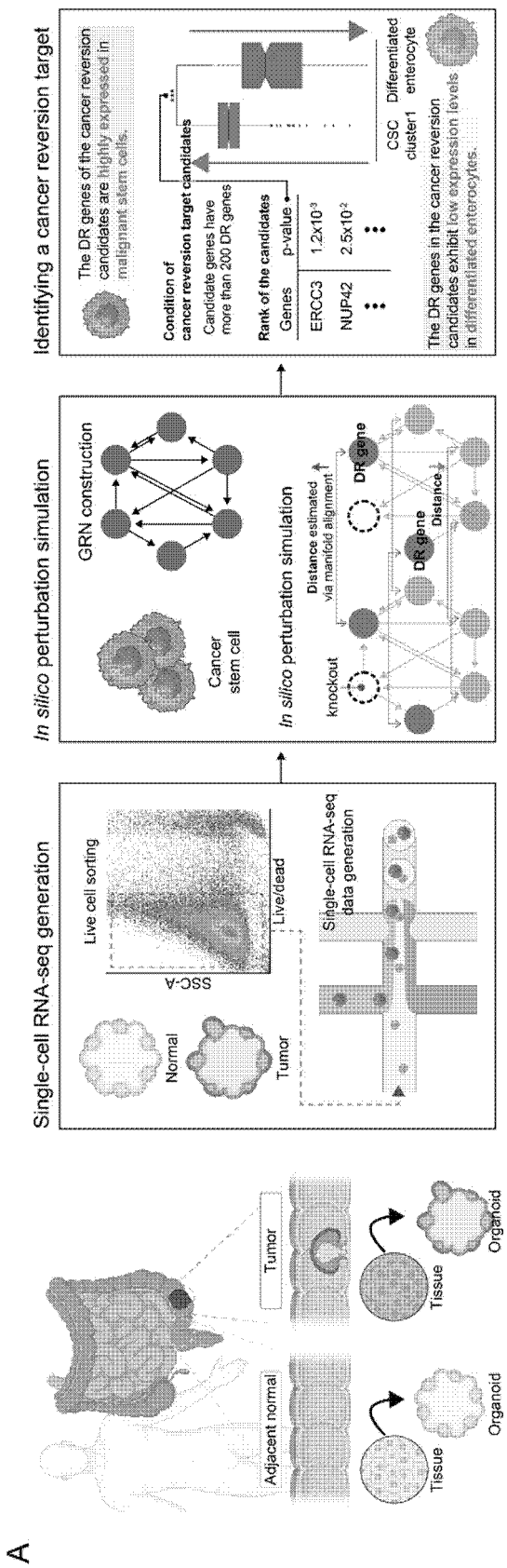
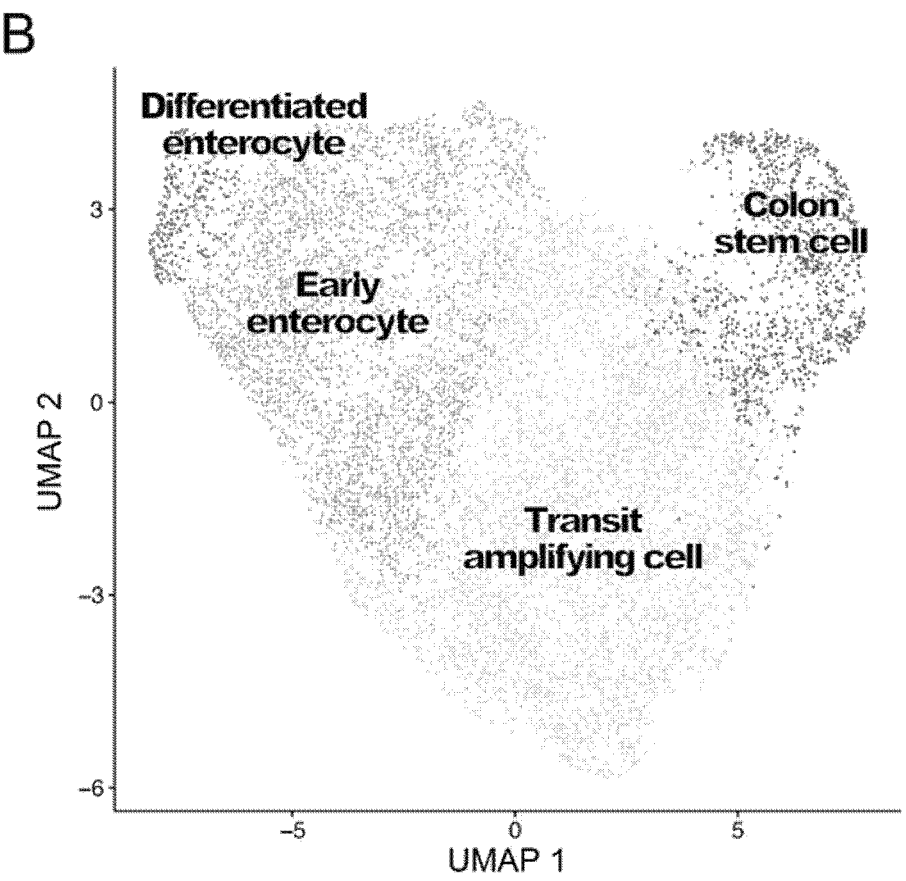
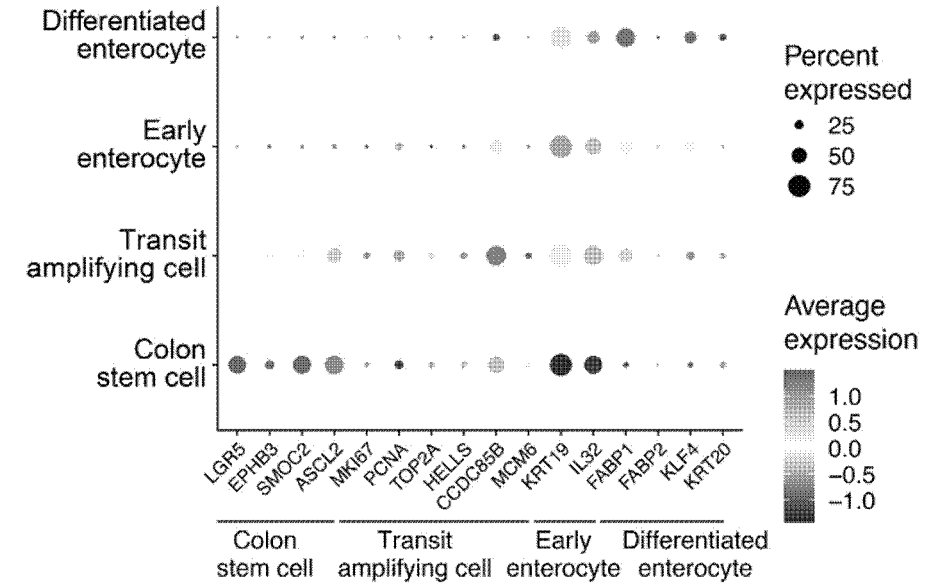
[Fig. 1A]


A
Adjacent normal
Tumor
Tissue
Organoid
Tissue
Organoid
Single-cell RNA-seq generation
Normal
Tumor
Live cell sorting
SSC-A
Live/dead
Single-cell RNA-seq data generation
In silico perturbation simulation
GRN construction
Cancer stem cell
In silico perturbation simulation
Distance estimated via manifold alignment
knockout
DR gene
DR gene
Distance
Identifying a cancer reversion target
The DR genes of the cancer reversion candidates are highly expressed in malignant stem cells.
Condition of cancer reversion target candidates
Candidate genes have more than 200 DR genes
Rank of the candidates
Genes
p-value
ERCC3
1.2x10-3
NUP42
2.5x10-2
CSC cluster1
Differentiated enterocyte
The DR genes in the cancer reversion candidates exhibit low expression levels in differentiated enterocytes.

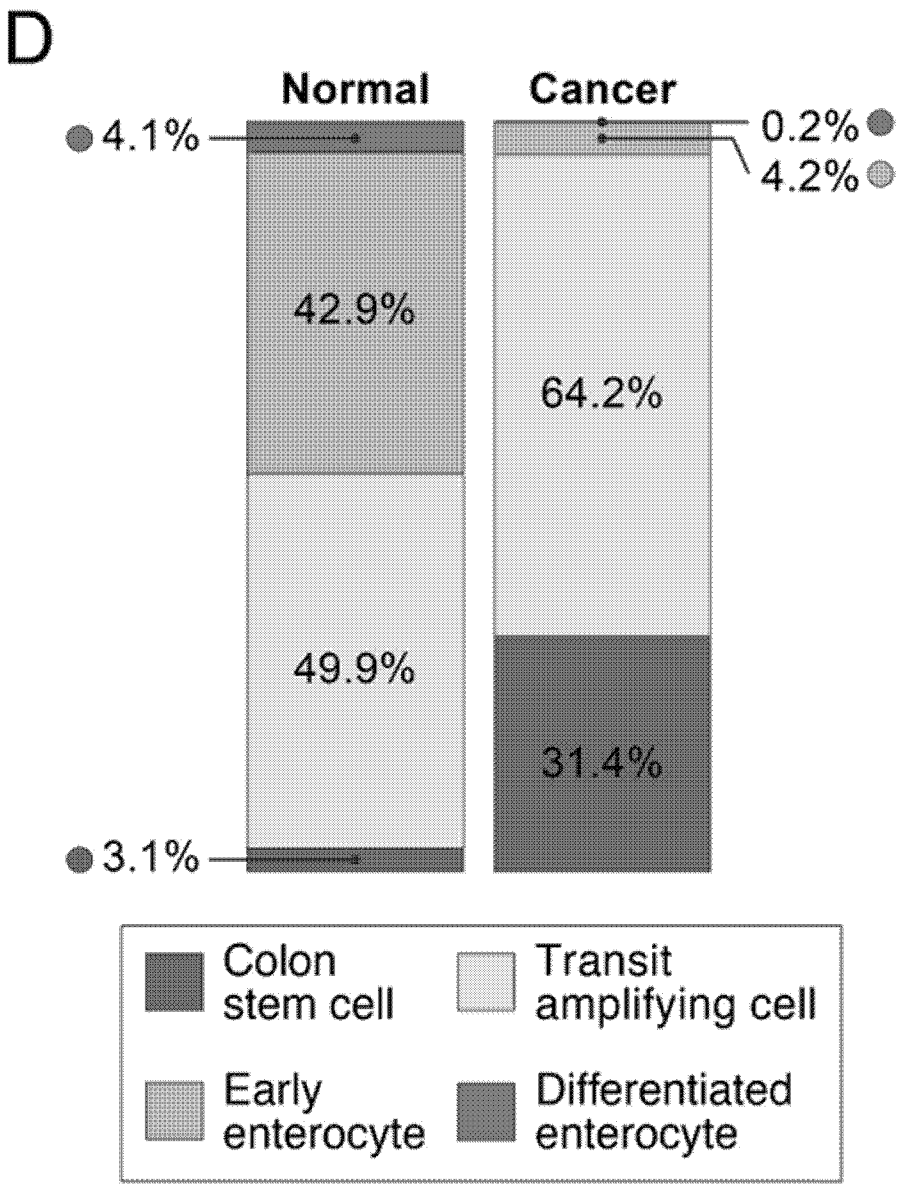
[Fig. 1B]


B
Differentiated enterocyte
Colon stem cell
Early enterocyte
Transit amplifying cell
UMAP 2
UMAP 1
3
0
−3
−6
−5
0
5

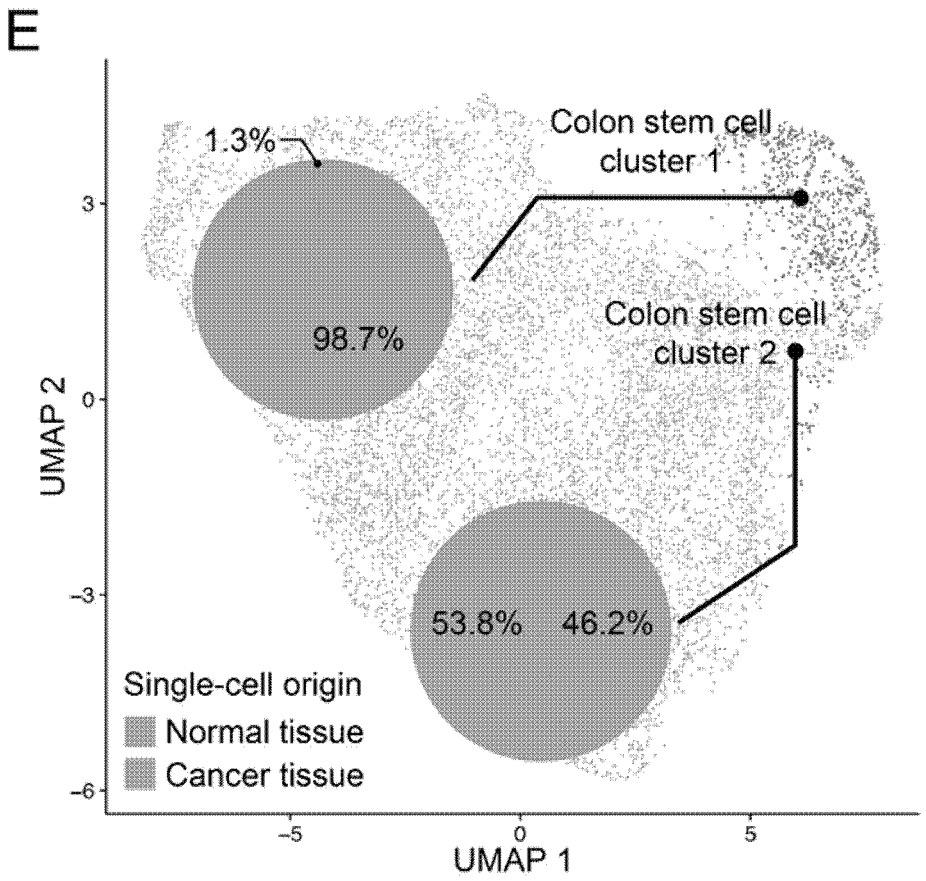
[Fig. 1C]


C
Differentiated enterocyte
Early enterocyte
Transit amplifying cell
Colon stem cell
LGR5
EPHB3
SMOC2
ASCL2
MKI67
PCNA
TOP2A
HELLS
CCDC85B
MCM6
KRT19
IL32
FABP1
FABP2
KLF4
KRT20
Colon stem cell
Transit amplifying cell
Early enterocyte
Differentiated enterocyte
Percent expressed
25
50
75
Average expression
1.0
0.5
0.0
−0.5
−1.0

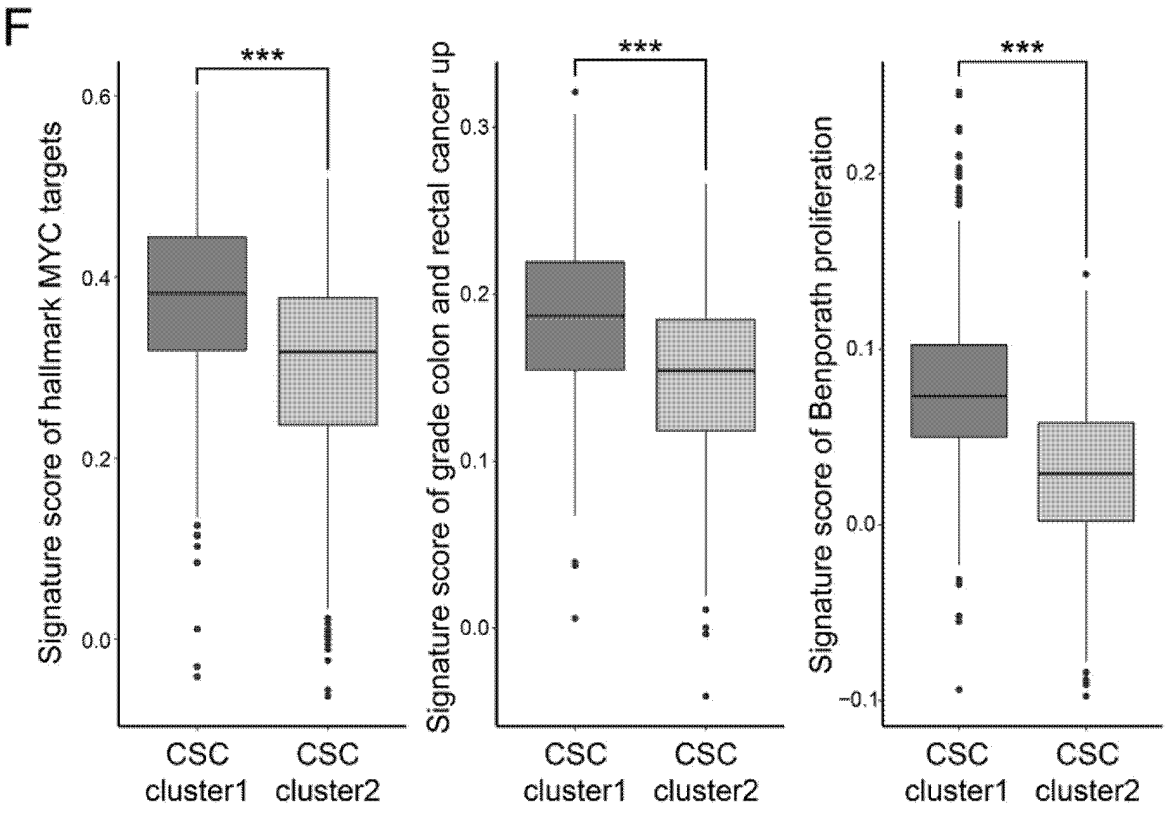
[Fig. 1D]


D
Normal
Cancer
4.1%
42.9%
49.9%
3.1%
0.2%
4.2%
64.2%
31.4%
Colon stem cell
Transit amplifying cell
Early enterocyte
Differentiated enterocyte

[Fig. 1E]

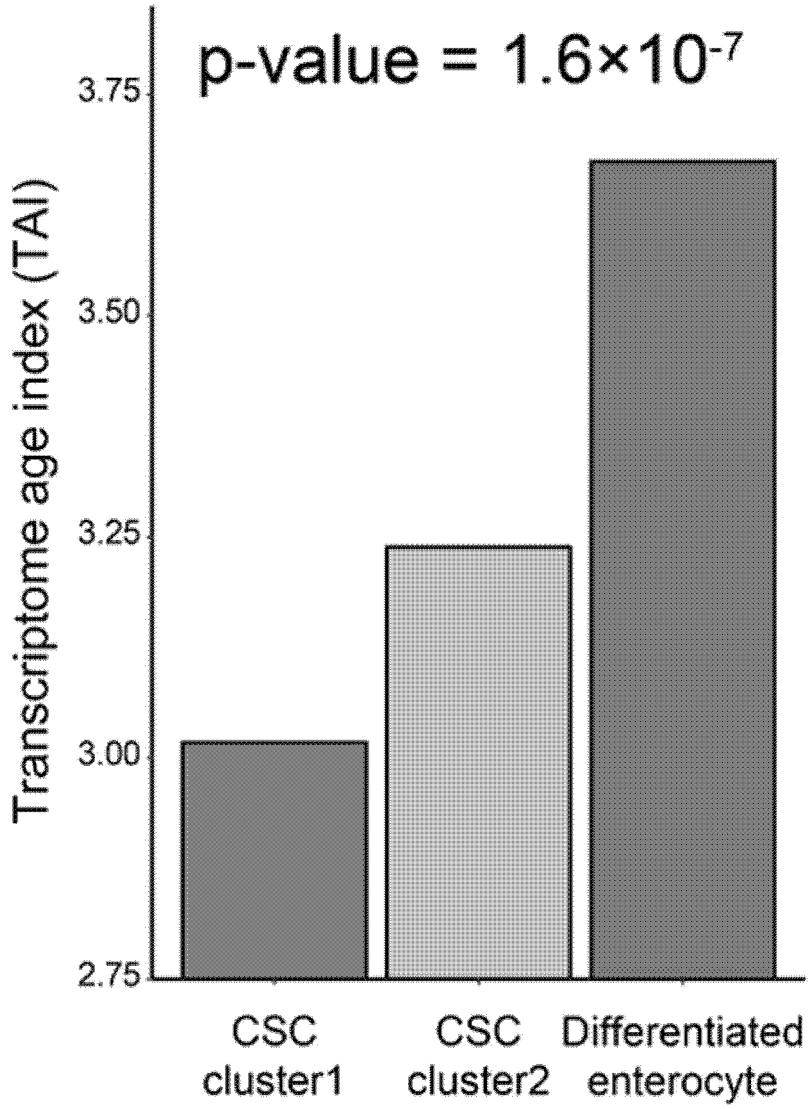

E
1.3%
98.7%
Colon stem cell cluster 1
Colon stem cell cluster 2
53.8%
46.2%
Single-cell origin
Normal tissue
Cancer tissue
UMAP 2
UMAP 1
3
0
−3
−6
−5
0
5

[Fig. 1F]

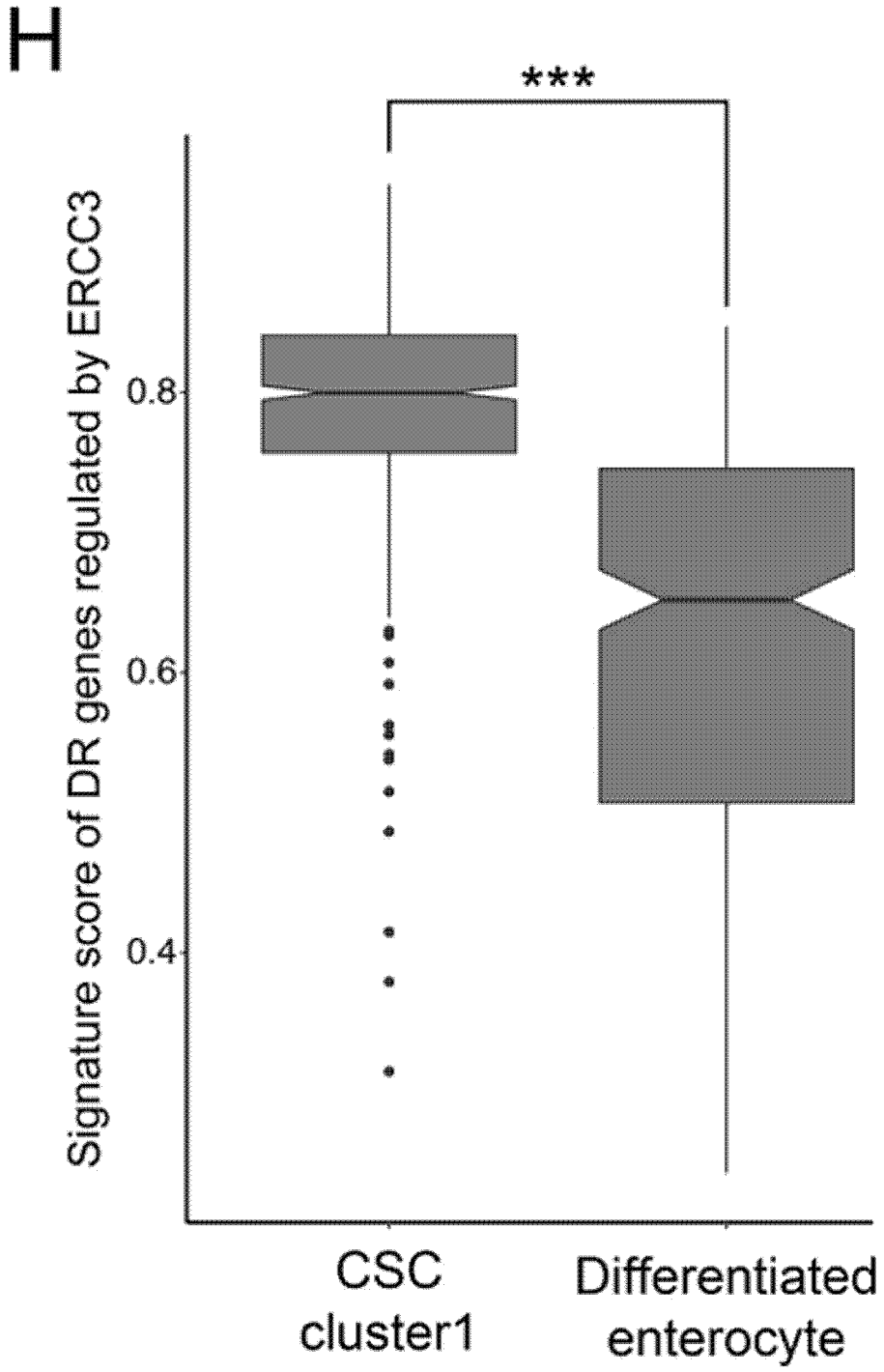

F
Signature score of hallmark MYC targets
Signature score of grade colon and rectal cancer up
Signature score of Benporath proliferation
***
CSC cluster1
CSC cluster2
0.6
0.4
0.2
0.0
0.3
0.1
−0.1

[Fig. 1G]

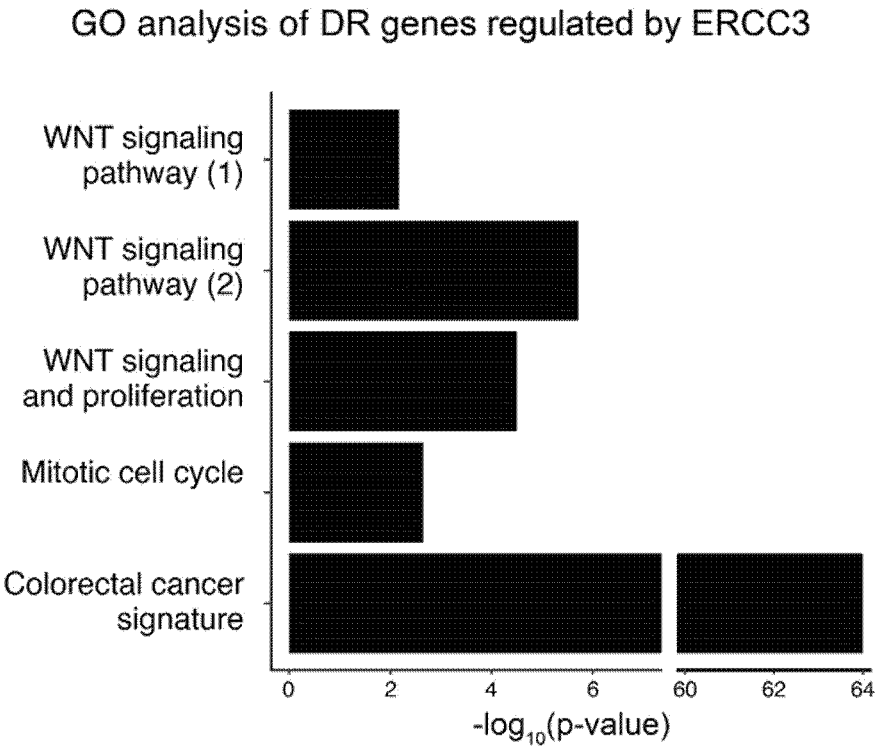
G


p-value = 1.6×10-7
Transcriptome age index (TAI)
3.75
3.50
3.25
3.00
2.75
CSC cluster1
CSC cluster2
Differentiated enterocyte

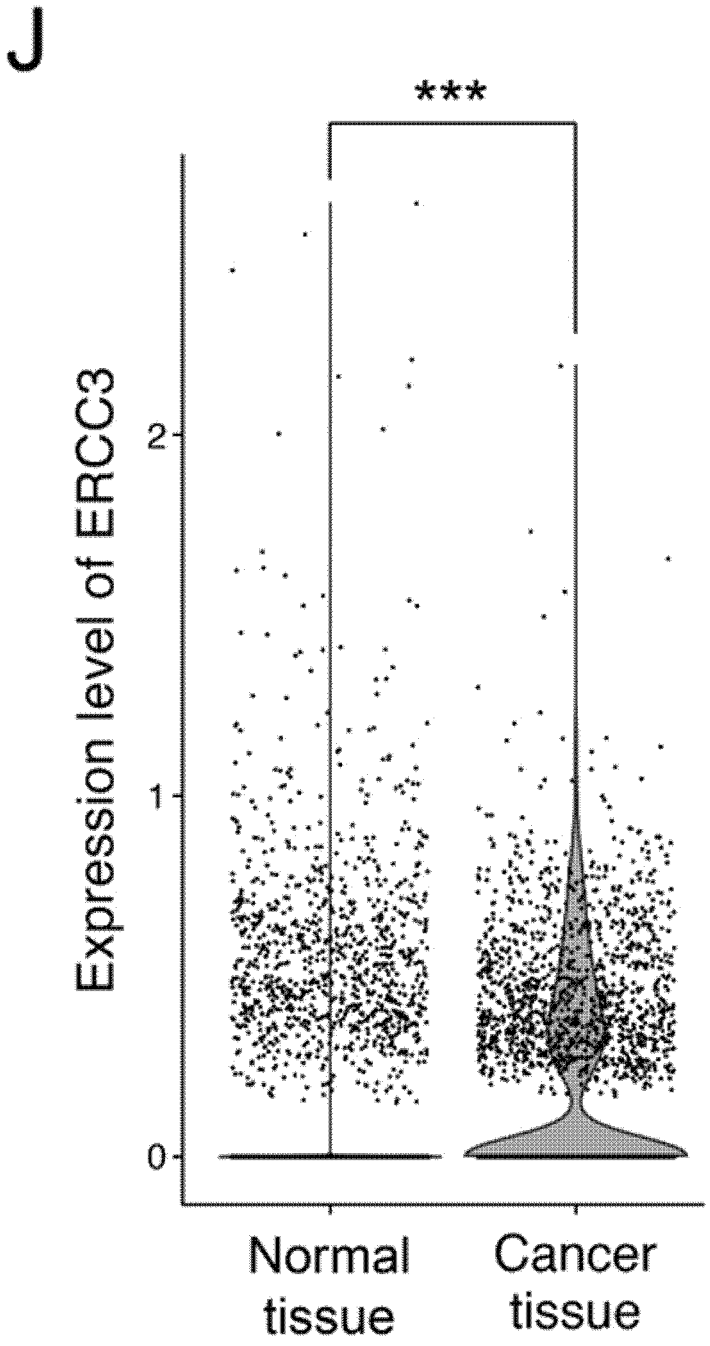
[Fig. 1H]


H
***
Signature score of DR genes regulated by ERCC3
0.8
0.6
0.4
CSC cluster1
Differentiated enterocyte

[Fig. 1l]

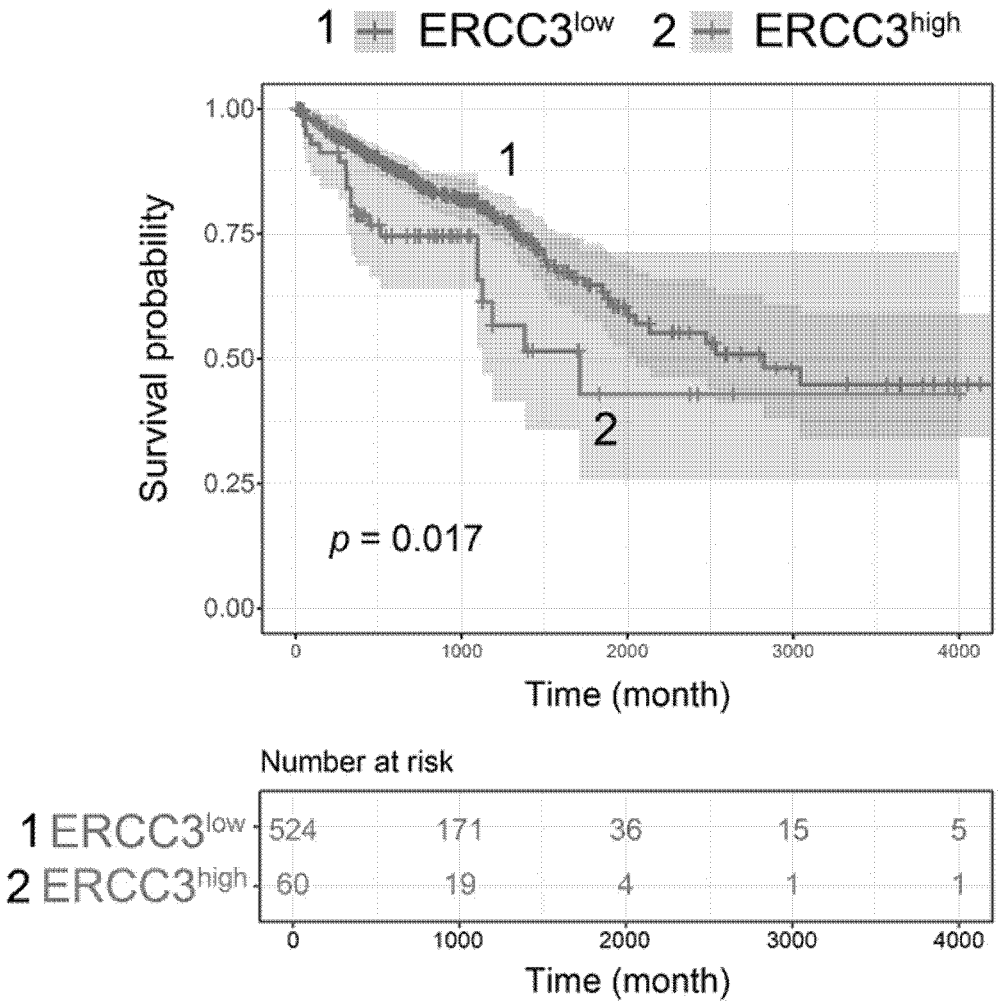

GO analysis of DR genes regulated by ERCC3
WNT signaling pathway (1)
WNT signaling pathway (2)
WNT signaling and proliferation
Mitotic cell cycle
Colorectal cancer signature
0
2
4
6
60
62
64
-log10(p-value)

[Fig. 1J]


J
***
Expression level of ERCC3
2
1
0
Normal tissue
Cancer tissue

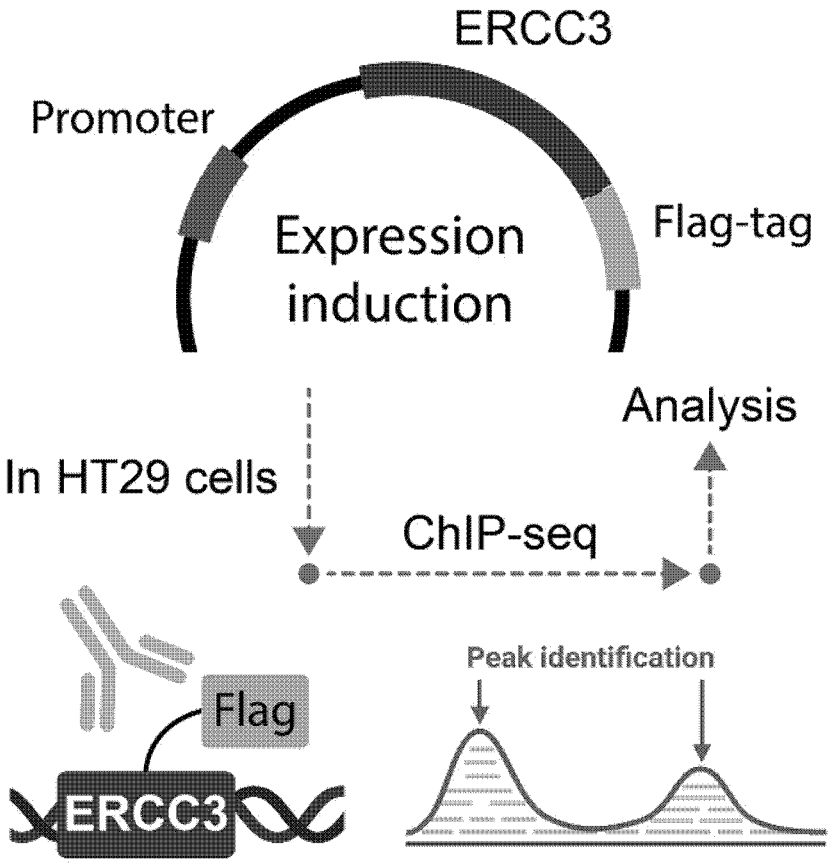
[Fig. 1K]

K


1 ERCC3low 2 ERCC3high
Survival probability
1.00
0.75
0.50
0.25
0.00
1
2
p = 0.017
0
1000
2000
3000
4000
Time (month)
Number at risk
1 ERCC3low 524 171 36 15 5
2 ERCC3high 60 19 4 1 1
0
1000
2000
3000
4000
Time (month)

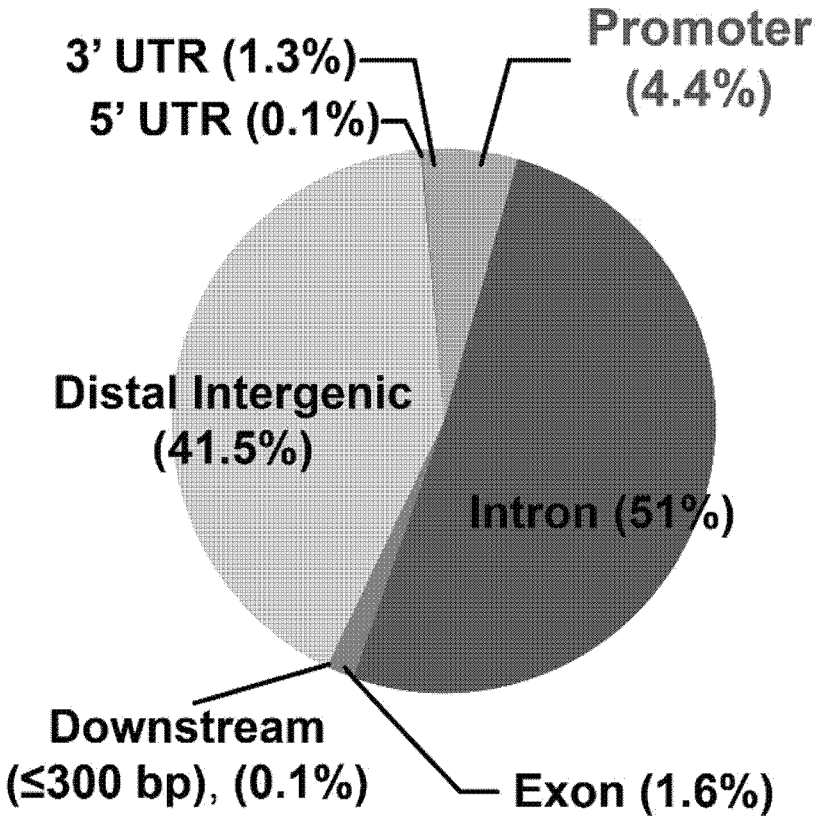
[Fig. 2A]

A


ERCC3
Promoter
Flag-tag
Expression induction
Analysis
In HT29 cells
ChIP-seq
Peak identification
Flag
ERCC3

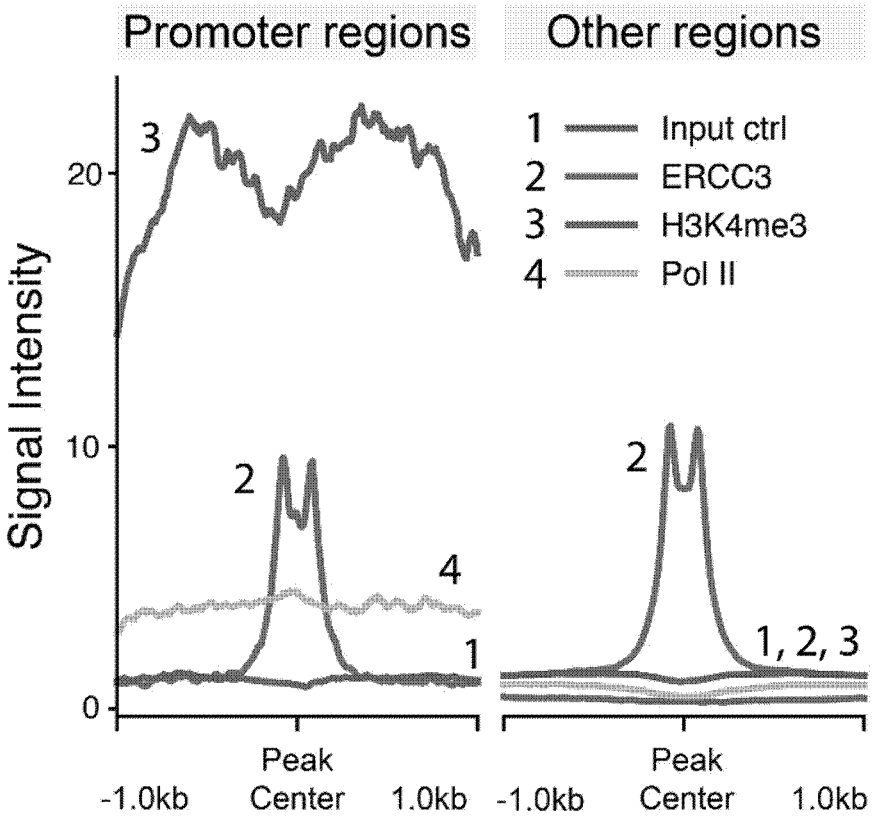
[Fig. 2B]

B

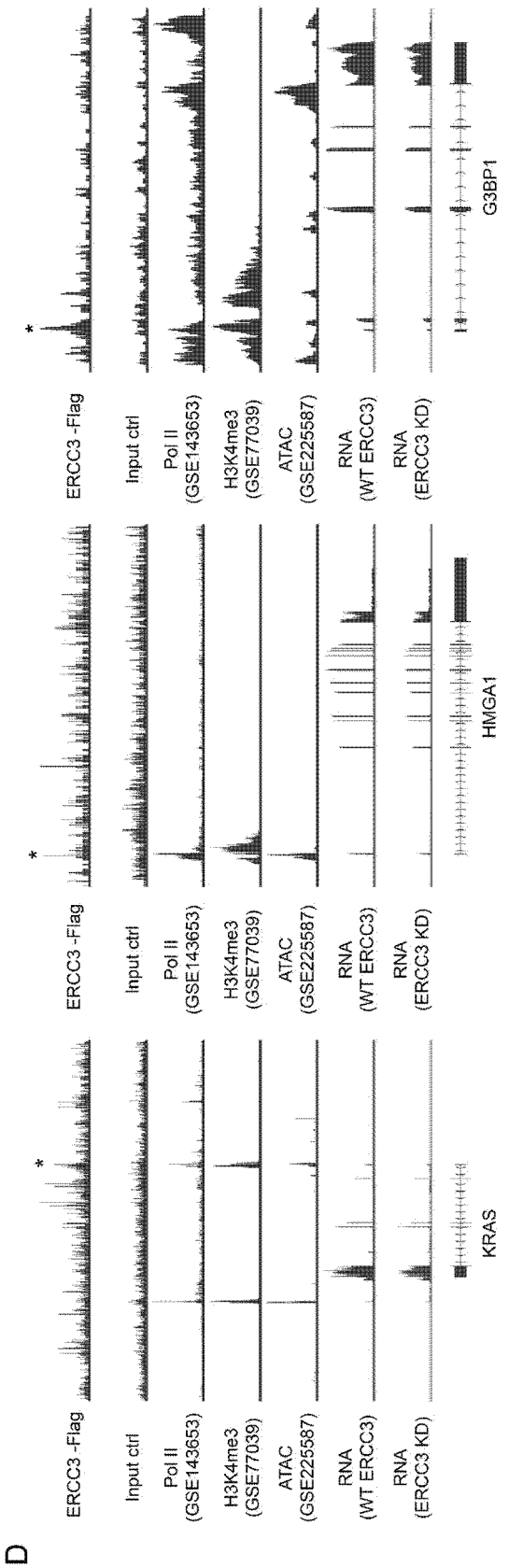

3' UTR (1.3%)
5' UTR (0.1%)
Promoter (4.4%)
Distal Intergenic (41.5%)
Intron (51%)
Downstream (≤300 bp), (0.1%)
Exon (1.6%)

[Fig. 2C]

C

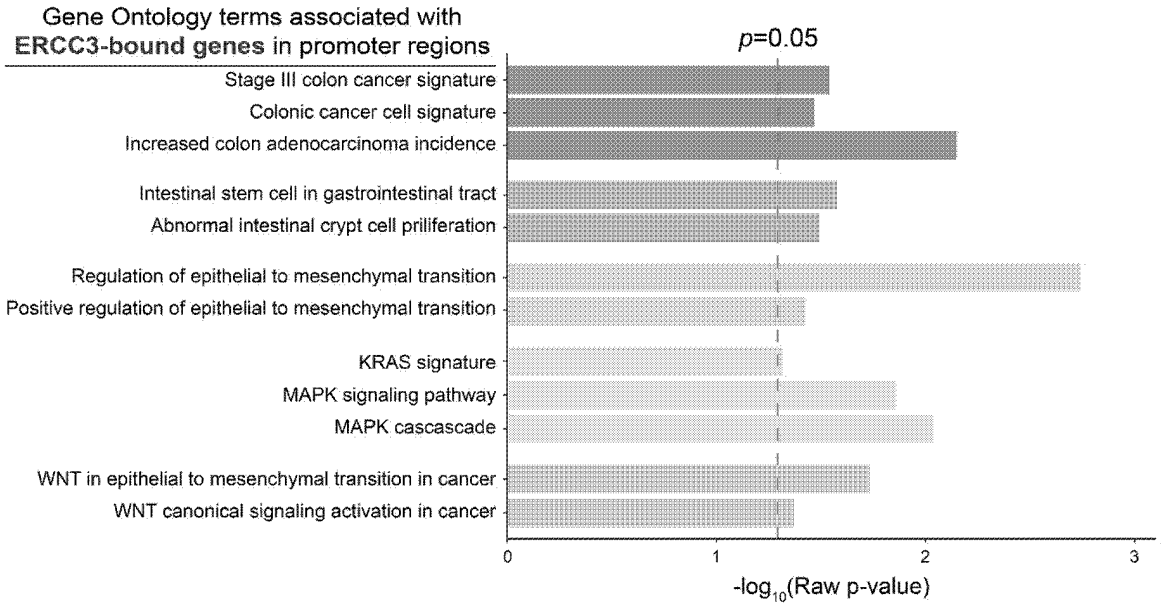

Promoter regions
Other regions
1 Input ctrl
2 ERCC3
3 H3K4me3
4 Pol II
Signal Intensity
20
10
0
3
2
4
1
2
1, 2, 3
Peak
Center
-1.0kb
1.0kb
Peak
Center
-1.0kb
1.0kb

[Fig. 2D]


D
ERCC3 -Flag
Input ctrl
Pol II (GSE143653)
H3K4me3 (GSE77039)
ATAC (GSE225587)
RNA (WT ERCC3)
RNA (ERCC3 KD)
KRAS
HMGA1
G3BP1

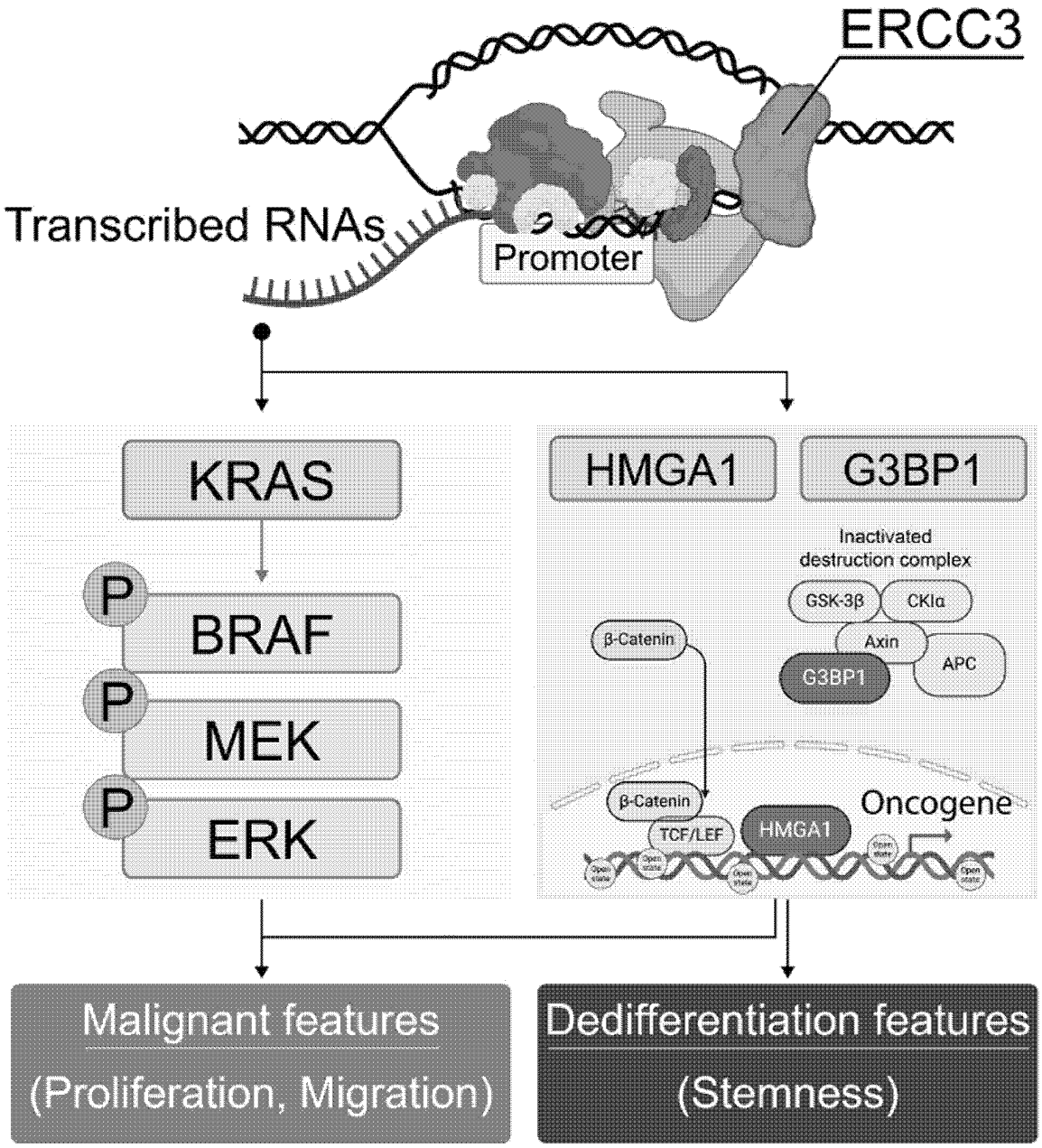
[Fig. 2E]

E


Gene Ontology terms associated with
ERCC3-bound genes in promoter regions
p=0.05
Stage III colon cancer signature
Colonic cancer cell signature
Increased colon adenocarcinoma incidence
Intestinal stem cell in gastrointestinal tract
Abnormal intestinal crypt cell priliferation
Regulation of epithelial to mesenchymal transition
Positive regulation of epithelial to mesenchymal transition
KRAS signature
MAPK signaling pathway
MAPK cascascade
WNT in epithelial to mesenchymal transition in cancer
WNT canonical signaling activation in cancer
0
1
2
3
-log10(Raw p-value)

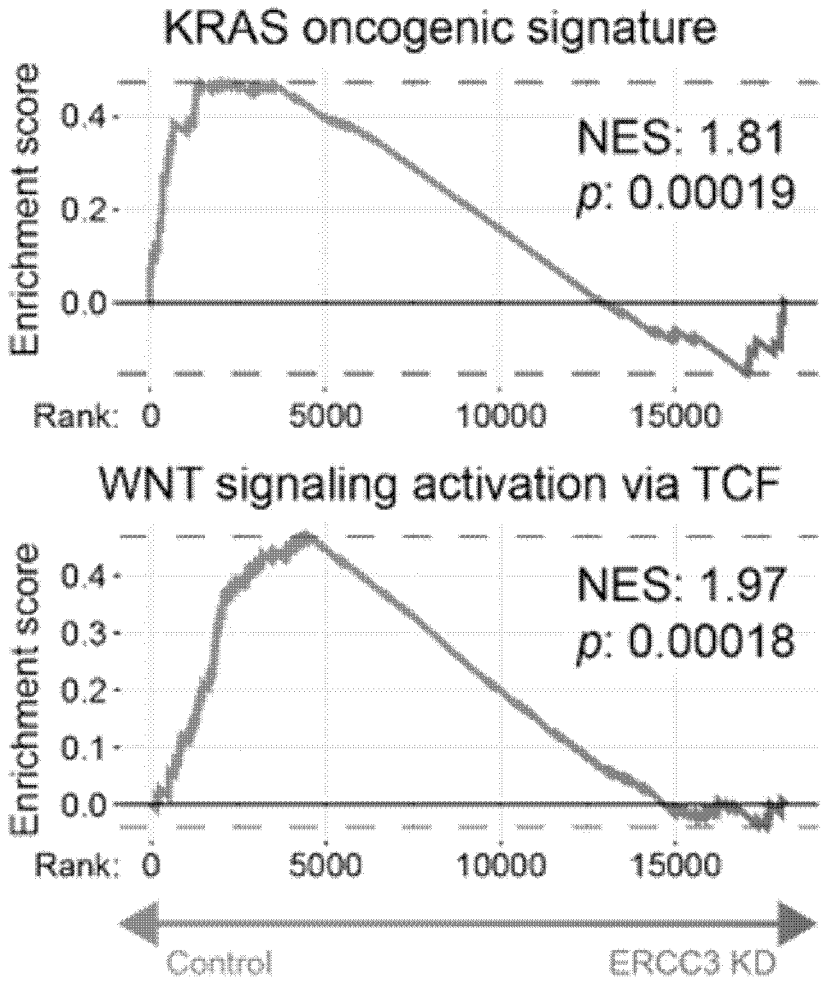
[Fig. 2F]

F

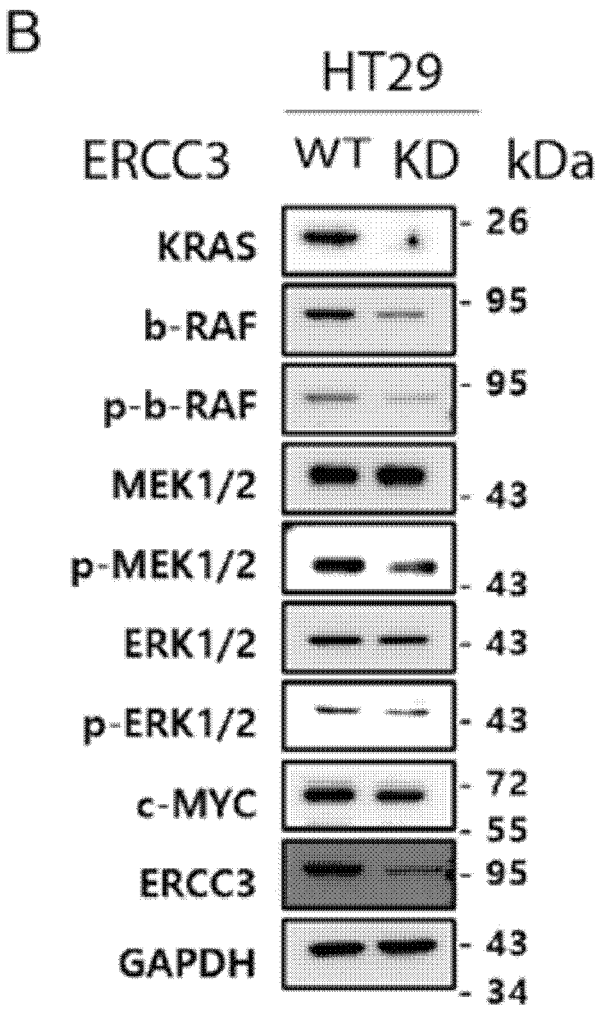

ERCC3
Transcribed RNAs
Promoter
KRAS
P
BRAF
P
MEK
P
ERK
HMGA1
G3BP1
Inactivated
destruction complex
GSK-3β
CKIα
Axin
APC
G3BP1
β-Catenin
β-Catenin
TCF/LEF
HMGA1
Oncogene
Malignant features
(Proliferation, Migration)
Dedifferentiation features
(Stemness)

[Fig. 3A]

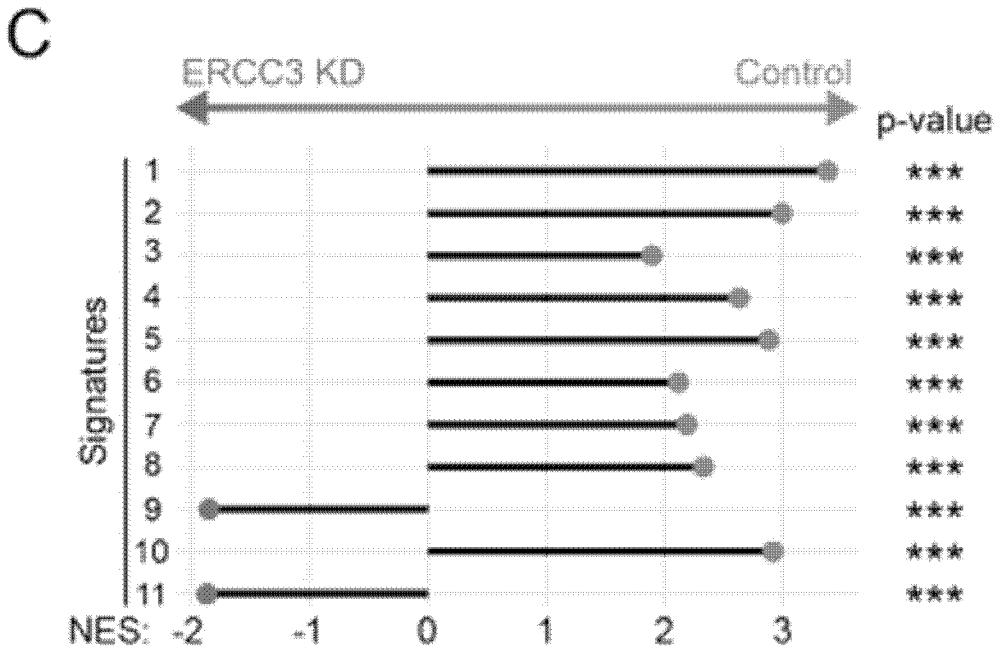
A


KRAS oncogenic signature
Enrichment score
0.4
0.2
0.0
NES: 1.81
p: 0.00019
Rank: 0
5000
10000
15000
WNT signaling activation via TCF
Enrichment score
0.4
0.3
0.2
0.1
0.0
NES: 1.97
p: 0.00018
Rank: 0
5000
10000
15000
Control
ERCC3 KD

[Fig. 3B]

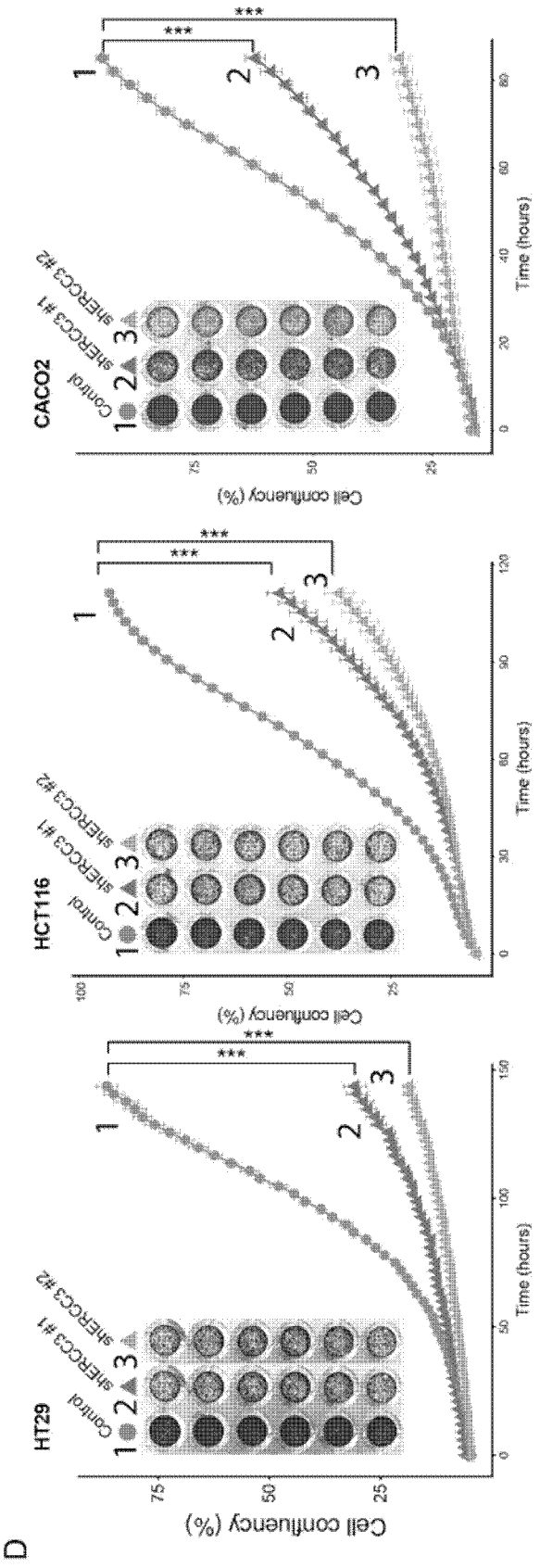

B
HT29
ERCC3
WT
KD
kDa
KRAS
b-RAF
p-b-RAF
MEK1/2
p-MEK1/2
ERK1/2
p-ERK1/2
c-MYC
ERCC3
GAPDH
26
95
95
43
43
43
43
72
55
95
43
34

[Fig. 3C]

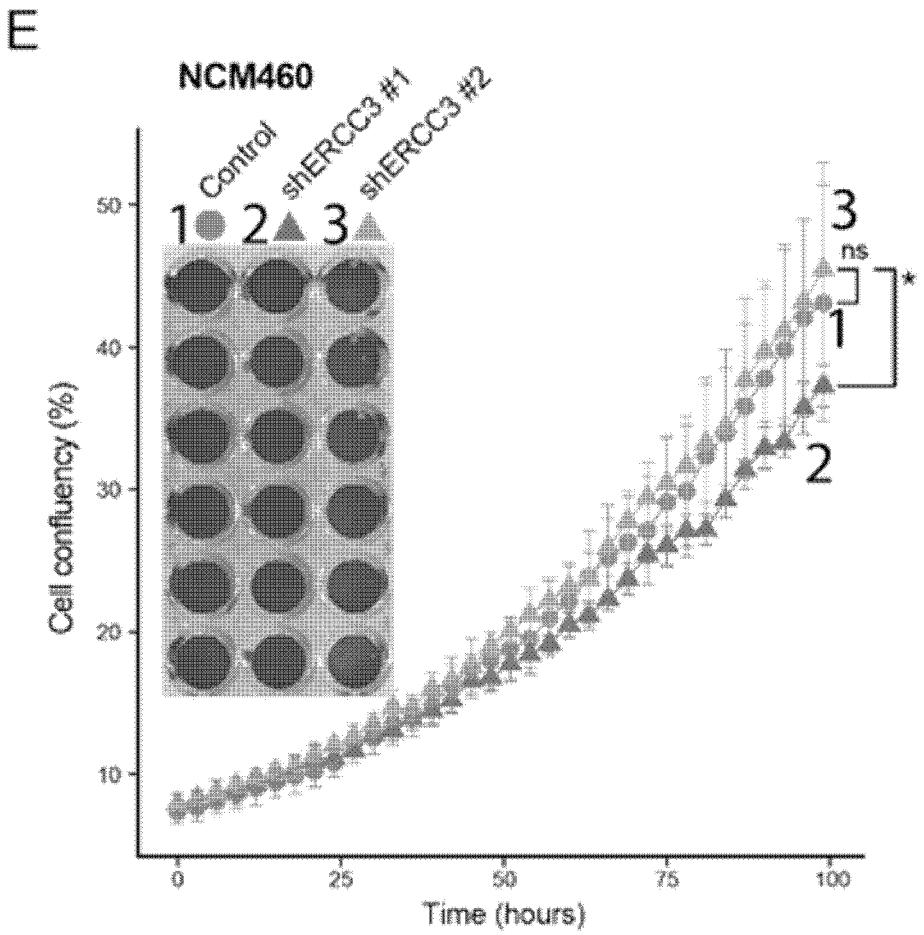


1. Hallmark MYC Target V1
2. DANG MYC target genes (Up)
3. BILD MYC oncogenic signature
4. BENPORATH proliferation
5. PCNA signature
6. MKI67 signature
7. SARRIO EMT up signature
8. BIDUS metastasis (UP)
9. BIDUS metastasis (Down)
10. GRADE colorectal cancer (Up)
11. GRADE colorectal cancer (Down)

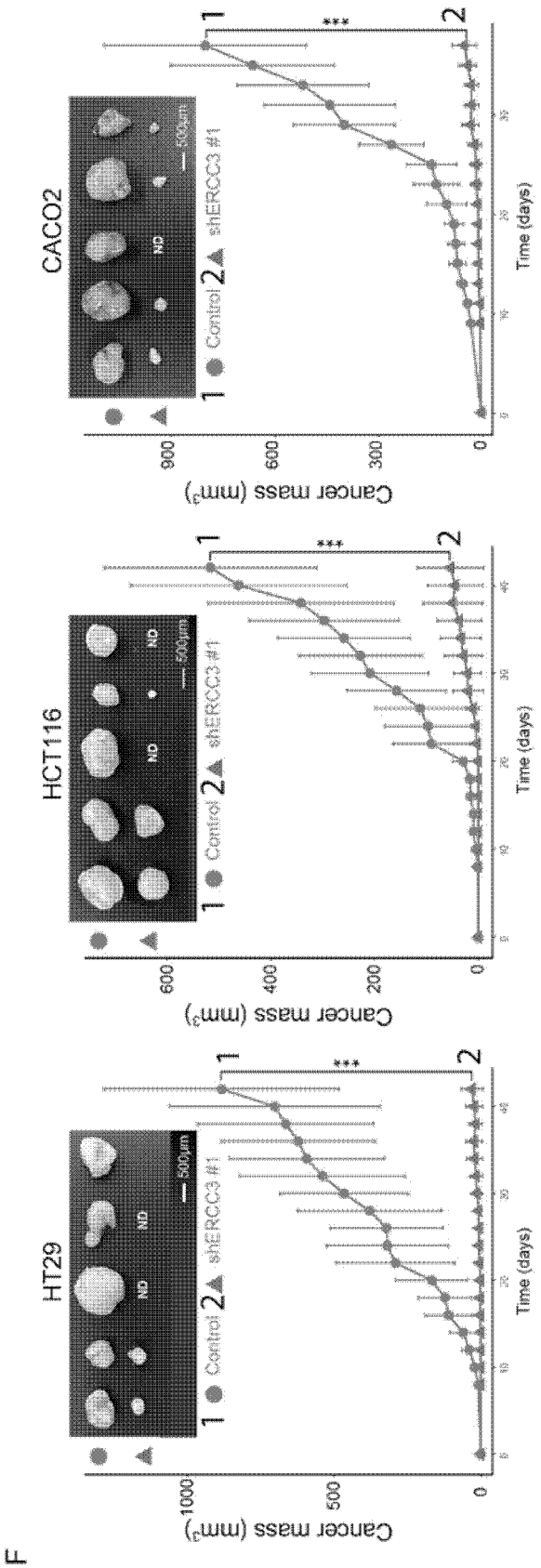
[Fig. 3D]


D
HT29
HCT116
CACO2
Control
shERCC3 #1
shERCC3 #2
Cell confluency (%)
Time (hours)
***

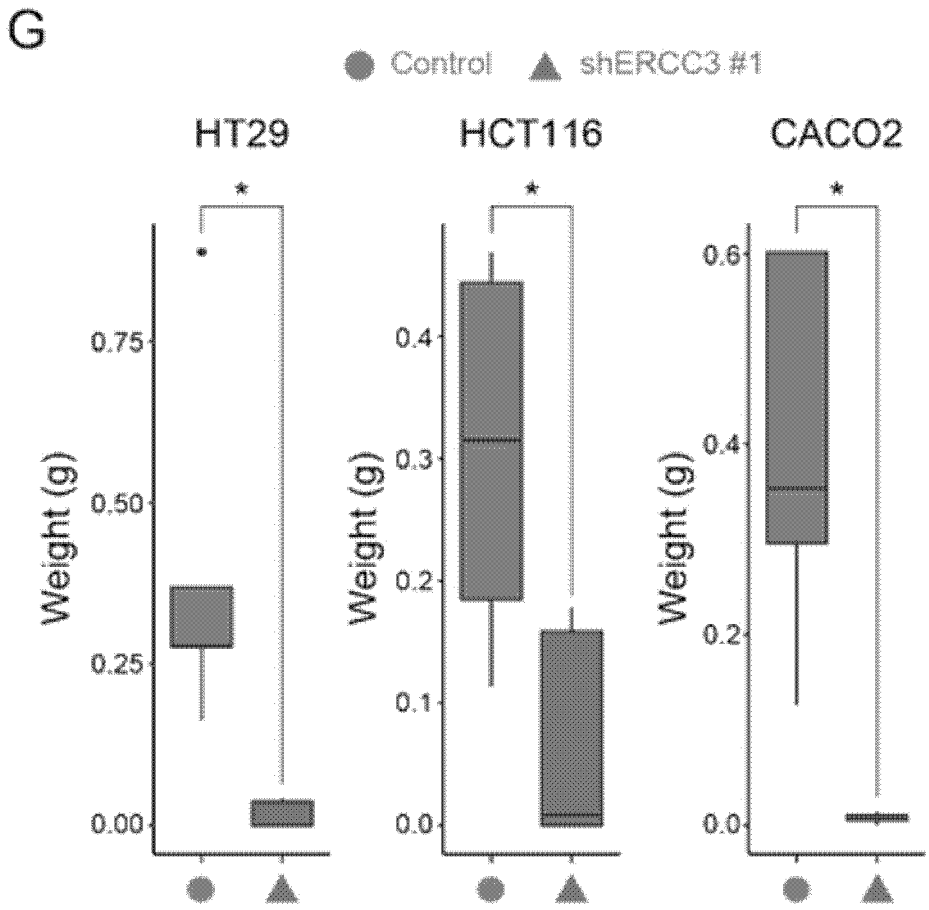
[Fig. 3E]


E
NCM460
Control
shERCC3 #1
shERCC3 #2
1
2
3
ns
*
Cell confluency (%)
Time (hours)
10
20
30
40
50
0
25
50
75
100

[Fig. 3F]

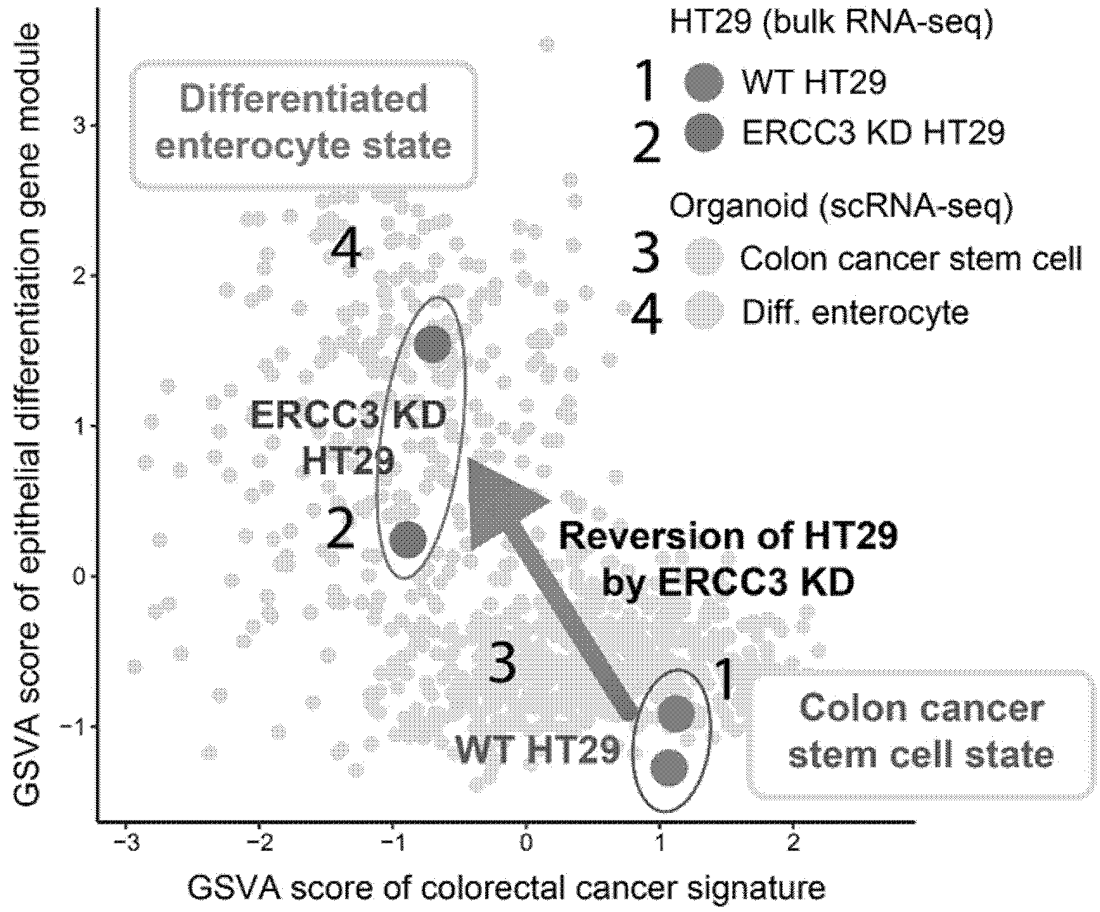

F
HT29
HCT116
CACO2
Control
shERCC3 #1
ND
500μm
***
Cancer mass (mm³)
Time (days)
1
2

[Fig. 3G]


G
Control
shERCC3 #1
HT29
HCT116
CACO2
Weight (g)
0.00
0.25
0.50
0.75
0.0
0.1
0.2
0.3
0.4
0.2
0.4
0.6
*

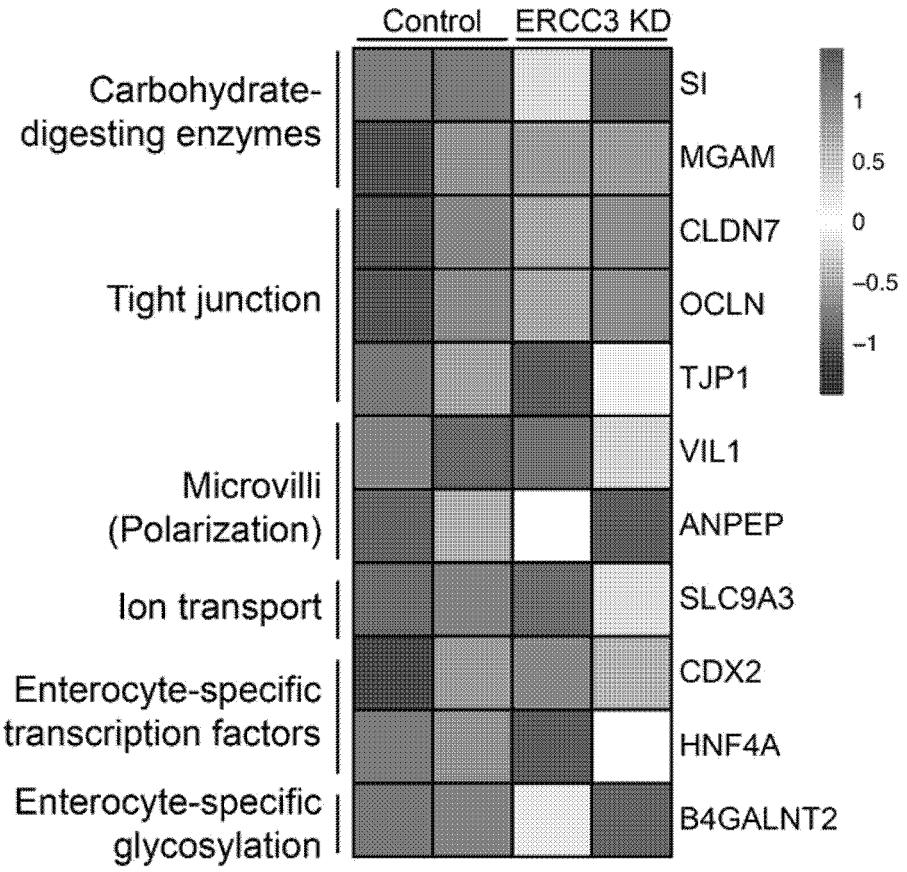
[Fig. 4A]

A


HT29 (bulk RNA-seq)
1 WT HT29
2 ERCC3 KD HT29
Organoid (scRNA-seq)
3 Colon cancer stem cell
4 Diff. enterocyte
Differentiated enterocyte state
4
ERCC3 KD HT29
2
Reversion of HT29 by ERCC3 KD
3
1
WT HT29
Colon cancer stem cell state
GSVA score of epithelial differentiation gene module
GSVA score of colorectal cancer signature
3
2
1
0
−1
−3
−2
−1
0
1
2

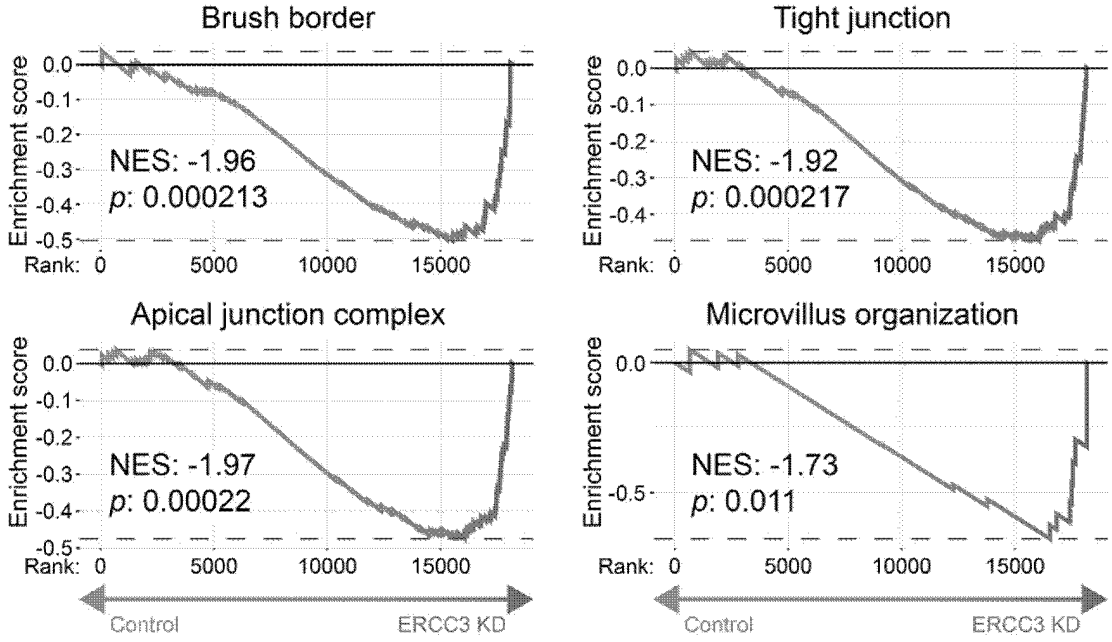
[Fig. 4B]

B

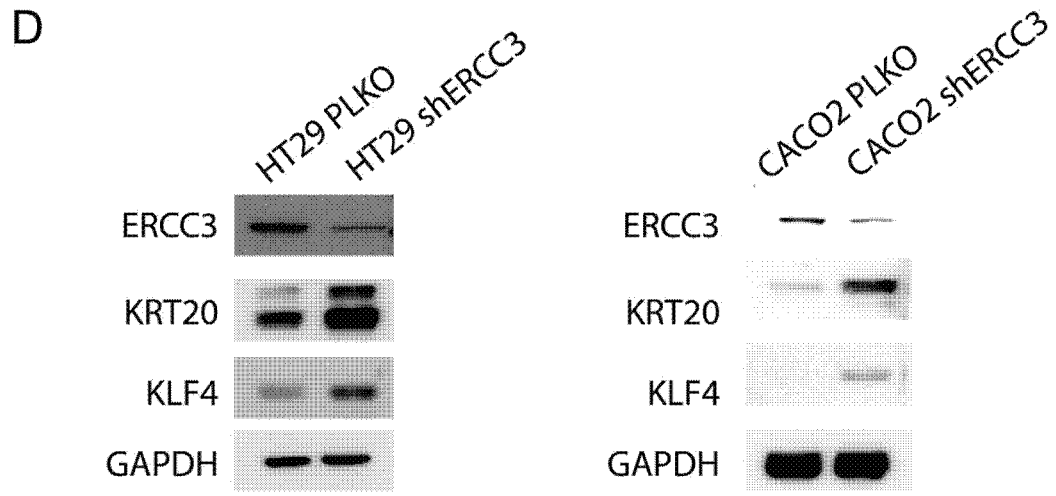


[Fig. 4C]

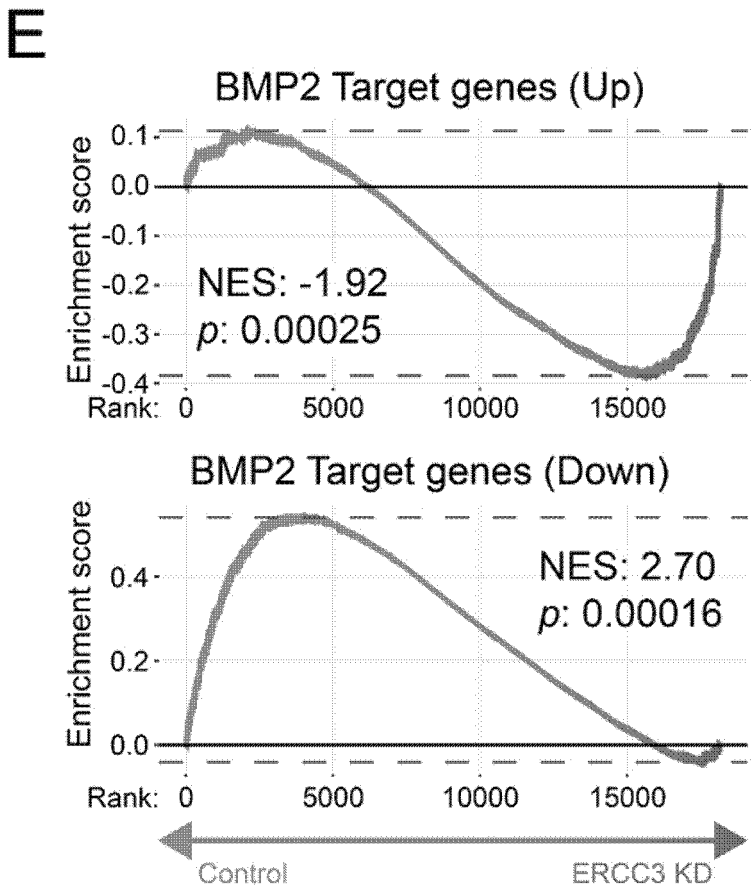
C



[Fig. 4D]

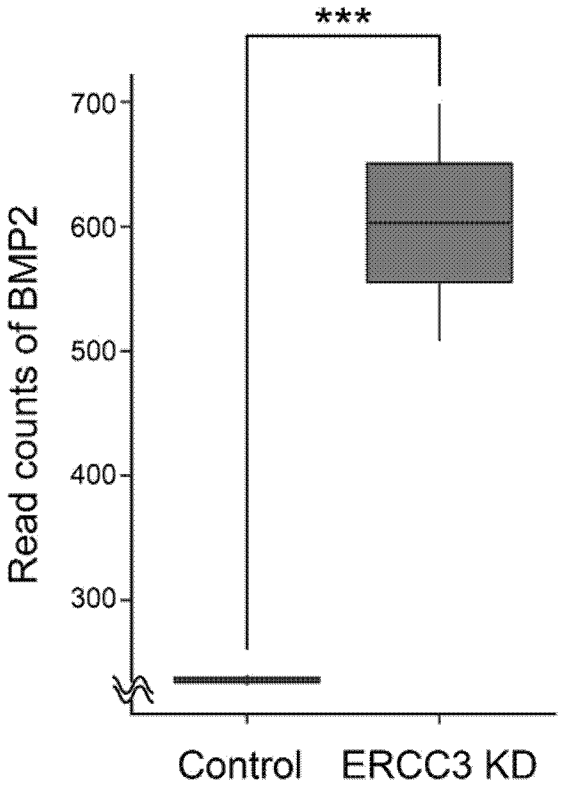

D
HT29 PLKO
HT29 shERCC3
CACO2 PLKO
CACO2 shERCC3
ERCC3
KRT20
KLF4
GAPDH
ERCC3
KRT20
KLF4
GAPDH

[Fig. 4E]


E
BMP2 Target genes (Up)
Enrichment score
0.1
0.0
-0.1
-0.2
-0.3
-0.4
NES: -1.92
p: 0.00025
Rank: 0
5000
10000
15000
BMP2 Target genes (Down)
Enrichment score
0.4
0.2
0.0
NES: 2.70
p: 0.00016
Rank: 0
5000
10000
15000
Control
ERCC3 KD

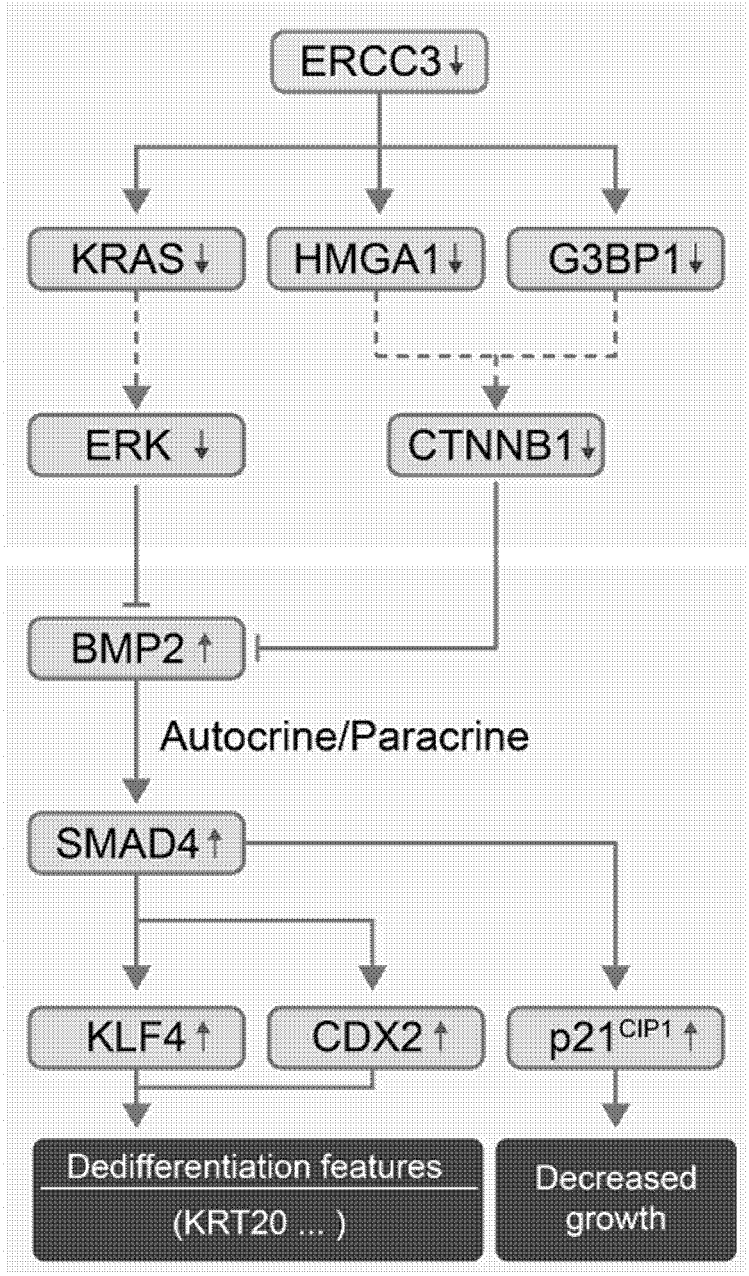
[Fig. 4F]

F

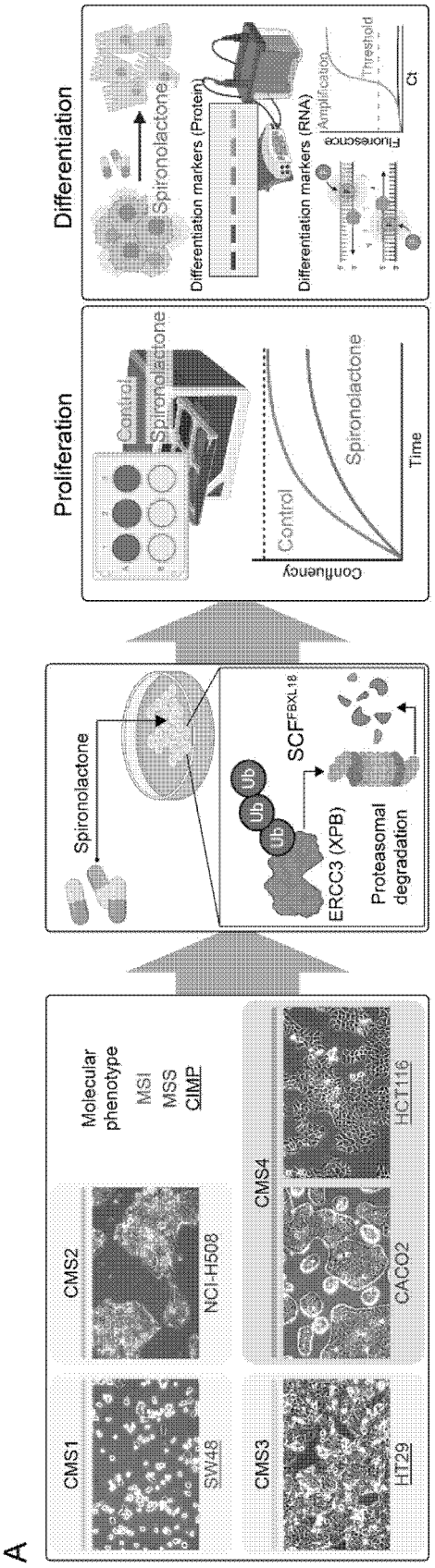


[Fig. 4G]

G


ERCC3↓
KRAS↓
HMGA1↓
G3BP1↓
ERK ↓
CTNNB1↓
BMP2↑
Autocrine/Paracrine
SMAD4↑
KLF4↑
CDX2↑
p21CIP1↑
Dedifferentiation features
(KRT20 ...)
Decreased growth

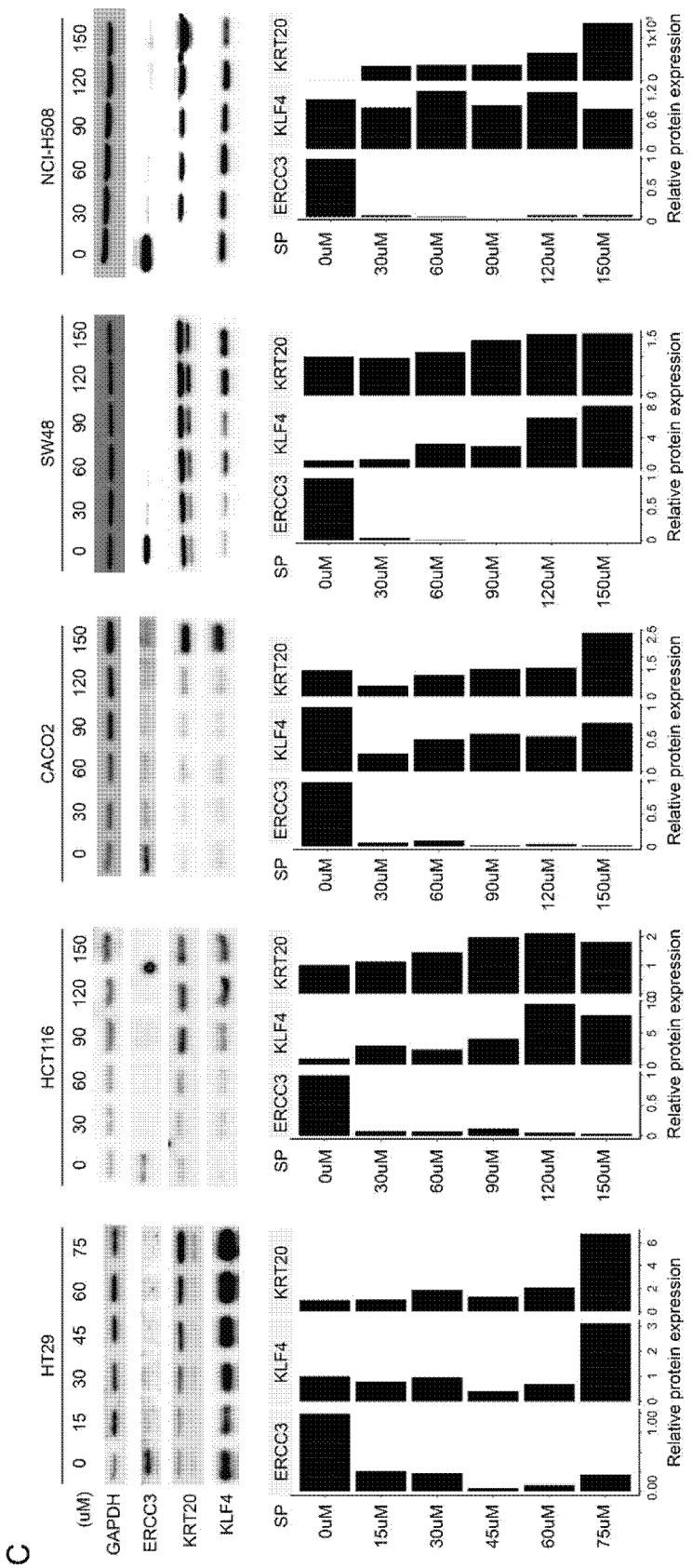
[Fig. 5A]


A
CMS1
CMS2
CMS3
CMS4
Molecular phenotype
MSI
MSS
CIMP
SW48
NCI-H508
HT29
HCT116
CACO2
Spironolactone
SCF^FBXL18
ERCC3 (XPB)
Ub
Proteasomal degradation
Proliferation
Control
Spironolactone
Confluency
Time
Differentiation
Differentiation markers (Protein)
Differentiation markers (RNA)
Fluorescence
Amplification
Threshold
Ct

[Fig. 5B]

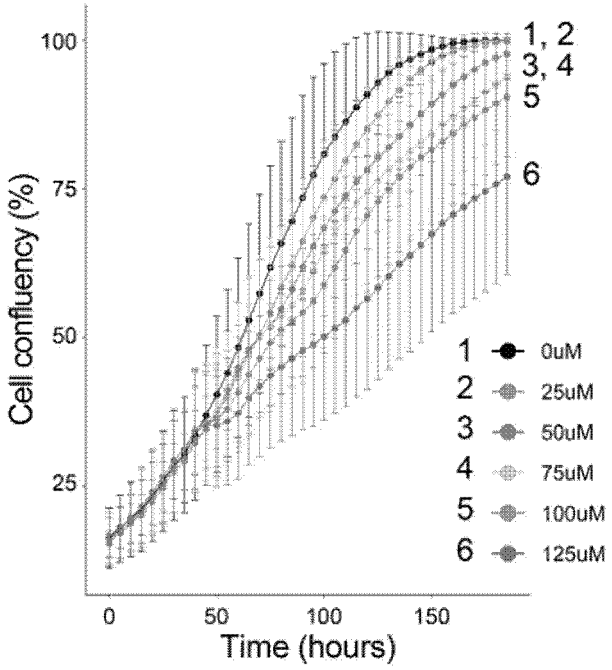
B
HT29
HCT116
CACO2
SW48
NCI-H508
Cell confluency (%)
Time (hours)
0uM
15uM
30uM
45uM
60uM
75uM
90uM
120uM
150uM

[Fig. 5C]


C
HT29
HCT116
CACO2
SW48
NCI-H508
(uM)
GAPDH
ERCC3
KRT20
KLF4
SP
ERCC3
KLF4
KRT20
0uM
15uM
30uM
45uM
60uM
75uM
90uM
120uM
150uM
Relative protein expression

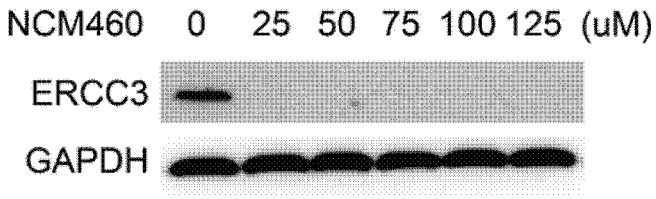
[Fig. 5D]

D



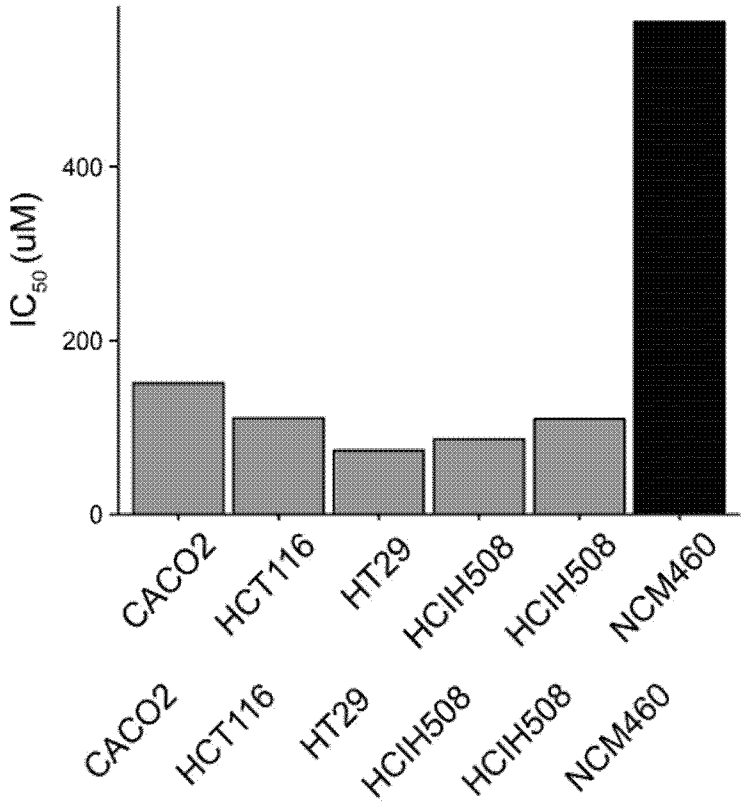
[Fig. 5E]

E

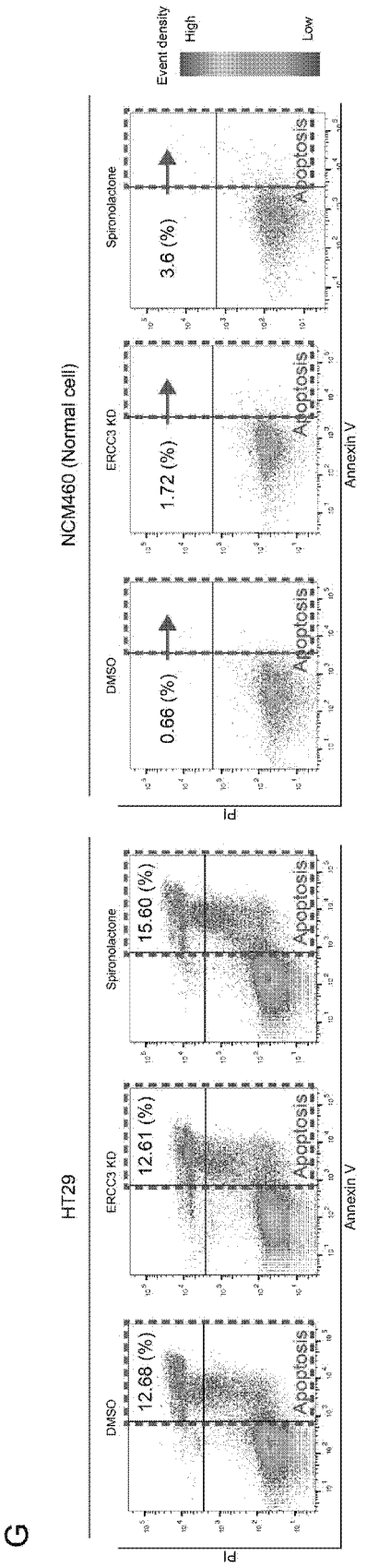


[Fig. 5F]

F

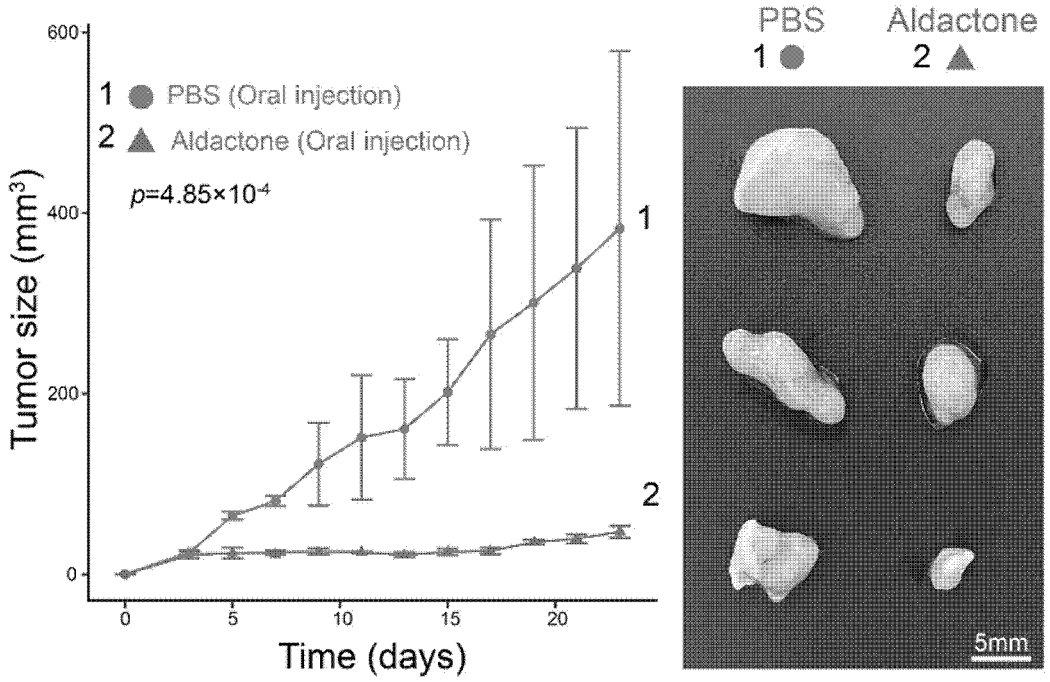

400
200
0
IC50 (uM)
CACO2
HCT116
HT29
HCIH508
HCIH508
NCM460
CACO2
HCT116
HT29
HCIH508
HCIH508
NCM460

[Fig. 5G]


G
HT29
NCM460 (Normal cell)
DMSO
ERCC3 KD
Spironolactone
12.68 (%)
12.61 (%)
15.60 (%)
0.66 (%)
1.72 (%)
3.6 (%)
Apoptosis
PI
Annexin V
Event density
High
Low

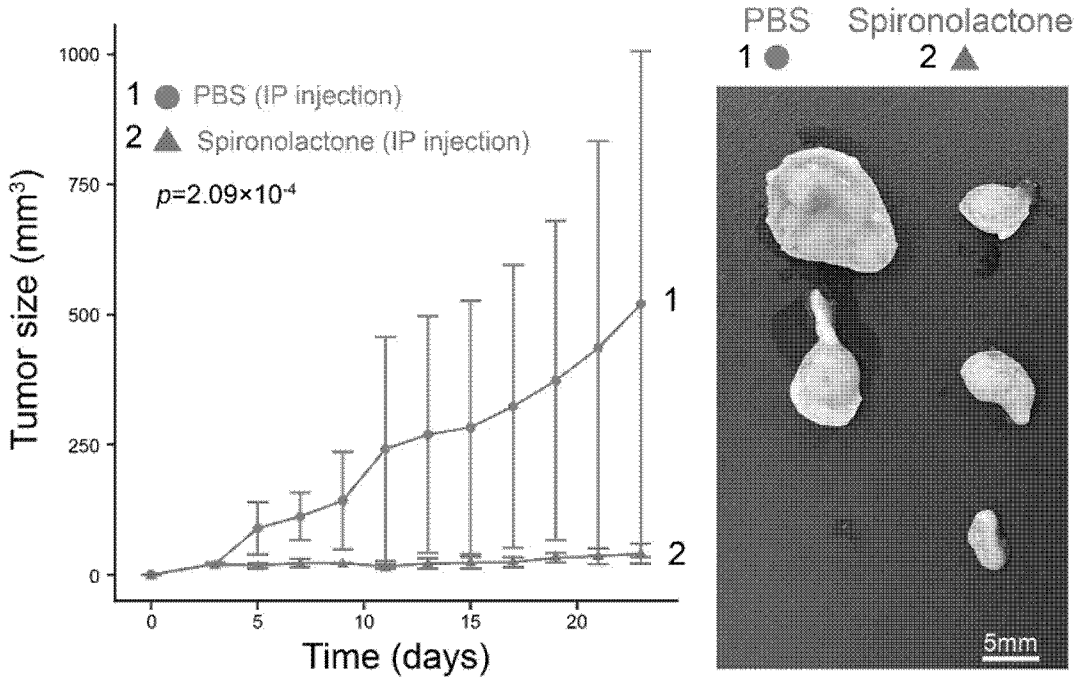
[Fig. 5H]

H



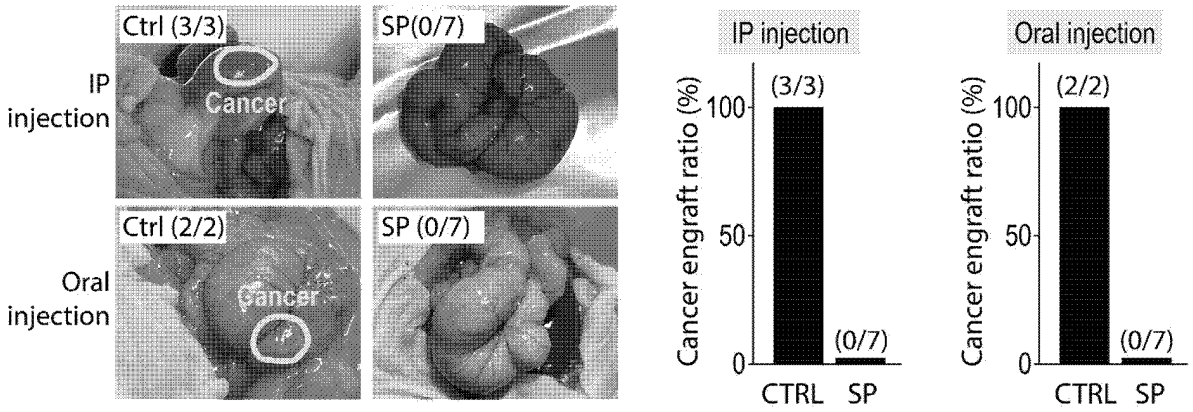
[Fig. 5I]

I



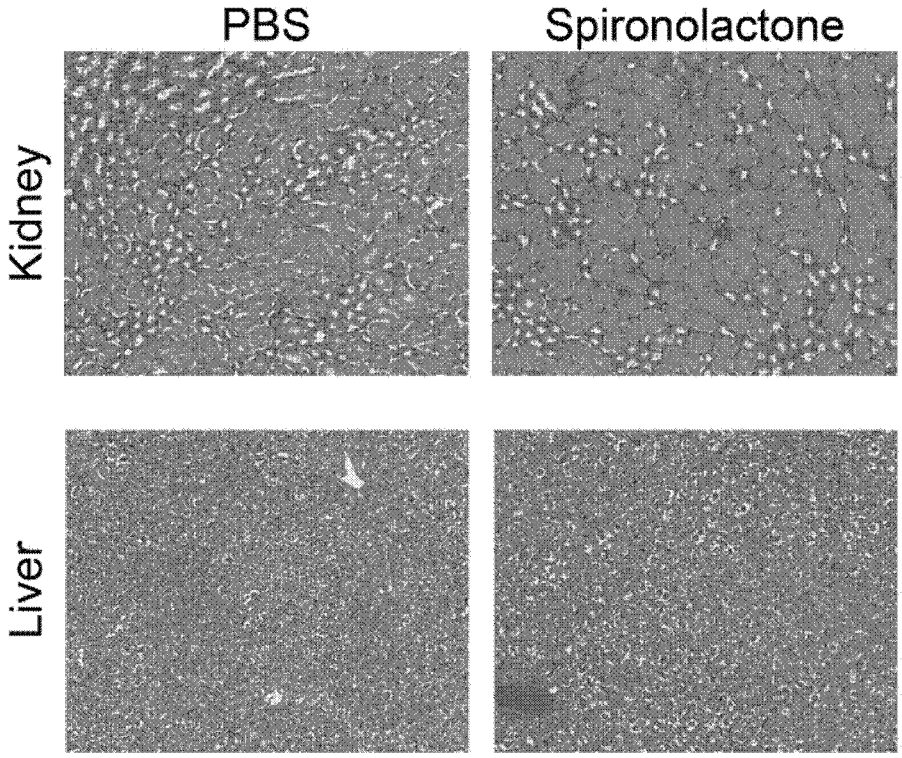
[Fig. 5J]

J



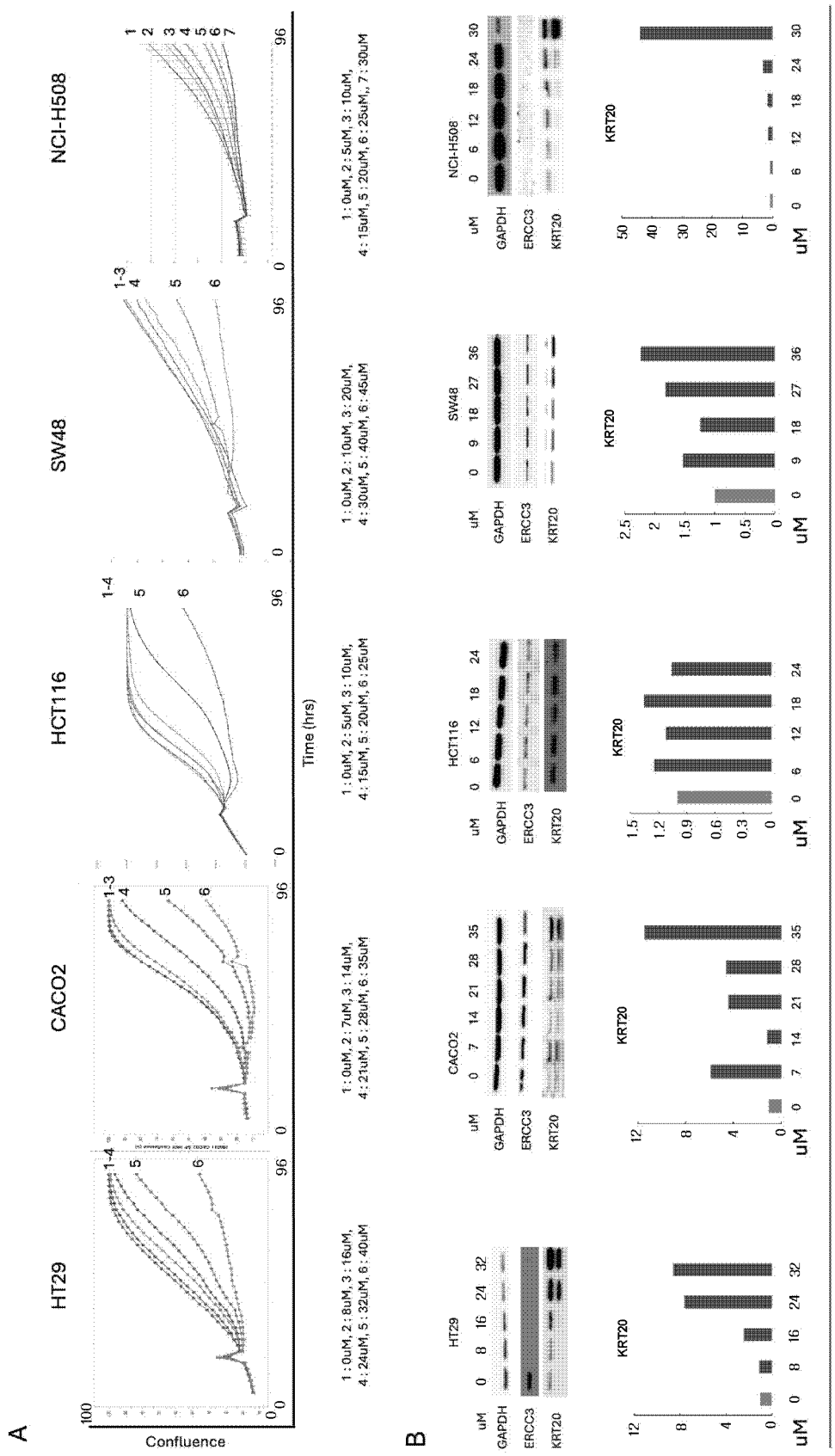
[Fig. 5K]

K



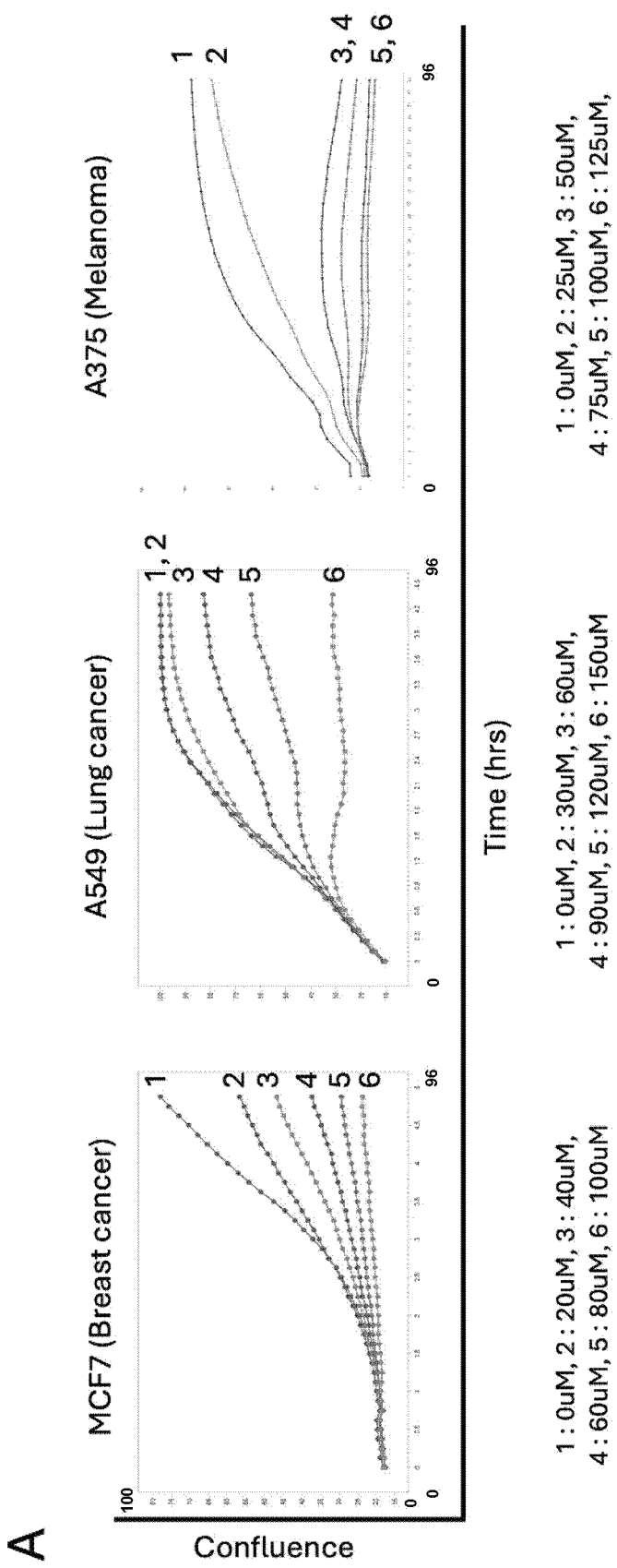
[Fig. 6A]

[Fig. 6B]

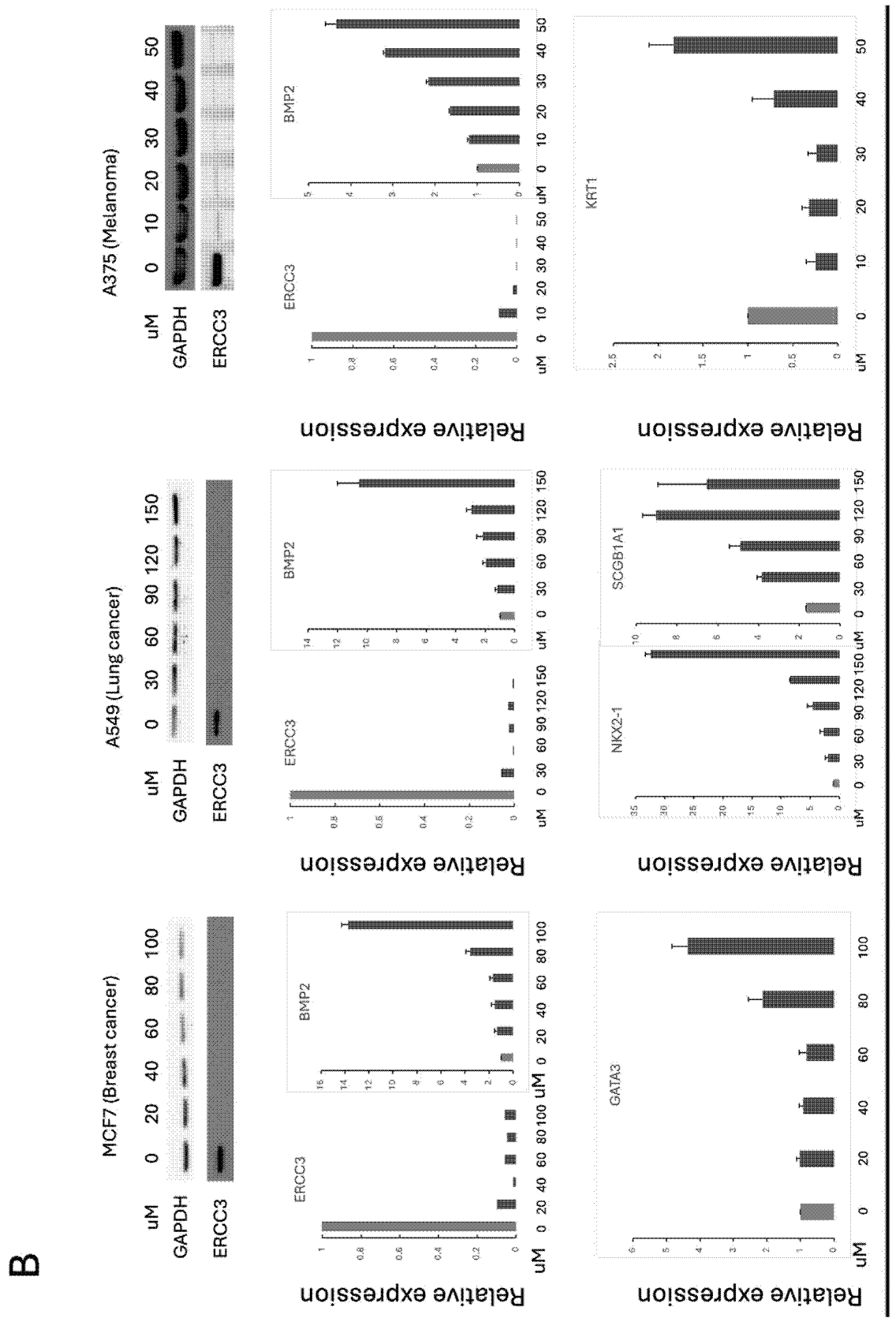


BPK-21

[Fig. 7A]


A
MCF7 (Breast cancer)
A549 (Lung cancer)
A375 (Melanoma)
Confluence
Time (hrs)
100
0
96
1
2
3
4
5
6
1, 2
3, 4
5, 6
1 : 0uM, 2 : 20uM, 3 : 40uM,
4 : 60uM, 5 : 80uM, 6 : 100uM
1 : 0uM, 2 : 30uM, 3 : 60uM,
4 : 90uM, 5 : 120uM, 6 : 150uM
1 : 0uM, 2 : 25uM, 3 : 50uM,
4 : 75uM, 5 : 100uM, 6 : 125uM,

[Fig. 7B]


B
MCF7 (Breast cancer)
A549 (Lung cancer)
A375 (Melanoma)
uM
GAPDH
ERCC3
BMP2
GATA3
NKX2-1
SCGB1A1
KRT1
Relative expression
Spironolactone