Agonist-dependent coupling of the promiscuous adenosine A_{2B} receptor to Gα protein subunits

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Table S1: Potency and efficacy of $A_{2B}AR$ agonists and CCh at HEK cells recombinantly expressing $G\alpha_{q/11}$ proteins determined in calcium mobilization assays.

Cell line	**HEK-$G\alpha_q$**	**HEK-$G\alpha_{11}$**	**HEK-$G\alpha_{14}$**	**HEK-$G\alpha_{15}$**
Adenosine				
Potency (pEC_{50})	4.84 ± 0.17[b]	partial activation	partial activation	5.52 ± 0.29[c]
Efficacy (AU)	4398 ± 1290[b]	1800 ± 350[a]	3570 ± 680[b]	6480 ± 410[c]
NECA				
Potency (pEC_{50})	4.37 ± 0.34[b]	n.a.	n.a.	5.79 ± 0.21[c]
Efficacy (AU)	4820 ± 840[b]	n.a.	n.a.	5450 ± 900[c]
BAY 60-6583				
Potency (pEC_{50})	n.a.	n.a.	n.a.	n.a.
Efficacy (AU)	n.a.	n.a.	n.a.	n.a.
CCh				
Potency (pEC_{50})	6.61 ± 0.24	6.55 ± 0.18	5.12 ± 0.83	5.28 ± 0.32[a]
Efficacy (AU)	6160 ± 1370	6790 ± 890	8060 ± 2930	4750 ± 900[a]

Data are presented as means ± SEM from 3 independent experiments performed in duplicates if not otherwise indicated.
[a] Data from four independent experiments.
[b] Data from five independent experiments.
[c] Data from six independent experiments.
n.a. no activation at the highest tested concentration

Table S2: Potency (pEC_{50}) of $A_{2B}AR$ agonists at Gα proteins determined by TRUPATH BRET2 assays.

TRUPATH Biosensor	Adenosine	NECA -ADA	NECA +ADA	BAY -ADA	BAY 60-6583 + ADA
$G\alpha_s$ subfamily					
$G\alpha_{s\text{-}s}$	4.99 ± 0.22	6.73 ± 0.18	6.50 ± 0.19[c]	7.46 ± 0.55	7.91 ± 0.45[a]
$G\alpha_{i/o}$ subfamily					
$G\alpha_{i1}$	5.19 ± 0.20	n.d.	7.97 ± 0.30	n.d.	n.a., <5
$G\alpha_{i2}$	5.38 ± 0.09	n.d.	7.43 ± 0.22	n.d.	n.a., <5
$G\alpha_{i3}$	5.58 ± 0.07	n.d.	8.00 ± 0.28	n.d.	n.a., <5
$G\alpha_{oA}$	5.18 ± 0.19[a]	n.d.	6.36 ± 0.71[a]	n.d.	n.a., <5
$G\alpha_{oB}$	5.39 ± 0.11[a]	n.d.	6.29 ± 0.49[b]	n.d.	n.a., <5
$G\alpha_{gust}$	n.a., <4	n.d.	n.a., <4	n.d.	n.a., <5
$G\alpha_{z}$	6.19 ± 0.48[a]	n.d.	7.17 ± 0.28[a]	n.d.	n.a., <5
$G\alpha_q$ subfamily					
$G\alpha_{q}$	6.48 ± 0.34[a]	7.05 ± 0.63[c]	5.90 ± 0.29[c]	n.a., <5	n.a., <5
$G\alpha_{11}$	5.85 ± 0.32[b]	7.89 ± 0.55[c]	6.90 ± 0.39[d]	n.a., <5	n.a., <5
$G\alpha_{15}$	6.51 ± 0.32[d]	7.56 ± 0.25[d]	8.67 ± 0.23[b]	7.14 ± 0.69[c]	9.23 ± 0.08
$G\alpha_{12/13}$ subfamily					
$G\alpha_{12}$	5.66 ± 0.03	n.d.	7.06 ± 0.48[a]	n.d.	8.61 ± 0.24[a]
$G\alpha_{13}$	5.66 ± 0.17	n.d.	6.67 ± 0.36[b]	n.d.	n.a., <5

Data are presented as means ± SEM from three independent experiments (unless otherwise noted) performed in duplicates.
pEC_{50} values calculated from experiments conducted with NECA and BAY 60-6583 were performed in the presence of ADA.
[a] Data from four independent experiments.
[b] Data from five independent experiments.
[c] Data from six independent experiments.
[d] Data from seven independent experiments.
n.a., no activation at the highest tested concentration
n.d., not determined

Table S3: Efficacy (E_{max}, % of maximum activation of a model receptor) of A_{2B}AR agonists at respective Gα proteins determined by TRUPATH BRET2 assays.

TRUPATH Biosensor	Adenosine	NECA -ADA	NECA +ADA	BAY -ADA	BAY 60-6583 + ADA
$G\alpha_s$ subfamily					
$G\alpha_{s\text{-}s}$	120 ± 15	110 ± 23	63 ± 6[c]	113 ± 38	32 ± 12[a]
$G\alpha_{i/o}$ subfamily					
$G\alpha_{i1}$	22 ± 5	n.d.	36 ± 6	n.d.	n.a., <10
$G\alpha_{i2}$	49 ± 18	n.d.	76 ± 12	n.d.	n.a., <10
$G\alpha_{i3}$	29 ± 2	n.d.	27 ± 4	n.d.	n.a., <10
$G\alpha_{oA}$	28 ± 4[a]	n.d.	33 ± 9[a]	n.d.	n.a., <10
$G\alpha_{oB}$	44 ± 4[a]	n.d.	37 ± 3[b]	n.d.	n.a., <10
$G\alpha_{gust}$	n.a., <4	n.d.	n.a., <4	n.d.	n.a., <10
$G\alpha_{z}$	47 ± 11[a]	n.d.	44 ± 11[a]	n.d.	n.a., <10
$G\alpha_{q/11}$ subfamily					
$G\alpha_{q}$	25 ± 6[a]	29 ± 6[c]	22 ± 2[c]	n.a., <10	n.a., <10
$G\alpha_{11}$	34 ± 4[b]	28 ± 3[c]	22 ± 4[d]	n.a., <10	n.a., <10
$G\alpha_{15}$	88 ± 24[d]	49 ± 9[c]	95 ± 18[b]	64 ± 20[c]	131 ± 13
$G\alpha_{12/13}$ subfamily					
$G\alpha_{12}$	64 ± 10	n.d.	51 ± 10[a]	n.d.	23 ± 2[a]
$G\alpha_{13}$	18 ± 6	n.d.	31 ± 2[b]	n.d.	n.a., <10

E_{max} values were calculated by dividing the maximum ΔBRET shift measured for the A_{2B}AR by the maximum ΔBRET shift observed by a control receptor (β_2 adrenoceptor for the $G\alpha_s$ family, μ opioid receptor for the $G\alpha_{i/o}$ family, neurotensin 1 receptor for the $G\alpha_{q/11}$ and $G\alpha_{12/13}$ family). Means ± SEM from three independent experiments unless otherwise noted.

[a] Data from four independent experiments.

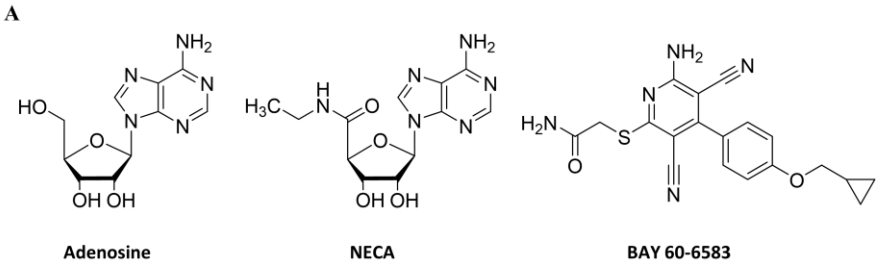
[b] Data from five independent experiments.

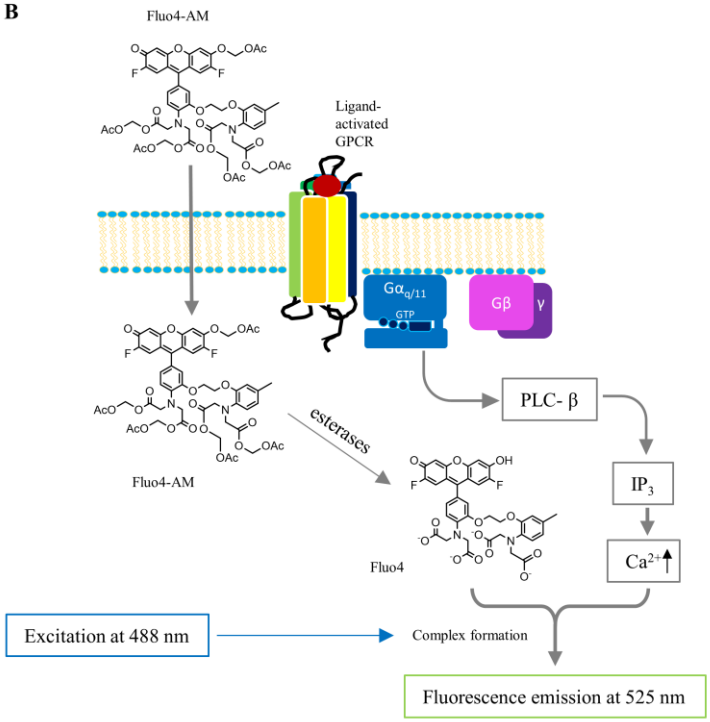
[c] Data from six independent experiments.

[d] Data from seven independent experiments.

n.a., no activation at the highest tested concentration

n.d., not determined


A
Adenosine
NECA
BAY 60-6583


B
Fluo4-AM
Ligand-activated GPCR
Gαq/11
GTP
Gβ
γ
PLC- β
esterases
Fluo4-AM
IP3
Ca2+
Fluo4
Excitation at 488 nm
Complex formation
Fluorescence emission at 525 nm

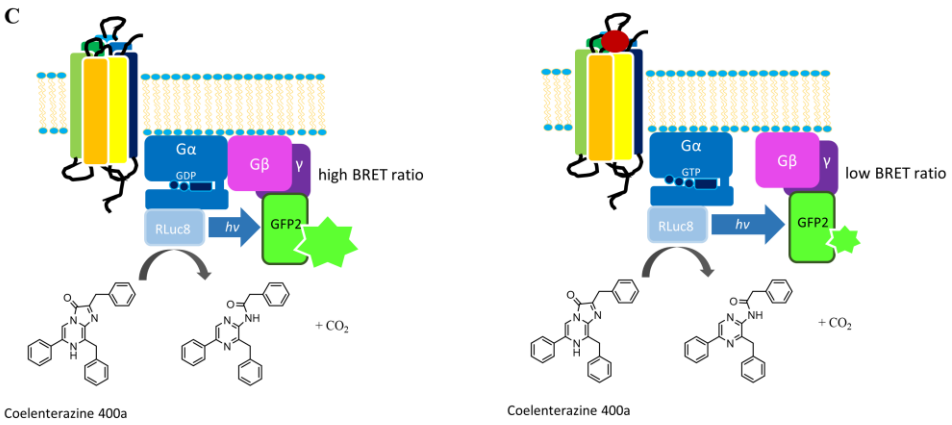

C
Gα
GDP
Gβ
γ
high BRET ratio
RLuc8
hv
GFP2
+ CO2
Coelenterazine 400a
Gα
GTP
Gβ
γ
low BRET ratio
RLuc8
hv
GFP2
+ CO2
Coelenterazine 400a

Figure S1: Structures of the investigated A_{2B}AR agonists, and assay principles of the calcium mobilization assay and the TRUPATH BRET2 assay. **A**. Structural formulas of investigated A_{2B}AR agonists. **B**. Calcium mobilization assay: Fluo-4-acetoxymethylester (Fluo-4-AM) penetrates the cell membrane. In the cytosol, the ester bonds are cleaved by esterases and the resulting anionic Fluo-4 is thereby trapped in the cytosol. Upon GPCR activation, $G_{q/11}$-family proteins release GDP and bind GTP instead, leading to calcium release from the endoplasmic reticulum via the phospholipase C-β (PLC-β) – inositol trisphosphate (IP_3) pathway. Free Ca^{2+} binds to Fluo-4 forming a fluorescent complex, which allows the monitoring of changes in the intracellular calcium concentration by fluorescence measurement at an emission wavelength of 520 nm after excitation of the complex at 488 nm. **C**. TRUPATH BRET2 assay: in the inactive receptor state, the RLuc8-tagged Gα subunit is bound to GDP and associated to the Gβγ dimer. The RLuc8 domain oxidizes coelenterazine 400a, emitting a constant signal of blue light, which in turn excites the proximate Gγ-GFP2 fusion protein resulting in a high BRET2 ratio (luminescence / fluorescence). In the active receptor state, the active, GTP-bound Gα-RLuc8 fusion protein separates from the Gβγ-GFP2 dimer. Due to the increased distance between RLuc8 and GFP2, energy transfer is reduced and the BRET2 ratio decreases.

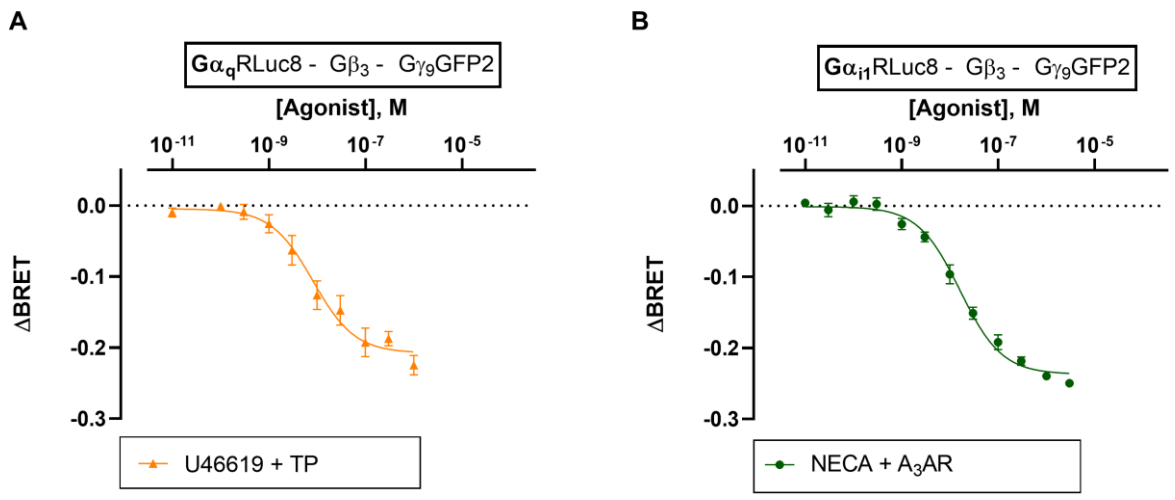


Figure S2: Establishment of TRUPATH assay: concentration-response curves of standard agonists at control receptors. **A**. $G\alpha_q$ biosensor, **B**. $G\alpha_{i1}$ biosensor, transiently expressed in HEK293 cells together with the investigated control GPCR. *p*EC_{50} values: $G\alpha_q$ - U46619 - TP = 8.02; $G\alpha_{i1}$ – NECA - A_3AR = 7.72. Data points are means ± SEM of three independent experiments performed in duplicates.

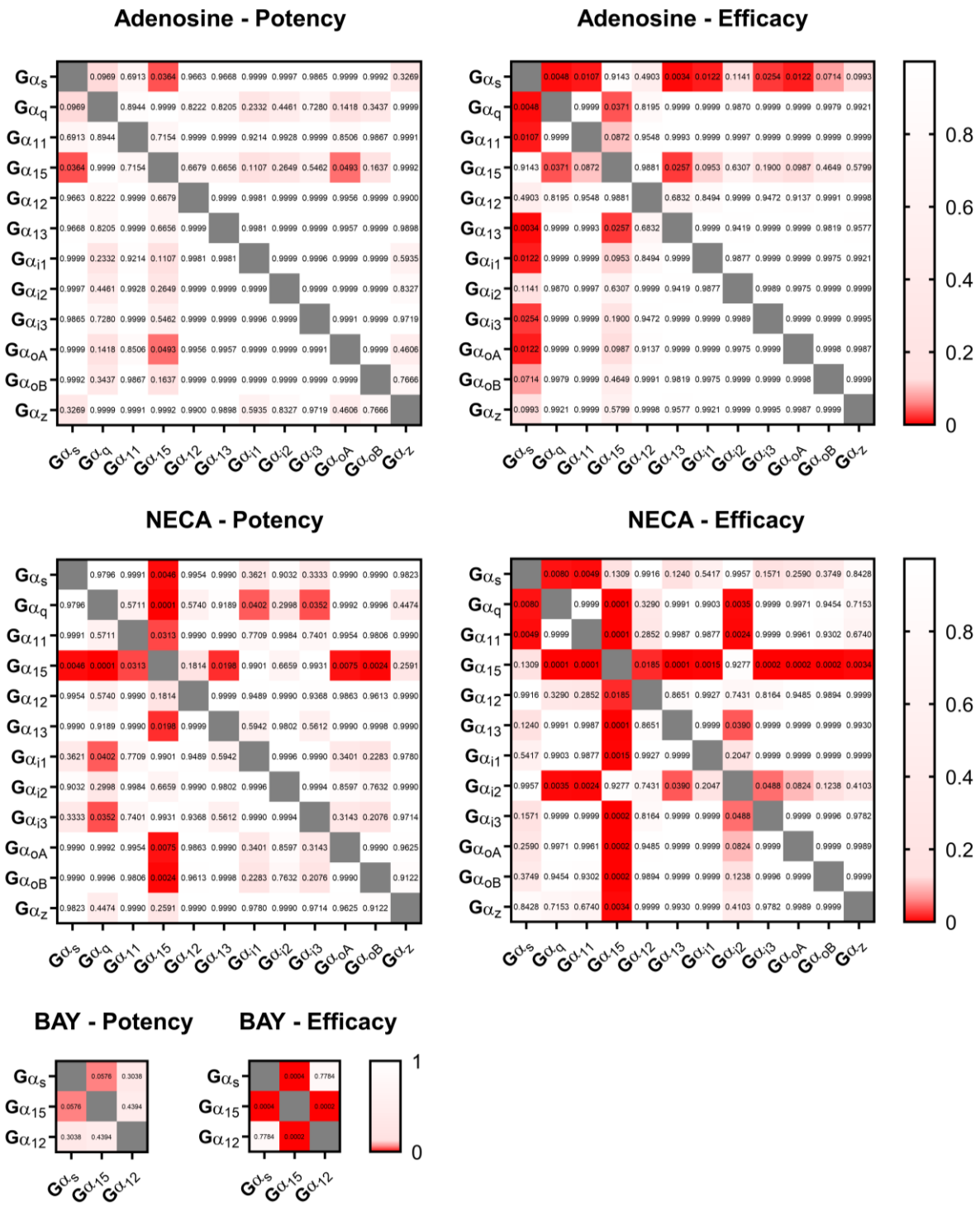


Figure S3: Statistical analysis of potency and efficacy values obtained from TRUPATH BRET2 assays. P-values were obtained from one-way analysis of variance (corrected for multiple comparisons using Turkey's test) comparing mean potencies and efficacies of adenosine (**A**, **B**), NECA (**C**, **D**), and BAY 60-6583 (**E**, **F**) at each investigated Gα subunit. High significance levels between means are indicated in red color.