# Supplementary Information

# MicroRNA-33b knock-in mice for an intron of sterol regulatory element-binding factor 1 (*Srebf1*) exhibit reduced HDL-C *in vivo*

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Supplementary Table S1

Serum lipid profiling of WT and miR-33b KI$^{+/+}$ mice by HPLC

| Lipoprotein<br>Major (Fraction No.)<br>Diameter | Sub-class (Fraction No.) | WT (n=4) | KI$^{+/+}$ (n=5) |
|---|---|---|---|
| TC (mg/dl) | | 72.71 ± 5.42 | 52.56 ± 3.91* |
| CM (1-2) >80nm | | 0.73 ± 0.14 | 0.36 ± 0.06* |
| VLDL (3-7) 30-80nm | | 3.93 ± 0.22 | 3.76 ± 0.41 |
| LDL (8-13) 16-30nm | | 10.86 ± 0.60 | 9.85 ± 0.78 |
| | large LDL (8) | 3.10 ± 0.09 | 3.39 ± 0.24 |
| | medium LDL (9) | 2.85 ± 0.11 | 3.15 ± 0.21 |
| | small LDL (10) | 1.77 ± 0.14 | 1.63 ± 0.18 |
| | very small LDL (11-13) | 2.73 ± 0.60 | 1.68 ± 0.26 |
| HDL (14-20) 8-16nm | | 57.20 ± 4.92 | 38.59 ± 3.23* |
| | very large HDL (14-15) | 2.30 ± 0.50 | 0.68 ± 0.14* |
| | large HDL (16) | 18.49 ± 2.44 | 8.89 ± 1.38** |
| | medium HDL (17) | 24.90 ± 1.46 | 18.97 ± 1.29* |
| | small HDL (18) | 8.45 ± 0.43 | 7.74 ± 0.47 |
| | very small HDL (19-20) | 3.05 ± 0.25 | 2.32 ± 0.25 |
| TG (mg/dl) | | 22.04 ± 2.48 | 19.27 ± 2.66 |

Values are mean ± SEM. Blood was obtained from chow-fed 8 weeks old male mice, after 4 h fasting.

The serum was analyzed by HPLC, as described in material and methods. *p<0.05, **p<0.01 compared with WT mice.

TC, total cholesterol; TG, triglyceride; CM, chylomicrons; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.
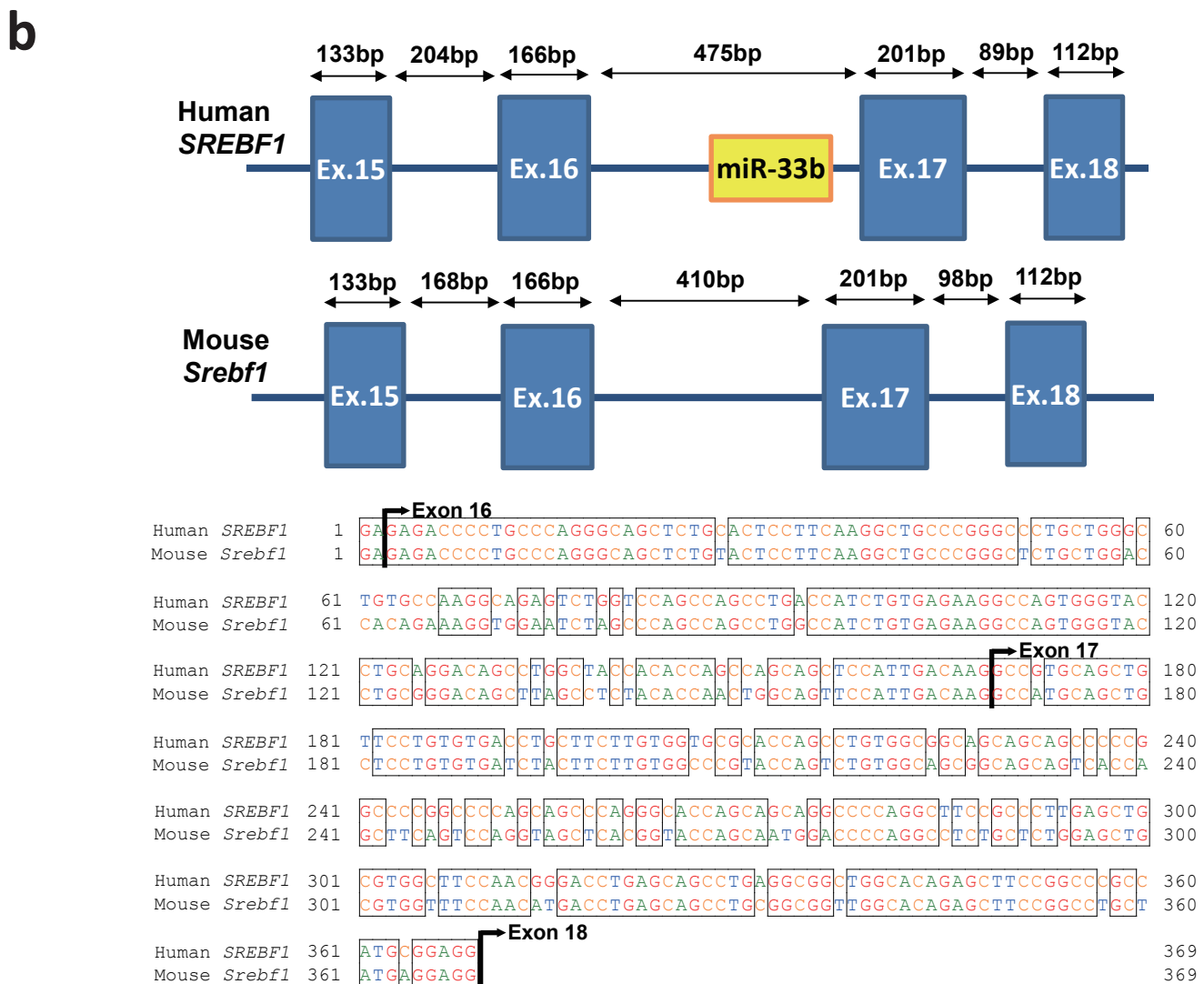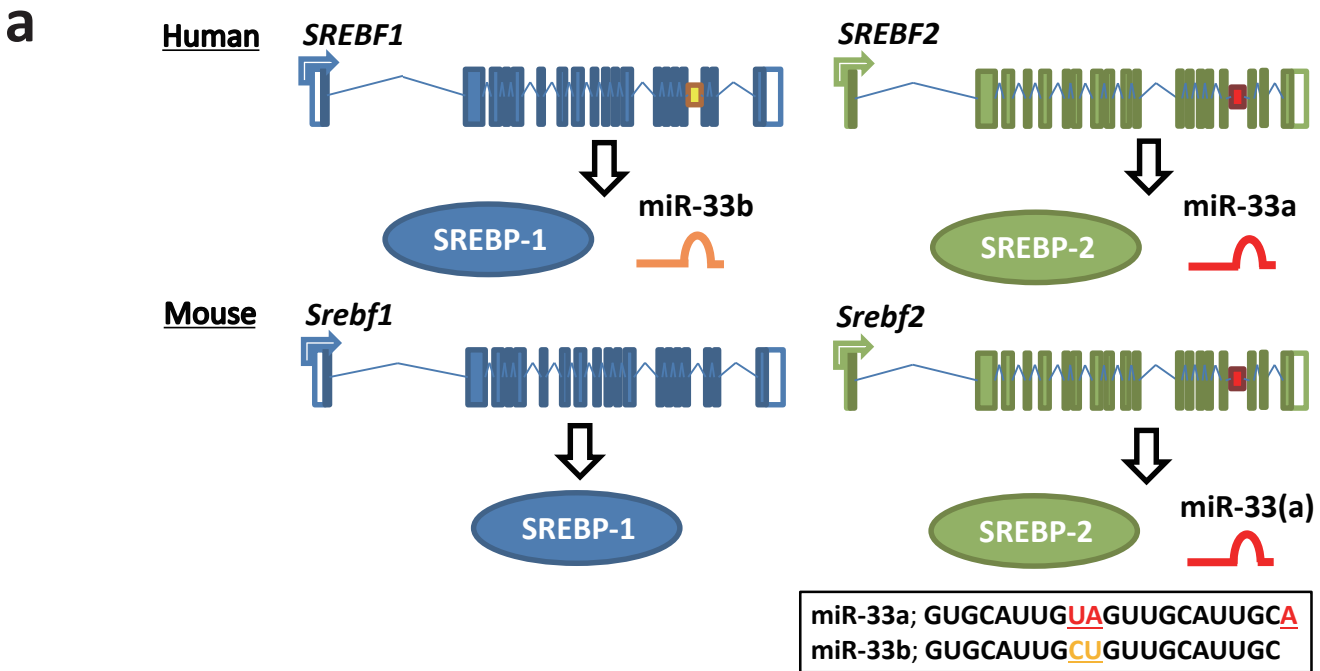
**Supplementary Figure S1. Scheme of *SREBFs* and miR-33s and homologies in exons 16 and 17 of *SREBF1* between human and mouse.** (a) miR-33b is lacking in intron of mouse *Srebf1*. Mature sequences of miR-33a and miR-33b are indicated in the inset. (b) There are high homologies in exons 16 and 17 between human *SREBF1* and mouse *Srebf1* (82.6% of nucleotides and 79.7% of amino acids).
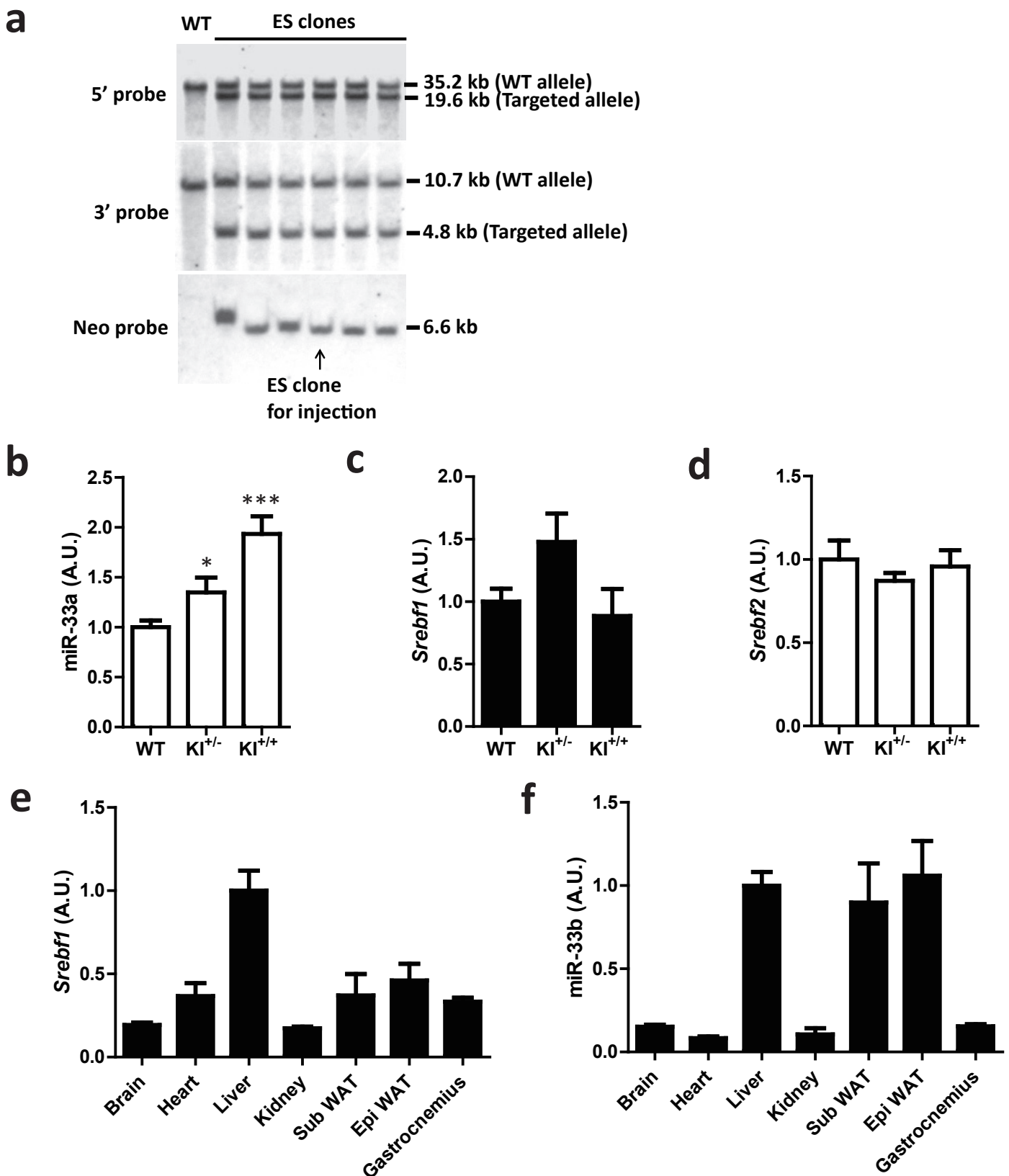
**Supplementary Figure S2. Generation of miR-33b knock-in mice.** (a) Southern blotting analysis of genomic DNA from ES cells that were successfully targeted by a KI vector. Representative images are shown. (b-d) Quantitative real-time PCR analysis of miR-33a (b), *Srebf1* (c) and *Srebf2* (d) in the liver of 8-wk-old WT, KI^{+/-} and KI^{+/+} male mice (n=6). Values are mean ± s.e.m. *p < 0.05 ***p < 0.001 by one-way analysis of variance. (e, f) Quantitative real-time PCR analysis of *Srebf1* (e) and miR-33b (f) in 8-wk-old KI^{+/+} male mice (n=3). Values are mean ± s.e.m. Sub WAT indicates subcutaneous white adipose tissue. Epi WAT indicates epididymal white adipose tissue.
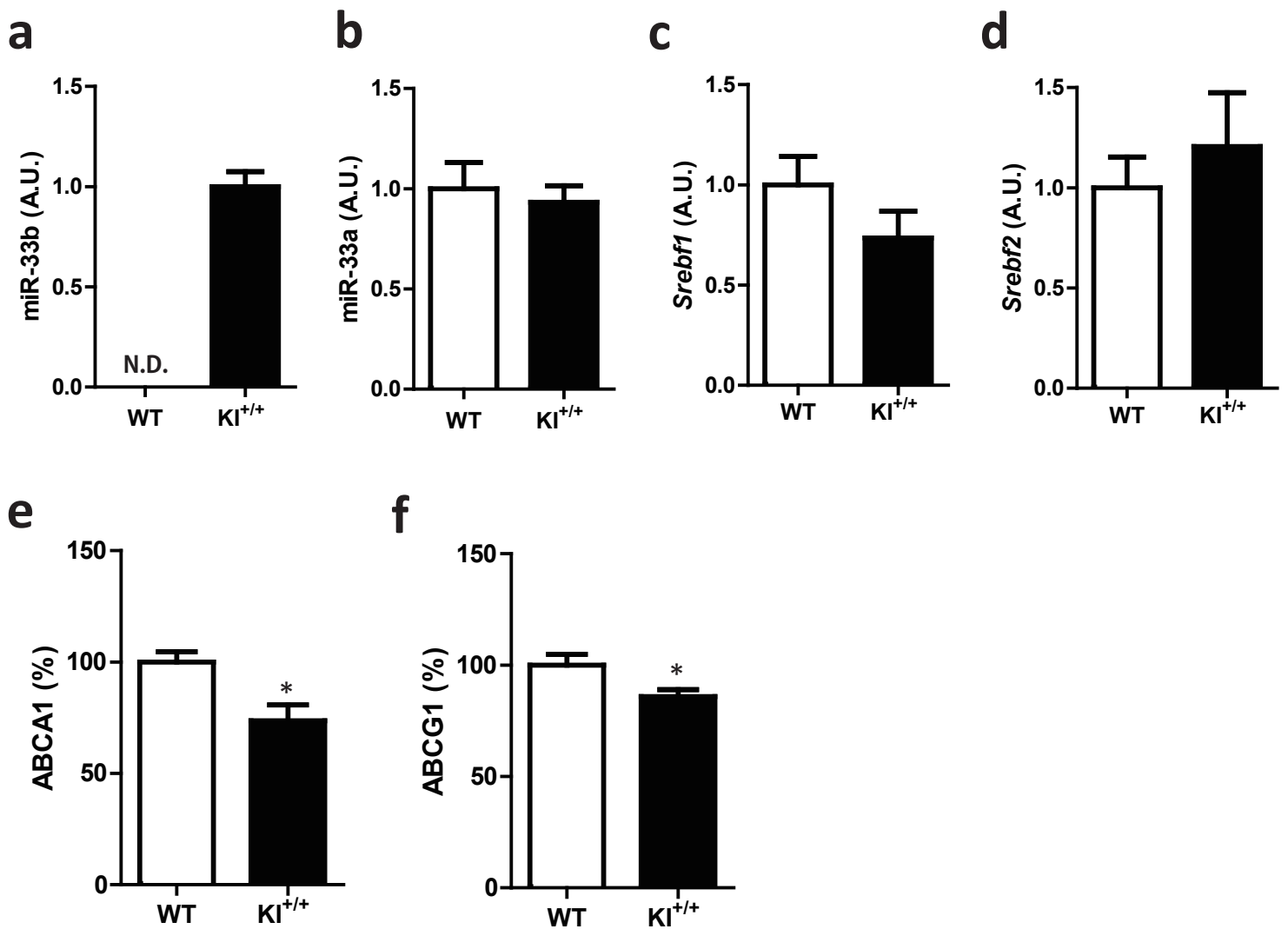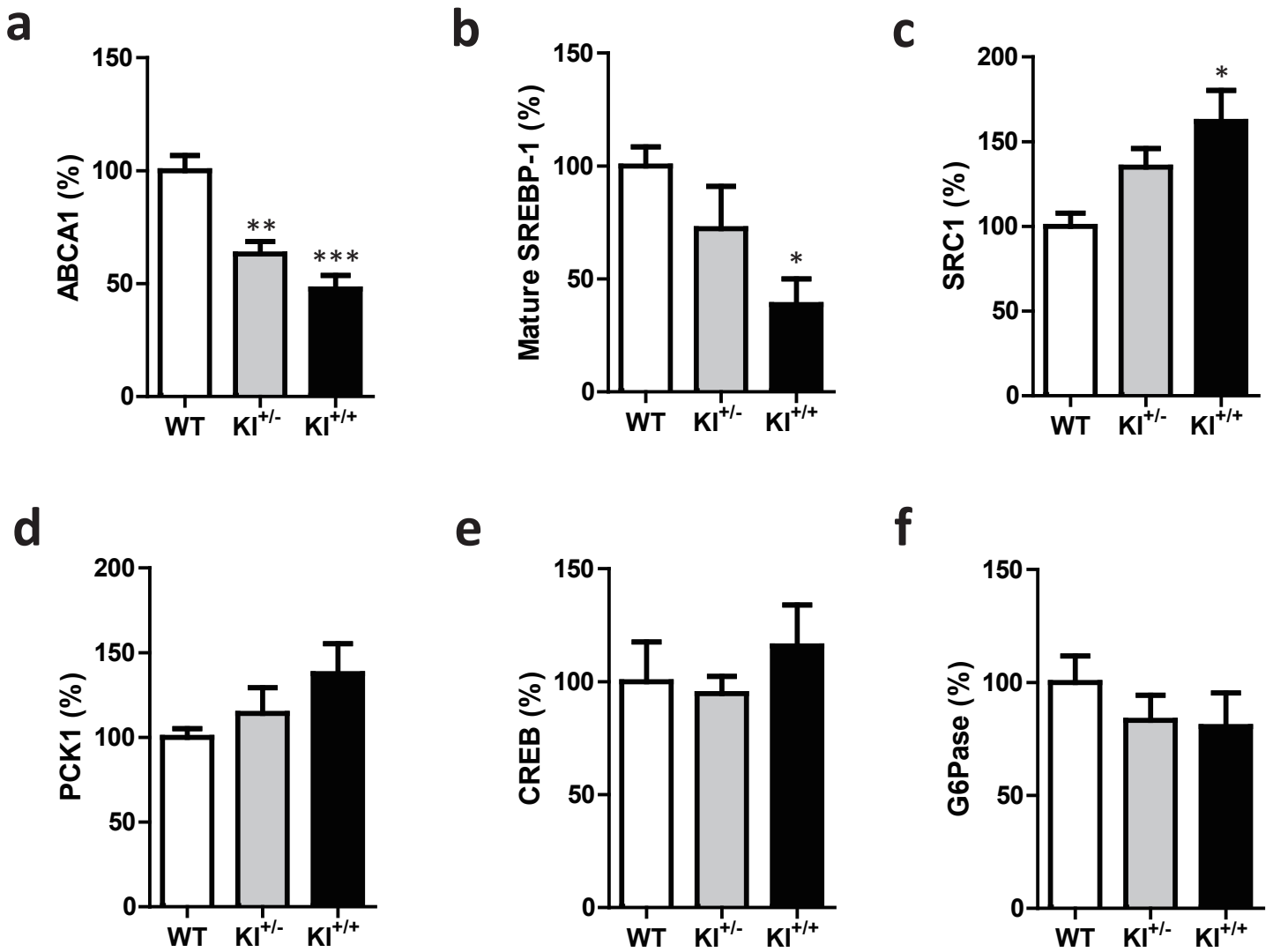
**Supplementary Figure S3. The expression levels of miR-33s and *Srebfs* in KI macrophages.** (a-d)

Quantitative real-time PCR analysis of miR-33b (a), miR-33a (b), *Srebf1* (c) and *Srebf2* (d) in peritoneal

macrophages from 8-wk-old WT and KI$^{+/+}$ male mice (n=3). Values are mean ± s.e.m. N.D., not determined. (e,

f) Densitomeric analysis of ABCA1 (e) and ABCG1 (f) in peritoneal macrophages from 8-wk-old WT and KI$^{+/+}$

male mice. (n = 4). Values are mean ± s.e.m. *p < 0.05 by Student's t-test.
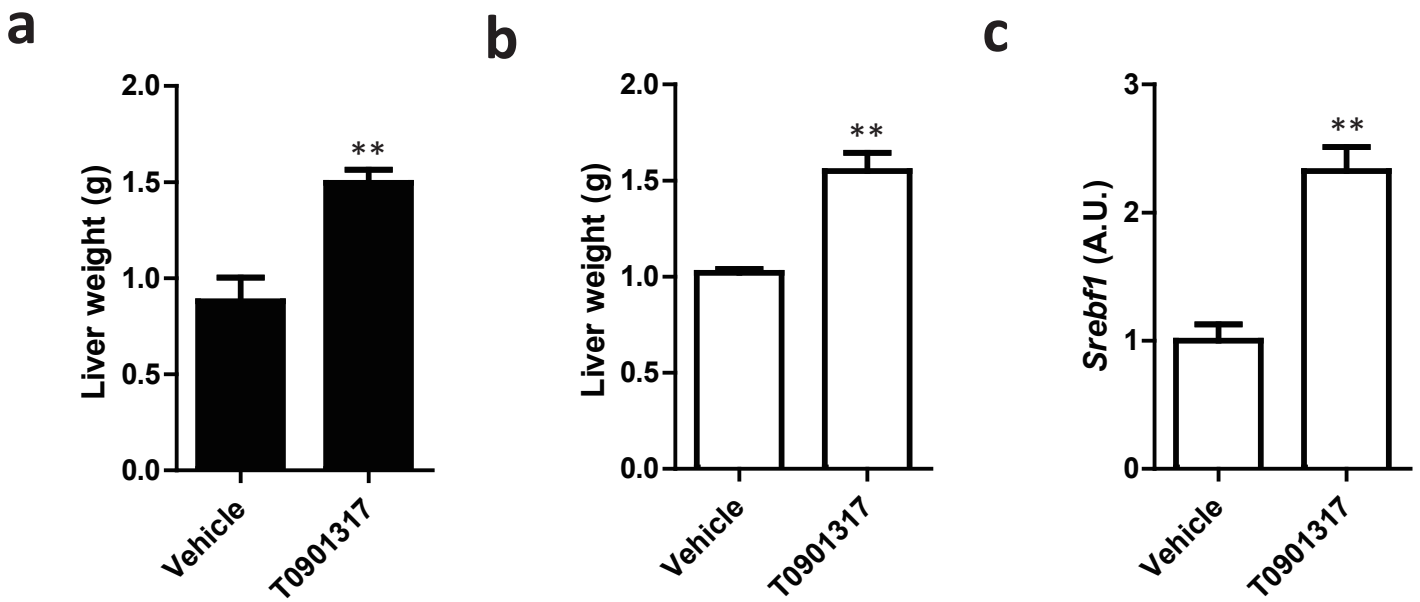
**Supplementary Figure S4. Liver weight and *Srebf1* expression of T0901317-treated mice.** (a) Liver weight

of vehicle or T0901317-treated 8-wk-old KI$^{+/+}$ male mice. Values are the means ± s.e.m. (n = 6, **p<0.01 by

Student's t-test). (b) Liver weight of vehicle or T0901317-treated 8-wk-old WT male mice (n=4, **p<0.01 by

Student's t-test). (c) Quantitative real-time PCR analysis of *Srebf1* in the liver of vehicle or T0901317-treated

8-wk-old WT male mice (n=4, **p< 0.01 by Student's t-test).

**Supplementary Figure S5. The expression levels of cholesterol and glucose metabolic genes.** The protein expression levels of ABCA1 (a), SREBP-1 (b), SRC1 (c), PCK1 (d), CREB (e) and G6Pase (f) in the liver of 8-wk-old WT, $KI^{+/-}$ and $KI^{+/+}$ male mice by densitometry. The expression levels were normalized with β-actin or TF2B expressions. The expression levels of WT mice were set at 100%. Values are mean ± s.e.m. *p < 0.05, **p<0.01 ***p < 0.001 compared with WT by one-way analysis of variance (a-d; n=6, e and f; n=3).
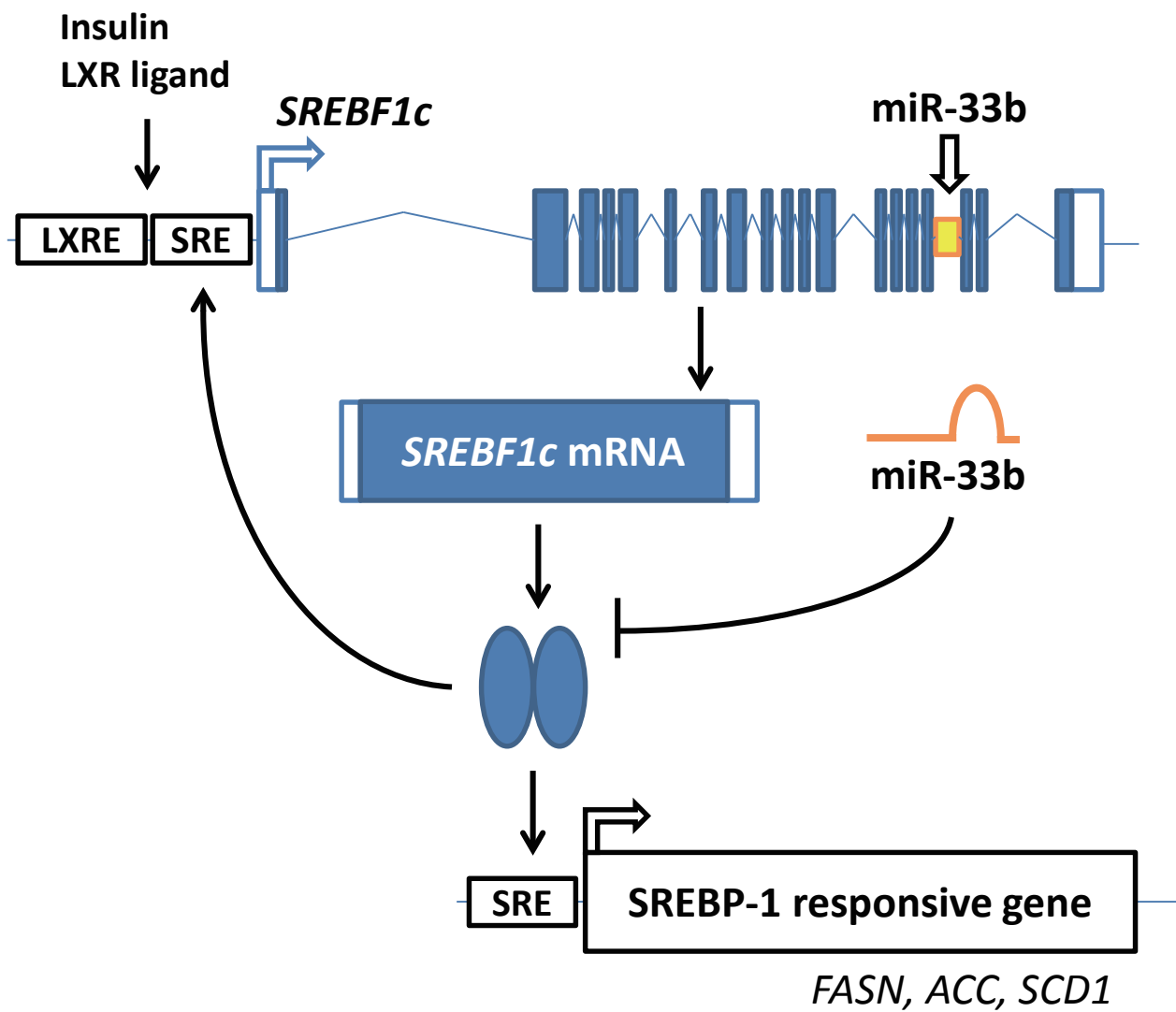
**Supplementary Figure S6. Proposed feedback mechanism to regulate SREBP-1 level by intronic miR-33b.**
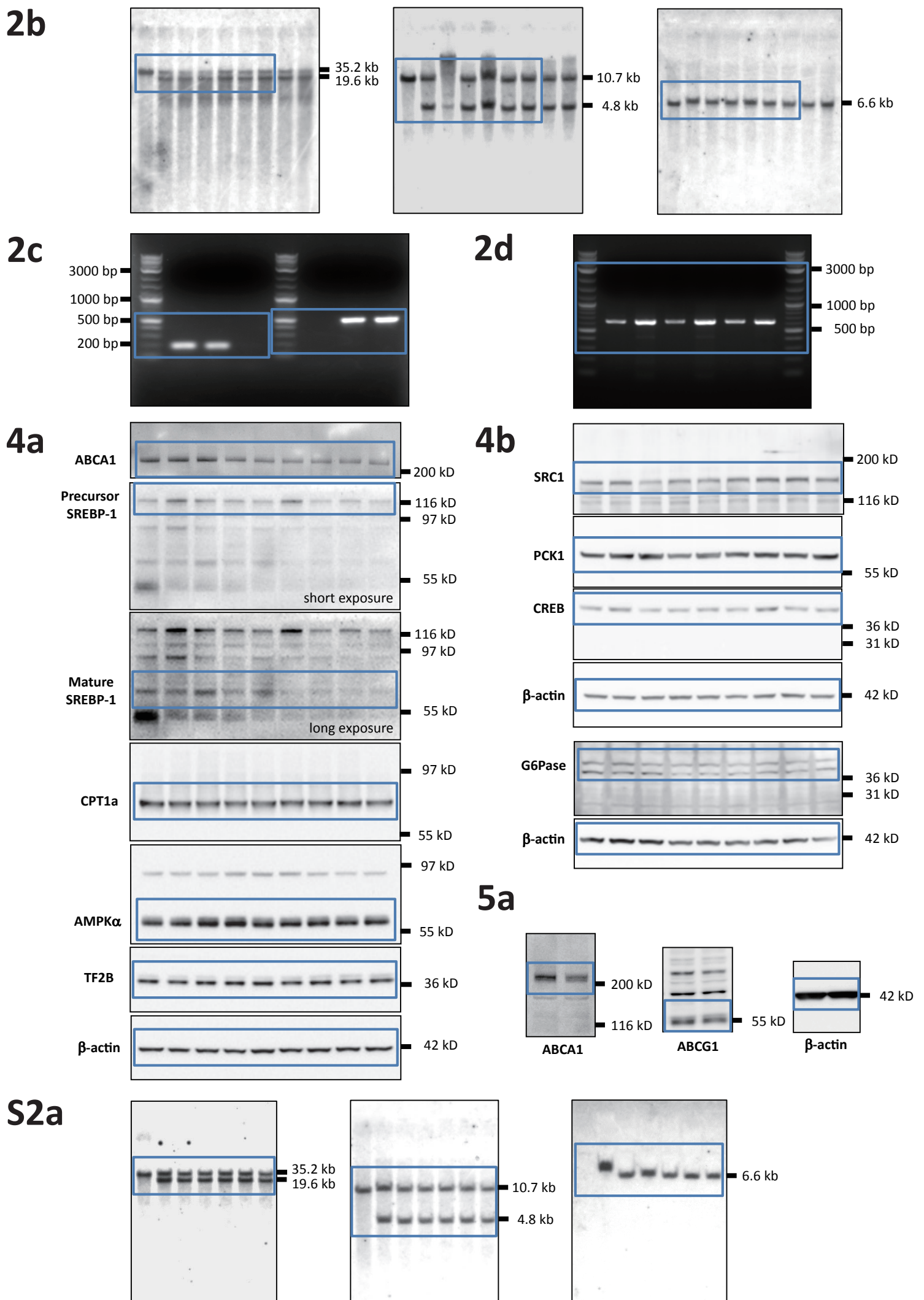
**Supplementary Figure S7. Scans of the original blots in figure 2b, 2c, 2d, 4a, 4b, 5a and S2a.**