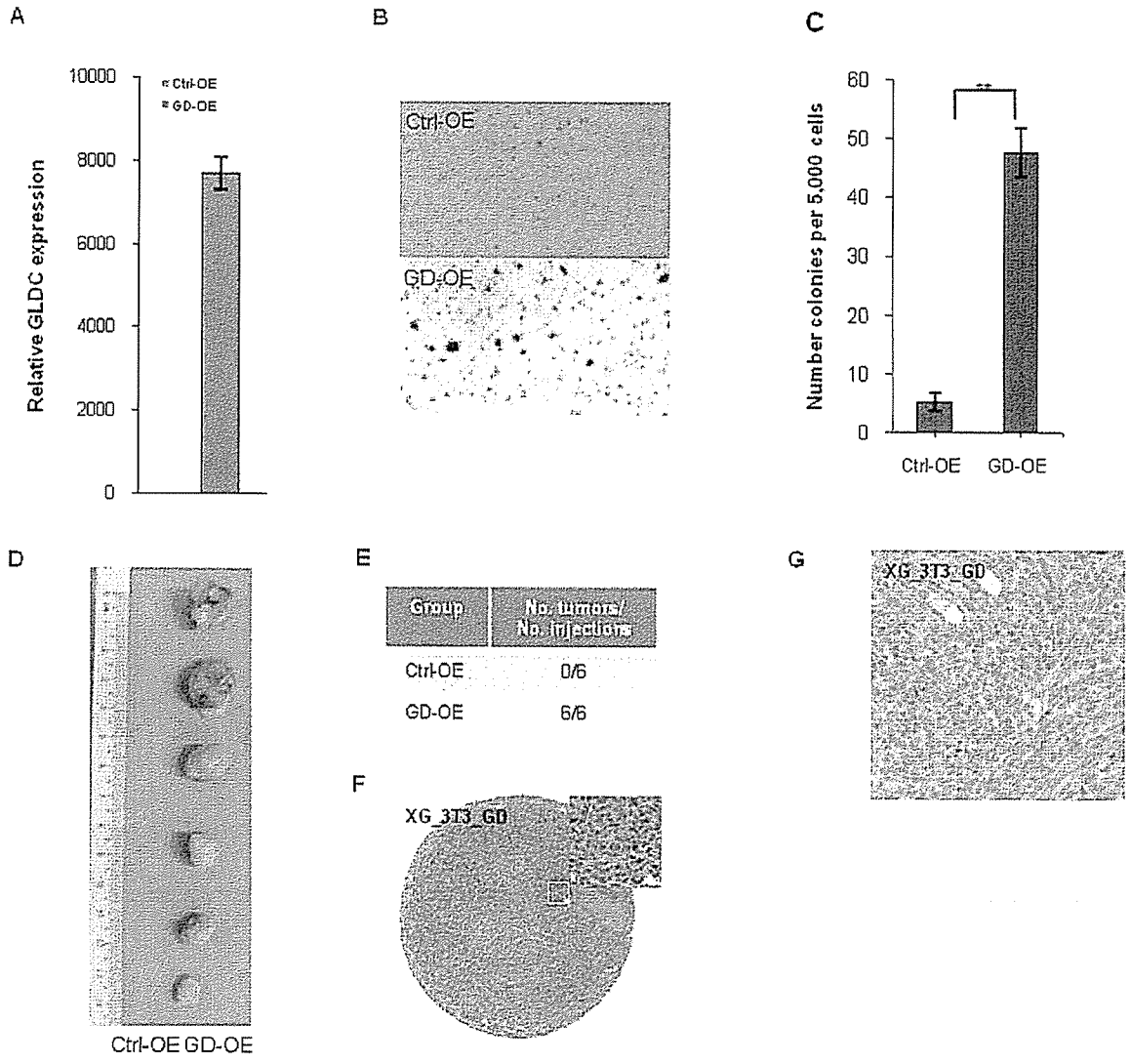
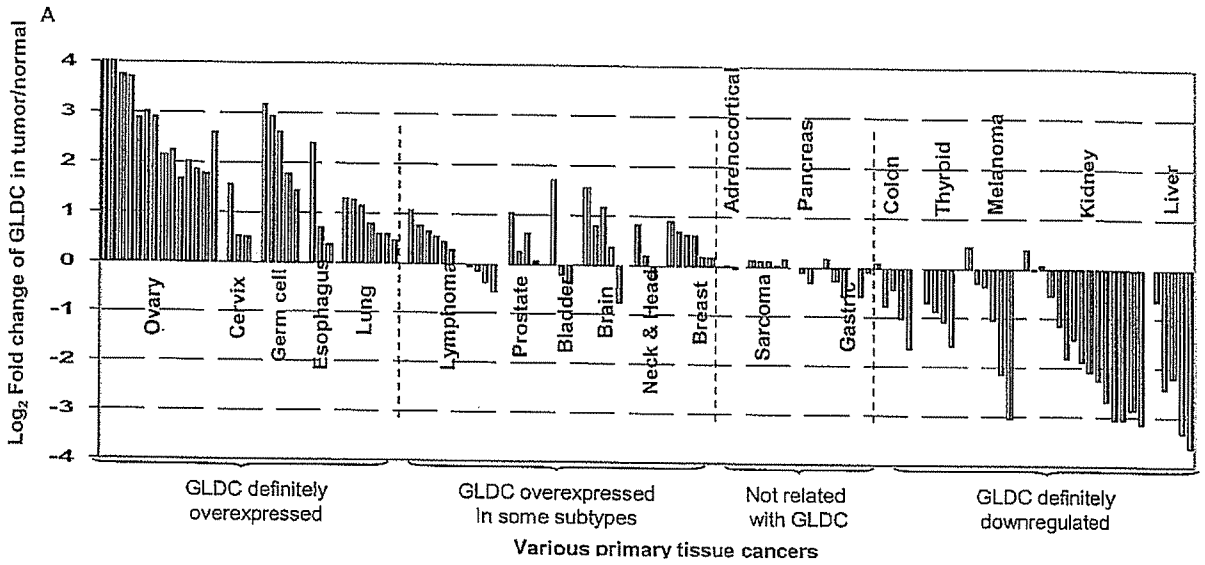

A
10000
8000
6000
4000
2000
0
Relative GLDC expression
Ctrl-OE
GD-OE
B
Ctrl-OE
GD-OE
C
60
50
40
30
20
10
0
Number colonies per 5,000 cells
Ctrl-OE
GD-OE
D
Ctrl-OE GD-OE
E
Group
No. tumors/ No. injections
Ctrl-OE
0/6
GD-OE
6/6
F
XG_3T3_GD
G
XG_3T3_GD

Figure 1


A
Log2 Fold change of GLDC in tumor/normal
4
3
2
1
0
-1
-2
-3
-4
Ovary
Cervix
Germ cell
Esophagus
Lung
Lymphoma
Prostate
Bladder
Brain
Neck & Head
Breast
Adrenocortical
Sarcoma
Pancreas
Gastric
Colon
Thyroid
Melanoma
Kidney
Liver
GLDC definitely overexpressed
GLDC overexpressed In some subtypes
Not related with GLDC
GLDC definitely downregulated
Various primary tissue cancers

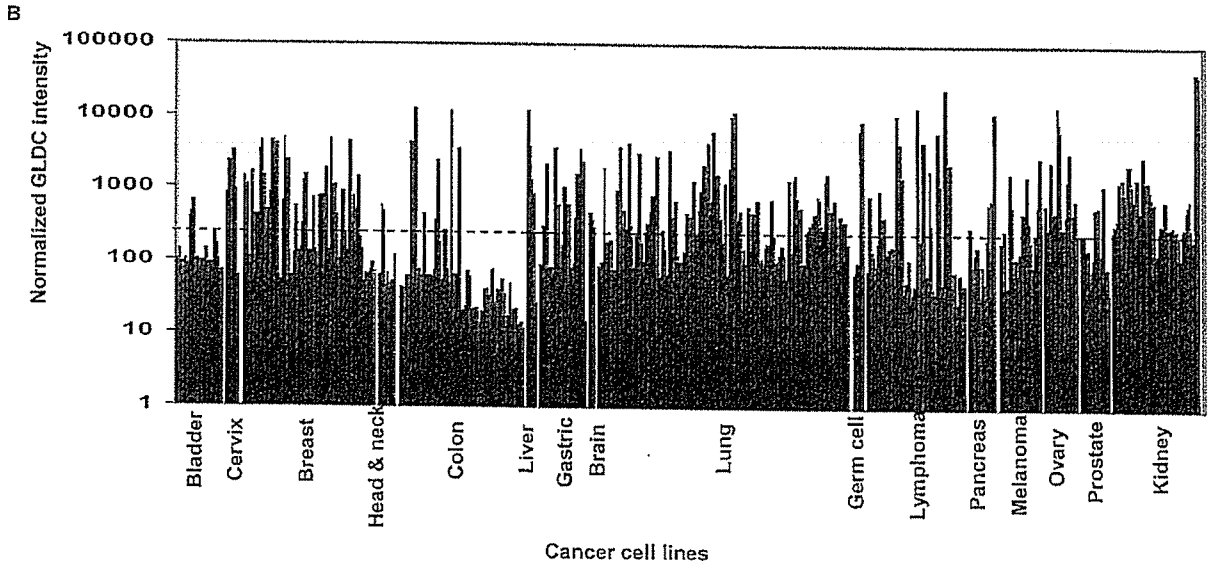

B
Normalized GLDC intensity
100000
10000
1000
100
10
1
Bladder
Cervix
Breast
Head & neck
Colon
Liver
Gastric
Brain
Lung
Germ cell
Lymphoma
Pancreas
Melanoma
Ovary
Prostate
Kidney
Cancer cell lines

Figure 2

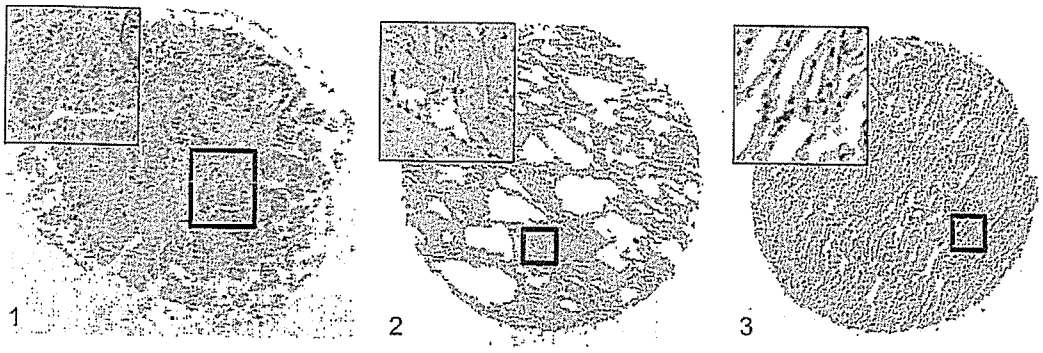


Figure 3

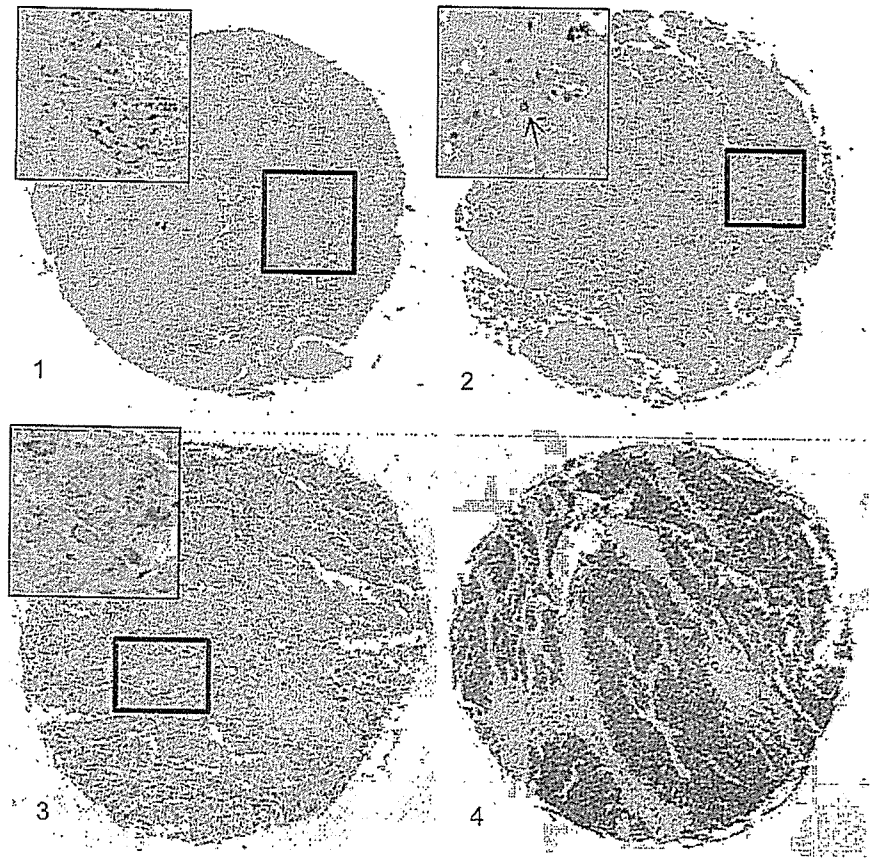


Figure 4

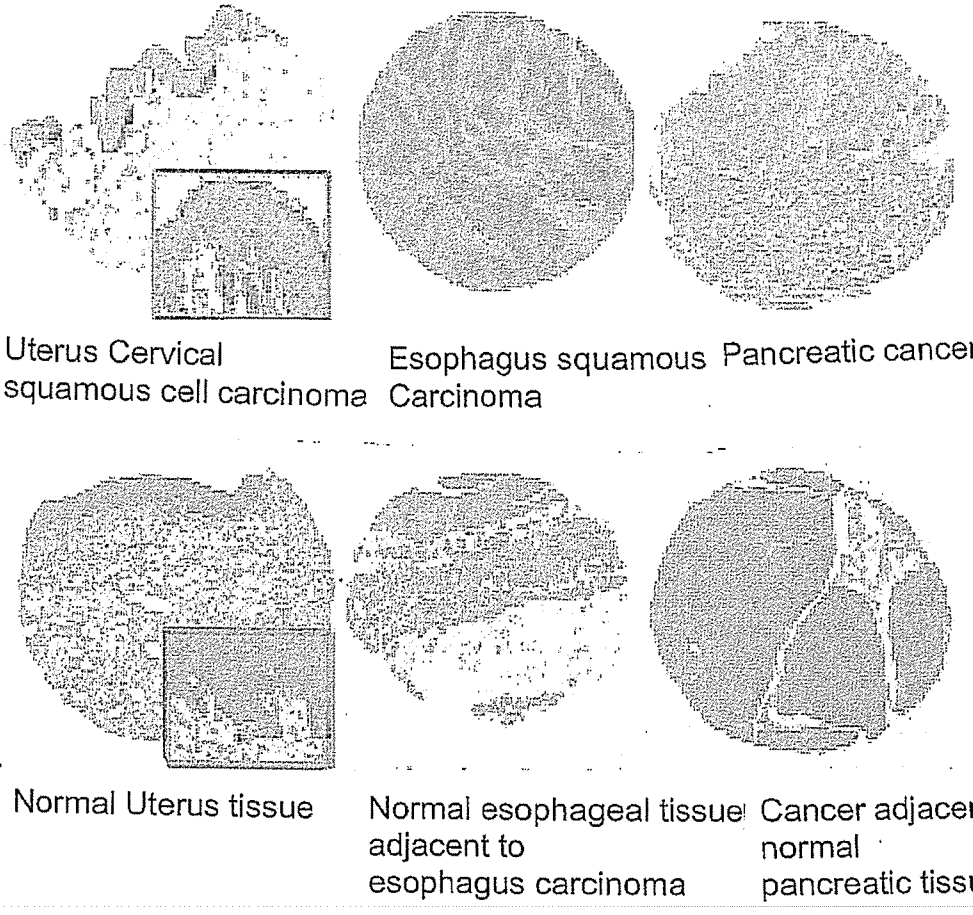

Uterus Cervical squamous cell carcinoma
Esophagus squamous Carcinoma
Pancreatic cancer
Normal Uterus tissue
Normal esophageal tissue adjacent to esophagus carcinoma
Cancer adjacent normal pancreatic tissue

Figure 5

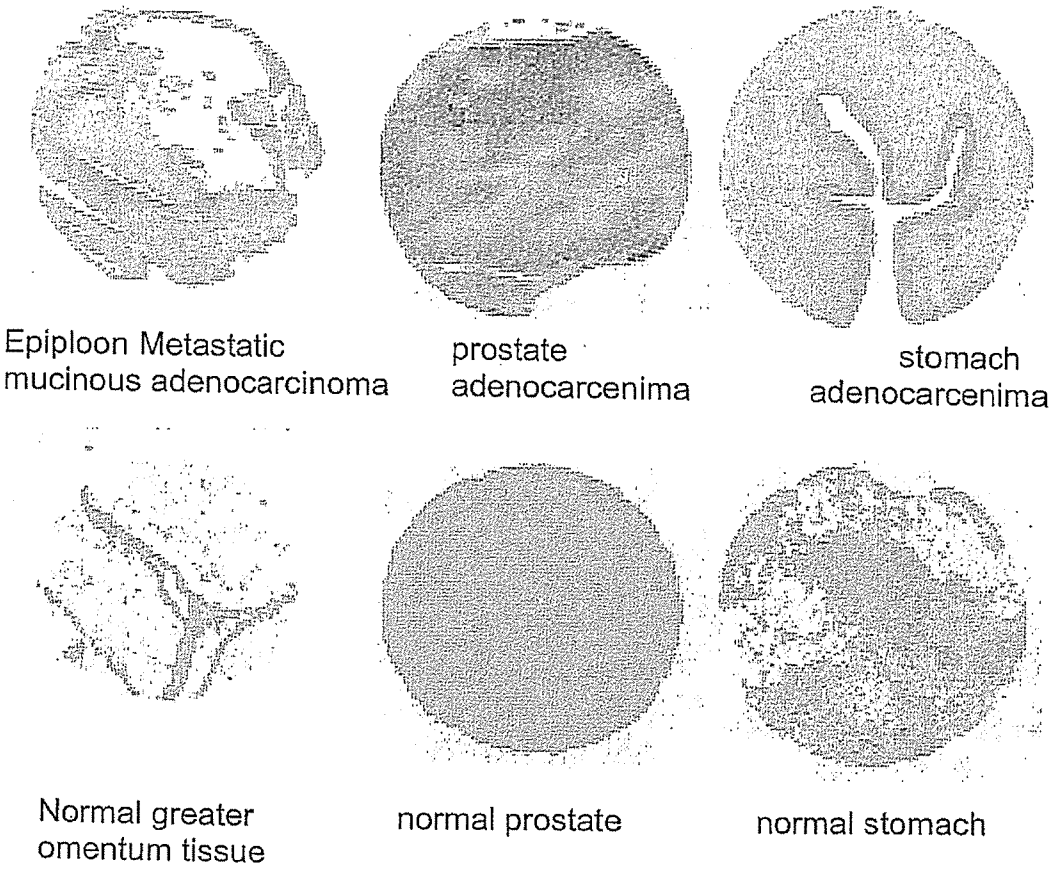


Figure 5 Continued

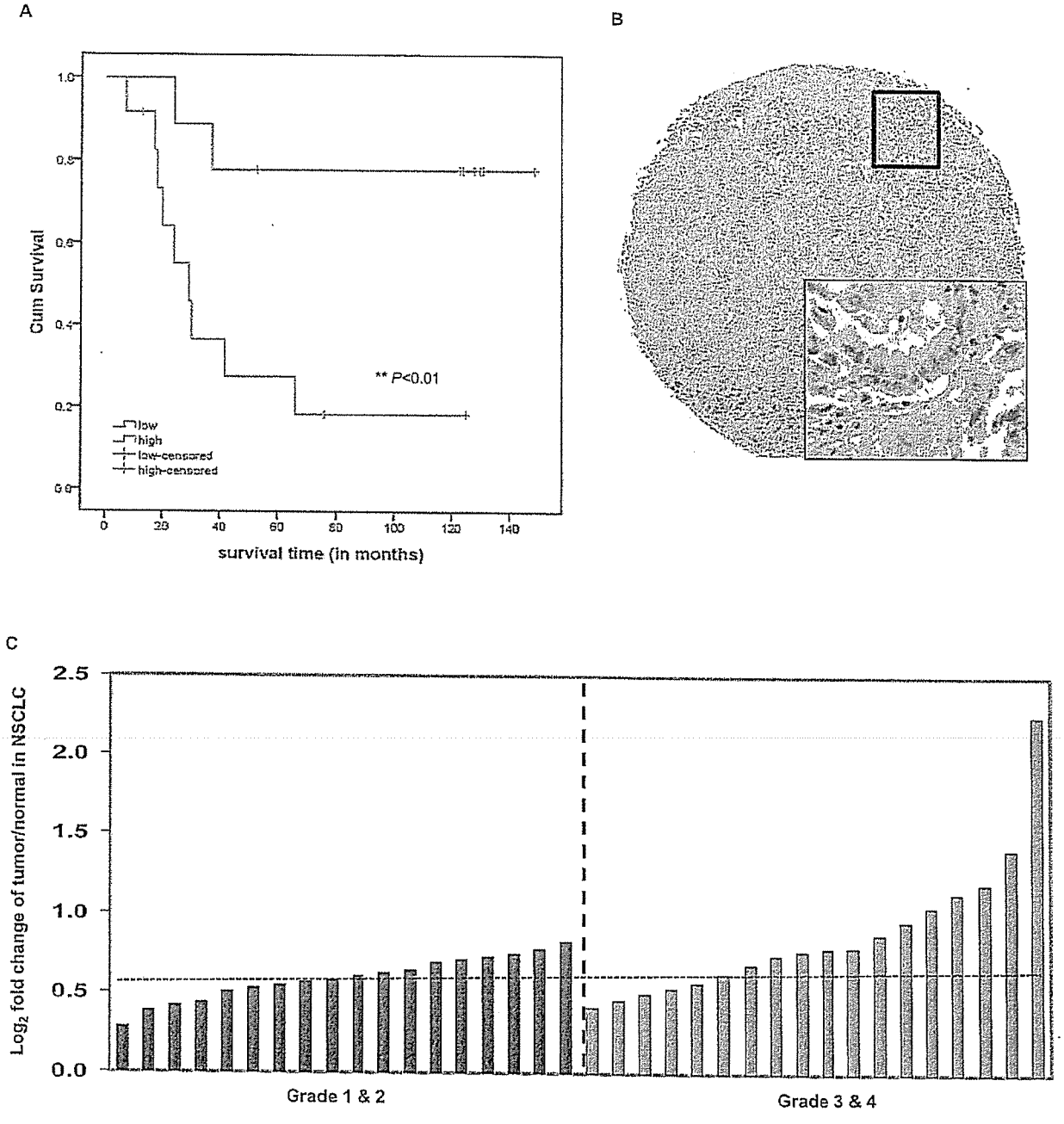

A
B
C
Cum Survival
1.0
0.8
0.6
0.4
0.2
0.0
** P<0.01
low
high
low-censored
high-censored
0
20
40
60
80
100
120
140
survival time (in months)
Log2 fold change of tumor/normal in NSCLC
2.5
2.0
1.5
1.0
0.5
0.0
Grade 1 & 2
Grade 3 & 4

Figure 6

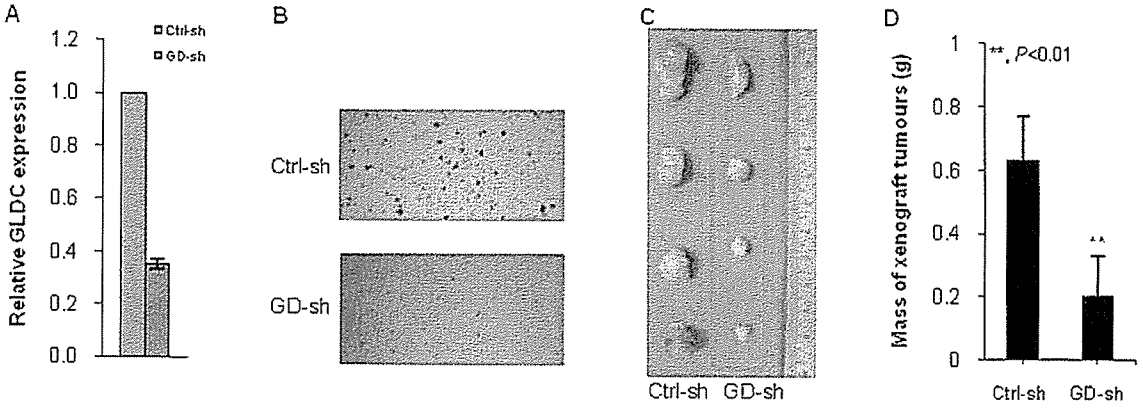
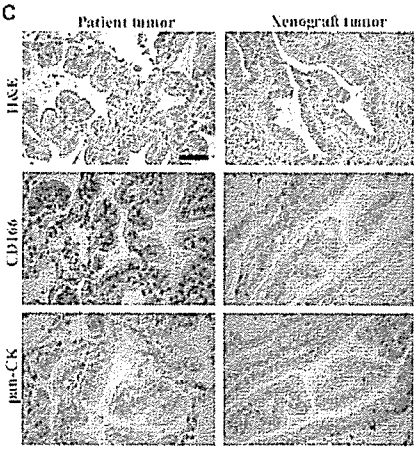

A
Ctrl-sh
GD-sh
Relative GLDC expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
B
Ctrl-sh
GD-sh
C
Ctrl-sh GD-sh
D
**, P<0.01
Mass of xenograft tumours (g)
1
0.8
0.6
0.4
0.2
0
Ctrl-sh
GD-sh

Figure 7

A

Cells	No. cells per injection	No. tumors / No. injections	LTICs frequency (95% CI)	Enrichment factor (x)	*P* value
Unsorted	≤100,000	2/31	1/403,971 (1/255,949 - 1/637,598)	1	
	150,000	2/4			
	200,000	1/10			
	300,000	4/5			
	500,000	3/5			
	1,000,000	9/9			
	2,000,000	2/2			
CD166$^+$Lin$^-$	≤500	1/12	1/5,175 (1/2,837 - 1/9,441)	78	1.37E-23
	1,000	2/8			
	5,000	2/3			
	10,000	7/9			
	23,000	2/2			
	25,000	4/4			
	50,000	1/1			
CD166$^-$Lin$^-$	≤100,000	0/18	1/3,297,891 (1/460,464 - 1/23,608,384)	0.1	4.82E-03
	210,000	0/1			
	260,000	1/3			
	500,000	0/3			

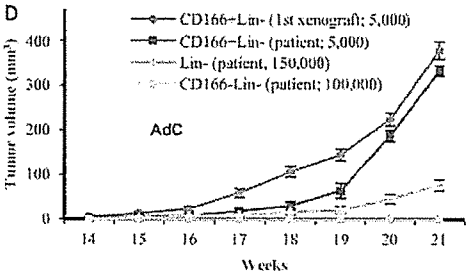
C



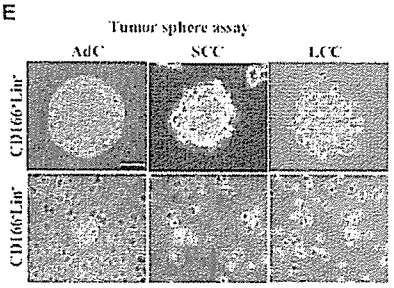
B

Population	No. tumors/ No. injections
CD166$^+$	12/12
CD166$^-$	0/10
CD44$^+$	6/8
CD44$^-$	6/8
CD133$^+$	6/11
CD133$^-$	7/13
EpCAM$^+$	10/13
EpCAM$^-$	8/14

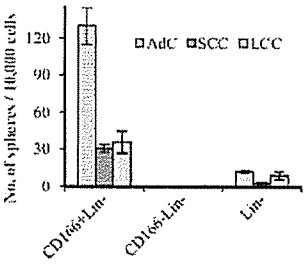
D



E



F



G

No. Cells	No. tumors/ No. injections 1st passage	10th passage
2500	12/12	N.D.
500	18/18	18/18
50	24/24	24/24
10	12/12	12/12
5	16/18	17/18
1	5/18	5/18

Figure 8

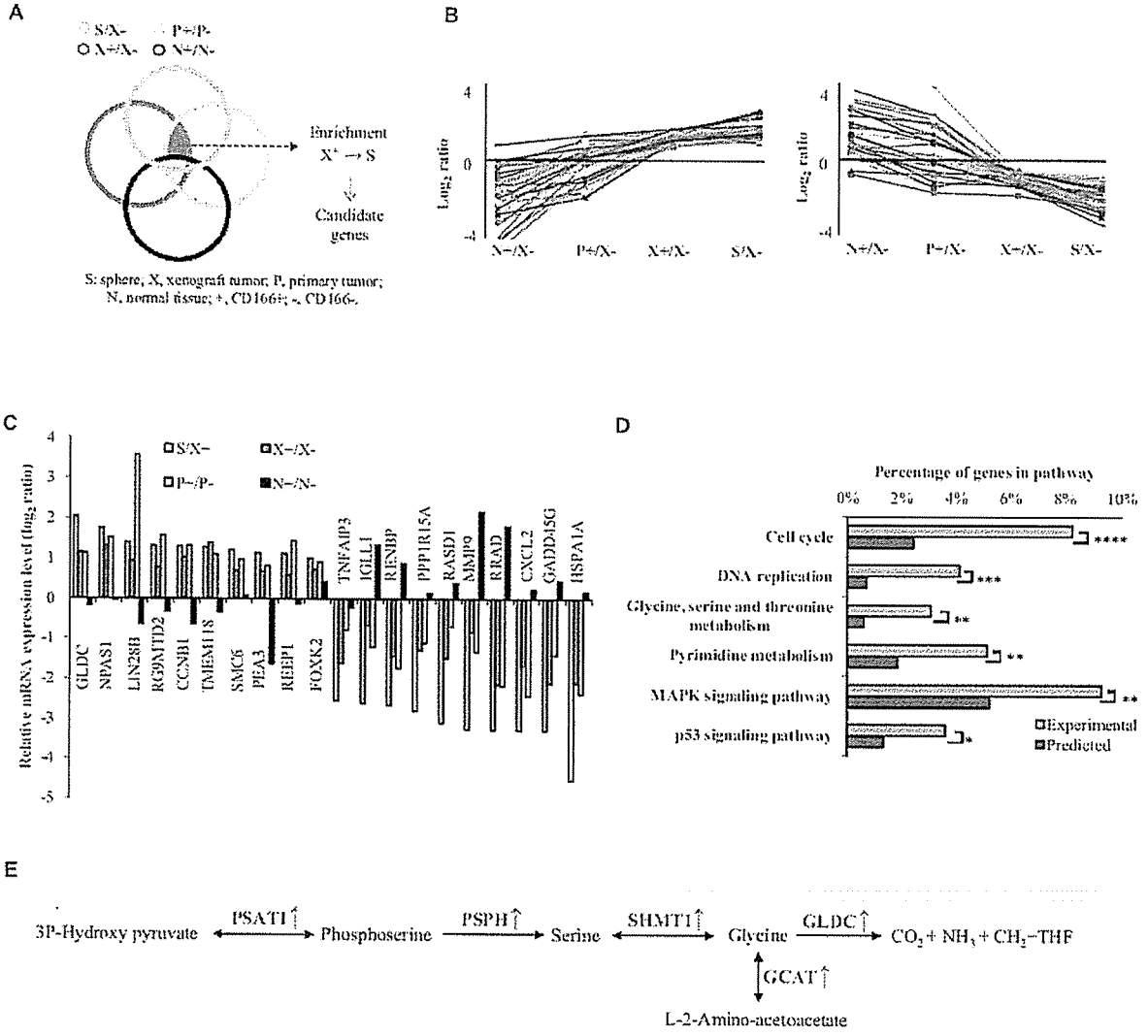

A
S/X-
P+/P-
X+/X-
N+/N-
Enrichment
X+ → S
Candidate genes
S: sphere; X, xenograft tumor; P, primary tumor;
N, normal tissue; +, CD166+; -, CD166-.
B
Log2 ratio
N-/X-
P+/X-
X+/X-
S/X-
N+/X-
P-/X-
X-/X-
S/X-
C
Relative mRNA expression level (log2 ratio)
S/X-
X-/X-
P-/P-
N-/N-
GLDC
NPAS1
LIN28B
RGMNTTD2
CCNB1
TMEM118
SMC6
PEA3
REEP1
FOXK2
TNFAIP3
IGLL1
RENBP
PPP1R15A
RASD1
MMP9
RRAD
CXCL2
GADD45G
HSPA1A
D
Percentage of genes in pathway
0%
2%
4%
6%
8%
10%
Cell cycle
DNA replication
Glycine, serine and threonine metabolism
Pyrimidine metabolism
MAPK signaling pathway
p53 signaling pathway
Experimental
Predicted
E
3P-Hydroxy pyruvate
PSAT1↑
Phosphoserine
PSPH↑
Serine
SHMT1↑
Glycine
GLDC↑
CO2 + NH3 + CH2-THF
GCAT↑
L-2-Amino-acetoacetate

Figure 9

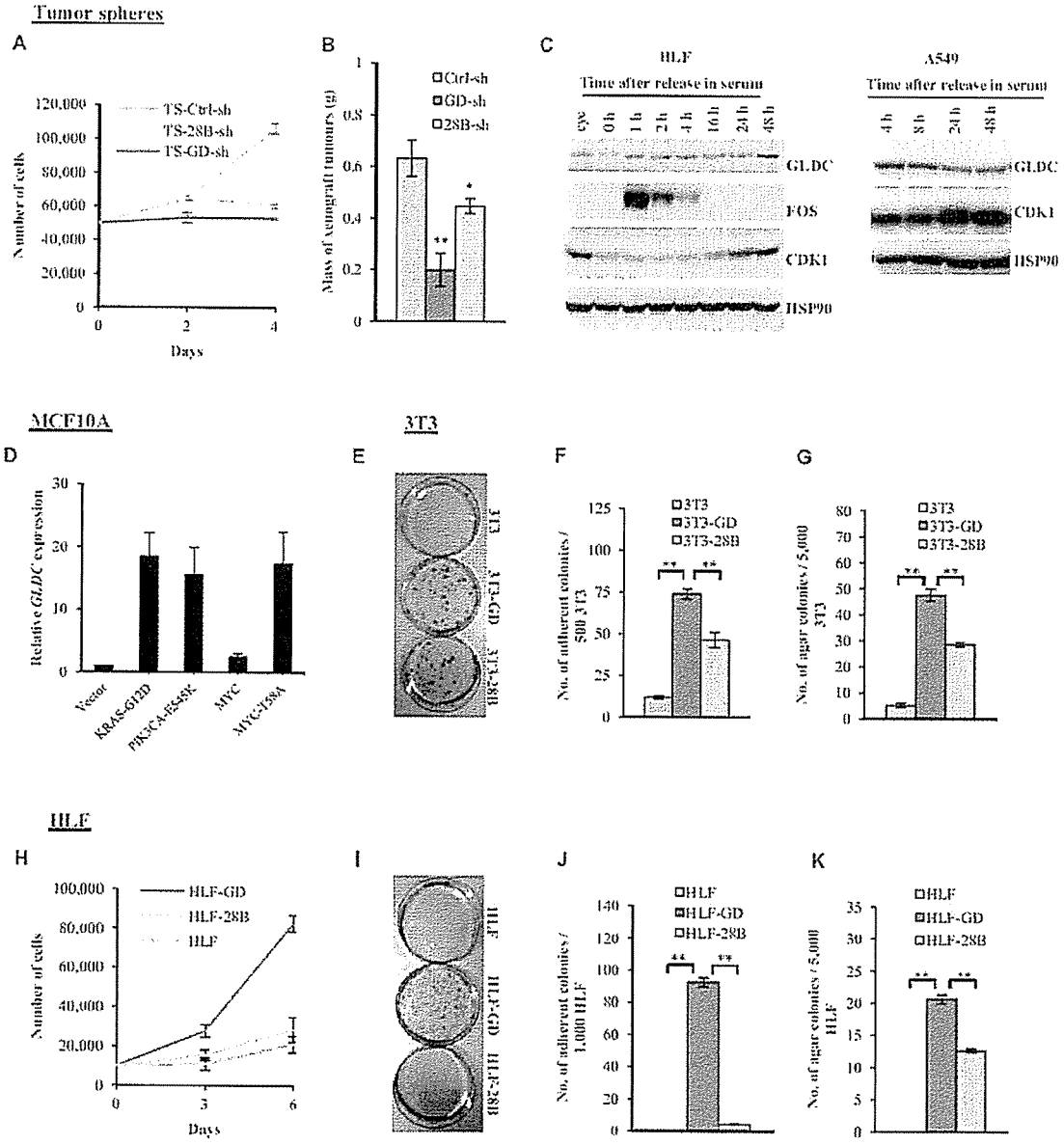

Tumor spheres
A
TS-Ctrl-sh
TS-28B-sh
TS-GD-sh
Number of cells
Days
B
Ctrl-sh
GD-sh
28B-sh
Mass of xenograft tumours (g)
C
HLF
Time after release in serum
A549
Time after release in serum
GLDC
FOS
CDK1
HSP90
MCF10A
Relative GLDC expression
Vector
KRAS-G12D
PIK3CA-E545K
MYC
MYC-T58A
3T3
3T3-GD
3T3-28B
F
No. of adherent colonies / 500 3T3
G
No. of agar colonies / 5,000 3T3
HLF
HLF-GD
HLF-28B
H
I
J
No. of adherent colonies / 1,000 HLF
K
No. of agar colonies / 5,000 HLF

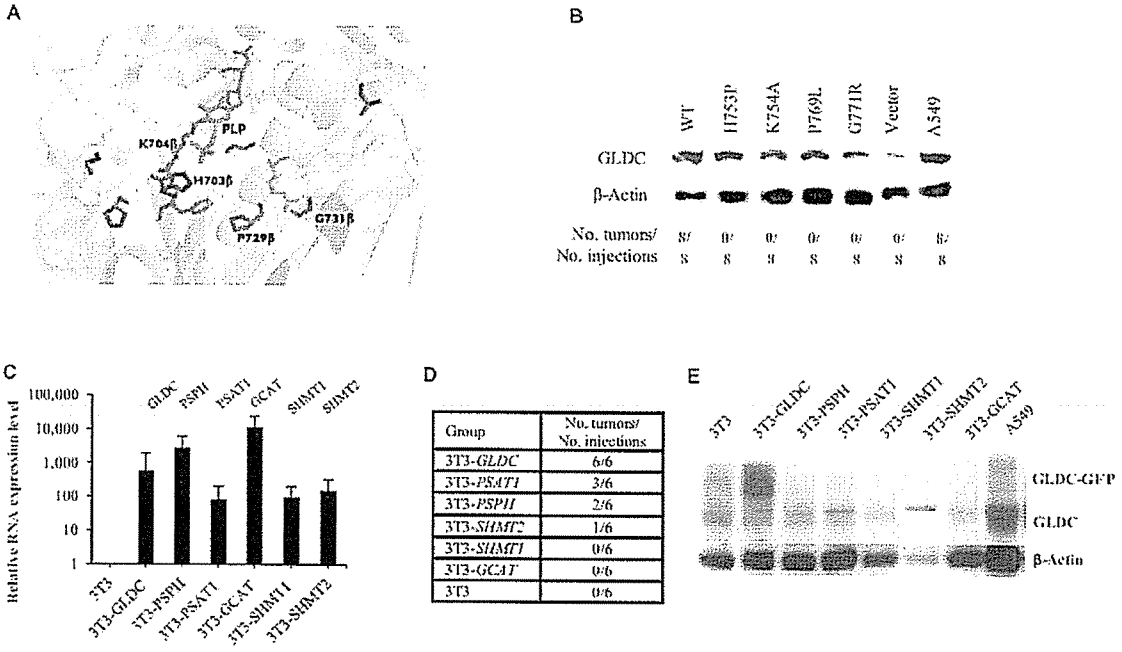
Figure 10

Xenograft

L

Group	No. cells / injection	No. tumors/ No. injections
CD166$^+$	200,000	0/12
	100,000	0/12
CD166$^+$ GD	200,000	4/12
	100,000	1/12

Figure 10 continued



Group	No. tumors/ No. injections
3T3-*GLDC*	6/6
3T3-*PSAT1*	3/6
3T3-*PSPH*	2/6
3T3-*SHMT2*	1/6
3T3-*SHMT1*	0/6
3T3-*GCAT*	0/6
3T3	0/6

Figure 11

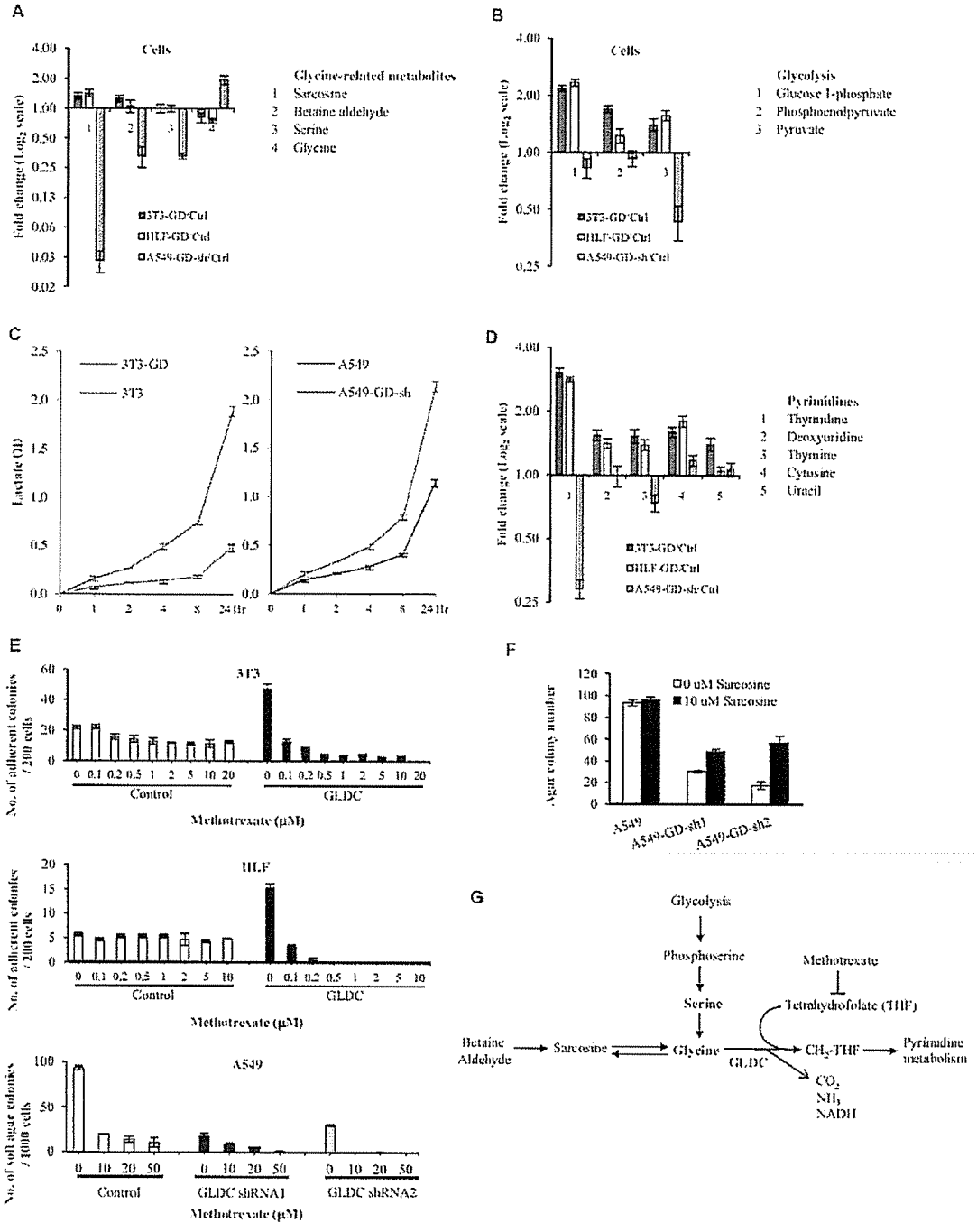
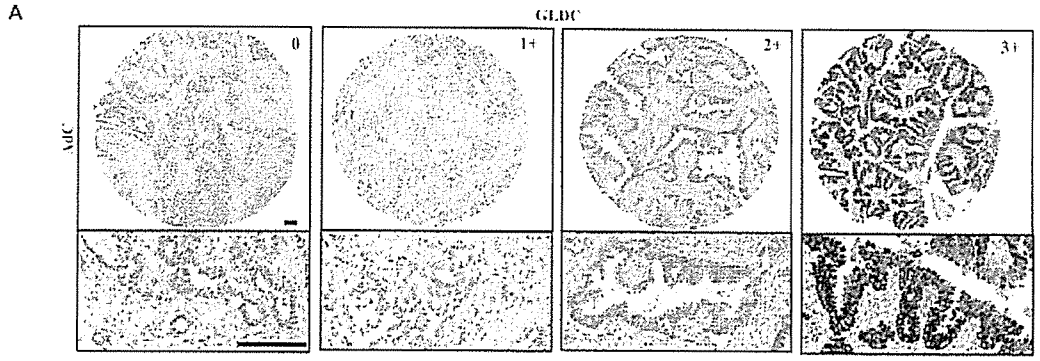
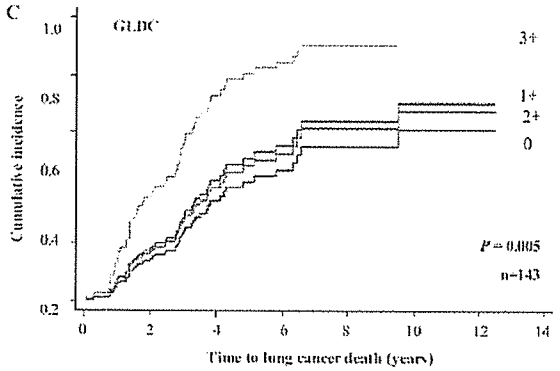

A
Cells
Glycine-related metabolites
1 Sarcosine
2 Betaine aldehyde
3 Serine
4 Glycine
3T3-GD/Ctrl
HLF-GD/Ctrl
A549-GD-sh/Ctrl
B
Cells
Glycolysis
1 Glucose 1-phosphate
2 Phosphoenolpyruvate
3 Pyruvate
C
3T3-GD
3T3
A549
A549-GD-sh
Lactate OD
D
Pyrimidines
1 Thymidine
2 Deoxyuridine
3 Thymine
4 Cytosine
5 Uracil
E
3T3
HLF
A549
Control
GLDC
GLDC shRNA1
GLDC shRNA2
Methotrexate (μM)
F
0 uM Sarcosine
10 uM Sarcosine
A549
A549-GD-sh1
A549-GD-sh2
G
Glycolysis
Phosphoserine
Serine
Methotrexate
Tetrahydrofolate (THF)
Betaine Aldehyde
Sarcosine
Glycine
GLDC
CH2-THF
Pyrimidine metabolism
CO2
NH3
NADH

Figure 12



B

GLDC Intensity	SHR (95% CI)	*P*-value
		0.005
0	1.00	-
1	1.28 (0.61 – 2.71)	0.514
2	1.19 (0.50 – 2.84)	0.700
3	3.01 (1.48 – 6.10)	0.002



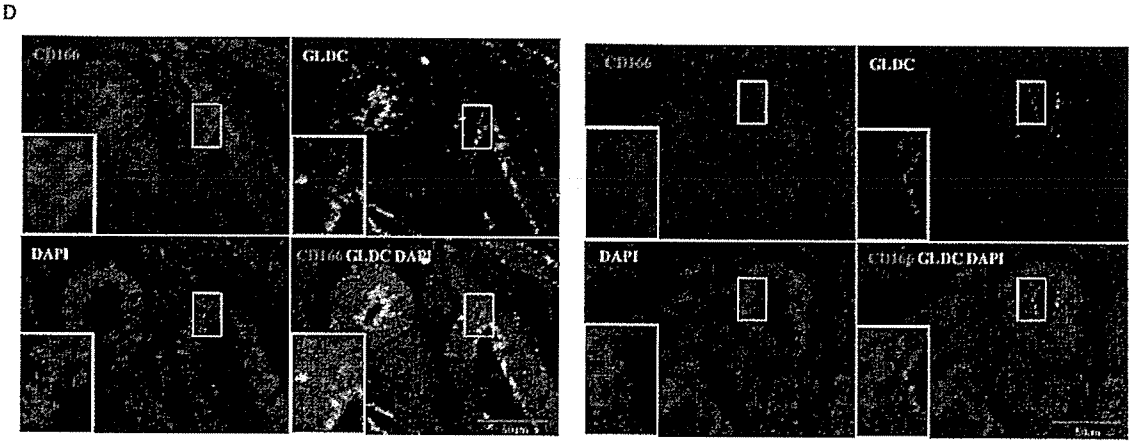


Figure 13

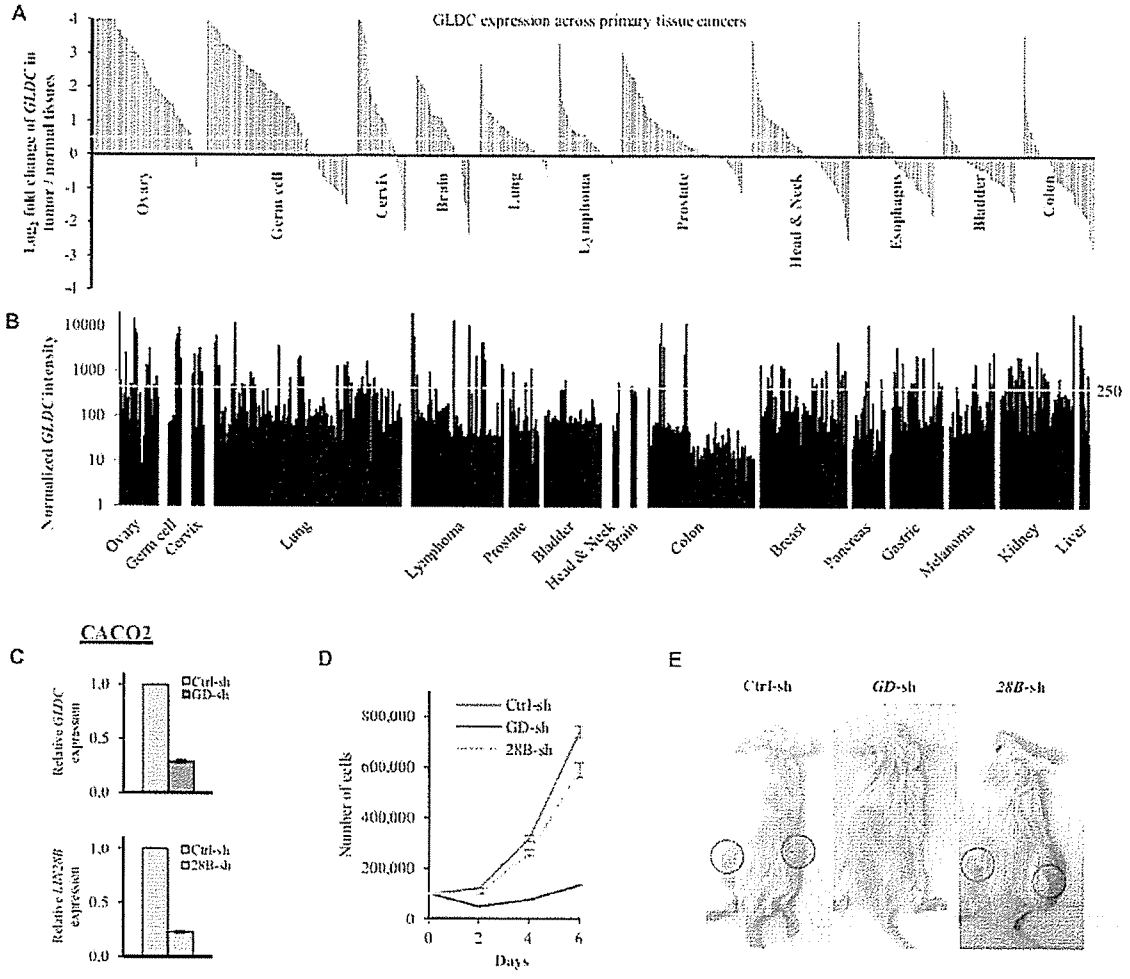
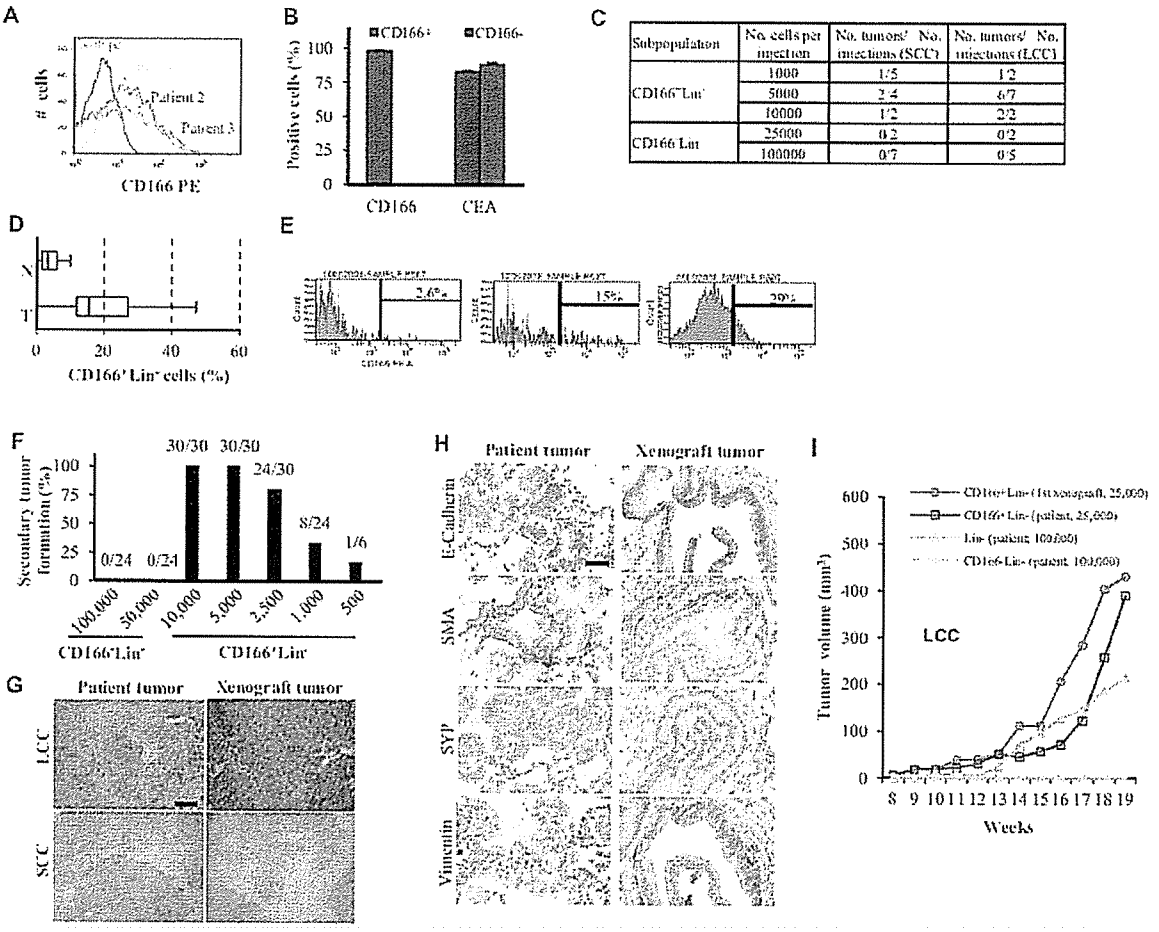
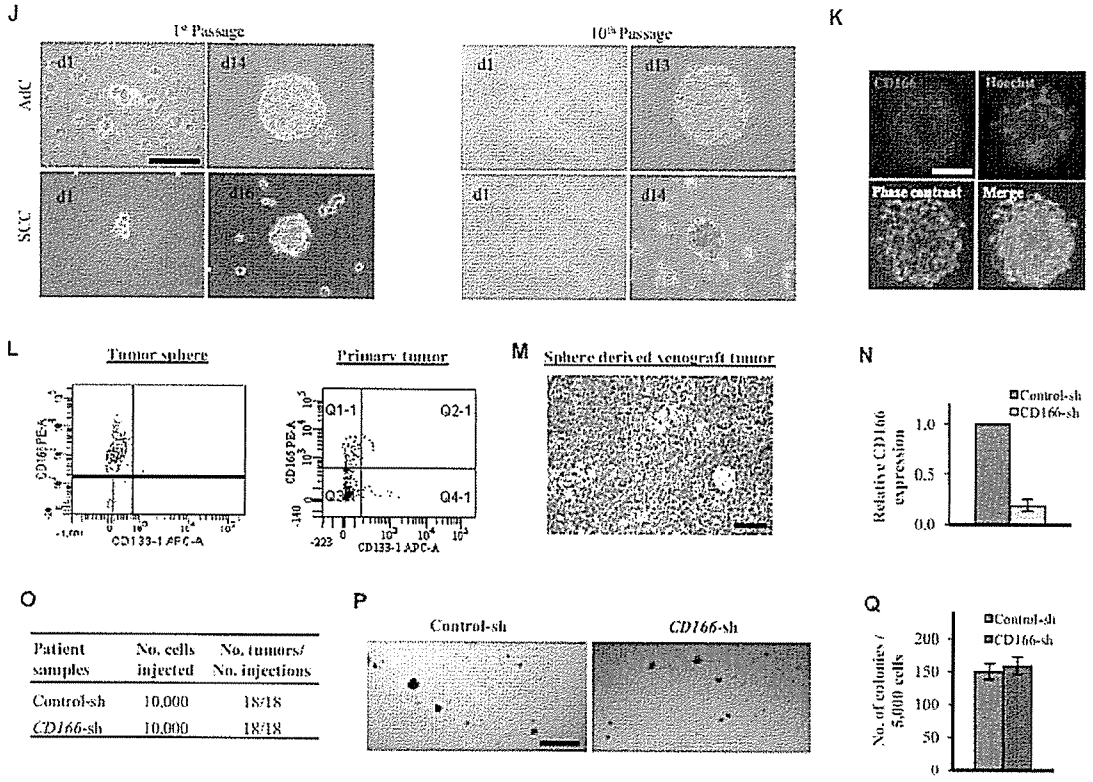

A
GLDC expression across primary tissue cancers
Log2 fold change of GLDC in tumor / normal tissues
Ovary
Germ cell
Cervix
Brain
Lung
Lymphoma
Prostate
Head & Neck
Esophagus
Bladder
Colon
B
Normalized GLDC intensity
10000
1000
100
10
1
250
Ovary
Germ cell
Cervix
Lung
Lymphoma
Prostate
Bladder
Head & Neck
Brain
Colon
Breast
Pancreas
Gastric
Melanoma
Kidney
Liver
C
CACO2
Relative GLDC expression
Ctrl-sh
GD-sh
Relative LIN28B expression
Ctrl-sh
28B-sh
D
Number of cells
Ctrl-sh
GD-sh
28B-sh
Days
E
Ctrl-sh
GD-sh
28B-sh

Figure 14



Subpopulation	No. cells per injection	No. tumors/ No. injections (SCC)	No. tumors/ No. injections (LCC)
CD166+Lin-	1000	1/5	1/2
	5000	2/4	6/7
	10000	1/2	2/2
CD166-Lin-	25000	0/2	0/2
	100000	0/7	0/5

Figure 15



Patient samples	No. cells injected	No. tumors/ No. injections
Control-sh	10,000	18/18
CD166-sh	10,000	18/18

Figure 15 Continued

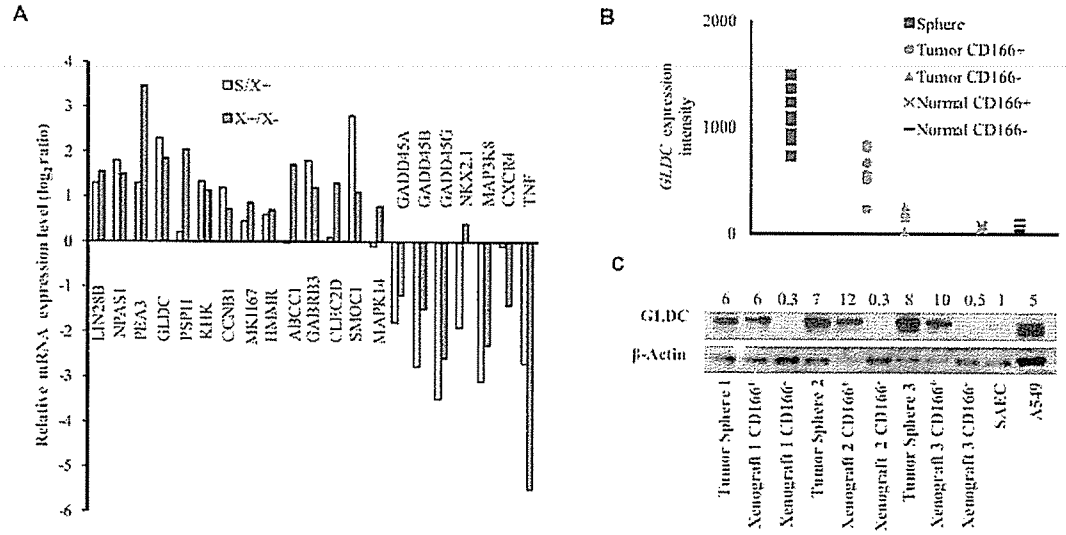


Figure 16

D

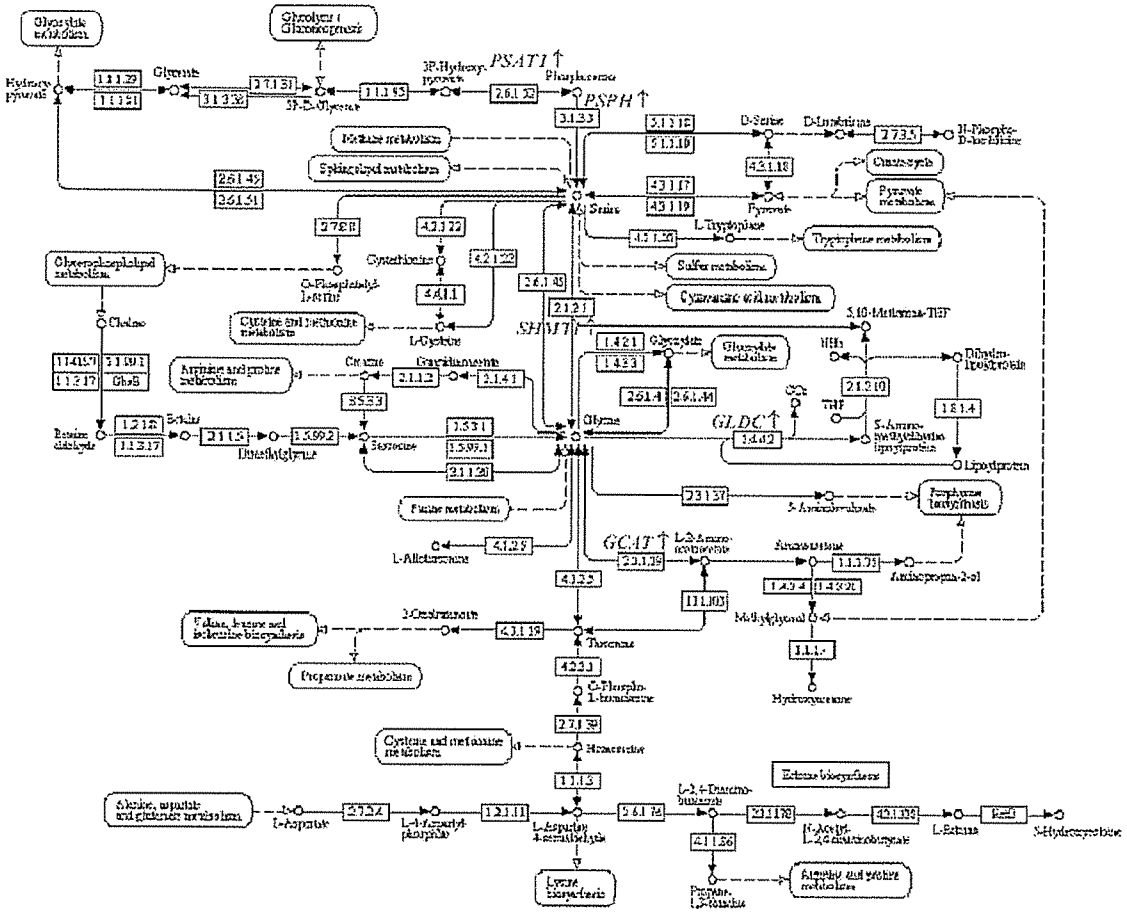
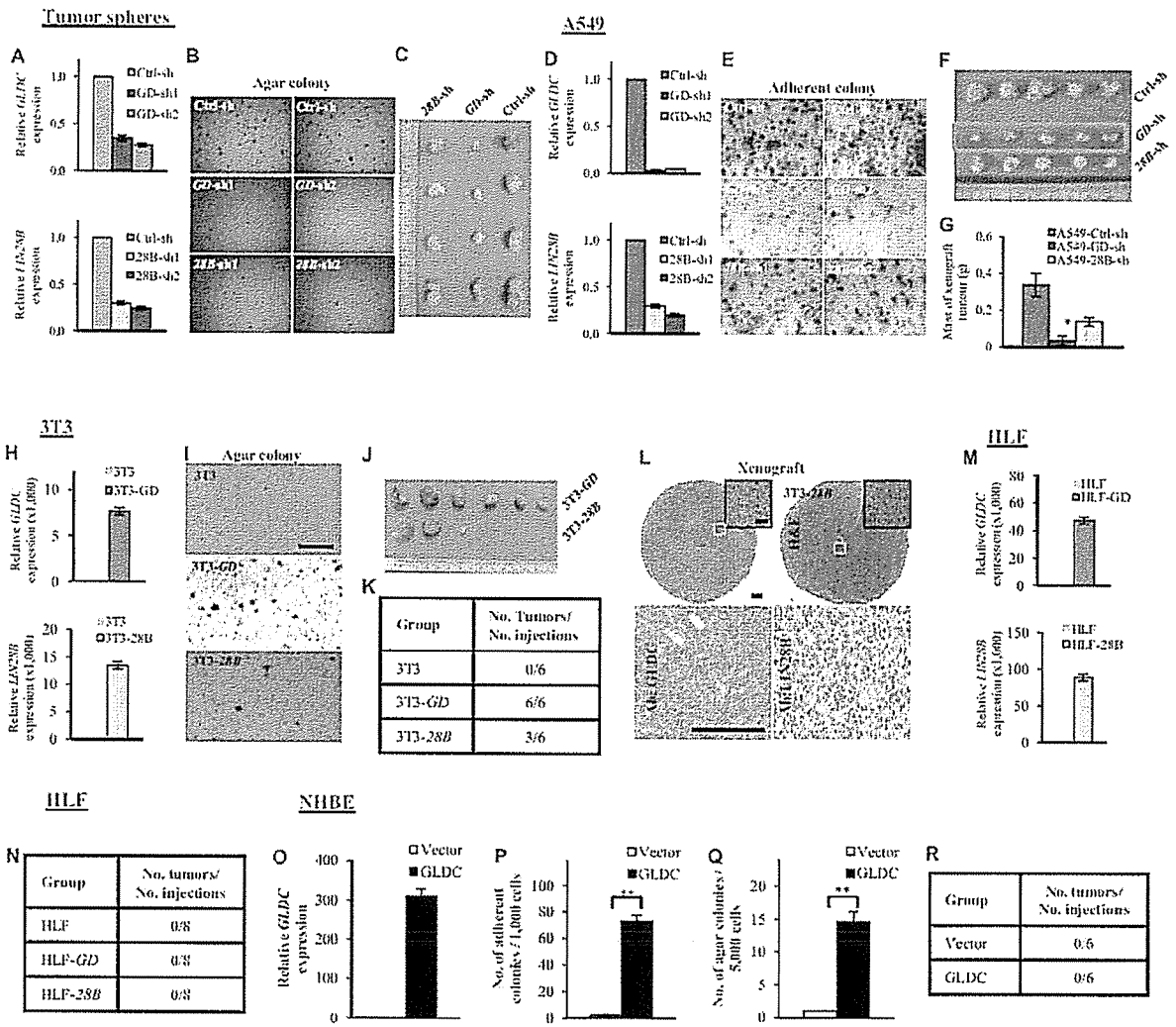


Figure 16 Continued



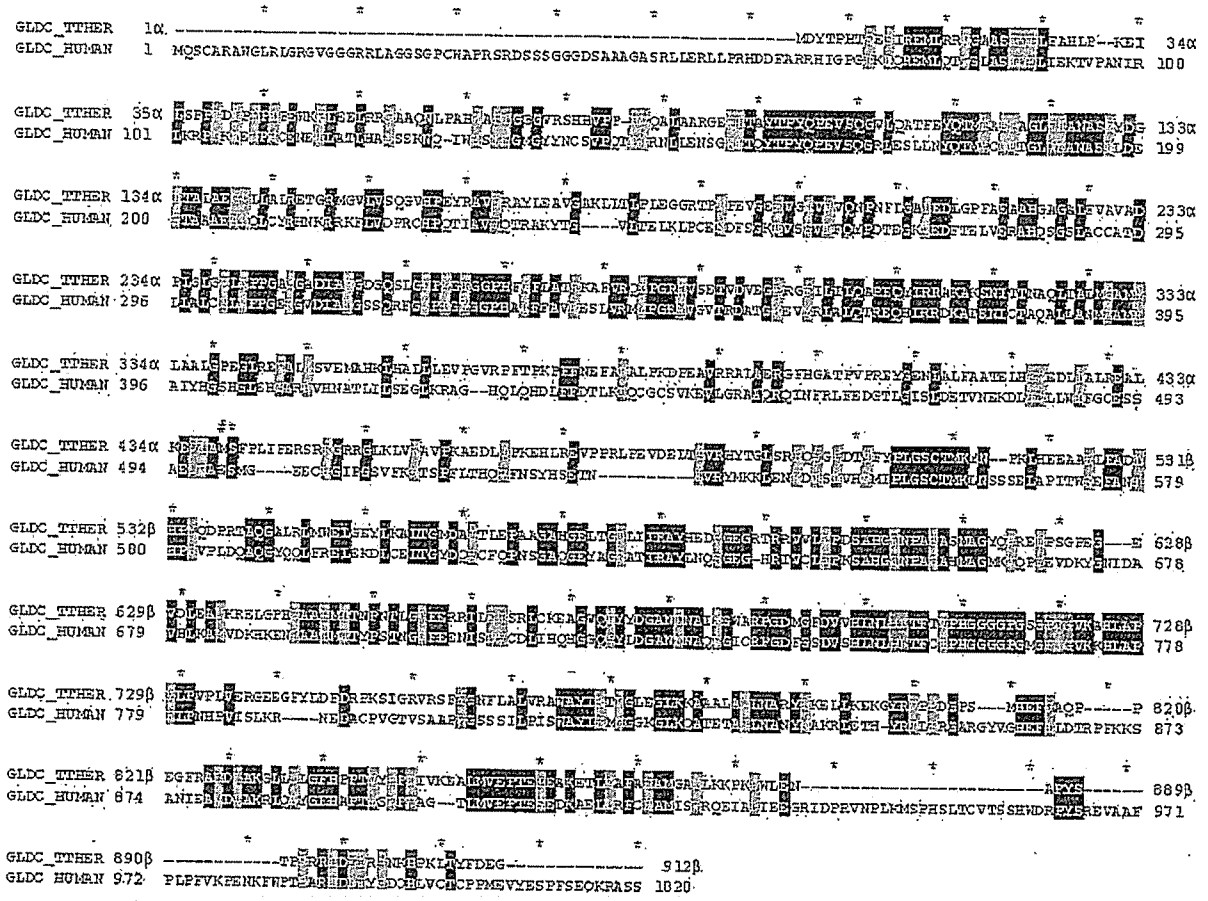
Group	No. Tumors/ No. injections
3T3	0/6
3T3-*GD*	6/6
3T3-*28B*	3/6

Group	No. tumors/ No. injections
HLF	0/8
HLF-*GD*	0/8
HLF-*28B*	0/8

Group	No. tumors/ No. injections
Vector	0/6
GLDC	0/6

Figure 17

A


GLDC_TTHER 1α
GLDC_HUMAN 1
GLDC_TTHER 35α
GLDC_HUMAN 101
GLDC_TTHER 134α
GLDC_HUMAN 200
GLDC_TTHER 234α
GLDC_HUMAN 296
GLDC_TTHER 334α
GLDC_HUMAN 396
GLDC_TTHER 434α
GLDC_HUMAN 494
GLDC_TTHER 532β
GLDC_HUMAN 580
GLDC_TTHER 629β
GLDC_HUMAN 679
GLDC_TTHER 729β
GLDC_HUMAN 779
GLDC_TTHER 821β
GLDC_HUMAN 874
GLDC_TTHER 890β
GLDC_HUMAN 972

B

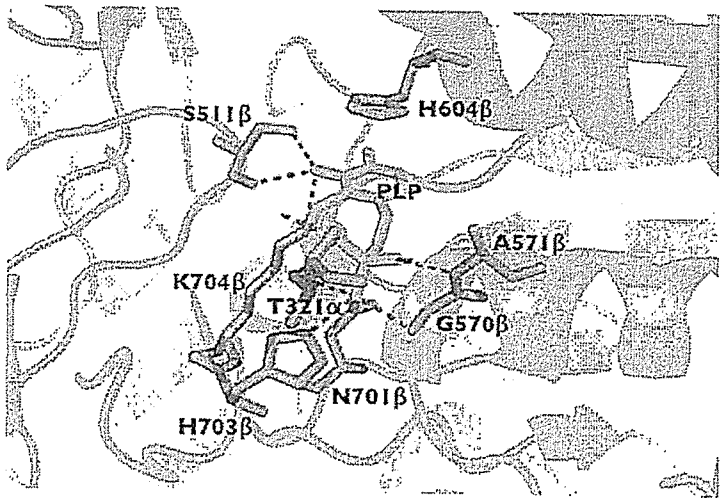
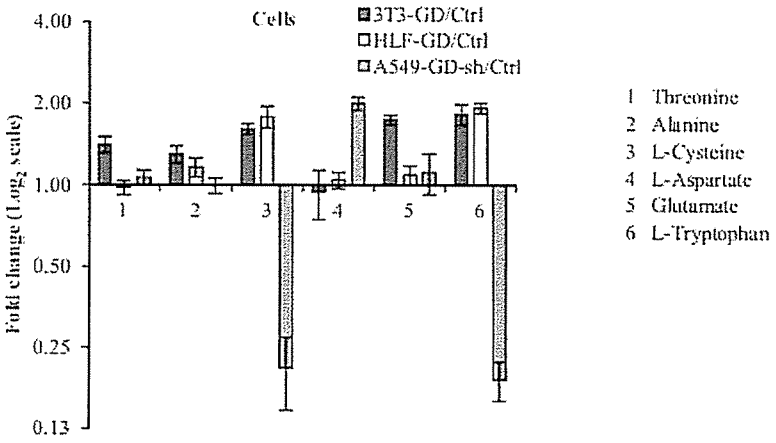
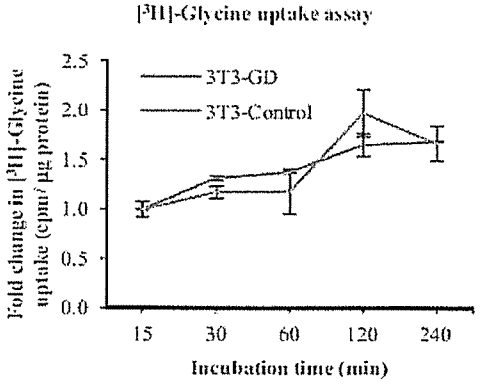

S511β
H604β
PLP
A571β
K704β
T321α
G570β
N701β
H703β

Figure 18

A



B



C

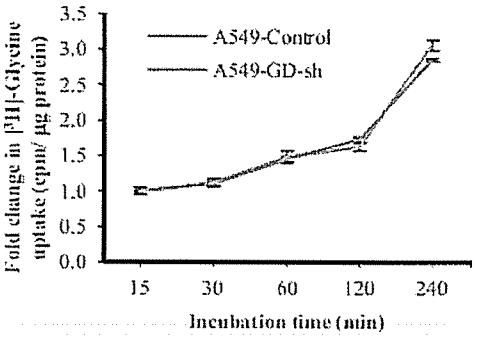
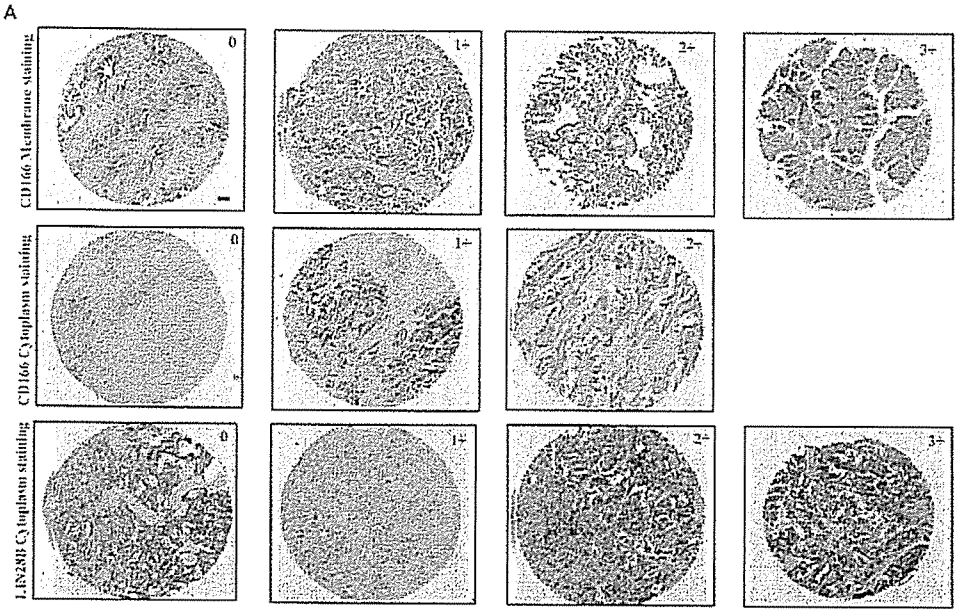


Figure 19

A



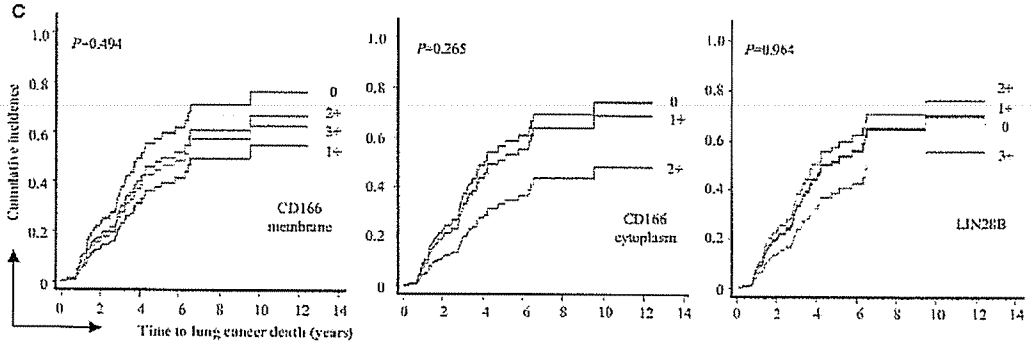
B

CD166 membrane Intensity	SHR (95% CI)	*P*-value
		0.494
0	1.00	-
1	0.55 (0.24 – 1.29)	0.178
2	0.76 (0.29 – 2.00)	0.578
3	0.69 (0.27 – 1.77)	0.437

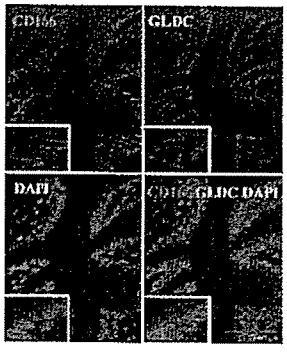
CD166 cytoplasm Intensity	SHR (95% CI)	*P*-value
		0.265
0	1.00	-
1	0.86 (0.46 – 1.59)	0.628
2	0.48 (0.20 – 1.16)	0.103
3	-	-

LIN28B Intensity	SHR (95% CI)	*P*-value
		0.964
0	1.00	-
1	1.02 (0.57 – 1.82)	0.955
2	1.19 (0.41 – 3.48)	0.752
3	0.67 (0.09 – 5.10)	0.703

C



D



E

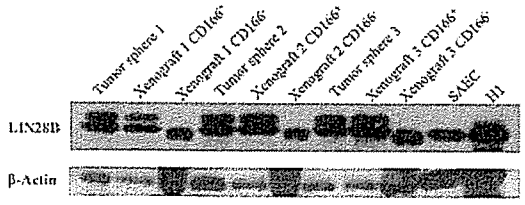


Figure 20

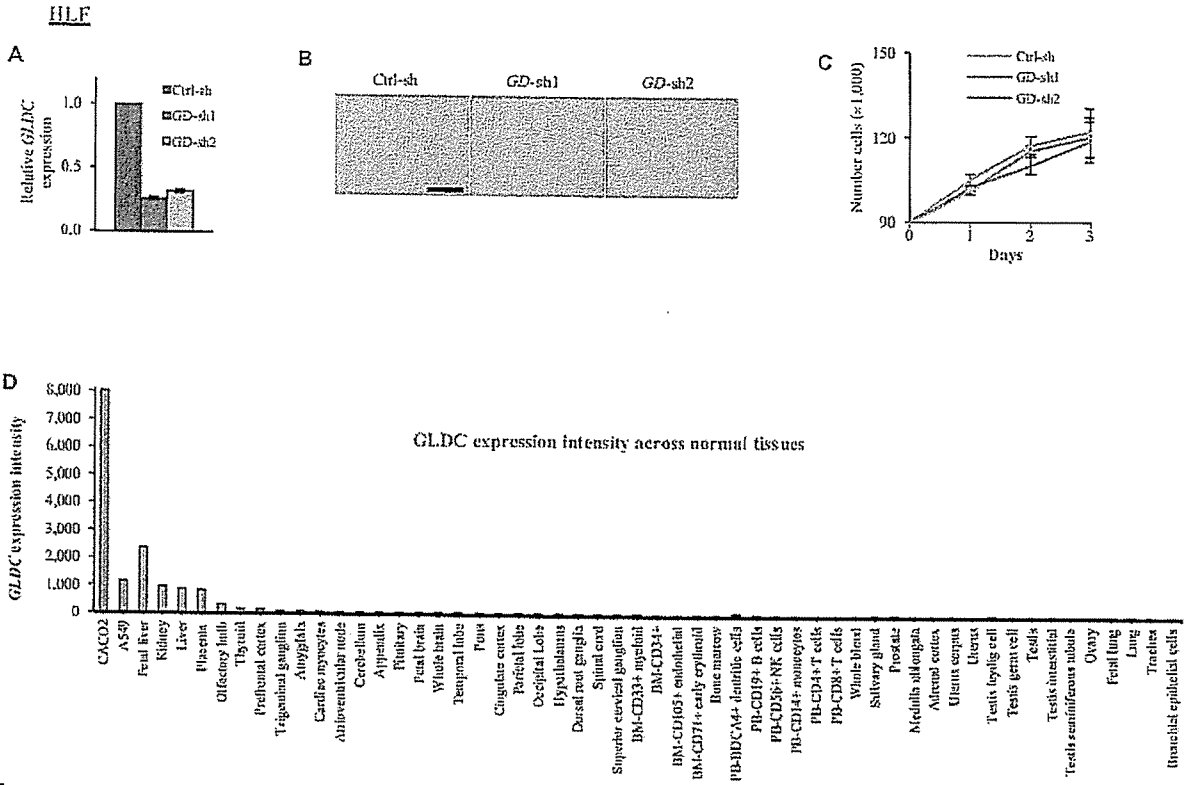


Figure 21

A

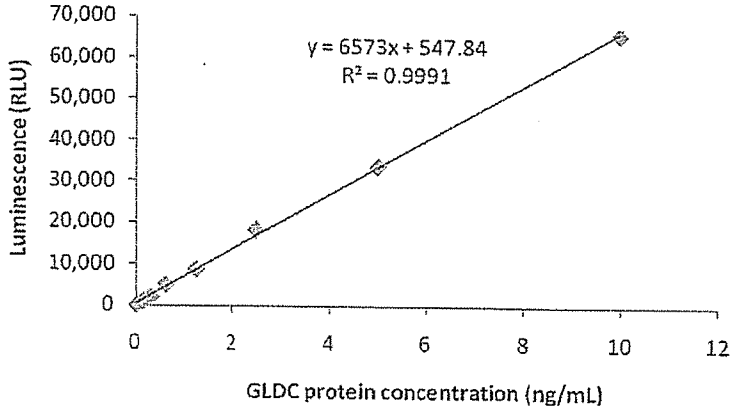
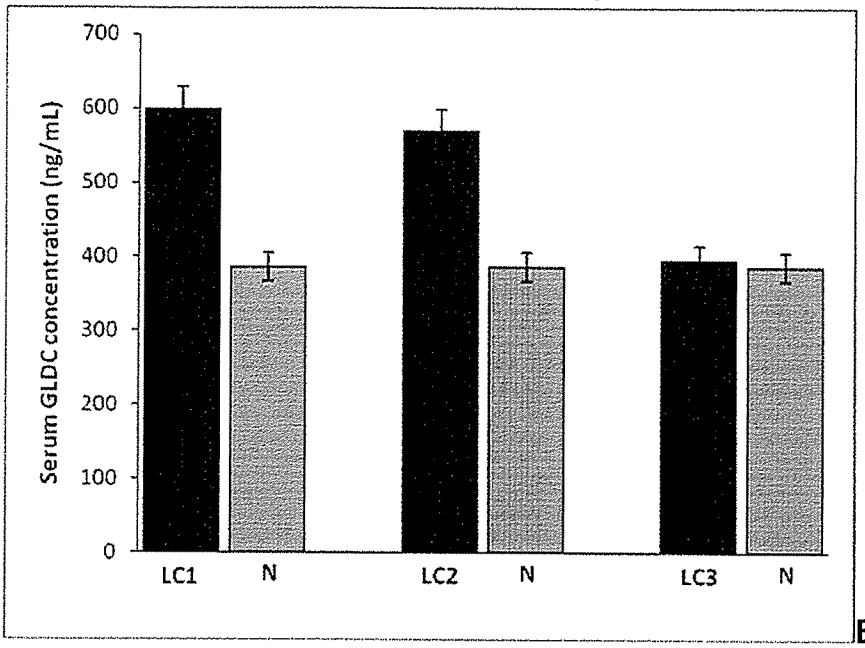
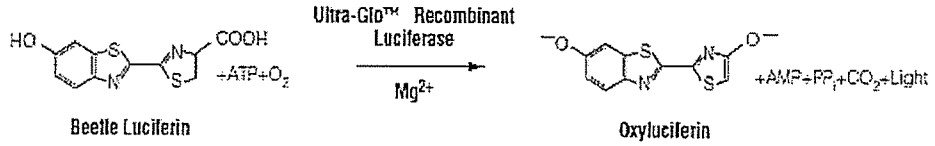


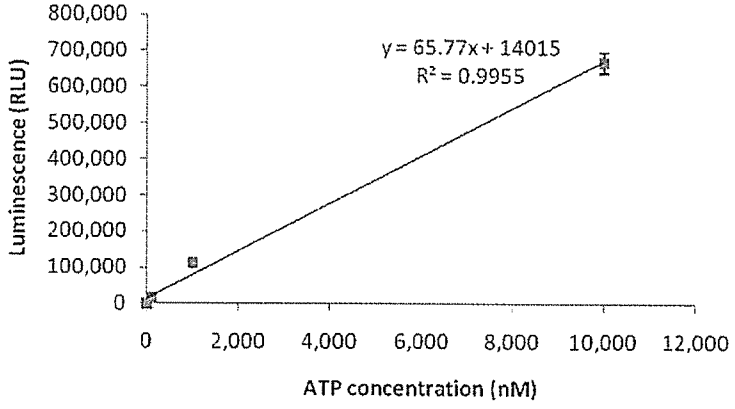
Figure 22



B

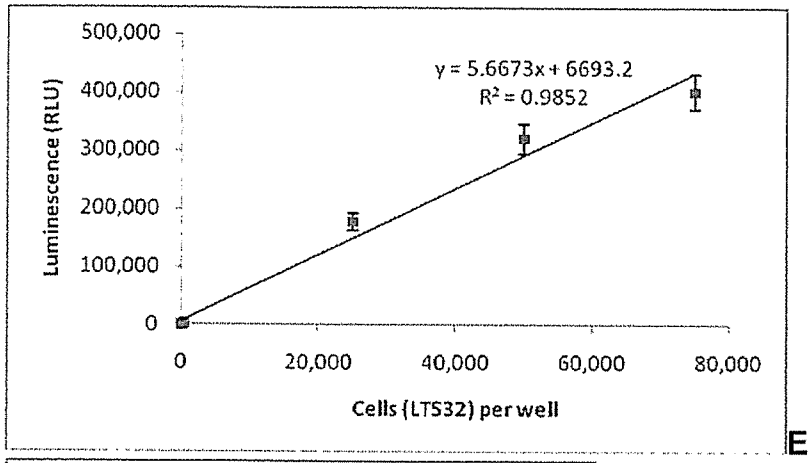


C

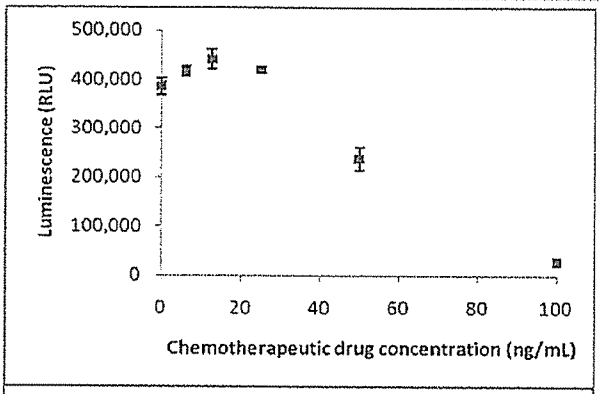


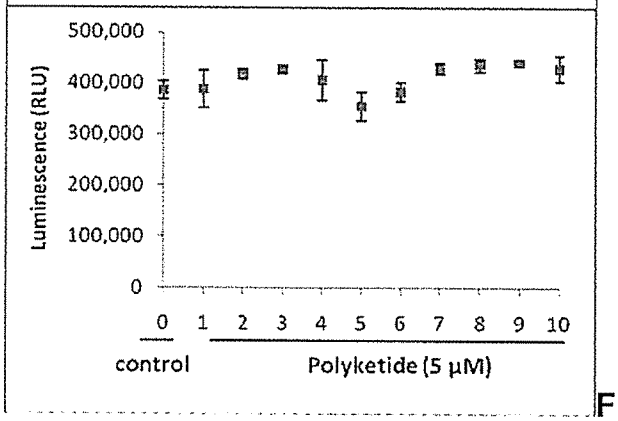
D

Figure 22 continued



E





F

Figure 22 continued