Supplementary Information

GPCRs regulate the assembly of a multienzyme complex for purine biosynthesis

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1. Supplementary Methods

**DMR profiling of HeLa cells using small molecules.** To screen functional GPCRs in HeLa cells, a library of GPCR agonists consisting of 113 known GPCR agonists and two control compounds (forskolin and IBMX) was assembled. These agonists include endogenous and synthetic agonists, covering > 150 known GPCRs. Forskolin is an activator for adenylyl cyclases, and IBMX is non-selective phosphodiesterase inhibitors. Each compound with the highest possible purity was purchased individually from commercial vendors (**Supplementary Table 1**). All compounds were dissolved in DMSO, except for epinephrine, ATP, ADP, UTP and UDP which were dissolved in water. The compound storage plate was made by dispensing 2 µl of corresponding solution at a desired concentration into 96well deep volume compound source plates (Corning Inc.). The storage concentration was 10 mM for small organic molecules, and 1 mM for peptides and lipid molecules. All compound storage plates were stored at -80 °C. For screening, all compounds were directly diluted using 1x HBSS assay buffer, except for peptide and lipid molecules which were diluted using 1x HBSS containing 0.25% bovine serum albumin. All compound solutions were then transferred into 384well compound source plates (Corning Inc). The screening dose was 10 µM for small organic molecules and 1 µM for both peptide and lipid agonists. At least two independent measurements were carried out. For each independent measurement, each compound was assayed in duplicate. The rest 154 wells were used as controls. The real time DMR responses were obtained for each compound.

**Supplementary Table 2** lists the information of all other compounds used in the present study.

**RNA Interference.** A small interfering RNA (siRNA) expression system was prepared using the psiRNAhH1GFPzeo vector (InvivoGen) according to the manufacturer’s protocol. To
specifically knock down the human CK2α catalytic subunit in HeLa cells, a DNA sequence of 5'-GTACCAGACGTTAACAGACTA-3' (siCK2α-1) generating short hairpin RNAs were inserted into the siRNA expression vector using restriction enzymes Acc65I and HindIII. siRNA transfection was monitored by a GFP marker present in the plasmid under fluorescence microscopy. The mock transfection was used as the controls. The efficiency of the siRNA knockdown was examined using immunoblotting of HeLa lysates obtained after 24 hr transfection and lysed in mammalian protein extraction reagent solution (Pierce) containing both protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Roche). Cell lysates then were loaded on SDS-PAGE gels and enhanced chemiluminescence signals were captured with the Fluor-S imaging system (Bio-Rad). The Western blotting was probed with commercially available anti-hCK2α antibody (C-18; Santa Cruz Biotechnology).

**Supplementary Table 1.** Information of a compound library used for identification of receptors whose signaling regulates purinosome dynamics.

| Compound name                          | Receptor                  | Source | Catalog # | M.W.   |
|----------------------------------------|---------------------------|--------|-----------|--------|
| Serotonin                              | 5-HT receptors            | Sigma  | H9523     | 212.68 |
| (S)-WAY 100135 dihydrochloride         | 5-HT1A                    | Tocris | 1253      | 468.47 |
| Ipsapirone                             | 5-HT1A                    | Tocris | 1869      | 401.48 |
| L-694247                               | 5-HT1D                    | Tocris | 781       | 411.48 |
| LY 334370 hydrochloride                | 5-HT1F                    | Tocris | 3079      | 387.88 |
| DOI hydrochloride                      | 5-HT2A, 5-HT2B, 5HT2C     | Tocris | 2643      | 357.62 |
| Ro 60-0175 fumarate                    | 5-HT2B, 5-HT2C            | Tocris | 1854      | 342.75 |
| RS 67506 hydrochloride                 | 5-HT4                     | Tocris | 990       | 454.41 |
| CPA (2-Chloro-N6-cyclopentyl-adenosine)| Adenosine A1 receptor     | Sigma  | A9251     | 267.24 |
| CGS 21680                              | Adenosine A2A receptor    | Tocris | 1063      | 535.99 |
| IB-MECA                                | Adenosine A3 receptor     | Tocris | 1066      | 510.29 |
| Adenosine                              | Adenosine receptors       | Sigma  | A9251     | 267.24 |
| Forskolin                              | Adenylyl cyclases         | Tocris | 1099      | 410.51 |
| (R)-(−)-Phenylephrine hydrochloride    | Adrenergic α1 receptors   | Tocris | 2838      | 203.67 |
| Compound                                      | Receptor Type                  | Supplier    | EC50  |
|-----------------------------------------------|--------------------------------|-------------|-------|
| A 61603 hydrobromide                          | Adrenergic α1A receptor        | Tocris      | 1052  |
| Clonidine                                     | Adrenergic α2 receptors        | Sigma       | 1660  |
| Salmeterol                                    | Adrenergic β2 receptor         | Tocris      | 1660  |
| (-)-epinephrine                               | Adrenergic receptors           | Sigma       | 2450  |
| (R)-(+-)-m-Nitrobiphenyline                   | Adrenergic α2C receptor        | Tocris      | 2948  |
| Adrenomedullin                                | Adrenomedullin receptor        | Bachem      | 2569  |
| Amylin                                        | Amylin receptor                | Bachem      | 2569  |
| L-162313                                      | Angiotensin AT1 receptor       | Sigma       | 582.78|
| Angiotensin                                   | Angiotensin receptors          | Bachem      | 1296.5|
| CGP 42112                                     | Angiotensin AT2 receptor       | Tocris      | 1052.2|
| Apelin                                        | APJ receptor                   | Bachem      | 1550.85|
| Neuramomin B                                  | BB1 receptor                   | Bachem      | 1132.31|
| Bombesin                                      | BB1, BB2, BB3                  | Bachem      | 1619.87|
| Gastrin releasing peptide (GRP) (human)       | BB2 receptor                   | Bachem      | 2859.42|
| Leukotriene B4                                | BLT1, BLT2                     | Tocris      | 336.47|
| Bradykinin                                    | Bradykinin receptors           | Bachem      | 1060.22|
| (Trp^63, Trp^64)-C3a(63-77)                   | C3A receptor                   | Bachem      | 1820.17|
| (Tyr^65, Phe^67)-C5a (65-74)                  | C5A receptor                   | Bachem      | 1244.44|
| Calcitonin                                    | Calcitonin receptor            | Bachem      | 3417.90|
| ACEA                                          | Cannabinoid CB1 receptor       | Tocris      | 365.99|
| Anadamide                                     | Cannabinoid CB2 receptor       | Tocris      | 347.54|
| CB65                                          | Cannabinoid CB2 receptor       | Tocris      | 417.93|
| spermidine                                    | Ca-sensing receptor            | Sigma       | 145.25|
| spermine                                      | Ca-sensing receptor            | Sigma       | 202.34|
| Cholecystokinin-33 (human)                    | CCK1, CCK2                     | Bachem      | 3945.45|
| ZK 756326                                     | CCR8 chemokine receptor        | Tocris      | 429.38|
| α-Calctonin gene-related peptide (human)      | CGRP                           | Tocris      | 3789.33|
| A-71623                                       | Cholecystokinin CCK1 receptor  | Tocris      | 840.97|
| Corticotropin-releasing factor                 | CRF1, CRF2                     | Tocris      | 4758.00|
| R(+)-SKF38393                                 | D1-like dopamine receptor      | Tocris      | 336.23|
| Cabergoline                                   | D2-like dopamine receptor      | Tocris      | 451.6 |
| Dopamine hydrochloride                        | Dopamine receptors             | Sigma       | 189.64|
| Endothelin-1                                  | ET₆, ET₈                      | Bachem      | 2491.94|
| N-Formyl-Met-Leu-Phe                          | Formyl peptide receptor 1      | Tocris      | 437.55|
| WKYMVM                                        | Formyl peptide receptors       | Tocris      | 855.41|
| SKF 97541                                     | GABAab                         | Tocris      | 137.12|
| GABA (γ-aminobutyric acid)                    | GABAab                         | Tocris      | 103.12|
| Galanin                                       | GAL1, GAL2                     | Bachem      | 3157.45|
| Name                                      | Receptor                           | Company    | Code | Price   |
|-------------------------------------------|------------------------------------|------------|------|---------|
| Ghrelin                                   | Ghrelin receptor                   | Tocris     | 1463 | 3370.90 |
| L-692,585                                 | GHRH receptor                      | Bachem     | H-3112 | 5107.84 |
| Growth hormone-releasing factor (ovine)   | GIP receptor                       | Tocris     | 2257 | 4633.21 |
| Gastric inhibitory polypeptide (1-39)    | GLP1, GLP2 receptor                | Tocris     | 1851 | 4169.52 |
| Glucagon                                  | Glucagon receptor                  | Bachem     | H6790.0001 | 3482.80 |
| Nafarelin                                 | GPR109 receptor agonist            | Tocris     | 2544 | 1322.00 |
| Nicotinic acid                            | GPR109A, GPR109B                   | Tocris     | 1762 | 218.21  |
| ICI182780                                 | GPR30                              | Tocris     | 1047 | 606.77  |
| β-Estradiol 17-cypionate                  | GPR30                              | BioMOL     | E8004 | 396.56  |
| NPPB                                      | GPR40                              | Sigma      | E4637 | 282.46  |
| Elaidic acid                              | Histamine H1, H2 receptors         | Tocris     | 646  | 614.57  |
| HTMT dimaleate                            | Histamine H2 receptor              | Tocris     | 668  | 319.06  |
| Amphetamine dihydrobromide                | Histamine H3, H4 receptor          | Tocris     | 729  | 332.06  |
| Imetit dihydrobromide                     | Histamine H4 receptor              | Tocris     | 2342 | 198.09  |
| 4-Methylhistamine dihydrochloride         | Histamine receptors                | Sigma      | H7125 | 111.15  |
| Histamine                                 | Histamine receptors                | Sigma      | H4437 | 1302.45 |
| Kisspeptin 10 (human)                     | KISS1 receptor (GPR54)             | Tocris     | 1067 | 322.19  |
| Oleoyl-L-α-lysophosphatidic acid sodium   | LPA1, LPA2, LPA4                   | Sigma      | L7260 | 436.52  |
| α-Melanotropin (human)                     | MC1, MC2, MC3, M4, MC5             | Bachem     | H-1075 | 1664.91 |
| Melanin-concentrating hormone             | MCH1, MCH2                         | Phoenix    | 070-47 | 2385.10 |
| L-aspartate acid                          | mGlur receptors                    | Sigma      | A9506 | 144.25  |
| L-Glutamic acid                           | mGlur receptors                    | Tocris     | 218  | 147.13  |
| L-Cysteinesulfonic acid                   | mGlur1a and mGlur5a                | Tocris     | 216  | 153.15  |
| L-serine-O-phosphate                      | mGLUR4 receptor                    | Tocris     | 238  | 185.07  |
| Motilin                                   | MOT receptor                       | Tocris     | 2264 | 2699.07 |
| Melatonin                                 | MT1, MT2, MT3                      | Bachem     | Q-1300 | 232.28 |
| McN-A 343                                 | Muscrunic M1 receptor              | Tocris     | 1384 | 317.21  |
| Acetylcholine chloride                    | Muscrunic M1-M5 receptor           | Sigma      | A2661 | 181.66  |
| Oxtremorine M                             | Muscrunic M1-M5 receptors          | Tocris     | 1067 | 322.19  |
| Substance P                               | Neurokinnin receptor NK1           | Bachem     | H1890 | 1347.65 |
| Neurokinnin A                             | NK2, NK3                           | Bachem     | H3745 | 1133.34 |
| Neuromedin U                              | NMU1, NMU2                         | Bachem     | H-5538 | 3080.42 |
| Neuropeptide B (NPB-23)                   | NPBW1, NPBW2                      | Phoenix    | 005-53 | 2348.66 |
| Neuropeptide Y                            | NPY1, Y2, Y4, Y5, Y6              | Bachem     | H6375 | 4271.74 |
| Neutropeptide                             | NTS1, NTS2                         | Bachem     | h4435 | 1672.95 |
| Drug Name                              | Receptor Type                                      | Vendor     | Catalog No. | Purity |
|----------------------------------------|----------------------------------------------------|------------|-------------|--------|
| SB 205607 dihydrobromide               | Opioid delta receptor                              | Tocris     | 921         | 506.28 |
| Endomorphin 1                          | Opioid mu receptor                                 | Bachem     | H-4002      | 610.71 |
| Dynorphin A                            | Opioid receptors                                   | Bachem     | H2620       | 2147.52|
| Nociceptin/orphanin FQ                 | ORL1                                               | Bachem     | H3036       | 1809.06|
| Orexin-A                               | OX1, OX2 receptors                                 | Tocris     | 1455        | 3561.12|
| 5-oxo-ETE                              | Oxoeicosanoid receptor                             | Tocris     | 1796        | 318.46 |
| ADP                                    | P2Y1, 12, 13                                       | Sigma      | A2754       | 427.20 |
| ATP                                    | P2Y2                                              | Sigma      | A6419       | 551.14 |
| UTP                                    | P2Y4                                              | Sigma      | U6875       | 484.14 |
| UDP                                    | P2Y6                                              | Sigma      | U4125       | 404.16 |
| Platelet activating factor PAF (C16)   | PAF receptor                                       | Calbiochem | 511075      | 523.70 |
| SFLLR-amide                            | PAR1                                              | Bachem     | H-2938      | 633.79 |
| SLIGKV-amide                           | PAR2                                              | Bachem     | H-4624      | 614.79 |
| 3-Isobutyl-1-methylxanthine (IBMX)     | phosphodiesterases                                | Sigma      | I7018       | 222.24 |
| Prostaglandin D2                       | Prostaglandin DP receptor                          | Sigma      | P5172       | 352.47 |
| Prostaglandin E2                       | Prostaglandin receptors                            | Tocris     | 2296        | 352.47 |
| Epoprostenol                           | Prostaglandin IP receptor                          | Tocris     | 2989        | 374.45 |
| Sphingosine-1-phosphate                | S1P receptors                                      | Sigma      | S9666       | 379.47 |
| SEW 2871                               | S1P1 receptor                                      | Tocris     | 2284        | 440.36 |
| Secretin (human)                       | Secretin receptor                                  | Tocris     | 1918        | 3039.44|
| Somatostatin                           | SST receptors                                      | Bachem     | J-1490      | 1637.90|
| tyramine                               | TA1, TA2                                           | Sigma      | T2879       | 173.64 |
| Thyrotropin-releasing hormone           | TRH1, TRH2                                         | Bachem     | H-4915      | 362.39 |
| Urotensin II                           | Urotensin receptor                                 | Tocris     | 1642        | 1388.57|
| (Arg8)-vasopressin                     | Vasopressin receptors                              | Bachem     | H-1780      | 1084.25|
| Vasoactive intestinal peptide          | VIP receptors VPAC1 and VPAC2                      | Bachem     | H-3775      | 3325.84|
Supplementary Table 2. Information of other compounds used in the present study.

| Compound name | Source       | Catalog # | Purity |
|---------------|--------------|-----------|--------|
| DMAT          | Tocris       | 3686      | >99%   |
| TBI           | Tocris       | T6951     | ≥98%   |
| TBB           | Sigma        | T0826     | ≥98%   |
| TBCA          | EMD Biosciences | 218710  | ≥95%   |
| Oxymetazoline | Tocris       | 1142      | >99%   |
| UK14304       | Tocris       | 0425      | >99%   |
| Salmeterol    | Tocris       | 1660      | >99%   |
| Isoproterenol | Tocris       | 1747      | >99%   |
| Betaxolol     | Tocris       | 0906      | >99%   |
| Propranolol   | Tocris       | 0834      | >99%   |
| Phentolamine  | Sigma        | P7547     | ≥98%   |
| Yohimbine     | Tocris       | 1127      | >99%   |
| Prazosin      | Tocris       | 0623      | >99%   |

2. Supplementary Results

Figure S1 shows the DMR characteristics of distinct known CK2 inhibitors. Figure S2 shows the dose responses of both DMAT and TBB. Figure S3 shows the dose responses of adrenergic receptor agonists. Figure S4 and S5 show the impact of known adrenergic receptor antagonists on the epinephrine response as well as the epinephrine-induced potentiation of the TBB response. Figure S6 shows the impact of toxin treatment on both DMAT and TBB responses. Figure S7 shows the DMR characteristics of endogenous GPCRs, other than adrenergic receptors, and their impacts on the TBB and DMAT responses.
Supplementary Figure 1. DMR signatures of four CK2 inhibitors in HeLa cells. Observed DMR signals were induced at 32 μM of DMAT (a), TBB (b), 4,5,6,7-tetrabromo-1H-benzimidazole (TBI, c) and tetrabromocinnamic acid (TBCA, d). All error bars represent the standard deviations of at least 4 measurements.
Supplementary Figure 2. Dose-dependent responses of DMAT and TBB measured by DMR assays in HeLa cells. EC50 values were calculated from corresponding DMR traces induced by DMAT (a-c) and TBB (d-e). All error bars represent the standard deviations of at least 4 measurements.
Supplementary Figure 3. Dose-dependent responses of AR agonists measured by DMR assays in HeLa cells. (a-e) DMR traces were monitored for various concentrations of oxymetazoline (OXY, a), UK14304 (b), salmeterol (SAL, c), isoproterenol (d), and epinephrine (EPI, e). (f) EC$_{50}$ values were calculated from the corresponding DMR traces. All error bars represent the standard deviations of at least 4 measurements.
Supplementary Figure 4. Effects of epinephrine on TBB responses with or without antagonists. The dose dependent DMR for epinephrine showed an EC$_{50}$ of 15.0 ± 3.7 nM to trigger its respective DMR (Fig. 3a, and Supplementary Fig. 3e) and an EC$_{50}$ of 4.0 ± 0.4 nM (n =4) to potentiate the TBB-induced DMR (a-b). Various AR blockers, each at 10 µM, were included with the epinephrine to test for their ability to interfere with epinephrine enhancements of the TBB response. Neither the potent $\alpha_1$-AR blocker, prazosin, nor the $\beta$-blockers, betaxolol or propranolol, altered the potency of epinephrine to increase the TBB response (b). In contrast, the potent $\alpha_2$-blocker yohimbine suppressed and shifted the epinephrine DMR titration (c-d) as well as completely blocking the epinephrine increased TBB response (e-f). EC$_{50}$ values were calculated from the corresponding DMR traces. All error bars represent the standard deviations of at least 4 measurements.
Supplementary Figure 5. Effect of phentolamine on DMR assays. An $\alpha_2$-blocker phentolamine that is less potent than yohimbine (Supplementary Fig. 4c-f) only slightly suppressed and right-shifted the epinephrine DMR (a-b), and also caused the right-shift in the potency of epinephrine to increase the TBB DMR (c-d). EC$_{50}$ values were calculated from the corresponding DMR traces. All error bars represent the standard deviations of at least 4 measurements.
Supplementary Figure 6. DMAT and TBB responses in the absence and the presence of toxin. PTX or CTX showed little effect on either DMAT (20 μM) or TBB (25 μM) responses, relative to DMR signals obtained from untreated HeLa cells. All error bars represent the standard deviations of at least 4 measurements. An arrow indicates the addition of DMAT (a) or TBB (b).
Supplementary Figure 7. DMR assays of additional endogenous GPCRs. (a) A purinergic P2Y receptor agonist, ATP, resulted in robust DMR signals, which were sensitive to both PTX and CTX pretreatments. Similar to α2A-AR agonists, the activation of P2Y receptors by ATP led to a decreased DMAT signal and an increased TBB signal relative to the positive control (d-e). Pretreatment of cells with PTX but not CTX blocked the alteration of the DMAT signal by ATP. (b) A LPA receptor agonist, LPA, led to a distinct DMR, which was also sensitive to both PTX and CTX pretreatments. PTX but not CTX rescued the suppressed DMAT signal and diminished the increased TBB signal by LPA (d-e). (c) A prostaglandin receptor agonist PGE2 triggered a complicated DMR, which was also sensitive to both PTX and CTX pretreatments. Pre-stimulation of cells with PGE2 increased the DMAT signal, but decreased the TBB signal (d-e). Both PTX and CTX pretreatments blocked the increased DMAT signal by PGE2, but had a little effect on the decreased TBB signal by PGE2. (d and e) Each type of HeLa cells was pre-
stimulated with 10 μM of agonists listed on the x-axis for 1 hr. Positive controls indicate DMAT (20 μM) or TBB (25 μM) responses from buffer-pretreated HeLa cells. Negative controls mean no treatment with DMAT or TBB. The % change of the DMAT response was calculated based on the difference between the positive control and the DMAT signal in a treated cell (e.g., EPI-treated cell, or PTX-EPI-treated cell). The % change of the TBB response was obtained similarly. All error bars represent the standard deviations of 4 or 6 measurements.