

Supplementary Materials: *STAT3* Mutation Is Associated with *STAT3* Activation in CD30⁺ ALK⁻ ALCL

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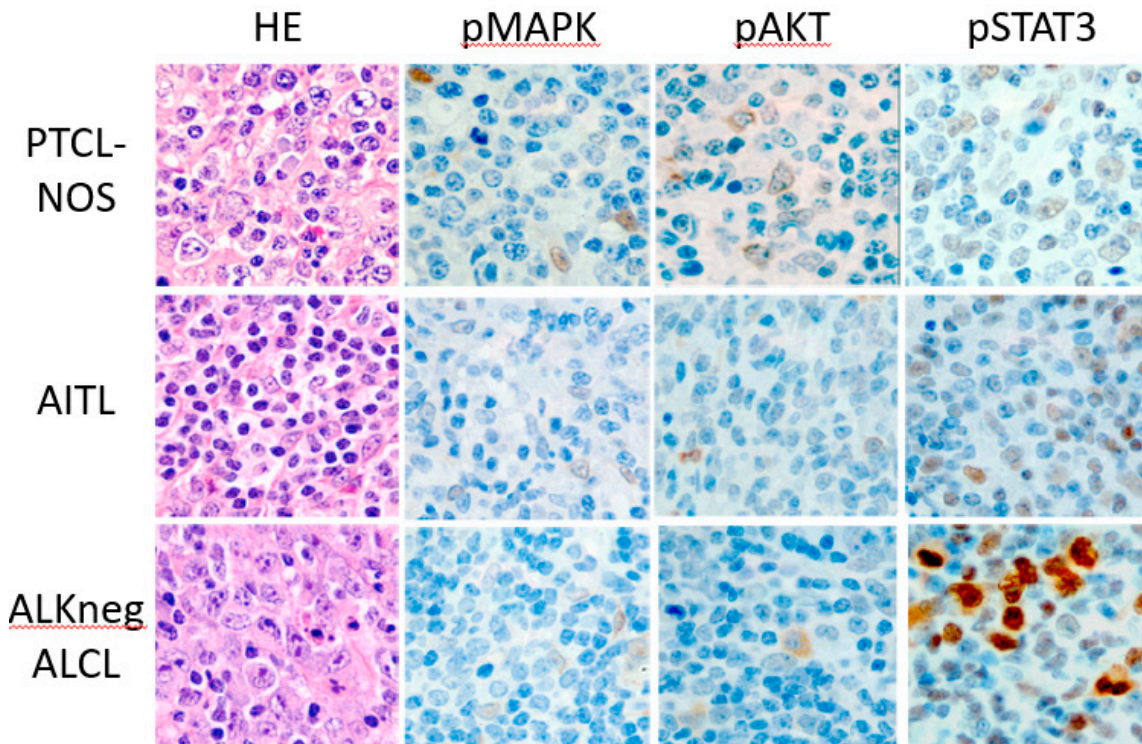


Figure S1. pMAPK, pAKT and pSTAT3 staining of peripheral T-cell lymphomas (PTCLs); not otherwise specified (NOS), angioimmunoblastic T-cell lymphoma (AITL) and ALK negative anaplastic large cell lymphoma (ALCL).

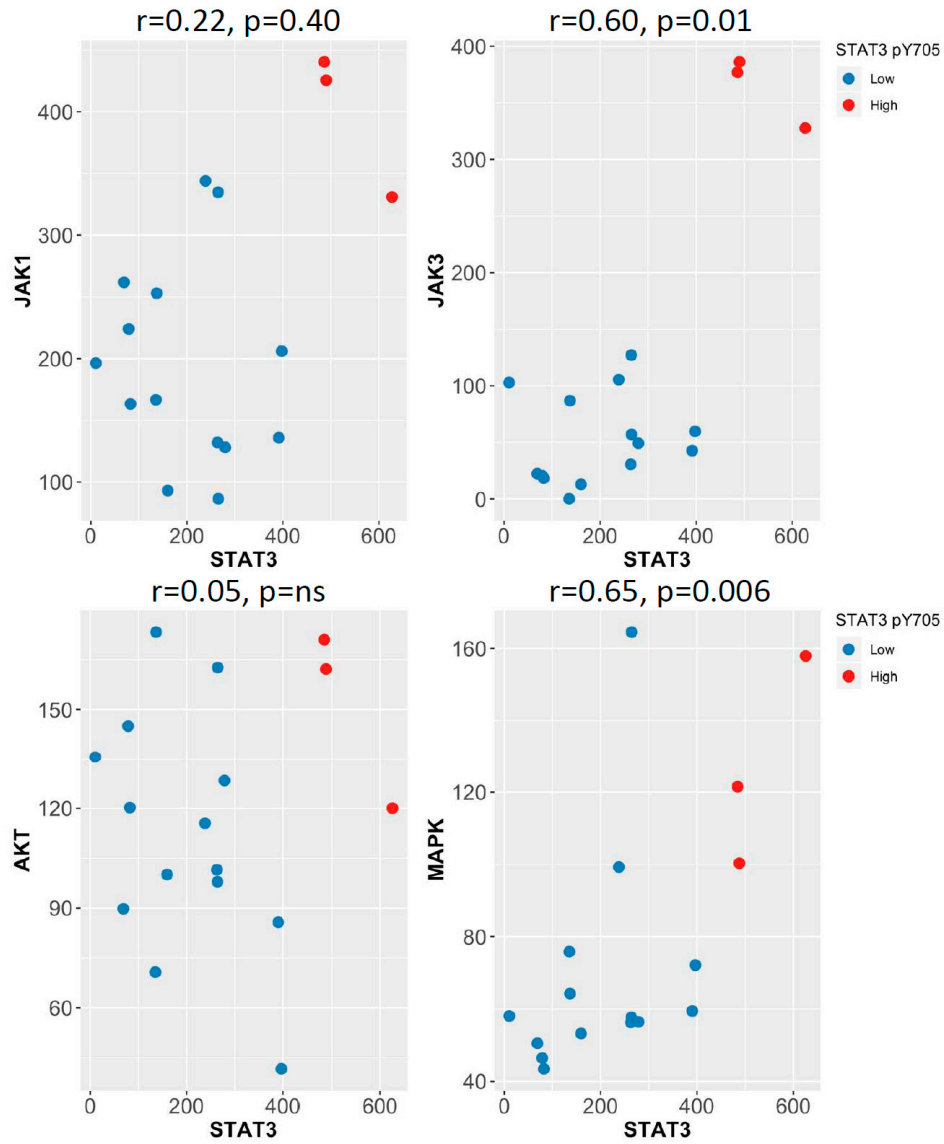


Figure S2. STAT3 expression was correlated (Spearman correlation) with JAK1, JAK3, AKT (arithmetic mean of AKT1 and AKT3) and MAPK (arithmetic mean of MAPK6, MAPK11, MAPK12 and MAPK14) expression (CPM) in T-cell neoplasia cell lines from the CCLE dataset. Cell lines are labelled based on STAT3 phosphorylation defined with RPPA.

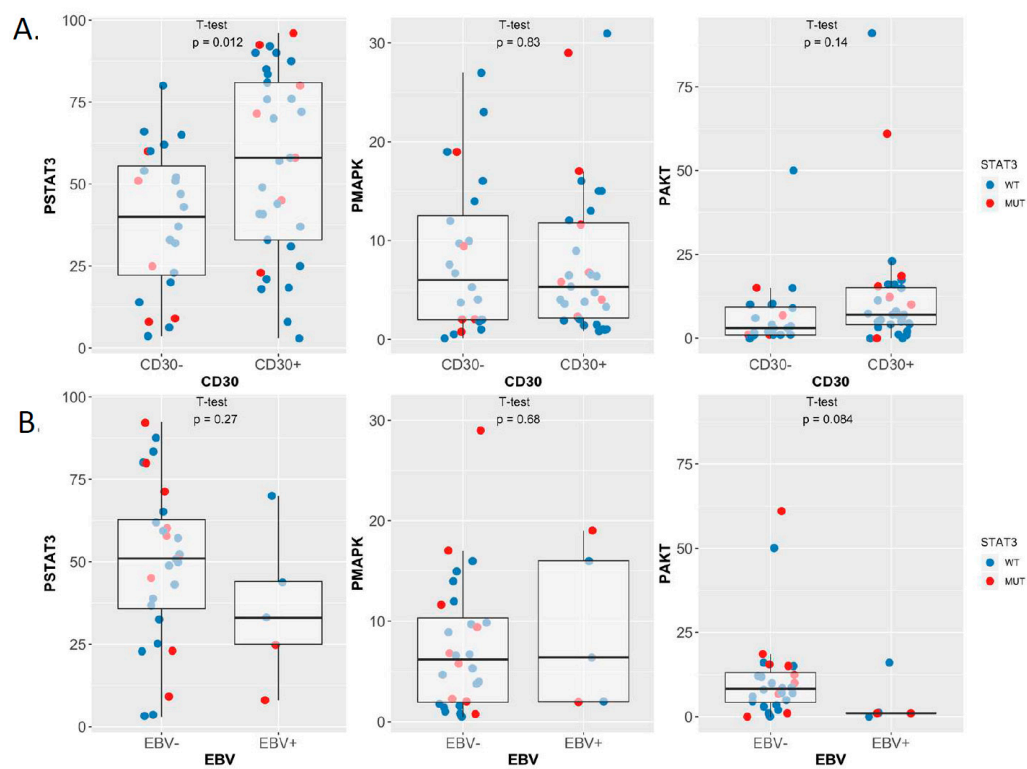


Figure S3. (A) Comparison between pSTAT3, pMAPK and pAKT expression and tumor CD30 and (B) EBV phenotype with unpaired t-test.

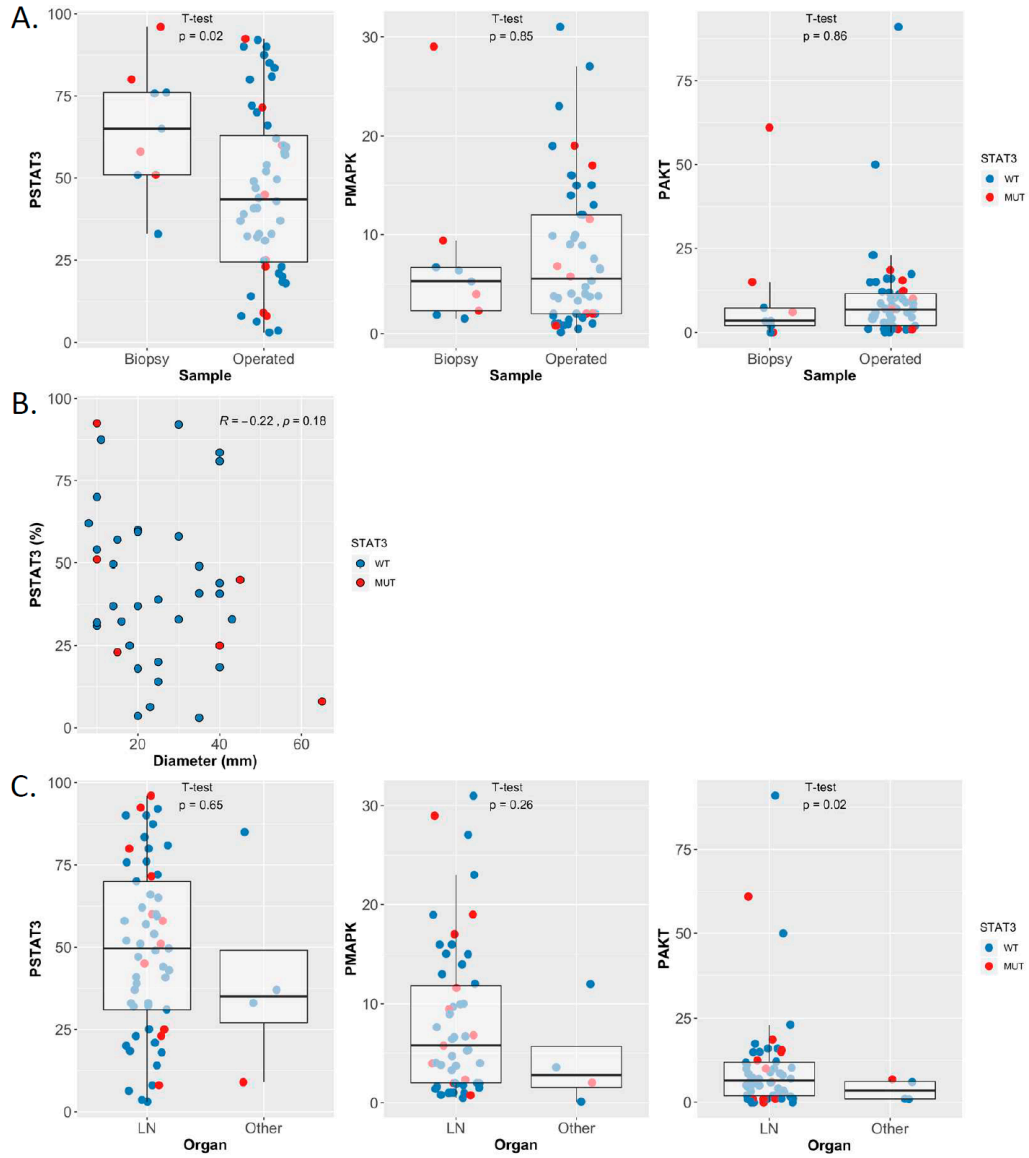


Figure S4. (A) Comparison between pSTAT3, pMAPK and pAKT expression and sample type. (B) Pearson correlation between pSTAT3 expression and sample diameter. (C) Comparison between pSTAT3, pMAPK and pAKT expression and organ origin of the sample. Unpaired T-test has been used for comparison.

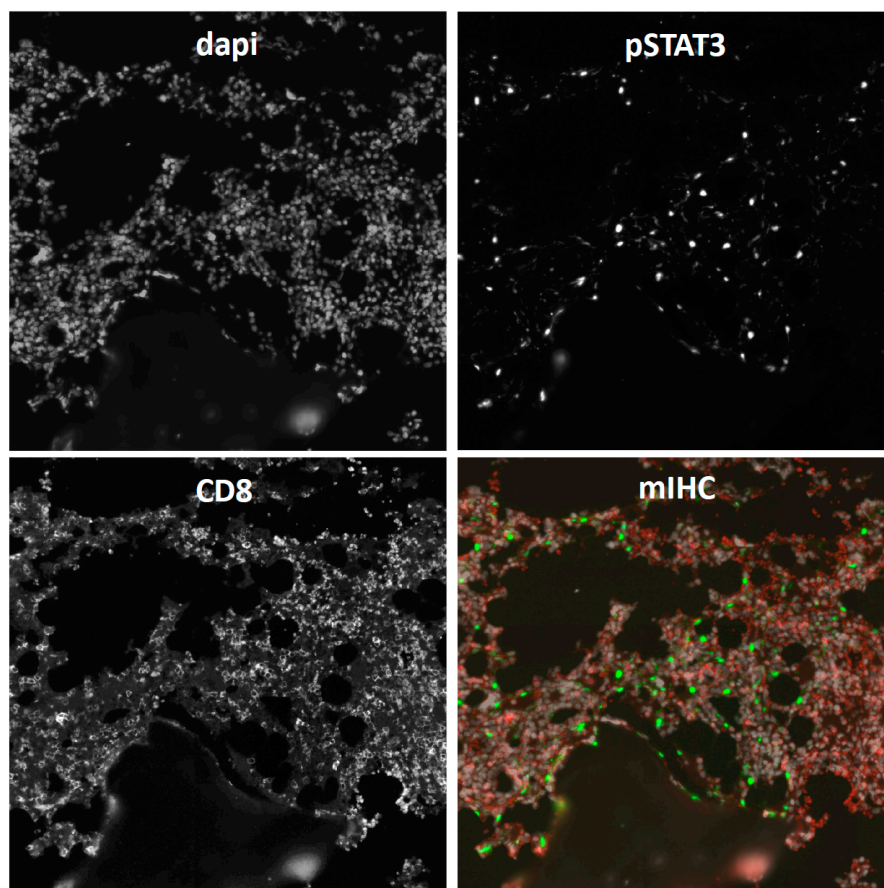


Figure S5. Multiplex immunohistochemistry (mIHC) staining of pSTAT3^{Tyr705} in CD8⁺ large granular lymphocyte (LGL) leukemia as a positive control stain for pSTAT3 antibody. pSTAT3 and CD8 were stained and registered in Cy3 and Cy5 channels, respectively. Dapi was used as nuclear counterstain. The first three images represent single stainings and the last figure their composite image.

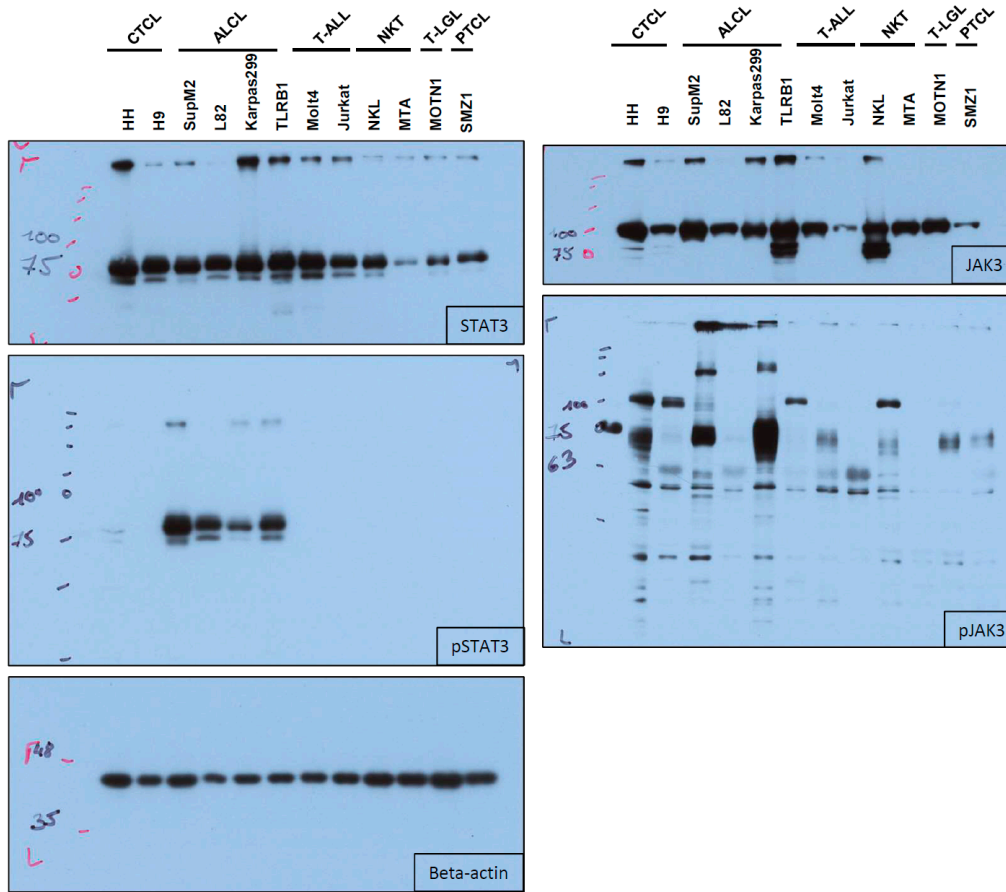


Figure S6. Full western blot of p-STAT3 (Tyr705), STAT3, p-JAK3 (Tyr980/981), and JAK3 in T cell malignancy cell lines. Beta-actin is used as a reference protein. Molecular weights are annotated in the left for each corresponding protein as the staining was digitalized with colorimetric detection.

Table S1. Cell lines and *JAK/STAT* mutation status.

Disease	Cell lines	Phenotype	JAK/STAT mutation status
CTCL	HH	CTCL, representing aggressive leukemic CD30+ Mycosis Fungoides and/or ALCL	no mutations
	H9	CTCL	missense JAK1/3
ALCL	SUP-M2	ALCL, ALK+	no mutations
	L82	ALCL, ALK+	no mutations
	Karpas 299	ALCL cell line (of T-cell type) derived from a lymphoma termed at the time “histiocytic high grade	no mutations
	TLBR1	ALCL, ALK-	missense STAT3 (S614R)
T-ALL	MOLT4	T-ALL	nonsense JAK1 missense STAT3 (A406V)
	Jurkat	Immature TCR $\alpha\beta$ + T-cell line (type T-III cortical) derived from relapsed childhood T-ALL	nonsense/missense STAT5B (T724M, L165L, L142P and Q368fs) missense STAT3 (R729fs and L78F)
NKT	NKL	NKT	no mutations
	MTA	NKT	no mutations
T-LGL	MOTN-1	T-LGL	missense JAK1
PTCL	SMZ-1	PTCL-NOS	no mutations

Table S2. Clinical and pathological characteristics of PTCL patients.

Dg	T %	P-Stat3%	P-MAPK %	P-AKT%	P-Stat5%	Organ	Sample type	Sample size (mm)	CD 3	CD 4	CD 5	CD 7	CD 8	CD 21	CD 30	EB V	MIB %	Gender	Age	Stage	LD	ECOG	CHOP	HDC T	Relapse/progression	Death	OS time
AITL	70	52	14	3	1	LN	Oper		1	1			0	1	0	0	60	F	59	3	496	3	1	0	0	1	572
AITL	80	33	27	3	2	LN	Oper	30	1	0	1		0	1	0		50	M	63	4	350	3	1	1	1	1	528
AITL	65	57	12	8	9	LN	Oper	15	1	1	1		0	1	1	0	55	F	51	4	634	4	1	1	0	0	2185
AITL	75	66	4	10	10	LN	Oper		1	1	1			0	1	0		F	85	3	238	4	0	0	0	1	26
AITL	85	54	19	2	15	LN	Oper	10	1	1	1		0				50	M	57	4	526		1	1	1	1	1717
AITL	55	31	15	91	7	LN	Oper	10	1	1	1		1	0	1	1	40	M	69	3	245	2	1	0	1	1	1115
AITL	40	25	2	1	0	LN	Oper	40	1	1	1		0	1	0	1		M	68	4	530	3	0	0	0	0	49
AITL	35	32	10	1	1	LN	Oper	10	1	1								M	80	3	534	2	0	0	1	1	218
AITL	75	47	23	9	3	LN	Oper		1	1	1	0	0	1	0		60	M	61	4	406	2	1	0	1	0	218
AITL	60	23	16	15	10	LN	Oper		1	1	1		0	1	0	0	40	F	63	4	235	1	1	1	1	1	2621
AITL	80	21	13	5	5	LN	Oper		1	1	1	1	0	1	1		70	F	77	3	272	2	1	0	1	1	2601
AITL	75	49	15	16	2	LN	Oper	35	1	1	1	1	0	1	1	0	70	M	74	3	254	1	1	0	0	1	259
AITL	70	43	1	1	2	LN	Oper		1	1	1	1	0	1	0	0	30	M	78	3	360	1	0	0	0	1	308
AITL	70	25	1	5	4	LN	Oper	18	1	1	1	0	0	1	1	0	60	M	64	4	263	1	1	1	1	1	445
AITL	90	58	31	23	0	LN	Oper	30	1	1	1	0	0	1	1		70	M	84	3	266	3	0	0	0	1	79
AITL	40	45	17	10	4	LN	Oper	45	1	1	1	0	0	1	1	0	90	M	57	3	237	2	1	0	1	1	698
AITL	70	44	16	16	1	LN	Oper	40	1	1	1	0	0	1	1	1	30	M	67	3	210	1	1	0	1	1	4739
AITL	60	60	1	1	1	LN	Oper	20	1	1	1	0	0	1	0	0	50	F	86	3	355	1	0	0	0	1	62
AITL	75					LN	Oper	35	1	1	1		0				40	M	31	3	372	0	0	1	1	1	1238
AITL	50	33	12	6	2	Oropharynx	Oper		1	1	1		0	1	0			F	74	1	194	1	1	0	1	1	2339
AITL	95	60	4	1	1	LN	Oper	20	1	1	1	1	1		0		85	F	63	3	386	2	1	1	1	1	1466
AITL	60	65	5	4	1	LN	Biopsy		1	1	1	1	0	1	0	0	30	F	68	3	229	1	1	0	0	1	125
AITL	60	51	9	15	1	LN	Biopsy	10		1	1	1	0	1	0	0	50	M	69	3	326	1	0	0	0	1	282
AITL	60	62	2	10	1	LN	Oper	8	1	1	1		0	0	0	0	40	F	45	4	448	1	1	1	0	0	2284
AITL	60	51	7	2	1	LN	Biopsy		1	1	1	1	0	1	0	0	60	F	79	2	212	0	0	0	1	1	392
AITL	18.4	3.3	5.4			LN	Oper	40	1	1	1	1	0		1			M	67	2	397	1	1	1	0	0	909
AITL	32.3	8.9	5.9			LN	Oper	16	1	1	1	0	0	1		0	35	F	78	4	303	2	1	0	1	0	1322
AITL	39.0	9.9	8.5			LN	Oper	25	1	1	1	0	1	0		0	70	F	67	3	353	3	1	0	1	1	260
AITL	49.6	3.8	8.6			LN	Oper	14	1	1	1	1	1	1		0	50	M	66	4	265	1	1	1	0	1	603
AITL	40.9	3.8	4.1			LN	Oper	35	1	1	1	1		1	1			M	40	4	241	1	1	1	0	0	2059
ALCL-ALK-	90	3	4	7	1	LN	Oper	35	0	1	0		0		1	0	90	M	70	3	268	1	1	0		1	1254
ALCL-ALK-	96	8	1	2	0	LN	Oper		1	0	0		0		1		90	F	46	2	178	0	1	0	1	0	4743
ALCL-ALK-	90	96	4	6	1	LN	Biopsy		0	0	0	0	0		1		90	F	40	4	229	2	1	1	0	0	3237
ALCL-ALK-	90	90	1	11	4	LN	Oper			1	1	0	0		1		90	F	84	4	169	4	0	0		1	54
ALCL-ALK-	90	80	10	50	28	LN	Oper		0	1		0			0	0	95	F	73	4	175	3	1	0	1	1	298
ALCL-ALK-	50	92	1	0	69	LN	Oper	30	0	1	0	0	0		1		60	F	44	2	212	1	1	1	0	0	1982
ALCL-ALK-	60	58	29	61	6	LN	Biopsy		0	1	0	0	0		1	0		M	60	2	364		0	0	1	1	2008
ALCL-ALK-	85	72	7		9	LN	Oper		0	1	1		0		1		90	M	59	4		4	0	0		1	13
ALCL-ALK-		83.5	1.4	4.5		LN	Oper	40	0	1		0	0	0	1	0		F	50	4	279	1	1	0	0	0	1519
ALCL-ALK-		23.0	6.8	18.6		LN	Oper	15	1	1	1	1	0		1	0	90	M	86	N/D	N/D	3	0	0		1	245
ALCL-ALK-		71.5	11.6	15.5		LN	Oper		0	0	0	0	0		1	0	75	M	66	4	366	2	1	0	0	1	75
ALCL-ALK-		92.4	5.8	12.4		LN	Oper	10	0	1	0	1	0		1	0	75	M	53	4	136	1	1	1	1	1	696
ALCL-ALK-		40.8	3.9	14.9		LN	Oper	40	1	1	1	1	0		1		90	M	49	4	774		1	0	0	0	1225
ALCL-ALK+	90	85	4	1	1	Oropharynx	Oper		0	1	0		1		1			F	30	4	187	1	1	0	0	0	2166
ALCL-ALK+	20					LN	Oper		0	1		0			1		90	M	29	4	172	4	1	0	0	0	2059

ALCL ALK+	75	90	5	4	7	LN	Oper		0	1	0	0	0	1	80	F	42	3	186	1	1	0	0	0	3694		
ALCL ALK+	90	80	2	0	0	LN	Biopsy		0	1	1		1	1	0	80	F	13	3	151	0	0	0	0	2002		
ALCL ALK+	90	70	2	1	0	LN	Oper	10	0	1	1	1	1	1	1	75	M	8	4					1	97		
ALCL ALK+		75.8	1.9	3.3		LN	Biopsy		0	1	1	0	0	1	80	F	71	3	191	1	1	0	0	0	1479		
ALCL ALK+		80.9		17.4		LN	Oper	40	0	1	0	1	0	1	70	F	31	3	168	1	1	0	0	0	1594		
ALCL ALK+		76.0	1.5	7.3		LN	Biopsy		0	1	0	0	0	1	70	M	67	2	152	0	1	0		0	1526		
PTCL NOS	90	8	19	1	1	LN	Oper	65	1	1	1		0	0	1	70	M	70	4	468	3	1	0	1	1	213	
PTCL NOS	85	4	1	0	0	LN	Oper	20	1	1	1		0	0	0	0	M	47	4	673	1	1	1	1	0	819	
PTCL NOS	80	14	2	4	5	LN	Oper	25	1	1	1		0	0	0		F	79	2	248	1	0	0	0	1	4199	
PTCL NOS	70	37	0	1	0	Pancreas	Oper	14	1	1	1		0	0	0		F	52	2	265	1	1	1	0	0	5193	
PTCL NOS	75	18	9	7	3	LN	Oper	20	1	1	1		0	1		M	47	3	388	0	1	1	0	1	197		
PTCL NOS	30	9	2	7	10	Oropharynx	Oper		1	1	1	1	1	0	0	50	M	41	2	220	1	1	1	0	0	3655	
PTCL NOS	80	37	5	7	2	LN	Oper	20	1	1	1	1	0	1	1	0	80	F	51	4	404	3	1	1	0	0	3196
PTCL NOS	80	33	6	0	5	LN	Biopsy	43	1	1	1	1	0	0	1	1	70	F	80	4	435	0	0	0	1	1	538
PTCL NOS		87.4	6.6	12.1		LN	Oper	11	1	1	1	1	0	1	1	0	70	M	47	3	333	1	1	1	1	0	1729
PTCL NOS		6.3	7.6	1.6		LN	Oper	23	1	1	1	1	0	0		70	M	64	4	320 0	3	1	1	1	1	378	
PTCL NOS		59.4	1.6	11.8		LN	Oper	20	1	1	1	1	1		0		M	42	4	349	3	1	1	0	1	207	
PTCL NOS		20.0	2.0	10.3		LN	Oper	25	1	1	1	1	0	0		60	M	79	1	121	2	0	0		1	1407	

Abbreviations. Diagnosis (Dg). Tumor proportion (T%). Lymph node (LN). Lactate dehydrogenase from blood (LD). Overall survival time (OS time).

Table 3. Locus-specific primers for *STAT3* and *STAT5B* exons and RHOA G17V mutation hotspot.

Site	Forward primer	Reverse primer
STAT3 exon 2	TCCCCATCACCTGTACCCAT	TGACACCTGTGTTGGGCAAT
STAT3 exon 3	ACACTAACACCCGACTCTGC	TGTATGCGTCGGCTTCAGAG
STAT3 exon 4	TCCATTCCCTCCAGACCAGG	GCTCTGAAGCCTTTGTTCCG
STAT3 exon 5	CCGAGGCTTGTAACCTTGCAT	TCCCTTCCTCTTGTGATGG
STAT3 exon 6	GACCAGGCTCCTTTGAGGAC	CTCTTGGGGATACTGCCTGC
STAT3 exon 7	CCGATCTAGGCAGATGTTGG	TCCCTCAGGTCAAGGAGTTT
STAT3 exon 8	CTGTGGGCCTGCAGTTAAGA	GTTCTGCTCTGGAGTTGACT
STAT3 exon 9	AAGAGAAGATGGGCTCACGC	TCCCTTCTCCATCTCACCT
STAT3 exon 10	TGGAAAGAATGACCCTGGCC	CACGTGGTAGAGTGAGAGGC
STAT3 exon 11	AATGCACCCCAAGGCTTTTG	CCTCCACAGTGCTGAGATT
STAT3 exon 12-14	CAAGGAAAACACCCAGTTG	AAATAACAGGTGGTCAAAGTAGGC
STAT3 exon 15	ATTGCCAGATGGGATGCCAA	CCACACCTGGCCTAAGAGTG
STAT3 exon 16	GAGGAGAACTGCCAGCTCAG	CCTTTCATTCTGAGCCCCGT
STAT3 exon 17	AGGGAGAAGGGGTGAAATGC	TGCCCCCTCCTTTTAGTTGG
STAT3 exon 18	CCTTGCCAGCCATGTTTTCC	AACCTCTTGACCCCAAGCTG
STAT3 exon 19	GTGCACACTCTGTCCAACCT	GCTTGAAGGCCCTGAACTCT
STAT3 exon 20	GGAGTCAAGGCCATCTCCAC	TGGATGCCCTGTTAGCAATA
STAT3 exon 21	CCAAAAAATTAATGCCAGGA	GGTTCCATGATCTTTCCTTCC
STAT3 exon 22	CTCACCCAGTGTCCTTCC	GGCAGATGGAGCTTTCAGA
STAT3 exon 23	GACCAGCTCTCGGTGTGTAC	TGGAGACCAGAGTTTGTGGC
STAT3 exon 24	GGCACTTGTCTAAGAACAACAACA	AGTTGCAGAGGGTGGACAAC
STAT5B exons 14-15	CTGACCTCAACAAATAGTAAGTACCC	TTCCAAGTGTTACTCTGGTGTTT
STAT5B exon16	TGTTGGGGTTTTAAGATTTCC	CAAATCAGAAATGCCAACATTG
STAT5B exon 17	CCCAGGGCTGAGACAGTTT	AGATTGCACCACCGTACTCC
STAT5B exon 18	TGGATTCCCTTGACCCAGC	AGATACCCCTTGGTCCCCTC
STAT5B exon 19	CTGGTGGCCTGTGGGGCTTG	TCTGTCTGTGCCCCCTCTGCT
RHOA G17V	TGGAAAATGGCATCAGTTGTTATG	GCATTGCAGGTAATATCTGTGTTT
JAK1 exon 14	CCACCCACCCCTTTGAAAGA	ATTGATGTTTCAGGGCCTGGG
JAK1 exon 20	CCACCAGCTAGCATGTCAGA	CTGTGTGTTCCGTGGCCTA
JAK1 exon 24	ACCAGGCACACCTTTGTTCA	AGGGGATGAAGGAGAGGACC
JAK3 exon 11	TCTCTGGACCCAGACTGAG	GTCTCAACAGCAGCAGCAAC

Table 4. Densitometry readings of western blot stainings.

	β-Actin	JAK3	STAT3	pJAK3	pSTAT3
HH	14546719	17062962	17023719	6057477	355163
H9	9331062	9270477	15436477	4239406	53192
SupM2	13836376	17276012	13283305	1165577	18000619
L82	6454355	11489012	13808598	106778	13158012
Karpas299	8757012	13082891	17198012	680749	6562891
TLRB1	8803426	17857841	16716062	2462820	12840083
Molt4	9240719	12835719	15543184	54657	0
Jurkat	11107305	3284891	11095184	91778	0
NKL	15404719	14683305	9410012	3615941	0
MTA	15076891	14215719	2003355	96485	0
MOTN1	19614669	14053426	5048305	37243	0
SMZ1	12446477	3447891	7313962	66071	0



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