SUPPLEMENTARY INFORMATION

Freeman et al.

Molecular determinants of outcomes in relapsed or refractory mantle cell lymphoma treated with ibrutinib or temsirolimus in the MCL3001 (RAY) trial

Contents

Sequencing method

Nucleotides were extracted from FFPE-slides, as outlined in the methods section. Normal samples were extracted from peripheral blood lymphocytes using QIAGEN Blood & Tissue Kits (QIAGEN, Hilden, Germany) after MACS separation with CD3 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) from corresponding patient blood samples.

Samples were pipetted into four 96-well plates (Tumor samples: Plates 1 and 2 and Matched normal samples: Plates 3 and 4) together with CEPH control samples in each plate. All samples were quantified using Pico Green. FFPE samples were normalized to 200 ng in 50 µL of 1× TE and all other samples were normalized to 100 ng in 50 uL 1× TE.

To construct the library, normalized DNA was fragmented (Covaris sonication [Covaris Inc., Woburn, MA, USA]) to 250 bp. Post-fragmentation sample quality was assessed using the Agilent TapeStation (Santa Clara, CA, USA). Samples were ligated to specific adapters during automated library preparation (Roche/KAPA Hyper KK8504) using the Beckman FXp liquid handling robot (Beckman Coulter, Indianapolis, IN, USA). Libraries were pooled in equal volume and sequenced on an Illumina Miseq to estimate the concentration based on the number of index reads per sample. Library construction was considered successful if the yield was ≥250 ng.

Libraries were pooled in equal mass into 750 ng for the enrichment of genes of interest ($N = 179$) (Table S1) using the XT HS Agilent SureSelect hybrid capture kit (Santa Clara, CA, USA). Captures were pooled by plate, and pools for each plate were sequenced on 2 lanes of the HiSeq3000 (Illumina, San Diego, CA, USA).

Table S1 Target genes of interest.

Genes of interest (N = 179)				
ABCA3	*CHMP4C*	*IRF8*	*PAX5*	*STAT3*
ABL1	*CIITA*	*IRS2*	*PCLO*	*STAT6*
AKT1	*CREBBP*	*ITK*	*PCSK2*	*SYK*
ANKRD17	*CSK*	*ITPKB*	*PHIP*	*TAB2*
ARID1A	*CYLD*	*JAK2*	*PIK3CA*	*TAB3*
ARID1B	*DAZAP1*	*JAK3*	*PIK3CD*	*TBC1D26*
ARID5B	*DGKB*	*KIAA1671*	*PIM1*	*TBL1XR1*
ATM	*DHDH*	*LCK*	*PLCG1*	*TEC*
B2M	*DLC1*	*LUZP4*	*PLCG2*	*TET2*
BCL10	*DLGAP2*	*LYN*	*PLXNA1*	*TLR2*
BCL2	*DNAJC6*	*MALT1*	*PLXNB3*	*TNF*
BCL6	*DNMT3A*	*MAP3K14*	*POT1*	*TNFAIP3*
BCL7A	*DUSP2*	*MAP3K7*	*PPM1D*	*TNFRSF11A*
BIRC2	*EGFR*	*MAPK1*	*PRDM1*	*TNFRSF13B*
BIRC3	*EIF2AK4*	*MAPK3*	*PRKCB*	*TNFRSF13C*
BIRC6	*EP300*	*MCL1*	*PTEN*	*TNFRSF14*
BLK	*ERBB2*	*MDM2*	*PTPN6*	*TNFSF13B*
BLNK	*ERBB4*	*MEF2B*	*PTPRD*	*TNRC6B*
BMX	*ETS1*	*MET*	*RB1*	*TP53*
BTG1	*EWSR1*	*MLL2*	*REL*	*TPMT*
BTG2	*EZH2*	*MLL3*	*RELB*	*TRAF1*
BTK	*FAS*	*MRGPRF*	*RET*	*TRAF2*
CARD11	*FAT4*	*MTOR*	*RGS4*	*TRAF3*
CCND1	*FOXO1*	*MUC4*	*RHOA*	*TRAF4*
CCND3	*GNA13*	*MYC*	*RIPK1*	*TRAF5*
CD28	*GON4L*	*MYD88*	*ROBO2*	*TRAF6*
CD40	*GRB2*	*NFKB1*	*S1PR1*	*TRPM6*
CD58	*HIST1H1B*	*NFKB2*	*SALL3*	*TXK*
CD70	*HIST1H1C*	*NFKBIA*	*SF3B1*	*UBR5*
CD79A	*HIST1H1D*	*NFKBIB*	*SGK1*	*UGT1A1*
CD79B	*HIST1H1E*	*NFKBIE*	*SMARCA2*	*VEGFA*
CDKN2A	*HNRNPH1*	*NFKBIZ*	*SMARCA4*	*VRK2*
CDKN2B	*IKBKB*	*NOTCH1*	*SMC1A*	*YLPM1*
CEBPA	*IKBKG*	*NOTCH2*	*SOCS1*	*WHSC1*
CHD8	*IKZF3*	*NSD2*	*SP140*	*ZNF117*
CHEK2	*IRF4*	*OGDHL*	*SPEN*	

Table S2 Influence of the site of origin on MCL35 risk groups.

MCL35 score	Lymph node and tonsils vs extranodal			Total (*N*)
	Lymph node and tonsils	Extranodal	Unknown	
Low risk, *n* (%)	39 (46)	11 (38)	5 (24)	55 (41)
Standard risk, *n* (%)	37 (44)	15 (52)	11 (52)	63 (47)
High risk, *n* (%)	8 (10)	3 (10)	5 (24)	16 (12)
Total, *n* (%)	84 (100)	29 (100)	21 (100)	134 (100)

Pearson's chi-squared test (4) = 5.5546; $p = 0.235$

Table S3 MYC expression in MCL35 risk groups.

Distribution of MYC expression[a] *across MCL35 risk groups*			
MCL35 score	MYC levels		Total (*N*)
	Low or no MYC	High MYC levels	
Low risk, *n* (%)	40 (73)	15 (27)	55 (100)
Standard risk, *n* (%)	48 (76)	15 (24)	63 (100)
High risk, *n* (%)	7 (44)	9 (56)	16 (100)
Total, *n* (%)	95 (71)	39 (29)	134 (100)
Association of the MCL35 assay and MYC expression[b]			
MCL35 high vs low-standard risk	MYC expression		Total (*N*)
	Low or no MYC	High MYC levels	
Low + standard risk, *n* (%)	88 (75)	30 (25)	118 (100)
High MCL35, *n* (%)	7 (44)	9 (56)	16 (100)
Total, *n* (%)	95 (71)	39 (29)	134 (100)

[a]MYC expression levels obtained divided into quartiles, with uppermost quartile only considered "high" levels of expression.

[b]1-sided Fisher's exact test: $p = 0.015$.

Table S4 *TP53* mutation status in MCL35 risk groups.

TP53 *mutation/deletion in MCL35 risk groups*			
MCL35 score			Total (*N*)
	Wild-type	*TP53* gene mutation/deletion	
Low risk, *n* (%)	49 (89)	6 (11)	55 (100)
Standard risk, *n* (%)	50 (79)	13 (21)	63 (100)
High risk, *n* (%)	7 (44)	9 (56)	16 (100)
Total, *n* (%)	106 (79)	28 (21)	134 (100)
Association of the MCL35 assay and TP53 *mutation/deletion*			
MCL35 high vs low-standard risk			Total (*N*)
	Wild-type	*TP53* gene mutation/deletion	
Low plus standard risk, *n* (%)	99 (84)	19 (16)	118 (100)
High MCL35, *n* (%)	7 (44)	9 (56)	16 (100)
Total, *n* (%)	106 (79)	28 (21)	134 (100)

1-sided Fisher's exact test: $p = 0.001$.

Table S5 Blastoid morphology in MCL35 risk groups.

Blastoid morphology in MCL35 risk groups			
MCL35 score	Blastoid morphology		Total (*N*)
	Non-blastoid	Blastoid	
Low risk, *n* (%)	54 (98)	1 (2)	55 (100)
Standard risk, *n* (%)	58 (92)	5 (8)	63 (100)
High risk, *n* (%)	11 (69)	5 (31)	16 (100)
Total, *n* (%)	123 (92)	11 (8)	134 (100)
Association of the MCL35 assay and blastoid morphology			
MCL35 high vs low-standard risk	Blastoid morphology		Total (*N*)
	Non-blastoid	Blastoid	
Low plus standard risk, *n* (%)	112 (95)	6 (5)	118 (100)
High MCL35, *n* (%)	11 (69)	5 (31)	16 (100)
Total, *n* (%)	123 (92)	11 (8)	134 (100)

1-sided Fisher's exact test: $p = 0.004$.

Table S6 Truncated CCND1 3′UTR expression in MCL35 risk groups.

Truncated CCND1 3'UTR in MCL35 risk groups			
MCL35 score	$\log_2$*CCND1*_8 probe minus $\log_2$*CCND1*		Total (*N*)
	< −0.59[a]	> −0.59	
Low risk, *n* (%)	1 (2)	53 (98)	54 (100)
Standard risk, *n* (%)	3 (5)	59 (95)	62 (100)
High risk, *n* (%)	9 (56)	7 (44)	16 (100)
Total, *n* (%)	13 (10)	119 (90)	132 (100)
Association of the MCL35 assay and truncated CCND1 *3'UTR*			
MCL35 high vs low-standard risk	$\log_2$*CCND1*_8 probe minus $\log_2$*CCND1*		Total (*N*)
	< −0.59[a]	> −0.59	
Low plus standard risk, *n* (%)	4 (3)	112 (97)	116 (100)
High MCL35, *n* (%)	9 (56)	7 (44)	16 (100)
Total, *n* (%)	13 (10)	119 (90)	132 (100)

1-sided Fisher's exact test: $p < 0.0001$.

[a]Truncated.

Table S7 *SOX11* expression in MCL35 risk groups.

SOX11 *expression in MCL35 risk groups*			
MCL35 score	*SOX11* expression[a]		Total (*N*)
	Low expression	High expression	
Low risk, *n* (%)	37 (67)	18 (33)	55 (100)
Standard risk, *n* (%)	51 (81)	12 (19)	63 (100)
High risk, *n* (%)	13 (81)	3 (19)	16 (100)
Total, *n* (%)	101 (75)	33 (25)	134 (100)
Association of SOX11 *expression in MCL35 risk groups*			
MCL35 high vs low-standard risk	*SOX11* expression		Total (*N*)
	Low expression	High	
Low plus standard risk, *n* (%)	37 (67)	18 (33)	55 (100)
High MCL35, *n* (%)	64 (81)	15 (19)	79 (100)
Total, *n* (%)	101 (75)	33 (25)	134 (100)

[a]SOX11 expression levels obtained divided into quartiles, with uppermost quartile only considered "high" level of expression,

1-sided Fisher's exact test: $p = 0.05$.

Table S8 Univariate analysis of variables on PFS ($N = 134$).

Variable	Hazard ratio	Confidence interval	*p* value
MCL35 risk group	1.74	1.26–2.41	<0.001
Treatment (temsirolimus vs ibrutinib)	0.21[a]	0.12–0.37	<0.001
sMIPI[b] 0 1 2	 – 1.15 2.40	 – 0.68–1.96 1.36–4.25	 – 0.6 0.003
Truncated *CCND1* 3'UTR (<0.25–0.59 vs >0.59)	0.58	0.03–1.13	0.11[c]
Blastoid morphology	2.26	1.13–4.54	0.02
Deletion/mutation *TP53*	1.93	1.18–3.16	0.01
BIRC3 mutation/deletion	1.83	1.16–2.88	0.01
ARID1A mutation/deletion	2.97	0.93–9.50	0.07
ARID1B mutation/deletion	1.57	0.98–2.52	0.06
SYK mutation/deletion	1.34	0.79–2.27	0.27[c]
CDKN2A mutation/deletion	1.40	0.79–2.50	0.25[c]
Deletion 8p23.3	1.48	0.84–2.61	0.17[c]

[a]In favor of ibrutinib.

[b]Treated as categorical given the lack of difference between low- and standard-risk groups.

[c]Not included in multivariate analysis.

Fig. S1 Flow diagram outlining the samples received and included in the analysis.

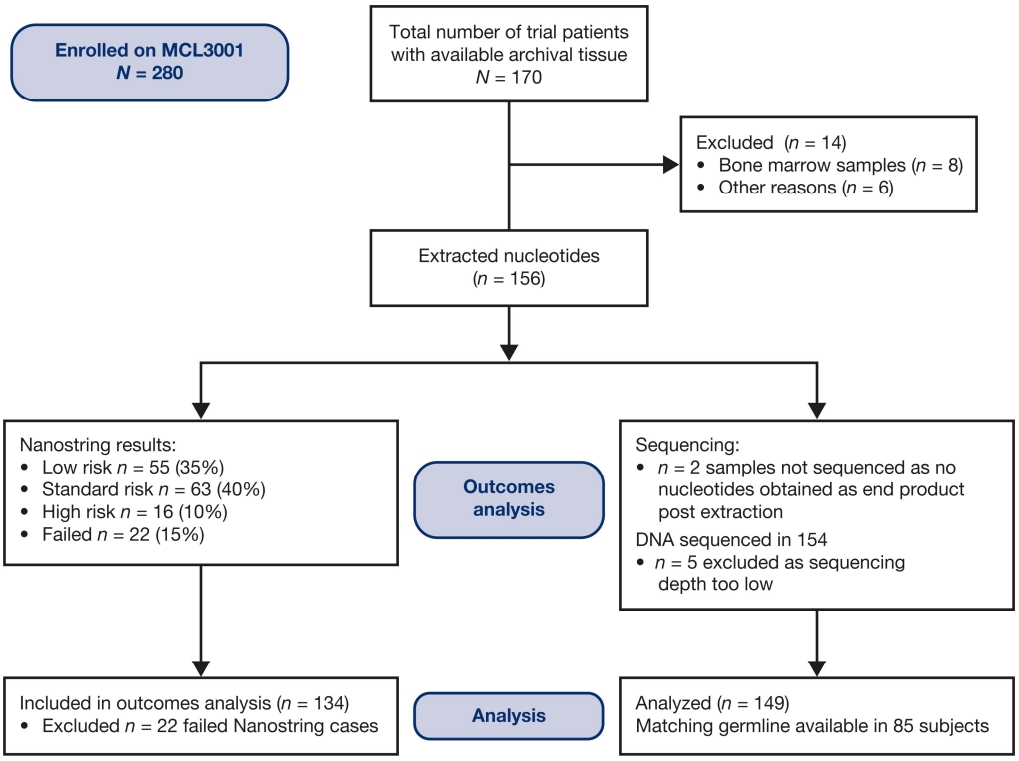
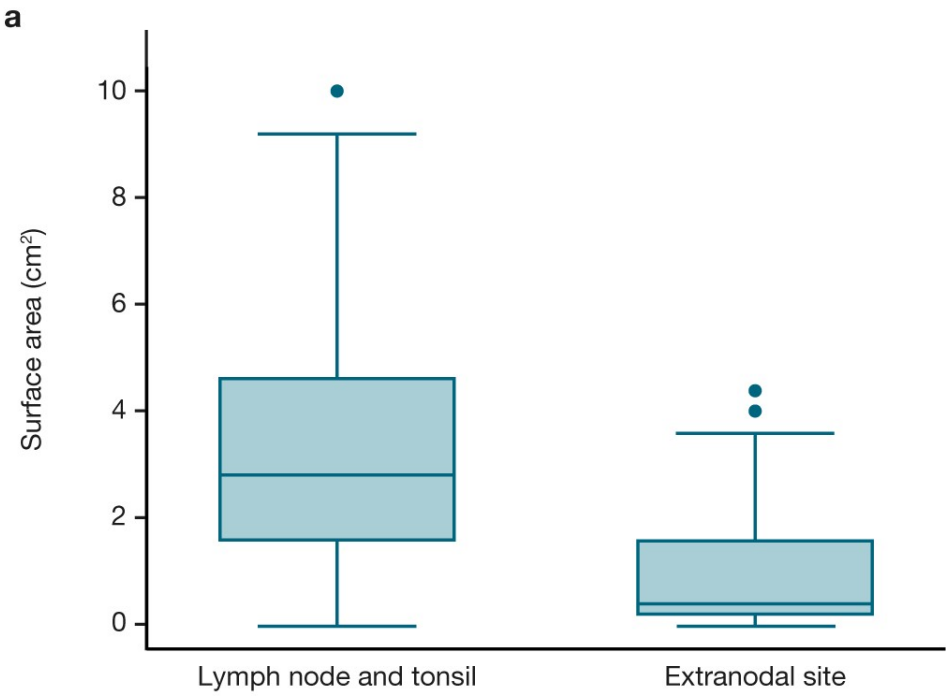


Fig. S2 (a) Surface area of lymph node or tonsil versus surface area of extranodal site (**b**) RNA concentration of lymph node or tonsil versus RNA concentration of extranodal site.



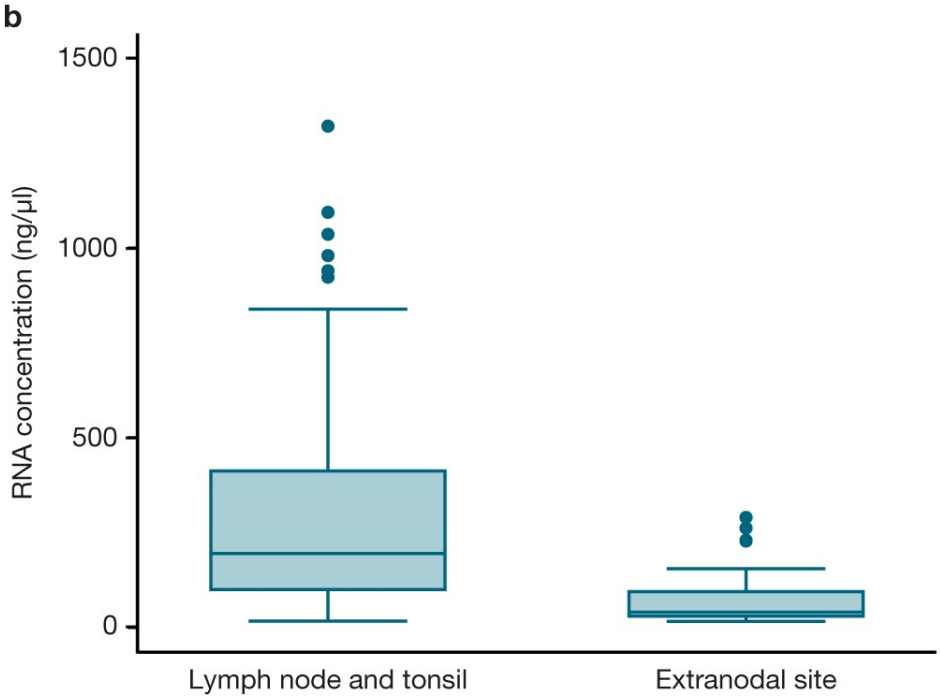
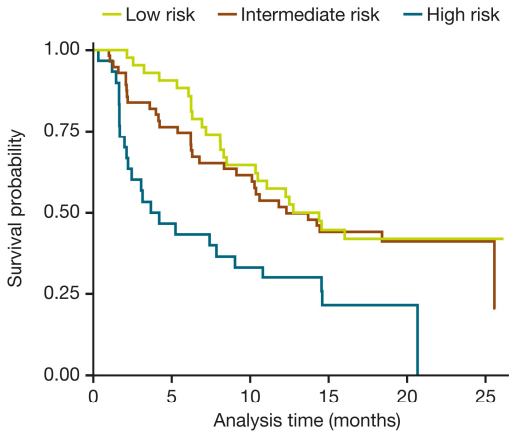
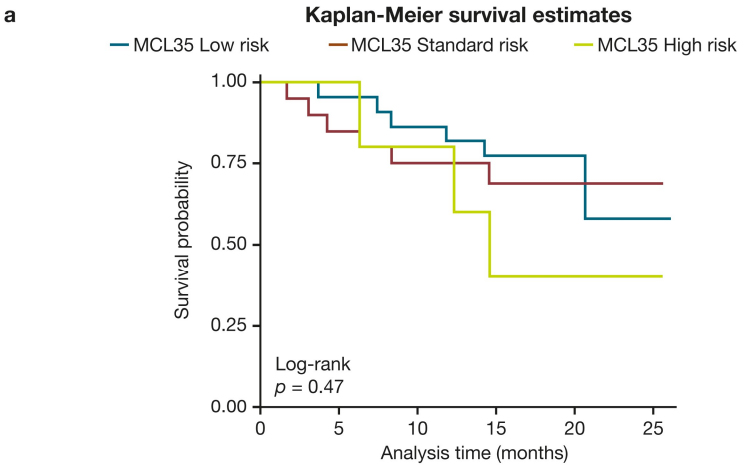


Fig. S3 IRC-assessed PFS split by sMIPI score.

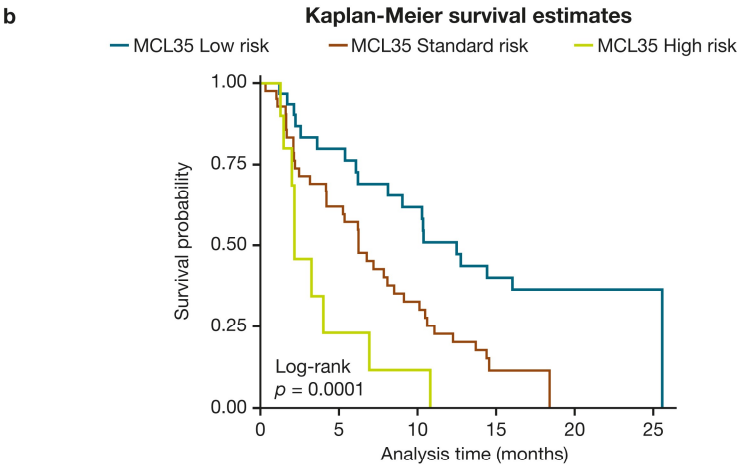


Patients at risk						
Low risk	43	38	27	16	8	2
Intermediate risk	60	41	32	18	12	2
High risk	31	14	10	4	2	0

Fig. S4 IRC-assessed PFS split by MCL35 risk limited to ibrutinib-only treated patients (**a**) ($N = 48$) and to temsirolimus-only treated patients (**b**) ($N = 86$).



Patients at risk						
MCL35 Low risk	22	21	19	13	10	1
MCL35 Standard risk	20	17	15	10	6	1
MCL35 High risk	6	5	4	2	2	1

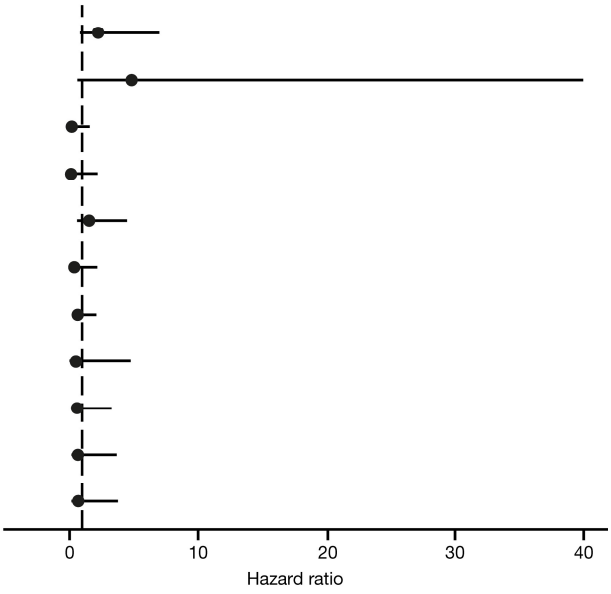


Patients at risk						
MCL35 Low risk	33	22	17	11	4	1
MCL35 Standard risk	43	26	13	2	0	0
MCL35 High risk	10	2	1	0	0	0

Fig. S5 Forest plot of mutation/deletions versus IRC-assessed PFS (**a**) and OS (**b**) in patients who received ibrutinib.

a

	HR (95% CI)	*p*	Q	Wild-type	Mutated
TP53	2.4 (0.85–7)	0.098	0.63	43	9
ARID1A	5 (0.64–40)	0.13	0.63	50	2
S1PR1	0.36 (0.082–1.6)	0.18	0.63	38	14
RB1	0.29 (0.038–2.2)	0.23	0.63	42	10
BIRC3	1.7 (0.63–4.5)	0.3	0.66	35	17
ARID1B	0.5 (0.11–2.2)	0.36	0.66	41	11
ATM	0.78 (0.29–2.1)	0.61	0.85	23	29
SYK	0.63 (0.083–4.8)	0.66	0.85	47	5
8p23.3 Del	0.74 (0.17–3.3)	0.7	0.85	43	9
CDKN2A	0.83 (0.19–3.7)	0.8	0.85	43	9
EWSR1	0.86 (0.19–3.8)	0.85	0.85	45	7



b

	HR (95% CI)	*p*	Q	Wild-type	Mutated
ARID1A	12 (1.3–110)	0.026	0.29	50	2
TP53	1.8 (0.67–4.9)	0.24	0.82	43	9
ARID1B	0.55 (0.16–1.9)	0.34	0.82	41	11
CDKN2A	1.6 (0.6–4.4)	0.34	0.82	43	9
8p23.3 Del	1.6 (0.57–4.2)	0.39	0.82	43	9
ATM	0.73 (0.32–1.7)	0.47	0.82	23	29
SYK	1.5 (0.43–4.9)	0.55	0.82	47	5
BIRC3	1.2 (0.52–2.9)	0.64	0.82	35	17
S1PR1	0.8 (0.3–2.2)	0.67	0.82	38	14
RB1	0.92 (0.31–2.7)	0.88	0.93	42	10
EWSR1	0.95 (0.28–3.2)	0.93	0.93	45	7

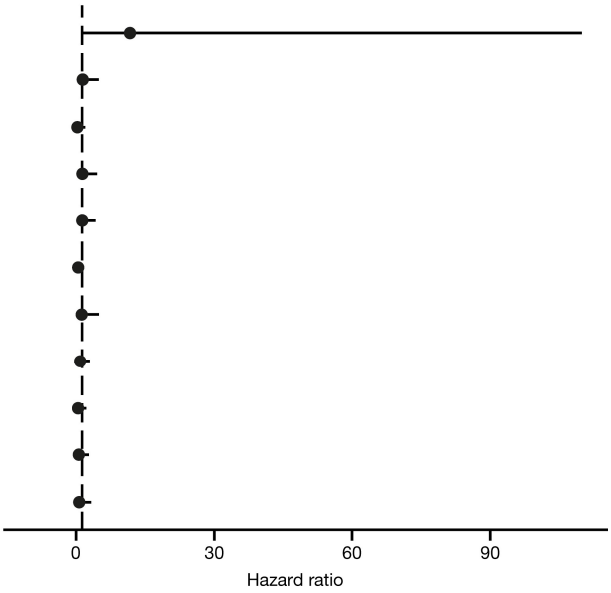


Fig. S6 PFS curve for patients with *TP53* mutation/deletion (**a**) (*N* = 33) and OS curve for patients with *TP53* mutation/deletion (**b**) (*N* = 33).