Supplementary information

Phenylalanine sensitive K562-D cells for the analysis of the biochemical impact of excess amino acid

Yoshitami Sanayama^{1,2}, Akio Matsumoto^{1*}, Naoki Shimojo³, Yoichi Kohno³, and Haruaki Nakaya¹

¹ Department of Pharmacology, Graduate School of Medicine, Chiba University, Chiba,

² Department of Pediatrics, National Hospital Organization, Shimoshizu Hospital, Chiba,

³ Department of Pediatrics, Graduate School of Medicine, Chiba University, Chiba, Japan

*Address for correspondence:

Akio Matsumoto, M.D. Ph.D.

Department of Pharmacology, Graduate School of Medicine, Chiba University 1-8-1 Inohana Chuo-ku Chiba 260-8670, Japan Tel: +81-43-226-2051 Fax: +81-43-226-2052 Email: akio@faculty.chiba-u.jp



Supplementary figure 1. A treatment with phenylalanine reduced mTOR activity in K562-D cells and valine restored the activity (Full-length blots).

A treatment with phenylalanine reduced mTOR activity in K562-D cells and valine restored the activity. K562-D cells were cultured for 3 days with phenylalanine (5 mM) alone or in combination with valine (1 mM). Thirty μ g of total cell lysate was separated on 10% SDS-PAGE followed by western blotting with a pThr389-specific p70S6 kinase antibody (left side panel) followed by reprobing with p70S6 kinase antibody (right side panel). For more details please see the legend to Figure 4E. The two lanes on the far left are not shown in Figure 4E as they received compound X for our purpose.