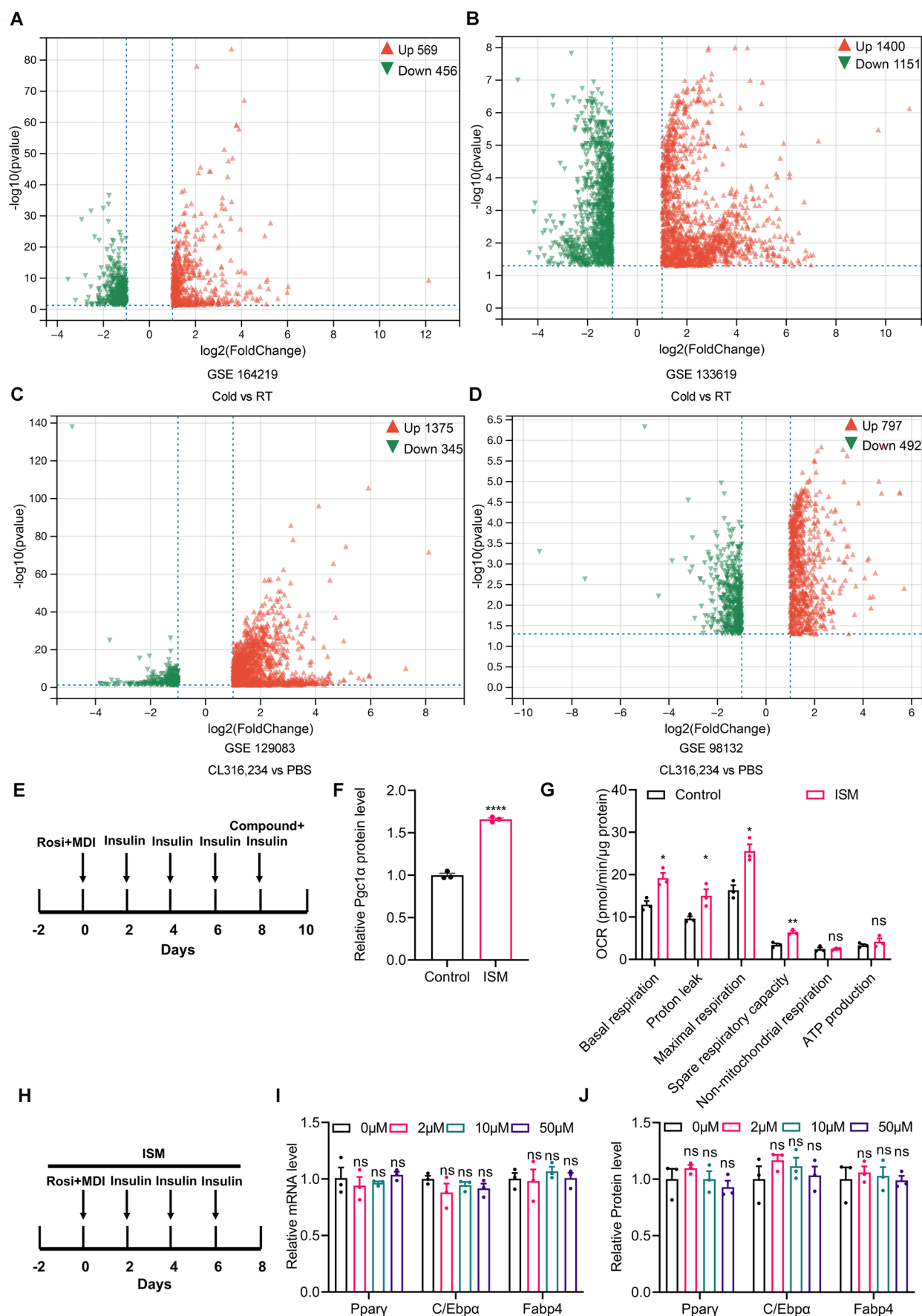


Expanded View Figures

Figure EV1. Identification of small-molecule compounds promoting adipocyte browning using CMap.

(A) Volcano plot of differentially expressed genes in the RNA-seq dataset [GSE164219](#) from cold exposure-induced browning of iWAT in mice ($n = 3$). Statistical test: Moderated t-tests and Benjamini-Hochberg procedure. (B) Volcano plot of differentially expressed genes in the RNA-seq dataset [GSE133619](#) from cold exposure-induced browning of iWAT in mice ($n = 3$). Statistical test: Moderated t-tests and Benjamini-Hochberg procedure. (C) Volcano plot of differentially expressed genes in the RNA-seq dataset [GSE129083](#) from β -adrenergic agonist CL316,243-induced browning of iWAT in mice ($n = 4$). Statistical test: Moderated t-tests and Benjamini-Hochberg procedure. (D) Volcano plot of differentially expressed genes in the RNA-seq dataset [GSE98132](#) from β -adrenergic agonist CL316,243-induced browning of iWAT in mice ($n = 3$). Statistical test: Moderated t-tests and Benjamini-Hochberg procedure. (E) Schematic illustration of the treatment method for maturation and differentiation induction in primary inguinal adipocytes, along with the administration of candidate compounds (10 μ M) screened from CMap. (F) Quantitative analysis of relative protein expression levels of Pgc1 α in mature primary inguinal adipocytes from control and ISM (50 μ M) treated groups ($n = 3$). Statistical test: Unpaired Student's two-tailed t-tests. $p < 0.0001$. (G) Quantitative analysis of OCR in mature primary inguinal adipocytes from control and ISM (50 μ M) treated groups in Fig. 2O ($n = 3$). Statistical test: Unpaired Student's two-tailed t-tests. Basal respiration, $p = 0.0142$; Proton leak, $p = 0.0299$; Maximal respiration, $p = 0.0100$; Spare respiratory capacity, $p = 0.0043$; Non-mitochondrial respiration, $p = 0.8941$; ATP production, $p = 0.3578$. (H) Schematic illustration of the treatment method for adipogenesis in adipocytes with ISM. (I) Relative mRNA expression levels of Pparg, C/EBP α , and Fabp4 in mature primary inguinal adipocytes treated with different concentrations of ISM ($n = 3$). Statistical test: one-way ANOVA followed by Dunnett's multiple comparisons test. Pparg: 0 μ M vs. 2 μ M, $p = 0.6106$; 0 μ M vs. 10 μ M, $p = 0.6716$; 0 μ M vs. 50 μ M, $p = 0.7857$; C/EBP α : 0 μ M vs. 2 μ M, $p = 0.2238$; 0 μ M vs. 10 μ M, $p = 0.2367$; 0 μ M vs. 50 μ M, $p = 0.1733$; Fabp4: 0 μ M vs. 2 μ M, $p = 0.8648$; 0 μ M vs. 10 μ M, $p = 0.3654$; 0 μ M vs. 50 μ M, $p = 0.9361$. (J) Quantitative analysis of relative protein expression levels of Pparg, C/EBP α , and Fabp4 in mature primary inguinal adipocytes treated with varying concentrations of ISM ($n = 3$). Statistical test: one-way ANOVA followed by Dunnett's multiple comparisons test. Pparg: 0 μ M vs. 2 μ M, $p = 0.3690$; 0 μ M vs. 10 μ M, $p = 0.9934$; 0 μ M vs. 50 μ M, $p = 0.5491$; C/EBP α : 0 μ M vs. 2 μ M, $p = 0.2496$; 0 μ M vs. 10 μ M, $p = 0.4520$; 0 μ M vs. 50 μ M, $p = 0.8255$; Fabp4: 0 μ M vs. 2 μ M, $p = 0.6430$; 0 μ M vs. 10 μ M, $p = 0.8337$; 0 μ M vs. 50 μ M, $p = 0.9254$. n presents biological replicates. Data are presented as mean $\pm$ SEM. Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and ns indicates no significant difference.



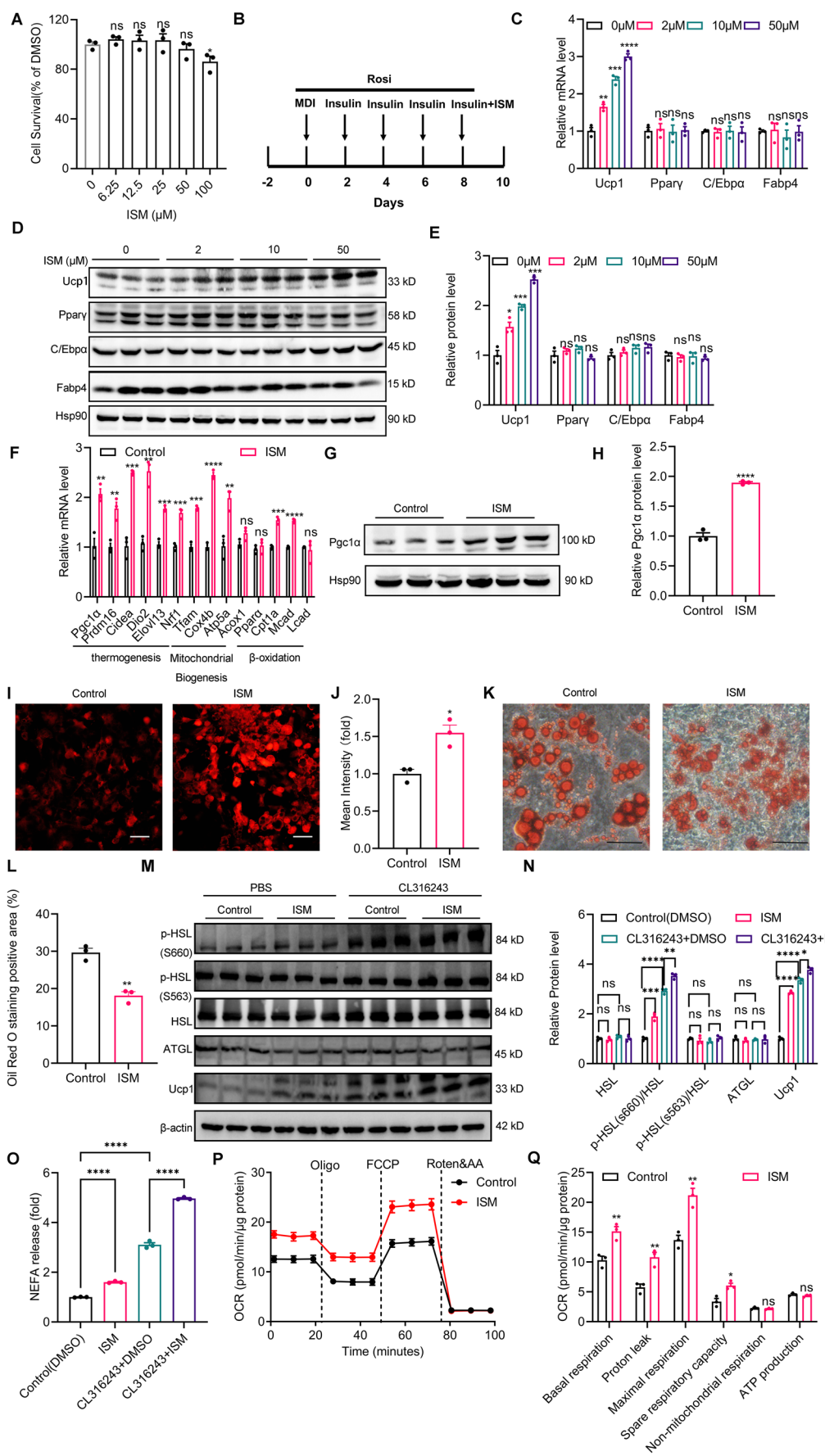


Figure EV2. Validation of ISM as a Novel Thermogenic Inducer.

(A) CCK8 assay measuring cell viability in human mesenchymal stem cells (HMSC) treated with different concentrations of ISM ($n = 3$). Statistical test: one-way ANOVA followed by Dunnett's multiple comparisons test. 0 vs. 6.25, $p = 0.9070$; 0 vs. 12.5, $p = 0.9608$; 0 vs. 25, $p = 0.9549$; 0 vs. 50, $p = 0.9422$; 0 vs. 100, $p = 0.0420$. (B) Schematic illustration of the treatment method for maturation and differentiation induction in HMSC and the promotion of browning in mature human adipocytes by ISM. (C) Relative mRNA expression levels of Ucp1, Ppar γ , C/EBP α , and Fabp4 in mature human adipocytes derived from HMSC treated with different concentrations of ISM ($n = 3$). Statistical test: one-way ANOVA followed by Dunnett's multiple comparisons test. Ucp1: 0 μ M vs. 2 μ M, $p = 0.0042$; 0 μ M vs. 10 μ M, $p = 0.0003$; 0 μ M vs. 50 μ M, $p < 0.0001$; Ppar γ : 0 μ M vs. 2 μ M, $p = 0.3909$; 0 μ M vs. 10 μ M, $p = 0.9130$; 0 μ M vs. 50 μ M, $p = 0.8935$; C/EBP α : 0 μ M vs. 2 μ M, $p = 0.6652$; 0 μ M vs. 10 μ M, $p = 0.6192$; 0 μ M vs. 50 μ M, $p = 0.8497$; Fabp4: 0 μ M vs. 2 μ M, $p = 0.8454$; 0 μ M vs. 10 μ M, $p = 0.4445$; 0 μ M vs. 50 μ M, $p = 0.9231$. (D) Representative immunoblot images of Ucp1, Ppar γ , C/EBP α , and Fabp4 in mature human adipocytes derived from HMSCs treated with varying concentrations of ISM. (E) Quantitative analysis of relative protein expression levels of Ucp1, Ppar γ , C/EBP α , and Fabp4 in mature human adipocytes derived from HMSCs treated with varying concentrations of ISM ($n = 3$). Statistical test: one-way ANOVA followed by Dunnett's multiple comparisons test. Ucp1: 0 μ M vs. 2 μ M, $p = 0.0134$; 0 μ M vs. 10 μ M, $p = 0.0007$; 0 μ M vs. 50 μ M, $p = 0.0001$; Ppar γ : 0 μ M vs. 2 μ M, $p = 0.3452$; 0 μ M vs. 10 μ M, $p = 0.2204$; 0 μ M vs. 50 μ M, $p = 0.5461$; C/EBP α : 0 μ M vs. 2 μ M, $p = 0.4425$; 0 μ M vs. 10 μ M, $p = 0.1332$; 0 μ M vs. 50 μ M, $p = 0.1133$; Fabp4: 0 μ M vs. 2 μ M, $p = 0.6264$; 0 μ M vs. 10 μ M, $p = 0.8377$; 0 μ M vs. 50 μ M, $p = 0.3627$. (F) Relative mRNA expression levels of thermogenesis, mitochondrial biogenesis and β -oxidation related genes in mature human adipocytes derived from HMSCs from control and ISM (50 μ M) treated groups ($n = 3$). Statistical test: Unpaired Student's two-tailed t-tests. Pgc1 α , $p = 0.0045$; Prdm16, $p = 0.0060$; Cidea, $p = 0.0004$; Dio2, $p = 0.0013$; Elovl3, $p = 0.0004$; Nr1f1, $p = 0.0008$; Tfam, $p = 0.4859$; Cox4b, $p = 0.0004$; Atp5a, $p = 0.0067$; Acox1, $p = 0.0745$; Ppara, $p = 0.4859$; Cpt1a, $p = 0.0004$; Mcad, $p < 0.0001$; Lcad, $p = 0.6934$. (G) Representative immunoblot images of Pgc1 α in mature human adipocytes derived from HMSC in control and ISM (50 μ M) treatment groups. (H) Quantitative analysis of relative protein expression levels of Pgc1 α in mature human adipocytes derived from HMSC in control and ISM (50 μ M) treatment groups ($n = 3$). Statistical test: Unpaired Student's two-tailed t-tests. $p < 0.0001$. (I) Representative MitoTracker staining images of mature human adipocytes derived from HMSC from control and ISM (50 μ M) treated groups; scale bar = 50 μ m. (J) Quantitative analysis of MitoTracker staining images in mature primary inguinal adipocytes from control and ISM (50 μ M) treated groups ($n = 3$). Statistical test: Unpaired Student's two-tailed t-tests. $p = 0.0105$. (K) Representative Oil Red O staining images of mature human adipocytes derived from HMSC from control and ISM (50 μ M) treated groups; scale bar = 50 μ m. (L) Quantitative analysis of Oil Red O staining images in mature human adipocytes derived from HMSC from control and ISM (50 μ M) treated groups ($n = 3$). Statistical test: Unpaired Student's two-tailed t-tests. $p = 0.0017$. After 24 h of ISM treatment, the cells were stimulated with 1 μ M CL316243 for an additional 24 h. (M) Representative immunoblot images of p-HSL(Ser660), p-HSL(Ser563), HSL, ATGL and Ucp1 in mature human adipocytes derived from HMSC from different groups. (N) Quantitative analysis of relative protein expression levels of p-HSL(Ser660), p-HSL(Ser563), HSL, ATGL and Ucp1 in mature human adipocytes derived from HMSC from different groups ($n = 3$). Statistical test: one-way ANOVA followed by Tukey's multiple comparisons. HSL: Control (DMSO) vs. ISM, $p = 0.5801$; Control (DMSO) vs. CL316243 + DMSO, $p = 0.2296$; CL316243 + DMSO vs. CL316243 + ISM, $p = 0.4517$; p-HSL(S660)/HSL: Control (DMSO) vs. ISM, $p = 0.0009$; Control (DMSO) vs. CL316243 + DMSO, $p < 0.0001$; CL316243 + DMSO vs. CL316243 + ISM, $p = 0.0032$; p-HSL(S563)/HSL: Control (DMSO) vs. ISM, $p = 0.5010$; Control (DMSO) vs. CL316243 + DMSO, $p = 0.1447$; CL316243 + DMSO vs. CL316243 + ISM, $p = 0.1580$; ATGL: Control (DMSO) vs. ISM, $p = 0.3646$; Control (DMSO) vs. CL316243 + DMSO, $p = 0.5620$; CL316243 + DMSO vs. CL316243 + ISM, $p = 0.8974$; Ucp1: Control (DMSO) vs. ISM, $p < 0.0001$; Control (DMSO) vs. CL316243 + DMSO, $p < 0.0001$; CL316243 + DMSO vs. CL316243 + ISM, $p = 0.0146$. (O) NEFA levels in the mature human adipocytes derived from HMSC culture medium from different groups ($n = 3$). Statistical test: one-way ANOVA followed by Tukey's multiple comparisons. Control (DMSO) vs. ISM, $p < 0.0001$; Control (DMSO) vs. CL316243 + DMSO, $p < 0.0001$; CL316243 + DMSO vs. CL316243 + ISM, $p < 0.0001$. (P) Oxygen consumption rates (OCR) in mature human adipocytes derived from HMSC from control and ISM (50 μ M) treated groups ($n = 3$). (Q) Quantitative analysis of OCR in mature human adipocytes derived HMSC from control and ISM (50 μ M) treated groups ($n = 3$). Statistical test: Unpaired Student's two-tailed t-tests. Basal respiration, $p = 0.0095$; Proton leak, $p = 0.0070$; Maximal respiration, $p = 0.0058$; Spare respiratory capacity, $p = 0.0147$; Non-mitochondrial respiration, $p = 0.5466$; ATP production, $p = 0.3115$. n presents biological replicates. Data are presented as mean $\pm$ SEM. Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and ns indicates no significant difference.

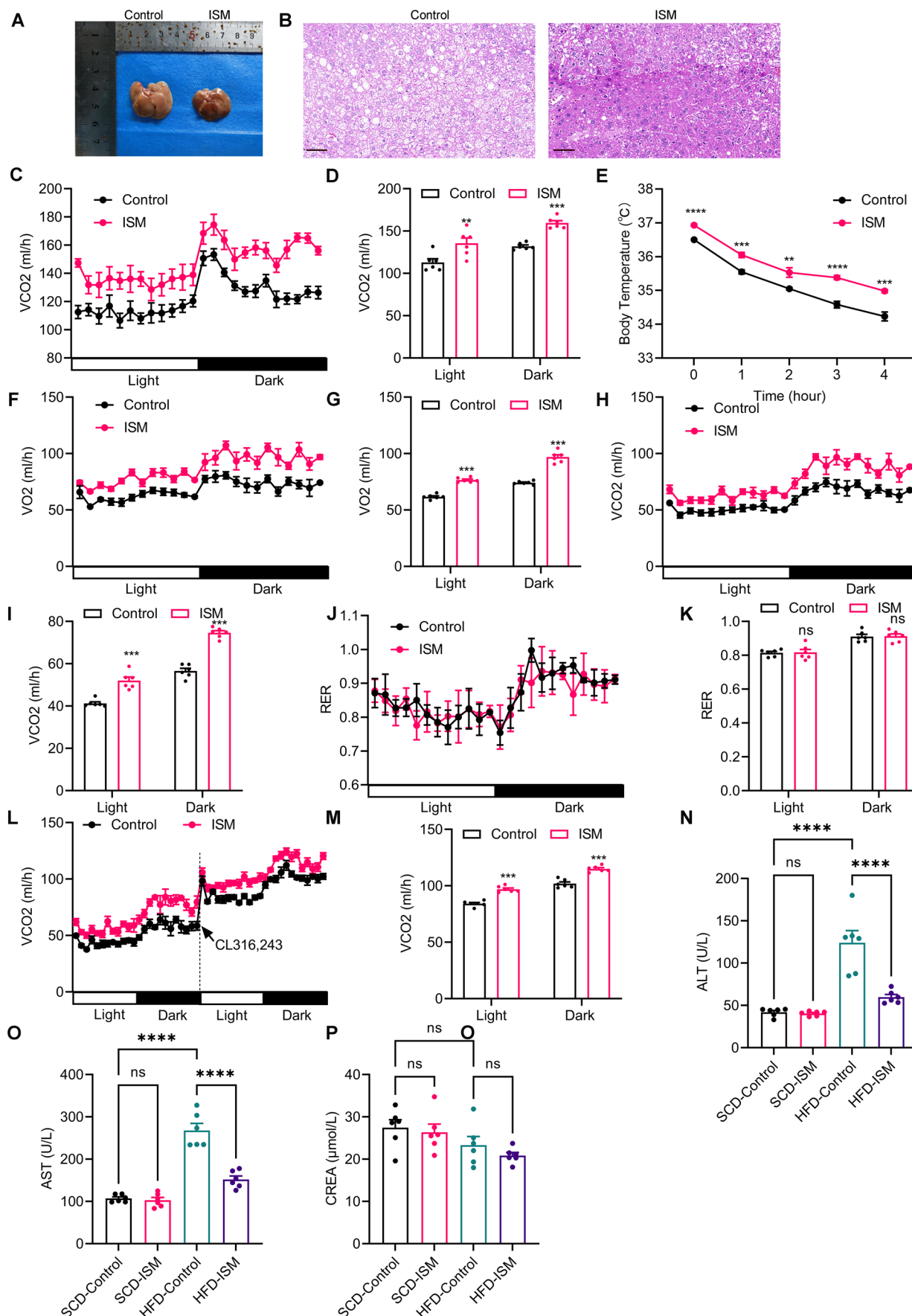
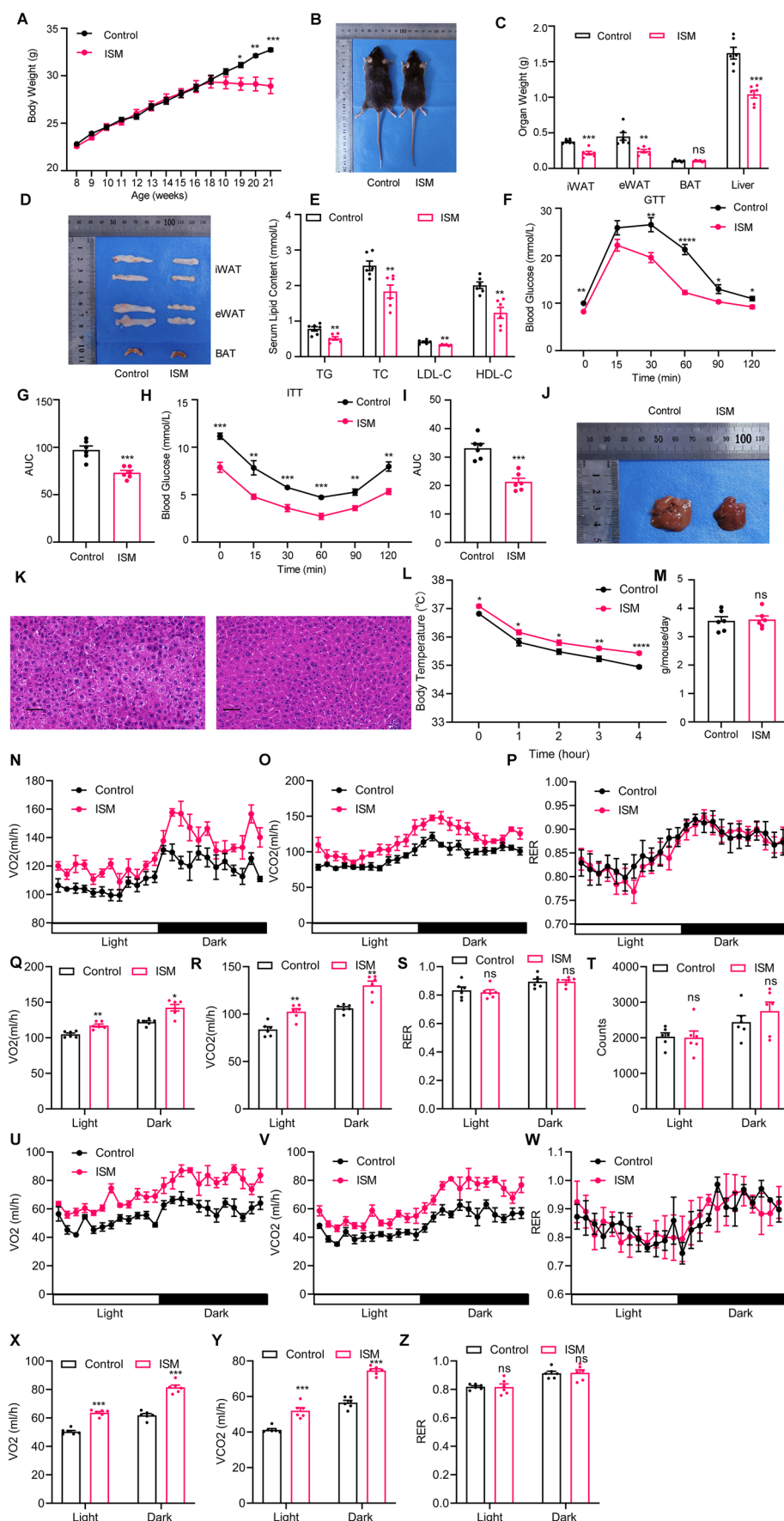


Figure EV3. ISM protects against obesity induced by high-fat diet and improves obesity-related metabolic dysfunction through promoting thermogenesis in mice.

(A) Representative images of the general appearance of livers from HFD mice. (B) Representative H&E staining images of liver sections; scale bar = 50 μ m. (C) VCO_2 of HFD mice. (D) Quantitative analysis of VCO_2 in (C). Statistical test: ANCOVA, with body weight as a covariate. VCO_2 -Light, $p = 0.004$; VCO_2 -Dark, $p < 0.001$. (E) Rectal temperature of HFD mice under different treatments measured at specified times at 4 $^{\circ}$ C ($n = 6$). Statistical test: Unpaired Student's two-tailed t-tests. 0 h, $p < 0.0001$; 1 h, $p = 0.0005$; 2 h, $p = 0.0075$; 3 h, $p < 0.0001$; 4 h, $p = 0.0003$. (F, G) VO_2 of mice injected with either vehicle or ISM and fed a high-fat diet for 4 weeks ($n = 6$). Statistical test: ANCOVA, with body weight as a covariate. VO_2 -Light, $p < 0.001$; VO_2 -Dark, $p < 0.001$. (H, I) VCO_2 of mice injected with either vehicle or ISM and fed a high-fat diet for 4 weeks ($n = 6$). Statistical test: ANCOVA, with body weight as a covariate. VCO_2 -Light, $p < 0.001$; VCO_2 -Dark, $p < 0.001$. (J, K) Respiratory exchange ratio (RER) of mice injected with either vehicle or ISM and fed a high-fat diet for 4 weeks ($n = 6$). Statistical test: Unpaired Student's two-tailed t-tests. RER-Light, $p = 0.9647$; RER-Dark, $p = 0.3803$. (L) VCO_2 of mice treated with [CL316243](#). (M) Quantitative analysis of VCO_2 in mice after [CL316243](#) treatment ($n = 6$). Statistical test: ANCOVA, with body weight as a covariate. VCO_2 -Light, $p < 0.001$; VCO_2 -Dark, $p < 0.001$. (N) Quantitative analysis of serum ALT levels in mice from different groups ($n = 6$). Statistical test: one-way ANOVA followed by Tukey's multiple comparisons. SCD-Control vs. SCD-ISM, $p = 0.9989$; SCD-Control vs. HFD-Control, $p < 0.0001$; HFD-Control vs. HFD-ISM, $p < 0.0001$. (O) Quantitative analysis of serum AST levels in mice from different groups ($n = 6$). Statistical test: one-way ANOVA followed by Tukey's multiple comparisons. SCD-Control vs. SCD-ISM, $p = 0.9903$; SCD-Control vs. HFD-Control, $p < 0.0001$; HFD-Control vs. HFD-ISM, $p < 0.0001$. (P) Quantitative analysis of serum CREA levels in mice from different groups ($n = 6$). Statistical test: one-way ANOVA followed by Tukey's multiple comparisons. SCD-Control vs. SCD-ISM, $p = 0.9681$; SCD-Control vs. HFD-Control, $p = 0.3478$; HFD-Control vs. HFD-ISM, $p = 0.7391$. n presents biological replicates. Data are presented as mean $\pm$ SEM. Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and ns indicates no significant difference.



◀ **Figure EV4. Administration of ISM also improves glucose tolerance and insulin sensitivity in mice fed a standard chow diet (SCD).**

Mice were intraperitoneally injected with either vehicle or ISM and fed a standard chow diet for 13 weeks ($n = 6$). n presents biological replicates. (A) Body weight of mice in different groups after feeding on a standard chow diet. 19w, $p = 0.0173$; 20w, $p = 0.0025$; 21w, $p = 0.0009$. (B) Representative images of the general appearance of mice prior to euthanasia. (C) Weights of tissue organs (inguinal subcutaneous adipose tissue, epididymal visceral adipose tissue, brown adipose tissue, and liver) from mice. iWAT, $p = 0.0001$; eWAT, $p = 0.0058$; BAT, $p = 0.9624$; Liver, $p = 0.0002$. (D) Representative images of the general appearance of adipose tissues (iWAT, eWAT, and BAT) from mice. (E) Serum lipid content (triglycerides, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol) after feeding on a standard chow diet for 13 weeks. TG, $p = 0.0059$; TC, $p = 0.0090$; LDL-C, $p = 0.0037$; HDL-C, $p = 0.0015$. (F) Glucose tolerance test (GTT) after feeding on SCD for 11 weeks. 0 min, $p = 0.0023$; 15 min, $p = 0.0881$; 30 min, $p = 0.0034$; 60 min, $p < 0.0001$; 90 min, $p = 0.0205$; 120 min, $p = 0.0134$. (G) Quantitative analysis of the glucose tolerance test (GTT). $p = 0.0005$. (H) Insulin tolerance test (ITT) after feeding on SCD for 12 weeks. 0 min, $p = 0.0003$; 15 min, $p = 0.0033$; 30 min, $p = 0.0008$; 60 min, $p = 0.0002$; 90 min, $p = 0.0021$; 120 min, $p = 0.0010$. (I) Quantitative analysis of the insulin tolerance test (ITT). $p = 0.0002$. (J) Representative images of the general appearance of livers from SCD mice. (K) Representative H&E staining images of liver sections; scale bar = 50 μm . (L) Rectal temperature of SCD mice under different treatments measured at specified times at 4 °C. 0 h, $p = 0.0267$; 1 h, $p = 0.0426$; 2 h, $p = 0.0324$; 3 h, $p = 0.0059$; 4 h, $p < 0.0001$. (M) Food intake in mice. $p = 0.8306$. (N–P) VO_2 , VCO_2 and Respiratory exchange ratio (RER) of SCD mice. (Q–S) Quantitative analysis of VO_2 , VCO_2 and RER of SCD mice in (N–P). VO_2 -Light, $p = 0.007$; VO_2 -Dark, $p = 0.018$; VCO_2 -Light, $p = 0.001$; VCO_2 -Dark, $p = 0.001$; RER-Light, $p = 0.6621$; RER-Dark, $p = 0.9868$. (T) Mean physical activity in mice. Light, $p = 0.9077$; Dark, $p = 0.3431$. (U–W) VO_2 , VCO_2 and RER of mice injected with either vehicle or ISM and fed a standard chow diet for 4 weeks ($n = 6$). n presents biological replicates. (X–Z) Quantitative analysis of VO_2 , VCO_2 and RER of SCD mice in (U–W) ($n = 6$). VO_2 -Light, $p < 0.001$; VO_2 -Dark, $p < 0.001$; VCO_2 -Light, $p < 0.001$; VCO_2 -Dark, $p < 0.001$; RER-Light, $p = 0.8021$; RER-Dark, $p = 0.7714$. Data are presented as mean $\pm$ SEM. Statistical test: The energy metabolism data, including VO_2 and VCO_2 were analyzed by ANCOVA, with body weight as a covariate. The others: Unpaired Student's two-tailed t-tests. Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and ns indicates no significant difference.

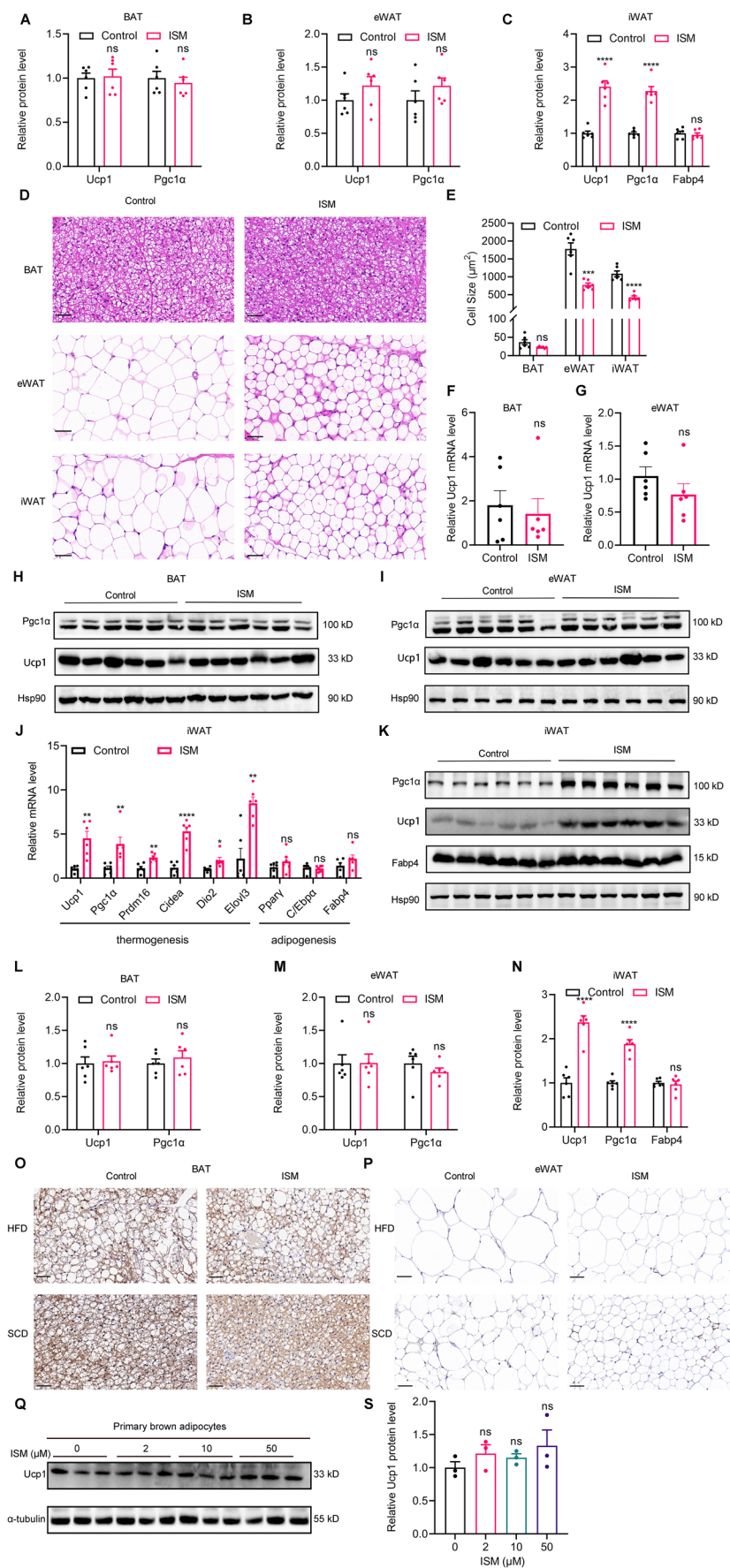


Figure EV5. ISM administration also promotes subcutaneous adipose browning and increases thermogenesis in mice fed a standard chow diet (SCD).

(A) Quantitative analysis of relative protein expression levels for the proteins shown in Fig. 4E ($n = 6$). Ucp1, $p = 0.8490$; Pgc1 α , $p = 0.6006$. (B) Quantitative analysis of relative protein expression levels for the proteins shown in Fig. 4F ($n = 6$). Ucp1, $p = 0.2182$; Pgc1 α , $p = 0.2610$. (C) Quantitative analysis of relative protein expression levels for the proteins shown in Fig. 4H ($n = 6$). Ucp1, $p < 0.0001$; Pgc1 α , $p < 0.0001$; Fabp4, $p = 0.6071$. (D) Representative H&E staining images of BAT, eWAT, and iWAT sections; scale bar = 50 μ m. (E) Quantitative analysis of adipocyte size in BAT, eWAT, and iWAT ($n = 6$). BAT, $p = 0.0708$; eWAT, $p = 0.0002$; iWAT, $p < 0.0001$. (F) Relative mRNA expression levels of Ucp1 in BAT ($n = 6$). $p = 0.6939$. (G) Relative mRNA expression levels of Ucp1 in eWAT ($n = 6$). $p = 0.2254$. (H) Representative immunoblot images of Pgc1 α and Ucp1 in BAT. (I) Representative immunoblot images of Pgc1 α and Ucp1 in eWAT. (J) Relative mRNA expression levels of thermogenesis and adipogenesis related genes in iWAT ($n = 6$). Ucp1, $p = 0.0015$; Pgc1 α , $p = 0.0063$; Prdm16, $p = 0.0016$; Cidea, $p < 0.0001$; Dio2, $p = 0.0150$; Elovl3, $p = 0.0010$; Pparg, $p = 0.2402$; C/EBP α , $p = 0.7316$; Fabp4, $p = 0.1663$. (K) Representative immunoblot images of Pgc1 α , Ucp1, and Fabp4 in iWAT. (L) Quantitative analysis of relative protein expression levels for the proteins shown in (H) ($n = 6$). Ucp1, $p = 0.8064$; Pgc1 α , $p = 0.4848$. (M) Quantitative analysis of relative protein expression levels for the proteins shown in (I) ($n = 6$). Ucp1, $p = 0.9714$; Pgc1 α , $p = 0.3209$. (N) Quantitative analysis of relative protein expression levels for the proteins shown in (K) ($n = 6$). Ucp1, $p < 0.0001$; Pgc1 α , $p < 0.0001$; Fabp4, $p = 0.6702$. (O) Representative immunohistochemical staining images of Ucp1 in BAT sections; scale bar = 50 μ m. (P) Representative immunohistochemical staining images of Ucp1 in eWAT sections; scale bar = 50 μ m. (Q) Representative immunoblot images of Ucp1 in mature primary brown adipocytes treated with varying concentrations of ISM. (S) Quantitative analysis of relative protein expression levels of Ucp1 in mature primary brown adipocytes treated with varying concentrations of ISM ($n = 3$). 0 vs. 2, $p = 0.6256$; 0 vs. 10, $p = 0.8106$; 0 vs. 50, $p = 0.3200$. n presents biological replicates. Data are presented as mean $\pm$ SEM. Statistical test: (S): one-way ANOVA followed by Dunnett's multiple comparisons test. The others: unpaired Student's two-tailed t-tests. Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and ns indicates no significant difference.

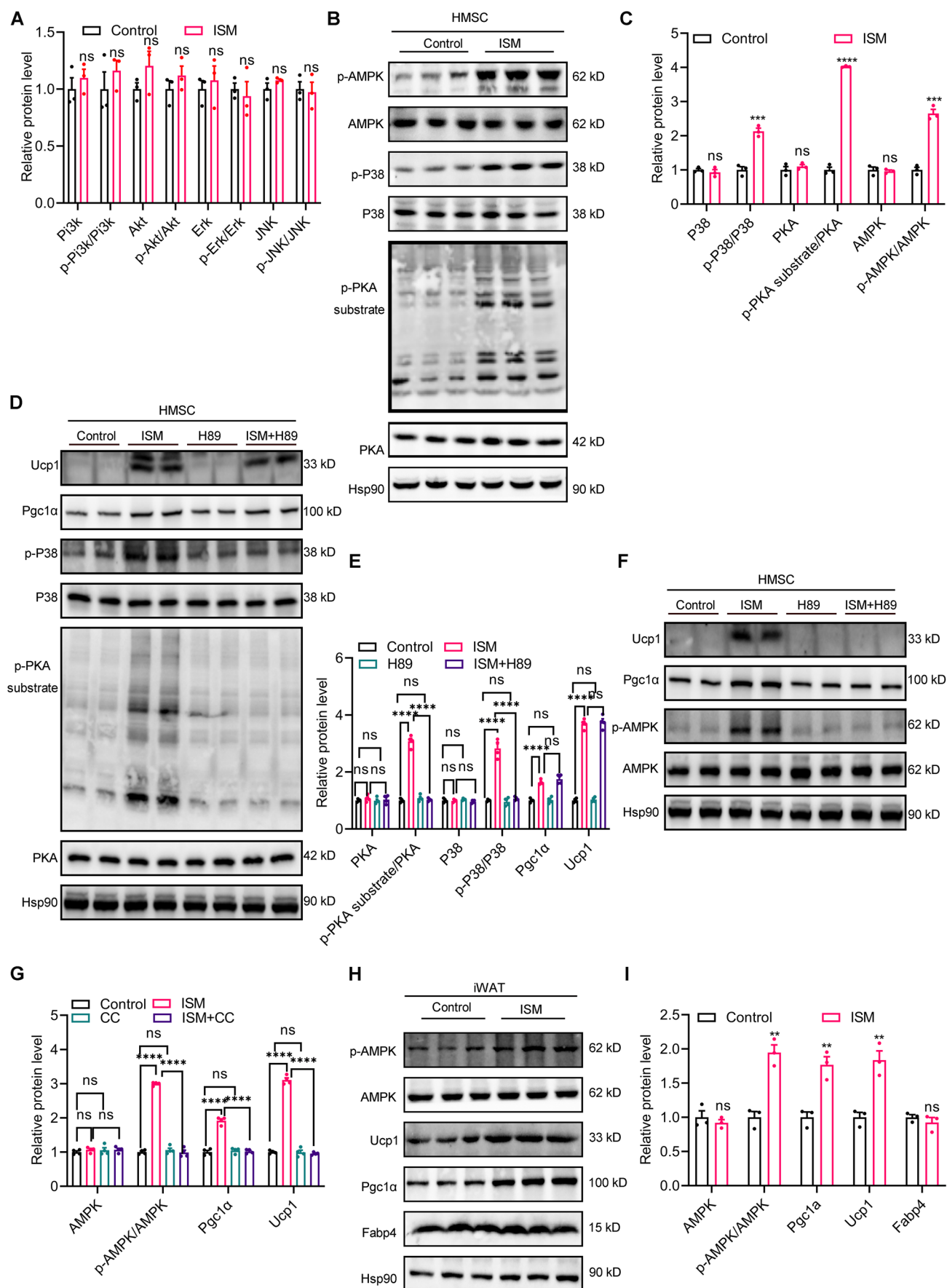


Figure EV6. ISM promotes browning by activating the AMPK pathway.

(A) Quantitative analysis of relative protein expression levels for the signaling pathway proteins shown in Fig. 5C ($n = 3$). Unpaired Student's two-tailed t-tests. Pi3k , $p = 0.4749$; p-Pi3k/Pi3k , $p = 0.4071$; Akt , $p = 0.2135$; p-Akt/Akt , $p = 0.3419$; Erk , $p = 0.6300$; p-Erk/Erk , $p = 0.6766$; JNK , $p = 0.2356$; p-JNK/JNK , $p = 0.8187$. (B) Representative immunoblot images of signaling pathway proteins in mature human adipocytes from control and ISM (50 μM) treatment groups. (C) Quantitative analysis of relative protein expression levels for the signaling pathway proteins shown in (B) ($n = 3$). Unpaired Student's two-tailed t-tests. P38 , $p = 0.4837$; p-P38/P38 , $p = 0.0010$; PKA , $p = 0.4066$; $\text{p-PKA substrate/PKA}$, $p < 0.0001$; AMPK , $p = 0.6970$; p-AMPK/AMPK , $p = 0.0003$. (D) Representative immunoblot images of signaling pathway proteins in mature human adipocytes treated with or without PKA inhibitor H89 (30 μM) for 2 h, followed by ISM or DMSO treatment for 48 h. (E) Quantitative analysis of relative protein expression levels for the signaling pathway proteins shown in (D) ($n = 3$). One-way ANOVA followed by Tukey's multiple comparisons. PKA : Control vs. ISM, $p = 0.3360$; Control vs. H89, $p = 0.9175$; ISM vs. ISM + H89, $p = 0.7420$; $\text{p-PKA substrate/PKA}$: Control vs. ISM, $p < 0.0001$; Control vs. H89, $p = 0.2637$; ISM vs. ISM + H89, $p < 0.0001$; P38 : Control vs. ISM, $p = 0.7647$; Control vs. H89, $p = 0.4293$; ISM vs. ISM + H89, $p = 0.4467$; p-P38/P38 : Control vs. ISM, $p < 0.0001$; Control vs. H89, $p = 0.5466$; ISM vs. ISM + H89, $p < 0.0001$; $\text{Pgc1}\alpha$: Control vs. ISM, $p < 0.0001$; Control vs. H89, $p = 0.8756$; ISM vs. ISM + H89, $p = 0.2669$; Ucp1 : Control vs. ISM, $p < 0.0001$; Control vs. H89, $p = 0.5887$; ISM vs. ISM + H89, $p = 0.6622$. (F) Representative immunoblot images of signaling pathway proteins in mature human adipocytes treated with or without AMPK inhibitor CC (10 μM) for 2 h, followed by ISM or DMSO treatment for 48 h. (G) Quantitative analysis of relative protein expression levels for the signaling pathway proteins shown in (F) ($n = 3$). One-way ANOVA followed by Tukey's multiple comparisons. AMPK : Control vs. ISM, $p = 0.2372$; Control vs. CC, $p = 0.5120$; ISM vs. ISM + CC, $p = 0.9842$; p-AMPK/AMPK : Control vs. ISM, $p < 0.0001$; Control vs. CC, $p = 0.3807$; ISM vs. ISM + CC, $p < 0.0001$; $\text{Pgc1}\alpha$: Control vs. ISM, $p < 0.0001$; Control vs. CC, $p = 0.4606$; ISM vs. ISM + CC, $p < 0.0001$; Ucp1 : Control vs. ISM, $p < 0.0001$; Control vs. CC, $p = 0.9252$; ISM vs. ISM + CC, $p < 0.0001$. (H) Representative immunoblot images of AMPK, p-AMPK, Ucp1, Pgc1 α , and Fabp4 in iWAT from mice fed a normal diet and injected with ISM or vehicle. (I) Quantitative analysis of relative protein expression levels for the proteins shown in (H) ($n = 3$). Unpaired Student's two-tailed t-tests. AMPK , $p = 0.4924$; p-AMPK/AMPK , $p = 0.0026$; $\text{Pgc1}\alpha$, $p = 0.0055$; Ucp1 , $p = 0.0056$; Fabp4 , $p = 0.4072$. n presents biological replicates. Data are presented as mean $\pm$ SEM. Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and ns indicates no significant difference.

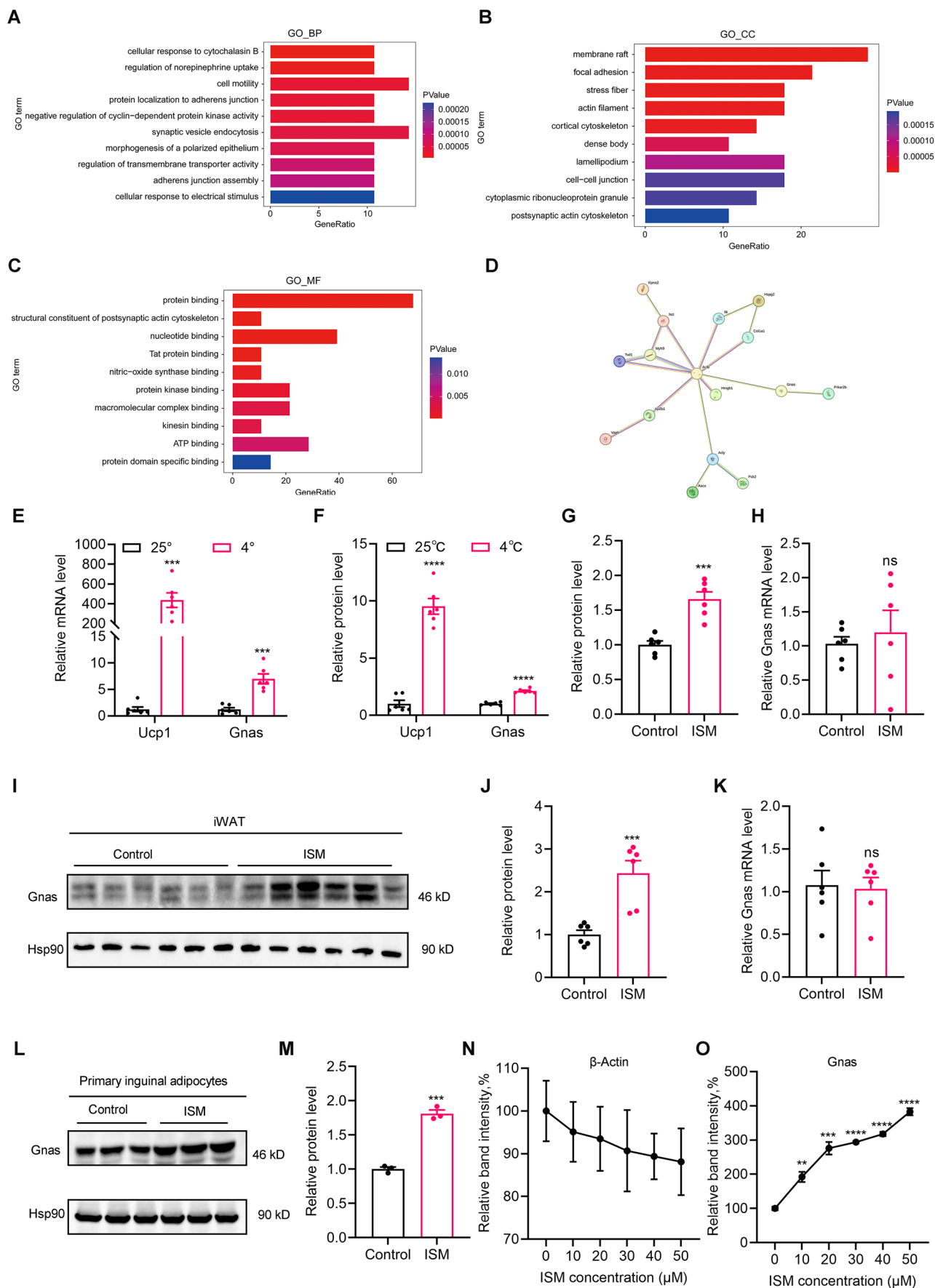


Figure EV7. Discovery of direct binding targets of ISM.

(A–C) Bar charts of GO enrichment analysis for 26 potential target proteins. (D) Protein–protein interaction (PPI) network diagram of 26 potential target proteins. (E) Relative mRNA expression levels of Gnas and Ucp1 in iWAT of 8-week-old male C57BL/6J mice exposed to 25 °C or 4 °C environments for 3 days ($n = 6$). Ucp1, $p = 0.0001$; Gnas, $p = 0.0002$. (F) Quantitative analysis of relative expression levels for the proteins shown in Fig. 6D ($n = 6$). Ucp1, $p < 0.0001$; Gnas, $p < 0.0001$. (G) Quantitative analysis of relative protein levels for the Gnas shown in Fig. 6E ($n = 6$). $p = 0.0003$. (H) Relative mRNA levels of Gnas in iWAT of mice fed a high-fat diet and injected with ISM or vehicle ($n = 6$). $p = 0.6227$. (I) Representative immunoblot images of Gnas in iWAT of mice fed a normal diet and injected with ISM or vehicle. (J) Quantitative analysis of relative protein levels for the Gnas shown in (I) ($n = 6$). $p = 0.0009$. (K) Relative mRNA levels of Gnas in iWAT of mice fed a normal diet and injected with ISM or vehicle ($n = 6$). $p = 0.8522$. (L) Representative immunoblot images of Gnas in mature primary inguinal adipocytes from control and ISM (50 μ M) treatment groups. (M) Quantitative analysis of relative protein levels for the Gnas shown in (L) ($n = 3$). $p = 0.0001$. (N) Quantitative analysis of relative grayscale values for β -actin in Fig. 6J ($n = 3$). 0 vs. 10, $p = 0.9860$; 0 vs. 20, $p = 0.9547$; 0 vs. 30, $p = 0.8439$; 0 vs. 40, $p = 0.7713$; 0 vs. 50, $p = 0.6964$. (O) Quantitative analysis of relative grayscale values for Gnas in Fig. 6J ($n = 3$). 0 vs. 10, $p = 0.0003$; 0 vs. 20, $p < 0.0001$; 0 vs. 30, $p < 0.0001$; 0 vs. 40, $p < 0.0001$; 0 vs. 50, $p < 0.0001$. n presents biological replicates. Data are presented as mean $\pm$ SEM. Statistical test: (N and O): one-way ANOVA followed by Dunnett's multiple comparisons test. The others: unpaired Student's two-tailed t-tests. Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and ns indicates no significant difference.