Supplementary Figure Legends

Fig. S1 The information of rs155386776 and The IC_{50} of 5-fluorouracil in CRC cells.

(A) The information of rs1553867776 in database. **(B)** The genotype of rs1553867776 in seven common CRC cell lines (HCT8, HCT116, RKO, SW480, SW620, SW1116, and LoVo), one gastric cancer cell line (MGC-803) and one liver cell line (HepG2). **(C-D)** The IC_{50} of 5-fluorouracil (5-FU) in HCT116 (C) and HCT8 (D) cells. NC, NC mimics; miR [del], deletion-type miR-4274 mimics; miR [ins], insertion-type miR-4274 mimics.

Fig. S2 Relative expression of miR-4274 mRNA in CRC cells transfected with miR-4274 mimics and inhibitors.

(A, B) Relative expression of miR-4274 mRNA in HCT116 **(A)** and HCT8 **(B)** cells transfected with miR-4274 mimics. **(C, D)** Relative expression of miR-4274 mRNA in HCT116 **(C)** and HCT8 **(D)** cells transfected with miR-4274 inhibitors. ***, $P < 0.001$ and ****, $P < 0.0001$ of Student's *t*-test. NC, NC mimics; miR [del], deletion-type miR-4274 mimics; miR [ins], insertion-type miR-4274 mimics; NC inh, NC inhibitor; miR inh [del], deletion-type miR-4274 inhibitor; miR inh [ins], insertion-type miR-4274 inhibitor.

Fig. S3 Colony formation assays in CRC cells with miR-4274 overexpression or downregulation.

(A, B) Colony formation assays in HCT116 (**A**) and HCT8 (**B**) cells with miR-4274 overexpression with or without IR treatment. Right panels represent data (means ± SEM) from 3 independent experiments. **(C, D)** Colony formation assays in HCT116 (**C**) and HCT8 (**D**) cells with miR-4274 downregulation with or without IR treatment. Right panels represent data (means ± SEM) from 3 independent experiments. *, $P < 0.05$; **, $P < 0.01$;

***, $P < 0.001$ and ns, not significant of Student's *t*-test, NC mimics; miR [del], deletion-type miR-4274 mimics; miR [ins], insertion-type miR-4274 mimics; NC inh, NC inhibitor; miR inh [del], deletion-type miR-4274 inhibitor; miR inh [ins], insertion-type miR-4274 inhibitor.

Fig. S4 Cell viability assays of BGC-803 and HepG2 cells

(A) Transfection miR-4274 mimics in BGC-803 (left) and HepG2 (right) cells significantly increase the cell proliferation and induced radioresistance. (B) Transfection miR-4274 inhibitor in BGC-803 (left) and HepG2 (right) cells significantly inhibit cell proliferation and increased the radiosensitivity. Growth curves data are mean ± SEM from 3 independent experiments and most error bars are with the symbols. **, $P < 0.01$; ***, $P < 0.001$ and ****, $P < 0.0001$ of Student's *t*-test. NC, NC mimics; miR [del], deletion-type miR-4274 mimics; miR [ins], insertion-type miR-4274 mimics; NC inh, NC inhibitor; miR inh [del], deletion-type miR-4274 inhibitor; miR inh [ins], insertion-type miR-4274 inhibitor. IR, irradiation.

Fig. S5 Cell migration assays of HCT8 cells

(A) Cell migration assays of HCT8 cells transected miR-4274 and NC mimics. **(B)** Cell migration assays of HCT8 cells transfected miR-4274 and NC inhibitors. NC, NC mimics; miR [del], deletion-type miR-4274 mimics; miR [ins], insertion-type miR-4274 mimics; NC inh, NC inhibitor; miR inh [del], deletion-type miR-4274 inhibitor; miR inh [ins], insertion-type miR-4274 inhibitor. Scale bar, 100 μm. ns, not significant. **(C)** Cell migration assays of NC and PEX5-KO HCT8 cells. **(D)** Cell migration assays of Vector and PEX5-OE HCT8 cells.

Fig. S6 The western blot results of γ-H2AX, PEX5, Ku70, Ku80 in different time after IR.

(A) The western blot results of γ-H2AX at 2, 6, 12, 24 h after IR and without IR in HCT116 and HCT8 wild-type cells. **(B)** The western blot results of PEX5, Ku70, Ku80 at 2, 12, 24 h after IR and without IR in PEX5-KO and NC HCT116 cells.

Fig. S7 The psiCHECK-2 vector map.

Fig. S8 Kaplan–Meier estimates of survival time by expression of PEX5 from TCGA.

(A) Kaplan–Meier estimates of survival time by expression of PEX5 from TCGA-KIRC, n = 533. **(B)** Kaplan–Meier estimates of survival time by expression of PEX5 from TCGA-LGG, n = 516.

Fig. S9 Relative expression of PEX5 mRNA in PEX5-KO or PEX5-OE CRC cells.

(A, B) Relative expression of PEX5 mRNA in PEX5-KO HCT116 **(A)** and HCT8 **(B)** cells. **(C, D)** Relative expression of PEX5 mRNA in PEX5-OE HCT116 **(C)** and HCT8 **(D)** cells. *, $P < 0.05$; **, $P < 0.01$ and ****, $P < 0.0001$ of Student's *t*-test.

Fig. S10 Colony formation assays in PEX5-KO or PEX5-OE CRC cells.

(A) Colony formation assays in PEX5-KO HCT116 cells with or without IR treatment. Right panels represent data (means ± SEM) from 3 independent experiments. **(B)** Colony formation assays in PEX5-KO HCT8 cells with or without IR treatment. Right panels represent data (means ± SEM) from 3 independent experiments. **(C)** Colony formation assays in PEX5-OE HCT116 cells with or without IR treatment. Right panels represent data (means ± SEM) from 3 independent experiments. **(D)** Colony formation assays in PEX5-OE HCT8 cells with or without IR treatment. Right panels represent data (means ±

SEM) from 3 independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ of the Student's *t*-test.

Fig. S11 PEX5 related to response to radiation in CRC cells.

(A) Fractions of cell survival by limiting dilution assays of PEX5-OE HCT116 cells with or without IR treatment. **(B)** Fractions of cell survival by limiting dilution assays of PEX5-OE HCT8 cells with or without IR treatment. **(C)** The images of comet assays of PEX5-KO and PEX5-OE HCT8 cells with or without IR treatment. Scale bar, 100 μm. **(D)** Bar charts show the statistics of tail moments in comet assays. Data are mean ± SEM from 3 replicate experiments and 4 fields were randomly selected for each experiment. ****, $P < 0.0001$ of Student's *t*-test.

Fig. S12 The RNA-seqing results of PEX5-KO cells after IR

(A) Volcano plot of differential genes in PEX5-KO vs NC cells after IR. **(B)** Metascape gene enrichment analysis of differential genes.

Fig. S13 Immunofluorescence analysis of Ku70 co-staining in PEX5-KO and PEX5-OE HCT116 cells with or without IR.

Scale bar, 100 μm.

Fig. S14 The effects of miR-4274 and PEX5 on CRC progression and radiosensitivity *in vivo*.

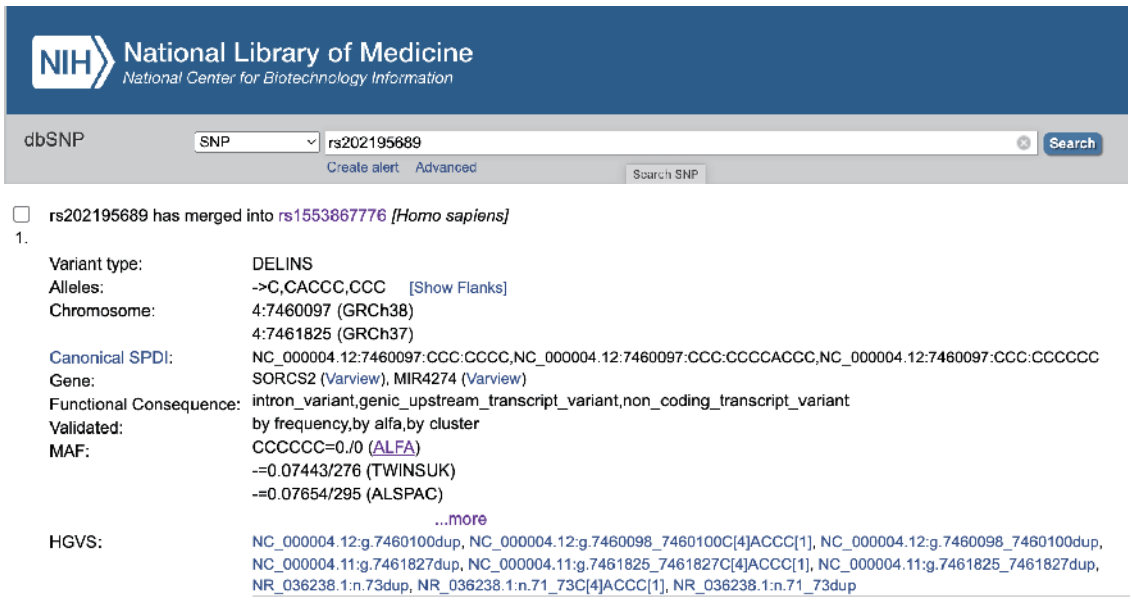
(A, B) The effects of miR-4274 on the volume of HCT116 xenograft tumors, n = 3. **(C)** The effects of miR-4274 on the weights of CRC tumors, n=3. **(D, E)** The effects of PEX5-KO on the volume of CRC tumors, n = 5. **(F)** The effects of PEX5-KO on the weights of CRC tumors, n = 5. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ and ns, not significant of

Student's *t*-test. NC, NC ago-mimics; miR [del], deletion-type miR-4274 ago-mimics; miR [ins], insertion-type miR-4274 ago-mimics.

Fig. S15 The effects of PEX5 overexpression on CRC progression and radiosensitivity *in vivo*.

(A, B) The effects of PEX5 OE on the volume of HCT116 xenograft tumors, n = 5. **(C, D)** The effects of PEX5-OE on the volume of HCT8 xenograft tumors, n = 5. **(E, F)** The effects of PEX5-OE on the weights of HCT116(E) and HCT8(F) xenograft tumors, n = 5. **, $P < 0.01$, ***, $P < 0.001$ and ****, $P < 0.0001$ of Student's *t*-test.

A


National Library of Medicine
National Center for Biotechnology Information
dbSNP
SNP
rs202195689
Search
Create alert
Advanced
Search SNP
rs202195689 has merged into rs1553867776 [Homo sapiens]
1.
Variant type: DELINS
Alleles: ->C,CACCC,CCC [Show Flanks]
Chromosome: 4:7460097 (GRCh38)
4:7461825 (GRCh37)
Canonical SPDI: NC_000004.12:7460097:CCC:CCCC,NC_000004.12:7460097:CCC:CCCCACCC,NC_000004.12:7460097:CCC:CCCCCC
Gene: SORCS2 (Varview), MIR4274 (Varview)
Functional Consequence: intron_variant,genic_upstream_transcript_variant,non_coding_transcript_variant
Validated: by frequency,by alfa,by cluster
MAF: CCCCCC=0./0 (ALFA)
-=0.07443/276 (TWINSUK)
-=0.07654/295 (ALSPAC)
...more
HGVS: NC_000004.12:g.7460100dup, NC_000004.12:g.7460098_7460100C[4]ACCC[1], NC_000004.12:g.7460098_7460100dup,
NC_000004.11:g.7461827dup, NC_000004.11:g.7461825_7461827C[4]ACCC[1], NC_000004.11:g.7461825_7461827dup,
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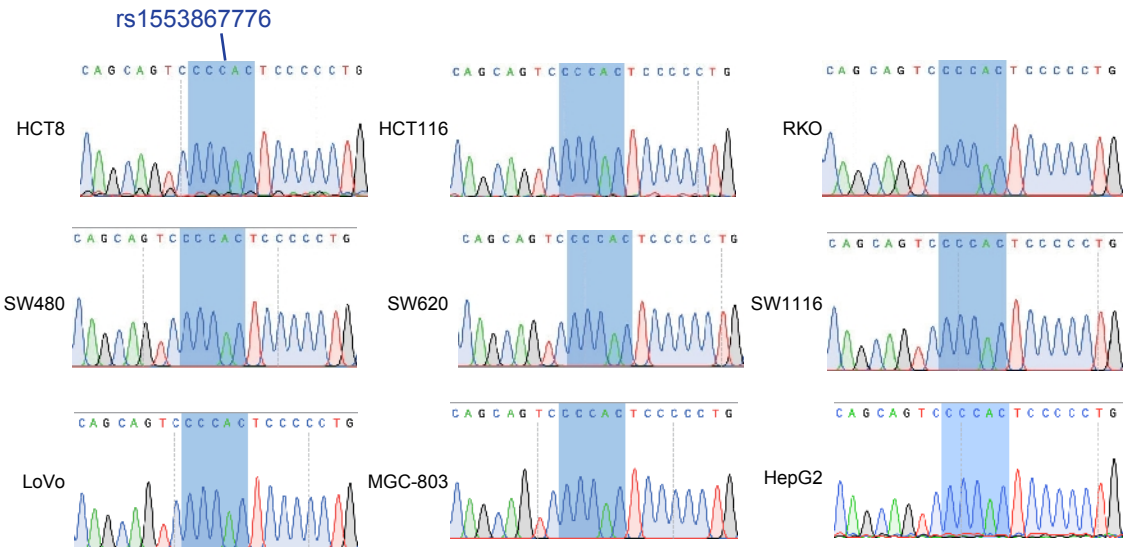
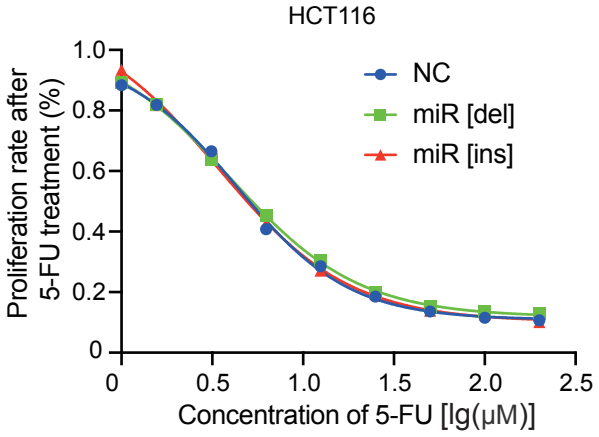
B


rs1553867776
HCT8
HCT116
RKO
SW480
SW620
SW1116
LoVo
MGC-803
HepG2

C


HCT116
NC
miR [del]
miR [ins]
Proliferation rate after 5-FU treatment (%)
Concentration of 5-FU [lg(μM)]
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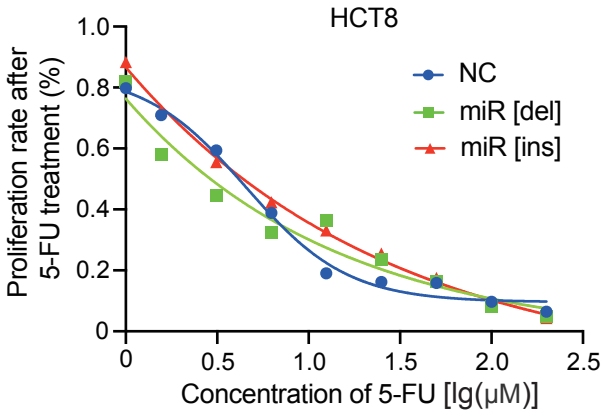
0.2
0.4
0.6
0.8
1.0
0.5
1.0
1.5
2.0
2.5

D


HCT8
NC
miR [del]
miR [ins]
Proliferation rate after 5-FU treatment (%)
Concentration of 5-FU [lg(μM)]
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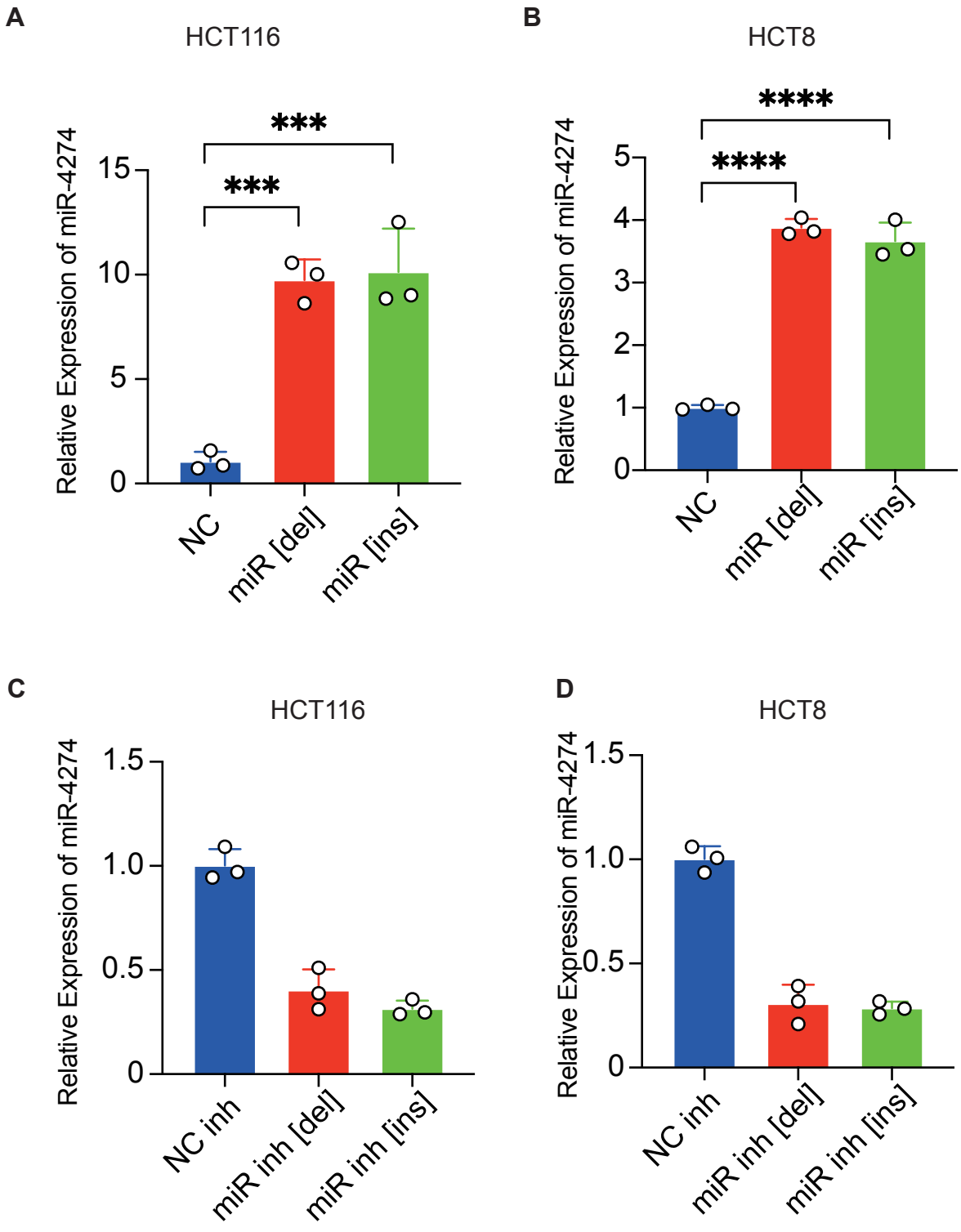
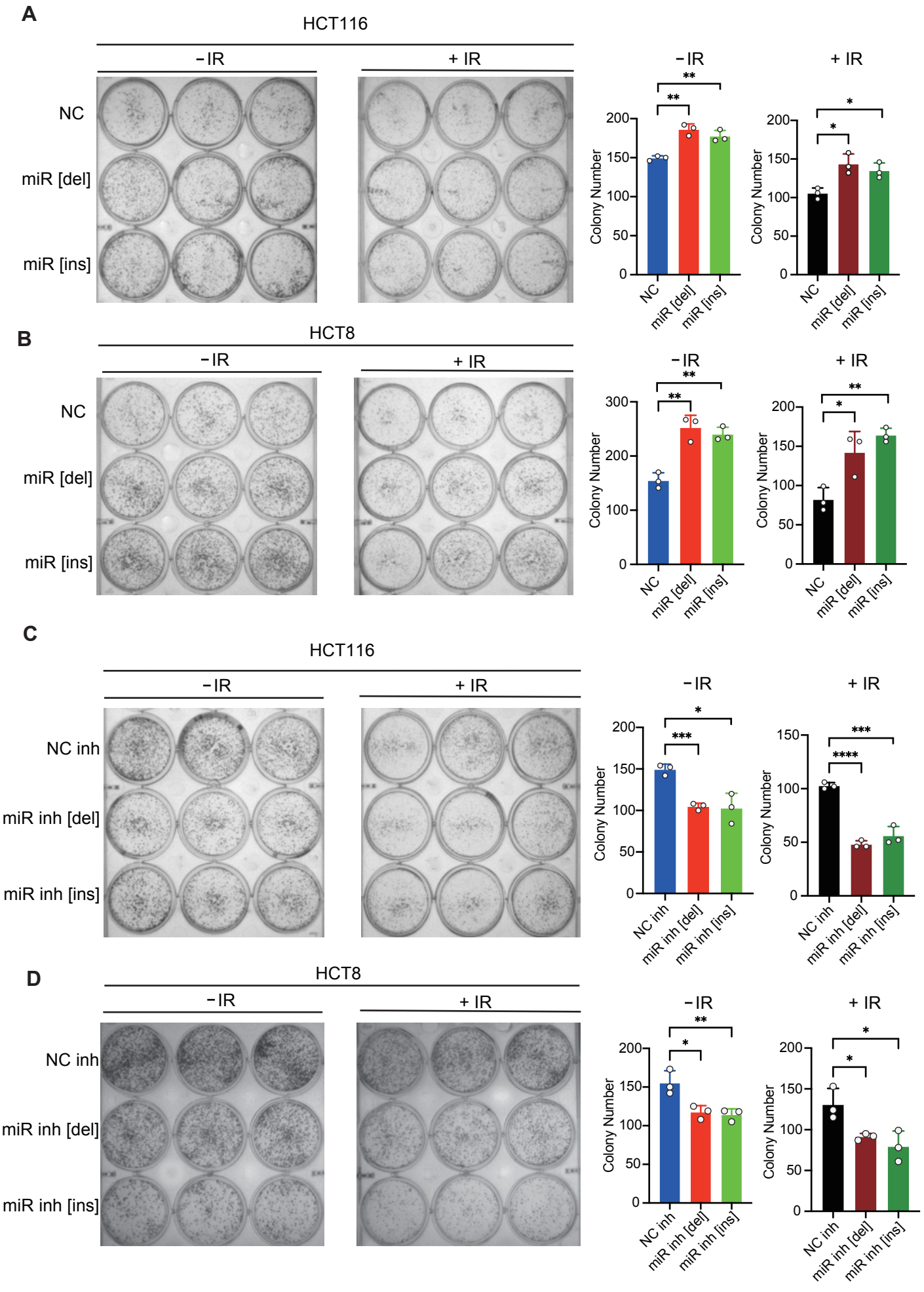
0.2
0.4
0.6
0.8
1.0
0.5
1.0
1.5
2.0
2.5


A
HCT116
Relative Expression of miR-4274
15
10
5
0
***
***
NC
miR [del]
miR [ins]
B
HCT8
Relative Expression of miR-4274
5
4
3
2
1
0
****
****
NC
miR [del]
miR [ins]
C
HCT116
Relative Expression of miR-4274
1.5
1.0
0.5
0
NC inh
miR inh [del]
miR inh [ins]
D
HCT8
Relative Expression of miR-4274
1.5
1.0
0.5
0
NC inh
miR inh [del]
miR inh [ins]


A
HCT116
−IR
+ IR
NC
miR [del]
miR [ins]
Colony Number
B
HCT8
C
HCT116
NC inh
miR inh [del]
miR inh [ins]
D
HCT8

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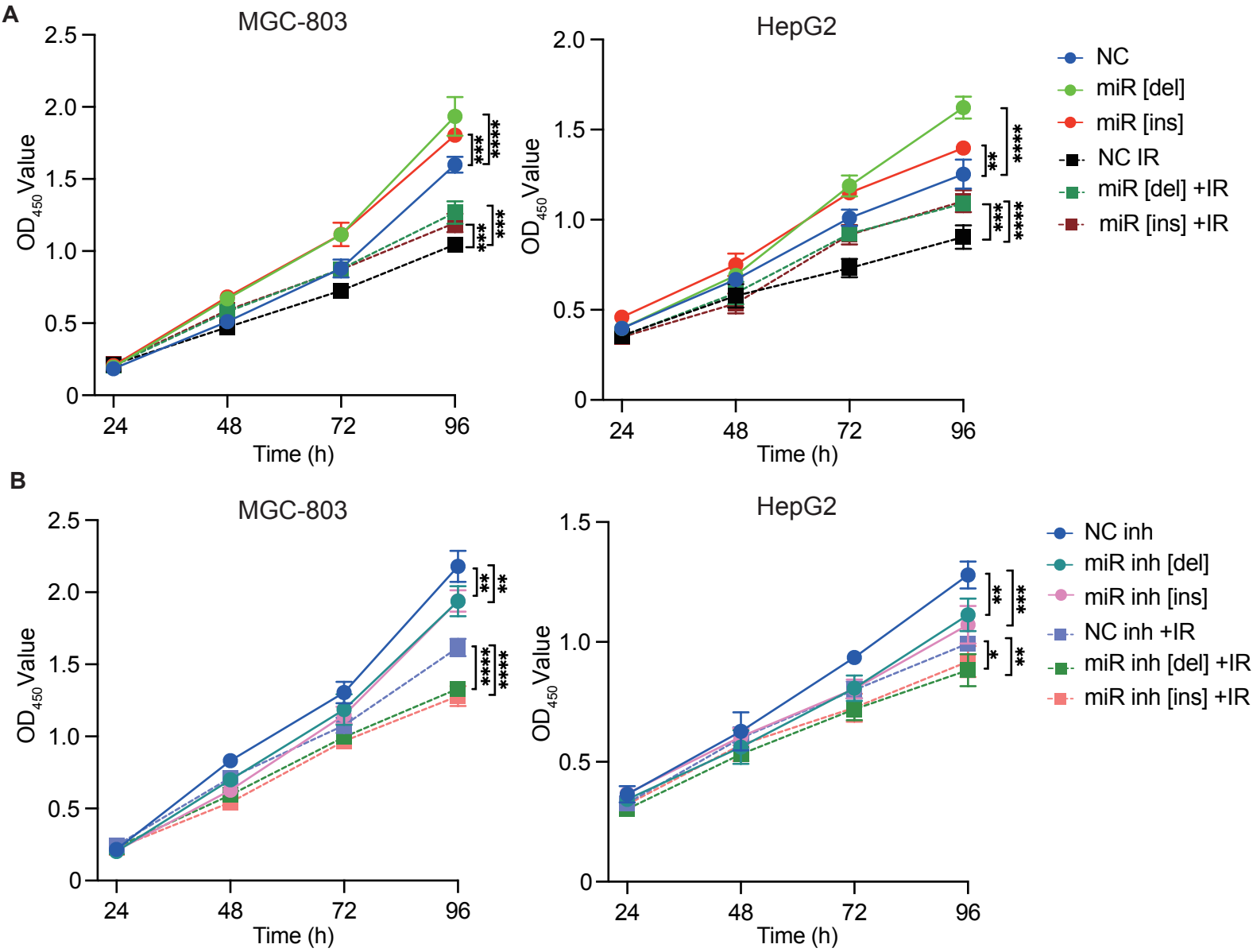
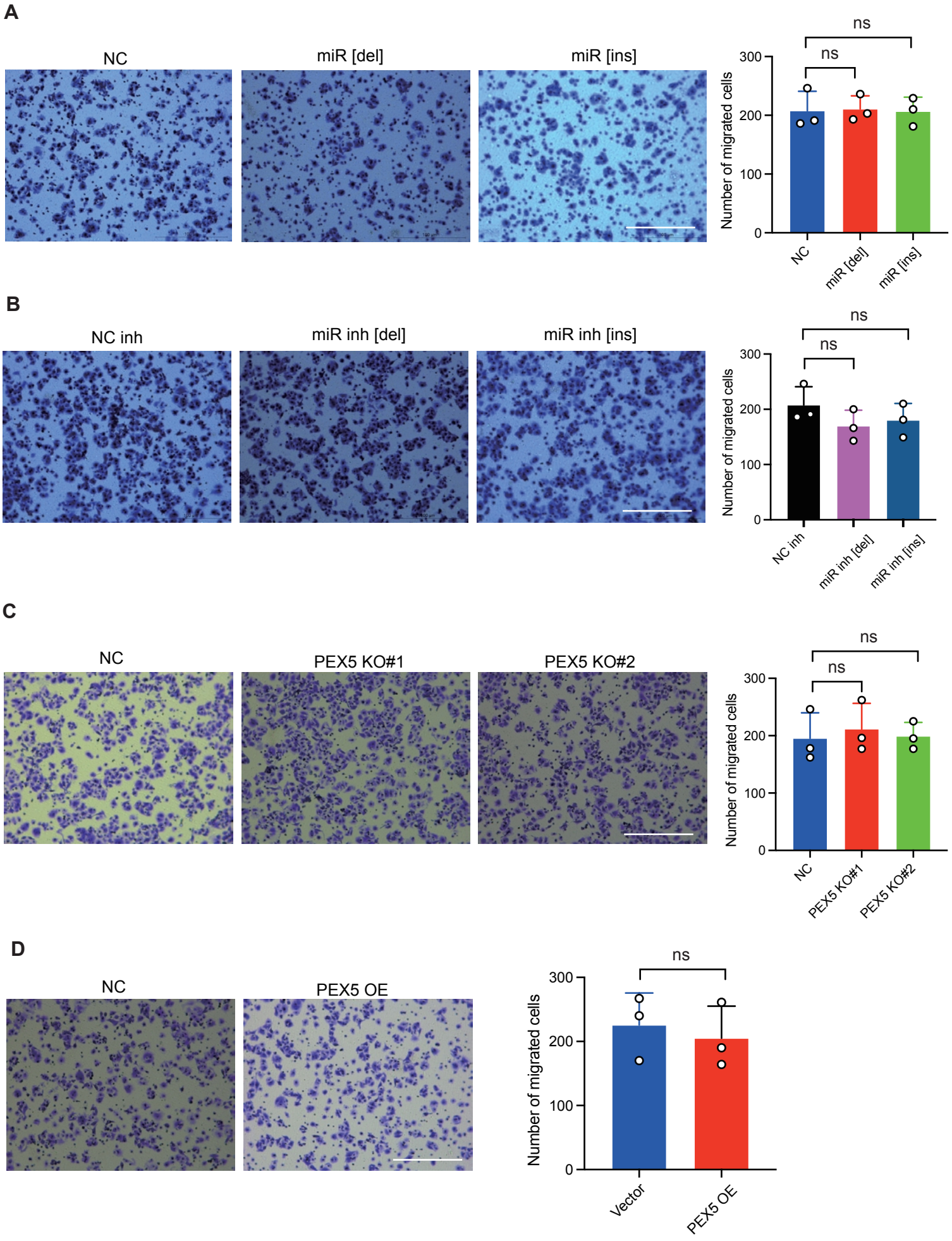
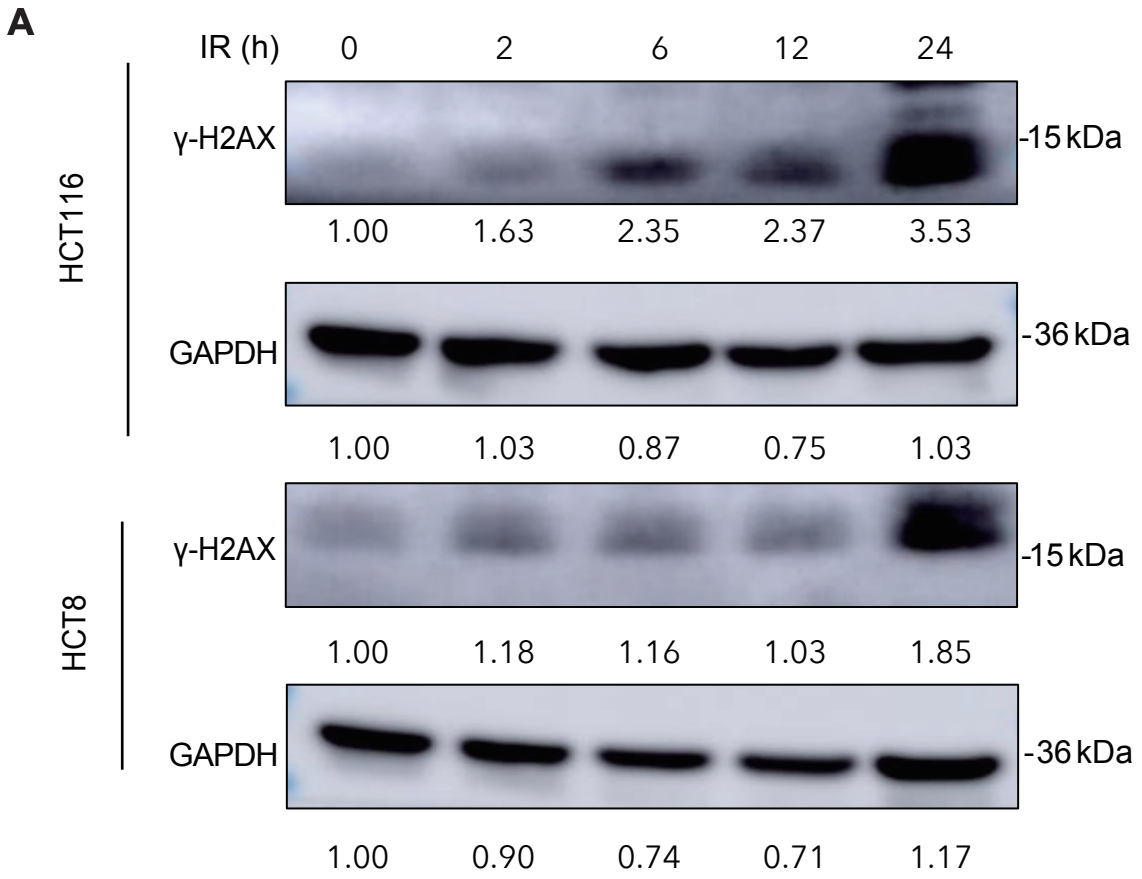
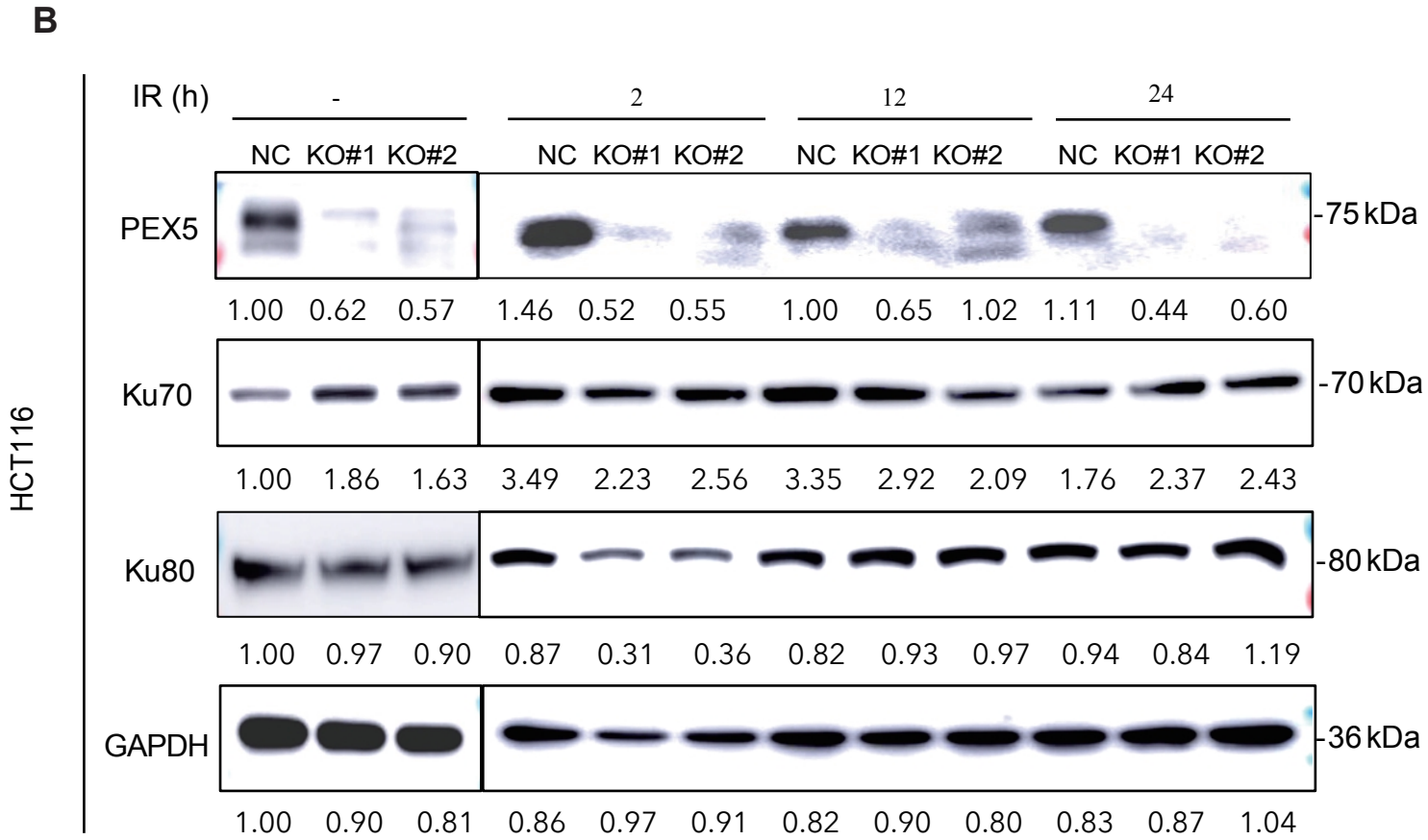



A
NC
miR [del]
miR [ins]
ns
ns
Number of migrated cells
300
200
100
0
NC
miR [del]
miR [ins]
B
NC inh
miR inh [del]
miR inh [ins]
ns
ns
Number of migrated cells
300
200
100
0
NC inh
miR inh [del]
miR inh [ins]
C
NC
PEX5 KO#1
PEX5 KO#2
ns
ns
Number of migrated cells
300
200
100
0
NC
PEX5 KO#1
PEX5 KO#2
D
NC
PEX5 OE
ns
Number of migrated cells
300
200
100
0
Vector
PEX5 OE


A
IR (h) 0 2 6 12 24
HCT116
γ-H2AX
-15 kDa
1.00 1.63 2.35 2.37 3.53
GAPDH
-36 kDa
1.00 1.03 0.87 0.75 1.03
HCT8
γ-H2AX
-15 kDa
1.00 1.18 1.16 1.03 1.85
GAPDH
-36 kDa
1.00 0.90 0.74 0.71 1.17


B
HCT116
IR (h) - 2 12 24
NC KO#1 KO#2 NC KO#1 KO#2 NC KO#1 KO#2 NC KO#1 KO#2
PEX5
-75 kDa
1.00 0.62 0.57 1.46 0.52 0.55 1.00 0.65 1.02 1.11 0.44 0.60
Ku70
-70 kDa
1.00 1.86 1.63 3.49 2.23 2.56 3.35 2.92 2.09 1.76 2.37 2.43
Ku80
-80 kDa
1.00 0.97 0.90 0.87 0.31 0.36 0.82 0.93 0.97 0.94 0.84 1.19
GAPDH
-36 kDa
1.00 0.90 0.81 0.86 0.97 0.91 0.82 0.90 0.80 0.83 0.87 1.04

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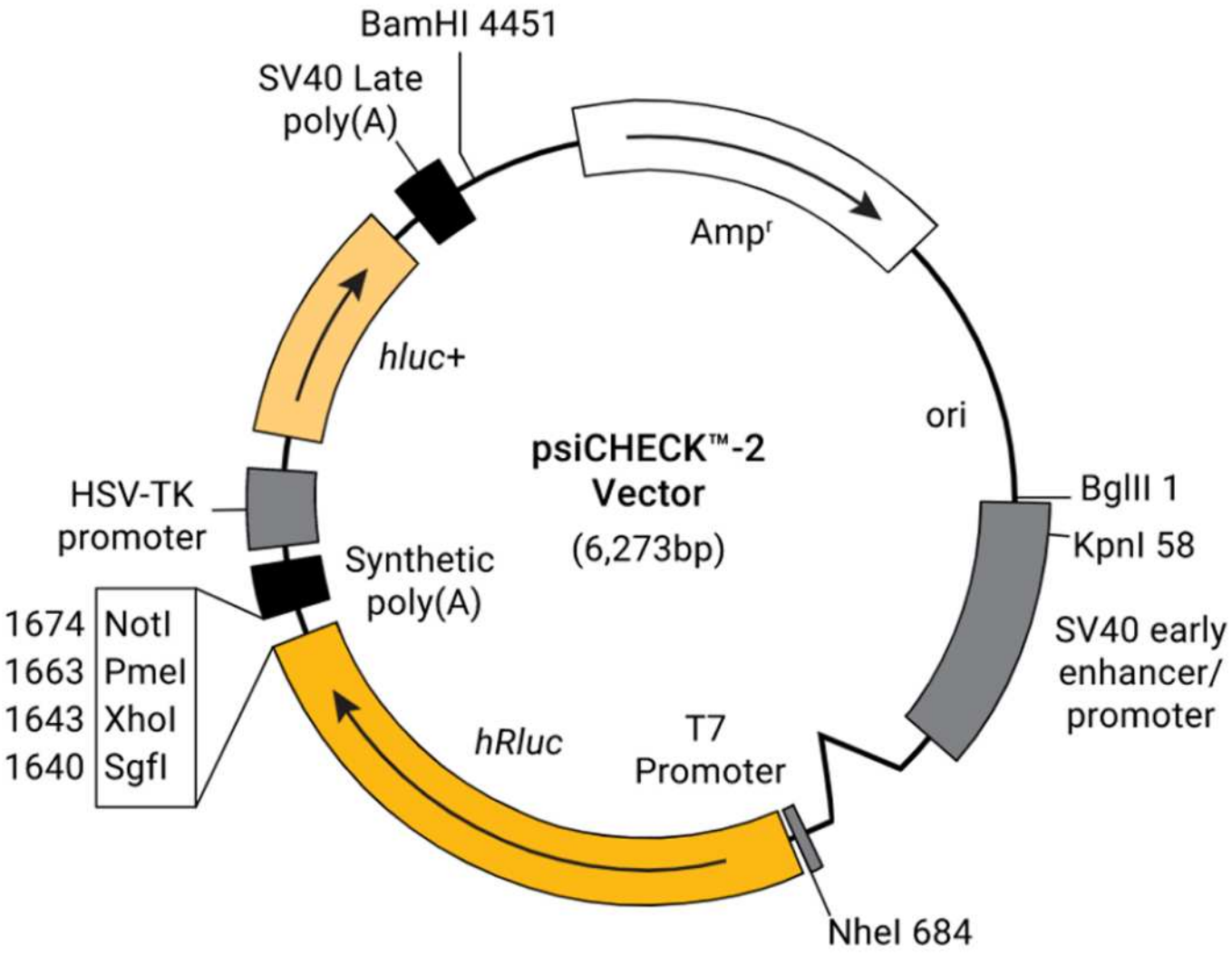
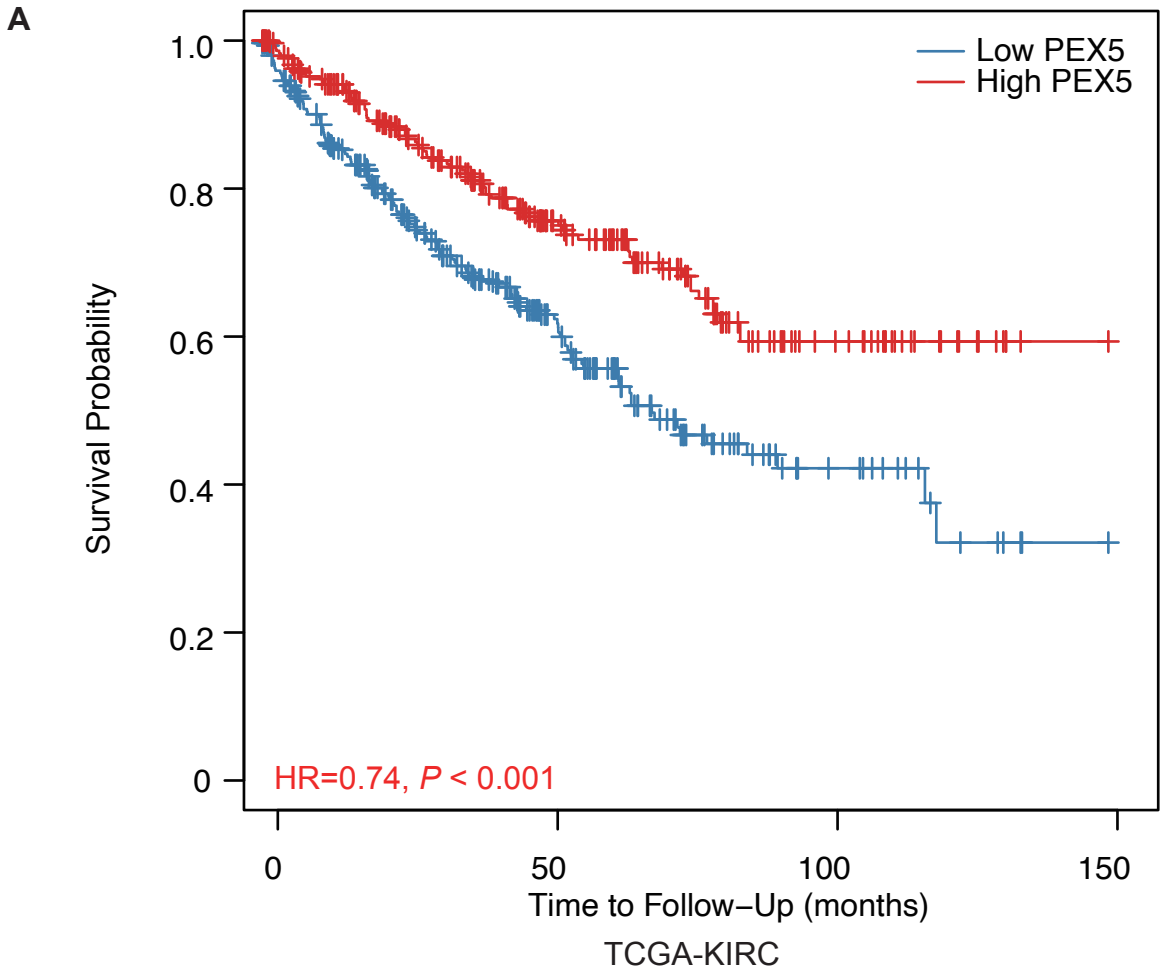
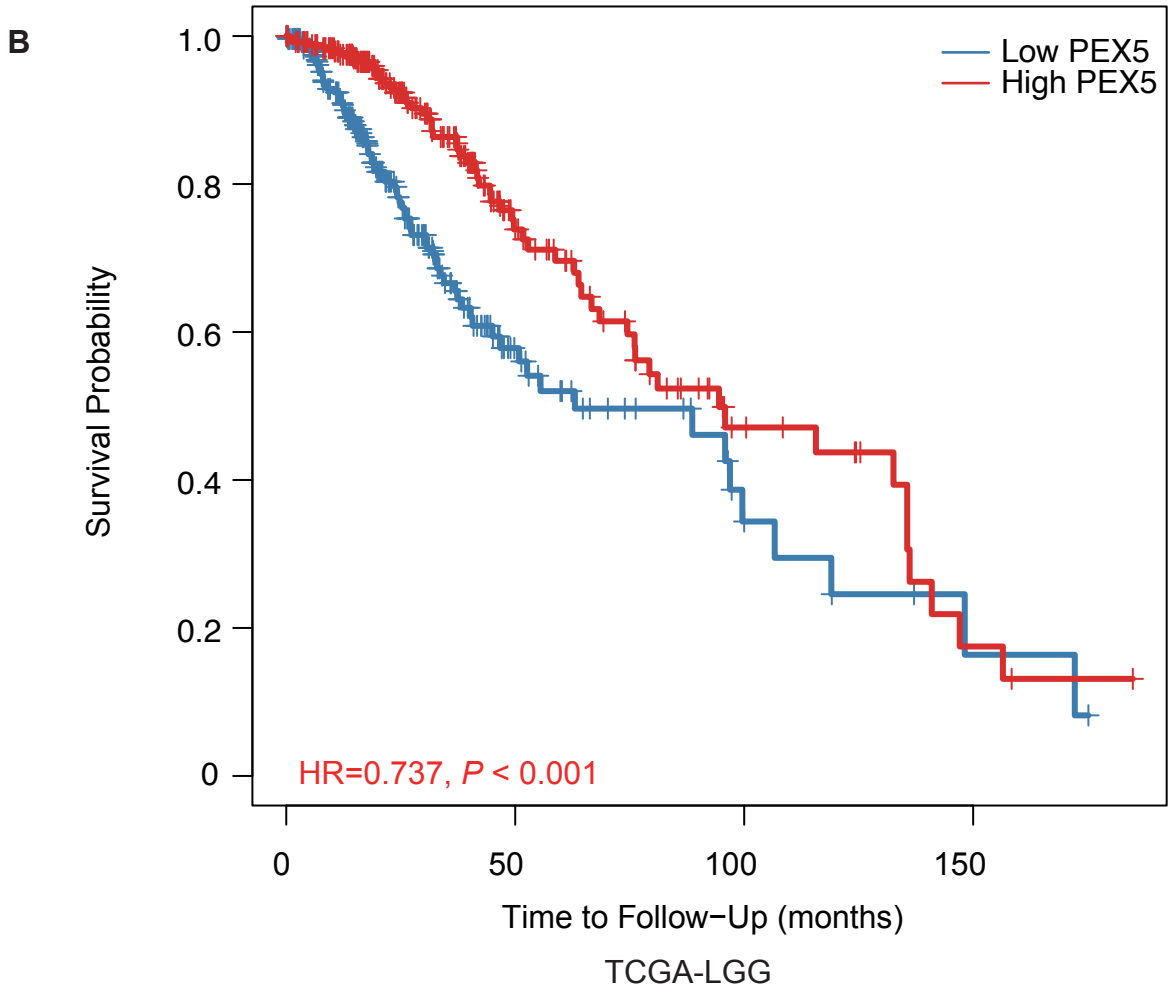
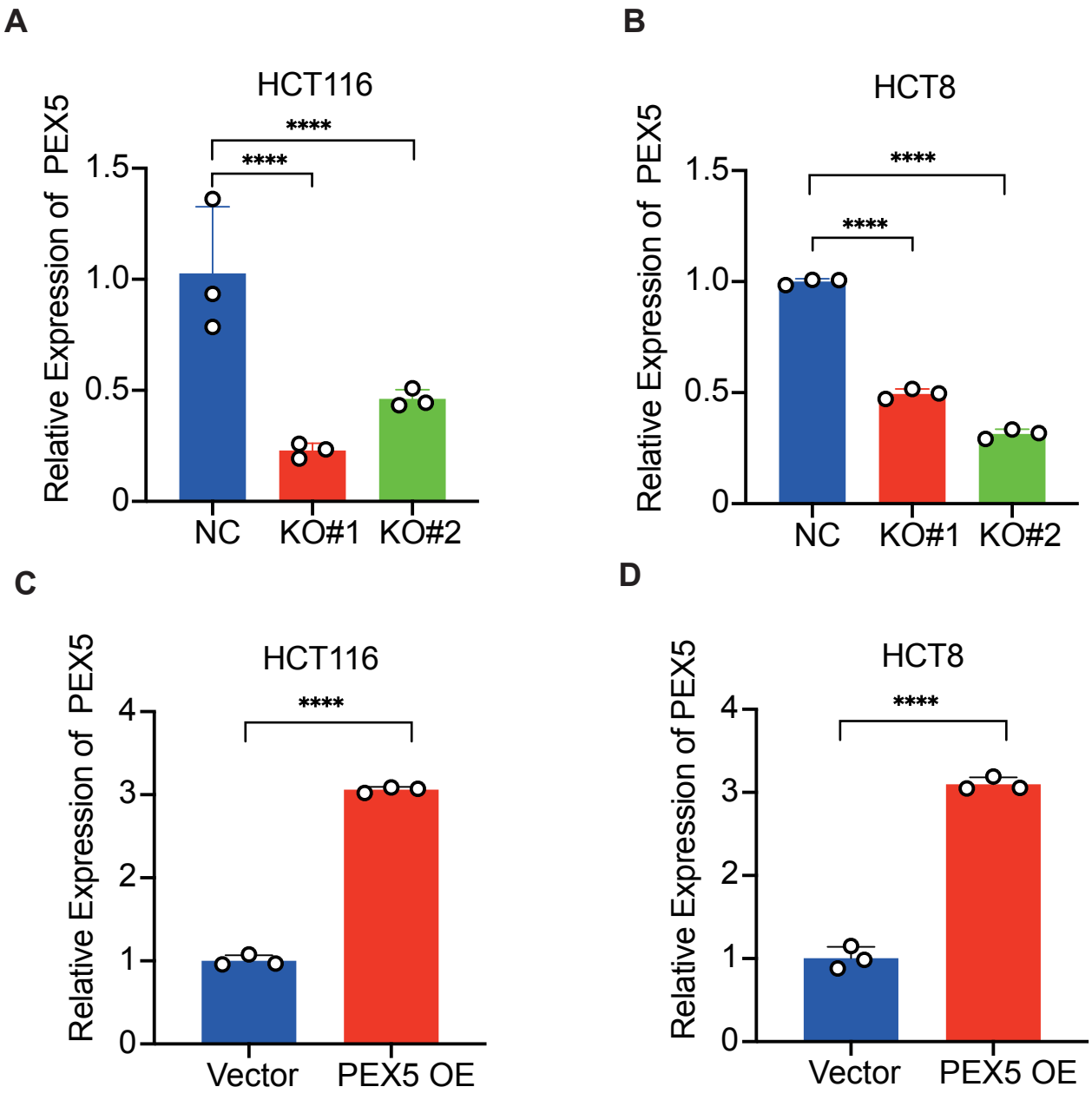
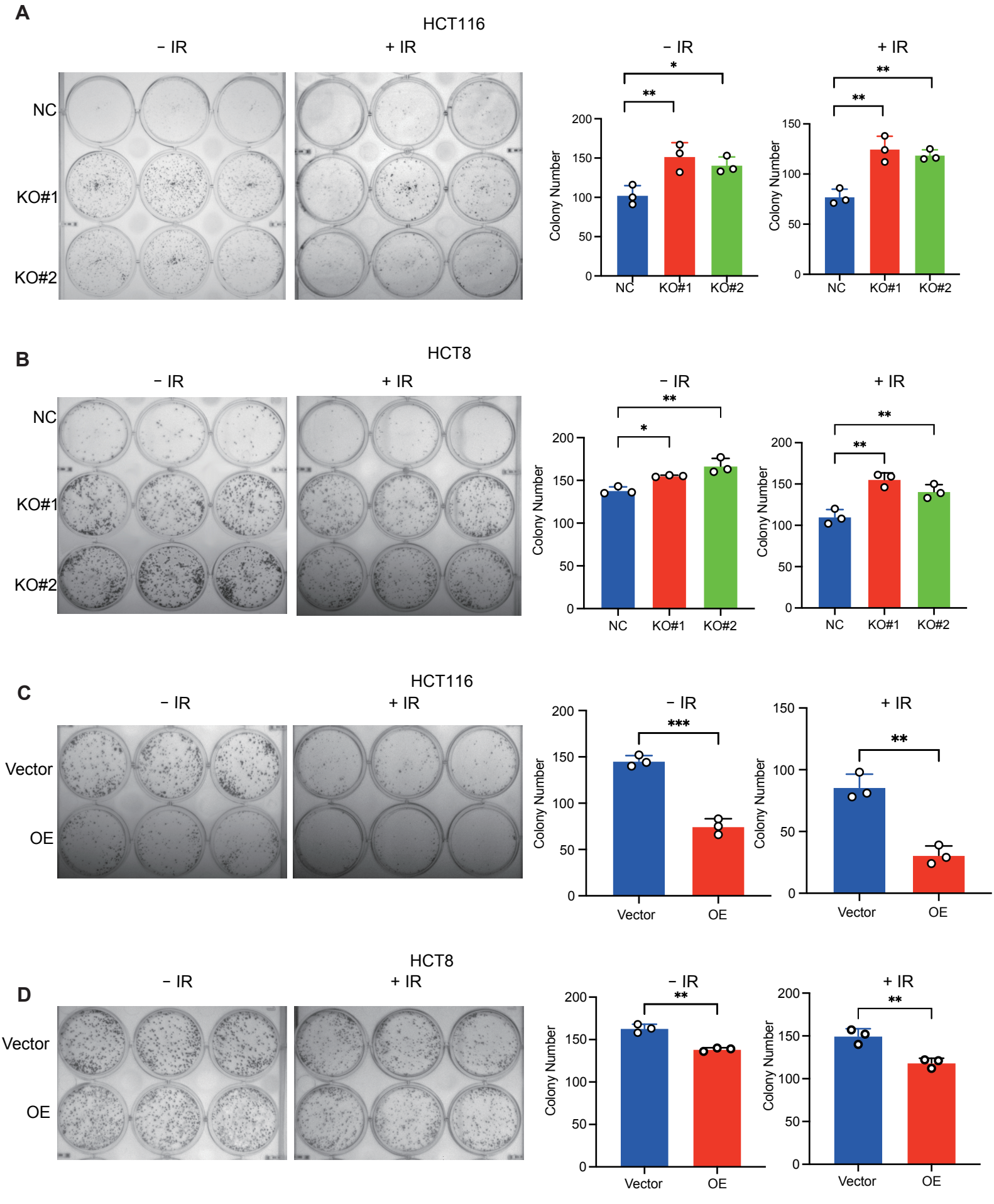
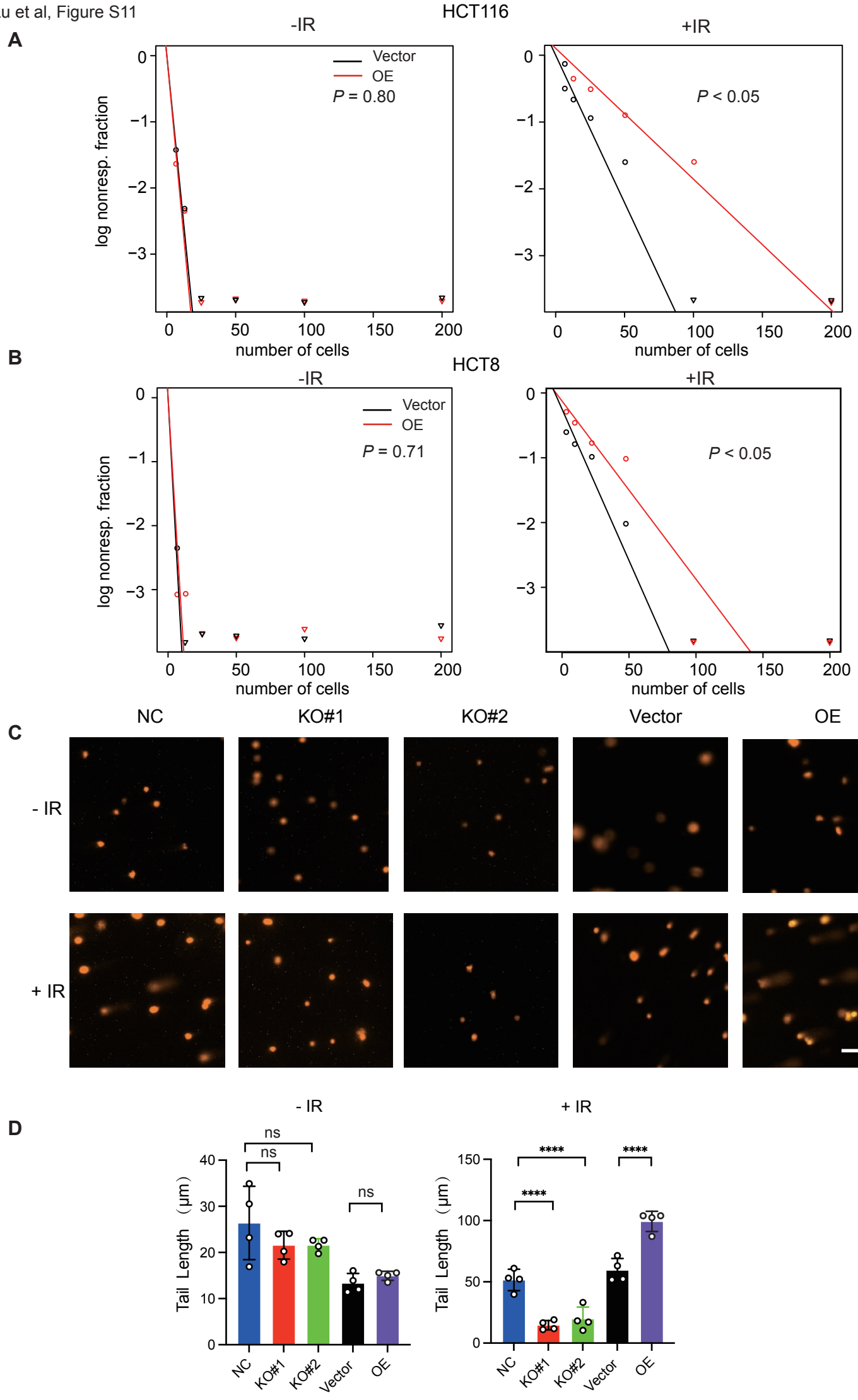



A
Low PEX5
High PEX5
Survival Probability
1.0
0.8
0.6
0.4
0.2
0
HR=0.74, P < 0.001
0
50
100
150
Time to Follow−Up (months)
TCGA-KIRC

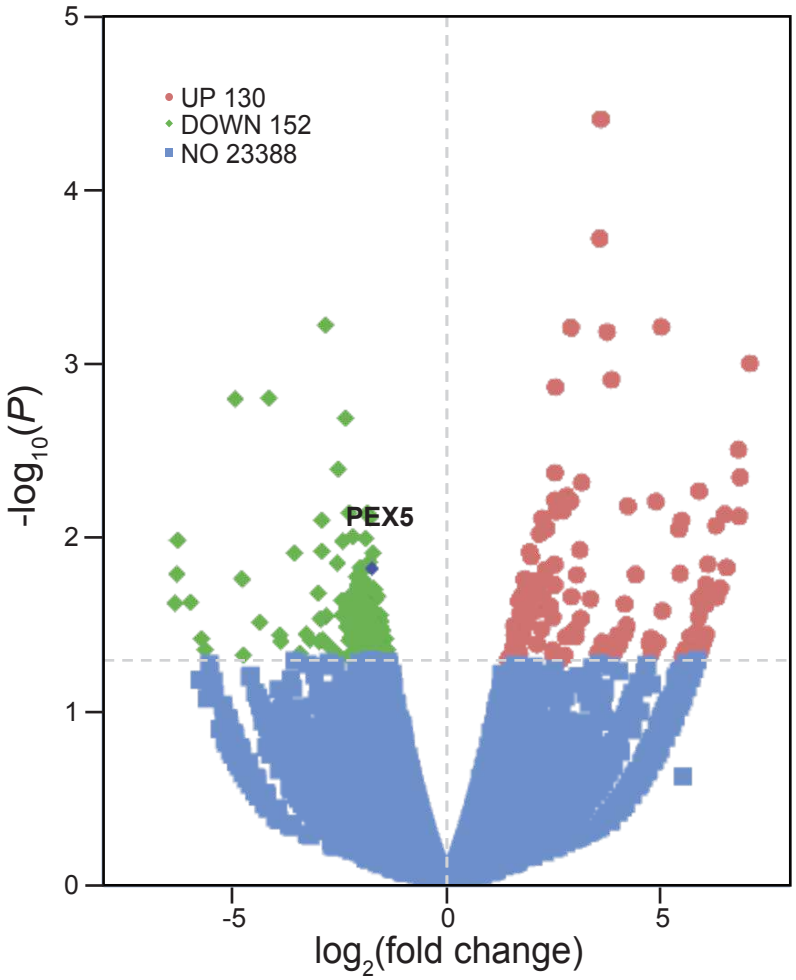

B
Low PEX5
High PEX5
Survival Probability
1.0
0.8
0.6
0.4
0.2
0
HR=0.737, P < 0.001
0
50
100
150
Time to Follow−Up (months)
TCGA-LGG


A
HCT116
Relative Expression of PEX5
****
****
1.5
1.0
0.5
0
NC
KO#1
KO#2
B
HCT8
Relative Expression of PEX5
****
****
1.5
1.0
0.5
0
NC
KO#1
KO#2
C
HCT116
Relative Expression of PEX5
****
4
3
2
1
0
Vector
PEX5 OE
D
HCT8
Relative Expression of PEX5
****
4
3
2
1
0
Vector
PEX5 OE

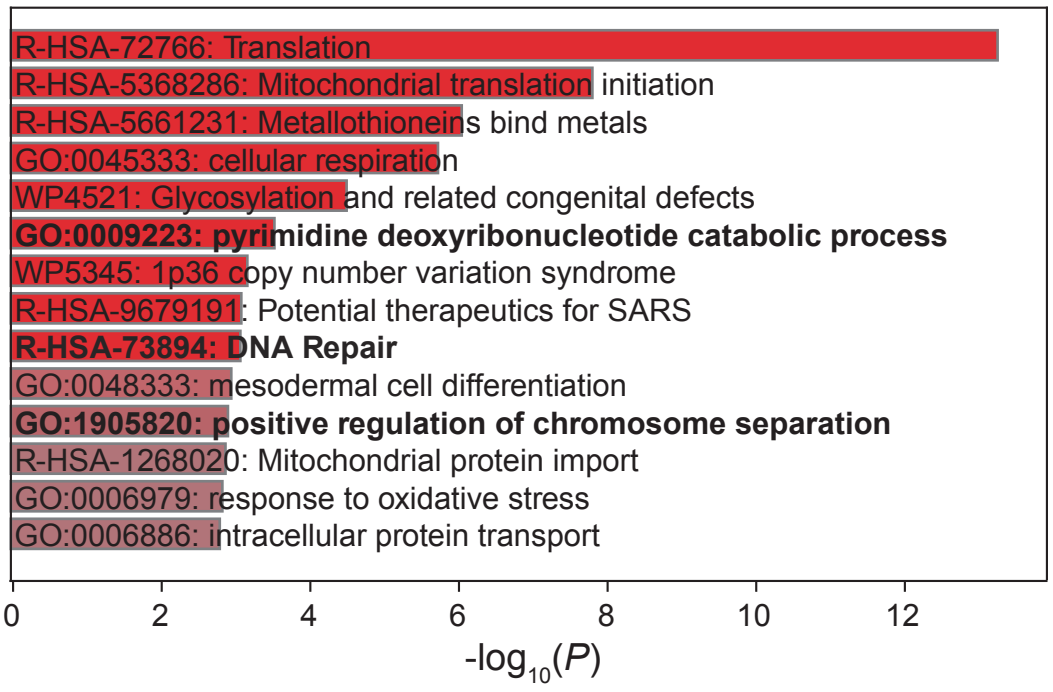

A
HCT116
− IR
+ IR
NC
KO#1
KO#2
− IR
+ IR
Colony Number
NC
KO#1
KO#2
B
HCT8
C
HCT116
Vector
OE
D
HCT8


A
HCT116
-IR
+IR
Vector
OE
P = 0.80
P < 0.05
log nonresp. fraction
number of cells
B
HCT8
-IR
+IR
Vector
OE
P = 0.71
P < 0.05
log nonresp. fraction
number of cells
C
NC
KO#1
KO#2
Vector
OE
- IR
+ IR
D
- IR
+ IR
ns
****
Tail Length (μm)
NC
KO#1
KO#2
Vector
OE

A


UP 130
DOWN 152
NO 23388
PEX5
-log10(P)
log2(fold change)
5
4
3
2
1
0
-5
0
5

B


R-HSA-72766: Translation
R-HSA-5368286: Mitochondrial translation initiation
R-HSA-5661231: Metallothioneins bind metals
GO:0045333: cellular respiration
WP4521: Glycosylation and related congenital defects
GO:0009223: pyrimidine deoxyribonucleotide catabolic process
WP5345: 1p36 copy number variation syndrome
R-HSA-9679191: Potential therapeutics for SARS
R-HSA-73894: DNA Repair
GO:0048333: mesodermal cell differentiation
GO:1905820: positive regulation of chromosome separation
R-HSA-1268020: Mitochondrial protein import
GO:0006979: response to oxidative stress
GO:0006886: intracellular protein transport
0
2
4
6
8
10
12
-log10(P)

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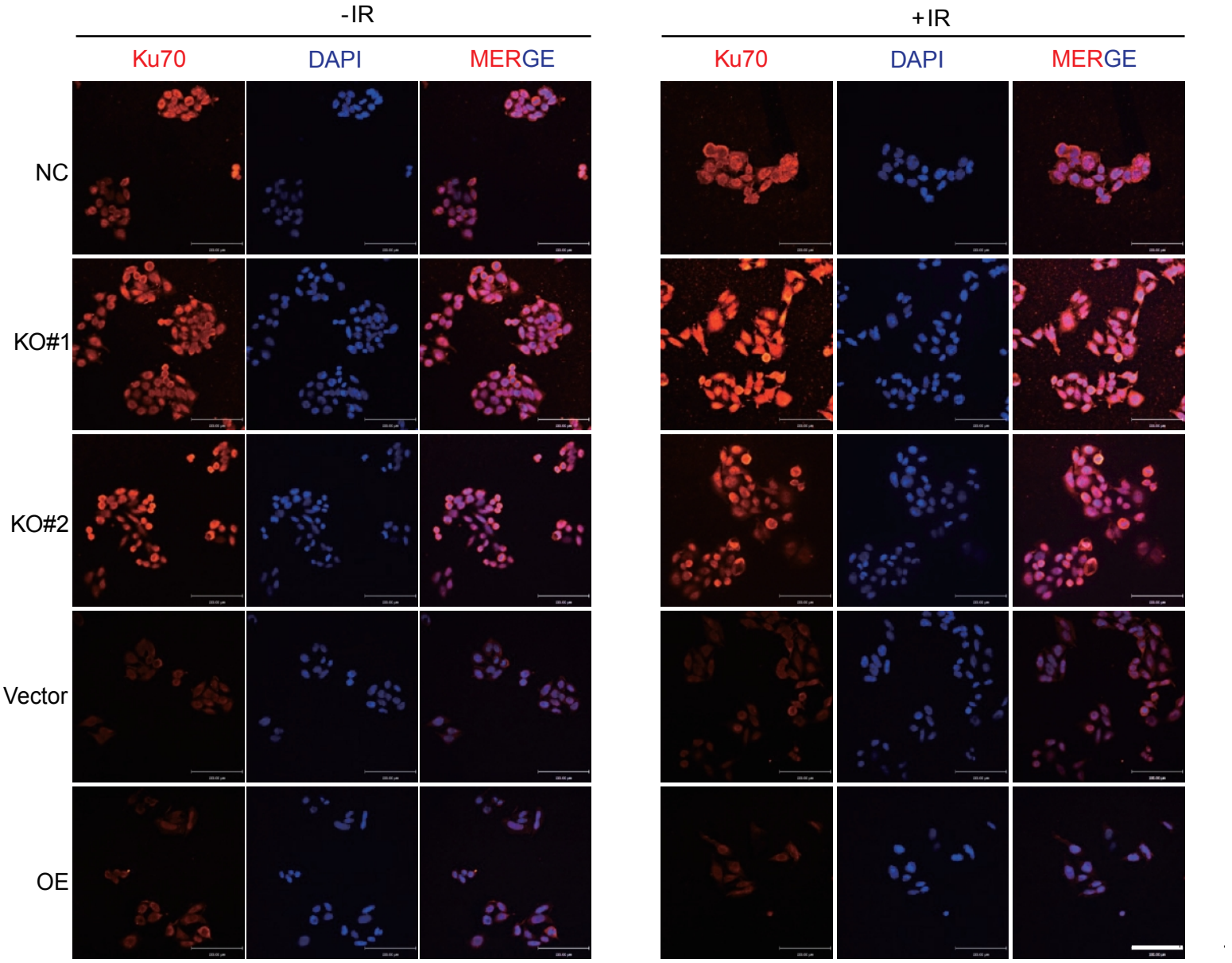
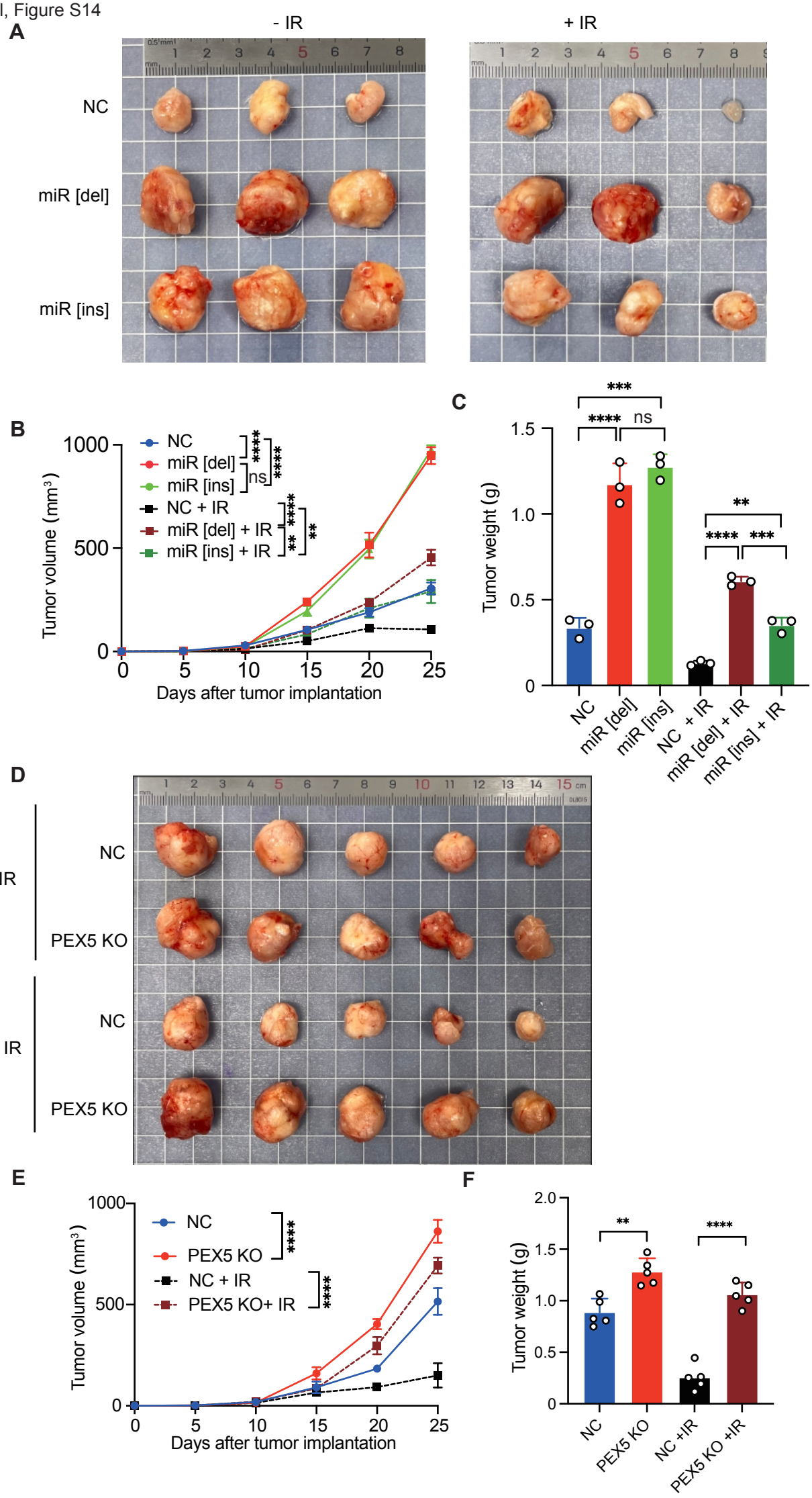
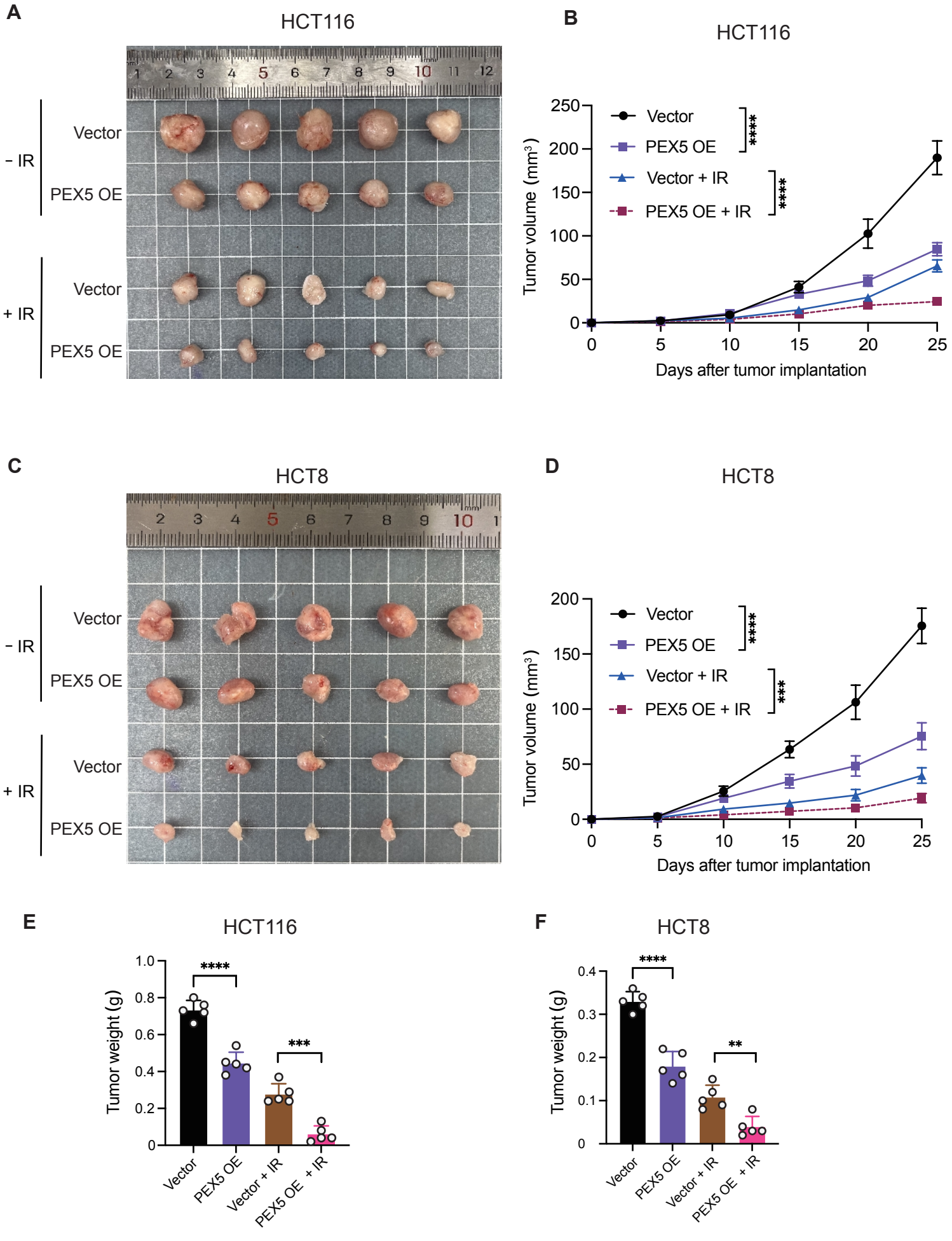


Lu et al, Figure S14




A
HCT116
- IR
+ IR
Vector
PEX5 OE
Vector
PEX5 OE
B
HCT116
Vector
PEX5 OE
Vector + IR
PEX5 OE + IR
****
****
Tumor volume (mm³)
Days after tumor implantation
C
HCT8
- IR
+ IR
Vector
PEX5 OE
Vector
PEX5 OE
D
HCT8
Vector
PEX5 OE
Vector + IR
PEX5 OE + IR
****
***
Tumor volume (mm³)
Days after tumor implantation
E
HCT116
Tumor weight (g)
****
***
Vector
PEX5 OE
Vector + IR
PEX5 OE + IR
F
HCT8
Tumor weight (g)
****
**
Vector
PEX5 OE
Vector + IR
PEX5 OE + IR