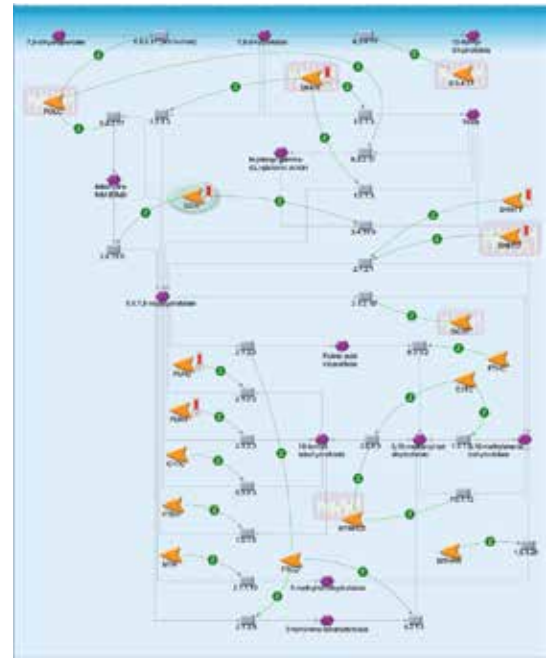


A

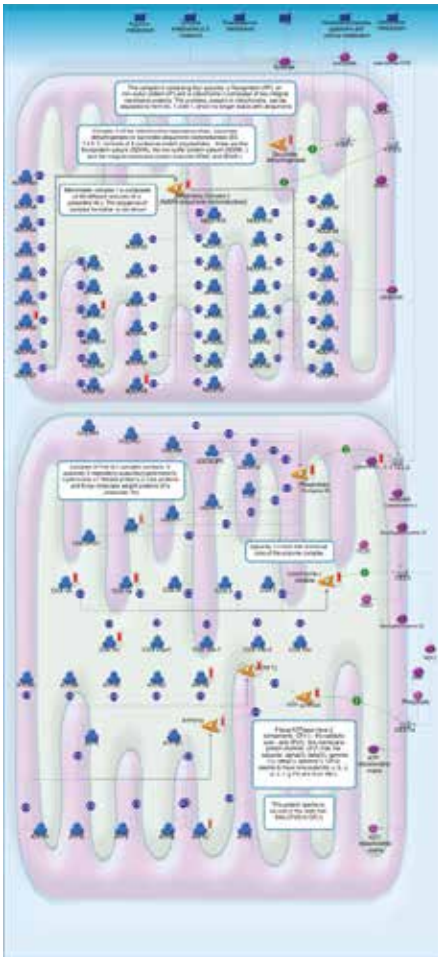
Significantly altered metabolic pathways

Pathway maps	p-values	Ratios (altered gene/total genes)
Oxidative phosphorylation	1.210E-6	10/105
Folic acid metabolism	6.858E-5	6/53
Histidine-glutamate -glutamine metabolism	2.648E-4	7/95

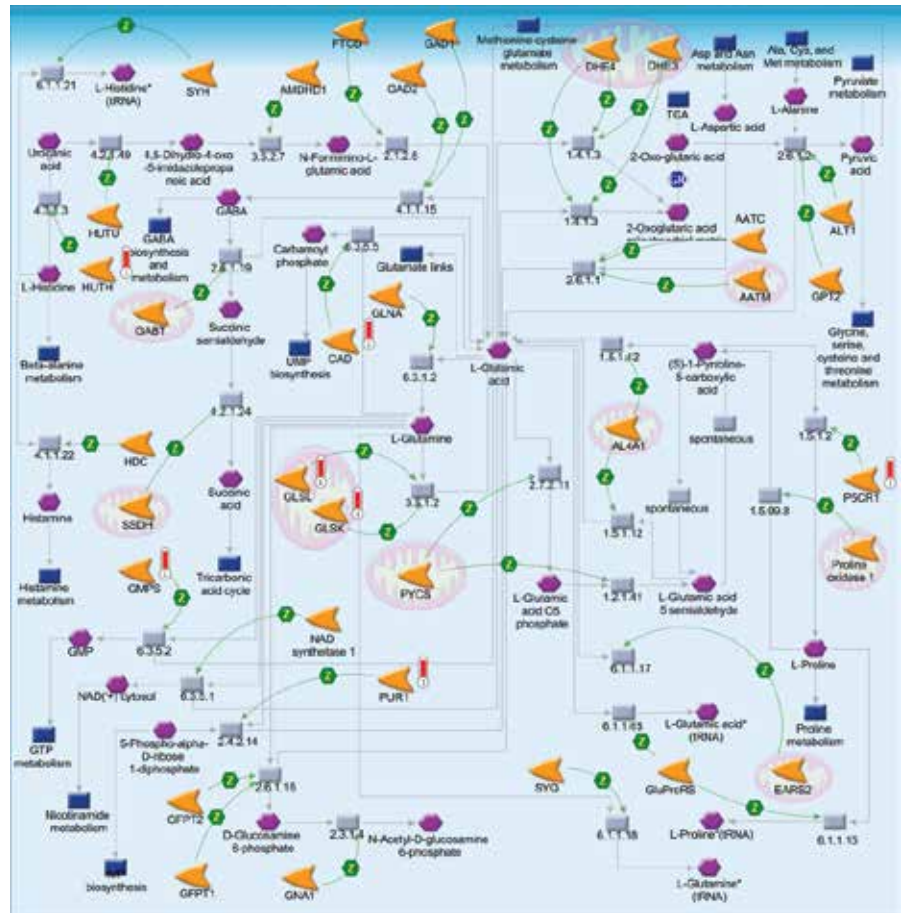
B

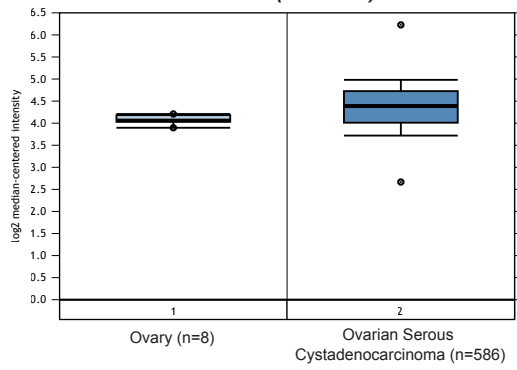


C

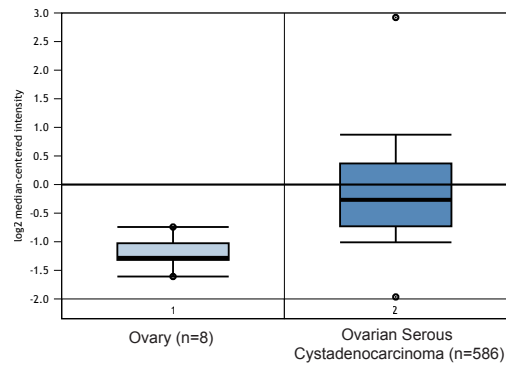


D

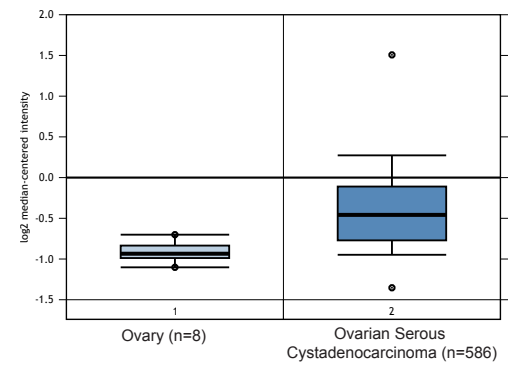


ATIC (PUR9)

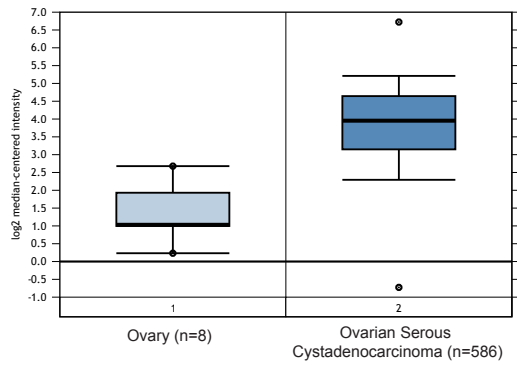
ATIC Fold change = +1.227
p-value = 8.49E-6

DHFR

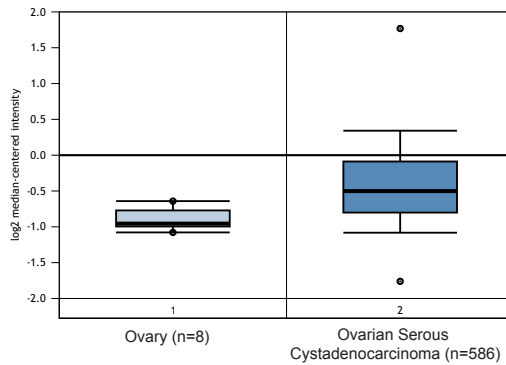
DHFR Fold change = +2.084
p-value = 1.27E-6

GART (PUR2)

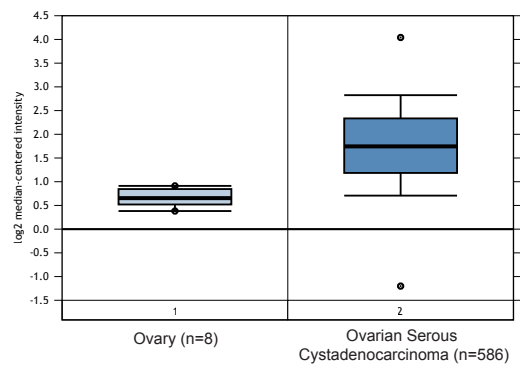
GART Fold change = +1.446
p-value = 4.73E-7

GGH

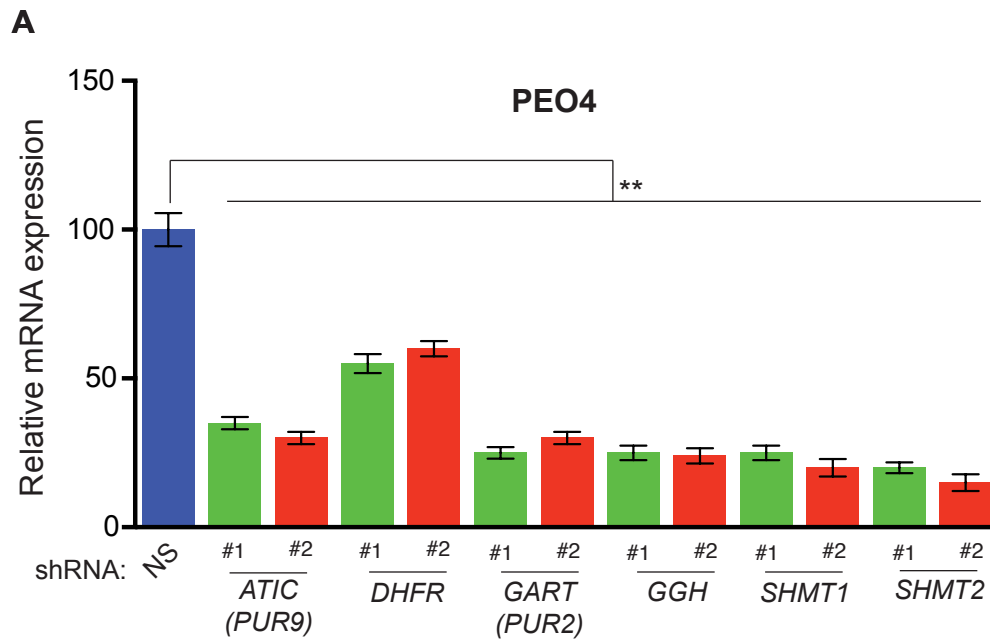
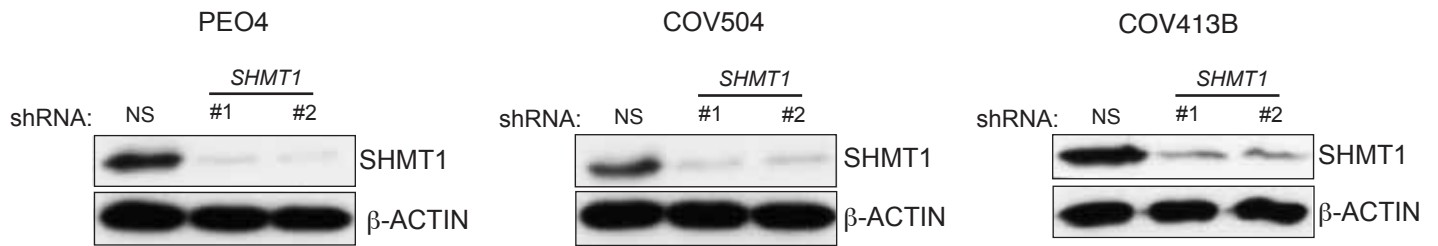
GGH Fold change = +6.21
p-value = 1.03E-5

SHMT1

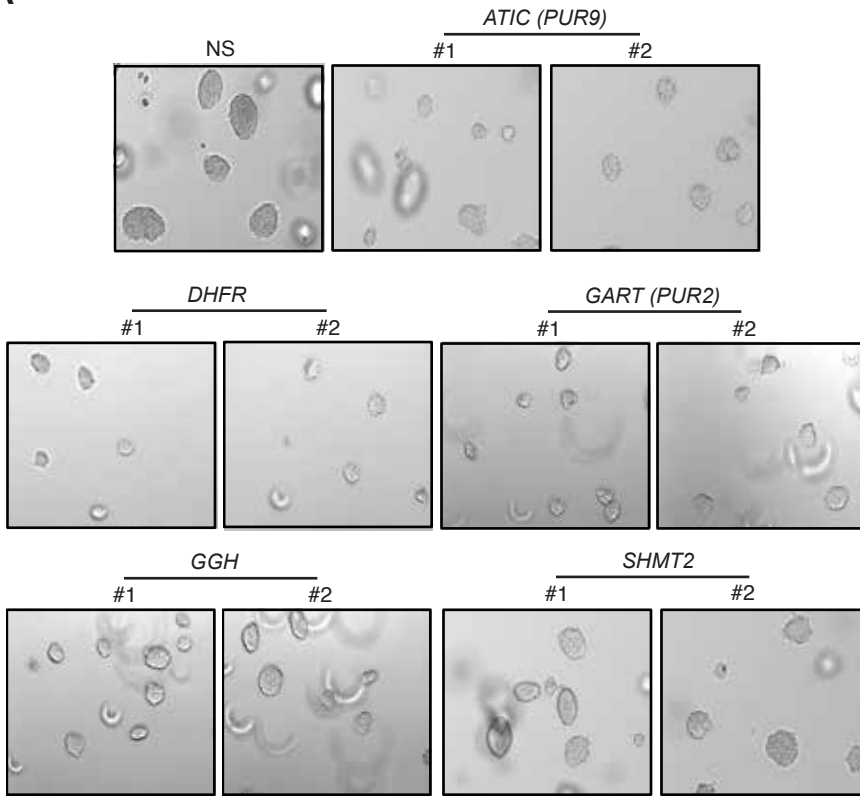
SHMT1 Fold change = +1.41
p-value = 2.26E-6

SHMT2

SHMT2 Fold change = +2.163
p-value = 1.13E-8

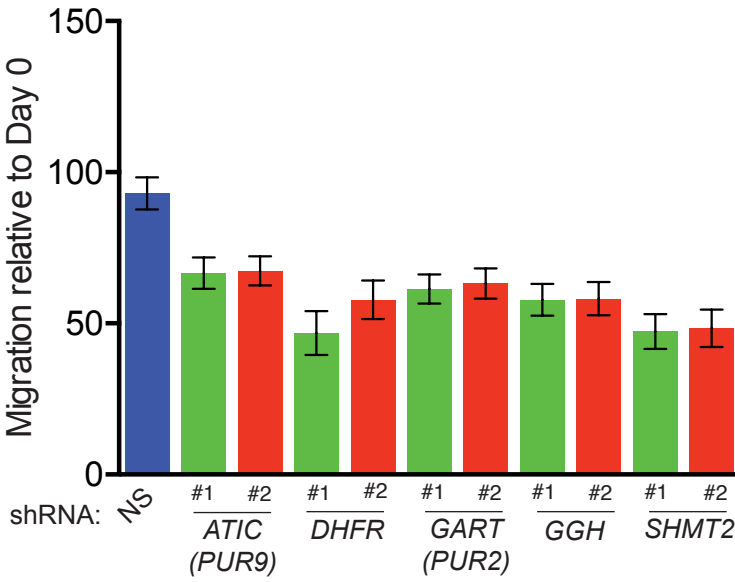
**B**

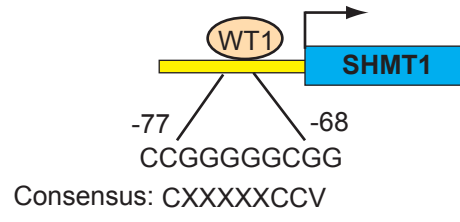
A



B

Cell line	shRNA	Colony number	Average Colony size
PEO4	NS	93±8.2	100±4.8
	ATIC (PUR9)#1	60±6	34.2±3.2
	ATIC (PUR9)#2	50±7.3	38.4±3
	DHFR#1	50±6.3	34.6±4.1
	DHFR#2	70±7.2	35.5±3.4
	GART (PUR2)#1	70±5.6	30.7±3.5
	GART (PUR2)#2	60±6.6	33.8±4.2
	GGH#1	90±11	53.8±3.8
	GGH#2	70±10	65.3±4.8
	SHMT2#1	80±9	92.3±4
	SHMT2#2	60±8	96.1±4.3





Supplementary Figure Legends

Supplementary Figure 1. Identification of key metabolic pathway alterations in ovarian cancer. **A.** Ingenuity pathway analysis (IPA) identifies oxidative phosphorylation, folic acid metabolism, and histidine-glutamate-glutamine metabolism as three key metabolic pathways that were altered in ovarian cancer cells. The table lists p-values and the number of genes in each pathway in ratio with genes that are altered in indicated metabolic pathways. **B.** Folic acid metabolic pathway showing six genes marked with red stick image (**inset**) that are significantly upregulated in TCGA ovarian cancer datasets. **C.** Oxidative phosphorylation pathway showing 10 genes marked with red stick image (**inset**) that are significantly upregulated in TCGA ovarian cancer datasets. **D.** Histidine-glutamate-glutamine metabolic pathway showing seven genes marked with red stick image (**inset image**) that are significantly upregulated in TCGA ovarian cancer datasets.

Supplementary Figure 2. Genes in folic acid metabolic pathway are upregulated in ovarian cancer cells. TCGA ovarian cancer dataset analysis shows relative fold-upregulation and p-values for indicated genes.

Supplementary Figure 3. Knockdown of genes of folic acid metabolic pathway in multiple ovarian cancer cell lines. **A.** PEO4 cells expressing either control nonspecific (NS) shRNA or shRNA targeting the indicated mRNAs were analyzed for expression of indicated mRNAs by RT-qPCR. Expression level relative to that of cells expressing NS shRNA is shown. **B.** PEO4,

COV504, and COV413B cells expressing NS or *SHMT1* shRNAs were analyzed for SHMT1 and β -ACTIN expression by immunoblotting. Data are presented as mean \pm SEM; **p<0.005.

Supplementary Figure 4. Effect of knockdown of folic acid metabolic pathway genes on ovarian cancer growth. PEO4 cells expressing either control nonspecific (NS) shRNA or shRNA targeting the indicated mRNAs were analyzed for the ability to grow in an anchorage-independent manner in a soft-agar assay. Representative soft-agar assay images (A) and colony number and size relative to those of cells expressing NS shRNA (B) are shown.

Supplementary Figure 5. Effect of knockdown of folic acid metabolic pathway genes on ovarian cancer cell migration. PEO4 expressing either a control nonspecific (NS) or indicated targeted shRNAs were analyzed for the ability to migrate in a wound-healing assay. Representative cell migration for indicated shRNAs at day 4 relative to day 0 is shown.

Supplementary Figure 6. Schematic representation of transcription factor Wilms tumor 1 (WT1) binding site on *SHMT1* promoter.

Supplementary Table 1. List of 6 genes regulating folic acid metabolic shortlisted for functional validation in ovarian cancer samples

Gene Symbol	Gene Name	Functional Category	Fold upregulation in TCGA dataset
ATIC (PUR9)	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase	Folic Acid Metabolism	+1.227 p-value=8.49E-6
DHFR	Dihydrofolate reductase	Folic Acid Metabolism	+2.084 p-value=1.27E-6
GART (PUR2)	phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase	Folic Acid Metabolism	+1.446 p-value=4.73E-7
GGH	Gamma-glutamyl hydrolase	Folic Acid Metabolism	+6.21 p-value=1.03E-5
SHMT1	Serine Hydroxymethyltransferase 1	Folic Acid Metabolism	+1.41 p-value=2.26E-6
SHMT2	Serine Hydroxymethyltransferase 2	Folic Acid Metabolism	+2.163 p-value= 1.13E-8

Supplementary Table 2. Fold change for significantly altered genes in PEO4 cells expressing *SHMT1* shRNAs compared to the cells expressing non-silencing shRNA

Gene Symbol	Fold change <i>SHMT1</i> shRNA#1	Fold Change <i>SHMT1</i> shRNA#2
ADM	-1.51119176	-1.36910868
ATOH8	1.32527115	1.715921298
AVPI1	-1.282157373	-1.512357676
BDNF	-1.606796987	-1.338330091
BLACAT1	-1.334442404	-1.331317824
C3orf14	-1.483782624	-1.67738232
CAMK2N1	-1.932333413	-1.825005487
CD163L1	-1.400289792	-1.574268376
CD24	-1.531860014	-1.997061865
CD24	-1.531860014	-1.492119753
CFI	-1.518543288	-1.365039684
CXCL8	-1.342219044	-2.201465452
CXCL8	-1.342219044	-2.164724636
DKAKD	1.475621422	1.294620178
DEFB1	-1.730575	-1.77585153
DHRS9	-1.451458765	-1.563242937
FAM83D	1.382984166	1.260764423
FGFR3	1.466252262	1.372789166
FMOD	1.346585943	1.357794286
GFRA2	3.237919393	2.026628216
ICAM2	-1.338111374	-1.379860057
IER3	-1.903510432	-1.899100239
IL24	-1.485749921	-1.703134679
IL6	-1.528866418	-1.316266015
KANK4	-1.51939388	-1.554746992
KANK4	-1.51939388	-1.545027157
KRT17	-1.385708821	-1.25350592
KRT81	-1.811243849	-1.865098126
LEMD1	-1.576916891	-1.398655882
MAL	-2.606955795	-1.441467059
MGAT3	-1.255031755	-1.546480376
MT1M	-1.786099952	-1.507042657
NDN	-1.273268363	-1.442652115
NT5E	-1.491530971	-1.560153367
PBX3	-1.370465743	-1.521449612
PDE4B	-1.387581731	-2.273554584
PDE4B	-1.387581731	-1.438673871
PHLDA1	-1.391449138	-1.608509464
PTGES	-1.600225599	-1.322914701
PXDN	-1.311852644	-1.604810459
RARRES1	-1.862862733	-2.006348736
RARRES1	-1.862862733	-1.697142428
RASD1	1.724253894	1.360291829
S100P	-1.936451328	-1.878168746
SHMT1	-1.726555704	-1.238954893
SHMT1	-1.223038773	-1.025780892

SLC2A3	-2.620649702	-1.389633758
SRPX2	-1.484815179	-1.268622988
SST	-1.835290759	-1.499521006
TFAP2A	-2.367339503	-1.826434342
TFAP2A	-2.367339503	-1.796460918
UCP2	1.409043271	1.462641205
UPP1	-1.280808019	-1.298120582

Supplementary Table 3. Significantly altered biological pathways identified in PEO4 cells expressing *SHMT1* shRNAs compared to the cells expressing nonsilencing shRNA

S.No.	Enriched pathway map	p-value	FDR	In data	Network objects from active data
1	Immune response HMGB1/TLR signaling pathway	1.339E-04	1.206E-02	3	IL-6, IL-8, TIRAP (Mal)
2	Immune response Bacterial infections in normal airway	3.373E-04	1.206E-02	3	IL-6, IL-8, TIRAP (Mal)
3	PDE4 regulation of cyto/chemokine expression in arthritis	3.373E-04	1.206E-02	3	PDE4, IL-6, IL-8
4	PDE4 regulation of cyto/chemokine expression in inflammatory skin diseases	3.582E-04	1.206E-02	3	PDE4, IL-6, IL-8
5	Immune response_HSP60 and HSP70/ TLR signaling pathway	4.499E-04	1.206E-02	3	IL-6, IL-8, TIRAP (Mal)
6	Immune response_TLR2 and TLR4 signaling pathways	5.277E-04	1.206E-02	3	IL-6, IL-8, TIRAP (Mal)
7	Immune response_Role of PKR in stress-induced antiviral cell response	5.277E-04	1.206E-02	3	IL-6, IL-8, TIRAP (Mal)
8	Multiple myeloma (general schema)	1.140E-03	2.279E-02	2	IL-6, FGFR3
9	Immune response_MIF-mediated glucocorticoid regulation	1.709E-03	3.038E-02	2	IL-6, IL-8
10	Immune response_Role of HMGB1 in dendritic cell maturation and migration	2.574E-03	4.025E-02	2	IL-6, IL-8
11	Immune response_Antigen presentation by MHC class I	2.767E-03	4.025E-02	2	CD8 alpha, CD8
12	Colorectal cancer (general schema)	3.174E-03	4.231E-02	2	IL-6, IL-8
13	Complement pathway disruption in thrombotic microangiopathy	4.800E-03	4.604E-02	2	IL-8, Factor I
14	Immune response_Role of integrins in NK cells cytotoxicity	5.058E-03	4.604E-02	2	IL-8, ICAM2
15	Substance P-mediated inflammation and pain in Sickle cell disease	5.058E-03	4.604E-02	2	IL-6, IL-8
16	Immune response_TSLP signalling	5.322E-03	42.604E-02	2	IL-6, IL-8
17	Immune response_MIF in innate immunity response	5.593E-03	4.604E-02	2	IL-6, IL-8
18	Immune response_Substance P-stimulated expression of proinflammatory cytokines via MAPKs	6.441E-03	4.604E-02	2	IL-6, IL-8
19	Role of platelets in the initiation of in-stent restenosis	6.441E-03	4.604E-02	2	IL-8, ICAM2
20	Immune response_IL-1 signaling pathway	6.736E-03	4.604E-02	2	IL-6, IL-8
21	Immune response_PGE2 signaling in immune response	7.038E-03	4.604E-02	2	PGES, IL-8
22	Immune response_Inhibitory action of Lipoxins on pro-inflammatory TNF-alpha	7.345E-03	4.604E-02	2	IL-6, IL-8

	signaling				
23	Chemotaxis_CCR1 signaling	7.977E-03	4.604E-02	2	IL-6, IL-8
24	Immune response_C3a signaling	7.977E-03	4.604E-02	2	IL-6, IL-8
25	Immune response_Histamine H1 receptor signaling in immune response	7.977E-03	4.604E-02	2	IL-6, IL-8
26	Immune response_TLR5, TLR7, TLR8 and TLR9 signaling pathways	7.977E-03	4.604E-02	2	IL-6, IL-8
27	Role and regulation of Prostaglandin E2 in gastric cancer	8.302E-03	4.604E-02	2	PGES, IL-8
28	Development_PEDF signaling	8.302E-03	4.604E-02	2	IL-6, BDNF
29	Huntington's disease (general schema)	8.443E-03	4.604E-02	2	BDNF
30	Immune response_Histamine signaling in dendritic cells	8.633E-03	4.604E-02	2	IL-6, IL-8
31	Inflammatory factors-induced expression of mucins in normal and asthmatic epithelium	8.970E-03	4.630E-02	2	IL-6, IL-8
32	Immune response_Inhibitory PD-1 signaling in T cells	9.661E-03	4.684E-02	2	CD8 alpha, CD8
33	Immune response_HMGB1/RAGE signaling pathway	9.661E-03	4.684E-02	2	IL-6, IL-8
34	Th17 cells in CF	1.002E-02	4.713E-02	2	IL-6, IL-8

Supplementary Table 4. Comparative mRNA (Common genes) fold change between SHMT1 Knockdown and CMAS Knockdown (Kohnz et.al., 2016 PMID: 27380425)

Gene Symbol	Fold Change-SHMT1 shRNA#1	Fold Change-SHMT1 shRNA#2	Fold Change-(CMAS knockdown)
KRT81	-1.8	-1.9	-0.920299329
SLC2A3	-2.6	-1.4	-0.5326393
BDNF	-1.6	-1.3	-0.691482534
IL6	-1.5	-1.3	-0.30881392
KANK4	-1.5	-1.6	-0.93313234
CFI	-1.5	-1.4	-0.81327496
ADM	-1.5	-1.4	-0.845720677
NT5E	-1.5	-1.6	-0.558947094
SRPX2	-1.5	-1.3	-0.840808638
PDE4B	-1.4	-1.4	-0.99759763
KRT17	-1.4	-1.3	-0.726446341
CXCL8 (IL8)	-1.3	-2.2	-0.744391055
UPP1	-1.3	-1.3	-0.793003389
ATOH8	1.3	1.7	1.353736295
FAM83D	1.4	1.3	1.263891559
FGFR3	1.5	1.4	1.199907978
DKAKD	1.5	1.3	1.036156948
RASD1	1.7	1.4	2.45188495

Supplementary Table 5: Primer sequences for RT-qPCR analysis; clone ID and catalog numbers for shRNAs (Open Biosystems); antibodies used; source and concentration of chemical inhibitors used- one

Application	Gene symbol	Forward primer (5'-3')	Reverse primer (5'-3')
RT-qPCR	<i>SHMT1</i>	TCCCGAGGACTTTTGAAGA	TGCCACAGCACTCTGGATCT
	<i>SHMT2</i>	GCTCAACCTGGCACTGACTG	CACTGATGTGGGCCATGTCT
	<i>DHFR</i>	TGAGGGATGATGGTTGCTTG	TGCCTGGCTGGTTTTTCTTT
	<i>GGH</i>	GCACATGCCTTGGATTTGAA	CGGAGAGGCTCCACTTATGG
	<i>PUR9 (ATIC)</i>	GTGTCCACGGAGATGCAGAG	AGCTTGGGCTGCAGTGTGA
	<i>PUR2 (GART)</i>	TTTGGTTGTGAAGGCCAGTG	TCCATTCCCCTGTGTTAG
	<i>WT1</i>	AGCAGGAAGCACACTGGTGA	ACCGGGCAAACCTTTTTCTGA
	<i>IL6</i>	TGTAACCGCCCCACACAGAC	TTCTGCCAGTGCCTCTTTGC
	<i>IL8</i>	TAAAAAGCCACCGGAGCACT	ATCAGGAAGGCTGCCAAGAG
CHIP assay			
	WT1 Promoter <i>SHMT1</i> ChIP	GAGAAACAGGCCCTGCCC	GAACTTGGCGCTGGACCG
	Gene symbol	Clone ID	Catalog number
shRNA	<i>SHMT1</i>	RHS3979-9602172	TRCN0000034764
		RHS3979-9602173	TRCN0000034765
	<i>SHMT2</i>	RHS3979-9602213	TRCN0000034805
		RHS3979-9602214	TRCN0000034806
	<i>DHFR</i>	RHS3979-9606408	TRCN0000039000
		RHS3979-9606411	TRCN0000039003
	<i>GGH</i>	RHS3979-9618537	TRCN0000051338
		RHS3979-9618541	TRCN0000051342
	<i>PUR9 (ATIC)</i>	RHS3979-9602372	TRCN0000034964
		RHS3979-9602375	TRCN0000034967
	<i>PUR2 (GART)</i>	RHS3979-9602315	TRCN0000034907
		RHS3979-9602316	TRCN0000034908
	<i>WT1</i>	RHS3979-9569562	TRCN0000001114
		RHS3979-9569563	TRCN0000010596
Immunoblotting / CHIP	Protein symbol	Antibody source	Dilution
	<i>SHMT1</i>	Cell signaling	1:5000
	<i>WT1</i>	Cell signaling	1:5000
	Actin	Cell signaling	1:5000
	<i>IL6</i>	Santa cruz	1:500
	<i>IL8</i>	Santa cruz	1:500
	Compounds	Concentration	Source
	P-3Fax- Neu5Ac	Different concentrations as indicated	Tocris
	Neu5Ac	Different concentrations as indicated	Santa Cruz