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Inhibition of the Wnt/b-catenin pathway using PNU-74654 reduces tumor growth in in vitro and in vivo models of colorectal cancer

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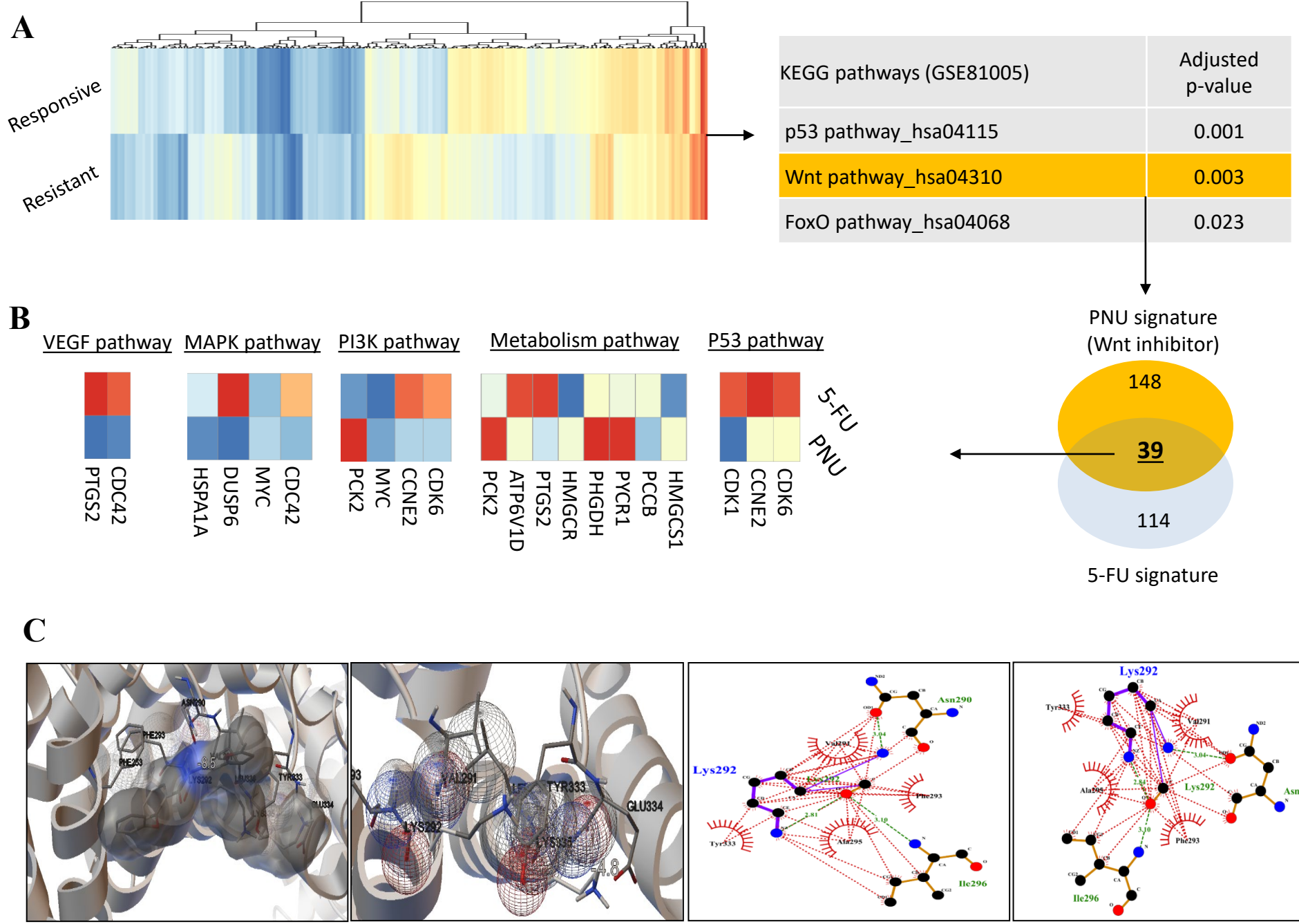

A
Responsive
Resistant
KEGG pathways (GSE81005)
Adjusted p-value
p53 pathway_hsa04115
0.001
Wnt pathway_hsa04310
0.003
FoxO pathway_hsa04068
0.023
B
VEGF pathway
MAPK pathway
PI3K pathway
Metabolism pathway
P53 pathway
PTGS2
CDC42
HSPA1A
DUSP6
MYC
CDC42
PCK2
MYC
CCNE2
CDK6
PCK2
ATP6V1D
PTGS2
HMGCR
PHGDH
PYCR1
PCCB
HMGCS1
CDK1
CCNE2
CDK6
5-FU
PNU
PNU signature
(Wnt inhibitor)
148
39
114
5-FU signature
C
Lys292
Asn290
Phe293
Ile296
Tyr333
Ala295
Val291
GLU334

Fig 1

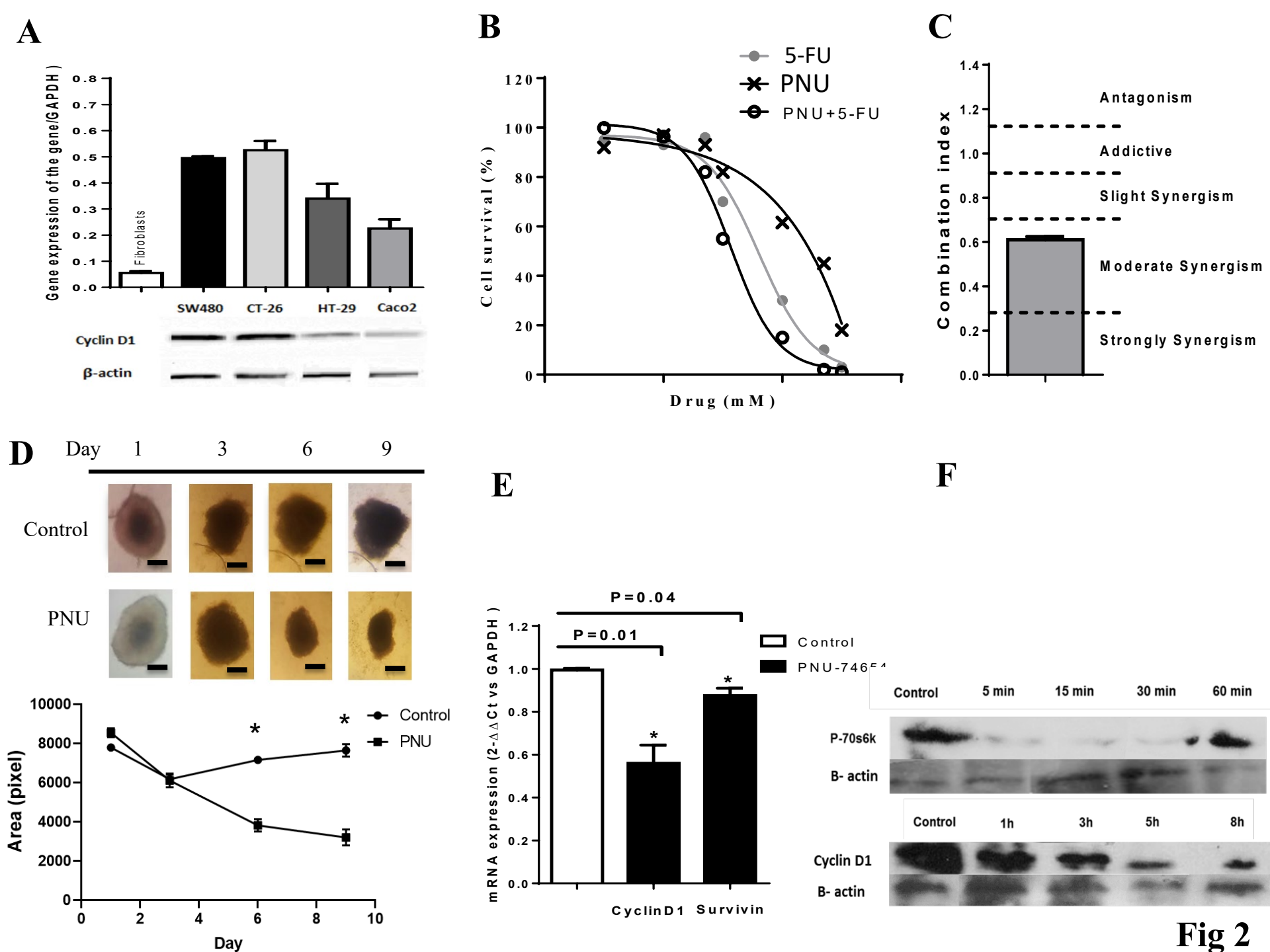

A
Gene expression of the gene/GAPDH)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Fibroblasts
SW480
CT-26
HT-29
Caco2
Cyclin D1
β-actin
B
5-FU
PNU
PNU+5-FU
Cell survival (%)
120
100
80
60
40
20
0
Drug (mM)
C
Combination index
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Antagonism
Addictive
Slight Synergism
Moderate Synergism
Strongly Synergism
D
Day 1 3 6 9
Control
PNU
Area (pixel)
10000
8000
6000
4000
2000
0
Control
PNU
Day
E
P=0.04
P=0.01
mRNA expression (2-ΔΔCt vs GAPDH)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Control
PNU-74654
CyclinD1
Survivin
F
Control
5 min
15 min
30 min
60 min
P-70s6k
B- actin
Control
1h
3h
5h
8h
Cyclin D1
B- actin

Fig 2

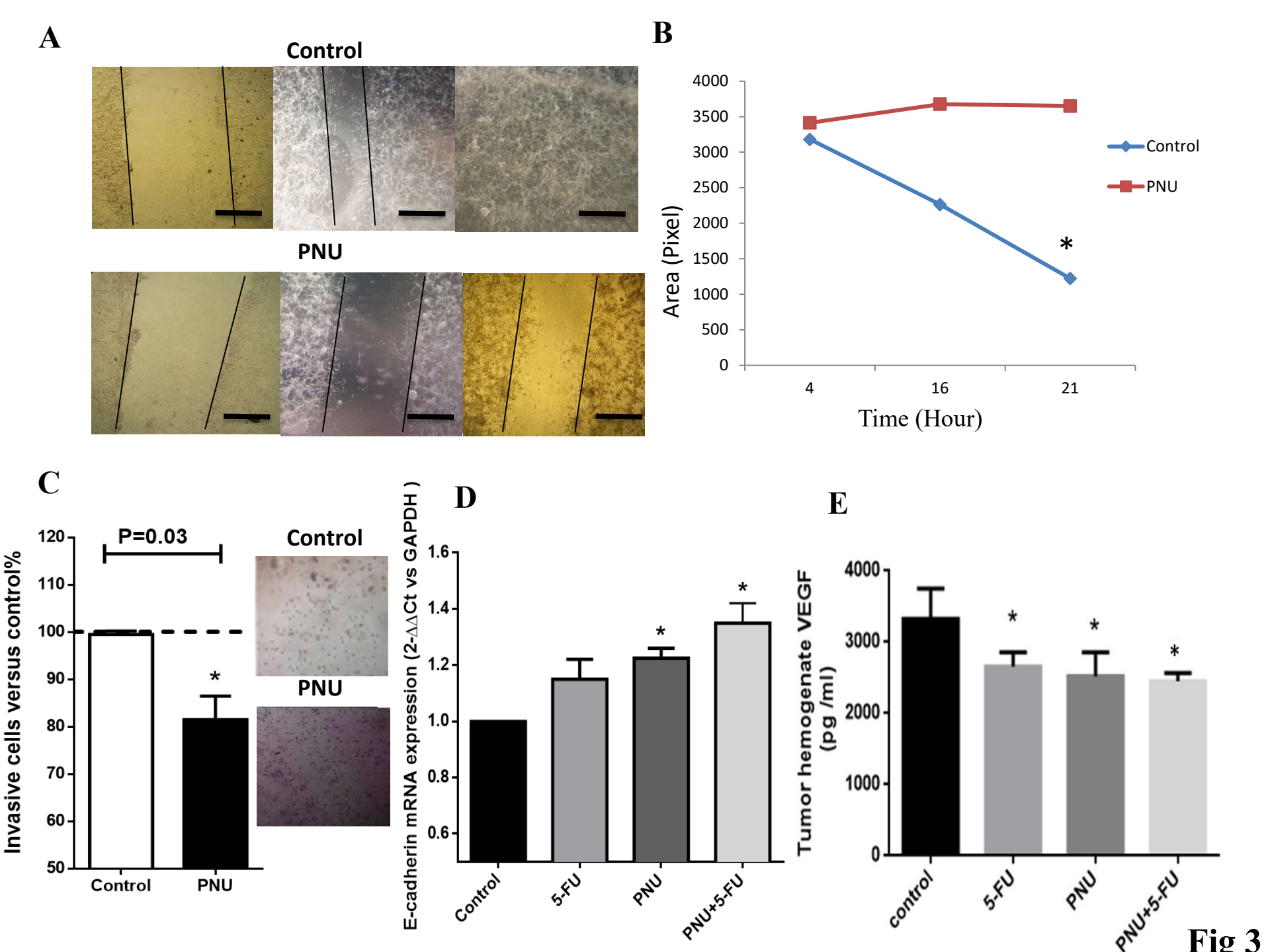
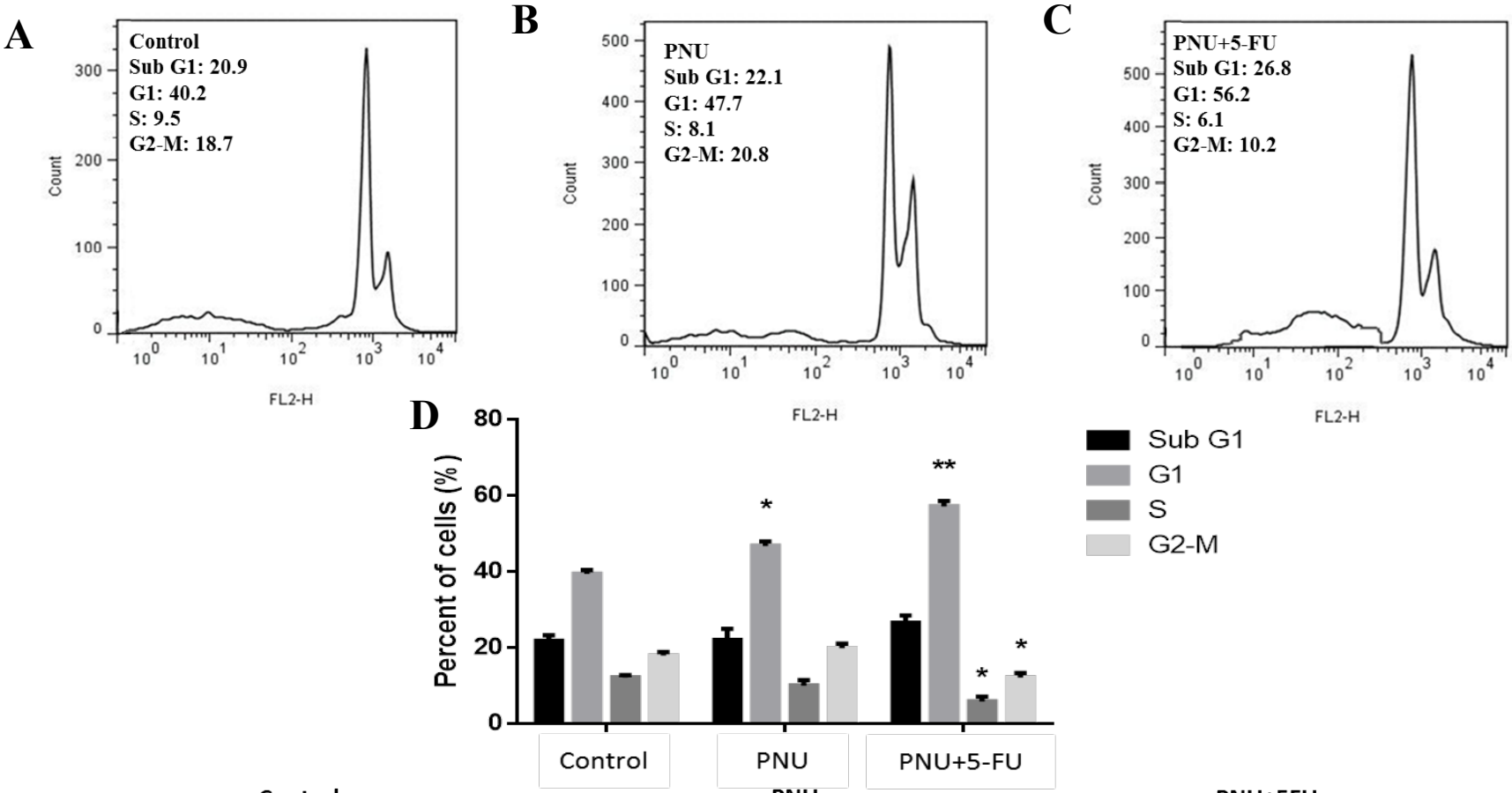

A
Control
PNU
B
Area (Pixel)
4000
3500
3000
2500
2000
1500
1000
500
0
4
16
21
Time (Hour)
Control
PNU
*
C
P=0.03
Invasive cells versus control%
120
110
100
90
80
70
60
50
Control
PNU
*
Control
PNU
D
E-cadherin mRNA expression (2-ΔΔCt vs GAPDH)
1.6
1.4
1.2
1.0
0.8
0.6
Control
5-FU
PNU
PNU+5-FU
E
Tumor hemogenate VEGF (pg /ml)
4000
3000
2000
1000
0
control
5-FU
PNU
PNU+5-FU

Fig 3


A
Control
Sub G1: 20.9
G1: 40.2
S: 9.5
G2-M: 18.7
Count
FL2-H
B
PNU
Sub G1: 22.1
G1: 47.7
S: 8.1
G2-M: 20.8
Count
FL2-H
C
PNU+5-FU
Sub G1: 26.8
G1: 56.2
S: 6.1
G2-M: 10.2
Count
FL2-H
D
Percent of cells (%)
Sub G1
G1
S
G2-M
Control
PNU
PNU+5-FU

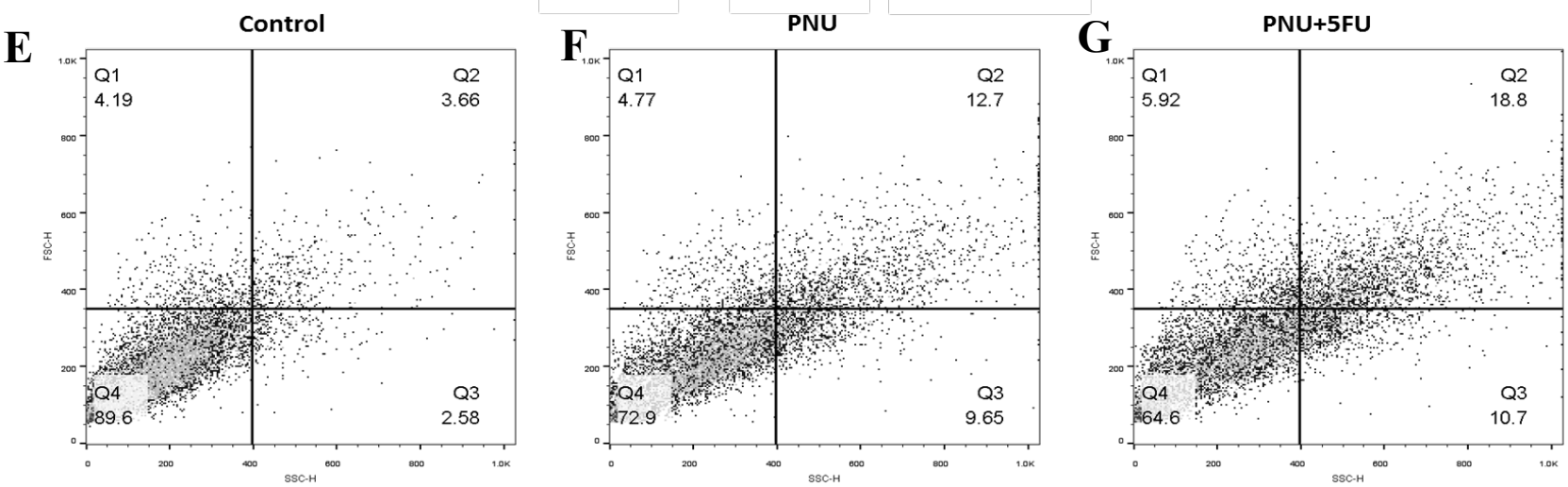

E
Control
Q1
4.19
Q2
3.66
Q4
89.6
Q3
2.58
F
PNU
Q1
4.77
Q2
12.7
Q4
72.9
Q3
9.65
G
PNU+5FU
Q1
5.92
Q2
18.8
Q4
64.6
Q3
10.7
FSC-H
SSC-H

Fig 4

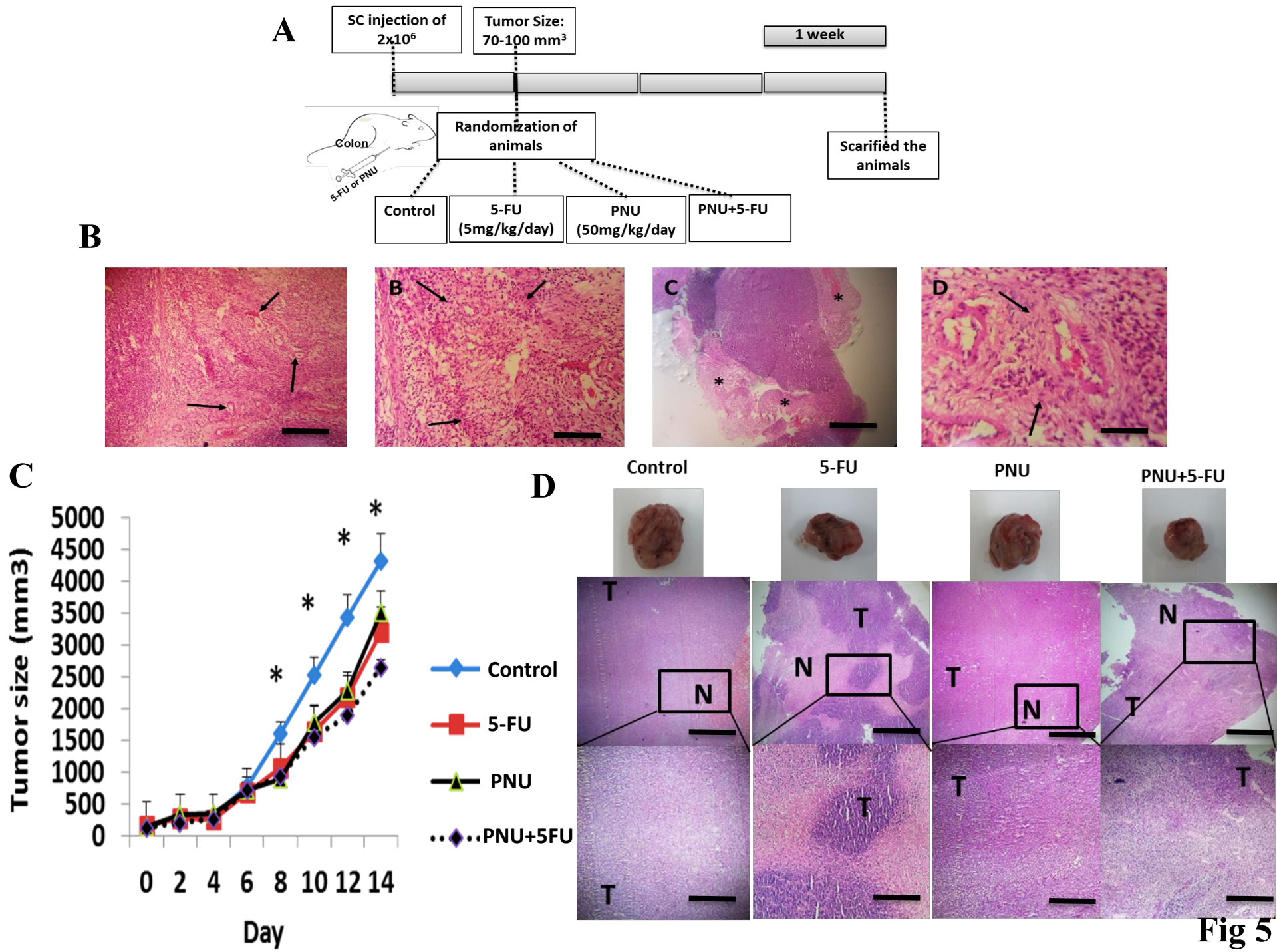

A
SC injection of 2x10⁶
Tumor Size: 70-100 mm³
1 week
Colon
5-FU or PNU
Randomization of animals
Scarified the animals
Control
5-FU (5mg/kg/day)
PNU (50mg/kg/day
PNU+5-FU
B
B
C
D
C
Tumor size (mm3)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0 2 4 6 8 10 12 14
Day
Control
5-FU
PNU
PNU+5FU
D
Control
5-FU
PNU
PNU+5-FU
T
N

Fig 5

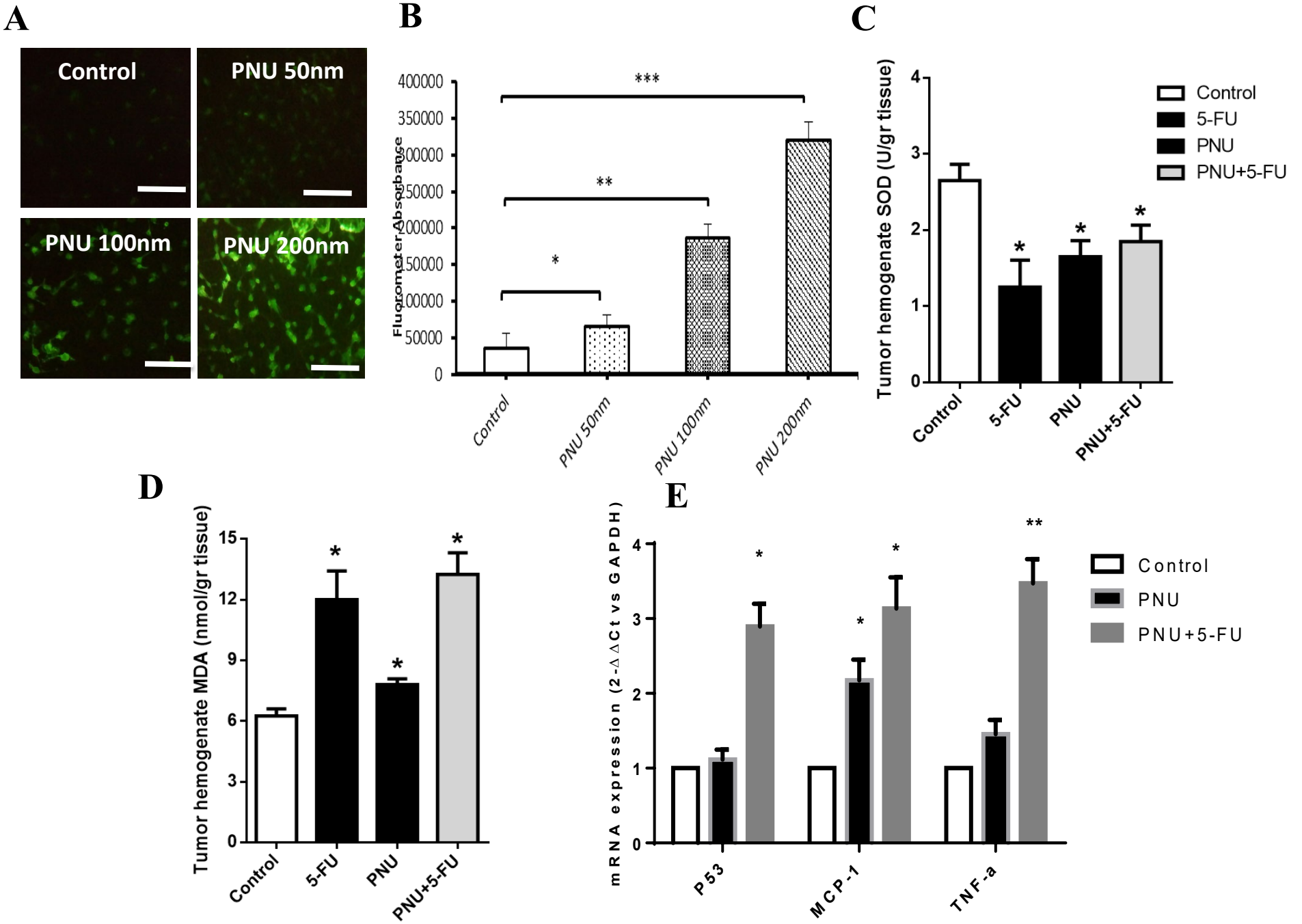

A
Control
PNU 50nm
PNU 100nm
PNU 200nm
B
Fluorometer Absorbance
400000
350000
300000
250000
200000
150000
100000
50000
0
***
**
*
Control
PNU 50nm
PNU 100nm
PNU 200nm
C
Tumor hemogenate SOD (U/gr tissue)
4
3
2
1
0
Control
5-FU
PNU
PNU+5-FU
*
*
*
Control
5-FU
PNU
PNU+5-FU
D
Tumor hemogenate MDA (nmol/gr tissue)
15
12
9
6
3
0
*
*
*
Control
5-FU
PNU
PNU+5-FU
E
mRNA expression (2-ΔΔCt vs GAPDH)
4
3
2
1
0
*
*
*
**
Control
PNU
PNU+5-FU
P53
MCP-1
TNF-a

Fig 6

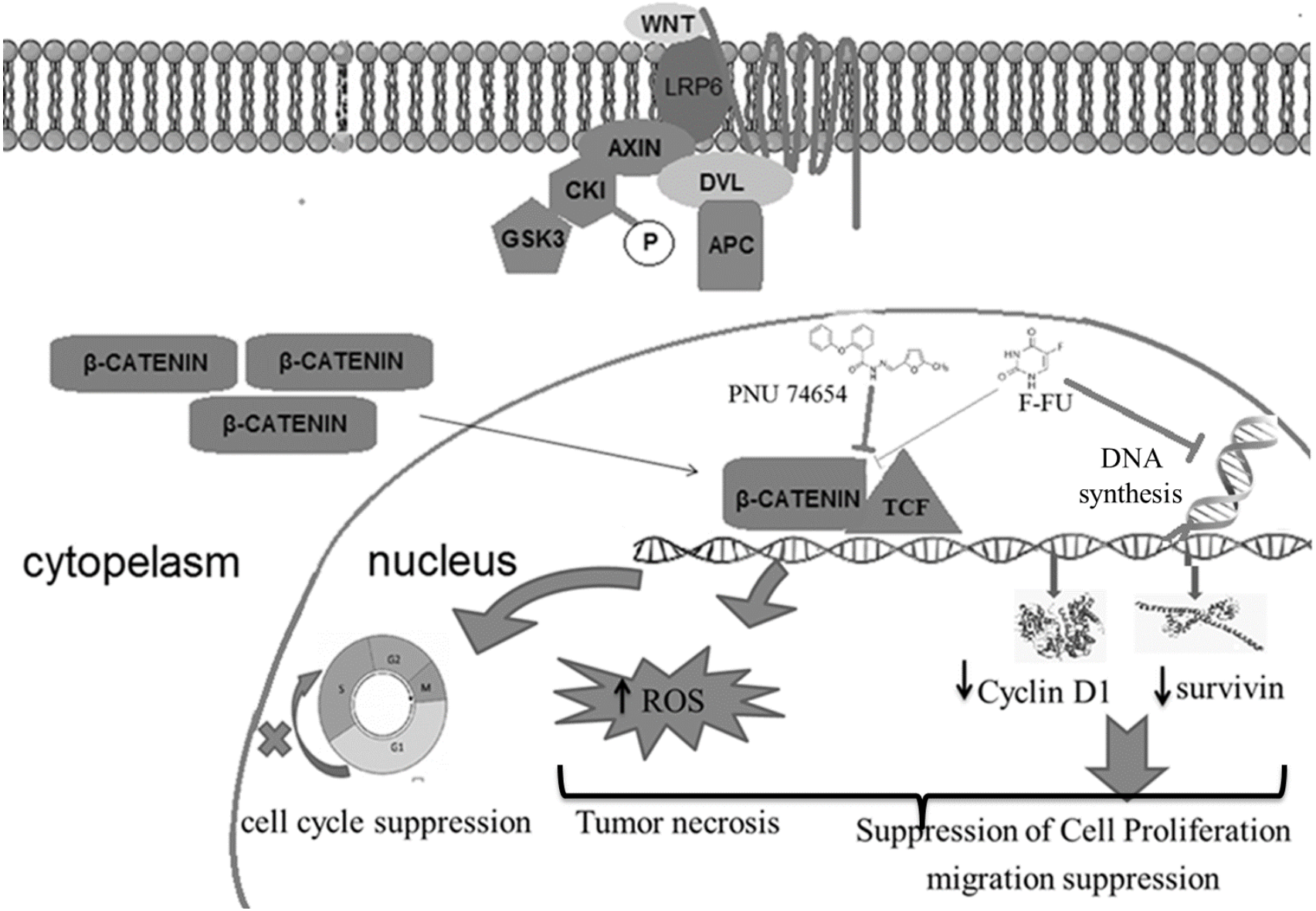

WNT
LRP6
AXIN
CKI
DVL
GSK3
P
APC
β-CATENIN
β-CATENIN
β-CATENIN
PNU 74654
F-FU
DNA
synthesis
β-CATENIN
TCF
cytopelasm
nucleus
G2
S
M
G1
ROS
Cyclin D1
survivin
cell cycle suppression
Tumor necrosis
Suppression of Cell Proliferation
migration suppression

Fig 7