

Title:

The landscape of genetic aberrations in myxofibrosarcoma

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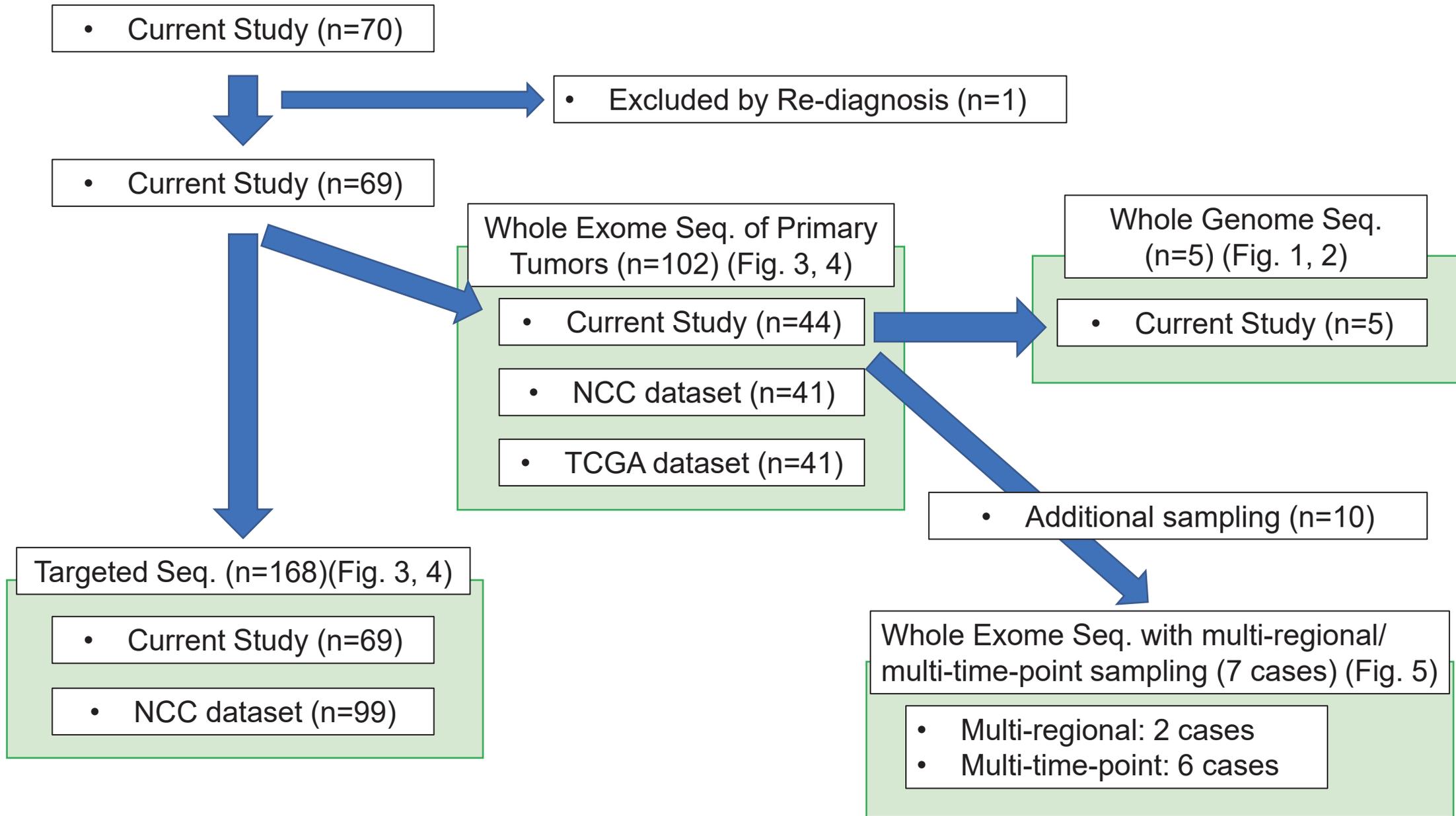
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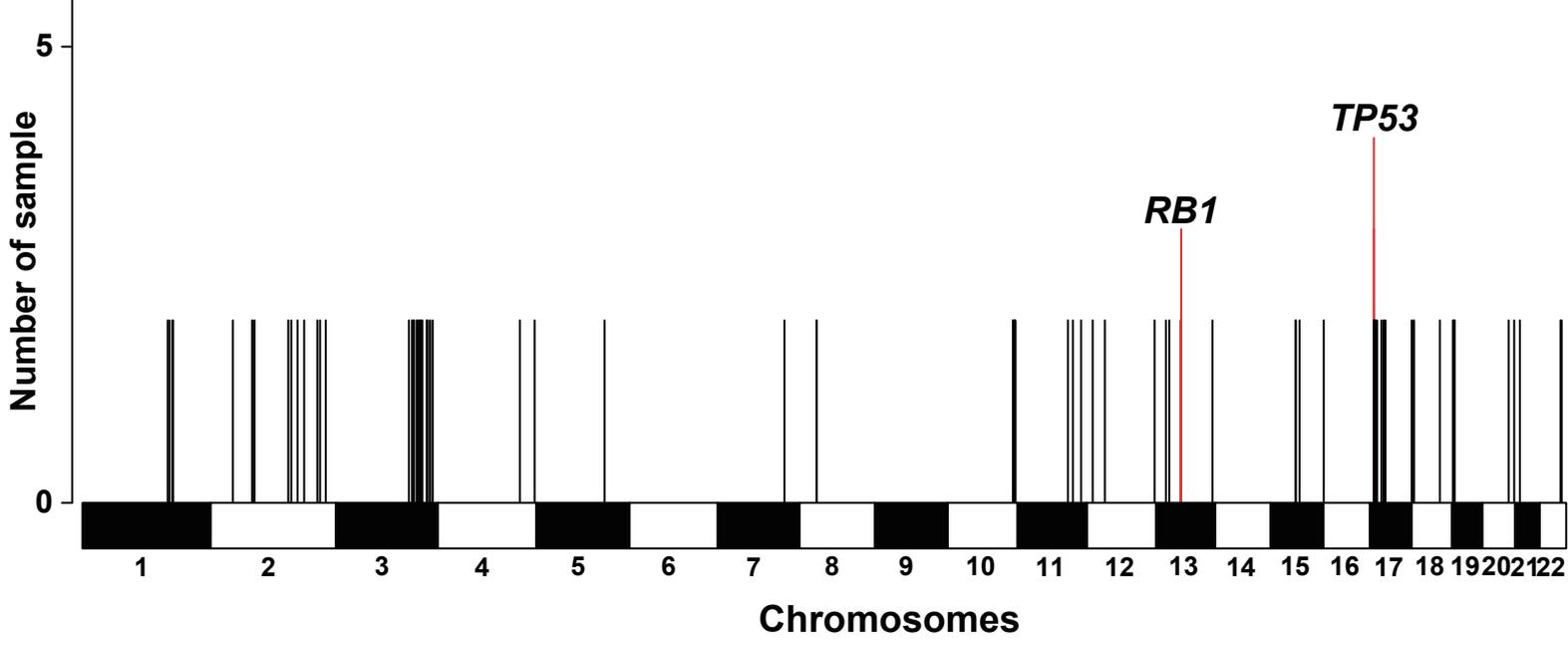
Supplementary table 6. Mutation calls by targeted capture sequence (TS)



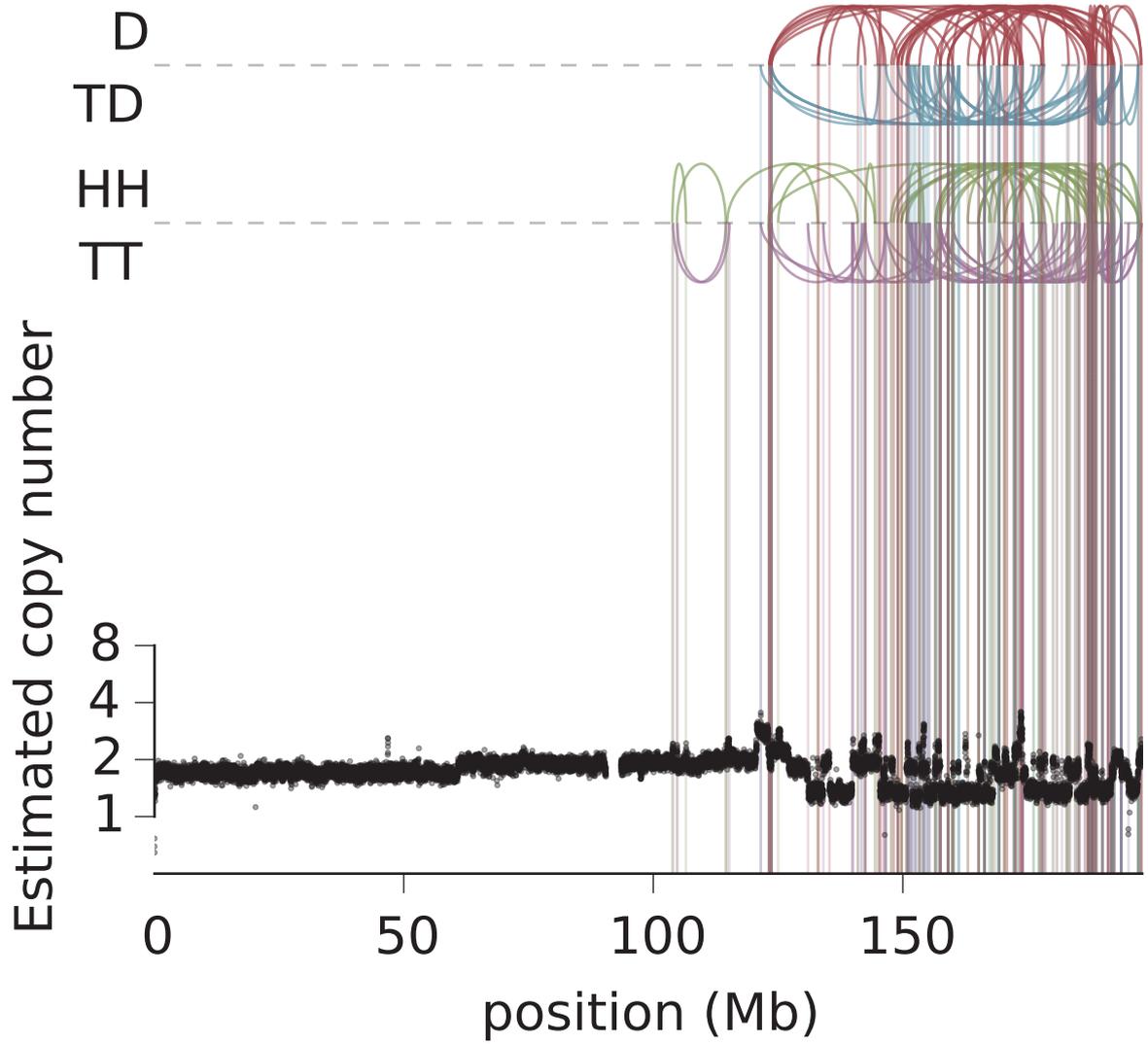
Supplementary Figure 1. Flowchart of the analysis

A total of 186 cases were enrolled to this study, among which one case was excluded because of re-diagnosis as myxoid liposarcoma with co-amplification of MDM2/CDK4. Seq: Sequencing, NCC: National Cancer Center Japan, TCGA: The Cancer Genome Atlas (TCGA) data

(A)



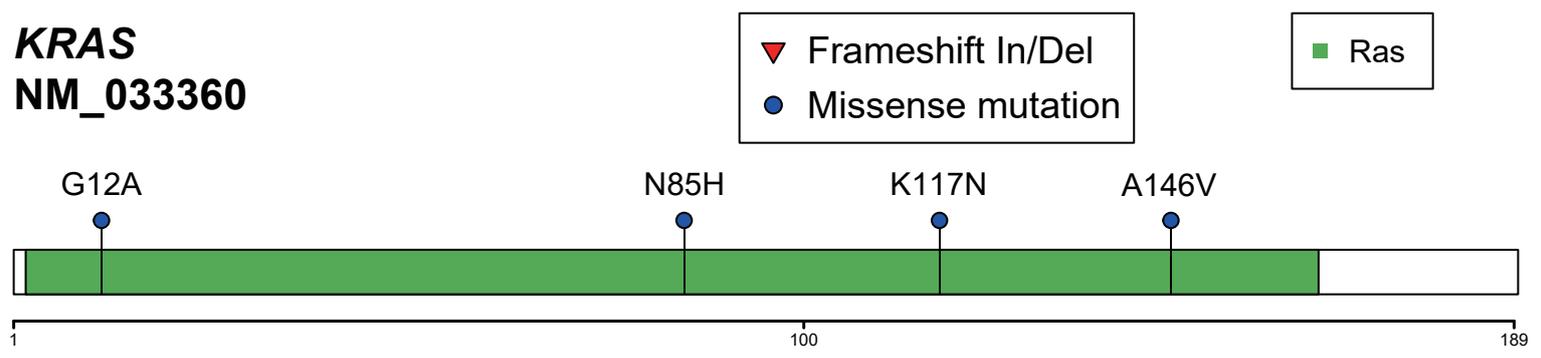
(B)



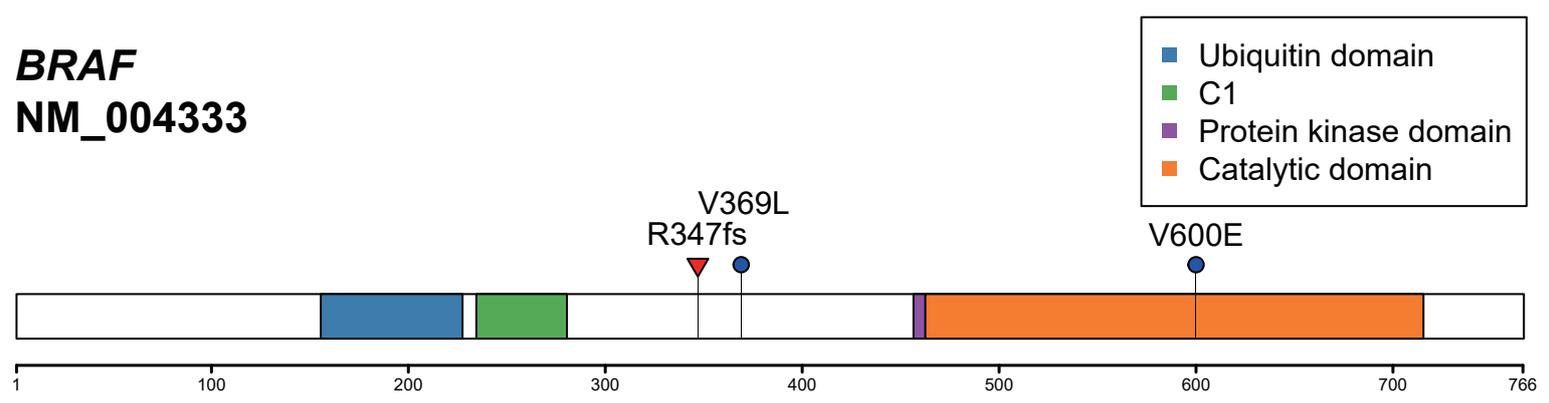
Supplementary Figure 2. SVs detected by WGS.

In 5 WGS analysis, a total of 2,298 somatic structural variations (median, 495 per case) were detected. a: Positions of SVs were binned into 100kb resolution and plotted on the genome. Summed number of samples are shown in the left. Among 5 samples, 4 samples harbored SV around the TP53 locus, and 3 harbored around the RB1 locus. b: Detailed analysis on SVs revealed that In the 5 cases, a total of 10 chromothripsis (range: 0 – 3, median: 2 per sample) were detected, and representative image of chromothripsis is depicted. Estimated copy number are plotted at the bottom of the figure, and intra-chromosomal SVs are shown in the indicated colors (D: deletion, TD: tandem duplication, HH: head to head translocation, TT: tail to tail translocation)

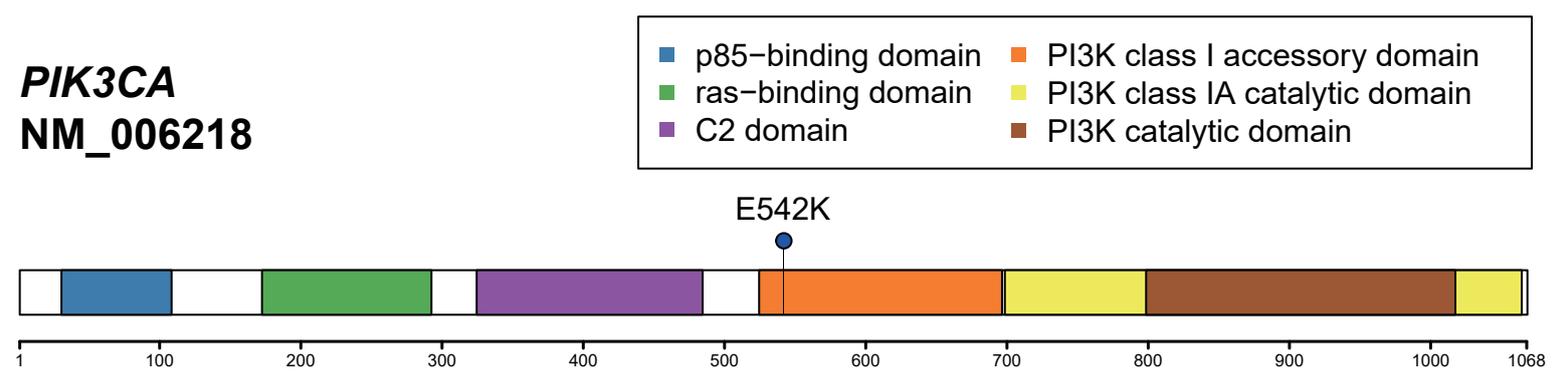
KRAS
NM_033360



BRAF
NM_004333

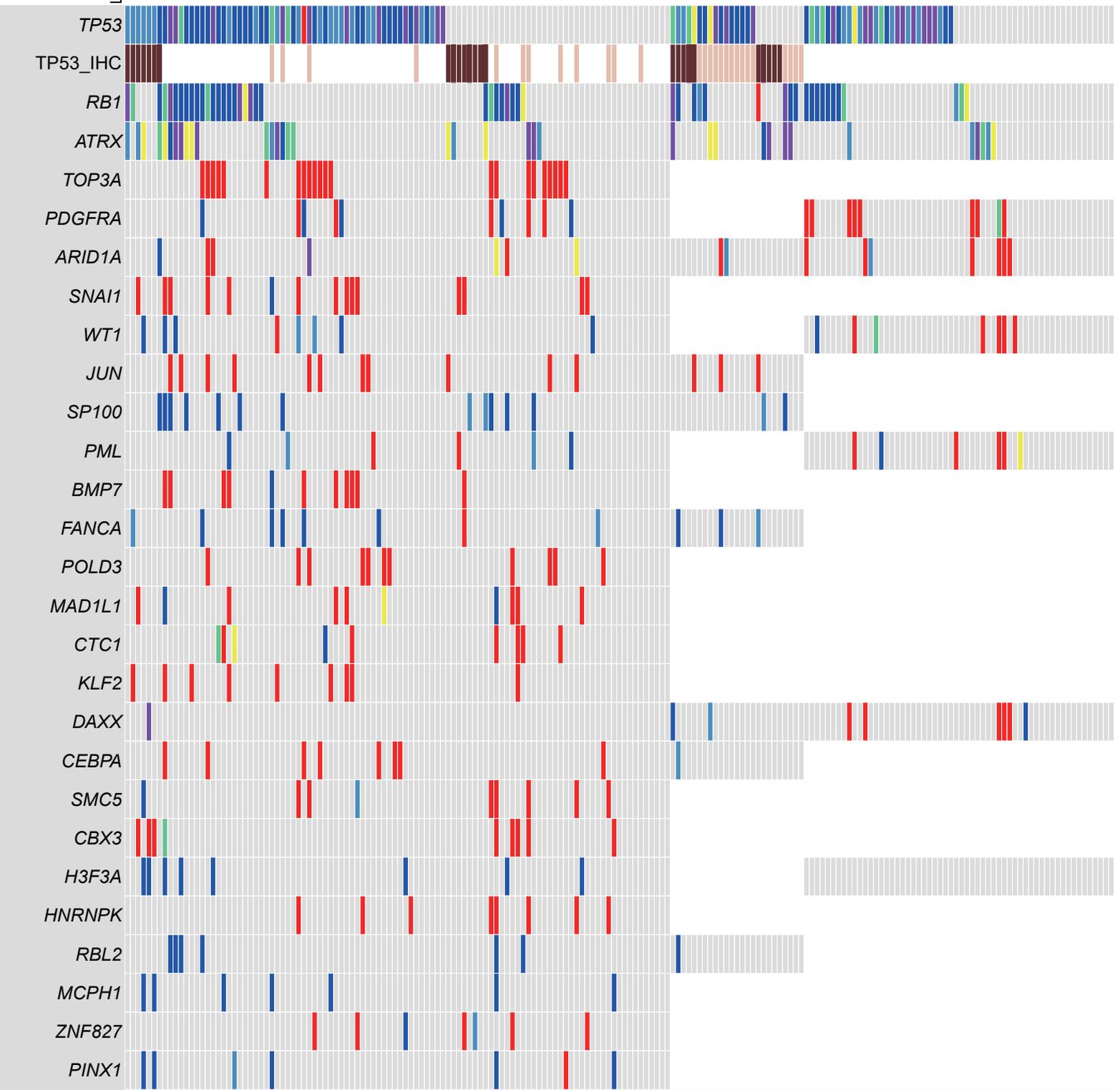
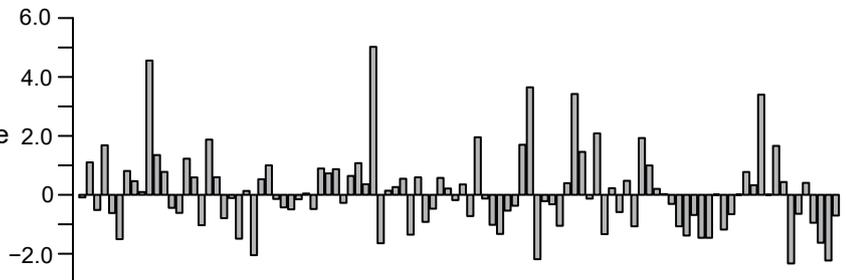


PIK3CA
NM_006218



Supplementary Figure 3. Distribution and mutational profile of KRAS, BRAF, and PIK3CA.
 Lollipop plot showing the distribution and mutation profile in *KRAS*, *BRAF*, and *PIK3CA*. The types and status of the somatic alterations are shown by the indicated colors. On the horizontal axis the amino acid position of each mutation is shown.

relative
telomere
length
(log₂)



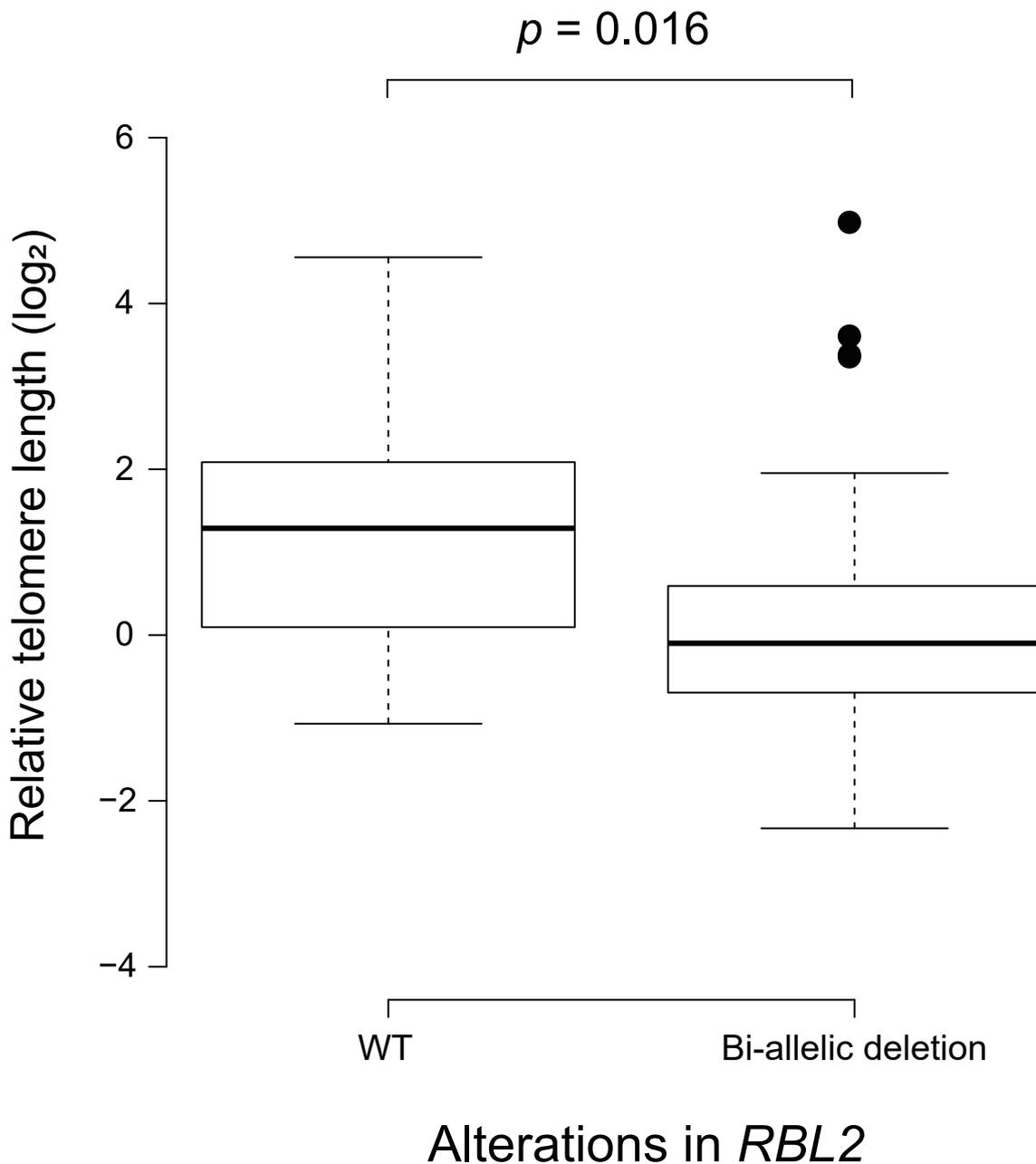
Alterations

- TP53 IHC Positive
- Nonsense / Frameshift InDel / Splice Site
- Missense / In-Frame InDel
- Deletion
- Amplification
- Structural Variation (SV)
- Multiple Mutations / SVs / Copy Number Alterations
- Wild Type
- Negative
- Not Assessed

Supplementary Figure 4. Deep targeted analysis of telomere alterations in MFS.

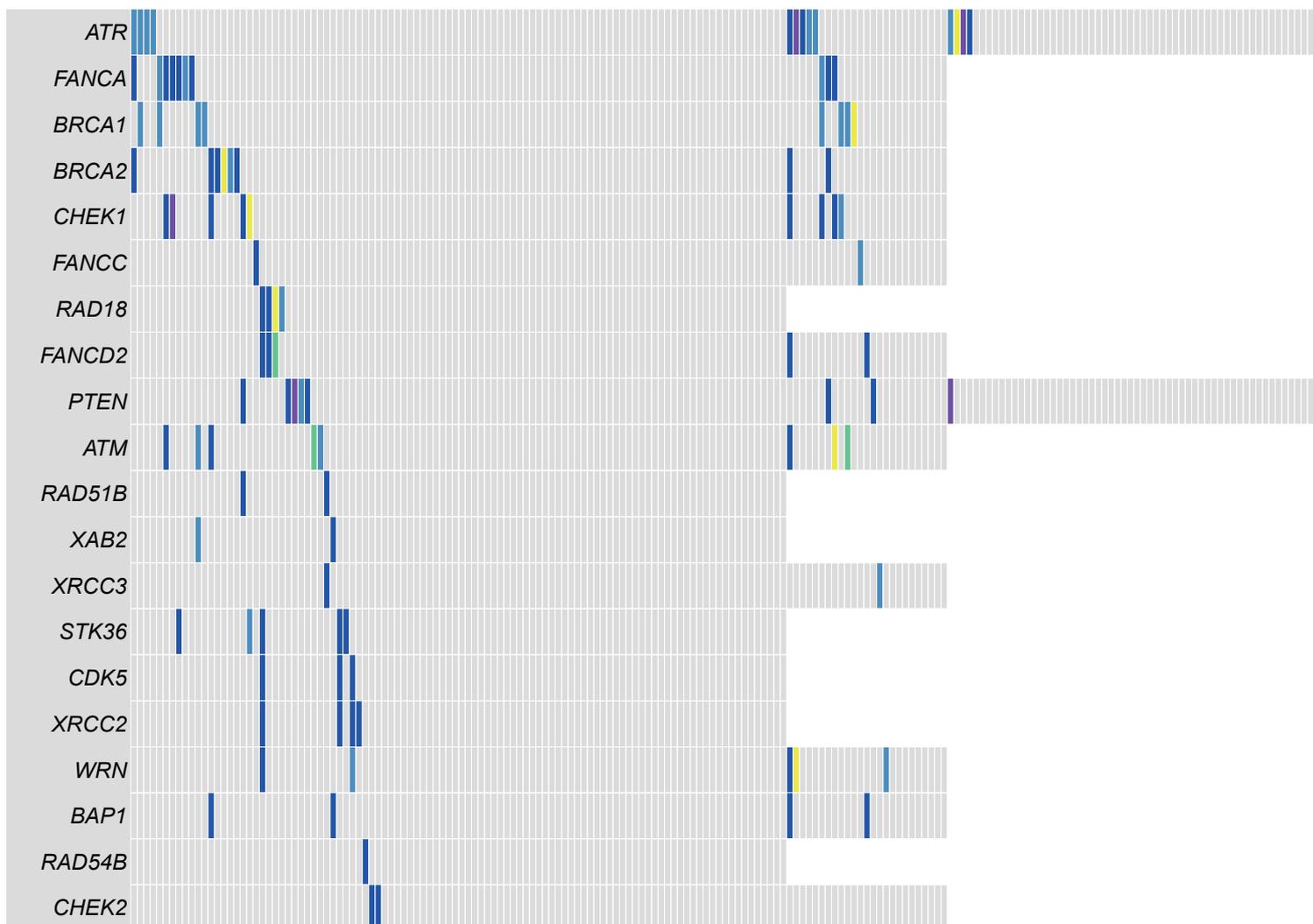
Based on the combined results from previous studies and this study, the spectrum of somatic mutations, somatic structural variations, and copy number alterations in telomere-associated genes in MFS are shown. Bar graph in the upper panel shows relative telomere length (\log_2) in each sample calculated by TelSeq. Lower panel shows landscape of genetic alterations in telomere maintenance associated genes listed in TelNet database. Each column represents an individual case, and each row represents the indicated gene with alteration. The types and status of the somatic alterations are shown by the indicated colors.

Bi-allelic deletions in *RBL2* and relative telomere length (\log_2)



Supplementary Figure 5. Alterations in *RBL2* and relative telomere length.

Genetic alteration states are shown in horizontal axis, and relative telomere length (\log_2) in each sample calculated by TelSeq are shown in vertical axis. Each dot shows each sample. WT: wild-type



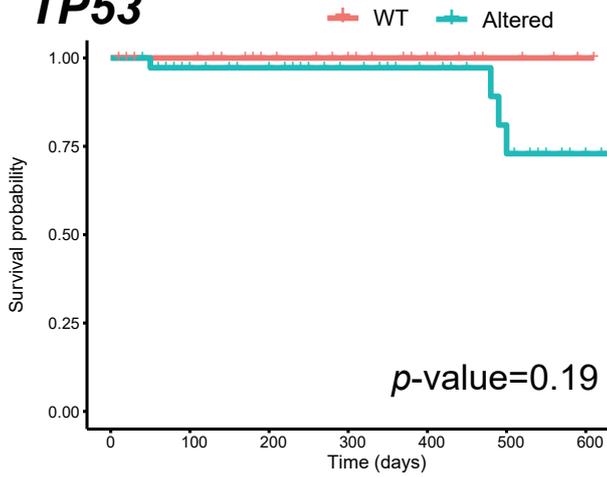
Alterations

- Nonsense / Frameshift InDel / Splice Site
- Missense / In-Frame InDel
- Deletion
- Structural Variation (SV)
- Multiple Mutations / SVs / Copy Number Alterations
- Negative / Wild Type
- Not Assessed

Supplementary Figure 6. Deep targeted analysis of BRCAness in MFS.

Based on the combined results from previous studies and this study, the spectrum of somatic mutations, somatic structural variations, and copy number alterations in BRCAness-associated genes in MFS are shown. Each column represents an individual case, and each row represents the indicated gene with alteration. Genes were assigned to the functional categories shown on the left. The types and status of the somatic alterations are shown by the indicated colors.

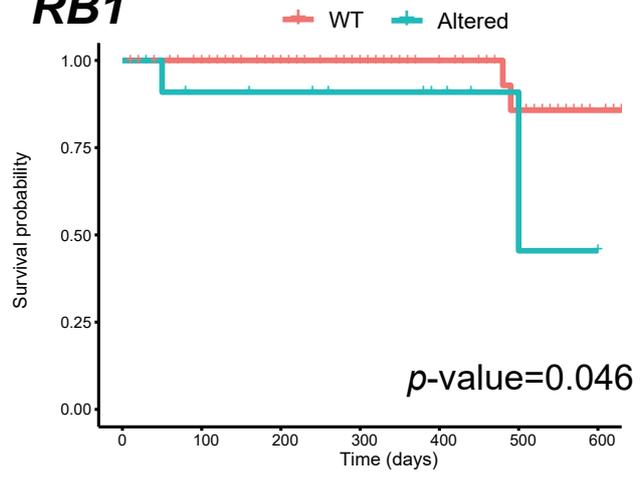
TP53



Number at risk

Strata	WT	26	23	17	14	9	4	1
	Altered	37	31	27	20	15	10	3
		0	100	200	300	400	500	600

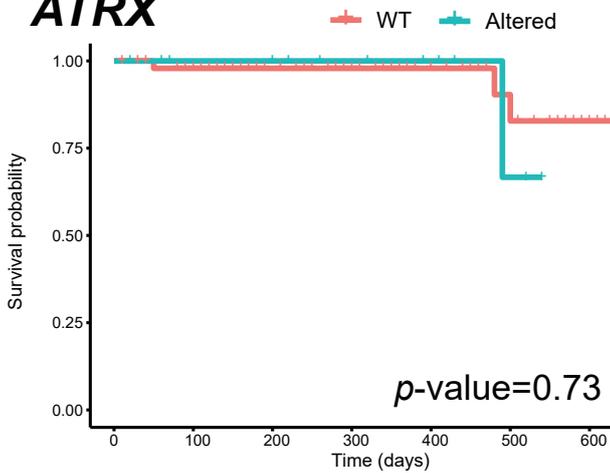
RB1



Number at risk

Strata	WT	51	45	36	28	20	12	3
	Altered	12	9	8	6	4	2	1
		0	100	200	300	400	500	600

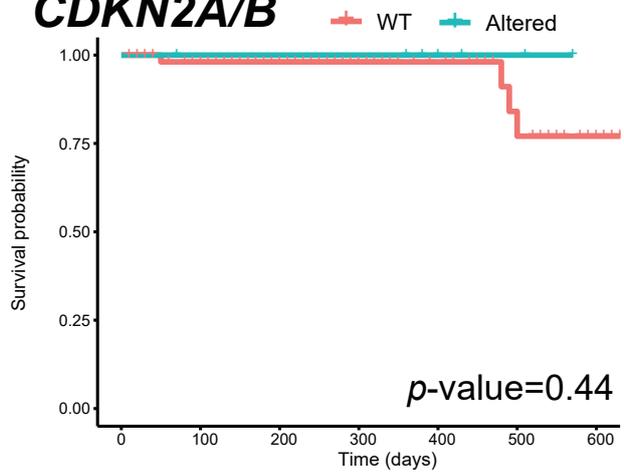
ATRX



Number at risk

Strata	WT	50	44	34	27	19	12	4
	Altered	13	10	10	7	5	2	0
		0	100	200	300	400	500	600

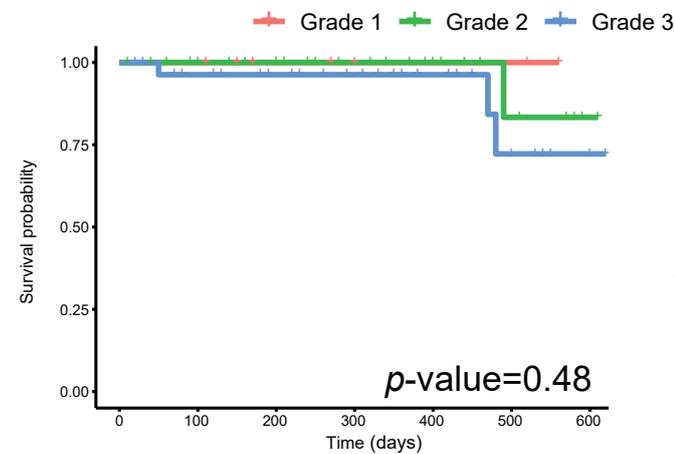
CDKN2A/B



Number at risk

Strata	WT	56	48	38	28	20	12	4
	Altered	7	6	6	6	4	2	0
		0	100	200	300	400	500	600

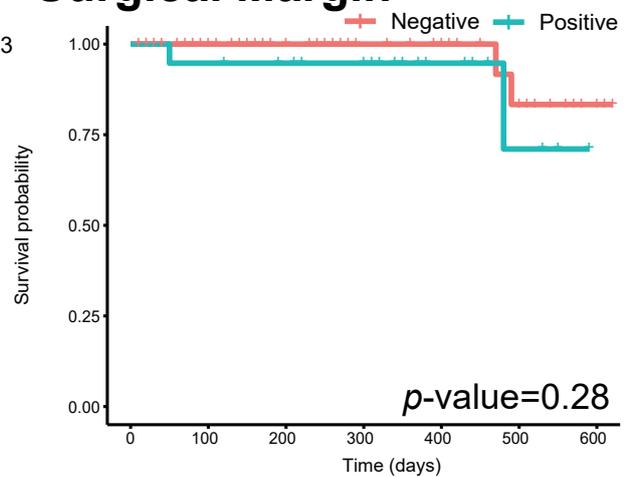
Histological Grade



Number at risk

Strata	Grade 1	7	7	4	3	2	2	0
	Grade 2	26	22	19	14	10	5	1
	Grade 3	29	24	20	16	11	6	2
		0	100	200	300	400	500	600

Surgical Margin

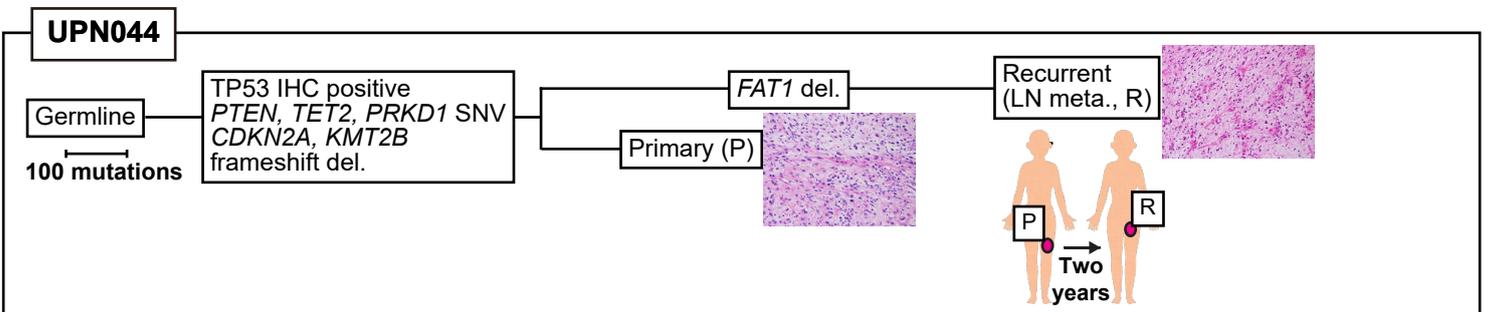
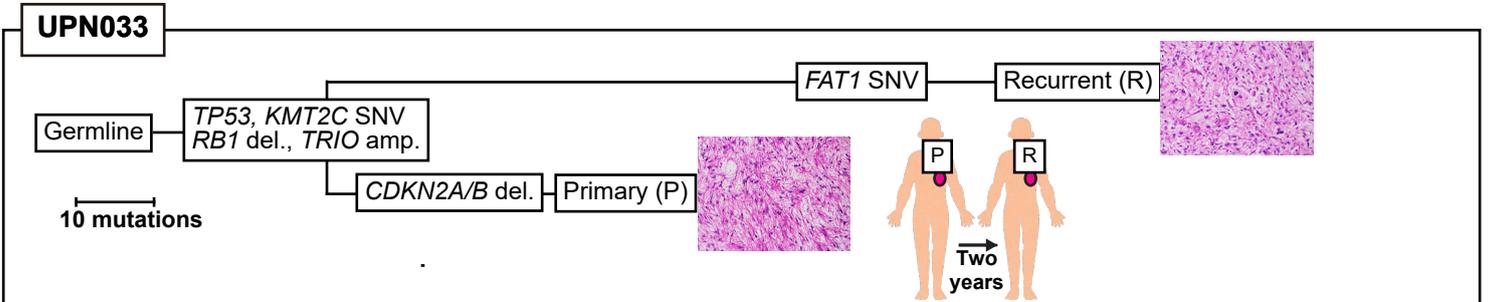
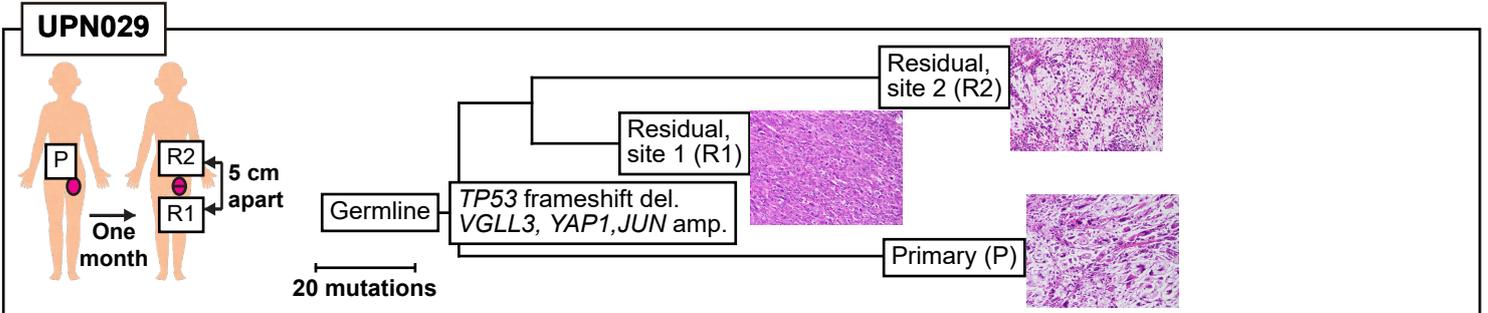
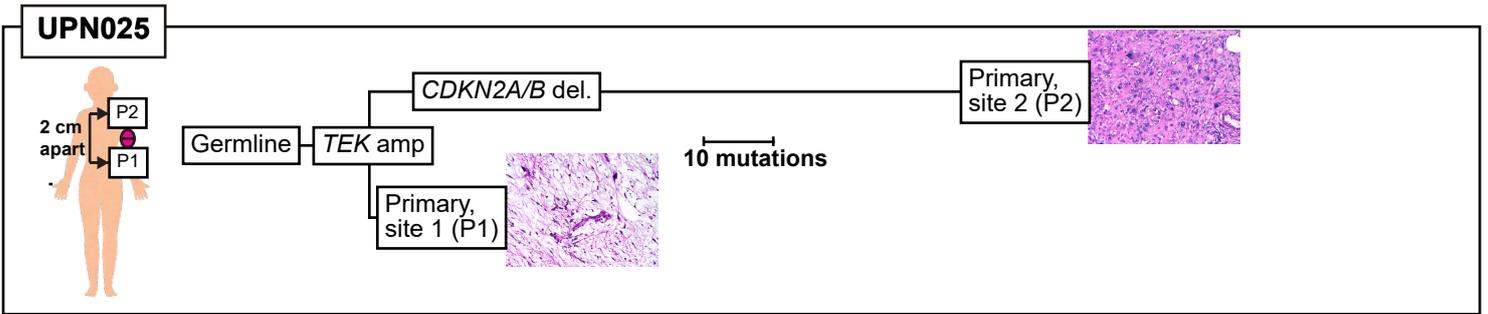
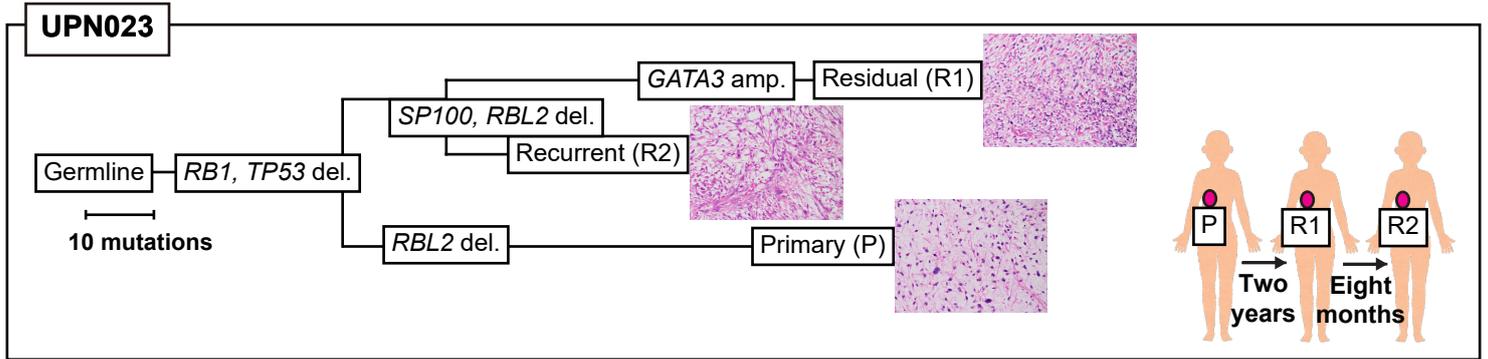
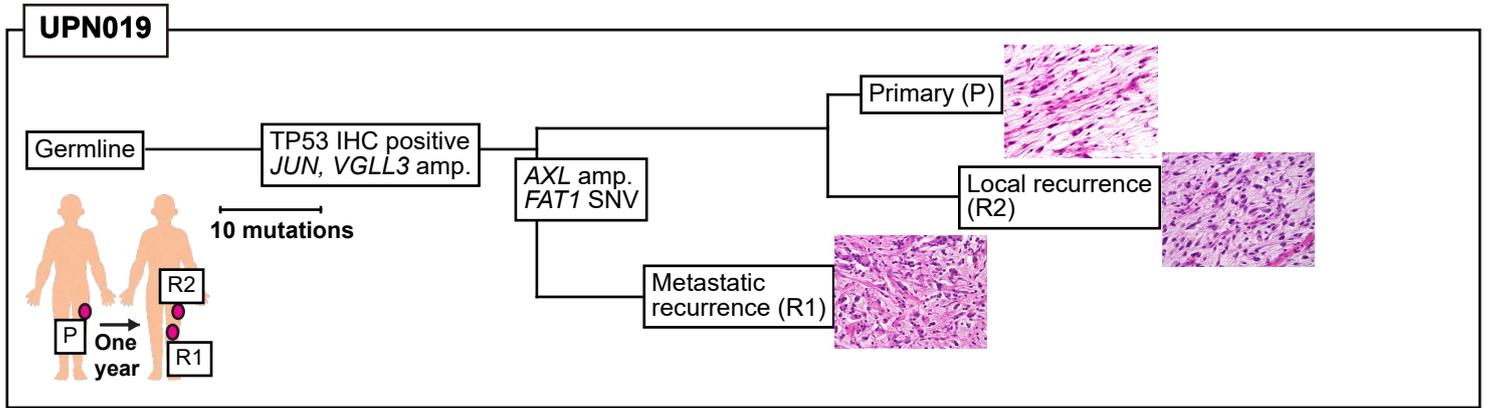


Number at risk

Strata	Margin Negative	43	35	27	19	16	10	3
	Margin Positive	19	18	16	14	7	3	0
		0	100	200	300	400	500	600

Supplementary Figure 7. Kaplan-Meier survival plots of overall survival stratified by absence or presence of gene alterations in each gene.

WT: wild-type



Supplementary Figure 8. Phylogenetic tree analysis of tumor heterogeneity in MFS.

Phylogenetic trees were illustrated by MEGAX (<https://www.megasoftware.net/>), and representative genetic alterations are annotated. Representative microscopic images are inserted in each sample.

Supplementary Text 1. Clinical course of multi-regional and/or multi-time-point sampling cases.

Case 1 (UPN019)

The patient was 80 years old male and presented with swelling of left lower leg. The patient underwent first surgery (**UPN019 primary site**), with the surgical margin positive state. The radiotherapy was planned but canceled by the patient by himself. One year after the first surgery, the tumor recurred with the swelling of the scar of the first surgery, with the swelling of the left popliteal fossa. Both the recurrent tumor of the primary site (**UPN019 Recurrent site 2**) and the popliteal fossa (**UPN019 Recurrent site 1**) were resected. The surgical margin of the former was negative, meanwhile the latter was positive. The patient is still alive for 4 years after the second surgery.

Case 2 (UPN021)

The patient was 70 years old male and presented with swelling of the left axilla. The patient underwent first surgery, and on the microscopic examination, the tumor harbored two histological components: myxoid area (**UPN021 primary site 1**) and solid area (**UPN021 primary site 2**), 5 cm apart from each site. The tumor was resected with the surgical margin positive state. The patient was followed up without additional chemo/radiotherapy, and local recurrence was found one year after the first surgery. The recurrent tumor was resected (**UPN021 Recurrent site**), and on the microscopic examination, the tumor again harbored myxoid and solid area. The patient underwent radiotherapy on the recurrent site.

Case 3 (UPN023)

The patient was 73 years old female and presented with swelling of the right back. The patient underwent first surgery (**UPN023 primary site**). The tumor was resected with the surgical margin positive state. The patient was followed up with radiotherapy, and local recurrence was found two year after the first surgery. The recurrent tumor was resected (**UPN023 Recurrent site**), with the surgical margin positive state. Additional radiotherapy was performed, and 8 months after the second surgery the patient underwent third surgery on the residual tumor (**UPN023 residual site**). The patient underwent additional radiotherapy, and the tumor recurred 4 months after the third surgery.

Case 4 (UPN025)

The patient was 72 years old female and presented with swelling of the left upper arm. The patient underwent first surgery, and on the microscopic examination, the tumor harbored two

histological components: myxoid area (**UPN025 primary site 1**) and solid area (**UPN025 primary site 2**). Samples were taken from each site, 2 cm apart each other. The tumor was resected with the surgical margin positive state. The patient was followed up with additional resection of the surgical site, with which no residual tumor was identified.

Case 5 (UPN029)

The patient was 85 years old female and presented with swelling and bleeding of the left buttock. The patient underwent first surgery (**UPN029 primary site**). The tumor was resected with the surgical margin negative state. One months after the first surgery, two metastatic tumors in the left inguinal area were resected (**UPN029 residual site 1 and 2**, 5 cm apart from each other).

Case 6 (UPN033)

The patient was 73 years old male and presented with a mass lesion in the chest wall. The patient underwent first surgery (**UPN033 primary site**). The tumor was resected with the surgical margin negative state and no additional therapy was performed. Two years after the first surgery, recurrent tumor in the primary site was resected (**UPN033 recurrent site**). The tumor was resected with the surgical margin positive state and radiotherapy was performed.

Case 7 (UPN044)

The patient was 75 years old female and presented with the swelling of left thigh. The patient underwent first surgery (**UPN044 primary site**). The tumor was resected with the surgical margin positive state and no additional therapy was performed. Two years after the first surgery, recurrent tumor in the left inguinal area was resected (**UPN044 recurrent site**). The tumor was resected with the surgical margin positive state and radiotherapy was performed.