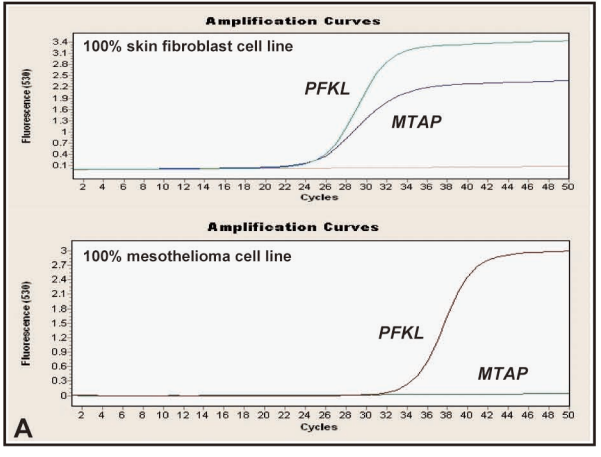

Amplification Curves
100% skin fibroblast cell line
PFKL
MTAP
Fluorescence (530)
Cycles
Amplification Curves
100% mesothelioma cell line
PFKL
MTAP
Fluorescence (530)
Cycles
A

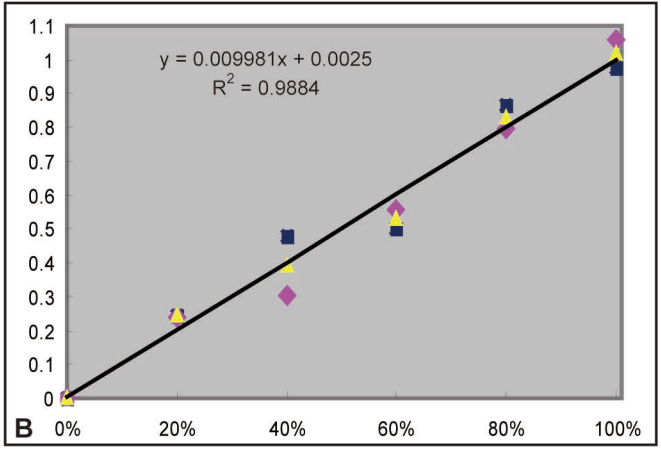
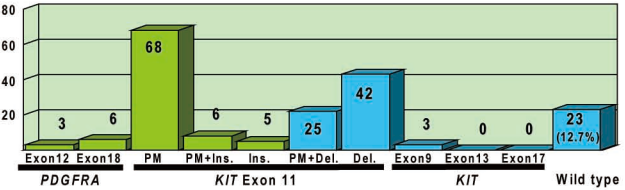
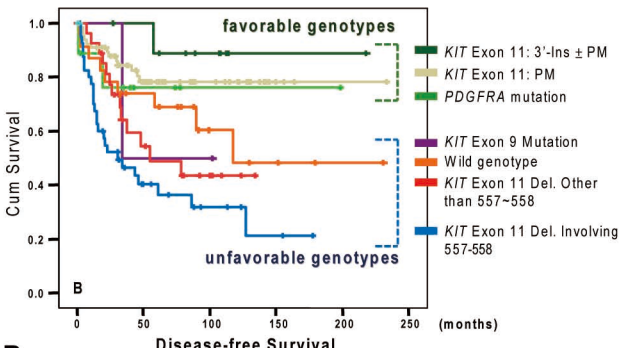

y = 0.009981x + 0.0025
R2 = 0.9884
B
0%
20%
40%
60%
80%
100%

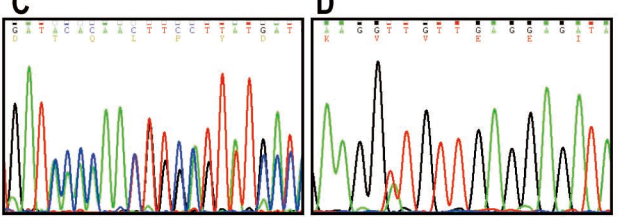
Fig. S1



A

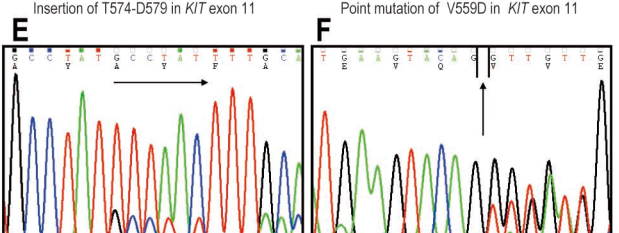


B



Insertion of T574-D579 in *KIT* exon 11

Point mutation of V559D in *KIT* exon 11



Insertion of A502-Y503 in *KIT* exon 9

Deletion of W557-K558 in *KIT* exon 11

Fig. S2

SUPPORTING DOCUMENTS (online only)

Supporting methods

Method-S1 Conditions of real-time quantitative PCR to measure MTAP gene dosage. The sequences of primer pairs and probes targeting *PFKL* and exon 8 of *MTAP* gene, concentrations of reagents, and thermal conditions for qPCR were as follows: ***PFKL***: GGGAAATCCCAGGTAGAACC, UPL hybridization probe #34 (AGAGGCAG, Roche, molecular diagnostics, Mannheim, Germany), and antisense: ACACTGGGCCTTCCATCC; ***MTAP***: sense: ATTACCAAGACATTAAAGTAGCATGG, UPL hybridization probe #2 (CAGGAGAA, Roche), and antisense: GGAATTCCCAAAATGACTGG. Amplification was conducted with LightCycler TaqMan MasterMix , using 10 ng of genomic DNAs, 100 nmol/l of the probes, and 500 nmol/l of the primers in a final 20 μl of reaction mixture. After 2 min incubation at 40°C to allow for uracil *N*-glycosylase cleavage, Taq DNA polymerase was activated by incubation for 10 min at 95°C. Each reaction of the 45 PCR cycles consisted of 10 sec of denaturation at 95°C and hybridization of the probe and primers for 30 sec at 60°C and was performed in duplicate.

Method-S2 Conditions of methylation-specific PCR to detect MTAP promoter hypermethylation

The first PCR was performed with primers specific for bisulfite-converted DNA (sense primer, 5′- ATTTTTGAGTTTTGGGTTAAGTTTATTT-3′; antisense primer, 5′-AAAAAACAACACTCCCTACTTAACC-3′), which did not discriminate between methylated **(M)** and unmethylated **(U)** sequences. At the annealing temperature of 59℃, PCR amplification was first run for 30 cycles in a reaction mixture of 15 μl containing 15 ng bisulfite-converted DNA, 0.1 μM of each primer, and 0.3 U HotStar Taq DNA polymerase (Qiagen, Gmb Hilden, Germany). The nested PCR for **M** and **U** promoters were both performed for 40 cycles at the annealing temperature of 61°C in a reaction mixture of 1 μl of 10-fold diluted, first PCR products, 0.1 μM of each primer, and 0.3 U HotStar Taq DNA polymerase (Qiagen, Gmb. Hilden, Germany) with the following specific primers:

M, sense primer, 5′-GTTGTTTAAGAGAATTTTCGTGGTC-3′; antisense primer, 5′-ACTTAACCCAATATTAATAACGTCG-3′).

U, sense primer, 5′-AGTTGTTTAAGAGAATTTTTGTGGTT-3′; antisense primer, 5′-CCCTACTTAACCCAATATTAATAACATCA-3′.

Supporting Table-S1: Clinicopathological characteristics and genotypes of *KIT* and *PDGFRA* genes in 22 GISTs subjected to genomic profiling by array comparative genomic hybridization (online only)

Case ID No.	Age (years)	Location	Size (cm)	Histological type	Mitotic count (50 hpf)	NIH risk level	RTK Genotype
553	51	Stomach	3.5	Spindle	0	Low	*KIT* Exon 11 PM
612	71	Stomach	3.0	Spindle	2	Low	*KIT* Exon 11 PM
S2091	62	Stomach	4.5	Spindle	2	Low	*KIT* Exon 11 DEL
439	40	Small intestine	7.0	Spindle	4	Intermediate	*KIT* Exon 9 DUP
914	48	Small intestine	8.5	Spindle	4	Intermediate	*KIT* Exon 11 PM
1414	50	Small intestine	8.0	Spindle	2	Intermediate	*KIT* Exon 11 DEL
1235	57	Stomach	7.5	Mixed	2	Intermediate	WT
1052	62	Stomach	5.5	Spindle	2	Intermediate	*KIT* Exon 11 PM
358	53	Stomach	8.0	Spindle	1	Intermediate	*KIT* Exon 11 PM
S2390	65	Stomach	7.0	Spindle	2	Intermediate	*KIT* Exon 11 DEL
1252	61	Stomach	3.3	Spindle	6	Intermediate	*KIT* Exon 11 PM+3'-DUP
897	46	Stomach	4.5	Spindle	7	Intermediate	*KIT* Exon 11 PM
861	71	Stomach	12.0	Mixed	10	High	*KIT* Exon 11 DEL
1205	48	Small intestine	8.5	Mixed	15	High	*KIT* Exon 11 PM+DEL
769	60	Peritoneum	8.5	Mixed	9	High	*KIT* Exon 11 PM.
S1936	40	Small intestine	6.5	Spindle	24	High	*KIT* Exon 9 DUP
S2507	59	Stomach	13.0	Mixed	37	High	*KIT* Exon 11 PM+DEL
1375	63	Stomach	12.5	Spindle	50	High	*KIT* Exon 11 PM+DEL
579	60	Stomach	12.5	Spindle	17	High	*KIT* exon 11 PM+DEL
32	64	Stomach	5.0	Spindle	51	High	*KIT* Exon 11PM+DEL
231	49	Stomach	7.0	Mixed	30	High	*KIT* Exon 11 PM+DEL
1499	57	Stomach	16.0	Mixed	158	High	*KIT* Exon 11 DEL

Abbreviations: PM, point mutation; DEL, deletion; DUP, duplication; WT, wild type

Supporting Table-S2: Independent validation sets of formalin-fixed GIST cases for assessing MTAP immunoexpression (n=306) and *MTAP* gene dosage (n=146)

(online only)

	Cohorts for MTAP immunostain (percentage)	**Cohorts for *MTAP* gene qPCR** (percentage)
Gender		
Male	153 (50.0%)	73 (50.0%)
Female	153 (50.0%)	73 (50.0%)
Age (years) (23 to 88, median 61)		
<70	227 (74.2%)	104 (71.2%)
>=70	79 (25.8%)	42 (28.8%)
Location		
Gastric	180 (58.8%)	97 (66.4%)
Non-gastric	126 (41.2%)	49 (33.6%)
Histological Type		
Spindle	235 (76.8%)	110 (75.3%)
Epithelioid & Mixed	71 (23.2%)	36 (24.7%)
Tumor Size (cm)		
=<5 cm	145 (47.4%)	61 (41.8%)
>5; =<10 cm	112 (36.6%)	59 (40.4%)
>10 cm	49 (16.0%)	26 (17.8%)
Mitotic Count (50HPFs)		
0-5	223 (72.9%)	98 (67.1%)
6-10	39 (12.7%)	11 (13.0%)
>10	44 (14.4%)	29 (19.9%)
NIH Risk		
Low/Very low	115 (37.6%)	44 (31.3%)
Intermediate	96 (31.4%)	49 (33.6%)
High	95 (31.0%)	53 (36.3%)
Ki-67 labeling index		
=<5	205 (71.9%)	99 (68.8%)
>5	80 (28.1%)	45 (31.3%)
Mutation Type		
Favorable Type	88 (48.6%)	70 (53.4%)
Unfavorable Type	93 (51.4%)	61 (46.6%)
MTAP expression		
Expressed	248 (81.0%)	116 (79.5%)
Deficient	58 (19.0%)	30 (20.5%)