Supplemental material

CCND2 and *CCND3* hijack immunoglobulin light chain enhancers in cyclin D1-negative mantle cell lymphoma

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SUPPLEMENTAL TABLES

Supplemental Table S1. Commercial and bacterial artificial chromosomes (BAC)-labeled probes used for FISH analyses.

BAC/Probe	Region	Chrom. location (hg19)	Reference	Fluorescent dye
CCND2* BAP-1				
RP11-578L13	5'	chr12:4002511-4203622	[1,2]	Spectrum Orange
RP11-388F6	3'	chr12:4443393-4613592	[1,2]	Spectrum Green
CCND2* BAP-2				
RP11-358F19	5'	chr12:3991712-4180381	Unpublished	Spectrum Red
RP11-8E02	3'	chr12:4434027-4604218	Unpublished	Spectrum Green
***CCND3* BAP**				
RP1-321B9	5'	chr6:42159875-42243431	[3]	Spectrum Orange
RP1-139D8	5'	chr6:41992892-42159974	[3]	Spectrum Orange
RP5-973N23	3'	chr6:41694188-41810693	[3]	Spectrum Green
RP11-298J23	3'	chr6:41564073-41743535	[3]	Spectrum Green
***CCNE1* BAP**				
RP11-17N20	3'	chr19:30370277-30526346	Unpublished	Spectrum Red
CTD-2063E21	5'	chr19:30097638-30224016	-	Spectrum Green
LSI IGH BAP		14q32.3	Abbott Molecular	Spectrum Green/Orange
XL IGL BAP		22q11.22	Metasystems	Spectrum Green/Orange
XL IGK BAP		2p11.2	Metasystems	Spectrum Green/Orange
Locus specific				
RP11-928N17	*CCND2*	chr12:4274557-4482113	-	Spectrum Red
CTD-3053I1	*CCND3*	chr6:41751478-41951375	-	Spectrum Red
RP11-104J24	*CCNE1*	chr19:30250326-30400791	-	Spectrum Red
RP11-272K10	*CCNE2*	chr8:95874697-96025074	-	Spectrum Red
RP11-1134E24	IGK-enh[#]	chr2:89135166-89284041	-	Spectrum Green
RP11-15J7	IGK-enh[#]	chr2:89140332-89302455	-	Spectrum Green
XCE 8	cen	Centromere chr8	Metasystems	Spectrum Orange

BAP: break-apart probe, cen: centromere, enh: enhancer.
*The design of both *CCND2* BAP probes was virtually the same, only the BACs selected were different.
[#]The BACs used do not cover exclusively the enhancer region of IGK.

Supplemental Table S2. Next-generation sequencing and molecular techniques applied to each sample.

Case	DNA origin	MP-WGS	WGS	WES	Sanger	FISH	CN array	GEP	qPCR	IGHV identity/ V-gene
ID3_1	FT	X		X	X	X	SNP-500K	X		95.85% IGHV3-48
ID3_2	FFPE					X	OncoScan			
ID5	FT			X	X	X	SNP-500K	X		97.11% IGHV4-39
ID6	FT	X		X	X	X	SNP-500K	X		
ID7	FFPE					X				
ID9	FFPE					X	Agilent 1M	X	X	
ID10	FFPE					X			X	
ID12_1	FFPE					X	Agilent 1M		X	100% IGHV3-21
ID12_2	FFPE					X	Agilent 1M	X	X	
ID13	FFPE					X	Agilent 1M			
ID14	FFPE					X	Agilent 1M	X	X	
ID15	FFPE					X	Agilent 1M			
ID16	FFPE					X	Agilent 1M			
ID17	FFPE					X	Agilent 1M		X	
ID18	FFPE					X	Agilent 1M			97.21% IGHV4-34
ID24	FFPE					X			X	99.59% IGHV1-3
ID26	FFPE					X	Agilent 1M	X	X	99.15% IGHV3-74
ID28	FFPE					X	Agilent 1M	X	X	100% IGHV1-8
ID29	FFPE					X	Agilent 1M		X	
ID31	FFPE					X	Agilent 1M		X	
ID32	FFPE					X	Agilent 1M			
ID34	FFPE					X	Agilent 1M	X	X	
ID35	FFPE					X	Agilent 1M		X	
ID36	FFPE					X	Agilent 1M			
ID37	FFPE					X	Agilent 1M			97.17% IGHV4-39
ID39	FFPE					X	Agilent 1M	X	X	97.44% IGHV3-48
ID40	FFPE					X	Agilent 1M	X	X	98.74% IGHV3-9
ID42	FFPE					X	Agilent 1M			
ID43	FFPE					X			X	
ID44	FFPE					X	OncoScan		X	
ID55	FFPE					X	Agilent 1M	X	X	
ID56	FFPE					X	Agilent 1M	X	X	
ID59	FFPE					X	Agilent 1M	X	X	95.07% IGHV4-69
ID60	FFPE					X	Agilent 1M	X	X	95.93% IGHV4-34
ID61	FFPE					X	Agilent 1M	X	X	
ID62	FFPE					X				
ID64	FFPE					X				

Case	DNA origin	MP-WGS	WGS	WES	Sanger	FISH	CN array	GEP	qPCR	IGHV identity/ V-gene
ID65_1	FFPE					X	Agilent 1M	X	X	99.60% IGHV3-21
ID65_2	FFPE					X	Agilent 1M			
ID66_1	FFPE					X	Agilent 1M			
ID66_2	FT					X	SNP- 6.0			
ID67	FFPE					X	Agilent 1M		X	96.39% IGHV3-23
ID68	FFPE					X			X	99.19% IGHV3-30
ID70	FFPE					X	Agilent 1M		X	
ID73_1	FT					X	SNP- 6.0			97.58% IGHV3-21
ID73_2	FT	X	X			X	SNP- 6.0		X	
ID75	FFPE					X	OncoScan			
ID76	FT	X				X	SNP- 6.0		X	
ID77	FFPE					X	OncoScan		X	
ID79	FFPE					X				
ID80	FFPE					X				
ID83	FFPE					X	OncoScan		X	
ID84	FFPE					X			X	99.60% IGHV3-21
ID85	FFPE					X			X	100% IGHV4-34
ID86	FFPE					X	OncoScan		X	
ID87	FFPE					X	OncoScan		X	
ID88	FFPE					X			X	
ID90	FFPE					X	OncoScan		X	100% IGHV3-21
ID91	FFPE					X			X	99.20% IGHV3-21
ID92	FFPE					X	OncoScan		X	100% IGHV4-59
ID93	FFPE					X			X	95.65% IGHV1-3

CN: copy number, FT: frozen tissue, FFPE: formalin-fixed paraffin embedded tissue, GEP: gene expression profile, IGHV: immunoMP-WGS: mate-pair whole-genome sequencing, WES: whole-exome sequencing, WGS: whole genome sequencing, qPCR: quantitative PCR.

Supplemental Table S3. Primers used for verification of IG and *CCND3* breakpoints by Sanger sequencing.

Primer name	Data	Sequence (5‘- 3‘)
ID3v2_ tel _F	MP-WGS	AATTGCATTGGCTCATTCCT
ID3v2_ tel _R	MP-WGS	CCTCCCTTGAATTGTCCAATAA
ID3_ cen _F	MP-WGS	AGATCAGCTGGGGTAAACGA
ID3_ cen _R	MP-WGS	AATTCCAGCCTCCCTTCATT
ID6-F	WES	TACTGGCCATCAGACCCAAA
ID6-R	WES	CCAAGAACCGAAGACAGCTC
ID5-left*	WES	CGGTCTTGGGACATTCAGAAA
ID5-right	WES	GGAGGCGTCAAGATCCACTA

cen: centromeric break, F: forward, MP-WGS: mate-pair whole-genome sequencing, R: reverse, tel: telomeric break, WES: whole-exome sequencing.

*This primer includes the breakpoint.

Supplemental Table S4. TaqMan assays (Thermo Fisher Scientific Inc.) used for quantitative PCR analyses.

Gene Symbol	Assay ID	Amplicon size (base pairs)	Reference sequence
CCND2	Hs_00153380_m1	69	MN_001759.3
CCND3	Hs_00236949_m1	67	MN_01136017.3
CCNE1	Hs_01026536_m1	64	MN_001322262.1
CCNE2	Hs_00180319_m1	92	MN_057749.2
GUSB	Hs_00939627_m1	96	MN_001293104.1

Supplemental Table S5. Complete FISH and gene expression results of *CCND2/D3, CCNE1/E2,* and IG genes of the 56 cyclin D1⁻ MCL.

ID	Group	Transl. Status	*CCND2* BAP	*CCND3* BAP	*CCNE1* #	*CCNE2* #	IGH BAP	IGK BAP	IGL BAP	IGK-*CCND2* cryptic ins.	IGK *CCND3* cryptic ins.	NGS/ Sanger
ID3	D3	D3-K	neg	neg			neg	neg	neg		pos	MP-WGS/ Sanger
ID5	D3	D3-L	neg	neg			neg	neg	neg			WES/ Sanger
ID6	D3	D3-K	neg	neg			neg	neg	neg		pos	MP-WGS/ WES/ Sanger
ID7	D2	D2-K	pos				neg	pos	neg			
ID9	D3	D3-K	neg	neg			neg	neg	neg		pos	
ID10	D2	D2-K	pos	neg			neg	pos	neg			
ID12	D2	D2-K	neg	neg				neg	neg	pos		
ID13	D2	D2-K	pos	neg			neg	pos				
ID14	D2	D2 -H	pos				pos					
ID15	D2	D2-K	pos					pos				
ID16	D2	D2-break	pos				neg	neg	n.e.			
ID17	D2	D2-K	pos				neg	pos				
ID18	D2	D2-K	pos	neg			neg	pos	neg			
ID24	D2	D2-H	pos	neg			pos	neg	neg			
ID26	E1/ E2	neg	neg	neg	BAP neg IGKe neg	IGKe neg	neg	neg	neg			
ID28	D2	D2-K	pos				neg	pos	neg			
ID29	D2	D2-break	pos				neg	neg	neg			
ID31	D2	D2-break	pos	neg			neg	neg	neg			
ID32	D2	D2-K	pos					pos				
ID34	D2	D2-break	pos	neg								
ID35	D2	D2-L	pos				neg	neg	pos			
ID36	D2	D2-L	pos				neg	neg	pos			
ID37	D2	D2-L	pos				neg	neg	pos			
ID39	D2	D2-L	pos				neg	neg	pos			
ID40	D2	D2-K	pos				neg	pos	neg			
ID42	D2	D2-L	pos						pos			
ID43	D2	D2-H	pos				pos	neg				
ID44	D2	D2-K	pos†					pos				
ID55	D2	D2-break	pos†				n.e.	neg	neg			
ID56	D2	D2-K	neg	n.e			neg	neg		pos		
ID59	D2	D2-break	pos				neg	neg				
ID60	E1/ E2	neg	neg	n.e.	BAP neg IGKe neg	IGKe n.e	neg	neg	neg			
ID61	D2	D2-K	pos				neg	pos	neg			
ID62	D2	D2-H*	pos									
ID64	D2	D2-break	pos				neg					
ID65	D2	D2-K	neg†	neg			neg	neg	neg	pos		
ID66	D2	D2-K	pos					pos				
ID67	D2	D2-K	pos					pos				
ID68	D2	D2-K	neg	n.e.						pos		
ID70	D3	D3-K	neg†	neg			neg	neg	neg		pos	
ID73	E1/ E2	neg	neg	neg	BAP n.e.	neg†		n.e.				
ID75	D2	D2-K	pos					pos				
ID76	D2	D2-K*	pos									
ID77	E1/ E2	neg	neg	n.e.	BAP neg IGKe neg	IGKe n.e						
ID79	D2	D2-K	pos				neg	pos	neg			
ID80	D2	D2-K	pos					pos				
ID83	D2	D2-break	pos					neg	n.e.			

ID	Group	Transl. Status	*CCND2* BAP	*CCND3* BAP	*CCNE1* #	*CCNE2* #	IGH BAP	IGK BAP	IGL BAP	IGK-*CCND2* cryptic ins.	IGK *CCND3* cryptic ins.
ID84	D2	D2-K	pos					pos	neg		
ID85	D2	D2-K	pos				neg	pos			
ID86	D3	D3-K	neg	neg			neg				pos
ID87	D3	D3-K	neg	neg†			neg				pos†
ID88	D2	D2-K	pos					pos			
ID90	D3	D3-K	neg	neg							pos
ID91	D2	D2-break	pos					neg	n.e.		
ID92	D3	D3-K	neg	neg							pos
ID93	D2	D2-K	pos					pos			

Amp: amplification, BAP: break-apart probe, D2: MCL with *CCND2* rearrangement and overexpression, D3: MCL with *CCND3* rearrangement and overexpression, D2-break: *CCND2* break with unknown partner, E1/E2: MCL with *CCNE1* and *CCNE2* concomitant overexpression, GEP: gene expression profiling, IGKe: IGK enhancer, ins: insertion, neg: negative, n.e.: non-evaluable, pos: positive, transl: translocation.

*Positive for *CCND2/*IGH or IGK/*CCND2* by FISH with locus specific probes and/or by karyotype.

#The different hybridizations performed with *CCNE1* or *CCNE2* probes are detailed for the 4 cases, including FISH with *CCNE2* break and FISH using the combination of IGK-enhancer (IGKe) with *CCNE1* or *CCNE2*.

†Gain of the locus detected by FISH.

Supplemental Table S6. Interchromosomal structural variants detected by mate-pair WGS analysis in 3 cyclin D1$^-$ MCL cases (hg19).

Case	Chr1	Position 1	Position 1b	Chr 2	Position 2	Position 2b	CNA 1	CNA 2	Primary SV
ID3	2	89130000	89140000	6	41960000	41970000			IGK/*CCND3*
ID3	3	154520000	154540000	6	107500000	107540000	Yes	Yes	
ID3	6	114510000	114520000	9	3700000	3710000	Yes	Yes	
ID3	6	124310000	124320000	9	112440000	112450000	Yes	Yes	
ID6	1	33510000	33520000	7	24450000	24460000			
ID6	1	160860000	160870000	4	112598000	112600000			
ID6	2	89150000	89160000	6	41920000	41930000			IGK/*CCND3*
ID6	5	43080000	43090000	7	80530000	80540000			
ID6	6	7310000	7320000	13	51620000	51640000			
ID6	6	51840000	51850000	10	92244400	92253000			
ID76	1	106680000	106690000	13	91408684	91418684			
ID76	1	106670000	106680000	13	91414411	91424411			
ID76	2	89150000	89160000	12	4342915	4352915			IGK/*CCND2*
ID76	2	89180000	89190000	12	4333595	4343595			IGK/*CCND2*
ID76	2	29980000	29990000	10	48415075	48425075			
ID76	3	123930000	123940000	13	92051732	92061732	Yes	Yes	
ID76	3	124220000	124230000	13	91192485	91202485			
ID76	3	124200000	124210000	13	91300059	91310059			
ID76	3	133550000	133560000	13	58884223	58894223		Yes	
ID76	3	124320000	124330000	13	91258208	91268208			
ID76	3	124520000	124530000	13	80886856	80896856	Yes	Yes	
ID76	5	430000	440000	11	63714793	63724793			
ID76	5	420000	430000	11	63717789	63727789			
ID76	9	105810000	105820000	15	100910062	100920062	Yes	Yes	
ID76	10	48410000	48420000	2	29983938	29993938			
ID76	11	63720000	63730000	5	424856	434856			
ID76	13	80890000	80900000	3	124525613	124535613	Yes	Yes	
ID76	13	90870000	90880000	3	124620979	124630979	Yes	Yes	
ID76	13	91260000	91270000	3	124318783	124328783			
ID76	13	91300000	91310000	3	124197467	124207467			
ID76	13	58880000	58890000	3	133549364	133559364	Yes		
ID76	13	91200000	91210000	3	124218054	124228054			
ID76	15	100910000	100920000	9	105806865	105816865	Yes	Yes	

Chr 1, Chr 2: the 2 chromosomes involved in the rearrangement, CNA 1, CNA 2: copy number alterations present in the breakpoint 1 and 2, respectively, Pos 1, Pos 1b: interval of the breakpoint in the first (Chr 1), Pos 2, Pos 2b: interval of the breakpoint in the second chromosome involved in the structural variant (Chr 2).

Supplemental Table S7. Structural variants detected by WGS (mate-pair and paired-end) in the cyclin D1$^-$ and cyclin D2$^-$ MCL case ID73 (hg19).

Chr 1	Position 1	Chr 2	Position 2	Library	Algorithm*	Type	CNA 1	CNA 2	Affected genes
chr2	32605430	chr2	32460793	PE	S	Intra			*BIRC6- NLRC4*
chr2	78796419	chr2	78799187	PE	S	Intra			
chr2	85242613	chr12	32610437	PE	S	Inter			*KCMF1-FGD4*
chr2	85242658	chr9	123193506	PE-MP	S-H	Inter			*KCMF1-CDK5RAP2*
chr2	111623747	chr16	89636965	PE-MP	L-H	Inter			*ACOXL-*
chr2	111875807	chr2	112004073	PE	S	Intra			*-MIR4435-1HG*
chr2	111920331	chr16	89637012	PE-MP	S-L-H	Inter			*BCL2L11-*
chr2		chr2	231315686	PE	L	Intra			*SP100*
chr3	22471929	chr6	145300900	PE	S-L	Inter	Yes	Yes	
chr3	24278915	chr6	157176493	PE	S-L	Inter	Yes	Yes	*THRB-ARID1B*
chr3	29890000 29900000	chr6	114870066 114880066	MP	H	Inter		Yes	*RBMS3-*
chr3	49260945	chr3	50655165	PE	L	Intra	Yes	Yes	*CCDC36-MAPKAPK3*
chr3	105443572	chr6	132820773	PE	S	Inter	Yes	Yes	*CBLB-STX7*
chr3	105507564	chr6	125420725	PE-MP	S-L-H	Inter	Yes		*CBLB-*
chr3	106852345	chr3	106924776	PE	S	Intra			*LINC00882*
chr3	106924705	chr3	106852236	PE	S	Intra			*LINC00882*
chr3	122462393	chr6	156403077	PE-MP	S-L-H	Inter		Yes	*HSPBAP1-*
chr3	122460000 122470000	chr6	135536311 135546311	MP	H	Inter			*HSPBAP1-*
chr3	132104159	chr3	115338256	PE	S	Intra			
chr3	187489719	chr6	104184816	PE-MP	L-H	Inter		Yes	
chr4	91150655	chr4	91749027	PE	S-L	Intra	Yes	Yes	*CCSER1*
chr5	25797188	chr5	25752663	PE	S	Intra			
chr6	127507698	chr18	78014475	PE	L	Inter		Yes	*RSPO3*
chr6	130869466	chr6	157310615	PE	S-L	Intra		Yes	*-ARID1B*
chr6	134910000 134920000	chr3	29890453 29900453	MP	H	Inter			*-RBMS3*
chr6	135530000 135540000	chr3	122460197 122470197	MP	H	Inter			*MYB-HSPBAP1*
chr6	136891590	chr3	132104583	PE-MP	S-H	Inter			*MAP3K5-*
chr6	136960484	chr18	77205507	PE-MP	S-L-H	Inter		Yes	*MAP3K5-NFATC1*
chr6	141883015	chr3	115335294	PE	S	Inter			
chr6	142796723	chr3	105507547	PE	S-L	Inter	Yes	Yes	*-CBLB*
chr6	145332773	chr6	145332871	PE	S	Intra	Yes	Yes	
chr6	147753309	chr6	127509761	PE	S	Intra			*-RSPO3*
chr8	89690000 89700000	chr18	48581050 48591050	MP	H	Inter	Yes	Yes	*-SMAD4*
chr8	90875850	chr8	91735970	PE	S	Intra	Yes		*-TMEM64*
chr8	90898425	chr18	44102601	PE	S	Inter	Yes		*-LOXHD1*
chr8	90920000 90930000	chr18	44095027 44105027	MP	H	Inter	Yes		*OSGIN2-LOXHD1*
chr8	90930000 90940000	chr18	44092192 44102192	MP	H	Inter	Yes		*OSGIN2-LOXHD1*
chr8	90969950	chr8	91032543	PE	S-L	Intra	Yes	Yes	*NBN-DECR1*
chr8	91593682	chr18	55055800	PE-MP	S-L-H	Inter	Yes	Yes	
chr8	91742645	chr8	91768347	PE	S-L	Intra	Yes	Yes	*TMEM64*
chr8	94917360	chr8	91032535	PE	S	Intra		Yes	*PDP1-DECR1*
chr8	95053197	chr8	89658766	PE	S	Intra	Yes	Yes	
chr9	21906122	chr9	22007374	PE	L	Intra	Yes	Yes	*MTAP-CDKN2B-AS1*
chr9	21906123	chr9	21998682	PE	L	Intra	Yes	Yes	*MTAP-CDKN2B-AS1*
chr9	21998774	chr9	22007374	PE	L	Intra	Yes	Yes	*CDKN2B-AS1*
chr9	37810261	chr9	37810213	PE	S	Intra			*DCAF10*
chr11	61841813	chr14	81786774	PE	L	Inter			*-STON2*

Chr 1	Position 1	Chr 2	Position 2	Library	Algorithm*	Type	CNA 1	CNA 2	Affected genes
chr12	32610366	chr9	25880494	PE	S	Inter			*FGD4-*
chr12	96340000 96350000	chr2	50762177 50772177	MP	H	Inter			*AMDHD1-NRXN1*
chr13	31724986	chr11	83807112	PE-MP	S-L-H	Inter	Yes		*HSPH1-DLG2*
chr13	48724010	chr13	48896200	PE	L	Intra	Yes	Yes	*-RB1*
chr13	48920363	chr13	49689000	PE	L	Intra	Yes	Yes	*RB1-FNDC3A*
chr14	45610000 45620000	chr2	70733462 70743462	MP	H	Inter			*FANCM-TGFA*
chr18	44090000 44100000	chr8	90924821 90934821	MP	H	Inter		Yes	*LOXHD1-OSGIN2*
chr18	44102584	chr18	48544346	PE	S-L	Intra		Yes	*LOXHD1-SMAD4*
chr18	44141025	chr18	54708286	PE	S	Intra		Yes	*LOXHD1-*
chr18	48584623	chr8	91025448	PE-MP	S-H	Inter	Yes	Yes	*SMAD4-DECR1*
chr18	55050000 55060000	chr8	91577816 91587816	MP	H	Inter	Yes	Yes	
chr18	77205553	chr6	134906326	PE-MP	S-H	Inter	Yes		*NFATC1-*
chr18	78014430	chr6	130899751	PE-MP	S-L-H	Inter	Yes		
chr19	1227924	chr19	1428961	PE	L	Intra			*STK11-DAZAP1*
chr19	2117444	chr19	2260245	PE	L	Intra			*AP3D1-JSRP1*
chr19	12676128	chr19	13368794	PE	L	Intra	Yes	Yes	*-CACNA1A*
chr19	18021099	chr19	19035828	PE	L	Intra	Yes	Yes	*-DDX49*
chr19	18229064	chr19	18256841	PE	S-L	Intra	Yes	Yes	*MAST3*
chr22	46580000 46590000	chr19	33277912 33287912	MP	H	Inter	Yes	Yes	*PPARA-TDRD12*
chrX	79669626	chrX	79825980	PE	S-L	Intra			*FAM46D-*

Chr 1, Chr 2: the 2 rearrangemed chromosomes, CNA 1, CNA 2: copy number alteration detected in both breakpoints, Inter: interchromosomal rearrangement, Intra: intrachromosomal rearrangement, MP: mate-pair whole-genome sequencing, PE: paired-end whole-genome sequencing.

In the last column the presence of " - " denotes position 1 or position 2 of the affected gene.

*The abbreviations S, L indicate "Smufin and Lumpy", respectively, used for SV in the WGS analysis; whereas H indicates "in-house script" used for SV in the MP-WGS analysis.

Supplemental Table S8. Protein-coding mutations of cyclin D1$^-$ and cyclin D2$^-$ MCL case ID73 (hg19).

Chr	Position	Ref	Obs	Type	Gene	Codon	Protein	VAF (%)	Predict.	Sanger *
chr1	156105059	C	T	missense	*LMNA*	c.892C>T	p.Arg298Cys	39.4	D/D/D	
chr1	248185661	A	T	stop gained	*OR2L5*	c.412A>T	p.Arg138	39.5	B/./D	
chr3	73433538	G	A	missense	*PDZRN3*	c.2179C>T	p.Arg727Cys	41.7	D/D/D	
chr4	94377059	G	A	missense	*GRID2*	c.1792G>A	p.Gly598Arg	38.6	B/D/D	
chr5	133496711	T	G	missense	*SKP1*	c.282A>C	p.Lys94Asn	28.6	B/D/D	
chr6	44147780	A	T	missense	*CAPN11*	c.1520A>T	p.Glu507Val	38.1	D/D/D	
chr6	128306918	G	A	stop gained	*PTPRK*	c.3217C>T	p.Arg1073*	36.4	B/./D	
chr7	100085885	C	T	missense	*NYAP1*	c.541C>T	p.Pro181Ser	33.3	B/B/B	
chr7	122757622	G	A	missense	*SLC13A1*	c.1553C>T	p.Pro518Leu	28.6	D/D/D	
chr8	77618608	A	T	missense	*ZFHX4*	c.2285A>T	p.Lys762Ile	47.6	D/D/D	
chr8	103845398	A	G	missense	*AZIN1*	c.790T>C	p.Ser264Pro	16.1	D/B/D	Yes
chr8	110509210	T	A	missense	*PKHD1L1*	c.10390T	p.Tyr3464Asp	17	D/D/D	Yes
chr8	110509217	A	C	missense	*PKHD1L1*	c.10397A	p.Asn3466Thr	17.5	B/D/D	Yes
chr9	7174671	C	T	missense	*KDM4C*	c.2174C>T	p.Pro1038Leu	20.0	D/D/D	
chr9	12694226	C	T	missense	*TYRP1*	c.230C>T	p.Pro77Leu	32.3	B/B/D	
chr10	123274833	G	A	missense	*FGFR2*	c.1088C>T	p.Ala363Val	40.7	B/B/D	
chr11	17627673	C	T	missense	*OTOG*	c.3964C>T	p.Arg1395Cys	42.4	D/D/D	
chr11	18369431	G	A	missense	*GTF2H1*	c.82G>A	p.Gly340Arg	25.6	B/B/D	
chr11	31327808	C	A	missense	*DCDC1*	c.562G>T	p.Ala188Ser	32.6	B/D/B	
chr11	70544862	C	T	missense	*SHANK2*	c.677G>A	p.Arg227His	25.9	D/D/D	Yes
chr12	7647946	C	A	missense	*CD163*	c.1151G>T	p.Gly384Val	23.5	D/D/D	Yes
chr14	23842422	C	T	missense	*IL25*	c.95C>T	p.Thr32Ile	30.8	D/B/B	
chr15	71188227	G	C	missense	*LRRC49*	c.145G>C	p.Gly49Arg	40.0	B/D/D	
chr15	75015000	T	C	missense	*CYP1A1*	c.439A>G	p.Ile147Val	37.5	B/B/B	
chr15	79750279	G	A	missense	*KIAA1024*	c.1790G>A	p.Cys597Tyr	40.0	./D/D	
chr16	71683304	G	A	missense	*PHLPP2*	c.3461C>T	p.Pro1154Leu	31.8	D/D/D	
chr17	57758724	A	T	missense	*CLTC*	c.3134A>T	p.Asn1045Ile	45.9	D/D/D	
chr20	30436646	G	A	missense	*DUSP15*	c.689C>T	p.Pro230Leu	34.4	D/D/B	Yes
chr20	60903462	C	T	missense	*LAMA5*	c.4487G>A	p.Gly1496Asp	24.0	D/D/D	
chrX	66937326	G	A	missense	*AR*	c.584G>A	p.Arg727His	38.2	D/D/D	
chrX	69456990	A	C	missense	*AWAT1*	c.352A>C	p.Thr118Pro	22.2	D/D/D	Yes

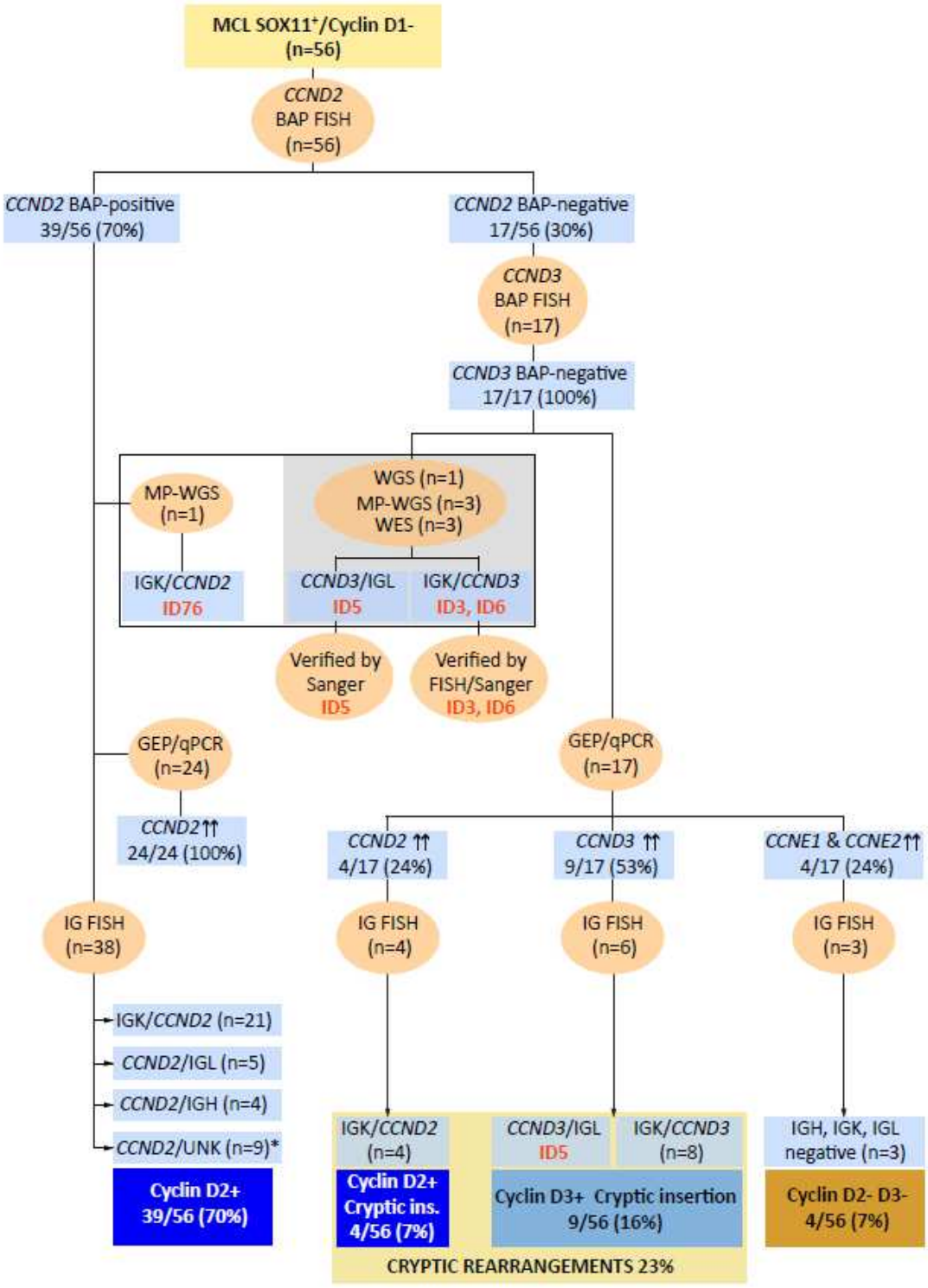
Chr: chromosome, Obs: observed, Ref: reference, SNV: single nucleotide variants, VAF: variant allele frequency.
The functional predictors (Predict.) used are SIFT/Polyphen2/MutationTaster and the functional effects are categorized as D: Damaging, B: Benign, .: NA.
*The selected 7 mutations (in 6 genes) detected by Sidrón and Mutect2 and not by Smufin were verified by Sanger sequencing.

Supplemental Table S9. Copy number alterations of 42 cyclin D1$^-$ MCL cases (47 samples) (hg19)(in excel format).

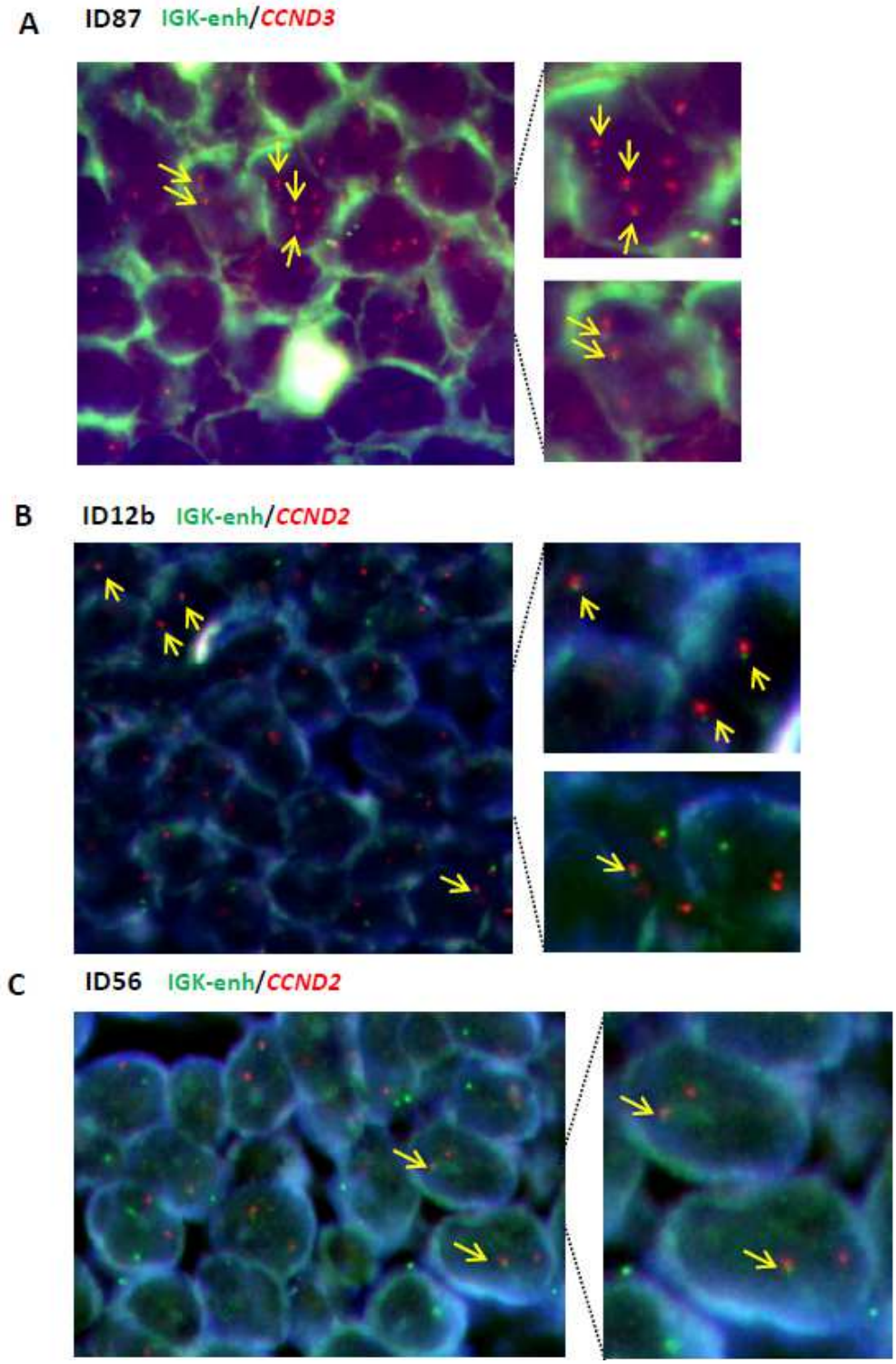
SUPPLEMENTAL FIGURES

Supplemental Figure S1. Schematic representation of the experimental design and cyclin D1$^-$ MCL subgroups. Flowchart diagram of cases, techniques, and results. The gray square highlights cases analyzed by NGS that lead to the discovery of the cryptic rearrangements. *In 1/9 cases with *CCND2* break no IGH, IGK, IGL FISH was performed to test the partner.

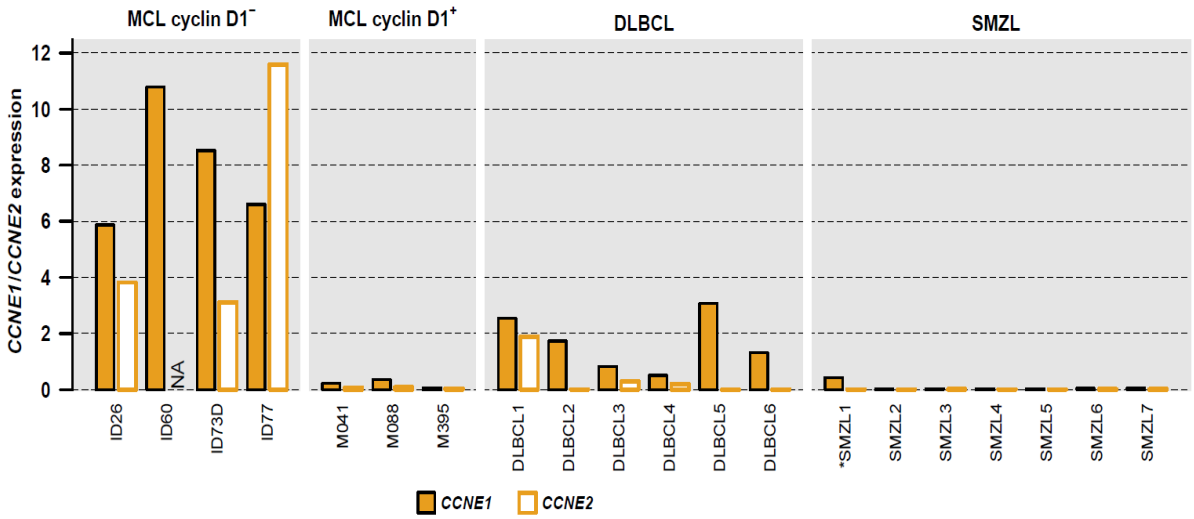
BAP: break-apart probes, IG FISH: FISH experiments to determine the partner gene, MP-WGS: mate-pair whole-genome sequencing, WES: whole-exome sequencing, ↑↑: overexpression.



Supplemental Figure S2. FISH analyses of *CCND2/D3* cryptic insertions. (**A**) Cryptic IGK/*CCND3* in case ID87, 2 or 3 fusion signals were found in each cell, concordant with a gain of *CCND3*, both by FISH and array. (**B-C**) Cryptic IGK/*CCND2* in cases ID12b and ID56. Fusion probes IGK-enh/*CCND3* and IGK-enh/*CCND2* were used, with *CCND3* and *CCND2* labeled in red and IGK-enh in green. Two red and two green signals are detected in normal cells, and yellow arrows highlight the insertion (one red and one small green signals juxtaposed. Magnification of cells with the rearrangement at the right side of each picture (enh: enhancer).

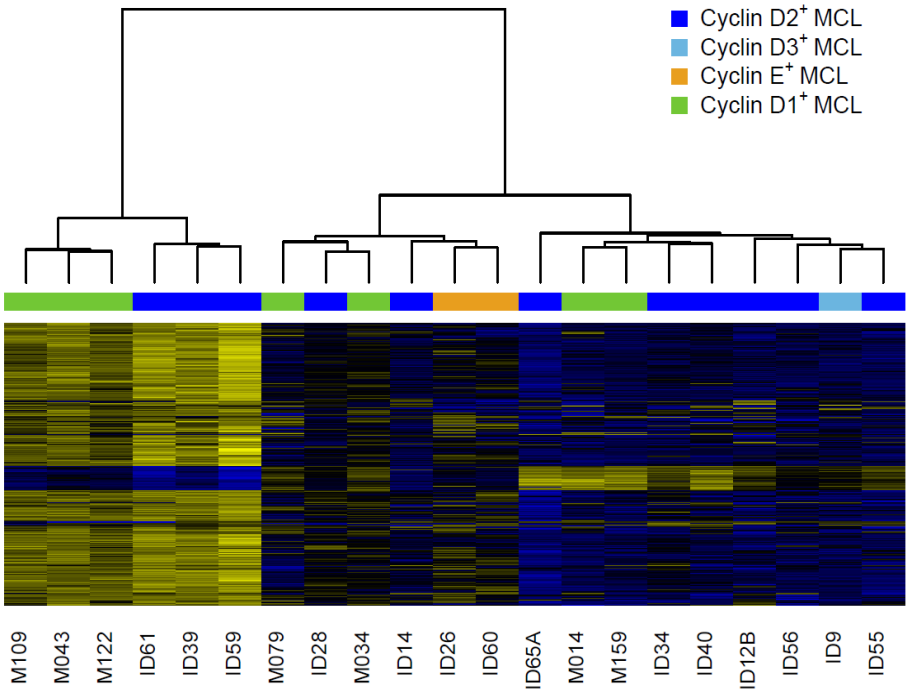


Supplemental Figure S3. *CCNE1* and *CCNE2* expression in cyclin D1$^-$ MCL, blastoid cyclin D1$^+$ MCL, DLBCL, and SMZL. (*SMZL1 corresponds to the case with high protein expression).

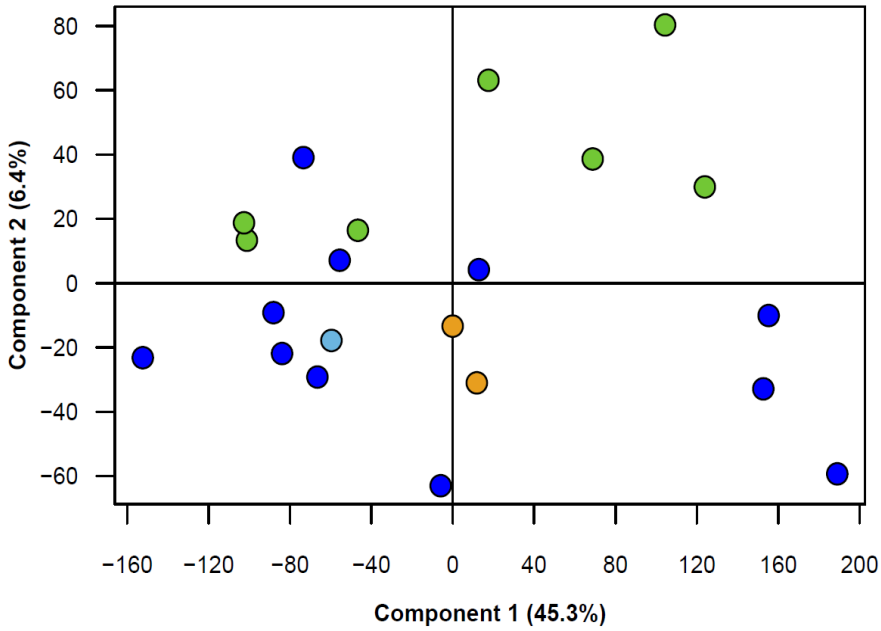


Supplemental Figure S4. Gene expression profiling analysis in cyclin D1$^-$ MCL. (**A**) Heatmap representation comparing cyclin D1$^-$ (cyclin D2$^+$, cyclin D3$^+$, or cyclin E$^+$) and conventional cyclin D1$^+$ MCL cases using GeneChip Human Genome U133 Plus 2.0 Arrays. Each column represents one case and each row one gene. The samples were randomly distributed along the generated clusters. (**B**) By principal component analysis (PCA) no differences were observed between cyclin D1$^-$ and cyclin D1$^+$ MCL cases. (**C**) Differentially expressed genes between cyclin D1$^+$ MCL and cyclin D1$^-$ MCL.

A



B



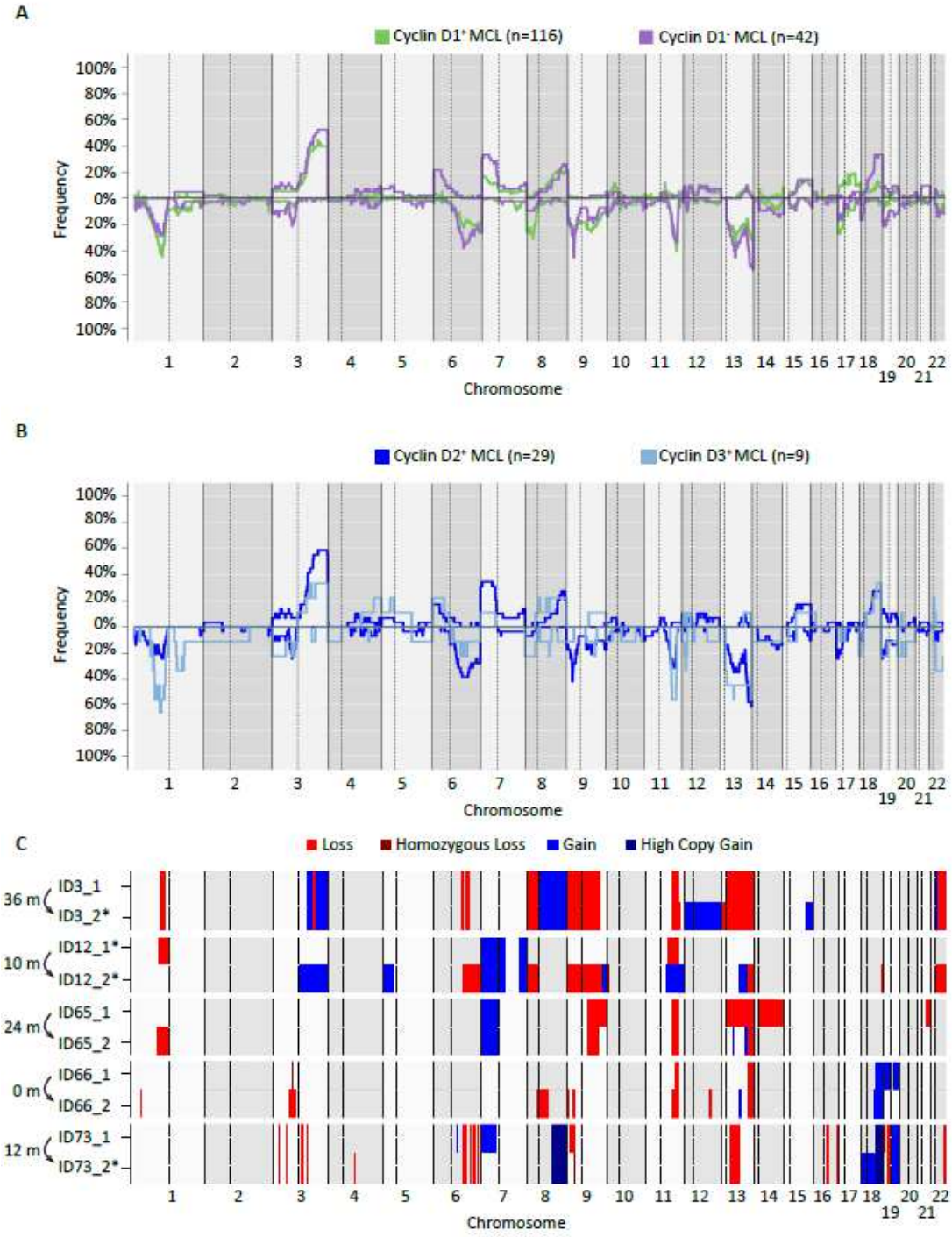
C

Genes differentially expressed between cyclin D1^{+} MCL versus cyclin D1^{-} MCL.

Probe	Gene	Order	log(FC)	t	*P*-value	Adjusted *P*-value
Top 20 genes						
208711_s_at	*CCND1*	1	4.363	15.112	<0.001	<0.001
222131_x_at	*RHOT2*	2	0.690	5.200	<0.001	0.244
214498_at	*ASIP*	3	0.602	5.152	<0.001	0.244
238097_at	*GAS6-AS1*	4	0.997	4.592	<0.001	0.715
211358_s_at	*CIZ1*	5	0.435	4.371	<0.001	0.801
220080_at	*FBXL8*	6	0.638	4.241	<0.001	0.801
227587_at	*KRI1*	7	0.680	4.232	<0.001	0.801
230664_at	*H2BFXP*	8	-0.922	-4.212	<0.001	0.801
221679_s_at	*ABHD6*	9	-0.960	-4.152	<0.001	0.801
225224_at	*NOL4L*	10	-0.712	-4.151	<0.001	0.801
213660_s_at	*TOP3B*	11	0.585	4.086	<0.001	0.801
226689_at	*CISD2*	12	-0.601	-4.065	<0.001	0.801
208461_at	*HIC1*	13	0.798	4.053	<0.001	0.801
218227_at	*NUBP2*	14	0.496	3.970	<0.001	0.801
228263_at	*GRASP*	15	0.480	3.969	<0.001	0.801
1552632_a_at	*ARSG*	16	-0.659	-3.947	<0.001	0.801
230633_at	*TMEM102*	17	0.461	3.928	<0.001	0.801
221704_s_at	*VPS37B*	18	0.876	3.917	<0.001	0.801
221992_at	*NPIPB15*	19	0.673	3.881	<0.001	0.801
218909_at	*RPS6KC1*	20	-0.645	-3.833	<0.001	0.801
Selected genes						
200951_s_at	*CCND2*	32	-2.541	-3.662	0.001	0.787
204914_s_at	*SOX11*	3486	-0.626	-1.378	0.182	0.999
211814_s_at	*CCNE2*	11919	-0.220	-0.537	0.597	0.999
201700_at	*CCND3*	16302	0.113	0.243	0.810	0.999
213523_at	*CCNE1*	17607	-0.024	-0.163	0.872	0.999

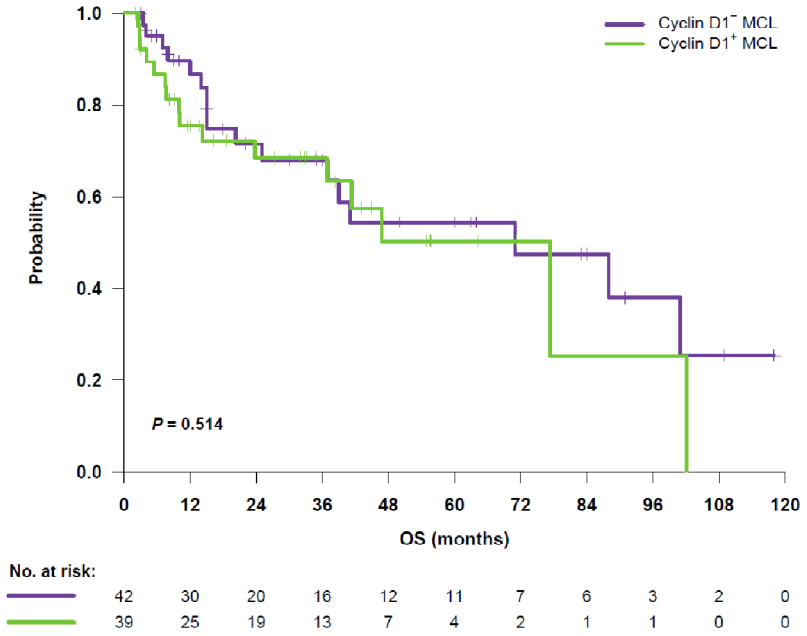
FC, fold-change.

Supplemental Figure S5. Copy number alterations in cyclin D1$^-$ MCL. Chromosomes are depicted on the X-axes and the frequency of altered cases is depicted on Y-axes; gains and losses are represented in the upper and lower parts, respectively. (**A**) Comparison of copy number alterations (CNA) between cyclin D1$^-$ and cyclin D1$^+$ MCL. (**B**) Comparison of CNA between cyclin D2$^+$ and cyclin D3$^+$ MCL. (**C**) Representation of CNA in 5 cases with 2 different samples (1 synchronous and 4 sequential). Chromosomes are depicted in columns and each row represents one sample. For each pair of samples the time interval is indicated in months and the asterisks highlight the post-treatment samples.

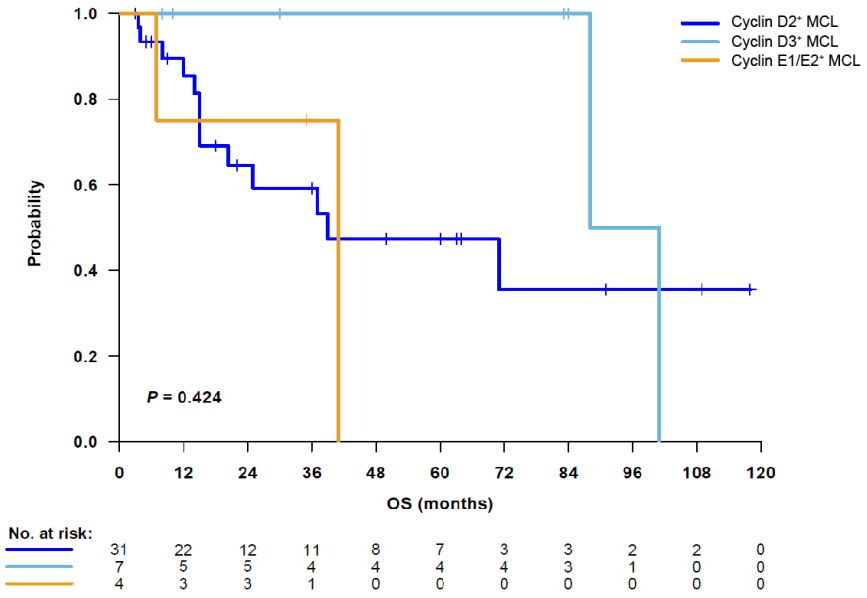


Supplemental Figure S6. Clinical outcome of cyclin D1$^-$ MCL patients. (A) Kaplan-Meier curve showing the similar overall survival (OS) of cyclin D1$^+$/SOX11$^+$ MCL and cyclin D1$^-$ MCL patients. **(B)** Kaplan-Meier curve comparing the OS of cyclin D2$^+$ MCL, cyclin D3$^+$ MCL, and cyclin E1$^+$/E2$^+$ MCL patients. The *P*-value was calculated using a Log-rank test.

A



B



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3. Sonoki T, Harder L, Horsman DE et al. Cyclin D3 is a target gene of t(6;14)(p21.1;q32.3) of mature B-cell malignancies. Blood 2001;98:2837-2844.