Supplementary information

Distribution and characterization of extrachromosomal circular DNA in colorectal cancer

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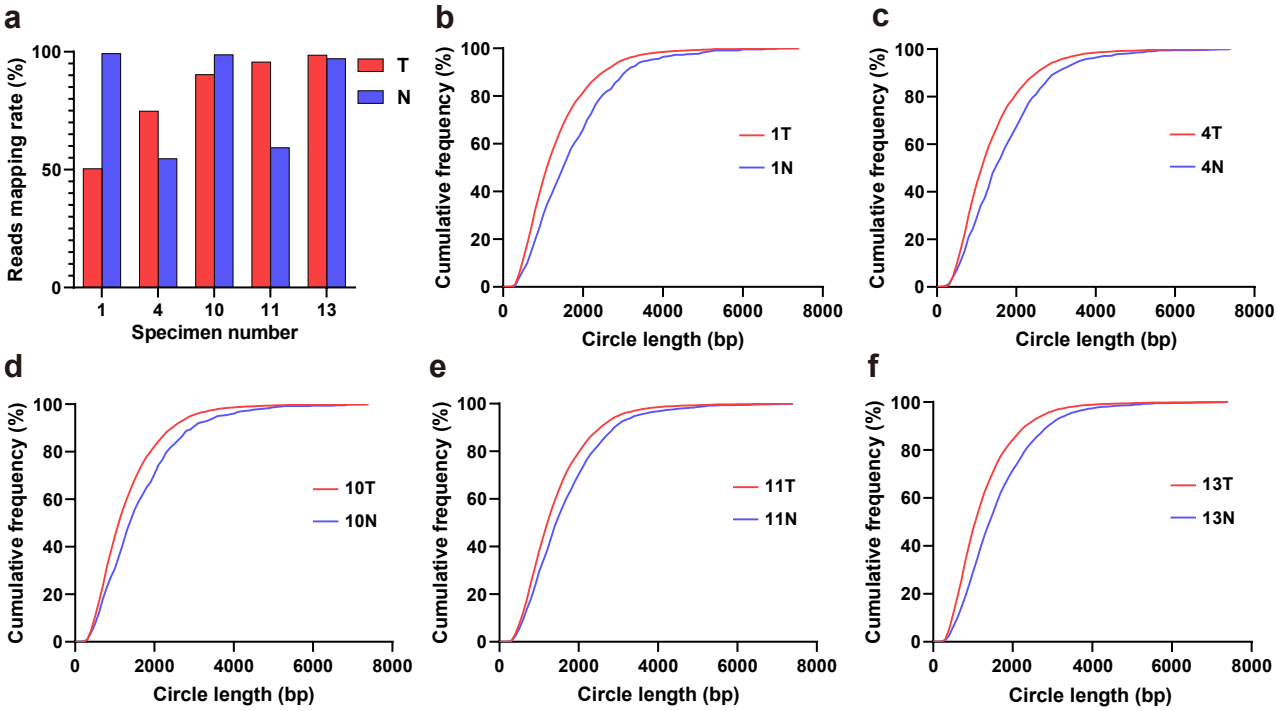
Supplementary Fig. 1-3

Supplementary Fig. 1 Length distribution of eccDNA in colorectal cancer and normal tissues. a Histogram of the reads mapping rate of eccDNA in each tumor group and normal group. **b-f** Cumulative frequency plots of eccDNA in tumor group and normal group.

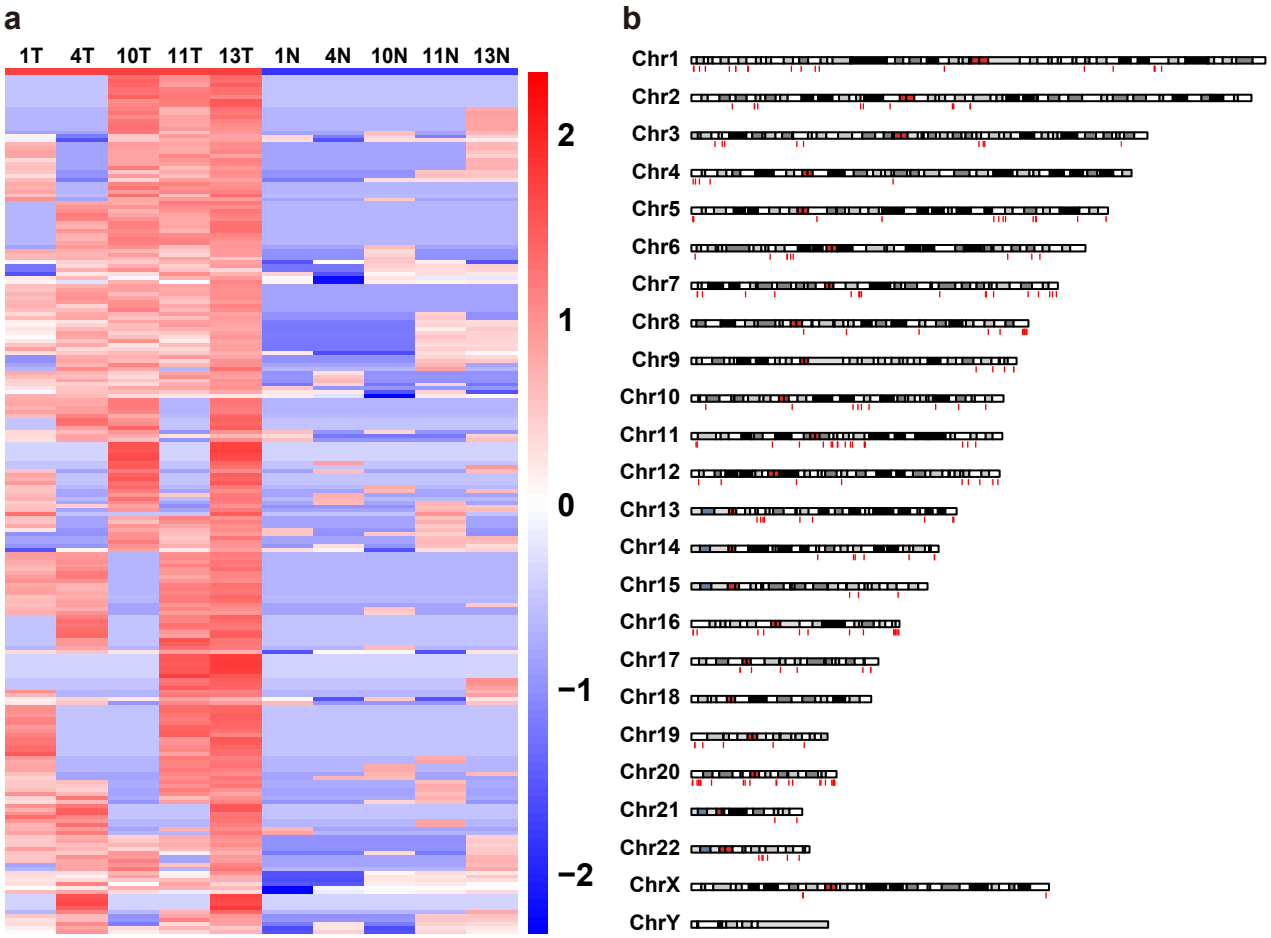
Supplementary Fig. 2 Chromosomal distribution and origin of genes highly-expressed both in Circle-seq and RNA-seq. a Heatmap of 219 genes highly-expressed both in Circle-seq and RNA-seq in each group. **b** Genomic distribution of 219 genes highly-expressed both in Circle-seq and RNA-seq**.**

Supplementary Fig. 3 Verification of eccDNA in colorectal cancer cell lines. a Gel electrophoresis result of the total DNA. HG: genome DNA of HCT116. HLE: total DNA with linear DNA elimination of HCT116. LG: genome DNA of LoVo. LLE: total DNA with linear DNA elimination of LoVo. **b** Schematic representation of the sequencing results of the PCR products of gene TP53I3 in samples after RCA with matching the sequence in chr2. **c** DNA electrophoresis of inverse PCR for junction sites of synthetic eccDNAs to prove transfection effectivity in HCT116. G: genome DNA. LE: total DNA with linear DNA elimination. RCA: total DNA with linear DNA elimination after RCA.

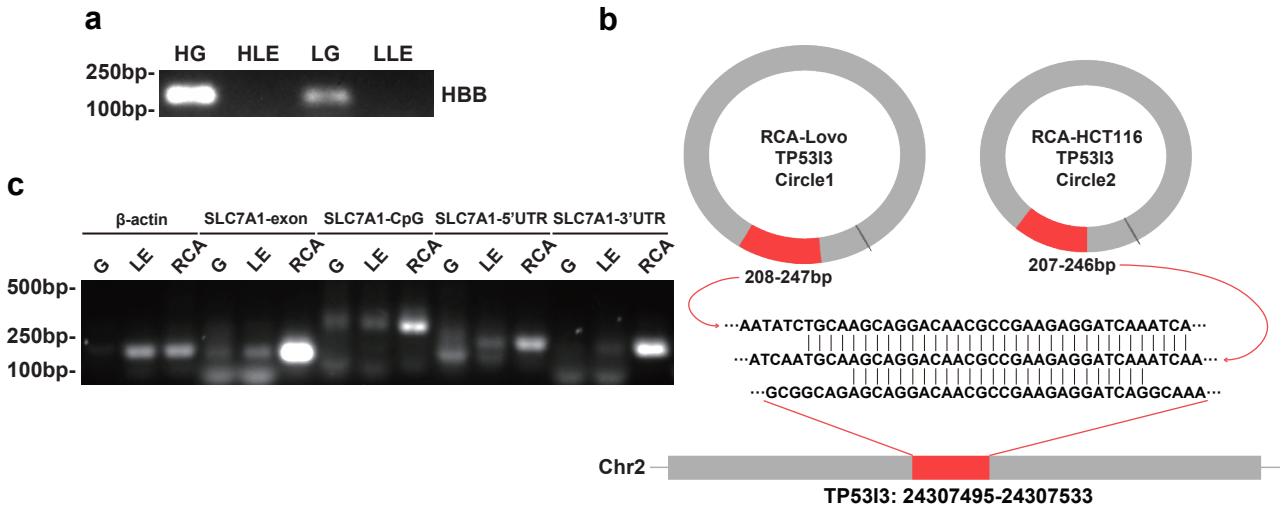
Supplementary Fig. 1



Supplementary Fig. 2



Supplementary Fig. 3



Supplementary table 1-5

Supplementary Table 1

Sequences of inverse primers[1] and PCR reaction[2]

Gene	Primer	Sequences	PCR conditions
ERBB2	FRD:	5’-GCCGTGGCTCACACCTGTAATC-3’	1. 98℃ for 2 min 2. 35 cycles of: 98℃ for 10 sec 60℃ for 15 sec 72℃ for 10 sec 3. 72℃ for 5 min 4. 4℃ for save
	REV:	5’-AGCAAGTTCCCAGACCTCCAGG-3’	
MYCBPAP	FRD:	5’-TTCGGCAGAGTGGCGGCAAT-3’	
	REV:	5’-AGAGCAAACAACCCAGCAGAGC-3’	
TP53I3	FRD:	5’-AACCTCTTGGCGGGCGGATT-3’	
	REV:	5’-TGATCCTCTTCGGCGTTGTCCT-3’	
EGFR	FRD:	5’-ACCTCCATCAGTGGCGATCTCC-3’	
	REV:	5’-GCCGCAATGTGGACAATACAGG-3’	
SLC7A1	FRD:	5’-GGCAGGCTTGCTTTAGCATTGA-3’	
	REV:	5’-TGTGAGAGGCATGTTCGTGTCT-3’	
SLC29A1	FRD:	5’-CCTGCCCTGGGTGGTATGAACT-3’	
	REV:	5’-GCTAAAGGCTTGGTTCGGAGGC-3’	

[1] Oligonucleotide primers were designed by Primer Premier 6.0

[2] PCR reaction was performed on ProFlex™ PCR System (Thermo Fisher)

Supplementary Table 2

Sequences of the synthetic eccDNAs

eccβ-actin: 358bp
GCTCATTGCCAATGGTGATGACCTGGCCGTCAGGCAGCTCGTAGCTCTTCTCCAGGG AGGAGCTGGAAGCAGCCGTGGCCATCTCTTGCTCGAAGTCCAGGGCGACGTAGCAC AGCTTCTCCTTAATGTCACGCACGATTTCCCGCTCGGCCGTGGTGGTGAAGCTGTAG CCGCGCTCGGTGAGGATCTTCATGAGGTAGTCAGTCAGGTCCCGGCCAGCCAGGTC CAGACGCAGGATGGCATGGGGGAGGGCATACCCCTCGTAGATGGGCACAGTGTGGG TGACCCCGTCACCGGAGTCCATCACGATGCCAGTGGTACGGCCAGAGGCGTACAGG GATAGCACAGCCTGGATAGC
eccSLC7A1-exon: 335bp
CCCCACCACCCTGCAATTTCCCCGTCCTCGGAACTGTCTCACGAGACCACCAGCGC ACAGACACGAACATGCCTCTCACACACCCCAGAACGCAGCTGTGCTGGAGTTGGGC ACAAAGGCCCCTCACTCCCTGTCTTCATGGTGTGGGTTCTTGGCTGCCAACAGAACT GTCAGCCTTTATTAAGAAGGAAGGGTCTGTGTAAAAATGGGATGTGTGAATCAGAA GAGGGGCCCTGGCACAGAGCCAGGCAGGCTTGCTTTAGCATTGAGTCCTGCGTCGC GCAATGCCCAGGACCTTGCCTGTTAAGTCTGGGTGGGGCCCCTATGGGGCATGT
eccSLC7A1-CpG: 382bp
CCCGGAGAACCGGCTGCAGAGCCGAGGGTGGGCTGGGGCTGCAGGTCAAGTAATT GCACCTTTGGCTGCAGTGAGGGTGTGGACGCAGGTGGTTTCTGTTGCACTGGTGGG GAGGGTGGGGTGCCTCCCGGTCCTCTGGGGGCGTCCCTCGGGGCTGCTGCCACCTC CGGGGGGCGGGGCTGTGCGTCACTTGCACTGGTCCAAGTTGCCGTCAGGAGTCCTT GCTTGGTCGGCATCCAGGGACGCCTCCTCGCTGTGCCACAGGCCATAGCCAAAGTA GATGATGAAGCCTGCGGGCCGACAGCAGAGACGGGCGTGAACAGACCGCCGGTTG CACCACCGCAGGCAGGGCTCAGAGCCCAGGGCGGTCCCACAGCAAACC
eccSLC7A1-5'UTR: 387bp
GCACTGCTGATGAAACCTGGCGCCGGAACCCGCCAGCCCTCGGCGCCCATTCAGTC CGCGCAGGCAGGTGTGAGCAGCGGGTCAACTACCTGGCAGGCGCGCACGCGGCCG CGGGCTCCCGCTAACCGCAGCCTCCACTCCTCTCCCCGCGCGCCGCGCCCCCGCCCC GCCCCGCCCCGCCCGGTCTCGCCGGCCGAGCGTCCGTTGGTCCTTGAGCGCGTCCG ACAGTCTGTCTGTTCGCGATCCTGCCGGAGCCCCGCCGCCGCCGGCTTGGATTCTGA AACCTTCCTTGTATCCCTCCTGAGACATCTTTGCTGCAAGATCGAGGCTGTCCTCTGG TGAGAAGGTGGTGAGGCTTCCCGTCATATTCCAGCTCTGAACAGCAAC
eccSLC7A1-3'UTR: 385bp
TTCTTCTACTGAACATGGAGCCATTATTAAGAGTTGTGTGTTTTTTATTATGTACATTT GTATATTTTTTTGCTTGTTTGATGTTCTATTTTTCTAATAGTTTTCTTTTAGTTTCTTAAA GTTGTGATACTAGATTTAGATTCTGATGCTAACTGCAAATCAGGTTGGTCTCTGCTGG GTCTCTCCTGCTTTTATTTTACTTTAAGGACAAGTGTAGTTGTCGTCCACCACCTTTC AAAAAATGTGAAACTGCCCTGCCTCCCCTTTTTGCTGACAACACTGTGTACATTGAC CACTTCCTACCATACTTTATGTTGTAAAATCAAACTCTTTTGTGGTACATTATCTCATG CTTCTGCAAATTCGAATAAATTCTATGGCTTCC

Supplementary Table 3

Sequences of primers[1] and PCR reaction[2]

Name	Primer	Sequences	PCR conditions
eccβ-actin	FRD:	5’ CCAGAGGCGTACAGGGATAGCA 3’	1. 98℃ for 2 min 2. 50 cycles of: 98℃ for 10 sec 60℃ for 15 sec 72℃ for 10 sec 3. 72℃ for 5 min 4. 4℃ for save
	REV:	5’ GCGGGAAATCGTGCGTGACAT 3’	
eccSLC7A1-exon	FRD:	5’ CCCCACCACCCTGCAATTTCCC 3’	
	REV:	5’ TGTGAGAGGCATGTTCGTGTCT 3’	
eccSLC7A1-CpG	FRD:	5’ CACTGGTCCAAGTTGCCGTCAG 3’	
	REV:	5’ CAGAAACCACCTGCGTCCACAC 3’	
eccSLC7A1-5’UTR	FRD:	5’ TTGCTGCAAGATCGAGGCTGTC 3’	
	REV:	5’ AGAGGAGTGGAGGCTGCGGTTA 3’	
eccSLC7A1-3’UTR	FRD:	5’ CAAGTGTAGTTGTCGTCCACCA 3’	
	REV:	5’ CACAACTCTTAATAATGGCTCC 3’	

[1] Oligonucleotide primers were designed by Primer Premier 6.0

[2] PCR reaction was performed on ProFlex™ PCR System (Thermo Fisher)

Supplementary Table 4

Sequences of inverse primers[1] and PCR reaction[2]

Name	Primer	Sequences	PCR conditions
eccβ-actin	FRD:	5’ GCTCATTGCCAATGGTGATGAC 3’	1. 98℃ for 2 min 2. 50 cycles of: 98℃ for 10 sec 60℃ for 15 sec 72℃ for 10 sec 3. 72℃ for 5 min 4. 4℃ for save
	REV:	5’ GCTATCCAGGCTGTGCTATCCC 3’	
eccSLC7A1-exon	FRD:	5’ GGCAGGCTTGCTTTAGCATTGA 3’	
	REV:	5’ ACATGCCCCATAGGGGCCCCAC 3’	
eccSLC7A1-CpG	FRD:	5’ CCCGGAGAACCGGCTGCAGAGC 3’	
	REV:	5’ GGTTTGCTGTGGGACCGCCCTG 3’	
eccSLC7A1-5’UTR	FRD:	5’ GCACTGCTGATGAAACCTGGCG 3’	
	REV:	5’ GTTGCTGTTCAGAGCTGGAATA 3’	
eccSLC7A1-3’UTR	FRD:	5’ TTCTTCTACTGAACATGGAGCC 3’	
	REV:	5’ GGAAGCCATAGAATTTATTCGA 3’	

[1] Oligonucleotide primers were designed by Primer Premier 6.0

[2] PCR reaction was performed on ProFlex™ PCR System (Thermo Fisher)

Supplementary Table 5

Sequences of primers[1] and RT-qPCR reaction[2]

Gene	Primer	Sequences	RT-qPCR conditions
SLC7A1	FRD:	5'GCCTGTGCTATGGCGAGTTT3'	1. 95℃ for 3 min 2. 50 cycles of: 95℃ for 5 sec 60℃ for 30 sec 3. 95℃ for 5 sec 60℃ for 1 min 4. 50℃ for 30 sec
	REV:	5'ACGCTTGAAGTACCGATGATGTA3'	
xβ-actin	FRD:	5'TGCTGTCCCTGTATGCCTCTGG3'	
	REV:	5'GGAACCGCTCGTTGCCAATAGT3'	
GAPDH	FRD:	5'CGGATTTGGTCGTATTGGG3'	
	REV:	5'CTGGAAGATGGTGATGGGATT3'	

[1] Oligonucleotide primers were designed by Primer Premier 6.0

[2] RT-qPCR reaction was performed on ROCHE LightCycler480 System (Rotor gene 6000 Software, Sydney, Australia).