

Supplemental Figures and Figure Legends

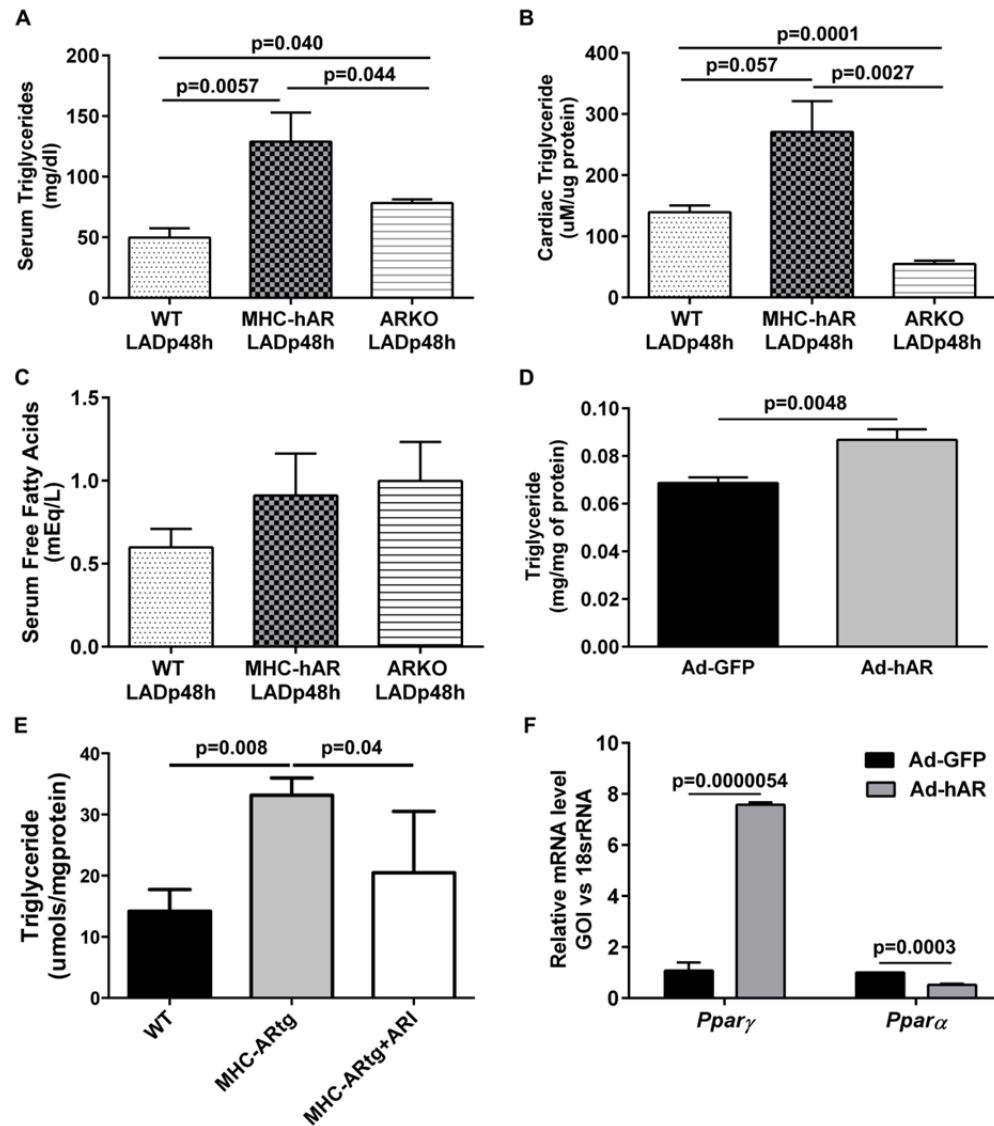


Figure S1, Related to Figure 1: AR expression enhances lipid accumulation. Serum were collected from fed wild type, MHC-hAR and ARKO mice subjected to left ascending coronary artery ligation post 48hrs (LAD p48h) (A). serum triglycerides (n=5 mice/ group). (B). cardiac tissue triglycerides (n=6 mice/group). (C). serum free fatty acids (n=5 mice/group). (D). Triglycerides measured from H9c2 cells adenovirally transduced with Ad-GFP/Ad-hAR at 48hrs post transfection. (n=6). (E). Cardiac triglycerides were measured in MHC-hAR mice treated with the AR inhibitor (ARI) or vehicle, and WT mice (n=5/group). (F). qRT-PCR analysis on the *Ppara* α and γ transcripts in transduced H9c2 cells. (n=5).

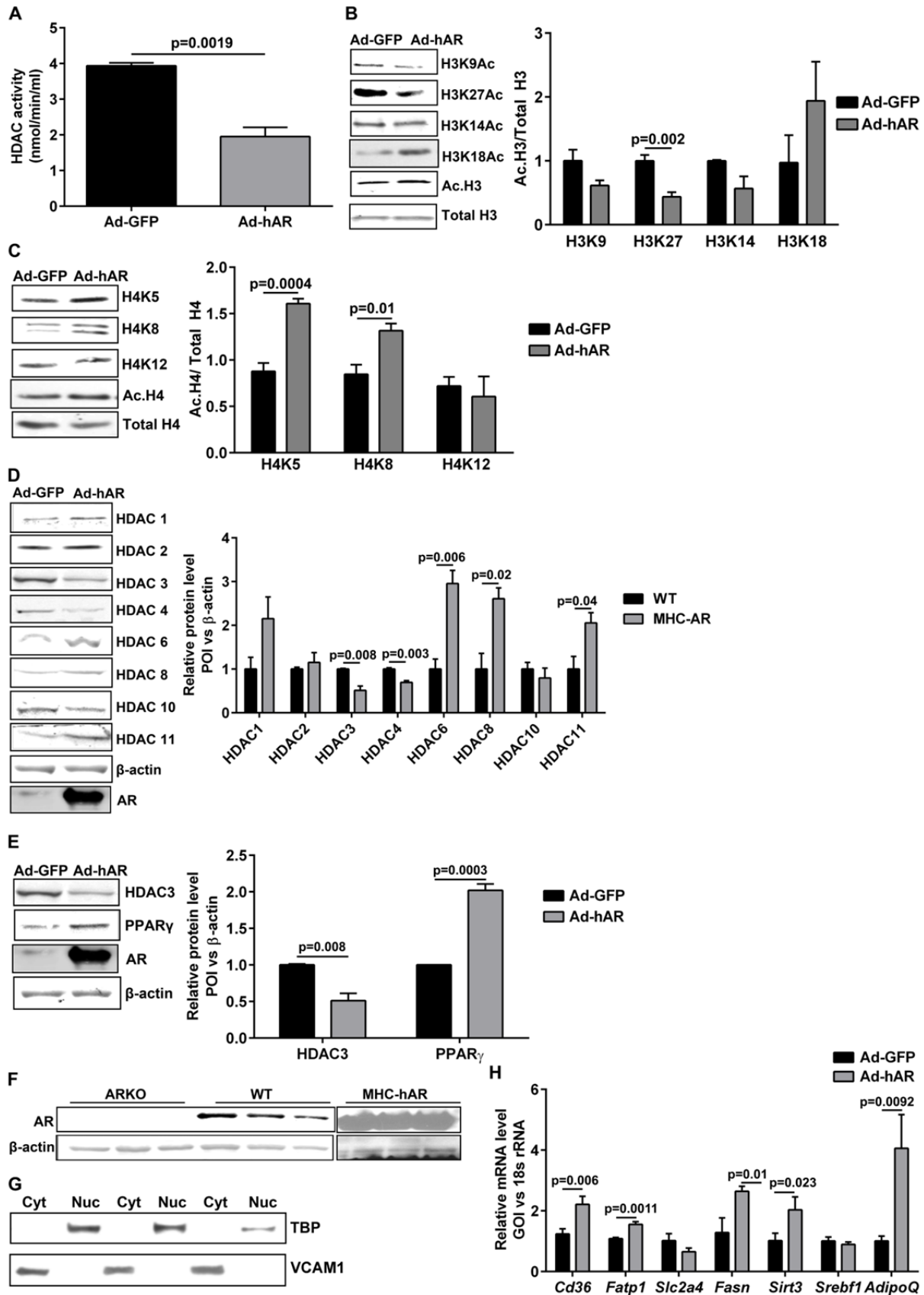


Figure S2, Related to Figure 2: PPAR γ is activated in AR expressing cells. Lysates from adenovirally transduced H9c2 cells with GFP/hAR were used post 48hrs transduction. (A). HDAC activity was measured from the nuclear fraction. (n=6). (B). Acetylated histone H3 modifications normalized against total H3. (C). Acetylated histone H4 modifications normalized against total H4. (D). Profiling of histone deacetylases, normalized against β -actin. (E). Western blot analysis of total cell lysates using HDAC3, AR and PPAR γ . β -actin used as loading control. B-E. (n=3). (F). AR expression in the cardiac tissue lysates from wild type, MHC-hAR and ARKO mice. (n=6). (G). Nuclear and cytoplasmic fractions were tested for purity using VCAM1 and TBP as cytoplasmic and nuclear specific proteins by western blot. (H). qRT-PCR analysis of PPAR γ target genes in adenovirally transduced H9c2 cells. (n=6).

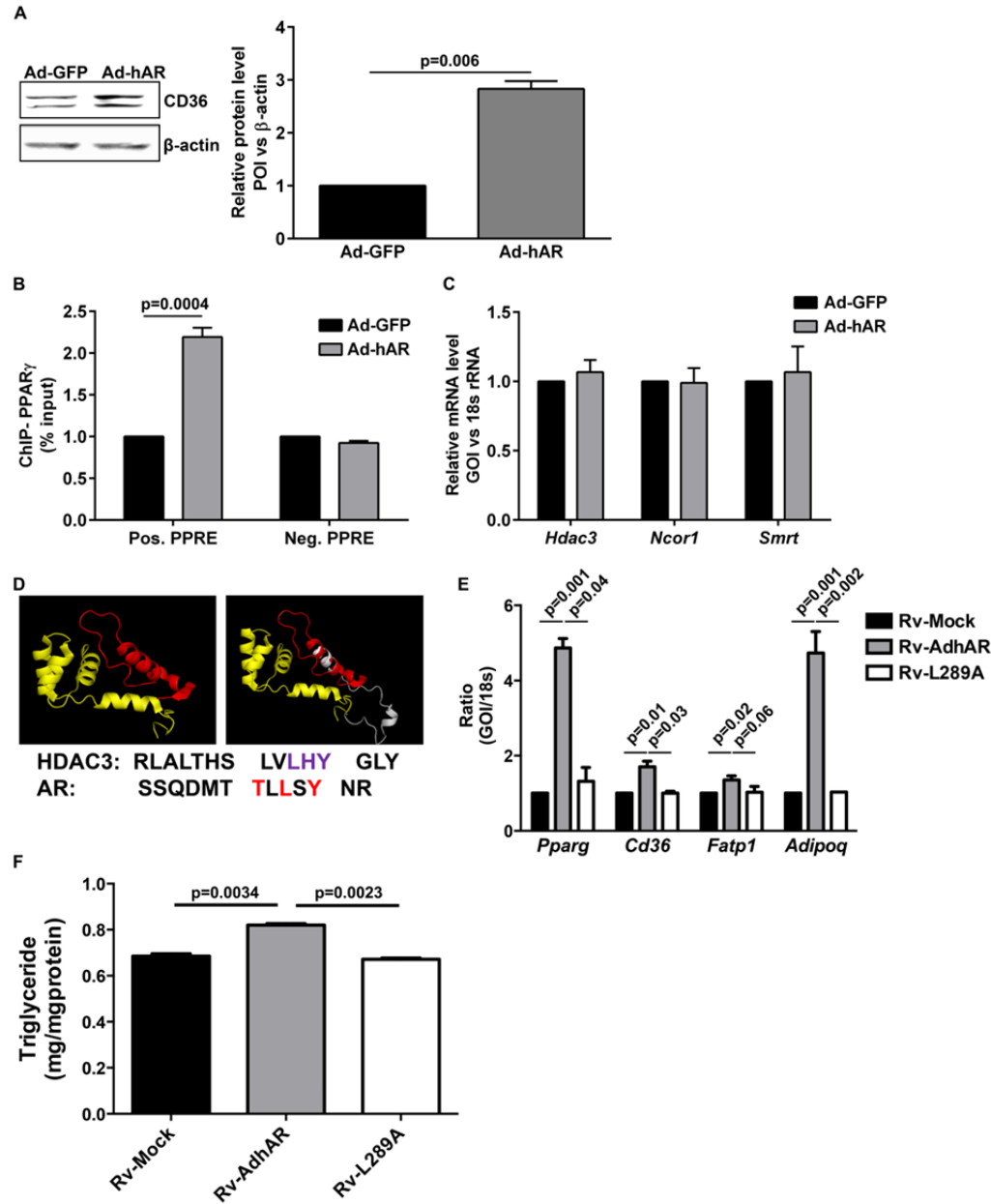


Figure S3, Related to Figure 2: AR activates PPAR γ in cells. Lysates from Ad-GFP/Ad-hAR cells, 48hrs post transduction were used for (A). Western blot analysis and quantification of CD36, normalized to β -actin. (n=3). (B). ChIP analysis done using PPAR γ antibody and RT-PCR were done using Sybrgreen with primers spanning the PPAR response element (PPRE) in the *Cd36* promoter region. Upstream to that region, devoid of PPRE, was used as negative control. (n=3). (C). qRT-PCR analysis on expression of *Hdac3* and nuclear corepressors. (D). Overlapping of secondary structures of HDAC3-DAD complex and AR using Pymol program. Alignment of protein sequences of HDAC3 and AR, within the overlapping region. Red- HDAC3 (9-49 aa); Yellow- SMRT-DAD; White- AR (266-312 aa). (E). qRT-PCR analysis performed on retrovirally transduced H9c2 cells (n=5/group). (F). Triglycerides were measured in H9c2 cells retrovirally transduced with mock/hAR/L289A (n=3/group).

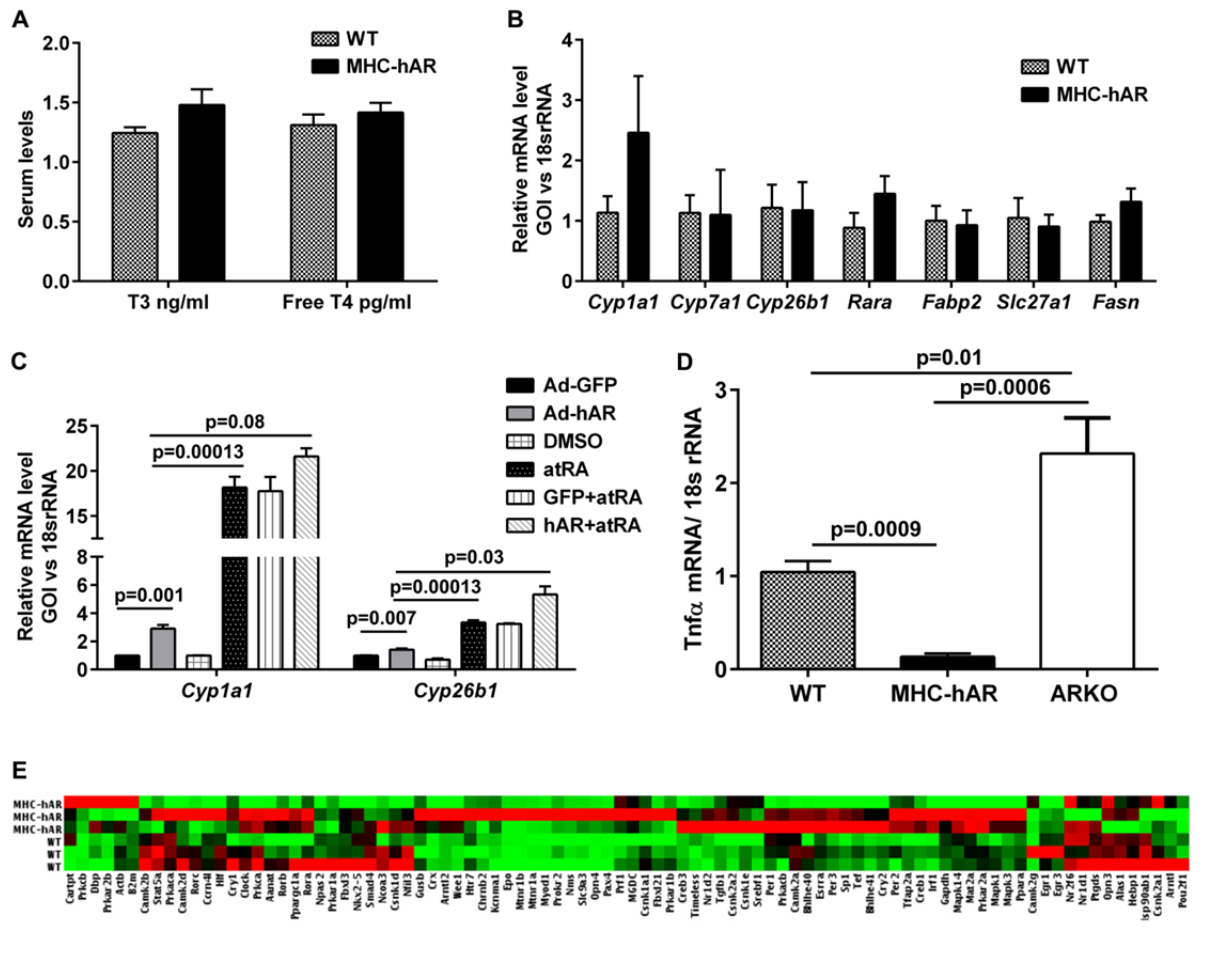


Figure S4, Related to Figure 6: AR expression hyper-activates RAR target genes. (A). serum T3 and T4 measurements made from wild type and MHC-hAR mice. (n=6 mice/group). (B). qRT-PCR analysis from the liver tissues of wild type and MHC-hAR mice. (C). qRT-PCR analysis for *Cyp1a1* and *Cyp26b1* in transduced H9c2 cells treated with/without at RA (all-trans retinoic acid) (n=3). (D). qRT-PCR analysis for *Tnf-α* in cardiac tissue (n=6 mice/group). E. PCR array results for genes involved in the Circadian rhythm on RNA isolated from MHC-hAR and WT hearts (n=3/group).

Supplemental Table S1. Details of primers used for ChIP assay and qRT-PCR

Sybr green primers

RT mqrttb11 fp	TGAGGTCTTCGTCTCGATCA
RT mqrptb11rp	CCTGGACTTTGTGAGCACCT
RT mqrtblr1 fp	CCACTTAAACCATGAGGTCACA
RT mqrtblr1rp	CGCCAAGCAGCTTCAGTAAC
RT mPpard FP	CTGTGGCTGTTCCATGACTG
RT mPpard RP	AGATGAAGACAAACCCACGG
RT mNCOR FP	AGATTTACGCGAGTTGGTCAG
RT mNCOR RP	CCTCCATCAGCCCATTTCATATT
RT mSMRT FP	GTTCCATCATCCAGCCACA
RT mSMRT RP	AGCAGGGTATCGGGTAGTAG
RT mFabp4 FP	TCCCCATTTACGCTGATGAT
RT mFabp4 RP	TGGAAGCTTGTCTCCAGTGA
RTmAdfp FP	CAATTTGTGGCTCCAGCTTC
RT mAdfp RP	CCCGTATTTGAGATCCGTGT
RT mFitm1 FP	CCTCTGCCTTACTGTACTTTGG
RT mFitm1 RP	TAGCGAAGATCGTCCGAGAGT
RT m18S FP	AGTCCCTGCCCTTTGTACACA
RT m18s RP	CGATCCGAGGGCCTCACTA
RT mFsp27 FP	ACGATTGTGCCATCTTCCTC
RT mFsp27 RP	AGGTTTCGCAAAGGCATCAT
RT mMGAT1 FP	GCTCAGGTCCCCACACTAAG
RT mMGAT1 RP	AGTATTAGGTTGGGGGAGGG
RT mAdipoQ FP	AGTAAACGTCATCTTCGGCAT
RT mAdipoQ RP	CTCTAAAGATTGTCAGTGGATCTG
RT mGpam FP	CAACACCATCCCCGACATC
RTmGpam RP	GTGACCTTCGATTATGCGATCA
Chip Cyp1a1 fp	CTACCCCTTCCTTCCTTCC
Chip Cyp1a1 rp	ACACTGTTACCTCTGAGCCA
Chip Cyp1a2 fp	CTGGCAGCTACTACCATCCA
Chip Cyp1a2 rp	GTACCCCATGCCTACCTGAA
Chip Cyp1b1 fp	CTCAGCTGACTTCCTCGTGA
Chip Cyp1b1 rp	CAGTCTTACTTGGAAGCGCG
Chip Cyp3a4fp	TGATCTGGCCTCCTATTGGG
Chip Cyp3a4 rp	GAGGCCAACATTGCATACTGT
Chip Cyp26b1 set 1 fp	AAGTGGTCTTCAGCTGTCCA
Chip Cyp26b1 set 1 rp	CAGCCTTCTCCTGTCATCGT
Chip Cyp26b1 set 2 fp	CCTGCACCTTTCTCCTCTGT

Chip Cyp26b1 set 2 rp	AAGCCTGCCTTCCTCAAGTT
chip sercafp	TCTGGTCCTCAGGGATCATT
chip sercarp	GACCTCCTGTGGTGAGTGCT
ChiP NCX FP	TGAAGCCATACTGGGGAAAG
ChiP NCX RP	TTGCTCTGCTGGAGAATGAA

Taqman primers:

Mouse specific Taqman primers

mGps2	Mm00517238_g1
mMyh6	Mm00440359_m1
mMyh7	Mm01319006_g1
mSlc8a1	Mm01232254_m1
mSerca2a	Mm01201431_m1
mLXRa	Mm00443451_m1
mSrebf1	Mm00550338_m1
mAbca1	Mm00442646_m1
mAbcg1	Mm00437390_m1
mCyp1a1	Mm00487218_m1
mCyp1a2	Mm00487224_m1
mCyp1b1	Mm00487229_m1
mCyp26b1	Mm00558507_m1
mRara	Mm01296312_m1
mSlc27a1	Mm01232254_m1
mCpt1a	Mm01231183_m1
mCpt2	Mm00487205_m1
mCd36	Mm01135198_m1
mAcsl1	Mm04207567_g1
mPPara	Mm00440939_m1
mPParg	Mm01184322_m1

Rat specific Taqman primers

rCd36	Rn01442638_m1
rFatp1	Rn00585821_m1
rSlc2a4	Rn01752377_m1
rFasn	Rn01463550_m1
rSirt3	Rn01501410_m1
rSrebf1	Rn01495769_m1
rAdipoq	Rn00595250_m1
rNcor1	Rn01399460_m1

Supplemental Experimental Procedures:

Surgical procedures related to coronary artery ligation (LAD) were done as previously described (Hwang et al., 2004). LAD was done on 4 months old mice and was sacrificed 48 hrs post-surgery. For the experiments conducted at basal level, 2 months old mice were used. For aging studies, young (4 months) and old (24 months) wild type C57B/6 mice were used. Diabetic mice were generated using STZ injection as described earlier (Vedantham et al., 2014). 6 months old diabetic mice with blood glucose >250mg/dl at time of experiment, along with age matched controls were used.

For cell culture experiments, cells were harvested post 48hrs after transduction. Chemicals including MG132 (5uM), bortezomib (1uM) and epoxomicin (1uM) were purchased from Sigma chemicals and cells were treated for overnight. For retroviral transduction, human NCOR1 cDNA (Transomic) was subcloned into retroviral vector pQXCIN (Clontech) and clones were verified through DNA sequencing. pQXCIN-NCOR1 plasmid was transfected using Lipofectamine 2000 (Invitrogen) in HEK293T cells. Supernatant media was collected for 3 days and were transduced in H9c2 using polybrene. 3 days after transfection, cells were selected with G418 for 15 days. The stably expressing NCOR1 cells were then adenovirally transduced with GFP or hAR.

Western Blot analysis: Total lysates from heart tissues or cultured cells were prepared using lysis buffer (Cell Signaling) for the detection of protein using the following antibodies: HDAC3 (Activemotif (N-terminal specific ab), Biovision (C-terminal specific ab), Abcam (N-terminal specific ab), PPAR γ (Caymen Chemicals), SMRT (Santacruz), NCOR1 (Abcam), CD36 (Novus Biologicals), Smooth muscle alpha Actin, β -actin (Sigma) TBP (Abcam), Acetyl histone H3 and acetyl histone H4 antibody sampler kit (Cell signaling).

qPcr: cDNA was prepared using iScriptcDNA synthesis kit (BioRad). Taqman probes (Life Tech) were used for quantification and the data were normalized using 18s rRNA. For TR, LXR and RAR target genes, primers were obtained from IDT. Sybr green quantification was done using Fast Sybr green master mix (Applied Biosystems) and normalized using 18s rRNA.

Chromatin immunoprecipitation: For chromatin immunoprecipitation, ChIP grade HDAC3 and PPAR γ antibodies were purchased from Abcam. ChIP was performed using ChIP-IT kit (ActiveMotif). Briefly, heart tissue was homogenized and crosslinked with 1% formaldehyde for 10min at room temperature and chromatin was obtained as per protocol. 150ug of chromatin was immunoprecipitated using 15ug HDAC3 antibody. Primers were designed using the nuclear receptors binding site data available from Applied Biosciences website and Primer3 software. The details of the primers were given in the Table S1. For ChIP in cells, primers were designed in the region containing PPRE and 2 Kb upstream regions were used as Negative PPRE. For HDAC3 ChIP, primer sequences corresponding to Serca2a and Nex.1 were used as negative control. Real time PCR were setup using Sybrgreen (Applied Biosystems) and results were normalized using input DNA. Data were represented as % of total input and fold change.

Supplemental References

Hwang, Y.C., Kaneko, M., Bakr, S., Liao, H., Lu, Y., Lewis, E.R., Yan, S., Ii, S., Itakura, M., Rui, L., *et al.* (2004). Central role for aldose reductase pathway in myocardial ischemic injury. *FASEB J* 18, 1192-1199.
Vedantham, S., Thiagarajan, D., Ananthakrishnan, R., Wang, L., Rosario, R., Zou, Y.S., Goldberg, I., Yan, S.F., Schmidt, A.M., and Ramasamy, R. (2014). Aldose reductase drives hyperacetylation of Egr-1 in hyperglycemia and consequent upregulation of proinflammatory and prothrombotic signals. *Diabetes* 63, 761-774.