SUPPLEMENTAL FIGURES

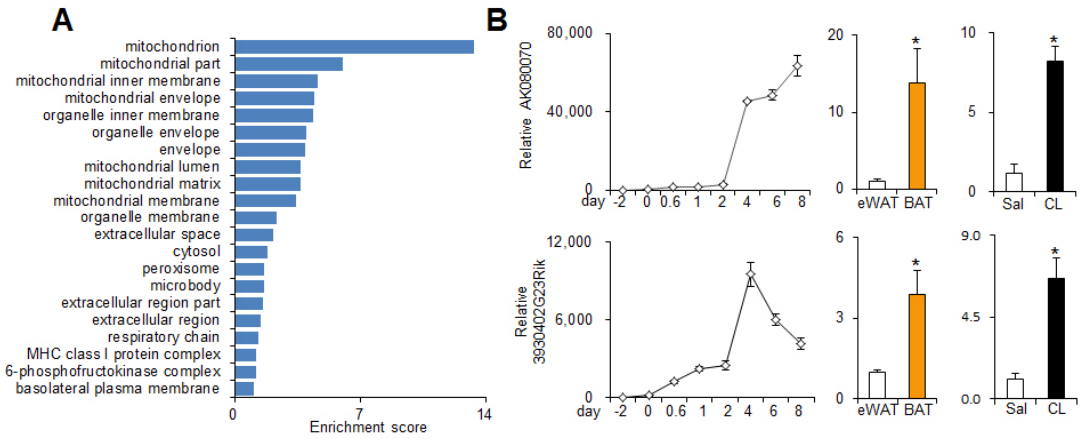


Figure S1, related to Figure 1. (**A**)**.** Enrichment of genes involved in mitochondrial function in the protein-coding geneset shown in Figure 1A. Gene ontology analysis was performed using DAVID (david.abcc.ncifcrf.gov). (**B**)**.** qPCR analysis of *AK080070* and *3930402G23Rik* expression during adipogenesis (left), in eWAT and BAT (center), and in eWAT from mice treated with saline (Sal) or CL (right). Data represent mean ± sd. *p<0.05.

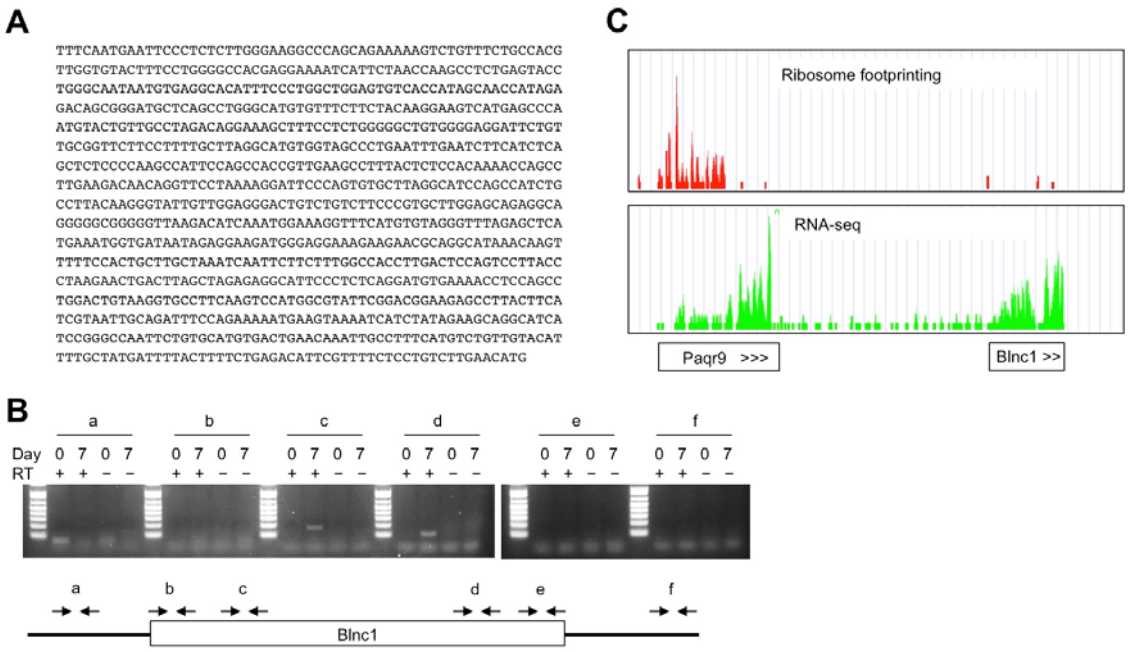


Figure S2, related to Figure 1. (**A**)**.** Nucleotide sequence of Blnc1. (**B**)**.** RT-PCR analysis with indicated primer pairs. RT reactions were performed using total RNA isolated from brown preadipocytes (0) or day 7 differentiated BAC (7) in the presence (+) or absence (-) of reverse transcriptase (RT). (**C**)**.** Ribosome footprinting and RNA sequencing traces near the mouse Blnc1 gene locus. Note that, Paqr9 mRNA strongly associates with ribosomes, whereas Blnc1 RNA shows modest ribosome association. Raw data were generated by *Ingolia NT* et al.

A

Category	Term	P-Value
GOTERM_CC_FAT	mitochondrion	7.40E-10
GOTERM_BP_FAT	glucose metabolic process	1.80E-05
GOTERM_BP_FAT	hexose metabolic process	5.30E-05
GOTERM_CC_FAT	mitochondrial part	1.10E-04
GOTERM_BP_FAT	fatty acid metabolic process	8.00E-04

B

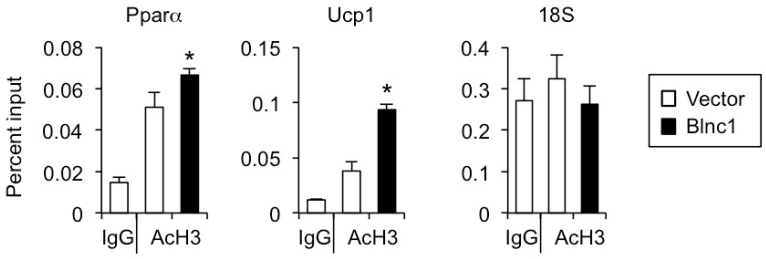
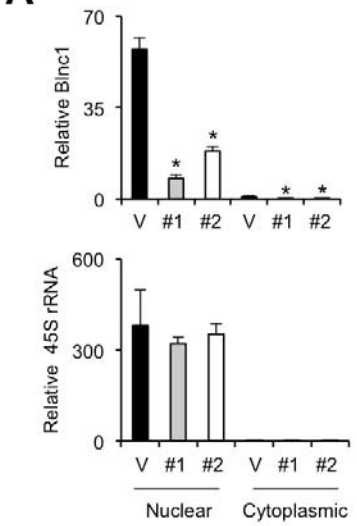


Figure S3, related to Figure 2. Effects of Blnc1 overexpression on BAC gene expression. (**A**)**.** Pathway analysis of genes upregulated by Blnc1. Gene ontology analysis was performed using DAVID (david.abcc.ncifcrf.gov). (**B**)**.** ChIP assay of differentiated BAC expressing vector or Blnc1. IgG and antibodies against Acetyl-Histone 3 (AcH3) were used. *p<0.05 Blnc1 vs. Vector.

A



B

Category	Term	P-Value
GOTERM_CC_FAT	mitochondrion	6.40E-56
GOTERM_CC_FAT	mitochondrial part	8.20E-28
GOTERM_CC_FAT	mitochondrial envelope	8.90E-19
GOTERM_CC_FAT	mitochondrial membrane	8.30E-18
GOTERM_CC_FAT	mitochondrial inner membrane	6.80E-17
GOTERM_CC_FAT	organelle inner membrane	2.90E-16
GOTERM_CC_FAT	organelle envelope	5.10E-15
GOTERM_CC_FAT	envelope	5.70E-15
GOTERM_CC_FAT	ribosome	1.00E-12
GOTERM_CC_FAT	organelle membrane	1.40E-11
GOTERM_BP_FAT	translation	1.30E-09
GOTERM_CC_FAT	ribonucleoprotein complex	1.30E-09
GOTERM_MF_FAT	structural constituent of ribosome	1.50E-09
GOTERM_CC_FAT	organellar ribosome	7.50E-09
GOTERM_CC_FAT	mitochondrial ribosome	7.50E-09
GOTERM_CC_FAT	mitochondrial matrix	2.20E-08
GOTERM_CC_FAT	mitochondrial lumen	2.20E-08
GOTERM_CC_FAT	respiratory chain	5.50E-06
GOTERM_BP_FAT	brown fat cell differentiation	3.40E-05

Figure S4, related to Figure 3. Effects of Blnc1 RNAi knockdown on BAC gene expression. (**A**). Nuclear and cytoplasmic levels of Blnc1 following RNAi knockdown. *p<0.05 vs. Vector. (**B**). Pathway analysis of genes downregulated by knockdown of Blnc1. Gene ontology analysis was performed using DAVID (david.abcc.ncifcrf.gov).

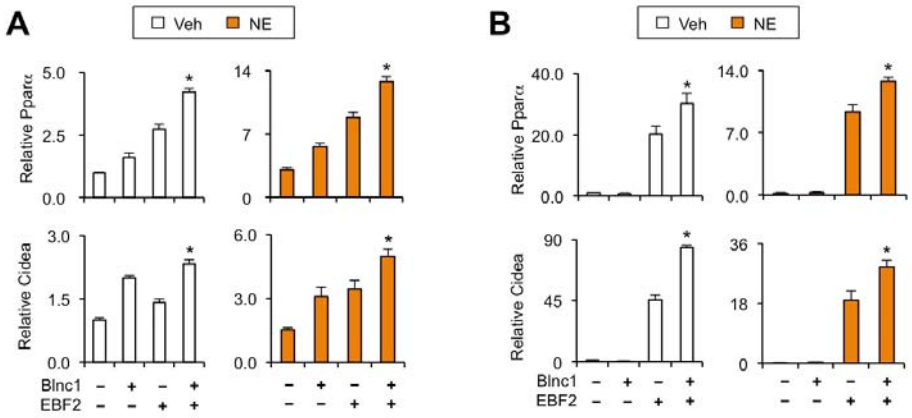


Figure S5, related to Figure 5. QPCR analyses of *Ppara* and *Cidea* mRNA expression in adipocytes differentiated from brown preadipocytes (**A**) or 10T1/2 cells (**B**) transduced with retroviruses indicated below. Differentiated adipocytes were treated with vehicle (open) and norepinephrine (NE, orange) for 4 hrs. Data represent mean ± sd. *p<0.05 EBF2/Blnc1 vs. EBF2 alone.

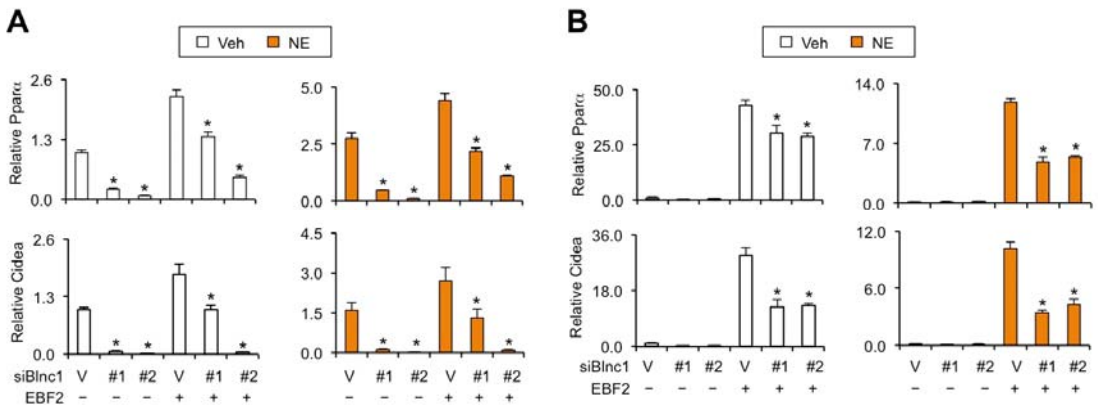


Figure S6, related to Figure 7. QPCR analyses of Ppara and Cidea mRNA expression in adipocytes differentiated from brown preadipocytes (**A**) or 10T1/2 cells (**B**) transduced with retroviruses indicated below. Differentiated adipocytes were treated with vehicle (open) and norepinephrine (NE, orange) for 4 hrs. Data represent mean ± sd. *p<0.05 vector vs. siBlnc1.

Table S1, related to Figure 1. List of protein-coding and lncRNA genes that are highly regulated in brown fat.

Table S2, related to Figure 3. List of genes altered by Blnc1 overexpression and knockdown in BAC.

Table S3. List of PCR primers

Gene	5' Primer	3' Primer
qPCR		
Blnc1	CAAGGAAGTCATGAGCCCAATG	TAAAGGCTTCAACGGTGGCTG
Cidea	GCAGCCTGCAGGAACTTATCAGC	GATCATGAAATGCGTGTTGTCC
Ucp1	GGCATTCAGAGGCAAATCAGCT	CAATGAACACTGCCACACCTC
Cox7a1	GTCTCCCAGGCTCTGGTCCG	CTGTACAGGACGTTGTCCATTC
Pgc-1a	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
Ppara	GCAGTGCCCTGAACATCGA	CGCCGAAAGAAGCCCTTAC
Fabp4	TGCCTTTGTGGGAACCTG	GCTTGTCACCATCTCGTTTTC
Tbx1	AATCAGCTGGGCACCGAG	AGCTTCACTTGGAACGTGGG
Pparg	CCGTAGAAGCCGTGCAAGAG	GGAGGCCAGCATCGTGTAGA
EBF2	GGAACCGGAACGAGACCCCT	TCCCTTGGGTTTCCCGCTGT
45S	GTGCCCTCACGTGTTTCACTTT	TAGGAGACAAACCTGGAACGCT
12S	TCGATAAACCCCGCTCTACCT	TGGCTACACCTTGACCTAACGTT
Prdm16	CGGAAGAGCGTGAGTACAAATG	TCCGTGAACACCTTGACACAGT
Dio2	GATGCTCCCAATTCCAGTGT	TGAACCAAAGTTGACCACCA
ChIP		
Blnc1-ChIP (EBF2)	GGATTGTCAGCATTTGTTGACATG	CCTACAAATTCGTGAGAAATCCACA
Ucp1-ChIP (EBF2)	CAAATGGTGACCGGGTGCCCT	GGGTGACTGACCCTCTGTGACG
Ucp1-ChIP (AceH3)	CTTGGAAAGAAAGCCAGGCTG	GAGGACATTGGGTGTCCTTGAG
Ppara-ChIP (EBF2)	AAGAGCATGGGACAGTGGCCG	TGGCCAGCTGAAGGTCACCAC
Ppara-ChIP (AceH3)	TGGGCAGGAAGATGGAAATC	GGGAGGAAGTGCCAGAATGA
18S-ChIP	AGTCCCTGCCCTTTGTACACA	CGATCCGAGGGCCTCACT
Mitochondrial DNA		
mtND1	CCTATCACCCTTGCCATCAT	GAGGCTGTTGCTTGTGTGAC
mtCox1	CTACTATTCGGAGCCTGAGC	GCATGGGCAGTTACGATAAC
PECAM	ATGGAAAGCCTGCCATCATG	TCCTTGTTGTTCAGCATCAC

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

In vitro Transcription/Translation Assay

In vitro transcription/translation assay was performed using a kit from Promega. Briefly, expression plasmids for luciferase and Blnc1 were mixed with a Coupled Reticulocyte Lysate System and ^{35}S-Methionine. After incubating at 30^{o} C for 60 minutes, translated products were separated on 4-20% gradient SDS-polyacrylamide gel and transferred to PVDF membrane for X-ray autoradiography.

Rapid Amplification of cDNA Ends (RACE)

RACE experiment was performed using RLM-RACE kit (Invitrogen). Briefly, total RNA was incubated with calf intestinal phosphatase (CIP) and tobacco acid pyrophosphatase (TAP) to remove 5' phosphates and cap structure. A specific RNA oligo with a 5' priming site was subsequently ligated to the 5' end of RNA transcripts using T4 RNA ligase. Reverse transcription was performed using MMLV-RT method with Oligo dT containing 3' Race primer sequence followed by nested PCR. The ends of Blnc1 cDNA were mapped by sequencing.

Plasmid construction

Blnc1 cDNA was amplified by PCR and cloned into pMSCV retroviral vector for retroviral production and transduction. To facilitate affinity capture, Blnc1 cDNA was fused to minimal S1 aptamer sequence (StA-Blnc1) at the 5' end (Walker et

al., 2011). The expression cassette was subsequently cloned into pcDNA3 expression vector. For shRNA knockdown of Blnc1, two target sequences (#1: *CCTTGACTCCAGTCCTTAC* and #2: *GGGCCAATTCTGTGCATGT*) were selected. Similarly, two independent sequences were selected for EBF2 knockdown (#1: *GACAAAGAGCAAGGCAA* and #2: *CATGAACGGCTACA GCAAT*). These shRNA expression cassettes were inserted into pSuper Retro-puro vector retroviral production and transduction. For Blnc1 reporters, the proximal Blnc1 promoters (1.5 kb and 1.0 kb) were amplified by PCR and cloned into pGL3 Basic luciferase vector. EBF2 binding site mutant reporter was generated by site-directed mutagenesis.