FIG. 1

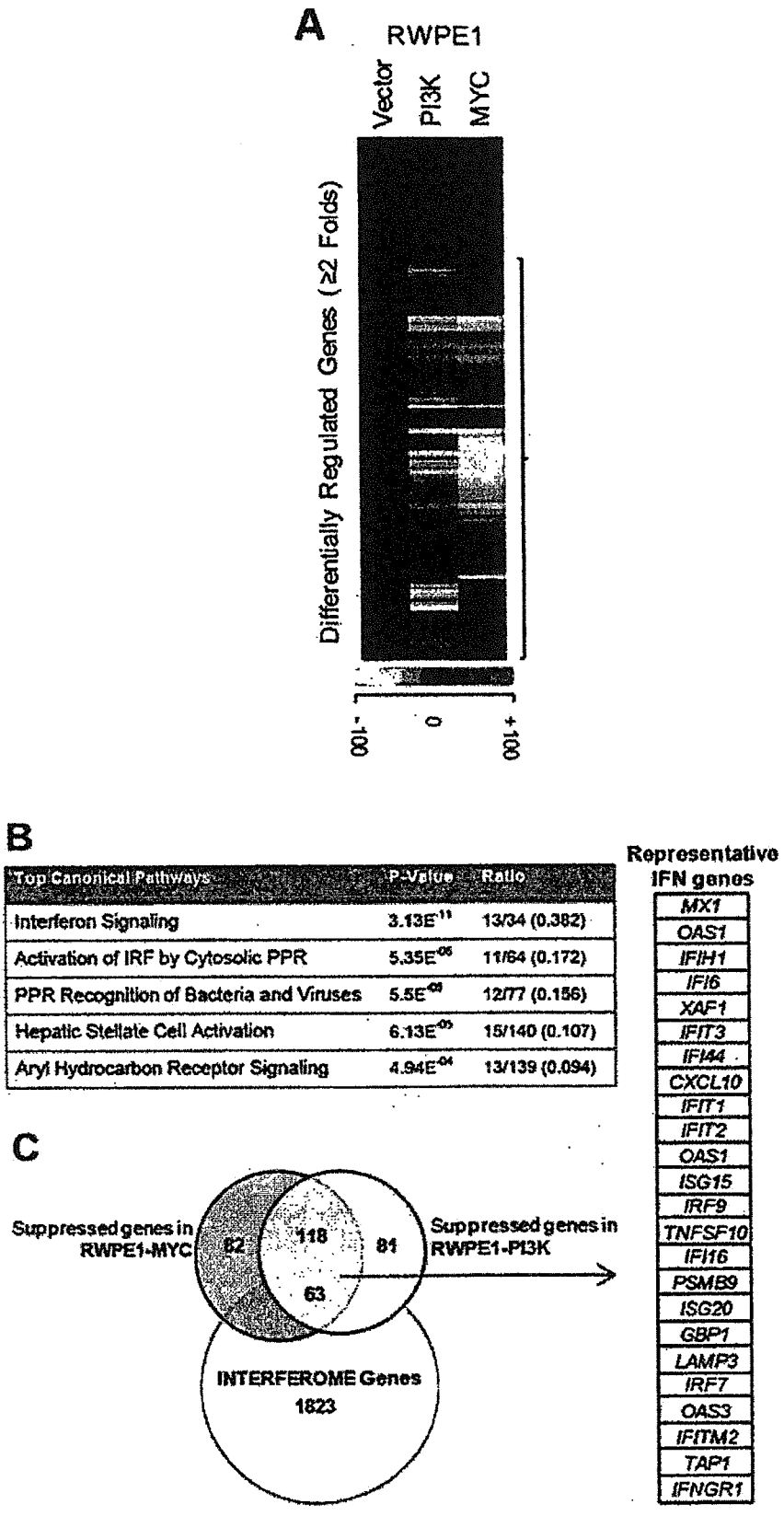

A
RWPE1
Vector
PI3K
MYC
Differentially Regulated Genes (≥2 Folds)
-100
0
+100
B
Top Canonical Pathways
P-Value
Ratio
Interferon Signaling
3.13E-11
13/34 (0.382)
Activation of IRF by Cytosolic PPR
5.35E-06
11/64 (0.172)
PPR Recognition of Bacteria and Viruses
5.5E-05
12/77 (0.156)
Hepatic Stellate Cell Activation
6.13E-05
15/140 (0.107)
Aryl Hydrocarbon Receptor Signaling
4.94E-04
13/139 (0.094)
Representative IFN genes
MX1
OAS1
IFIH1
IFI6
XAF1
IFIT3
IFI44
CXCL10
IFIT1
IFIT2
OAS1
ISG15
IRF9
TNFSF10
IFI16
PSMB9
ISG20
GBP1
LAMP3
IRF7
OAS3
IFITM2
TAP1
IFNGR1
C
Suppressed genes in RWPE1-MYC
82
118
81
Suppressed genes in RWPE1-PI3K
63
INTERFEROME Genes
1823

FIG. 1 (Cont'd)

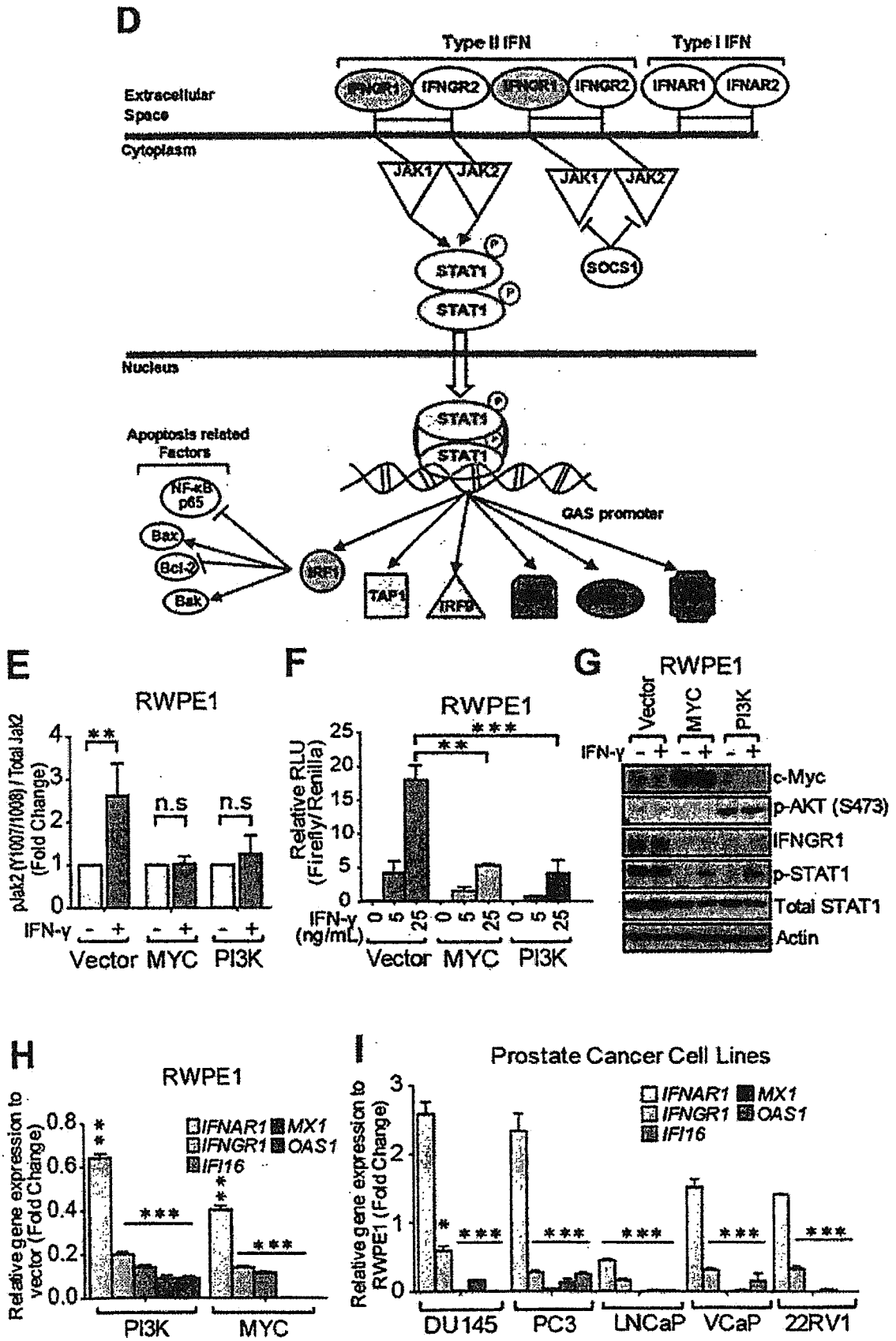

D
Type II IFN
Type I IFN
IFNGR1
IFNGR2
IFNGR1
IFNGR2
IFNAR1
IFNAR2
Extracellular Space
Cytoplasm
JAK1
JAK2
JAK1
JAK2
STAT1
STAT1
SOCS1
Nucleus
STAT1
STAT1
Apoptosis related Factors
NF-κB p65
Bax
Bcl-2
Bak
IRF1
GAS promoter
TAP1
IRF9
E
RWPE1
pJak2 (Y1007/1008) / Total Jak2 (Fold Change)
n.s
n.s
IFN-γ
Vector MYC PI3K
F
RWPE1
Relative RLU (Firefly/Renilla)
IFN-γ (ng/mL)
Vector MYC PI3K
G
RWPE1
Vector
MYC
PI3K
IFN-γ
c-Myc
p-AKT (S473)
IFNGR1
p-STAT1
Total STAT1
Actin
H
RWPE1
Relative gene expression to vector (Fold Change)
IFNAR1 MX1
IFNGR1 OAS1
IFI16
PI3K
MYC
I
Prostate Cancer Cell Lines
Relative gene expression to RWPE1 (Fold Change)
IFNAR1 MX1
IFNGR1 OAS1
IFI16
DU145
PC3
LNCaP
VCaP
22RV1

FIG. 2

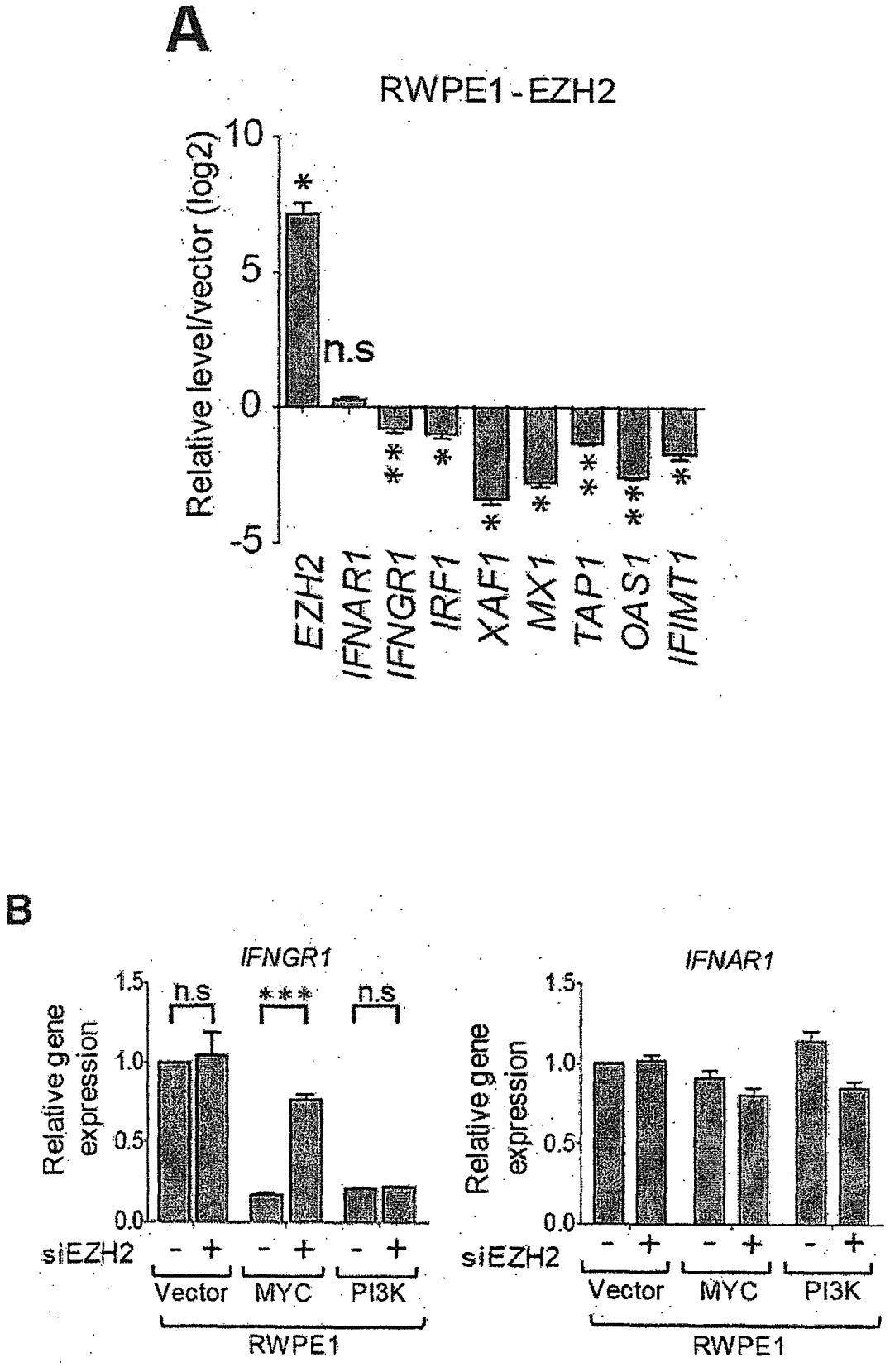
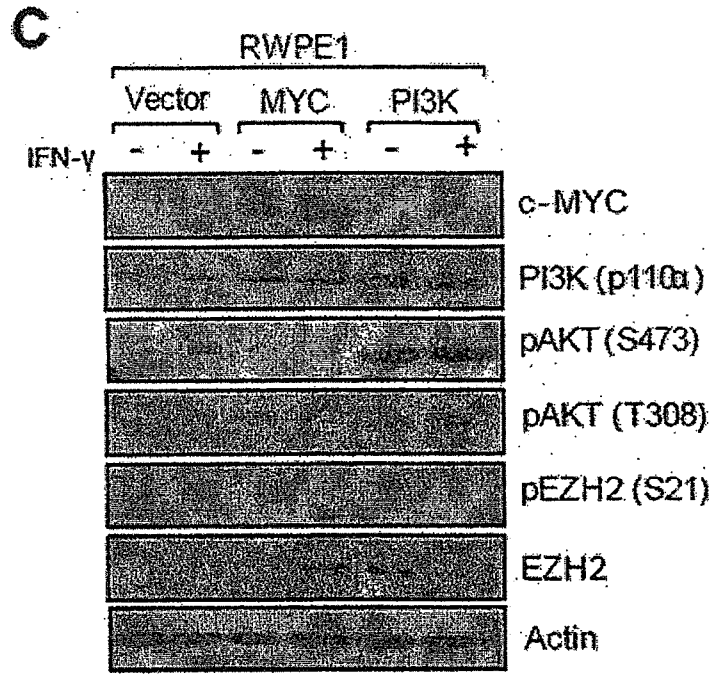
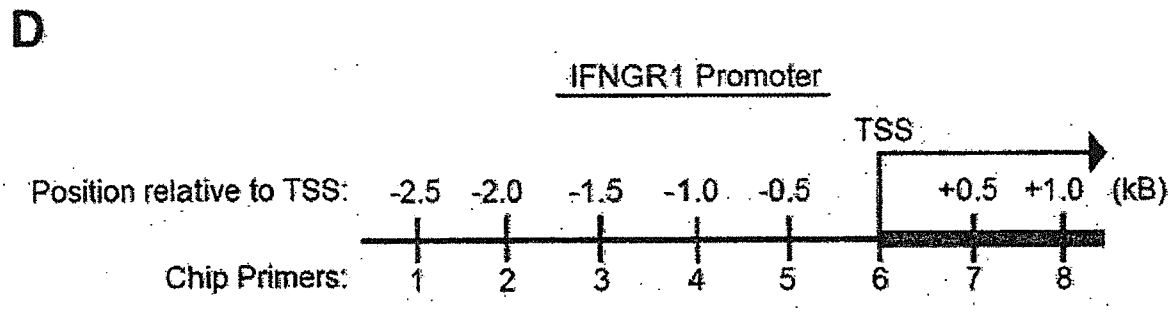

A
RWPE1-EZH2
Relative level/vector (log2)
10
5
0
-5
n.s
EZH2
IFNAR1
IFNGR1
IRF1
XAF1
MX1
TAP1
OAS1
IFIMT1
B
IFNGR1
IFNAR1
Relative gene expression
1.5
1.0
0.5
0.0
n.s
***
n.s
siEZH2
- + - + - +
Vector
MYC
PI3K
RWPE1

FIG. 2


C
RWPE1
Vector
MYC
PI3K
IFN-γ
- + - + - +
c-MYC
PI3K (p110α)
pAKT (S473)
pAKT (T308)
pEZH2 (S21)
EZH2
Actin


D
IFNGR1 Promoter
TSS
Position relative to TSS:
-2.5 -2.0 -1.5 -1.0 -0.5 +0.5 +1.0 (kB)
Chip Primers:
1 2 3 4 5 6 7 8

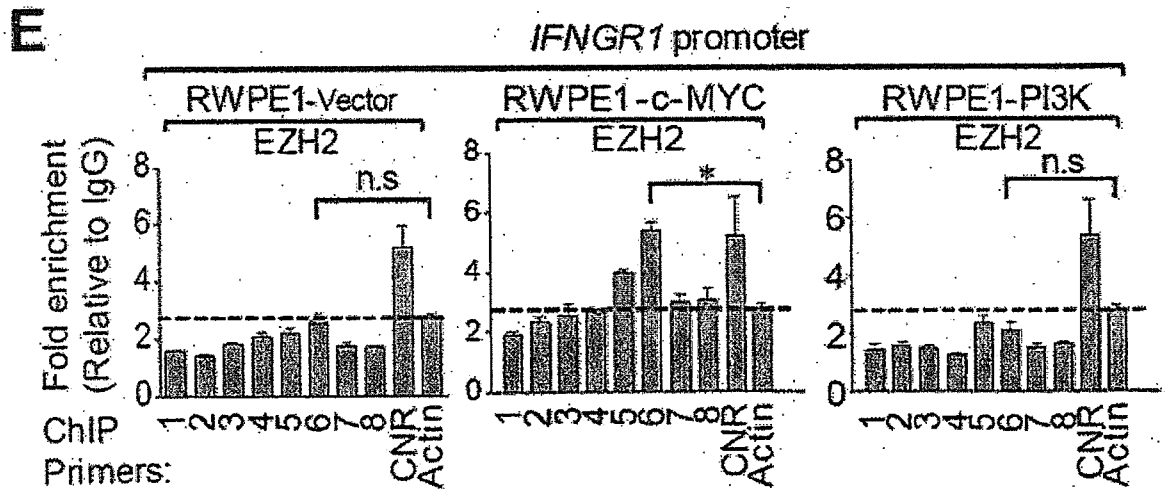
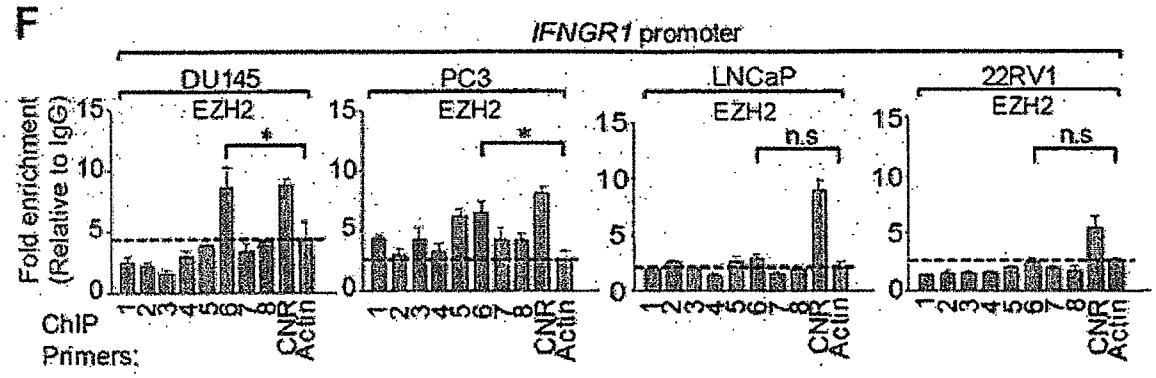

E
IFNGR1 promoter
RWPE1-Vector
RWPE1-c-MYC
RWPE1-PI3K
EZH2
n.s
*
Fold enrichment (Relative to IgG)
ChIP Primers:
1 2 3 4 5 6 7 8 CNR Actin

FIG. 2 (Cont'd)


F
IFNGR1 promoter
DU145
PC3
LNCaP
22RV1
EZH2
Fold enrichment (Relative to IgG)
15
10
5
0
*
n.s
1 2 3 4 5 6 7 8 CNR Actin
ChIP Primers:

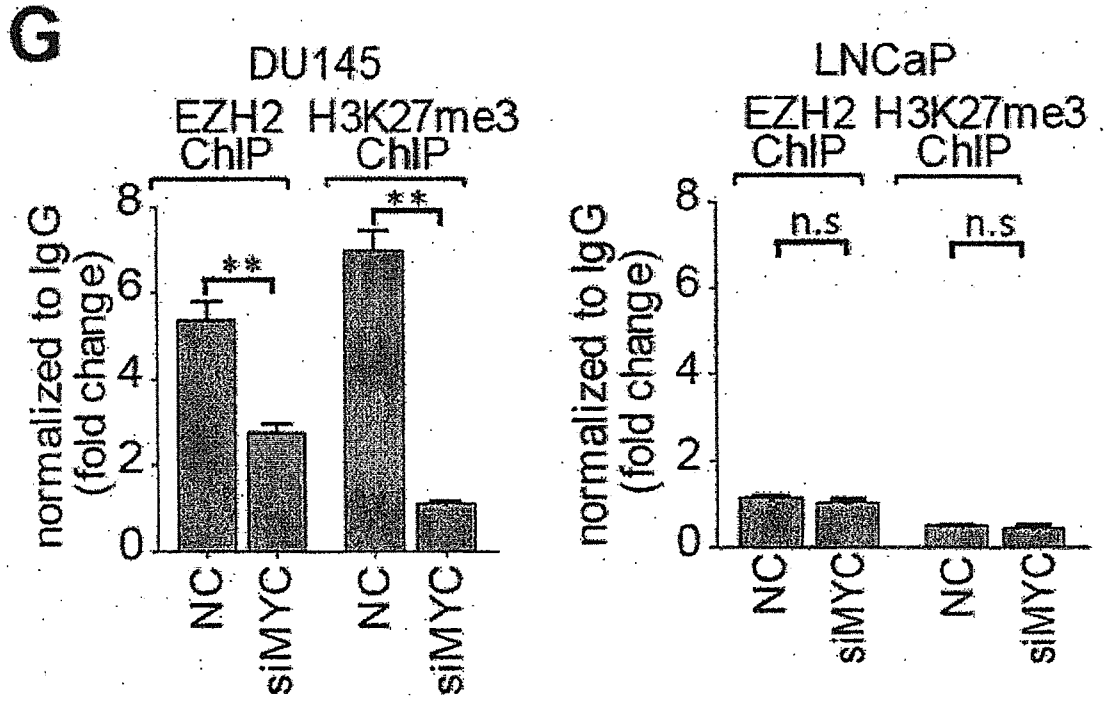

G
DU145
LNCaP
EZH2 ChIP
H3K27me3 ChIP
normalized to IgG (fold change)
8
6
4
2
0
**
n.s
NC
siMYC

FIG. 3

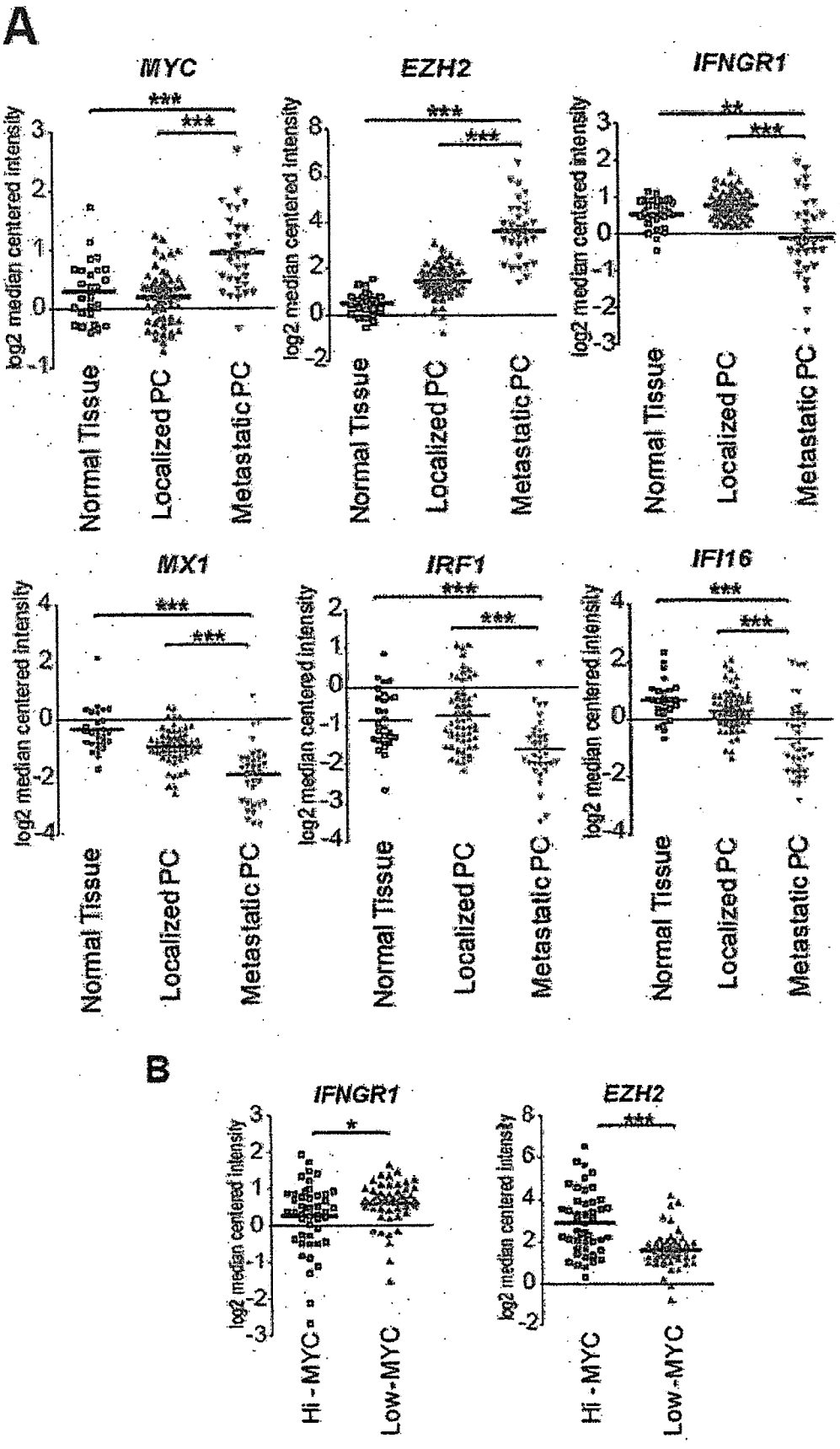

A
MYC
EZH2
IFNGR1
MX1
IRF1
IFI16
log2 median centered intensity
Normal Tissue
Localized PC
Metastatic PC
B
IFNGR1
EZH2
Hi -MYC
Low-MYC

FIG. 3 (cont'd)

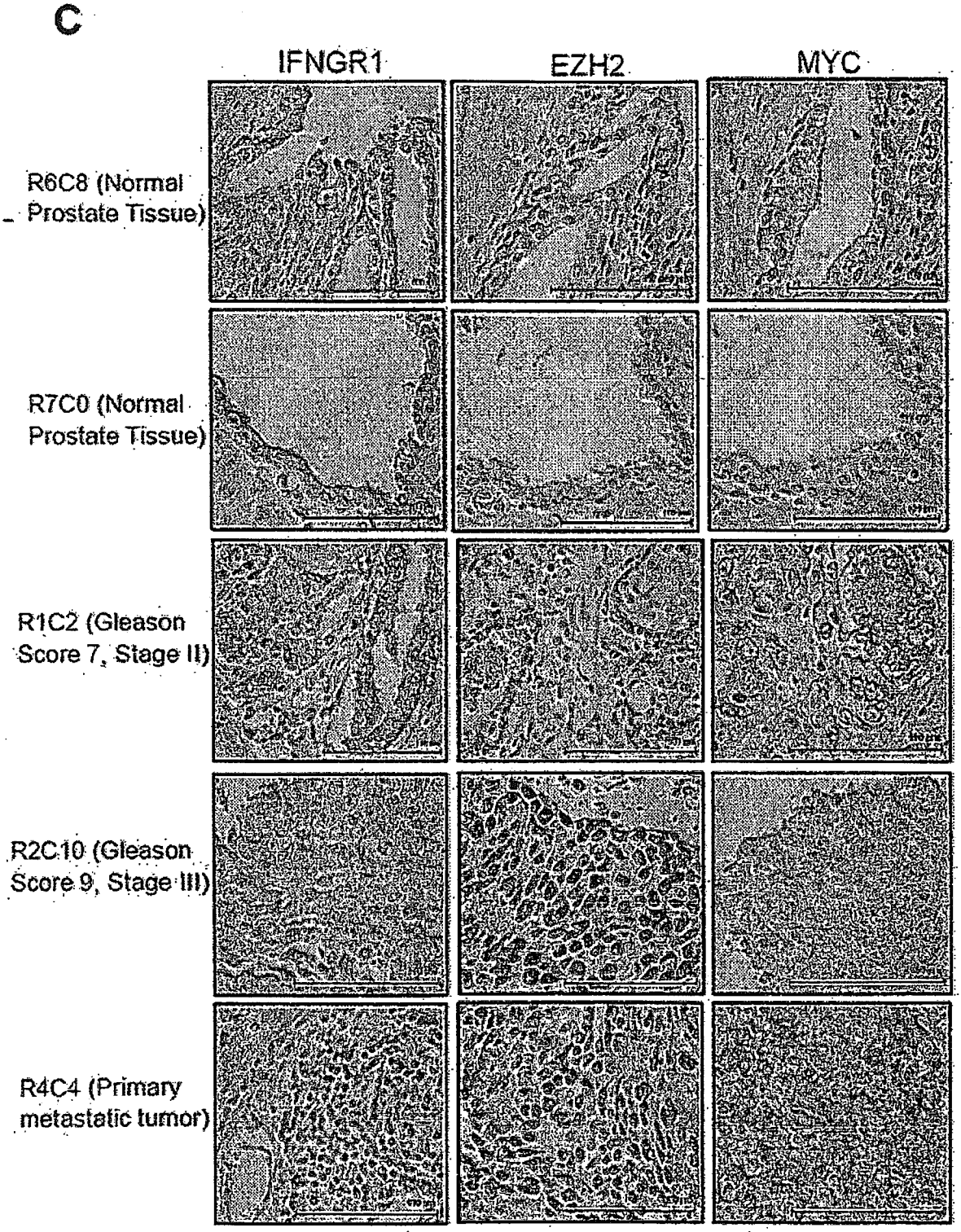

C
IFNGR1
EZH2
MYC
R6C8 (Normal Prostate Tissue)
R7C0 (Normal Prostate Tissue)
R1C2 (Gleason Score 7, Stage II)
R2C10 (Gleason Score 9, Stage III)
R4C4 (Primary metastatic tumor)

FIG. 4

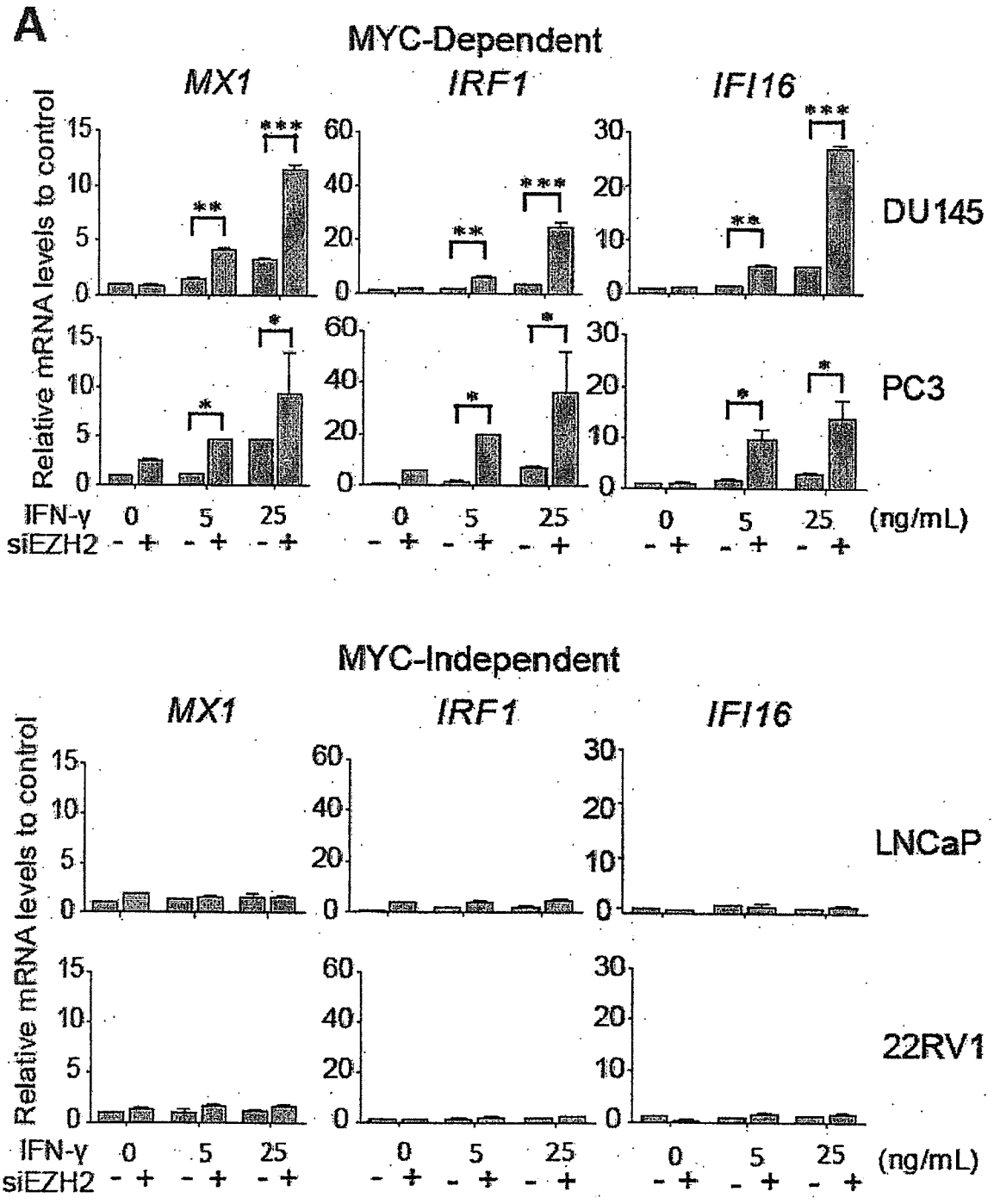

A
MYC-Dependent
MX1
IRF1
IFI16
Relative mRNA levels to control
DU145
PC3
IFN-γ 0 5 25 (ng/mL)
siEZH2 - + - + - +
MYC-Independent
MX1
IRF1
IFI16
Relative mRNA levels to control
LNCaP
22RV1
IFN-γ 0 5 25 (ng/mL)
siEZH2 - + - + - +

FIG. 4 (Cont'd)

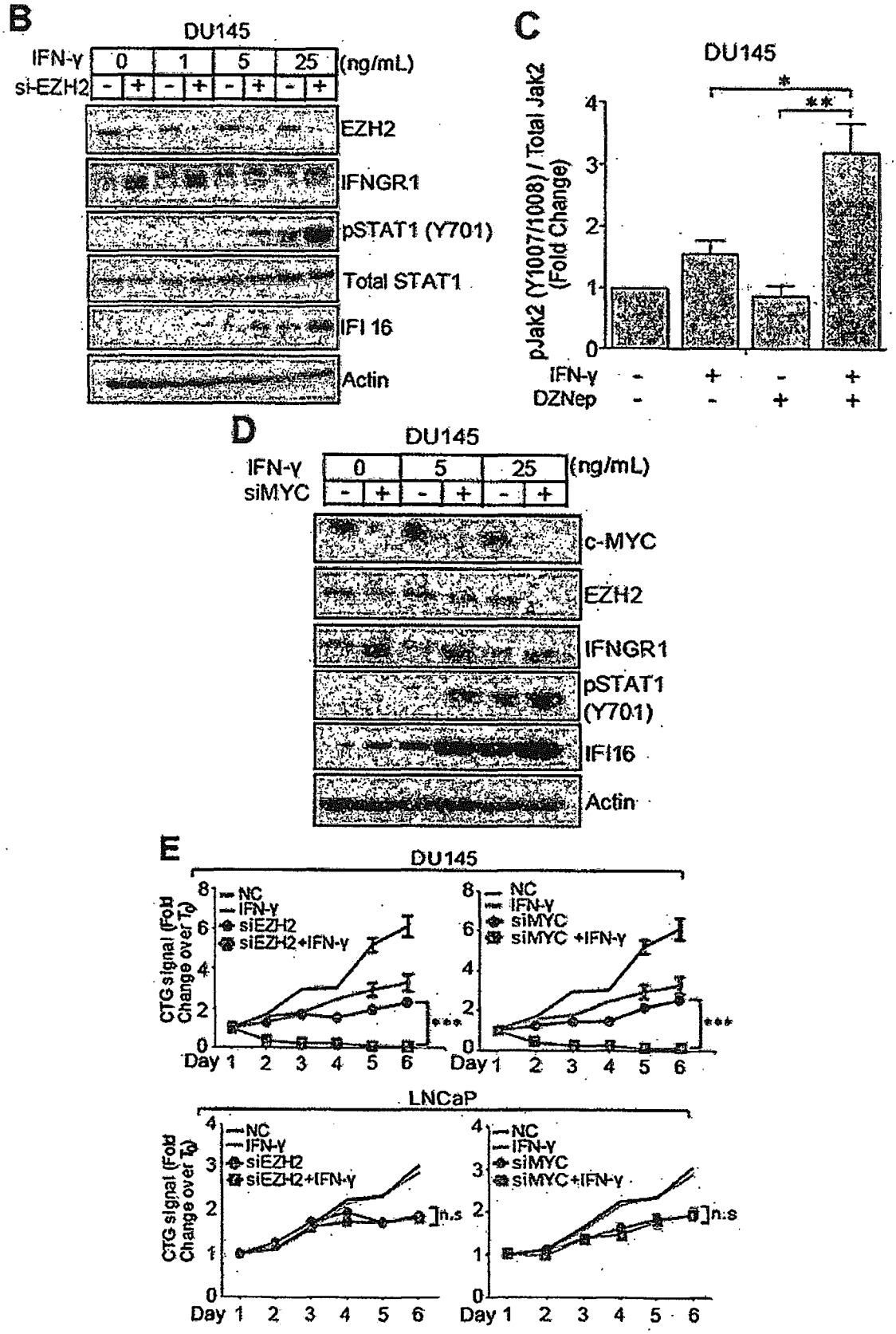
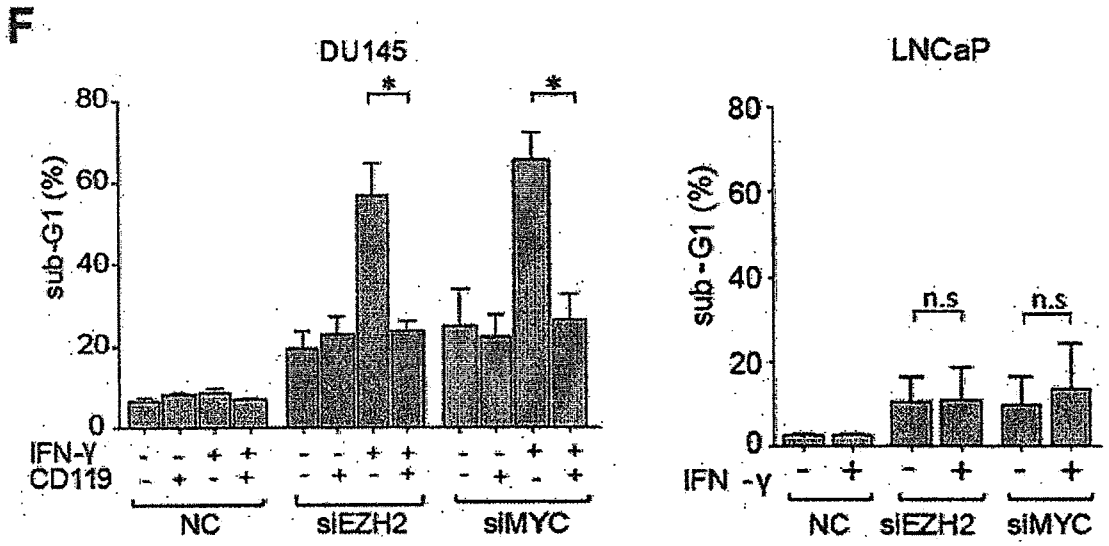

B
DU145
IFN-γ 0 1 5 25 (ng/mL)
si-EZH2 - + - + - + - +
EZH2
IFNGR1
pSTAT1 (Y701)
Total STAT1
IFI 16
Actin
C
DU145
pJak2 (Y1007/1008) / Total Jak2 (Fold Change)
*
**
IFN-γ - + - +
DZNep - - + +
D
DU145
IFN-γ 0 5 25 (ng/mL)
siMYC - + - + - +
c-MYC
EZH2
IFNGR1
pSTAT1 (Y701)
IFI16
Actin
E
DU145
CTG signal (Fold Change over T0)
NC
IFN-γ
siEZH2
siEZH2+IFN-γ
siMYC
siMYC +IFN-γ
***
Day 1 2 3 4 5 6
LNCaP
n.s

FIG. 4 (cont'd)


F
DU145
LNCaP
sub-G1 (%)
80
60
40
20
0
*
*
n.s
n.s
IFN-γ
CD119
IFN -γ
NC
siEZH2
siMYC

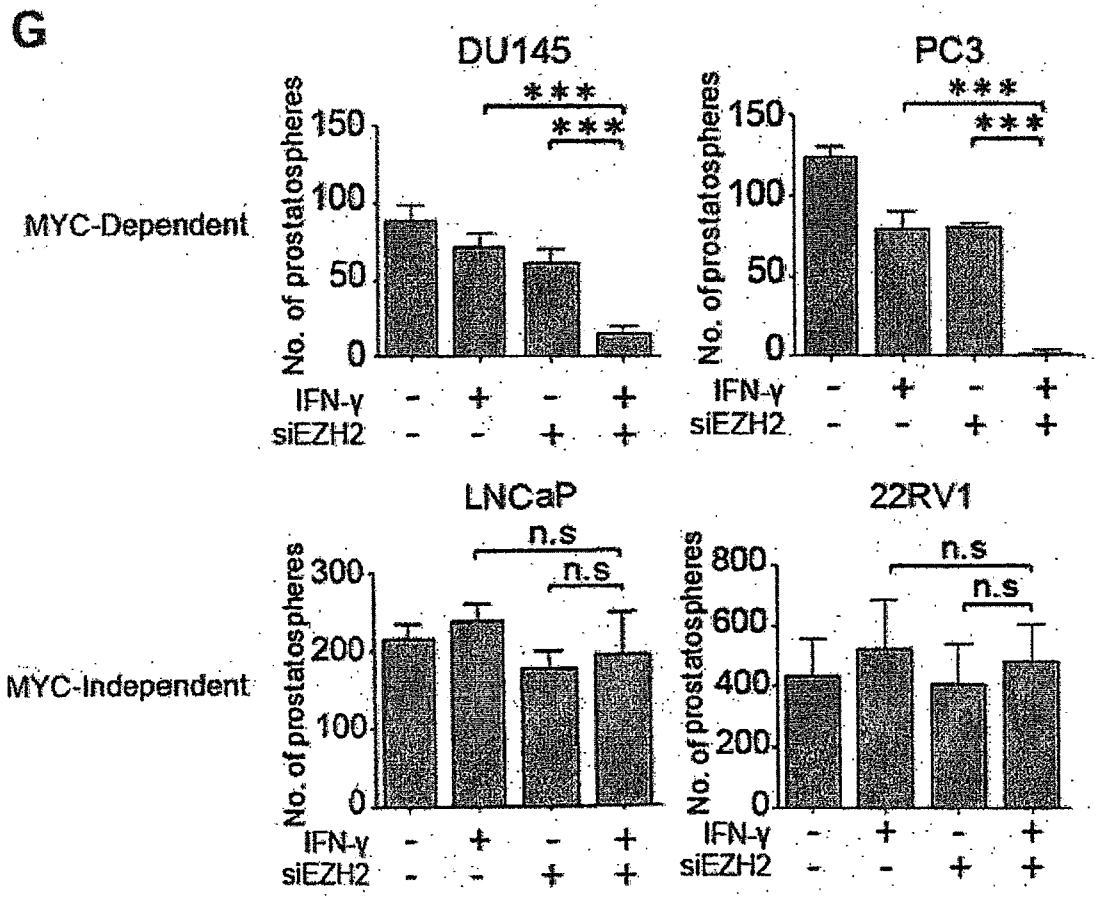

G
MYC-Dependent
MYC-Independent
DU145
PC3
LNCaP
22RV1
No. of prostatospheres
150
100
50
0
300
200
800
600
400
***
n.s
IFN-γ
siEZH2

FIG. 4 (Cont'd)

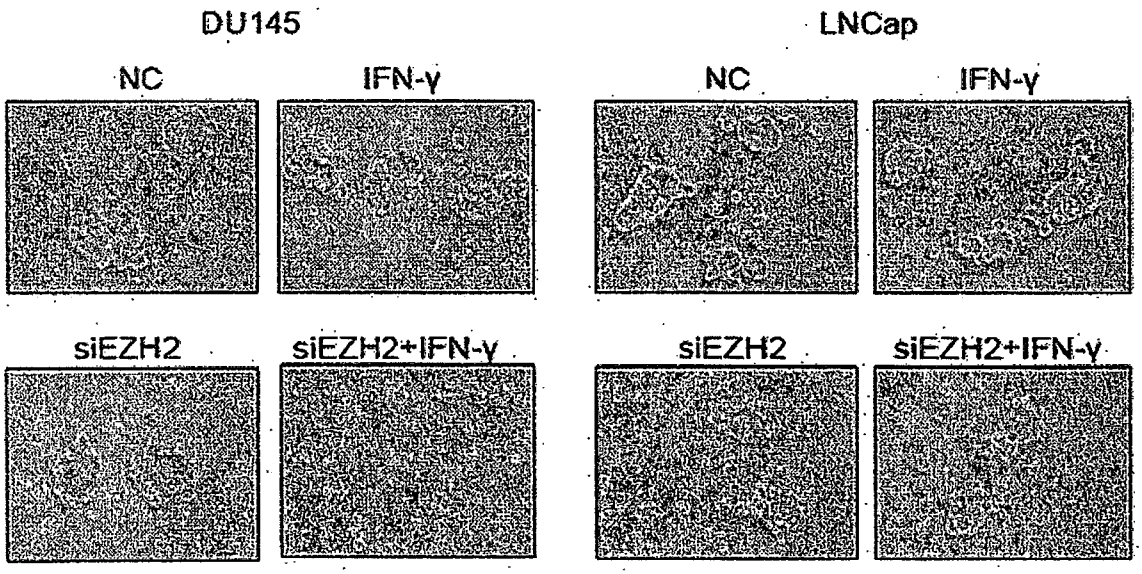
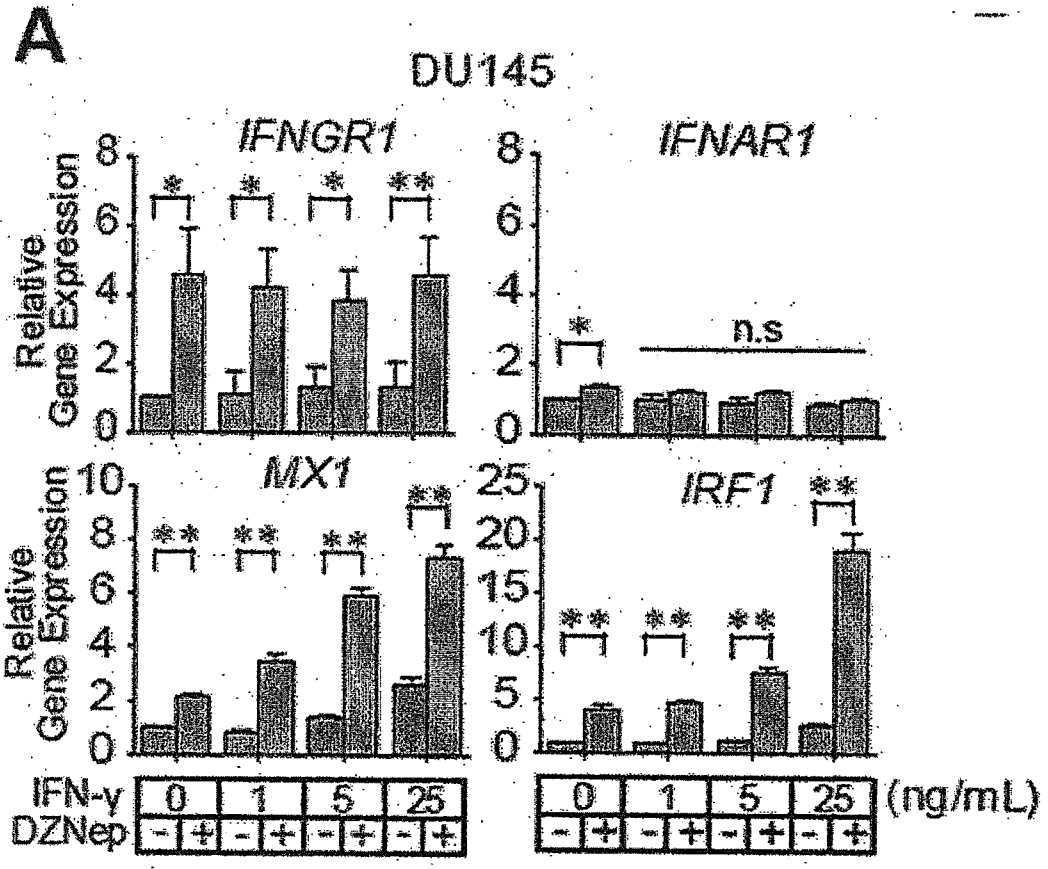

DU145
NC
IFN-γ
siEZH2
siEZH2+IFN-γ
LNCap
NC
IFN-γ
siEZH2
siEZH2+IFN-γ

FIG. 5


A
DU145
IFNGR1
IFNAR1
MX1
IRF1
Relative Gene Expression
n.s
IFN-γ 0 1 5 25 (ng/mL)
DZNep - + - + - + - +

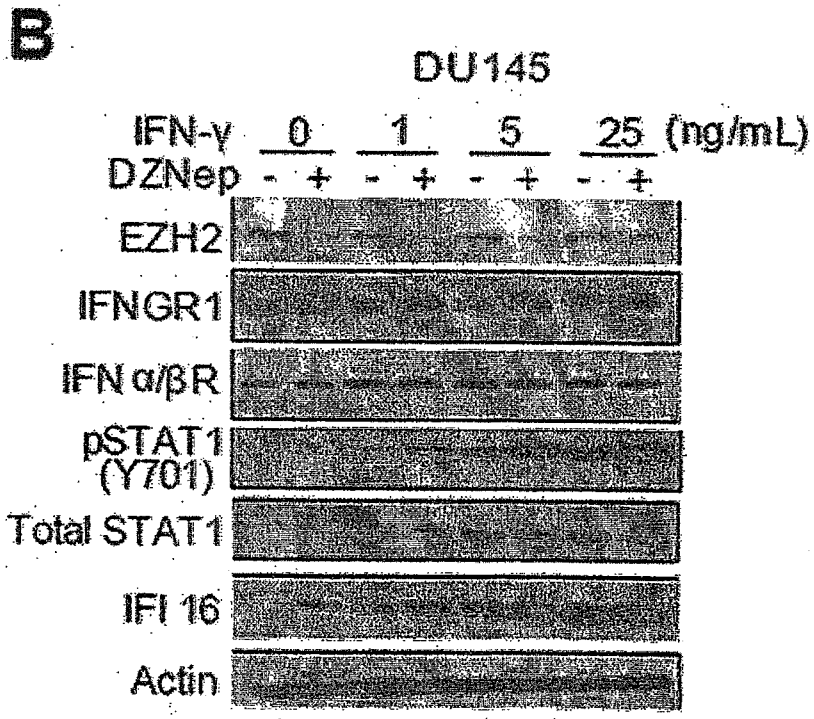

B
DU145
IFN-γ 0 1 5 25 (ng/mL)
DZNep - + - + - + - +
EZH2
IFNGR1
IFN α/βR
pSTAT1 (Y701)
Total STAT1
IFI 16
Actin

FIG. 5 (Cont'd)

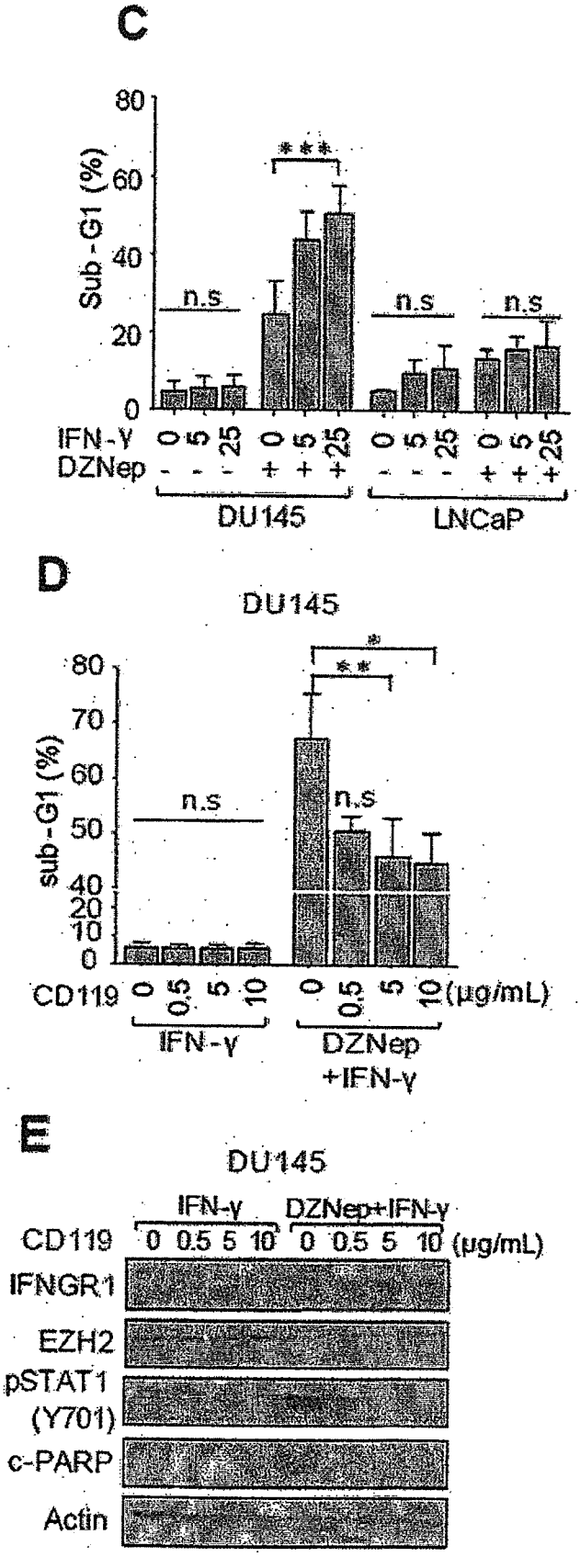

C
Sub-G1 (%)
80
60
40
20
0
***
n.s
n.s
n.s
IFN-γ 0 5 25 0 5 25 0 5 25 0 5 25
DZNep - - - + + + - - - + + +
DU145
LNCaP
D
DU145
sub-G1 (%)
80
70
60
50
40
20
10
0
*
**
n.s
n.s
CD119 0 0.5 5 10 0 0.5 5 10 (μg/mL)
IFN-γ
DZNep +IFN-γ
E
DU145
IFN-γ
DZNep+IFN-γ
CD119 0 0.5 5 10 0 0.5 5 10 (μg/mL)
IFNGR1
EZH2
pSTAT1 (Y701)
c-PARP
Actin

FIG. 5 (cont'd)

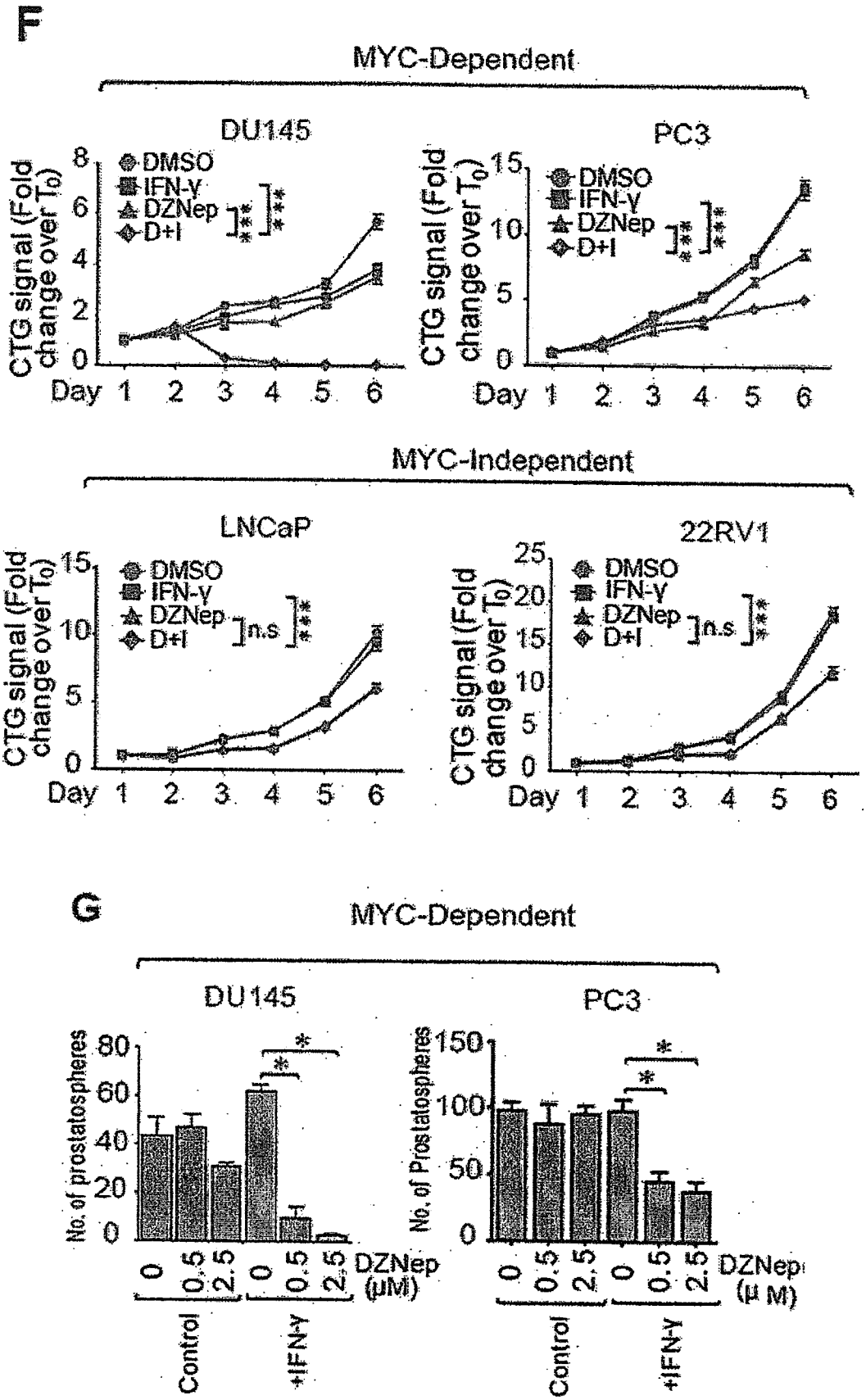
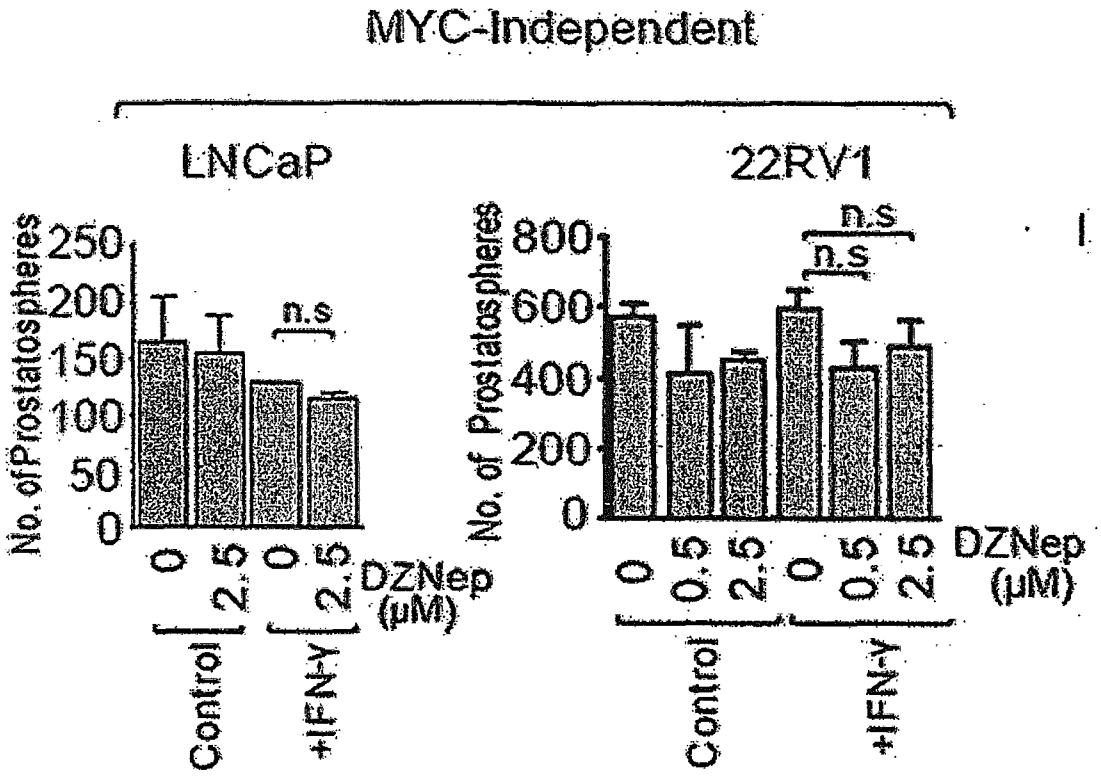

F
MYC-Dependent
DU145
PC3
DMSO
IFN-γ
DZNep
D+I
CTG signal (Fold change over T0)
Day 1 2 3 4 5 6
MYC-Independent
LNCaP
22RV1
n.s
G
MYC-Dependent
DU145
PC3
No. of prostatospheres
No. of Prostatospheres
0 0.5 2.5 0 0.5 2.5 DZNep (μM)
Control
+IFN-γ

FIG. 5 (cont'd)


MYC-Independent
LNCaP
22RV1
No. of Prostatospheres
250
200
150
100
50
0
n.s
800
600
400
200
0
0
2.5
0.5
DZNep (μM)
Control
+IFN-γ

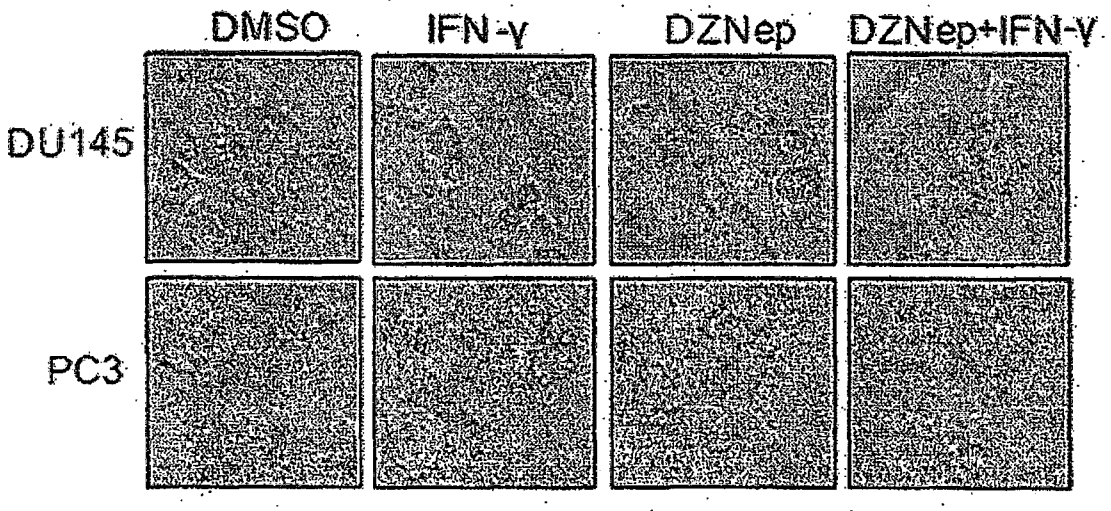

DMSO
IFN-γ
DZNep
DZNep+IFN-γ
DU145
PC3

FIG. 6

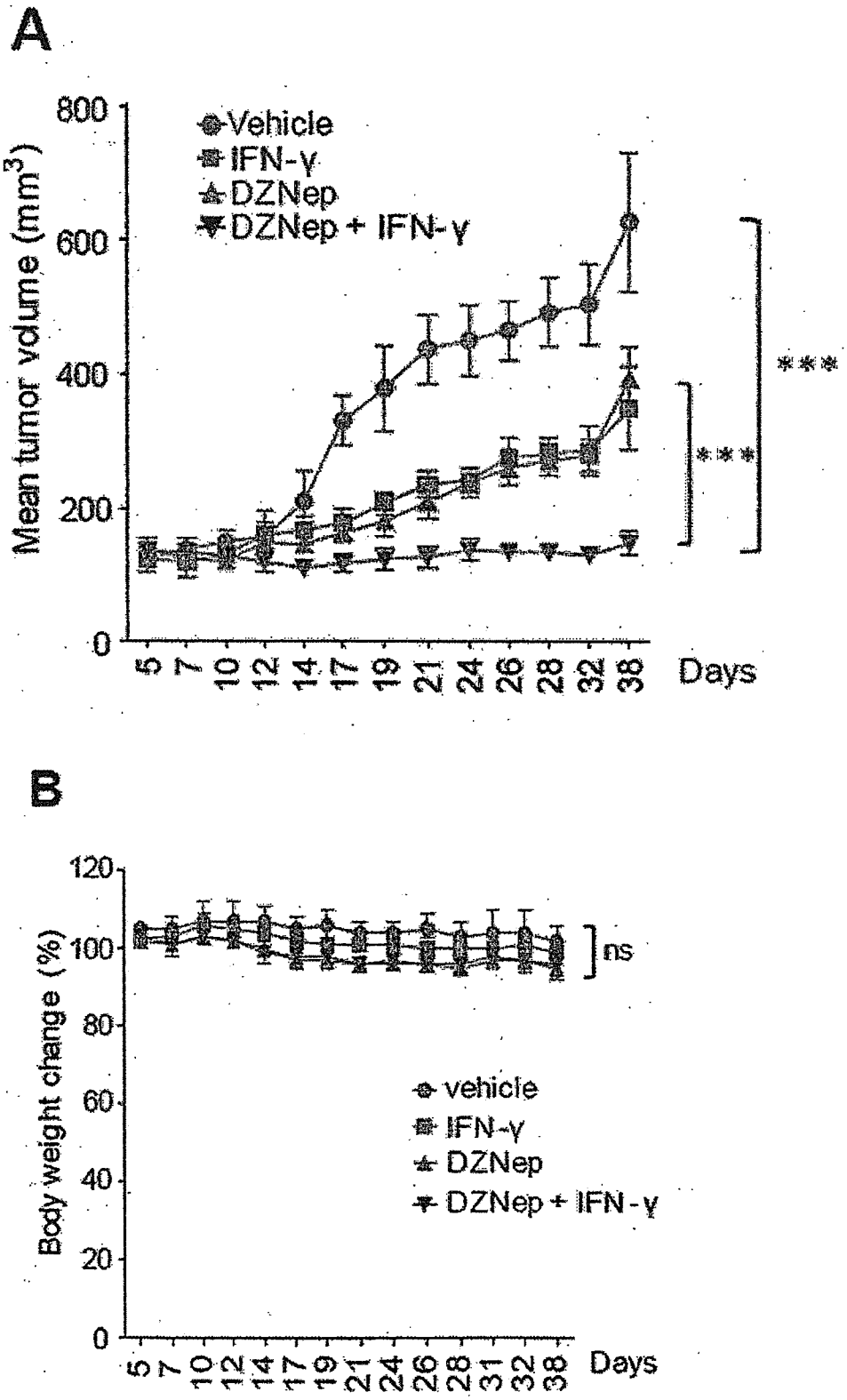

A
Mean tumor volume (mm3)
800
600
400
200
0
Vehicle
IFN-γ
DZNep
DZNep + IFN-γ
***
***
5 7 10 12 14 17 19 21 24 26 28 32 38
Days
B
Body weight change (%)
120
100
80
60
40
20
0
ns
vehicle
IFN-γ
DZNep
DZNep + IFN-γ
5 7 10 12 14 17 19 21 24 26 28 31 32 38
Days

FIG. 6 (Cont'd)

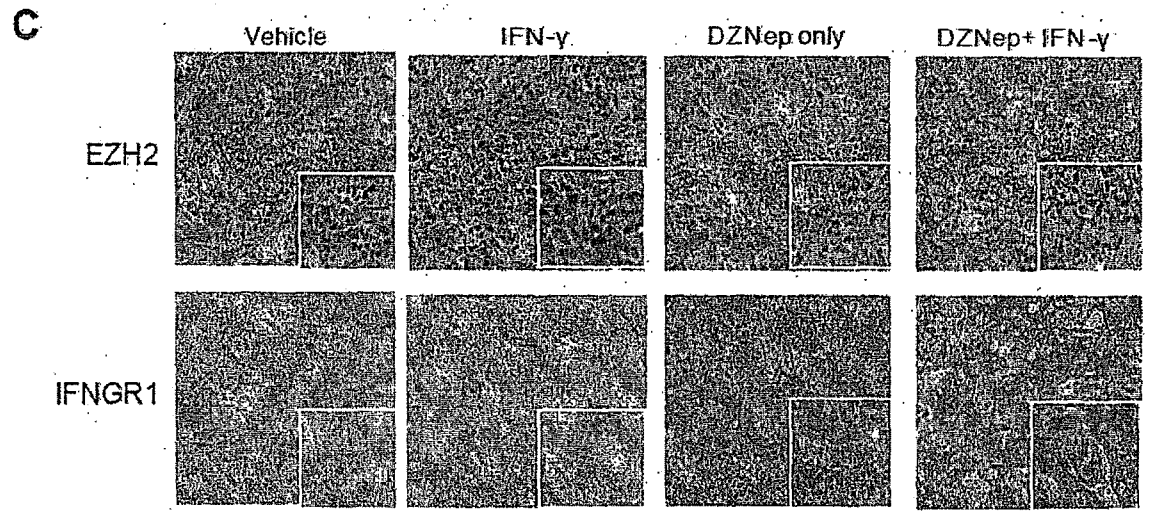


FIG. 7

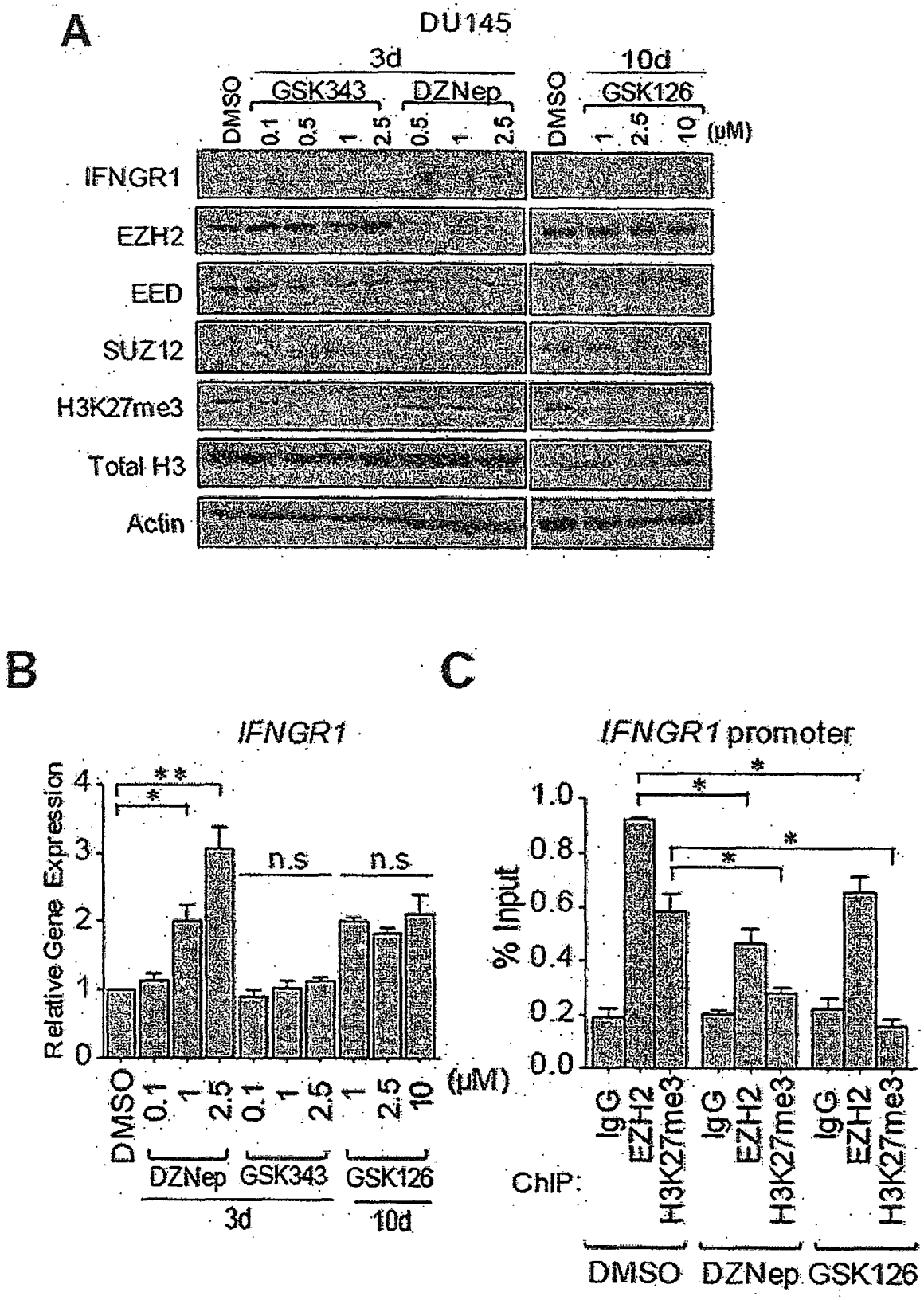

A
DU145
3d
10d
DMSO
GSK343
DZNep
GSK126
0.1
0.5
1
2.5
0.5
1
2.5
DMSO
1
2.5
10
(μM)
IFNGR1
EZH2
EED
SUZ12
H3K27me3
Total H3
Actin
B
IFNGR1
Relative Gene Expression
n.s
n.s
DMSO
0.1
1
2.5
0.1
1
2.5
1
2.5
10
(μM)
DZNep
GSK343
GSK126
3d
10d
C
IFNGR1 promoter
% Input
ChIP:
IgG
EZH2
H3K27me3
DMSO
DZNep
GSK126

FIG. 7 (Cont'd)

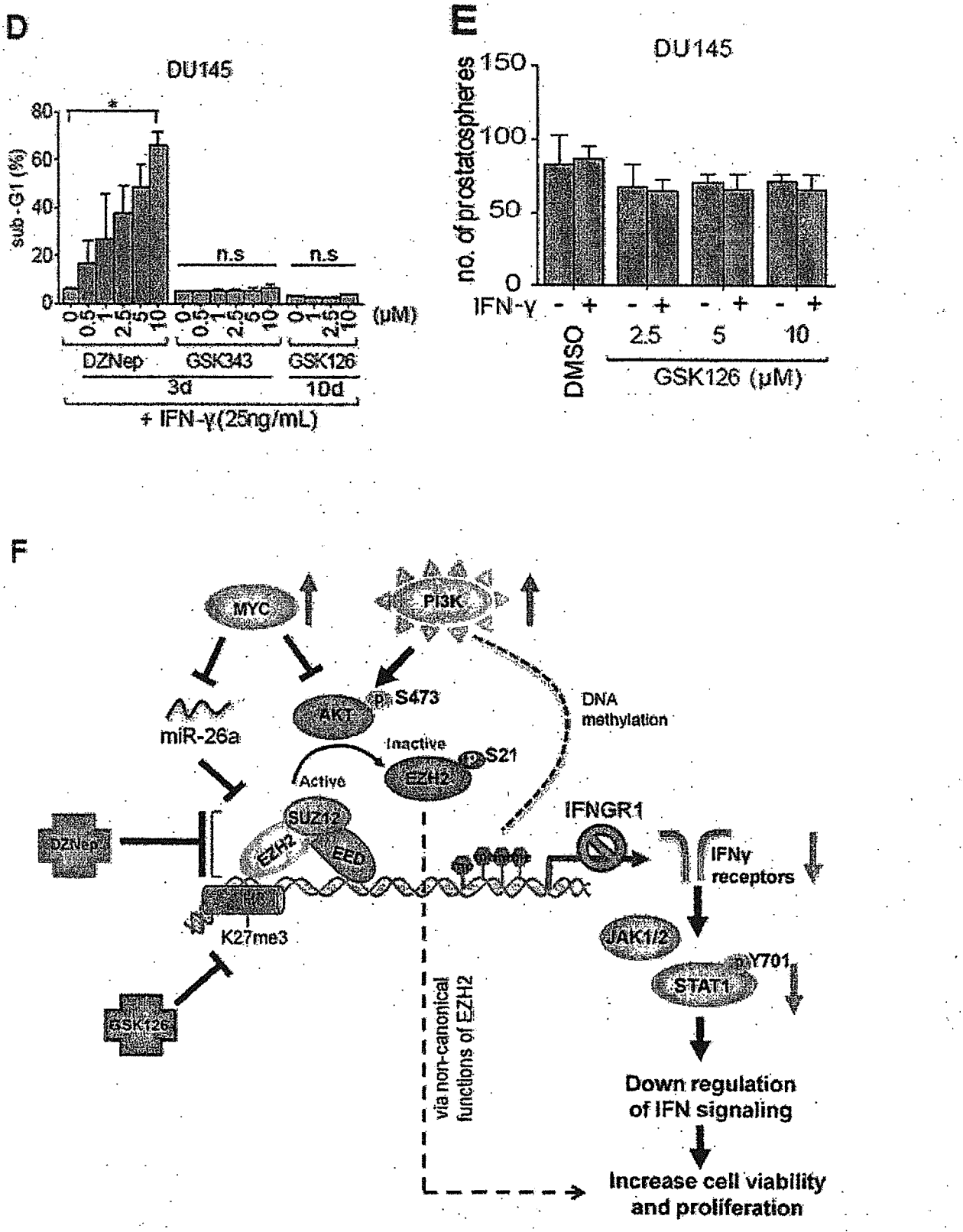

D
DU145
sub-G1 (%)
n.s
n.s
(μM)
DZNep
GSK343
GSK126
3d
10d
+ IFN-γ(25ng/mL)
E
DU145
no. of prostatospheres
IFN-γ
DMSO
GSK126 (μM)
F
MYC
PI3K
AKT
P S473
miR-26a
Inactive
P S21
Active
EZH2
DNA methylation
SUZ12
EZH2
EED
DZNep
IFNGR1
IFNγ receptors
K27me3
JAK1/2
STAT1
Y701
GSK126
via non-canonical functions of EZH2
Down regulation of IFN signaling
Increase cell viability and proliferation

FIG. 8

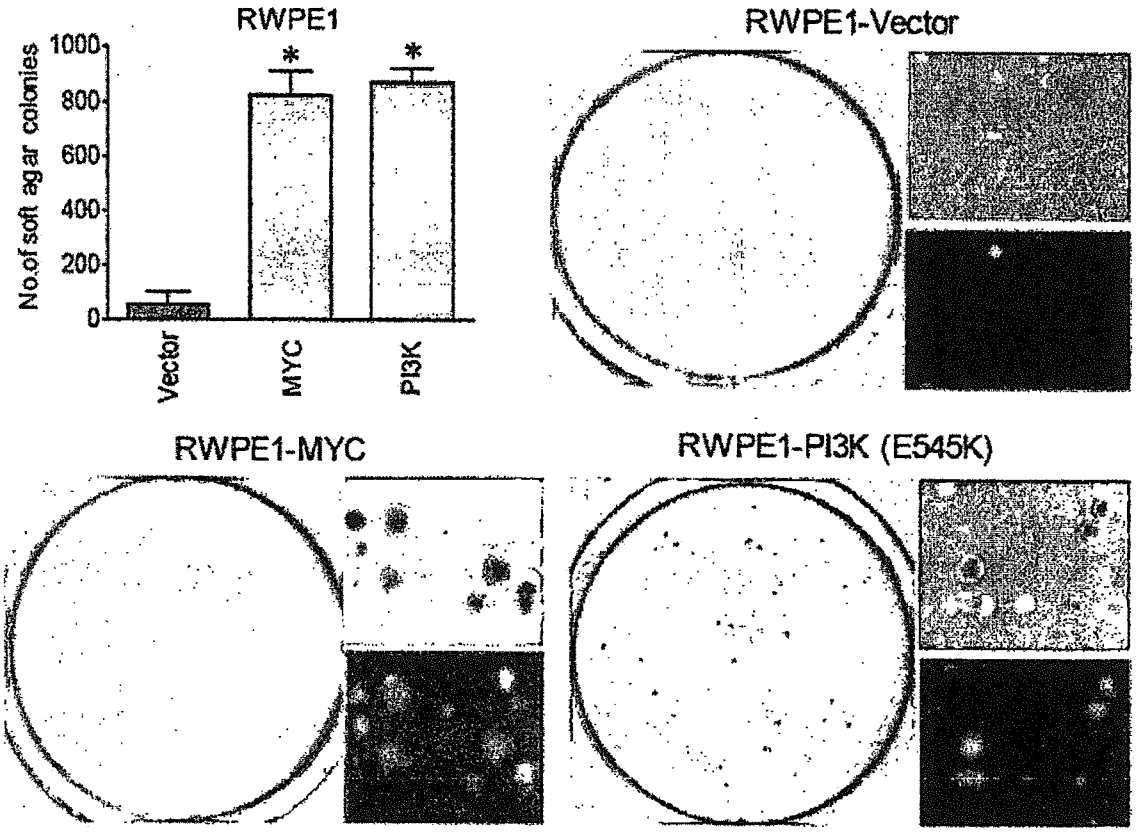

RWPE1
No.of soft agar colonies
1000
800
600
400
200
0
*
*
Vector
MYC
PI3K
RWPE1-Vector
RWPE1-MYC
RWPE1-PI3K (E545K)

FIG. 9

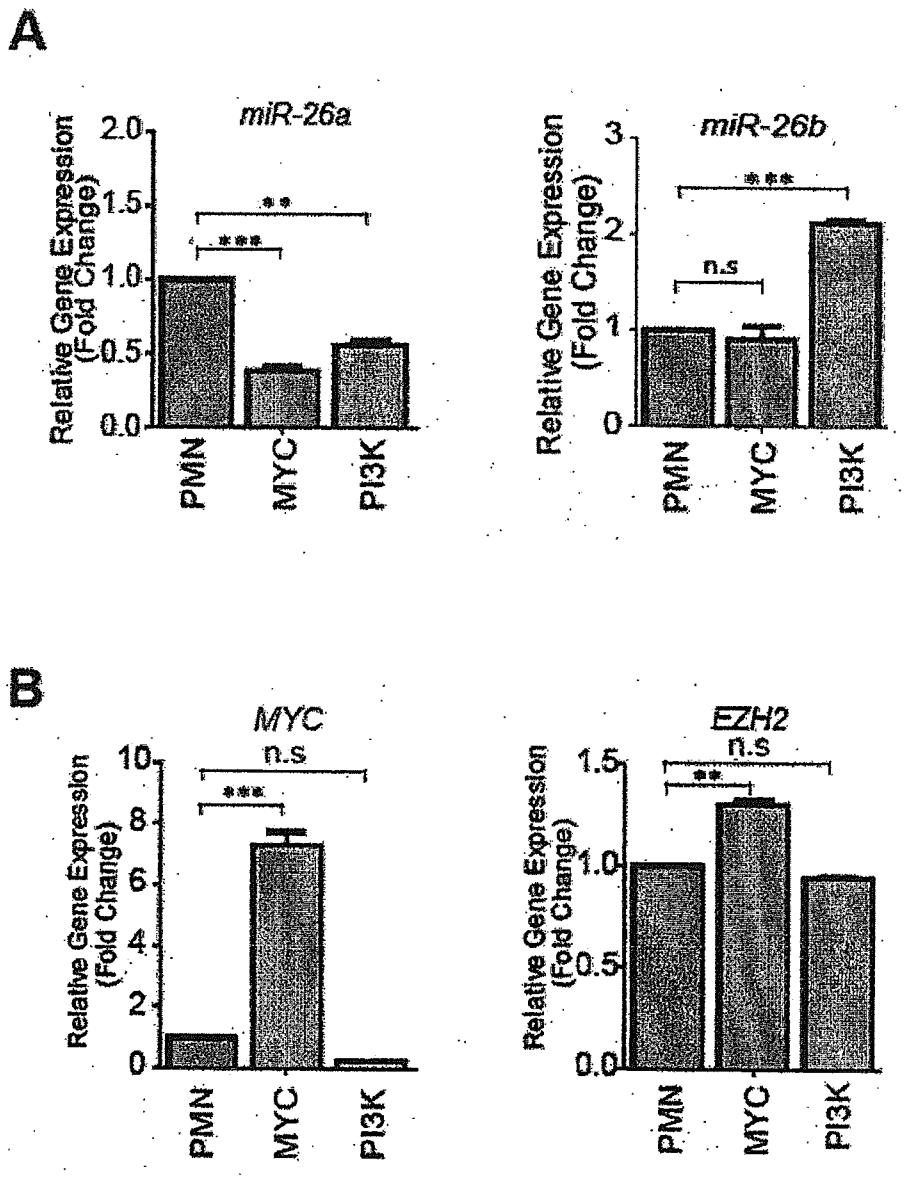

A
miR-26a
Relative Gene Expression (Fold Change)
2.0
1.5
1.0
0.5
0.0
**
***
PMN
MYC
PI3K
miR-26b
3
2
1
0
***
n.s
B
MYC
10
8
6
4
2
0
n.s
EZH2
1.5
1.0
0.5
0.0

FIG. 9 (Cont'd)

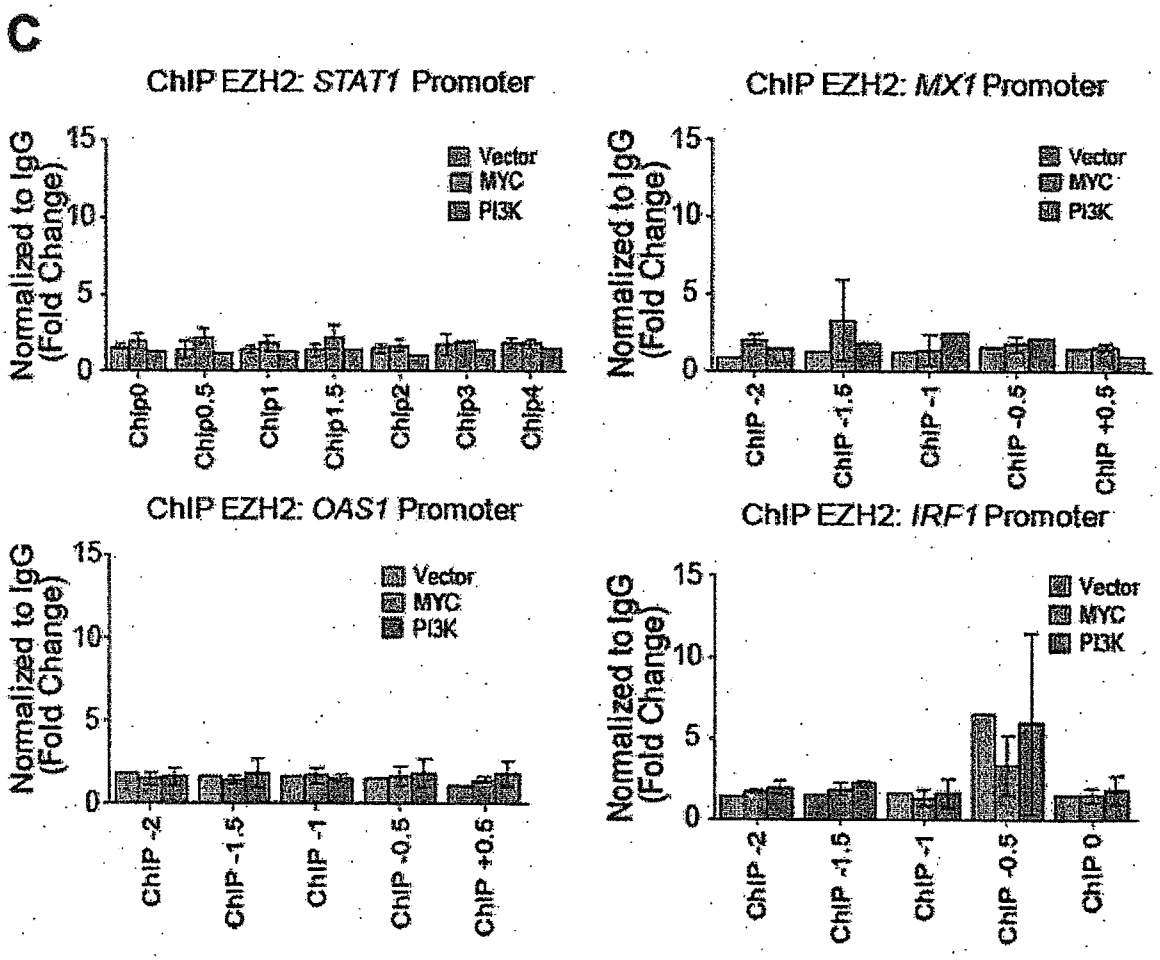
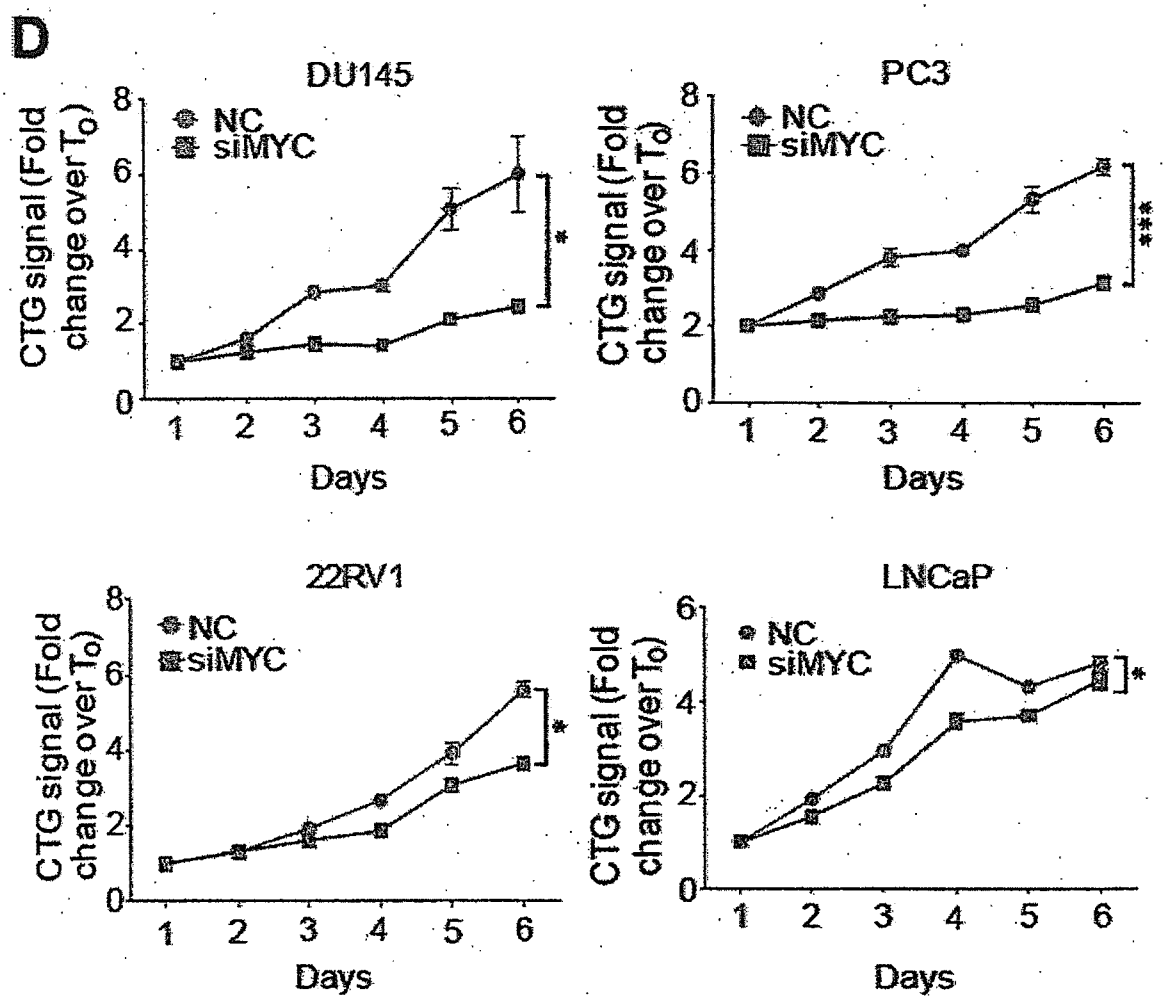

C
ChIP EZH2: STAT1 Promoter
Normalized to IgG (Fold Change)
15
10
5
0
Vector
MYC
PI3K
Chip0
Chip0.5
Chip1
Chip1.5
Chip2
Chip3
Chip4
ChIP EZH2: MX1 Promoter
Normalized to IgG (Fold Change)
15
10
5
0
Vector
MYC
PI3K
ChIP -2
ChIP -1.5
ChIP -1
ChIP -0.5
ChIP +0.5
ChIP EZH2: OAS1 Promoter
Normalized to IgG (Fold Change)
15
10
5
0
Vector
MYC
PI3K
ChIP -2
ChIP -1.5
ChIP -1
ChIP -0.5
ChIP +0.5
ChIP EZH2: IRF1 Promoter
Normalized to IgG (Fold Change)
15
10
5
0
Vector
MYC
PI3K
ChIP -2
ChIP -1.5
ChIP -1
ChIP -0.5
ChIP 0

FIG. 9 (Cont'd)


D
DU145
PC3
22RV1
LNCaP
NC
siMYC
CTG signal (Fold change over T0)
Days
*
***

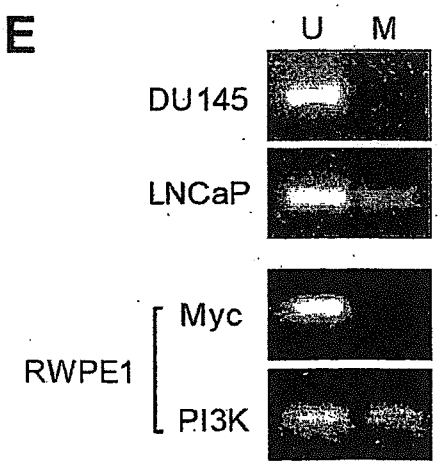

E
U
M
DU145
LNCaP
RWPE1
Myc
PI3K

FIG. 10

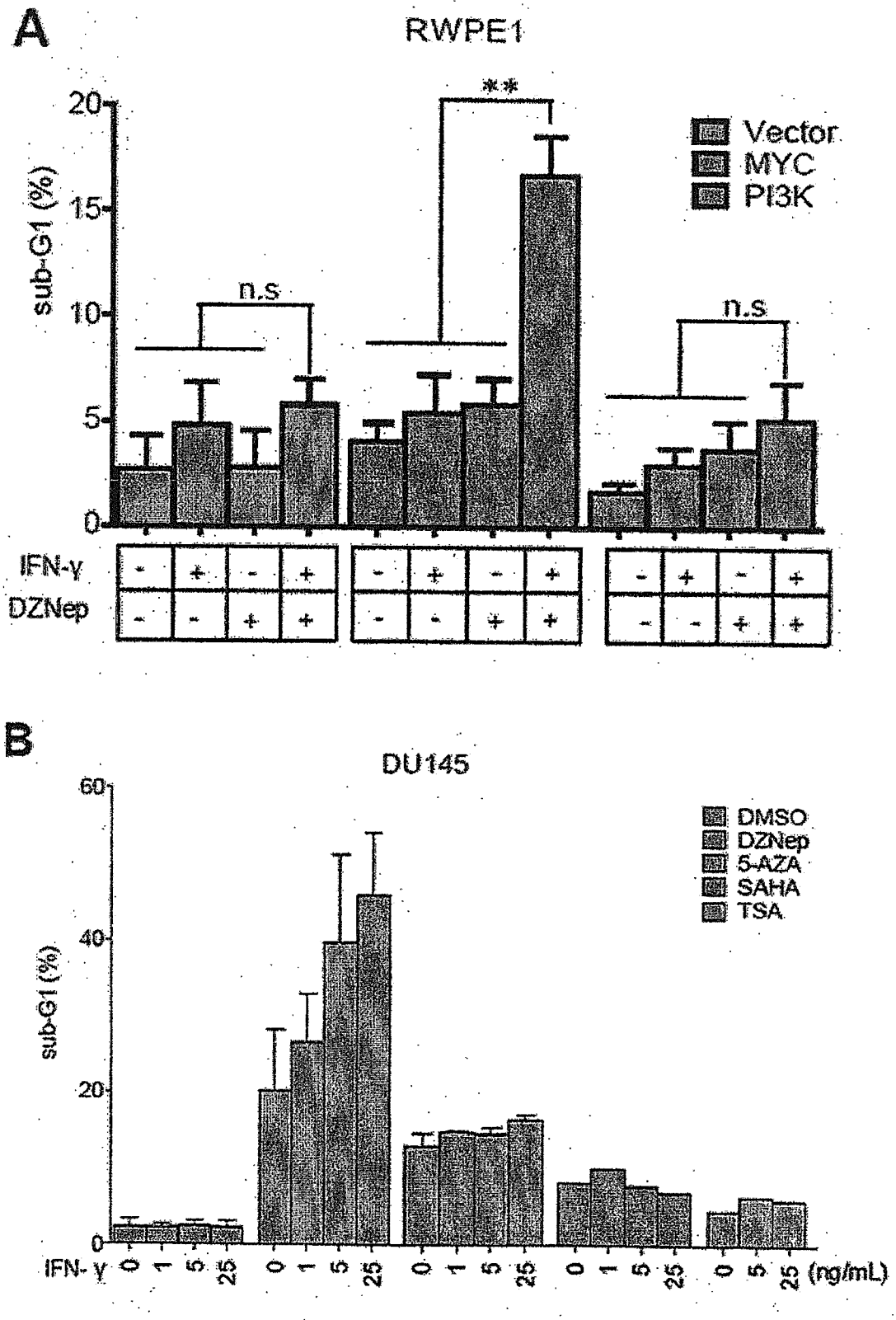
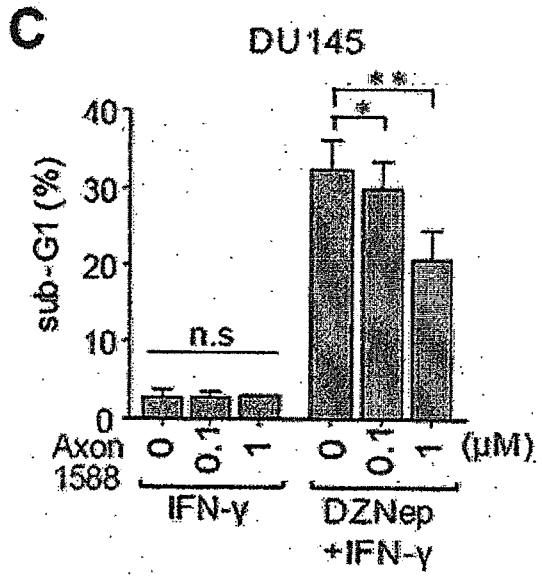

A
RWPE1
sub-G1 (%)
20
15
10
5
0
**
n.s
n.s
Vector
MYC
PI3K
IFN-γ
DZNep
B
DU145
sub-G1 (%)
60
40
20
0
DMSO
DZNep
5-AZA
SAHA
TSA
IFN-γ 0 1 5 25 0 1 5 25 0 1 5 25 0 1 5 25 0 5 25 (ng/mL)

FIG. 10 (Cont'd)


C
DU145
sub-G1 (%)
40
30
20
10
0
n.s
**
*
Axon 1588
0
0.1
1
0
0.1
1
(μM)
IFN-γ
DZNep +IFN-γ

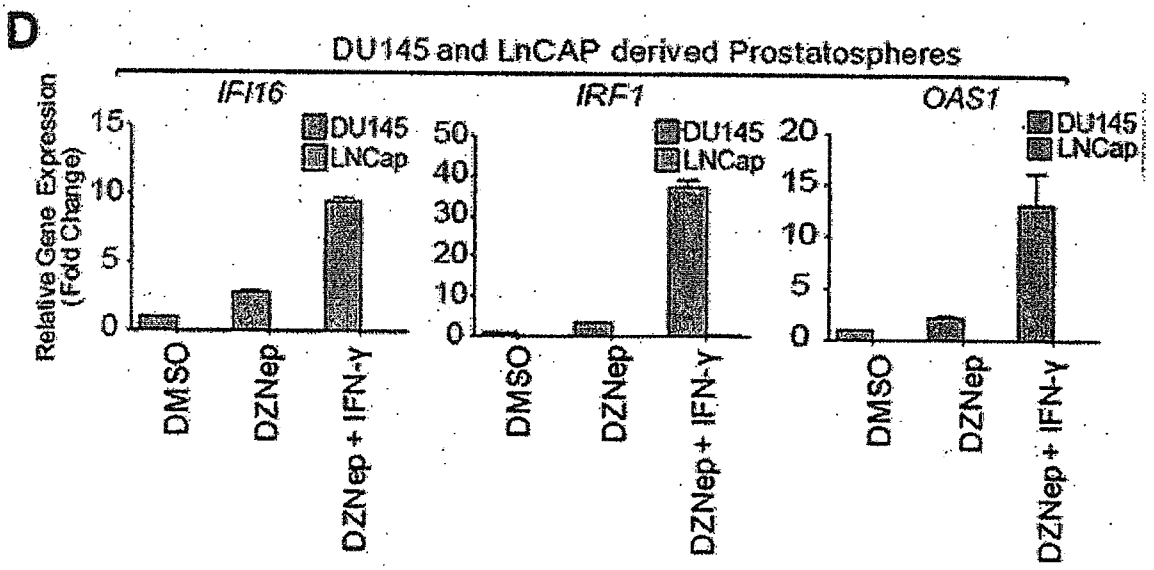

D
DU145 and LnCAP derived Prostatospheres
IFI16
IRF1
OAS1
Relative Gene Expression (Fold Change)
DU145
LNCap
DMSO
DZNep
DZNep + IFN-γ

FIG. 11

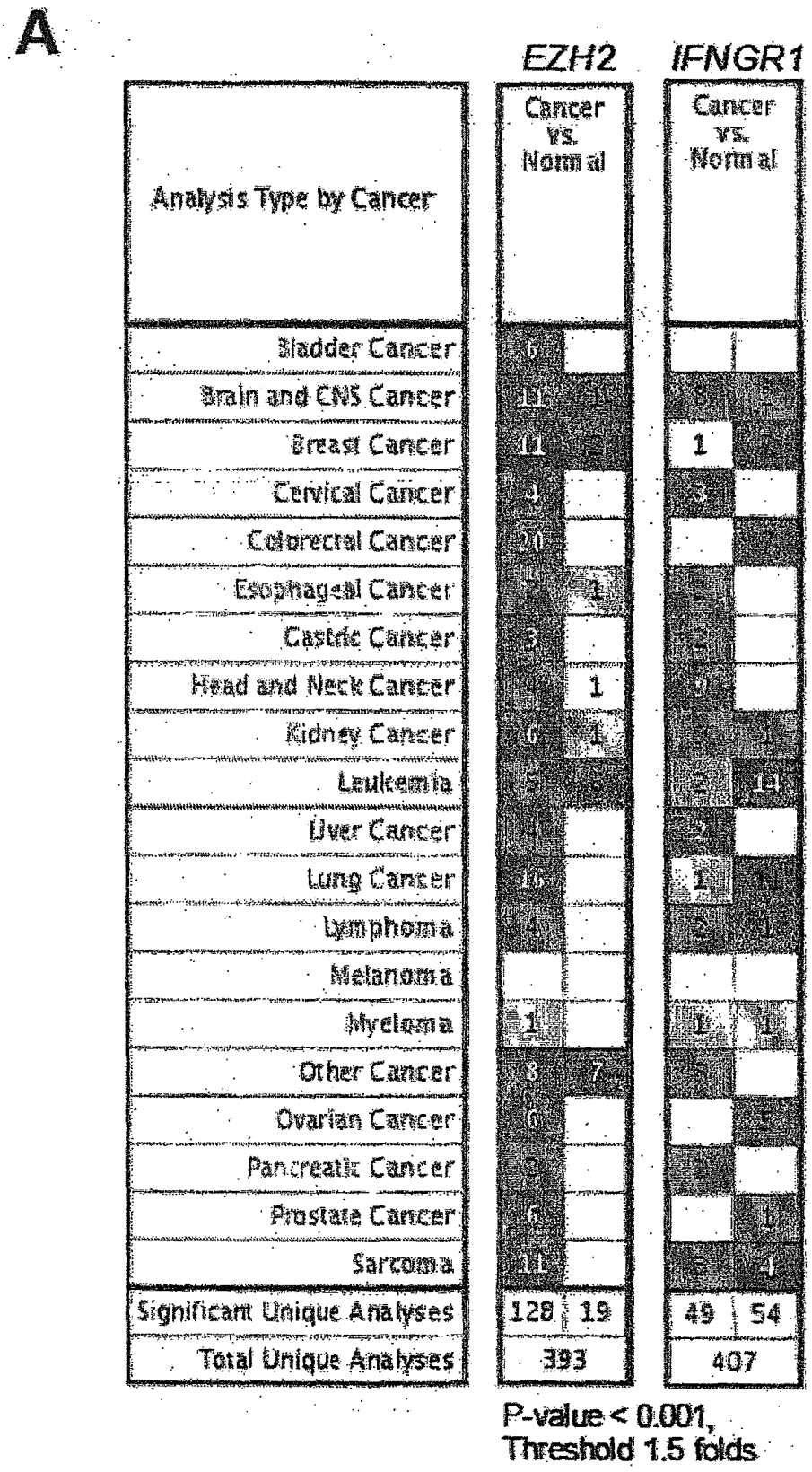

A
EZH2
IFNGR1
Analysis Type by Cancer
Cancer vs. Normal
Cancer vs. Normal
Bladder Cancer
Brain and CNS Cancer
Breast Cancer
Cervical Cancer
Colorectal Cancer
Esophageal Cancer
Gastric Cancer
Head and Neck Cancer
Kidney Cancer
Leukemia
Liver Cancer
Lung Cancer
Lymphoma
Melanoma
Myeloma
Other Cancer
Ovarian Cancer
Pancreatic Cancer
Prostate Cancer
Sarcoma
Significant Unique Analyses
128 19
49 54
Total Unique Analyses
393
407
P-value < 0.001,
Threshold 1.5 folds

FIG. 11 (Cont'd)

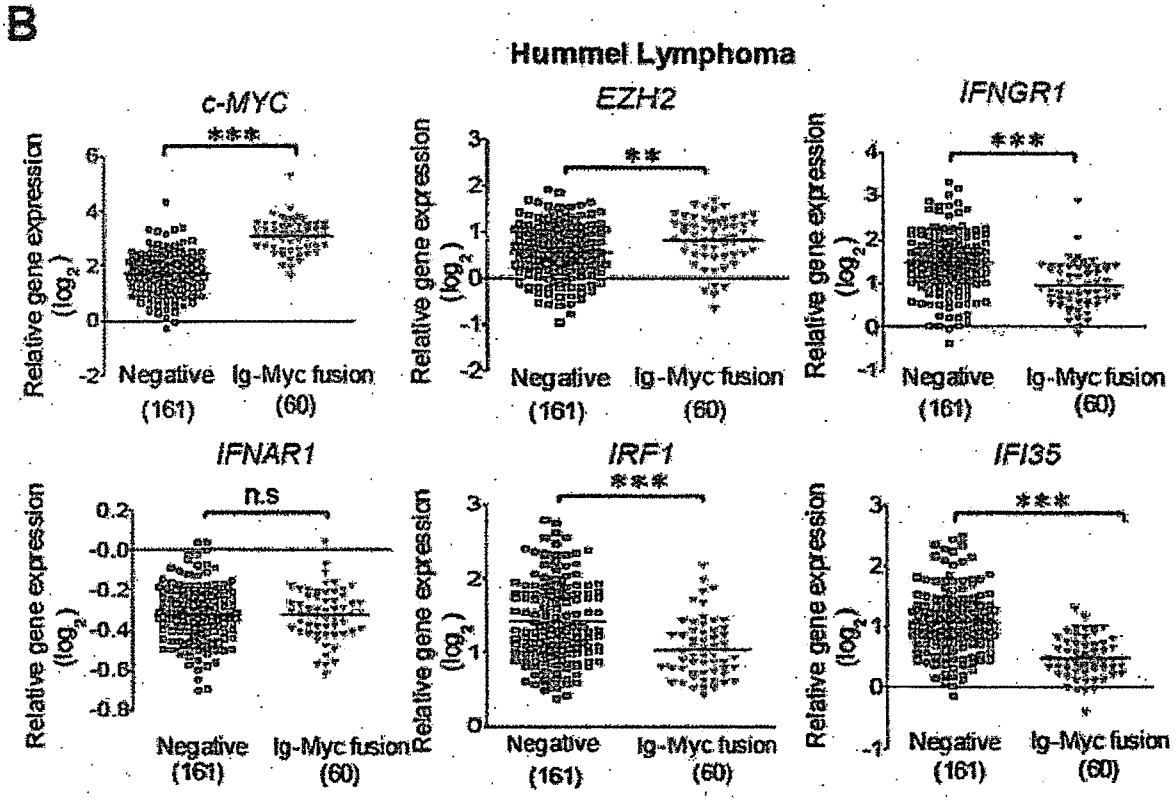

B
Hummel Lymphoma
c-MYC
EZH2
IFNGR1
IFNAR1
IRF1
IFI35
Relative gene expression (log2)
***
**
n.s
Negative (161)
Ig-Myc fusion (60)

FIG. 12

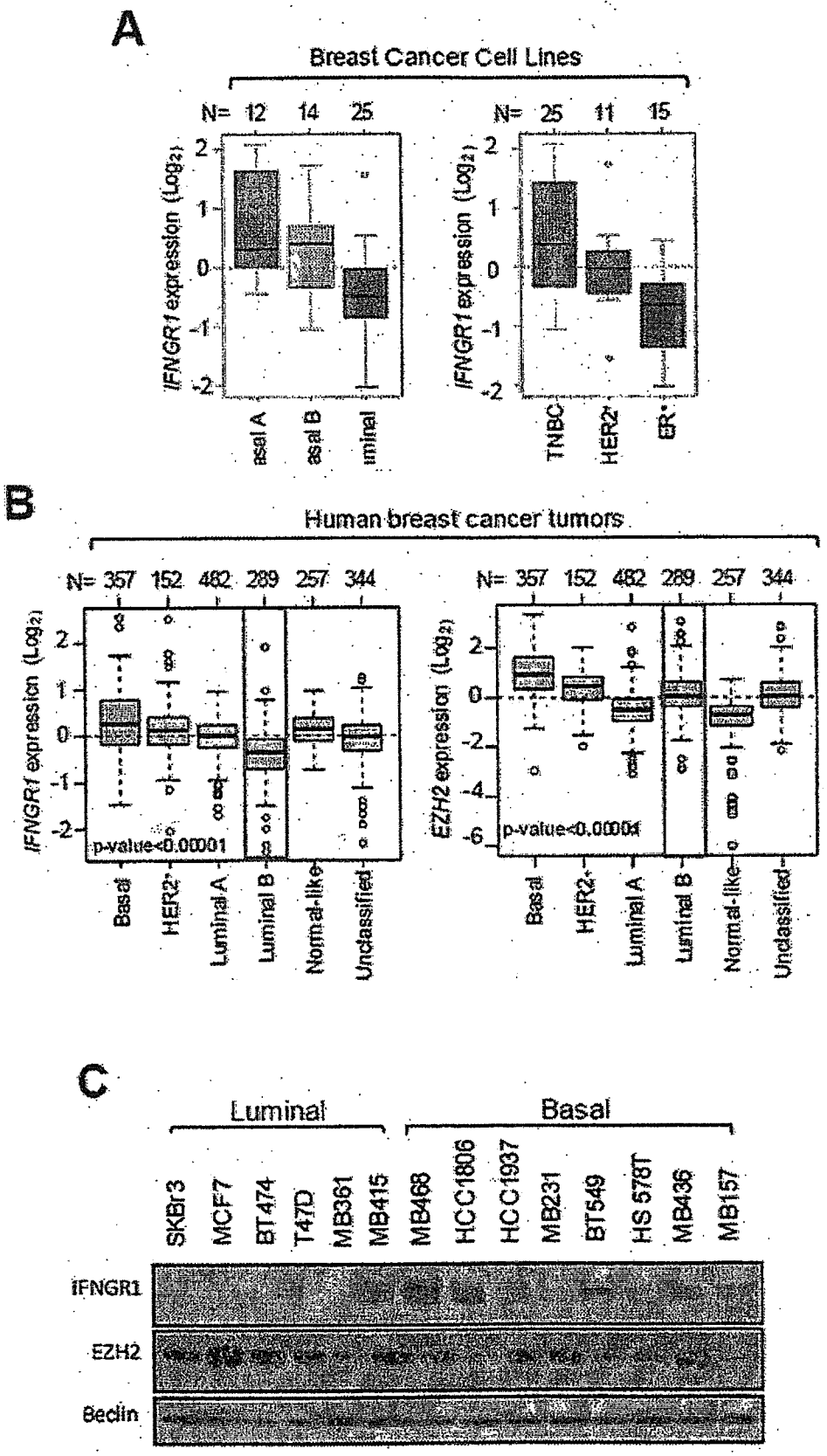

A
Breast Cancer Cell Lines
N= 12 14 25
IFNGR1 expression (Log2)
basal A
basal B
luminal
N= 25 11 15
TNBC
HER2+
ER+
B
Human breast cancer tumors
N= 357 152 482 289 257 344
IFNGR1 expression (Log2)
p-value<0.00001
Basal
HER2+
Luminal A
Luminal B
Normal-like
Unclassified
EZH2 expression (Log2)
p-value<0.00001
C
Luminal
Basal
SKBr3
MCF7
BT474
T47D
MB361
MB415
MB468
HCC1806
HCC1937
MB231
BT549
HS 578T
MB436
MB157
IFNGR1
EZH2
Beclin

FIG. 12 (Cont'd)

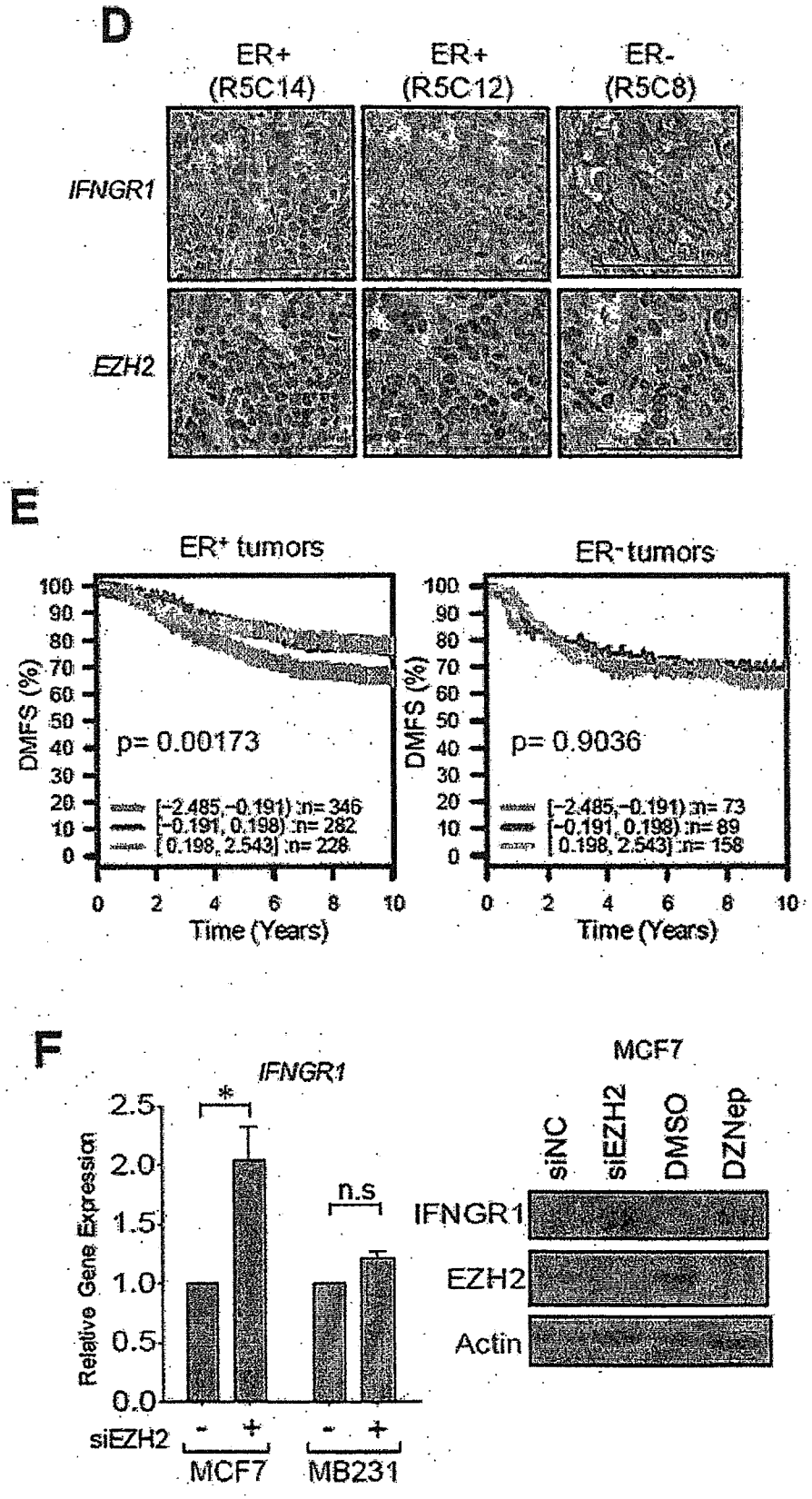

D
ER+ (R5C14)
ER+ (R5C12)
ER- (R5C8)
IFNGR1
EZH2
E
ER+ tumors
ER- tumors
DMFS (%)
p= 0.00173
p= 0.9036
[-2.485,-0.191) :n= 346
[-0.191, 0.198) :n= 282
[0.198, 2.543] :n= 228
[-2.485,-0.191) :n= 73
[-0.191, 0.198) :n= 89
[0.198, 2.543] :n= 158
Time (Years)
F
IFNGR1
Relative Gene Expression
siEZH2
MCF7
MB231
n.s
MCF7
siNC
siEZH2
DMSO
DZNep
IFNGR1
EZH2
Actin

FIG. 12 (Cont'd)

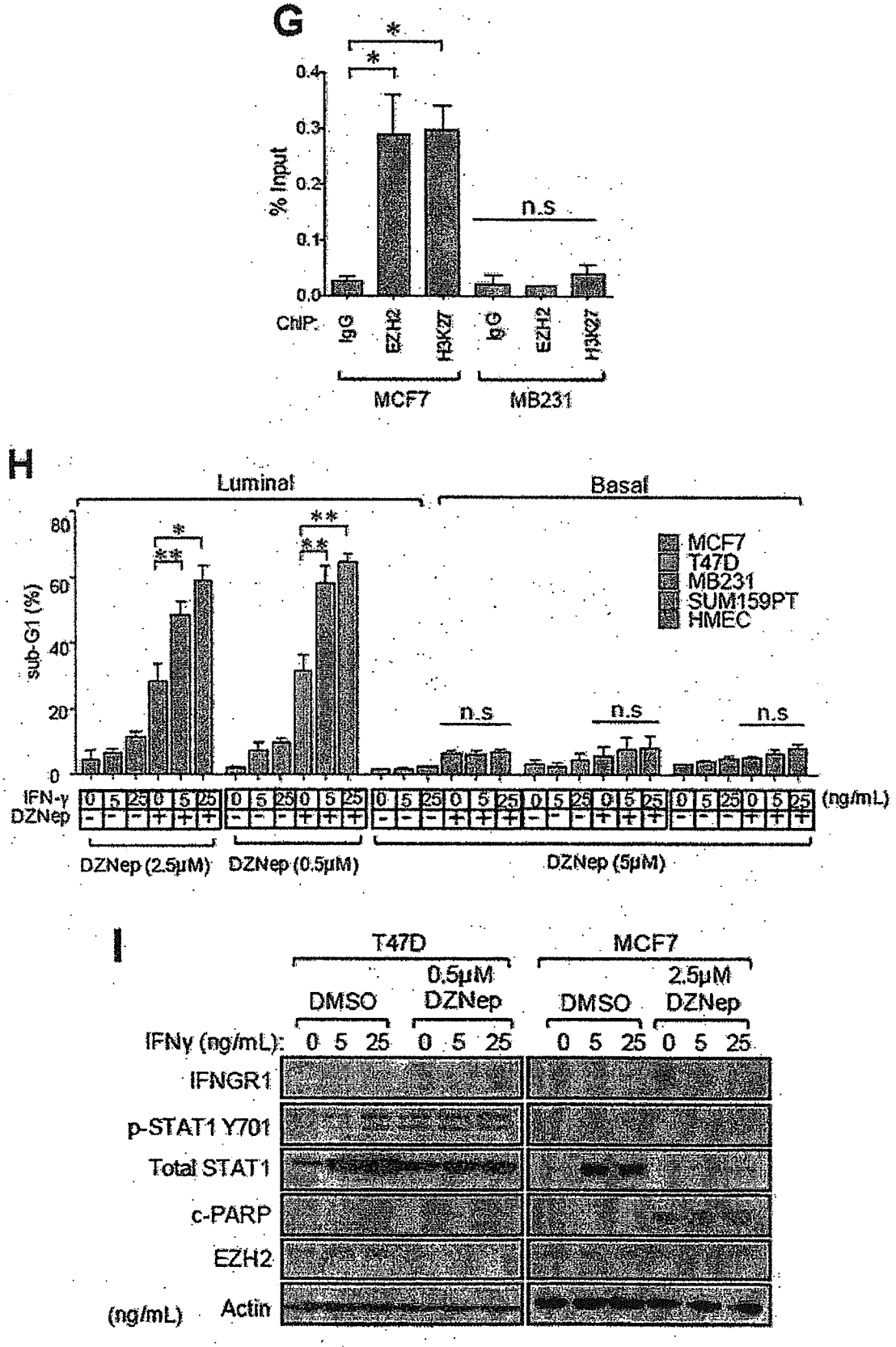

G
% Input
0.4
0.3
0.2
0.1
0.0
*
*
n.s
ChIP:
IgG
EZH2
H3K27
IgG
EZH2
H3K27
MCF7
MB231
H
Luminal
Basal
sub-G1 (%)
80
60
40
20
0
MCF7
T47D
MB231
SUM159PT
HMEC
n.s
n.s
n.s
IFN-γ
DZNep
(ng/mL)
DZNep (2.5μM)
DZNep (0.5μM)
DZNep (5μM)
I
T47D
MCF7
DMSO
0.5μM DZNep
DMSO
2.5μM DZNep
IFNγ (ng/mL):
IFNGR1
p-STAT1 Y701
Total STAT1
c-PARP
EZH2
(ng/mL)
Actin

FIG. 13

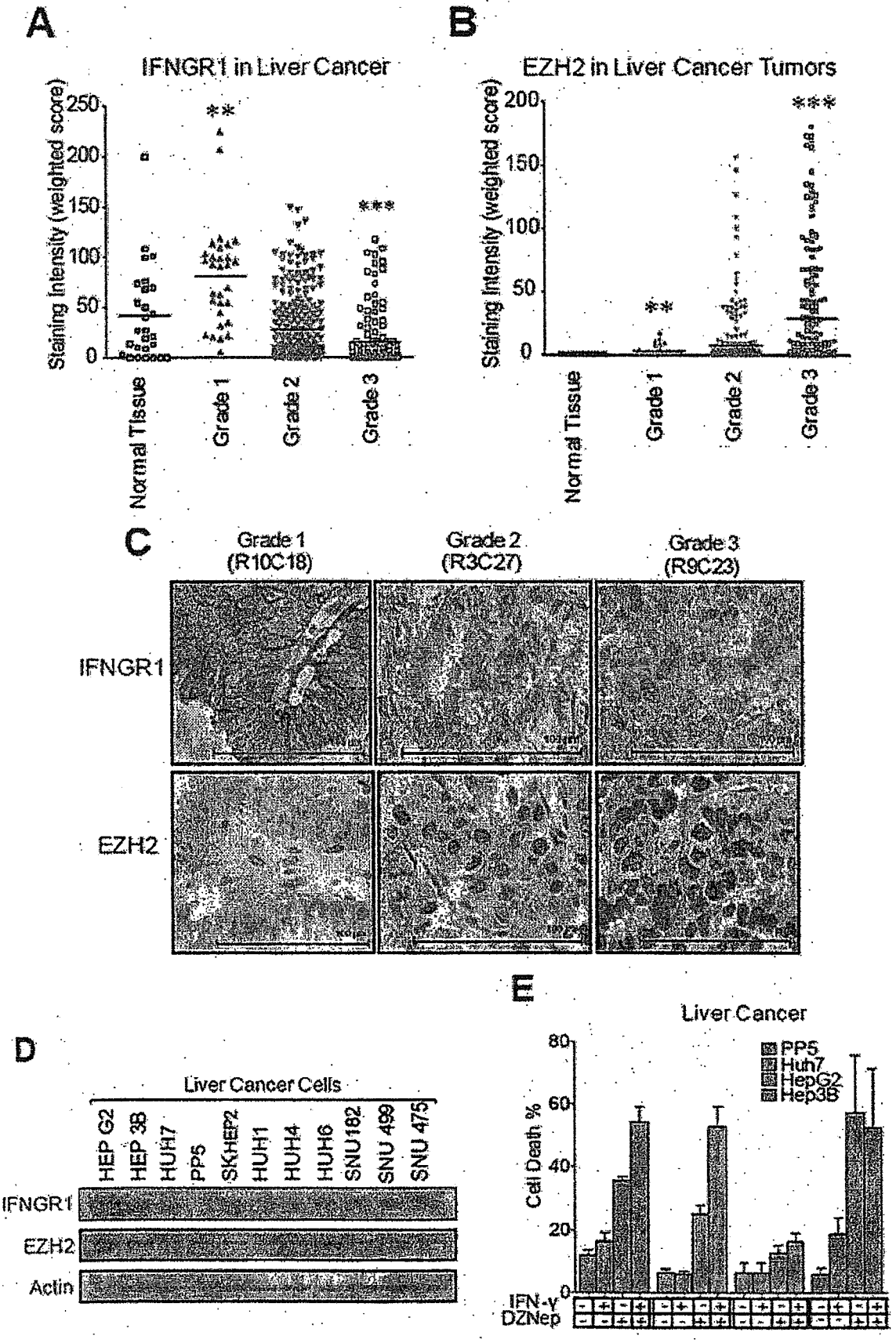

A
IFNGR1 in Liver Cancer
Staining Intensity (weighted score)
250
200
150
100
50
0
Normal Tissue
Grade 1
Grade 2
Grade 3
B
EZH2 in Liver Cancer Tumors
Staining Intensity (weighted score)
200
150
100
50
0
Normal Tissue
Grade 1
Grade 2
Grade 3
C
Grade 1 (R10C18)
Grade 2 (R3C27)
Grade 3 (R9C23)
IFNGR1
EZH2
D
Liver Cancer Cells
HEP G2
HEP 3B
HUH7
PP5
SKHEP2
HUH1
HUH4
HUH6
SNU182
SNU 499
SNU 475
IFNGR1
EZH2
Actin
E
Liver Cancer
PP5
Huh7
HepG2
Hep3B
Cell Death %
80
60
40
20
0
IFN-γ
DZNep

FIG. 14

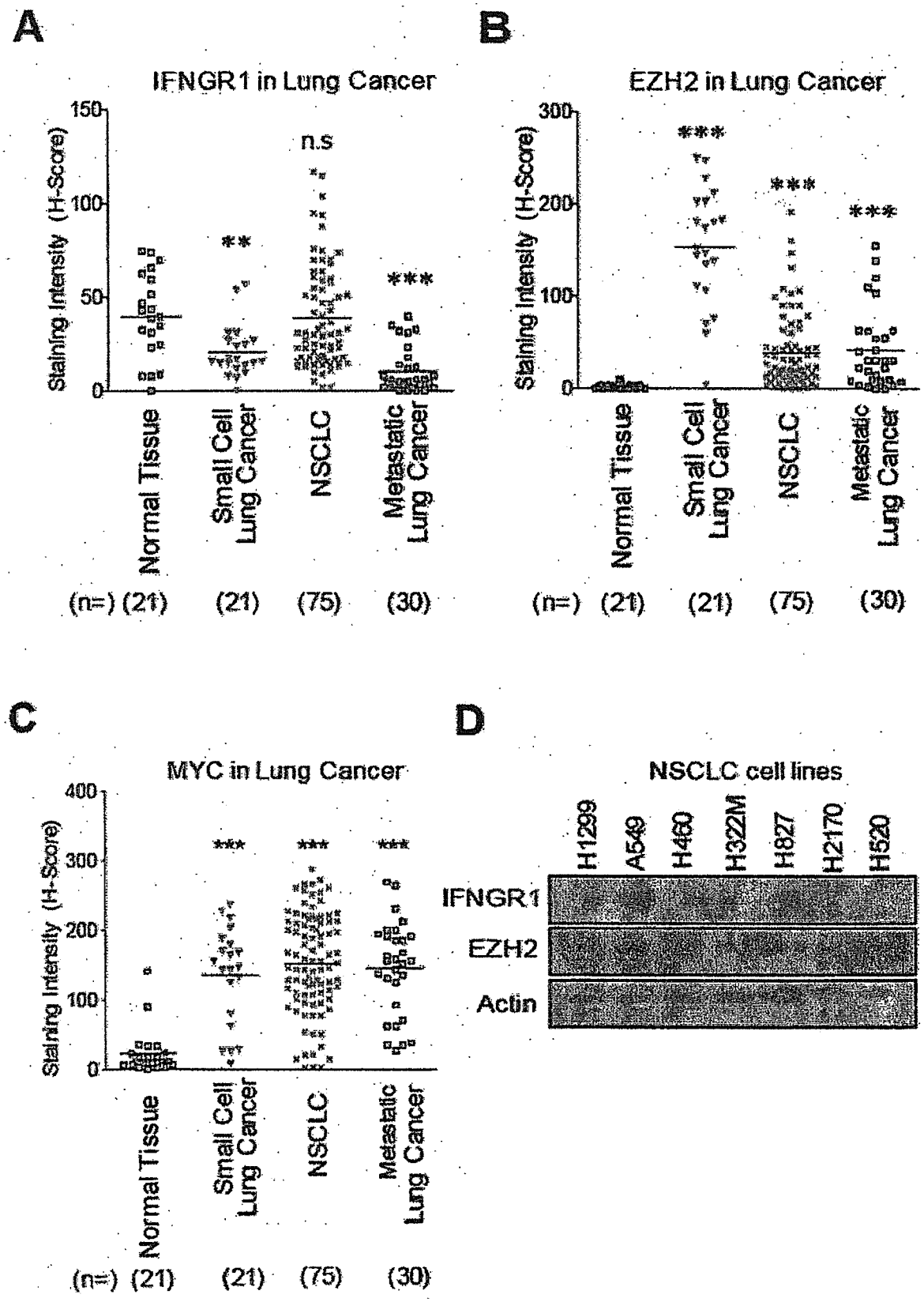
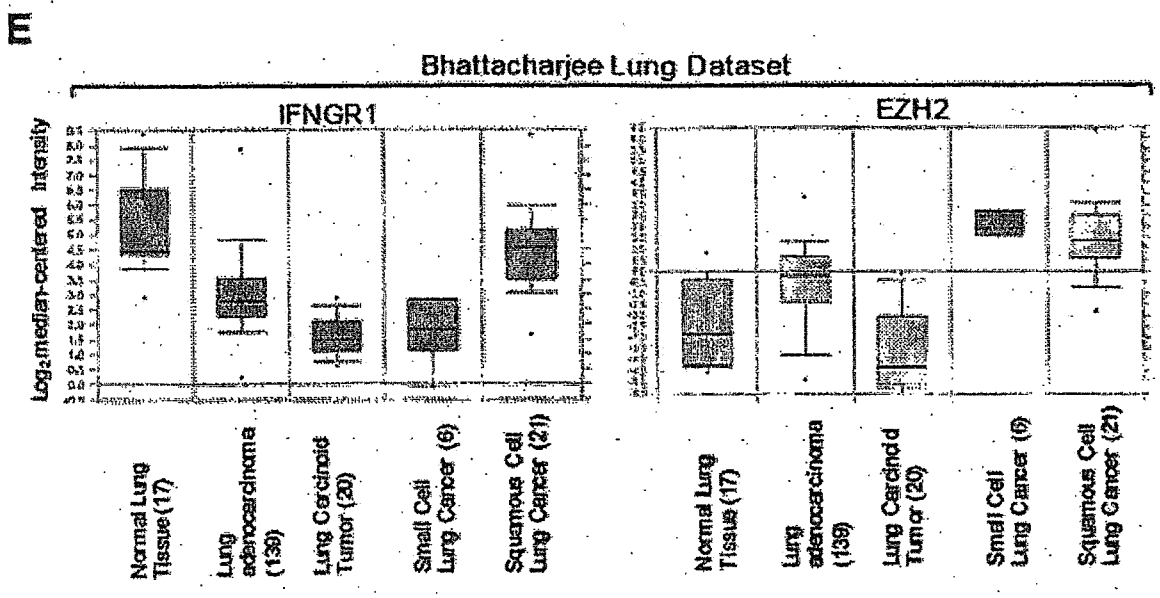

A
IFNGR1 in Lung Cancer
Staining Intensity (H-Score)
150
100
50
0
n.s
**
***
Normal Tissue
Small Cell Lung Cancer
NSCLC
Metastatic Lung Cancer
(n=) (21) (21) (75) (30)
B
EZH2 in Lung Cancer
Staining Intensity (H-Score)
300
200
100
0
***
***
***
Normal Tissue
Small Cell Lung Cancer
NSCLC
Metastatic Lung Cancer
(n=) (21) (21) (75) (30)
C
MYC in Lung Cancer
Staining Intensity (H-Score)
400
300
200
100
0
***
***
***
Normal Tissue
Small Cell Lung Cancer
NSCLC
Metastatic Lung Cancer
(n=) (21) (21) (75) (30)
D
NSCLC cell lines
H1299
A549
H460
H322M
H827
H2170
H520
IFNGR1
EZH2
Actin

FIG. 14 (Cont'd)


E
Bhattacharjee Lung Dataset
IFNGR1
EZH2
Log2 median-centered Intensity
Normal Lung Tissue (17)
Lung adenocarcinoma (139)
Lung Carcinoid Tumor (20)
Small Cell Lung Cancer (6)
Squamous Cell Lung Cancer (21)

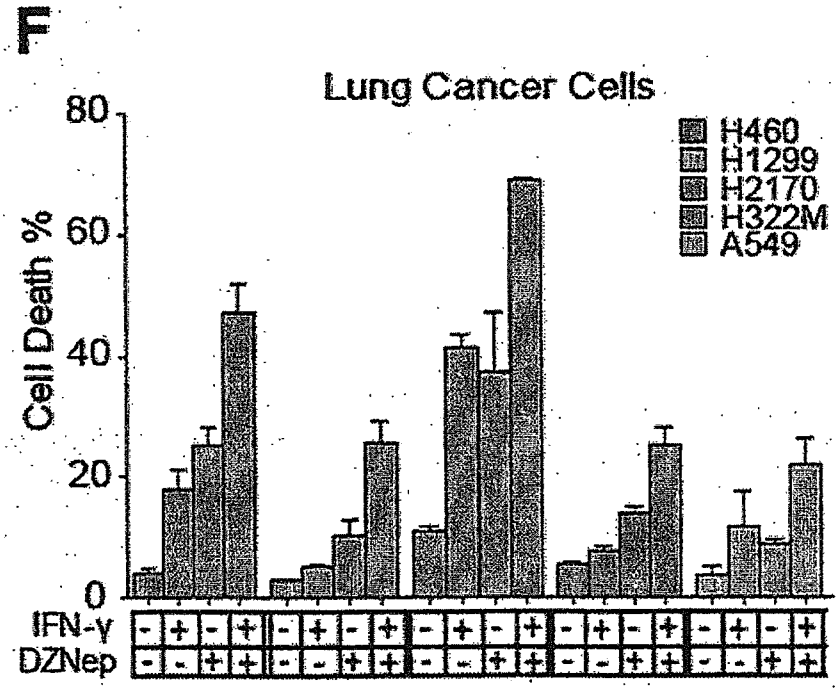
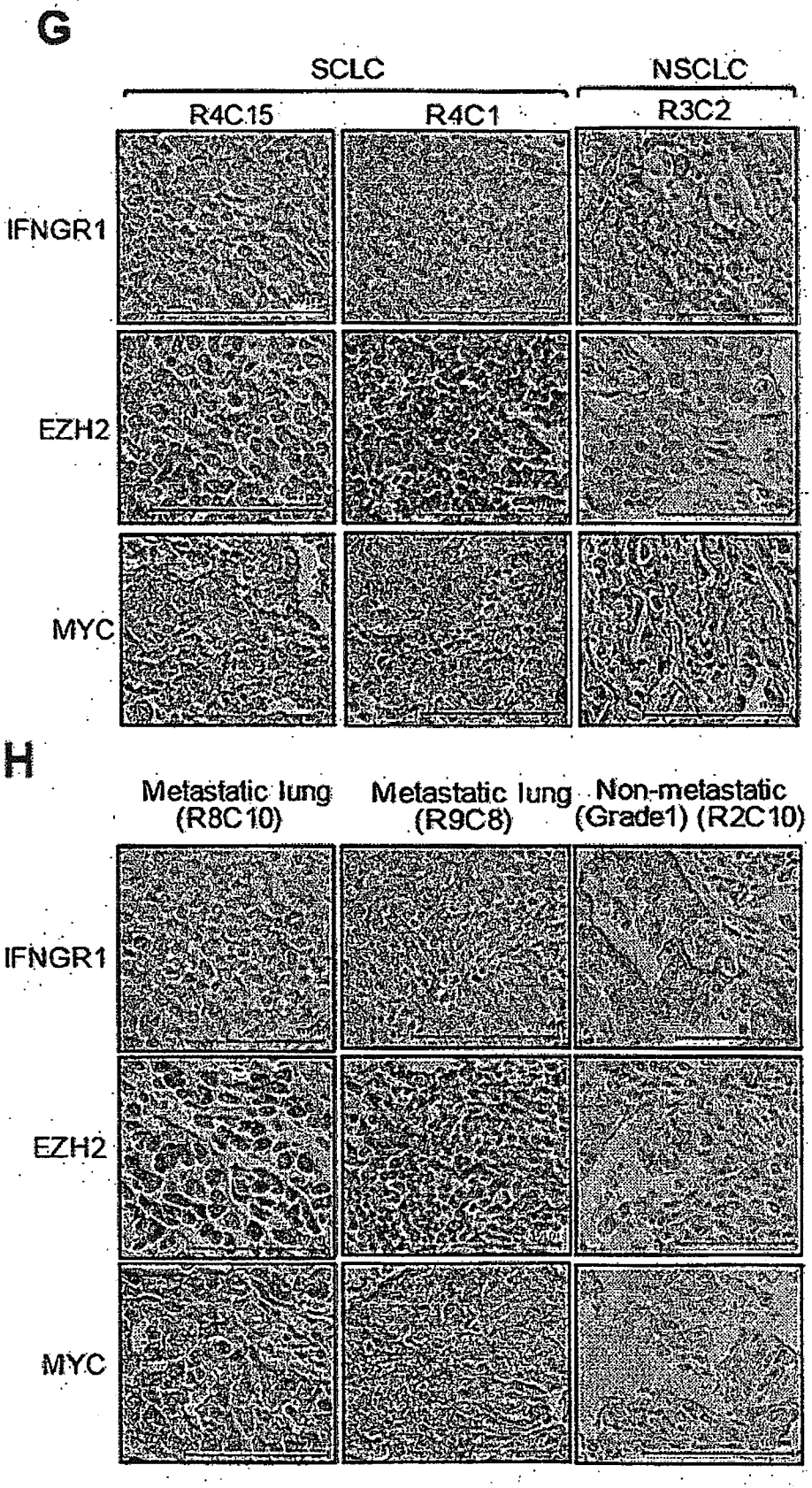

F
Lung Cancer Cells
H460
H1299
H2170
H322M
A549
Cell Death %
80
60
40
20
0
IFN-γ
DZNep

FIG. 14 (Cont'd)


G
SCLC
NSCLC
R4C15
R4C1
R3C2
IFNGR1
EZH2
MYC
H
Metastatic lung (R8C10)
Metastatic lung (R9C8)
Non-metastatic (Grade1) (R2C10)
IFNGR1
EZH2
MYC