

Supplemental information.

TITLE: Using metabolomics and gene regulatory networks to define metabolic stages during osteogenic differentiation of mesenchymal stromal cells

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SUPPLEMENTAL TABLES

Table S1. Primer pairs used in RT-qPCR. Forward and reverse primers used for RT-qPCR analysis of SPP1, RUNX2 and COL1A2 with TBP as a referencing gene.

Gene	Forward Primer	Reverse Primer
RUNX2	5'-CTTCACAAATCCTCCTCCCAAGT	5'-AGGCGGTCAGAGAACAAAC
SPP1	5'-CCCCACAGTAGACACATATGATG	5'-TTCCAACCTCTCGCTTTCCAT
COL1A2	5'-CCTTCAATCCATCCAGACCAT	5'-GTGAGAGAGGAGTTGTTGGAC
TBP	5'-CAGTGAATCTTGGTTGTAACTTGA	5'-TCGTGGCTCTCTTATCCTCAT

Table S2. Akaike Information Criteria from models fitted with the gamm4 function in R, all models are for each metabolite varying by day with the combinations of random effects considered below.

	Donor + Analysis Batch	PIPL/Donor + Analysis Batch	Passage/Donor + Analysis Batch	PIPL/Passage/Donor + Analysis Batch	Donor + Media + Analysis Batch	Donor
Glucose	1543.795	1545.795	1545.795	1547.795	1545.795	1558.992
Lactate	1168.993	1170.993	1170.993	1172.993	1167.776	1227.897
Glutamine	587.1644	589.1644	588.3329	591.1644	595.5849	591.5849
Glutamate	292.9485	294.9485	293.2039	296.9485	381.4727	377.9853
Ammonia	757.6984	759.3254	758.5992	761.3254	820.5771	816.5771

Table S3. ALP activities for 4 different donors during 28 days of osteogenic differentiation. SEM is the standard error of the mean. Related to Figure S1A.

Donor	ALP Activity (nmol/min)			
	Day 0	Day 7	Day 14	Day 28
D12	0.98	2.08	3.61	1.44
D13	2.80	4.40	4.89	3.35
D14	0.70	3.21	3.77	1.95
D15	1.92	4.49	4.93	5.20
Mean (SEM)	1.60 (0.48)	3.55 (0.57)	4.30 (0.35)	2.98 (0.84)

Table S4. Alizarin Red S staining measurement results (both optical density and percentage increase in relation to Day 0), validating mineralization, for 4 different donors during 28 days of osteogenic differentiation. SEM is the standard error of the mean. Related to Figure S1B.

Donor	Optical Density after Alizarin Red Staining (-)			% increase in mineralization	
	Day 0	Day 14	Day 28	Day 14	Day 28
D12	0.099	0.141	0.148	42.4	49.40
D13	0.103	0.155	1.720	50.4	1570
D14	0.127	0.685	4.000	439	3049
D15	1.956	0.908	5.203	-46.4	166
Mean (SEM)	0.571 (0.462)	0.472 (0.193)	2.768 (1.133)	121.35 (108.13)	1208.6 (704.06)

Table S5. Real-time qPCR results showing gene expression levels of RUNX2 and COL1A2. For each transcription factor, a relative expression level (compared to the housekeeping gene, TBP) is shown for Day 0 (the beginning of osteogenic differentiation) and Day 28 (the end of the differentiation). The calculated fold change between Days 0 and 28 is also shown. SEM is the standard error of the mean.

Donor	RUNX2			COL1A2		
	Relative Expression		Fold change	Relative Expression		Fold change
	Day 0 ^b	Day 28		Day 0 ^b	Day 28	
D13	2.43	2.68	1.10	0.03	17.35	0.08
D14	0.88	1.95	2.22	207.70	28.13	0.07
D15	1.28	3.69	2.89	379.06	152.20	0.64
Mean						
(SEM)	1.53 (0.47)	2.77 (0.50)	2.07 (0.52)	195.60 (109.59)	65.89 (43.27)	0.26 (0.19)

^b Measurements of the transcription factors used to determine relative expression levels for day 0 were obtained from a parallel study done over the same time period, in the same environment, and using the same material and cells from the same donors.

Supplemental Figures

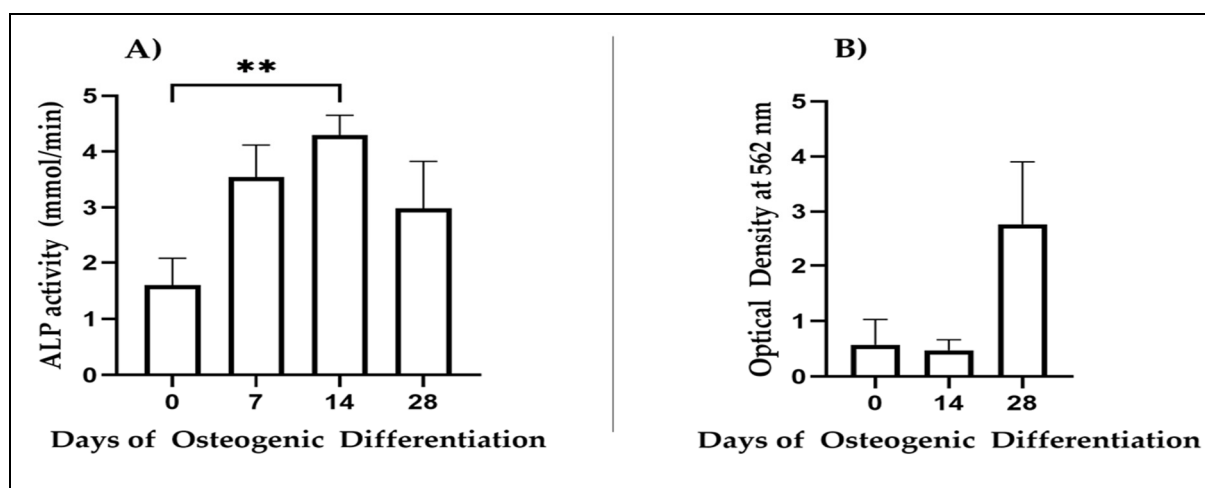
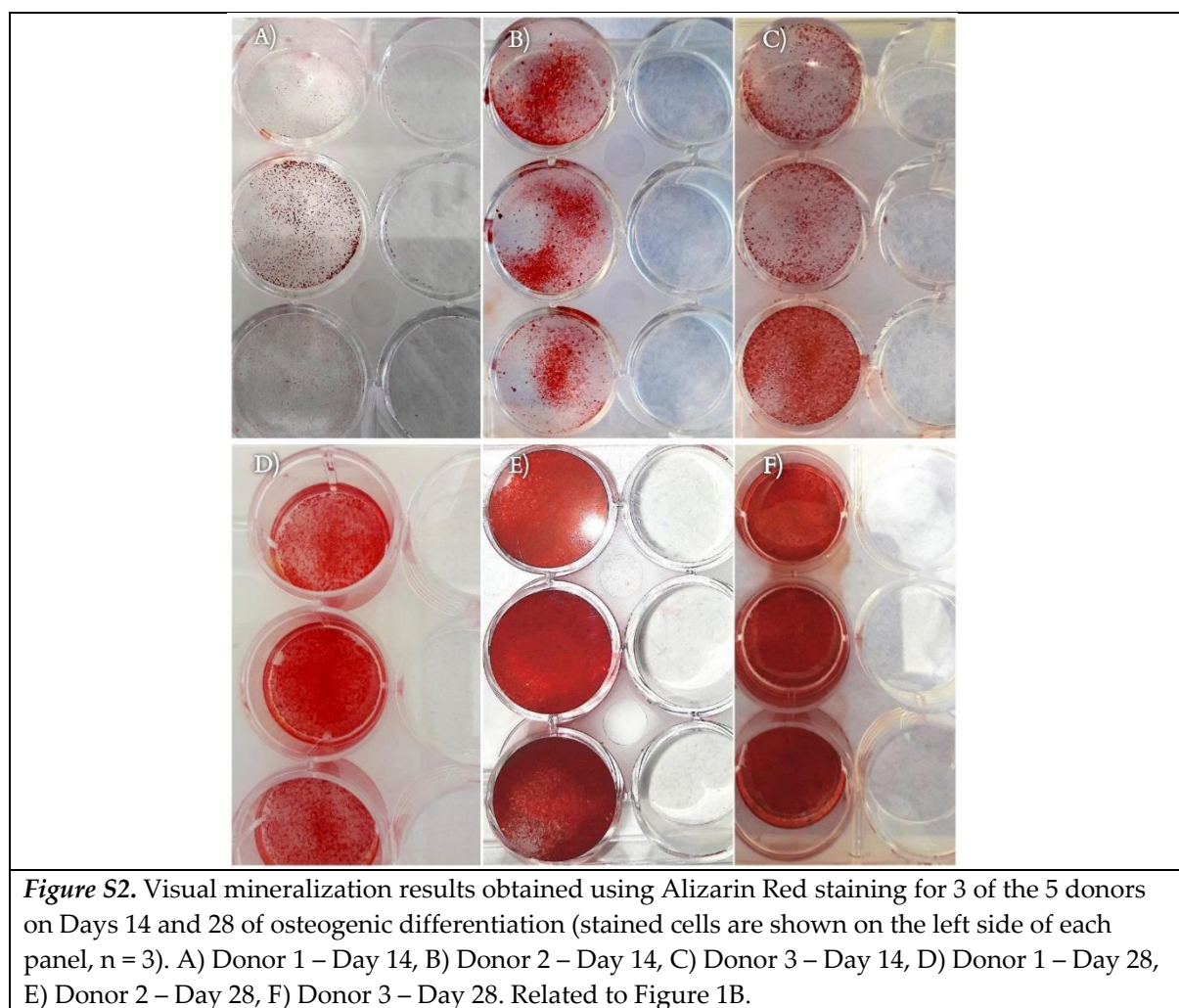


Figure S1. Indicators of osteogenic differentiation. (A) Alkaline phosphatase (ALP) activity for Days 0, 7, 14 and, 28. ALP is shown as nmol/min of p-nitrophenyl phosphate converted into p-nitrophenol. (B) Alizarin Red quantification for Days 0, 14, and 28. Mean and standard error of the mean (SEM) are shown ($n = 4$).



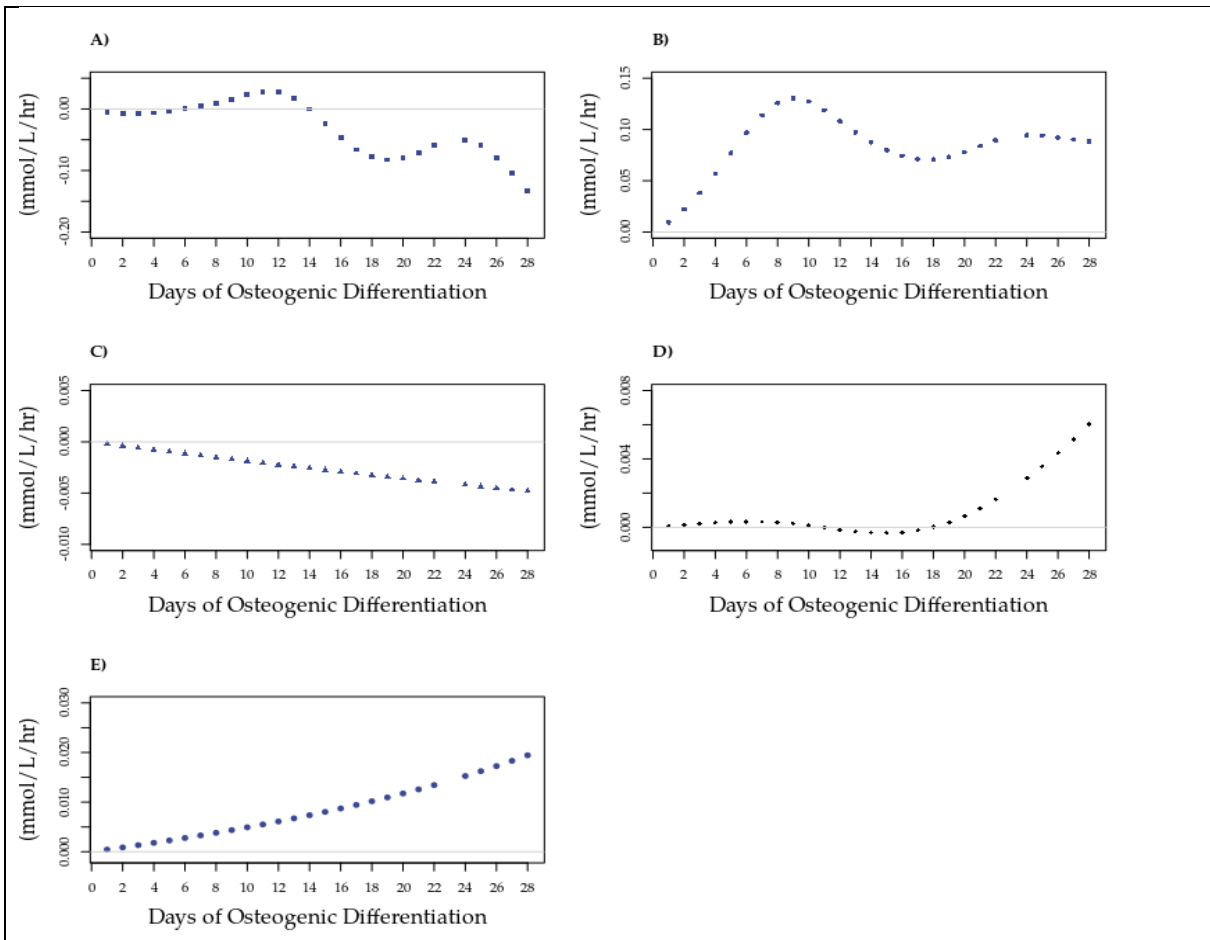
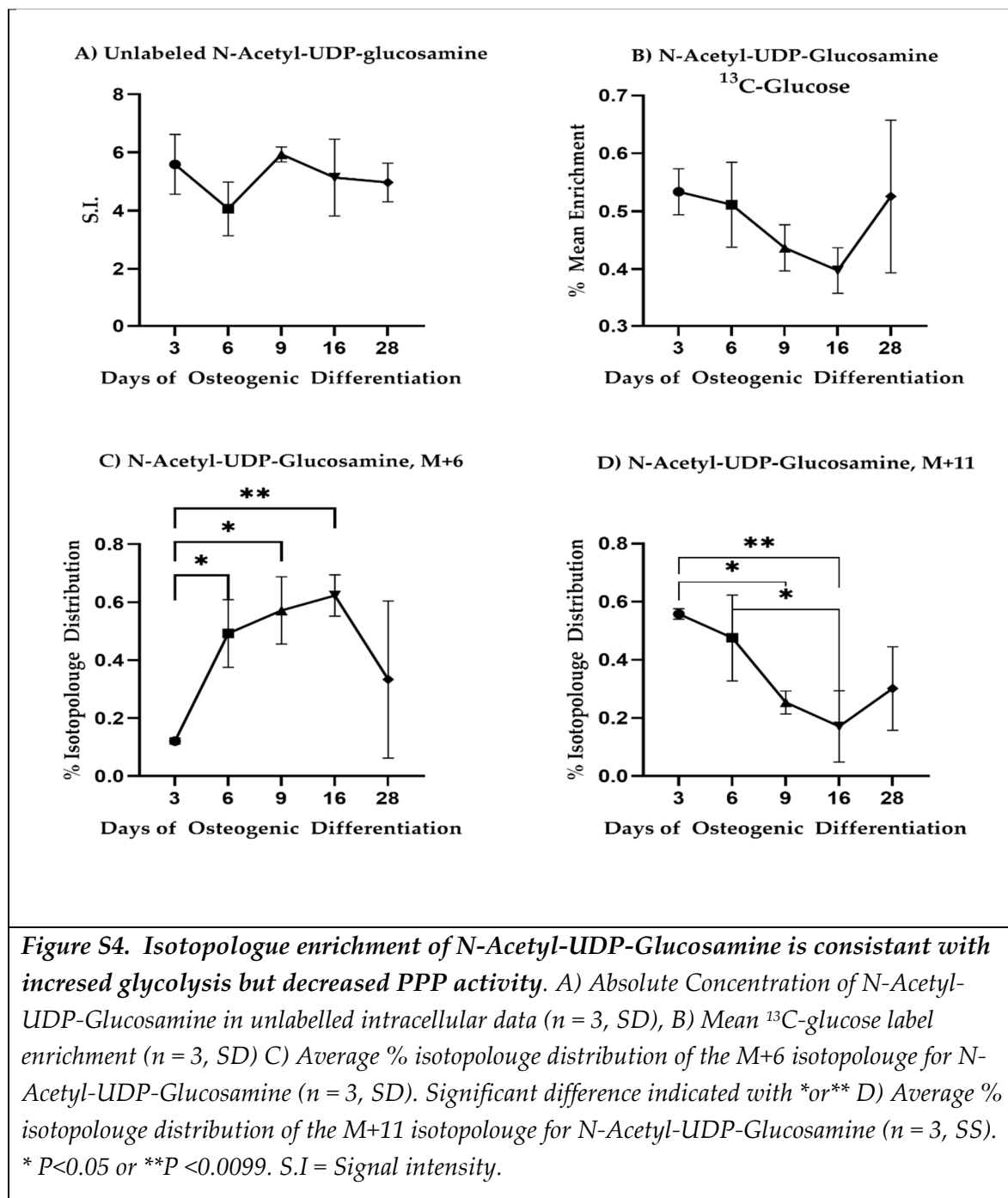


Figure S3. Rate of concentration change (mmol/l/hr) in spent medium for extracellular (A) glucose ($n = 4$), (B) lactate ($n = 4$), (C) glutamine ($n = 4$), (D) ammonia ($n = 4$), and (E) glutamate ($n = 4$) for Days 1 to 28 of osteogenic differentiation. Dots (various shapes) represent the model predicted value subsequently used in change point analysis.



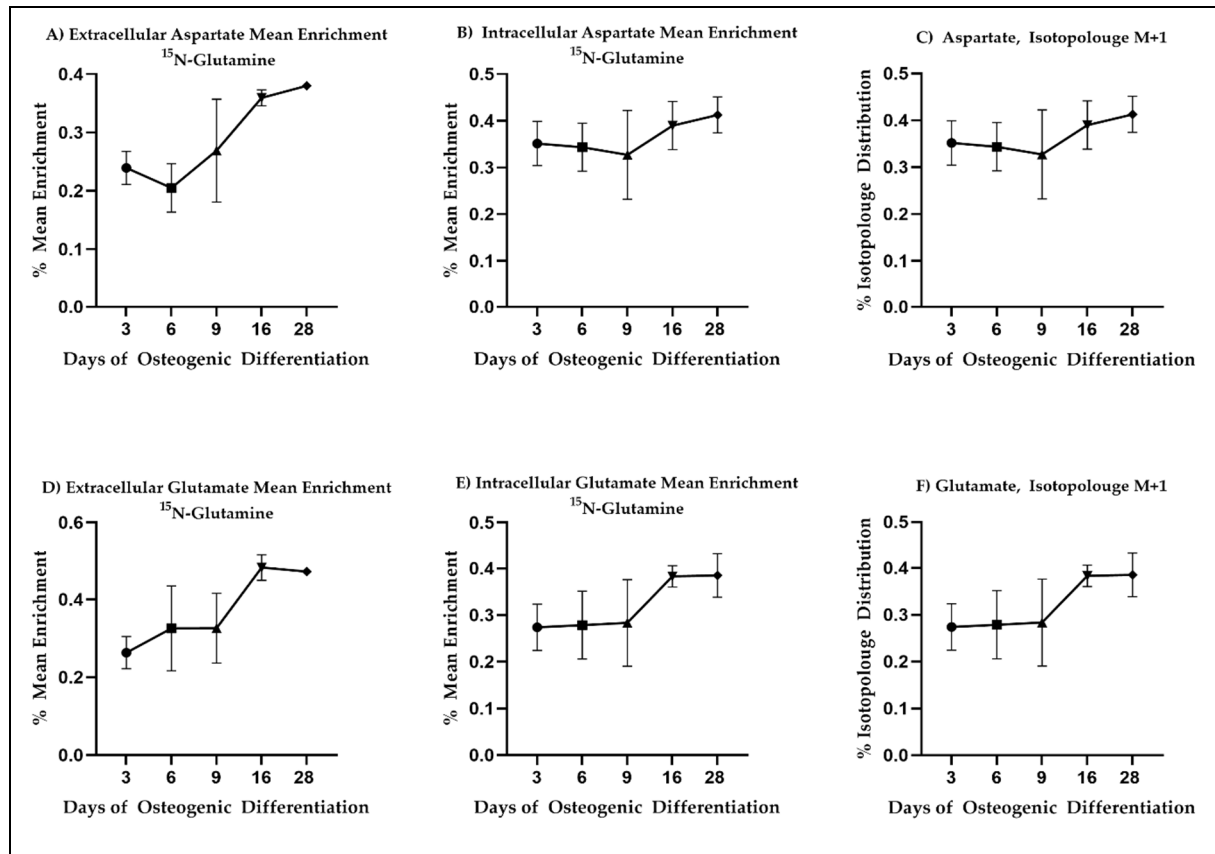


Figure S5. ^{15}N - glutamine label incorporation and isotopologue enrichment of aspartate and glutamate. A) Extracellular mean label enrichment of aspartate ($n = 3$, SD), B) Intracellular mean label enrichment of aspartate ($n = 3$, SD) C) Average % isotopologue distribution of the M+1 isotopologue for aspartate ($n = 3$, SD) D) Extracellular mean label enrichment of glutamate ($n = 3$, SD), E) Intracellular mean label enrichment of glutamate ($n = 3$, SD) (n = 3, SD) F) Average % isotopologue distribution of the M+1 isotopologue for glutamate ($n = 3$, SD)

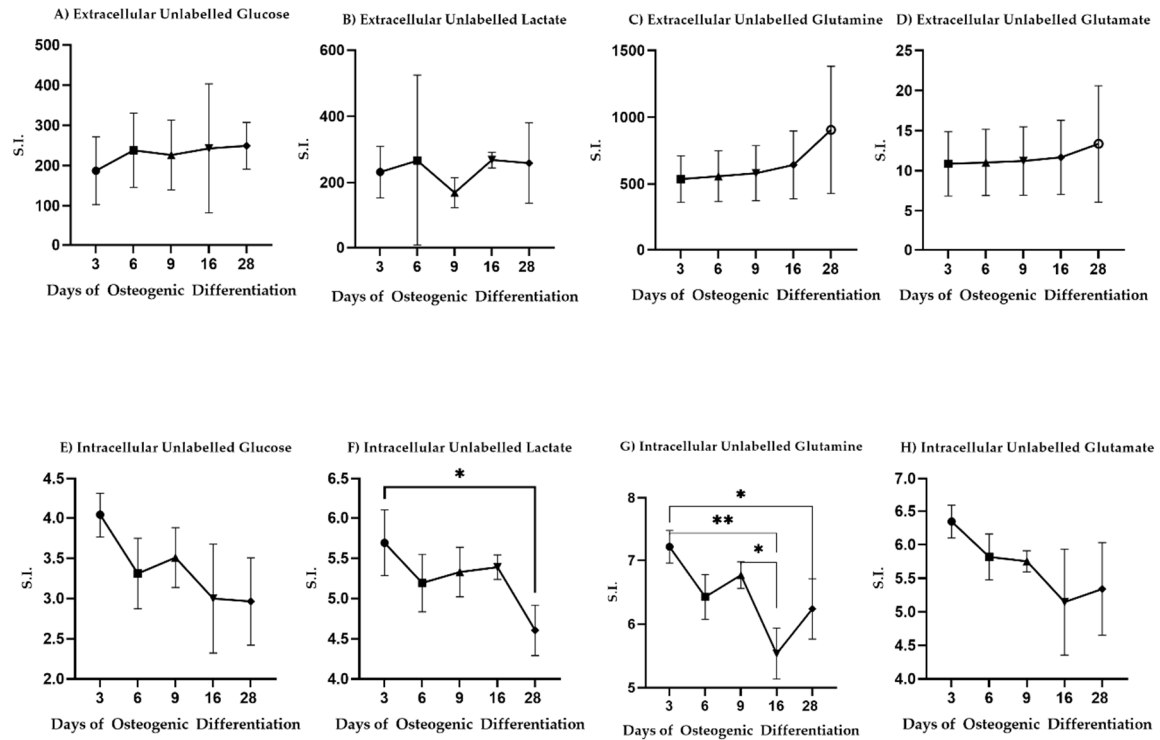


Figure S6. Intracellular and extracellular absolute concentration levels for A) and E) glucose, B) and F) lactate, C) and G) glutamine, and D) and H) glutamate ($n = 3$, SD in all instances). S.I = signal intensity * $P < 0.05$ or ** $P < 0.0099$.