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Supplementary Materials for

Suppression of nuclear GSK3 signaling promotes serine/one-carbon metabolism and confers metabolic vulnerability in lung cancer cells

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

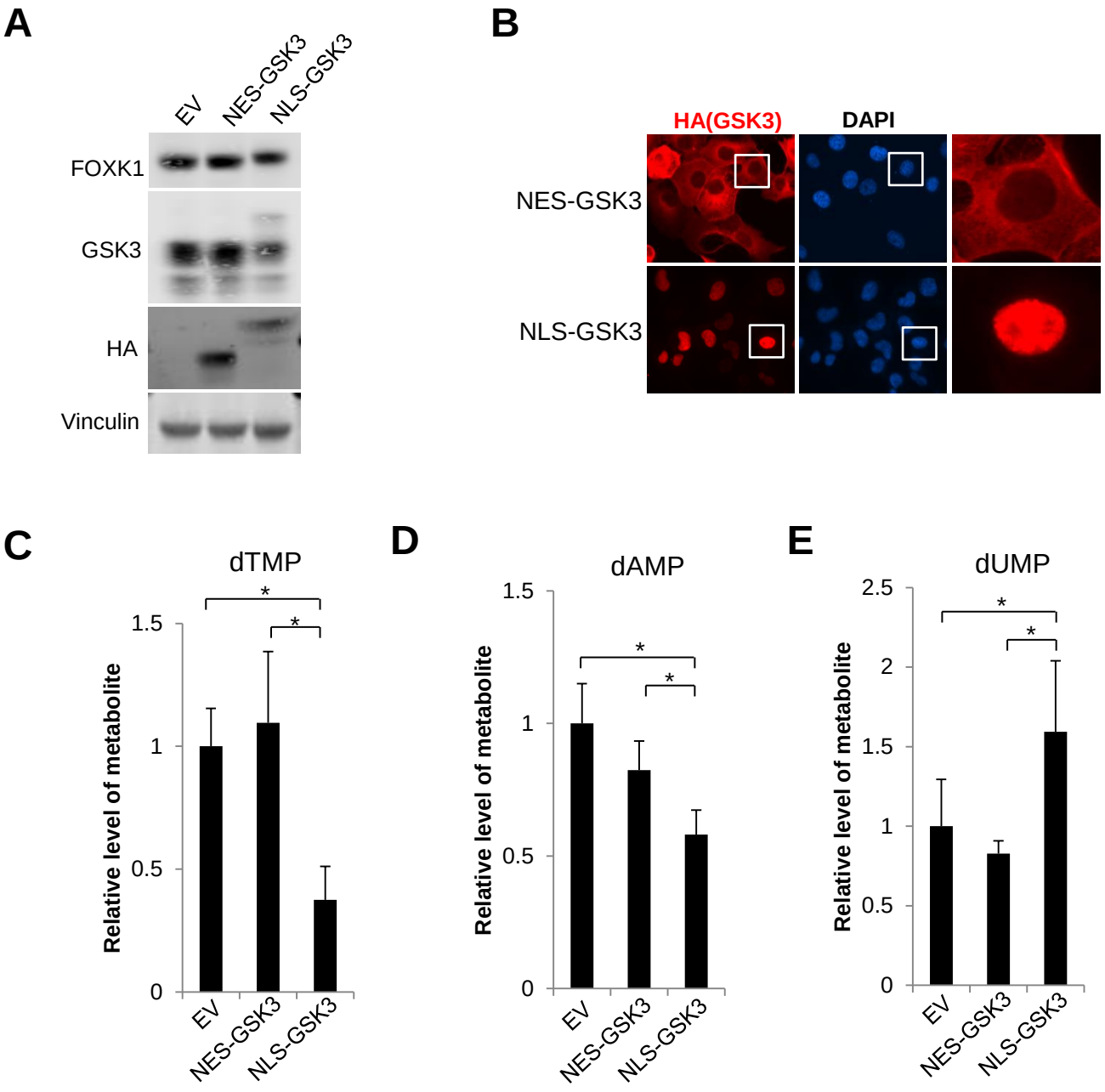

Figure S1 Cell lines were established as described in Figure 1 and exposed to 1mM Doxycycline for 24 hrs. (A) Whole cell lysate was subjected to immunoblot analysis with the indicated antibodies. (B) Immunostaining was performed with anti-HA antibody. (C-E) Metabolomic analysis was performed as described in *Materials and Methods* and alteration in levels of the indicated metabolites is shown.

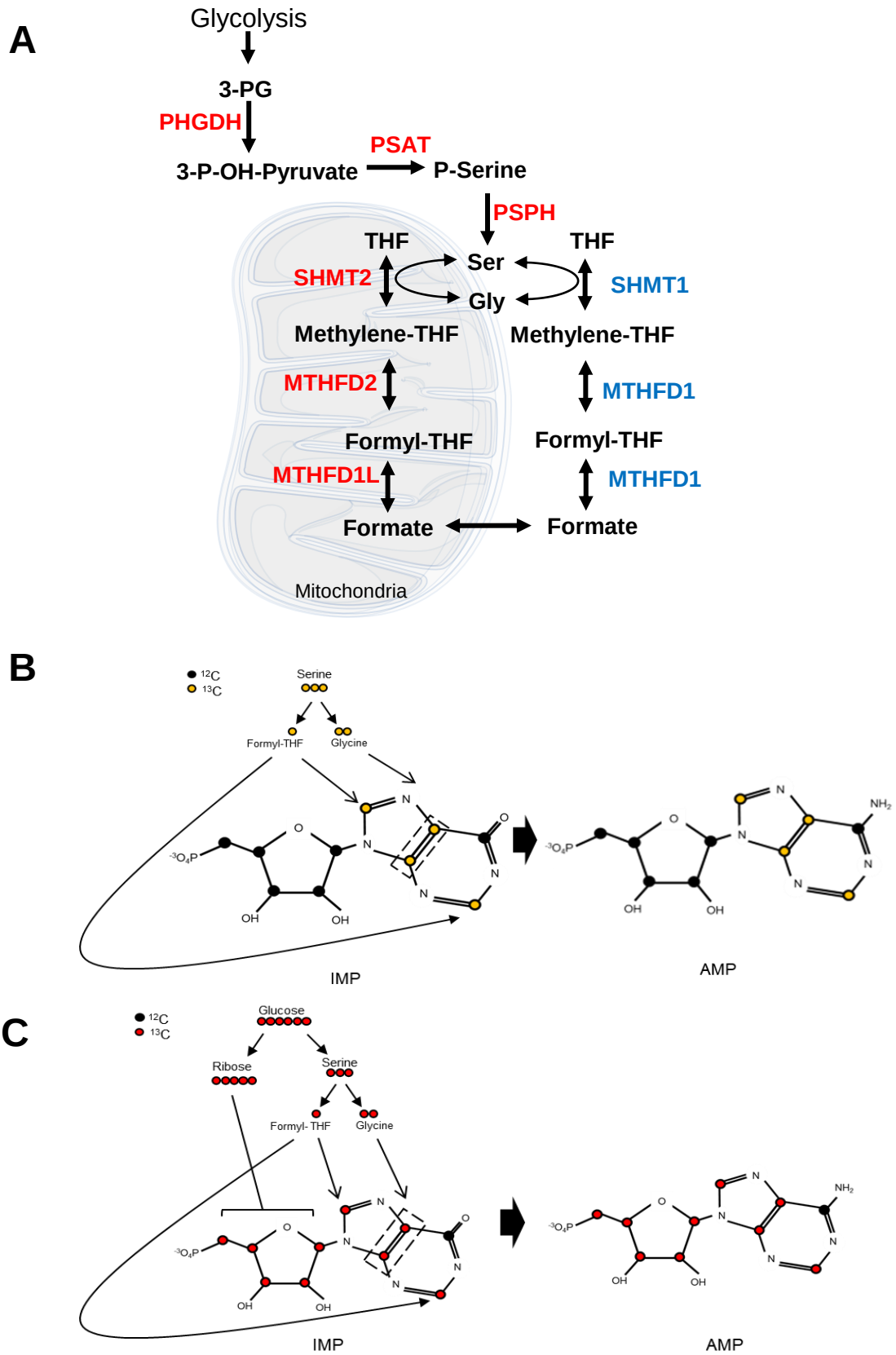

Figure S2 (A) Diagram of serine/one-carbon metabolism. Enzymes indicated in red are suppressed upon NLS-GSK3 overexpression whereas levels of enzymes indicated in blue remain unaltered. (B, C) Incorporation of ^{13}C from U-^{13}C-Serine or U-^{13}C-Glucose into nucleotides.

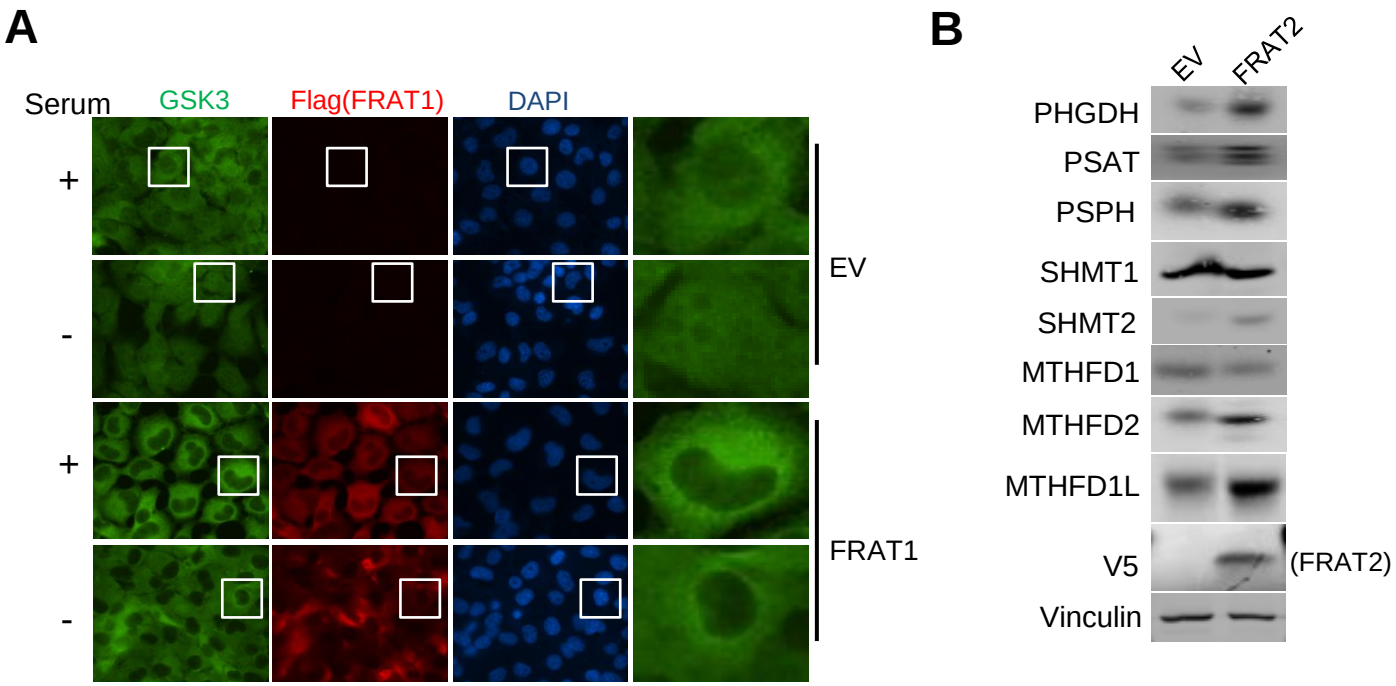

Figure S3 Cell lines were established with overexpression of either EV or FRAT2 in NCI-H1299 cells. (A) Cells were subjected to serum starvation for 24hrs where indicated and immunostaining was performed with antibodies against HA and Flag. (B) Whole cell lysate was subjected to immunoblot analysis with the indicated antibodies.

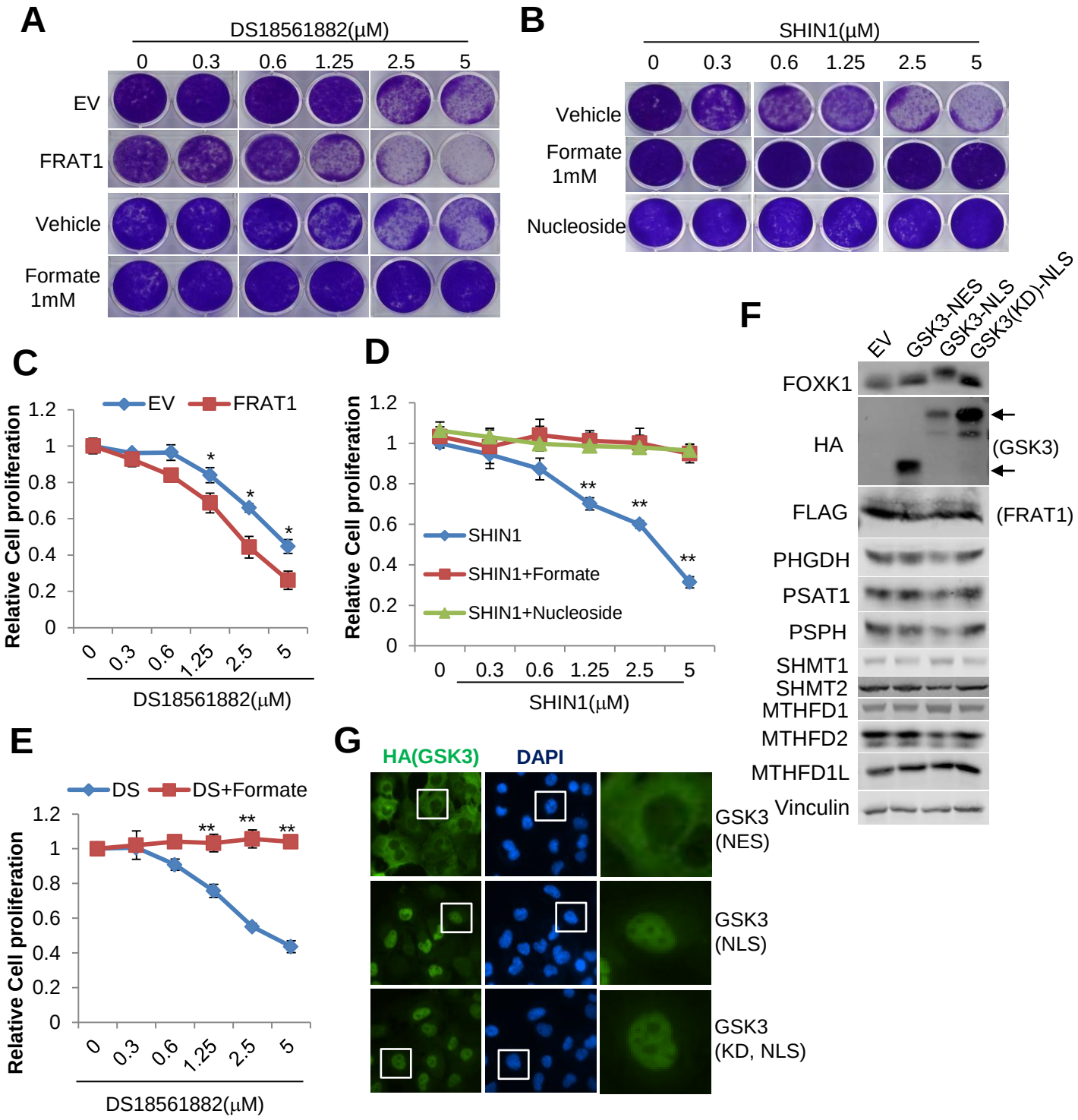

Figure S4 (A-E) Cell lines were established as described in Figure 4A. (A, B) Clonogenic assay was performed using the indicated cell lines and with the indicated concentrations of either DS18561882 or SHIN1. 1mM Formate or 1x nucleoside (ES-008-D, Sigma) was added to the media where indicated (n=3). (C-E) Cell proliferation was estimated by quantification of clonogenic cell growth as described in *Materials and Methods*. Values are expressed as mean ± SD. Two-tailed Student T-Test,*p<0.05, **p<0.01. (F,G) Cell lines were established as described in Figure 4C. (F) Whole cell lysate was subjected to immunoblot analysis with the indicated antibodies. (G) Immunostaining was performed with anti-HA antibodies.

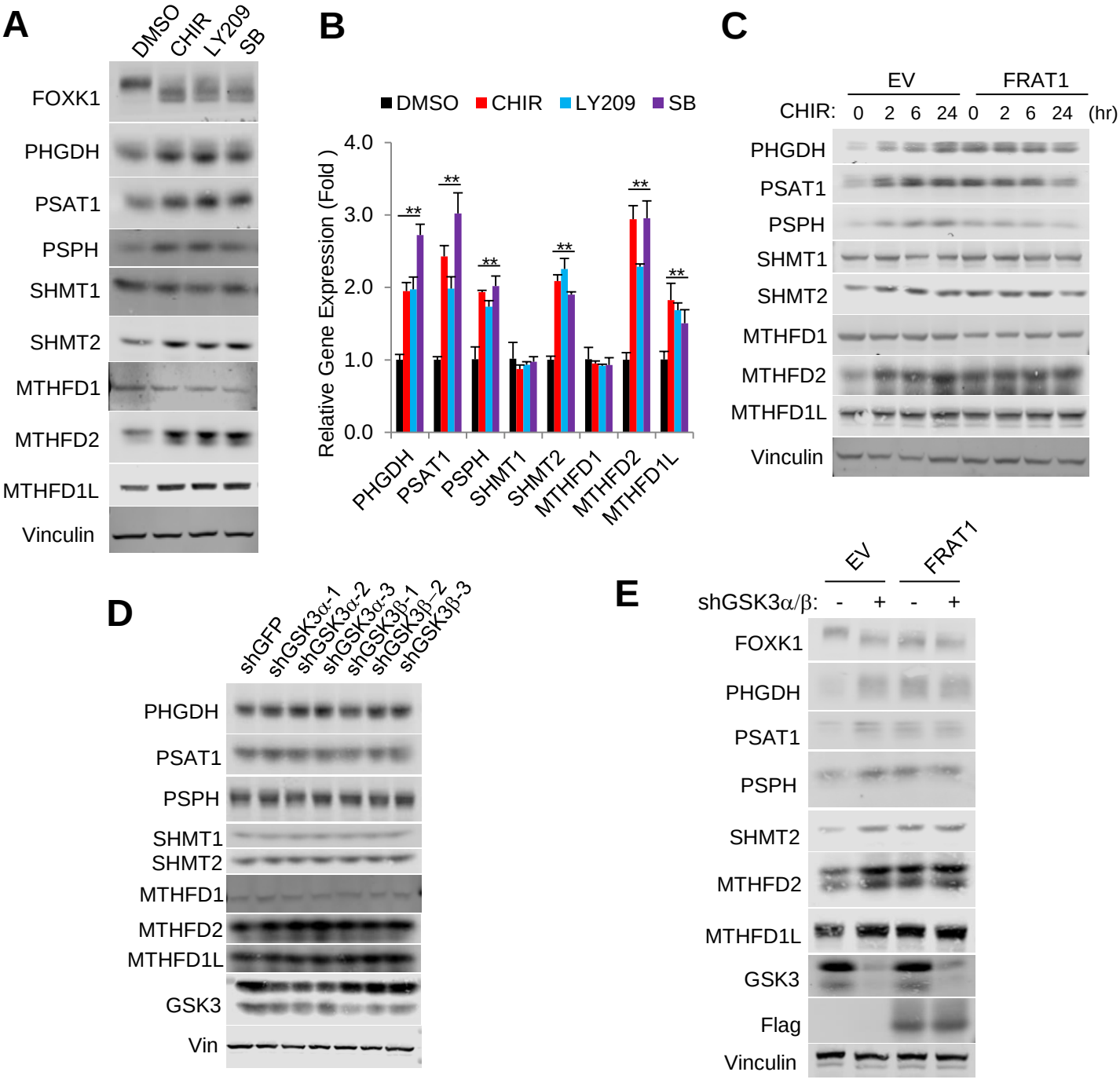

Figure S5 NCI-H1299 cells were exposed to 1mM of the indicated inhibitors for 24 hrs. (A) Whole cell lysate was subjected to immunoblot analysis with the indicated antibodies. (B) Total RNA was extracted and subjected to RT-qPCR analysis with the indicated primers. (C) EV and FRAT1 expressing Cell lines were established as in Figure 4. The indicated cells were exposed to 1mM CHIR99021 for the indicated time. Whole cell lysate was subjected to WB analysis with the indicated antibodies. (D) Cell lines were established with knockdown of the indicated genes using short-hairpin RNAs and the whole cell lysate was subjected to immunoblot analysis with the indicated antibodies. (E) Cell lines were established with either knockdown of GSK3α/β, FRAT1 overexpression, or both. Whole cell lysate was subjected to WB analysis with the indicated antibodies.

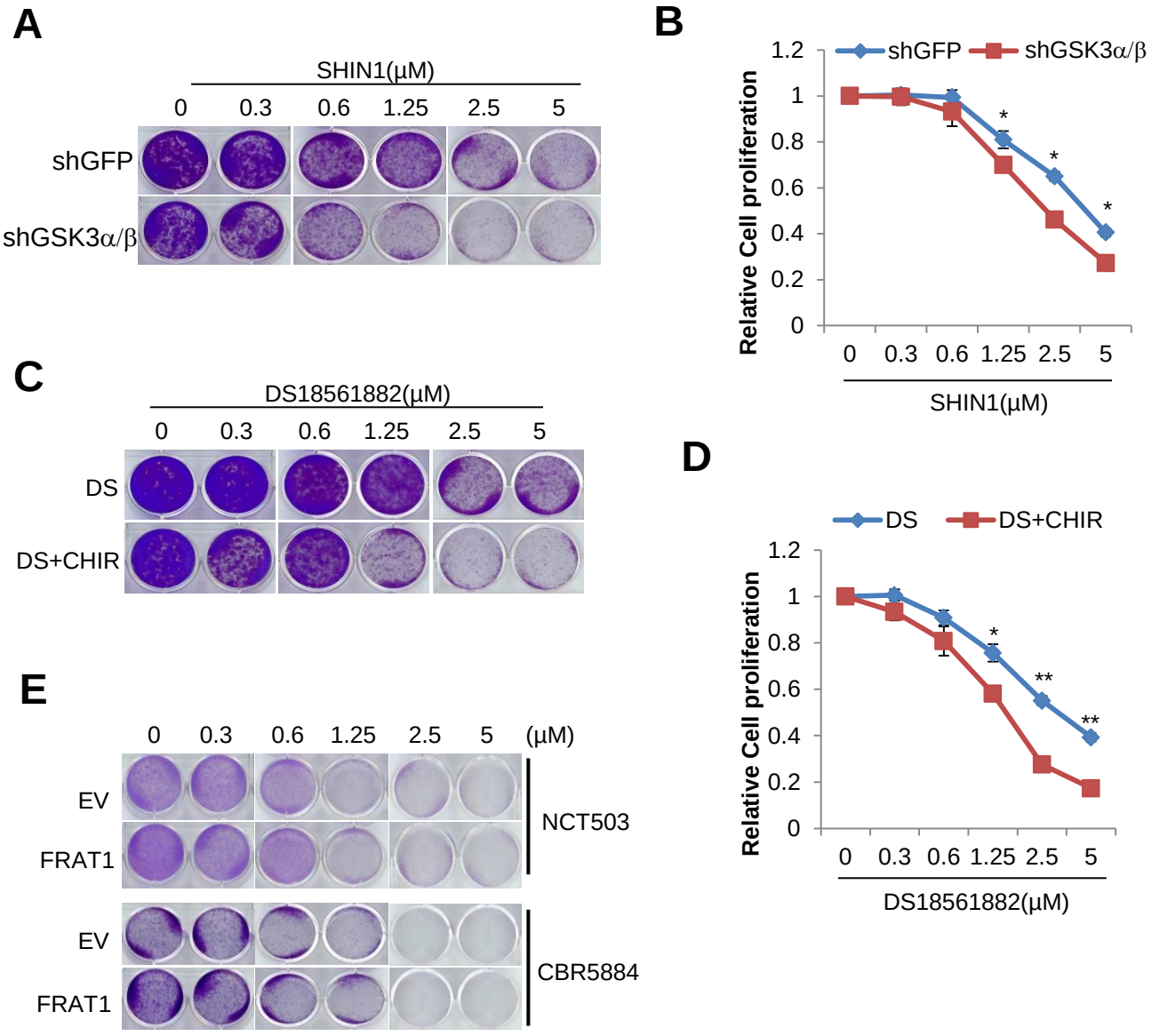

Figure S6(A,B) Cell lines were established as described in Figure 5C. (A) Clonogenic assay was performed with the indicated concentrations of SHIN1. (B) Cell proliferation was estimated by quantification of clonogenic cell growth as described in *Materials and Methods*. (C) Clonogenic assay was performed with the indicated concentrat ion of DS18561882 at the presence or absence of 1mM CHIR99021. (D) Cell proliferation was estimated by quantification of clonogenic cell growth as described in *Materials and Methods*. Values are expressed as mean ± SD. Two-tailed Student T-Test,*p<0.05, ***p*<0.01. (E) Cell lines were established as described in Figure 3A. Clonogenic assay was performed with the indicated concentrations of NCT503 or CBR5884.

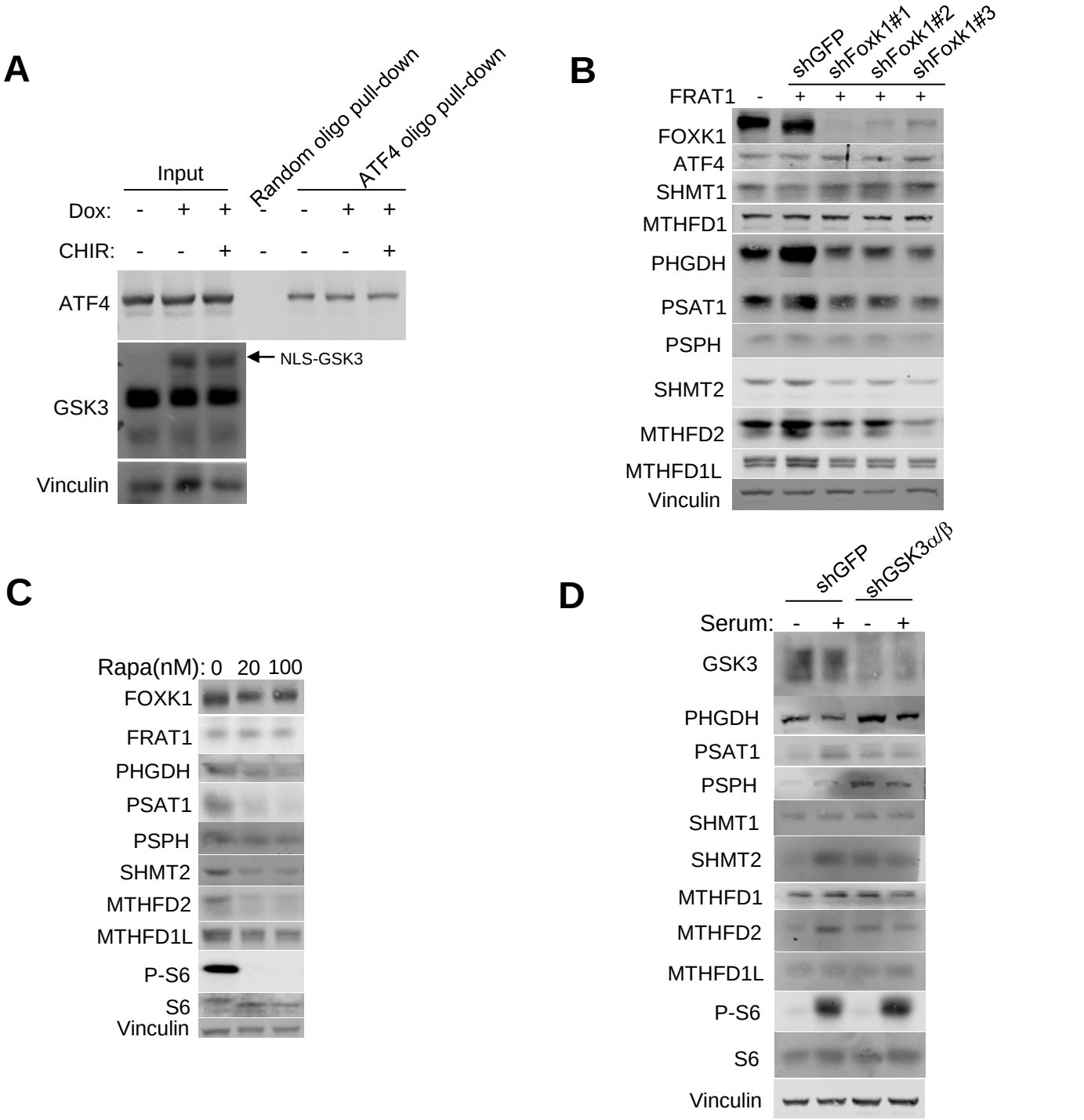

Figure S7 (A) Biotinylated DNA fragments including ATF4 binding oligo or scrambled control in lysates from cells established with doxycycline-inducible NLS-GSK3 exposed to the indicated reagents for 24hrs. WB analysis with the indicated antibodies was performed after immunoprecipitation (n=3). (B) Cell lines were established with either EV or FRAT1 overexpression plus hairpins targeting control (shGFP) or FOXK1 (shFOXK1#1, shFOXK1#2, and shFOXK1#3) in NCI-H1299 cells. Whole cell lysate was extracted and subjected to WB analysis with the indicated antibodies (n=3). (C) NCI-H1299 cells were exposed to 100nM rapamycin for 24hrs and whole cell lysate was subjected to WB analysis with the indicated antibodies (n=3). (D) Cells were established as in Figure 5C. Cells were subjected to serum starvation for 24hrs where indicated and whole cell lysate was extracted and used for WB analysis with the indicated antibodies (n=2).

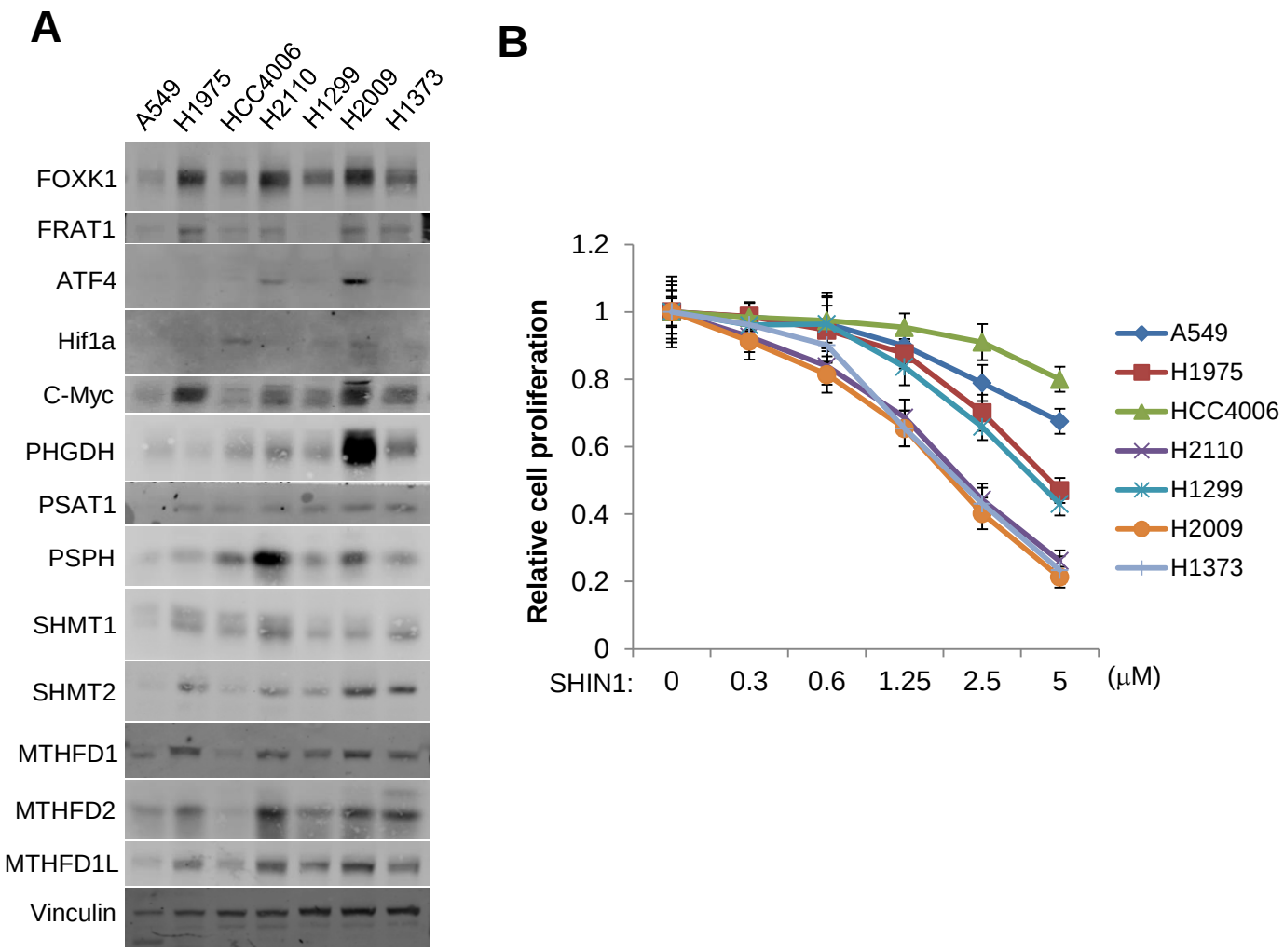

Figure S8 (A) Whole cell lysate from the indicated cell lines was subjected to WB analysis with the indicated antibodies. (B) Clonogenic assay was performed with the indicated cell lines and cell proliferation was estimated by quantification of clonogenic cell growth as described in *Materials and Methods.*

Supplemental Table 1
Fractionation of metabolites for U-^{13}C-Glucose and U-^{13}C-Serine tracing experiments.