**Supplementary data for article:**

**Administration of thyrotropin-releasing hormone (TRH) in the hypothalamic paraventricular nucleus (PVN) of male rats mimics the metabolic cold defence response**

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**Table legends**

**Table S1**. Primers used in qPCR.

**Table S2**. Basal sample values for figures 1, 3, 5 and S1. Basal sample values for figures 1, 3 , 5 and S1. Data are expressed as mean±SEM. Statistical *P* values were analysed by student *t*-test for comparison between two groups and one-way ANOVA for comparison between three groups.

**Figure legends**

**Figure S1** TRH intracerebroventricular (ICV), but not intravenous (IV) increases blood glucose concentrations and endogenous glucose production (EGP). In experiment-A (TRH IV, n=10) animals received a jugular vein cannula for stable glucose isotope infusion and a carotid artery cannula for blood sampling. In experiment-B (TRH ICV, n=12), in addition an ICV probe was placed into the lateral cerebral ventricle (Anteroposterior: -0.8 mm, lateral: +2.0 mm, depth: -3.3 mm) using a stereotaxic apparatus. During the experiment, at time 0 (between 12:00 and 13:45), a bolus infusion (1 µl/min) of 40 mM TRH or Ringer was given, using a multichannel microinjection pump. After 5 min, the injection speed was switched to 5 µl/h until the end of the experiment. Blood sampling and EGP measurement were performed as described in the manuscript. TRH ICV significantly increased blood glucose (*Time* *P=*0.010, *Treatment* *P*=0.008, *Time\*Treatment* *P*<0.001) (A) and EGP (*Time* *P=*0.009, *Treatment* *P*=0.009, *Time\*Treatment* *P*<0.0001) (B). TRH IV administration elicited no significant differences in blood glucose concentrations between the TRH group and the control group (*Time* *P*<0.001, *Treatment* *P*=0.067, *Time\*Treatment* *P*=0.209) (C). EGP was not measured in TRH IV experiment. Data are shown as the absolute increase (delta) compared to the basal samples before treatments. Basal values are shown in supplementary Table S1. N=5-6 per group. Stars indicate significant differences between groups at individual time points. Post-hoc \* P<0.05, \*\* P<0.01, \*\*\* P<0.001.

**Figure S2** Effects of hepatic sympathectomy (HSX) and parasympathectomy (HPX) on TRH-mediated changes in plasma corticosterone concentrations. Corticosterone concentrations were measured with radio-immunoassay kits (Millipore, Billerica, USA and MP Biomedicals, Orangeburg, USA). N=6-10 per group.

**Figure S3** Histological localization of microdialysis probe placement sites in the paraventricular nucleus (PVN). A, Placement locations of probes included for analysis (n=8) are plotted in right side on a drawing of a coronal brain slice at bregma −1.8 mm (adapted from Paxinos and Watson, 2006). B, a histological section containing representative microdialysis probes in the PVN. Abbreviations: mt, mamillothalamic tract; AH, anterior hypothalamus; 3V, third ventricle; f, fornix; VMH, ventromedial hypothalamic nucleus; ME, median eminence; D3V, dorsal third ventricle; opt, optic tract; BMA, basal medial amygdala; ic, internal capsule; LV, lateral ventricle.

**Figure S4** Reference gene expression for normalization of targeted genes expression. Two reference genes: *Gapdh and Hprt* were selected based on their stable expression across treatments. Geometric mean (geoMean) of these two genes were used to normalize targeted genes expression in BAT and liver during cold (A) and TRH treatment (B). ns indicates a non-significant difference (p=0.136).