Supporting information

Mannose Promotes Metabolic Discrimination of Osteosarcoma Cells at Single-Cell Level by Mass Spectrometry

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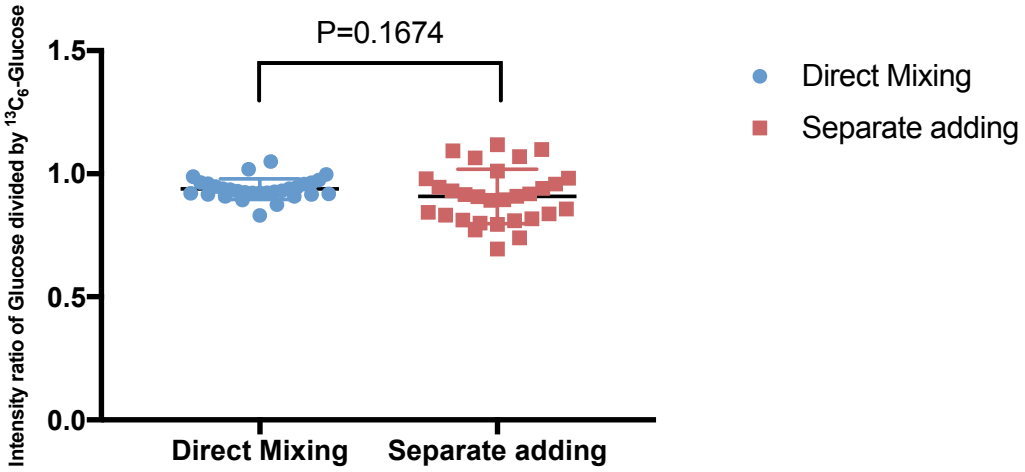

Figure S1. Droplet extraction repeatability of manual operation evaluation using standard solution. The operation error was evaluated using 500 μM ^{13}C glucose internal standard solution and 500 μM glucose solution. In the direct mixing group, 500 μM ^{13}C glucose solution was mixed with 500 μM glucose solution in the same volume before droplet extraction to the sample, so the relative standard deviation (RSD) in the direct mixing group was mainly caused by system error. Meanwhile, in the separate addition group, we first added 500 μM ^{13}C glucose internal standard solution to the sample, and then adding same volume of 500 μM glucose solution to the same sample to mix the two solutions. Due to unstable manual operation, there were errors in operation. The RSD of the direct mixing group was 4.4%, and that of the single adding group was 12.17%. This showed that operation error did exist in the droplet extraction method. However, since the two-tailed

student test showed no significant difference in the ratio between the two groups, it can be concluded that the operational errors in our method were within acceptable limits and would not cause measurement distortion.

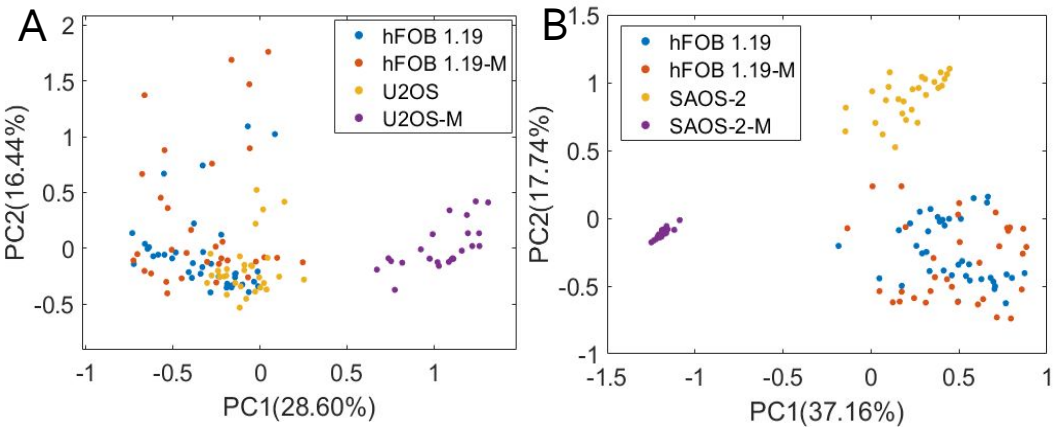

Figure S2. PCA of osteosarcoma cells and normal human osteoblasts with and without mannose in the same picture. It showed that mannose set osteosarcoma cells apart while normal osteoblasts stayed in the same region with no mannose treatment. (A) PCA of U2OS and hFOB 1.19 cells with and without mannose added in the same picture (orange dots: U2OS cells without mannose, blue dots: hFOB 1.19 cells without mannose, purple dots: U2OS cells with 25mM mannose, yellow dots: hFOB 1.19 cells with 25mM mannose). (B) PCA of SAOS-2 and hFOB 1.19 cells without and with mannose added in the same picture (orange dots: SAOS-2 cells without mannose, blue dots: hFOB 1.19 cells without mannose, purple dots: SAOS-2 cells with 25mM mannose, yellow dots: hFOB 1.19 cells with 25mM mannose).

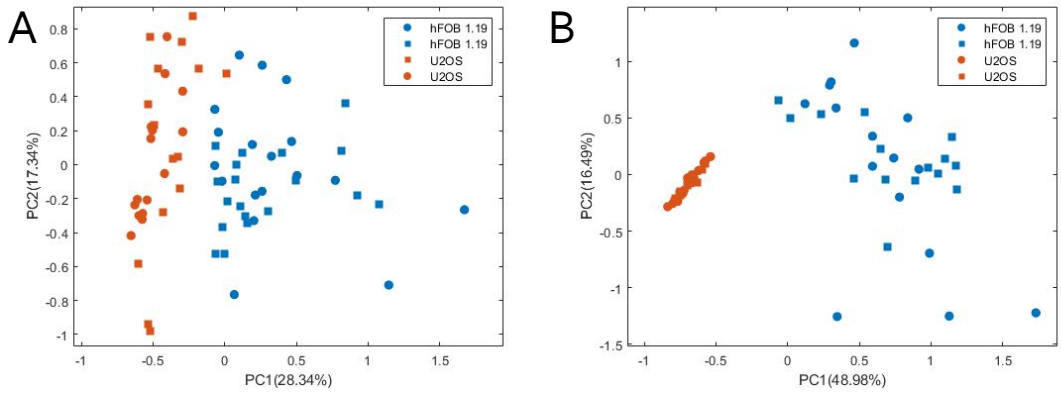

Figure S3. First repeating discrimination of osteosarcoma cells U2OS and normal human osteoblasts hFOB 1.19. Each single-cell sample was extracted in two batches represented by squares (first batch) and dots (second batch), respectively. (A) PCA without mannose added (U2OS cells: orange squares and dots; hFOB 1.19 cells: blue squares and dots). (B) PCA with 25mM mannose added (U2OS cells: orange squares and dots; hFOB 1.19 cells: blue squares and dots).

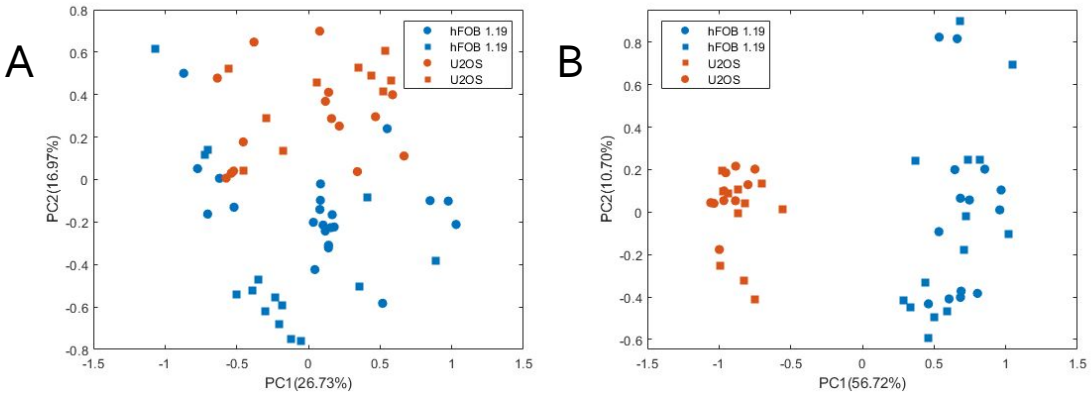

Figure S4. Second repeating discrimination of osteosarcoma cells U2OS and normal human osteoblasts hFOB 1.19. Each single-cell sample was extracted in two batches represented by squares (first batch) and dots (second batch), respectively. (A) PCA without mannose added (U2OS cells: orange squares and dots; hFOB 1.19 cells: blue squares and dots). (B) PCA with 25mM mannose added (U2OS cells: orange squares and dots; hFOB 1.19 cells: blue squares and dots).

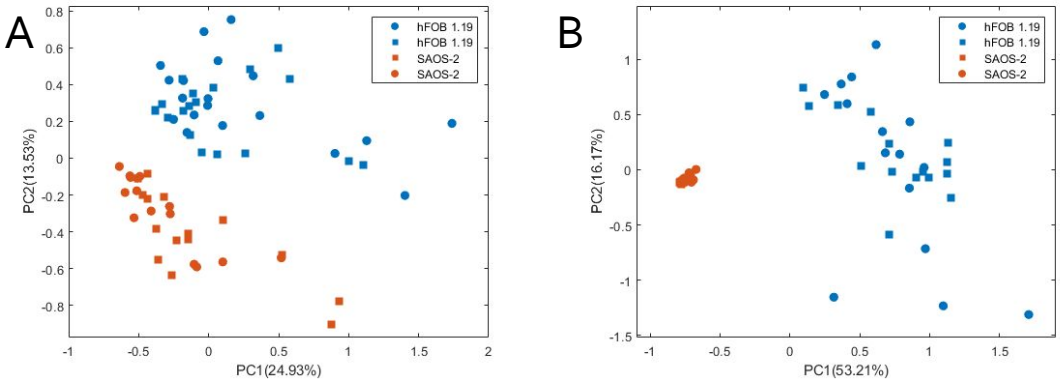

Figure S5. First repeating discrimination of osteosarcoma cells SAOS-2 and normal human osteoblasts hFOB 1.19. Each single-cell sample was extracted in two batches represented by squares (first batch) and dots (second batch), respectively. (A) PCA without mannose added (SAOS-2 cells: orange squares and dots; hFOB 1.19 cells: blue squares and dots). (B) PCA with 25mM mannose added (SAOS-2 cells: orange squares and dots; hFOB 1.19 cells: blue squares and dots).

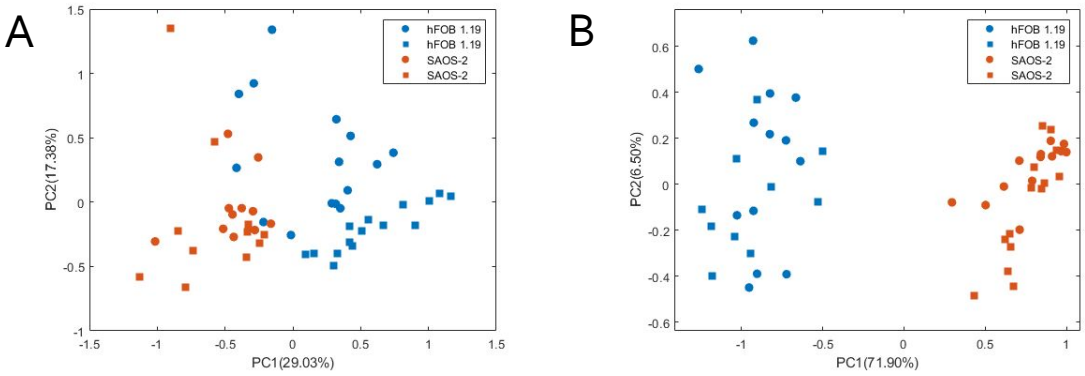

Figure S6. Second repeating discrimination of osteosarcoma cells SAOS-2 and normal human osteoblasts hFOB 1.19. Each single-cell sample was extracted in two batches represented by squares (first batch) and dots (second batch), respectively. (A) PCA without mannose added (SAOS-2 cells: orange squares and dots; hFOB 1.19 cells: blue squares and dots). (B) PCA with 25mM mannose added (SAOS-2 cells: orange squares and dots; hFOB 1.19 cells: blue squares and dots).

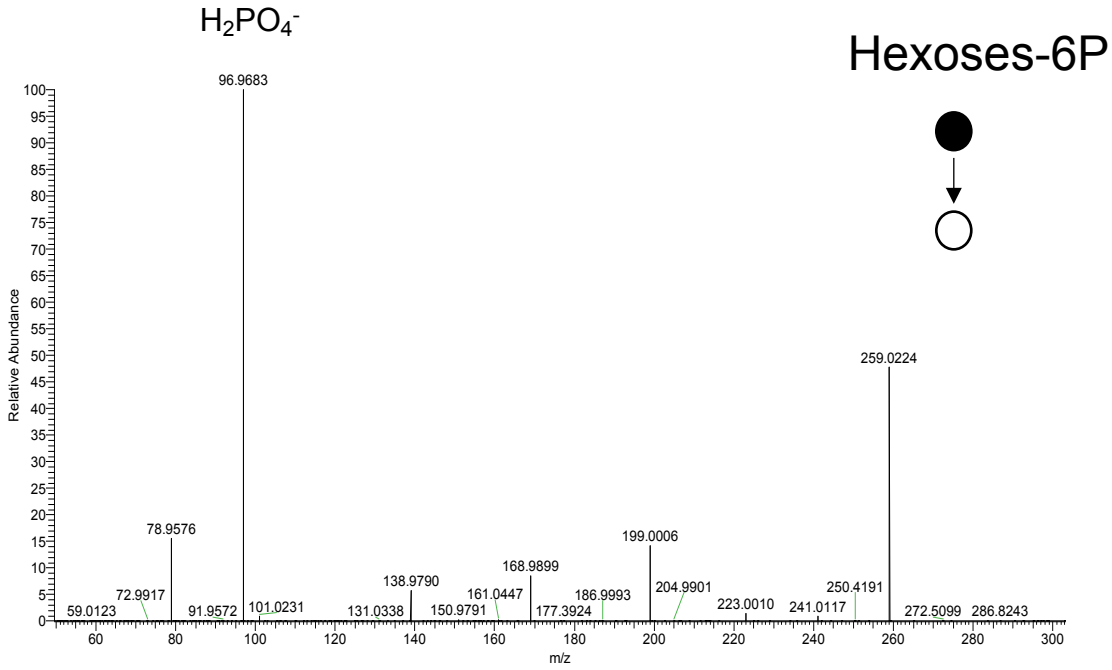

Figure S7. MS^2 data of hexoses-6P.

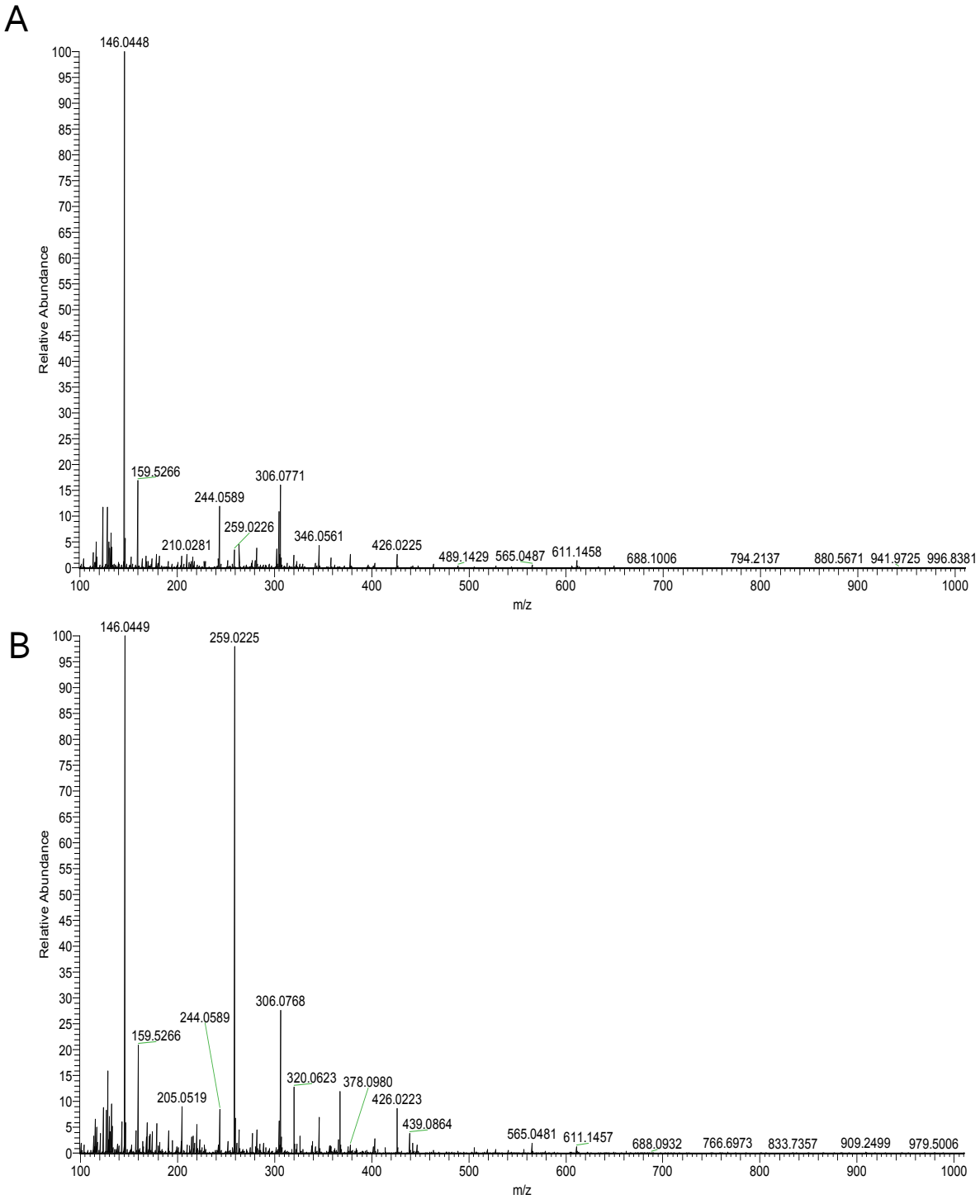

Figure S8. Representative single-cell level mass spectra of U2OS cells (A) without mannose treatment. (B) with 25mM mannose treatment.

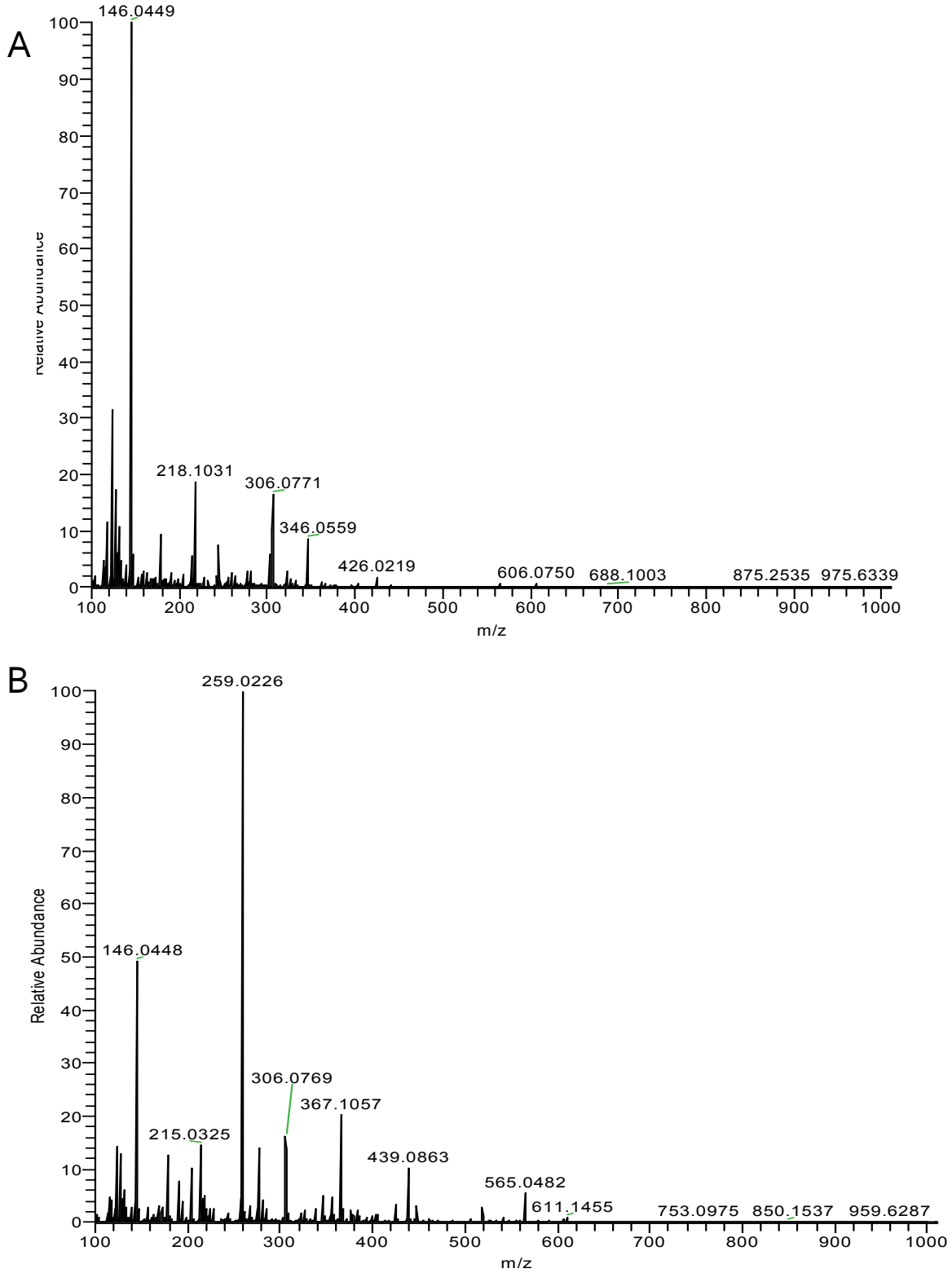

Figure S9. Representative single-cell level mass spectra of SAOS-2 cells (A) without mannose treatment. (B) with 25mM mannose treatment.

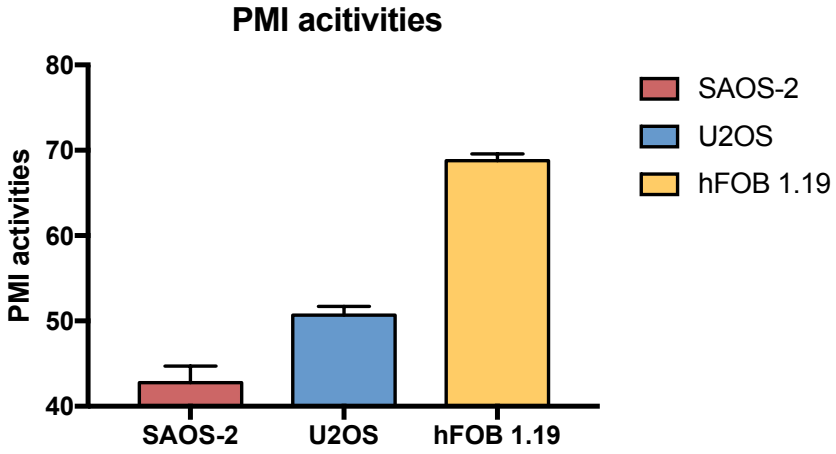

Figure S10. PMI activities in SAOS-2 cells, U2OS cells and hFOB 1.19 cells.

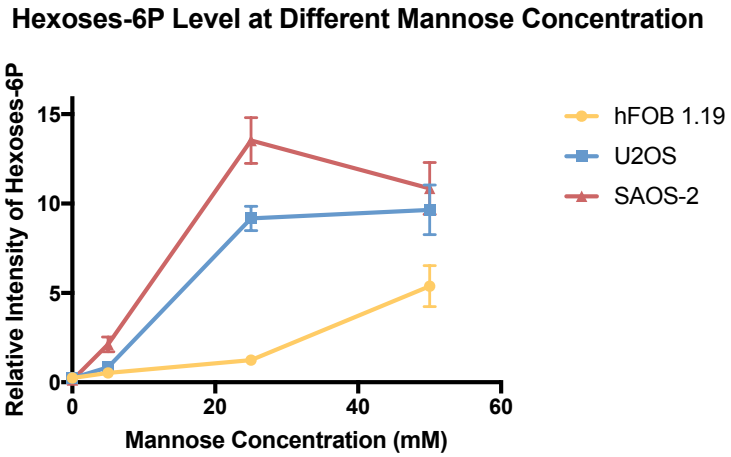

Figure S11. Hexoses-6P level at different mannose concentration.

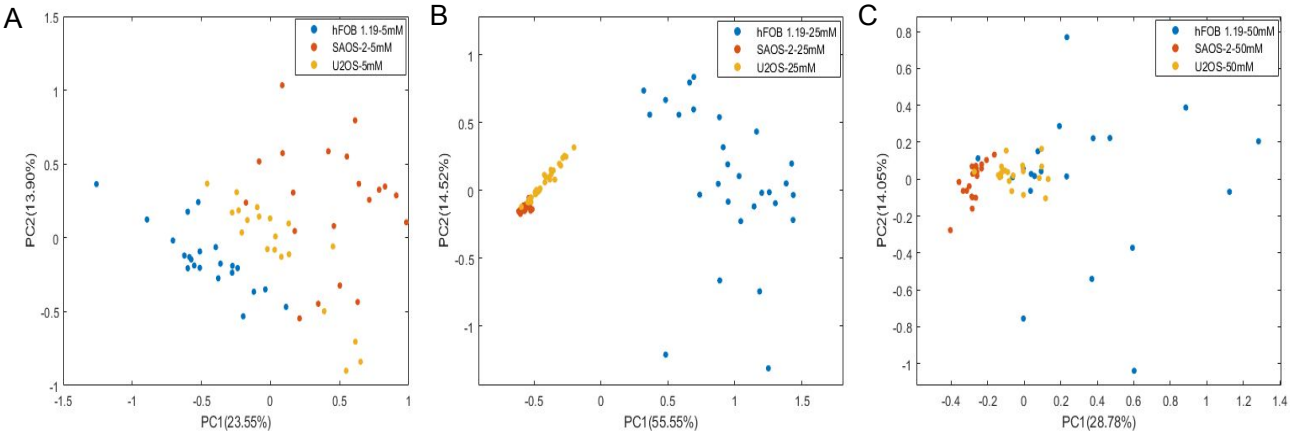

Figure S12. PCA of three cell lines at different mannose concentration (A) 5 mM mannose treatment. (B) 25 mM mannose treatment. (C) 50 mM mannose treatment.

Number	Measured M/Z	-logP	Assigned compound	Chemical formula	Monoisotopic Mass	Adduct	Adduct M/Z	Delta (ppm)	Absolute Intensity (order of magnitude)
1	259.0225	14.26733	Hexoses-6-phosphate	$C_6H_{13}O_9P$	260.0297	M-H	259.0224	0	10^8
2	260.0256	12.71007028	O-Sulfotyrosine	$C_{12}H_{14}ClNO$	223.0764	M+K-2H	260.025	2	10^7
3	261.0273	12.37905	Hydroxykynurenine	$C_{10}H_{12}N_2O_4$	224.0797	M+K-2H	261.0283	4	10^6
4	323.0291	10.44858749	Uridine 5'-monophosphate (UMP)	$C_9H_{13}N_2O_9P$	324.0359	M-H	323.0286	2	10^6
5	220.9826	8.548634958	D-Erythrose 4-phosphate	$C_4H_9O_7P$	200.0086	M+Na-2H	220.9833	3	10^6
6	281.0043	8.492730973	Hexoses-6-phosphate	$C_6H_{13}O_9P$	260.0297	M+Na-2H	281.0044	0	10^6
7	184.0011	8.108332046	Glutamic acid	$C_3H_8NO_6P$	185.0089	M+K-2H	184.0016	4	10^6
8	302.9866	7.765363777	Oxidized dithiothreitol	$C_4H_8O_2S_2$	151.9966	2M-H	302.9859	2	10^5
9	483.0501	7.756055065	Thiamine pyrophosphate	$C_{12}H_{19}N_4O_7P_2S$	425.045	M+HAc-H	483.0515	3	10^5
10	178.0713	7.718930199	Fructosamine	$C_6H_{13}NO_5$	179.0794	M-H	178.0721	4	10^5
11	205.1594	7.588028462	Goshuyic acid	$C_{14}H_{24}O_2$	224.1776	M-H_2O-H	205.1592	1	10^5
12	168.9899	7.469326643	Fructose 1,6-bisphosphate	$C_6H_{14}O_{12}P_2$	339.996	M-2H	168.9907	5	10^6
13	240.0905	7.326379047	Hexanoylglycine	$C_8H_{15}NO_3$	173.1052	M-H+HCOONa	240.0854	1	10^6
14	223.9937	7.128445837	(S)-2-amino-6-oxohexanoate	$C_6H_{11}NO_3$	145.0739	M+Br	223.9928	4	10^6
15	373.9484	7.027178765	Iodotyrosine	$C_9H_{10}INO_3$	306.9705	M-H+HCOONa	373.9507	3	10^4
16	284.0222	6.973787112	Tyramine-O-sulfate	$C_8H_{11}NO_4S$	217.0409	M-H+HCOONa	284.0211	4	10^6
17	368.1089	6.743839966	Dihydro-2,4,6-tris(2-methylpropyl)-4h-1,3,5-dithiazine	$C_{15}H_{31}NS_2$	289.1898	M+Br	368.1087	1	10^6
18	287.0243	6.699503794	[4-(3-acetyloxiran-2-yl)-2-methoxyphenyl]oxidanesulfonic acid	$C_{11}H_{12}O_7S$	288.0304	M-H	287.0231	4	10^5
19	137.0400	6.413049031	Glutamylglutamic acid	$C_{10}H_{16}N_2O_7$	276.0958	M-2H	137.0406	4	10^3
20	353.0267	6.325648816	dIMP	$C_{10}H_{13}N_4O_7P$	332.0522	M+Na-2H	353.0269	0	10^5

Table S1. The 20 metabolites that changed the most in ANOVA between mannose treated and untreated SAOS-2 cells.

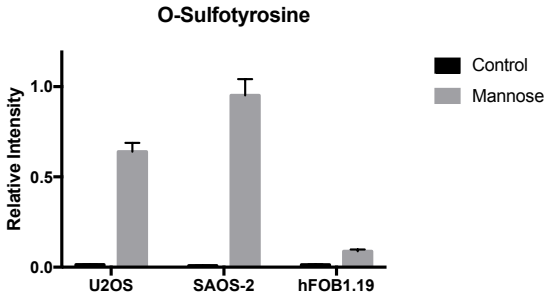

Figure S13. O-Sulfotyrosine level in three cell lines.

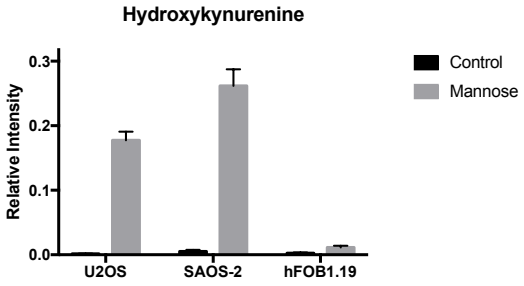

Figure S14. Hydroxykynurenine level in three cell lines.

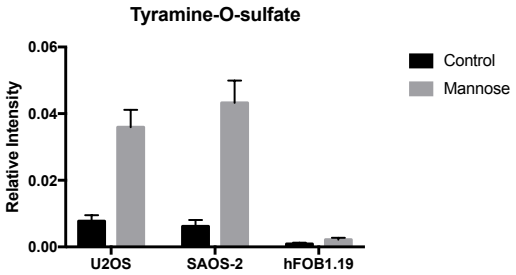

Figure S15. Tyramine-O-sulfate level in three cell lines.