

# Product Datasheet

## CHREBP Antibody - BSA Free

### NB400-135

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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**NB400-135**

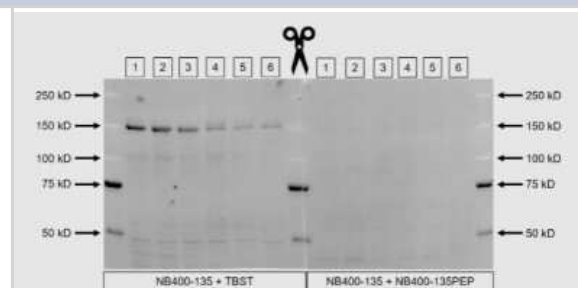
CHREBP Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	95 kDa
Product Description	
Description	Novus Biologicals Knockout (KO) Validated Rabbit CHREBP Antibody - BSA Free (NB400-135) is a polyclonal antibody validated for use in IHC, WB, ICC/IF, IP and ChIP. Anti-CHREBP Antibody: Cited in 157 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	51085
Gene Symbol	MLXIPL
Species	Human, Mouse, Rat
Reactivity Notes	Use in Mouse reported in scientific literature (PMID: 33812059).
Immunogen	A C-terminal synthetic peptide made to the human CHREBP protein sequence (between residues 800-852). [UniProt# Q9NP71, Isoform 1/Alpha]
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Chromatin Immunoprecipitation, Gel Super Shift Assays, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation, SDS-Page, Chromatin Immunoprecipitation (ChIP), Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1:1000, Chromatin Immunoprecipitation 1:10 - 1:500. Use reported in scientific literature (PMID 21282101), Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:100 - 1:500, Immunoprecipitation 1:10 - 1:500, Immunohistochemistry-Paraffin 1:100, Immunoblotting reported in scientific literature (PMID 26181104), Gel Super Shift Assays reported in scientific literature (PMID 20025850), SDS-Page reported in scientific literature (PMID 35041621), Chromatin Immunoprecipitation (ChIP) 1:10-1:500, Knockout Validated reported in scientific literature (PMID 31668386), Knockdown Validated

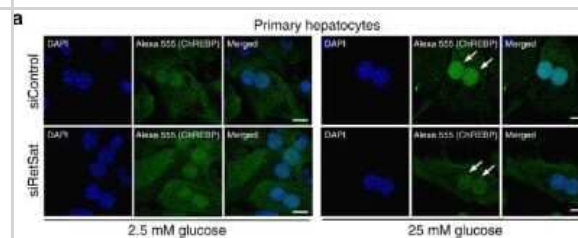


## Images

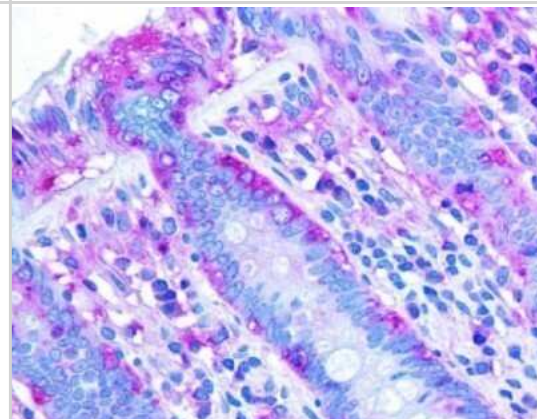
**Western Blot: CHREBP Antibody - BSA Free [NB400-135] - CHREBP Antibody [NB400-135] - Mouse liver tissue lysate, 10 ug total protein per lane, probed with CHREBP antibody. Specific bands at ~150 kDa (some wells as doublets). Weaker bands at ~45 kDa, very faint bands at ~100 kDa. These bands were absent after exposure to ChREBP blocking peptide, although some faint non-specific banding remained at ~90, ~60, and ~40 kDa. WB image submitted by a verified customer review.**



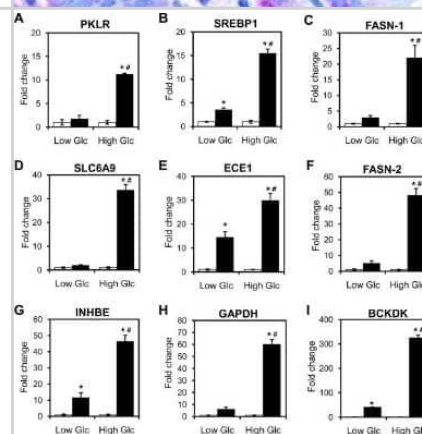
**Immunocytochemistry/Immunofluorescence: CHREBP Antibody - BSA Free [NB400-135] - CHREBP Antibody [NB400-135] - RetSat depletion prevents the glucose-induced nuclear accumulation of ChREBP independent of 13,14-dihydroretinol generation. Hepatocytes were treated with Control or RetSat siRNA overnight. The next morning, cells were incubated with vehicle (DMSO) or 1  $\mu$ M 13,14-dhretinol for 24 h at the indicated glucose concentrations and mRNA expression of Txnip determined by qPCR. Data are shown as mean  $\pm$  s.d., n = 4. Two-way ANOVA with Bonferroni post test revealed significances between low and high glucose (#P < 0.05) and between siControl and siRetSat (\*P < 0.05), treatment with 13,14-dhretinol had no effect. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41467-017-00430-w>), licensed under a CC-BY license.**



**Immunohistochemistry-Paraffin: CHREBP Antibody - BSA Free [NB400-135] - CHREBP Antibody [NB400-135] - Staining of human colon, epithelium at a 40X magnification.**



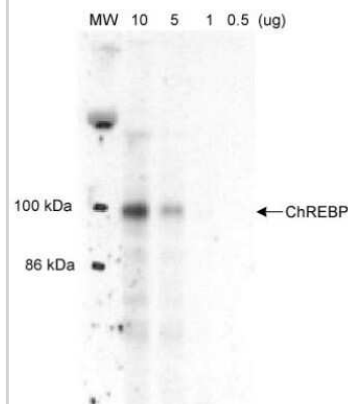
**Chromatin Immunoprecipitation: CHREBP Antibody - BSA Free [NB400-135] - CHREBP Antibody [NB400-135] - Effects of glucose on ChREBP binding. HepG2 cells were treated with low (2.7 mM) and high (25 mM) glucose for 8 h. Chromatin was isolated and fragmented, and ChIP was performed with control IgG or anti-ChREBP antibody. Validated primers for each gene were used for quantitative real-time PCR. The data presented as fold increase for the signal from anti-ChREBP relative to control IgG. The negative control, Cyclo, showed no enrichment (data not shown). Values represent the mean  $\pm$  S.D. of three independent samples. \*p<0.005 vs. IgG, #p<0.0001 vs. 2.7 mM glucose with anti-ChREBP. Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0022544>), licensed under a CC-BY license.**



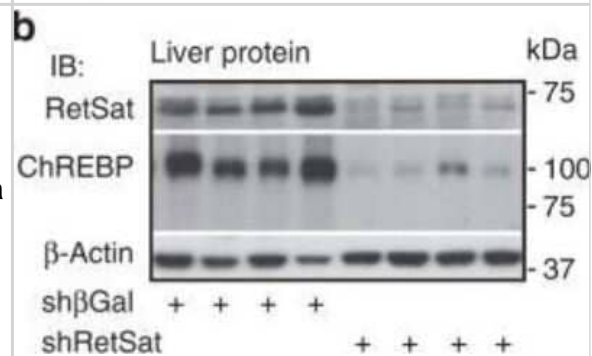
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - CHREBP Antibody [NB400-135] - Detection of ChREBP in 20 ug of human hepatocyte lysate using NB400-135. 5-10 second film exposure.



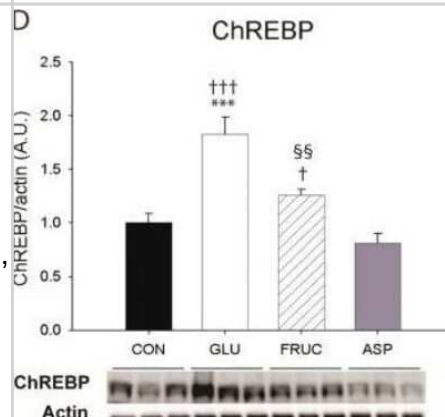
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - CHREBP Antibody [NB400-135] - Detection of ChREBP in liver nuclear extracts from well-fed rats. 7% SDS-PAGE gel, 1:1000 dilution of NB400-135. Photo courtesy of Dr. Uyeda, UT Southwestern University.



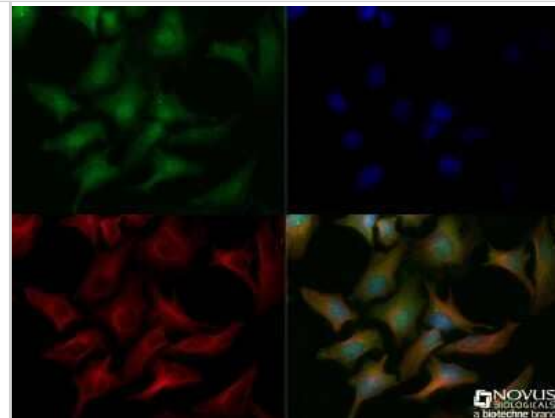
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - CHREBP Antibody [NB400-135] - RetSat depletion in mouse liver reduces protein levels and target gene expression of ChREBP. RetSat and ChREBP protein expression by immunoblotting. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41467-017-00430-w>), licensed under a CC-BY license.



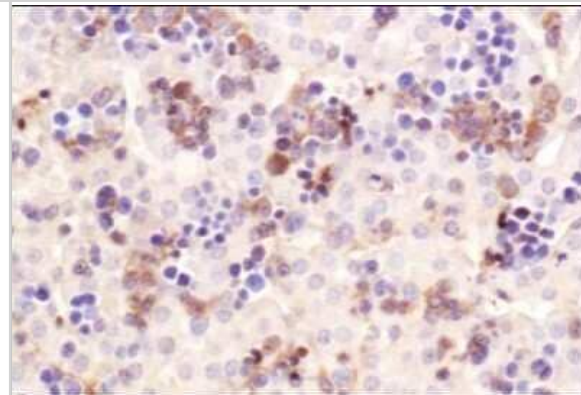
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - CHREBP Antibody [NB400-135] - Protein expression levels of carbohydrate-responsive element-binding protein (ChREBP), in livers of rats receiving normal water (CON), a 13% (w/v) glucose solution (GLU), a 13% (w/v) fructose solution (FRUC), or a 0.4% (w/v) aspartame solution (n = 6 per diet group). All data were normalized to beta-actin expression levels and are expressed relative to the controls (CON). Data are expressed as means  $\pm$  SEM. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 vs. CON; +p < 0.05, ++p < 0.01, +++p < 0.001 vs. ASP; p < 0.01, p < 0.001 vs. GLU. Image collected and cropped by CiteAb from the following publication (<https://www.mdpi.com/2072-6643/9/5/476>), licensed under a CC-BY license.



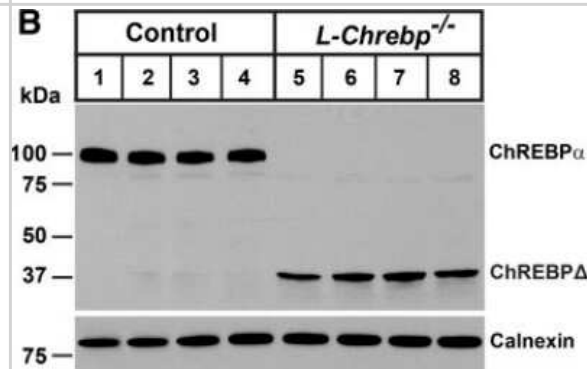
**Immunocytochemistry/Immunofluorescence: CHREBP Antibody - BSA Free [NB400-135] - CHREBP Antibody [NB400-135] - HeLa cells were fixed for 10 minutes using 10% formalin and permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-ChREBP (NB400-135) at a 1:200 dilution overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Alpha tubulin was used as a co-stained at 1:1000 and detected with an anti-mouse DyLight 550 (Red) at a 1:500. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.**



**Immunohistochemistry: CHREBP Antibody - BSA Free [NB400-135] - CHREBP Antibody [NB400-135] - Analysis of CHREBP in mouse liver using DAB with hematoxylin counterstain.**



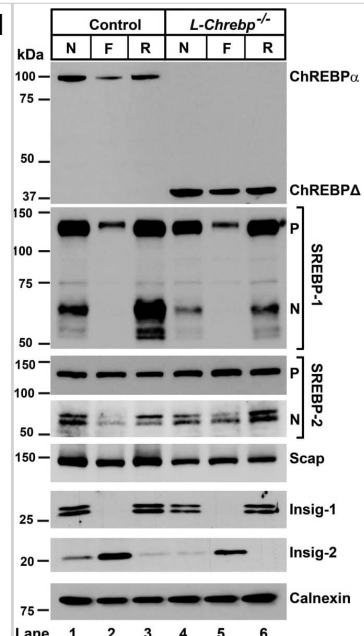
**Western Blot: CHREBP Antibody - BSA Free [NB400-135] - Immunoblot analysis of ChREBP in liver lysates of control and L-Chrebp -/- mice. Aliquots (60 ug of protein) of liver whole-cell lysates were subjected to SDS-PAGE and immunoblot analysis with anti-ChREBP and anti-calnexin antibodies. ChREBP delta denotes a truncated aberrant ChREBP protein present only in lysates prepared from L-Chrebp -/- livers. Image collected and cropped by CiteAb from the following publication (<http://pubmed.ncbi.nlm.nih.gov/29335275/>) licensed under a CC-BY license.**



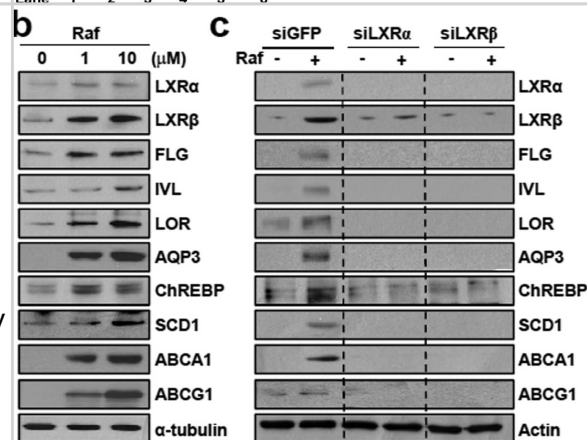
**Analysis of protein levels in livers transfected with GK and GKA456V. Livers excised from mice in postabsorptive state were homogenized and resolved by Western blot. (a, b) Representative blots from three independent experiments. The densitometric analysis is presented (N = 4, \*P < 0.05 versus pControl, \*\*\*P < 0.001 versus pGKA456V). (c) Liver sections of 5-hour fasted mice injected with pControl, pGK, or pGKA456V were immunostained with Glc6Pase antibody. TO-PRO-3 was used to visualize nuclei.**



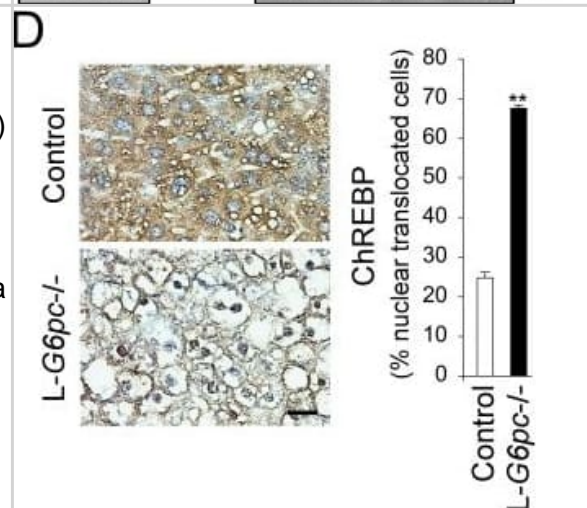
Immunoblot analysis of livers from control & L-Chrebp<sup>-/-</sup> mice subjected to fasting & refeeding with a high-sucrose diet. Littermate control & L-Chrebp<sup>-/-</sup> mice (same as those described in supplemental Tables S2A & S2B) were subjected to fasting & refeeding. The nonfasted (N) groups were fed chow diet ad libitum. The fasted (F) group was fasted 12 h, & the refeed (R) group was fasted for 12 h & then refeed with 60% (w/w) high-sucrose diet for 12 h prior to study. Liver whole-cell lysates & membrane fractions were prepared individually, & equal amounts of protein from each mouse of the same group (four per group) were pooled. Aliquots (40 µg for whole-cell lysates & 30 µg for membrane fractions) of the pooled protein were subjected to SDS-PAGE & immunoblot analysis. Immunoblot analysis of Insig-1 & Insig-2 were carried out using membrane fractions. Whole-cell lysates were used to detect other proteins. The precursor & nuclear forms of SREBPs are denoted as P & N, respectively. Calnexin was used as loading control. Image collected & cropped by CiteAb from the following publication (<https://linkinghub.elsevier.com/retrieve/pii/S0022227520331369>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



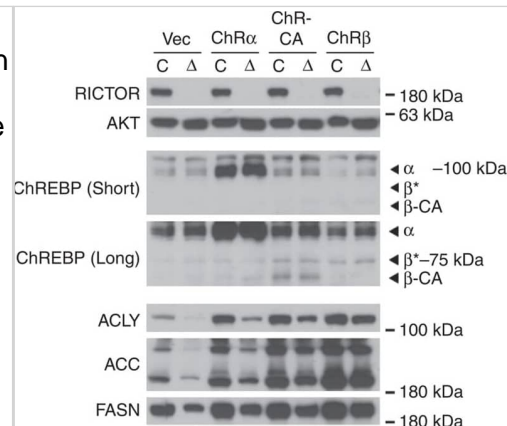
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - Raffinose stimulates transcription of genes involved in epidermal barrier function in HaCaT cells. (a & b) HaCaT cells were treated with vehicle, 1 µM or 10 µM raffinose (Raf), or 1 µM TO901317 (T17) for 24 h. Expressions of transcripts (a) & proteins (b) were analyzed by qRT-PCR or western blotting, respectively. FLG; filaggrin. IVL; involucrin, LOR; loricrin, AQP3; aquaporin3. (c) HaCaT cells were transfected with siGFP control, siLXRα, or siLXRβ, & then treated with 1 µM raffinose for 24 h. Expression of proteins was analyzed by western blotting. The original blots are shown in Supplementary Fig. S9. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28266648>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



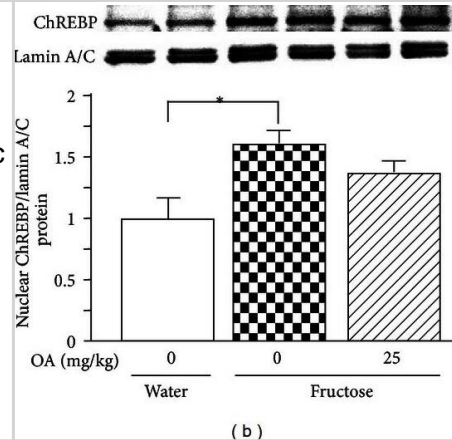
Immunohistochemistry-Paraffin: CHREBP Antibody - BSA Free [NB400-135] - Impaired hepatic SIRT1-FoxO signaling in L-G6pc<sup>-/-</sup> mice. (A) Western blots & densitometry analysis (n = 5), & quantification of mRNA for hepatic SIRT1 & FoxO3a (n = 8). (B) Hepatic NAD<sup>+</sup> levels (n = 9). (C) Western blots & densitometry analysis of PPAR-γ, PPAR-α & β-actin (n = 5). (D) Immunohistochemical analysis of hepatic ChREBP & quantification of nuclear ChREBP-translocated cells (n = 4). Scale bar, 25 µm. (E) Quantification of mRNA for hepatic Acaca, Fasn & Elovl6 by real-time RT-PCR (n = 6). (F) Western blots of acetylated & total FoxO3a after immunoprecipitation of nuclear extracts using anti-FoxO3a, & quantification of the acetylated FoxO3a/total FoxO3a (n = 5). Data represent the mean ± SEM. \*P < 0.05, \*\*P < 0.005. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28558013>), licensed under a CC0-1.0 license. Not internally tested by Novus Biologicals.



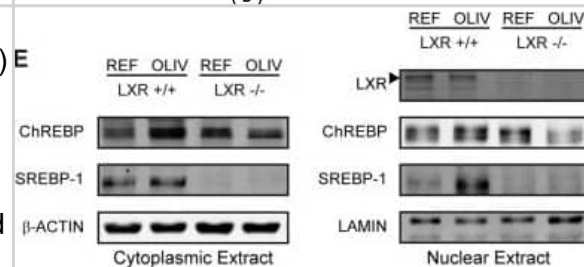
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - Expressing recombinant ChREBP $\beta$  in Rictor-deficient adipocytes rescues expression of DNL enzymes. Western blot of indicated proteins in differentiated adipocytes with or without Rictor deletion transfected with various rescue constructs that were stably expressed in cells before differentiation. <sup>\*\*</sup> indicates ChREBP $\beta$  based on molecular weight. Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/ncomms11365>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



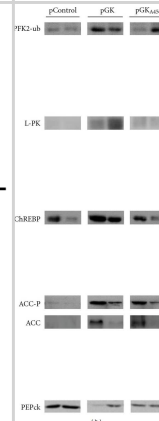
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - Hepatic expression of mRNAs encoding carbohydrate response element-binding protein (ChREBP) (a), liver pyruvate kinase (LPK) (c), & microsomal triglyceride transfer protein (MTTP) (d), & nuclear ChREBP protein (b) in water-control, 10% fructose solution-control, & fructose pair-fed oleonic acid- (OA-) treated rats at week 10. Animals were administered with OA (25 mg/kg/day) or vehicle (OA: 0 mg/kg, 5% Gum Arabic) by oral gavage daily for 10 weeks. mRNA was determined by real-time PCR. Protein expression was determined by Western blot. Data are means  $\pm$  SEM (n = 6 each group). \*P < 0.05. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/23737835>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



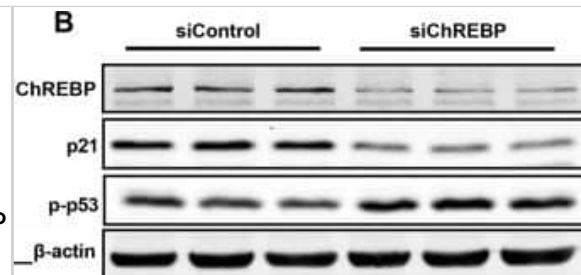
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - LXR mediate the induction of lipogenesis induced by an oleic acid-rich diet. (A) Hepatic *Acy*, *Acaca*, *Acacb*, *Fasn*, *Elovl6*, *Scd1* mRNA levels quantified by qPCR. (B) Cytoplasmic protein expression levels of P-ACLY, ACLY, ACC, ELOVL6, SCD1, FASN &  $\beta$ -ACTIN assayed by Western Blotting. (C) *Fads1*, *Fads2*, *Elovl5*, *Gpat*, *Pnpla3* & *Lpk* mRNA quantification assayed by qPCR. (D) *Srebp-1c* & *Chrebp* mRNA quantification assayed by qPCR. (E) Cytoplasmic & nuclear expression levels of LXR, SREBP-1c & ChREBP assayed by Western Blotting. Data are the mean  $\pm$  SEM of values measured in LXR+/+ & LXR-/- mice fed REF or OLIV diet. a Significant genotype effect. b Significant difference versus REF diet (n = 6 mice per group). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28732092>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



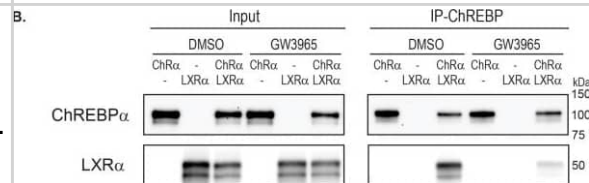
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - Analysis of protein levels in livers transfected with GK & GKA456V. Livers excised from mice in postabsorptive state were homogenized & resolved by Western blot. (a, b) Representative blots from three independent experiments. The densitometric analysis is presented (N = 4, \*P < 0.05 versus pControl, \*\*\*P < 0.001 versus pGKA456V). (c) Liver sections of 5-hour fasted mice injected with pControl, pGK, or pGKA456V were immunostained with Glc6Pase antibody. TO-PRO-3 was used to visualize nuclei. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/22194744>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



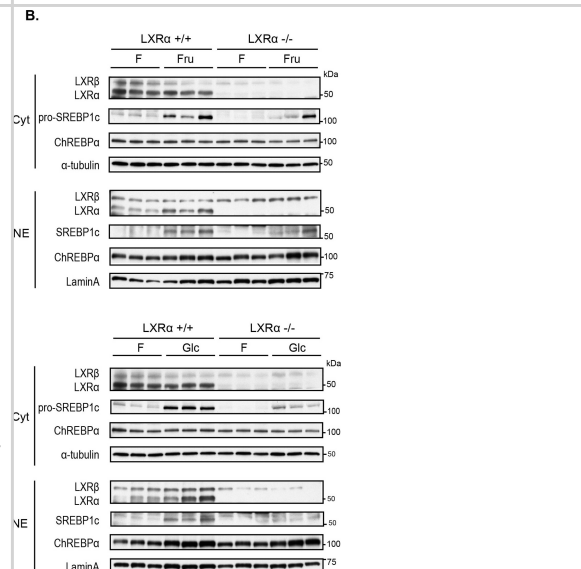
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - ChREBP knockdown inhibited glycolysis, lipogenesis & p21 in HT29 cells. (A) Inhibited relative mRNA expression of ChREBP  $\alpha$  & ChREBP  $\beta$  normalized to B2M after control or ChREBP siRNA transfection for 48 hours. (B) Western-blot of ChREBP, phospho-p53 & p21 & their quantification on the right.  $\beta$ -actin was served as a loading control. Proteins were extracted from cells transfected with sicontrol & siChREBP after 48 hours. The quantification of western blot was normalized to  $\beta$ -actin. (C) Decreased relative mRNA expression of glycolytic & lipogenic genes, normalized to B2M. (D) Relative mRNA expression of p53 & p21, normalized to B2M. (E) Relative mRNA expression of cell Cyclins, normalized to B2M.  $n = 4$ . \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32144313>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



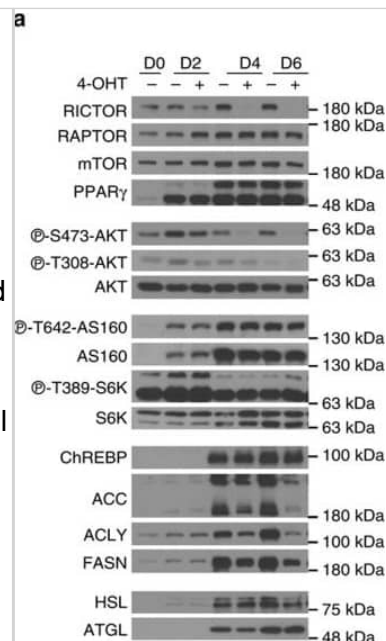
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - Ligand-activated LXR reduces ChREBP binding to chromatin. (A). Left panels: Local pattern of LXR-ChREBP co-occupancy. Browser view of LXR & ChREBP tracks in the promoter region of Lpk (Pklr), Txnip, Fasn & Scd1. Square brackets indicate the scale maxima of ChIP/input ratios. Arrows indicate the genomic locations of quantitative RT-PCR primers. Right panel: AML12 cells transfected with ChREBP $\alpha$ /Mlx $\gamma$  & LXR $\alpha$ /RXR $\alpha$  were treated with DMSO (0.1%) or GW3965 (10  $\mu$ M) for 18 h. ChREBP or LXR binding to genomic location indicated in the right panels were detected by ChIP using antibodies against ChREBP, LXR or IgG as negative control. Data are presented as mean  $\pm$  SEM ( $n = 3-5$ ). Significant differences are shown as \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  compared to ChIP-IgG & # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  between DMSO & GW3965 groups. ns, not significant. (B). CoIP of LXR $\alpha$  & ChREBP $\alpha$ , expressed in COS-1 cells cultured in 25 mM glucose, treated with DMSO (0.1%) or GW3965 (1  $\mu$ M) for 18 h. Lysates were immunoprecipitated with ChREBP antibody ( $n = 3$ ). Input & immunoprecipitated proteins were immunoblotted with ChREBP or LXR $\alpha$  antibodies. One representative western blot is shown. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32414201>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



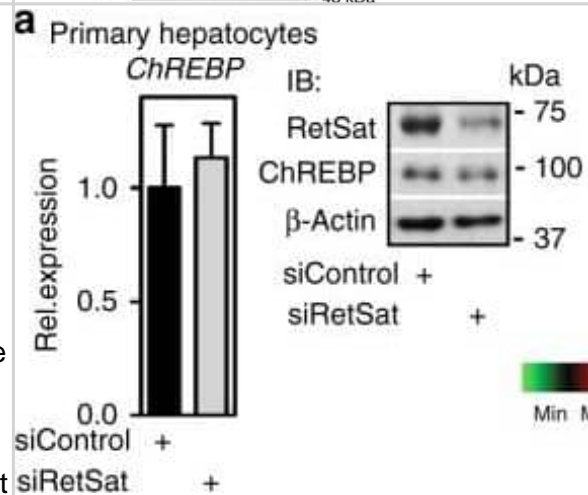
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - Induction of hepatic Srebp-1 & carbohydrate response element-binding protein (Chrebp) $\beta$  expression by dietary glucose is reduced in LXR $\alpha$  $^{-/-}$  mice. (A) Hepatic gene expression of Lxr $\alpha$ / $\beta$ , Srebp-1, & Chrebp $\alpha$ / $\beta$  was analyzed by quantitative RT-PCR & normalized to Tbp; (B) Cytosolic & nuclear lysates were immunoblotted with antibodies against LXR, SREBP-1, & ChREBP with  $\alpha$ -Tubulin & Lamin A as loading controls. Each lane represents independent mice from each group. One representative western blot is shown ( $n = 3$ ). Data represent the mean  $\pm$  SEM ( $n = 5$ ). Significant differences were found using two-way ANOVA followed by Tukey's multiple comparison test (fasted vs. fructose fed & fasted vs. glucose fed RNA data were analyzed separately). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  compared to fasted. # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  compared to LXR $\alpha$  $^{+/+}$  mice. Image collected & cropped by CiteAb from the following publication (<http://www.mdpi.com/2072-6643/9/7/678>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



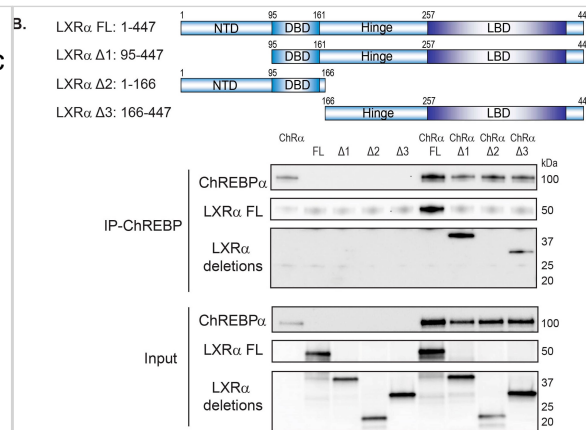
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - Decreased glucose uptake & ChREBP-driven DNL is a primary consequence of Rictor loss in adipocytes. (a) Western blots of indicated proteins at different days of differentiation using Rictor iKO primary adipocytes (described in Methods). (b) Oil Red O staining of differentiated adipocytes. (c) The relative mRNA level of ChREBP in differentiated cells at various time points.  $n=3$ . (d) 2-DG uptake in differentiated adipocytes without or with insulin stimulation.  $n=3$ . (e) The C14-glucose-derived FA in differentiated adipocytes.  $n=3$ . (f) The C14-glucose derived triglyceride (TAG) in differentiated adipocytes.  $n=3$ . (g) Relative GLUT4 expression in differentiated cells at various time points.  $n=3$ . (h) Relative GLUT4 expression in sWAT.  $n=8$ . (i) Glycerol release in differentiated adipocytes under basal & isoproterenol (Iso) stimulation.  $n=4$ . (j) Glycerol release in ex vivo pgWAT under basal & isoproterenol stimulation.  $n=6$ . Data were analysed by Student's t-test. Values are expressed as mean  $\pm$  s.e.m. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/ncomms11365>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



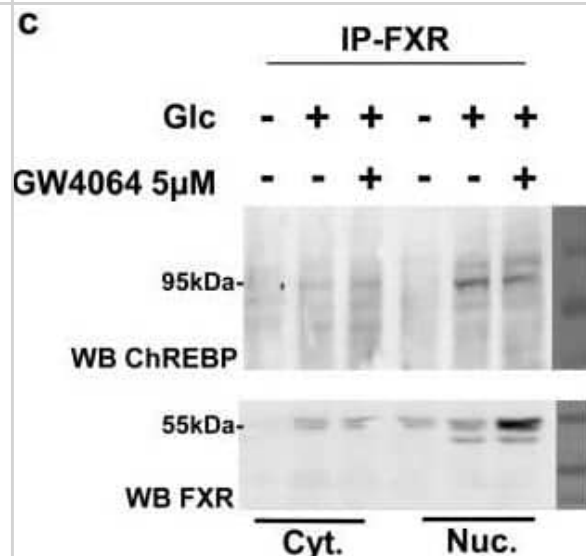
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - RetSat controls ChREBP activity & glucose sensing in primary hepatocytes. Primary mouse hepatocytes were treated with Control or RetSat siRNA for 48 h, (a, left) ChREBP mRNA expression determined by qPCR, & RetSat & ChREBP protein levels determined by immunoblotting (a, right). left, Data are shown as mean  $\pm$  1 s.d.,  $n = 3$  independent transfections of hepatocyte cultures from the same mouse. Two independent experiments yielded similar results. b Hepatocytes were treated as described in a & mRNA expression of a selection of known ChREBP target genes visualized in a heatmap. c Primary hepatocytes were depleted of RetSat using two siRNA's targeting different sites of the RetSat transcript for 48 h, & expression of the indicated genes analyzed by qPCR. Data are shown as mean  $\pm$  s.d.,  $n = 6$  independent transfections of hepatocyte cultures from two different mice; \* $P < 0.05$  between siControl und siRetSat by one-way ANOVA with Bonferroni post test. An independent experiment yielded similar results. d Hepatocytes treated with Control or RetSat siRNA were transfected with a ChoRE-Luc reporter, exposed to low & high glucose concentrations as indicated, & analyzed for luciferase activity. e Hepatocytes treated with Control or RetSat siRNA were exposed to low & high glucose concentrations as indicated, & mRNA expression determined by qPCR. In d, e, data are shown as mean  $\pm$  s.d.,  $n = 6$  independent transfections of hepatocyte cultures from two mice; two-way ANOVA with Bonferroni post test revealed significances between low & high glucose concentrations (# $P < 0.05$ ) & between siControl & siRetSat (\* $P < 0.05$ ). An independent experiment yielded similar results Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41467-017-00430-w>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



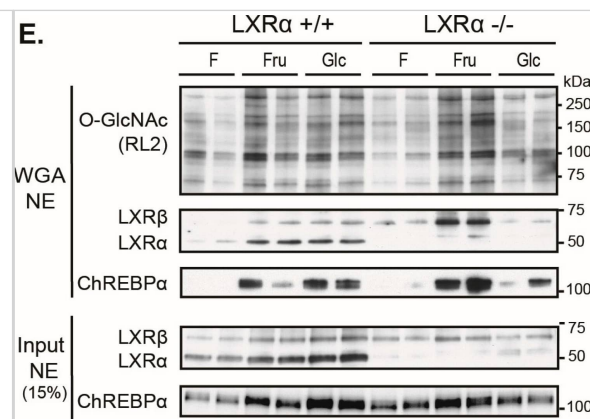
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - LXR $\alpha$  & ChREBP $\alpha$  interact via key activation domains. (A). Top panel: Schematic representation of the ChREBP $\alpha$  full-length (FL), ChREBP $\beta$  & the low glucose inhibitory domain (LID) protein. Bottom panel: CoIP of LXR $\alpha$  & ChREBP $\alpha$ , ChREBP $\beta$  or LID, expressed in COS-1 cells cultured in 25 mM glucose. The ChREBP expression plasmids were transfected with a DNA ratio of ChREBP $\alpha$ :ChREBP $\beta$ :LID = 1:6:1, to obtain comparable protein levels. Lysates were immunoprecipitated with ChREBP, FLAG (for LID) or LXR $\alpha$  antibodies & input & immunoprecipitated proteins immunoblotted with the same antibodies (n = 3). One representative western blot is shown. LID, low-glucose inhibitory domain; GRACE, glucose-response activation conserved element; bHLH, basic helix-loop-helix domain; ZIP, leucine zipper. (B). Top panel: Schematic representation of the LXR $\alpha$  FL & truncations. Bottom panel: CoIP of ChREBP $\alpha$  & LXR $\alpha$  FL or truncations expressed in COS-1 cells cultured in 25 mM glucose. Lysates were immunoprecipitated with ChREBP antibody (n = 3). Input & immunoprecipitated proteins were immunoblotted with ChREBP or FLAG (for LXR $\alpha$  FL & truncations) antibodies. One representative western blot is shown. NTD, N-terminal domain; DBD, DNA-binding domain; LBD, ligand-binding domain. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32414201>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



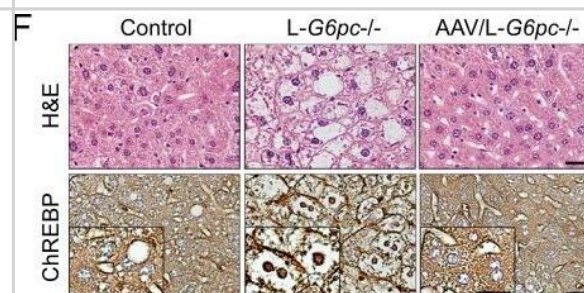
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - FXR inhibits glucose-induced proglucagon expression (a) Proglucagon qPCR on cDNA from GLUTag cells starved for 12h with lactate (10 mmol L<sup>-1</sup>) & then incubated for 24h in lactate (10 mmol L<sup>-1</sup>), glucose (5.6 mmol L<sup>-1</sup>) or 2-deoxyglucose (5.6 mmol L<sup>-1</sup>) media containing DMSO, GW4064 (5  $\mu$ mol L<sup>-1</sup>) or CDCA (100  $\mu$ mol L<sup>-1</sup>) (n=3; performed 3 times). Data are represented as mean  $\pm$  SD. Two-Way ANOVA analysis followed by Bonferroni's posthoc test. \*\*\*P $\leq$ 0.001: effect of GW4064 & CDCA on proglucagon mRNA levels in each medium conditions. §§§P $\leq$ 0.001: effect of glucose on proglucagon mRNA levels in DMSO, GW4064 & CDCA conditions. (b) ChREBP qPCR on cDNA from FACS-sorted proglucagon-negative & proglucagon-positive cells from the ileum (ileum L<sup>-</sup>; ileum L<sup>+</sup>) & colon (colon L<sup>-</sup>; colon L<sup>+</sup>) of GLU-VENUS mice (lower panel; n=3) & ChREBP protein expression from cytoplasm & nucleus extract from GLUTag cells (upper panel; performed 3 times). Data are represented as mean  $\pm$  SD. Student t-test. \*P $\leq$ 0.05 & \*\*P $\leq$ 0.01 (c) ChREBP & FXR western-blots after FXR immunoprecipitation on lysates from cytoplasm & nucleus of GLUTag cells treated or not with GW4064 (5  $\mu$ mol L<sup>-1</sup>) in presence or not of glucose (5.6 mmol L<sup>-1</sup>) (performed 2 times). (d) Proglucagon qPCR on cDNA from GLUTag cells electroporated with a siCtrl or siChREBP, starved for 12h with lactate (10 mmol L<sup>-1</sup>) & then incubated for 24h in lactate 10 mmol L<sup>-1</sup> (Glc -) or glucose 5.6 mmol L<sup>-1</sup> (Glc +) media supplemented with DMSO or GW4064 (5  $\mu$ mol L<sup>-1</sup>) (n=3; performed 3 times). Data are represented as mean  $\pm$  SD. Two-Way ANOVA analysis followed by Bonferroni's posthoc test. \*P $\leq$ 0.05 & \*\*P $\leq$ 0.01: effect of treatments on each transfection condition. §§§P $\leq$ 0.001: effect of siChREBP in each treatment condition. Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/ncomms8629>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



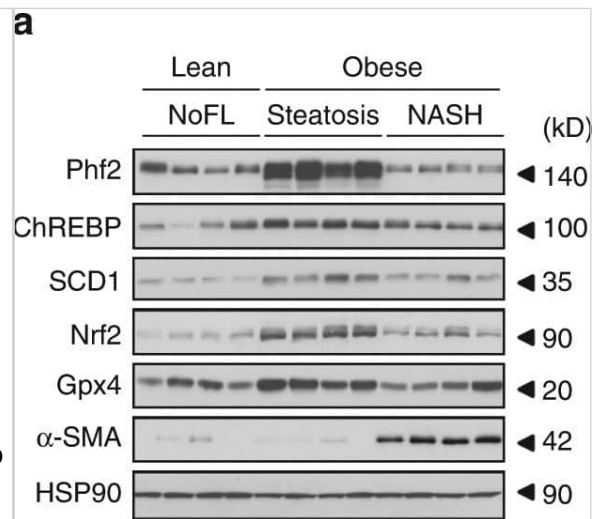
Western Blot: CHREBP Antibody - BSA Free [NB400-135] - Dietary fructose induces nuclear O-GlcNAc signaling. (A) Simplified schematic overview of the hexosamine signaling pathway including the rate limiting enzyme GFAT & O-GlcNAc transferase (OGT); (B) High-performance liquid chromatography (HPLC) analysis of hepatic UDP-GlcNAc levels. Left panel: Example of a typical running profile w/ or w/out injection of UDP-GlcNAc standard (Std.) is shown. Right panel: Quantification of the HPLC data normalized to protein concentration; (C) Hepatic expression of the *Ogt* gene & cytosolic & nuclear OGT protein analyzed by quantitative RT-PCR & western blotting/Image J & normalized to *Tbp*,  $\alpha$ -tubulin, & Lamin A, respectively. Data represent the mean  $\pm$  SEM (n = 5); (D) Cytosolic & nuclear lysates immunoblotted w/ anti-O-GlcNAc antibody (RL2) w/  $\alpha$ -Tubulin & Lamin A as loading controls. Each lane represents independent mice from each group. One representative western blot is shown (n = 2); (E) Nuclear lysates subjected to wheat germ agglutinin (WGA) beads to precipitate O-GlcNAcylated proteins. WGA enriched samples (upper panel) & input lysates (bottom panel) immunoblotted w/ antibodies detecting O-GlcNAcylated proteins (RL2), LXR & ChREBP. Each lane represents independent mice from experimental groups. Representative western blots are shown (n = 2); (F) ChREBP binding to carbohydrate response element (ChoRE) containing region of the L-pk promoter & negative control sequence (NC) 2216–2288 bp into L-pk gene after ChoRE sequence detected by chromatin immunoprecipitation (ChIP) using antibodies against ChREBP or IgG as a control. Data represent the mean  $\pm$  SEM (n = 5). Significant differences found using two-way ANOVA followed by Tukey's multiple comparison test. \*\* p < 0.01 compared to fasted. Image collected & cropped by CiteAb from the following publication (<http://www.mdpi.com/2072-6643/9/7/678>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunohistochemistry-Paraffin: CHREBP Antibody - BSA Free [NB400-135] - Correction of hepatic G6Pase- $\alpha$  deficiency normalizes autophagy. L-G6pc-/- mice were treated with 1 x 10<sup>12</sup> vp/kg of rAAV-G6PC at 4 WP & analyzed at 12 WP. (A) Hepatic G6Pase- $\alpha$  activity in control (n = 7), L-G6pc-/- (n = 7), & rAAV-treated L-G6pc-/- (AAV/ L-G6pc-/-, n = 8) mice. (B) Liver weights in control (n = 10), L-G6pc-/- (n = 5), & AAV/ L-G6pc-/- (n = 8) mice. (C) The levels of hepatic metabolites in control, L-G6pc-/- & AAV/ L-G6pc-/- (n = 8) mice. (D) Fasting glucose test (FGT) profile of control (n = 13), L-G6pc-/- (n = 6) & AAV/ L-G6pc-/- (n = 8) mice. (E) Western blots of hepatic SIRT1, FoxO3a, LC3B, p62 &  $\beta$ -actin & densitometry analysis (n = 8). (F) Hematoxylin & eosin (H&E) stained liver sections, & immunohistochemical analysis of hepatic ChREBP & quantification of nuclear ChREBP-translocated cells in control, L-G6pc-/-, & rAAV-treated L-G6pc-/- (AAV/ L-G6pc-/-) mice (n = 4). The insets present higher magnification views. Scale bar, 25  $\mu$ m. Data represent the mean  $\pm$  SEM. \*P < 0.05, \*\*P < 0.005. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28558013>), licensed under a CC0-1.0 license. Not internally tested by Novus Biologicals.



Western Blot: CHREBP Antibody - BSA Free [NB400-135] - Phf2 expression is increased in the liver of obese patients with benign hepatic steatosis & positively correlates with insulin sensitivity. Human liver biopsies from lean subjects with no fatty liver (NoFL) or obese patients with simple steatosis or NASH were obtained from the ABOS cohort. a Representative Western blot analysis of Phf2, ChREBP, SCD1, Nrf2, Gpx4, &  $\alpha$ -SMA expression (n = 10 per group). b ChIP experiments for H3K9me2 levels & for the recruitment of ChREBP & the RNA polIII at the SCD1 & Nrf2 promoter (n = 10 per group). c MUFA/SFA ratio reflecting SCD1 activity (n = 10 per group). d Expression of Nrf2 & Nrf2-regulated genes (n = 10 per group). e Levels of carbonylated proteins (n = 10 per group). f Expression of coll-1a1,  $\alpha$ -SMA, & TIMP-1 (n = 10 per group). All error bars represent mean  $\pm$  SEM. Statistical analyses were made using Anova, followed by Bonferonni's test. \*Obese with steatosis compared to lean with noFL;  $P < 0.01$ . \*\*Obese with steatosis compared to obese with NASH;  $P < 0.05$  Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41467-018-04361-y>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

An J, Astapova I, Zhang G et al. Integration of metabolomic and transcriptomic analyses reveals regulatory functions of the ChREBP transcription factor in energy metabolism. *Cell reports* 2025-02-07 [PMID: 39921857]

Cheong, MC;Mackowiak, B;Kim, HB;Hernandez, G;Nandu, T;Vale, K;Zhang, Y;Zacharias, LG;Mathews, TP;Gao, B;Kraus, WL;Kliwer, SA;Mangelsdorf, DJ; Ethanol induction of FGF21 in the liver is dependent on histone acetylation and ligand activation of ChREBP by glycerol-3-phosphate *Proceedings of the National Academy of Sciences of the United States of America* 2025-06-03 [PMID: 40440069]

Petricek K, Kirchner M, Sommerfeld M et al. An acetylated lysine residue of its low-glucose inhibitory domain controls activity and protein interactions of ChREBP. *Journal of molecular biology* 2025-05-06 [PMID: 40339981]

Chang M, Zhao M, Whang E et al. The sphingosine-1-phosphate receptor 2 S1PR2 mediates chronic glucocorticoid exposure-induced hepatic steatosis and hypertriglyceridemia. *The Journal of Biological Chemistry* 2025-06-07 [PMID: 40490140]

Yamaguchi F, Akieda-Asai S, Nakamura E et al. Continuous exposure of non-obese adult male rats to a soft-textured, readily absorbable diet induces insulin resistance and derangements in hepatic glucose and lipid metabolism. *The Journal of nutrition* 2025-03-10 [PMID: 40074175]

Zhang Y, Tang D, Yang C et al. Deficiency of SCAMP5 Triggers Pancreatic  $\beta$ -Cell Secretory Dysfunction and Apoptosis. *Advanced science (Weinheim, Baden-Wuerttemberg, Germany)* 2025-09-15 [PMID: 40953307]

Takeuchi A, Tsujimoto K, Aoki J et al. Sex difference in BAT thermogenesis depends on PGC-1 $\alpha$ -mediated phospholipid synthesis in mice. *Nature Communications* 2025-07-14 [PMID: 40659621]

Bae J, Lee JY, Shin E et al. The effects of the voglibose on non-alcoholic fatty liver disease in mice model *Scientific reports* 2022-08-10 [PMID: 35948569] (Chemotaxis, Western Blot, Rat)

Senatus L, Egaña-Gorroño L, López-Díez R et al. DIAPH1 mediates progression of atherosclerosis and regulates hepatic lipid metabolism in mice *Communications Biology* 2023-03-17 [PMID: 36932214] (Chemotaxis, Western Blot, Rat)

Li L, Sakiyama H, Eguchi H et al. Activation of the mitogen-activated protein kinase ERK1/2 signaling pathway suppresses the expression of ChREBP $\beta$  and  $\gamma$  in HepG2 cells *FEBS Open Bio* 2021-07-01 [PMID: 34051057] (Chemotaxis, Western Blot, Rat)

Thevkar-Nagesh P, Habault J, Voisin M et al. Transcriptional regulation of *Acs11* by CHREBP and NF-kappa B in macrophages during hyperglycemia and inflammation *PLOS ONE* 2022-09-02 [PMID: 36054206] (Chemotaxis, Western Blot, Rat)

Li, L;Long, J;Mise, K;Poungavrin, N;Lorenzi, PL;Mahmud, I;Tan, L;Saha, PK;Kanwar, YS;Chang, BH;Danesh, FR; The Transcription Factor ChREBP Links Mitochondrial Lipidomes to Mitochondrial Morphology and Progression of Diabetic Kidney Disease *The Journal of biological chemistry* 2023-08-21 [PMID: 37611830] (Chemotaxis, Western Blot, Rat)

More publications at <http://www.novusbio.com/NB400-135>

## Procedures

### Western Blot Protocol for CHREBP Antibody (NB400-135)

#### Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

### Immunocytochemistry/ Immunofluorescence Protocol for CHREBP Antibody (NB400-135)

#### Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.

**Immunohistochemistry Whole-Mount Protocol for CHREBP Antibody (NB400-135)**

## Immunohistochemistry-Paraffin Embedded Sections

## Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer all the time).

## Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.





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### **Products Related to NB400-135**

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NB400-135PEP	CHREBP Antibody Blocking Peptide
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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