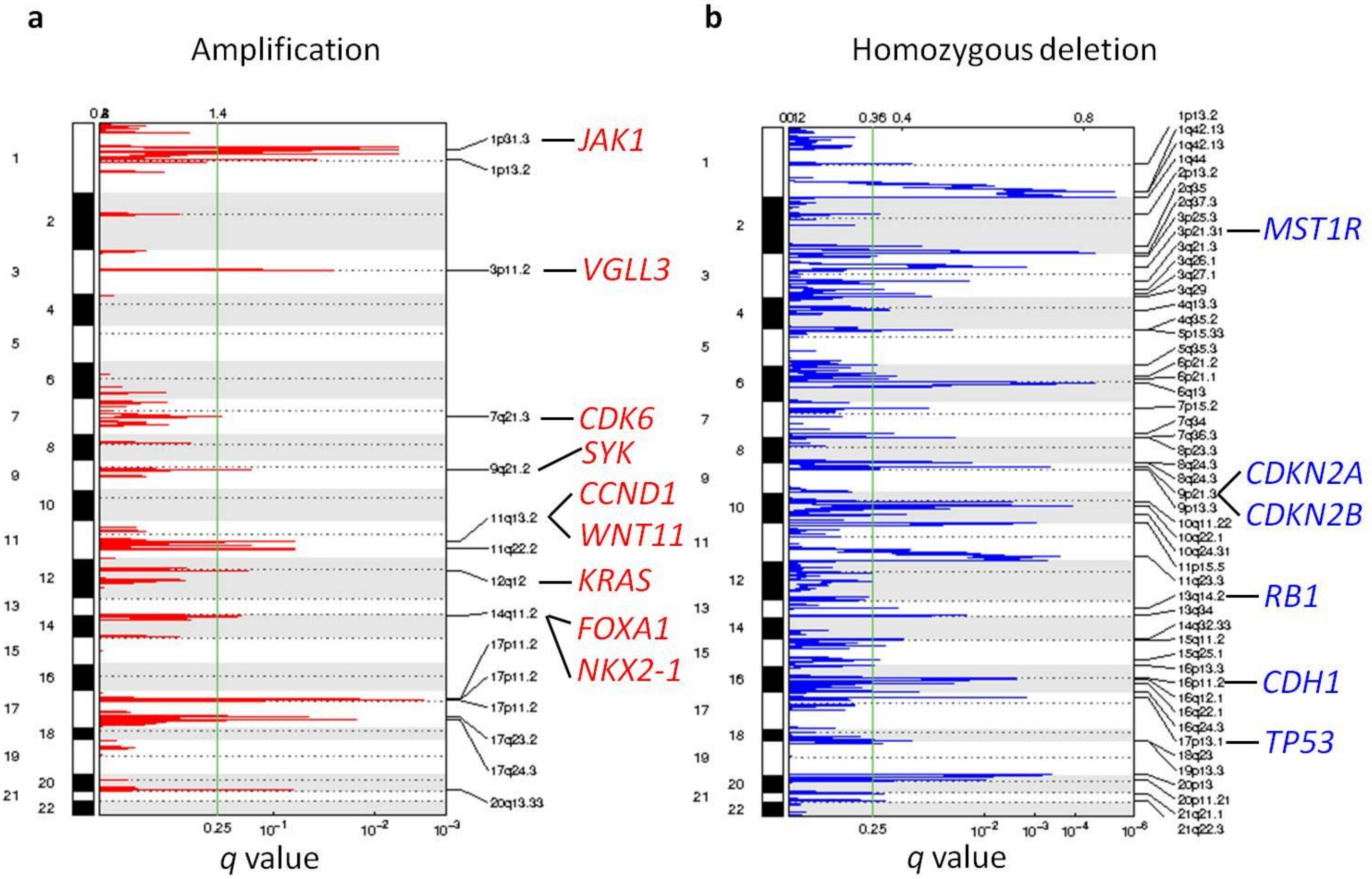
Supplementary Information

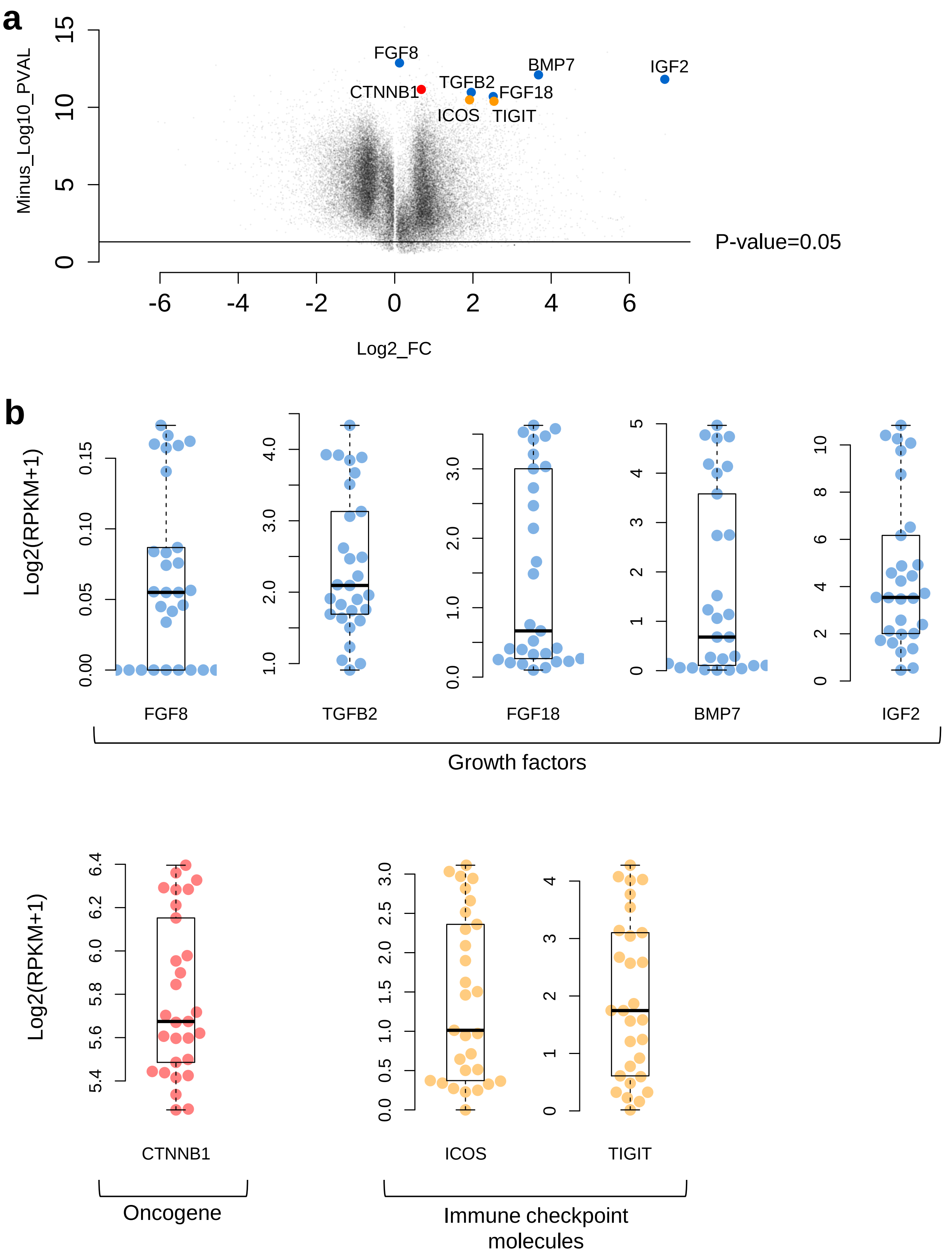
Integrated genetic and epigenetic analysis of myxofibrosarcoma

Ogura et al.

1. **Supplementary Figures 1-8**
2. **Supplementary Tables 1-3**



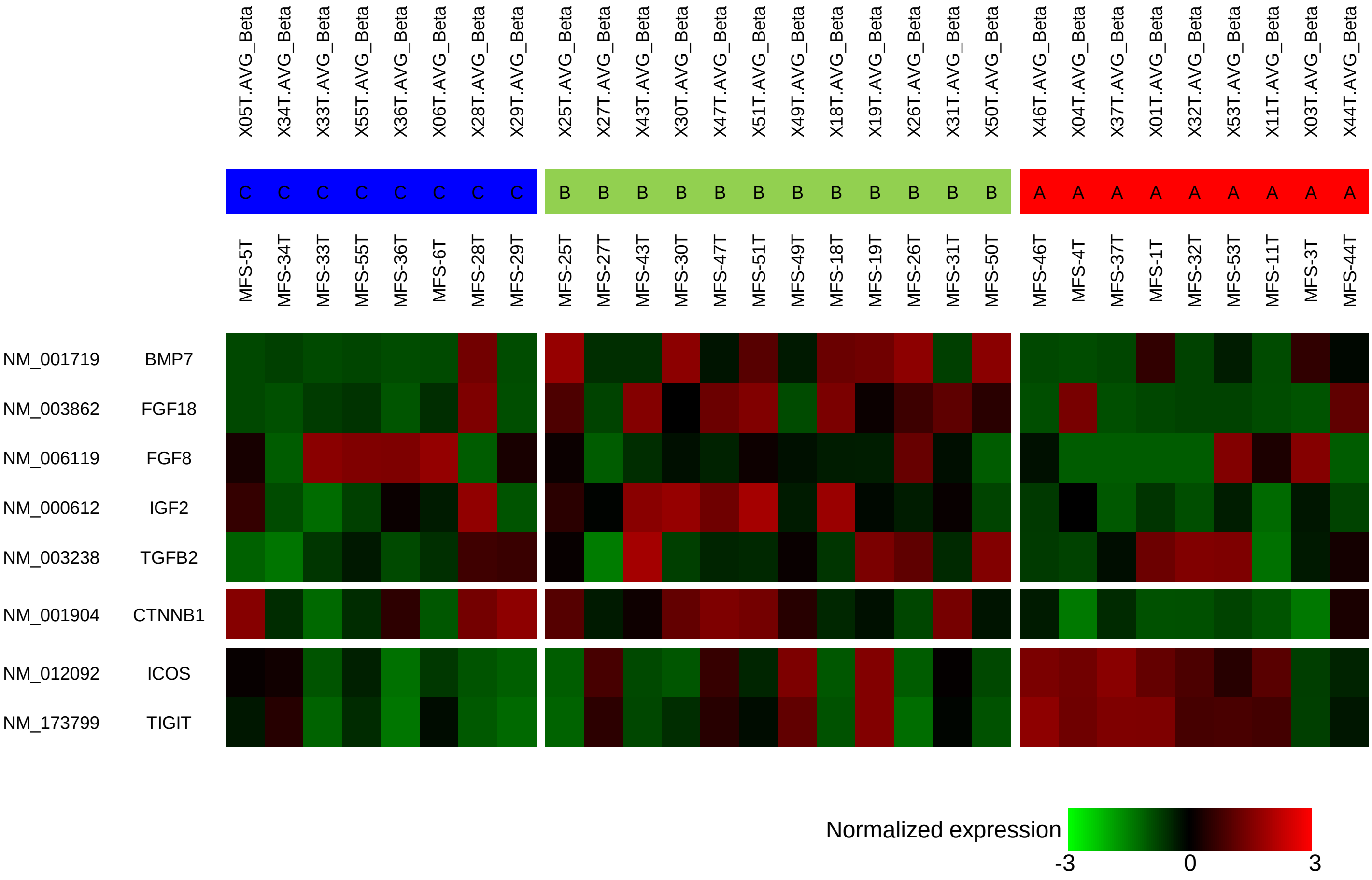
Supplementary Figure 1. Significant copy number alterations in 41 MFSs
Regions showing statistically significant focal amplifications (a) and homozygous deletions (b) detected using the GISTIC 2.0 algorithm. For each *q*-value peak, putative gene targets are listed.



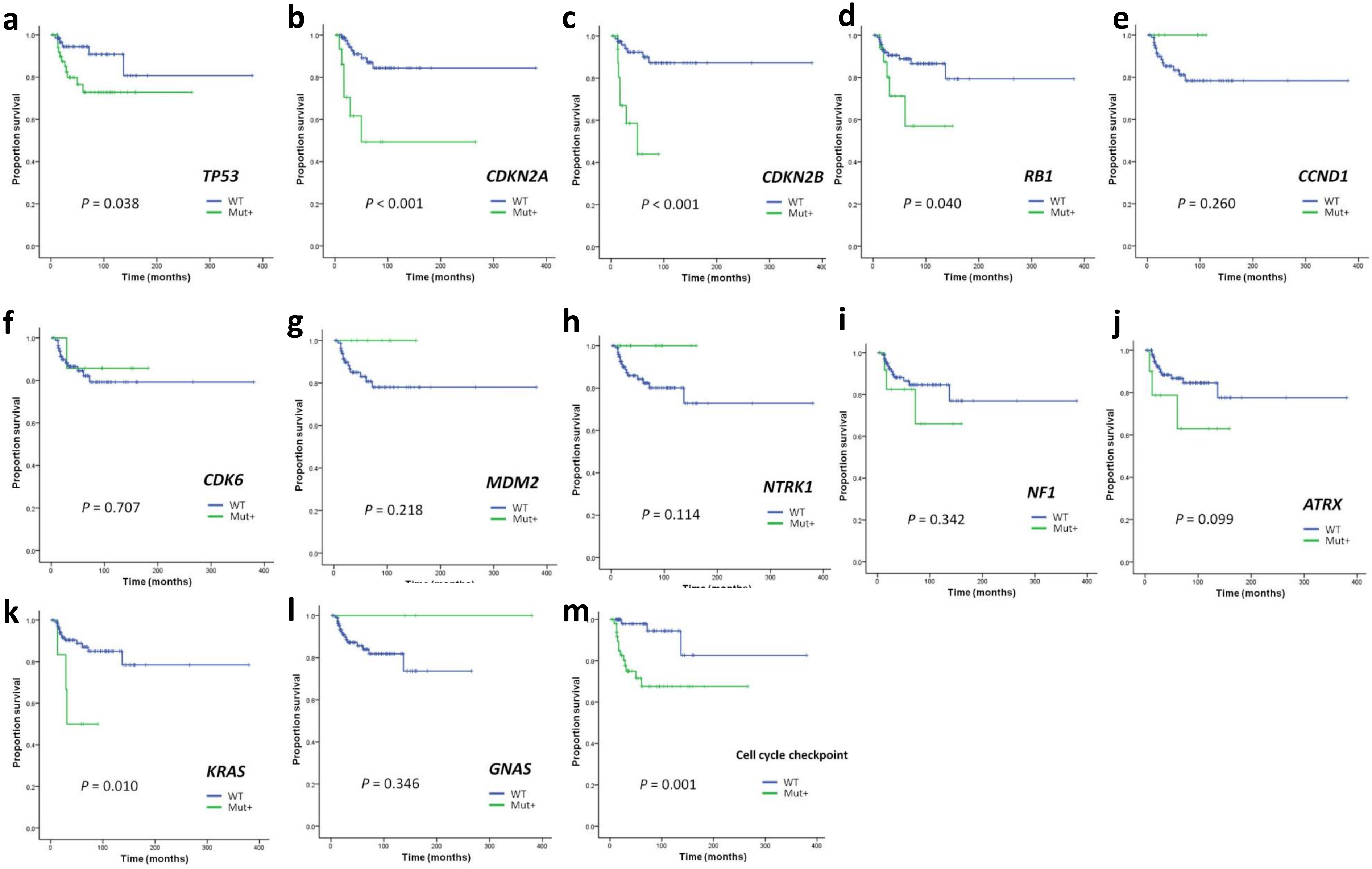
Supplementary Figure 2. Significantly up-regulated genes among 29 MFSs

(a) Distribution of significantly up-regulated genes. Interesting molecules with the top-ranked genes were shown: growth factors (*FGF8*, *BMP7*, *IGF2*, *TGFB2*, and *FGF18*), oncogenes (*CTNNB1*), and immune-related molecules (*ICOS* and *TIGIT*).

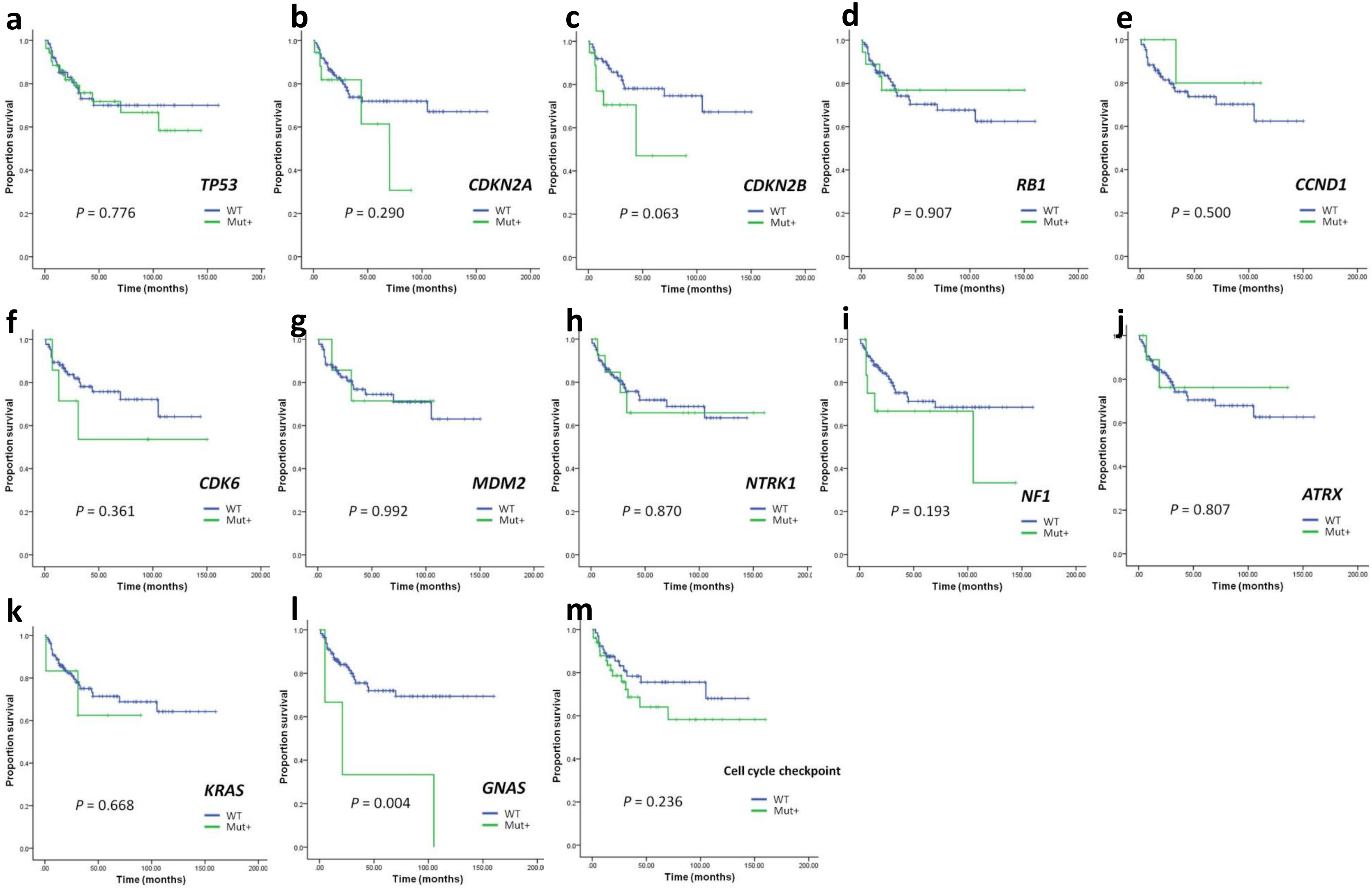
(b) Distribution of 29 MFSs in each gene.



Supplementary Figure 3. Relation between methylation clusters and expression of significantly up-regulated genes

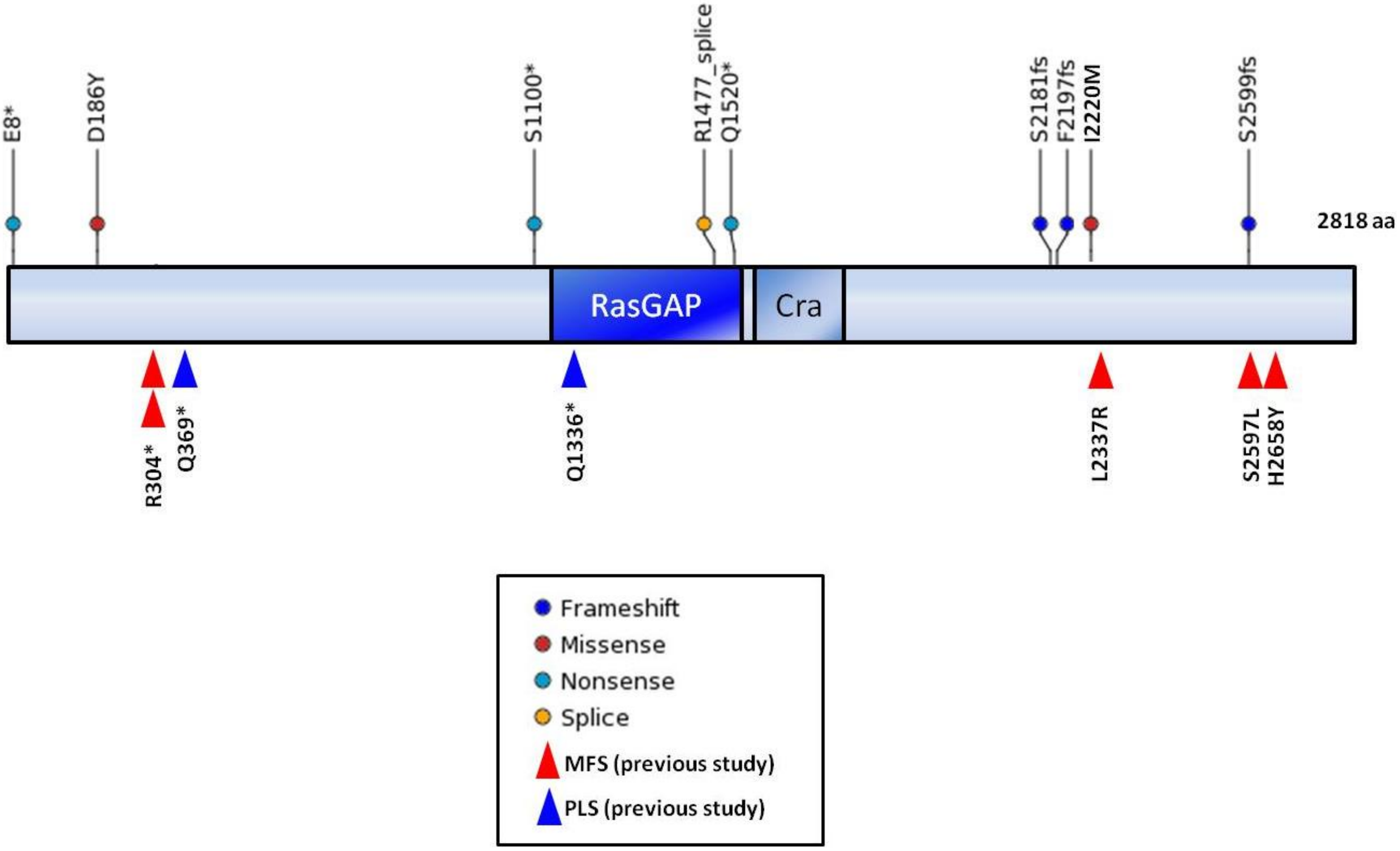


Supplementary Figure 4. Kaplan-Meier survival plots of overall survival stratified by absence or presence of gene alterations
TP53 (a), *CDKN2A* (b), *CDKN2B* (c), *RB1* (d), *CCND1* (e), *CDK6* (f), *MDM2* (g), *NTRK1* (h), *NF1* (i), *ATRX* (j), amplification of *KRAS* (k), somatic mutation of *GNAS* (l), and cell cycle checkpoints overall (m).

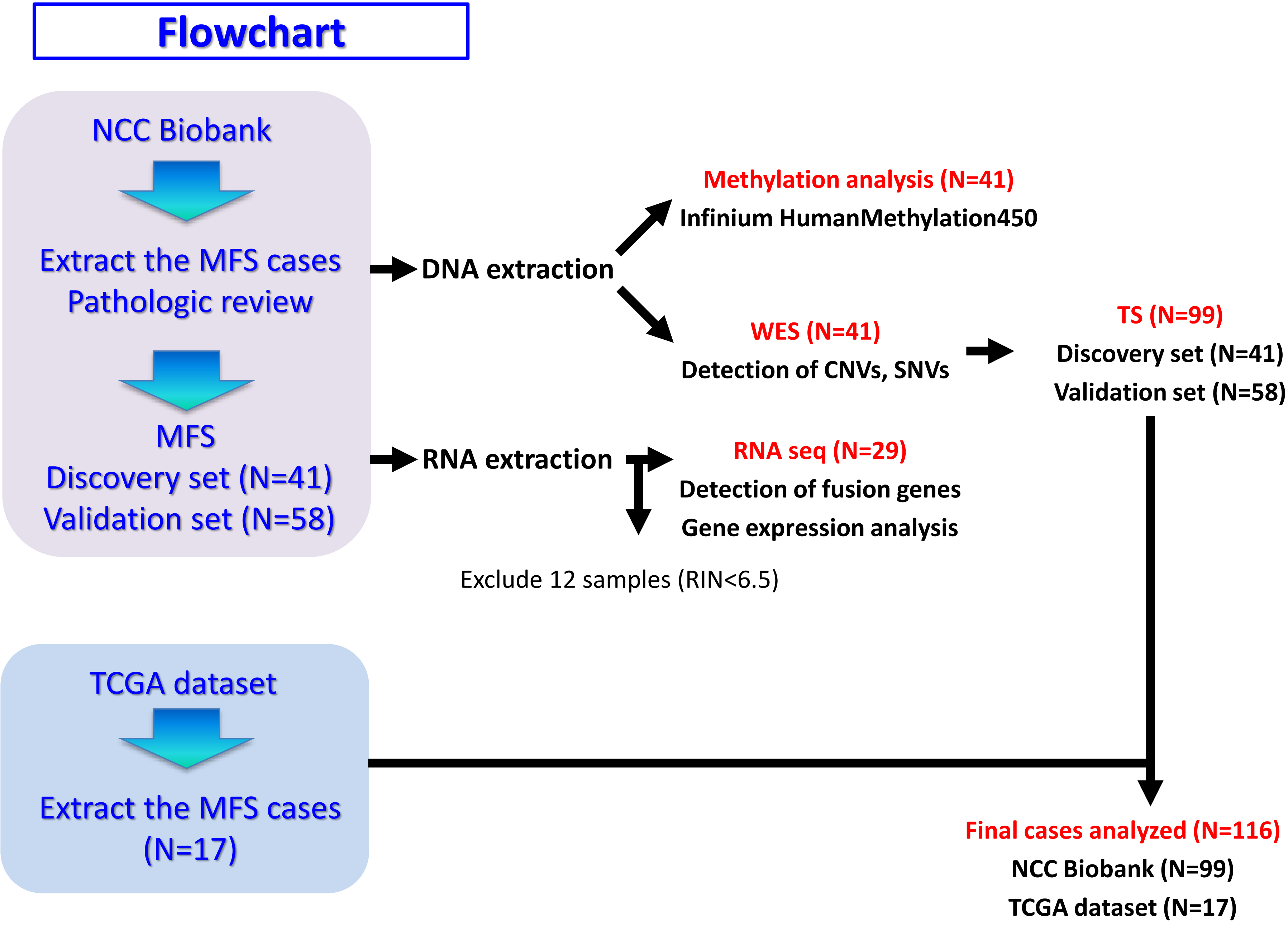


Supplementary Figure 5. Kaplan-Meier survival plots of local recurrence-free survival stratified by absence or presence of gene alterations

TP53 (a), *CDKN2A* (b), *CDKN2B* (c), *RB1* (d), *CCND1* (e), *CDK6* (f), *MDM2* (g), *NTRK1* (h), *NF1* (i), *ATRX* (j), amplification of *KRAS* (k), somatic mutation of *GNAS* (l), and cell cycle checkpoints overall (m).



Supplementary Figure 6. NF1 mutations in the present and previous studies in MFS
The location of somatic mutations in NF1 observed in this study is indicated above a schematic of the NF1 protein, and those previously reported in MFS (blue triangles) and PLS (red triangles) are indicated below.
Types of mutations are indicated by color: frameshift indel, blue; missense, red; nonsense, light blue; and splice site, orange.



Flowchart

NCC Biobank

Extract the MFS cases
Pathologic review

MFS
Discovery set (N=41)
Validation set (N=58)

DNA extraction

Methylation analysis (N=41)
Infinium HumanMethylation450

WES (N=41)
Detection of CNVs, SNVs

TS (N=99)
Discovery set (N=41)
Validation set (N=58)

RNA extraction

RNA seq (N=29)
Detection of fusion genes
Gene expression analysis

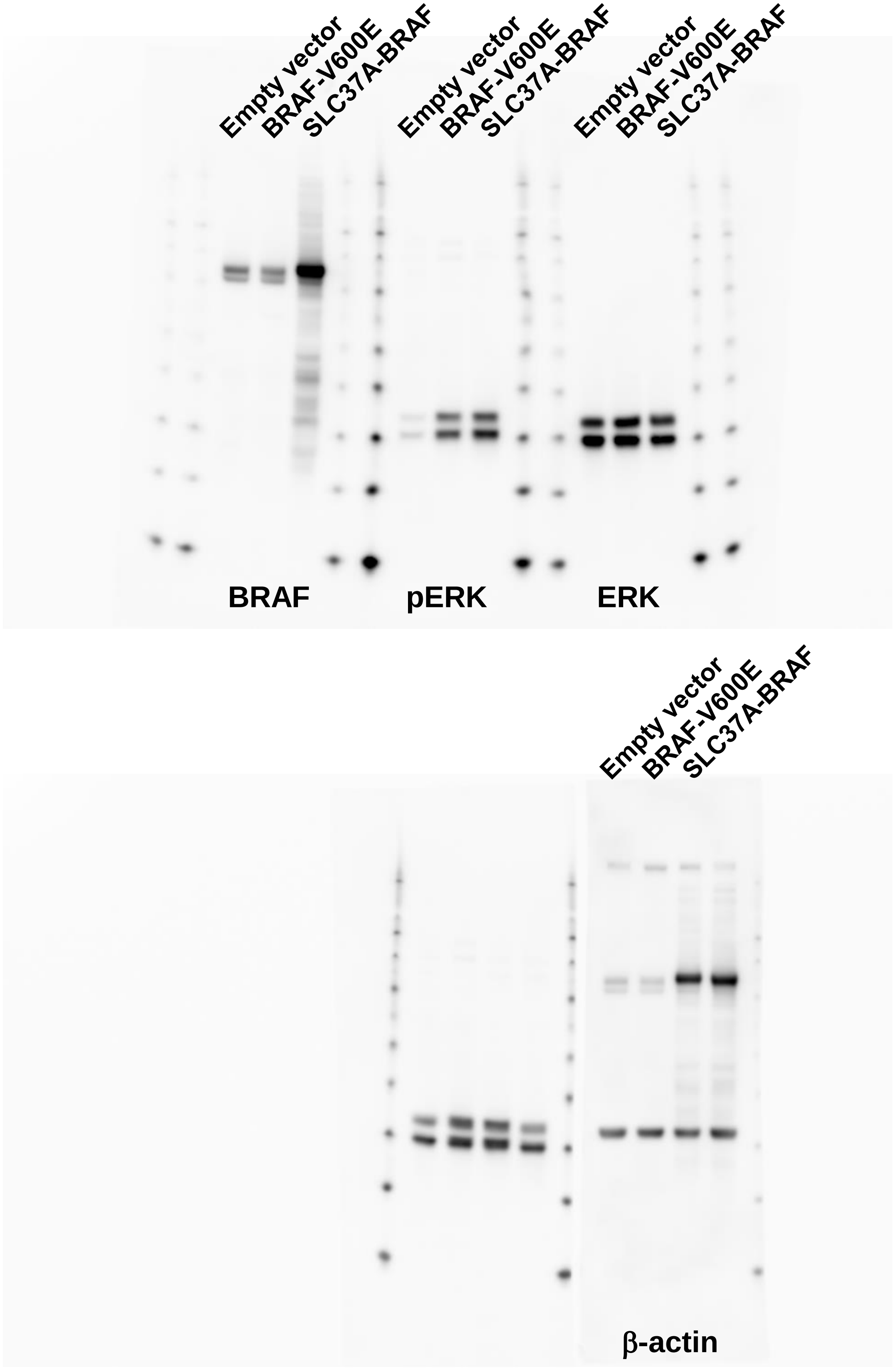
Exclude 12 samples (RIN<6.5)

TCGA dataset

Extract the MFS cases
(N=17)

Final cases analyzed (N=116)
NCC Biobank (N=99)
TCGA dataset (N=17)

Supplementary Figure 7. Flowchart of the analysis



Supplementary Figure 8. Original images of the immunoblots shown in Fig. 3b

Supplementary Table 1. List of 140 genes included in the targeted capture sequencing

ABCA13
AEN
ALK
🟩 *AMACR*
🟩 *ANK1*
ARID1A
ARID1B
ARID2
🟩 *ASS1*
ATP1A3
ATR
ATRX
BRAF
CAMTA1
CCND1
CDC73
CDH1
🟩 *CDK6*
CDKN2A
CDKN2B
CIC
CLIP2
CTNNB1
DAXX
DCC
DNMT1
DNMT3A
EGFR
EPHA1
EPHA5
EPHA7
EPHA8
ERBB2
ERBB3
ERBB4
ERC2
EXT1
EZH2
FBXW7
FGFR1
FGFR2
FGFR3
FGFR4
FLT3
FLT4
FOXA1
FRYL
GAS8
GATA3
GNAS
🟩 *GPR64*
GRIA1
GRIN2B
H3F3A
H3F3B
HIST1H3B
HOXA11
HOXA13
HOXA9
HRAS
HYDIN
IDH1
IDH2
INPPL1
JAK1
JAK2
JAK3
KDM5C
KDM6A
KIAA1199
KIAA1549
KIAA2022
KIT
KRAS
LAMA4
LTK
MAP2
MARVELD3
MCYN
MDM2
MEP1A
🟩 *MET*
MMEL1
MST1R
🟩 *MTAP*
MTHFR
MUC17
MYC
MYCL1
MYOCD
NACA
NBPF1
NF1
NKX2-1
NLRP9
NOTCH1
NOTCH2
NOTCH3
NPM1
NRAS
NTRK1
OR4X1
PCDH19
PDGFRA
PDGFRB
PI4KA
PIK3CA
PIK3R1
PIK3R3
PLEKHA7
PML
POF1B
PRAMEF1
PRDM1
PTEN
PTK2B
PTPRB
RB1
RET
RTL1
🟩 *S100A3*
SAMSN1
SETD2
🟩 *SKP2*
SMARCA4
SMARCB1
STAT3
STK11
SYK
TDRD5
🟩 *TERT*
TET2
TIGD2
TMPRSS11F
🟩 *TNXB*
TP53
UNC80
🟩 *WISP2*
WT1
ZCCHC6

🟩 Promoter

These genes were chosen on the basis of the WES data of 41 MFSs (*p* -values) and the associated genes. Genes of interest from a review of the published literature were also chosen.

WES, whole-exome sequencing; MFS, myxofibrosarcoma.

Supplementary Table 2. Comparison of major alterations in various soft tissue sarcomas with complex karyotypes using our data and TCGA dataset (N = 290)

Altered gene	DDLS (N = 50), %	LMS (N = 80), %	MFS (N = 116), %	UPS (N = 44), %	*P*- value
TP53	4.0	61.3	45.7	63.6	<0.001
MDM2	94.0	0.0	6.0	0.0	<0.001
CDKN2A	6.0	7.5	15.5	18.2	0.104
CDKN2B	6.0	7.5	15.5	18.2	0.104
RB1	0.0	37.5	18.1	36.4	<0.001
CCND1	8.0	2.5	6.0	0.0	0.180
CDK6	0.0	0.0	6.9	9.1	0.014
CDK4	86.0	0.0	0.0	2.3	<0.001
NF1	8.0	3.8	11.2	4.5	0.218
PTEN	0.0	18.8	3.4	6.8	<0.001
KRAS	8.0	1.3	6.9	2.3	0.170
NTRK1	8.0	2.5	9.5	11.4	0.216
SYK	4.0	1.3	3.4	0.0	0.456
PIK3CA	0.0	2.5	0.9	6.8	0.076
GNAS	8.0	1.3	8.6	6.8	0.184
JAK1	14.0	3.8	4.3	4.5	0.063
FOXA1	0.0	1.3	6.9	4.5	0.089
NKX2-1	0.0	0.0	6.0	0.0	0.013
ATRX	34.0	16.3	8.6	31.8	<0.001
TET2	2.0	1.3	3.4	2.3	0.795
SETD2	0.0	2.5	2.6	4.5	0.551
FLT4	10.0	1.3	4.3	0.0	0.036
CDH1	0.0	0.0	2.6	0.0	0.208

Supplementary Table 3. Hazard ratio stratified by presence or absence of gene alteration (Cox proportional hazards model, univariate analysis)

Altered gene	Frequency (%)	Overall survival		Metastasis-free survival		Local recurrence-free survival	
		Hazard ratio (95% CI)	*P*- value	Hazard ratio (95% CI)	*P*- value	Hazard ratio (95% CI)	*P*- value
TP53	46%	2.92 (1.01–8.42)	0.048	1.38 (0.65–2.94)	0.405	1.11 (0.54–2.30)	0.777
RB1	18%	2.91 (1.00–8.42)	0.049	2.32 (0.98–5.52	0.056	0.94 (0.33–2.70)	0.907
CDKN2A	16%	10.85 (3.69–31.93)	<0.001	3.42 (1.43–8.17)	0.006	1.90 (0.69–5.24)	0.394
CDKN2B	16%	7.52 (2.51–22.50)	<0.001	3.06 (1.27–7.37)	0.012	2.41 (0.92–6.32)	0.073
NF1	11%	1.82 (0.52–6.40)	0.350	0.99 (0.30–3.30)	0.992	1.88 (0.72–4.93)	0.201
NTRK1	9%	0.04 (0.00–20.67)	0.312	2.12 (0.85–5.25)	0.106	1.09 (0.38–3.14)	0.870
GNAS	9%	0.04 (0.00–1044.34)	0.540	0.04 (0.00–185.69)	0.463	4.86 (1.46–16.19)	0.010
ATRX	9%	2.75 (0.78–9.66)	0.114	1.37 (0.41–4.57)	0.604	0.84 (0.20–3.52)	0.808
KRAS	7%	4.58 (1.29–16.30)	0.019	4.82 (1.65–14.06)	0.004	1.00 (1.00–1.00)	0.351
CDK6	7%	0.68 (0.09–5.21)	0.709	1.70 (0.50–5.75)	0.394	1.75 (0.52–5.92)	0.369
MDM2	6%	0.04 (0.00–99.91)	0.425	0.51 (0.07–3.78)	0.508	0.99 (0.23–4.26)	0.992
CCND1	6%	0.04 (0.00–189.51)	0.463	0.47 (0.06–3.47)	0.456	0.51 (0.07–3.79)	0.509

CI, confidence interval.