# Supplemental information

# SREBP1 regulates *Lgals3*

# activation in response to cholesterol loading

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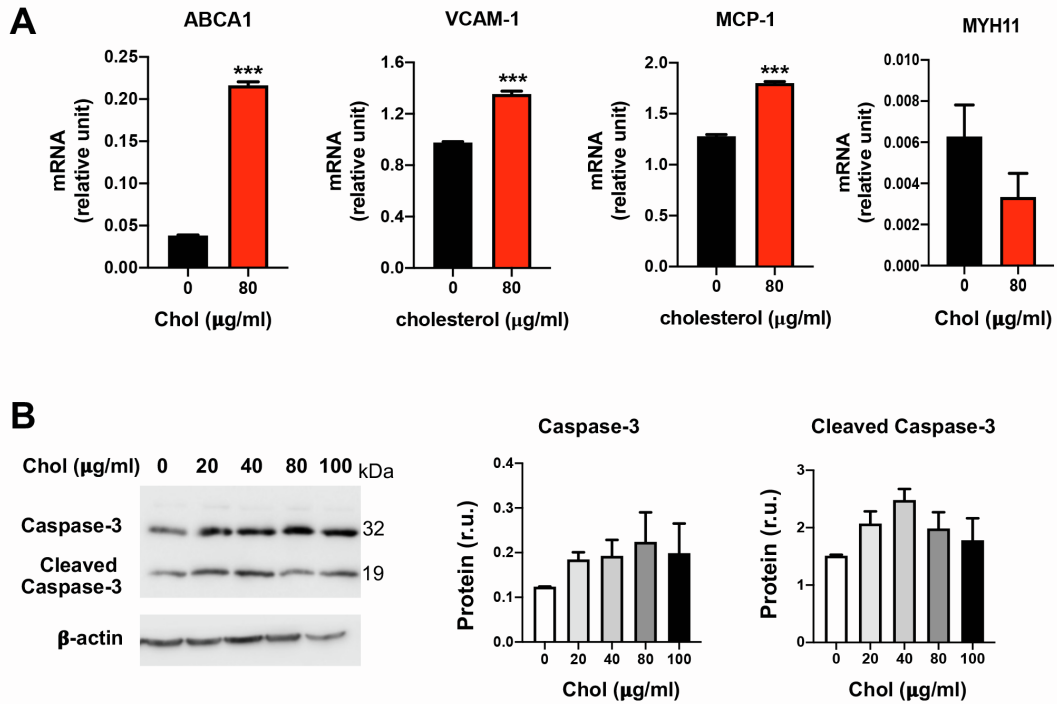
# Supplemental Figures



**Figure S1. Cholesterol loading stimulates inflammatory marker gene expression without causing apoptosis**

MOVAS cell culture, cholesterol feeding, and data quantification were performed as described for Figure 1. qRT-PCR data are presented as mean $\pm$ SD, n = 3 replicates. Western blot data are presented as mean $\pm$ SEM, n = 3 independent repeat experiments. Statistics: ANOVA followed by Tukey test; **P<0.01, ***P<0.001, compared to the basal condition (the 1st bar in each plot); non-significant comparisons are not labeled.

- **A.** Upregulation of inflammatory marker mRNAs (qRT-PCR) due to cholesterol feeding (80 μg/ml).
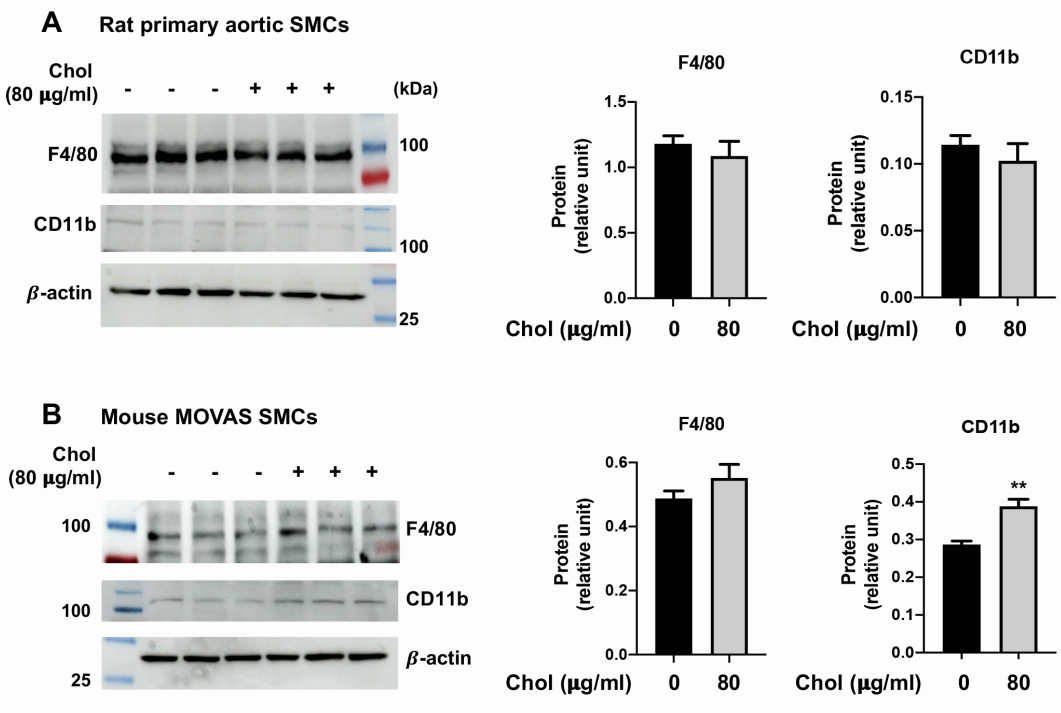- **B.** Full length and cleaved caspase-3 protein (Western blot).

**Figure S2. Effect of cholesterol loading on macrophage markers**

Rat primary SMCs (A) and the MOVAS mouse SMC line (B) were used. Cell culture, cholesterol loading, and data quantification were performed as described for Figure 1. Data quantification for Western blots: Mean ± SD, n = 3 replicates. Statistics: Student's t-test. **P<0.01.
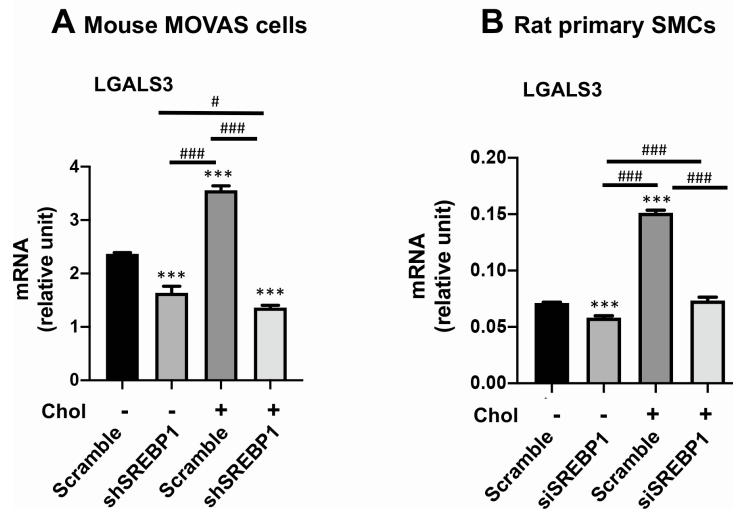
**A** Mouse MOVAS cells

LGALS3

**B** Rat primary SMCs

LGALS3

**Figure S3. Effect of SREBP1 silencing on LGALS3 mRNA levels.**

Both the MOVAS mouse SMC line (A) and rat primary SMCs (B) were used. Cell culture, cholesterol loading, and data quantification were performed as described for Figure 1.
Data quantification: Mean ± SD, n = 3 replicates. Statistics: one-way ANOVA followed by Tukey test; ###P<0.001 (between paired bars); ***P<0.001, compared to the basal condition (the 1st bar in each plot); non-significant comparisons are not labeled.

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**Figure S4. LGALS3 gain-of-function upregulates the full length and N-terminal half molecules of the SREBP1 protein**

MOVAS cells (A) or rat primary aortic SMCs (B) were used. Experiments were performed as in Figure 5B. Data are presented as mean ± SEM, n = 3 independent repeat experiments. Statistics: ANOVA followed by Tukey test; *P<0.05, **P<0.01, ***P<0.001, compared to EVec (empty vector control).

**A  Mouse MOVAS cells**

LGALS3, CD68, ABCA1, MCP-1 (mouse MOVAS cells)

**B  Rat primary SMCs**

LGALS3, CD68, ABCA1, MCP-1 (rat primary SMCs)

**Figure S5. Inhibitory effect of the BETs inhibitor JQ1 on LGALS3 mRNA levels**

Both the MOVAS mouse SMC line (A) and rat primary SMCs (B) were used. Cell culture, cholesterol loading, and data quantification were performed as described in detail for Figure 1. Data quantification: Mean $\pm$ SD, n = 3 replicates. Statistics: one-way ANOVA followed by Tukey test; ##$P<0.01$, ###$P<0.001$ (between paired bars); ***$P<0.001$, compared to the basal condition (the 1st bar in each plot); non-significant comparisons are not labeled.
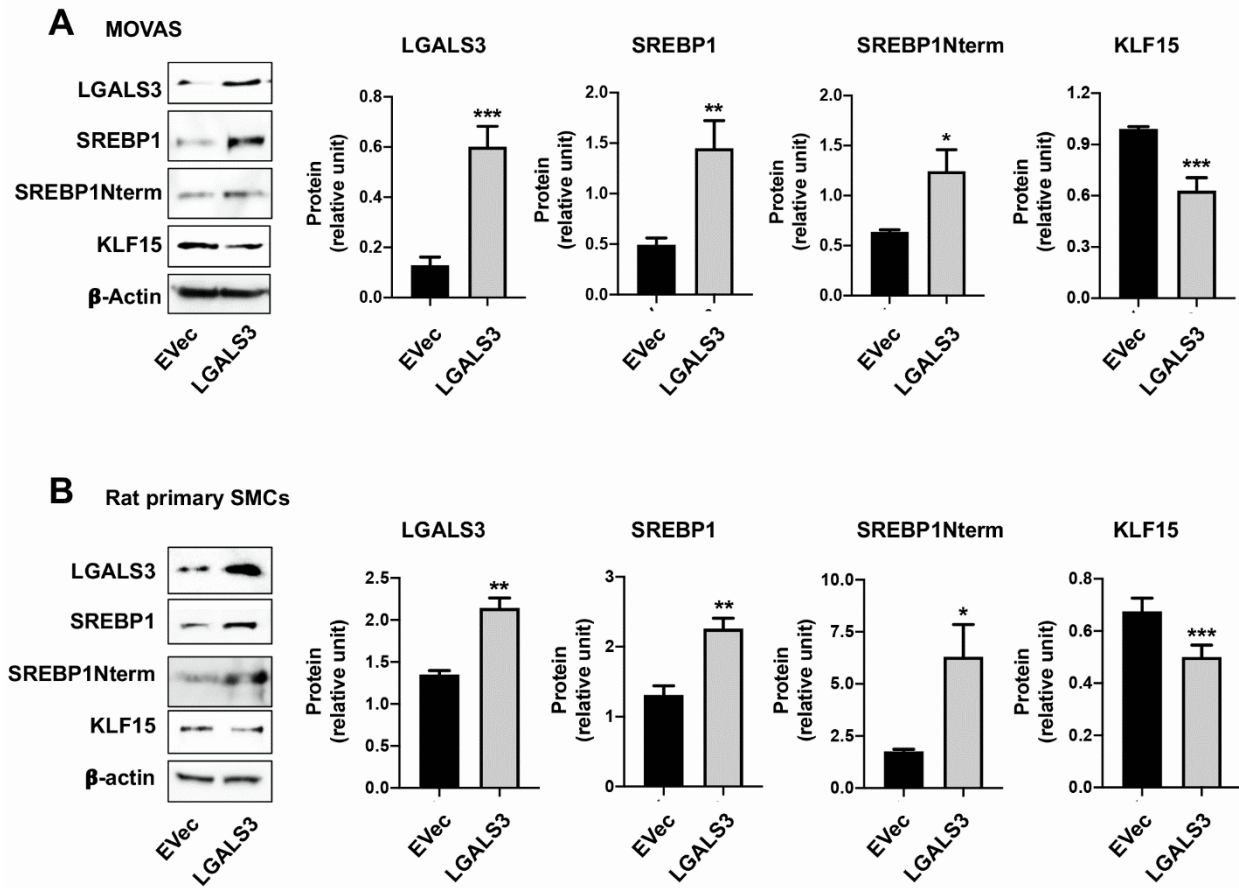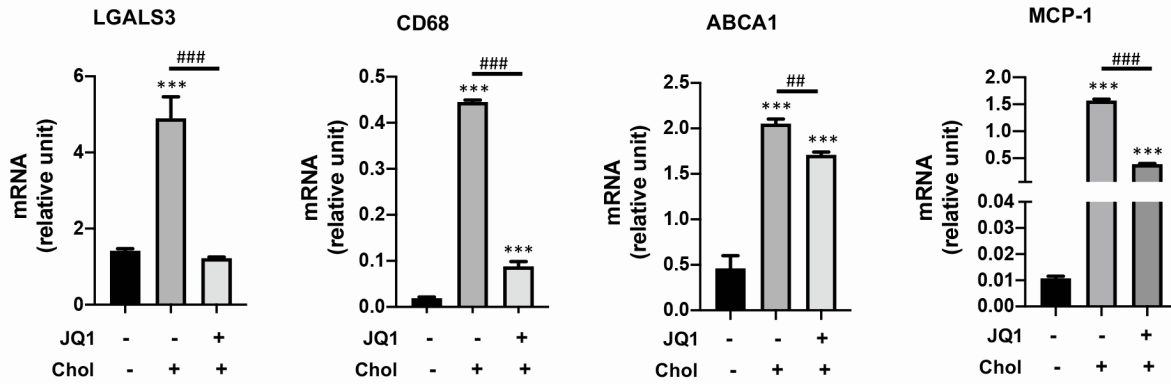
**Figure S6. Effect of LGALS3 loss-of-function on cleaved caspase-3**

Experiments were performed as in Figure 5A. Data are presented as mean ± SEM, n = 3 independent repeat experiments. Statistics: ANOVA followed by Tukey test; [#]P<0.05, [##]P<0.01 (between paired bars); *P<0.05, compared to the basal condition (the 1st bar in the plot); non-significant comparisons are not labeled; r.u., relative unit.

**Figure S7. Chol loading alters the levels of proteins involved in lipid storage**

Experiments were performed as in Figure 7D but in rat primary SMCs instead. Western blot data are presented as mean ± SEM, n = 3 independent repeat experiments. qRT-PCR data are presented as mean ± SD, n = 3 replicates. Statistics: ANOVA followed by Tukey test; *P<0.05, **P<0.01, ***P<0.001, compared to the control without cholesterol loading.
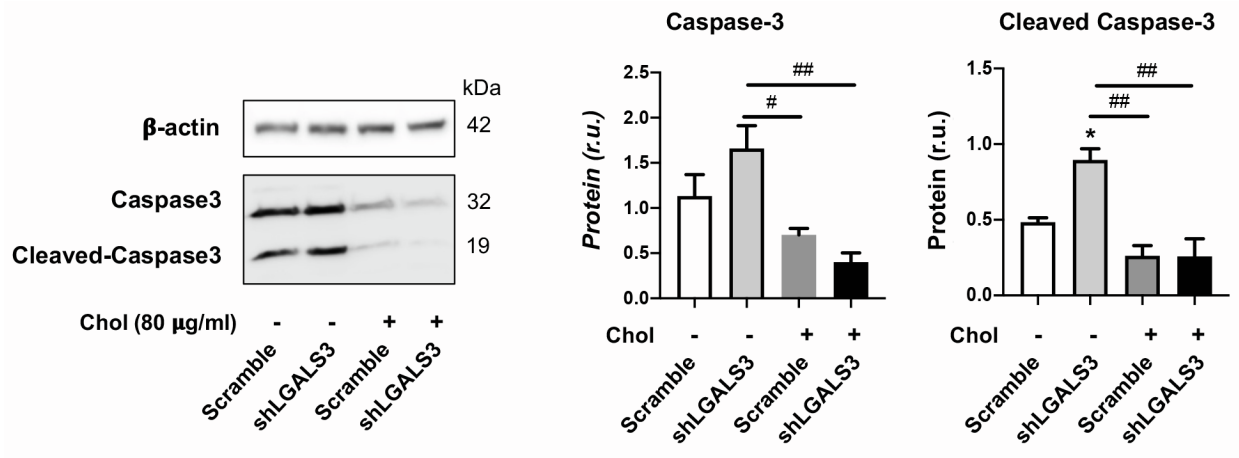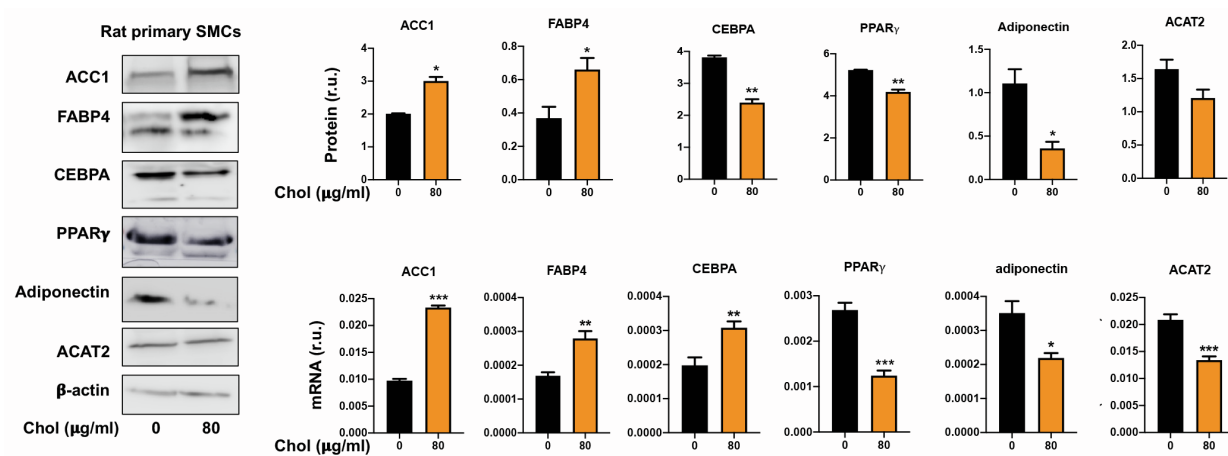
# Supplemental Tables

## Table S1. Kits and reagents

| Product | Manufacturer | Catalog Number |
|---|---|---|
| JetPRIME Transfection reagent | Polyplus transfection | 114-07 |
| Lipofectamine RNAiMAX Transfection Reagent | Invitrogen | 3778150 |
| PageRuler™ PLUS Prestained Protein Ladder | Thermo Scientific | 26620 |
| Clarity Western ECL Substrate | Bio-Rad | 170-5060 |
| QIAGEN Plasmid MIDIprep Kit | QIAGEN | 12143 |
| PureLink™ Quick Plasmid Miniprep Kit | Invitrogen | K210011 |
| PureLink™ Quick Gel Extraction and PCR Purification Combo Kit | Invitrogen | K220001 |
| NE-PER™ Nuclear and Cytoplasmic Extraction Reagents | Invitrogen | 78835 |
| LightShift™ Chemiluminescent EMSA Kit | Invitrogen | 20148 |
| Pierce™ Protein A/G Magnetic Agarose Beads | Invitrogen | 78610 |
| UltraPure™ Salmon Sperm DNA Solution | Invitrogen | 15632011 |
| Mouse IgG | Invitrogen | 10400C |
| PerfeCTa SYBR® Green SuperMix | Quantabio | 95054 |
| V5-TAG MAGNETIC BEADS | Fisher Scientific | NC0777490 |
| Pierce™ Anti-DYKDDDDK Magnetic Agarose | Thermofisher | A36797 |

**Table S2. shRNAs and siRNAs for gene silencing**

| Name | Sequence (5'-3') |
|---|---|
| Rat *Srebf1* siRNA (ID: s134945) | Sense: CCUGCGAAGUGCUCACAAATT |
| | Antisense: UUUGUGAGCACUUCGCAGGGT |
| Rat *Lgals3* siRNA (ID: s136243) | Sense: GCAAACCAUUCAAAAUACATT |
| | Antisense: UGUAUUUUGAAUGGUUUGCCG |
| Human *Srebf1* siRNA (ID: 5140) | Sense: GGCAAAGCUGAAUAAAUCUTT<br>Antisense: AGAUUUAUUCAGCUUUGCCTC |
| Human *Lgals3* siRNA (ID: s8149) | Sense: CGGUGAAGCCCAAUGCAAATT<br>Antisense: UUUGCAUUGGGCUUCACCGTG |
| Rat *Klf15* shRNA#1 | CCGGCTACCCTGGAGGAGATTGAAGCTCGAGCTTCAATCTCCTCCAGGGTAGTTTTTG |
| | AATTCAAAAACTACCCTGGAGGAGATTGAAGCTCGAGCTTCAATCTCCTCCAGGGTAG |
| Rat *Klf15* shRNA#2 | CCGGCCAAACCTATTGGCTCAGGATCTCGAGATCCTGAGCCAATAGGTTTGGTTTTTG |
| | AATTCAAAAACCAAACCTATTGGCTCAGGATCTCGAGATCCTGAGCCAATAGGTTTGG |
| Rat KLF15 shRNA#3 | CCGGCCAGGGCAGCATCTTGGATTTCTCGAGAAATCCAAGATGCTGCCCTGGTTTTTG |
| | AATTCAAAAACCAGGGCAGCATCTTGGATTTCTCGAGAAATCCAAGATGCTGCCCTGG |
| Mouse *Srebf1* shRNA#1 | CCGGGCGGCTGTTGTCTACCATAAGCTCGAGCTTATGGTAGACAACAGCCGCTTTTTG |
| | AATTCAAAAAGCGGCTGTTGTCTACCATAAGCTCGAGCTTATGGTAGACAACAGCCGC |
| Mouse *Srebf1* shRNA#2 | CCGGGCCTGCTATGAGGAGGGTATTCTCGAGAATACCCTCCTCATAGCAGGCTTTTTG |
| | AATTCAAAAAGCCTGCTATGAGGAGGGTATTCTCGAGAATACCCTCCTCATAGCAGGC |
| Mouse *Srebf1* shRNA#3 | CCGGCCTGCACTTCTTGACACGTTTCTCGAGAAACGTGTCAAGAAGTGCAGGTTTTTG |
| | AATTCAAAAACCTGCACTTCTTGACACGTTTCTCGAGAAACGTGTCAAGAAGTGCAGG |
| Mouse *Srebf1* shRNA#4 | CCGGGCTGAATAAATCTGCTGTCTTCTCGAGAAGACAGCAGATTTATTCAGCTTTTTG |
| | AATTCAAAAAGCTGAATAAATCTGCTGTCTTCTCGAGAAGACAGCAGATTTATTCAGC |
| Mouse *Lgals3* shRNA#1 | CCGGGGAGCTTATCCTGGCTCAACTCTCGAGAGTTGAGCCAGGATAAGCTCCTTTTTG |
| | AATTCAAAAAGGAGCTTATCCTGGCTCAACTCTCGAGAGTTGAGCCAGGATAAGCTCC |
| Mouse *Lgals3* shRNA#2 | CCGGCCGCATGCTGATCACAATCATCTCGAGATGATTGTGATCAGCATGCGGTTTTTG |

| | |
|---|---|
| | AATTCAAAAACCGCATGCTGATCACAATCATCTCGAGATGATTGT GATCAGCATGCGG |
| Mouse *Lgals3* shRNA#3 | CCGGGCAGTACAACCATCGGATGAACTCGAGTTCATCCGATGGT TGTACTGCTTTTTG |
| | AATTCAAAAAGCAGTACAACCATCGGATGAACTCGAGTTCATCC GATGGTTGTACGCC |
| Mouse *Klf15* shRNA#1 | CCGGACCGAAATGCTCAGTGGGTTACTCGAGTAACCCACTGAG CATTTCGGTTTTTTG |
| | AATTCAAAAAACCGAAATGCTCAGTGGGTTACTCGAGTAACCCA CTGAGCATTTCGGT |
| Mouse *Klf15* shRNA#2 | CCGGGCGATCTCACTCGGGTGTGAACTCGAGTTCACACCCGAG TGAGATCGCTTTTTG |
| | AATTCAAAAAGCGATCTCACTCGGGTGTGAACTCGAGTTCACAC CCGAGTGAGATCGC |
| Mouse *Klf15* shRNA#3 | CCGGCTACCCTGGAGGAGATTGAAGCTCGAGCTTCAATCTCCTC CAGGGTAGTTTTTG |
| | AATTCAAAAACTACCCTGGAGGAGATTGAAGCTCGAGCTTCAAT CTCCTCCAGGGTAG |
| Mouse *Klf15* shRNA#4 | CCGGGCGATCTCACTCGGGTGTGAACTCGAGTTCACACCCGAG TGAGATCGCTTTTTG |
| | AATTCAAAAAGCGATCTCACTCGGGTGTGAACTCGAGTTCACAC CCGAGTGAGATCGC |
| Mouse *Klf15* shRNA#5 | CCGGACCGAAATGCTCAGTGGGTTACTCGAGTAACCCACTGAG CATTTCGGTTTTTTG |
| | AATTCAAAAAACCGAAATGCTCAGTGGGTTACTCGAGTAACCCA CTGAGCATTTCGGT |
| Mouse *Klf15* shRNA#6 | CCGGCCCTCAAAGTTTGTGCGAATTCTCGAGAATTCGCACAAAC TTTGAGGGTTTTTG |
| | AATTCAAAACCCTCAAAGTTTGTGCGAATTCTCGAGAATTCGCA CAAACTTTGAGGG |
| Mouse *Brd4* shRNA#1 | CCGGGCCATCTACACTACGAGAGTTCTCGAGAACTCTCGTAGTG TAGATGGCTTTTTG |
| | AATTCAAAAAGCCATCTACACTACGAGAGTTCTCGAGAACTCTCG TAGTGTAGATGGC |
| Mouse *Brd4* shRNA#2 | CCGGGATGTGTTTGAAATGCGCTTTCTCGAGAAAGCGCATTTCA AACACATCTTTTTG |
| | AATTCAAAAAGATGTGTTTGAAATGCGCTTTCTCGAGAAAGCGCA TTTCAAACACATC |
| Mouse *Brd4* shRNA#3 | CCGGGCAGCAGCAGCAGCAGCAACCCTCGAGGGTTGCTGCTGC TGCTGCTGCTTTTTG |
| | AATTCAAAAAGCAGCAGCAGCAGCAGCAACCCTCGAGGGTTGCT GCTGCTGCTGC |
| Mouse *Brd4* shRNA#4 | CCGGCCCAGCTCCTCTGACAGTGAAGCTCGAGCTTCACTGTCA GAGGAGCTGGATTTTTG |
| | AATTCAAAAACCCAGCTCCTCTGACAGTGAAGCTCGAGCTTCAC TGTCAGAGGAGCTGGA |
| Mouse *Brd3* shRNA#1 | CCGGAGCTGAACCTGCCTGATTATCCTCGAGGATAATCAGGCAG GTTCAGCTTTTTTG |

| | AATTCAAAAAAGCTGAACCTGCCTGATTATCCTCGAGGATAATCAGGCAGGTTCAGCT |
|---|---|
| Mouse *Brd3* shRNA#2 | CCGGCCACAGATGATATAGTGCTAACTCGAGTTAGCACTATATCATCTGTGGTTTTTG |
| | AATTCAAAAACCACAGATGATATAGTGCTAACTCGAGTTAGCACTATATCATCTGTGG |
| Mouse *Brd2* shRNA#1 | CCGGCAGCCCAAGAAATCTAAGAAACTCGAGTTTCTTAGATTTCTTGGGCTGTTTTTG |
| | AATTCAAAAACAGCCCAAGAAATCTAAGAAACTCGAGTTTCTTAGATTTCTTGGGCTG |
| Mouse *Brd2* shRNA#2 | CCGGCCTCAGAATGTATGCAGGATTCTCGAGAATCCTGCATACATTCTGAGGTTTTTG |
| | AATTCAAAAACCTCAGAATGTATGCAGGATTCTCGAGAATCCTGCATACATTCTGAGG |
| Scrambled-sequence Control shRNA | CCGGTAGCGACTAAACACATCAATCCTCGAGGATTGATGTGTTTAGTCGCTATTTTTG |
| | AATTCAAAAATAGCGACTAAACACATCAATCCTCGAGGATTGATGTGTTTAGTCGCTA |

**Table S3. Primers for cloning to overexpress a gene**

| Name | Sequence (5'-3') | Application |
|---|---|---|
| Mouse *Srebf1* cDNA | Forward: GTGGAATTCTATGGACTACAAGGATGAC GATGAC | Lentiviral vector (inserted into pLenti-puro) |
| | Reverse: CTCTAGACTGCTGGAAGTGACGGTGGTTCCGCC | |
| Mouse *Klf15* cDNA | Forward: CCGCTAGCGATGGATTACAAGGATGACGACG | Lentiviral vector (inserted into pLenti-puro) |
| | Reverse: CGACCGGTAGGTTGATGGCGCGTACTGCGC | |
| Mouse *Lgals3* cDNA | Forward: GTGGATCCAATGGCAGACAGCTTTTCGCTT | Lentiviral vector (inserted into pLenti-puro) |
| | Reverse: CACTCGAGCGGATCATGGCGTGGTTAGCGC | |
| Rat *Lgals3* cDNA | Forward: GTGGATCCAATGGCAGACGGCTTCTCACTT | Lentiviral vector (inserted into pLenti-puro) |
| | Reverse: CGAATTCCAGATCATGGCGTGGGAAGCGCT | |

**Table S4. Primers for qRT-PCR**

| | |
|---|---|
| Mouse GAPDH | Forward: AAGGTCGGTGTGAACGGATTT |
| | Reverse: CTTTGTCACAAGAGAAGGCAGC |
| Mouse ACTA2 | Forward: ACTCTGTCTGGATCGGTGGC |
| | Reverse: TTCGTCGTATTCCTGTTTGCT |
| Mouse VCAM1 | Forward: TGGAAATGTGCCCGAAAC |
| | Reverse: GCCTGGCGGATGGTGTAC |
| Mouse ABCA1 | Forward: CCGTCTTTCCAGGACAGTAT |
| | Reverse: CAGGGTGGCTCTTCTCATC |
| Mouse CD68 | Forward: ACCGTGACCAGTCCCTCTT |
| | Reverse: AAGGCGATGAGCACCAGGGTGAGGA |
| Mouse LGALS3 | Forward: CTGGAGGCTATCCTGCTGC |
| | Reverse: AACAATCCTGTTTGCGTTGG |
| Mouse KLF15 | Forward: CCGAAATGCTCAGTGGGTTAC |
| | Reverse: GAGTCAGGGCTGGCACAAGA |
| Mouse SREBP1 C-terminal domain (primer 1) | Forward: CCAGGTGACCCGGCTATTC |
| | Reverse: CCAAGGGCATCTGAGAACTCC |
| Mouse SREBP1 N-terminal domain (primer 2) | Forward: CGGCACCCGCTGCTTTA |
| | Reverse: TGGCACTGGCTCCTCTTTGA |

**Table S5. Antibodies for Western blot (WB) or ChIP or IP**

| Antigen | Manufacturer | Catalog Number | Dilution Ratio | Application |
|---|---|---|---|---|
| BRD2 | Proteintech | 22236-1-AP | 1:1000 | WB, ChIP |
| Beta-actin | Proteintech | 60008-1-Ig | 1:10000 | WB |
| FLAG tag | Sigma Aldich | F3165 | 1:1000 | WB |
| Alpha-SMA | Proteintech | 55135-1-AP | 1:5000 | WB |
| LGALS3 | Cell Signaling Technology | 87985 | 1:1000 | WB |
| CD68 | Santa Cruz | sc-17832 | 1:250 | WB |
| KLF15 | Santa Cruz | sc-271675 | 1:1000 | WB, ChIP |
| SREBP1(2A4) (for full-length and N-term) | Santa Cruz | sc-13551 | 1:1000 | WB, ChIP |
| MRTF-A/MKL1 | Cell Signaling Technology | 14760 | 1:1000 | WB |
| Histone-3 | Cell Signaling Technology | 14269 | 1:1000 | WB |
| GAPDH | Cell Signaling Technology | 5174 | 1:1000 | WB |
| Caspas3/P17/P19 | Proteintech | 19677-1-AP | 1:1000 | WB |
| ACC1 | Proteintech | 21923-1-AP | 1:1000 | WB |
| FABP4 | abcam | ab23693 | 1:1000 | WB |
| CEBPA | Proteintech | 18311-1-AP | 1:1000 | WB |
| PPAR gamma | Proteintech | 16643-1-AP | 1:1000 | WB |
| Adiponectin | Cell Signaling Technology | 2789s | 1:1000 | WB |
| ACAT2 | Proteintech | 14755-1-AP | 1:1000 | WB |

**Table S6.  Primers for ChIP-qPCR**

| | |
|---|---|
| ChIP: Anti-SREBPNterm or anti-KLF15, <br> S1 site in *Lgals3* promoter | Forward: TGGGTGCGGTCGGCTAGGGCCC |
| | Reverse: CCCCACGGACCACGGAGCTTC |
| ChIP: Anti-SREBPN1term or anti-KLF15, <br> S2 site in *Lgals3* promoter | Forward: GCTCCTCCCTTCCCTCACAC |
| | Reverse: CACCCAGACTCTCAGACTCACCC |
| ChIP: Anti-SREBP1Nterm or Anti-KLF15, <br> S3 site in *Lgals3* promoter | Forward: CAGGGATCAAAGTTAGGCGTC |
| | Reverse: GTCGCTGTGCCCTTGCTTAC |
| ChIP: Anti-BRD2 or anti-H3K27ac, <br> Primer #1 for *Srebf1* promoter | Forward: CTGCCACCCAAGTGCTGG |
| | Reverse: CTGAAGGGCCAGTGGGCTC |
| ChIP: Anti-BRD2 or anti-H3K27ac, <br> Primer #2 for *Srebf1* promoter | Forward: GAACCTCTCCTCCCTCCC |
| | Reverse: CTGTAACAGAGGTCCTGAG |
| ChIP: Anti-BRD2 and anti-H3K27ac, <br> Primer S1 for *Lgals3* promoter | Forward: TGGGTGCGGTCGGCTAGGGCCC |
| | Reverse: CCCCACGGACCACGGAGCTTC |
| ChIP: Anti-BRD2 and anti-H3K27ac, <br> Primer S2 for *Lgals3* promoter | Forward: GCTCCTCCCTTCCCTCACAC |
| | Reverse: CACCCAGACTCTCAGACTCACCC |

**Table S7. Oligo sequences for EMSA**

| Name | Sequence (5'-3') | Biotin label |
|---|---|---|
| S1 WT F (SREBP1) | CTGTGGGGAAGCTCCGTGGTCCGTGGGGCGCGGTCCAGCCAGGCGCCTGC | 5' labeling |
| S1 WT R (SREBP1) | GCAGGCGCCTGGCTGGACCGCGCCCCACGGACCACGGAGCTTCCCCACAG | |
| S1-mu1 F (SREBP1) | CTGTGGGGAAGCTCCGTGGTCCGTGTGGTGCGGTCCAGCCAGGCGCCTGC | 5' labeling |
| S1-mu1 R (SREBP1) | GCAGGCGCCTGGCTGGACCGCACCACACGGACCACGGAGCTTCCCCACAG | |
| S1-mu2 F (SREBP1) | CTGTGGGGAAGCTCCGTGGTCCTTGTGGTGCGGTCCAGCCAGGCGCCTGC | 5' labeling |
| S1-mu2 R (SREBP1) | GCAGGCGCCTGGCTGGACCGCACCACAAGGACCACGGAGCTTCCCCACAG | |
| S1-mu3 F (SREBP1) | CTGTGGGGAAGCTCCGTGGTCCTTGTGTTGCGGTCCAGCCAGGCGCCTGC | 5' labeling |
| S1-mu3 R (SREBP1) | GCAGGCGCCTGGCTGGACCGCAACACAAGGACCACGGAGCTTCCCCACAG | |
| | | |
| S2 WT F (KLF15) | GTAAAGCCCTAGGCATAGAGTGGGGTGCATAAGTGTTTGGTAGATATTAG | 5' labeling |
| S2 WT R (KLF15) | CTAATATCTACCAAACACTTATGCACCCCACTCTATGCCTAGGGCTTTAC | |
| S3- F | CATTCCCCTGCCCCGGGTGTGGGGAGAAGTCAGCAGAATGGGGGGC | |
| S3- R | GCCCCCCATTCTGCTGACTTCTCCCCACACCCGGGGCAGGGGAATG | |
| S2-mu1 F (KLF15) | GTAAAGCCCTAGGCATAGAGTGTGTTGCATAAGTGTTTGGTAGATATTAG | 5' labeling |
| S2-mu1 R (KLF15) | CTAATATCTACCAAACACTTATGCAACACACTCTATGCCTAGGGCTTTAC | |
| S2-mu2 F (KLF15) | GTAAAGCCCTAGGCATAGATTGTGTTGCATAAGTGTTTGGTAGATATTAG | 5' labeling |
| S2-mu2 R (KLF15) | CTAATATCTACCAAACACTTATGCAACACAATCTATGCCTAGGGCTTTAC | |
| S2-mu3 F (KLF15) | GTAAAGCCCTAGGCATAGATTGTGTTTCATAAGTGTTTGGTAGATATTAG | 5' labeling |
| S2-mu3 R (KLF15) | CTAATATCTACCAAACACTTATGAAACACAATCTATGCCTAGGGCTTTAC | |
| | | |

Note: The oligo sequences are within the respective S1 and S2 Lgals3 promoter regions.