Supplementary Information

Table S1. Differentially expressed microRNAs between imatinib-naïve and imatinib-resistant gastrointestinal stromal tumors. Listed are 35 miRNAs ($p < 0.01$; FDR < 20%) that are differentially expressed between imatinib-naïve (IM-n) ($n = 33$) and imatinib-resistant (IM-r) GIST ($n = 20$) samples and that were used in the supervised hierarchical clustering (Figure 1). *p*-values of *t*-test (parametric P) and false discovery rate *p*-values (FDR) are listed, as well as the fold-change upregulation of miRNA expression (Up in IM-r and Up in IM-n).

	miRNA	Up in IM-r	Up in IM-n	Parametric P	FDR
1	miR-186		1.3495141	5.98286×10^{-5}	0.038568
2	miR-526a;miR-520c-5p; miR-518d-5p	1.2326554		0.000106836	0.038568
3	miR-188-5p	2.0378778		0.000345567	0.083167
4	miR-675		1.6985022	0.000863845	0.15053
5	miR-1296		1.2730468	0.001263595	0.15053
6	miR-708*	1.2024122		0.001281441	0.15053
7	miR-24		1.4884172	0.001632288	0.15053
8	miR-455-3p		1.5224393	0.002012757	0.15053
9	miR-335	1.9508284		0.002150029	0.15053
10	miR-92a	1.3374119		0.002282718	0.15053
11	miR-18b		1.3447477	0.002293387	0.15053
12	miR-144	1.6089693		0.00322487	0.173452
13	miR-629	1.2127094		0.003348348	0.173452
14	miR-376b		1.3186401	0.003363339	0.173452
15	miR-885-5p	1.3111764		0.003899124	0.18163
16	miR-143*		1.4948171	0.004266206	0.18163
17	miR-891a	1.2480536		0.00428809	0.18163
18	miR-662	1.1984868		0.004690577	0.18163
19	miR-539		1.2473837	0.004810446	0.18163
20	miR-212	1.3433722		0.005607983	0.18163
21	miR-30c		1.3199678	0.005845988	0.18163
22	miR-18a*	1.185796		0.005863012	0.18163
23	miR-505*	1.2471201		0.006096226	0.18163
24	miR-542-5p	1.201965		0.006223562	0.18163
25	miR-148b		1.2824882	0.006527379	0.18163
26	miR-181a		1.3637022	0.006540709	0.18163
27	miR-516a-3p;miR-516b*	1.1586478		0.007321078	0.190193
28	miR-99a		1.5850102	0.00762179	0.190193
29	miR-548b-5p		1.1389362	0.007639334	0.190193
30	miR-23b		1.3400315	0.008718018	0.199662
31	miR-452		1.3033082	0.008822402	0.199662
32	miR-641	1.1238858		0.009550755	0.199662
33	miR-144*	1.1791067		0.009642671	0.199662
34	miR-195*	1.3858141		0.009665876	0.199662
35	miR-595	1.3852625		0.009678903	0.199662

Table S2. Differentially expressed microRNAs between imatinib-resistant gastrointestinal stromal tumors with and without secondary KIT mutations. Listed are 22 miRNAs ($p < 0.01$) that distinguish between IM-r GIST samples ($n = 9$) with resistance causing secondary KIT mutations (S^+) and IM-r GIST ($n = 11$) samples without secondary mutations (S^-) and that were used in a supervised hierarchical clustering (Figure 2). *p*-values of *t*-test (Parametric P) and false discovery rate p-values (FDR) are listed, as well as the fold-change upregulation of miRNA expression (Up in S^+ and Up in S^-).

	miRNA	Up in S^-	Up in S^+	Parametric P	FDR
1	miR-550*	1.499389		0.000429914	0.310398
2	miR-618		1.274545	0.00102463	0.349131
3	miR-196b		1.393345	0.001450684	0.349131
4	miR-181b		1.44845	0.003302656	0.441227
5	miR-578		1.396228	0.003455634	0.441227
6	miR-302d		1.146457	0.003666707	0.441227
7	miR-380*		1.432246	0.004278633	0.44131
8	miR-581		1.316223	0.005066014	0.457208
9	miR-621	1.120689		0.009139269	0.481995
10	miR-32		1.199577	0.009151744	0.481995
11	miR-566	1.173189		0.009257969	0.481995
12	miR-26b*		1.124953	0.009916984	0.481995
13	miR-519c-3p		1.277479	0.009945044	0.481995
14	miR-301b		1.18747	0.010807049	0.481995
15	miR-640	1.239771		0.010978027	0.481995
16	miR-496		1.204576	0.01099503	0.481995
17	miR-125b		1.824483	0.011348924	0.481995
18	miR-335*		1.30212	0.01270197	0.50949
19	miR-191*		1.231575	0.014321017	0.522105
20	miR-515-5p	1.33432		0.014630895	0.522105
21	miR-652	2.117462		0.015185871	0.522105
22	miR-609		1.259427	0.016334917	0.531633

Table S3. List of 352 differentially expressed genes between imatinib-resistant and imatinib-naïve GIST samples. Comparison of mRNA expression data from IM-r ($n = 15$) and IM-n ($n = 14$) GIST samples identified 352 genes, defined by 475 different Affymetrix probe sets, that were differentially expressed ($p < 0.008$; FDR < 10%) (Supplementary Figure 1). Listed for each gene in the table are the Affymetrix probes set identifiers (NAME), fold-differences in expression (IM-r vs IM-n), p-values (Parametric P), False Discovery Rates (FDR), gene ID (Representation) and gene symbols.

Table S4. Differentially expressed genes associated with the top deregulated canonical pathways. Among 352 most differentially expressed genes, 8 genes were among the most differentially expressed ones in the top deregulated canonical pathways associated with the cell cycle (Supplementary Figure 2). Listed are gene symbols and the corresponding Entrez gene names. The *p* values—based on two-sample *t*-test—indicate the statistical significance of the genes between IM-r and IM-n GIST samples. The depicted fold changes (FC) were calculated from the comparison of the Geomean of the gene expression in IM-r and IM-n groups. Minus and plus values signify the lower and higher expression of the corresponding genes in the IM-r setting, respectively.

Symbol	Entrez Gene Name	*p*-value	FC IM-r vs IM-n
CCNA2	cyclin A2	0.000615	2.632
CCNB1	cyclin B1	0.0066	2.326
CCNB2	cyclin B2	0.00811	2.128
CCND2	cyclin D2	0.000645	-5.36
CCNE2	cyclin E2	0.0000975	2.941
CDK1	cyclin dependent kinase 1	0.00322	3.03
E2F7	E2F transcription factor 7	0.00216	2.174
E2F8	E2F transcription factor 8	0.00102	2.041

Table S5. Ingenuity Pathway Analysis Symbols.

Table S6. Differentially expressed microRNAs between imatinib-naïve and imatinib-resistant gastrointestinal stromal tumors in the samples that were used for mRNA profiling. Eighty-eight significantly ($p < 0.03$; FDR < 30%) differentially expressed miRNAs were detected in 29 GIST samples (IM-r, $n = 15$ vs IM-n, $n = 14$) that were also used for mRNA profiling. The 88 differentially expressed miRNAs were used as input for the integrative IPA of Figure 5. *p*-values of *t*-test (Parametric P) and false discovery rate p-values (FDR) are listed, as well as the fold-change upregulation of miRNA expression (Up in IM-r and Up in IM-n).

	miRNA	Up in IM-r	Up in IM-n	Parametric P	FDR
1	miR-186		1.465364413	0.000142123	0.065297868
2	miR-23b		1.600990618	0.000315439	0.065297868
3	miR-30c		1.569388509	0.000406921	0.065297868
4	miR-374a		1.399173478	0.000481105	0.065297868
5	miR-1296		1.36939107	0.000587714	0.065297868
6	miR-99a		2.295847867	0.000652128	0.065297868
7	miR-30e		1.360999043	0.000755959	0.065297868
8	miR-708*	1.220111545		0.000836728	0.065297868
9	miR-24		1.70013927	0.000882683	0.065297868
10	miR-374b		1.238498341	0.000903152	0.065297868
11	miR-662	1.263511315		0.000999104	0.065668409
12	miR-338-3p	1.290656116		0.001370818	0.077562569
13	miR-511		1.132611196	0.001394624	0.077562569
14	miR-629	1.328014266		0.001785574	0.092212146
15	miR-192*		1.185132714	0.002217836	0.096316515
16	miR-34c-5p	1.304273962		0.002251631	0.096316515
17	miR-455-5p		1.591962841	0.002264704	0.096316515
18	miR-891a	1.304766558		0.002702779	0.108561636
19	miR-524-3p		1.340586356	0.003121461	0.114789794
20	miR-125a-5p		1.471858759	0.003389134	0.114789794
21	miR-526a; miR-520c-5p; miR-518d-5p	1.213370815		0.003425339	0.114789794
22	miR-425*	1.313142787		0.003492912	0.114789794
23	miR-661	1.213995299		0.003922538	0.120973061
24	miR-675		1.661244547	0.004015703	0.120973061
25	miR-885-5p	1.399970729		0.004224052	0.122159595
26	miR-455-3p		1.66339195	0.004585885	0.127522877
26	miR-493*	1.39876134		0.007619979	0.203048448
28	miR-635	1.232138082		0.007863564	0.203048448
29	miR-30a		1.588884872	0.008520114	0.212415261
30	miR-202*		1.284870076	0.0094041	0.218726806
31	miR-501-3p		1.447609026	0.009424178	0.218726806
32	miR-106a*	1.410743402		0.009904977	0.218726806
33	miR-595	1.622629452		0.010108567	0.218726806
34	miR-204		1.145140088	0.010576345	0.218726806
35	miR-132*	1.252451393		0.010788021	0.218726806
36	miR-29c		1.435810249	0.011432348	0.218726806
37	miR-181a		1.387856624	0.011456468	0.218726806
38	miR-641	1.128133915		0.011496015	0.218726806
39	miR-525-5p	1.310391793		0.012919296	0.230343614
40	miR-524-5p	1.237620101		0.013002086	0.230343614
41	miR-584		1.22054574	0.013062363	0.230343614
42	miR-190		1.309696336	0.014025091	0.241431918
43	miR-220c	1.287503569		0.015363205	0.249776498
44	miR-135b*	1.431042466		0.015727909	0.249776498
45	miR-144	1.56789822		0.015855044	0.249776498
46	miR-330-5p	1.333775505		0.015891727	0.249776498
47	miR-136	1.351326777		0.016292595	0.250628642
48	miR-657	1.311175418		0.017027307	0.253034294
49	miR-520c-3p	1.371534698		0.017148936	0.253034294

50	miR-625*		1.803149424	0.017805656	0.257469782
51	let-7f		1.753102735	0.018310513	0.259578453
52	miR-30c-1*		1.28614592	0.019764184	0.27479818
53	miR-144*	1.194803304		0.020216862	0.275788508
54	miR-541	1.198409739		0.020768723	0.278070128
55	miR-520a-5p	1.228522565		0.02186297	0.279390831
56	miR-518f*	1.194516641		0.022728614	0.279390831
57	miR-491-3p		1.655054252	0.022914672	0.279390831
58	miR-26b		1.53087878	0.023108316	0.279390831
59	miR-146b-3p	1.282473253		0.023670193	0.279390831
60	miR-577		1.200769137	0.024221263	0.279390831
61	miR-155	1.375105134		0.024332148	0.279390831
62	miR-101		1.441474426	0.024625483	0.279390831
63	miR-516a-3p; miR516b*	1.2039334		0.025414319	0.279390831
64	miR-30b		1.367010087	0.025901015	0.279390831
65	miR-582-3p	1.157495228		0.02623892	0.279390831
66	miR-431*		1.218489135	0.026409191	0.279390831
67	miR-659	1.203371358		0.026979188	0.279390831
68	miR-885-3p	1.376404732		0.027352065	0.279390831
69	miR-337-3p		1.135486605	0.027634654	0.279390831
70	miR-29b		1.388370529	0.027731949	0.279390831
71	miR-92a	1.305186792		0.027804244	0.279390831
72	miR-890		1.25279541	0.02894404	0.279390831
73	miR-184	1.363654376		0.029674786	0.279390831
74	miR-182*		1.11840109	0.029871354	0.279390831
75	miR-96*	1.13454567		0.030353262	0.279390831
76	miR-505*	1.325496038		0.030411543	0.279390831
77	miR-18b		1.32288215	0.03049196	0.279390831
78	miR-220b	1.270376441		0.03112976	0.279390831
79	miR-548b-5p		1.239066827	0.031162015	0.279390831
80	miR-510	1.273879596		0.031657895	0.279390831
81	miR-379*		1.139422237	0.031668059	0.279390831
82	miR-26a-2*		1.152867809	0.032021038	0.279390831
83	miR-155*		1.328898801	0.032936027	0.279390831
84	miR-18a*	1.158960147		0.033168697	0.279390831
85	miR-150		1.35609573	0.033285129	0.279390831
86	miR-920	1.41268658		0.033486091	0.279390831
87	miR-616		1.11727855	0.033619644	0.279390831
88	miR-877*	1.342330426		0.035692417	0.293245652

Table S7. Overview of the microRNA expression levels measured in the imatinib-naïve and imatinib-resistant gastrointestinal stromal tumor samples. The colors in the top row indicate the IM-n samples (blue) and the IM-r samples (brown). The ID numbers in first column refer to specific miRNA capture probes as indicated in the second column. The ID annotation can also be found in the platform description in the Gene Expression Omnibus (GEO) data repository under accession number GPL16851. The sample designation corresponds to the annotation used throughout the manuscript. The values depicted are normalised miRNA expression values that were used for the analyses described in the manuscript.

Figure S1. Differentially expressed mRNAs between imatinib-naïve and imatinib-resistant GIST samples. Depicted is a heat map of a supervised hierarchical clustering based on the 475 Affymetrix probes sets that represent 352 most significant ($p < 0.008$; FDR < 10%) differentially expressed genes in fresh frozen GIST samples from IM-n ($n = 14$) and IM-r ($n = 15$) patients. In the heat map red indicates relative high expression and green indicates relative low expression.

Figure S2. Top deregulated canonical pathways between imatinib-naïve and imatinib-resistant gastrointestinal stromal tumors. The most deregulated canonical pathways in the IM-r samples were derived, using Ingenuity Pathway Analyses, from the 475 most differentially expressed mRNAs ($p < 0.008$; FDR < 10%) between IM-n and IM-r GIST samples. The significance of the top deregulated pathways, which is displayed on the top X-axis as ''-log(p-value)'' is an indication of the probability of the association of genes represented in our mRNA dataset with the canonical pathways by random chance alone. The orange colored bars are indicative of

predicted pathway activation (positive Z-score) and the blue ones indicate predicted inhibition (negative Z-score). White and gray bars are those with Z-score close to 0 or no available prediction, respectively. The ratio, indicated on the bottom X-axis, represents the number of genes in a given pathway that pass the cut-off criteria/number of total genes that constitute the pathway, and is visualized by orange points.

Figure S3. Quantitative RT-PCR validation of differentially expressed microRNAs and mRNAs in imatinib-naïve and imatinib-resistant gastrointestinal stromal tumors. **A**) A standardized RT-PCR procedure was used to validate the expression levels of differentially expressed microRNAs in the IM-n (n = 33) and IM-r GIST (n = 20) samples sets. A standard dilution series of a cDNA sample-pool was used to determine absolute quantification of the miRNA expression. **B**) A standardized RT-PCR procedure was used to validate the expression levels of differentially expressed mRNAs in the IM-n (n = 33) and IM-r (n = 20) GIST samples. Expression levels were analyzed using GAPDH, HPRT and PPIA for normalization purposes. Boxplots depict 1st and 3rd quartile with the median indicated as a horizontal line. Outliers designate measurements > 1.5 IQR (interquartile range). * indicates $p < 0.05$.