Supporting Information for:

Glucose limitation alters glutamine metabolism in MUC1 overexpressing pancreatic cancer cells

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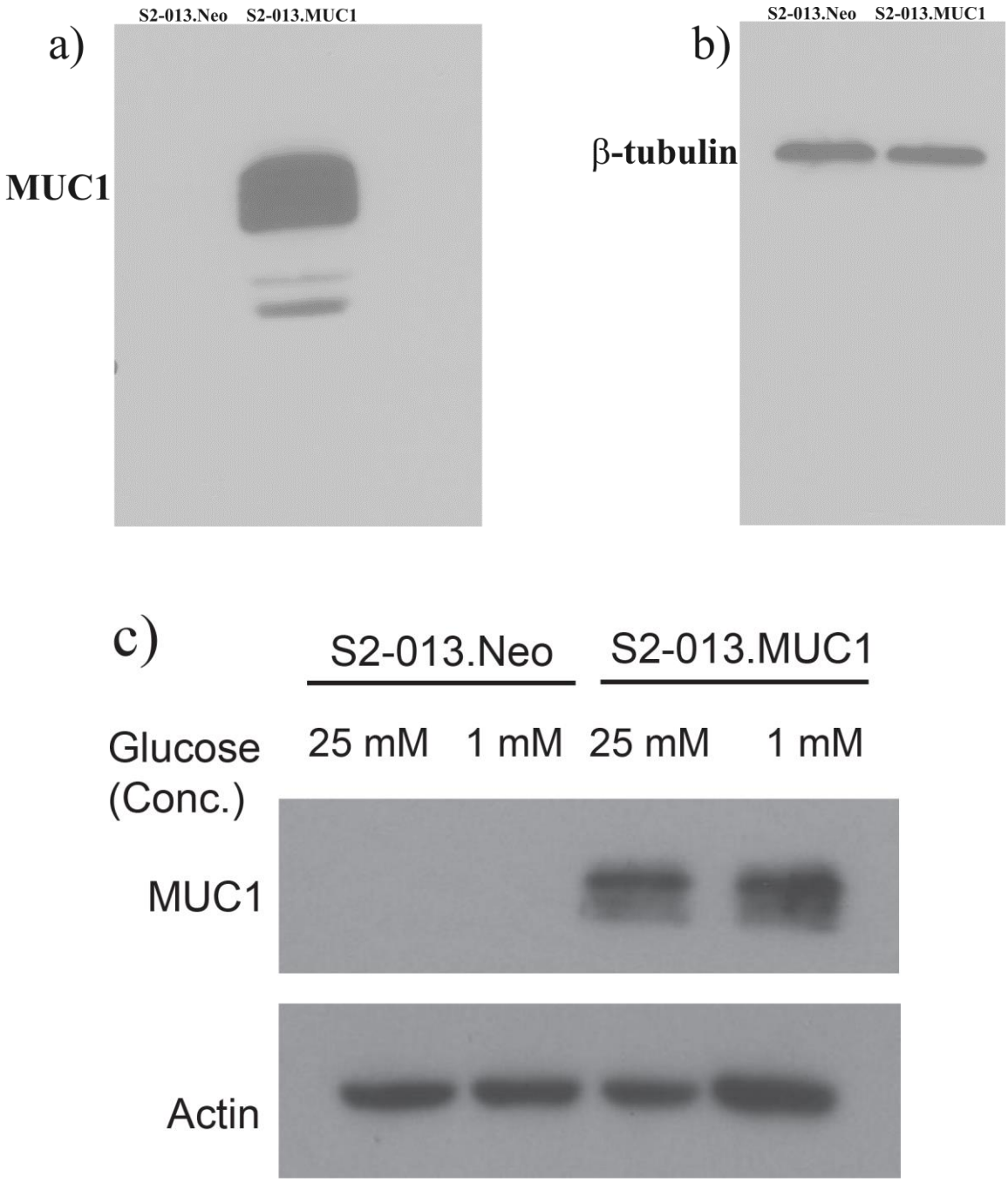

Figure S1: Western blot images of MUC1 overexpression in S2-013.Neo and S2-013.MUC1 cells. Gels were probed with either a (**a**) MUC1 antibody or a (**b**) β-tubulin antibody or antibodies against MUC1 and actin (**c**). Total protein was extracted from the cells using a radio immuno-precipitation assay buffer [10 mM Tris-Cl (pH 8.0), 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 140 mM NaCl, and 1 mM phenylmethanesulfonyl

fluoride], 30 µg of the total protein for each of the cell lines were electrophoresed, transferred to polyvinylidene difluoride membrane and probed with the respective antibodies. The Western blot images in a and b were zoomed in to regions of interest and then combined to create the composite image in Figure 1a.

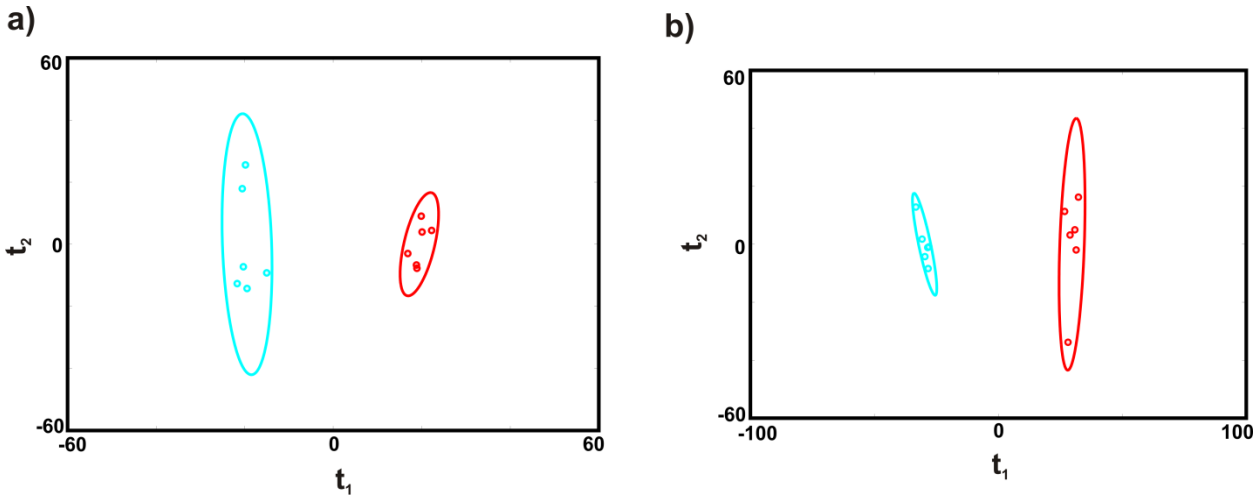

Figure S2: OPLS-DA scores plot generated from 1D ^{1}H NMR spectra of S2-013.Neo (red) and S2-013.MUC1 (cyan) cell extracts. **(a)** The cells were cultured in 25 mM glucose containing media. The OPLS-DA model has R^2 (0.99), Q2 (0.87) and CV-ANOVA *p*-value ($3.92x10^{-04}$). **(b)** The cells were cultured in 1 mM glucose supplemented media. The OPLS-DA model has R^2 (0.99), Q^2 (0.84) and CV-ANOVA *p*-value ($4.48x10^{-05}$).

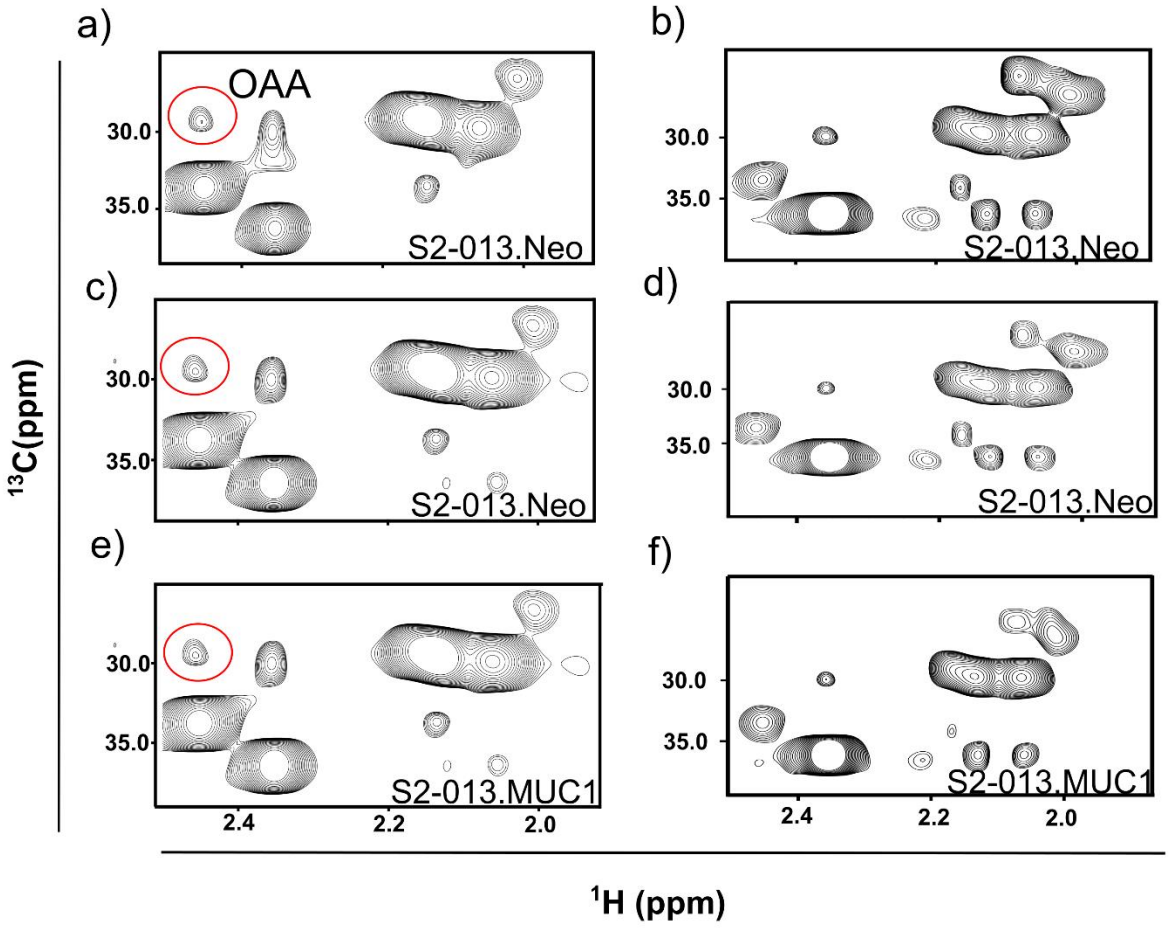

Figure S3: $^{13}C_3$ oxaloacetate originates from ^{13}C-labeled glutamine. Left panel; Expanded view of 2D ^{1}H-^{13}C HSQC spectra of S2-013.Neo cells cultured for 12 h in 2 mM U-$^{13}C_5$ glutamine and (a) 25 mM or (c) 1mM $^{12}C_6$ glucose; or (e) S2-013.MUC1 cells cultured for 12 h in 2 mM U-$^{13}C_5$ glutamine and 1 mM $^{12}C_6$ glucose. The OAA ^{1}H-$^{13}C_3$ NMR peaks are circled. Right panel**;** Same expanded view as left panel of 2D ^{1}H-^{13}C HSQC spectra of S2-013.Neo cells cultured for 12 h in 2 mM $^{12}C_5$ glutamine and (b) 25 mM or (d) 1mM $^{13}C_6$ glucose; or (f) S2-013.MUC1 cells cultured for 12 h in 2 mM $^{12}C_5$ glutamine and 1 mM $^{13}C_6$ glucose.

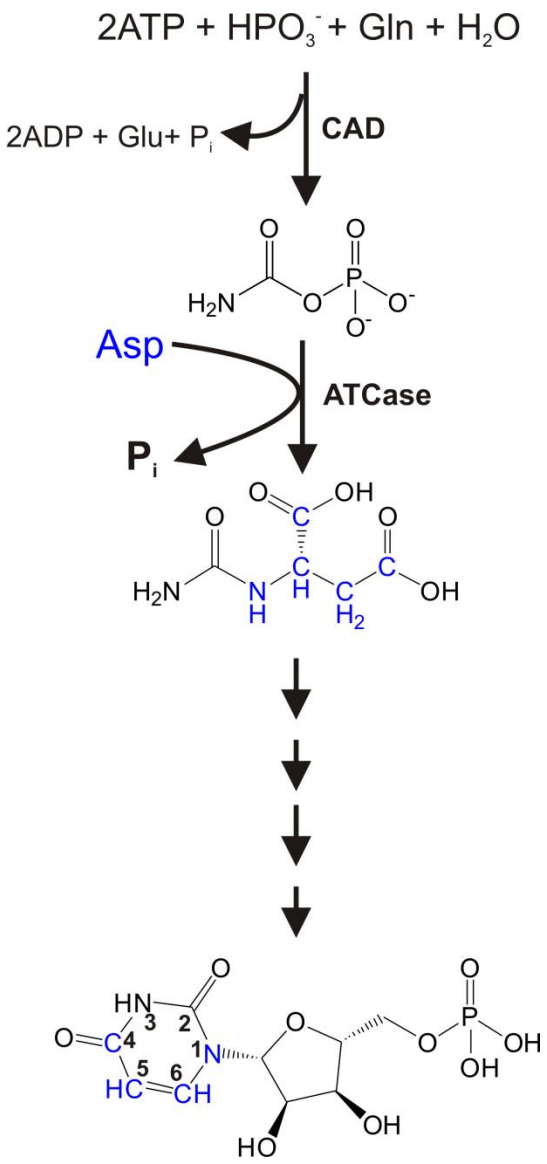

Figure S4: Metabolic scheme illustrating the incorporation of aspartate derived carbon atoms into a pyrimidine nucleotide. The C_5 and C_6 carbons of the nucleotide ring have an attached hydrogen and are observable in a 2D ^{1}H-^{13}C HSQC experiment.

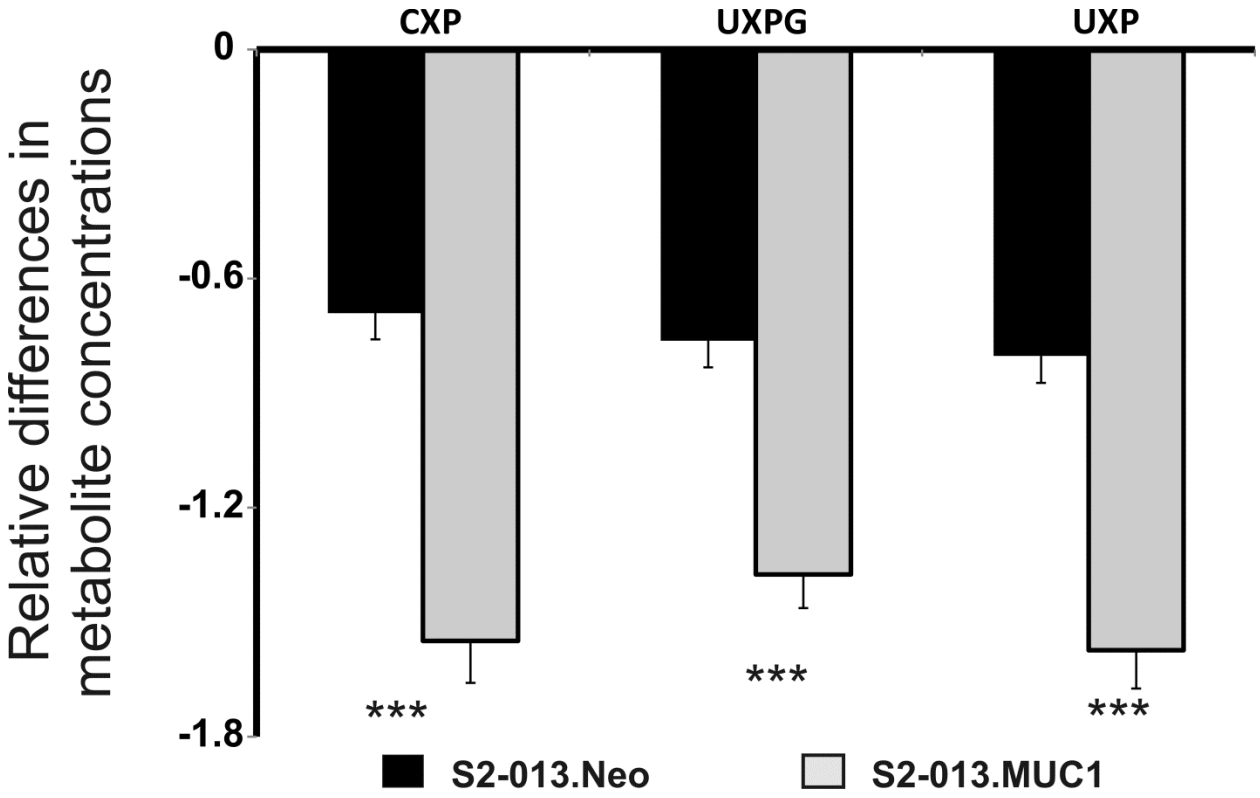

Figure S5: The incorporation of glutamine-derived carbons into pyrimidine nucleotides decreases significantly when S2-013.MUC1 cells are cultured under glucose limitation (1 mM glucose). The bar graph depicts the relative difference in pyrimidine nucleotide concentrations based on peak heights in 2D ^{1}H-^{13}C HSQC spectra after the cells were cultured with U-$^{13}C_5$ glutamine. The mean difference in the relative pyrimidine nucleotide concentrations is plotted between cells cultured under glucose limitation and steady state (25 mM glucose) conditions. The means are the results of triplicate experiments. The error bars are calculated using error propagation for two means. (*** $P < 0.001$).