Supporting Data

Supplementary Methods

DNA sequencing analysis of *CDKN2A (p16)*

Sequencing of the *CDKN2A* (NM_00077.4) coding region was performed using dye termination chemistry. PCR was carried out using AmpliTaq Gold (Applied Biosystems, Foster City, CA) according to the standard protocol. After the PCR reaction, the amplicons were treated with ExoSAP-IT (USB Corp., Cleveland, OH) to degrade the unincorporated PCR primers and deoxynucleotide triphosphates. The ddNTP terminator reactions were carried out with ABI BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Sequencing products were loaded and the data were collected on the ABI 3130xl genetic analyzer (Applied Biosystems). The Mutation Surveyor (SoftGenetics, State College, PA) was the main tool used in the data analysis.

Supplementary Tables

Table S1. High copy number amplification/gain segments and genes and ESCC samples

Chr. Region	Amp #	Gain #	SRO (bp) (hg 18)	Size (kb)	Interesting gene (s)	Cases
1p36.21	1	2	13,656,262-14,300,247	644	*PDPN, PRDM2, CSF3R*	74T*, TL0124, TL0128
1p34.3	1	2	36,168,792-36,712,519	544	*ADPRHL2, TRAPPC3*	74T*, TL0124, TL0128
1p34.3-p34.1	1	2	37,750,211-44,556,498	6806		74T*, TL0124, TL0128
1p32.2-p31.3	1	2	58,018,913-65,106,407	7087	*MYSM1, INADL, RAVER2*	74T*, TL0124, TL0128
1q21.1	1	9	144,131,366-	339	*POLR3GL, ANKRD34A, RBM8A, PIAS3,*	TL0123*, 74T, 79T, 80T, 97T, TL0110, TL0122, TL0124,

			144,470,025		NUDT17	TL0129, TL0134
1q22	1	10	154,193,863-154,412,749	219	ARHGEF2, RAB25, LMNA	TL0123*, 74T, 79T, 80T, 97T, TL0110, TL0122, TL0124, TL0128, TL0129, TL0134
1q31.1	1	9	184,200,166-184,231,458	31	HMCN1	TL0123*, 74T, 80T, 97T, TL0110, TL0122, TL0124, TL0128, TL0129, TL0134
3q28.1	2	13	190,425,032-190,675,215	250	TPRG1	33T, 39T, 44T*, 74T, 79T, 80T, TL0105, TL0110, TL0122, TL0124, TL0127, TL0128*, TL0129, TL0134, TL0140
3q28	2	13	191,312,603-191,762,732	450	LEPREL1, CLDN1, CLDN16, TMEM207, IL1RAP	33T, 39T, 44T, 74T, 79T, 80T, TL0105*, TL0110, TL0122, TL0124, TL0127, TL0128*, TL0129, TL0134, TL0140
4q22.1	1	1	90,562,750-93,568,852	3006	SNCA, MMRN1, TMSL3, GRID2	79T*, TL0110
5p15.33-p15.32	1	9	3,250,143-4,531,397	1281	IRX1	57T, 74T*, 80T, TL0105, TL0110, TL0123, TL0124, TL0127, TL0128, TL0129
6q11.1	1	3	62,325,013-62,625,246	300	KHDRBS2	44T, 74T, 79T*, TL0124
7p11.2	3	7	54,800,197-55,431,257	631	EGFR	39T*, 80T*, TL0128*, 57T, TL0105, TL0123, TL0122, TL0129, TL0124, TL0127
	1	9	55,925,047-57,356,329	1431	ZNF713, MRPS17, GBAS, PSPH, SUMF2, PHKG1, CHCHD2, CCT6A, LOC389493	80T*, TL0128, 57T, TL0105, TL0123, TL0122, TL0129, TL0124, TL0127, TL0110
7q11.21-q11.22	1	6	64,837,590-67,950,195	3112	GUSB, ASL, CRCP, TPST1, KCTD7, RABGEF1, STAG3L4	80T, TL0105, TL0123, TL0122, TL0129, TL0110, TL0128*
7q21.11-q21.12	2	5	85,968,783-87,250,029	1281	GRM3, KIAA1324L, DMTF1, CROT, P53TG1, ABCB4, ABCB1, RUNDC3B	61T*, 80T*, TL0105, TL0123, TL0122, TL0129, TL0110
7q21.13-q21.2	2	5	90,550,106-92,212,635	1663	PFTK1, FZD1, MTERF, AKAP9, CYP51A1, KRIT1, GATAD1, ERVWE1, PEX1, CDK6	80T*, TL0128*, TL0105, TL0122, TL0129, TL0110, TL0123
7q21.2-q21.3	1	4	92,218,874-97,506,257	5287	TFPI2, PPP1R9A, PON1, PDK4, SLC25A13, SHFM1, DLX5, TAC1, OCM2	80T*, TL0110, TL0122, TL0123, TL0129
7q21.3-q22.1	1	6	97,693,786-99,262,531	1569	NPTX2, TMEM130, TRRAP, SMURF1, ARPC1A, PDAP1, CPSF4, ZKSCAN5, CYP3A4	80T*, TL0122, TL0129, TL0110, TL0123, TL0134, TL0128
8p22	1	3	16,493,823-18,375,005	1881	FGF20, CNOT7, SLC7A2, NAT1, NAT2	80T*, 44T, TL0123, TL0140
8q22.3	1	14	101,881,344-102,800,006	919	YWHAZ, GRHL2, NCALD	TL0105*, TL0123, TL0140, 44T, TL0122, TL0134, 61T, 74T, TL0128, TL0127, TL0124, 80T, 33T, TL0110, TL129
8q23.3-q24.13	1	15	116,400,027-126,100,075	9700	TRPS1, EIF3H, RAD21, MED30, EXT1, SAMD12, TNFRSF11B, SNTB1, ZHX2,	80T*, TL0105, TL0123, TL0140, 44T, TL0122, TL0134, 61T, 74T, TL0128, TL0127, TL0124, 57T, 33T, TL0110, TL129

					WDR67, ZHX1, TMEM65, RNF139	
8q24.21	1	15	128,237,732-128,925,222	688	MYC	80T*, TL0140, TL0123, 44T, TL0122, TL0134, 61T, 74T, TL0128, TL0127, TL0124, 33T, TL0110, TL0105, TL0129, 57T
10q21.2-q21.3	1	1	61,456,296-66,443,911	4988	CDC2, TMEM26, ARID5B, EGR2	TL0124*, TL0128
11q13.3	10	4	69,787,502-70,193,941	406	PPFIA1, CTTN, SHANK2	33T*, 74T*, 79T*, 80T*, TL0123*, TL0124*, TL0127*, TL0128*, TL0134*, TL0140*, 39T, 57T, 97T, TL0122
12q14.1-q14.2	1	0	61,237,534-61,431,255	194	MON2, PPM1H	61T*
12q15	2	0	67,475,003-69,231,418	1756	MDM2, CPM, CPSF6, LYZ, YEATS4, FRS2, CCT2, BEST3, RAB3IP, CNOT2, KCNMB4, PTPRB	61T*, TL0105*
13q22.1	1	2	72,968,865-73,287,548	319	KLF12	44T*, TL0127, TL0134
14q13.3-q21.1	1	5	35,793,858-38,768,934	2975	MBIP, NKX2-1, SLC25A21, PAX9, MIPOL1, TTC6, CLEC14A, SEC23A, SIP1, TRAPPC6B, PNN	61T*, TL0127, TL0123, TL0128, TL0124, 33T
14q32.2[n]	1	7	97,731,373-98,918,992	1188	BCL11B	TL0124*, TL0127, TL0123, TL0128, TL0110, TL0134, 33T, 57T
17q12	1	4	35,100,238-35,281,299	181	ERBB2, GRB7, IKZF3	44T*, TL0124, TL0123, 97T, 74T
17q21.2	1	3	36,812,713-37,062,706	250	KRT37, KRT38, KRT32, KRT35, KRT36, KRT13, KRT15, KRT19, KRT9, KRT14, KRT17	TL0124*, 44T, TL0123, 97T
18p11.32-p11.31	1	2	2,656,359-4,968,947	2313	SMCHD1, EMILIN2, LPIN2, MYOM1, MRCL3, MRCL2, TGIF1, DLGAP1	TL140*, TL0105, TL0127
18p11.21	1	1	13,262,584-14,150,145	888	RNMT	TL128*, TL0105
18q12.3-q21.1	1	2	40,331,315-42,037,504	1706	SETBP1, SLC14A2, SLC14A1, SIGLEC15, KIAA1632, PSTPIP2, CCDC5, ATP5A1	TL0140*, TL0110, 79T

18q21.1	1	2	42,043,968-43,006,268	962	RNF165, PIAS2, KATNAL2, TCEB3C, TCEB3B, HDHD2, IER3IP1	TL0140*, TL0110, 79T
20p12.2	1	5	9,612,691-10,731,253	1119	PAK7, ANKRD5, SNAP25	79T*, TL0105, TL0128, TL0127, 80T, 44T
20p11.22-p11.21	1	4	21,681,469-22,975,201	1294	FOXA2, THB	79T*, TL0105, TL0128, TL0127, 44T
21q11.2-q21.1	1	0	14,818,882-15,456,445	638	SAMSN1, NRIP1	80T*
21q22.3	1	1	42,743,983-43,037,602	294	RSPH1, SLC37A1, PDE9A	80T*, TL0124
	1	1	44,637,562-45,575,195	938	TRPM2, UBE2G2, ITGB2, ADARB1, POFUT2	80T*, TL0124
22q11.21[n]	2	7	18,537,622-19,793,876	1256	RTN4R, RIMBP3, MED15, SERPIND1, CRKL	80T*, TL0128*, TL0123, TL0124, TL0110, 44T, 33T, TL0127, TL0140
Xp22.2	1	1	15,056,486-15,556,357	500	ASB9, ASB11, PIGA, FIGF, PIR, BMX, ACE2	61T*, TL0128

Based on the array CGH data, log2 Ratio >0.5 was considered to be a high-level amplification. *: case has high-level amplification.

Abbreviations: Amp, amplification; Chr, chromosome; ESCC, esophageal squamous cell carcinoma; SRO, smallest region of overlap

Table S2. Possible homozygous loss that is smaller than 1.0 Mb

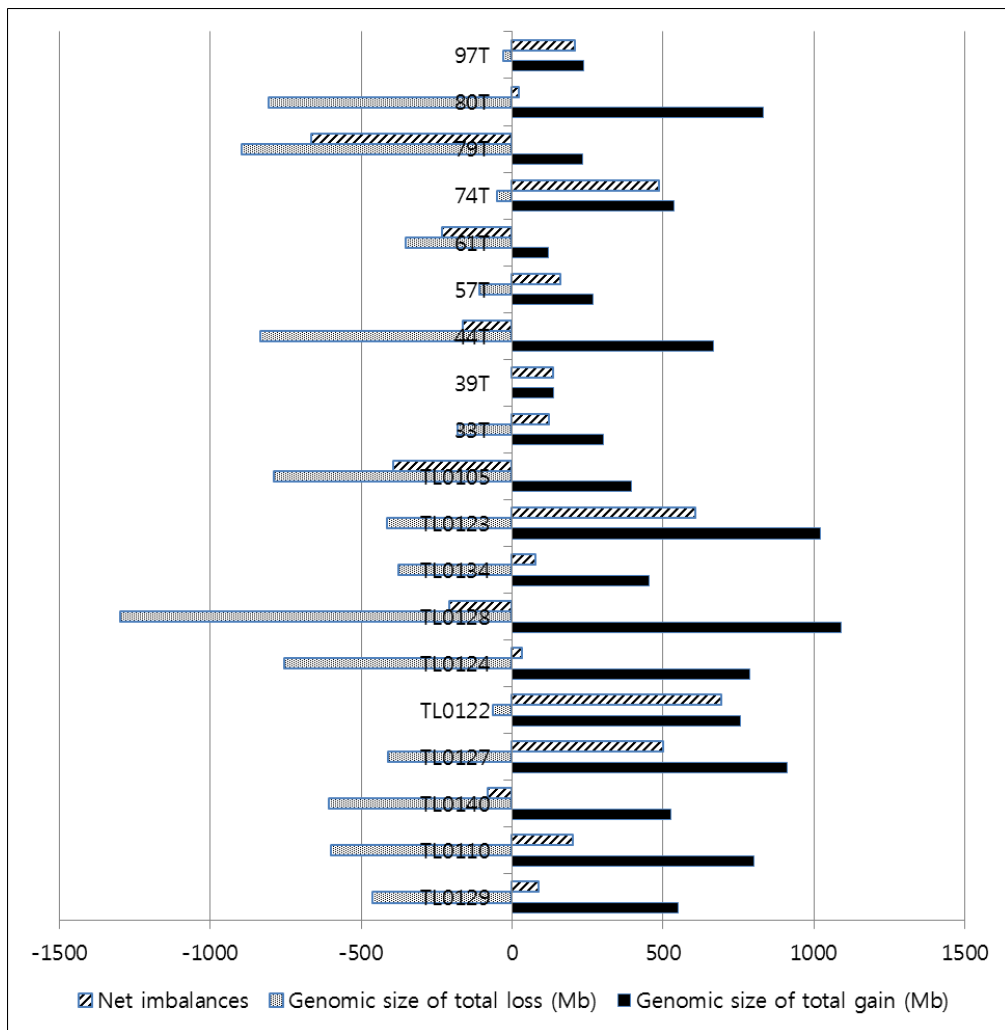| Chromosome	Target genes	Genomic coordinates (NCBI Build 36.3; hg18)	Size	Log 2 Ratio	Case no.
3p14.2	*FHIT*	60,681,464-61,181,306	500kb	-0.5	TL0127
	FHIT	60,181,428-60,731,422	550kb	-0.6	TL0124
9p21.3	*CDKN2A, CDKN2B*	21,856,489-22,075,247	219kb	-0.5	TL0124

Supplementary Figures

Figure S1. Net genomic imbalances in 19 ESCC samples. The left side indicates genomic losses and the right side indicates genomic gains. Net genomic gains are more common than net genomic losses.
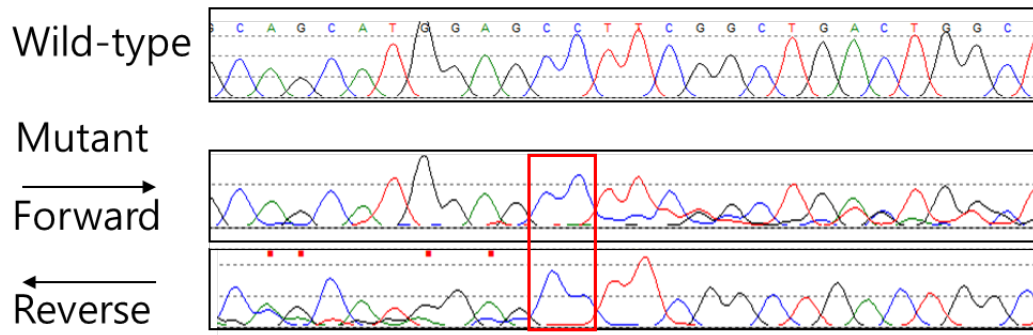
Figure S2. A somatic mutation in exon2 of *CDKN2A* c.331_32dupCC (p.S12Lfs*15) was detected in one ESCC tumor tissue (red box) but not in the adjacent normal tissue. The accession number of *CDKN2A* is NM_00077.4.