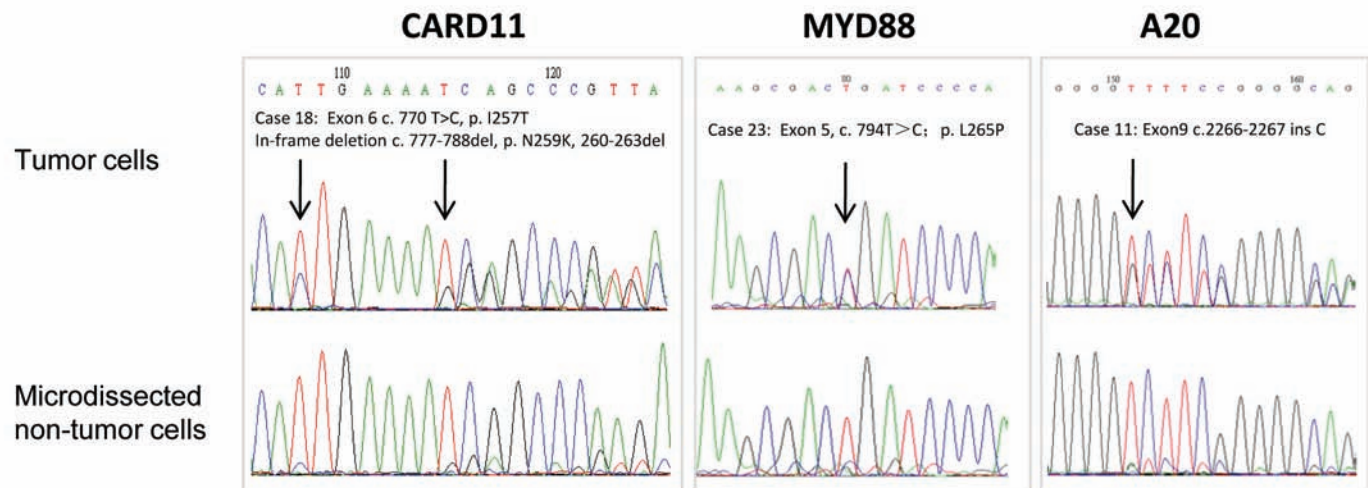


BCR and TLR signaling pathways are recurrently targeted by genetic changes in splenic marginal zone lymphomas

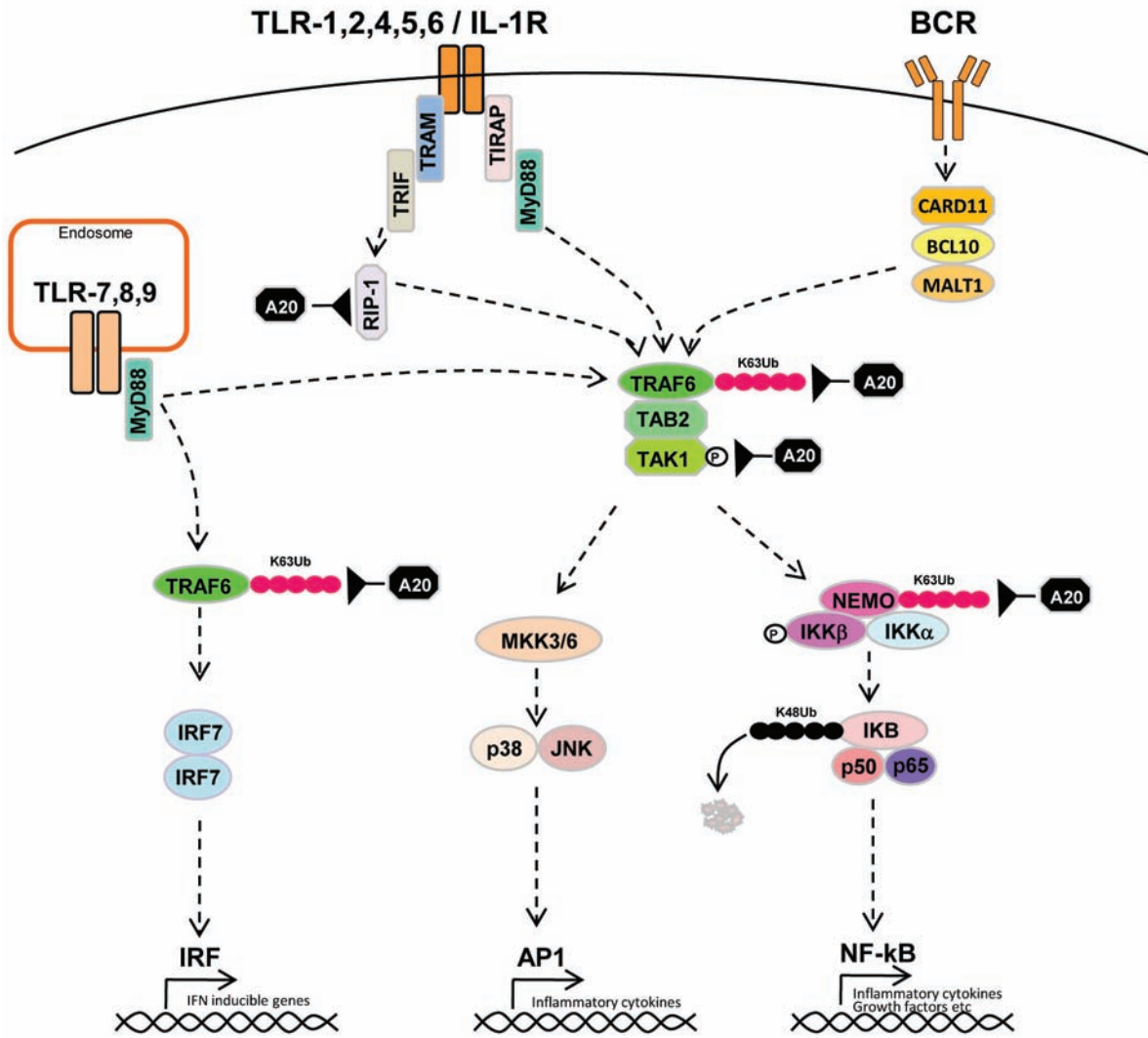
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Online Supplementary Figure S1. Examples of CARD11, MYD88 and A20 mutation seen in SMZL.



Online Supplementary Figure S2. *CARD11*, *MYD88* and *A20* in BCR and TLR signaling pathways. *CARD11* links the BCR signaling to the canonical NF-κB activation pathway. *MYD88* is a universal adaptor and couples TLR/IL-R signaling to the NF-κB, AP1 and IRF activation pathway. *A20*, an ubiquitinmodification enzyme, inactivates RIP-1, TRAF6, TAK1 and NEMO, important signaling molecules mediating NF-κB, AP1 and IRF activation.

Online Supplementary Table S1. Primers and PCR conditions used for amplification of the CARD11, MYD88, CD79A, CD79B and A20 and ABIN-1 coding exons.

Genes	Exon	Primer name	Sequence (5'-3')	Amplicon size (bp)	PCR annealing temperature (°C)	PCR condition
CARD11	E5-1	Forward	GTGCCCCCTCCACAGT	200	62	PCR was carried out in a 10µl reaction mixture with 5-10ng template DNA and AmpliTaq Gold 360 (Applied Biosystem) master mix plus GC-enhancer according to the manufacturer's instructions. The PCR conditions were 95°C for 10 min to activate the enzyme, followed by 40 cycles of denaturation at 95°C for 20 sec, annealing at 58-62°C (depending on the primer set) for 20 sec, and extension at 72°C for 30-45 sec (depending on the amplicon size).
		Reverse	AGTACCGCTCCTGGAAGGTT			
	E5-2	Forward	GAAGAAGCAGATGACGCTGA	235	62	
		Reverse	GTCACCCTGGCGGAGTAG			
	E6	Forward	CACCTTGGGGTATTTTCAGA	210	59	
		Reverse	CAGGCCCTCACCTGGATG			
	E7	Forward	CCTGACCCTCTGAAACCTCCT	204	62	
		Reverse	GCGATCCCCACTCCAC			
	E8	Forward	TCGATGCGCATATTGATTTTC	181	62	
		Reverse	CTGCAGGTGGTGCCTGTA			
	E9	Forward	CCCAAAGCAGCCTTCGTC	234	62	
		Reverse	CCTGGTCCAGTTGTTGCTGTCC			
MYD88	E1-1	Forward	CTCGGGGCTCCAGATTGTA	327	58	
		Reverse	GCCGGATCTCCAAGTACTCA			
	E1-2	Forward	GCTGCTCTCAACATGCGAGT	317	62	
		Reverse	GGAAAGTCAGCCTCCTCACC			
	E2	Forward	CTGGATCCTGACTGTGGGTAA	281	62	
		Reverse	GCTTCAAACCCATGCTCT			
	E3	Forward	TCTGACCACCACCCTTGTG	264	62	
		Reverse	CAGGGCAGGGCTTCATGC			
	E4	Forward	GGCCCTCCTGAAGCTATTC	270	62	
		Reverse	TGGTACTGCATCCACAGTCC			
E5	Forward	GTTGAAGACTGGGCTTGTC	292	59		
	Reverse	AGGAGGCAGGGCAGAAGTA				
CD79A	E5	Forward	ATGAAGTGAAGGGTGGG	326	58	
		Reverse	AGAATGTCCCAGGGAAGTGAG			
CD79B	E5	Forward	TAGGTGGCTGTCTGGTCAATG	306	58	
		Reverse	TGTTCTTGAGAATGCACCTC			
	E6	Forward	CTGGAGACAAATGGCAGCTC	362	58	
		Reverse	CACCTACGAGGTAAGGAGAGGG			
A20	E2-1	Forward	CTGCAGGCAGCTATAGAGGAG	272	58	
		Reverse	CGAAACTGAGGACAAAACCTGG			
	E2-2	Forward	GCAATATGCGGAAAGCTGTG	300	58	
		Reverse	GCTATCACCCAGGCAAAAGA			
	E3	Forward	TTGCTGGGTCTTACATGCAG	271	58	
		Reverse	TTAGGGGGAAAAACCTACCC			
	E4	Forward	GGGAGTACAGGATACATTCAAGC	251	58	
		Reverse	AAGGCATAAGGCTGAAAGCA			
	E5	Forward	ACCTAAGGGCCTCATTTTCC	275	58	
		Reverse	GCAAAAAGGAAAACCTGATG			
	E6	Forward	TGAGATCTACTTACCTATGGCCTTG	315	58	
		Reverse	TCAGGTGGCTGAGGTTAAAGA			
	E7-1	Forward	ACAGGCCTGCATTTTCAGTG	282	58	
		Reverse	GGAAGGTTCCATGGGATTC			
E7-2	Forward	GCAGGAAAACAGCGAGCA	272	58		
	Reverse	CCAAGGGCTCATAGGCTTCT				
E7-3	Forward	ACTCCAAAGCTGAACTCCA	304	58		

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		Reverse	GGGATCCAAGTGCCTTGT		
	E7-4	Forward	ACTGCCATGAAGTGCAGGAG	279	58
		Reverse	ATCTGACTTGGAACGCTGGT		
	E7-5	Forward	TGCAGTACTTGCTTCAAAAGGA	313	58
		Reverse	CCACTTCACTCACGTTTGT		
	E8	Forward	GGGGTGACCCTATGTGGTACT	293	58
		Reverse	CCAGTTGCTCTTCTGTCCTTTT		
	E9-1	Forward	GTGCTCTCCCTAAGAAATGTGAG	205	58
		Reverse	CTGGTTGGGATGCTGACACT		
	E9-2	Forward	CTCTGCATGGAGTGCAGCAT	257	58
		Reverse	GGGTTTCAGAGGATAGCACCA		
ABIN-1	E2	Forward	GGCATTGACAGACTAGAGCTTC	315	58
		Reverse	GACTGGCATCACAGTCTGC		
	E3	Forward	GGGAGAAGCAGCACACTG	272	58
		Reverse	GAAGGGAGTTCAGTGAAGG		
	E4	Forward	CCTGTCTGAGAACCCTTTTG	280	58
		Reverse	GACCTCAACCCTCTTCTTC		
	E5	Forward	GCTTTGTGATCTCATGTGAGATG	327	58
		Reverse	GGGATCTGAACAGGTCTGTG		
	E6	Forward	GAGGAAGCAGAGGGAGAATG	346	58
		Reverse	GAGCAGGAAGGTGGGAAG		
	E7	Forward	CCCACCATCCTCTAGCTCAG	252	58
		Reverse	CCAGAAATCAGTGTGCAC		
	E8	Forward	CAACTCATGCGATAGTGAGC	332	58
		Reverse	GAACCTTCTACCACTGGCAC		
	E9	Forward	GGTCTTAGAGGAGCACCGAG	236	58
		Reverse	AGCCTTTGTGCTGCTGGT		
	E10	Forward	TAGCCACTATTACACACACC	265	58
		Reverse	CCCAAGGTCAAAGCTG		
	E11	Forward	CACATCCTGCAAGTGTCTAC	290	58
		Reverse	GCTTGTTGCTCCACAGAAC		
	E12	Forward	CCAGGCAGGACAGAGAATC	302	58
		Reverse	CCTGAGTCACTCCAGTGTG		
	E13	Forward	GAAGGAGTCCCTGAGGATG	273	58
		Reverse	GAAAGCTCCAGTCCCACAG		
	E14	Forward	AGGAGGCATGGGAGTCTG	251	58
		Reverse	GAGGACAGGCCAGTTGC		
	E15	Forward	CCAGAGGGAAGCATCAG	259	58
		Reverse	CACACACTGTGCATCCATC		
E16	Forward	AGAGGGTGATGAGATGGGT	328	58	
	Reverse	CTCTCTGGAAGGTGTCTGG			
E17	Forward	TTGTCTGGCACAGTAGGTG	238	58	
	Reverse	AGGCAATGCTGGCAGATAAG			
E18	Forward	ACTGTTCTGCACTGCATTC	272	58	
	Reverse	TCAGGGACTGGGTACAAGC			

Online Supplementary Table S2. Summary of mutations found in SMZL.

Case reference	CARD11 mutation	MYD88 mutation	A20 mutation
C08	n/a	n/a	Exon7 c.1246-1250 del AACAA
C09	No	No	Exon4 c.553G>T, p.G185X (somatic origin confirmed)
C10	No	No	Exon9 c.2209C>T, p. Q737X
C11	No	No	Exon9 c.2266-2267insC (somatic origin confirmed)
C17	Exon6 c.746A>C, p.Q249P	No	No
C18	Exon6,c. 770 T>C, p. I257T;In-frame deletion c. Δ12bp(777-788del), p. N259K,260-263del; (both mutations occurred on the same allele and somatic origin confirmed)	No	No
C19	Exon5 c.572A>G, p. N191S	Exon5 c.794T>C, p.L265P	No
C20	No	Exon5 c.794T>C, p.L265P (somatic origin confirmed)	No
C21	No	Exon5 c.794T>C, p.L265P	No
C22	No	Exon5 c.794T>C, p.L265P	No
C23	n/a	Exon5 c.794T>C, p.L265P (somatic origin confirmed)	n/a
C24	No	Exon5 c.794T>C, p.L265P	No
C28	No	No	Exon3 c.400-401 del GA (somatic origin confirmed)
C29	n/a	n/a	Exon6 c.872 del T

n/a: not applicable.

Online Supplementary Table S3. Univariate analysis for prognosis by the Kaplan-Meier method.

Factor		OS			EFS		
		2 yrs	5 yrs	P =	2 yrs	5 yrs	P =
Age	<60 (n= 7)	1	1	0.210	1	1	0.117
	>=60 (n=17)	0.941	0.739		0.729	0.574	
Stage	<IV (n=1)	1	1	0.675	1	1	0.517
	IV (n= 24)	0.958	0.834		0.781	0.558	
Arcaini prognostic index	Low (n=4)	1	1	0.204	0.667	0.333	0.510
	Moderate (n=16)	1	0.923		0.909	0.701	
	High (n=7)	0.857	0.643		0.686	0.686	
Lymphocytosis	Yes (n=18)	0.944	0.850	0.767	0.791	0.562	0.800
	No (n= 10)	1	0.889		0.875	0.750	
Villous lymphocytes	Yes (n=15)	1	1	0.121	0.917	0.625	0.641
	No (n= 9)	1	0.800		0.833	0.833	
7q deletion	Yes (n=10)	1	0.857	0.689	0.714	0.714	0.200
	No (n=19)	0.947	0.806		0.821	0.559	
TP53 status	Abnormal (n=5)	1	0.800	0.954	0.800	0.800	0.759
	Normal (n=24)	0.958	0.834		0.771	0.551	
A20 mutation	Yes (n=1)	1	1	0.686	1	1	0.541
	No (n=24)	0.958	0.846		0.797	0.607	
CARD11 mutation	Yes (n=3)	1	1	0.571	1	1	0.471
	No (n=18)	0.944	0.872		0.807	0.588	
MYD88 mutation	Yes (n=5)	1	0.800	0.713	0.8	0.533	0.514
	No (n=20)	0.950	0.871		0.793	0.634	
A20 or CARD11 or MYD88 mutation	Yes (n=7)	1	1	0.281	1	0.667	0.675
	No (n=14)	0.929	0.825		0.734	0.554	

2 yrs: 2-year cumulative probability of survival

5 yrs: 5-year cumulative probability of survival

P value by log rank (Mantel-Cox)