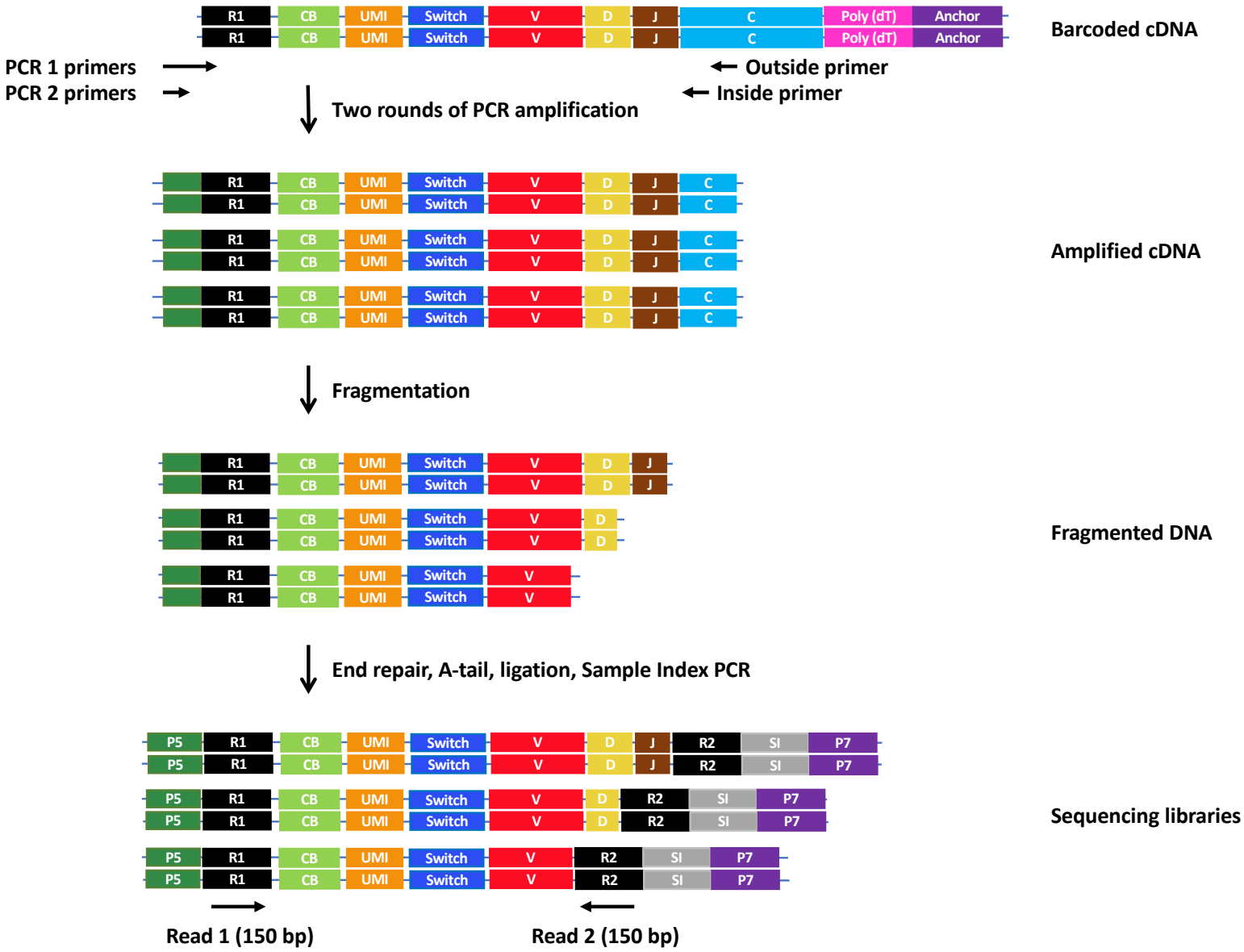
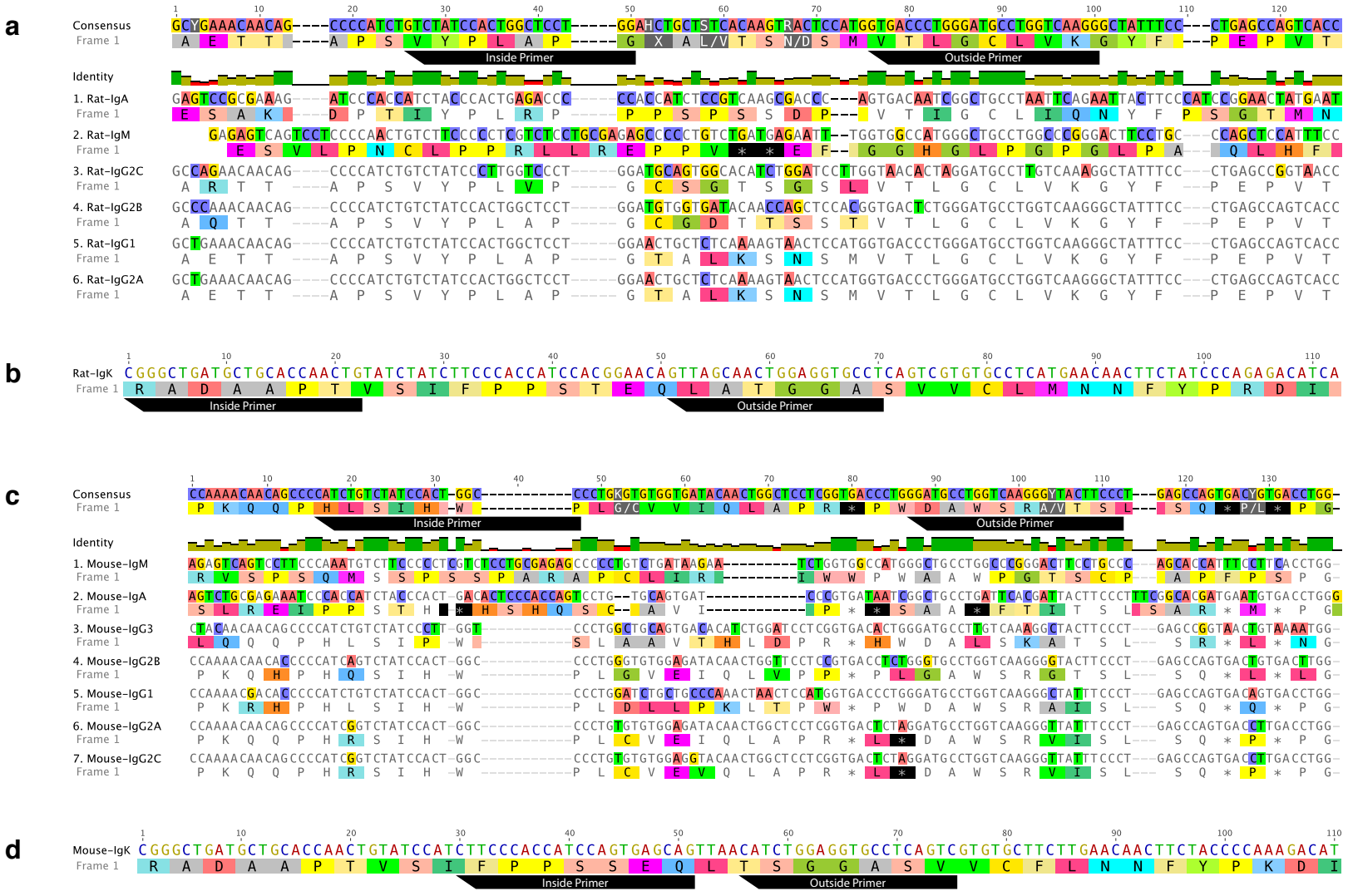
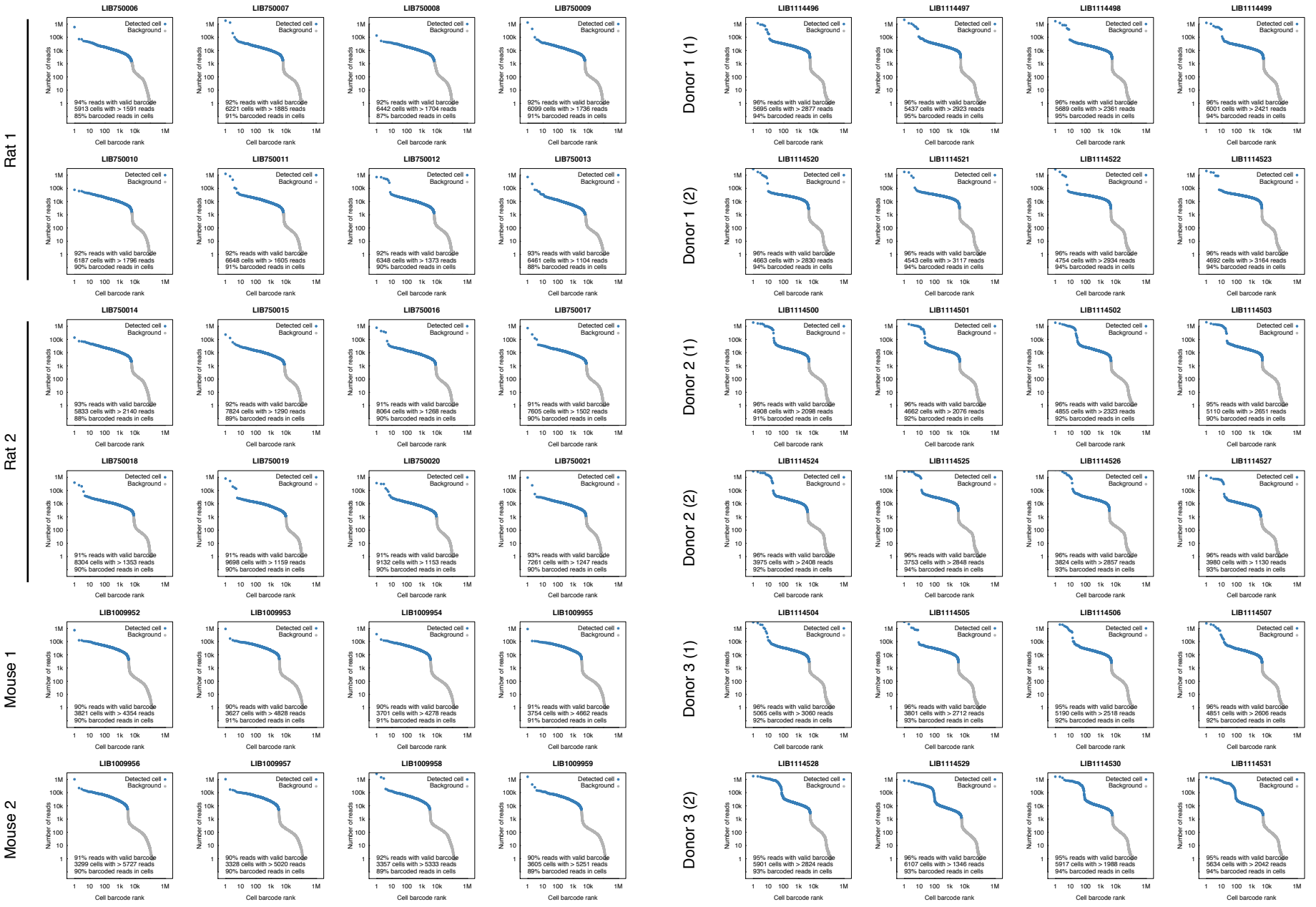


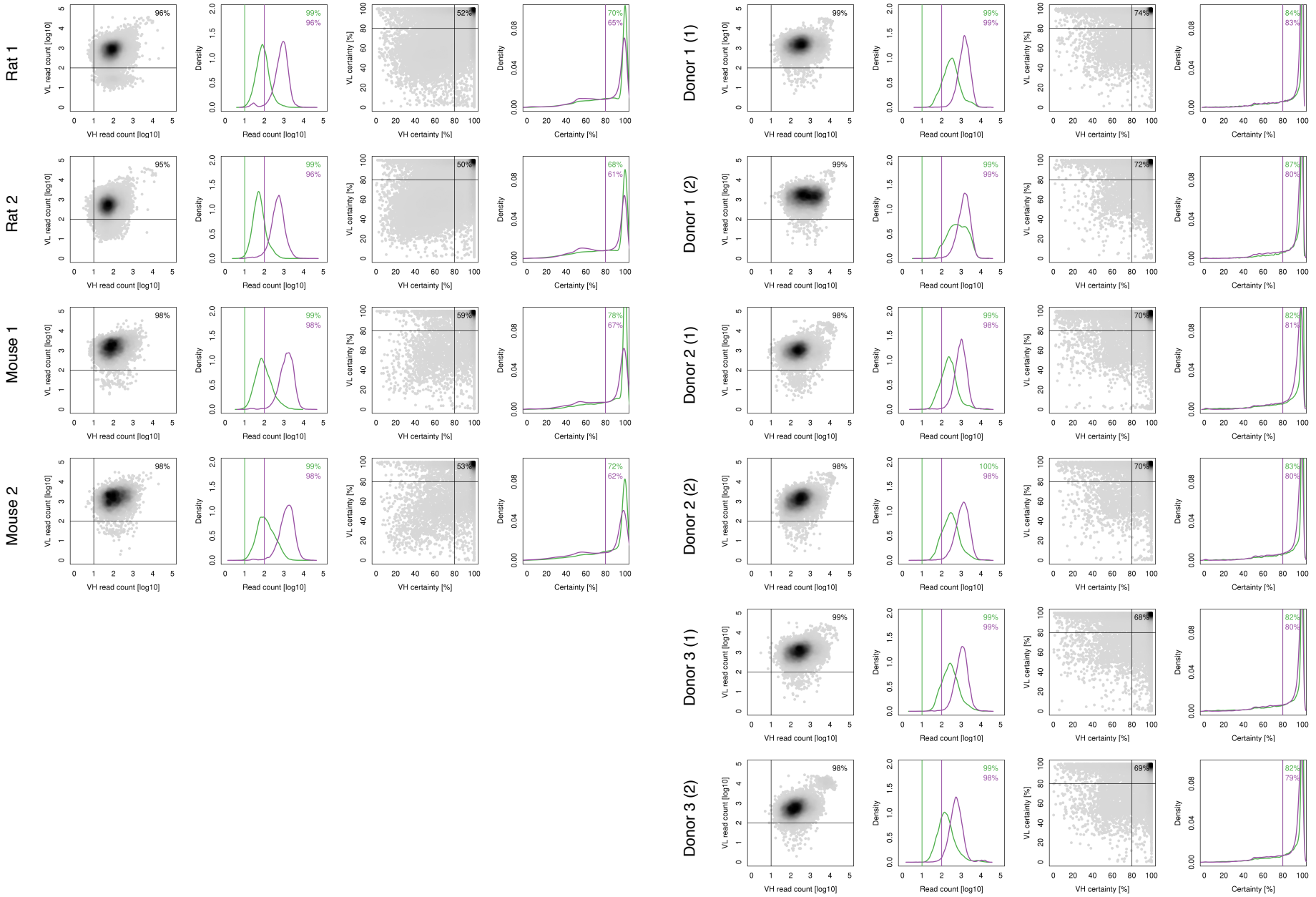
Supplementary Figure 1. Schematic of single-cell cDNA generation and library construction following the manufacturer's user guide (10x Genomics, Pleasanton, CA, CG000086_SingleCellVDJReagentKitsUserGuide_RevB). Only heavy chain is shown.



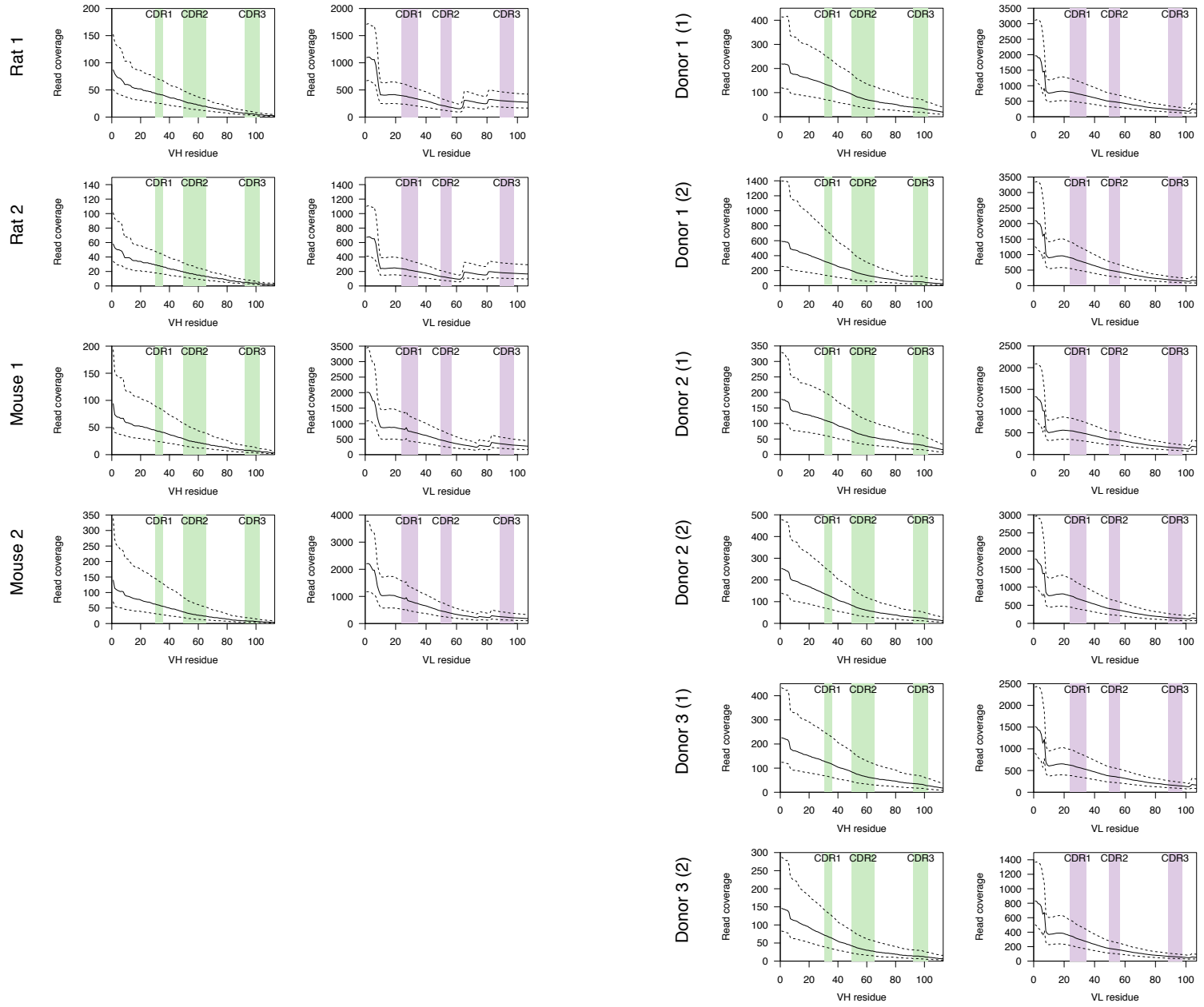
Supplementary Figure 2. Custom PCR primers targeting the rat and mouse heavy- and light-chain constant regions. (a) Multiple sequence alignment of rat heavy-chain isotype constant regions. (b) Rat κ light-chain constant region. (c) Multiple sequence alignment of mouse heavy-chain isotype constant regions. (d) Mouse κ light-chain constant region.



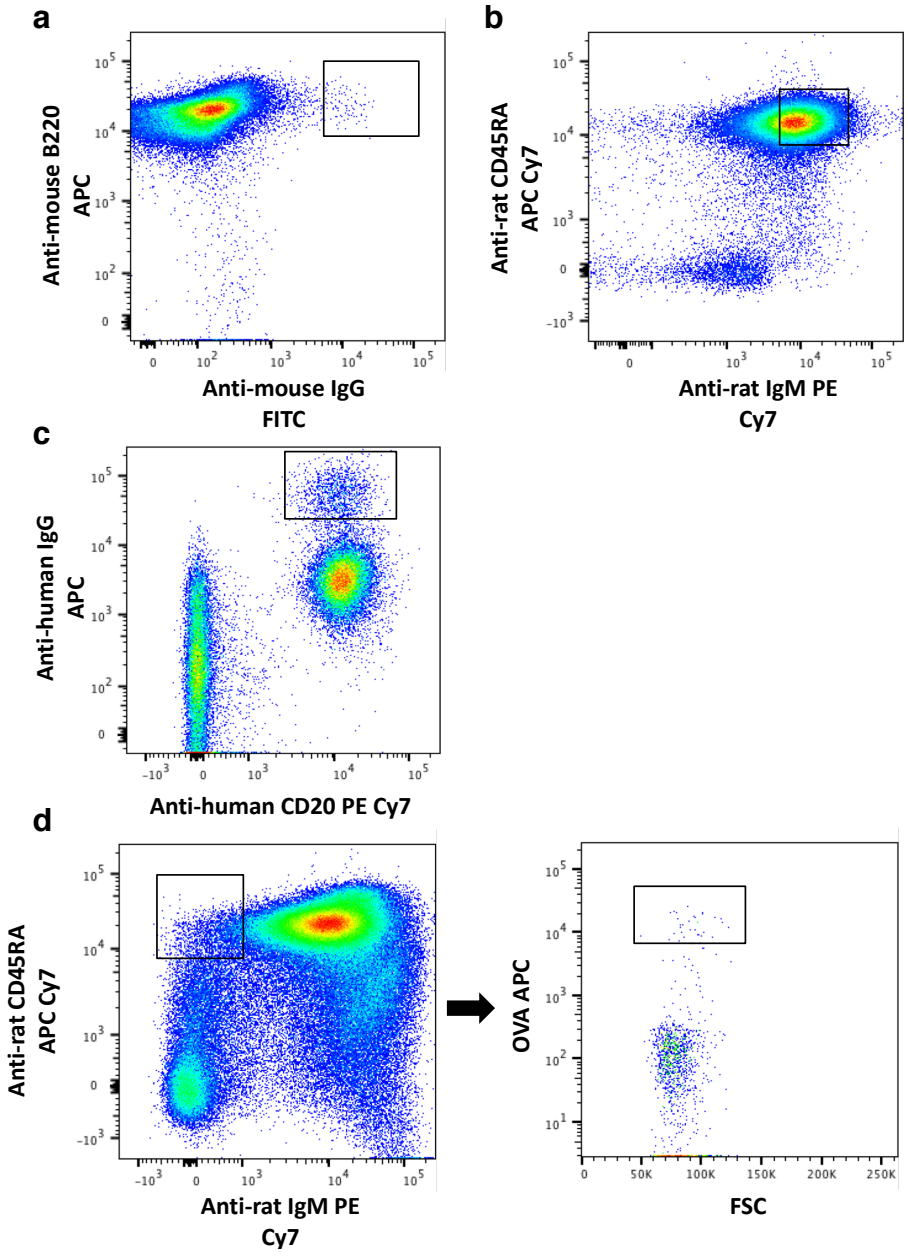
Supplementary Figure 3. Cell detection. Number of reads per barcode plotted against barcode rank for each library, barcodes for detected cells are colored in blue.



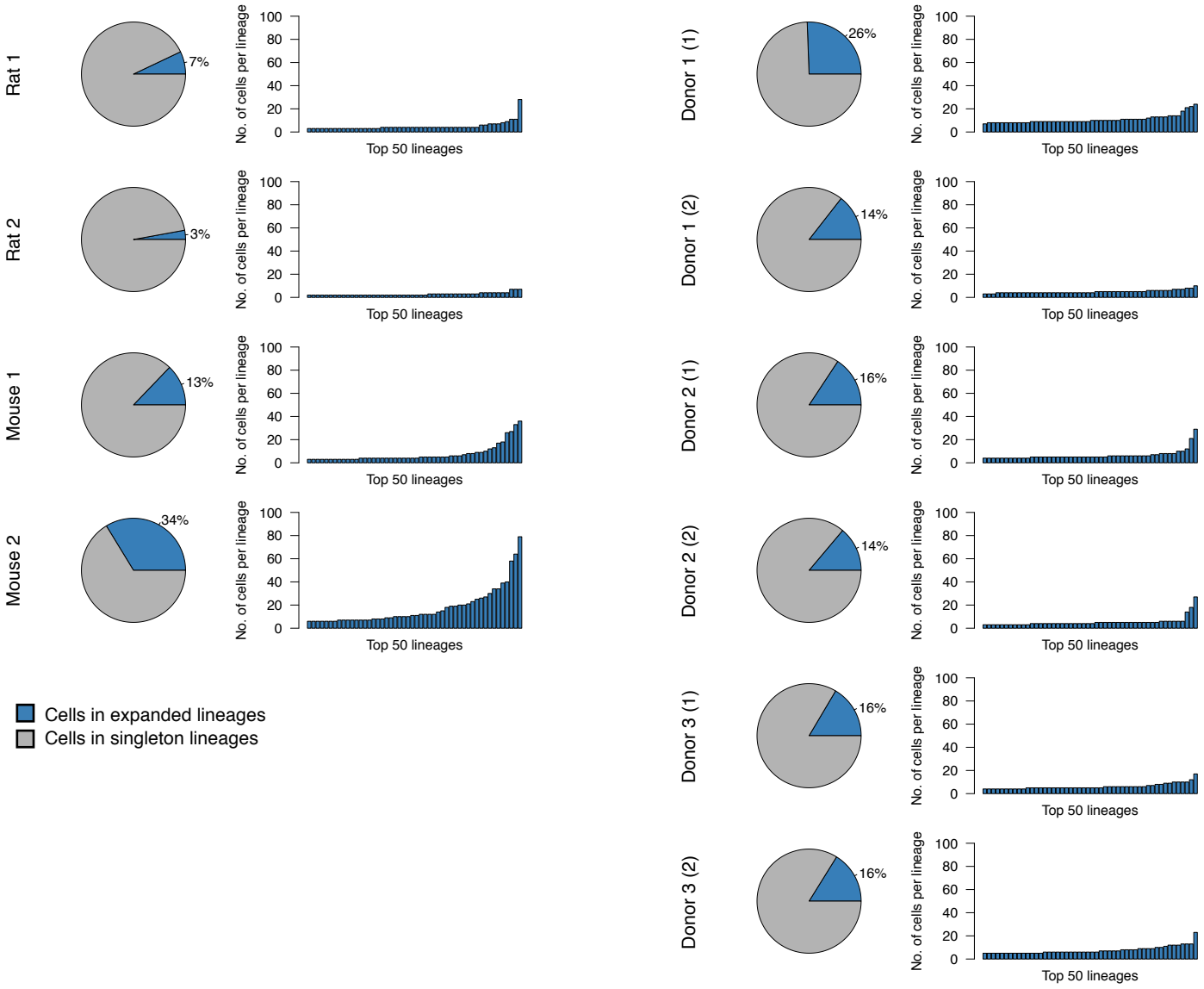
Supplementary Figure 4. Cell quality filtering. Otherwise as in Figure 2b.



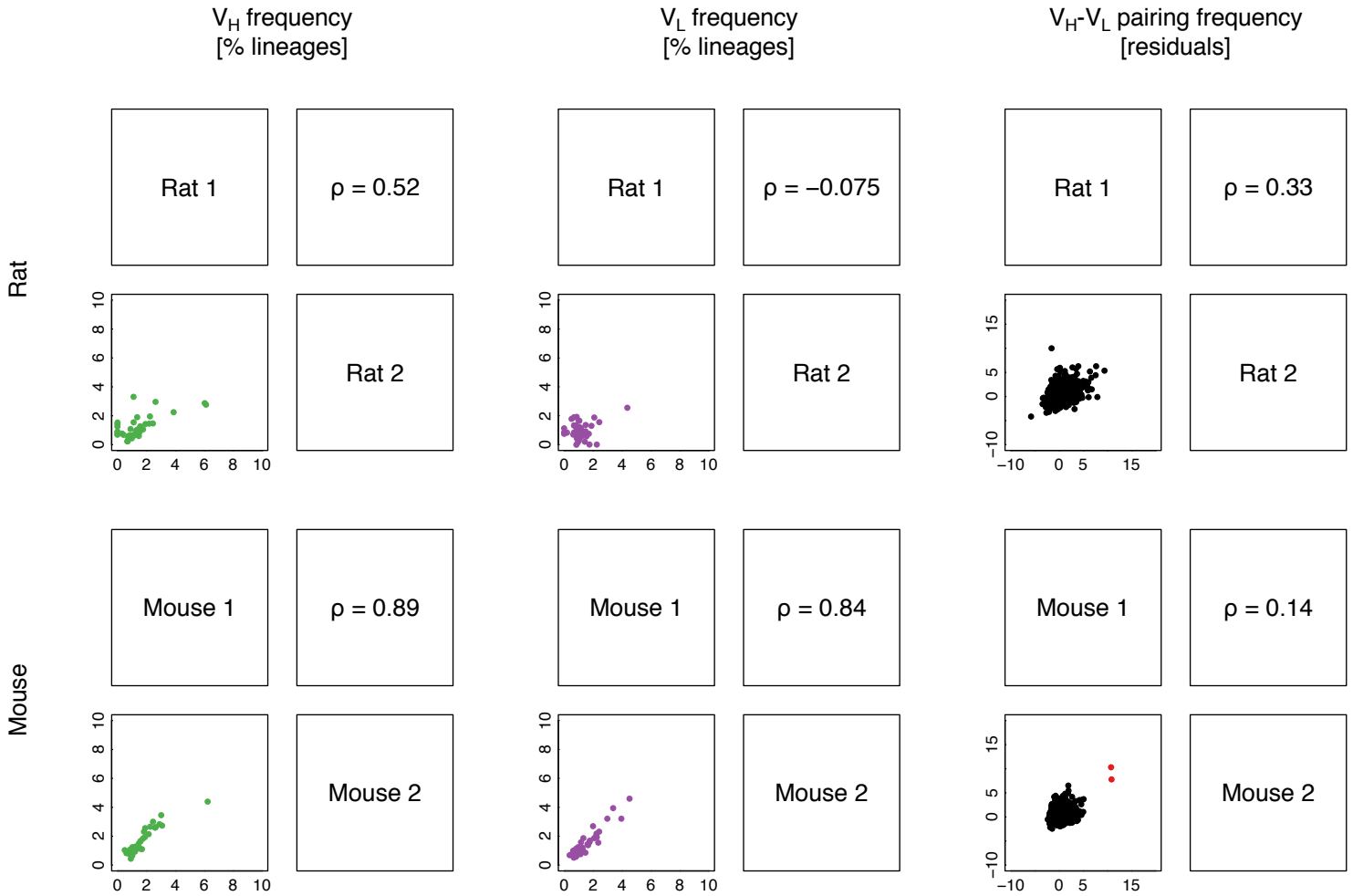
Supplementary Figure 5. Read coverage for quality-filtered VH and VL assemblies. Otherwise as in Figure 2c.



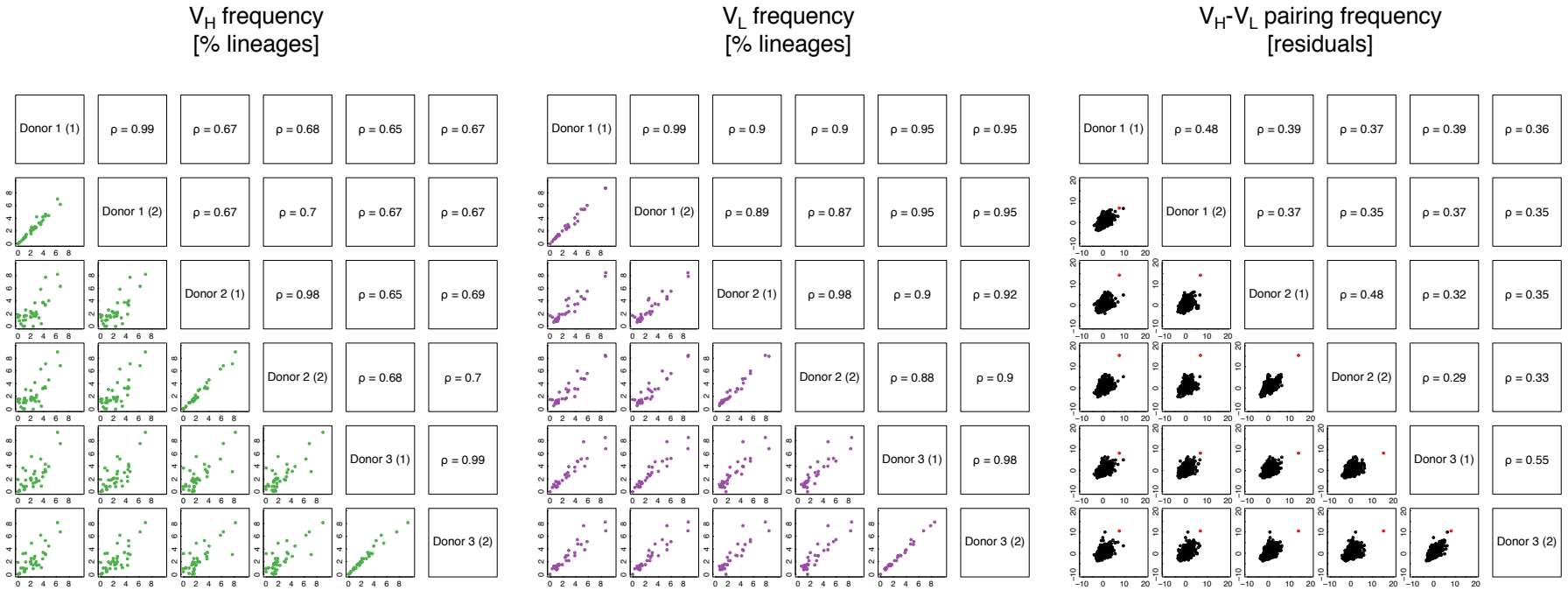
Supplementary Figure 6. Representative B cell sorting gates for naïve mice (a), naïve rat (b), human (c) and pooled OVA-immunized rats (d).



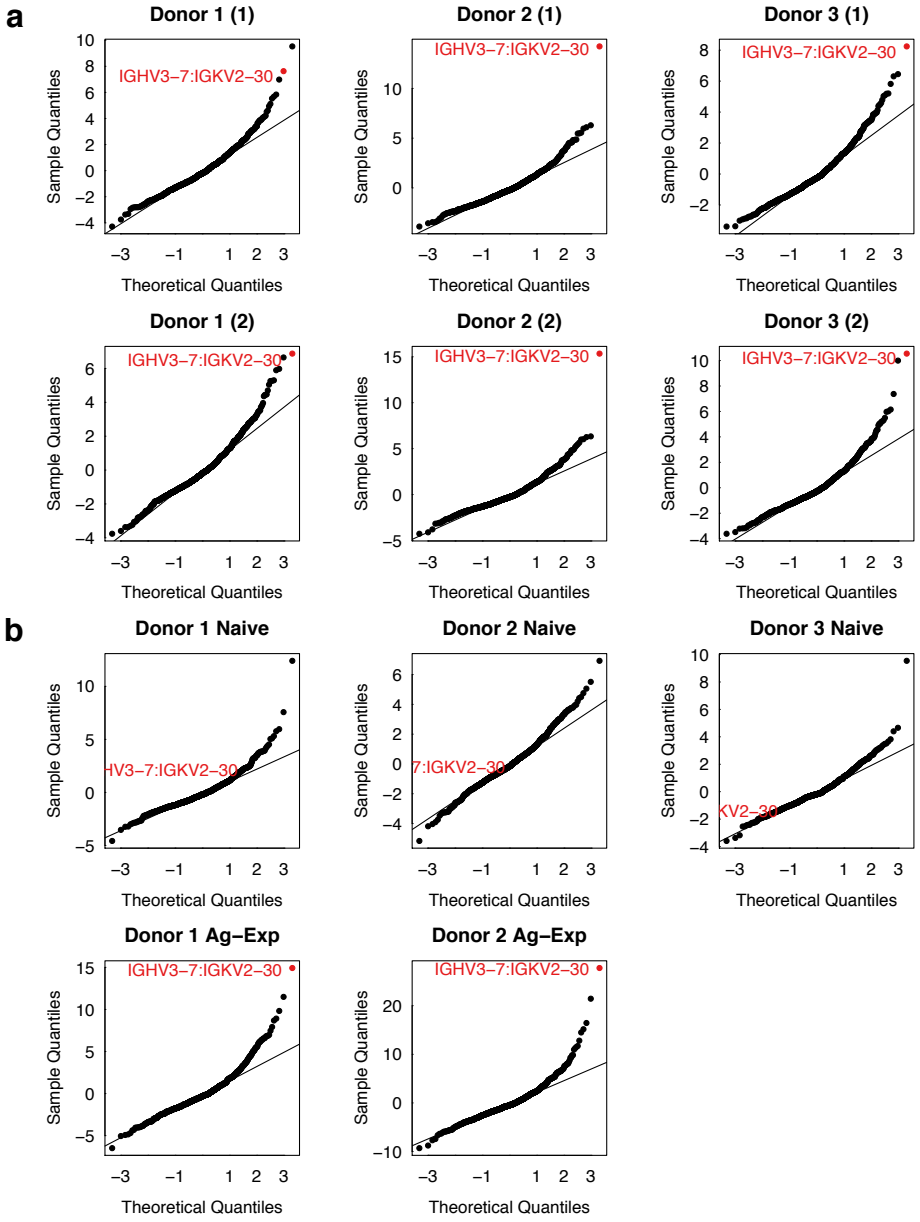
Supplementary Figure 7. Lineage expansions. Otherwise as in Figure 6a,b.



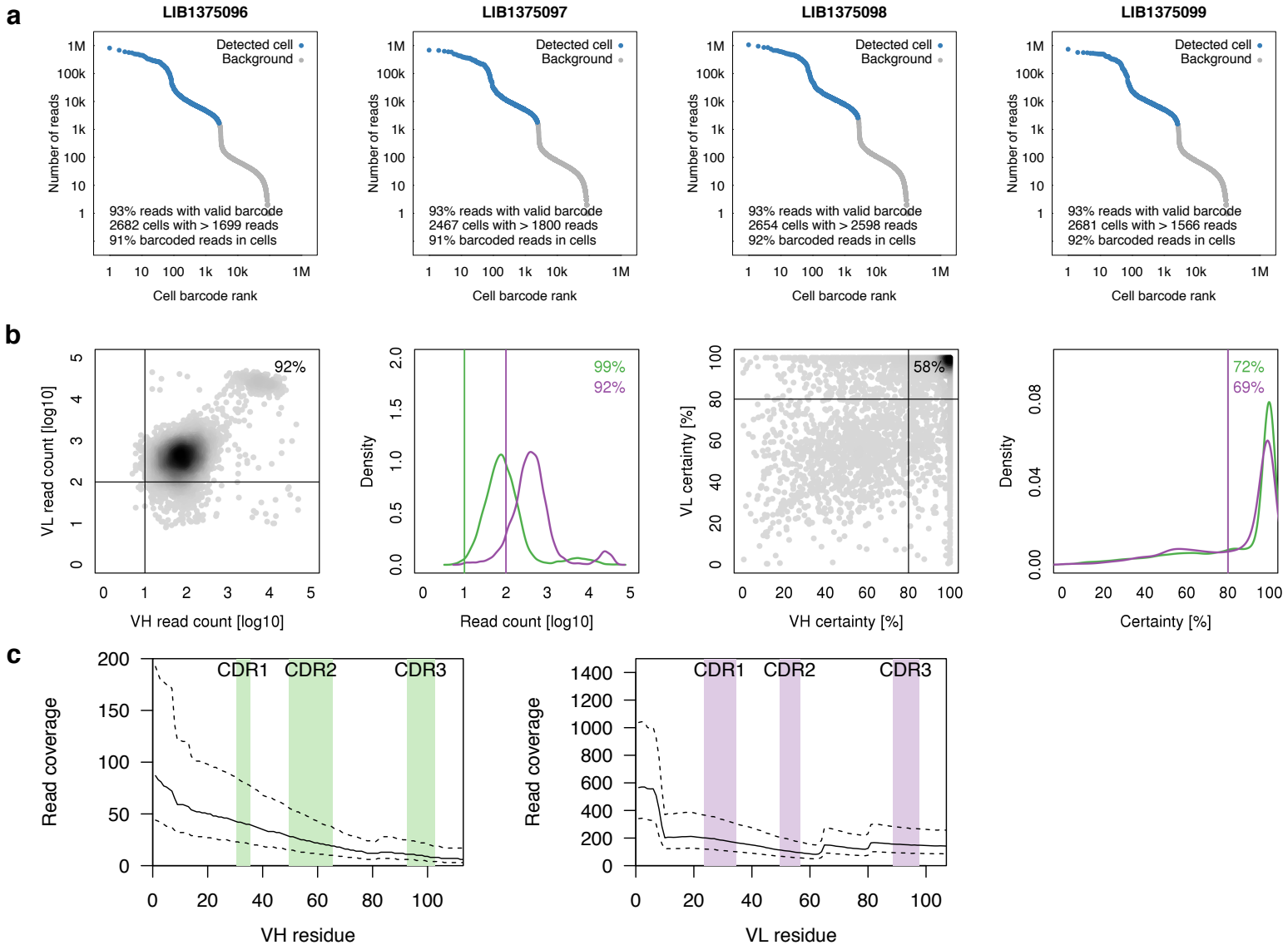
Supplementary Figure 8. Pairwise scatter plots for V_H frequencies, V_L frequencies, and standardized residuals for V_H-V_L pairing frequencies for two rats (top) and two mice (bottom). Frequencies are for lineages with a particular germline gene segment or pairing. *IGHV8-12:IGKV3-7* and *IGHV3-2:IGKV5-43* are highlighted in red.



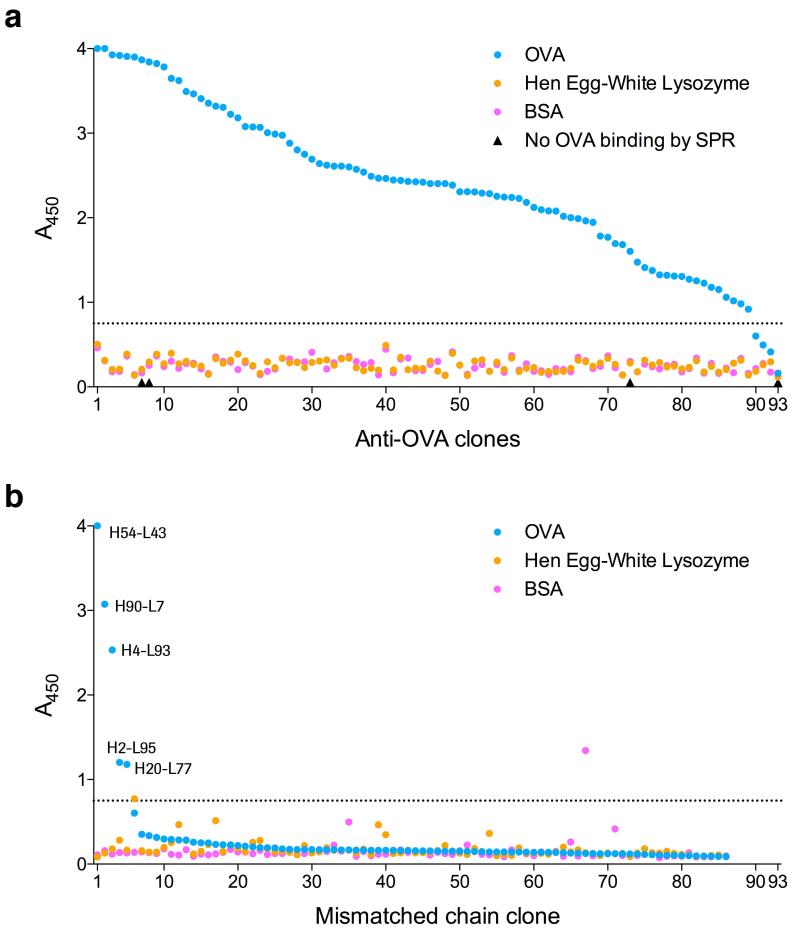
Supplementary Figure 9. Pairwise scatter plots for V_H frequencies, V_L frequencies, and standardized residuals for V_H-V_L pairing frequencies for three human donors, each profiled at two different time points. Frequencies are for lineages with a particular germline gene segment or pairing. *IGHV3-7:IGKV2-30* is highlighted in red.



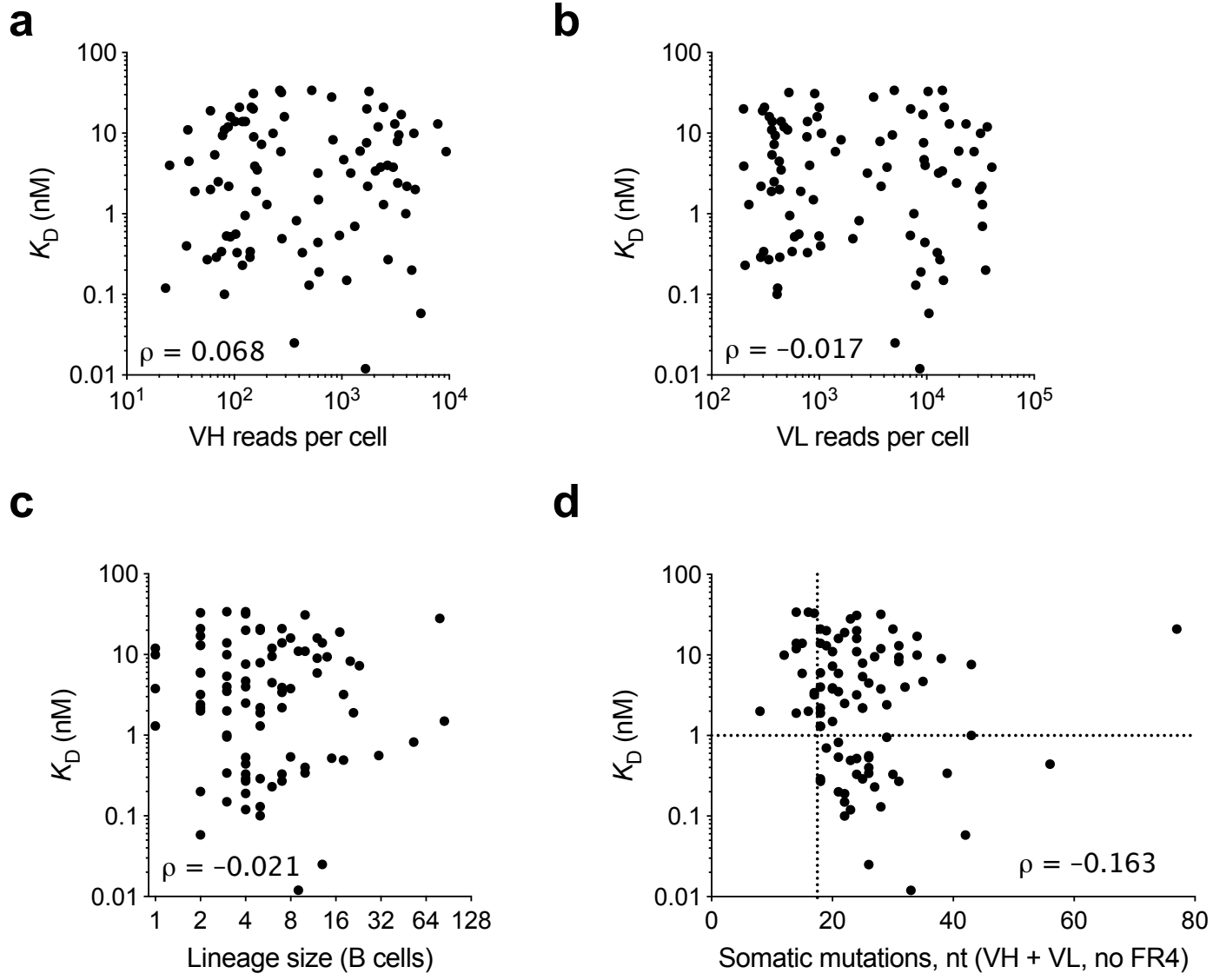
Supplementary Figure 10. QQ-plots of standardized residuals for V_H-V_L pairing frequencies. (a) Data from three human donors, each profiled at two different time points. (b) Data for naïve and antigen-experienced human B cells published in DeKosky, B.J. et al. Large-scale sequence and structural comparisons of human naive and antigen-experienced antibody repertoires. Proc Natl Acad Sci U S A 113, E2636-2645 (2016).



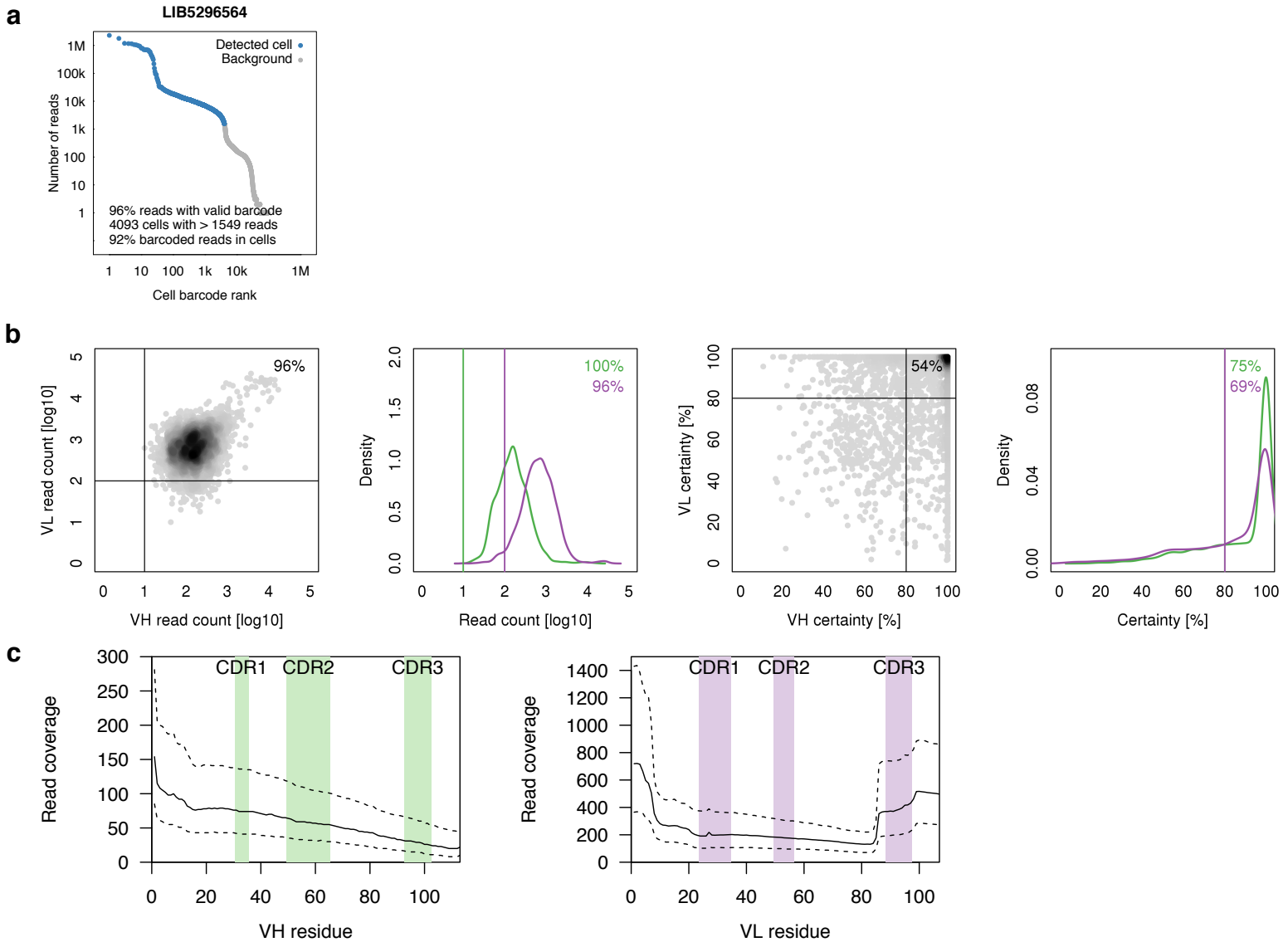
Supplementary Figure 11. Quality assessment of IgM^{neg}/OVA^{pos} lymph node B cell data. (a) Cell detection. (b) Cell quality filtering. (c) Read coverage for quality-filtered VH and VL assemblies.



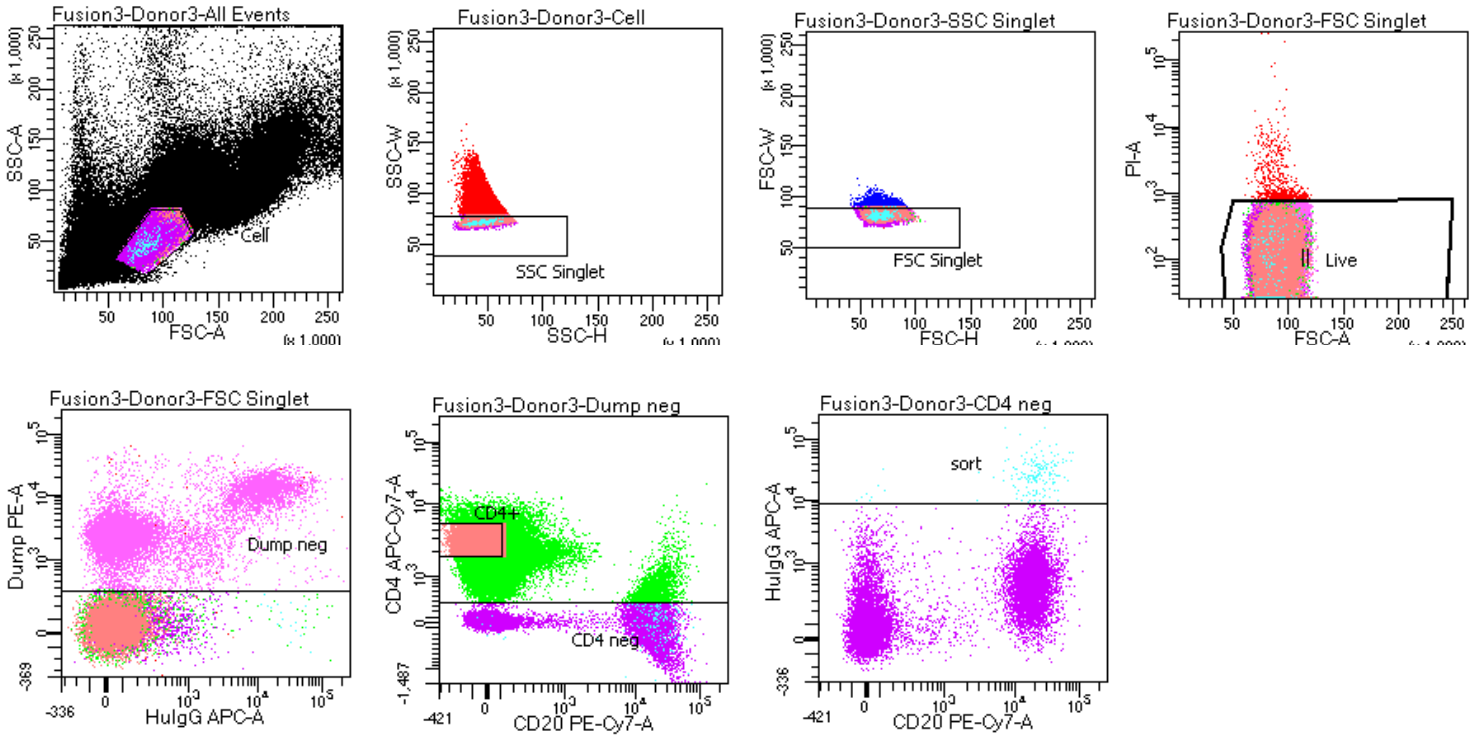
Supplementary Figure 12. ELISA results for predicted OVA antigen-reactive B cell clones (a) and negative controls obtained by mispairing VH and VL chains (b). Clones are ranked by OVA ELISA signal separately in (a) and (b).



Supplementary Figure 13. Binding affinity (K_D) did not show obvious associations with VH read count (a), VL read count (b), lineage size (c), or SHM load (d).



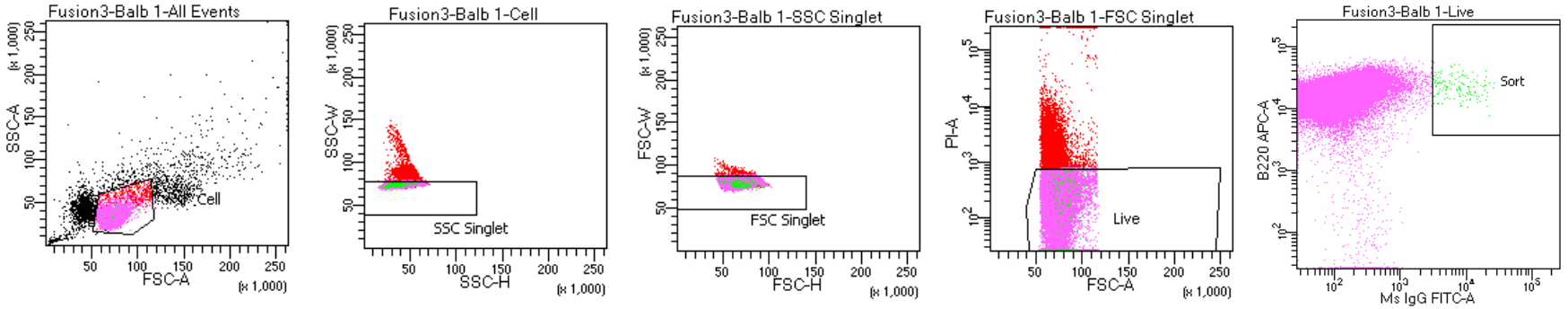
Supplementary Figure 14. Quality assessment of mouse data generated by 10x Genomics (B cells isolated from C57BL/6 mouse splenocytes - Direct Ig enrichment). (a) Cell detection. (b) Cell quality filtering. (c) Read coverage for quality-filtered VH and VL assemblies.



Tube: Donor3

Population	#Events	%Parent	%Total
All Events	510,250	####	100.0
Cell	250,147	49.0	49.0
SSC Singlet	233,951	93.5	45.9
FSC Singlet	231,564	99.0	45.4
Live	230,891	99.7	45.3
Dump neg	113,173	49.0	22.2
CD4 neg	11,722	10.4	2.3
sort	178	1.5	0.0
CD4+	49,323	43.6	9.7

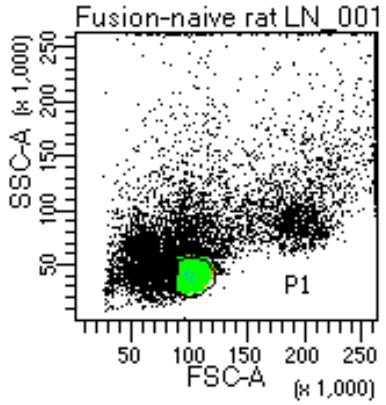
Supplementary Figure 15. Human IgG^{pos} B cell sort gating strategy. 1) FSC vs SSC to gate lymphocytes, 2/3) SSC-H/SSC-W, FSC-H/FSC-W gates to exclude cell doublets, 4) FSC/PI for dead cell exclusion (PI-negative gate), 5) IgG-APC/Dump-PE (CD11b, CD11c, CD14, CD16, CD56, CD64, CD8) to exclude PE-positive non-B cells, 6) CD20-PE Cy7/CD4 APC-Cy7 to exclude CD4+ cells, 7) CD20-PECy7/IgG-APC to sort for IgG^{pos} B cells.

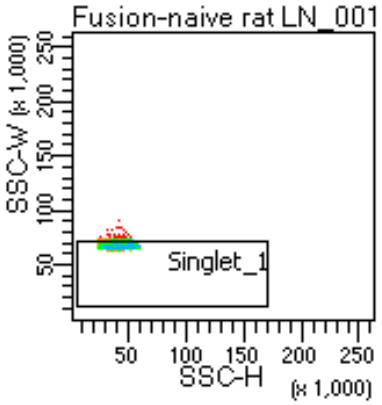


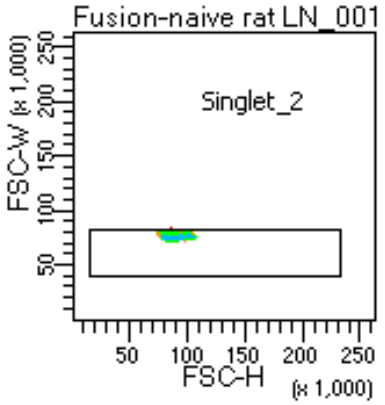
Tube: Balb 1

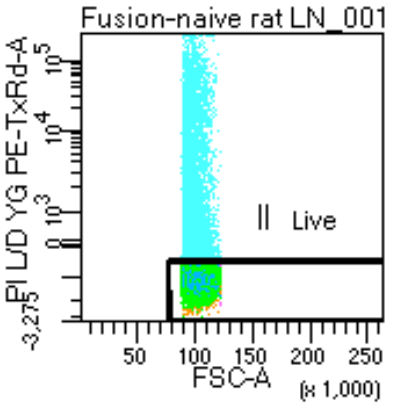
Population	#Events	%Parent	%Total
All Events	100,000	####	100.0
Cell	78,224	78.2	78.2
SSC Singlet	74,841	95.7	74.8
FSC Singlet	74,547	99.6	74.5
Live	69,670	93.5	69.7
Sort	141	0.2	0.1

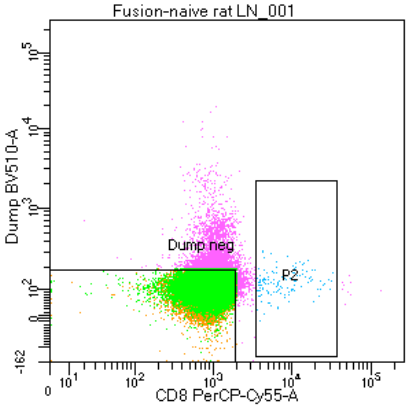
Supplementary Figure 16. Naïve Balb/c mouse B cell sort gating strategy. 1) FSC vs SSC to gate lymphocytes, 2/3) SSC-H/SSC-W, FSC-H/FSC-W gates to exclude cell doublets, 4) FSC/PI for dead cell exclusion (PI-negative gate), 5) IgG FITC/B220-APC to sort for IgG^{pos} B cells.

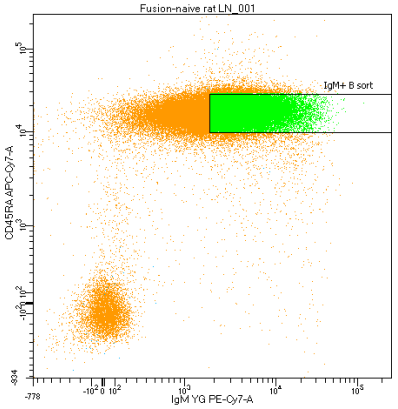








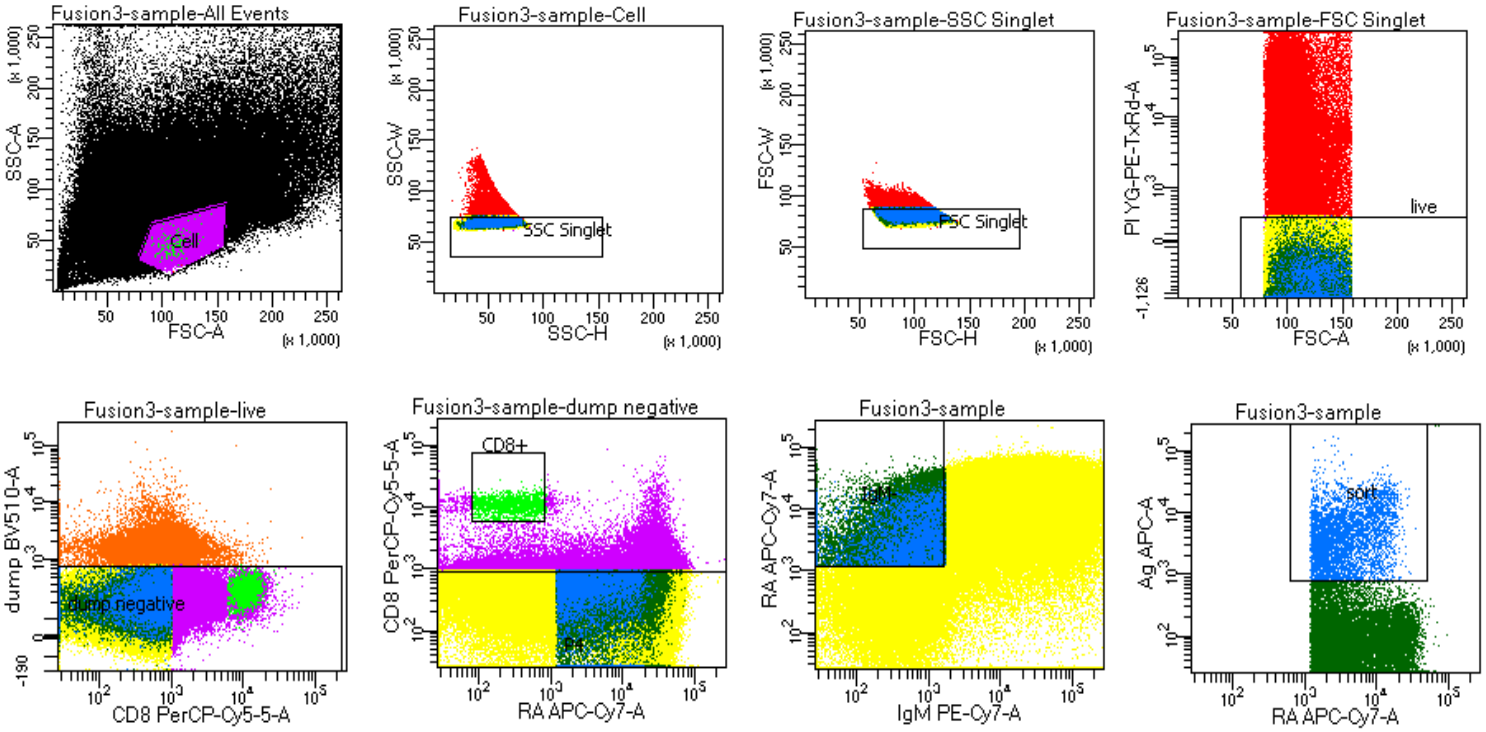




Tube: naive rat LN_001

Population	#Events	%Parent	%Total
All Events	243,300	####	100.0
P1	96,263	39.6	39.6
Singlet_1	96,219	100.0	39.5
Singlet_2	96,218	100.0	39.5
Live	87,696	91.1	36.0
Dump neg	83,716	95.5	34.4
IgM+ B sort	49,498	59.1	20.3
P2	133	0.2	0.1

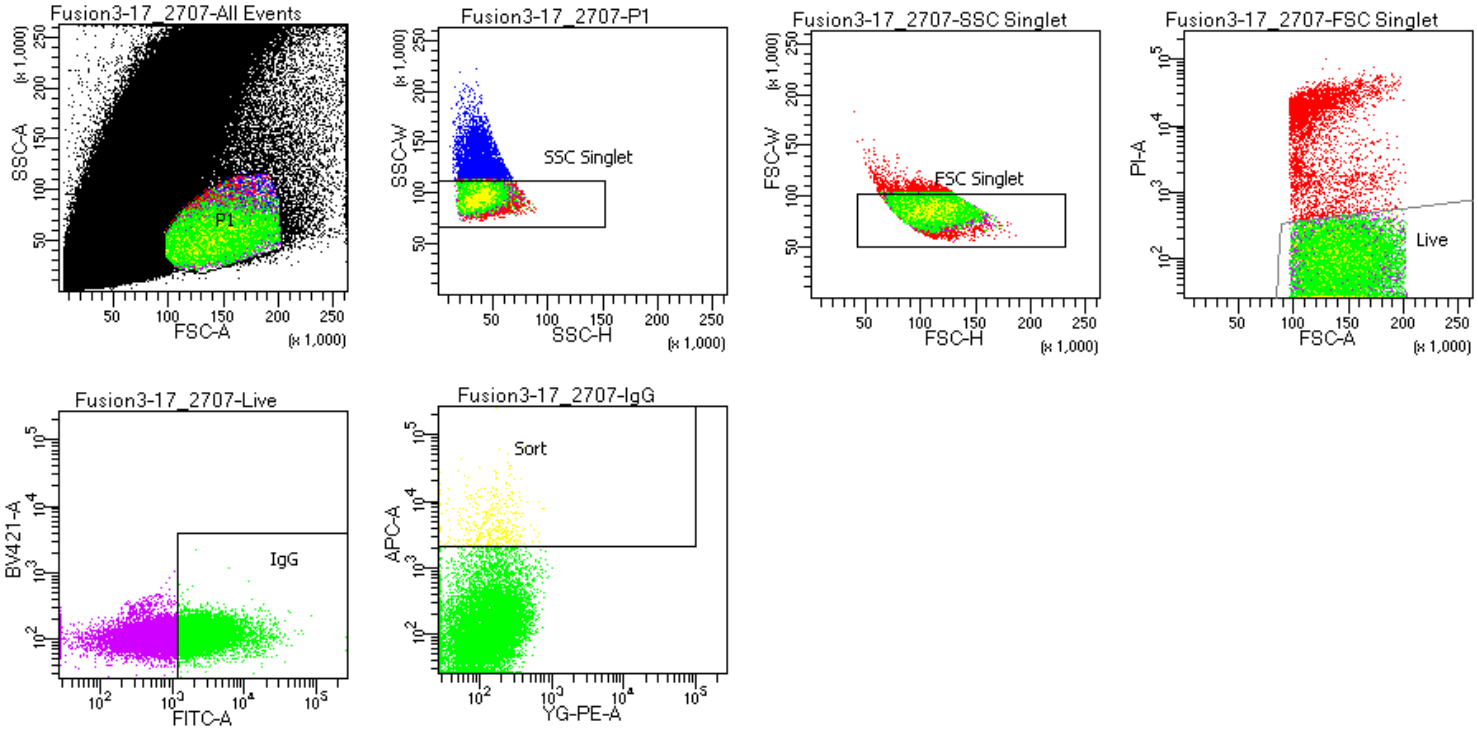
Supplementary Figure 17. Naïve SD rat B cell sort gating strategy. 1) FSC vs SSC to gate lymphocytes, 2/3) SSC-H/SSC-W, FSC-H/FSC-W gates to exclude cell doublets, 4) FSC/PI for dead cell exclusion (PI-negative gate), 5) CD8-PerCP Cy5.5/Dump-BV510 (CD4, CD11b, CD161a, granulocyte marker) to exclude CD8+BV510+ non-B cells, 6) IgM-PECy7/CD45RA-APC Cy7 to sort for B cells.



Tube: sample

Population	#Events	%Parent	%Total
All Events	4,225,000	####	100.0
Cell	1,328,095	31.4	31.4
SSC Singlet	1,304,252	98.2	30.9
FSC Singlet	1,287,995	98.8	30.5
live	1,194,887	92.8	28.3
dump negative	1,141,436	95.5	27.0
CD8+	1,391	0.1	0.0
P4	1,013,025	88.8	24.0
IgM-	39,402	3.9	0.9
sort	4,356	11.1	0.1

Supplementary Figure 18. Immunized SD rat, OVA^{pos}IgM^{neg} B cell sort gating strategy. 1) FSC vs SSC to gate lymphocytes, 2/3) SSC-H/SSC-W, FSC-H/FSC-W to exclude cell doublets, 4) FSC/PI for dead cell exclusion (PI-negative gate), 5) CD8-PerCP Cy5.5/Dump-BV510 (CD4, CD11b, CD161a, granulocyte marker) to exclude BV510+ non-B cells, 6) CD45R-APC Cy7/CD8-PerCP Cy5.5 to exclude CD8+ cells, 7) IgM-PE Cy7/CD45R-APC Cy7 to gate for IgM^{neg} B cells, 8) CD45R-APC Cy7/OVA-APC to sort for OVA^{pos}IgM^{neg} B cells.



Tube: 17_2707

Population	#Events	%Parent	%Total
All Events	4,875,000	####	100.0
P1	36,297	0.7	0.7
SSC Singlet	32,718	90.1	0.7
FSC Singlet	31,846	97.3	0.7
Live	27,820	87.4	0.6
IgG	13,787	49.6	0.3
Sort	429	3.1	0.0
NOT(P1)	4,838,703	99.3	99.3

Supplementary Figure 19. Immunized SD rat, OVA^{pos}IgG^{pos} hybridoma sort gating strategy. 1) FSC vs SSC to gate hybridoma cells, 2/3) SSC-H/SSC-W, FSC-H/FSC-W gates to exclude cell doublets, 4) FSC/PI for dead cell exclusion (PI-negative gate), 5) IgG FITC/BV421 (empty channel) to gate for IgG^{pos} hybridomas, 6) PE (empty channel)/OVA-APC to sort for OVA^{pos}IgG^{pos} hybridomas.

PCR primers (reverse strand, constant region)

Rat CH1, outside PCR primer: YYTKGACCAGGCAKCCCAKDGTCAC
Rat CH1, inside PCR primer: CCAGGAGCCAGTGGATAGAC
Rat Cκ, outside PCR primer: AGGCACCTCCAGTTGCTAAC
Rat Cκ, inside PCR primer: CAGTTGGTGCAGCATCAGCCCG

Mouse CH1, outside PCR primer: GGGAARTAVYCCTTGACCAGGCABCC
Mouse CH1, inside PCR primer: GRCCARKGGATAGACHGATG
Mouse Cκ, outside PCR primer: GACTGAGGCACCTCCAGATG
Mouse Cκ, inside PCR primer: CTGCTCACTGGATGGTGGGAAG

List of additional Rat VH germlines not in IMGT database

>rIGHV2U2 ; wgs
CAGGTGCAGCTGAAGGAGTCAGGACCTGGTCTGGTGCAGCCCTCAGAGACCCTGTCCCTCACCTGCACTGTCTCTGGGTTCTCATT
AACCAGCAATAGTGTACACTGGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGGATGGGAATAATACGGAGTGGTGGAAGCA
CAGATTATAATTCAGCTCTCAAATCCCGACTGAGCATCAGCAGGGACACCTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTG
CAAACTGAAGACACAGCCATTTACTACTGTACCAGA

>rIGHV2U3 ; wgs
CAGGTGCAGCTGAAGGAGTCAGGACCTGGTCTGGTGCAGCCCTCACAGACCCTGTCCCTCACCTGCACTGTCTCTGGATTCTCATT
AACCAGCTATCATGTAAGCTGGGTTTGCCAGCCTCCAGGAAAGGGTCTGGAGTGGATGGGAAGAATACAGAGTGGTGGAAGCAC
AGATTATAATTCAGCTCTCAAATCCCGACTGAGCATCAGCAGGGACACCTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGC
AAACTGAAGACACAGCCATTTACTACTGTACCAGAGA

>rIGHV2U4 ; wgs
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AACCAGCTATCATGTAAGCTGGGTTCGCCAGCCTCCAGGAAAAGGTCTGGAGTGGATGGGAGTAATATGGACTGGTGGAAGCAC
AGCATATAATTCACTTCTCAAATCCCGACTGAGCATCAGCAGGGACATCTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGC
AAACTGAAGACACAGCCACTTACTACTGTGCCAGAGA

>rIGHV2U5 ; wgs
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CAGCATATAATTCACTTCTCAAATCCCGACTGAGCATCAGCAGGGACACCTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCAG
CAAACTGAAGACACAGCCATTTACTTCTGTACCAGA

>rIGHV2U6 ; wgs
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AAGCAGCTATGGTGTAATCTGGGTTCACCAGCCTCCAGGAAAGGGTCTGGAGTGGATGGGAGGAATATGGGGTGATGGAAGCA
CAGATTATAATTCAGCTCTCAAATCCCGACTGAGCATCAGCAGGGACACCTCCAAGAGTCAAGTTTTCTTAAAAATGAACAGTCTG
CAAACTGAAGACACAGCCATTTACTTCTGTACCAGA

>rIGHV2U8 ; wgs
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AAACTGAAGACACAGCCATGTACTTCTGTGCCAGAAA

>rIGHV2U9 ; wgs

CAGGTACAACTGAAGGAGACAGGACCTGACCTAGTGCAACTGACACAGACCCTGTCCATCACATGCACTGTCTCTGGGTTCTCATT
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AAACTGATGACACAGCCAAGTACTTCTGTGCCAGAA

>rIGHV2U12 ; wgs
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AAACTGATGACACAGGCACTTACTACTGTACCAGAGA

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AACCAGCTATCATGTAAGCTGGGTTCGACAGCCTCCAGGAAAGGGTCTGGAGTGGATGGGAGTAATATGGGGTGATGGAAGCAC
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>rIGHV2U16 ; wgs
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CAAACTGAAGACACAGCCATTTACTTCTGTACCAGAGA

>rIGHV5U21 ; wgs
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CTGAGGTCTGAGGACACGGCCACTTATTACTGTACAACAGA

>rIGHV5U22 ; wgs
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TGAGGTCTGAGGACACGTCCACTTATTACTGTGCAAAAGACA

>rIGHV5U23 ; wgs
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GCACTTACTATCGAGACCCCGTGAAGGGCCGATTCACTATCTCCAGAGATAATGCAAAAAGCACCCTATACCTGCAAATGGACAG
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>rIGHV5U25 ; wgs
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GCACTTACTATCCAGATTCCGTGAAGGGCAGATTTACTATCTCCAGAGATAATGCAAAAAGCACCCTATACCTGCAAATGGACAGT
CTGAGATCTGAGGACACGGCCAGTTACTATTGTGCAAGACA

>rIGHV2U17d ; repertoire NGS
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AACCAGCAATAGTGTACACTGGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGGGTTGGAGCAATATGGAGTGGTGGAAGCAC
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>rIGHV2U18d ; repertoire NGS
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CACCAGCTATGGTGTAAGCTGGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGGATTGCAGCAATATGGAGTGGTGGAAGTAC
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AAACTGAAGACACAGCCATGTACTTCTGTGCCAGA

>rIGHV3U1d ; repertoire NGS
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>rIGHV5U27d ; repertoire NGS
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>rIGHV5U28d ; repertoire NGS
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>rIGHV5U29d ; repertoire NGS
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>rIGHV5U30d ; repertoire NGS
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>rIGHV5U31d ; repertoire NGS
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List of additional Rat Vκ germlines not in IMGT database

>rIGKV1U5 ; wgs
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