Supplement 1

Lysis with proteinase K (specific activity of the solution > 600 mAU/mL): CFs were equilibrated to room temperature and then treated with 400 µL of proteinase K. Cyst tissues were removed from Allprotect Tissue Reagent, cut into small pieces and then placed into 360 µL of ATL Buffer with 40 µL of proteinase K. Samples were mixed and incubated at 56°C overnight. AL buffer (4 mL for cyst fluid and 400 µL for neoplastic tissue) was added at 70°C for 10 min and then completed with an equal volume of pure ethanol.

Purification on column: mixtures were applied to the QIAamp spin columns and centrifuged at 6,000 g for 1 min. This operation was repeated until all samples passed. Residual contaminants were removed by using two different washing buffers: 500 µL of AW1 Buffer was first added to the column, which was centrifuged at 6,000 g for 1 min, and then 500 µL of AW2 Buffer was added and the column was centrifuged at 20,000 g for 3 min. The column was completely dried by a supplementary centrifugation at full speed for 1 min.

Elution: Columns were placed into a 1.5 mL microcentrifuge tube. DNAs were eluted in 40 µl nuclease-free water, incubated at room temperature for 3 min and then centrifuged at 6,000 g for 1 min. Genomic DNA was extracted from peripheral blood collected in EDTA tubes using a FlexiGene DNA kit (Qiagen, Germany) following the manufacturer's recommendations. DNA was quantified using a Qubit dsDNA HS Assay on a Qubit 2.0 fluorometer (Life Technologies, CA, USA) and a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, USA).

Supplement 2

List of genes for sequencing:

ABCB1	CD302	ERG	IL7R	MLL	PHF10	RNF43	TET2
ABCC1	CDA	ESR1	IMP4	MLL2	PHLPP2	ROBO1	TEX15
ABCC2	CDH1	ESR2	IMPAD1	MLL3	PHOX2B	ROBO2	TGFBR2
ABCC4	CDH13	ETV1	INSIG2	MLL4	PIK3C2G	ROPN1L	TLR3
ABCG1	CDH20	ETV4	INSR	MMP16	PIK3C3	ROS1	TMPRSS2
ABCG2	CDH5	ETV5	IRAK1	MOB1A	PIK3CA	RPS6KA3	TNFSF10
ABL1	CDK12	ETV6	IRF2	MOB1B	PIK3CG	RPS6KA5	TNKS
ABL2	CDK4	EWSR1	IRS1	MORC1	PIK3R1	RPS6KB1	TNKS2
ACTL6A	CDK6	EZH2	IRS2	MPL	PIK3R2	RPS6KC1	TOP1
ACTL6B	CDKN1B	FAM123B	ISOC1	MPP6	PIM1	RPTN	TP53
ACVR1B	CDKN2A	FAM190A	JAK1	MRPS9	PIM2	RPTOR	TP73
AFF2	CDKN2B	FANCA	JAK2	MSH2	PIM3	RSPO1	TPK1
AKT1	CDKN2C	FBXW7	JAK3	MSH3	PKHD1	RSPO2	TPMT
AKT2	CHD4	FCGR2A	JUN	MSH6	PKM	RSPO3	TPR
AKT3	CHD5	FCGR3A	KAT6A	MST1	PKM2	RSPO4	TRAF2
ALK	CHEK1	FGF2	KAT6B	MST1R	PLAT	RUNX1	TRAF3
ALKBH3	CHEK2	FGF5	KDM2A	MTHFR	PLK2	SAV1	TRAF7
ANO1	CHFR	FGFR1	KDM2B	MTOR	PLXDC2	SELL	TSC1
APC	CHODL	FGFR2	KDM3B	MYB	PMS2	SETD2	TSC2
APCDD1	CHUK	FGFR3	KDM4A	MYC	POLD1	SETD6	TSHR
AR	CIC	FGFR4	KDM4B	MYCL1	POLE	SF3B1	TTK
ARAF	CREBBP	FH	KDM4C	MYCN	PORCN	SFRP1	TYMS
ARID1A	CRKL	FKBP3	KDM5A	MYH9	PPARA	SIM1	U2AF1
ARID1B	CTCF	FLG	KDM5C	NAALADL2	PPIF	SLC19A1	UBE2A
ARID2	CTLA4	FLG2	KDM6A	NAF1	PPM1L	SLC35F3	UGT1A1
ARRDC3	CTNNB1	FLI1	KDR	NAT2	PPP1R3A	SLCO1B1	UGT8
ATAD2	CYP11B2	FLRT2	KEAP1	NCOR1	PPP2R1A	SLIT2	USP8
ATM	CYP17A1	FLRT3	KHDRBS3	NDE1	PPP6C	SLITRK6	VEGFA
ATR	CYP19A1	FLT1	KIF5B	NDFIP2	PRDM1	SMAD2	VHL
ATRX	CYP1B1	FLT3	KIT	NEGR1	PRDM9	SMAD3	VPREB1
AURKA	CYP2A6	FLT4	KLF13	NF1	PREX2	SMAD4	WDFY4
AURKB	CYP2B6	FOXA1	KLF4	NF2	PRKAR1A	SMARCA1	WHSC1
AXIN1	CYP2C19	FOXC1	KLHL1	NFE2L2	PRKCA	SMARCA2	WHSC1L1
AXL	CYP2C8	FOXL2	KMT2A	NFKBIA	PRKCB	SMARCA4	WSB1
B3GAT1	CYP2C9	FOXM1	KMT2B	NIPA1	PRKCG	SMARCA5	WT1
BACH1	CYP2D6	FXN	KMT2D	NIPAL1	PRKDC	SMARCAD1	WWOX
BACH2	CYP3A4	G6PD	KRAS	NKX2-1	PRLR	SMARCAL1	XRCC1
BAGE	CYP3A5	GAB2	KYNU	NOTCH1	PROZ	SMARCB1	YAF2
BAP1	DACH2	GABRA6	LATS1	NOTCH2	PRSS1	SMARCC1	ZC3H13
BARD1	DAXX	GATA1	LATS2	NOTCH3	PRX	SMARCC2	ZNF217
BARHL2	DCC	GATA3	LDHC	NOTCH4	PTCH1	SMARCD1	ZNF324
BBOX1	DCX	GNA11	LHFP	NPM1	PTCH2	SMARCD2	ZNF704

BCL10	DDR2	GNAQ	LIN7A	NQO1	PTEN	SMARCD3	ZSWIM2
BCL2	DDX11	GNAS	LMO1	NR1I2	PTGS2	SMARCE1	
BCL2A1	DICER1	GPC6	LTK	NRAS	PTP4A1	SMO	
BCL2L1	DKK1	GRB2	MAGEC3	NSD1	PTPN11	SNTG2	
BCL2L11	DLGAP2	GRM3	MAP2K1	NTNG1	PTPN22	SOCS1	
BCL3	DNMT3A	GSK3B	MAP2K2	NTRK1	PTPRD	SOCS6	
BCL6	DOT1L	GSTM1	MAP2K4	NTRK2	RAB5A	SOX2	
BCOR	DPF1	GSTP1	MAP3K1	NTRK3	RABGEF1	SPOP	
BHLHE40	DPF2	GSTT1	MAP3K13	OLFM4	RAC1	SRFBP1	
BIRC2	DPF3	H3F3A	MAPK1	PAK7	RAD21	SS18	
BIRC3	DPYD	HCCS	MAPK3	PALB2	RAD50	ST8SIA4	
BIRC7	DSG3	HIST1H3B	MAPK7	PARD6G	RAD51	STAT3	
BLM	DTX2	HPGD	MAX	PARK2	RAD51B	STK11	
BOK	DUSP2	HRAS	MBD4	PARP1	RAD51C	STK19	
BRAF	DUSP4	HRNR	MBNL1	PARP2	RAD51D	STK3	
BRCA1	EGFR	HS3ST1	MCL1	PARP3	RAD52	STXBP5L	
BRCA2	ELK3	HSP90AA1	MDM2	PARP4	RAF1	SUFU	
BRD7	EML4	HSP90AB1	MDM4	PAX7	RB1	SULT1A1	
CASP8	EP300	HSP90B1	MECOM	PBRM1	RBFOX1	TAB3	
CBFB	EPHA3	IDH1	MED12	PCDH10	RBM10	TBC1D7	
CBL	EPHA5	IDH2	MELK	PCDH15	REG4	TBPL2	
CCDC89	EPHA6	IFNG	MEN1	PCDH7	RET	TBX20	
CCNB2	ERBB2	IGF1R	MET	PDGFRA	RICTOR	TBX3	
CCND1	ERBB3	IGF2	MGMT	PDGFRB	RIOK3	TCF7L1	
CCND2	ERBB4	IGF2R	MITF	PDK1	RND3	TCF7L2	
CCND3	ERCC1	IGFBP3	MLH1	PDPK1	RNF19A	TDRD3	
CCNE1	ERCC2	IKBKE	MLH3	PEA15	RNF32	TERT	

The protocol consisted of four steps: i) Digestion of genomic DNA (50 ng per sample) in 8 different restriction reactions, each containing two restriction enzymes. ii) Overnight hybridization of restricted fragments with probes whose ends were complementary to the target fragments and sample barcoding. During hybridization, fragments were circularized, and sequencing motifs, including index sequences, were incorporated. iii) Capture of targeted DNA using Dynabeads MyOne Streptavidin T1 (Thermo Scientific, Wilmington, USA) and ligation of circularized fragments. iv) Nineteen cycles of PCR amplification to enrich captured target libraries. Libraries were quantified using a Qubit dsDNA HS Assay on a Qubit 2.0 fluorometer (Life Technologies, CA, USA) and qualified using a 2100 Bioanalyzer instrument and the

High Sensitivity DNA Kit (Agilent Technologies, CA, USA) to enable equimolar pooling of barcoded samples.

Samples were mixed equimolarly in two pools, denatured with an equal volume of NaOH 0.1 M solution, and then diluted to 20 pM. Sequencing was performed using a NextSeq Mid Output kit (2 x 150 cycles) on the NextSeq 500 System (Illumina, CA, USA).

Supplement 3

Sequencing general features

Coverage summary	**Cyst fluid**	**Neoplastic Tissue**	**Blood**
Mean passing filter reads (paired-ends) (mean +/-standard deviation)	6.0 ± 1.7 E6	6.7 ± 2.8 E6	3.4 ± 0.7 E6
Mean coverage (mean +/- standard deviation)	1206 ± 339	1354 ± 561	688 ± 140
% of coverage at 50 X (mean+/- standard deviation)	> 98 ± 2		

Supplement 4: Mutations detected by NGS (in vcf format)

Patient #-Sample	Gene	Variant	Chr	Coordinate	Alt Variant Freqency	Read Depth	Alt Read Depth	Allelic Depth	HGVSc	HGVSp
P5-CF	GNAS	G>G/A	20	57484421	12,2	803	98	705,98	NM_080425.2:c.2531G>A	NP_536350.2:p.Arg844His
P5-CF	KRAS	C>C/A	12	25398284	40,93	215	88	127,88	NM_033360.2:c.35G>T	NP_203524.1:p.Gly12Val
P5-NT	KRAS	C>C/A	12	25398284	30,87	230	71	159,71	NM_033360.2:c.35G>T	NP_203524.1:p.Gly12Val
P6-CF	GNAS	C>C/T	20	57484420	31,42	837	263	574 263	NM_080425.2:c.2530C>T	NP_536350.2:p.Arg844Cys
P6-CF	KRAS	C>C/T	12	25398284	12,35	170	21	149,21	NM_033360.2:c.35G>A	NP_203524.1:p.Gly12Asp
P6-CF	KRAS	C>C/G	12	25398285	17,54	171	30	141,30	NM_033360.2:c.34G>C	NP_203524.1:p.Gly12Arg
P9-NT	KRAS	C>A/A	12	25398284	100	16	16	0,16	NM_033360.2:c.35G>T	NP_203524.1:p.Gly12Val
P9-NT	PTPRD	C>T/T	9	8319853	100	11	11	0,11	NM_002839.3:c.5648G>A	NP_002830.1:p.Arg1883Gln
P9-NT	RAF1	G>G/A	3	12633282	25,58	86	22	64,22	NM_002880.3:c.1118C>T	NP_002871.1:p.Ala373Val
P10-CF	KRAS	C>C/A	12	25398284	4,78	545	26	518,26	NM_033360.2:c.35G>T	NP_203524.1:p.Gly12Val
P10-CF	POLD1	C>C/T	19	50909765	50,11	908	455	453 455	NM_002691.3:c.1485C>T	NM_002691.3:c.1485C>T(p.=)
P10-NT	POLD1	C>C/T	19	50909765	23,53	34	8	26,8	NM_002691.3:c.1485C>T	NM_002691.3:c.1485C>T(p.=)
P13-CF	KRAS	C>C/T	12	25398284	3,1	160	5	130,5	NM_033360.2:c.35G>T	NP_203524.1:p.Gly12Val
P13-CF	TP53	A>A/C	17	7577128	1,23	1060	13	1047,13	NM_000546.5:c.810T>G	NP_000537.3:p.Phe270Leu
P13-NT	KRAS	C>C/T	12	25398284	34,84	155	54	101,54	NM_033360.2:c.35G>A	NP_203524.1:p.Gly12Asp
P13-NT	TP53	A>A/C	17	7577128	21,64	610	132	478 132	NM_000546.5:c.810T>G	NP_000537.3:p.Phe270Leu
P14-CF	GNAS	G>G/A	20	57484421	37,36	1167	436	731 436	NM_080425.2:c.2531G>A	NP_536350.2:p.Arg844His
P14-NT	GNAS	G>G/A	20	57484421	18,73	1276	239	1 037 239	NM_080425.2:c.2531G>A	NP_536350.2:p.Arg844His
P15-CF	GNAS	G>G/A	20	57484421	49,62	1064	528	536 528	NM_080425.2:c.2531G>A	NP_536350.2:p.Arg844His
P15-CF	KRAS	C>C/A	12	25398285	32,48	157	51	106,51	NM_033360.2:c.34G>T	NP_203524.1:p.Gly12Cys
P15-NT	GNAS	G>G/A	20	57484421	19,38	1104	214	890 214	NM_080425.2:c.2531G>A	NP_536350.2:p.Arg844His
P15-NT	KRAS	C>C/A	12	25398285	8,96	268	24	244,24	NM_033360.2:c.34G>T	NP_203524.1:p.Gly12Cys
P17-CF	CTNNB1	A>A/T	3	41268766	56,12	237	133	104 133	NM_001904.3:c.1004A>T	NP_001895.1:p.Lys335Ile

P17-CF	GNAS	G>G/A	20	57484421	48,17	764	368	396 368	NM_080425.2:c.2531G>A	NP_536350.2:p.Arg844His
P17-CF	KRAS	T>T/A	12	25380275	25,42	533	135	396 135	NM_033360.2:c.183A>T	NP_203524.1:p.Gln61His
P17-NT	CTNNB1	A>A/T	3	41268766	37,09	151	56	95,56	NM_001904.3:c.1004A>T	NP_001895.1:p.Lys335Ile
P17-NT	GNAS	G>G/A	20	57484421	42,02	533	224	309 224	NM_080425.2:c.2531G>A	NP_536350.2:p.Arg844His
P17-NT	KRAS	T>T/A	12	25380275	21,15	312	66	246,66	NM_033360.2:c.183A>T	NP_203524.1:p.Gln61His
P18-CF	KRAS	C>C/G	12	25398285	40,66	91	37	54,37	NM_033360.2:c.34G>C	NP_203524.1:p.Gly12Arg
P18-CF	RNF43	C>C/CGGCCT	17	56439905	38,64	88	34	54,34	NM_017763.4:c.682_686dupAGGCC	NP_060233.3:p.Asp230GlyfsTer191
P18-NT	KRAS	C>C/G	12	25398285	32,14	84	27	57,27	NM_033360.2:c.34G>C	NP_203524.1:p.Gly12Arg
P18-NT	RNF43	C>C/CGGCCT	17	56439905	35,48	31	11	20,11	NM_017763.4:c.682_686dupAGGCC	NP_060233.3:p.Asp230GlyfsTer191
P19-CF	KRAS	C>C/A	12	25398284	23,36	321	75	246,75	NM_033360.2:c.35G>T	NP_203524.1:p.Gly12Val
P19-CF	TP53	A>A/C	17	7579349	31,47	2577	811	1 766 811	NM_000546.5:c.338T>G	NP_000537.3:p.Phe113Cys
P19-NT	TP53	A>A/C	17	7579349	17,63	1748	308	1 439 308	NM_000546.5:c.338T>G	NP_000537.3:p.Phe113Cys
P20-CF	GNAS	G>G/A	20	57484421	25,88	2280	590	1 690 590	NM_080425.2:c.2531G>A	NP_536350.2:p.Arg844His
P20-NT	GNAS	G>G/A	20	57484421	6,57	2800	184	2 607 184	NM_080425.2:c.2531G>A	NP_536350.2:p.Arg844His

CF: cyst fluid

NT: neoplastic tissue

Chr: the chromosome number in which the gene occurs.

Variant: lists the reference allele and the diploid genotype call for the sample as Reference > AlleleA/AlleleB. AlleleA and AlleleB are explicitly defined from the REF, ALT, and GT fields of the VCF file. For example, at a heterozygous position noted as GT=0/1 is represented as REF > REF/ALT, and a homozygous non-reference position noted as GT=1/1 is represented as REF > ALT/ALT.

Coordinate: The genomic location of the variant (1-based).

Alt Variant Freq: the frequency of the Alt Allele.

Read Depth: the total number of reads passing quality filters at this position.

Alt Read Depth: the number of reads called at this position.

Allelic Depth: the number of reads called for the Ref Allele and the Alt Allele

HGVSc: human genome variation society (HGVS) notation in the cDNA.

HGVSp: human genome variation society (HGVS) notation in the protein.