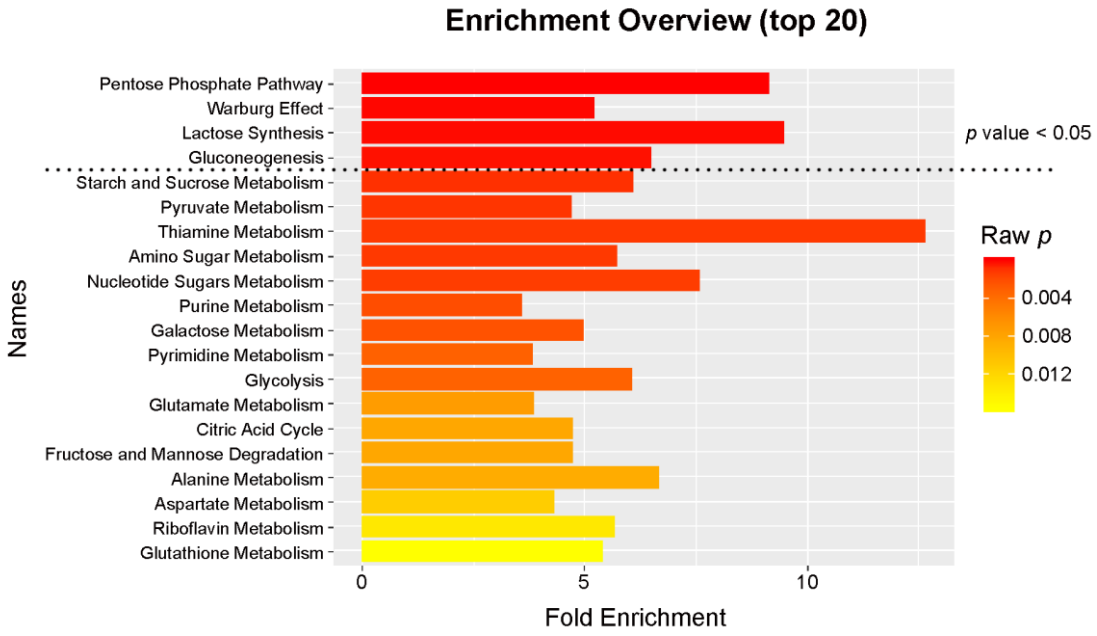

Figure S7. Metabolic enrichment analysis demonstrated major impact in pentose phosphate pathway and Warburg effect. The summary plot for metabolite set enrichment analysis (MSEA) ranks the metabolite sets according to *p* value with a dashed line indicating Holm *p* value threshold. The top twenty metabolite sets are shown.

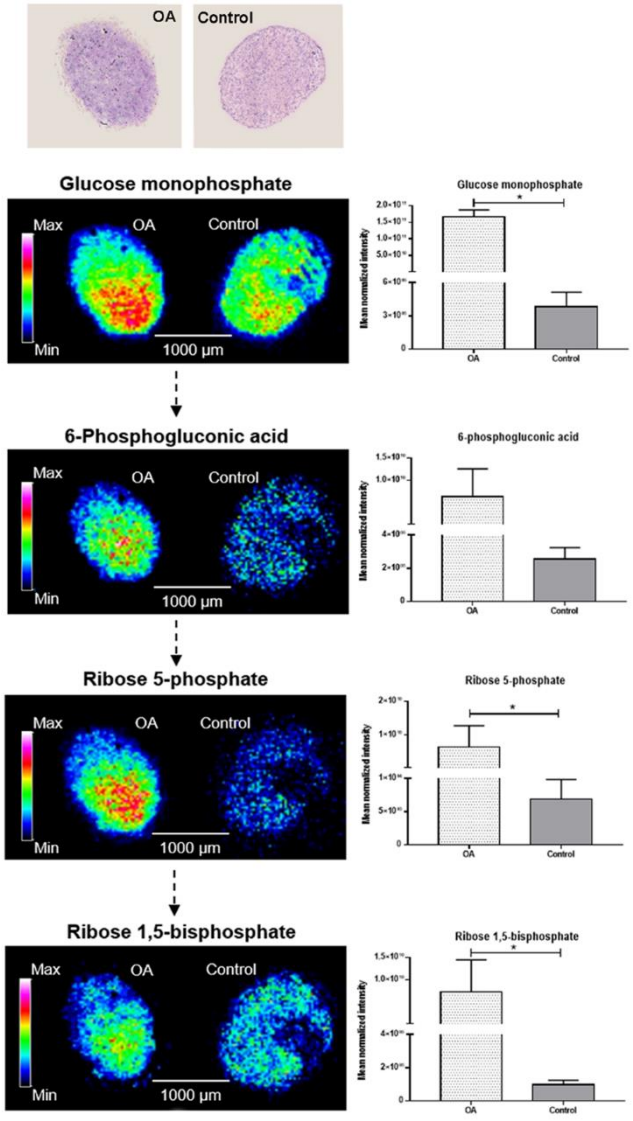

Figure S8. Spatial distribution and abundance of metabolites involved in pentose phosphate pathway in OA and control micromasses at the undifferentiated chondrogenic stage. Ribose-5-phosphate and Ribose-1,5-bisphosphate are localized in the core zone of OA micromasses according to molecular images and histology. Two sections of OA (n=5) and control (n=3) micromasses collected at day 2 were analyzed and compared. Graphs depict mean normalized intensity (arbitrary units) ± standard mean error (Mann-Whitney test) **p* value <0.05, compared to control.

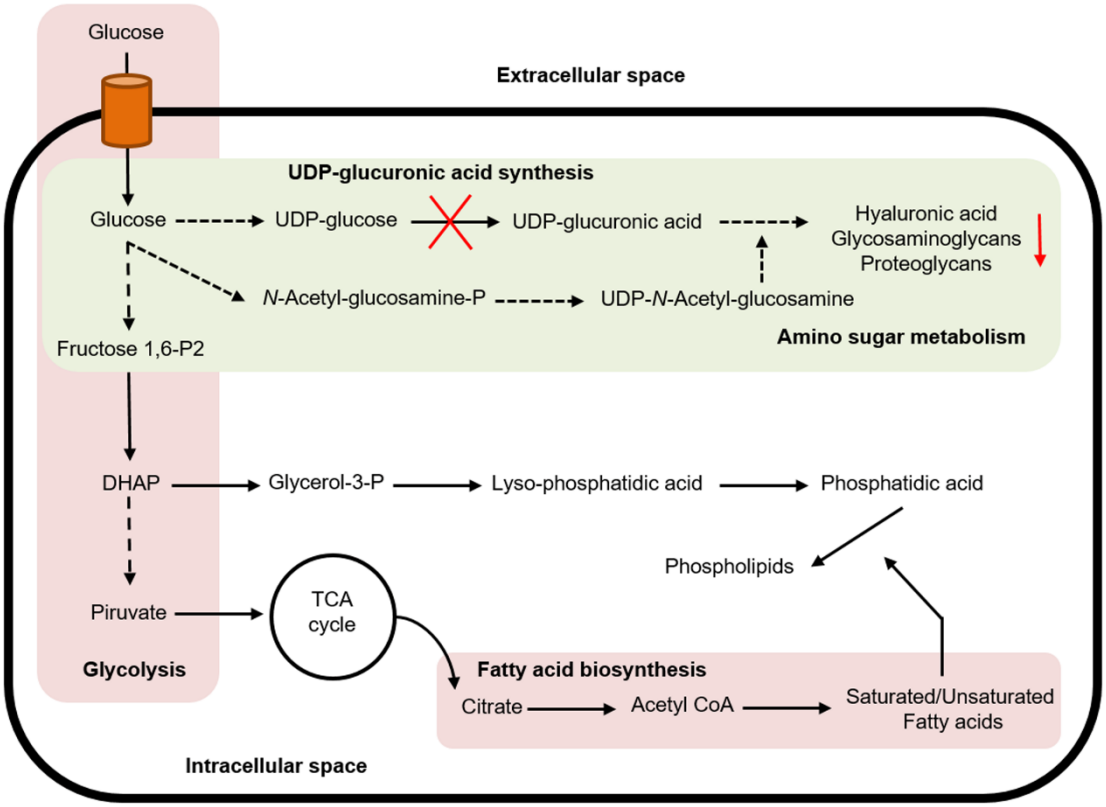

Figure S9. Altered metabolism in OA hBMSCs undergoing chondrogenesis. The glycolysis pathway and unsaturated fatty acid biosynthesis are upregulated in OA hBMSCs during chondrogenesis (pathways in red), whereas amino sugar metabolism and UDP-glucuronic acid synthesis are downregulated (pathways in green).

Supplementary Tables

Table S1. Overview of the clinical data of the OA patients and healthy control donors included in this study.

Patient diagnosis	age range (mean ±sd)	weight range in kg (mean ±sd)	height range in cm (mean ±sd)	BMI range (mean ±sd)
Osteoarthritis (n=13)	65-76 (71±5,5)	53-97 (75,5±20)	151-177 (162±11,5)	20-36 (29±6,7)
Controls (n=11)	69-84 (76,5±10,5)	52-82 (67,5±21)	163-169 (166±4)	20-29 (24±6,5)

BMI: body mass index; sd: standard deviation

Table S2. Metabolites detected in hBMSCs undergoing chondrogenesis with MALDI-FT-ICR-MSI in negative ion mode. Provided separately as an excel file.

Table S3. Targeted genes, primer details and PCR conditions used for quantitative Real-Time PCR.

Primers details

Gene	Sequence (5' - 3')	Melting Temp (°C)	Probe	GC%	Amplicon Length (nt)
UGDH Fw	GAATTTGCCAGAAGTAGCTCGT	63.40	#62	45.4	73
UGDH Rv	AGCAAACCTCCTCCTCTGGT	64.10		55	
UGP2 Fw	CAAAATGCCATTGACATGGA	64.30	#58	40	90
UGP2 Rv	TTTGATGGCAGCCCCTACT	64.50		52.6	
RPL13 Fw	GGATAAGAAACCCTGCGACA	60	#63	50	91
RPL13 Rw	GCCTCGACCATCAAGCAC	60		61	

RT-qPCR details

Program	Cycles	Temp (°C)	Time
Pre-Incubation	1	95	10 min
Amplification	45	95	10 sec
		60	30 sec
		72	1 sec
Cooling	1	40	20 sec

Table S4. List of identified proteins in OA hBMSCs undergoing chondrogenesis after a SILAC-based proteomic analysis. Provided separately as an excel file.

Table S5. List of identified peptides in OA hBMSCs undergoing chondrogenesis after a SILAC-based proteomic analysis. Provided separately as an excel file.

Table S6. Pathway analysis results of altered metabolic pathways involved in the chondrogenesis of control human bone marrow mesenchymal stem cells using MetaboAnalyst.

	Pathway name	Total [a)]	Hits [b)]	Raw p [c)]	-log(p)	Holm p[d)]	FDR [e)]	Impact[f)]
1	Pyrimidine metabolism	60	3	0,003	5,892	0,218	0,110	0,141
2	Purine metabolism	92	4	0,001	7,153	0,062	0,062	0,127
3	Glycerophospholipid metabolism	39	1	0,178	1,724	1,000	1,000	0,037
4	Amino sugar and nucleotide sugar metabolism	88	1	0,361	1,019	1,000	1,000	0,026
5	Nitrogen metabolism	39	1	0,178	1,724	1,000	1,000	0,000
6	Glycine, serine and threonine metabolism	48	1	0,215	1,536	1,000	1,000	0,000
7	Steroid hormone biosynthesis	99	1	0,397	0,925	1,000	1,000	0,000

hBMSCs, human bone marrow mesenchymal stem cells

[a)] Total is the total number of compounds in the pathway.

[b)] Hits is the actually matched number of compounds from the user uploaded data.

[c)] Raw p is the original p value calculated from the enrichment analysis.

[d)] Holm p is the p value adjusted by Holm-Bonferroni method.

[e)] FDR is the p value adjusted using False Discovery Rate.

[f)] Impact is the pathway impact value calculated from pathway topology analysis.

Table S7. Pathway analysis results of altered metabolic pathways involved in the chondrogenesis of OA human bone marrow mesenchymal stem cells using MetaboAnalyst.

	Pathway name	Total [a]	Hits [b]	Raw p [c]	-log(p)	Holm p [d]	FDR [e]	Impact [f]
1	Pyrimidine metabolism	60	6	0,000	10,213	0,003	0,003	0,185
2	Purine metabolism	92	5	0,003	5,799	0,239	0,121	0,142
3	Galactose metabolism	41	3	0,010	4,595	0,788	0,269	0,035
4	Amino sugar and nucleotide sugar metabolism	88	4	0,015	4,175	1,000	0,278	0,288
5	Starch and sucrose metabolism	50	3	0,017	4,053	1,000	0,278	0,296
6	Glycolysis or Gluconeogenesis	31	2	0,046	3,076	1,000	0,559	0,047
7	Glycerolipid metabolism	32	2	0,049	3,018	1,000	0,559	0,012
8	Glycerophospholipid metabolism	39	2	0,070	2,665	1,000	0,696	0,175
9	Fatty acid biosynthesis	49	2	0,103	2,270	1,000	0,918	0,000
10	Pentose and glucuronate interconversions	53	2	0,118	2,139	1,000	0,942	0,006
11	Pentose phosphate pathway	32	1	0,305	1,189	1,000	1,000	0,012
12	Glutathione metabolism	38	1	0,351	1,048	1,000	1,000	0,237
13	Inositol phosphate metabolism	39	1	0,358	1,027	1,000	1,000	0,000
14	Nitrogen metabolism	39	1	0,358	1,027	1,000	1,000	0,000
15	Ascorbate and aldarate metabolism	45	1	0,401	0,914	1,000	1,000	0,000
16	Cysteine and methionine metabolism	56	1	0,472	0,750	1,000	1,000	0,007
17	Arachidonic acid metabolism	62	1	0,508	0,678	1,000	1,000	0,217

OA hBMSCs, osteoarthritis human bone marrow mesenchymal stem cells

[a] Total is the total number of compounds in the pathway.

[b] Hits is the actually matched number of compounds from the user uploaded data.

[c] Raw p is the original p value calculated from the enrichment analysis.

[d] Holm p is the p value adjusted by Holm-Bonferroni method.

[e] FDR is the p value adjusted using False Discovery Rate.

[f] Impact is the pathway impact value calculated from pathway topology analysis.

Table S8. Integrated pathway analysis results of metabolites/proteins involved in the chondrogenesis of control human bone marrow mesenchymal stem cells using MetaboAnalyst.

	Pathway name	Total [a)]	Hits [b)]	Raw p [c)]	Topology[d)]
1	**Glycolysis / Gluconeogenesis**	91	6	0,003	0,407
2	Galactose metabolism	55	2	0,246	0,143
3	Fructose and mannose metabolism	55	2	0,246	0,216
4	Pyruvate metabolism	64	2	0,305	0,174
5	Starch and sucrose metabolism	78	2	0,395	0,148
6	Phenylalanine metabolism	29	1	0,400	0,091
7	Pyrimidine metabolism	142	3	0,452	0,239
8	Nicotinate and nicotinamide metabolism	39	1	0,499	0,054
9	Pentose phosphate pathway	48	1	0,574	0,390
10	Purine metabolism	234	4	0,597	0,230
11	Propanoate metabolism	52	1	0,603	0,049
12	Pentose and glucuronate interconversions	52	1	0,603	0,276
13	Cysteine and methionine metabolism	63	1	0,675	0,036
14	Glycine, serine and threonine metabolism	68	1	0,704	0,032
15	Glycerolipid metabolism	72	1	0,725	0,162
16	Glutathione metabolism	75	1	0,740	0,113
17	Amino sugar and nucleotide sugar metabolism	84	1	0,780	0,025
18	Inositol phosphate metabolism	90	1	0,803	0,031
19	Arachidonic acid metabolism	100	1	0,837	0,051
20	Glycerophospholipid metabolism	119	1	0,886	0,053
21	Steroid hormone biosynthesis	137	1	0,919	0,011
22	Metabolism of xenobiotics by cytochrome P450	139	1	0,922	0,018

hBMSCs, human bone marrow mesenchymal stem cells

[a)] Total is the total number of compounds in the pathway.

[b)] Hits is the actually matched number of compounds from the user uploaded data.

[c)] Raw p is the original p value calculated from the enrichment analysis.

[d)] Topology analysis score.

Table S9. Integrated pathway analysis results of metabolites/proteins involved in the chondrogenesis of OA human bone marrow mesenchymal stem cells using MetaboAnalyst.

	Pathway name	Total [a]	Hits [b]	Raw p [c]	Topology [d]
1	**Glycolysis / Gluconeogenesis**	91	8	0,002	0,542
2	**Pentose and glucuronate interconversions**	52	5	0,009	0,621
3	**Galactose metabolism**	55	5	0,012	0,571
4	**Starch and sucrose metabolism**	78	6	0,013	0,463
5	**Amino sugar and nucleotide sugar metabolism**	84	6	0,018	0,457
6	**Biosynthesis of unsaturated fatty acids**	27	3	0,031	0,094
7	Pentose phosphate pathway	48	3	0,125	0,683
8	Pyruvate metabolism	64	3	0,229	0,174
9	Ascorbate and aldarate metabolism	35	2	0,230	0,250
10	Pyrimidine metabolism	142	5	0,305	0,398
11	Fatty acid biosynthesis	49	2	0,366	0,022
12	Propanoate metabolism	52	2	0,394	0,049
13	Fructose and mannose metabolism	55	2	0,422	0,216
14	Cysteine and methionine metabolism	63	2	0,494	0,036
15	Phenylalanine metabolism	29	1	0,537	0,091
16	Glycerolipid metabolism	72	2	0,567	0,243
17	Glutathione metabolism	75	2	0,590	0,264
18	Nicotinate and nicotinamide metabolism	39	1	0,647	0,108
19	Inositol phosphate metabolism	90	2	0,691	0,047
20	Purine metabolism	234	5	0,753	0,310
21	Glycerophospholipid metabolism	119	2	0,830	0,107
22	Glycine, serine and threonine metabolism	68	1	0,840	0,032
23	Arachidonic acid metabolism	100	1	0,935	0,244

OA hBMSCs, osteoarthritis human bone marrow mesenchymal stem cells

[a] Total is the total number of compounds in the pathway.

[b] Hits is the actually matched number of compounds from the user uploaded data.

[c] Raw p is the original p value calculated from the enrichment analysis.

[d] Topology analysis score.

Table S10. Metabolites modulated at day 2 in OA human bone marrow mesenchymal stem cells undergoing chondrogenesis *versus* control cells detected by MALDI-FT-ICR-MSI in negative ion mode.

Metabolite assignment [a]	**Parent ion** [b]	**Experimental *m/z***
Upregulated at day 2 in OA		
Fatty acids		
Oleic acid	$[M-H]^-$	281,2483
Stearic acid	$[M-H]^-$	283,2643
Nucleotides		
Adenosine monophosphate (AMP)	$[M-H]^-$	346,0563
Adenosine diphosphate (ADP)	$[M-H]^-$	426,0218
Adenosine diphosphate (ADP)	$[M-H2O-H]^-$	408,0113
Adenosine triphosphate (ATP)	$[M-H]^-$	505,9878
Adenosine triphosphate (ATP)	$[M-H2O-H]^-$	487,9778
Cytidine diphosphate (CDP)	$[M-H]^-$	402,0098
Guanosine monophosphate (GMP)	$[M-H]^-$	362,0503
Guanosine diphosphate (GDP)	$[M-H]^-$	442,0163
Guanosine triphosphate (GTP)	$[M-H]^-$	521,9828
Uridine monophosphate (UMP)	$[M-H]^-$	323,0283
Uridine monophosphate (UMP)	$[M-H2O-H]^-$	305,0173
Uridine diphosphate (UDP)	$[M-H2O-H]^-$	384,9838
Uridine triphosphate (UTP)	$[M-H]^-$	482,9603
Sugar nucleotides		
UDP-*N*-Acetyl-glucosamine	$[M-H]^-$	606,0738
Sugar phosphates		
Glucose monophosphate	$[M-H]^-$	259,0218
N-Acetyl-glucosamine-phosphate	$[M-H2O-H]^-$	282,0378
N-Acetyl-glucosamine-phosphate	$[M-H]^-$	300,0478
Fructose 1,6-bisphosphate	$[M-H]^-$	338,9874
Ribose 5-phosphate	$[M-H2O-H]^-$	211,0003
Ribose 1,5-bisphosphate	$[M-H2O-H]^-$	290,9668
6-Phosphogluconic acid	$[M-H]^-$	275,0163
Inositol-related		
Inositol cyclic phosphate	$[M-H]^-$	241,0113
1-(sn-Glycero-3-phospho)-1D-myo-inositol	$[M-H2O-H]^-$	315,0483
Stearoylglycerophosphoinositol	$[M-H]^-$	599,3213

PA-related		
LysoPA(P-16:0e)	$[M-H2O-H]^-$	375,2303
Miscellaneous		
PPPi	$[M-H2O-H]^-$	238,8903
Cytidine	$[M-H]^-$	242,0793
Glutathione	$[M-H]^-$	306,0763
Downregulated at day 2 in OA		
PA-related		
CPA (18:0)	$[M-H]^-$	419,2568
LysoPA(18:0)	$[M-H]^-$	437,2673
PA (16:0_18:1)	[M-H]-	673,4793
PA (18:1_18:2)	[M-H]-	699,4993
Miscellaneous		
NNAL-N-glucuronide	$[M-H]^-$	384,1433
Cholesterol sulfate	$[M-H]^-$	465,3048

CPA, cyclic phosphatidic acid; LysoPA, lysophosphatidic acid; PA, phosphatidic acid.

[a)] Assignments of *m/z* values according to tandem MS analysis and high mass accuracy.

[b)] Adduct ions: deprotonated ions $[M-H]^-$ and deprotonated ions with loss of water $[M-H2O-H]^-$.

Table S11. Pathway analysis results of altered metabolic pathways in control human bone marrow mesenchymal stem cells versus OA at day 2 of chondrogenesis using MetaboAnalyst.

	Pathway name	Total [a]	Hits [b]	Raw p [c]	-log(p)	Holm p [d]	FDR [e]	Impact [f]
1	**Purine metabolism**	92	7	0,000	10,558	0,002	0,002	0,193
2	**Pentose phosphate pathway**	32	4	0,000	8,225	0,021	0,011	0,246
3	Pyrimidine metabolism	60	4	0,003	5,815	0,233	0,080	0,135
4	Fatty acid biosynthesis	49	2	0,091	2,402	1,000	1,000	0,000
5	Riboflavin metabolism	21	1	0,198	1,621	1,000	1,000	0,000
6	Ether lipid metabolism	23	1	0,214	1,540	1,000	1,000	0,002
7	Amino sugar and nucleotide sugar metabolism	88	2	0,232	1,462	1,000	1,000	0,107
8	Glycolysis or Gluconeogenesis	31	1	0,278	1,280	1,000	1,000	0,047
9	Glycerolipid metabolism	32	1	0,286	1,253	1,000	1,000	0,012
10	Glutathione metabolism	38	1	0,330	1,110	1,000	1,000	0,237
11	Nitrogen metabolism	39	1	0,337	1,089	1,000	1,000	0,000
12	Glycerophospholipid metabolism	39	1	0,337	1,089	1,000	1,000	0,101
13	Galactose metabolism	41	1	0,351	1,048	1,000	1,000	0,005
14	Folate biosynthesis	42	1	0,357	1,029	1,000	1,000	0,000
15	Starch and sucrose metabolism	50	1	0,410	0,892	1,000	1,000	0,019
16	Cysteine and methionine metabolism	56	1	0,447	0,806	1,000	1,000	0,007
17	Steroid hormone biosynthesis	99	1	0,652	0,428	1,000	1,000	0,000

[a] Total is the total number of compounds in the pathway.

[b] Hits is the actually matched number of compounds from the user uploaded data.

[c] Raw p is the original p value calculated from the enrichment analysis.

[d] Holm p is the p value adjusted by Holm-Bonferroni method.

[e] FDR is the p value adjusted using False Discovery Rate.

[f] Impact is the pathway impact value calculated from pathway topology analysis.