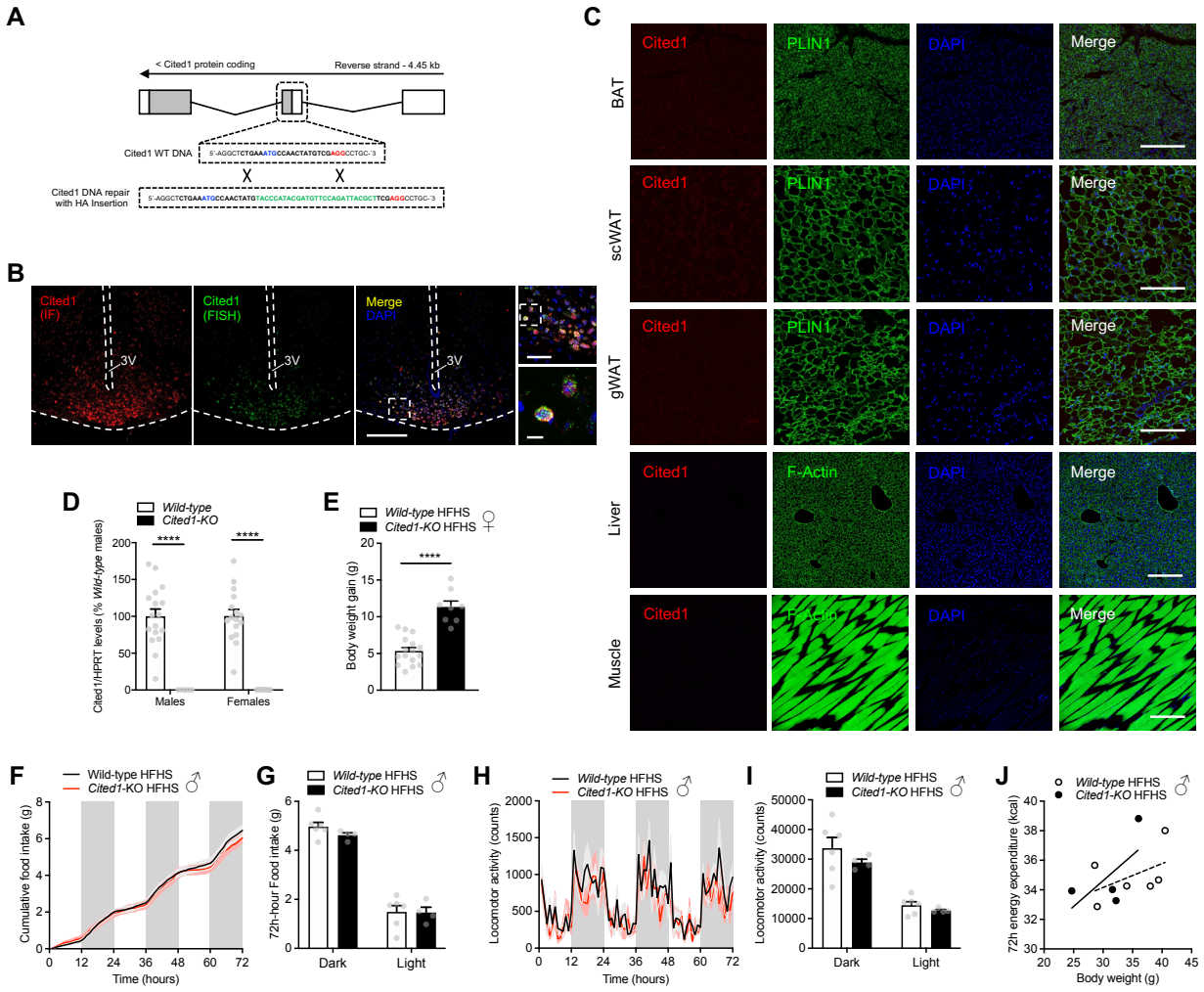

Figure S1. Cited1 characterization and metabolic phenotype of whole body Cited1 knock out, related to Figure 1.

(**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 scope™. Scale bar = 200 µm (left micrograph), 50 µm (right top micrograph) and 10 µm (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 µm.

(**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 ± 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.

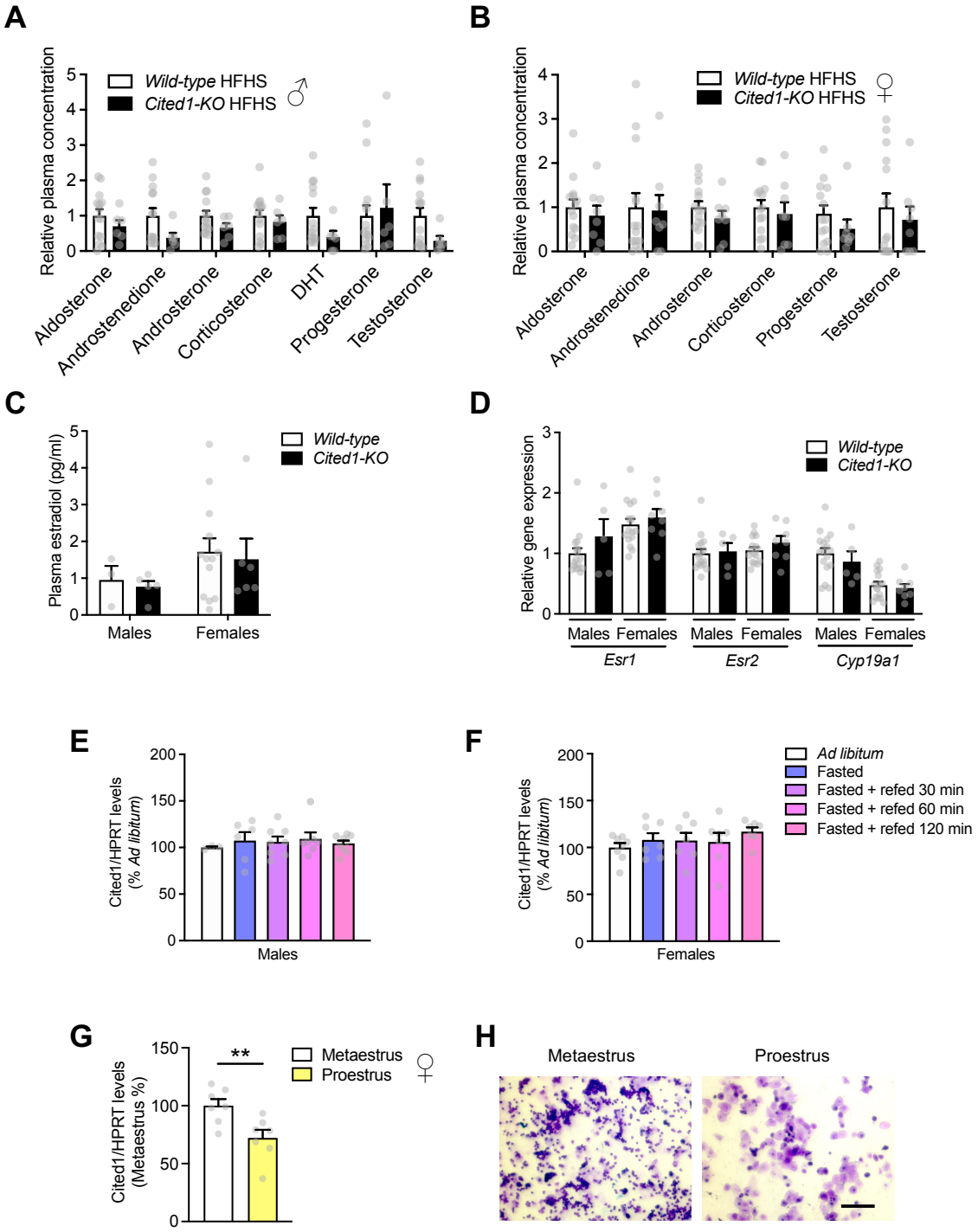

Figure S2. Endocrine characterization of whole body Cited1 knock out, related to Figure 2.

(**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 µm.

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

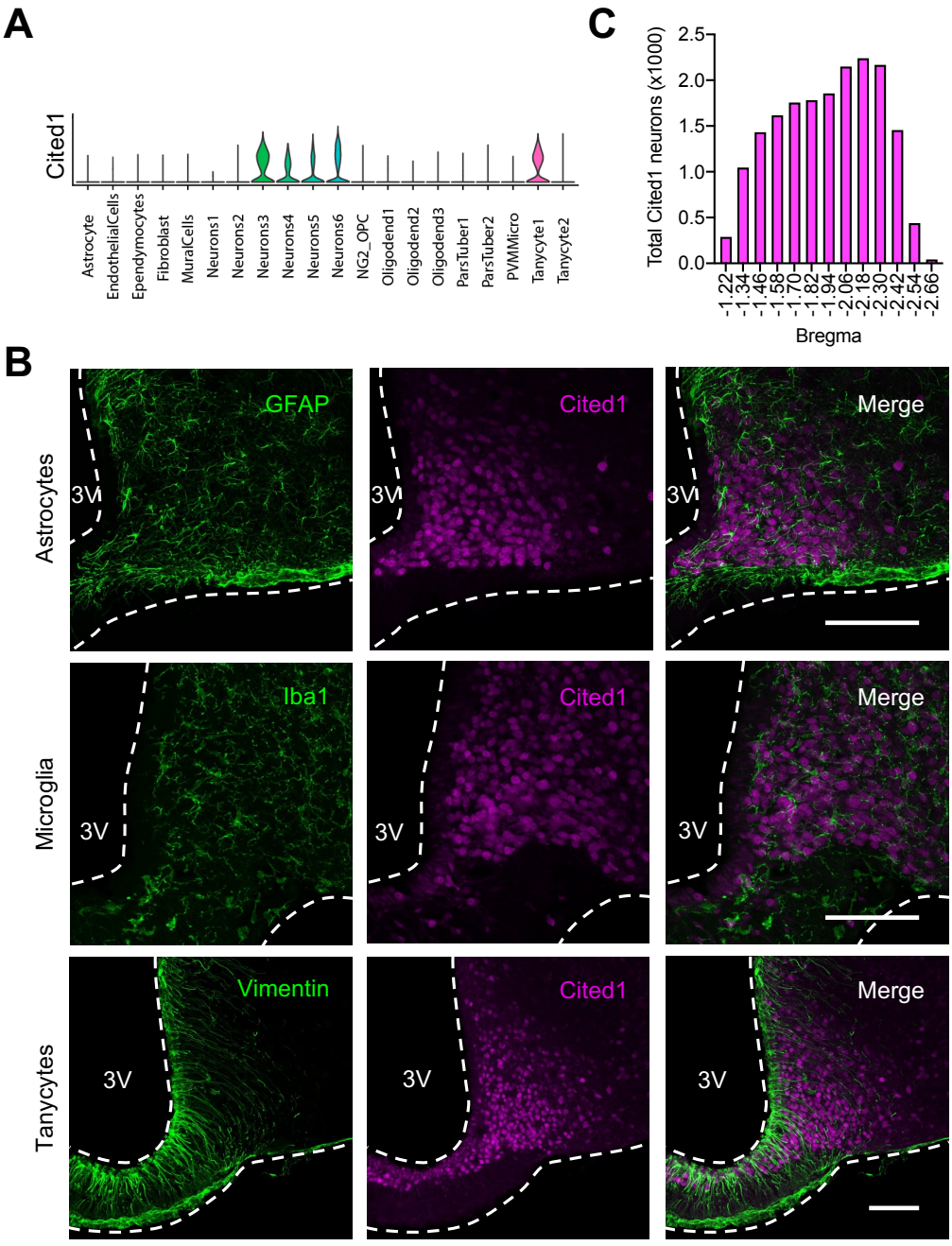

Figure S3. Cellular characterization of Cited1 in the hypothalamus, related to Figure 3.

(**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 µm.

(**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.

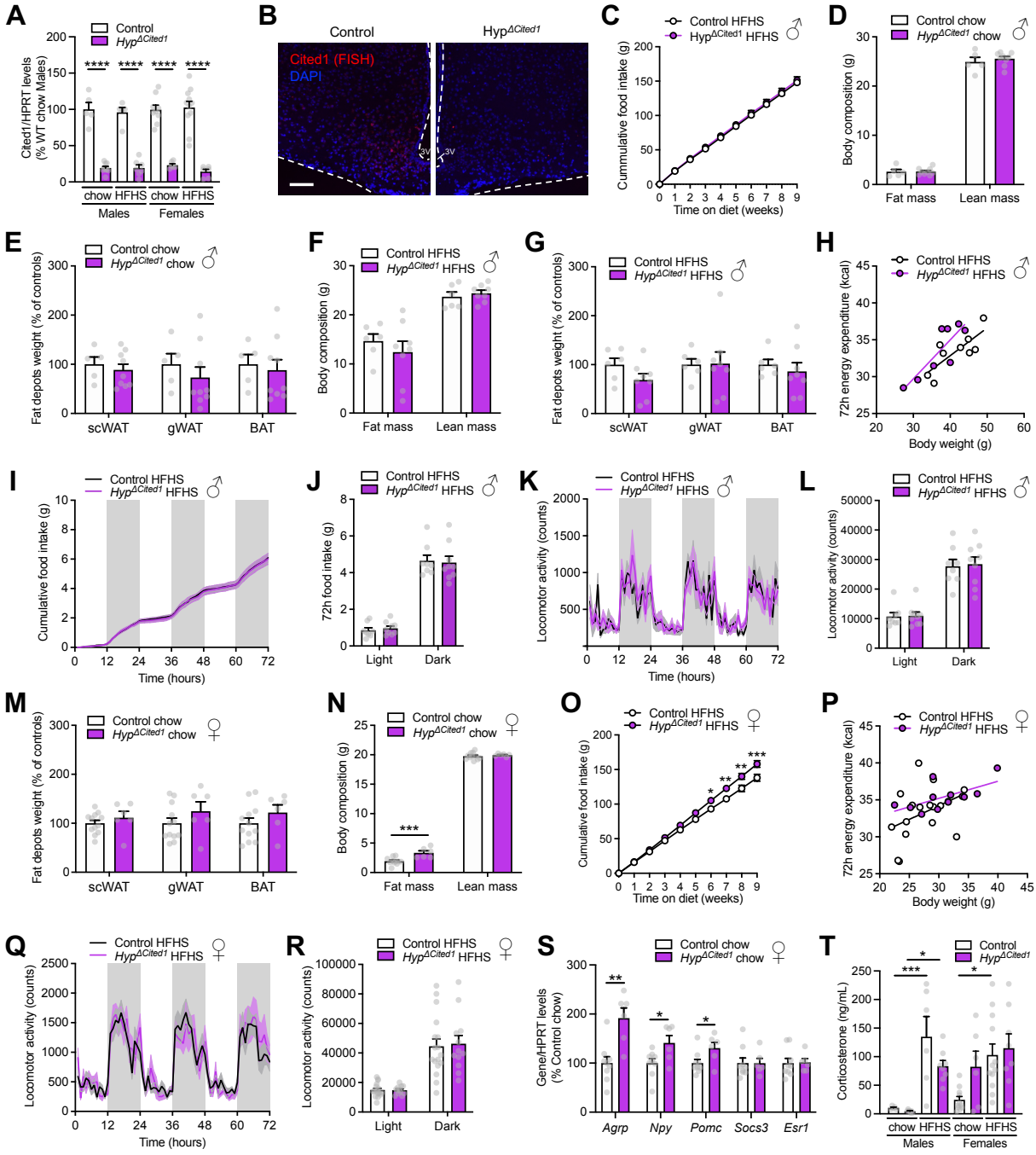

Figure S4. Metabolic phenotype of the hypothalamic Cited1 knock out mice, related to Figure 3.

(**A**) *Cited1* expression levels in the hypothalamus of control or *Hyp*$^{\Delta Cited1}$ 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*$^{\Delta Cited1}$ mice obtained using RNA Scope^{TM}. Scale bar = 200 µm.

(**C**) Cumulative food intake of control or *Hyp*$^{\Delta Cited1}$ 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*$^{\Delta Cited1}$ 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*$^{\Delta Cited1}$ 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*$^{\Delta Cited1}$ 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*$^{\Delta Cited1}$ female mice fed with HFHS diet. n = 8-17 mice per group.

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

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

Data are expressed as mean ± 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.

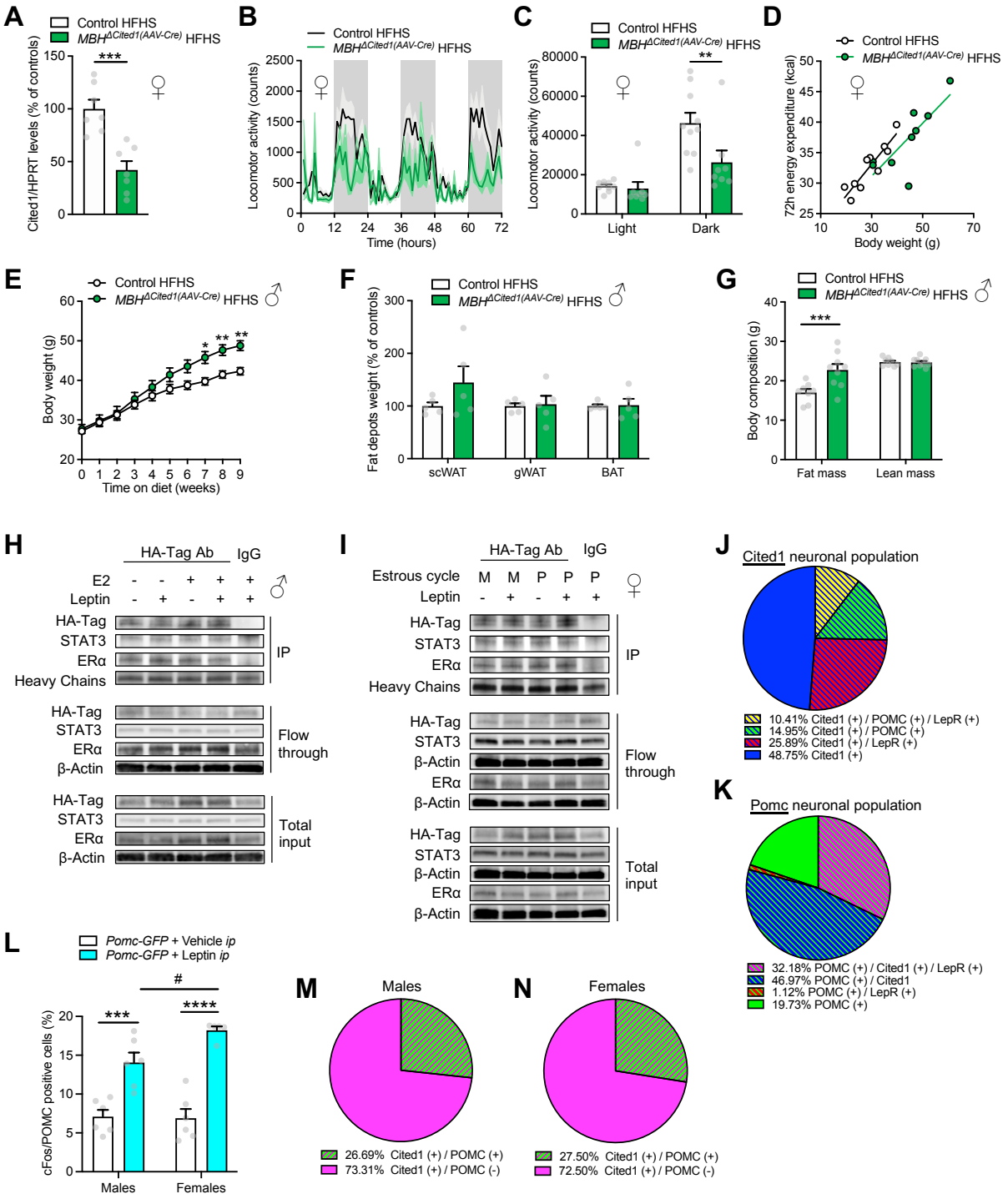

Figure S5. Metabolic phenotype of the virogenetic Cited1 knockout mice, molecular and cellular characterization of Cited1, and sex-dimorphic leptin action in the ARC, related to Figures 3, 5 and 6.

(**A**) *Cited1* expression levels in the hypothalamus of control or *MBH*$^{\Delta Cited1(AAV\text{-}Cre)}$ 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*$^{\Delta Cited1(AAV\text{-}Cre)}$ 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*$^{\Delta Cited1(AAV\text{-}Cre)}$ female mice fed with HFHS diet. n = 9-10 mice per group.

(**E**) Body weight of control or *MBH*$^{\Delta Cited1(AAV\text{-}Cre)}$ 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*$^{\Delta Cited1(AAV\text{-}Cre)}$ 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 ± 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.

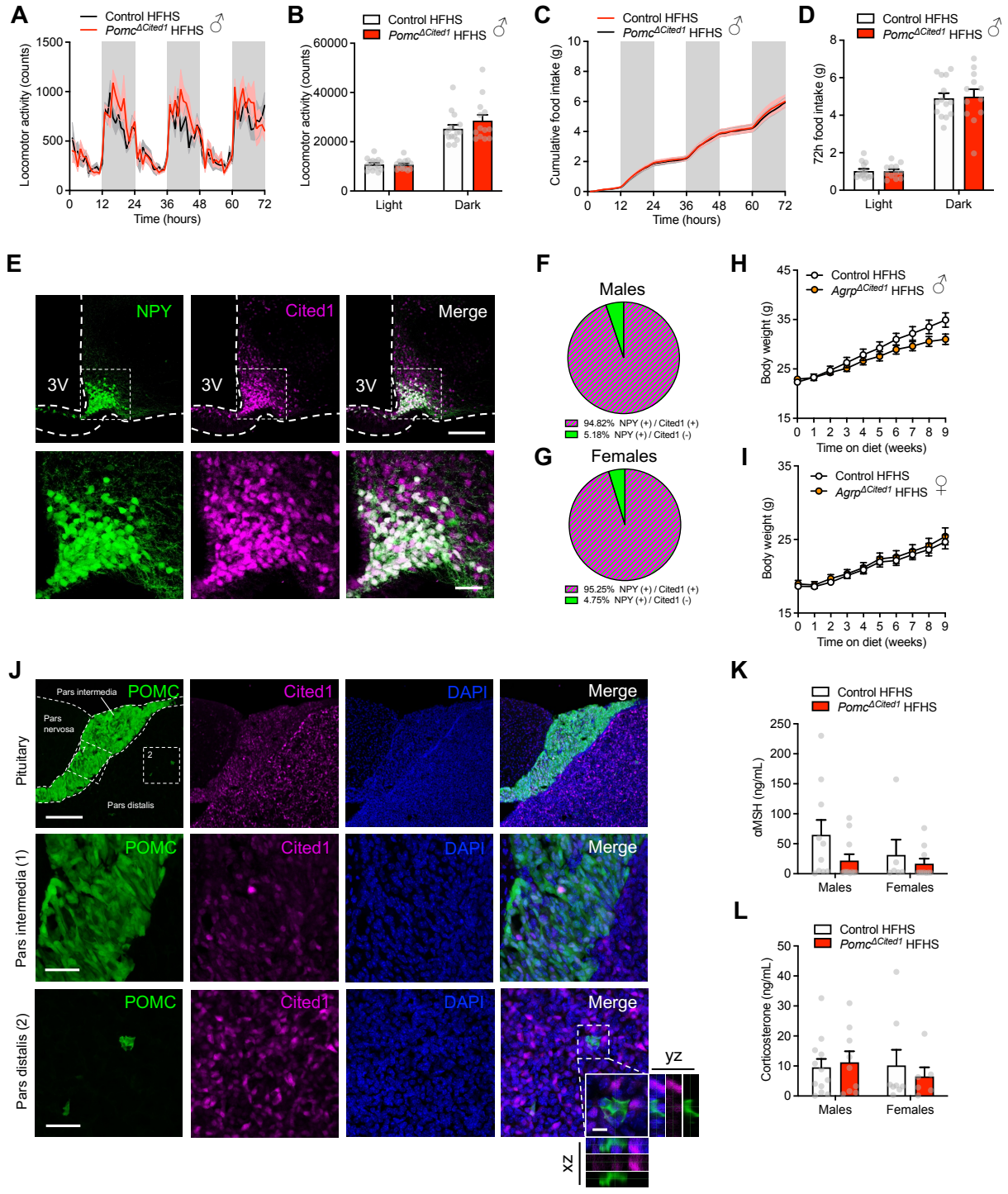

Figure S6. Cited1 characterization in Npy neurons and metabolic phenotype of conditional Cited1 knockout mouse models in Agrp and Pomc neurons, related to Figure 7.

(**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*$^{\Delta Cited1}$ 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 µm (top micrograph), 50 µm (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*$^{\Delta Cited1}$ 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 µm (left top micrograph), 50 µm (left middle and left bottom micrographs) and 5 µm (right bottom micrograph).

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

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

Data are expressed as mean ± 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.

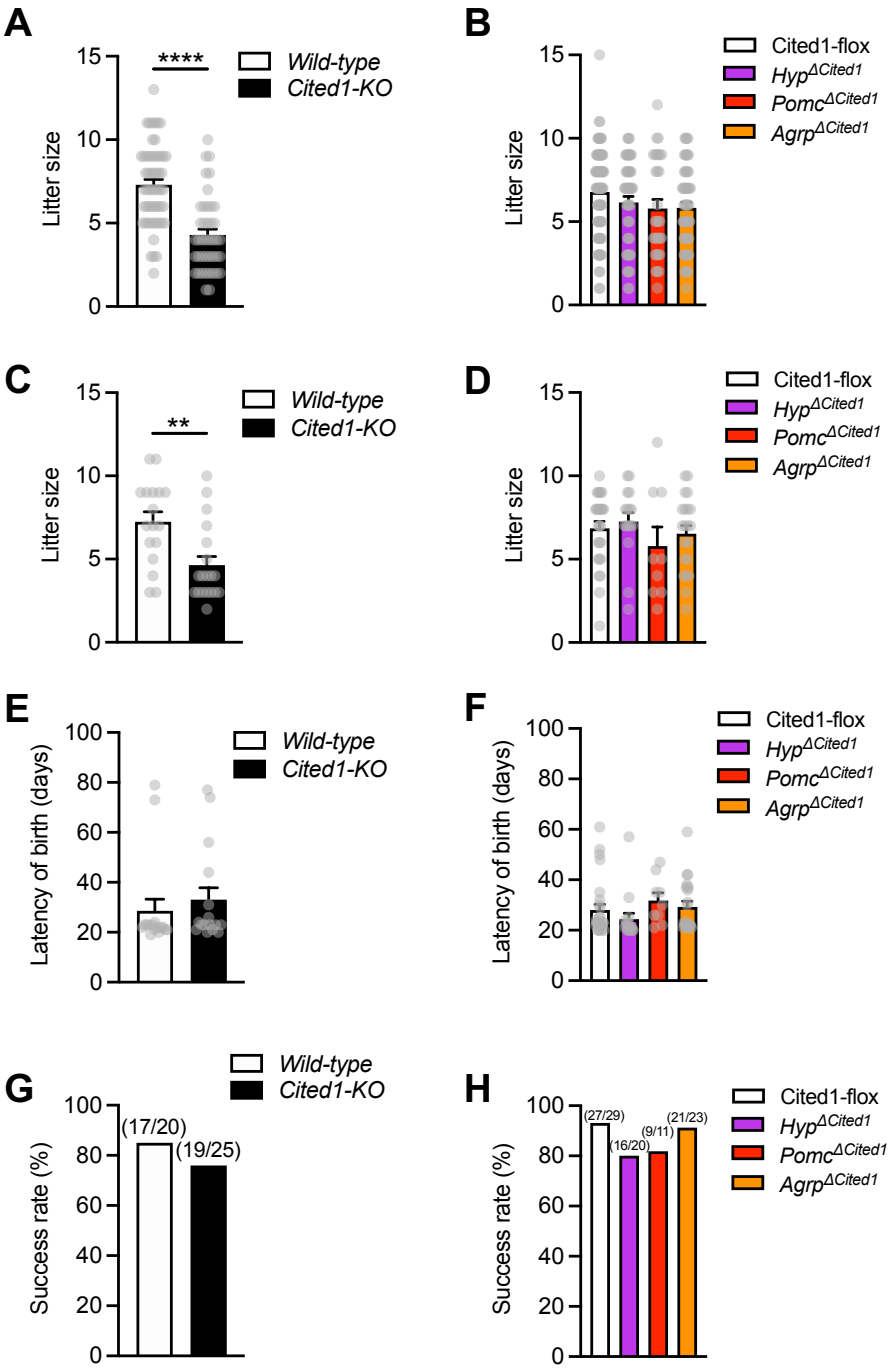

Figure S7. Fertility assessment of the Cited1 knockout mouse lines, related to Figures 1, 3 and 7.

(**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*$^{\Delta Cited1}$, *Pomc*$^{\Delta Cited1}$ *and Agrp*$^{\Delta Cited1}$ 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*$^{\Delta Cited1}$, *Pomc*$^{\Delta Cited1}$ *and Agrp*$^{\Delta Cited1}$ 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*$^{\Delta Cited1}$, *Pomc*$^{\Delta Cited1}$ *and Agrp*$^{\Delta Cited1}$ 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*$^{\Delta Cited1}$, *Pomc*$^{\Delta Cited1}$ *and Agrp*$^{\Delta Cited1}$ 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.