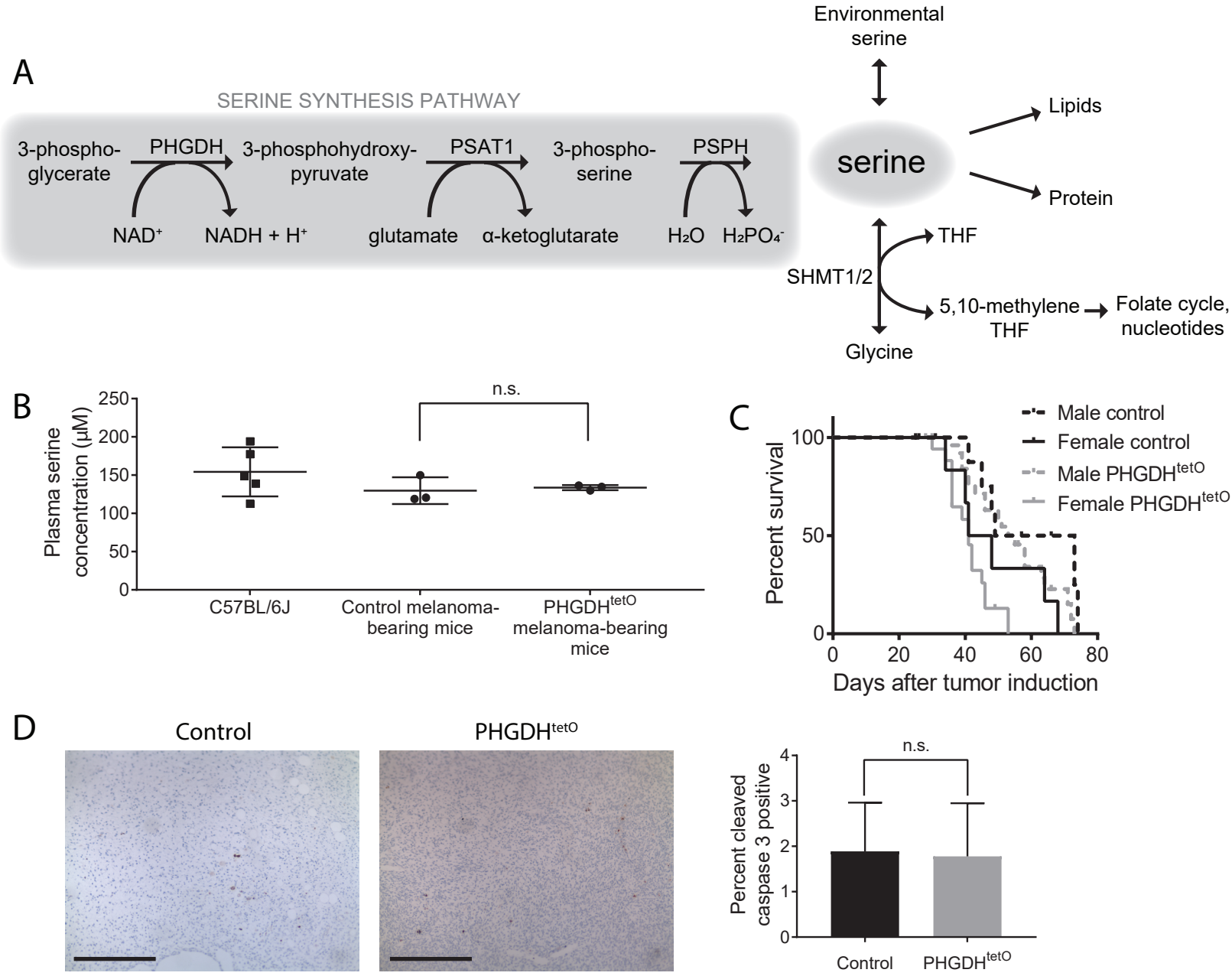

Figure S1. Related to Figure 1; PHGDH expression does not alter circulating serine levels or apoptotic cell death in *Braf*^{CA}; *PTEN* $^{-/-}$ melanoma-bearing mice. **(A)** Schematic showing the serine synthesis pathway from glucose and how serine contributes to biomass in cells. PHGDH, phosphoglycerate dehydrogenase; PSAT1, phosphoserine amino transferase 1; PSPH, phosphoserine phosphatase; SHMT1/2, serine hydroxymethyltransferase 1/2; THF, tetrahydrofolate. **(B)** Plasma serine concentration in C57BL/6J mice, control *Braf*^{CA}; *PTEN* $^{-/-}$ mice bearing melanomas, and PHGDH^{tetO} *Braf*^{CA}; *PTEN* $^{-/-}$ mice bearing melanomas as measured by GC/MS. Plasma from tumor bearing mice was collected 1 month after tumor induction. There is no statistically significant difference in plasma serine concentration between control and PHGDH^{tetO} tumor bearing mice based on an unpaired, two-tailed Welch's t test with a p-value of 0.7330. n = 3 mice for control and PHGDH^{tetO} mice and n = 5 for C57BL/6J mice. **(C)** Kaplan-Meier plot showing survival of control and PHGDH expressing (PHGDH^{tetO}) mice bearing *Braf*^{CA}; *PTEN* $^{-/-}$ tumors as described in Figure 1, with survival of animals separated by gender. **(D)** Representative immunohistochemistry staining and quantitation of cleaved caspase 3 (CC3) in the same *Braf*^{CA}; *PTEN* $^{-/-}$ melanomas analyzed in Figure 1H that arose in mice without (control) or with (PHGDH^{tetO}) a PHGDH transgene. Images were obtained at 10x magnification, scale bar = 300 μm. CC3 staining was quantitated by scoring 300 cells as CC3 positive or negative for each of 5 tumors of each genotype. No statistically significant difference was observed based on a Welch's t test that yielded a p value of 0.9094. Values represent the mean +/- SD.

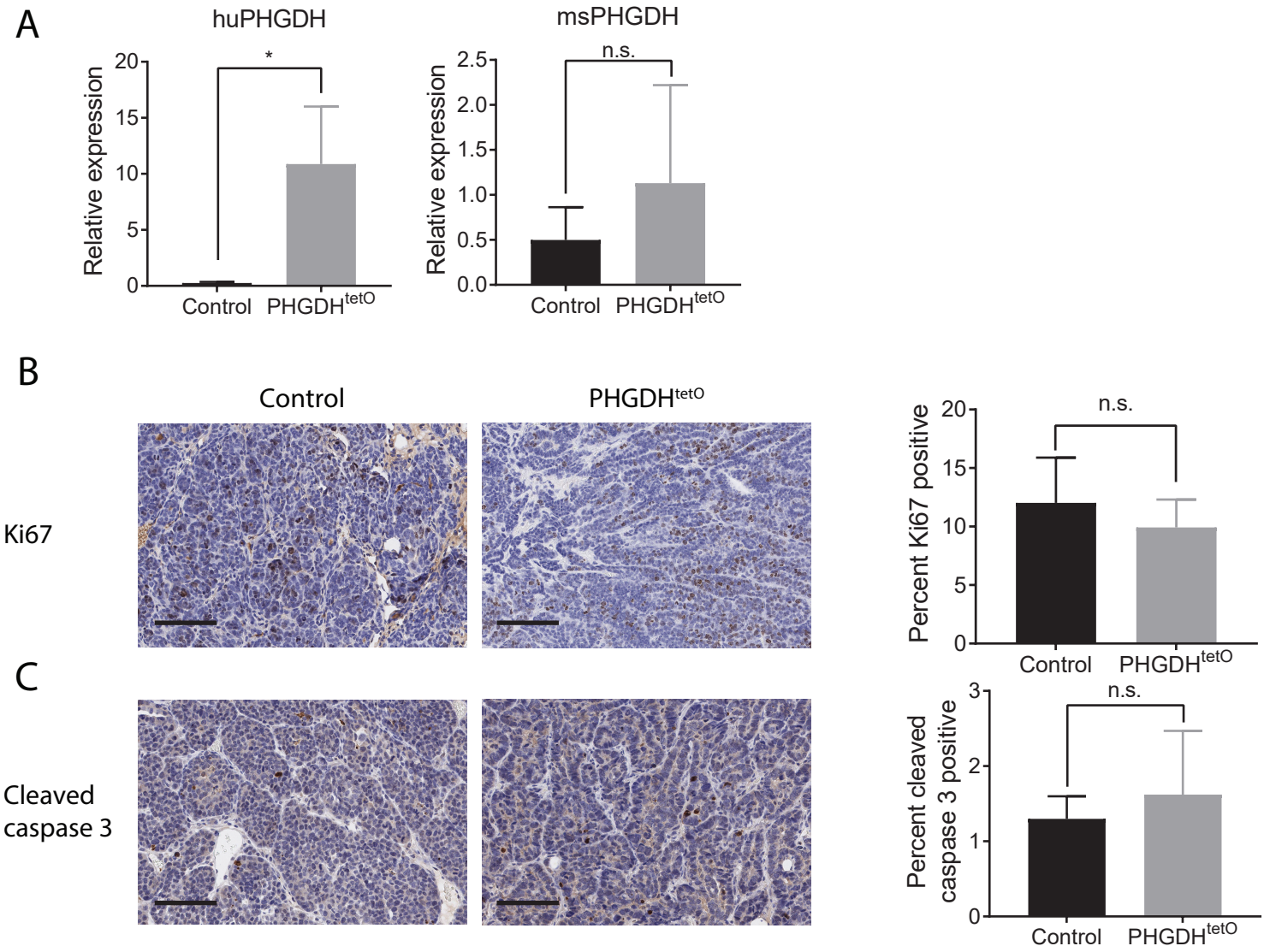

Figure S2. Related to Figure 1; End stage *BRCA*$^{fl/fl}$; *Trp53*$^{+/-}$ breast tumors have similar rates of proliferation and apoptosis regardless of PHGDH^{tetO} status. (A) Species specific RT-qPCR for human (huPHGDH) and mouse (msPHGDH) PHGDH in *BRCA*$^{fl/fl}$; *Trp53*$^{+/-}$ breast tumors described in Figure 2. The difference in huPHGDH expression is statistically significant with p = 0.0497 by an unpaired, two-tailed Welch's t test, and the difference in msPHGDH expression is not statistically significant, with p = 0.1101 by an unpaired, two-tailed Welch's t test. n=10 tumors per genotype (B) Representative immunohistochemistry staining for Ki67 in control and PHGDH^{tetO} *BRCA*$^{fl/fl}$; *Trp53*$^{+/-}$ breast tumors. Images were obtained at 20x magnification, scale bar = 100 µm. Ki67 staining in an area of 1.5 mm by 0.7 mm was quantitated for 5 tumors of each genotype, and no statistically significant difference was observed based on a Welch's t test that yielded a p value of 0.6598. (C) Representative immunohistochemistry staining for cleaved caspase 3 (CC3) in control and *PHGDH*^{tetO}; *BRCA*$^{fl/fl}$; *Trp53*$^{+/-}$ breast tumors. Images were obtained at 20x magnification, scale bar = 100 µm. CC3 staining was quantitated by scoring 300 cells as CC3 positive or negative for each of 5 tumors of each genotype. No statistically significant difference was observed based on a Welch's t test that yielded a p value of 0.7345. For all panels, the values represent the mean +/- SD.

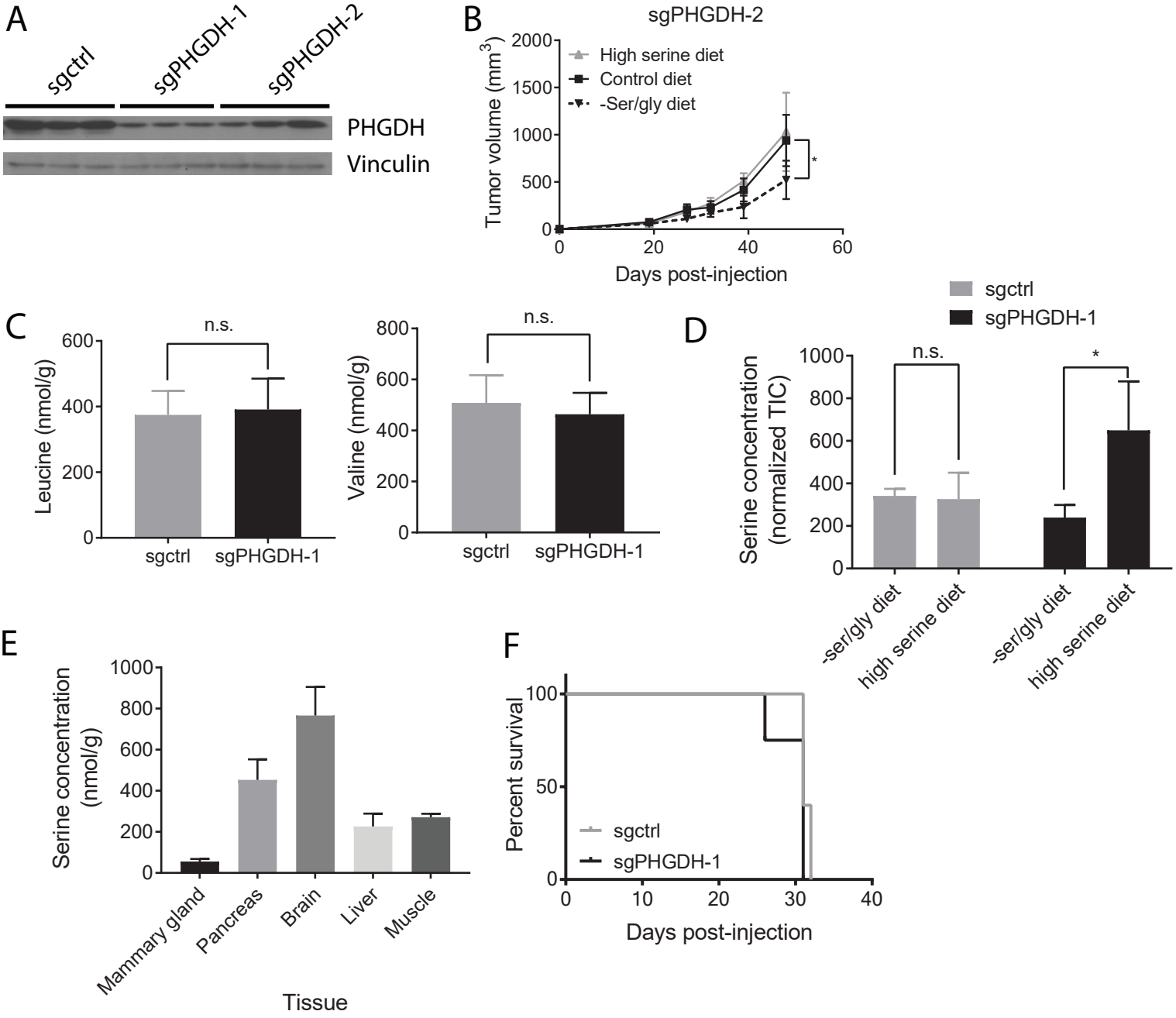

Figure S3. Related to Figure 3; Dependence on dietary serine for tumor growth is negatively correlated with both PHGDH expression level and tissue serine levels. (A) Breast cancer cells from an autochthonous *BRCA*$^{fl/fl}$; *Trp53*$^{+/-}$ *MMTV-Cre* breast tumor with either a non-targeting sgRNA (sgctrl), or an sgRNA targeting PHGDH (sgPHGDH-1, sgPHGDH-2) were injected into the mammary fat pad of female NSG mice. Western blot analysis of PHGDH expression in tumors derived from these mice is shown. **(B)** Measurement of the tumor size over time in mice fed the specified diets, for tumors derived from injecting *BRCA*$^{fl/fl}$; *Trp53*$^{+/-}$ *MMTV-Cre* breast tumor cells with either a non-targeting sgRNA (sgctrl) or an sgRNA targeting PHGDH (sgPHGDH-2) into the mammary fat pad of female NSG mice. There was a statistically significant difference in final tumor volume between mice fed –ser/gly and control diets based on an unpaired, two-tailed Welch's t test which yielded a p value of 0.0345. n=5 mice on each diet. **(C)** Concentration of the essential amino acids leucine and valine in control tumors (sgctrl) compared to PHGDH knockdown tumors (sgPHGDH-1) as measured by GC/MS. There was no statistically significant change in leucine or valine concentration based on an unpaired, two-tailed Welch's t test that produced p values of 0.7919 for leucine and 0.5803 for valine. n=4 tumors for each genotype. **(D)** LC/MS measurement of total ion counts (TIC) of serine normalized to tumor weight in control (sgctrl) and PHGDH knockdown (sgPHGDH-1) mammary fat pad orthotopic tumors formed from murine cell lines in mice fed either a –ser/gly diet or a high serine diet. No statistically significant change in serine levels were detected between sgctrl tumors fed a –ser/gly diet or a high serine diet as determined by an unpaired, two-tailed Welch's t test that yielded a p value of 0.8208. There was a statistically significant difference in serine levels between sgPHGDH-1 tumors in mice fed either a –ser/gly diet or a high serine diet as determined by an unpaired, two-tailed Welch's t test that yielded a p value of 0.0134. n = 4 tumors for each genotype. **(E)** Total serine amount measured in tissues from C57BL/6J mice by GC/MS normalized to tissue weight. n = 4 mice for each tissue. **(F)** Kaplan-Meier plot showing survival of NSG mice for which the same number of control (sgctrl) or PHGDH knockdown (sgPHGDH-1) cells derived from autochthonous breast tumors were implanted into the pancreas. There was no statistically significant difference in survival based on a Mantel-Cox log-rank test with a p-value of 0.1050. n = 5 mice per condition. For all panels, the values represent the mean +/- SD.

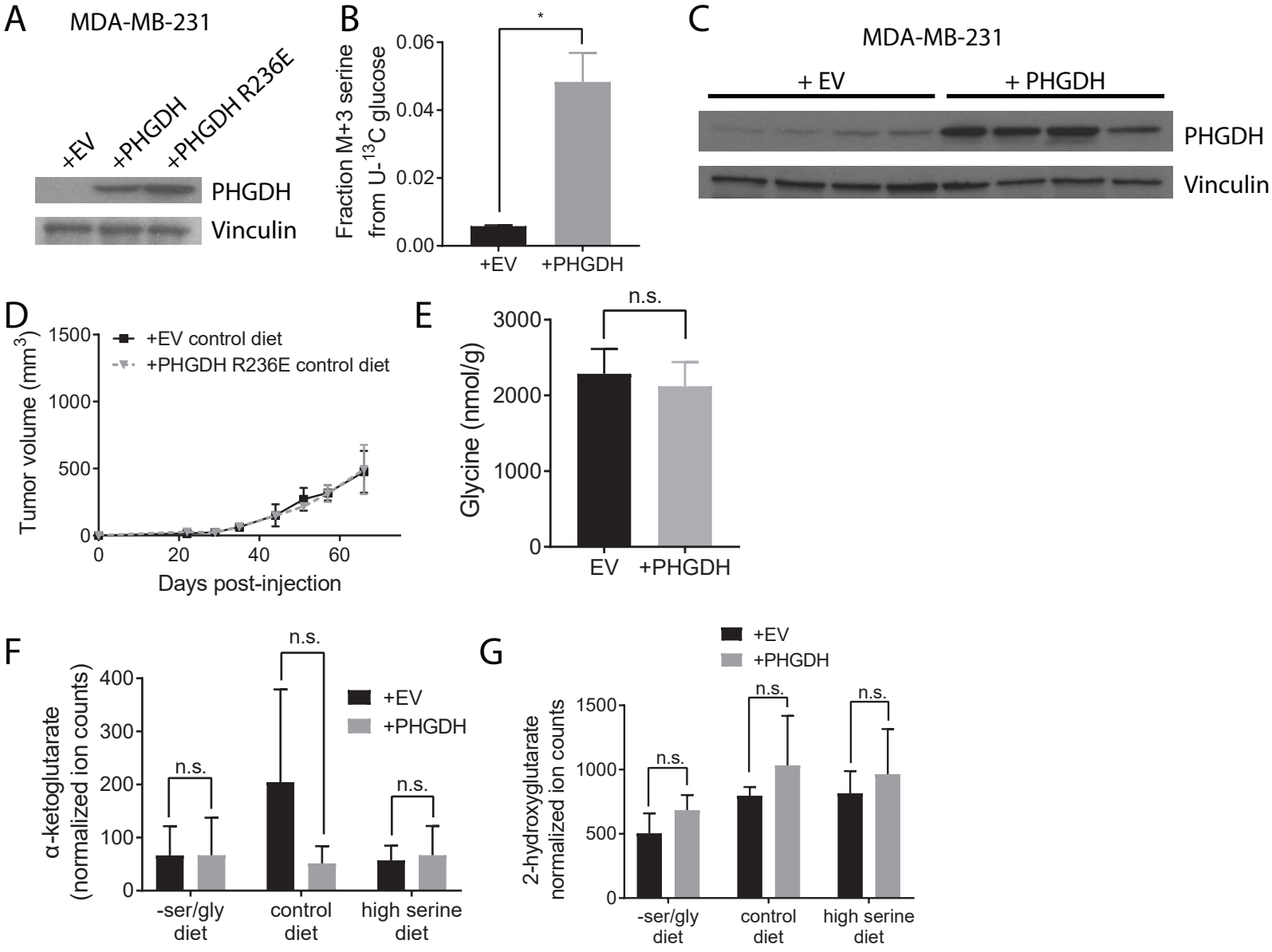

Figure S4. Related to Figure 4; **(A)** Western blot of MDA-MB-231 breast cancer cell lines with an empty vector control (+EV), PHGDH (+PHGDH), or PHGDH with an R236E mutation (PHGDH R236E) cultured *in vitro*. **(B)** Fraction of ^{13}C-labeled serine (fully labeled, M+3) in in control (+EV) or PHGDH expressing (+PHGDH) MDA-MB-231 cells cultured in U-^{13}C glucose as measured by GC/MS. There is a statistically significant difference in the fraction of glucose-derived serine between the cell types with a p-value of 0.0130 derived from an unpaired, Welch's t test. **(C)** Western blot of PHGDH expression in empty vector control (+EV) and PHGDH expressing (+PHGDH) MDA-MB-231 mammary fat pad orthotopic tumors. **(D)** Empty vector control (+EV) or PHGDH R236E expressing (+PHGDH R236E) MDA-MB-231 breast cancer cells were injected into the mammary fat pad of female NSG mice fed a control diet. Tumor size over time is shown. n=5 mice for each genotype. **(E)** GC/MS measurement of glycine concentration in empty vector control (EV) and PHGDH expressing (+PHGDH) MDA-MB-231 mammary fat pad orthotopic tumors. No statistically significant change in glycine concentration was detected as determined by an unpaired, two-tailed Welch's t test that yielded a p value of 0.7330. **(F)** Amount of α-ketoglutarate in empty vector control (EV) and PHGDH expressing (+PHGDH) MDA-MB-231 mammary fat pad orthotopic tumors in mice fed either a –ser/gly diet, a control diet, or a high serine diet as measured by LC/MS. No statistically significant change in α-ketoglutarate levels were detected between +EV and +PHGDH tumors as determined by unpaired, two-tailed Welch's t tests that yielded p values of 0.9965, 0.1356, and 0.7359, respectively. **(G)** Amount of 2-hydroxyglutarate in empty vector control (EV) and PHGDH expressing (+PHGDH) MDA-MB-231 mammary fat pad orthotopic tumors in mice fed either a –ser/gly diet, a control diet, or a high serine diet as measured by LC/MS. No statistically significant change in 2-hydroxyglutarate levels were detected between +EV and +PHGDH tumors as determined by unpaired, two-tailed Welch's t tests that yielded p values of 0.0726, 0.2707, and 0.4290, respectively. n=5 tumors for each genotype for **(E)**, **(F)**, and **(G)**. For all panels, the values represent the mean +/- SD.

Table S2. Related to STAR Methods; Nutrient composition of serine diets.

Nutrient	Serine and glycine free diet (g/kg)	Control diet (g/kg)	High serine diet (g/kg)
L-Alanine	3.5	3.5	3.5
L-Arginine HCl	12.1	12.1	12.1
L-Asparagine	6.0	6.0	6.0
L-Aspartic Acid	3.5	3.5	3.5
L-Cystine	3.5	3.5	3.5
L-Glutamic Acid	40.0	40.0	40.0
Glycine	0	23.3	23.3
L-Histidine HCl, monohydrate	4.5	4.5	4.5
L-Isoleucine	8.0	8.0	8.0
L-Leucine	12.0	12.0	12.0
L-Lysine HCl	18.0	18.0	18.0
L-Methionine	8.2	8.2	8.2
L-Phenylalanine	7.5	7.5	7.5
L-Proline	3.5	3.5	3.5
L-Serine	0	3.5	20.0
L-Threonine	8.2	8.2	8.2
L-Tryptophan	1.8	1.8	1.8
L-Tyrosine	5.0	5.0	5.0
L-Valine	8.0	8.0	8.0
Sucrose	100.0	100.0	100.0
Corn Starch	407.88	381.18	364.58
Maltodextrin	150.0	150.0	150.0
Soybean Oil	80.0	80.0	80.0
Cellulose	50.0	50.0	50.0
Mineral Mix, AIN-93M-MX (94049)	35.0	35.0	35.0
Calcium Phosphate, monobasic, monohydrate	8.2	8.2	8.2
Vitamin Mix, AIN-93-VX (94047)	13.0	13.0	13.0
Choline Bitartrate	2.5	2.5	2.5
TBHQ, antioxidant	0.02	0.02	0.02