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Supplemental Information

Mouse Thermoregulation: Introducing the Concept of the Thermoneutral Point

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Table S1. Summary of experimental parameters. Key to abbreviations and parameters is on next page. Data are mean ± SEM. Related to Figures 1 - 4.

Ta range of 16 - 34 °C	Control C57BL/6J pooled from 4 experiments	Control C57BL/6J	RTX	Control C57BL/6J	TKO	Control C57BL/6J	*ob/ob*	Control C57BL/6J	DIO	24h C57BL/6J light	24h C57BL/6J dark
Figure reference	**1**	**2A**	**2A**	**2B**	**2B**	**2C**	**2C**	**2D**	**2D**	**Abreu-Vieira 2015**	**Abreu-Vieira 2015**
Phase	Light	Light	Light	Light	Light	Light	Light	Light	Light	Light	Dark
Ta setup protocol (Table S2)	1	1	1	1	1	1	1	1	1	-	-
n of mice	21	5	6	5	6	6	5	5	6	19	19
n of data points in each regression	2320	325	390	575	690	690	575	650	741	8310	8982
Body weight (g)	32.27 ± 0.97	38.02 ± 1.19	32.90 ± 0.48	30.92 ± 1.18	27.72 ± 0.95	32.77 ± 0.49	56.50 ± 2.02	30.58 ± 0.95	48.80 ± 0.74	26.92 ± 0.5	26.92 ± 0.5
Age (wks)	15, 16, 39, 45	45	45	39	39	16	16	25	25	10 to 12	10 to 12
Sex	male	male	male	male	male	male	male	male	male	male	male
Tlc_{EE} (°C)	28.90 ± 0.15	28.87 ± 0.29	26.59 ± 0.48	28.57 ± 0.24	29.16 ± 0.25	27.11 ± 0.26	25.00 ± 0.34	28.58 ± 0.24	29.99 ± 0.20	29.03 ± 0.12	32.36 ± 0.17
Tlc_{cond} (°C)	28.78 ± 0.08	28.56 ± 0.23	25.77 ± 0.48	28.79 ± 0.18	28.83 ± 0.17	28.80 ± 0.14	27.04 ± 0.20	28.45 ± 0.13	29.21 ± 0.12	28.73 ± 0.17	not defined
Tb_{inc} (°C)	28.92 ± 0.11	27.58 ± 0.24	not defined	28.75 ± 0.17	28.81 ± 0.18	28.99 ± 0.16	25.61 ± 0.19	29.22 ± 0.15	29.55 ± 0.13	27.49 ± 0.38	not seen
Tb_{inc} - Tlc_{EE} (°C)	0.02	-1.29	-	0.18	-0.35	1.88	0.61	0.64	-0.44	-1.53	-
TEE, at Tlc_{EE} (kcal/h)	0.246 ± 0.006	0.245 ± 0.013	0.330 ± 0.010	0.239 ± 0.010	0.227 ± 0.007	0.272 ± 0.005	0.388 ± 0.009	0.235 ± 0.006	0.280 ± 0.009	0.235 ± 0.006	0.265 ± 0.006
Cond, <Tlc_{cond} (kcal/h/Δ°C)	0.0328 ± 0.0006	0.0349 ± 0.0014	0.0351 ± 0.0012	0.0315 ± 0.0019	0.0314 ± 0.0009	0.0339 ± 0.0007	0.0430 ± 0.0020	0.0319 ± 0.0008	0.0410 ± 0.0011	0.0310 ± 0.0006	-
Tb, < Tb_{inc} (°C)	35.60 ± 0.07	35.44 ± 0.09	varies	35.66 ± 0.12	35.70 ± 0.16	35.49 ± 0.09	33.91 ± 0.07	35.45 ± 0.06	35.87 ± 0.08	35.80 ± 0.11	36.89 ± 0.13
defended Tb (°C)	37.80 ± 0.36	36.77 ± 0.65	38.67 ± 1.32	37.12 ± 0.53	37.51 ± 0.47	35.39 ± 0.52	35.28 ± 1.13	36.83 ± 0.47	38.36 ± 0.37	38.26 ± 0.29	43.04 ± 0.32
defended Tb - Tb, <Tb_{inc} (°C)	2.20	1.33	-	1.46	1.81	-0.09	1.37	1.38	2.49	2.45	6.15
TEE Slope, <Tlc (kcal/h/°C)	-0.0277 ± 0.0007	-0.0310 ± 0.0011	-0.0273 ± 0.0020	-0.0279 ± 0.0008	-0.0272 ± 0.0008	-0.0328 ± 0.0012	-0.0378 ± 0.0030	-0.0285 ± 0.0008	-0.0335 ± 0.0007	-0.0255 ± 0.0004	-0.0248 ± 0.0003
Cond Slope, >Tlc_{cond} (kcal/h/Δ°C/°C)	0.0070 ± 0.0005	0.0054 ± 0.0003	0.0032 ± 0.0002	0.0061 ± 0.0004	0.0056 ± 0.0003	0.0079 ± 0.0003	0.0072 ± 0.0003	0.0069 ± 0.0003	0.0077 ± 0.0003	0.0073 ± 0.0004	-
Tb Slope, >Tb_{inc} (°C Tb/°C)	0.337 ± 0.029	0.274 ± 0.015	0.488 ± 0.017	0.368 ± 0.021	0.319 ± 0.020	0.390 ± 0.019	0.557 ± 0.017	0.287 ± 0.016	0.333 ± 0.016	0.145 ± 0.014	-

Ta range of 16 - 38 °C	Control C57BL/6J pooled from 3 experiments	Control C57BL/6J	C57BL/6J 30 °C acclimated	Control C57BL/6J	129 male	129 male	129 female	C57BL/6J dark phase pooled from 2 expts	Control C57BL/6J	Control C57BL/6J home cages	Control C57BL/6J Dark	Control C57BL/6J home cages, dark
Figure reference	**3D, 4B, S3**	**3A**	**3A**	**3B**	**3B**	**3C**	**3C**	**3D, 4C**	**3E**	**3E**	**3F**	**3F**
Phase	Light	Light	Light	Light	Light	Light	Light	Dark	Light	Light	Dark	Dark
Ta setup protocol (Table S2)	2	2	3	2	2	2	2	4	2	2	4	4
n of mice	15	5	5	5	6	7	4	17	6	11	6	11
n of data points in each regression	2175	585	725	705	846	1001	572	2636	809	4213	881	4114
Body weight (g)	30.13 ± 0.53	29.78 ± 1.37	31.32 ± 1.44	30.02 ± 0.33	31.82 ± 1.16	27.14 ± 0.60	21.05 ± 0.50	33.42 ± 1.02	28.62 ± 0.71	31.18 ± 0.62	28.35 ± 0.75	32.08 ± 0.71
Age (wks)	25, 27, 33	33	28	27	26	14	14	10, 46	12	15	10	18
Sex	male	male	male	male	male	male	female	male	male	male	male	male
Tlc_{EE} (°C)	28.83 ± 0.12	28.92 ± 0.19	28.60 ± 0.01	28.77 ± 0.23	29.29 ± 0.22	28.80 ± 0.20	29.90 ± 0.20	same as TEE_R	30.44 ± 0.25	30.92 ± 0.12	same as TEE_R	same as TEE_R
Tlc_{cond} (°C)	28.30 ± 0.10	28.70 ± 0.21	28.19 ± 0.22	28.75 ± 0.16	29.16 ± 0.15	29.16 ± 0.14	28.89 ± 0.38	not defined	27.36 ± 0.41	27.58 ± 0.14	not defined	not defined
Tb_{inc} (°C)	28.76 ± 0.13	28.74 ± 0.33	27.69 ± 0.19	27.23 ± 0.33	29.35 ± 0.18	30.43 ± 0.01	30.11 ± 0.04	same as Tb_R	28.19 ± 0.02	27.77 ± 0.45	same as Tb_R	same as Tb_R
Tb_{inc} - Tlc_{EE} (°C)	-0.07	-0.18	-0.91	-1.54	0.06	1.63	0.21	-1.17	-2.25	-3.15	-1.22	-0.09
TEE, at Tlc_{EE} (kcal/h)	0.234 ± 0.004	0.221 ± 0.004	0.230 ± 0.008	0.246 ± 0.007	0.253 ± 0.006	0.181 ± 0.006	0.185 ± 0.008	0.286 ± 0.008	0.226 ± 0.005	0.226 ± 0.007	0.257 ± 0.010	0.267 ± 0.007
Cond, <Tlc_{cond} (kcal/h/Δ°C)	0.0326 ± 0.0004	0.0335 ± 0.0012	0.0310 ± 0.0022	0.0327 ± 0.0016	0.0350 ± 0.0014	0.0232 ± 0.0012	0.0273 ± 0.0011	-	0.0335 ± 0.0027	0.0340 ± 0.0020	-	-
Tb, < Tb_{inc} (°C)	35.40 ± 0.06	35.27 ± 0.08	35.15 ± 0.08	35.46 ± 0.10	35.83 ± 0.10	36.09 ± 0.12	36.31 ± 0.17	36.52 ± 0.06	35.35 ± 0.10	35.31 ± 0.06	36.51 ± 0.14	36.35 ± 0.06
defended Tb (°C)	37.21 ± 0.34	36.20 ± 0.47	37.86 ± 0.41	38.11 ± 0.49	37.81 ± 0.43	37.62 ± 0.47	37.23 ± 0.43	45.40 ± 0.39	39.50 ± 0.42	39.76 ± 0.31	44.72 ± 0.51	44.50 ± 0.34
defended Tb - Tb, <Tb_{inc} (°C)	1.81	0.93	2.95	2.65	1.97	1.53	0.92	8.88	4.15	4.44	8.21	8.15
TEE Slope, <Tlc_{EE} (kcal/h/°C)	-0.0279 ± 0.0008	-0.0304 ± 0.0013	-0.0248 ± 0.0006	-0.0264 ± 0.0008	-0.0297 ± 0.0008	-0.0205 ± 0.0005	-0.0252 ± 0.0006	-0.0249 ± 0.0004	-0.0250 ± 0.0006	-0.0256 ± 0.0003	-0.0246 ± 0.0005	-0.0248 ± 0.0003
Cond Slope, >Tlc_{cond} <Cond_R (kcal/h/Δ°C/°C)	0.0063 ± 0.0004	0.0063 ± 0.0004	0.0061 ± 0.0003	0.0086 ± 0.0004	0.0074 ± 0.0003	0.0057 ± 0.0002	0.0039 ± 0.0006	-	0.0048 ± 0.0006	0.0069 ± 0.0003	-	-
Tb Slope, >Tb_{inc} <Tb_R (°C Tb/°C)	0.237 ± 0.025	0.256 ± 0.030	0.272 ± 0.021	0.154 ± 0.013	0.300 ± 0.024	0.335 ± 0.017	0.265 ± 0.026	-	0.130 ± 0.010	0.068 ± 0.008	-	-
TEE_R (°C)	33.91 ± 0.29	33.78 ± 0.37	35.24 ± 0.39	34.38 ± 0.84	33.33 ± 0.04	not seen	not seen	33.94 ± 0.14	33.65 ± 0.85	34.50 ± 0.20	34.28 ± 0.22	33.74 ± 0.12
Cond_R (°C)	32.95 ± 0.10	33.47 ± 0.24	35.83 ± 0.11	33.51 ± 0.02	not seen	not seen	32.80 ± 0.42	not defined	31.94 ± 0.17	32.43 ± 0.08	not defined	not defined
Tb_R (°C)	33.15 ± 0.08	32.85 ± 0.16	33.31 ± 0.15	33.26 ± 0.11	33.19 ± 0.13	33.15 ± 0.14	32.88 ± 0.02	32.77 ± 0.13	33.44 ± 0.12	33.36 ± 0.05	33.06 ± 0.35	33.65 ± 0.04
TEE Slope, >TEE_R (kcal/h/°C)	0.0158 ± 0.0041	0.0275 ± 0.0058	0.0181 ± 0.0037	0.0112 ± 0.0071	0.0125 ± 0.0028	-	-	0.0215 ± 0.0032	0.0125 ± 0.0044	0.0212 ± 0.0016	0.0252 ± 0.0065	0.0143 ± 0.0017
Cond Slope, >Cond_R (kcal/h/Δ°C/°C)	0.0134 ± 0.0011	0.0131 ± 0.0007	0.0296 ± 0.0014	0.0125 ± 0.0006	-	-	0.0081 ± 0.0006	-	0.0190 ± 0.0005	0.0214 ± 0.0002	-	-
Tb Slope, >Tb_R (°C Tb/°C)	0.780 ± 0.035	0.841 ± 0.033	0.728 ± 0.049	0.800 ± 0.029	0.891 ± 0.027	0.805 ± 0.025	0.576 ± 0.033	0.620 ± 0.030	0.784 ± 0.028	0.841 ± 0.010	0.558 ± 0.083	0.800 ± 0.011

Table S1 continued: Key to abbreviations and parameters

DIO	diet induced obese
ob/ob	leptin-deficient
RTX	neonatal treatment with resiniferatoxin
TKO	*Trpv1*$^{-/-}$;*Trpm8*$^{-/-}$;*Trpa1*$^{-/-}$ triple knockout
Tlc_{EE}	lower critical temperature, the (first) breakpoint of the TEE vs Ta graph
Tlc_{cond}	lower critical temperature, the (first) breakpoint of the conductance vs Ta graph
Tb_{inc}	Ta above which the Tb first increases
Tb_{inc} - Tlc_{EE}	difference between the Tb_{inc} and Tlc_{EE}
TEE at Tlc_{EE}	mean TEE at Ta =Tlc_{EE}
Cond, <Tlc	mean conductance at Ta <Tlc_{cond}
Tb, <Tb_{inc}	mean Tb at Ta < Tb_{inc}
defended Tb	X intercept of TEE vs Ta line (using only Ta <Tlc_{EE})
defended Tb - Tb, <Tb_{inc}	defended Tb minus the measured Tb <Tb_{inc}
TEE Slope, <Tlc_{EE}	TEE vs Ta slope for Ta <Tlc_{EE}
Cond Slope, >Tlc_{cond} <Cond_R	conductance slope in the region >Tlc_{cond} and <Cond_R
Tb Slope, >Tb_{inc} <Tb_R	Tb slope in the region >Tb_{inc} and <Tb_R
TEE_R	breakpoint of the TEE vs Ta graph, where TEE starts to rise with Ta
Cond_R	second breakpoint of the conductance vs Ta graph
Tb_R	breakpoint of the Tb vs Ta graph, where Tb starts to steeply rise with Ta
TEE Slope, >TEE_R	TEE vs Ta slope for Ta >TEE_R
Cond Slope, >Cond_R	conductance vs Ta slope for Ta >Cond_R
Tb Slope, >Tb_R	Tb vs Ta slope for Ta >Tb_R

Table S2. Ta setup protocols. Related to STAR methods.

Experimental outline showing Ta changes setup in calorimetry systems used for data acquisition. Data from gray-shaded areas were used for regression analysis.

Protocol number 1
Ta range 16 - 34 °C

Day time (h)	16 - 8	8 - 9	9 - 10	10 -11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	
Day 1	22 °C	22 °C	22 °C	19 °C change at 10:00	19 °C	19 °C	16 °C change at 13:00	16 °C	16 °C	change to 25 °C at 16:00
Day 2	25 °C	25 °C	25 °C	27 °C change at 10:00	27 °C	28 °C change at 12:00	28 °C	29 °C change at 14:00	29 °C	
Day 3	25 °C	30 °C change at 8:00	30 °C	30 °C	30 °C	31 °C change at 12:00	31 °C	32 °C change at 14:00	32 °C	
Day 4	25 °C	33 °C change at 8:00	33 °C	33 °C	33 °C	34 °C change at 12:00	34 °C	35 °C change at 14:00	35 °C	

Protocol number 2
Ta range 16 - 38 °C

Day time (h)	16 - 8	8 - 9	9 - 10	10 -11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	
Day 1	22 °C	22 °C	22 °C	19 °C change at 10:00	19 °C	19 °C	16 °C change at 13:00	16 °C	16 °C	change to 25 °C at 16:00
Day 2	25 °C	25 °C	25 °C	27 °C change at 10:00	27 °C	28 °C change at 12:00	28 °C	29 °C change at 14:00	29 °C	
Day 3	25 °C	30 °C change at 8:00	30 °C	30 °C	30 °C	31 °C change at 12:00	31 °C	32 °C change at 14:00	32 °C	
Day 4	25 °C	33 °C change at 8:00	33 °C	33 °C	33 °C	34 °C change at 12:00	34 °C	35 °C change at 14:00	35 °C	
Day 5	25 °C	36 °C change at 8:00	36 °C	36 °C	36 °C	37 °C change at 12:00	37 °C	38 °C change at 14:00	38 °C	

Protocol number 3
30 °C acclimated mice

Day time (h)	16 - 8	8 - 9	9 - 10	10 -11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	
Day 1	30 °C	30 °C	30 °C	31 °C change at 10:00	31 °C	32 °C change at 12:00	32 °C	33 °C change at 14:00	33 °C	change to 30 °C at 16:00
Day 2	30 °C	34 °C change at 8:00	34 °C	34 °C	34 °C	35 °C change at 12:00	35 °C	36 °C change at 14:00	36 °C	
Day 3	30 °C	37 °C change at 8:00	37 °C	37 °C	37 °C	38 °C change at 12:00	38 °C	40 °C change at 14:00	40 °C	
Day 4	30 °C	29 °C change at 8:00	29 °C	28 °C change at 10:00	28 °C	27 °C change at 12:00	27 °C	25 °C change at 14:00	25 °C	
Day 5	30 °C	22 °C	22 °C	22 °C	22 °C	19 °C change at 12:00	19 °C	19 °C change at 14:00	19 °C	

Protocol number 4
Dark phase

Day time (h)	04 - 18	18 - 19	19 - 20	20 - 21	21 - 22	22 - 23	23 - 00	00 - 01	01 - 02	02 - 03	03 - 04	
Day 1	22 °C	22 °C	22 °C	22 °C	22 °C	19 °C change at 22:00	19 °C	19 °C	16 °C change at 1:00	16 °C	16 °C	change to 25 °C at 4:00
Day 2	25 °C	25 °C	25 °C	27 °C change at 20:00	27 °C	28 °C change at 22:00	28 °C	29 °C change at 00:00	29 °C	30 °C change at 2:00	30 °C	
Day 3	25 °C	31 °C change at 18:00	31 °C	31 °C	31 °C	32 °C change at 22:00	32 °C	33 °C change at 00:00	33 °C	34 °C change at 2:00	34 °C	
Day 4	25 °C	35 °C change at 18:00	35 °C	35 °C	35 °C	36 °C change at 22:00	36 °C	37 °C change at 00:00	37 °C	38 °C change at 2:00	38 °C	

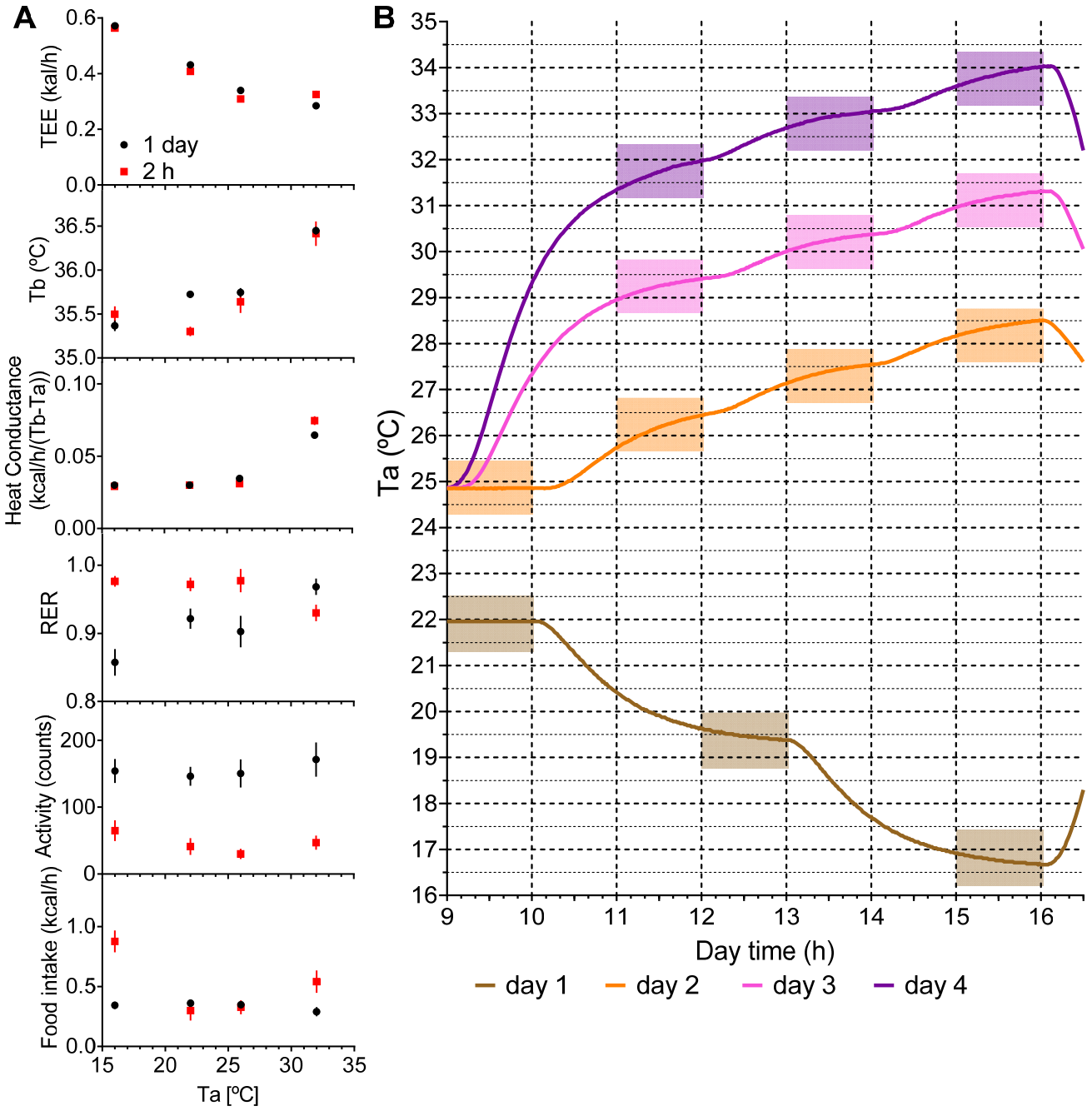

Figure S1. Measuring thermal biology parameters. Related to Figures 1 – 3 and STAR methods. (A) TEE, Tb, heat conductance, RER, activity, and food intake in mice using 65-min intervals (red squares) and the same mice housed 24 h (black circles) at the indicated Ta. In the 24 h dataset, only light phase data from 1 h after lights on to 1 h before lights off were used. Data are mean ± SEM, n = 11. (B) Experimental outline showing environmental chamber Ta (showing Ta setup protocol 1 from Table S2). Shaded areas are the 65-min intervals used for the points in Figures 1, 2, 3, and S3. Regression analyses used all data from days 1, 2 (0900 – 1600) and days 3, 4 (1100 – 1600).

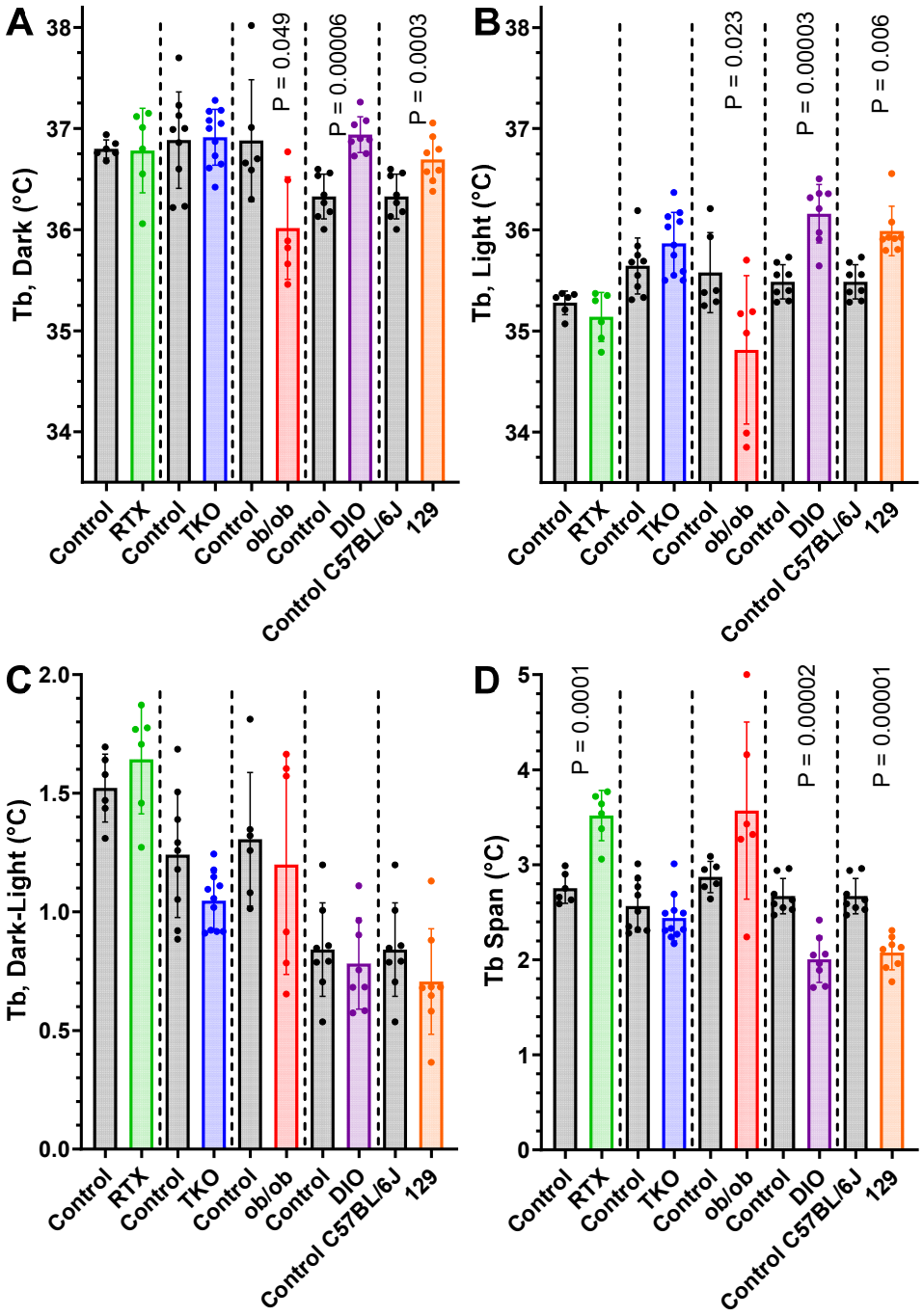

Figure S2. Baseline body temperature (Tb). Related to Figures 2, 3. Tb was measured continuously at an ambient temperature (Ta) of 22 °C by telemetry in singly-housed mice in their home cages for 72 consecutive hours. (A) Dark phase Tb. (B) Light phase Tb. (C) Difference between dark and light phase Tb. (D) Tb span, the difference between 5th and 95th percentiles of the full dataset. Experiments done at different times are separated by dashed lines. Data are mean ±SD, n=6-11/group, *P* values are t-test vs controls studied simultaneously.

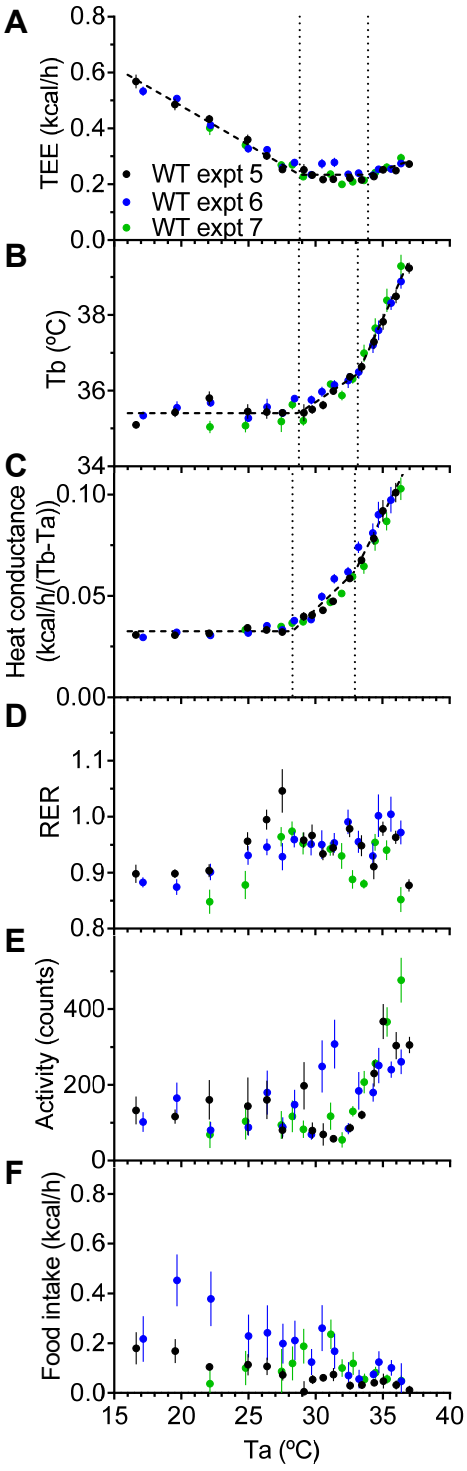

Figure S3. Energy metabolism and thermal physiology at high ambient temperatures (Ta). Related to Figures 3, 4. Male C57BL/6J mice were studied in three independent experiments during light phase, with measurement of (A) total energy expenditure (TEE), (B) body temperature (Tb), (C) heat conductance, (D) respiratory exchange ratio (RER), (E) physical activity, and (F) food intake. Lines and breakpoints (indicated by vertical lines) were calculated by mixed model regression analysis. For visual clarity, only Ta plateau mean ± SEM data points are depicted (each from 65 min, 5 sampling cycles). See Table S1 for regression parameters and n.