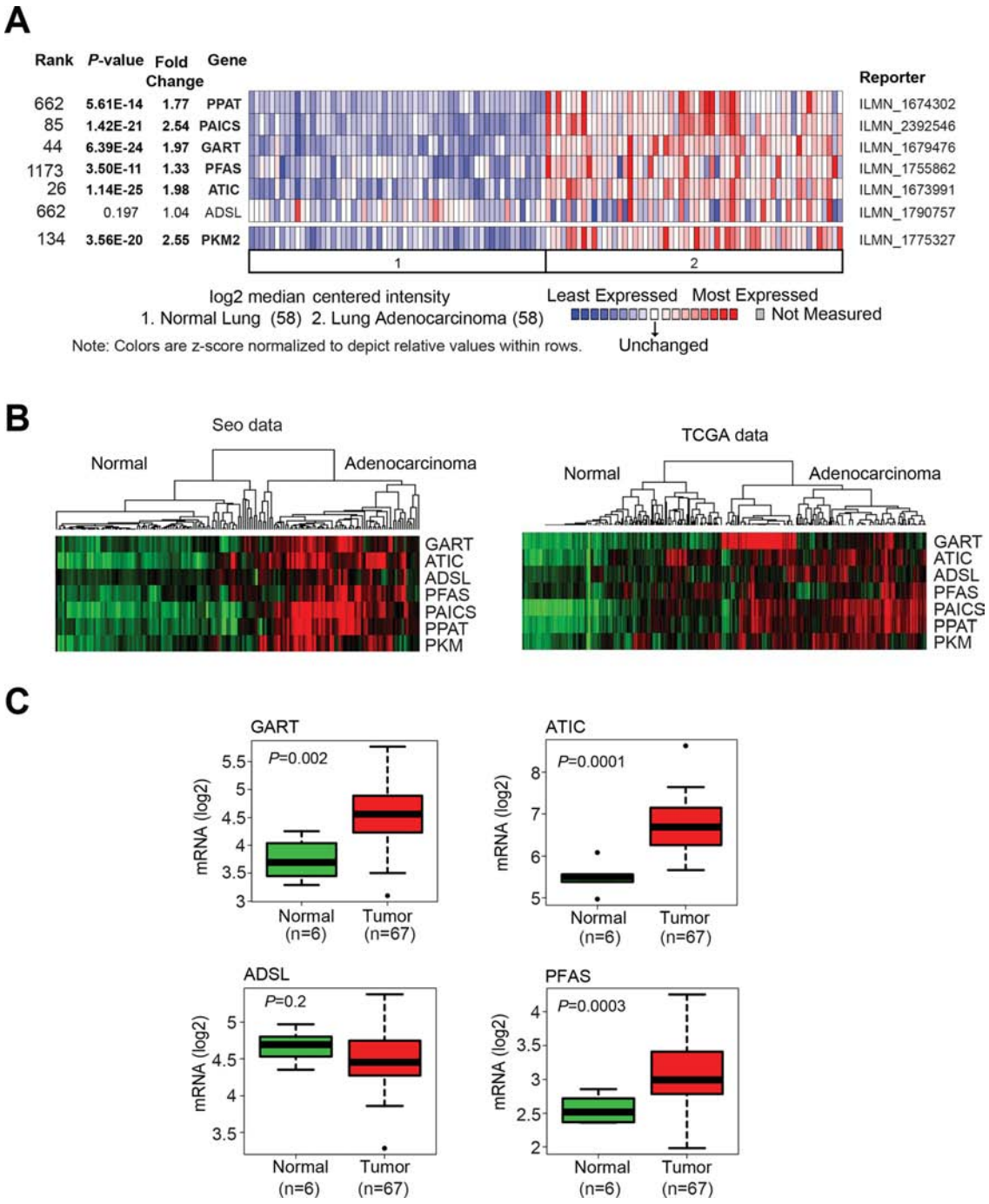
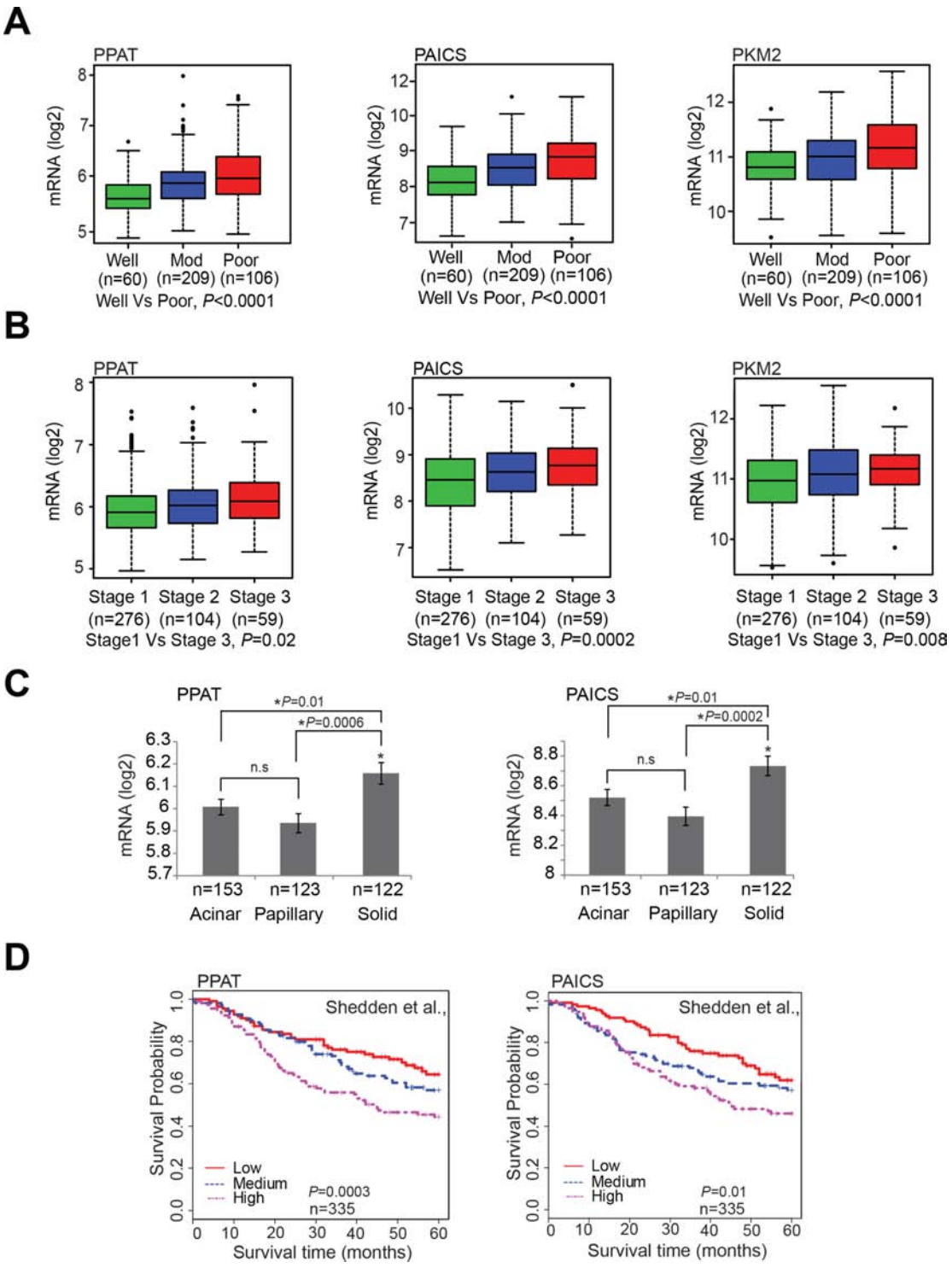
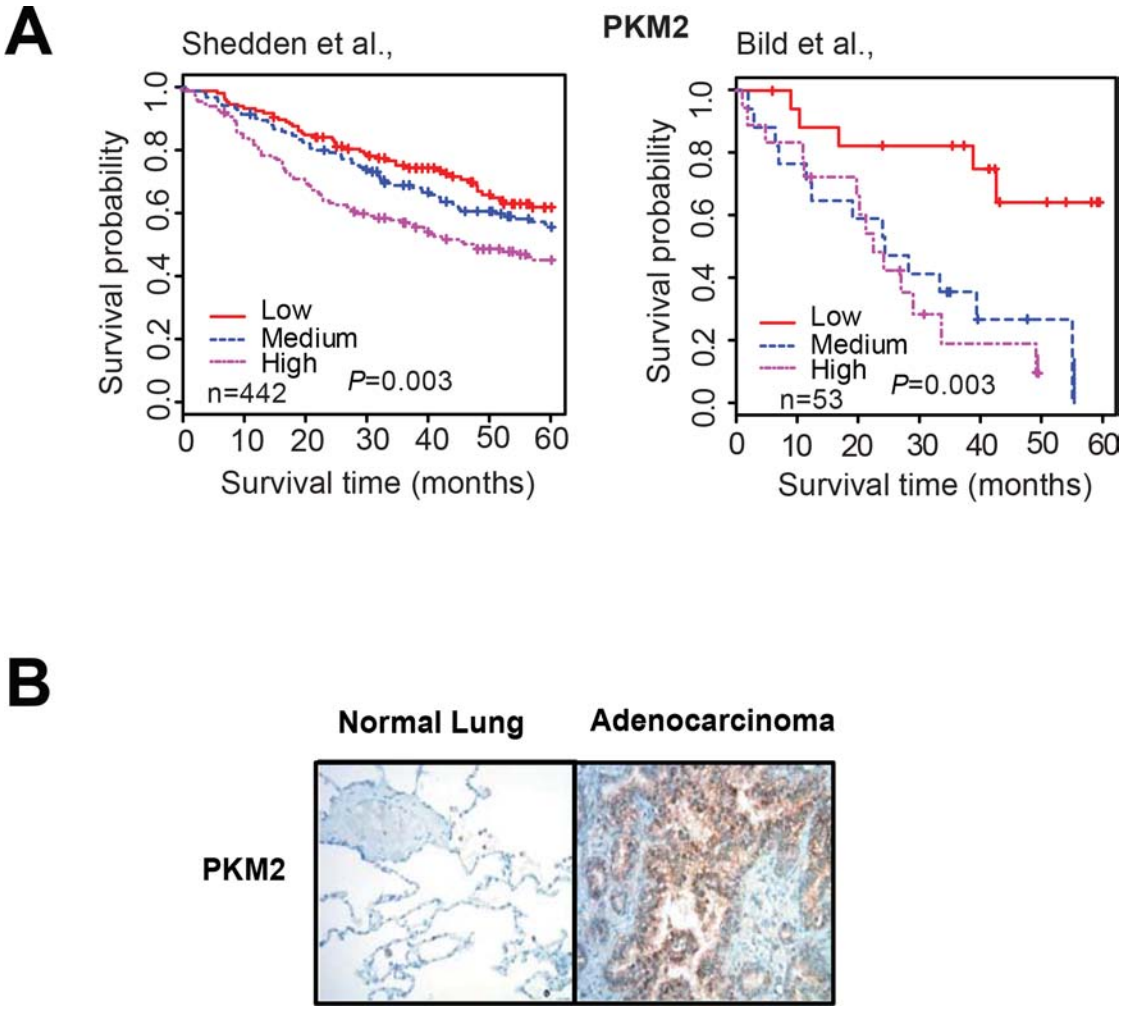
## SUPPLEMENTARY DATA



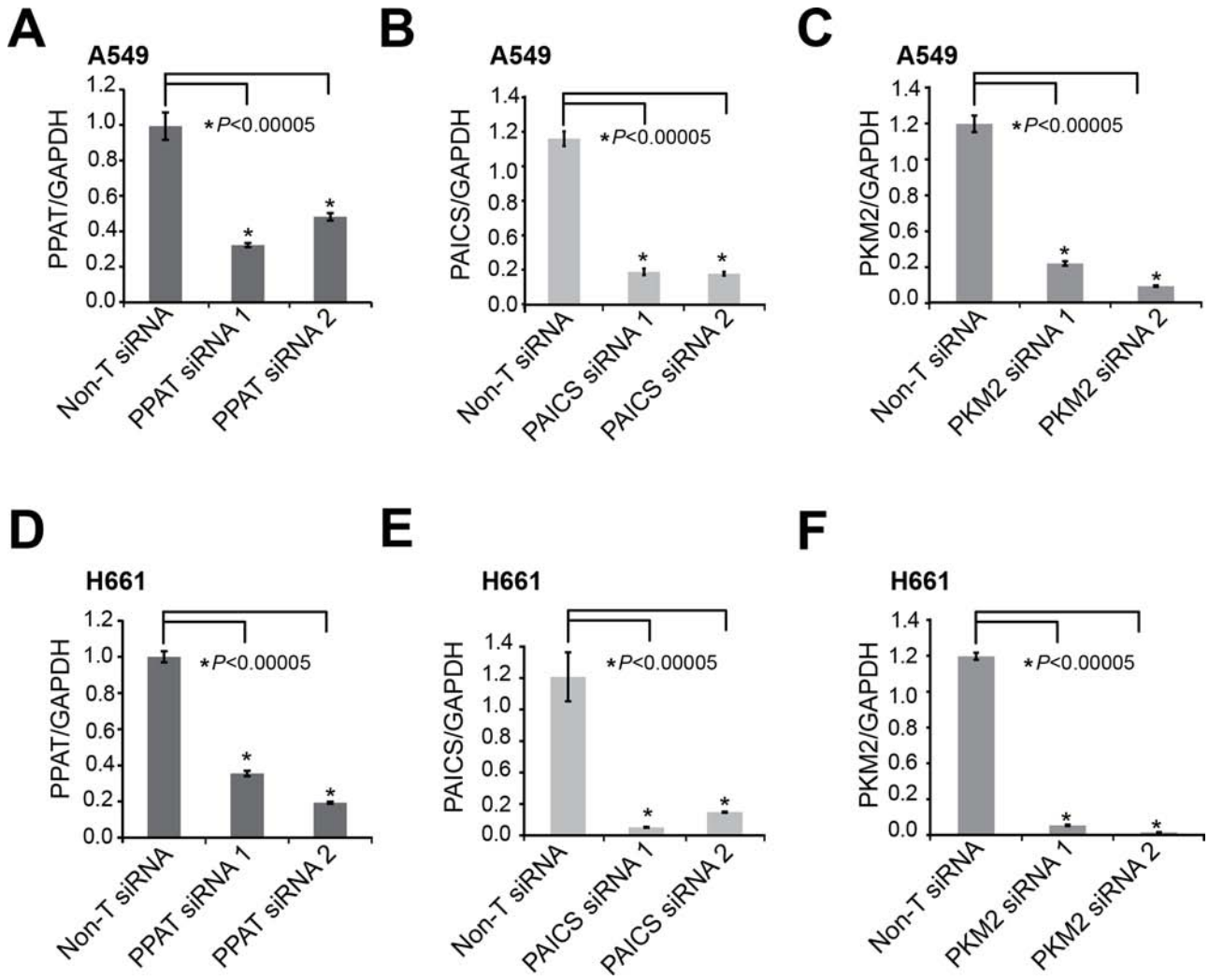

**Supplementary Figure S1: *De novo* purine biosynthetic enzymes and pyruvate kinase M expression in lung adenocarcinoma. A.** Gene expression profiling studies by Oncomine [1] and **B.** Seo et al(85 AD vs 77 normal) [2] and TCGA(309 AD vs 73 normal) [3, 4] datasets suggest enhanced expression of multiple *de novo* purine biosynthetic enzymes and pyruvate kinase M in lung adenocarcinoma tissues. Expression is represented by a color scale highlighting down-regulation (blue), no alteration (white), and up-regulation (red) of transcripts in S1A. In Figure S1B, down-regulation is represented in green while up-regulation is represented in red. **C.** Quantitative measurement of *GART*, *ATIC, ADSL* and *PFAS* by next-generation RNA sequencing [RPKM (log2)] in lung adenocarcinomas [5]. Related to Figure 1.



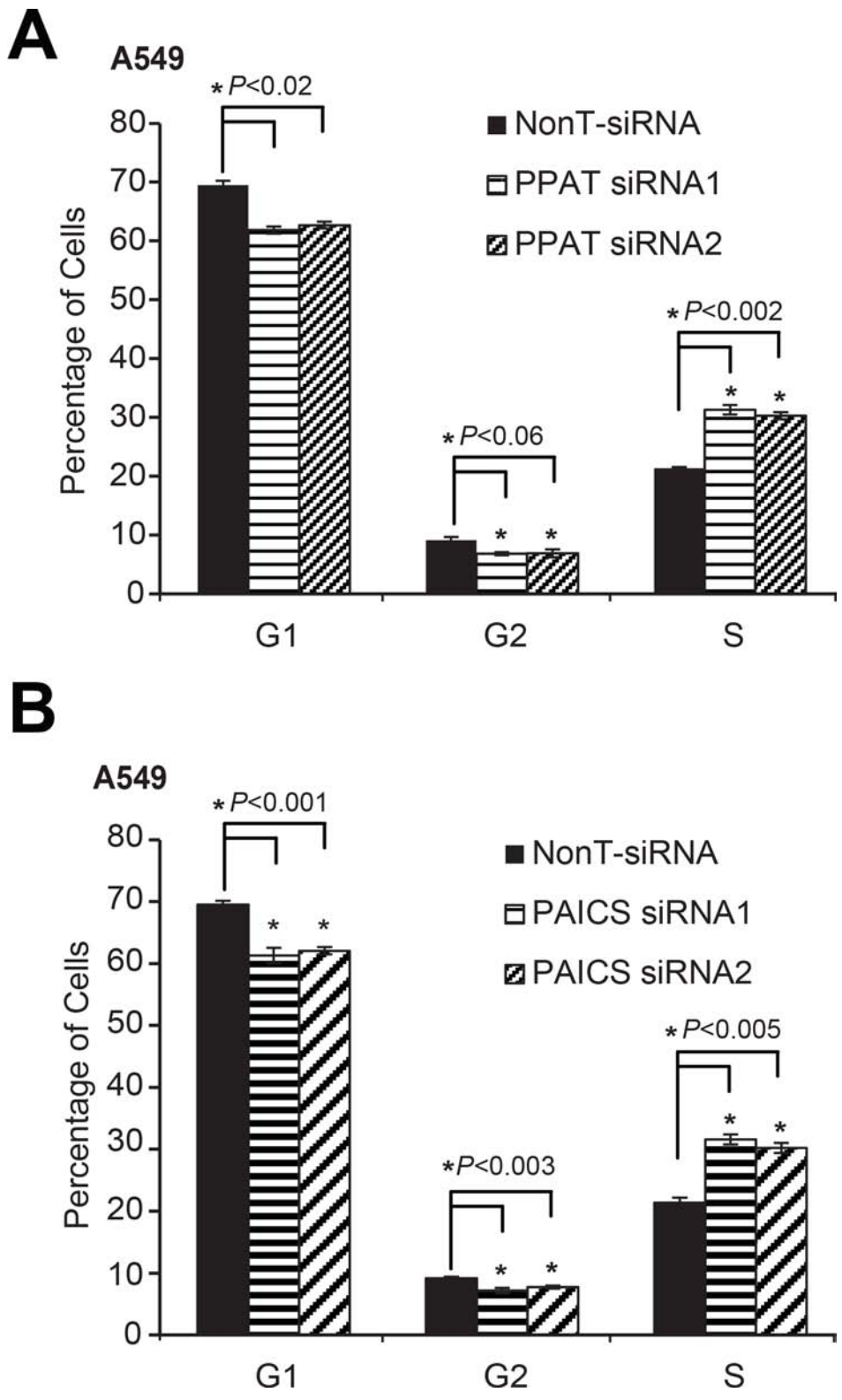

**Supplementary Figure S2: Increased expression of *de novo* purine biosynthesis pathway genes, *PPAT* and *PAICS*, and *PKM2* correlates with poor differentiation status and advanced stage of lung cancer. A.** Transcript levels of *PPAT*, *PAICS* and *PKM2* based on differentiation levels (well, moderate or poorly differentiated) and **B.** stage specific manner in lung cancers [6]. **C.** *PPAT* and *PAICS* transcript expression based on morphological type of invasive adenocarcinoma (acinar, papillary or solid). **D.** Kaplan–Meier (K-M) analysis of disease free survival (DFS) time according to the *PPAT* and *PAICS* transcript levels as measured using Affymetrix oligonucleotide microarray datasets (data retrieved from $n = 335$ out of 442 adenocarcinomas which have disease free survival information) by Shedden *et al* [6]. Related to Figures 1 and 2.



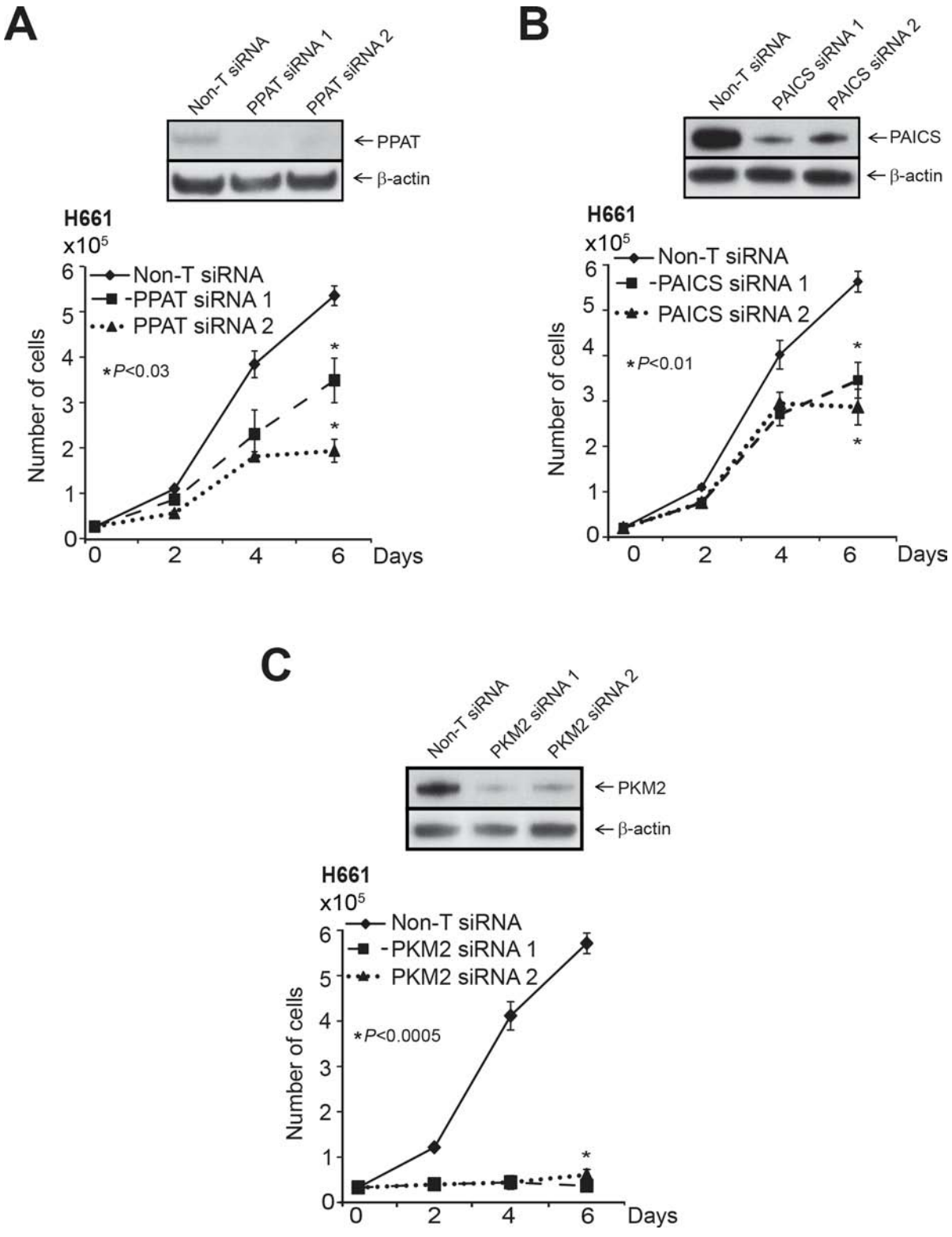

**Supplementary Figure S3: Over-expressed *PKM2* correlates with poor prognosis of lung cancer patients and immunohistochemical analyses show increased *PKM2* expression in lung adenocarcinomas. A.** Kaplan-Meier analysis of survival time according to *PKM2* transcript levels as measured using affymetrix oligonucleotide datasets by Shedden *et al* [6] and Bild *et al* [7]. **B.** Photomicrographs of *PKM2* immunostaining in Normal Lung (Left) and Adenocarcinoma (Right) using *PKM2* specific antibodies. Related to Figure 2.



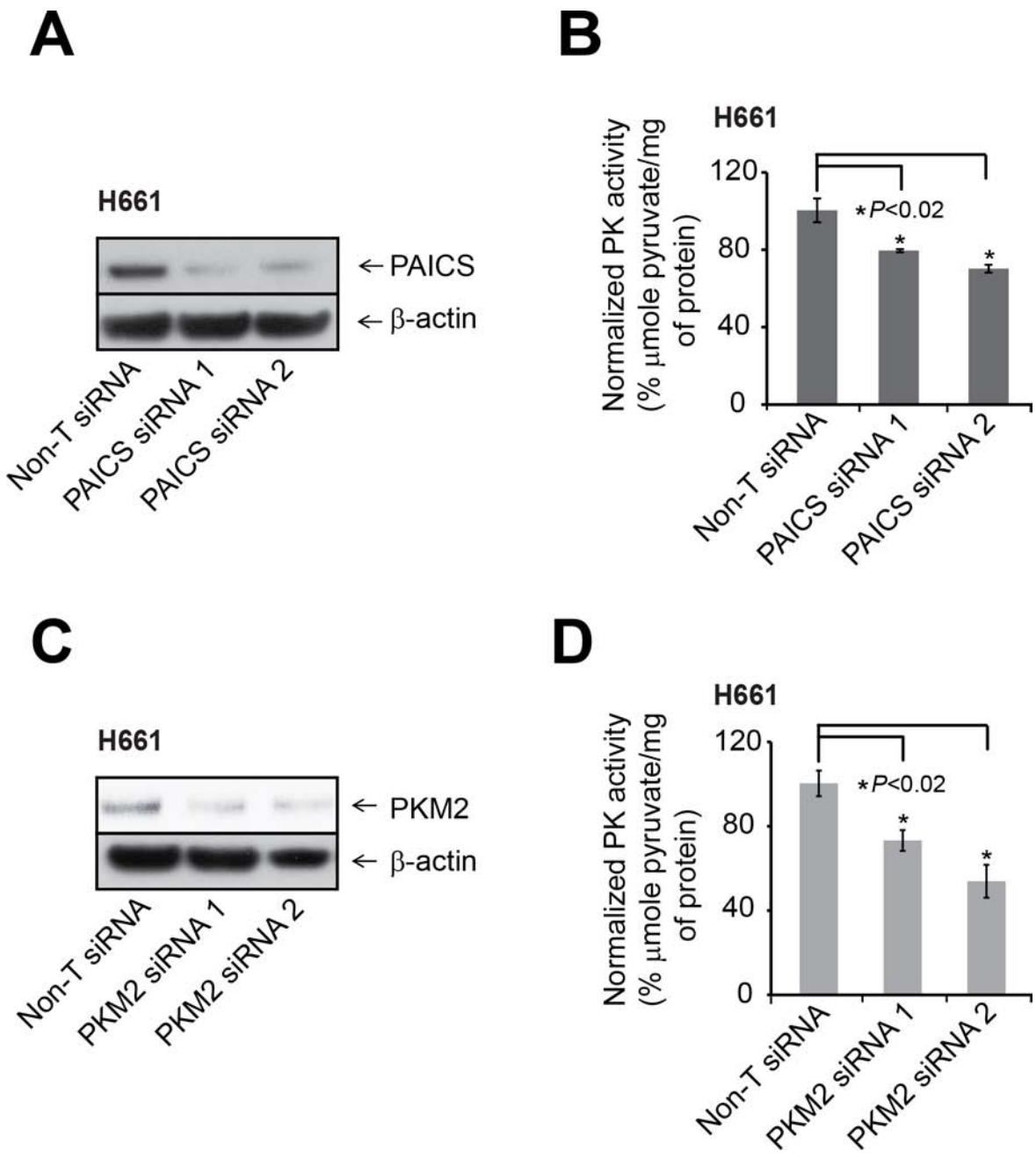

**Supplementary Figure S4: RNA interference using siRNA duplex against *PPAT*, *PAICS* and *PKM2* decreases respective transcripts in multiple lung cancer cell lines.** qRT-PCR analyses of **A, D.** *PPAT*, **B, E.** *PAICS* and **C, F.** *PKM2* transcripts levels in A549 and H661 cells using *GAPDH* for normalization of data. Two independent duplexes were used for the knockdown and measurement of transcript expression. Related to Figure 3.



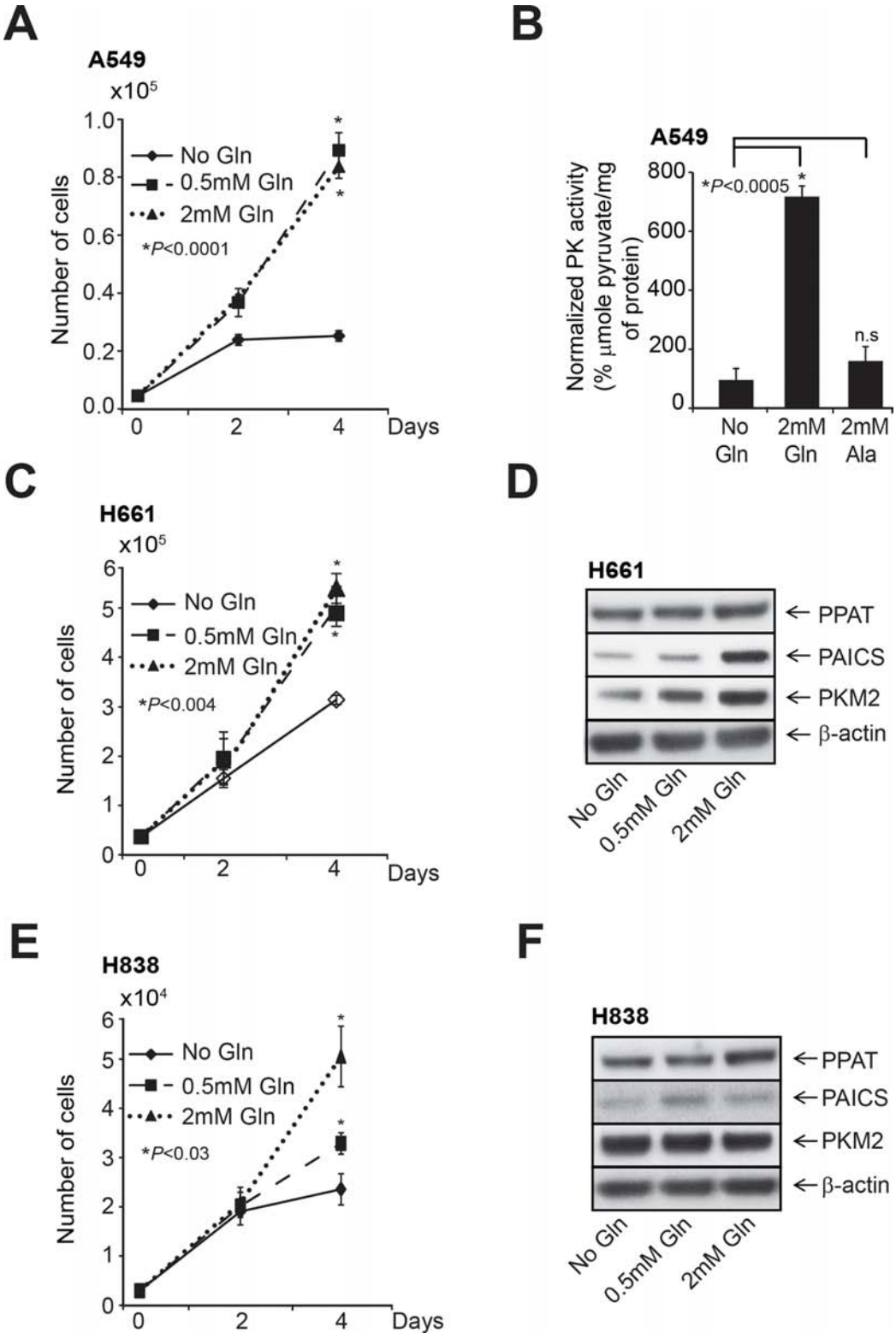

**Supplementary Figure S5: Cell cycle analyses in *PPAT* and *PAICS* knockdown cells show arrest and increase in S-phase.** Cell cycle analyses of A549 cells using siRNA against **A.** *PPAT* and **B.** PAICS genes respectively using two independent duplexes. Asterisk indicates data is statistically significant ($P$ <0.05). Related to Figure 3.



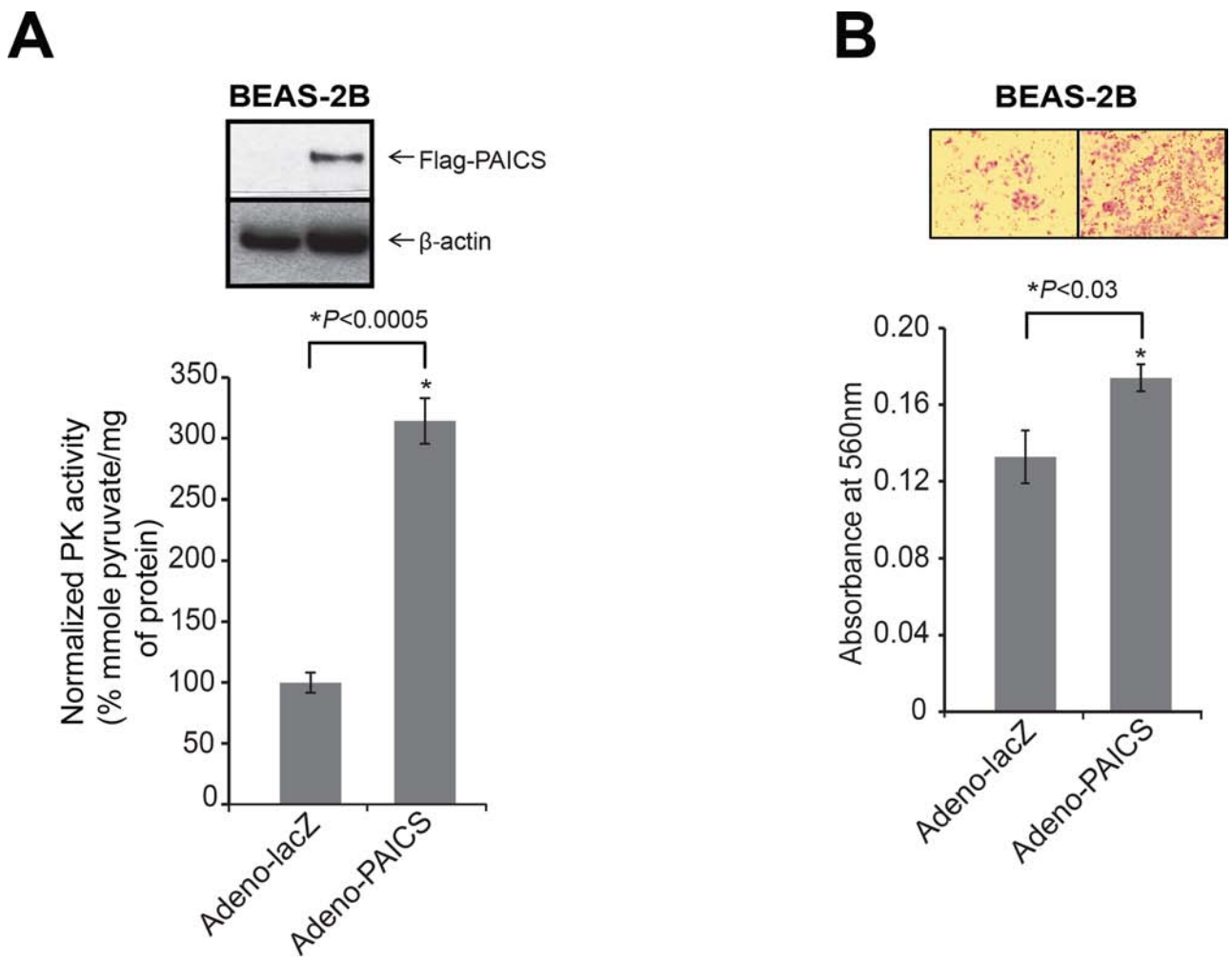

**Supplementary Figure S6: RNA interference using specific siRNA duplex against *PPAT*, *PAICS* and *PKM2* decreases cell proliferation in H661, a large cell carcinoma cell line. A.** *PPAT*, **B.** *PAICS* and **C.** *PKM2* knockdown was achieved using two independent duplexes followed by measurement of cell proliferation. The inset shows immunoblot analyses of the respective genes after transfection with the duplexes. β-actin was used as a loading control. Related to Figure 3.



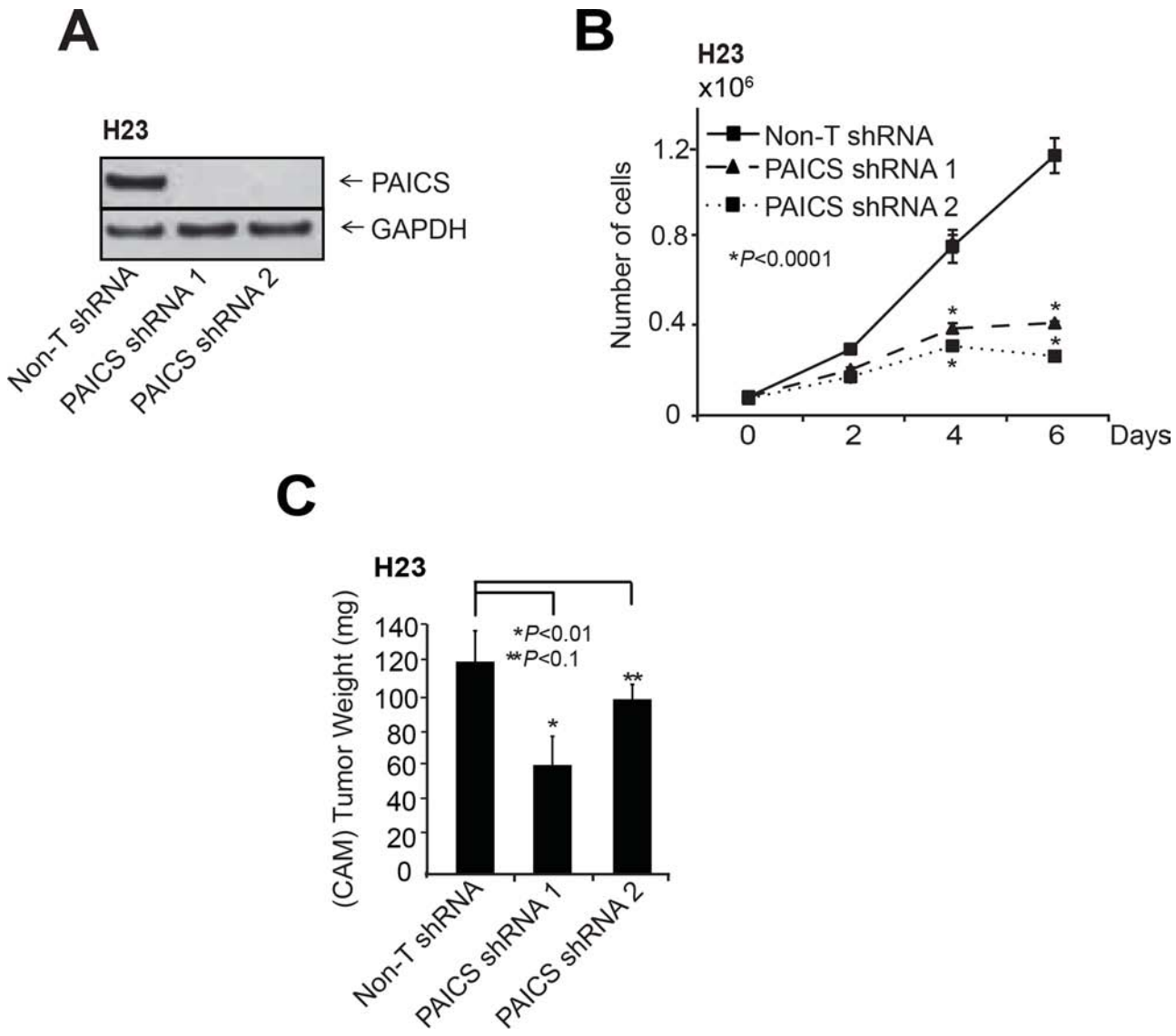

**Supplementary Figure S7: PK activity is modulated on altering *PAICS* and *PKM2* levels in H661 cells.** Knockdown of **A.** *PAICS* and **C.** *PKM2* was achieved using two independent duplexes tested using immunoblot analyses. β-actin was used as loading control. PK activity was measured in **B.** PAICS and **D.** *PKM2* knockdown cells. Related to Figure 4.




**Supplementary Figure S8: Glutamine triggers cell proliferation and induction of *PPAT* and *PAICS* in multiple lung cancer cells.** Glutamine triggered cell proliferation was measured in **A.** A549, **C.** H661 and **E.** H838 cells. **B.** PK activity was measured in presence of 2 mM glutamine and 2 mM alanine. Minus glutamine was used as a negative control. Glutamine induced *PPAT* and *PAICS* was measured in **D.** H661 and **F.** H838 using immunoblot analyses. β-actin was used as a loading control. Related to Figure 4.




**Supplementary Figure S9: *PAICS* overexpression induces pyruvate kinase activity and increased invasion in benign lung epithelial cells BEAS-2B. A.** BEAS-2B cells were infected with adenovirus containing PAICS-flag (shown as inset immunoblot; flag-antibody#F1804, Sigma-Aldrich, USA) followed by measurement of PK activity. **B.** Following PAICS-Adenovirus transfections, BEAS-2B cell invasion was measured using Boyden Chamber Matrigel invasion assay. Related to Figure 4.




**Supplementary Figure S10: Stable knockdown of *PAICS* in H23 decreases cell proliferation and tumor formation by CAM assay.** Knockdown of *PAICS* in H23 was achieved **A.** by using *PAICS*-specific shRNAs and tested using immunoblot analyses followed by **B.** measuring cell proliferation. **C.** *PAICS* knockdown decreased tumor formation as evaluated by CAM assay. Related to Figure 5.

**Supplementary Table S1. Correlation of PPAT, PAICS, PKM2 mRNA and clinical characteristics in 442 lung adenocarcinomas**

| Variable | Number | PKM2* | *P* value** |
|---|---|---|---|
| Age average (ys) | 64.4 | | |
| <60 | 128 | 6.02 | 0.5 |
| > = 70 | 149 | 6.13 | |
| Gender | | | |
| Female | 219 | 5.93 | 0.0001 |
| Male | 223 | 6.11 | |
| N status | | | |
| N0 | 299 | 5.99 | 0.1 |
| N1-2 | 141 | 6.07 | |
| T status | | | |
| T1 | 150 | 5.93 | |
| T2 | 251 | 6.03 | 0.03+ |
| T3-4 | 39 | 6.29 | 0.0002+ |
| Smoking | | | |
| Never | 49 | 5.79 | |
| Past | 268 | 5.98 | 0.002++ |
| Current | 32 | 6.21 | 0.0004++ |
| | | **PAICS*** | ***P* value**** |
| Age average (ys) | 64.4 | | |
| <60 | 128 | 8.52 | 0.9 |
| > = 70 | 149 | 8.64 | |
| Gender | | | |
| Female | 219 | 8.4 | 0.0002 |
| Male | 223 | 8.64 | |
| N status | | | |
| N0 | 299 | 8,45 | 0.003 |
| N1-2 | 141 | 8.66 | |
| T status | | | |
| T1 | 150 | 8.44 | |
| T2 | 251 | 8.52 | 0.2+ |
| T3-4 | 39 | 8.82 | 0.001+ |
| Smoking | | | |
| Never | 49 | 8.17 | |
| Past | 268 | 8.47 | 0.007++ |
| Current | 32 | 8.85 | 0.0006++ |

(*Continued*)

| Variable | Number | PKM2* | *P* value** |
|---|---|---|---|
| Age average (ys) | 64.4 | | |
| <60 | 128 | 11.09 | 0.04 |
| > = 70 | 149 | 10.87 | |
| Gender | | | |
| Female | 219 | 10.98 | 0.1 |
| Male | 223 | 11.05 | |
| N status | | | |
| N0 | 299 | 10.97 | 0.005 |
| N1-2 | 141 | 11.12 | |
| T status | | | |
| T1 | 150 | 11 | |
| T2 | 251 | 11.02 | 0.6+ |
| T3-4 | 39 | 11.08 | 0.4+ |
| Smoking | | | |
| Never | 49 | 10.98 | |
| Past | 268 | 11.01 | 0.7++ |
| Current | 32 | 11.02 | 0.7++ |

AD, adenocarcinoma;
*mean value (data from Shedden, 2008 Nature Med, U133A Affymetrix, RMA normalized and log2 transformed);
***t* test;
+compared to T1;
++compared to Never

**Supplementary Table S2. List of antibodies used in this study**

| Antibody | Application | Dilution | Supplier | Cat. No. |
|---|---|---|---|---|
| **PPAT** | IB | IB, 1:1000 | Origene, Rockville, MD | TA504559 |
| | IHC | IHC, 1:100 | | |
| **PAICS** | IB | IB, 1:10,000 | Genetex, Irvine, CA | GTX83950 |
| | IHC | IHC, 1:10000 | | |
| **PKM2** | IB (Tissue) | IB, 1:1000 | Cell Signaling Technology | 4053S |
| | IHC | IHC, 1:500 | Danvers, MA | |
| | IB | IB, 1:1000 | PTG lab, Chicago, IL | 60268-1-Ig |
| **PKM1** | IB | IB, 1:1000 | Cell Signaling Technology Danvers, MA | 7067 |
| **β -actin** | IB | IB, 1:20000 | PTG lab, Chicago, IL | HRP-60008 |
| **Total H3** | IB | IB, 1:1000 | Cell Signaling Technology Danvers, MA | 9715 |
| **GAPDH** | IB | IB, 1:5000 | PTG lab, Chicago, IL | HRP-60004 |
| **Anti-mouse HRP** | IHC | Manufacturer instructions | Ventana Medical Systems Roche Diagnostics, USA | 760-4313 |
| **Anti-Rabbit HRP** | IHC | Manufacturer instructions | Ventana Medical Systems Roche Diagnostics, USA | 760-4315 |

**Supplementary Table S3. List and sequence of primers used in this study**

| Gene | Primers | Sequence | Reference |
|---|---|---|---|
| PAICS | Forward | GTGGCAGGCAGAAGTAATGG | Custom designed |
| PAICS | Reverse | CACATCCTGAACTCCCCAGT | Custom designed |
| PKM1 | Forward | CGAGCCTCAAGTCACTCCAC | [43] |
| PKM1 | Reverse | GTGAGCAGACCTGCCAGACT | [43] |
| PKM2 | Forward | ATTATTTGAGGAACTCCGCCGCCT | [43] |
| PKM2 | Reverse | ATTCCGGGTCACAGCAATGATGG | [43] |
| PPAT | Forward | GCGATTGAAGCACCTGTGGATG | Custom designed |
| PPAT | Reverse | CGGTTTTTACACAGCACCTCCAC | Custom designed |
| GAPDH | Forward | TGCACCACCAACTGCTTAGC | Custom designed |
| GAPDH | Reverse | GGCATGGACTGTGGTCATGAG | Custom designed |

**Supplementary Table S4.** List and sequences of si and shRNAs used in this study

| Gene | Sequence | Supplier | Cat. No. |
|---|---|---|---|
| PPAT si1 | GAAAUGGUCUGGAAUGUUU | Dharmacon, CO, Thermo Fisher Scientific, Pittsburgh, PA | J-006003-06 |
| si2 | GGAAAUAUCCAGACACAAU | Dharmacon, CO, Thermo Fisher Scientific, Pittsburgh, PA | J-006003-07 |
| PAICS si1 | GUACACUGGUUGAUAUGAA | Dharmacon, CO, Thermo Fisher Scientific, Pittsburgh, PA | J-003980-07 |
| si2 | GAAGGGCUCCAAAUGGUAA | Dharmacon, CO, Thermo Fisher Scientific, Pittsburgh, PA | J-003980-09 |
| PKM2 si1 | AGGCAGAGGCUGCCAUCUA | Dharmacon, CO, Thermo Fisher Scientific, Pittsburgh, PA | Custom made |
| si2 | CCAUAAUCGUCCUCACCAA | Dharmacon, CO, Thermo Fisher Scientific, Pittsburgh, PA | Custom made |
| PPAT sh1 | TTGTAGGAATGTTTATTCC | Open Biosystems, Thermo Fisher Scientific, Pittsburgh, PA | V2LHS_170267 |
| sh2 | ATTCAAATGCCAATTTGCC | Open Biosystems, Thermo Fisher Scientific, Pittsburgh, PA | V2LHS_170269 |
| PAICS sh1 | GUACACUGGUUGAUAUGAA | System Biosciences, Mountain View, CA | Custom made |
| sh2 | GAAGGGCUCCAAAUGGUAA | System Biosciences, Mountain View, CA | Custom made |

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