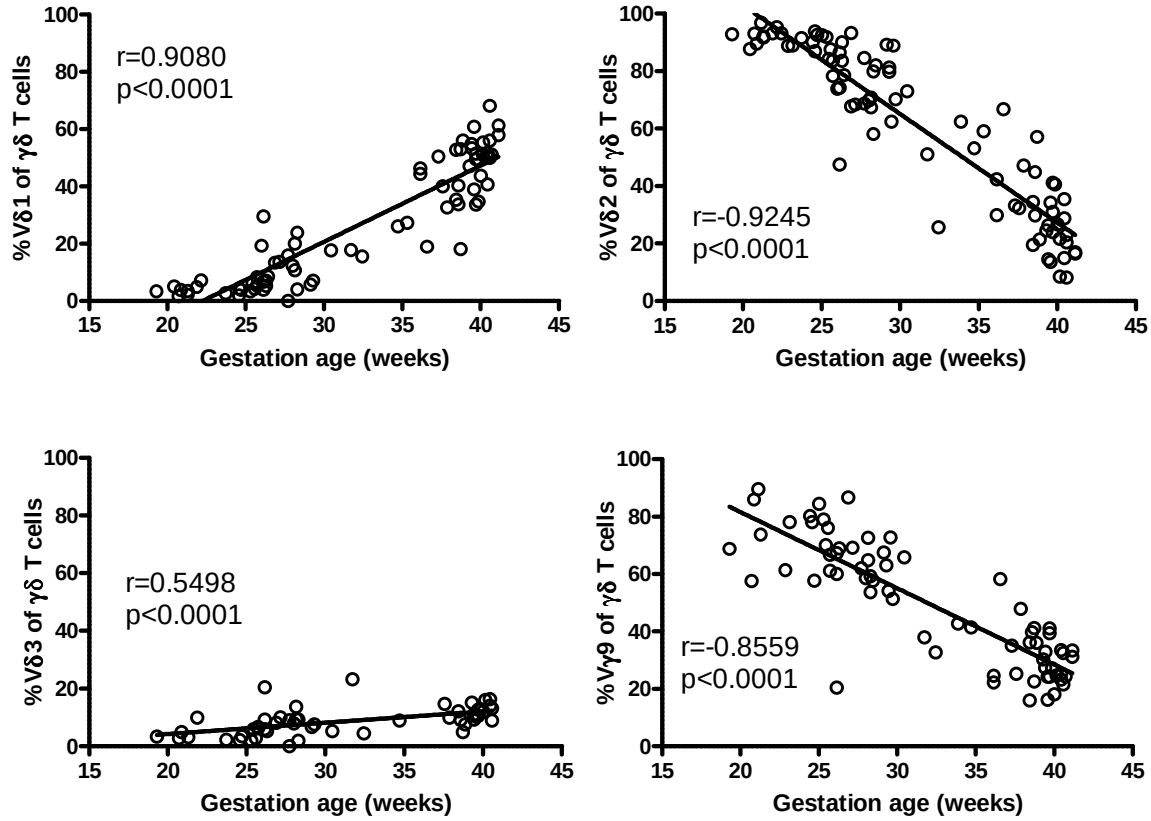

Figure S1. Percentages of γδ T cells positive for Vδ1, Vδ2, Vδ3 and Vγ9 according to gestation age.

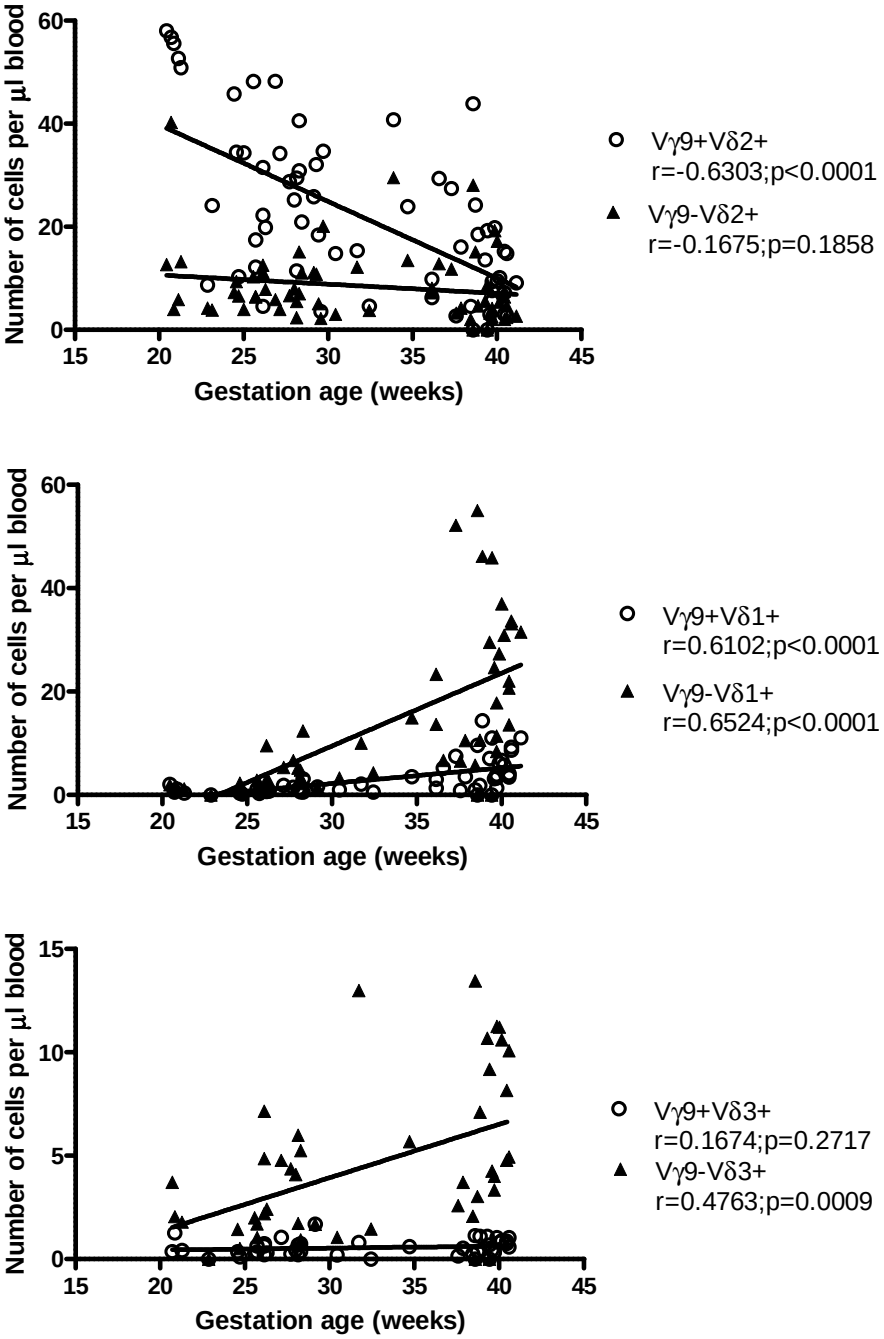

Figure S2. Absolute number counts of γδ T cell subsets according to gestation age.

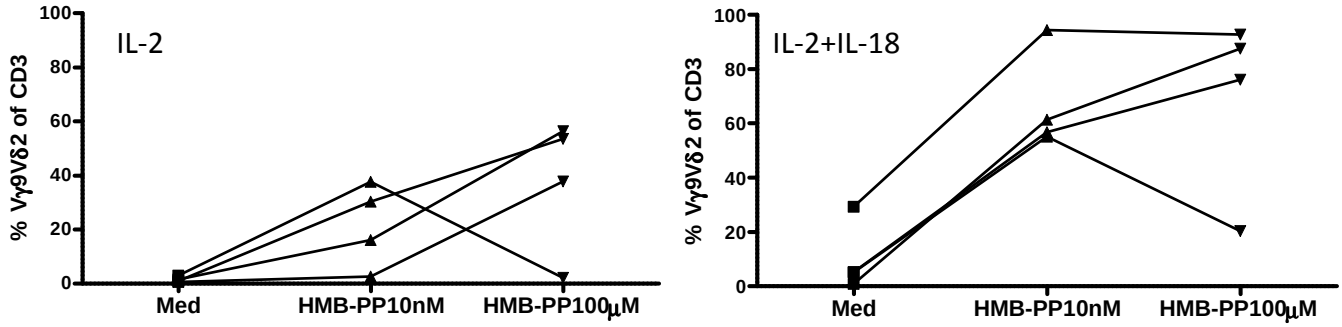

Figure S3. Incubation with 100 μM HMB-PP in the presence of IL-2+IL-18 results in the highest expansion of fetal Vγ9Vδ2 T cells. Fetal PBMC (<30 weeks of gestation) were cultured for 10 days with HMB-PP (10 nM or 100 μM) in the presence of IL-2 (left panel) or in the presence of IL-2+IL-18 (right panel). Each line represents data obtained from one fetal sample.

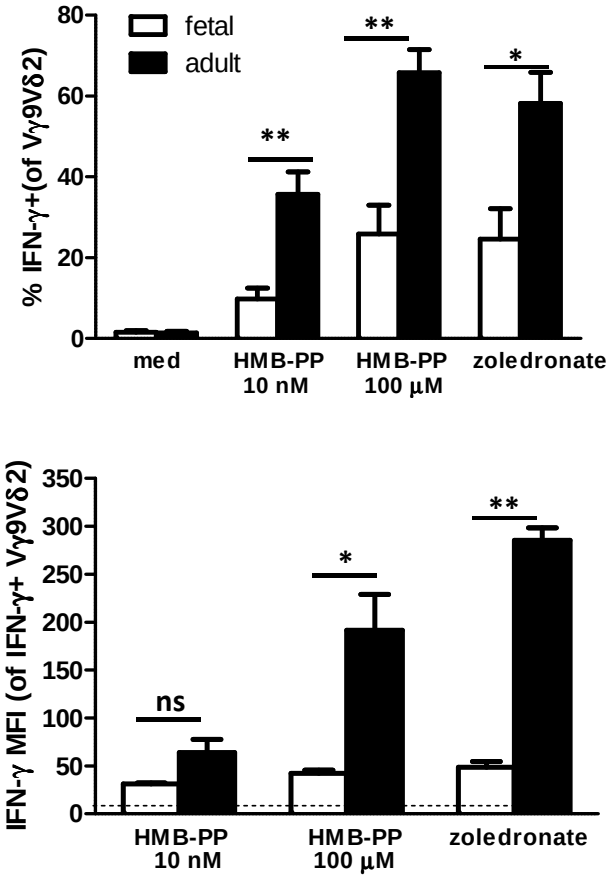

Figure S4. HMB-PP and zoledronate can induce IFN-γ production in fetal Vγ9Vδ2 T cells but significantly less than in adult Vγ9Vδ2 T cells. Fetal (<30 weeks of gestation) and adult PBMC were stimulated for 3 days with HMB-PP and zoledronate in the presence of IL-2. Top panel: percentage of Vγ9Vδ2 T cells positive for IFN-γ (fetal: n=5, adult: n=6); bottom panel: MFI of IFN-γ from the IFN-γ positive Vγ9Vδ2 T cells (fetal and adult: n=3). The total MFI for IFN-γ in the medium control was around 8, as indicated by the dashed line. Gate was put on CD3+γδ+Vγ9+Vδ2+ cells; IFN-γ induction was only observed in the Vγ9Vδ2 T cell subset. Mean values with SEM are shown.

Table S1A. Primer sequences used for spectratyping and sequencing

	'5-'3
Cδ	GTAGAATTCCTTCACCAG-ACAAG
Vδ1	CTGTCAACTTCAAGAAA-GCAGCGAAATC
Vδ2	ATACCGAGAAAAG GACATCTATG
Vδ3	GTACCGGATAAGGCCAGATTA
Cγ	CAAGAA GACAAAGGTATGTTCCAG
Vγ2	GCAAGCACAAGGAASAACTTGAG
Vγ3	GTACTATGACGTCTCCACCG
Vγ4	ATGACTCCTACACCTCCAGC
Vγ5/3	CCCAGGAGGTGGAGCTGGAT
Vγ9	ATCAACGCTGGCAGTCC
Run-off reaction (spectratyping):	
Cδ-FAM	ACGGATGGTTTGGTATGAG GCTGA
Cγ-FAM	AATAGTGGGCTTGGGGGAAAC

Table S1B. Primer sequences used to quantify gene expression within sorted fetal blood Vγ9Vδ2 and αβ T cells

	5'-3'
GZMA-F	ATT-CTT-GGG-GCT-CAC-TCA-ATA-AC
GZMA-R	GGG-TCA-TAG-CAT-GGA-TAG-GGA-AA
GZMB-F	CCC-TGG-GAA-AAC-ACT-CAC-ACA
GZMB-R	GCA-CAA-CTC-AAT-GGT-ACT-GTC-G
GZMH-F	CTG-GCT-GGG-GTT-ATG-TCT-CAA
GZMH-R	GGC-TAC-GTC-CTT-ACA-CAC-GAG
GZMK-F	GGG-GCT-TAT-ATG-ACT-CAT-GTG-TG
GZMK-R	GTG-GAT-CAA-TCA-GAA-CAC-CTC-C
GZMM-F	ACA-CCC-GCA-TGT-GTA-ACA-ACA
GZMM-R	GGA-GGC-TTG-AAG-ATG-TCA-GTG
IFNG-F	TCG-GTA-ACT-GAC-TTG-AAT-GTC-CA
IFNG-R	TCG-CTT-CCC-TGT-TTT-AGC-TGC
IL2-F	AAC-TCC-TGT-CTT-GCA-TTG-CAC
IL2-R	GCT-CCA-GTT-GTA-GCT-GTG-TTT
TNFa-F	CCT-CTC-TCT-AAT-CAG-CCC-TCT-G
TNFa-R	GAG-GAC-CTG-GGA-GTA-GAT-GAG
IL4-F	CCA-ACT-GCT-TCC-CCC-TCT-G
IL4-R	TCT-GTT-ACG-GTC-AAC-TCG-GTG
IL17A-F	AGA-TTA-CTA-CAA-CCG-ATC-CAC-CT
IL17A-R	GGG-GAC-AGA-GTT-CAT-GTG-GTA
Tbet-F	TTG-AGG-TGA-ACG-ACG-GAG-AG
Tbet-R	CCA-AGG-AAT-TGA-CAG-TTG-GGT
eomes-F	GTG-CCC-ACG-TCT-ACC-TGT-G
eomes-R	CCT-GCC-CTG-TTT-CGT-AAT-GAT
Runx3-F	AGC-ACC-ACA-AGC-CAC-TTC-AG
Runx3-R	GGG-AAG-GAG-CGG-TCA-AAC-TG
PLZF-F	GAA-GCG-GTT-CCT-GGA-TAG-TTT-G
PLZF-R	CAC-CGC-ACT-GAT-CAC-AGA-CAA
IL18RAP-F	CTG-GAT-TGA-AAT-AGT-GCT-GCT-GTA-C
IL18RAP-R	TGC-ATA-GGA-TAC-GAA-AGC-ATC-AAA
NKp30-F	TGG-ATT-CTA-TGC-TGT-CAG-CTT-TCT
NKp30-R	GGT-CAG-ACA-TTT-GCC-CTG-GTA
perforin-F	GAC-TGC-CTG-ACT-GTC-GAG-G
perforin-R	TCC-CGG-TAG-GTT-TGG-TGG-AA
CCR5-F	TTC-TGG-GCT-CCC-TAC-AAC-ATT
CCR5-R	TTG-GTC-CAA-CCT-GTT-AGA-GCT-A
CXCR3-F	CCA-CCT-AGC-TGT-AGC-AGA-CAC
CXCR3-R	AGG-GCT-CCT-GCG-TAG-AAG-TT
CCR7-F	TGA-GGT-CAC-GGA-CGA-TTA-CAT
CCR7-R	GTA-GGC-CCA-CGA-AAC-AAA-TGA-T
cyclophilin-F	TGC-TGG-ACC-CAA-CAC-AAA-TG
cyclophilin-R	TGC-CAT-CCA-ACC-ACT-CAG-TCT
actin-F	CCC-AGC-CAT-GTA-CGT-TGC-TA
actin-R	TCA-CCG-GAG-TCC-ATC-ACG-AT

Table S2
Sequencing data of the CDR3γ9 from PBMC derived from four different fetuses before 30 weeks of gestation

	CDR3 length (aa)	Freq.	3' Vγ9-region	P	N	P	5' Jγ-region
			tgt gcc ttg tgg gag gtg **C A L W E V**				**1** g aat tat tat aag aaa ctc ttt **N Y Y K K L F**
							2 g aat tat tat aag aaa ctc ttt **N Y Y K K L F**
							P t ggg caa gag ttg ggc aaa aaa atc aag gta ttt **G Q E L G K K I K V F**
							P1 at acc act ggt tgg ttc aag ata ttt **T T G W F K I F**
							P2 at agt agt gat tgg atc aag acg ttt **S S D W I K T F**
GD-002 25w5d	9	1/18	tgt gcc ttg tgg gag gtg **C A L W E V**		gga tg G W		g aaa ctc ttt **K L F**
	10	2/18	tgt gcc ttg **C A L**		cgt gt **R V**		c act ggt tgg ttc aag ata ttt **T G W F K I F**
	11	1/18	tgt gcc ttg tg **C A L**				a gag ttg ggc aaa aaa atc aag gta ttt **E L G K K I K V F**
	12	1/18	tgt gcc ttg tgg gag **C A L W E**		cct tca P S		aat tat tat aag aaa ctc ttt **N Y Y K K L F**
	12	1/18	tgt gcc ttg tgg **C A L W**		caa gag ttg ggc ag Q E L G R		a aaa atc aag gta ttt **K I K V F**
	13	1/18	tgt gcc ttg tgg gag g **C A L W E**				aa gag ttg ggc aaa aaa atc aag gta ttt **E E L G K K I K V F**
	13	1/18	tgt gcc ttg tgg gag **C A L W E**				caa gag ttg ggc aaa aaa atc aag gta ttt **Q E L G K K I K V F**
	13	1/18	tgt gcc ttg tgg gag gtg **C A L W E V**	c			ag ttg ggc aaa aaa atc aag gta ttt **Q L G K K I K V F**
	13	1/18	tgt gcc ttg tgg gag gtg **C A L W E V**				gag ttg ggc aaa aaa atc aag gta ttt **E L G K K I K V F**
	14	1/18	tgt gcc ttg tgg gag gtg **C A L W E V**	c	gg R		gag ttg ggc aaa aaa atc aag gta ttt **E L G K K I K V F**
	14	5/18	tgt gcc ttg tgg gag gtg **C A L W E V**				caa gag ttg ggc aaa aaa atc aag gta ttt **Q E L G K K I K V F**
	14	1/18	tgt gcc ttg tgg gag gtg **C A L W E V**	ca **Q**	ag		at acc act ggt tgg ttc aag ata ttt **D T T G W F K I F**
	15	1/18	tgt gcc ttg tgg gag gtg **C A L W E V**		ga E		g caa gag ttg ggc aaa aaa atc aag gta ttt **Q E L G K K I K V F**
GD-003 25w3d	11	1/18	tgt gcc ttg tgg gag gtg **C A L W E V**				aat tat tat aag aaa ctc ttt **N Y Y K K L F**
	11	1/18	tgt gcc ttg tgg gag gtg **C A L W E V**	ca **Q**	a gag ttg ggc **E L G**		aa aaa atc aag gta ttt # K I K V F
	13	3/18	tgt gcc ttg tgg gag **C A L W E**				caa gag ttg ggc aaa aaa atc aag gta ttt **Q E L G K K I K V F**
	14	5/18	tgt gcc ttg tgg gag gtg **C A L W E V**				caa gag ttg ggc aaa aaa atc aag gta ttt **Q E L G K K I K V F**
	14	1/18	tgt gcc ttg tgg gag gtg **C A L W E V**		gga **G**		gag ttg ggc aaa aaa atc aag gta ttt **E L G K K I K V F**
	14	1/18	tgt gcc ttg tgg gaa **C A L W** E		ata **I**		caa gag ttg ggc aaa aaa atc aag gta ttt **Q E L G K K I K V F**
	14	1/18	tgt gcc **C A**		tcg tgg gag tgt **S W E V**		caa gag ttg ggc aaa aaa atc aag gta ttt **Q E L G K K I K V F**
	14	1/18	tgt gcc ttg tgg gag gtg **C A L W E V**		aga gg **R G**		g ttg ggc aaa aaa atc aag gta ttt **L G K K I K V F**
	14	1/18	tgt gcc t **C A**		cg tgg gag **S W E**		gaa gag ttg ggc aaa aaa atc aag gta ttt **E E L G K K I K V F**
	15	1/18	tgt gcc **C A**		tcg tgg gag tgt cct **S W E V P**		caa gag ttg ggc aaa aaa atc aag gta ttt **Q E L G K K I K V F**
	15	1/18	tgt gcc ttg tgg gag gtg **C A L W E V**	c			gg caa gag ttg ggc aaa aaa atc aag gta ttt **R Q E L G K K I K V F**
	16	1/18	tgt gcc ttg tgg gag **C A L W E**		gcg cac ttc **A H F**		caa gag ttg ggc aaa aaa atc aag gta ttt **Q E L G K K I K V F**

(Table S2, continued)

GD-006	10	1/16	tgt	gcc	ttg	tgg	gag	gtg									tat	tat	aag	aaa	cta	ttt
29w0d			**C**	**A**	**L**	**W**	**E**	**V**									**Y**	**Y**	**K**	**K**	**L**	**F**
	11	1/16	tgt	gcc	ttg	tgg	ga			a gt					a	aat	tat	tat	aag	aaa	ctc	ttt
			C	**A**	**L**	**W**	**E**			**V**						**N**	**Y**	**Y**	**K**	**K**	**L**	**F**
	11	1/16	tgt	gcc	ttg	tgg	gag			tt					c	act	ggt	tgg	ttc	aag	ata	ttt
			C	**A**	**L**	**W**	**E**			**F**						**T**	**G**	**W**	**F**	**K**	**I**	**F**
	12	1/16	tgt	gcc	ttg	tgg	gag	gtg		cac c	c					t	tat	tat	aag	aaa	ctc	ttt
			C	**A**	**L**	**W**	**E**	**V**		**H**	**P**						**Y**	**Y**	**K**	**K**	**L**	**F**
	12	1/16	tgt	gcc	ttg	tgg	gag	gtg		gaa aat tat tat aaa										aaa	ctc	ttt
			C	**A**	**L**	**W**	**E**	**V**		**E N Y Y K**										**K**	**L**	**F**
	13	1/16	tgt	gcc	ttg	tgg	gag						gaa	gag	ttg	ggc	aaa	aaa	atc	aag	gta	ttt
			C	**A**	**L**	**W**	**E**						**E**	**E**	**L**	**G**	**K**	**K**	**I**	**K**	**V**	**F**
	13	1/16	tgt	gcc	ttg	tgg	gag						caa	gag	ttg	ggc	aaa	aaa	atc	aag	gta	ttt
			C	**A**	**L**	**W**	**E**						**Q**	**E**	**L**	**G**	**K**	**K**	**I**	**K**	**V**	**F**
	14	6/16	tgt	gcc	ttg	tgg	gag	gtg					caa	gag	ttg	ggc	aaa	aaa	atc	aag	gta	ttt
			C	**A**	**L**	**W**	**E**	**V**					**Q**	**E**	**L**	**G**	**K**	**K**	**I**	**K**	**V**	**F**
	14	1/16	tgt	gcc	ttg	tgg	gag	gtg		a			aa	gag	ttg	ggc	aaa	aaa	atc	aag	gta	ttt
			C	**A**	**L**	**W**	**E**	**V**					**K**	**E**	**L**	**G**	**K**	**K**	**I**	**K**	**V**	**F**
	14	1/16	tgt	gc						t tcg tgg gag gag cag				gag	ttg	ggc	aaa	aaa	atc	aag	gta	ttt
			C	**A**						**S W E E Q**				**E**	**L**	**G**	**K**	**K**	**I**	**K**	**V**	**F**
	15	1/16	tgt	gcc	ttg	tgg	gag			caa att			caa	gag	ttg	ggc	aaa	aaa	atc	aag	gta	ttt
			C	**A**	**L**	**W**	**E**			**Q I**			**Q**	**E**	**L**	**G**	**K**	**K**	**I**	**K**	**V**	**F**
GD-012	9	1/18	tgt	gcc	ttg	tgg				ttg tgc a								at	aag	aaa	ctc	ttt
30w3d			**C**	**A**	**L**	**W**				**L C**								**N**	**K**	**K**	**L**	**F**
	10	1/18	tgt	gcc	ttg	tgg	gag	gtg		g							at	tat	aag	aaa	ctc	ttt
			C	**A**	**L**	**W**	**E**	**V**		**D**								**Y**	**K**	**K**	**L**	**F**
	10	1/18	tgt	gcc	ttg	tgg	g			g					c	act	ggt	tgg	ttc	aag	ata	ttt
			C	**A**	**L**	**W**				**V**						**T**	**G**	**W**	**F**	**K**	**I**	**F**
	10	1/18	tgt	gcc	ttg	tgg				gtc aat							ggt	tgg	ttc	aag	ata	ttt
			C	**A**	**L**	**W**				**V N**							**G**	**W**	**F**	**K**	**I**	**F**
	11	1/18	tgt	gcc	ttg	tgg	gag	gtg	c	gg							gat	tgg	atc	aag	acg	ttt
			C	**A**	**L**	**W**	**E**	**V**		**R**							**D**	**W**	**I**	**K**	**T**	**F**
	13	3/18	tgt	gcc	ttg	tgg	gag						caa	gag	ttg	ggc	aaa	aaa	atc	aag	gta	ttt
			C	**A**	**L**	**W**	**E**						**Q**	**E**	**L**	**G**	**K**	**K**	**I**	**K**	**V**	**F**
	14	5/18	tgt	gcc	ttg	tgg	gag	gtg					caa	gag	ttg	ggc	aaa	aaa	atc	aag	gta	ttt
			C	**A**	**L**	**W**	**E**	**V**					**Q**	**E**	**L**	**G**	**K**	**K**	**I**	**K**	**V**	**F**
	14	1/18	tgt	gcc	ttg	tgg	gag	gtg	cac					gag	ttg	ggc	aaa	aaa	atc	aag	gta	ttt
			C	**A**	**L**	**W**	**E**	**V**	**H**					**E**	**L**	**G**	**K**	**K**	**I**	**K**	**V**	**F**
	14	1/18	tgt	gcc	ttg	tgg	ga			t at		g	caa	gag	ttg	ggc	aaa	aaa	atc	aag	gta	ttt
			C	**A**	**L**	**W**	**D**			**M**			**Q**	**E**	**L**	**G**	**K**	**K**	**I**	**K**	**V**	**F**
	14	1/18	tgt	gcc	ttg	tgg	gag	gtg	c	cc				gag	ttg	ggc	aaa	aaa	atc	aag	gta	ttt
			C	**A**	**L**	**W**	**E**	**V**	P					**E**	**L**	**G**	**K**	**K**	**I**	**K**	**V**	**F**
	14	1/18	tgt	gcc	ttg	tgg				ga		g	caa	gag	ttg	ggc	aaa	aaa	atc	aag	gta	ttt
			C	**A**	**L**	**W**				**E**			**Q**	**E**	**L**	**G**	**K**	**K**	**I**	**K**	**V**	**F**
	16	1/18	tgt	gcc	ttg	tgg	gag	g		gt cga tat tcc tat acc acg							ggt	tgg	ttc	aag	ata	ttt
			C	**A**	**L**	**W**	**E**			**G R Y S Y T T**							G	W	F	K	I	F

Grey shading indicates the highly enriched public/invariant CDR3γ9 CALWEVQELGKKIKVF sequence.

Table S3

Sequencing data of the CDR3γ9 of sorted Vγ9Vδ2 γδ T cells and nonVγ9Vδ2 γδ T cells from peripheral blood of three different fetuses before 30 weeks of gestation

	CDR3g9 length (aa)	Freq.	3'V-REGION	P3'V	N-REGION	P5'J	5'J-REGION	JUNCTION (aa)	J-GENE
GD-018 Vγ9Vδ2	9	1/10	tgtgccttgtgggaggt		cc		ggttcaagatattt	CALWEVRFKIF	TRGJP1
	11	1/10	tgtgccttgtgggag				ttgggcaaaaaaatcaaggtattt	CALWELGKKIKVF	TRGJP
	12	1/10	tgtgccttgtgggaggtg		aa		gaattattataagaaactcttt	CALWEVKNYYKKLF	TRGJ1
	13	1/10	tgtgccttgtgggag		aag		gagttgggcaaaaaaatcaaggtattt	CALWEKELGKKIKVF	TRGJP
	13	1/10	tgtgccttgtgggagg				aagagttgggcaaaaaaatcaaggtattt	CALWEEELGKKIKVF	TRGJP
	14	5/10	tgtgccttgtgggaggtg				caagagttgggcaaaaaaatcaaggtattt	CALWEVQELGKKIKVF	TRGJP
	out of frame		tgtgccttgtgggagg		gggggaagttgggc		aaaaaatcaaggtattt	CALWEGGKLGKKSRY	TRGJP
GD-018 nonVγ9Vδ2	9	1/13	tgtgccttgtgggagg		ag		tataagaaactcttt	CALWEEYKKLF	TRGJ1
	10	1/13	tgtgccttgtgg				aattattataagaaactcttt	CALWNYYKKLF	TRGJ1
	10	1/13	tgtgccttgtgggaggtg				tattataagaaactcttt	CALWEVYYKKLF	TRGJ1 or TRGJ2
	14	1/13	tgtgccttgtgggaggtg		agg		gagttgggcaaaaaaatcaaggtattt	CALWEVRELGKKIKVF	TRGJP
	14	1/13	tgtgccttgtgggaggtg		agg		gagttgggcaaaaaaatcaaggtattt	CALWEVRELGKKIKVF	TRGJP
	14	1/13	tgtgccttgtgggaggtg		agggagttgggcaag		aaaatcaaggtattt	CALWEVRELGKKIKVF	TRGJP
	14	1/13	tgtgccttgtggg		cga		ggcaagagttgggcaaaaaaatcaaggtattt	CALWARQELGKKIKVF	TRGJP
	14	1/13	tgtgccttgtggg		cga		ggcaagagttgggcaaaaaaatcaaggtattt	CALWARQELGKKIKVF	TRGJP
	14	1/13	tgtgccttgtggg		cga		ggcaagagttgggcaaaaaaatcaaggtattt	CALWARQELGKKIKVF	TRGJP
	15	2/13	tgtgccttgtgggag		aatcaagt		agagttgggcaaaaaaatcaaggtattt	CALWENQVELGKKIKVF	TRGJP
	out of frame		tgtgccttg		caagagttgggca		aaaaaaatcaaggtattt	CALQELGKKNQGI	TRGJP
	out of frame		tgtgccttgtgggag				taccactggttggttcaagatattt	CALWEYHWLVQDI	TRGJP1
GD-019 Vγ9Vδ2	11	2/8	tgtgccttgtgggaggt		ctc		ttattataagaaactcttt	CALWEVSYYKKLF	TRGJ1
	12	1/8	tgtgccttgtgggag			t	ataccactggttggttcaagatattt	CALWEYTTGWFKIF	TRGJP1
	13	1/8	tgtgccttgtgggag				caagagttgggcaaaaaaatcaaggtattt	CALWEQELGKKIKVF	TRGJP
	14	2/8	tgtgccttgtgggaggtg				caagagttgggcaaaaaaatcaaggtattt	CALWEVQELGKKIKVF	TRGJP
	14	1/8	tgtgccttgtgggaggtg	c	g		agagttgggcaaaaaaatcaaggtattt	CALWEVRELGKKIKVF	TRGJP
	15	1/8	tgtgccttgtgggag		ccagga		caagagttgggcaaaaaaatcaaggtattt	CALWEPGQELGKKIKVF	TRGJP
GD-019 nonVγ9Vδ2	9	1/8	tgtgccttgtggga		cctgg		ataagaaactcttt	CALWDLDKKLF	TRGJ1 or TRGJ2
	10	1/8	tgtgccttgtgggaggt		aa		attataagaaactcttt	CALWEVNYKKLF	TRGJ1 or TRGJ2
	16	1/8	tgtgccttgtgggaggt		tgggccc		caagagttgggcaaaaaaatcaaggtattt	CALWEVGPQELGKKIKVF	TRGJP
	out of frame		tgtgccttgtgggagg		g		agtagtgattggatcaagacgttt	CALWEGVVIGSRR	TRGJP2
	out of frame		tgtgccttgtgggaggtg	c	gatgcaa		tattataagaaactcttt	CALWEVRCNIIRNS	TRGJ1
	out of frame		tgtgccttgtgggagg		cgcaacctc	at	ataccactggttggttcaagatattt	CALWEAQPHIPLVGSRY	TRGJP1
	out of frame		tgtgccttgtgggag		acgcccaaactttc		aattattataagaaactcttt	CALWETPKLSIIIRNS	TRGJ1
	out of frame		tgtgccttgtgggagg		g		agtagtgattggatcaagacgttt	CALWEGVVIGSRR	TRGJP2
GD-023 Vγ9Vδ2	11	1/7	tgtgccttgtgggag				ttgggcaaaaaaatcaaggtattt	CALWELGKKIKVF	TRGJP
	13	1/7	tgtgccttgtgggaggtg				gagttgggcaaaaaaatcaaggtattt	CALWEVELGKKIKVF	TRGJP
	13	1/7	tgtgccttgtgggaggt				agagttgggcaaaaaaatcaaggtattt	CALWEVELGKKIKVF	TRGJP
	13	1/7	tgtgccttgtgggag				caagagttgggcaaaaaaatcaaggtattt	CALWEQELGKKIKVF	TRGJP
	14	1/7	tgtgccttgtgggaggtg		atcgg		gttgggcaaaaaaatcaaggtattt	CALWEVIGLGKKIKVF	TRGJP
	14	2/7	tgtgccttgtgggaggtg				caagagttgggcaaaaaaatcaaggtattt	CALWEVQELGKKIKVF	TRGJP
	out of frame		tgtgccttgtgggag		caagagttgggca		aaaaaaatcaaggtattt	CALWEQELGKKNQGI	TRGJP
GD-023 nonVγ9Vδ2	11	1/10	tgtgccttgtgggaggtg		agg		tattataagaaactcttt	CALWEVRYYKKLF	TRGJ2
	11	1/10	tgtgcct				ggcaagagttgggcaaaaaaatcaaggtattt	CAWQELGKKIKVF	TRGJP
	12	2/10	tgtgccttgtgggaggtg		agaggc		tattataagaaactcttt	CALWEVRGYYKKLF	TRGJ1
	12	2/10	tgtgccttgtgggaggtg	ca			tagtgattggatcaagacgttt	CALWEVHSDWIKTF	TRGJP2
	15	2/10	tgtgccttgtgggag		ccatt		gcaagagttgggcaaaaaaatcaaggtattt	CALWEPLQELGKKIKVF	TRGJP
	16	2/10	tgtgccttgtgggaggtg	ca	gacggaga		agttgggcaaaaaaatcaaggtattt	CALWEVQTEKLGKKIKVF	TRGJP
	out of frame		tgtgccttgtgggag		tg		aagagttgggcaaaaaaatcaaggtattt	CALWE*RVGQKNQGI	TRGJP
	out of frame		tgtgccttgtgggaggtg		acca		aagaaactcttt	CALWEVTKETL	TRGJ1 or TRGJ2
	out of frame		tgtgccttgtgggaggtg		acca		aagaaactcttt	CALWEVTKETL	TRGJ1 or TRGJ2
	out of frame		tgtgccttgtgggaggtg		acca		aagaaactcttt	CALWEVTKETL	TRGJ1 or TRGJ2
	out of frame		tgtgccttgtgggaggtg		acca		aagaaactcttt	CALWEVTKETL	TRGJ1 or TRGJ2

Grey shading: public/invariant CDR3γ9 sequence CALWEVQELGKKIKVF

Table S4
Sequencing data of the CDR3γ9 of three different fetuses (< 30 weeks gestation) before (ex-vivo) and after HMB-PP-induced proliferation

	CDR3 length (aa)	Freq.	3'V-REGION	P3'V	N-REGION	P5'J	5'J-REGION	JUNCTION (aa)	J-GENE
GD-002	9	1/18	tgtgccttgtgggaggtg		ggatg		gaaactcttt	CALWEVGWKLF	TRGJ1
Ex-vivo	10	2/18	tgtgccttg		cgtgt		cactggttggttcaagatattt	CALRVTGWFKIF	TRGJP1
	11	1/18	tgtgccttgtg				agagttgggcaaaaaaatcaaggtattt	CAL*ELGKKIKVF	TRGJP
	12	1/18	tgtgccttgtgggag		ccttca		aattattataagaaactcttt	CALWEPSNYYKKLF	TRGJ2
	12	1/18	tgtgccttgtgg		caagagttgggcag		aaaaatcaaggtattt	CALWQELGRKIKVF	TRGJP
	13	1/18	tgtgccttgtgggagg				aagagttgggcaaaaaaatcaaggtattt	CALWEEELGKKIKVF	TRGJP
	13	1/18	tgtgccttgtgggag				caagagttgggcaaaaaaatcaaggtattt	CALWEQELGKKIKVF	TRGJP
	13	1/18	tgtgccttgtgggaggtg	c			agttgggcaaaaaaatcaaggtattt	CALWEVQLGKKIKVF	TRGJP
	13	1/18	tgtgccttgtgggaggtg				gagttgggcaaaaaaatcaaggtattt	CALWEVELGKKIKVF	TRGJP
	14	1/18	tgtgccttgtgggaggtg	c	gg		gagttgggcaaaaaaatcaaggtattt	CALWEVRELGKKIKVF	TRGJP
	14	5/18	tgtgccttgtgggaggtg				caagagttgggcaaaaaaatcaaggtattt	CALWEVQELGKKIKVF	TRGJP
	14	1/18	tgtgccttgtgggaggtg	ca	ag		ataccactggttggttcaagatattt	CALWEVQDTTGWFKIF	TRGJP1
	15	1/18	tgtgccttgtgggaggtg		ga		gcaagagttgggcaaaaaaatcaaggtattt	CALWEVEQELGKKIKVF	TRGJP
GD-002	10	1/11	tgtgccttgtgggag		cgcg		attataagaaactcttt	CALWERDYKKLF	TRGJ1
After HMB-PP-	11	1/11	tgtgccttgtgggaggtg				aattattataagaaactcttt	CALWEVNYYKKLF	TRGJ2
induced proliferation	13	1/11	tgtgccttgtgggaggt		t		gagttgggcaaaaaaatcaaggtattt	CALWEVELGKKIKVF	TRGJP
	14	4/11	tgtgccttgtgggaggtg				caagagttgggcaaaaaaatcaaggtattt	CALWEVQELGKKIKVF	TRGJP
	14	1/11	tgtgccttgtgggaggt		t		caagagttgggcaaaaaaatcaaggtattt	CALWEVQELGKKIKVF	TRGJP
	14	1/11	tgtgccttgtgggaggtg	ca	ggg		gttgggcaaaaaaatcaaggtattt	CALWEVQGLGKKIKVF	TRGJP
	15	1/11	tgtgccttgtgggaggtg	ca	tac		agagttgggcaaaaaaatcaaggtattt	CALWEVHTELGKKIKVF	TRGJP
	15	1/11	tgtgccttgtgggaggtg	c			ggcaagagttgggcaaaaaaatcaaggtattt	CALWEVRQELGKKIKVF	TRGJP
	15	1/11	tgtgccttgtgggag		cccccc		caagagttgggcaaaaaaatcaaggtattt	CALWEPPQELGKKIKVF	TRGJP
GD-011	9	1/9	tgtgccttg		ag		gaattattataagaaactcttt	CALRNYYKKLF	TRGJ2
Ex-vivo	10	1/9	tgtgccttgtgggag		cctg		attataagaaactcttt	CALWEPDYKKLF	TRGJ1
	11	1/9	tgtgccttg		g		aagagttgggcaaaaaaatcaaggtattt	CALEELGKKIKVF	TRGJP
	13	1/9	tgtgccttgtgggaggt				agagttgggcaaaaaaatcaaggtattt	CALWEVELGKKIKVF	TRGJP
	13	1/9	tgtgccttgtggg		ca		caagagttgggcaaaaaaatcaaggtattt	CALWAQELGKKIKVF	TRGJP
	14	3/9	tgtgccttgtgggaggtg				caagagttgggcaaaaaaatcaaggtattt	CALWEVQELGKKIKVF	TRGJP
	15	1/9	tgtgccttgtgggaggtg	ca	ggg		agagttgggcaaaaaaatcaaggtattt	CALWEVQGELGKKIKVF	TRGJP
	out of frame		tgtgccttgtggga		agggtc		aagaaactcttt	CALWEGSRNS	TRGJ1 or TRGJ2
	out of frame		tgtgccttgtggga		tc		aagaaactcttt	CALWDQETL	TRGJ1 or TRGJ2
	out of frame		tgtgccttgtgggaggtg	ca	agag		actcttt	CALWEVQETL	TRGJ1 or TRGJ2
GD-011	12	1/11	tgtgccttgtggg		gt		gagttgggcaaaaaaatcaaggtattt	CALWGELGKKIKVF	TRGJP
After HMB-PP-	12	1/11	tgtgccttgtgg		a		aagagttgggcaaaaaaatcaaggtattt	CALWKELGKKIKVF	TRGJP
induced proliferation	12	1/11	tgtgccttgtgggagg				agttgggcaaaaaaatcaaggtattt	CALWEELGKKIKVF	TRGJP
	13	1/11	tgtgccttgtgggagg				aagagttgggcaaaaaaatcaaggtattt	CALWEEELGKKIKVF	TRGJP
	14	4/11	tgtgccttgtgggaggtg				caagagttgggcaaaaaaatcaaggtattt	CALWEVQELGKKIKVF	TRGJP
	14	1/11	tgtgccttgtgggaggtg	ca	g		gagttgggcaaaaaaatcaaggtattt	CALWEVQELGKKIKVF	TRGJP
	14	1/11	tgtgccttgtgggagg		at		caagagttgggcaaaaaaatcaaggtattt	CALWEDQELGKKIKVF	TRGJP
	14	1/11	tgtgccttgtgggaggtg		a		aagagttgggcaaaaaaatcaaggtattt	CALWEVKELGKKIKVF	TRGJP
GD-013	11	1/12	tgtgccttgtggga				taccactggttggttcaagatattt	CALWDTTGWFKIF	TRGJP1
Ex-vivo	11	1/12	tgtgccttgtgggaggtg	ca	ggga		tataagaaactcttt	CALWEVQGYKKLF	TRGJ1
	11	1/12	tgtgccttgtgggaggtg	c			attattataagaaactcttt	CALWEVHYYKKLF	TRGJ1
	12	1/12	tgtgccttgtgg				caagagttgggcaaaaaaatcaaggtattt	CALWQELGKKIKVF	TRGJP
	13	1/12	tgtgccttgtgggaggtg				gagttgggcaaaaaaatcaaggtattt	CALWEVELGKKIKVF	TRGJP
	14	6/12	tgtgccttgtgggaggtg				caagagttgggcaaaaaaatcaaggtattt	CALWEVQELGKKIKVF	TRGJP
	14	1/12	tgtgccttgtgggaggtg	c	cc		gagttgggcaaaaaaatcaaggtattt	CALWEVPELGKKIKVF	TRGJP
	out of frame	1/12	tgtgccttgtg		agttgggcaaaaaag		tcaaggtattt	CAL*VGQKSQGI	TRGJP
GD-013	13	1/11	tgtgccttgtgggaggtg	c			agttgggcaaaaaaatcaaggtattt	CALWEVQLGKKIKVF	TRGJP
After HMB-PP-	13	1/11	tgtgccttgtgggaggtg				gagttgggcaaaaaaatcaaggtattt	CALWEVELGKKIKVF	TRGJP
induced proliferation	14	6/11	tgtgccttgtgggaggtg				caagagttgggcaaaaaaatcaaggtattt	CALWEVQELGKKIKVF	TRGJP
	14	2/11	tgtgccttgtgggaggtg	c	g		agagttgggcaaaaaaatcaaggtattt	CALWEVRELGKKIKVF	TRGJP
	14	1/11	tgtgccttgtgggaggtg	c	gg		gagttgggcaaaaaaatcaaggtattt	CALWEVRELGKKIKVF	TRGJP

Grey shading: public/invariant CDR3γ9 sequence CALWEVQELGKKIKVF

Table S5. Percentages of activation (HLA-DR) and differentiation (CD27, CD28, CD45RO) markers, NKRs (CD94, NKG2A, NKG2C, CD158, NKG2D, CD161 and KLRG1), granule-associated molecules (perforin, granzyme A, granzyme B, granzyme K and granulysin), chemokine receptors (CCR5, CCR6, CCR7, CCR9 and CX3CR1), transcription factors (T-bet and eomes) and cytokines (IFN-γ, IL-2, TNF-α after 4h stimulation with PMA/ionomycin) on fetal blood γδ and αβ T cells at mid-gestation.

Marker	γδ T cells - mean±SD (min-max)	αβ T cells - mean±SD (min-max)	p value (γδ vs αβ), n
HLA-DR	1.28±0.82 (0.47-3.00)	0.45±0.26 (0.18-1.04)	p=0.031, n=9
CD27-CD28-	0.58±0.47 (0.00-1.22)	0.80±0.90 (0.04-2.05)	p>0.05, n=5
CD45RO	32.13±12.37 (18.04-55.60)	9.04±4.96 (5.00-19.68)	p<0.001, n=11
Ki-67	15.02±4.02 (8.40-22.03)	11.83±4.96 (4.70-21.00)	p=0.042, n=12
CD94	9.22±5.39 (3.07-19.11)	0.29±0.17 (0.10-0.56)	p<0.01, n=7
NKG2A	18.03± 17.70(5.00-63.45)	3.44±5.86 (0.21-18.20)	p<0.01, n=10
NKG2C	2.23±2.19 (0.14-7.64)	0.24±0.10 (0.12-0.44)	p =0.016, n=10
CD158a	6.14±5.39 (1.55-16.70)	0.64±0.60 (0.09-1.85)	p<0.01, n=11
CD158b	6.24±5.93 (1.28-17.40)	0.90±0.68 (0.19-2.24)	p=0.013, n=11
NKG2D	50.29±19.80 (15-83.32)	25.08±14.18 (3.00-46.15)	p<0.001, n=10
CD161	67.24±9.55 (57.14-84.05)	8.48±3.16 (3.01-12.26)	p<0.001, n=6
KLRG1	49.77±15.41 (25.53-67.34)	27.85±14.29 (4.31-47.23)	p<0.001, n=8
perforin	1.64±0.86 (0.40-2.54)	0.70±0.77 (0.15-2.34)	p=0.029, n=7
Granzyme A	47.75±21.35 (11.73-94.15)	2.61±3.67 (0.44-14.25)	p<0.001, n=19
Granzyme B	1.54±0.90 (0.60-3.49)	1.50±2.01 (0.18-6.70)	p>0.05, n=11
Granzyme K	45.35±10.50 (29.97-61.61)	1.71±1.62 (0.39-4.03)	p<0.001, n=6
Granulysin	1.17±0.76 (0.10-2.56)	1.07±1.12 (0.13-4.00)	p>0.05, n=11
CCR5	19.62±8.15 (10.48-28.44)	1.06±0.89 (0.38-2.52)	p<0.01, n=5
CCR6	12.40±5.03 (6.44-18.51)	2.87±0.59 (2.26-3.63)	p=0.023, n=4
CCR7	66.65±4.13 (60.79-70.47)	94.39±2.48 (91.04-96.32)	p<0.01, n=4
CCR9	4.36±1.70 (2.25-6.40)	0.90±0.62 (0.34-1.77)	P<0.01,n=4
CX3CR1	4.45±2.46 (1.62-6.08)	0.78±0.45 (0.35-1.25)	P> 0.05, n=3
T-bet	42.44±10.72 (23.00-61.27)	3.73±4.65 (0.41-12.00)	p<0.001, n=12
Eomes	45.35±3.23 (41.87-51.86)	6.97±5.04 (0.48-17.20)	p<0.001, n=12
IFN-γ	26.45±5.44 (17.54-32.42)	1.58±2.01 (0.34-7.11)	p<0.001, n=10
IL-2	7.39±1.52 (5.19-9.50)	32.66±7.27 (20.61-40.35)	p<0.001, n=7
TNF-α	5.66±4.12 (0.10-11.83)	2.85±1.65 (1.18-5.78)	p=0.051, n=6
IL-4	Not detected	Not detected	
IL-17	Not detected	Not detected	

Table S6. Gene expression analysis (microarray) of sorted fetal blood Vγ9Vδ2 T cells and αβ T cells (<30 weeks of gestation).

Symbol	Illumina ID	M	p	
Chemokines, cytokines and receptors				
IL18RAP	ILMN_1721762	**2.47**	0.0000001	
CCL5 (RANTES)	ILMN_1773352	**1.84**	0.0000001	
CXCR4	ILMN_1801584	**1.22**	0.0000439	
CXCR3	ILMN_1797975	**0.86**	0.0000299	
CD84 (SLAMF5)	ILMN_1698367	**0.69**	0.0005255	
CCR4	ILMN_2086143	**0.62**	0.0003042	
TGFBR3	ILMN_1784287	**0.81**	0.0000155	
EBI2	ILMN_1798706	**0.71**	0.0005299	
IFNG	ILMN_2207291	**0.42**	0.0007387	
NK: receptors, signaling and granule-associated molecules				
GMP-17 (NKG7)	ILMN_1682993	**2.74**	0.0000005	
GZMK	ILMN_1710734	**2.32**	0.0000001	
S1PR5	ILMN_2073184	**2.00**	0.0000237	
KLRB1, CD161	ILMN_2079655	**1.79**	<0.0000001	
PRF1	ILMN_1740633	**1.51**	0.0009307	
PIK3AP1	ILMN_1652787	**1.40**	0.0000061	
CST7 (cystatin F)	ILMN_1679826	**1.36**	0.0000823	
STX11	ILMN_1720771	**1.25**	0.0000149	
ELF4 (MEF)	ILMN_1652082	**1.22**	0.0000025	
PILRB	ILMN_1768754	**1.15**	0.0003007	
PLCG2	ILMN_1815719	**1.12**	0.0000023	
NCR3 (NKp30)	ILMN_2044471	**1.00**	0.0000441	
HCST (DAP10)	ILMN_1699931	**0.94**	0.0000913	
PIK3R1 (p85; GRB1)	ILMN_2398235	**0.94**	0.0003928	
PIK3CG	ILMN_1770433	**0.86**	0.0006724	
TYROBP (DAP12)	ILMN_1778977	**0.84**	0.0008443	
FCRL3	ILMN_1797428	**0.84**	0.0000429	
GZMA	ILMN_1779324	**0.79**	0.0006917	
FNBP1	ILMN_1797342	**0.76**	0.0001454	
TXNIP (VDUP1)	ILMN_1697448	**0.69**	0.0000386	
KLRG1	ILMN_2124920	**0.67**	0.0003379	
CD247 (CD3zeta)	ILMN_2377669	**0.54**	0.0001315	
FCRL3	ILMN_1699599	**0.51**	0.0004603	
GAB3	ILMN_1805979	**0.45**	0.0002846	
RAC2	ILMN_1709795	**0.45**	0.000586	
JAKMIP1 (Marlin 1)	ILMN_1784141	**0.40**	0.0007327	

Symbol	Illumina ID	M	p	
Transcription factors				
PLZF (ZBTB16)	ILMN_1750496	**2.12**	<0.0000001	
BHLHB2 (STRA13)	ILMN_1768534	**1.84**	0.0000018	
NR4A2 (NURR1)	ILMN_2339955	**1.36**	0.0000025	
BHLHA38 (TWIST1)	ILMN_1672908	**1.32**	0.0010628	
MAF	ILMN_1719543	**1.29**	0.0000022	
NR4A3	ILMN_1807298	**1.29**	0.0002119	
FOSL2	ILMN_1725175	**1.25**	0.0000022	
MYBL1	ILMN_3241046	**1.18**	0.0007205	
FOXP4	ILMN_1773809	**1.15**	0.0001531	
LITAF	ILMN_1713934	**0.92**	0.0002150	
KLF4	ILMN_2137789	**0.92**	0.0000836	
JUNB	ILMN_2086077	**0.86**	0.0001723	
DDX3X (C/EBP delta)	ILMN_1794392	**0.84**	0.0000454	
BZW1	ILMN_1793846	**0.81**	0.0000529	
HNRNPUL1	ILMN_3257430	**0.81**	0.0004457	
RUNX3	ILMN_1787461	**0.79**	0.0000287	
EGR1	ILMN_1762899	**0.76**	0.0006082	
KLF10	ILMN_1659122	**0.74**	0.0000611	
MAFF	ILMN_1680139	**0.74**	0.0004494	
REL	ILMN_2124064	**0.74**	0.0003637	
NFIL3	ILMN_1707312	**0.64**	0.0008908	
KLF2	ILMN_1735930	**0.62**	0.0007467	
KLF11	ILMN_1751656	**0.62**	0.0003109	
MYC	ILMN_1680618	**0.60**	0.0000732	
CDK9	ILMN_1747556	**0.58**	0.0002243	
HHEX	ILMN_1762712	**0.56**	0.0002216	
BZW1	ILMN_1704760	**0.49**	0.0009906	
MLL3	ILMN_2295183	**0.47**	0.0009164	
SERTAD2	ILMN_1651347	**0.45**	0.0005114	
Adhesion				
ITGAM (CD11b)	ILMN_1685009	**1.15**	0.0000027	
CD97	ILMN_1676718	**0.45**	0.0002600	
AMICA1 (JAML)	ILMN_1778723	**0.45**	0.0004771	

Lists of increased expressed genes in fetal blood Vγ9Vδ2 T cells compared to fetal blood αβ T cells (same fetuses) related to 'chemokines, cytokines and their receptors', 'NK cells', 'transcription factors' and 'adhesion molecules'. M (log2 fold change Vγ9Vδ2/αβ) reflects the differential expression of a gene. N= 4 fetuses.

: gene is also enriched in adult blood Vγ9Vδ2 T cells compared to adult αβ T cells; based on Pont *et al* (1) comparing four sorted adult blood Vγ9+ T cell samples (NCBI GEO data set under accession numberGSE27291) with six sorted adult blood αβ T cell samples (GSE15659 and GSE8059).

Table S7. Gene expression data of 16 genes from sorted Vγ9Vδ2 T cells compared to the expression within sorted αβ T cells derived from the same fetuses, as determined by real-time PCR.

Gene	Fetus			
	GD-018	GD-019	GD-020	GD-023
PLZF	5.28	4.65	2.89	3.04
Foxp1	-2.83	-4.49	-2.88	-4.54
T-bet	2.25	11.83	8.13	14.46
Eomes	3.23	2.07	6.10	7.50
Runx3	2.53	0.70	2.59	1.35
IFN-γ	3.76	-2.10	2.56	2.31
IL-2	2.78	-0.34	-4.79	-1.33
TNF-α	0.05	-0.20	-0.18	-0.43
IL18RAP	2.56	10.21	3.98	6.71
NKp30	0.36	2.98	2.69	2.97
perforin	2.83	1.03	2.38	-0.03
granzyme K	4.23	5.53	3.87	3.69
granzyme A	2.38	1.58	2.11	-0.64
CCR5	13.06	7.92	7.08	10.82
CXCR3	2.62	5.19	3.28	1.98
CCR7	-1.11	-2.67	-0.12	-2.48
IL-4	not detected			
IL-13	not detected			
IL-17	not detected			

Data are expressed as log2 of fold change Vγ9Vδ2/αβ for each individual fetus.

Table S8. Gene expression data of selected genes from sorted Vγ9Vδ2 T cells compared to the expression within sorted nonVγ9Vδ2 γδ T cells derived from the same fetuses, as determined by real-time PCR.

Gene	Fetus			
	GD-018	GD-019	GD-020	GD-023
T-bet	4.97	1.44	5.64	3.32
Eomes	13.27	5.10	8.43	1.50
IL18RAP	3.86	1.87	-0.31	2.24
perforin	6.46	3.42	1.19	-0.02
granzyme K	1.96	0.89	0.27	2.23
granzyme A	3.95	2.04	1.99	0.84
CCR5	-0.6	8.33	2.04	11.01
CXCR3	1.37	1.16	1.15	1.36

Data are expressed as log2 of fold change Vγ9Vδ2/nonVγ9Vδ2 for each individual fetus.

Table S9. Comparison between gene expression enriched in adult (Pont *et al.*, (1)) and fetal (this study) Vγ9Vδ2 T cells

Pont et al: cytokine/receptor	This study
CCL3	
CCL5, RANTES	
CCR2	
CCR5	
CSF1	
CXCL11	
CXCL9	
CXCR3	
CXCR6	
FASL G	
IFNG	
IL12RB2	
IL15	M=0.40;p=0.005
IL18R1	M=0.40;p=0.021
IL23R	
IL2RA	
IL2RB	M=0.71;p=0.002
IL4	
IL9R	
LIF	
TNFRSF11A	
TNFRSF12A	
TNFRSF1B	
TNFSF14	M=0.45;p=0.019
TNFSF4	
TNFSF9	
Pont et al: Jak/STAT	This study
IFNG	
IL12RB2	
IL15	M=0.40;p=0.005
IL23R	
IL2RA	
IL2RB	
IL4	
IL9R	
LIF	
PIK3R3	
SOCS1	
STAT4	M=0.51; p=0.008

Pont et al: NK cytotoxicity	This study
CASP3	
FASLG	
FCGR3A	
FCGR3B	
GZMB	
IFNG	
KLRC1, NKG2A	
KLRC2, NKG2C	
KLRD1, CD94	
KLRK1, NKG2D	
PIK3R3	
PLCG2	
PRF1	
SHC4	
TYROBP, DAP12	
ULBP2	
NKG7	
Pont et al: Th1	This study
AUTS2	
CCL3	
CCL3L1	
CCL5, RANTES	
CD38	
CXCL9	
CCR5	
CXCR3	
DNAJC6	
GOS2	
GNLY	
GZMB	
GZMH	
GZMK	
IFNG	
IL12RB2	
IL18RAP	
IL18R1	M=0.40;p=0.021
IRF8	
LRP8	
PLAUR	
PRF1	
TNFRSF1B	

Tables are based on enriched genes in adult Vγ9Vδ2 T cells vs adult αβ T cells by Pont *et al*. (1)

:also enriched expression in fetal Vγ9Vδ2 T cells vs fetal αβ T cells (as analyzed by microarray analysis and/or real-time PCR)

:indication of enriched expression by microarray analysis (but not meeting the criteria of M>0.4 and p<0.001)

References Appendix

1. Pont F, Familiades, J., Dejean, S., Fruchon, S., Cendron, D. *et al.* (2012) The gene expression profile of phosphoantigen-specific human gammadelta T lymphocytes is a blend of alphabeta T-cell and NK-cell signatures. *Eur. J. Immunol* 42(1):228-240.