

Supplement Material

Supplemental Materials and Methods

Plasma parameters

Plasma concentrations of total cholesterol (TC) and triglycerides (TG) were measured using commercially available kits (Biomerieux, France). HDL-cholesterol (HDL-C) concentrations were determined in plasma after precipitation of apolipoprotein (apo) B-containing lipoproteins using phosphotungstic acid/Mg (Boehringer Mannheim, Germany). Non-HDL-cholesterol (N-HDL-C) was calculated by subtraction of HDL-C values from TC.

Hepatic lipid analysis

Frozen liver tissue (50 mg) was homogenized in SET buffer (1 mL ; sucrose 250 mM, EDTA 2 mM and Tris 10 mM), followed by two freeze-thaw cycles and three times passing through a 27-gauge syringe needle and a final freeze-thaw cycle. Protein content was determined with the BCA method and TG and cholesterol measured as described above.

RNA extraction and quantitative PCR analysis

RNA, isolated from the liver using the acid guanidinium thiocyanate/phenol/chloroform method (Chomczynski et al.), was reverse transcribed using random hexamer primers and Moloney murine leukemia virus-reverse transcriptase (Invitrogen, France). RNA levels were determined by real-time quantitative PCR on the MX-4000 apparatus (Stratagene) using the Brilliant SYBR Green QPCR master mix (Stratagene) and specific primers listed in Supplemental Table 1 (Eurogentec). Results are expressed normalized to 28S RNA.

Reference:

Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem.* 1987;162:156-159.

Chromatin Immunoprecipitation (ChIP) Assay

For each ChIP experiment, chromatin was prepared from approximately 30 mg of liver as described (Nielsen et al.). Chromatin IP was carried out as described using anti-RXR (sc553, Santa Cruz

biotechnologies) and anti-RNA polymerase II antibodies (AC-0555-100, Diagenode). Precipitated DNA was analyzed by quantitative PCR. Primer sequences can be acquired upon request. DR4 elements in adjacent to the ABCG5 and 8 genes were carried out using the NHR scan algorithm (www.cisreg.ca/cgi-bin/NHR-scan/nhr_scan.cgi ; Sandelin and Wasserman)

References:

Nielsen R, Grontved L, Stunnenberg HG, Mandrup S. Peroxisome proliferator-activated receptor subtype- and cell-type-specific activation of genomic target genes upon adenoviral transgene delivery. *Mol Cell Biol.* 2006;26:5698-5714.

Sandelin A, Wasserman W. Prediction of nuclear hormone receptor response elements. *Mol Endo.* 2005;19:595-606.

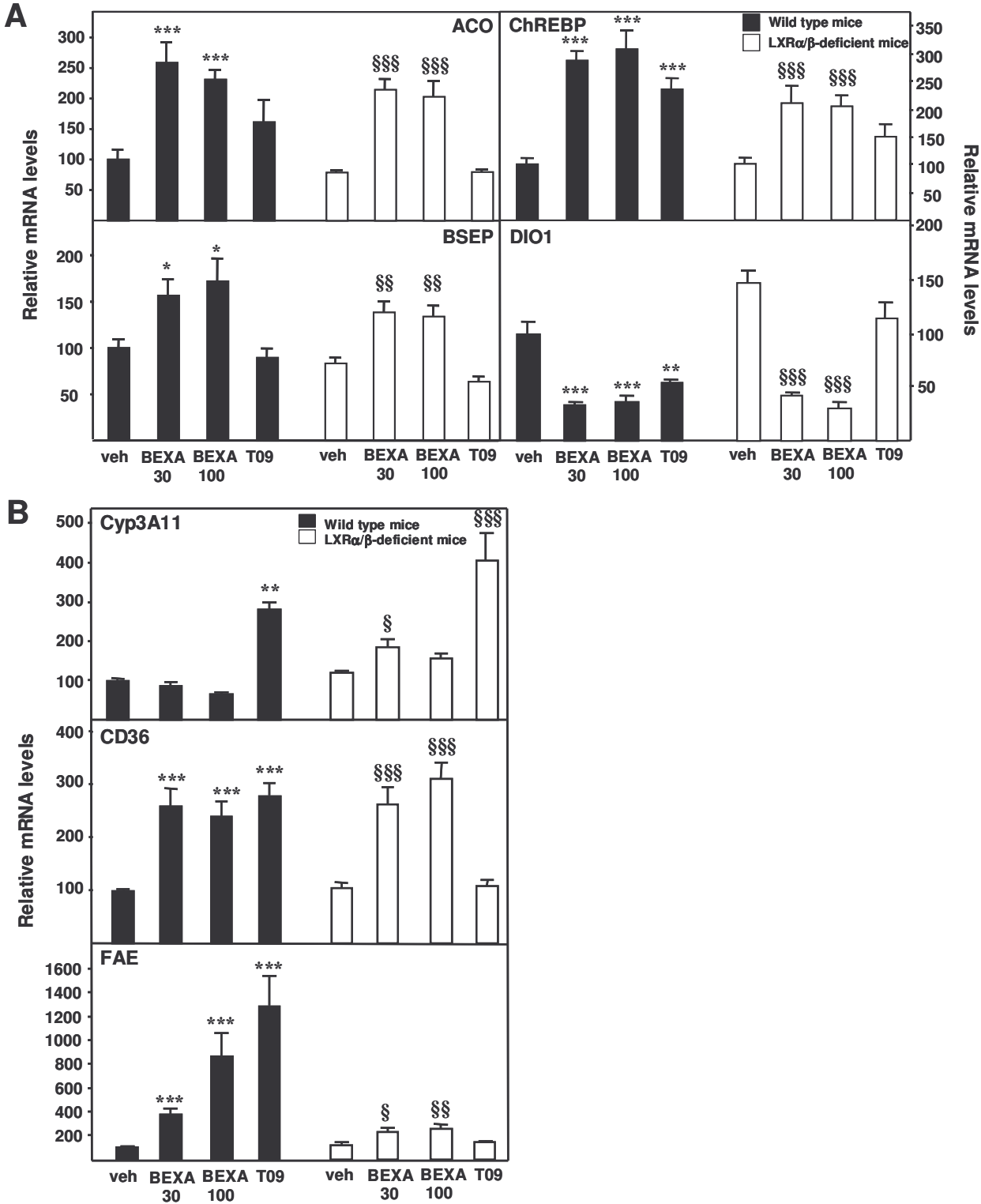
Statistical analysis

Values are presented as mean \pm sem. Data comparison between more than two groups was determined by ANOVA, using the SPSS software. Significant differences were then subjected to post-hoc analysis by using the Scheffe-test. A value of $p < 0.05$ was accepted as statistically significant.

Supplemental Table 1 : Primers used for real-time PCR

Gene	Forward primer	Reverse primer
28S	5'-AAACTCTGGTGGAGGTCCGT-3'	5'-CTTACCAAAAGTGGCCCACTA-3'
SREBP1C	5'-GTTACTCGAGCCTGCCTTCAGG-3'	5'-CAAGCTTTGGACCTGGGTGTG-3'
FAS	5'-GGTATGTCC GGGAAATTGCC-3'	5'-ATTGTGTGTGCCTGCTTGGG-3'
SCD1	5'-TTCCGTCCTGCAAGCTCTACACCTG-3'	5'-AGCCGTGCCTTGTAAATTCTGTGG-3'
ABCA1	5'-ACACCTGACACACCAGCTACAAGGC-3'	5'-TGAGTTCTTCCCACATGCCTTCC-3'
ABCG1	5'-CCTTCAGCATCATGCG-3'	5'-CCGATCCCAATGTGCGA-3'
ABCG5	5'-TGGCCCTGCTCAGCATCTCTG-3'	5'-GGATTTTTAAAGGAATGGGCATCTCTTG-3'
ABCG8	5'-CCGTCGTCAGATTTCCAATGA-3'	5'-GGCTTCCGACCCATGAATG-3'
ACO	5'-ACGTCTTGGATGGTAGTCCG-3'	5'-TAACGCTGGCTTCGAGTGAG-3'
BSEP	5'-GTCTGACTCAGTGATTCTTCGC-3'	5'-GAGCAATGCGCACACACTTC-3'
ChREBP	5'-CTGGGGACCTAACAGGAGC-3'	5'-GAAGCCACCCTATAGCTCCC-3'
Cyp3A11	5'-TAAGCAGAAGCACCGAGTGG-3'	5'-ATTGACTGGGCTGTGATCTCC-3'
CD36	5'-GCACCACTGTGTACAGACAG-3'	5'-GTGCAGCTGCTACAGCCAG-3'
FAE	5'-CATGCCGTCATGTA CTCTTAC-3'	5'-ATCTGAGTGATCTGGGACAAG-3'
DIO1	5'-CAGCTATGGCTGTGGCTG-3'	5'-CCTACAGCCACCTCCAAG-3'
NPC1L1	5'-ATCCTCATCCTGGGCTTTGC-3'	5'-GCAAGGTGATCAGGAGGTTGA-3'

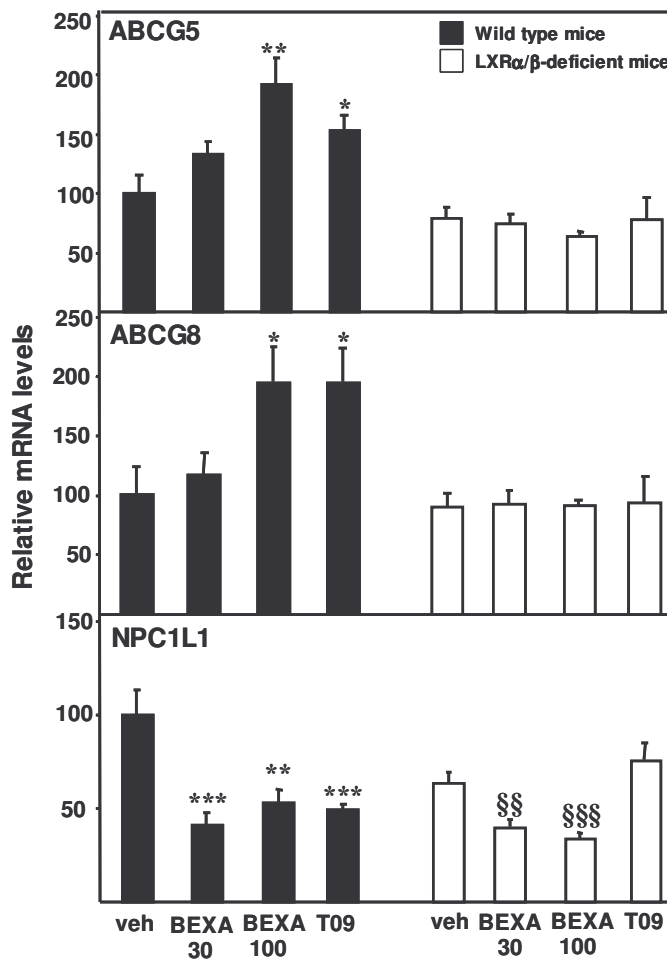
Supplemental Figures



Supplemental Figure 1 : Influence of bexarotene and T0901317 treatment on the expression of target genes of different RXR partners in the liver

Hepatic mRNA levels of ACO, BSEP, ChREBP, DIO1 (A), Cyp3A11, CD36 and FAE (B) were measured in LXRα/β-deficient mice (□) and wild type mice (■) gavaged with the RXR agonist

(bexarotene, 30 and 100 mpk), the LXR agonist (T0901317, 30 mpk) or vehicle alone for 14 days (N=7/group). Data normalized to 28S RNA are expressed as a percentage of the wild type mice group treated with vehicle alone, to which an arbitrary value of 100 was attributed. *p<0.05, **p<0.01, ***p<0.001 versus wild type mice treated with vehicle, ^sp<0.05, ^{ss}p<0.01, ^{sss}p<0.001 versus LXR α / β -deficient mice treated with vehicle.



Supplemental Figure 2 : Influence of bexarotene and T0901317 treatment on the expression of ABCG5, ABCG8 and NPC1L1 in the jejunum

mRNA levels of ABCG5, ABCG8 and NPC1L1 were measured in the jejunum of LXR α / β -deficient mice (□) and wild type mice (■) gavaged with the RXR agonist (bexarotene, 30 and 100 mpk), the LXR agonist (T0901317, 30 mpk) or vehicle alone for 14 days (N=7/group). Data normalized to 28S RNA are expressed as a percentage of the wild type mice group treated with vehicle alone, to which an arbitrary value of 100 was attributed. *p<0.05, **p<0.01, ***p<0.001 versus wild type mice treated with vehicle, ^{ss}p<0.01, ^{sss}p<0.001 versus LXR α / β -deficient mice treated with vehicle.