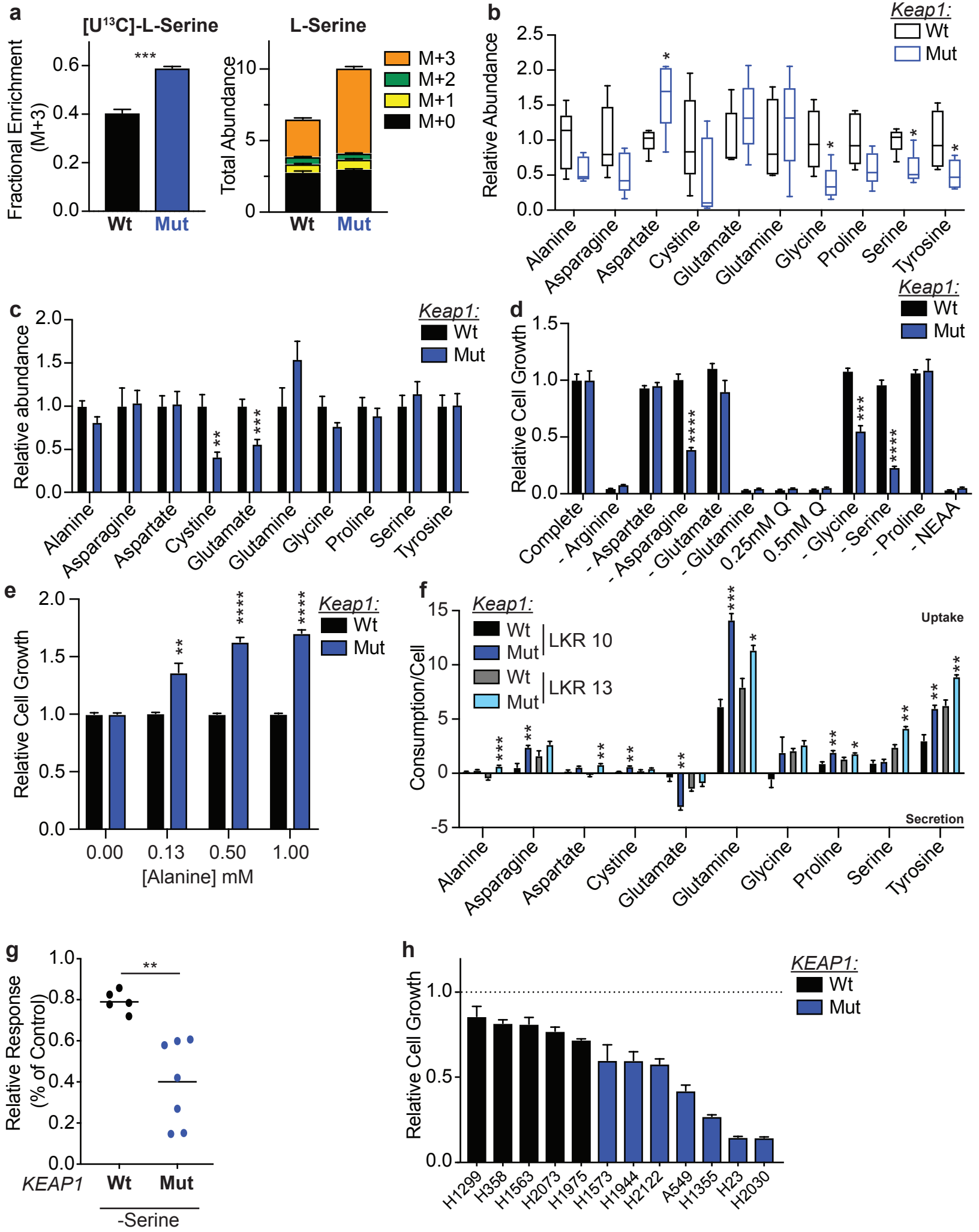
Supplementary Figure 1 - Related to Figure 1

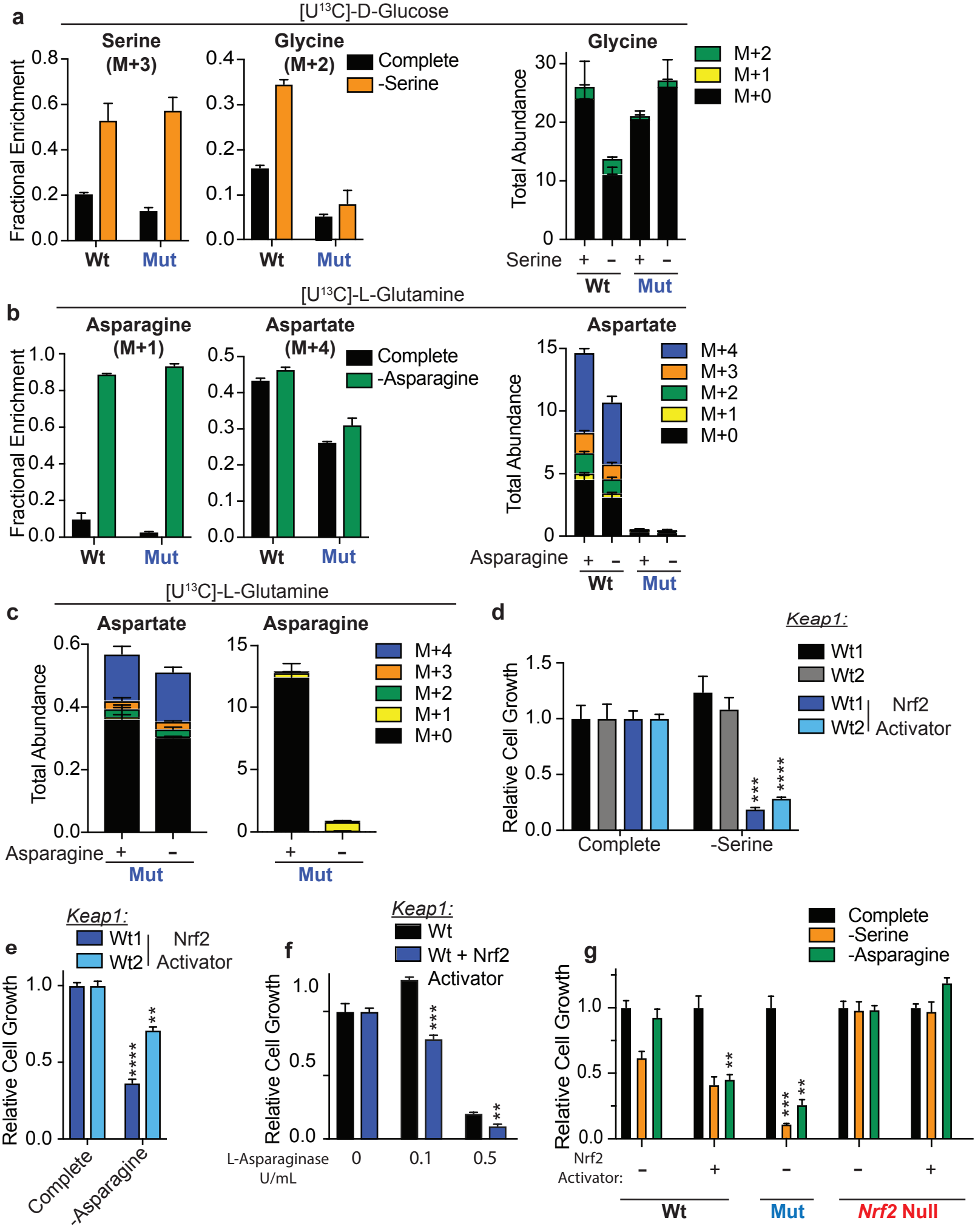


Supplementary Figure Legends

Supplementary Figure 1 – Related to Figure 1

a) Mass isotopomer analysis of serine in wildtype (Wt) and *Keap1* mutant (Mut) cells cultured for 3 hours with [$U^{13}C$]**-**L-serine (Left). Total abundance of serine in wildtype and *Keap1* mutant cells depicted in left panel (right). **b)** Measurement of serum level of non-essential amino acids in mice bearing wildtype (Wt) or *Keap1* mutant (Mut) subcutaneous tumors. Full panel of detectable metabolites that are represented in Figure 1b. **c)** Measurement of non-essential amino acids in wildtype (Wt) or *Keap1* mutant (Mut) tumors. **d)** Proliferation of wildtype (Wt) vs *Keap1* mutant (Mut) cells in RPMI media lacking specified amino acid. Full panel of deprived amino acids that is represented in Figure 1c. Data is represented as relative to growth in complete media condition for each cell line. **e)** Proliferation of wildtype (Wt) vs *Keap1* mutant (Mut) cells in RPMI (10% dialyzed FBS) supplemented with alanine. Data is represented as relative to growth in complete media condition for each cell line. **f)** *In vitro* uptake assay comparing uptake of amino acids in wildtype (black/grey) or *Keap1* mutant (dark/ light blue) LKR ($Kras^{G12D/+}$;$p53^{+/+}$) murine lung adenocarcinoma cell lines after 24 hours in RPMI media. **g)** Proliferation of a panel of human lung adenocarcinoma cell lines in RPMI lacking serine Each individual point represents an independent cell line. Response is represented as relative to growth in complete media conditions for each cell line. **h)** Proliferation of human lung adenocarcinoma cell lines shown in panel e in RPMI lacking serine. Data is represented as relative to proliferation in complete media for each cell line. All error bars depict s.e.m. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

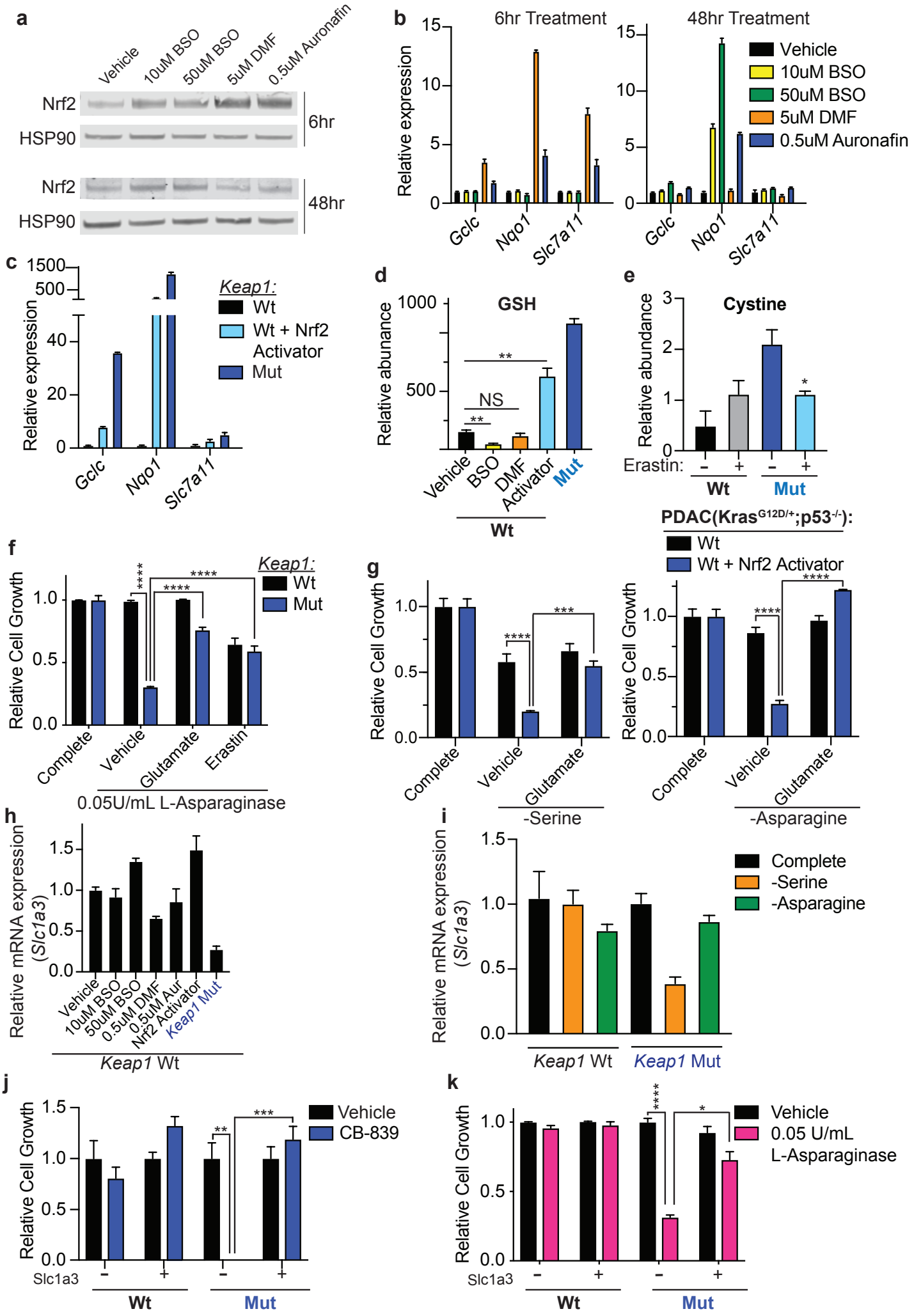
Supplementary Figure 2 - Related to Figure 1 & 2



Supplementary Figure 2 – Related to Figure 1 & 2

a) Left panels: Mass isotopomer analysis of serine and glycine in wildtype vs *Keap1* mutant cells in complete media vs RPMI lacking serine. Cells were cultured in [$U^{13}C$]-D-glucose for 1 hour. Right panel: Relative abundance of glycine in wildtype vs *Keap1* mutant cells in complete media vs RPMI lacking serine (from left panel). Relative pool sizes are normalized to cell counts for each condition. **b)** Left panels: Mass isotopomer analysis of asparagine and aspartate in wildtype vs *Keap1* mutant cells in complete media vs RPMI lacking asparagine. Cells were cultured in [$U^{13}C$]-L-glutamine for 1 hour. Right panel: Relative abundance of aspartate in wildtype vs *Keap1* mutant cells in complete media vs RPMI lacking asparagine (from left panel). Relative pool sizes are normalized to cell counts for each condition. **c)** Relative abundance of aspartate in *Keap1* mutant cells from panel b and relative abundance of asparagine in *Keap1* mutant cells from Figure 1h. **d)** Proliferation of wildtype cells in complete media or RPMI lacking serine. Cells were pretreated with 1μM of Nrf2 activator (KI696) where indicated. Data is represented as relative to proliferation in complete media for each cell line. **e)** Proliferation of wildtype cells that were pretreated with 1μM of Nrf2 activator (KI696) in complete media or RPMI lacking asparagine. Data is represented as relative to proliferation in complete media for each cell line. **f)** Proliferation of wildtype cells treated with L-Asparaginase. Cells were pretreated with 1μM Nrf2 activator (KI696) where indicated. Data is represented as relative to proliferation in complete media for each cell line. **g)** Proliferation of wildtype (Wt), *Keap1* mutant (Mut), and *Nrf2* null cells in complete media or media lacking serine or asparagine. Where indicated, cells were treated with 1μM of Nrf2 activator (KI696). Data is represented as relative to proliferation in complete media for each cell line. All error bars depict s.e.m. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

Supplementary Figure 3 - Related to Figure 2 & 3

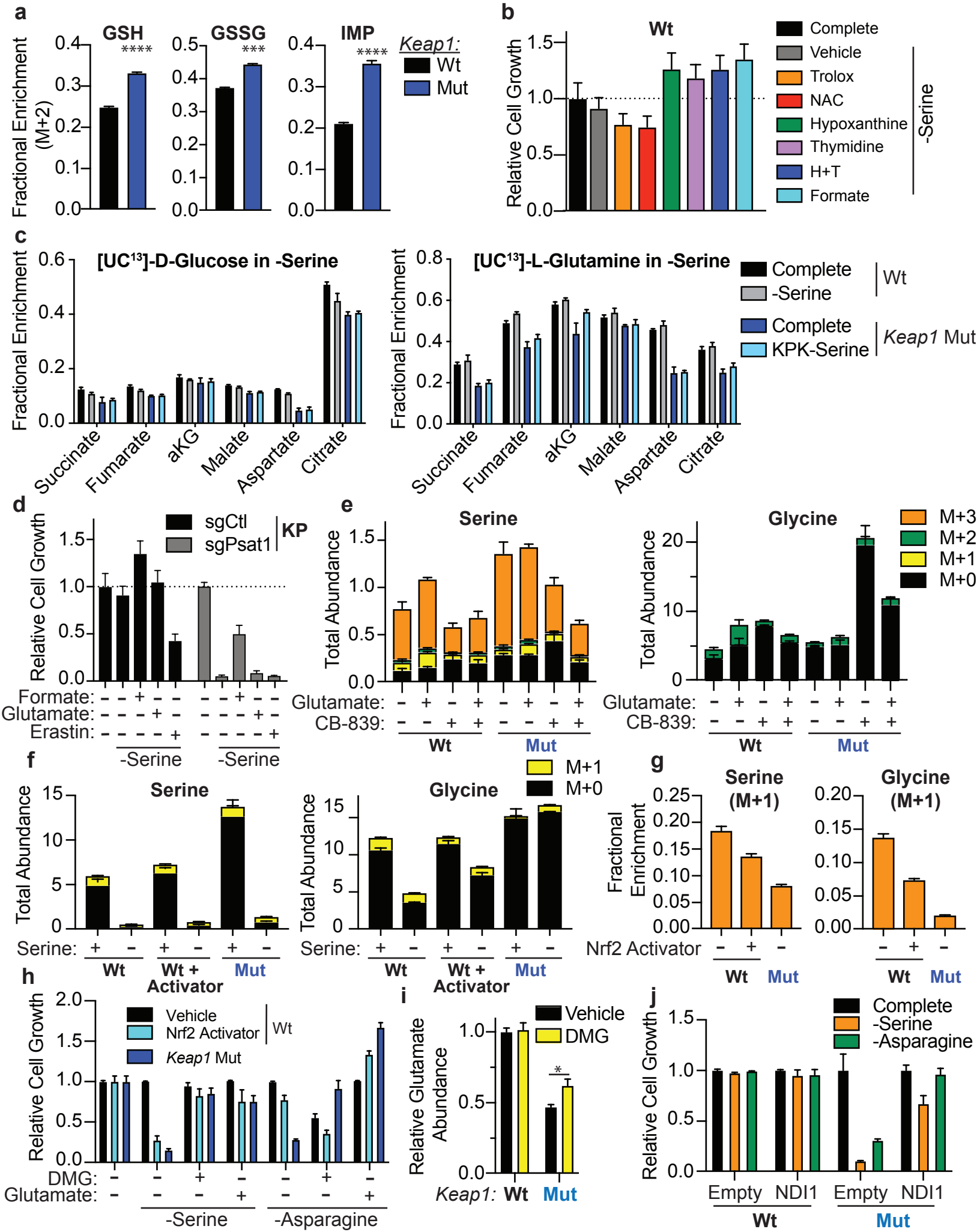


Supplementary Figure 3 – Related to Figure 2 & 3

a) Western blot of wildtype cells treated with vehicle,10μM BSO, 50μM BSO, 5μM DMF, or 0.5μM auronafin after 6 or 48 hours of treatment. HSP90 is used as a loading control. **b)** qPCR of Nrf2 target genes in wildtype cells after treatment with vehicle,10μM BSO, 50μM BSO, 5μM DMF, or 0.5μM auronafin after 6 or 48 hours of treatment. Data is represented as relative to the house keeping gene β-actin and gene expression in vehicle treated controls. **c)** qPCR of Nrf2 target genes in wildtype and *Keap1* mutant cells. Wildtype cells were pretreated with 1μM of Nrf2 activator (KI696) where indicated. Data is represented as relative to the house keeping gene β-actin and gene expression in wildtype cells. **d)** Measurement of relative glutathione levels in wildtype cells treated with vehicle, 50μM BSO or 5μM DMF for 6 hours and *Keap1* mutant cells. Where indicated, wildtype cells were pretreated with1μM of Nrf2 activator (KI696). **e)** Relative abundance of intracellular cystine in wildtype (Wt) or *Keap1* mutant (Mut) cells treated with 500nM Erastin. Total metabolite pool sizes are normalized to cell counts for each condition. **f)** Proliferation of wildtype (Wt) vs *Keap1* mutant (Mut) cells treated with L-asparaginase. Cells were supplemented with 6mM glutamate or 500nM Erastin where indicated. Data is represented as relative to proliferation in complete media for each cell line. **g)** Proliferation of murine PDAC ($Kras^{G12D/+};p53^{-/-}$) cell line in media lacking serine (left) or asparagine (right). Cells were pre-treated with 6mM glutamate and 1μM of Nrf2 activator (KI696) where indicated. Data is represented as relative to proliferation in complete media. **h)** qPCR of *Slc1a3* in wildtype and *Keap1* mutant cells. Wildtype cells were treated with vehicle,10μM BSO, 50μM BSO, 5μM DMF, or 0.5μM auronafin for 48 hours or pretreated with 1μM of Nrf2 activator (KI696) where indicated. Data is represented as relative to the house keeping gene β-actin and gene expression in vehicle treated controls. **i)** qPCR of *Slc1a3* in wildtype and *Keap1* mutant cells after being cultured in complete media or media lacking serine or asparagine. Data is represented as relative to the house keeping gene β-actin and gene expression in vehicle

treated controls. **j)** Proliferation of wildtype (Wt) vs Keap1 mutant (Mut) cells expressing Slc1a3 or an empty vector control after treatment with 250nM CB-839. Data is represented as relative to proliferation in complete media for each cell line. **k)** Proliferation of wildtype (Wt) vs *Keap1* mutant (Mut) cells expressing Slc1a3 or an empty vector control after treatment with L-asparaginase. Data is represented as relative to proliferation in complete media for each cell line. All error bars depict s.e.m. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Supplementary Figure 4 - Related to Figure 4

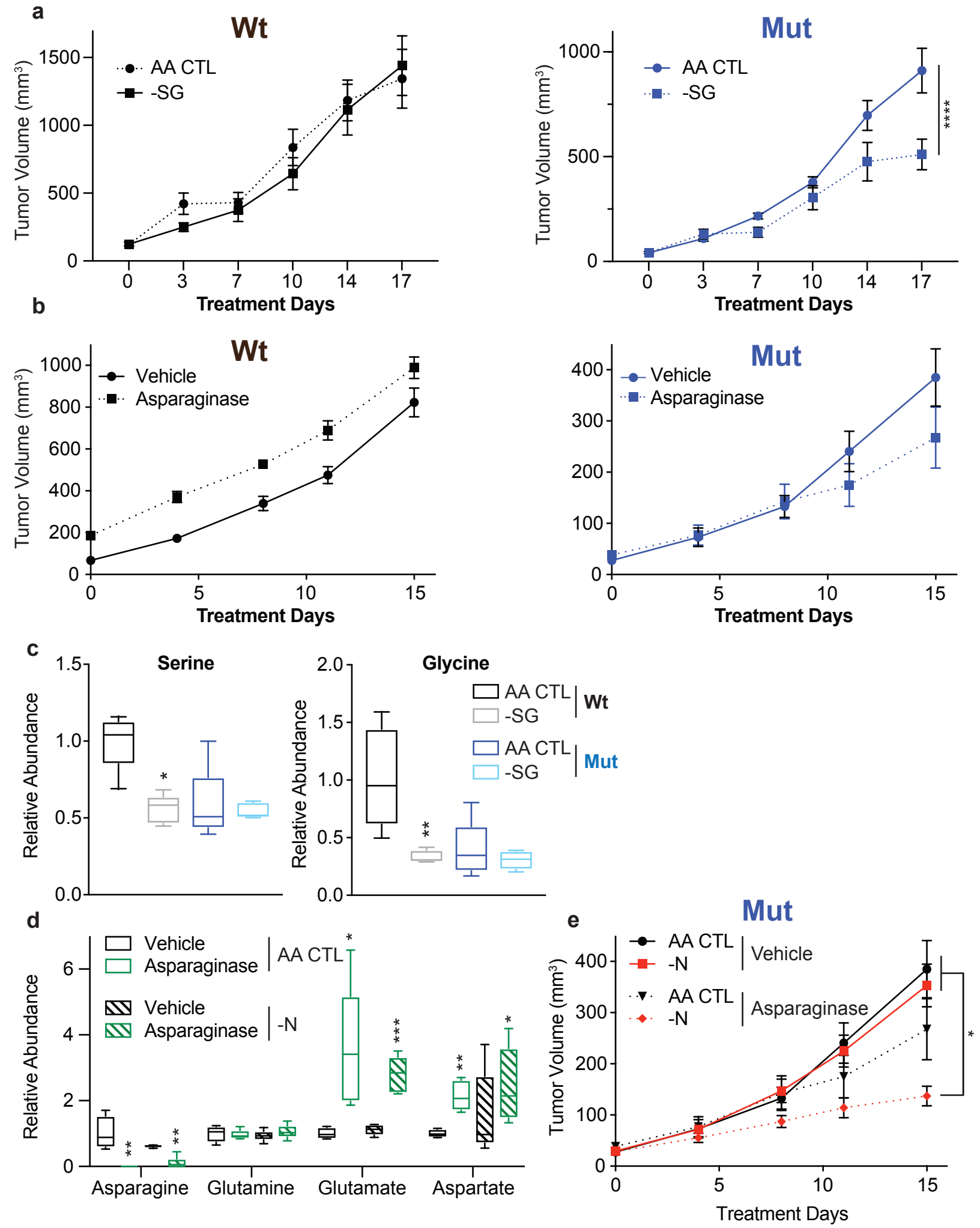


Supplementary Figure 4 – Related to Figure 4

a) Mass isotopomer analysis of glutathione (GSH), glutathione disulfide (GSSG, oxidized form of GSH) and inosine mono-phospate (IMP) in wildtype (Wt) and *Keap1* mutant (Mut) cells. Cells were cultured for 3 hours with [UC^{13}]-L-glycine. **b)** Proliferation of wildtype cells in RPMI lacking serine. Where indicated cells were supplemented with 3mM Formate, 30 μM hypoxanthine, 16 μM thymidine, 30 μM hypoxanthine and 16 μM thymidine (H+T), 50 μM Trolox, or 0.5 mM NAC. Data is represented as relative proliferation in vehicle treated complete media. **c)** Mass isotopomer analysis of TCA cycle intermediates in wildtype (Wt) and *Keap1* mutant (Mut) cells in complete media or RPMI lacking serine. Cells were cultured with [UC^{13}]-D-glucose (left) or [UC^{13}]-L-glutamine (right) for 1 hour. **d)** Proliferation wildtype cells expressing an sgRNA against PSAT or a non-targeting control (sgCtl) in media lacking serine. Cells were supplemented with 3mM Formate, 6mM glutamate, or 500nM Erastin where indicated. Data is represented as relative proliferation in vehicle treated complete media for each cell line. **e)** Relative abundance of serine and glycine in wildtype (Wt) vs *Keap1* (Mut) mutant cells depicted from Figure 4d. Relative pool sizes are normalized to cell counts for each condition. **f)** Relative abundance of serine and glycine in wildtype vs *Keap1* mutant cells depicted from Figure 4f. Relative pool sizes are normalized to cell counts for each condition. **g)** Mass isotopomer analysis of serine and glycine in wildtype (Wt) vs *Keap1* mutant (Mut) cells cultured in complete media. Wildtype cells were pre-treated with 1μM of Nrf2 activator (KI696) where indicated. **h)** Proliferation of wildtype (Wt) and *Keap1* mutant (Mut) cells in media lacking serine or asparagine. Cells were pretreated with 2mM DMG, 6mM Glutamate or 1μM of Nrf2 activator (KI696) where indicated. Data is represented as relative proliferation in vehicle treated complete media. **i)** Total abundance of intracellular glutamate in wildtype (Wt) vs *Keap1* mutant (Mut) cells cultured with 2mM DMG. Total metabolite pool sizes are normalized to cell counts for each condition. **j)** Proliferation of wildtype (Wt) vs *Keap1* mutant (Mut) cells expressing NDI1 or an empty vector control in media

lacking serine or asparagine. Data is represented as relative proliferation in vehicle treated complete media. All error bars depict s.e.m. *p<0.05, **p<0.01, ***p<0.001

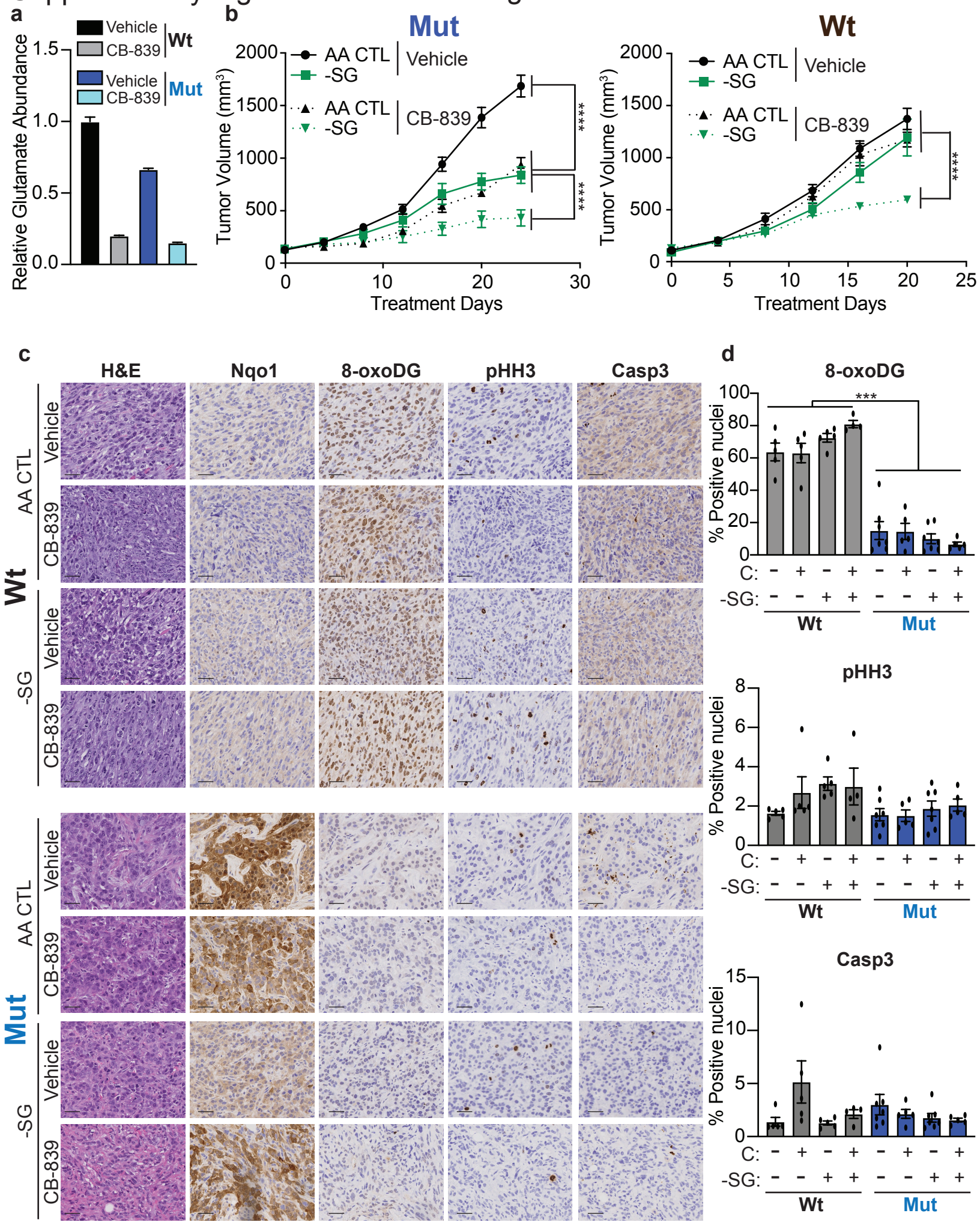
Supplementary Figure 5 - Related to Figure 5



Supplementary Figure 5 – Related to Figure 5

a) Tumor volumes of subcutaneous wildtype (left) and *Keap1* mutant (right) tumors on AA CTL or -SG diet from Figure 5a. **b)** Tumor volumes of subcutaneous wildtype (left) and *Keap1* mutant (right) tumors treated with vehicle or L-asparaginase from Figure 5b. **c)** Measurement of serum level of serine and glycine in mice bearing wildtype (black/grey) or *Keap1* mutant (blue/light blue) subcutaneous tumor receiving amino acid control (AA CTL) diet or a diet lacking serine and glycine (-SG). Serum analyzed from mice from Figure 5a. **d)** Measurement of serum level of asparagine, glutamine, glutamate and aspartate in mice bearing *Keap1* mutant subcutaneous tumors treated vehicle or L-asparaginase and receiving either AA CTL or -N diet. Serum analyzed from mice from Figure 5c & d. **e)** Tumor volumes of subcutaneous wildtype and *Keap1* mutant tumors on AA CTL or -N diet. All error bars depict s.e.m. *$p<0.05$, **$p<0.01$, ****$p<0.0001$

Supplementary Figure 6 - Related to Figure 5



Supplementary Figure 6 – Related to Figure 5

a) Relative abundance of intracellular glutamate in wildtype (Wt) vs *Keap1* mutant (Mut) cells treated with CB-839. Total metabolite pool sizes are normalized to cell counts for each condition. **b)** Tumor volumes of subcutaneous *Keap1* mutant (left) and wildtype (right) tumors on AA CTL or -SG diet and treated with CB-839 or vehicle from Figure 5e & f. **c)** Representative images from wildtype or *Keap1* mutant subcutaneous tumors from mice on AA CTL or -SG diet and either treated with vehicle or CB-839 (from figure 5e & f). Paraffin embeded sections were stained with hematoxylin and eosin (H&E), anti-Nqo1, andti-8-oxo-deoxiguanosine (8-oxoDG), anti-phospho-histone-H3 (pHH3) or anti-cleaved caspase 3 (Casp3). Scale bar represents 40μM **c)** Quantification of percentage of positive nuclei for indicated stains. All error bars depict s.e.m. *p<0.05, **p<0.01, ****p<0.0001

Table S1. Oligo Sequences, Related to STAR methods section

Description: oligonucleotides table

qPCR oligos		
Gene Name	**Forward Sequence**	**Reverse Sequence**
Nqo1	agcgttcggtattacgatcc	agtacaatcagggctcttctcg
Slc7a11	gattcatgtccacaagcacac	gagcatcaccatcgtcagag
Gclc	agatgatagaacacgggaggag	accagattgggagggaacat
ActB	ctaaggccaaccgtgaaaag	accagaggcatacagggaca

CRISPR sgRNA oligos		
gRNA Target	**Forward Sequence**	**Reverse Sequence**
Non Targeting (sgCtl)	cacccgccctcgatctcgaactcg	aaaccgagttcgagatcgagggcg
Psat (sgPsat)	caccgcaatacagagaatcttgtga	aaactcacaagattctctgtattgc