

Supplemental information

**Estradiol regulates leptin sensitivity
to control feeding via hypothalamic Cited1**

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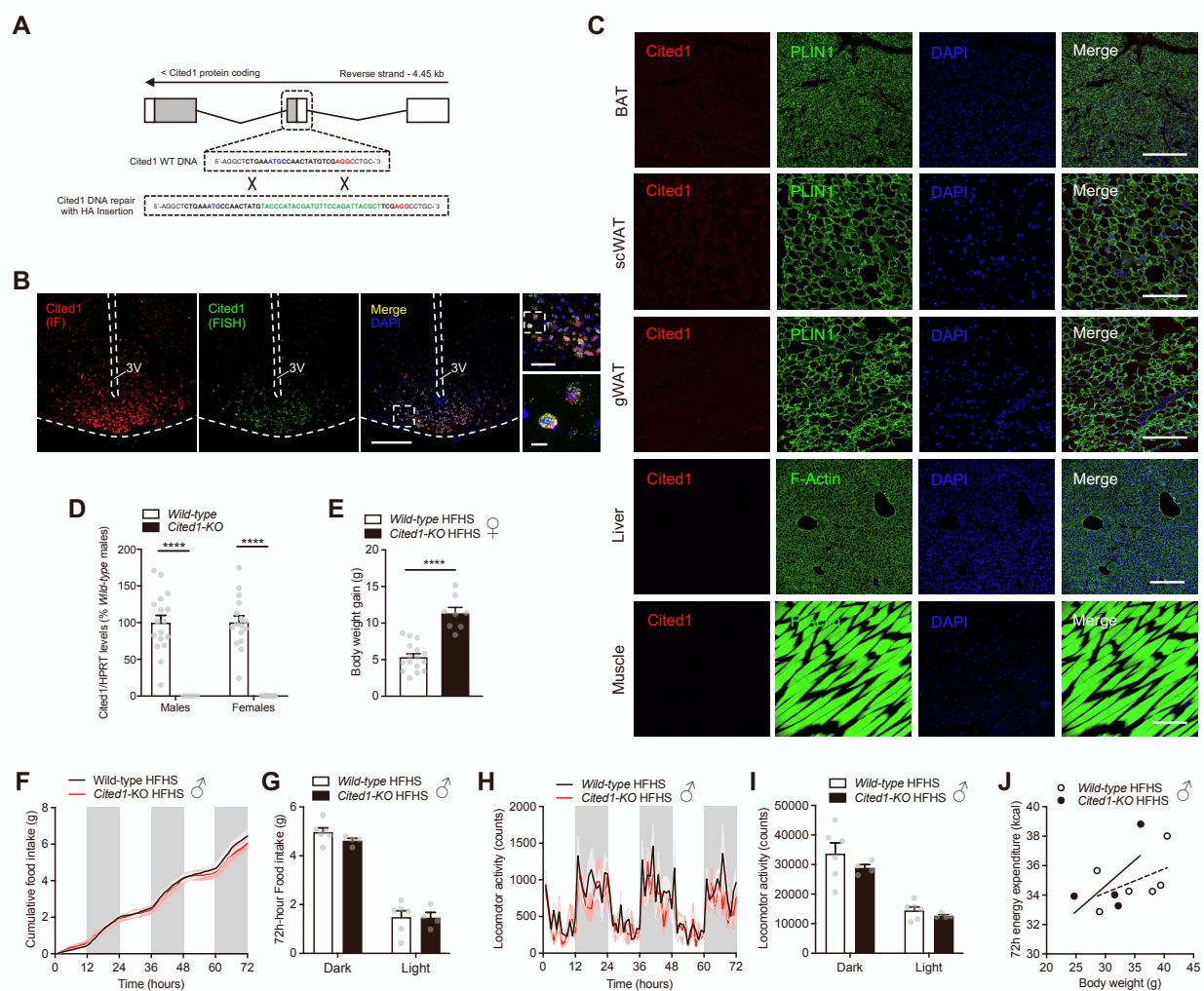


Figure S1. Cited1 characterization and metabolic phenotype of whole body Cited1 knock out, related to Figure 1.

(A) Graphical scheme of the targeted knock-in strategy followed to generate the *Cited1*-HA mice. An HA sequence (green) was inserted downstream the start codon (blue). Bold: gRNA sequence. Red: Pam sequence.

(B) Representative confocal micrographs depicting Cited1 HA-Tag protein (red), *Cited1* mRNA (green) and DAPI (blue) fluorescence in the ARC of *Cited1*-HA female mice subjected to a

combined immunofluorescence-RNA scopeTM. Scale bar = 200 μ m (left micrograph), 50 μ m (right top micrograph) and 10 μ m (right bottom micrograph).

(C) Representative confocal images depicting HA-Tag (red), PLIN1 or F-Actin (green), and DAPI (blue) immunoreactivity in brown adipose tissue (BAT), subcutaneous white adipose tissue (scWAT), gonadal adipose tissue (gWAT), liver and muscle (gastrocnemius) of *Cited1-HA* mice. Scale bars = 200 μ m.

(D) *Cited1* expression levels in the hypothalamus of *wild-type* or *Cited1-KO* male and female mice fed with HFHS diet. n = 5-17 mice per group.

(E) Body weight gain of *wild-type* or *Cited1-KO* female mice fed with HFHS diet. n = 8-16 mice per group.

(F-J) Cumulative and total (light vs. dark phase) food intake, time dependent and total (light vs. dark phase) locomotor activity, ANCOVA analysis of the total energy expenditure (72 hours) vs. body weight of *wild-type* or *Cited1-KO* male mice fed with HFHS diet. n = 4-6 mice per group.

Data are expressed as mean $\pm$ SEM **(D-I)** and individual values **(J)**. Statistical analysis included two-way ANOVA **(D-I)** and ANCOVA **(J)**. ****p < 0.0001. 3V, third ventricle; IF, immunofluorescence; FISH, fluorescence in situ hybridization.

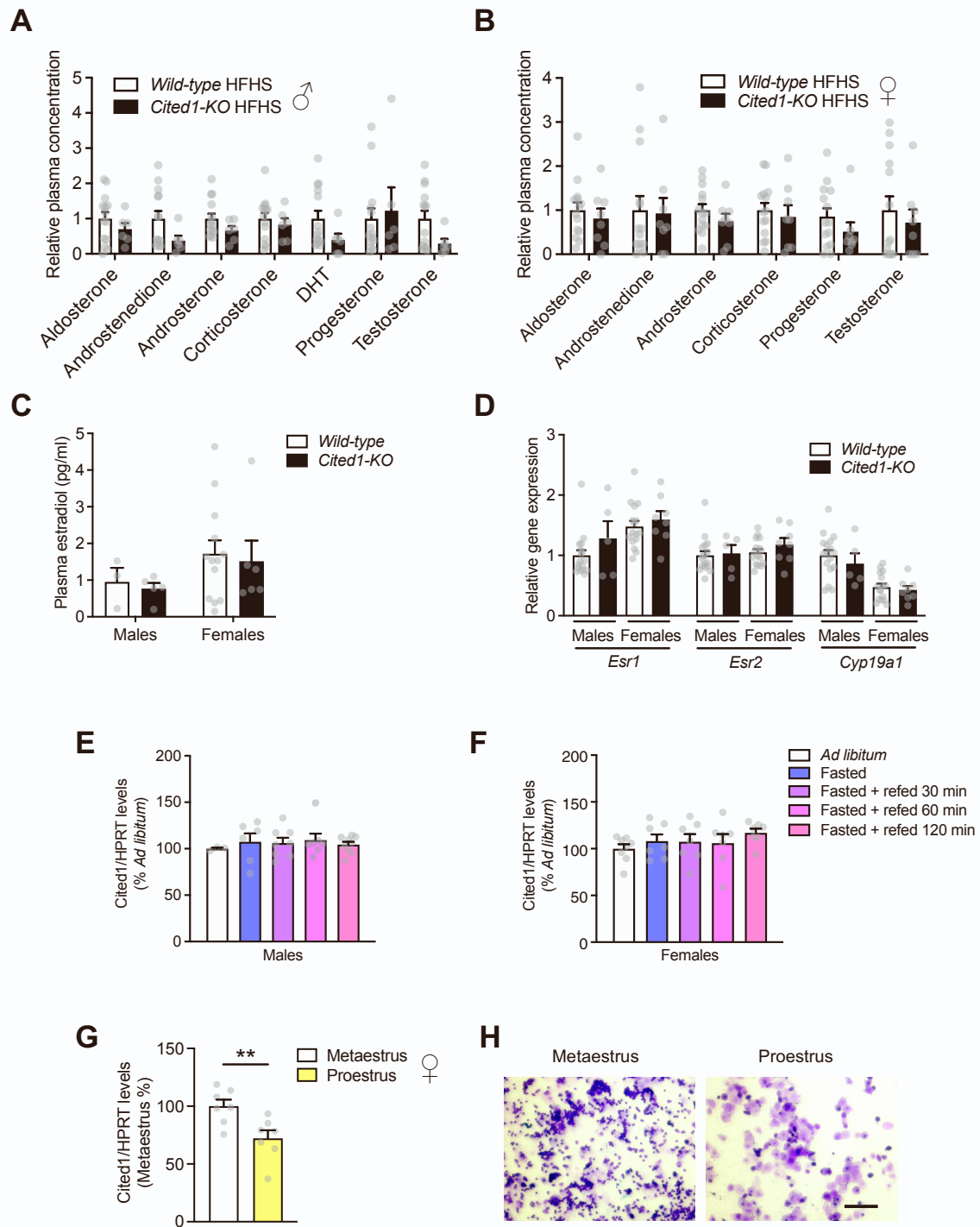


Figure S2. Endocrine characterization of whole body *Cited1* knock out, related to Figure 2.

(A-C) Plasma steroid hormones levels of *wild-type* or *Cited1*-KO male and female mice fed with HFHS diet. n = 3-14 mice per group.

(D) Gene expression levels of *wild-type* or *Cited1-KO* male and female mice fed with HFHS diet.
n = 5-17 mice per group.

(E and F) *Cited1* expression levels in the hypothalamus of *wild-type* male and female mice under different feeding paradigms. n = 3-9 mice per group.

(G) *Cited1* expression levels in the hypothalamus of *wild-type* female mice in metaestrus and proestrus cycle stage from *wild-type* mice. n = 7 mice per group.

(H) Representative microscopy images depicting vaginal smear cytology in metaestrus and proestrus cycle stage from *wild-type* mice. Scale bar = 500 μ m.

Data are expressed as mean $\pm$ SEM **(A-E)**. Statistical analysis included two-way ANOVA **(A-D)** and unpaired Student's t test **(E)**. **p < 0.01.

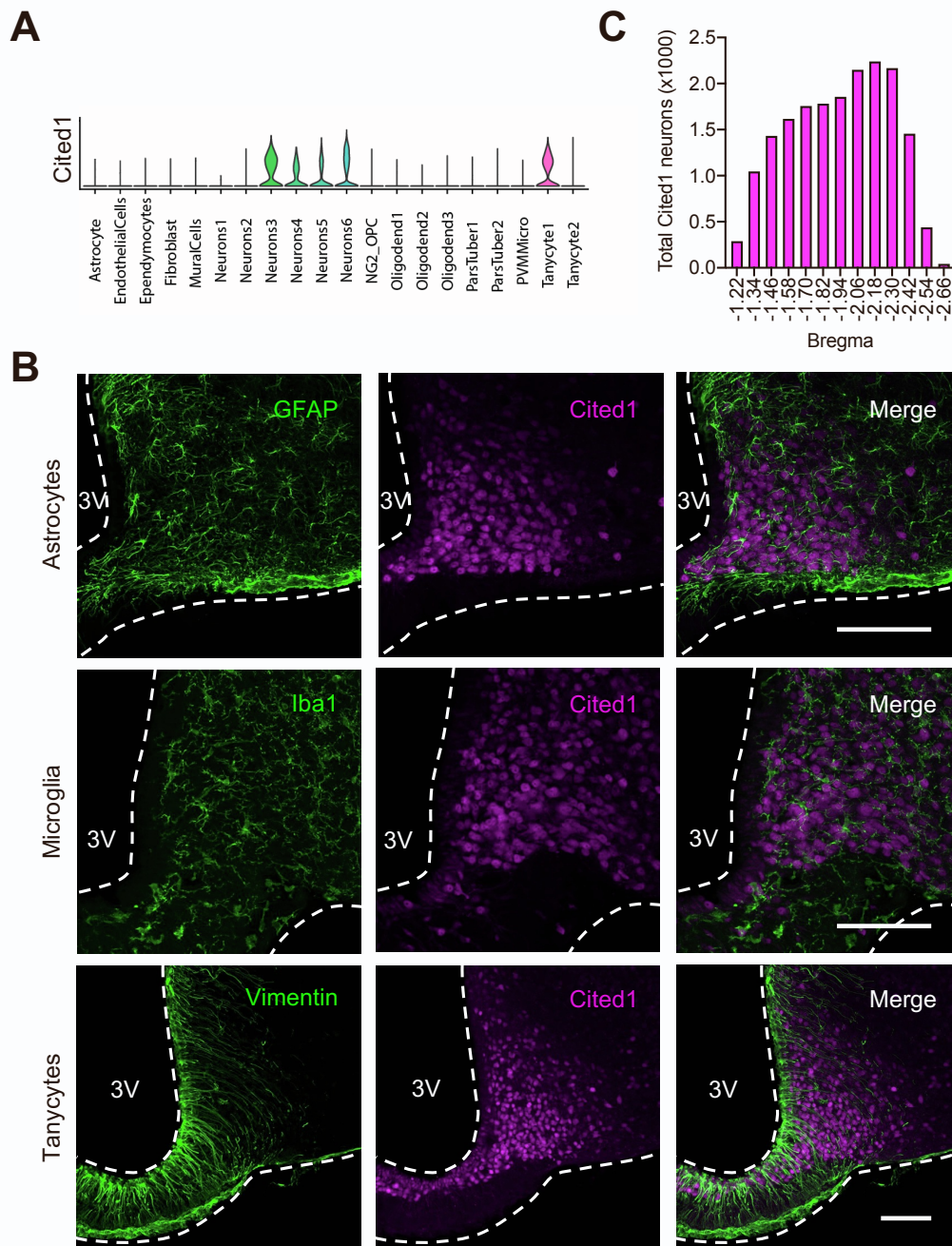


Figure S3. Cellular characterization of Cited1 in the hypothalamus, related to Figure 3.

(A) Violin plots depict the expression of *Cited1* across cell clusters identified by Campbell et al.²⁵. The shape of the violin plot indicates the distribution of cells depending on *Cited1* mRNA expression levels in the corresponding cell clusters.

(B) Representative confocal micrographs depicting Gfap, Iba1, Vimentin (green) and HA-Tag (magenta) immunoreactivity in the ARC of *Cited1-HA* mice. Scale bar = 100 μ m.

(C) Cited1 positive cells distribution along the coronal plane in the ARC region of *Cited1-HA* female mice.

Data are expressed as violin plot **(A)** or total numbers **(B)**. 3V, third ventricle.

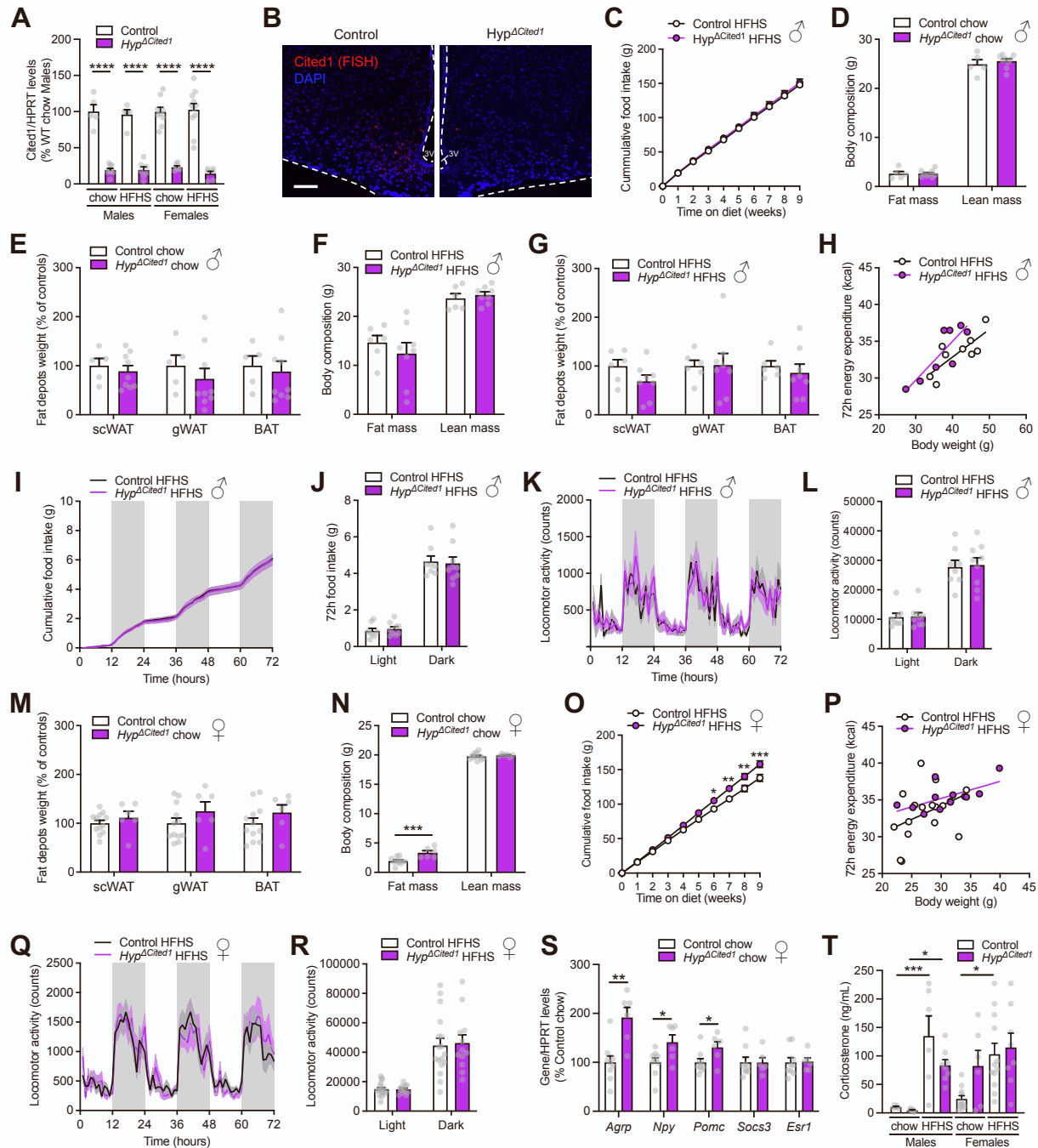


Figure S4. Metabolic phenotype of the hypothalamic *Cited1* knock out mice, related to Figure 3.

(A) *Cited1* expression levels in the hypothalamus of control or *Hyp^{ΔCited1}* male and female mice fed with chow or HFHS diet. n = 5-11 mice per group.

(B) Representative confocal micrographs depicting *Cited1* mRNA (red) and DAPI (blue) in the ARC of control or *Hyp* ^{Δ *Cited1*} mice obtained using RNA Scope™. Scale bar = 200 μ m.

(C) Cumulative food intake of control or *Hyp* ^{Δ *Cited1*} male mice fed with HFHS diet. n = 7-9 mice per group.

(D and E) Fat and lean mass and relative scWAT, gWAT and BAT weights of control or *Hyp* ^{Δ *Cited1*} male mice fed with chow diet. n = 5-9 mice per group.

(F-L) Fat and lean mass and relative scWAT, gWAT and BAT weights, ANCOVA analysis of the total energy expenditure (72 hours) vs. body weight, cumulative and total (light vs. dark phase) food intake, time dependent and total (light vs. dark phase) locomotor activity of control or *Hyp* ^{Δ *Cited1*} male mice fed with HFHS diet. n = 6-8 mice per group.

(M and N) Relative scWAT, gWAT and BAT weights and fat and lean mass of control or *Hyp* ^{Δ *Cited1*} female mice fed with chow diet. n = 6-12 mice per group.

(O-R) Cumulative food intake, ANCOVA analysis of the total energy expenditure (72 hours) vs. body weight, time-dependent and total (light vs. dark phase) locomotor activity of control or *Hyp* ^{Δ *Cited1*} female mice fed with HFHS diet. n = 8-17 mice per group.

(S) Gene expression levels in the hypothalamus of control or *Hyp* ^{Δ *Cited1*} female mice fed with chow diet. n = 6-9 mice per group.

(T) Serum corticosterone levels of control or *Hyp* ^{Δ *Cited1*} male and female mice fed with chow or HFHS diet. n = 5-12 mice per group.

Data are expressed as mean $\pm$ SEM (**A, C-G, I-O, Q-T**) and individual values (**H** and **O**). Statistical analysis included two-way ANOVA (**A, C-G, I-O, Q-T**), and ANCOVA (**H** and **O**). *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001. 3V, third ventricle, FISH, fluorescence in situ hybridization.

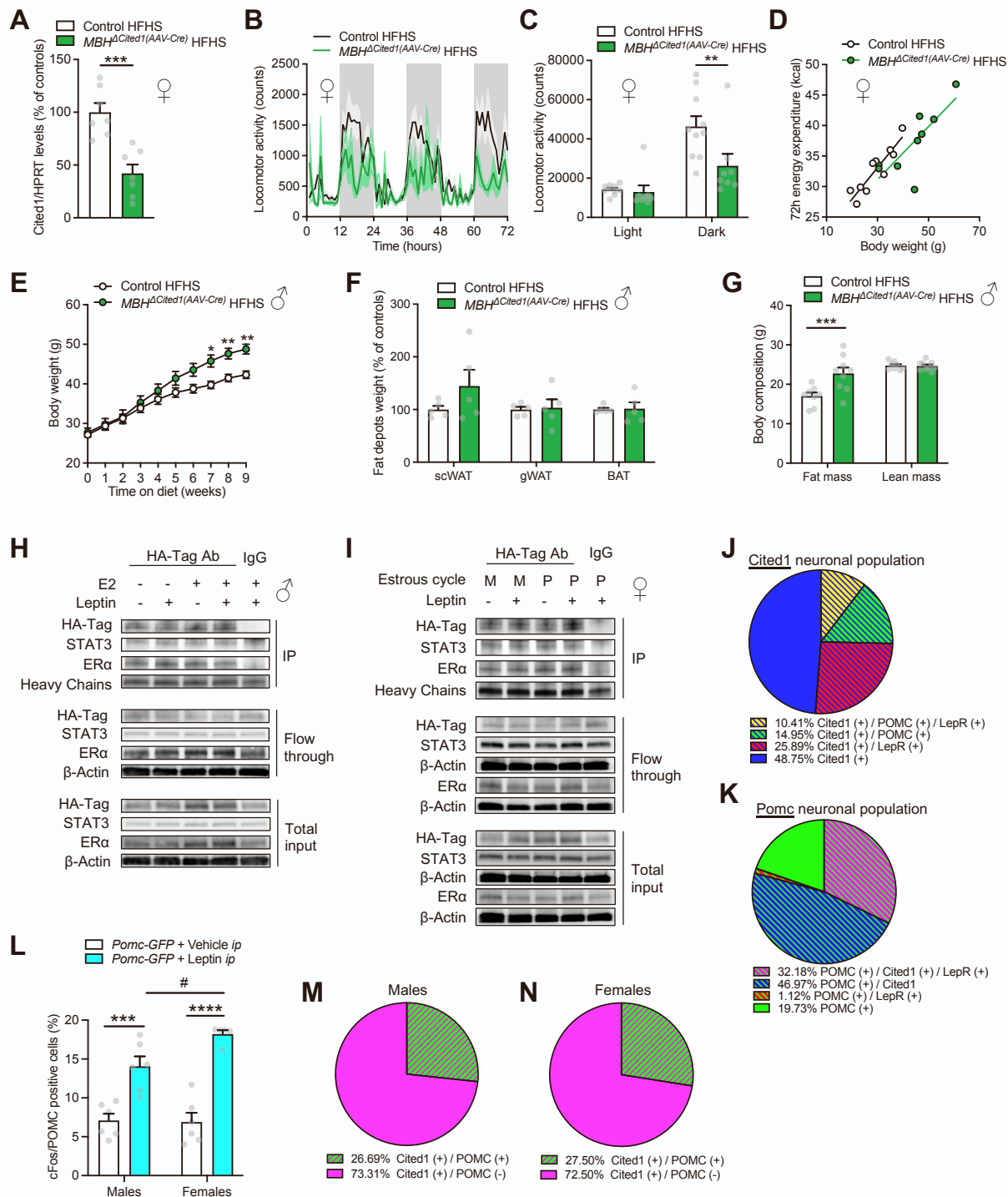


Figure S5. Metabolic phenotype of the virogenetic *Cited1* knockout mice, molecular and cellular characterization of *Cited1*, and sex-dimorphic leptin action in the ARC, related to Figures 3, 5 and 6.

(A) *Cited1* expression levels in the hypothalamus of control or *MBH^{ΔCited1(AAV-Cre)}* female mice fed with HFHS diet. n = 7 mice per group.

(B and C) Time dependent and total (light vs. dark phase) locomotor activity of control or *MBH^{ΔCited1(AAV-Cre)}* female mice fed with HFHS diet. n = 8-10 mice per group.

(D) ANCOVA analysis of the total energy expenditure (72 hours) vs. body weight of control or *MBH^{ΔCited1(AAV-Cre)}* female mice fed with HFHS diet. n = 9-10 mice per group.

(E) Body weight of control or *MBH^{ΔCited1(AAV-Cre)}* male mice fed with HFHS diet. n = 9-10 mice per group.

(F and G) Relative scWAT, gWAT and BAT weights and fat and lean mass of control or *MBH^{ΔCited1(AAV-Cre)}* female mice fed with HFHS diet. n = 5-9 mice per group.

(H) Western blot chemiluminescence images of HA-Tag, Stat3, ERα and heavy chains after a HA-Tag co-IP, compared to the flow through and total input of hypothalami from *Cited1-HA*, male mice fed with chow diet and treated with either vehicle sc or E2 sc (1μg/mice) and vehicle ip or leptin ip (3 mg/kg). n=1 mice per group.

(I) Western blot chemiluminescence images of HA-Tag, Stat3, ERα and heavy chains after a HA-Tag co-IP, compared to the flow through and total input of hypothalami from *Cited1-HA* female mice fed with chow diet during metaestrus (M) or proestrus (P) and treated either with vehicle ip or leptin ip (3 mg/kg). n=1 mouse per group.

(J) Quantification of the relative number of hypothalamic *Cited1* neurons which co-express *Pomc*, leptin receptor (*LepR*) or both in *Cited1-HA;Pomc-GFP;LepR-Cre;Ai14-tdTomato* mice. n=4 mice per group; 6 sections/mouse.

(K) Quantification of the relative number of hypothalamic *Pomc* neurons which co-express *Cited1*, leptin receptor (*LepR*) or both in *Cited1-HA;Pomc-GFP;LepR-Cre;Ai14-tdTomato* mice. n=4 mice per group; 6 sections/mouse.

(L) Percentage of cFos/GFP+ neurons in the ARC of *Pomc-GFP* male and female mice fed with chow diet and treated either with vehicle or leptin *ip* (3 mg/kg). n=5-6 mice per group; 6 sections/mouse.

(M and N) Quantification of the relative number of hypothalamic *Cited1* neurons which co-express *Pomc* in *Cited1-HA;Pomc-GFP* male or female mice. n=3 mice per group; 6 sections/mouse.

Data are expressed as mean $\pm$ SEM (**A-C**, **E-G** and **L**), mean (**J-K** and **M-N**) and individual values (**D**). Statistical analysis included two-way ANOVA (**B** and **C**, **E-G** and **L**), unpaired Student's t test (**A**) and ANCOVA (**D**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ and # $p < 0.05$. 3V, third ventricle. IgG, Ab, antibody; immunoglobulin G, IP, immunoprecipitation.

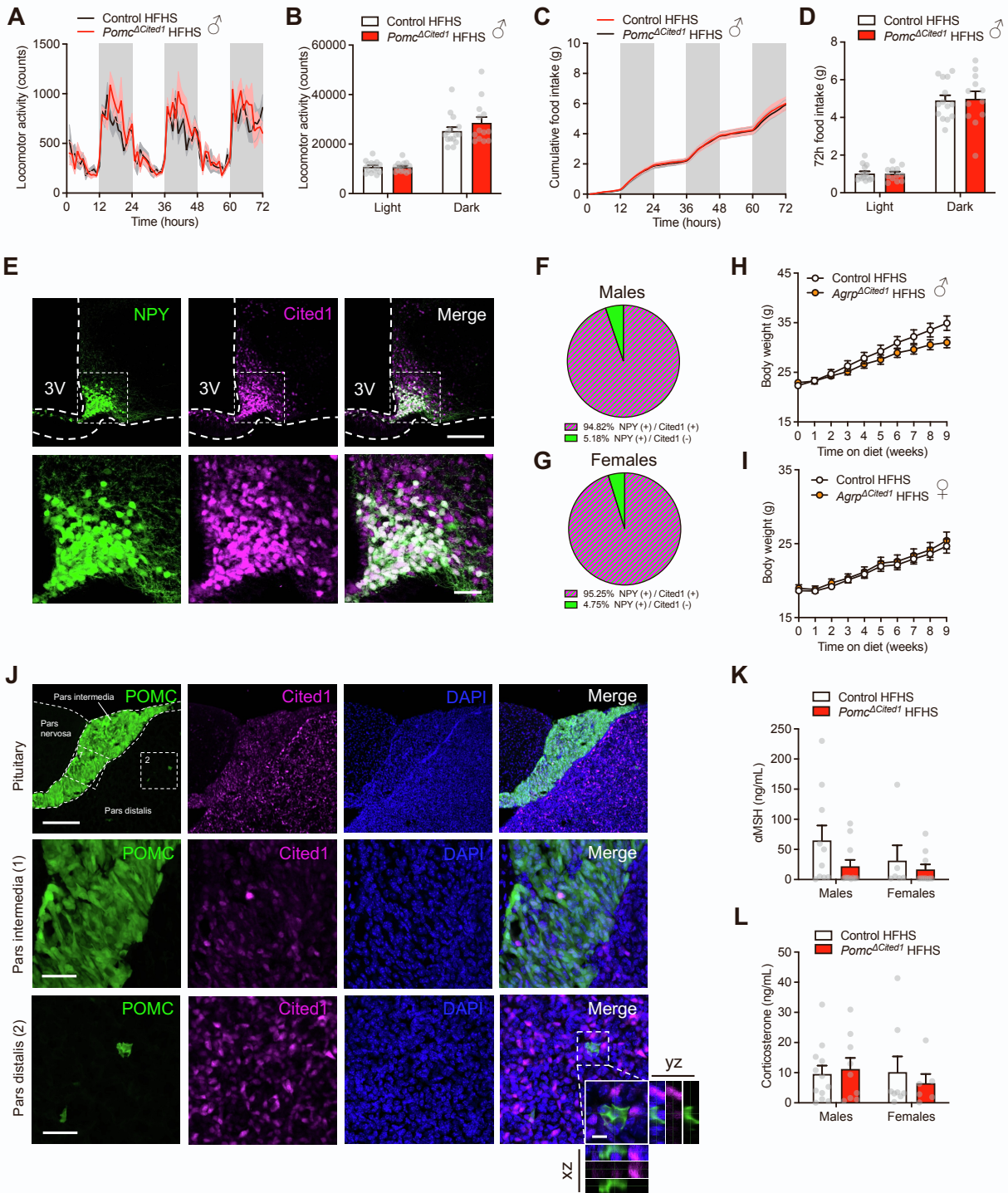


Figure S6. Cited1 characterization in Npy neurons and metabolic phenotype of conditional Cited1 knockout mouse models in AgRP and PomC neurons, related to Figure 7.

(A-D) Time-dependent and total (light vs. dark phase) locomotor activity, cumulative and total (light vs. dark phase) food intake of control or *Pomc* ^{Δ Cited1} male mice fed with HFHS diet. n = 12-15 mice per group.

(E) Representative confocal micrographs depicting GFP (green) and HA-Tag (magenta) immunoreactivity in the ARC of *Cited1-HA;Npy-GFP* mice. Scale bars = 200 μ m (top micrograph), 50 μ m (bottom micrograph).

(F and G) Quantification of the relative number of hypothalamic Npy neurons which co-express Cited1 in the *Cited1-HA;Npy-GFP* male or female mice. n=3 mice per group; 6 sections/mouse.

(H and I) Body weight of control or *Agrp* ^{Δ Cited1} male and female mice fed with HFHS diet. n = 12-21 mice per group.

(J) Representative confocal micrographs depicting GFP (green), HA-Tag (magenta) and DAPI (blue) immunoreactivity in the pars intermedia and pars distalis pituitary of *Cited1-HA;Pomc-GFP* mice. Orthogonal xz and yz planes of the corticotrope cell high magnification are displayed to allow a precise visualization of the tridimensional space. Scale bars = 200 μ m (left top micrograph), 50 μ m (left middle and left bottom micrographs) and 5 μ m (right bottom micrograph).

(K) Serum α -MSH levels of control or *Hyp* ^{Δ Cited1} male and female mice fed with HFHS diet. n = 6-11 mice per group.

(L) Serum corticosterone levels of control or *Hyp* ^{Δ Cited1} male and female mice fed with HFHS diet. n = 6-12 mice per group.

Data are expressed as mean $\pm$ SEM (**A-D, H, I, K and L**) and mean (**F-G**). Statistical analysis included two-way ANOVA (**A-D, H, I, K and L**). 3V, third ventricle.

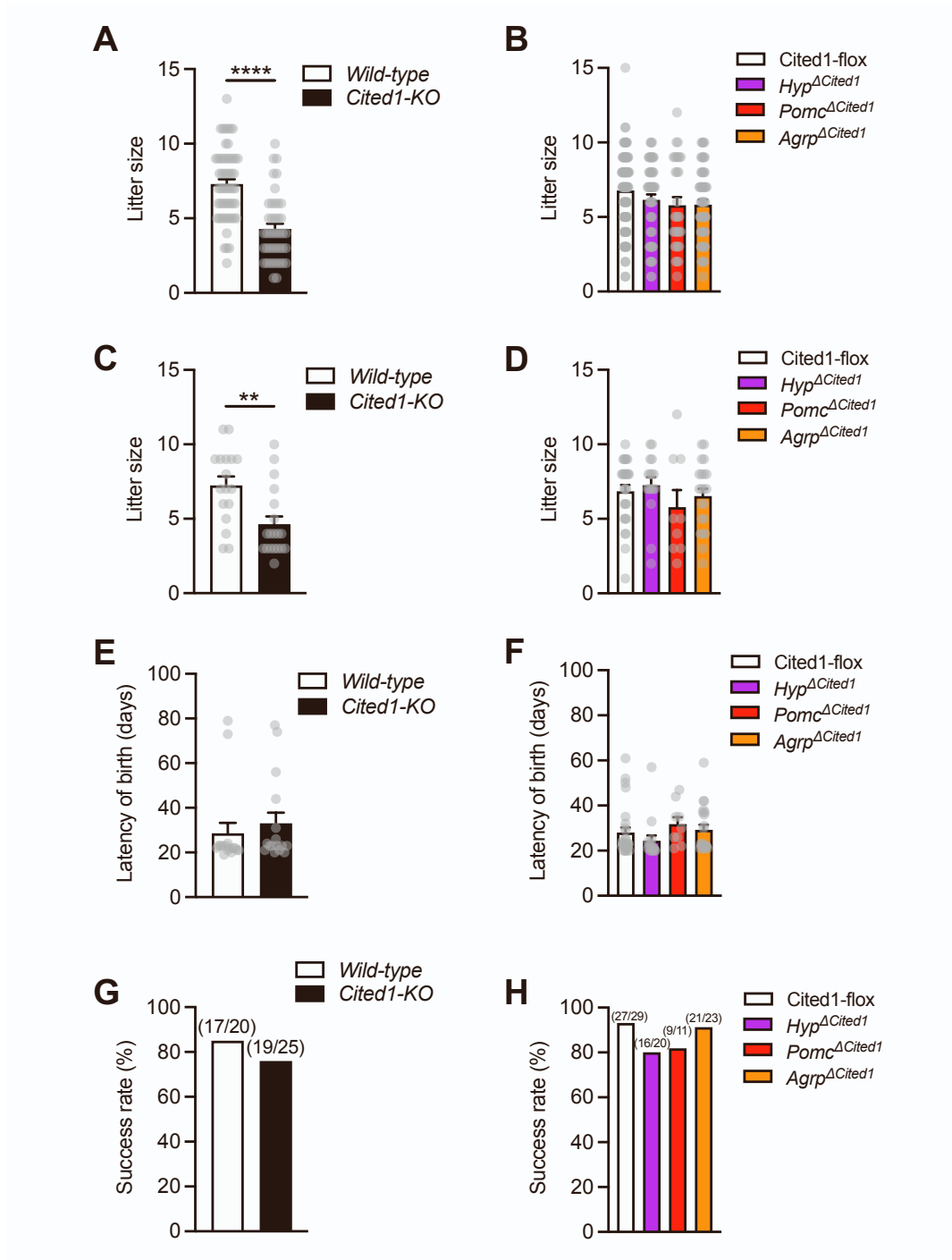


Figure S7. Fertility assessment of the *Cited1* knockout mouse lines, related to Figures 1, 3 and 7.

(A) Average litter size considering all litters of *wild-type* or *Cited1*-KO colonies. n = 42-56 mating pairs.

(B) Average litter size considering all litters of *Cited1*^{loxP/loxP}, *Hyp*^{ΔCited1}, *Pomc*^{ΔCited1} and *Agrp*^{ΔCited1} colonies. n = 31-84 mating pairs.

(C) Average size of the first litter of *wild-type* or *Cited1*-KO colonies. n = 17-19 mating pairs.

(D) Average size of the first litter of *Cited1*^{loxP/loxP}, *Hyp*^{ΔCited1}, *Pomc*^{ΔCited1} and *Agrp*^{ΔCited1} colonies. n = 9-27 mating pairs.

(E) Latency of birth considered as the average time period between mating and delivery day of pups of *wild-type* or *Cited1*-KO colonies. n = 16 mating pairs.

(F) Latency of birth considered as the average time period between mating and delivery day of *Cited1*^{loxP/loxP}, *Hyp*^{ΔCited1}, *Pomc*^{ΔCited1} and *Agrp*^{ΔCited1} colonies. n = 9-27 mating pairs.

(G) Success rate considered as the ratio of mating pairs that successfully delivered pups of *wild-type* or *Cited1*-KO colonies. n = 20-25 mating pairs.

(H) Success rate considered as the ratio of mating pairs that successfully delivered pups of *Cited1*^{loxP/loxP}, *Hyp*^{ΔCited1}, *Pomc*^{ΔCited1} and *Agrp*^{ΔCited1} colonies. n = 11-29 mating pairs.

Data are expressed as mean ± SEM (A-H) and total number (G and H). Statistical analysis included unpaired Student's t test (A, C and E), two-way ANOVA (B, D, and F) and ChiSquare (G and H). **p < 0.01 and ****p < 0.0001.

Oligonucleotide	Sequence
gRNA	5'-GCTGTATCAACCGTAAAGTC-3'
HA knock-in repair template	5'-CAACTGCCACCGATTTATCGGACTTCTGCCCA GGCTCTGAAATGCCAACTATGTACCCATACGATG TTCCAGATTACGCTTCGAGACCTGCACTTGATGT CAAGGGTGGCACCACCTCTGGGAAGGAGGTGA GTTTTAT-3'
Cited1 Fw genotyping primer	5'-CAGACGAAGCCCTGCTGTAT-3'
Cited1 Rv genotyping primer	5'-GTCACCCCTACCTCTGGACT-3'

Cited1 HA Fw genotyping primer 5'-TGCAGGTCTCGAAGCGTAAT-3'

Table S1: Guide RNA, knock-in template and primers used for the generation and genotyping of *Cited1-HA* mice, related to the generation of a Cited1-HA knock in mouse in the STAR Methods section.