Supplemental Materials

Article

Ablation of mouse selenium-binding protein 1 and 2 elevates LDL by disruption of cholesterol efflux and lipid metabolism

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Table S1. Sequences of primers used for genotyping

	Primer sequence	Product size
Genotyping-SeBP1-WT	Forward: 5′-TTGTTTCCCATCCACTGTCA-3′ Reverse: 5′-CATAGCTGAGTGTTGGGGGT-3′	829 bp
Genotyping-SeBP1-KO	Forward: 5′-GAACAAGATGGATTGCACGCAGGTTCTCCG-3′ Reverse: 5′-GTAGCCAACGCTATGTCCTGATAG-3′	668 bp
Genotyping-SeBP2-WT	Forward: 5′-ACAAAGTGTGGTCCAGGTTAC-3′ Reverse: 5′-TGGCTGGTGTGCGTATTG-3′	1297 bp
Genotyping-SeBP2-KO	Forward: 5′-CAGTCTTGACCAAATACACACACTC-3′ Reverse: 5′-TGTCCTACATACAGGAGTATTTTGC-3′	777 bp

Table S2. Sequences of primers used for PCR amplification of mRNAs

Target mRNA (Genebank Accession)	Primer sequence	Product size
SeBP1 (NM_009150)	Forward: 5′-CTGATACTGCCTGGTCTCA-3′ Reverse: 5′-AGTGGCTGGTGTGCAAAC-3′	142 bp
SeBP2 (NM_019414)	Forward: 5′-CTGATACTGCCTGGTCTCA-3′ Reverse: 5′-AGTGGCTGGTGTGCGTAT-3′	142 bp
Ppara (NM_011144)	Forward: 5′-CATCACAGACACCCTCTCTC-3′ Reverse: 5′-AAGCCCTTACAGCCTTCAC-3′	174 bp
Pparg (NM_011146)	Forward: 5′-AGACCACTCGCATTCCTTTGAC-3′ Reverse: 5′-TTTATCCCCACAGACTCGGCAC-3′	274 bp
Ppard (NM_011145)	Forward: 5′-AACACACGTTCCTTCCAG-3′ Reverse: 5′-GATCGCACTTCTCATACTCG-3′	237 bp
Rxra (NM_011305)	Forward: 5′-CTCCTTCACCAAGCACATC-3′ Reverse: 5′-GTCTTTGCGTACTGTCCTC-3′	115 bp
Cpt1a (NM_013495)	Forward: 5′-CTCCGCCTGAGCCATGAAG-3′ Forward: 5′-CACCAGTGATGATGCCATTCT-3′	100 bp
Cpt2 (NM_009949)	Forward: 5′-CCTGCTCGCTCAGGATAAACA-3′ Forward: 5′-GTGTCTTCAGAAACCGCACTG-3′	203 bp
Cyp4a12a (NM_177406)	Forward: 5′-GACTTCTATCACCTGGAATGAC-3′ Reverse: 5′-AGCTCTCTGCTCACACTTG-3′	105 bp
Cyp4a12b (NM_172306)	Forward: 5′-TACTCAGCAGTTCCCATCC-3′ Reverse: 5′-TCTCCCCAGAATCAGCTTC-3′	187 bp
Sod1 (NM_011434)	Forward: 5′-ATGGGTTCCACGTCCATCAG-3′ Reverse: 5′-GTCTCCAACATGCCTCTCTTC-3′	122 bp
Sod2 (NM_013671)	Forward: 5′-ACAACTCAGGTCGCTCTTC-3′ Reverse: 5′-ATAGCCTCCAGCAACTCTC-3′	128 bp
Acox1	Forward: 5′-TAACTTCCTCACTCGAAGCCA-3′	283 bp

(NM_001271898)	Reverse: 5′- AGTTCCATGACCCATCTCTGTC-3′	
Acox3	Forward: 5′-GGACAGGACTGGGAATATCAC-3′	125 bp
(NM_030721)	Reverse: 5′-CAGACATGCTGATGATGGAG-3′	
Lxra	Forward: 5′-CTCAATGCCTGATGTTTCTCCT-3′	150 bp
(NM_001177730)	Forward: 5′-TCCAACCCTATCCCTAAAGCAA-3′	
Abcg1	Forward: 5′-GCTCCATCGTCTGTACCATCC-3′	88 bp
(NM_009593)	Forward: 5′-ACGCATTGTCCTTGACTTAGG-3′	
Abca1	Forward: 5′-GCTTGTTGGCCTCAGTTAAGG-3′	135 bp
(NM_013454)	Forward: 5′-GTAGCTCAGGCGTACAGAGAT-3′	
Abcg5	Forward: 5′-CGCGAGACGTTGCGATACA-3′	128 bp
(NM_031884)	Forward: 5′-CTGCCAATCATTTGGTCCGC-3′	
Abcg8	Forward: ATACCCTGGAGGTCTCATAGCA-3′	134 bp
(NM_001286005)	Forward: ACGTCGAGTAGTGAGGCTCTC-3′	
Hmgcr	Forward: 5′-CTGGAATTATGAGTGCCCCAAA-3′	101 bp
(NM_001360165.1)	Forward: 5′-ACGACTGTACTGAAGACAAAGC-3′	
Acaca	Forward: 5′-ATGGGCGGAATGGTCTCTTTC-3′	148 bp
(NM_133360.3)	Forward: 5′-TGGGGACCTTGTCTTCATCAT-3′	
Fasn	Forward: 5′-GGAGGTGGTGATAGCCGGTAT-3′	140 bp
(NM_007988.3)	Forward: 5′-TGGGTAATCCATAGAGCCCAG-3′	
Srebp1	Forward: 5′-GATGTGCGAACTGGACACAG-3′	104 bp
(NM_011480.4)	Forward: 5′-CATAGGGGGCGTCAAACAG-3′	
Srebp2	Forward: 5′-GCAGCAACGGGACCATTCT-3′	200 bp
(NM_033218.2)	Forward: 5′-CCCCATGACTAAGTCCTTCAACT-3′	
β-actin	Forward: 5′-GATTACTGCTCTGGCTCCTA-3′	135 bp
(NM_007393)	Reverse: 5′-TCCTGCTTGCTGATCCAC-3′	
hSeBP1	Forward: 5′-TCCTCTACTTCAGCAACTGG-3′	183 bp
(NM_001258288)	Reverse: 5′-GTTTTCCCTTGACCACTAGG-3′	

PPARA (NM_001001928)	Forward: 5′-TTCGCAATCCATCGGCGAG-3′ Reverse: 5′-CCACAGGATAAGTCACCGAGG-3′	146 bp
ACOX1 (NM_001185039)	Forward: 5′-GGAACTCACCTTCGAGGCTTG-3′ Reverse: 5′-TTCCCCTTAGTGATGAGCTGG-3′	164 bp
ACOX3 (NM_001101667.2)	Forward: 5′-CGAGCTGAACTTCCTTCGATG-3′ Reverse: 5′- CCAGGCACTGAATCAAGGC-3′	107 bp
CPT1A (NM_001031847)	Forward: 5′-ATCAATCGGACTCTGGAAACGG-3′ Reverse: 5′-TCAGGGAGTAGCGCATGGT-3′	121 bp
SOD1 (NM_000454)	Forward: 5′-GGTGGGCCAAAGGATGAAGAG-3′ Reverse: 5′- CCACAAGCCAAACGACTTCC-3′	227 bp
SOD2 (NM_000636)	Forward: 5′-GGAAGCCATCAAACGTGACTT-3′ Reverse: 5′-CCCGTTCCTTATTGAAACCAAGC-3′	116 bp
β-ACTIN (NM_001101)	Forward: 5′-GGCACCACACCTTCTACAATG-3′ Reverse: 5′-AGCACAGCCTGGATAGCAAC-3′	163 bp

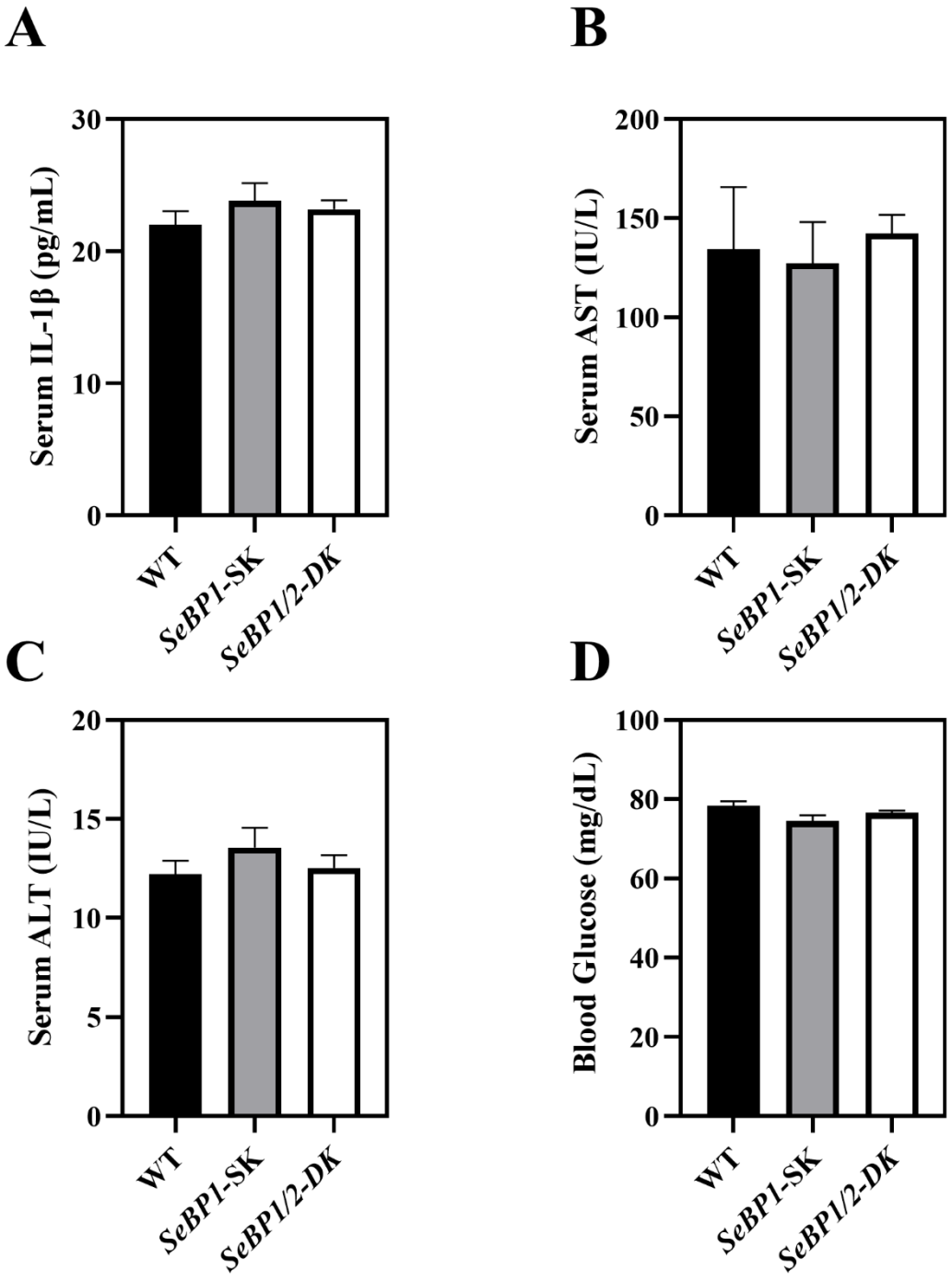


Figure S1. Effect of SeBP1/2 deletion on serum parameters. Each bar represents the mean ± SEM for 5 samples. IL-1β levels (A), serum AST (B), ALT(C) activity, and blood glucose level in WT, *SeBP1*-SK, and *SeBP1/2*-DK female mice were determined using commercially available kits at 8 weeks. WT, wild type; *SeBP1*-SK, *SeBP1* knockout mice; *SeBP1/2*-DK, *SeBP1* and *SeBP2* double-knockout mice.

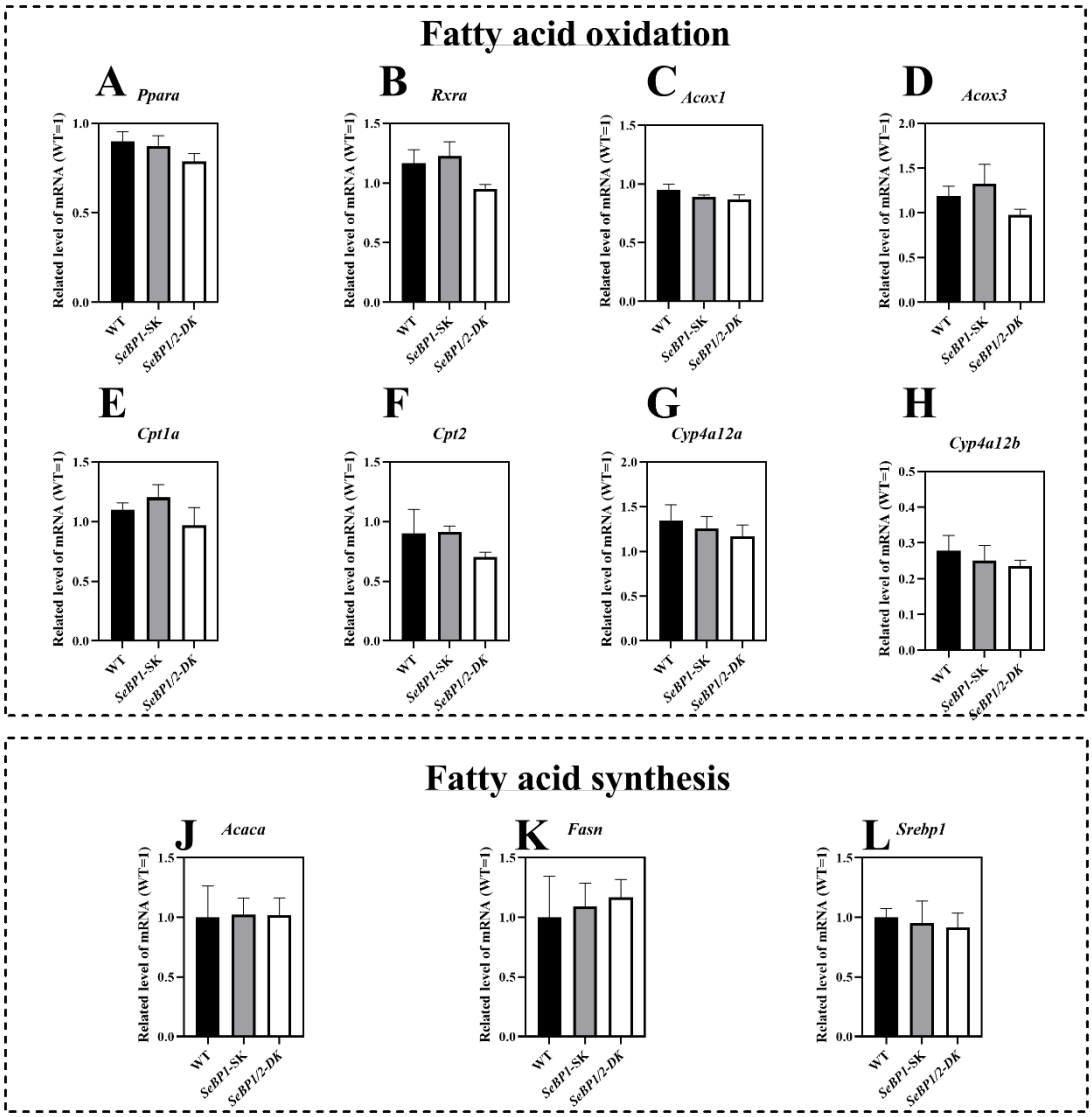


Figure S2. Key genes associated with β-oxidation of fatty acids in the liver. WT, *SeBP1*-SK, and *SeBP1/2*-DK female mice were fasted for 20 h at 8-weeks of age and euthanized by CO_2 inhalation. The livers were snap-frozen and stored at −80°C. Total RNA was isolated from the liver using a RNeasy Mini Kit (Qiagen, GmbH, Hilden, Germany). Values represent the means ± SEM for 5 mice. *Ppara*, peroxisome proliferator-activated receptor alpha; *Rxra*, retinoid X receptor alpha; *Acox1*, acyl-CoA oxidase 1; *Acox3*, acyl-CoA oxidase 3; *Cpt1a*, carnitine palmitoyltransferase 1A; *Cpt2*, carnitine palmitoyltransferase 2; *Cyp4a12a*, cytochrome P450, family 4, subfamily a, polypeptide 12A; *Cyp4a12b*, cytochrome P450, family 4, subfamily a, polypeptide 12B; *Acaca*, acetyl-coA carboxylase alpha; *Fasn*, fatty acid synthase; *Srebp1*, sterol regulatory element-binding protein 1; WT, wild type; *SeBP1*-SK, *SeBP1* knockout mice; *SeBP1/2*-DK, *SeBP1* and *SeBP2* double-knockout mice.

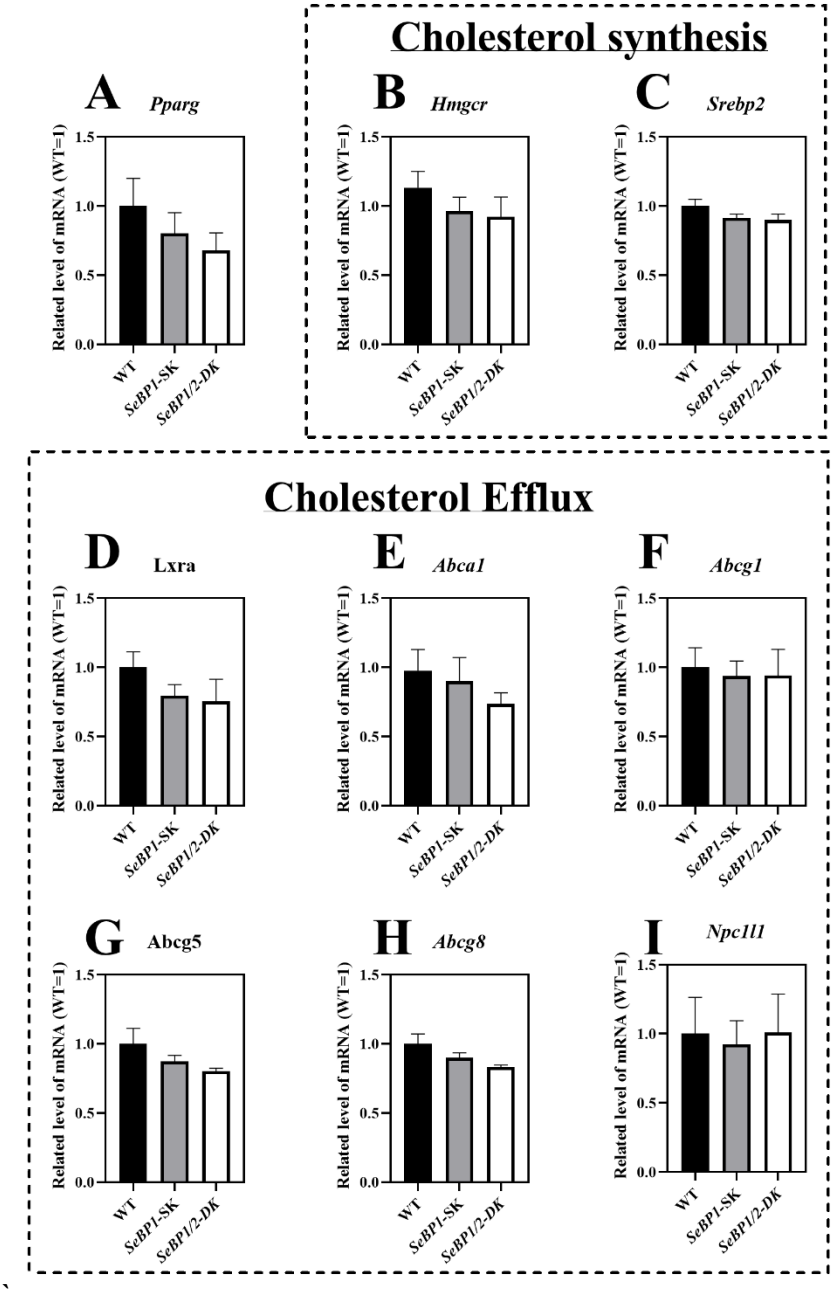


Figure S3. Key genes associated with cholesterol transport from the liver. WT, *SeBP1*-SK, and *SeBP1/2*-DK female mice were fasted for 20 h at 8-weeks of age and euthanized by CO_2 inhalation. The livers were snap-frozen and stored at −80°C. Total RNA was isolated from the liver using a RNeasy Mini Kit (Qiagen, GmbH, Hilden, Germany). Values represent the means ± SEM for 5 mice. *Pparg*, peroxisome proliferator-activated receptor gamma; *Hmgcr,* 3-hydroxy-3-methylglutaryl-CoA reductase; *Srebp2*, sterol regulatory element-binding protein 2; *Lxra*, liver X receptor alpha; *Abca1*, ATP-binding cassette transporter A1; *Abcg1*, ATP-binding cassette transporter G1; *Abcg5*, ATP-binding cassette transporter G5; *Abcg8*, ATP-binding cassette transporter G8; *Npc1l1*, niemann-pick c1-like 1; WT, wild type; *SeBP1*-SK, *SeBP1* knockout mice; *SeBP1/2*-DK, *SeBP1* and *SeBP2* double-knockout mice.

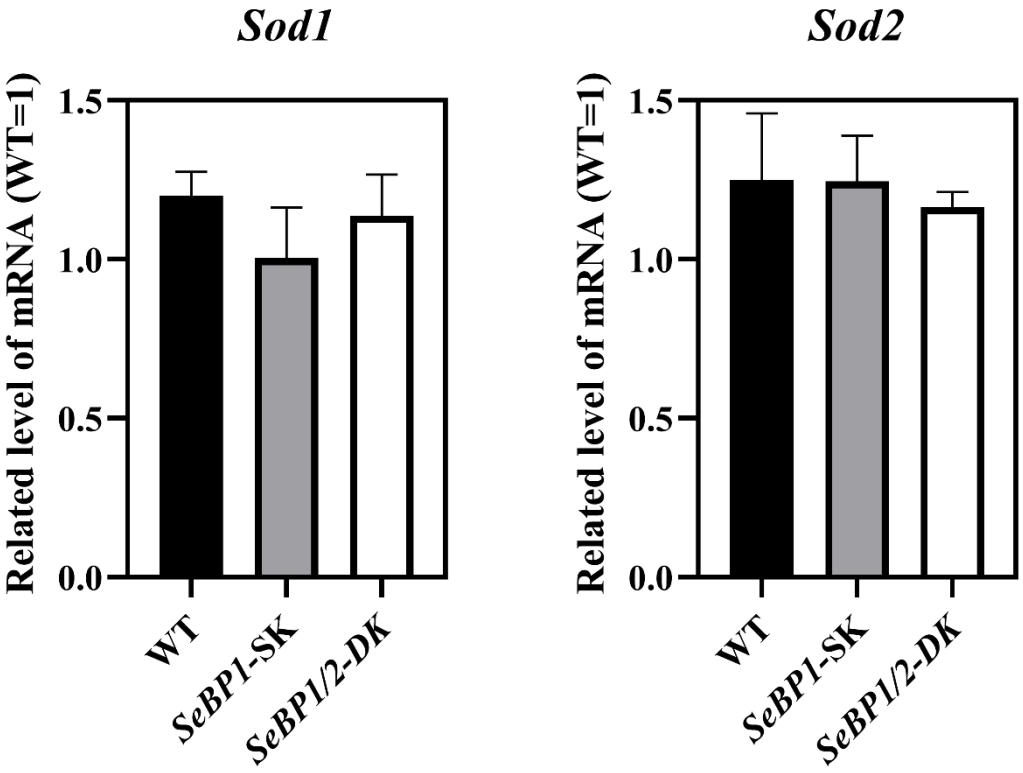


Figure S4. **Key genes associated with redox reaction in the liver.** WT, *SeBP1*-SK, and *SeBP1/2*-DK female mice were fasted for 20 h at 8-weeks of age and euthanized by CO_2 inhalation. The livers were snap-frozen and stored at −80°C. Total RNA was isolated from the liver using a RNeasy Mini Kit (Qiagen, GmbH, Hilden, Germany). Values represent the means ± SEM for 5 mice. *Sod1*, superoxide dismutase 1; *Sod2*, superoxide dismutase 2.