SI Appendix

Genomic alterations in BCL2L1 and DLC1 contribute to drug sensitivity in gastric cancer

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SI Materials and Methods

Gastric Cancer Patient Sample Collection and Genomic DNA Extraction. Frozen tissue samples of gastric cancer and paired normal gastric tissue samples were obtained from individuals who underwent gastrectomies at Seoul National University Hospital. Total DNA was extracted from sections using the QIAamp DNA Mini kit (Qiagen). All samples were obtained with informed consent at the Seoul National University Hospital, and the study was approved by the institutional review board in accordance with the Declaration of Helsinki.

Array Comparative Genomic Hybridization (aCGH). The probes for each chromosome were assigned to each corresponding array in a linear order. To remove repetitive sequences such as SINEs and LINEs, we removed all sequences with over 50% repeat content. The custom-designed 1 million CNV genotyping array was designed using 5,197 CNVs from our data set, 8,599 CNVs reported in Conrad *et al*. (2009) (1), 556 CNVs from the 1000 Genomes project, and 5,608 segmental duplication regions. After aCGH experiments, images were analyzed with Feature Extraction Software 10.5.1.1 (Agilent Technologies), using the CGH-105_Jan09 protocol for background subtraction and normalization. The ADM2 statistical algorithm was used to identify CNVs based on the combined $\log_2$ ratios (i.e., the statistical threshold of the ADM2 algorithm (p-value < 10^{-15}), the minimum ± $\log_2$ ratio = 0.4, and the minimum number of probes in a CNV interval ≥ 5, minimum size of altered region = 10 kb). We calculated positive predictive values based on the comparison CNV calls from our platform against droplet digital PCR validation results.

Droplet Digital PCR (ddPCR). The extracted genomic DNA was restricted with EcoRI (New England Biolabs) enzyme for 1 hr at 37°C. The PCR mixture was assembled in 20-μL

solution containing 1X ddPCR supermix (Bio-Rad), 1X probe and primer premix for determining target gene and internal control gene, RNase P (final concentration of 250 nM for probe and 900 nM for each primer; Applied Biosystems), and 10 ng of the restricted DNA. The reaction mixture and droplet generation oil (Bio-Rad) were loaded into the droplet generator (QX-200; Bio-Rad). The droplets were transferred to a 96-well PCR plate and PCR reaction was performed as follows: enzyme activation for 10 min at 95°C, 40 cycles of 94°C for 30 sec, 60°C for 1 min, and 98°C for 10 min, followed by enzyme deactivation for 10 min at 98°C and 4°C hold (performed with a ramp rate of 2°C/sec in all steps). The PCR plate was placed in a droplet reader (Bio-Rad). After the reading, the copy number variation of target genes was analyzed by Quanta software (Bio-Rad) accompanied by the droplet reader. The amplification-threshold value was set at 3.0 for patient tissues and cell lines.

Exome Sequencing Processing and Variant Calling. FASTQ files from 55 normal and 55 tumor samples were aligned to the human reference genome GRCh37/hg19 by Burrows-Wheeler Aligner (BWA) mem. Marking of duplicate reads was performed by Picard tools. Indel realignment and base recalibration were performed using Genome Analysis Tool Kit (GATK). After processing the BAM files, somatic mutations were called by Mutect and Indelocator, and annotated with Annovar. To select rare functional variants, variants within coding regions were retained and filtered by minor allele frequency if lower than 0.01 or not reported, based on the 1000 Genome Project Asian frequency and NHLBI Exome sequencing project 6500. Nonflagged-SNPs were filtered out, based on dbSNP138, to sort out passenger calls, and possible false positive calls in segmental duplicate region were also removed. Variants with total read depths below 8 and alternate allele depth lower than 4 were not passed (Fig. S1*B*).

Identification of Significantly Altered Genes and Pathway Analysis. We listed significantly altered genes using the following criteria: 1) genes with high mutation frequency (> 10%), 2) amplified or deleted genes with high frequency (> 10% for amplification and > 20% for deletion), 3) genes annotated in cancer gene databases including TARGET (Tumor Alterations Relevant for GEnomics-driven Therapy; http://www.broadinstitute.org/cancer/cga/target), Cancer Gene Census (http://cancer.sanger.ac.uk/cancergenome/projects/census), and Vogelstein Cancer Gene (http://www.sciencemag.org/content/339/6127/1546)), 4) genes that are not annotated in Gene Ontology (GO) Biological Processes (http://geneontology.org) were excluded. Significantly altered pathways were analyzed with the above gene list using the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis tool in DAVID bioinformatics resources (http://david.abcc.ncifcrf.gov; $p < 0.1$).

Cell Culture. Gastric cancer cells were obtained from the Korean Cell Line Bank and maintained in RPMI 1640 medium (Life Technologies) containing 10% fetal bovine serum (Life Technologies). Penicillin (100 U/ml; Life Technologies) and streptomycin sulfate (100 μg/ml; Life Technologies) were supplemented to all cell culture media. All cells were maintained in a humidified incubator with 5% CO_2 at 37°C.

Western Blot Analysis. Cells were lysed in RIPA buffer (Thermo Scientific) containing protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Roche), and were centrifuged at 20,000 g for 10 min at 4°C. After determination of protein concentration in the cell extract by the BCA method (Thermo Scientific), 20 μg of protein were resolved by SDS-PAGE and transferred to a polyvinyl difluoride membrane. Membranes were blocked for 1 hr with 5% skim milk in Tris-buffered saline, and were incubated with anti-BCL2L1 (Cell

Signaling Technology), anti-cleaved caspase 3 (Cell Signaling Technology), anti-PARP (Cell Signaling Technology), anti-Myc tag (Cell Signaling Technology), and anti-Actin (Sigma-Aldrich Corporation) antibody. The membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibody, followed by enhanced chemiluminescence development according to the manufacturer's instructions (Pierce). Western blot quantification was performed by ImageJ software (http://rsb.info.nih.gov/ij/index.html).

Quantitative Real-Time PCR. Total RNA was purified using RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's instructions. One microgram of total RNA was transcribed into cDNA using Maxime RT PreMix (Intron Biotechnology) for 1 hr at 45°C. Quantitative real-time PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems). β-2-Microglobulin (B2M) was used as the internal control for normalization. The sequences of primers for DLC1 were 5'-CTGTGTGGATGGCCTGTTTA-3' and 5'-GTCCTTCGC TCACCTTCTTATAG-3', the sequences for HM13 were 5'- GGCCAAGGGAGAAGTGA CAG-3' and 5'-ATGCCTCTGTTCCCTCTTTG-3', the sequences for COX4I2 were 5'-ACTACCCCATGCCAGAAGAG-3' and 5'- TCATTGGAGCGACGGTTCATC-3', and the sequences for B2M were 5'- TGAGTATGCCTGCCGTGTGAAC-3' and 5'-TGCTGCTTA CATGTCTCGATCCC-3'.

Transfection of DNA and siRNA. Specific siRNA targeting BCL2L1 and DLC1 was purchased from Santa Cruz Biotechnology. Transfection experiments were performed using Lipofectamine 2000 Transfection reagent according to the manufacturer's protocol (Invitrogen). After 24 - 48 hr, cells were harvested and plated for appropriate assays. The effects of siRNA knock-down were measured by western blot analysis with the indicated antibodies or real-time PCR.

Cell Proliferation and Cell Viability Assay. For the cell proliferation assays, 2,000 - 4,000 cells were plated into 96-well plates and viable cells were estimated using EzCytox WST assay kit at 24, 48 and 72 hr according to the manufacturer's instructions (Daeil Lab). The relative cell proliferation was estimated compared to viable cells assayed immediately after plating. For the cell viability assays, 2,000 - 4,000 cells in 96-well plated were treated with indicated drugs for 72 hr and cell viabilities were estimated using the EzCytox WST assay kit. Cell viabilities were estimated as relative values compared to untreated controls. To quantify the potency of drugs for cancer cell treatments, half maximal inhibitory concentration (IC_{50}) for cell viability was calculated using CompuSyn Software (ComboSyn Inc.).

Colony Formation Assay. Specific siRNA-transfected cells were plated onto 100-mm culture dishes (2,000 cells/dish) and incubated for 14 days. Cell seeding was performed in duplicates. After washing twice with PBS, cultures were stained with crystal violet solution (0.5% (w/v) crystal violet, 30% ethanol, 3% formaldehyde) for 10 min. Colonies were examined and counted using ImageJ software.

Bromodeoxyuridine (BrdU) Incorporation Assay. Specific siRNA-transfected cells were seeded at 2,000 - 4,000 cells per well in 96-well plates and allowed to attach for 24 hr. The incorporation of BrdU into genomic DNA was determined using the Cell Proliferation ELISA BrdU Kit (Roche). BrdU (10 μM) was added to the culture medium 16 hr before fixation.

Combination Index (CI) Analysis. Drug synergism was quantified by calculating CI based on the multiple drug effect equation of Chou-Talalay (2). CI for each concentration of drugs was calculated by CompuSyn Software. A CI lower than 0.9 indicates synergism; a CI of 0.9

to 1.1 indicates additive; and a CI higher than 1.1 indicates antagonism.

Site-directed Mutagenesis. The expression construct of Myc-tagged WT DLC1 was kindly provided by Dr. J. W. Ping Yam. The QuickChange site-directed mutagenesis kit (Stratagene) was used to make point mutations, and the resulting mutations were verified by Sanger sequencing. The primers used in constructing the point mutations were the following (the underlined sequences indicate the mutated bases): R549W, GCAAGAGGTGGTCC TGGCTTGAAGAGTTTG; G845V, GAAGCTTCCACGTCCCTGGCCACATC; K1060E, CTGGGCCGTGCCCGAGTTCATGAAGAG; and P1475S, GATTGAACCCTGTGGG TCAGGAAAATCCAAAC.

Protein Stability Prediction. We predicted protein stability using the web-based database i-Mutant (http://folding.biofold.org/i-mutant/i-mutant2.0.html), which predicts a change in protein stability as 'Increase' or 'Decrease' upon single point mutation from protein sequence. Environmental conditions for the mutant protein were set at 37°C and pH 7.4, generally regarded as the normal physical state of the human body. Higher reliability index represents less stable mutant protein.

Animal Experiments. For patient-derived xenograft (PDX) models, the surgically resected tissues were minced into pieces approximately ~2 mm in size and injected into the flanks of 4-week-old NOD/SCID/IL-2γ-receptor null (NSG) female mice. For cell line xenograft models, the flanks of 4-week-old NSG female mice were injected subcutaneously with 1×10^6 MKN74 cells in 100 μL phosphate-buffered saline. Drug treatments began after tumors reached approximately 200 mm^3. Mice were randomly divided into four treatment groups consisting of 5-6 mice in each group: 1) vehicle only, 2) irinotecan only (Kwang Dong

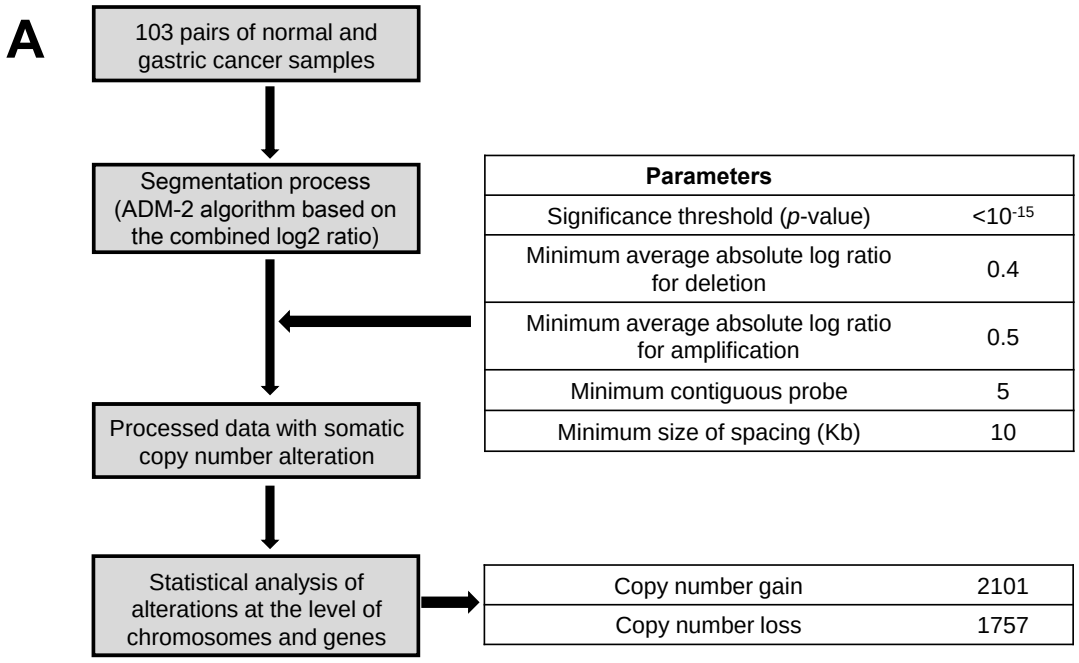
Pharmaceutical Co., 50 mg/kg, weekly), 3) ABT-737 only (Selleckchem, 100 mg/kg, daily), and 4) irinotecan plus ABT-737. The vehicle for ABT-737 was 30% (v/v) propylene glycol, 5% (v/v) Tween-80, and 65% (v/v) of 5% (w/v) dextrose in water. Irinotecan, ABT-737, and vehicle were administered via intraperitoneal injection.

Statistical Analysis. Statistical calculations were performed using Prism 4.0 (GraphPad). Differences between two variables and multiple variables were assessed by unpaired Student's *t* test and one-way ANOVA with Tukey's multiple comparison test, respectively. Associations between two discrete variables were estimated by Fisher's exact test or Pearson's chi-square test. Correlations of two continuous variables were estimated by linear regression analysis. The difference was considered significant if the *p*-value was less than 0.05.

References for SI Appendix

1. Conrad DF, et al. (2010) Origins and functional impact of copy number variation in the human genome. *Nature* 464(7289):704-712.
2. Chou TC (2006) Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacol Rev* 58(3):621-681.

Supplementary Figures



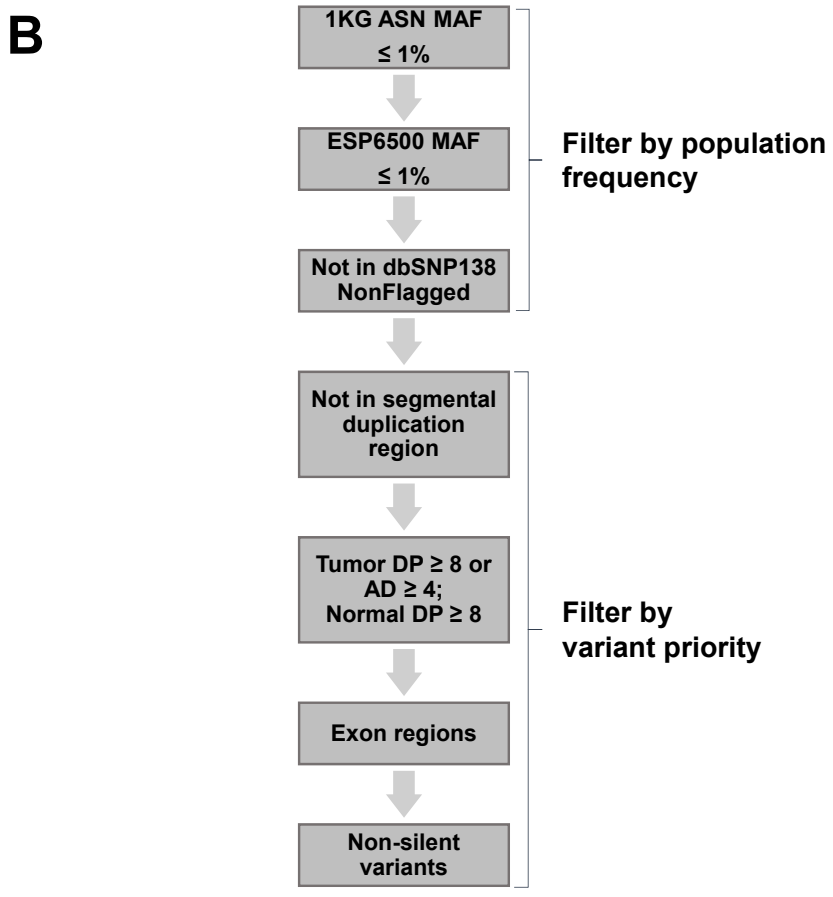


Fig. S1. Pipelines for somatic alteration discovery in array Comparative Genomic Hybridization (aCGH) and whole exome sequencing. (*A*) The procedure for processing aCGH data. (*B*) Detailed filtering criteria for variants from whole exome sequencing on 55 paired normal-tumor GC samples. 1KG ASN MAF: 1000 genome project Asian Minor Allele Frequency, ESP6500: NHLBI Exome Sequencing Project 6500 frequency, DP: Depth, AD: Alternate allele depth.

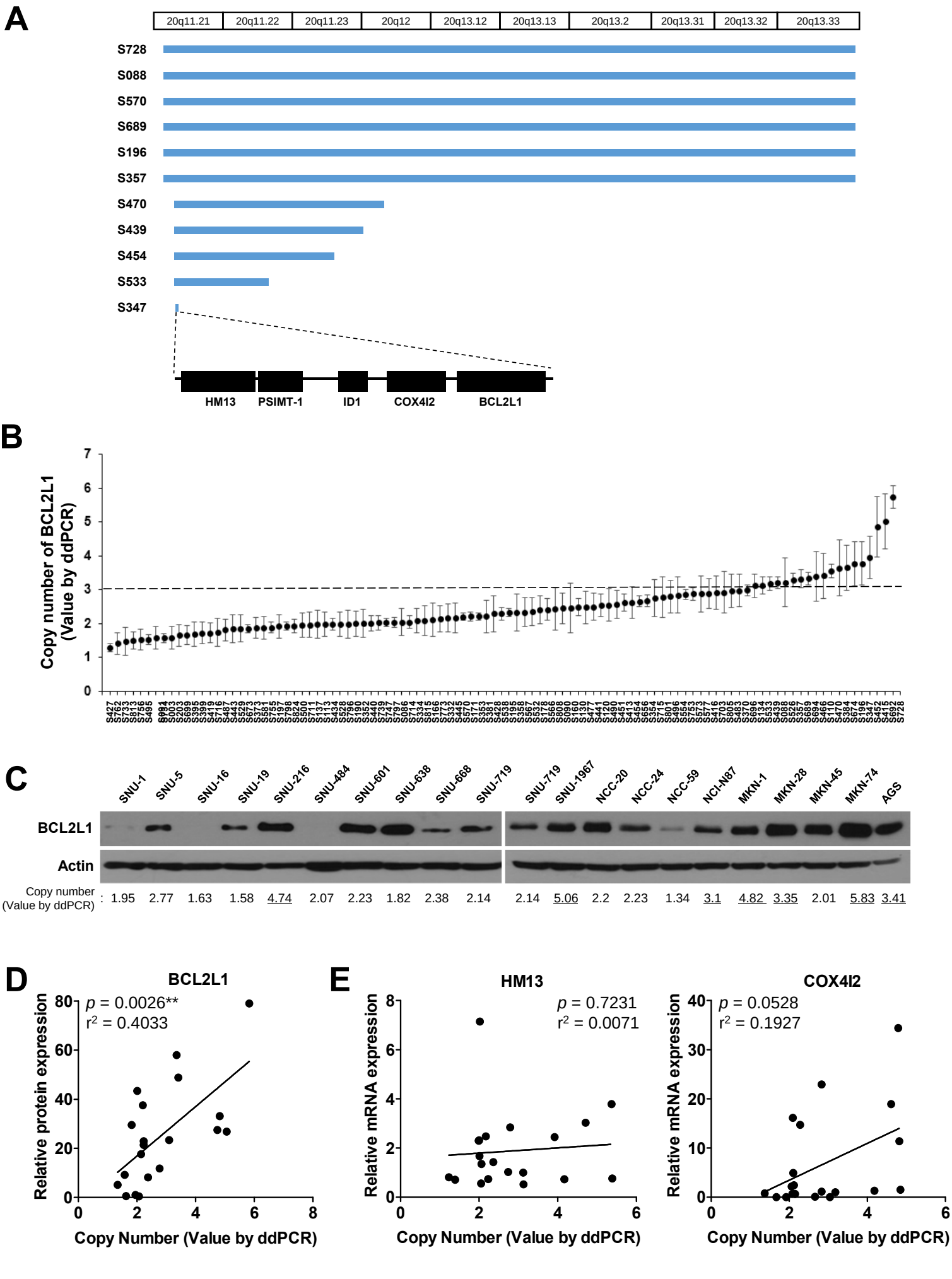
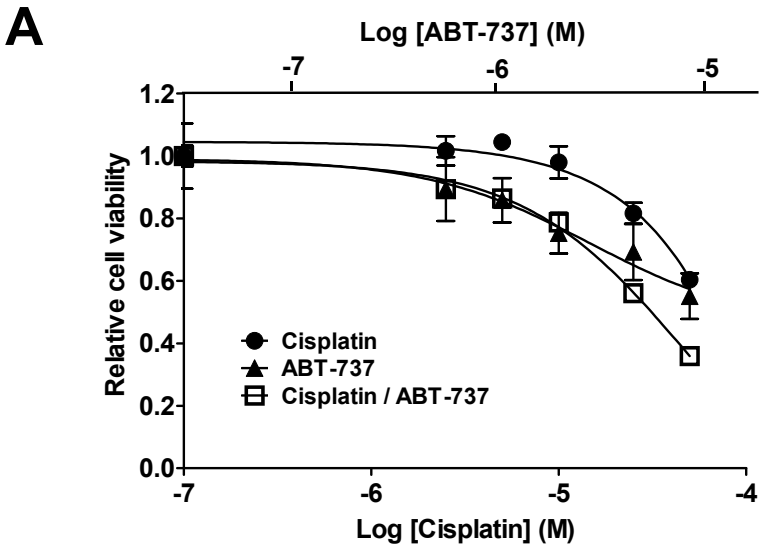
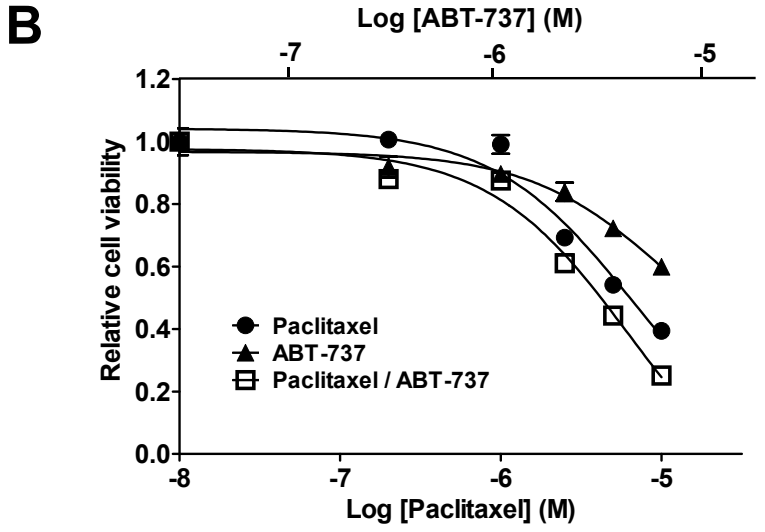

A
20q11.21
20q11.22
20q11.23
20q12
20q13.12
20q13.13
20q13.2
20q13.31
20q13.32
20q13.33
S728
S088
S570
S689
S196
S357
S470
S439
S454
S533
S347
HM13
PSIMT-1
ID1
COX4I2
BCL2L1
B
Copy number of BCL2L1
(Value by ddPCR)
C
SNU-1
SNU-5
SNU-16
SNU-19
SNU-216
SNU-484
SNU-601
SNU-638
SNU-668
SNU-719
SNU-719
SNU-1967
NCC-20
NCC-24
NCC-59
NCI-N87
MKN-1
MKN-28
MKN-45
MKN-74
AGS
BCL2L1
Actin
Copy number
(Value by ddPCR) : 1.95 2.77 1.63 1.58 4.74 2.07 2.23 1.82 2.38 2.14 2.14 5.06 2.2 2.23 1.34 3.1 4.82 3.35 2.01 5.83 3.41
D
BCL2L1
p = 0.0026**
r2 = 0.4033
Relative protein expression
Copy Number (Value by ddPCR)
E
HM13
p = 0.7231
r2 = 0.0071
Relative mRNA expression
Copy Number (Value by ddPCR)
COX4I2
p = 0.0528
r2 = 0.1927
Relative mRNA expression
Copy Number (Value by ddPCR)

Fig. S2. Amplification of BCL2L1 in gastric cancer (GC) tissues and cell lines. (*A*) The chromosomal locations of BCL2L1-containing amplicons in 11 BCL2L1-amplified patients estimated by array Comparative Genomic Hybridization (aCGH). (*B*) Estimated copy numbers of BCL2L1 of 103 GC patients by droplet digital PCR (ddPCR). Error bars indicate the Poisson 95% confidence intervals for each determination. The dashed line indicates the ddPCR threshold cut-off of 3.0 copies for calling a sample BCL2L1-amplified. (*C*) Protein expression levels and copy numbers of BCL2L1 in 20 GC cell lines. Copy number values of BCL2L1 were estimated by ddPCR and the underlined numbers represent the BCL2L1-amplified samples (threshold cut-off of 3.0 copies). (*D*) The correlation between protein expression levels and copy numbers of BCL2L1 in GC cell lines. Protein expression levels were quantified by western blotting and densitometric analysis using ImageJ, and copy number values were estimated by ddPCR. (*E*) The correlation between mRNA expression levels and copy numbers of HM13 and COX4I2 in GC cell lines. Messenger RNA expression levels were quantified by real-time PCR and copy number values were estimated by ddPCR. Correlation between gene expression and copy number was estimated by linear regression analysis (**, $p < 0.01$).



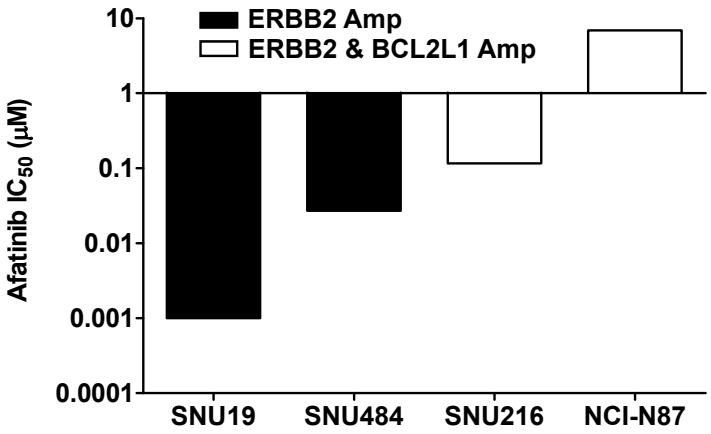
Cisplatin (μM)	2.5	5.0	10.0	25.0	50.0
ABT-737 (μM)	0.5	1.0	2.0	4.0	8.0
Combination Index	1.09	1.49	1.46	0.82	0.62



Paclitaxel (μM)	0.2	1.0	2.5	5.0	10.0
ABT-737 (μM)	0.5	1.0	2.0	4.0	8.0
Combination Index	0.57	1.37	0.72	0.82	0.85

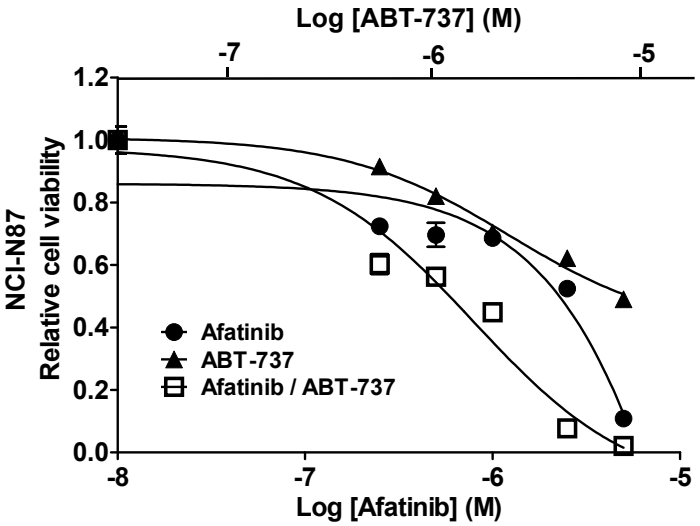
Fig. S3. Combinational effects of BCL2L1 inhibitor and conventional chemotherapeutic drugs in BCL2L1-amplified MKN74 cells. (*A*, *B*) Combination cytotoxicity of ABT-737 with cisplatin (*A*) and paclitaxel (*B*). WST assays were used to examine the cell growth inhibitory effect in MKN74 cells. Lower panels represent the calculated combination index values at the applied concentration. Gray boxes represent synergistic effect of the two drugs (combination index < 0.9).

A



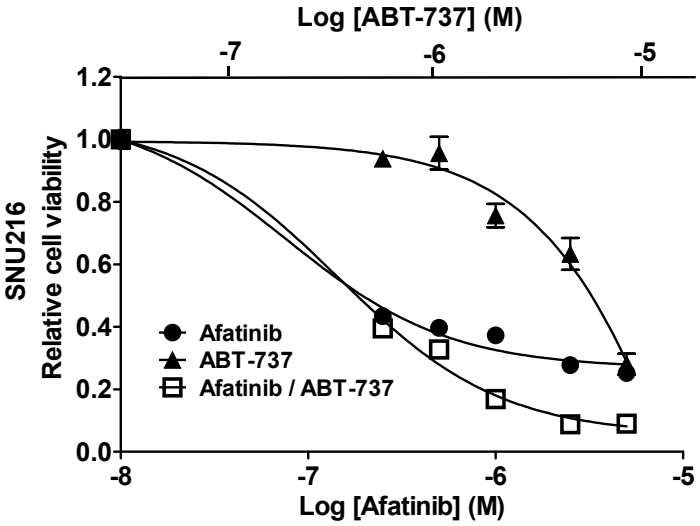
	SNU19	SNU484	SNU216	NCI-N87
ERBB2 (copies) :	5	3	9	57
BCL2L1 (copies) :	2	2	5	3

B



Afatinib (μM)	0.25	0.5	1.0	2.5	5.0
ABT-737 (μM)	0.5	1.0	2.0	4.0	8.0
Combination Index	0.44	0.73	0.87	0.16	0.08

C



Afatinib (μM)	0.25	0.5	1.0	2.5	5.0
ABT-737 (μM)	0.5	1.0	2.0	4.0	8.0
Combination Index	0.57	0.48	0.17	0.16	0.33

Fig. S4. Combinational effects of BCL2L1 and ERBB2 inhibitors in both BCL2L1 and ERBB2-amplified cells. (*A*) Growth inhibitory effects of afatinib were determined by calculating IC_{50} values in ERBB-amplified cells (SNU19 and SNU484) and ERBB/BCL2L1-amplified cells (SNU216 and NCI-N87). Amp: amplification. (*B*, *C*) Combination cytotoxicity of afatinib and ABT-737 in NCI-N87 (*B*) and SNU216 cells (*C*). WST assays were used to examine the cell growth inhibitory effect. Lower panels represent the calculated combination index values at the applied concentration. Gray boxes represent synergistic effect of the two drugs (combination index < 0.9).

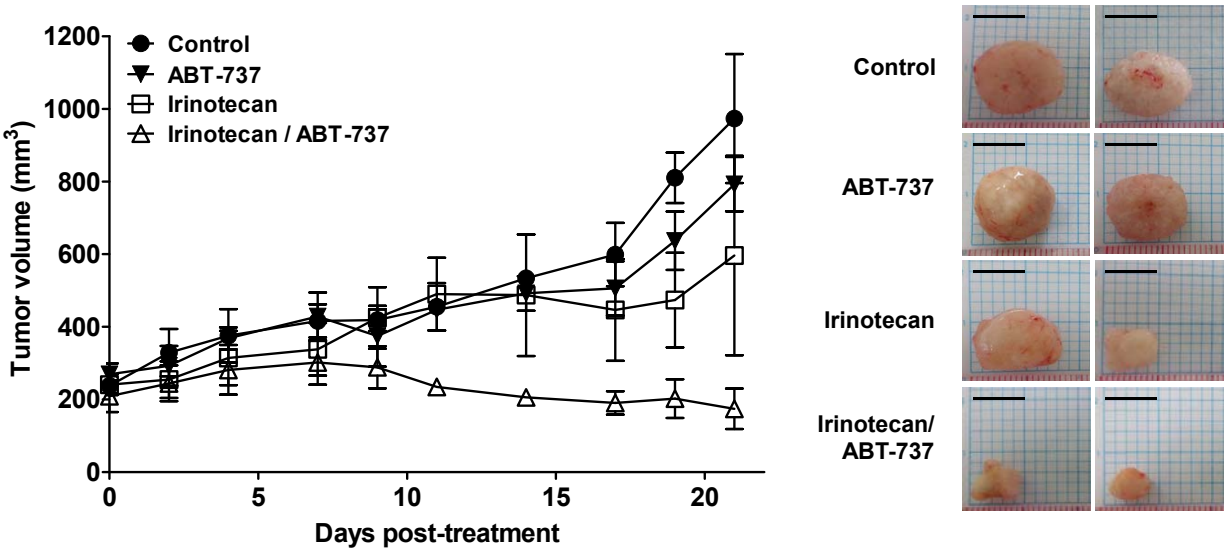


Fig. S5. The *in vivo* efficacy of irinotecan and ABT-737 in a gastric cancer cell xenograft model. MKN74 cells were injected into the flanks of NOD/SCID/IL-2γ-receptor null (NSG) mice. The mice were treated with irinotecan (50 mg/kg/week), ABT-737 (100 mg/kg/day), or the combination of the two drugs for 21 days (n = 5). Average tumor sizes for each group are plotted (left panel) and representative tumors after treatment are shown (right panel). Scale bar: 10 mm.

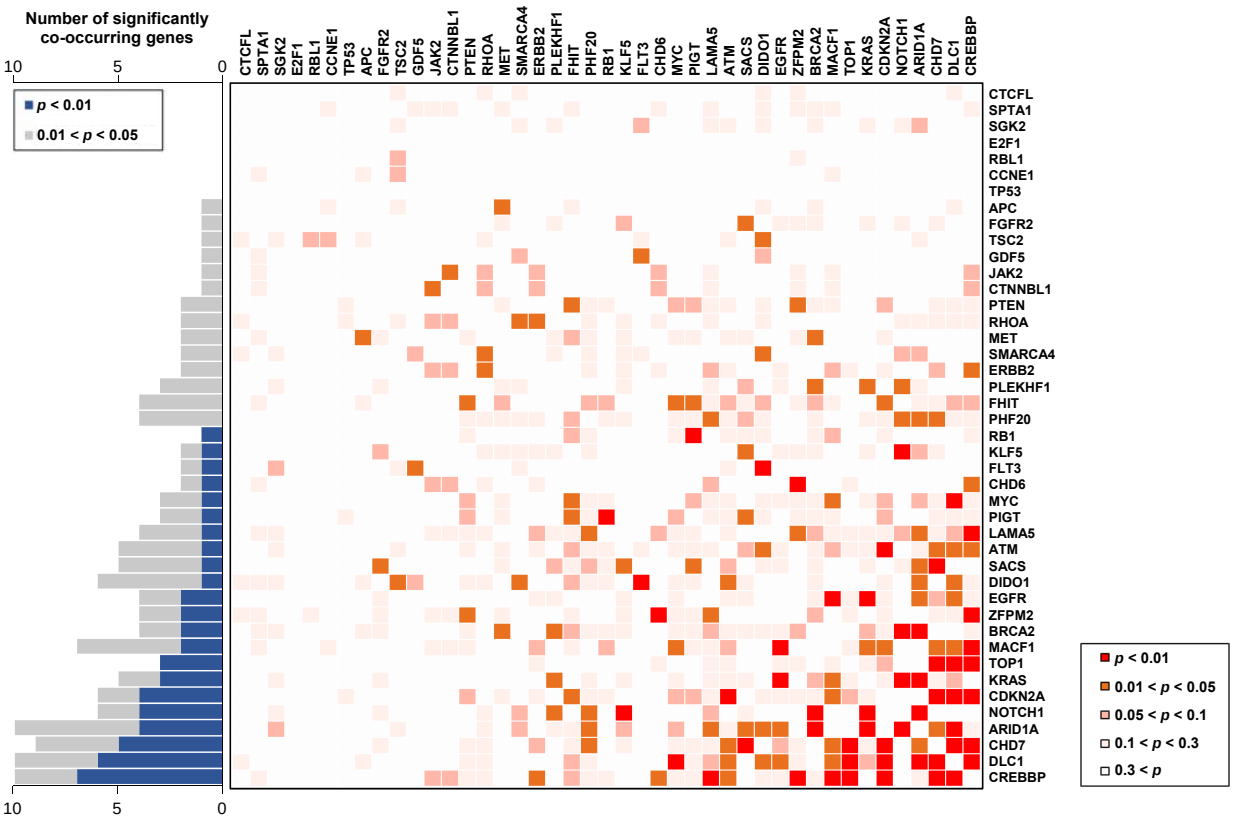


Fig. S6. Potential cooperative interactions of mutations in gastric cancer. The matrix displays possible tendency towards co-occurrence of somatic mutations. Colors represent *p*-values estimated by a Fisher's exact test. Left panel shows the number of significantly co-occurring genes for each gene.

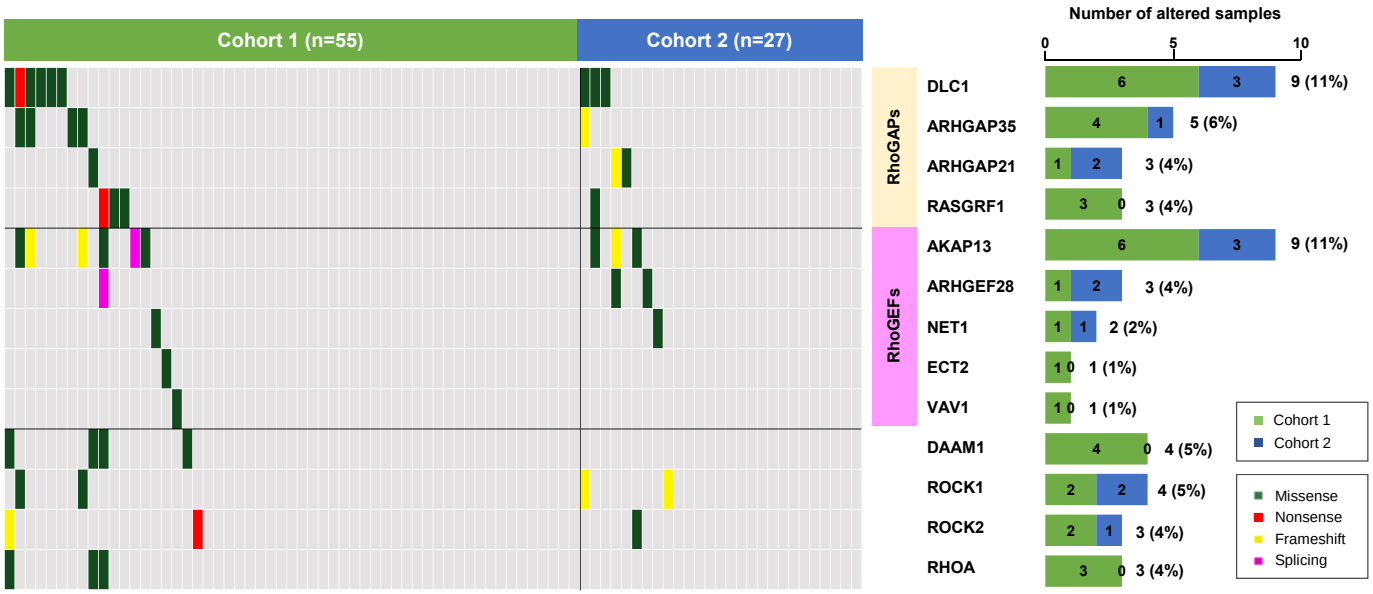


Fig. S7. Mutation profiles for genes closely linked to RhoA in total 82 gastric cancer (GC) samples. Cohort 1 comprises 55 GC samples in our study and cohort 2 is an additional cohort comprising 27 GC samples. The left matrix shows mutated genes colored by the type of mutation in the RhoA pathway (see key at right), and the right graph shows the number of samples with mutations for each gene in the RhoA pathway from the total of 82 patients in the two cohorts.

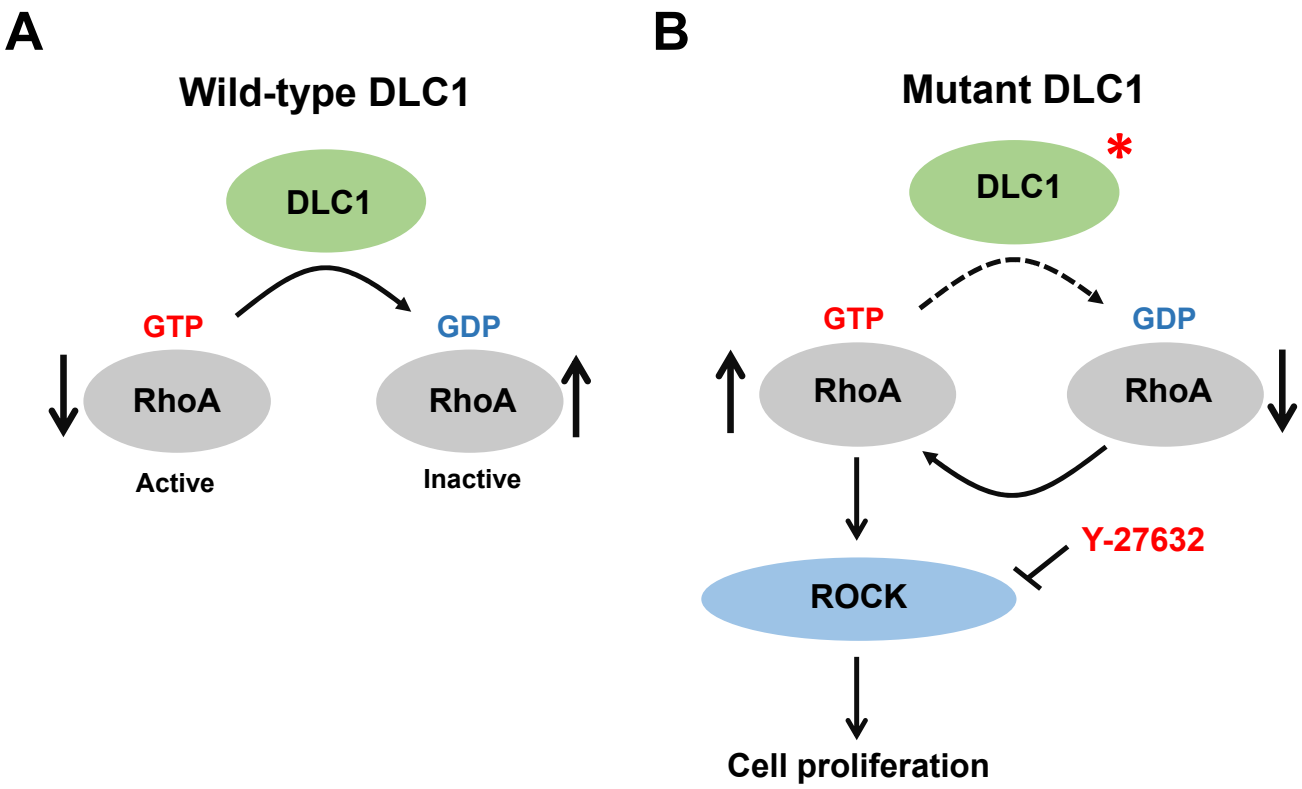


Fig. S8. Regulation of the Rho-ROCK signaling pathway by DLC1. (*A*) Wild-type DLC1 inhibits RhoA activity through its GTPase activating activity for RhoA. (*B*) Mutation of DLC1 decreases DLC1 protein stability, resulting in activation of the RhoA-ROCK signaling pathway. Y-27632 is a ROCK inhibitor.

Supplementary Tables

Table S1. Clinical information for the 103 gastric cancer patients in this study.

Table S2. Comparison of the clinical information of 103 gastric cancer patients in this study with 220 stomach adenocarcinoma cases in the TCGA database.

Table S3. Summary of droplet digital PCR (ddPCR) validation for 20 randomly chosen copy number altered genes among the 103 patients.

Table S4. Summary of FASTQ data quality for each normal and tumor sample from whole exome sequencing.

Table S5. Summary of on-target coverage quality for each normal and tumor sample from whole exome sequencing.

Table S6. Summary of somatic variants in the coding regions of 55 gastric cancer cases examined by exome sequencing in this study.

Table S7. Summary of Sanger sequencing validation for 73 randomly chosen variants from whole exome sequencing.

Table S8. Comparison of mutation signatures according to subtypes of gastric cancer.

Table S9. Summary of alteration profiles for eight known cancer-associated genetic driver genes among the 55 exome-sequenced gastric cancer cases in this study.

Table S10. RhoA mutation status according to Lauren histological type and differentiation status of gastric cancer.

Table S11. List of altered genes in gastric cancer with significant differences based on Lauren histology.

Table S12. Detailed summary of significantly altered KEGG pathways in 103 gastric cancers.

Table S13. Comparison of BCL2L1 amplifications estimated by droplet digital PCR (ddPCR) and array Comparative Genomic Hybridization (aCGH).

Table S14. Comparison of BCL2L1 amplification frequencies between Asian and non-Asian populations in TCGA stomach adenocarcinoma cases.

Table S15. Potential cooperative interactions of mutations in genes closely linked to RHOA from 82 gastric cancers (cohort 1 (n= 55) and cohort 2 (n= 27)).

Table S16. Predicted protein stability of DLC1 mutations using the i-Mutant database.

Table S17. Predicted functional impacts of DLC1 mutations using three bioinformatic algorithms.

Table S1. Clinical information for the 103 gastric cancer patients in this study.

Patient ID	Age (years)	Gender	Site of tumor	Diagnosis	WHO classification	Lauren classification	Cellularity	Stage (T)	Stage (N)	Stage (M)	TNM 7th stage	MSI status	Recurrence	Platform
S090	67	M	Circ	AGC	4	Diffuse	80%	T3	N2	M0	3A	MSS	No	WES+aCGH
S110	64	M	Circ	AGC	3	Intestinal	70%	T4	N3B	M0	3C	MSS	No	WES+aCGH
S113	52	M	GC	AGC	4	Diffuse	90%	T2	N0	M0	1B	MSI	No	WES+aCGH
S120	54	M	LC	AGC	3	Intestinal	90%	T3	N3A	M1	4	MSS	Initial Metastasis	WES+aCGH
S130	57	M	Circ	AGC	4	Intestinal	80%	T3	N1	M0	2B	MSS	Yes	WES+aCGH
S134	37	M	Circ	AGC	4	Intestinal	80%	T3	N3A	M0	3B	MSS	No	WES+aCGH
S137	59	F	Circ	AGC	4	Intestinal	70%	T3	N1	M0	2B	MSS	No	WES+aCGH
S160	73	M	LC	AGC	2	Intestinal	70%	T2	N3A	M0	3A	MSS	No	WES+aCGH
S166	68	F	AW	AGC	3	Intestinal	80%	T3	N3A	M0	3B	MSS	No	WES+aCGH
S195	67	M	Circ	AGC	2	Intestinal	70%	T3	N2	M0	3A	MSS	No	WES+aCGH
S332	64	M	PW	AGC	3	Intestinal	70%	T3	N2	M0	3A	MSI	Yes	WES+aCGH
S334	56	F	Circ	AGC	6	Diffuse	70%	T4	N3B	M1	4	MSI	Initial Metastasis	WES+aCGH
S353	51	M	LC	AGC	4	Diffuse	80%	T4	N3A	M0	3C	MSS	No	WES+aCGH
S354	48	M	GC	AGC	3	Intestinal	70%	T4	N3A	M0	3C	MSS	Yes	WES+aCGH
S357	62	M	LC	AGC	4	Mixed	90%	T3	N3B	M0	3B	MSS	Yes	WES+aCGH
S358	68	M	Circ	AGC	3	Intestinal	80%	T4	N3B	M1	4	MSI	Initial Metastasis	WES+aCGH
S381	55	M	PW	AGC	3	Intestinal	70%	T4	N3B	M1	4	MSS	Initial Metastasis	WES+aCGH
S384	68	M	LC	AGC	3	Intestinal	70%	T3	N3B	M0	3B	MSS	Yes	WES+aCGH
S395	77	M	GC	AGC	3	Intestinal	80%	T3	N3B	M0	3B	MSI	No	WES+aCGH
S399	77	M	LC	AGC	4	Intestinal	70%	T2	N3A	M0	3A	MSS	No	WES+aCGH
S413	70	M	LC	AGC	3	Intestinal	70%	T3	N3A	M0	3B	MSS	No	WES+aCGH
S415	66	M	LC	AGC	3	Intestinal	70%	T3	N2	M1	4	MSI	Initial Metastasis	WES+aCGH
S419	78	F	LC	AGC	4	Diffuse	70%	T3	N0	M0	2A	MSI-H	No	WES+aCGH
S427	72	M	LC	AGC	3	Intestinal	90%	T3	N3B	M1	4	MSS	Initial Metastasis	WES+aCGH
S434	46	M	Circ	AGC	4	Diffuse	90%	T4	N3B	M0	3C	MSS	Yes	WES+aCGH
S451	66	M	LC	AGC	6	Diffuse	80%	T3	N0	M0	2A	MSS	Yes	WES+aCGH
S452	65	M	LC	AGC	3	Intestinal	80%	T3	N2	M0	3A	MSS	No	WES+aCGH

S466	86	F	LC	AGC	4	Diffuse	90%	T3	N0	M0	2A	MSI-H	No	WES+aCGH
S477	82	M	LC	AGC	Unknown	Intestinal	70%	T4	N3A	M0	3C	MSI-H	No	WES+aCGH
S487	48	F	GC	AGC	6	Diffuse	90%	T4	N0	M0	2B	MSS	Yes	WES+aCGH
S496	68	F	LC	Unknown	4	Diffuse	90%	T3	N0	M0	2A	MSI-H	No	WES+aCGH
S500	65	M	LC	AGC	4	Intestinal	90%	T3	N2	M0	3A	MSS	No	WES+aCGH
S529	57	F	LC	AGC	2	Intestinal	80%	T3	N3A	M1	4	MSI-H	Initial Metastasis	WES+aCGH
S533	66	M	LC	AGC	3	Intestinal	70%	T3	N0	M0	2A	MSS	No	WES+aCGH
S538	66	M	LC	AGC	5	Diffuse	80%	T3	N3B	M0	3B	MSS	No	WES+aCGH
S554	69	F	GC	AGC	3	Intestinal	70%	T3	N2	M0	3A	MSS	Yes	WES+aCGH
S566	61	F	LC	AGC	3	Diffuse	80%	T2	N0	M0	1B	MSI-H	No	WES+aCGH
S567	66	M	PW	AGC	3	Intestinal	90%	T3	N2	M0	3A	MSS	Yes	WES+aCGH
S570	85	M	Circ	AGC	5	Diffuse	80%	T4	N3A	M1	4	MSS	Initial Metastasis	WES+aCGH
S573	70	M	AW	AGC	2	Intestinal	70%	T3	N3B	M1	4	MSS	Initial Metastasis	WES+aCGH
S673	65	M	PW	AGC	3	Intestinal	80%	T2	N1	M0	2A	MSS	No	WES+aCGH
S689	74	F	PW	AGC	3	Intestinal	90%	T3	N2	M0	3A	MSS	No	WES+aCGH
S703	71	M	AW	AGC	3	Intestinal	70%	T3	N3A	M0	3B	MSI	No	WES+aCGH
S711	72	M	LC	AGC	3	Intestinal	80%	T4	N3B	M0	3C	MSS	yes	WES+aCGH
S714	60	M	LC	AGC	4	Diffuse	80%	T4	N2	M0	3B	MSS	No	WES+aCGH
S715	55	M	PW	AGC	1	Intestinal	70%	T4	N2	M0	3B	MSS	No	WES+aCGH
S716	73	M	LC	AGC	3	Intestinal	90%	T3	N2	M0	3A	MSS	No	WES+aCGH
S728	57	M	LC	AGC	2	Intestinal	90%	T3	N2	M1	4	MSS	Initial Metastasis	WES+aCGH
S747	53	M	LC	AGC	3	Intestinal	70%	T4	N3A	M0	3C	MSS	No	WES+aCGH
S753	46	M	GC	AGC	4	Diffuse	70%	T4	N0	M0	2B	MSS	No	WES+aCGH
S796	65	F	GC	AGC	3	Mixed	90%	T3	N1	M0	2B	MSI-H	No	WES+aCGH
S797	85	M	LC	AGC	3	Diffuse	80%	T4	N3A	M0	3C	MSI-H	Yes	WES+aCGH
S801	73	M	GC	AGC	3	Intestinal	90%	T4	N2	M0	3B	MSS	Yes	WES+aCGH
S805	67	M	LC	AGC	7	Intestinal	80%	T4	N1	M0	3A	MSS	Yes	WES+aCGH
S813	85	F	LC	AGC	4	Intestinal	70%	T3	N2	M0	3A	MSI-H	No	WES+aCGH
S003	56	F	LC	AGC	4	Diffuse	80%	T3	N3A	M0	3B	MSI	Yes	aCGH
S086	48	F	LC	AGC	4	Diffuse	90%	T4	N3B	M1	4	MSS	Initial Metastasis	aCGH
S088	60	M	AW	AGC	3	Intestinal	70%	T4	N0	M0	2B	MSS	No	aCGH

S091	81	M	LC	AGC	6	Diffuse	80%	T3	N2	M0	3A	MSS	No	aCGH
S171	51	M	GC	AGC	4	Diffuse	70%	T3	N3B	M0	3B	MSS	Yes	aCGH
S178	54	M	LC	AGC	5	Diffuse	70%	T4	N3B	M0	3C	MSS	Yes	aCGH
S190	64	M	GC	AGC	2	Intestinal	70%	T3	N0	M0	2A	MSS	No	aCGH
S196	43	M	LC	AGC	4	Diffuse	70%	T4	N3A	M1	4	MSS	Initial Metastasis	aCGH
S197	56	F	LC	AGC	2	Intestinal	70%	T1	N0	M0	1A	MSS	No	aCGH
S203	86	F	Circ	AGC	4	Diffuse	70%	T3	N3B	M0	3B	MSS	Yes	aCGH
S347	69	F	LC	AGC	3	Intestinal	80%	T1	N0	M0	1A	MSS	No	aCGH
S352	78	M	LC	AGC	4	Mixed	80%	T3	N3B	M0	3B	MSS	Yes	aCGH
S370	67	M	LC	AGC	4	Mixed	70%	T3	N3A	M1	4	MSS	Initial Metastasis	aCGH
S373	61	M	LC	AGC	3	Intestinal	90%	T3	N0	M0	2A	MSS	No	aCGH
S416	74	M	LC	AGC	4	Diffuse	70%	T3	N3A	M1	4	MSS	Initial Metastasis	aCGH
S428	55	F	LC	AGC	6	Diffuse	90%	T3	N3A	M0	3B	MSS	No	aCGH
S439	48	M	LC	AGC	3	Intestinal	80%	T2	N2	M0	2B	MSS	No	aCGH
S440	70	F	LC	AGC	3	Intestinal	90%	T3	N0	M0	2A	MSS	No	aCGH
S441	78	M	LC	AGC	3	Intestinal	70%	T2	N1	M0	2A	MSS	No	aCGH
S443	80	M	LC	AGC	3	Intestinal	80%	T1	N0	M0	1A	MSI-H	No	aCGH
S445	65	M	LC	AGC	3	Intestinal	80%	T1	N0	M0	1A	MSI-H	Yes	aCGH
S454	84	M	LC	AGC	3	Mixed	70%	T3	N2	M0	3A	MSS	No	aCGH
S470	52	F	GC	AGC	3	Intestinal	70%	T3	N1	M0	2B	MSS	No	aCGH
S483	75	M	LC	AGC	3	Intestinal	80%	T3	N0	M0	2A	MSI-H	No	aCGH
S490	40	M	AW	AGC	4	Diffuse	80%	T3	N3B	M1	4	MSS	Initial Metastasis	aCGH
S495	61	M	LC	AGC	3	Intestinal	70%	T2	N0	M0	1B	MSS	Yes	aCGH
S526	68	M	Circ	AGC	5	Diffuse	80%	T4	N0	M0	2B	MSS	No	aCGH
S528	74	M	AW	AGC	3	Intestinal	70%	T3	N1	M0	2B	MSI-H	No	aCGH
S532	62	M	LC	AGC	3	Intestinal	90%	T2	N0	M0	1B	MSI-H	No	aCGH
S577	73	M	GC	AGC	4	Diffuse	70%	T3	N2	M0	3A	MSS	No	aCGH
S581	57	M	PW	AGC	5	Intestinal	90%	T2	N1	M0	2A	MSI-H	No	aCGH
S656	68	M	AW	AGC	4	Unknown	70%	T2	N0	M0	1B	MSI-H	No	aCGH
S674	62	M	GC	AGC	3	Intestinal	80%	T2	N0	M0	1B	MSS	No	aCGH
S692	68	F	LC	EGC	3	Intestinal	80%	T1	N0	M0	1A	MSI-H	No	aCGH

S694	75	F	AW	EGC	3	Intestinal	80%	T1	N1	M0	1B	MSS	No	aCGH
S696	66	M	PW	AGC	4	Diffuse	80%	T3	N3A	M0	3B	MSS	Yes	aCGH
S699	26	F	LC	AGC	4	Diffuse	70%	T4	N3B	M1	4	MSS	Initial Metastasis	aCGH
S733	67	M	AW	AGC	2	Intestinal	80%	T1	N0	M0	1A	MSI	No	aCGH
S734	46	F	AW	AGC	2	Intestinal	80%	T3	N0	M0	2A	MSS	No	aCGH
S739	75	F	LC	AGC	4	Diffuse	90%	T4	N3B	M1	4	MSS	Initial Metastasis	aCGH
S755	52	M	GC	AGC	3	Intestinal	70%	T2	N0	M0	1B	MSS	No	aCGH
S756	46	M	Circ	AGC	3	Intestinal	90%	T2	N0	M0	1B	MSI-H	No	aCGH
S762	66	F	Circ	AGC	6	Diffuse	90%	T4	N3A	M0	3C	MSS	no	aCGH
S773	57	M	LC	AGC	5	Diffuse	80%	T4	N2	M0	3B	MSS	No	aCGH
S798	75	M	Circ	AGC	4	Diffuse	80%	T2	N2	M1	4	MSS	Initial Metastasis	aCGH
S808	42	M	Circ	AGC	6	Diffuse	80%	T4	N3A	M0	3C	MSS	No	aCGH
S815	59	M	GC	AGC	4	Diffuse	90%	T4	N3A	M0	4	MSS	Yes	aCGH
S824	58	M	LC	AGC	4	Intestinal	80%	T3	N1	M0	2B	MSS	No	aCGH

* F: Female, M: Male

* AW: Anterior wall, PW: Posterior wall, LC: Lesser curvature, GC: Greater curvature, Circ: Circular

* AGC: Advanced gastric cancer, EGC: Early gastric cancer

* MSS: Microsatellite stable, MSI: Microsatellite instable, MSI-H: Microsatellite instable-high

* WES: Whole exome sequencing, aCGH: Array comparative genomic hybridization

Table S2. Comparison of the clinical information of 103 gastric cancer patients in this study with 220 stomach adenocarcinoma cases in the TCGA database.

Variables	Korean (n = 103)		TCGA (n = 220)		*p*-value[#]
	No.	%	No.	%	
Age, years					0.1420
< 50	13	12.6%	13	5.9%	
≥ 50, < 60	22	21.4%	50	22.7%	
≥ 60, < 70	37	35.9%	62	28.2%	
≥ 70, < 80	22	21.4%	62	28.2%	
≥ 80	9	8.7%	25	11.4%	
N/A	0	0.0%	8	3.6%	
Gender					0.0185*
Male	76	73.8%	128	58.2%	
Female	27	26.2%	87	39.5%	
N/A	0	0.0%	5	2.3%	
Race					< 0.001***
Asian	103	100.0%	43	19.5%	
Non-Asian	0	0.0%	131	59.6%	
N/A	0	0.0%	46	20.9%	
Pathology					0.1210
Diffuse	36	35.0%	51	23.2%	
Intestinal	61	59.2%	145	65.9%	
Mixed	5	4.9%	14	6.4%	
N/A	1	0.9%	10	4.5%	
Tumor stage					0.1419
Ⅰ	15	14.6%	29	13.2%	
Ⅱ	24	23.3%	70	31.8%	
Ⅲ	45	43.7%	77	35.0%	
Ⅳ	19	18.4%	24	10.9%	
N/A	0	0.0%	20	9.1%	
T stage					0.1212
T1	7	6.8%	6	2.7%	
T2	15	14.5%	63	28.6%	
T3	52	50.5%	90	40.9%	
T4	29	28.2%	47	21.4%	
TX	0	0.0%	14	6.4%	
N stage					< 0.001***
N0	28	27.2%	69	31.4%	
N1	11	10.7%	63	28.6%	
N2	21	20.4%	33	15.0%	
N3	43	41.7%	38	17.3%	
NX	0	0.0%	17	7.7%	
MSI status					0.0733
MSS	76	73.8%	135	61.4%	
MSI	9	8.7%	35	15.9%	
MSI-H	18	17.5%	50	22.7%	

* MSS: Microsatellite stable, MSI: Microsatellite instable, MSI-H: Microsatellite instable-high

Calculated by Pearson’s chi-square test (*: $p < 0.05$, ***: $p < 0.001$)

Table S3. Summary of droplet digital PCR (ddPCR) validation for 20 randomly chosen copy number altered genes among the 103 patients.

Gene	Probe ID	Genomic position (Chromosome number: start nucleotide-end nucleotide, hg19)	No. of tested samples	Validation rate (%)
RPRD2	Hs05734909_cn	1:150,341,703-150,341,809	3	100.0 (3/3)
PRPF3	Hs06581144_cn	1:150,300,067-150,300,157	4	75.0 (3/4)
MYC	Hs03660964_cn	8:128,748,315-128,753,680	16	100.0 (16/16)
CWF19L2	Hs06263469_cn	11:107,197,071-107,328,572	2	100.0 (2/2)
KLF12	Hs06393785_cn	13:74,260,149-74,708,066	10	60.0 (6/10)
ERBB2	Hs05499477_cn	17:37,852,477-37,852,552	11	81.8 (9/11)
GRB7	Hs05479918_cn	17:37,895,348-37,895,445	11	81.8 (9/11)
GSDMB	Hs05512397_cn	17:38,064,326-38,064,426	7	71.4 (5/7)
ORMDL3	Hs06432347_cn	17:38,082,104-38,082,204	6	66.7(4/6)
PPP1R1B	Hs05522423_cn	17:37,786,435-37,786,526	9	77.8 (7/9)
STARD3	Hs05516905_cn	17:37,811,132-37,811,217	12	75.0 (9/12)
ZPBP2	Hs05501179_cn	17:38,029,995-38,030,097	9	77.8 (7/9)
ROCK1	Hs06489567_cn	18:18,616,283-18,616,353	8	62.5 (5/8)
BCL2L1	Hs07224221_cn	20:30,255,626-30,255,733	11	81.8 (9/11)
COX4I2	Hs07180784_cn	20:30,228,541-30,228,628	8	87.5 (7/8)
HM13	Hs07215941_cn	20:30,140,159-30,140,246	9	77.8 (7/9)
B4GALT5	Hs07204471_cn	20:48,258,005-48,258,101	11	81.8 (9/11)
KCNB1	Hs07173996_cn	20:48,038,610-48,038,718	9	77.8 (7/9)
PTGIS	Hs07176147_cn	20:48,125,120-48,125,212	10	70.0 (7/10)
SPATA2	Hs07200145_cn	20:48,523,978-48,524,064	12	75.0 (9/12)
Total			178	78.7 (140/178)

Table S4. Summary of FASTQ data quality for each normal and tumor sample from whole exome sequencing.

Sample ID	Total bases	Read count	GC (%)	Q20 (%)	Q30 (%)
S090N	10,780,080,672	106,733,472	45.3	95.7	90.1
S110N	9,371,905,746	92,791,146	56.5	94.4	88.4
S113N	9,582,170,172	94,872,972	45.3	92.8	85.4
S120N	10,673,050,972	105,673,772	47.7	97.2	93.7
S130N	3,047,065,364	30,168,964	48.4	97.0	92.3
S134N	8,740,276,996	86,537,396	45.4	92.8	85.3
S137N	16,198,982,970	160,385,970	45.1	93.2	86.0
S160N	10,882,270,250	107,745,250	50.0	95.6	90.3
S166N	10,852,271,432	107,448,232	54.4	93.1	86.9
S195N	9,989,564,176	98,906,576	49.7	95.9	91.0
S332N	8,829,996,710	87,425,710	47.3	96.4	92.1
S334N	10,517,734,990	104,135,990	49.4	95.7	90.5
S353N	19,076,357,224	188,874,824	44.7	93.3	86.2
S354N	10,026,036,084	99,267,684	47.7	96.6	92.2
S357N	3,133,362,390	31,023,390	48.6	96.7	91.9
S358N	8,674,633,864	85,887,464	47.9	96.8	92.6
S381N	9,113,509,164	90,232,764	47.7	97.4	94.0
S384N	9,442,460,508	93,489,708	45.4	92.9	85.5
S395N	17,184,114,548	170,139,748	45.1	93.3	86.1
S399N	8,794,381,080	87,073,080	47.4	96.0	91.2
S413N	10,554,144,682	104,496,482	47.1	96.2	91.7
S415N	11,670,647,364	115,550,964	47.9	96.6	92.2
S419N	7,565,950,804	74,910,404	47.4	97.1	93.6
S427N	11,202,689,114	110,917,714	48.0	97.2	93.6
S434N	11,477,127,324	113,634,924	44.9	93.0	85.6
S451N	10,740,265,866	106,339,266	45.3	95.7	90.2
S452N	12,905,799,998	127,780,198	46.8	96.5	92.1

S466N	10,949,120,938	108,407,138	47.9	96.5	91.8
S477N	10,888,312,070	107,805,070	53.1	94.7	88.8
S487N	10,020,853,774	99,216,374	44.0	93.0	85.7
S496N	11,060,844,512	109,513,312	53.8	94.4	88.1
S500N	9,235,446,868	91,440,068	44.7	95.8	90.3
S529N	9,542,642,206	94,481,606	44.2	93.0	85.5
S533N	8,444,258,722	83,606,522	43.7	93.3	86.1
S538N	4,074,520,184	40,341,784	48.1	95.7	88.8
S554N	9,274,307,224	91,824,824	47.6	96.8	92.6
S566N	11,015,671,858	109,066,058	47.5	96.8	92.5
S567N	10,193,149,270	100,922,270	45.1	95.8	90.3
S570N	10,004,160,898	99,051,098	44.6	95.7	90.1
S573N	9,418,880,240	93,256,240	43.9	93.3	86.0
S673N	3,443,737,814	34,096,414	48.5	96.8	92.0
S689N	11,208,599,028	110,976,228	47.8	96.8	92.6
S703N	12,808,796,972	126,819,772	47.7	96.0	91.1
S711N	9,873,881,402	97,761,202	47.3	96.7	92.4
S714N	8,011,731,272	79,324,072	44.1	93.1	85.8
S715N	10,781,802,318	106,750,518	47.9	96.0	91.1
S716N	11,997,374,890	118,785,890	47.4	96.5	91.9
S728N	9,003,926,992	89,147,792	48.1	96.8	92.5
S747N	10,696,154,924	105,902,524	46.5	96.8	92.5
S753N	3,051,652,986	30,214,386	47.8	97.0	92.4
S796N	9,735,328,794	96,389,394	48.1	96.0	90.9
S797N	11,461,414,956	113,479,356	48.0	95.9	90.7
S801N	11,629,236,556	115,140,956	47.8	96.0	91.1
S805N	12,562,332,126	124,379,526	47.5	96.4	91.6
S813N	21,841,689,552	216,254,352	44.6	93.3	86.0
S090T	8,591,437,336	85,063,736	45.2	95.7	90.2

S110T	9,784,714,360	96,878,360	47.6	96.7	92.4
S113T	9,223,951,654	91,326,254	46.0	92.8	85.3
S120T	9,936,722,188	98,383,388	47.8	96.7	92.5
S130T	9,777,489,022	96,806,822	47.6	92.0	84.1
S134T	9,380,220,066	92,873,466	48.6	91.7	83.7
S137T	3,095,651,010	30,650,010	48.4	97.0	92.3
S160T	8,922,800,560	88,344,560	47.7	96.0	91.0
S166T	11,050,581,498	109,411,698	48.0	96.7	92.4
S195T	10,813,506,016	107,064,416	47.8	95.8	90.8
S332T	10,311,661,256	102,095,656	47.7	96.6	92.1
S334T	10,746,832,886	106,404,286	55.6	94.4	88.2
S353T	18,898,926,282	187,118,082	44.7	93.2	86.0
S354T	8,115,141,132	80,347,932	47.8	96.7	92.4
S357T	3,037,137,266	30,070,666	49.3	96.6	91.7
S358T	9,347,100,954	92,545,554	47.6	96.9	92.7
S381T	10,812,249,374	107,051,974	47.9	96.7	92.1
S384T	8,498,256,150	84,141,150	45.4	92.8	85.4
S395T	13,333,270,580	132,012,580	45.1	93.2	85.8
S399T	9,784,682,646	96,878,046	47.4	96.5	92.1
S413T	9,354,199,234	92,615,834	49.7	95.7	90.8
S415T	12,417,728,608	122,947,808	50.5	95.8	90.8
S419T	8,619,717,740	85,343,740	48.9	96.5	92.1
S427T	10,306,390,066	102,043,466	53.3	95.4	89.9
S434T	25,889,023,566	256,326,966	44.8	93.2	85.8
S451T	10,355,931,576	102,533,976	45.2	95.7	90.2
S452T	11,967,104,786	118,486,186	47.5	96.7	92.4
S466T	10,800,969,896	106,940,296	47.9	96.4	91.6
S477T	8,057,127,338	79,773,538	46.9	96.7	92.3
S487T	8,792,517,428	87,054,628	44.0	93.1	85.8

S496T	11,900,817,678	117,829,878	47.8	96.4	91.6
S500T	10,180,950,288	100,801,488	45.1	95.8	90.3
S529T	3,555,159,196	35,199,596	48.4	97.0	92.4
S533T	10,043,117,810	99,436,810	45.3	95.7	90.1
S538T	3,101,872,812	30,711,612	48.3	95.6	88.7
S554T	11,675,494,354	115,598,954	47.6	96.5	91.8
S566T	8,592,654,992	85,075,792	50.4	96.9	93.1
S567T	3,178,808,148	31,473,348	48.7	96.7	91.8
S570T	3,384,022,372	33,505,172	49.1	96.5	91.5
S573T	2,928,296,838	28,993,038	48.6	96.7	92.0
S673T	3,164,033,464	31,327,064	47.9	96.8	92.1
S689T	10,126,559,162	100,262,962	47.7	95.7	90.5
S703T	8,797,096,364	87,099,964	47.9	97.1	93.5
S711T	10,922,551,474	108,144,074	47.9	96.0	91.0
S714T	10,642,902,674	105,375,274	44.4	93.1	85.8
S715T	11,795,486,798	116,786,998	47.4	96.0	90.9
S716T	10,436,266,168	103,329,368	47.8	96.9	92.7
S728T	10,709,549,746	106,035,146	47.7	96.8	92.4
S747T	10,651,180,634	105,457,234	47.9	96.7	92.4
S753T	3,564,958,418	35,296,618	49.0	96.7	91.9
S796T	11,038,171,022	109,288,822	46.8	96.5	91.9
S797T	8,853,068,342	87,654,142	48.1	96.7	92.2
S801T	9,923,520,478	98,252,678	47.8	95.7	90.5
S805T	9,284,920,102	91,929,902	48.2	96.5	91.8
S813T	8,524,982,366	84,405,766	44.6	93.2	85.9

* Software: Illumina Pipeline (CASAVA) v1.8.2

* Fastq Quality Encoding: Sanger Quality (ASCII Character Code = Phred Quality Value + 33)

* N: Normal, T: Tumor

Table S5. Summary of on-target coverage quality for each normal and tumor sample from whole exome sequencing.

Sample ID	Read mapping stat	Total base depth	Mean coverage	On-target coverage			
				8X≥bases (%)	15X≥bases (%)	30X≥bases (%)	50X≥bases (%)
S090N	49,828,370 (99.89%)	3,897,272,231	77.4	94.2	89.6	77.1	59.4
S113N	46,440,447 (99.78%)	3,504,585,237	69.6	93.8	88.6	74.4	55.3
S130N	22,651,565 (99.94%)	1,851,516,354	36.8	88.6	79.2	55.3	27.4
S134N	42,753,246 (99.77%)	3,225,117,884	64.0	93.3	87.4	71.8	51.5
S137N	66,883,901 (99.78%)	5,071,818,811	100.7	95.3	92.3	83.6	69.9
S353N	74,797,270 (99.76%)	5,678,681,534	112.8	95.7	93.0	85.3	73.1
S357N	22,243,733 (99.91%)	1,809,950,398	35.9	88.7	78.9	54.0	26.0
S384N	46,424,747 (99.76%)	3,515,075,570	69.8	93.5	88.2	74.2	55.2
S395N	70,503,256 (99.81%)	5,357,231,426	106.4	95.5	92.6	84.3	71.2
S434N	53,141,067 (99.74%)	4,037,117,389	80.2	93.9	89.3	77.0	59.8
S451N	50,322,909 (99.90%)	3,933,475,782	78.1	94.3	89.7	77.4	60.0
S487N	45,463,826 (99.74%)	3,431,671,771	68.1	93.2	87.2	71.9	52.4
S500N	42,458,709 (99.87%)	3,315,280,261	65.8	93.1	86.9	71.2	51.4
S529N	43,572,066 (99.73%)	3,294,771,294	65.4	92.8	86.6	70.8	51.0
S533N	33,976,390 (99.74%)	2,566,621,025	51.0	91.4	82.7	62.1	39.6
S538N	29,454,605 (99.94%)	2,369,509,577	47.1	91.3	84.8	66.8	41.5
S567N	47,561,582 (99.91%)	3,713,342,775	73.7	94.0	89.0	75.7	57.3
S570N	46,787,699 (99.91%)	3,635,881,292	72.2	93.8	88.5	74.5	55.8
S573N	40,953,123 (99.76%)	3,103,602,589	61.6	92.3	85.5	68.5	47.9
S673N	25,152,663 (99.93%)	2,043,833,350	40.6	90.1	82.0	60.4	32.8
S714N	35,367,738 (99.73%)	2,674,574,716	53.1	91.4	83.0	63.2	41.3

S753N	21,960,028 (99.94%)	1,791,758,269	35.6	88.3	78.6	54.0	25.7
S813N	90,702,281 (99.74%)	6,889,992,105	136.8	95.8	93.7	88.0	78.5
S110N	52,812,755 (99.70%)	4,289,554,897	85.2	82.2	73.9	62.4	51.7
S120N	63,584,181 (99.88%)	5,214,250,908	103.5	98.8	97.8	93.6	82.5
S160N	69,213,879 (99.85%)	5,587,130,627	110.9	99.0	98.2	94.2	83.7
S166N	35,165,223 (99.62%)	2,326,286,194	46.2	89.0	73.4	43.5	24.1
S195N	64,831,882 (99.86%)	5,312,349,261	105.5	98.8	97.8	92.9	81.2
S332N	54,651,490 (99.78%)	4,433,875,207	88.0	98.6	97.2	91.2	76.5
S334N	70,826,508 (99.82%)	5,763,688,068	114.5	98.2	96.9	92.4	82.8
S354N	69,825,081 (99.88%)	5,729,968,765	113.8	98.7	97.7	93.6	83.4
S358N	56,767,283 (99.88%)	4,638,924,564	92.1	98.7	97.3	91.3	76.7
S381N	60,030,157 (99.90%)	4,990,435,753	99.1	98.5	97.2	91.4	78.2
S399N	63,019,128 (99.85%)	5,099,760,025	101.3	98.5	97.1	91.6	78.9
S413N	52,393,264 (99.87%)	4,274,440,548	84.9	98.5	96.9	89.4	72.9
S415N	75,087,848 (99.88%)	6,101,945,961	121.2	99.0	98.3	95.2	86.8
S419N	47,704,639 (99.90%)	3,863,360,629	76.7	98.5	96.8	88.5	69.2
S427N	72,321,211 (99.91%)	5,917,464,670	117.5	98.8	98.1	94.8	85.9
S452N	77,429,999 (99.77%)	6,284,645,935	124.8	98.8	98.0	95.0	87.2
S466N	75,231,927 (99.90%)	6,148,306,940	122.1	98.8	98.0	94.8	86.3
S477N	70,161,497 (99.77%)	5,764,312,711	114.5	98.6	96.9	89.9	76.9
S496N	70,020,619 (99.79%)	5,772,032,827	114.6	98.6	97.0	90.1	77.0
S554N	61,880,181 (99.88%)	5,039,458,416	100.1	98.7	97.6	92.9	80.5
S566N	71,077,477 (99.89%)	5,765,711,572	114.5	98.8	98.0	94.7	85.6
S689N	70,503,469 (99.87%)	5,750,688,411	114.2	98.8	98.1	94.9	86.0
S703N	86,606,479 (99.86%)	7,001,117,192	139.0	99.0	98.3	95.8	89.4

S711N	69,612,988 (99.84%)	5,686,301,965	112.9	98.6	97.5	93.0	82.3
S715N	75,692,486 (99.86%)	6,147,976,484	122.1	98.8	98.0	94.5	85.9
S716N	68,567,733 (99.93%)	5,561,250,119	110.4	98.8	97.9	93.7	83.2
S728N	60,788,226 (99.89%)	4,973,237,367	98.8	98.7	97.6	92.4	79.4
S747N	59,096,325 (99.88%)	4,813,097,236	95.6	98.7	97.5	92.0	78.5
S796N	66,558,124 (99.88%)	5,505,379,596	109.3	98.5	97.4	92.8	81.9
S797N	80,153,159 (99.88%)	6,612,579,361	131.3	98.7	97.9	94.6	86.7
S801N	81,556,482 (99.85%)	6,629,860,604	131.7	98.9	98.1	95.1	87.6
S805N	76,391,016 (99.86%)	6,226,072,343	123.6	98.9	98.2	95.3	87.9
N average	57,799,344 (99.84%)	4,617,057,214	91.7	95.8	92.3	82.5	67.7
S090T	40,264,616 (99.88%)	3,145,751,673	62.5	92.8	86.4	69.9	49.3
S113T	45,905,957 (99.78%)	3,474,896,163	69.0	93.6	88.4	74.2	54.9
S130T	48,665,101 (99.76%)	3,682,396,858	73.1	94.1	89.4	76.2	57.6
S134T	47,871,854 (99.76%)	3,622,207,228	71.9	93.8	88.5	74.1	54.7
S137T	22,978,197 (99.93%)	1,870,049,012	37.1	89.1	80.0	56.1	27.6
S353T	75,414,110 (99.76%)	5,723,615,412	113.7	95.5	92.6	84.4	71.7
S357T	22,052,924 (99.94%)	1,793,290,845	35.6	88.0	77.3	51.5	24.7
S384T	41,441,140 (99.77%)	3,135,210,474	62.3	92.7	86.3	69.8	49.1
S395T	62,228,419 (99.79%)	4,738,152,963	94.1	94.6	90.9	80.8	65.9
S434T	109,338,463 (99.73%)	8,338,479,920	165.6	96.0	94.3	89.7	82.1
S451T	48,188,374 (99.88%)	3,760,672,354	74.7	94.0	89.1	75.9	57.6
S487T	40,214,816 (99.74%)	3,037,614,469	60.3	92.2	85.3	67.9	47.1
S500T	47,690,279 (99.90%)	3,728,392,901	74.0	93.9	88.8	75.3	57.0
S529T	25,056,823 (99.94%)	2,043,249,221	40.6	89.8	81.7	60.0	32.5
S533T	47,140,553 (99.90%)	3,676,395,653	73.0	93.6	88.2	73.8	54.8

S538T	23,240,861 (99.95%)	1,865,668,062	37.1	89.1	79.7	55.6	27.6
S567T	22,951,138 (99.93%)	1,869,514,598	37.1	87.8	77.4	52.6	26.5
S570T	24,768,651 (99.92%)	2,010,103,854	39.9	90.0	81.7	59.5	31.8
S573T	20,773,860 (99.93%)	1,692,769,409	33.6	87.4	76.1	48.9	21.8
S673T	22,517,508 (99.93%)	1,832,951,506	36.4	88.8	79.2	54.6	26.4
S714T	44,700,363 (99.73%)	3,384,909,764	67.2	93.3	87.5	72.2	52.4
S753T	26,583,929 (99.93%)	2,163,827,231	43.0	90.2	82.6	62.1	35.6
S813T	40,397,951 (99.75%)	3,068,045,090	60.9	92.3	85.7	69.0	48.2
S110T	67,775,303 (99.88%)	5,539,988,437	110.0	98.6	97.4	92.4	80.5
S120T	67,926,858 (99.87%)	5,634,884,839	111.9	98.6	97.5	92.7	81.5
S160T	62,491,958 (99.85%)	5,041,080,106	100.1	98.6	97.3	92.0	79.2
S166T	71,876,877 (99.89%)	5,866,439,624	116.5	98.7	97.6	92.9	81.7
S195T	72,519,539 (99.83%)	5,900,800,937	117.2	98.8	97.8	93.9	84.2
S332T	64,754,824 (99.89%)	5,254,793,077	104.3	98.7	97.5	92.1	79.1
S334T	66,151,629 (99.78%)	5,347,384,560	106.2	85.1	77.2	67.6	58.5
S354T	51,431,378 (99.90%)	4,184,985,072	83.1	98.4	96.4	87.4	68.6
S358T	62,229,920 (99.89%)	5,094,322,631	101.2	98.7	97.6	92.5	80.1
S381T	72,762,698 (99.86%)	6,080,751,847	120.7	98.6	97.5	93.0	82.9
S399T	61,817,370 (99.81%)	5,034,689,452	100.0	98.7	97.5	92.6	80.5
S413T	55,470,815 (99.81%)	4,534,491,971	90.0	98.5	96.7	88.7	72.4
S415T	76,427,130 (99.74%)	6,298,622,155	125.1	98.7	97.7	93.3	83.6
S419T	53,097,449 (99.81%)	4,389,866,740	87.2	97.4	94.6	86.6	72.3
S427T	69,420,337 (99.85%)	5,708,900,044	113.4	89.9	86.1	79.5	70.4
S452T	76,120,166 (99.88%)	6,199,199,657	123.1	98.9	98.0	94.4	85.3
S466T	69,911,759 (99.87%)	5,697,842,346	113.1	98.8	98.0	94.5	85.1

S477T	54,456,380 (99.87%)	4,436,953,293	88.1	98.5	97.0	90.2	74.5
S496T	75,968,523 (99.87%)	6,169,642,624	122.5	98.9	98.2	95.3	87.3
S554T	77,118,380 (99.88%)	6,291,227,394	124.9	98.8	98.1	95.0	86.9
S566T	56,840,332 (99.90%)	4,704,239,681	93.4	96.9	94.0	86.5	73.8
S689T	64,098,701 (99.76%)	5,246,851,318	104.2	98.5	97.4	92.8	81.8
S703T	58,595,750 (99.91%)	4,766,265,963	94.6	98.8	97.6	91.9	77.7
S711T	76,702,579 (99.85%)	6,379,405,860	126.7	98.6	97.6	93.8	85.1
S715T	69,020,957 (99.84%)	5,724,431,131	113.7	98.4	97.2	92.6	82.0
S716T	69,881,339 (99.89%)	5,727,012,449	113.7	98.8	97.9	94.1	84.3
S728T	68,471,626 (99.89%)	5,584,747,759	110.9	98.7	97.6	92.9	81.2
S747T	67,895,685 (99.88%)	5,541,168,586	110.0	98.9	98.0	94.1	83.8
S796T	73,774,241 (99.86%)	6,043,481,267	120.0	98.7	97.9	94.4	85.4
S797T	57,681,386 (99.90%)	4,666,577,859	92.7	98.8	97.6	91.9	77.6
S801T	69,130,268 (99.85%)	5,589,132,477	111.0	98.6	97.4	92.5	81.2
S805T	61,517,798 (99.87%)	5,058,428,199	100.4	98.6	97.2	91.2	77.5
T average	55,885,924 (99.85%)	4,498,123,164	89.3	95.4	91.6	81.3	65.5

* N: Normal, T: Tumor

Table S6. Summary of somatic variants in the coding regions of 55 gastric cancer cases examined by exome sequencing in this study.

MSI status	Patient ID	Splice SNVs	Missense SNVs	Nonsense SNVs	Total non-silent SNVs	Out-frame indel	In-frame indel	Total non-silent variants	Non-silent somatic variants per Mb	Synonymous mutations	Synonymous somatic variants per Mb
MSS	**S090**	30	664	25	719	16	2	737	14.74	268	5.36
	S110	4	134	9	147	0	0	147	2.94	41	0.82
	S120	2	75	5	82	1	1	84	1.68	24	0.48
	S130	1	84	2	87	0	0	87	1.74	41	0.82
	S134	1	69	3	73	0	2	75	1.50	22	0.44
	S137	15	551	29	595	142	8	745	14.90	255	5.10
	S160	2	168	11	181	0	1	182	3.64	63	1.26
	S166	2	132	10	144	1	2	147	2.94	81	1.62
	S195	2	95	3	100	0	0	100	2.00	39	0.78
	S353	1	55	5	61	0	0	61	1.22	23	0.46
	S354	3	118	5	126	2	1	129	2.58	37	0.74
	S357	0	58	3	61	2	1	64	1.28	19	0.38
	S381	3	173	8	184	1	2	187	3.74	63	1.26
	S384	1	109	10	120	1	4	125	2.50	25	0.50
	S399	2	140	12	154	1	2	157	3.14	64	1.28
	S413	0	123	6	129	1	4	134	2.68	52	1.04
	S427	2	295	7	304	7	8	319	6.38	151	3.02
	S434	4	85	6	95	0	0	95	1.90	28	0.56
	S451	8	202	13	223	0	1	224	4.48	64	1.28
	S452	2	61	2	65	2	0	67	1.34	27	0.54
	S487	0	4	1	5	0	0	5	0.10	2	0.04
	S500	0	5	0	5	0	0	5	0.10	3	0.06

	S533	0	57	4	61	2	2	65	1.30	24	0.48
	S538	0	2	0	2	0	0	2	0.04	1	0.02
	S554	2	76	7	85	1	1	87	1.74	27	0.54
	S567	2	82	5	89	2	0	91	1.82	31	0.62
	S570	0	27	1	28	0	0	28	0.56	12	0.24
	S573	0	62	4	66	2	0	68	1.36	27	0.54
	S673	0	20	0	20	0	1	21	0.42	11	0.22
	S689	4	212	6	222	0	0	222	4.44	68	1.36
	S711	1	72	7	80	2	1	83	1.66	30	0.60
	S714	0	29	3	32	0	1	33	0.66	14	0.28
	S715	2	106	3	111	0	2	113	2.26	35	0.70
	S716	1	52	3	56	0	0	56	1.12	26	0.52
	S728	1	41	2	44	4	0	48	0.96	12	0.24
	S747	1	31	3	35	0	1	36	0.72	6	0.12
	S753	1	78	8	87	0	0	87	1.74	25	0.50
	S801	1	84	2	87	0	1	88	1.76	28	0.56
	S805	10	242	17	269	5	4	278	5.56	105	2.10
Average		2.85	119.82	6.41	129.08	5.00	1.36	135.44	2.71	48.05	0.96
	S113	17	904	48	969	60	2	1031	20.62	415	8.30
	S332	1	66	5	72	0	2	74	1.48	26	0.52
	S334	1	34	4	39	5	1	45	0.90	16	0.32
MSI	**S358**	2	71	3	76	0	0	76	1.52	18	0.36
	S395	12	551	35	598	76	2	676	13.52	235	4.70
	S415	2	125	17	144	3	0	147	2.94	63	1.26
	S703	1	107	5	113	0	1	114	2.28	33	0.66
Average		5.14	265.43	16.71	287.29	20.57	1.14	309.00	6.18	115.14	2.30
	S419	9	689	37	735	86	8	829	16.58	323	6.46

MSI-H	**S466**	7	692	44	743	44	1	788	15.76	301	6.02
	S477	66	2674	125	2865	88	5	2958	59.16	1624	32.48
	S496	14	605	35	654	49	6	709	14.18	309	6.18
	S529	15	532	30	577	167	13	757	15.14	274	5.48
	S566	2	173	5	180	7	9	196	3.92	96	1.92
	S796	34	1035	46	1115	219	8	1342	26.84	464	9.28
	S797	17	639	30	686	2	2	690	13.80	258	5.16
	S813	17	490	29	536	5	1	542	10.84	234	4.68
Average		20.11	836.56	42.33	899.00	74.11	5.89	970.00	19.58	431.44	8.63

* MSS: Microsatellite stable, MSI: Microsatellite instable, MSI-H: Microsatellite instable-high, SNV: single nucleotide variant

Table S7. Summary of Sanger sequencing validation for 73 randomly chosen variants from whole exome sequencing.

Patient ID	Gene	Genomic position (Chromosome number: nucleotide position, hg19)	Reference	Alternate	Mutation type	Validation
S813	CLCA1	1:86,951,098	C	T	stopgain	Yes
S113	CLCA1	1:86,954,728	C	T	nonsyn	Yes
S434	CLCA1	1:86,954,825	A	C	nonsyn	Yes
S113	SF3B1	2:198,266,128	C	T	nonsyn	Yes
S753	SF3B1	2:198,299,710	G	C	nonsyn	Yes
S813	IDH1	2:209,101,886	G	A	nonsyn	Yes
S813	MAP2	2:210,559,779	C	T	nonsyn	Yes
S384	MAP2	2:210,561,385	G	T	nonsyn	Yes
S529	MAP2	2:210,574,821	C	T	nonsyn	Yes
S113	PAIP2B	2:71,417,076	A	G	nonsyn	Yes
S130	PAIP2B	2:71,429,678	T	G	nonsyn	Yes
S813	ALMS1	2:73,717,154	C	T	nonsyn	No
S113	ALMS1	2:73,717,722	G	A	nonsyn	Yes
S395	ALMS1	2:73,777,542	A	C	nonsyn	Yes
S090	ALMS1	2:73,800,244	C	T	nonsyn	Yes
S451	ALMS1	2:73,836,724	A	C	nonsyn	Yes
S137	SETD2	3:47,163,356	C	T	nonsyn	Yes
S529	ROBO1	3:78,667,090	G	A	nonsyn	Yes
S090	ROBO1	3:78,795,895	T	C	nonsyn	Yes
S451	ROBO1	3:78,987,903	C	T	nonsyn	Yes
S090	DCBLD2	3:98,518,517	T	G	nonsyn	Yes
S487	DCBLD2	3:98,518,569	C	A	nonsyn	No
S434	DCBLD2	3:98,530,089	T	C	nonsyn	Yes
S451	DCBLD2	3:98,538,144	T	G	nonsyn	Yes
S137	TET2	4:106,197,563	C	T	nonsyn	Yes
S113	MAP3K1	5:56,168,549	G	T	nonsyn	Yes
S570	ITPR3	6:33,630,325	C	A	nonsyn	No
S538	ITPR3	6:33,632,706	T	C	nonsyn	No
S090	ITPR3	6:33,643,509	G	A	nonsyn	Yes
S538	ITPR3	6:33,654,822	A	G	nonsyn	No
S813	ITPR3	6:33,656,142	G	A	nonsyn	Yes
S813	ITPR3	6:33,656,175	C	T	nonsyn	Yes
S137	DLC1	8:12,943,842	G	A	nonsyn	Yes
S529	DLC1	8:12,952,744	T	C	nonsyn	Yes
S090	PCM1	8:17,823,511	G	A	stopgain	Yes
S529	PCM1	8:17,843,562	T	C	nonsyn	Yes
S813	CSMD1	8:2,857,552	G	A	nonsyn	Yes
S113	CSMD1	8:2,967,843	T	C	nonsyn	Yes
S567	CSMD1	8:3,889,610	G	T	nonsyn	No
S113	CSMD1	8:4,851,931	G	A	nonsyn	Yes
S451	OSR2	8:99,961,449	T	C	nonsyn	Yes
S529	OSR2	8:99,963,848	C	T	nonsyn	Yes
S137	OSR2	8:99,963,901	T	C	stoploss	Yes
S353	PDZRN4	12:41,966,268	C	T	stopgain	Yes
S137	PDZRN4	12:41,966,460	T	A	nonsyn	Yes
S813	PDZRN4	12:41,967,474	C	T	stopgain	Yes
S113	PDZRN4	12:41,967,580	A	G	nonsyn	Yes
S113	RNF6	13:26,788,215	G	A	stopgain	Yes
S113	TRAF7	16:2,215,901	G	A	nonsyn	Yes
S567	TRAF7	16:2,221,329	T	A	nonsyn	Yes
S529	CYLD	16:50,826,572	T	A	nonsyn	Yes
S573	SALL1	16:51,171,162	G	A	nonsyn	Yes
S137	SALL1	16:51,174,461	G	A	nonsyn	Yes
S529	SALL1	16:51,175,811	C	T	nonsyn	Yes
S113	NF1	17:29,483,110	G	A	nonsyn	Yes
S137	NF1	17:29,576,073	C	T	nonsyn	Yes

S090	SETBP1	18:42,530,000	C	T	nonsyn	Yes
S113	ZBTB14	18:5,291,008	T	C	nonsyn	No
S813	ZBTB14	18:5,291,212	G	T	nonsyn	Yes
S384	ZBTB14	18:5,291,275	G	T	nonsyn	Yes
S353	SALL3	18:76,753,943	G	C	nonsyn	Yes
S113	SALL3	18:76,755,209	C	T	nonsyn	Yes
S090	DNMT1	19:10,248,600	C	A	stopgain	No
S090	DNMT1	19:10,248,600	C	A	stopgain	No
S813	KMT2B	19:36,213,522	C	T	nonsyn	Yes
S137	KMT2B	19:36,216,441	G	A	nonsyn	Yes
S529	KMT2B	19:36,218,627	C	T	nonsyn	Yes
S487	KMT2B	19:36,219,012	C	A	nonsyn	No
S570	PTPRT	20:40,877,405	A	G	nonsyn	Yes
S137	PTPRT	20:41,408,885	C	T	nonsyn	Yes
S573	GNAS	20:57,430,085	C	T	nonsyn	Yes
S567	PHF6	X:133,547,544	G	T	stopgain	No
S500	MED12	X:70,343,481	C	A	nonsyn	No

* stopgain: stop gain mutation, stoploss: stop loss mutation, nonsyn: nonsynonymous mutation

Table S8. Comparison of mutation signatures according to subtypes of gastric cancer.

(a) Lauren classification ($p = 0.6544^{\#}$)

Type	No. of samples	C>T	C>A	C>G	A>G	A>T	A>C
Diffuse	16	50.8%	13.0%	8.2%	14.8%	3.6%	9.6%
Intestinal	37	45.9%	18.0%	7.4%	12.7%	5.8%	10.2%
Mixed	2	56.9%	12.7%	5.1%	17.4%	1.2%	6.6%

Calculated by Pearson's chi-square test

(b) TCGA classification ($p = 0.3529^{\#}$)

Type	No. of samples	C>T	C>A	C>G	A>G	A>T	A>C
MSI	16	52.0%	20.4%	3.3%	15.5%	3.8%	5.0%
GS	22	43.9%	13.4%	10.1%	13.3%	5.2%	14.1%
CIN	17	48.7%	16.2%	8.3%	11.8%	6.0%	9.0%

Calculated by Pearson's chi-square test

* MSI: microsatellite instable, GS: genomically stable, CIN: chromosomal instability

Table S9. Summary of alteration profiles for eight known cancer-associated genetic driver genes among the 55 exome-sequenced gastric cancer cases in this study.

Patient ID	ARID1A MT	ERBB2 MT/Amp	FAT4 MT	KRAS MT	PIK3CA MT	TP53 MT	CTNNB1 MT	MYC Amp
S090	L1080P			G13D				
S113	P1018S	T694M			R88Q			
S130								
S134								
S137	M274fs						L497P	
S353						R337C		Amp
S357		Amp				E286K	S33F	Amp
S384						H214fs		
S395	Q1741X		P12Q		C420R			
S434					E81K			
S451							S37F	
S487	W1073X							
S500								
S529	A1304V							Amp
S533		Amp				E285V		Amp
S538								
S567		S310F						
S570			N1225H					
S573						V143fs		
S673		Amp				V272M		
S714						R273H		
S753								
S813	A578V			G13D	H1047R			
S110						G244C		
S120						R273H		
S160								
S166			E4616G			Y220C		
S195					R93L			
S332								
S334						S20fs		
S354						R213X		
S358								
S381			V1526F			I251S		
S399								
S413	G624R					G245S		
S415	I1173fs		R405C			R273C		
S419	E1783fs			G12D		R282W		
S427						P191fs		
S452						P27fs		
S466	P549fs		E2571K	A146T		R248W		
S477	G458fs		P2152H			V157A		
S496		M611I		G12D	R88Q	R342X		
S554			E3799X			C141G		
S566								
S689						V272M		
S703						R175H		
S711						G266E		
S715						C176W		
S716								
S728								
S747								
S796	D2157G			G12D	H1047R			
S797		D277Y						
S801								
S805						Y234C		

* MT: Mutation, Amp: Amplification, X: stop codon, fs: frame shift indel

Table S10. RhoA mutation status according to Lauren histological type and differentiation status of gastric cancer.

(a) Lauren histological type ($p = 0.3331^{\#}$)

RhoA	Diffuse	Intestinal	Mixed
WT	14	36	2
MT	2	1	0

Calculated by Pearson's chi-square test

* WT: Wild-type, MT: Mutation

(b) Differentiation status ($p = 0.7764^{\#}$)

RhoA	Well differentiated	Moderately differentiated	Poorly differentiated	Others
WT	5	24	14	9
MT	0	2	1	0

Calculated by Pearson's chi-square test

* WT: Wild-type, MT: Mutation

Table S11. List of altered genes in gastric cancer with significant differences based on Lauren histology.

(a) Mutations

Gene	Diffuse		Intestinal		*p*-value[#]
	MT	WT	MT	WT	
DCBLD2	4	12	0	37	0.006
PER1	4	12	0	37	0.006
RNF207	4	12	0	37	0.006
DYNC1H1	5	11	1	36	0.007
TCHH	5	11	1	36	0.007
DNAH10	6	10	3	34	0.016
NEB	5	11	2	35	0.021
ANKRD13B	3	13	0	37	0.024
CDH22	3	13	0	37	0.024
SMO	3	13	0	37	0.024
AKAP9	3	13	0	37	0.024
ALDH1B1	3	13	0	37	0.024
ATXN7L1	3	13	0	37	0.024
CASKIN2	3	13	0	37	0.024
CD36	3	13	0	37	0.024
CDH7	3	13	0	37	0.024
COL6A1	3	13	0	37	0.024
DDC	3	13	0	37	0.024
DDHD1	3	13	0	37	0.024
DOPEY2	3	13	0	37	0.024
FAM160B2	3	13	0	37	0.024
FARS2	3	13	0	37	0.024
IGSF9B	3	13	0	37	0.024
ITGA3	3	13	0	37	0.024
KIF24	3	13	0	37	0.024
NLGN2	3	13	0	37	0.024
NR1H4	3	13	0	37	0.024
PCDHGB1	3	13	0	37	0.024
PHF2	3	13	0	37	0.024
PRRT4	3	13	0	37	0.024
RNF220	3	13	0	37	0.024
RUSC2	3	13	0	37	0.024
SLC4A3	3	13	0	37	0.024
SRCIN1	3	13	0	37	0.024
TBX3	3	13	0	37	0.024
UGGT2	3	13	0	37	0.024
CACNA1G	4	12	1	36	0.025
KIF26A	4	12	1	36	0.025
USP42	4	12	1	36	0.025
KRAS	4	12	1	36	0.025

ABHD16B	4	12	1	36	0.025
ALPK2	4	12	1	36	0.025
CFAP46	4	12	1	36	0.025
COL2A1	4	12	1	36	0.025
GUCY2D	4	12	1	36	0.025
KCNB2	4	12	1	36	0.025
MYH7	4	12	1	36	0.025
RAB37	4	12	1	36	0.025
PCDH17	5	11	3	34	0.045
ALMS1	5	11	3	34	0.045

Calculated by Fisher's exact test

* MT: Mutation, WT: Wild-type

(b) Amplifications

Gene	**Diffuse**		**Intestinal**		***p*-value**[#]
	Amp	**WT**	**Amp**	**WT**	
ZPBP2	0	35	10	52	0.012
GSDMB	0	35	9	53	0.024
ERBB2	1	34	12	50	0.028
STARD3	1	34	12	50	0.028
C17orf37	1	34	12	50	0.028
GRB7	1	34	12	50	0.028
IKZF3	1	34	12	50	0.028
PERLD1	1	34	12	50	0.028
PNMT	1	34	12	50	0.028
TCAP	1	34	12	50	0.028
MYCBP2	0	35	7	55	0.047
KCTD12	0	35	7	55	0.047
BTF3L1	0	35	7	55	0.047
CLN5	0	35	7	55	0.047
COMMD6	0	35	7	55	0.047
FBXL3	0	35	7	55	0.047
LMO7	0	35	7	55	0.047
LOC647288	0	35	7	55	0.047
UCHL3	0	35	7	55	0.047
MED1	0	35	7	55	0.047
FBXL20	0	35	7	55	0.047
CRKRS	0	35	7	55	0.047
ORMDL3	0	35	7	55	0.047

Calculated by Fisher's exact test

* Amp: Amplification, WT: Wild-type

(c) Deletions

Gene	Diffuse		Intestinal		*p*-value[#]
	Del	WT	Del	WT	
WWOX	1	34	14	48	0.009
FHIT	4	31	22	40	0.016

Calculated by Fisher's exact test

* Del: Deletion, WT: Wild-type

Table S12. Detailed summary of significantly altered KEGG pathways in 103 gastric cancers.

Patient ID	Platform	Jak STAT signaling pathway	Cell cycle	p53 signaling pathway	Wnt signaling pathway	Neurotrophin signaling pathway	Huntingtons disease	Focal adhesion	Apoptosis	ErbB signaling pathway	Adherens junction	Endocytosis	TGF beta signaling pathway	Tight junction	VEGF signaling pathway	Dorso ventral axis formation
No. of altered sample		**63**	**61**	**55**	**54**	**51**	**49**	**48**	**45**	**44**	**42**	**37**	**35**	**30**	**28**	**16**
S090	WES+aCGH	V	V	V	V	V	V	V		V	V	V	V	V	V	V
S113	WES+aCGH	V			V	V		V	V	V	V		V	V	V	V
S130	WES+aCGH			V												
S134	WES+aCGH				V											
S137	WES+aCGH	V	V		V		V	V			V		V			
S353	WES+aCGH	V	V	V	V	V	V		V	V			V			
S357	WES+aCGH	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
S384	WES+aCGH		V	V	V	V	V		V							
S395	WES+aCGH	V		V	V	V		V	V	V	V		V	V	V	
S434	WES+aCGH	V				V		V	V	V					V	
S451	WES+aCGH	V	V								V					
S487	WES+aCGH	V														
S500	WES+aCGH															
S529	WES+aCGH	V	V	V	V	V	V		V	V		V	V			
S533	WES+aCGH	V	V	V	V	V	V	V	V	V	V	V	V	V		V
S538	WES+aCGH															

S567	WES+aCGH								V		V	V						
S570	WES+aCGH	V	V	V	V	V	V	V	V	V	V	V	V	V	V			
S573	WES+aCGH	V	V	V	V	V	V		V									
S673	WES+aCGH		V	V	V	V	V	V	V	V	V					V		
S714	WES+aCGH		V	V	V	V	V		V									
S753	WES+aCGH		V	V	V	V				V	V	V			V	V		
S813	WES+aCGH	V	V	V	V	V		V	V	V	V	V	V	V	V	V		
S110	WES+aCGH		V	V	V	V	V	V	V	V	V	V						
S120	WES+aCGH	V	V	V	V	V	V	V	V	V	V							
S160	WES+aCGH		V	V									V					
S166	WES+aCGH	V	V	V	V	V	V	V	V	V	V	V	V					V
S195	WES+aCGH	V	V	V	V	V	V	V	V	V	V	V				V		V
S332	WES+aCGH		V					V			V	V						
S334	WES+aCGH		V	V	V	V	V	V	V						V			
S354	WES+aCGH	V	V	V	V	V	V	V	V	V	V	V						
S358	WES+aCGH														V			
S381	WES+aCGH		V	V	V	V	V		V									
S399	WES+aCGH	V	V	V		V		V	V				V					
S413	WES+aCGH		V	V	V	V	V	V	V	V	V	V						
S415	WES+aCGH	V	V	V	V	V	V		V	V			V	V	V	V		
S419	WES+aCGH	V	V	V	V	V	V	V	V	V				V	V	V		
S427	WES+aCGH	V	V	V	V	V	V	V	V	V	V	V	V	V				
S452	WES+aCGH	V	V	V	V	V	V		V	V			V		V			
S466	WES+aCGH	V	V	V	V	V	V	V	V	V	V	V		V	V	V		
S477	WES+aCGH	V	V	V	V	V	V	V	V	V	V	V	V	V				
S496	WES+aCGH	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V		

S554	WES+aCGH	V	V	V	V	V	V	V	V								
S566	WES+aCGH	V						V			V	V					
S689	WES+aCGH	V	V	V	V	V	V	V	V	V	V	V	V	V	V		
S703	WES+aCGH		V	V	V	V	V		V					V			
S711	WES+aCGH		V	V	V	V	V		V			V					
S715	WES+aCGH		V	V	V	V	V	V	V	V	V						
S716	WES+aCGH	V															
S728	WES+aCGH	V	V	V	V	V	V	V	V	V	V	V	V	V	V		
S747	WES+aCGH	V															
S796	WES+aCGH	V		V		V		V	V	V	V	V	V	V	V	V	
S797	WES+aCGH	V	V		V	V	V	V		V	V	V	V	V			
S801	WES+aCGH	V	V		V	V	V	V		V	V	V	V	V	V	V	
S805	WES+aCGH	V	V	V	V	V	V	V	V		V	V		V		V	
S003	aCGH	V															
S086	aCGH	V															
S091	aCGH	V															
S171	aCGH		V	V								V					
S178	aCGH																
S196	aCGH	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
S203	aCGH				V		V	V				V	V		V		
S352	aCGH	V	V	V	V	V	V			V			V				
S370	aCGH	V				V		V	V	V	V				V		
S373	aCGH																
S416	aCGH	V															
S428	aCGH																
S443	aCGH	V					V	V		V	V						

S445	aCGH	V															
S490	aCGH		V	V	V												
S495	aCGH																
S526	aCGH	V															
S696	aCGH	V	V	V	V	V	V	V		V	V	V	V	V	V		
S699	aCGH																
S739	aCGH		V	V								V					
S762	aCGH	V	V	V	V	V	V	V		V	V	V	V	V	V	V	V
S798	aCGH	V	V	V	V			V									
S815	aCGH	V	V	V	V			V									
S088	aCGH	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
S190	aCGH		V	V			V										
S197	aCGH							V			V	V					
S347	aCGH	V	V	V			V		V				V				
S439	aCGH	V	V		V	V	V	V	V	V	V	V	V	V	V		
S440	aCGH		V	V			V										
S441	aCGH	V															
S454	aCGH	V	V		V	V	V	V	V	V	V	V	V	V	V		
S470	aCGH	V	V	V	V	V	V	V	V	V	V	V	V	V	V		
S483	aCGH																
S528	aCGH	V															
S532	aCGH	V															
S577	aCGH	V															
S581	aCGH	V	V		V					V			V				
S656	aCGH	V	V	V	V	V	V	V		V	V	V	V				
S674	aCGH																

S692	aCGH				
S694	aCGH	V	V		
S733	aCGH	V			
S734	aCGH		V	V	
S755	aCGH				
S756	aCGH				
S773	aCGH				
S808	aCGH				
S824	aCGH				

Table S13. Comparison of BCL2L1 amplifications estimated by droplet digital PCR (ddPCR) and array Comparative Genomic Hybridization (aCGH).

Patient ID	BCL2L1 copy number estimated by ddPCR	BCL2L1 amplification in aCGH
S088	3.21	Amplified
S110	3.41	Normal
S134	3.12	Normal
S196	3.77	Amplified
S347	3.77	Amplified
S357	3.28	Amplified
S384	4.64	Normal
S415	4.87	Normal
S439	3.18	Amplified
S452	3.96	Normal
S454#	2.62	Amplified
S466	3.39	Normal
S470	3.56	Amplified
S526	3.21	Normal
S533	3.13	Amplified
S570#	2.20	Amplified
S674	3.65	Normal
S689	3.32	Amplified
S692	5.01	Normal
S694	3.33	Normal
S728	5.73	Amplified
No. of BCL2L1-amplified cases	19	11

Amplifications of BCL2L1 were detected only by aCGH.

Table S14. Comparison of BCL2L1 amplification frequencies between Asian and non-Asian populations in TCGA stomach adenocarcinoma cases.

BCL2L1 copy number	2 copies	3-5 copies	High level amplification (> 5 copies)	*p*-value
Asian (n=42)	12	30	0	0.6862
Non-Asian (n=131)	49	79	3	

Table S15. Potential cooperative interactions of mutations in genes closely linked to RHOA from 82 gastric cancers (cohort 1 (n= 55) and cohort 2 (n= 27)).

	DLC1	ARHGAP35	DAAM1	ARHGAP21	ROCK1	ROCK2	RASGRF1	ARHGEF28	RHOA
AKAP13	3	3†	1	1	2	1	2†	2†	1
DLC1		3†	1	0	2	0	1	0	1
ARHGAP35			0	0	2†	0	0	0	0
DAAM1				1	0	1	1	1	3†
ARHGAP21					0	0	0	1	1
ROCK1						0	0	0	0
ROCK2							0	0	1
RASGRF1								1	1
ARHGEF28									1

* Numbers indicate the number of co-occurring samples of two genes

† Significant tendency to co-occur in 82 samples (cohort 1 and 2, Fisher exact test, $p < 0.05$)

Table S16. Predicted protein stability of DLC1 mutations using the i-Mutant database.

Mutation	Stability	Reliability index	pH	Temperature (°C)
G75W	Decrease	5	7.4	37
E450X	-	-	7.4	37
R501M	Decrease	6	7.4	37
R549W	Decrease	7	7.4	37
G845V	Decrease	3	7.4	37
K1060E	Decrease	1	7.4	37
R1086H	Decrease	7	7.4	37
P1475S	Decrease	7	7.4	37

Table S17. Predicted functional impacts of DLC1 mutations using three bioinformatic algorithms.

Mutation	SIFT	PolyPhen-2	MutationTaster
G75W	D	D	N
E450X	D	B	D
R501M	-	D	D
R549W	D	D	D
G845V	D	D	D
K1060E	D	D	D
R1086H	D	D	D
P1475S	T	B	D

* B: benign, N: neutral, T: tolerant, D: damaging