Supplementary Materials and Methods

Morphological observation of spheroids

Phase-contrast images of spheroids were captured using an Olympus IX70 microscope equipped with an Olympus DP70 digital camera (Olympus, Tokyo, Japan).

Preparation of DNA and histology specimens of spheroids

Spheroids in Matrigel were suspended in Cell Recovery Solution (Corning), and they were incubated at 4°C for 30–60 min. Spheroids in collagen type-I were suspended in collagenase solution, and they were incubated at 37°C for 30 min. Next, the spheroids were centrifuged at 200 × *g* for 5 min and washed with PBS. Genomic DNA was purified using a DNeasy Blood & Tissue Kit (Qiagen, Venlo, the Netherlands). For histological analyses, spheroids were embedded in iPGell (Genostaff, Tokyo, Japan) and fixed with 4% paraformaldehyde in PBS at 4°C for 3 days.

Patient-derived spheroid xenograft (PDSX)

Four- to six-week-old female nude and NSG mice were purchased from The Jackson Laboratory Japan (Yokohama, Japan). The spheroid suspension was subcutaneously injected into mice as

described previously.[34]

Histopathological classification of gastric cancer

Formalin-fixed, paraffin-embedded specimens were sectioned into 4-µm thick sections, and they were stained with H&E. Histological images were captured using a Leica DM2000 microscope (Leica, Wetzlar, Germany) equipped with an Olympus DP73 digital camera (Olympus), and histological grades of primary tumors and spheroids were determined according to the 5th edition of WHO guidelines.[4]

Immunohistochemistry

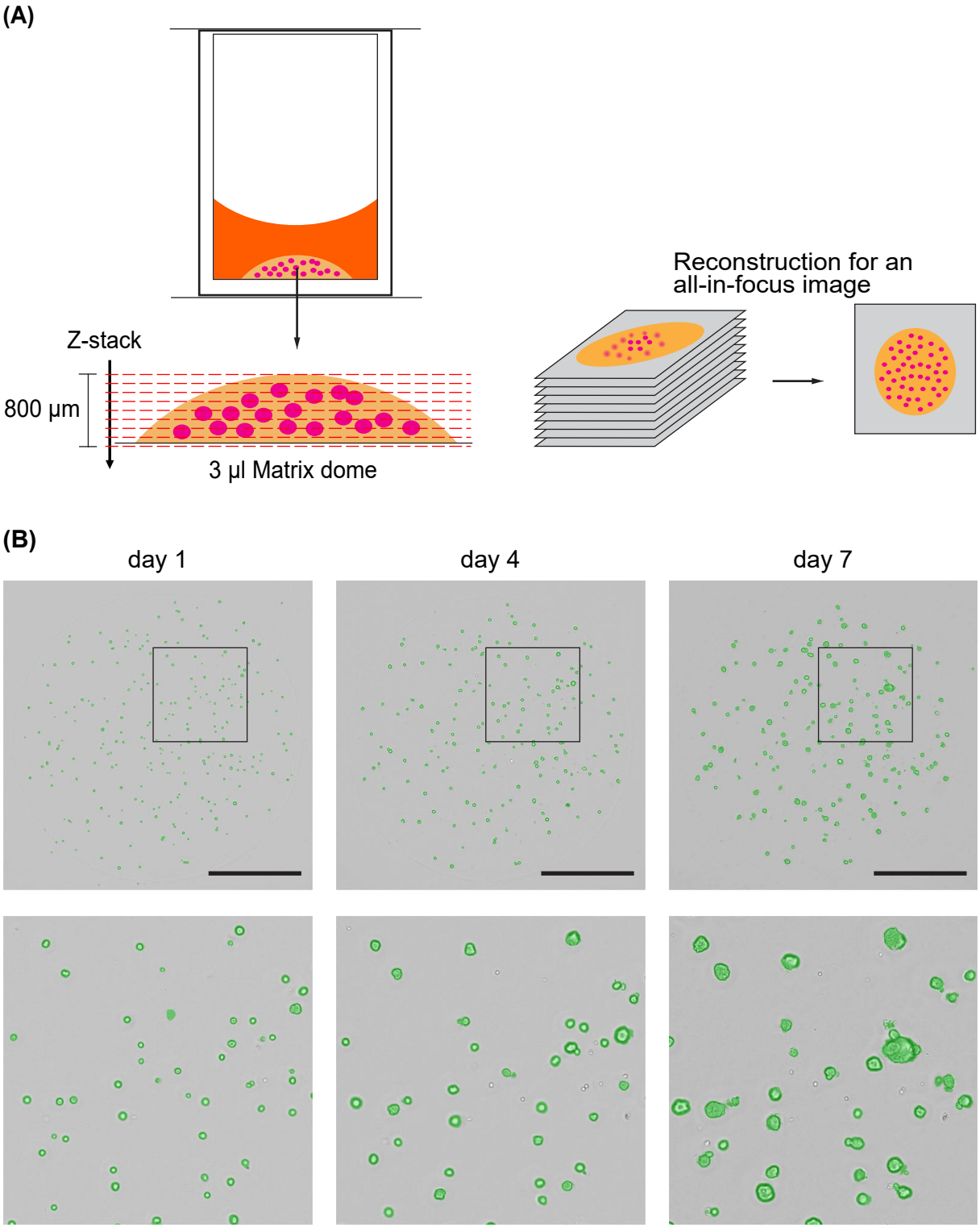
Primary antibodies against MLH-1 (M1, Ventana, Oro Valley, AZ, USA), PMS2 (EPR 3947, Ventana), MSH2 (G219-1129, Ventana), MSH6 (EPR3945, Abcam, Cambridge, UK), Ki67 (SP6, ThermoFisher, Waltham, MA, USA), CDX2 (SM392-5M, BioiGenex, Fremont, CA, USA), and MUC2 (CCP58, Agilent, Santa Clara, CA, USA) were purchased from commercial sources. Deparaffinized sections were incubated in Trilogy solution (Sigma-Aldrich) at 95°C for 60 min for unmasking target antigens, and they were further incubated in 0.3% H_2O_2 in methanol to inactivate endogenous peroxidases at room temperature for 15 min. Primary antibodies diluted in the blocking buffer [5% goat serum (Vector Laboratories, Burlingame, CA, USA) and 3%

bovine serum albumin (Sigma-Aldrich) in PBS] were applied on the sections. Specific signals were visualized using OptiView DAB IHC Detection Kit (Ventana) or the VECTASTAIN Elite ABC Kit (Vector Laboratories).

Quantitative RT-PCR (qRT-PCR)

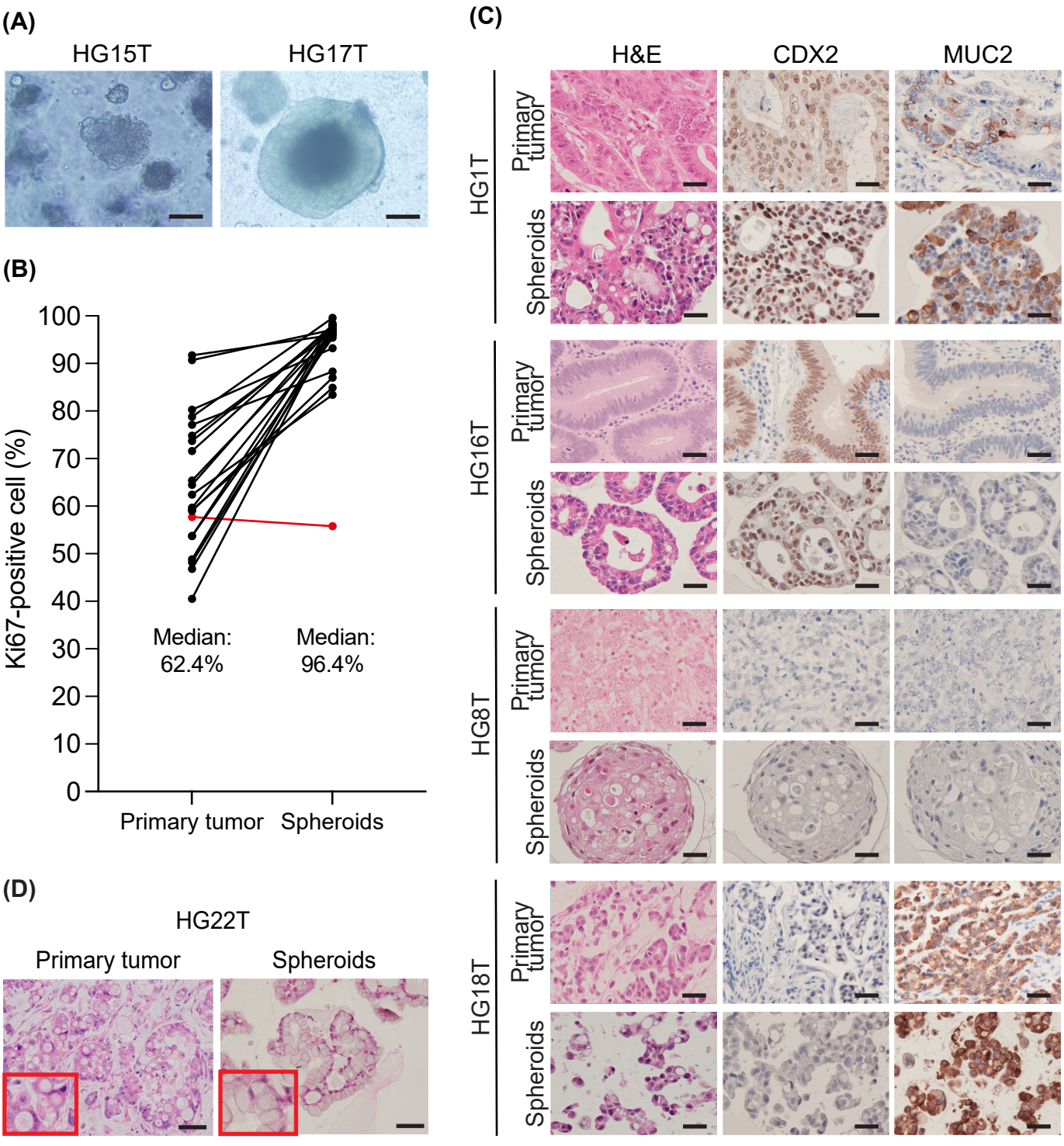
First strand cDNA was synthesized using ReverTra Ace kit (TOYOBO, Osaka, Japan), and qPCR was performed using SYBR qPCR Mix (TOYOBO) on an StepOnePlus thermal cycler (ThermoFisher). Expression levels were normalized relative to those of *ACTB*. Sequences of primer pairs were as follows: *MKI67*, TCCTTTGGTGGGCACCTAAGACCTG and TGATGGTTGAGGTCGTTCCTTGATG; *LGR5*, CCTTCATAAGAAAGATGCTGGAA and GTTTAATGGGGGAAATGTACAGA; *ACTB*, GGGGTGTTGAAGGTCTCAAA and GGCATCCTCACCCTGAAGTA.

Supplementary figure 1: A schematic workflow for monitoring cell growth using optical cell imaging.



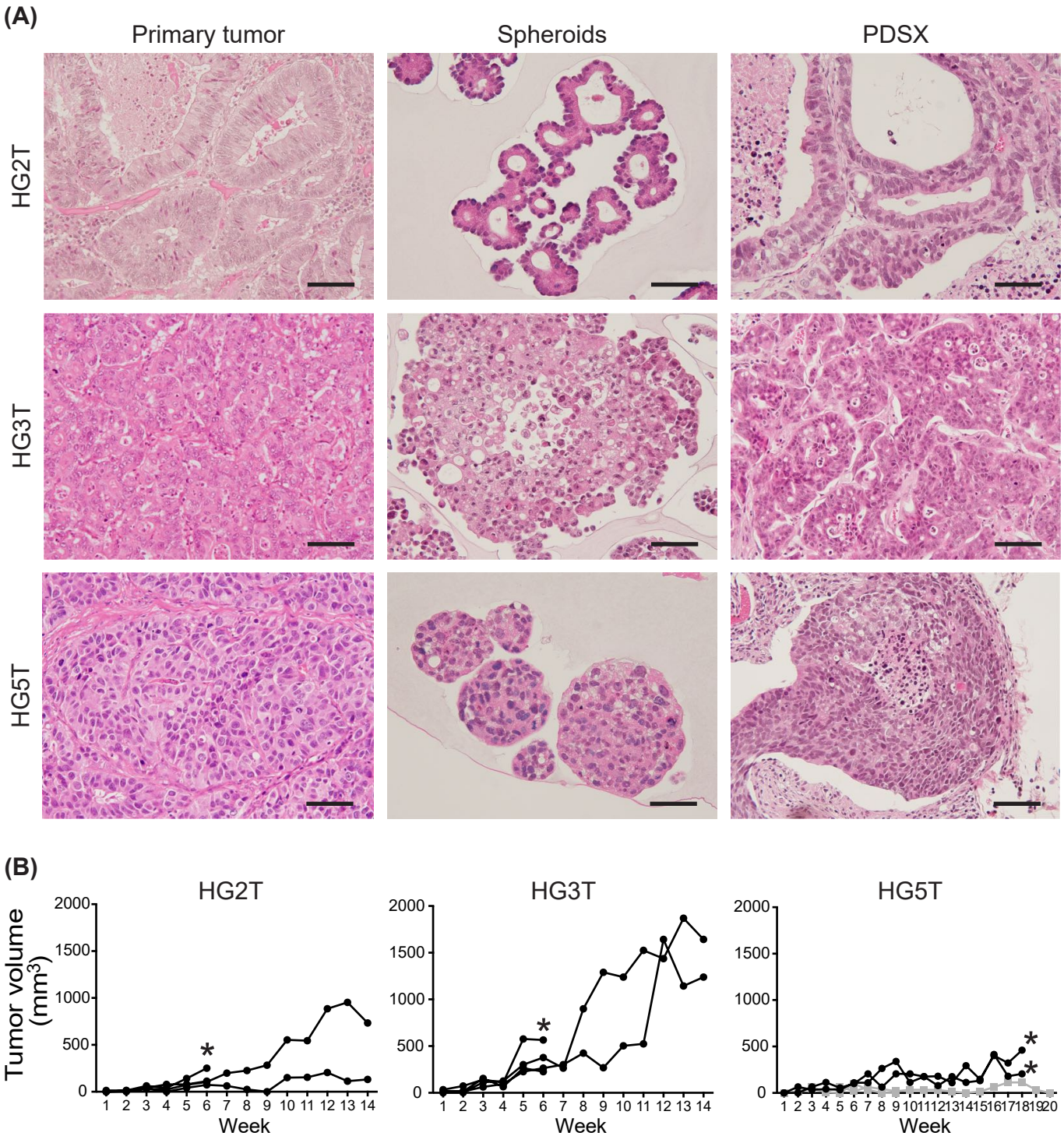
(**A**) Outline of the cell scanning procedures. Nine Z-stack images were acquired for each matrix dome (3 μL), and they were reconstructed to generate an all-in-focus image. (**B**) Time course of the spheroid growth. Shown are the reconstituted images of HG16T spheroids (top) and their higher magnification (bottom) at 1, 4, and 7 days after passage. Object areas recognized as spheroids are shown in green. Scale bar, 1 mm.

Supplementary figure 2: Histopathological characterization of patient-derived gastric cancer stem cell (PD–GC-SC) spheroids.



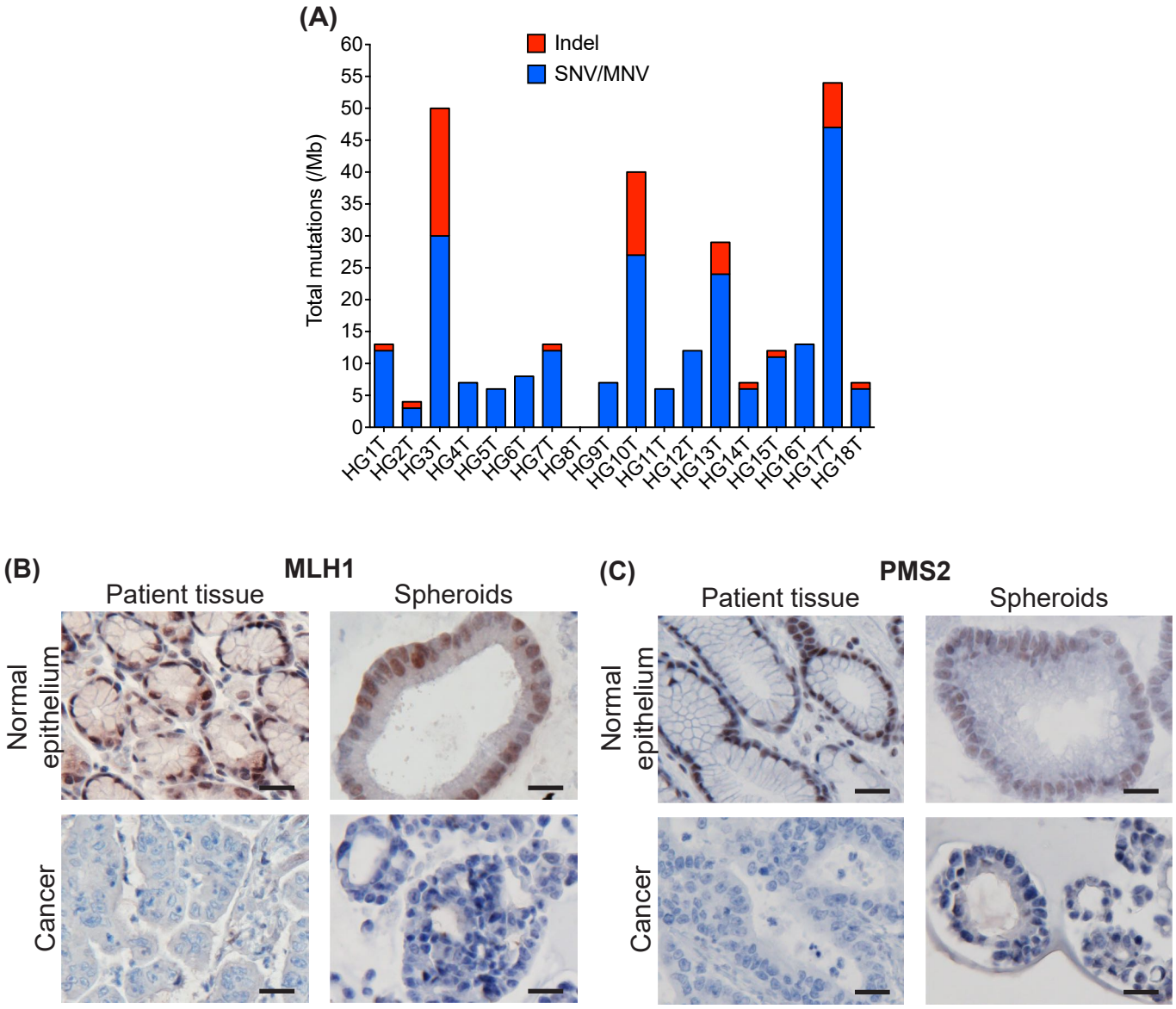
(**A**) Representative phase-contrast micrographs of GC-SC spheroids. Scale bar, 200 μm. (**B**) The fraction of proliferating cells monitored by immunohistochemistry for Ki67. The average percentages of Ki67-positiive cells in three microscopic fields are shown as raw data points with lines connecting the pairs of the primary tumors and spheroids from the same GC patients (n = 21). Note that proliferating cells were enriched in spheroids more than in primary tumors in all cases except for one diffuse-type tumor (HG8T, red). $P < 0.0001$, statistical significance of the data difference (Wilcoxon test). (**C**) Pairs of the primary tumor (top) and spheroid specimens (bottom) from the same GC patients analyzed by H&E staining (left), and by immunohistochemistry for CDX2 (center) and MUC2 (right). HG1T, tubular adenocarcinoma containing CDX2-positive moderately differentiated tumor cells forming the tubular structure. HG16T, tubular adenocarcinoma containing CDX2-positive well-differentiated tumor cells forming the tubular structure. MUC2-positive cells were heterogeneously distributed in HG1T but rarely observed in HG16T. HG8T, poorly cohesive carcinoma containing CDX2-negative/ MUC2-negative poorly differentiated tumor cells. HG18T, mucinous adenocarcinoma containing CDX2-low/MUC2-positive poorly differentiated tumor cells. Scale bar, 50 μm. (**D**) A pair of H&E-stained specimens of the primary tumor (left) and spheroids (right) from the same GC patient (HG22T, poorly cohesive carcinoma with signet-ring cells). Spheroids were cultured in the cancer medium without L-WRN conditioned medium for three days. Insets indicate cells with large vacuoles and compressed nuclei. Scale bar, 50 μm.

Supplementary figure 3: Characterization of tumor-initiating gastric cancer (GC) cells in patient-derived spheroid xenograft (PDSX) tumors.



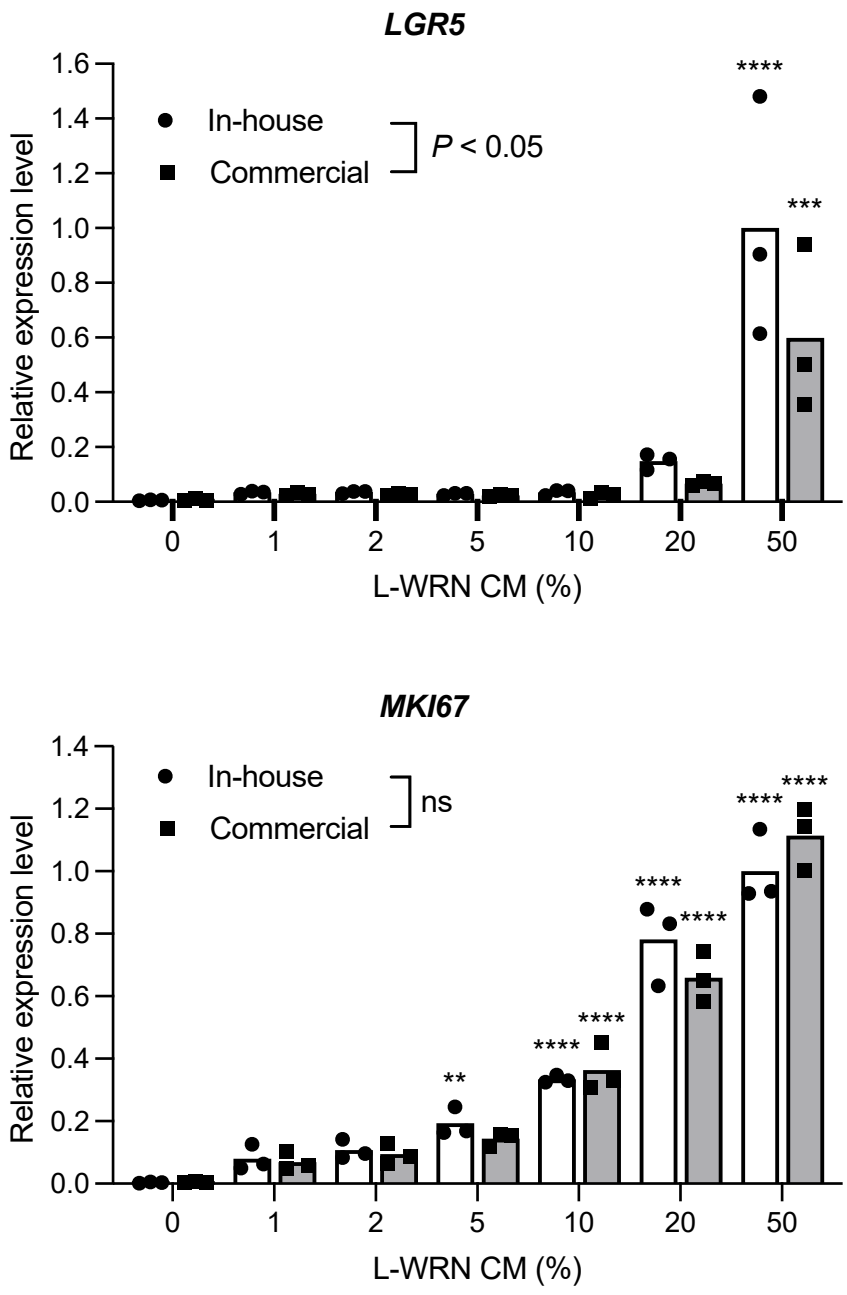
(**A**) Pairs of the primary tumor (left), spheroids (center), and PDSX specimens (right) from the same patients analyzed by H&E staining. HG2T, tubular adenocarcinoma containing well-differentiated tumor cells forming the tubular structure. HG3T, tubular adenocarcinoma containing moderately differentiated cells forming the tubular structure. HG5T, tubular adenocarcinoma containing poorly differentiated cells forming the solid structure. Scale bar, 50 μm. (**B**) Growth curves of PDSX tumors derived from GC spheroids, HG2T (n = 3), HG3T (n = 3), and HG5T (n = 4). Mice were sacrificed when they became moribund (asterisks). The tumor regressed in two PDSX mice for HG5T (gray lines).

Supplementary figure 4: Detection of mismatch repair deficiency in gastric stem cell (GC-SC) spheroids.



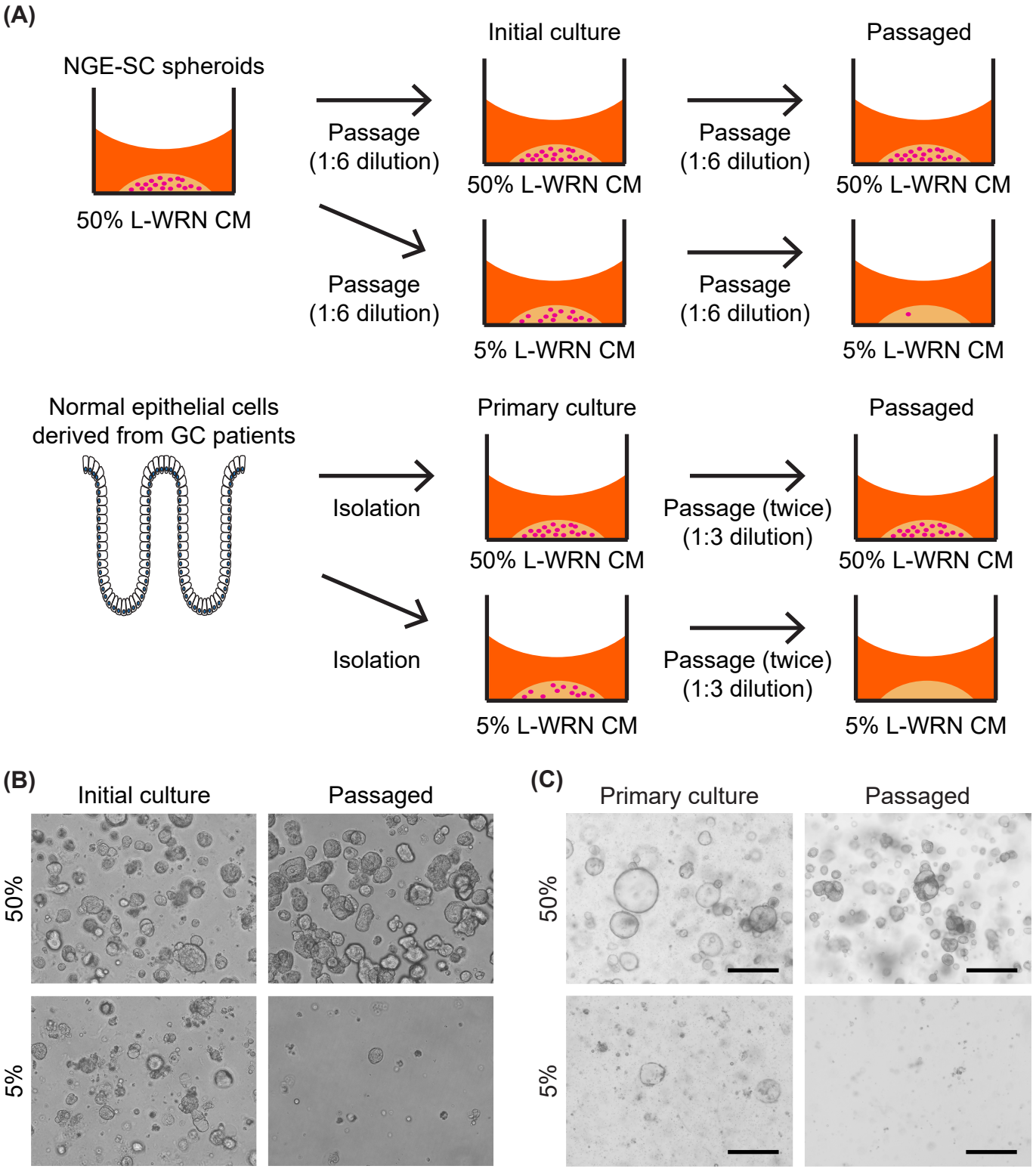
(**A**) Mutational burden estimated by exonic sequencing of 409 cancer-related genes spanning 1.29 Mb. Indel, insertion/deletion variant. SNV/MNV, single nucleotide variant/multi-nucleotide variant. (**B, C**) Immunohistochemistry for mismatch repair proteins, MLH1 (B) and PMS2 (C). Shown are paired specimens of the patient tissue (left) and spheroids (right) of the normal gastric epithelium (top) and GC (bottom) derived from the same patient (HG10T). Scale bar, 25 μm.

Supplementary figure 5: Dose-dependent effects of L-WRN conditioned media (CM) on gene expression in normal colonic epithelial stem cell (NCE-SC) spheroids.



NCE-SC spheroids were cultured for 3 days in the eL-WRN media containing indicated percentages of L-WRN CM from two different sources (in-house and commercial). Plots with the means of relative expression levels of LGR5 (top) and MKI67 (bottom) were determined by qRT-PCR analysis. ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001, statistical significance of the data difference between untreated (0%) and treated groups in three independent experiments (two-way ANOVA followed by Šidák's post-test).

Supplementary figure 6: Effects of L-WRN conditioned medium (CM) concentrations on the growth of normal gastric epithelial stem cells (NGE-SCs).



(**A**) Schematic of the experimental procedure. (**B**) NGE-SC spheroids were cultured in the cancer medium containing 50% (top) or 5% (bottom) L-WRN CM for 3 days (left; Initial culture), passaged at a split ratio of 1:6, and cultured for another 3 days (right; Passaged). Scale bar, 200 μm. (**C**) Normal gastric epithelial cells isolated from patient samples were cultured in the cancer medium containing 50% (top) or 5% (bottom) L-WRN CM for 6 days (left; Primary culture). Then spheroids were cultured for another 12 days with two serial passages at a split ratio of 1:3 (right; Passaged). Scale bar, 200 μm.

Supplementary table 1: Composition of media.

Reagent	Final conc.	Source
Washing medium		
DMEM/F12 with HEPES and L-glutamine		Nacalai Tesque
Penicillin-streptomycin solution (100 x)	1 x	Nacalai Tesque
Calf serum	10%	Sigma-Aldrich
Collagenase solution		
Washing medium		
Collagenase type I	0.2%	Thermo Fisher
Gentamicin	50 µg/ml	Thermo Fisher
Cancer medium		
Advanced DMEM/F-12		Thermo Fisher
Penicillin-streptomycin solution (100 x)	1 x	Nacalai Tesque
L-Glutamine	2 mM	Nacalai Tesque
Y27632	10 µM	R&D systems
SB431542	1 µM	R&D systems
Plasmocin	5 µg/ml	Invivogen
Fetal bovine serum	5%	Thermo Fisher
Epidermal growth factor (EGF)	50 ng/ml	Peprotech
Fibroblast growth factor 2 (FGF2)	100 ng/ml	Peprotech
5'-(*N*-Ethyl-carboxamido)-adenosine (NECA)	1 µM	Sigma-Aldrich
B27 supplement (50 x)	1 x	Thermo Fisher
L-WRN conditioned medium[a]	5%	In-house[b]
[a]L-WRN conditioned medium was added after the protocol revision.		
[b]Also available from Sigma-Aldrich (SCM105)		
eL-WRN medium		
Advanced DMEM/F-12		Thermo Fisher
Penicillin-streptomycin solution (100 x)	1 x	Nacalai Tesque
L-Glutamine	2 mM	Nacalai Tesque
L-WRN conditioned medium	50%	In-house[b]
Y27632	10 µM	R&D systems
SB431542	1 µM	R&D systems
Plasmocin	5 µg/ml	Invivogen
Fetal bovine serum	20%	Thermo Fisher
Epidermal growth factor (EGF)	50 ng/ml	Peprotech

Supplementary table 2: Success rates for spheroid establishment according to the clinicopathological characteristics of patients in the first patient cohort.

	Succeeded	Failed	Success rate (%)	*P* value[a]
Total (n = 71)	18	53	25	
Age, median (range)	73.6 (59–87)	69.5 (39–88)		*P* = 0.40
<60	1	11	8	
60–69	5	13	28	
70–79	8	15	35	
≥80	4	14	22	
Sex				*P* = 0.78
Male	10	32	24	
Female	8	21	28	
Stage				*P* = 0.92
IA	2	6	25	
IB	0	2	0	
IIA	5	9	36	
IIB	4	10	29	
IIIA	3	9	25	
IIIB	2	12	14	
IIIC	2	5	29	
IV	0	0	NA	
Tumor invasion				*P* = 0.73
T1	3	8	27	
T2	2	4	33	
T3	9	22	29	
T4	4	19	17	
LN metastasis				*P* = 0.55
N0	5	18	22	
N1	6	9	40	
N2	3	8	27	
N3a	2	13	13	
N3b	2	5	29	
Lauren's classification				*P* = 0.02
Intestinal	16	30	35	
Diffuse	2	23	8	
Location				*P* = 0.06
GE junction	2	0	100	
Fundus	4	6	40	
Corpus	5	25	17	
Antrum	7	22	24	
Metastasis at initial diagnosis				*P* = 1.00
Yes	2	5	29	
No	16	48	25	
Chemotherapy				*P* = 1.00
Before surgery	2	8	20	
No treatment	16	45	26	

[a]Fisher's exact test

Supplementary table 3: Success rates for spheroid establishment according to the clinicopathological characteristics of patients in the second patient cohort.

	Succeeded	Failed	Success rate (%)	*P* value[a]
Total (n = 33)	29	4	88	
Age, median (range)	79 (60-86)	76.5 (72-93)		*P* = 1.00
<60	0	0	NA	
60–69	5	0	100	
70–79	11	3	79	
≥80	13	1	93	
Sex				*P* = 1.00
Male	19	3	86	
Female	10	1	91	
Stage				*P* = 1.00
IA	6	0	100	
IB	1	0	100	
IIA	8	2	80	
IIB	6	0	100	
IIIA	1	1	50	
IIIB	5	1	83	
IIIC	2	0	100	
IV	0	0	NA	
Tumor invasion				*P* = 1.00
T1	8	0	100	
T2	7	1	88	
T3	8	3	73	
T4	6	0	100	
LN metastasis				*P* = 1.00
N0	12	2	86	
N1	4	0	100	
N2	6	0	100	
N3a	5	2	71	
N3b	2	0	100	
Lauren's classification				*P* = 1.00
Intestinal	23	3	88	
Diffuse	6	1	86	
Location				*P* = 1.00
GE junction	1	0	100	
Fundus	6	0	100	
Corpus	8	2	80	
Antrum	14	2	88	
Metastasis at initial diagnosis				*P* = 0.23
Yes	1	1	50	
No	28	3	90	
Chemotherapy				*P* = 0.33
Before surgery	2	1	67	
No treatment	27	3	90	

[a]Fisher's exact test

Supplementary table 4: Mutational status of 409 cancer-related genes in each gastric cancer stem cell (GC-SC) spheroid line in the first patient cohort detected using targeted next-generation sequencing.

Chrom	Position	Ref	Variant	Frequency	Type	Allele Name	Gene Symbol[a]	AAChange.refGene
HG1T								
chr3	187451403	T	C	41.2	SNV	---	BCL6	NM_001706:exon3:c.79A>G:p.S27G
chr4	62845473	A	C	63.6	SNV	---	ADGRL3	NM_015236:exon17:c.2794A>C:p.N932H
chr5	112163677	A	T	37.2	SNV	COSM18768	APC	NM_000038:exon13:c.1600A>T:p.K534X
chr5	112175303	C	T	62.8	SNV	COSM13129	APC	NM_000038:exon16:c.4012C>T:p.Q1338X
chr6	134492239	A	-	67.6	DEL	---	SGK1	NM_005627:exon10:c.960delT:p.I320fs
chr6	152476033	T	A	69.5	SNV	---	SYNE1	NM_033071:exon132:c.23910A>T:p.K7970N
chr6	166826287	C	T	35.4	SNV	---	RPS6KA2	NM_021135:exon21:c.2165G>A:p.R722H
chr7	151879573	T	C	30.8	SNV	---	KMT2C	NM_170606:exon36:c.5372A>G:p.Q1791R
chr9	134067664	C	A	45.8	SNV	---	NUP214	NM_005085:exon27:c.3644C>A:p.S1215X
chr11	3723971	G	C	51.2	SNV	---	NUP98	NM_016320:exon23:c.3234C>G:p.F1078L
chr12	56488301	A	G	33.9	SNV	---	ERBB3	NM_001982:exon15:c.1820A>G:p.Q607R
chr17	7577538	C	T	97.7	SNV	COSM10662	TP53	NM_000546:exon7:c.743G>A:p.R248Q
chr22	28195386	C	A	52.5	SNV	---	MN1	NM_002430:exon1:c.1146G>T:p.Q382H
HG2T[b]								
chr5	112175952	-	A	100.0	INS	COSM19695	APC	NM_000038:exon16:c.4662dupA:p.E1554fs
chr10	88649927	T	A	95.2	SNV	COSM9548662	BMPR1A	NM_004329:exon4:c.176T>A:p.L59X
chr17	7578223	C	T	100.0	SNV	COSM45995	TP53	NM_000546:exon6:c.626G>A:p.R209K
chr18	50278486	A	C	21.6	SNV	---	DCC	NM_005215:exon2:c.154A>C:p.M52L
HG3T[b]								
chr1	27023831	G	-	52.6	DEL	---	ARID1A	NM_006015:exon1:c.937delG:p.G313fs
chr1	27105617	C	T	52.9	SNV	---	ARID1A	NM_006015:exon20:c.5228C>T:p.T1743M
chr1	145532152	G	A	49.6	SNV	---	ITGA10	NM_003637:exon8:c.796G>A:p.E266K
chr1	147092593	T	C	49.2	SNV	---	BCL9	NM_004326:exon8:c.2632T>C:p.S878P
chr1	220808833	TC	-	50.1	DEL	---	MARK1	NM_018650:exon12:c.1238_1239del:p.I413fs
chr2	29451873	C	T	50.8	SNV	---	ALK	NM_004304:exon16:c.2692G>A:p.E898K
chr3	30691872	A	-	NA	DEL	---	TGFBR2	NM_003242:exon3;c.657Adel:p.K128fs
chr3	52442539	G	A	97.7	SNV	---	BAP1	NM_004656:exon4:c.206C>T:p.T69M
chr3	138461565	C	T	44.0	SNV	---	PIK3CB	NM_006219:exon3:c.456G>A:p.M152I
chr3	178952085	A	G	48.5	SNV	COSM94986	PIK3CA	NM_006218:exon21:c.3140A>G:p.H1047R
chr4	55152009	G	A	50.9	SNV	---	PDGFRA	NM_006206:exon18:c.2441G>A:p.C814Y
chr4	62936601	C	T	48.7	SNV	---	ADGRL3	NM_015236:exon25:c.4385C>T:p.P1462L
chr5	226052	C	T	50.3	SNV	---	SDHA	NM_004168:exon5:c.511C>T:p.R171C
chr5	176524337	G	T	49.5	SNV	---	FGFR4	NM_002011:exon17:c.2198G>T:p.R733M
chr6	31132590	C	T	57.5	SNV	---	POU5F1	NM_002701:exon5:c.871G>A:p.D291N
chr6	31138020	CTT	-	48.1	DEL	---	POU5F1	NM_002701:exon1:c.376_378del:p.126_126del
chr6	33287889	TCT	-	46.1	DEL	---	DAXX	NM_001350:exon5:c.1362_1364del:p.454_455del
chr6	41555186	C	-	56.0	DEL	---	FOXP4	NM_138457:exon7:c.805delC:p.P269fs
chr6	56327860	G	A	51.0	SNV	---	DST	NM_015548:exon82:c.15113C>T:p.P5038L
chr6	56462752	A	-	52.2	DEL	---	DST	NM_015548:exon28:c.4112delT:p.L1371fs
chr6	135518423	C	T	50.0	SNV	---	MYB	NM_001130173:exon10:c.1528C>T:p.R510C

chr7	2968323	G	-	58.8	DEL	---	CARD11	NM_032415:exon13:c.1663delC:p.R555fs
chr8	145737431	C	T	35.0	SNV	---	RECQL4	NM_004260:exon20:c.3256G>A:p.G1086R
chr8	145738671	T	C	29.3	SNV	---	RECQL4	NM_004260:exon15:c.2393A>G:p.Y798C
chr8	145739409	T	C	37.9	SNV	---	RECQL4	NM_004260:exon12:c.1961A>G:p.Q654R
chr9	120476084	-	A	29.4	INS	---	TLR4	NM_003266:exon4:c.1558dupA:p.S519fs
chr9	134039290	T	-	53.3	DEL	---	NUP214	NM_005085:exon20:c.2757delT:p.A919fs
chr9	135801117	C	A	45.8	SNV	---	TSC1	NM_000368:exon5:c.220G>T:p.D74Y
chr9	136901405	T	C	48.1	SNV	---	BRD3	NM_007371:exon10:c.1685A>G:p.D562G
chr10	76739022	A	G	51.1	SNV	---	KAT6B	NM_012330:exon10:c.2156A>G:p.Y719C
chr12	46246012	G	A	44.1	SNV	---	ARID2	NM_152641:exon15:c.4106G>A:p.G1369D
chr12	49431874	C	-	61.7	DEL	---	KMT2D	NM_003482:exon34:c.9265delG:p.V3089fs
chr13	110435906	C	T	47.8	SNV	---	IRS2	NM_003749:exon1:c.2495G>A:p.R832H
chr14	92471207	-	T	47.2	INS	---	TRIP11	NM_004239:exon11:c.3113dupA:p.K1038fs
chr15	40913546	AC	-	55.3	DEL	---	KNL1	NM_144508:exon10:c.1084_1085del:p.T362fs
chr16	23647028	T	-	52.0	DEL	---	PALB2	NM_024675:exon4:c.839delA:p.N280fs
chr16	50828253	T	C	49.9	SNV	---	CYLD	NM_015247:exon19:c.2600T>C:p.I867T
chr16	68857418	G	A	51.6	SNV	---	CDH1	NM_004360:exon13:c.2053G>A:p.V685M
chr17	29556478	G	T	49.2	SNV	---	NF1	NM_000267:exon21:c.2845G>T:p.G949X
chr17	78262019	-	C	28.2	INS	---	RNF213	NM_020954:exon4:c.667dupC:p.G222fs
chr17	78301694	A	G	49.9	SNV	---	RNF213	NM_001256071:exon19:c.3272A>G:p.K1091R
chr18	22807087	-	A	53.1	INS	---	ZNF521	NM_015461:exon4:c.795dupT:p.A266fs
chr18	50976892	C	-	78.7	DEL	---	DCC	NM_005215:exon23:c.3253delC:p.P1085fs
chr19	11141427	G	-	50.5	DEL	---	SMARCA4	NM_003072:exon25:c.3404delG:p.R1135fs
chr19	18870879	-	G	28.7	INS	---	CRTC1	NM_015321:exon8:c.727dupG:p.G243fs
chr19	45856398	G	A	48.0	SNV	---	ERCC2	NM_000400:exon19:c.1774C>T:p.R592C
chr19	52725449	A	G	37.3	SNV	---	PPP2R1A	NM_014225:exon13:c.1616A>G:p.N539S
chrX	44928908	C	T	99.2	SNV	---	KDM6A	NM_021140:exon17:c.2008C>T:p.Q670X
chrX	48121199	A	C	100.0	SNV	---	SSX1	NA (splicing)
chrX	66765779	G	-	100.0	DEL	---	AR	NM_000044:exon1:c.791delG:p.R264fs

HG4T

chr2	29543662	AT	GC	56.1	MNV	---	ALK	NM_004304:exon7:c.1500_1501GC
chr7	92734452	T	A	53.5	SNV	---	SAMD9	NM_017654:exon3:c.959A>T:p.Y320F
chr9	93627378	C	T	35.0	SNV	---	SYK	NM_003177:exon6:c.845C>T:p.A282V
chr10	43600607	C	A	61.2	SNV	COSM95173	RET	NM_020630:exon4:c.833delC:p.T278fs
chr11	47259484	G	A	29.0	SNV	---	DDB2	NM_000107:exon8:c.1120G>A:p.V374M
chr17	7577120	C	G	100.0	SNV	COSM43896	TP53	NM_000546:exon8:c.818G>C:p.R273P
chr22	41564594	T	C	100.0	SNV	---	EP300	NM_001429:exon24:c.4016T>C:p.M1339T

HG5T[b]

chr5	112174494	C	A	100.0	SNV	COSM4166493	APC	NM_000038:exon16:c.3203C>A:p.S1068X
chr8	48776032	T	A	21.9	SNV	---	PRKDC	NM_006904:exon42:c.5688A>T:p.E1893V
chr9	134019700	C	G	26.2	SNV	---	NUP214	NM_005085:exon12:c.1328C>G:p.A443G
chr11	102195701	TT	CA	56.5	MNV	---	BIRC3	NM_001165:exon2:c.461_462TT>CA:pF154S
chr17	7577117	A	G	100.0	SNV	COSM44393	TP53	NM_000546:exon8:c.821T>C:p.V274A
chrX	76939580	G	A	50.4	SNV	---	ATRX	NM_000489:exon9:c.1168C>T:p.R390C

HG6T

chr1	19018313	C	T	50.3	SNV	---	PAX7	NM_013945:exon5:c.646C>T:p.R216X

chr3	46490413	C	T	53.8	SNV	---	LTF	NM_002343:exon9:c.1153G>A:p.E385K
chr6	41564936	A	G	38.9	SNV	---	FOXP4	NM_138457:exon15:c.1642A>G:p.M548V
chr14	81609793	G	T	63.0	SNV	---	TSHR	NM_000369:exon10:c.1391G>T:p.G464V
chr17	7578212	G	A	99.2	SNV	COSM10654	TP53	NM_000546:exon6:c.637C>T:p.R213X
chr17	37881332	G	A	96.9	SNV	COSM14065	ERBB2	NM_004448:exon21:c.2524G>A:p.V842I
chrX	110366374	C	T	42.3	SNV	---	PAK3	NM_002578:exon5:c.43C>T:p.P15S

HG7T[b]

chr2	216272884	A	C	46.7	SNV	---	FN1	NM_002026:exon17:c.2465T>G:p.V822G
chr3	41266113	C	A	46.4	SNV	COSM5666	CTNNB1	NM_001904:exon3:c.110C>A:p.S37Y
chr6	117687341	T	C	72.0	SNV	---	ROS1	NM_002944:exon18:c.2710A>G:p.I904V
chr6	152540143	A	C	22.3	SNV	---	SYNE1	NM_033071:exon119:c.21826T>G:p.L7276V
chr7	2987388	G	A	78.9	SNV	COSM452940	CARD11	NM_032415:exon3:c.41C>T:p.T14M
chr7	106509517	C	G	28.0	SNV	---	PIK3CG	NM_002649:exon2:c.1511C>G:p.S504C
chr7	128851988	C	T	24.2	SNV	COSM5020286	SMO	NM_005631:exon12:c.2060C>T:p.P687L
chr9	21971007	CAGGTCCACGGG	-	100.0	DEL	---	CDKN2A	NM_000077:exon2:c.340_351del:p.114_117del
chr14	95569756	G	A	48.4	SNV	---	DICER1	NM_030621:exon23:c.3977C>T:p.A1326V
chr16	15931862	T	C	32.2	SNV	---	MYH11	NM_002474:exon2:c.248A>G:p.K83R
chr17				100.0			TP53	Large deletion[c]
chr19	18280013	C	A	45.2	SNV	---	PIK3R2	NM_005027:exon16:c.2096C>A:p.A699D
chr20	57480528	G	A	59.1	SNV	---	GNAS	NM_000516:exon6:c.523G>A:p.A175T
chrX	100608246	C	T	99.0	SNV	---	BTK	NM_000061:exon18:c.1844G>A:p.R615H

HG8T

No mutations in 409 cancer-related genes.

HG9T

chr5	7875383	C	A	30.6	SNV	---	MTRR	NM_002454:exon4:c.296C>A:p.S99X
chr8	71053424	T	A	74.0	SNV	---	NCOA2	NM_006540:exon14:c.3023A>T:p.N1008I
chr11	3752675	A	G	51.9	SNV	---	NUP98	NM_005387:exon14:c.1727T>C:p.F576S
chr15	41797675	G	A	94.5	SNV	---	LTK	NM_002344:exon14:c.1751C>T:p.T584I
chr17	7577126	T	A	95.0	SNV	COSM44469	TP53	NM_000546:exon8:c.812A>T:p.E271V
chr19	11143994	G	A	50.2	SNV	---	SMARCA4	NM_003072:exon26:c.3575G>A:p.R1192H
chr20	57430299	G	A	24.0	SNV	---	GNAS	NM_080425:exon1:c.1979G>A:p.R660H

HG10T

chr1	145532231	G	A	38.3	SNV	---	ITGA10	NM_003637:exon8:c.875G>A:p.C292Y
chr1	145537735	AG	-	38.7	DEL	---	ITGA10	NA (splicing)
chr1	179090821	A	G	48.8	SNV	---	ABL2	NM_005158:exon5:c.824T>C:p.M275T
chr1	220835187	G	T	46.9	SNV	---	MARK1	NM_018650:exon18:c.2067G>T:p.K689N
chr2	148672772	C	-	52.1	DEL	---	ACVR2A	NM_001616:exon5:c.541delC:p.P181fs
chr2	223161776	C	T	49.9	SNV	---	PAX3	NM_000438:exon2:c.242G>A:p.G81D
chr3	142280211	A	G	33.6	SNV	---	ATR	NM_001184:exon5:c.1223T>C:p.I408T
chr3	195593784	C	T	33.3	SNV	---	TNK2	NM_005781:exon14:c.3086G>A:p.G1029D
chr4	55151647	A	-	52.0	DEL	---	PDGFRA	NM_006206:exon17:c.2433delA:p.S811fs
chr4	153247175	-	T	49.8	INS	---	FBXW7	NM_018315:exon9:c.1387dupA:p.R463fs
chr5	180058748	G	-	43.1	DEL	---	FLT4	NM_002020:exon2:c.89delC:p.P30fs
chr6	41555186	C	-	44.1	DEL	---	FOXP4	NM_138457:exon7:c.805delC:p.P269fs

chr6	51612648	G	A	50.4	SNV	---	PKHD1	NM_170724:exon58:c.9766C>T:p.P3256S
chr6	70048837	C	T	49.0	SNV	---	ADGRB3	NM_001704:exon25:c.3218C>T:p.T1073M
chr6	106547205	A	G	38.8	SNV	---	PRDM1	NM_001198:exon4:c.442A>G:p.I148V
chr6	135507123	C	T	52.3	SNV	---	MYB	NM_005375:exon2:c.106C>T:p.R36C
chr6	152472716	T	A	48.6	SNV	---	SYNE1	NM_033071:exon134:c.24209A>T:p.D8070V
chr8	41791555	C	T	29.8	SNV	---	KAT6A	NM_006766:exon17:c.4183G>A:p.D1395N
chr8	48689466	G	-	22.1	DEL	---	PRKDC	NM_006904:exon85:c.12127delC:p.P4040fs
chr8	92983013	G	A	34.0	SNV	COSM33136	RUNX1T1	NM_004349:exon10:c.1331C>T:p.A444V
chr8	92983068	C	T	34.8	SNV	---	RUNX1T1	NM_004349:exon10:c.1276G>A:p.V426I
chr9	133760106	C	T	49.4	SNV	---	ABL1	NM_005157:exon11:c.2429C>T:p.P810L
chr12	46215214	T	-	26.5	DEL	---	ARID2	NM_152641:exon6:c.649delT:p.F217fs
chr12	46243853	T	-	45.6	DEL	---	ARID2	NM_152641:exon15:c.1947delT:p.H649fs
chr12	49430935	GCT	-	34.3	DEL	---	KMT2D	NM_003482:exon34:c.10202_10204del:p.3401_3402del
chr12	49443503	G	A	49.9	SNV	---	KMT2D	NM_003482:exon11:c.3868C>T:p.R1290W
chr12	49444842	G	T	51.9	SNV	---	KMT2D	NM_003482:exon10:c.2624C>A:p.P875H
chr12	121432117	GC	-	43.5	DEL	---	HNF1A	NM_000545:exon4:c.864_865del:p.G288fs
chr12	121432118	CC	-	56.5	DEL	---	HNF1A	NM_000545:exon4:c.865_866del:p.P289fs
chr13	110435136	C	G	43.2	SNV	---	IRS2	NM_003749:exon1:c.3265G>C:p.A1089P
chr14	102551189	CTT	-	48.4	DEL	---	HSP90AA1	NM_005348:exon5:c.806_808del:p.269_270del
chr15	88726662	G	T	47.5	SNV	---	NTRK3	NM_002530:exon5:c.382C>A:p.H128N
chr17	8110652	C	T	49.3	SNV	---	AURKB	NM_001313955:exon4:c.17G>A:p.G6D
chr17	41607297	G	A	49.1	SNV	---	ETV4	NM_001986:exon10:c.910C>T:p.R304X
chr17	75478295	C	T	52.8	SNV	---	SEPT9	NM_006640:exon3:c.737C>T:p.A246V
chr18	59195372	A	T	100.0	SNV	---	CDH20	NM_031891:exon7:c.1190A>T:p.E397V
chr19	11144149	C	T	54.2	SNV	---	SMARCA4	NM_003072:exon26:c.3730C>T:p.R1244C
chr20	31022281	C	A	52.5	SNV	---	ASXL1	NM_015338:exon12:c.1766C>A:p.P589H
chrX	41075161	G	A	46.3	SNV	---	USP9X	NM_001039590:exon35:c.5341G>A:p.V1781I
chrX	153762700	C	T	44.5	SNV	---	G6PD	NM_000402:exon6:c.587G>A:p.R196H

HG11T

chr1	45795081	G	A	68.7	SNV	---	MUTYH	NM_012222:exon16:c.1538C>T:p.P513L
chr2	24952578	T	C	27.4	SNV	---	NCOA1	NM_003743:exon15:c.3095T>C:p.F1032S
chr4	62936411	C	A	47.2	SNV	---	ADGRL3	NM_015236:exon25:c.4195C>A:p.Q1399K
chr15	66729181	A	G	62.2	SNV	---	MAP2K1	NM_002755:exon3:c.389A>G:p.Y130C
chr17	7578263	G	A	100.0	SNV	COSM10705	TP53	NM_000546:exon6:c.586C>T:p.R196X
chr17	37880257	C	G	100.0	SNV	COSM51317	ERBB2	NM_004448:exon19:c.2301C>G:p.I767M

HG12T

chr1	162725039	G	A	40.8	SNV	---	DDR2	NM_006182:exon6:c.511G>A:p.D171N
chr2	141459833	C	T	49.0	SNV	---	LRP1B	NM_018557:exon39:c.6179G>A:p.R2060H
chr6	152129391	C	T	56.5	SNV	---	ESR1	NM_000125:exon1:c.344C>T:p.P115L
chr6	152129451	A	G	41.8	SNV	---	ESR1	NM_000125:exon1:c.404A>G:p.E135G
chr6	152472810	G	A	47.2	SNV	---	SYNE1	NM_033071:exon134:c.24115C>T:p.R8039C
chr6	152532711	C	T	46.0	SNV	---	SYNE1	NM_033071:exon123:c.22294G>A:p.E7432K
chr7	2946337	C	T	59.7	SNV	---	CARD11	NM_032415:exon25:c.3400G>A:p.V1134I
chr16	65005506	T	G	24.2	SNV	---	CDH11	NM_001797:exon11:c.1618A>C:p.N540H
chr16	66426109	G	A	53.8	SNV	---	CDH5	NM_001795:exon7:c.1040G>A:p.R347Q
chr16	68863616	C	G	51.4	SNV	---	CDH1	NM_004360:exon15:c.2355C>G:p.N785K
chr17	37868208	C	A	58.1	SNV	---	ERBB2	NM_004448:exon8:c.929C>A:p.S310Y

chr19	18279669	G	A	57.0	SNV	---	PIK3R2	NM_005027:exon15:c.1942G>A:p.E648K

HG13T

chr1	162724541	C	T	44.1	SNV	---	DDR2	NM_006182:exon5:c.313C>T:p.R105C
chr1	185069410	G	A	40.2	SNV	---	RNF2	NM_007212:exon7:c.988G>A:p.A330T
chr1	241680541	C	T	44.0	SNV	---	FH	NM_000143:exon2:c.208G>A:p.A70T
chr2	141294155	T	A	51.5	SNV	---	LRP1B	NM_018557:exon46:c.7637A>T:p.Y2546F
chr2	148683686	A	-	57.7	DEL	---	ACVR2A	NM_001616:exon10:c.1304delA:p.K435fs
chr2	216292965	G	A	57.6	SNV	---	FN1	NM_054034:exon6:c.782C>T:p.T261I
chr2	223163270	C	T	31.6	SNV	---	PAX3	NM_000438:exon1:c.65G>A:p.R22H
chr3	37090443	T	C	89.9	SNV	---	MLH1	NM_000249:exon18:c.2038T>C:p.C680R
chr3	187447511	G	A	26.1	SNV	---	BCL6	NM_001706:exon5:c.682C>T:p.R228W
chr5	180047947	G	A	45.7	SNV	---	FLT4	NM_002020:exon15:c.2228C>T:p.A743V
chr6	56476324	A	-	58.3	DEL	---	DST	NM_015548:exon24:c.3518delT:p.L1173fs
chr6	117609728	C	T	52.2	SNV	---	ROS1	NM_002944:exon43:c.6971G>A:p.C2324Y
chr7	116409799	C	T	47.8	SNV	---	MET	NM_000245:exon12:c.2684C>T:p.T895M
chr8	113267554	A	T	49.8	SNV	---	CSMD3	NM_052900:exon60:c.9458T>A:p.I3153K
chr9	136913503	G	A	78.4	SNV	---	BRD3	NM_007371:exon6:c.788C>T:p.S263L
chr10	89693007	A	-	65.2	DEL	COSM5847	PTEN	NM_000314:exon5:c.487delA:p.K163fs
chr10	89725051	T	G	55.7	SNV	---	PTEN	NM_000314:exon9:c.1034T>G:p.L345R
chr11	32456771	C	T	53.4	SNV	---	WT1	NM_000378:exon1:c.121G>A:p.A41T
chr13	110436710	G	A	57.4	SNV	---	IRS2	NM_003749:exon1:c.1691C>T:p.A564V
chr14	99642275	G	A	44.4	SNV	---	BCL11B	NM_022898:exon3:c.685C>T:p.R229W
chr14	99642286	C	-	44.4	DEL	---	BCL11B	NM_022898:exon3:c.674delG:p.G225fs
chr17	7577121	G	A	92.8	SNV	COSM99933	TP53	NM_000546:exon8:c.817C>T:p.R273C
chr17	29661945	C	T	64.1	SNV	COSM30766	NF1	NM_000267:exon39:c.5839C>T:p.R1947X
chr18	45394825	A	-	26.4	DEL	---	SMAD2	NM_005901:exon5:c.524delT:p.L175fs
chr19	18278020	G	A	48.8	SNV	---	PIK3R2	NM_005027:exon13:c.1640G>A:p.R547Q
chr20	57484420	C	T	55.6	SNV	COSM123397	GNAS	NM_000516:exon8:c.601C>T:p.R201C
chrX	48544188	G	T	45.2	SNV	---	WAS	NM_000377:exon4:c.426G>T:p.Q142H
chrX	63411537	G	A	21.0	SNV	---	AMER1	NM_152424:exon2:c.1630C>T:p.P544S
chrX	70627470	C	T	44.2	SNV	---	TAF1	NM_004606:exon27:c.4214C>T:p.T1405M

HG14T

chr2	24914529	G	T	48.7	SNV	---	NCOA1	NM_003743:exon7:c.712G>T:p.D238Y
chr3	30713544	AGA	-	51.8	DEL	---	TGFBR2	NM_003242:exon4:c.869_871del:p.290_291del
chr3	52442567	G	A	98.7	SNV	---	BAP1	NM_004656:exon4:c.178C>T:p.R60X
chr3	178936091	G	A	51.0	SNV	COSM125370	PIK3CA	NM_006218:exon10:c.1633G>A:p.E545K
chr16	68844179	A	G	98.4	SNV	---	CDH1	NM_004360:exon6:c.767A>G:p.N256S
chr17	7578496	A	C	97.3	SNV	COSM45351	TP53	NM_000546:exon5:c.434T>G:p.L145R
chrX	70674025	G	C	37.3	SNV	---	TAF1	NM_004606:exon33:c.4819G>C:p.E1607Q

HG15T

chr2	219562333	C	T	59.4	SNV	---	STK36	NM_015690:exon24:c.2909C>T:p.A970V
chr3	89259092	A	C	28.0	SNV	---	EPHA3	NM_005233:exon3:c.236A>C:p.N79T
chr5	112170745	C	-	100.0	DEL	---	APC	NM_000038:exon15:c.1841delC:p.A614fs
chr6	152675840	C	T	75.5	SNV	---	SYNE1	NM_182961:exon67:c.10880G>A:p.R3627H
chr11	71729920	C	T	62.1	SNV	---	NUMA1	NM_006185:exon10:c.691G>A:p.D231N
chr11	106810667	G	T	63.0	SNV	---	GUCY1A2	NM_000855:exon4:c.725C>A:p.P242H

chr12	25398285	C	T	38.2	SNV	COSM517	KRAS	NM_004985:exon2:c.34G>A:p.G12S
chr16	14028150	G	C	35.3	SNV	---	ERCC4	NM_005236:exon7:c.1204G>C:p.G402R
chr17	7577094	G	A	100.0	SNV	COSM10704	TP53	NM_000546:exon8:c.844C>T:p.R282W
chr17	11958269	C	T	46.8	SNV	---	MAP2K4	NM_003010:exon2:c.179C>T:p.T60I
chr18	48573628	G	T	54.9	SNV	COSM7410653	SMAD4	NM_005359:exon2:c.212G>T:p.C71F
chrX	110391010	A	C	45.6	SNV	---	PAK3	NM_002578:exon7:c.322A>C:p.T108P

HG16T

chr3	3209379	G	A	50.8	SNV	---	CRBN	NM_016302:exon5:c.626C>T:p.P209L
chr5	112176017	G	T	100.0	SNV	COSM236691	APC	NM_000038:exon16:c.4726G>T:p.E1576X
chr6	152599391	T	A	50.6	SNV	---	SYNE1	NM_033071:exon97:c.18193A>T:p.K6065X,
chr8	71036145	C	G	54.7	SNV	---	NCOA2	NM_006540:exon21:c.4267G>C:p.G1423R
chr8	113651126	A	C	51.3	SNV	---	CSMD3	NM_052900:exon20:c.3013T>G:p.F1005V
chr8	37697642	G	A	49.2	SNV	---	ADGRA2	NM_032777:exon17:c.2515G>A:p.G839S
chr10	104159195	CA	TG	42.2	MNV	---	NFKB2	NM_002502:exon13:c.1268_1269TG
chr11	106680767	T	G	48.9	SNV	---	GUCY1A2	NM_000855:exon5:c.1644A>C:p.E548D
chr17	5424974	A	G	100.0	SNV	---	NLRP1	NM_033004:exon13:c.3653T>C:p.L1218P
chr17	7578449	C	A	100.0	SNV	COSM43549	TP53	NM_000546:exon5:c.481G>T:p.A161S
chr18	59217341	A	C	30.9	SNV	---	CDH20	NM_031891:exon11:c.1779A>C:p.Q593H
chr20	40980892	T	G	50.4	SNV	---	PTPRT	NM_007050:exon10:c.1594A>C:p.S532R
chr21	39817504	T	G	91.1	SNV	---	ERG	NM_004449:exon4:c.80A>C:p.E27A

HG17T

chr1	47691173	C	T	73.3	SNV	---	TAL1	NM_003189:exon4:c.388G>A:p.A130T
chr1	145537512	G	A	50.1	SNV	---	ITGA10	NM_003637:exon20:c.2522G>A:p.S841N
chr1	237058733	G	A	50.3	SNV	---	MTR	NM_000254:exon31:c.3481G>A:p.A1161T
chr2	5833692	C	T	56.5	SNV	---	SOX11	NM_003108:exon1:c.839C>T:p.T280M
chr2	140990847	G	A	53.1	SNV	---	LRP1B	NM_018557:exon91:c.13708C>T:p.Q4570X
chr2	141114024	T	A	47.3	SNV	---	LRP1B	NM_018557:exon75:c.11417A>T:p.E3806V
chr2	141625795	G	A	44.0	SNV	---	LRP1B	NM_018557:exon26:c.4207C>T:p.R1403C
chr2	219544700	G	A	51.5	SNV	---	STK36	NM_015690:exon9:c.1033G>A:p.G345R
chr3	138664876	G	A	61.2	SNV	---	FOXL2	NM_023067:exon1:c.689C>T:p.A230V
chr4	1807388	C	T	47.0	SNV	---	FGFR3	NM_000142:exon12:c.1637C>T:p.T546M
chr4	1962801	G	A	49.1	SNV	---	NSD2	NM_133330:exon20:c.3295G>A:p.E1099K
chr4	1978254	C	T	46.4	SNV	---	NSD2	NM_133330:exon23:c.3674C>T:p.T1225M
chr4	55138644	C	T	54.2	SNV	---	PDGFRA	NM_006206:exon9:c.1321C>T:p.P441S
chr4	55970882	C	T	49.7	SNV	---	KDR	NM_002253:exon13:c.1915G>A:p.D639N
chr4	87968244	G	A	44.9	SNV	---	AFF1	NM_005935:exon3:c.536G>A:p.R179Q
chr5	176524292	G	T	53.3	SNV	---	FGFR4	NA (splicing)
chr6	51612675	C	T	52.0	SNV	---	PKHD1	NM_138694:exon58:c.9739G>A:p.V3247I
chr6	69348958	C	T	37.5	SNV	---	ADGRB3	NM_001704:exon3:c.391C>T:p.R131C
chr6	152461296	C	A	50.5	SNV	---	SYNE1	NM_033071:exon140:c.25103G>T:p.G8368V
chr6	152539487	C	T	48.7	SNV	---	SYNE1	NM_033071:exon120:c.21883G>A:p.A7295T
chr7	13971195	G	A	48.2	SNV	---	ETV1	NM_004956:exon9:c.734C>T:p.A245V
chr7	98547355	C	T	46.4	SNV	---	TRRAP	NM_003496:exon35:c.4951C>T:p.R1651C
chr7	98608684	G	A	50.4	SNV	---	TRRAP	NM_003496:exon69:c.10819G>A:p.D3607N
chr7	126544156	T	-	99.3	DEL	---	GRM8	NM_000845:exon4:c.887delA:p.K296fs
chr7	128845518	C	T	49.2	SNV	---	SMO	NM_005631:exon4:c.815C>T:p.A272V
chr7	152055732	C	G	100.0	SNV	---	KMT2C	NM_170606:exon2:c.190G>C:p.E64Q

chr8	145739598	C	T	31.1	SNV	---	RECQL4	NM_004260:exon11:c.1853G>A:p.R618Q
chr10	76789461	G	A	53.7	SNV	---	KAT6B	NM_012330:exon18:c.4879G>A:p.A1627T
chr10	104160958	ACG	-	53.6	DEL	---	NFKB2	NM_002502:exon19:c.2093_2095del:p.698_699del
chr10	104160962	G	T	53.4	SNV	---	NFKB2	NM_002502:exon19:c.2097G>T:p.E699D
chr11	32456494	G	A	69.1	SNV	---	WT1	NM_024426:exon1:c.398C>T:p.P133L
chr11	118377154	G	A	53.0	SNV	---	KMT2A	NM_005933:exon27:c.10538G>A:p.G3513E
chr12	46123699	A	G	51.4	SNV	---	ARID2	NM_152641:exon1:c.80A>G:p.H27R
chr12	49434492	G	-	55.1	DEL	---	KMT2D	NM_003482:exon31:c.7061delC:p.P2354fs
chr12	56481660	C	T	46.8	SNV	---	ERBB3	NM_001982:exon6:c.695C>T:p.A232V
chr12	121432115	G	-	55.3	DEL	---	HNF1A	NM_000545:exon4:c.862delG:p.G288fs
chr13	28959144	C	T	42.6	SNV	---	FLT1	NM_002019:exon14:c.1994G>A:p.R665Q
chr13	110435129	G	A	64.2	SNV	---	IRS2	NM_003749:exon1:c.3272C>T:p.P1091L
chr14	23776992	T	G	51.6	SNV	---	BCL2L2	NM_004050:exon3:c.16T>G:p.S6A
chr14	99642359	C	-	100.0	DEL	---	BCL11B	NM_022898:exon3:c.601delG:p.E201fs
chr15	88420264	G	A	47.5	SNV	---	NTRK3	NM_002530:exon19:c.2380C>T:p.Q794X
chr15	91295095	A	G	55.5	SNV	---	BLM	NM_000057:exon4:c.878A>G:p.D293G
chr16	3807902	G	A	50.1	SNV	---	CREBBP	NM_004380:exon18:c.3517C>T:p.R1173X
chr17	8110651	G	A	52.5	SNV	---	AURKB	NM_004217:exon5:c.241C>T:p.R81C
chr17	45360843	G	A	51.3	SNV	---	ITGB3	NM_000212:exon3:c.289G>A:p.D97N
chr19	42795811	C	T	44.3	SNV	---	CIC	NM_015125:exon11:c.2800C>T:p.R934W
chr19	57744888	G	T	46.7	SNV	---	AURKC	NM_003160:exon5:c.394G>T:p.D132Y
chr20	31017181	G	A	50.0	SNV	---	ASXL1	NM_015338:exon6:c.512G>A:p.R171Q
chr20	31017747	-	CAG	52.3	INS	---	ASXL1	NM_015338:exon7:c.608_609insCAG:p.S203delinsSS
chr20	31019407	C	T	49.9	SNV	---	ASXL1	NM_015338:exon9:c.904C>T:p.R302C
chr20	57415336	C	T	65.0	SNV	---	GNAS	NM_016592:exon1:c.175C>T:p.Q59X
chr20	57415354	C	T	65.1	SNV	---	GNAS	NM_016592:exon1:c.193C>T:p.L65F
chr20	57429959	C	T	46.3	SNV	---	GNAS	NM_080425:exon1:c.1639C>T:p.R547C
chr22	33198077	C	-	52.1	DEL	---	TIMP3	NM_000362:exon1:c.90delC:p.H30fs

HG18T

chr3	3214610	C	A	45.8	SNV	---	CRBN	NA (splicing)
chr3	128204594	G	A	50.9	SNV	---	GATA2	NM_032638:exon3:c.847C>T:p.R283C
chr3	134851696	C	T	51.0	SNV	---	EPHB1	NM_004441:exon5:c.1102C>T:p.R368W
chr8	114111160	A	G	28.5	SNV	---	CSMD3	NM_052900:exon5:c.742T>C:p.S248P
chr11	94194148	-	A	61.6	INS	---	MRE11	NM_005590:exon12:c.1280dupT:p.L427fs
chr17	7578418	T	C	100.0	SNV	COSM44732	TP53	NM_000546:exon5:c.512A>G:p.E171G
chr21	46313417	C	T	47.6	SNV	---	ITGB2	NM_000211:exon10:c.1126G>A:p.D376N

Abbreviations: SNV, single nucleotide variant; MNV, multiple nucleotide variant; INS, insertion; DEL, deletion.

[a]The list of 409 genes is available at http://assets.thermofisher.com/TFS-Assets/CSD/Reference-Materials/ion-ampliseq-cancer-panel-gene-list.pdf.

[b]The cancer-specific mutations were detected referring to the profiles of matched normal DNA.

[c]The gene deletion was detected with Integrative Genomics Viewer.

Supplementary table 5: Mutational status of 409 cancer-related genes in 25 gastric cancer stem cell (GC-SC) spheroid lines in the second patient cohort detected using RNA sequencing (RNA-seq).

Chrom	Position	Ref	Variant	Frequency	Type	Allele Name	Gene Symbol[a]	AAChange.refGene
HG19T								
chr9	134053745	G	A	36.4	SNV	---	NUP214	NM_005085:exon24:c.3367G>A:p.V1123I
chr17	7577120	C	T	100.0	SNV	COSM10660	TP53	NM_001126115:exon4:c.422G>A:p.R141H
HG20T								
chr6	56566690	C	T	54.5	SNV	---	DST	NM_183380:exon4:c.317G>A:p.R106H
chr17	7577094	G	A	100.0	SNV	COSM10704	TP53	NM_000546:exon8:c.844C>T:p.R282W
chr19	11170854	A	C	100.0	SNV	---	SMARCA4	NM_003072:exon34:c.4902A>C:p.E1634D
HG21T								
chr3	30732970	G	A	65.2	SNV	COSM33076	TGFBR2	NM_003242:exon7:c.1583G>A:p.R528H
chr17	37880261	G	T	97.6	SNV	COSM1251412	ERBB2	NM_004448:exon19:c.2305G>T:p.D769Y
chr17				100.0		---	TP53	Splicing[b]
chr19	11101959	AGA	-	47.2	DEL	COSM30583	SMARCA4	NM_003072:exon8:c.1379_1381del:p.460_461del
HG22T								
No detectable mutations in 409 cancer-related genes.								
HG23T								
chr1	27106320	-	G	33.3	INS	COSM6916114	ARID1A	NM_006015:exon20:c.5932dupG:p.L1977fs
HG24T								
No detectable mutations in 409 cancer-related genes.								
HG25T								
chr13	48934188	T	C	60.0	SNV	---	RB1	NM_000321:exon7:c.643T>C:p.S215P
chr16	3828111	G	A	30.4	SNV	COSM7347140	CREBBP	NM_004380:exon10:c.2014C>T:p.R672C
chr17	37682291	C	T	50.0	SNV	---	CDK12	NM_015083:exon13:c.3482C>T:p.T1161M
HG26T								
chr8	57079350	T	C	55.0	SNV	---	PLAG1	NM_002655:exon5:c.955A>G:p.I319V
chr20	39795470	G	A	47.0	SNV	COSM3291377	PLCG1	NM_002660:exon19:c.2272G>A:p.E758K
HG28T								
chr17	7577100	T	C	100.0	SNV	COSM11123	TP53	NM_000546:exon8:c.838A>G:p.R280G
HG29T								
chr1	27106804	C	-	42.1	DEL	---	ARID1A	NM_006015:exon20:c.6415delC:p.P2139fs
chr2	148683686	A	-	54.2	DEL	---	ACVR2A	NM_001616:exon10:c.1303delA:p.K435fs
chr3	30691872	AA	-	100.0	DEL	COSM5989666	TGFBR2	NM_001024847:exon4:c.449_450del:p.E150fs
chr3	30691873	-	A	100.0	INS	---	TGFBR2	NM_001024847:exon4:c.450dupA:p.E150fs
chr3	66023896	C		37.5	DEL	---	MAGI1	NM_004742:exon1:c.88delG:p.V30X

chr3	69928320	C	T	62.5	SNV	---	MITF	NM_006722:exon2:c.137C>T:p.P46L
chr3	187447663	C	T	55.8	SNV	---	BCL6	NM_001706:exon5:c.530G>A:p.S177N
chr3	195595423	C	A	41.1	SNV	---	TNK2	NM_005781:exon12:c.1701G>T:p.E567D
chr3	195615342	A	G	36.7	SNV	---	TNK2	NM_005781:exon2:c.118T>C:p.Y40H
chr5	138223183	G	A	36.2	SNV	COSM6369106	CTNNA1	NM_001903:exon9:c.1148G>A:p.R383H
chr5	176722087	C	-	35.7	DEL	---	NSD1	NM_022455:exon23:c.7718delC:p.S2573fs
chr6	52876605	G	A	40.9	SNV	---	ICK	NM_014920:exon11:c.1454C>T:p.A485V
chr6	56434717	T	-	41.9	DEL	---	DST	NM_015548:exon35:c.5946delA:p.K1982fs
chr6	56600064	T	C	33.3	SNV	---	DST	NM_183380:exon2:c.115A>G:p.K39E
chr7	116395528	T	A	48.7	SNV	---	MET	NM_000245:exon6:c.1821T>A:p.N607K
chr9	120475384	TTC	-	42.1	DEL	---	TLR4	NM_003266:exon4:c.858_860del:p.286_287del
chr9	133759541	C	T	47.5	SNV	---	ABL1	NM_005157:exon11:c.1864C>T:p.R622W
chr9	133759623	C	-	31.6	DEL	---	ABL1	NM_005157:exon11:c.1946delC:p.T649fs
chr9	133760108	C	T	25.9	SNV	---	ABL1	NM_005157:exon11:c.2431C>T:p.P811S
chr10	76735496	G	C	56.3	SNV	---	KAT6B	NM_012330:exon8:c.1401G>C:p.K467N
chr10	76788342	C	T	36.5	SNV	COSM257480	KAT6B	NM_012330:exon18:c.3760C>T:p.R1254C
chr11	69456196	G	A	48.3	SNV	---	CCND1	NM_053056:exon1:c.115G>A:p.A39T
chr11	95825682	C	T	40.0	SNV	---	MAML2	NM_032427:exon2:c.1513G>A:p.G505S
chr12	25398284	C	T	53.3	SNV	COSM521	KRAS	NM_004985:exon2:c.35G>A:p.G12D
chr12	132510328	C	T	34.8	SNV	---	EP400	NM_015409:exon25:c.4993C>T:p.P1665S
chr15	74315557	G	A	34.1	SNV	COSM1937940	PML	NM_002675:exon3:c.991G>A:p.A331T
chr16	14029219	G	A	46.2	SNV	COSM8194527	ERCC4	NM_005236:exon8:c.1430G>A:p.R477Q
chr17	5436192	G	-	28.6	DEL	---	NLRP1	NM_014922:exon11:c.3246delC:p.P1082fs
chr17	12043184	A	G	27.0	SNV	---	MAP2K4	NM_003010:exon10:c.1069A>G:p.K357E
chr17	75484906	G	A	38.0	SNV	---	SEPT9	NM_006640:exon6:c.1168G>A:p.V390I
chr19	18870855	C	A	40.5	SNV	---	CRTC1	NM_015321:exon8:c.703C>A:p.L235M
chr19	45855781	T	C	28.9	SNV	COSM1630983	ERCC2	NM_000400:exon21:c.2029A>G:p.M677V
chr20	54958077	T	C	26.5	SNV	---	AURKA	NM_003600:exon5:c.530A>G:p.Q177R
chr20	54961519	G	T	38.3	SNV	COSM6274846	AURKA	NM_003600:exon3:c.113C>A:p.P38H
chrX	44732910	C	-	41.2	DEL	---	KDM6A	NM_021140:exon1:c.113delC:p.S38fs

HG32T

Chr1	2493196	C	G	51.1	SNV	---	TNFRSF14	NM_003820:exon6:c.636C>G:p.I212M
Chr2	148672848	T	C	58.1	SNV	---	ACVR2A	NM_001616:exon5:c.617T>C:p.V206A
Chr3	142188286	T	G	32.4	SNV	---	ATR	NM_001184:exon38:c.6445A>C:p.I2149L
Chr20	36030983	C	T	32.6	SNV	COSM4430704	SRC	NM_005417:exon12:c.1262C>T:p.A421V

HG33T

chr3	10084304	T	C	28.6	SNV	---	FANCD2	NM_033084:exon11:c.845T>C:p.I282T
chr6	56434742	G	A	33.3	SNV	---	DST	NM_015548:exon35:c.5921C>T:p.S1974L
chr6	56434780	C	A	100.0	SNV	---	DST	NM_015548:exon35:c.5883G>T:p.Q1961H
chr6	56505257	A	G	28.6	SNV	---	DST	NM_015548:exon4:c.563T>C:p.L188S
chr11	108117816	G	A	40.0	SNV	---	ATM	NM_000051:exon8:c.1027G>A:p.E343K
chr15	99251312	T	C	50.0	SNV	---	IGF1R	NM_000875:exon2:c.616T>C:p.W206R
chr17	7576873	C	A	100.0	SNV	COSM307331	TP53	NM_000546:exon9:c.973G>T:p.G325X
chr22	23652547	G	A	35.0	SNV	---	BCR	NM_004327:exon18:c.3109G>A:p.E1037K

HG34T

chr5	112173917	C	T	50.0	SNV	COSM18852	APC	NM_000038:exon16:c.2626C>T:p.R876X

chr5	112175639	C	T	58.3	SNV	COSM13127	APC	NM_000038:exon16:c.4348C>T:p.R1450X
chr11	71726490	C	T	47.8	SNV	COSM9833905	NUMA1	NM_006185:exon15:c.2059G>A:p.A687T
chr12	46230707	C	T	66.7	SNV	COSM6955940	ARID2	NM_152641:exon8:c.956C>T:p.S319F
HG35T								
chr1	27106861	C	T	56.3	SNV	COSM51432	ARID1A	NM_006015:exon20:c.6472C>T:p.R2158X
chr5	112175799	C	A	100.0	SNV	COSM5732639	APC	NM_000038:exon16:c.4508C>A:p.S1503X
chr10	49612963	A	C	50.0	SNV	---	MAPK8	NM_139046:exon5:c.191A>C:p.Q64P
chr12	56489535	G	A	54.5	SNV	COSM1677075	ERBB3	NM_001982:exon17:c.2000G>A:p.R667H
chr17				100.0		---	TP53	Splicing[b]
HG36T								
chr16	50830391	A	G	48.9	SNV	---	CYLD	NM_015247:exon20:c.2843A>G:p.Q948R
chr17	29509642	G	T	30.4	SNV	COSM3179569	NF1	NM_000267:exon8:c.847G>T:p.D283Y
HG37T								
chr2	47690192	T	C	52.4	SNV	---	MSH2	NM_000251:exon9:c.1409T>C:p.V470A
chr7	116422120	G	T	37.1	SNV	---	MET	NM_000245:exon18:c.3601G>T:p.V1201F
chr17	7577547	C	A	100.0	SNV	COSM11196	TP53	NM_000546:exon7:c.734G>T:p.G245V
HG38T								
chr10	102891485	G	A	100.0	SNV	---	TLX1	NM_005521:exon1:c.187G>A:p.A63T
chr14	92482072	C	T	47.6	SNV	COSM6279187	TRIP11	NM_004239:exon6:c.791G>A:p.R264Q
chr17	7577574	T	C	100.0	SNV	COSM10731	TP53	NM_000546:exon7:c.707A>G:p.Y236C
HG39T								
chr15	90633765	A	G	48.0	SNV	---	IDH2	NM_002168:exon3:c.319T>C:p.Y107H
chr17	48264477	G	A	51.9	SNV	---	COL1A1	NM_000088:exon47:c.3430C>T:p.P1144S
HG40T								
chr1	226564855	G	A	60.0	SNV	COSM1219296	PARP1	NM_001618:exon13:c.1895C>T:p.T632M
chr6	160468835	C	A	39.6	SNV	---	IGF2R	NM_000876:exon17:c.2241C>A:p.N747K
chr22	41554449	G	A	40.0	SNV	---	EP300	NM_001429:exon19:c.3535G>A:p.G1179S
HG42T								
chr1	27087503	C	T	100.0	SNV	COSM184236	ARID1A	NM_006015:exon5:c.2077C>T:p.R693X
chr3	178936091	G	A	42.9	SNV	COSM763	PIK3CA	NM_006218:exon10:c.1633G>A:p.E545K
chr5	112176008	G	T	43.8	SNV	COSM4167225	APC	NM_000038:exon16:c.4717G>T:p.E1573X
chr22	23655131	C	T	32.8	SNV	---	BCR	NM_004327:exon20:c.3380C>T:p.T1127M
chr22	41546045	C	T	56.5	SNV	---	EP300	NM_001429:exon14:c.2660C>T:p.T887I
HG43T								
chr7	2956956	G	C	41.3	SNV	---	CARD11	NM_032415:exon20:c.2671C>G:p.R891G
HG44T								
chr6	51890782	T	C	50.0	SNV	---	PKHD1	NM_170724:exon32:c.3826A>G:p.R1276G
chr6	152461248	T	C	33.3	SNV	---	SYNE1	NM_033071:exon140:c.25151A>G:p.E8384G
chr7	2959046	C	G	29.3	SNV	COSM452935	CARD11	NM_032415:exon18:c.2470G>C:p.D824H
chr9	22006138	G	A	100.0	SNV	COSM6983462	CDKN2B	NM_004936:exon2:c.265C>T:p.R89W

chr15	91292605	C	T	25.0	SNV	---	BLM	NM_000057:exon3:c.107C>T:p.T36I
HG45T								
chr7	2977555	G	A	31.3	SNV	COSM3027901	CARD11	NM_032415:exon8:c.1129C>T:p.R377W
HG46T								
chr11	64572285	G	A	68.8	SNV	COSM8474098	MEN1	NM_000244:exon10:c.1369C>T:p.R457W
chr17	7579311	C	T	100.0	SNV	---	TP53	Splicing[b]
HG47T								
chr1	179077046	T	A	50.0	SNV	---	ABL2	NM_007314:exon12:c.3356A>T:p.Y1119F
chr7	91630394	G	C	44.4	SNV	---	AKAP9	NM_005751:exon8:c.1163G>C:p.R388T
chr8	118825130	G	A	54.8	SNV	COSM1454473	EXT1	NM_000127:exon8:c.1703C>T:p.T568M
chr8	42166476	G	A	38.5	SNV	COSM1099990	IKBKB	NM_001556:exon8:c.625G>A:p.G209S
chr17	7577106	G	A	100.0	SNV	COSM10939	TP53	NM_000546:exon8:c.832C>T:p.P278S
chr19	18856733	A	G	40.0	SNV	---	CRTC1	NM_015321:exon3:c.344A>G:p.H115R

Abbreviations: SNV, single nucleotide variant; INS, insertion; DEL, deletion.

[a]Only mutations in 409 cancer-related genes (see Table S4) were listed.

[b]Aberrant splicing was detected with Integrative Genomics Viewer.

Supplementary table 6: Summary of immunohistochemistry analysis for mismatch repair proteins in the primary tumor and spheroids in four hypermutated gastric cancer (GC) cases.

	MSH2				MSH6			
	Normal epithelium		Cancer		Normal epithelium		Cancer	
	Primary	Spheroids	Primary	Spheroids	Primary	Spheroids	Primary	Spheroids
HG3T	+	+	+	+	+	+	+	+
HG10T	+	+	+	+	+	+	+	+
HG13T	+	+	+	+	+	+	+	+
HG17T	+	+	+	+	+	+	+	+

	MLH1				PMS2			
	Normal epithelium		Cancer		Normal epithelium		Cancer	
	Primary	Spheroids	Primary	Spheroids	Primary	Spheroids	Primary	Spheroids
HG3T	+	+	–	–	+	+	–	–
HG10T	+	+	–	–	+	+	–	–
HG13T	+	+	–	–	+	+	–	–
HG17T	+	+	–	–	+	+	–	–

Abbreviations: +, positive; –, negative.