Loss of 5-hydroxymethylcytosine is a frequent event in peripheral T-cell lymphomas

François Lemonnier,[1,2] Elsa Poullot,[2,3*] Aurélie Dupuy,[1,2] Lucile Couronné,[4] Nadine Martin,[1,2] Laurianne Scourzic,[4] Virginie Fataccioli,[1,2,3] Julie Bruneau,[5] Rob A Cairns,[6] Tak W. Mak,[6] Olivier A. Bernard,[4] Laurence de Leval[7] and Philippe Gaulard[1,2,3]*

[1]INSERMU955 équipe 9, Institut Mondor de Recherche Biomédicale, Créteil, France; [2]Université Paris-Est, Faculté de Médecine, Créteil, France; [3]Département de Pathologie, Groupe Henri-Mondor Albert-Chenevier, AP-HP, Créteil, France; [4]INSERMU1170, Institut Gustave Roussy, Université Paris-Sud, Villejuif, France; [5]Département de Pathologie, Hôpital Necker Enfants Malades, APHP, Paris, France; [6]Campbell Family Institute for Breast Cancer Research, Princess Margaret Hospital, Toronto, Canada and [7]Institute of Pathology, Lausanne University Hospital, Lausanne, Switzerland

**These authors contributed equally to this paper*

Correspondence: philippe.gaulard@aphp.fr
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Supplemental methods

PTCL samples

Seventy one PTCL samples with formalin fixed paraffin embedded and/or frozen tissues representative of various histology and mutational status, as well as 2 reactive lymph nodes and 4 reactive tonsils were selected within the framework of a multicenter T-cell lymphoma consortium (TENOMIC) and included in this study. The study was approved by the local ethics committee (CPP Ile de France IX 08-009).

Immunohistochemistry

5hmC and 5mC level was evaluated by IHC using rabbit anti 5hmC (Active motif 39770) and mouse anti 5mC (Active motif, clone 33D3) antibodies on deparrafinized tissue sections. Double immunoenzymatic methods were used, as previously described[1] to evaluate 5hmC levels in PD1 positive TFH cells (ABCAM, clone NAT105), or IDH2R172K (EwEast, 26163) mutated cells.

Genotyping.

TET2, *DNMT3A* and *IDH2* was genotyped by Sanger sequencing after DNA extraction from frozen tissue. Methods and results of the *TET2* and *IDH2* sequence analysis have been previously reported[2,3].

Five samples have been studied by target deep sequencing using the PGM technology. We sequenced all exons of TET2 gene (excepted codons 1-25, 95-119, 401-420, 720-748, 1373-1394, 1560-1589, 1783-1806), exon 4 of IDH2 and exons 3, 7, 9, 10, 11, 13, 15 to 23 of DNMT3A, among a wider panel.

Briefly 20 ng DNA was extracted from FFPE samples and amplified using an Ampliseq custom panel. Amplicons were then digested, barcoded, and amplified using the Ion Ampliseq Library kit 2.0 and Ion Xpress barcode adapter kit (ThermoFisher), according to the manufacturer's instructions. After quantification of DNA, 8 pM of each library was multiplexed and clonally amplified on Ion sphere particles (ISP) by emulsion PCR performed on an Ion One Touch 2 instrument with the Ion PGM template OT2 200 kit (ThermoFisher), according to the manufacturer's instructions. After quality control, the ISP templates were enriched, loaded on an Ion 316 chip, and sequenced on a PGM sequencer with the Ion PGM Hi-Q Sequencing Kit, according to the manufacturer's instructions. The raw data were analyzed using torrent suite software v5.0. A mean coverage depth of 1277X was obtained. Mutations were detected using the Variant Caller plug-in v 5.0.0.7. Each mutation in the

resulting variant list was verified using the Integrative genome viewer (IGV) from the Broad Institute (http://www.broadinstitute.org/igv/).

SETD2 sequencing methods and results were previously published[4]

Reference

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Supplemental Table 1: Description of mutational status and 5hmC and 5mC results of patients included in the study (MIETL cases are described in Table 3)

sample	diagnosis	TFH derivation	TET2	IDH2 mut	DNMT3A	5hmC in tumor cells	5mC in tumor cells
112	AITL	TFH	WT	R172K	WT	negative	positive
215	AITL	TFH	mut	R172K	mut	negative	positive
4	AITL	TFH	WT	R172K	WT	negative	positive
414	AITL	TFH	WT	R172K	WT	negative	positive
281	AITL	TFH	WT	R172K	WT	negative	positive
397	AITL	TFH	mut	R172K	mut	negative	positive
60	AITL	TFH	mut	WT	WT	negative	positive
391	AITL	TFH	mut	R172G	mut	negative	positive
166	AITL	TFH	mut	R172S	mut	negative	positive
288	AITL	TFH	WT	R172S	WT	negative	positive
527	AITL	TFH	mut	WT	mut	negative	positive
568	AITL	TFH	mut	R140Q	WT	negative	positive
81	AITL	TFH	mut	R172G	mut	negative	positive
39	AITL	TFH	mut	R172K	WT	negative	positive
7	AITL	TFH	WT	WT	WT	negative	positive
8	AITL	TFH	WT	WT	WT	negative	positive
9	AITL	TFH	WT	WT	WT	negative	positive
50	AITL	TFH	WT	WT	WT	negative	positive
233	AITL	TFH	WT	WT	WT	negative	positive
483	AITL	TFH	WT	WT	WT	negative	positive
56	AITL	TFH	WT	WT	WT	negative	positive
252	AITL	TFH	WT	WT	WT	negative	positive
250	AITL	TFH	WT	WT	WT	negative	positive

59	AITL	TFH	WT	WT	WT	negative	positive
58	AITL	TFH	WT	WT	WT	negative	positive
12774	AITL	TFH	WT	WT	WT	negative	positive
13207	AITL	TFH	WT	WT	WT	negative	positive
13637	AITL	TFH	WT	WT	WT	negative	positive
12773	AITL	TFH	WT	WT	WT	negative	positive
12512	AITL	TFH	WT	WT	WT	negative	positive
287	PTCL, NOS	TFH	WT	R172K	WT	negative	positive
23	PTCL, NOS	TFH	mut	WT	WT	negative	positive
425	PTCL, NOS	TFH	WT	WT	WT	negative	Positive
234	PTCL, NOS	TFH	mut	WT	WT	negative	Positive
450	PTCL, NOS	non TFH	mut	WT	WT	negative	Positive
19	PTCL, NOS	non TFH	WT	WT	WT	negative	Positive
25	PTCL, NOS	nonTFH	WT	WT	WT	negative	Positive
303	PTCL, NOS	non TFH	WT	WT	WT	negative	Positive
225	PTCL, NOS	non TFH	WT	WT	WT	negative	Positive
103	PTCL, NOS	non TFH	WT	WT	WT	negative	Positive
419	NKTCL	non TFH	WT	WT	WT	negative	Positive
12	NKTCL	non TFH	WT	WT	WT	negative	Positive
299	NKTCL	non TFH	WT	WT	WT	negative	Positive
454	NKTCL	non TFH	WT	WT	WT	negative	Positive
418	EATL	non TFH	WT	WT	WT	negative	Positive
141	EATL	non TFH	WT	WT	WT	negative	Positive
37	HSTL	non TFH	WT	WT	WT	positive	Positive
179	HSTL	non TFH	WT	WT	WT	positive	Positive
180	HSTL	non TFH	WT	WT	WT	positive	Positive
238	ALK-ALCL	non TFH	mut	WT	WT	negative	Positive

105	ALK-ALCL	non TFH	WT	WT	WT	negative	Positive
217	ALK-ALCL	non TFH	WT	WT	WT	negative	Positive
ALK1	ALK+ ALCL	non TFH	WT	WT	WT	negative	Positive
ALK2	ALK+ ALCL	non TFH	WT	WT	WT	negative	Positive
ALK3	ALK+ ALCL	non TFH	WT	WT	WT	negative	Positive

Supplemental Table 2: Description of *TET2*, *IDH2,* and *DNMT3A* mutations in patients included in this study

Sample	Diagnosis	TET2		IDH2		DNMT3A	
112	AITL	WT		c601G>A	p.Arg172Lys		
215	AITL	c.1384delC,	p.Gln440fs	c.601G>A	p.Arg172Lys	Splice c.2660+1G>A	
4	AITL	WT		c.601G>A	p.Arg172Lys	WT	
414	AITL	WT		c.601G>A	pArg172Lys	WT	
281	AITL	WT		c.601G>A	pArg172Lys	WT	
397	AITL	c.[3929G>A (+)4730_4731delAG]	p.[Gly1288Asp(+) Glu1555fs]	c.601G>A	pArg172Lys	c.2181C>T	p.Gln615X
60	AITL	c.3877T>G	p.Cys1271Gly	WT		WT	
391	AITL	c.3851G>C	c.3851G>C p.Arg1262Pro	c.600A>G	p. Arg172Gly	c.3049C>T	p.Pro904Leu
166	AITL	c.[1918C>T (+)3661delG]	p.[Gln618X (+) Val1199fs]	c.602G>T	p.Arg172Ser	c. 2983G>A	p.Arg882His
288	AITL	WT		c.601G>C	pArg172T	WT	
527	AITL	c.4353delT	p.Phe1429fs	WT		splice ex17	
568	AITL	c.587delC	p.Pro174fs	c.505G>A	pArg140Gln	WT	
81	AITL	c. 1516insG	p.Cys484fs	c.600A>G	p.Arg172Gly	c. 2982C>T	p.Arg882Cys
39	AITL	c.[2116C>T(+)4020+1G>A]	p.Gln685X	c.601G>A	p.Arg172lys	WT	

Sample	Diagnosis	TET2		IDH2		DNMT3A
287	PTCL, NOS	WT		c.601G>A	pArg172Lys	WT
23	PTCL, NOS	c.[3831C>A(+)4205A>T]	p.[Tyr1255X(+)His1380Leu]	WT		WT
234	PTCL, NOS	c.4835delG	p.Gly1590fs	WT		WT
425	PTCL, NOS	WT		WT		WT
450	PTCL, NOS	c.[1403T>G (+)2932_2933delCT]	p.[Leu446X(+)Leu956fs]	WT		WT
238	ALK-ALCL	c.1918_1919insG	p.Leu615fs	WT		WT

Supplemental Table 3: Description of MEITL cases used in this study. * indicate cases where <20% tumor cells retain a decreased but detectable 5hmC staining.

	5hmC	MIB/Ki67	SETD2 mutation	SETD2 deletion	TCR αβ	TCR γδ
Case 1	Neg	85%	WT	Del	Neg	Pos
Case 9	Neg	30%	Mut	Del	Neg	NI
Case 10	Neg	40-50%	Mut	NC	Pos	Neg
Case 14	Neg *	80%	Mut	Not del	Neg	Pos
Case 17	Neg	80%	Mut	Not del	Pos	Neg
Case 20	Neg	85%	Mut	Not del	NI	Neg
Case 23	Neg	75%	NA	Not del	Neg	Neg
Case 24	Neg	50%	Mut	Del	Neg	NI
Case 26	Neg	20-30%	Mut	Not del	Pos	Neg
Case 28	Neg *	95%	WT	Del	Neg	Pos
Case 29	Neg	80%	NA	NA	NA	NA
Case 30	Neg *	80%	Mut	Del	NA	NA
Case 31	NE	90%	NA	NA	Neg	Pos
Case 33	Neg *	NA	NA	NA	NA	NA
Case 34	Neg	NA	NA	NA	NA	NA
Case 143	Neg	NA	NA	NA	NA	NA

Supplemental appendix: TENOMIC investigators

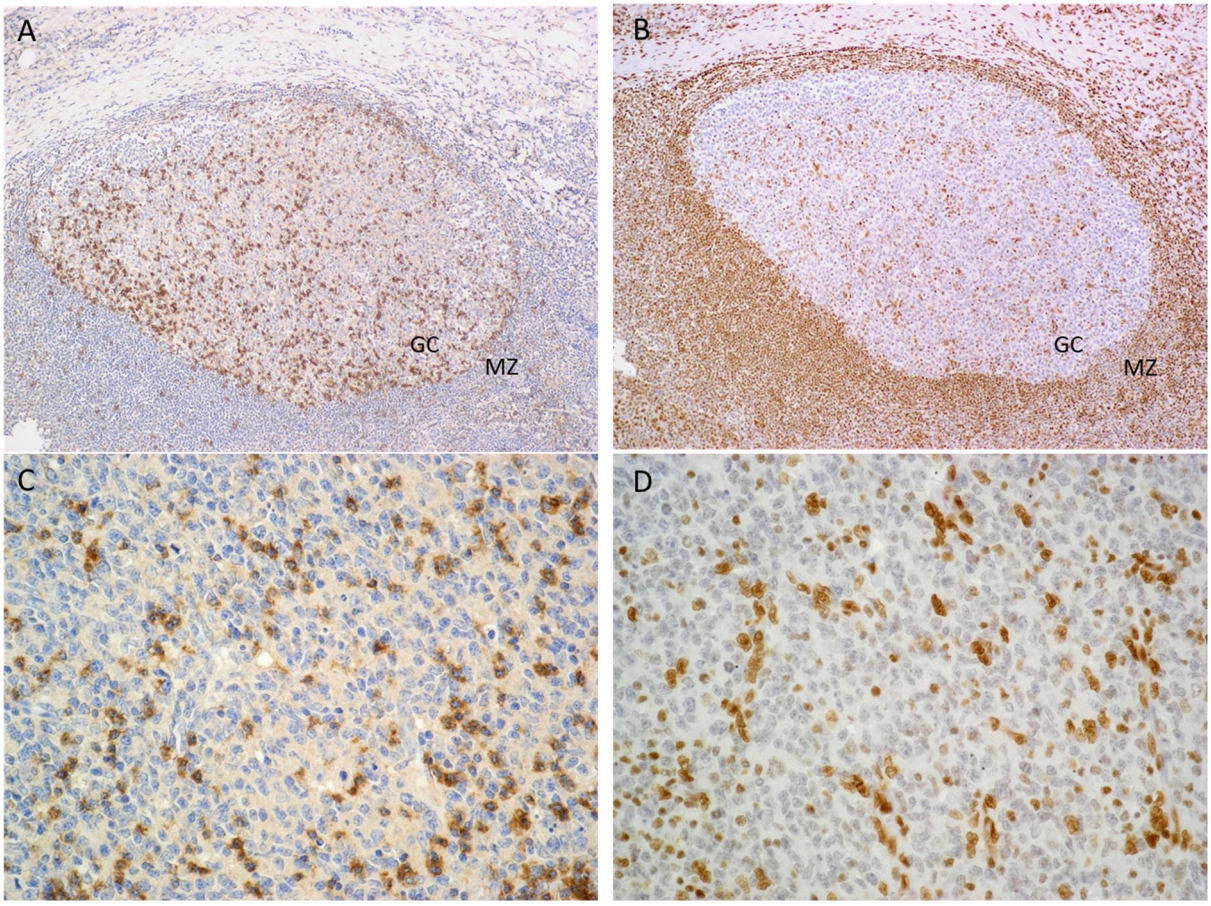
TENOMIC Consortium members: A. Martin, Hôpital Avicenne, Bobigny ; I. Soubeyran, P. Soubeyran, Institut Bergonié, Bordeaux; P. Dechelotte, A. Pilon, O.Tournilhac, CHU Estaing, Clermont Ferrand ; K. Leroy, P. Gaulard, MH Delfau, A Plonquet, C. Haïoun, F. Lebras, Hôpital Henri Mondor, Créteil; T. Petrella, L. Martin, JN Bastié, O Casasnovas, CHU, Dijon; B. Fabre, R. Gressin, D. Leroux, MC Jacob, M Calannan, CHU, Grenoble ; L. de Leval, D. Vallois, AL Roberti, A. Cairoli, CHUV, Lausanne, Suisse ; B. Bisig, G. Fillet, C. Bonnet, CHU Sart-Tilman, Liège ; M.C. Copin, B. Bouchindhomme, F. Morschhauser, CHU, Lille ; B. Petit, A. Jaccard, Hôpital Dupuytren, Limoges ; A. traverse-Glehen, E. Bachy, L. Genestier, B. Coiffier, CHU Sud, Lyon ; T. Rousset, P. Quittet, G. Cartron, Hôpital Gui de Chauliac-St Eloi, Montpellier ; B. Drenou, Hôpital E. Muller, Mulhouse ; K. Montagne, C. Bastien, S. Bologna, CHU de Brabois, Nancy ; C. Bossard, S. Le Gouill, Hôtel-Dieu, Nantes ; T. Molina, Hôtel-Dieu, Paris ; V. Meignin, J. Brière, C. Gisselbrecht, Hôpital St Louis, Paris ; B. Fabiani, S. Amorim, P. Coppo, Hôpital Saint-Antoine, Paris ; F. Charlotte, J. Gabarre, Hôpital Pitié-Salpétrière, Paris ; J. Bruneau, D. Canioni, V. Verkarre, E Macintyre, V. Asnafi, O. Hermine, R. Delarue, F Suarez, D. Sibon, JP Jaïs, Hôpital Necker, Paris ; M. Parrens, JP Merlio, K. Bouabdallah, Hôpital Haut Lévêque, Bordeaux ; F. LLamas-Gutierez, P. Tas, S. Maugendre-Caulet, T. Lamy, R. Houot, CHU Pontchaillou, Rennes ; JM Picquenot, L. Verezevan, P. Ruminy, F. Drieux, F. Jardin, C. Bastard, Centre H Becquerel, Rouen ; M. Peoc'h, J. Cornillon, CHU, Saint Etienne ; L. Lamant, C. Laurent, G. Laurent, L. Oberic, Hôpital Purpan, Toulouse ; J.Bosq, P. Dartigues, V. Ribrag, Institut G Roussy, Villejuif ; M. Patey, A. Delmer, Hôpital R. Debré, Reims ; JF Emile, K. Jondeau, Hôpital Ambroise Paré, Boulogne ; MC Rousselet, M. Hunault, CHU, Angers ; C. Badoual, Hôpital Européen Georges Pompidou, Paris ; C. Legendre, F. Boidart, S. Castaigne, AL Taksin, CH Versailles, Le Chesnay ; J. Vadrot, A. Devidas, B. Joly, CH Sud francilien, Corbeil ; Dr Gandhi DAMAJ, CHU Amiens.

F Lemonnier, A Dupuy, N. Martin-Garcia, INSERM U955, Créteil ; P Dessen, G Meurice, Institut G Roussy, Villejuif ; M Delorenzi, E Missiaglia, P. Dobay, Swiss Institute of Bioinformatics, Lausanne, Switzerland ; F Radvanyi, E Chapeaublanc, Institut Curie, Paris ; P. Ferrier, S. Spicuglia, CIML, Marseille ; J Soulier, Hôpital St Louis, Paris ; C Thibault, IGBMC, Illkirsch

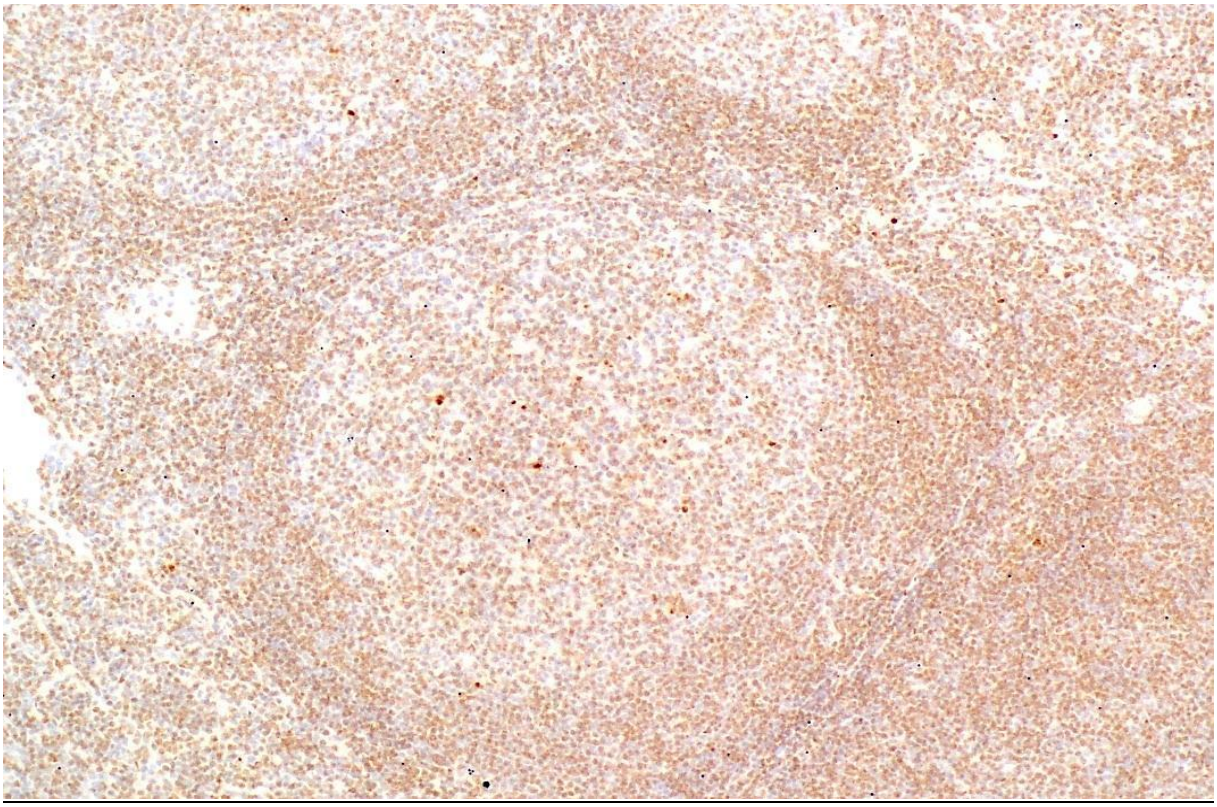
the LYSA (the Lymphoma Study Association)

V Fataccioli, Project Manager, Hôpital H Mondor, Créteil

Supplemental figure 1: Staining of PD1 (A and C) and 5hmC in serial slides of reactive tonsils. Original magnification X10 and X40



Supplemental figure 2: Repartition of 5mC in a reactive tonsil Original magnification objective: X10



Supplemental Figure 3: Illustration of 5hmC and 5mC level in various nodal and extranodal PTCL entities.

A-C) TET2 mutated PTCL-NOS (sample 234), respectively HES, 5hmC and 5mC X 20.

D-F) TET2, IDH2 and DNMT3a PTCL-NOS (sample 303), respectively HES, 5hmC and 5mC X 20

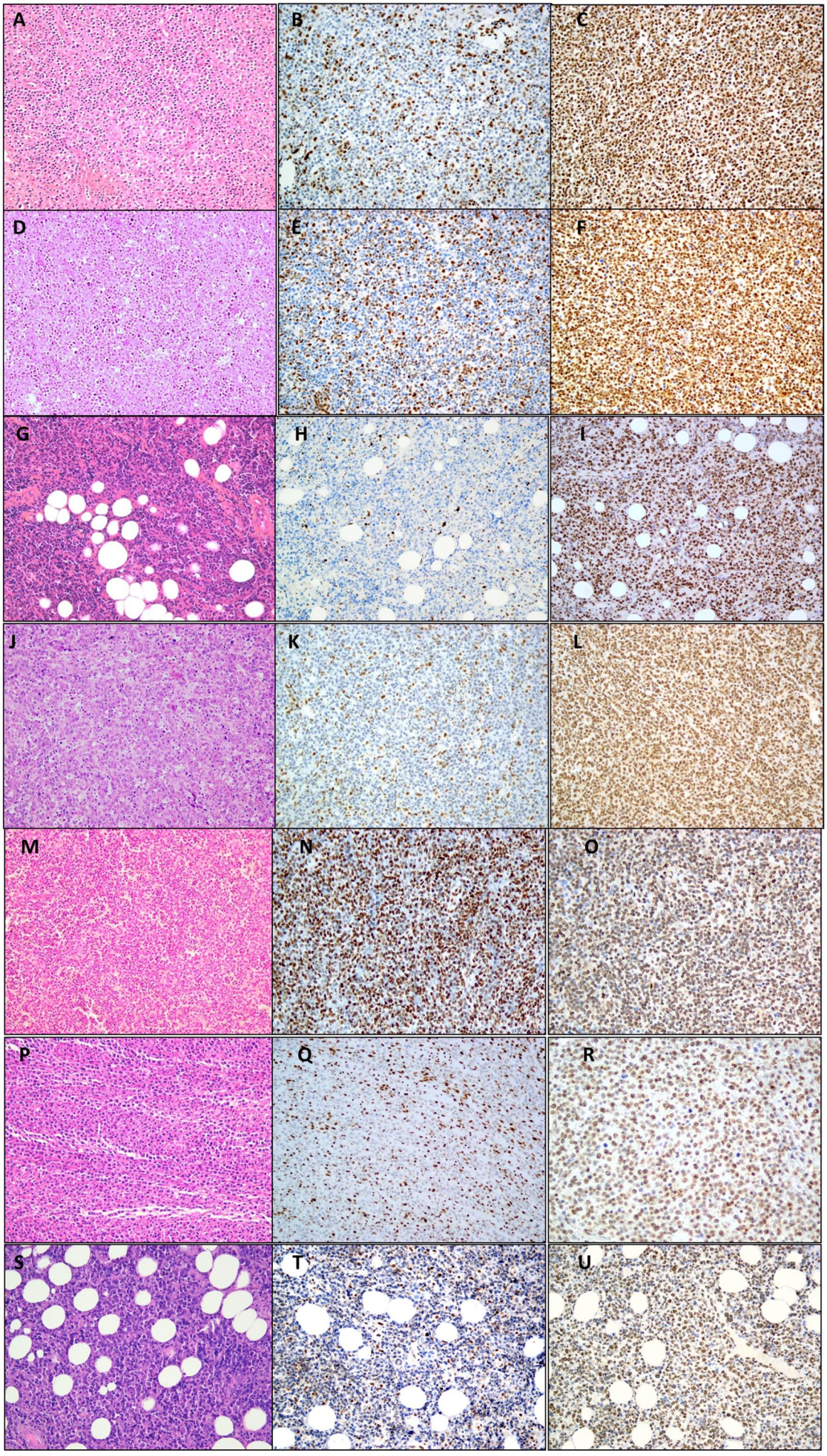
G-I) TET2 mutated ALK-ALCL (sample 238) respectively HES, 5hmC and 5mC X 20

J-L) WT ALK+ALCL, respectively HES, 5hmC and 5mC X 20

M-O) WT HSTL, (sample 37) respectively HES, 5hmC and 5mC X 20

P-R) WT EATL (sample 418) respectively HES, 5hmC and 5mC X 20

S-U) WT NKTCL (sample 12) respectively HES, 5hmC and 5mC X 20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Supplemental Figure 4: Absence of relationship between 5hmC level and proliferation level in MEITL. A) Ki67/MIB1 and B) 5hmC staining of case MEITL 29 which have a MIB1/Ki67 estimated at 80% show that contrary to endothelial cells, which are strongly positive for 5hmC, tumor cells, which are mainly proliferative are negative for 5hmC. C) Ki67/MIB1 and D) 5hmC staining of case MEITL 9, which have a MIB1/Ki67 estimated at 30%, show that 5hmC is negative in tumor cells, even in lymphomas with low proliferation index estimated by MIB1/Ki67.