Loss of 5-hydroxymethylcytosine expression is near-universal in B-cell lymphomas with variable mutations in epigenetic regulators

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Letter to the Editor

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Tanager et al.

Supplementary

Supplementary Tables & Figures

Table S1. Cases by COO status and predicted significance of observed mutations. Predicted significance of observed mutations with SIFT, Polyphen 2 and COSMIC. Read alignment, mutation calling, and annotation were performed using a pipeline modified from the Genomic Data Commons (GDC) data harmonization pipelines. Reads were aligned to the GRCh38.d1.vd1 reference sequence using bwa-mem (version 0.7.17). According to GenomeAnalysisToolkit (GATK) best practice protocol, duplicates were marked and removed, followed by base quality score recalibration (BQSR) using GATK4 (version 4.1.3.0).

COO	Group	WES Sample	Hugo_Symbol	SIFT	PolyPhen	COSMIC
GC	HG	UCMC-111				
GC	HG	UCMC-117	EZH2	deleterious(0.04)	possibly_damaging(0.75)	
NGC	HG	UCMC-14	CREBBP	deleterious(0.05)	benign(0.021)	N/A
INDETERMINATE	HG	UCMC-145	WT1	deleterious_low_confidence(0.02)	possibly_damaging(0.815)	N/A
GC	HG	UCMC-19				
NGC	HG	UCMC-2	CREBBP	deleterious(0.01)	possibly_damaging(0.838)	N/A
NGC	HG	UCMC-2	IDH2	deleterious_low_confidence(0)	possibly_damaging(0.908)	N/A
NGC	HG	UCMC-2	IDH2	deleterious_low_confidence(0)	probably_damaging(1)	N/A
NGC	HG	UCMC-20				
NGC	HG	UCMC-21	CREBBP	deleterious(0)	probably_damaging(0.997)	Pathogenic
NGC	HG	UCMC-21	TET2	deleterious(0)	probably_damaging(0.963)	N/A
NGC	HG	UCMC-23	TET2	tolerated(0.22)	benign(0.012)	N/A
NGC	HG	UCMC-26				
UNKNOWN	HG	UCMC-29				
GC	HG	UCMC-4				

NGC	HG	UCMC-57	TET1	tolerated(0.16)	benign(0.039)	N/A
NGC	HG	UCMC-8				
GC	HG	UCMC-85	CREBBP	tolerated_low_confidence(0.73)	benign(0.022)	Pathogenic
GC	HG	UCMC-86	IDH1	deleterious_low_confidence(0)	benign(0.148)	N/A
GC	HG	UCMC-86	WT1	deleterious_low_confidence(0)	benign(0.003)	N/A
GC	HG	UCMC-87	CREBBP	deleterious(0)	probably_damaging(0.999)	Pathogenic

Figure S1: Large B-cell lymphoma lacking 5hmC in lymphoma cells and microenvironment lymphoid cells: A. Low-power H&E depicting diffuse lymphoid infiltrate of a non-GCB DLBCL rich in histiocytes with scattered small clusters of large cells (inset). Focus rich in small lymphocytes (*) corresponding to reactive T-cells is noted at the periphery. B. The marked area is devoid of small B-cells while background malignant large B-cells are positive for CD20. C. The marked area comprises predominantly CD3+ T-cells with a prominent T-cell rich background elsewhere. D. The marked area (rich in T-cells) is largely negative for 5hmC. Most of the lymphoma cells are also negative with staining restricted to scattered histiocytes and endothelial cells. Only occasional tumor cells were positive in this case. Tumor cells marked with black arrow in inset. Specifically endothelial cells showed the strongest staining while most T-cells showed weak to negative staining in this case.

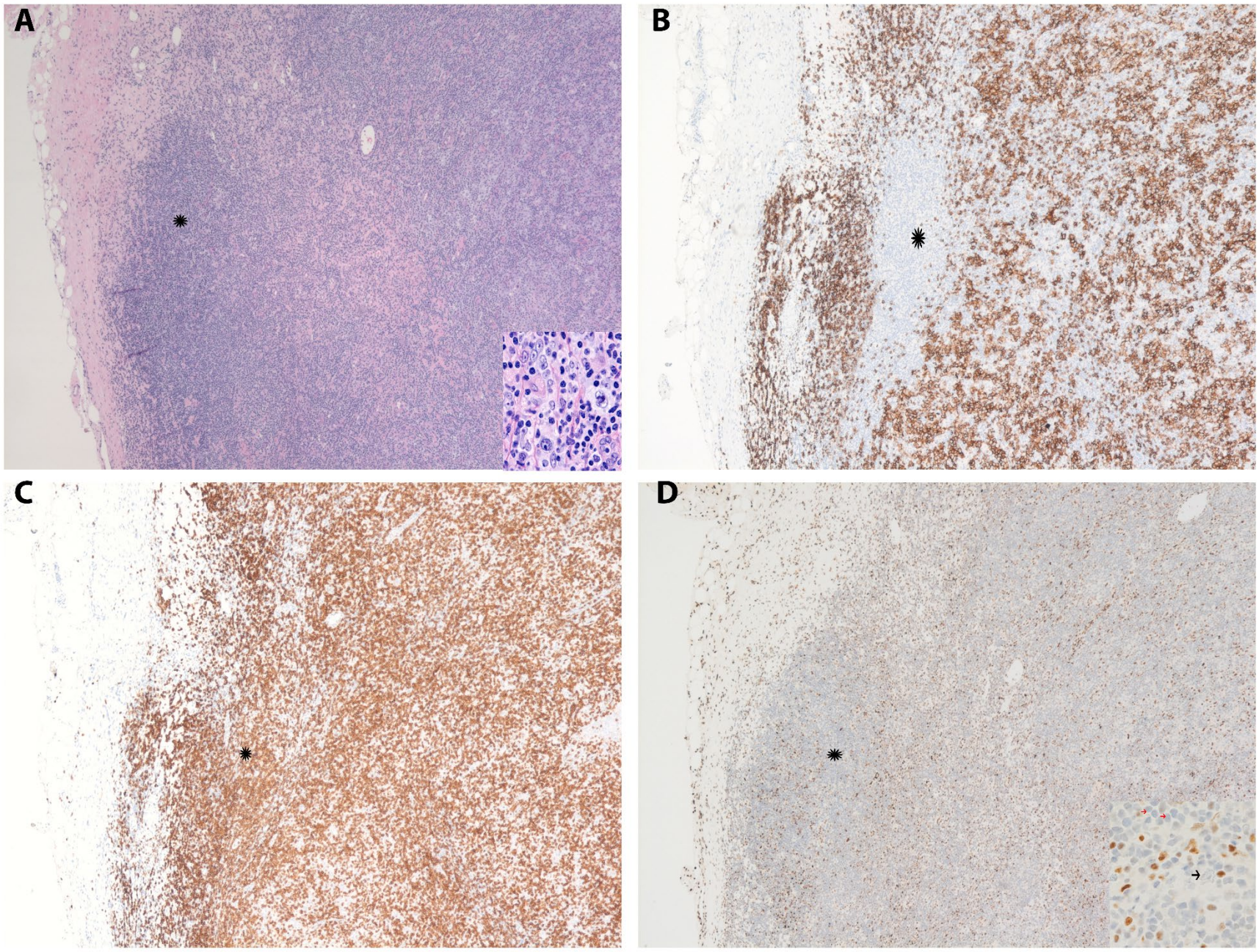
A
B
C
D

Figure S2. Predicted functional significance of mutations and their locations.