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Supporting information for article:

Crystal structure of N-terminal degron-truncated human glutamine synthetase

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Figure S1 Sequence alignment of GS. Amino acid sequence alignment of mammalian and bacterial GS proteins; human: *Homo sapiens* (NP_001028216), dog: *Canis lupus* (Q8HZM5), mouse: *Mus musculus* (NP_032157.2), *Bacillus subtilis* (NP_389628), *Helicobacter pylori* (WP_000637150), *Salmonella typhimurium* (NP_462887), and *Mycobacterium tuberculosis* (NP_216736). Sequence identity values of dog, mouse, *Bs*, *Hp*, *St*, and *Mt* GS compared to human GS are 97.3, 94.6, 23.1, 24.5, 24.4 and 22.1%, respectively. Conserved acidic, basic and non-polar residues are colored red, blue and green, respectively. The secondary structure, based on the human GS (23-373) structure determined in this study, is shown over the sequence alignment as pink cylinders (α -helices and 3₁₀ helices) and yellow arrows (β -strands). The disordered regions are shown as dashed lines. Boxed regions: Glu-flap (green box), glycerol-binding residues (blue boxes), MSO-binding residues (black boxes), and K11, K14 of the N-degron (orange boxes).



Figure S2 Pentamer rings interact through a loop. The pentamer rings interacts through the $\beta6-\alpha5$ loop. Hydrogen bonds are formed between the mainchain carbonyl oxygen (C=O) of P155 from mol B and the mainchain amide group (NH) of F154 from mol J, and *vice versa*.



Figure S3 Structural comparison of N-degron-deleted and full-length GS. (*a*) A top view of the overlay between the $GS^{\Delta N}$ decamer (cyan and green, molecule units labeled red) and the *CfGS* decamer (yellow, molecule units labeled black). A side-view is displayed on the right. (*b*) A top view of the overlay between the $GS^{\Delta N}$ decamer (cyan and green, molecule units labeled red) and the GS^{ADP} decamer (pink, molecule units labeled black). A side-view is displayed on the right. (*c*) A top view of the overlay between the $GS^{\Delta N}$ decamer (cyan and green, molecule units labeled in red) and the $GS^{ADP+MSO}$ decamer (magenta, molecule units labeled black). A side-view is displayed on the right. (*d*) A top view of the overlay between the $GS^{\Delta N}$ pentamer (cyan and green) and the *CfGS* pentamer (yellow). (*e*) A top view of the $GS^{\Delta N}$ pentamer (cyan and green). (*f*) A top view of the *CfGS* pentamer (yellow).