

	Vδ6.3	P/N	Dδ1	P/N	Dδ2	P/N	Jδ1
	GCT CTC TGG GAG CTG G		ATGGCATAT		ATCGGAGGGATACGAG		CT ACC GAC AAA
	A L W E L V				I G G I R A		T D K

WT liver V6⁺ cells (n=17)

fetal	(9)	GCT CTC TGG GAG C	AT		ATCGGAGGGATACGAG		CT ACC GAC AAA
		A L W E H			I G G I R A		T D K
	(1)	GCT CTC TGG GAG C	AT		ATCGGAGGGATACGAG		CT ACC GAC
		A L W E H			I G G I R A		T D
	(6)	GCT CTC TGG GAG CT	T		ATCGGAGGGATACGAG		CT ACC GAC AAA
		A L W E L			I G G I R A		T D K
	(1)	GCT CTC TGG GAG CT	T		ATCGGAGGGATACGAG		CT
		A L W E L			I G G I R A		

Itk^{-/-} liver V6⁺ cells (n=14)

fetal	(4)	GCT CTC TGG GAG C	AT		ATCGGAGGGATACGAG		CT ACC GAC AAA	
		A L W E H			I G G I R A		T D K	
	(3)	GCT CTC TGG GAG CTG G			TGGGAGGGATACGAG		CT ACC GAC AAA	
		A L W E L V			G G I R A		T D K	
	(3)	GCT CTC TGG GAG CTG G	C		CGGAGGGATACGAG		CT ACC GAC AAA	
		A L W E L A			G G I R A		T D	
adult	(4)	GCT CTC TGG GAG CTG G	TCCCATAT	ATGGC	CCTTT	ATCGGAGGGATACG	GCA	ACC GAC AAA
	A L W E L V	P Y	M A	L Y	R R D T	A	T D K	

WT-4Get thymocyte stage 1 (CD122⁻GFP⁺; n=28)

fetal	(4)	GCT CTC TGG GAG C	AT		ATCGGAGGGATACGAG		CT ACC GAC AAA	
		A L W E H			I G G I R A		T D K	
	(5)	GCT CTC TGG GAG CTG G			TGGGAGGGATACGAG		CT ACC GAC AAA	
		A L W E L V			G G I R A		T D K	
	(2)	GCT CTC TGG GAG CTG G			TGGGAGGGATACGAG		CT	
		A L W E L V			G G I R A			
	(5)	GCT CTC TGG GAG CTG G	C		CGGAGGGATACGAG		CT ACC GAC AAA	
		A L W E L A			G G I R A		T D K	
adult	(2)	GCT CTC TGG G	GCCT	A	ATCGGAGG	AATAT	CT ACC GAC AAA	
		A L W G	L		I G G	I S	T D K	
	(1)	GCT CTC TGG GAG CTG G	T	TGGC	TT	TCCAG	CT ACC GAC AAA	
		A L W E L V		G	L	E G	P A	T D K
	(1)	GCT CTC TGG GAG CTG G	CCT		CGGAGG	CCCA	ACC GAC AAA	
		A L W E L A	P		E G	P	T D K	
	(1)	GCT CTC TGG GAG CTG G	AG	GG	GGAT	ATCGGAGGGATAC	CTG	CT ACC GAC AAA
		A L W E L E		G	D	I G G I P	A	T D K
	(1)	GCT CTC TGG GAG CTG	AAG	GG	AGAT	ATCGGAGGGAT	TCCCG	CT ACC GAC AAA
		A L W E L	K	G	D	I G G I	P A	T D K
	(1)	GCT CTC TGG GAG CT	C	CAT		ATCGGAGGGATACGAG	CTT	CC GAC AAA
		A L W E L		H		I G G I R A	S	D K
	(1)	GCT CTC TGG GAG C	CAG	ATAT	C	GGG	G	CT ACC GAC AAA
		A L W E P	D	I		G	A	T D K
(1)	GCT CTC TGG GAG C	CCT	AT		ATCGGAGGGATA	GTCTTCT	CT ACC GAC AAA	
	A L W E P	Y			I G G I	V F S	T D K	
(1)	GCT CTC TGG GAG		TGGC		CGGAGGGATACGAG	CTTAGG	ACC GAC AAA	
	A L W E		W P		E G Y E	L R	T D K	
(1)	GCT CTC TGG GAG CT	TGAT			ATCGGAGGGATACG	TCCCG	CT ACC GAC AAA	
	A L W E L	D			I G G I R	P A	T D K	
(1)	GCT CTC TGG GAG CT	T			ATCGGAGGGATACG	GGGTCCG	CT ACC GAC AAA	
	A L W E L				I G G I R	G S A	T D K	

WT-4Get thymocyte stage 2 (CD122⁺GFP⁺; n=25)

fetal	(1)	GCT CTC TGG GAG C	GT		ATCGGAGGGATACGAG		CT
		A L W E R			I G G I R A		
	(1)	GCT CTC TGG GAG C	AG		ATCGGAGGGATACGAG		CT ACC GAC AAA
		A L W E Q			I G G I R A		T D K
	(2)	GCT CTC TGG GAG CTG G			TGGGAGGGATACGAG		CT ACC GAC AAA
		A L W E L V			G G I R A		T D K
adult	(1)	GCT CTC TGG GAG C	AT		ATCGGAGGGATACG	GG	CT ACC GAC AAA
		A L W E H			I G G I R	A	T D K
	(1)	GCT CTC TGG GAG CTG G	C		CGGAGGGATA	ACGG	CT ACC GAC AAA
		A L W E L A			G G I	T A	T D K
	(1)	GCT CTC TGG GAG	TTCG		AGGGATACGAG	CTTG	CC GAC AAA
		A L W E	F E		G Y E	L A	D K

adult	(1)	GCT CTC TGG GAG C	AGT	ATGGCA	GAAT	ATCGGAGGGAT		CC GAC AAA
		A L W E Q	Y	G R	I	S E G S		D K
	(1)	GCT CTC TGG GAG C		ATGGCATAT	CGTCT	ATCGGAGGGATACGAG		CT ACC GAC AAA
		A L W E H		G I S	S	I G G I R A		T D K
	(1)	GCT CTC TGG GAG C	ACAT	ATGGCAT		ATCGGAGGGATACGA		ACC GAC AAA
		A L W E H	I	W H		I G G I R		T D K
	(1)	GCT CTC TGG GAG C			ACCTT	ATCGGAGGGATACGAG	CCCCGTA	T ACC GAC AAA
		A L W E H			L	I G G I R A	P Y	T D K
	(1)	GCT CTC TGG GAG C	AT	ATGGCATAT	GA	CGGAGGGATACGAG		CC GAC AAA
		A L W E H		M A Y	D	G G I R A		D K
	(1)	GCT CTC TGG GAG CTG G	ACGGA	ATGGCATAT	T	GGATACGAG		CT ACC GAC AAA
		A L W E L D	G	M A Y	W	I R A		T D K
	(1)	GCT CTC TGG GAG CTG G	CGAGAGGCAT			ATCGGAGGGATACG	GGGGAT	CC GAC AAA
		A L W E L A	R G I			S E G Y G	G S	D K
	(1)	GCT CTC TGG GAG CTG G			AGGGA	CGGAGGGATACG	C	CC GAC AAA
		A L W E L E			G	R R D T	P	D K
	(1)	GCT CTC TGG GAG C	CTCAT			ATCGGAGGGATACGAG	CTC	CC GAC AAA
		A L W E P	H			I G G I R A	P	D K
(1)	GCT CTC TGG GAG C	CTCAT			ATCGGAGGGATAC	CCTCCC	CC GAC AAA	
	A L W E P	H			I G G I P	S P	D K	
(2)	GCT CTC TGG GAG CTG G	GGGA	GG	T	ATCGGAG	CC	ACC GAC AAA	
	A L W E L G	G	G		I G A		T D K	
(2)	GCT CTC TGG GAG CTG G			GGG	CGGAGGGATACG	GGG	CT ACC GAC AAA	
	A L W E L G			A	E G Y G	A	T D K	
(1)	GCT CTC TGG GAG CTG G	CGGCCCT			ATA	TGGCCCTC	CT ACC GAC AAA	
	A L W E L A	A L			Y	G P P	T D K	
(1)	GCT CTC TGG GAG CTG G	GG			GGAGGGATACGAG	CCC	CT ACC GAC AAA	
	A L W E L G				G G I R A	P	T D K	
(1)	GCT CTC TGG GAG CT	AGGG	GGC	TTCTT	ATCGGAGGGATAC	C	CC GAC AAA	
	A L W E L	G	G	F L	S E G Y	P	D K	
(1)	GCT CTC TGG GAG CT	C	TAT		ATCGGAGGGATACGAG	CTC	CC GAC AAA	
	A L W E L		Y		I G G I R A	P	D K	

WT-4Get thymocyte stage 3 (CD122⁺GFP⁻; n=22)

fetal	(3)	GCT CTC TGG GAG C	AT			ATCGGAGGGATACGAG		CT ACC GAC AAA
		A L W E H				I G G I R A		T D K
adult	(2)	GCT CTC TGG GAG CTG G				TCGGAGGGATACGAG		CT ACC GAC AAA
		A L W E L V				G G I R A		T D K
	(1)	GCT CTC TGG GAG CTG G	C			CGGAGGGATACGA	A	CT ACC GAC AAA
		A L W E L A				G G I R	T	T D K
	(1)	GCT CTC TGG GAG CTG G				GGGATA		CT ACC GAC AAA
		A L W E L G				D T		T D K
	(1)	GCT CTC TGG GAG	AC			CGGAGGG	GTACGGT	CT
		A L W E L	T			G G	V R S	
	(1)	GCT CTC TGG GAG	T			TCGG		T ACC GAC AAA
		A L W E				G		T D K
	(1)	GCT CTC TGG GAG C	CTCAT			ATCGGAGGGATACGAG	CTC	CC GAC AAA
		A L W E P	H			I G G I R A	P	D K
	(1)	GCT CTC TGG GAG C	C	ATGGCA	ACTCC	GAGGGATACGAG		CT ACC GAC AAA
		A L W E P		W Q	L R	G I R A		T D K
	(1)	GCT CTC TGG GAG CTG G	GGTTGGG	TGG	CCCCAT	ATCGGAGGG	GT	C GAC AAA
		A L W E L G	L G	G	P H	I G G	V	D K
	(1)	GCT CTC TGG GAG CTG G	AG	GG	GGAT	ATCGGAGGGATAC	CTG	CT ACC GAC AAA
		A L W E L E		G	D	I G G I P	A	T D K
(1)	GCT CTC TGG GAG		TGG		CGGAGGGATAC	CCCTC	CT ACC GAC AAA	
	A L W E		W		R R D T	P P	T D K	
(1)	GCT CTC TGG GA	CAAT			ATCGGAGGGATACG	GG	CT ACC GA	
	A L W D	N			I G G I R	A	T D K	
(1)	GCT CTC TGG GA	CCAGGT	ATGGC	CCCCACC	CGGAGGGATACGAG		CT ACC GAC AAA	
	A L W D	Q V	W P	P P	G G I R A		T D K	
(1)	GCT CTC TGG GAG CT	T			ATCGGAGGGATACGAG	CTCCCC	CT ACC GAC AAA	
	A L W E L				I G G I R A	P P	T D K	
(1)	GCT CTC TGG GAG CTG G	GG			GGAGGGATACGAG	CCC	CT ACC GAC AAA	
	A L W E L G				G G I R A	P	T D K	
(1)	GCT CTC TGG GAG CTG G	CGGGG			CGGAGGGATA	CCCCCG	CT ACC GAC AAA	
	A L W E L A	G			R R D T	P A	T D K	
(1)	GCT CTC TGG GAG CTG		TAT		ATCGGAGGG	GTTG	CT ACC GAC AAA	
	A L W E L		Y		I G G	V A	T D K	
(1)	GCT CTC TGG GAG CTG		TAT		ATCGGAGGGATACGAG	CTA	CT ACC GAC AAA	
	A L W E L		Y		I G G I R A	T	T D K	
(1)	GCT CTC TGG GAG CT	CCG			CGGAGGGATACGAG		CT ACC GAC AAA	
	A L W E L	R			G G I R A		T D K	

Itk^{-/-}-4Get thymocyte stage 1 (CD122⁻GFP⁺; n=25)

fetal	(4)	GCT CTC TGG GAG C	AT		ATCGGAGGGATACGAG		CT ACC GAC AAA
		A L W E H			I G G I R A		T D K
	(1)	GCT CTC TGG GAG C	AT		ATCGGAGGGATACGAG		CT ACC
		A L W E H			I G G I R A		T
	(1)	GCT CTC TGG GAG C	GT		ATCGGAGGGATACGAG		CT ACC GAC AAA
		A L W E R			I G G I R A		T D K
adult	(1)	GCT CTC TGG GAG CTG G			TCCGAGGGATACGAG		CT ACC GAC AAA
		A L W E L V			G G I R A		T D K
	(1)	GCT CTC TGG GAG CTG			ATCGGAGGGATACGAG		CT ACC GAC AAA
		A L W E L			I G G I R A		T D K
	(2)	GCT CTC TGG GAG CTG G	C		CGGAGGGATACGAG		CT
		A L W E L A			G G I R A		
	(1)	GCT CTC TGG GAG CT	TGAT		ATCGGAGGGATACGAG		CT
		A L W E L	D		I G G I R A		
	(2)	GCT CTC TGG GAG CTG G			GGGATACG	G	CT ACC GAC AAA
		A L W E L G			D T	A	T D K
	(1)	GCT CTC TGG GAG CTG G	T		GGG	TC	CT ACC GAC AAA
		A L W E L V			G	P	T D K
	(1)	GCT CTC TGG GAG CT	TCATCG		AGGGATACGAG		CT ACC GAC AAA
		A L W E L	H R		G I R A		T D K
(1)	GCT CTC TGG GAG CTG G	TCCTCTA		CGGAG		CT ACC GAC AAA	
	A L W E L V	L Y		G A		T D K	
(1)	GCT CTC TGG GAG CTG G		CATAT	ATCGGAGGGATAC		CC GAC AAA	
	A L W E L A		Y	I G G I P		D K	
(1)	GCT CTC TGG GAG CTG G		ATGGC	GGATCGGGC	A	ACC GAC AAA	
	A L W E L D		G	I G A		T D K	
(1)	GCT CTC TGG GAG CTG G	AGCCGG	TGGCATAT	ATGTGG	A	ACC GAC AAA	
	A L W E L E	P V	A Y	M W		T D K	
(2)	GCT CTC TGG GAG	TTCAAT		CGGAGGGATACGAG		CT ACC GAC AAA	
	A L W E	F N		R R D T R		T D K	
(1)	GCT CTC TGG GAG	GAAT		ATCGGAGGGGT		CT ACC GAC AAA	
	A L W E	E Y		R R G	G	T D K	
(1)	GCT CTC TGG GAG C	CCTAT		ATCGGAGGGATA	GTCTTCT	CT ACC GAC AAA	
	A L W E P	Y		I G G I	V F S	T D K	
(1)	GCT CTC TGG GAG CTG	TCAT	ATGGC	ATAGGGGGATACGAG		CT ACC GAC AAA	
	A L W E L	S Y	G	I G G I R A		T D K	
(1)	GCT CTC TGG GAG CT		ATGGCAT	ATCGGAGGGATACGAG	AAC	CC GAC AAA	
	A L W E L		W H	I G G I R E	P	D K	

Itk^{-/-}-4Get thymocyte stage 2 (CD122⁺GFP⁺; n=33)

fetal	(9)	GCT CTC TGG GAG C	AT		ATCGGAGGGATACGAG		CT ACC GAC AAA
		A L W E H			I G G I R A		T D K
	(6)	GCT CTC TGG GAG CTG G			TCCGAGGGATACGAG		CT ACC GAC AAA
	A L W E L V			G G I R A		T D K	
adult	(1)	GCT CTC TGG GAG CT	T		ATCGGAGGGATACGAG		CT ACC GAC AAA
		A L W E L			I G G I R A		T D K
	(1)	GCT CTC TGG GAG CTG			ATCGGAGGGATA	TATG	CT ACC GAC AAA
		A L W E L			I G G I Y A		T D K
	(1)	GCT CTC TGG GAG CTG G	C		CGGAGGGATAC	CCG	CT ACC GAC AAA
		A L W E L A			G G I P	A	T D K
	(2)	GCT CTC TGG GAG CT	CGC		CGGAGGGATACG	GG	CT ACC GAC AAA
		A L W E L	A		G G I R	A	T D K
	(1)	GCT CTC TGG GAG C	CGGAT		ATCGGAGGGATACGAG		CT
		A L W E P D			I G G I R A		
	(2)	GCT CTC TGG GAG CTG G	AT		ATCGGAGGG	T	CT ACC GAC AAA
		A L W E L D			I G G	S	T D K
	(1)	GCT CTC TGG GAG CTG G			AAA GAGGGATACGAG		CT ACC GAC AAA
		A L W E L E			R G I R A		T D K
	(1)	GCT CTC TGG GAG CTG G		AGGGGG AT	ATCGGAGGGATACCTG		CT ACC GAC AAA
		A L W E L E		G D	I G G I P A		T D K
	(2)	GCT CTC TGG GAG C		TC CAT	ATCGGAGGGATACGAG		CT GCC GAC AAA
		A L W E L		H	I G G I R A		D K
(2)	GCT CTC TGG GAG C		TG TAT	ATCGGAGGGATACGAG		CT ACC GAC AAA	
	A L W E L		Y	I G G I R A		T D K	
(1)	GCT CTC TGG GA	CCAG	ATAT	GGCCCCACC		CT ACC GAC AAA	
	A L W D	Q	I W	P P P		T D K	
(2)	GCT CTC TGG GAG CTG G			TCCCAGCC GAG	CTCG	CC GAC AAA	
	A L W E L V			P A E	L A	D K	
(1)	GCT CTC TGG GAG CT			CGGAGGGATACCC	CT G	CT ACC GCC AGA	
	A L W E L			G G I P F	A	T A R	

Itk^{-/-}-4Get thymocyte stage 3 (CD122⁺GFP⁻; n=18)

fetal	(8)	GCT CTC TGG GAG C	AT		ATCGGAGGGATACGAG		CT ACC GAC AAA	
		A L W E H			I G G I R A		T D K	
	(3)	GCT CTC TGG GAG CTG G	C		CGGAGGGATACGAG		CT ACC GAC AAA	
	A L W E L A			G G I R A		T D K		
adult	(1)	GCT CTC TGG GAG	T		GGAGGGATACGAG	CC	CC GAC AAA	
		A L W E	W		R D T S	P	D K	
	(1)	GCT C C	AT		ATCGGAGGGATACG	GAA		
		A L	W		E L P Y	R		
	(2)	GCT CTC TGG GAG CT		CCCAT	ATCGGAGGGATACG	GAA		
		A L W E L		P Y	R R D T	E		
	(1)	GCT CTC TGG GAG CT	C	T ATAT	GGCCCT	ATCGGAGGGATACGAG		CT ACC GAC AAA
		A L W E L		Y M	A P	I G G I R A		T D K
	(1)	GCT CTC TGG GAG C	CAG	ATAT	C	GGG	G	CT ACC GAC AAA
		A L W E P	D	I		G	A	T D K
(1)	GCT CTC TGG GAG C			CTTCT	ATCGGAGGGATACGA	AT	C GAC AAA	
	A L W E P			S	I G G I R	I	D K	

Supplemental Figure 1: Sequence analysis of Vδ6-Jδ1 junctions in γδNKT cells from the liver and thymus of WT and *Itk*^{-/-} mice.

At the top, the germline sequences are shown. Below, the junctional regions of Vδ6 (variable region), Dδ1 and 2 (diversity region) and Jδ1 (joining region) rearrangements in liver and thymus subsets are shown. Only rearrangements resulting in in-frame junctions are included. The frequency (left) indicates the number of independent clones with each sequence. Junctions were characterized as fetal or adult based on the absence or presence of Dδ1 sequences and N-region nucleotides, respectively. In addition, sequences classified as fetal were confirmed based on comparison to those reported by Grigoriadou, et al (1) as being derived from isolated fetal V6 cells.

1. Grigoriadou, K., L. Boucontet, and P. Pereira. 2003. Most IL-4-producing gamma delta thymocytes of adult mice originate from fetal precursors. *J Immunol* 171: 2413–2420.