Supplemental Data (Rittiner et al.)

EXPERIMENTAL PROCEDURES

Molecular Biology

Full-length expression constructs for human A_1R (GenBank accession #AY136746) and human $A_{2B}R$ (GenBank accession #AY136748) were obtained from the Missouri S&T Clone Collection (www.cdna.org). Human A_1R point mutants were generated by PCR-based mutagenesis. Chimeric G protein constructs ($G\alpha_{q\text{-}i5}$ and $G\alpha_{q\text{-}s5}$) and the mouse transmembrane (TM)-PAP (nt 64-1317 from GenBank accession # NM_207668) expression construct were previously described (1-3). Full-length expression constructs of mouse NT5E (nt 47-1777 from GenBank accession # NM_011851.3) and mouse A_1R (nt 1070-2053 from GenBank accession # NM_001008533) were generated by RT-PCR using C57BL/6 dorsal root ganglia cDNA as template. The TM-PAP and NT5E expression constructs hydrolyzed AMP when transfected into HEK293 cells (assessed using enzyme histochemistry; data not shown for NT5E and previously shown for TM-PAP (1)). PCR-generated constructs have a Kozak consensus sequence, were cloned into pcDNA3.1(+) and were sequence verified.

Compounds

Adenosine (A9251), AMP (01930), inosine (I4125), 2-chloro-N^6-cyclopentyladenosine (C7938), N^6-cyclopentyladenosine (C8031), α,β-methylene adenosine 5'-diphosphate (αβ-met-ADP; M3763), and pertussis toxin (P7208) were purchased from Sigma-Aldrich. Pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS; 0625) and suramin (1472) were

purchased from Tocris Bioscience. Stock solutions of adenosine (10 mM), AMP (10 mM), αβ-met-ADP (50 μM), PPADS (10 mM), and suramin (10 mM) were made in Hank's balanced salt solution assay buffer (HBSS, Gibco catalog #14025, supplemented with 9 mM HEPES, 11 mM D-glucose, 0.1% fatty-acid free bovine serum albumin, pH 7.3) and frozen at -80°C in single use aliquots. All other compounds were dissolved in HBSS assay buffer at final concentration immediately before use.

Chemical synthesis of deoxyadenosine 5'-monophosphonate (ACP; compound 14)

Other designations for this non-hydrolyzable analog include 5'-deoxyadenosine 5'-methylenephosphonate, 6'-deoxyhomoadenosine-6'-phosphonate or 9-[5,6-dideoxy-6'-(hydroxyphosphinyl)-β-D-ribo-hexofuranosyl]-adenine, CAS Registry Number 22257-15-4 (4,5). ACP is structurally equivalent to AMP but contains a non-hydrolyzable methylene group between the 5' ribose ring position and the phosphorous atom. The charge of the phosphate and phosphonate groups also differ at neutral pH, with ACP having one negative charge and AMP having two. These charges are based on pKa values of 3.8 and 6.2 for the phosphate group of AMP and calculated pKa values of 2.3 and 7.7 for the phosphonate group of ACP (6,7).

HPLC data of all compounds were acquired using an Agilent 6110 Series system with the UV detector set to 220 nm. Samples were injected (<10 μL) onto an Agilent Eclipse Plus 4.6 × 50 mm, 1.8 μM, C-18 column at room temperature. A mobile phase of A being H_2O + 0.1% acetic acid and B being MeOH + 0.1% acetic acid was used. A linear gradient from 10% to 100% B in 5.0 min was followed by pumping 100% B for another 2 minutes with a flow rate of 1.0 mL/min. Mass spectra (MS) data were acquired in positive ion mode using an Agilent 110 single quadrupole mass spectrometer with an electrospray ionization (ESI) source. High-

resolution (negative ion) mass spectra (HRMS) were acquired using a Shimadzu LCMS-IT-TOF time-of-flight mass spectrometer. NMR spectra were recorded on a Varian Mercury spectrometer at 400 MHz for proton (^{1}H NMR) and 100 MHz for carbon (^{13}C NMR); chemical shifts are reported in ppm (δ) relative to the solvent peaks (8). Preparative HPLC was performed using an Agilent Prep 1200 series with the UV detector set to 220 nm. Samples were injected onto a Phenomenex Luna 75 × 30 mm, 5 μM, C-18 column at room temperature. A mobile phase of A being H_2O + 0.1% TFA and B being MeOH was used with a flow rate of 30 mL/min. A linear gradient from 10% to 100% B in 17.0 min was followed by pumping 100% B for another 3 minutes.

Sodium 9-[5',6'-Dideoxy-6'-(hydroxyphosphinyl)-*β*-D-ribo-hexofurnosyl]adenine (ACP; **14**) was synthesized using a convergent route (**Fig. S6**) similar to the published procedure (4), employing dibenzyl ((triphenylphosphoranylidene)methyl)phosphonate in the key oxidation/Wittig reaction sequence (9).

5'-*O*-(4,4'-Dimethoxytrityl)-N^6-benzoyl-D-adenosine (2). To a solution of N^6-benzoyl-D-adenosine (**1**) (2.97 g, 8.0 mmol) in pyridine (40 mL) was added 4,4'-dimethoxytritylchloride (3.25, 9.6 mmol) and the reaction mixture was stirred at rt for 1 h. Ethanol (4 mL) was then added and the volatiles were removed under reduced pressure. The residue was dissolved in chloroform (100 mL), washed with saturated aqueous $NaHCO_3$ (30 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel (10 - 50% ethyl acetate/hexanes gradient) to afford the title compound **2** as a white solid (4.74 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.25 (d, J = 23.5 Hz, 1H), 9.95 (d, J = 46.1 Hz, 1H), 9.30 (dd, J = 111.0, 60.2 Hz, 4H), 8.82 (dd, J = 27.9, 7.2 Hz, 4H), 8.47 (dd, J = 46.6, 16.0 Hz, 6H), 7.98 (d, J = 8.6 Hz, 2H), 7.29 (d, J = 5.6 Hz, 1H), 6.94 (d,

J = 29.2 Hz, 1H), 6.70 (s, 1H), 6.21 – 5.56 (m, 1H), 5.00 (s, 1H), 4.79 – 4.30 (m, 1H), 2.88 (s, 1H). LRMS-ES$^+$ *m/z* (relative intensity) 674 (MH^+, 100).

***N*^{6}-Benzoyl-9-(2',3'-di-*O*-benzoyl-*β*-D-ribofuranosyl)adenine (4).** To a solution of nucleoside **2** (5.34 g, 7.92 mmol) in CH_2Cl_2 (47 mL) were added benzoic anhydride (5.34g, 23.6 mmol) and dimethylaminopyridine (575 mg, 4.72 mmol). The reaction mixture was stirred at rt for 1 h, and ethanol (4 mL) was added. The solution was diluted with chloroform (50 mL), washed with saturated aqueous $NaHCO_3$ (30 mL), 0.5 M aqueous KH_2PO_4 (50 mL) and dried over Na_2SO_4. The volatiles were removed under reduced pressure, the remaining residue was dissolved in chloroform (140 mL) and the solution was cooled to 0 ˚C. A solution of benzenesulfonic acid in methanol (6.67%, 60 mL) was then added and the reaction mixture was stirred at 0 ˚C for 15 min. Following addition of saturated aqueous $NaHCO_3$ (50 mL), the reaction mixture was diluted with chloroform, washed with saturated aqueous $NaHCO_3$ (50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel (10 - 50% ethyl acetate/hexanes gradient) to afford the title compound **4** as a yellow oil (3.96 g, 86 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H), 8.84 (s, 1H), 8.18 – 7.92 (m, 3H), 7.82 (d, *J* = 7.3 Hz, 1H), 7.69 – 7.37 (m, 6H), 7.36 – 7.17 (m, 5H), 6.38 – 6.31 (m, 2H), 6.06 (m, 1H), 4.61 (m, 1H), 4.08 – 3.98 (m, 2H); LRMS-ES$^+$ *m/z* (relative intensity) 580 (MH^+, 100).

Dibenzyl(hydroxymethyl)phosphonate (7). To a mixture of paraformaldehyde (1.9 g, 63.3 mmol) and dibenzyl phosphate (**6**) (15.4 g, 59.0 mmol) was added anhydrous triethylamine (1.0 g, 63.0 mmol), and the reaction mixture was heated for 15 min at 50 ˚C. The reaction was then heated for an additional 2 h at 90 ˚C, cooled to rt and diluted with chloroform (20 mL). The solvent was removed under reduced pressure and the residue was purified by flash column

chromatography on silica gel (CH_2Cl_2 100% - Et_2O 100%) to afford the title compound **7** as a yellow oil (8.64 g, 50%). LRMS-ES^+ *m/z* (relative intensity) 293.1 (MH^+, 100).

(Bis(benzyloxy)phosphoryl)methyl Trifluoromethanesulfonate (8). To a mixture of dibenzyl (hydroxymethyl)phosphonate (**7**) (2.31 g, 7.90 mmol) and 2,6-lutidine (1.01 g, 9.48 mmol) in anhydrous CH_2Cl_2 (50 mL) was added triflic anhydride 1 M solution in CH_2Cl_2 (9.48 mL) at -50 ˚C dropwise. The resulting mixture was allowed to warm to 0 ˚C over 1.5 h and diluted with diethyl ether (100 mL). The precipitates were removed by filtration and the ethereal solution was successively washed with water (50 mL), 1 N aqueous HCl (50 mL), brine (50 mL) and then dried over Na_2SO_4. The solvent was removed under reduced pressure to give the title compound **8** as yellow oil (3.32 g, 99%) which was used in the next step without further purification. NMR conforms to literature values (10); LRMS-ES^+ *m/z* (relative intensity) 425 (MH^+, 100).

((Bis(benzyloxy)phosphoryl)methyl)triphenylphosphonium (9). To a stirred solution of triphenylphosphine (5.94 g, 22.64 mmol) in anhydrous CH_2Cl_2 (100 mL) was added triflate **8** (8.02 mg, 18.87 mmol) in CH_2Cl_2 (100 mL) dropwise at 0 ˚C. The reaction mixture was allowed to warm to rt and stirred for an additional 16 h. The solvent was removed under reduced pressure to about 1/3 of the volume and the remaining mixture was triturated with diethyl ether (200 mL). The resulting white solid was collected by filtration and washed with diethyl ether (2 x 50mL) to yield the title compound **9** as a white solid following recrystallization from ethyl acetate/hexanes (12.0 g, 99%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.07 (m, 25H), 4.93 – 4.72 (m, 2H), 4.26 (dd, J = 20.2, 16.1 Hz, 1H), 3.97 (dd, J = 19.7, 16.0 Hz, 1H), 3.58 – 3.26 (m, 2H); LRMS-ES^+ *m/z* (relative intensity) 538 (MH^+, 100).

Dibenzyl ((triphenylphosphoranylidene)methyl)phosphonate (10). To a suspension of NaH (122 mg, 4.65 mmol) in anhydrous THF (10 mL) was added triphenylphosphonium triflate salt (**9**) (1.00 mg, 1.86 mmol) in anhydrous THF (10 mL) at 0 ˚C. The reaction mixture was stirred at 0 ˚C for 30 min. The solvent was removed under reduced pressure, the residue extracted with anhydrous CH_2Cl_2 (50 mL) and filtered through a sintered funnel. The solvent was removed under reduced pressure and the resulting colorless oil was triturated with hexanes to yield the title compound **10** as an off-white solid (400 mg, 40%). LRMS-ES$^+$ *m/z* (relative intensity) 537.2 (MH^+, 100).

9-[5',6'-Dideoxy-6'-(hydroxyphosphinyl)-*β*-D-ribo-hexofurnosyl]adenine (13). To a solution of N^6-benzoyl-9-(2',3'-di-*O*-benzoyl-*β*-D-ribofuranosyl)adenine (**4**) (100 mg, 0.173 mmol; (11)) in DMSO (3 mL) were added 1,3-dicyclohexylcarbodiimide (142 mg, 0.69 mmol) and pyridinetrifluoride (66.3 mg, 0.35 mmol). The reaction mixture was stirred at rt for 22 h followed by addition of phosphonate **10** (105 mg, 0.196 mmol). Stirring was continued for 24 h. Excess carbodiimide was hydrolyzed by careful addition of a solution of oxalic acid dehydrate (26 mg, 0.206 mmol) in methanol (10 mL). After 10 min, the volatiles were removed under reduced pressure and the formed urea was removed by vacuum filtration. The filtrate was extracted with ethyl acetate (50 mL), the organic phase was washed with brine (2 x 50 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by preparative HPLC chromatography to give **11** (32 mg, 0.038 mmol). To a solution of vinyl phosphonate **11** (32 mg, 0.038 mmol) in methanol (10 mL) was then added Pd-C (10%, 30 mg) and the reaction mixture stirred for 12 h at rt in a pressure vessel under 60 psi of hydrogen gas. After 12 h, the mixture was filtered through Celite to remove the catalyst, the solvent was removed under reduced pressure and 6 N methanolic ammonia (25 mL) was added

at 0 ˚C. The reaction was again sealed in a pressure vessel and stirred for 12 h at rt. The volatiles were removed under reduced pressure and the residue purified *via* preparative HPLC chromatography to give the title compound **13** (10 mg, 22%) as a white solid. NMR conforms to literature values (4); LRMS-ES$^+$ *m/z* (relative intensity) 346 (MH^+, 100).

Sodium 9-[5',6'-Dideoxy-6'-(hydroxyphosphinyl)-*β*-D-ribo-hexofurnosyl]adenine (14) To a solution of 9-[5',6'-Dideoxy-6'-(hydroxyphosphinyl)-*β*-D-ribo-hexofurnosyl]adenine (**13**) (10 mg, 0.034 mmol) in water (1 mL) was added 1 N aqueous NaOH (0.034 mL) and the solvents were removed under reduced pressure to give the title compound **14** (14 mg, 99%) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ 8.52 (s, 1H), 8.57-8.40 (m, 1H), 6.14 (d, J = 5.3 Hz, 1H), 4.94-4.79 (m, 1H), 4.37-4.25 (m, 1H), 4.26 – 4.21 (m, 1H), 4.15-3.96 (m, 1H) 2.16 – 1.99 (m, 2H), 1.99-1.77 (m, 2H); LRMS-ES$^+$ *m/z* (relative intensity) 346 (MH^+, 100).

Cyclic AMP GloSensor Assay

Cyclic AMP determinations were made using a modified GloSensor luciferase detection system (Promega). Low passage, subconfluent HEK293T cells (ATCC CRL-11268) grown in DMEM without phenol red (Gibco #31053) and supplemented with 10% Fetal Bovine Serum (Gibco #26140) were reverse transfected by spotting a calcium phosphate DNA complex mixture containing 12.5 ng each of GloSensor 22F plasmid (Promega #E2301) and human A_1R plasmid in 25 mM HEPES at pH 7.1, 140 mM sodium chloride, 0.75 mM disodium monophosphate and 250 mM calcium chloride. Cells were immediately added at a density of 20,000 cells per well using a Multidrop 384 (Titertek) to 384 well white, clear bottom tissue culture plates (Corning #3707). Cell plates were incubated for 24 hours at 37^{o}C and 5% CO_2. Sixteen point, 1:3 dilutions curves of test compounds starting at 100 μM final concentration were diluted to 4x final

concentration in HBSS (Gibco #14175) supplemented with 2 mM HEPES, pH 7.5 and then added to the cell plates with a Multimek automated liquid handling device (Nanoscreen, Charleston, SC). Following a 10 minute incubation at room temperature, 50 μM 3-isobutyl-1-methylxanthine (Sigma) and 175 nM (-)-isoproterenol hydrochloride (Sigma) were added by Multimek. Seven minutes later, GloSensor cAMP reagent (Promega #E1291) containing 2% luciferin and supplemented with 0.2% NP40 (Tergitol, Sigma) to permeabilize the cells was added by Multimek along with a final 5 μL addition of 100% Ethanol (Decon Labs) to eliminate bubbles. Luminescence was read on an Envision plate reader (Perkin Elmer) for 15 minutes. Data from 95% of Vmax for isoproterenol (~10 minutes post GloSensor reagent addition) were normalized for scale to 100% response equivalent to the response of 1 μM 2-chloro-N^6-cyclopentyladenosine and 0% response equal to the response from the isoproterenol alone.

Cortical Neuron Dissociation and Culture

Embryonic cortical neurons were cultured as previously described (12). Briefly, cortices from ~E16.5 embryos were dissected and digested in dissociation medium (DM) (98 mM Na_2SO_4, 30 mM K_2SO_4, 5.8 mM $MgCl_2$, 0.25 mM $CaCl_2$, 1 mM HEPES, 20 mM D-glucose, 0.125 mN NaOH, and 0.001% phenol red) containing 0.32 mg/mL L-cysteine (Sigma, W326305) and 20 U/mL papain (Roche, 10108014001) at 37°C for 20 minutes with occasional mixing. After digestion, cortices were washed twice with DM containing 1 mg/mL BSA (Sigma, A3912) and 1 mg/mL trypsin inhibitor (Sigma, T9128), followed by incubation in DM containing 10 mg/mL BSA and 10 mg/mL trypsin inhibitor for 2 minutes. Prepared cortices were then suspended in plating media (Neurobasal-A, Gibco, 10888) containing 4.5% FBS, 2% B27 (Gibco, 17504), 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine) and

gently disrupted by pipetting 15-20 times. The dissociated neurons were counted in a hemocytometer and plated at 1 x 10^6 cells/well in polylysine/laminin-coated 6-well plates containing plating media.

Neuron Treatment and cAMP ELISA Assay

After 1 day in vitro, neuron plating media was replaced with serum-free plating media (otherwise identical) for 1 hour. Afterwards, the media was replaced with Neurobasal-A containing 1 mM ACP or 1 μM N^6-cyclopentyladenosine (CPA) (Sigma, C8031) for 30 minutes. For untreated conditions, media was replaced with Neurobasal-A containing no additives. For antagonist conditions, 100 μM 8-cyclopentyl-1,3-dipropylxanthine (CPX) (Tocris, 0439) was added 15 minutes before agonist addition, and agonist solutions also contained 100 μM CPX. Following incubation, forskolin (Sigma, F6886) was added, to a final concentration of 10 μM. After a final 15 minute incubation, the media was aspirated, and the neurons were washed twice in ice-cold Dulbecco's phosphate buffered saline (PBS) (Sigma, D8537). Then, lysis buffer (provided with cAMP ELISA Kit) (R&D Systems, KGE002B) was added, and the neurons were scraped and collected.

The cAMP ELISA assay was performed according to manufacturer's instructions. Briefly, cells were subjected to 2 freeze/thaw cycles from -20°C to room temperature to ensure cell lysis. Cells were then centrifuged at 600 x g for 10 minutes and the supernatant was isolated. Neuron samples and cAMP standards were added to a microplate containing immobilized cAMP antibody, followed by a labeled cAMP conjugate. After incubation and washes, a substrate solution was added, causing a colorimetric reaction proportional to the quantity of bound cAMP conjugate. After incubation, stop solution was added, and absorbance

was measured on a BioTek Synergy HT plate reader. Standard curve fitting and sample analysis was performed using GraphPad Prism.

Immunohistochemistry

HEK293 cells were grown on poly-D-lysine (Sigma, P0899)-coated coverslips and transfected using Lipofectamine Plus with 1 μg (per coverslip) of wild-type or mutant hA_1R. 24 hours after transfection, the cells were washed twice with PBS and fixed for 15 minutes with 4% paraformaldehyde in PBS. The cells were then washed 3 times with PBS for 5 minutes and permeablized with 0.05% Triton-X-100 (Fisher, BP151) in PBS for 20 minutes. The cells were washed three times (5 minutes/wash) and blocked with 5% normal goat serum (NGS) in PBS for 30 minutes. Cells were then incubated with a 1:250 dilution of rabbit anti-hA_1R primary antibody (Santa Cruz Biotechnology, sc-28995) in 10% NGS for 2 hours, and washed three times (10 minutes/wash) with 10% NGS. The cells were then incubated in the dark for 1 hour with a 1:1000 dilution of Alexa 546-conjugated Goat anti-rabbit secondary antibody (Invitrogen, A11010) in 10% NGS. Cells were washed three times with PBS and mounted on glass slides. The following day, the slides were imaged on an Olympus FV1000 confocal microscope. Dissociated cortical neurons were immunostained for mA_1R in the same way, except that incubation with primary antibody was conducted at 4°C overnight.

SUPPLEMENTAL FIGURES

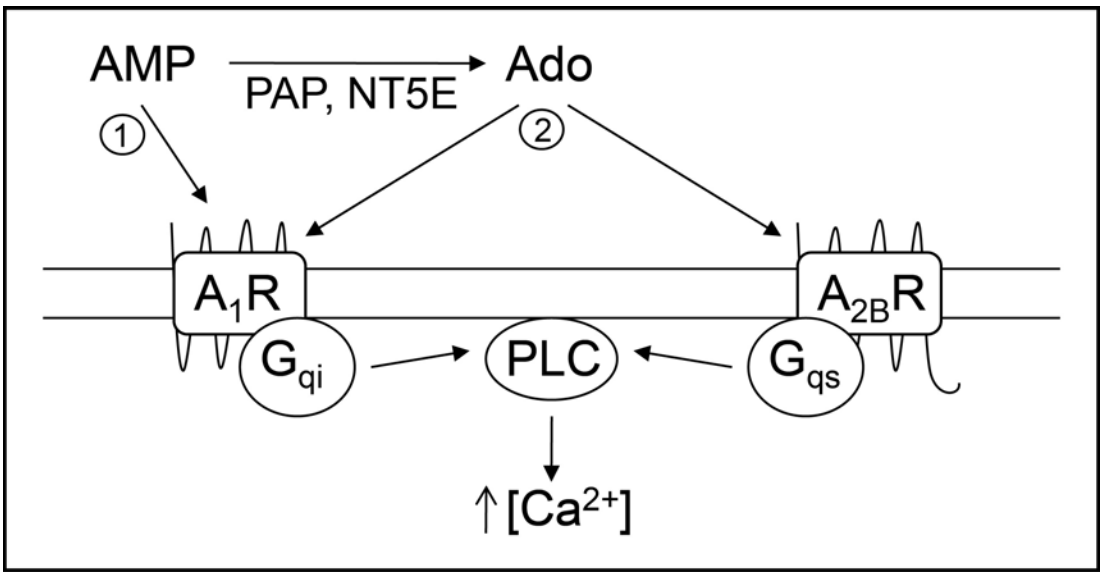

Fig. S1. Real-time visualization of adenosine receptor activation and ectonucleotidase activity. G_i-coupled A_1R and G_s-coupled $A_{2B}R$ do not mobilize intracellular calcium when activated in most cell types, including HEK293 cells. However, when A_1R or $A_{2B}R$ are co-expressed with chimeric G proteins that couple to phospholipase C (PLC; G_{qi}, G_{qs}, respectively), receptor stimulation can be visualized in real-time using the calcium-sensitive dye Fura-2. PAP and NT5E hydrolyze extracellular AMP to adenosine. Real-time visualization allowed us to show that (1) AMP directly activates A_1R whereas (2) AMP activates $A_{2B}R$ indirectly via ectonucleotidase-catalyzed hydrolysis to adenosine.

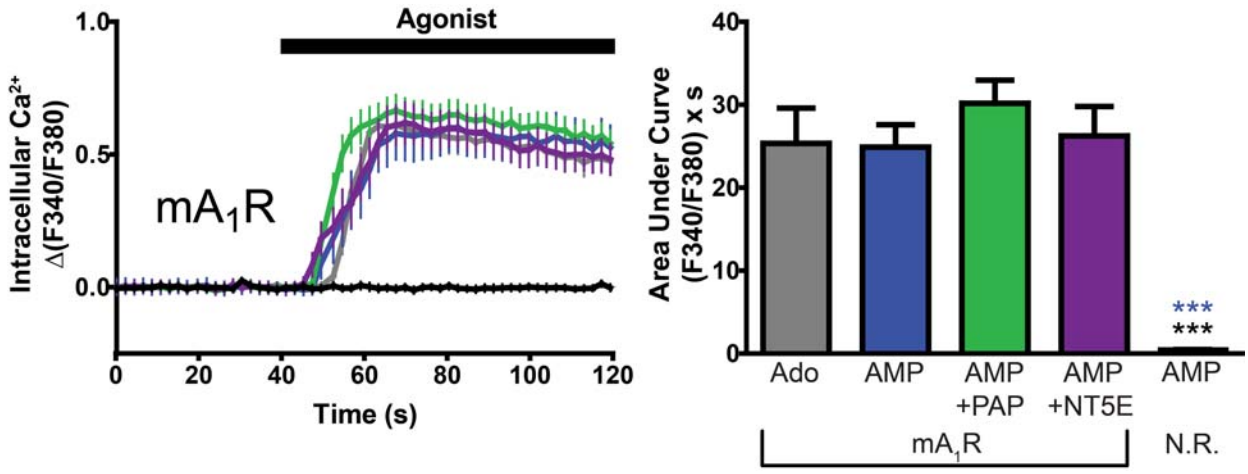

Fig. S2. AMP activates mouse (m)A_1R. Calcium mobilization responses in HEK293 cells expressing Gqi ± mA_1R. Agonists: (Grey) 1 mM adenosine. (Blue) 1 mM AMP. (Green) 1 mM AMP in PAP co-expressing cells. (Purple) 1 mM AMP in NT5E co-expressing cells. (Black) 1 mM AMP in the absence of a transfected adenosine receptor, but in the presence of Gqi. Area under curve (AUC) measurements extended for 1 minute from agonist addition. Paired t tests were used to compare AUC data. Black asterisks, significant difference compared to adenosine stimulation. Blue asterisks, significant difference compared to AMP stimulation (in A_1R-expressing cells). ***$p < 0.0005$. All data are the average of three experiments. n=18-34 cells per condition. All data, including calcium traces, are presented as means ± s.e.m.

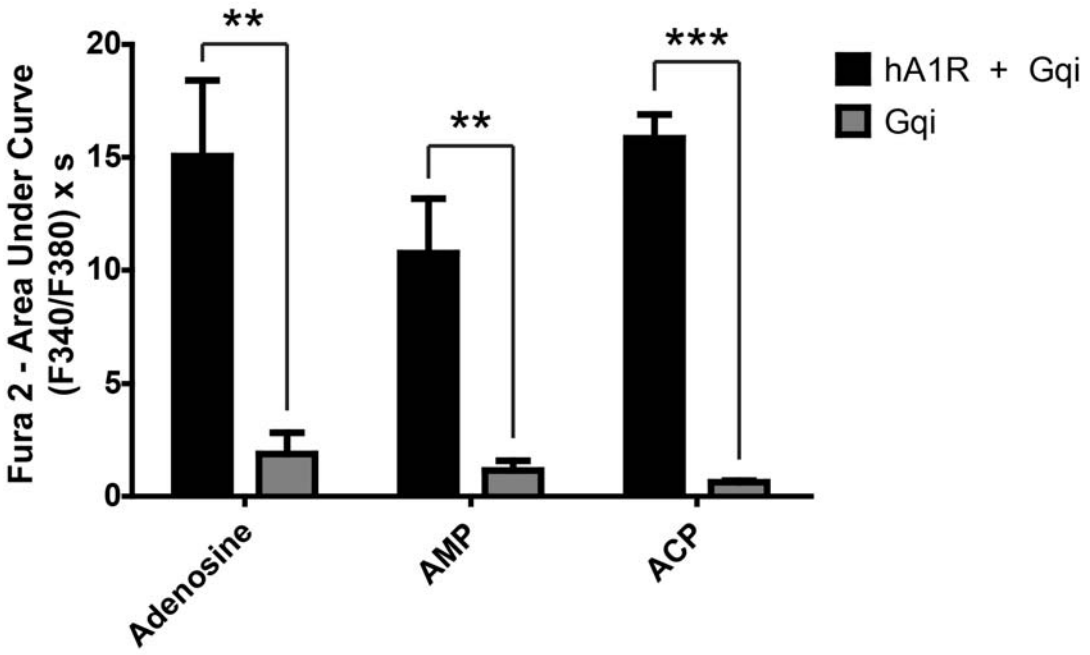

Fig. S3. Adenosine, AMP and ACP stimulate calcium mobilization in COS7 cells. Calcium mobilization in COS7 cells expressing (black) hA_1R + Gqi or (grey) Gqi alone, and stimulated with the indicated compounds (at 1 mM). AUC measurements extended for 1 minute from agonist addition. Paired t tests were used to compare AUC data in the presence and absence of transfected hA_1R, $^{**}p < 0.005$, $^{***}p < 0.0005$. All experiments performed in duplicate. n=15-21 cells per condition. All data are presented as means ± s.e.m.

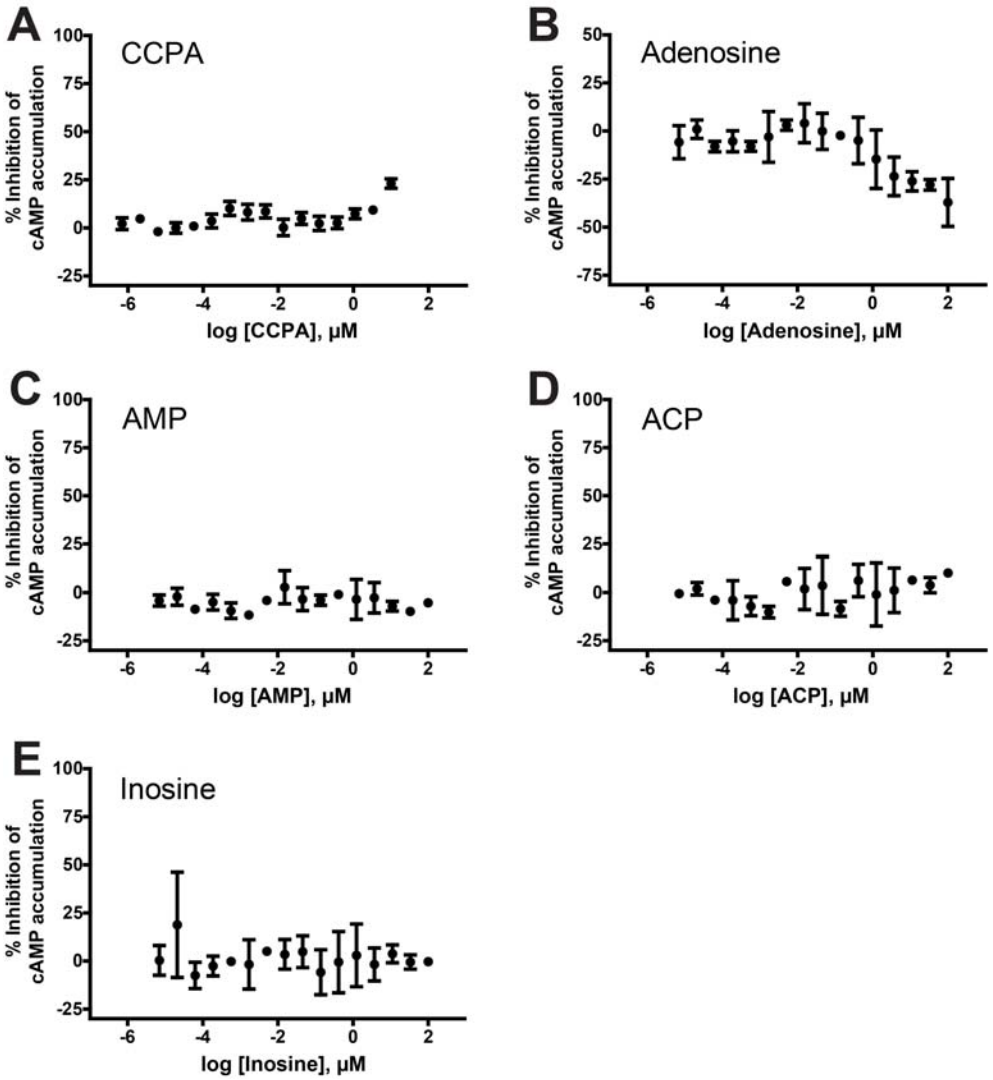

Fig. S4. Adenosine, AMP and related analogs do not inhibit cAMP accumulation in cells lacking hA_1R. HEK293T cells were co-transfected with empty expression vector and GloSensor 22F plasmid then were stimulated with (A) CCPA, (B) adenosine, (C) AMP, (D) ACP, and (E) inosine. Cells were incubated with test compound for 10 minutes, then 175 nM (-)-isoproterenol was added for 7 minutes to stimulate cAMP accumulation. Following incubation, GloSensor cAMP reagent was added and luminescence was measured. All data were normalized such that 0% is equal to the response from isoproterenol alone. All experiments performed in duplicate. All data are presented as means ± standard deviation.

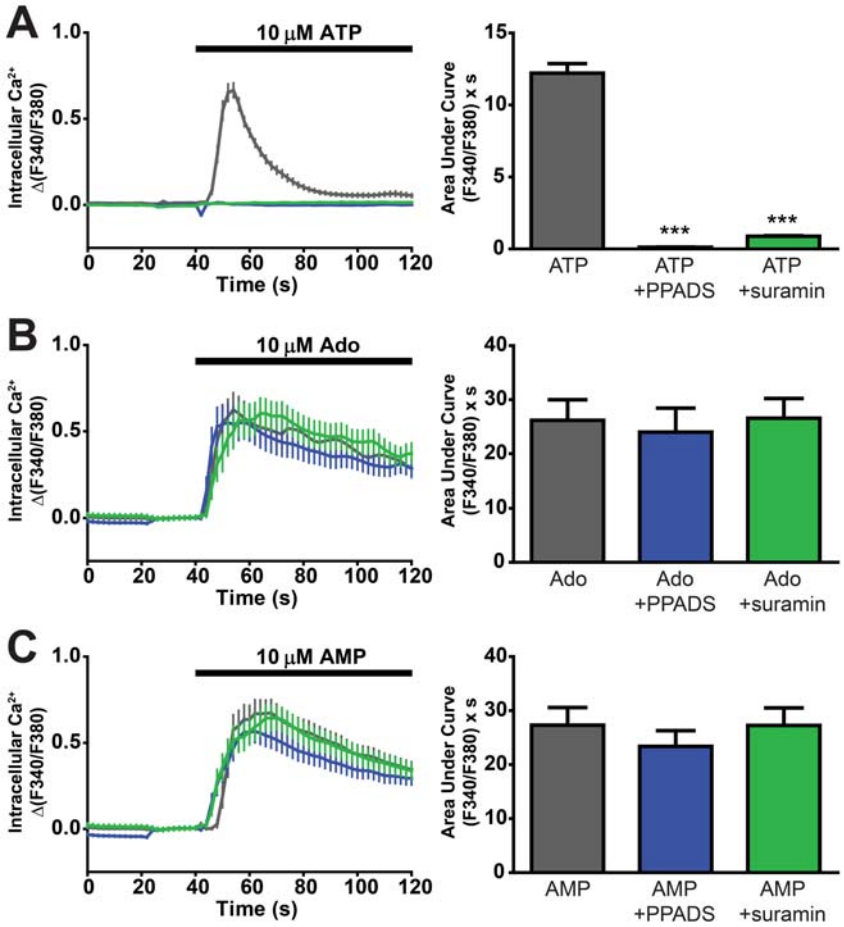

Fig. S5. AMP activates hA$_1$R independent of P2Y receptor activity. (A) Calcium mobilization responses in untransfected HEK293 cells stimulated with 10 μM ATP in the absence or presence of P2Y receptor antagonists (PPADS or suramin). (B, C) Calcium mobilization responses in HEK293 cells expressing hA$_1$R + Gqi following stimulation with (B) 10 μM adenosine or (C) 10 μM AMP, in the absence or presence of 100 μM PPADS or 100 μM suramin. Cells were incubated in 100 μM PPADS/suramin for 3 minutes prior to experiments. AUC measurements extended for 1 minute from agonist addition. Paired t tests were used to compare AUC data relative to agonist alone, ***$p < 0.0005$. All data are the average of (A) one or (B, C) two experiments performed in duplicate. n=21-74 cells per condition. All data, including calcium traces, are presented as means ± s.e.m.

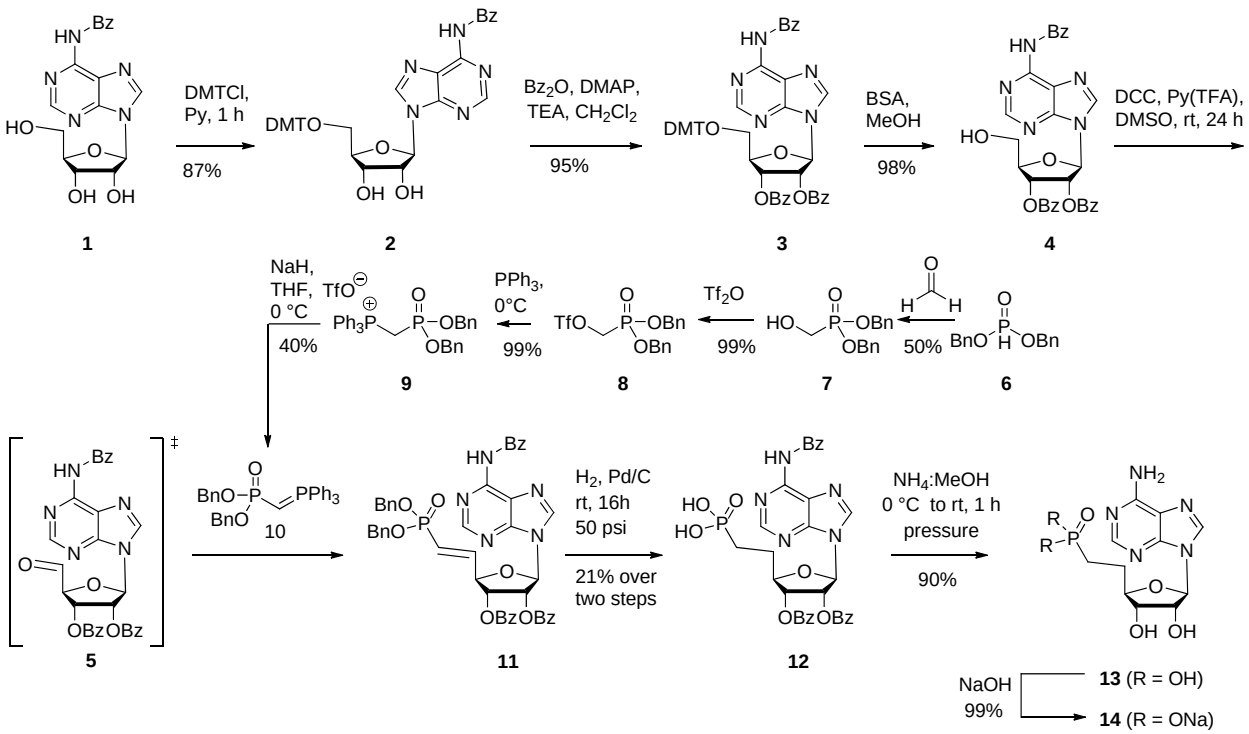

Fig. S6. Chemical synthesis of deoxyadenosine 5'-monophosphonate (ACP; compound 14).

SUPPLEMENTAL REFERENCES

1. Zylka, M. J., Sowa, N. A., Taylor-Blake, B., Twomey, M. A., Herrala, A., Voikar, V., and Vihko, P. (2008) *Neuron* **60**, 111-122
2. Zhang, J. Y., Nawoschik, S., Kowal, D., Smith, D., Spangler, T., Ochalski, R., Schechter, L., and Dunlop, J. (2003) *Eur J Pharmacol* **472**, 33-38
3. Coward, P., Chan, S. D., Wada, H. G., Humphries, G. M., and Conklin, B. R. (1999) *Anal Biochem* **270**, 242-248
4. Koh, Y. H., Shim, J. H., Wu, J. Z., Zhong, W., Hong, Z., and Girardet, J. L. (2005) *J Med Chem* **48**, 2867-2875
5. Naito, Y., and Lowenstein, J. M. (1985) *Biochem J* **226**, 645-651
6. McElroy, W. D., and Glass, B. (1951) *Phosphorus metabolism*, Johns Hopkins University Press, Baltimore
7. Shelley, J. C., Cholleti, A., Frye, L. L., Greenwood, J. R., Timlin, M. R., and Uchimaya, M. (2007) *J Comput Aided Mol Des* **21**, 681-691
8. Gottlieb, H. E., Kotlyar, V., and Nudelman, A. (1997) *J Org Chem* **62**, 7512-7515
9. Suk, D. H., Bonnac, L., Dykstra, C. C., Pankiewicz, K. W., and Patterson, S. E. (2007) *Bioorg Med Chem Lett* **17**, 2064-2067
10. Xu, Y., Flavin, M. T., and Xu, Z. Q. (1996) *J Org Chem* **61**, 7697-7701
11. Urata, H., Hara, H., Hirata, Y., Ohmoto, N., and Akagi, M. (2005) *Tetrahedron: Asymmetry* **16**, 2908-2917
12. Polleux, F., and Ghosh, A. (2002) *Sci STKE* **2002**, pl9