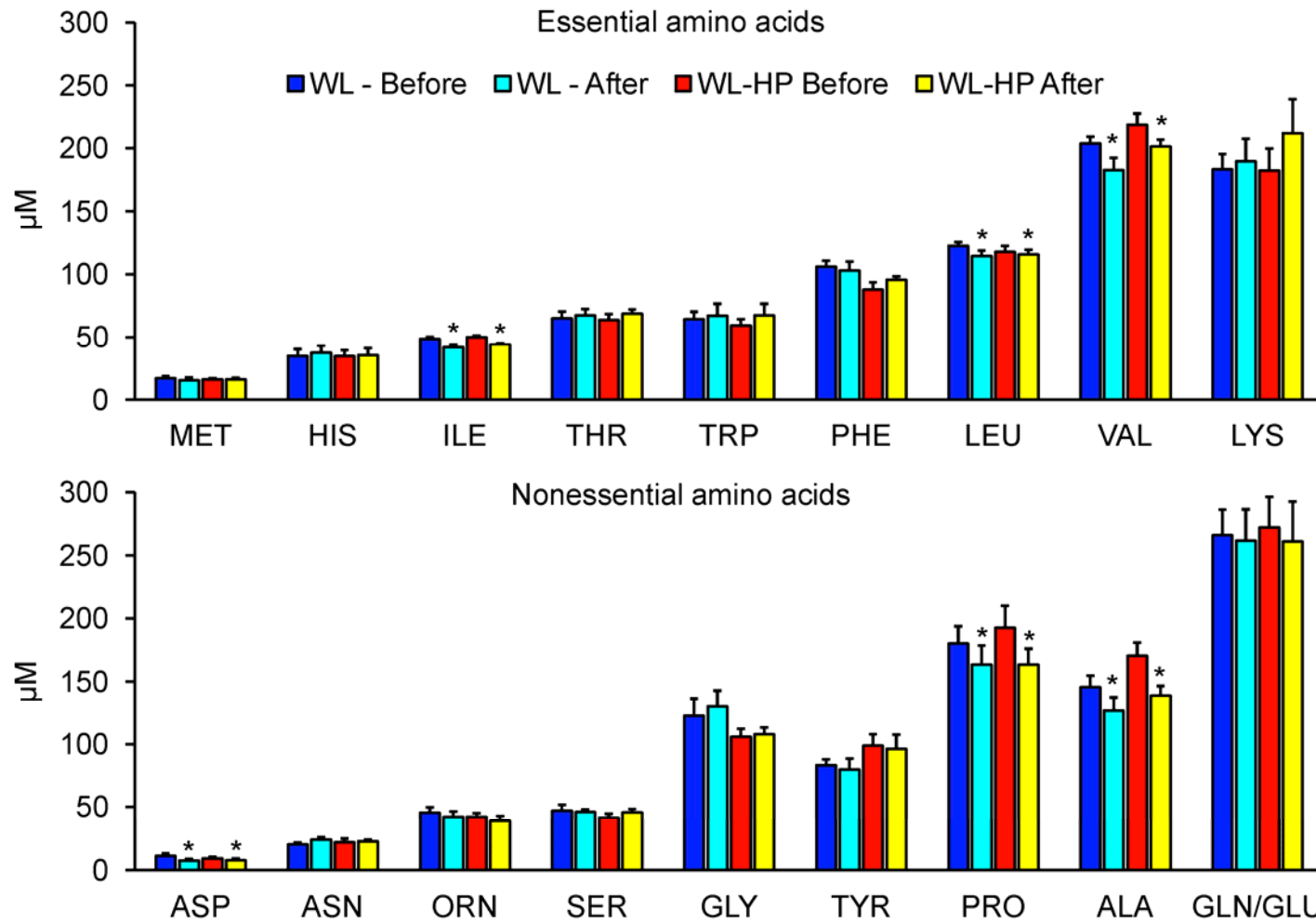
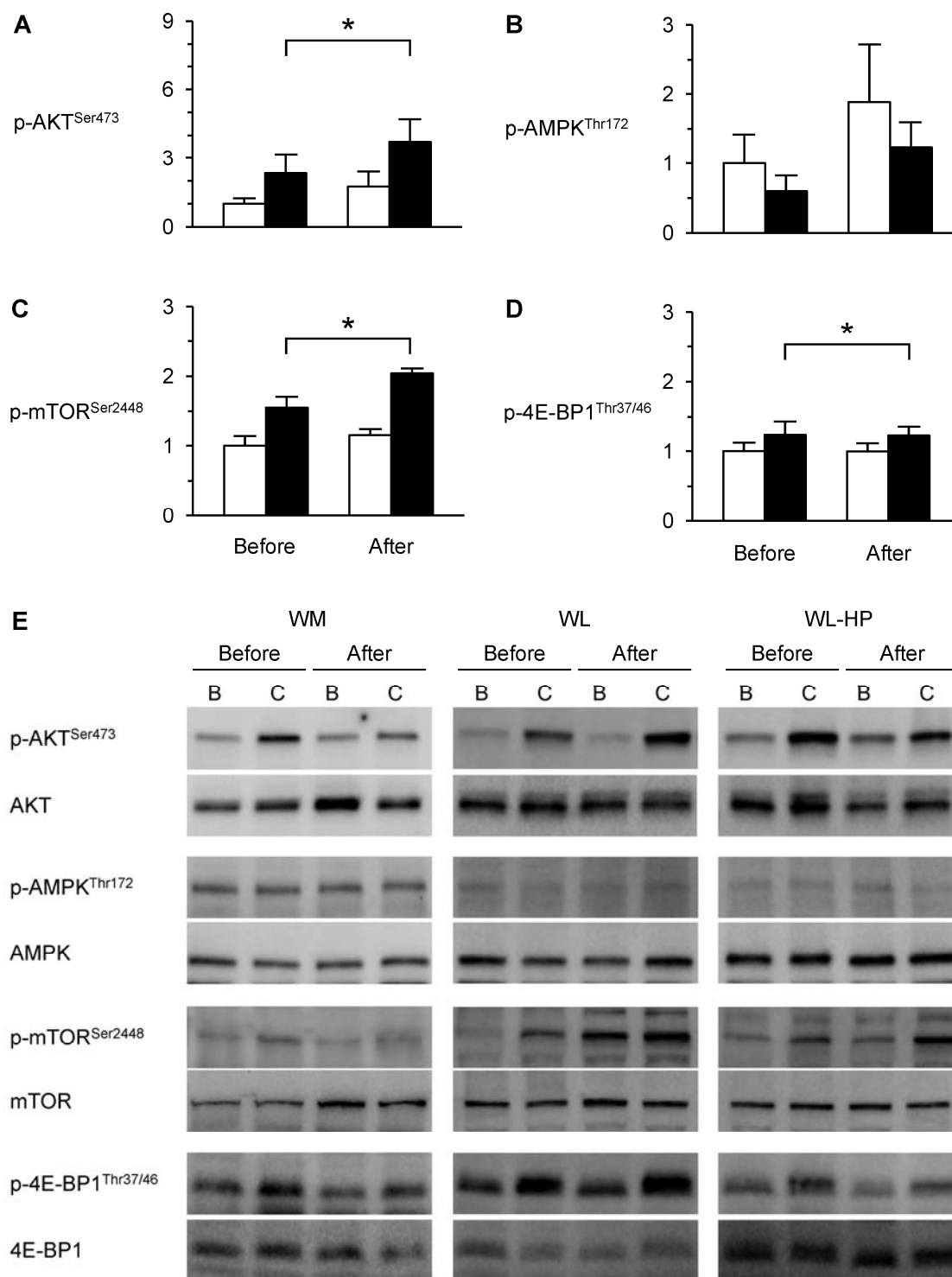


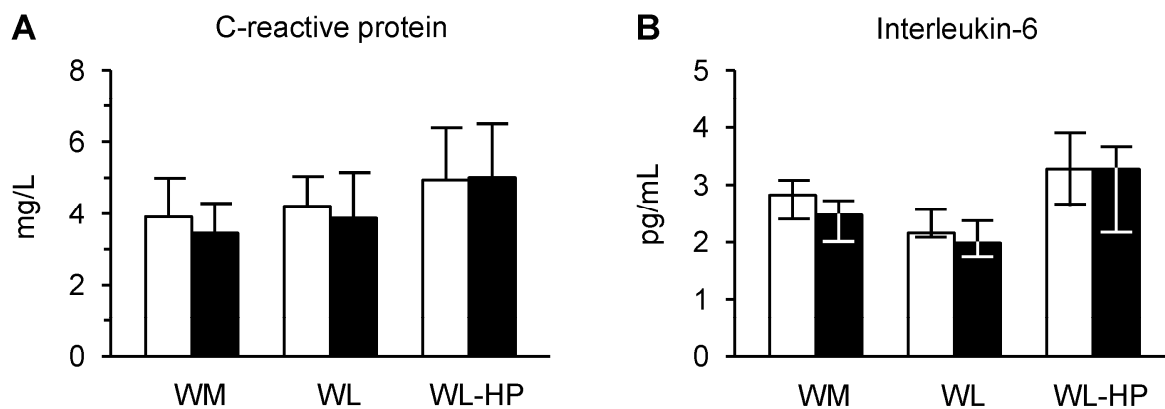
**SUPPLEMENTAL FIGURES AND LEGENDS**



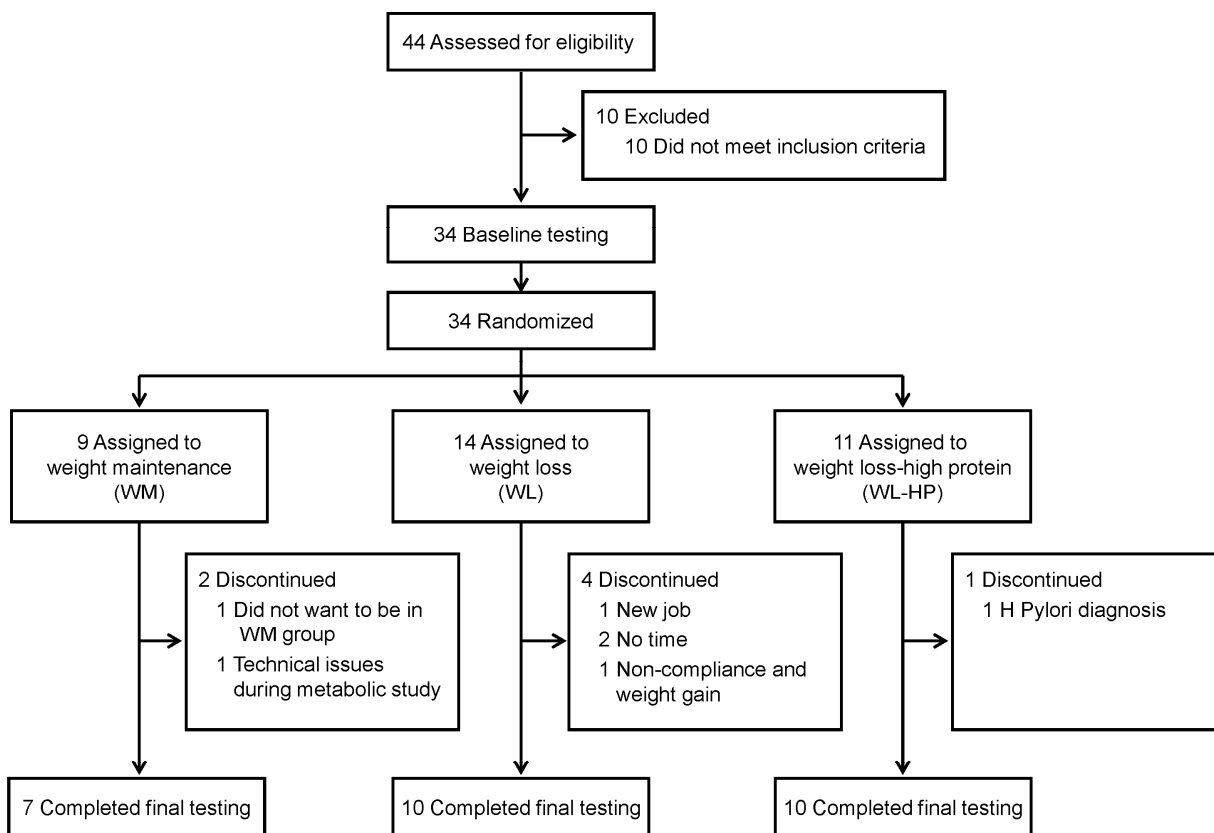
**Figure S1, related to Table 2. Plasma amino acid concentration profile.** Plasma amino acid concentrations during basal, postabsorptive conditions before and after weight loss in subjects consuming the standard weight loss (WL, n = 10) and weight loss high-protein (WL-HP, n = 10) diets. Data are expressed as mean  $\pm$  SEM. \* Value significantly different from corresponding value before weight loss ( $P < 0.05$ ).



**Figure S2, related to Figure 2. Intramyocellular signaling elements in the weight maintenance group and representative Western blots.** p-AKT<sup>Ser473</sup> (A), p-AMPK<sup>Thr172</sup> (B), p-mTOR<sup>Ser2448</sup> (C), and p-4E-BP1<sup>Thr37/46</sup> (D) contents in muscle during basal, postabsorptive conditions (white bars) and the hyperinsulinemic-euglycemic clamp (black bars) in subjects consuming the weight maintenance (WM; n = 5) diet and representative Western blots for the WM, and the standard weight loss (WL) and weight loss high-protein (WL-HP) diet groups (E). Data in panels A-D are expressed as mean  $\pm$  SEM. \* Significant main effect of the hyperinsulinemic-euglycemic clamp ( $P < 0.05$ ). In panel E, B = basal, postabsorptive conditions and C = hyperinsulinemic-euglycemic clamp.




**Figure S3, related to Figure 4. Pro-inflammatory marker concentrations in plasma.** Plasma C-reactive protein (A) and interleukin-6 (B) concentrations before (white bars) and after (black bars) the diet intervention in subjects consuming the weight maintenance (WM, n = 7) and the standard weight loss (WL, n = 10) and weight loss high-protein (WL-HP, n = 10) diets. C-reactive protein data are expressed as mean  $\pm$  SEM; interleukin-6 data are expressed as medians (quartiles).



**Figure S4, related to Table 1. Flow of study participants.**

## SUPPLEMENTAL TABLES

Table S1, related to Table 3. Additional gene set pathways that were differently affected by WL and WL-HP.

Gene set name	WL		WL-HP		Heat map	
	Z-score	P-value	Z-score	P-value	WL	WL-HP
	Heat Map Key					
						
	-7		0		+6	
GLYCOSAMINOGLYCAN_BINDING	5.61	< <b>0.001</b>	-1.65	0.099		
REGULATION_OF_HEART_CONTRACTION	5.34	< <b>0.001</b>	-1.19	0.235		
POLYSACCHARIDE_BINDING	5.27	< <b>0.001</b>	-1.83	0.068		
COLLAGEN	5.08	< <b>0.001</b>	-1.75	0.080		
HEPARIN_BINDING	4.96	< <b>0.001</b>	-2.38	0.017		
PATTERN_BINDING	4.48	< <b>0.001</b>	-1.59	0.112		
REGULATION_OF_MULTICELLULAR_ORGANISMAL_PROCESS	4.41	< <b>0.001</b>	-0.22	0.828		
SKELETAL_DEVELOPMENT	4.20	< <b>0.001</b>	0.61	0.539		
CARBOHYDRATE_BINDING	4.07	< <b>0.001</b>	-1.07	0.283		
LEUKOCYTE_CHEMOTAXIS	4.02	< <b>0.001</b>	-0.50	0.616		
CYSTEINE_TYPE_ENDOPEPTIDASE_ACTIVITY	3.93	< <b>0.001</b>	-0.88	0.379		
LEUKOCYTE_MIGRATION	3.93	< <b>0.001</b>	-0.87	0.384		
ORGAN_DEVELOPMENT	3.61	< <b>0.001</b>	0.83	0.406		
EXTERNAL_SIDE_OF_PLASMA_MEMBRANE	3.50	< <b>0.001</b>	-0.65	0.514		
NICOTINIC_ACETYLCHOLINE_GATED_RECEPTOR_CHANNEL_COMPLEX	3.50	< <b>0.001</b>	0.35	0.725		
NICOTINIC_ACETYLCHOLINE_ACTIVATED_CATION_SELECTIVE_CHANNEL_ACTIVITY	3.50	< <b>0.001</b>	0.35	0.725		
CALCIUM_ION_BINDING	3.50	< <b>0.001</b>	-0.39	0.694		
CYSTEINE_TYPE_PEPTIDASE_ACTIVITY	3.29	< <b>0.001</b>	-2.00	0.046		
CYTOPLASM	-0.95	0.340	-5.60	< <b>0.001</b>		
CYTOPLASMIC_PART	-0.97	0.331	-6.23	< <b>0.001</b>		

WL: weight loss (n = 6); WL-HP: weight loss high-protein (n = 9). A P-value  $\leq 0.001$  in either the WL or WL-HP group and a difference in Z-scores  $> 1.96$  between the WL and WL-HP groups was considered a statistically significant treatment effect that differed between the two groups.

**Table S2, related to Table 3. Gene set pathways that were similarly affected by WL and WL-HP.**

Gene set name	WL		WL-HP		Heat map	
	Z-score	P-value	Z-score	P-value	WL	WL-HP
	Heat Map Key					
	-7		0		+6	
MYELOID_LEUKOCYTE_DIFFERENTIATION	3.54	< <b>0.001</b>	2.71	<b>0.007</b>		
CARBOXYLIC_ACID_METABOLIC_PROCESS	-3.29	< <b>0.001</b>	-2.25	<b>0.025</b>		
ORGANIC_ACID_METABOLIC_PROCESS	-3.43	< <b>0.001</b>	-2.20	<b>0.028</b>		
ORGANELLAR_RIBOSOME	-3.49	< <b>0.001</b>	-2.97	<b>0.003</b>		
MITOCHONDRIAL_RIBOSOME	-3.49	< <b>0.001</b>	-2.97	<b>0.003</b>		
ENVELOPE	-3.64	< <b>0.001</b>	-3.28	<b>0.001</b>		
ORGANELLE_ENVELOPE	-3.64	< <b>0.001</b>	-3.28	<b>0.001</b>		
RIBOSOMAL_SUBUNIT	-3.66	< <b>0.001</b>	-2.92	<b>0.004</b>		
CELLULAR_RESPIRATION	-3.72	< <b>0.001</b>	-2.67	<b>0.007</b>		
ENERGY_DERIVATION_BY_OXIDATION_OF_ORGANIC_COMPOUNDS	-3.83	< <b>0.001</b>	-2.56	<b>0.010</b>		
NADH_DEHYDROGENASE_COMPLEX	-4.17	< <b>0.001</b>	-4.13	< <b>0.001</b>		
MITOCHONDRIAL_RESPIRATORY_CHAIN_COMPLEX_I	-4.17	< <b>0.001</b>	-4.13	< <b>0.001</b>		
RESPIRATORY_CHAIN_COMPLEX_I	-4.17	< <b>0.001</b>	-4.13	< <b>0.001</b>		
MITOCHONDRIAL_RESPIRATORY_CHAIN	-4.21	< <b>0.001</b>	-4.36	< <b>0.001</b>		
OXIDOREDUCTASE_ACTIVITY	-4.26	< <b>0.001</b>	-2.76	<b>0.006</b>		
COFACTOR_CATABOLIC_PROCESS	-4.39	< <b>0.001</b>	-2.53	<b>0.011</b>		
MITOCHONDRIAL_MATRIX	-4.48	< <b>0.001</b>	-3.07	<b>0.002</b>		
MITOCHONDRIAL_LUMEN	-4.48	< <b>0.001</b>	-3.07	<b>0.002</b>		
ORGANELLE_INNER_MEMBRANE	-4.48	< <b>0.001</b>	-4.91	< <b>0.001</b>		
MITOCHONDRIAL_MEMBRANE_PART	-4.52	< <b>0.001</b>	-4.72	< <b>0.001</b>		
MITOCHONDRIAL_MEMBRANE	-4.56	< <b>0.001</b>	-4.78	< <b>0.001</b>		
MITOCHONDRIAL_INNER_MEMBRANE	-4.73	< <b>0.001</b>	-4.90	< <b>0.001</b>		
MITOCHONDRIAL_ENVELOPE	-4.80	< <b>0.001</b>	-4.45	< <b>0.001</b>		
GENERATION_OF_PRECURSOR_METABOLITES_AND_ENERGY	-5.04	< <b>0.001</b>	-3.13	<b>0.002</b>		
MITOCHONDRIAL_PART	-6.46	< <b>0.001</b>	-5.38	< <b>0.001</b>		
MITOCHONDRION	-7.04	< <b>0.001</b>	-6.84	< <b>0.001</b>		

WL: weight loss (n = 6); WL-HP: weight loss high-protein (n = 9). A P-value  $\leq 0.001$  in either the WL or WL-HP or both groups and a difference in Z-score  $< 1.96$  between the two groups was considered a statistically significant effect of weight loss without a difference in the effects of WL and WL-HP.

**Table S3, related to Figures 3 and 4. Sequence of primers used for RT-PCR.**

Gene name	Accession No.	Forward (F) and reverse (R) primer
<i>ACADM</i>	NM_000016	F: 5'- ACAGGGGTTTCAGACTGCTATT -3' R: 5'- TCCTCCGTTGGTTATCCACAT -3'
<i>CAT</i>	NM_001752	F: 5'- TGGAGCTGGTAACCCAGTAGG -3' R: 5'- CCTTTGCCTTGGAGTATTTGGTA -3'
<i>CD68</i>	NM_001251	F: 5'- CTTCTCTCATTCCCCTATGGACA -3' R: 5'- GAAGGACACATTGTACTCCACC -3'
<i>CHREBP</i>	NM_032951	F: 5'- AAGATCCGCTGAACAACG -3' R: 5'- CACTTGTGGTATCCCCGCATC -3'
<i>COX4I1</i>	NM_001861	F: 5'- CAGGGTATTTAGCCTAGTTGGC -3' R: 5'- GCCGATCCATATAAGCTGGGA -3'
<i>CPT1B</i>	NM_004377	F: 5'- GCGCCCCTTGTGGATGAT -3' R: 5'- CCACCATGACTTGAGCACCAG -3'
<i>ELOVL6</i>	NM_024090	F: 5'- AACGAGCAAAGTTTGAAGTACTGAGG -3' R: 5'- TCGAAGAGCACCGAATATACTGA -3'
<i>FADS1</i>	NM_013402	F: 5'- CTACCCCGCGCTACTTCAC -3' R: 5'- CGGTCGATCACTAGCCACC -3'
<i>FASN</i>	NM_004104	F: 5'- TGGAAAGTCACCTATGAAGCCA -3' R: 5'- ACGAGTGTCTCGGGGTCTC -3'
<i>GSTA4</i>	NM_001512	F: 5'- CCGGATGGAGTCCGTGAGAT -3' R: 5'- GGGCACTTGTGGAACAGC -3'
<i>IL6</i>	NM_000600	F: 5'- ACTCACCTCTTCAGAACGAATTG -3' R: 5'- CCATCTTTGGAAGGTTTCAGGTTG -3'
<i>MCPI</i>	NM_002982	F: 5'- CAGCCAGATGCAATCAATGCC -3' R: 5'- TGGAATCCTGAACCCACTTCT -3'
<i>PDK4</i>	NM_002612	F: 5'- GGAGCATTTCTCGCGCTACA -3' R: 5'- ACAGGCAATTCTTGTGCGCAA -3'
<i>PPARGC1A</i>	NM_013261	F: 5'- TCTGAGTCTGTATGGAGTGACAT -3' R: 5'- CCAAGTCGTTACATCTAGTTCA -3'
<i>PRDX3</i>	NM_006793	F: 5'- ACAGCCGTTGTCAATGGAGAG -3' R: 5'- ACGTCGTGAAATTCGTTAGCTT -3'
<i>RPLP0</i>	NM_001002	F: 5'- GTGATGTGCAGCTGATCAAGACT -3' R: 5'- GATGACCAGCCCAAAGGAGA -3'
<i>SCD</i>	NM_005063	F: 5'- AACTTGGGAGCCCTGTATG -3' R: 5'- GCAGCCGAGCTTTGTAAGA -3'
<i>SOD1</i>	NM_000454	F: 5'- GGTGGGCCAAAGGATGAAGAG -3' R: 5'- CCACAAGCCAAACGACTTCC -3'
<i>SREBF1</i>	NM_001005291	F: 5'- ACAGTGACTTCCCTGGCCTAT -3' R: 5'- GCATGGACGGGTACATCTTCAA -3'
<i>TNF</i>	NM_000594	F: 5'- GAGGCCAAGCCCTGGTATG -3' R: 5'- CGGGCCGATTGATCTCAGC -3'
<i>UCP2</i>	NM_003355	F: 5'- CCCCGAAGCCTCTACAATGG -3' R: 5'- CTGAGCTTGAATCGGACCTT -3'