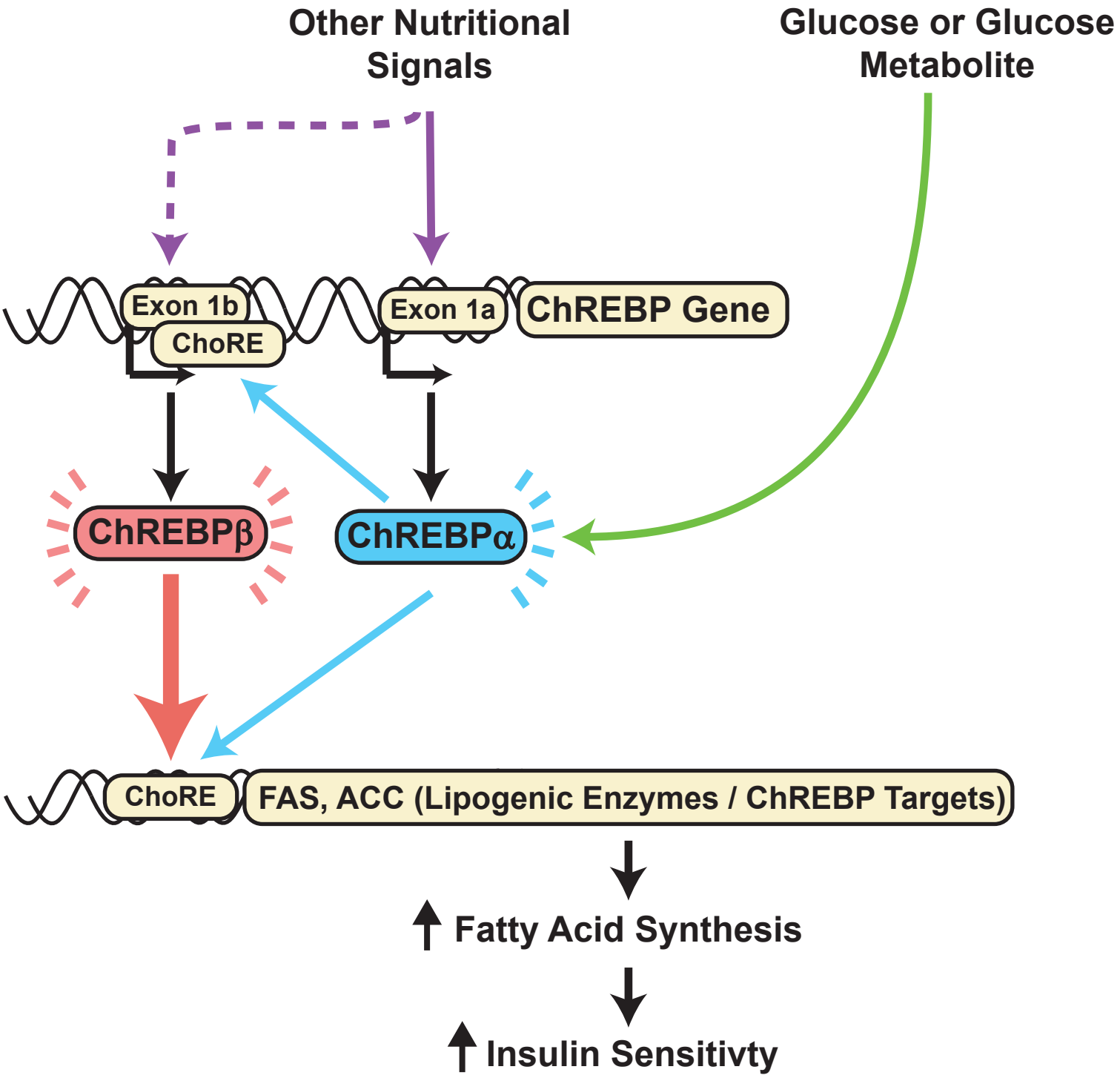
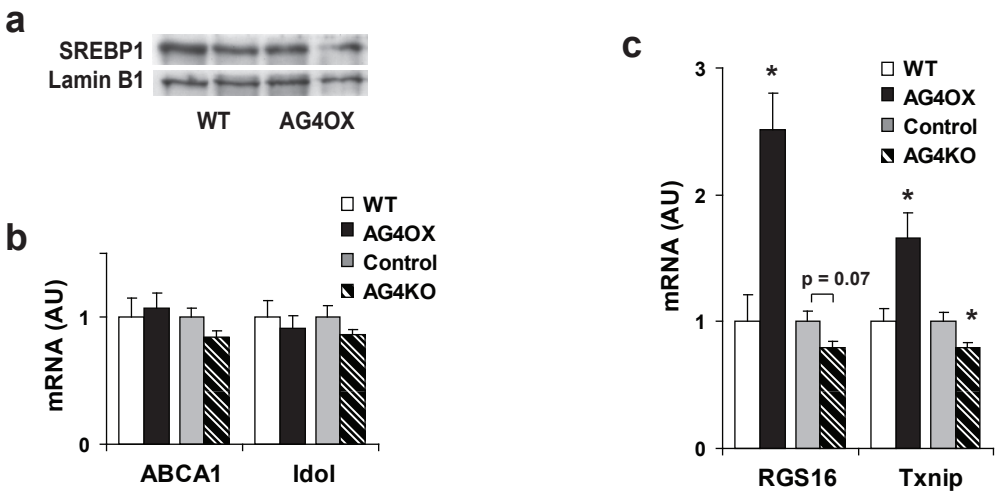
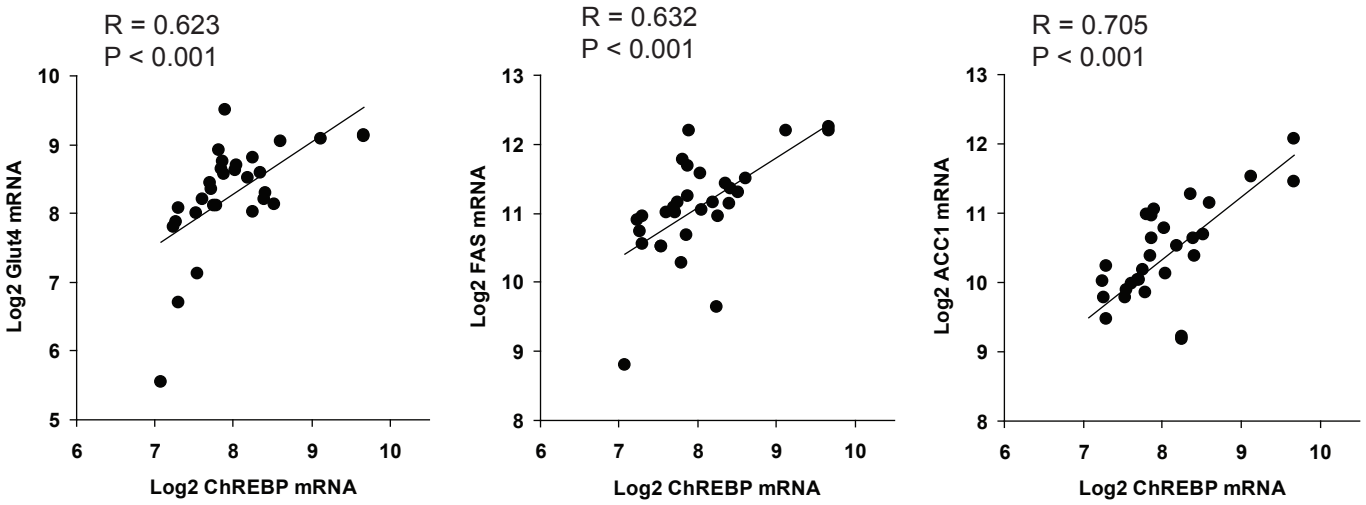


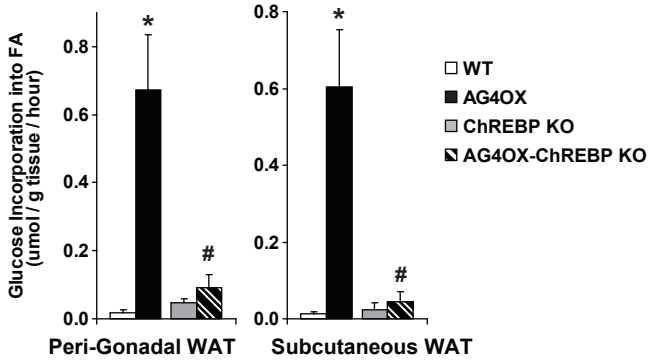
Supplementary Figure 1. Glucose-mediated activation of ChREBPα induces expression of ChREBPβ. Glucose or a glucose metabolite stimulates the transcriptional activity of ChREBPα which binds to ChoREs in its lipogenic targets and in ChREBPβ resulting in increased gene expression. The increased ChREBPβ protein further activates expression of ChREBP lipogenic target genes by binding to ChoREs. Whereas glucose regulates ChREBPα transcriptional activity, other nutritional signals regulate ChREBPα expression. Other nutritional signals may also regulate ChREBPβ expression. The activation of ChREBPα and induction of ChREBPβ expression increase fatty acid synthesis in adipose tissue which improves systemic insulin sensitivity.



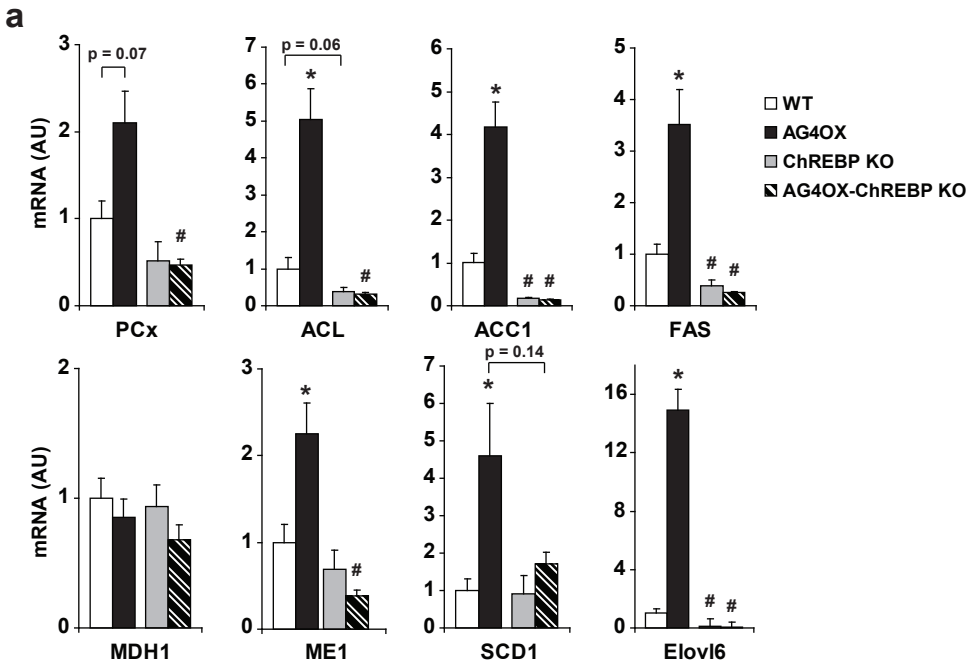
Supplementary Figure 2. Changes in ChREBP and lipogenic enzyme expression in AG4OX and AG4KO adipose tissue is not due to changes in SREBP1c nuclear abundance or LXR activity. a, Nuclear abundance of mature Srebp1 and Lamin B1 (loading control) protein in WT and AG4OX perigonadal adipose tissue from fed 6-week-old females. Each lane comprises nuclear lysates pooled from 3-4 mice. mRNA of, b, LXR transcriptional targets (ABCA1 and Idol) and, c, ChREBP transcriptional targets (RGS16 and Txnip) in perigonadal fat from 6-week-old female AG4OX, AG4KO, and littermate control mice (n=10-14 per group).*P<0.05 compared to the respective controls.



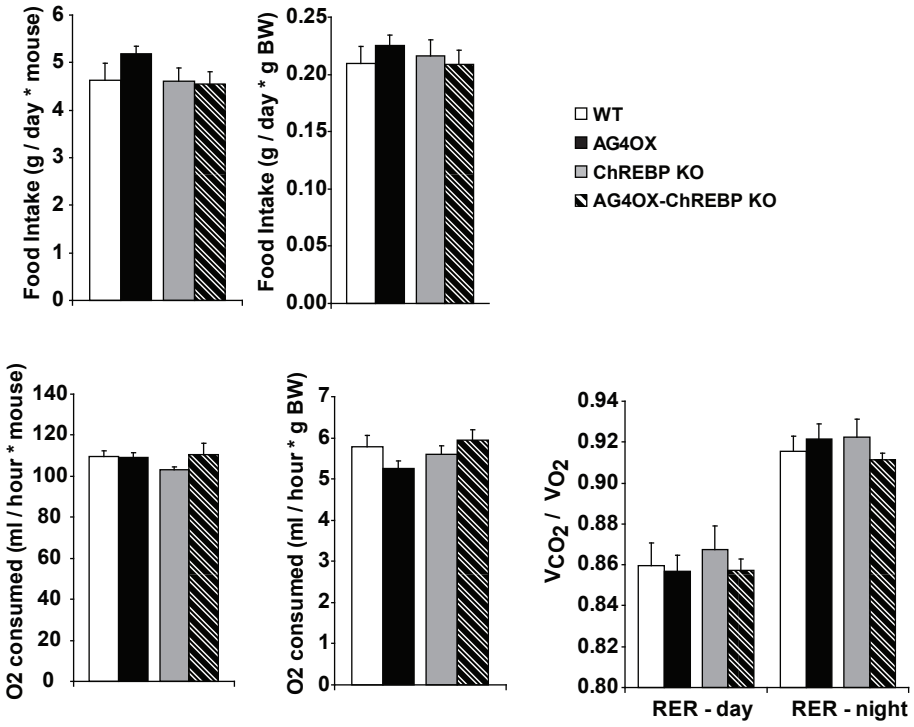
Supplementary Figure 3. Adipose tissue Glut4, FAS, and ACC1 mRNA expression correlate with ChREBP mRNA expression across 30 mouse strains. Each point on the graphs represents a different mouse strain. Expression data was obtained from the GNF1M Gene Atlas Data set via the BioGPS website (http://biogps.gnf.org) [Su, A. I. et al. Proc Natl Acad Sci U S A 99, 4465-70 (2002); Wu, C. et al. Genome Biol 10, R130 (2009)]. n=30 mouse strains. Statistics: two-tailed Pearson Correlation.



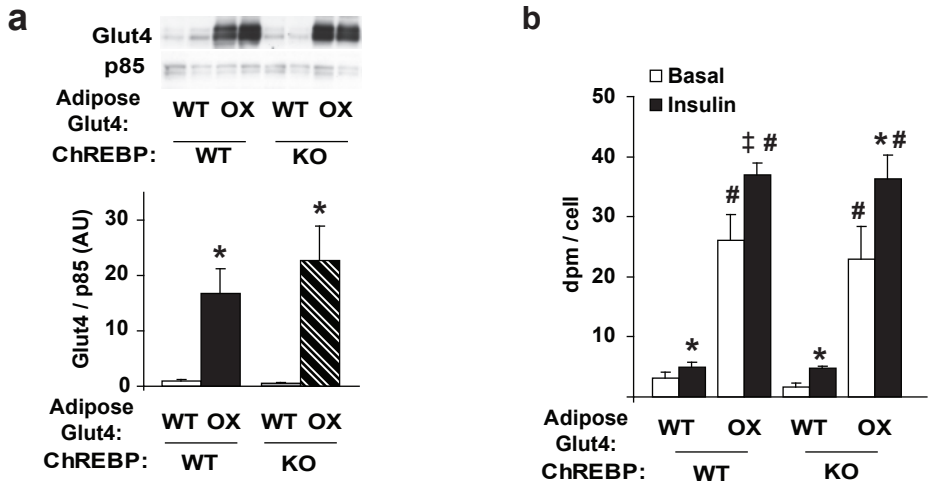
Supplementary Figure 4. Glucose incorporation into newly synthesized fatty acids is normalized in AG4OX adipose tissue lacking ChREBP. Values are means ± SE. n = 5 - 6 per group. *P<0.05 versus WT. #P<0.05 versus AG4OX.



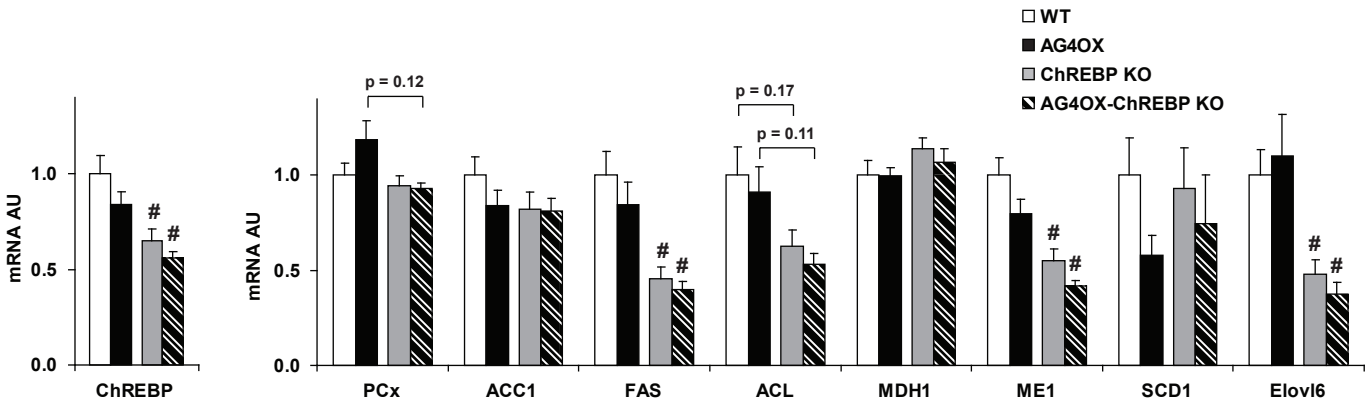
Supplementary Figure 5. a, mRNA expression of fatty acid synthetic enzymes and b, western blots of FAS, ACC, and p85 (loading control) proteins in SC fat from fed, 6-month-old females (n=9-10 per group), *P<0.05 versus WT. #P<0.05 versus same AG4OX genotype, different ChREBP genotype.



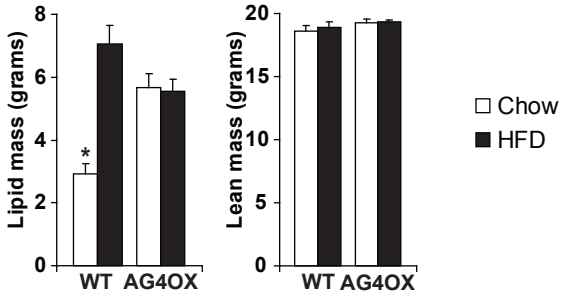
Supplementary Figure 6. Food Intake, energy expenditure, and respiratory exchange ratio (RER) measured in AG4OX mice crossbred with ChREBP KO mice. Food intake was measured in individually caged two-month-old female mice over two weeks. Metabolic measurements were made using the Comprehensive Lab Animal Monitoring System (Columbus Instruments). Food intake and O2 consumption are expressed per mouse (left panels) and per gram body weight (right panels). Values are means ± SE. n=7-8 per group. Statistical comparisons were performed by ANOVA with pairwise comparisons by Tukey's test. No statistically significant changes were observed.



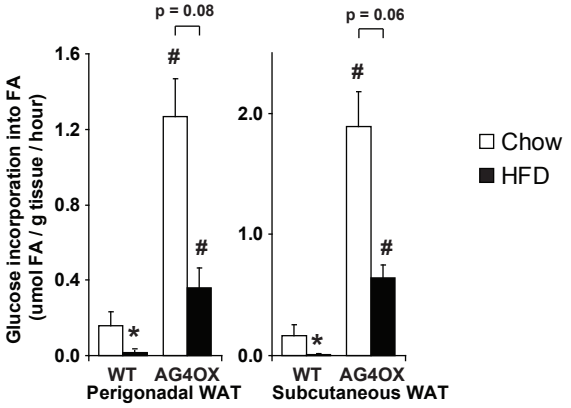
Supplementary Figure 7. a, Glut4 protein levels in perigonadal-fat of 6-month-old females (n=6-8 per group). *P<0.001 within same ChREBP genotype. b, Basal and insulin-stimulated glucose uptake into adipocytes isolated from perigonadal fat (n=6 per group) from male mice. *P<0.05 and ‡P=0.09 by paired t-test for the effect of insulin within genotype. #P<0.05 versus WT and ChREBP KO within same insulin treatment condition. OX, overexpressor.



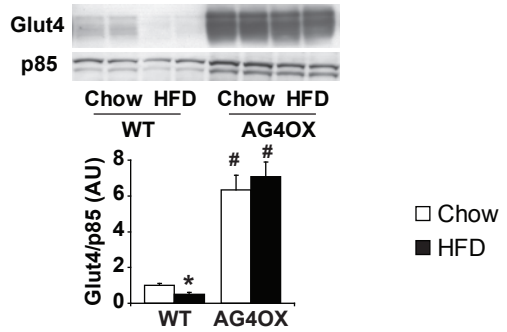
Supplementary Figure 8. Expression of ChREBP and lipogenic enzymes in liver of fed 6-month-old female mice. Values are means ± SE. n=9-10 per group. #P<0.05 comparing same AG4OX genotype, different ChREBP genotype. Using qPCR primers which are proximal to the deleted exons in ChREBP KO mice, ChREBP mRNA can be detected in ChREBP KO tissues.



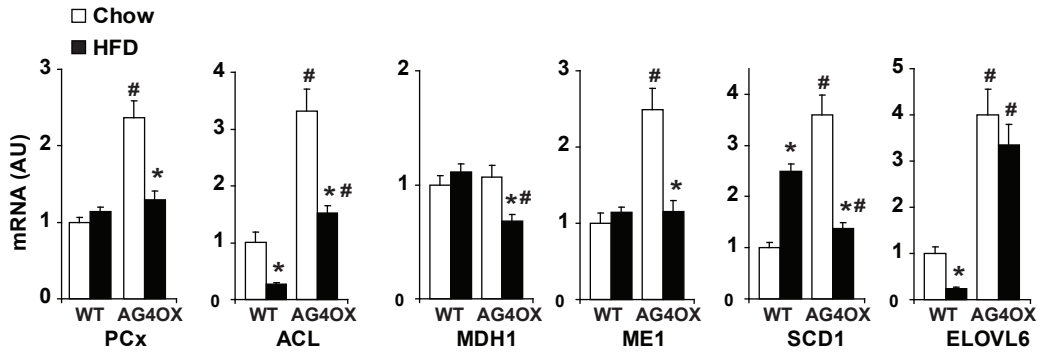
Supplementary Figure 9. Body composition in chow- and HFD-fed WT and AG4OX mice (9-week-old, n=8 per group),* P<0.01 for WT-Chow compared to all others.



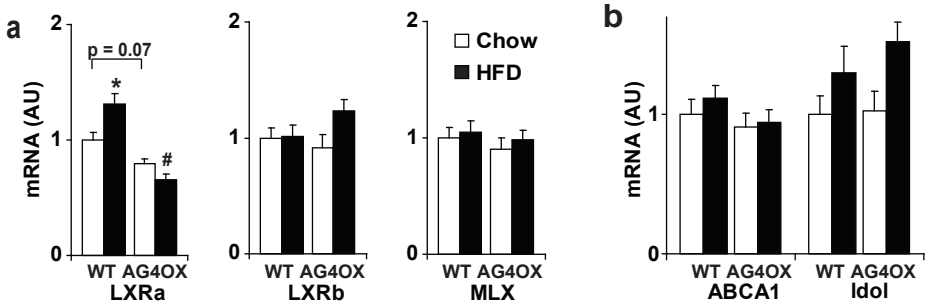
Supplementary Figure 10. Glucose incorporation into newly synthesized fatty acids is reduced in adipose tissue of WT and AG4OX mice on HFD. Values are means ± SE. n=7 per group. * P<0.05 compared to WT chow. #P<0.05 compared within diet, WT to AG4OX.



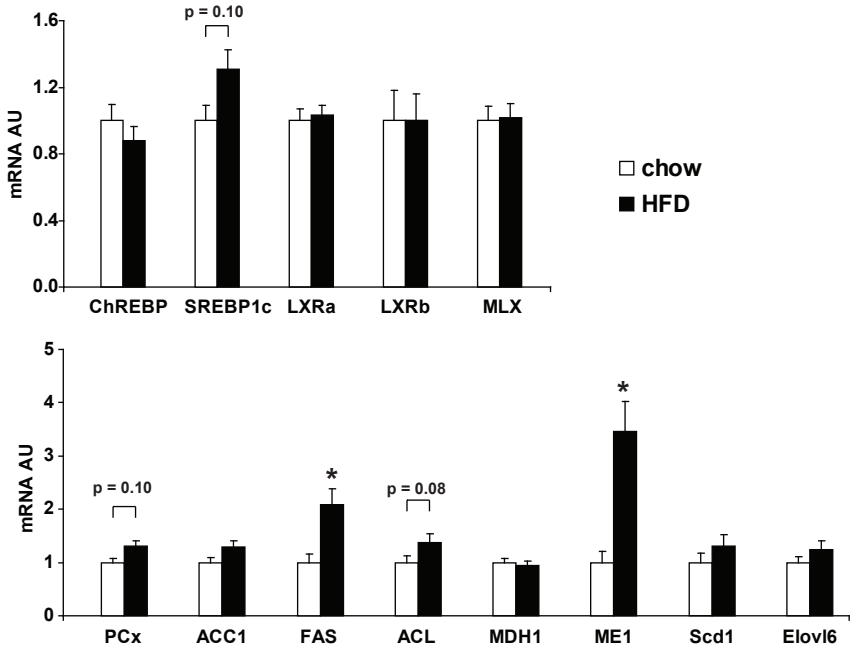
Supplementary Figure 11. Glut4 protein levels in PG fat from 4-month-old-males (n=6-8 per group), * P<0.05 compared to WT chow. #P<0.05 compared within diet, WT to AG4OX. p85 is a loading control.



Supplementary Figure 12. mRNA expression of fatty acid synthetic enzymes in SC fat from 4-month-old male mice (n=8-14 per group). *P<0.05 compared to same genotype, different diet. #P<0.05 compared to same diet, different genotype.



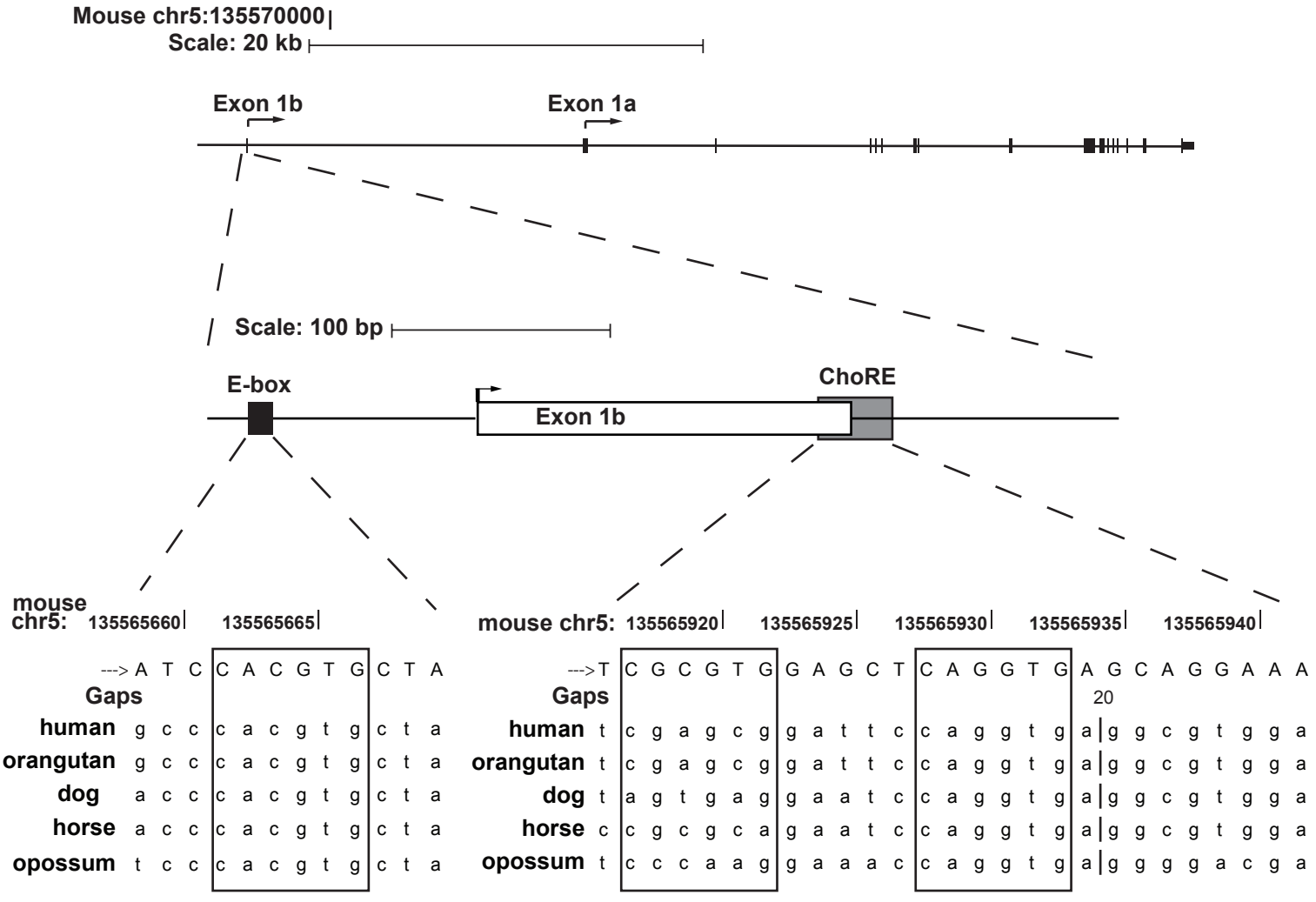
Supplementary Figure 13. a, mRNA expression of LXRs and Mlx, and b, LXR transcriptional targets in SC fat from 4-month-old male mice (n=8-14 per group). * P<0.05 compared to WT chow. #P<0.05 compared to WT HFD diet.



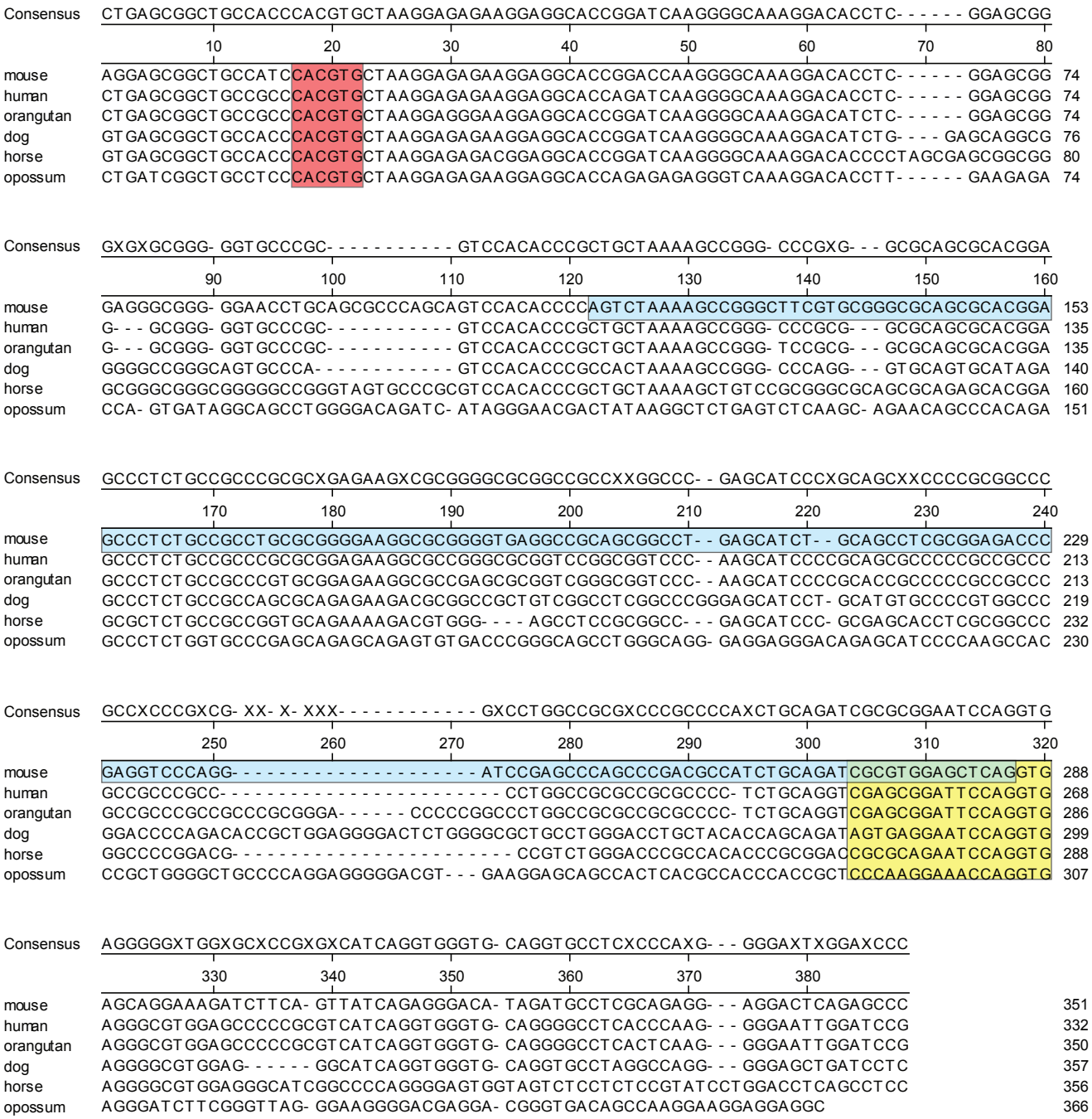
Supplementary Figure 14. Liver mRNA expression in 4-month-old male WT mice on chow versus HFD. Values are means ± SE. n = 10-14 per group. * P< 0.05 comparing chow to HFD.

Nucleotide	Position 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A	1	13	0	0	3	0	3	3	5	8	5	0	6	1	5	3	0
C	13	0	12	0	3	0	5	5	10	3	2	12	4	13	0	0	0
G	2	3	0	16	1	16	8	3	1	1	7	4	3	0	11	0	16
T	0	0	4	0	9	0	0	5	0	4	2	0	3	2	0	13	0
Consensus ChoRE	C	A	C	G	T	G						C	A	C	G	T	G

Supplementary Figure 15. Position weight matrix for ChoREs. This matrix is generated by counting the frequency of each nucleotide species at each position in the aligned ChoREs of 16 reported and experimentally validated ChoREs in human, mouse, or rat ChREBP targets. [Minn, A. H., et al. Endocrinology 146, 2397-405 (2005); O'Callaghan, B. L., et al. J Biol Chem 276, 16033-9 (2001); Pedersen, K. B., et al. Biochem J 426, 159-70 (2009); Shih, H. M., et al. J Biol Chem 267, 13222-8 (1992)]

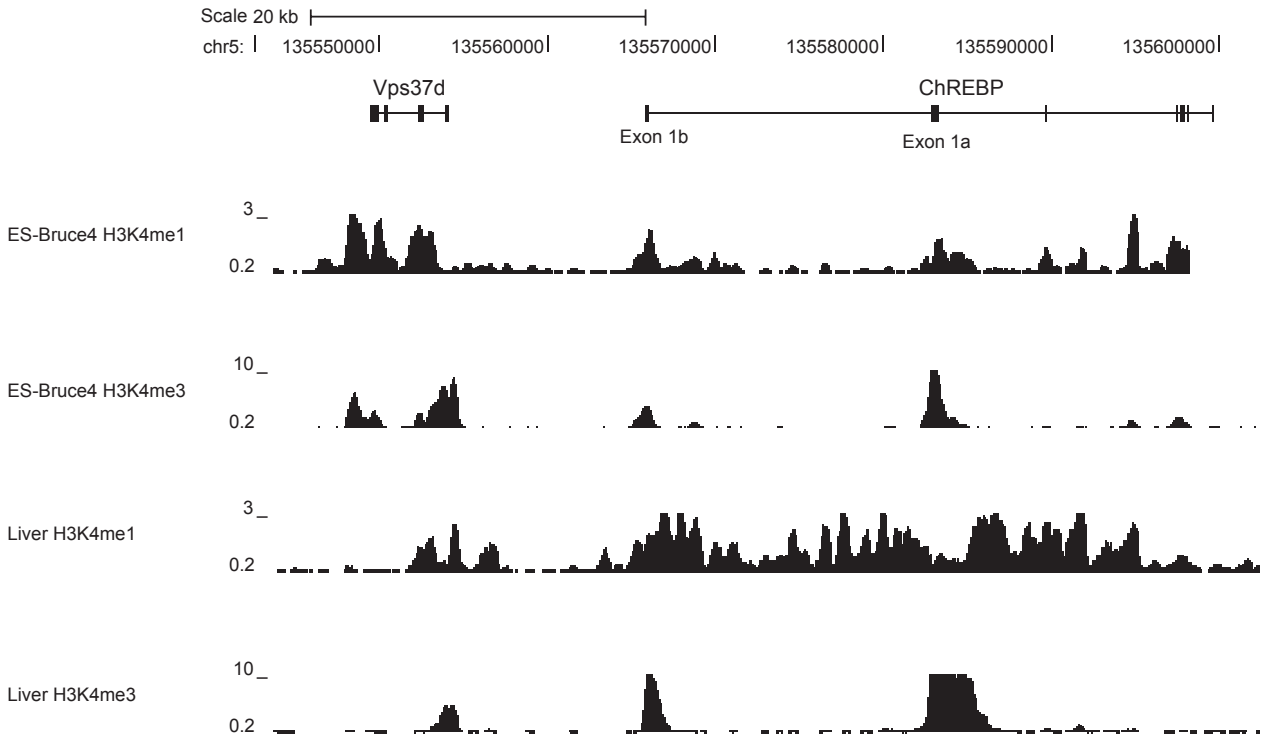


Supplementary Figure 16. Conservation of E-box and ChoRE in ChREBP Gene. Sequence is obtained from, and elements of the image are adapted, from http://genome.ucsc.edu[43,44].

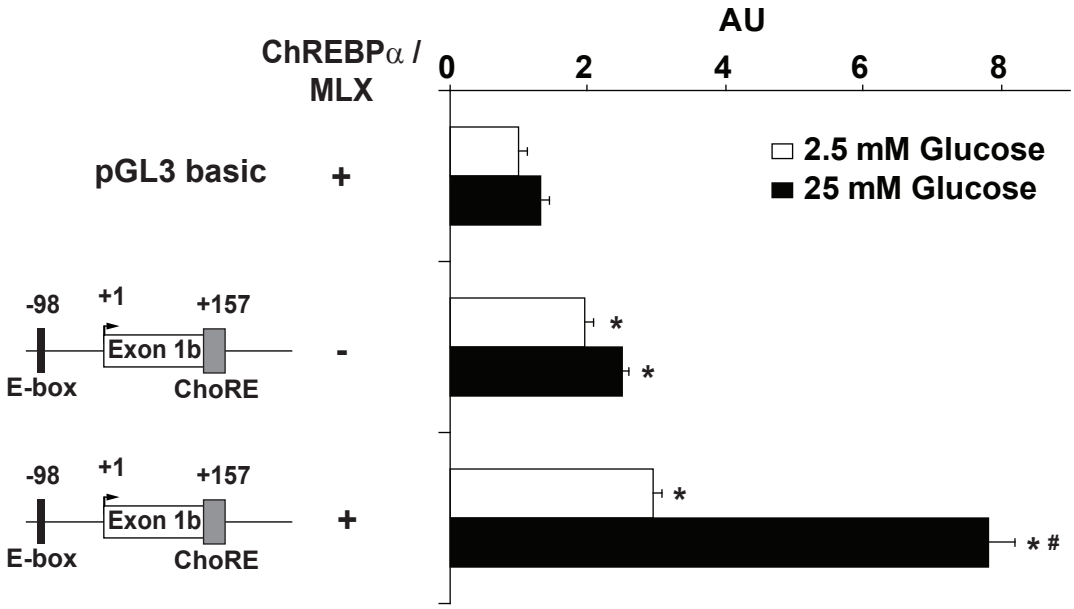


Supplementary Figure 17. Alignment of genomic sequence obtained from http://genome.ucsc.edu[43,44]. Alignment performed by Clustal W algorithm using Megalign (Dnastar) software. Sequence highlighted in blue corresponds to mouse exon 1b. Sequence highlighted in red corresponds to the upstream E-box. Sequence highlighted in yellow corresponds to the ChoRE. Sequence assemblies and coordinates are as follows:

Mouse: July 2007 (NCBI37/mm9) Assembly; Chr5(+):135565646-135565996.
Human: Feb. 2009 (GRCh37/hg19) Assembly; Chr7(-):73062038 -73062369.
Orangutan: July 2007 (WUGSC 2.0.2/ponAbe2) Assembly; Chr7(+):14424980 -14425329.
Dog: May 2005 (Broad/canFam2) Assembly; Chr6(+):9619418-9619774.
Horse: Sep. 2007 (Broad/equCab2) Assembly; Chr13(-):11282385 -11282740.
Opossum: Oct. 2006 (Broad/monDom5) Assembly; Chr2(-):530122703-530123068.

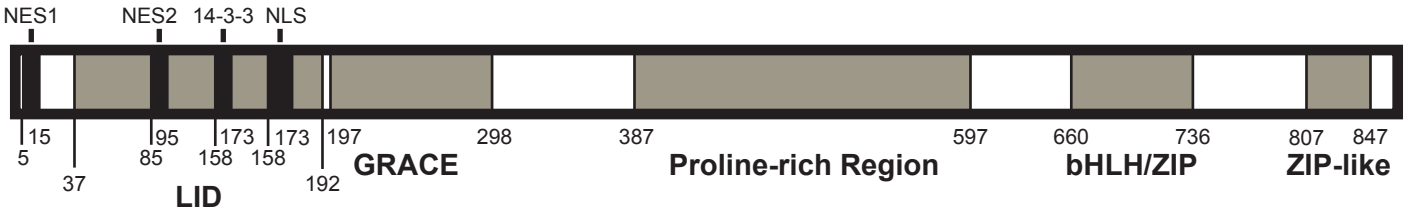


Supplementary Figure 18. Mono-methylation of lysine 4 of histone H3 (H3k4me1) and tri-methylation of lysine 4 of histone H3 (H3k4me3) is suggestive of the presence of an active promoter[45,46]. The peaks in the above graph indicate genomic regions with increased H3K4me1 or H3k4me3. Peaks align under ChREBP exon 1a which is the canonical transcriptional start site for ChREBP. H3k4me1 and H3k4me3 marks are also increased in the genomic region 17 kb 5' of the canonical ChREBP exon 1a in the Bruce4 embryonic stem cell line and in liver tissue suggesting the presence of an active promoter in this genomic region. ChREBP exon 1b aligns with this region. Image adapted from http://genome.ucsc.edu[43].



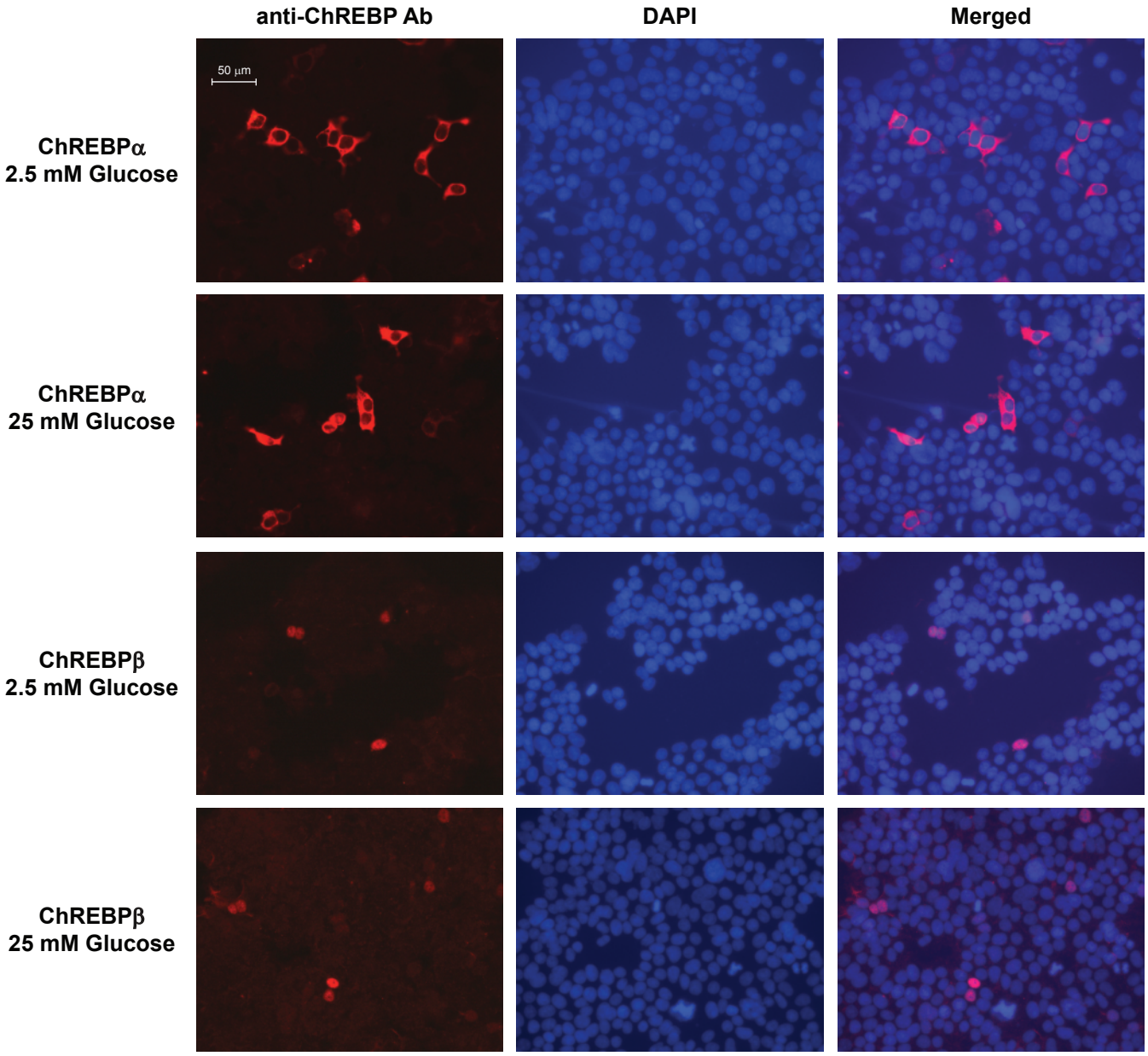
Supplementary Figure 19. Glucose-dependent expression of the exon 1b-promoter-luciferase construct requires co-transfection of ChREBPα and Mlx in HEK293T cells. (n=3/group). *P<0.05 compared to pGL3 basic in the same glucose condition. #P<0.05 compared to exon1b-luciferase construct in low glucose. This is representative of three independent experiments.

ChREBPα



ChREBPβ

Supplementary Figure 20. Mouse ChREBPα (864 amino acids) and ChREBPβ (687 amino acids) protein structure. NES1 and NES2: nuclear export signals 1 and 2. 14-3-3: binding site for 14-3-3 protein. NLS: nuclear localization sequence. LID: low glucose inhibitory domain. GRACE: glucose response conserved element. Proline-rich region. bHLH/ZIP: basic helix-loop-helix-leucine-zipper. ZIP-like: leucine zipper-like domain [the location of domains within the ChREBP protein adapted from and reviewed in Poupeau, A. and Postic, C. Biochimica et Biophysica Acta 1182, 995-1006 (2011)].



Supplementary Figure 21. ChREBPα is localized in the nucleus in low and high glucose conditions. In contrast, ChREBPβ is predominantly localized in the cytoplasm in low and high glucose conditions. The panels labeled anti-ChREBP Ab show ChREBPα or ChREBPβ localization in red. The panels labeled Dapi show nuclei in blue. The panels labeled Merged show the merged images of ChREBP and nuclear staining. HEK392T cells were transfected with Flag-tagged ChREBPα or ChREBPβ and co-transfected with Mlx overnight in DMEM + 10% FBS. Cells were subsequently incubated in DMEM with 2.5 mM glucose and 10% FBS. Cells were either maintained in 2.5 mM glucose or, after 4 hours, changed to 25 mM glucose. Cells were fixed in methanol after 2 hours (shown above) or 18 hours incubation (data not shown). ChREBP localization at both glucose concentrations was similar after 2 or 18 hours incubation. Immunofluorescence was performed using a rabbit polyclonal anti-ChREBP antibody (Novus Biologicals) and an Alexa-Fluor 594-conjugated goat anti-rabbit antibody (Invitrogen). Nuclei were stained with Dapi (Vector Systems). Similar findings were also obtained by Western blotting nuclear and extra-nuclear lysates after subcellular fraction of HEK293T cells transfected with ChREBPα or ChREBPβ (data not shown). Transfection with the same molar concentrations of ChREBPα and β plasmids results in markedly lower levels of ChREBPβ protein (data not shown).

a

NAME	SIZE	NES	NOM p-val	FDR q-val
FATTY_ACID_SYNTHESIS	8	-1.92	0.00	0.15
MALATEXPATHWAY	6	-1.80	0.00	0.25
MATRIX_METALLOPROTEINASES	19	-1.76	0.08	0.24
CYTOKINEPATHWAY	22	-1.70	0.03	0.25
ST_INTERLEUKIN_13_PATHWAY	8	-1.67	0.08	0.25
ST_IL_13_PATHWAY	8	-1.56	0.07	0.43
ROS	9	-1.52	0.08	0.47
MAP00480_GLUTATHIONE_METABOLISM	26	-1.48	0.10	0.53
KREBS-TCA_CYCLE	26	-1.46	0.00	0.51
GPCRS_CLASS_B_SECRETIN-LIKE	16	-1.43	0.10	0.55

b

PROBE	GENE SYMBOL	GENE NAME	RANK IN GENE LIST
93308_s_at	PCX	pyruvate carboxylase	12245
98575_at	FASN	fatty acid synthase	12140
162288_f_at	PCX	pyruvate carboxylase	12044
160207_at	ACLY	ATP citrate lyase	12038
100593_at	TNNT2	troponin T2, cardiac	11794
162077_f_at	SCD5	stearoyl-CoA desaturase 5	4857
95758_at	SCD5	stearoyl-CoA desaturase 5	4219
95485_at	HADH	hydroxyacyl-Coenzyme A dehydrogenase	3839

SupplementaryTable 1. Gene Set Enrichment Analysis (GSEA) in adipose tissue of AG4OX mice.

Total RNA from epididymal adipose tissue was extracted using the RNeasy Mini Kit from Qiagen from three mice from each of two genotypes: AG4OX and FVB littermate controls. RNA from each mouse was hybridized on an Affymetrix MG-U74-A.v2 Genechip microarray containing (12,421 probes). Genome-wide expression analysis of the microarray data was performed using gene set enrichment analysis (GSEA) software version 2.05 available from the Broad Institute (http://www.broadinstitute.org/gsea/downloads.jsp)[11] and gene set database version s2.mgu74av2.gmt containing 522 distinct gene sets.

In Supplemental Table 1a, the top ten up-regulated gene sets ranked by "NES" - normalized enrichment score - in AG4OX adipose tissue compared to controls are listed. "Name" indicates the name of the gene set as listed in the s2.mgu74av2.gmt database. "Size" refers to the number of genes in the gene set. "Nom p-val" and "FDR q-val" indicate the nominal p-values and false discovery rates for the individual sets, respectively, as calculated by the GSEA analysis software.

In Supplemental Table 1B, the Affymetrix Probe number, Gene Symbol, and Gene Name are listed for each gene included in the fatty acid synthesis gene set according to the s2.mgu74av2.gmt database. 'Rank in Gene List' refers to the position of the gene in the ranked list of all genes present in our expression dataset and ranked by signal to noise ratio. The GSEA algorithm calculates an enrichment score reflecting the degree to which the genes included in a gene set are overrepresented at the top or bottom of the ranked list of all genes present in the expression dataset.

	WT	AG4OX	ChREBP KO	AG4OX-ChREBP KO
Glucose (mg/dl)	**193 ± 9**	**148 ± 6 ***	**211 ± 9**	**205 ± 7 #**
Insulin (ng/ml)	**1.02 ± .13**	**0.82 ± 0.10**	**1.46 ± 0.21**	**1.26 ± 0.19 †**
Glucose * Insulin Product	**196 ± 24**	**124 ± 17 ‡**	**314 ± 51 §**	**261 ± 42 #**
Leptin (ng/ml)	**4.37 ± 1.17**	**2.82 ± 0.78**	**4.30 ± 1.14**	**2.35 ± 0.36**
NEFA (mM)	**0.66 ± 0.05**	**0.77 ± 0.05**	**0.56 ± 0.04**	**0.59 ± 0.03 #**
Triglyceride (mg/dl)	**62.9 ± 8.06**	**91.4 ± 10.73 £**	**50.2 ± 6.42**	**52.9 ± 5.47 #**

Supplementary Table 2. Serum metabolites in AG4OX mice crossbred with ChREBP KO mice on chow diet. Serum was collected from 6-month-old female mice in the fed state at 9 AM. Values are means ± SE. n = 9-10 per group. Statistical comparisons were performed by ANOVA with pairwise comparisons by Tukey's test. * $P<0.05$ comparison between different AG4OX genotypes with same ChREBP genotype; # $p < 0.05$ comparison between different ChREBP genotypes with same AG4OX genotype. † $P=0.15$ compared to AG4OX; ‡ $P=0.12$ compared to WT; § $P=0.20$ compared to WT; £ $P=0.07$ compared to WT.

	WT-Chow	WT-HFD	AG4OX-Chow	AG4OX-HFD
Glucose (mg/dl)	**225 ± 7**	**253 ± 7 ***	**159 ± 6 #**	**215 ± 11 *#**
Insulin (ng/ml)	**1.65 ± 0.12**	**2.96 ± 0.28 ***	**1.14 ± 0.17 †**	**3.27 ± 0.56 ***
Glucose * Insulin Product	**376 ± 28**	**682 ± 75 ***	**248 ± 51 #**	**755 ± 169 ***
Leptin (ng/ml)	**4.86 ± 1.17**	**13.01 ± 0.78 ***	**3.41 ± 1.14**	**5.73 ± 0.36 *#**
NEFA (mM)	**0.77 ± 0.03**	**0.77 ± 0.04**	**1.14 ± 0.05 #**	**0.98 ± 0.06 *#**
Triglyceride (mg/dl)	**196 ± 12**	**137 ± 11 ***	**242 ± 13 #**	**190 ± 11 *#**

Supplementary Table 3. Fed serum metabolites in WT vs AG4OX mice on chow vs HFD. Serum was collected from 4-month-old male mice in the fed state from 9 AM to 11 AM. Values are means ± SE. n = 11-17 per group. Statistical comparisons were performed by ANOVA with pairwise comparisons by Tukey's test. * $P<0.05$ comparison within genotype (chow compared to HFD); # $P<0.05$ comparison within diet (WT compared to AG4OX); † $P=0.054$ compared to WT-chow.

Anthropometric characteristics of cross-sectional study subjects (Fig. 3f-g)	
Number (men/women)	123 (64/59)
Age (years)	57 ± 15
BMI (kg/m^2)	29.8 ± 6.5
% Body Fat	31 ± 10

Anthropometric characteristics of obese case-control subjects (Fig. 3h-i and Fig. 5b)	
Number (men/women)	38 (28/10)
Age (years)	41 ± 11
BMI (kg/m^2)	36.6 ± 4.6
% Body Fat	41 ± 9

Supplementary Table 4. Anthropometric characteristics of human study subjects. All values are Means ± SD

Model Summary[b]

R	R Square	Adjusted R Square	Std. Error of the Estimate
.554[a]	.307	.259	80.905

a. Predictors: (Constant), Log AT ChREBP Beta (AU), Log AT ChREBP Alpha (AU)

b. Dependent Variable: Glucose Rd % stim

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
	Regression	84139.554	2	42069.777	6.427	.005[a]
	Residual	189821.946	29	6545.584		
	Total	273961.500	31			

a. Predictors: (Constant), Log AT ChREBP Beta (AU), Log AT ChREBP Alpha (AU)

b. Dependent Variable: Glucose Rd % stim

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations		
		B	Std. Error	Beta			Zero-order	Partial	Part
	(Constant)	609.126	116.106		5.246	.000			
	Log AT ChREBP Alpha (AU)	102.333	109.030	.166	.939	.356	.388	.172	.145
	Log AT ChREBP Beta (AU)	80.457	31.422	.454	2.560	.016	.535	.429	.396

a. Dependent Variable: Glucose Rd % stim

Supplementary Table 5. Linear regression analysis for ChREBPα and ChREBPβ as predictors of insulin sensitivity as measured by insulin stimulated glucose uptake (% over basal) in 32 obese non-diabetic individuals.

Murine

Gene Name	Gene Symbol	Abbreviation in Text and Figures	Forward Primer	Reverse Primer
18s ribosomal RNA	18s	18s	TTGACTCAACACGGGAAACC	AGACAAATCGCTCCACCAAC
acetyl-Coenzyme A carboxylase alpha	Acaca	ACC1	TGTACAAGCAGTGTGGGCTGGCT	CCACATGGCCTGGCTTGGAGGG
ATP citrate lyase	Acly	ACL	GCCAGCGGGAGCACATC	CTTTGCAGGTGCCACTTCATC
MLX interacting protein-like	Mlxipl	Total ChREBP	CACTCAGGGAATACACGCCTAC	ATCTTGGTCTTAGGGTCTTCAGG
		ChREBPα	CGACACTCACCCACCTCTTC	TTGTTCAGCCGGATCTTGTC
		ChREBPβ	TCTGCAGATCGCGTGGAG	CTTGTCCCGGCATAGCAAC
ELOVL family member 6, elongation of long chain fatty acids	Elovl6	Elovl6	TCAGCAAAGCACCCGAAC	AGCGACCATGTCTTTGTAGGAG
fatty acid synthase	Fasn	FAS	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT
nuclear receptor subfamily 1, group H, member 3	Nr1h3	LXRα	AGGAGTGTCGACTTCGCAAA	CTCTTCTTGCCGCTTCAGTTT
nuclear receptor subfamily 1, group H, member 2	Nr1h2	LXRβ	ATAGTGGGTCACGAAGCAGC	AGGGCAACAGAGTCGGAGAC
malate dehydrogenase 1, NAD (soluble)	Mdh1	MDH1	AAGGCATGGAGAGGAAGGAC	AGTTCGTATTGGCTGGGTTTC
malic enzyme 1, NADP(+)-dependent, cytosolic	Me1	ME1	ATCACTTTGGATGTGGGAACAG	CAGGAAGGCGTCATACTCAGG
MAX-like protein X	Mlx	MLX	GGAGCTCTCAGCTTGTGTCTTCA	CACCGATCACAATCTCTCGTAGAGT
pyruvate carboxylase	Pcx	PCx	GGAGCTTATCCCGAACATCC	CGGAAGACGTCCATACCATTC
stearoyl-Coenzyme A desaturase 1	Scd1	SCD1	CCCTGCGGATCTTCCTTATC	TGTGTTTCTGAGAACTTGTGGTG
sterol regulatory element binding transcription factor 1 (isoform c)	Srebpf1	SREBP1c	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
TATA box binding protein	Tbp	Tbp	CCCTATCACTCCTGCCACAC	ACGAAGTGCAATGGTCTTTAGG

Human

Gene Name	Gene Symbol	Abbreviation in Text and Figures	Forward Primer	Reverse Primer
MLX interacting protein-like	Mlxipl	ChREBPα	AGTGCTTGAGCCTGGCCTAC	TTGTTCAGGCGGATCTTGTC
		ChREBPβ	AGCGGATTCCAGGTGAGG	TTGTTCAGGCGGATCTTGTC

Supplementary Table 6. Gene names, symbols, abbreviations and qPCR primer sequences.