Supplementary Materials

Fig. S1. Protection against renal IR by dietary protein deficiency in the absence of reduced calorie intake. Wildtype male B6D2F1 mice were given ad libitum (AL) access to complete or protein (prot) free chow, or pair-fed (PF) to the protein free chow group with complete chow, for 14 days (A, B, D) or 6 days (E-G) prior to induction renal ischemia reperfusion (IR) injury. (A, E) Total food intake corrected for body weight (top, expressed as weight of food eaten per total weight of animals in the cage) or expressed on a per mouse basis (bottom) (A, n=2 cages/group; E, n=3-7 cages/group). (B, F) Blood glucose prior to induction of renal IR (B, n=9/group; F, n=8/group). (C) Total activity of individually housed mice with ad libitum access to the indicated diet (n=4/group) measured in the X axis using an animal activity meter (Opto M3, Colombus Instruments) over a 48 hour period and separated according to phase. (D, G) Change in weight 2 days after renal IR expressed as a percentage of pre-operative weight (E, n=8-9/group; F, n=7-8/group). Error bars indicate SEM. Asterisks indicate the significance of the difference between the indicated groups by one-way ANOVA followed by Tukey's multiple comparison test comparing all pairs of values; *p<0.05, **p<0.01, ***p<0.001.

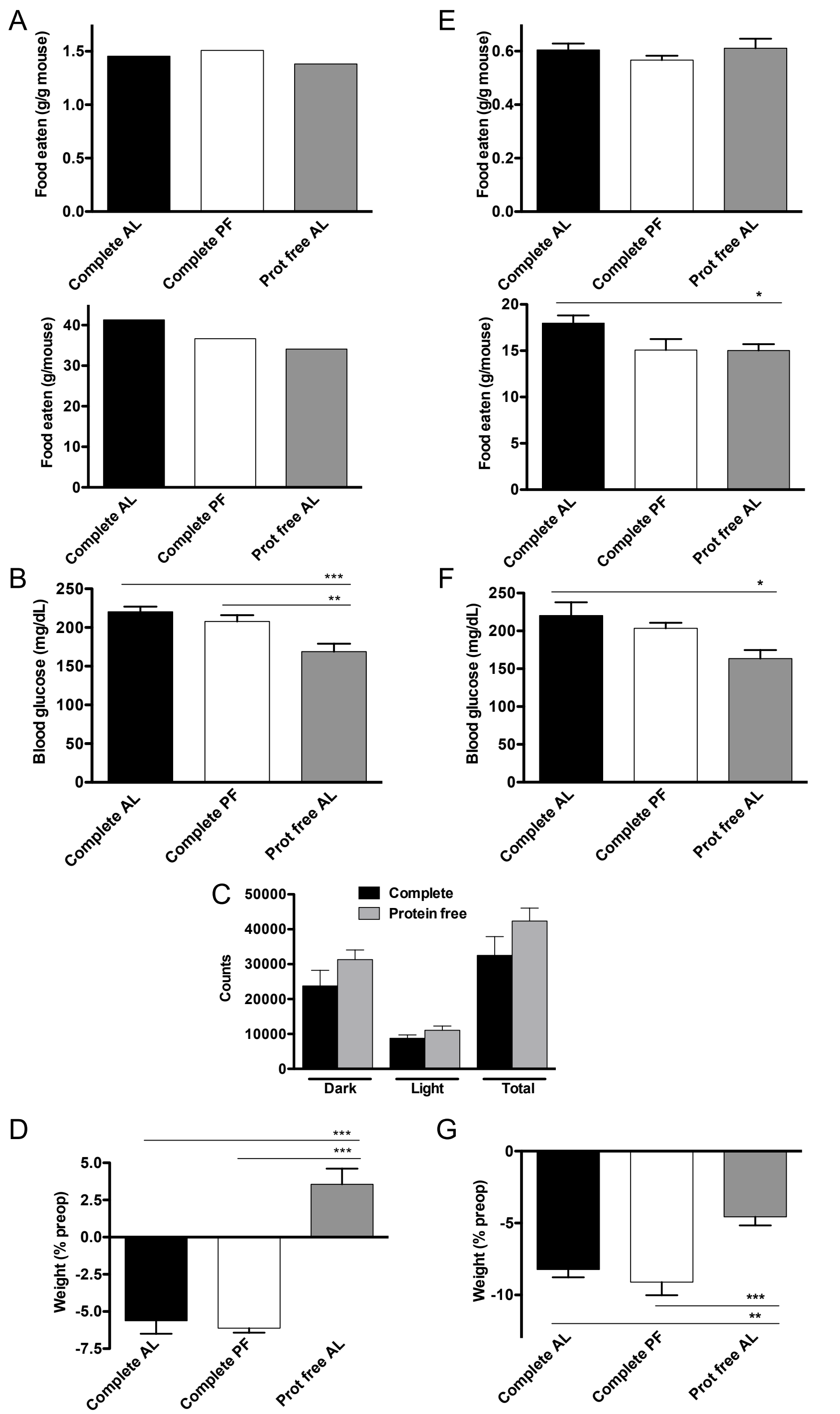
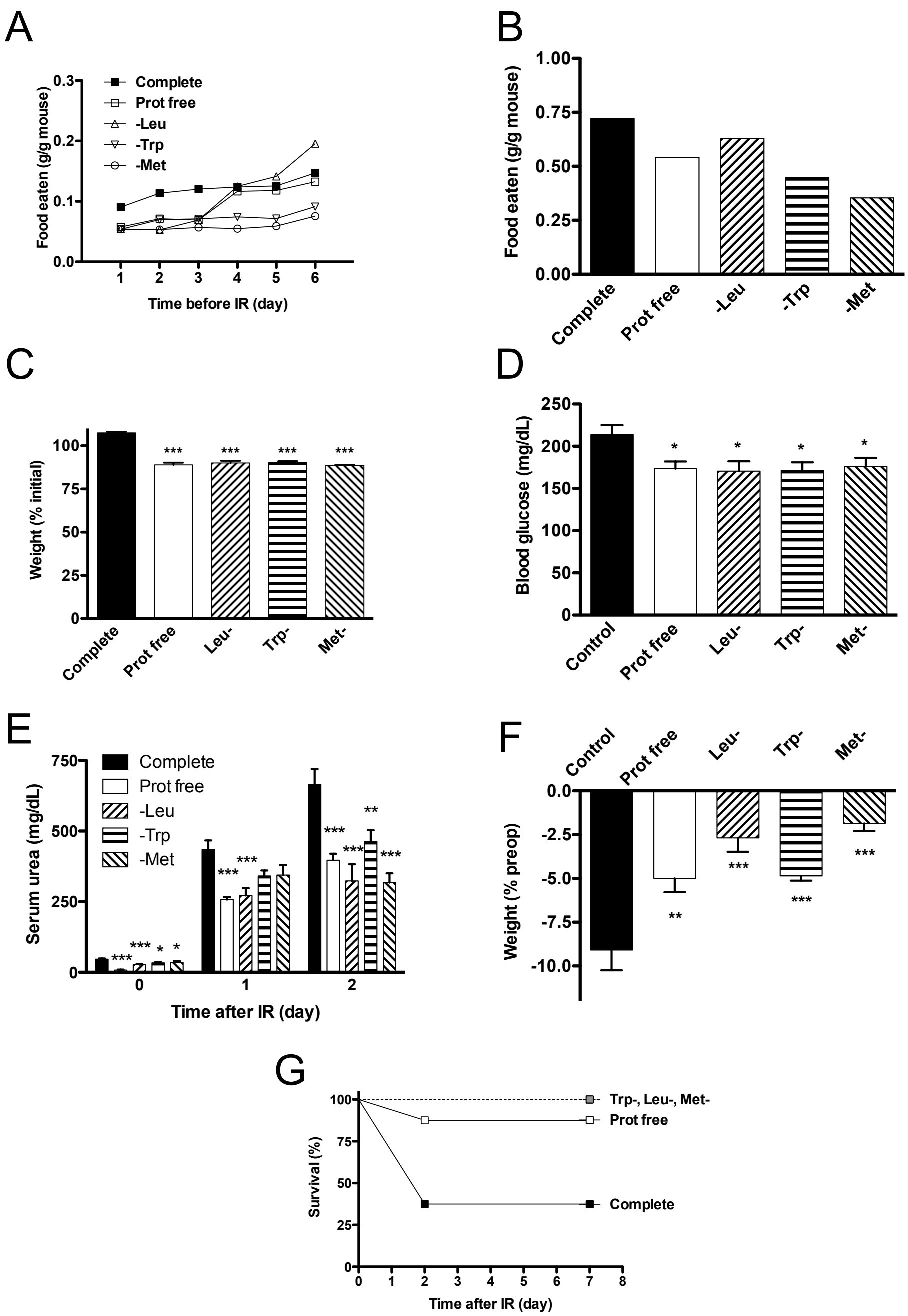


Fig. S2. Protection against renal IR by isolated essential amino acid deprivation. Ad libitum feeding of isocaloric protein or single essential amino acid deficient diets protected against 35 minutes renal IR. (A) Daily food intake of mice (n=2 cages/group) expressed as weight of food eaten per total weight of animals in that cage. (B) Total food intake over 6 day preoperative period. (C) Body weights after 6 days of preconditioning expressed as a percentage of initial body weight (n=8 per group). (D) Blood glucose after 6 days of preconditioning (n=8/group). (E) Serum urea on the indicated day prior to (day 0) or following renal IR (n=7-8 animals/group). (F) Post-operative weight loss 2 days after 25 minutes bilateral renal IR (n=7-8/group) expressed as a percentage of pre-operative weight. Error bars indicate SEM. Asterisks indicate significance of the difference between the indicated group and complete AL by one-way ANOVA followed by Dunnett's multiple comparison test within a given time point; *p<0.05, **p<0.01, ***p<0.001. (G) Kaplan-Meier survival curves of the indicated groups up to 7 days after renal IR (n=7-8/group). Survival in all groups was significantly better than in the complete diet group (log rank test p=0.009 for met- and trp-, p=0.0133 for leu- and 0.0455 for protein deficiency vs. complete).



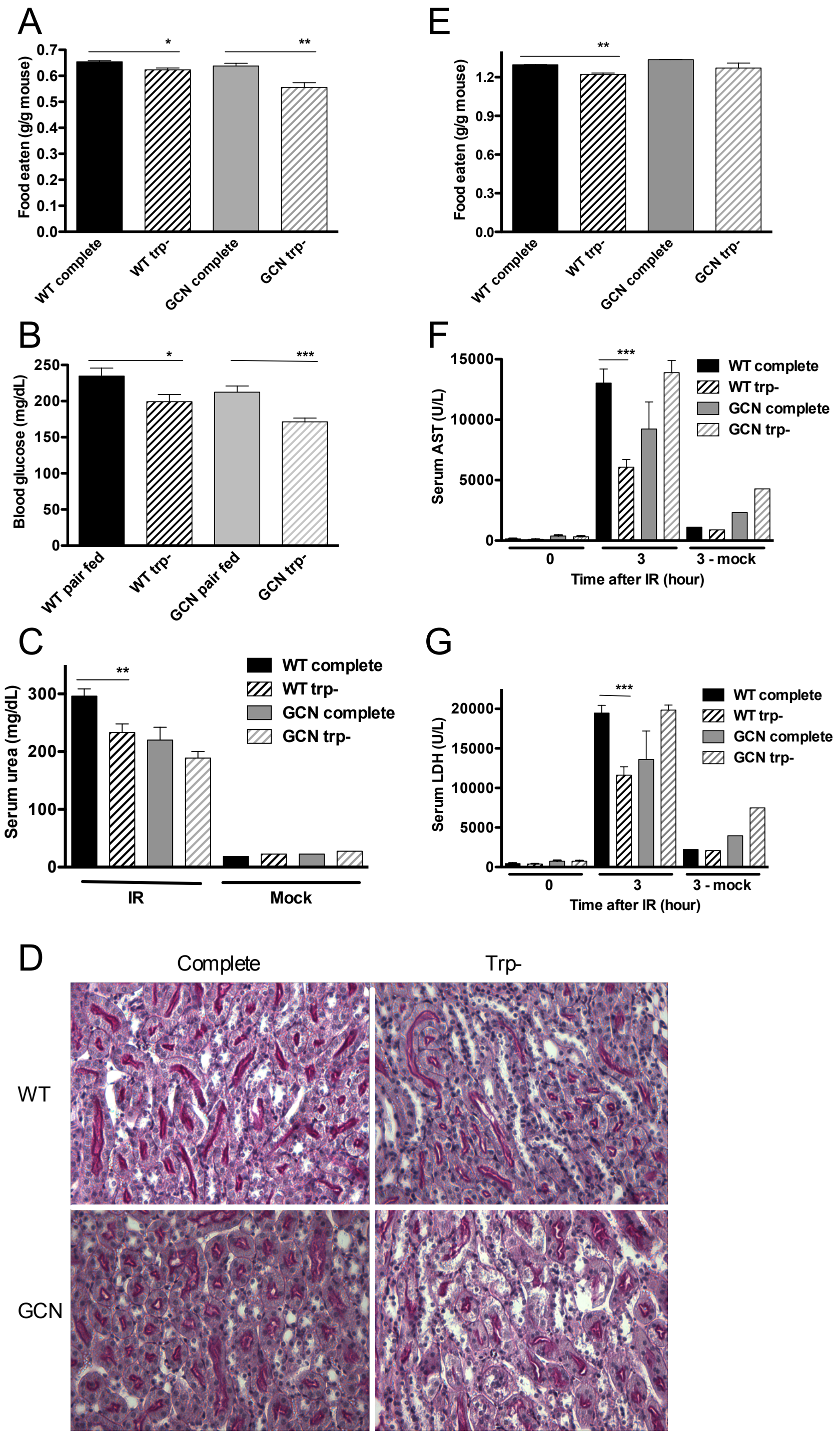


Fig. S3. Requirement for *Gcn2* in tryptophan deficiency-mediated protection against renal and hepatic IR. (A-D) *Gcn2*-mediated protection by tryptophan deficiency against renal IR. (A) Total food intake of mice (n=4 cages/group) expressed as weight of food eaten per total weight of animals in that cage. (B) Blood glucose after 6 days on the indicated diet (n=16-17/group). (c) Renal function as indicated by serum urea 1 day after renal IR (n=9-10/group) or mock ischemia (n=2-3/group). (D) Representative images of periodic acid/Schiff base-stained kidney sections 1day after mock IR showing tubules with intact brush borders (purple) in the corticomedullary junction. (E-G) *Gcn2*-mediated protection by tryptophan deficiency against hepatic IR. (E) Total food intake of mice (n=3 cages/group) expressed as weight of food eaten per total weight of animals in that cage. (F-G) Serum AST (F) and LDH (G) prior to (0 hour) or 3 hours after 45 minutes of hepatic ischemia (n=5-10/group) or mock IR (n=2-3/group). Error bars indicate SEM. Asterisks indicate the significance of the difference between the indicated groups by Student's t test for effect of diet within the same genotype; *p<0.05, **p<0.01, ***p<0.001.

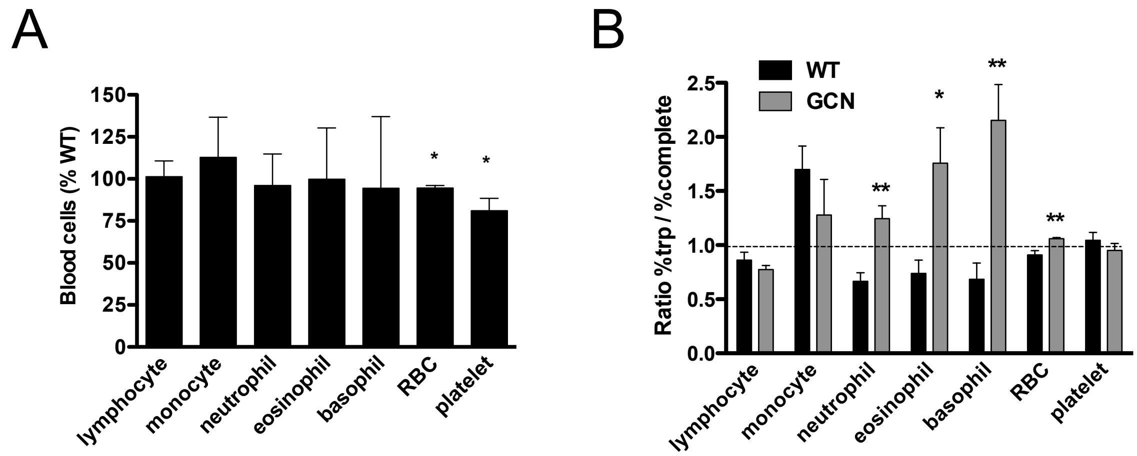


Fig. S4. Effects of tryptophan deficiency on peripheral blood counts.
(A) Complete blood cell counts of ad libitum, complete diet-fed GCN animals expressed as a percentage of WT (n=4-5/group). (B) Ratio of blood cell numbers from 6 days of trp- vs. complete pair-fed WT or GCN mice (n=5/group). Error bars indicate SEM. Asterisks indicate significance of the difference between WT and GCN by Student's t test; *p<0.05, **p<0.01, ***p<0.001.

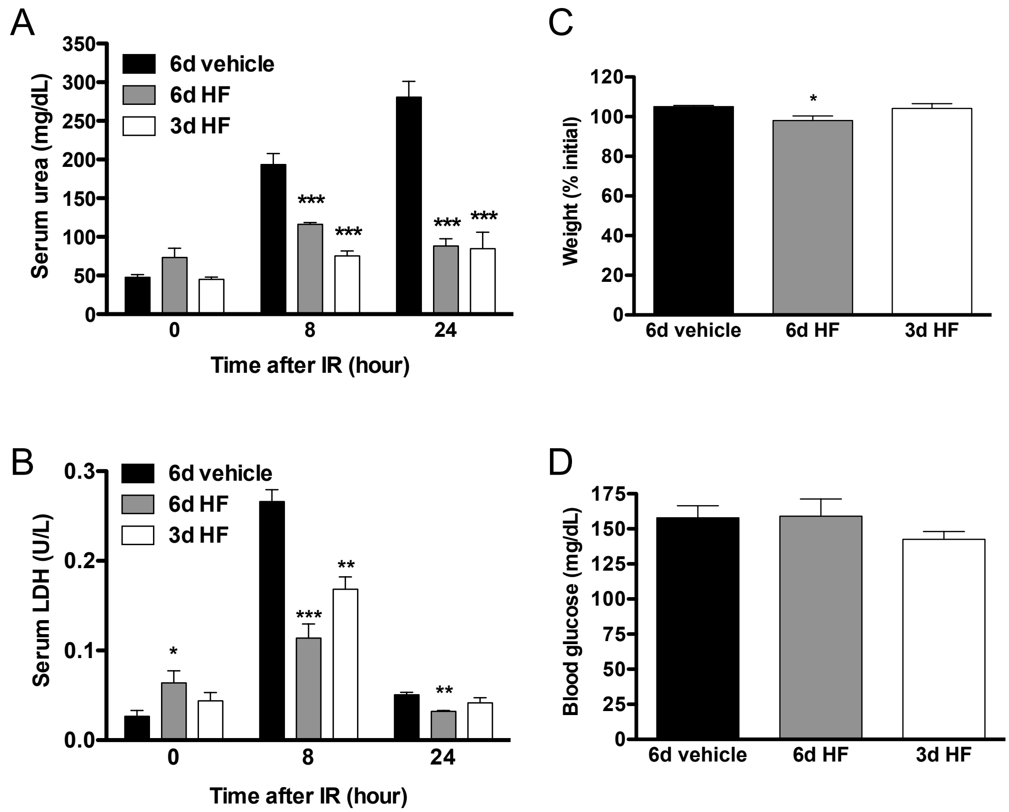


Fig. S5. Protection against IR by pharmacological activation of the amino acid starvation response. Preconditioning with halofuginone (HF) for 3-6 days protected against renal IR. (A-B) Serum urea (A) and LDH (B) prior to (0 hour) or 8-24 hours after 25 minutes of bilateral renal IR (n=5/group). (C) Body weights of male mice (n=5/group) after 3 days of the indicated treatment expressed as % initial weight. (D) Blood glucose after 3 days of the indicated treatment (n=5/group). Error bars indicate SEM. Asterisks indicate the significance of the difference between the indicated group and the vehicle-treated group according to a one-way ANOVA followed by Dunnett's multiple comparison test; *p<0.05, **p<0.01, ***p<0.001.

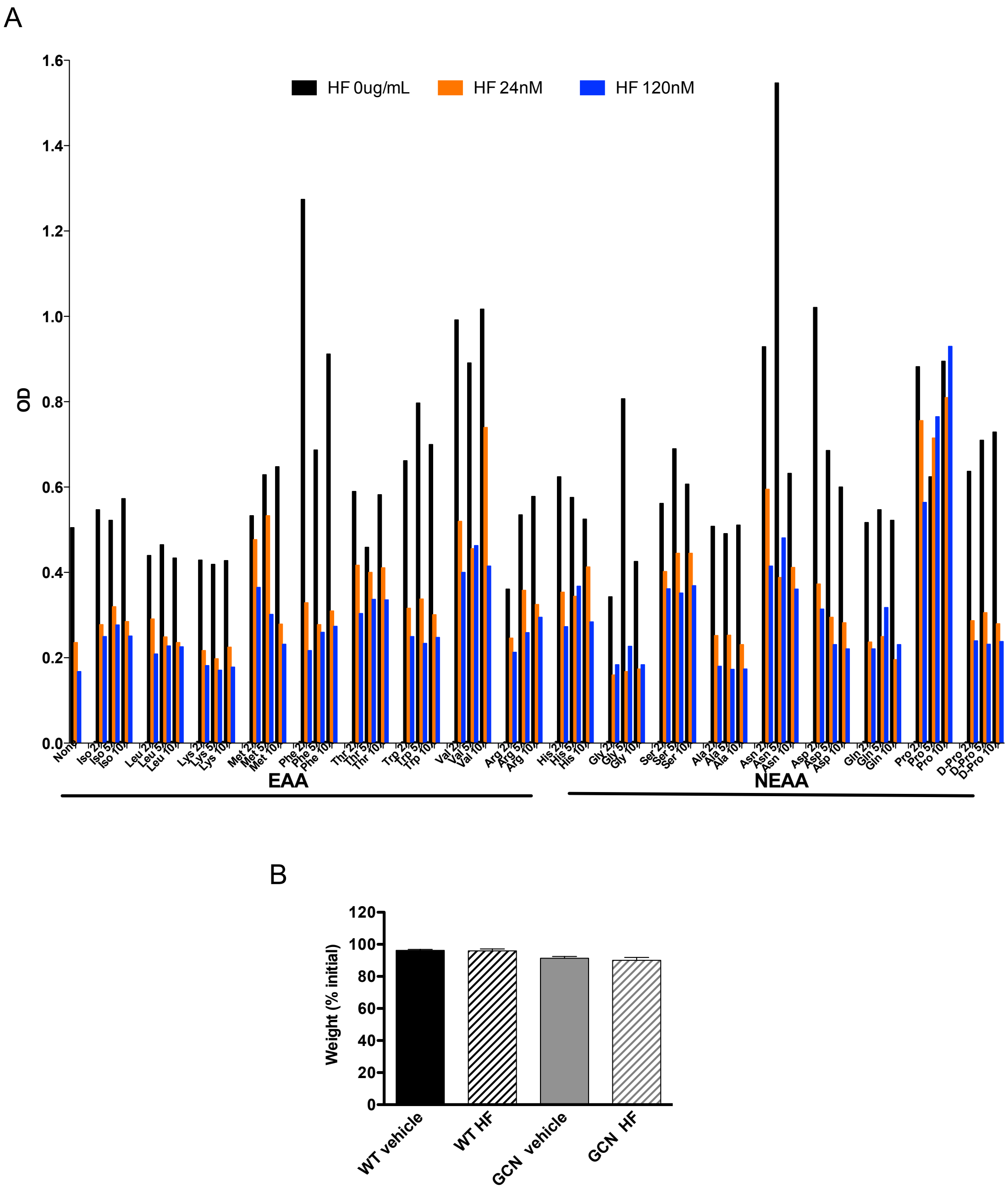


Fig. S6. **Halofuginone activity blocked by excess proline *in vitro* and requirement for *Gcn2 in vivo*.** (A) Representative experiment showing titration of individual amino acids against two doses of HF in cultured mouse embryo fibroblasts. OD from the MTT assay is proportional to cell viability. All amino acids tested were L-enantiomers except D-proline and were included at concentrations 2, 5 or 10 fold above that already present in DMEM. (B) Body weights of male mice (n=4-9/group) after 3 days of vehicle or HF treatment expressed as % initial weight.