SUPPLEMENTARY INFORMATION

Yaghootkar H, Zhang Y, Spracklen CN, Karaderi T, Huang LO, Bradfield J, et al.
Genetic studies of leptin concentrations implicate leptin in the regulation of early adiposity

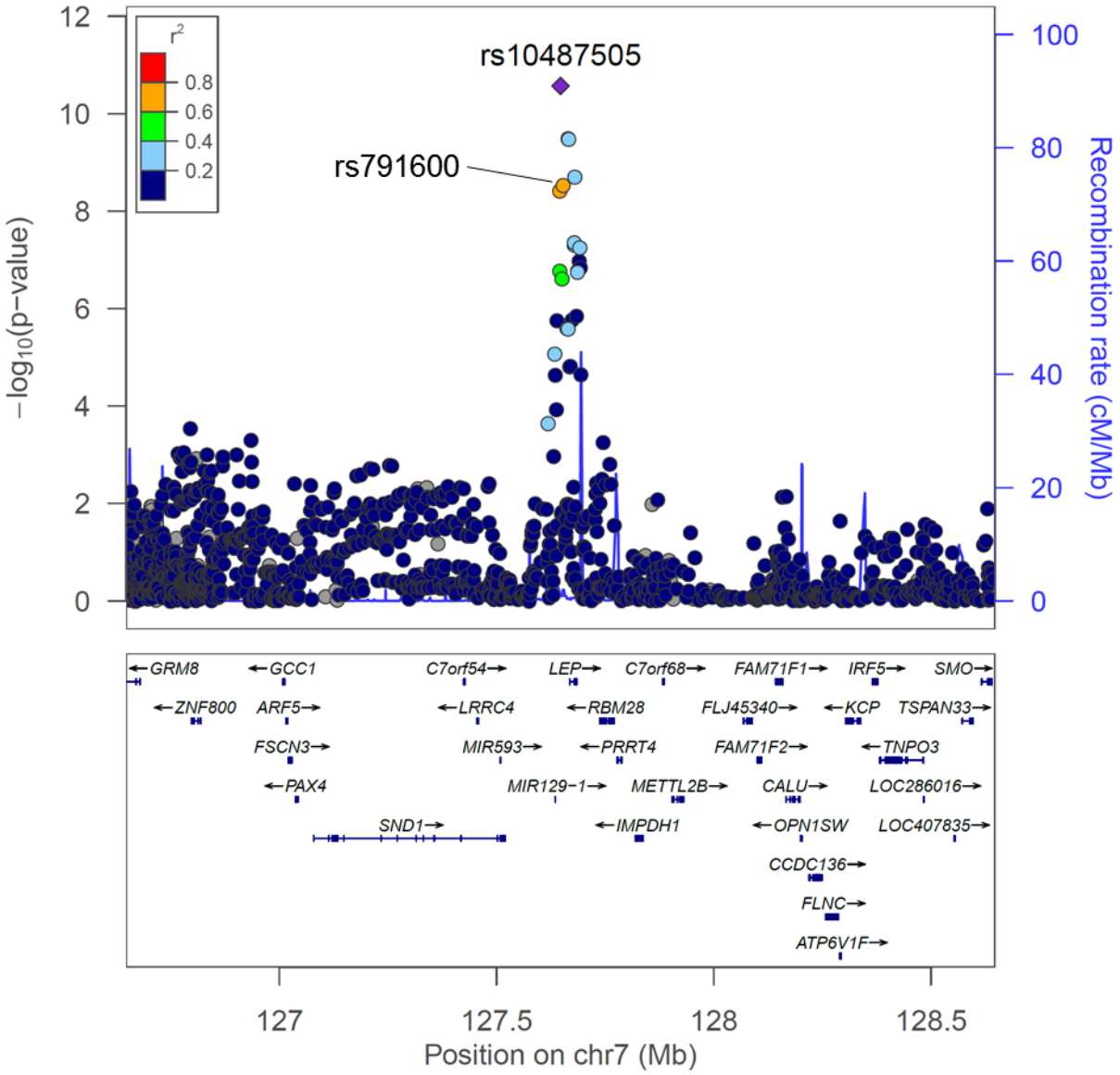


Figure S1. Association of rs10487505 and rs791600 variants near *LEP* with leptin concentrations adjusted for BMI in a genome-wide association study of up to 32,161 individuals of European ancestry (Kilpeläinen et al., 2016).

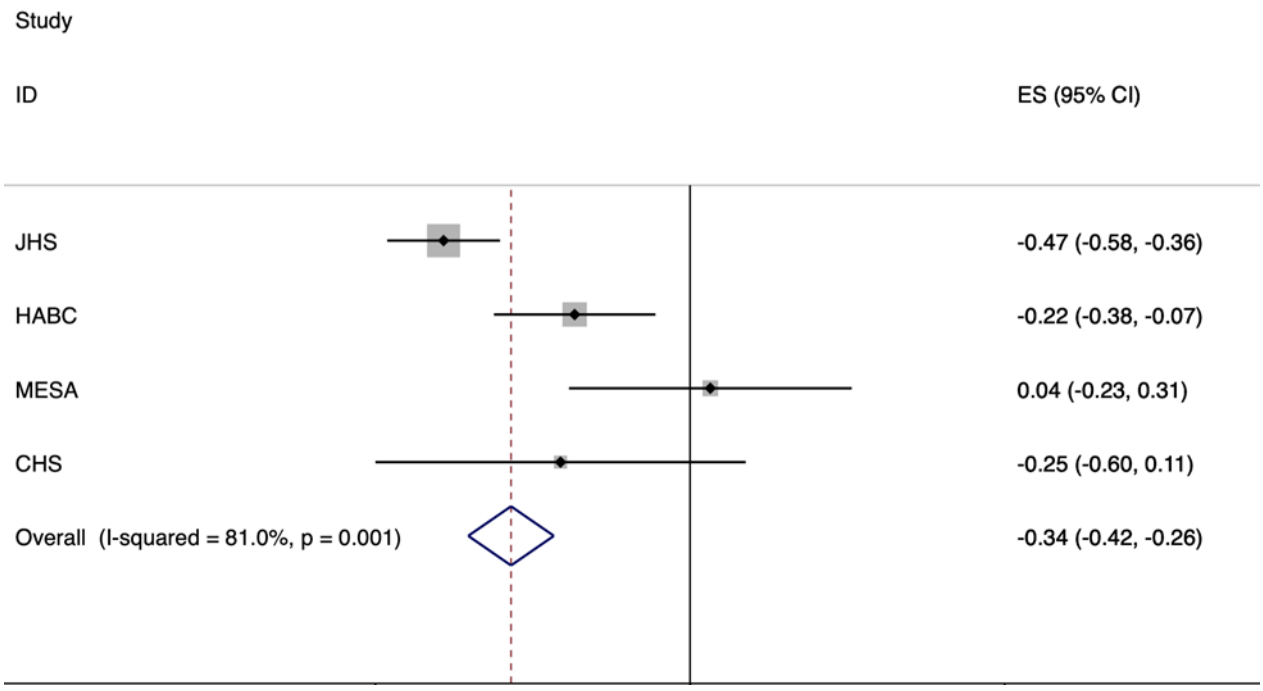


Figure S2. Meta-analysis of the association of the Met94 allele of rs17151919 with leptin concentrations adjusted for BMI in cohorts of African ancestry.

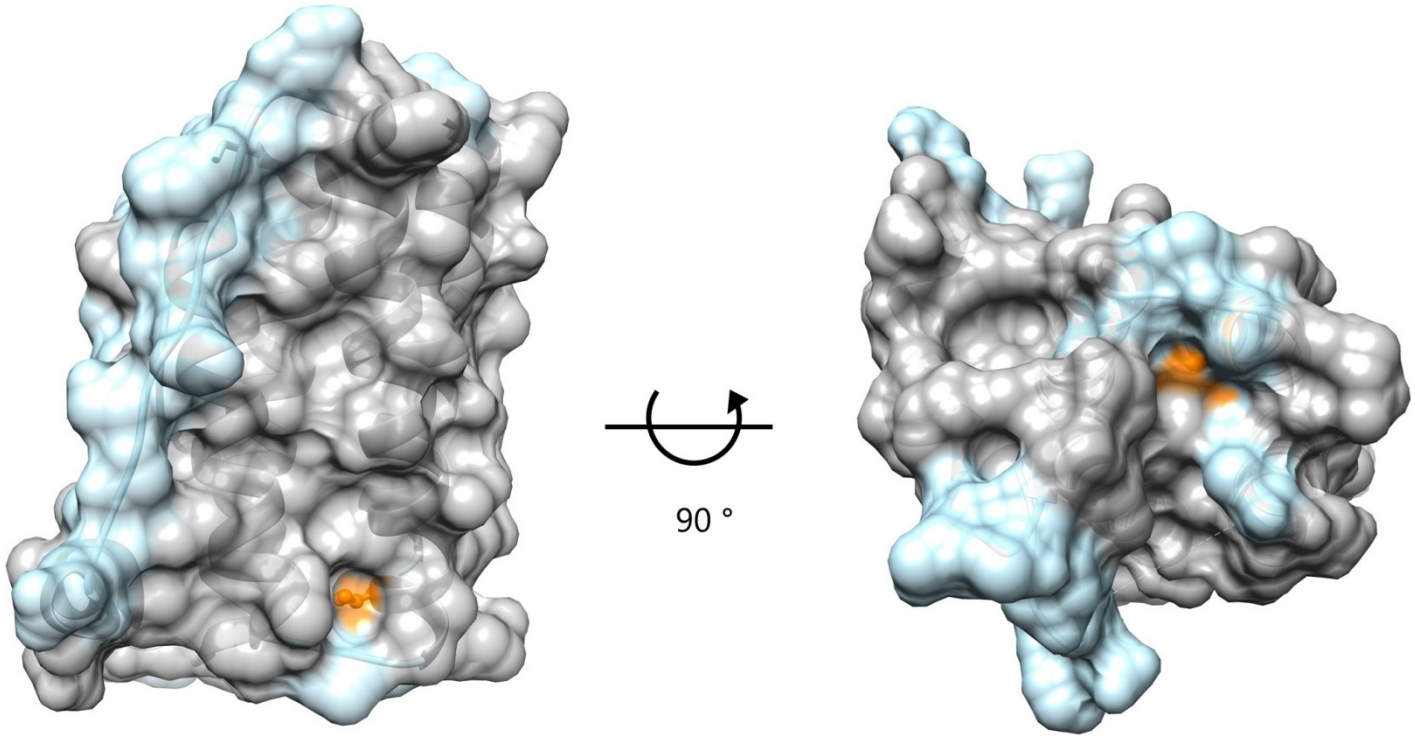
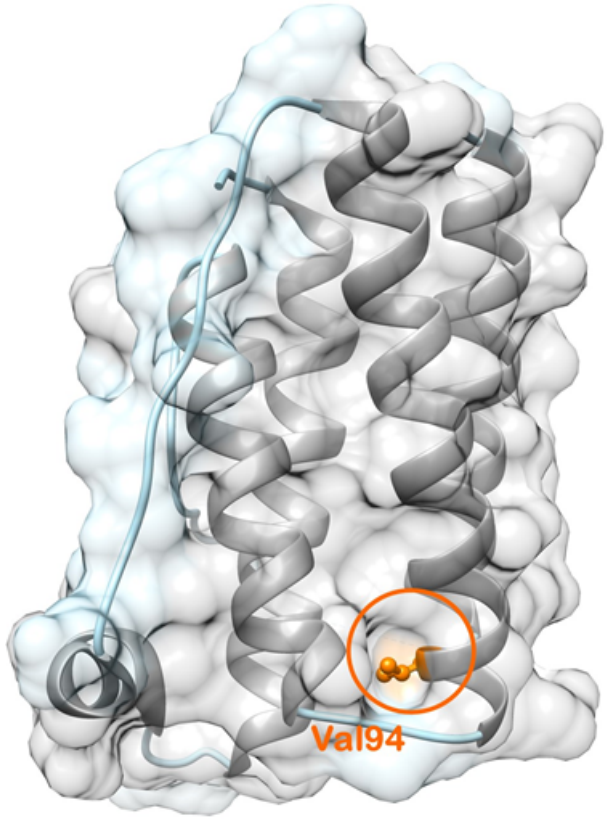
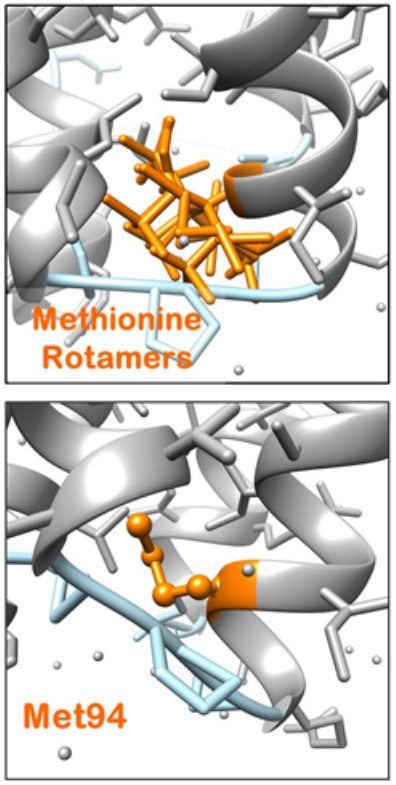


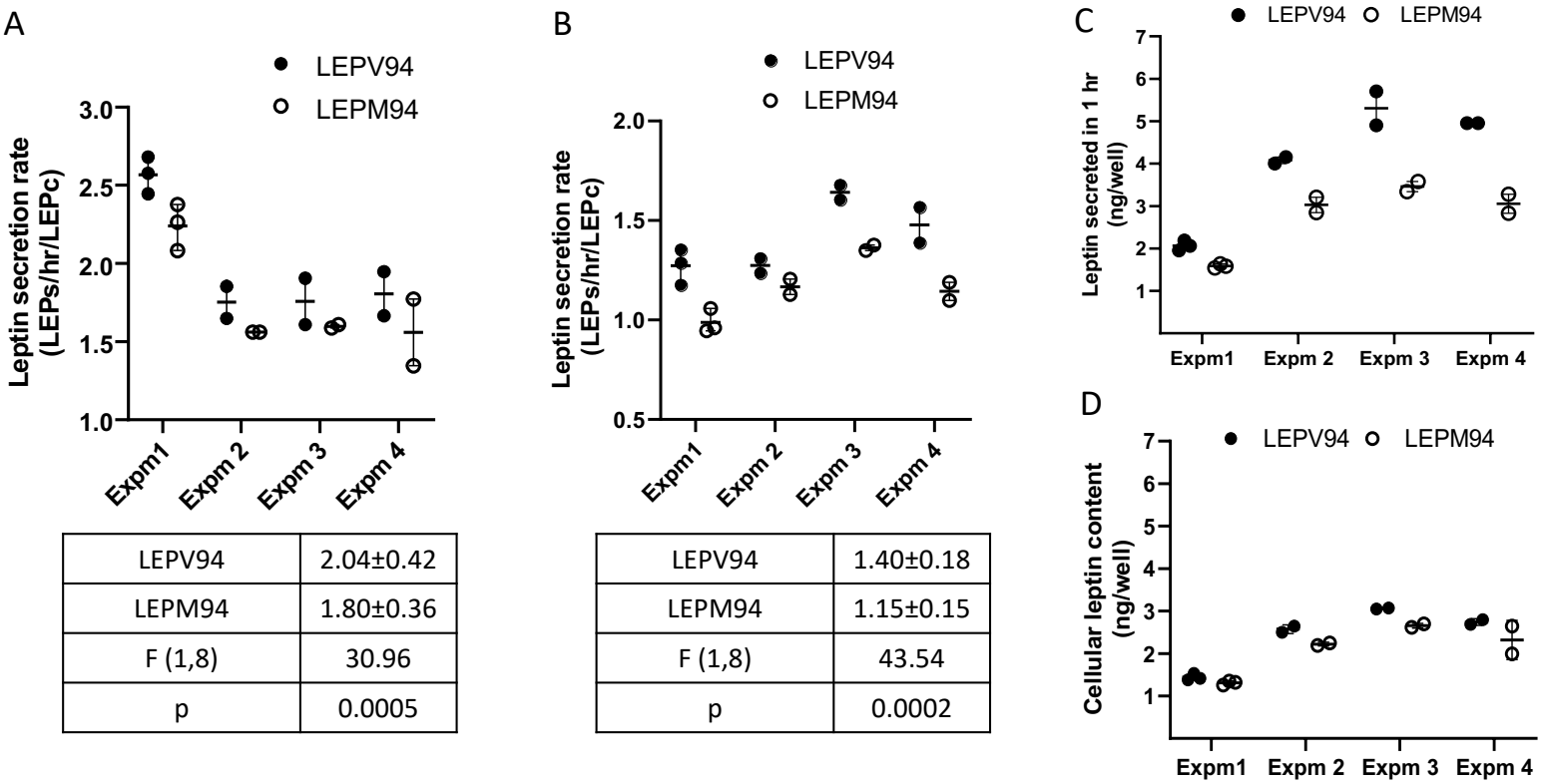
Figure S3: Surface region of the leptin protein with the Val94Met position (Val73Met in the mature protein) highlighted.





Chi 1	Chi 2	Probability	Clashes
-68.8	-59.1	0.2023	4
-68.3	175.6	0.1882	5
-70.1	-171.9	0.1331	3
-174.5	60.7	0.0947	5
-68.7	178.8	0.0923	2
-65.7	-60.3	0.0562	5
-179.2	-179.3	0.0534	9
-173.6	176.5	0.0512	5
-66.7	-63.1	0.0412	5
-170.7	65.2	0.0223	8
-175.7	176.7	0.0218	11
179.8	-84.1	0.0182	5
-169.7	67.0	0.0086	6
69.3	-176.2	0.0042	14

Figure S4: Leptin structure and the predicted impact of mutagenesis in position 73 from valine to methionine. The Rotamer list on the left shows sidechain torsions (Chi 1 and 2), with the probability and number of interatomic clashes, i.e. unfavourable interactions where atoms are too close together. On the right, the lower picture shows all possibilities for sidechain torsions when methionine is substituted with valine, whereas the upper picture displays the substitution with the highest probability (marked with red square in the Rotamer list).



LEPV94	2.04±0.42
LEPM94	1.80±0.36
F (1,8)	30.96
p	0.0005

LEPV94	1.40±0.18
LEPM94	1.15±0.15
F (1,8)	43.54
p	0.0002

Fig S5. Impact of Val94Met transversion at *LEP* rs17151919 on leptin secretion rate in HEK293 cells in different conditions. A) Leptin secretion rates for Val94 and Met94 during a 24-hr incubation period (48-72 hr post-transfaction), expressed as the amount of leptin secreted in ng per hour over 24 hrs (LEPs/hr) normalized by the respective cellular leptin content (LEPc, ng) at the end of incubation. B) Leptin secretion rates for Val94 and Met94 during a 1-hour incubation (72-73 hr post-transfection) in the presence of cycloheximide (CHX, 20 µg/ml) expressed as the amount of leptin secreted in ng during the 1-hour incubation (LEPs/hr), normalized by the respective cellular leptin content (LEPc, ng). Individual data points from four separate experiments (each with 2-3 technical replicates) are plotted. All data passed D'Agostino & Pearson normality test and repeated measures one-way ANOVA was performed to assess the difference in secretion rate between the genotypes. Mean ± SD and AVOVA results (F and p values) are reported in the table below each graph. C-D. The amounts of leptin secreted (LEPs) during a 1 hr incubation (72-73 hr post-transfection) in untreated control cells (C), and the corresponding cellular leptin content (LEPc) at the end of the

incubation (D). Leptin secretion rates shown in Fig 2B were ratios of the amounts of leptin secreted (LEPs) over the corresponding cellular leptin contents (LEPc) shown here.

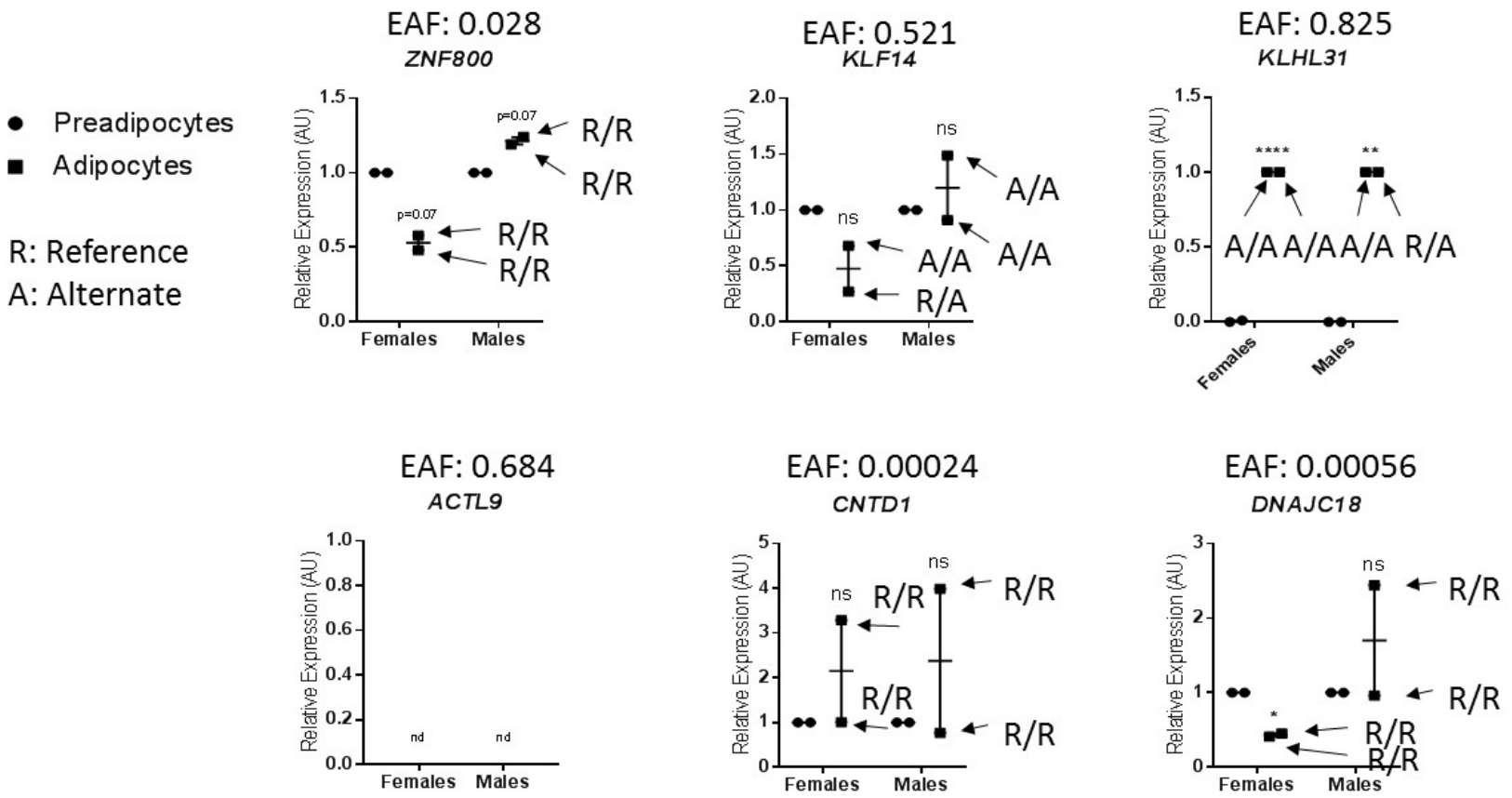


Figure S6. Expression of leptin modifiers in human preadipocytes and mature adipocytes. De-identified human subcutaneous adipose stromal cells were generously provided by the Boston NORC and were cultured and differentiated as previously described (Lee and Fried, 2014). Preadipocytes and *in vitro*-differentiated adipocytes from two females and two males were studied. Lipid-laden cells were assayed between 10-14 days after initial treatment with differentiation factors. Transcript levels were determined by RT-qPCR, normalized to the geometric mean of *RPLP0* and *PPIA*, and expressed relative to levels in preadipocytes. Two-way repeated measures ANOVA with post-hoc Sidak's multiple comparison tests were performed *: $p<0.05$, **: $p<0.01$, ****: $p<0.0001$, ns (no statistical difference) are indicated, comparing the transcript levels between preadipocytes and mature adipocytes. There was an interaction

between sex and differentiation stage for ZNF800 ($p<0.01$). No *ACTL9* transcript was detected (nd: none detected). Genotypes of the individuals were marked as R-reference allele and A-alternative allele.

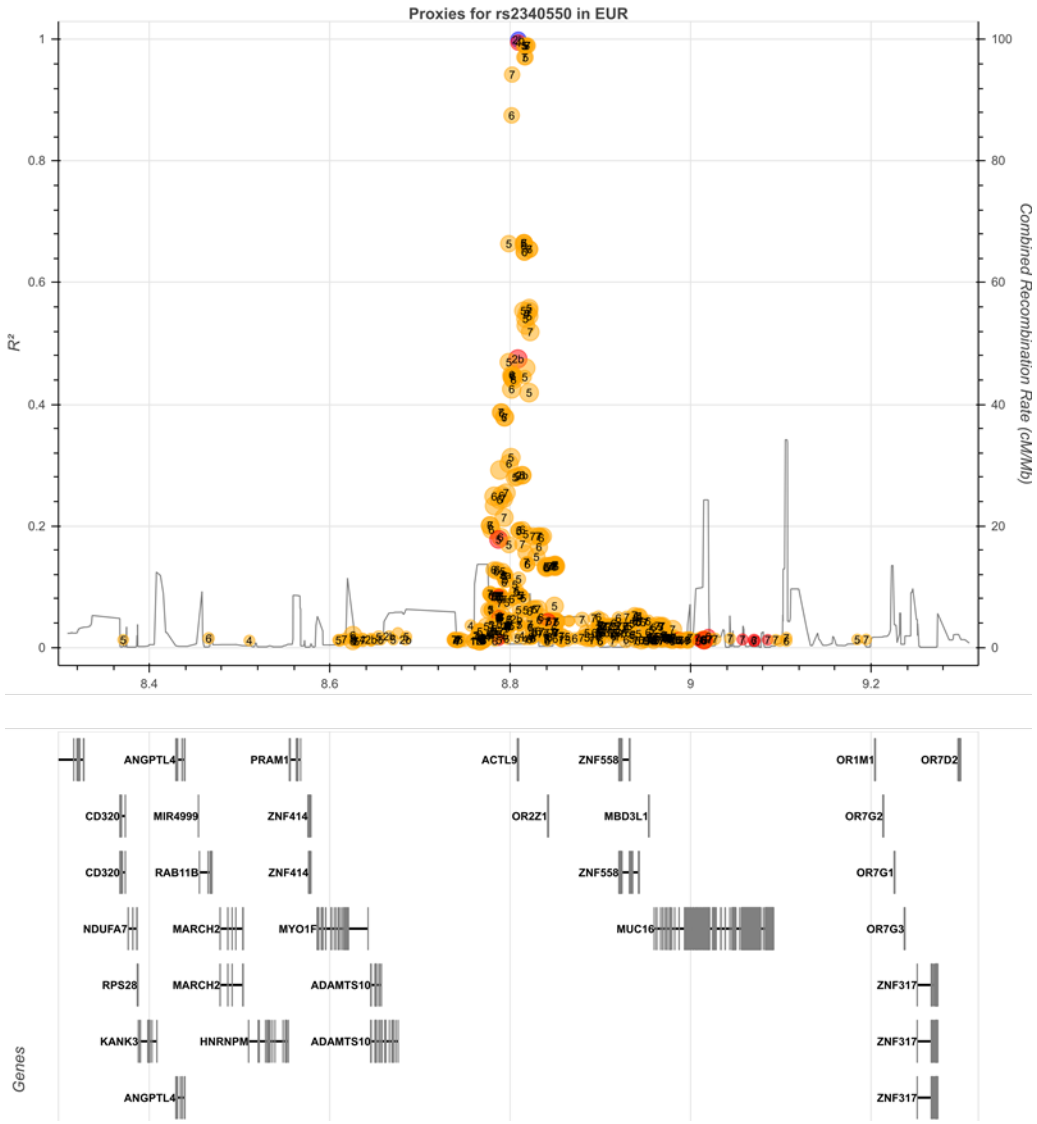


Figure S7. Linkage disequilibrium between the Ser37Phe (rs2340550) variant in *ACTL9* and variants within ±500 kb in the 1000 Genomes European ancestry reference panel. The numbering refers to Regulome DB score of the variants (www.regulomedb.org). Non-coding variants are marked in orange color and coding variants in red. The plot was produced using LDlink (https://ldlink.nci.nih.gov).

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The **Framingham Heart Study (FHS)** was initiated in 1948 and is comprised of 5,209 participants from Framingham, MA (US), who have undergone examinations every other year to evaluate cardiovascular disease and related risk factors. The Offspring cohort was recruited in 1971 and includes 5,124 children of the Original cohort and the children’s spouses. Participants from the Offspring cohort have attended exams roughly every four years. The current analysis includes 2,223 individuals with available phenotypic and genotypic information.

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2. Lee, M.J., and Fried, S.K. (2014). Optimal protocol for the differentiation and metabolic analysis of human adipose stromal cells. Methods Enzymol *538*, 49-65.