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Supplemental information

Altered brown fat thermoregulation and enhanced cold-induced thermogenesis in young, healthy, winter-swimming men

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Table S1. Subject characterization. *Related to Table 1.*

Subject characterization	Winter swimmers (n= 7)	Control group (n=8)	P
Erytrocytes volume fraction (MHz)	0.4 (0.02)	0.4 (0.02)	0.91
Erytrocytes (MCV)	83.9 (3.2)	83.0 (2.1)	0.05
Erytrocytes	4.8 (0.3)	5.0 (0.2)	0.28
Hemoglobin (mmol/L)	9.0 (0.5)	9.0 (0.5)	1.0
Iron (µmol/L)	19.1 (8.0)	20.9 (7.4)	0.61
Leukocytes (10^9/L)	5.1 (0.5)	5.3 (0.7)	0.47
Basophil (10^9/L)	0.03 (0.02)	0.03 (0.02)	0.80
Eosinophil (10^9/L)	0.1 (0.05)	0.2 (0.1)	0.35
Lymphocytes (10^9/L)	1.9 (0.4)	1.9 (0.4)	0.95
Monocytes (10^9/L)	0.4 (0.07)	0.5 (0.14)	0.29
Neutrophil (10^9/L)	2.6 (0.6)	2.8 (0.5)	0.69
Metamyelo myelo promyelocytes(10^9/L)	0.008 (0.0)	0.01 (0.0)	0.17
Transferrin (g/L)	2.3 (0.3)	2.6 (0.1)	0.02
Thrombocytes (10^9/L)	201.1 (42.6)	129.1 (57.3)	0.51
Bilirubin (µmol/L)	12.4 (6.8)	11.4 (4.7)	0.74
Calcium (µmol/L)	2.4 (0.06)	2.4 (0.06)	0.84
Creatinine (µmol/L)	79.8 (7.2)	91.0 (15.1)	0.08
Carbamid (mmol/L)	4.0 (0.8)	4.7 (1.7)	0.28
Potassium (mmol/L)	4.2 (0.2)	3.8 (0.3)	0.03
Sodium (mmol/L)	141.5 (1.3)	140.6 (1.3)	0.20
Phosphate (mmol/L)	1.1 (0.09)	1.01 (0.09)	0.23
Albumin (g/L)	39.5 (2.9)	40.6 (2.0)	0.38
Myoglobin (	43.8 (7.5)	44.3 (4.9)	0.85
Protein (g/L)	69.4 (4.5)	71.4 (2.1)	0.27
Amylase (µg/L)	58.8 (19.6)	50.4 (12.6)	0.33
Alkaline phosphotase (U/L)	53.0 (16.1)	74.4 (14.0)	0.01
Gamma glutamyl transferase (U/L)	12.6 (4.9)	18.5 (8.1)	0.10
Lactatdehydrogenase (U/L)	177.2 (24.5)	190.1 (41.7)	0.52
Thyrotropin (TSH) (x10^-3IU/L)	2.3 (1.1)	2.0 (0.4)	0.43
Triiodothyronine free (T3) (pmol/L)	5.5 (0.7)	5.5 (0.7)	0.83
Thyroxin (T4) (pmol/L)	14.6 (2.0)	17.0 (1.6)	0.02
Total cholesterol (mmol/l)	4.3 (0.9)	4.0 (0.4)	0.48
HDL cholesterol (mmol/l)	1.5 (0.4)	1.4 (0.2)	0.53
LDL cholesterol (mmol/l)	2.7 (0.9)	2.5 (0.5)	0.57
Triglycerides (pmol/l)	0.7 (0.2)	0.7 (0.2)	0.89
Alanine transaminase (ALAT) (U/l)	20.9 (8.5)	25.1 (12.1)	0.43
Aspartattransaminase (ASAT) (U/l)	25.4 (13.2)	27.1 (7.8)	0.76
Testosteron (nmol/L)	18.2 (6.6)	17.8 (2.3)	0.86
Heartrate during VO_{2max} (mean beats/min)	166.8 (6.1)	169.0 (9.2)	0.65
Workload (VO_{2max})	345.7 (27.7)	323.6 (31.1)	0.42

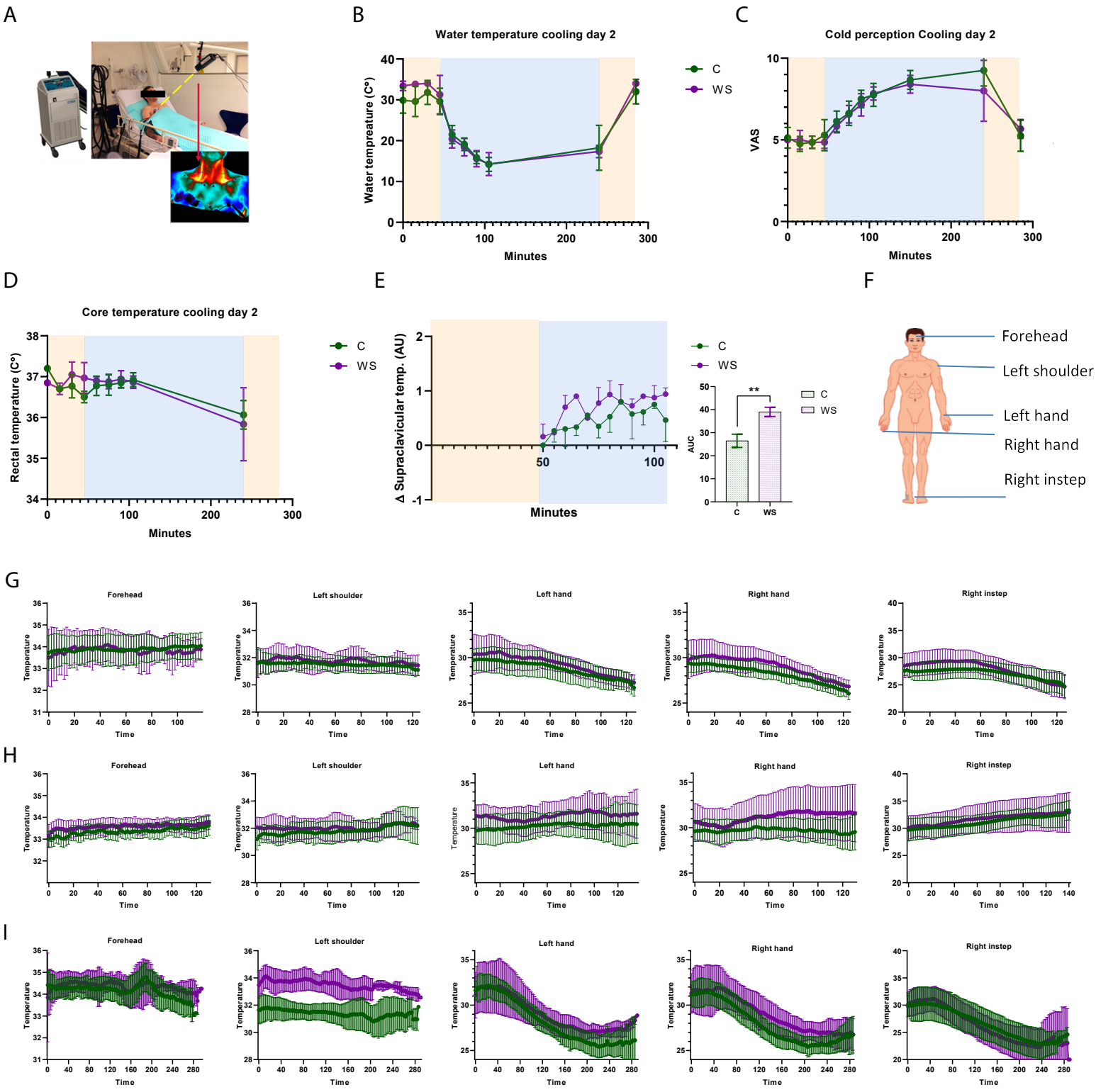

Figure S1. Thermoregulation during perception based cooling and thermal comfort protocols. Winter swimmers (n=7) and control subjects (n=8), were subjected to cooling and thermal comfort protocols. The n-numbers represent number of human individuals in each group and are consistent throughout this figure unless otherwise stated. **A)** Experimental set-up showing the water perfused blanket used for temperature regulation (BlanketRoll III) and the FLIR infrared thermocamera set-up. **B)** Water temperature on the second cooling day. **C)** Cold perception on the second cooling day. **D)** Core temperature on the second cooling day, as measured by rectal thermometers. **E)** Supraclavicular BAT skin temperature on the second cooling day, as measured by IRT, normalized to thermal comfort state directly prior to cooling and related to sternum skin temperature. Data are mean +/- SD analyzed using area under the curve (AUC), tested with unpaired t-test. * P<0.05, ** P<0.01. **F)** Illustration of temperature measure points. **G-I)** Skin temperature at 5 body sites using iButtons with a sampling rate of two minutes. **G)** Cooling day 1. **H)** Thermal comfort day. **I)** Cooling day 2. Data are mean +/- SD. *Related to Figure 2.*

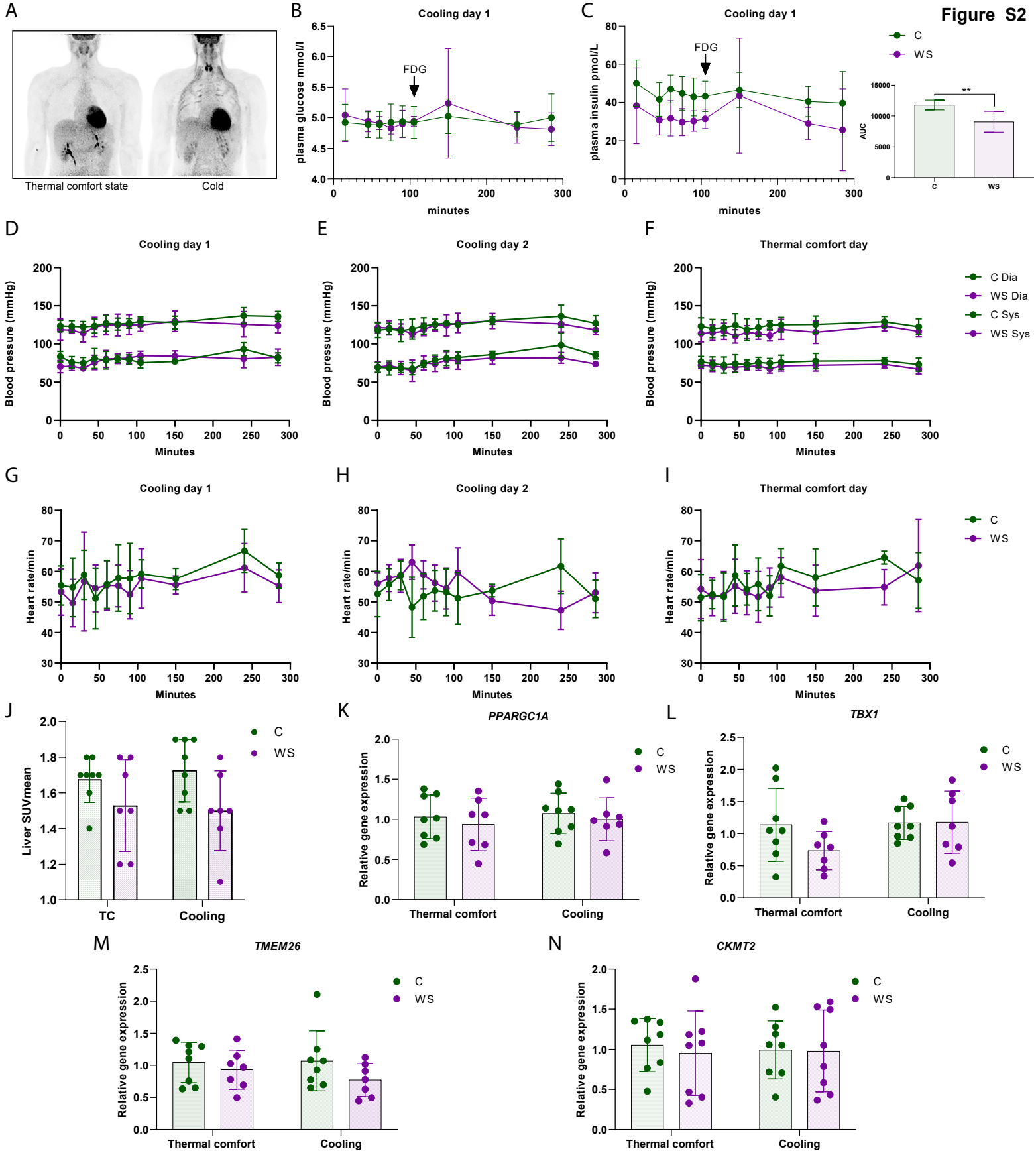

Figure S2. **Physiological responses to cooling and thermoneutrality.** Winterswimmers (n=7) and control subjects (n=8), were subjected to cooling and thermoneutral protocols. The n-numbers represent number of human individuals in each group and are consistent throughout this figure unless otherwise stated. **A)** Initially, n=8 winter swimmers were included. One subject (winter swimmer), shown here, had no detectable BAT during cold or thermal comfort state. This subject was therefore excluded, explaining the n=7 winter swimmers. **B)** Plasma glucose levels during cooling. **C)** Plasma insulin levels during cooling. Data are mean +/- SD analyzed using area under the curve (AUC), tested with unpaired t-test. * P<0.05, ** P<0.01. **D-F)** Blood pressure during cooling day 1, cooling day 2 and thermal comfort day **G-I)** Heart rate during cooling day 1, cooling day 2 and thermal comfort day. **J)** Liver SUVmean between groups and thermal comfort versus cooling state. **(K-N)** Subcutaneous adipose tissue biopsies were obtained from all subjects before and after cooling. RNA was isolated and relative gene expression of PPARGC1A, TBX1, TMEM26 and CKMT2 was assessed by using qPCR. Differences were assessed using two-way repeated-measures ANOVA. Data are mean +/- SD. *Related to Figure 3.*

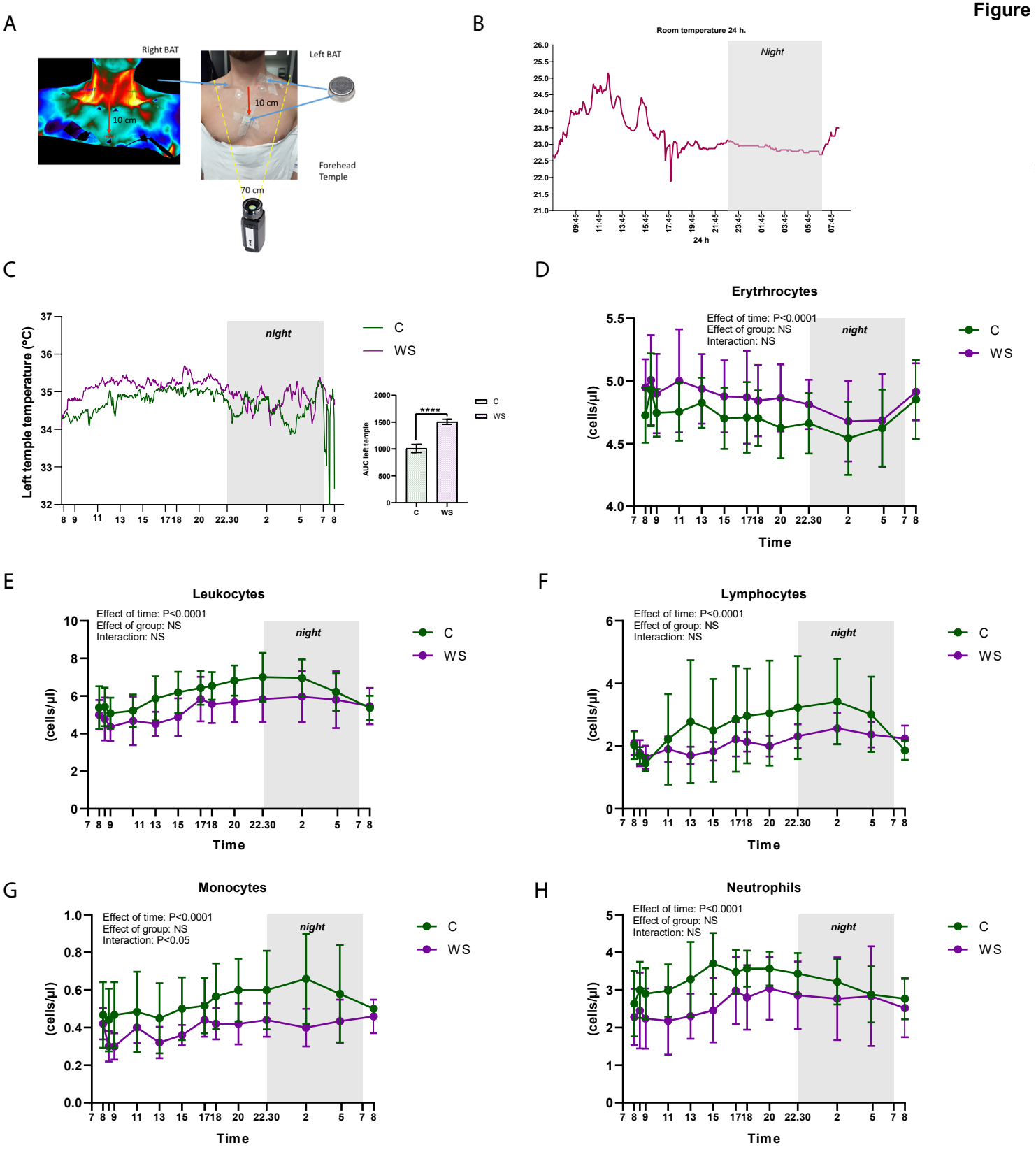

Figure S3. Diurnal experiment and blood cell profiling. Winterswimmers (n=5) and control subjects (n=6) were studied during for body temperature during 24 hours. The n-numbers represent number of human individuals in each group and are consistent throughout this figure unless otherwise stated. **A)** Illustration of the placement of iButtons for the Diurnal experiment. **B)** Room temperature measured with an iButton during the study. **C)** Left temple temperature recorded using iButtons **D-H)** Blood cell profile including Erythrocytes, Leukocytes, Lymphocytes, Monocytes and Neutrophils during the 24h experiment. Differences were assessed using two-way repeated-measures ANOVA. Data are mean +/- SD. * P<0.05, ** P<0.01, *** P<0.001, ****P<0.0001. *Related to Figure 4.*