LETTER TO THE EDITOR

# The glutaminase activity of ASNS fuels glutamine metabolism in leukemia


## Authors

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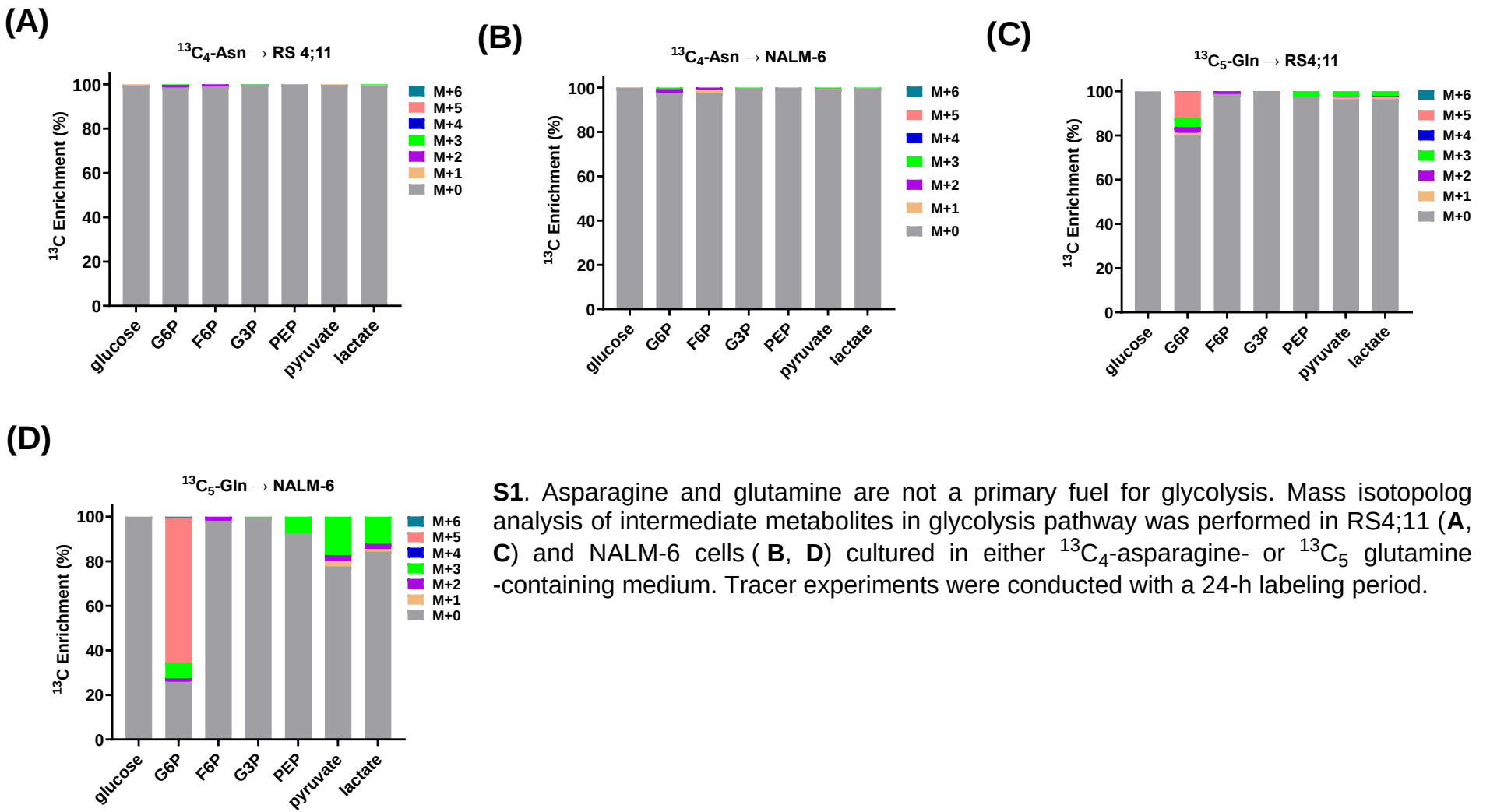
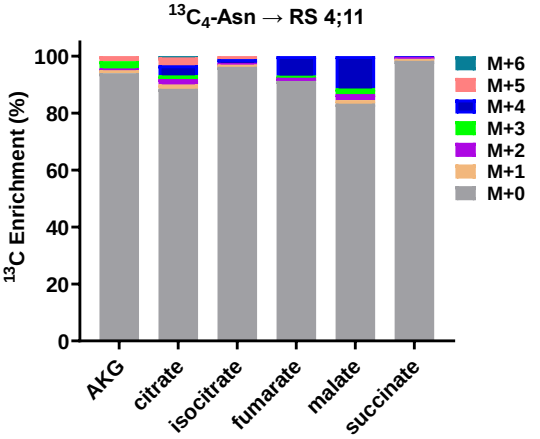
# S1



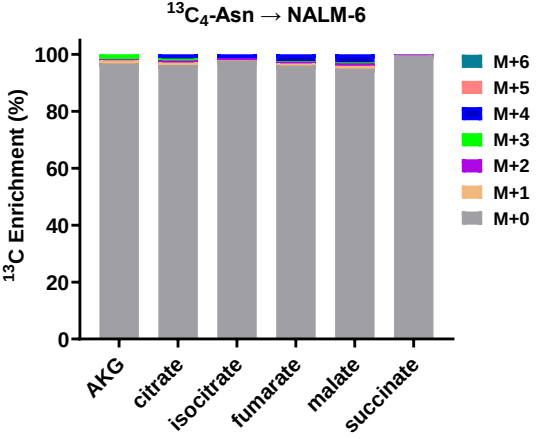

**S1**. Asparagine and glutamine are not a primary fuel for glycolysis. Mass isotopolog analysis of intermediate metabolites in glycolysis pathway was performed in RS4;11 (**A**, **C**) and NALM-6 cells ( **B**, **D**) cultured in either $^{13}C_4$-asparagine- or $^{13}C_5$ glutamine -containing medium. Tracer experiments were conducted with a 24-h labeling period.

# S2

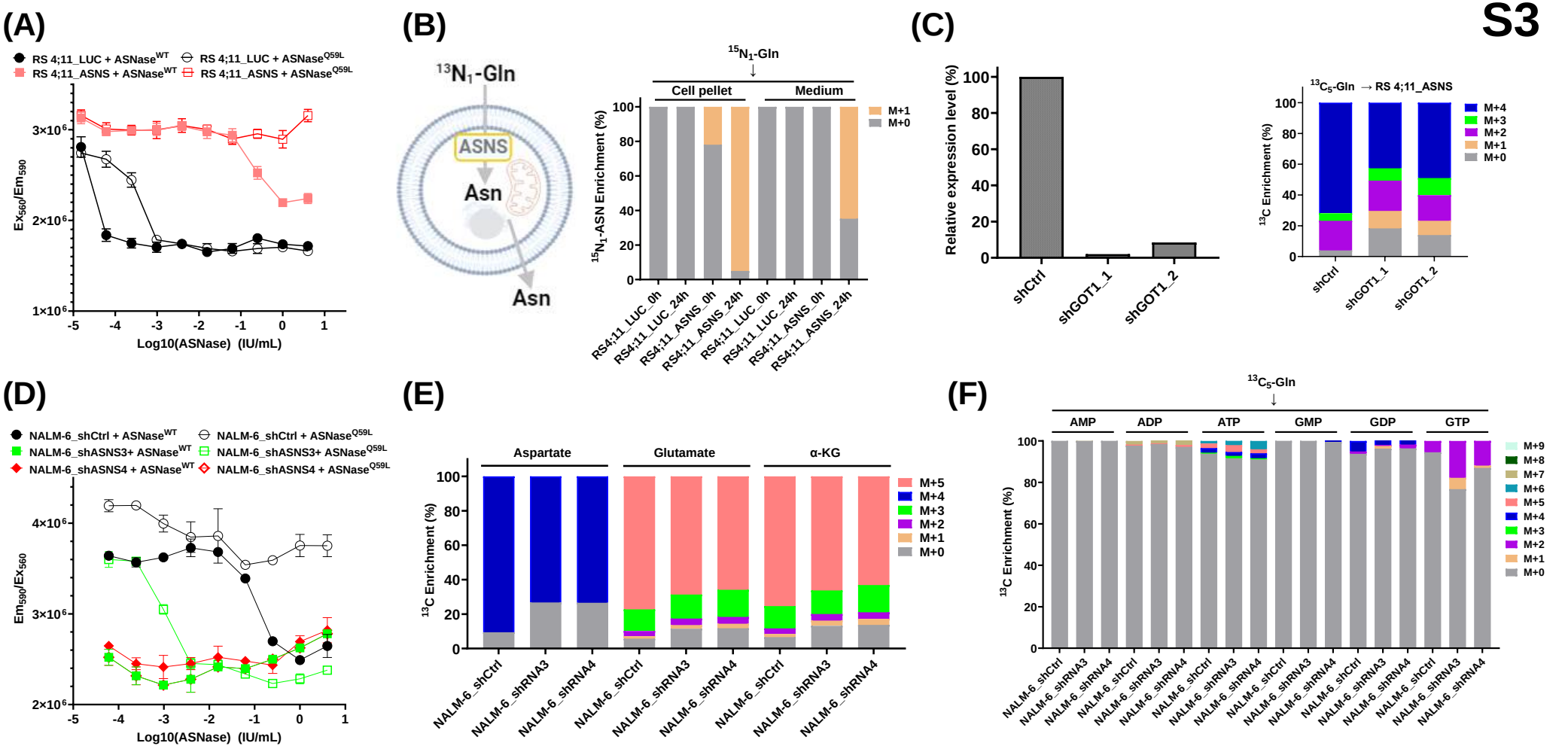
(A)




(B)




**S2**. Asparagine is not a primary fuel for TCA cycel. (**A**, **B**) Mass isotopolog analysis of intermediate metabolites in TCA cycle was performed in RS4;11 (**A**) and NALM-6 cells (**B**) cultured in $^{13}C_4$-asparagine-containing medium. Tracer experiments were conducted with a 24-h labeling period.




**S3.** ASNS-overexpressing RS 4:11 cell line and ASNS-knockdown NALM-6 cell lines. (**A**) RS4;11_LUC and RS4;11_ASNS cells in **Figure 2B** were seeded in 96-well plates and incubated overnight, then treated with indicated concentrations of E coli ASNase$^{WT}$ or ASNase$^{Q59L}$ mutant for 72 hours. Cell viability was assayed with CellTiter-Blue using fluorescence excitation at 560 nm and emission at 590 nm. (**B**) RS4;11 cells expressing ASNS (RS4;11_ASNS) but not the control cells expressing luciferase (RS4;11_LUC) efficiently synthesize ASN using $^{15}N_1$-GLN as a nitrogen donor. Cell pellet and media samples were collected at the indicated times. Time zero measurements were made on samples collected immediately after the tracer was administered to the cells. (**C**) Depletion of GOT1 decreases conversion efficiency from $^{13}C_5$-glutamine to aspartate. Left panel: To knock down GOT1 expression, RS 4;11_ASNS cells were infected with shGOT1 lentiviral supernatants for 48 hours, followed by selection with puromycin at a final concentration of 1 µg/mL for 3 days. Total RNA was extracted from the cell pellets. RNA was used for reverse transcription to cDNA, and the resulting cDNA was then used for digital PCR to quantify the relative mRNA expression levels of GOT1 normalized to β-actin, utilizing the QuantStudio™ Absolute Q™ Digital PCR System. Right panel: Mass isotopolog analysis of aspartate was performed in the cells in (**A**) after puromycin selection. $^{13}C_5$-glutamine was used as a tracer.(**D**) CellTiter-Blue assay was performed on the cells in **Figure 2F** with experiment setup as in panel (**A**). (**E**) Depletion of ASNS decreases conversion efficiency from $^{13}C_5$-glutamine to aspartate, glutamate and α-KG in NALM-6 cells depleted of ASNS, as described in **Figure 2F**. (**F**) ASNS does not modulate purine synthesis. Mass isotopolog analysis of purine nucleotides was performed in indicated NALM-6 cells cultured in $^{13}C_5$-glutamine-containing medium. All tracer experiments were conducted with a 24-h labeling period.