

## Supplementary Information

### Estrogen-Sensitive Medial Preoptic Area Neurons Coordinate Torpor in Mice

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#### **Supplementary Information provided in this file:**

Supplementary Figure 1. Activating ER $\alpha$ + MPA neurons alters temperature and heat loss

Supplementary Figure 2. Chemogenetic activation of ER $\alpha$ + MPA neurons induces a torpor-like state

Supplementary Figure 3. Neuronal activity of ER $\alpha$ + MPA neurons during temperature challenge

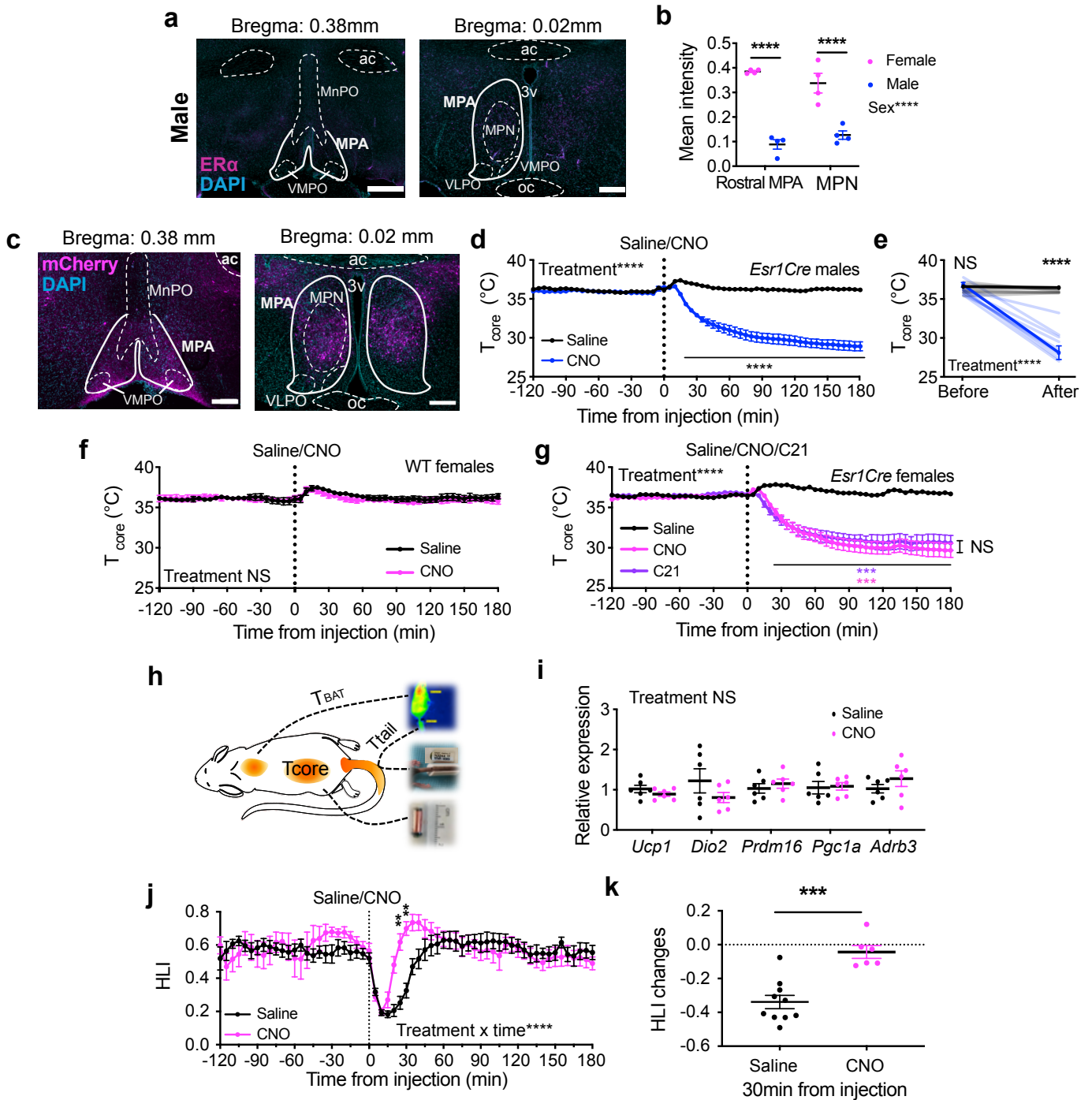
Supplementary Figure 4. Sex differences after ablating ER $\alpha$ + MPA cells in baseline activity and fasting-induced torpor

Supplementary Figure 5. Projections of ER $\alpha$ + neurons from the MPA

Supplementary Table 1. qPCR primer sequences

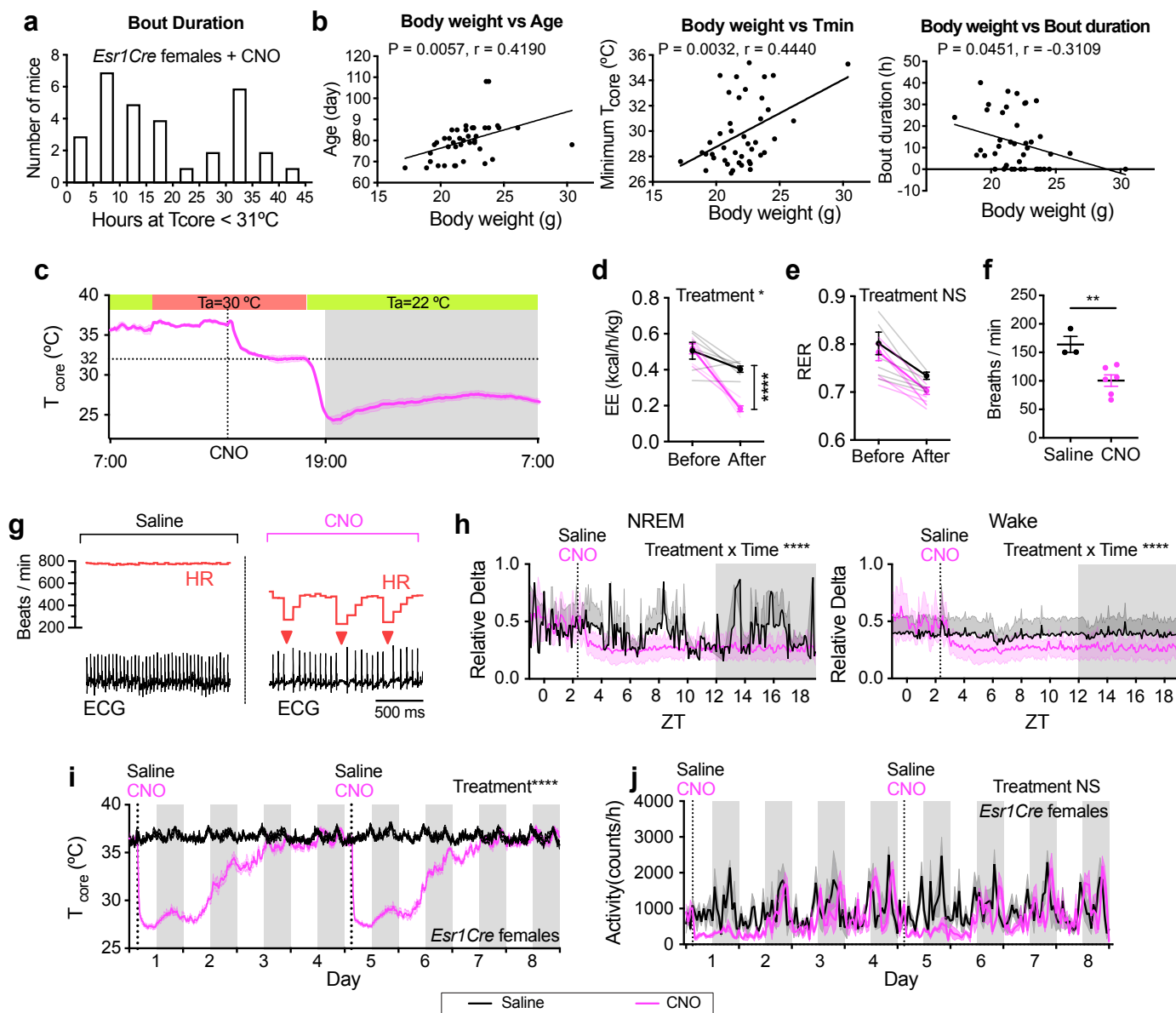
#### **Supplementary Files available separately:**

Source\_Data.xlsx



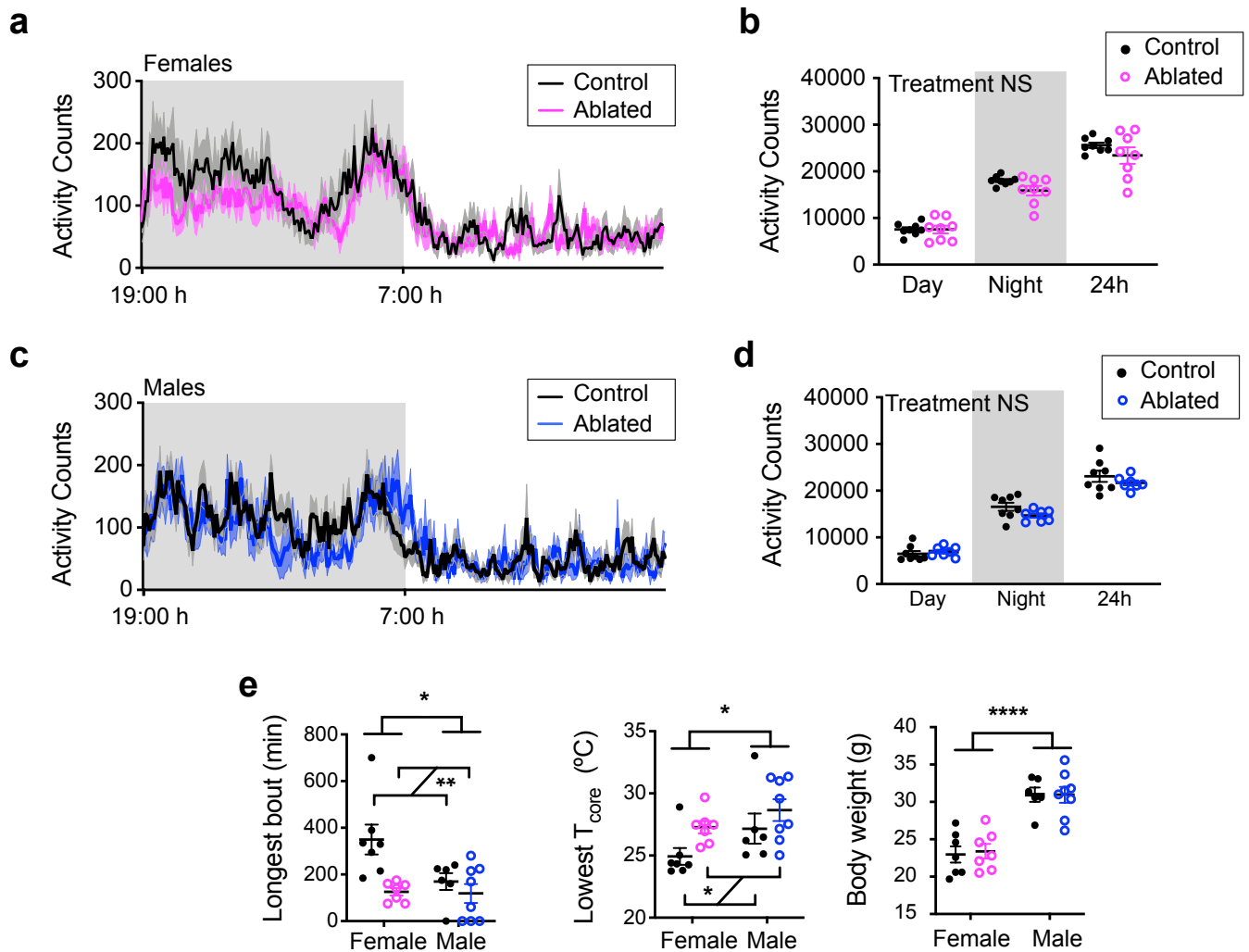
**Supplementary Figure 1. Activating ER $\alpha$ + MPA neurons alters temperature and heat loss.** **a**, ER $\alpha$  immunoreactivity (magenta) in the MPA of adult male mice. Image representative of  $n = 4$  mice. Scale bar: 200  $\mu\text{m}$ . 3v, third ventricle; ac, anterior commissure; MnPO, median preoptic nucleus; MPA, medial preoptic area; MPN, medial preoptic nucleus; oc, optic chiasm; VLPO, ventrolateral preoptic nucleus; VMPO, ventromedial preoptic nucleus. **b**, Quantification of mean ER $\alpha$  intensity in rostral MPA and MPN; **c**, mCherry reporter expression in the MPA of *Esr1Cre* males following stereotaxic delivery of AAV-flex-hM3Dq-mCherry to the MPA. Images representative of  $n=9$  mice. Scale bar: 200  $\mu\text{m}$ . **d**, Core body temperature measured every 5 minutes before and after injection (dotted line at  $x=0$ ) of saline (black) or CNO (blue) in  $n=9$  *Esr1Cre* male mice. **e**, Per-animal averages of core temperature before (-120 min to 0 min) and after (120 min to 180 min) saline or CNO injection for animals shown in **d**. **f**, Core body temperature before and after injection of saline (black) or CNO (pink) in wide-type females ( $n=6$ ). **g**, Core body temperature before and after injection of saline (black), CNO (pink) or C21

(purple) in *Esr1Cre* females (n=6). **h**, Schematic showing the strategy of measuring core ( $T_{\text{core}}$ ), brown adipose tissue ( $T_{\text{BAT}}$ ) and tail ( $T_{\text{tail}}$ ) temperature. **i**, qPCR analysis of thermogenic gene expression in BAT 90 min after saline or CNO injection in *Esr1Cre* females expressing hM3Dq in the MPA (n=6 per group). **j**, heat loss index (HLI) calculated by attached tail thermo-logger before and after injection (dotted line at x = 0) of saline (black, n=10 mice) or CNO (pink, n=6 mice) in *Esr1Cre* females expressing hM3Dq in the MPA. **k**, Changes in HLI from baseline (-120 min to 0 min) to after (25 min to 35 min) saline or CNO injection. Statistical significance denoted by NS, not significant; \*\*, p<0.05; \*\*\*, p<0.01; \*\*\*\*, p<0.0001 for Sidak's multiple comparison tests following a significant effect of treatment or the interaction of treatment with region (**b**) time (**d-g**, **i**) or gene (**h**) in a two-way ANOVA (**b**, **i**) or RM ANOVA (**d-g**, **j**). \*\*\*\*, p<0.001 for (**k**) paired, two-tailed student's t-test. All error bars show SEM.

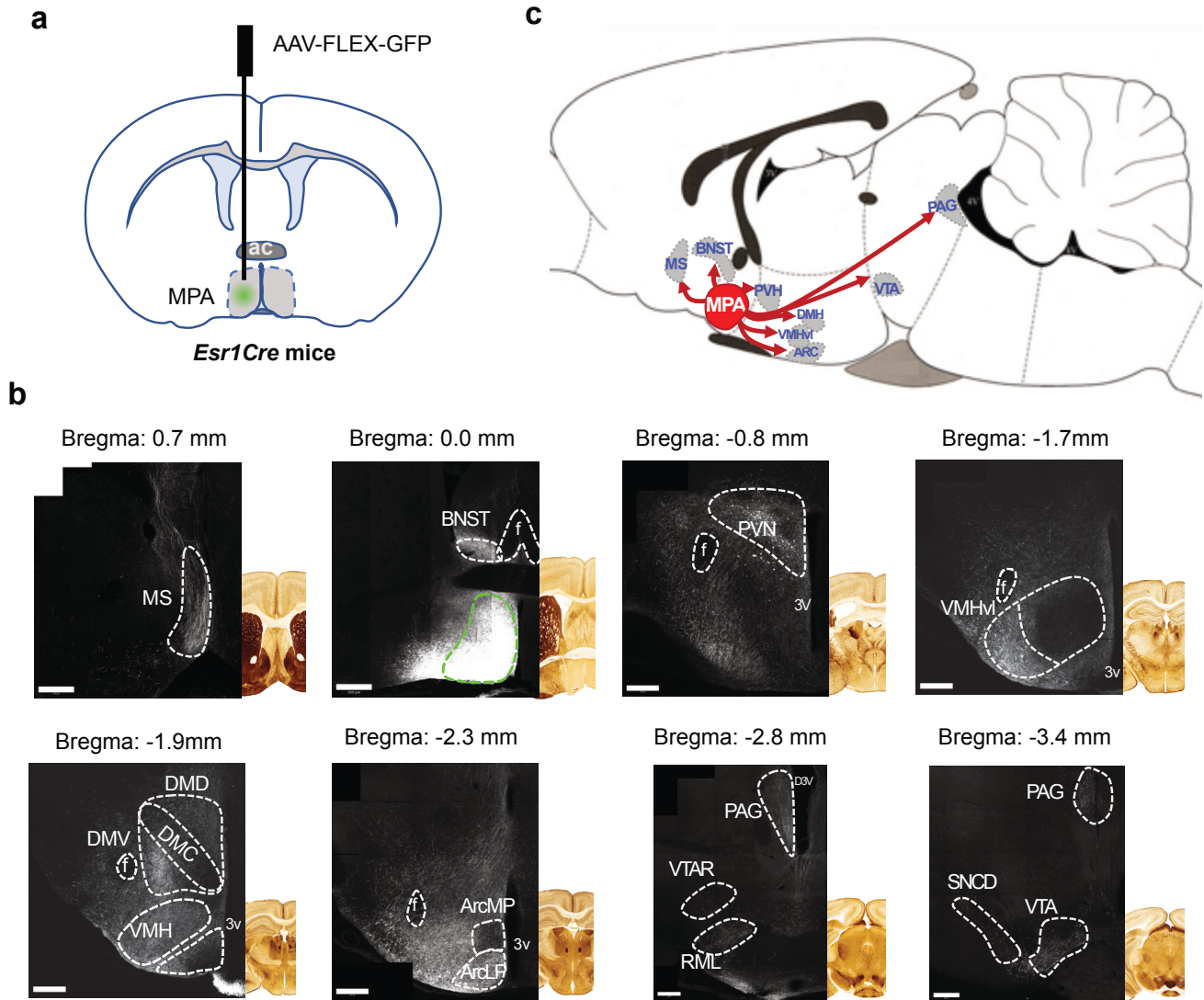


**Supplementary Figure 2. Chemogenetic activation of ER $\alpha$ + MPA neurons induces a torpor-like state.** **a**, Frequency distribution showing duration of hypothermia bouts ( $T_{\text{core}} < 31^{\circ}\text{C}$ ) after CNO injection ( $n=31$  mice). **b**, Linear regressions for body weight vs. age, body weight vs. minimum core temperature ( $T_{\text{min}}$ ), and BW vs. bout duration after CNO injection ( $n=42$  female *Esr1Cre* mice). **c**, Core temperature after CNO injection in mice ( $n=4$  *Esr1Cre* females) during exposure to an ambient temperature ( $T_{\text{a}}$ ) of  $30^{\circ}\text{C}$  (red bar) then  $22^{\circ}\text{C}$  (green bar). **d**, **e**, Energy expenditure (EE or heat generation kcal/h/kg) (**d**) and respiratory exchange ratio (RER) (**e**) before (-40 min to 0) and after (120 min to 180 min) saline or CNO injection calculated from indirect calorimetry data and normalized to lean body mass ( $n = 6$  *Esr1Cre* females). **f**, Respiratory rate (breaths/min) quantified from >1 min videos of *Esr1Cre* mice 3 hours after saline ( $n = 3$  females) or CNO ( $n = 5$  females, 1 male) injection. **g**, Representative ECG in mice 4 h after saline or CNO injection. Red arrowheads indicate skipped heart beats. **h**, Relative delta power from 4-day consecutive EEG recordings with saline NREM vs. CNO and saline wake vs. CNO. White and grey bars represent light and dark periods. ZT, zeitgeber time. **i-j**, Eight-day consecutive recordings of core temperature (**i**) and movement (**j**) after two single injections of CNO (dotted line) in a cohort of *Esr1Cre* female mice showing long torpor bouts ( $n = 4$  *Esr1Cre* females). Lines show group means, error bars or shaded area (**c**, **h-j**) show SEM, faded lines (**d**, **e**) show individual animals. \*\*\*\*,  $p < 0.0001$  for Sidak's multiple comparison tests comparing saline and CNO following a significant effect of treatment (**d** and **i**) in a two-way RM ANOVA or the interaction between treatment and time in a linear mixed effects model (**h**). \*\*,  $p < 0.01$  for unpaired, 2-tailed student's t-test (**f**).





**Supplementary Figure 4. Sex differences after ablating ER $\alpha$ + MPA cells in baseline activity and fasting-induced torpor.** **a** and **c**, Physical activity over 24 h, measured every 5 min for 3 days. Group averages shown for control (black, n=8) and ablated (pink, n=8 female; blue, n=7 male) mice. Shading along the curve denotes the SEM. **b** and **d**, Sum of activity from mice shown in panel **a** and **c** highlighting per animal in light (7:00 to 19:00), dark (19:00 to 7:00), and total 24 h periods. **e**, Duration of the longest bout of  $T_{core} \leq 31^{\circ}C$ , lowest  $T_{core}$  and body weight during the fasting period for control females (n=7 mice), ablated females (n=7 mice), control males (n=6 mice), and ablated males (n=8 mice). Error bars show SEM. Statistical significance denoted by NS, not significant effect of treatment in a two-way RM ANOVA (**b** and **d**), \*,  $p < 0.05$  and \*\*,  $p < 0.01$ , \*\*\*\*,  $p < 0.0001$  for two-way ANOVA (**e**).



**Supplementary Figure 5. Projections of ER $\alpha$ + neurons from the MPA.** **a**, Schematic strategy for visualizing descending target structures from the MPA using Cre-dependent virus expressing GFP. **b**, GFP expression in ER $\alpha$ + cell bodies at the injection site (green dashed outline in Bregma: 0.0 mm) and in fibers from the major projection targets. Projection profile is shown 2 weeks after GFP injection. Images representative of  $n=3$  *Esr1Cre* female mice. 3v, third ventricle; ac, anterior commissure; ArcMP or ArcLP, arcuate nucleus, medial posterior part (ArcMP) or lateroposterior part (ArcLP); BNST, bed nucleus of stria terminalis; f, fornix; D3V, dorsal third ventricle; DMC (DMD/DMV), dorsal medial hypothalamus, compact part (or dorsal/ventral parts); MPA, medial preoptic area; MS, medial septum nucleus; PAG, periaqueductal gray; PVH, paraventricular hypothalamus; RML, retromammillary nucleus, lateral part; SNCD, substantia nigra, compact part, dorsal tier; VMH (vl), ventromedial hypothalamus (ventral lateral part); VTA (R), ventral tegmental area (rostral part). Scale bars, 250 $\mu$ m. **c**, Schematic summary of the major projection brain areas from ER $\alpha$ + MPA neurons.

**Supplementary Table 1.** qPCR primer sequences

<b>Gene name</b>	<b>Symbol</b>	<b>Forward</b>	<b>Reverse</b>
Uncoupling protein 1	<i>Ucp1</i>	CACGGGGACCTACAATGCTT	TAGGGGTCGTCCCTTTCCAA
Deiodinase, iodothyronine, type II	<i>Dio2</i>	CCTCAGAAGGGCTGCGCTGTG	TCAGCGGTCTTCTCCGAGGC
PR domain containing 16	<i>Prdm16</i>	GAAGTCACAGGAGGACACGG	TCATTGCATATGCCTCCGGG
Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	<i>Pgc1a</i>	CAGTACAGCCCCGATGACTC	GAAAGCTCGTCCACGTCAGAC
Adrenergic receptor, beta 3	<i>Adrb3</i>	GGAAGCTTGCTTGATCCCCA	GCCGTTGCTTGTCTTTCTGG