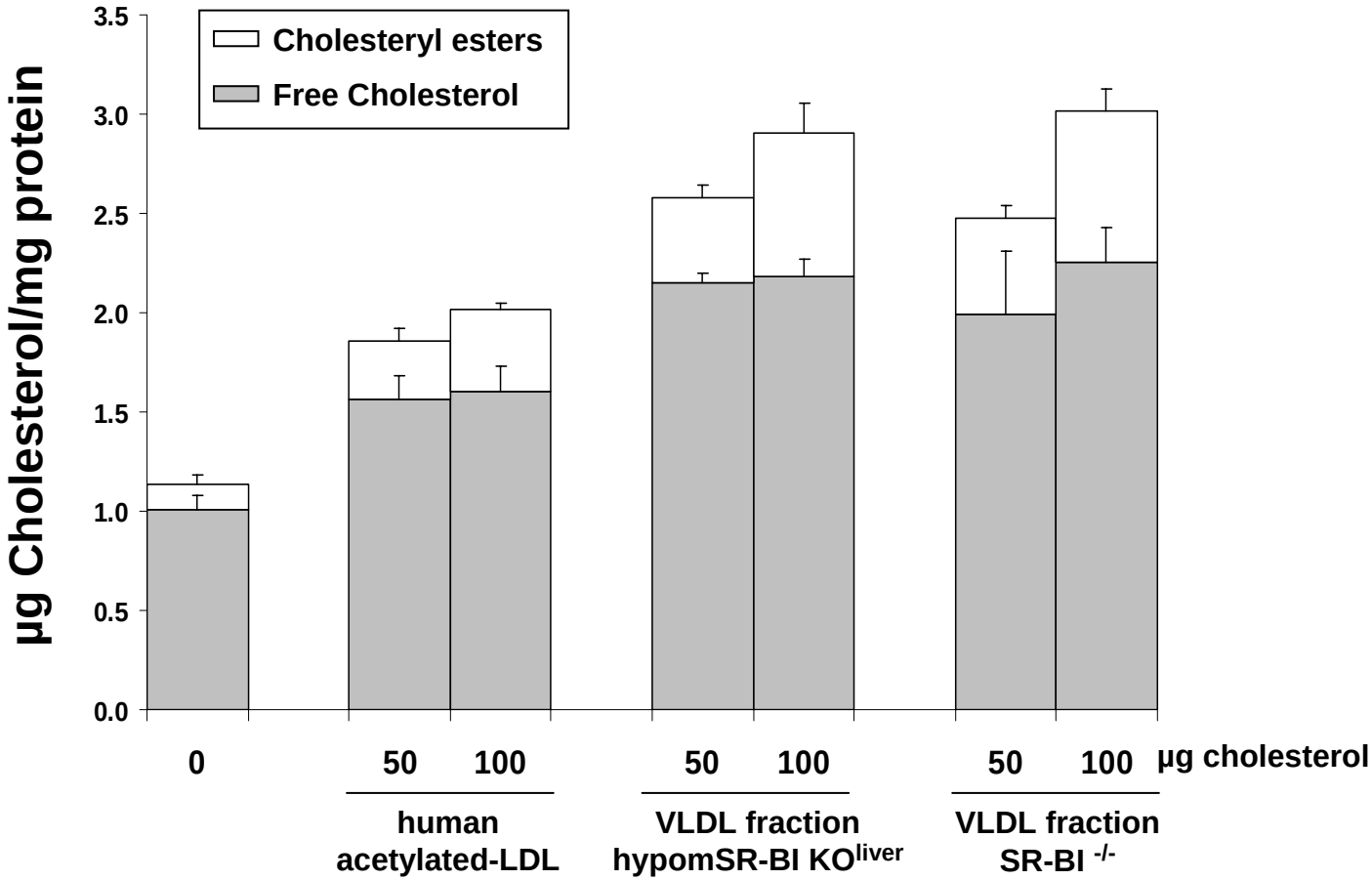
Supplemental table I

Real time quantitative PCR : Gene specific primer pairs

Gene Accession numb.	**Full name**	**Sense primer**	**Antisense primer**	**Amplicon (bp)**
SR-BI NM_016741	Scavenger Receptor Class B Type I	**5'GCTGCGCTCGGCGTTGTCAT3'**	**5'GGGACGGGGATCTCCTTCCA3'**	122
ABCG1 NM_009593	ATP-binding cassette, sub-family G (WHITE), member 1	**5'AGGTCTCAGCCTTCTAAAGTTCCTC3'**	**5'TCTCTCGAAGTGAATGAAATTTATCG3'**	85
F4/80 NM_010130	EGF-like module containing, mucin-like, hormone receptor-like sequence 1 (Emr1)	**5'CTTTGGCTATGGGCTTCCAGTC3'**	**5'GCAAGGAGGACAGAGTTTATCGTG3'**	165
HPRT NM_013556	hypoxanthine guanine phosphoribosyl transferase 1	**5'TGACACTGGTAAAACAATGC3'**	**5'AACACTTCGAGAGGTCCTTT3'**	106

Supplemental figure I



Legend of supplemental figure I: *Mouse macrophage-like RAW cells accumulate similar amount of cholesterol following incubation with VLDL-sized lipoproteins isolated either from hypomSR-BI KO^{liver} or SR-BI$^{-/-}$ female mice fed the atherogenic HFC diet.*

Pooled plasma samples (n=5) from hypomSR-BI KO^{liver} or SR-BI$^{-/-}$ female mice fed the atherogenic HFC diet were first ultracentrifuged (100,000 g for 5.5 h at 15°C in a fixed-angle rotor in a TL100 ultracentrifuge (Beckman Instruments)) at a density of 1.21 g/ml and lipoproteins recovered in the supernatant were then fractionated by gel filtration on Superose 6 columns. Fractions corresponding to VLDL-sized particles were pooled, concentrated using Microcon YM-30 centrifugal filter units (Millipore) and sterilized using 0.2 µm filters. RAW264.7 cells were plated in 96-well dishes with 40,000 cells per well. The cells were loaded either with human acetylated-LDL or VLDL-sized lipoproteins (50 or 100 µg cholesterol/ml) isolated from HFC-fed hypomSR-BI KO^{liver} or SR-BI$^{-/-}$ mice in serum-free DMEM supplemented with 50 mM glucose, 2 mM glutamine, and 0.2% BSA, as previously described (1). After 24-hours, the medium was removed and cellular lipids were extracted in hexane-isopropanol (3:2, v/v). After solvent evaporation, cellular free and esterified cholesterol were quantitated using the Amplex Red Cholesterol Assay Kit (Invitrogen). Values were normalized for cellular protein concentration.

SUPPLEMENTARY METHODS

Generation of SR-BI conditional knock-out and SR-BI$^{-/-}$ mouse models.

A genomic DNA clone of mouse SR-BI was isolated from a 129/Sv genomic library. The fragments used for constructing the targeting vector were a ~12.0-kb SR-BI/*Xba*I fragment encompassing the first exon of the SR-BI gene and spanning ~2.2- and 9.5-kb of sequence upstream and downstream of exon 1 respectively, and a ~8-kb SR-BI/*BamH*I fragment internal to the 12.0-kb *Xba*I fragment (Figure 1). The left homology arm of the targeting vector was generated by PCR amplification of the SR-BI/*Xba*I fragment cloned in pBluescript with forward primer SRBI-f1 5'-TGAAGGTGGTCTTCAAGAGCAGTCCT-3' and reverse primer SRBI-r1 5'-ACCTACACGGGGATCACGTTC-3' located 1.2-kb upstream and 3.5-kb downstream of SR-BI exon 1, respectively. The PCR fragment generated was subsequently cloned in a TA-cloning vector (Invitrogen). A *lox*P sequence 5'-GCAACTTCGTATAGCATACATTATACGAAGTTAT-3' (the outer 2 underlined bases were modified from the original *lox*P sequence in order to destabilize the loxP hairpin structure) was then introduced at position -85 bp (relative to the initiation translation codon) by a PCR-based mutagenesis approach (Stratagene Excite mutagenesis kit). The left arm cassette containing the *lox*P site was then excised from the TA-vector using *Spe*I and *Xba*I restriction sites located in the multiple cloning sites region of the vector backbone and subsequently cloned into the *Xba*I-digested pPN2T vector (2) to yield the pPN2T-Larm. The right homology arm was PCR-amplified using the ~8-kb SR-BI/*BamH*I fragment cloned in pBluescript as template with the forward SRBI/*Xho*I-*lox*P-f2 primer 5'-GCCGctcgagATAACTTCGTATAGCATACATTATACGAAGTTATGACAGTTTTCAGAGCTCAGGG-3' (complementary sequence adjacent to the SRBI-r1 primer sequence in intron 1)

containing an introduced 5' restriction site for *Xho*I (lower letters) and a 5'-*lox*P sequence (underlined), and M13 reverse primer 5'-CAGGAAACAGCTATGACC-3' (M13 reverse sequence located in pBluescript). The resulting PCR product was cloned into the *Xba*I site of pBluescript. Finally, the *Xho*I and *Not*I sites, located 5' of the *lox*P site and in the multiple cloning site region of the vector respectively, were used for directional cloning of the right arm into the *Xho*I/*Not*I-digested pPN2T-Larm plasmid to yield the final vector pPN2T-floxSRBI.

Mouse embryonic stem (ES) cells (129/SvJ; GoGermline, Genome Systems, Inc) were electroporated with 20-40 µg of targeting pPN2T-floxSRBI construct and plated onto mitotically inactivated, neomycin-resistant mouse embryonic fibroblast feeder layers. Selective medium containing 150 µg/ml G418 and 0.5 µg/ml 1-2'-deoxy-2'-fluoro- 3 -D-arabinofuranosyl-5-iodouracil was applied two days later. Individual clones were isolated after 10 to 12 days, expanded, and screened by Southern blot analysis of *Xba*I-digested DNA. The 511-bp 3' probe A (Figure 1) was amplified by PCR with primers SRBIprobe-f3 5'-GACAGTTTTCAGAGCTCAGGG-3' and SRBIprobe-r3 5'-GGGGAAGAGGGACTTTAATAG-3'. Several targeted clones containing an SR-BI allele with a floxed exon 1 (SR-BI$^{flox/+}$ ES) were further transfected with a circular pBS185 CRE plasmid (Invitrogen) modified to contain the hygromycin B resistance gene under the control a PGK promoter, and subsequently selected in hygromycin B (150 µg/ml)-containing medium for 8 days. CRE-mediated deletion of exon 1 of the floxed SR-BI allele in hygromycin B-resistant ES clones (SR-BI$^{+/-}$ ES) was confirmed by PCR and Southern blot analysis. SR-BI$^{flox/+}$ ES and derived-SR-BI$^{+/-}$ ES clones were injected into C57BL/6 blastocysts and chimeric offspring were bred to C57BL/6 mice to test for germ line transmission of the floxed SR-BI allele or the exon 1-deleted SR-BI allele, respectively. SR-BI$^{flox/+}$ and SR-BI$^{+/-}$ mouse lines selected for the study were

derived from the same ES clone (i.e. one SR-BI$^{flox/+}$ ES clone and a derived-SR-BI$^{+/-}$ ES clone). Alb-Cre transgenic mice (3) (provided by Dr. M.A. Magnuson, Vanderbilt University School of Medicine) were bred with mice carrying the SR-BI floxed allele to obtain liver-deficient SR-BI gene animals. The presence of the floxed or wild-type SR-BI alleles was determined by PCR amplification with primers SRBI-f4 5'-ACGCCTCACCATCAGAGC-3' and SRBI-r4 5'-CCTTGAGCACCTGCTGCTTG-3' that flank the *lox*P integration site in the 5'-region of the SR-BI gene (Figure 1). Genotyping of the animals for the Alb-Cre transgene was performed by PCR using primers Cre-F and Cre-R (4). SR-BI$^{-/-}$ mice were generated by breeding heterozygous or homozygous animals (on a C57BL/6J genetic background for six generations) fed a sterilized regular chow (A03 (SAFE)) containing 0.5% probucol (Sigma). The presence of the exon 1-deleted SR-BI allele in the offspring was assessed by PCR amplification of DNA extracted from tail biopsies with SRBI-f4, SRBI-r4 (see above) and SR-BI-r5 5'-GGCTCTCTTGAGTGATGAACC-3' primers.

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