SUPPLEMENTAL INFORMATION

Sequencing of *DICER1* in sarcomas identifies biallelic somatic *DICER1* mutations in an adult-onset embryonal rhabdomyosarcoma

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SUPPLEMENTAL MATERIALS AND METHODS

Patients and Samples:

We collected a total of 73 sarcomas as follows: 56 sarcoma samples (53 fresh frozen, and 3 Formalin-fixed paraffin-embedded (FFPE)) were obtained from the Victorian Cancer Biobank, Melbourne, Australia. Matched normal genomic DNA (gDNA) was obtained where possible [cases 1 to 43 and 45 to 56]; 4 cases of embryonal sarcoma of the liver (FFPE-derived) were acquired from Siriraj Hospital, Bangkok, Thailand [cases 57 to 60]; a single multicystic sarcoma of the thigh (FFPE-derived) was acquired from The Hospital for Sick Children, Toronto, Canada [case 61]; 11 Ewing sarcomas (5 fresh frozen and 6 FFPE-derived) were obtained from the Universitair Medisch Centrum in Utrecht, The Netherlands [cases 62 to 72]; and 1 additional case of Ewing sarcoma (FFPE-derived) was obtained from Erasmus MC University Medical Center, Rotterdam, The Netherlands [case 73] (see Supplemental Tables S2a and S2b).

Rationale for inclusion of embryonal sarcomas of the liver:

The four cases of embryonal sarcoma of the liver were included because this malignancy often arises from a benign cystic lesion, known as mesenchymal hamartoma. This development sequence could be regarded as analogous to that of other DICER1-related tumours, including DICER1 anaplasic sarcoma of the kidney arising from cystic nephroma (Wu *et al*, 2016), and Type II and Type III pleuropulmonary blastoma (PPB) arising within a pre-existing Type I PPB lesion.

Bioinformatics methods:

We performed SNP and INDEL discovery on Fluidigm-derived sequencing data using the Freebayes variant caller software v0.9.21 (Garrison & Gabor, 2012). All variants with alternate allele frequencies ≥10% were called and subsequently annotated with functional prediction using SnpEff v.4.1 (Cingolani *et al*, 2012b). Additionally, functional annotation of variants present in two public databases, NCBI dbSNP (Sherry *et al*, 2001) and dbNSFP (Liu *et al*, 2013), was added using SnpSift (Cingolani *et al*, 2012a). Depth of coverage was calculated for all samples using bedtools 2.25.0 (Quinlan & Hall, 2010) and at least 80% of the target region was covered at a depth of 10 or more reads in all 67 samples (Supplementary Figure S2).

Cloning experiments:

To determine the phase and effect of the mutations identified in Case 1, DNA and RNA were extracted from the fresh frozen tumour using the Qiagen DNeasy Blood & Tissue kit and the RNeasy Mini kit, respectively. cDNA was synthesised from RNA using Superscript III (Invitrogen). PCR amplification was performed using LongAmp Hot Start Taq DNA Polymerase (New England Biolabs Inc.) and the following primer pairs: *cDNA (exon 10 to 25):* 5'-CCTATGTTCAATCTAAAGGAAGAGC-3; and 5'-ATTAGTGGCCGCATCATGG-3'; *DNA (exon 11 to intron 12)*: 5'-GGCAGACAGCATACAGCAGA-3; and 5'-TGAACATGTAGATGACTACAAAAGC-3'. PCR fragments were TA cloned into pCR-XL-TOPO (Invitrogen) following the manufacturer recommendations. DNA from 48 cDNA clones and 24 DNA clones was collected using the QIAprep Spin Miniprep Kit (Qiagen) and Sanger sequenced (McGill University and Génome Québec Innovation Centre (MUGQIC)).

Droplet Digital PCR (ddPCR) experiment to investigate DICER1 mosaicism:

Investigation of the mosaic origin of the two *DICER1* mutations identified in case 1 was performed using the QX200 Droplet Digital PCR system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Custom TaqMan® probes (part 4331349, Life Technologies, Carlsbad, CA, USA) aimed to target the mutant alleles (c.5439G>T, p.E1813D (exon 25) and c.1785_1786insA, p.T596Nfs*3 (exon 11)) were designed for this purpose using the Custom TaqMan® Assay Design Tool (Life Technologies) and are listed in the table below:

***DICER1* Mutation**	**Target assay**	**Probe & Primer Sequences**
c.1785_1786insA (exon 11)	T596Nfs_ANEPR9P_A	F primer: TGGGAAAACGTCATCATCATCCAT R primer: TCAGATCTTGAGAAACAAGTGTTCCAA VIC probe: AGTCTCACCAG**T**ATCAAC FAM probe: TCTCACCAG**TT**ATCAAC
c.5439G>T (exon 25)	E1813D_ANKA3JE_T	F primer: CAGTGACATCCCACTATCCATGTAA R primer: GGAGGATGAAGAGAAAGAAGAGGATATTG VIC probe: TTTTTGA**G**TCGCTTGCTG FAM probe: ATTTTTGA**T**TCGCTTGCTG

The 20 µl reaction mix consisted of 10 µl of 2x ddPCR SuperMix for Probes (Bio-Rad Laboratories), 0.5 µl of the 40X SNP genotyping assay (T596Nfs_ANEPR9P or E1813D_ANKA3JE), 8.5 µl of water and 1 µl of

genomic DNA (50ng/µl). Assays were validated by temperature gradient to ensure optimal separation of alternate and reference-allele-containing droplets. Cycling conditions for the reaction were 95°C for 10 min, followed by 45 cycles of 94°C for 30 sec and 60°C for 1 min, 98°C for 10 minutes and finally a 10°C hold on a Life Technologies Veriti thermal cycler. Data was analysed using QuantaSoft v1.6.6 (Bio-Rad Laboratories) with default parameters. Each experiment was performed in duplicate on a set of samples including the tumour and adjacent normal DNA's from patient 1, 6 negative controls which were known not to harbour either of the mutations in question, 5 to 6 positive control DNAs into which we spiked DNA harbouring the target mutations at varying proportions, and two reference DNA samples (NA10843 and HuRef) plus 1 non-template control. This experiment was performed by The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Canada.

The results of the experiment are presented in Supplemental Table S4. In summary, we detected both the exon 11 and exon 25 mutations in ~1.10% frequency in the adjacent normal DNA sample. Our interpretation of the mutations being present at almost exactly the same frequency is that there is low-level tumour DNA contamination of the adjacent normal sample. Unfortunately, no additional non-tumourous DNA samples were available from the now-deceased patient. Nevertheless, we believe that the results of the ddPCR experiment suggest that it is unlikely that either of the pathogenic mutations from case 1 are mosaic in origin.

TruSight Tumor 15 Panel Sequencing of Case 1:

Given the young age of sarcoma onset in case 1 (at 23 years of age), a *TP53* germ-line mutation may be suspected. We therefore performed targeted sequencing of *TP53* in the patient's tumour and adjacent normal DNA samples using the TruSight Tumor 15 panel from Illumina. The panel targets the full coding region of *TP53* in addition to regions of 14 other genes that are frequently somatically-mutated in solid tumours (additional information on the capture design and protocol can be found at the following address: https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/trusight-tumor-15-data-sheet-1170-2015-003.pdf). DNA quantification, library preparation and sequencing in a MiSeq Sequencer were performed following manufacturer specifications. The obtained sequencing data was aligned to the Human

reference sequence (Human_v37p10_dbsnp135) and was analysed using Soft Genetics software (version 2.4.2) using default parameters. All variants with alternate allele frequencies ≥5% were called. Subsequent variant annotation was performed using the web-based wANNOVAR software (http://wannovar.wglab.org/) and then manually evaluated using the Integrative Genomics Viewer (version 2.3) (https://software.broadinstitute.org/software/igv/download). Final results are presented in Supplemental Table S5. In summary, 12 variants were called (all germ-line in origin) in 6 genes, including 3 silent missense variants (*PDGFRA* (n = 2); *KIT* (1)), 6 intronic variants (*EGFR* (3); *MET* (2); *ERBB2* (1)), and 3 and low-frequency variants were identified in the 3'UTR of *TP53.* In addition, loss of heterozygosity (LOH) is evident in the tumour on chromosome 7, involving *EGFR* (extent of LOH not known). No pathogenic germ-line or somatic *TP53* mutations were identified.

Copy Number Variation (CNV) experiment (ddPCR):

A total of 59 tumours samples and 52 normal samples (52 pairs and 7 non-paired tumours) for which sufficient, good quality DNA was available were investigated for the presence of copy number variations involving the *DICER1* locus. Given the plausible presence of somatic CNVs in the sarcomas, two different experiments were performed using a reference probe in either the *TERT* locus on chromosome 5 or within the *AMOT* locus on the X chromosome. Copy number estimation of *DICER1* was performed using the QX200 Droplet Digital PCR system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using the primers and probe assays outlined in the table below:

Target Assay		**Reference Assay**		**TUMOUR (n = 59)**	**NORMAL (n = 52)**
DICER1-Hs00237483_cn	vs	TaqMan® Copy Number Reference Assay, human, TERT	→	Done	Done
DICER1-Hs00237483_cn	vs	PrimePCR™ ddPCR™ Expression Probe Assay: AMOT, Human dHsaCPE5035959	→	Done	Done

Prior to the copy number experiment, 50 ng of genomic DNA was digested with 2.5U of NspI in a 5 µl reaction (New England Biolabs, Ipswich, Massachusetts, United States), 1 h x 37°C incubation and no enzyme

denaturation. The 20 µl copy number reaction mix consisted of 10 µl of 2x ddPCR SuperMix for Probes (Bio-Rad Laboratories), 1 µl of the copy number target assay (DICER1- Hs00237483_cn (localized within the RNase IIIb domain of *DICER1*) labelled with FAM), 1ul of the copy number reference assay (*TERT* (Life Technologies part 4403316, labelled with VIC) or *AMOT* (Bio-Rad Laboratories, AMOT_dHsaCPE5035959 (assay is exonic) labelled with HEX)), 3 µl water and 5 µl of 10 ng/µl digested genomic DNA. All assays were validated by temperature gradient to ensure optimal separation of target and reference-containing droplets. Cycling conditions for the reaction were 95°C for 10 min, followed by 45 cycles of 94°C for 30 sec and 60°C for 1 min, 98°C for 10 minutes and finally a 10°C hold on a Life Technologies Veriti thermal cycler. Data was analysed using QuantaSoft v1.6.6 (Bio-Rad Laboratories) with default parameters and the *DICER1* vs *AMOT* CNV ratios were calculated taking the patients' gender (and therefore X load) into account. Two reference DNA samples (NA10843 and HuRef, both male) plus 1 to 2 non-template controls were included with the study samples. Experiments were performed at the Genetic Analysis Facility, The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Canada.

For the interpretation of CNVs, samples in which a copy number alteration was only evident in one of the two experiments (*DICER1* vs *TERT*; or *DICER1* vs *AMOT*) were considered as having a copy number alteration encompassing the reference gene locus (*TERT* or *AMOT*); samples for which both experiments resulted in a *DICER1*/reference copy number ratio of <1.25 or >2.7 were considered to be positive, representing a loss or gain in *DICER1*, respectively. Five cases were found to have a CNV involving the *DICER1* locus (8.5%). This is a minimal estimation given our resolution power. A summary of the findings is presented in Supplemental Table S6.

SUPPLEMENTAL TABLES

SUPPLEMENTAL TABLE S1a. The 20 most frequently mutated genes in 811 sarcomas from TCGA

	Gene	# Mutations in gene	# Samples with mutation in gene	Mutation frequency
1	***TP53***	191	179	23.55%
2	***PIK3CA***	52	47	6.41%
3	***ATRX***	52	45	6.41%
4	***PCLO***	49	29	6.04%
5	***LRP1B***	45	28	5.55%
6	***PTEN***	44	30	5.43%
7	***EWSR1***	43	43	5.30%
8	***RB1***	40	35	4.93%
9	***KMT2D***	38	29	4.69%
10	***STAG2***	36	34	4.44%
11	***OBSCN***	36	24	4.44%
12	***FBXW7***	35	32	4.32%
13	***SYNE1***	35	25	4.32%
14	***FRG1BP***	31	21	3.82%
15	***NF1***	30	25	3.70%
16	***PLEC***	30	18	3.70%
17	***XIRP2***	29	10	3.58%
18	***ARID1A***	28	22	3.45%
19	***DMD***	28	19	3.45%
20	***RYR2***	28	17	3.45%

Method of TCGA data retrieval and analysis: A list of mutated genes** from each of 7 sarcoma-specific studies (more information in Table S3b) was downloaded from The Cancer Genome Altas Network (TCGA) database via the cBioportal (http://www.cbioportal.org/index.do). The data were consolidated to obtain the number of mutations in each gene and the number of samples with a mutation in the gene in question. The mutation frequency was then calculated based on the number of mutations identified within a gene and the total number of samples sequenced (n = 811). After sorting by mutation frequency, a list of the top 20 most frequently somatically-mutated genes in sarcomas was compiled.

**genes that are in the top 500 recurrently-mutated (≥2 mutations), are known cancer genes, or are detected by MutSig (http://www.cbioportal.org/)

SUPPLEMENTARY TABLE S1b. Sarcoma subtypes comprising TCGA cohort

	Histology	#Samples	Institute(s)	PubMed Reference ID
1	Pediatric Ewing sarcoma	105	DFCI	PMID: 25186949
2	Ewing Sarcoma	112	Institut Curie	PMID: 25223734
3	Leiomyosarcoma	134	MSKCC (n = 27); TCGA (n = 107)	PMID: 20601955 (MSKCC)
4	Dedifferentiated liposarcoma	109	MSKCC (n = 50); TCGA (n = 29)	PMID: 20601955 (MSKCC)
5	Pleomorphic liposarcoma	26	MSKCC (n = 24); TCGA (n = 2)	PMID: 20601955 (MSKCC)
6	Myxofibrosarcoma	63	MSKCC (n = 38); TCGA (n = 25)	PMID: 20601955 (MSKCC)
7	Malignant Peripheral Nerve Sheath Tumour	10	TCGA	--
8	Synovial sarcoma	34	MSKCC (n = 24); TCGA (n = 10)	PMID: 20601955 (MSKCC)
9	Embryonal Rhabdomyosarcoma	29	NIH	PMID: 24436047
10	Alveolar Rhabdomyosarcoma	18	NIH	PMID: 24436047
11	Mixed Alveolar/Embryonal Rhabdomyosarcoma	3	NIH	PMID: 24436047
12	Rhabdomyosarcoma-NOS	3	NIH	PMID: 24436047
13	Myxoid/round cell liposarcoma	21	MSKCC	PMID: 20601955
14	Gastrointestinal stromal tumour	22	MSKCC	PMID: 20601955
15	Desmoid/Aggressive Fibromatosis	2	TCGA	--
16	Undifferentiated Pleomorphic Sarcoma/High-Grade Spindle Cell Sarcoma	50	TCGA	--
17	Uterine carcinosarcoma/uterine malignant mixed Mullerian	70	Johns Hopkins (n = 22); TCGA (n = 57)	PMID: 25233892 (JHU)
	Total:	811		

Abbreviations: NOS, not otherwise specified; DFCI, Dana-Farber Cancer Institute; MSKCC, Memorial Sloan Kettering Cancer Center; TCGA, The Cancer Genome Atlas; NIH, National Insitutes of Health.

SUPPLEMENTAL TABLE S1c. *DICER1* variants in TGCA sarcoma cohort

Cases	Tumour Histology	Protein Change	DNA Change	DICER1 Domain	Mutation Status	#Mutations in Sample	Insitute	PubMed Reference ID
1	Ewing sarcoma	p.Q1832R	c.5495A>G	DUF	Unknown	276	DFCI	25186949
2	Ewing sarcoma	p.P365T	c.1093C>A	TRBPBD	Unknown	75	DFCI	25186949
3	Leiomyosarcoma	p.N477S	c.1430A>?	HELICc	Somatic	24	TCGA	
4	Malignant Peripheral Nerve Sheath Tumour	p.D1709N	c.5125G>?	RNase IIIb (hotspot)	Somatic	38	TCGA	
5	Undifferentiated Pleomorphic Sarcoma/High-Grade Spindle Cell Sarcoma	p.A1560T	c.4678G>?	RNase IIIb (non-hotspot)	Somatic	511	TCGA	
6	Uterine Carcinosarcoma/Uterine Malignant Mixed Mullerian Tumour	p.P986S	c.2956C>T	PAZ	Unknown	5502	Johns Hopkins	25233892
		p.L1164F	c.3490C>T	Between connector helix & RNase IIIa	Unknown			
7	Uterine Carcinosarcoma/Uterine Malignant Mixed Mullerian Tumour	p.N1609H	c.4825A>C	Between RNase IIIa & RNase IIIb	Unknown	5639	Johns Hopkins	25233892
8	Uterine Carcinosarcoma/Uterine Malignant Mixed Mullerian Tumour	p.D1709N	c.5125G>A	RNase IIIb (hotspot)	Unknown	30	Johns Hopkins	25233892
9	Uterine Carcinosarcoma/Uterine Malignant Mixed Mullerian Tumour	p.D1810V	c.5429A>?	RNase IIIb (hotspot)	Somatic	66	TCGA	
10	Uterine Carcinosarcoma/Uterine Malignant Mixed Mullerian Tumour	p.D1709N	c.5125G>?	RNase IIIb (hotspot)	Somatic	3863	TCGA	

Abbreviations: DFCI, Dana-Farber Cancer Institute; TCGA, The Cancer Genome Atlas Research Network

SUPPLEMENTAL TABLE S2a. Sarcoma (various subtypes) clinical information

Case #	DEMOGRAPHICS				DIAGNOSIS & PATHOLOGY					SURGERY	PERSONAL AND FAMILY HISTORY		MATERIALS & METHODS			
	Age at Dx	Sex	Age at Death	Cause of Death	Diagnosis	Tumour Site	Stage	Differentiation or Grade	Bone vs ST	Surgical Procedure	Personal History of Cancer?	Family History of Cancer?	Sample Type	*DICER1* Sequencing Method	Germline *DICER1* MLPA	Tumour CNV Analysis
1	23.0	F	27.8	Disease	Recurrent embryonal rhabdomyosarcoma	Retroperitoneum (broad ligament)	4	High grade	ST	Ileo-colic resection	NR	NR	FFT	Fluidigm	Done	Done
2	53.3	F	57.1	Disease	Metastatic Leiomyosarcoma (with non caseating granulomas)	R upper lobe of lung	4	High grade	ST	R metastasectomy	High grade leiomyosarcoma	NR	FFT	Fluidigm	Done	Done
3	22.0	F	26.1	Disease	Undifferentiated sarcoma	L lower lobe of lung	4	Large moderately pleomorphic	ST	L lower lobectomy	Vulval neural ectodermal carcinoma (13y), Ewing sarcoma	NR	FFT	Fluidigm	Done	Done
4	36.9	F	NA	NR	Parosteal osteosarcoma	Long bones of lower limb	NR	Low grade	Bone	Biopsy of L femur	NR	NR	FFT	Fluidigm	Done	Done
5	54.0	M	NA	NR	Undifferentiated pleomorphic sarcoma	L proximal femur	3	High grade	ST	L proximal femur	None	None	FFT	Fluidigm	Done	Done
6	47.2	M	NA	NR	High-grade intravascular sarcoma	R lung	4	High grade	ST	R pneumonectomy	None	NR	FFT	Fluidigm	Done	Done
7	55.3	M	56.4	NR	Undifferentiated pleomorphic sarcoma	Soft tissue of pelvis	3	NR	ST	L hemipelvectomy	None	NR	FFT	Fluidigm	Done	Done
8	37.7	M	NA	NR	Undifferentiated pleomorphic sarcoma	Soft tissue of L thigh	2A	High Grade (4)	ST	L thigh excision	NR	NR	FFT	Fluidigm	Done	Done
9	46.0	M	NA	NA	Pleomorphic liposarcoma	L distal thigh	4	With myxoid and pleomorphic areas	ST	L distal thigh excision	Cutaneous leiomyosarcoma (pectoral)	NR	FFT	Fluidigm	Done	Done
10	73.9	F	76.4	NR	Solitary fibrous tumour	Ischiorectal fossa (anus)	1A	Relatively benign	ST	Laparotomy	Bladder tumour	NR	FFT	Fluidigm	Done	Done
11	24.3	F	26.4	Disease	Angiosarcoma	R breast	4	High grade	ST	R mastectomy	NR	NR	FFT	Fluidigm	Done	Done
12	26.1	F	NA	NA	Epitheloid haemangioendothelioma	R foot	1B	Low grade	ST	Lower leg amputation	None	NR	FFT	Fluidigm	Done	Done
13	19.8	M	NA	NA	Synovial sarcoma	R knee	4	Monophasic	ST	Wide excision	NR	NR	FFT	Fluidigm	Done	Done
14	71.0	M	81.3	Disease	Undifferentiated pleomorphic sarcoma	R thigh	4	NR	ST	R thigh excision	None	NR	FFT	Fluidigm	Done	Done
15	32.0	M	NA	NA	Angiomatoid fibrous histiocytoma	L groin	4	Low grade	ST	L groin/anterior pelvic wall excision	NR	Grandfather: Lung	FFT	Fluidigm	Done	Done
16	21.9	M	NA	NA	Ewing sarcoma	L clavicle	4	NA	Bone	L clavicle excision	Lumber spinal Ewing sarcoma (11y)	NR	FFT	Fluidigm	Done	Done
17	30.4	F	NA	NR	Undifferentiated pleomorphic sarcoma	L thigh	4	Grade 3	ST	L thigh excision	NR	None significant	FFT	Fluidigm	Done	Done
18	75.6	F	NA	NA	Low grade chondrosarcoma	L femoral shaft	1B	Low grade (2)	Bone	L femoral shaft biopsy	NR	NR	FFT	Fluidigm	Done	Done
19	81.2	F	NA	NA	Undifferentiated pleomorphic sarcoma	R thigh	2B	Intermediate grade	ST	R thigh excision	None	Brother: Liver ca.	FFT	Fluidigm	Done	Done
20	77.9	M	78.2	Disease	Undifferentiated pleomorphic sarcoma	Ilium	4	High grade pleomorphic	ST	Hindquarter amputation	NR	NR	FFT	Fluidigm	Done	Done
21	40.4	M	NA	NR	Myxoid liposarcoma	R popliteal fossa	2B/3	NR	ST	R popliteal fossa excision	None	NR	FFT	Fluidigm	Done	Done

SUPPLEMENTAL TABLE S2a Continued. Sarcoma (various subtypes) clinical information

Case #	DEMOGRAPHICS				DIAGNOSIS & PATHOLOGY					SURGERY	PERSONAL AND FAMILY HISTORY		MATERIALS & METHODS			
	Age at Dx	Sex	Age at Death	Cause of Death	Diagnosis	Tumour Site	Stage	Differentiation or Grade	Bone vs ST	Surgical Procedure	Personal History of Cancer?	Family History of Cancer?	Sample Type	*DICER1* Sequencing Method	Germline *DICER1* MLPA	Tumour CNV Analysis
22	21.1	M	NA	NA	Desmoplastic small round cell tumour	Ascending colon	4	NR	ST	R colon and small bowel resection	None	NR	FFT	Fluidigm	Done	Done
23	70.3	F	NA	NA	Adamantinoma	Tibia	1	NR	Bone	Tibia excision	NR	Mother: Bowel; Father: NR cancer	FFT	Fluidigm	Done	Done
24	47.9	F	NA	NR	Undifferentiated pleomorphic sarcoma	R lower lobe of lung	4	High grade	ST	R lower lobectomy	Retroperitoneal sarcoma; L kidney cancer; 5x BCCs	NR	FFT	Fluidigm	Done	Done
25	64.3	F	NA	NR	Undifferentiated pleomorphic sarcoma	L lower lobe of lung	4	High grade	ST	L lower lobectomy	Osteosarcomatous lesion in chest wall	NR	FFT	Fluidigm	Done	Done
26	60.3	M	NA	NA	Solitary fibrous tumour	Rectum	3	High grade. Malignant haemangiopericytoma	ST	High anterior resection	NR	None	FFT	Fluidigm	Done	Done
27	47.3	M	NA	NA	Dedifferentiated liposarcoma	Transverse colon	1	High grade (3), features are of a spindle cell sarcoma	ST	En bloc R hemicolectomy	None	NR	FFT	Fluidigm	Done	Done
28	87.4	M	NA	NR	Undifferentiated pleomorphic sarcoma	L forearm	2B	High grade	ST	L forearm excision	None	NR	FFT	Fluidigm	Done	Done
29	26.5	F	NA	NA	Undifferentiated sarcoma of the breast	L breast	2	High grade/undifferentiated	ST	L breast/chest wall resection	None	None	FFT	Fluidigm	Done	Done
30	50.6	M	NA	NA	Metastatic epithelioid sarcoma	L inguinal lymph node	4	High grade/undifferentiated	ST	Inguinal lymphadenectomy	Skin cancer	Mother: Brain tumour	FFT	Fluidigm	Done	Done
31	45.3	F	46.8	Disease	Periacetabular osteosarcoma	L pelvis	4	Pleomorphic spindle cells	Bone	L periacetabular wider resection	None	None	FFT	Fluidigm	Done	Done
32	43.0	M	NA	Disease	Ewing sarcoma/ PNET	L chest wall	4	NA	Bone	Wide en bloc resection	None	NR	FFT	Fluidigm	Done	Done
33	38.4	M	40.8	Disease	Metastatic clear cell sarcoma	Tibia soft tissue	4	NR	ST	Wide en bloc resection	Sarcoma	NR	FFT	Fluidigm	Done	Done
34	64.8	M	NA	NR	Chordoma (post radiotherapy)	Sacrococcygeal	2A	Locally advanced	Bone	En bloc resection including rectum	NR	No	FFT	Fluidigm	Done	Done
35	64.5	F	NA	NR	Myxofibrosarcoma	Back of L thigh	2B	Intermediate grade	ST	L thigh excision	None	NR	FFT	Fluidigm	Done	Done
36	48.8	M	50.3	Disease	Myxoid liposarcoma (post radiotherapy)	R thigh	4	NR	ST	Wide resection of R quadriceps	Small cell lung cancer	NR	FFT	Fluidigm	Done	Done
37	18.8	F	NA	NR	Ewing sarcoma (post-chemotherapy)	Femur	3	NA	Bone	En bloc resection of L distal femur	NR	NR	FFT	Fluidigm	Done	Done
38	68.1	M	70.0	Disease	Myxofibrosarcoma	R chest wall	4	High-grade/pleomorphic undifferentiated sarcoma	ST	Wide excision chest wall	Diffuse Large B-cell Lymphoma	NR	FFT	Fluidigm	Done	Done
39	61.0	M	NA	NA	Leiomyosarcoma	R inguinal	1A	Low grade	ST	R inguinal excision	NR	NR	FFT	Fluidigm	Done	Done
40	50	F	52	NR	Undifferentiated pleomorphic sarcoma	Small bowel	4	Ulcerated, high grade	ST	Resection of small bowel	Cardiac sarcoma	NR	FFT	Fluidigm	Done	Done
41	39.2	F	NA	NR	Liposarcoma	Retroperitoneum	2B	Components of well diff and de-diff liposarcoma, with low grade de-diff showing metaplastic bone formation	ST	Laparotomy	NR	NR	FFT	Fluidigm	Done	Done
42	73.0	M	NA	NR	Myxofibrosarcoma	R shoulder	1B	Low grade	ST	Wide excision, R shoulder	Skin SCCs and TURP	Brother: Prostate ca.	FFT	Fluidigm	Done	Done

SUPPLEMENTAL TABLE S2a Continued. Sarcoma (various subtypes) clinical information

Case #	DEMOGRAPHICS				DIAGNOSIS & PATHOLOGY					SURGERY	PERSONAL AND FAMILY HISTORY		MATERIALS & METHODS			
	Age at Dx	Sex	Age at Death	Cause of Death	Diagnosis	Tumour Site	Stage	Differentiation or Grade	Bone vs ST	Surgical Procedure	Personal History of Cancer?	Family History of Cancer?	Sample Type	*DICER1* Sequencing Method	Germline *DICER1* MLPA	Tumour CNV Analysis
43	47.5	F	49.9	Disease	Leiomyosarcoma	Retroperitoneum	4	Moderate pleomorphism	ST	Resection of retroperitoneum	Breast cancer; Low grade glioma	No	FFT	Fluidigm	Done	Done
44	33.8	F	NA	NA	Liposarcoma associated with radiotherapy changes	L retroperitoneum	3	High grade, well differentiated.	ST	Laparotomy	None	No	FFT	Fluidigm	Done	Done
45	62.9	M	NA	NR	Liposarcoma with radiation effect	L axilla	Benign	NR	ST	L wide axillary dissection	Long history of benign lipomas	NR	FFT	Fluidigm	Done	Done
46	79.0	F	NA	NR	Pleomorphic sarcoma with giant cells	L adductor magnus	4	Undifferentiated	ST	L adductor magnus excision	NR	NR	FFT	Fluidigm	Done	Done
47	77.5	F	NA	NA	Liposarcoma (post radiotherapy)	R thigh	T2b	Well differentiated. Low grade	ST	R thigh excision	NR	NR	FFT	Fluidigm	Done	Done
48	72.8	F	NA	NA	Undifferentiated pleomorphic sarcoma	R tibia	3	High grade	ST	Wide en bloc resection	NR	NR	FFT	Fluidigm	Done	Done
49	39.3	F	NA	NA	Low grade fibromyxoid sarcoma	L thigh	1A	Low grade	ST	L thigh excision	None	NR	FFT	Fluidigm	Done	Done
50	38.3	M	39.4	Disease	Epithelioid angiosarcoma	Small intestine	4	Moderately to poorly differentiated	ST	Abdominoperineal resection	None	None	FFT	Fluidigm	Done	Done
51	18.3	F	NA	NA	Metastatic malignant peripheral nerve sheath tumour	L lower lobe of lung	4	Low grade, cartilaginous differentiation	ST	L VATS wedge	Malignant peripheral nerve sheath tumour	Bowel cancer	FFT	Fluidigm	Done	Done
52	76.6	M	77.0	Disease	Undifferentiated pleomorphic sarcoma	R buttock	4	High grade	ST	R buttock excision	Leiomyosarcoma	NR	FFT	Fluidigm	Done	Done
53	68.1	F	NA	NA	Gastrointestinal stromal tumour (GIST)	Stomach	NR	Low risk for aggressive behaviour	ST	Laparoscopy and resection	No	No	FFPE	Fluidigm	Not Done	Not Done
54	60.1	F	NA	NA	Gastrointestinal stromal tumour (GIST)	Small bowel	NR	Low malignant potential	ST	Laparoscopic small bowl resection and gastroscopy	No	Father: Lung ca.	FFPE	Fluidigm	Not Done	Not Done
55	66.2	F	NA	NA	Gastrointestinal stromal tumour (GIST)	Stomach	NR	Low malignant potential	ST	Laparoscopic excision	No	Father: Colon ca.	FFPE	Fluidigm	Not Done	Not Done
56	NR	F	NA	NR	Myxoid liposarcoma	NR	NR	NR	ST	NR	NR	NR	FFT	Fluidigm	Done	Done
57	13.0	F	NR	NR	Embryonal sarcoma of the liver	Liver	NR	NR	ST	NR	NR	NR	FFPE	Sanger	Not Done	Not Done
58	9.0	F	NR	NR	Embryonal sarcoma of the liver	Liver	NR	NR	ST	NR	NR	NR	FFPE	Sanger	Not Done	Not Done
59	29.0	M	NR	NR	Embryonal sarcoma of the liver	Liver	NR	NR	ST	NR	NR	NR	FFPE	Sanger	Not Done	Not Done
60	6.5	M	NR	NR	Embryonal sarcoma of the liver	Liver	NR	NR	ST	NR	NR	NR	FFPE	Sanger	Not Done	Not Done
61	0.3	M	NA	NA	Multicystic sarcoma of the thigh (undifferentiated sarcoma)	Thigh	NR	NR	ST	NR	NR	NR	FFPE	Sanger	Not Done	Not Done

Abbreviations: BCC, basal cell carcinoma; Ca, carcinoma; CNV, copy number variation; Diff, differentiation; Dx, diagnosis; F, female; FFPE, formalin-fixed paraffin-embedded; FFT, fresh frozen tissue; L, left; M, male; Mets, metastasis; MLPA, Multiplex Ligation-dependent Probe Amplification assay; NA, not applicable; NR, not reported; PNET, primitive neuroectodermal tumour; R, right; SCC, squamous cell carcinoma; ST, soft tissue; TURP, Transurethral resection of the prostate; VATS, Video-assisted thoracoscopic surgery.

SUPPLEMENTAL TABLE S2b. Ewing sarcoma clinical information

Case	Age at Dx (years)	Sex	Diagnosis	Sample Type	*DICER1* Sequencing Method	Tumour CNV Analysis (ddPCR)
62	48.0	F	Ewing sarcoma	FFT	Fluidigm	Done
63	46.5	M	Ewing sarcoma	FFT	Fluidigm	Done
64	49.5	M	Ewing sarcoma	FFPE	Fluidigm	Not Done
65	25.0	F	Ewing sarcoma	FFPE	Fluidigm	Not Done
66	39.8	F	Ewing sarcoma	FFT	Fluidigm	Done
67	19.0	M	Ewing sarcoma	FFPE	Fluidigm	Not Done
68	25.0	M	Ewing sarcoma	FFT	Fluidigm	Done
69	19.0	F	Ewing sarcoma	FFT	Fluidigm	Done
70	0.7	F	Ewing sarcoma	FFPE	Fluidigm	Not Done
71	5.0	M	Ewing sarcoma	FFT	Fluidigm	Done
72	1.7	M	Ewing sarcoma	FFPE	Fluidigm	Not Done
73	3.0	F	Ewing sarcoma	FFPE	Sanger	Not Done

Abbreviations: CNV, copy number variation; ddPCR, droplet digital polymerase chain reaction; Dx, diagnosis; F, female; FFT, fresh frozen tumour; FFPE, formalin-fixed paraffin-embedded; M, male.

SUPPLEMENTAL TABLE S3. *DICER1* variants identified

Patient Information					Variant Information					Variant Frequency Data		Variant Effect Prediction	
Case #	Age at Dx (y)	Sex	Diagnosis	Tumour Site	DNA Change	Protein Change	Exon	Variant ID	Origin	ExAC MAF % (allele count)	EVS MAF %	PolyPhen-2 (score)	SIFT (score)
1	23	F	Recurrent embryonal rhabdomyosarcoma	Retroperitoneum	c.1786_1787insA	p.T596Nfs*3	11	--	Somatic	Not available	Not available	NA	NA
					c.2040+53_2040+54insT	--	12	rs397807177	Germ-line	Not available	Not available	NA	NA
					c.5439G>T	p.E1813D	25	--	Somatic	Not available	Not available	Probably Damaging (0.997)	Damaging (0)
11	24.3	F	Angiosarcoma	Right breast	c.5145C>T	p.L1715L	24	rs139500905	Germ-line	0.1475 (179/121396)	0.1538	NA	Tolerated (1)
14	71	M	Undifferentiated pleomorphic sarcoma	Right thigh	c.5145C>T	p.L1715L	24	rs139500905	Germ-line	0.1475 (179/121396)	0.1538	NA	Tolerated (1)
20	77.9	M	Undifferentiated pleomorphic sarcoma	Ilium	c.884C>G	p.S295C	7	rs548231008	Somatic	0.0952 (115/120792)	Not available	Probably Damaging (0.989)	Tolerated (0.06)
28	87.4	M	Undifferentiated pleomorphic sarcoma	Left forearm	c.4014G>A	p.A1338A	21	rs143454689	Germ-line	0.1043 (126/120760)	0.0384	NA	Tolerated (0.52)
38	68.1	M	Myxofibrosarcoma	Right chest wall	c.2040+29T>C	--	Int. 12	rs370866625	Germ-line	0.01571 (19/120918)	Not available	NA	NA
39	61	M	Leiomyosarcoma	Right inguinal	c.1377-4T>G	--	Int. 8	rs192490028	Germ-line	0.333 (401/120416)	0.3153	NA	NA
46	79	F	Pleomorphic sarcoma with giant cells	Left adductor magnus	c.3208C>G	p.L1070V	20	--	Somatic	Not available	Not available	Probably Damaging (1.000)	Damaging (0.01)
65	25	F	Ewing Sarcoma	Not available	c.2614G>A	p.A872T	16	COSM959266; rs149242330	Not known	0.08432 (102/120974)	0.0846	Probably Damaging (0.8)	Tolerated (0.5)
73	3	F	Ewing Sarcoma	Thorax	c.2257-7A>G	--	14	--	Germ-line	Not available	Not available	NA	NA

Abbreviations: Dx, diagnosis; EVS, Exome Variant Server (available at http://evs.gs.washington.edu/EVS/); ExAC, Exome Aggregation Consortium (available at http://exac.broadinstitute.org/); F, female; Int., intron; NA, not applicable; M, male; MAF, minor allele frequency.

SUPPLEMENTALTABLE S4. ddPCR Results: Investigation of mosaic origin of *DICER1* mutations in Case 1

	Exon 11 Mutation: c.1786_1787insA			Exon 25 Hotspot Mutation: c.5439G>T		
	Replicate 1 Fractional Abundance (%)	Replicate 2 Fractional Abundance (%)	Mean Fractional Abundance (%)	Replicate 1 Fractional Abundance (%)	Replicate 2 Fractional Abundance (%)	Mean Fractional Abundance (%)
CASE 1 TUMOUR	46.4%	48.6%	47.5%	49.3%	49%	49.15%
CASE 1 NORMAL	0.89%	1.34%	1.12%	0.87%	1.30%	1.09%

Interpretation: Both the exon 11 and exon 25 mutations were detected in approximately 1.1% in the patient's adjacent normal DNA sample. Our interpretation of the mutations being present at almost exactly the same frequency is that there is low-level tumour DNA contamination of the adjacent normal sample. The results of the ddPCR experiment suggest that it is unlikely that either of the pathogenic mutations from case 1 are mosaic in origin.

SUPPLEMENTAL TABLE S5. Results of TruSight Tumour 15 Panel Sequencing of Case 1

Variant Information										Variant Frequency Data			Case 1 - NORMAL					Case 1 - TUMOUR					Notes
Chr	Start	End	Ref	Alt	Gene	Region	Variant Information	Variant ID	COSMIC ID	1000G MAF %	ExAC MAF %	ESP MAF %	Total Reads	# Ref Reads	# Alt Reads	Ref % Freq.	Alt % Freq.	Total Reads	# Ref Reads	# Alt Reads	Ref % Freq.	Alt % Freq.	
4	55141055	55141055	A	G	*PDGFRA*	exon 12	NM_006206:c.A1701G,p.P567P	rs1873778	COSM1430082	0.96	0.988	0.96	39557	28	39506	0.1	99.9	29646	29	29610	0.1	99.9	Germ-line (homozygous)
4	55152040	55152040	C	T	*PDGFRA*	exon 18	NM_006206:c.C2472T,p.V824V	rs2228230	COSM22413	0.24	0.182	0.2	22046	11306	10728	51.3	48.7	15282	7648	7626	50.0	49.9	Germ-line (heterozygous)
4	55602765	55602765	G	C	*KIT*	exon 18	NM_000222:c.G2586C,p.L862L	rs3733542	COSM1325	0.16	0.1159	0.19	35183	17595	17511	50.0	49.8	30476	14873	15536	48.8	51.0	Germ-line (heterozygous)
7	55128207	55128207	A	G	*EGFR*	intronic	NM_005228.3:c.88+41149A>G	rs729969	--	0.85	--	--	5815	8	5803	0.1	99.8	4704	9	4692	0.2	99.7	Germ-line (homozygous)
7	55220177	55220177	A	G	*EGFR*	intronic	NM_005228.3:c.629-62A>G	rs11506105	--	0.55	--	--	12445	6348	6093	51.0	49.0	9686	9497	183	98.0	1.9	Germ-line (heterozygous): Chr7 LOH in tumour
7	55228053	55228053	A	T	*EGFR*	intronic	NM_005228.3:c.1498+22A>T	rs1558544	--	0.77	0.7697	0.66	4238	2207	2027	52.1	47.8	3541	3457	80	97.6	2.3	Germ-line (heterozygous): Chr7 LOH in tumour
7	116312986	116312986	T	C	*MET*	intronic	NM_000245.3:c.-15+355T>C	rs38840	--	0.88	--	--	7201	10	7186	0.1	99.8	6061	12	6046	0.2	99.8	Germ-line (homozygous)
7	116319002	116319002	A	G	*MET*	intronic	NM_000245.3:c.-15+6371A>G	rs714180	--	0.63	--	--	4379	6	4371	0.1	99.8	3351	1	3350	0.0	100.0	Germ-line (homozygous)
17	7572024	7572024	T	A	*TP53*	3'UTR	NM_001126112:c.*903A>T	--	--	--	--	--	13188	12349	827	93.6	6.3	8111	7585	517	93.5	6.4	Germ-line (low frequency)
17	7572026	7572026	T	A	*TP53*	3'UTR	NM_001126112:c.*901A>T	--	--	--	--	--	13188	12230	945	92.7	7.2	8111	7554	553	93.1	6.8	Germ-line (low frequency)
17	7572029	7572029	T	A	*TP53*	3'UTR	NM_001126112:c.*898A>T	--	--	--	--	--	13187	12451	729	94.4	5.5	8111	7711	397	95.1	4.9	Germ-line (low frequency)
17	37870837	37870837	T	C	*ERBB2*	intronic	NM_001005862.2:c.1059-702T>C	rs191397129	--	0.0066	--	--	3261	1635	1620	50.1	49.7	1916	950	963	49.6	50.3	Germ-line (heterozygous)

Genes targeted on panel: *TP53, NRAS, FOXL2, PIK3CA, KIT, PDGFRA, BRAF, EGFR, MET, GNAQ, RET, KRAS, AKT1, ERBB2, GNA11.* **Abbreviations:** Alt, alternate allele; Freq. Frequency; LOH, loss of heterozygosity; MAF, minor allele frequency; Ref, reference allele.

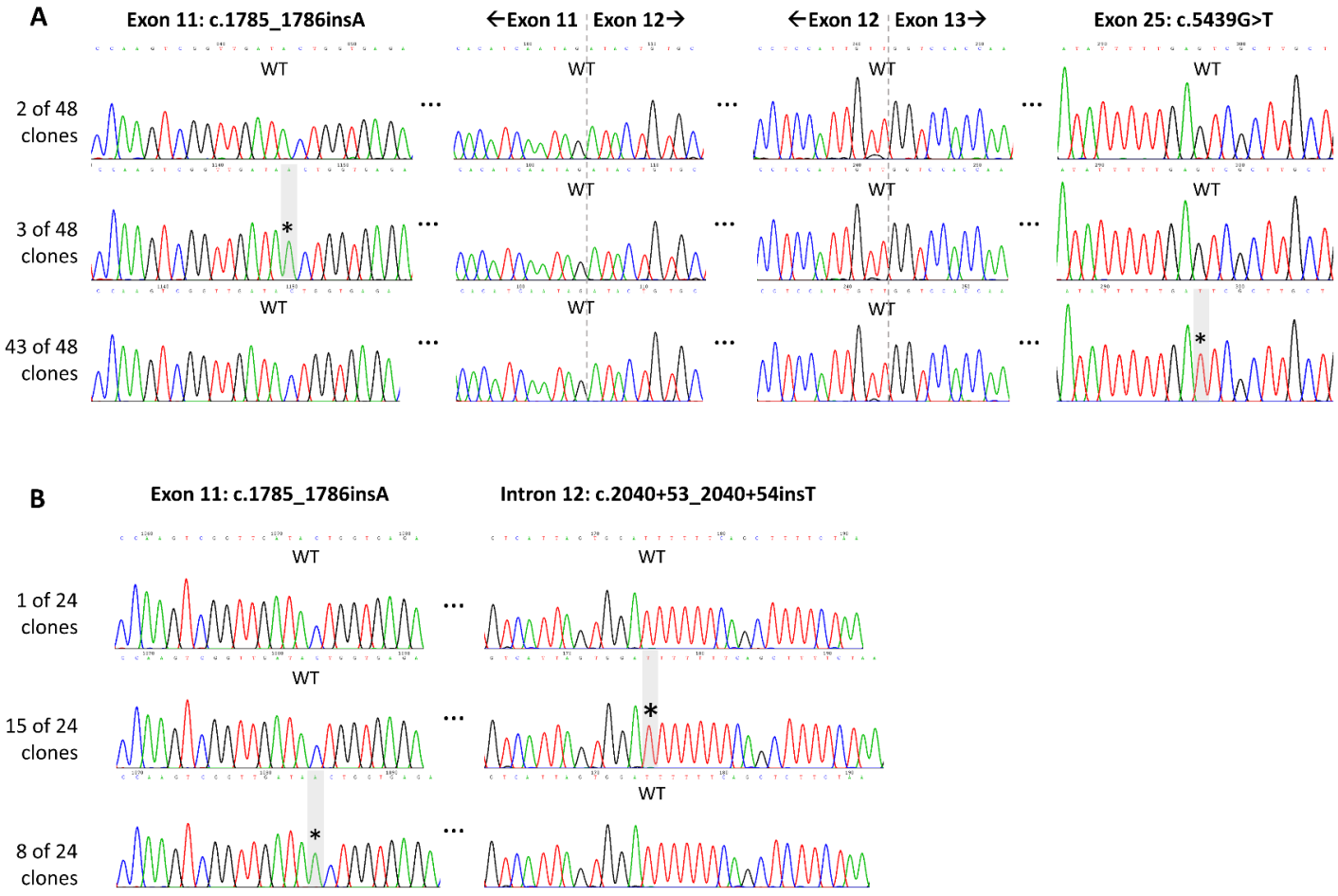
SUPPLEMENTAL TABLE S6. *DICER1* Copy Number Variation Results

Case #	Gender	Sarcoma Subtype	*DICER1* Copy Number Variation Identified? *
1	Female	Recurrent embryonal rhabdomyosarcoma	No
2	Female	Metastatic Leiomyosarcoma	No
3	Female	Undifferentiated sarcoma	No
4	Female	Parosteal osteosarcoma	No
5	Male	Undifferentiated pleomorphic sarcoma	No
6	Male	High-grade intravascular sarcoma	No
7	Male	Undifferentiated pleomorphic sarcoma	No
8	Male	Undifferentiated pleomorphic sarcoma	No
10	Female	Solitary fibrous tumour	No
11	Female	Angiosarcoma	No
12	Female	Epitheloid haemangioendothelioma	No
14	Male	Undifferentiated pleomorphic sarcoma	No
15	Male	Angiomatoid fibrous histiocytoma	No
16	Male	Ewing sarcoma	No
17	Female	Undifferentiated pleomorphic sarcoma	No
18	Female	Low grade chondrosarcoma	No
19	Female	Undifferentiated pleomorphic sarcoma	Yes (Gain)
20	Male	Undifferentiated pleomorphic sarcoma	No
21	Male	Myxoid liposarcoma	No
22	Male	Desmoplastic small round cell tumour	No
23	Female	Adamantinoma	No
24	Female	Undifferentiated pleomorphic sarcoma	No
25	Female	Undifferentiated pleomorphic sarcoma	No
26	Male	Solitary fibrous tumour	No
27	Male	Dedifferentiated liposarcoma	Yes (Gain)
28	Male	Undifferentiated pleomorphic sarcoma	No
30	Male	Metastatic epithelioid sarcoma	No
31	Female	Periacetabular osteosarcoma	Yes (Gain)
32	Male	Ewing sarcoma/ PNET	No
33	Male	Metastatic clear cell sarcoma	No
34	Male	Chordoma (post radiotherapy)	No
35	Female	Myxofibrosarcoma	No
36	Male	Myxoid liposarcoma (post radiotherapy)	No
37	Female	Ewing sarcoma (post-chemotherapy)	No
38	Male	Myxofibrosarcoma	No
39	Male	Leiomyosarcoma	No
40	Female	Undifferentiated pleomorphic sarcoma	No
41	Female	Liposarcoma	No
42	Male	Myxofibrosarcoma	Yes (Loss)
43	Female	Leiomyosarcoma	No
44	Female	Liposarcoma associated with radiotherapy changes	No
45	Male	Liposarcoma with radiation effect	No
46	Female	Pleomorphic sarcoma with giant cells	No
47	Female	Liposarcoma (post radiotherapy)	No
48	Female	Undifferentiated pleomorphic sarcoma	No
49	Female	Low grade fibromyxoid sarcoma	No
50	Male	Epithelioid angiosarcoma	No
51	Female	Metastatic malignant peripheral nerve sheath tumour	No
52	Male	Undifferentiated pleomorphic sarcoma	No
56	Female	Myxoid liposarcoma	No
62	Female	Ewing sarcoma	Yes (Gain)
63	Male	Ewing sarcoma	No
66	Female	Ewing sarcoma	No
68	Male	Ewing sarcoma	No
69	Female	Ewing sarcoma	No
71	Male	Ewing sarcoma	No

Abbreviations: CNV, copy number variation; F, female; M, Male. **Notes: *** CNV analysis was performed using the droplet digital PCR system. Two different experiments were performed using a reference probe in either the TERT locus on Chromosome 5 or within the AMOT locus on Chromosome X. See Supplemental Information, Materials and Methods section for more details.

SUPPLEMENTAL FIGURES

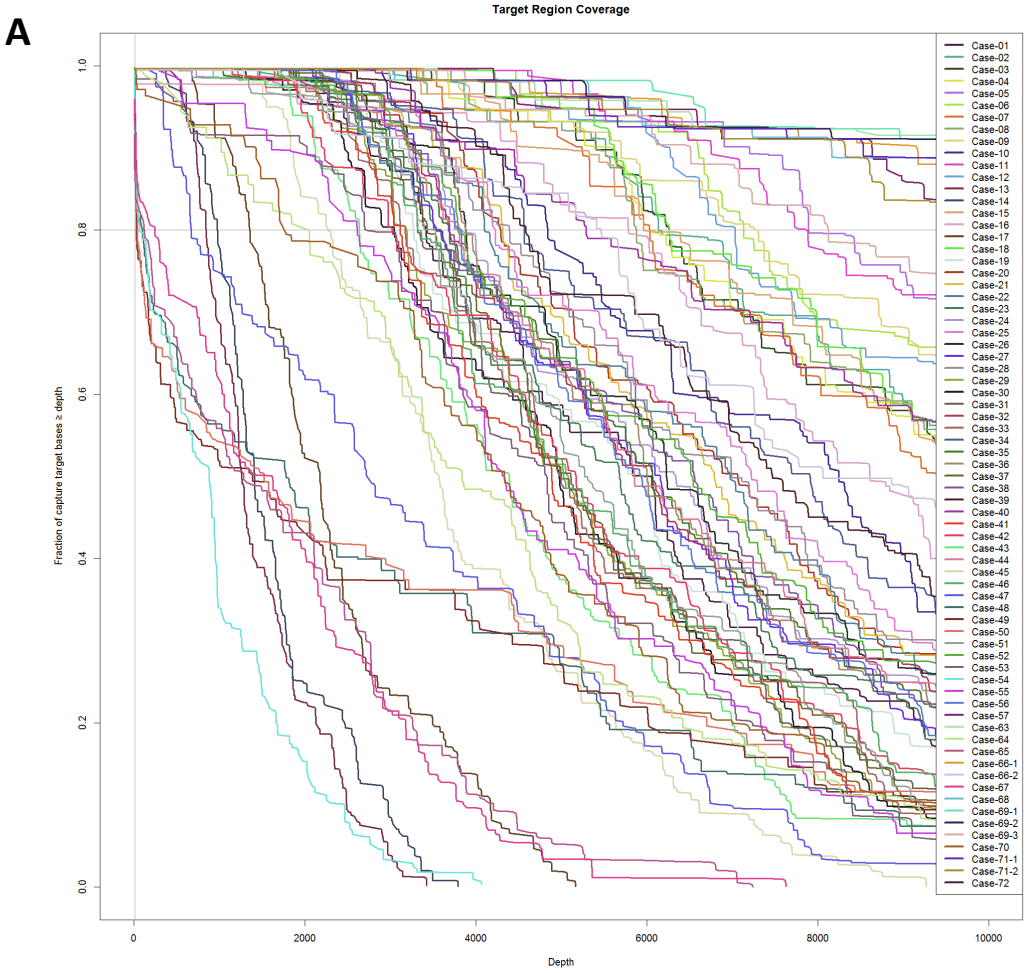
Supplemental Figure S1

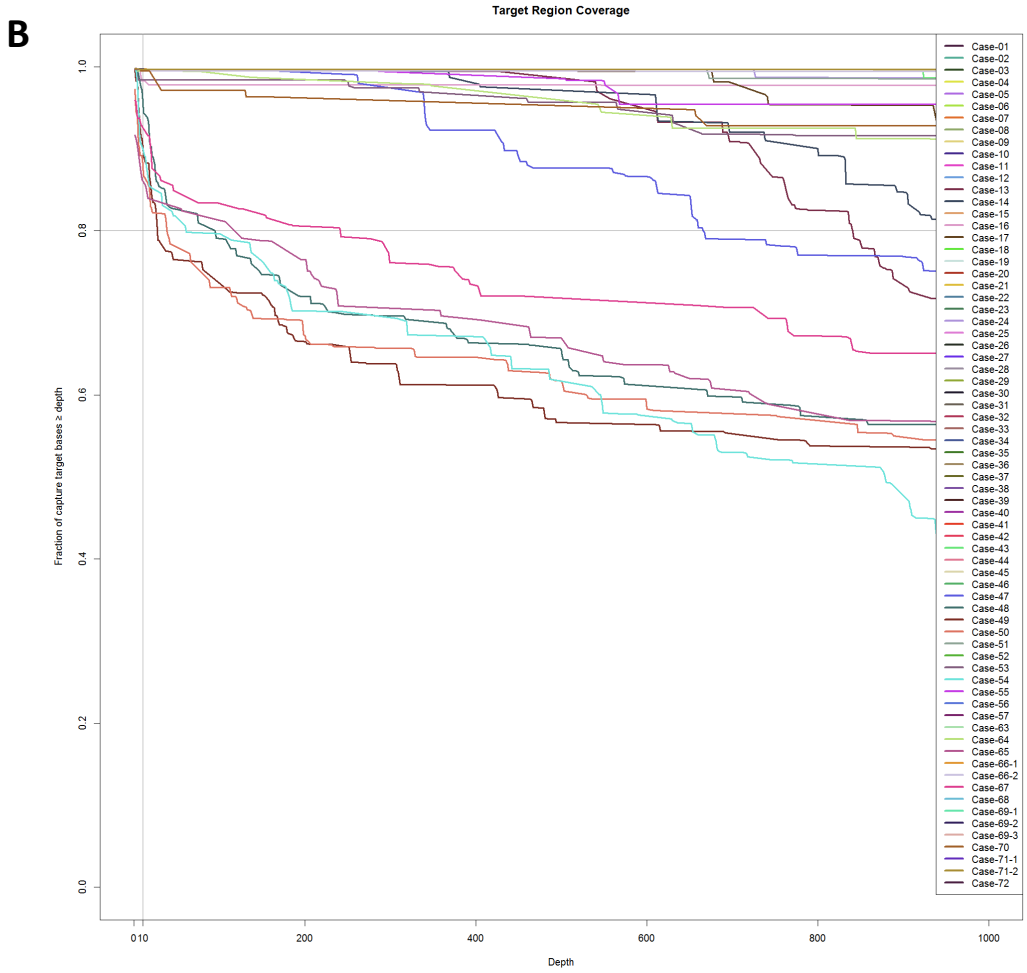


Supplemental Figure S1. Cloning results – Case 1. A) The phase and effect of the three identified *DICER1* mutations was assessed by cloning a fragment of complementary DNA (cDNA), synthesised from tumour RNA that spanned from exon 10 to exon 25 of *DICER1*, and sequencing of the resulting products. The chromatograms depict the three scenarios observed in cDNA clones. 2 of the 48 clones were wild-type at the position of both the exon 11 and exon 25 mutations. These cDNA fragments were likely derived from non-tumourous cells. 3 of 48 clones expressed the c.1785_1786insA mutation, but were wild-type at the c.5439 position. The remaining 43 clones were found to be wild-type at the position of the exon 11, c.1785_1786insA mutation, but mutated at position c.5439 in exon 25. This indicates that the c.1785_1786insA and c.5439G>T mutations are present *in trans*. It is also evident that the majority of transcripts carrying the c.1785_1786insA mutation are degraded by nonsense-mediated decay, since only 3/48 clones were found to express the mutation.

The intron 12 mutation (c.2040+53_2040+54insT) did not seem to affect splicing of exons in the sequenced clones, as the exons 11 to 12 and 12 to 13 splice junctions were normal. **B)** The phase of the exon 11 and intron 13 mutations was assessed by cloning a DNA fragment that spanned from exon 11 to intron 12, and sequencing of the resulting products. The chromatograms depict the three scenarios observed in the DNA clones. 1 of the 24 clones was wild-type at the position of both the exon 11 and intron 12 mutations. This DNA fragment was likely derived from a non-tumourous cell. 15/24 clones carried only the intron 12, c.2040+53_2040+54insT mutation. The remaining 8/24 clones carried the exon 11 c.1785_1786insA mutation only. The exon 11 and intron 12 mutations are therefore present *in trans*. It can be deduced that the intron 12 (c.2040+53_2040+54insT) and exon 25 (c.5439G>T) mutations are present *in cis*. Mutations are indicated by an asterisk; ellipses indicate omitted sequence; vertical dashed line denotes an exon-exon boundary. Abbreviation: WT, wild-type.

Supplemental Figure S2

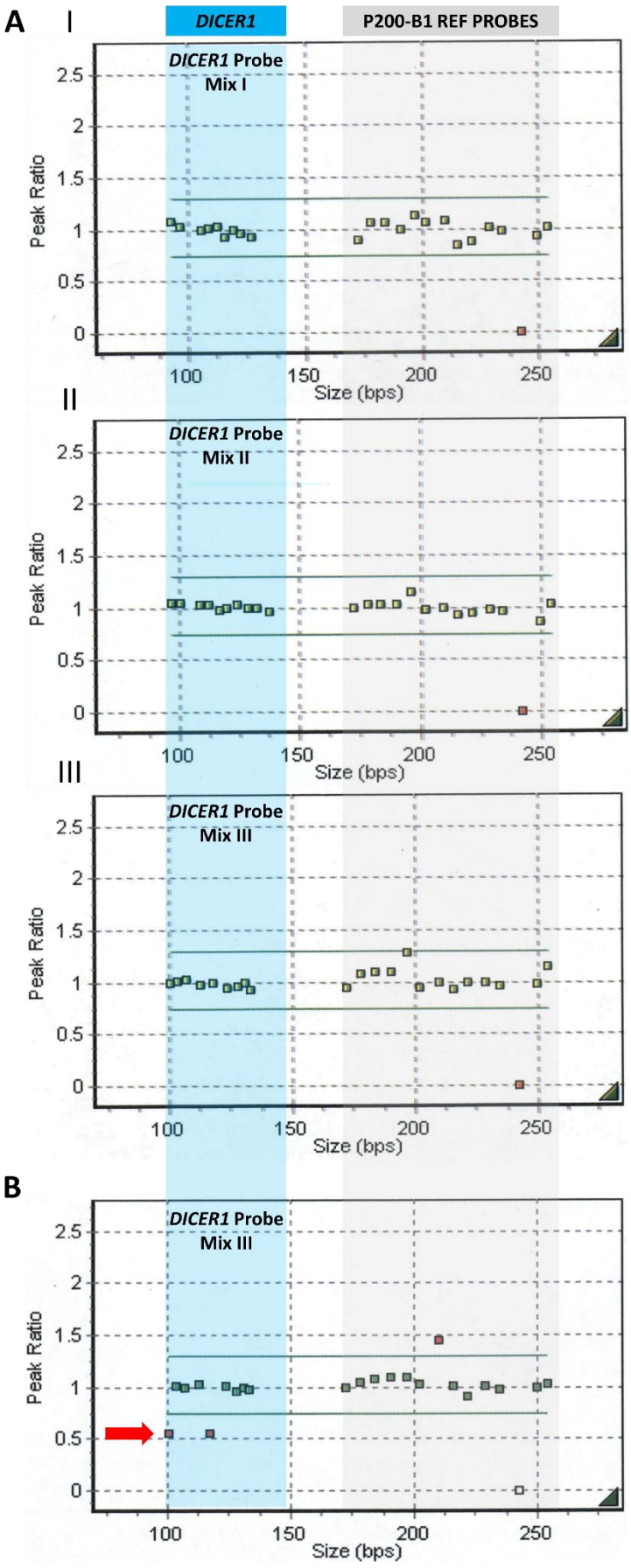




Supplemental Figure S2. Depth of coverage plots. Depth of coverage achieved for all 67 samples sequenced following capture with a custom Fluidigm Access Array (see Supplemental Tables S2a and S2b), viewed at a depth of 10,000 reads in plot **A** and 1,000 reads in plot **B**. The target region of the capture comprises all exons and exon-intron boundaries of *DICER1*. For all samples, ≥80% of the target region was covered at a depth of ≥10 reads.

Supplemental Figure S3.

Representation of the germ-line *DICER1* MLPA results. Three probe mixes for *DICER1* are used (A, panels I, II and III respectively), in combination with the P200-B1 set of control and reference probes from MRC-Holland (Amsterdam, The Netherlands) (Sabbaghian *et al*, 2014). All panels were generated using GeneMarker v. 1.70 (SoftGenetics, LLC). The blue zone indicates the region of peak ratio values for *DICER1* probes, and that for the P200-B1 probes is shown in grey. **A)** All 53 germ-line DNA samples screened for deletions or duplications in *DICER1* were not found to harbour any such alterations, with all ratios for *DICER1* exons (green squares, blue zone) falling within the normal peak ratio range of 0.7 to 1.3 (green horizontal lines). The sample used in this representation is a female, hence the peak ratio value of 0 for the Y chromosome (red square, grey zone, panel I-III). **B)** A sample with a known large *DICER1* exon deletion was used as an internal positive control. Affected exons have lower peak ratio values and are indicated by a red arrow.



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