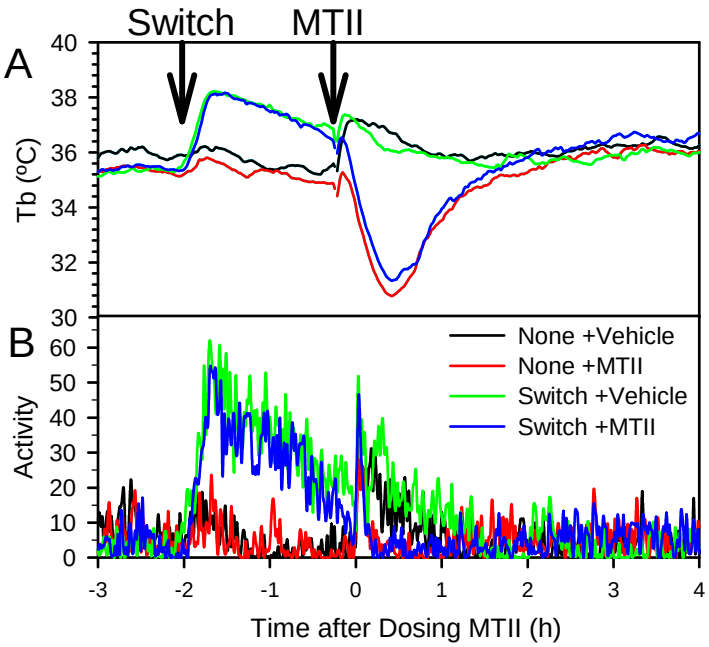

Figure S1, Related to Figure 2. Cage switch does not affect response to MTII. (A) Tb and (B) activity after cage switch (Lee et al., 2004), in which chow-fed male mice (body weight 30.6 ±0.3 g) were placed in a cage containing the bedding of another male mouse (green, blue) or no intervention (red, black) followed two hours later by treatment with MTII (blue, red) or vehicle (green, black). The expected stress Tb and activity increases occurred and did not prevent hypothermia caused by MTII. Activity is in arbitrary units via Mini Mitter. Data are means from n=6/group; SEMs omitted for visual clarity.

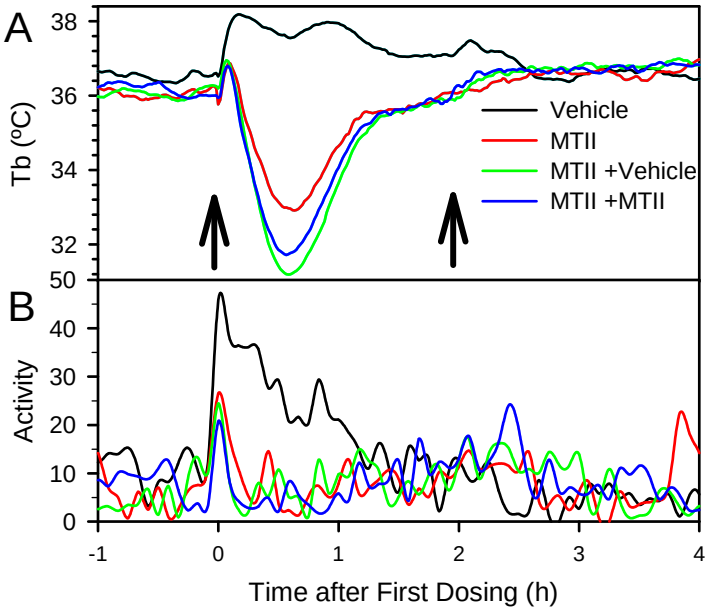

Figure S2, Related to Figure 3. Attenuation of MTII effect with repeated dosing. (A) Tb and (B) activity in DIO mice treated with vehicle or MTII at time 0 and again with vehicle, MTII, or not handled at all 120 minutes later. Dosing times are indicated by arrows. The treatments are vehicle once (black), MTII once (red), MTII and vehicle (green), and MTII and MTII (blue). Activity is in arbitrary units via Mini Mitter. Data are mean, n=3-4/group; body weight 52.1 ±1.3 g).

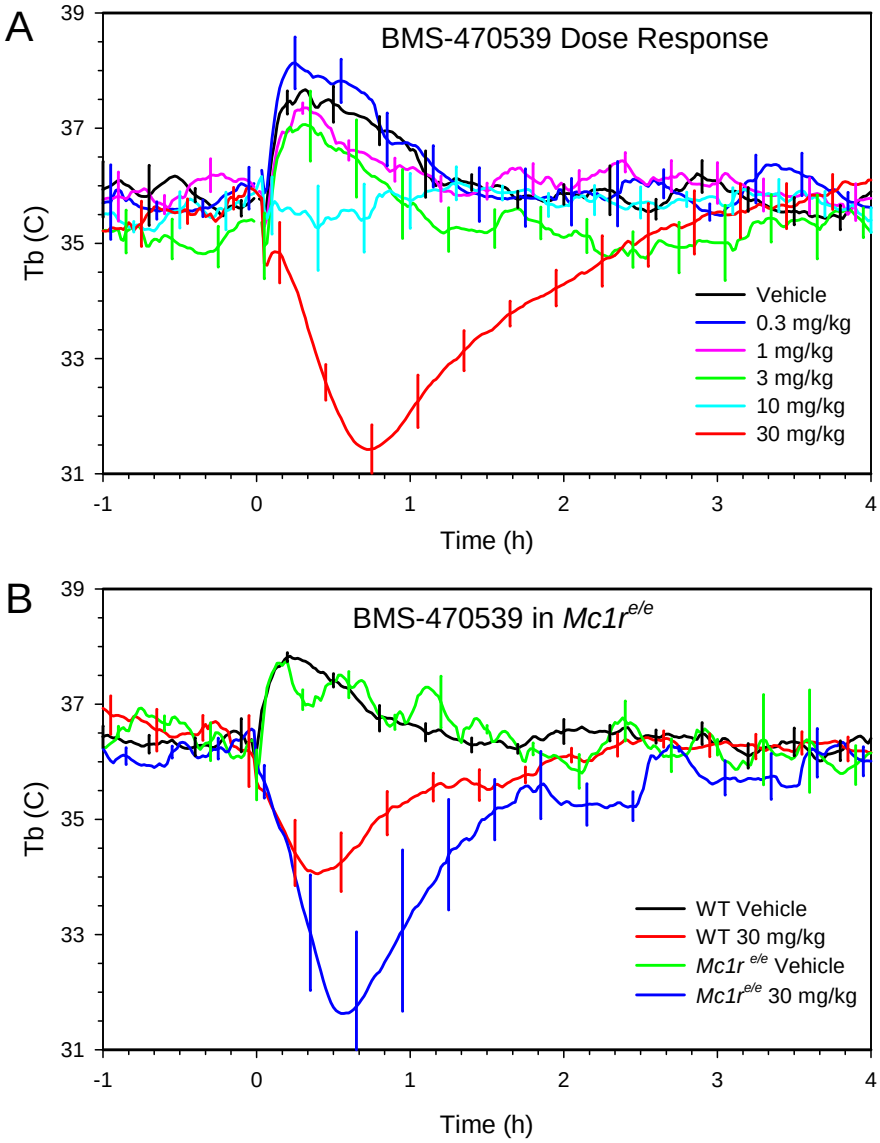

Figure S3, Related to Figure 5. Effects of other melanocortin agonists. (A) BMS-470539 was administered at the indicated ip dose at time 0 to C57BL/6J mice (n=4/group body weight 32.2 ±0.3 g). (B) BMS-470539 (30 mg/kg ip) or vehicle was administered at time 0 to wild type (n=6/group, 27.8 ±1.6 g) or *Mc1r* $^{e/e}$ mice (n=4/group, 26.5 ±0.2 g). Data are mean, with every tenth SEM shown. (C) Compound 2B dose response: Compound 2B was administered at the indicated ip dose at time 0 to DIO C57BL/6J mice (body weight 53.5 ±0.8 g). Data are mean, n=8-9/group. (D) Effect of compound 2B in *Mc4r-/-* mice. Compound 2B (40 mg/kg ip) or vehicle was administered at time 0 to wild type (26.5 ±1.4 g) or *Mc4r-/-* mice (52.9 ±2.2 g). Data are mean, n=6/group (3 male and 3 female). (E) Effect of NDP-MSH. NDP-MSH was administered at the indicated ip dose at time 0 to C57BL/6J mice (vehicle, 27.7 ±1.6g, N=10; 100 µg, 30.3 ±1.8g, N=8 (3.3 mg/kg); 400 µg, 33.8 ±2.9g, N=5 (11.8 mg/kg)) and *Mc3r-/-; A*^{vy}/+ mice (400 µg is 10.6 mg/kg, see Figure 5G). Data are mean, with every tenth SEM shown.

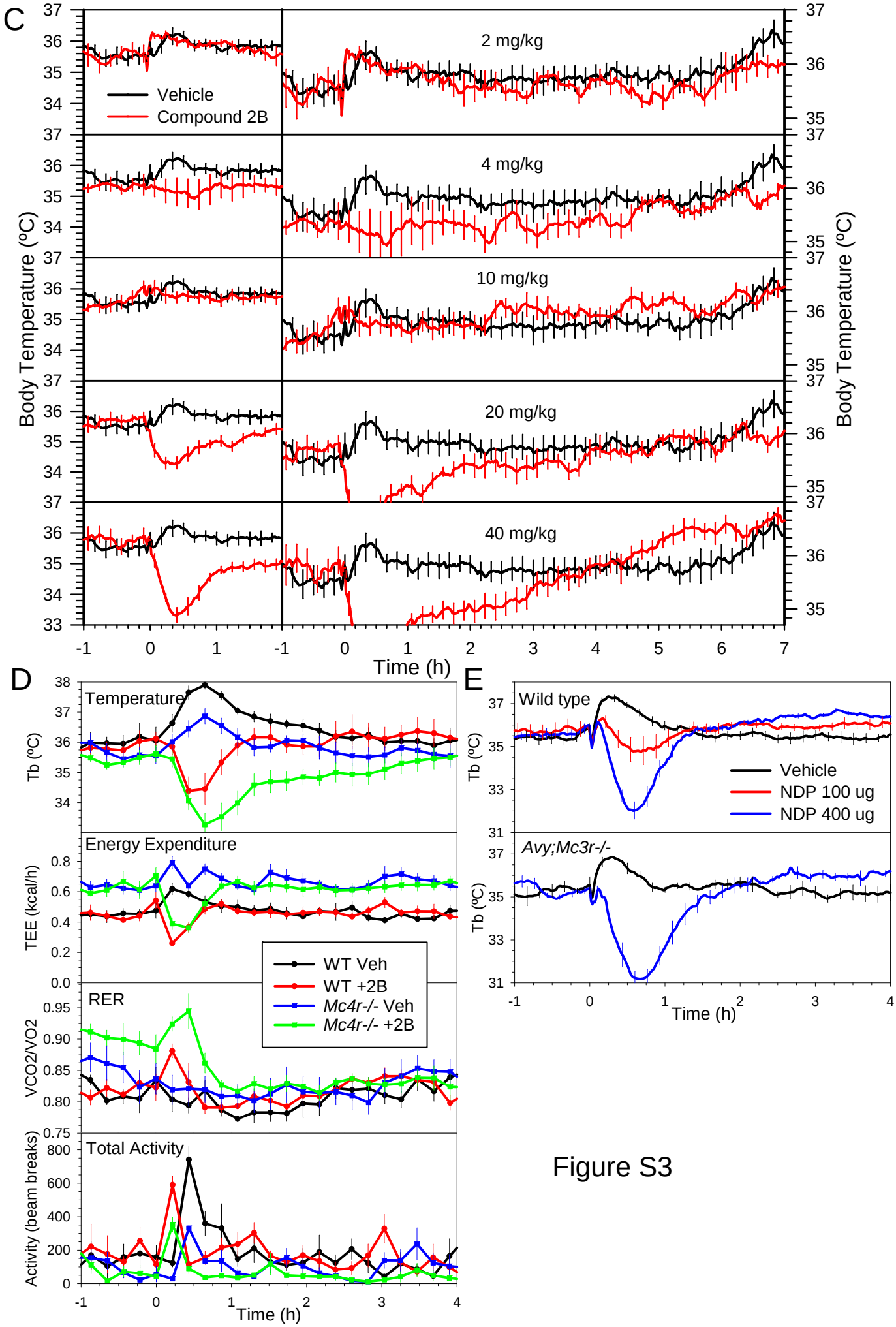
C
Vehicle
Compound 2B
2 mg/kg
4 mg/kg
10 mg/kg
20 mg/kg
40 mg/kg
Body Temperature (ºC)
Time (h)
D
Temperature
Tb (ºC)
Energy Expenditure
TEE (kcal/h)
WT Veh
WT +2B
Mc4r-/- Veh
Mc4r-/- +2B
RER
VCO2/VO2
Total Activity
Activity (beam breaks)
Time (h)
E
Wild type
Vehicle
NDP 100 ug
NDP 400 ug
Avy;Mc3r-/-
Tb (ºC)
Time (h)

Figure S3

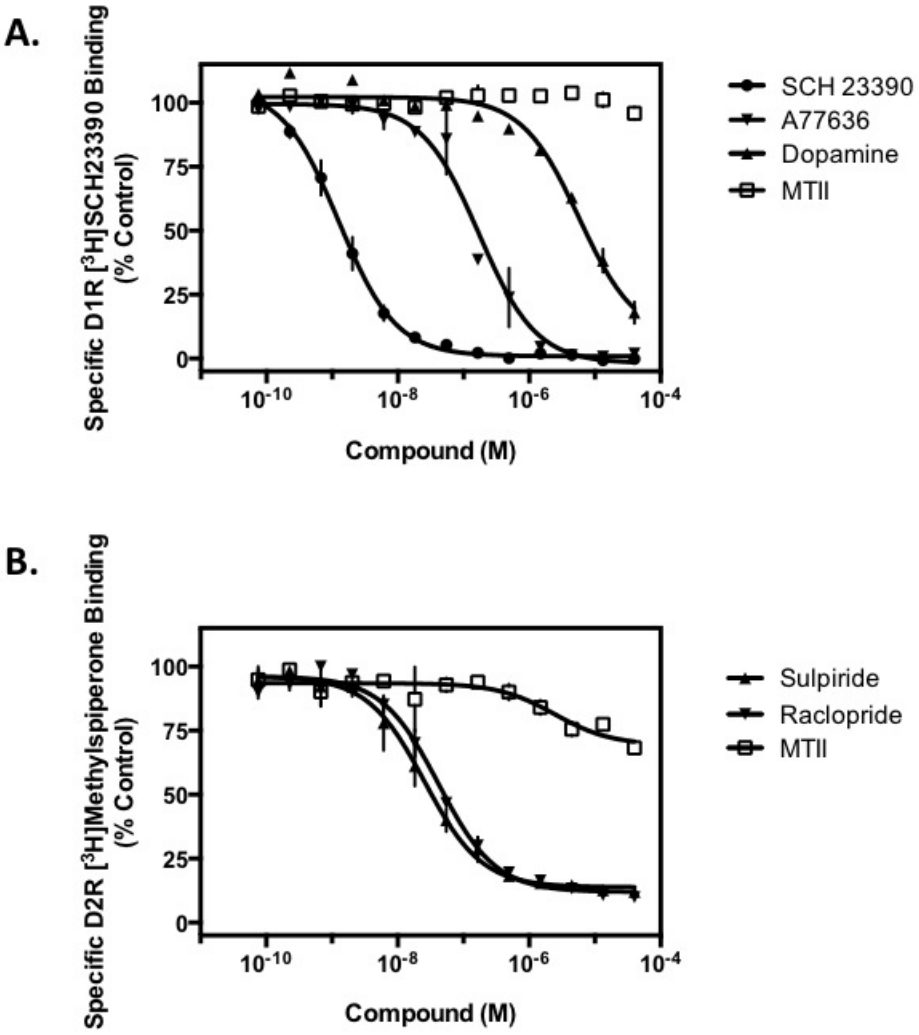

Figure S4, Related to Figure 6. MTII does not bind with high affinity to either D1 or D2 dopamine receptors. Human D1R **(A)** or D2R **(B)** containing membranes were incubated with the D1R- or D2R-selective radioligands [^{3}H]SCH23390 or [^{3}H]methylspiperone, respectively, along with unlabeled test compounds. Ki values of the test compounds were derived from their IC_{50} values using the Cheng-Prusoff equation and binding parameters of the radioligands determined via independent saturation isotherms (data not shown), **A**, Ki affinity values were as follows: MTII >100 μM; SCH23390, 0.46 ± 0.09 nM; dopamine, 2.0 ± 0.06 μM, A77636, 77.1 ± 35.0 nM. **B**, Ki affinity values were as follows: MTII >100 μM; sulpiride, 10.0 ± 3.4 nM; raclopride, 16.3 ± 2.0 nM. Competition binding assays were conducted as previously described (Chun et al., 2013). Data are means of two independent experiments expressed as a percentage of the specific binding seen in the absence of competing ligand.

Chun LS, Free RB, Doyle TB, Huang XP, Rankin ML and Sibley DR (2013) D1-D2 dopamine receptor synergy promotes calcium signaling via multiple mechanisms. *Molecular Pharmacology* **84**(2): 190-200.