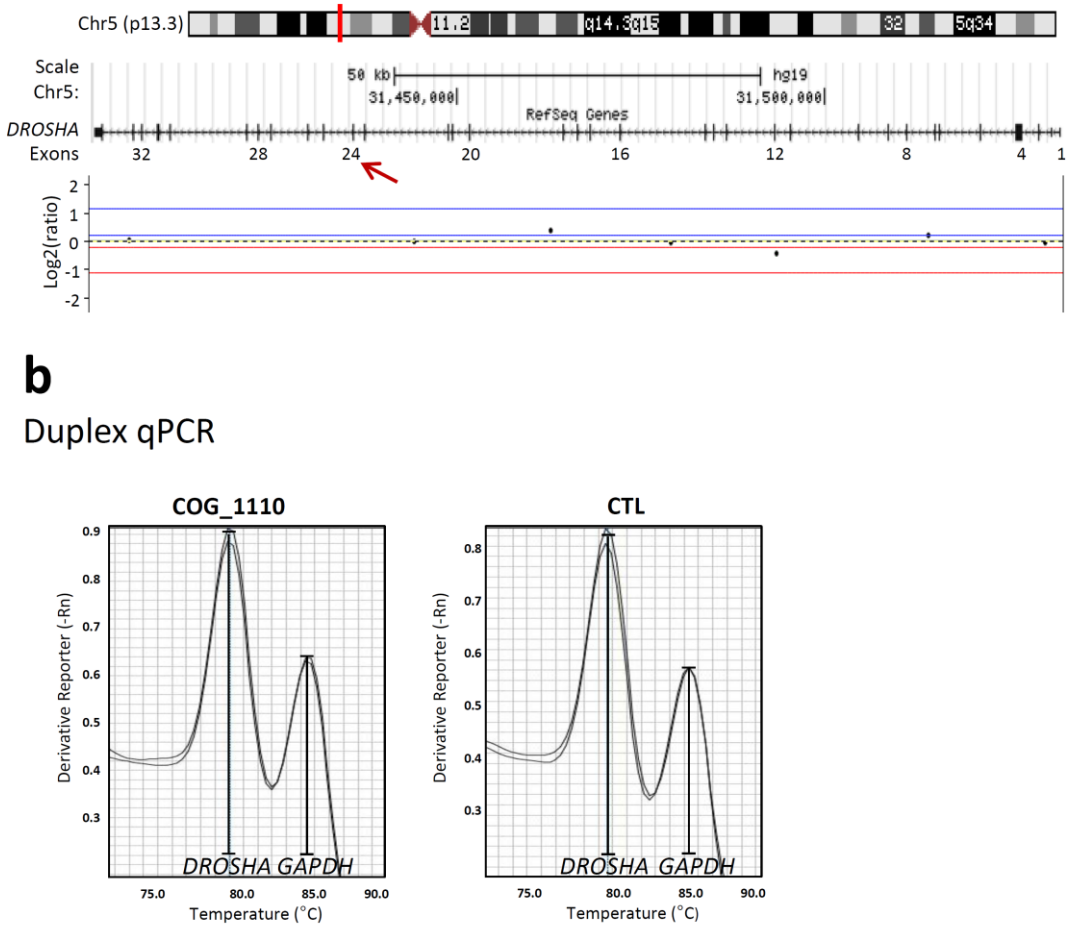
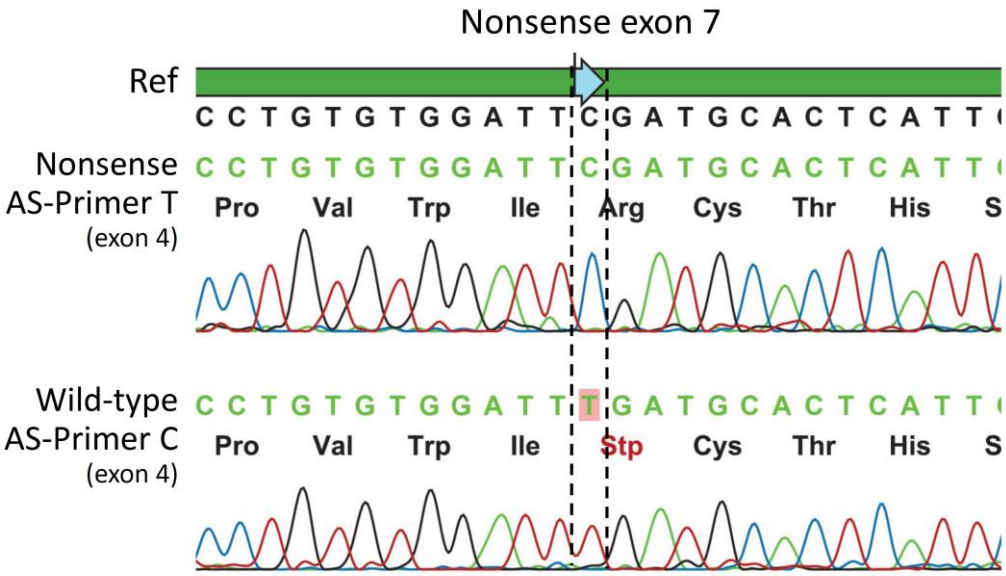
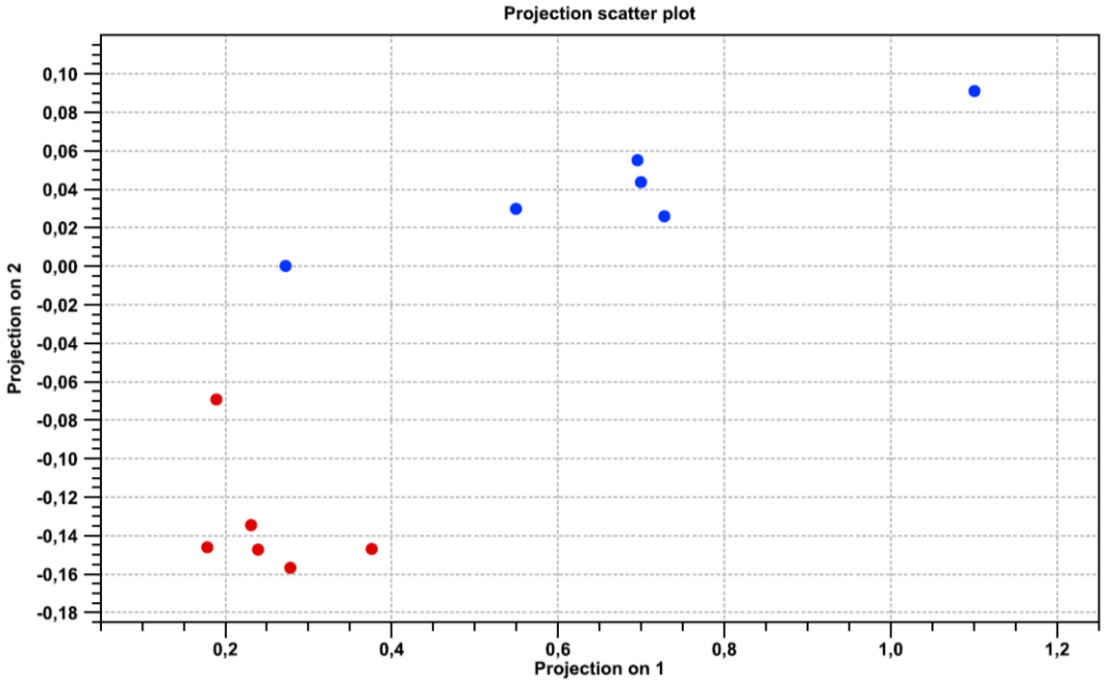
SUPPLEMENTARY FIGURES



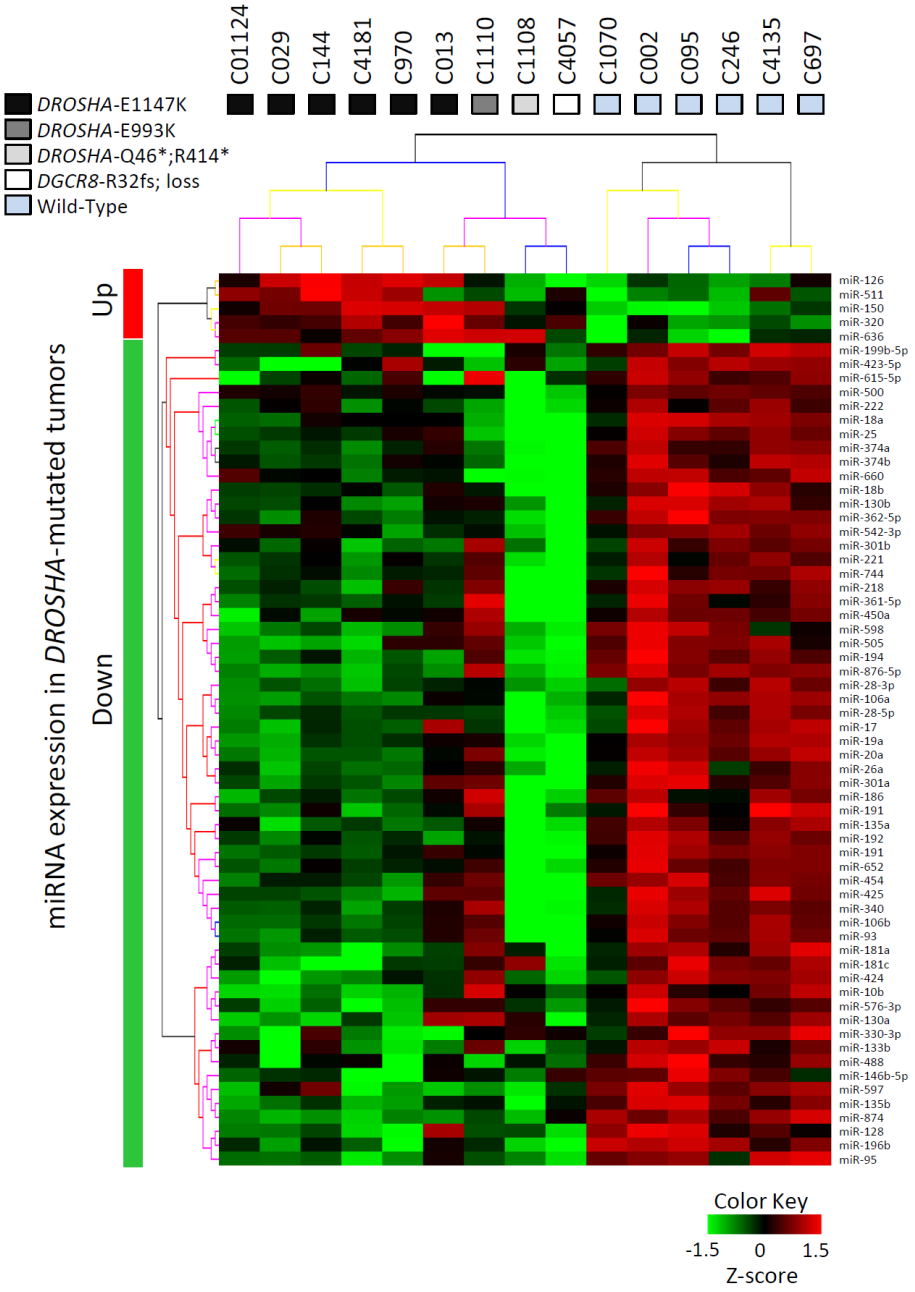
Supplementary Figure 1: Copy number analysis of *DROSHA* gene in patient COG_1110. **(a)** Top panel displays a schematic representation of chromosome 5 and the *DROSHA* locus; middle panel displays the *DROSHA* exons (both modified from UCSC genome browser). Bottom panel presents the array-CGH profile of *DROSHA* genomic region. Seven probes were located within this gene and all probes presented normal log2 ratio values. **(b)** Duplex qPCR for the *DROSHA* exon 24 (location of E993K mutation) and a reference gene (*GAPDH)*; duplex qPCR was performed as previously described[1] (primer sequences are available at Supplementary Table 12).The ratio between the peaks of the melting curve of the patient (COG_1110) and a control sample (CTL) was 0.93, which confirmed the absence of copy number alterations in this region.



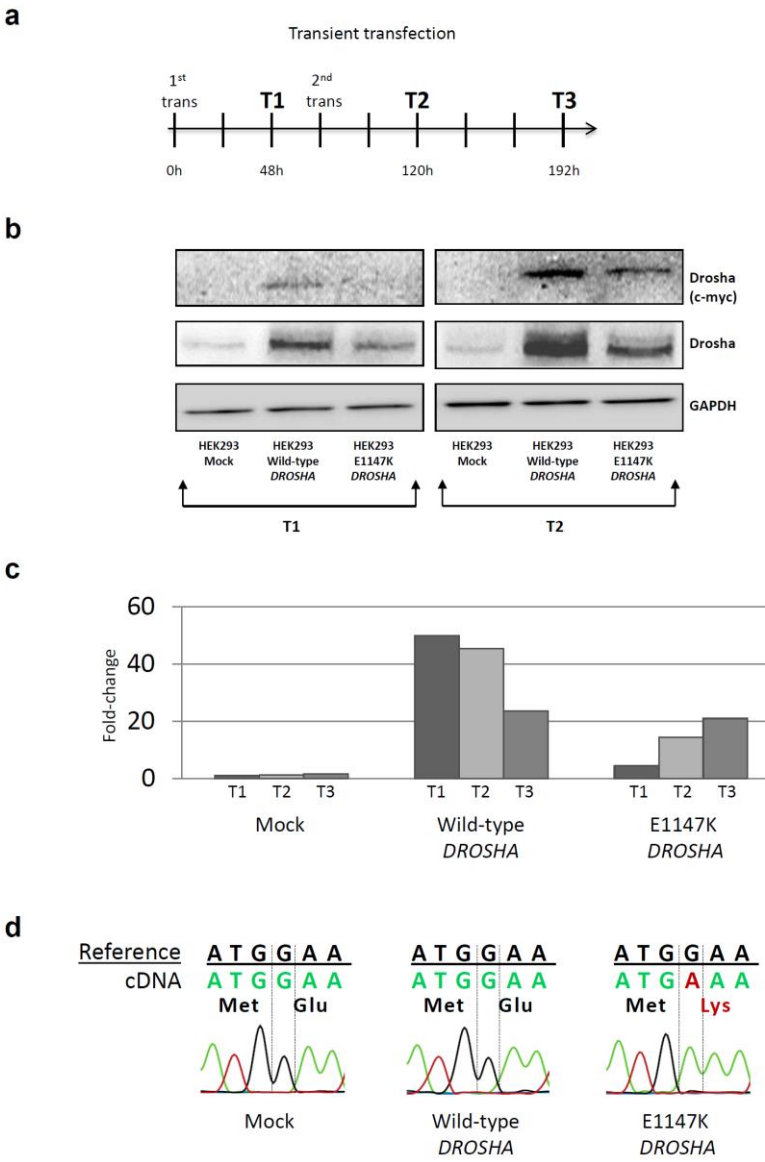
Supplementary Figure 2: Allele specific (AS) PCR of patient COG_1108. To evaluate whether the two nonsense mutations (c.136C>T; p.Q46* at exon 4 and c.1240C>T; p.R414* at exon 7) were affecting each one of the alleles of the patient, allele specific forward primers were design for the c.136C>T nonsense mutation at exon 4, either to align with the mutated (T) or the wild-type (C) base at the 3' end. A common reverse primer located at exon 11 was used for amplification of cDNA (primer sequences are available at Supplementary Table 12). Amplification performed with AS-primer for the mutated allele at exon 4 resulted in amplification of the wild-type allele at exon 7 (top sequence). Amplification performed with AS-primer for the wild-type allele at exon 4 resulted in amplification of the mutated allele at exon 7 (bottom sequence). These results confirm that the two nonsense mutations are located in distinct alleles (biallelic mutations).



Supplementary Figure 3: Principal component analysis of global miRNAs expression levels. A combination of PC2 with PC1 allowed a linear separation between *DROSHA*-E1147K mutated (red circles) and non-mutated (blue circles) tumors.

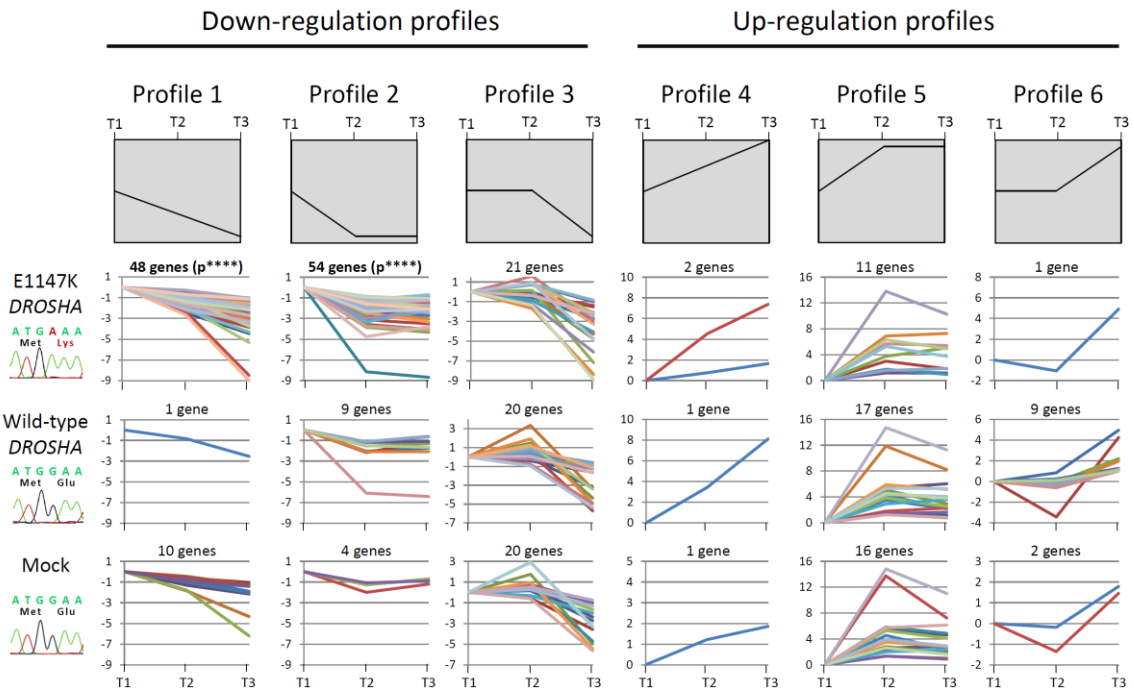


Supplementary Figure 4: Unsupervised hierarchical clustering analysis. The expression profile of 64 miRNAs affected by DROSHA-E1147K mutation were investigated in three additional WT samples with other mutations in *DROSHA* or *DGCR8* genes (COG_1110: DROSHA homozygous E993K; COG_1108: two nonsense mutations in *DROSHA* – Q46* and R414* and COG_4057: *DGCR8* frameshift and genomic loss). Clustering analysis confidently separated miRNA core genes mutated samples from non-mutated samples. Interestingly, the two tumors with double hits (COG_1108 and COG_4057) presented a more distinct miRNA expression profile, showing a remarkable lower expression of the affected miRNAs.

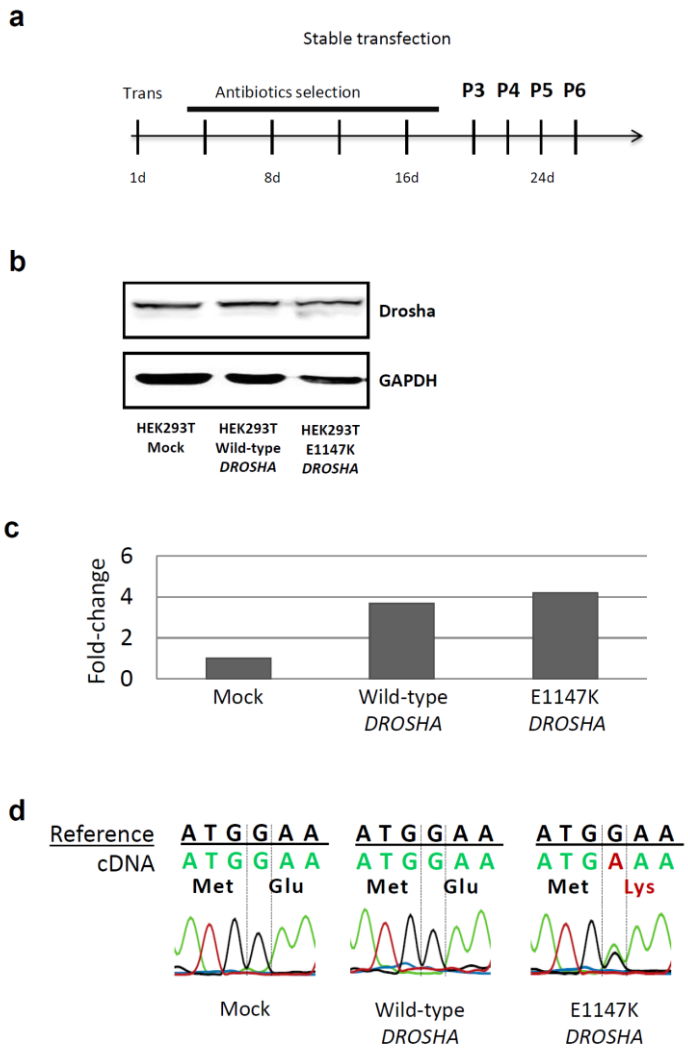


Supplementary Figure 5: Validation of plasmids expression in HEK293 wild-type and E1147K *DROSHA* transiently transfected cells. **(a)** Schematic representation of the time course experiment: transfections were performed twice in a 72-h interval (1st trans and 2nd trans); cell extracts were collected at three time points (T1, T2 and T3). **(b)** Expression of proteins extracted from HEK293 transfected with mock, wild-type *DROSHA*, and E1147K *DROSHA* plasmids were analyzed by Western blotting using anti-c-Myc antibody targeted against plasmids (pcDNA4/TO/cmycDrosha). GAPDH antibody was used as a control. In first transfection (T1), densitometry quantification of WB bands resulted in normalized Drosha expression value (calculated by DROSHA/GAPDH ratio) of 0.27 for mock, 3.42 for wild-type, and 1.25 for E1147K transfected cells. In second transfection (T2), normalized Drosha expression was 0.40 for mock, 4.90 for wild-type, and 3.49 for E1147K transfected cells. **(c)** RT-qPCR was performed for confirming *DROSHA* over-expression in wild-type and E1147K *DROSHA* transfected cells. *DROSHA*

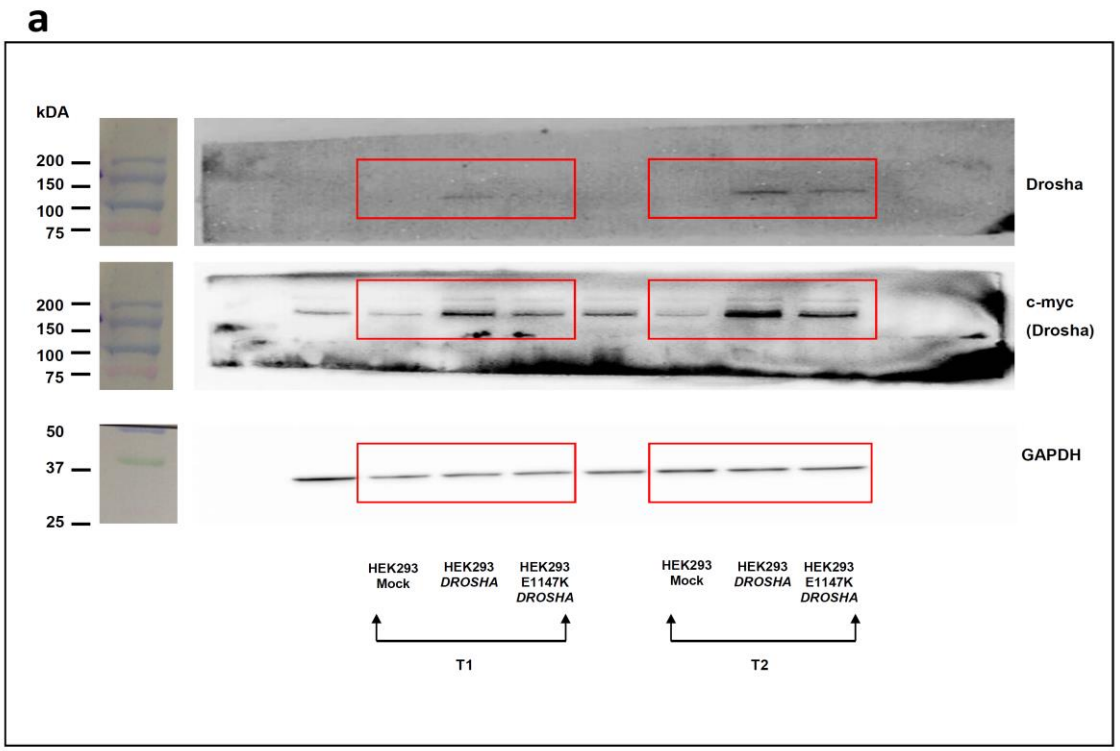
expression in times T1, T2, and T3 of the three transfection conditions are presented as fold-changes normalized by the expression of HEK293 mock cells (T1). **(d)** Sanger sequencing of cDNA from T3 time point of mock, wild-type *DROSHA*, and E1147K *DROSHA* transfected cells showing the expression of the mutated transcript in E1147K cells.

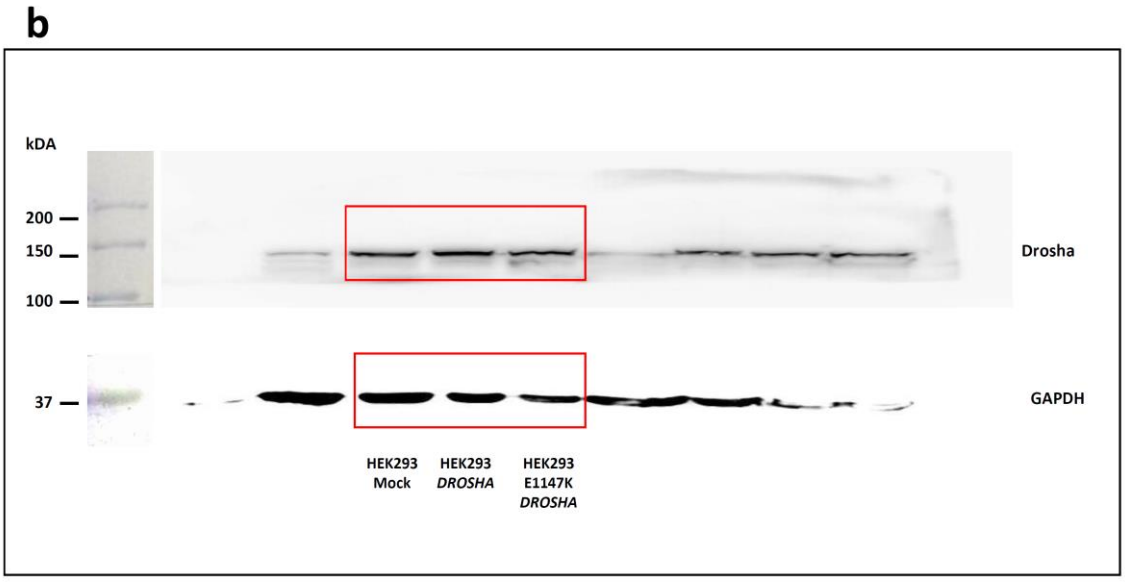


Supplementary Figure 6: STEM result of time course experiment in HEK293 cells expressing wild-type or E1147K Drosha. STEM analysis was used for clustering and analyzing the expression data. Plasmid transfections were performed twice in HEK293 cells in a 72-h interval and miRNA expression levels were measured at three time points using the TaqMan Array platform (T1, T2 and T3). Top panel presents the three possible down-regulation and the three possible up-regulation profiles produced by STEM analysis. It shows that E1147K-transfected cells presented two statistically significant profiles (profiles 1 and 2) harboring more genes (48 and 54, respectively) than expected by chance. These two profiles are those that represent a reduction of mature miRNA levels from time point 1 to 3.



Supplementary Figure 7: Validation of plasmids expression in HEK293T wild-type and E1147K *DROSHA* stably transfected cells. **(a)** Schematic representation of the experiment, showing the time length of selection and the passages when cell extracts were collected for miRNA expression analysis**. (b)** Expression of proteins extracted from HEK293T transfected with mock, wild-type *DROSHA*, and E1147K *DROSHA* plasmids were analyzed by Western blotting using anti-Drosha (WB with anti-c-Myc antibody did not result in reliable results due to the low expression of exogenous protein in this assay). GAPDH antibody was used as a control. Densitometry quantification of WB bands resulted in normalized Drosha expression value (calculated by DROSHA/GAPDH ratio) of 0.28 for mock, 0.46 for wild-type, and 0.71 for E1147K stably transfected cells. **(c)** *DROSHA* expression of the three stable cell lines was assessed by RT-qPCR and is presented as fold-changes normalized by the expression of HEK293T mock cells. **(d)** Sanger sequencing of cDNA from mock, wild-type *DROSHA*, and E1147K *DROSHA* stably transfected cells showing the heterozygous expression of the mutated transcript in E1147K-stably transfected cells.





Supplementary Figure 8: Full pictures of western blots presented in the manuscript. Red boxed regions correspond to cropped fragment used in the figures. **(a)** Western blot from transient transfection experiment. **(b)** Western blot from stably transfection experiment.

SUPPLEMENTARY TABLES

Supplementary Table 1: Sequence coverage by WES.

Sample	Total reads	% of mapped reads (hg19)	Target coverage	Mean coverage depth	Targets covered >20X	Reads on target
ACC_12T	194,425,183	40.4%	90.58%	41X	48.3%	76.9%
ACC_12B	620,978,245	47.9%	93.68%	178X	73.4%	72.9%
ACC_12M	431,293,170	45.5%	93.42%	122X	72.3%	76.3%
ACC_12F	288,505,016	55.3%	93.18%	100X	73.4%	75.0%

ACC = sample obtained from AC Camargo Cancer Center; 12T = index case tumor DNA; 12B = proband germline DNA; 12M = proband's mother germline DNA; 12F = proband's father germline DNA.

Supplementary Table 2: Somatic SNVs and indels identified by WES.

Chrom	Position	Ref base	Var base	Cov Ref	Cov Var	Strand	Allele	Freq	Gene	Codon Ref	Codon Var	aa Ref	aa Var	Alteration type	Sanger Validation
SNVs															
Chr19	52537713	C	T	24	8	+/-	Heterozigous	33.3	*ZNF432*	GGA	AGA	G	R	Nonsynonymous	Not confirmed
Chr8	110463332	A	C	28	6	+	Heterozigous	21.4	*PKHD1L1*	AGT	CGT	S	R	Nonsynonymous	Not confirmed
Chr12	11286336	T	G	29	5	+/-	Heterozigous	17.2	*TAS2R30*	AGT	CGT	S	R	Nonsynonymous	Not confirmed
Chr5	**31421465**	**C**	**T**	**32**	**5**	**-**	**Heterozigous**	**15.6**	***DROSHA***	**GAA**	**AAA**	**E**	**K**	**Nonsynonymous**	**Validated**
ChrX	12906217	T	C	26	5	+	Heterozigous	19.2	*TLR7*	TTC	CTC	F	L	Nonsynonymous	Not confirmed
Indels															
Chr1	7858661	C	CGA	15	3	-	Heterozigous	20.0	*PER3*	-	-	-	-	Frameshift	NE
Chr8	56699534	T	TG	14	5	+	Heterozigous	35.7	*TGS1*	-	-	-	-	Frameshift	NE
Chr22	26769476	A	AA	8	3	+/-	Heterozigous	37.5	*SEZ6L*	-	-	-	-	Frameshift	NE
Chr6	57398190	A	AA	32	9	+/-	Heterozigous	28.1	*PRIM2*	-	-	-	-	Frameshift	NE
Chr2	46711400	G	GG	7	3	+	Heterozigous	42.9	*LOC388946*	-	-	-	-	Frameshift	NE

Chr = chromosome; Ref = reference base; Var = variant base; Cov = coverage; Freq = frequency of variant base; aa = Amino acid; NE = not-evaluated. Validated *DROSHA* E1147K mutation is represented in bold letters.

Supplementary Tables 3a, b and c: Clinical features of investigated cohorts. Mutated samples are highlighted in bold letters.

a) Fresh-frozen COG cohort

ID	Predominant histologic pattern	Stage	Relapse	QTneo
COG1_13	**Blastema**	**III**	**No**	**No**
COG1_29	**Blastema**	**IV**	**No**	**No**
COG_1110	**Blastema**	**IV**	**No**	**No**
COG_1124	**Blastema**	**III**	**No**	**No**
COG_1144	**Blastema**	**IV**	**No**	**No**
COG_128	**Blastema**	**III**	**Yes**	**No**
COG_4181	**Blastema**	**III**	**No**	**No**
COG_970	**Blastema**	**III**	**Yes**	**No**
COG_526	**Blastema**	**II**	**Yes**	**No**
COG_1108	**Blastema**	**II**	**Yes**	**No**
COG1_1	Blastema	III	Yes	No
COG1_10	Blastema	III	No	No
COG1_12	Blastema	III	No	No
COG1_14	Blastema	III	No	No
COG1_15	Blastema	III	No	No
COG1_17	Blastema	III	No	No
COG1_18	Blastema	III	No	No
COG1_19	Blastema	IV	Yes	No
COG1_2	Blastema	III	Yes	No
COG1_20	Blastema	IV	Yes	No
COG1_21	Blastema	IV	Yes	No
COG1_22	Blastema	IV	Yes	No
COG1_23	Blastema	IV	Yes	No
COG1_24	Blastema	IV	Yes	No
COG1_25	Blastema	IV	No	No
COG1_26	Blastema	IV	No	No
COG1_27	Blastema	IV	No	No
COG1_28	Blastema	IV	No	No
COG1_30	Blastema	IV	No	No
COG1_4	Blastema	III	Yes	No
COG1_5	Blastema	III	Yes	No
COG1_7	Blastema	III	Yes	No
COG1_8	Blastema	III	Yes	No
COG1_9	Blastema	III	Yes	No
COG_001	Blastema	III	No	No
COG_002	Blastema	III	Yes	No
COG_031	Blastema	IV	No	No
COG_032	Blastema	III	No	No

COG_095	Blastema	III	No	No
COG_1060	Blastema	IV	No	No
COG_1065	Blastema	III	No	No
COG_1068	Blastema	IV	No	No
COG_1070	Blastema	IV	No	No
COG_1099	Blastema	III	No	No
COG_1104	Blastema	I	Yes	No
COG_1088	Blastema	III	No	No
COG_117	Blastema	IV	Yes	No
COG_1232	Blastema	III	Yes	No
COG_1247	Blastema	IV	No	No
COG_1290	Blastema	III	No	No
COG_178	Blastema	IV	No	No
COG_201	Blastema	III	Yes	No
COG_2050	Blastema	III	No	No
COG_2063	Blastema	III	No	No
COG_2081	Blastema	III	No	No
COG_2113	Blastema	IV	No	No
COG_2182	Blastema	III	No	No
COG_219	Blastema	III	Yes	No
COG_246	Blastema	III	No	No
COG_277	Blastema	II	Yes	No
COG_321	Blastema	IV	No	No
COG_329	Blastema	IV	No	No
COG_341	Blastema	II	Yes	No
COG_342	Blastema	IV	Yes	No
COG_353	Blastema	III	No	No
COG_396	Blastema	IV	Yes	No
COG_399	Blastema	III	No	No
COG_4057	Blastema	III	Yes	No
COG_4094	Blastema	III	No	No
COG_4135	Blastema	IV	No	No
COG_4159	Blastema	III	No	No
COG_4196	Blastema	IV	No	No
COG_420	Blastema	III	Yes	No
COG_4272	Blastema	III	No	No
COG_4305	Blastema	III	No	No
COG_472	Blastema	IV	No	No
COG_495	NA	NA	NA	No
COG_497	Blastema	II	Yes	No
COG_575	Blastema	IV	No	No
COG_6000	Blastema	III	No	No
COG_6011	Blastema	III	Yes	No
COG_697	Blastema	II	Yes	No

b) Fresh-frozen ACC cohort

ID	Predominant histologic pattern	Stage	Relapse	QTneo
ACC_08	**Biphasic - B+E**	**II**	**No**	**Yes**
ACC_12	**Biphasic - E+B**	**I**	**Yes**	**Yes**
ACC_01	Stromal	V	Yes	Yes
ACC_02	NA	NA	NA	NA
ACC_04	Nephrogenic rests	V	No	Yes
ACC_05	Stromal	III	No	Yes
ACC_06	Triphasic	I	No	Yes
ACC_07	Triphasic	III	No	Yes
ACC_10	Diffuse Anaplasia	II	No	Yes
ACC_13	Stromal	II	No	Yes
ACC_15	Blastema	III	Yes	Yes
ACC_17	Blastema	V	No	Yes
ACC_19	Epithelial	NA	NA	NA
ACC_20	Triphasic	III	No	Yes
ACC_21	Triphasic	III	No	Yes

c) FFPE ACC cohort

ID	Predominant histologic pattern	Stage	Relapse	QTneo
ACCp_19	**Blastema**	**II**	**No**	**No**
ACCp_34	**Blastema**	**I**	**No**	**No**
ACCp_37	**Blastema**	**III**	**NA**	**Yes**
ACCp_72	**Biphasic B+E**	**NA**	**NA**	**NA**
ACCp_74	**Biphasic B+E**	**III**	**No**	**Yes**
ACCp_76	**Triphasic**	**I**	**No**	**No**
ACCp_84	**Blastema**	**II**	**No**	**No**
ACCp_100	**NA**	**I**	**No**	**No**
ACCp_101	**Triphasic**	**III**	**No**	**No**
ACCp_102	**Biphasic S+E; Rhab diff**	**III**	**No**	**Yes**
ACCp_115	**Epithelial**	**I**	**No**	**No**
ACCp_136	**Blastema**	**I**	**No**	**No**
ACCp_151	**Tubular diff**	**I**	**No**	**No**
ACCp_154	**Blastema**	**III**	**NA**	**No**
ACCp_6	Biphasic B+E	II	No	No
ACCp_10	Biphasic B+E	I	No	No
ACCp_11	Biphasic B+E	II	No	Yes
ACCp_12	Blastema	III	Yes	No
ACCp_13	NA	NA	NA	NA
ACCp_14	Blastema	II	No	No

ACCp_15	Triphasic	V	Yes	Yes
ACCp_17	NA	I	No	No
ACCp_18	Blastema	II	No	No
ACCp_21	Undifferentiated	III	Yes	No
ACCp_23	NA	II	Yes	Yes
ACCp_24	Biphasic B+E	II	No	No
ACCp_25	NA	NA	NA	NA
ACCp_26	Focal Anaplasia	II	Yes	Yes
ACCp_27	Triphasic	I	No	Yes
ACCp_29	Triphasic	II	No	No
ACCp_32	Epithelial	I	No	No
ACCp_33	NA	NA	NA	NA
ACCp_36	Tubular Diff	IV	Yes	No
ACCp_38	Triphasic	I	No	No
ACCp_39	Triphasic	IV	No	Yes
ACCp_41	Triphasic	II	Yes	Yes
ACCp_42	Epithelial	II	No	No
ACCp_44	NA	II	Yes	No
ACCp_46	NA	III	No	Yes
ACCp_47	Triphasic	III	No	Yes
ACCp_48	Triphasic	III	No	No
ACCp_52	Triphasic	I	No	No
ACCp_53	Triphasic	II	No	No
ACCp_54	Triphasic	III	No	No
ACCp_55	Blastema	I	No	No
ACCp_57	NA	NA	NA	NA
ACCp_58	Epithelial	III	No	No
ACCp_59	Blastema	III	Yes	Yes
ACCp_60	Blastema	III	Yes	Yes
ACCp_61	Blastema	I	No	No
ACCp_63	Triphasic	III	No	No
ACCp_65	Blastema	I	No	No
ACCp_68	Triphasic	III	No	No
ACCp_70	Blastema	II	No	No
ACCp_71	Blastema	IV	Yes	NA
ACCp_73	Epithelial	I	No	No
ACCp_75	Triphasic	I	No	No
ACCp_78	Blastema	IV	No	Yes
ACCp_79	Triphasic	III	No	Yes
ACCp_80	Triphasic	IV	No	No
ACCp_81	Stromal	II	Yes	No
ACCp_82	NA	IV	No	No
ACCp_83	NA	NA	NA	NA
ACCp_86	NA	IV	Yes	Yes
ACCp_87	Triphasic	I	No	No

ACCp_88	Triphasic	III	No	No
ACCp_89	Triphasic	IV	No	Yes
ACCp_90	Triphasic	I	No	No
ACCp_91	Triphasic	I	No	No
ACCp_93	Triphasic	III	Yes	Yes
ACCp_94	NA	IV	No	Yes
ACCp_95	Blastema	I	No	No
ACCp_96	NA	III	Yes	No
ACCp_97	Stromal	II	No	Yes
ACCp_99	NA	NA	NA	NA
ACCp_103	Blastema	II	No	No
ACCp_104	Blastema	III	No	No
ACCp_105	NA	III	No	Yes
ACCp_107	Blastema	III	No	Yes
ACCp_108	Blastema	III	Yes	No
ACCp_109	Blastema	V	Yes	Yes
ACCp_110	Blastema	I	No	Yes
ACCp_111	Triphasic	I	No	No
ACCp_112	NA	III	No	Yes
ACCp_113	Blastema	II	Yes	No
ACCp_116	Blastema	II	Yes	No
ACCp_117	NA	V	Yes	Yes
ACCp_118	Stromal	V	Yes	Yes
ACCp_119	Blastema	II	No	No
ACCp_120	Triphasic	I	Yes	No
ACCp_121	Blastema	III	Yes	No
ACCp_123	Blastema	I	No	No
ACCp_124	Blastema	I	No	No
ACCp_125	Blastema	I	No	No
ACCp_126	Stromal	III	Yes	Yes
ACCp_127	Blastema	III	No	No
ACCp_128	Triphasic	II	No	No
ACCp_130	Blastema	II	No	No
ACCp_131	Blastema	III	Yes	No
ACCp_132	Blastema	IV	No	Yes
ACCp_133	NA	II	No	Yes
ACCp_134	Blastema	III	NA	Yes
ACCp_135	Blastema	III	Yes	No
ACCp_137	Epithelial	I	No	No
ACCp_138	Epithelial	I	No	No
ACCp_139	Stromal	V	Yes	Yes
ACCp_140	Stromal	V	Yes	Yes
ACCp_141	Biphasic S+E	I	No	Yes
ACCp_142	Stromal	V	No	Yes
ACCp_143	Cystic nephroblastoma	I	No	Yes

ACCp_144	NA	III	No	Yes
ACCp_145	Blastema	IV	No	Yes
ACCp_146	Biphasic B+E	I	No	No
ACCp_147	Biphasic B+E	I	No	No
ACCp_148	Focal Anaplasia	II	No	No
ACCp_149	Blastema	II	No	No
ACCp_150	Tubular Diff	II	No	No
ACCp_152	Blastema	II	No	No
ACCp_153	Blastema	II	No	No
ACCp_155	Blastema	III	No	No
ACCp_156	Rhabdoide Tumor	III	Yes	Yes
ACCp_159	Blastema	IV	Yes	Yes
ACCp_160	Blastema	V	Yes	Yes
ACCp_161	Blastema	II	No	No

QTneo = neoadjuvant chemotherapy; NA = information not available; B = blastema; E = epithelial; S = stromal; Diff = differentiation; Rhab Diff = rhabdomyoblastic differentiation

Supplementary Table 4. Description of point mutations detected in miRNA core processing genes and WT-associated genes by targeted sequencing in 66 WT frozen samples. Genomic imbalances detected in the same genes displaying point mutations in any of the samples are also shown (10 samples). Clinical characteristics, such as the predominant histology of each tumor, clinical stage, the occurrence of relapse and the use of neoadjuvant chemotherapy are described.

ID	Point mutations	Genomic imbalances	Predominant Histology	Stage	Relapse	QTneo
ACC_8	*DROSHA* p.E1147K; *TP53* p.P72A; *FBXW7* p.R35C		Biphasic - Blastema	II	No	Yes
COG_4181	*DROSHA* p.E1147K	*WT1* loss	Blastema	III	No	No
COG_1124	*DROSHA* p.E1147K	*WT1* loss	Blastema	III	No	No
COG_970	*DROSHA* p.E1147K	*WT1* loss	Blastema	III	Yes	No
COG_1144	*DROSHA* p.E1147K		Blastema	IV	No	No
ACC_12	*DROSHA* p.E1147K		Biphasic - Epithelial	I	Yes	Yes
COG_128	*DROSHA* p.E1147K		Blastema	III	Yes	No
COG_1110	*DROSHA p.E1147K*		Blastema	IV	No	No
COG_526	*DROSHA* p.P211T	*WT1* loss	Blastema	II	Yes	No
COG_1108	*DROSHA* p.Q46*; *DROSHA* p.R414*		Blastema	II	Yes	No
COG_032	*DGCR8* p.E518K; *FBXW7* p.E117del	*DGCR8* loss	Blastema	III	No	No
COG_353	*DGCR8* p.Y721H	*WTX* loss; *DIS3L2* loss	Blastema	III	No	No
COG_219	*DGCR8* p.S92R		Blastema	III	Yes	No
COG_4159	*DGCR8* p.A558T		Blastema	III	No	No
ACC_15	*DGCR8* p.G55S	NA	Blastema	III	Yes	Yes
COG_4057	*DGCR8* p.R32fs	*DGCR8 loss*	Blastema	III	Yes	No

COG_117	*DICER1* p.I85M; *WT1* p.P183fs; *CTNNB1* P.Y333F		Blastema	IV	Yes	No
ACC_13	*DICER1* p.D1810N	NA	Stromal	II	No	Yes
COG_396	*DICER1* p.Q48E		Blastema	IV	Yes	No
ACC_4	*XPO5* p.V832I; *TARBP2* p.R296H	NA	Nephrogenic rests	V	No	Yes
COG_2050	*TARBP2* p.R353fs		Blastema	III	No	No
ACC_5	*CTNNB1* p.T41A*;* *WT1* p.D367_ splice		Stromal	III	No	Yes
ACC_7	*CTNNB1* p.N387K	NA	Triphasic - Stromal	III	No	Yes
ACC_1	*CTNNB1* p.S45F	NA	Stromal	V	Yes	Yes
COG_2081	*CTNNB1* p.S45P	*XPO5* gain; *TARBP2* gain; *DIS3L2* gain	Blastema	III	No	No
COG_6000	*WT1* p.F391fs; *WT1* p.K316fs		Blastema	III	No	No
COG_1290	*WT1* p.V379fs		Blastema	III	No	No
COG_031	*WTX* p.D354fs	*CTNNB1* gain	Blastema	IV	No	No
COG_4135	*WTX* p.R353*		Blastema	IV	No	No
COG_1065	*WTX* p.R497*		Blastema	III	No	No
ACC_10	*TP53* p.339_340EM>V	NA	Anaplasia	II	No	Yes
COG_497	*DIS3L2* p.Q230*		Blastema	II	Yes	No
COG_4196	*DIS3L2* p.R483G		Blastema	IV	No	No
COG_321		*DICER1* loss	Blastema	IV	No	No
COG_1232		*DICER1* gain; *FBXW7* loss	Blastema	III	Yes	No
COG_246		*DICER1* loss	Blastema	III	No	No
COG_4272		*XPO5* gain; *TARBP2* gain; *WTX* loss	Blastema	III	No	No
COG_4305		*XPO5* gain; *TARBP2* gain	Blastema	III	No	No
COG_420		*XPO5* gain; *TARBP2* gain	Blastema	III	Yes	No
COG_1104		*XPO5* gain	Blastema	I	Yes	No

COG_201	*WT1* loss; *WTX* loss	Blastema	III	Yes	No
COG_001	*WTX* loss	Blastema	III	No	No
COG_2113	*DIS3L2* loss	Blastema	IV	No	No

QTneo = neoadjuvant chemotherapy; *NA* – not available.

Supplementary Table 5: Detailed description of point mutations detected by targeted sequencing in 66 WT frozen samples. Sequencing metrics, such as variant frequency and coverage, as well as pathogenicity prediction software results are described.

ID	Gene ID	Chr	Position	cDNA change	Protein change	Var freq	Cov	Polyphen2 (score)	SIFT (score)	MutationTaster (score)
ACC_8	*DROSHA*	5	31421465	c.3439G>A	p.E1147K	41.7	144	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
	TP53	17	7579473	c.214C>G	p.P72A	7.8	51	benign (0)	tolerated (0.353)	polymorphism (p> 0.999)
	FBXW7	4	153332853	c.103C>T	p.R35C	49.4	87	probably damaging (0.994)	damaging (0.000)	disease causing (p> 0.999)
COG_4181	*DROSHA*	5	31421465	c.3439G>A	p.E1147K	51.4	208	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
COG_1124	*DROSHA*	5	31421465	c.3439G>A	p.E1147K	53.0	219	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
COG_970	*DROSHA*	5	31421465	c.3439G>A	p.E1147K	45.4	196	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
COG_1144	*DROSHA*	5	31421465	c.3439G>A	p.E1147K	47.8	46	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACC_12	*DROSHA*	5	31421465	c.3439G>A	p.E1147K	35.2	230	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
COG_128	*DROSHA*	5	31421465	c.3439G>A	p.E1147K	18.8	329	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
COG_1110	*DROSHA*	5	31435937	c.2977G>A	p.E993K	93.6	311	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
COG_526	*DROSHA*	5	31526409	c.631C>A	p.P211T	50.9	379	benign (0)	damaging (0.030)	polymorphism (p> 0.999)
COG_1108	*DROSHA*	5	31526904	c.136C>T	p.Q46*	43.5	269			
	DROSHA	5	31515145	c.1240C>T	p.R414*	47.2	316			
COG_032	*DGCR8*	22	20079439	c.1552G>A	p.E518K	88.1	159	probably damaging (1)	damaging (0.002)	disease causing (p> 0.999)
	FBXW7	4	153332605	c.349_351delGAG	p.E117del	44.4	342			
COG_353	*DGCR8*	22	20096449	c.2161T>C	p.Y721H	5.1	39	possibly damaging (0.481)	tolerated (0.461)	disease causing (p> 0.999)
COG_219	*DGCR8*	22	20073762	c.276T>A	p.S92R	40.6	165	benign (0.148)	damaging (0.003)	disease causing (p> 0.999)
COG_4159	*DGCR8*	22	20080397	c.1672G>A	p.A558T	7.8	205	benign (0.151)	damaging (0.004)	disease causing (p> 0.999)
ACC_15	*DGCR8*	22	20073649	c.163G>A	p.G55S	7.0	201	probably damaging (1)	damaging (0.000)	disease causing (p> 0.999)
COG_4057	*DGCR8*	22	20073569	c.83_93dup	p.R32fs	68.0	122			
COG_117	*DICER1*	14	95598904	c.255C>G	p.I85M	51.0	727	benign (0.047)	tolerated (0.344)	disease causing (p> 0.999)

	WT1	11	32456342	c.549_550insTC	p.P183fs	53.3	165			
	CTNNB1	3	41268760	c.998A>T	p.Y333F	8.4	431	probably damaging (0.992)	damaging (0.008)	disease causing (p> 0.999)
ACC_13	*DICER1*	14	95557639	c.5428G>A	p.D1810N	91.8	195	probably damaging (1)	damaging (0.000)	disease causing (p> 0.999)
COG_396	*DICER1*	14	95599654	c.142C>G	p.Q48E	48.9	233	probably damaging (0.998)	tolerated (0.066)	disease causing (p> 0.999)
ACC_4	*XPO5*	6	43499263	c.2494G>A	p.V832I	54.9	204	benign (0.002)	tolerated (0.437)	polymorphism (p> 0.999)
	TARBP2	12	53899578	c.887G>A	p.R296H	51.0	102	benign (0)	tolerated (0.131)	polymorphism (p> 0.999)
COG_2050	*TARBP2*	12	53899890	c.1059delC	p.R353fs	52.2	295			
ACC_5	*WT1*	11	32417935	c.1099_splice	p.D367_splice	81.4	188			
	CTNNB1	3	41266124	c.121A>G	p.T41A	48.0	204	possibly damaging (0.94)	damaging (0.003)	disease causing (p> 0.999)
ACC_7	*CTNNB1*	3	41274911	c.1161T>G	p.N387K	44.8	669	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACC_1	*CTNNB1*	3	41266137	c.134C>T	p.S45F	55.3	94	probably damaging (0.996)	damaging (0.000)	disease causing (p> 0.999)
COG_2081	*CTNNB1*	3	41266136	c.133T>C	p.S45P	5.0	788	probably damaging (0.988)	damaging (0.002)	disease causing (p> 0.999)
COG_6000	*WT1*	11	32439126	c.946_947insAA	p.K316fs	45.7	374			
	WT1	11	32417879	c.1172_1173insC	p.F391fs	27.2	279			
COG_1290	*WT1*	11	32417917	c.1135delG	p.V379fs	52.1	217			
COG_031	*WTX*	X	63412106	c.1061delA	p.D354fs	45.7	324			
COG_4135	*WTX*	X	63412110	c.1057C>T	p.R353*	91.7	36			
COG_1065	*WTX*	X	63411678	c.1489C>T	p.R497*	8.2	110			
ACC_10	*TP53*	17	7574009	c.1016_1018delAGA	p.339_340EM>V	82.1	123			
COG_497	*DIS3L2*	2	232995415	c.688C>T	p.Q230*	93.2	192			
COG_4196	*DIS3L2*	2	233127938	c.1447C>G	p.R483G	52.3	842	probably damaging (1)	damaging (0.000)	disease causing (p> 0.999)

Chr = chromosome; Var freq = variant frequency; Cov = coverage; Polyphen2: the classification of the variants are given as “benign”, “possibly damaging”, “probably damaging” along with the probability of the variation being damaging (from 0 to 1); SIFT: the substitution is predicted as damaging or tolerated if the score is <= 0.05 or > 0.05, respectively; Mutation Taster: a prediction is given as either ‘disease-causing’ or ‘polymorphism’ along with a P value indicating the security of the prediction (with 1 being most secure).

Supplementary Table 6: Clinical features and mutation classification of 26 *DROSHA* mutated samples.

Sample ID	Predominant histology	Relapse	Clinical stage	QTneo	*DROSHA* mutation	Polyphen2 (score)	SIFT (score)	MutationTaster (score)
COG cohort								
COG_128	Blastema	Yes	III	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
COG_970	Blastema	Yes	IIII	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
COG_1124	Blastema	No	III	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
COG_1144	Blastema	No	IV	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
COG_4181	Blastema	Yes	II	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
COG_13	Blastema	No	III	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
COG_29	Blastema	No	IV	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
COG_1110	Blastema	No	IV	No	E993K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
COG_526	Blastema	Yes	II	No	P211T	benign (0)	damaging (0.030)	polymorphism (p> 0.999)
COG_1108	Blastema	Yes	II	No	Q46*; R414*	-	-	-
ACC fresh frozen cohort								
ACC_08	Biphasic - B+E	No	II	Yes	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACC_12	Biphasic - E+B	Yes	I	Yes	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)

ACC FFPE cohort								
ACCp_19	Blastema	No	II	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACCp_34	Blastema	No	I	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACCp_37	Blastema	NA	III	Yes	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACCp_72	Biphasic B+E	NA	NA	NA	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACCp_74	Biphasic B+E	No	III	Yes	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACCp_84	Blastema	No	II	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACCp_100	NA	No	I	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACCp_101	Triphasic	No	III	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACCp_102	Biphasic S+E Rhab diff	No	III	Yes	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACCp_115	Epithelial	No	I	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACCp_136	Blastema	No	I	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACCp_151	Tubular diff	No	I	No	E1147K	probably damaging (1)	damaging (0.001)	disease causing (p> 0.999)
ACCp_76	Triphasic	No	I	No	D1151G	probably damaging (1)	damaging (0.004)	disease causing (p> 0.999)
ACCp_154	Blastema	NA	III	No	D1151G	probably damaging (1)	damaging (0.004)	disease causing (p> 0.999)

QTneo = neoadjuvant chemotherapy; NA = information not available; B = blastema; E = epithelial; S = stromal; Rhab diff = rhabdomyoblastic differentiation; diff = differentiation.

Supplementary Table 7: Clinical features and RNA quality of WTs samples used in miRNA expression analysis.

Sample ID	*DROSHA* status	Histological components	First treatment	Relapse	Clinical stage	RNA RIN
COG_970	E1147K	80% Blastema and 20% Epithelium+ Stroma (removed by macrodissection)	Surgery	Yes	IIII	NA
COG_1124	E1147K	Blastema	Surgery	No	III	8.2
COG_1144	E1147K	Blastema	Surgery	No	IV	6.6
COG_4181	E1147K	Blastema	Surgery	Yes	II	7.5
COG_13	E1147K	NA	Surgery	No	III	8.5
COG_29	E1147K	NA	Surgery	No	IV	7.3
COG_1110	E993K	50% Blastema and 50% Epithelium+ Stroma (removed by macrodissection)	Surgery	No	IV	4.5
COG_1108	Q46*; R414*	Blastema	Surgery	Yes	II	5.2
COG_4057	R32fs; *DGCR8* loss	Blastema	Surgery	Yes	III	6.3
COG_002	Wild-type	Blastema	Surgery	Yes	III	6.4
COG_095	Wild-type	Blastema	Surgery	No	III	8.2
COG_246	Wild-type	Blastema	Surgery	No	III	3.1
COG_697	Wild-type	Blastema	Surgery	Yes	II	5.6
COG_1070	Wild-type	Blastema	Surgery	No	IV	6.5
COG_4135	Wild-type	Blastema	Surgery	No	III	4.8

RIN = RNA integrity number; NA = information not available.

Supplementary Table 8: Differentially expressed miRNAs in *DROSHA*-E1147K mutated and non-mutated WTs. DROSHA-E1147K tumors had a predominant reduction of mature miRNAs compared to non-mutated tumors, as 59 miRNAs were down-regulated and only 5 were up-regulated (underlined miRNAs).

miRNA	Fold-change	p-value FDR-corrected	Mutated means	Non-mutated means
miR-511	13.03	0.05	0.0000110	0.0000008
miR-150	4.25	0.003	0.0012704	0.0002991
miR-636	3.89	0.02	0.0000300	0.0000077
miR-320	2.65	0.02	0.0268548	0.0101420
miR-126	2.64	0.003	0.1494239	0.0566215
miR-500	-2.04	0.03	0.0000403	0.0000823
miR-222	-2.15	0.05	0.0122761	0.0263598
miR-28-3p	-2.16	0.02	0.0007771	0.0016779
miR-660	-2.16	0.02	0.0009309	0.0020133
miR-191	-2.25	0.03	0.0176693	0.0397207
miR-362-5p	-2.35	0.05	0.0001051	0.0002470
miR-425	-2.37	0.03	0.0003782	0.0008955
miR-26a	-2.45	0.05	0.0103445	0.0253326
miR-374a	-2.60	0.01	0.0007793	0.0020277
miR-301a	-2.65	0.02	0.0013006	0.0034502
miR-454	-2.76	0.01	0.0064733	0.0178931
miR-106a	-2.81	0.01	0.1270424	0.3573796
miR-28-5p	-2.86	0.02	0.0001966	0.0005620
miR-186	-2.94	0.03	0.0024706	0.0072657
miR-17	-2.99	0.02	0.1548721	0.4632929
miR-146b-5p	-3.01	0.04	0.0072702	0.0218677
miR-576-3p	-3.15	0.05	0.0000031	0.0000097
miR-374b	-3.15	0.02	0.0029104	0.0091730
miR-361-5p	-3.15	0.03	0.0000829	0.0002614
miR-221	-3.17	0.04	0.0007485	0.0023708
miR-93	-3.21	0.01	0.0173274	0.0556616
miR-106b	-3.22	0.01	0.0048635	0.0156397
miR-19a	-3.27	0.01	0.0102394	0.0335059
miR-598	-3.28	0.02	0.0002171	0.0007116
miR-128	-3.29	0.05	0.0000510	0.0001681
miR-130b	-3.33	0.02	0.0006281	0.0020933

miR-19b	-3.34	0.01	0.0832017	0.2779872
miR-301b	-3.37	0.01	0.0004749	0.0016001
miR-196b	-3.49	0.01	0.0041953	0.0146420
miR-340	-3.57	0.02	0.0003645	0.0013031
miR-25	-3.59	0.02	0.0010624	0.0038123
miR-744	-3.62	0.03	0.0003679	0.0013328
miR-542-3p	-3.69	0.03	0.0000082	0.0000303
miR-218	-3.77	0.02	0.0040355	0.0152324
miR-130a	-3.79	0.03	0.0016740	0.0063521
miR-20a	-3.87	0.00	0.0346136	0.1339278
miR-18b	-4.30	0.02	0.0000388	0.0001667
miR-194	-4.37	0.003	0.0000656	0.0002870
miR-181a	-4.39	0.01	0.0002431	0.0010679
miR-652	-4.44	0.01	0.0002272	0.0010086
miR-18a	-4.55	0.02	0.0006195	0.0028208
miR-10b	-4.65	0.01	0.0062117	0.0289072
miR-135a	-4.70	0.01	0.0002408	0.0011309
miR-199b-5p	-4.84	0.04	0.0000059	0.0000284
miR-330-3p	-4.96	0.03	0.0000009	0.0000047
miR-615-5p	-5.16	0.03	0.0000023	0.0000119
miR-192	-5.20	0.003	0.0002430	0.0012648
miR-95	-5.47	0.01	0.0000876	0.0004791
miR-181c	-6.20	0.01	0.0000026	0.0000163
miR-450a	-7.98	0.04	0.0000076	0.0000607
miR-424	-8.38	0.01	0.0000099	0.0000826
miR-597	-9.97	0.02	0.0000048	0.0000478
miR-488	-11.84	0.05	0.0000037	0.0000434
miR-135b	-12.49	0.003	0.0013583	0.0169711
miR-505	-13.95	0.02	0.0000016	0.0000220
miR-423-5p	-14.65	0.03	0.0000031	0.0000449
miR-133b	-16.12	0.02	0.0000017	0.0000277
miR-874	-36.34	0.00003	0.0000002	0.0000070
miR-876-5p	-37.67	0.00003	0.0000002	0.0000089

Supplementary Table 9: Expression of miRNAs with 5p and 3p-derived strands. DROSHA-E1147K affected both 5p and 3p-derived miRNAs. From 7 differentially expressed (at least one strand differentially expressed) and 15 non-differentially expressed miRNAs that presented data for both strands, all of them had fold-changes in the same direction for the two variants.

Differentially expressed miRNAs			
Feature ID	Strand	Fold	p-value FDR-corrected
miR-146b	3p	-8.58	0.17
	5p	-3.01	0.04
miR-28	3p	-2.16	0.02
	5p	-2.86	0.02
miR-362	3p	-1.33	0.57
	5p	-2.35	0.05
miR-532	3p	-1.34	0.62
	5p	-1.91	0.05
miR-542	3p	-3.69	0.03
	5p	-3.90	0.10
miR-615	3p	-3.41	0.18
	5p	-5.16	0.03
miR-876	3p	-1.82	0.17
	5p	-37.67	0.00003

Non-differentially expressed miRNAs			
Feature ID	Strand	Fold	p-value FDR-corrected
miR-125a	3p	-1.30	0.44
	5p	-1.07	0.83
miR-140	3p	-1.66	0.25
	5p	-2.13	0.07
miR-142	3p	1.21	0.84
	5p	1.79	0.85
miR-193a	3p	-1.56	0.33
	5p	-1.51	0.36
miR-199a	3p	-2.82	0.48
	5p	-1.16	0.79
miR-296	3p	-17.67	0.14
	5p	-29.52	0.08
miR-324	3p	-1.80	0.26
	5p	-1.66	0.35
miR-331	3p	-2.42	0.08
	5p	-2.37	0.16
miR-339	3p	-1.26	0.41
	5p	-2.08	0.08
miR-342	3p	-1.56	0.19
	5p	-2.18	0.19
miR-455	3p	-3.02	0.17
	5p	-2.05	0.13
miR-486	3p	2.76	0.88
	5p	8.26	0.13
miR-502	3p	-1.95	0.13
	5p	-1.49	0.37
miR-654	3p	-2.26	0.74
	5p	-3.07	0.85
miR-886	3p	1.43	0.84
	5p	3.64	0.92

Supplementary Table 10: Expression of primary and mature miRNA pairs. The expression of 15 primary and mature miRNA pairs was assessed with TaqMan individual assays. From eight differentially expressed miRNA pairs, all mature miRNAs were validated as differentially expressed between *DROSHA*-E1147K and wild-type groups; none of the eight pri-miRNAs presented any significant difference. For all six non-differentially expressed miRNA pairs used as controls, neither mature miRNAs nor pri-miRNAs presented any significant difference between the groups.

	TLDA miRNA card		Individual Taqman assay		Primary miRNA Individual Taqman assay		
miRNA	Fold	p value (FDR)	Fold	p value	pri-miRNA	Fold	p value
Differentially expressed miRNAs pairs							
miR-95	-4.47	0.01	-3.16	0.01	pri-95	-4.28	0.19
miR-128a	-3.29	0.04	-3.24	0.007	pri-128	-2.38	0.60
miR-135b	-12.49	0.003	-9.00	0.0001	pri-135b	-10.83	0.28
miR-874	-36.34	0.00003	-4.00	0.005	pri-874	-2.37	0.79
miR-876-5p	-37.67	0.00003	-5.83	0.001	pri-876	3.55	0.38
miR-126	2.64	0.003	2.14	0.006	pri-126	-6.10	0.76
miR-150	4.25	0.003	2.43	0.02	pri-150	-1.39	0.98
miR-636	3.89	0.01	3.34	0.001	pri-636	1.24	0.63
Non-differentially expressed miRNAs pairs							
miR-26b	-2,08	0.06	-1.53	0.11	pri-26b	4.90	0.14
Let-7b	-1.04	0.61	-2.36	0.63	pri-Let-7b	-10.21	0.76
Let-7c	-1.07	0.64	1.18	0.93	pri-Let-7c	-1.04	0.65
Let-7d	-1.81	0.68	-2.43	0.81	pri-Let-7d	-1.45	0.69
Let-7e	-1.90	0.60	-1.87	0.23	pri-Let-7e	-2.19	0.75
Let-7g	-2.07	0.87	-2.14	0.72	pri-Let-7g	-3.98	0.07

Supplementary Table 11: Concordant down-regulated miRNAs in *DROSHA*-mutated tumors and HEK293-E1147K transiently transfected cells. 31 of 59 down-regulated miRNAs in DROSHA-mutated tumors also had a decreased expression in HEK293-E1147K cells.

miRNA	Tumor samples		HEK293-E1147K Cell line			
	Fold	p-value FDR-corrected	STEM profile	T1	T2	T3
miR-106a	-2.81	0.01	2	0.00	-1.38	-1.34
miR-106b	-3.22	0.01	2	0.00	-1.43	-1.50
miR-128	-3.29	0.05	1	0.00	-2.45	-3.57
miR-130b	-3.33	0.02	2	0.00	-2.09	-1.72
miR-17	-2.99	0.02	2	0.00	-1.63	-1.48
miR-18a	-4.55	0.02	2	0.00	-2.24	-2.73
miR-18b	-4.30	0.02	1	0.00	-1.16	-2.14
miR-191	-2.25	0.03	2	0.00	-1.05	-0.95
miR-194	-4.37	0.003	2	0.00	-3.19	-1.93
miR-19a	-3.27	0.01	1	0.00	-0.84	-1.30
miR-19b	-3.34	0.01	2	0.00	-1.82	-1.82
miR-20a	-3.87	0.00	1	0.00	-1.53	-2.16
miR-218	-3.77	0.02	2	0.00	-1.27	-1.37
miR-221	-3.17	0.04	2	0.00	-2.47	-3.04
miR-25	-3.59	0.02	2	0.00	-2.19	-2.73
miR-26a	-2.45	0.05	1	0.00	-0.96	-1.65
miR-28-3p	-2.16	0.02	2	0.00	-2.74	-1.99
miR-301a	-2.65	0.02	2	0.00	-2.13	-2.03
miR-301b	-3.37	0.01	2	0.00	-2.05	-2.16
miR-340	-3.57	0.02	1	0.00	-1.00	-1.58
miR-361-5p	-3.15	0.03	2	0.00	-3.34	-2.22
miR-362-5p	-2.35	0.05	2	0.00	-2.47	-3.34
miR-425	-2.37	0.03	2	0.00	-1.88	-1.25
miR-500	-2.04	0.03	2	0.00	-1.73	-1.11
miR-576-3p	-3.15	0.05	1	0.00	-0.78	-1.17
miR-597	-9.97	0.02	1	0.00	-0.28	-1.06
miR-598	-3.28	0.02	1	0.00	-0.88	-1.92
miR-652	-4.44	0.01	2	0.00	-2.31	-1.86
miR-660	-2.16	0.02	2	0.00	-1.41	-1.01
miR-744	-3.62	0.03	2	0.00	-0.87	-1.14
miR-93	-3.21	0.01	2	0.00	-2.33	-2.13

T1, T2 and T3 represent the log2 transformed expression value of each time point normalized according to STEM method, in which the expression values are normalized to start at 0 (T1=0).

Supplementary Table 12: Primer sequences used in Sanger sequencing, pyrosequencing and duplex qPCR analyses.

Template	Region	Forward sequence	Reverse sequence
Sanger sequencing			
DNA	*DROSHA* RIIIb_1	GAAATTTCTGCTCATGGGAGAAGG	CCAATTCCTTCGAAGGGCATG
DNA	*DROSHA* RIIIb_2*	ATCGAGGGGCCTTAGGGAAT	ACCCCTCTAATCTTCACTCTGC
DNA	*DROSHA* RIIIb_3	CTCTCAGGCTAGTTGGGTC	ACAAGGTTTCTTTAGCCAGAAC
DNA	*DROSHA* RIIIb_4	GAGGGCCTGGAAGTTGAAAC	GGGCTCCTGTCATGATCACC
cDNA	*DROSHA* RIIIa_1	CGATCAACTGGATCGTGAACAG	CATGTGATGAACTTTTCTGTCTCC
cDNA	*DROSHA* RIIIa_2	GGAGACAGAAAAGTTCATCACATG	GATAATTGAGCCAGACTTCGC
cDNA	*DROSHA* RIIIb*	GCGAAGTCTGGCTCAATTATC	GGGTCATTCCAATCCTGATTC
Pyrosequencing			
FFPE DNA	*DROSHA* RIIIb* (PCR)	5BIO-CCAGAGGCCACAATCAGAG	ACCCCTCTAATCTTCACTCTGC
FFPE DNA	*DROSHA* RIIIb* (Seq)		TTATGGAGTCACCTAGGAAT
Allele specific PCR			
cDNA	*DROSHA* Exon 4-11 (PCR)	GGCTGCTTCACCCTCAG**T**	GCATTGTTGGTCATAGGACGAC
cDNA	*DROSHA* Exon 4-11 (PCR)	GCTGCTTCACCCTCAG**C**	
cDNA	*DROSHA* Exon 7 (Seq)	GTTGGAGTGACAACCAGAGTTC[#2]	
Duplex qPCR			
	DROSHA Exon 24	GTAGCGTCCATTTGTACTATTTG	TGCTGCAGATGTCTTCTATACC
	GAPDH Intron 7	GCTCCCACCTTTCTCATCC	CTGCAGCGTACTCCCCAC

***** = regions spanning the hotspot E1147K *DROSHA* mutation; Seq = sequencing

SUPPLEMENTARY METHODS

STEM analysis

For the transiently transfected cell lines, the regulation of miRNA expression of each population of cells (mock, Drosha wild-type or Drosha-E1147K) during the time course experiment was evaluated with STEM analysis (http://www.cs.cmu.edu/~jernst/stem/). The STEM clustering method initially defines a set of distinct and representative model of temporal expression profiles that correspond to possible profiles of a gene's expression change over time, independent of the data. All model profiles start at 0, and between two time points a model profile can either hold steady, increase or decrease an integral number of units up to a parameter value. In our analysis, the parameter value was defined as fold-change $\geq |2|$. Then, the $\log_2$ gene expression values of the time series experiment for each miRNA are normalized, so that T1 corresponds to 0 (T1=0), and T2 and T3 are normalized accordingly. Each miRNA is assigned to the model profile to which its time series most closely matches based on the correlation coefficient. The number of miRNAs assigned to each model profile is then computed. Also, the number of miRNAs expected by chance to be assigned to a profile is calculated by randomly permuting the original time point values, renormalizing the expression values, then assigning miRNAs to their most closely matching model profiles, and repeating for a large number of permutations. The average number of all permutations is used as the estimate of the expected number of miRNAs assigned to the profile. The statistical significance of the number of miRNAs assigned to each profile versus the number expected is then computed, indicating if the profile presents more or less miRNAs than expected by chance.

Supplementary Reference

1. Torrezan, G. T. *et al.* A novel SYBR-based duplex qPCR for the detection of gene dosage: detection of an APC large deletion in a familial adenomatous polyposis patient with an unusual phenotype. *BMC Med. Genet.* **13,** 55 (2012).