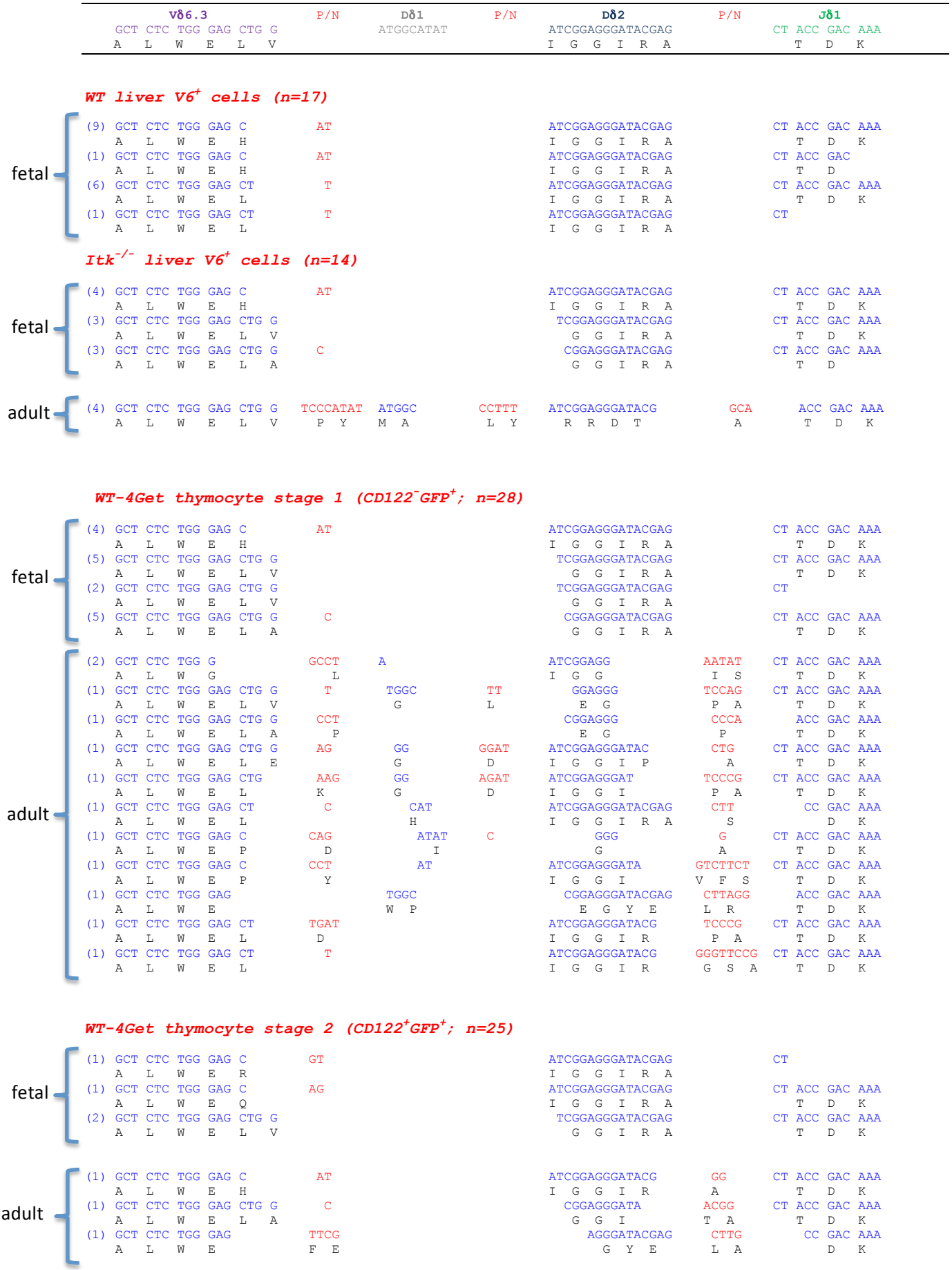
Vδ6.3 P/N Dδ1 P/N Dδ2 P/N Jδ1
GCT CTC TGG GAG CTG G ATGGCATAT ATCGGAGGGATACGAG CT ACC GAC AAA
A L W E L V I G G I R A T D K
WT liver V6+ cells (n=17)
fetal
(9) GCT CTC TGG GAG C AT ATCGGAGGGATACGAG CT ACC GAC AAA
A L W E H I G G I R A T D K
(1) GCT CTC TGG GAG C AT ATCGGAGGGATACGAG CT ACC GAC
A L W E 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 TCGGAGGGATACGAG 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 TCGGAGGGATACGAG 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 TCGGAGGGATACGAG 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 GGAGGG 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 CGGAGGG 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
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(1) GCT CTC TGG GAG C CCT AT ATCGGAGGGATA GTCTTCT CT ACC GAC AAA
A L W E P Y I 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 GGGTTCCG 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 TCGGAGGGATACGAG 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
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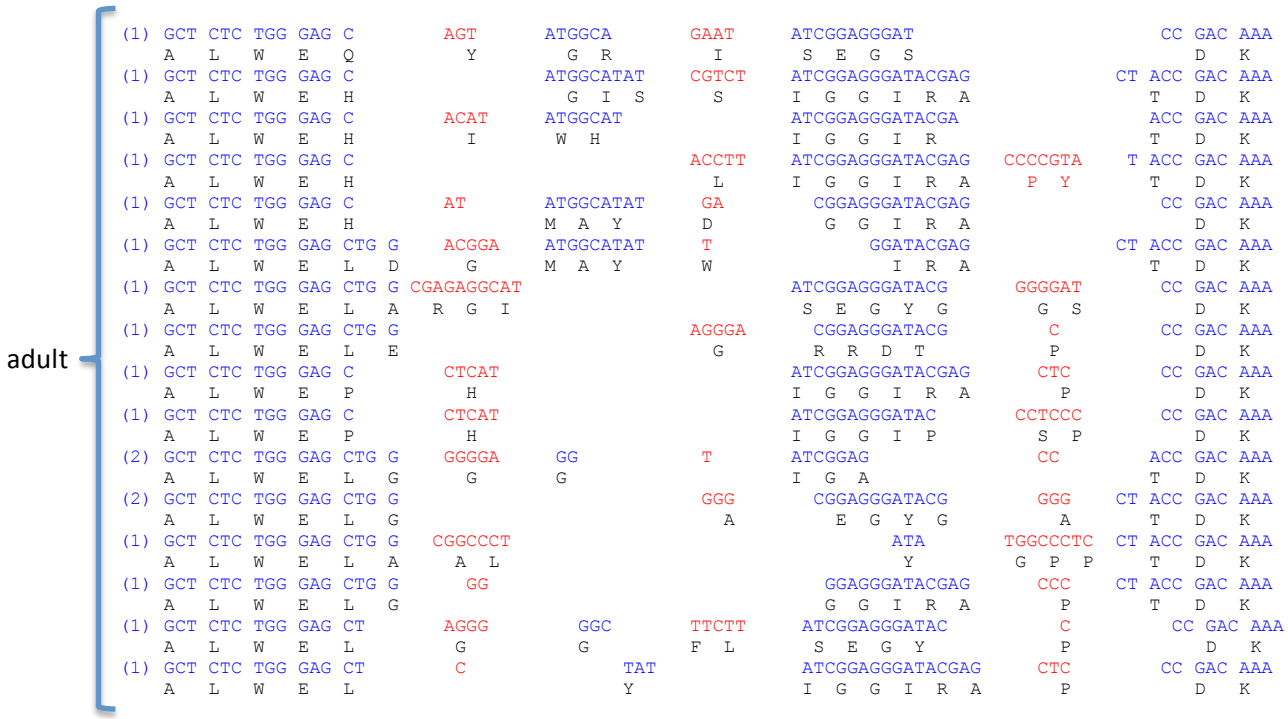
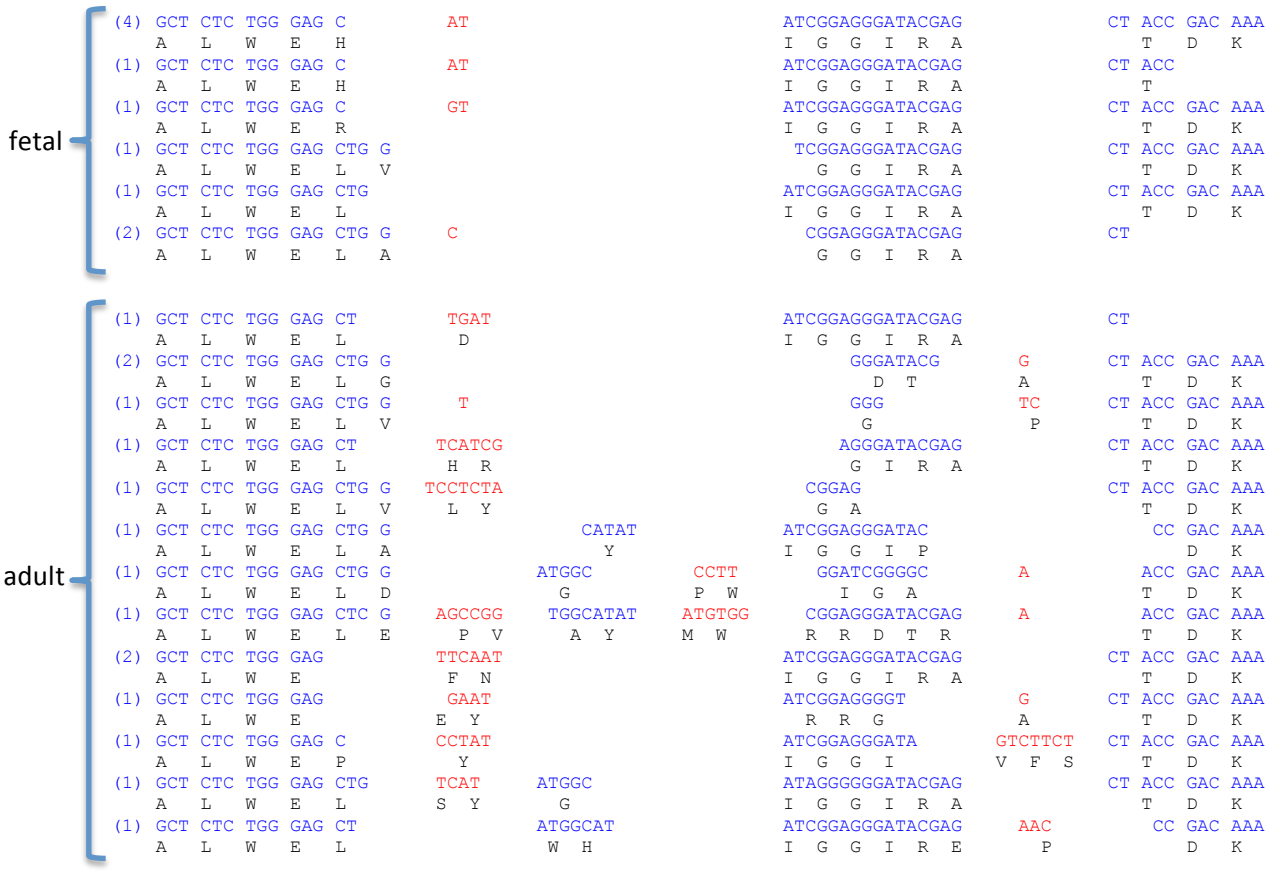
A L W E F E G Y E L A D K

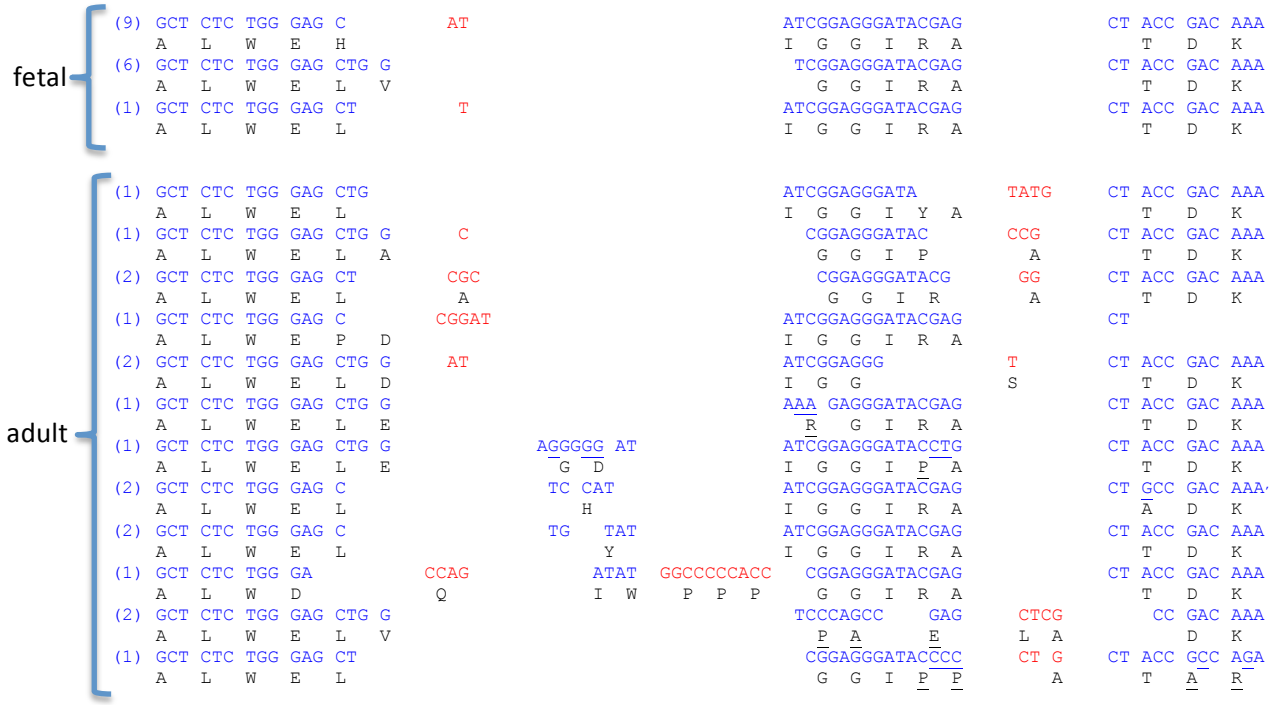


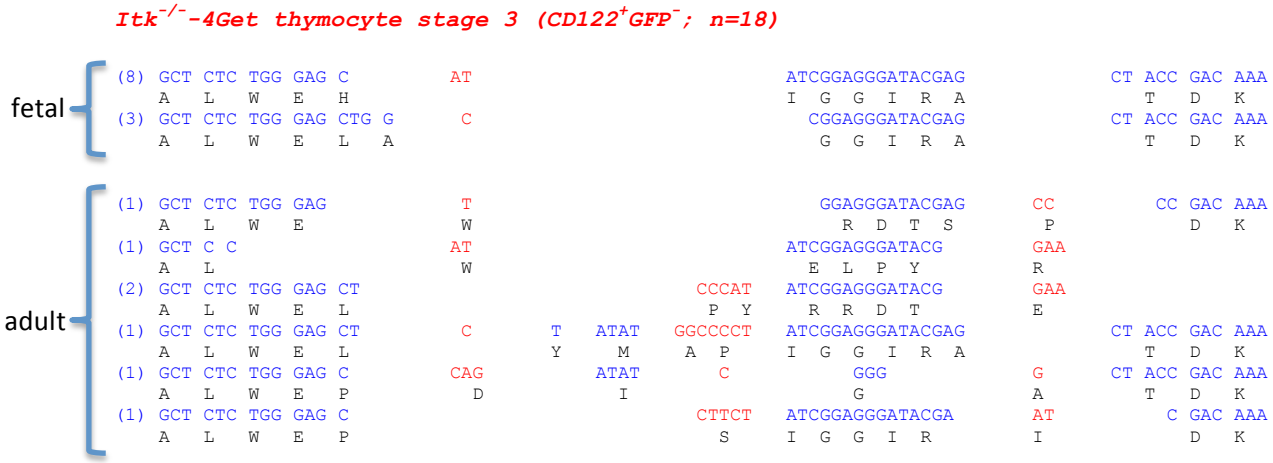

***WT-4Get thymocyte stage 3 (CD122$^{+}$GFP$^{-}$; n=22)***

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




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






**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.