Expanded View Figures

Figure EV1. *Cited4* is a target of rosiglitazone in murine adipocyte progenitors promoting beige differentiation.

A mRNA expression in C3H10T1/2 cells, differentiated in the presence of 1 μM Rosi or vehicle for the indicated time, as determined by qRT–PCR (day 0, $n = 3$; day 4, $n = 4$; day 6, Ctrl, $n = 2$, Rosi, $n = 4$; day 10, $n = 4$). ***$P = 0.001$, **$P = 0.007$ (Day 6), **$P = 0.005$ (Day 10), in 2 × 2 ANOVA with Bonferroni's posttests (Rosi vs. vehicle).

B mRNA expression in 3T3-L1 cells, differentiated in the presence of 1 μM Rosi or vehicle for the indicated time, as determined by qRT–PCR ($n = 3$). 2 × 2 ANOVA with Bonferroni's posttests, $P > 0.05$ (Rosi vs. vehicle).

C mRNA expression in female Lin^-Sca1^+ cells, differentiated in the presence of 0.1 or 1 μM pioglitazone (Pio) or vehicle for 8 days, as determined by qRT–PCR ($n = 4$ for Day 2, $n = 4/2/6$ for Day 8). ***$P = 1*10^{-10}$, **$P = 0.003$, in 2 × 2 ANOVA with Bonferroni's posttests (Pio vs. vehicle).

D mRNA expression in female Lin^-Sca1^+ cells, differentiated in the presence of the indicated substances for 8 days, as determined by qRT–PCR ($n = 3$). **$P = 0.0035$, ***$P = 4 \times 10^{-10}$, ***$P = 7 \times 10^{-10}$ (*Ucp1*), *$P = 0.036$, ***$P = 0.0002$, ***$P = 1 \times 10^{-10}$, ***$P = 1 \times 10^{-10}$ (*Cpt1b*), ***$P = 2 \times 10^{-5}$ (*Cidea*), ***$P = 1 \times 10^{-6}$, ***$P = 4 \times 10^{-9}$, ***$P = 1 \times 10^{-8}$, (*Elovl3*), ***$P = 9 \times 10^{-8}$, ***$P = 2 \times 10^{-7}$ (*Cox7a1*), ***$P = 0.0002$, ***$P = 1 \times 10^{-8}$, ***$P = 4 \times 10^{-5}$, ***$P = 0.0002$ (*Cox8b*), *$P = 0.032$, **$P = 0.006$, ***$P = 0.0007$ (*Dio2*), ***$P = 1 \times 10^{-6}$, ***$P = 2 \times 10^{-8}$ (*Cyc1*), ***$P = 8 \times 10^{-5}$, ***$P = 3 \times 10^{-6}$ (*Ndufb3*) in 2 × 2 ANOVA with Holm–Sidak posttests ($Cited4^{-/-}$ vs. $Cited4^{+/+}$).

E mRNA expression in male Lin^-Sca1^+ progenitor cells, differentiated in the presence of 100 nM Rosi or vehicle for the indicated time, as determined by qRT–PCR ($n = 3$). ****$P = 1 \times 10^{-10}$ (Days 2 and 4), ****$P = 4 \times 10^{-8}$ (Day 8) in 2 × 2 ANOVA with Bonferroni's posttests (Rosi vs. vehicle).

F mRNA expression in male Lin^-Sca1^+ cells, differentiated in the presence of 100 nM Rosi or vehicle for 8 days, as determined by qRT–PCR ($n = 3$, t-test).

Data information: Data are presented as mean ± SEM except for (C) (individual data).

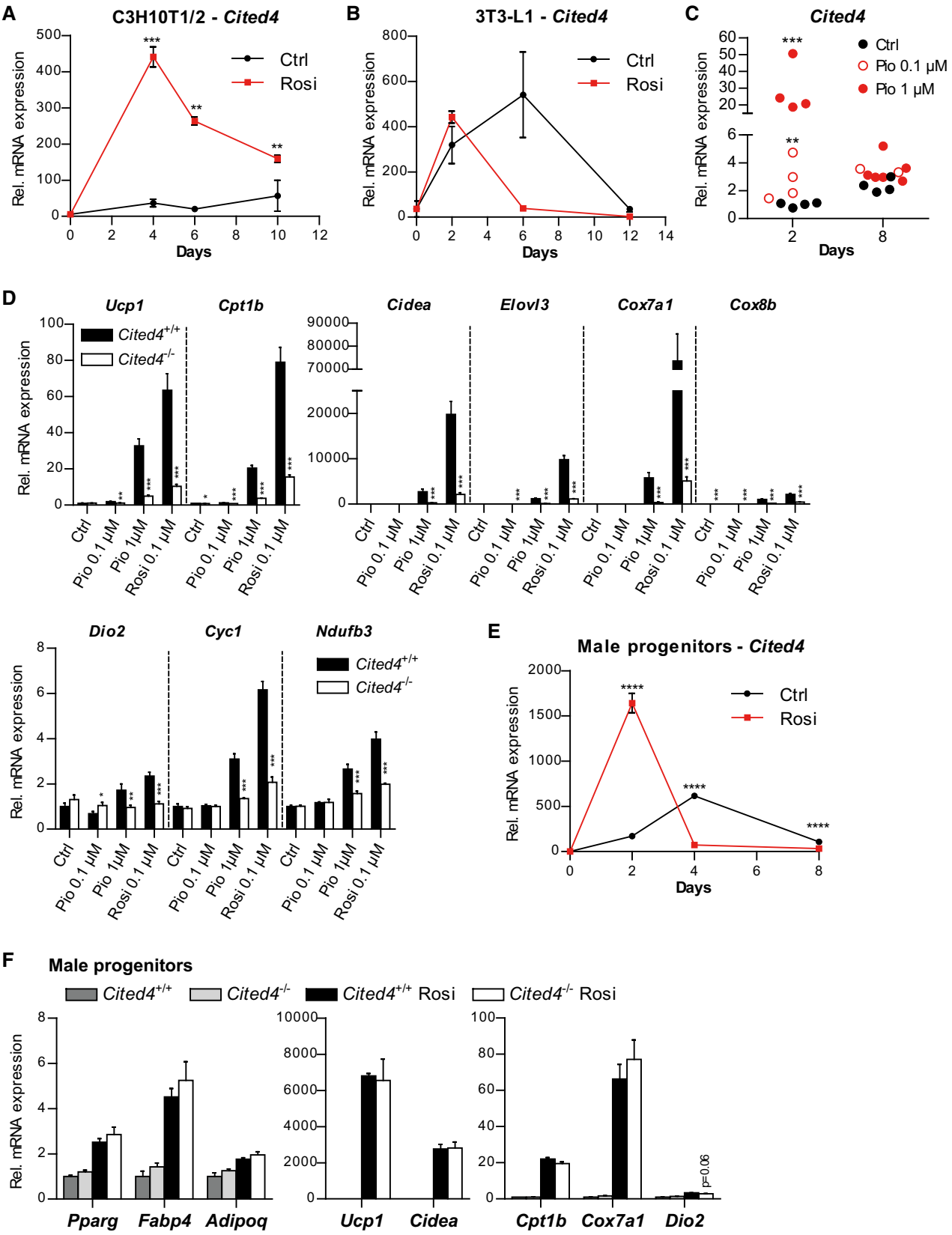

Figure EV1.

Figure EV2. *Cited4* is a target of rosiglitazone in murine and human adipocyte progenitors promoting beige differentiation.

A Phase contrast microscopy of primary SVF cells from human subcutaneous fat, differentiated in the presence of 100 nM Rosi or vehicle for 14 days (representative of $n = 5$ patients). Scale bar is 100 μm.

B, C mRNA expression in primary SVF cells from human subcutaneous fat, differentiated in the presence of 100 nM Rosi or vehicle, as determined by qRT–PCR ($n = 5$ patients). (B) ****$P = 1 \times 10^{-10}$ (Day 2), ****$P = 5 \times 10^{-8}$ (Day 6), ****$P = 1 \times 10^{-9}$ (Days 10 and 14), (C) ****$P = 7 \times 10^{-7}$ (Day 6), ****$P = 1 \times 10^{-9}$ (Days 10 and 14) in 2 × 2 ANOVA with Bonferroni's posttests (Rosi vs. vehicle).

D mRNA expression in primary SVF cells from human male subcutaneous fat transfected with the indicated siRNA prior to differentiation in the presence of 100 nM Rosi for 9 days, as determined by qRT–PCR ($n = 3$). ***$P = 0.0002$ (*siCITED4.1 CITED4*), ***$P = 0.0003$ (*siCITED4.2 CITED4*), **$P = 0.005$ (*UCP1*), *$P = 0.01$ (*CIDEA*), ***$P = 0.0006$ (*CITED4.1 CPT1B*), **$P = 0.007$ (*CPT1B*), ***$P = 0.0006$ (*CITED4.1 SLC2A4*),**$P = 0.009$ (*ADIPOQ*) in ANOVA with Tukey's posttests (vs. *siCtrl*).

E GFP fluorescence intensity distribution of Lin^-Sca1^+ progenitor cells 24 hours after transfection with GFP mRNA, determined by flow cytometry (compared to non-transfected cells).

F mRNA expression in female *Cited4*$^{F/F}$ Lin^-Sca1^+ progenitor cells transfected with Cre or control mRNA prior to differentiation in the presence of 100 nM Rosi or vehicle for 8 days, as determined by qRT–PCR ($n = 3$). *t*-test Cre vs. Ctrl (Rosi), **$P = 0.002$ (*Cited4*), *$P = 0.039$ (*Adipoq*), *$P = 0.015$ (*Ucp1*), *$P = 0.011$ (*Cidea*), **$P = 0.004$ (*Cpt1b*), *$P = 0.011$ (*Dio2*).

G mRNA expression in female *Cited4*$^{F/F}$ Lin^-Sca1^+ progenitor cells transfected with Cre or control mRNA 3 days after induction of differentiation in the presence of 100 nM Rosi or vehicle for 8 days, as determined by qRT–PCR ($n = 4$). *t*-test Cre vs. Ctrl, ***$P = 0.0005$ (*Cited4*), **$P = 0.009$ (*Cpt1b*).

Data information: Data are presented as mean ± SEM except for (E) (individual data).

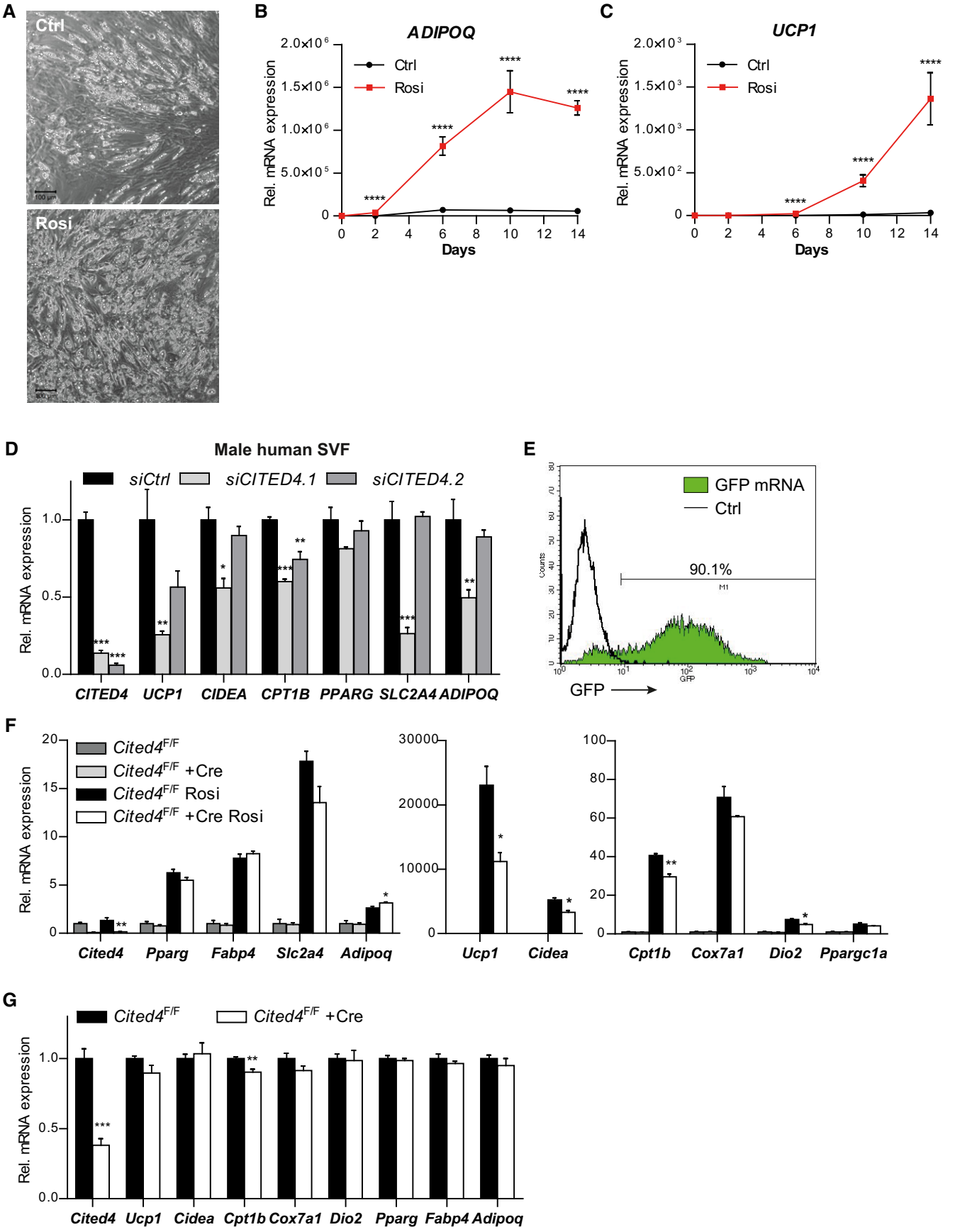

Figure EV2.

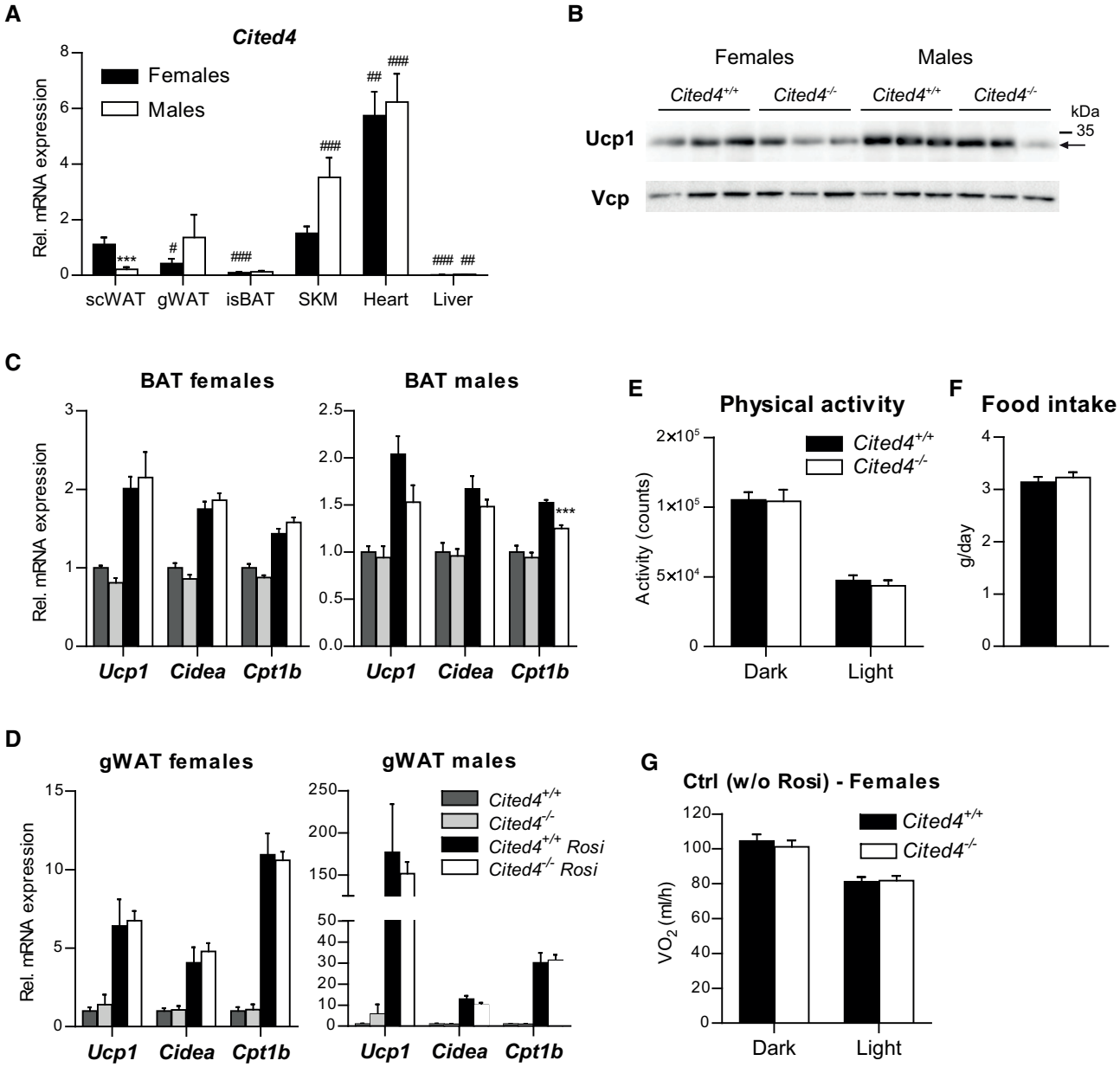

Figure EV3. *Cited4* deficiency specifically affects rosiglitazone-mediated thermogenic expression in subcutaneous fat.

A mRNA expression in wild-type mice without treatment, as determined by qRT–PCR (n = 6). scWAT: subcutaneous white adipose tissue; gWAT: gonadal WAT; isBAT: interscapular brown adipose tissue; SKM: gastrocnemius skeletal muscle. 2 × 2 ANOVA with Holm–Sidak posttests, ***P = 0.0004 (female vs. male), $^{\#\#\#}P$ = 0.0004, $^{\#\#\#}P = 7 \times 10^{-6}$, $^{\#\#\#}P = 6 \times 10^{-9}$, $^{\#\#\#}P = 3 \times 10^{-10}$, (isBAT, SKM, heart, liver, respectively, vs. scWAT within indicated sex), $^{\#}P$ = 0.032 (females: gWAT vs. scWAT), $^{\#\#}P$ = 0.006 (females: Heart vs. scWAT), $^{\#\#}P$ = 0.006 (males: Liver vs. scWAT).

B Ucp1 expression in scWAT of mice fed a diet with 0.0075% Rosi or control diet for 2.5 weeks, determined by Western blot with VCP as loading control (n = 3, same samples as in Fig 4C and D).

C mRNA expression in BAT of mice treated as in (A), determined by qRT–PCR. t-test *Cited4*$^{-/-}$ vs. *Cited4*$^{+/+}$ (Rosi), females: n = 5/5/6/6, males: n = 5/4/5/5, ***P = 0.0004.

D mRNA expression in gWAT of mice treated as in (A), determined by qRT–PCR. t-test *Cited4*$^{-/-}$ vs. *Cited4*$^{+/+}$ (Rosi), females: n = 5/5/6/6, males: n = 5/4/5/5.

E Three-day averages of total activity counts per day and mouse of female mice fed a diet with 0.0075% Rosi for 2.5 weeks, determined by Phenomaster (n = 9/10, t-test).

F Three-day averages of food intake per day and mouse of female mice shown in (E) (n = 9/10, t-test).

G Oxygen consumption rate of female mice fed control diet, determined by indirect calorimetry and adjusted for body weight by ANCOVA. Three-day averages of VO_2 were calculated for each mouse (n = 10). ANCOVA with Bonferroni's posttest (*Cited4*$^{-/-}$ vs. *Cited4*$^{+/+}$).

Data information: Data are presented as mean ± SEM.
Source data are available online for this figure.

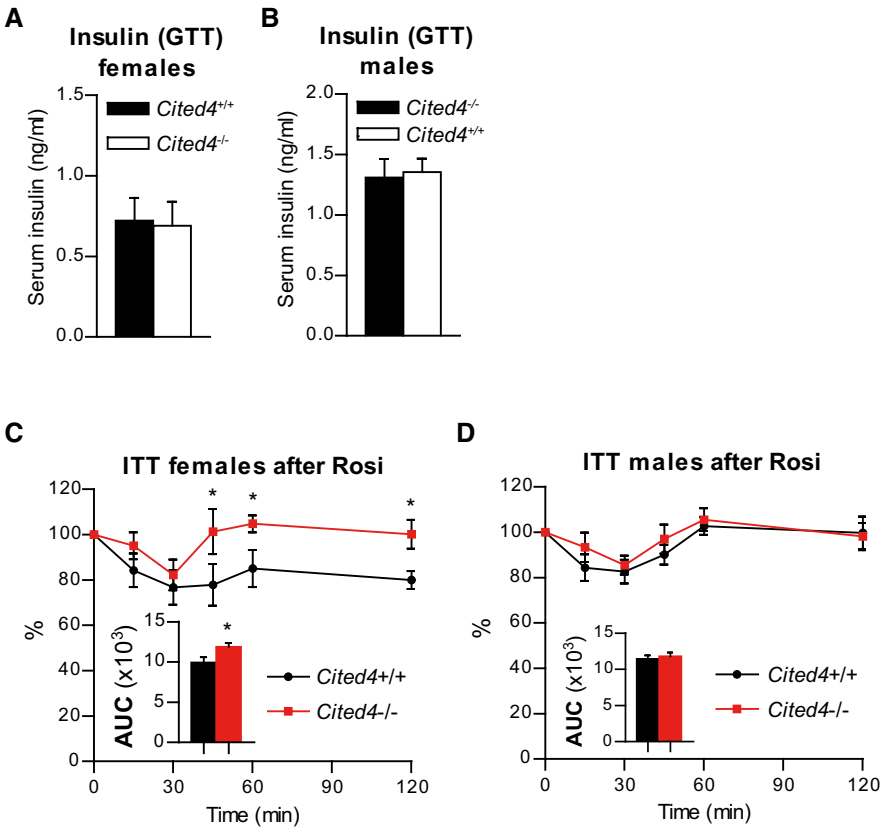

Figure EV4. Sex-specific involvement of *Cited4* insulin sensitization upon therapeutic rosiglitazone treatment.

A, B Serum insulin concentration 60 min after glucose injection in the GTT in mice after 11 weeks of HFD and 4 weeks of HFD + Rosi. *t*-test $Cited4^{-/-}$ vs. $Cited4^{+/+}$, (A) $n = 8$, (B) $n = 7/8$.

C Blood glucose during insulin tolerance test (ITT) with 1 U insulin per kg lean mass on female mice after 11 weeks of HFD and 4 weeks of HFD + Rosi, expressed as % of the 0-time point value ($n = 8$). $^*P = 0.015$ (45 min), $^*P = 0.039$ (60 min), $^*P = 0.035$ (120 min) in repeated-measures 2 × 2 ANOVA with Holm–Sidak posttests; Inlet: AUC *t*-test $Cited4^{-/-}$ vs. $Cited4^{+/+}$ $^*P = 0.041$.

D Blood glucose during ITT on male mice as in (C) ($n = 7/8$). Repeated-measures 2 × 2 ANOVA with Holm–Sidak posttests; Inlet: AUC *t*-test.

Data information: Data are presented as mean ± SEM.