**Supplementary methods**

**Folate extraction and determination**

Folate extraction and determination was extracted using the method of Riaz et al. (2019) , with some modifications.The 50 mg of powder was transferred into a 1.5 mL screw cap tube (Axygen, ST-150) and 1 mL of freshly prepared extraction solution [5 mM phosphate (Sigma–Aldrich, Missouri, USA) buffer, pH 7.2; 1% sodium ascorbate (Sigma–Aldrich, Missouri, USA) and 0.2% β–mercaptoethanol (Biotechnology grade, Amresco, Ohio, USA)] was added. After homogenization, the mixture was immediately boiled for 10 min in a water bath using electromagnetic oven, cooled on ice for 10 min, and centrifuged at 13,000×g at 4 °C for 10 min. The supernatant (0.5 mL) was transferred to a new tube, and 50 μL of rat serum was added, followed by incubating at 37 °C for 4 h to deconjugate the polyglutamylated tails. Subsequently, the samples were boiled for 10 min, cooled on ice for 10 min, and centrifuged at 13,000×g at 4 °C for 10 min. The supernatants were moved to 3 kDa ultra-filtration tubes (Millipore) for cleanup and centrifuged at 13,000 × g at 4 °C for 25 min. Finally, the resulting solution was collected and 100 μL was transferred into new tubes for direct folate detection, and the remaining solution was stored at −80 °C. There were three biological repeats for each sample. Folate extraction was performed under subdued light to minimize light-induced degradation.

Chromatographic analyses were performed on an SCIEX ExionLC™ HPLC system (Foster City, CA, USA) using an Akzo Nobel analytical column (Kinetex 2.6µm F5 C18, 50×4.6 mm) at a flow rate of 0.30 mL/min. The injection volume was 2.0 μL. The temperature of the injector and column oven was separately maintained at 4 °C and 30 °C, respectively. The mobile phase was 0.1% (v/v) formic acid in water (phase A) and 0.1% (v/v) formic acid in acetonitrile (phase B), respectively. The gradient program ran for a total of 8 min. The proportion of mobile phase B increased linearly from 2 to 98% over 3 min. After holding steady at 98% for 1 min, the proportion of phase B decreased to 2% in 0.1 min followed by a subsequent equilibration. An SCIEX Triple Quad™ 5500 triple quadruple tandem MS coupled with an ESI (electron spray ionization) interface was used for mass analyses and quantification of target analysis. The mass spectrometer was operated in positive ion mode. The source temperature was adjusted to 550 ºC and the ion spray voltage to 5500 V. Nitrogen was used as gas 1 (55 psig), gas 2 (55 psig), curtain gas (35 psig) and collision-activated dissociation gas (8 psig). Interface heater was on. The sum of contents of different folate derivatives represented as the total folate levels. Compound parameters are given in Table 1.

**Table 1 Compound parameters for folate standards**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Folate | Precursor  ion (m/z) | Product ion  (m/z) | DP (V) | EP(V) | CE (V) | CXP(V) |
| THF | 446.2 | 299.3 | 80 | 10 | 25 | 10 |
| 446.2 | 166.3 | 80 | 10 | 59 | 10 |
| 5-CH3-THF | 460.3 | 313.2 | 70 | 10 | 27 | 10 |
| 460.3 | 194.2 | 60 | 10 | 47 | 10 |
| 5-CHO-THF | 474.2 | 299.3 | 61 | 10 | 43 | 10 |
| 474.2 | 166.3 | 120 | 10 | 54 | 10 |
| 10-CHO-FA | 470.1 | 295.3 | 65 | 10 | 35 | 10 |
| 470.1 | 176.2 | 86 | 10 | 59 | 10 |
| FA | 442.1 | 295.3 | 79 | 10 | 24 | 10 |
| 442.1 | 176.2 | 79 | 10 | 50 | 10 |

Note: DP: declustering potential; EP: entrance potential; CE: collision energy; CXP: collision cell exit potential; V: volt.

**Determination of pteridine and *p*ABA**

The determination of pteridine and *p*ABA followed the method of Ramírez Rivera et al. (2016). Briefly, tissue (0.5g FW) was ground in liquid N2, suspended in 5 mL methanol : chloroform : water (12:5:1, v/v/v), heated to 50 °C for 5 min, and vortex mixed for 2 min. After clearing by centrifugation, extracts were split into aqueous and organic phases by adding 0.5 mL chloroform and 0.75 mL water, mixed thoroughly, and centrifuged. The aqueous phase was frozen-dried, taken up in 200 µL water. Samples were oxidized by adding 20 µL solution of 1% I2 and 2% KI (w/v) in 1 M HCl and incubating in darkness for 1 h. Excess I2 was then removed by adding 10 µL of 5% (w/v) Na-ascorbate. The oxidized samples were separated by HPLC with a Zorbax SB, C18, column 250×4.6 mm, 5μm (Agilent Technologies, Santa Clara, CA, USA) eluted isocratically with solution of 0.1% formic acid and 5% acetonitrile in water at 0.6 mL·min-1. The column temperature was 40 °C and injection volume was 20 µL. Peaks were detected by a Waters 2475 fluorescence detector (350 nm excitation and 450 nm emission) and identified by reference to standards and by spectral properties. The 6-hydroxymethylpterin peaks were quantified relative to standard pterin.

For *p*ABA analysis, tissue (0.5g FW) pulverized in liquid N2 and homogenized with 7 mL of methanol. The methanol extract was used for analysis of total *p*ABA (i.e., free *p*ABA plus *p*ABA glucose ester) by acid hydrolysis, ethyl acetate partitioning, and fluorometric HPLC according to the method of Ramírez Rivera et al. (2016).

**GCH1 and ADCS activity assay**

GCH1 activity assays was conducted according to McIntosh et al. (2008a) and McIntosh et al. (2008b). Tissue (1 g FW) was ground in 2 mL of 50 mM Tris-HCl buffer [0.2% polyvinyl-polypyrrolidone (w/v), 0.1M KCl, 1 mM DTT, and 5 mM EDTA, pH 8.0, freshly prepared). Extract was centrifuged at 10,000×g for 15 min. The supernatant was used for analysis of GCH1 activity. Each assay contained 200 µL extract and 800 µL 0.5 mM Tris-HCl (1 mM DTT, 0.1% BSA, and 1 mM GTP, 0.1 M KCl, pH 8.0, freshly prepared), and was incubated for 60 min at 37 ºC. The product of the reaction, dihydroneopterin triphosphate, was subsequently oxidized and dephosphorylated to neopterin prior to HPLC analysis. The detection of reaction products referred to the determination of pterin above. One unit of the activity was defined as producing 1 nmol of 6-hydroxymethylpterin/60 min of the reaction.

ADCS activity was determined according to Basset et al. (2004). Briefly, tissue (1 g FW) was ground in 5 mL of 0.1 M Tris-HCl buffer (10 mM MgCl2, 10 mM DTT, 10 mM sodium ascorbate, 10% glycerin, pH 7.5, freshly prepared). Extract was centrifuged (13,000×g, 4 ºC, 10 min). The supernatant was used for the next analysis of ADCS activity. Each assay contained 50 µL extract and 100 µL of 0.5 mM Tris-HCl (10 mM MgCl2, 10 mM DTT, 5 mM L-glutamine, 100 µM chorismate, pH 7.5), and was run at 37 ºC for 60 min. Reactions were stopped with 20 µL of 75% (v/v) acetic acid, incubated on ice for 60 min, and centrifuged (13,000×g, 4 ºC, 10 min). The product of the reaction (*p*ABA) was separated by HPLC with a Zorbax SB C18 column (250×4.6 mm, 5 μm) eluted isocratically with solution of 0.5% acetic acid and 20% methanol in water at 0.7 mL·min-1. The column temperature was 30 ºC and the injection volume was 20 µL. The *p*ABA peak was detected by a Waters 2475 fluorescence detector (290 nm excitation and 340 nm emission) and quantified relative to standards. One unit of activity was defined as producing 1 nmol of *p*ABA/60 min of the reaction.

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