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.

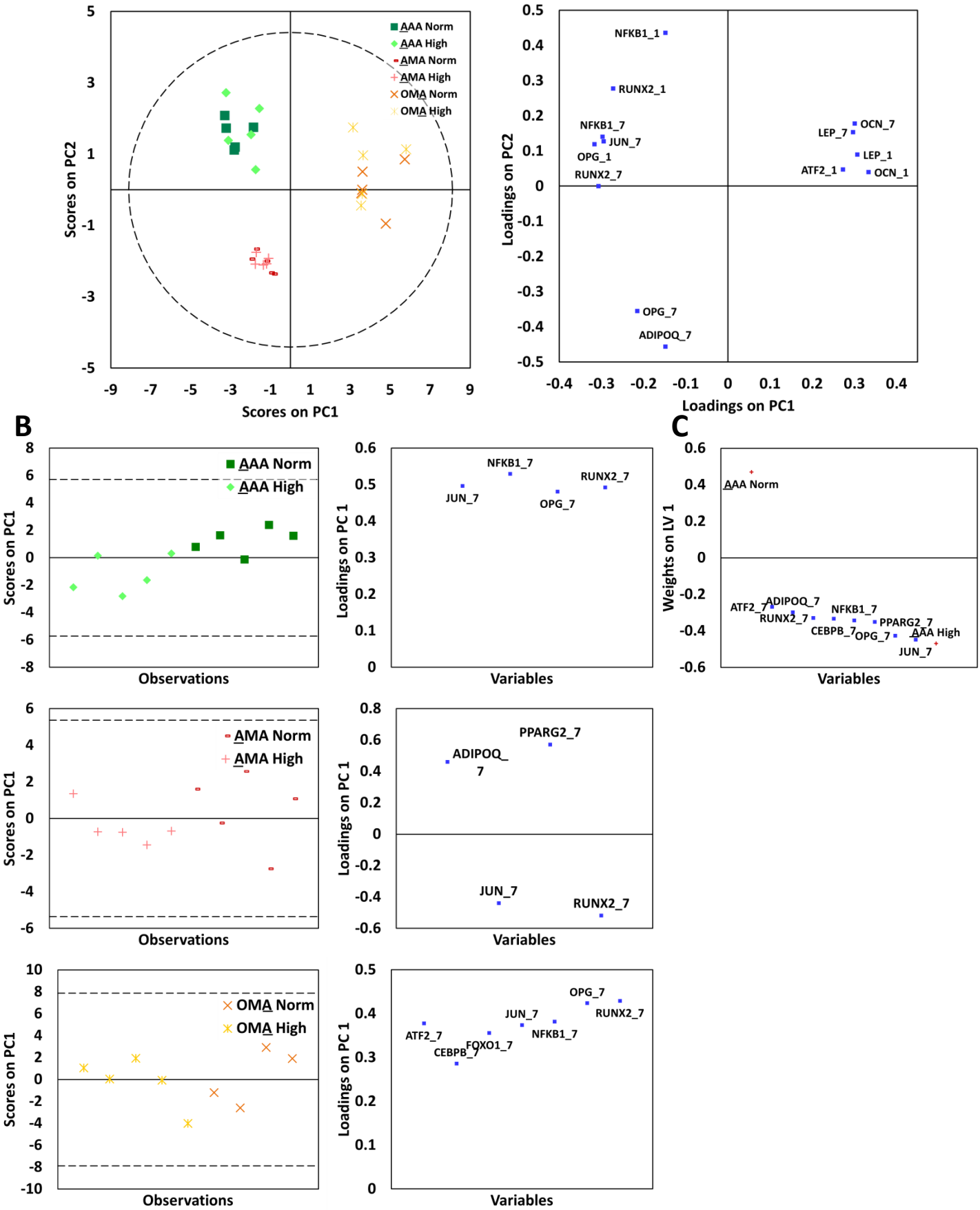
Fig. S3 PCA was conducted within culture configuration datasets to determine if difference in gene expression resulted in clustering glucose levels prior to further assigning of classes for PLS-DA. (A) PCA score and loading plots for OOO (R^2X = 0.71, Q^2 = 0.52), OMO (R^2X = 0.72, Q^2 = 0.51), and OMA (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).

Figure S1


A
AAA Norm
AAA High
AMA Norm
AMA High
OMA Norm
OMA High
Scores on PC2
Scores on PC1
Loadings on PC2
Loadings on PC1
NFKB1_1
RUNX2_1
NFKB1_7
JUN_7
OPG_1
RUNX2_7
OPG_7
ADIPOQ_7
LEP_7
OCN_7
LEP_1
ATF2_1
OCN_1
B
Scores on PC1
Observations
Loadings on PC 1
Variables
NFKB1_7
RUNX2_7
JUN_7
OPG_7
C
Weights on LV 1
AAA Norm
ATF2_7
ADIPOQ_7
RUNX2_7
NFKB1_7
CEBPB_7
PPARG2_7
OPG_7
AAA High
JUN_7
AMA Norm
AMA High
PPARG2_7
ADIPOQ_7
JUN_7
RUNX2_7
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NFKB1_7
ATF2_7
FOXO1_7
CEBPB_7

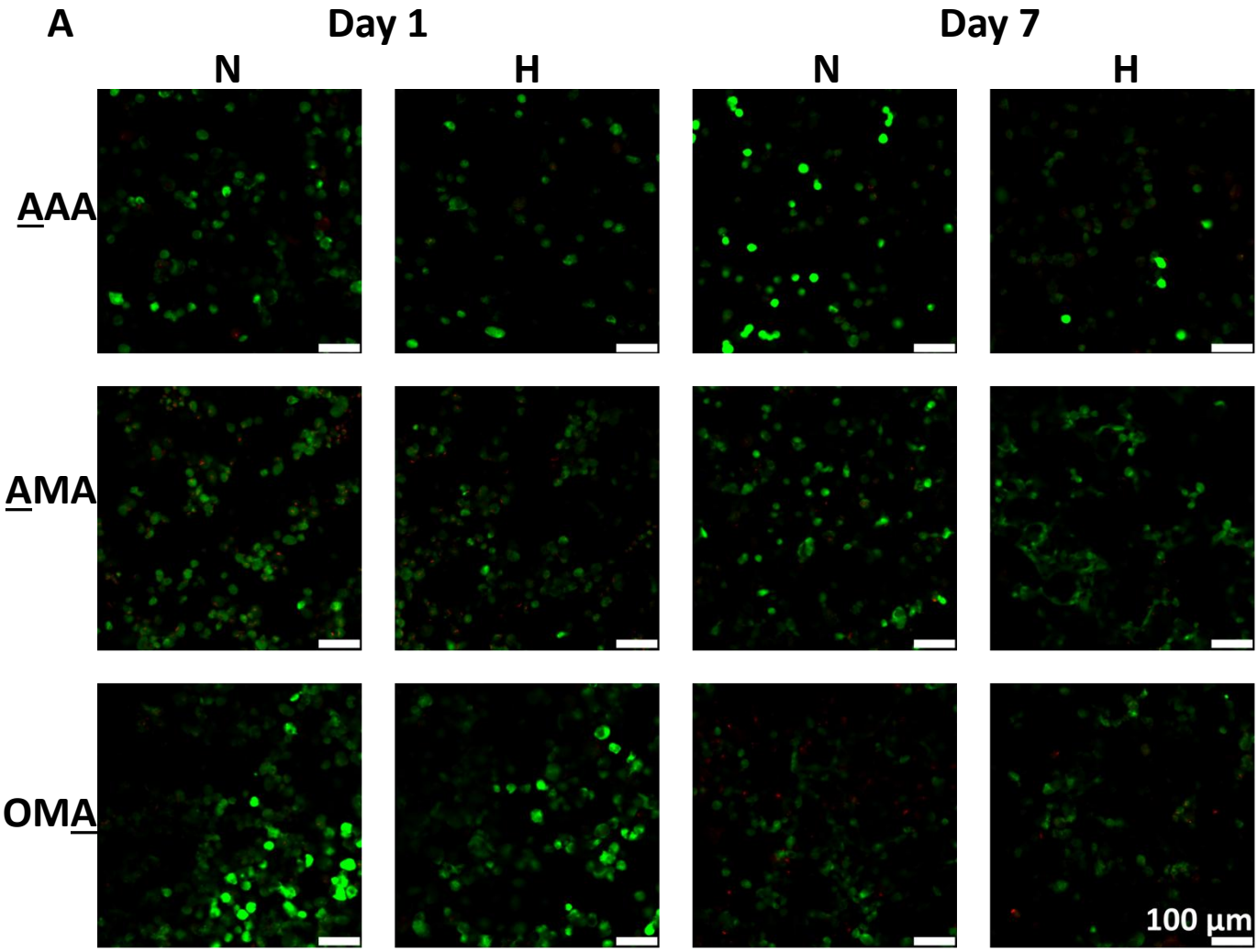

A
Day 1
Day 7
N
H
N
H
AAA
AMA
OMA
100 µm

Figure S2

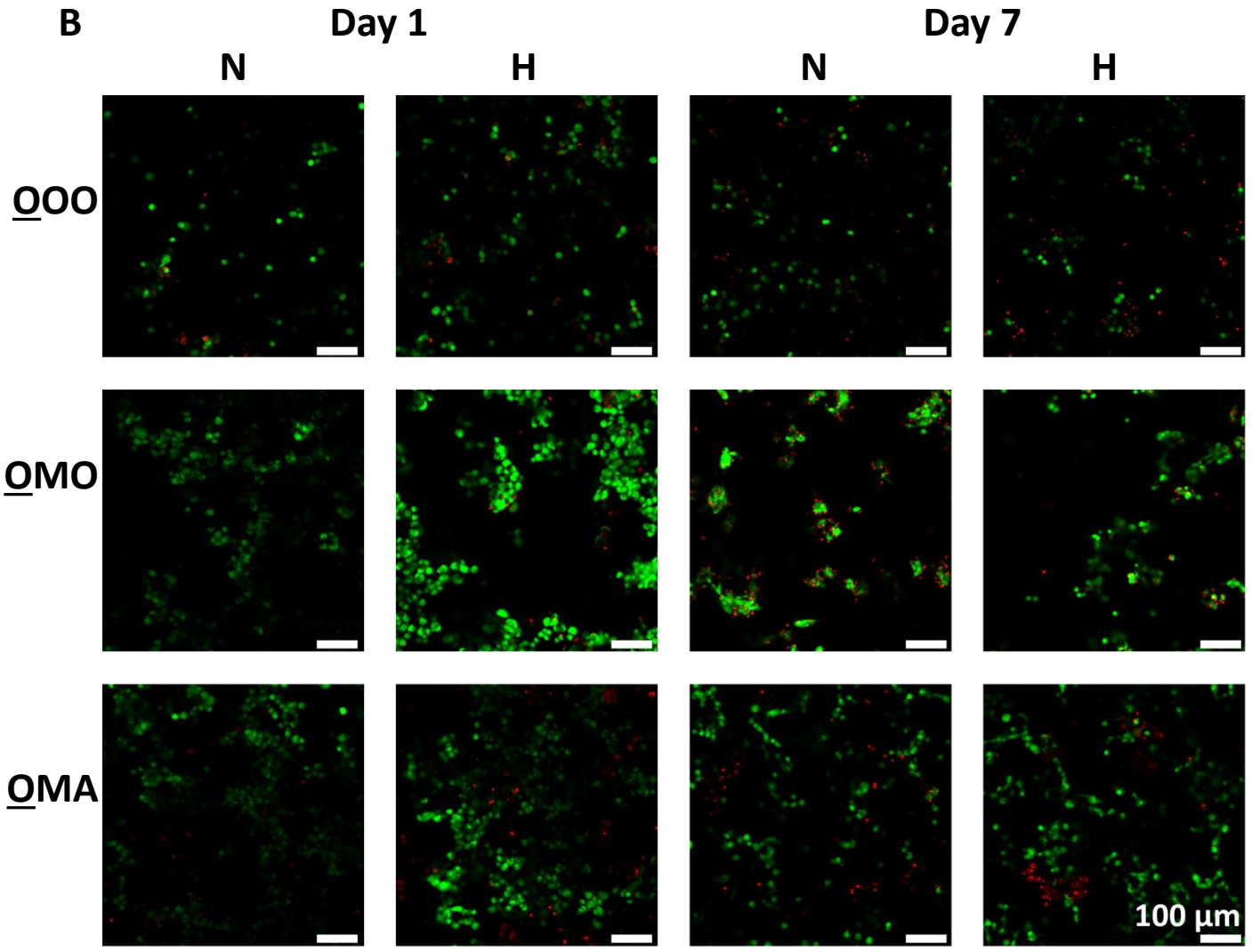


Figure S2

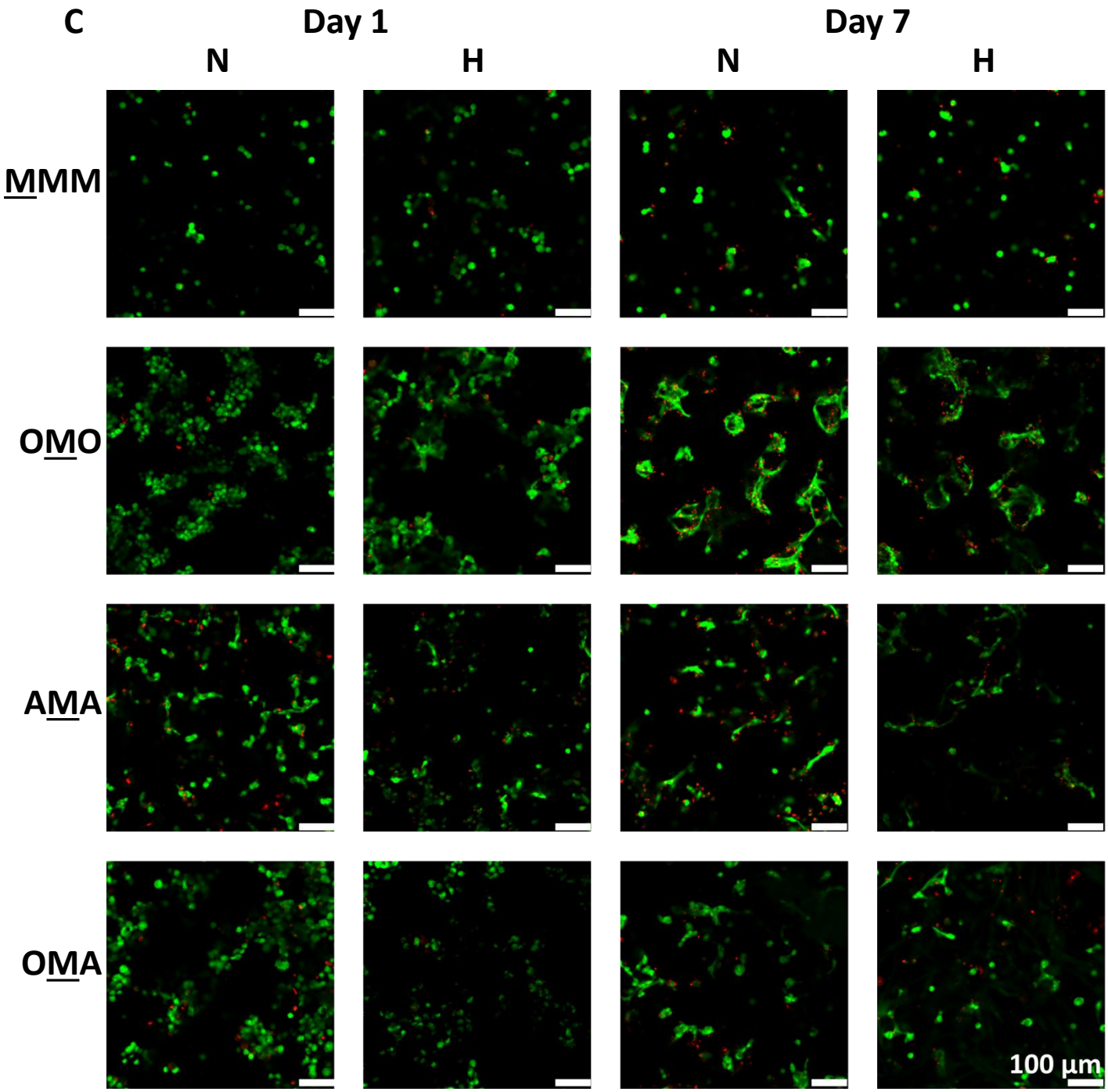


Figure S3

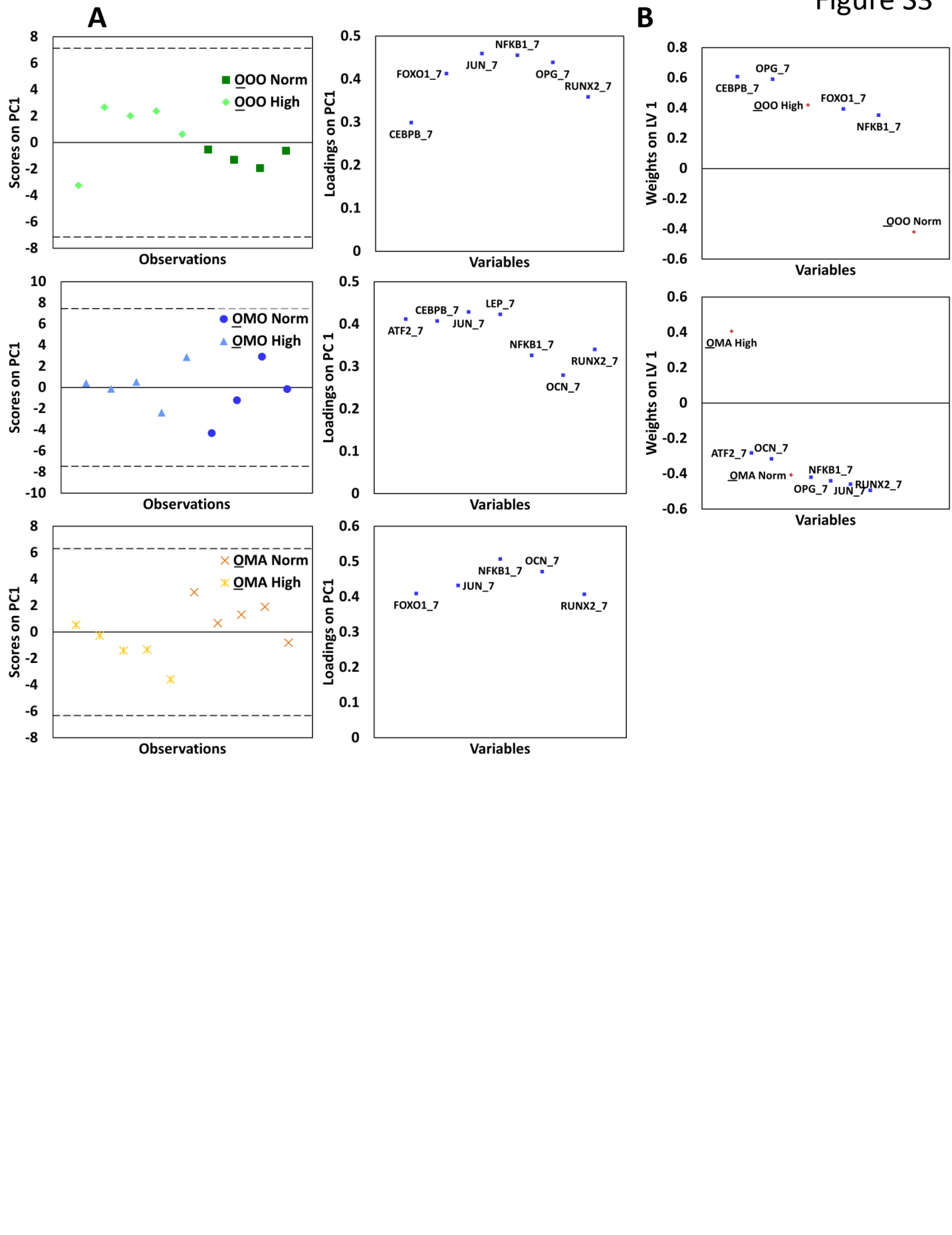

A
B
Scores on PC1
Observations
OOO Norm
OOO High
Loadings on PC1
Variables
NFKB1_7
JUN_7
FOXO1_7
OPG_7
RUNX2_7
CEBPB_7
Weights on LV 1
OMO Norm
OMO High
LEP_7
ATF2_7
OCN_7
OMA Norm
OMA High

Figure S4

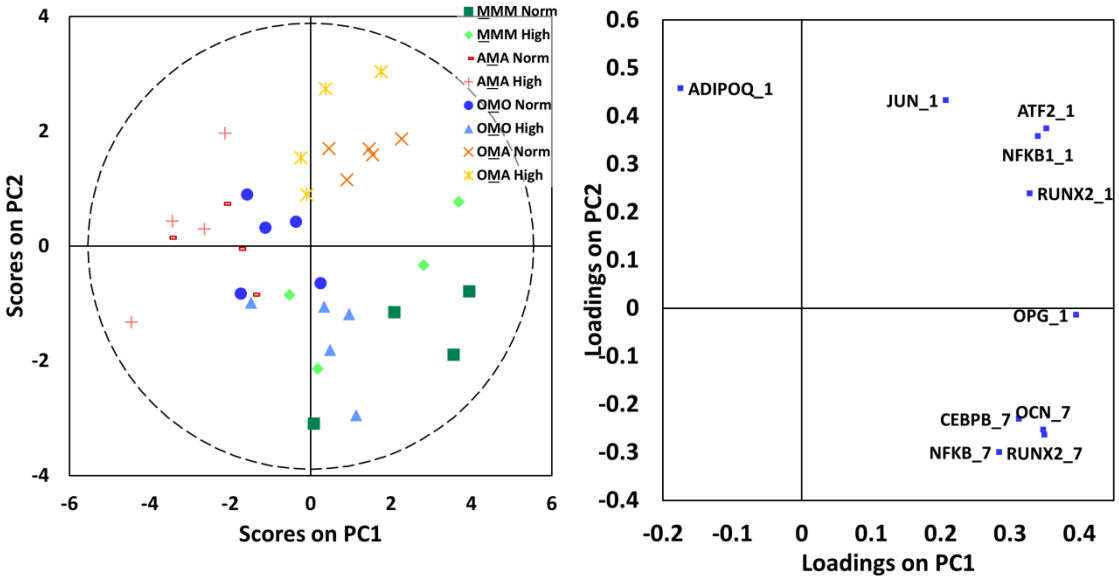
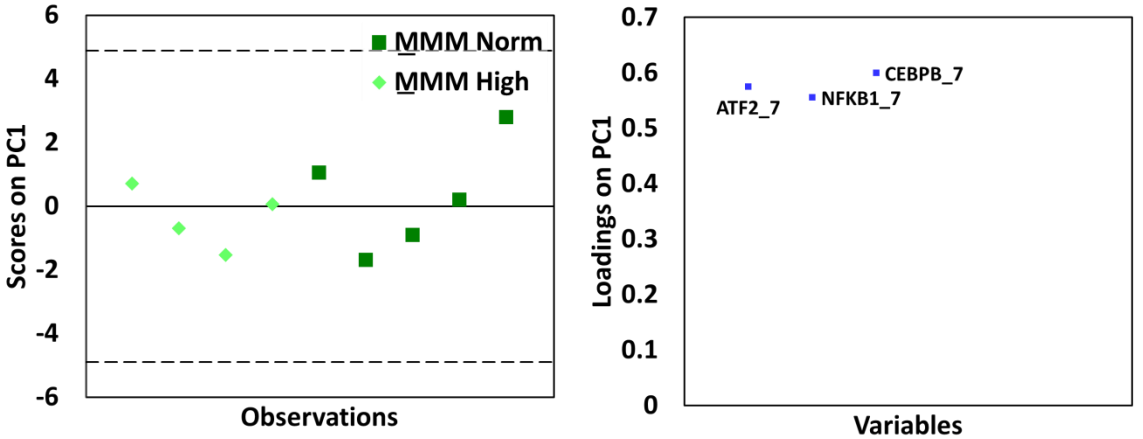
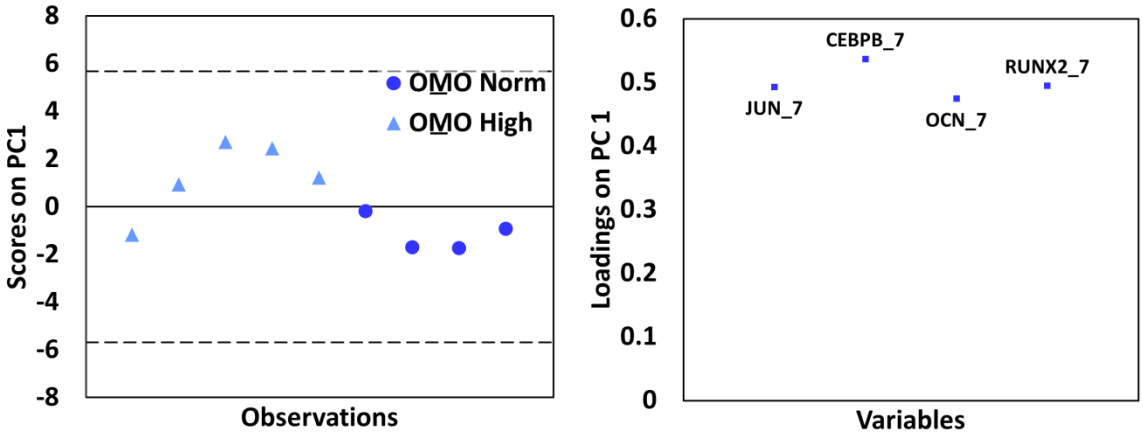

MMM Norm
MMM High
AMA Norm
AMA High
OMO Norm
OMO High
OMA Norm
OMA High
Scores on PC2
Scores on PC1
4
2
0
-2
-4
-6
-4
-2
0
2
4
6
Loadings on PC2
Loadings on PC1
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.2
-0.1
0
0.1
0.2
0.3
0.4
ADIPOQ_1
JUN_1
ATF2_1
NFKB1_1
RUNX2_1
OPG_1
CEBPB_7
OCN_7
NFKB_7
RUNX2_7

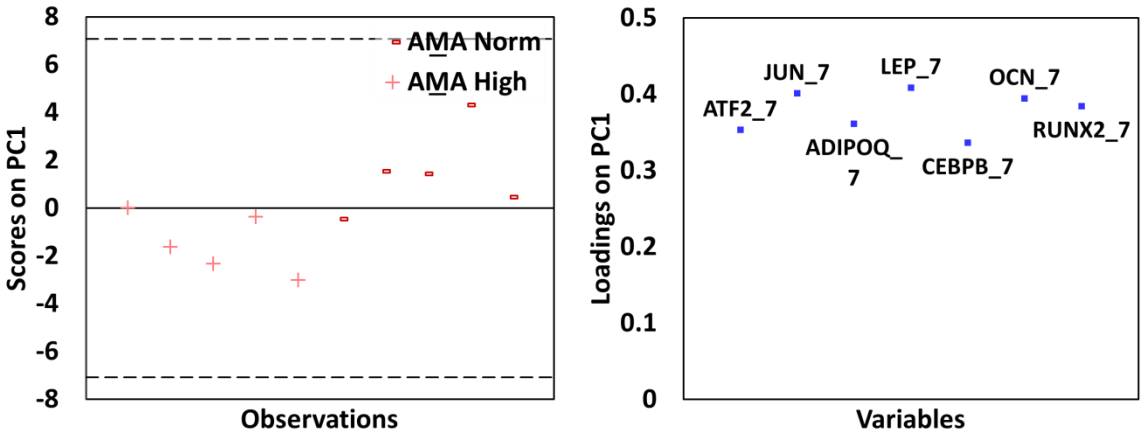
Figure S5


MMM Norm
MMM High
Scores on PC1
Observations
6
4
2
0
-2
-4
-6

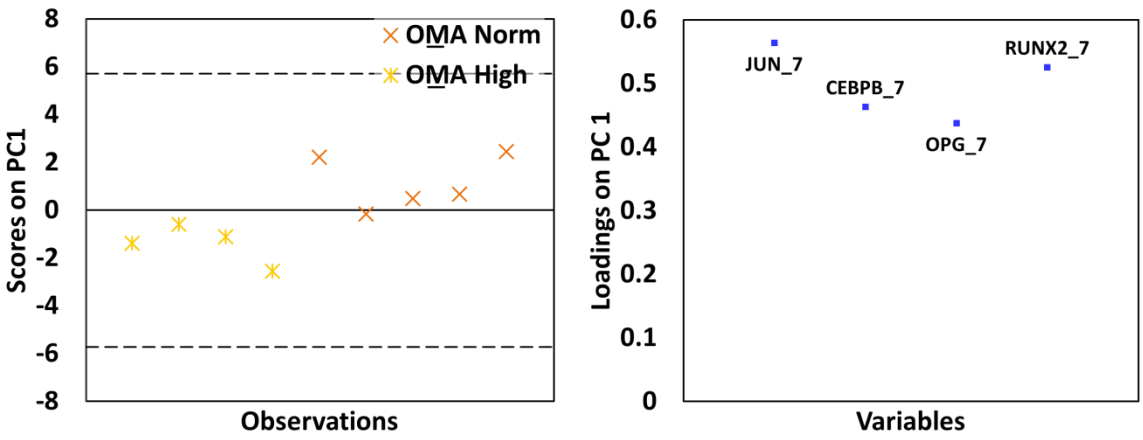
CEBPB_7
ATF2_7
NFKB1_7
Loadings on PC1
Variables
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0


OMO Norm
OMO High
Scores on PC1
Observations
8
6
4
2
0
-2
-4
-6
-8

CEBPB_7
RUNX2_7
JUN_7
OCN_7
Loadings on PC 1
Variables
0.6
0.5
0.4
0.3
0.2
0.1
0

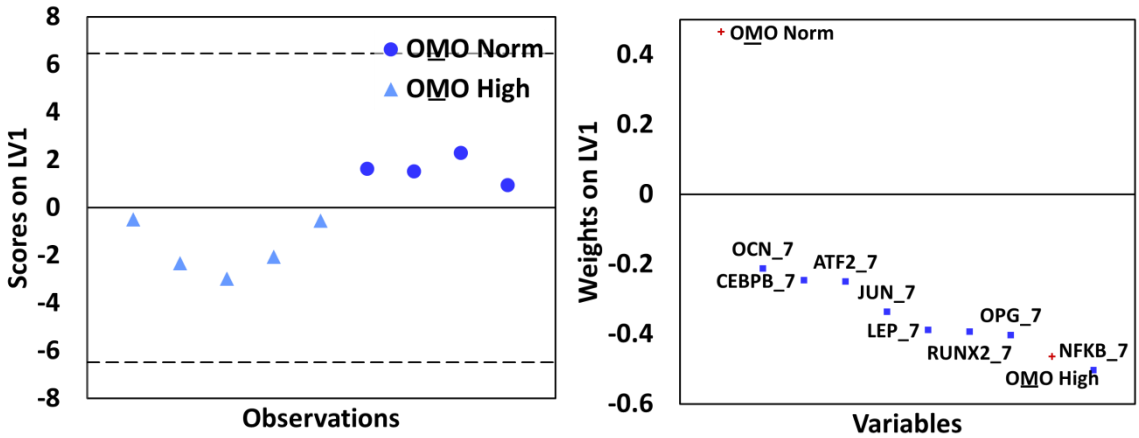

AMA Norm
AMA High
Scores on PC1
Observations
8
6
4
2
0
-2
-4
-6
-8

JUN_7
LEP_7
OCN_7
ATF2_7
RUNX2_7
ADIPOQ_7
CEBPB_7
Loadings on PC1
Variables
0.5
0.4
0.3
0.2
0.1
0


OMA Norm
OMA High
Scores on PC1
Observations
8
6
4
2
0
-2
-4
-6
-8

JUN_7
RUNX2_7
CEBPB_7
OPG_7
Loadings on PC 1
Variables
0.6
0.5
0.4
0.3
0.2
0.1
0

Figure S6


Scores on LV1
Observations
OMO Norm
OMO High
Weights on LV1
Variables
OMO Norm
OCN_7
ATF2_7
CEBPB_7
JUN_7
OPG_7
LEP_7
RUNX2_7
NFKB_7
OMO High

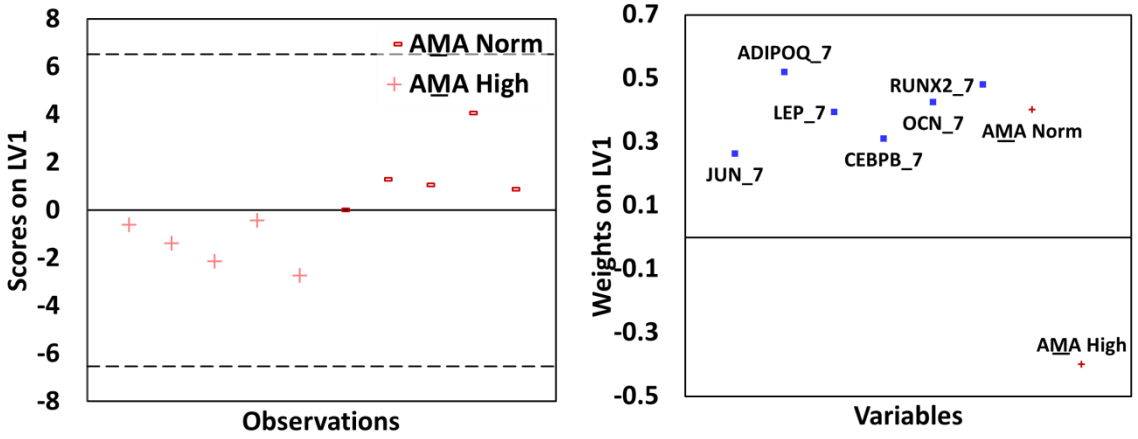

Scores on LV1
Observations
AMA Norm
AMA High
Weights on LV1
Variables
ADIPOQ_7
RUNX2_7
LEP_7
OCN_7
AMA Norm
CEBPB_7
JUN_7
AMA High

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	TTTGCACTGGGTCATGTGTT
		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	GGTCCTTCCTCTCCCCAACCAGTA
		CTGTAGTGGATGTGGCTGGCTGT
JUN	NM_002228	GACAGACACAGCCAGCCAGCCAG
		GGACACTCCCGAAACACCAGCCC
FOXO1	NM_002015	GCTACCAATAACCCCAGCCCCAA
		AATGCCAGGTTGGTCTGTTCGCA
NFKB1	NM_003998	AGACAAAAACTGGGCTACTCTGGCG
		TGAGAGGTGGTCTTCACTGGGCT
RPS18	NM_022551	CGATGGGCGGCGGAAAATAGCCTTGC
		CAGTGGTCTTGGTGTGCTGGCCTCGG
ACTB	NM_001101	GCAGTCGGTTGGAGCGAGCATCCCC
		TCCCCTGTGTGGACTTGGGAGAGGAC