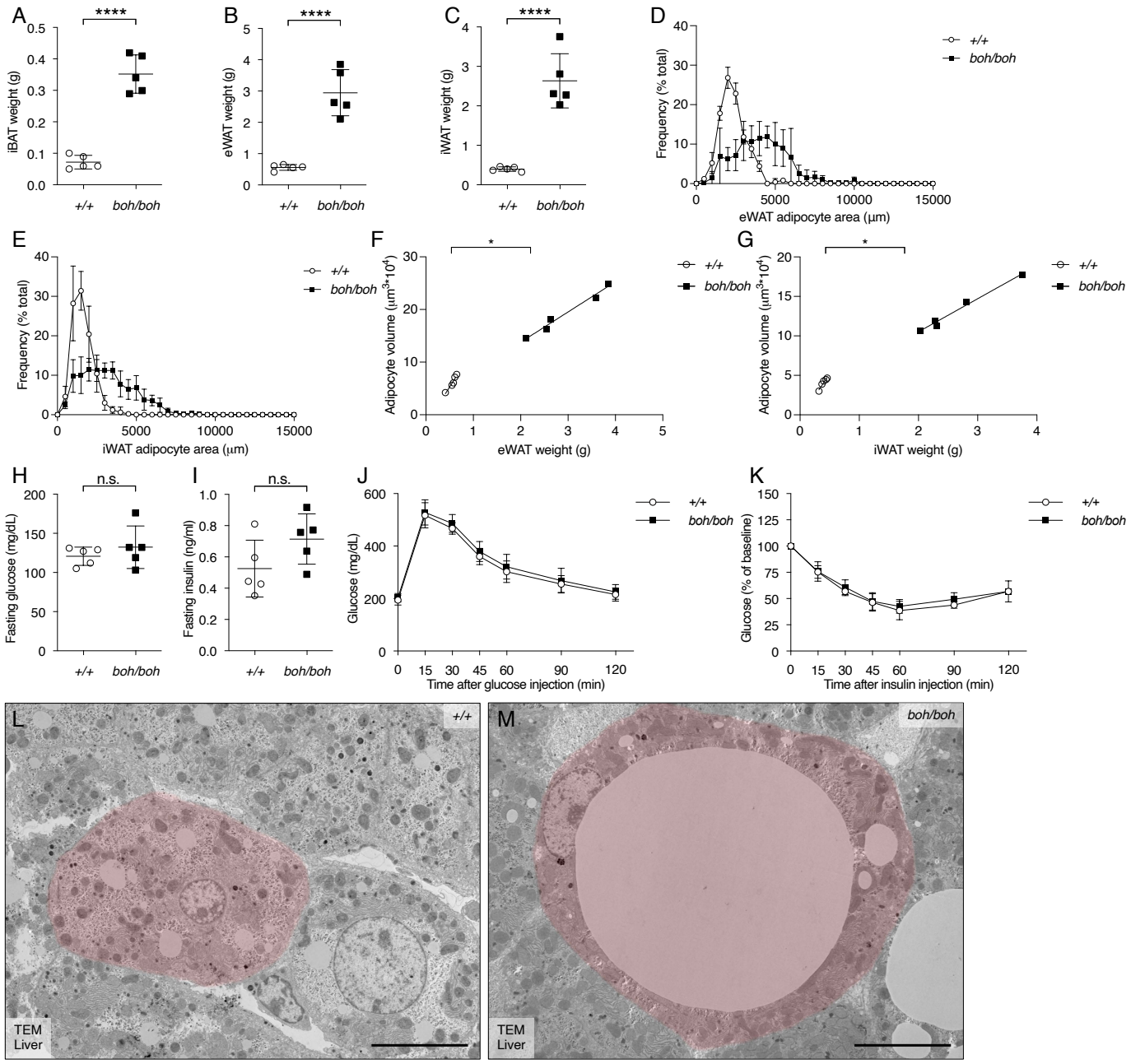


Figure S1. The body weight, adipocyte quantification, and lipid accumulation in livers of *boh* mice, related to Figure 1

(A-C) Weight of iBAT (A), eWAT (B), and iWAT (C) from 16-week-old male mice.

(D-G) Quantification of adipocyte area and adipocyte volume from H&E sections of 16-week-old male homozygous *boh* mice and WT littermates.

(H and I) Serum glucose (H) and insulin (I) in 5-week-old male mice after a 6-h fast.

(J) Glucose tolerance test. Blood glucose was measured at indicated times after i.p. glucose injection in 5-week-old male mice (n=5).

(K) Insulin tolerance test. Blood glucose was measured at indicated times after i.p. insulin injection in 5-week-old male mice (n=5). The baseline blood glucose levels (0 min) of *boh/boh* and WT littermates were 211 ± 28 mg/dL and 219 ± 23 mg/dL, respectively.

(L and M) Representative TEM images of liver sections from an 18-week-old female homozygous *boh* mouse (M) and a WT littermate (L). The boundary of a single liver cell is highlighted in red color. Scale bar: 10 μm.

Data are presented as means ± SD. *P* values were determined by Student's *t* test (A-C, H, and I). A linear correlation with a two-tailed comparison of slope and intercept was calculated and compared between different mouse groups (F and G). *P* values are denoted by * $P \leq 0.05$; **** $P \leq 0.0001$; ns, not significant with $P > 0.05$. Data points represent individual mice (A-C, F-I). Data are representative of two independent experiments (A-K) or one experiment (L and M).

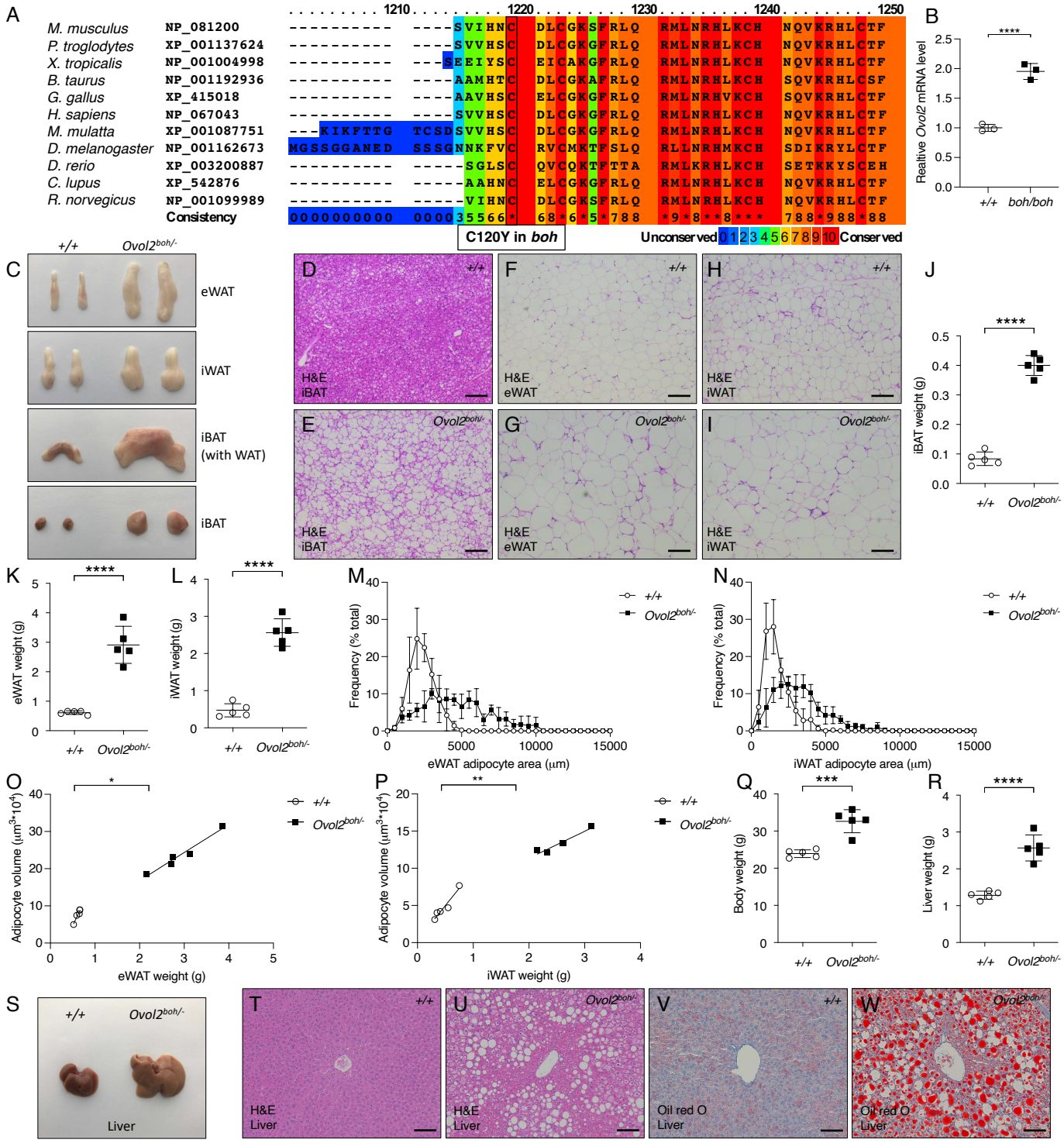


Figure S2. The *boh* phenotype is caused by a conserved mutation in *Ovol2*, related to Figure 2

(A) *Boh* mutation is highly conserved among different species.

(B) Relative *Ovol2* mRNA level in iWAT of 12-week-old male *Ovol2*$^{boh/boh}$ mice and WT littermates (mRNA level was normalized to *Polr2a*).

(C) Representative photographs of eWAT, iWAT, iBAT with WAT, and iBAT from 16-week-old male mice.

(D-I) H&E staining of sections from different adipose tissues of 16-week-old male mice. Scale bar: 100 μm.

(J-L) Weight of iBAT (J), eWAT (K), and iWAT (L) from 16-week-old male mice.

(M-P) Quantification of adipocyte area and adipocyte volume from H&E sections of 16-week-old male mice.

(Q and R) Body weight (Q) and liver weight (R) of 16-week-old male mice.

(S) Representative photographs of liver from 16-week-old male mice.

(T-W) Liver sections of 16-week-old male mice stained with H&E (T and U) and Oil Red O (V and W). Scale bar: 100 μm.

Data are presented as means ± SD. *P* values were determined by Student's *t* test (B, J-L, Q, and R). A linear correlation with a two-tailed comparison of slope and intercept was calculated and compared between different mouse groups (O and P). *P* values are denoted by * $P \le 0.05$; ** $P \le 0.01$; *** $P \le 0.001$; **** $P \le 0.0001$. Data points represent individual mice (B, J-L, O-R). Data are representative of two independent experiments (B-S) or one experiment (T-W).

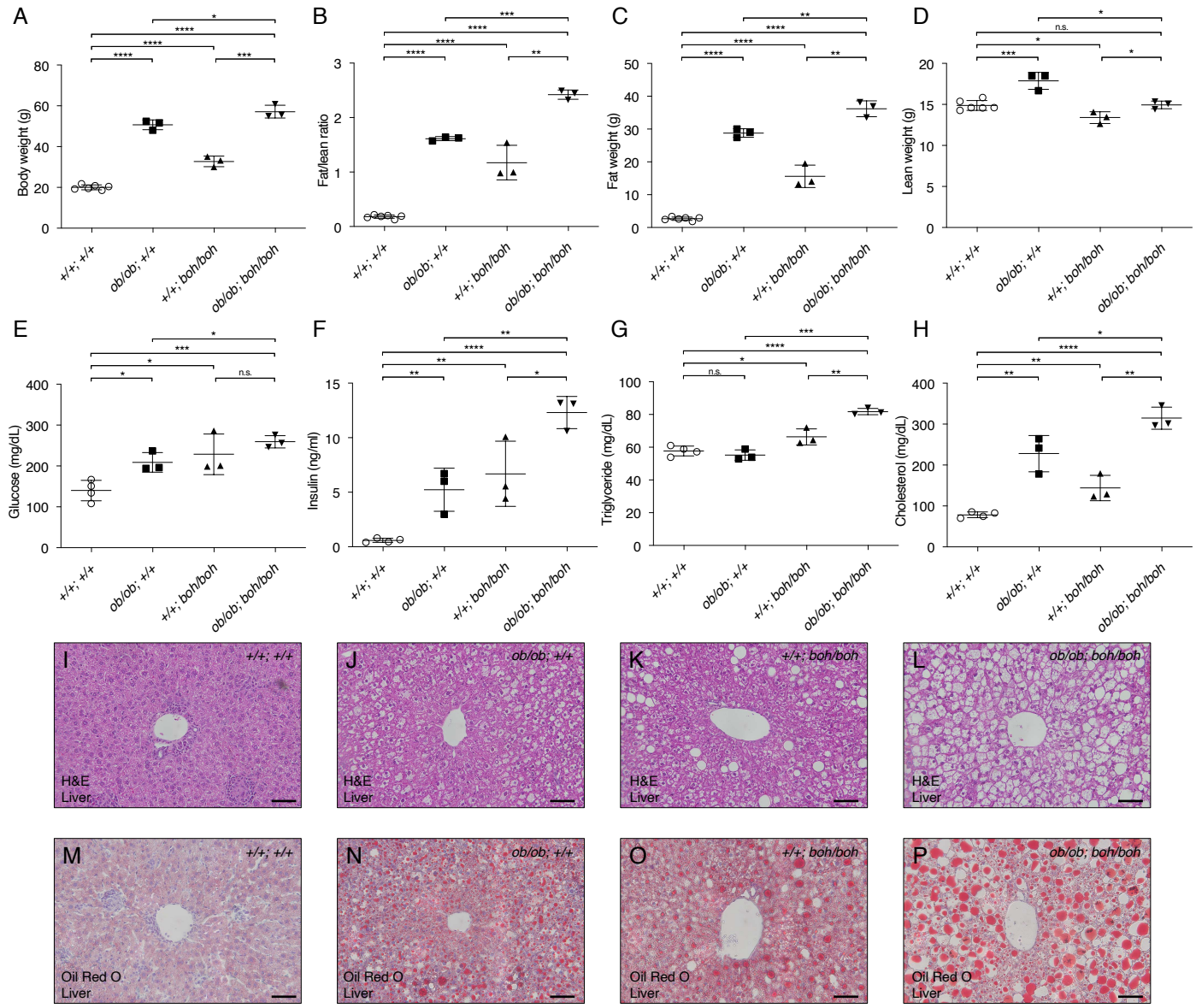


Figure S3. The *boh* mutation further increases obesity in *ob* mutant mice, related to Figure 3

(A-D) Body weight (A), fat to lean weight ratio (B), fat weight (C), and lean weight (D) of 16-week-old female mice.

(E-H) Serum glucose (E), insulin (F), triglyceride (G), and cholesterol (H) in 16-week-old female mice after a 6-h fast.

(I-P) Liver sections of 16-week-old female mice stained with H&E (I-L) and Oil Red O (M-P). Scale bar: 100 μm.

Data are presented as means ± SD. *P* values were determined by one-way ANOVA with Tukey's multiple comparison test. *P* values are denoted by * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$; ns, not significant with $P > 0.05$. Data points represent individual mice (A-H). Data are representative of two independent experiments (A-H) or one experiment (I-P).

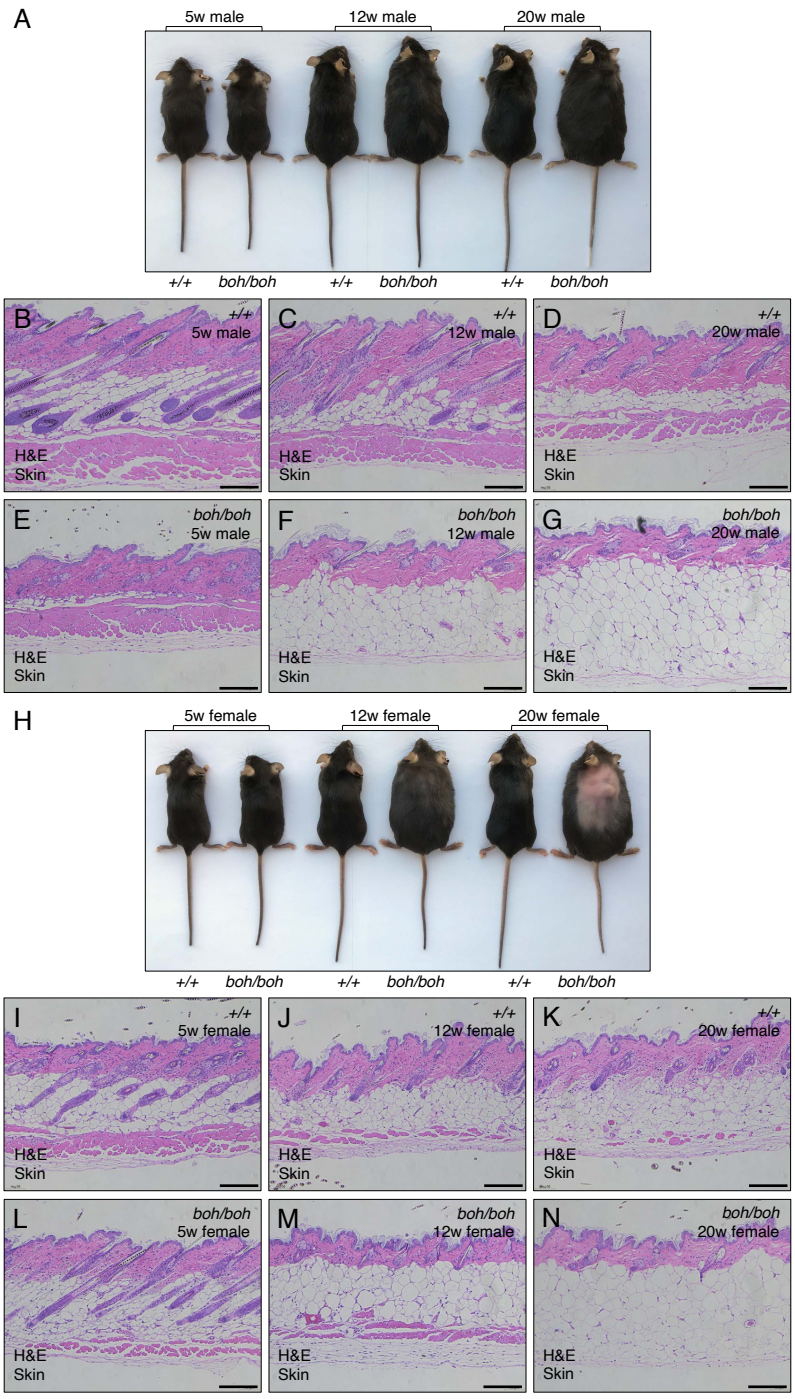


Figure S4. Changes of skin from WT and *Ovol2*$^{boh/boh}$ mice at different ages, related to Figure 4

(A) Photograph of male *Ovol2*$^{boh/boh}$ mice and WT littermates at different ages (week).

(B-G) H&E staining of skin sections from male *Ovol2*$^{boh/boh}$ mice and WT littermates at different ages (week).

(H) Photograph of female *Ovol2*$^{boh/boh}$ mice and WT littermates at different ages (week).

(I-N) H&E staining of skin sections from female *Ovol2*$^{boh/boh}$ mice and WT littermates at different ages (week).

Data are from one experiment.

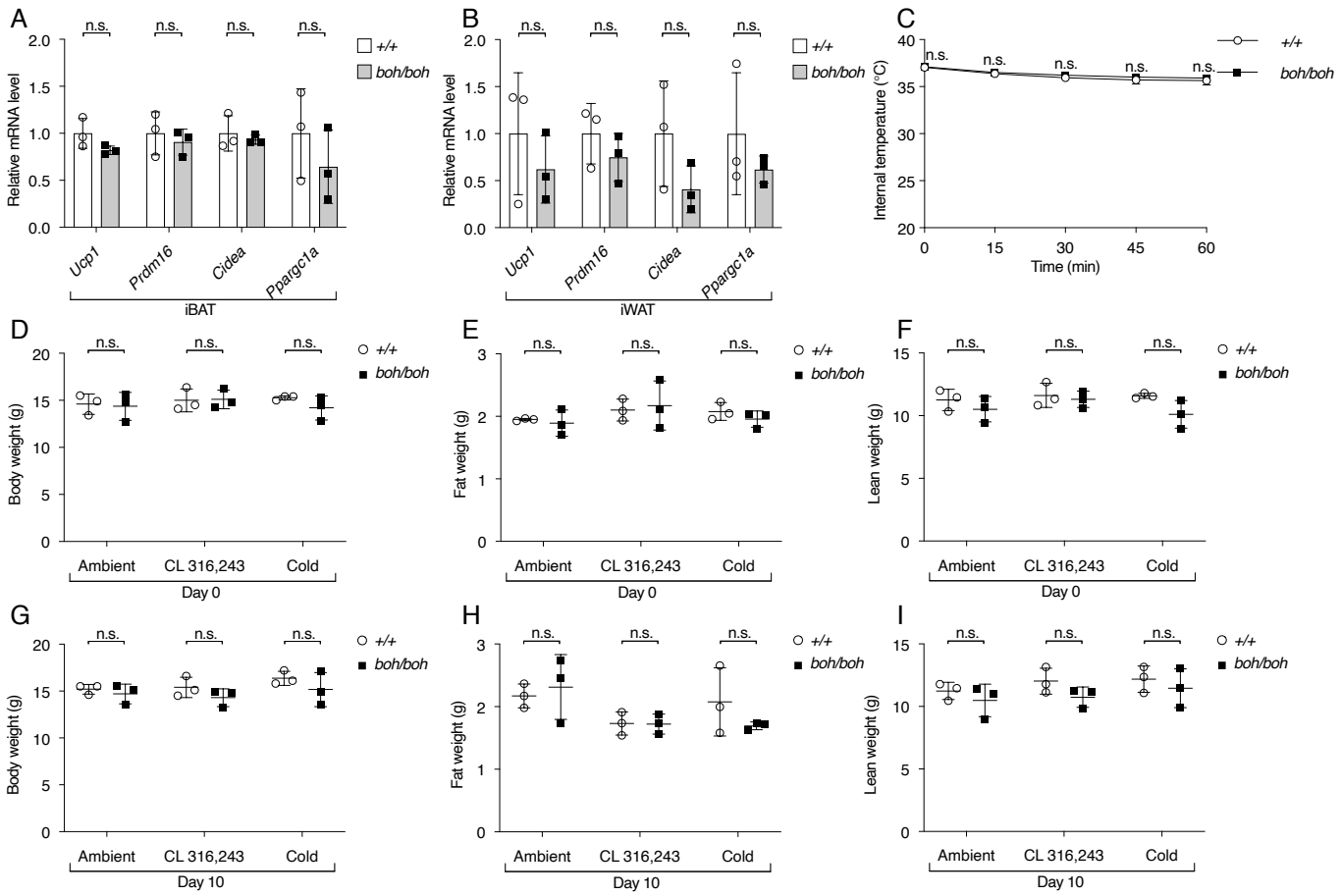


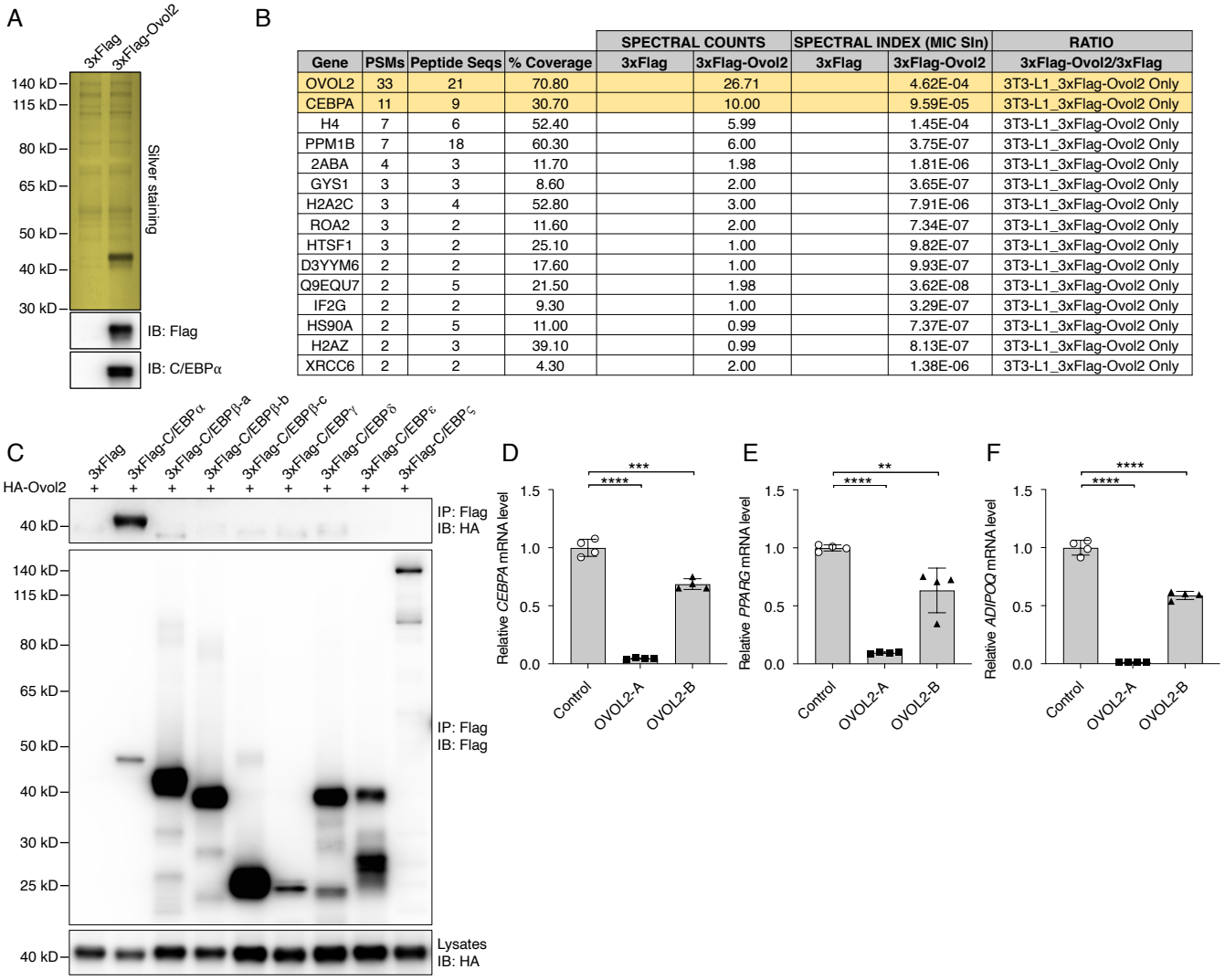
Figure S5. The expression of thermogenic genes, acute cold tolerance, and body composition of 5-week-old *Ovol2*$^{boh/boh}$ mice after chronic cold challenge, related to Figure 5

(A and B) Relative mRNA level of thermogenic genes in iBAT (A) and iWAT (B) of 5-week-old female mice (mRNA level was normalized to *Polr2a* and shown as fold change to WT controls).

(C) Internal temperature change of 5-week-old female mice housed at cold environment in the absence of food (n=4).

(D-I) Body weight, fat weight, and lean weight of 5-week-old female mice that were untreated (D-F) or treated with different conditions for 10 days (G-I).

Data are presented as means ± SD. *P* values were determined by Student's *t* test. *P* values are denoted by ns, not significant with $P > 0.05$. Data points represent individual mice (A, B, and D-I). Data are representative of two independent experiments (A-C) or one experiment (D-I).



Gene	PSMs	Peptide Seqs	% Coverage	SPECTRAL COUNTS 3xFlag	SPECTRAL COUNTS 3xFlag-Ovol2	SPECTRAL INDEX (MIC SIn) 3xFlag	SPECTRAL INDEX (MIC SIn) 3xFlag-Ovol2	RATIO 3xFlag-Ovol2/3xFlag
OVOL2	33	21	70.80		26.71		4.62E-04	3T3-L1_3xFlag-Ovol2 Only
CEBPA	11	9	30.70		10.00		9.59E-05	3T3-L1_3xFlag-Ovol2 Only
H4	7	6	52.40		5.99		1.45E-04	3T3-L1_3xFlag-Ovol2 Only
PPM1B	7	18	60.30		6.00		3.75E-07	3T3-L1_3xFlag-Ovol2 Only
2ABA	4	3	11.70		1.98		1.81E-06	3T3-L1_3xFlag-Ovol2 Only
GYS1	3	3	8.60		2.00		3.65E-07	3T3-L1_3xFlag-Ovol2 Only
H2A2C	3	4	52.80		3.00		7.91E-06	3T3-L1_3xFlag-Ovol2 Only
ROA2	3	2	11.60		2.00		7.34E-07	3T3-L1_3xFlag-Ovol2 Only
HTSF1	3	2	25.10		1.00		9.82E-07	3T3-L1_3xFlag-Ovol2 Only
D3YYM6	2	2	17.60		1.00		9.93E-07	3T3-L1_3xFlag-Ovol2 Only
Q9EQU7	2	5	21.50		1.98		3.62E-08	3T3-L1_3xFlag-Ovol2 Only
IF2G	2	2	9.30		1.00		3.29E-07	3T3-L1_3xFlag-Ovol2 Only
HS90A	2	5	11.00		0.99		7.37E-07	3T3-L1_3xFlag-Ovol2 Only
H2AZ	2	3	39.10		0.99		8.13E-07	3T3-L1_3xFlag-Ovol2 Only
XRCC6	2	2	4.30		2.00		1.38E-06	3T3-L1_3xFlag-Ovol2 Only

Figure S6. Mass spectrometry identified C/EBPα as a specific interacting protein of OVOL2 among C/EBP family proteins, related to Figure 6

(A) Mass spectrometry identification of OVOL2 interacting proteins. Silver staining (Top) and immunoblot analysis (Middle and Bottom) of immunoprecipitates of 3T3-L1 adipocytes expressing 3xFLAG-tagged OVOL2.

(B) Mass spectrometry identified hits that were only found in 3xFLAG-OVOL2 immunoprecipitates.

(C) Immunoblot analysis of immunoprecipitates (Top and Middle) or lysates (Bottom) of 293T cells expressing HA-tagged Ovol2 and 3xFLAG-tagged C/EBP family proteins.

(D-F) Relative mRNA level of human adipocyte markers 14 days after primary human subcutaneous pre-adipocyte differentiation with overexpression of control or OVOL2 isoforms.

Data are presented as means ± SD. *P* values were determined by one-way ANOVA with Tukey's multiple comparison test. *P* values are denoted by ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$. Data are representative of two independent experiments (C-F) or one experiment (A and B).

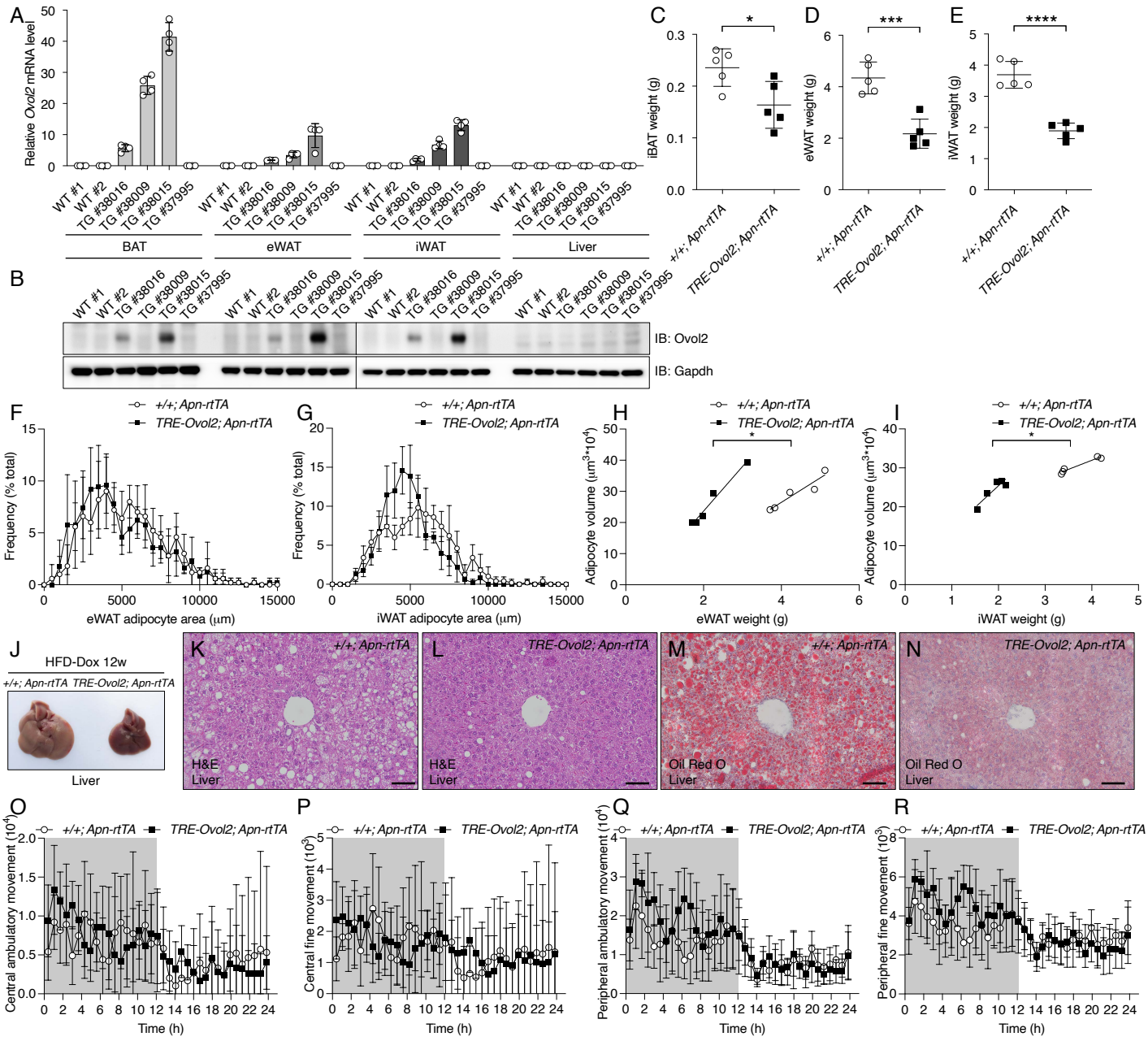


Figure S7. Verification and testing of inducible adipocyte-specific OVOL2 expression mouse model, related to Figure 7

(A) *Ovol2* mRNA levels normalized to *Polr2a* mRNA in different tissues of male mice after 4 weeks high-fat diet doxycycline feeding.

(B) Immunoblots of lysates of different tissues of male mice after 4 weeks high-fat diet doxycycline feeding.

(C-E) Weight of iBAT (C), eWAT (D), and iWAT (E) from 12 weeks HFD-Dox feeding mice.

(F-I) Quantification of adipocyte area and adipocyte volume from H&E sections of 12 weeks HFD-Dox feeding mice.

(J) Representative photographs of liver from 12 weeks HFD-Dox feeding mice.

(K-N) Liver sections of 12 weeks HFD-Dox feeding mice stained with H&E (K and L) and Oil Red O (M and N). Scale bar: 100 μm.

(O-R) Metabolic cage measurements of central ambulatory movement (O), central fine movement (P), peripheral ambulatory movement (Q), and peripheral fine movement (R) of 12 weeks HFD-Dox feeding mice (n=6) housed at 23 °C.

Data are presented as means ± SD. *P* values were determined by Student's *t* test (C-E). A linear correlation with a two-tailed comparison of slope and intercept was calculated and compared between different mouse groups (H and I). *P* values are denoted by * $P \le 0.05$; *** $P \le 0.001$; **** $P \le 0.0001$. Data points represent individual mice (C-E, H, and I). Data are representative of two independent experiments (A-E) or one experiment (F-R).

Table S1. Primers used for quantitative PCR, related to STAR Methods

Target name	Forward primer (5'-3')	Reverse primer (5'-3')
Ovol2	AATCAAGTTTACCACCGGCA	CTCTTCAGGTCGAAGGTGTC
Polr2a	CAAGATGCAAGAGGAGGAAGAG	TGTTGTCTGTCTGAGGTAAGTG
Ucp1	AAATACTGGCAGATGACGTC	GTACAATCCACTGTCTGTCTG
Prdm16	CCATTCATATGCGAGGTCTG	GTGTAATGGTTCTTGCCCTC
Cidea	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG
Ppargc1a	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
Fabp4	CCAGGGAGAACCAAAGTTGAG	CTGACCATGTGACTGTAGGAG
Glut4	GGCTCTATGTCATCCTGCTG	CTTGGACTGCGAAATTTCTG
ACTB	CACCACACCTTCTACAATGAG	GTCTCAAACATGATCTGGGTC
CEBPA	GTTCTACATGAAGGTGGAGG	CTAGCTTTCTGGTGTGACTC
PPARG	GTAATGGAAGACCACTCCCAC	CACTTTGATTGCACTTTGGTACTC
ADIPOQ	CAGGAAACCACGACTCAAGG	GGACCAATAAGACCTGGATCTC