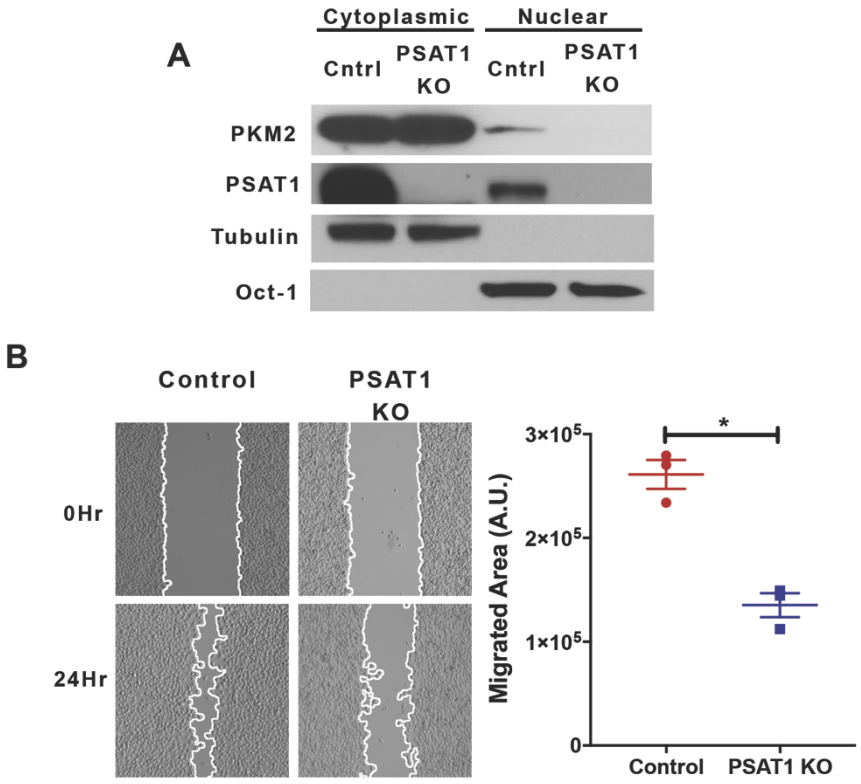
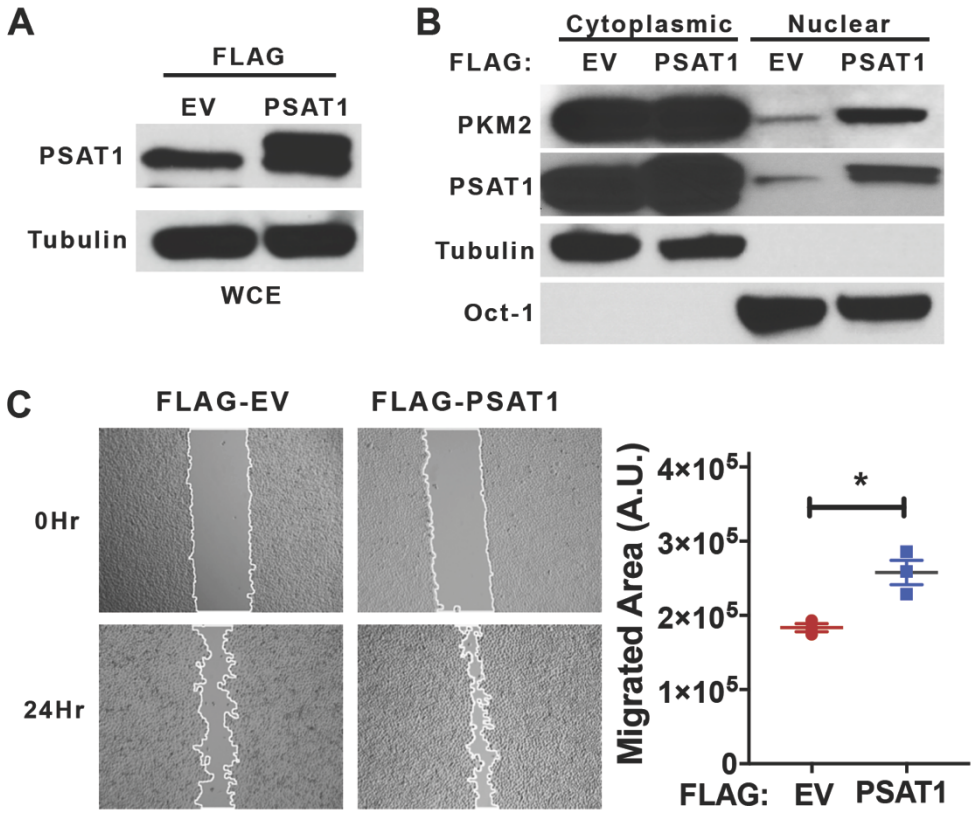
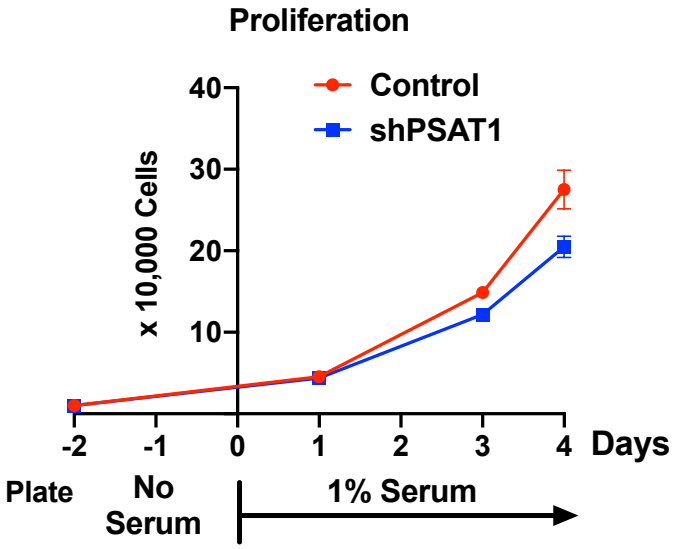


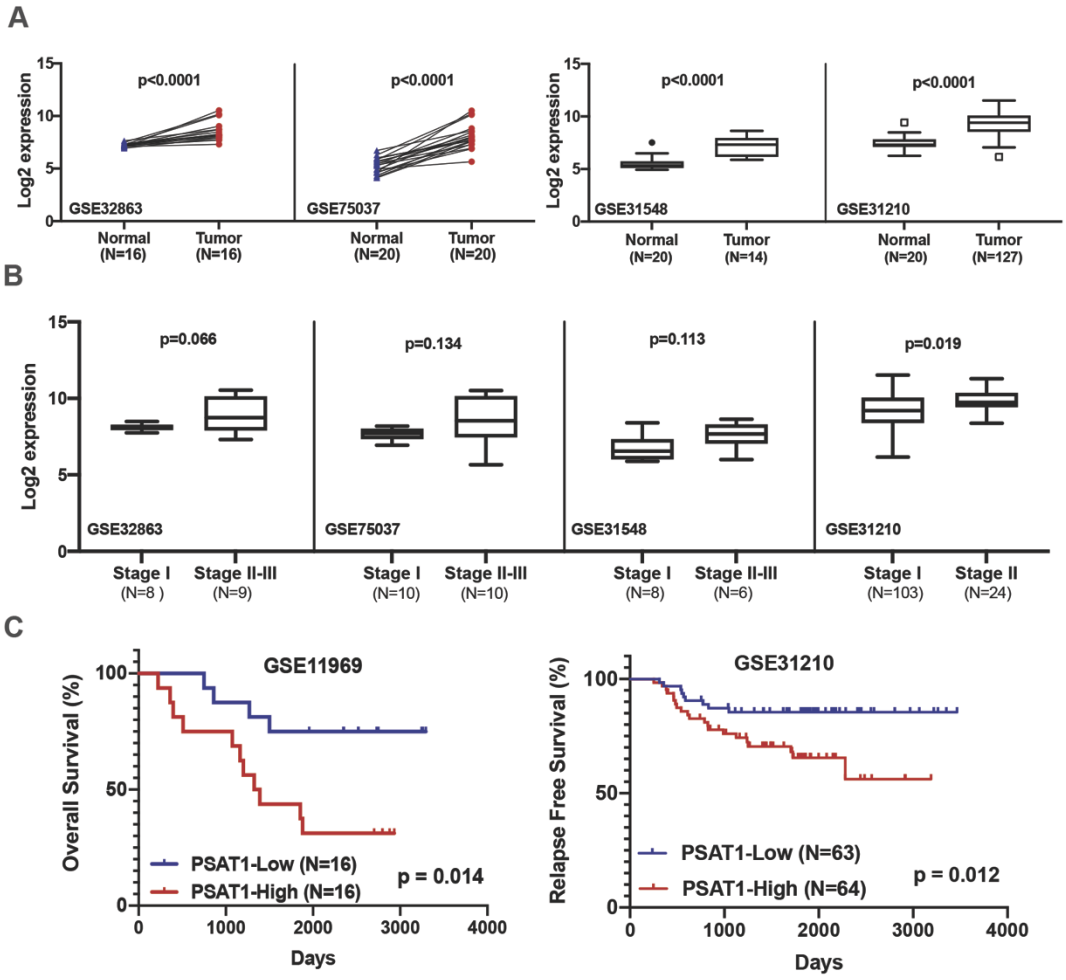
Supplemental Figure S1. Genomic deletion of PSAT1 recapitulates the phenotype of shRNA-mediated PSAT1 silencing in PC9 cells. A) Immunoblot analysis of PKM2 and PSAT1 localization after PSAT1 knockout. Cytoplasmic and nuclear fractions from Control and PSAT1 KO PC9 cells were analyzed using anti-PKM2 or anti-PSAT1 antibodies. Oct-1 and α-tubulin served as loading controls for nuclear and cytoplasmic compartments, respectively. Shown is representative image from three independent experiments. B) Wound healing assay of serum-starved Control and PSAT1 KO PC9. Shown are representative images at 0 hr and 24 hr with migrating cells demarcated by continuous white lines. Data is presented as mean ± SE migrated area after 24 hours from three independent experiments. Statistical significance was determined by paired t-test analysis. *, $p<0.05$ and A.U.: arbitrary unit.



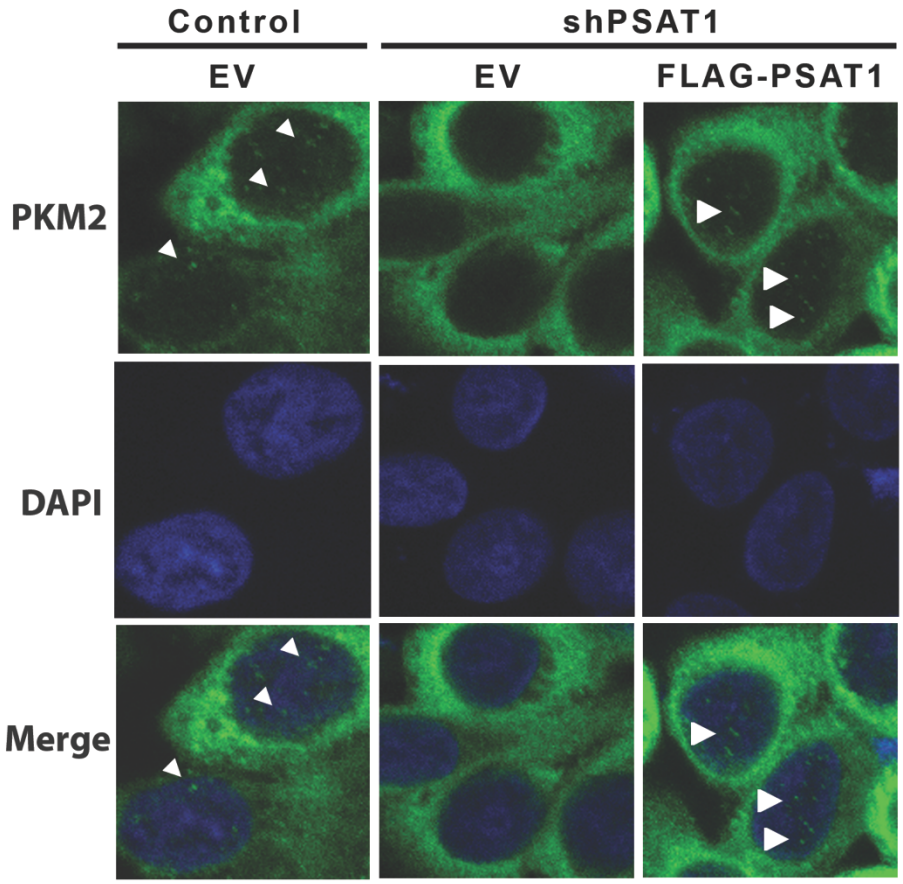
Supplemental Figure S2. Ectopic expression of PSAT1 induces PKM2 nuclear localization and cell migration in PC9 cells. A) Immunoblot analysis of FLAG-PSAT1 expression in PC9 cells. Endogenous and tagged PSAT1 was analyzed using anti-PSAT1 antibody in lysates from PC9 cells expressing empty vector (FLAG-EV) or vector encoding FLAG-PSAT1. Tubulin expression was used for loading control. Shown are representative images from three independent experiments. B) PKM2 and PSAT1 localization in PC9 cells ectopically expressing empty vector (FLAG-EV) or FLAG-PSAT1. Cytoplasmic and nuclear fractions from FLAG-EV and FLAG-PSAT1 expressing cells were analyzed using anti-PKM2 or anti-PSAT1 antibodies. Oct-1 and α-tubulin served as loading controls for nuclear and cytoplasmic compartments, respectively. Shown are representative images from three independent experiments. C) Wound healing assay of serum-starved PC9 cells expressing FLAG-EV or FLAG-PSAT1. Shown are representative images at 0 hr and 24 hr with migrating cells demarcated by continuous white lines. Data is presented as mean ± SE migrated area after 24 hours from three independent experiments. Statistical significance was determined by paired t-test analysis. *, $p<0.05$. EV: Empty Vector. A.U.: arbitrary unit.



Supplemental Figure S3. Stable PSAT1 suppression does not impact PC9 cell proliferation under medium conditions and timing used within cell migration assays. PC9 cells (plated at low density ~10,000 cells) were serum-starved for 24-hours prior to addition of 1% serum. Proliferation was monitored by cell enumeration at the indicated time points. Shown are mean ± SE from two independent experiments. No effect on cell proliferation was observed at 24 hours and PSAT1 loss had moderate impact at three and four days.



Supplemental Figure S4. *PSAT1* is elevated in EGFR-mutant NSCLC compared to normal lung and correlates with poorer patient outcomes. A) Expression analysis of *PSAT1* in EGFR mutant lung tumors compared with paired (GSE32863 and GSE75037) and unpaired (GSE31548 and GSE31210) normal lung tissue. Statistical significance were determined by paired and unpaired t-test, respectively. B) Expression analysis of *PSAT1* in late-stage (Stage II and/ or Stage III) compared with early-stage (Stage I) EGFR mutant lung tumor. Statistical significance were determined by unpaired -test. C) Kaplan-Meier analysis for *PSAT1* expression with overall survival [Hazard Ratio = 3.77 (1.35 – 10.52)] and relapse-free survival [Hazard Ratio = 2.615 (1.28 – 5.36)] in EGFR-mutant lung cancer patients. ***p*** values are indicated for each comparison.



Supplemental Figure S5. PSAT1 loss abrogates nuclear PKM2 expression, which is rescued upon PSAT1 restoration. Immunofluorescence analysis of PKM2 localization in either Control PC9 cells or PSAT1-depleted cells stably expressing either empty vector (EV) or FLAG-PSAT1. Analysis was performed using anti-PKM2 primary antibody followed by AlexaFluor 488-conjugated secondary. DAPI served as a control for nuclear staining. Arrowheads indicate nuclear PKM2 staining in representative images from three independent experiments.

Primer ID	Sequence (5′-3′)	Mutagenesis Kit
MT1-PKM2-F **MT1-PKM2-R**	CAGAGGCTGCCATCTACCAC**AG**G**A**AATTATTTGAGGAACTCCGC GCGGAGTTCCTCAAATAATT**T**C**CT**GTGGTAGATGGCAGCCTCTG	QuikChange mutagenesis XL kit
MT2-PKM2-F **MT2-PKM2-R**	CTCCGCCGCCTGGCG**AG**C**CA**TACCAGCGACCCCAC GTGGGGTCGCTGGTA**TG**G**CT**CGCCAGGCGGCGGAG	
MT3-PKM2-F **MT3-PKM2-R**	**GCGAGCCAT**ACCAGCGACCCCACAGAA **CAGGCGGAC**GAGTTCCTCAAATAATTGCAAGTGGTAG	Q5 Site-Directed Mutagenesis
MT4-PKM2-F **MT4-PKM2-R**	TGAGGAACTC**GTC**CGCCTGGCGC AATAATTGCAAGTGGTAGATGGC	
K433Q-PKM2-F **K433Q-PKM2-R**	CGTCCTCACC**C**AGTCTGGCAG ATTATGGCCCCACTGCAG	
shRNA-resistant-PSAT1-F **shRNA-resistant-PSAT1-R**	**TAC**TGTTAGAGATACAAAAGGAATTATTAGACTACAAAGGAGTTGGCATTAG **CAC**TGTGCGGCAGCTTGGCGGG	

Supplemental Table S1: PCR primers for PKM2 site-directed mutagenesis. Point mutations are indicated in bold letters and underlined.