

## Additional File 1

### **NF- $\kappa$ B Upregulates Glutamine-Fructose-6-Phosphate Transaminase 2 to Promote Migration in Non-Small Cell Lung Cancer**

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This document contains the following additional information:

Supplementary Tables (5 total)  
Supplementary Figures (5 total)

**Supplementary Table 1: shRNA and siRNA sequences**

<b>Company</b>	<b>Target</b>	<b>siRNA Target Sequence</b>
GE Dharmacon (D-001206-13)	siRNA Non-targeting Control Pool 1	UAGCGACUAAACACAUCAA UAAGGCUAUGAAGAGAUAC AUGUAUUGGCCUGUAUUAG AUGAACGUGAAUUGCUCAA
GE Dharmacon (M-019111-00)	Human <i>OGT</i> SMART pool	GCAGUUCGCUUGUAUCGUA CGACAUGCAUUGCGUCUCA GAUUAAGCCUGUUGAAGUC CCGAGAAAUUGGCUUAUUAU
GE Dharmacon (M-010390-01)	Human <i>GFPT2</i> SMART pool	UCGCCAAGCUGAUUAAAUA GAUCAUCCGUGGCUUGAGA AGACAAAGGCAACGAAUUU CGAUGGGAAUAAUCACGAA

<b>Company</b>	<b>Target</b>	<b>Mature Target Sequence</b>
GE Dharmacon (V2THS_50314)	TRIPZ human GFPT2 shRNA	TCAGATGAAAGGTGGTGAT

## Supplementary Table 2: RT-qPCR primer sequences

### Primer Sequences for *H. sapiens*

Gene	Sequence
<i>GAPDH</i> F	GAAGGTGAAGGTCGGAGTC
<i>GAPDH</i> R	GAAGATGGTGATGGGATTTTC
<i>SNAI1</i> F	CACTATGCCGCGCTCTTTC
<i>SNAI1</i> R	GGTCGTAGGGCTGCTGGAA
<i>SNAI2</i> F	ATGAGGAATCTGGCTGCTGT
<i>SNAI2</i> R	CAGGAGAAAATGCCTTTGGA
<i>ZEB2</i> F	CAATACCGTCATCCTCAGCA
<i>ZEB2</i> F	CCAATCCCAGGAGGAAAAAC
<i>TWIST1</i> F	CGGGAGTCCGCAGTCTTA
<i>TWIST1</i> R	CTTGAGGGTCTGAATCTTGCT
<i>OGT</i> F	AGGAAATGTCTTGAAAGAGGCAC
<i>OGT</i> R	TCGTAGTACACACAAGCCCAGG
<i>SLC2A1</i> F	CATCAACGCTGTCTTCTATTACTC
<i>SLC2A1</i> R	ATGCTCAGATAGGACATCCA
<i>SLC1A4</i> F	CTGTGGACTGGATTGTGGAC
<i>SLC1A4</i> R	TCCACTTTCACCTCAGCAAG
<i>NT5E</i> F	TGGAGATGGGTTCAGATGATAA
<i>NT5E</i> R	GGATAAATTACTTTTCATTTTGGAGATAT
<i>UUP1</i> F	ACTGCCCAGGTAGAGACTATC
<i>UUP1</i> R	CTGCACCAGCTTCTTGTTAAG
<i>GFPT1</i> F	CCAGCCAGTTTGTATCCCTT
<i>GFPT1</i> R	CAAGCATGATCTCTTTGCGT
<i>GFPT2</i> F	AGGTGCATTCGCGCTGGTT
<i>GFPT2</i> R	TGTGGAGAGCTTGTATTTGCTCCCGGG
<i>UAP1</i> F	AATGACCTCAAACCTCACGTTGT
<i>UAP1</i> R	GCTCTGCATAAAGTTCTACCTGT
<i>MGEA5</i> F	TCCATAACCCAAGGTCTTCCA
<i>MGEA5</i> R	TTGGAGGAGCGGGAGAGCGA
<i>TNFAIP3</i> F	TTGACCAGGACTTGGGACTT
<i>TNFAIP3</i> R	ACAGCTTTCGCGCATATTGCT
<i>BIRC3</i> F	GCTGTGATGGTGGACTCAGG
<i>BIRC3</i> R	TGGCTTGAACCTTGACGGATG
<i>HPRT</i> F	TTGGAAAGGGTTATTCCTCA
<i>HPRT</i> R	TCCAGCAGCTCAGCAAAGAA

**Supplementary Table 3: Antibodies**

<b>Antibody Target</b>	<b>Company</b>	<b>Product Number</b>
$\alpha$ -Tubulin	Sigma	T6793
E-Cadherin	Santa Cruz	sc-7870
Fibronectin	BD Bioscience	610078
Flag M2	Sigma	F1804
GAPDH	GeneTex	GTX627408
GFPT1	Cell Signaling	3818
GFPT2	Cell Signaling	6917
GFPT2 (IHC)	Atlas	HPA059910
MMP9	R&D Systems	MAB936
N-Cadherin	BD Transduction Labs	610920
Normal IgG (ChIP)	Santa Cruz	sc-2025 (mouse) Rabbit sc-2027
OGA	Sigma	SAB4200311
OGT	Sigma	O6264
O-GlcNAc RL-2	Thermo Scientific	MA1-072
O-GlcNAc CTD10.1	Cell Signaling	98705s
p65 C-20 (ChIP)	Santa Cruz	Sc-372
V5	Invitrogen	R960-25
Vimentin	Sigma	V6630

## Supplementary Table 4: ChIP-PCR primer sequences corresponding to the *GFPT2* gene

### *GFPT2* Locus

### Sequence

<i>H. sapiens</i> ChIP site A F	TCCTGCCCTTCCCAGTGATAAA
<i>H. sapiens</i> ChIP site A R	CTTTGCTTATTGTCCTGTTG
<i>H. sapiens</i> ChIP site B F	AGTGGCAAGGGTGAGCTTCT
<i>H. sapiens</i> ChIP site B R	TTGCACAATGCCTGCCTG

### Site A:

TCCTGCCCTTCCCAGTGATAAAATGAACCTCATTGGAAAATCCCCCACTTCAGTGTAGATGATGATTCTCATTG  
TGTCCCCAAAGCAGGGAGGCTCCAGGAGGCCACCCACACCCAAACTGGACTCCAGCCCTGACTCAGGAT  
GAGTAATTGAAAAGAAAACAACAGGACAATAAGCAAAG

### Site B:

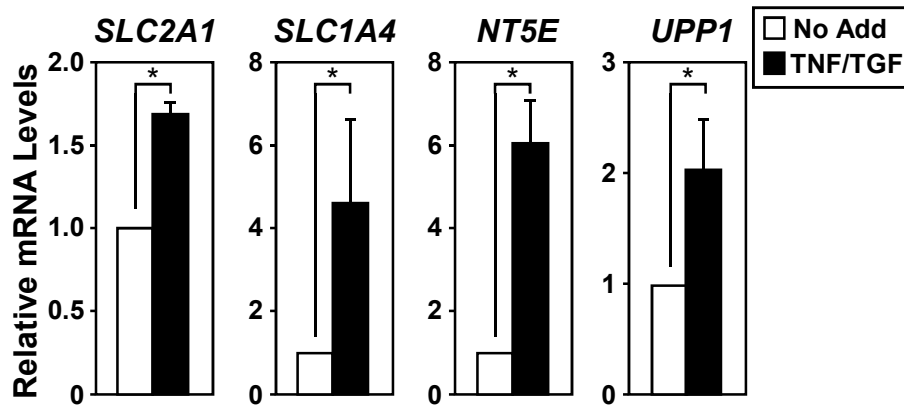
AGTGGCAAGGGTGAGCTTCTGAGGTGGGATGATCTCAGGGCCTCCGGGAGTGACTCAGAGGTATTTGGCTGTGA  
TGCTGGCAAACATTTTTCCCCCTTCCCGGTTTTCCCTGTTCTTGACTTAAGTCACTCCAAGGGCAGATCACCA  
GGGAGGAGAGGGAGTTGGCCAGGCAGGCATTGTGCAA

## SUPPLEMENTARY TABLES

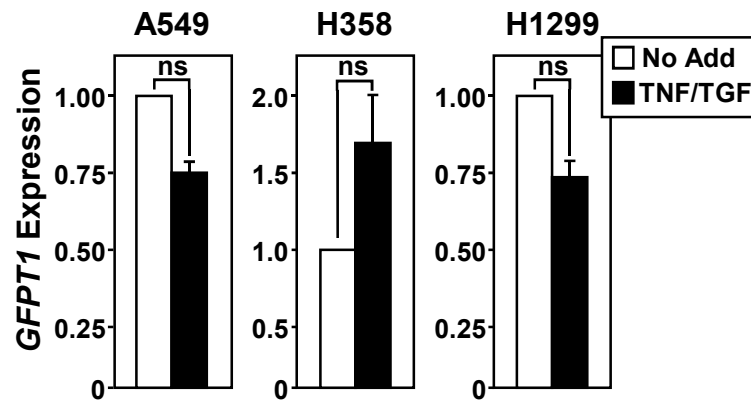
### Supplementary Table 5: Metabolic genes upregulated during EMT

#### Genes

ABAT	GLS1	SLC2A1
ACO1	GPAM	SLC4A7
ACSL5	HK2	SLC6A6
ADA	HKDC1	SLC6A8
AGPAT4	HLCS	SLC6A10P
AGPAT6	HMOX2	SLC16A2
AK8	HS3ST3B1	SLC22A4
ATP5C1	IDS	SLC25A37
B4GALT1	INPP4B	SLC39A8
BCAT1	ITPKC	SLC43A3
BMP2	LSS	SMAD5
BPGM	MAP2K1	SMAD7
CDA	MBOAT2	SMOX
CHKB	MTAP	SMS
CHST11	MTHFD1L	SOD2
CHST2	NAMPT	SPHK1
CPT1B	NDST1	SPTLC2
CYP2B6	NEDD4L	SQLE
CYP2R1	NT5E	ST3GAL1
DHRS3	ODC1	SYNJ2
DIO2	PANK2	TDO2
DSE	PECR	UAP1
ENO3	PGK2	UBA6
FA2H	PGM2	UBE2D1
FSTL1	PIK3CD	UBE2D3
GAD1	PLCB4	UBE2E2
GALNT12	PLD1	UBE2O
GALNT5	PNPLA8	UPP1
GCH1	PPAP2B	UXS1
GFPT2	PTGES	XDH
GGH	PTGS2	XYLT1
GK	PYGL	
GK3P	SAT1	

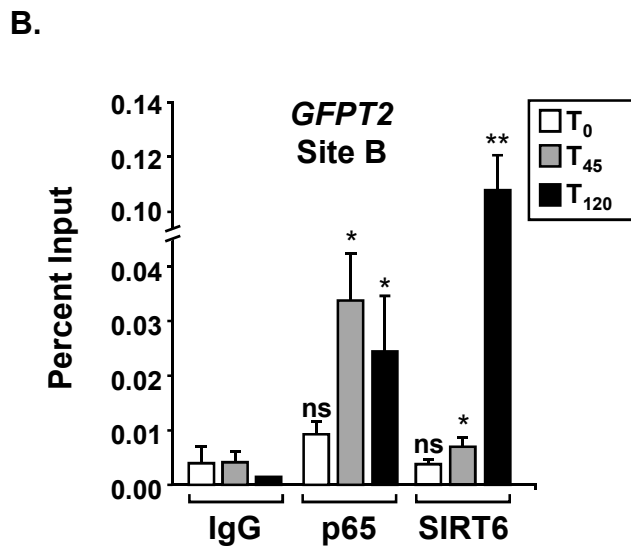
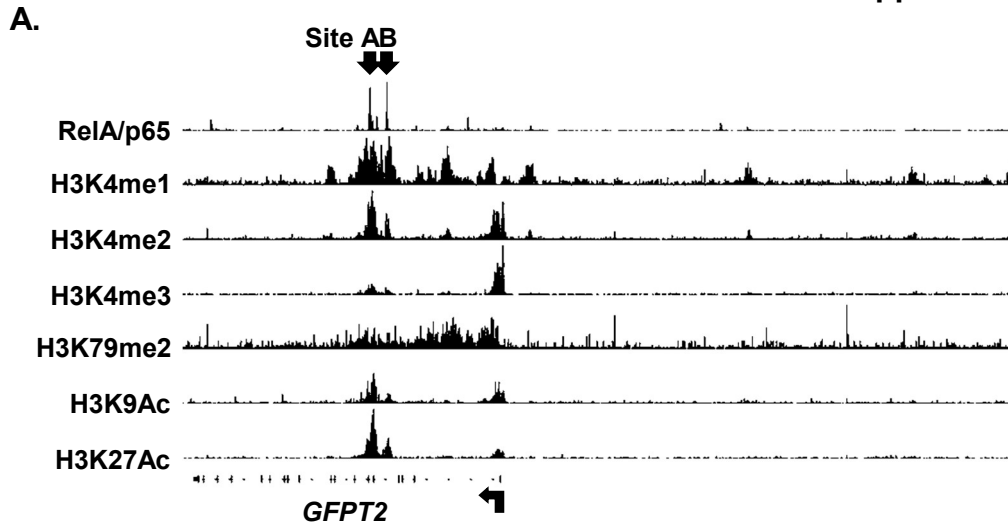


**Supplementary Figure 1: RT-qPCR analysis confirms the upregulation of metabolic genes.** Data represents three independent experiments performed in triplicate; mRNA levels were calculated relative to *HPRT* mRNA expression. Mean +SD are shown; \*  $p < 0.05$ .

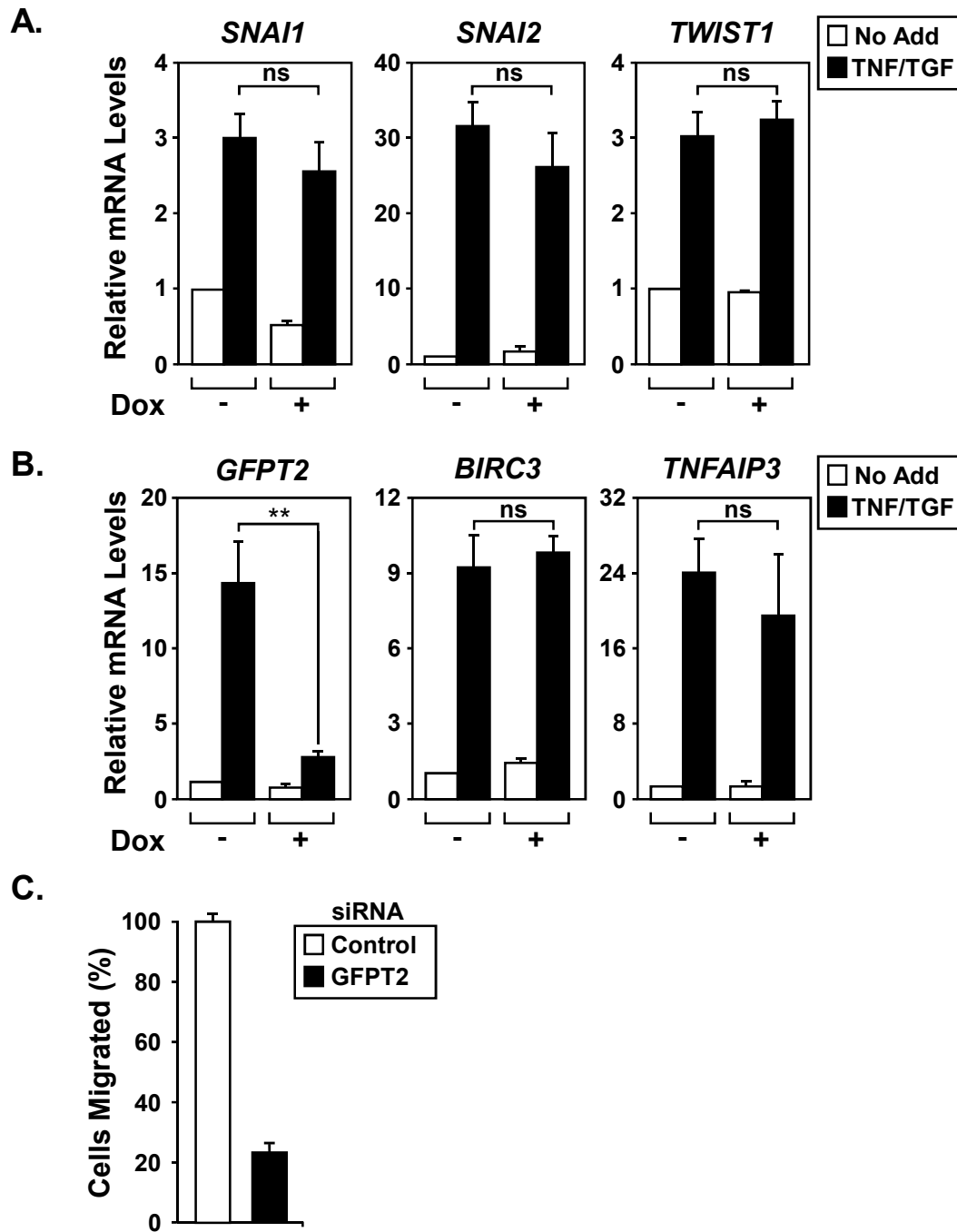


**Supplementary Figure 2: Unlike *GFPT2*, *GFPT1* mRNA expression is not upregulated in NSCLC spheroid cultures following TNF and TGF $\beta$  stimulation, as detected by RT-qPCR analysis.** Data represents three independent experiments performed in triplicate; mRNA levels were calculated relative to *HPRT* mRNA expression. Mean  $\pm$  SD are shown; ns – not significant.





**Supplementary Figure 3: The *GFPT2* gene is regulated by NF- $\kappa$ B and SIRT6.** (A) A screen shot of the *GFPT2* gene showing ChIP-seq enrichments for RelA/p65 are localized to Site A and B, which correspond to the image shown in Figure 6A. (B) ChIP-qPCR analysis across the Site B of the *GFPT2* locus indicates elevated chromatin occupancy of p65 and SIRT6 in TNF stimulated A549 cells. Data represent changes in ChIP-qPCR relative to percent input. Mean and SD  $\pm$  are shown; \*  $p < 0.05$ , \*\*  $p < 0.01$ , ns - not significant;  $n = 3$ .



**Supplementary Figure 4: Knockdown of *GFPT2* fails to impact the expression of NF- $\kappa$ B-regulated genes as well as mesenchymal markers, but is required for cell migration.** (A & B) Knockdown of *GFPT2* following Dox treatment of A549:sh*GFPT2* cells fails to show changes in mesenchymal markers *SNAI1*, *SNAI2*, or *TWIST1*, as well as NF- $\kappa$ B transcriptional targets, *BIRC3* and *TNFAIP3* following stimulation. (C) siRNA-mediated silencing of *GFPT2* abolished H1299 cell migration, in a similar manner as data shown in Figure 7D. Mean and SD  $\pm$  are shown; \*\*  $p < 0.01$ , ns - not significant;  $n = 3$ .

**A.**

**Disease Free Survival**

<b>GFPT2 Expression</b>	<b>Total Cases</b>	<b>Relapsed/Progressed Cases</b>	<b>Median Months Survival</b>
High	63	36	22.9
Low	82	34	44.02

**B.**

**Overall Survival**

<b>GFPT1 Expression</b>	<b>Total Cases</b>	<b>Deceased Cases</b>	<b>Median Months Survival</b>
High	101	44	39.32
Low	133	45	39.72

**Disease Free Survival**

<b>GFPT1 Expression</b>	<b>Total Cases</b>	<b>Relapsed/Progressed Cases</b>	<b>Median Months Survival</b>
High	82	39	26.94
Low	118	52	28.42

**Supplementary Figure 5: Elevated *GFPT2*, but not *GFPT1*, mRNA expression correlates with poor overall and disease free survival.**(A) As shown in Table 3 elevated *GFPT2* expression is associated with reduced overall survival and with reduced median months disease free survival. (B) Unlike *GFPT2*, *GFPT1* expression is not predictive of overall or disease free survival.