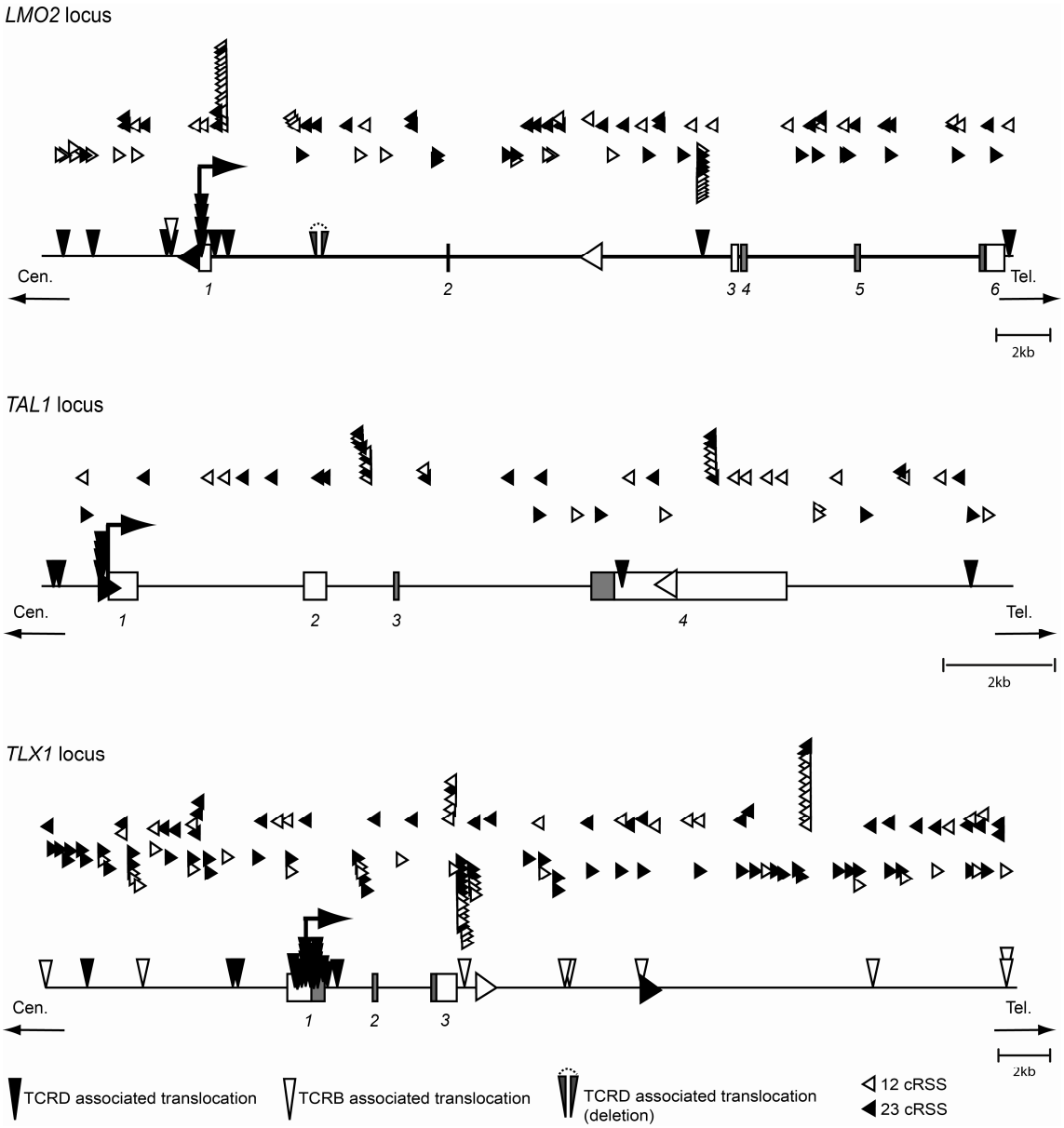
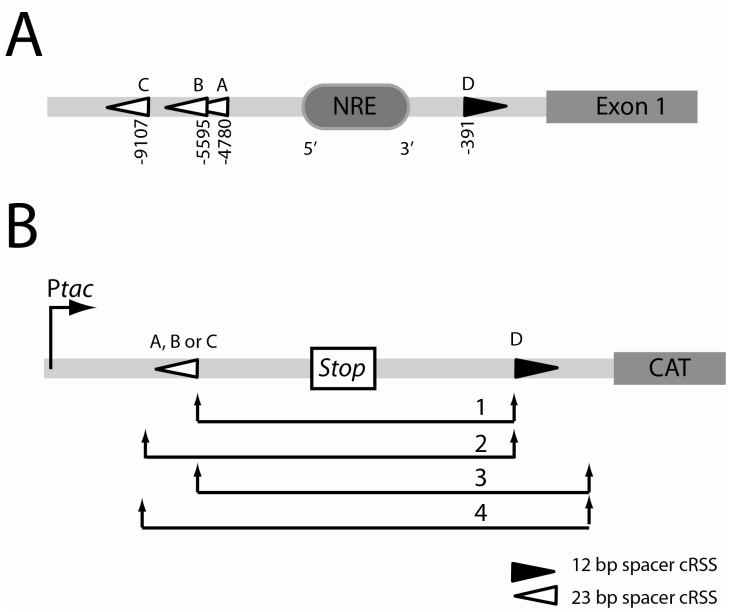
Supplementary Figure 1



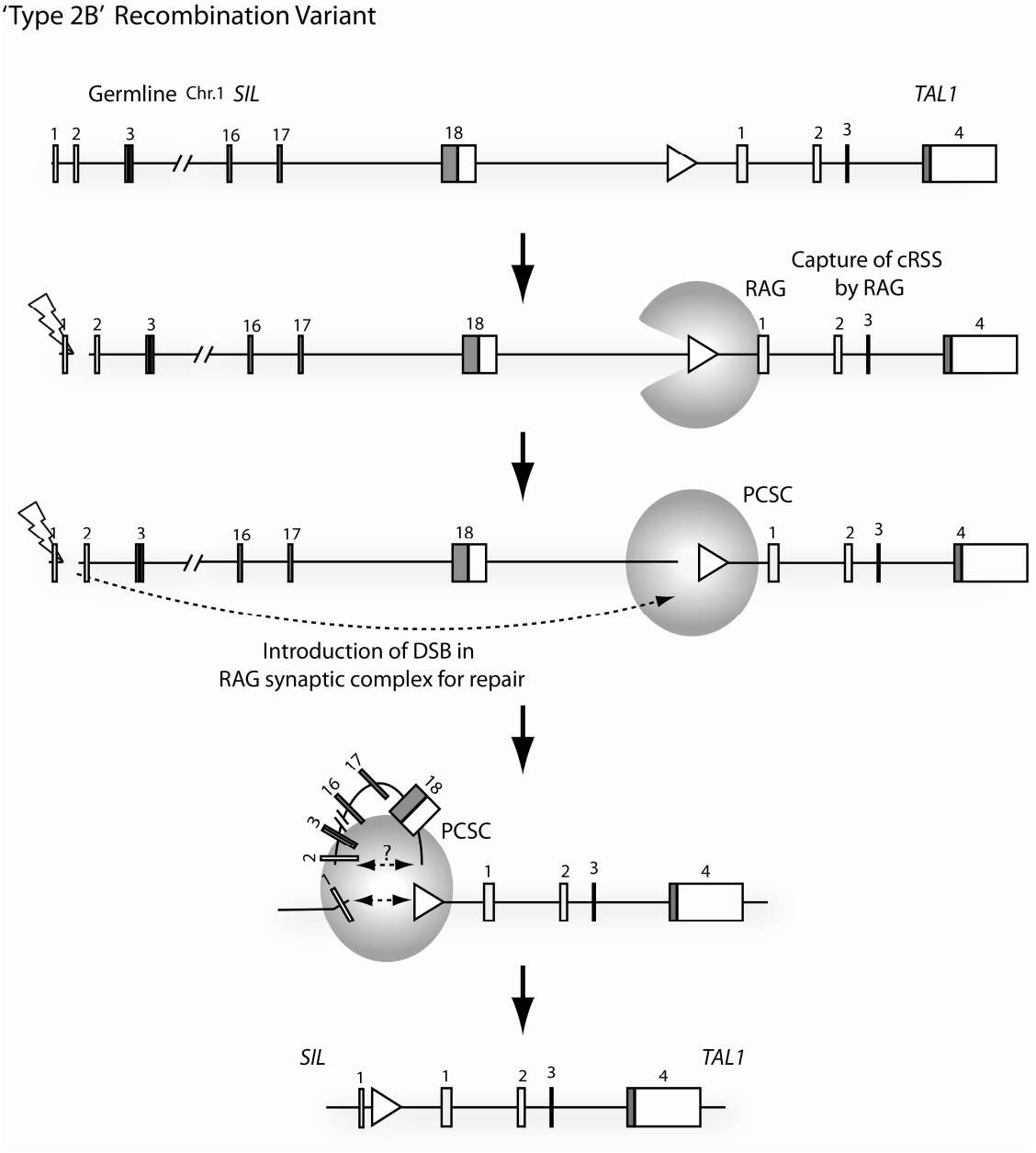
Overview of the relative position of all functional cRSSs within the *LMO2*, *TAL1* and *TLX1* locus as determined by recombination information content (RIC) algorithm tool (http://www.itb.cnr.it/rss/). The larger arrows indicate the highest RIC scoring cRSSs.

Supplementary Figure 2



A) Overview of position of cRSSs with respect to the NRE in the *LMO2* locus. Black triangle represents the 12 bp spacer cRSS at position 391 upstream of the TSS 3'of the NRE. White triangle represents the three 23 bp spacer cRSSs at positions 4780, 5594 and 9107 upstream of the TSS 5'of the NRE. Shown exon 1 of *LMO2*. B) The *LMO2* NRE 23 bp spacer cRSS were cloned in the upstream *Mlu*I-*Sal*I cassette, and the *LMO2* NRE 12 bp spacer cRSS was cloned in the downstream *SpeI-SacII* cassette. P*tac*: promoter; CAT: chloramphenicol acetyltransferase gene; Stop: transcriptional terminator. Black triangle represents the 12 bp spacer cRSS and the white triangle represents one of the three 23 bp spacer cRSSs. Pathway 1: V(D)J-mediated recombination between 12 bp spacer RSS and 23 bp spacer cRSS; pathway 2: V(D)J-mediated recombination between 12 bp spacer RSS and other BP ; pathway 3: V(D)J-mediated recombination between 23 bp spacer RSS and other BP; pathway 4: break repair mediated recombination (defined as non-V(D)J recombination mediated. At recombination via any of these pathways, the transcription termination sequence is removed enabling the activation of the CAT gene, the selection marker for plasmids having undergone recombination events.

Supplementary Figure 3



Basic representation of the 'Type 2' Recombination Variant involving the *SIL-TAL1* deletion (del(1p)).

DSB: double strand break, PCSC; post cleavage synaptic complex.

Supplementary Table 1 BP site specific primers used to produce insert sequence for cloning into the recombination substrate assay cassette

Oncogene	cRSS	BP or cRSS Position to TSS	Forward Primer	Reverse primer
Primers for cRSS functionality test against an authentic RSS				
[A] *LMO2*	23 bp spacer	-6,902	[E] 5' ACGCGT / GGCACTGTTATCTCTTGA 3'	[F] 5' GTCGAC / TGGGGAAAATTGTACTC 3'
[B] *TLX1*	-	-168/-176	[E] 5' ACGCGT / CTTCCCCTCTCTGGCTTCT 3'	[F] 5' GTCGAC / CTCCTTGGGTTTGTCTGTCT 3'
[B] *TLX1*	-	-191/ -196	[E] 5' ACGCGT / CCTCCTTGGGTTTGTCTGTCT 3'	[F] 5' GTCGAC / AGAGGAGCGGGGTGAATGA 3'
[B] *TLX1*	-	+ 11,372	[E] 5' ACGCGT / GGCTGTTACTGACTAGGTT 3'	[F] 5' GTCGAC / GGCGCTGAAACACACAATTAC 3'
[B] *TLX1*	-	+30,526/+30,539	[E] 5' ACGCGT / AAACGAGGGTCCATAGGTGAA 3'	[F] 5' GTCGAC / GGCGCTGAAACACACAATTAC 3'
[B] *LMO1*	-	-9,644	[E] 5' ACGCGT / CACATTTATTTATTTTCTTTTGGGTTTTG 3'	[F] 5' GTCGAC / AGTTTGTTTTATGAGCCAGCATTTT 3'
[B] *LMO3*	-	+224,944	[E] 5' ACGCGT / TTCTTGCCTACATTAGTCTTACTTGGAG 3'	[F] 5' GTCGAC / GAAATCAGAGACCTTACCCAGTGTC 3'
[B] *LYL1*	-	-8,444	[E] 5' ACGCGT / GGAGGGAGAGAATGGGGATG 3'	[F] 5' TGCGAC / CTGGGCTGGGGGATTTTT 3'
Primers for LMO2 12bp spacer-cRSS functionality test against an LMO2 23bp spacer-cRSS				
[B] *LMO2*	12 bp spacer	-391	[C] 5' ACTAGT / GACAGCCGGAGTCCCTTTTATT 3'	[D] 5' CCGCGG / CACCTCACCCCCTCATTCATA 3'
[B] *LMO2*	23 bp spacer	-9107	[E] 5' ACGCGT / TACATTGATCCTCCCGCCTC 3'	[F] 5' GTCGAC / AATAACCACAGAGCCCAAC 3'
[B] *LMO2*	23 bp spacer	-5594	[E] 5 'ACGCGT / AGTGATGCTCCCACAGTGTGA 3'	[F] 5' GTCGAC / GAATAGTGACTGCCCTTTCAACC 3'
[B] *LMO2*	23 bp spacer	-4780	[E] 5' ACGCGT / GCTTGAGCCAGGAGTTGTAGTCT 3'	[F] 5' GTCGAC / GATTTACAGGCCCAGAAATGTT 3'

[A] Dik *et al*[8], [B] This study, [C] *SpeI* restriction site linker, [D] *SacI* restriction site linker, [E] *MluI* restriction site linker, [F] *SalI* restriction site linker, Reference numbering according to Supplementary Reference list,

Supplementary Table 2 An extensive overview of oncogene, TCR locus, BP site and translocation type involvement in TCR translocations of 117 BP sites [§]

Gene	Chr. #	BP Distance to TSS (nt)	1st TCR Derivative (Containing coding oncogene region)	2nd TCR Derivative	Presumed Coupling	Reference*	# of breaks	*in silico* determined cRSS present	RIC-score (strand)	[B] Functionality	Translocation type
LMO2	*11*	-6,902**	nd	5'of Dδ2	(Vδ?)-Dδ2Dδ3Jδ1	T064(1)	?	CACTGTG-23-CTTATTCAC	-57.17(-)	yes[C(1)]	1
		-5,654/-5,644	3'of Dδ2	5'of Vδ3	Vδ3-Dδ2Jδ1	UPN4395(1)	3	no	-	no	2
		-1,849/-1,846**	nd nd nd	5'of Jδ1 3'of Dδ2 3'of Dδ2	(Dδ?)-Jδ1 Dδ3-(Jδ?) (Dδ?)-Jδ1	6206(1) EF455600.1[F], Patient 121 EF450258.1[F], case 906	?	no	-	yes[C(1)]	2
		-1,659**	3'of Dδ3 nd	5'of 3'RSS[Dδ3] 5'of Jβ2.3	Vδ1Dδ3-Jδ (Dβ?)-Jβ2.3	T121(1) (2)	2 ?	no	-	yes[C(1)]	2
		[D]-390 -390 -392 -384	3'of Dδ2 3'of Dδ3 3'of Dδ3 3'of Dδ2	3'of RSS[Dδ3] 3'of RSS[Dδ3] 5'of 3'RSS[Dδ3] nd	Dδ2-Jδ2 Dδ1-Jδ2 Dδ2Dδ3-Jδ2 Dδ2-(Dδ/Jδ?)	1114(3, 4) 2114(3) 647(1) T068(1)	3 3 2 ?	CACAGTA-12-GCAATAATT	-29.21(-)	yes[C(1)]	1
		+169	nd	5'of Dδ1	(Vδ?)-Dδ1Dδ2	LALW-2(5)	?	no	-	nt	2
		+716	5'of Dδ2	3'of 5'RSS[Dδ3]	(Vδ?)-Dδ2Dδ3Jδ2	8511(HA)(5, 6)	2	CACCGTG-23-TGAATAGAT	-57.56(-)	yes[C(7)]	1
		[A]+4,284 +4,778	3'of Dδ2	5'of Jδ1	Dδ2-Jδ1	UPN1589(1)	4	CACAGCA-23-CCCCAACCC CACAATA-23-CCCATAATC	-55.32(-) -58.47(-)	nt nt	1? 1?
		+8,645	nd	3'of Dδ2	Dδ2-(Jδ?)	EF450768.1[F] Patient 185	?	no	-	nt	2
		+20,600	3'of Dδ3	5'of 3'RSS[Dδ3]	Dδ2-Dδ3	T024(1)	2	CACACTA-12-ACAGAAATG	-38.74(+)	yes[C](1)	1
		+34,160	3'of Dδ2	5'of Dδ3	Dδ2-Dδ3	TALL-104(1)	3	no	-	yes[C(1)]	2
TAL1	*1*	-3,208	nd	5'of RSS[Dδ2]	((8)?/Dδ?)-Dδ2	S65911.1[F]	?	no	-	nt	2
		-932**	nd	3'of Jδ3	Jδ3[(intron)]*	Patient 1(9)	?	no	-	nt	2
		-817**	nd	5'of Jδ1	(Dδ?)-Jδ1	Patient 5(9)	?	no	-	nt	?
		+431 [D]+431 +431	nd nd nd	5'of RSS[Dδ2] 5'of Dδ3 5'of Dδ3	(Vδ?/Dδ?)-Dδ2 (Vδ?)-Dδ3Jδ1 (Vδ?)-Dδ3Jδ1	S65910.1[F] (8)Patient 4(9) Patient 2(10)	? ? ?	CACACCG-23-CGAAAAAGG	-46.33(+)	nt	1?
		+431*	nd	5'of Dδ3	(Vδ?)-Dδ3Jδ1	L23(11)	?	CACACCG-23-CGAAAAAGG	-46.33(+)	nt	1?
		+420	nd	5'of Dδ2	(Vδ?)-Dδ2Dδ3Jδ1	Patient 0(10)	?	CACACCG-23-CGAAAAAGG	-46.33(+)	nt	1?
		+427	nd	5'of Dδ2	(Vδ?)-Dδ2Jδ1	S65909.1[F] (8)	?	CACACCG-23-CGAAAAAGG	-46.33(+)	nt	1?
		+10,786	5'of Dδ3	3'of Dδ3	Dδ2-Dδ3	DU.528(12, 13)	3	no	-	nt	2

		+17,719	5'of Jδ3	3'of Dδ2	Dδ2-Jδ3	JU(14)	3	no	-		2
		+36,633	nd	5'of Dδ2	(Vδ?)-Dδ2Jδ1	Patient 6 (15)	?	no	-	nt	2
TAL1	*1*	+53,085	3'of 5'RSS$^{Jβ2.1}$	3'of 5'RSS$^{Jβ2.7}$	(Dβ2?)-Jβ2.7	(16)	?	CACACAC-12-ACACACACA CACACAC-23-AGAGAACCC	-32.09(-) -49.42(-)	nt	1?
TLX1		-11,129**	nd	5'of Dβ2.1	(Vβ?)-Dβ2.1Jβ2.4	PER-255(6)	?	no	-	nt	2
		-9,781**	5'of Dδ3	nd	(Vδ/Dδ?)-Dδ3Jδ1	UPN494(17)	?	no	-	nt	2
		-7,324**	nd	3'of 5'RSS^{Dβ2}	(Vβ?)-Dβ2	UPN57(17)	?	CACAGGT-12-ACCTAACCA	-34.28(+)	nt	1?
		-3,385**	nd	5'of Dδ3	(Vδ/Dδ?)-Dδ3Jδ1	UPN480(17)	?	no	-	no	2
		-3,158**	nd	5'of Dδ3	?-Jδ1Dδ3$^{(inverted)}$	(18)	?	no	-	nt	2
		-509/-505	3'of Dδ3	5' of Jδ1	Dδ2Dδ3-Jδ1	UPN200(17)	3	no	-	no	2
		-224/-220	3'of Dδ3	5'of Jδ1	Dδ2Dδ3-Jδ1	UPN528(17)	3	no	-	nt	2
		^{D}-191/-180	3'of 5'RSS^{Dδ3}	5'of Dδ2	Dδ2-Dδ3	T605046(17)	3				
		-197	5'of Dδ2	5'of Dδ3	Dδ2-Dδ3	UPN503(17)	3	no	-	yes	2
		-192	nd	nd	TCRD	BA(19)	?				
		^{D}-181	3'of 5'RSS^{Dδ2}	5'of Dδ3	Dδ2-Dδ3	UPN443(17)	3	no	-	no	2
		-188/-181	3'of Dδ2	5'of Dδ3	Dδ2-Dδ3	UPN553(17)	3				
		-178**	3'of Dδ2	nd	Dδ2-(Dδ/Jδ?)	UPN48(17)	?	no	-	nt	2
		^{D}-167	3'of Dδ3	5'of Jδ1	Dδ2Dδ3-Jδ1	UPN103(17)	3				
		-167	3'of Dδ2	5'of Dδ3	Dδ2-Dδ3	UPN242(17)	3				
		-509/-168	3'of Dδ2	5'of Dδ3	Dδ2-Dδ3	^{A}UPN12(17)	4				
		-167	3'of Dδ2	5'of Dδ3	Dδ2-Dδ3	UPN226(17)	3				
		-167	3'of Dδ3	nd	Dδ2Dδ3-(Jδ?)	UPN346(17)	?	no	-	no	2
		-167	3'of Dδ2	5'of Dδ3	Dδ2-Dδ3	T6329(17)	3				
	10	-166	3'of Dδ2	5' of Dδ3	Dδ2-Dδ3	T7001(17)	3				
		-169/-167	3'of Dδ2	5' of Dδ3	Dδ2-Dδ3	UPN499(17)	3				
		-167	3'of Dδ2	5' of Dδ3	Dδ2-Dδ3	UPN460(17)	3				
		-152**	3'of Dδ2	nd	Dδ2-(Dδ/Jδ?)	UPN461(17)	?	no	-	nt	2
		-139	3'of 5'RSS^{Dδ2}	3'of 5'RSS^{Dδ3}	Dδ2-Dδ3	UPN259(17)	3	no	-	no	2
		^{A,D}-131/ -107	3'of Dδ2	5'of Dδ3	Dδ2-Dδ3	UPN496(17)	3	no	-	no	2
		-107**	3'of Dδ2	nd	Dδ2-(Dδ/Jδ?)	UPN501(17)	?	no	-	nt	2
		^{D}-79	3'of Dδ3	5'of Jδ1	Dδ2Dδ3-Jδ1	UPN273(17)	3				
		-80	3'of Dδ2	5'of Dδ3	Dδ2-Dδ3	UPN281(17)	3				
		-85	3'of Dδ2	5'of Dδ3	Dδ2-Dδ3	UPN9(17)	3				
		-87	3'of Dδ2	5'of Dδ3	Dδ2-Dδ3	UPN269(17)	3				
		-81	3'of Dδ3	5'of Jδ1	Dδ2Dδ3-Jδ1	UPN362(17)	3	no	-	yes	2
		-87	3'of Dδ3	5'of Jδ1	Dδ2Dδ3-Jδ1	UPN506(17)	3				
		-81	3'of Dδ2	nd	Dδ2-(Dδ/Jδ?)	UPN452(17)	?				
		-88	3'of Dδ2	5'of Dδ3	Dδ2-Dδ3	BO(20)	3				
		-86	nd	3'of 5'RSS^{Dδ2}	(Vδ/Dδ?)-Dδ2	JM(20, 21)	?				
		-86	nd	3'of 5'RSS^{Dδ2}	(Vδ/Dδ?)-Dδ2	^{A}DW(20, 21)	?				
		-83	nd	3'of 5'RSS^{Dδ2}	(Vδ/Dδ?)-Dδ2	VB(21)	?				

		-86	nd	3'of 5'RSS$^{D\delta2}$	(Vδ/Dδ?)-Dδ2	AR(21)	?				
		-80	nd	3'of 5'RSS$^{D\delta2}$	(Vδ/Dδ?)-Dδ2	BN(21)	?				
		-67	nd	3'of 5'RSS$^{D\delta2}$	(Vδ/Dδ?)-Dδ2	JS(21)	?				
		+19	nd	5'of Dδ3	(Vδ/Dδ?)-Dδ3Jδ1	UPN430(17)	?	no	-	no	2
		+1,313**	nd	5'of (Dδ3)Jδ1	(Vδ?)-(Dδ3)Jδ1	T-ALL1143(22)	?	no	-	nt	2
		+6,839**	5'of Jβ2.5	nd	(Dβ?)-Jβ2.5	UPN43(17)	?	CACACCA-12-GCATACAAT	-32.87(-)	nt	1?
	10	+11,177	5'of Jβ2.1	3'of Dβ1	Dβ1-Jβ2.7	UPN537(17)	3	no	-	nt	2
		+11,369/ +11,372	5'of Jβ2.5	3'of Dβ1	Dβ1-Jβ2.5	T178(17)	3	no	-	yes	2
		+14,531	5'of Jβ1.5	3'of Dβ1	Dβ1-Jβ1.5	UPN474(17)	3	CACAGAG-23-AGGGAAGCG	-47.75(-)	nt	1?
		+24,671**	nd	3'of Dβ1	Dβ1-(Jβ?)	UPN546(17)	?	no	-	nt	2
		^{D}+30,540	nd	3'of Dβ1	Dβ1-(Jβ?)	T051(17)	?	no	-	yes	2
		+30,538	5'of Jβ2.1	3'of Dβ2	Dβ2-Jβ2.1	UPN280(17)	3				
TAL2	9	^{D}+31,795/+31,766 (us TMEM38B)	5'of Jβ2.3	3'of Dβ1.1	Dβ1.1-Jβ2.3	SUP-T3(23)	2	CACTGTG-12-TATAAAAAT	-28.39(-)	nt	1?
			5'of Jβ2.7	3'of Dβ1.1	Dβ1.1-Jβ2.7	SUP-T6(23)	2	CACACTG-12-GATATAAAA	-36.38(-)	nt	
			5'of Jβ2.7	3'of Dβ1.1	Dβ1.1-Jβ2.7	BT(23)	2				
LYL1	19	+8,444	5'of Jβ1.1	3'of Dβ1	Dβ1-Jβ1.1	(24)	3	no	-	nt	2
		+787**	5'of Jβ1.1	nd	(Dβ?)-Jβ1.1	(25)	?	no	-	nt	2
CCND2	*12*	-61,328**	nd	3'of Dβ1	Dβ1-(Jβ?)	[46]	?	CATAGTC-12-ATCTTCCCC	-38.70(-)	nt	1?
MYC	8	^{A}+10,318	5'of Jα34	5'of Jα38	Jα38-Jα34	(26, 27)	3	no	-	nt	1?
		+1165,291						CACAATC-23-TGAGTATTC	-56.02(+)		
		+7,906**	5'of Jα31	nd	(Vα?)-Jα31	(26, 27)	?	no	-	nt	2
		+5,978	5'of 3'RSS$^{V\alpha36V\delta7}$	3'of Vα36Vδ7	Vα36Vδ7-(Jα?)	(26, 28)	2	no	-	nt	2
		+239,565**	nd	5'of Dδ3	(Vδ?/Dδ?)-Dδ3Jδ1	(26, 29)	?	no	-	nt	2
		+5,964**	3'of Jα59	nd	Jα59intr.58	(30)	?	no	-	nt	2
NOTCH 1	9	^{D}+39,552**	5'of Dβ2.2 (1tm24)	nd	(Vβ?)-Dβ2.2Jβ2.2	(31, 32)	?	CACTGAA-23-CTGCTAACC	-55.91(-)	nt	1?
			5'of Jβ2.2 (1tm24)	nd	(Dβ?)-Jβ2.2	Case 1(31)	?				
		^{D}+39,653/+39,649 **	5'of Jβ1.1 5'of Jβ1.1	nd	(Dβ?)-Jβ1.1	Case 2(31)	?	no	-	nt	2
				nd	(Dβ?)-Jβ1.1	Case 3(31)	?				
		+42,969**	3'of Jβ2.7^{int}	nd	(Dβ?)-Jβ2.7	(33)	?	no	-	nt	2

LMO1	11	-9,644	3'of Dδ2	5'of Jδ1	Dδ2-Jδ1	(24)	3	no	-	no	2
		[D]-5,258	5'of3'RSS[Dδ2] 5'of3'RSS[Dδ2]	5'of Dδ2/Dδ3 5'of Dδ1	Dδ2-Dδ3Jδ1 Vδ-Dδ1Dδ2Jδ1	(34) (35)	3	CACAGTG-12-ACACAGCCC CACAGTG-23-ACTCTGGCA	-29.44(+) -44.10(+)	no no	1?
LMO3	12	+224,944	5'of Jβ2.1	3'of Vβ2.3	Vβ2.3-Jβ2.1	(36)	3	no	-	no	2
NKX2-4	20	-494	3'of Dδ2	3'of 5'RSSDδ3	Dδ2-Dδ3	Unpub.	3	no	-	no	2
NKX2-1	14	+4,286	5'of Jδ1 3'of Dβ1[int]	nd nd	(Dδ?)-Jδ1 Dβ1-(Vβ?)	T033 unpub. 9989(37)	2 2	no	-	nd	2
NKX2-5	5	+35,271**	nd	3'of Dδ3	Dδ3-(Jδ?)	(38)	?	no	-	nd	2
C-MYB	6	[D]+53,891/+53,903	5'of Jβ1.2 5'of Dβ2	3'of 5'RSSDβ1 3'of Dβ1	(Dβ1?)-Jβ1.2 Dβ1-Dβ2	T124(39) UPN5846(39)	3 3	no	-	nt	2
		+144,584 (DS AHI1)	3'of Dβ1	5'of Jβ1.2	Dβ1-Jβ1.2	TL34(39)	3	CACAGTG-12-GTAGGATTT CACAGTG-23-TAAGTGATT	-33.04(-) -53.27(-)	yes	1
BMI1	10	-277,473	5'of Jβ2.3	3'of Dβ2.2	Dβ2.2-Jβ2.3	T003(40)	3	no	-	nt	2
LCK	1	-20,286	5'of Jβ1.2	3'of Dβ1.1	Dβ1.1-Jβ1.2	(41)	3	CACACAC-12-GCCAAAACA CACAGAC-23-AGCCAAAAC	-27.16(+) -53.83(+)	yes[C](7)	1
		-25,085	5'of Dβ1.1	3'of Vβ11.2	Vβ11.2-Dβ1.1Jβ2.7	(42)	3	no	-	nt	2
TLX3	5	-50,034** (DS RANBP17)	nd	5'of Dδ2	(Vδ?)-Dδ2Dδ3Dδ2Jδ1	(43)	?	no	-	nt	2
OLIG2 (BHLHB 1)	21	+84,224 (DS OLIG1)	5'of Jα53	3'of Vα29Vδ5	Vα29Vδ5-Jα53	(44)	3	no	-	nt	2
HOXA6	7	-2,179	3'of Dδ3	5'of Jδ1	Dδ2Dδ3-Jδ1	(45)	3	no	-	nt	2
HOXA9	7	-2,992	3'of Dβ1	5'of Jβ2.7	Dβ1-Jβ2.7	(46)	3	no	-	nt	2
		-501[E]	5'of Jβ2.7	-	(Dβ)-Jβ2.7	(36)	2	no	-	nt	2
BCL11B	14	+48.651**[E]	5'of Dδ2	-	(Vδ)-Dδ2	(47)	2	no	-	nt	2

TCL1A	14	-99,998[E]	5'of Jα42-1	-	(Vα?)-Jα42-1	(48)	3	no	-	nt	2
		-145,630	5'of Cβ1	nd	-	(49)	?	no	-	nt	2

[§] Comprehensive overview of the different TCR translocations identified in T-ALL and their corresponding translocation partners.
Only those translocations, of which complete sequences and BPs were available, were used in this study. For each BP the distance (nt) relative to its corresponding TSS was determined and the presence of a cRSSs was *in silico* determined with the recombination information content (RIC) algorithm tool (http://www.itb.cnr.it/rss/)(50)For a few cases the functionality of possible cRSS was also tested *ex vivo*.
* Reference numbering according to Supplementary Reference list, **The precise BP position is not known due to type of break, [A] Deletion of > 10nt, [B] If functionality of the cRSS or BP associated sequence was *ex vivo* tested; yes, no or not tested (nt) , [C] Reference to article where functionality of that particular cRSS was tested, [D] More than one break was identified to be associated to this BP site in different T-ALL patients, [E] Inversion, [F] Accession number GenBank, *Brake in intron of TCR locus, nd: not determined due to absence of reciprocal translocation sequence, No: no functional cRSS found at that BP position according to the RIC algorithm analysis, -: no RIC score, ?: not known

Supplementary Table 3 Ex vivo analysis of functionality BP site associated sequences involved in LMO1, LMO3 and LYL1 TCR translocations.

Oncogene	Position BP (nt)	[A]cRSS	[B]Vector used	[C]No. Colonies	Recombination Pathway			
					[D]1	[E]2	[F]3	[G]4
LMO2	-6,902	[H]CACAGTG-23-ACGCTCAAC	Dδ3	7	4	0	2	1
			$Dδ3_{inverted}$	10	0	1	2	6
TLX1	-168/-176	no cRSS	Dδ3	9	0	0	0	7
			$Dδ3_{inverted}$	8	0	0	0	8
TLX1	-191/-196	no cRSS	[I] Dδ3	1	0	0	1	0
TLX1	+11,372	no cRSS	[I] Dδ3	5	0	0	0	5
TLX1	+30,526/+30,539	no cRSS	Dδ3	1	0	0	0	1
			$Dδ3_{inverted}$	8	0	0	0	7
LMO1	-9,644	no cRSS	Dδ3	0	0	0	0	0
			$Dδ3_{inverted}$	1				
LMO3	+224,944	no cRSS	Dδ3	0	0	0	0	0
			$Dδ3_{inverted}$	0				
LYL1	-8,444	no cRSS	Dδ3	0	0	0	0	0
			$Dδ3_{inverted}$	0				

[A] Presence of cRSS determined by in silico analysis, result yes or no
[B] Cassette used as described by Dik et al (1)
[C] Total number of clones obtained and analyzed (total number of colonies obtained from one transfections)
[D] Total number of clones which had a V(D)J-mediated recombination between authentic Dδ3 RSS and the oncogene BP sequence
[E] Total number of clones which had a V(D)J-mediated recombination between authentic Dδ3 RSS and other cRSS in the cloned oncogene BP sequence
[F] Total number of clones which had a V(D)J-mediated recombination between authentic Dδ3 RSS and other cRSS in the core vector
[G] Total number of clones which had a break repair mediated recombination (defined as non V(D)J recombination mediated
[H] cRSS sequence from Dik et al (1)
[I] $Dδ3_{inverted}$ not tested

Supplementary Table 4 Ex vivo and in silico analysis of LMO2 cRSS functionality at the NRE site.

12 bp spacer cRSS	RIC score	cRSS position to TSS (nt)	23 pb spacer cRSS	RIC score	cRSS position to TSS (nt)	No. Colonies[A]	Recombination Pathway			
							1[B]	2[C]	3[D]	4[E]
CACAGTA-12-GCAATAATT	-29.20	-391	CACACCA-23-GGCAAGACC	-56.93	-4780	0	0	0	0	0
			CACAGTG-23-GGCTGTCAG	-58.68	-5595	0	0	0	0	0
			CAAAGTG-23-AGAGCCTGG	-57.22	-9107	5	0	0	0	5

[A] Total number of clones obtained and analyzed (total number of colonies obtained from two independent transfections)
[B] Total number of clones which had a V(D)J-mediated recombination between 12 bp spacer RSS and 12 bp spacer cRSS
[C] Total number of clones which had a V(D)J-mediated recombination between 12 bp spacer RSS and other BP
[D] Total number of clones which had a V(D)J-mediated recombination between 23 bp spacer RSS and other BP
[E] Total number of clones which had a break repair mediated recombination (defined as non V(D)J recombination mediated)

Supplementary Design and Methods

Human material and DNA isolation

Diagnostic bone marrow or peripheral blood samples from T-ALL patients were used. All samples were used after informed consent was obtained in accordance with Institutional Review Board guidelines (IRB; project MEC 2007-394) and the Declaration of Helsinki. The tumor load of the samples was in most cases approximately 90%. DNA isolation was carried out using the Qiagen DNA isolation kit (Qiagen, CA, USA) according to manufacturer's protocol.

Ligation-mediated polymerase chain reaction (LM-PCR)

Translocation junctions were identified by means of ligation-mediated polymerase chain reaction (LM-PCR) (50). In short, 1µg of T-ALL DNA and HeLa DNA were digested with either *DraI, PvuII* (Invitrogen, Grand Island, NY*) HincII,* or *StuI* (New England BioLabs, Ipswich, MA) blunt-end enzymes. After O/N digestion and phenol-chloroform (Sigma, Switzerland) extraction, DNA was precipitated. 50µM of adaptor was ligated to both ends of the DNA fragments. TCR translocation partners were detected via nested PCR with adaptor-specific AP1 and AP2 reverse primers and TCRD- or TCRB-specific primers (1, 47). PCR products of T-ALL samples that give a dissimilar band size compared to products of the HeLa germline control samples were isolated using the QIAquick gel extraction kit (Qiagen) and sequenced with the BigDye Terminator V3.1 cycle sequencing kit (Applied Biosystems, Warrington, UK).

Literature search and definition of TCR translocation BP sites

To create a comprehensive overview of all different TCR-associated translocations in T-ALL including the corresponding translocation partners and to determine the nature of these translocations, a broad survey was conducted on TCR translocation BP sites in human T-ALL. Sequence information on TCR translocations from our T-ALL cohort and T-ALL TCR translocations described in literature were used. First articles which give an overview of TCR and non-TCR associated aberrations seen in T-ALL were enquired (7, 51-55). Based on these results a search for each gene was done in the MEDLINE database through the PubMed search engine (http://www.ncbi.nlm.nih.gov/pubmed/), using a combination of the following search terms: <oncogene name>, and/or T-cell acute lymphoblastic leukemia, T-ALL, and translocation. Furthermore, the GenBank data base was searched. In total, 117 BP sites (from 22 different TCR translocation partners) and 118 BP sites from non-TCR aberrations (involving 3 different genes) were

used in this study. In ~40% of the cases both derivate sequences were known. In cases lacking one derivate sequence, a region of 50 bp flanking the known fusion site was considered as potential BP site. The position of every BP site was determined relative to the transcription starting site (TSS) of each particular oncogene involved, using TSS positions as given by the UCSC database (http://genome.ucsc.edu).

***In silico* determination of cRSS functionality**

The online recombination information content (RIC) algorithm tool (http://www.itb.cnr.it/rss/) was used to predict the functionality of regions of translocation junctions identified in our T-ALL patients and those found in the literature. Regions of 100 nucleotides upstream and downstream of the BP sites were analyzed for the presence of functional 12 and 23 nucleotide spacer cRSSs. Pass/fail thresholds given by the RIC tool were used (12 RSS: pass with RIC > -38.81, 23 RSS: pass with RIC >-58.45).

***Ex vivo* recombination substrate assay**

Regions surrounding BP sites involving different oncogenes, or regions surrounding the negative regulatory element (NRE) of the *LMO2* locus were cloned into recombination-constructs as described previously (1). Primers were designed with restriction enzyme linkers for cloning into the *MluI-SalI* or *SpeI-SacII* cassette (Supplementary Table 1). In total 22 new, different constructs were made. Fourteen inserts were cloned into the *MluI-SalI* cassette as previously described (1) (Supplementary Table 1). The remaining inserts were cloned to produce *LMO2* constructs each of which carried the 12 bp spacer cRSS (391 nt upstream of the *LMO2* TSS) cloned within the *SpeI-SacII* cassette with one of the three 23 bp cRSSs (at positions 478, 5594 or 9107 upstream *LMO2* TSS) cloned within the *MluI-SalI* cassette (Supplementary Figure 2). *LMO2* 23bp-spacer cRSS (BP position -6,902) with a RIC score of -57.17, which in our previous study was proven to function as cRSS (1), was used as a positive control in the recombination substrate assay. To make sure that we had no bias with respect to the 12/23 rule, recombination vectors carrying an authentic Dδ3 RSS with both a 12 and a 23 bp spacer were used which was also tested in an inverted orientation (1). The recombination substrate assay was performed as previously described (1).

References to the translocations in the Supplementary Table 2 and Supplementary Design and Methods

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