Mutations in the 3′ untranslated region of *NOTCH1* are associated with low CD20 expression levels in chronic lymphocytic leukemia

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Mutations in the 3′ untranslated region (3′ UTR) of *NOTCH1* are associated with low CD20 expression levels in chronic lymphocytic leukemia.

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Supplemental Materials and Methods

CLL cases
Primary neoplastic cells of the 662 CLL cases entering the study were separated by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation, and used either directly or cryopreserved until use, as reported.[1]

Next generation sequencing (NGS)
NOTCH1 mutational status was investigated by NGS with an amplicon based strategy. Specific primers for *NOTCH1* exon 34 and part of 3′ untranslated region (3′ UTR), carrying new mutation sites described by Puente *et al*.,[2] were designed with the ION Ampliseq designer (Thermo Scientific, Milan, Italy, https://www.ampliseq.com/), checked using the Primer3 program,[3,4] and modified according to Illumina (San Diego, CA) protocol adding specific adapter sequences (Table S1). Amplicon libraries were generated using a modified Illumina protocol. Briefly, multiplex PCR products were generated using Phusion High-Fidelity DNA Polymerase (Thermo Scientific, Milan, Italy) and subsequently tagged with specific index according to modified procedures for NexteraXT (DNALibrary Preparation kit, Illumina). Purified libraries were pooled, and paired-end sequenced in a MiSeq instrument (Illumina). The average sequencing coverage was >6000X. Across the whole target region, a coverage >1000x was obtained in >93% of the sequence in all samples.

Bioinformatic workflow
Sequencing reads were mapped to the human reference genome (GRCh37/hg19) using the Burrows-Wheeler Aligner–MEM algorithm (version 0.7.10).[5] Coverage along the targeted regions was analyzed using SAMtools (version 1.1).[6] Reads were filtered with a quality score >30. Variant calling was performed using the entire pipeline established on the MiSeq Reporter software (MSR; version 2.4.60). Variants found were annotated using ANNOVAR (version 2014Jul14).[7] Data were analyzed with MiSeq reporter (Illumina) and Integrative Genomics Viewer (IGV) software[8,9] against human genome assembly GRCh37/hg19. The *NOTCH1* mutational status was indicated as variant allele frequency (VAF, i.e. percentage of *NOTCH1* mutated DNA). Synonymous variants and polymorphisms described in the Single Nucleotide Polymorphism Database (dbSNP138), with a European population frequency higher than 1% (1000 Genomes Project database), were removed. *NOTCH1* mutations truncating the NOTCH1 protein were considered somatic mutations. Cases with truncating *NOTCH1* mutations, with a VAF between 45% and 55% were subjected to the cell sorting of CD3-positive cell population. In this sorted CD3-positive cell population, *NOTCH1* mutational status was investigated to further confirm the somatic origin of the mutation. The sensitivity of the NGS methodology was verified by performing four independent dilution experiments (3 somatic mutations and 1 SNP) to define the minimum detectable VAF. The approach was able to detect a minimum VAF of 1.3% (Figure S1A-B).

Verification of detected *NOTCH1* mutations
The presence of c.7541-7542delCT was verified by using an ARMS-PCR strategy detecting the presence of the mutation (see Table S2 for primer sequences, and Figure S1C).[1] The presence of coding *NOTCH1* mutations other than the c.7541-7542delCT was verified by Sanger sequencing when clonal (i.e. with a VAF≥12%, Table S3 for primer sequences and Figure S1B),[10] otherwise, when subclonal (i.e. with a VAF<12%) with a second independent NGS experiment. The 3′ UTR *NOTCH1* mutations were verified by a RT-PCR strategy detecting the presence of the aberrant splicing in 23/26 cases (Table S4 for primer sequences and Figure S1D). In the remaining 3/26 cases, a second independent NGS experiment was performed to verify the presence of the 3′ UTR *NOTCH1* mutation.

Quantitative real-time PCR (QRT-PCR)
Transcript expression levels the of genes of interest (i.e. *MS4A1*, *B2M*) were evaluated as reported.[1] Hydrolysis probe for *MS4A1* (Hs.PT.56a.24784282) was from Integrated DNA Technologies (IDT, Leuven, Belgium). Taqman Gene Expression assays for *B2M* (Hs00984230_m1), was from Life Technologies (Monza, Italy). Reactions were done in triplicate from the same cDNA reaction (technical replicates) with FastStart Universal Probe Master (Roche, Milan, Italy) on a CFX96 (Bio-Rad, Milan, Italy) instrument. The relative amount of each gene was calculated utilizing the expression of *B2M* as internal control using the equation $2^{-\Delta Ct}$ where $\Delta Ct=(Ct^{gene}-Ct^{B2M})$.[1]

CD20 expression
CD20 expression was evaluated by flow cytometry with a fluorescein isothiocyanate (FITC)-conjugated anti-CD20 antibody (clone L27, BD Biosciences, Milan, Italy, Table S5).[1] CD20 levels were expressed as Mean Fluorescence Intensity (MFI). Irrelevant isotype-matched antibodies were used to determine background fluorescence. Data reproducibility was ensured using as instrumental set-up an application setting linked to Cytometer Setup & Tracking Beads (CS&T Beads, BD Biosciences) ran daily. All the experiments were analyzed with FACSDiva (BD Biosciences) or FlowJo (FlowJo LLC, Ashland, USA) softwares.[1]

Western blot (WB)
WB was performed as reported,[1] using the following antibodies: anti-NOTCH1 intracytoplasmic domain (NICD, Val1744, clone D3B8, CST-Cell Signaling Technology, Leiden, The Netherlands), anti-NOTCH1 (clone D1E11, CST), anti-CD20 (clone L26, Abcam, Cambridge, UK), and anti-β-actin (clone AC-74, Sigma, Milan, Italy) as control (Table S5). Total proteins were extracted in RIPA lysis buffer (Santa Cruz Biotechnology, Heidelberg, Germany), quantified through Bradford assay (Bio-Rad) and ran in 4-15% SDS-PAGE precast gels (Bio-Rad) prior to transfer to nitrocellulose membranes (Trans-Blot Turbo pack, Bio-Rad). Detection was performed with HRP-conjugated antibodies (GE Healthcare) and ClarityECL (Bio-Rad, Milan, Italy) or ECLprime (GE Healthcare, for high sensitivity conditions to detect CD20 expression) on a ChemiDoc Touch Imaging System (Bio-Rad). Image analysis was performed with ImageLab software (Bio-Rad).

Cell Sorting
CLL cells from selected *NOTCH1* mutated cases were sorted according to CD20 expression by using the phycoerythrin (PE) -conjugated anti-CD20 antibody (clone L27, BD Biosciences, Milan, Italy). The CD20^{low} or CD20^{high} fractions were selected below the 25th percentile or above the 75th percentile of CD20 expression, respectively. Sorting was performed utilizing a FACSAriaIII cell sorter (BD Biosciences), as described.[1]

In-vitro treatment with γ-secretase inhibitor (GSI)
Purified primary CLL cells ($2x10^6$cells/ml) were treated with the γ-secretase inhibitor (GSI L-685,458, Sigma, 10μM for 6-24 hours). In control experiments, equal volume of dimethyl sulfoxide (DMSO) was added.

Complement-dependent-citotoxicity (CDC) assay
CDC assay was performed in primary CLL, as described.[11] Residual viable cells were evaluated by staining cells with 7-amino-actinomycin-D (BD Biosciences), as described.[11,12] In particular, $2x10^5$ primary CLL cells were incubated with rituximab (5μg/ml) or with ofatunumab (5μg/ml) in a final volume of 150 μl for 10 min at room temperature prior to the addition of PNHS (25%) and a further incubation at 37° C for 1 hour.

Statistical analysis

All statistical analyses were performed with Medcalc software (Medcalc Software, Ostend, Belgium). Data were compared using two-sided Student's t-test for independent or paired (GSI treated/untreated) samples, as reported.[1] Variance similarity between compared groups was tested with Fisher's exact-test. Distribution normality was tested with D'Agostino-Pearson test. A p-value smaller than 0.05 was considered as significant.[1]

References

1 Pozzo F, Bittolo T, Arruga F et al. NOTCH1 mutations associate with low CD20 level in chronic lymphocytic leukemia: evidence for a NOTCH1 mutation-driven epigenetic dysregulation. Leukemia. 2016;30(1):182-189.

2 Puente XS, Bea S, Valdes-Mas R et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. Nature. 2015;526(7574):519-524.

3 Koressaar T, Remm M. Enhancements and modifications of primer design program Primer3. Bioinformatics. 2007;23(10):1289-1291.

4 Untergasser A, Cutcutache I, Koressaar T et al. Primer3--new capabilities and interfaces. Nucleic Acids Res. 2012;40(15):e115.

5 Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25(14):1754-1760.

6 Li H, Handsaker B, Wysoker A et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25(16):2078-2079.

7 Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38(16):e164.

8 Robinson JT, Thorvaldsdottir H, Winckler W et al. Integrative genomics viewer. Nat Biotechnol. 2011;29(1):24-26.

9 Thorvaldsdottir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Brief Bioinform. 2013;14(2):178-192.

10 Nadeu F, Delgado J, Royo C et al. Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1 and ATM mutations in chronic lymphocytic leukemia. Blood. 2016.

11 Macor P, Tripodo C, Zorzet S et al. In vivo targeting of human neutralizing antibodies against CD55 and CD59 to lymphoma cells increases the antitumor activity of rituximab. Cancer Res. 2007;67(21):10556-10563.

12 Zucchetto A, Vaisitti T, Benedetti D et al. The CD49d/CD29 complex is physically and functionally associated with CD38 in B-cell chronic lymphocytic leukemia cells. Leukemia. 2012;26(6):1301-1312.

Table S1. Primers for targeted ultra-deep NGS.

Target Gene	Target region	Forward primer (5'-3')	Reverse primer (5'-3')
NOTCH1	**exon_34**	GGGTGGGTTTCAGAAGATGTATCAAAG	TGCGCACCAGGTTGTACTC
NOTCH1	**exon_34**	AGCGCATGCATCACGACATC	CACGTCTGACAGGTAGCCAT
NOTCH1	**exon_34**	CTCAAGGCACGGAGGAAGAAGTC	GAGGTGGGCCAGTCTCAAAG
NOTCH1	**exon_34**	CATCGGGCACCTGAACGT	GCAGAGGGTTGTATTGGTTCG
NOTCH1	**exon_34**	CAGTTTGAATGGTCAATGCGAGT	ACTTTGCTGCTGCTGGATGTTT
NOTCH1	**exon_34**	CAGCCACAAAACTTACAGATGCA	GTCCACAGGCGAGGAGTAGCT
NOTCH1	**exon_34**	ATGGCGGTGCACACTATTCTG	ACCGAAGGCTTGGGAAAGGAAG
NOTCH1	**exon_34**	TGAGAGCCAGGGTTTTCCTA	CCAGGAGCTTTTTGGACTATGC

Forward primer adapter: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG

Reverse primer adapter: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG

Forward and reverse primer adapters are designed according to Illumina.

Table S2. Primers for ARMS-PCR.

Target Gene	chr	Genomic position (hg19)	DNA change	Control forward primer (5'-3')	Mutant forward primer (5'-3')	Control reverse primer (5'-3')
NOTCH1	chr9	139390648	CAG>C	AACTGGGCTGCGGTCAC	TCGGGACGGGGTGAGGA	GCTTCCTTTCCCAAGCCTT

Table S3. Primers for Sanger sequencing of the *NOTCH1* gene.

Forward primer (5'-3')	Reverse primer (5'-3')	Experiment
GGCCTGCAGTTGGTTAGATTTTC	GGGTGCACTCTTGGCATACACA	DNA amplification
AAGGCTTGGGAAAGGAAGC	CTCAACCACCTGCCTGGGAT	Sequencing
GGTAGCTCATCATCTGGGACA	GCTGCTTCCTCTGGTGATGG	Sequencing

Table S4. Primers for detection of *NOTCH1* aberrant splicing in cases with mutations in the 3' UTR.

Target Gene	Target region	Forward primer (5'-3')	Reverse primer (5'-3')
NOTCH1	3'_UTR	CCTAACAGGCAGGTGATGCT	TACTCCTCGCCTGTGGACAA

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Table S5. Antibodies for western blotting and flow cytometry.

Target Protein	Clone	Species	Conjugation	Vendor	ID	Dilution
NOTCH1	D1E11	Rabbit		CST	#3608	1/3000 (WB)
NICD	D3B8	Rabbit		CST	#4147	1/1000 (WB)
CD20	L26	Mouse		Abcam	ab9475	1/500-1/2000 (WB)
β-Actin	AC-15	Mouse	HRP	Sigma	A3854	1/100 000 (WB)
Anti-Rabbit		Donkey	HRP	GE Healthcare	NA-934	1/3000 (WB)
Anti-Mouse		Sheep	HRP	GE Healthcare	NA-931	1/1000-1/3000 (WB)
Mouse Isotype	MOPC-21	Mouse	PE	BD	555749	20ul/test (Flow)
CD20	L27	Mouse	PE	BD	345793	20ul/test (Flow)
CD20	L27	Mouse	FITC	BD	345792	20ul/test (Flow)
CD19	HIB19	Mouse	APC	BD	555415	20ul/test (Flow)
CD3	SK7	Mouse	APC-H7	BD	560275	20ul/test (Flow)

Table S6. Biological characterization of the CLL cohort (662 cases).

ID #	NOTCH1 status[a]	IGHV status[b]	FISH status[c]	CD49d[d]	CD38[d]	ZAP-70[d]	CD20 MFI[e]
1	wt	m	del13p	neg	neg	neg	1996
2	mut	um	norm	pos	pos	pos	4665
3	wt	m	del13p	neg	neg	neg	3329
4	wt	um	del13p	neg	pos	neg	2580
5	wt	m	del13p	pos	neg	neg	5523
6	wt	um	norm	neg	neg	neg	2298
7	mut	na	del17p	pos	pos	neg	4253
8	mut	m	del17p	pos	neg	neg	4322
9	mut	um	norm	pos	pos	pos	1437
10	mut	um	tris12	pos	pos	pos	2807
11	wt	um	norm	neg	neg	pos	3449
12	wt	um	del11q	neg	pos	neg	1671
13	mut	um	del17p	pos	pos	pos	5597
14	wt	um	norm	pos	neg	neg	31016
15	wt	m	tris12	pos	neg	neg	36209
16	wt	um	tris12	pos	pos	neg	22099
17	wt	m	del13p	neg	neg	pos	2113
18	mut	um	tris12	pos	pos	pos	2313
19	wt	m	del17p	neg	neg	pos	679
20	wt	um	del11q	neg	neg	neg	2315
21	wt	m	norm	neg	neg	pos	1296
22	wt	na	del13p	neg	neg	neg	2640
23	wt	um	del13p	pos	pos	pos	1595
24	wt	um	norm	neg	pos	pos	2254
25	wt	m	norm	neg	neg	neg	1801
26	wt	m	norm	pos	neg	neg	2877
27	wt	m	del11q	neg	neg	neg	1133
28	wt	m	del11q	pos	neg	neg	1741
29	wt	m	norm	neg	neg	neg	1242
30	mut	um	del17p	pos	pos	pos	1857
31	wt	na	norm	neg	neg	neg	3815
32	wt	um	norm	pos	pos	pos	12058
33	wt	m	norm	neg	neg	neg	1812
34	mut	um	tris12	pos	pos	pos	3655
35	wt	m	del13p	neg	neg	neg	8044
36	wt	m	del13p	neg	neg	neg	2439
37	wt	m	norm	neg	neg	neg	4822
38	mut	um	tris12	pos	pos	neg	6212
39	wt	m	norm	pos	pos	neg	15099
40	wt	um	norm	neg	pos	neg	1475
41	wt	m	del13p	neg	neg	neg	2556
42	wt	m	norm	pos	neg	neg	1387
43	wt	m	del13p	neg	neg	neg	939
44	wt	m	del13p	neg	neg	neg	2200
45	mut	um	tris12	pos	neg	pos	2080
46	wt	um	norm	neg	neg	neg	2432
47	wt	um	norm	neg	neg	pos	2110
48	wt	m	del13p	neg	neg	neg	3342
49	wt	m	norm	neg	neg	neg	2478
50	wt	m	del13p	pos	neg	neg	12184
51	wt	um	tris12	pos	pos	neg	12555
52	wt	m	del13p	neg	neg	neg	2393
53	wt	m	del13p	neg	neg	neg	901
54	wt	m	tris12	pos	neg	neg	32914
55	mut	m	norm	neg	neg	neg	1366
56	wt	um	del13p	pos	neg	pos	5099
57	wt	um	del11q	neg	pos	neg	1004
58	wt	m	del13p	neg	neg	neg	1663

ID #	NOTCH1 status[a]	IGHV status[b]	FISH status[c]	CD49d[d]	CD38[d]	ZAP-70[d]	CD20 MFI[e]
59	wt	m	del13p	neg	neg	neg	2553
60	wt	um	del13p	neg	neg	pos	4944
61	wt	m	del13p	neg	neg	neg	3057
62	wt	m	del13p	neg	neg	neg	1636
63	wt	um	tris12	neg	neg	pos	2892
64	wt	m	del13p	neg	neg	pos	4821
65	wt	m	tris12	neg	neg	pos	3308
66	mut	um	del13p	pos	pos	pos	3756
67	wt	m	del11q	neg	neg	neg	2707
68	wt	m	tris12	pos	neg	pos	11727
69	wt	um	tris12	pos	neg	pos	7689
70	wt	m	del13p	neg	neg	neg	5431
71	wt	m	del13p	neg	neg	pos	2469
72	mut	um	norm	pos	neg	pos	3098
73	wt	m	del13p	neg	neg	neg	3411
74	wt	m	norm	neg	neg	neg	1213
75	wt	m	norm	neg	neg	neg	3012
76	wt	m	norm	neg	neg	neg	2963
77	wt	um	del13p	neg	neg	neg	3579
78	wt	um	del11q	neg	pos	neg	4502
79	wt	m	del13p	neg	neg	neg	1925
80	wt	um	del17p	neg	neg	neg	3886
81	wt	m	del13p	neg	neg	neg	4178
82	mut	um	norm	neg	pos	pos	4342
83	wt	m	tris12	pos	pos	neg	11480
84	wt	m	del13p	pos	pos	neg	4468
85	wt	um	del17p	pos	neg	pos	1294
86	mut	m	del13p	neg	neg	neg	5091
87	wt	m	del13p	neg	neg	neg	5373
88	wt	m	del13p	pos	neg	pos	8169
89	wt	m	del13p	neg	neg	neg	1668
90	wt	m	tris12	pos	neg	pos	25468
91	wt	um	del13p	neg	neg	pos	4815
92	wt	um	tris12	neg	neg	pos	2987
93	wt	m	del13p	neg	neg	neg	3115
94	wt	m	norm	neg	neg	pos	2769
95	mut	m	norm	neg	neg	pos	2925
96	wt	m	del13p	pos	neg	pos	4822
97	wt	m	norm	pos	pos	pos	28751
98	wt	na	del17p	pos	neg	neg	24530
99	wt	m	tris12	pos	neg	pos	29097
100	wt	m	norm	neg	neg	neg	2290
101	wt	m	del13p	neg	neg	neg	4124
102	wt	m	del11q	neg	neg	pos	1588
103	wt	na	del17p	pos	neg	neg	690
104	wt	m	del13p	neg	neg	pos	5276
105	mut	m	del13p	pos	neg	pos	979
106	wt	na	del17p	pos	neg	pos	2431
107	wt	m	del13p	neg	neg	pos	2093
108	wt	m	del17p	neg	neg	neg	2368
109	wt	m	del13p	pos	pos	neg	24048
110	wt	um	norm	pos	pos	pos	2695
111	wt	um	norm	pos	pos	pos	1767
112	mut	na	norm	neg	neg	neg	1248
113	wt	um	del13p	pos	neg	pos	7866
114	wt	m	del13p	neg	neg	neg	7431
115	wt	m	del13p	neg	neg	neg	2082
116	wt	na	del13p	neg	neg	neg	1288
117	NOTwt1 status[a]	IGHV status[b] m	del17p	na	na	pos	5932
118	wt	m	del13p	neg	neg	neg	1954
119	wt	na	del17p	neg	pos	neg	1189

ID #	NOTCH1 status[a]	IGHV status[b]	FISH status[c]	CD49d[d]	CD38[d]	ZAP-70[d]	CD20 MFI[e]
120	wt	na	del17p	neg	neg	neg	4230
121	mut	um	norm	pos	pos	pos	1835
122	wt	m	del13p	pos	neg	pos	3912
123	wt	m	del13p	neg	neg	pos	3213
124	wt	m	del17p	neg	neg	pos	3064
125	wt	na	del17p	neg	neg	neg	1235
126	mut	na	del13p	neg	pos	pos	1471
127	wt	na	del13p	neg	neg	pos	2091
128	wt	na	del13p	neg	neg	pos	2315
129	wt	um	norm	pos	neg	pos	3720
130	wt	na	norm	pos	neg	pos	3552
131	wt	m	norm	pos	pos	pos	4600
132	wt	m	norm	pos	neg	pos	2797
133	wt	m	del13p	neg	neg	pos	3520
134	wt	m	norm	neg	neg	pos	2380
135	mut	m	del13p	neg	neg	pos	3951
136	wt	m	del13p	neg	neg	pos	3354
137	wt	na	del13p	neg	neg	pos	1916
138	wt	um	tris12	pos	pos	pos	2065
139	wt	na	del17p	neg	neg	pos	1640
140	mut	na	tris12	pos	neg	pos	1844
141	wt	na	del13p	neg	neg	pos	2470
142	wt	m	del13p	neg	neg	neg	2320
143	wt	um	del13p	pos	neg	pos	1108
144	wt	m	norm	neg	neg	pos	2386
145	wt	m	del13p	neg	neg	pos	3109
146	wt	um	del13p	neg	neg	neg	2172
147	wt	um	del17p	pos	pos	pos	1945
148	wt	m	del13p	neg	neg	pos	3069
149	wt	m	del11q	pos	neg	pos	20820
150	wt	m	del13p	neg	neg	neg	2808
151	wt	m	del13p	neg	neg	pos	1741
152	mut	um	del13p	pos	pos	pos	2264
153	mut	um	norm	neg	pos	pos	3504
154	wt	m	norm	neg	neg	pos	1584
155	wt	um	del11q	pos	pos	pos	3152
156	wt	um	norm	neg	neg	pos	2506
157	wt	m	norm	neg	neg	pos	2300
158	mut	um	del17p	neg	pos	pos	2297
159	wt	m	norm	pos	pos	pos	10889
160	wt	m	norm	pos	pos	neg	6564
161	wt	um	del17p	neg	neg	pos	3595
162	wt	m	del17p	neg	neg	pos	2027
163	mut	um	del13p	pos	pos	pos	3233
164	wt	m	del13p	pos	neg	pos	2615
165	wt	m	del13p	neg	neg	pos	1380
166	wt	m	del17p	pos	neg	pos	9638
167	mut	um	tris12	pos	pos	pos	2126
168	wt	um	norm	pos	pos	pos	4138
169	wt	m	norm	neg	neg	pos	2378
170	wt	um	del17p	neg	neg	pos	1773
171	wt	m	tris12	pos	pos	pos	26131
172	wt	m	del13p	neg	neg	neg	1939
173	wt	na	del13p	neg	neg	neg	1727
174	wt	um	del11q	neg	neg	pos	3177
175	mut	na	del11q	neg	neg	pos	3054
176	wt	um	del11q	neg	neg	neg	2481
177	wt	m	tris12	pos	pos	pos	16810
178	mut	um	tris12	pos	neg	pos	2477
179	wt[a]	um[b]	del11q	neg	pos	pos	3181
180	wt	m	tris12	neg	neg	pos	3489

ID #	NOTCH1 status[a]	IGHV status[b]	FISH status[c]	CD49d[d]	CD38[d]	ZAP-70[d]	CD20 MFI[e]
181	wt	um	del13p	neg	neg	pos	1600
182	mut	m	del13p	neg	neg	pos	4406
183	wt	m	del17p	neg	neg	neg	1894
184	wt	um	tris12	pos	neg	pos	1276
185	wt	m	del13p	neg	neg	na	3512
186	mut	um	tris12	pos	neg	na	3282
187	wt	um	del13p	neg	pos	na	1721
188	wt	um	norm	neg	neg	pos	1651
189	wt	m	del13p	neg	neg	pos	1356
190	wt	m	del13p	neg	neg	pos	3865
191	wt	m	del13p	neg	neg	pos	1583
192	mut	um	tris12	pos	pos	pos	5283
193	wt	m	tris12	pos	pos	pos	25248
194	wt	um	del13p	neg	neg	pos	804
195	wt	m	del13p	neg	neg	pos	5983
196	wt	m	del13p	neg	neg	pos	3812
197	wt	m	del13p	pos	neg	pos	3528
198	wt	na	del17p	neg	neg	neg	4950
199	wt	m	del17p	pos	neg	pos	20018
200	wt	m	del13p	neg	neg	pos	2549
201	wt	m	del11q	neg	neg	pos	4420
202	wt	m	tris12	pos	pos	pos	14291
203	wt	m	del17p	neg	neg	pos	1833
204	wt	m	del17p	neg	neg	pos	2054
205	mut	m	tris12	pos	neg	pos	4162
206	wt	m	tris12	pos	pos	pos	5402
207	mut	um	tris12	neg	pos	pos	1222
208	wt	um	del13p	neg	neg	pos	1037
209	wt	m	tris12	pos	neg	pos	7621
210	mut	um	norm	neg	neg	pos	1160
211	wt	m	del17p	neg	neg	pos	1995
212	mut	um	del17p	pos	pos	pos	1814
213	mut	um	norm	pos	pos	pos	472
214	mut	um	del17p	pos	pos	pos	1470
215	wt	um	norm	pos	pos	pos	573
216	mut	m	norm	neg	pos	pos	588
217	wt	m	del11q	neg	neg	pos	1377
218	wt	um	del17p	neg	neg	pos	1844
219	wt	m	del13p	pos	neg	pos	2090
220	wt	m	norm	pos	neg	pos	1359
221	wt	m	norm	pos	neg	pos	963
222	mut	um	norm	pos	neg	pos	228
223	wt	m	norm	pos	neg	neg	3255
224	wt	m	norm	pos	pos	pos	9336
225	wt	um	del13p	pos	pos	pos	1085
226	wt	um	norm	pos	pos	pos	8508
227	wt	m	del13p	neg	neg	pos	984
228	wt	m	del17p	neg	neg	pos	3804
229	wt	m	norm	pos	neg	pos	1541
230	mut	um	tris12	pos	neg	pos	2661
231	wt	m	del17p	neg	neg	pos	1602
232	mut	um	tris12	pos	pos	pos	772
233	wt	um	del11q	neg	neg	pos	478
234	mut	um	tris12	pos	pos	pos	4192
235	wt	m	norm	neg	neg	pos	3234
236	wt	na	del13p	neg	neg	neg	2426
237	wt	m	norm	neg	neg	neg	825
238	wt	um	del11q	pos	pos	pos	521
239	NOTCH1 status[a] wt	IGHV status[b] m	del17p	pos	neg	pos	11921
240	wt	m	del13p	pos	pos	pos	13057
241	wt	m	del17p	neg	neg	pos	1514

ID #	NOTCH1 status[a]	IGHV status[b]	FISH status[c]	CD49d[d]	CD38[d]	ZAP-70[d]	CD20 MFI[e]
242	wt	m	tris12	neg	neg	pos	1734
243	wt	m	tris12	neg	neg	pos	901
244	wt	m	tris12	pos	pos	pos	5737
245	wt	m	del13p	neg	neg	neg	837
246	wt	um	del13p	neg	pos	pos	3699
247	wt	m	del11q	pos	neg	neg	963
248	wt	m	del13p	pos	pos	pos	11136
249	wt	m	del11q	pos	pos	pos	6412
250	wt	m	del13p	pos	neg	neg	1272
251	wt	m	del13p	neg	neg	neg	1885
252	wt	m	norm	pos	neg	pos	6778
253	mut	um	tris12	pos	neg	pos	1299
254	wt	m	del13p	pos	neg	pos	561
255	wt	m	norm	na	na	pos	11484
256	wt	um	tris12	pos	neg	pos	10704
257	wt	um	del17p	pos	neg	pos	1326
258	wt	m	del13p	neg	neg	pos	1218
259	mut	um	del11q	neg	neg	pos	995
260	wt	m	del13p	neg	neg	pos	1472
261	wt	m	del17p	pos	neg	pos	17614
262	wt	m	del13p	neg	neg	pos	801
263	wt	m	del13p	neg	neg	pos	2076
264	wt	um	tris12	pos	pos	pos	2395
265	wt	m	del17p	pos	neg	pos	834
266	wt	um	del17p	neg	pos	pos	525
267	wt	m	norm	neg	neg	pos	695
268	wt	um	norm	pos	neg	pos	904
269	wt	m	del13p	neg	neg	neg	1621
270	mut	um	norm	pos	pos	pos	1702
271	wt	m	del13p	neg	neg	pos	1296
272	wt	m	del17p	neg	neg	pos	568
273	wt	m	del13p	neg	neg	pos	434
274	wt	m	del13p	neg	neg	pos	2049
275	wt	um	tris12	pos	neg	pos	5533
276	mut	m	del13p	pos	neg	pos	2646
277	wt	m	del13p	neg	neg	pos	996
278	wt	m	del17p	pos	neg	pos	1469
279	wt	um	del17p	pos	neg	pos	2647
280	wt	um	tris12	pos	pos	pos	5670
281	wt	na	del13p	neg	neg	pos	1540
282	wt	m	del17p	neg	neg	pos	3321
283	wt	m	norm	neg	neg	pos	711
284	wt	um	norm	neg	pos	pos	1194
285	wt	um	del11q	neg	pos	pos	1121
286	mut	um	tris12	pos	pos	pos	1686
287	mut	um	del13p	neg	neg	pos	803
288	wt	m	norm	pos	neg	pos	646
289	mut	um	tris12	pos	pos	pos	1945
290	mut	na	norm	neg	pos	pos	796
291	mut	um	del13p	pos	pos	pos	1007
292	wt	um	norm	pos	pos	pos	642
293	mut	m	tris12	neg	neg	pos	3437
294	wt	um	norm	pos	pos	pos	2158
295	wt	um	tris12	neg	neg	pos	186
296	wt	m	del13p	neg	neg	neg	205
297	wt	m	del13p	neg	neg	pos	1957
298	wt	m	del13p	neg	neg	neg	3616
299	wt	m	del13p	pos	neg	pos	8324
300	NOTCH1 status[a]	IGHV status[b]	del13p	neg	neg	neg	698
301	wt	m	del13p	neg	neg	neg	1255
302	wt	m	tris12	pos	neg	neg	10801

ID #	NOTCH1 status[a]	IGHV status[b]	FISH status[c]	CD49d[d]	CD38[d]	ZAP-70[d]	CD20 MFI[e]
303	wt	um	del17p	neg	pos	pos	1904
304	wt	na	del13p	neg	neg	neg	141
305	mut	um	del13p	pos	pos	neg	54
306	wt	m	del13p	neg	neg	neg	713
307	wt	m	del13p	neg	neg	pos	1038
308	mut	um	tris12	pos	neg	pos	1516
309	wt	m	norm	neg	neg	pos	1132
310	mut	um	del13p	neg	pos	pos	2205
311	wt	m	norm	pos	neg	pos	18411
312	wt	m	tris12	pos	neg	neg	6021
313	mut	m	del13p	neg	neg	neg	1783
314	wt	um	del11q	neg	neg	pos	1956
315	wt	m	del13p	neg	neg	neg	419
316	wt	um	del13p	neg	neg	neg	2437
317	wt	m	del13p	neg	neg	neg	2184
318	wt	um	norm	neg	neg	neg	752
319	wt	m	del13p	neg	neg	neg	1408
320	wt	um	tris12	pos	pos	neg	3187
321	wt	um	del11q	neg	pos	pos	1413
322	mut	um	tris12	pos	pos	pos	709
323	mut	um	del11q	neg	pos	pos	2355
324	wt	m	del13p	pos	neg	neg	17032
325	wt	um	norm	neg	pos	pos	1478
326	wt	m	del13p	neg	neg	neg	993
327	wt	m	norm	neg	pos	pos	1207
328	wt	m	del13p	neg	neg	neg	1639
329	wt	m	del13p	neg	neg	neg	3053
330	wt	m	del13p	neg	neg	pos	2415
331	mut	um	del13p	neg	neg	pos	853
332	wt	um	norm	pos	pos	pos	957
333	wt	m	tris12	neg	neg	neg	1339
334	wt	m	norm	neg	neg	neg	1094
335	wt	m	del13p	neg	neg	neg	739
336	wt	um	del11q	neg	neg	pos	411
337	wt	m	norm	neg	neg	neg	578
338	wt	m	del13p	neg	neg	neg	1962
339	wt	m	norm	neg	neg	neg	1233
340	mut	um	tris12	pos	pos	pos	1455
341	wt	m	norm	neg	pos	neg	751
342	mut	um	del13p	neg	pos	pos	610
343	wt	m	del13p	neg	neg	pos	1647
344	wt	m	tris12	pos	neg	neg	2080
345	wt	m	del13p	neg	neg	neg	615
346	mut	um	del17p	pos	pos	pos	2434
347	wt	m	del13p	neg	neg	pos	2598
348	wt	um	del11q	pos	pos	pos	2314
349	wt	m	norm	neg	neg	pos	358
350	wt	m	del13p	neg	neg	pos	2481
351	wt	um	norm	neg	neg	pos	588
352	wt	m	del13p	neg	neg	pos	1330
353	wt	m	del13p	neg	neg	neg	910
354	wt	um	del11q	neg	neg	pos	1630
355	wt	m	norm	neg	neg	pos	3940
356	wt	um	del11q	neg	neg	pos	554
357	wt	m	norm	neg	neg	pos	258
358	wt	m	norm	pos	neg	neg	1112
359	wt	um	del13p	pos	neg	pos	2046
360	wt	na	del13p	neg	neg	pos	918
361	NOTCH1 wt status[a]	m	del13p	neg	neg	pos	2356
362	status[a]	m	del13p	neg	neg	pos	1615
363	wt	na	del17p	neg	neg	neg	690

ID #	NOTCH1 status[a]	IGHV status[b]	FISH status[c]	CD49d[d]	CD38[d]	ZAP-70[d]	CD20 MFI[e]
364	wt	um	norm	pos	pos	pos	4609
365	mut	um	tris12	pos	pos	pos	3793
366	wt	um	del13p	neg	pos	pos	3434
367	wt	m	tris12	pos	neg	pos	4501
368	wt	um	norm	pos	pos	pos	2256
369	mut	um	norm	pos	pos	pos	2302
370	wt	m	norm	neg	neg	pos	1716
371	wt	m	del13p	neg	neg	pos	591
372	wt	m	del17p	pos	neg	pos	6978
373	wt	um	del17p	pos	neg	pos	2738
374	wt	um	del11q	neg	neg	pos	802
375	wt	m	del13p	neg	neg	pos	2553
376	wt	um	del11q	neg	neg	neg	1796
377	wt	m	tris12	pos	pos	neg	10857
378	wt	m	del13p	pos	neg	pos	2254
379	wt	m	del17p	neg	neg	pos	2526
380	wt	m	norm	pos	neg	pos	12610
381	wt	m	tris12	neg	neg	pos	3331
382	wt	m	del13p	pos	neg	pos	14634
383	wt	m	norm	neg	neg	pos	779
384	wt	m	del13p	neg	neg	neg	1230
385	wt	um	norm	pos	neg	pos	793
386	wt	m	del13p	neg	neg	pos	1511
387	wt	m	norm	pos	neg	pos	22258
388	wt	m	del13p	pos	neg	pos	10987
389	wt	m	del13p	pos	neg	pos	2003
390	mut	um	del11q	neg	neg	pos	3086
391	wt	um	del13p	neg	neg	pos	1391
392	wt	m	del13p	neg	neg	pos	1524
393	wt	m	norm	pos	neg	pos	17394
394	wt	m	tris12	pos	pos	pos	10278
395	wt	m	del13p	neg	neg	pos	2707
396	wt	m	del13p	neg	neg	neg	2260
397	wt	m	del13p	neg	neg	pos	3220
398	wt	m	del13p	neg	neg	neg	1155
399	wt	m	del11q	neg	pos	pos	3981
400	mut	um	tris12	pos	pos	pos	2443
401	mut	um	del17p	pos	neg	neg	1765
402	mut	m	tris12	neg	pos	pos	2683
403	wt	m	del13p	neg	neg	neg	2038
404	wt	m	norm	neg	neg	neg	1414
405	wt	um	norm	pos	pos	pos	3447
406	wt	m	tris12	pos	pos	pos	6231
407	wt	m	del13p	neg	neg	pos	2422
408	wt	m	norm	neg	neg	neg	2969
409	wt	m	del13p	neg	neg	neg	3289
410	wt	m	del13p	neg	neg	neg	1572
411	wt	m	del17p	neg	neg	neg	548
412	wt	m	norm	neg	pos	pos	989
413	wt	m	del13p	neg	neg	neg	1205
414	wt	m	del13p	pos	pos	pos	2020
415	wt	m	del13p	neg	neg	neg	2950
416	wt	m	norm	neg	neg	neg	1185
417	wt	m	del13p	na	na	neg	4602
418	wt	m	norm	pos	pos	pos	2251
419	wt	um	tris12	pos	pos	pos	1953
420	wt	m	del17p	pos	pos	neg	2754
421	wt	m	del13p	neg	neg	neg	1883
422	wt1	umv	norm	neg	neg	pos	1163
423	stmut[a]	stum[b]	del11q	neg	pos	pos	2334
424	mut	um	del17p	neg	neg	pos	697

ID #	NOTCH1 status[a]	IGHV status[b]	FISH status[c]	CD49d[d]	CD38[d]	ZAP-70[d]	CD20 MFI[e]
425	wt	m	del13p	neg	neg	neg	1659
426	wt	m	del13p	neg	neg	neg	3500
427	mut	um	tris12	pos	pos	pos	554
428	wt	m	norm	neg	neg	neg	1638
429	wt	um	del13p	neg	neg	pos	1586
430	mut	um	tris12	pos	pos	pos	2404
431	wt	um	norm	neg	neg	neg	2743
432	mut	um	del11q	neg	neg	neg	2366
433	wt	um	del13p	neg	neg	neg	1700
434	wt	um	tris12	neg	neg	neg	622
435	wt	m	del13p	pos	pos	neg	1808
436	wt	um	tris12	pos	pos	pos	3374
437	wt	m	tris12	pos	pos	neg	10364
438	wt	m	norm	pos	neg	neg	3822
439	wt	m	del13p	neg	neg	neg	3175
440	wt	um	del13p	neg	neg	pos	968
441	wt	um	del11q	neg	pos	pos	1076
442	wt	m	del13p	neg	neg	neg	1029
443	wt	m	norm	pos	neg	neg	7829
444	wt	um	del13p	neg	neg	neg	628
445	wt	um	del11q	neg	pos	pos	1800
446	wt	m	del13p	neg	neg	neg	1226
447	wt	m	del13p	neg	neg	neg	1128
448	wt	m	del13p	neg	neg	pos	2453
449	wt	um	del13p	neg	pos	pos	3874
450	wt	um	del17p	neg	pos	pos	2227
451	wt	na	del13p	neg	neg	neg	1650
452	wt	m	norm	neg	neg	pos	9282
453	wt	m	norm	neg	neg	neg	2168
454	wt	m	del13p	neg	neg	pos	1680
455	mut	m	del17p	neg	neg	pos	4669
456	mut	m	tris12	pos	pos	neg	4053
457	mut	m	del13p	neg	neg	neg	1581
458	wt	um	norm	neg	neg	pos	1068
459	mut	um	tris12	pos	neg	pos	1450
460	wt	um	norm	neg	neg	neg	2305
461	mut	um	norm	pos	pos	pos	1711
462	mut	um	del11q	pos	pos	pos	1471
463	wt	na	norm	neg	neg	neg	1450
464	wt	m	norm	neg	neg	neg	27
465	wt	um	norm	pos	neg	neg	2847
466	mut	m	norm	pos	pos	neg	2177
467	wt	m	del13p	neg	neg	neg	1362
468	mut	um	tris12	pos	neg	pos	897
469	wt	m	norm	pos	pos	neg	12362
470	mut	um	del13p	pos	pos	pos	2242
471	wt	um	tris12	pos	pos	pos	3111
472	wt	um	norm	neg	neg	pos	391
473	wt	m	tris12	pos	pos	neg	11468
474	wt	m	del13p	neg	neg	neg	1801
475	wt	m	del17p	neg	neg	neg	949
476	wt	m	tris12	pos	neg	pos	5943
477	mut	m	del13p	neg	neg	neg	1709
478	wt	m	norm	neg	neg	neg	3772
479	wt	m	norm	pos	neg	neg	15720
480	wt	um	del13p	neg	pos	neg	655
481	wt	um	del13p	pos	neg	neg	1350
482	wt	um	del17p	pos	pos	neg	15505
483	mut	um	tris12	pos	pos	neg	8648
484	wt	um	del11q	neg	neg	pos	1085
485	wt	m	del13p	neg	neg	neg	679

ID #	NOTCH1 status[a]	IGHV status[b]	FISH status[c]	CD49d[d]	CD38[d]	ZAP-70[d]	CD20 MFI[e]
486	wt	m	del13p	neg	pos	pos	3246
487	wt	m	del13p	neg	neg	pos	1572
488	wt	m	tris12	pos	pos	pos	8510
489	wt	m	del13p	neg	neg	neg	1285
490	wt	m	del13p	neg	neg	neg	4678
491	wt	um	norm	pos	neg	pos	11253
492	wt	m	norm	pos	neg	neg	1968
493	wt	m	tris12	pos	neg	neg	9784
494	wt	um	del13p	neg	neg	pos	1430
495	wt	m	tris12	pos	neg	neg	12251
496	wt	na	norm	pos	neg	neg	2014
497	mut	um	tris12	pos	pos	pos	1062
498	wt	m	norm	neg	neg	neg	1129
499	mut	um	del13p	neg	neg	pos	2484
500	wt	um	del13p	neg	neg	pos	2216
501	wt	m	del13p	pos	pos	pos	2664
502	wt	m	del13p	pos	neg	neg	1661
503	wt	m	norm	neg	neg	neg	1469
504	wt	m	del13p	neg	neg	pos	2075
505	wt	m	norm	pos	neg	neg	2263
506	mut	um	del13p	pos	neg	pos	579
507	wt	m	norm	pos	pos	neg	1350
508	wt	m	norm	pos	neg	neg	15024
509	wt	m	del13p	neg	neg	neg	772
510	wt	um	tris12	pos	pos	pos	3031
511	mut	um	tris12	pos	neg	pos	7355
512	wt	um	del17p	neg	neg	neg	890
513	mut	na	del13p	neg	neg	neg	2681
514	wt	m	norm	neg	neg	pos	1281
515	wt	um	del13p	neg	neg	neg	234
516	wt	m	norm	pos	neg	pos	11740
517	wt	m	del13p	neg	neg	neg	3385
518	wt	um	del11q	pos	pos	pos	1720
519	wt	m	norm	neg	neg	neg	2423
520	wt	m	del13p	neg	neg	neg	1750
521	wt	um	del13p	neg	neg	neg	1335
522	wt	m	del13p	neg	neg	neg	1543
523	wt	m	norm	pos	neg	neg	12313
524	wt	um	norm	pos	pos	pos	2466
525	wt	um	tris12	pos	neg	pos	4008
526	wt	m	del13p	neg	neg	neg	2030
527	mut	um	del13p	neg	pos	pos	2289
528	wt	um	tris12	pos	pos	pos	2382
529	wt	m	del13p	neg	neg	neg	1817
530	wt	m	del13p	pos	neg	na	882
531	wt	um	norm	pos	pos	pos	1786
532	wt	m	del13p	neg	neg	neg	3301
533	wt	um	norm	pos	neg	pos	1542
534	wt	um	del11q	neg	pos	pos	2212
535	mut	um	tris12	pos	neg	pos	989
536	wt	um	del11q	neg	neg	neg	3664
537	wt	m	del13p	pos	neg	pos	5189
538	mut	um	norm	pos	pos	pos	2778
539	wt	um	tris12	pos	neg	neg	6035
540	wt	um	del17p	pos	pos	pos	653
541	wt	um	norm	pos	pos	pos	2328
542	wt	m	del13p	neg	neg	neg	759
543	wt	m	norm	neg	neg	neg	1644
544	wt[NOTCH1]	m[IGHV]	tris12	na	na	neg	16209
545	mut[status a]	m[status b]	del13p	neg	neg	neg	1554
546	wt	m	tris12	neg	neg	neg	1683

ID #	NOTCH1 status[a]	IGHV status[b]	FISH status[c]	CD49d[d]	CD38[d]	ZAP-70[d]	CD20 MFI[e]
547	mut	um	tris12	pos	neg	pos	1931
548	wt	m	norm	neg	neg	neg	1467
549	wt	m	norm	neg	neg	neg	19299
550	wt	um	tris12	pos	pos	pos	10444
551	wt	m	del13p	neg	neg	neg	1730
552	mut	um	tris12	pos	pos	pos	2255
553	wt	m	del13p	neg	neg	neg	2125
554	wt	um	tris12	pos	neg	pos	2469
555	mut	m	del11q	neg	pos	neg	1095
556	wt	m	del13p	neg	neg	neg	996
557	wt	um	del17p	neg	neg	neg	480
558	wt	um	norm	neg	neg	pos	234
559	mut	um	del11q	neg	neg	pos	2188
560	wt	m	tris12	pos	pos	neg	7258
561	wt	m	del13p	neg	neg	neg	1026
562	wt	um	norm	pos	neg	neg	1569
563	wt	m	del11q	pos	pos	neg	13620
564	wt	m	del13p	neg	neg	pos	2356
565	wt	m	del13p	neg	neg	neg	4434
566	wt	m	del13p	pos	neg	pos	12399
567	wt	m	del13p	neg	neg	pos	1846
568	wt	m	norm	neg	neg	neg	864
569	wt	um	del13p	pos	pos	neg	320
570	wt	m	del13p	neg	neg	na	1695
571	mut	m	tris12	pos	neg	na	4270
572	wt	um	norm	pos	neg	na	697
573	wt	um	tris12	pos	pos	pos	2776
574	mut	um	norm	pos	pos	pos	419
575	wt	m	norm	pos	neg	pos	21743
576	mut	um	tris12	neg	neg	pos	522
577	mut	um	del17p	pos	neg	neg	21
578	wt	um	del11q	pos	neg	pos	81
579	mut	um	tris12	pos	neg	na	1575
580	wt	um	del11q	neg	neg	na	3486
581	wt	m	del13p	neg	neg	pos	1632
582	wt	m	norm	neg	neg	pos	1374
583	wt	m	del17p	pos	neg	pos	12828
584	wt	um	del11q	neg	pos	pos	2806
585	mut	um	del13p	pos	neg	neg	1830
586	mut	um	tris12	pos	pos	pos	1665
587	wt	m	tris12	pos	pos	na	9848
588	wt	m	del13p	pos	na	neg	17024
589	wt	m	del17p	pos	pos	pos	2356
590	wt	m	del13p	neg	neg	neg	2875
591	mut	um	del17p	pos	pos	pos	3091
592	wt	na	del17p	pos	neg	pos	7630
593	wt	m	norm	neg	neg	pos	2287
594	wt	m	del13p	neg	neg	pos	3160
595	wt	m	tris12	pos	pos	neg	19559
596	wt	m	del17p	neg	neg	neg	615
597	mut	um	tris12	pos	pos	pos	1202
598	wt	um	del13p	neg	neg	neg	1655
599	mut	um	del11q	neg	pos	na	2407
600	wt	um	del13p	neg	neg	neg	2624
601	wt	m	del13p	neg	neg	pos	2644
602	wt	na	norm	neg	neg	pos	1068
603	wt	um	del11q	neg	neg	neg	621
604	wt	um	del11q	neg	pos	pos	2127
605	wt[a]	m	tris12	pos	neg	neg	2861
606	wt[a]	m[b]	del13p	neg	neg	neg	1159
607	mut	um	tris12	pos	pos	pos	1950

ID #	NOTCH1 status[a]	IGHV status[b]	FISH status[c]	CD49d[d]	CD38[d]	ZAP-70[d]	CD20 MFI[e]
608	wt	m	del13p	neg	neg	neg	632
609	wt	na	norm	neg	neg	neg	1784
610	wt	m	del17p	pos	neg	neg	1874
611	wt	na	norm	pos	pos	neg	22500
612	wt	m	del13p	neg	neg	na	1048
613	wt	um	tris12	pos	neg	na	904
614	wt	m	norm	pos	neg	na	8337
615	wt	um	del13p	neg	neg	na	1964
616	wt	m	del13p	neg	neg	na	1435
617	wt	um	del13p	neg	neg	na	2933
618	wt	m	del13p	neg	neg	neg	1435
619	wt	um	norm	neg	neg	na	10098
620	mut	um	del11q	pos	pos	na	2396
621	wt	m	del13p	neg	neg	na	2906
622	wt	m	del13p	neg	neg	na	2052
623	wt	m	norm	neg	neg	na	2686
624	wt	m	del13p	pos	pos	na	3610
625	wt	m	del17p	neg	neg	na	3626
626	wt	um	del17p	neg	neg	na	1023
627	wt	m	del13p	pos	pos	na	1994
628	wt	m	del11q	pos	pos	na	11905
629	mut	um	del13p	neg	pos	na	1277
630	wt	um	del13p	pos	neg	na	1064
631	wt	m	del13p	neg	neg	na	1798
632	wt	m	norm	neg	neg	na	809
633	wt	um	del11q	neg	neg	na	2844
634	wt	m	norm	neg	neg	na	2141
635	wt	um	tris12	pos	pos	na	41
636	wt	m	del13p	neg	neg	na	654
637	wt	um	norm	neg	pos	na	3043
638	wt	m	del13p	neg	neg	na	2140
639	wt	m	del13p	neg	neg	na	1853
640	mut	um	tris12	pos	pos	na	2438
641	wt	m	del13p	neg	neg	na	1566
642	wt	m	tris12	pos	pos	na	3470
643	wt	m	norm	neg	neg	na	1471
644	wt	um	del11q	neg	neg	na	1820
645	wt	m	del13p	pos	neg	na	10082
646	wt	na	del11q	pos	neg	na	3243
647	wt	um	tris12	pos	neg	na	1801
648	wt	um	del11q	neg	pos	na	1840
649	wt	um	del13p	neg	neg	pos	2508
650	mut	um	norm	pos	pos	na	1957
651	mut	um	del13p	pos	pos	pos	2077
652	mut	m	norm	neg	neg	na	2960
653	mut	m	norm	neg	neg	na	1448
654	wt	m	tris12	pos	neg	na	9933
655	mut	um	tris12	pos	pos	na	1801
656	wt	m	tris12	pos	neg	na	13955
657	mut	um	del11q	pos	neg	na	1610
658	wt	um	del13p	neg	neg	na	2632
659	wt	m	del13p	pos	neg	na	5602
660	wt	m	del13p	neg	neg	na	2898
661	wt	m	del13p	neg	neg	na	1189
662	mut	m	del11q	pos	neg	pos	2360

a: as determined by next generation sequencing (NGS). b: IGHV status was established according to the conventional cut-off, as reported in ref. 9. c: FISH status was determined according to ref. 13. d: CD49d, CD38 and ZAP70 expression are reported as percentage of positive cells (cutoff of CD49d and CD38 expression: ≥ 30% of positive cells; cutoff of ZAP70 expression: ≥ 20% of positive cells), as in ref. 9. e: MFI values are substracted from the values of irrelevant isotype-matched antibody.

Abbreviations: ID #: identification number, NOTCH1 status: wt, NOTCH1 wild type, mut, NOTCH1 mutated; IGHV status: um, IGHV unmutated, m, IGHV mutated; FISH: norm, normal karyotype; CD49d, CD38, ZAP70 expression: pos, positive, neg, negative; na, not available.

Table S7. *NOTCH1* mutation features of the *NOTCH1* mutated cohort (116 cases).

ID#	Mutation_1	Mutation_2	Mutation_3	Verification
2	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 8.1%			ARMS-PCR
7	g.139390152T>C; c.*7668+371A>G; 49.4%			RT-PCR
8	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 48.3%			ARMS-PCR
9	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 11.5%			ARMS-PCR
10	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 3.5%			ARMS-PCR
13	g.139390765CG>C; c.7425delC; p.V2476*; 35.8%			Sanger
18	g.139390152T>C; c.*7668+371A>G; 36.5%			RT-PCR
30	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 3.2%			ARMS-PCR
34	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 10.5%			ARMS-PCR
38	g.139390797G>GGC; c.7392_7393insGC; p.P2465Afs*13; 43.3%			Sanger
45	g.139390152T>C; c.*7668+371A>G; 35.6%			RT-PCR
55	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 2.0%			ARMS-PCR
66	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 3.7%			ARMS-PCR
72	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 33.4%			ARMS-PCR
82	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 19.5%			ARMS-PCR, Sanger
86	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 15.4%			ARMS-PCR
95	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 4.3%			ARMS-PCR
112	g.139390152T>C; c.*7668+371A>G; 46.3%			NGS
121	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 8.1%			ARMS-PCR
126	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 1.5%			ARMS-PCR
135	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 83.0%			ARMS-PCR
140	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 54.5%			ARMS-PCR
152	g.139390152T>C; c.*7668+371A>G; 44.0%			RT-PCR
153	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 39.0%			ARMS-PCR
158	g.139390152T>C; c.*7668+371A>G; 22.5%	g.139390897AC>A; c.7293delG; p.R2431fs*4; 12.9%		NGS, Sanger
167	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 34.9%			ARMS-PCR, Sanger
175	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 4.6%			ARMS-PCR
178	g.139390152T>C; c.*7668+371A>G; 33.9%			RT-PCR
182	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 4.6%			ARMS-PCR
186	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 41.6%			ARMS-PCR
192	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 2.5%			ARMS-PCR
205	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 19.1%			ARMS-PCR
207	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 3.5%			ARMS-PCR, Sanger
212	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 43.0%			ARMS-PCR
213	g.139390929AC>A; c.7261delG; p.V2421*; 32.7%			Sanger
214	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 46.9%			ARMS-PCR
216	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 3.2%			ARMS-PCR
222	g.1139913369GGA>G; c.6820_6821delTC; p.S2274Pfs*79; 50.0%			Sanger
230	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 18.9%			ARMS-PCR
232	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 38.6%			ARMS-PCR
234	g.139390145T>C; c.*7668+378A>G; 5.8%	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*; 3.2%		RT-PCR, ARMS-PCR
253	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 18.7%			ARMS-PCR
259	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 47.3%			ARMS-PCR
270	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 41.4%			ARMS-PCR, Sanger
276	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 1.2%			NGS*
286	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 31.1%			ARMS-PCR, Sanger
287	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 5.2%			ARMS-PCR
289	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 39.6%			ARMS-PCR
290	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 6.4%	g.139390791GA>G c.7399delT p.S2467Rfs*10 5.7%		ARMS-PCR, NGS
291	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 40.2%			ARMS-PCR
293	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 1.1%			NGS*
305	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 2.9%			ARMS-PCR
308	g.139390684G>A; c.7507C>T; p.Q2503*; 2.1%			NGS
310	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 36.6%			ARMS-PCR
313	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 1.3%			ARMS-PCR
322	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 3.0%			ARMS-PCR

ID#	Mutation_1	Mutation_2	Mutation_3	Verification
323	g.139390145T>C; c.*7668+378A>G; 4.1%			RT-PCR
331	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 23.3%			ARMS-PCR
340	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 1.0%			NGS*
342	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 1.1%			NGS*
346	g.139390861G>A; c.7330C>T; p.Q2444*; 12.5%			Sanger
365	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 35.8%			ARMS-PCR
369	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 1.3%	g.139391645CTTCTTCCTCCGTGCCT>C; c.6530_6545delAGGCACGGAGGAAGAA; p.K2177Sfs*66; 5.5%		ARMS-PCR, NGS
390	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 2.9%			ARMS-PCR
400	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 26.2%			ARMS-PCR, Sanger
401	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 77.0%			ARMS-PCR
402	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 33.0%			ARMS-PCR
423	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 31.3%			ARMS-PCR
424	g.139390152T>C; c.*7668+371A>G; 14.1%			RT-PCR
427	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 45.7%			ARMS-PCR, Sanger
430	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 37.9%			ARMS-PCR, Sanger
455	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 2.2%			ARMS-PCR
456	g.139390152T>C; c.*7668+371A>G; 49.9%			NGS
457	g.139390152T>C; c.*7668+371A>G; 20.4%			RT-PCR
459	g.139390152T>C; c.*7668+371A>G; 49.3%			RT-PCR
461	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 41.3%			ARMS-PCR, Sanger
462	g.139390152T>C; c.*7668+371A>G; 20.5%			RT-PCR
466	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 24.2%	g.139390861G>A; c.7330C>T; p.Q2444*; 25.8%		ARMS-PCR, Sanger
468	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 3.5%			ARMS-PCR
470	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 42.0%			ARMS-PCR
477	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 1.8%			ARMS-PCR
483	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 5.2%			ARMS-PCR
497	g.139390152T>C; c.*7668+371A>G; 49.9%			RT-PCR
499	g.139390145T>C; c.*7668+378A>G; 44.8%			RT-PCR
506	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 16.7%			ARMS-PCR
511	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 1.8%			ARMS-PCR
513	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 1.6%			ARMS-PCR
527	g.139390152T>C; c.*7668+371A>G; 6.7%			RT-PCR
535	g.139390861G>A; c.7330C>T; p.Q2444*; 18.3%			Sanger
538	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 36.6%			ARMS-PCR
545	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 12.9%			ARMS-PCR
547	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 13.8%			ARMS-PCR
552	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 5.6%			ARMS-PCR, Sanger
555	g.139390152T>C; c.*7668+371A>G; 12.1%			RT-PCR
559	g.139390734G>T; c.7457C>A; p.S2486*; 1.6%			NGS
571	g.139390873G>A; c.7318C>T; p.Q2440*; 39.2%			Sanger
574	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 2.4%	g.139390648_2C>A; c.7543G>T; p.E2515*; 35.9%		ARMS-PCR, Sanger
576	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 1.5%			ARMS-PCR
577	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 33.4%			ARMS-PCR
579	g.139390861G>A; c.7330C>T; p.Q2444*; 30.1%			Sanger
585	g.139390145T>C; c.*7668+378A>G; 40.1%			RT-PCR
586	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 1.4%	g.139390846TG>T; c.7344delC; p.S2449Afs*28; 19.1%		ARMS-PCR, Sanger
591	g.139390145T>C; c.*7668+378A>G; 27.7%	g.139390675C>A; c.7516G>T; p.E2506*; 12.6%		RT-PCR, Sanger
597	g.139390873G>A; c.7318C>T; p.Q2440*; 7.5%			NGS
599	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 46.3%			ARMS-PCR, Sanger
607	g.139390152T>C; c.*7668+371A>G; 18.0%	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 2.9%		RT-PCR, NGS
620	g.139390145T>C; c.*7668+378A>G; 13.0%			RT-PCR
629	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 1.7%			ARMS-PCR
640	g.139390152T>C; c.*7668+371A>G; 45.2%			RT-PCR
650	g.139390145T>C; c.*7668+378A>G; 19.6%	g.139390684G>A; c.7507C>T; p.Q2503*; 5.5%	g.139390690G>A; c.7501C>T; p.Q2501*; 5.1%	RT-PCR, NGS
651	g.139390152T>C; c.*7668+371A>G; 47.4%			RT-PCR
652	g.139390659GTGAGGAAGGGGTGCTCAGGCACC>G; c.7509_7532delGGTGCCTGAGCACCCCTTCCTCA; p.T2511Rfs*24; 2.6%			NGS

ID#	Mutation_1	Mutation_2	Mutation_3	Verification
653	g.139390152T>C; c.*7668+371A>G; 31.4%			NGS
655	g.139390716G>T; c.7475C>A; p.S2492*; 3.6%			NGS
657	g.139390648CAG>C; c.7541_7542delCT; p.P2514Rfs*4; 3.4%			ARMS-PCR
662	g.139390721G>C; c.7470C>G; p.Y2490*; 2.9%			NGS

ID#: identification number.

Mutation description: genomic ("g" relative to human genome assembly hg19), mRNA ("c"), aminoacidic ("p") substitutions and variant allele frequency (VAF, "%") are reported.

ARMS-PCR, mutation verified by Amplification Refractory Mutation System; NGS, mutation verified by an independent round of NGS (amplification and sequencing); Sanger, mutation verified by Sanger sequencing; RT-PCR, mutation verified by specific PCR for aberrant splicing on cDNA.

NGS*: preliminarily detected by ARMS-PCR; VAF computed by analysis of raw NGS data.

Supplemental figure legends

Figure S1. Validation of NGS strategy to detect *NOTCH1* mutations. (A) Dilution experiments to determine the sensitivity of NGS approach to call low frequency mutations. Serial dilutions were made by mixing with normal DNA from a healthy donor either the DNA of one of 3 CLL cases carrying *NOTCH1* mutations (i.e. 1 case with g.139390152T>C, 1 case with g.139390648CAG>C, and 1 case with g.139390873G>A) or the DNA of a CLL case carrying a SNP in the *NOTCH1* gene (i.e. g.139391636G>A, rs2229974). As reported in supplemental materials and methods, only somatic mutations truncating the NOTCH1 protein were included in the analysis. **(B)** Threshold of Sanger sequencing detection. Shown are screenshots of Integrative Genomics Viewer (IGV), and Sanger sequencing electropherograms (inset), of 3 representative *NOTCH1* mutations detected by NGS, and investigated by Sanger sequencing. *NOTCH1* mutations with a VAF<12% were confirmed by a second independent NGS experiment (see Table S7). ID, mutation VAF and coverage (depth), corresponding to each *NOTCH1*-mut cases is reported. **(C)** Representative gel image of ARMS-PCR verification for c.7541-7542delCT mutation (g.139390648CAG>C). Representative results of the ARMS PCR assay showing 3 CLL samples that scored positive and 1 sample that scored negative for c.7541-7542delCT mutation. Negative sample shows a normal band of 284 base pair (bp, single arrow). Positive samples show an additional band of 183 bp (double arrow). Molecular weight is the 100 bp ladder. Camera: Gel Doc EZ, BioRad; image acquisition software: Image Lab 3.0, BioRad. ID and mutation VAF corresponding to each *NOTCH1*-mut cases is reported. **(D)** RT-PCR to detect the presence of the aberrant spliced transcript in 3′ UTR *NOTCH1*-mut cases. RT-PCR amplification showing the expected 719-base pair (bp) band in a *NOTCH1*-wt case, and an additional smaller band in cases carrying 3′ UTR *NOTCH1* mutations. This amplification was performed in 23/26 3′ UTR *NOTCH1*-mut cases. In 3/26 3′ UTR *NOTCH1*-mut cases, in which RNA sample was not available, a second independent NGS experiment was performed to confirm the presence of the 3′ UTR *NOTCH1* mutations (see Table S7). Molecular weight is the 100 bp ladder. Camera: Gel Doc EZ, BioRad; image acquisition software: Image Lab 3.0, BioRad. ID and mutation VAF corresponding to each *NOTCH1*-mut cases is reported. **(E)** Frequencies of clonal (i.e. with VAF≥12%, subdivided in <35% or ≥35%) and subclonal (i.e. with VAF<12%) mutations, among the group of 26 3′ UTR *NOTCH1* mutations or the group of 101 coding *NOTCH1* mutations. Data were analyzed using chi-square test.
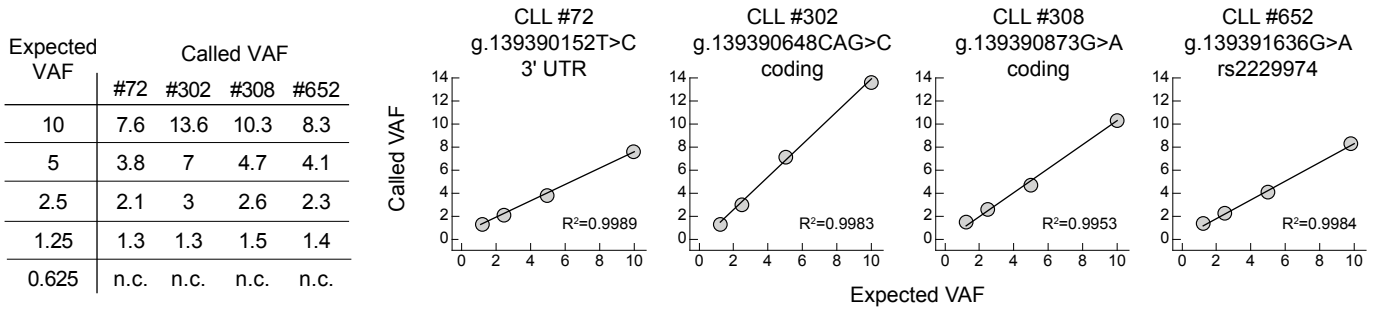
Figure S2. Western blot of NOTCH1 and CD20 protein expression according to *NOTCH1* mutational status. (A) Western blot showing total NOTCH1 protein expression (transmembrane NOTCH1 and NICD) in representative CLL cases, i.e. in 4 *NOTCH1*-wt cases, 3 coding *NOTCH1*-mut cases (2 cases with the g.139390648CAG>C, c.7541-7542delCT, p.P2514Rfs*4; 1 case with the g.139390929AC>A, c.7261delG, p.V2421*), and 7 3′ UTR *NOTCH1*-mut cases (5 cases with the g.139390152T>C, c.*7668+371A>G; 2 cases with the g.139390145T>C, c.*7668+378A>G). β-actin was used as loading control. **(B)** Western blot showing CD20 (L26 epitope) protein expression by using high antibody concentrations (anti-CD20, dilution 1/500, and anti-mouse secondary antibody, dilution 1/1000, see Table S5) at three different exposure times (i.e. 15 seconds, 4 minutes and 20 minutes). CD20 expression was investigated in 3 *NOTCH1*-wt cases, 3 coding *NOTCH1*-mut cases (2 cases with the g.139390648CAG>C, c.7541-7542delCT, p.P2514Rfs*4; 1 case with the g.139390929AC>A, c.7261delG, p.V2421*), and 7 3′ UTR *NOTCH1*-mut cases (5 cases with the g.139390152T>C, c.*7668+371A>G; 2 cases with the g.139390145T>C, c.*7668+378A>G).

Figure S3. CD20 expression by flow cytometry in CLL cases. (A) Bar graphs showing CD20 protein expression levels, in CLL cases according to cytogenetic abnormalities as evaluated by flow cytometry. Bar graphs represent mean values, error bars represent 95% CI. Norm, normal
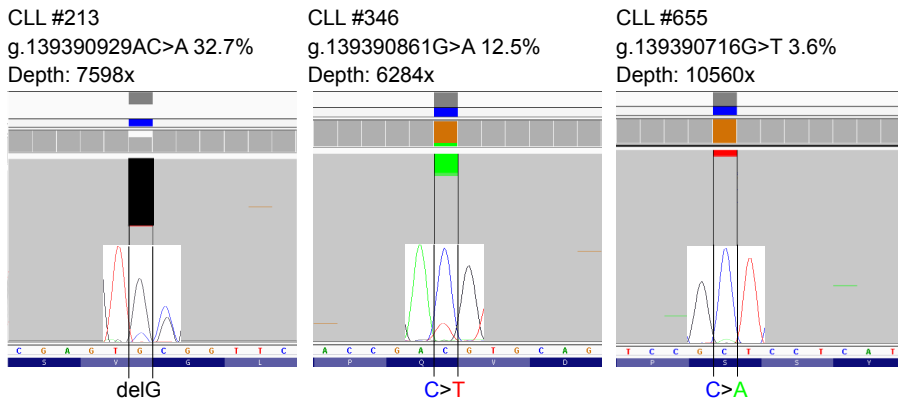
karyotype; del13q, 13q deletion; tris12, trisomy 12; del11q, 11q deletion; del17p, 17p deletion. **(B)** Dot plots showing CD20 expression, as evaluated by flow cytometry using a FITC-conjugated anti-CD20 antibody, in prototypic 3′ UTR *NOTCH1*-mut cases, coding *NOTCH1*-mut cases and *NOTCH1*-wt cases of trisomy 12 and non-trisomy 12 CLL categories.
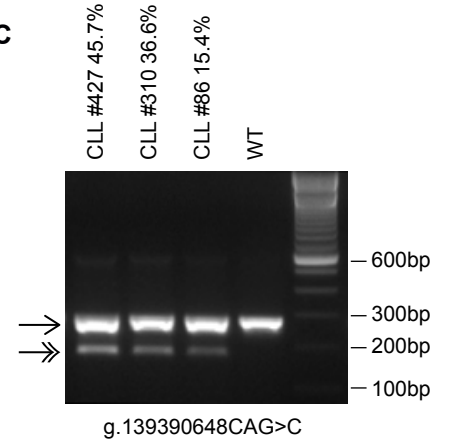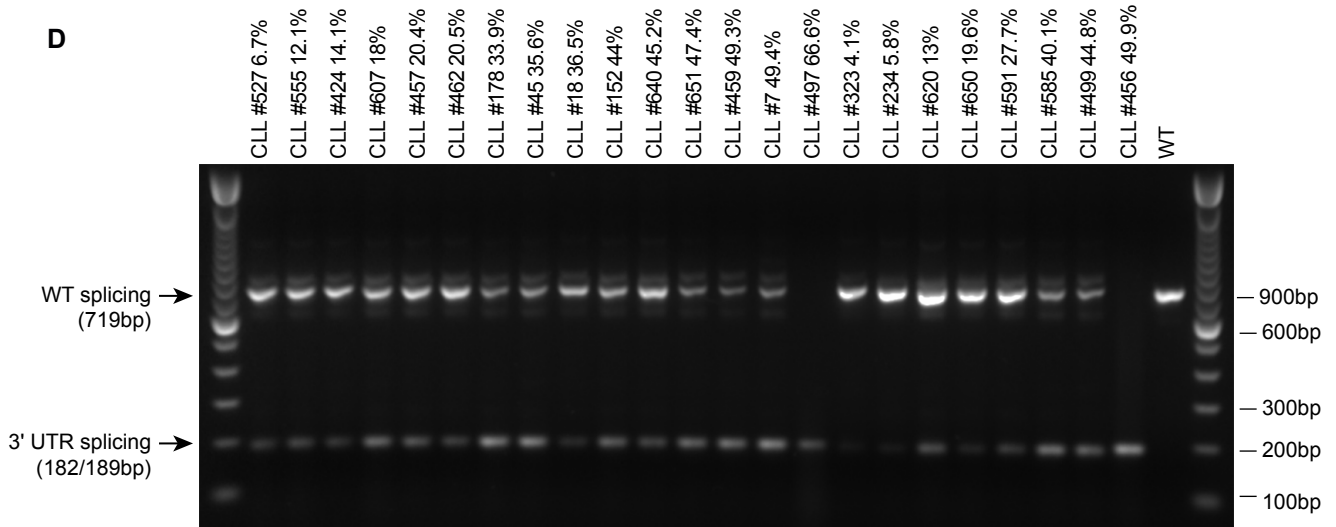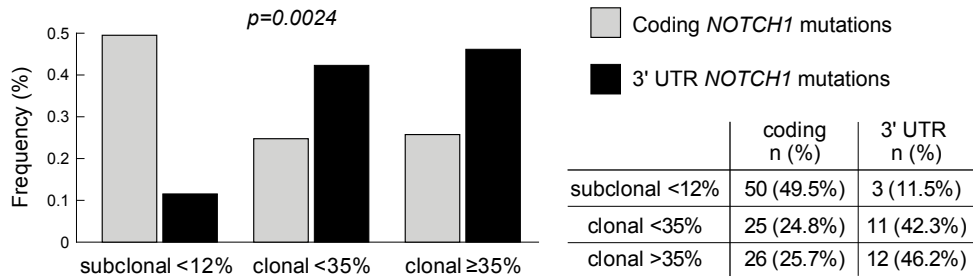
A

Expected VAF	Called VAF			
	#72	#302	#308	#652
10	7.6	13.6	10.3	8.3
5	3.8	7	4.7	4.1
2.5	2.1	3	2.6	2.3
1.25	1.3	1.3	1.5	1.4
0.625	n.c.	n.c.	n.c.	n.c.

CLL #72
g.139390152T>C
3' UTR

R²=0.9989

CLL #302
g.139390648CAG>C
coding

R²=0.9983

CLL #308
g.139390873G>A
coding

R²=0.9953

CLL #652
g.139391636G>A
rs2229974

R²=0.9984

Called VAF

Expected VAF

B

CLL #213
g.139390929AC>A 32.7%
Depth: 7598x

delG

CLL #346
g.139390861G>A 12.5%
Depth: 6284x

C>T

CLL #655
g.139390716G>T 3.6%
Depth: 10560x

C>A

C

CLL #427 45.7%
CLL #310 36.6%
CLL #86 15.4%
WT

— 600bp
— 300bp
— 200bp
— 100bp

g.139390648CAG>C

D

CLL #527 6.7%
CLL #555 12.1%
CLL #424 14.1%
CLL #607 18%
CLL #457 20.4%
CLL #462 20.5%
CLL #178 33.9%
CLL #45 35.6%
CLL #18 36.5%
CLL #152 44%
CLL #640 45.2%
CLL #651 47.4%
CLL #459 49.3%
CLL #7 49.4%
CLL #497 66.6%
CLL #323 4.1%
CLL #234 5.8%
CLL #620 13%
CLL #650 19.6%
CLL #591 27.7%
CLL #585 40.1%
CLL #499 44.8%
CLL #456 49.9%
WT

WT splicing
(719bp)

3' UTR splicing
(182/189bp)

— 900bp
— 600bp

— 300bp
— 200bp

— 100bp

E

p=0.0024

Frequency (%)

subclonal <12%   clonal <35%   clonal ≥35%

Coding *NOTCH1* mutations

3' UTR *NOTCH1* mutations

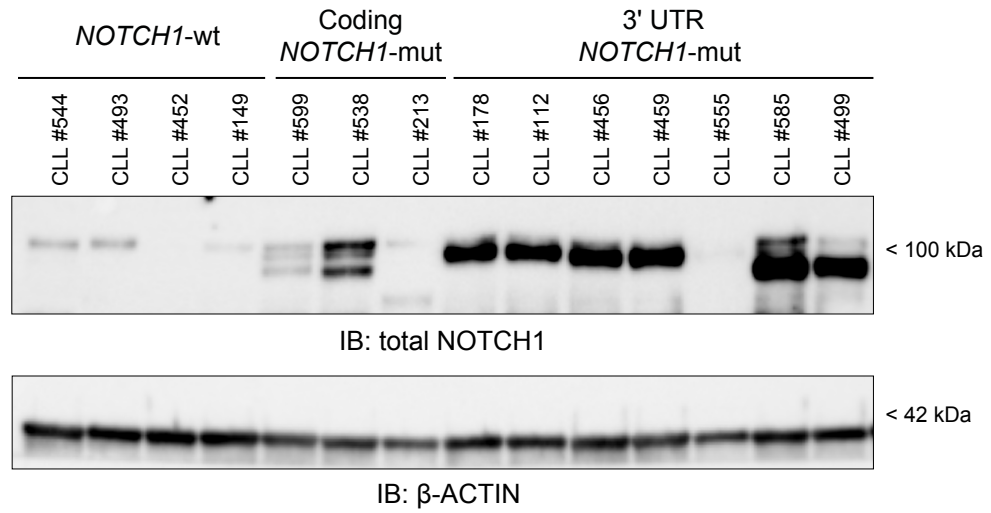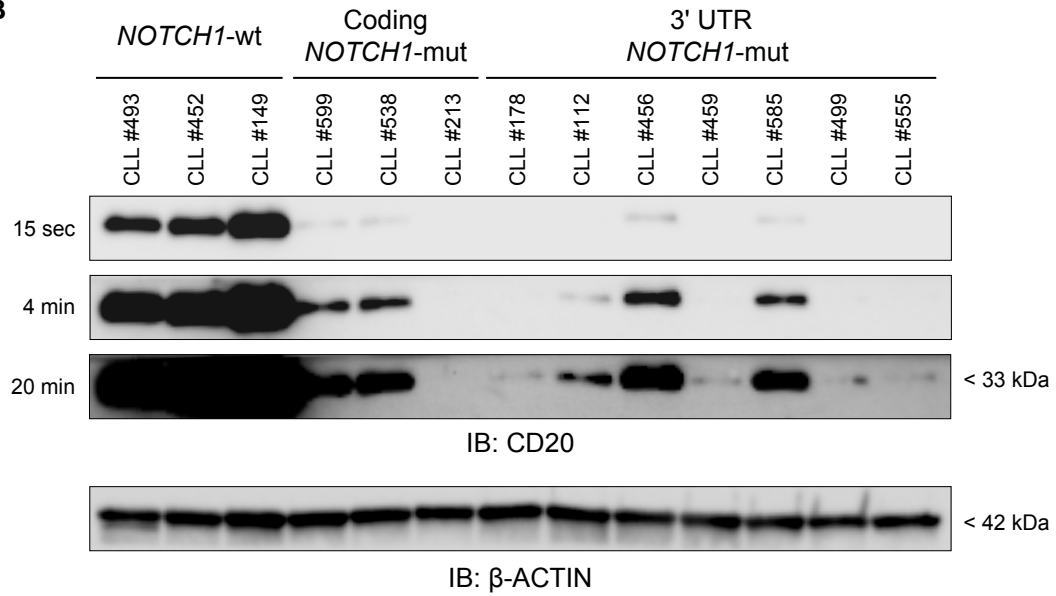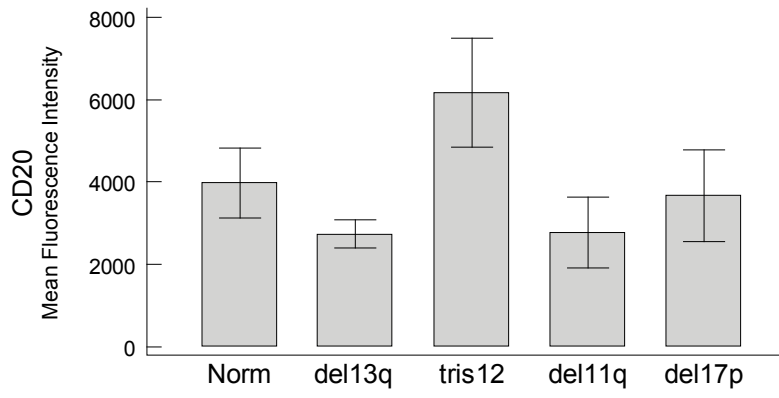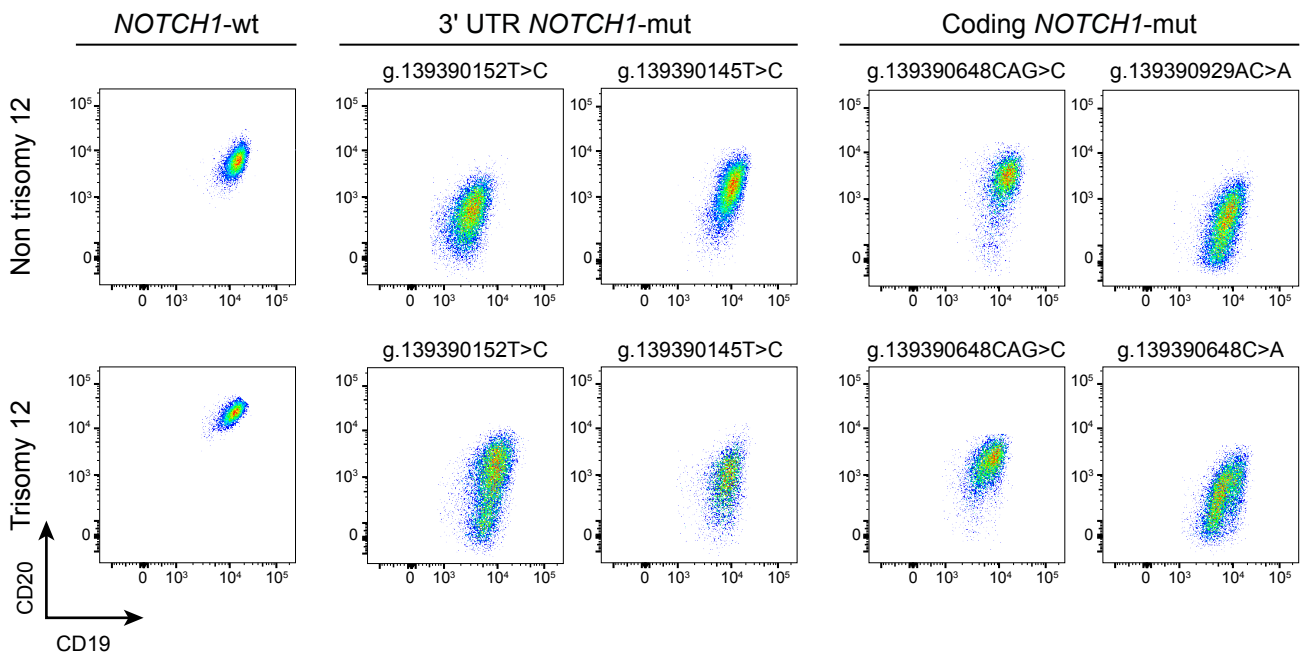|	coding n (%)	3' UTR n (%)
subclonal <12%	50 (49.5%)	3 (11.5%)
clonal <35%	25 (24.8%)	11 (42.3%)
clonal >35%	26 (25.7%)	12 (46.2%)

Figure S1

A

B

Figure S2

A

B

NOTCH1-wt | 3' UTR *NOTCH1*-mut | Coding *NOTCH1*-mut

Figure S3