

# Cell Type-Specific Metabolic Response to Amino Acid Starvation Dictates the Role of Sestrin2 in Regulation of mTORC1

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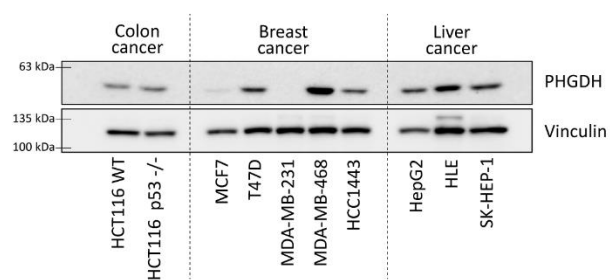
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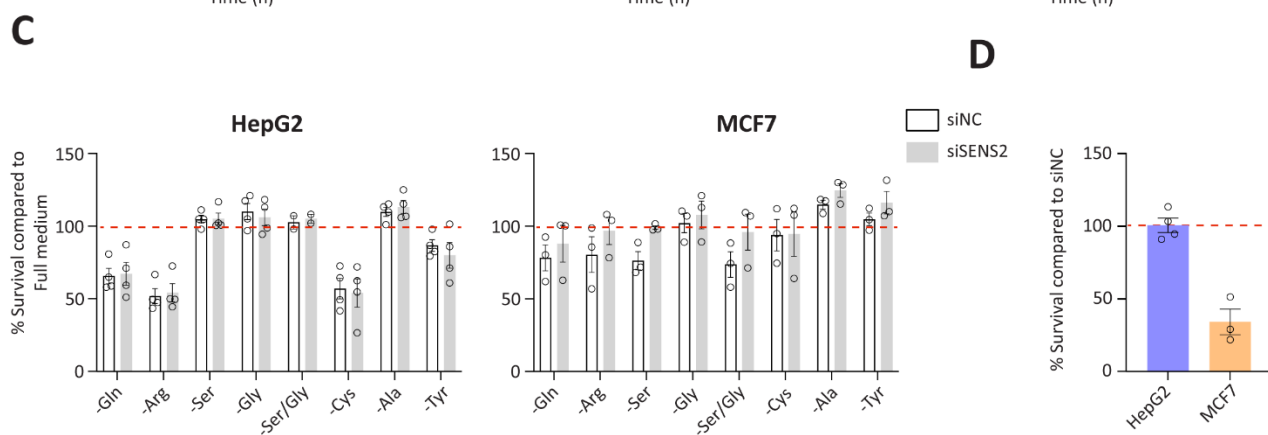
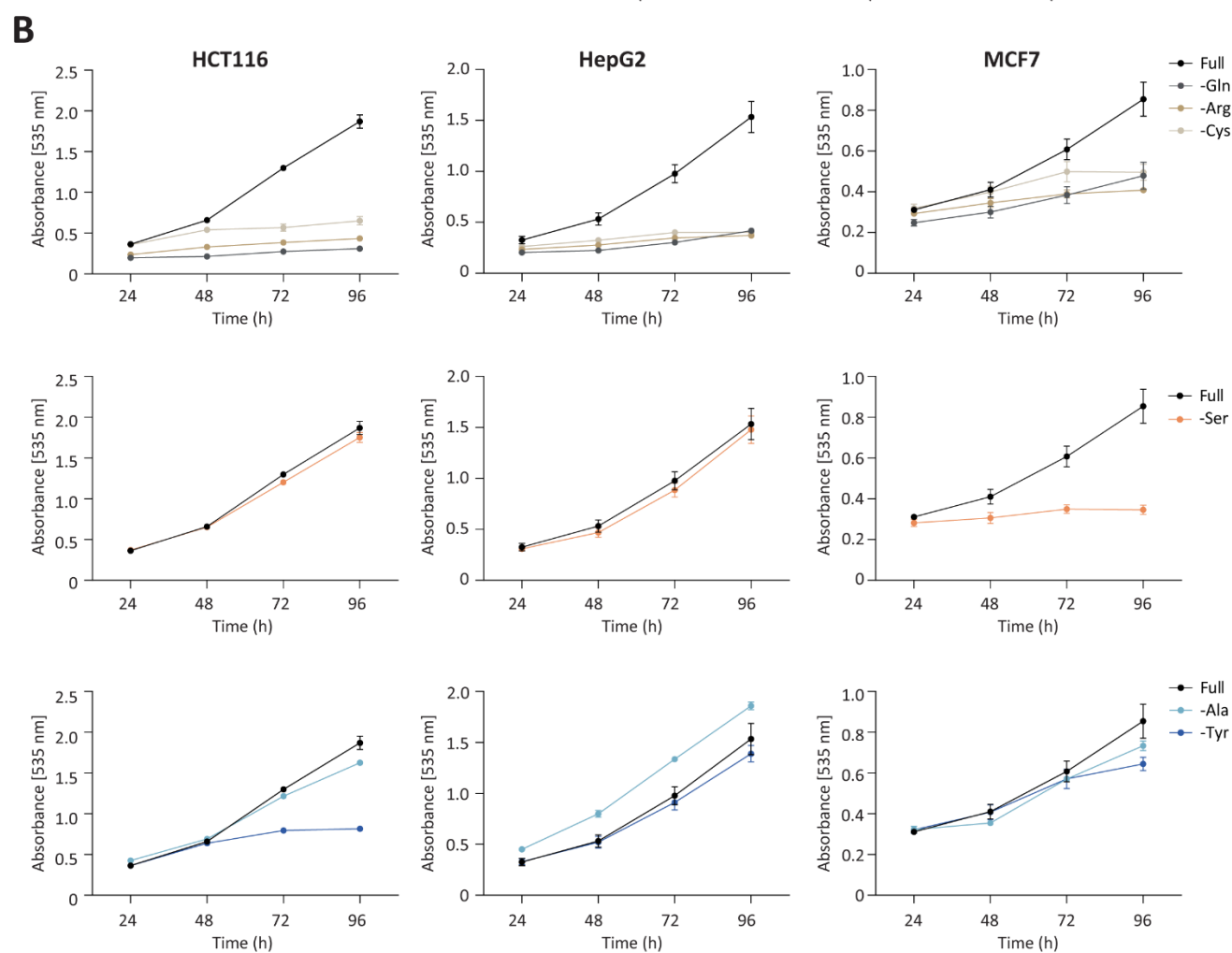
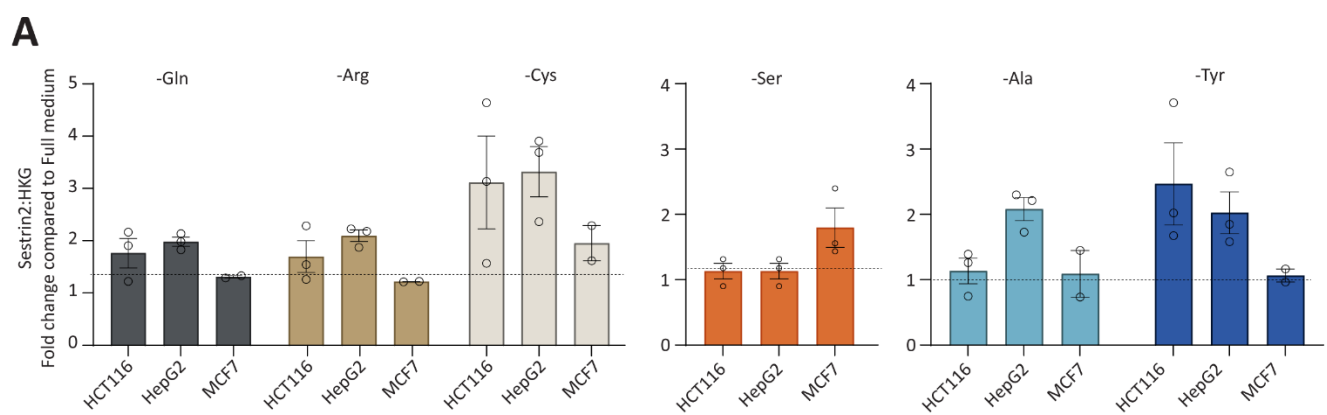
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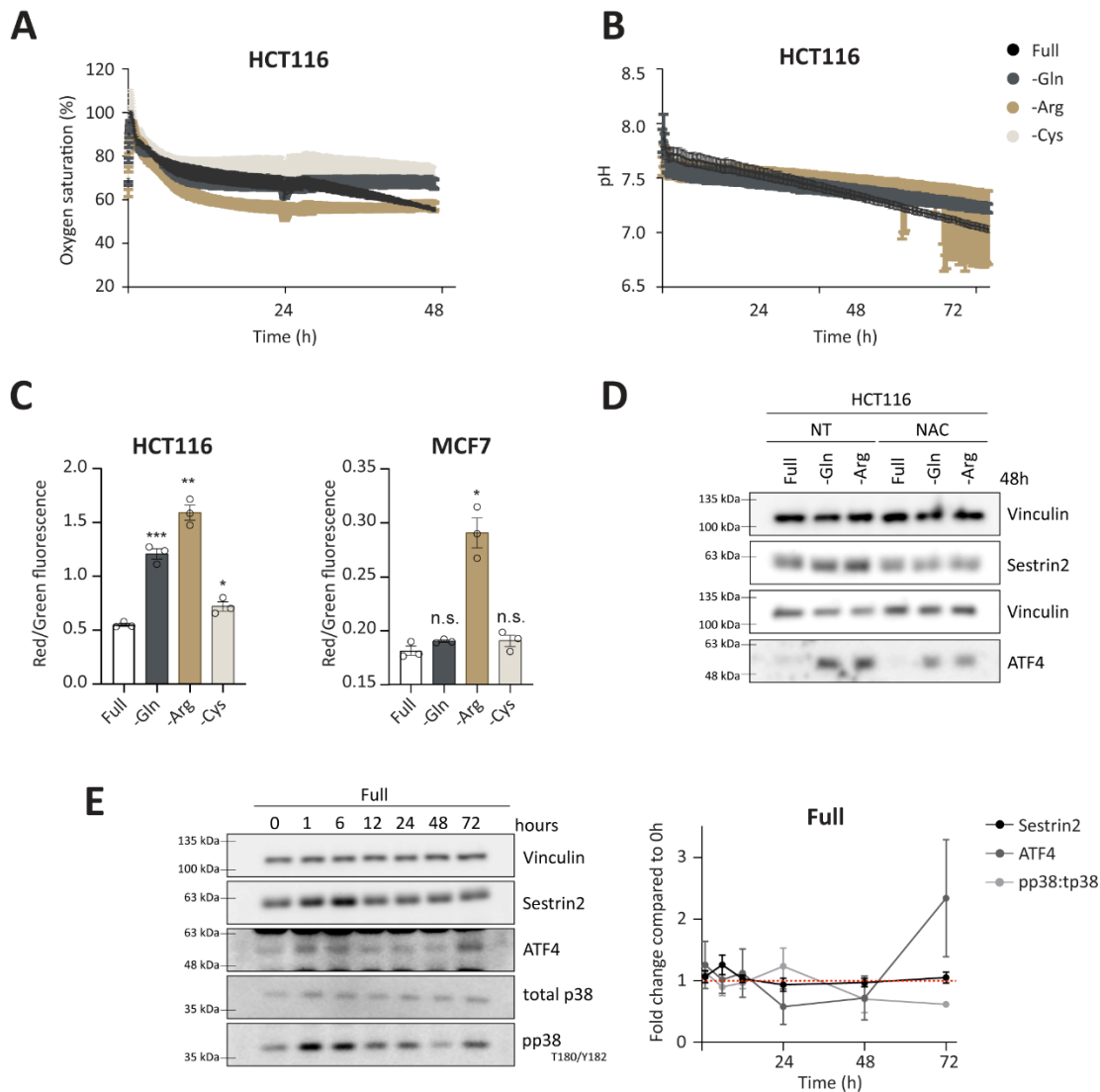
**Supplementary Materials:** The following supporting information can be downloaded at: [www.mdpi.com/xxx/s1](http://www.mdpi.com/xxx/s1), Figure S1: Basal PHGDH levels in various cell lines; Figure S2: Sestrin2 regulation and proliferation rate upon amino acid deprivations; Figure S3: Group 1 of amino acid deprivations causes metabolic, oxidative, and nutritional stress; Figure S4: Group 2 of amino acid deprivations causes solely nutritional stress; Figure S5: Group 3 of amino acid deprivations causes solely nutritional stress; Figure S6: Sestrin2-dependent regulation of mTORC1 upon amino acid deprivation.



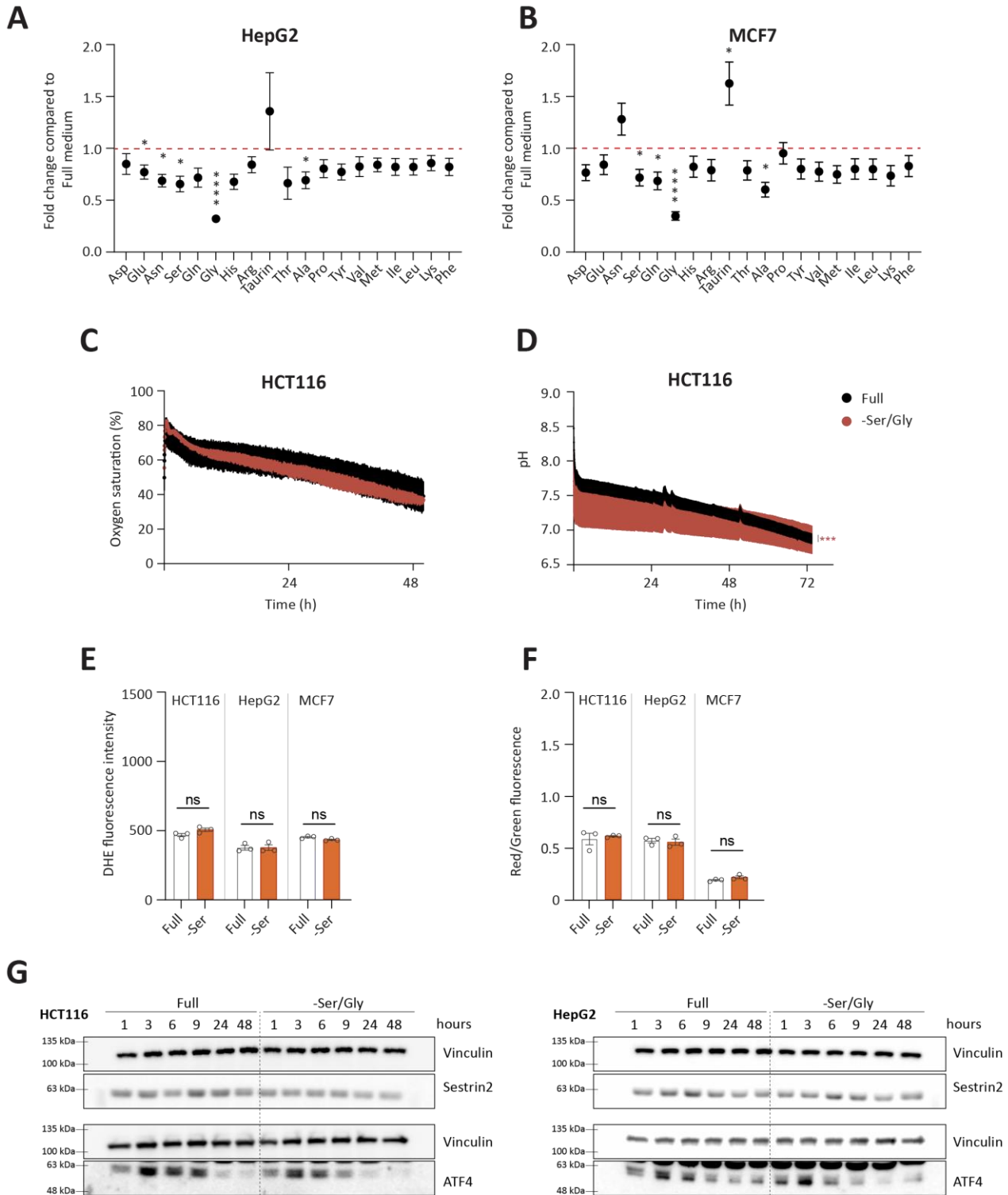
**Figure S1.** Basal PHGDH levels in various cell lines.



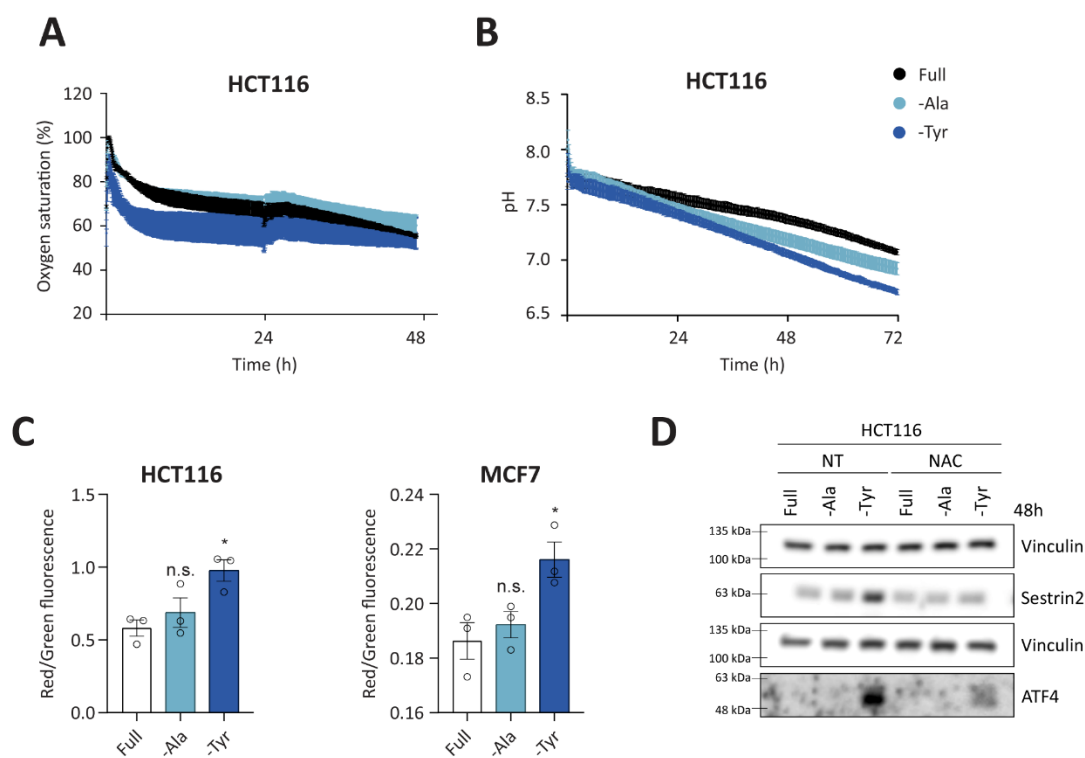
**Figure S2.** Sestrin2 regulation and proliferation rate upon amino acid deprivations. (A) Densitometric quantification of western blots from three independent experiments; (B) time-dependent survival measurements upon amino acid deprivations; (C) cellular survival in various media lacking an indicated amino acid(s) upon silencing SESN2; (D) cellular survival upon SESN2 knock-down compared to siNC. Dots represent individual values.



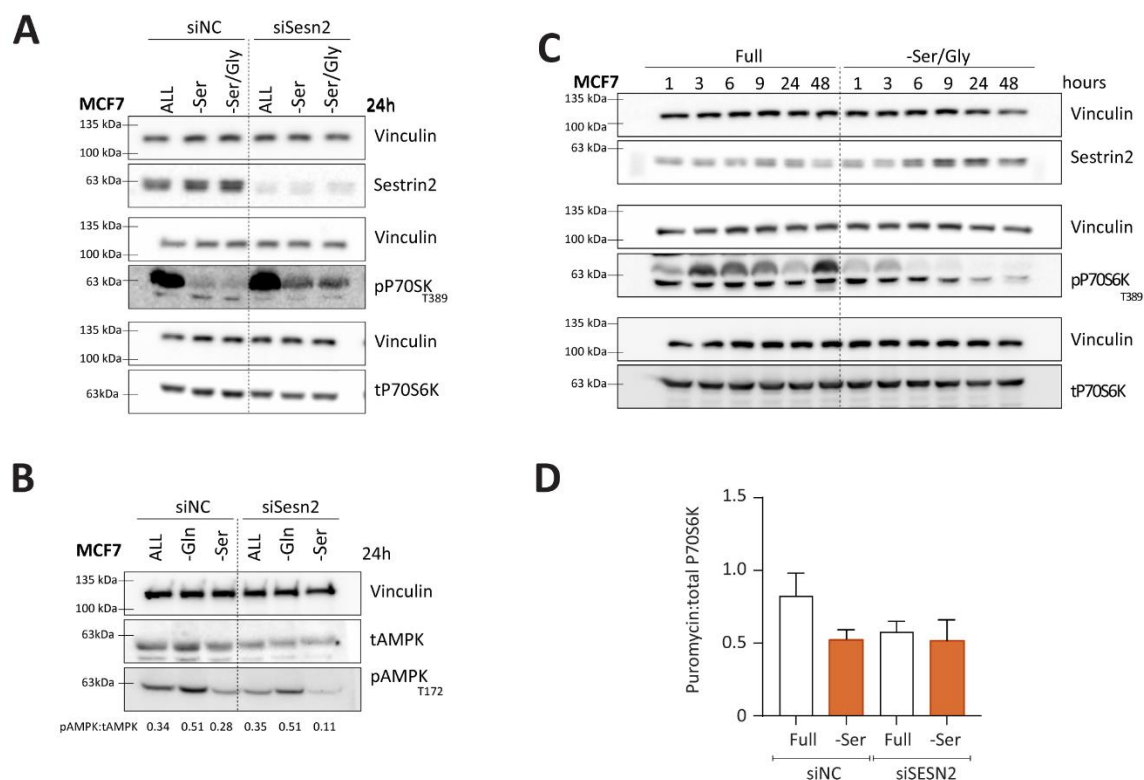
**Figure S3.** Group 1 of amino acid deprivations causes metabolic, oxidative, and nutritional stress. (A-B) Continuous measurements of oxygen saturation (A) and pH (B) values in extracellular medium; (C) mitochondrial membrane potential measured 48h of amino acid deprivations; (D) Sestrin2 and ATF4 levels upon 10 mM N-acetyl cysteine co-treatment; (E) time-dependent regulation of Sestrin2, ATF4, and phosphorylation of p38 in the full medium with densitometric quantification. Dots represent individual values. \*, \*\*, and \*\*\* indicate  $p \leq 0.05$ , 0.01, and 0.001, respectively.



**Figure S4.** Group 2 of amino acid deprivations causes solely nutritional stress. (A-B) Intracellular amino acid levels upon 48h of serine/glycine deprivation in comparison to full medium. One-tailed t-test was used to measure statistical significance; (C-D) continuous measurements of oxygen saturation (C) and pH (D) values in extracellular medium; (E-F) intracellular ROS levels (E) and mitochondrial membrane potential (F) measured 48h of amino acid deprivations; (G) time-dependent regulation of Sestrin2 and ATF4 upon the serine/glycine deprivation. Dots represent individual values. \* and \*\*\*\* indicate  $p \leq 0.05$  and 0.0001, respectively. n.s. indicates statistical insignificance.



**Figure S5.** Group 3 of amino acid deprivations causes solely oxidative stress. **(A-B)** Continuous measurements of oxygen saturation **(A)** and pH **(B)** values in extracellular medium; **(C)** mitochondrial membrane potential measured 48h of amino acid deprivations; **(D)** Sestrin2 and ATF4 levels upon 10 mM N-acetyl cysteine co-treatment. Dots represent individual values. \* indicates  $p \leq 0.05$ . n.s. indicates statistical insignificance.



**Figure S6.** Sestrin2-dependent regulation of mTORC1 upon amino acid deprivation. (A) Phosphorylation of P70S6K upon SESN2 silencing in the presence or absence of indicated amino acids; (B) phosphorylation of AMPK $\alpha$  upon SESN2 silencing in the presence or absence of indicated amino acids; (C) time-dependent regulation of Sestrin2 and phosphorylation of P70S6K upon the serine/glycine deprivation; (D) densitometric quantification of puromycin experiment shown in Figure 6G (n = 2).