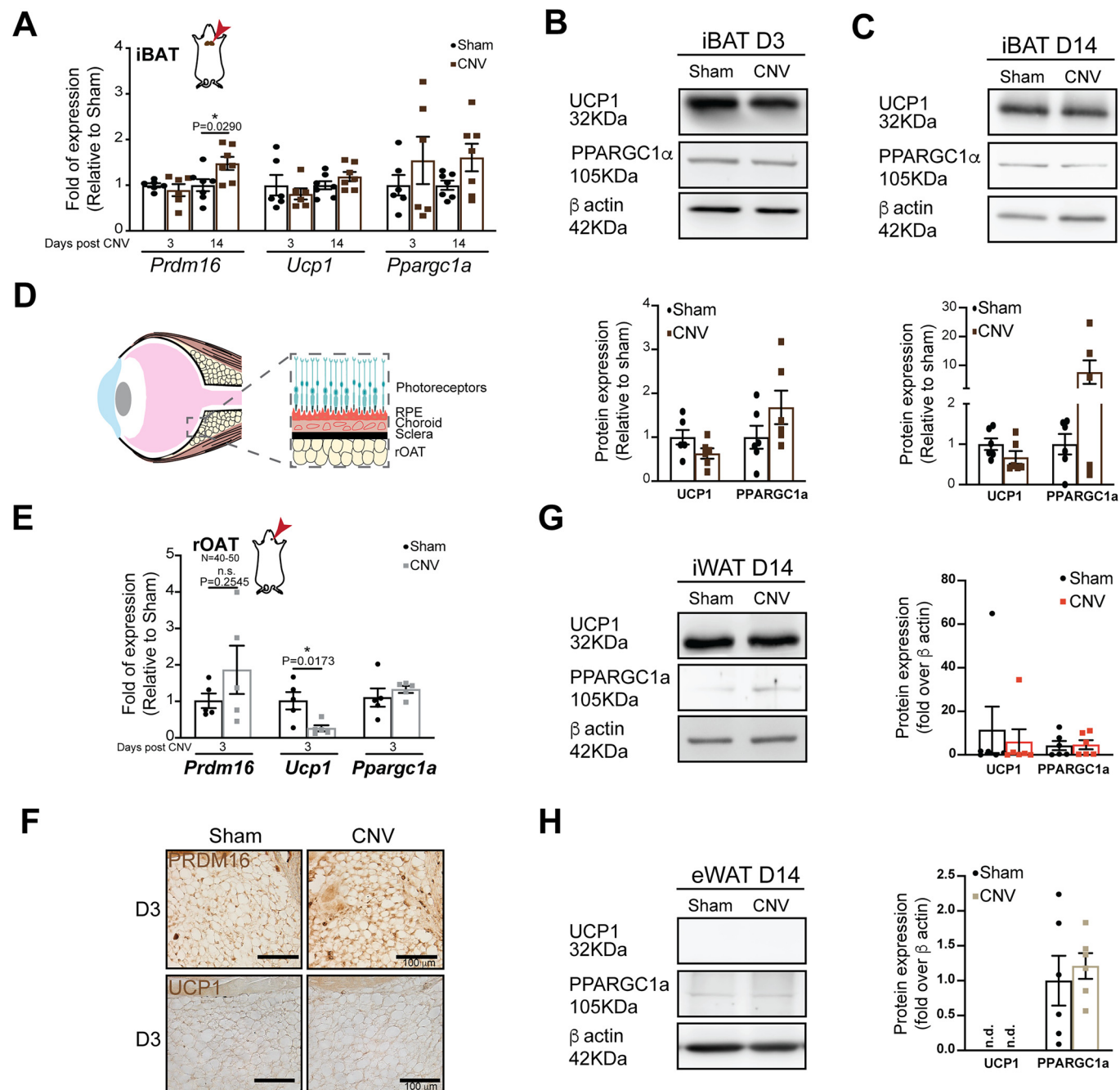


Expanded View Figures

**Figure EV1. Thermogenic factor expression in iBAT is not affected by CNV.**

(A) iBAT relative mRNA expression of *Prdm16*, *Ucp1* and *Pparg1a* from laser CNV and sham C57BL/6J mice 3- and 14-days post CNV, $n = 7$ mice per group. Gene expression was normalized to sham mice 3- and 14-days post CNV. (B) Representative immunoblots and their corresponding quantification for UCP1 and PPARGC1 α protein levels of iBAT at 3-days in laser CNV and sham group ($n = 6$ per group). (C) Representative immunoblots and their corresponding quantification for UCP1 and PPARGC1 α protein levels in iBAT 14-days in laser CNV and sham group ($n = 6$ per group). β -actin was used as a loading control. (D) Schematic representation of the anatomical location of rOAT. The rOAT is located around the optic nerve behind the posterior pole of the eyeball in contact with the sclera. (E) rOAT relative mRNA expression of *Prdm16*, *Ucp1* and *Pparg1a* from laser CNV and sham C57BL/6J mice at 3 days post CNV. Gene expression was normalized to sham mice, $n = 40$ –50 mice per group. (F) Representative immunohistochemistry staining images for PRDM16 and UCP1 in rOAT from laser CNV and sham C57BL/6J mice at 3 days post CNV, $n = 5$ mice per group. (G, H) Representative immunoblots and their corresponding quantification for UCP1 and PPARGC1 α protein levels in (G) iWAT and (H) eWAT 14-days in laser CNV and sham group ($n = 6$ per group). β -actin was used as a loading control. Data is presented as mean $\pm$ SEM. Statistical significance was assessed using unpaired two-tailed Student's t test. Exact P values are indicated in the figure, with $*P < 0.05$. Source data are available online for this figure.

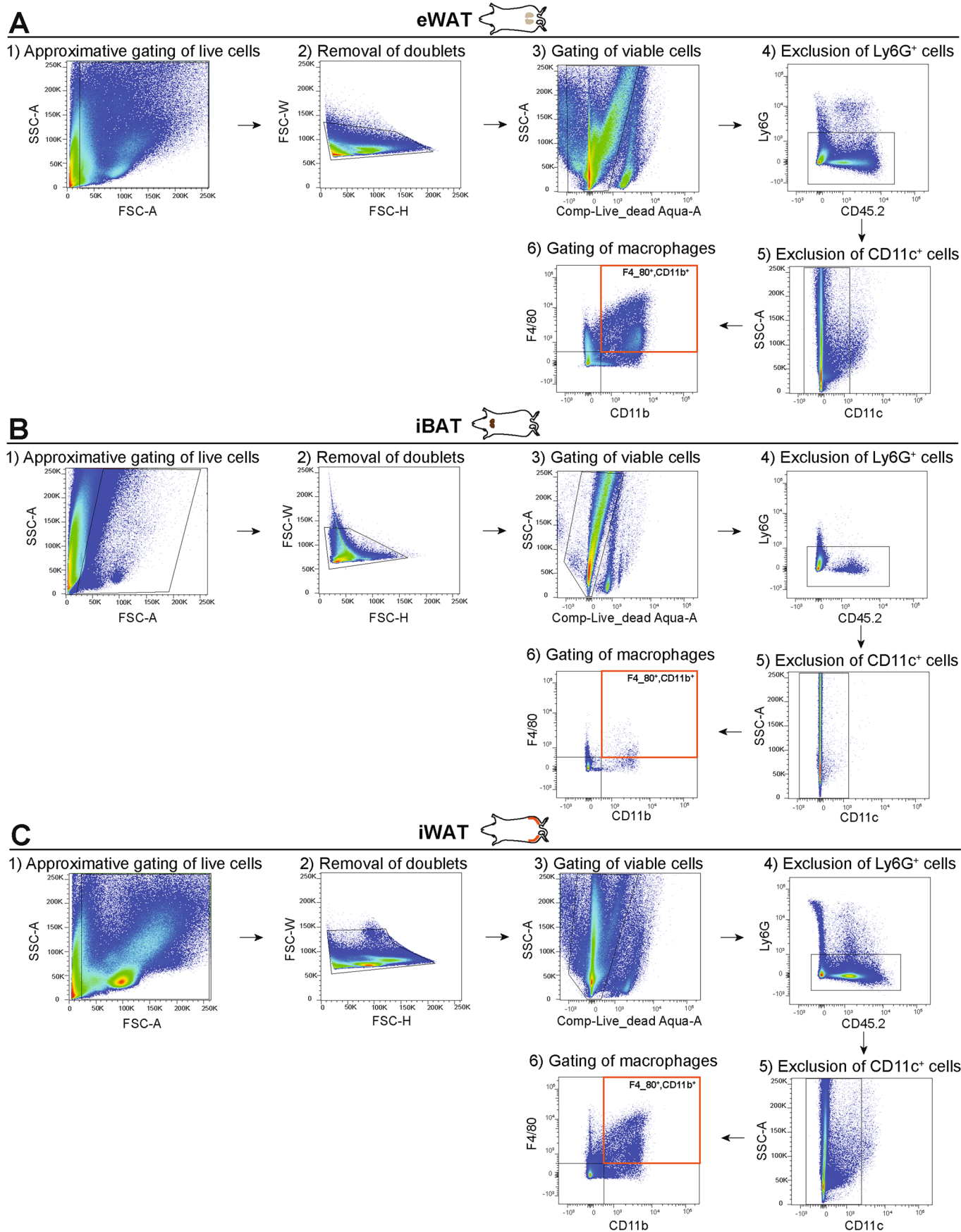


**Figure EV2. Flow cytometry gating strategies of ATMs from ATs.**

(A–C) Gating strategy for ATMs in the SVF of (A) eWAT, (B) iBAT, and (C) iWAT: (1) approximative gating of live cells, (2) gating to remove doublets, (3) selection of viable cells, (4) exclusion of Ly6G-positive cells, (5) exclusion of CD11c-positive cells, and (6) gating of ATMs.

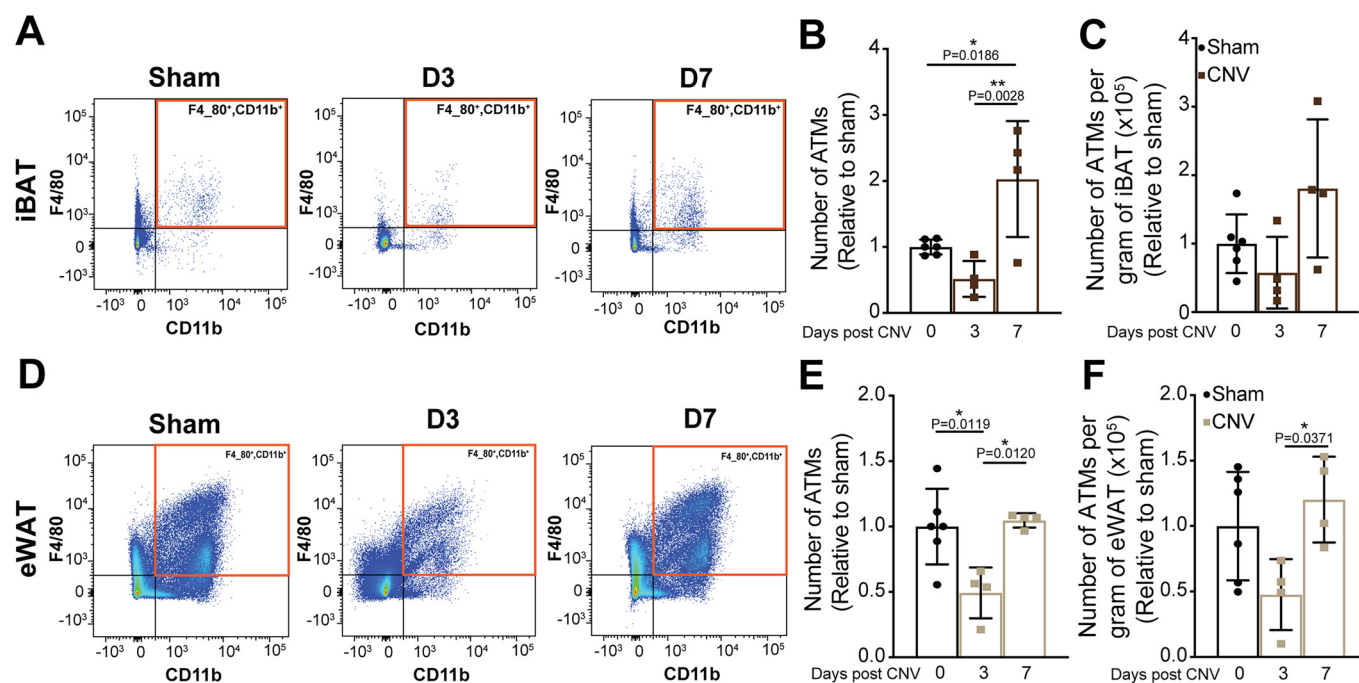


Figure EV3. ATMs increase in iBAT during CNV formation.

(A) Representative flow cytometry analysis time course of iBAT ATMs, (B) quantification of the number of ATMs, and (C) total number of ATMs per gram of iBAT normalized to sham mice from laser CNV mice at 0, 3, and 7 days after laser burn ($n = 4-6$ per group). (D) Representative flow cytometry analysis time course of eWAT ATMs, (E) quantification of the number of ATMs, and (F) total number of ATMs per gram of eWAT normalized to sham mice from laser CNV mice at 0, 3, and 7 days after laser burn ($n = 4-6$ per group). Data are presented as mean $\pm$ SEM. Statistical analysis was performed using ordinary one-way ANOVA followed by Tukey's multiple comparisons post hoc test. Exact P values are indicated in the figure, with * $P < 0.05$ and ** $P < 0.01$.

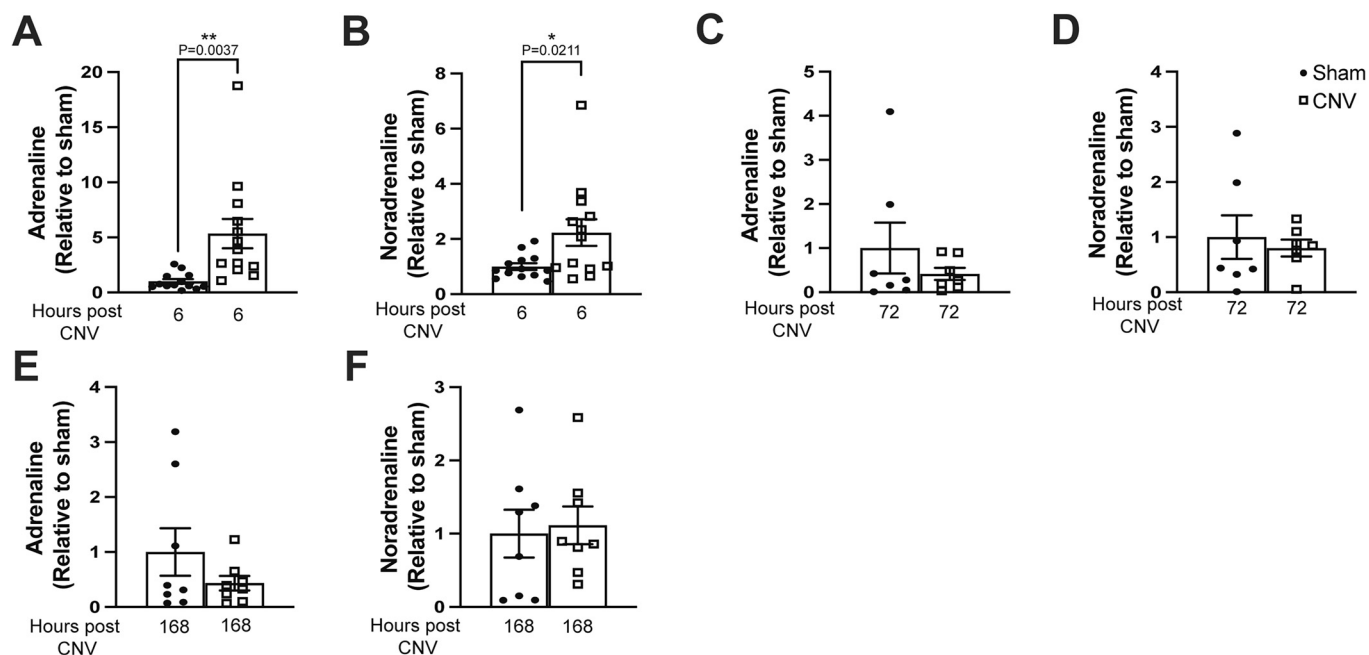
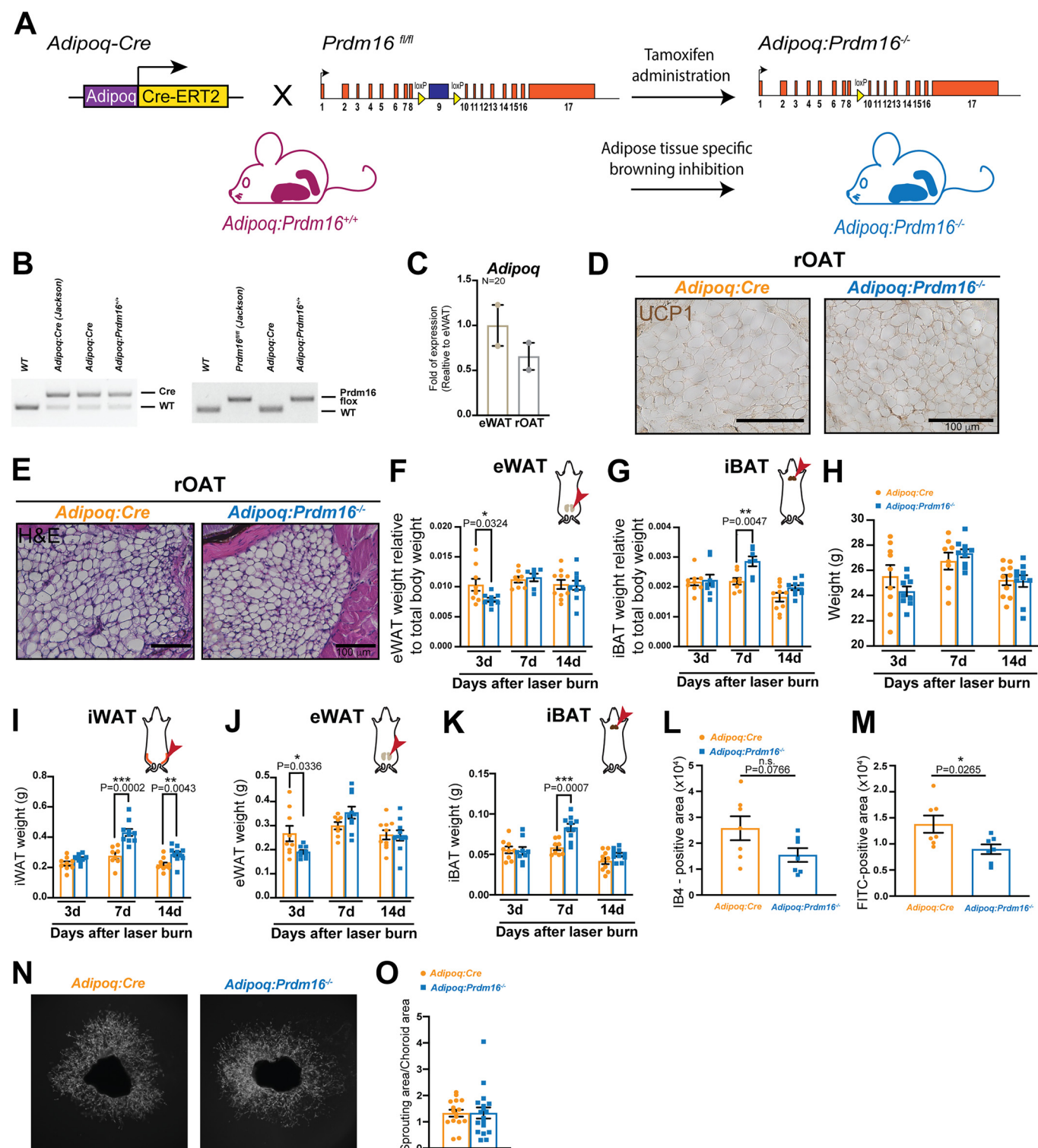


Figure EV4. Plasma catecholamines are upregulated 6 h after laser treatment.

(A) Adrenaline and (B) noradrenaline plasma levels from laser CNV and sham C57BL/6J mice 6 h post CNV, $n = 13$. (C) Adrenaline and (D) noradrenaline plasma levels from laser CNV and sham C57BL/6J mice 72 h post CNV, $n = 7$. (E) Adrenaline and (F) noradrenaline plasma levels from laser-burned and sham C57BL/6J mice 168 h post CNV, $n = 8$. Data are presented as mean $\pm$ SEM. Statistical significance was assessed using unpaired two-tailed Student's t test. Exact P values are indicated in the figure, with $*P < 0.05$ and $**P < 0.01$. Source data are available online for this figure.



◀ **Figure EV5. Knockout of *Prdm16* is specific to AT in *Adipoq:Prdm16*^{+/-} mice.**

(A) Schematic representation of *Adipoq:Prdm16*^{+/-} mice generation. *Adipoq:Cre* mice expressing Cre-ERT2 specifically in the ATs were crossed with *Prdm16*^{lox/lox} mice that have loxP-flanked exon 9 of *Prdm16*, thus generating *Adipoq:Prdm16*^{+/-} mice. Following tamoxifen administration, the exon 9 of *Prdm16* is removed generating *Adipoq:Prdm16*^{-/-} mice that have a specific browning inhibition in the ATs from the *Adipoq:Prdm16*^{+/-} mice. (B) Genotyping of *Adipoq:Cre* and *Adipoq:Prdm16*^{+/-} mice for Cre and *Prdm16* loxP-flanked regions. (C) eWAT and rOAT relative mRNA expression of *Adipoq* from C57BL/6J mice. Gene expression was normalized to eWAT expression, *n* = 2–20 mice per group (as per graph). (D, E) Representative immunohistochemistry staining images for (D) UCP1 and (E) H&E in rOAT from gavaged *Adipoq:Cre* and *Adipoq:Prdm16*^{+/-} mice, *n* = 4 mice per group. (F) eWAT and (G) iBAT masses were calculated as a ratio from tissue-specific weight over total body weight from *Adipoq:Cre* and *Adipoq:Prdm16*^{+/-} mice at 3, 7, and 14 days after laser burn (*n* = 8–10 mice per group). (H) Comparison of body weight from *Adipoq:Cre* and *Adipoq:Prdm16*^{+/-} mice, at 3, 7, and 14 days after laser burn (*n* = 8–10 mice per group). (I) iWAT, (J) eWAT, and (K) iBAT masses of *Adipoq:Cre* and *Adipoq:Prdm16*^{+/-} mice at 3, 7, and 14 days after laser burn (*n* = 8–10 mice per group). (L) Quantification of isolectin B₄-positive area and (M) quantification of FITC-dextran-labeled CNV area per laser burned *Adipoq:Cre* and *Adipoq:Prdm16*^{+/-} mouse 14 days after laser burn; *n* = 7 mice per group. (N) Representative images of choroid explants and (O) quantification of sprouting area over choroid explant area from *Adipoq:Cre* (*n* = 16 explants) and *Adipoq:Prdm16*^{+/-} (*n* = 18 explants) mice gavaged with tamoxifen. Data were normalized to *Adipoq:Cre* mice choroid explants. Data are presented as mean ± SEM. Statistical significance was assessed using unpaired two-tailed Student's *t* test. Exact *P* values are indicated in the figure, with **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. Source data are available online for this figure.

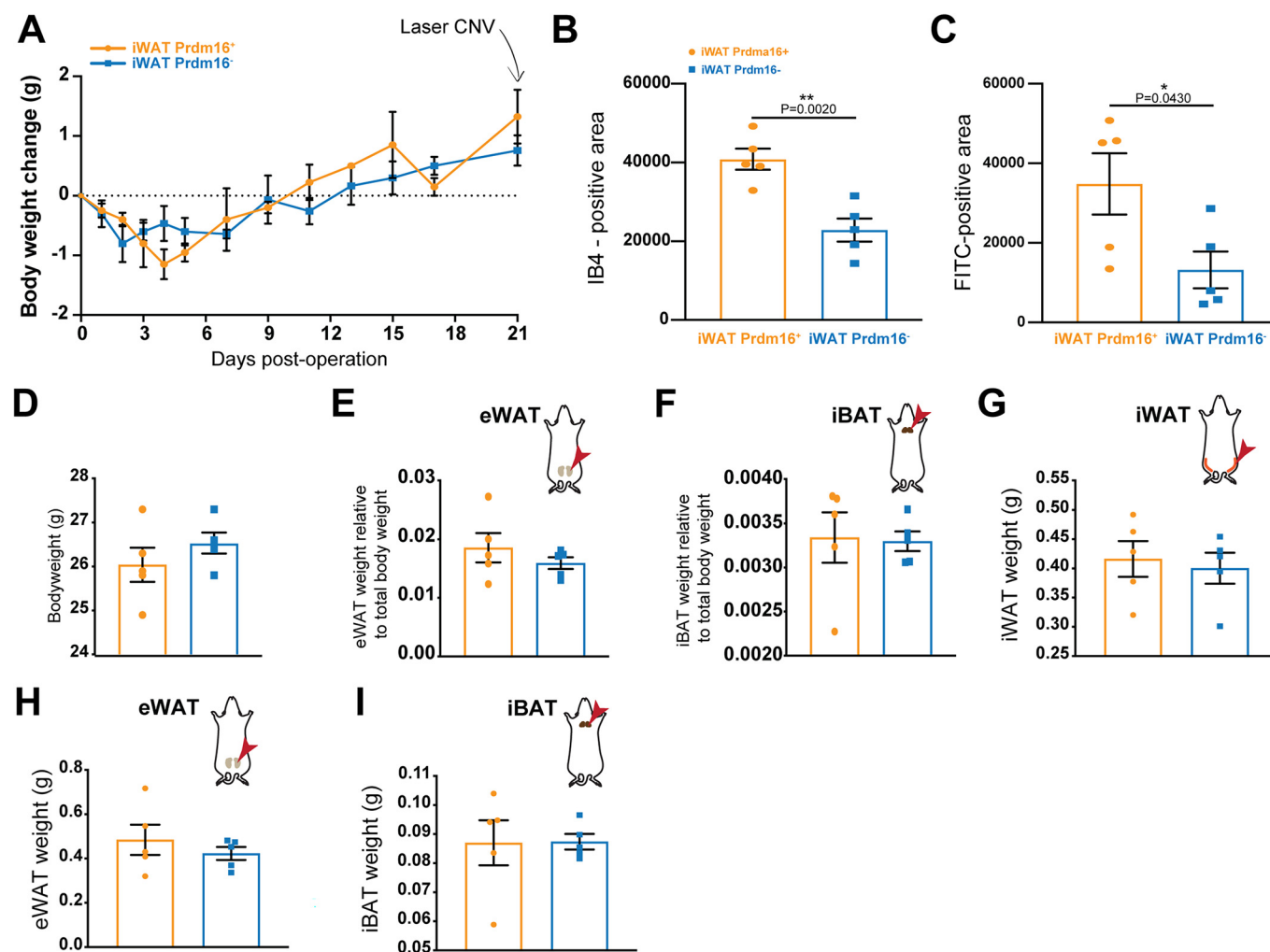


Figure EV6. Changes in AT weight in *Adipoq:Prdm16*^{-/-} mice following ATT.

(A) Body weight change from *Adipoq:Prdm16*^{-/-} recipient mice transplanted with either *Prdm16*-positive or *Prdm16*-negative iWAT for 21 days following the ATT ($n = 5$). (B) Quantification of isolectin B4-positive area and (C) quantification of FITC-dextran-labeled CNV area per *Adipoq:Prdm16*^{+/+} mice transplanted with *Prdm16*-positive or *Prdm16*-negative iWAT 14 days after laser burn; $n = 5$ mice per group. (D) Comparison of the body weight of *Adipoq:Prdm16*^{+/+} transplanted mice with either *Prdm16*-positive or *Prdm16*-negative iWAT. (E) eWAT and (F) iBAT masses were calculated as a ratio from tissue-specific weight over total body weight from *Adipoq:Prdm16*^{+/+} mice transplanted with *Prdm16*-positive or *Prdm16*-negative iWAT at 14 days after laser burn ($n = 5$ mice per group). (G) iWAT, (H) eWAT, and (I) iBAT masses of *Adipoq:Prdm16*^{+/+} transplanted mice with either *Prdm16*-positive or *Prdm16*-negative iWAT at 14 days after laser burn ($n = 5$ mice per group). Data are presented as mean $\pm$ SEM. Statistical significance was assessed using unpaired two-tailed Student's *t* test. Exact *P* values are indicated in the figure, with $*P < 0.05$ and $**P < 0.01$. Source data are available online for this figure.