

Figure S1: Tb span dependence on Tb. Using 24-hour measurement periods, the Tb span (the 95th percentile minus the 5th percentile) and 24-h mean Tb were analyzed by linear regression in the indicated groups of male mice at 23 °C. The regression lines are: WT group, $Tb\ span = 0.5271 * Tb - 16.51$, $R^2 = 0.80$; WT single, $Tb\ span = 0.6048 * Tb - 19.61$, $R^2 = 0.88$; *Brs3*^{-/-} group, $R^2 < 0.1$, and *Brs3*^{-/-} single, $Tb\ span = 0.7672 * Tb - 24.86$, $R^2 = 0.74$. The regression line slopes are different from zero at $P < 0.0001$, $P = 0.018$, $P = 0.22$, and $P = 0.028$, respectively.

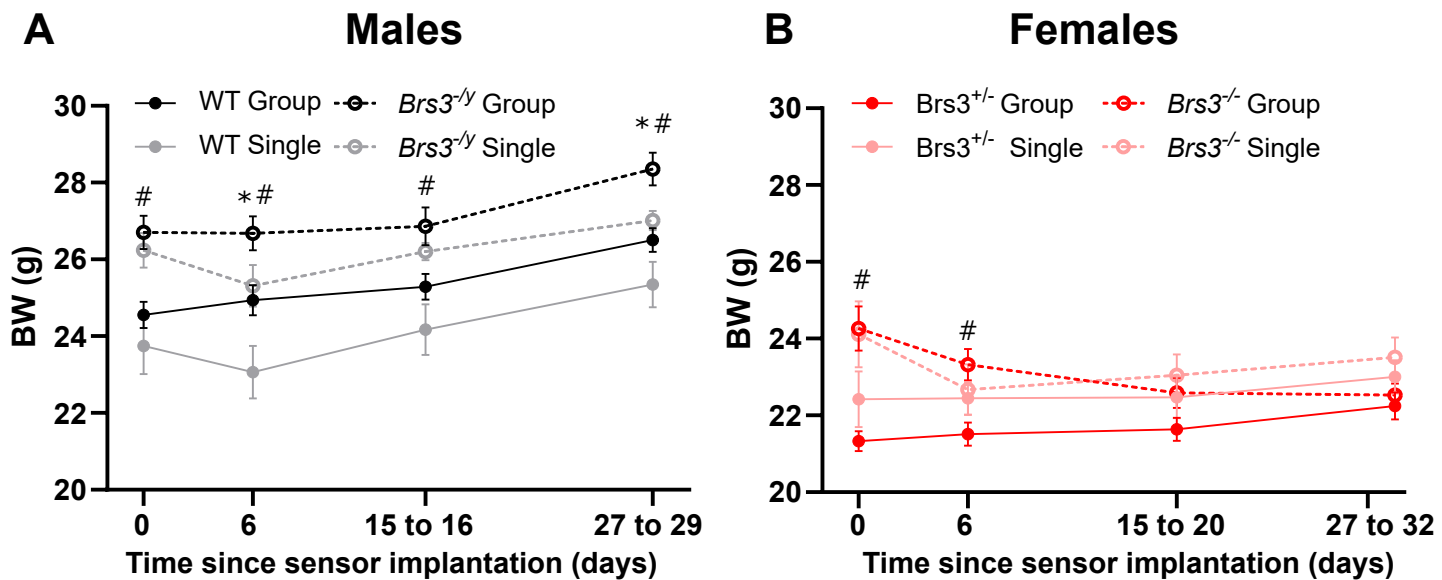
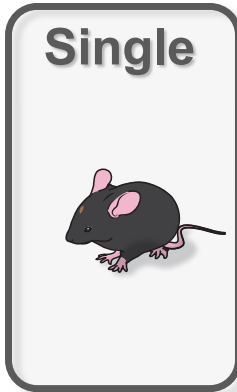


Figure S2: Body weights during study. Body weights were measured on the indicated day after telemeter implantation in (A) male mice implanted at 10 weeks and (B) female mice implanted at 15 weeks of age. 2-way ANOVA: * $P < 0.05$ effect of housing, # $P < 0.05$ effect of genotype, $n = 5 - 18/\text{group}$.

Housing

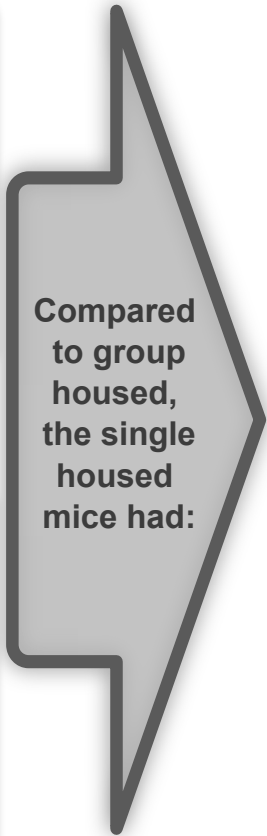


Single

VS



Group



Compared to group housed, the single housed mice had:

		♂	♀
Below thermoneutrality (8 °C, 23 °C)	Energy expenditure	↑	↑
	Body temperature	↓	NO Δ
	Heat conductance	↑	↑
Above thermoneutrality (35 °C)	Energy expenditure	NO Δ	NO Δ
	Body temperature	↓	NO Δ
	Heat conductance	NO Δ	↑
Fasting (23 °C)	Body temperature	↓	↓
	Torpor episode number	↑	NO Δ
	Torpor episode duration	NO Δ	↑

Figure S3: Thermal physiology is different in single versus group housed mice, with different strategies used in male and female mice.

Table S1: Summary of measured parameters, continued

	Males										Females									
	WT group		WT single	WT	Brs3-/y group		Brs3-/y single	Brs3-/y	2-way ANOVA P value		Brs3+/- group		Brs3+/- single	Brs3+/-	Brs3-/- group		Brs3-/- single	Brs3-/-	2-way ANOVA P value	
				P value*				P value	housing	genotype	interaction				P value				housing	genotype
35 °C Ambient temperature (Figure 5, continued)																				
Light water intake (ml/h)	0.095 ± 0.008	0.334 ± 0.030	<0.0001	0.082 ± 0.005	0.299 ± 0.026	<0.0001	<0.0001	0.25	0.60		0.081 ± 0.027	0.317 ± 0.053	0.0084	0.066 ± 0.020	0.324 ± 0.082	0.0040	0.0001	0.94	0.83	
Dark water intake (ml/h)	0.099 ± 0.025	0.135 ± 0.014	0.29	0.068 ± 0.011	0.150 ± 0.016	0.0067	0.0029	0.64	0.20		0.084 ± 0.019	0.204 ± 0.032	0.0122	0.098 ± 0.014	0.235 ± 0.039	0.0045	0.0002	0.43	0.76	
24 h water intake (ml/h)	0.097 ± 0.015	0.216 ± 0.012	<0.0001	0.074 ± 0.007	0.198 ± 0.015	<0.0001	<0.0001	0.13	0.83		0.083 ± 0.006	0.244 ± 0.012	<0.0001	0.083 ± 0.009	0.200 ± 0.022	<0.0001	<0.0001	0.12	0.11	
Light PA (counts/min)	57 ± 4	55 ± 6	0.95	56 ± 3	60 ± 5	0.85	0.88	0.74	0.57		27 ± 3	29 ± 3	0.94	29 ± 4	34 ± 5	0.66	0.43	0.37	0.71	
Dark PA (counts/min)	52 ± 3	65 ± 9	0.34	66 ± 2	72 ± 9	0.80	0.18	0.14	0.60		45 ± 3	53 ± 6	0.38	54 ± 3	68 ± 5	0.07	0.0200	0.0180	0.49	
24 h PA (counts/min)	54 ± 3	60 ± 8	0.68	61 ± 2	66 ± 6	0.78	0.32	0.24	0.91		37 ± 2	42 ± 5	0.47	43 ± 3	52 ± 3	0.09	0.0308	0.0248	0.48	
Fasting, CLAMS (Figure 6)																				
Mean Tb (°C)	35.65 ± 0.11	35.27 ± 0.30	0.19	35.62 ± 0.06	35.24 ± 0.24	0.16	0.02	0.84	0.99		35.73 ± 0.09	35.47 ± 0.21	0.37	35.93 ± 0.08	35.14 ± 0.27	0.0007	0.0007	0.67	0.07	
Minimum Tb (°C)	33.38 ± 0.16	32.38 ± 0.23	0.0037	33.05 ± 0.10	31.26 ± 0.37	<0.0001	<0.0001	0.0011	0.06		33.05 ± 0.16	30.77 ± 0.64	<0.0001	32.67 ± 0.15	30.41 ± 0.50	<0.0001	<0.0001	0.22	0.97	
Tb span (°C)	3.46 ± 0.19	3.84 ± 0.42	0.45	3.91 ± 0.09	5.45 ± 0.27	<0.0001	0.0001	<0.0001	0.0136		4.03 ± 0.15	6.32 ± 0.66	<0.0001	4.43 ± 0.13	6.80 ± 0.46	<0.0001	<0.0001	0.13	0.88	
Tb < 34 °C (% of time)	7.8 ± 1.6	17.9 ± 3.5	0.0118	10.6 ± 1.4	21.7 ± 3.8	0.0027	<0.0001	0.17	0.82		11.6 ± 1.8	19.7 ± 3.2	0.05	11.5 ± 1.5	25.1 ± 3.8	0.0007	<0.0001	0.30	0.27	
Torpor episodes (number)	1.9 ± 0.3	4.6 ± 0.7	0.0005	2.9 ± 0.2	4.5 ± 0.7	0.0366	<0.0001	0.31	0.22		2.7 ± 0.3	3.0 ± 0.5	0.88	2.9 ± 0.3	2.7 ± 0.6	0.92	0.95	0.85	0.57	
Torpor episode duration (min)	53.5 ± 9.0	49.8 ± 7.2	0.87	47.4 ± 5.9	57.3 ± 4.9	0.70	0.87	0.92	0.39		44.5 ± 5.9	70.1 ± 13.2	0.25	51.6 ± 8.5	108.3 ± 24.3	0.0032	0.0012	0.06	0.20	
Longest torpor episode (min)	83.4 ± 0.0	102.1 ± 0.0	0.70	73.2 ± 0.0	126.6 ± 0.0	0.05	0.0397	0.68	0.31		77.1 ± 11.6	132.0 ± 38.2	0.06	76.6 ± 9.8	145.8 ± 13.1	0.0143	0.0009	0.70	0.68	
Conductance (kcal/h/Δ°C)	0.025 ± 0.001	0.028 ± 0.002	0.18	0.026 ± 0.001	0.031 ± 0.002	0.0123	0.0025	0.17	0.48		0.024 ± 0.000	0.031 ± 0.002	<0.0001	0.025 ± 0.000	0.030 ± 0.000	<0.0001	<0.0001	0.28	0.06	
Mean TEE (kcal/h)	0.308 ± 0.010	0.353 ± 0.017	0.22	0.331 ± 0.015	0.374 ± 0.030	0.25	0.0355	0.27	0.95		0.286 ± 0.006	0.370 ± 0.020	0.0003	0.315 ± 0.006	0.365 ± 0.012	0.0217	<0.0001	0.35	0.19	
Minimum TEE (kcal/h)	0.145 ± 0.008	0.125 ± 0.007	0.30	0.147 ± 0.006	0.125 ± 0.016	0.24	0.0438	0.93	0.92		0.121 ± 0.005	0.113 ± 0.009	0.74	0.117 ± 0.007	0.111 ± 0.010	0.85	0.40	0.72	0.89	
Mean RER	0.741 ± 0.006	0.701 ± 0.010	0.0008	0.739 ± 0.003	0.694 ± 0.006	0.0002	<0.0001	0.53	0.68		0.744 ± 0.005	0.729 ± 0.012	0.39	0.751 ± 0.005	0.728 ± 0.009	0.12	0.0319	0.76	0.62	
Mean PA (counts/min)	76 ± 9	156 ± 13	0.0025	69 ± 4	169 ± 25	0.0003	<0.0001	0.87	0.50		77 ± 7	178 ± 33	0.0021	94 ± 5	176 ± 15	0.0114	<0.0001	0.70	0.61	
24 h water intake (ml/h)	0.057 ± 0.010	0.087 ± 0.012	0.25	0.051 ± 0.014	0.092 ± 0.018	0.10	0.0190	0.99	0.72		0.066 ± 0.012	0.102 ± 0.021	0.37	0.096 ± 0.024	0.139 ± 0.018	0.25	0.06	0.10	0.86	
Fasting, telemetry (section 3.5)																				
Mean Tb (°C)	35.52 ± 0.12	35.29 ± 0.32	0.62	35.52 ± 0.09	34.76 ± 0.29	0.0057	0.0076	0.14	0.14		35.11 ± 0.15	35.60 ± 0.14	0.08	35.45 ± 0.10	35.09 ± 0.13	0.25	0.69	0.59	0.0137	
Minimum Tb (°C)	32.39 ± 0.16	32.57 ± 0.53	0.89	32.45 ± 0.11	30.03 ± 0.61	<0.0001	0.0003	<0.0001	<0.0001		30.31 ± 0.40	29.84 ± 0.52	0.71	31.40 ± 0.20	29.25 ± 0.56	0.0026	0.0049	0.57	0.06	
Tb span (°C)	4.59 ± 0.20	3.53 ± 0.41	0.05	4.94 ± 0.13	6.47 ± 0.72	0.0021	0.48	<0.0001	0.0002		6.61 ± 0.43	6.74 ± 0.50	0.97	5.48 ± 0.17	7.84 ± 0.56	0.0014	0.0092	0.98	0.0188	
Tb < 34 °C (% of time)	15.9 ± 1.4	14.3 ± 4.4	0.89	19.3 ± 1.9	31.9 ± 3.7	0.0018	0.0395	0.0002	0.0085		24.2 ± 2.1	19.6 ± 2.7	0.35	21.6 ± 1.6	23.1 ± 0.9	0.89	0.53	0.84	0.22	
Torpor episodes (number)	3.9 ± 0.4	3.0 ± 0.5	0.39	3.4 ± 0.2	5.2 ± 0.7	0.0217	0.37	0.09	0.0092		3.1 ± 0.2	2.8 ± 0.5	0.88	3.7 ± 0.4	3.7 ± 0.6	0.99	0.70	0.11	0.80	
Torpor episode duration (min)	57.7 ± 5.0	54.7 ± 12.9	0.98	81.1 ± 10.1	84.8 ± 13.7	0.97	0.99	0.0241	0.77		97.9 ± 7.5	89.1 ± 16.4	0.8111	84.6 ± 6.3	85.9 ± 16.3	0.99	0.73	0.44	0.64	
Longest torpor episode (min)	113.8 ± 7.3	78.4 ± 18.9	0.63	144.9 ± 13.0	245.9 ± 81.4	0.0232	0.25	0.0010	0.0194		164.6 ± 17.6	129.6 ± 10.2	0.36	147.1 ± 10.9	154.1 ± 18.6	0.96	0.47	0.86	0.28	

*adjusted P value from post hoc Sidák's test for group vs single housing.

Abbreviations:

Tb, core body temperature

TEE, total energy expenditure

RER, respiratory exchange ratio

PA, physical activity

Table S2. Effect of a range of ambient temperatures on thermal physiology of C57BL/6J mice

	Males, single housed	Females, single housed	Males, group housed	Females, group housed
n of cages	6	6	6	6
n of data points in regression	3654	4832		
Body weight (g)	28.0 ± 0.5	23.6 ± 0.3	29.8 ± 0.5	21.8 ± 0.3
TEE analysis:				
TEE Slope, <Tl_{C_{EE}} (kcal/h/°C)	-0.0291 ± 0.0004	-0.0218 ± 0.0004		
Tl_{C_{EE}} (TN_{PL}, °C)	28.45 ± 0.01	30.06 ± 0.19		
TEE, at Tl_{C_{EE}} (kcal/h)	0.229 ± 0.006	0.204 ± 0.003		
TEE_R (°C)	35.52 ± 0.39	36.63 ± 0.28		
TEE Slope, >TEE_R (kcal/h/°C)	0.0350 ± 0.0240	0.0188 ± 0.0033		
defended Tb (°C)	36.31 ± 0.24	39.42 ± 0.36		
Tb analysis:				
Tb, <Tb_{inc} (°C)	35.59 ± 0.07	36.16 ± 0.13		
Tb_{inc} (°C)	30.39 ± 0.28	29.10 ± 0.20		
Tb Slope, >Tb_{inc} <Tb_R (°C Tb/°C)	0.191 ± 0.026	0.272 ± 0.015		
Tb_R (°C)	33.94 ± 0.08	34.65 ± 0.14		
Tb Slope, >Tb_R (°C Tb/°C)	0.967 ± 0.030	0.649 ± 0.014		
Conductance analysis:				
n of data points in regression	658	651	665	680
Conductance slope, Ta<27°C (cal/h/°C/°C)	0.013 ± 0.081	0.246 ± 0.063	0.838 ± 0.087	0.758 ± 0.076

Key to parameters:

TEE Slope, <Tl _{C_{EE}} (kcal/h/°C)	TEE vs. Ta slope for Ta <Tl _{C_{EE}}
Tl _{C_{EE}} (TN _{PL} , °C)	lower critical temperature, the (first) breakpoint of the TEE vs. Ta graph; TN _{PL}
TEE, at Tl _{C_{EE}} (kcal/h)	mean TEE at Ta = Tl _{C_{EE}}
TEE _R (°C)	breakpoint of the TEE vs. Ta graph, where TEE starts to rise with Ta; TN _{PD}
TEE Slope, >TEE _R (kcal/h/°C)	TEE vs. Ta slope for Ta >TEE _R
defended Tb (°C)	defended Tb, X intercept of TEE vs. Ta line (using only Ta <Tl _{C_{EE}})
Tb, <Tb _{inc} (°C)	mean Tb at Ta < Tb _{inc}
Tb _{inc} (°C)	Ta above which the Tb first increases; TN _{PL}
Tb Slope, >Tb _{inc} <Tb _R (°C Tb/°C)	Tb slope in the region >Tb _{inc} and <Tb _R
Tb _R (°C)	second breakpoint of Tb vs. Ta graph, where Tb starts to rise steeply; TN _{PD}
Tb Slope, >Tb _R (°C Tb/°C)	Tb vs. Ta slope for Ta >Tb _R
Conductance slope, Ta<27°C	Heat conductance vs. Ta slope for Ta<27°C

TEE and Tb parameters were determined by mixed model segmented linear regression [6]. The TEE model has three segments with the slope of the middle one fixed at zero. The Tb model has three segments with the slope of the first one fixed at zero. In both models, the two breakpoints and other two slopes are determined by the model. Conductance slope was obtained by linear regression. Data are means ± SE.

Table S3: Summary of adipose tissue weight, *UCP1* mRNA expression, and hormone levels

	Males									Females								
	WT group	WT single	WT <i>P</i> value*	Brs3-/y group	Brs3-/y single	Brs3-/y <i>P</i> value*	2 way ANOVA			Brs3+/- group	Brs3+/- single	Brs3+/- <i>P</i> value*	Brs3-/- group	Brs3-/- single	Brs3-/- <i>P</i> value*	2 way ANOVA		
								housing	genotype	interaction								housing
BAT weight (mg)	97.2 ± 4.8	81.9 ± 5.5	0.89	118.7 ± 6.5	114.2 ± 2.8	0.26	0.18	0.0005	0.46	74.9 ± 3.4	88.1 ± 5.3	0.52	83.1 ± 5.5	91.9 ± 5.4	0.24	0.075	0.33	0.72
iWAT weight (g)	0.289 ± 0.013	0.288 ± 0.022	0.62	0.334 ± 0.016	0.360 ± 0.032	1.00	0.56	0.0071	0.52	0.304 ± 0.015	0.316 ± 0.009	0.25	0.382 ± 0.038	0.302 ± 0.027	0.97	0.36	0.39	0.22
eWAT weight (g)	0.270 ± 0.012	0.267 ± 0.013	0.52	0.338 ± 0.015	0.311 ± 0.019	0.99	0.42	0.0031	0.52	0.204 ± 0.009	0.212 ± 0.020	0.70	0.292 ± 0.039	0.253 ± 0.023	0.98	0.67	0.09	0.52
BAT <i>Ucp1</i> mRNA	1.00 ± 0.19	1.75 ± 0.20	0.54	0.28 ± 0.04	0.49 ± 0.09	0.0035	0.0039	<0.0001	0.08	0.65 ± 0.07	0.97 ± 0.07	0.37	0.77 ± 0.15	0.95 ± 0.06	0.058	0.018	0.62	0.47
iWAT <i>Ucp1</i> mRNA	0.00245 ± 0.00116	0.00188 ± 0.00122	0.67	0.00064 ± 0.00034	0.00174 ± 0.00085	0.90	0.78	0.32	0.40	0.00050 ± 0.00023	0.00228 ± 0.00151	0.43	0.00016 ± 0.00005	0.00016 ± 0.00001	0.40	0.10	0.71	0.97
Serum T3 (ng/ml)	1.35 ± 0.06	1.54 ± 0.10	0.018	1.42 ± 0.07	1.11 ± 0.05	0.17	0.47	0.028	0.0030	0.80 ± 0.08	0.70 ± 0.09	0.085	0.96 ± 0.08	0.42 ± 0.08	0.98	0.11	0.91	0.19
Serum T4 (µg/dl)	4.67 ± 0.11	4.84 ± 0.14	0.920	4.64 ± 0.20	4.54 ± 0.18	0.79	0.87	0.388	0.4961	4.67 ± 0.23	4.57 ± 0.32	0.001	5.19 ± 0.30	4.13 ± 0.55	0.71	0.00	0.55	0.03
Serum leptin (ng/ml)	2.22 ± 0.20	1.19 ± 0.15	0.460	3.21 ± 0.29	2.70 ± 0.36	0.05	0.02	0.000	0.4238	2.58 ± 0.35	1.19 ± 0.15	0.756	3.87 ± 0.61	3.26 ± 0.40	0.98	0.54	0.10	0.74

* *P* value from post hoc Šidák multiple comparison test for group vs single housing