Table S1. Semi-nested PCR Primers

PCR1 Primer name	Primer Sequence
IGHV1	CCTCAGTGAAGGTCTCCTGCAAGG
IGHV2	TCCTGCGCTGGTGAAACCCACACA
IGHV3	GGTCCCTGAGACTCTCCTGTGCA
IGHV4	TCGGAGACCCTGTCCCTCACCTGC
IGHV5	CAGTCTGGAGCAGAGGTGAAA
IGHV6	CCTGTGCCATCTCCGGGGACAGTG
CHA''	GGCTCCTGGGGGAAGAAGCC
CHG''	GAGTTCCACGACACCGTCAC
CHM''	GGGGAATTCTCACAGGAGAC
IGHJ	ACCTGAGGAGACGGTGACCAGGGT
PCR2 Primer name	**Primer Sequence†**
MID1: IGHV1	acgagtgcgtCCTCAGTGAAGGTCTCCTGCAAGG
MID1: IGHV2	acgagtgcgtTCCTGCGCTGGTGAAACCCACACA
MID1: IGHV3	acgagtgcgtGGTCCCTGAGACTCTCCTGTGCA
MID1: IGHV4	acgagtgcgtTCGGAGACCCTGTCCCTCACCTGC
MID1: IGHV5	acgagtgcgtCAGTCTGGAGCAGAGGTGAAA
MID1: IGHV6	acgagtgcgtCCTGTGCCATCTCCGGGGACAGTG
MID1: CHA	acgagtgcgtGGAAGAAGCCCTGGACCAGGC
MID1: CHG	acgagtgcgtCACCGTCACCGGTTCGGGG
MID1: CHM	acgagtgcgtCAGGAGACGAGGGGGAAAAGG
MID2: IGHV1	acgctcgacaCCTCAGTGAAGGTCTCCTGCAAGG
MID2: IGHV2	acgctcgacaTCCTGCGCTGGTGAAACCCACACA
MID2: IGHV3	acgctcgacaGGTCCCTGAGACTCTCCTGTGCA
MID2: IGHV4	acgctcgacaTCGGAGACCCTGTCCCTCACCTGC
MID2: IGHV5	acgctcgacaCAGTCTGGAGCAGAGGTGAAA
MID2: IGHV6	acgctcgacaCCTGTGCCATCTCCGGGGACAGTG
MID2: CHA	acgctcgacaGGAAGAAGCCCTGGACCAGGC
MID2: CHG	acgctcgacaCACCGTCACCGGTTCGGGG
MID2: CHM	acgctcgacaCAGGAGACGAGGGGGAAAAGG
MID3: IGHV1	agacgcactcCCTCAGTGAAGGTCTCCTGCAAGG
MID3: IGHV2	agacgcactcTCCTGCGCTGGTGAAACCCACACA
MID3: IGHV3	agacgcactcGGTCCCTGAGACTCTCCTGTGCA
MID3: IGHV4	agacgcactcTCGGAGACCCTGTCCCTCACCTGC
MID3: IGHV5	agacgcactcCAGTCTGGAGCAGAGGTGAAA
MID3: IGHV6	agacgcactcCCTGTGCCATCTCCGGGGACAGTG
MID3: CHA	agacgcactcGGAAGAAGCCCTGGACCAGGC
MID3: CHG	agacgcactcCACCGTCACCGGTTCGGGG
MID3: CHM	agacgcactcCAGGAGACGAGGGGGAAAAGG
MID4: IGHV1	agcactgtagCCTCAGTGAAGGTCTCCTGCAAGG
MID4: IGHV2	agcactgtagTCCTGCGCTGGTGAAACCCACACA
MID4: IGHV3	agcactgtagGGTCCCTGAGACTCTCCTGTGCA
MID4: IGHV4	agcactgtagTCGGAGACCCTGTCCCTCACCTGC
MID4: IGHV5	agcactgtagCAGTCTGGAGCAGAGGTGAAA
MID4: IGHV6	agcactgtagCCTGTGCCATCTCCGGGGACAGTG
MID4: VLJH	agcactgtagGTGACCAGGGTACCTTGGCCCCAG
MID4: CHA	agcactgtagGGAAGAAGCCCTGGACCAGGC
MID4: CHG	agcactgtagCACCGTCACCGGTTCGGGG

MID4: CHM	agcactgtagCAGGAGACGAGGGGGAAAAGG
MID5: IGHV1	atcagacacgCCTCAGTGAAGGTCTCCTGCAAGG
MID5: IGHV2	atcagacacgTCCTGCGCTGGTGAAACCCACACA
MID5: IGHV3	atcagacacgGGTCCCTGAGACTCTCCTGTGCA
MID5: IGHV4	atcagacacgTCGGAGACCCTGTCCCTCACCTGC
MID5: IGHV5	atcagacacgCAGTCTGGAGCAGAGGTGAAA
MID5: IGHV6	atcagacacgCCTGTGCCATCTCCGGGGACAGTG
MID5: CHA	atcagacacgGGAAGAAGCCCTGGACCAGGC
MID5: CHG	atcagacacgCACCGTCACCGGTTCGGGG
MID5: CHM	atcagacacgCAGGAGACGAGGGGGAAAAGG
MID6: IGHV1	atatcgcgagCCTCAGTGAAGGTCTCCTGCAAGG
MID6: IGHV2	atatcgcgagTCCTGCGCTGGTGAAACCCACACA
MID6: IGHV3	atatcgcgagGGTCCCTGAGACTCTCCTGTGCA
MID6: IGHV4	atatcgcgagTCGGAGACCCTGTCCCTCACCTGC
MID6: IGHV5	atatcgcgagCAGTCTGGAGCAGAGGTGAAA
MID6: IGHV6	atatcgcgagCCTGTGCCATCTCCGGGGACAGTG
MID6: CHA	atatcgcgagGGAAGAAGCCCTGGACCAGGC
MID6: CHG	atatcgcgagCACCGTCACCGGTTCGGGG
MID6: CHM	atatcgcgagCAGGAGACGAGGGGGAAAAGG
MID7: IGHV	cgtgtctctaCCTCAGTGAAGGTCTCCTGCAAGG
MID7: IGHV1	cgtgtctctaTCCTGCGCTGGTGAAACCCACACA
MID7: IGHV2	cgtgtctctaGGTCCCTGAGACTCTCCTGTGCA
MID7: IGHV3	cgtgtctctaTCGGAGACCCTGTCCCTCACCTGC
MID7: IGHV4	cgtgtctctaCAGTCTGGAGCAGAGGTGAAA
MID7: IGHV5	cgtgtctctaCCTGTGCCATCTCCGGGGACAGTG
MID7: CHA	cgtgtctctaGGAAGAAGCCCTGGACCAGGC
MID7: CHG	cgtgtctctaCACCGTCACCGGTTCGGGG
MID7: CHM	cgtgtctctaCAGGAGACGAGGGGGAAAAGG
MID8: IGHV1	ctcgcgtgtcCCTCAGTGAAGGTCTCCTGCAAGG
MID8: IGHV2	ctcgcgtgtcTCCTGCGCTGGTGAAACCCACACA
MID8: IGHV3	ctcgcgtgtcGGTCCCTGAGACTCTCCTGTGCA
MID8: IGHV4	ctcgcgtgtcTCGGAGACCCTGTCCCTCACCTGC
MID8: IGHV5	ctcgcgtgtcCAGTCTGGAGCAGAGGTGAAA
MID8: IGHV6	ctcgcgtgtcCCTGTGCCATCTCCGGGGACAGTG
MID8: CHA	ctcgcgtgtcGGAAGAAGCCCTGGACCAGGC
MID8: CHG	ctcgcgtgtcCACCGTCACCGGTTCGGGG
MID8: CHM	ctcgcgtgtcCAGGAGACGAGGGGGAAAAGG
MID9: IGHV1	tagtatcagcCCTCAGTGAAGGTCTCCTGCAAGG
MID9: IGHV2	tagtatcagcTCCTGCGCTGGTGAAACCCACACA
MID9: IGHV3	tagtatcagcGGTCCCTGAGACTCTCCTGTGCA
MID9: IGHV4	tagtatcagcTCGGAGACCCTGTCCCTCACCTGC
MID9: IGHV5	tagtatcagcCAGTCTGGAGCAGAGGTGAAA
MID9: IGHV6	tagtatcagcCCTGTGCCATCTCCGGGGACAGTG
MID9: CHA	tagtatcagcGGAAGAAGCCCTGGACCAGGC
MID9: CHG	tagtatcagcCACCGTCACCGGTTCGGGG
MID9:CHM	tagtatcagcCAGGAGACGAGGGGGAAAAGG
MID10: IGHV1	tctctatgcgCCTCAGTGAAGGTCTCCTGCAAGG
MID10: IGHV2	tctctatgcgTCCTGCGCTGGTGAAACCCACACA
MID10: IGHV3	tctctatgcgGGTCCCTGAGACTCTCCTGTGCA

MID10: IGHV4	tctctatgcgTCGGAGACCCTGTCCCTCACCTGC
MID10: IGHV5	tctctatgcgCAGTCTGGAGCAGAGGTGAAA
MID10: IGHV6	tctctatgcgCCTGTGCCATCTCCGGGGACAGTG
MID10: CHA	tctctatgcgGGAAGAAGCCCTGGACCAGGC
MID10: CHG	tctctatgcgCACCGTCACCGGTTCGGGG
MID10: CHM	tctctatgcgCAGGAGACGAGGGGGAAAAGG
MID11: IGHV1	tgatacgtctCCTCAGTGAAGGTCTCCTGCAAGG
MID11: IGHV2	tgatacgtctTCCTGCGCTGGTGAAACCCACACA
MID11: IGHV3	tgatacgtctGGTCCCTGAGACTCTCCTGTGCA
MID11: IGHV4	tgatacgtctTCGGAGACCCTGTCCCTCACCTGC
MID11: IGHV6	tgatacgtctCAGTCTGGAGCAGAGGTGAAA
MID11: IGHV6	tgatacgtctCCTGTGCCATCTCCGGGGACAGTG
MID11: CHA	tgatacgtctGGAAGAAGCCCTGGACCAGGC
MID11: CHG	tgatacgtctCACCGTCACCGGTTCGGGG
MID11: CHM	tgatacgtctCAGGAGACGAGGGGGAAAAGG
MID12: IGHV1	tactgagctaCCTCAGTGAAGGTCTCCTGCAAGG
MID12: IGHV2	tactgagctaTCCTGCGCTGGTGAAACCCACACA
MID12: IGHV3	tactgagctaGGTCCCTGAGACTCTCCTGTGCA
MID12: IGHV4	tactgagctaTCGGAGACCCTGTCCCTCACCTGC
MID12: IGHV5	tactgagctaCAGTCTGGAGCAGAGGTGAAA
MID12: IGHV6	tactgagctaCCTGTGCCATCTCCGGGGACAGTG
MID12: CHA	tactgagctaGGAAGAAGCCCTGGACCAGGC
MID12: CHG	tactgagctaCACCGTCACCGGTTCGGGG
MID12: CHM	tactgagctaCAGGAGACGAGGGGGAAAAGG

Table S1. Semi-nested PCR Primers

† Capital letters correspond to gene-specific sequences, and small letters to the 10-base pair MID sequence.

All sequences are written 5’ to 3’.

Volunteer		a								b								c							
Population		Transitional		Naïve		IgM		IgG or IgA		Transitional		Naïve		IgM		IgG or IgA		Transitional		Naïve		IgM		IgG or IgA	
Cell count		1700		39200		10400		20300		3700		35300		23600		6200		200		20300		2900		11000	
QC pass		16%		89%		93%		93%		70%		45%		32%		89%		80%		65%		95%		87%	
Sequence		121		669		395		1296		335		68		228		579		84		56		1088		265	
Clone count		95		561		289		734		285		55		177		309		53		46		743		250	
Variability*		nv	v	nv	v	nv	v	nv	v	nv	v	nv	v	nv	v	nv	v	nv	v	nv	v	nv	v	nv	v
Clone Size	1	76		477		232		534		251		46		143		210		33		37		583		205	
	2	9	5	46	25	29	6	59	49	18	6	5	1	19	6	29	22	9	3	6	2	69	42	13	10
	3		3	4	3	3	8	16	22	4	2	1	1	3	2	3	16	2	3	1		10	16	1	6
	4					1	2	5	11	2	1	1			1	3	8	1	2			4	7		5
	5	1		3	3	1	3	2	10						2		6					1	1		1
	6	1				1	1	1	2	1					1		1					1	3		3
	7	1					1	1	2								4								1
	8						0		5															1	2
	9						1		3								1								1
	10							1	3								1								
	11								1								1					1			
	12								1													1	1		
	13								1								1					1	1		
	15								1								1						1		
	17																								
	20																								1
	21																1								
	23																1								
	26																								
	27								1																
	28								2																
	29								1																
	37																								
	74																						1		
Total Clone		87	8	530	31	267	22	619	115	276	9	53	2	165	12	245	64	45	8	44	2	670	73	220	30

Table S2. Origins of data and clonal variability

* nv: non-variable, where the % IGHV gene similarity to the germline IGHV sequence varies by less than 1 within a clonal group. v: variable where the % IGHV gene similarity to the germline IGHV sequence varies by more than 1 within a clonal group. Variability indicates that a clone expanded i*n vivo* during an immune response involving somatic hypermutation. Non-variable clones may have been generated by PCR, or by sequencing of more than one copy of cDNA from one cell, rather than reflecting the *in vivo* situation, especially if the number of cells in the initial samples were low. However, non-variable clones may exist *in vivo*, as evidenced in our data by a number of instances where members of a non-variable clone have been isolated from different samples

(data not shown). Additionally, in all cases the majority of sequences are unique (clone size = 1), so the initial sample population does not appear to be limiting. Since there is no absolute test to distinguish between in vivo cell clonal groups and “clones” due to multiple cDNA copies or PCR amplification then the data for memory populations should be considered in comparison to that for the transitional and naïve groups where significant *in vivo* clonal expansion would not be expected.

Volunteer	a						b						c					
Comparison	T vs. N	T vs. S	T vs. M	N vs. S	N vs. M	S vs. M	T vs. N	T vs. S	T vs. M	N vs. S	N vs. M	S vs. M	T vs. N	T vs. S	T vs. M	N vs. S	N vs. M	S vs. M
IGHV1-18	*	**		*	***	***		*								*		***
IGHV1-2		*		**	**	***		**				*		***	***			
IGHV1-24																		
IGHV1-3	*		*	***		**												**
IGHV1-46		*		***		***		*				*						***
IGHV1-58	*			*														
IGHV1-69			***		***	***		**		*		***			***			***
IGHV1-8																		*
IGHV2-26														*				
IGHV2-5				*										***				***
IGHV2-70				*		*		*						*				*
IGHV3-11		*									*							
IGHV3-13								*								***	***	
IGHV3-15		**			*	*												
IGHV3-20	*																	
IGHV3-21				*														
IGHV3-22																		
IGHV3-23	*		***	*	**	***									*			**
IGHV3-30		***		**		*	*		*	*		*						
IGHV3-30-3		*		***		*			*					**		**	*	
IGHV3-33		*		***	**			*	*									
IGHV3-43		**																
IGHV3-48		*			***	***			**			**						
IGHV3-49																*		
IGHV3-53						*			**						*			
IGHV3-64	*	*			*	*										*		
IGHV3-66					**	**												*
IGHV3-7					*	**												
IGHV3-71																		
IGHV3-72	*				*													
IGHV3-73														*				
IGHV3-74			*		**	**												
IGHV3-9	*	*														**	**	
IGHV3-h																		
IGHV4-28																		
IGHV4-30-2		*		**	*													
IGHV4-30-4																		*
IGHV4-31								*		*		**						
IGHV4-34							**	*	*	***		***						
IGHV4-39																		
IGHV4-4																		*
IGHV4-55																		
IGHV4-59		*					*		*									
IGHV4-61								*		*							*	*
IGHV4-b																		
IGHV5-51																		
IGHV5-a					*	*										*		
IGHV6-1				*	**								*	***	*			*
IGHV7-4-1																		

Table S3. Chi-square comparisons of individual IGHV genes between four different populations of B cells in three separate donors[†]

[†] Transitional, naive, switched memory and IgM memory populations were compared with each other for each individual donor. Total numbers of sequences for each group are as in table S2. p values are indicated as *p<0.05, **p<0.005 and ***p<0.0005. p values for the pooled donors are in Table 2.

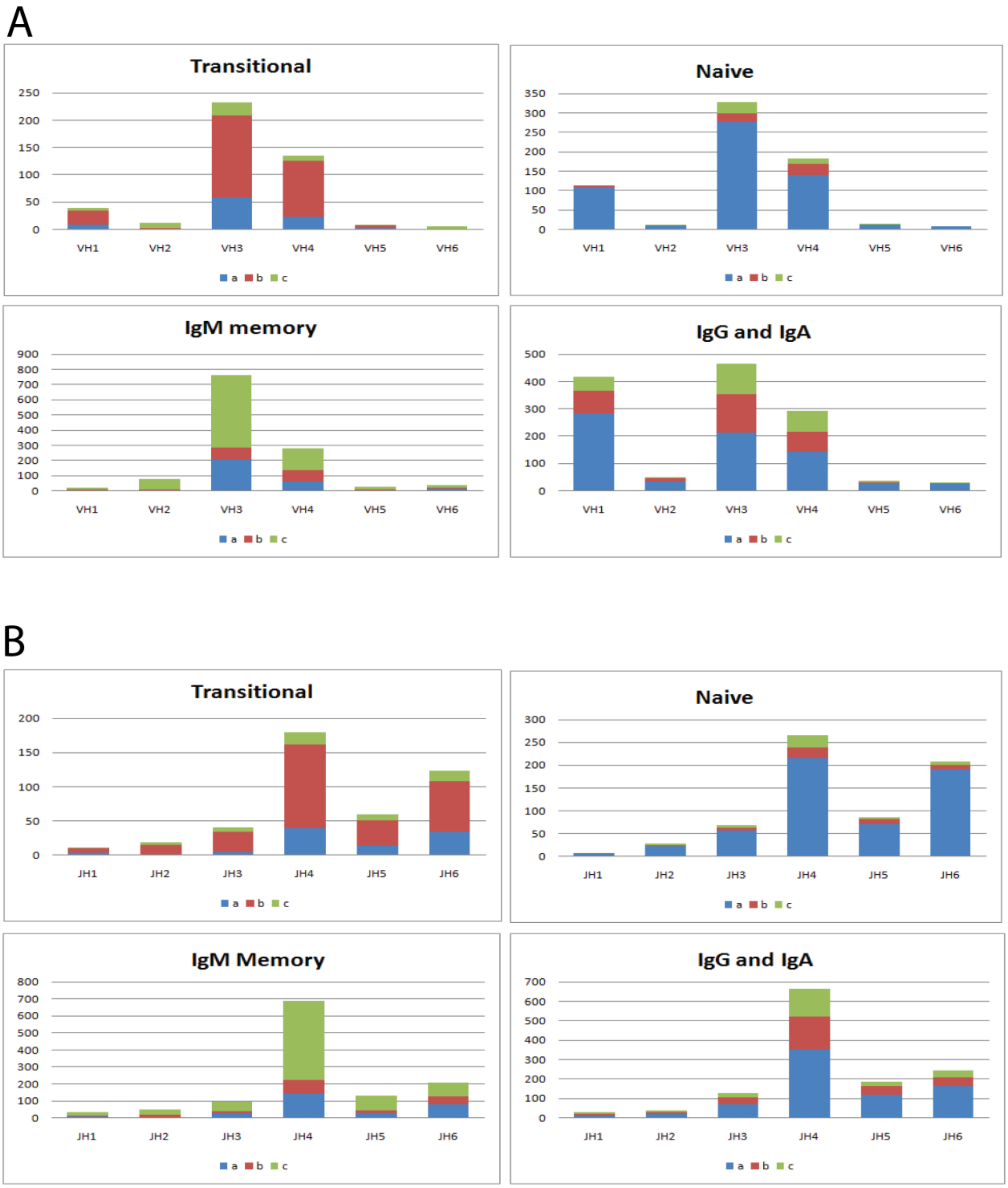


Figure S1. Individual donor contributions to the repertoire data. The absolute numbers of sequences in A) IGHV family groups and B) IGHJ family groups in four different B cell populations showing the contribution from donor a (blue) donor b (red) and donor c (green).

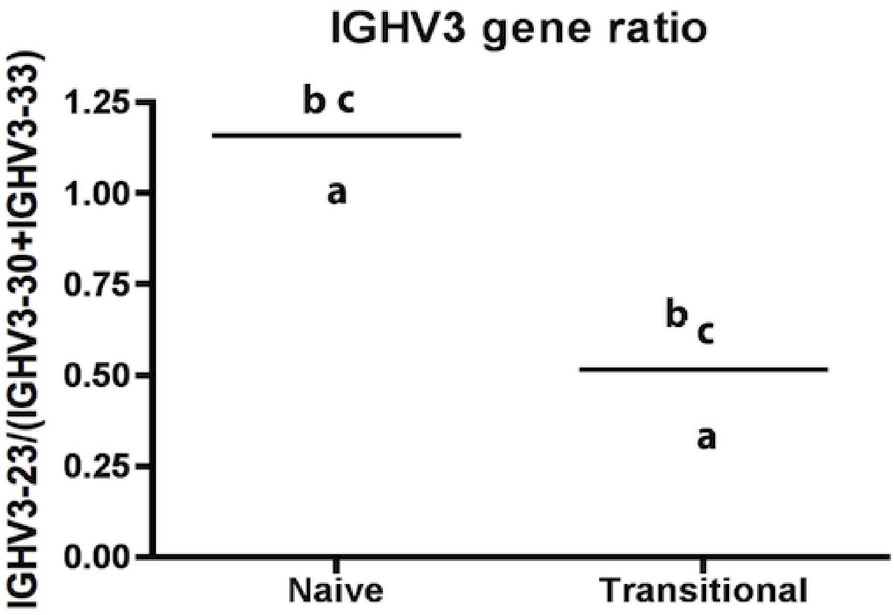
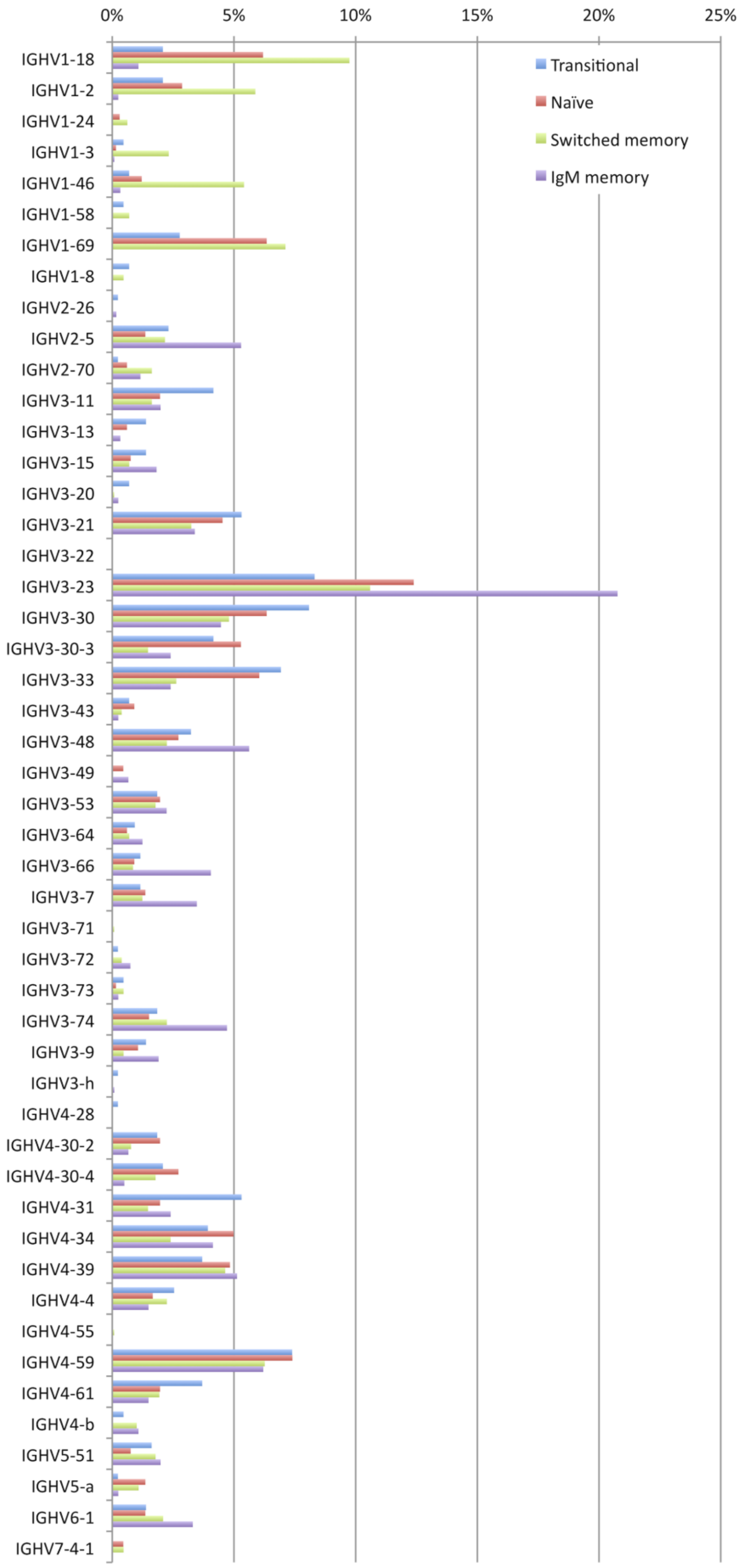


Figure S2. Individual donor contributions to the IGHV3 gene differences between naïve and transitional cells. The relative proportions of three common IGHV3 genes changes. The ratio of *IGHV3-23* to (*IGHV3-33* + *IGHV3-30*) is shown for the three donors individually (donors a, b and c as indicated) for naïve and transitional populations.



0%
5%
10%
15%
20%
25%
Transitional
Naïve
Switched memory
IgM memory
IGHV1-18
IGHV1-2
IGHV1-24
IGHV1-3
IGHV1-46
IGHV1-58
IGHV1-69
IGHV1-8
IGHV2-26
IGHV2-5
IGHV2-70
IGHV3-11
IGHV3-13
IGHV3-15
IGHV3-20
IGHV3-21
IGHV3-22
IGHV3-23
IGHV3-30
IGHV3-30-3
IGHV3-33
IGHV3-43
IGHV3-48
IGHV3-49
IGHV3-53
IGHV3-64
IGHV3-66
IGHV3-7
IGHV3-71
IGHV3-72
IGHV3-73
IGHV3-74
IGHV3-9
IGHV3-h
IGHV4-28
IGHV4-30-2
IGHV4-30-4
IGHV4-31
IGHV4-34
IGHV4-39
IGHV4-4
IGHV4-55
IGHV4-59
IGHV4-61
IGHV4-b
IGHV5-51
IGHV5-a
IGHV6-1
IGHV7-4-1

Figure S3. Individual IGHV gene usage in B cell populations. The usage frequency of individual IGHV genes in transitional (IgD+CD27-CD10+, n= 433), naïve (IgD+CD27-CD10-, n=662), class-switched memory (IgD- and either IgG+ or IgA+, n=1293) and IgM memory (IgD+CD27+, n=1209) populations. Where clonal expansions of IGHV genes occurred, only one example of each clone family was counted. Allelic variations of each IGHV gene are not shown. Results of Chi-square comparisons are given in Table 2. Usage frequency on an individual donor basis is shown in Figure S4.

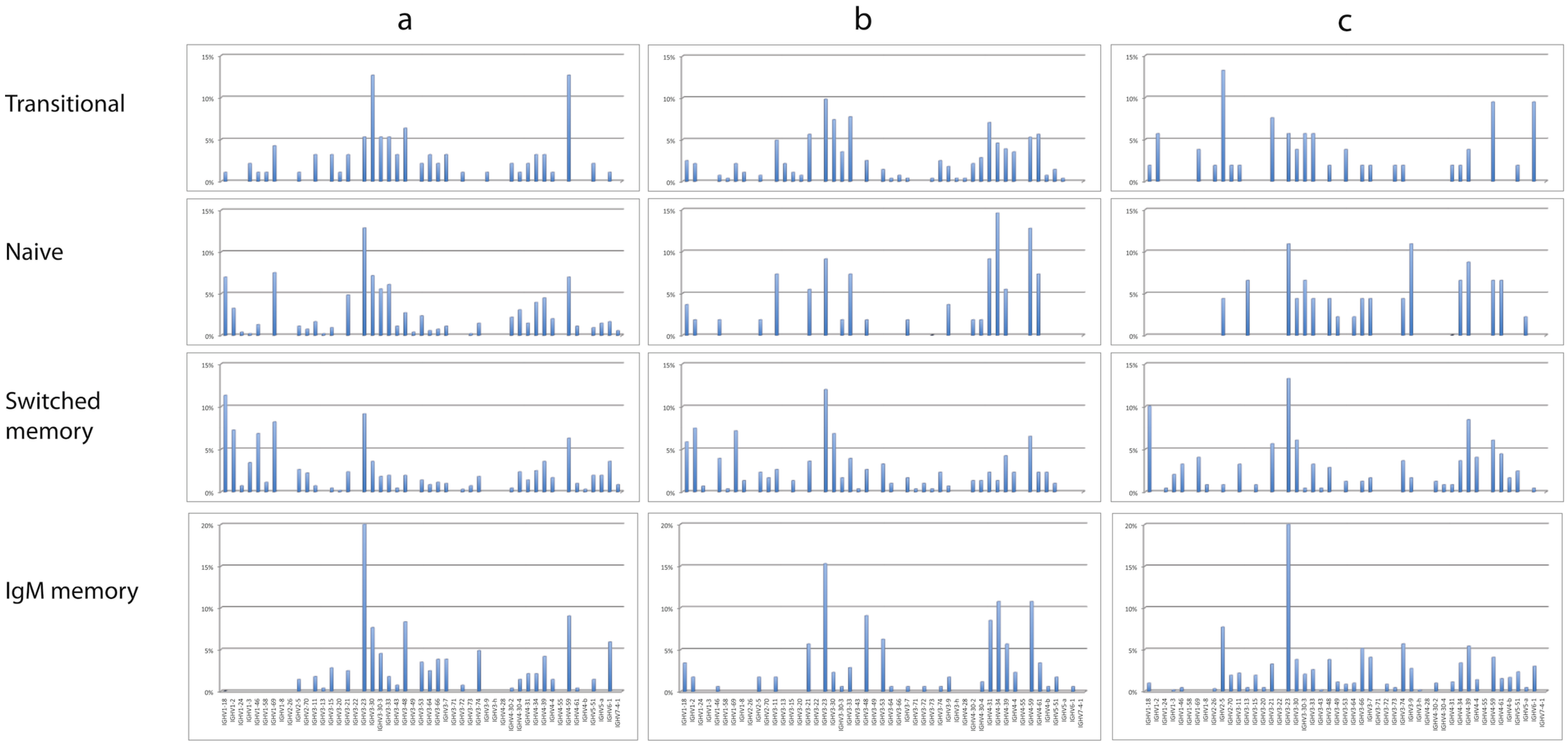


Figure S4. **Individual IGHV gene usage in B cell populations from individual donors.** The usage frequency of individual IGHV genes in transitional (IgD+CD27-CD10+, n= 433), naïve (IgD+CD27-CD10-, n=662), class-switched memory (IgD- and either IgG+ or IgA+, n=1293) and IgM memory (IgD+CD27+, n=1209) populations. Where clonal expansions of IGHV genes occurred, only one example of each clone family was counted. Allelic variations of each IGHV gene are not shown. Results of Chi-square comparisons are given in Table S3.

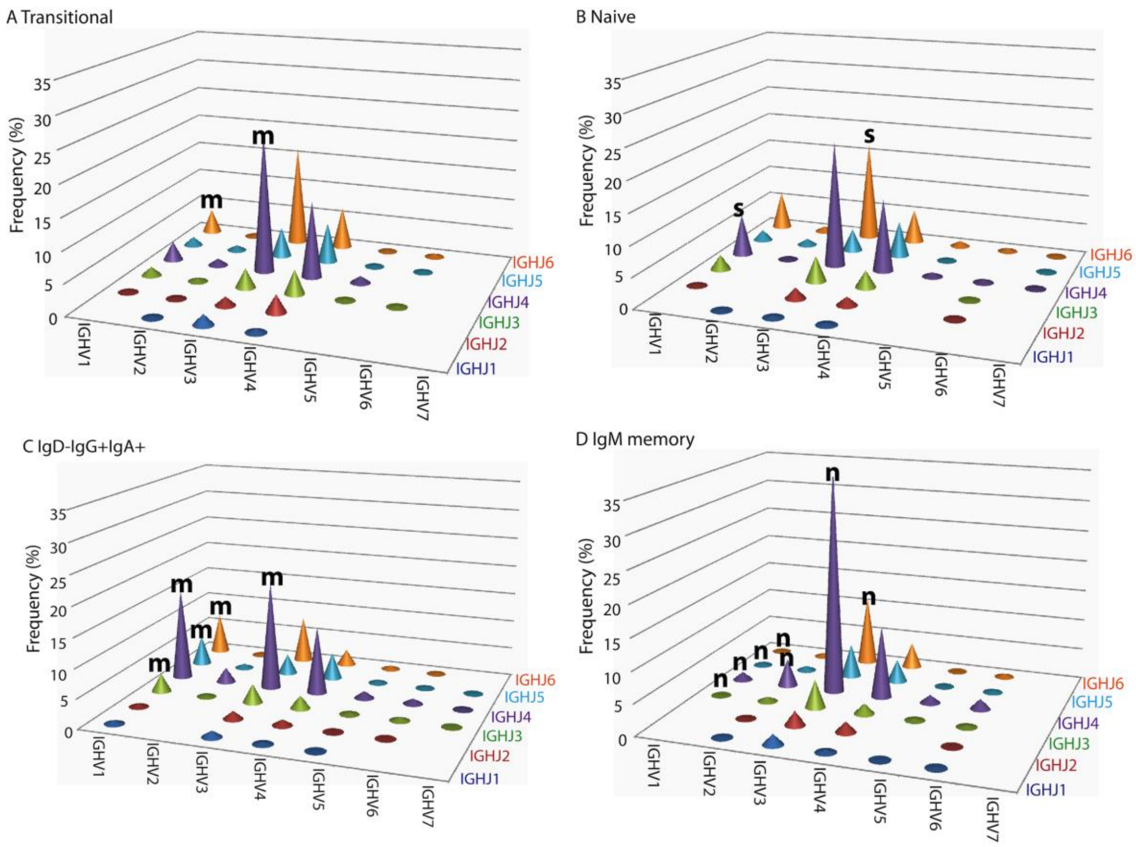


Figure S5. IGHV gene family frequency in combination with IGHJ gene usage. Individual genes were grouped into their IGHV gene families and separated by their IGHJ use. Frequency of usage for A) Transitional (IgD+CD27-CD10+, n= 433), B) Naïve (IgD+CD27-CD10-, n=662), C) Switched memory (IgD- and either IgG+ or IgA+, n=1293) and D) IgM memory (IgD+CD27+, n=1209) populations is given and groups marked with "m"," s"," n" show highly significant differences from IgM memory, Class-switched memory and Naïve cell populations respectively (p<0.0001).