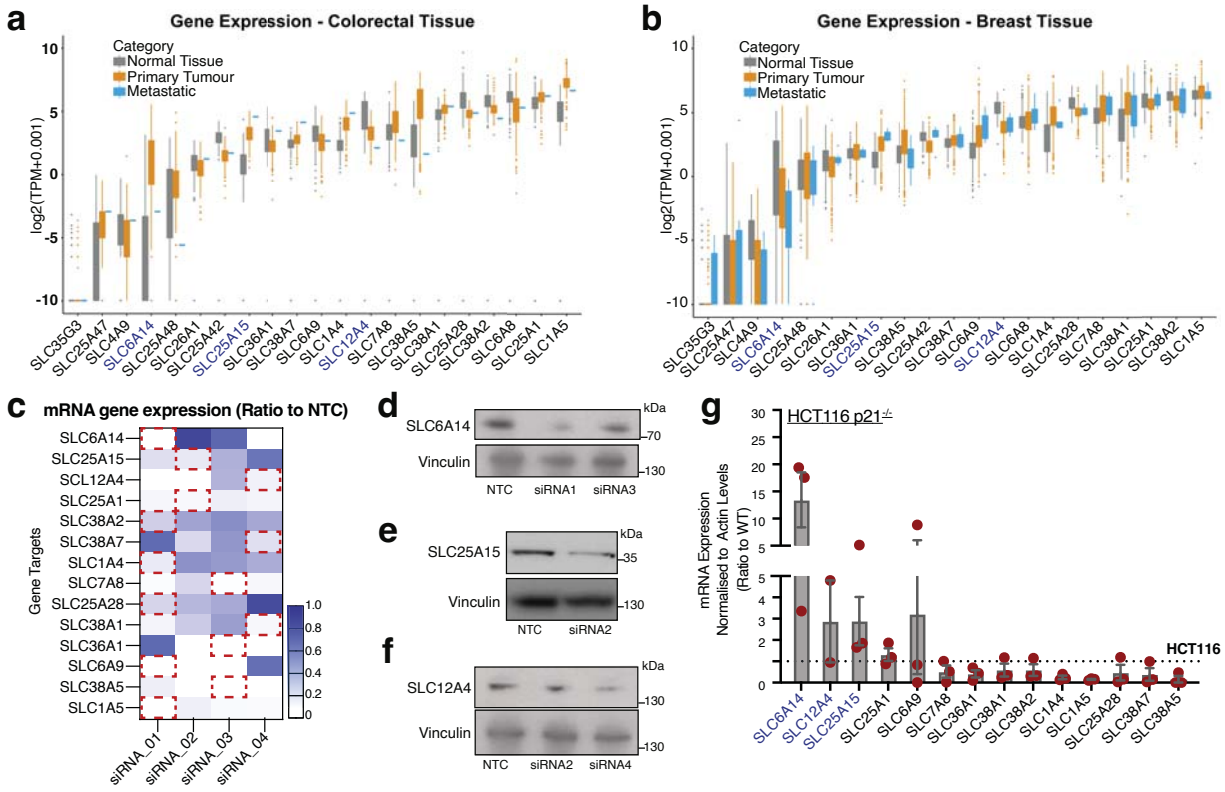

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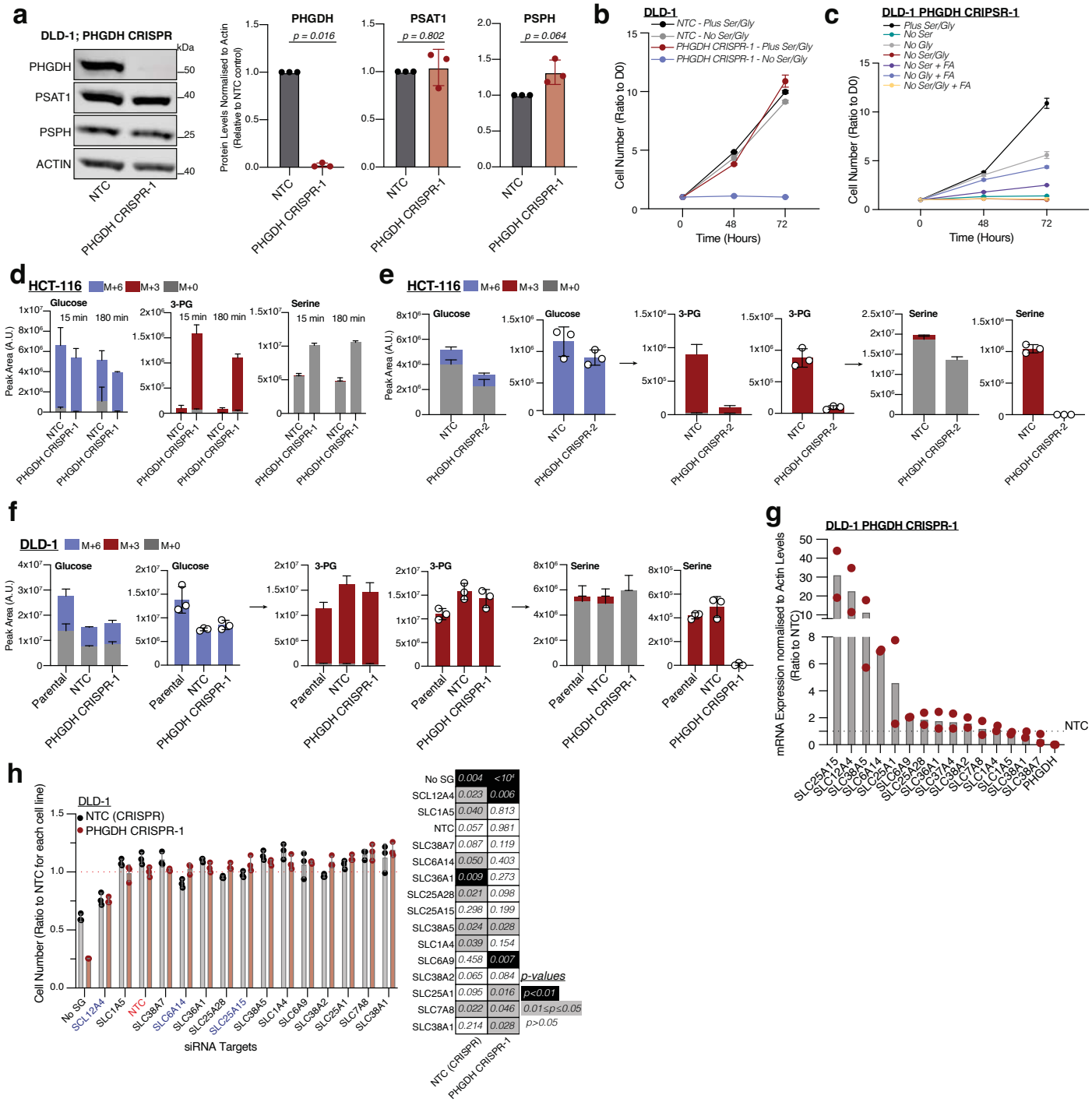

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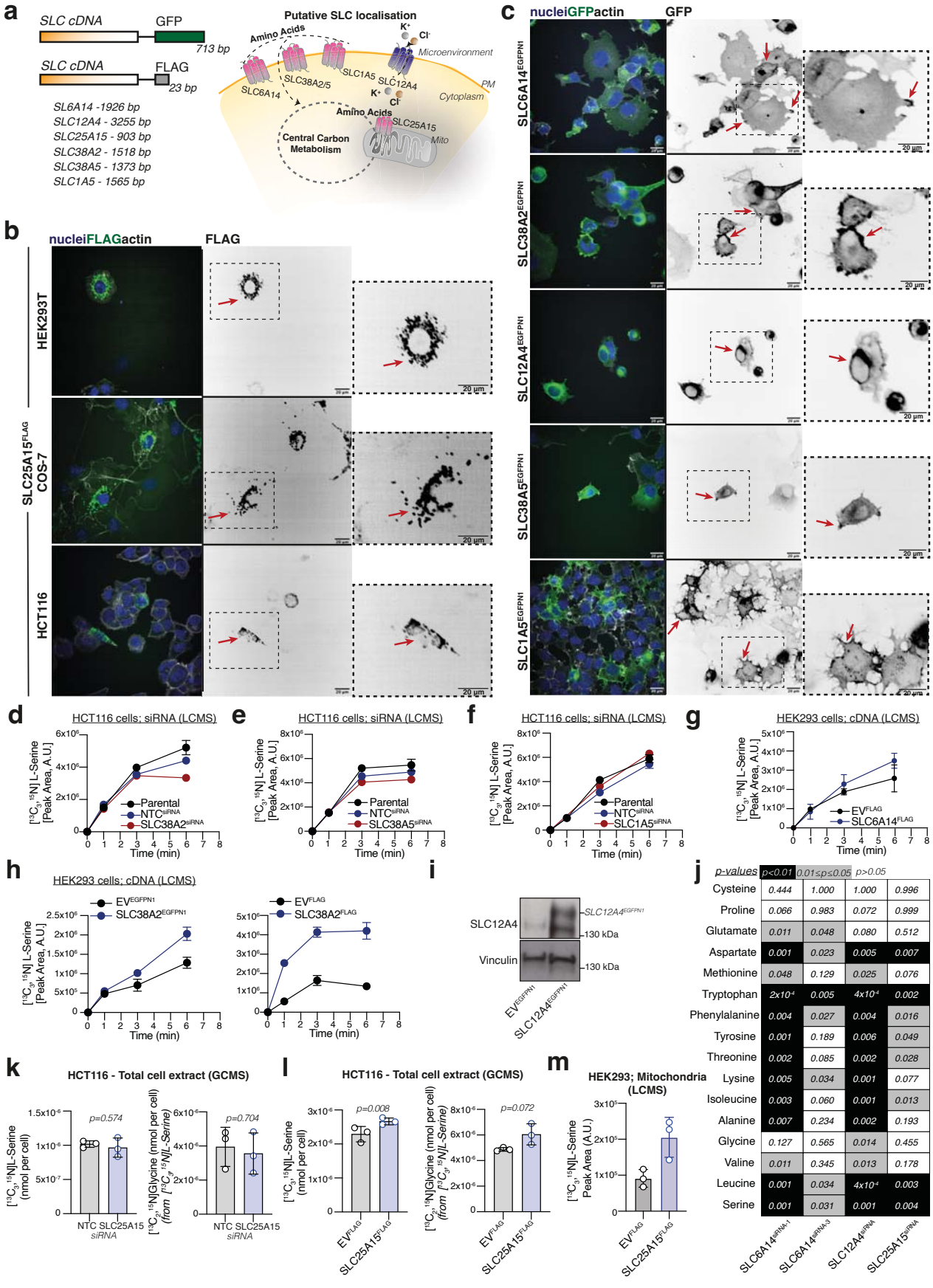

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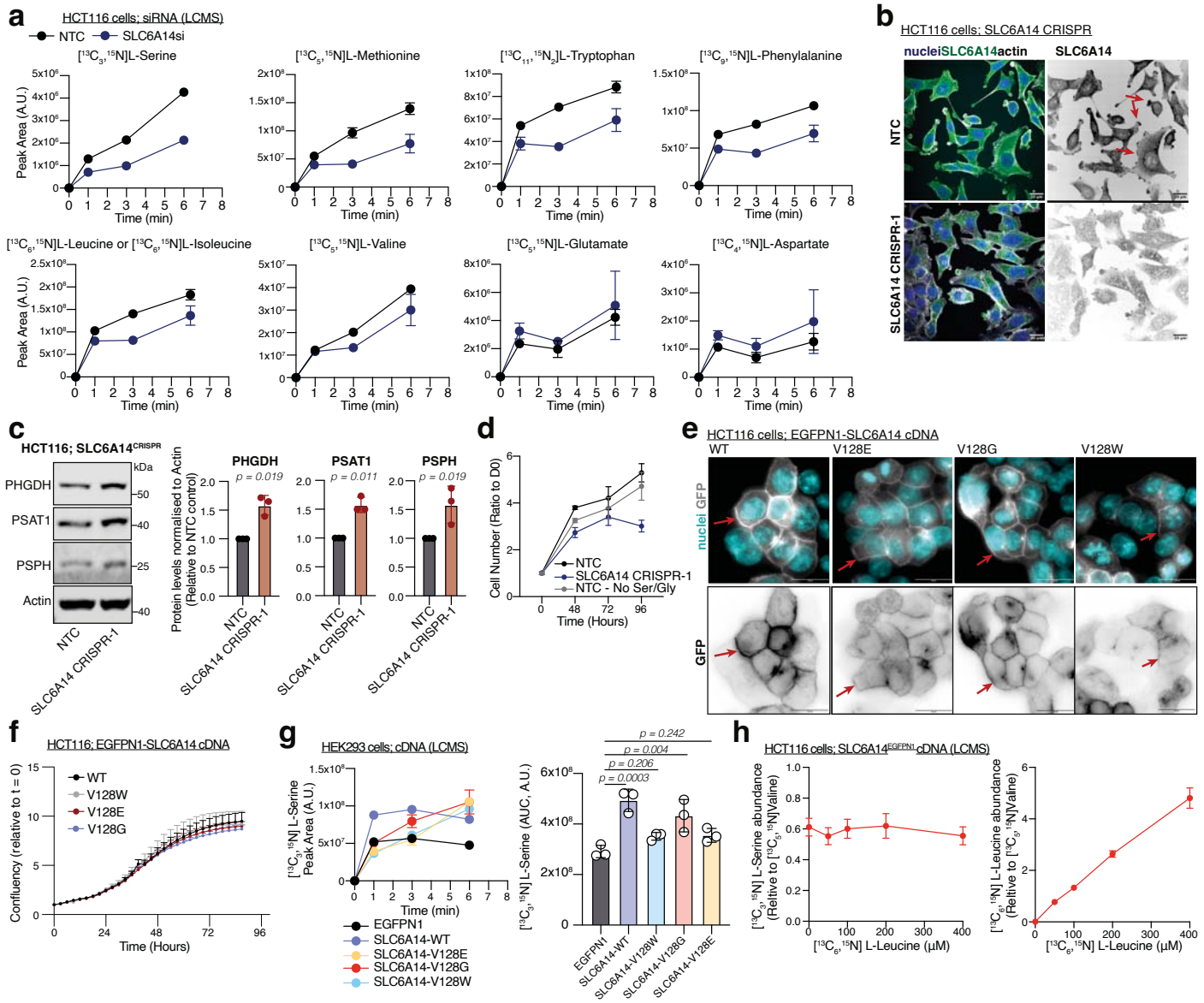

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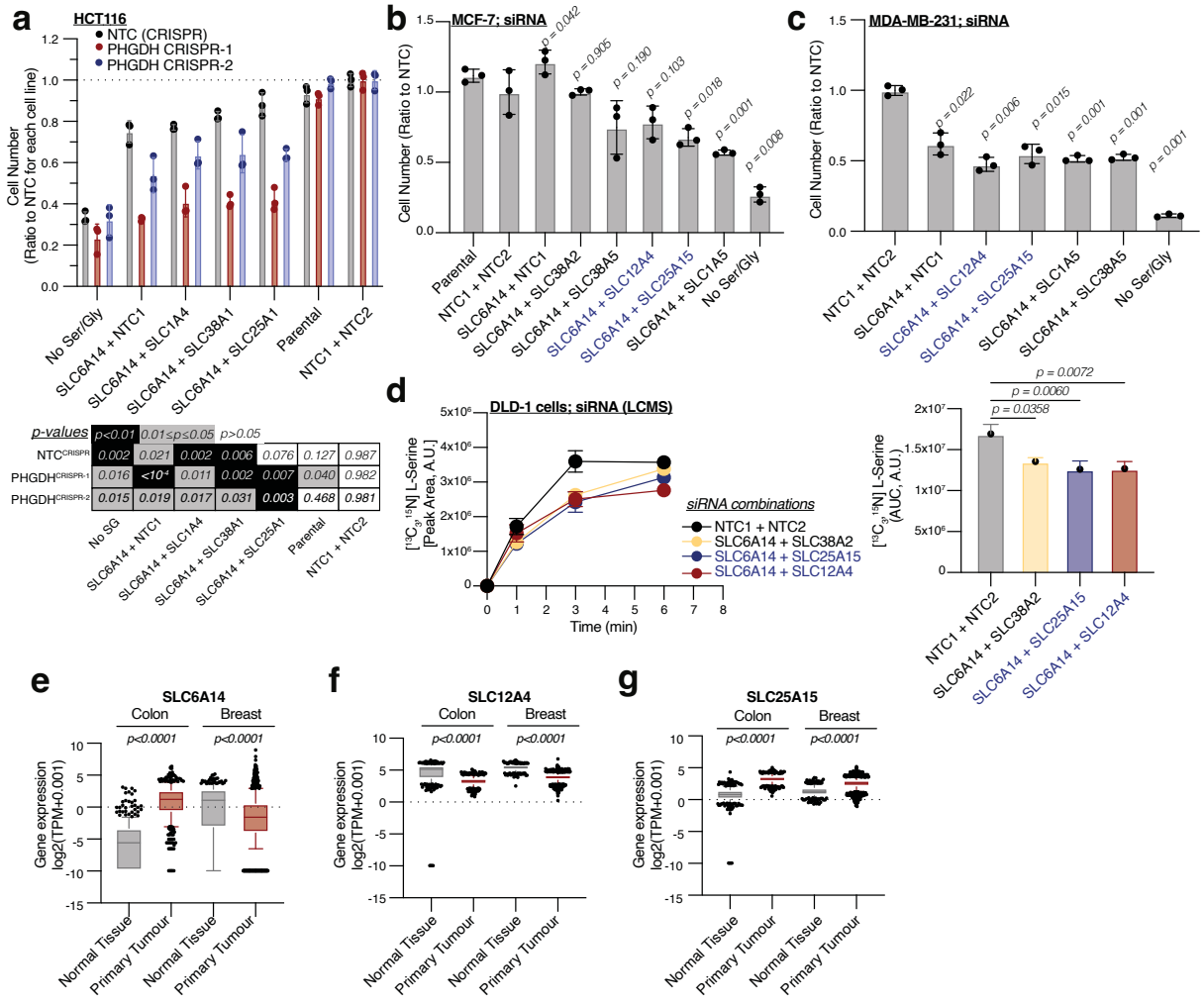

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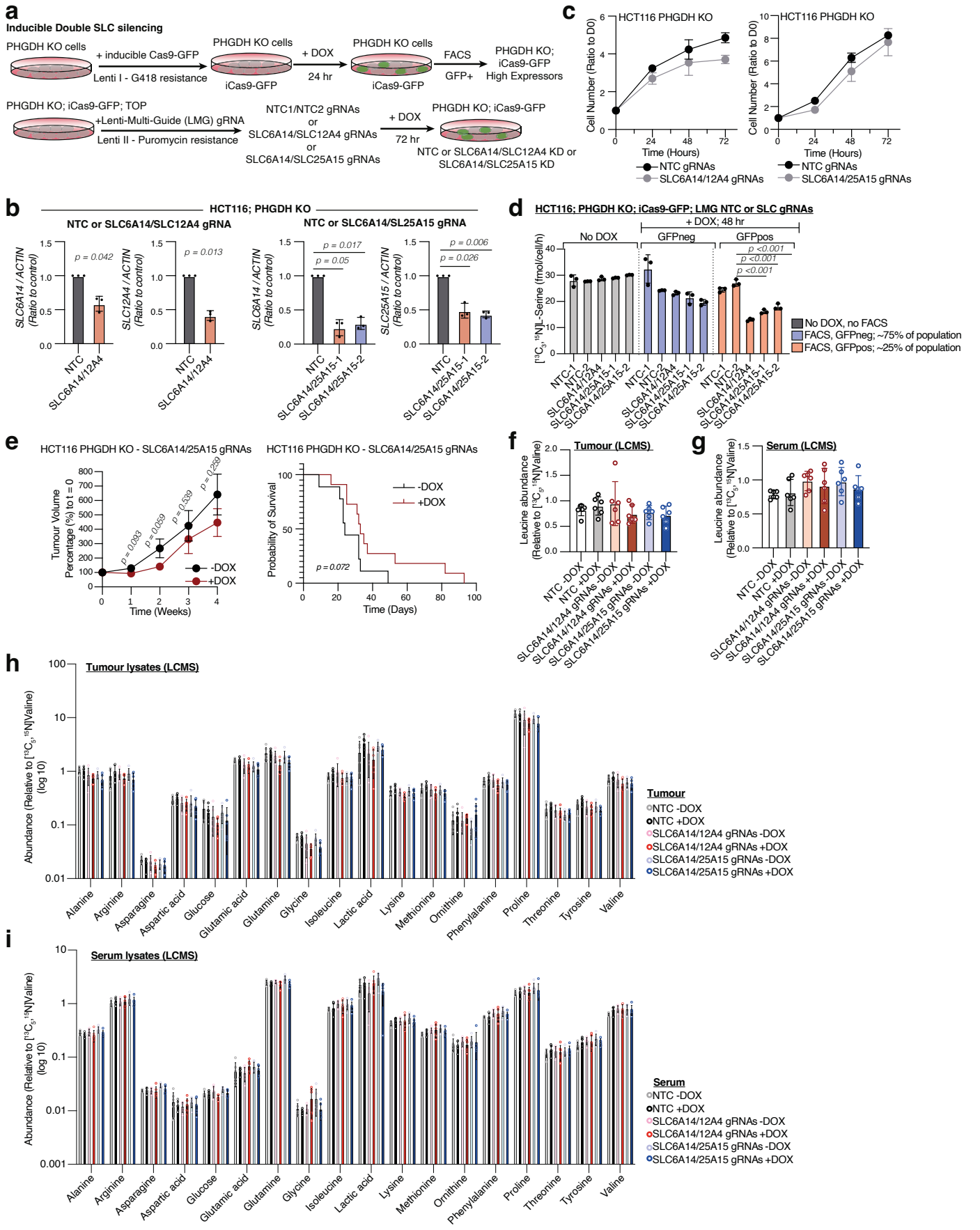
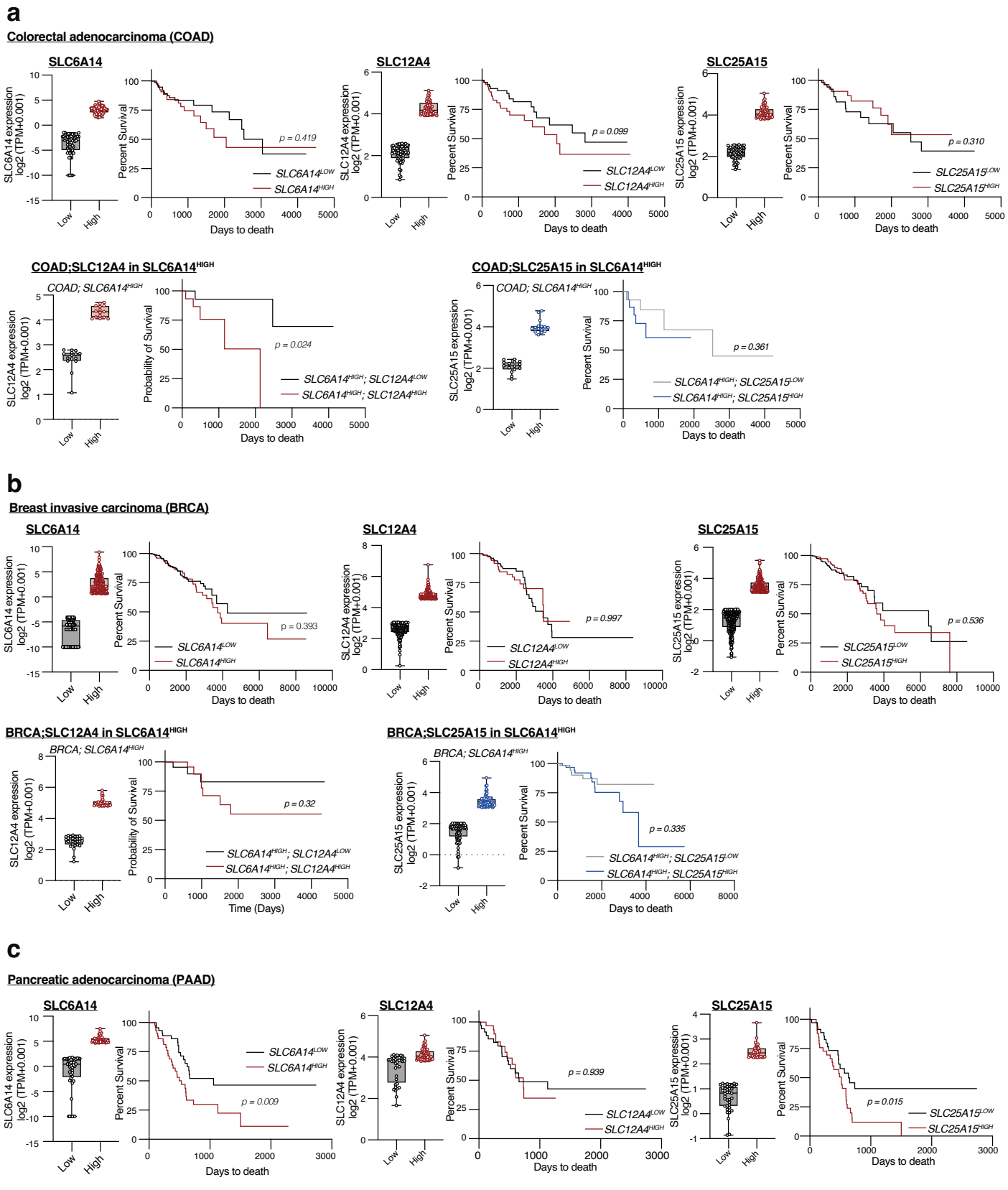


Extended Data Figure 6



Extended Data Figure 7

Extended Data Figure 1 - Phenotypic landscape of SLC superfamily reveals SLCs influencing serine transport in cancer cells.

a. Gene expression levels of selected SLC encoding genes in human colorectal tissues. Values are log2(TPM+0.001) from n = 359 normal tissue, n = 380 primary tumour and n = 1 metastatic samples.

b. Gene expression levels of selected SLC encoding genes in human breast tissues. Values are log2(TPM+0.001) from n = 292 normal tissue, n = 1092 primary tumour and n = 7 metastatic samples.

c. Heatmap showing gene silencing efficiency of 4 deconvoluted siRNAs against each SLC encoding gene. Values are mRNA expression normalised to *ACTIN* levels and relative to NTC control. Red boxes indicate selected siRNA for further experiments.

d. Immunoblots of SLC6A14 and Vinculin (loading control) in control (NTC) or SLC6A14-silenced HCT116 cells. Blots are representative of three independent experiments.

e. Immunoblots of SLC25A15 and Vinculin (loading control) in control (NTC) or SLC25A15-silenced HCT116 cells. Blots are representative of three independent experiments.

f. Immunoblots of SLC12A4 and Vinculin (loading control) in control (NTC) or SLC12A4-silenced HCT116 cells. Blots are representative of three independent experiments.

g. mRNA expression levels of indicated SLC encoding genes in HCT116 $p21^{-/-}$ cells versus HCT116 WT control. Values are mean±s.e.m. from n = 3 independent experiments.

Data in **a**,**b**: : Box and whiskers plots are log2 (TPM+0.001) with box limits representing 25^{th} and 75^{th} percentiles of the dataset. Whiskers extend from the hinge to the largest or smallest value, corresponding to the upper or lower 1.5x IQR (inter-quartile range). Individual dot points represent data beyond the end of the whiskers.

Extended Data Figure 2 – Serine synthesis deficient cells depend on SLC-mediated serine uptake

a. Left; Immunoblots of PHGDH, PSAT1, PSPH and ACTIN (loading control) in control (NTC) or PHGDH-depleted DLD-1 cells. Blots are representative of three independent experiments. **Right**; densitometric

quantification of protein. Values are mean±s.d. from n=3 independent experiments. Statistical significance assessed with two-tailed one-sample *t*-test on natural log-transformed values.

b. Growth curves of DLD-1 NTC (control) or PHGDH-depleted cells in media ± serine and glycine. Values are mean±s.e.m. from n=3 biological replicates and relative to t = 0.

c. Growth curves of DLD-1 PHGDH-depleted cells in media ± serine and/or glycine ± formic acid (FA). Values are mean±s.e.m. from n=3 biological replicates and relative to t = 0.

d-e. Incorporation of glucose-derived, labelled carbons into 3-phosphoglycerate (3-PG) and serine in HCT116 NTC (control) or PHGDH CRISPR-1 **(d)** and PHGDH CRISPR-2 **(e)** depleted cells following 15 min and 180 min incubation in media supplemented with [U-$^{13}C_6$]glucose. Values are mean±s.d. from n = 3 biological replicates.

f. Incorporation of glucose-derived, labelled carbons into 3-phosphoglycerate (3-PG) and serine in DLD-1 NTC (control) or PHGDH-depleted cells following 15 min and 180 min incubation in media supplemented with [U-$^{13}C_6$]glucose. Values are mean±s.d. from n = 3 biological replicates.

g. mRNA expression levels of indicated SLC genes in DLD-1 PHGDH-depleted cells versus DLD-1 NTC control. Values are mean±s.d. from n = 2 independent experiments.

h. Left; Cell number (Ratio to NTC) of DLD-1 NTC (CRISPR control) and PHGDH-depleted cells upon knockdown of indicated SLC, after 72 hours of growth. Values are mean±s.d. from n = 3 biological replicates. No SG; no Serine/Glycine condition. **Right**; Heatmap indicating *p*-values for each condition compared to NTC (siRNA control). Statistical significance was assessed by two-tailed one-sample *t*-test on natural log-transformed values.

Extended Data Figure 3 - Functional identification of shortlisted SLCs in serine uptake

a. Left; Schematic of SLC tagging for overexpression studies. **Right;** Schematic of observed and reported localisation/functionality of shortlisted SLCs.

b. Left; Immunofluorescence of HEK293 (top), COS7 (middle) and HCT116 (bottom) cells expressing SLC25A15 tagged with FLAG showing nuclei (blue), anti-FLAG (green) and actin (grey). Middle; anti-

FLAG staining (grey). **Right**; Magnification of areas indicated by black boxes. Red arrows indicate representative localisation. Scale bars; 20 µm. Images are representative from n = 3 biological replicates.

c. Left; Immunofluorescence of HEK293 cells expressing the indicated SLC proteins tagged with GFP showing nuclei (blue), anti-GFP (green) and actin (grey). Middle; anti-GFP staining (grey). **Right**; Magnification of areas indicated by black boxes. Red arrows indicate representative localisation for each SLC protein. Scale bars; 20 µm. Images are representative from n = 3 biological replicates.

d-f. [$^{13}C_{3,}{}^{15}N$]L-Serine uptake of HCT116 Parental, NTC siRNA (control) and cells silenced for SLC38A5 **(d)**, SLC1A5 **(e)** or SLC25A15 **(f).** Values are mean±s.e.m. from n = 3 biological replicates.

g. [$^{13}C_{3,}{}^{15}N$]L-Serine uptake of HEK293 EV^{FLAG} (control) or $SLC6A14^{FLAG}$ overexpressing cells. Values are mean±s.d. from n = 3 biological replicates.

h. [$^{13}C_{3,}{}^{15}N$]L-Serine uptake of HEK293 EV^{EGFPN1} (control) and $SLC38A2^{EGFPN1}$ (**left**) or EV^{FLAG} (control) or $SLC38A2^{FLAG}$ (**right**) cells. Values are mean±s.e.m. from n = 3 biological replicates.

i. Immunoblots of SLC12A4 and Vinculin (loading control) in HCT116 cells expressing GFP (EV^{EGFPN1}) or GFP-tagged SLC12A4. Blots are representative of three independent experiments.

j. Heatmap indicating *p*-values of amino acid uptake comparison between SLC6A14, SLC12A4, SLC25A15 to NTC (siRNA control) in HCT116 cells. Data linked to Figures **3i, 4h** and **5a**.

k. [$^{13}C_{3,}{}^{15}N$]L-Serine levels (nmol per cell) (**left**) and [$^{13}C_{2,}{}^{15}N$]Glycine levels (nmol per cell) (**right**) of HCT116 NTC (siRNA control) and HCT116 SLC25A15-silenced cells. Statistical significance was assessed with two-tailed unpaired *t*-test.

l. [$^{13}C_{3,}{}^{15}N$]L-Serine levels (nmol per cell) (**left**) and [$^{13}C_{2,}{}^{15}N$]Glycine levels (nmol per cell) (**right**) of HCT116 FLAG (EV^{FLAG}, control) and HCT116 FLAG-tagged SLC25A15-overexpressing cells. Statistical significance was assessed with two-tailed unpaired *t*-test.

m. [$^{13}C_{3,}{}^{15}N$]L-Serine uptake in mitochondria enriched fractions of HEK293 cells overexpressing EV^{FLAG} or $SLC25A15^{FLAG}$ and supplemented with [$^{13}C_{3,}{}^{15}N$]L-Serine for 60 min.

Data in k-m are mean±s.d. from n = 3 biological replicates.

Extended Data Figure 4 - Functional identification of SLC6A14 in serine uptake

a. Uptake curves SLC6A14-silenced HCT116 cells supplemented with a pool of the indicated labelled amino acids for -1, -3 and -6 min. Values are mean±s.d. from n = 3 biological replicates.

b. Left; Immunofluorescence of control (NTC) or SLC6A14-depleted HCT116 cells expressing showing nuclei (blue), anti-SLC6A14 (green) and actin (grey). **Right**; anti-SLC6A14 staining (grey). Red arrows indicate representative localisation of SLC6A14. Scale bars; 20 µm. Images are representative from n = 3 biological replicates.

c. Left; Immunoblots of PHGDH, PSAT1, PSPH and ACTIN (loading control) in control (NTC) or SLC6A14-depleted HCT116 cells. Blots are representative of three independent experiments. **Right**; densitometric quantification of protein. Values are mean±s.d. from n=3 independent experiments. Statistical significance assessed with two-tailed one-sample *t*-test on natural log-transformed values.

d. Growth curves of HCT116 NTC (CRISPR control) or SLC6A14-depleted cells. Values are mean±s.d. from n = 3 biological replicates. No Ser/Gly – No Serine/Glycine condition.

e. Top; Immunofluorescence of HCT116 cells expressing GFP-tagged WT SLC6A14 or V128E, V128G and V128W SLC6A14 mutants showing nuclei (blue) and gfp (grey). **Bottom;** anti-GFP staining (grey). Scale bars; 20 µm. Red arrows indicate representative localisation of SLC6A14.

f. Growth curves of cells from **(e)**.

g. Left; [$^{13}C_{3,}{}^{15}N$]L-Serine uptake over time of HET293 cells expressing EGFPN1 (EV^{EGFPN1}, control), GFP-tagged SLC6A14 WT or V128E, V128G, V128W mutants as indicated. Values are mean±s.e.m. from n = 3 biological replicates. **Right**; Area Under the Curve (AUC) of [$^{13}C_{3,}{}^{15}N$]L-Serine uptake curves. Values are mean±s.d. from n = 3 biological replicates. Statistical significance was assessed with ordinary one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons test.

h. Left; [$^{13}C_{3,}{}^{15}N$]L-Serine uptake after 15 min labelling over increasing concentrations of [$^{13}C_{6,}{}^{15}N$]L-Leucine and stable [$^{13}C_{3,}{}^{15}N$]L-Serine supply in HCT116 cells overexpressing GFP-tagged SLC6A14. Values are mean±s.d. from n = 3 biological replicates. **Right;** [$^{13}C_{6,}{}^{15}N$]L-Leucine uptake after 15 min labelling with increasing concentrations of [$^{13}C_{6,}{}^{15}N$]L-Leucine and stable [$^{13}C_{3,}{}^{15}N$]L-Serine supply in

HCT116 cells overexpressing GFP-tagged SLC6A14. Values are mean±s.d. from n = 3 biological replicates.

Extended Data Figure 5 - Synthetic lethality between paired serine transporter silencing and PHGDH loss

a. Top; Cell number (Ratio to NTC siRNA control) of HCT116 NTC (CRISPR control) and two PHGDH-depleted cell lines upon double knockdown of SLC6A14 and indicated SLC genes, after 72 hours of growth. Values are mean±s.d. from n = 3 biological replicates. Control data ('NTC1+NTC2', 'Parental', 'SLC6A14+NTC1' and 'No Ser/Gly') are replicated in **Fig. 6a**. **Bottom;** Heatmap indicating *p*-values for each condition compared to NTCs (siRNA control).

b. Cell number (Ratio to NTC siRNA control) of MCF7 cells upon double knockdown of SLC6A14 and indicated SLC genes, after 72 hours of growth. Values are mean±s.d. from n = 3 biological replicates.

c. Cell number (Ratio to NTC siRNA control) of MDA-MB-231 cells upon double knockdown of *SLC6A14* and indicated *SLC* genes, after 72 hours of growth. Values are mean±s.d. from n = 3 biological replicates.

d. Left; [$^{13}C_{3,}{}^{15}N$]L-Serine uptake of DLD-1 'NTC1 and NTC2' siRNA (control) and cells double-silenced for SLC6A14 and SLC12A4, SLC38A2 or SLC25A15 as indicated. Values are mean±s.e.m. from n = 3 biological replicates.

Right; Area Under the Curve (AUC) of [$^{13}C_{3,}{}^{15}N$]L-Serine uptake curves from cells on **Left**. Values are mean±s.e.m. and relative to NTC siRNA control from n = 3 biological replicates. Statistical significance was assessed with ordinary one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons test.

e-g. Gene expression (log2(TPM+0.001)) profiles of *SLC6A14* **(e)**, *SLC12A4* **(f)** and *SLC25A15* **(g)** in normal tissue vs primary tumour specimen of colon or breast tissue. Box plots represent median and 25th and 75th percentiles as the limits of the box and whiskers represent 10th and 90th percentiles of the data with individual dots representing outliers from n = 359 normal colon tissue, n = 380 primary colon tumours, n = 292 normal breast tissue and n = 1092 primary breast tumours. Statistical

significance was assessed with ordinary one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons test.

No Ser/Gly; no Serine/Glycine condition.

Data on a,b,c: Statistical significance was assessed by two-tailed one-sample *t*-test on natural log-transformed values.

Extended Data Figure 6 - Inducible knockout of *SLC6A14/12A4* and *SLC6A14/25A15* SLC transporters.

a. Schematic of DOX-inducible iCas9-GFP - NTCs, *SLC6A14/12A4* or *SLC6A14/25A15* gRNAs cell line generation.

b. Left; mRNA expression levels of *SLC6A14* and *SLC12A4* genes in HCT116 PHGDH-depleted cells expressing iCas9-GFP; NTC or *SLC6A14/SLC12A4* gRNAs following 72 hours of DOX. **Right**; mRNA expression levels of *SLC6A14* and *SLC25A15* genes in HCT116 PHGDH-depleted cells expressing iCas9-GFP; NTC or *SLC6A14/SLC25A15* gRNAs following 72 hours of DOX. Values are mean±s.d. from n = 3 independent experiments. Statistical significance assessed with two-tailed one-sample *t*-test on natural log-transformed values.

c. Cell number (Ratio to DO) over time (hours) of HCT116 PHGDH-depleted cells expressing iCas9-GFP; NTC or *SLC6A14/SLC12A4* gRNAs **(left)** or NTC or *SLC6A14/SLC25A15* gRNAs **(right)** following 72 hours of DOX. Values are mean±s.d. from n = 3 biological replicates.

d. [$^{13}C_{3,}{}^{15}N$]L-Serine consumption in DOX-inducible iCas9-GFP; PHGDH-depleted HCT116 cells expressing either a combination of NTCs, *SLC6A14/12A4* or *SLC6A14/25A15* gRNAs and treated with ±DOX for 48 hr and FACS sorted for selecting GFPneg and GFPpos populations**.** Values are mean±s.d. from n = 3 biological replicates. Statistical significance was assessed with ordinary one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons test comparing the GFPpos (+DOX 48hr) group.

e. Left; Tumour volume over time of tumours from PHGDH-depleted HCT116 cells expressing *SLC6A14/25A15* gRNAs ±DOX. Values are mean±s.e.m. from n = 9 -DOX and n = 11 +DOX mice. Statistical significance was assessed with two-tailed unpaired t-test. **Right;** Kaplan-Meier plot showing

probability of survival on mice from **left**. Statistical significance was assessed with Gehan-Breslow-Wilcoxon test.

f. Leucine levels in tumours formed by PHGDH-depleted HCT116 cells expressing NTC, SLC6A14/SLC12A4 or SLC6A14/SLC25A15 gRNAs ±DOX induction.

g. Leucine levels in circulating blood serum of mice with tumours formed by PHGDH-depleted HCT116 cells expressing NTC, SLC6A14/SLC12A4 or SLC6A14/SLC25A15 gRNAs ±DOX induction.

In **f, g:** Values are mean±s.d. from n = 6 mice per condition.

h. Levels of detected amino acids, glucose, lactic acid and ornithine in tumours formed by PHGDH-depleted HCT116 cells expressing NTC, SLC6A14/SLC12A4 or SLC6A14/SLC25A15 gRNAs ±DOX induction.

i. Levels of detected amino acids, glucose, lactic acid and ornithine in circulating blood serum of mice with tumours formed by PHGDH-depleted HCT116 cells expressing NTC, SLC6A14/SLC12A4 or SLC6A14/SLC25A15 gRNAs ±DOX induction.

In **h, i:** Values are log-scale of mean±s.d. from n = 6 mice per condition.

Extended Data Figure 7 - Assessment of the prognostic value of SLC6A14, SLC25A15 and SLC12A4 in cancer patients.

a. Colon adenocarcinoma (COAD) TCGA dataset: **TOP;** Prognostic value for overall survival of *SLC6A14* transcript levels comparing samples with 25% lowest (*SLC6A14^{LOW})* (n = 57) and 25% highest (*SLC6A14^{HIGH})* (n = 80) expression **(left)**, of *SLC12A4* comparing samples with 20% lowest (*SLC12A4^{LOW})* (n = 58) and 20% highest (*SLC12A4^{HIGH})* (n = 58) expression **(middle)** and of *SLC25A15* comparing samples with 20% lowest (*SLC25A15^{LOW})* (n = 58) and 20% highest (*SLC25A15^{HIGH})* (n = 58) expression **(right)**.

Bottom; Prognostic value for overall survival of *SLC12A4* transcript levels in *SLC6A14^{HIGH}* COAD patients with 20% lowest (SLC6A14^{HIGH};*SLC12A4^{LOW})* (n = 15) and 20% highest (*SLC6A14^{HIGH};SLC12A4^{HIGH})* (n = 15) expression **(left)** and of *SLC25A15* in *SLC6A14^{HIGH}* COAD patients with 20% lowest

(SLC6A14^{HIGH};*SLC25A15^{LOW})* (n = 15) and 20% highest (*SLC6A14^{HIGH};SLC25A15^{HIGH})* (n = 15) expression **(right)**.

b. Breast invasive carcinoma (BRCA) TCGA dataset: **TOP;** Prognostic value for overall survival of *SLC6A14* transcript levels comparing samples with 25% lowest (*SLC6A14^{LOW})* (n = 277) and 25% highest (*SLC6A14^{HIGH})* (n = 273) expression **(left)**, of *SLC12A4* comparing samples with 15% lowest (*SLC12A4^{LOW})* (n = 164) and 15% highest (*SLC12A4^{HIGH})* (n =164) expression **(middle)** and of *SLC25A15* comparing samples with 25% lowest (*SLC25A15^{LOW})* (n = 276) and 25% highest (*SLC25A15^{HIGH})* (n = 273) expression **(right)**.

Bottom; Prognostic value for overall survival of *SLC12A4* transcript levels in *SLC6A14^{HIGH}* BRCA patients with 10% lowest (SLC6A14^{HIGH};*SLC12A4^{LOW})* (n = 28) and 10% highest (*SLC6A14^{HIGH};SLC12A4^{HIGH})* (n = 30) expression **(left)** and of *SLC25A15* in *SLC6A14^{HIGH}* BRCA patients with 25% lowest (SLC6A14^{HIGH};*SLC25A15^{LOW})* (n = 70) and 25% highest (*SLC6A14^{HIGH};SLC25A15^{HIGH})* (n = 69) expression **(right)**.

c. Pancreatic adenocarcinoma (PAAD) TCGA dataset: **TOP;** Prognostic value for overall survival of *SLC6A14* transcript levels comparing samples with 25% lowest (*SLC6A14^{LOW})* (n = 45) and 25% highest (*SLC6A14^{HIGH})* (n = 45) expression **(left)**, of *SLC12A4* comparing samples with 20% lowest (*SLC12A4^{LOW})* (n = 36) and 20% highest (*SLC12A4^{HIGH})* (n =36) expression **(middle)** and of *SLC25A15* comparing samples with 20% lowest (*SLC25A15^{LOW})* (n = 37) and 20% highest (*SLC25A15^{HIGH})* (n = 40) expression **(right)**.

In **a-c**: Box and whiskers plots are log2 (TPM+0.001) with boxes representing median and 25th and 75th percentiles and whiskers extending from the smallest to the highest value of the data with individual dots showing each point of the dataset. Kaplan-Meier curves were statistically compared using log-rank (Mantel-Cox) test.