Supplementary

Table S1 The primers of candidate eccDNAs

Candidate eccDNAs	Forward primer	Reverse primer	Product size
O1 (chr3:153320590-153321556)	5'-ACTCTGGCTGGTAGGACCAA-3'	5'-GATTGGGCAAAGAGGTGGGA-3'	256 bp
O2 (chr14:65484268-65484970)	5'-GGGCCTTTCATTGGAAGCAATC-3'	5'-TGACATTGCCCCAGAAGCG-3'	679 bp
O3 (chr1:201630725-201631049)	5'-GAGTGCTGCATCTAGCGGAG-3'	5'-TGGGAGCTGGGGAGTCTAAT-3'	153 bp
O4 (chr5:167233287-167234692)	5'-AAAAGCCTGGCCTCCATTGT-3'	5'-GCTGGGGGCCATCTTCATTA-3'	677 bp
O5 (chr3:137490587-137491130)	5'-GTAAGCAGGATCCACCCGAG-3'	5'-CTCGCGTCTATGCGGGTATT-3'	278 bp
S1 (chr8:38223231-38223598)	5'-CGTTCACTCTGTTTCAGAGGCTT-3'	5'-CCAGCCTCTCTATATGACTGATTCC-3'	320 bp
S2 (chr1:68141078-68144108)	5'-TGGCCCACATCTAAGACCAG-3'	5'-GGTCCGTCCTTACTGCTGTG-3'	300 bp
S3 (chr10:70277601-70277938)	5'-ACGGAGAGCTATAATTGTGCGG-3'	5'-CCTCCAGCCTTAGTCTCTGAGT-3'	312 bp
S4 (chr14:31640553-31641482)	5'-AGTTCCAAAAGCAGAGGCCC-3'	5'-CCATGCCATCTCCATCCACT-3'	657 bp
S5 (chr13:41890332-41891079)	5'-GCAAGATGATTCTGTCGAACCCA-3'	5'-ATGGAGCAACTTCTCACAGCATC-3'	659 bp

Ox or Sx indicates the up-regulated or down-regulated candidate eccDNAs in ESCC relative to matched normal epithelium. In the form of ChrX: Y-Z, X indicates chromosome id, Y indicates start site and Z indicates end site. EccDNA, extrachromosomal circular DNA.

Table S2 The summary of reads and eccDNA count in each sample

Reads or count	C1	C2	C3	N1	N2	N3
Raw reads	141,510,152	138,644,094	148,054,446	136,927,808	137,162,048	142,572,110
Clean reads	141,495,966	138,630,264	148,034,092	136,914,164	137,144,340	142,555,544
EccDNA count	91,030	103,663	40,755	52,896	71,261	60,837

Cx indicated ESCC, while Nx indicated matched normal epithelium. EccDNA, extrachromosomal circular DNA; ESCC, esophageal squamous cell carcinoma.

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Table S3 The genes giving rise to more than 100 eccDNAs

Gene	Number of eccDNAs generated by the gene
LSAMP	192
CSMD1	159
MACROD2	149
MAGI2	142
CNTNAP2	138
RBFOX1	129
WWOX	125
NRXN3	121
LPP	116
NAALADL2	114
DAB1	108
AUTS2	106
LRP1B	106
SHANK2	104
PTPRD	103
IMMP2L	101

EccDNA, extrachromosomal circular DNA.

Table S4 The genes generating the most candidate eccDNAs at differential level (top 10)

Up-regulated eccDNAs in ESCC		Down-regulated eccDNAs in ESCC	
Gene	Count of eccDNAs generated by the gene	Gene	Count of eccDNAs generated by the gene
AUTS2	13	*LSAMP*	7
SHANK2	11	*CSMD1*	7
MACROD2	11	*BICD1*	7
ASIC2	10	*KALRN*	6
LPP	10	*ROBO2*	6
EYS	10	*FHOD3*	5
MAGI2	9	*GRID2*	5
MYO3B	9	*DOCK3*	5
TENM4	9	*MACROD2*	5
DMD	9	*RUNX1*	5

EccDNA, extrachromosomal circular DNA; ESCC, esophageal squamous cell carcinoma.

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Table S5 The nucleotide pattern flanking the eccDNAs junctional sites

Location	Start position				End position			
	A	C	G	T	A	C	G	T
−10	0.297100624739	0.195207984525	0.215521492005	0.29215906197	0.293638279773	0.201812990025	0.214827939336	0.289720790867
−9	0.292950145484	0.193176091939	0.2083150463	0.305547879517	0.293389034282	0.197109835986	0.217959763108	0.291541366624
−8	0.287894796729	0.18256148507	0.225063259589	0.304469621851	0.289585331361	0.191631853574	0.210655786559	0.308127028506
−7	0.265549396663	0.188521703322	0.210287336704	0.335630726551	0.285375249923	0.182160524933	0.223860379178	0.308603845966
−6	0.317939715102	0.16721121388	0.227078897035	0.287759337224	0.276971342187	0.177397768711	0.220717718645	0.324913170457
−5	0.376680375169	0.157230557497	0.216854413542	0.249223817032	0.332997393759	0.15799996749	0.242380402802	0.266622235949
−4	0.390210070601	0.140444415546	0.230172792146	0.239161884946	0.378062062127	0.148387760963	0.224304686357	0.249245490553
−3	0.301646645752	0.160237758524	0.271016542315	0.267088216649	0.358810557172	0.157219720737	0.237866892071	0.24610283002
−2	0.240586918946	0.217271628819	0.239313599593	0.302817015881	0.267207421014	0.203926158314	0.257047958083	0.271818462589
−1	0.274988215023	0.217271628819	0.246140758682	0.25012868653	0.235764560542	0.240966205563	0.233689320914	0.289579912981
0	0.282156732067	0.264162291325	0.226845906685	0.226835069924	0.287981490813	0.234567098512	0.226119843734	0.251331566941
1	0.247706670568	0.250513391527	0.161890364494	0.339889573411	0.279913522652	0.262574705917	0.17736525843	0.280141094621
2	0.239443640718	0.219401052249	0.146632205768	0.394523101264	0.248281018872	0.241096246688	0.145597295145	0.365020020915
3	0.24278678132	0.24751160888	0.151698391283	0.358003218518	0.250665106173	0.214844194477	0.152354015291	0.382131265679
4	0.320204598037	0.226146935635	0.169188922663	0.284459543664	0.273064690042	0.225047004448	0.161034260418	0.340848626712
5	0.316303364272	0.226699610418	0.181342349518	0.275654675791	0.325314130594	0.210991726133	0.183628905975	0.280059818918
6	0.30845213132	0.212774373229	0.191252566958	0.287520928494	0.313252816203	0.218274029162	0.183141251754	0.285326484501
7	0.293638279773	0.219265592744	0.197380754997	0.289715372487	0.306658647464	0.208510107988	0.19013096225	0.294694863917
8	0.287856868068	0.217683425717	0.202262715584	0.292196990632	0.294120515613	0.211750299366	0.196806406693	0.297317359948
9	0.284107348949	0.214383632157	0.206163949349	0.295345069545	0.292982655765	0.212481780696	0.19673596775	0.297794177409
10	0.282693151709	0.213148241465	0.212714771046	0.29144383578	0.294911599127	0.209929723608	0.199217585895	0.29593567299

The left column indicates the position of upstream (negative number) or downstream (positive number) of start/end site. The number in the main text indicates the percentage of one nucleotide at certain position. EccDNA, extrachromosomal circular DNA.

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