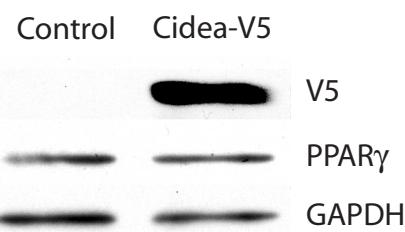
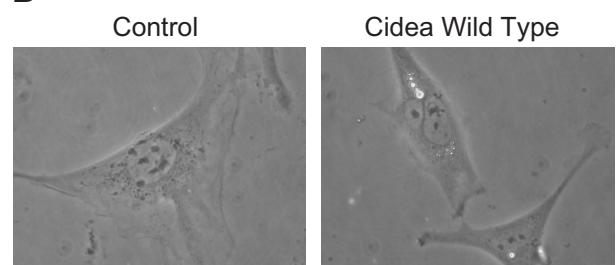
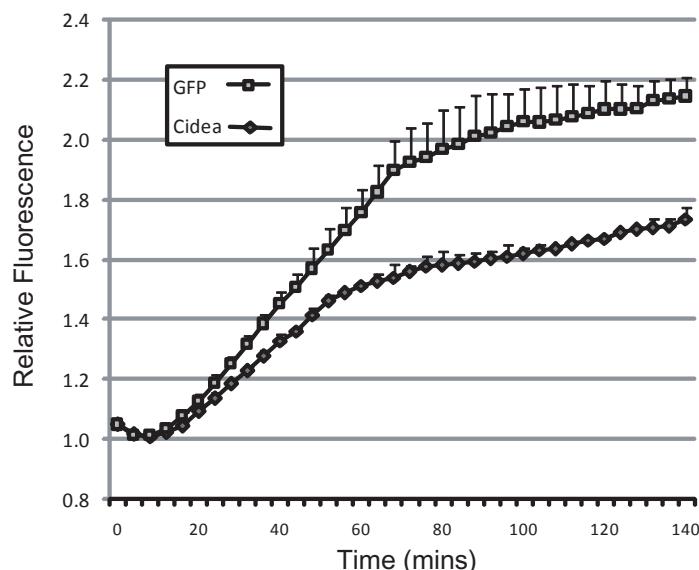


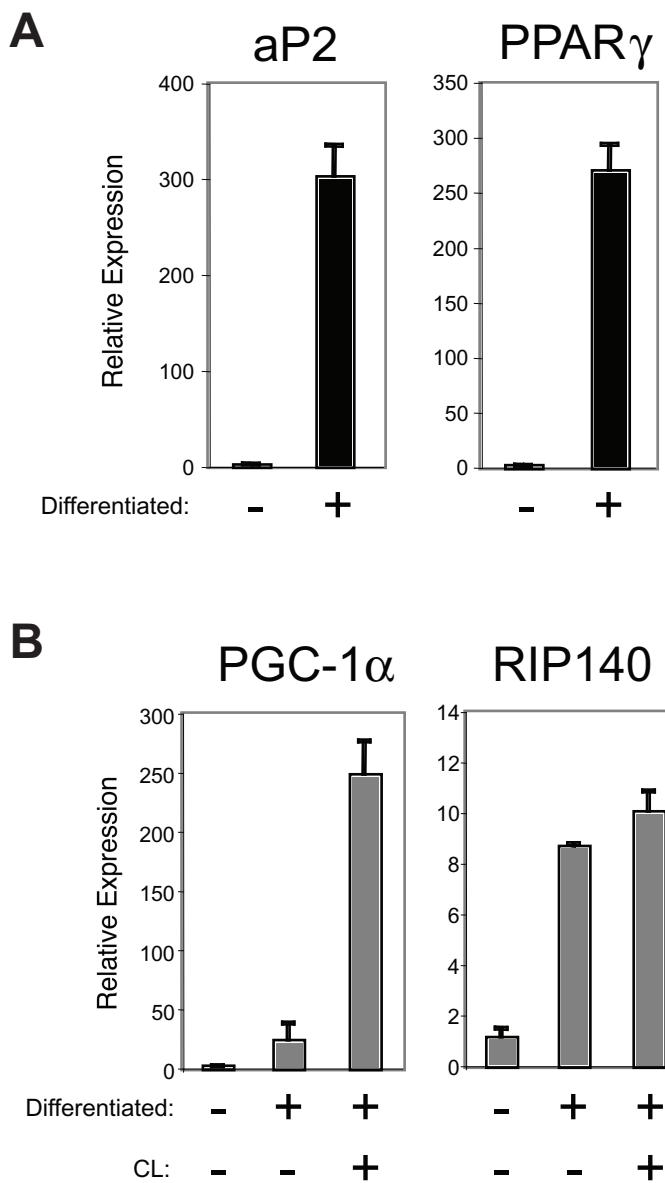
Comassie Blue staining of GST-fused PGC-1 α .

GST-PGC-1 α and GST-PGC-1 α fragments were separated on a pre-cast 4-12% SDS polyacrylamide gel (Invitrogen) and stained with Comassie Brilliant Blue (Bio-Rad). Arrows indicate the relevant proteins at the expected size.

A**B****C**

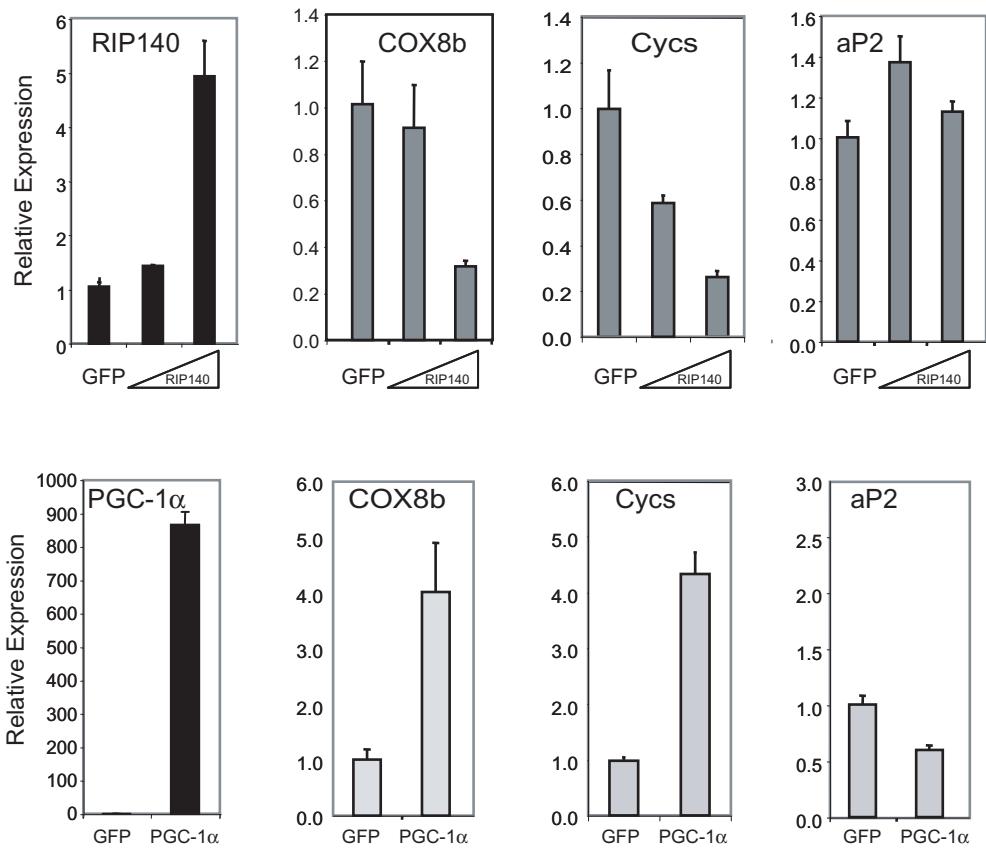
Ectopic Cidea expression in IMBAT-1 Cells.

IMBAT cells were transfected with pcDNA3 or pcDNA3-Cidea-V5. Protein extracts were prepared in laemmli buffer, subjected to Western Blotting and probed with antibodies against V5, PPAR γ , and GAPDH (A). Before protein extraction transfected cells were visualised by phase contrast microscopy (B). The absence of PPAR γ induction indicates that the ectopic Cidea expression does not induce adipogenesis. C, Oxygen consumption by differentiated IMBAT-1 cells infected with adenovirus expressing GFP or CIDEA was measured using the BD Oxygen Biosensor System (BD Biosciences, San Diego, CA) with 20,000 cells per well, in triplicate, using a fluorescence plate reader at 485 nm excitation and 630 nm emission. Fluorescence values for each well were normalized to their initial value and then to the values of no-cell controls at each time point. A representative graph is shown of three replicate experiments.



Gene expression in the brown fat cell line IMBAT-1

The expression of aP2, PPAR γ , PGC-1 α and RIP140 was analysed by real-time PCR in untreated or differentiated IMBAT-1 cells (A and B) in presence of CL316,243 (10 μ M) as indicated.



RIP140 and PGC-1 α target genes in the brown fat cell line IMBAT-1

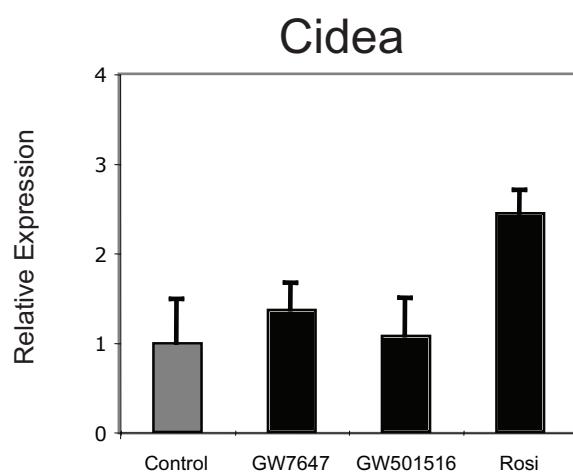
Gene expression in differentiated IMBAT-1 cells was assessed by real-time PCR following adenoviral-mediated expression of RIP140 or PGC-1 α .

Cidea Promoter Sequence

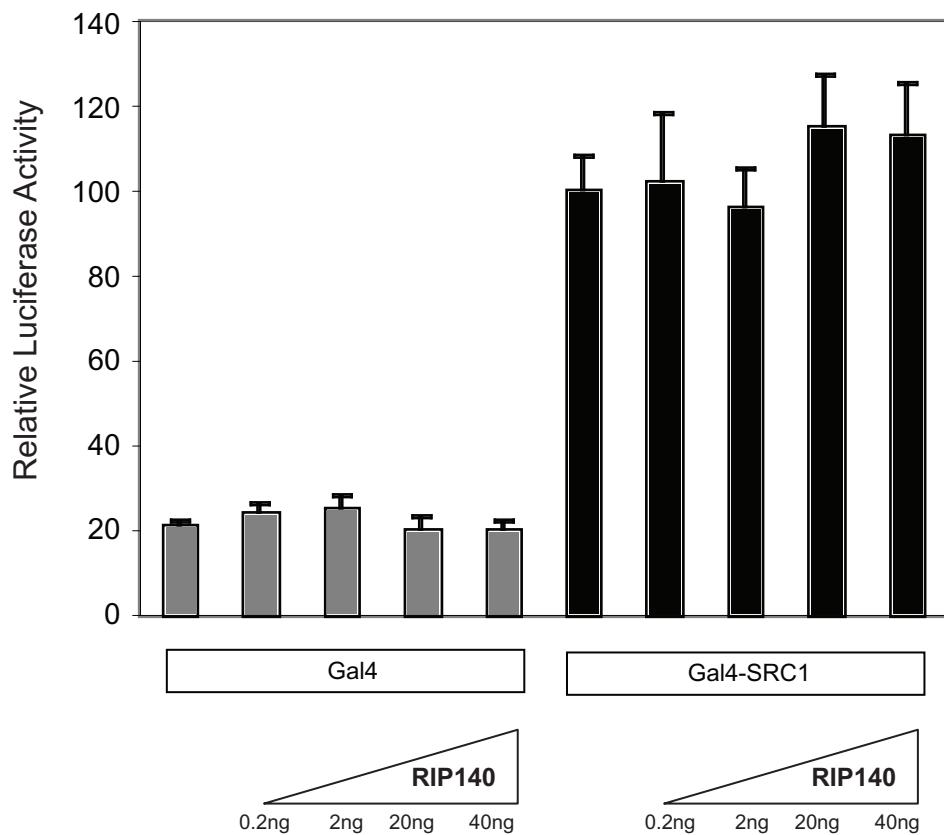
-1347 aatgcaaatttgcgtggaggagagacacaaacgttaaggacgtcactcaagaggaagagattacgttcttatctgtgagcttg
-1260 gaggccagactggctagaggggtggggagactaaggatataatcaaagggtttaaataacaagcgaatccatcagacgcgtgg
-1173 gggctctgagcctggctatgcag **ATGGTCA**actccgataccctgtgcacatctggacatgtgagttcc **AGGACA**gcagggtatacagg
-1086 gaaaccctgtctcgaaaaaaaagttctctacagttagacatttctaaccagggtggttgaatggattggattcccatttctca
-999 cctcctctgagagggtcagtaggggtccgtaatgagcc **AGGTGA**ctt **TGTCC**Accccatagctgtggcaggaaaggcagc
-912 tttgtgagcctttgcagggcgttcaactggaaactgcttttagcttacttcc **TGAAGGTCA**gtttaggtgtg **TGGAC**AccgaT
-825 **GACCC**cgaagaaggaggcagtctttgtcgttagatgtaccagagaagagggtggtagccgagcctggaacacagtaacaagtt
-738 ctggacagttagttagcagaaaaacttagcaactctaattccagacccctgttagcttgcgaagaaagtcatccaatttttagcga
-651 gctgtggtaatgctcgccaactcggttagggcagctgtttcacgaaaaccttcttaaatcttgctttcctTCTGTGtctc
-564 agaaactgcctaacctacaagtccaggattaaatgggtggcagcattatgttcctccactccctcgacgcactctacttacct
-477 acccacagggaaagccctggcacctctgagaacccgaagtgtcggttacgcg **TGTCCC**cagcaacttccct**TGTCCC**ccggaaac
-390 tcgctggccagcccccagccctgggtgggctttggagactccagcatgcagacagccgtt **AGGACA**ctccgtcgccccacgca
-303 cacctgcttctactgccaagccatatcgctgacattgtat **ATGTCA**c **CAGTCA**cccccagtctccagaagaaaaacaaactgac
-216 aagagccaccaacatca **C**CAAATCCTGGGTTTGGGCCCTCGGT **ACCGTTGCGCA**CGAAGGGCGTGGCCCCGGGACCCAGGCCA
-129 **GGGAGCCAGAACTATT**CGCTGCTCGCAGGAGCGCAGCCTGTGCCAAGGTGGGTCAAGTCGTCGCGGGCGTGGCTGATAGGGCAG
-42 **TGATT****T**AGAGACGCGGTTGGACAGG**A**GGACCCGCAC**A** **ATGGAGACCGCCAGGGACTACGCGGGAGCCCTCATCAG**gcagtg
+45 **ccgaccacgtacccctgcgccccgggt**acccttcttcagaataactctgctgttcatttctgtccccagttcttcctg
+132 agcccccaagtaccaactccccgcgttccctgtgaatccctcccttcacaccccatcacctacccgggtctctgtccctgg
+219 **gccatagtccccgtgcggcctgttctaccacgcagccagccag**ccaggttcccccggaaaaaggccacaggtgcggcagg
+306 **agtgtcccgaggtgcgtggagtcgc**aaacaagtgcacatttgtgaacgcggggcggggggtgggggggtggcgggaagggtgc

Sequence of the mouse CIDEA promoter

5'RACE was carried out using the FirstChoice RLM-RACE kit (Ambion) on RNA extracted from differentiated IMBAT-1 cells to identify the CIDEA transcription start site. The sequence 5' and 3' of the translational start site (in bold) in the first exon of the CIDEA gene is presented. The exon is capitalised, transcription start sites are boxed (█), and CpG islands, identified by UCSC Goldenpath are underlined. The binding sites identified for ERR and NRF-1 are indicated as are additional potential nuclear receptor half-sites.



Gene expression in the brown fat cell line IMBAT-1 in response to PPAR ligands
CIDEA mRNA expression in 9 day differentiated IMBAT-1 cells treated for the last 72h of culture with DMSO (control), GW7647 (10 μ M), GW501516 (10 μ M), or Rosiglitazone (5 μ M) was assessed by real-time PCR.



RIP140 Does Not Affect Transcription from Gal-SRC1

Transient transfection of Cos cells with pGal-DBDx5/luc in the presence of Gal4 or Gal-SRC1 with increasing amounts of pEF-RIP140.