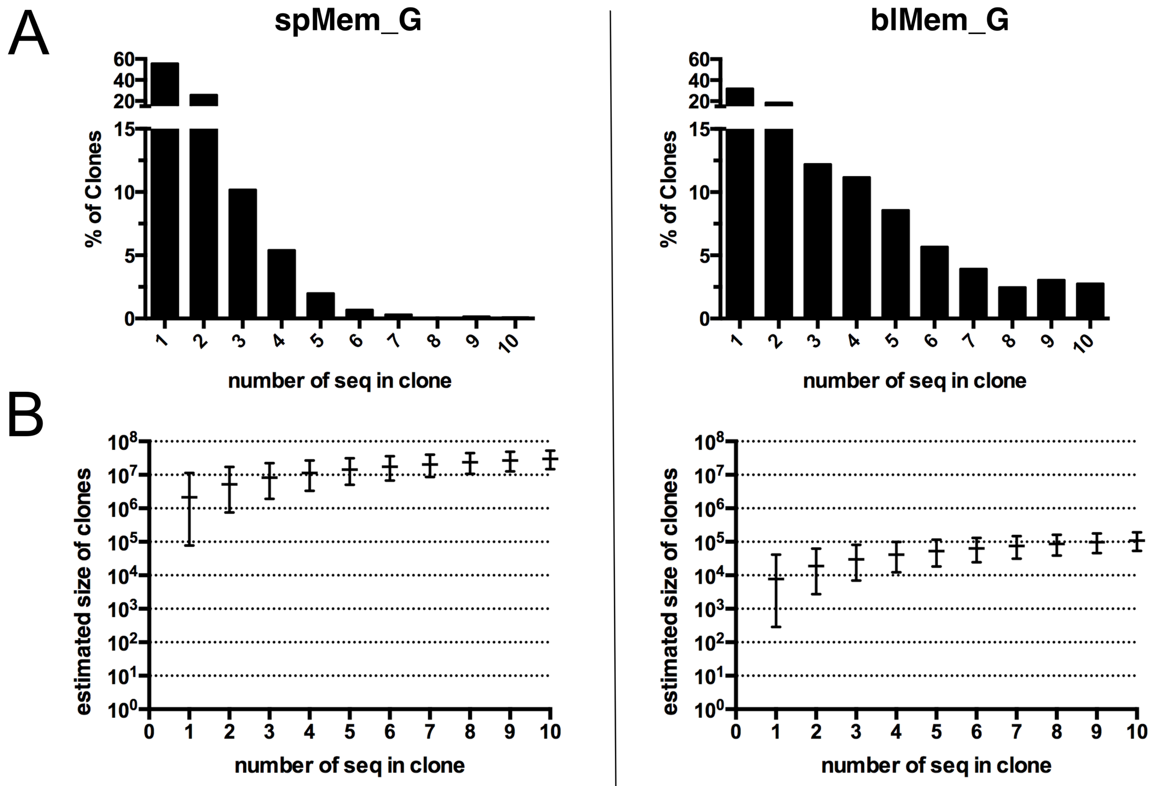

C

spMem_G

| # of seq in clone | lower 95% I.C. real size | # of clones | estimated min equivalent B cell per clone size | |
|---|---|---|---|---|
| 1 | 78195 | 733 | 5.7E+07 | "low 95% I.C." x "# of clones" |
| 2 | 7.48E+05 | 337 | 2.5E+08 | |
| 3 | 1.91E+06 | 134 | 2.6E+08 | |
| 4 | 3.36E+06 | 71 | 2.4E+08 | |
| 5 | 5.01E+06 | 26 | 1.3E+08 | |
| 6 | 6.79E+06 | 9 | 6.1E+07 | |
| 7 | 8.68E+06 | 4 | 3.5E+07 | |
| 8 | 1.06E+07 | | 0.0E+00 | |
| 9 | 1.27E+07 | 2 | 2.5E+07 | |
| 10 | 1.48E+07 | 1 | 1.5E+07 | |
| estimate # B cells | total # of clones | **1317** | **1.1E+09** | estimated minimun equivalent B cell |
| **7.20E+09** | | | **15%** | |

blMem_G

| # of seq in clone | lower 95% I.C. real size | # of clones | estimated min equivalent B cell per clone size | |
|---|---|---|---|---|
| 1 | 285 | 219 | 6.2E+04 | "low 95% I.C." x "# of clones" |
| 2 | 2722 | 127 | 3.5E+05 | |
| 3 | 6947 | 84 | 5.8E+05 | |
| 4 | 12240 | 77 | 9.4E+05 | |
| 5 | 18233 | 59 | 1.1E+06 | |
| 6 | 24714 | 39 | 9.6E+05 | |
| 7 | 31621 | 27 | 8.5E+05 | |
| 8 | 38797 | 17 | 6.6E+05 | |
| 9 | 46207 | 21 | 9.7E+05 | |
| 10 | 53890 | 19 | 1.0E+06 | |
| estimate # B cells | total # of clones | **689** | **7.5E+06** | estimated minimun equivalent B cell |
| **3.90E+07** | | | **19%** | |

**Figure S1. Estimate of clone sizes and fraction of B cell repertoire analyzed .**

This figure describes in three steps the calculation performed for one spleen (left part) and one blood (right part) subset chosen as an example:

**A.** Distribution of clone sizes obtained by CDR3 clustering within a given subset.

**B.** Using the procedure described in Material and Methods ("Estimate of clone sizes and B cell sampling" section), the values obtained in panel A are extrapolated for the total size of the B cell subset, with error bars representing the 95% interval of confidence of the estimate.

**C.** The lower 5% values of the estimate of B cell numbers obtained in panel B are tabulated and multiplied by the number of clones in each category. The total number of cells obtained in this calculation, reported to the total size of the B cell subset considered, represents the minimal fraction of B cells within this subset that is clonally related to the sequenced sample and is listed in Table S1 ("minimum equivalent B cell fraction").

Table S1. Estimate size of B cell subsets, number of unique sequence obtained, number of sequenced clones and minimal estimate of the corresponding fraction of the B cell repertoire analyzed for three adult donors.

| | **estimated population size** | **number of unique sequences** | **number of observed clones** | **minimum equivalent B cell fraction (%)** |
|---|---|---|---|---|
| spMem_G | $7.6*10^{9}$ | 2416 | 1662 | 10.7 |
| | $2.7*10^{9}$ | 2319 | 1309 | 21.0 |
| | $7.2*10^{9}$ | 2333 | 1317 | 15.0 |
| blMem_G | $1.2*10^{8}$ | 1519 | 615 | 23.3 |
| | $8.0*10^{7}$ | 2832 | 1783 | 18.6 |
| | $3.9*10^{7}$ | 3473 | 543 | 39.3 |
| spMem_A | $6.8*10^{9}$ | 2094 | 1498 | 10.2 |
| | $2.3*10^{9}$ | 1973 | 1263 | 13.3 |
| | $6.1*10^{9}$ | 1608 | 1184 | 9.9 |
| blMem_A | $1.9*10^{8}$ | 1636 | 835 | 17.6 |
| | $1.7*10^{8}$ | 2741 | 1488 | 18.7 |
| | $1.2*10^{8}$ | 2037 | 484 | 44.7 |
| spMem_M | $1.2*10^{9}$ | 167 | 108 | 11.3 |
| | $1.3*10^{9}$ | 1184 | 626 | 16.2 |
| | $6.8*10^{8}$ | 93 | 72 | 8.0 |
| blMem_M | $7.2*10^{7}$ | 336 | 136 | 23.5 |
| | $2.4*10^{7}$ | 1762 | 485 | 36.9 |
| | $2.3*10^{7}$ | 119 | 51 | 24.2 |
| spMZ | $4.5*10^{9}$ | 4580 | 2802 | 12.5 |
| | $1.5*10^{10}$ | 1812 | 1379 | 8.0 |
| | $1.4*10^{10}$ | 10077 | 5586 | 13.0 |
| blMZ | $5.4*10^{8}$ | 2122 | 1166 | 14.4 |
| | $3.9*10^{8}$ | 11715 | 5889 | 18.7 |
| | $5.5*10^{7}$ | 1547 | 534 | 28.0 |
| spDN_G | $5.6*10^{8}$ | 2297 | 1576 | 11.6 |
| | $4.8*10^{8}$ | 1477 | 1008 | 10.8 |
| | $1.5*10^{9}$ | 2755 | 1393 | 16.0 |
| blDN_G | $3.6*10^{7}$ | 453 | 130 | 22.3 |
| | $2.6*10^{7}$ | 3553 | 1257 | 22.0 |
| | $1.4*10^{7}$ | 686 | 236 | 20.7 |
| spDN_A | $3.6*10^{8}$ | 2106 | 1527 | 9.6 |
| | $1.6*10^{8}$ | 479 | 325 | 11.4 |
| | $4.3*10^{8}$ | 1919 | 1017 | 16.2 |
| blDN_A | $1.8*10^{7}$ | 606 | 134 | 46.3 |
| | $2.6*10^{7}$ | 2416 | 1186 | 28.3 |
| | $3.9*10^{7}$ | 1636 | 202 | 60.4 |
| spDN_M | $3.3*10^{8}$ | 460 | 222 | 19.2 |
| | $1.7*10^{8}$ | 182 | 156 | 5.7 |
| | $1.4*10^{8}$ | 41 | 39 | 3.5 |
| blDN_M | $4.7*10^{7}$ | 21 | 17 | 6.2 |
| | $1.3*10^{7}$ | 1291 | 76 | 13.6 |
| | $1.3*10^{7}$ | 59 | 24 | 12.7 |

Table S2. Number of clones obtained that are either unique to each subset or shared by two different ones*

| | **blMem_A** | **spMem_A** | **blMem_G** | **spMem_G** | **blMem_M** | **spMem_M** | **blMZ** | **spMZ** | **blDN_A** | **spDN_A** | **blDN_G** | **spDN_G** | **blDN_M** | **spDN_M** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **blMem_A** | 2806 | 190 | 34 | 12 | 7 | 8 | 29 | 24 | 472 | 79 | 2 | 2 | | |
| **spMem_A** | | 3945 | 16 | 31 | 8 | 8 | 36 | 37 | 67 | 189 | 3 | 3 | | 2 |
| **blMem_G** | | | 3161 | 161 | 5 | 5 | 25 | 22 | 27 | 7 | 36 | 42 | | 1 |
| **spMem_G** | | | | 4287 | 3 | 4 | 18 | 19 | 7 | 6 | 22 | 78 | 1 | |
| **blMem_M** | | | | | 672 | 43 | 85 | 37 | 3 | 2 | | | 4 | 13 |
| **spMem_M** | | | | | | 806 | 97 | 56 | 3 | | 1 | 1 | 1 | 13 |
| **blMZ** | | | | | | | 7589 | 552 | 14 | 6 | 4 | 4 | 2 | 30 |
| **spMZ** | | | | | | | | 9767 | 8 | 18 | 7 | 5 | 3 | 36 |
| **blDN_A** | | | | | | | | | 1522 | 44 | 12 | 1 | | |
| **spDN_A** | | | | | | | | | | 2869 | 8 | 17 | | |
| **blDN_G** | | | | | | | | | | | 1624 | 91 | | |
| **spDN_G** | | | | | | | | | | | | 3977 | | |
| **blDN_M** | | | | | | | | | | | | | 243 | 8 |
| **spDN_M** | | | | | | | | | | | | | | 417 |

Analysis is performed for each donor separately, and the number of clones obtained in each category is pooled for the three donors

*Numbers in the diagonal represent clones unique to this subset. Numbers outside of the diagonal are clones shared by the two corresponding subsets: since clones can be shared between more than two subsets, the sum of these numbers is bigger than the total number of shared clones.

# Table S3. Analysis of statistical significance of repertoire comparisons reported in Figures 5 and 6

| | | | **mut** | | **CDR3** | **VH** | **JH** |
|---|---|---|---|---|---|---|---|
| Fig 5A | | | MZ | Mem_M | | | |
| | **(Mem_M/MZ)** | **Mem_M** | ns | ns | ns | ns | ns |
| | | **MZ** | *** | *** | ns | *** | *** |
| | | | Mem_M | DN_M | | | |
| | **(Mem_M/DN_M)** | **Mem_M** | ns | ns | ns | ns | ns |
| | | **DN_M** | *** | *** | ns | ns | * |
| | | | MZ | DN_M | | | |
| | **(MZ/DN_M)** | **MZ** | ** | *** | ns | ns | *** |
| | | **DN_M** | *** | *** | ns | ns | ** |
| | | **Mem_M** | ns | ns | ns | ns | ns |
| | | | | | | | |
| Fig 5B | | | Mem_M | Mem_A-G | | | |
| | **(MemM/Mem_A-G)[a]** | **Mem_M** | ns | ** | ns | ns | ns |
| | | | Mem_M | Mem_A | | | |
| | **(MZ/Mem_A)** | **MZ** | *** | *** | ns | *** | ns |
| | | **Mem_A** | *** | ns | ns | * | ns |
| | | **Mem_M** | ns | ** | ns | ns | ns |
| | | | MZ | Mem_G | | | |
| | **(MZ/Mem_G)** | **MZ** | *** | *** | ns | *** | ns |
| | | **Mem_G** | *** | ns | * | ** | ns |
| | | **Mem_M** | ns | *** | ns | ns | ns |
| | | | | | | | |
| Fig 5C | | | MZ | DN_A | | | |
| | **(MZ/DN_A)** | **MZ** | ns | *** | ns | ns | ns |
| | | **DN_A** | *** | ns | ns | ns | ns |
| | | **Mem_M** | * | ns | ns | *** | ns |
| | | | MZ | DN_G | | | |
| | **(MZ/DN_G)** | **MZ** | ns[b] | ns[b] | ** | ns | ns |
| | | **DN_G** | ns[b] | ns[b] | ns | ns | ns |
| | | **Mem_M** | *[b] | ns[b] | * | ns | ns |

| | | | **mut** | | **CDR3** | **VH** | **JH** |
|---|---|---|---|---|---|---|---|
| Fig 6A | | | bl | sp | | | |
| | **Mem_A(bl/sp)** | **bl** | *** | *** | ns | *** | ns |
| | | **sp** | ns | ns | ns | ns | ns |
| | | | bl | sp | | | |
| | **Mem_G(bl/sp)** | **bl** | ns | ns | ns | *** | ns |
| | | **sp** | * | ns | ns | ns | ns |
| | | | | | | | |
| Fig 6B | | | bl | sp | | | |
| | **Mem_M(bl/sp)** | **bl** | ns | ns | ns | ns | ns |
| | | **sp** | ** | ns | ns | ns | ns |
| | | | bl | sp | | | |
| | **MZ(bl/sp)** | **bl** | *** | ns | ns | ns | ns |
| | | **sp** | *** | *** | ns | *** | ns |

[a] statistics are shown for pooled Mem_A and Mem_G samples

[b] statistics calculated on the mean mutation frequency and not on the mutation distribution because of the small sample size