

Electronic Supplementary Information (ESI)

Interactions between Mesenchymal Stem Cells, Adipocytes, and Osteoblasts in a 3D Tri-Culture Model of Hyperglycemic Conditions in the Bone Marrow Microenvironment

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Fig. S1 Principal Component Analysis (PCA) was conducted to determine if difference in gene expression resulted in clustering of culture configuration or glucose levels prior to further assigning of classes for PLS-DA. (A) PCA for the entire adipocyte gene expression data set yielded two principal components that discriminated adipocytes by culture configuration ($R^2X = 0.84$, $Q^2 = 0.74$). (B) PCA score and loading plots for AAA ($R^2X = 0.74$, $Q^2 = 0.45$), AMA ($R^2X = 0.65$, $Q^2 = 0.25$), and OMA ($R^2X = 0.74$, $Q^2 = 0.56$) cultures yielded one PC. Observed clustering by glucose level warranted further investigated with PLS-DA for AAA. (C) PLS-DA weight plot for AAA model in Fig. 2C.

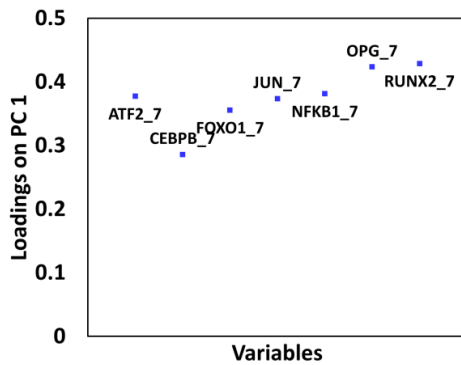
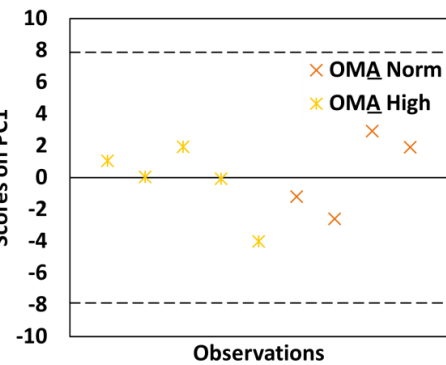
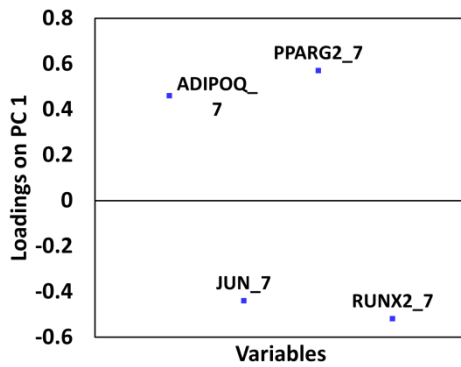
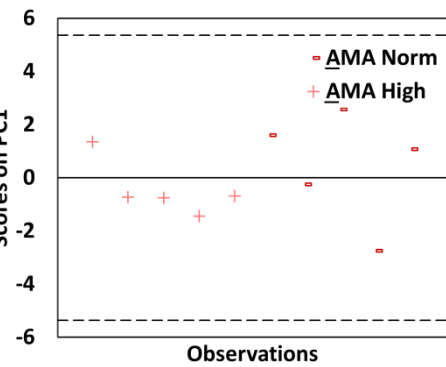
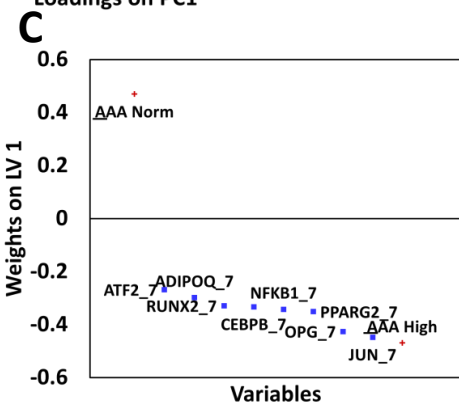
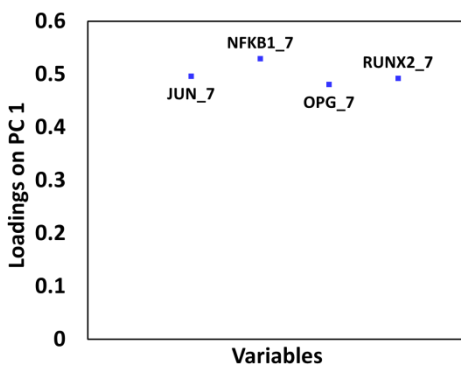
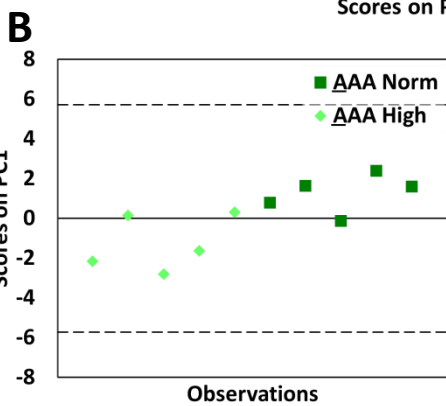
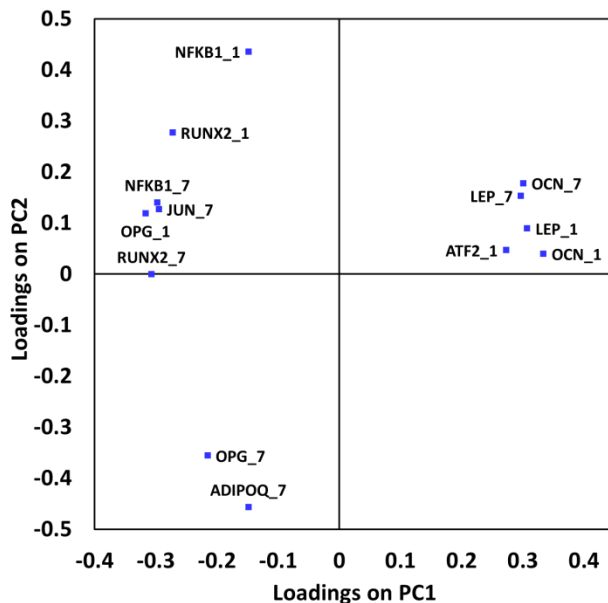
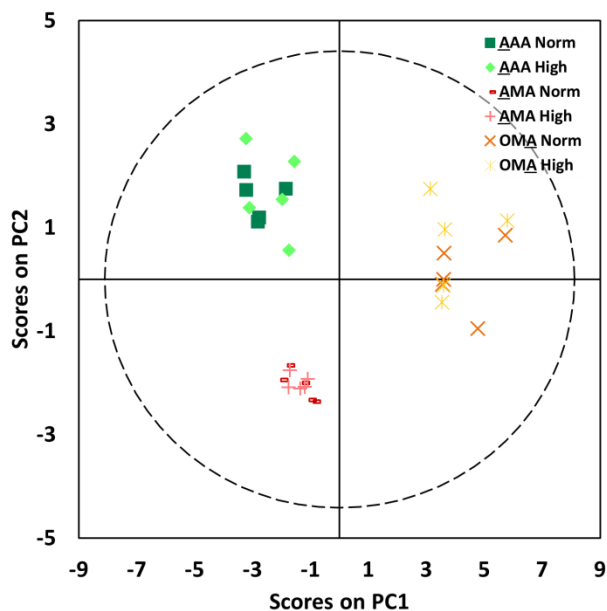
Fig. S2 Images were captured using confocal microscopy of calcein (green, live) and ethidium bromide (red, dead) staining in each experimental condition. Merged representative images are presented here. Representative images of adipocytes (A), osteoblasts (B), and MSCs (C) on days 1 and 7 at normal and high glucose conditions in each culture configuration.

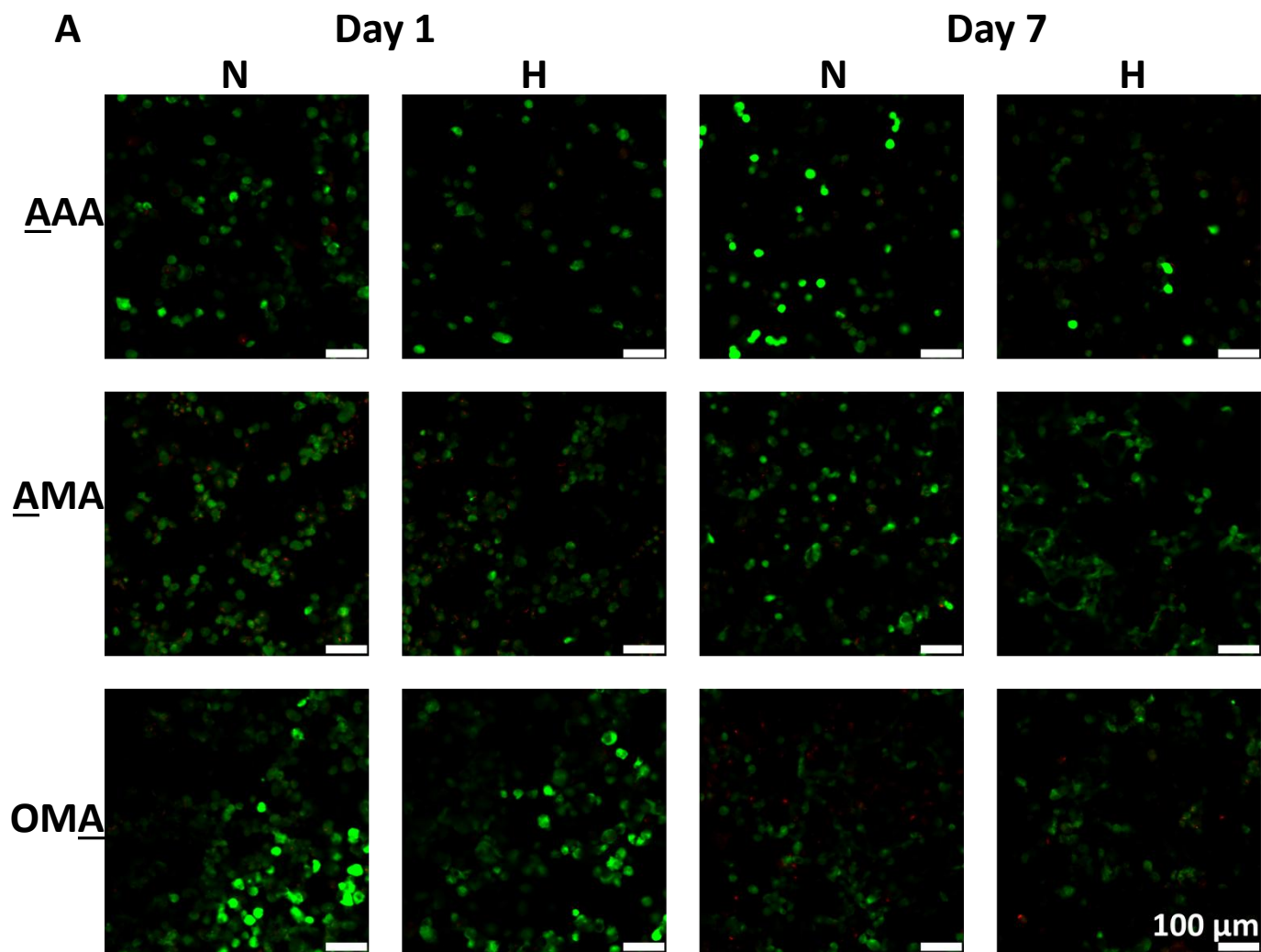
Fig. S3 PCA was conducted within culture configuration datasets to determine if difference in gene expression resulted in clustering glucose levels prior to further assigning of classes for PLS-DA. (A) PCA score and loading plots for OOO ($R^2X = 0.71$, $Q^2 = 0.52$), OMO ($R^2X = 0.72$, $Q^2 = 0.51$), and OMA ($R^2X = 0.72$, $Q^2 = 0.41$) cultures yielded one PC. Observed clustering by glucose level warranted further investigated with PLS-DA for OOO and OMA configuration for classes assigned according to glucose level. (B) PLS-DA weight plot for OOO and OMA models in Fig. 4C.

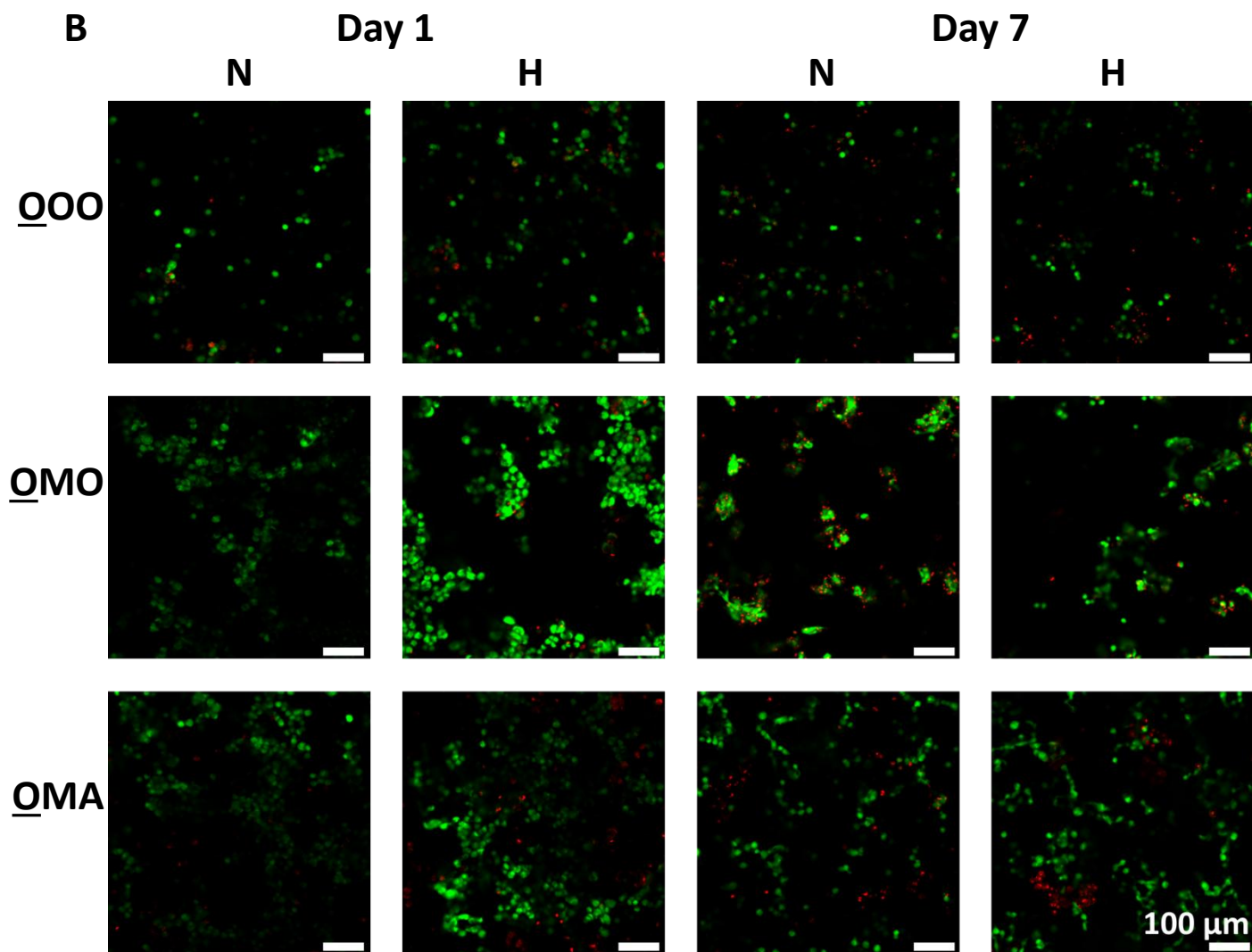
Fig. S4 PCA was conducted to determine if difference in gene expression resulted in clustering of culture configuration or glucose levels prior to further assigning of classes for PLS-DA. PCA for the entire MSC gene expression data set yielded two principal components and clustering by culture configuration ($R^2X = 0.66$, $Q^2 = 0.36$).

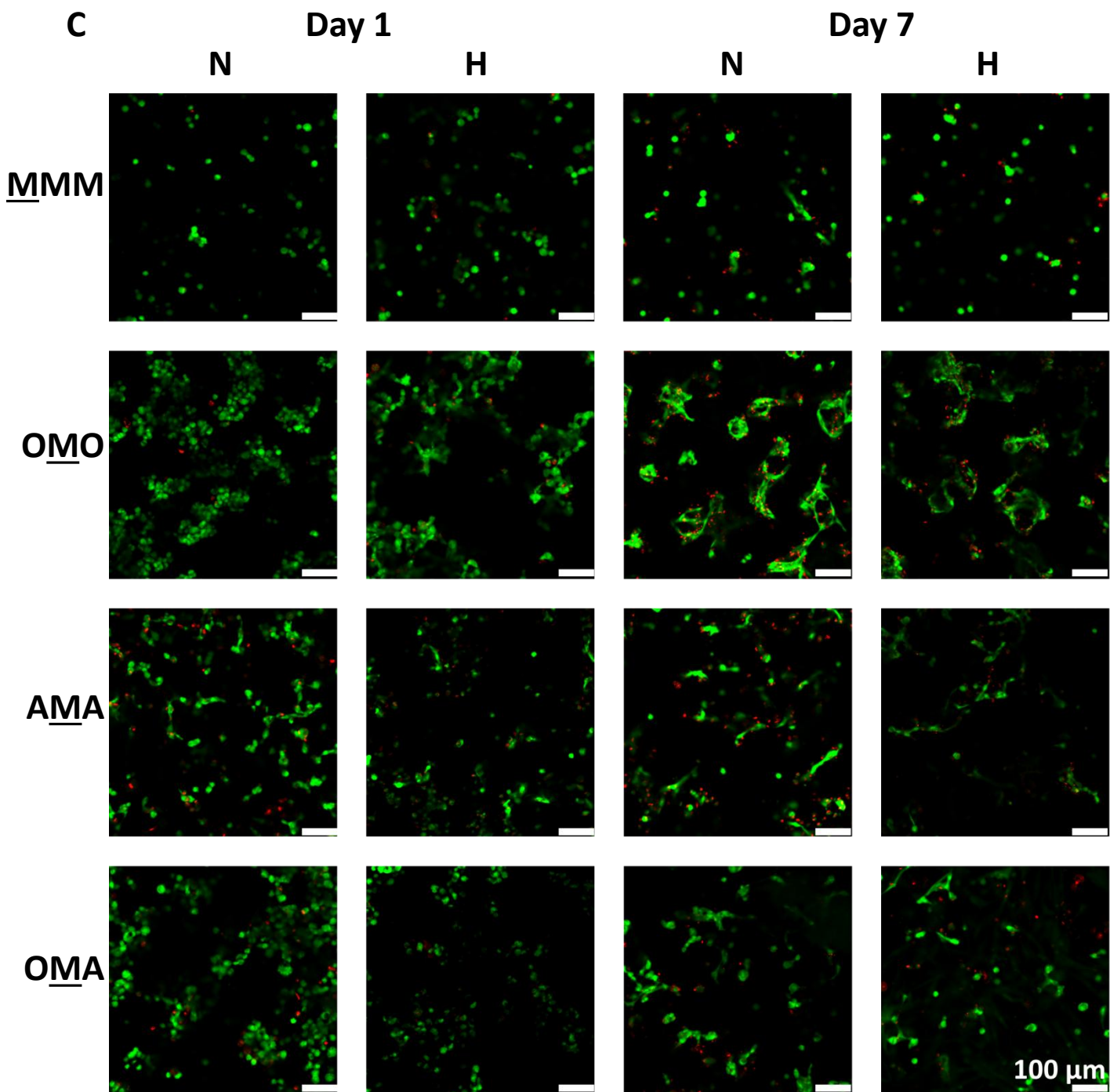
Fig. S5 PCA was conducted within culture configuration datasets to determine if difference in gene expression resulted in clustering glucose levels prior to further assigning of classes for PLS-DA. PCA score and loading plots for MMM ($R^2X = 0.66$, $Q^2 = -0.1$), OMO ($R^2X = 0.73$, $Q^2 = 0.43$), AMA ($R^2X = 0.65$, $Q^2 = 0.42$), and OMA ($R^2X = 0.68$, $Q^2 = 0.02$) cultures yielded one PC.

Fig. S6 Observed clustering by glucose level warranted further investigated with PLS-DA for OMO and AMA culture configurations with classes assigned according to glucose level. PLS-DA score and weight plot for OMO ($R^2Y = 0.82$, $Q^2 = 0.73$) and AMA ($R^2Y = 0.62$, $Q^2 = 0.41$).

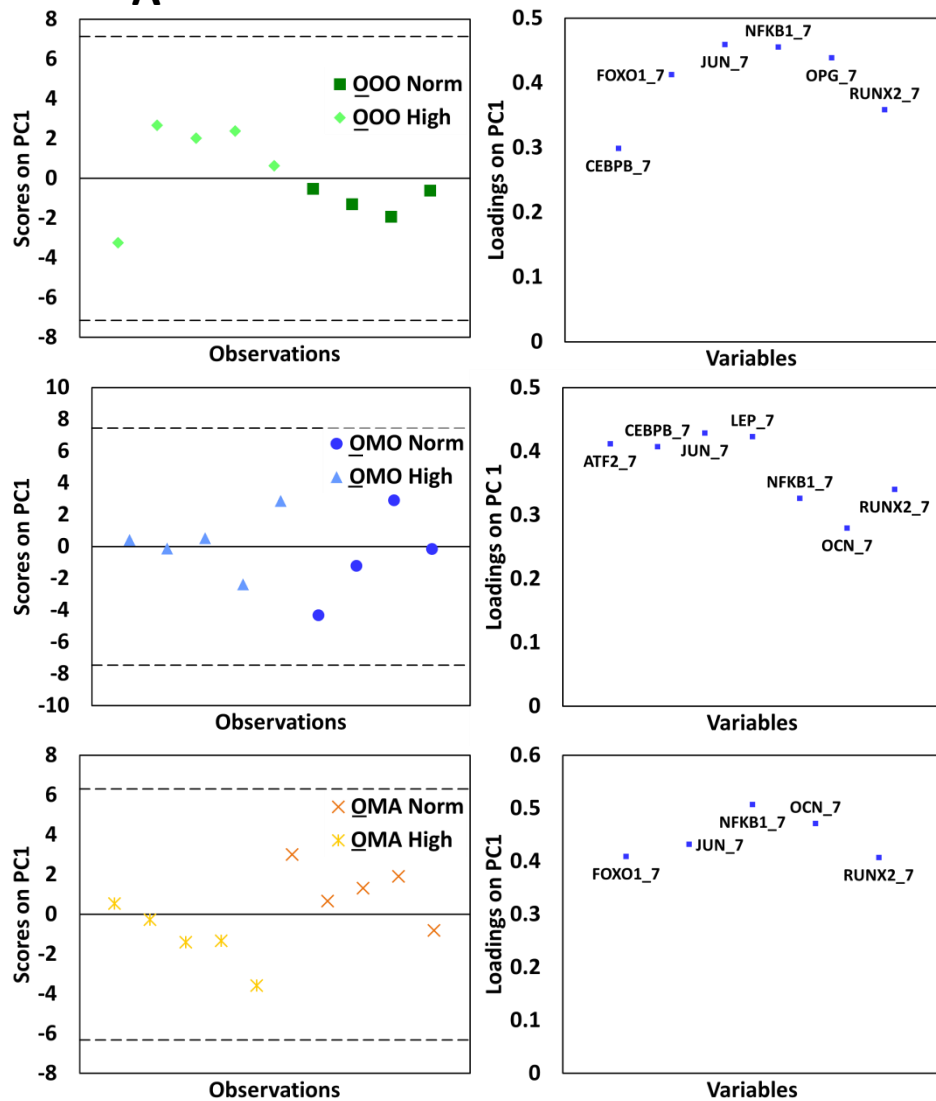




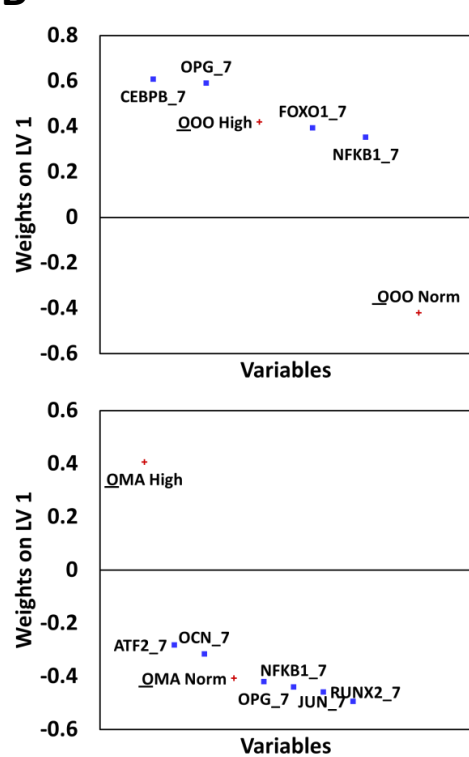


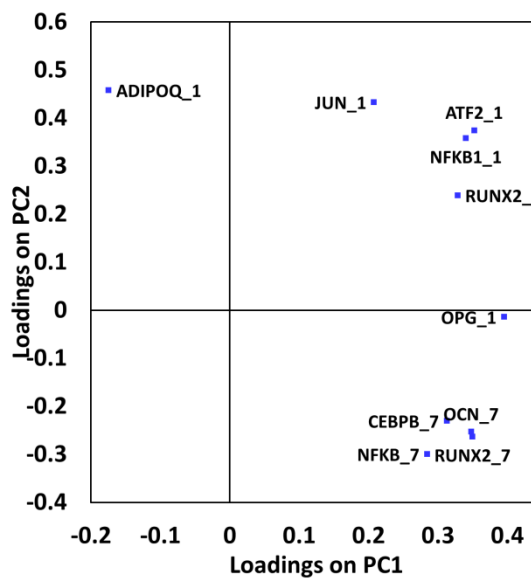
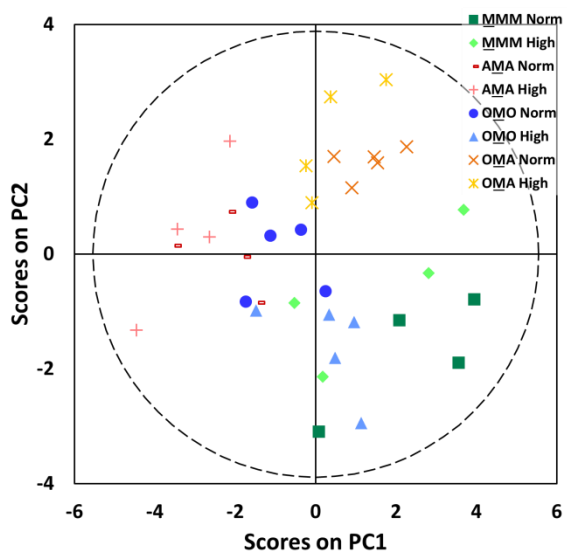


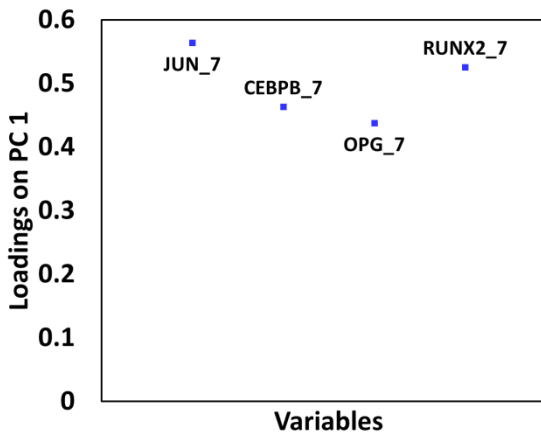
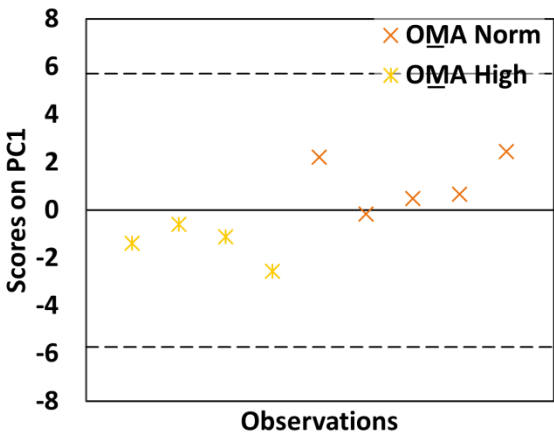
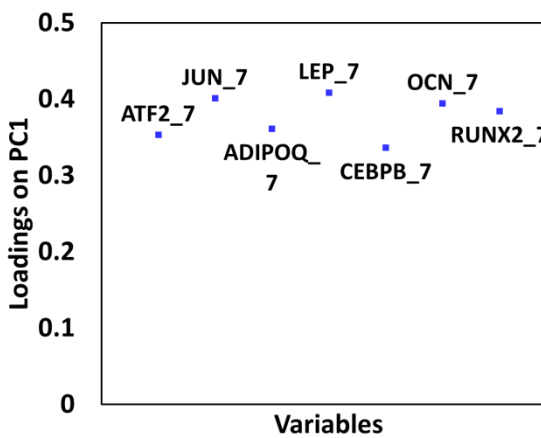
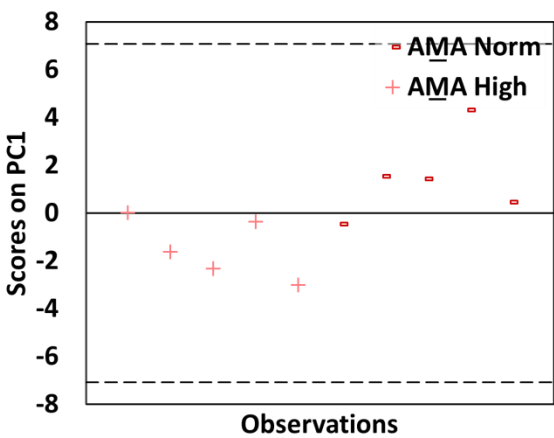
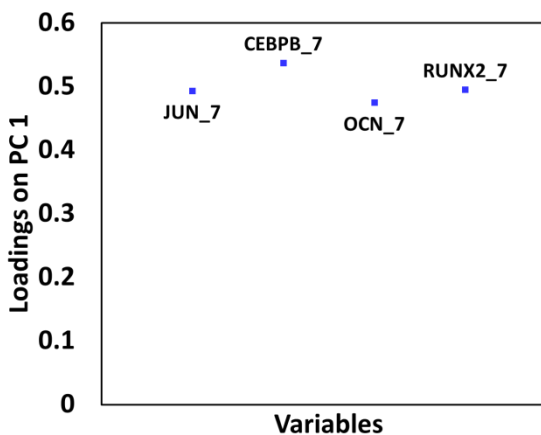
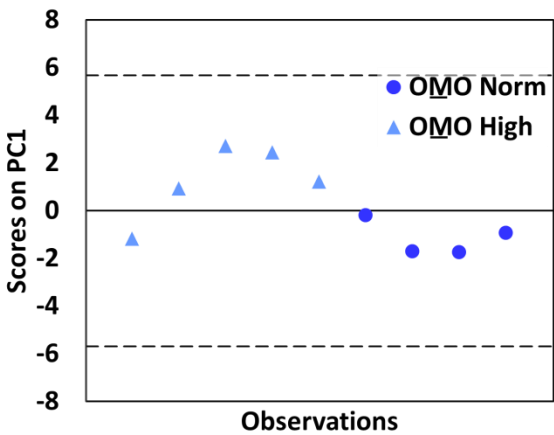
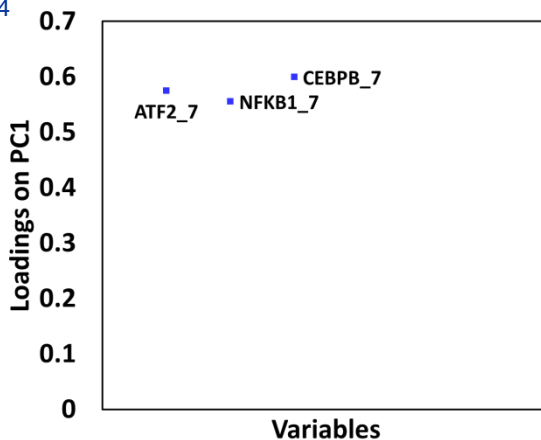
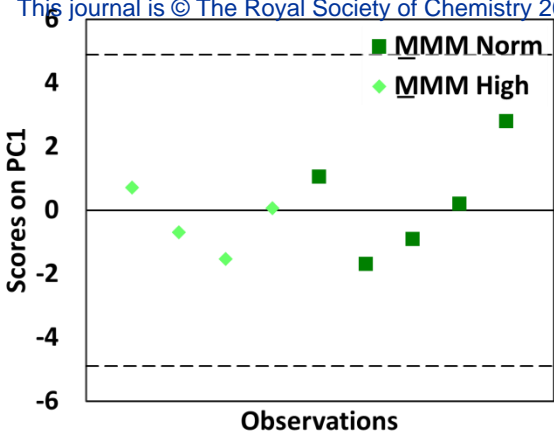
A



B







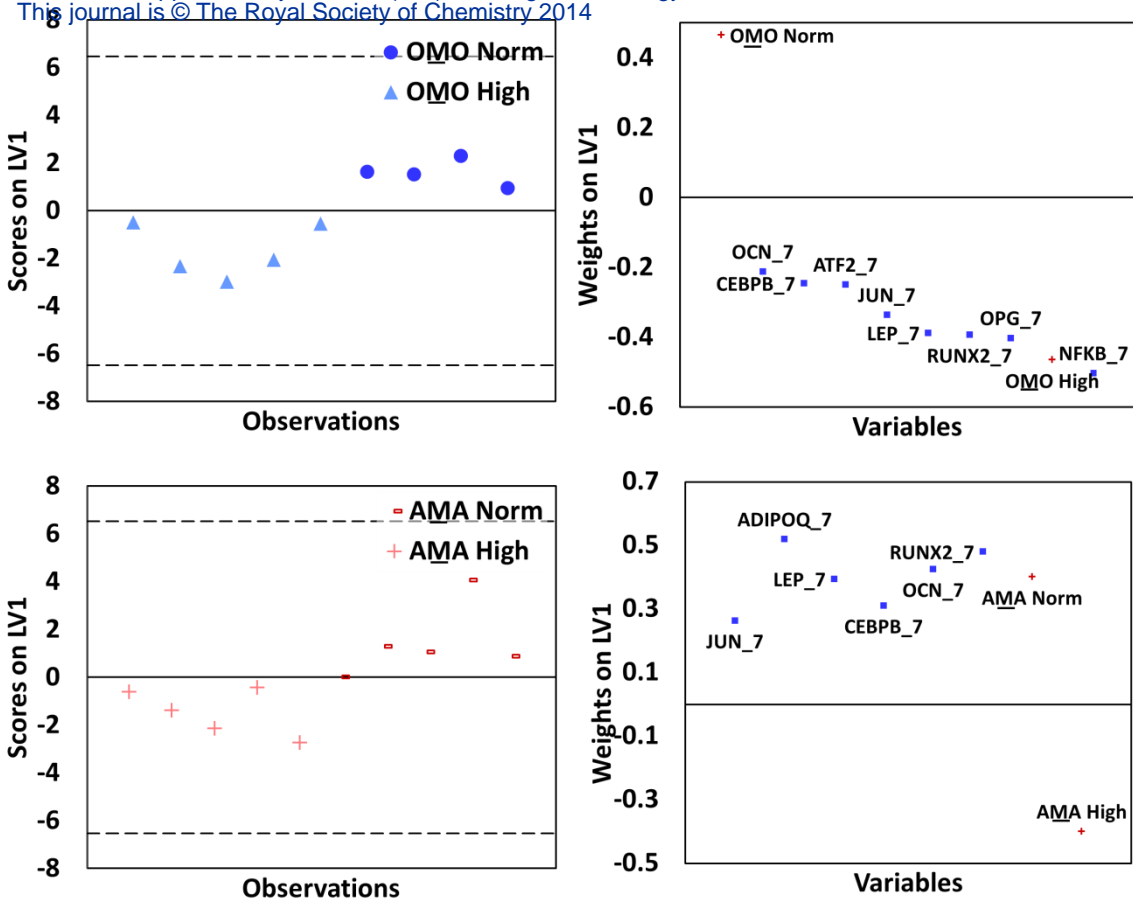


Table S1. National Library of Medicine accession number and primer sequences for target genes in qPCR.

Target Gene	Accession Number	Primer Set (Forward, Reverse)
RUNX2	NM_001024630	TTTGCACTGGGTTCATGTGTT
		TGGCTGCATTGAAAAGACTG
OCN	NM_199173	GTGCAGAGTCCAGCAAAGGT
		AGCAGAGCGACACCCTAGAC
OPG	NM_002546	CGGGAAAGAAAGTGGGAGCAGAAG
		CGTCTTTGAGTGCTTTAGTGCGTG
PPARG2	NM_015869	TCCATGCTGTTATGGGTGAA
		GGGAGTGGTCTTCCATTACG
CEBPB	NM_005194	CGAGTCAGAGCCGCGCAC
		GCAGGGGGAGACATGCTGGG
LEP	NM_000230	ACCCTGTGCGGATTCTTGTGGCTTTGG
		GGCTCTGCCTACCCCTCTGCCCT
ADIPOQ	NM_001177800	ATCTGGTTGGGGTGGGCTCCTTAC
		GTTGACTCTCTCTGTGCCTCTGGTT
ATF2	NM_001880	GGTCCTTCTCTCCCAACCAGTA
		CTGTAGTGGATGTGGCTGGCTGT
JUN	NM_002228	GACAGACACAGCCAGCCAGCCAG
		GGACTCTCCGAAACACCAGCCC
FOXO1	NM_002015	GCTACCAATAACCCAGCCCCAA
		AATGCCAGGTTGGTCTGTTGCA
NFKB1	NM_003998	AGACAAAAGTGGGCTACTCTGGCG
		TGAGAGGTGGTCTTCACTGGGCT
RPS18	NM_022551	CGATGGGCGGCGGAAAATAGCCTTGC
		CAGTGGTCTTGGTGTGCTGGCCTCGG
ACTB	NM_001101	GCAGTCGGTTGGAGCGAGCATCCCC
		TCCCCTGTGTGGACTTGGGAGAGGAC