

Different primary thyroid B-cell lymphomas show overlapping mutation profiles, suggesting involvement of a common pathogenic process

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SUPPLEMENTARY MATERIALS

SUPPLEMENTARY METHODS

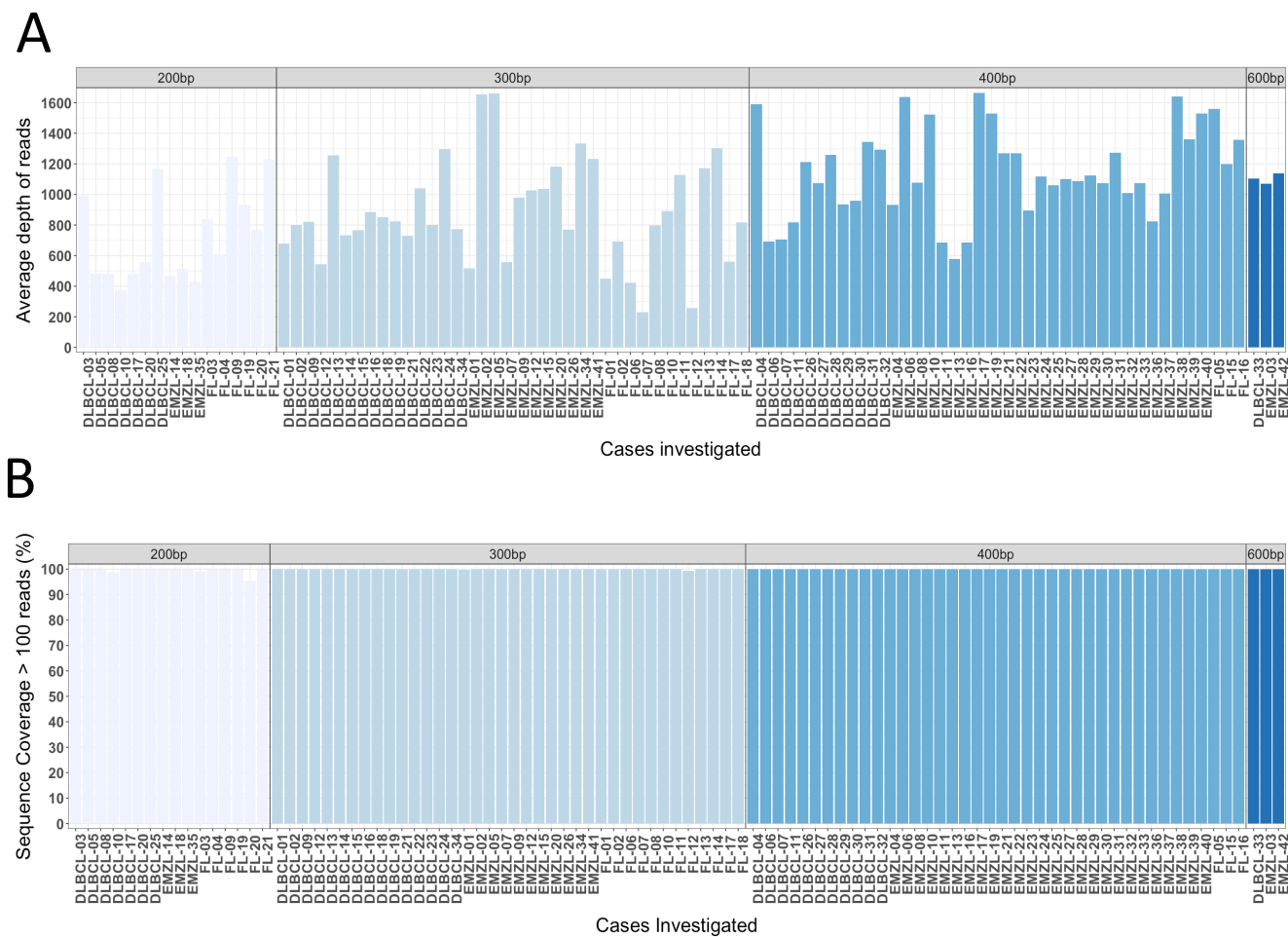
Detection of *BCL6* rearrangements by capture-based sequencing

Two cases (FL-09 and FL-16) were analysed using a bespoke capture panel designed to target genomic regions encompassing and flanking *BCL6* and the immunoglobulin loci (*IGH*, *IGK* and *IGL*), enabling detection of *BCL6* rearrangements (Supplementary Table 4).

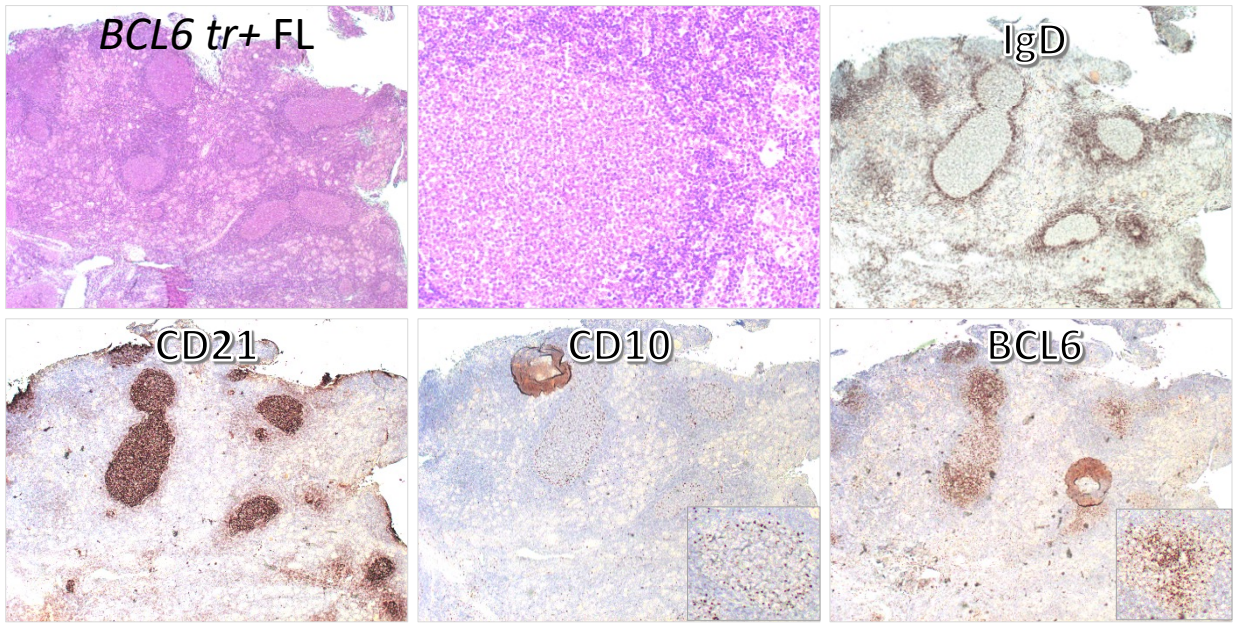
Library preparation and target enrichment were performed similarly as described in the targeted next-generation sequencing section for mutation analysis. Briefly, 100 ng of FFPE-derived DNA was fragmented by Covaris ultrasonication, indexed using xGen™ UDI-UMI adapters (IDT), and enriched using the custom translocation capture probes. Enriched libraries were PCR-amplified and sequenced using 2 × 150 bp paired-end reads on an Illumina Novaseq X 25B platform. Read alignment and processing were performed using the same bioinformatic pipeline applied to the mutation panel.

Chromosomal translocations were identified using a combination of three structural variant callers: GRIDDS¹, Manta² and Delly³. Only on-target translocations supported by more than five split reads and assigned a “PASS” quality status were retained for downstream analysis. All candidate translocations were subsequently reviewed manually using the Integrative Genomics Viewer (IGV)⁴.

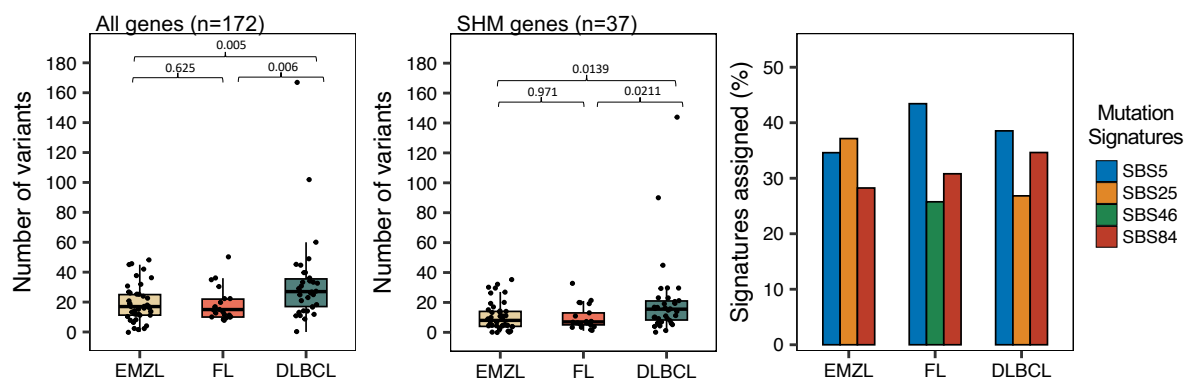
SUPPLEMENTARY FIGURES



Supplementary Figure S1. Sequencing coverage of targeted NGS. DNA quality as measured by PCR of variable-sized genomic fragments is indicated on top of each panel. All cases with DNA quality only amenable for PCR amplification up to 300bp were investigated in duplicates.



Supplementary Figure S2. Histopathology of a primary thyroid FL with *BCL6* translocation . The case (FL-11) displays follicular growth pattern with little involvement of the interfollicular region. The neoplastic B cells are CD10 negative but express BCL6.



Supplementary Figure S3. Comparison of mutation load and signature among thyroid EMZL, FL and DLBCL. For mutation load analysis, all somatic variants (synonymous, non-synonymous, indels and changes in UTRs) are included, while for mutation signature analysis, only somatic single nucleotide changes are used. SHM: somatic hypermutation; SBS: single base substitutions.



Supplementary Figure S4. Heatmap presentation of all potentially pathogenic mutations identified in all three lymphoma entities. The mutated genes are grouped according to their association with lymphoma entity and ordered according to frequency. * and ** denote known and predicted somatic hypermutation targets respectively. FL: follicular lymphoma; DLBCL: diffuse large B-cell lymphoma; EMZL: extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue; tr+ve: translocation positive, tr-ve: translocation negative; SNV: single nucleotide variation; SHM: somatic hypermutation.

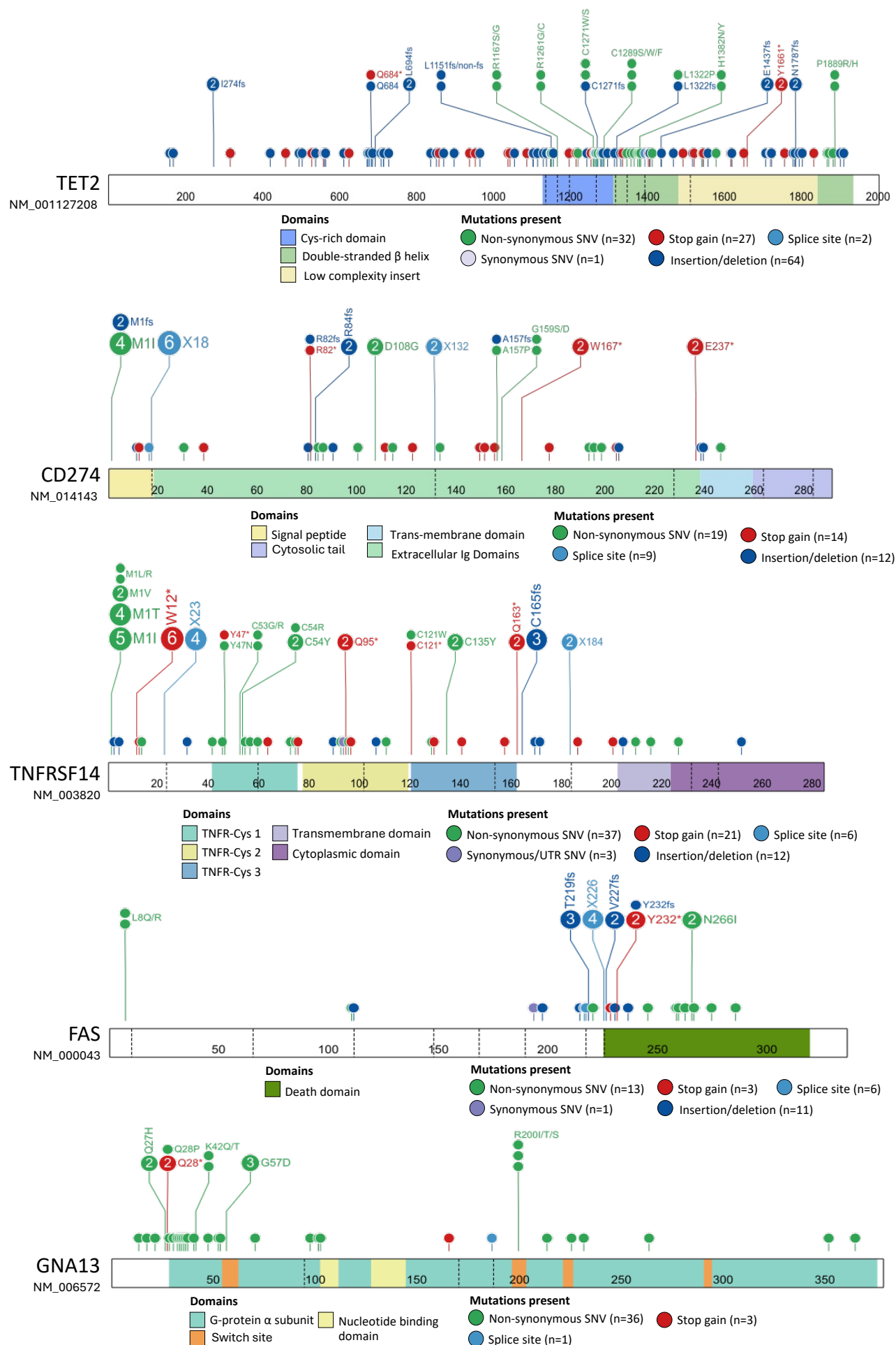
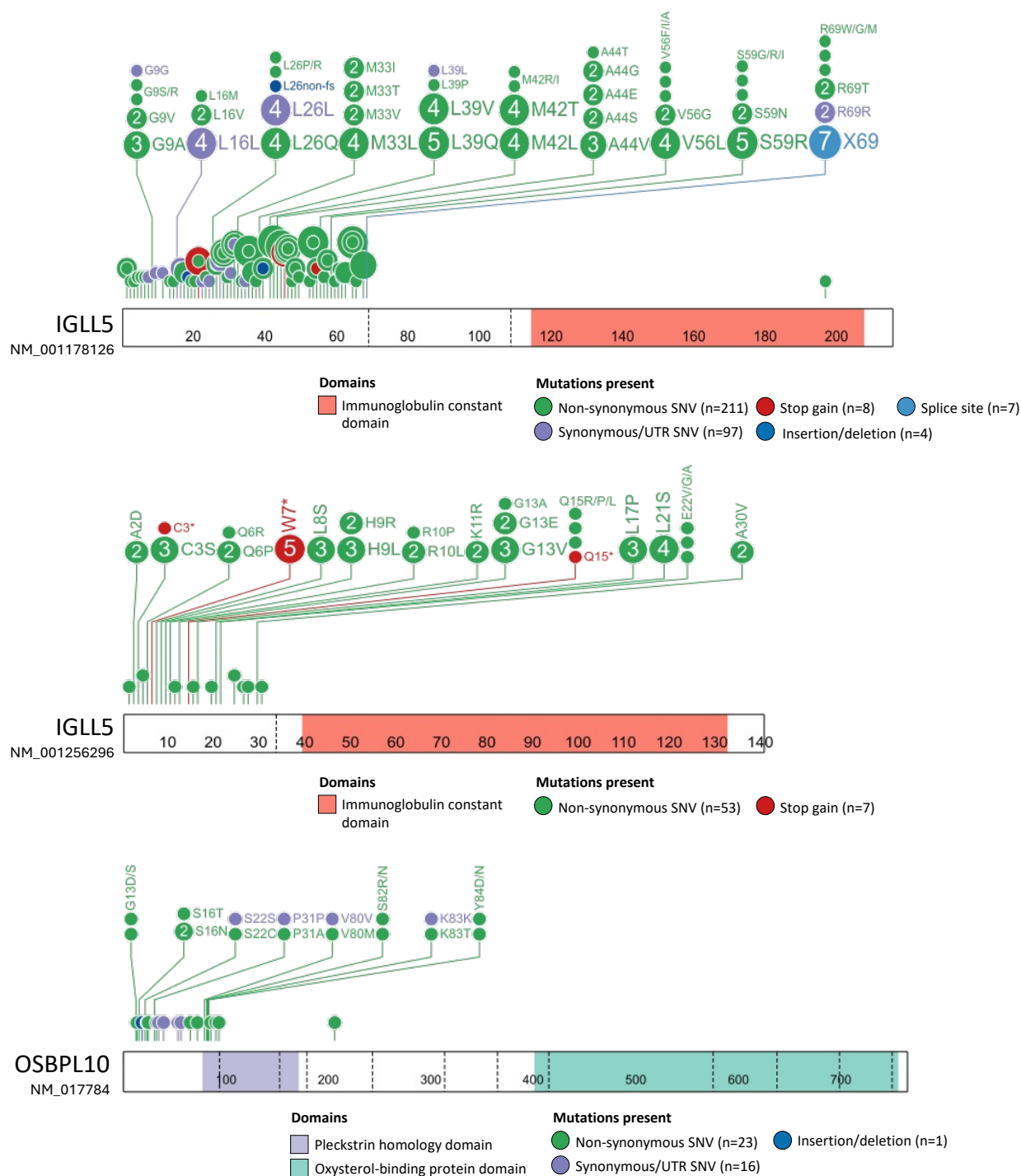
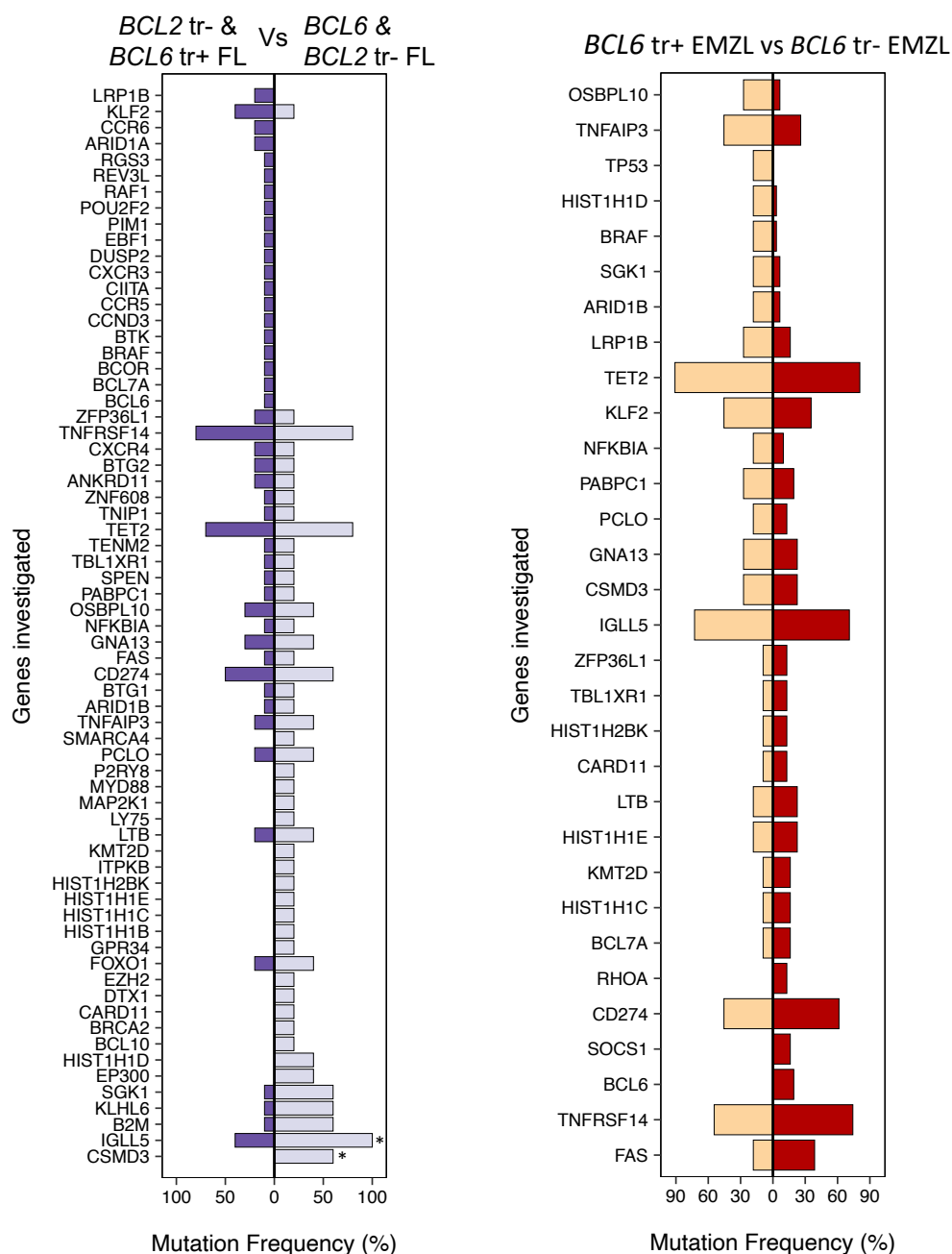


Figure S5: continue to the next page

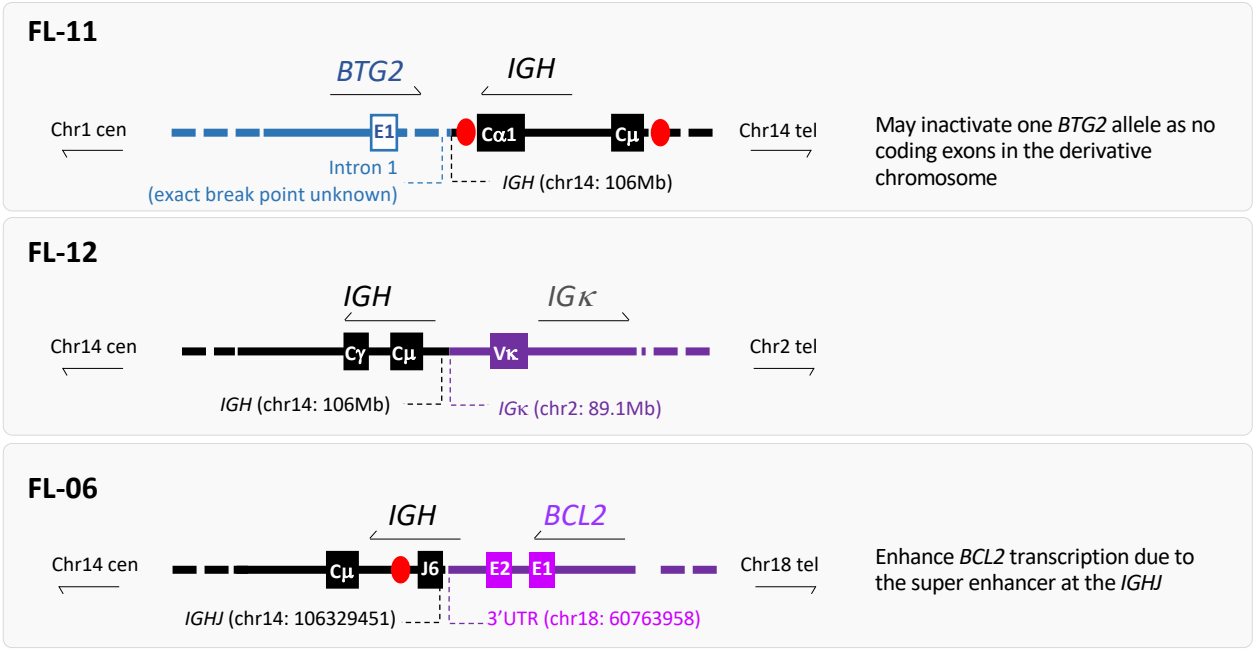


Supplementary Figure S5. Characteristics and distribution of mutations identified in genes frequently mutated in thyroid EMZL, DLBCL and *BCL2* translocation negative FL.

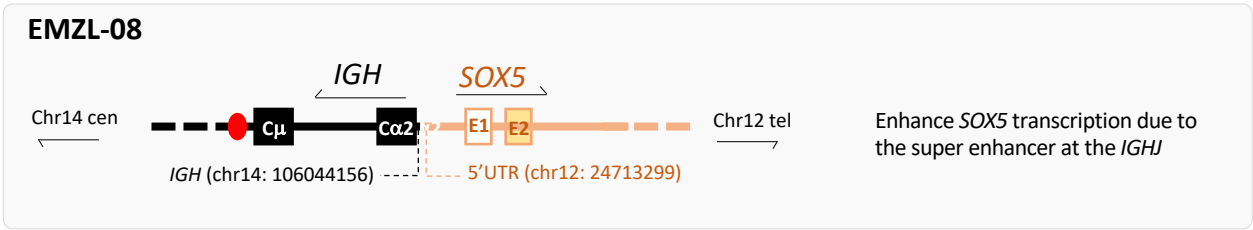


Supplementary Figure S6. Comparison of mutation frequency between subsets of thyroid EMZL and FL as indicated. FL: follicular lymphoma; EMZL: extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue; tr+ve: translocation positive, tr-ve: translocation negative. * denotes significant changes between subsets.

Thyroid FL



Thyroid EMZL



Supplementary Figure S7. Additional translocations detected in *BCL6* translocation positive thyroid FL and EMZL by targeted locus capture-based next generation sequencing (TLC-NGS). The sequence annotations are based on human genome (hg19) together with *IGH* super enhancers according to Mikulasova et al ⁵. FL: follicular lymphoma; EMZL: extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue; cen: centromeric; tel: telomeric.

SUPPLEMENTARY TABLES

Supplementary Table S1. Antibodies and DNA probes used for immunohistochemistry and FISH respectively.

Antibodies used for Immunohistochemistry	Antibody type	Clone name	Source
Primary antibodies			
CD10	Mouse Monoclonal	56C6	Leica Microsystems Ltd., Newcastle-upon-Tyne, U.K.
CD21	Mouse Monoclonal	2G9	Leica Microsystems Ltd., Newcastle-upon-Tyne, U.K.
BCL2	Rabbit Monoclonal	E17	Abcam, Cambridge, U.K.
BCL6	Mouse Monoclonal	LN22	Leica Microsystems Ltd., Newcastle-upon-Tyne, U.K.
STMN1	Rabbit Monoclonal	SP49	Sigma-Aldrich, St. Louis, Missouri, U.S.
GCET1	Mouse Monoclonal	RAM341	Abcam Limited, Cambridge, U.K.
HGAL	Rabbit Monoclonal	EPR14333	Abcam Limited, Cambridge, U.K.
LMO2	Rabbit Monoclonal	SP51	Sigma-Aldrich, St. Louis, Missouri, U.S.
Secondary antibodies			
HRP-conjugated secondary polymer		DS9800	Leica Microsystems Ltd., Newcastle-upon-Tyne, UK
Probes for FISH			
<i>BCL6</i> Dual Colour Break Apart FISH Probe	Cat no.	Source	
	01N23-020	Vysis	
<i>BCL2</i> Dual Colour Break Apart FISH Probe	05N51-020	Vysis	

Supplementary Table S2. Clinical and laboratory features of thyroid lymphoma cohort.

			Thyroid FL				Thyroid EMZL		Thyroid DLBCL
			<i>BCL2</i> translocation positive	<i>BCL6</i> translocation positive	<i>BCL2 /BCL6</i> translocation positive	<i>BCL2 /BCL6</i> translocation negative	<i>BCL6</i> translocation positive	<i>BCL6</i> translocation negative**	
Generic clinical data	Age	<60	3 (5)	1 (10)	0 (1)	1 (5)	3 (11)	3 (30)	5 (34)
		≥60, <70	1 (5)	1 (10)	1 (1)	1 (5)	5 (11)	8 (30)	11 (34)
		≥70, <80	1 (5)	4 (10)	0 (1)	3 (5)	2 (11)	15 (30)	11 (34)
		≥80	0 (5)	4 (10)	0 (1)	0 (5)	1 (11)	4 (30)	7 (34)
	Sex	male	0 (5)	2 (10)	0 (1)	1 (5)	0 (11)	27 (30)	7 (34)
		female	5 (5)	8 (10)	1 (1)	4 (5)	11 (11)	3 (30)	27 (34)
	Stage***	IE	2 (5)	8 (10)	0 (1)	2 (4)	8 (11)	18 (25)	6 (33)
		IIE	2 (5)	2 (10)	0 (1)	2 (4)	2 (11)	6 (25)	23 (33)
		IIIE	1 (5)	0 (10)	1 (1)	0 (4)	0 (11)	1 (25)	2 (33)
		IVE	0 (5)	0 (10)	0 (1)	0 (4)	1 (11)	0 (25)	2 (33)
	Hashimoto's thyroiditis	Yes	5 (5)	7 (10)	0 (1)	5 (5)	9 (11)	29 (29)	28 (34)
		No	0 (5)	3 (10)	1 (1)	0 (5)	2 (11)	0 (29)	6 (34)
	Average duration of HT to lymphoma (range), years	All cases [range] [median]	2.3 [0.02-9.9] [0.17] (5)	1.4 [0.03-8.5] [0.15] (7)	NA	8.1 [0.03-30.5] [0.09] (5)	1.39 [0.05-5.21] [0.18] (9)	7.2 [0.37.3] [2.9] (28)	6.7 [0.03-50] [0.15] (27)
		Exclude "simultaneous" HT diagnosis" [median][range]	5.5 [1.2-9.9] [5.5] (2)	4.6 [0.77-8.5] [4.6] (2)	NA	20 [9.6-30.5] [20] (2)	3.9 [3.2-5.2] [3.2] (3)	8.74 [0.09-37.3] [5.64] (23)	16.3 [0.44-50] [10.3] (11)
Clinical biochemical results	TgAb*	Normal (U/ml) [≤ 40]	1 (5)	3 (9)	1 (1)	0 (3)	2 (8)	3 (14)	6 (23)
		High	4 (5)	6 (9)	0 (1)	3 (3)	6 (8)	11 (14)	17 (23)
	TPOAb*	Normal (U/ml)[≤28]	3 (5)	5 (9)	1 (1)	2 (3)	3 (8)	4 (14)	12 (23)
		High	2 (5)	4 (9)	0 (1)	1 (3)	5 (8)	10 (14)	11 (23)
	FT3*	Normal (pg/ml) [2.2-4.3]	5 (5)	9 (9)	1 (1)	3 (3)	8 (8)	13 (13)	21 (23)
		Abnormal	0 (5)	0 (9)	0 (1)	0 (3)	0 (8)	0 (13)	2 (23)
	FT4*	Normal (ng/dl) [0.8-1.6]	5 (5)	8 (9)	1 (1)	3 (3)	8 (8)	12 (13)	19 (23)
		Abnormal	0 (5)	1 (9)	0 (1)	0 (3)	0 (8)	1 (13)	4 (23)
	TSH*	Normal (U/ml) [0.2-4.5]	4 (5)	6 (9)	1 (1)	3 (3)	5 (8)	8 (13)	11 (23)
		High	1 (5)	3 (9)	0 (1)	0 (3)	3 (8)	5 (13)	12 (23)
	LDH	Normal (IU/L) [124-222]	3 (5)	6 (10)	1 (1)	5 (5)	6 (11)	25 (27)	16 (33)
		High	2 (5)	4 (10)	0 (1)	0 (5)	5 (11)	2 (27)	17 (33)
	sIL2R	Normal (U/ml) [145-519]	3 (4)	6 (9)	1 (1)	1 (5)	8 (11)	17 (26)	6 (33)
		High	1 (4)	3 (9)	0 (1)	4 (5)	3 (11)	9 (26)	27 (33)
Treatment and outcome	Levothyroxine supplement	Yes	0 (5)	1 (10)	0 (1)	2 (5)	3 (11)	14 (27)	10 (33)
		No	5 (5)	9 (10)	1 (1)	3 (5)	8 (11)	13 (27)	23 (33)
	Lymphoma primary treatment	Surgery + Nothing	0 (4)	1 (10)	0 (1)	0 (3)	0 (11)	6 (29)	1 (19)
		Thyroid lobectomy + Nothing	1 (4)	0 (10)	0 (1)	0 (3)	1 (11)	4 (29)	0 (19)
		Surgery + Radiation therapy	1 (4)	8 (10)	0 (1)	0 (3)	10 (11)	19 (29)	2 (19)
		Surgery + Rituximab	0 (4)	1 (10)	0 (1)	0 (3)	0 (11)	0 (29)	0 (19)
		R-CHOP or similar regiment	0 (4)	0 (10)	1 (1)	1 (3)	0 (11)	0 (29)	8 (19)
		R-CHOP + Radiation therapy	2 (4)	0 (10)	0 (1)	2 (3)	0 (11)	0 (29)	8 (19)
		R-CHOP + BMT	0 (4)	0 (10)	0 (1)	0 (3)	0 (11)	0 (29)	0 (19)
	Response	No Change	1 (4)	0 (10)	0 (1)	0 (1)	0 (10)	0 (22)	0 (12)
		Partial Response	0 (4)	3 (10)	1 (1)	0 (1)	4 (10)	6 (22)	6 (12)
		Complete Response	3 (4)	7 (10)	0 (1)	1 (1)	6 (10)	16 (22)	6 (12)
	Survival	Stay remission	5 (5)	9 (10)	1 (1)	5 (5)	10 (11)	24 (26)	32 (34)
		Progressed/alive	0 (5)	1 (10)	0 (1)	0 (5)	1 (11)	1 (26)	2 (34)
		Progressed/died	0 (5)	0 (10)	0 (1)	0 (5)	0 (11)	0 (26)	0 (34)
		Died due to other reason	0 (5)	0 (10)	0 (1)	0 (5)	0 (11)	1 (26)	0 (34)

R-CHOP, rituximab cyclophosphamide, adriamycin, vincristine, and prednisolone; LDH, lactate dehydrogenase; TgAb, thyroglobulin antibody; TPO, thyroperoxidase antibody; TSH, thyroid stimulating hormone; BMT, bone marrow transplant; T3, Triiodothyronine; T4, thyroxine; sIL2R, soluble interleukin-2 receptor, sIL2R; HT, Hashimoto's thyroiditis; EMZL, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue; FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; NA, not available.

* Patients treated with levothyroxine were removed from the analysis

** Three cases were originally diagnosed as HT

*** Clinical staging was undertaken in accordance to the Ann Arbor staging system for lymphomas (6).

Supplementary Table S3. List of the genes investigated by TWIST capture and NGS.

MALT LYMPHOMA RELATED				GPCR GENE				CANDIDATE GENES -WES	
Apoptosis/DNA damage	ATM	NF-κB pathway	BCL10	Adenosine receptor	ADORA3	Class A orphan receptor	GPR83	BCR signaling	BANK1
	TP53		BIRC3	Chemokine receptor	ACKR3		GPR132	DNA damage and repair	BRCA2
	FAS		CARD11		ACKR4		GPR174		REV3L
B cell development	KLHL6		CD79A		CCR1		GPR183		POLK
	PIK3CD		CD79B		CCR10		GPR32	RIF1	
	PRDM1		MAP3K14		CCR3		GPR34	RG53	
POU2F2	MYD88		CCR4		GPR35		TIAM2		
FOXO1	TNFAIP3		CCR5		GPR55		LRRC32		
ARID1A	TNFRSF11A		CCR6		GPR87		LY75		
CREBBP	TNIP1		CCR7		LGR4	ANXA1			
EP300	TRAF3		CCR8		LGR5	MAPK-ERK signalling	RAF1		
KLF2	NFKBIA		CCR9		LGR6	NF-κB pathway	CPNE1		
KMT2D	TRAF6		CCR12		P2RY10	Transcription Factor EB	TFEB		
TBL1XR1	IKBKB		CX3CR1		P2RY8	Transcriptional regulator	BCOR		
TET2	ID3		CXCR3				AFF3		
YY1	Jak-STAT signalling pathway	PIM1	CXCR4		EIF3J				
Epigenetic/Translational regulator	KMT2C	Wnt signalling	APC		CXCR5	Ubiquitin Specific Protease	USP28		
	SETBP1	Cell cycle regulation	RB		XCR1	Zinc Finger	ZC3H18		
	HIST1H1E	/	CD36	Class A_lipid like	PTAFR	TENM2			
	MEF2B	/	MKI67	Class A receptor	SUCNR1				
	Immune related	SPEN		Leukotriene receptor	CYSLTR1				
		IGLL5		Neurotensin receptor	NTSR2				
		CIITA		Protease-activated receptor	F2R				
		B2M		Prostaglandin	PTGER4				
		CD58		S1P receptor	S1PR2				
		CD274			S1PR3				
TNFRSF14		S1PR4							
NOTCH pathway	NOTCH1			S1PR5					
	NOTCH2		/	GPR155					
49 GENES				43 GENES				19 GENES	
DLBCL RELATED				FL RELATED		THYROID LYMPHOMA RELATED		PANEL DETAILS	
B cell development	BCL6	Zinc Finger	ZNF608	ABC transporter	ABCA3	MAPK-ERK signalling	BRAF	IN TOTAL: 172 GENES	
	EBF1	/	DUSP2	Apoptosis/DNA damage	EZH2		KRAS	COVERAGE: 99.63 %	
	IRF8	/	MGA	B cell development	MAP2K1		NRAS	TARGET SIZE: 541,301bp	
	ITPKB	/	OSBPL10	cAMP mediated signalling	PAX5			SEQUENCEABLE: 539,323bp	
	ZFP36L1	/	PLEC	Cell Cycle	PCLO				
Cell apoptosis	BCL2	/	VMP1		CCND3				
Cell cycle	BTG1			Epigenetic Regulator	BCL7A				
	BTG2				HIST1H1D				
	CDKN2A				SMARCA4				
Epigenetic/Translational regulator	ANKRD11			mTORC1 signaling	ERBB4				
	HIST1H1B				ETV1				
	HIST1H1C				ATP6AP1				
	HIST1H2BK			NF-κB pathway	ATP6V1B2				
	SETD1B				RRAGC				
	SETD2				BCR				
Ga13 signalling	ARID1B			STAT family member	STAT6				
	ARID5B			Tumor suppressor	LRP1B				
	GNA13			Ras homolog	RHPN2				
Immune related	PDCD1LG2			Immune related	TMEM30A				
	CD70			/	CSMD3				
	IRF2BP2			/	FOXP1				
	IRF4			/	PABPC1				
	MPEG1			/	RHOA				
				/	XPO1				
JAK-STAT signaling	SOCS1								
Non-canonical NF-κB	LTB								
NOTCH signalling	DTX1								
	SGK1								
33 GENES				25 GENES		3 GENES			

Supplementary Table S4: List of the genes and coordinates investigated by TWIST capture translocation panel and NGS.

Chromosome	Start	Stop	TWIST Tiling strategy	Target gene area
chr3	187,721,376	188,032,725	4x	BCL6
chr14	105546276	105940295	4x	IGH
chr2	88686728	89330586	4x	IGK
chr22	22322441	23061520	4x	IGL

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

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