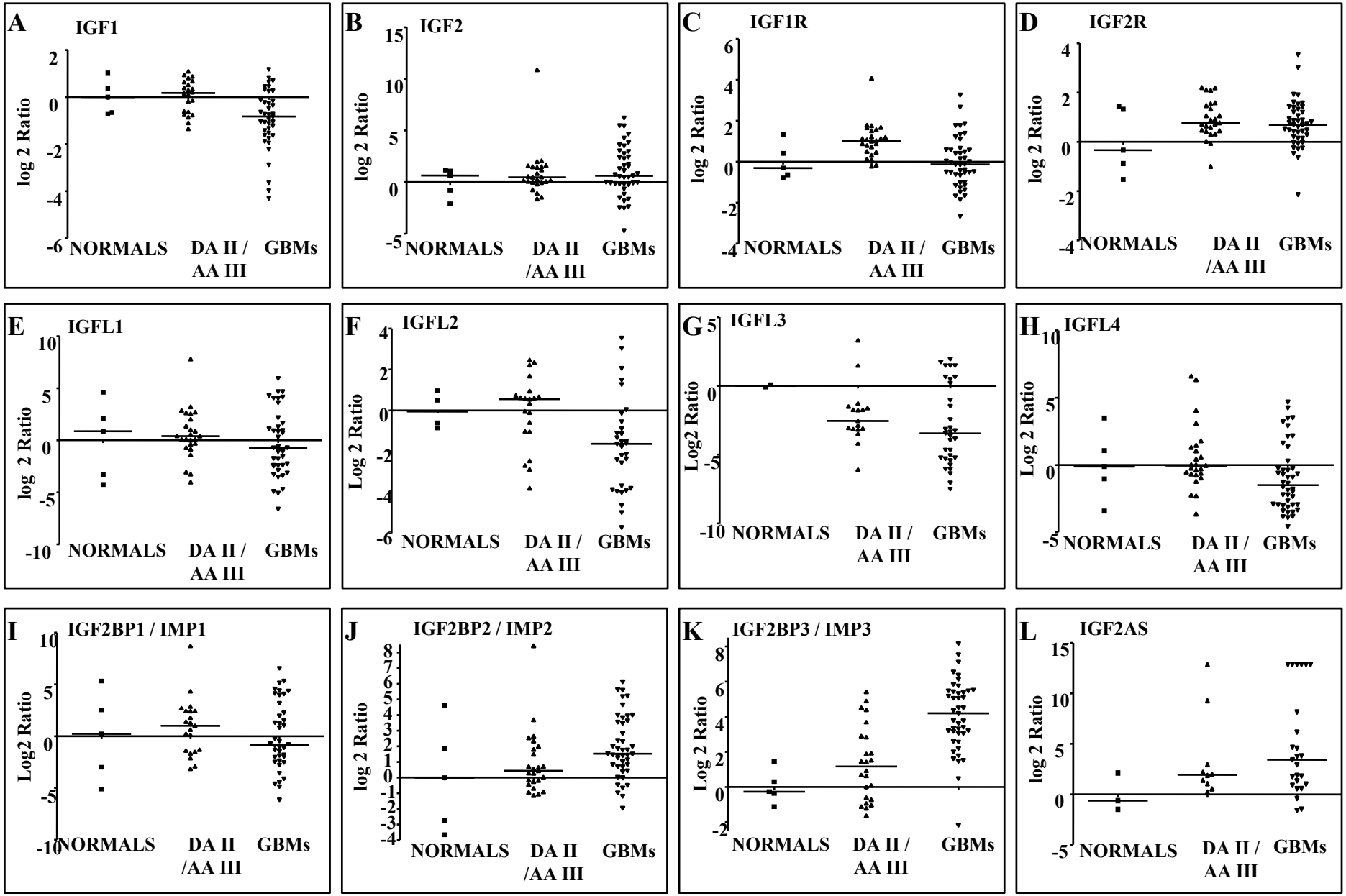
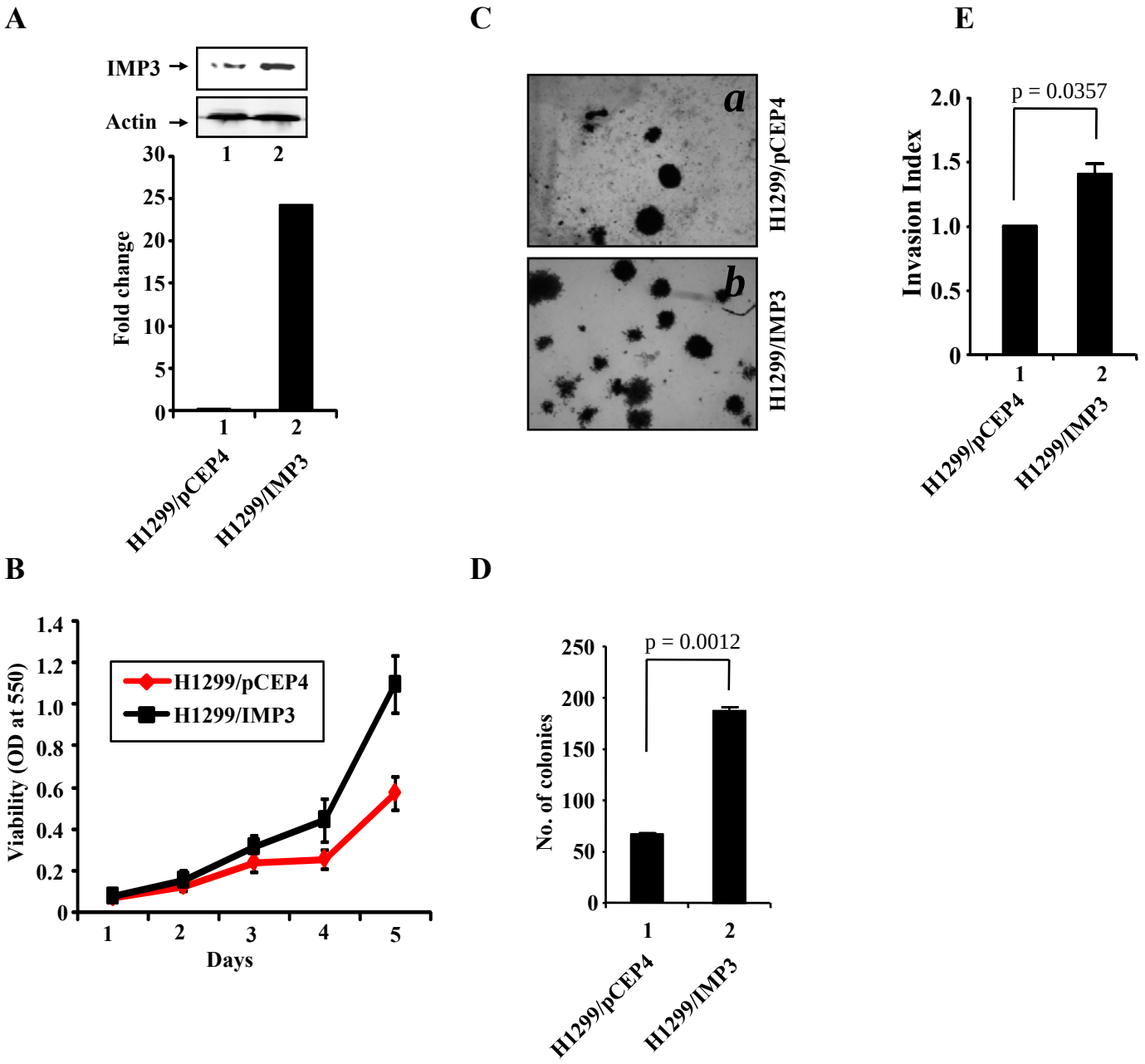
Supplementary Table and Figures (SF1-SF9)

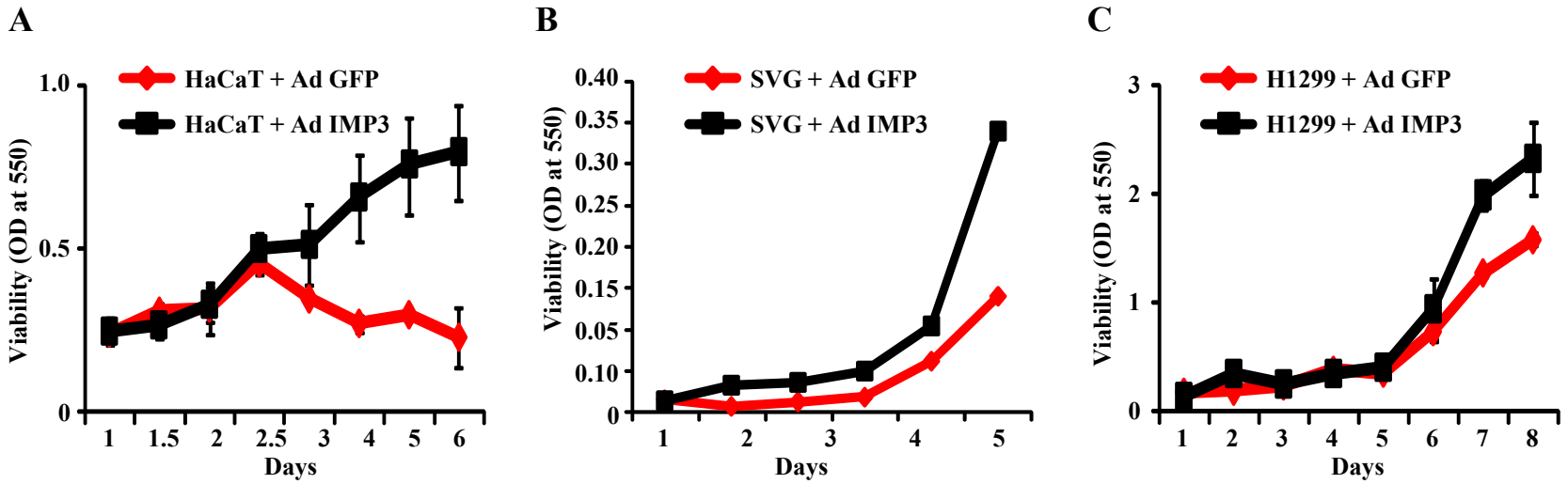
Significance analysis of differential regulation of IGF family members during glioma development*

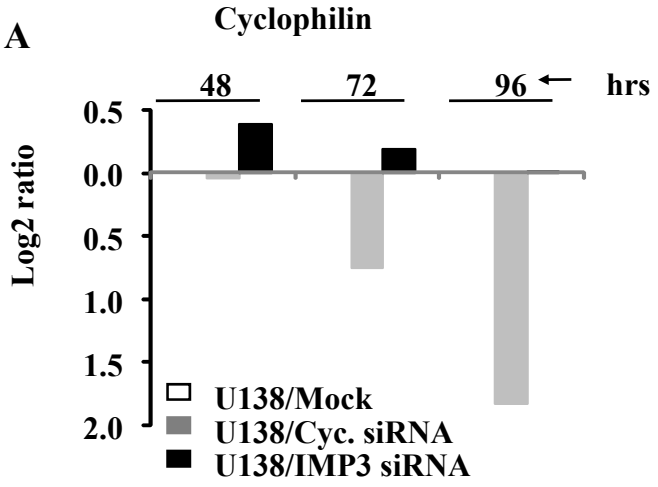
S. No	Gene ID	ANOVA p value followed by Dunnet's Post hoc test	Normals vs DAII/AAIII	Normals vs GBMs	DAII/AAIII vs GBMs
1	IGF1	p=0.0033	NS	NS	**
2	IGF2	p=0.6460	NS	NS	NS
3	IGF1R	p=0.0009	NS	NS	***
4	IGF2R	p=0.1400	NS	NS	NS
5	IGF2BP1	p=0.6022	NS	NS	NS
6	IGF2BP2	p=0.0446	NS	NS	NS
7	IGF2BP3	p≤0.0001	NS	***	***
8	IGFL1	p=0.4773	NS	NS	NS
9	IGFL2	p=0.0161	NS	NS	*
10	IGFL3	p=0.2385	NS	NS	NS
11	IGFL4	p=0.0590	NS	NS	*
12	IGF2AS	p=0.2314	NS	NS	NS

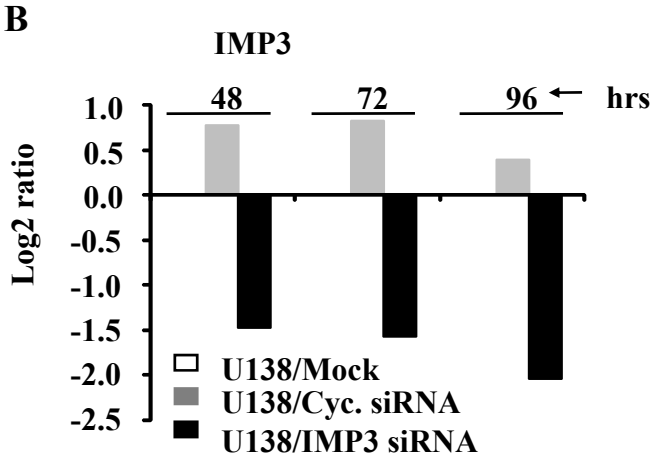
Log 2-transformed gene expression ratios obtained from RT-qPCR analysis of indicated samples were calculated for the transcript levels of the following genes - IGF-1, IGF-2, IGF-1R, IGF-2R, IGF2BP1, IGF2BP2, IGF2BP3/IMP3, IGF2AS, IGFL1, IGFL2, IGFL3, IGFL4. For each sample, fold change in gene expression is calculated over its mean expression in normal brain samples after normalization with six different internal reference genes - AGPAT, GARS, ATP5G1, GAPDH, ACTB and RPL35A. These log 2-transformed gene expression values were then subjected to one way analysis of variance (ANOVA) and a dunnet's post hoc test was carried out to test for significance. After analysis, no significant change in the transcript levels of the various IGF family members was found except for IGF2BP3/IMP3 which was significantly up regulated in the grade IV astrocytomas or glioblastomas compared to the normal brain samples and the grade II/grade III astrocytomas.

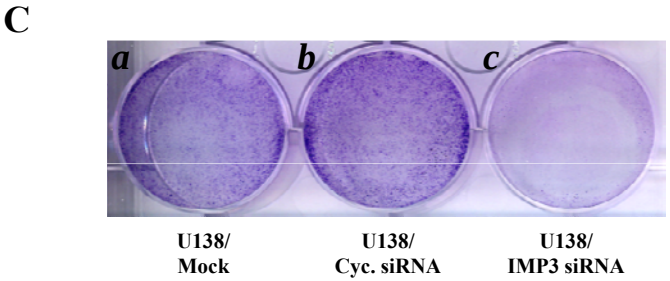

A
IGF1
log 2 Ratio
NORMALS
DA II / AA III
GBMs
B
IGF2
log 2 Ratio
NORMALS
DA II /AA III
GBMs
C
IGF1R
log 2 Ratio
NORMALS
DA II / AA III
GBMs
D
IGF2R
log 2 Ratio
NORMALS
DA II /AA III
GBMs
E
IGFL1
log 2 Ratio
NORMALS
DA II / AA III
GBMs
F
IGFL2
Log 2 Ratio
NORMALS
DA II / AA III
GBMs
G
IGFL3
Log2 Ratio
NORMALS
DA II / AA III
GBMs
H
IGFL4
Log 2 Ratio
NORMALS
DA II / AA III
GBMs
I
IGF2BP1 / IMP1
Log2 Ratio
NORMALS
DA II /AA III
GBMs
J
IGF2BP2 / IMP2
log 2 Ratio
NORMALS
DA II /AA III
GBMs
K
IGF2BP3 / IMP3
Log 2 Ratio
NORMALS
DA II / AA III
GBMs
L
IGF2AS
log 2 Ratio
NORMALS
DA II / AA III
GBMs

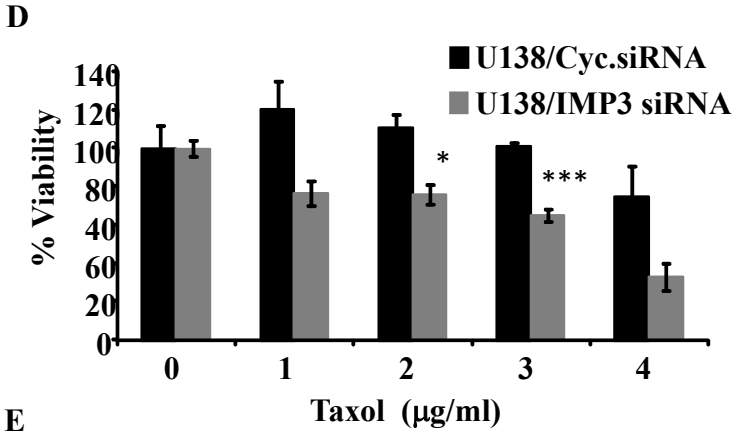

A
IMP3
Actin
1
2
30
25
20
15
10
5
0
Fold change
1
2
H1299/pCEP4
H1299/IMP3
B
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Viability (OD at 550)
H1299/pCEP4
H1299/IMP3
1
2
3
4
5
Days
C
a
b
H1299/pCEP4
H1299/IMP3
D
p = 0.0012
250
200
150
100
50
0
No. of colonies
1
2
H1299/pCEP4
H1299/IMP3
E
p = 0.0357
2.0
1.5
1.0
0.5
0
Invasion Index
1
2
H1299/pCEP4
H1299/IMP3

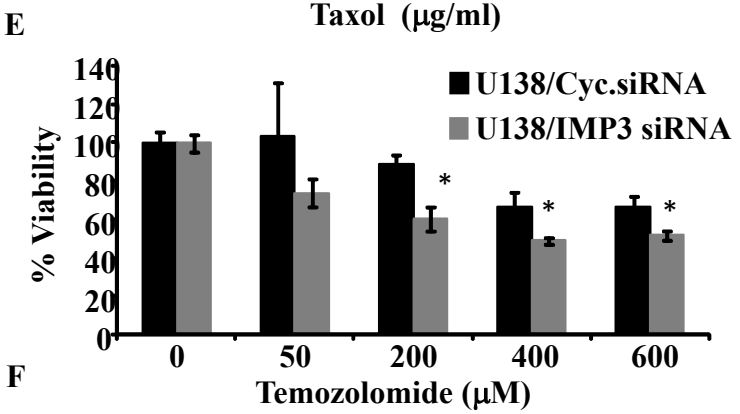

A
HaCaT + Ad GFP
HaCaT + Ad IMP3
Viability (OD at 550)
1.0
0.5
0
1
1.5
2
2.5
3
4
5
6
Days
B
SVG + Ad GFP
SVG + Ad IMP3
Viability (OD at 550)
0.40
0.35
0.30
0.25
0.20
0.15
0.05
0.10
0
1
2
3
4
5
Days
C
H1299 + Ad GFP
H1299 + Ad IMP3
Viability (OD at 550)
3
2
1
0
1
2
3
4
5
6
7
8
Days

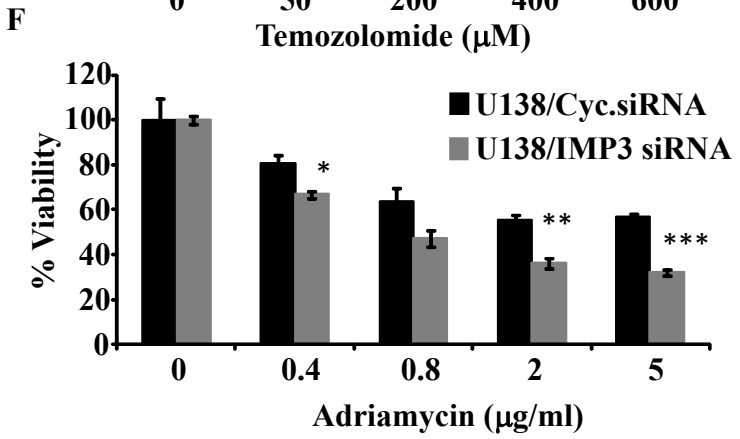

A
Cyclophilin
48
72
96
hrs
Log2 ratio
0.5
0.0
0.5
1.0
1.5
2.0
U138/Mock
U138/Cyc. siRNA
U138/IMP3 siRNA

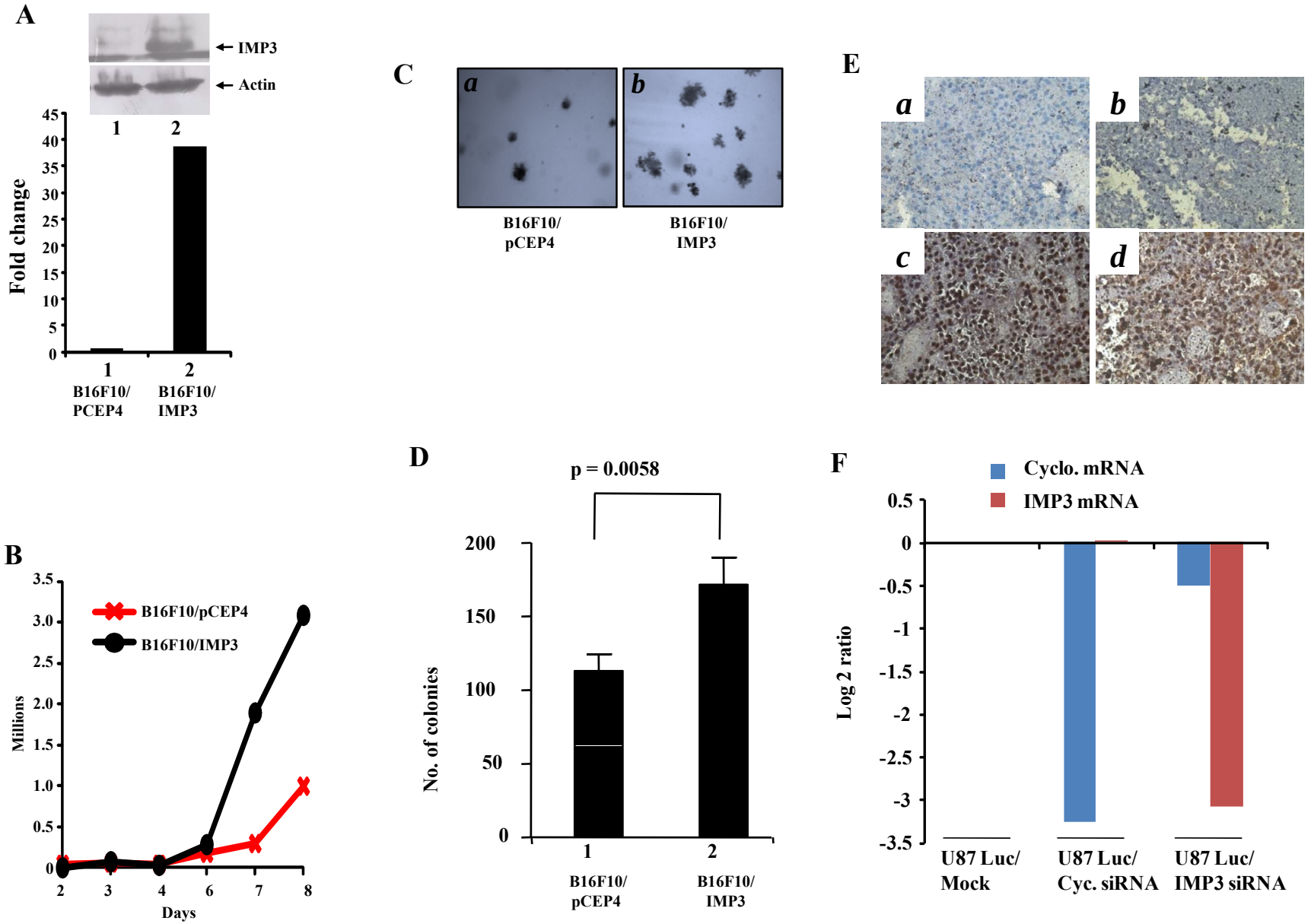

B
IMP3
48
72
96
hrs
Log2 ratio
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
U138/Mock
U138/Cyc. siRNA
U138/IMP3 siRNA


C
a
b
c
U138/
Mock
U138/
Cyc. siRNA
U138/
IMP3 siRNA

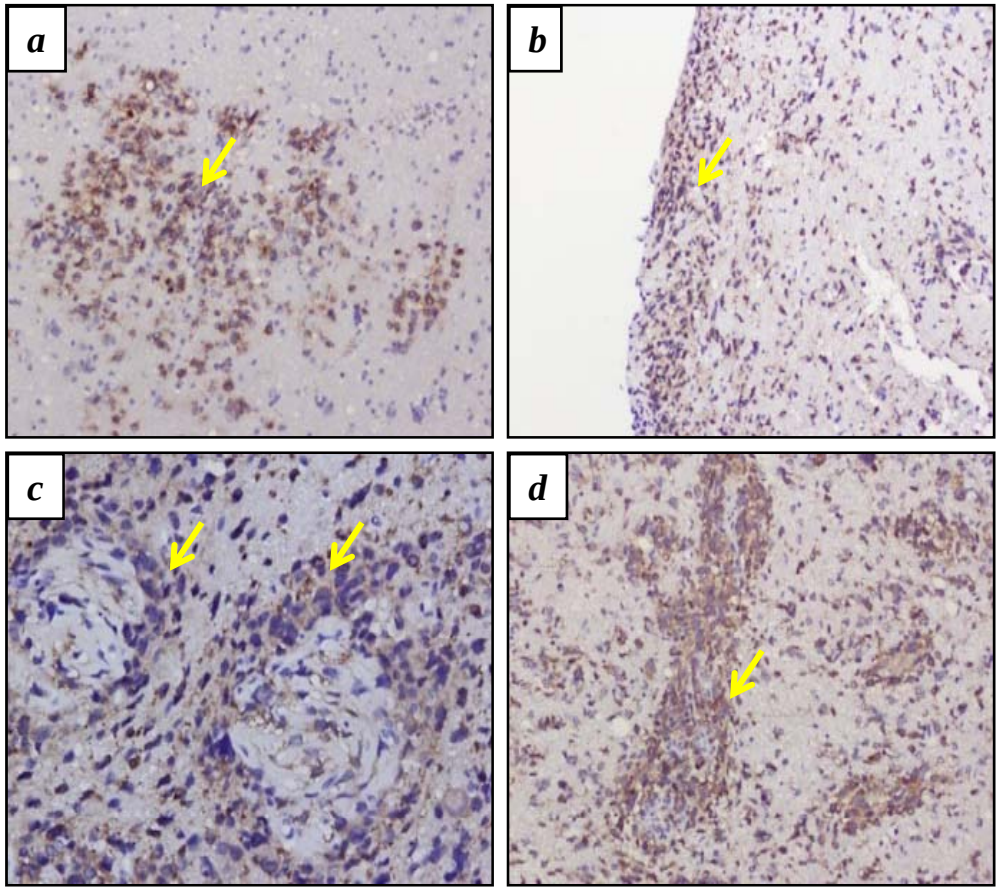

D
U138/Cyc.siRNA
U138/IMP3 siRNA
% Viability
140
120
100
80
60
40
20
0
*
***
0
1
2
3
4
Taxol (µg/ml)


E
U138/Cyc.siRNA
U138/IMP3 siRNA
% Viability
140
120
100
80
60
40
20
0
*
*
*
0
50
200
400
600
Temozolomide (µM)

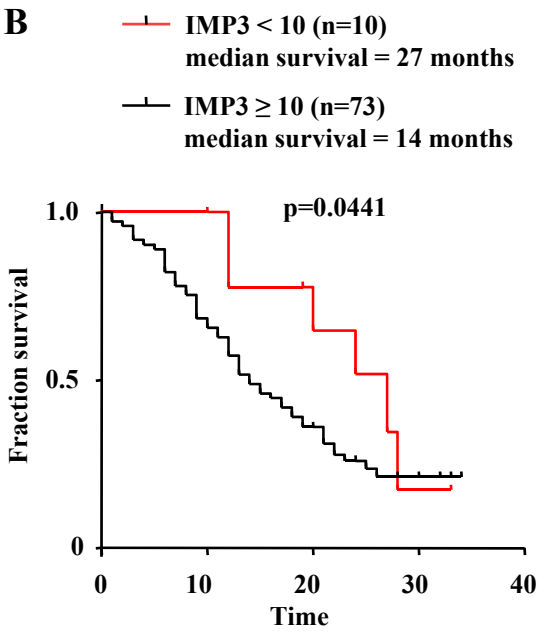

F
U138/Cyc.siRNA
U138/IMP3 siRNA
% Viability
120
100
80
60
40
20
0
*
**
***
0
0.4
0.8
2
5
Adriamycin (µg/ml)

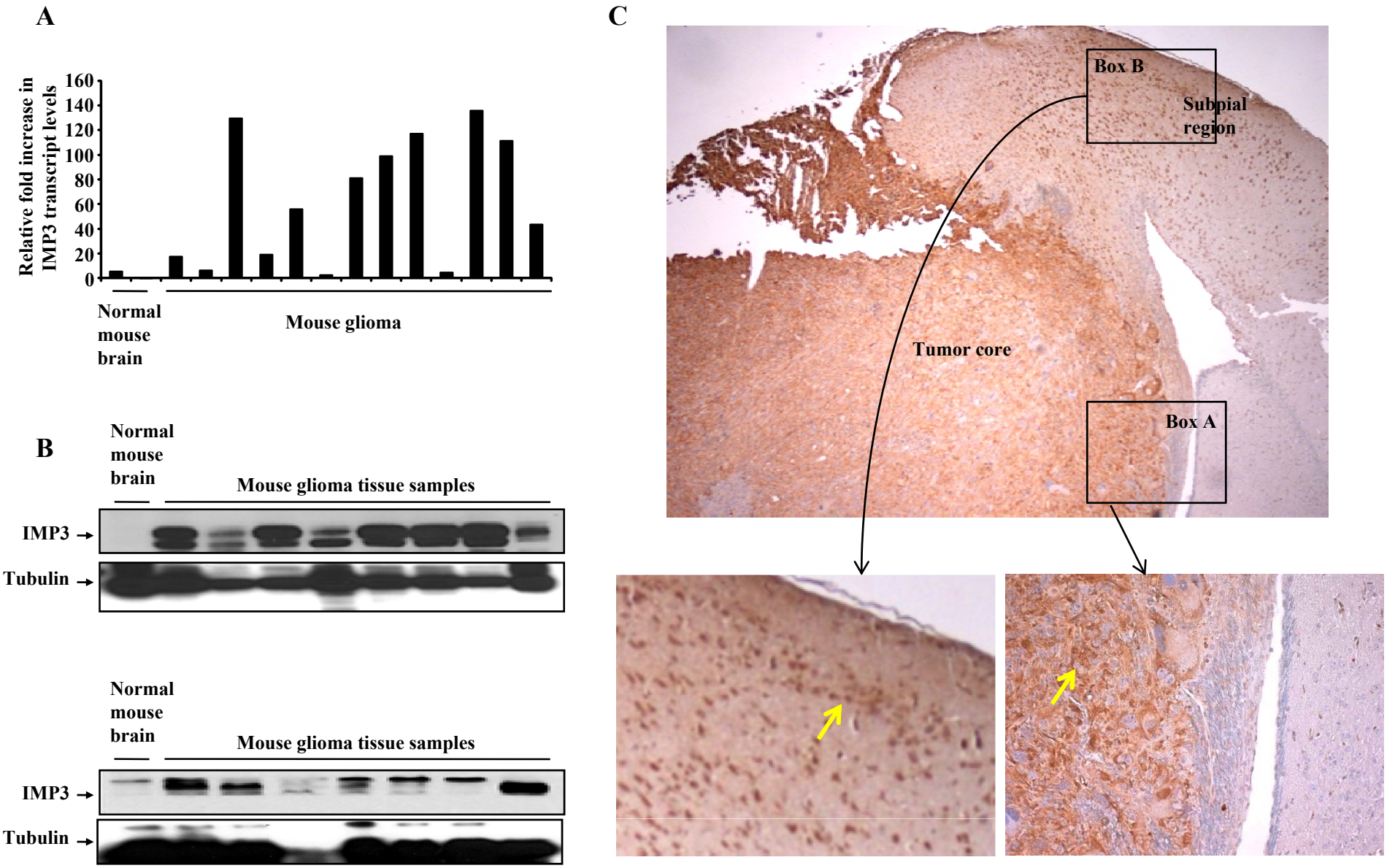

A
IMP3
Actin
1
2
Fold change
45
40
35
30
25
20
15
10
5
0
1
2
B16F10/
PCEP4
B16F10/
IMP3
B
B16F10/pCEP4
B16F10/IMP3
Millions
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
2
3
4
6
7
8
Days
C
a
b
B16F10/
pCEP4
B16F10/
IMP3
D
p = 0.0058
No. of colonies
200
150
100
50
0
1
2
B16F10/
pCEP4
B16F10/
IMP3
E
a
b
c
d
F
Cyclo. mRNA
IMP3 mRNA
Log 2 ratio
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
U87 Luc/
Mock
U87 Luc/
Cyc. siRNA
U87 Luc/
IMP3 siRNA

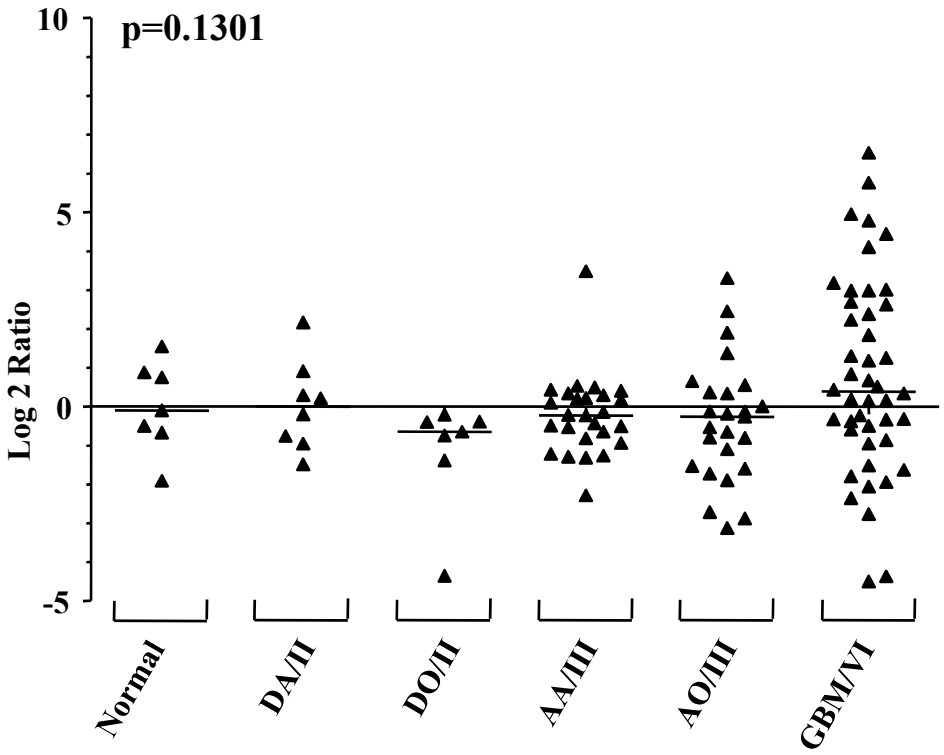
A

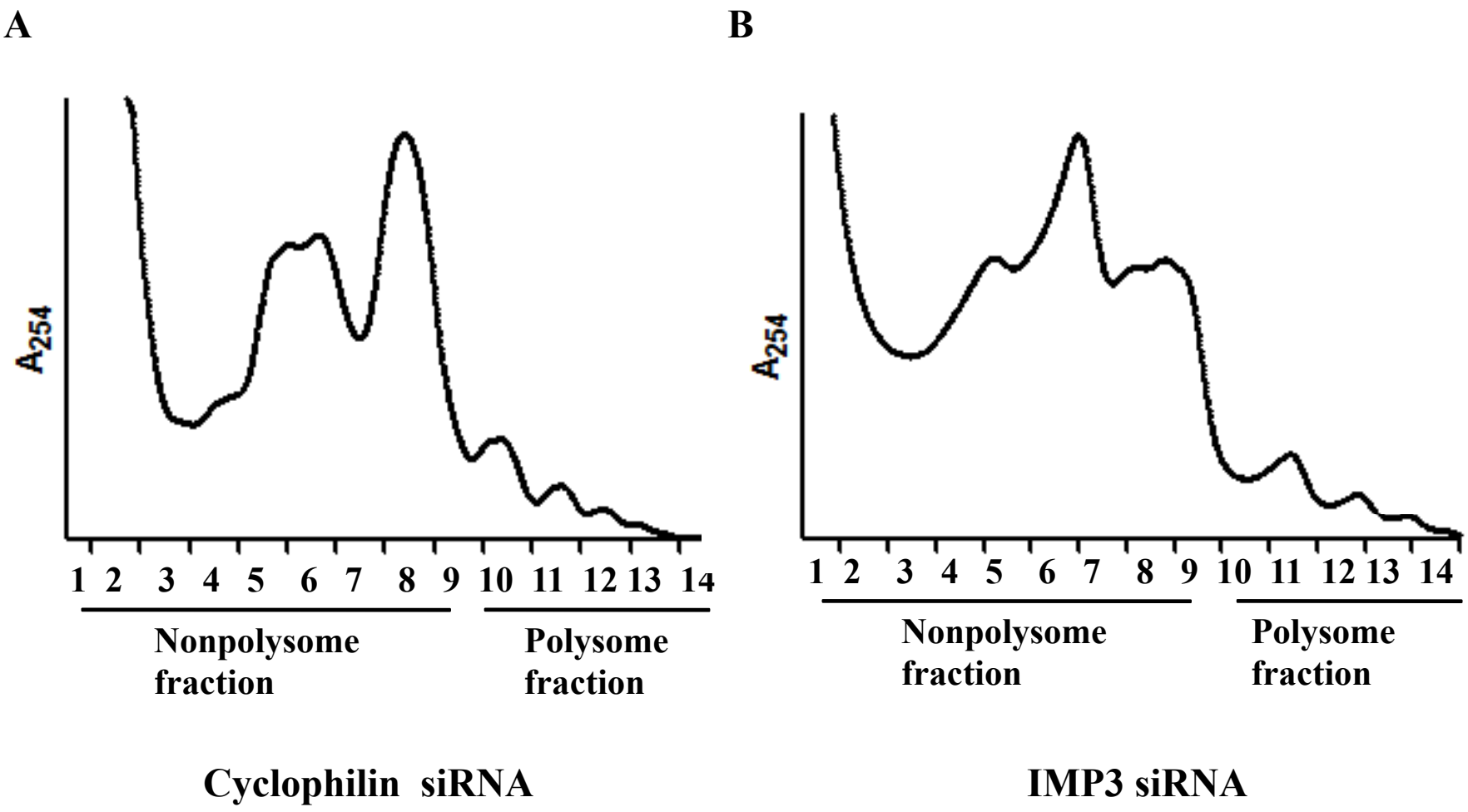

a
b
c
d

B


IMP3 < 10 (n=10)
median survival = 27 months
IMP3 ≥ 10 (n=73)
median survival = 14 months
p=0.0441
Fraction survival
1.0
0.5
0
0
10
20
30
40
Time


A
Relative fold increase in IMP3 transcript levels
160
140
120
100
80
60
40
20
0
Normal mouse brain
Mouse glioma
B
Normal mouse brain
Mouse glioma tissue samples
IMP3
Tubulin
Normal mouse brain
Mouse glioma tissue samples
IMP3
Tubulin
C
Box B
Subpial region
Tumor core
Box A


p=0.1301
10
5
0
-5
Log 2 Ratio
Normal
DA/II
DO/II
AA/III
AO/III
GBM/VI


A
B
A254
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Nonpolysome fraction
Polysome fraction
Cyclophilin siRNA
IMP3 siRNA