SUPPLEMENTARY APPENDIX

Highly sensitive and specific *in situ* hybridization assay for quantification of *SOX11* mRNA in mantle cell lymphoma reveals association of *TP53* mutations with negative and low *SOX11* expression

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Supplementary material
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Supplemental Material and Methods

RNAscope technology:

In brief, as pre-treatment, 2.5 μm FFPE tissue sections were dried in an incubator at 37C° overnight to ensure permeabilization of the cells. The RNAscope procedure was performed in the Ventana Discovery XT autostainer for open procedures using custom software (Ventana Medical Systems, USA) according to the protocol. The method is based on a specific probe hybridization to the target RNA and amplification of the signals. Evaluation was performed by light microscopy. Each dot visualized per cell corresponds to a single *SOX11* RNA target molecule.

RNA isolation and Real-time quantitative PCR (RT-qPCR)

RNA was transcribed into cDNA using Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA), a mix of Oligo(dT) primer (Promega, Madison, WI, USA) and random hexamers (Roche Applied Science, Penzberg, Germany) according to the manufacturer's instructions. *SOX11* (NM_003108.3) mRNA and the *TATA box-binding protein* (*TBP*) (NM_003194) mRNA as housekeeping gene were quantified by RT-qPCR using the TaqMan™Gene Expression Assay (FAM) (Hs00846583_s1, ThermoFisher Scientific) and the TaqMan™Gene Expression Assay (FAM) (Hs00427620_m1, ThermoFisher Scientific) respectively, the LightCycler 480 Probes Master and the LightCycler 480 System for detection (Roche Applied Science, Penzberg, Germany), was carried out in duplicates as previously described.[37] Data were analyzed using the $2^{-\Delta\Delta Cp}$ method and the mean of the SOX11 negative cases was defined as calibrator.

Next generation sequencing of *TP53* gene

The Ion AmpliSeq™ TP53 Panel covers all coding exons of *TP53*. The 24 amplicons were generated using two primer pools. Amplicon library preparation and semiconductor sequencing was done according to the manufacturers' manuals. In a primary step, the Ion AmpliSeq Library Kit v2.0 was applied to amplify target regions specified by the panel. Subsequently primer sequences were partially digested using the FuPA reagent. Each sample was marked by barcodes ligating Ion Xpress™ Barcode Adapters which include sequencing adapters. Libraries were purified and quantified applying Agencourt® AMPure XP (Beckman Coulter, Brea, CA, USA) magnetic beads and the Ion Library Quantitation Kit (Thermo Fisher Scientific) on the LightCycler 480 real-time PCR system (Roche Molecular Systems, Pleasanton, CA, USA). Libraries were diluted to 100 pM each and pooled. Afterwards the Ion Chef™ Instrument (Thermo Fisher Scientific) was used with the Ion 510 & Ion 520 & Ion 530 Kit-Chef (2 sequencing runs per initialization) (Thermo Fisher Scientific) to perform the template preparation and chip loading using the Ion 520 Chip Kit (Thermo Fisher Scientific). Finally sequencing was performed using the Ion GeneStudio™ S5 Prime System (Thermo Fisher Scientific).

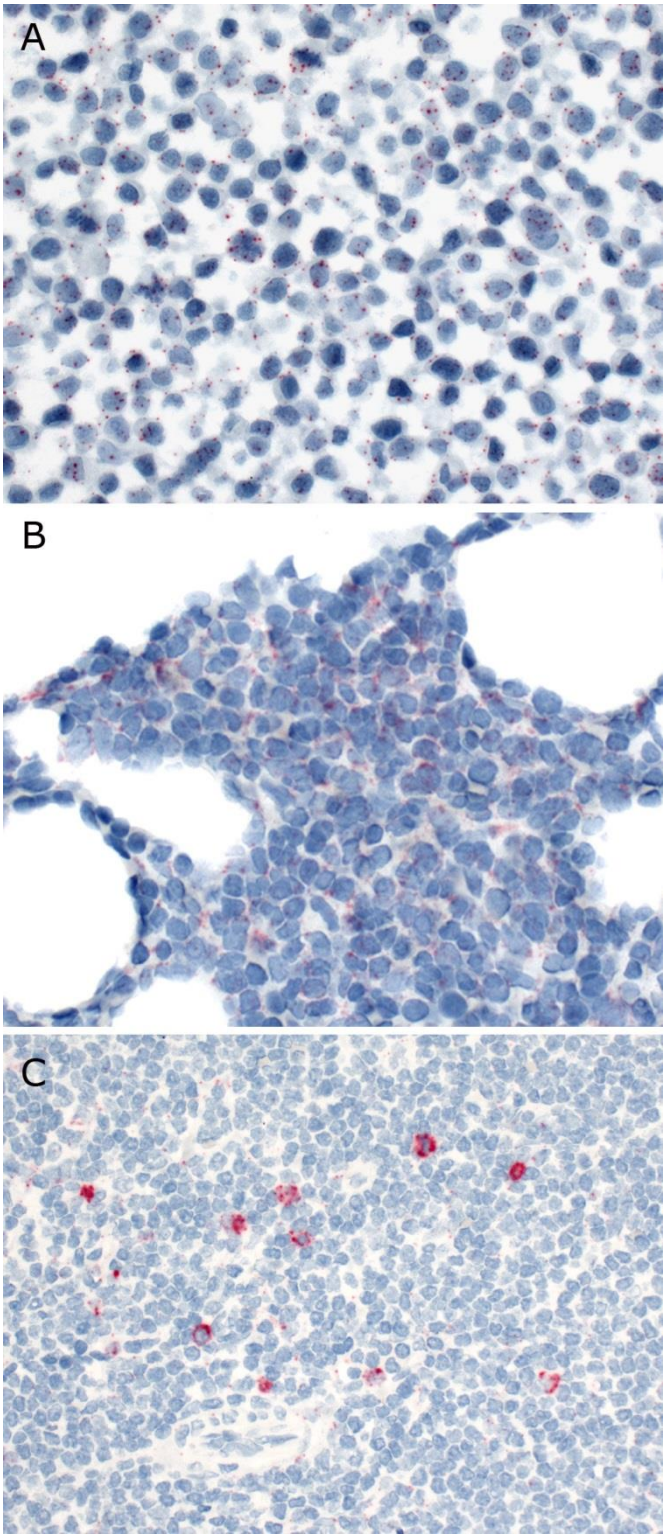
Detection of non-synonymous variants compared to the human reference sequence (hg19) was performed using the Torrent Suite™ and the Ion Torrent Variant Caller. Detection thresholds were set at an allele frequency of 5%. Variants were annotated and filtered against the dbSNP and COSMIC databases using the Annotate variants single sample workflow of the Ion Reporter Software (Thermo Fisher Scientific). Obtained variants were further visualized with the freely available program Integrative Genomics Viewer (IGV, Broad Institute) to discriminate artefacts and true variants. Prediction of the deleterious effect of the variants was done using the CADD (Combined Annotation Dependent Depletion) (http://cadd.gs.washington.edu/home) predictor.

Supplemental Table 1. Sox11, cyclin D1 and *TP53* status in MCL cases

#	Diagnosis	SOX11 IHC	Cyclin D1 IHC	RNA scope	TP53 IHC	TP53 SEQ
1	cMCL - classic	*pos*	*pos*	4	*Neg, 5%*	-
2	cMCL - classic	*pos*	*pos*	3	*Neg, 3%*	-
3	cMCL - classic	*pos*	*pos*	4	*Neg, 10%*	*WT*
4	cMCL - blastoid	*pos*	*pos*	4	*Neg, 3%*	*WT*
5	cMCL - classic	*pos*	*pos*	2	*Neg, 5%*	*WT*
6	cMCL - classic	*low**	*pos*	1	*Pos, 60%*	*MUT*
7	cMCL - classic	*pos*	*pos*	4	*Neg, 15%*	*WT*
8	cMCL - classic	*pos*	*pos*	3	#	*WT*
9	nnMCL	*low**	*pos*	1	*Pos, 90%*	*MUT*
10	cMCL - classic	*pos*	*neg*	4	*Neg, 5%*	*WT*
11	cMCL - blastoid	*pos*	*neg*	4	*Neg, 1%*	*WT*
12	cMCL - classic	*pos*	*neg*	2	-	-
13	cMCL - classic	*pos*	*neg*	3	-	-
14	nnMCL	*neg*	*pos*	0	-	-
15	nnMCL	*neg*	*pos*	0	*Pos, 80%*	*MUT*
16	nnMCL	*neg*	*pos*	0	*Pos, 90%*	*MUT*
17	cMCL - classic	*pos*	*pos*	3	*Neg, 10%*	*WT*
18	cMCL - blastoid	*pos*	*pos*	2	*Neg, 10%*	*WT*
19	cMCL - blastoid	*pos*	*pos*	3	*Neg, 5%*	*WT*
20	cMCL - classic	*pos*	*pos*	3	*Neg, 20%*	-
21	cMCL - blastoid	*pos*	*pos*	2	#	*WT*
22	cMCL - classic	*pos*	*pos*	2	*Neg, 5%*	*WT*
23	nnMCL - blastoid	*neg*	*pos*	0	*Neg, 3%*	*MUT*
24	cMCL - classic	*pos*	*pos*	3	*Neg, 10%*	-
25	cMCL - blastoid	*pos*	*pos*	3	*Pos, 70%*	*MUT*
26	cMCL - classic	*pos*	*pos*	3	*Neg,10%*	*WT*
27	cMCL - classic	*pos*	*pos*	4	*Neg, 3%*	-
28	cMCL - classic	*pos*	*pos*	3	*Neg, 15%*	-
29	cMCL - classic	*low**	*pos*	1	*Neg, 3%*	-
30	cMCL - classic	*pos*	*pos*	1	*Neg, 5%*	-
31	cMCL - classic	*pos*	*pos*	NI	*Neg, 4%*	-
32	nnMCL	*neg*	*pos*	0	*Neg, 5%*	-
33	cMCL - classic	*pos*	*pos*	2	*Neg, 3%*	-
34	pleura effusion - blastoid	*low**	*pos*	1	*Pos, 30%*	*MUT*

35	nnMCL	*neg*	*pos*	0	*Pos, 90%*	*MUT*
36	cMCL - classic	*pos*	*pos*	4	*#*	*WT*
37	cMCL - blastoid	*pos*	*neg*	4	*Pos, 80%*	*MUT*
38	cMCL - blastoid	*pos*	*pos*	4	*Neg, 10%*	*WT*
39	cMCL - classic	*pos*	*pos*	2	*#*	*WT*
40	cMCL - classic	*pos*	*pos*	4	*Neg, 5%*	*WT*
41	cMCL - blastoid	*pos*	*pos*	4	*Neg, 3%*	*WT*
42	cMCL - classic	*pos*	*pos*	4	*Neg, 3%*	*MUT*
43	cMCL - classic	*pos*	*pos*	NI	*Neg, 2%*	-
44	cMCL - classic	*pos*	*pos*	4	*#*	*WT*
45	cMCL - classic	*pos*	*pos*	2	*Neg, 15%*	
46	cMCL - classic	*pos*	*pos*	4	*Neg, 5%*	*WT*
47	cMCL - classic	*pos*	*pos*	3	*Neg, 3%*	*WT*
48	cMCL - classic	*pos*	*pos*	4	*Neg, 10%*	-
49	cMCL - classic	*pos*	*pos*	2	*Neg, 1%*	-
50	cMCL - classic	*pos*	*pos*	4	*Neg, 3%*	-
51	cMCL - classic	*pos*	*pos*	1	*Neg, 10%*	-
52	cMCL - classic	*pos*	*pos*	3	*Neg, 1%*	-
53	cMCL - classic	*pos*	*pos*	3	*Neg, 5%*	-
54	nnMCL	*neg*	*pos*	0	*Neg, 10%*	-
55	cMCL - classic	*pos*	*pos*	2	*Neg, 5%*	-
56	cMCL - classic	*pos*	*pos*	4	*Neg, 15%*	*WT*
57	cMCL - classic	*pos*	*pos*	2	*Neg, 5%*	-
58	cMCL - blastoid	*low**	*pos*	1	*Pos, 90%*	*MUT*
59	cMCL - classic	*pos*	*pos*	4	*Neg, 10%*	-
60	cMCL - classic	*pos*	*neg*	4	*Neg*, 15%	-
61	cMCL - classic	*pos*	*pos*	4	*Neg, 10%*	-
62	cMCL - classic	*pos*	*pos*	4	*Neg, 5%*	-
63	cMCL - classic	*pos*	*pos*	2	*Neg, 15%*	*WT*
64	cMCL - classic	*low**	*pos*	1	*Pos, 80%*	*MUT*
65	cMCL - classic	*low**	*pos*	NI	*Neg, 5%*	*WT*
66	cMCL - blastoid	*pos*	*pos*	3	*Pos, 90%*	*MUT*

cMCL conventional mantle cell lymphoma; nnMCL non-nodal leukemic mantle cell lymphoma
NI – not informative; WT – wild type; MUT – mutated; * low <10%
weak heterogenous positive in >20% considered equivocal



Supplemental Figure 1

RNAscope analysis with Peptidylpropyl isomerase B (PPIB) as housekeeping gene for measuring the quality of the assay. **A** HeLa cell-line shows crisp signals in most of the cells. **B** A bone marrow with heterogenous but crisp signals in the tumor cells in a case of nnMCL. **C** A lymph node with MCL infiltration (Case #16) shows circular clustering of dots representing the proper function of the assay.