# Supplementary information

**Title**

PAX5 P80R-mutated B-cell acute lymphoblastic leukemia with transformation to histiocytic sarcoma – clonal evolution assessment using NGS-based immunoglobulin clonality and mutation analysis

**Journal:**

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**Authors:**

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**Supplementary Tables**

**Supplementary Table 1**: Immunoflowcytometric phenotyping of the blasts in the bone marrow discriminated 2 B-cell precursor populations, that showed both expression of CD19, CD22, CD34, cytoplasmic CD179a and TdT, but the expression levels and some additional markers (CD10) differed.

| Population | CD19 | CD34 | CD33 | CD10 | CD20 | HLADR | CD38 | cytCD79a+ | cytCD179a+ | TdT | CD22 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | + | + | + (50%) | + (22%) | - | + | + | + | + | + | + (55%) |
| 2 | ++ | + | + | - | - | ++ | ++ | + | + | + | + |

**Supplementary Table 2**: SNP-array

| Array results | 1q21.1qter(143932350_249224684)x3[0.35] | [gain 105,3 Mb] |
|---|---|---|
| | 9pterp13.1(192129_38787480)x1 | [deletie 38,6 Mb; bevat CDKN2A/B en PAX5 gen] |
| | 16q11.2qter(46463673_90163275)x1[0.35] | [deletie 43,7 Mb] |
| | 20q11.21q13.2(31532616_50232497)x1 | [deletie 18,7 Mb] |
| | 20q13.2qter(54937543_62915556)x1 | [deletie 8 Mb] |
| Array-based karyotype | 46,XY,dup(1)(q21.1qter),del(9)(pterp13.1),del(16)(q11.2qter),del(20)(q11.21q13.2),del(20)(q13.2qter) | |

**Supplementary Table 3:** Immunohistochemical expression of the histiocytic sarcoma in the skin.

| Positive markers | Negative markers |
|---|---|
| CD68 | CD20 |
| CD163 | CD79a |
| CD14 | CD10 |
| CD4 (weak) | CD34 |
| Lysozyme (partly) | TdT |
| | CD117 |
| | MPO |
| | CD1a |
| | CD123 |
| | CD23 |
| | CD56 |
| | TCL-1 |
| | Tryptase |

**Supplementary Table 4**: Pathogenic mutations detected using TruSight oncology 500

| Gene | Transcript | Mutation | B-ALL (90% tumor cells) | HS (70% tumor cells) |
|------|-----------|----------|-------------------------|----------------------|
| | | | VAF | VAF |
| PAX5 | NM_016734.2 | c.239C>G p.(Pro80Arg) | 88% | 56% |
| KRAS | NM_004985.5 | c.35G>A p.(Gly12Asp) | 4% | 31% |
| CDKN2A | NM_000077.4 | c.151dup p.(Val51fs) | 14% | - |
| PTPN11^ | NM_002834.5 | c.218C>T p.(Thr73Ile) | 14% | - |
| PTPN11# | NM_002834.5 | c.1508G>C p.(Gly503Ala) | 9% | - |
| PTPN11# | NM_002834.5 | c.1507G>A p.(Gly503Arg) | 3% | - |
| PTPN11# | NM_002834.5 | c.1504T>C p.(Ser502Pro) | 3% | - |
| PTPN11^ | NM_002834.5 | c.215C>T p.(Ala72Val) | 2% | - |
| NRAS | NM_002524.5 | c.37G>C p.(Gly13Arg) | 1% | - |
| RAF1 | NM_002880.3 | c.1171A>T p.(Arg391Trp) | - * | 37% |
| Median unique coverage | | | 737x | 807x |

* Covered by 932 unique reads: not any T detected at this position in the B-ALL sample

# and ^: mutations close together, but present in different sequencing reads

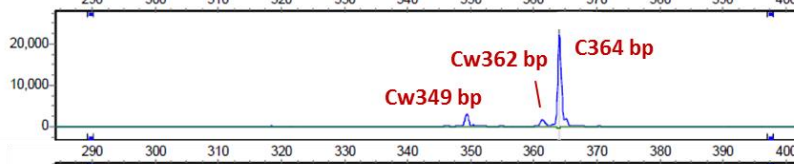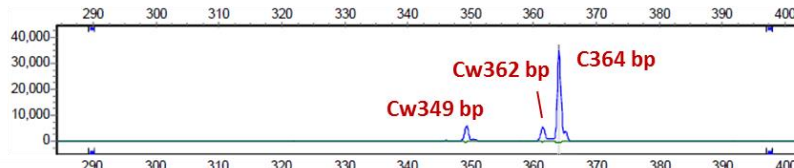**Supplementary Table 5**: Copy number variations detected using TruSight oncology 500

| | B-ALL (90% tumor cells) | HS (70% tumor cells) |
|------|-------------------------|----------------------|
| **CDKN2A** | mono-allelic loss* | bi-allelic loss |
| **PAX5** | mono-allelic loss | mono-allelic loss |

* It can not be excluded that bi-allelic loss is present in a small subclone

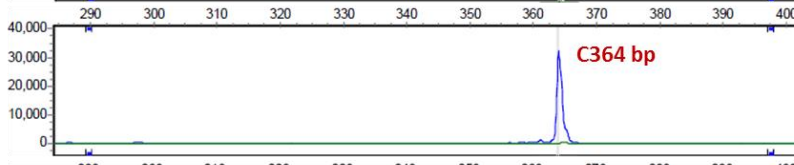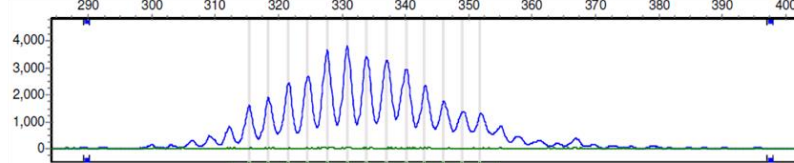# Supplementary Figures

## A: IGHV-IGHJ FR1



## B: IGHV-IGHJ FR2

## C: IGHV-IGHJ FR3

**B-ALL**
Clonal
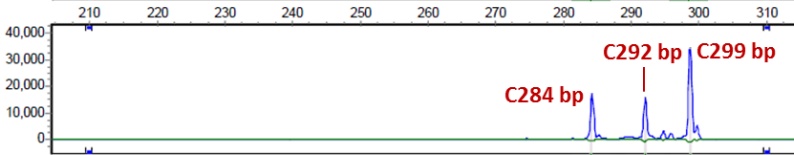


C149 bp
Cw161 bp
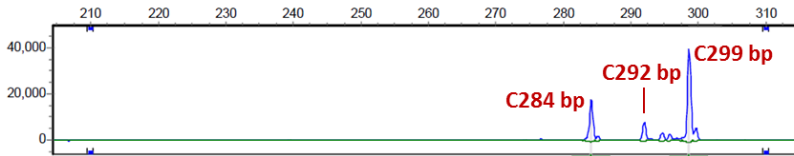Cw164 bp

C149 bp
Cw161 bp
Cw164 bp

**HS**
Clonal

C164 bp

*

C164 bp

*
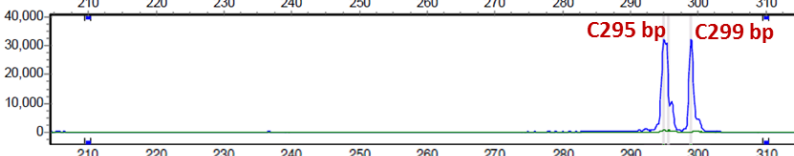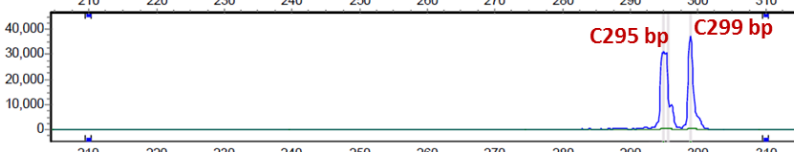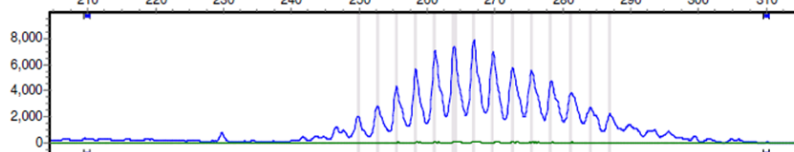
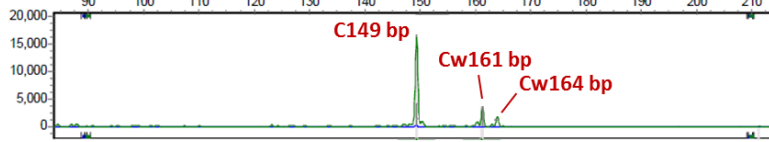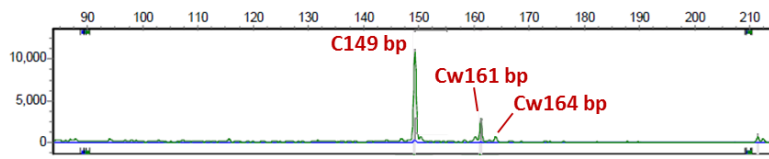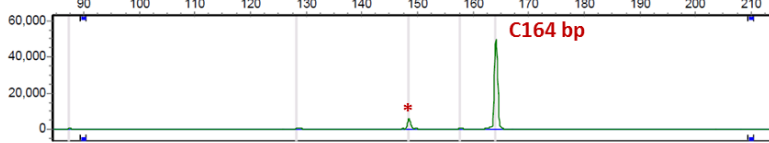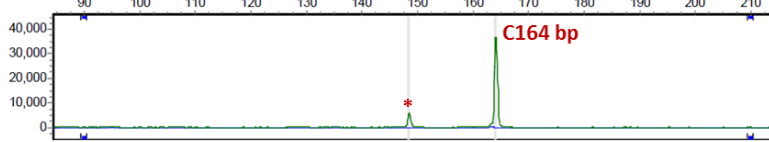**Polyclonal control**
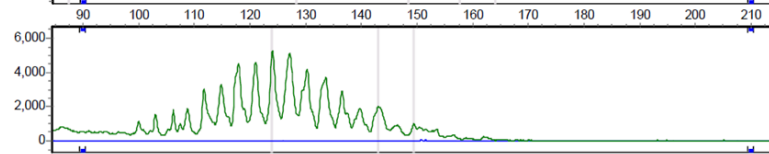
## D: IGHD-IGHJ

**B-ALL**
Clonal



C257 bp

C257 bp
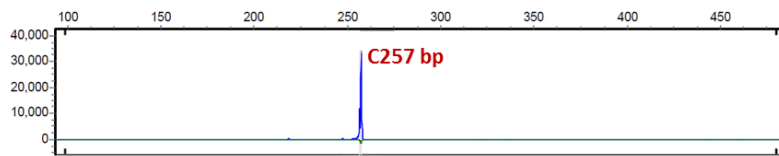
**HS**
No specific product
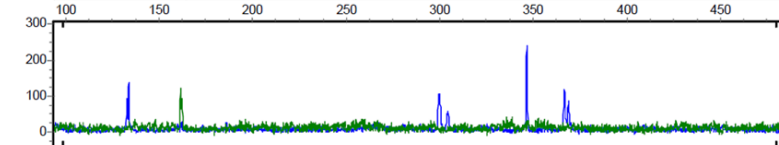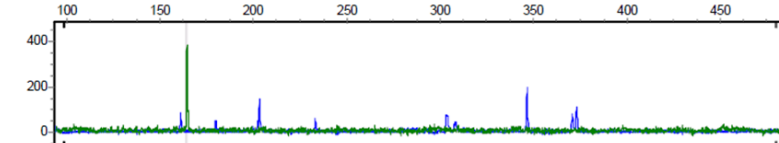
**Polyclonal control**
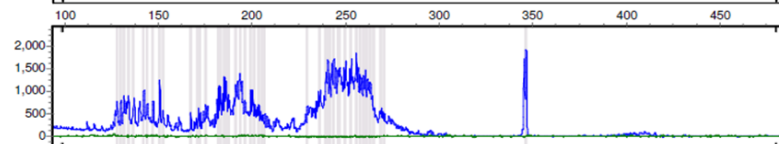
**E: IGKV-IGKJ**
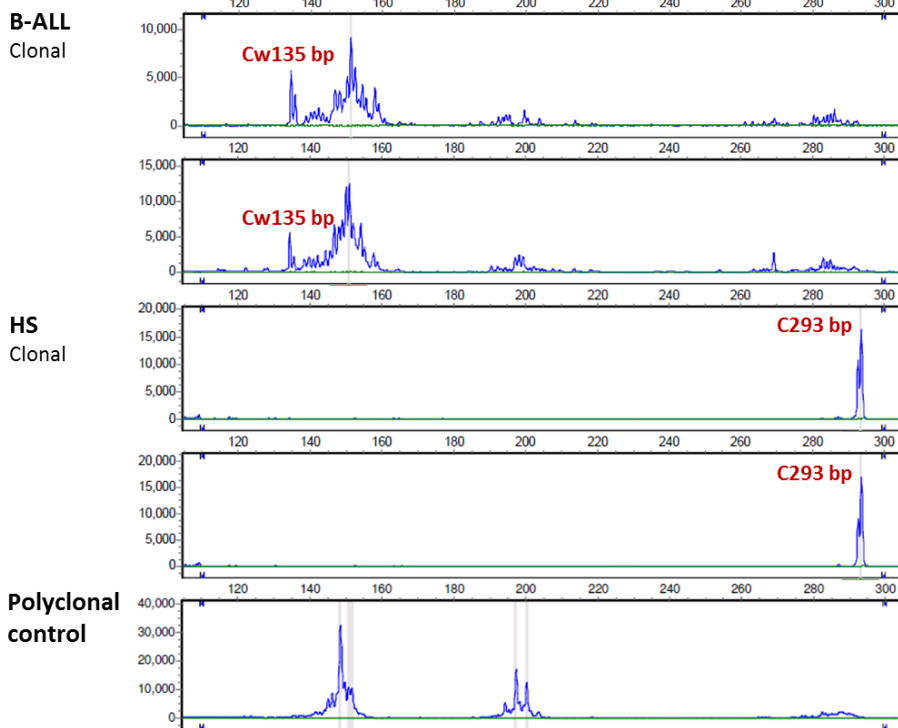


**F: IGKV/intron-IGKde**
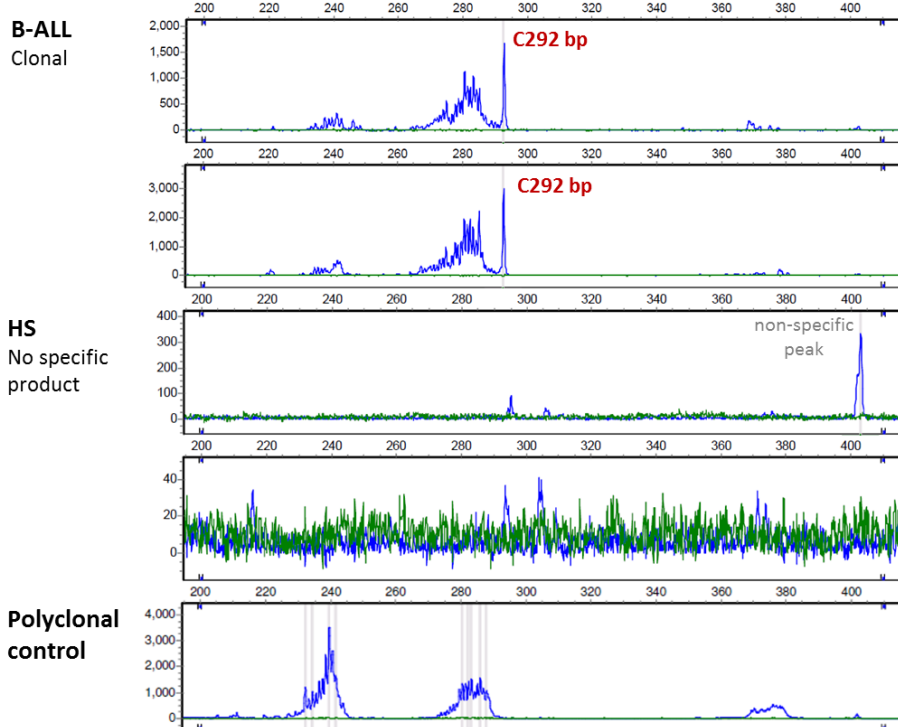


**Supplementary Fig. 1**: GeneScanning results of the EuroClonality/BIOMED-2 IG assays. For each target a duplicate analysis for the ALL and the HS sample is shown and in addition a polyclonal control. A) IGHV-IGHJ FR1; B) IGHV-I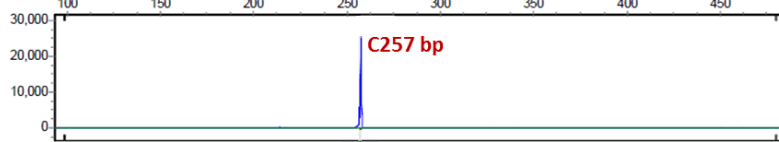GHJ FR2; C) IGHV-IGHJ FR3; D) IGHD-IGHJ; E) IGKV-IGKJ; F) IGKV/intron-IGKde. B-ALL, B-cell acute lymphoblastic leukemia; HS, histiocytic sarcoma; FR, framework; C, clonal; Cw, clonal weak; and bp, base pairs.

\* Sanger sequencing analysis of this product (Cw148 bp) showed that this is a result of cross-annealing of the IGH V4 primer to the V6-1 gene. Therefore, this small peak also represents the V6-1 rearrangement (similar as the C164 bp product resulting from the IGH V6 primer).

## A: IGHV-IGHJ FR3

### B-ALL: Clonal (122028 reads)
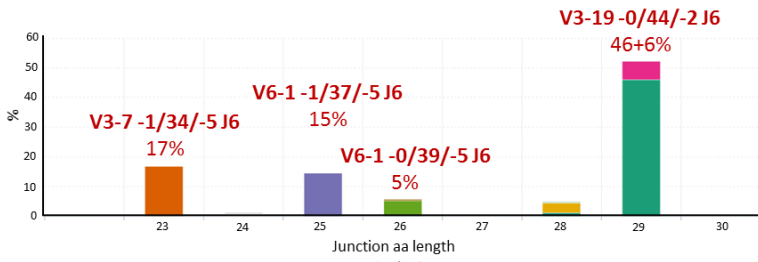


**V3-7 -1/34/-5 J6**
17%

**V6-1 -1/37/-5 J6**
15%

**V6-1 -0/39/-5 J6**
5%

**V3-19 -0/44/-2 J6**
46+6%

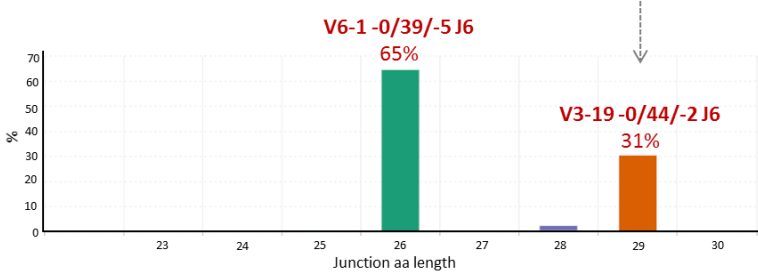| rearrangement type | clonotype | junction in amino acids | junction information | junction aa length | % |
|---|---|---|---|---|---|
| VJ:Vh-(Dh)-Jh | V3-19 -0/44/-2 J6 | CVRNG*GYYDILTGYPRR#YYYYYMDVW | unp | 29 | 46.2 |
| VJ:Vh-(Dh)-Jh | V3-7 -1/34/-5 J6 | CASPFQ*YQLLCFWYYYYGMDVW | unp | 23 | 16.9 |
| VJ:Vh-(Dh)-Jh | V6-1 -1/37/-5 J6 | CARGTCESSTSCYVS#YYYYGMDVW | unp | 25 | 14.5 |
| VJ:Vh-(Dh)-Jh | V3-19 -0/45/02 J6 | CVRNG*GY*RYFDWLSSP#YYYYYYMDVW | unp | 29 | 6.2 |
| VJ:Vh-(Dh)-Jh | V6-1 -0/39/-5 J6 | CARDGVYCSSTSCYVS#YYYYGMDVW | unp | 26 | 5.3 |
| VJ:Vh-(Dh)-Jh | V3-19 -0/43/-4 J6 | CVRNG*GYYDILTGYPRR#YYYYYMDVW | unp | 28 | 3.3 |
| VJ:Vh-(Dh)-Jh | V3-19 -0/44/-2 J6 | CVRNG*GYYDILTGYPRR#YYYYYMDVW | unp | 28 | 1.2 |
| VJ:Vh-(Dh)-Jh | V3-7 -1/43/-5 J6 | CARVPF*L**YQLLCFWYYYYGMDVW | unp | 26 | 0.4 |
| VJ:Vh-(Dh)-Jh | V3-7 -0/39/-5 J6 | CARRG*L**YQLLCFWYYYYGMDVW | unp | 25 | 0.2 |
| VJ:Vh-(Dh)-Jh | V1-2 -0/44/-5 J6 | CAREETKDIVVVPAAMFL#YYYYGMDVW | unp | 28 | 0.2 |

### HS: Clonal (105422 reads)



**V6-1 -0/39/-5 J6**
65%

**V3-19 -0/44/-2 J6**
31%

| rearrangement type | clonotype | junction in amino acids | junction information | junction aa length | % |
|---|---|---|---|---|---|
| VJ:Vh-(Dh)-Jh | V6-1 -0/39/-5 J6 | CARDGVYCSSTSCYVS#YYYYGMDVW | unp | 26 | 64.6 |
| VJ:Vh-(Dh)-Jh | V3-19 -0/44/-2 J6 | CVRNG*GYYDILTGYPRR#YYYYYMDVW | unp | 29 | 30.6 |
| VJ:Vh-(Dh)-Jh | V3-19 -0/43/-4 J6 | CVRNG*GYYDILTGYPRR#YYYYYMDVW | unp | 28 | 1.8 |
| VJ:Vh-(Dh)-Jh | V3-19 -0/44/-2 J6 | CVRNG*GYYDILTGYPRR#YYYYYMDVW | unp | 28 | 0.8 |
| VJ:Vh-(Dh)-Jh | V6-1 -0/32/-1 J6 | CARDGVYCSSTSCY#FWYYYGMDVW | unp | 25 | 0.4 |
| VJ:Vh-(Dh)-Jh | V6-1 -0/39/-5 J6 | CARDGVYCSSTSCYVS#YYYYGMDVW | unp | 25 | 0.1 |
| VJ:Vh-(Dh)-Jh | V3-19 -0/39/-5 J6 | CARDGVYCSSTSCYVS#YYYYGMDVW | unp | 26 | 0.1 |
| VJ:Vh-(Dh)-Jh | V3-19 -0/47/-2 J6 | CVRNG*GYYDILTGYPRRL#YYYYYYMDVW | unp | 30 | 0.08 |
| VJ:Vh-(Dh)-Jh | V3-19 -0/42/-2 J6 | CVRNG*GITIFDWLSSP#YYYYYMDVW | unp | 28 | 0.07 |
| VJ:Vh-(Dh)-Jh | V6-1 -0/39/-5 J6 | CAREWVYCSSTSCYVS#YYYYGMDVW | unp | 26 | 0.07 |

## B: IGHD-IGHJ

### B-ALL: Clonal (24106 reads)
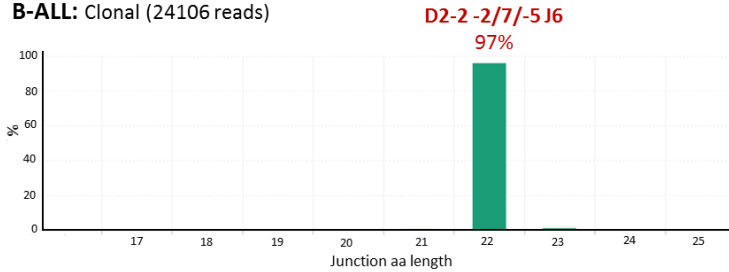


**D2-2 -2/7/-5 J6**
97%

| rearrangement type | clonotype | junction in amino acids | junction information | junction aa length | % |
|---|---|---|---|---|---|
| DJ:Dh-Jh | D2-2 -2/7/-5 J6 | VRIL**YQLLCFWYYYYGMDVW | pop | 22 | 97.2 |
| DJ:Dh-Jh | D2-2 -2/7/-5 J6 | VRIL**YQLLCFWYYYYGMDVW | pop | 23 | 1.1 |
| DJ:Dh-Jh | D2-2 -2/0/-1 J6 | VRIL**YQLLCFWYYYGMDVW | pop | 21 | 0.8 |
| DJ:Dh-Jh | D2-2 -2/12/-8 J6 | VRIL**YQLLCFWL#YYYYGMDVW | pop | 23 | 0.07 |
| DJ:Dh-Jh | D2-2 -2/2/-2 J5 | VRIL**YQLLW#NWFDPW | pop | 18 | 0.06 |
| DJ:Dh-Jh | D5-5=D5-18 -1/12/-3 J4 | VVDTAMVRGTAHFDYW | pop | 16 | 0.05 |
| DJ:Dh-Jh | D2-2 -2/7/-5 J6 | VRIL**YQLLCFW#LLLRYGTSW | pop | 23 | 0.05 |
| DJ:Dh-Jh | D2-2 -2/7/-5 J6 | VRIL**YQLLCFWYYYYGMDVR | pop | 22 | 0.03 |
| DJ:Dh-Jh | D2-2 -2/7/-2 J6 | VRIL**YQLLCFWYYYYGMDVW | pop | 23 | 0.03 |
| DJ:Dh-Jh | D1-26 -3/11/-5 J4 | VGIVGATPGV#FDYW | pop | 15 | 0.03 |

### HS: No specific product (5 reads)

## C: IGKV-IGKJ

**B-ALL:** Clonal (89337 reads)
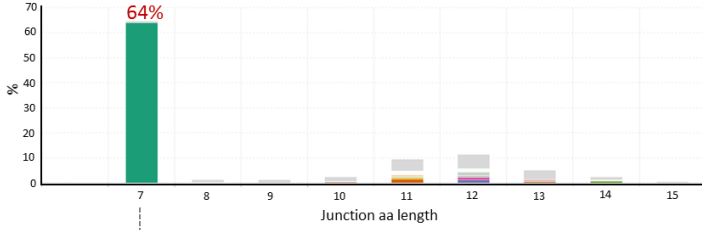


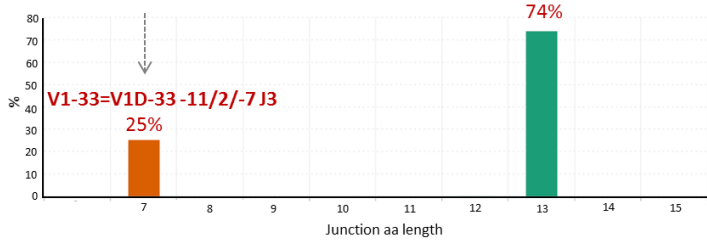| rearrangement type | clonotype | junction in amino acids | junction information | junction aa length | % |
|---|---|---|---|---|---|
| VJ:Vk-Jk | V1-33=V1D-33 -11/2/-7 J3 | CQQYD#F | unp | 7 | 64.1 |
| VJ:Vk-Jk | V2-30=V2D-30 -0/3/-7 J3 | CMQGTHWPP#F | unp | 11 | 1.4 |
| VJ:Vk-Jk | V1-33=V1D-33 -0/0/-1 J4 | CQQYDNLP#LTF | unp | 12 | 1.3 |
| VJ:Vk-Jk | V4-1 -0/0/-0 J4 | CQQYYSTPPLTF | pro | 12 | 0.8 |
| VJ:Vk-Jk | V1-39=V1D-39 -3/9/-0 J4 | CQQSYSTPGGSLTF | pro | 14 | 0.7 |
| VJ:Vk-Jk | V1-37=V1D-37 -0/0/-5 J4 | GQRTYNAP##F | unp | 11 | 0.6 |
| VJ:Vk-Jk | V1-33=V1D-33 -1/10/-6 J4 | CQQYDNLPLKAHF | pro | 13 | 0.5 |
| VJ:Vk-Jk | V4-1 -5/2/-0 J2 | CQQYYST##CSF | unp | 12 | 0.4 |
| VJ:Vk-Jk | V4-1 -1/0/-4 J4 | CQQYYSTP#TF | unp | 11 | 0.4 |
| VJ:Vk-Jk | V1-37=V1D-37 -2/1/-0 J4 | GQRTYNAP#LTF | unp | 12 | 0.4 |

**HS:** Clonal (124755 reads)



| rearrangement type | clonotype | junction in amino acids | junction information | junction aa length | % |
|---|---|---|---|---|---|
| VJ:Vk-Jk | V2D-26 -1/2/-0 J4 | CMQDAQDPQ#LTF | unp | 13 | 74.1 |
| VJ:Vk-Jk | V1-33=V1D-33 -11/2/-7 J3 | CQQYD#F | unp | 7 | 25.3 |
| VJ:Vk-Jk | V2D-26 -1/2/-0 J4 | CMQDAQDPQ#LTF | unp | 12 | 0.2 |
| VJ:Vk-Jk | V2D-26 -1/2/-0 J4 | CMARCTRSSGSLF | pro | 13 | 0.03 |
| VJ:Vk-Jk | V2D-26 -1/2/-0 J4 | CMARCTRSS#LTF | unp | 13 | 0.02 |
| VJ:Vk-Jk | V1-33=V1D-33 -11/2/-7 J3 | CQQYD#F | unp | 6 | 0.02 |
| VJ:Vk-Jk | V2D-26 -1/2/-0 J4 | RMARCTRSS#LTF | unp | 13 | 0.02 |

## D: IGKV/intron-IGKde

**B-ALL:** Clonal (11232 reads)
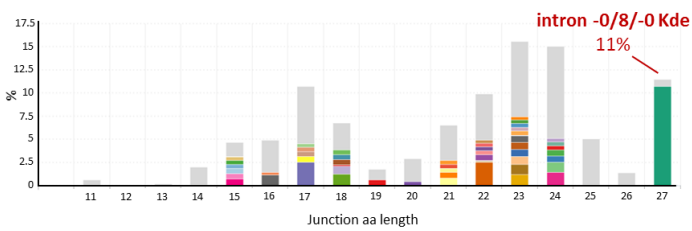


| rearrangement type | clonotype | junction in amino acids | junction information | junction aa length | % |
|---|---|---|---|---|---|
| intron-Kde | intron -0/8/-0 Kde | PCVCPIDAAVASFPDASN#SPSGSPGR | unk | 27 | 10.7 |
| Vk-Kde | V1-37=V1D-37 -4/3/-0 Kde | GQRTYNALRSPSGSPGR | unk | 17 | 2.5 |
| intron-Kde | intron -8/2/-2 Kde | PCVCPINAAVASF#SPSGSPGR | unk | 22 | 2.5 |
| intron-Kde | intron -3/3/-0 Kde | PCVCPINAAVASFPGRSPSGSPGR | unk | 24 | 1.4 |
| intron-Kde | intron -3/4/-20 Kde | PCVCPIDAAVASFPG#GR | unk | 18 | 1.2 |
| intron-Kde | intron -2/0/-3 Kde | PCVCPINAAVASFP##PSGSPGR | unk | 23 | 1.2 |
| Vk-Kde | V4-1 -10/4/-0 Kde | CQQYYT##SPSGSPGR | unk | 16 | 1.1 |
| intron-Kde | intron -2/0/-3 Kde | PCVCPIDAAVASFP##PSGSPGR | unk | 23 | 1.1 |
| intron-Kde | intron -0/0/-2 Kde | PCVCPIDAAVASFPD#SPSGSPGR | unk | 24 | 1.1 |
| Vk-Kde | V5-2 -2/10/-6 Kde | CLQHDNFPSSSSGSPGR | unk | 18 | 0.9 |

**HS:** No specific product (2 reads)

**Supplementary Fig. 2**: NGS-based clonality results. For each target the result from the B-ALL and HS sample are shown. On the left the top 50 most abundant clonotypes are visualised in colour, based on the amino acid (AA) junction length (X-axis) and frequency (Y-axis). On the right the top 10 most frequent clonotypes are shown in a table format. A) IGHV-IGHJ FR3; B) IGHD-IGHJ; C) IGKV-IGKJ; D) IGKV/intron-IGKde. B-ALL, B-cell acute lymphoblastic leukemia; HS, histiocytic sarcoma; FR, framework; unp, unproductive; pro, productive; pop, potentially productive; and unk, unknown.