Supplementary figure legends

Figure S1. Schematic of recruitment of study subjects, sampling of biospecimens, and biomarker analysis.

Figure S2. Evaluation of response of CRC to preoperative neoadjuvant chemotherapy using surgically removed tissue samples.

Tumor Regression Grade (TRG) was used: TRG1, no residual cancer; TRG2, rare residual cancer cells; TRG3, fibrosis outgrowing residual cancer; TRG4, residual cancer outgrowing fibrosis and TRG5, absence of regression changes. Representative images of some CRC samples having different response to the chemotherapy are shown. H&E, 200×.

Figure S3. Overexpression of piR-54265 in colorectal cancer.

(A) Top 20 highly expressed piRNAs in colorectal cancer based on the analysis of TCGA and GEO databases.

(B) The expression levels of piR-54265 in CRC and paired non-tumor tissues by qRT-PCR (mean ± SEM; ***, $P<0.001$).

(C) Northern blot of piR-54265 in CRC cell lines HCT116 and LoVo cells confirming its existence and molecular size of 29 nt as expected.

(D) Copy number per cell of piR-54265 in CRC cell lines (mean ± SEM).

(E) Distribution of piR-54265 in cytoplasm and nucleus of CRC cells with U6 and GAPDH as markers (% ± SEM).

Figure S4. Associations of piR-54265 levels with survival time of CRC patients by tumor location and stage.

(A) Associations of piR-54265 levels with progression-free survival time (*left panel*) and overall survival time (*right panel*) in CRC patients with different tumor locations. **(B)** Associations of piR-54265 levels with progression-free survival time (*left panel*) and overall survival time (*right panel*) in CRC patients with different TNM stage. HR, hazard ratio; CI, confident interval.

Figure S5. Effects of piR-54265 on cell cycle progression of CRC cells.

(A) Stable overexpression (OE) of piR-54265 in CRC cells (mean ± SEM, N=3; **, $P<0.01$).

(B) Stable knockdown (KD) of piR-54265 in CRC cells (mean ± SEM, N=3; ***, $P<0.001$; ****, $P<0.0001$).

(C) Effects of piR-54265 on cell cycle progression of CRC cells. Flow cytometry images (*left panel*) and quantitative statistics (mean ± SEM, N=3; NS, not significant) (*right panel*). OE, overexpression; KD, knockdown.

Figure S6. Metastatic cancers in the lung of mice implanted with CRC cells.

OE, overexpression; KD, knockdown.

Figure S7. Null effect of piR-54265 on expressions of nearby genes.

(A) Genomic location of piR-54265 gene and the nearby genes within about 2 mega-bases centering piR-54265.

(B) Null effects of piR-54265 knockdown on the expressions of nearby genes in CRC cells (mean ± SEM). KD, knockdown.

Figure S8. Inhibitory effects of PIWIL2 and STAT3 knockdown on oncogenic

functions of piR-54265.

(A) Efficient knockdown of PIWIL2 (*left panel*) and STAT3 (*right panel*) mRNA expressions in CRC cells by small interfering RNAs target to PIWIL2 or STAT3 gene (means ± SEM, N=3).

(B) Representative images of CRC cell migration (*upper panel*) and invasion (*lower panel*) in vitro in transwell assays. Scale bars, 100 μm. See also **Figure 4E** for the quantitative results.

(C) Flow cytometry images of CRC cell apoptosis. See also **Figure 4F** for the quantitative results.

Figure S9. Effect of piR-54265 on sensitivity of CRC cells to anticancer agents and effect of antagopiR54265 treatment on mouse bodyweight gain.

(A) Effects of piR-54265 overexpression (OE) or knockdown (KD) on apoptosis of CRC cells induced by 5-FU (*left panel*) or oxaliplatin (*right panel*). See also **Figure 5C-D** for the quantitative results.

(B) Effect of antagopiR54265 treatment on bodyweight gain of mice with subcutaneous xenograft of CRC cells (mean ± SEM for each point in the curves, N=5; NS, not significant).

(C) Effect of antagopiR54265 treatment on body weight gain of mice with metastatic tumors derived from plantation of CRC cells (mean ± SEM for each point in the curves, N=5; NS, not significant).

Figure S1

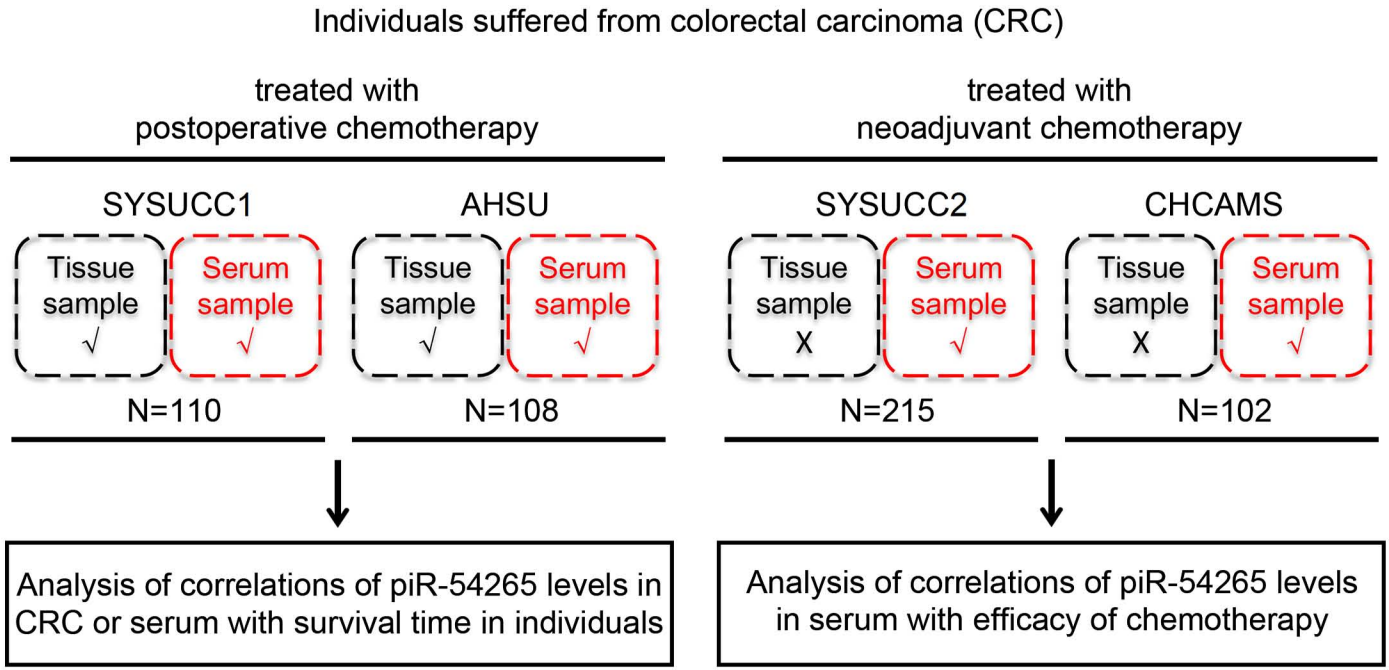
Individuals suffered from colorectal carcinoma (CRC)
treated with postoperative chemotherapy
treated with neoadjuvant chemotherapy
SYSUCC1
AHSU
SYSUCC2
CHCAMS
Tissue sample √
Serum sample √
Tissue sample √
Serum sample √
Tissue sample X
Serum sample √
Tissue sample X
Serum sample √
N=110
N=108
N=215
N=102
Analysis of correlations of piR-54265 levels in CRC or serum with survival time in individuals
Analysis of correlations of piR-54265 levels in serum with efficacy of chemotherapy

Figure S2

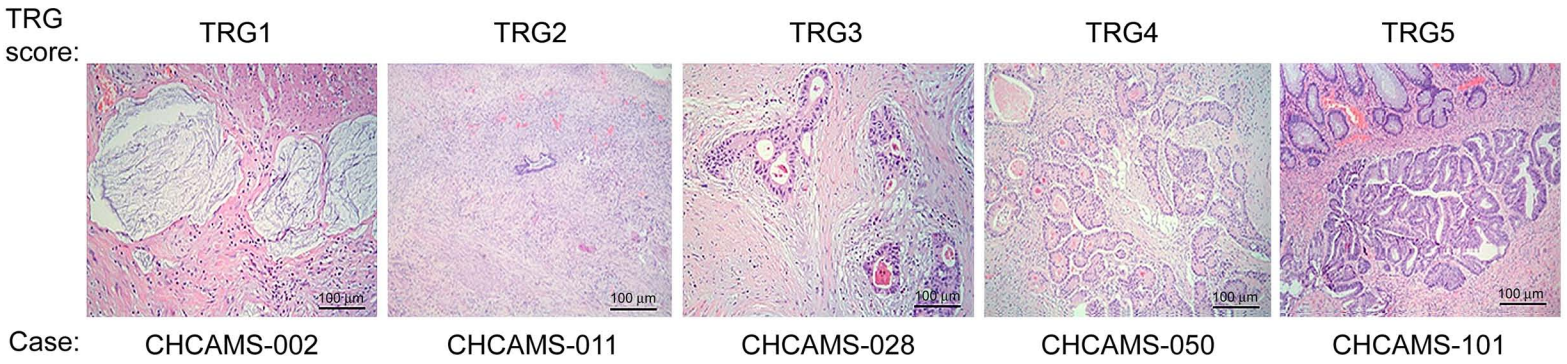
TRG score:
TRG1
TRG2
TRG3
TRG4
TRG5
100 µm
100 µm
100 µm
100 µm
100 µm
Case:
CHCAMS-002
CHCAMS-011
CHCAMS-028
CHCAMS-050
CHCAMS-101

Figure S3

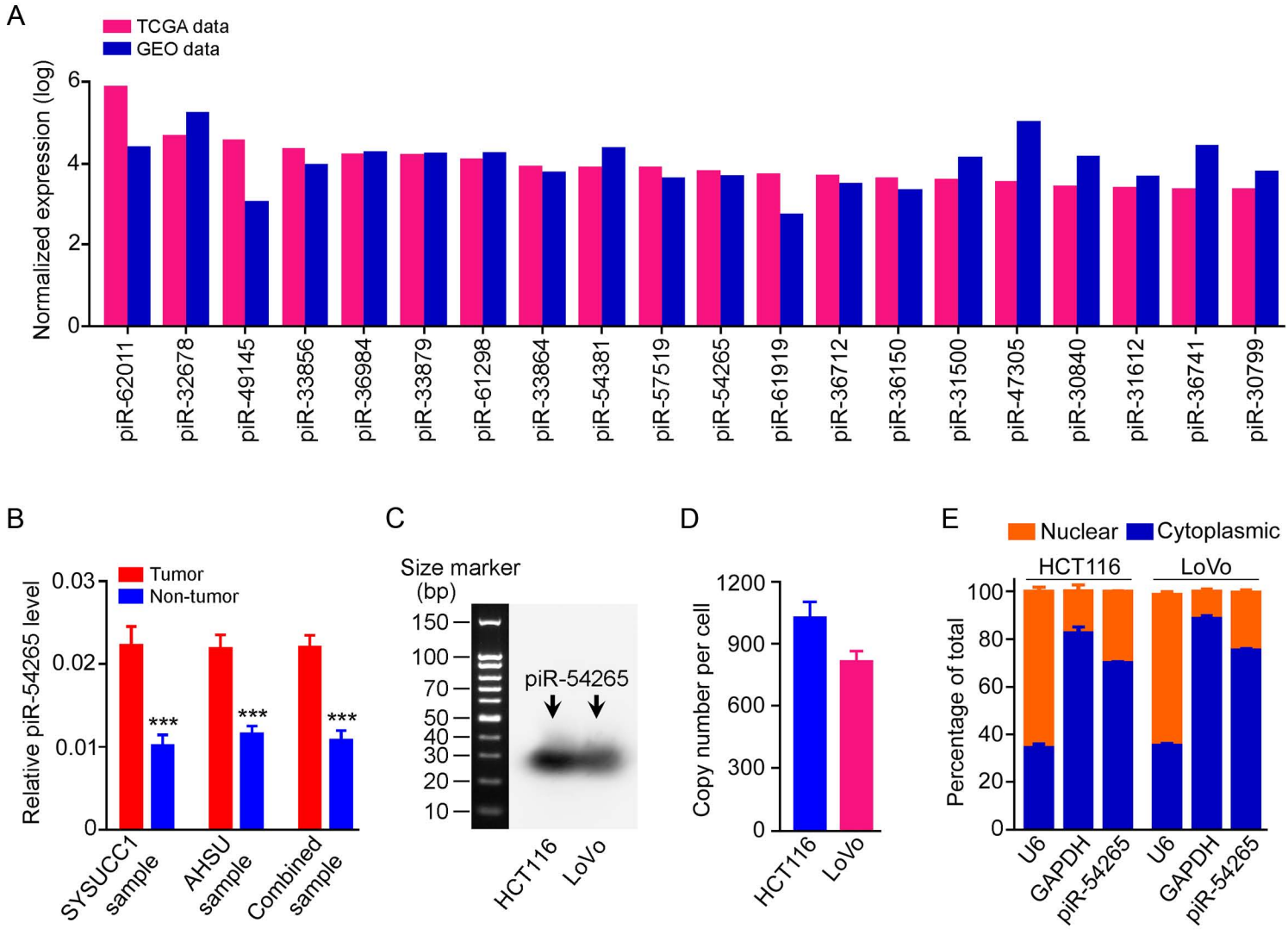
A
TCGA data
GEO data
Normalized expression (log)
0
2
4
6
piR-62011
piR-32678
piR-49145
piR-33856
piR-36984
piR-33879
piR-61298
piR-33864
piR-54381
piR-57519
piR-54265
piR-61919
piR-36712
piR-36150
piR-31500
piR-47305
piR-30840
piR-31612
piR-36741
piR-30799
B
Tumor
Non-tumor
Relative piR-54265 level
0
0.01
0.02
0.03
***
***
***
SYSUCC1 sample
AHSU sample
Combined sample
C
Size marker (bp)
150
100
70
50
40
30
20
10
piR-54265
HCT116
LoVo
D
Copy number per cell
0
300
600
900
1200
HCT116
LoVo
E
Nuclear
Cytoplasmic
HCT116
LoVo
Percentage of total
0
20
40
60
80
100
U6
GAPDH
piR-54265
U6
GAPDH
piR-54265

Figure S4

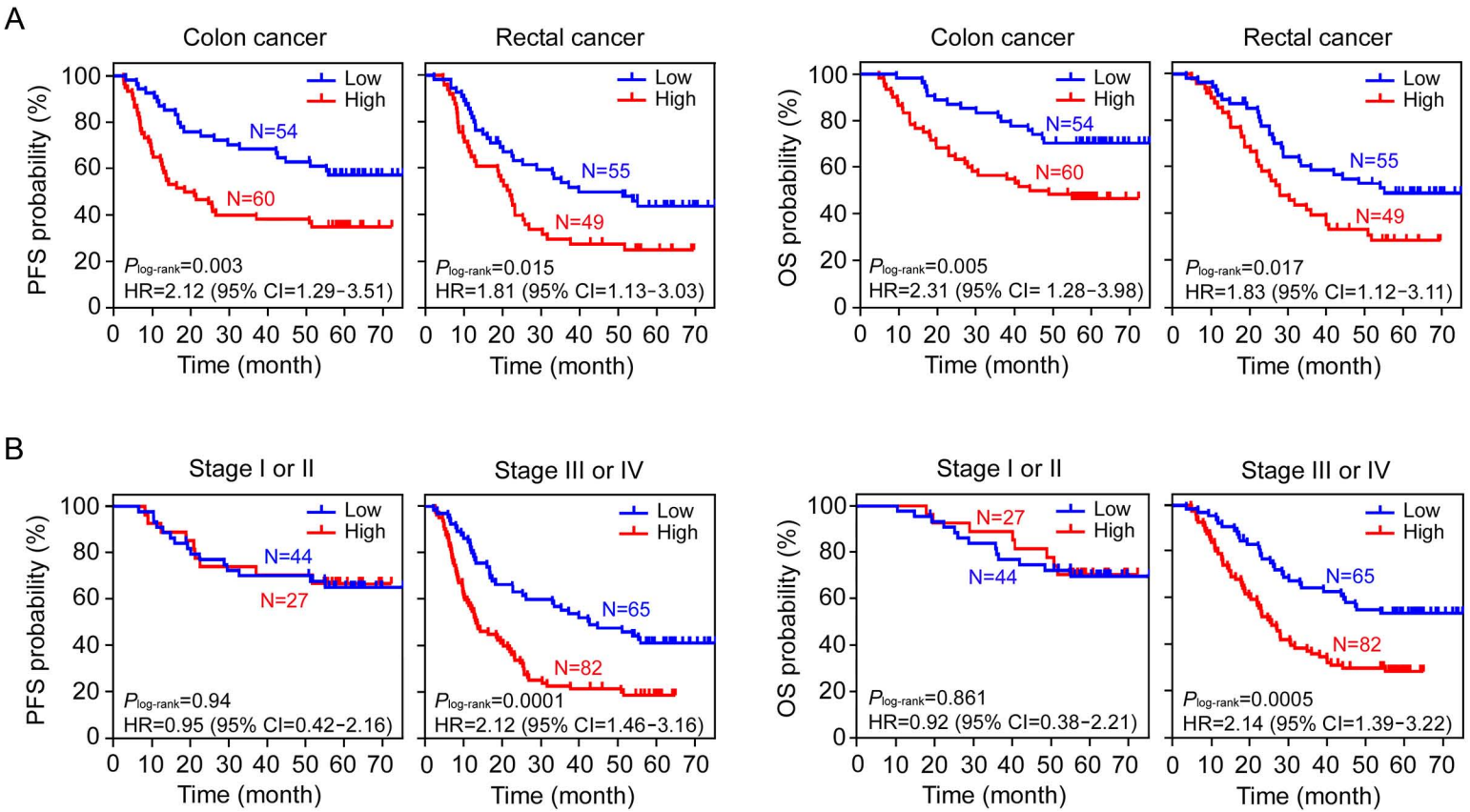
A
Colon cancer
Rectal cancer
Colon cancer
Rectal cancer
PFS probability (%)
OS probability (%)
Low
High
N=54
N=60
N=55
N=49
Plog-rank=0.003
HR=2.12 (95% CI=1.29-3.51)
Plog-rank=0.015
HR=1.81 (95% CI=1.13-3.03)
Plog-rank=0.005
HR=2.31 (95% CI= 1.28-3.98)
Plog-rank=0.017
HR=1.83 (95% CI=1.12-3.11)
Time (month)
B
Stage I or II
Stage III or IV
Stage I or II
Stage III or IV
N=44
N=27
N=65
N=82
N=27
N=44
N=65
N=82
Plog-rank=0.94
HR=0.95 (95% CI=0.42-2.16)
Plog-rank=0.0001
HR=2.12 (95% CI=1.46-3.16)
Plog-rank=0.861
HR=0.92 (95% CI=0.38-2.21)
Plog-rank=0.0005
HR=2.14 (95% CI=1.39-3.22)

Figure S5

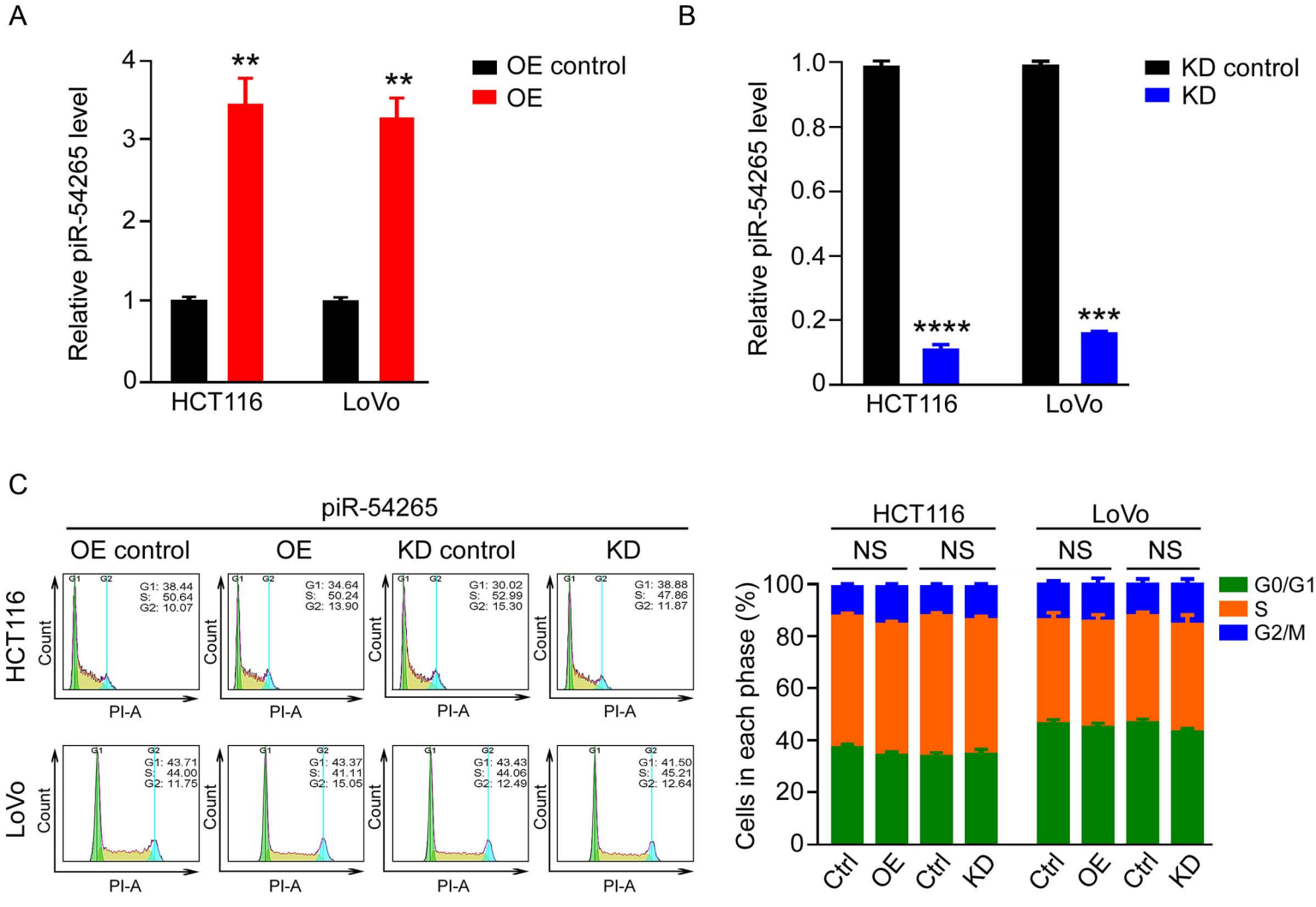
A
Relative piR-54265 level
OE control
OE
**
**
HCT116
LoVo
B
Relative piR-54265 level
KD control
KD
****
***
HCT116
LoVo
C
piR-54265
OE control
OE
KD control
KD
HCT116
LoVo
Count
PI-A
G1: 38.44
S: 50.64
G2: 10.07
G1: 34.64
S: 50.24
G2: 13.90
G1: 30.02
S: 52.99
G2: 15.30
G1: 38.88
S: 47.86
G2: 11.87
G1: 43.71
S: 44.00
G2: 11.75
G1: 43.37
S: 41.11
G2: 15.05
G1: 43.43
S: 44.06
G2: 12.49
G1: 41.50
S: 45.21
G2: 12.64
HCT116
LoVo
NS
Cells in each phase (%)
G0/G1
S
G2/M
Ctrl
OE
KD

Figure S6

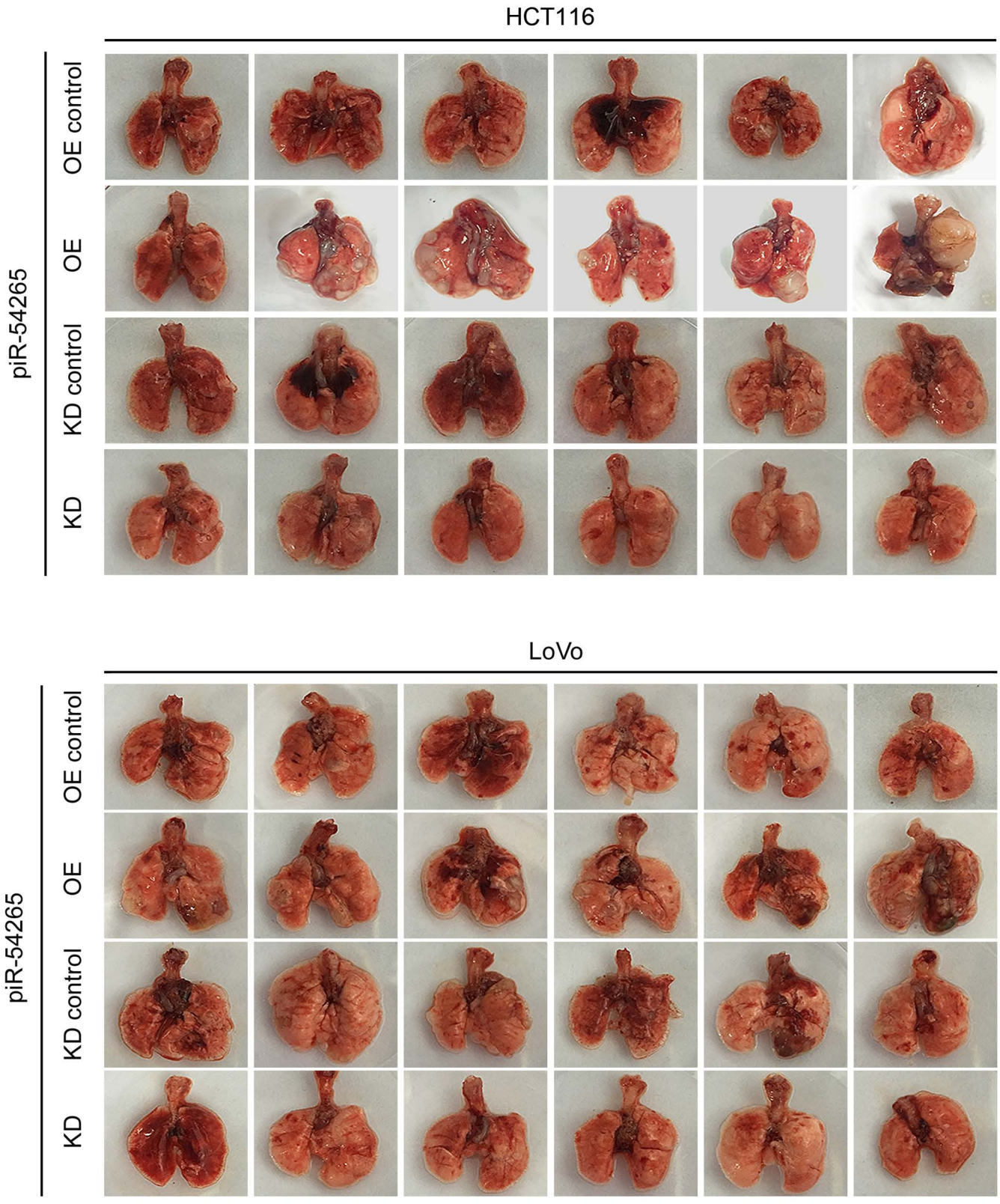
HCT116
piR-54265
OE control
OE
KD control
KD
LoVo
piR-54265
OE control
OE
KD control
KD

Figure S7

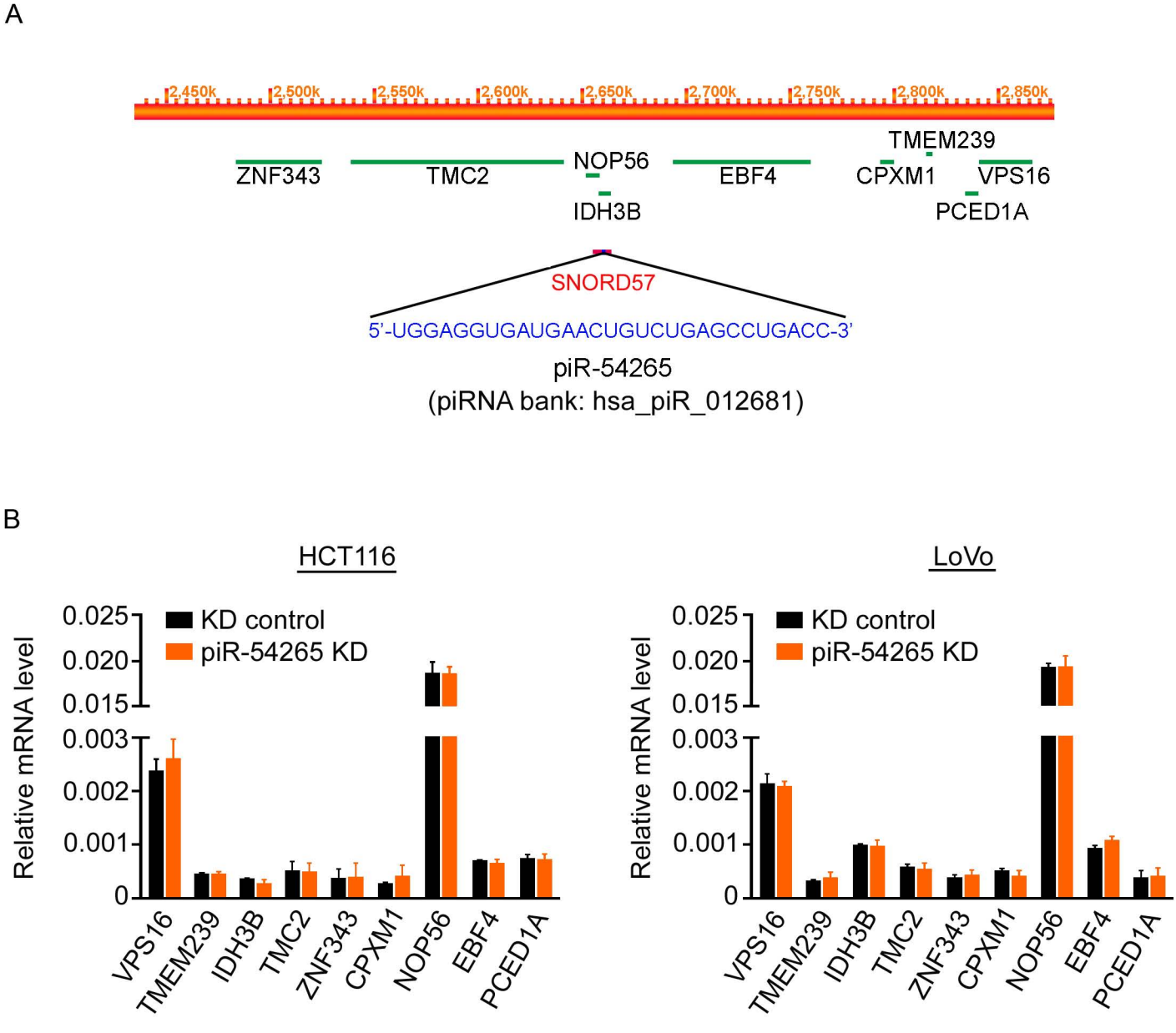
A
2,450k
2,500k
2,550k
2,600k
2,650k
2,700k
2,750k
2,800k
2,850k
ZNF343
TMC2
NOP56
EBF4
TMEM239
CPXM1
VPS16
IDH3B
PCED1A
SNORD57
5'-UGGAGGUGAUGAACUGUCUGAGCCUGACC-3'
piR-54265
(piRNA bank: hsa_piR_012681)
B
HCT116
LoVo
KD control
piR-54265 KD
Relative mRNA level
0.025
0.020
0.015
0.003
0.002
0.001
0
VPS16
TMEM239
IDH3B
TMC2
ZNF343
CPXM1
NOP56
EBF4
PCED1A

Figure S8

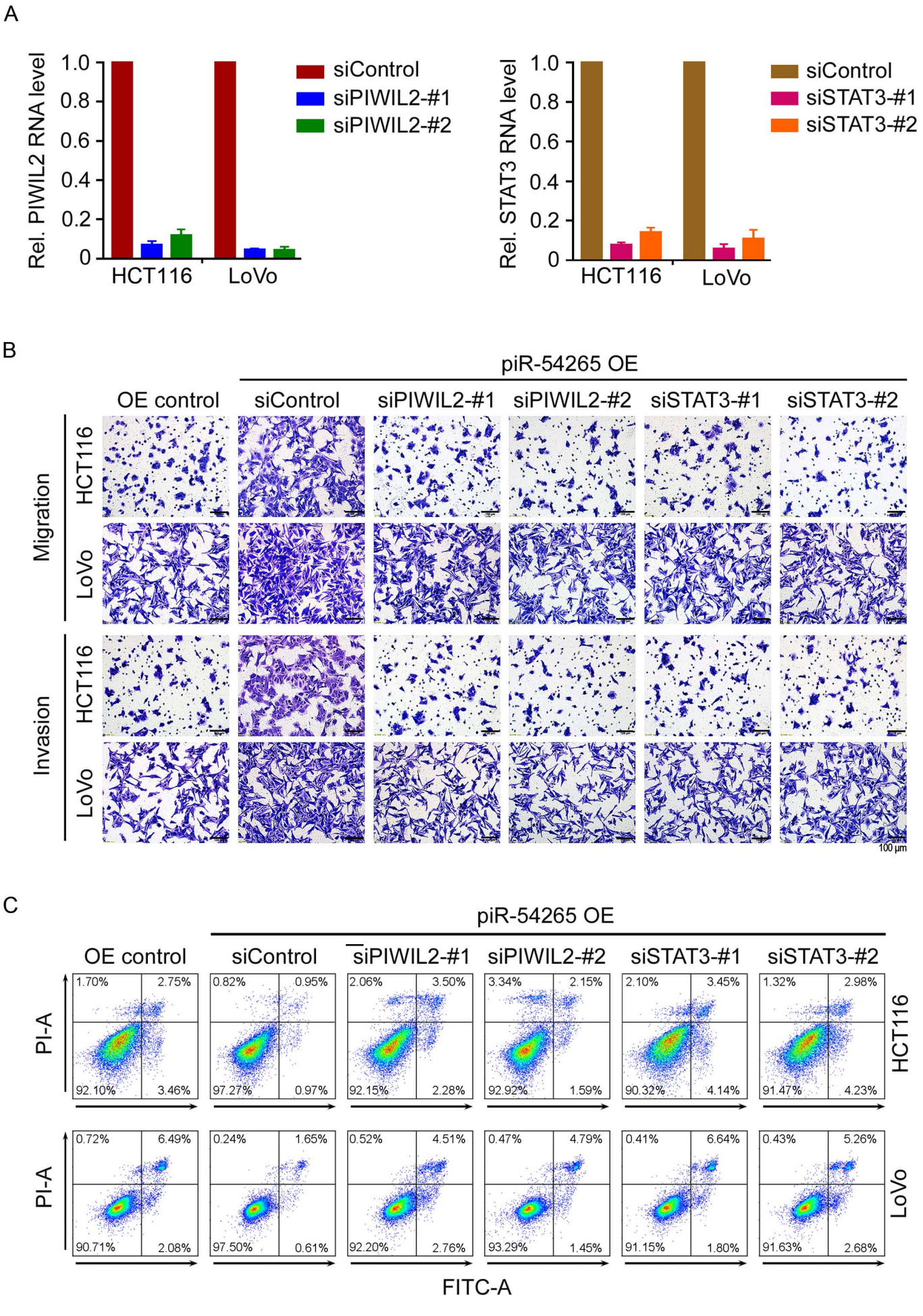
A
Rel. PIWIL2 RNA level
1.0
0.8
0.6
0.4
0.2
0
HCT116
LoVo
siControl
siPIWIL2-#1
siPIWIL2-#2
Rel. STAT3 RNA level
siControl
siSTAT3-#1
siSTAT3-#2
B
piR-54265 OE
OE control
siControl
siPIWIL2-#1
siPIWIL2-#2
siSTAT3-#1
siSTAT3-#2
Migration
Invasion
HCT116
LoVo
100 μm
C
piR-54265 OE
OE control
siControl
siPIWIL2-#1
siPIWIL2-#2
siSTAT3-#1
siSTAT3-#2
PI-A
HCT116
LoVo
1.70% 2.75% 92.10% 3.46%
0.82% 0.95% 97.27% 0.97%
2.06% 3.50% 92.15% 2.28%
3.34% 2.15% 92.92% 1.59%
2.10% 3.45% 90.32% 4.14%
1.32% 2.98% 91.47% 4.23%
0.72% 6.49% 90.71% 2.08%
0.24% 1.65% 97.50% 0.61%
0.52% 4.51% 92.20% 2.76%
0.47% 4.79% 93.29% 1.45%
0.41% 6.64% 91.15% 1.80%
0.43% 5.26% 91.63% 2.68%
FITC-A

Figure S9

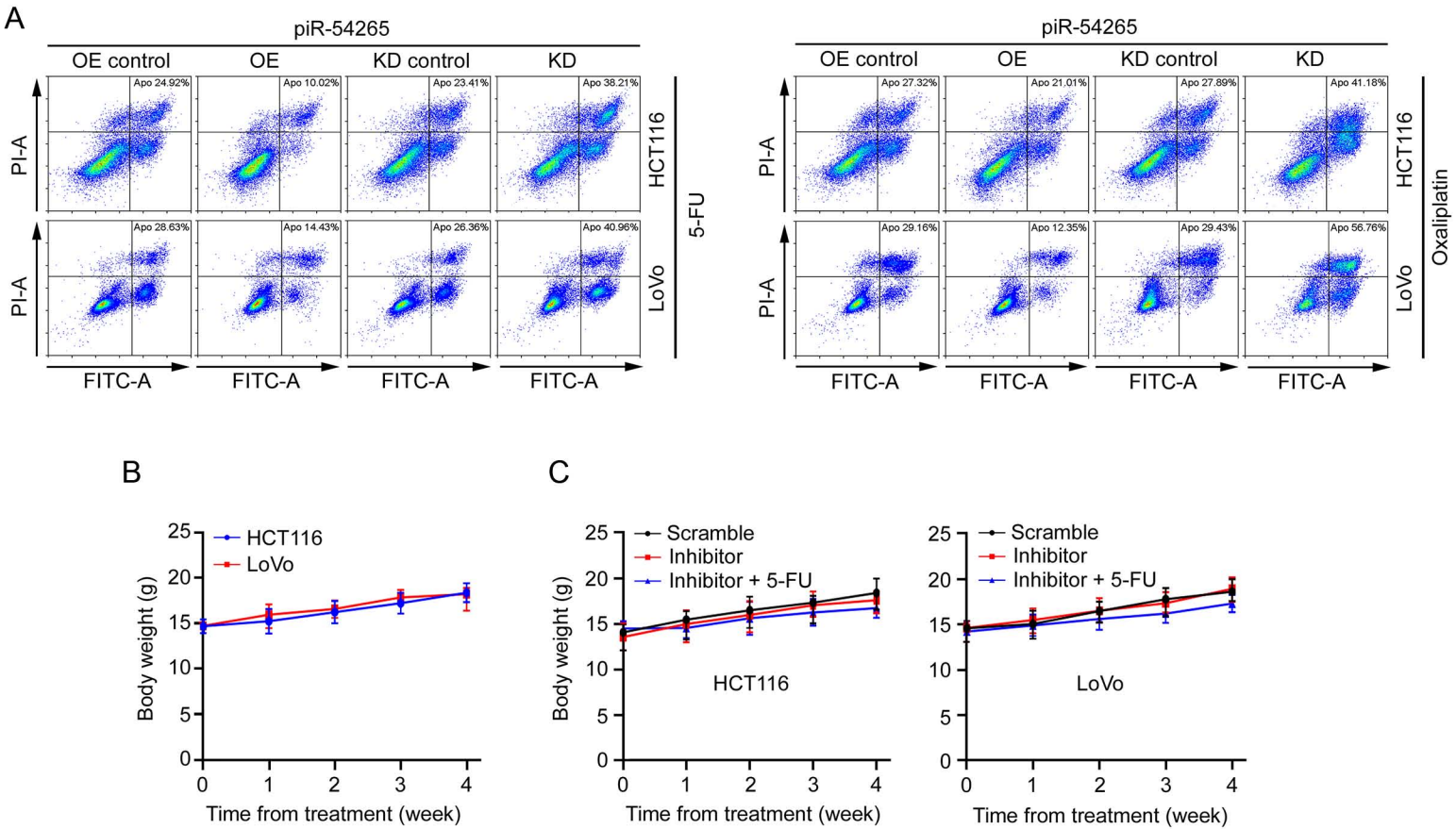
A
piR-54265
OE control
OE
KD control
KD
PI-A
FITC-A
Apo 24.92%
Apo 10.02%
Apo 23.41%
Apo 38.21%
Apo 28.63%
Apo 14.43%
Apo 26.36%
Apo 40.96%
HCT116
LoVo
5-FU
Apo 27.32%
Apo 21.01%
Apo 27.89%
Apo 41.18%
Apo 29.16%
Apo 12.35%
Apo 29.43%
Apo 56.76%
Oxaliplatin
B
HCT116
LoVo
Body weight (g)
Time from treatment (week)
C
Scramble
Inhibitor
Inhibitor + 5-FU
HCT116
LoVo

Table S1. Demographic and clinical characteristics of individuals with CRC and treated with 5-FU and oxaliplatin based postoperative chemotherapy

Sample ID	Sex*	Age (years)	Family history	Smoking status	Drinking status	TNM stage	Tumor location	Survival status	Tumor progression	PFS** (month)	OS** (month)	piR-54265 in tumor	piR-54265 in serum
SYSUCC-001	F	70	No	No	No	IVb	Colon	Dead	Yes	9.7	12.7	High	High
SYSUCC-002	F	43	No	No	No	Ib	Colon	Alive	No	57.3	57.3	Low	Low
SYSUCC-003	F	61	No	No	No	IVb	Colon	Dead	Yes	44.7	44.7	Low	Low
SYSUCC-004	M	37	No	No	No	IIb	Rectum	Dead	Yes	6.6	41.9	Low	Low
SYSUCC-005	F	41	No	No	No	IIb	Rectum	Alive	No	55.4	55.4	High	High
SYSUCC-006	M	78	No	No	No	IIb	Rectum	Alive	No	75.0	75.0	Low	High
SYSUCC-007	F	69	Yes	No	No	IIb	Rectum	Dead	Yes	18.9	40.6	High	High
SYSUCC-008	F	39	No	No	No	IIb	Rectum	Alive	No	75.0	75.0	Low	High
SYSUCC-009	M	57	No	Yes	No	IIb	Colon	Alive	Yes	29.6	75.0	Low	High
SYSUCC-010	M	73	No	No	Yes	IIb	Colon	Alive	Yes	10.5	75.0	Low	Low
SYSUCC-011	F	54	Yes	No	No	Ib	Rectum	Alive	No	69.1	69.1	High	Low
SYSUCC-012	M	60	No	No	No	IV	Colon	Alive	No	75.0	75.0	Low	High
SYSUCC-013	M	68	Yes	No	No	IV	Colon	Alive	Yes	8.2	75.0	Low	Low
SYSUCC-014	M	65	No	No	No	Ib	Rectum	Alive	No	75.0	75.0	Low	Low
SYSUCC-015	F	80	No	No	No	Ia	Rectum	Dead	Yes	25.2	51.2	Low	Low
SYSUCC-016	M	40	Yes	No	No	IV	Colon	Dead	Yes	9.8	41.0	High	High
SYSUCC-017	M	38	Yes	Yes	No	IV	Colon	Alive	No	72.4	72.4	Low	Low
SYSUCC-018	F	72	No	No	No	IIIb	Colon	Alive	No	72.3	72.3	High	High
SYSUCC-019	M	72	No	Yes	No	IV	Colon	Alive	No	73.8	73.8	Low	Low
SYSUCC-020	F	60	No	No	No	IV	Rectum	Alive	No	73.4	73.4	Low	Low
SYSUCC-021	F	73	No	No	No	Ib	Colon	Alive	No	68.3	68.3	Low	High
SYSUCC-022	F	45	No	No	No	Ib	Colon	Alive	No	65.5	65.5	Low	Low
SYSUCC-023	M	69	No	No	No	IV	Rectum	Alive	No	70.6	70.6	Low	Low
SYSUCC-024	M	47	No	No	No	IV	Colon	Dead	Yes	3.5	22.8	High	High
SYSUCC-025	F	78	No	No	No	Ib	Colon	Alive	No	69.7	69.7	Low	Low
SYSUCC-026	M	60	No	Yes	Yes	Ib	Rectum	Alive	No	69.6	69.6	Low	Low

SYSUCC-027	M	59	No	Yes	Yes	Ib	Rectum	Alive	No	69.7	69.7	High	High
SYSUCC-028	M	72	Yes	No	No	IIb	Rectum	Alive	No	69.9	69.9	Low	Low
SYSUCC-029	M	75	No	No	No	IV	Rectum	Dead	Yes	23.1	23.1	High	High
SYSUCC-030	M	72	No	Yes	Yes	IIb	Rectum	Dead	Yes	12.9	26.0	Low	Low
SYSUCC-031	M	58	No	No	No	IV	Colon	Alive	Yes	55.7	63.0	Low	High
SYSUCC-032	M	72	No	No	No	IV	Colon	Dead	Yes	22.5	22.5	Low	High
SYSUCC-033	F	57	Yes	No	No	IIb	Rectum	Alive	No	69.8	69.8	Low	Low
SYSUCC-034	F	52	No	Yes	Yes	IV	Rectum	Dead	Yes	20.2	20.2	High	High
SYSUCC-035	F	36	No	No	No	IIIa	Rectum	Dead	Yes	37.2	44.2	Low	High
SYSUCC-036	M	54	No	Yes	Yes	IVb	Rectum	Alive	Yes	16.8	59.8	Low	Low
SYSUCC-037	M	46	No	Yes	Yes	IIIb	Rectum	Alive	Yes	35.2	68.1	Low	Low
SYSUCC-038	F	61	No	No	No	IIIb	Rectum	Alive	Yes	39.8	58.9	Low	Low
SYSUCC-039	M	62	No	No	No	IIIc	Rectum	Dead	Yes	5.7	8.8	High	Low
SYSUCC-040	M	54	No	Yes	Yes	IIIc	Rectum	Dead	Yes	11.3	15.1	High	High
SYSUCC-041	M	60	No	No	No	IV	Rectum	Alive	No	66.8	66.8	Low	High
SYSUCC-042	F	71	No	No	No	IIIa	Rectum	Alive	Yes	37.6	57.6	High	Low
SYSUCC-043	M	49	No	No	No	IIIc	Colon	Alive	No	59.4	59.4	High	High
SYSUCC-044	M	62	No	No	No	IIIb	Colon	Dead	Yes	12.1	19.7	High	High
SYSUCC-045	M	38	No	Yes	Yes	IVb	Rectum	Dead	Yes	4.5	4.9	High	High
SYSUCC-046	M	76	No	Yes	Yes	IIIb	Colon	Alive	No	64.9	64.9	Low	Low
SYSUCC-047	F	60	Yes	No	No	IV	Rectum	Dead	Yes	22.3	22.3	High	High
SYSUCC-048	M	66	No	No	No	IIIb	Colon	Dead	Yes	37.1	40.1	High	Low
SYSUCC-049	M	71	No	No	No	IV	Colon	Dead	Yes	6.7	9.7	High	High
SYSUCC-050	F	45	No	No	No	IIb	Colon	Dead	Yes	12.4	29.0	High	High
SYSUCC-051	F	59	No	No	No	IV	Colon	Dead	Yes	24.6	24.6	High	Low
SYSUCC-052	M	62	No	Yes	Yes	IV	Rectum	Dead	Yes	12.8	12.8	Low	Low
SYSUCC-053	F	54	No	No	No	IV	Rectum	Dead	Yes	12.5	22.1	Low	Low
SYSUCC-054	M	72	No	Yes	Yes	IIIc	Colon	Dead	Yes	2.5	18.1	High	High
SYSUCC-055	M	40	No	No	No	IV	Rectum	Dead	Yes	9.7	11.7	High	High

SYSUCC-056	F	71	No	No	No	IIb	Colon	Alive	No	63.8	63.8	Low	Low
SYSUCC-057	F	56	No	No	No	IIIb	Colon	Alive	No	62.5	62.5	Low	Low
SYSUCC-058	M	65	No	Yes	Yes	IIIb	Rectum	Dead	Yes	53.7	53.7	Low	High
SYSUCC-059	M	46	No	Yes	Yes	IIb	Colon	Alive	No	69.0	69.0	High	High
SYSUCC-060	M	36	No	Yes	Yes	IVb	Colon	Dead	Yes	13.5	44.0	High	High
SYSUCC-061	F	67	No	No	No	IIIb	Rectum	Dead	Yes	8.1	27.9	High	High
SYSUCC-062	M	39	No	Yes	Yes	IIIb	Colon	Alive	No	58.1	58.1	High	High
SYSUCC-063	F	30	No	No	No	IIb	Colon	Alive	No	60.8	60.8	High	High
SYSUCC-064	F	77	No	No	No	IVb	Colon	Dead	Yes	26.5	30.5	High	High
SYSUCC-065	M	63	No	Yes	Yes	IIIa	Rectum	Alive	No	54.6	54.6	High	High
SYSUCC-066	M	46	No	No	No	Ib	Rectum	Dead	Yes	28.8	28.8	Low	Low
SYSUCC-067	M	66	No	No	No	IVa	Colon	Alive	No	61.3	61.3	High	High
SYSUCC-068	M	44	No	No	No	IIb	Colon	Alive	No	64.2	64.2	High	Low
SYSUCC-069	M	69	No	Yes	Yes	IV	Rectum	Dead	Yes	18.7	18.7	High	High
SYSUCC-070	M	43	No	Yes	Yes	IV	Colon	Alive	No	60.3	60.3	High	Low
SYSUCC-071	M	57	No	No	No	IVb	Colon	Dead	Yes	4.9	9.5	High	Low
SYSUCC-072	F	62	No	No	No	IIIb	Colon	Alive	No	59.9	59.9	High	Low
SYSUCC-073	M	65	No	No	No	IIIb	Colon	Alive	Yes	6.9	60.0	High	High
SYSUCC-074	F	63	No	No	No	IIb	Rectum	Dead	Yes	19.3	19.3	Low	Low
SYSUCC-075	F	44	No	No	No	IIIb	Colon	Alive	No	59.8	59.8	High	High
SYSUCC-076	F	68	No	No	No	IIb	Rectum	Dead	Yes	14.8	14.8	Low	Low
SYSUCC-077	F	46	No	No	No	IIIc	Rectum	Dead	Yes	25.6	25.6	High	High
SYSUCC-078	F	78	No	No	No	IIIb	Rectum	Dead	Yes	8.3	13.0	High	High
SYSUCC-079	F	61	No	No	No	IV	Colon	Alive	No	68.7	68.7	Low	Low
SYSUCC-080	M	51	No	No	No	IIIb	Rectum	Dead	Yes	19.5	34.8	High	High
SYSUCC-081	F	59	No	No	No	I	Rectum	Dead	Yes	33.3	33.3	Low	High
SYSUCC-082	M	70	No	No	No	IIIb	Colon	Alive	No	58.9	58.9	Low	Low
SYSUCC-083	M	39	No	Yes	Yes	IIb	Colon	Alive	No	58.9	58.9	High	High
SYSUCC-084	M	66	No	No	No	IIIc	Rectum	Alive	No	68.5	68.5	Low	Low

SYSUCC-085	F	65	No	No	No	IIb	Rectum	Dead	Yes	55.1	55.1	Low	Low
SYSUCC-086	F	52	No	No	No	IV	Rectum	Dead	Yes	31.6	31.6	High	Low
SYSUCC-087	M	33	No	No	No	IIb	Colon	Alive	No	65.3	65.3	Low	Low
SYSUCC-088	F	34	No	No	No	IIb	Colon	Alive	No	58.3	58.3	High	High
SYSUCC-089	F	74	No	Yes	Yes	IV	Colon	Dead	Yes	10.2	14.2	High	High
SYSUCC-090	F	58	No	No	No	IV	Colon	Dead	Yes	17.3	17.3	Low	Low
SYSUCC-091	M	49	No	Yes	Yes	IIb	Colon	Alive	No	58.0	58.0	High	Low
SYSUCC-092	M	44	No	No	No	IIIb	Rectum	Alive	No	63.8	63.8	Low	Low
SYSUCC-093	M	77	No	No	No	IV	Rectum	Dead	Yes	13.1	15.1	High	Low
SYSUCC-094	F	42	No	No	No	IIb	Rectum	Alive	No	62.8	62.8	Low	Low
SYSUCC-095	M	61	No	No	No	IV	Rectum	Dead	Yes	10.0	10.0	High	High
SYSUCC-096	M	59	No	No	No	IIb	Colon	Alive	No	57.8	57.8	High	High
SYSUCC-097	M	54	No	No	No	IV	Colon	Alive	Yes	25.6	56.4	High	High
SYSUCC-098	M	60	Yes	Yes	Yes	IV	Colon	Dead	Yes	6.0	11.0	High	High
SYSUCC-099	F	62	No	No	No	IIIb	Colon	Alive	Yes	12.6	57.4	High	Low
SYSUCC-100	M	76	No	No	No	IIIb	Rectum	Alive	No	58.1	58.1	Low	Low
SYSUCC-101	M	45	No	No	No	IV	Rectum	Dead	Yes	12.0	28.7	Low	High
SYSUCC-102	M	62	No	Yes	Yes	IIIb	Colon	Alive	No	66.9	66.9	Low	Low
SYSUCC-103	F	56	No	No	No	IIIb	Rectum	Dead	Yes	6.6	6.6	Low	High
SYSUCC-104	F	40	No	No	No	IV	Colon	Dead	Yes	16.7	16.7	Low	Low
SYSUCC-105	M	59	No	Yes	Yes	IIb	Colon	Alive	No	56.1	56.1	Low	Low
SYSUCC-106	M	52	No	Yes	Yes	IIb	Rectum	Dead	Yes	22.5	50.8	High	Low
SYSUCC-107	M	74	No	No	No	IV	Colon	Dead	Yes	6.1	6.1	High	High
SYSUCC-108	M	48	No	No	No	IVa	Colon	Dead	Yes	42.6	47.6	Low	High
SYSUCC-109	F	36	Yes	No	No	IIIb	Colon	Alive	Yes	51.4	61.4	High	High
SYSUCC-110	F	53	No	No	No	IIb	Colon	Dead	Yes	11.3	36.5	Low	Low
AHSU-001	M	26	Yes	Yes	No	IV	Colon	Dead	Yes	2.9	6.0	High	Low
AHSU-002	M	29	No	No	No	II	Colon	Alive	No	57.8	57.8	Low	Low
AHSU-003	M	30	No	No	No	IV	Colon	Dead	Yes	16.0	16.0	Low	High

AHSU-004	F	30	No	No	No	IIb	Colon	Alive	No	60.4	60.4	Low	High
AHSU-005	M	32	No	No	No	IV	Rectum	Alive	No	64.4	64.4	Low	High
AHSU-006	F	34	Yes	Yes	No	IV	Colon	Alive	No	57.9	57.9	High	High
AHSU-007	M	35	No	Yes	Yes	IIIb	Rectum	Dead	Yes	30.3	30.3	High	High
AHSU-008	M	36	Yes	Yes	Yes	IVb	Colon	Dead	Yes	13.1	43.6	Low	Low
AHSU-009	F	36	Yes	No	No	Ib	Colon	Alive	Yes	51.0	61.0	Low	Low
AHSU-010	M	38	No	No	Yes	IVb	Rectum	Dead	Yes	2.1	3.5	Low	High
AHSU-011	F	38	No	Yes	No	IIIb	Rectum	Dead	Yes	7.9	14.6	High	Low
AHSU-012	M	39	Yes	No	Yes	IIIb	Colon	Alive	No	57.7	57.7	Low	Low
AHSU-013	M	40	No	Yes	No	IV	Rectum	Dead	Yes	9.3	11.3	Low	Low
AHSU-014	F	40	Yes	No	No	IV	Colon	Dead	Yes	16.3	16.3	High	High
AHSU-015	M	40	No	No	No	IV	Rectum	Dead	Yes	11.8	21.8	High	High
AHSU-016	F	41	No	No	No	IIb	Rectum	Alive	No	55.0	55.0	Low	Low
AHSU-017	M	41	No	No	Yes	II	Colon	Alive	No	64.8	64.8	High	High
AHSU-018	F	42	No	No	No	IV	Colon	Alive	Yes	14.2	53.8	High	High
AHSU-019	F	42	No	No	No	IIb	Rectum	Alive	No	62.4	62.4	Low	Low
AHSU-020	M	43	No	Yes	Yes	III	Colon	Dead	Yes	16.7	39.0	Low	Low
AHSU-021	M	43	No	Yes	No	II	Colon	Alive	No	63.8	63.8	Low	Low
AHSU-022	F	44	No	No	No	IIIb	Colon	Alive	No	59.4	59.4	High	Low
AHSU-023	M	44	No	Yes	No	IIIb	Rectum	Alive	No	62.4	62.4	Low	Low
AHSU-024	F	45	Yes	No	No	IIb	Colon	Dead	Yes	9.0	19.6	High	Low
AHSU-025	M	45	Yes	No	No	IV	Rectum	Dead	Yes	11.6	28.3	Low	Low
AHSU-026	F	45	No	No	No	I	Rectum	Alive	No	51.9	51.9	Low	Low
AHSU-027	M	46	Yes	No	No	Ib	Rectum	Dead	Yes	22.4	22.4	Low	High
AHSU-028	F	46	Yes	No	No	IIIc	Rectum	Dead	Yes	25.2	25.2	High	Low
AHSU-029	M	46	No	No	Yes	IIb	Colon	Alive	No	64.6	64.6	Low	High
AHSU-030	M	47	No	Yes	No	IV	Rectum	Dead	Yes	7.5	27.8	High	High
AHSU-031	M	48	Yes	No	No	IVa	Colon	Dead	Yes	42.2	47.2	Low	High
AHSU-032	M	49	No	No	No	IIIc	Colon	Alive	No	59.0	59.0	Low	High

AHSU-033	M	50	No	No	Yes	IIIa	Colon	Dead	Yes	17.1	18.1	Low	Low
AHSU-034	M	50	No	Yes	Yes	IV	Rectum	Dead	Yes	8.6	39.9	High	High
AHSU-035	M	51	No	Yes	No	IIIb	Rectum	Dead	Yes	11.1	26.4	Low	High
AHSU-036	F	51	No	No	No	III	Rectum	Dead	Yes	23.2	40.0	High	Low
AHSU-037	F	51	Yes	No	No	Ib	Rectum	Alive	No	59.8	59.8	Low	Low
AHSU-038	F	52	No	No	No	IV	Rectum	Dead	Yes	25.2	25.2	Low	High
AHSU-039	M	52	No	Yes	Yes	IIb	Rectum	Dead	Yes	20.1	48.4	Low	High
AHSU-040	F	53	No	No	No	III	Rectum	Alive	No	60.8	60.8	High	Low
AHSU-041	M	54	No	Yes	Yes	IIIc	Rectum	Dead	Yes	7.9	11.7	Low	High
AHSU-042	F	54	No	No	No	IV	Rectum	Dead	Yes	7.1	17.7	High	High
AHSU-043	M	54	No	No	No	III	Rectum	Alive	No	45.9	45.9	High	High
AHSU-044	F	54	No	No	No	I	Rectum	Alive	No	60.9	60.9	High	Low
AHSU-045	M	55	No	No	No	IIIb	Colon	Alive	Yes	50.8	59.6	High	Low
AHSU-046	F	56	No	Yes	No	IIIb	Rectum	Dead	Yes	6.2	6.2	High	Low
AHSU-047	F	57	No	Yes	No	IIb	Rectum	Alive	No	62.4	62.4	Low	Low
AHSU-048	M	57	No	Yes	Yes	II	Colon	Alive	No	64.0	64.0	Low	High
AHSU-049	F	57	No	No	No	II	Colon	Alive	No	64.0	64.0	Low	High
AHSU-050	F	58	No	No	No	IV	Colon	Dead	Yes	12.9	12.9	High	High
AHSU-051	M	58	No	No	No	IV	Colon	Alive	Yes	55.3	62.6	Low	Low
AHSU-052	F	59	No	Yes	No	IV	Rectum	Dead	Yes	32.9	32.9	Low	High
AHSU-053	F	59	No	No	No	III	Rectum	Dead	Yes	12.7	35.9	High	Low
AHSU-054	M	59	No	Yes	Yes	IIb	Colon	Alive	No	55.7	55.7	High	High
AHSU-055	M	59	No	Yes	No	IIb	Colon	Alive	No	57.4	57.4	Low	Low
AHSU-056	M	60	No	No	Yes	IV	Colon	Dead	Yes	2.6	6.6	High	High
AHSU-057	M	60	No	No	No	IV	Colon	Dead	Yes	9.5	17.9	High	High
AHSU-058	M	61	No	Yes	No	IV	Rectum	Alive	No	9.6	9.6	High	Low
AHSU-059	M	61	No	No	No	I	Rectum	Alive	No	63.9	63.9	High	Low
AHSU-060	M	62	No	No	Yes	IV	Rectum	Dead	Yes	8.4	8.4	High	High
AHSU-061	M	62	No	Yes	No	IIIb	Colon	Dead	Yes	11.7	19.3	Low	High

AHSU-062	F	62	Yes	No	No	IV	Colon	Dead	Yes	21.4	27.0	High	High
AHSU-063	M	62	No	Yes	No	II	Rectum	Dead	Yes	16.0	36.0	Low	Low
AHSU-064	F	62	No	No	No	IIIb	Colon	Alive	Yes	3.1	57.0	Low	High
AHSU-065	F	62	No	No	No	IIIb	Colon	Alive	No	59.5	59.5	Low	Low
AHSU-066	M	62	No	No	Yes	IIIb	Colon	Alive	No	62.5	62.5	Low	Low
AHSU-067	M	62	No	Yes	Yes	III	Colon	Alive	No	64.9	64.9	High	Low
AHSU-068	F	63	No	No	No	IIb	Rectum	Alive	No	18.9	18.9	Low	Low
AHSU-069	M	63	No	No	Yes	IIIa	Rectum	Alive	No	54.2	54.2	Low	Low
AHSU-070	M	63	No	No	Yes	I	Colon	Alive	No	56.9	56.9	High	Low
AHSU-071	M	64	Yes	Yes	Yes	IV	Colon	Dead	Yes	5.3	27.8	High	High
AHSU-072	M	64	Yes	No	Yes	IV	Colon	Alive	Yes	25.5	58.8	High	High
AHSU-073	F	65	No	No	No	IV	Rectum	Dead	Yes	21.9	21.9	High	High
AHSU-074	F	65	No	No	No	II	Rectum	Dead	Yes	51.7	51.7	High	High
AHSU-075	M	66	No	No	No	III	Rectum	Dead	Yes	9.9	23.0	Low	Low
AHSU-076	M	66	No	No	No	IIb	Colon	Dead	Yes	32.7	35.7	Low	High
AHSU-077	M	66	No	No	No	IIIc	Rectum	Alive	No	64.1	64.1	High	Low
AHSU-078	F	67	No	No	No	IIIb	Rectum	Dead	Yes	4.7	18.5	High	Low
AHSU-079	M	67	No	Yes	No	III	Colon	Alive	No	64.9	64.9	Low	Low
AHSU-080	M	68	Yes	Yes	No	IV	Colon	Dead	Yes	7.9	7.9	High	High
AHSU-081	F	68	No	No	No	IIb	Rectum	Dead	Yes	10.4	10.4	Low	High
AHSU-082	M	69	No	No	Yes	IV	Rectum	Alive	No	18.3	18.3	Low	High
AHSU-083	F	69	No	No	No	III	Colon	Dead	Yes	18.4	55.0	High	Low
AHSU-084	M	69	No	No	No	II	Rectum	Alive	No	61.8	61.8	Low	High
AHSU-085	F	70	No	Yes	No	IVb	Colon	Dead	Yes	5.3	8.3	High	High
AHSU-086	M	71	No	Yes	No	IV	Colon	Dead	Yes	6.3	9.3	Low	Low
AHSU-087	M	72	No	No	Yes	IIc	Colon	Dead	Yes	7.2	12.7	High	High
AHSU-088	M	72	No	Yes	Yes	IIb	Rectum	Dead	Yes	8.2	17.9	High	High
AHSU-089	M	72	No	Yes	Yes	III	Rectum	Alive	No	42.8	42.8	High	High
AHSU-090	F	72	No	No	No	I	Rectum	Alive	No	54.8	54.8	High	Low

AHSU-091	F	72	No	No	No	IV	Rectum	Alive	No	55.9	55.9	High	High
AHSU-092	M	72	No	No	No	IIb	Rectum	Alive	No	64.5	64.5	Low	Low
AHSU-093	M	74	No	No	No	IV	Colon	Dead	Yes	4.7	4.7	High	High
AHSU-094	F	74	No	Yes	Yes	IV	Colon	Dead	Yes	6.8	10.8	High	Low
AHSU-095	M	74	No	No	Yes	IV	Rectum	Dead	Yes	10.9	10.9	High	High
AHSU-096	M	74	Yes	No	No	IV	Rectum	Dead	Yes	26.8	26.8	High	High
AHSU-097	F	74	No	No	No	II	Rectum	Alive	Yes	21.2	57.8	High	Low
AHSU-098	M	75	No	No	No	IV	Rectum	Dead	Yes	22.7	22.7	Low	Low
AHSU-099	F	75	No	No	No	I	Colon	Alive	No	50.8	50.8	Low	Low
AHSU-100	M	76	Yes	Yes	No	IV	Colon	Dead	Yes	13.6	22.9	High	High
AHSU-101	F	76	No	Yes	No	II	Colon	Dead	Yes	20.9	48.9	High	High
AHSU-102	F	76	No	No	No	III	Colon	Alive	No	56.9	56.9	High	High
AHSU-103	M	76	No	Yes	Yes	IIIb	Colon	Alive	No	63.5	63.5	Low	Low
AHSU-104	F	76	No	No	No	II	Colon	Alive	No	64.9	64.9	Low	Low
AHSU-105	F	77	No	No	No	IVb	Colon	Dead	Yes	26.1	30.1	Low	High
AHSU-106	F	77	No	No	No	III	Colon	Alive	No	61.8	61.8	Low	Low
AHSU-107	M	83	No	No	No	III	Colon	Dead	Yes	5.9	26.0	Low	Low
AHSU-108	M	85	No	No	No	III	Colon	Dead	Yes	6.5	38.0	High	Low

*F, female; M, male.

**Data are follow-up time for individuals who were alive until the last follow-up. The last follow-up date at SYSUCC was 2016/5/10 with a median follow-up time of 65.8 months, while the last follow-up date at AHSU was 2017/4/23 with a median follow-up time of 56.6 months. For combined sample, the median follow-up time was 60.4 months.

Table S2. Characteristics of individuals with CRC received preoperative neoadjuvant chemotherapy recruited at SYSUCC

Sample ID	Sex*	Age (year)	Tumor location	TNM stage	Neoadjuvant chemotherapy**	Response to treatment***	Serum piR-54265
SYSUCC-111	M	38	Colon	IV	FOLFOX6	SD	High
SYSUCC-112	F	53	Colon	IIIb	FOLFOXIRI	PD	High
SYSUCC-113	F	55	Colon	IV	XELOX	PD	High
SYSUCC-114	F	45	Colon	IV	XELOX	SD	High
SYSUCC-115	M	50	Colon	IV	XELOX	PR	High
SYSUCC-116	M	51	Colon	IV	XELOX	PD	High
SYSUCC-117	F	55	Colon	IIb	FOLFOX4	PR	High
SYSUCC-118	F	58	Colon	IV	FOLFOX6	SD	High
SYSUCC-119	F	43	Colon	IV	XELOX	PR	High
SYSUCC-120	F	62	Colon	IV	FOLFOX	PD	High
SYSUCC-121	M	52	Rectum	IIa	XELOX	SD	Low
SYSUCC-122	M	48	Rectum	IIa	XELOX	PR	Low
SYSUCC-123	M	72	Rectum	IIa	XELOX	PR	Low
SYSUCC-124	M	58	Rectum	IIa	XELOX	PR	Low
SYSUCC-125	M	40	Rectum	IIa	XELOX	SD	Low
SYSUCC-126	M	75	Rectum	IIa	XELOX	PR	Low
SYSUCC-127	M	61	Rectum	IIa	XELOX	PR	Low
SYSUCC-128	M	72	Rectum	IIa	XELODA	SD	Low
SYSUCC-129	M	54	Rectum	IIa	XELOX	PR	Low
SYSUCC-130	M	69	Rectum	IIa	XELOX	SD	Low
SYSUCC-131	M	48	Rectum	IIa	XELOX	SD	Low
SYSUCC-132	M	65	Rectum	IIa	XELOX	PR	Low
SYSUCC-133	M	59	Rectum	IIa	XELOX	PR	Low
SYSUCC-134	M	61	Rectum	IIa	XELOX	PR	Low
SYSUCC-135	M	52	Rectum	IIa	XELOX	PD	Low
SYSUCC-136	M	65	Rectum	IIb	XELOX	PR	Low

SYSUCC-137	M	63	Rectum	IIb	XELOX	PR	Low
SYSUCC-138	M	58	Rectum	IIb	XELOX	PR	Low
SYSUCC-139	M	50	Rectum	IIb	XELOX	PR	Low
SYSUCC-140	M	60	Rectum	IIb	XELOX	PD	Low
SYSUCC-141	M	54	Rectum	IIb	XELOX	PR	Low
SYSUCC-142	M	64	Rectum	IIb	XELOX	PR	Low
SYSUCC-143	M	34	Rectum	IIb	XELOX	SD	Low
SYSUCC-144	M	36	Rectum	IIIb	XELOX	CR	Low
SYSUCC-145	M	43	Rectum	IIIb	XELOX	CR	Low
SYSUCC-146	M	55	Rectum	IIIb	XELOX	CR	Low
SYSUCC-147	M	56	Rectum	IIIb	XELOX	CR	Low
SYSUCC-148	M	47	Rectum	IIIb	XELOX	SD	Low
SYSUCC-149	M	62	Rectum	IIIb	XELOX	PR	Low
SYSUCC-150	M	61	Rectum	IIIb	XELOX	PR	Low
SYSUCC-151	M	60	Rectum	IIIb	XELOX	PR	Low
SYSUCC-152	M	41	Rectum	IIIb	XELOX	SD	Low
SYSUCC-153	M	68	Rectum	IIIb	FOLFOX	PR	Low
SYSUCC-154	M	43	Rectum	IIIb	XELOX	PR	Low
SYSUCC-155	M	58	Rectum	IIIb	XELOX	PR	Low
SYSUCC-156	M	74	Rectum	IIIb	XELOX	SD	Low
SYSUCC-157	M	70	Rectum	IIIb	XELOX	PR	Low
SYSUCC-158	M	60	Rectum	IIIb	XELOX	PR	Low
SYSUCC-159	M	70	Rectum	IIIb	XELOX	PR	Low
SYSUCC-160	M	53	Rectum	IIIb	XELOX	PR	Low
SYSUCC-161	M	65	Rectum	IIIb	XELOX	PR	Low
SYSUCC-162	M	57	Rectum	IIIb	XELOX	PR	Low
SYSUCC-163	M	30	Rectum	IIIb	XELOX	SD	Low
SYSUCC-164	M	73	Rectum	IIIb	XELOX	PR	Low
SYSUCC-165	M	41	Rectum	IIIb	XELOX	PR	Low

SYSUCC-166	M	62	Rectum	IIIb	XELOX	PR	Low
SYSUCC-167	M	49	Rectum	IIIb	XELOX	SD	Low
SYSUCC-168	M	62	Rectum	IIIb	XELOX	SD	Low
SYSUCC-169	M	41	Rectum	IIIb	XELOX	SD	Low
SYSUCC-170	M	56	Rectum	IIIb	XELOX	SD	Low
SYSUCC-171	M	66	Rectum	IIIb	XELOX	PR	Low
SYSUCC-172	M	30	Rectum	IIIc	XELOX	PR	Low
SYSUCC-173	M	66	Rectum	IIIc	XELOX	CR	Low
SYSUCC-174	M	41	Rectum	IIIc	XELOX	PR	Low
SYSUCC-175	M	67	Rectum	IIIc	XELOX	PR	Low
SYSUCC-176	M	34	Rectum	IIIc	XELOX	PR	Low
SYSUCC-177	M	52	Rectum	IIIc	XELOX	PR	Low
SYSUCC-178	M	54	Rectum	IIIc	XELOX	SD	Low
SYSUCC-179	M	56	Rectum	IIIc	XELOX	PD	Low
SYSUCC-180	M	65	Rectum	IIIc	XELOX	PR	Low
SYSUCC-181	M	70	Rectum	IIIc	XELOX	PR	Low
SYSUCC-182	M	72	Rectum	IIIc	XELOX	SD	Low
SYSUCC-183	M	55	Rectum	IIIc	XELOX	SD	Low
SYSUCC-184	M	71	Rectum	IIIc	XELOX	PR	Low
SYSUCC-185	M	67	Rectum	IIIc	XELOX	PR	Low
SYSUCC-186	M	38	Rectum	IIIc	XELOX	PR	Low
SYSUCC-187	M	77	Rectum	IIIc	XELOX	PR	Low
SYSUCC-188	M	34	Rectum	IIIc	FOLFOX6	PR	Low
SYSUCC-189	M	69	Rectum	IIIc	XELOX	PR	Low
SYSUCC-190	M	23	Rectum	IIIc	XELOX	PR	Low
SYSUCC-191	M	67	Rectum	IIIc	XELOX	SD	Low
SYSUCC-192	M	43	Rectum	IIIc	XELOX	PR	Low
SYSUCC-193	M	67	Rectum	IV	XELOX	PR	Low
SYSUCC-194	M	60	Rectum	IV	XELOX	PR	Low

SYSUCC-195	M	53	Rectum	IV	XELOX	PR	Low
SYSUCC-196	M	60	Rectum	IV	XELOX	PR	Low
SYSUCC-197	M	60	Rectum	IV	FOLFIRI	SD	Low
SYSUCC-198	M	48	Rectum	IV	FOLFOX	SD	Low
SYSUCC-199	M	75	Rectum	IV	XELOX	PR	Low
SYSUCC-200	M	56	Rectum	IIa	XELOX	PR	High
SYSUCC-201	M	61	Rectum	IIa	XELOX	CR	High
SYSUCC-202	M	66	Rectum	IIa	XELOX	PR	High
SYSUCC-203	M	67	Rectum	IIa	XELOX	PR	High
SYSUCC-204	M	65	Rectum	IIa	XELOX	PR	High
SYSUCC-205	M	48	Rectum	IIa	XELOX	PR	High
SYSUCC-206	M	65	Rectum	IIa	XELOX	SD	High
SYSUCC-207	M	47	Rectum	IIa	XELOX	PR	High
SYSUCC-208	M	48	Rectum	IIa	XELOX	PR	High
SYSUCC-209	M	66	Rectum	IIa	XELOX	SD	High
SYSUCC-210	M	52	Rectum	IIa	XELOX	PR	High
SYSUCC-211	M	60	Rectum	IIa	XELOX	PR	High
SYSUCC-212	M	59	Rectum	IIa	XELOX	PD	High
SYSUCC-213	M	77	Rectum	IIa	XELOX	SD	High
SYSUCC-214	M	62	Rectum	IIa	XELOX	SD	High
SYSUCC-215	M	50	Rectum	IIa	XELOX	PR	High
SYSUCC-216	M	60	Rectum	IIa	XELOX	PD	High
SYSUCC-217	M	70	Rectum	IIb	XELOX	PR	High
SYSUCC-218	M	32	Rectum	IIb	XELOX	PR	High
SYSUCC-219	M	62	Rectum	IIb	XELOX	SD	High
SYSUCC-220	M	68	Rectum	IIb	XELODA	SD	High
SYSUCC-221	M	61	Rectum	IIIb	XELOX	PR	High
SYSUCC-222	M	62	Rectum	IIIb	XELOX	SD	High
SYSUCC-223	M	67	Rectum	IIIb	XELOX	SD	High

SYSUCC-224	M	71	Rectum	IIIb	XELOX	PR	High
SYSUCC-225	M	74	Rectum	IIIb	XELOX	SD	High
SYSUCC-226	M	45	Rectum	IIIb	XELOX	PR	High
SYSUCC-227	M	56	Rectum	IIIb	XELOX	PR	High
SYSUCC-228	M	66	Rectum	IIIb	XELOX	PR	High
SYSUCC-229	M	76	Rectum	IIIb	XELOX	PR	High
SYSUCC-230	M	62	Rectum	IIIb	XELOX	PR	High
SYSUCC-231	M	82	Rectum	IIIb	XELOX	SD	High
SYSUCC-232	M	35	Rectum	IIIb	XELOX	PR	High
SYSUCC-233	M	69	Rectum	IIIb	XELOX	PR	High
SYSUCC-234	M	32	Rectum	IIIb	XELOX	SD	High
SYSUCC-235	M	56	Rectum	IIIb	XELOX	SD	High
SYSUCC-236	M	60	Rectum	IIIb	XELOX	SD	High
SYSUCC-237	M	56	Rectum	IIIb	XELOX	SD	High
SYSUCC-238	M	73	Rectum	IIIb	XELOX	SD	High
SYSUCC-239	M	66	Rectum	IIIb	XELOX	PD	High
SYSUCC-240	M	52	Rectum	IIIc	XELOX	SD	High
SYSUCC-241	M	40	Rectum	IIIc	XELOX	SD	High
SYSUCC-242	M	60	Rectum	IIIc	XELOX	PR	High
SYSUCC-243	M	76	Rectum	IIIc	XELOX	SD	High
SYSUCC-244	M	50	Rectum	IIIc	XELOX	PR	High
SYSUCC-245	M	67	Rectum	IIIc	XELOX	PR	High
SYSUCC-246	M	60	Rectum	IIIc	XELOX	SD	High
SYSUCC-247	M	52	Rectum	IIIc	XELOX	SD	High
SYSUCC-248	M	27	Rectum	IIIc	XELOX	PR	High
SYSUCC-249	M	60	Rectum	IV	XELOX	SD	High
SYSUCC-250	M	58	Rectum	IV	FOLFOX6	PR	High
SYSUCC-251	M	54	Rectum	IV	XELOX	SD	High
SYSUCC-252	M	49	Rectum	IV	FOLFOX6	SD	High

SYSUCC-253	M	68	Rectum	IV	XELOX	PD	High
SYSUCC-254	M	60	Rectum	IV	FOLFOX	PR	High
SYSUCC-255	M	58	Rectum	IV	XELOX	PR	High
SYSUCC-256	M	54	Rectum	IV	XELOX	SD	High
SYSUCC-257	M	67	Rectum	IV	FOLFOX	SD	High
SYSUCC-258	M	68	Rectum	IV	XELOX	SD	High
SYSUCC-259	M	41	Rectum	IV	XELOX	PD	High
SYSUCC-260	M	49	Rectum	IV	XELOX	SD	High
SYSUCC-261	M	54	Rectum	IV	XELOX	PD	High
SYSUCC-262	M	67	Rectum	IV	FOLFOX	SD	High
SYSUCC-263	F	58	Rectum	IIa	XELOX	SD	Low
SYSUCC-264	F	58	Rectum	IIa	XELOX	PR	Low
SYSUCC-265	F	68	Rectum	IIa	XELOX	PR	Low
SYSUCC-266	F	62	Rectum	IIb	XELOX	PR	Low
SYSUCC-267	F	59	Rectum	IIb	XELOX	SD	Low
SYSUCC-268	F	77	Rectum	IIb	XELOX	PR	Low
SYSUCC-269	F	70	Rectum	IIb	XELOX	PR	Low
SYSUCC-270	F	43	Rectum	IIb	XELOX	PR	Low
SYSUCC-271	F	41	Rectum	IIIb	XELOX	PD	Low
SYSUCC-272	F	50	Rectum	IIIb	XELOX	PR	Low
SYSUCC-273	F	32	Rectum	IIIb	XELOX	PR	Low
SYSUCC-274	F	34	Rectum	IIIb	XELOX	SD	Low
SYSUCC-275	F	54	Rectum	IIIb	XELOX	PR	Low
SYSUCC-276	F	63	Rectum	IIIb	XELOX	PR	Low
SYSUCC-277	F	60	Rectum	IIIb	XELOX	SD	Low
SYSUCC-278	F	42	Rectum	IIIb	XELOX	PR	Low
SYSUCC-279	F	62	Rectum	IIIb	XELOX	PR	Low
SYSUCC-280	F	25	Rectum	IIIc	XELOX	PR	Low
SYSUCC-281	F	65	Rectum	IIIc	XELOX	PR	Low

SYSUCC-282	F	51	Rectum	IIIc	XELOX	PR	Low
SYSUCC-283	F	45	Rectum	IIIc	XELOX	PR	Low
SYSUCC-284	F	45	Rectum	IIIc	XELOX	PR	Low
SYSUCC-285	F	51	Rectum	IIIc	FOLFOX	PR	Low
SYSUCC-286	F	55	Rectum	IIIc	XELOX	SD	Low
SYSUCC-287	F	41	Rectum	IIIc	XELOX	CR	Low
SYSUCC-288	F	70	Rectum	IIIc	XELOX	PR	Low
SYSUCC-289	F	64	Rectum	IV	FOLFIRI	PR	Low
SYSUCC-290	F	36	Rectum	IV	XELOX	PR	Low
SYSUCC-291	F	59	Rectum	IIa	XELOX	PR	High
SYSUCC-292	F	35	Rectum	IIa	XELOX	PR	High
SYSUCC-293	F	68	Rectum	IIa	XELOX	PR	High
SYSUCC-294	F	42	Rectum	IIa	XELOX	SD	High
SYSUCC-295	F	69	Rectum	IIa	XELOX	PR	High
SYSUCC-296	F	35	Rectum	IIa	XELOX	PR	High
SYSUCC-297	F	74	Rectum	IIa	XELOX	PR	High
SYSUCC-298	F	65	Rectum	IIa	XELOX	SD	High
SYSUCC-299	F	65	Rectum	IIb	XELOX	SD	High
SYSUCC-300	F	46	Rectum	IIb	XELOX	PR	High
SYSUCC-301	F	41	Rectum	IIIb	XELOX	PR	High
SYSUCC-302	F	32	Rectum	IIIb	XELOX	SD	High
SYSUCC-303	F	42	Rectum	IIIb	XELOX	PR	High
SYSUCC-304	F	48	Rectum	IIIb	XELOX	SD	High
SYSUCC-305	F	64	Rectum	IIIb	XELOX	PR	High
SYSUCC-306	F	45	Rectum	IIIb	XELOX	PR	High
SYSUCC-307	F	30	Rectum	IIIb	XELOX	PR	High
SYSUCC-308	F	69	Rectum	IIIb	XELOX	SD	High
SYSUCC-309	F	49	Rectum	IIIb	XELOX	SD	High
SYSUCC-310	F	67	Rectum	IIIb	XELOX	PR	High

SYSUCC-312	F	41	Rectum	IIIb	XELOX	PR	High
SYSUCC-313	F	63	Rectum	IIIb	XELOX	PR	High
SYSUCC-314	F	71	Rectum	IIIb	XELOX	PR	High
SYSUCC-315	F	72	Rectum	IIIb	XELOX	PD	High
SYSUCC-316	F	55	Rectum	IIIc	XELOX	CR	High
SYSUCC-317	F	42	Rectum	IIIc	XELOX	PR	High
SYSUCC-318	F	67	Rectum	IIIc	XELOX	PR	High
SYSUCC-319	F	66	Rectum	IIIc	XELOX	PR	High
SYSUCC-320	F	58	Rectum	IIIc	XELOX	PR	High
SYSUCC-321	F	66	Rectum	IIIc	XELOX	PR	High
SYSUCC-322	F	37	Rectum	IIIc	XELOX	PR	High
SYSUCC-323	F	53	Rectum	IIIc	XELOX	SD	High
SYSUCC-324	F	19	Rectum	IV	FOLFOX	PR	High
SYSUCC-325	F	67	Rectum	IV	XELOX	PR	High
SYSUCC-326	F	60	Rectum	IV	XELOX	PR	High

SYSUCC, Sun Yat-sen University Cancer Center (Guangzhou, China).

*F, female; M, male.

**FOLFOX4/6, oxaliplatin + leucovorin + 5-FU; XELOX, xeloda (capecitabine) + oxaliplatin; FOLFOXIRI, irinotecan + oxaliplatin + leucovorin + 5-FU.

***CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Table S3. Characteristics of individuals with rectal cancer received preoperative neoadjuvant chemotherapy recruited at CHCAMS

Sample ID	Sex*	Age (year)	Tumor location	TNM stage	Neoadjuvant Chemotherapy**	Response to treatment (TRG)***	Serum piR-54265
CHCAMS-001	M	33	Rectum	II	XELOX	1	Low
CHCAMS-002	M	70	Rectum	III	XELOX	1	Low
CHCAMS-003	M	33	Rectum	III	XELOX	1	Low
CHCAMS-004	M	39	Rectum	III	XELOX	1	Low
CHCAMS-005	M	59	Rectum	III	XELOX	1	Low
CHCAMS-006	F	51	Rectum	III	XELOX	1	Low
CHCAMS-007	M	43	Rectum	I	XELOX	2	Low
CHCAMS-008	M	70	Rectum	II	XELOX	2	Low
CHCAMS-009	M	56	Rectum	II	XELOX	2	Low
CHCAMS-010	M	73	Rectum	II	XELOX	2	Low
CHCAMS-011	M	59	Rectum	II	XELOX	2	Low
CHCAMS-012	M	58	Rectum	II	XELOX	2	Low
CHCAMS-013	M	37	Rectum	III	XELOX	2	Low
CHCAMS-014	M	62	Rectum	III	XELOX	2	Low
CHCAMS-015	M	45	Rectum	III	XELOX	2	Low
CHCAMS-016	M	51	Rectum	III	XELOX	2	Low
CHCAMS-017	M	42	Rectum	III	XELOX	2	Low
CHCAMS-018	M	60	Rectum	III	XELOX	2	Low
CHCAMS-019	M	44	Rectum	III	XELOX	2	Low
CHCAMS-020	M	38	Rectum	III	XELOX	2	Low
CHCAMS-021	F	37	Rectum	III	XELOX	2	Low
CHCAMS-022	F	66	Rectum	III	XELOX	2	Low
CHCAMS-023	F	54	Rectum	III	XELOX	2	Low
CHCAMS-024	F	26	Rectum	III	XELOX	2	Low
CHCAMS-025	F	51	Rectum	III	XELOX	2	Low
CHCAMS-026	M	41	Rectum	II	XELOX	3	Low

CHCAMS-027	M	60	Rectum	II	XELOX	3	Low
CHCAMS-028	M	74	Rectum	II	XELOX	3	Low
CHCAMS-029	M	38	Rectum	II	XELOX	3	Low
CHCAMS-030	F	50	Rectum	II	XELOX	3	Low
CHCAMS-031	M	57	Rectum	III	XELOX	3	Low
CHCAMS-032	M	82	Rectum	III	XELOX	3	Low
CHCAMS-033	M	54	Rectum	III	XELOX	3	Low
CHCAMS-034	M	50	Rectum	III	XELOX	3	Low
CHCAMS-035	M	54	Rectum	III	XELOX	3	Low
CHCAMS-036	M	38	Rectum	III	XELOX	3	Low
CHCAMS-037	M	67	Rectum	III	XELOX	3	Low
CHCAMS-038	M	69	Rectum	III	XELOX	3	Low
CHCAMS-039	M	58	Rectum	III	XELOX	3	Low
CHCAMS-040	M	65	Rectum	III	XELOX	3	Low
CHCAMS-041	M	70	Rectum	III	XELOX	3	Low
CHCAMS-042	M	36	Rectum	III	XELOX	3	Low
CHCAMS-043	M	57	Rectum	III	XELOX	3	Low
CHCAMS-044	F	49	Rectum	III	XELOX	3	Low
CHCAMS-045	F	41	Rectum	III	XELOX	3	Low
CHCAMS-046	F	43	Rectum	III	XELOX	3	Low
CHCAMS-047	F	57	Rectum	III	XELOX	3	Low
CHCAMS-048	F	34	Rectum	III	XELOX	3	Low
CHCAMS-049	M	48	Rectum	III	XELOX	4	Low
CHCAMS-050	M	29	Rectum	III	XELOX	4	Low
CHCAMS-051	F	41	Rectum	III	XELOX	4	Low
CHCAMS-052	M	67	Rectum	II	XELOX	1	High
CHCAMS-053	M	71	Rectum	II	XELOX	1	High
CHCAMS-054	M	66	Rectum	III	XELOX	1	High
CHCAMS-055	M	47	Rectum	III	XELOX	1	High

CHCAMS-056	M	51	Rectum	II	XELOX	2	High
CHCAMS-057	F	54	Rectum	II	XELOX	2	High
CHCAMS-058	M	64	Rectum	III	XELOX	2	High
CHCAMS-059	M	75	Rectum	III	XELOX	2	High
CHCAMS-060	M	47	Rectum	III	XELOX	2	High
CHCAMS-061	M	47	Rectum	III	XELOX	2	High
CHCAMS-062	F	67	Rectum	III	XELOX	2	High
CHCAMS-063	F	39	Rectum	III	XELOX	2	High
CHCAMS-064	F	48	Rectum	III	XELOX	2	High
CHCAMS-065	F	72	Rectum	III	XELOX	2	High
CHCAMS-066	F	67	Rectum	III	XELOX	2	High
CHCAMS-067	M	44	Rectum	II	XELOX	3	High
CHCAMS-068	M	72	Rectum	II	XELOX	3	High
CHCAMS-069	M	29	Rectum	II	XELOX	3	High
CHCAMS-070	F	44	Rectum	II	XELOX	3	High
CHCAMS-071	F	51	Rectum	II	XELOX	3	High
CHCAMS-072	F	54	Rectum	II	XELOX	3	High
CHCAMS-073	F	62	Rectum	II	XELOX	3	High
CHCAMS-074	M	50	Rectum	III	XELOX	3	High
CHCAMS-075	M	55	Rectum	III	XELOX	3	High
CHCAMS-076	M	62	Rectum	III	XELOX	3	High
CHCAMS-077	M	34	Rectum	III	XELOX	3	High
CHCAMS-078	M	71	Rectum	III	XELOX	3	High
CHCAMS-079	M	34	Rectum	III	XELOX	3	High
CHCAMS-080	M	55	Rectum	III	XELOX	3	High
CHCAMS-081	M	58	Rectum	III	XELOX	3	High
CHCAMS-082	M	68	Rectum	III	XELOX	3	High
CHCAMS-083	M	59	Rectum	III	XELOX	3	High
CHCAMS-084	M	42	Rectum	III	XELOX	3	High

CHCAMS-085	M	53	Rectum	III	XELOX	3	High
CHCAMS-086	M	36	Rectum	III	XELOX	3	High
CHCAMS-087	F	72	Rectum	III	XELOX	3	High
CHCAMS-088	F	47	Rectum	III	XELOX	3	High
CHCAMS-089	F	56	Rectum	III	XELOX	3	High
CHCAMS-090	F	56	Rectum	III	XELOX	3	High
CHCAMS-091	M	73	Rectum	II	XELOX	4	High
CHCAMS-092	M	74	Rectum	II	XELOX	4	High
CHCAMS-093	M	58	Rectum	III	XELOX	4	High
CHCAMS-094	M	30	Rectum	III	XELOX	4	High
CHCAMS-095	M	60	Rectum	III	XELOX	4	High
CHCAMS-096	M	45	Rectum	III	XELOX	4	High
CHCAMS-097	M	70	Rectum	III	XELOX	4	High
CHCAMS-098	F	58	Rectum	III	XELOX	4	High
CHCAMS-099	F	56	Rectum	III	XELOX	4	High
CHCAMS-100	F	50	Rectum	III	XELOX	4	High
CHCAMS-101	M	56	Rectum	II	XELOX	5	High
CHCAMS-102	F	62	Rectum	III	XELOX	5	High

CHCAMS, Cancer Hospital, Chinese Academy of Medical Sciences (Beijing, China).
*F, female; M, male.
**XELOX, xeloda (capecitabine) plus oxaliplatin regimen.
***TRG, tumor regression grade.

Table S4. Sequences of synthesized probes, siRNAs and antagopiR54265 used in this study

Sequence used for pulldown assay	
piRNA-54265	5’Biotin-GGTCAGGCTCAGACAGTTCATCACCTCCA-3’
Probe used for Northern blot assay	
piRNA-54265	5’DIG-11-dUTP-GGTCAGGCTCAGACAGTTCATCACCTCCA-3’
Sequences of small interfering RNAs	
Scramble	5’-UUCUCCGAACGUGUCACGUTT-3’
PIWIL2-siRNA#1	5’-AGGUGGUAUCAGCAGAGAATT-3’
PIWIL2-siRNA#2	5’-CCCACGUGAUGAUCUCUAUTT-3’
STAT3-siRNA#1	5’-CCACUUUGGUGUUUCAUAATT-3’
STAT3-siRNA#2	5’-GCAACAGAUUGCCUGCAUUTT-3’
Synthesized Caenorhabditis elegant miRNA	
cel-miR-39-oligo	5’-UCACCGGGUGUAAAUCAGCUUG-3’
cel-miR-54-oligo	5’-UACCCGUAAUCUUCAUAAUCCGAG-3’
AntagopiR54265 used for in vivo assay	
AntagomiRNC22	5'chol-CAGUACUUUUGUGUAGUACAAA (2'OMe)-3'
AntagopiR54265	5'chol-GGUCAGGCUCAGACAGUUCAUCACCUCCA(2'OMe)-3'

Table S5. Primers used for quantitative real time-PCR in this study

Gene Symbol	Forward primer (5'→3')	Reverse primer (5'→3')
CPXM1	AATCAAGTGGATGAAGCGGA	AATGGGTAGGACACCACGA
NOP56	GCCAGAGCCTACACACTTAC	TGGAGTGTTACCCCTTGTCT
EBF4	CAGAGTGGCGTGGGTCT	TTGTTAGTCTTTTCCGCCCC
TMC2	TAAAGGGCCTGAAAGAGGAAGCA	CCTGTCACCTGTGTGTGGAGA
IDH3B	GGCAGTGGGCAGGAATATAG	CCATGAAAAGGGCAGTGGAT
TMEM239	CCTGGATAGAATTTTGGCCCT	GGCTTGCTGGGTCAGTTTTG
ZNF343	TGCTCCCGAGAACTTCCTAT	AATGTGAGTGAAAGCGACGA
VPS16	ACAGCATCACTCAAGGAGAAG	GGAGTCCTCATCCAGCACAAA
PCED1A	ACTGTGGGCAGCCTCACTTG	ACTTCCGTTCACCCATGTCC
PIWIL2	GGTCGAGGCTTGTCTGCTAA	CGGAGGTCTCTGCCATCTTG
STAT3	ATATAATCCCTGAAACGGGC	GAAACAACCTAGCCTCTGAA
GAPDH	CAAGGTCATCCATGACAACTTTG	GTCCACCACCCTGTTGCTGTAG
β-ACTIN	CAGGGCGTGATGGTGGGCATG	GTAGAAGGTGTGGTGCCAGATT

Primers used for stem-loop RT-PCR of piRNAs

Gene Symbol	Stem-loop reverse transcription primers (5'→3')	Forward primer (5'→3')	Reverse primer (5'→3')
piRNA-62011*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACACCTCACC	TTTGGCAATGGTAGAACTCACAC TGG	CAGTGCGTGTCGTGG AGT
piRNA-32678*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG	CATTGATCATCGACACTTCGAA	CAGTGCGTGTCGTGG

	CACTGGATACGACCAAGTGCG		AGT
piRNA-49145*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACTGTAACTC	tttTGAGGTAGTAGGTTGTATGGTT TAG	CAGTGCGTGTCGTGG AGT
piRNA-33856*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACCAAAGGTG	ttCTGCAGTGATGACTTTCTTAGG A	CAGTGCGTGTCGTGG AGT
piRNA-36984*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACGAGGACCA	TAAAGTGCTGACAGTGCAGATAG TG	CAGTGCGTGTCGTGG AGT
piRNA-33879*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACCAAAGGTG	CTGCGATGATGGCATTTCTTAGG	CAGTGCGTGTCGTGG AGT
piRNA-61298*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACCAAAGGTG	TTGCTGTGATGACTATCTTAGGA CA	CAGTGCGTGTCGTGG AGT
piRNA-33864*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACATTGTTCA	CTGCATCCACTGATAGACCTTG	CAGTGCGTGTCGTGG AGT
piRNA-54381*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACTCTCAGGT	ggggTGGATATGATGACTGATTAC	CAGTGCGTGTCGTGG AGT
piRNA-57519*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACTCCTTGTA	TGGTGTATTAGTTTATACTA	CAGTGCGTGTCGTGG AGT
piRNA-54265*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACGGTCAGGC	TGGAGGTGATGAACTGTCTGA	CAGTGCGTGTCGTGG AGT
piRNA-61919*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACTTTCAGAT	ttttTTTCTGTGTGGAATTTGAATAT	CAGTGCGTGTCGTGG AGT
piRNA-36712*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACTGGCTCAG	GTTCACTGATGAGAGCATTGTTC T	CAGTGCGTGTCGTGG AGT
piRNA-36150*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACATCAACAG	GGGAAGTGATGACACCTGTGAC T	CAGTGCGTGTCGTGG AGT
piRNA-31500*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACAGGGTCAT	AGGTGAGCGCTTTGCGCAGTGAT	CAGTGCGTGTCGTGG AGT
piRNA-47305*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTG CACTGGATACGACTAAGGGGT	TGAATCTGACAACAGAGGCTTAC GAC	CAGTGCGTGTCGTGG AGT

piRNA-30840*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGCTCAGTC	aaAGAACGTGTGGAAAACTAATGA	CAGTGCGTGTCGTGGAGT
piRNA-31612*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAATCAGAA	AGTTCGTGATGGATTTGCTTTTTT	CAGTGCGTGTCGTGGAGT
piRNA-36741*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTGTTTATG	GTTTAGACGGGCTCACATCACC	CAGTGCGTGTCGTGGAGT
piRNA-30799*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAATCAGAA	ACTGTGTGCTGATTGTCACG	CAGTGCGTGTCGTGGAGT
cel-miR-39	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACCAAGCTGA	TCACCGGGTGTAAATCAGCTTGGTCGTA	CAGTGCGTGTCGTGGAGT
cel-miR-54	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACCTCGGA	TACCCGTAATCTTCATAATCCGAGGTCG	CAGTGCGTGTCGTGGAGT
U6	AACGCTTCACGAATTTGCGT	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT

*Homo sapiens origin.