Supplementary Information

Distinct roles of GCN5/PCAF-mediated H3K9ac and CBP/p300-mediated H3K18/27ac in nuclear receptor transactivation

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Figure S1. GW-induced *Angptl4* and *PDK4* expression is PPARδ-dependent

(A) Immortalized *PPARδ*$^{flox/flox}$ MEFs were infected with retroviruses MSCVpuro expressing Cre or vector (Vec) alone. Cells were treated with PPARδ ligand GW501516 (GW) or DMSO for 48h, followed by qRT-PCR analysis of gene expression.

(B) *PPARδ*$^{flox/flox}$ MEFs were treated with GW and 100 μg/ml cycloheximide (CHX) for 6h, followed by qRT-PCR analysis of *Angptl4* and *PDK4* expression.

All results are representative of 3 independent experiments.

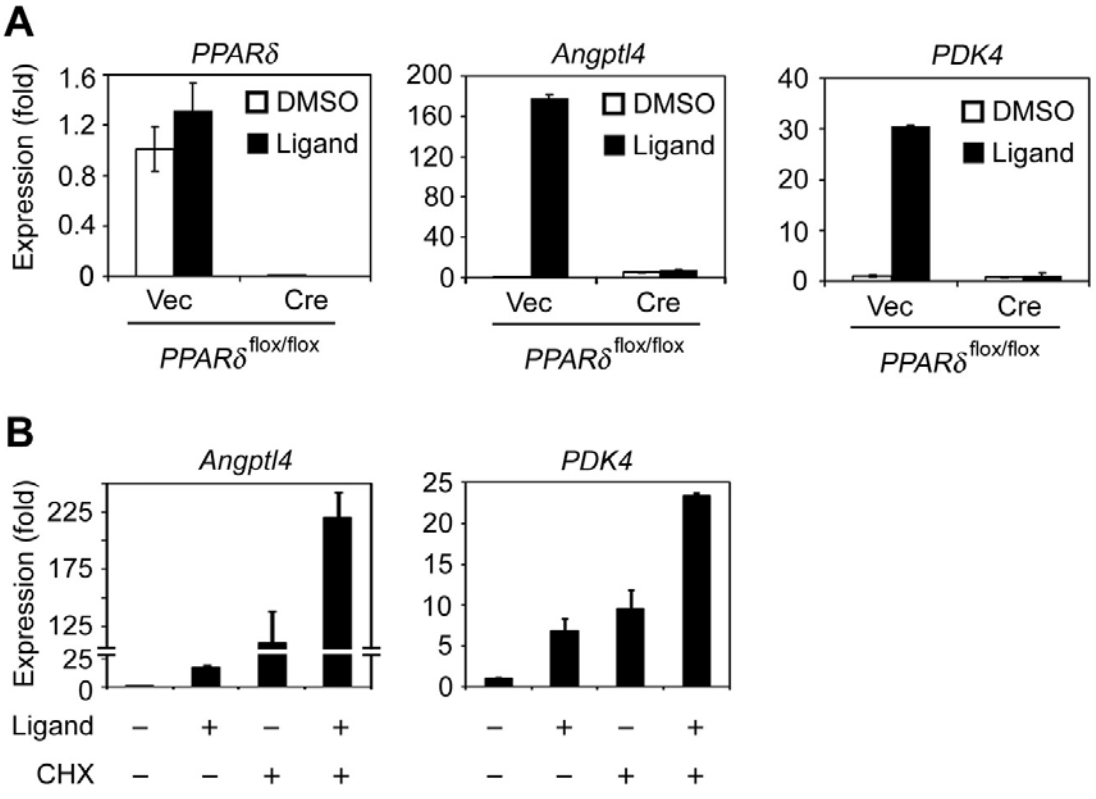

Figure S2. Knockout of GCN5 alone has little effect on PPARδ ligand-induced *Angptl4* expression

GCN5$^{flox/\Delta}$ MEFs were infected with MSCVpuro expressing Cre or Vec. Cells were treated with GW or DMSO for 24h, followed by qRT-PCR analysis of *GCN5* and *Angptl4* expression. All results are representative of 3 independent experiments.

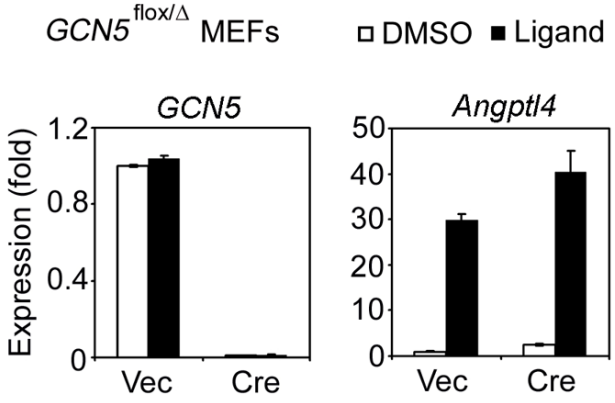

Figure S3. Double knockout of GCN5 and PCAF vastly reduces H3K9ac but has little effects on housekeeping gene expression

(A) Immortalized *PCAF*$^{-/-}$;*GCN5*$^{flox/\Delta}$ brown preadipocytes were infected with MSCVpuro expressing Vec or Cre. Nuclear extracts were prepared for Western blot analysis of H3K9ac, H3K14ac and H3.

(B - D) *PCAF*$^{-/-}$;*GCN5*$^{flox/\Delta}$ MEFs were infected with MSCVpuro expressing Vec or Cre.

(B) Western blot analysis of H3K9ac, H3K14ac and H3K18ac in the nuclear extracts. The sources of antibodies are indicated on the left.

(C) qRT-PCR of expression of housekeeping genes *GAPDH, β-actin* and *β-catenin.*

(D) ChIP of H3K9ac on the housekeeping gene promoters.

All results are representative of 2-4 independent experiments.

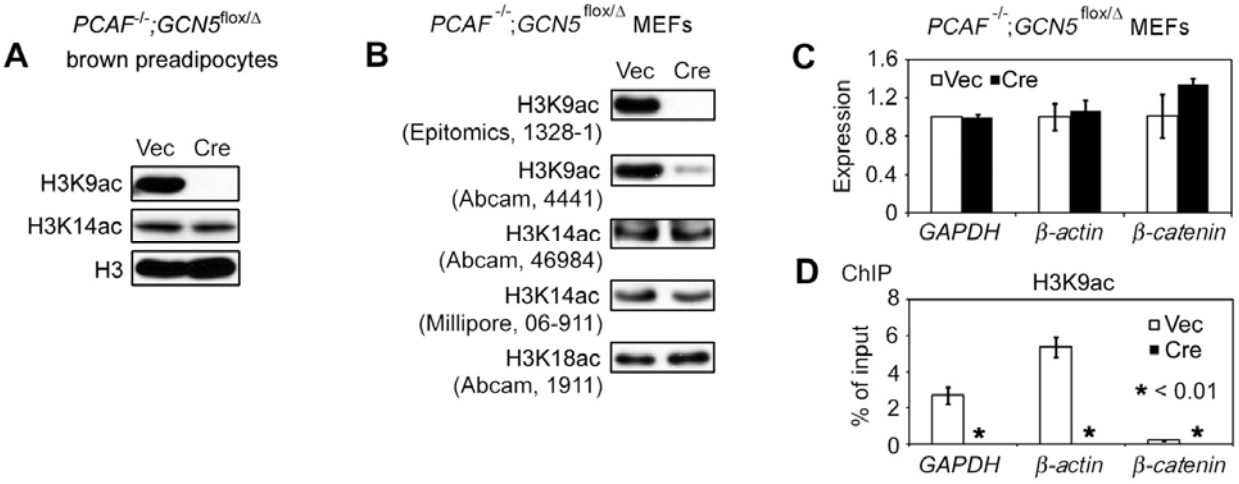

Figure S4. Purification of GST-GCN5 and GCN5-associated HAT complexes

(A) Coomassie blue staining of GST-fused mouse GCN5 protein (residues 400 - 830) purified from bacteria. The arrow indicates the purified protein. The asterisk indicates a degradation product. M, protein marker.

(B - C) Purification of GCN5-associated HAT complexes (GCN5.com) from MEFs. Nuclear extracts prepared from MEFs expressing FLAG-tagged full-length mouse GCN5 were subjected to affinity purification using anti-FLAG M2-agarose as described (Cho et al, 2007). The purified GCN5.com was resolved on SDS-PAGE, followed by silver staining (B) or Western blot analysis using antibodies indicated on the left (C). Ada2b and SPT3 are specific subunits of GCN5-associated SAGA complex while Ada2a and MBIP are specific subunits of GCN5-associated ATAC complex (Wang et al, 2008).

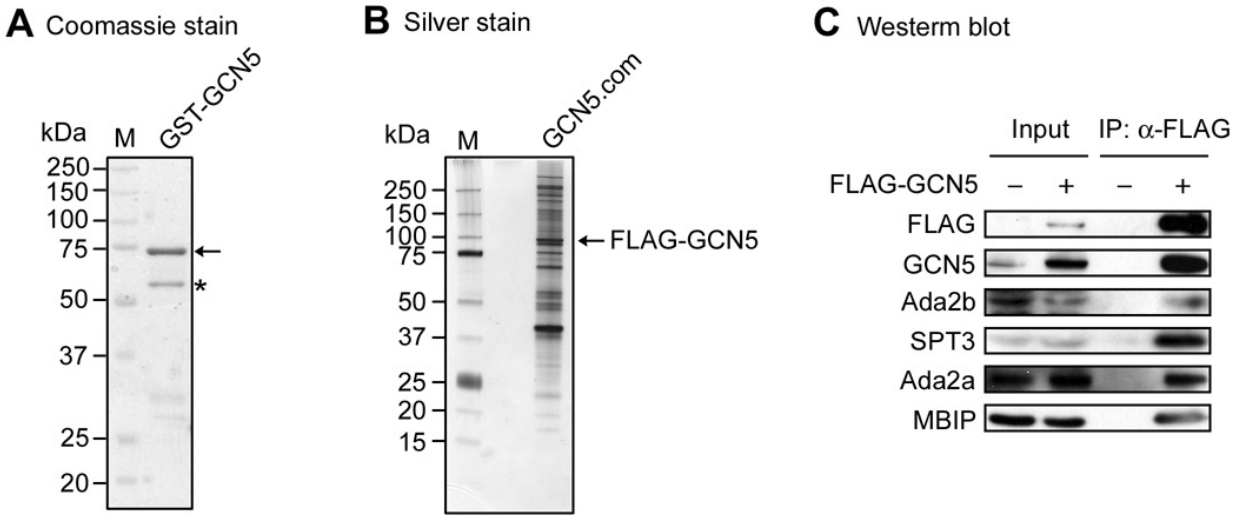

Figure S5. GCN5 and PCAF are dispensable for MyoD-stimulated myogenesis in MEFs and ligand-induced PPARδ target gene expression in myocytes

PCAF$^{-/-}$;*GCN5*$^{flox/\Delta}$ MEFs were infected with retrovirus MSCVpuro-MyoD. After puromycin selection, cells were infected with adenoviruses expressing GFP or Cre. Two days later, cells were induced to undergo myogenesis for 3 days, followed by treatment with PPARδ ligand GW for 24h.

(A – B) GCN5/PCAF are dispensable for MyoD-stimulated myogenesis in MEFs. Three days after induction of MyoD-stimulated myogenesis, cell morphology was observed under phase-contrast microscope (A). Expression of myogenesis marker genes *myogenin* and *myosin heavy chain* (*MHC*) was analyzed by qRT-PCR (B). Undiff, before differentiation; Diff, after differentiation.

(C) qRT-PCR analysis of GW-induced PPARδ target gene expression in myocytes.

All results are representative of 2-4 independent experiments.

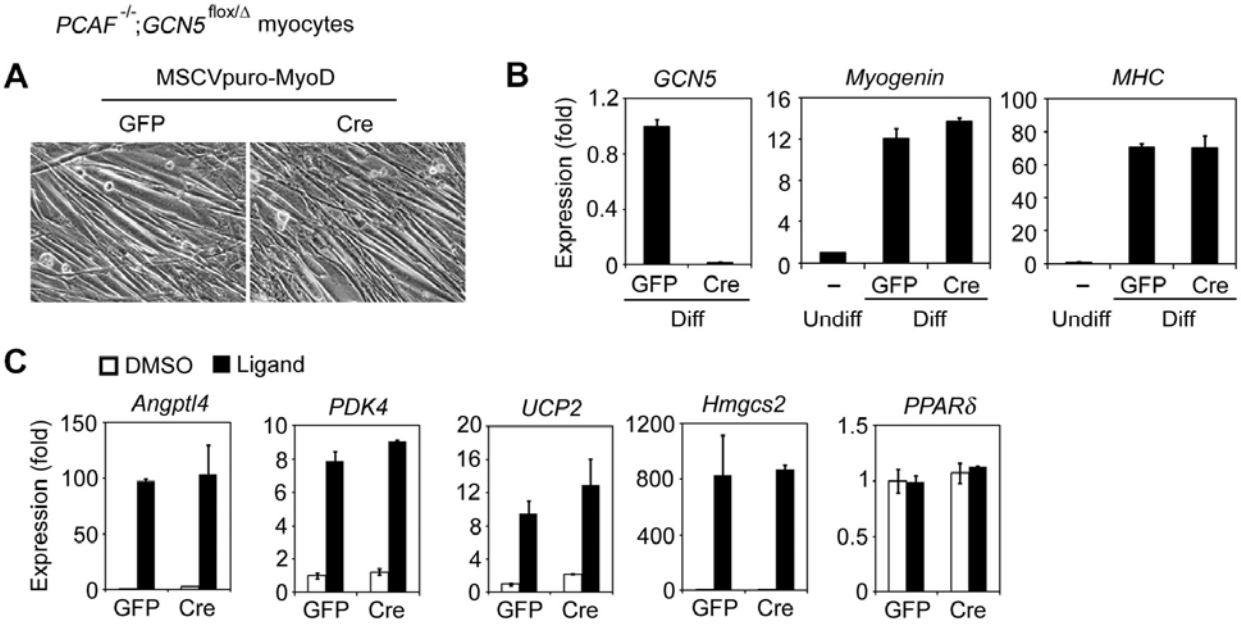

Figure S6. GCN5/PCAF and GCN5/PCAF-mediated H3K9ac are dispensable for ligand-induced expression of several LXRα and RARα target genes

Retroviral Vec- or Cre-infected *PCAF*$^{-/-}$;*GCN5*$^{flox/\Delta}$ MEFs were treated with either 1 μM LXRα ligand T0901317 (A – B) or 1 μM RARα ligand all-trans-retinoic acid (C – D) for 24h. (A) and (C), qRT-PCR of gene expression. (B) and (D), ChIP of H3K9ac and Pol II recruitment on indicated genes. All results are representative of 2 independent experiments.

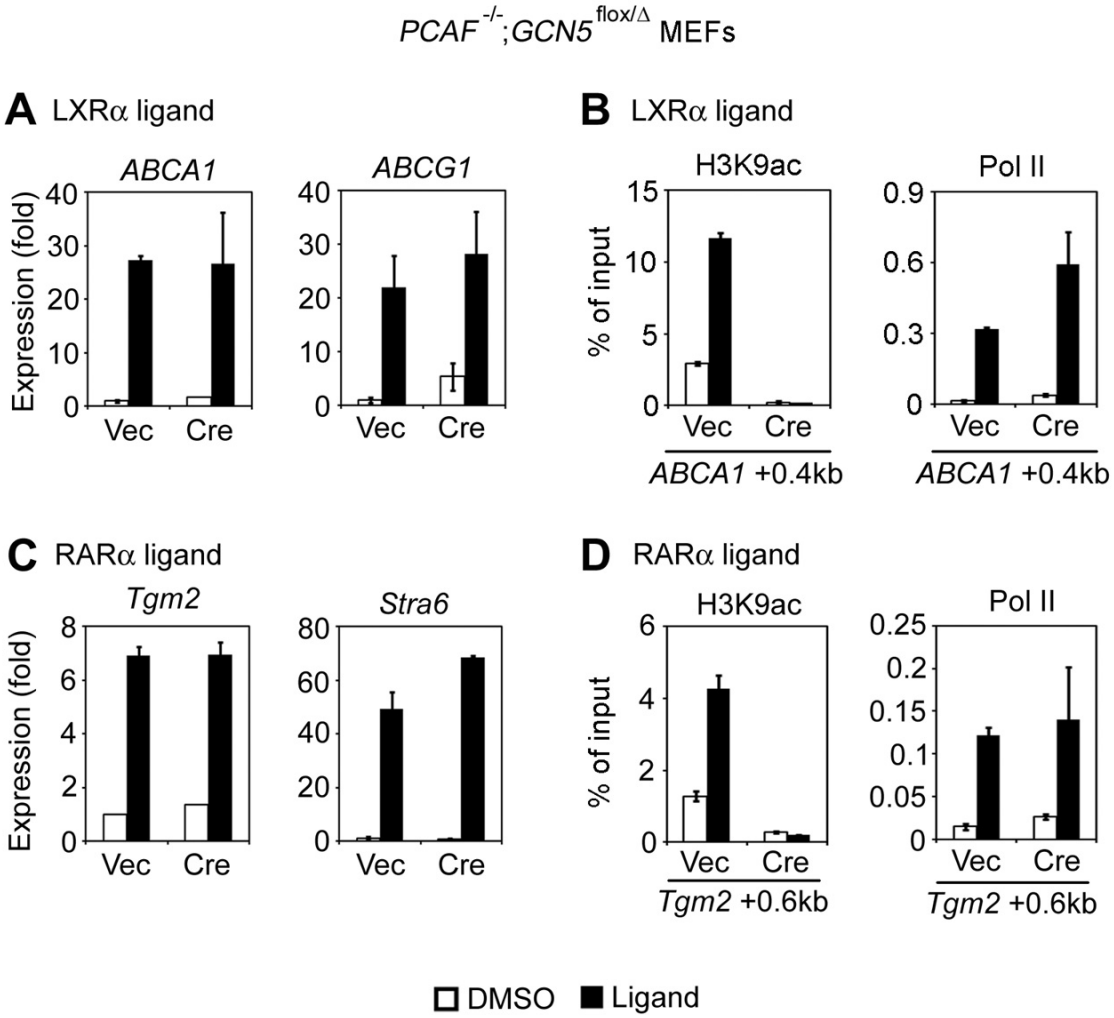

Figure S7. Single deletion of CBP or p300 has no effect on PPARδ ligand-induced *Angptl4* expression

Immortalized *CBP*$^{flox/flox}$ MEFs (A) or *p300*$^{flox/flox}$ MEFs (B) were infected with retroviruses MSCVpuro expressing Cre or Vec alone, followed by puromycin selection. Cells were treated with GW or DMSO for 24h, followed by qRT-PCR analysis of gene expression. All results are representative of 2 independent experiments.

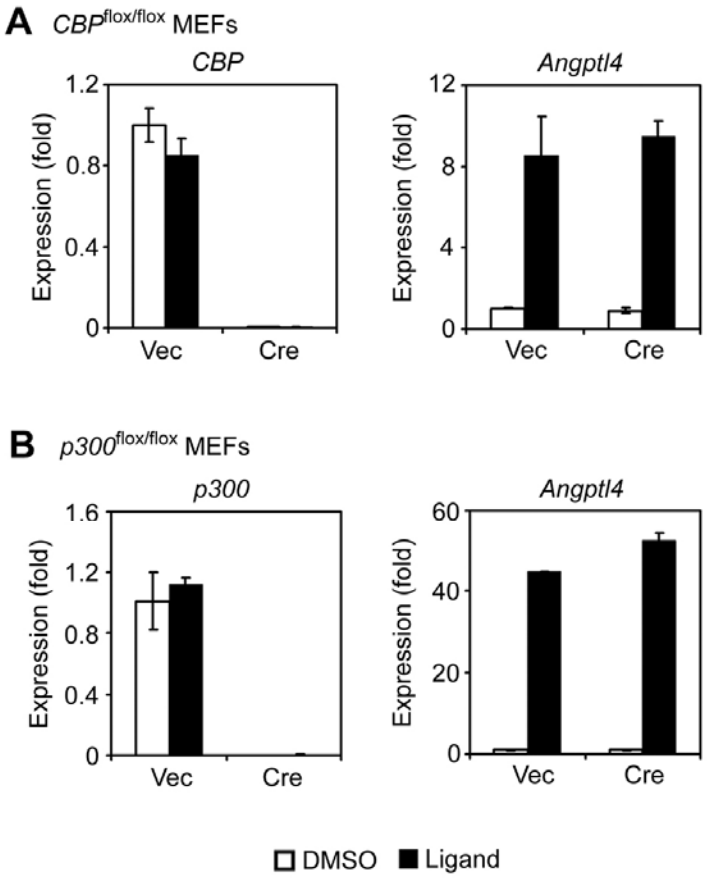

Figure S8. Expression of housekeeping genes and Western blot of H3K56ac in CBP/p300 double knockout MEFs

Immortalized *CBP*$^{flox/flox}$;*p300*$^{flox/flox}$ MEFs were infected with adenoviruses expressing Cre or GFP control as described in Figure 5.

(A) Expression of housekeeping genes.

(B) ChIP of H3K18ac and H3K27ac on housekeeping gene promoters.

(C) Nuclear extracts were prepared for Western blot analysis of H3K56ac.

All results are representative of 2-4 independent experiments.

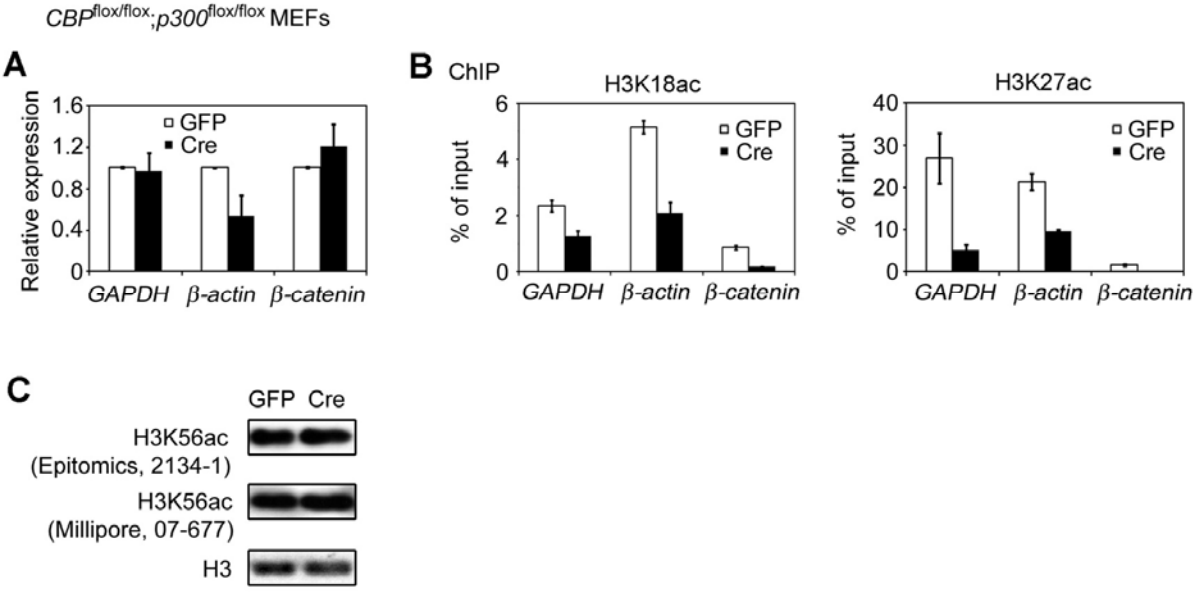

Figure S9. CBP/p300 are essential for ligand-induced expression of several LXRα and RARα target genes

Immortalized *CBP*$^{flox/flox}$;*p300*$^{flox/flox}$ MEFs were infected with adenoviruses expressing Cre or GFP control. Two days later, cells were replated. After 24h, cells were treated with either 1 µM LXRα ligand T0901317 (A – B) or 1 µM RARα ligand all-trans-retinoic acid (C – D) for 24h. (A) and (C), qRT-PCR analysis of gene expression. (B) and (D), ChIP of histone acetylations and Pol II recruitment on indicated gene promoters.

All results are representative of 2 independent experiments.

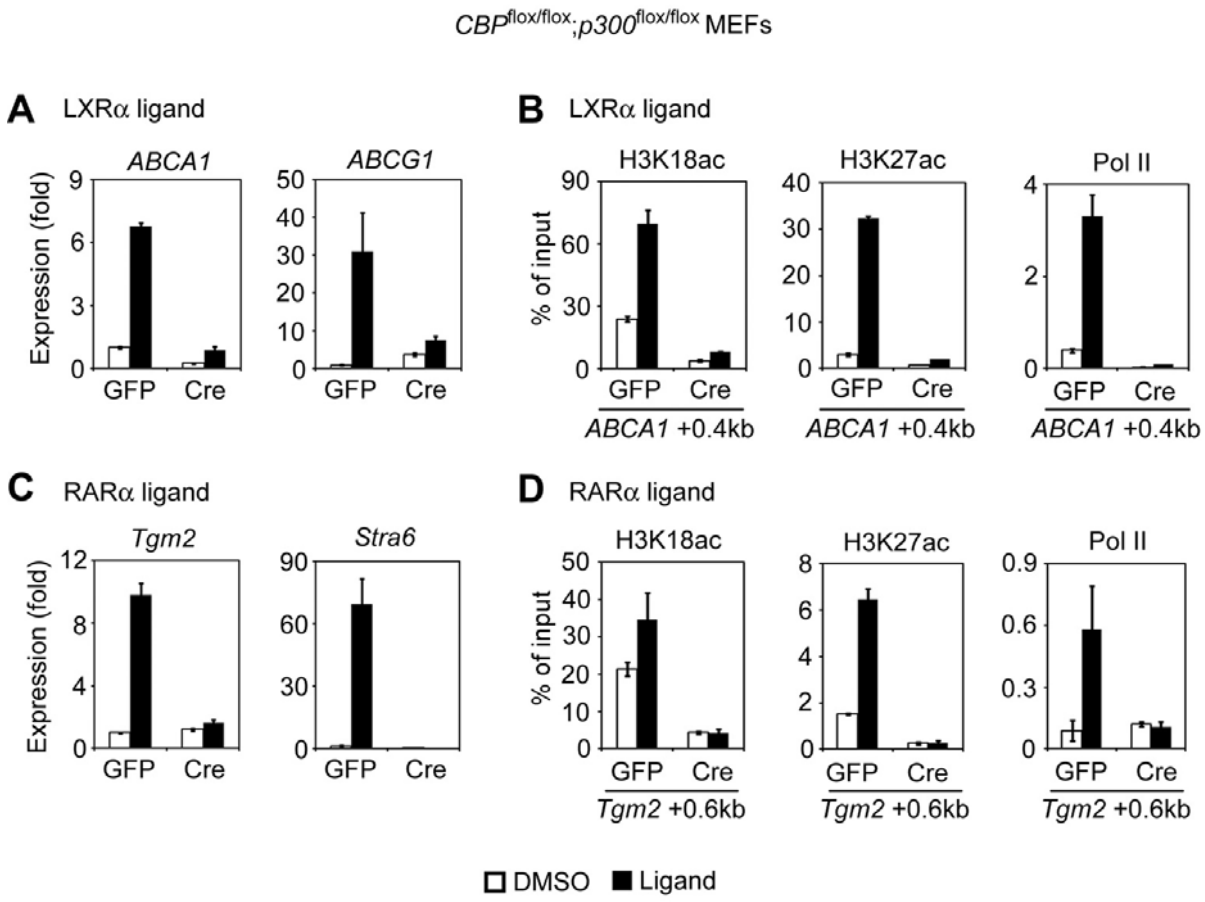

Table S1. List of commercial antibodies

Antibody	Company (catalog number)	Usage
Histone H3	Abcam (ab1791)	WB (Western blot), ChIP
H3K4ac	Millipore (07-539)	WB
H3K9ac	Epitomics (1328-1)	WB, ChIP
H3K14ac	Millipore (07-353)	WB, ChIP
H3K18ac	Abcam (ab1191)	WB, ChIP
H3K18ac	Epitomics (1766-1)	WB
H3K23ac	Millipore (07-355)	WB
H3K27ac	Abcam (ab4729)	WB, ChIP
H3K36ac	Millipore (07-540)	WB
H3K56ac	Millipore (07-677)	WB
H3R2me2a	Millipore (07-585)	WB
H3K4me2	Abcam (ab7766)	WB
H3K4me3	Abcam (ab8580)	WB, ChIP
H3K9me2	Abcam (ab1220)	WB, ChIP
H3K9me3	Diagenode (pAb-056-050)	WB
H3K27me2	Millipore (07-452)	WB
H3K27me3	Millipore (07-499)	WB, ChIP
H3K36me3	Abcam (ab9050)	WB, ChIP
H3K79me2	Abcam (ab3594)	WB, ChIP
H3K79me3	Abcam (ab2621)	WB
Histone H4	Abcam (ab7311)	WB
H4K5/8/12/16ac (H4ac)	Millipore (06-866)	WB, ChIP
H4K5ac	Millipore (07-327)	WB
H4K8ac	Millipore (07-328)	WB
H4K12ac	Millipore (07-595)	WB
H4K16ac	Santa Cruz (sc-8662R)	WB
H4K20me1	Abcam (ab9051)	WB
H4K20me3	Abcam (ab9053)	WB
Pol II	Abcam (ab5408)	ChIP
S5P Pol II	Abcam (ab5131)	ChIP
S2P Pol II	Abcam (ab5095)	ChIP
GCN5	Santa Cruz (sc-20698)	WB, ChIP
PCAF	Santa Cruz (sc-13124)	WB
PCAF	Abcam (ab12188)	ChIP
MBIP	ProteinTech (10685-1-AP)	WB
CBP	Santa Cruz (sc-369)	WB, ChIP
P300	Santa Cruz (sc-585)	WB

Table S2. List of Sybr Green primers for quantitative PCR

qRT-PCR primers for gene expression analysis:

Gene	Forward primer	Reverse primer
ABCA1	AAAACCGCAGACATCCTTCAG	CATACCGAAACTCGTTCACCC
ABCG1	CTTTCCTACTCTGTACCCGAGG	CGGGGCATTCCATTGATAAGG
Angptl4	CAGCCTCAACATGGAATGTC	TACCTGAAGCAGGCAAATCC
β-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
β-catenin	TTAAACTCCTGCACCCACCAT	GGCAAGGTTTCGAATCAATCC
CBP	AGACCCTGCAGCTCTGAAAGATC	TGTCTCCCTCCACTTTCTTAGCA
Cxcl1	CTGGGATTCACCTCAAGAACATC	CAGGGTCAAGGCAAGCCTC
Cxcl2	GCGCCCAGACAGAAGTCATAG	AGCCTTGCCTTTGTTCAGTATC
GCN5	CTTCTGTGCCGTCACCTCAA	TGGTACTCCTTTAGGTGGTTCATCA
Hmgcs2	GAAGAGAGCGATGCAGGAAAC	GTCCACATATTGGGCTGGAAA
Hsp70	TGGTGCAGTCCGACATGAAG	GCTGAGAGTCGTTGAAGTAGGC
MHC	TTGAAAAGACGAAGCAGCGAC	AGAGAGCGGGACTCCTTCTG
Myogenin	AGGCTGGGTGTGCATGTGA	TTAAAAGCCCCCTGCTACAGAAG
p300	CTCCGGATCCTGCTGCTTTA	CCCCTTCCACTTTACGAGCAT
PDK4	AGGGAGGTCGAGCTGTTCTC	GGAGTGTTCACTAAGCGGTCA
PCAF	GTGTCAGAGGAAGAGATGGACAGA	TGGACGCAGGTGAAGAGGTACT
PPARδ	GCAGACCTCTCCCAGAATTCC	ACACCCGACATTCCATGTTGA
Stra6	AGCCCCACAACCGTCATG	GACATCTATATGCTGCACGTTCAGA
Tgm2	TGTTCTCCAAGCCCAGATTCTC	CTGGTCCAACTGCCCCTAAC
UCP2	ATGGTTGGTTTCAAGGCCACA	CGGTATCCAGAGGGAAAGTGAT

qPCR primers for ChIP:

Genomic location	Forward primer	Reverse primer
ABCA1 +0.4kb	GGCATAAACAGGGAAAGAATGTTC	CGAGTGCGGCAGTTCTGA
Angptl4 -9.2kb	CAAAGTAGCCGCATTACTCAAAAA	CCCTAGGTGTGCGGCTTCT
Angptl4 -7.3kb	AATTATATCAAGAGCCGTGCTGTTTT	AGGCCTGTTACACAGGGAATGT
Angptl4 -5.5kb	AAGAGCAGTCGGTGCTTTCAAC	GAGTTCCAGGCCAGTCAAGAAT
Angptl4 -3.5kb	TTAGGATAAAAGCCAGAGCCAGTT	CCTGCTGGGTACAACCATGAC
Angptl4 -1.9kb	AGTCAATTTGCCCTAAGGGTCTT	TGGTGTTCCTTTTTCTGACAGTTC
Angptl4 -0.5kb	TCAGCCTACCAGGGAGAGAA	ACGTGGATGCCTTCTTGACT
Angptl4 -0.1kb	CCCCGCCTCCAATGCT	GCACCTAAAGCCCCACTTTATAAA
Angptl4 +0.1kb	CTGCTGGGTCTTGAACTCCT	AGTAGCCGCGCATAGCAC
Angptl4 +0.6kb	AGGGTAGAAGGGAGGGTGAA	TGAGCCTTGAGCTGAGTCTG
Angptl4 +1.0kb	CAAGATGACCCAGCTCATTG	TGCCAGATGACAGCAAAGAC
Angptl4 +2.3kb	GGACTTGTCAAGGCCAAGTTCTT	TGTGGGATACGGCTATGTCTGTT
Angptl4 +3.7kb	AGCGCTTCCATTGAATGTATGAA	TCCAGAGTTTTGGGTAGCAGACTT
Angptl4 +6.5kb	ATGGCGGACTCAGTCATATTGAC	CACCTACAACAGCACCATGAGTGT
Angptl4 +9.3kb	CTGAAGGGAAGGGAATTGGTT	CACGGCAT CTGCGTTGTG
β-catenin -0.4kb	GTTAATAGATGTTCGCAGACACTCTTG	CCGCTGTGCCTCTGGAAT
GAPDH +0.8kb	CGCCGCCATGTTGCA	GGAAGGCCTAAGCAAGATTTCA
Tgm2 +0.6kb	TGTTCTCCAAGCCCAGATTCTC	CTGGTCCAACTGCCCCTAAC

REFERENCES

Cho Y-W, Hong T, Hong S, Guo H, Yu H, Kim D, Guszczynski T, Dressler GR, Copeland TD, Kalkum M, Ge K (2007) PTIP Associates with MLL3- and MLL4-containing Histone H3 Lysine 4 Methyltransferase Complex. *J Biol Chem* **282:** 20395-20406

Wang Y-L, Faiola F, Xu M, Pan S, Martinez E (2008) Human ATAC Is a GCN5/PCAF-containing Acetylase Complex with a Novel NC2-like Histone Fold Module That Interacts with the TATA-binding Protein. *Journal of Biological Chemistry* **283:** 33808-33815