

Supplemental Information

**Malic Enzyme Couples Mitochondria
with Aerobic Glycolysis in Osteoblasts**

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Supplemental Materials

Supplemental Fig. S1

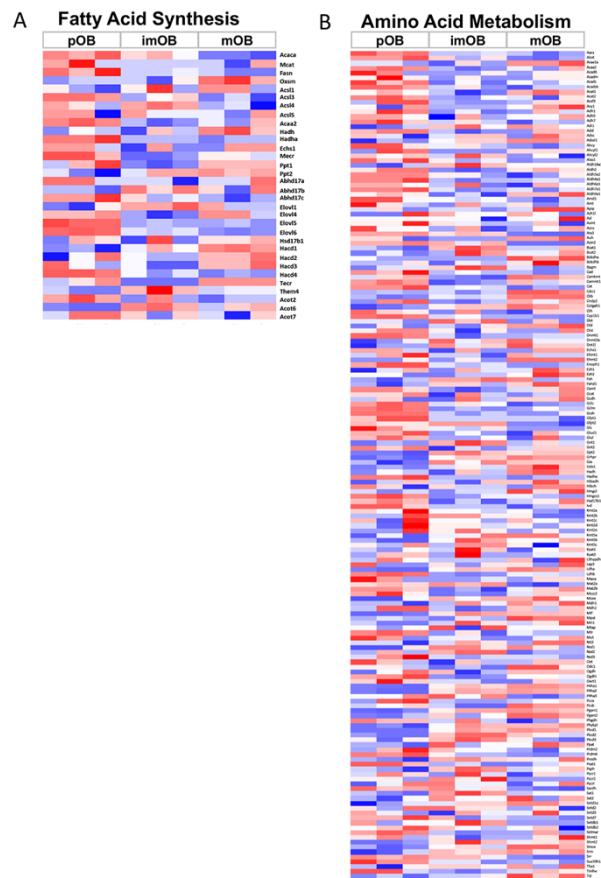


Figure S1. Heatmaps of gene expression levels by RNA-seq. Related to Figure 6. (A) Genes involved in amino acid metabolism. (B) Genes involved in fatty acid synthesis. pOB: preosteoblasts at d0; imOB: immature osteoblasts at d4 of differentiation; mOB: mature osteoblasts at d7 of differentiation. N=3 for each stage.

Supplemental Fig. S2

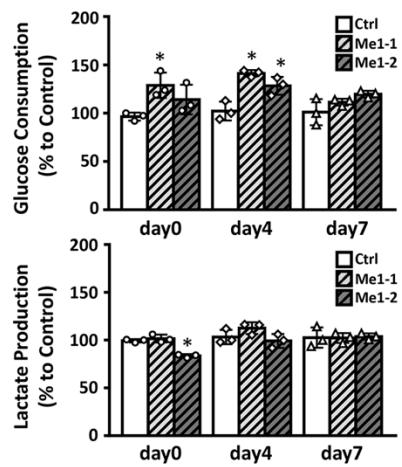


Figure S2. Effects of Me1 knockdown on glucose consumption or lactate production. Related to Figure 7.

Ctrl: shRNA against EGFP; Me1-1: shRNA #1 against Me1; Me1-2: shRNA #2 against Me1. *: p<0.05, n=3. Error bars: std.

Table S1. Equations for calculations of ATP production from Seahorse data. Related to STAR Methods section.

Calculated Values	Equations
OCR_{mito}	$= (OCR_{total} - OCR_{rot/myc})$
$OCR_{coupled}$	$= (OCR_{total} - OCR_{oligo}) * \text{hyperpolarization correction factor}$
PPR_{total}	$= (ECR_{total} / \text{buffering power})$
PPR_{resp}	$= (OCR_{mito} * (\text{max } H^+ / O_2) * ((10^{pH-pK1}) / (1 + 10^{pH-pK1}))) = (OCR_{mito} * K_{PPRresp})$
PPR_{glyc}	$= (PPR_{total} - PPR_{resp})$
Glycolytic ATP ($J_{ATPglyc}$)	$= (PPR_{glyc} * ATP/lactate) + (OCR_{mito} * 2 * ATP/[O]_{glyc})$
Oxidative ATP (J_{ATPox})	$= (OCR_{coupled} * 2 * ATP/[O]_{oxphos}) + (OCR_{mito} * 2 * ATP/[O]_{TCA})$

Parameters	Values	Parameters	Values
buffering power (mpH/pmol H ⁺)	0.045	$K_{PPRresp}$	0.953
pK ₁ at 37°C ($CO_2 \rightarrow HCO_3^-$)	6.093	$ATP/[O]_{glyc}$	0.167
hyperpolarization correction factor	0.908	$ATP/[O]_{oxphos}$	2.486
$\text{max } H^+ / O_2$	1.000	$ATP/[O]_{TCA}$	0.121
ATP/lactate	1.000		

Table S2. Primers for RT-qPCR. Related to STAR Methods section.

Primer	Sequence
Alpl_Mm_F	ACTGATGTGGAATACGAAGCTGG
Alpl_Mm_R	AGTCAGTGCCTCCAG
Atf4_Mm_F	ATGGCGTATTAGAGGCAGC
Atf4_Mm_R	CTTTGTCCGTTACAGAACAC
Bglap2_Mm_F*	CACCTAGCAGACACCATGAG
Bglap2_Mm_R*	GTTCACTACCTTATTGCCCTCC
Col1a1_Mm_F	AGCCGCAAAGAGTCTACATG
Col1a1_Mm_R	CTTAGGCCATTGTGTATGCAG
Ldha_Mm_F	GCTCCCCAGAACAGATTACAG
Ldha_Mm_R	TCGCCCTTGAGTTGTCTTC
Me1_Mm_F	AGTATCCATGACAAAGGGCAC
Me1_Mm_R	ATCCCATTACAGCCAAGGTC
Me2_Mm_F	TGTTATTCTCTGTGAAGCCCG
Me2_Mm_R	TGAATATTAGCCAGCGACGG
Rn18s_Mm_F	CGGCTACCACATCCAAGGAA
Rn18s_Mm_R	GCTGGAATTACCGCGGCT
Sp7_Mm_F	ATCTTCCACTTCGCCTGC
Sp7_Mm_R	AACCAATGGTCCACCGC

* By Primer-BLAST from NCBI, this primer set also recognize Bglap and Bglap3.

Table S3. Primers for DNA-qPCR. Related to STAR Methods section.

Primer	Sequence
Hk2_Mm_gDNA_F	GCCAGCCTCTCCTGATTTAGTGT
Hk2_Mm_gDNA_R	GGGAACACAAAAGACCTCTCTGG
mtNd4_Mm_gDNA_1F	CATCACTCCTATTCTGCCTAGC
mtNd4_Mm_gDNA_1R	GTGGCTATAAGTGGGAAGACC
mtNd4_Mm_gDNA_2F	AGCTCAATCTGCTTACGCCA
mtNd4_Mm_gDNA_2R	TGTGAGGCCATGTGCGATTA