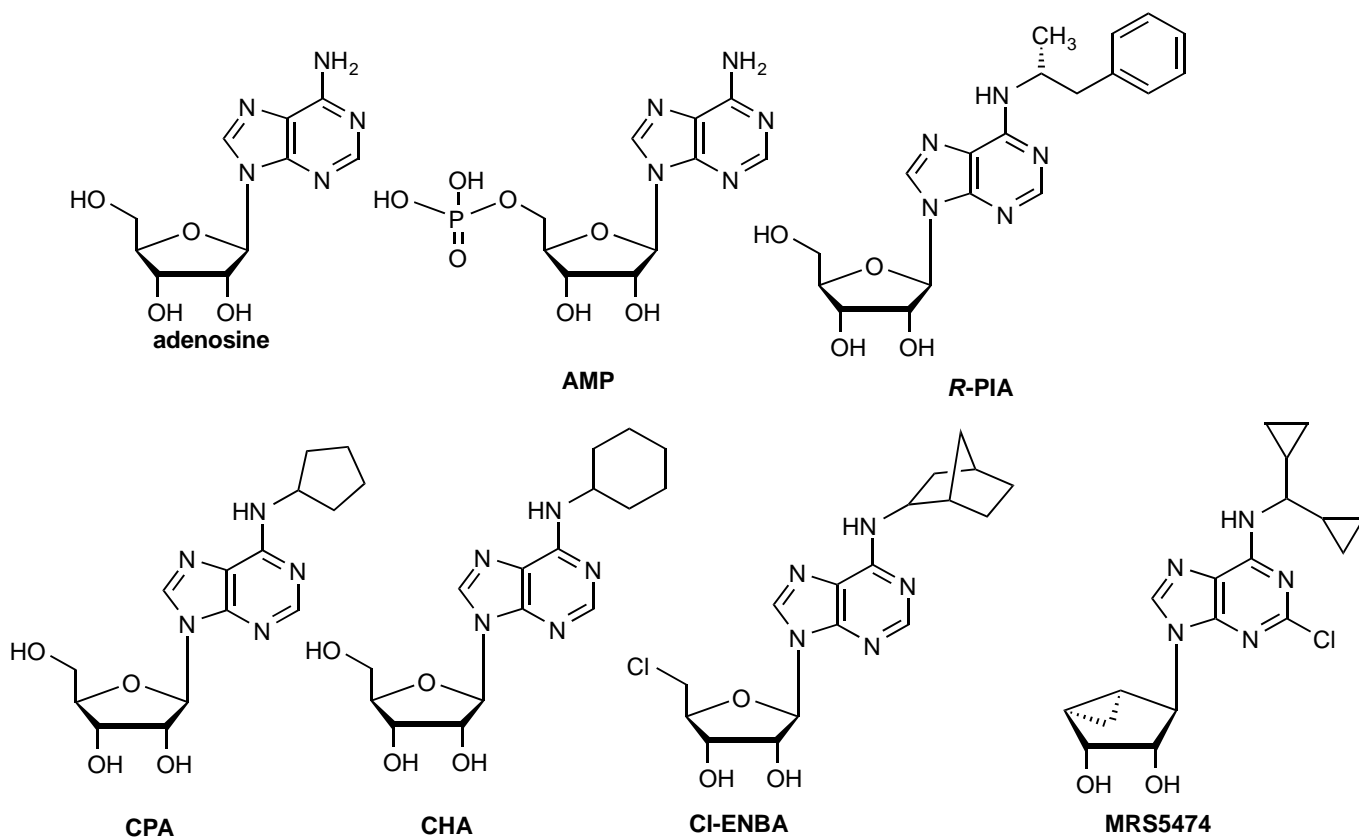


## Supplementary Material

### Hypothermia in mouse is caused by adenosine A<sub>1</sub> and A<sub>3</sub> receptor agonists and AMP via three distinct mechanisms

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**Fig. S1. Adenosine receptor ligands and related compounds.**

AMP, adenosine 5'-monophosphate

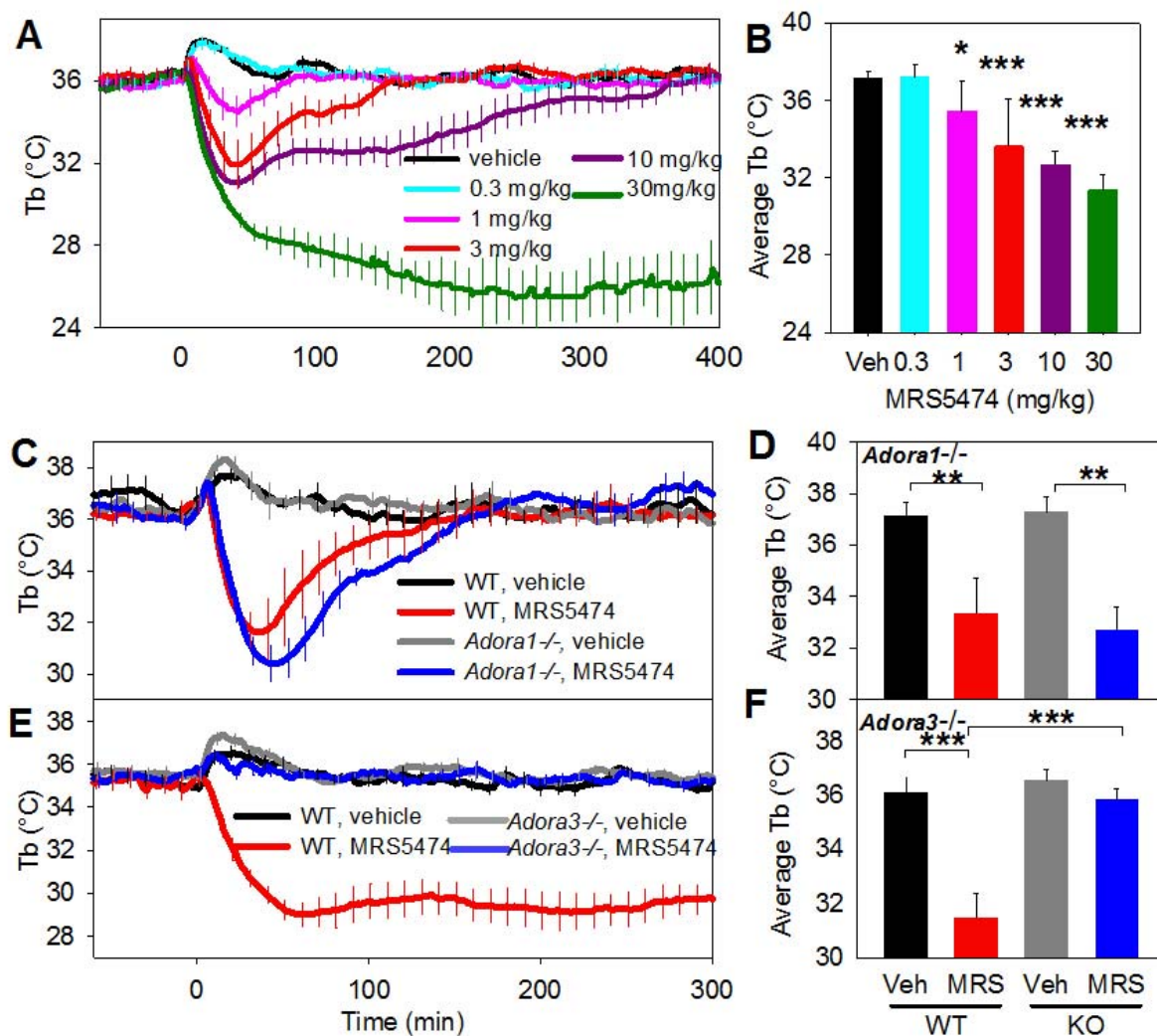
R-PIA, R(-)-N<sup>6</sup>-(2-phenylisopropyl) adenosine

CPA, N<sup>6</sup>-cyclopentyladenosine

CHA, N<sup>6</sup>-cyclohexyladenosine

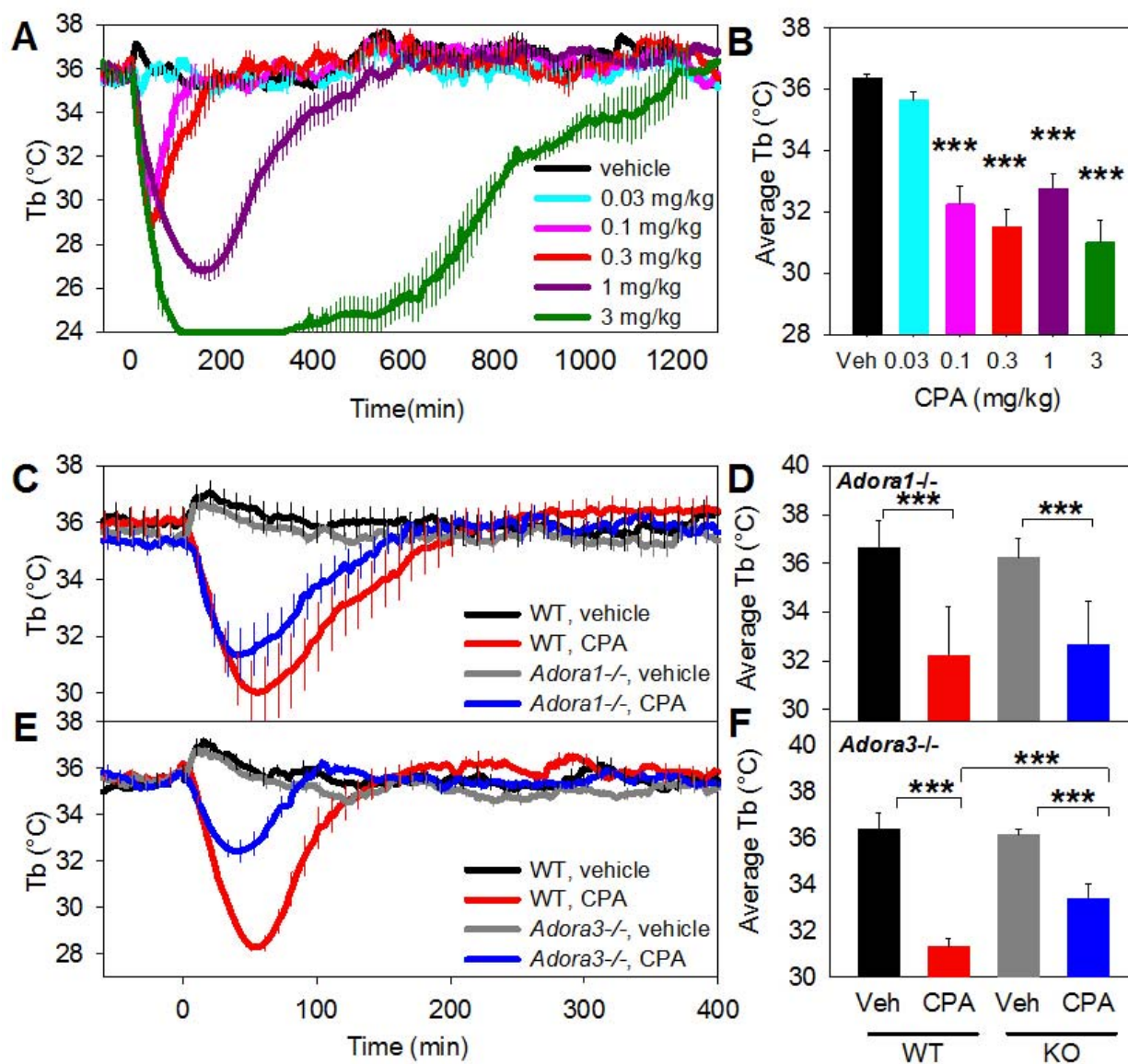
Cl-ENBA, (±)-5'-chloro-5'-deoxy-N<sup>6</sup>-endo-norbornyladenosine

MRS5474, (1R,2R,3S,5S)-4-(2-chloro-6-((dicyclopropylmethyl)amino)-9H-purin-9-yl)bicyclo[3.1.0]hexane-2,3-diol

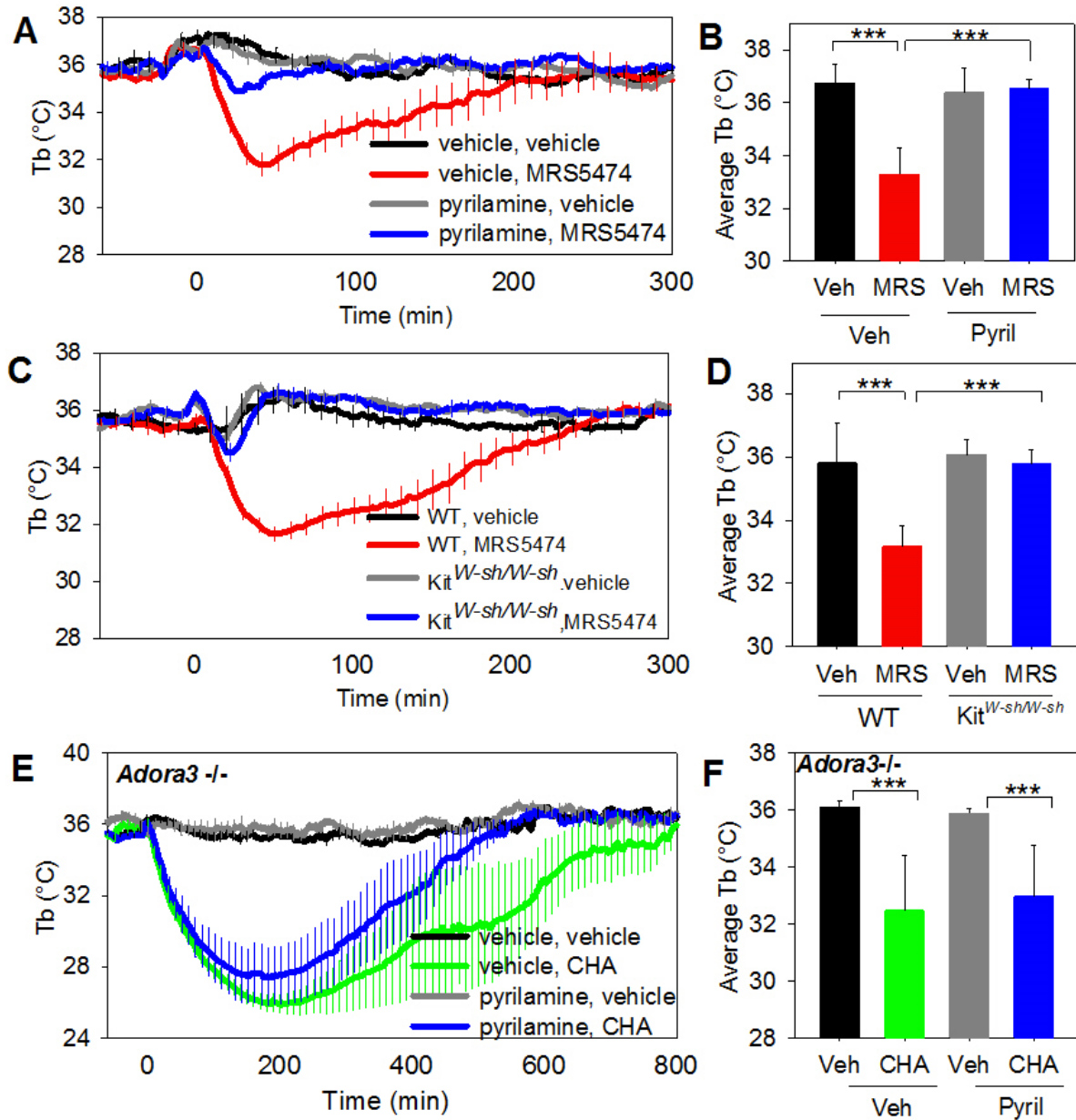


**Fig. S2. Systemic MRS5474 acts via A<sub>3</sub>AR to induce hypothermia and decrease activity.**

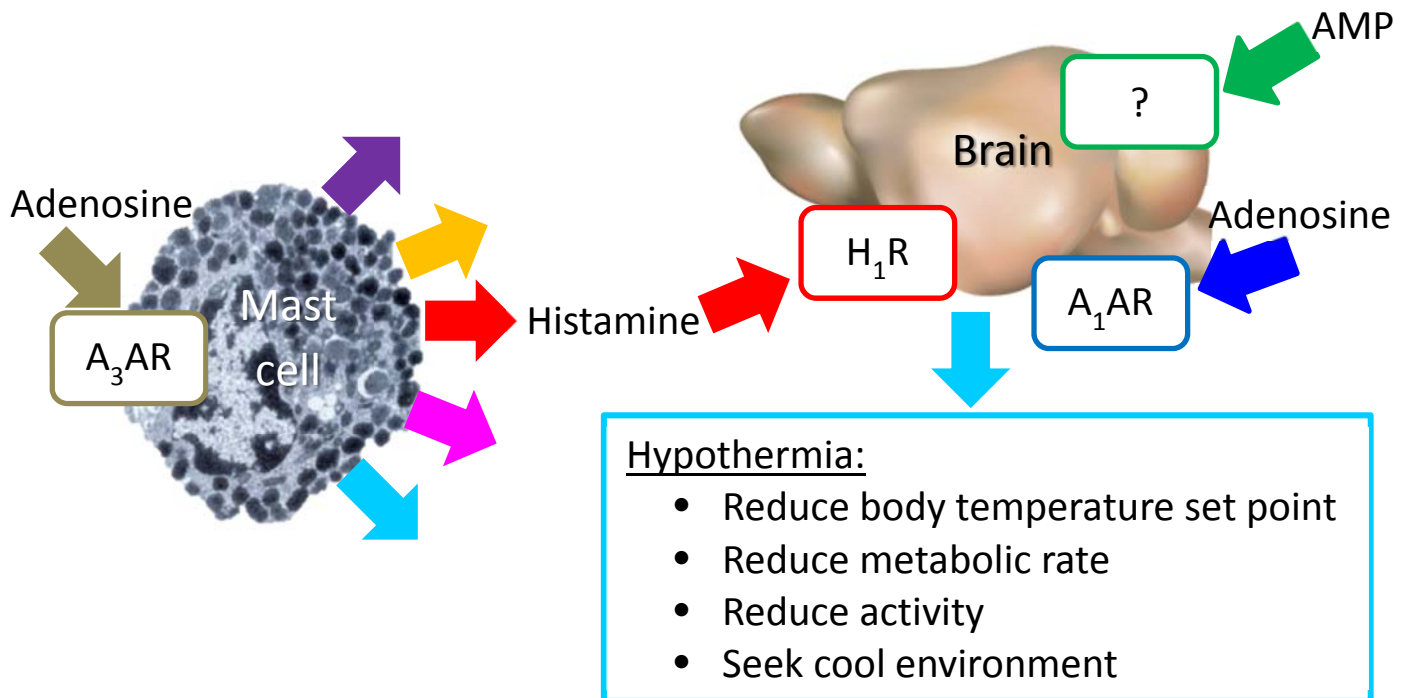
(A,B) Tb response to the indicated MRS5474 dose injected i.p. into C57BL/6J mice. Data are mean  $\pm$  SEM, n=4-5/group. (C,D) Tb response to MRS5474 (3 mg/kg, i.p.) or vehicle in C57BL/6J (WT) and *Adora1*<sup>-/-</sup> (KO) mice. (E,F) Tb response to MRS5474 (3 mg/kg, i.p.) or vehicle in C57BL/6J (WT) and *Adora3*<sup>-/-</sup> (KO) mice. Data are mean  $\pm$  SEM, n=6-10/group in a crossover design; every tenth SEM is shown in A, C, and E; \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.



**Fig. S3. Systemic CPA causes dose-dependent hypothermia and decreased physical activity through both A<sub>1</sub>AR and A<sub>3</sub>AR.** (A,B) Tb response to the indicated CPA doses injected i.p. into C57BL/6J mice. Data are mean ± SEM, n=3-6/group; every tenth SEM is shown in A. (C,D) Tb response to CPA (0.3 mg/kg, i.p.) or vehicle in C57BL/6J (WT) and *Adora1*<sup>-/-</sup> (KO) mice. (E,F) Tb response to CPA (0.3 mg/kg, i.p.) or vehicle in C57BL/6J (WT) and *Adora3*<sup>-/-</sup> (KO) mice. Data are mean ± SEM, n=3-7/group in a crossover design; every tenth SEM is shown in C and E; \*\*\* p<0.001.



**Fig. S4. Systemic A<sub>3</sub>AR-mediated hypothermia is blocked by H<sub>1</sub>R antagonist; A<sub>1</sub>AR-mediated hypothermia is not.** (A,B) Tb response to pretreatment with pyrilamine (10 mg/kg, i.p.) or vehicle followed by MRS5474 (10 mg/kg, i.p.) or vehicle in C57BL/6J mice (n=5/group). (C,D) Tb response to MRS5474 (10 mg/kg, i.p.) in C57BL/6J (WT) or *Kit<sup>W-sh/W-sh</sup>* mice (n=5-6/group). Data are mean ± SEM in a crossover design; every tenth SEM is shown in A and C; \*\*\* p<0.001



**Fig. S5. Three distinct adenosine-related routes to hypothermia in mice.** 1) activation of central  $A_1AR$ , 2) peripheral mast cell activation by  $A_3AR$  agonists, releasing histamine, which stimulates histamine  $H_1$  receptors, and 3) a central AMP-mediated mechanism.