Supplementary Material

Downregulation of N-Acetylglucosaminyltransferase GCNT3 by miR-302b-3p Decreases Non-Small Cell Lung Cancer (NSCLC) Cell Proliferation, Migration and Invasion

Qian Li[a,b] Pengzhan Ran[a] Xiyu Zhang[a] Xiaopeng Guo[a] Yuncang Yuan[a]
Tianqi Dong[a] Bei Zhu[a] Shangyong Zheng[a] Chunjie Xiao[a]

[a]School of Medicine, Yunnan University, Kunming, [b]Transfusion Medicine Research Department,Yunnan University, Kun ming, China

Figure S1

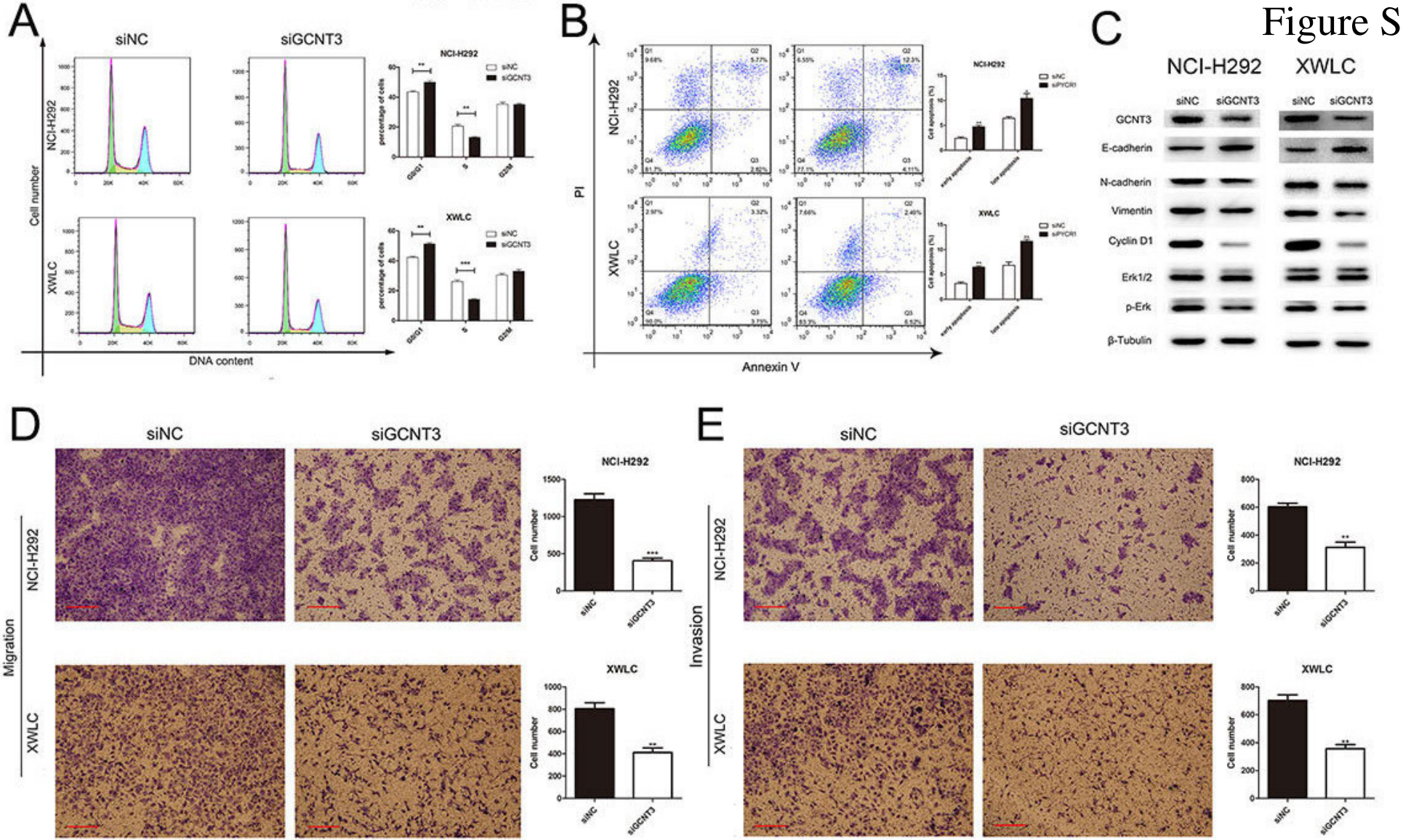
A
siNC
siGCNT3
NCI-H292
XWLC
Cell number
DNA content
percentage of cells
G0/G1
S
G2M
B
PI
Annexin V
Cell apoptosis (%)
early apoptosis
late apoptosis
C
NCI-H292
XWLC
GCNT3
E-cadherin
N-cadherin
Vimentin
Cyclin D1
Erk1/2
p-Erk
β-Tubulin
D
Migration
Cell number
E
Invasion

Figure S2

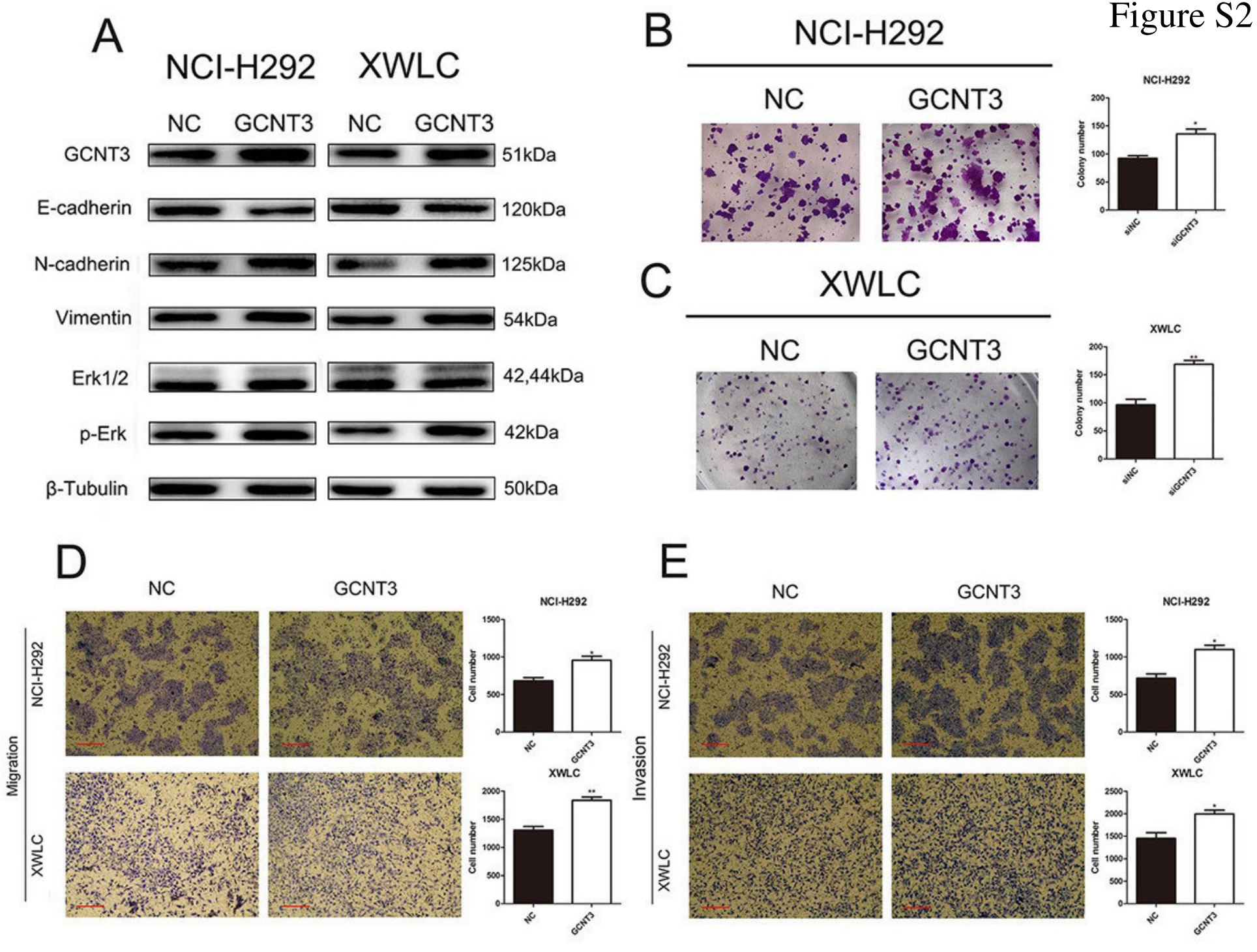
A
NCI-H292
XWLC
NC
GCNT3
NC
GCNT3
GCNT3
51kDa
E-cadherin
120kDa
N-cadherin
125kDa
Vimentin
54kDa
Erk1/2
42,44kDa
p-Erk
42kDa
β-Tubulin
50kDa
B
NCI-H292
NC
GCNT3
NCI-H292
Colony number
siNC
siGCNT3
C
XWLC
NC
GCNT3
XWLC
Colony number
siNC
siGCNT3
D
NC
GCNT3
Migration
NCI-H292
XWLC
NCI-H292
Cell number
NC
GCNT3
XWLC
Cell number
NC
GCNT3
E
NC
GCNT3
Invasion
NCI-H292
XWLC
NCI-H292
Cell number
NC
GCNT3
XWLC
Cell number
NC
GCNT3

Figure S3

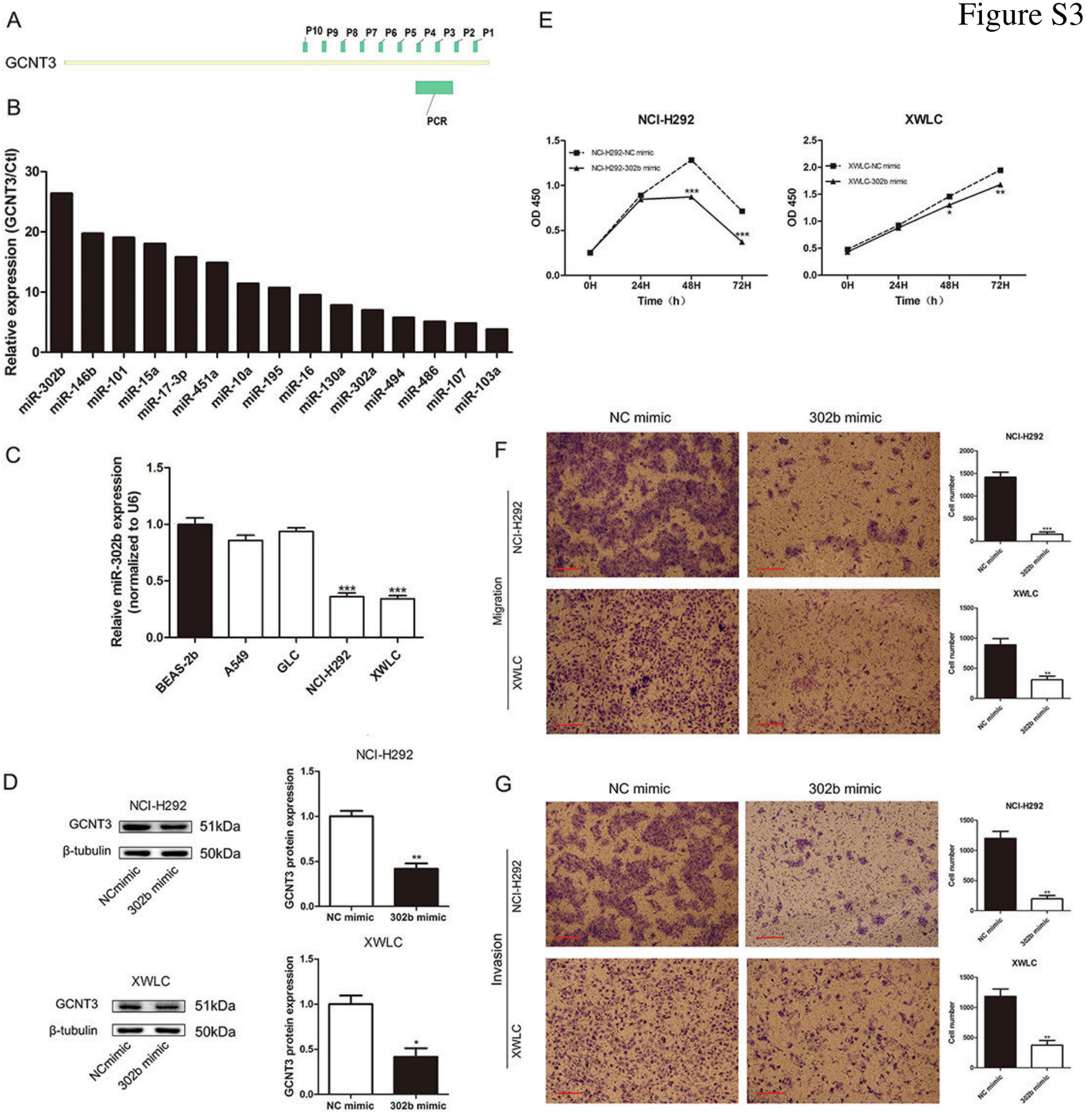
A
P10 P9 P8 P7 P6 P5 P4 P3 P2 P1
GCNT3
PCR
B
Relative expression (GCNT3/Ctl)
miR-302b
miR-146b
miR-101
miR-15a
miR-17-3p
miR-451a
miR-10a
miR-195
miR-16
miR-130a
miR-302a
miR-494
miR-486
miR-107
miR-103a
C
Relaive miR-302b expression (normalized to U6)
BEAS-2b
A549
GLC
NCI-H292
XWLC
D
NCI-H292
GCNT3
51kDa
β-tubulin
50kDa
NCmimic
302b mimic
XWLC
GCNT3 protein expression
NC mimic
302b mimic
E
NCI-H292
NCI-H292-NC mimic
NCI-H292-302b mimic
OD 450
0H
24H
48H
72H
Time (h)
XWLC
XWLC-NC mimic
XWLC-302b mimic
F
NC mimic
302b mimic
Migration
NCI-H292
XWLC
Cell number
G
Invasion

Figure S4

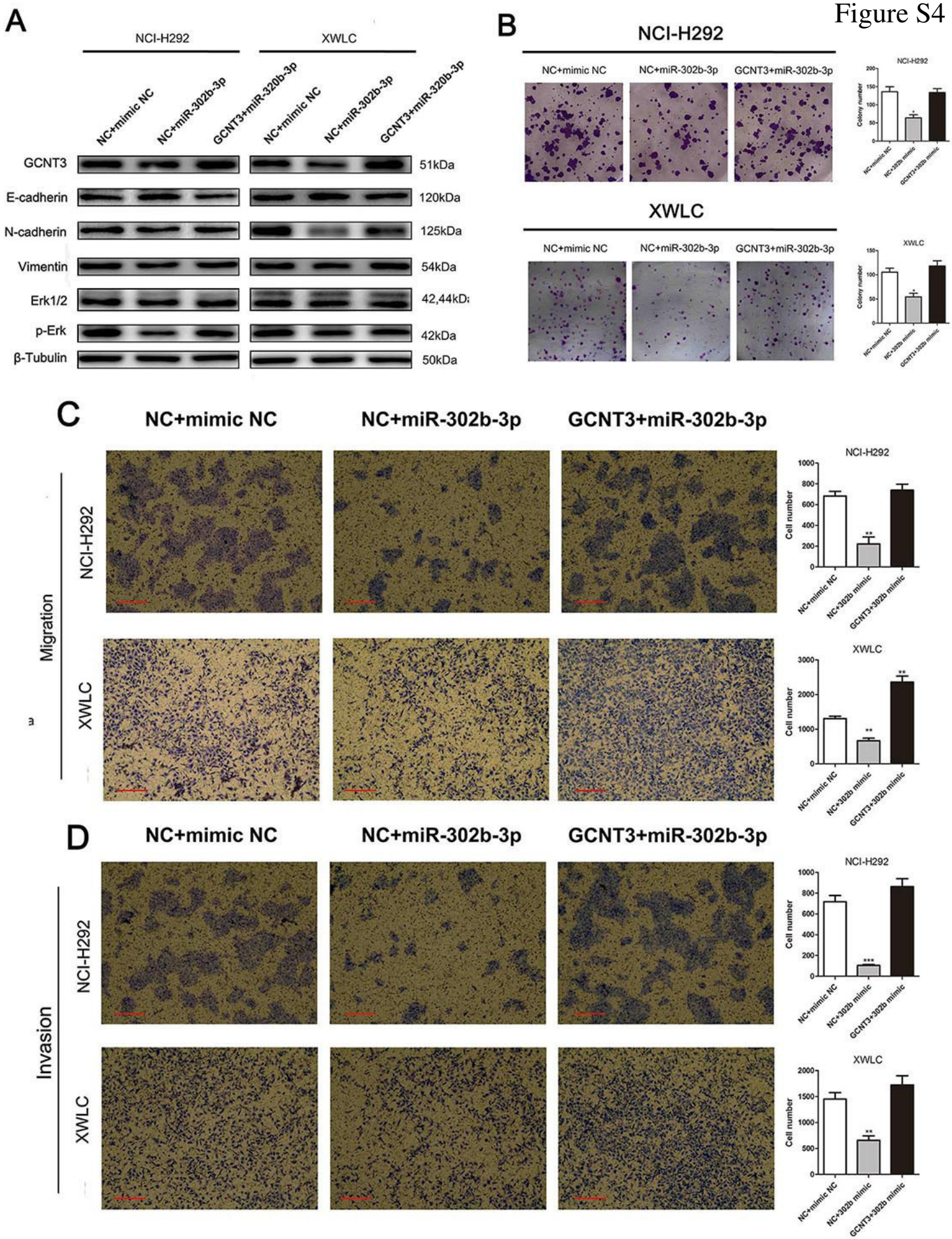
A
NCI-H292
XWLC
NC+mimic NC
NC+miR-302b-3p
GCNT3+miR-320b-3p
NC+mimic NC
NC+miR-302b-3p
GCNT3+miR-320b-3p
GCNT3
E-cadherin
N-cadherin
Vimentin
Erk1/2
p-Erk
β-Tubulin
51kDa
120kDa
125kDa
54kDa
42,44kDa
42kDa
50kDa
B
NCI-H292
NC+mimic NC
NC+miR-302b-3p
GCNT3+miR-302b-3p
Colony number
XWLC
C
NC+mimic NC
NC+miR-302b-3p
GCNT3+miR-302b-3p
Migration
NCI-H292
XWLC
Cell number
NC+mimic NC
NC+302b mimic
GCNT3+302b mimic
D
Invasion

Figure S1. GCNT3 knockdown inhibited NSCLC cell migration, invasion and induced cell cycle arrest and apoptosis. (A) The cell cycle progression of XWLC and NCI-H292 cell transfected with si-GCNT3 or siNC were measured by flow cytometry assay using PI staining. The bar chart represented the percentage of cell in G0/G1, S, or G2/M phase, as indicated. **(B)** Cell apoptosis of XWLC and NCI-H292 cell lines transfected with siGCNT3 or siNC was measured by Annexin V-PI double staining followed by flow cytometry analysis. **(C)** Western blotting assay detected E-cadherin, N-cadherin, Vimentin, Cyclin D1, Erk and p-Erk expression after GCNT3 knockdown in NSCLC cell, and β-Tubulin was used as the loading control. **(D, E)** Migration and invasion of XWLC and NCI-H292 cell lines transfected with siGCNT3 or siNC were measured by transwell assay after 48h. For invasion assay, the upper chamber was pre-coated with Matrigel. The cell was photographed and counted under a microscope in randomly selected fields. All data was presented as the mean ± standard error of experiments performed in triplicate, and comparisons between groups were performed using *t*-tests. (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; Scale bar: 250 μm)

Figure S2. GCNT3 overexpression promoted NSCLC cell proliferation, migration and invasion. (A) Western blotting assay detected E-cadherin, N-cadherin, Vimentin, Erk and p-Erk expression after GCNT3 overexpression in NSCLC cells, and β-Tubulin was used as the loading control. **(B, C)** Colony-forming assays were used to detect the proliferation of GCNT3-vector-transfected XWLC and NCI-H292 cells. All data was presented as the mean ± standard error of experiments performed in triplicate, and comparisons between groups were performed using *t*-tests. **(D, E)** Migration and invasion of XWLC and NCI-H292 cell lines transfected with GCNT3-vector

or NC were measured by transwell assay after 48h. For invasion assay, the upper chamber was pre-coated with Matrigel. The cells were photographed and counted under a microscope in randomly selected fields. Data was presented as mean ± SD based on at least three repeated experiments. (*P < 0.05; **P < 0.01; ***P < 0.001; Scale bar: 250 μm)

Figure S3. GCNT3 was the target of miR-302b-3p, and miR-302b-3p overexpression inhibited NSCLC cell proliferation, migration, invasion. (A) GCNT3 mRNA, probe binding sites and region analyzed by qRT-PCR. **(B)** qRT-PCR of multiplex miRNA data of GCNT3 probe affinity purification in XWLC cell. Shown data were normalized values for the enriched miRNAs (>3 fold). **(C)** Real-time RT-PCR analyzed miR-302b-3p expression in four human NSCLC cell lines (A549, GLC, XWLC and NCI-H292) and a human Normal pulmonary epithelial cell line (BEAS-2B). (**D**) Western blot determined GCNT3 expression after miR-302b-3p upregulated. β-Tubulin was used as the loading control. **(E)** CCK8 assay was performed to measure the proliferation of XWLC and NCI-H292 cell lines at 0, 24, 48 and 72 h after miR-302b-3p mimic or NC mimic (control) transfection. **(F, G)** Migration and invasion of XWLC and NCI-H292 cell lines transfected with miR-302b-3p mimic or NC mimic were measured by transwell assay after 48h. For invasion assay, the upper chamber was pre-coated with Matrigel. The cell were photographed and counted under a microscope in randomly selected fields. All data was presented as the mean ± standard error of experiments performed in triplicate, and comparisons between groups were performed using t-tests. (*P < 0.05; **P < 0.01; ***P < 0.001; Scale bar: 250 μm)

Figure S4. miR-302b-3p inhibited NSCLC cell proliferation, migration and invasion in a

GCNT3-dependent manner. (A) Western blotting assay detected E-cadherin, N-cadherin, Vimentin, Erk and p-Erk expression after NSCLC cell transfected with miR-302-3p alone or GCNT3 vector and miR-302b-3p in combination, and β-Tubulin was used as the loading control. **(B)** Colony-forming assays was used to detect the proliferation of XWLC and NCI-H292 cells transfected with miR-302b-3p alone or GCNT3 vector and miR-302b-3p in combination. Data were based on at least 3 independent experiments, and shown as mean ± SD. **(C, D)** Migration and invasion of XWLC and NCI-H292 cell lines transfected with miR-302b-3p alone or GCNT3 vector and miR-302b-3p in combination were measured by transwell assay after 48h. For invasion assay, the upper chamber was pre-coated with Matrigel. The cells were photographed and counted under a microscope in randomly selected fields. All data was presented as the mean ± standard error of experiments performed in triplicate, and comparisons between groups were performed using t-tests. (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; Scale bar: 250 μm)