Supplementary Figure legends

Figure S1. The 5' tail mirtron miR-6778-5p is a transcript splice derived from intron 5 of SHMT1

(A) Interference of Drosha in MGC-803, SGC-7901, NUGC-3 and MGC-823 GC cells was detected by qRT-PCR. (B, C) qRT-PCR was used to determine the expression levels of miR-6778-5p and miR-21 in MGC-803/Drosha KD gastric cancer cells and MGC-803/Drosha WT gastric cancer cells (B), and in Drosha high-expressing and Drosha low-expressing gastric tumour tissues (C). The relative expression levels of miR-6778-5p were normalized by miR-21 levels in gastric cancer cells and tumor tissues. U6 is an internal control. (D) c-MYC was predicted to be a potential transcription factor of SHMT1 using bioinformatics (Promoter 2.0, JASPAR database) (left panel). A schematic diagram of the c-MYC binding motif is shown (middle panel). A c-MYC binding site is present in the promoter region of SHMT1 (right panel). (E) c-MYC was confirmed to bind to the promoter of SHMT1 by a chromatin immunoprecipitation (ChIP) assay. BCAT1 was used as a positive control. (F) A luciferase assay was used to show the regulation of SHMT1 transcriptional activity by c-MYC in MGC-803 cells. (G, H) The expression levels of c-MYC in MGC-803 Drosha WT and knockdown (KD) cells were determined by qRT-PCR (G) and western blotting (H).

Figure S2. Effect of miR-6778-5p on gastric cancer stem cells (GCSCs)

(A). Cell proliferation in MGC-803 gastric cancer cells. (B) Image of spheres in Drosha WT and Drosha KD GCSCs. (C). CD44 expression in human gastric cancer tissues is shown using the data from GEPIA (http://gepia.cancer-pku.cn). (D) Expression of Drosha and CD44 in gastric cancer tissues and paired normal gastric tissues (n=60). T: gastric cancer tissues; N: normal gastric tissues. (E) CSC-associated gene expression in Drosha WT GCSCs and Drosha KD GCSCs was determined by qRT-PCR. (F) Expression of miR-6778-5p and CD44 in Drosha wild type and Drosha-knocked down GCSCs. (G) Images of GCSC spheres derived from MGC-803/Drosha WT/miR-6778-5p KD, SGC7901/Drosha WT/miR-6778-5p KD, MGC-803/Drosha KD/miR-6778-5p KD, SGC-7901/Drosha KD/miR-6778-5p KD and their controls (magnification: ×100; scale bar: 100 μm). (H) Images of GCSC spheres derived

from MGC-803/Drosha WT/miR-6778-5p inhibitor, SGC7901/Drosha WT/miR-6778-5p inhibitor, MGC-803/Drosha KD/miR-6778-5p inhibitor, SGC-7901/Drosha KD/miR-6778-5p inhibitor and their controls (magnification: ×100; scale bar: 100 μm). Histograms show the formation efficiency of CSC spheres.

Figure S3. YWHAE, a target of miR-6778-5p, regulates SHMT1through downregulation of c-MYC

(A) Schematic diagram of luciferase reporters with the wild-type (WT) YWHAE 3'-UTR or the mutant (Mut) YWHAE 3'-UTR binding sites for miR-6778-5p. (B) The effect of miR-Ctrl or miR-6778 on luciferase activity in HEK-293T cells co-transfected with either the wild-type YWHAE 3'-UTR reporter or the mutant miR-6778-5p binding site reporter. (C, D) Effect of YWHAE on the formation of gastric cancer stem cells. (C) Overexpression of YWHAE in Drosha KD cells (magnification: ×100; scale bar: 100 μm). (D) Knockdown of YWHAE in Drosha wild type gastric cancer cells (magnification: ×100; scale bar: 100 μm). (E) The decrease in c-MYC protein in YWHAE-overexpressing MGC-803 gastric cell was restored by MG132.

Figure S4. SHMT1 regulates the self-renewal capacity of GCSCs

(A, B) Expression of metabolic enzymes involved in mitochondrial carbon metabolism (SHMT2 and MTHFD2) in MGC-803/Drosha WT and MGC-803/Drosha KD cells was determined by qRT-PCR (A) and western blotting (B). (C) SHMT1 expression in human gastric cancer tissues using the data from GEPIA (http://gepia.cancer-pku.cn). (D) The expression levels of SHMT1 and CD44 in GC tissues as determined by qRT-PCR. (E) The expression levels of SHMT1, SHMT2 and CD44 in Drosha high-expressing and Drosha low-expressing gastric tumour tissues by qRT-PCR. (F-H). After Silencing of SHMT1 in Drosha-wild type MGC-803 and SGC-7901 cells, CSC sphere formation and CSC-related gene expression were evaluated. (F) Images of GCSC spheres (magnification: ×100; scale bar: 100 μm); (G) CSC sphere formation efficiency; (H) CSC-associated gene expression analysed by western blotting.

Figure S5. The miR-6778-5p/YWHAE/SHMT1 axis contributes to GCSC maintenance via regulation of cytoplasmic one-carbon metabolism

(A) Images of knocked down (KD) miR-6778-5p, SHMT1 and SHMT2 in gastric cancer Drosha WT or gastric cancer Drosha KD GCSCs after addition of 0.8 mM serine (magnification: ×100; scale bar: 100 μm). (B) Images of knocked down miR-6778-5p and SHMT1 in gastric cancer Drosha WT or gastric cancer Drosha KD GCSCs after addition of 30 μg/ml of 5-FU (magnification: ×100; scale bar: 100 μm).

Figure S6. Effects of miR-6778-5p and SHMT1 on gastric cancer tumourigenesis in vivo

The indicated GCSCs (1×10^5) were subcutaneously injected into nude mice. Images of tumour sizes and their corresponding tumour growth curve are shown. Mice were injected with MGC-803/Drosha WT/miR-6778-5p KD, MGC-803/Drosha WT /SHMT1 KD, and the control cells, MGC-803/Drosha WT. The mice were given serine (130 mg/kg) and 5-FU (30 mg/kg) at 5 days after cell injection. (A) Representative images of tumours in the indicated nude mice xenografts. (B) The tumour growth curve corresponding to (A).

Supplementary Table 1. Sequences that target specific genes

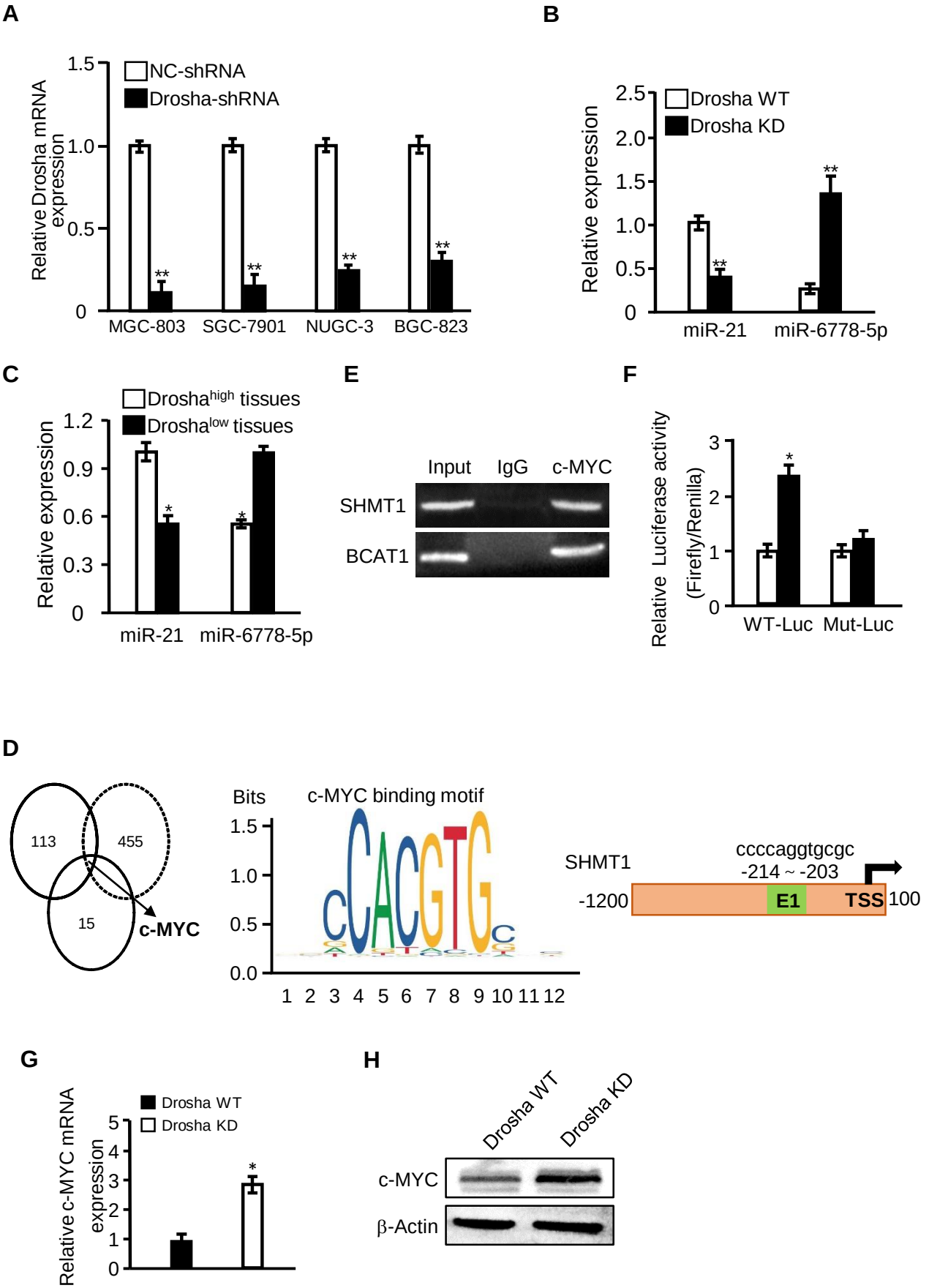
Gene name	Sequence
miR-6778-5p shRNA	5'-ACCTGCCTCCTGTCCTCCCACT-3'
SHMT1 shRNA 1#	5'-TAGGCTCTTGCTTAAATAATT-3'
SHMT1 shRNA 2#	5'-AAGCTATGACTCTGGAATTTT-3'
YWHAE shRNA	5'-CTGAGTGAAGAAAGCTATA-3'
Negative control	5'-CAGTACTTTTGTGTAGTACAA-3'
miR-6778-5p overexpression	FW 5'-AGTGGGAGGACAGGAGGCAGGT-3' REV 5'- CTGCCTCCTGTCCTCCCACTTT-3'
negative control	FW 5'-TTCTCCGAACGTGTCACGTTT-3' REV 5'-ACGTGACACGTTCGGAGAATT-3'

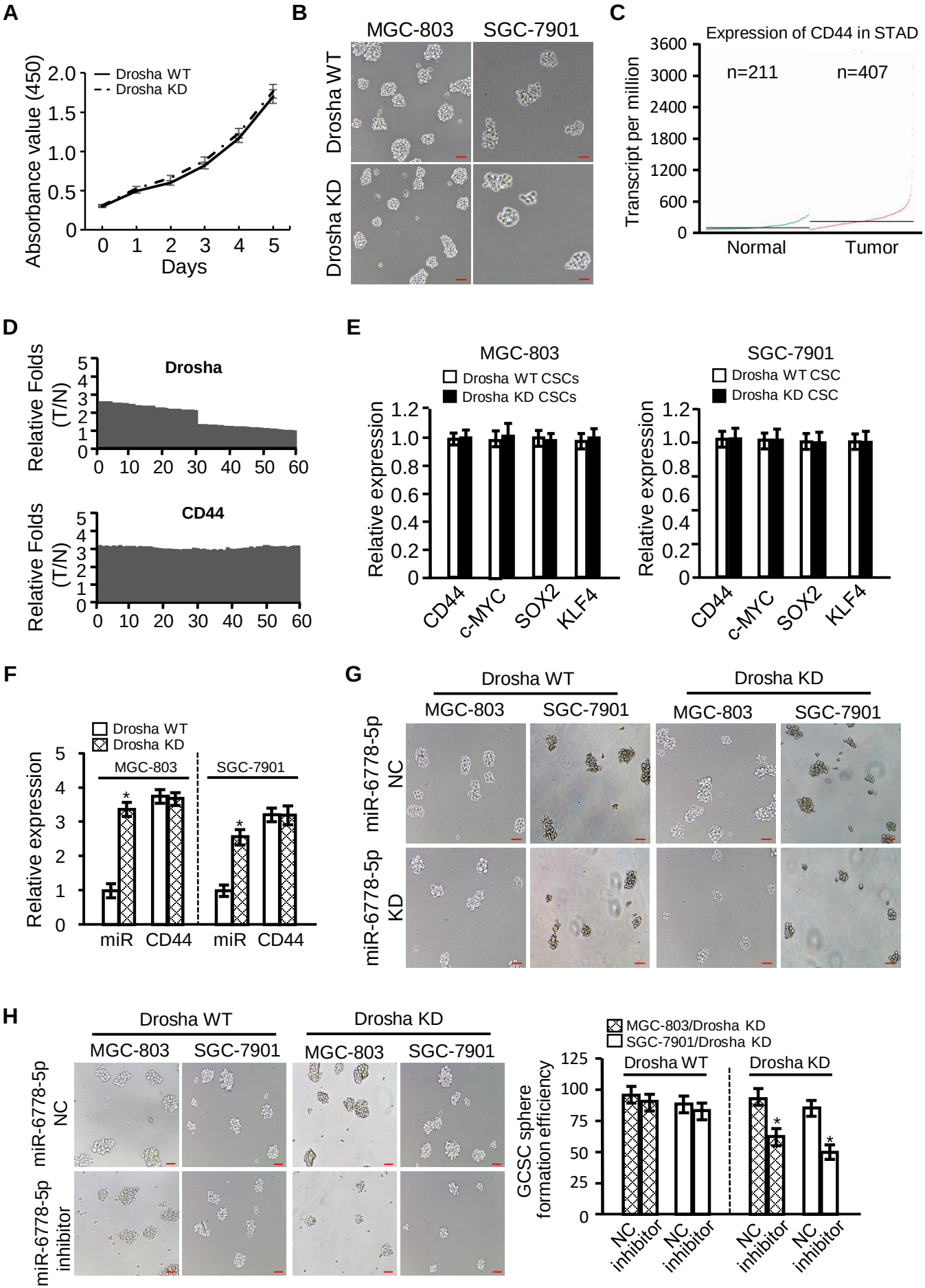
Supplementary Table 2. Primers used for SHMT1 gene amplification and 5' and 3' splicing sites mutagenesis

Gene name	Primer
SHMT1	FW 5'-GCTCCCCTGCAAACTTTGCT-3' REV 5'-CTGCGATGATCAGCTTCGGGT-3'
5'splice site mutation	FW 5'-CCTTGCCCTACAAGCTAAGCATGTGTTGG-3' REV 5'-TAGATTCAAAGAAGATGGACGTGGCAGAG-3'
3'splice site mutation	FW 5'-CCCTGACATTCCACACGTGAACCCAGATAC-3' REV 5'-AGGCCAGGTTCAGGCTGCTTCCTCC-3'

Supplementary Table 3. Primers used for qRT-PCR

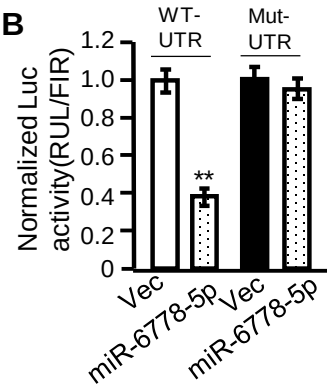
Gene name	Primer
miR-6778-5p	F 5’-GCGAGTGGGAGGACAGGAG-3’ R 5’-ATCCAGTGCAGGGTCCGAGG-3’ RT 5’-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCTGC-3’
SOX2	F 5’-GCACATGAACGGCTGGAGCAACG-3’ R 5’-TGCTGCGAGTAGGACATGCTGTAGG-3’
c-Myc	F 5’-AAAGGCCCCCAAGGTAGTTA-3’ R 5’-GCACAAGCGTTCCGTAGCTG-3’
CD44	F 5’-TCTGAATCAGATGGACACTCAC-3’ R 5’-CATTGCCACTGTTGATCACTAG-3’
KLF4	F 5’-GAACTGACCAGGCACTACCG-3’ R 5’-TTCTGGCAGTGTGGGTCATA-3’
SHMT1	F 5’-AGGAAAGGAGTGAAAAGTGTGGA-3’ R 5’-GACACCAGTGTCGCTCTGGATCTG-3’
SHMT2	F 5’-TGGCAAGAGATACTACGGAGG-3’ R 5’-GCAGGTCCAACCCCATGAT-3’
MTHFD2	F 5’-TACTCCATGGGGTGTGTGG-3’ R 5’-TGGGCATTCCAACGTTTT-3’
YWHAE	F 5’-TGTGTCGTCTCCGTGCCAGAT-3’ R 5’-AAGAGGTTGAGCGAGCGAAGGA-3’
EFNA3	F 5’-CTTGTGGCTCTGGTAATGTTTGG-3’ R 5’-GAGGAGGACGTGCTTATTGCTGT-3’
GSK3β	F 5’-CCTTAACCTGCTGCTGGACT-3’ R 5’-AGCTCTGGTGCCCTGCCAGAT-3’
β-Actin	F 5’-AGGGGCCGGACTCGTCATACT-3’ R 5’-GGCGGCACCACCATGTACCCT-3’
U6	F 5’-CTCGCTTCGGCAGCACA-3’ R 5’-AACGCTTCACGAATTTGCGT-3’


A
Relative Drosha mRNA expression
NC-shRNA
Drosha-shRNA
MGC-803
SGC-7901
NUGC-3
BGC-823
B
Relative expression
Drosha WT
Drosha KD
miR-21
miR-6778-5p
C
Drosha^high tissues
Drosha^low tissues
Relative expression
miR-21
miR-6778-5p
E
Input
IgG
c-MYC
SHMT1
BCAT1
F
Relative Luciferase activity (Firefly/Renilla)
WT-Luc
Mut-Luc
D
113
455
15
c-MYC
Bits
c-MYC binding motif
SHMT1
ccccaggtgcgc
-214 ~ -203
-1200
E1
TSS
100
G
Relative c-MYC mRNA expression
Drosha WT
Drosha KD
H
Drosha WT
Drosha KD
c-MYC
β-Actin

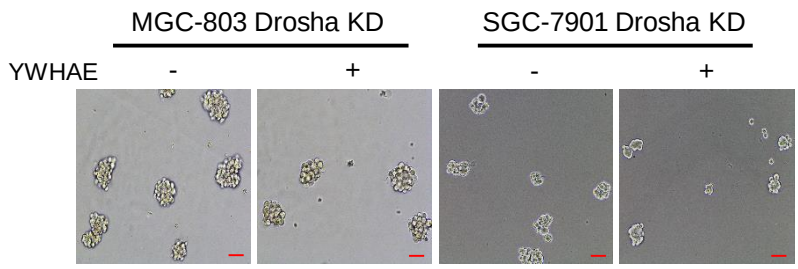

A
Absorbance value (450)
Drosha WT
Drosha KD
Days
B
MGC-803
SGC-7901
Drosha WT
Drosha KD
C
Expression of CD44 in STAD
Transcript per million
n=211
n=407
Normal
Tumor
D
Drosha
CD44
Relative Folds (T/N)
E
MGC-803
SGC-7901
Drosha WT CSCs
Drosha KD CSCs
Drosha WT CSC
Drosha KD CSC
Relative expression
CD44
c-MYC
SOX2
KLF4
F
Drosha WT
Drosha KD
MGC-803
SGC-7901
Relative expression
miR
CD44
G
Drosha WT
Drosha KD
MGC-803
SGC-7901
miR-6778-5p NC
miR-6778-5p KD
H
Drosha WT
Drosha KD
MGC-803
SGC-7901
miR-6778-5p NC
miR-6778-5p inhibitor
MGC-803/Drosha KD
SGC-7901/Drosha KD
GCSC sphere formation efficiency
NC
inhibitor

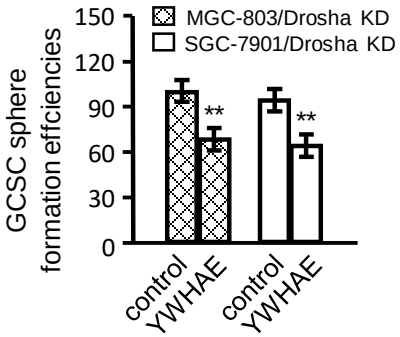
A

Mut YWHAE 3'UTR 5' …CU**ACGGA**ACUUUACU**GAGGGTG**U…
WT YWHAE 3'UTR 5' …CUUGCCUACUUUACUCUCCCACU…
Hsa-miR-6778-5p 3' …UGGACGGAGGACAG -- GAGGGUGA.

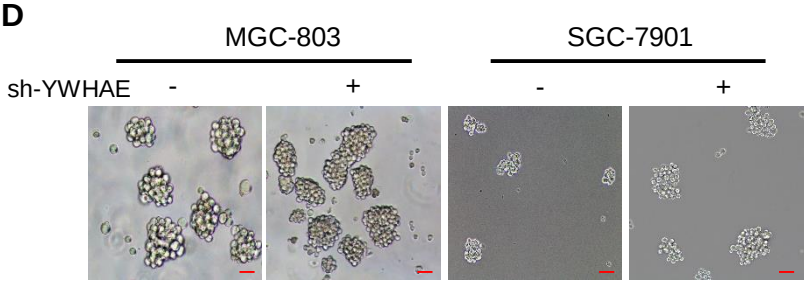


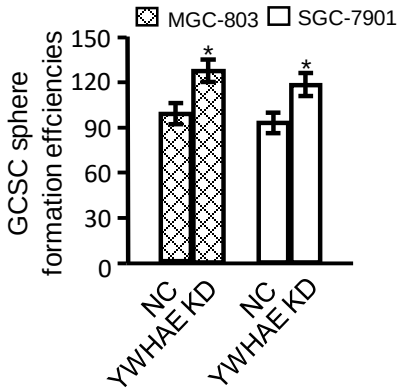
C



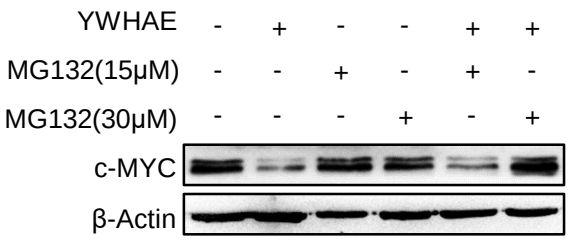


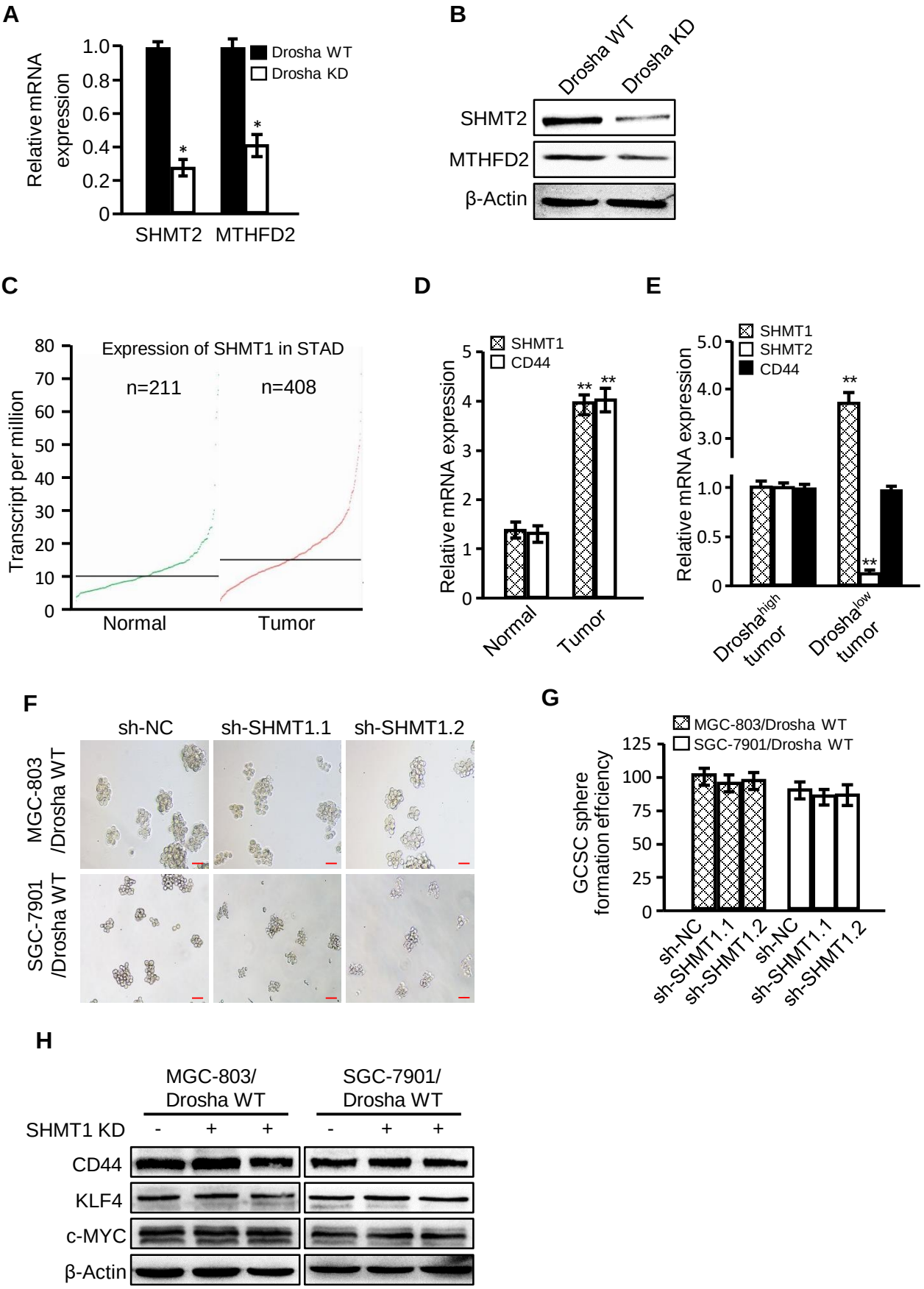
D



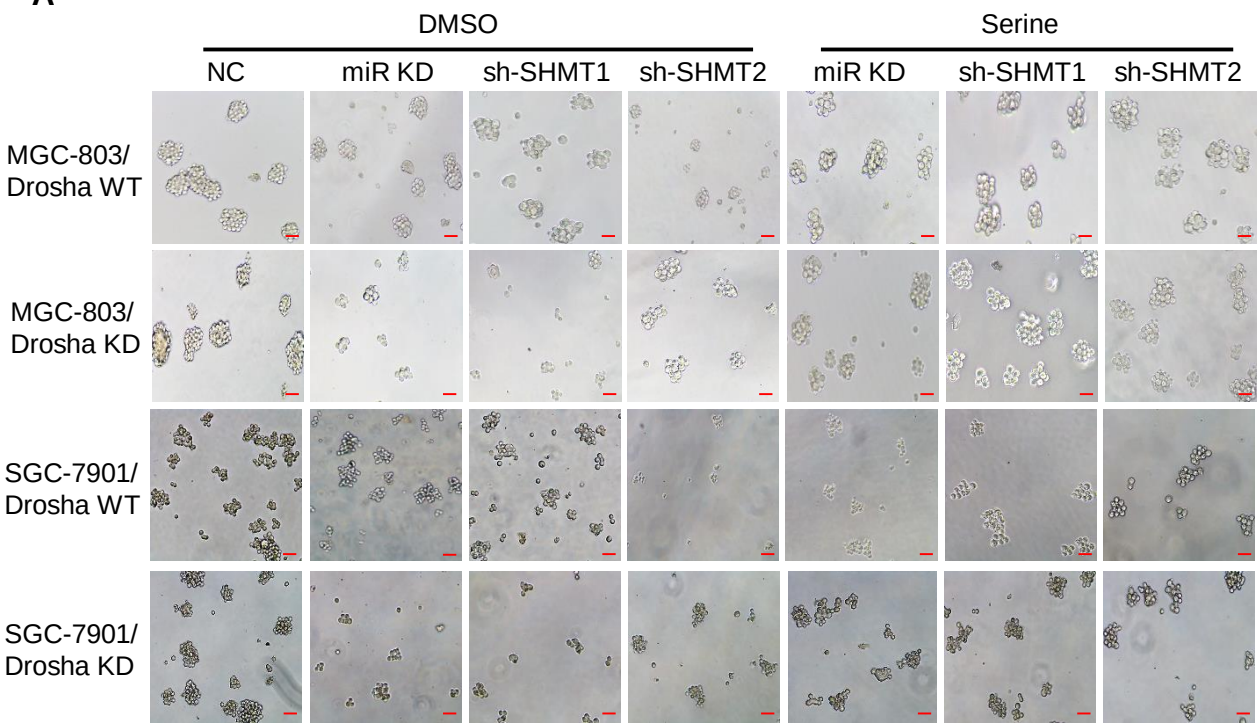


E

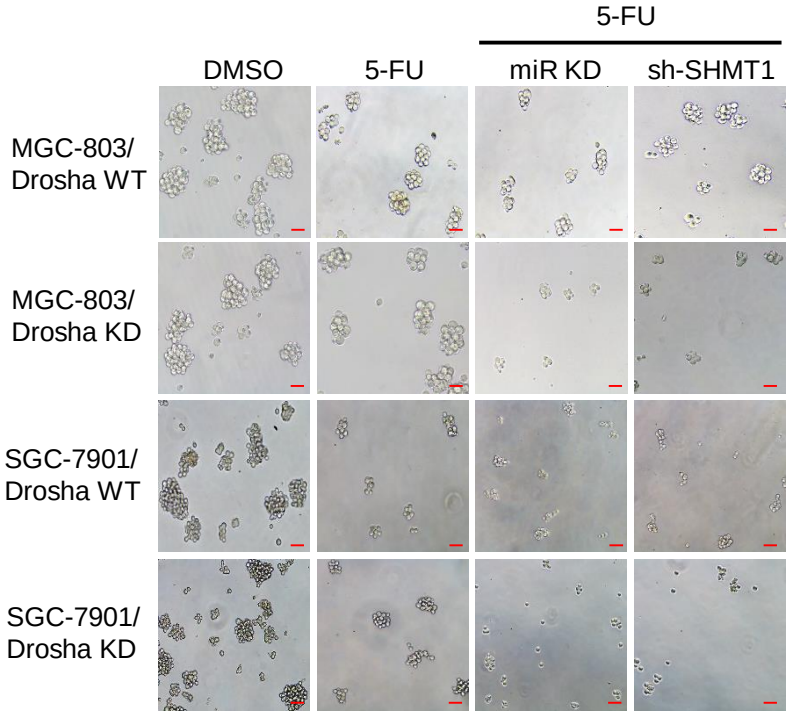



A
Relative mRNA expression
1.0
0.8
0.6
0.4
0.2
0
Drosha WT
Drosha KD
*
*
SHMT2
MTHFD2
B
Drosha WT
Drosha KD
SHMT2
MTHFD2
β-Actin
C
Expression of SHMT1 in STAD
n=211
n=408
Transcript per million
80
70
60
50
40
30
20
10
0
Normal
Tumor
D
SHMT1
CD44
Relative mRNA expression
5
4
3
2
1
0
**
**
Normal
Tumor
E
SHMT1
SHMT2
CD44
Relative mRNA expression
5.0
4.0
3.0
1.0
0.5
0
**
**
Drosha^high tumor
Drosha^low tumor
F
sh-NC
sh-SHMT1.1
sh-SHMT1.2
MGC-803 /Drosha WT
SGC-7901 /Drosha WT
G
MGC-803/Drosha WT
SGC-7901/Drosha WT
GCSC sphere formation effciency
125
100
75
50
25
0
sh-NC
sh-SHMT1.1
sh-SHMT1.2
sh-NC
sh-SHMT1.1
sh-SHMT1.2
H
MGC-803/ Drosha WT
SGC-7901/ Drosha WT
SHMT1 KD
- + + - + +
CD44
KLF4
c-MYC
β-Actin

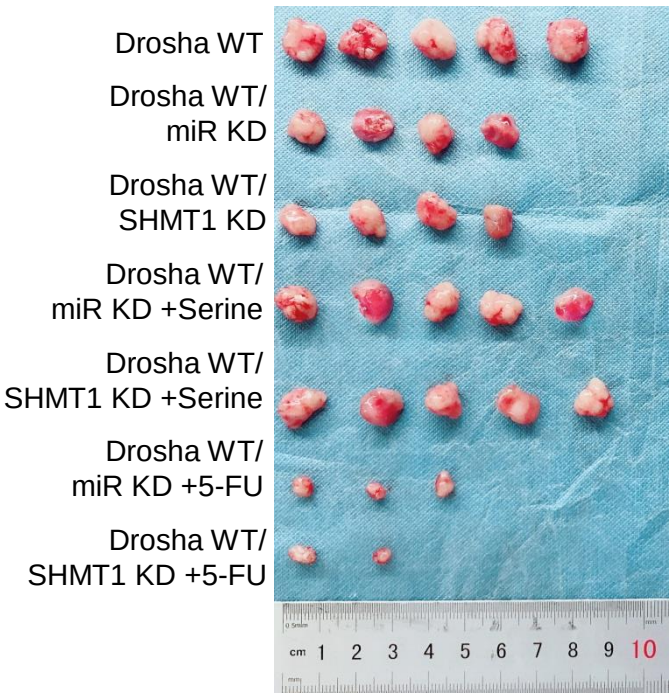
A


DMSO
Serine
NC
miR KD
sh-SHMT1
sh-SHMT2
miR KD
sh-SHMT1
sh-SHMT2
MGC-803/ Drosha WT
MGC-803/ Drosha KD
SGC-7901/ Drosha WT
SGC-7901/ Drosha KD

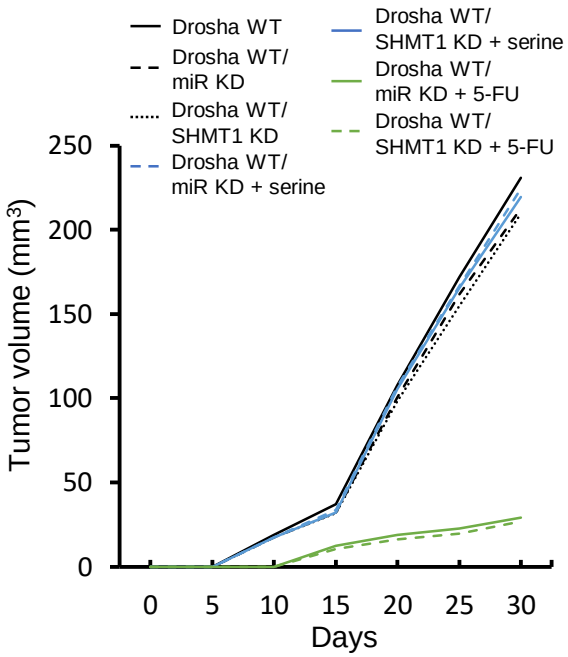
B


5-FU
DMSO
5-FU
miR KD
sh-SHMT1
MGC-803/ Drosha WT
MGC-803/ Drosha KD
SGC-7901/ Drosha WT
SGC-7901/ Drosha KD

A


Drosha WT
Drosha WT/ miR KD
Drosha WT/ SHMT1 KD
Drosha WT/ miR KD +Serine
Drosha WT/ SHMT1 KD +Serine
Drosha WT/ miR KD +5-FU
Drosha WT/ SHMT1 KD +5-FU
cm 1 2 3 4 5 6 7 8 9 10

B


Drosha WT
Drosha WT/ miR KD
Drosha WT/ SHMT1 KD
Drosha WT/ miR KD + serine
Drosha WT/ SHMT1 KD + serine
Drosha WT/ miR KD + 5-FU
Drosha WT/ SHMT1 KD + 5-FU
Tumor volume (mm3)
250
200
150
100
50
0
0 5 10 15 20 25 30
Days