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Supplemental Information

Serine Metabolism Supports the Methionine Cycle and DNA/RNA Methylation through De Novo ATP Synthesis in Cancer Cells

Oliver D.K. Maddocks, Christiaan F. Labuschagne, Peter D. Adams, and Karen H. Vousden

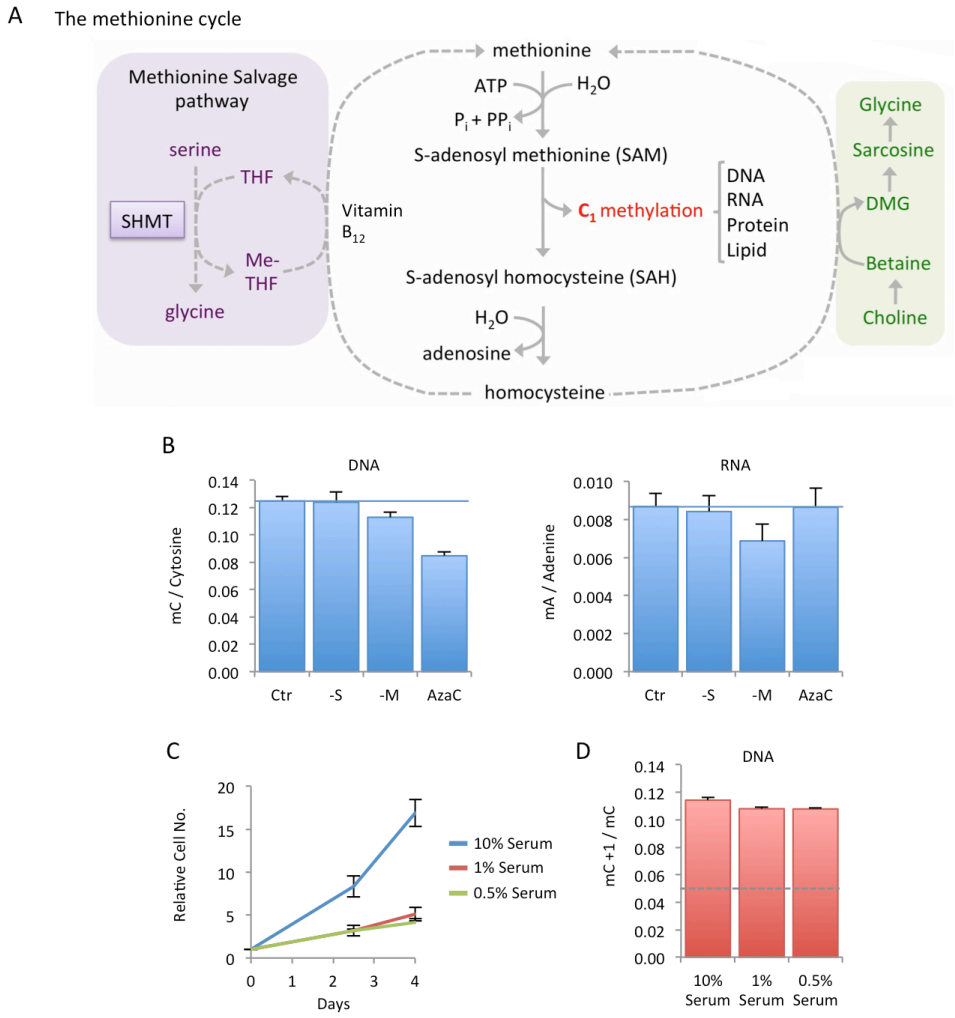

Figure S1 (related to Figure 1)
(A) Schematic diagram depicting the methionine cycle; S-adenosyl methionine (SAM) is the major cellular methyl (CH_3) donor in methylation reactions that modify and alter the function of a variety of substrates including proteins, nucleic acids and lipids. SAM can be regenerated by multiple pathways, here we focus on the serine/glycine/THF re-methylation pathway.
(B) RKO cells were grown for 24 hours in medium with serine and methionine (Ctr) or without serine (-S) or without methionine (-M). DNA and RNA were extracted and analysed by LCMS. The ratio of methyl cytosine (mC) to cytosine was calculated by dividing the peak areas of these metabolites. Azacytidine 0.5uM (AzaC) a DNA methyltransferase inhibitor was used as positive control for DNA specific demethylation. Data are averages of triplicate wells, error bars are SD.
(C) HCT116 cells were grown in varying levels of fetal calf serum and counted over time. Data are averages of triplicate wells, error bars are SD.
(D) HCT116 cells were grown in varying levels of fetal calf serum with unlabeled methionine for 6 hours followed by matched medium containing $^{13}C_5{}^{15}N_1$-labeled methionine for 3 hours. DNA was isolated and analysed by LCMS. Broken line indicates the background labeling expected due to natural ^{13}C carbon abundance. Data are averages of triplicate wells, error bars are SD.

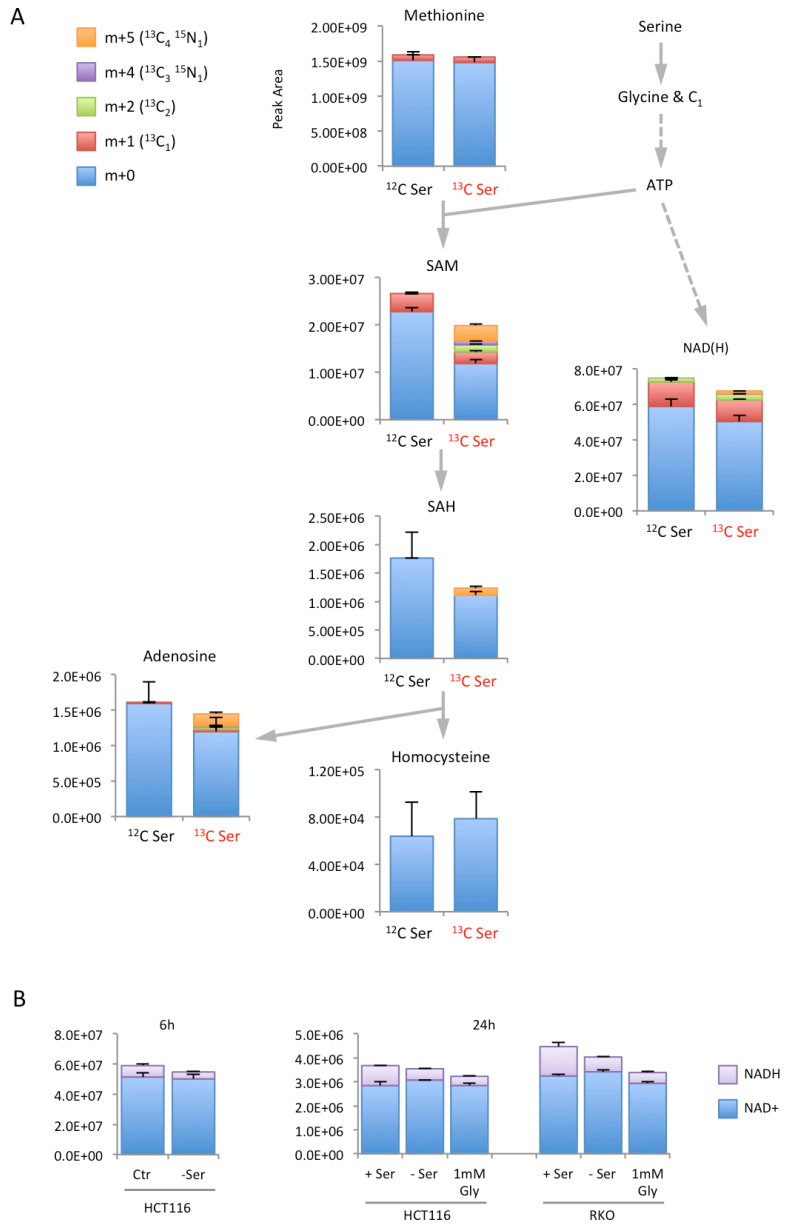

Figure S2 (related to Figure 4). Contribution of serine to the SAM cycle and NAD(H).
(A) HCT116 cells were either fed unlabeled or $^{13}C_3{}^{15}N_1$-labeled serine 0.4mM for three hours (in the presence of methionine 0.1mM). Metabolites were extracted and analysed by LCMS. The major isotopomers resulting from serine labeling are shown. Data are averages of triplicate wells, error bars are SD.
(B) HCT116 and RKO cells were grown with or without serine (+/-Ser) or without serine, plus high glycine (1mM Gly) for 6 to 24 hours and analysed by LCMS. Data are averages of triplicate wells, error bars are SD.

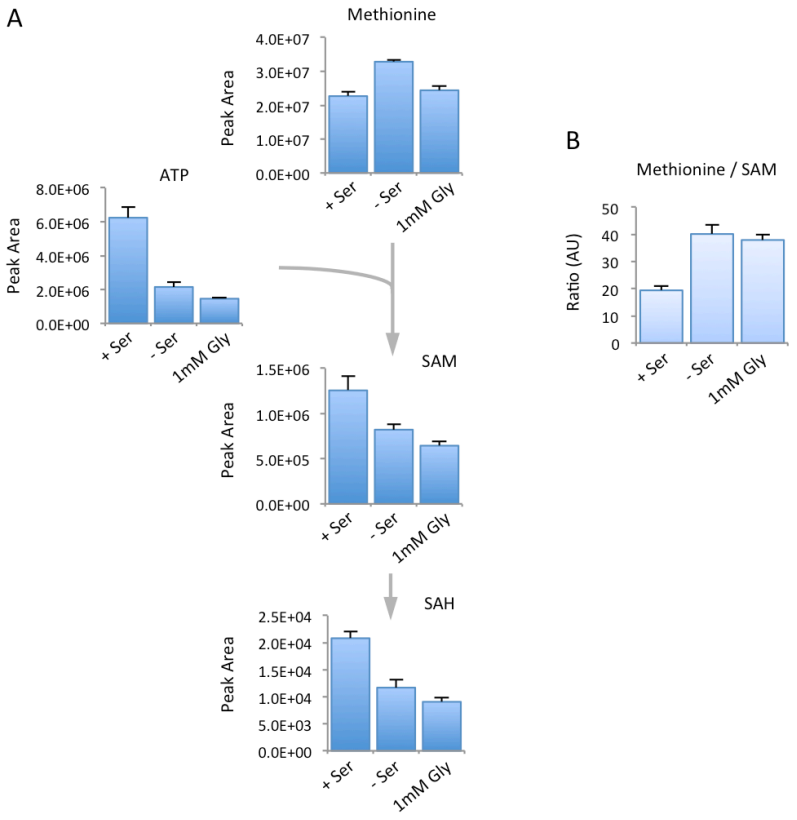

Figure S3 (related to Figure 5). Metabolic stresses which suppress *de novo* nucleotide synthesis increase the methionine / SAM ratio.
(A) RKO cells were grown with or without serine (+/-Ser) or without serine, plus high glycine (1mM Gly) for 24 hours and analysed by LCMS. Data are averages of triplicate wells, error bars are SD.
(B) The methionine / SAM ratio was calculated by dividing the peak areas of these metabolites. Data are averages of triplicate wells, error bars are SD.

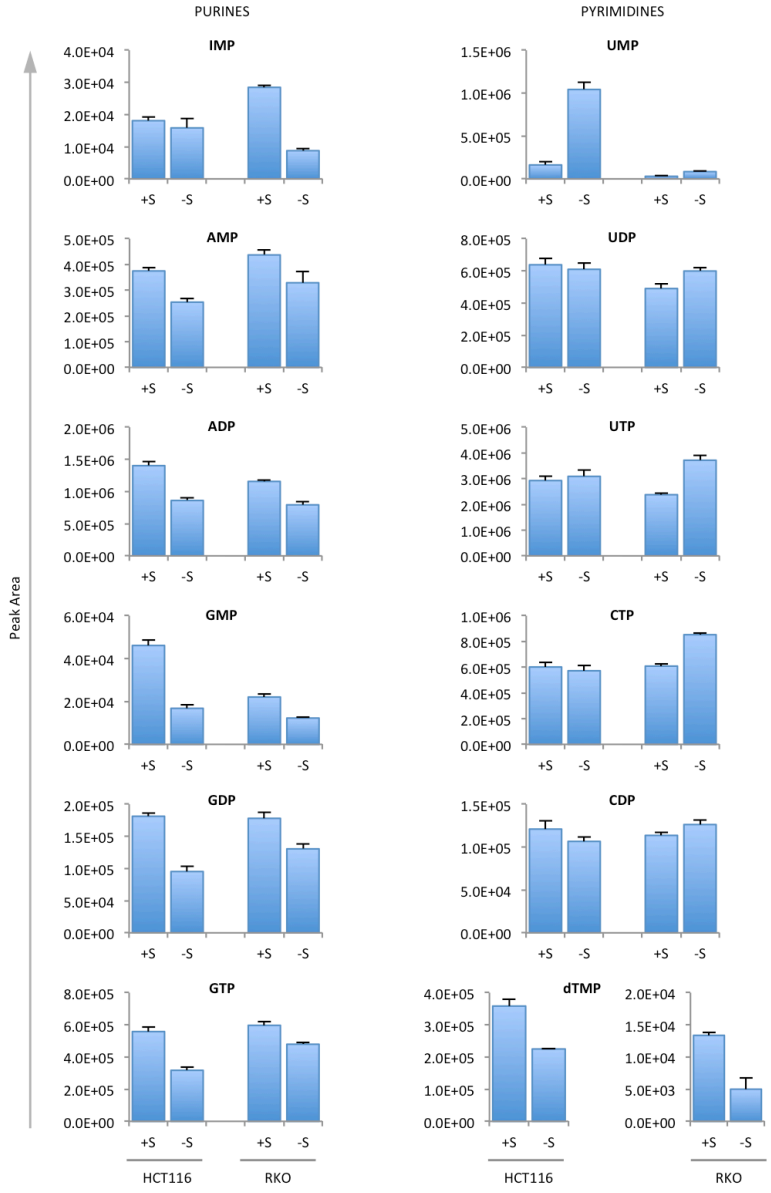

Figure S4 (related to Figure 5). Impact of serine starvation on purine and pyrimidine nucleotide levels.
HCT116 and RKO cells were grown with or without serine (+/-S 0.4mM) for 24 hours and analysed by LCMS. Data are averages of triplicate wells, error bars are SD.

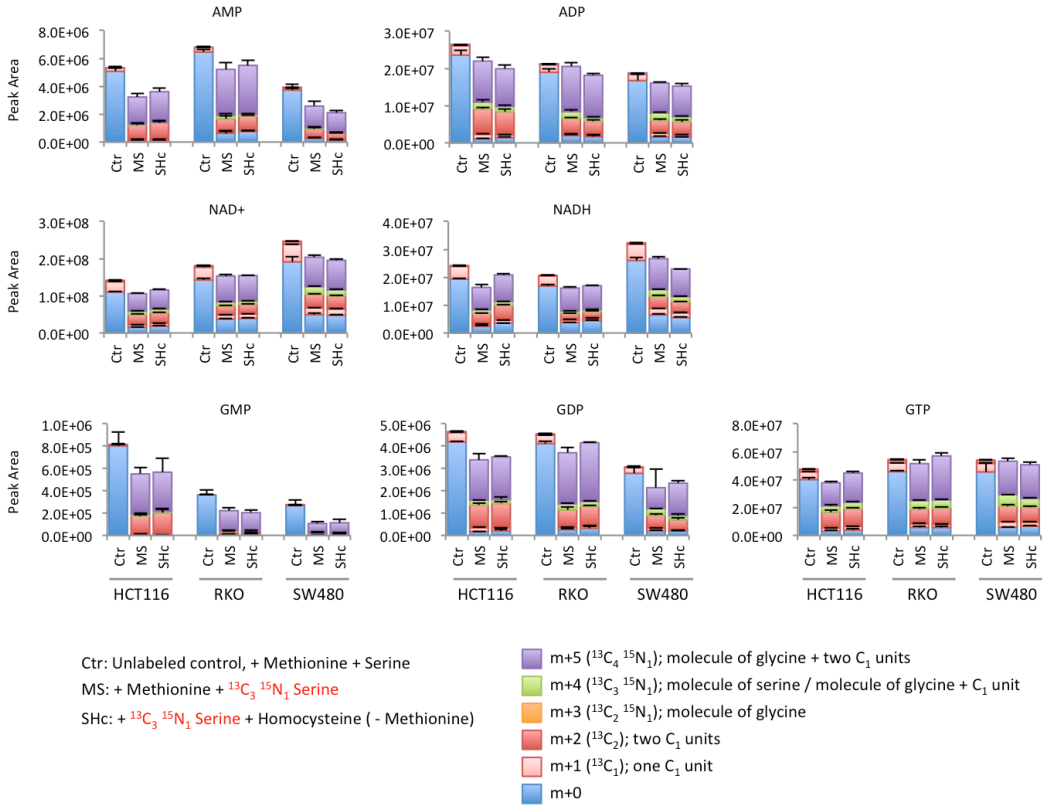

Figure S5 (related to Figure 7). Contribution of serine to *de novo* nucleotide synthesis.
(A) HCT116, RKO and SW480 cells were grown with unlabeled methionine 0.1mM plus $^{13}C_3{}^{15}N_1$-labeled serine 0.8mM (MS) or without methionine, with homocysteine 0.8mM, vitamin B_{12} 1uM, plus $^{13}C_3{}^{15}N_1$-labeled serine (SHc) for 24 hours, glycine was not included in any of the media. Metabolites were extracted and analysed by LCMS. The major isotopomers resulting from serine labeling are shown. Data are averages of triplicate wells, error bars are SD.

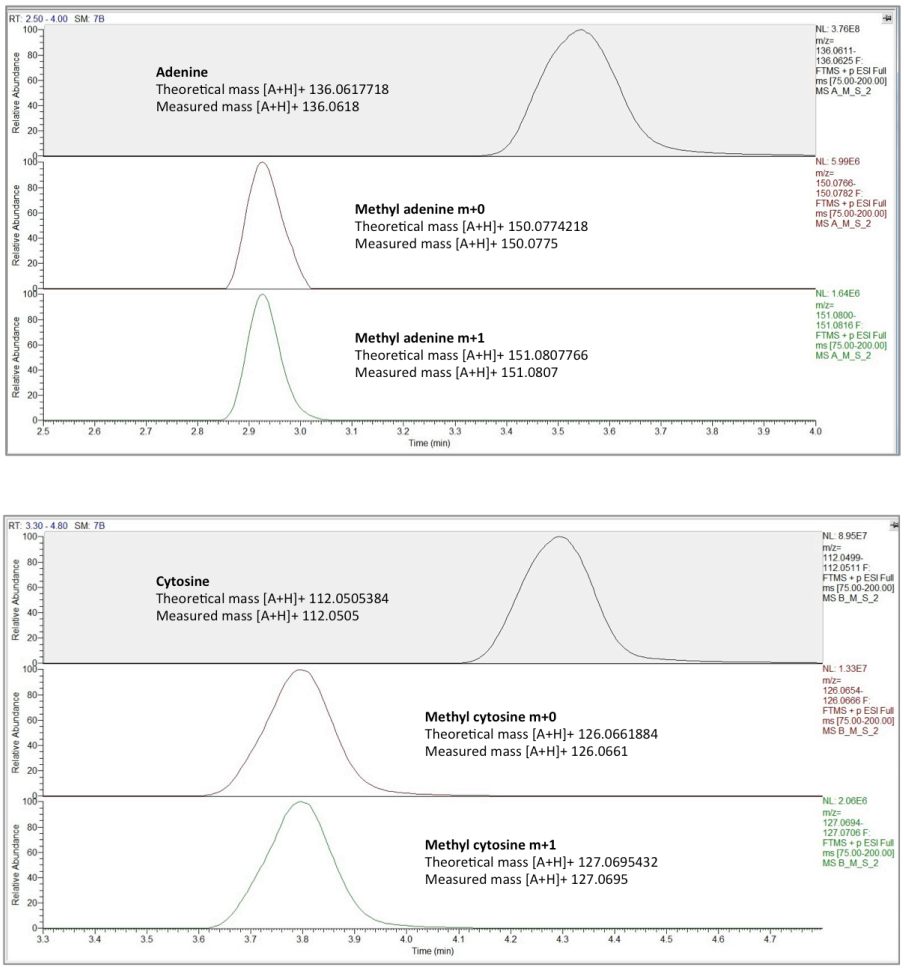

Figure S6 (related to experimental procedures). Chromatogram peaks for unmodified and methylated adenine and cytosine.
Example chromatogram peaks for adenine, methyl-adenine, cytosine and methyl cytosine, without (m+0) and with (m+1) ^{13}C carbon labeling. Adenine and methyl-adenine are the acid hydrolysis products of RNA-derived adenosine / methyl-adenosine, cytosine and methyl-cytosine are the acid hydrolysis products of DNA-derived cytidine / methyl-cytidine. For each molecule the theoretical (computed) mass and measured mass are shown next to the peak.

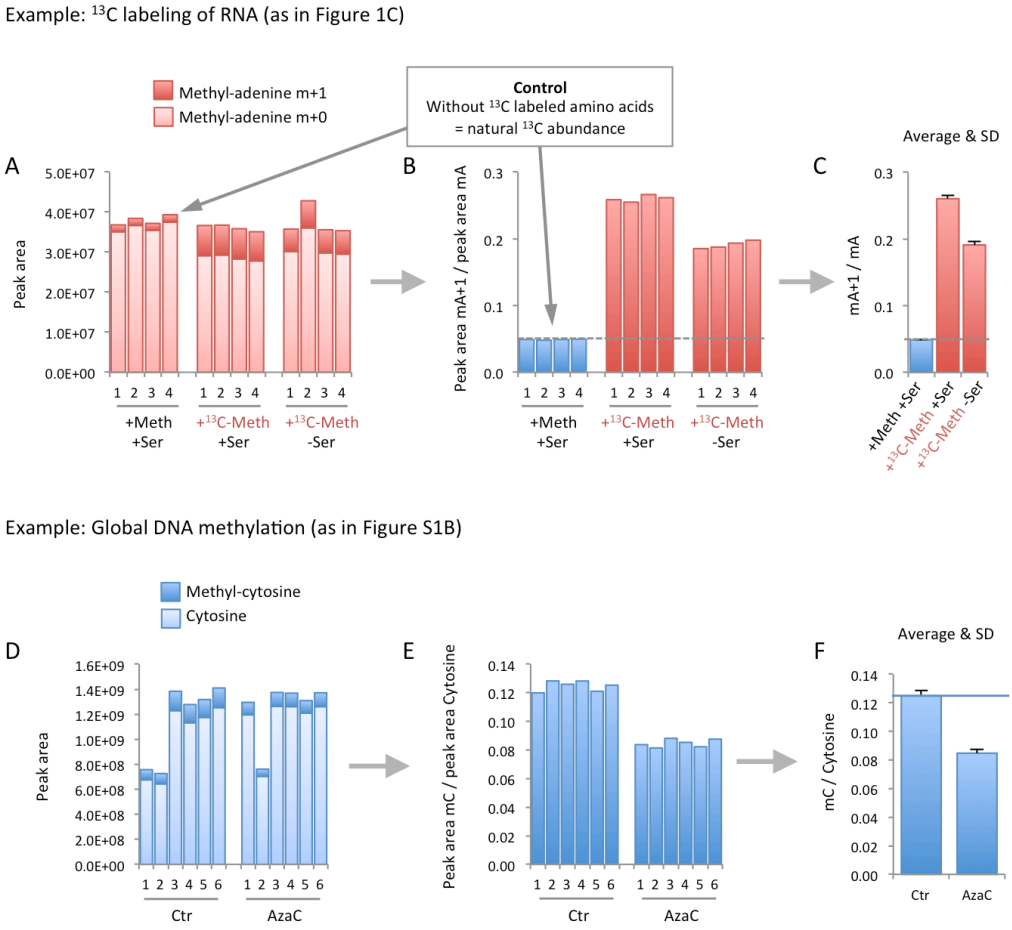

Figure S7 (related to experimental procedures). Presenting DNA / RNA methylation data
(A) Cells were grown in media either containing unlabeled methionine, or ^{13}C-labeled methionine with or without unlabeled serine for 3 hours. RNA was isolated and subjected to acid hydrolysis, followed by LCMS. The peak areas for m+0 (containing only ^{12}C) and m+1 (containing a single ^{13}C) methyl-adenine are plotted for four replicate samples (1-4) under each condition.
(B) The methyl-adenine m+1 (mA+1) peak area is divided by the methyl-adenine m+0 (mA) peak area to produce a ratio representing the relative abundance of the ^{13}C labeled (i.e. m+1) form. A small amount (~1%) of carbon in nature is the ^{13}C-isotope, hence an mA+1 peak is detected even when unlabeled methionine is used. This natural/background level of ^{13}C is presented as the control (blue) bars and the broken line.
(C) The data shown in (B) is averaged to produce the final presented data. In this example the cells fed labeled methionine show mA+1 abundance above natural levels (i.e. above the broken line) indicating transfer of ^{13}C methyl groups from ^{13}C-methionine onto RNA. The amount of ^{13}C is lower when cells are starved of serine (see Figure 1C).
(D) Cells were grown in media either with or without azacytidine (AzaC) for 24 hours. DNA was isolated and subjected to acid hydrolysis, followed by LCMS. The peak areas for cytosine and methyl cytosine are plotted for six replicate samples (1-6).
(E) The methyl-cytosine (mC) peak area is divided by the cytosine peak area to produce a ratio representing the relative abundance of methyl-cytosine.
(F) The data shown in (E) is averaged to produce the final presented data, in this example treatment with the DNA methyl-transferase inhibitor azacytidine caused a decrease in total methyl-cytosine levels in DNA (see Figure S1B).