

Phenotypic determinism and stochasticity in antibody repertoires of clonally expanded plasma cells

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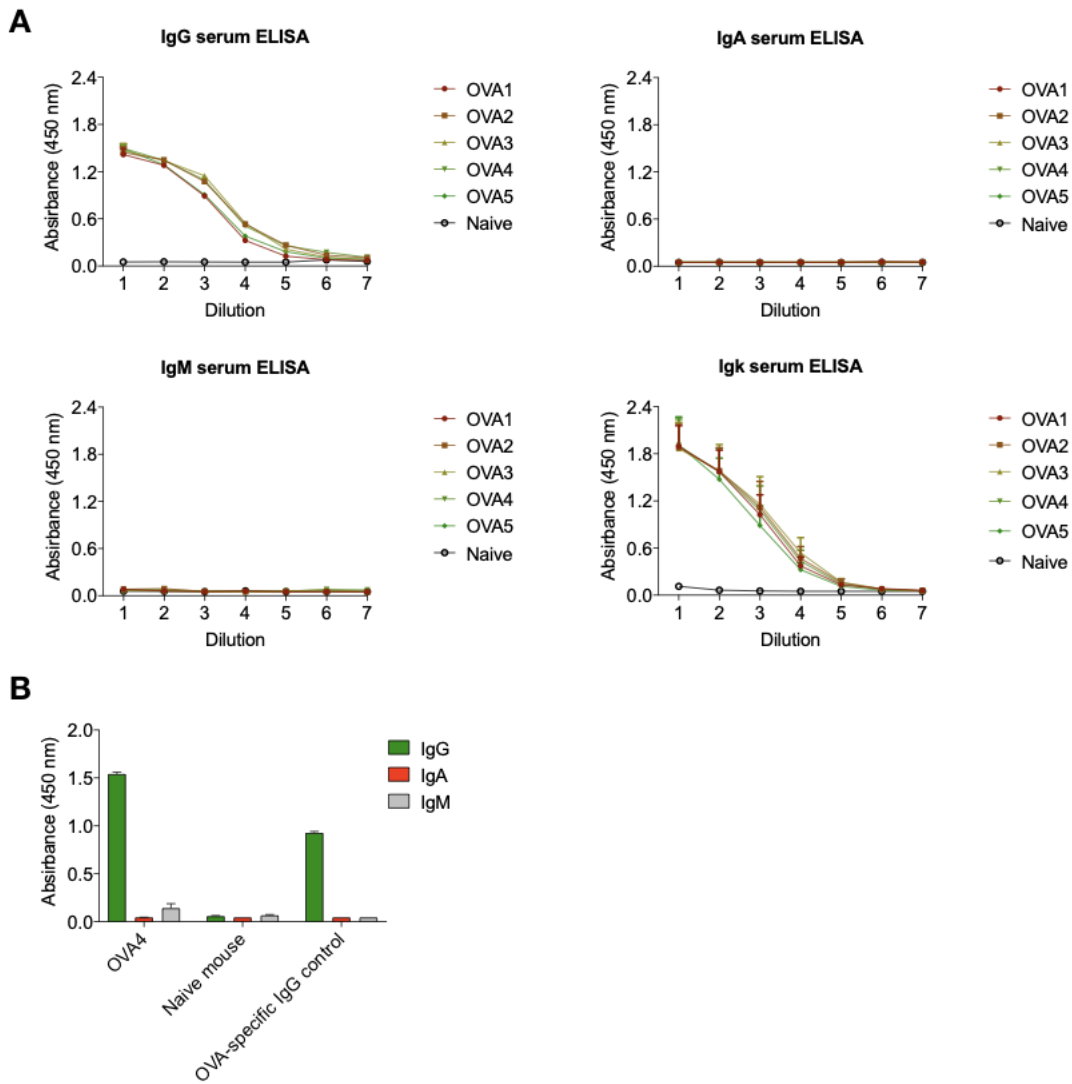
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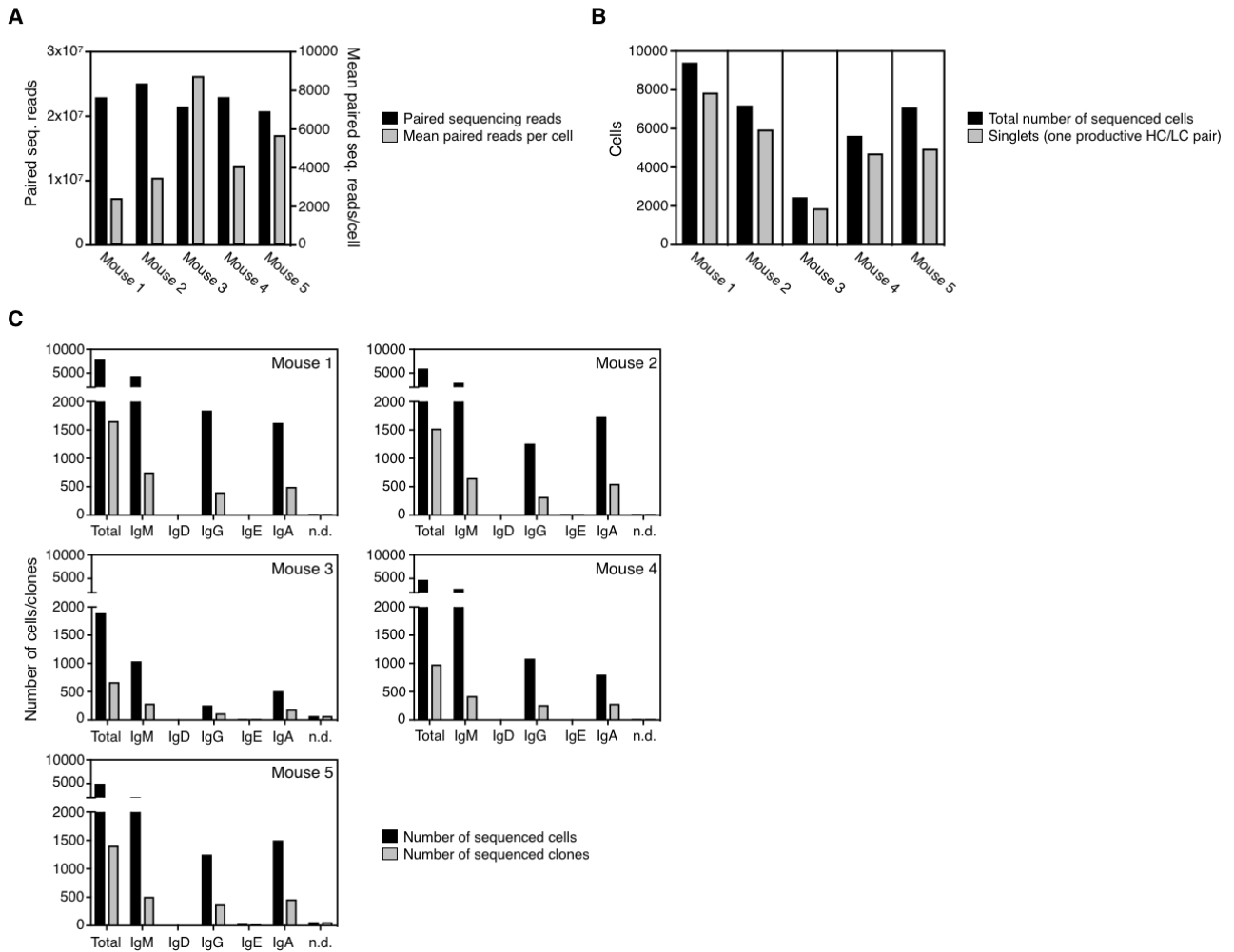
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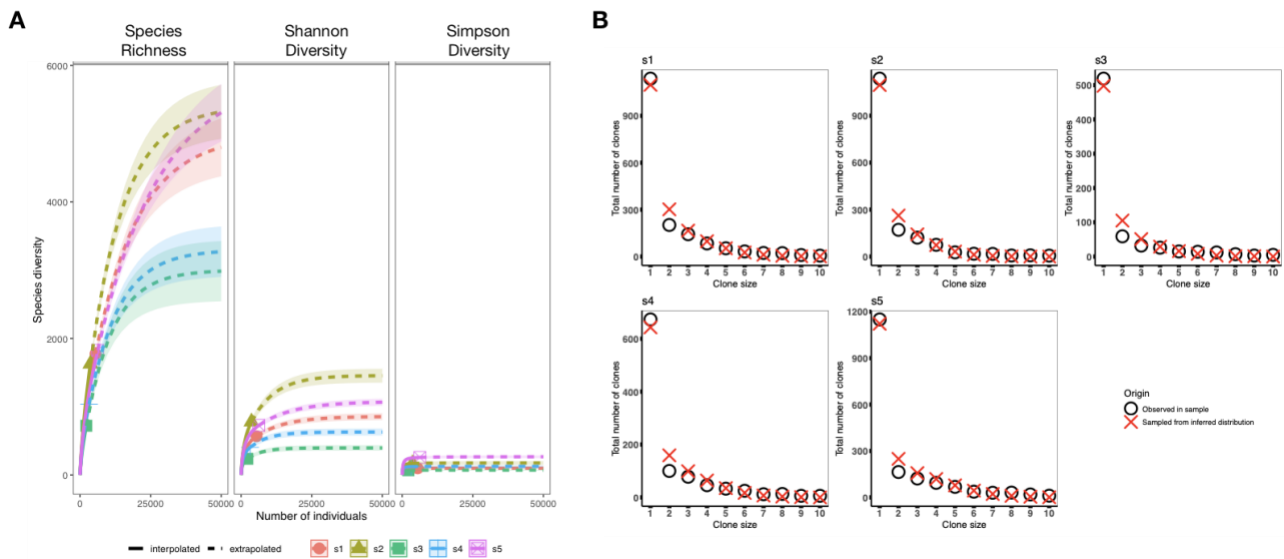
*Contributed equally to this work



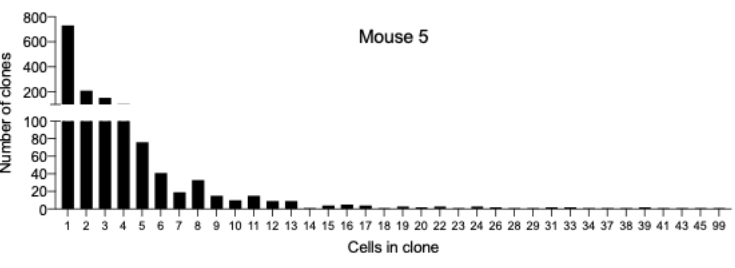
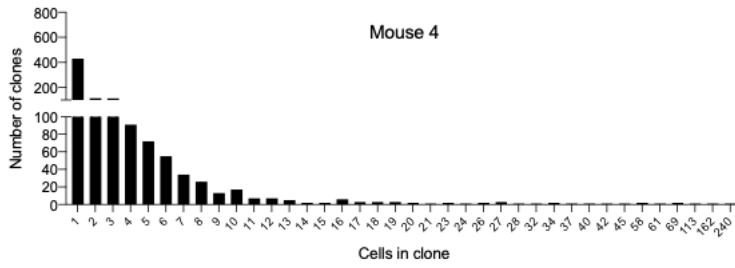
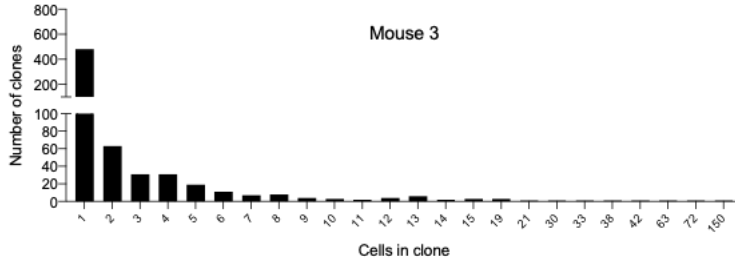
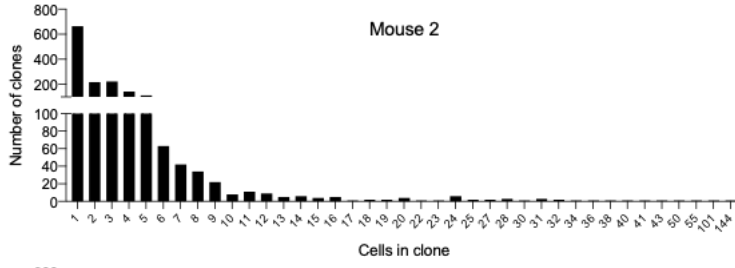
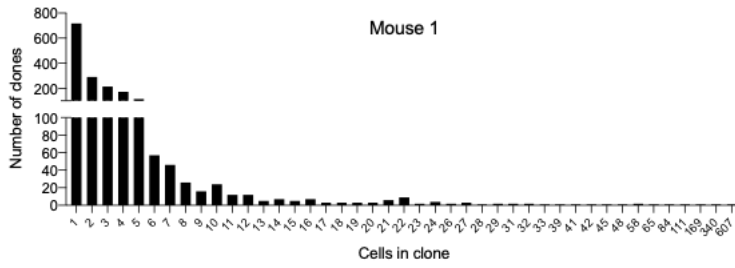
Extended Data Figure 1. Isotype-specific enzyme-linked immunosorbent assay (ELISA) on serum. A. OVA-specific ELISA was performed using IgG, IgA, IgM, and Igk detection antibodies. Serum was incubated with 1:5 serial dilutions of pre-diluted (1:100) serum. Unimmunized (naïve) serum served as control. B. OVA-specific ELISA on serum using 1:20 dilution. Supernatant from an IgG2c-expressing OVA-specific hybridoma cell line was used as a control.

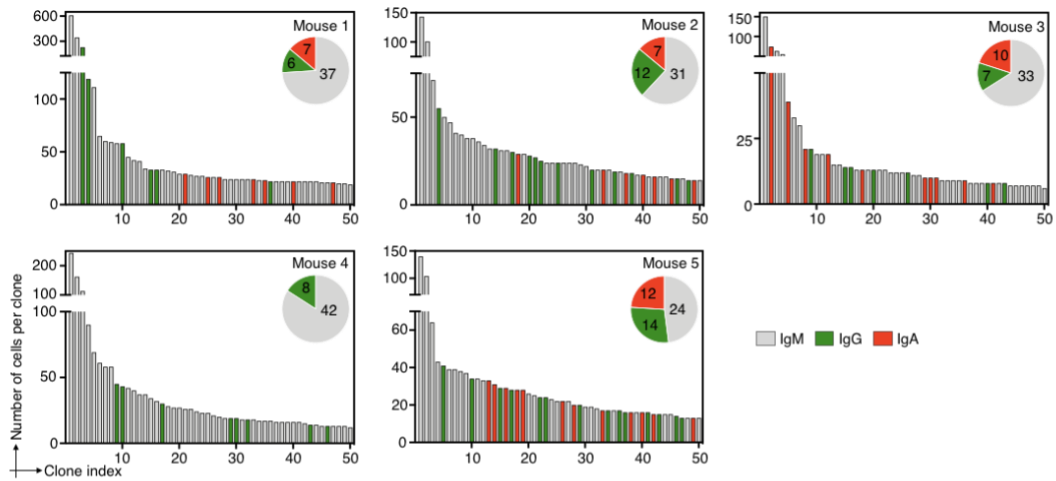
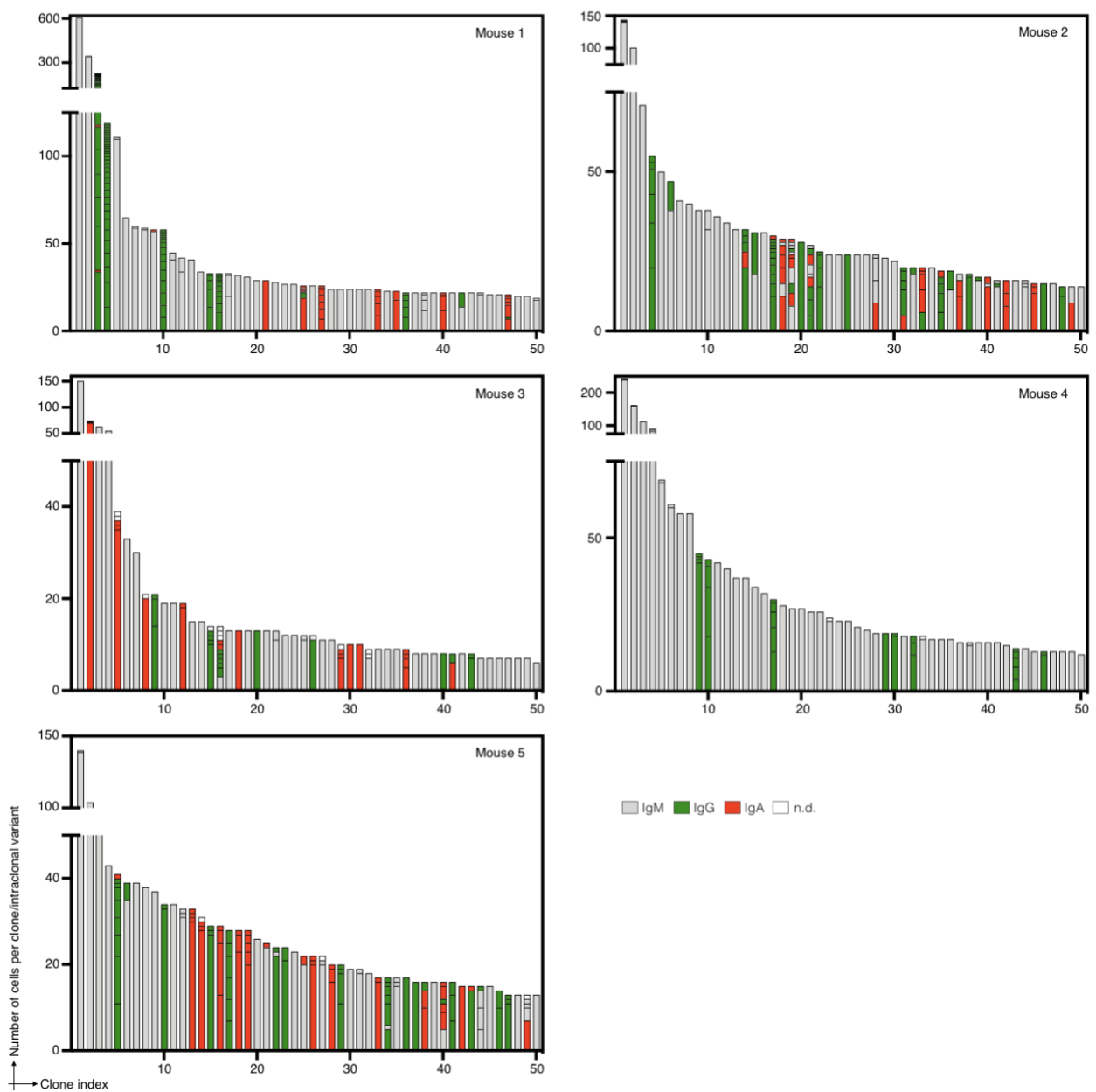


Extended Data Figure 2. Single-cell sequencing statistics. **a.** Total number of paired sequencing reads per mouse (black, left axis) and mean paired sequencing reads per cell (grey, right axis) for all mice. **b.** Number of all sequenced cells per mouse (black) and number of cells with one productive, full-length heavy- and light-chain pair (grey). **c.** Number of sequenced productive cells (black) and clones (grey) with isotype resolution for each mouse. Clonotype definition is based on unique CDRH3-CDRL3 amino acid combination.



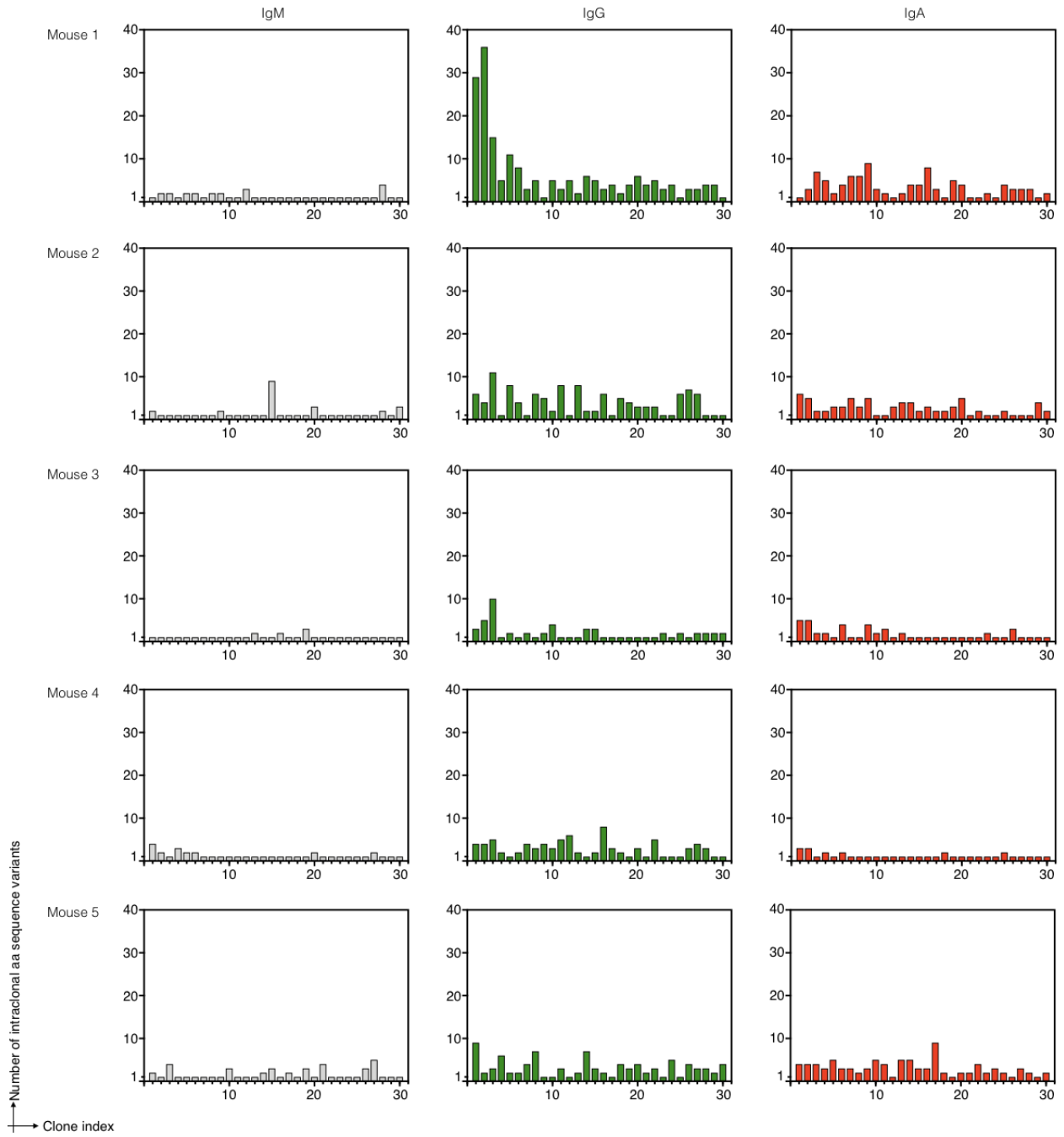
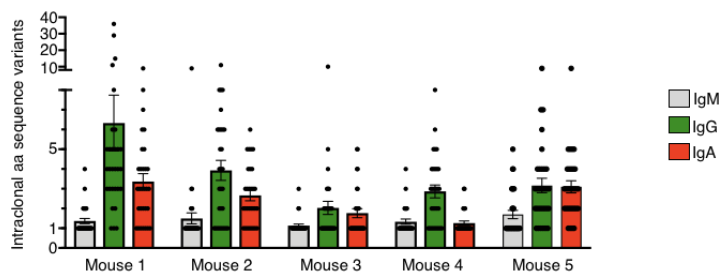
Extended Data Figure 3. Rarefaction and sampling estimates of profiled repertoire. **a.** Investigation of how under sampling the number of cells influences various species diversity metrics (species richness, Shannon diversity, Simpson diversity) for each immunized repertoire (s1-s5, indicated by color). Species richness refers to the number of unique clones. Dotted line indicates extrapolated diversity when sampling to 50,000 cells whereas solid line indicates sampled diversity. **b.** Recon estimates of the number of missing clones from each individual repertoire (s1-s5). Clone size refers to the number of cells within each clone. Recon suggests that sampled repertoire and predicted repertoire converge for clone sizes above approximately clone size = 2.



A**B**

Extended Data Figure 5. Clonal expansion profiles for the 50 most expanded clones per mouse. a. Clonal expansion profiles based on isotype majority. Isotypes are indicated in grey (IgM), green (IgG) and red

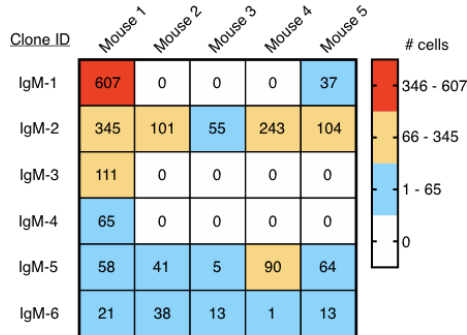
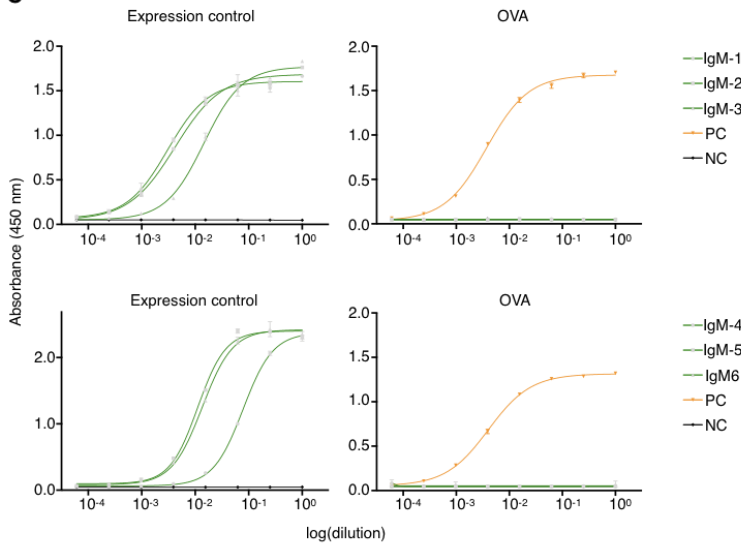
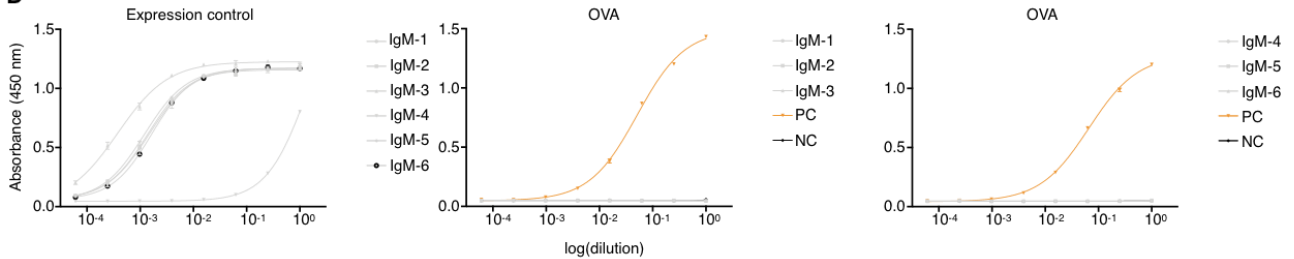
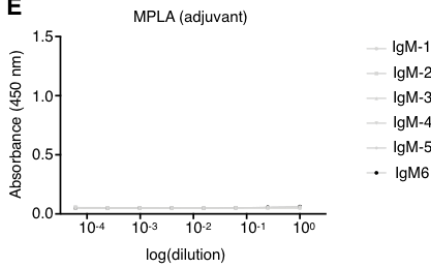
(IgA) respectively. Pie-chart inset indicates the numbers of isotype clones among the top 50 clones shown. **b.** Clonal expansion profiles indicating all clonal amino acid sequence variants per clone and their respective isotype assignment. Separate clonal sequence variants are shown in stacked bar plots ordered by their respective size from bottom to top. Whenever a smaller sized bar of different isotype origin intersects two bigger bars, this indicates that these cells belong to the clonal variant below. Coloring scheme according to **a** with unassigned isotype cells shown in white.

A**B**

Extended Data Figure 6. Intraclonal amino acid sequence variants for the 30 most expanded clones per isotype. a. Total number of intraclonal amino acid sequence variants per individual clone per isotype for all mice. **b.** Mean number of intraclonal amino acid sequence variants per isotype for all mice.

A

ID	Clone rank IgM	# cells	V _H	CDRH3	V _L	CDRL3	nt dist. (aa-changes)
IgM-1	1, public (2 mice)	607	IGHV3-1	CATMITTWFAYW	IGKV3-2	CQQSKEVPWTF	0
IgM-2	2, public (5 mice)	345	IGHV11-2	CMRYGNYWYFDVW	IGKV14-126	CLQHGESPFTF	0
IgM-3	3	111	IGHV12-3	CAGDRWGYWYFDVW	IGKV4-91	CQQGSSIPYTF	0
IgM-4	4	65	IGHV1-64	CANWDYW	IGKV5-48	CQQSNSWWTF	1 (0)
IgM-5	5, public (5 mice)	58	IGHV7-1	CARDNWDWYFDVW	IGKV15-103	CQQGQSYPLTF	0
IgM-6	27-33, public (5 mice)	21	IGHV7-1	CARDDYDWYFDVW	IGKV15-103	CQQGQSYPLTF	0

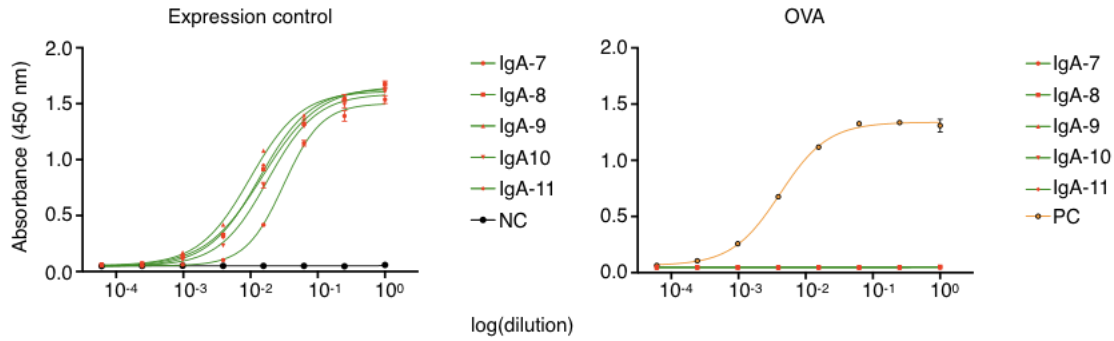
B**C****D****E**

Extended Data Figure 7. Profiling of expanded IgM clones of MS-1. **a.** Characteristics of chosen clones. **b.** Heatmap indicating the number of cells per mouse with identical clonal CDRH3-CDRL3 amino acid sequence per chosen clone. Clone ID follows **a.** **c.** Sandwich ELISA results of 3-fold serially diluted stable hybridoma cell culture supernatant of engineered cell lines expressing selected clones as IgGs. Plots on the left indicate IgG expression levels and plots on the right show binding to OVA. For each sample, two technical

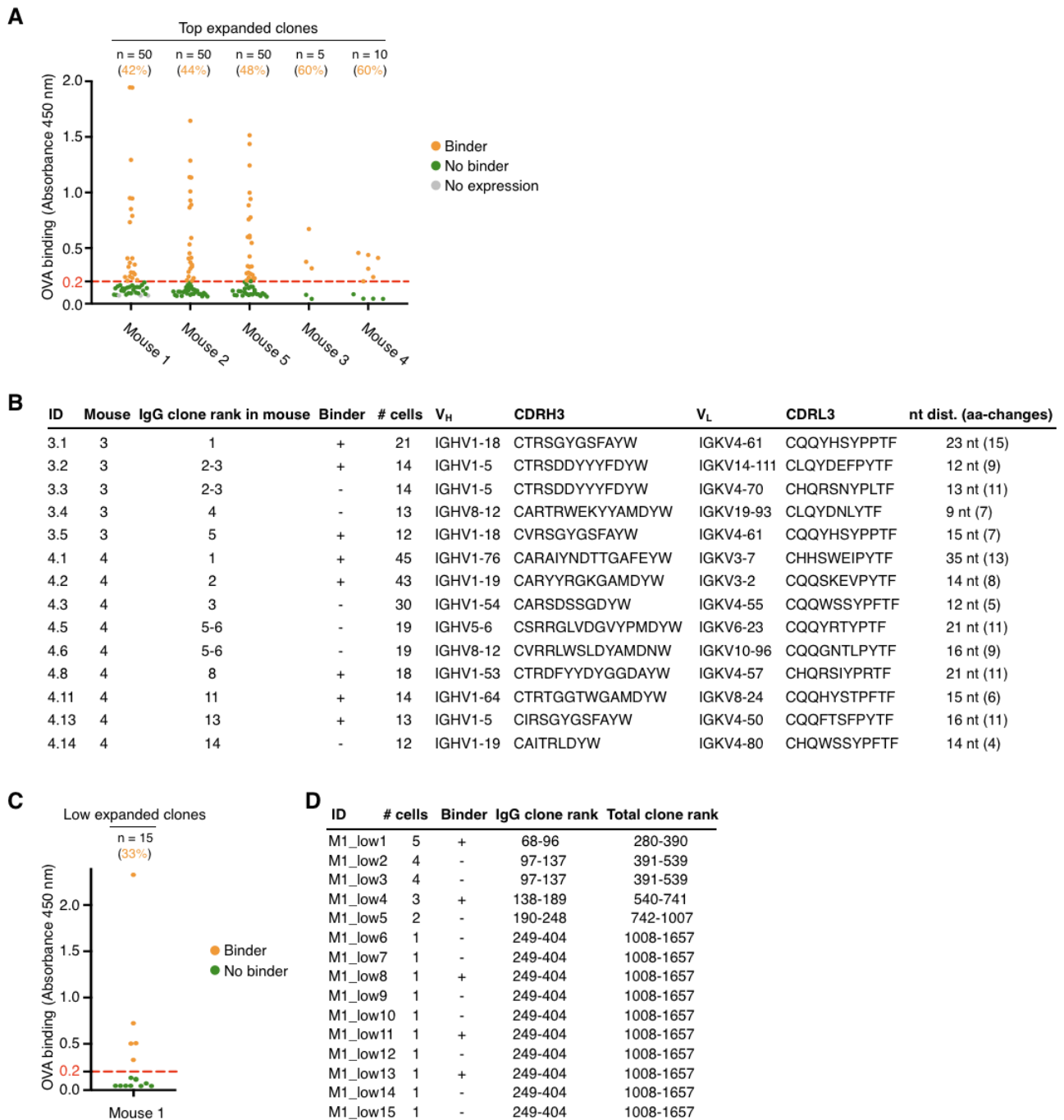
replicates were analysed and a four-parameter logistical curve was fitted to the data by nonlinear regression. Data are presented as the mean and error bars indicate standard deviation. Supernatant of a hybridoma cell line that does not express antibody served as negative control (NC, black) for the expression ELISA (left) and supernatant of an OVA specific inhouse cell line was used as positive control (PC, orange) for the antigen ELISA (right). **d.** Sandwich ELISA results of 3-fold serially diluted transient HEK-293 cell culture supernatant of cells expressing selected clones as IgM following **c.** Supernatant of IgM reformatted RSVF specific inhouse antibody served as negative control (NC, black) and supernatant of IgM reformatted positive clone from **c** served as positive control (PC, orange). **e.** Sandwich ELISA results on transient HEK-293 cell culture supernatant of cells expressing selected clones as IgM for binding to MPLA adjuvant.

A

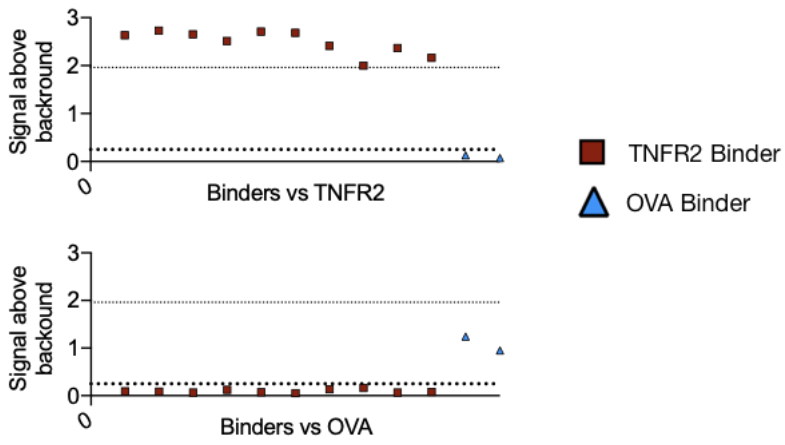
ID	Clone rank	IgA	# cells	V _H	CDRH3	V _L	CDRL3	nt dist. (aa-changes)
IgA-7	1		29	IGHV1-22	CVRRGSPDYYFDYW	IGKV5-43	CQQSNSWPLTF	24nt (18)
IgA-8	2-3		26	IGHV1-53	CARSLYDYGAYW	IGKV10-94	CQQYSKLPRTF	8nt (1)
IgA-9	2-3		26	IGHV3-1	CARSGTTPDYW	IGKV6-15	CQQYNSYPLTF	22nt (8)
IgA-10	5		23	IGHV7-3	CPREYFGSFAYW	IGKV15-103	CQQGQSYPWTF	12nt (9)
IgA-11	7		21	IGHV3-1	CARGGNYPYFDYW	IGKV4-61	CQQYHSYPPTF	22nt (15)

B

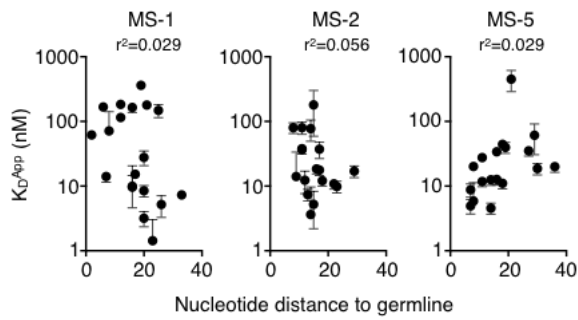
Extended Data Figure 8. Profiling of expanded IgA clones of MS-1. **a.** Characteristics of chosen clones. **b.** Sandwich ELISA results of 3-fold serially diluted stable hybridoma cell culture supernatant of engineered cell lines expressing selected clones as IgGs. Plot on the left indicates IgG expression levels and plot on the right shows binding to OVA. For each sample, two technical replicates were analysed and a four-parameter logistical curve was fitted to the data by nonlinear regression. Data are presented as the mean and error bars indicate standard deviation. Supernatant of a hybridoma cell line that does not express antibody served as negative control (NC, black) for the expression ELISA (left) and supernatant of an OVA specific inhouse cell line was used as positive control (PC, orange) for the antigen ELISA (right).



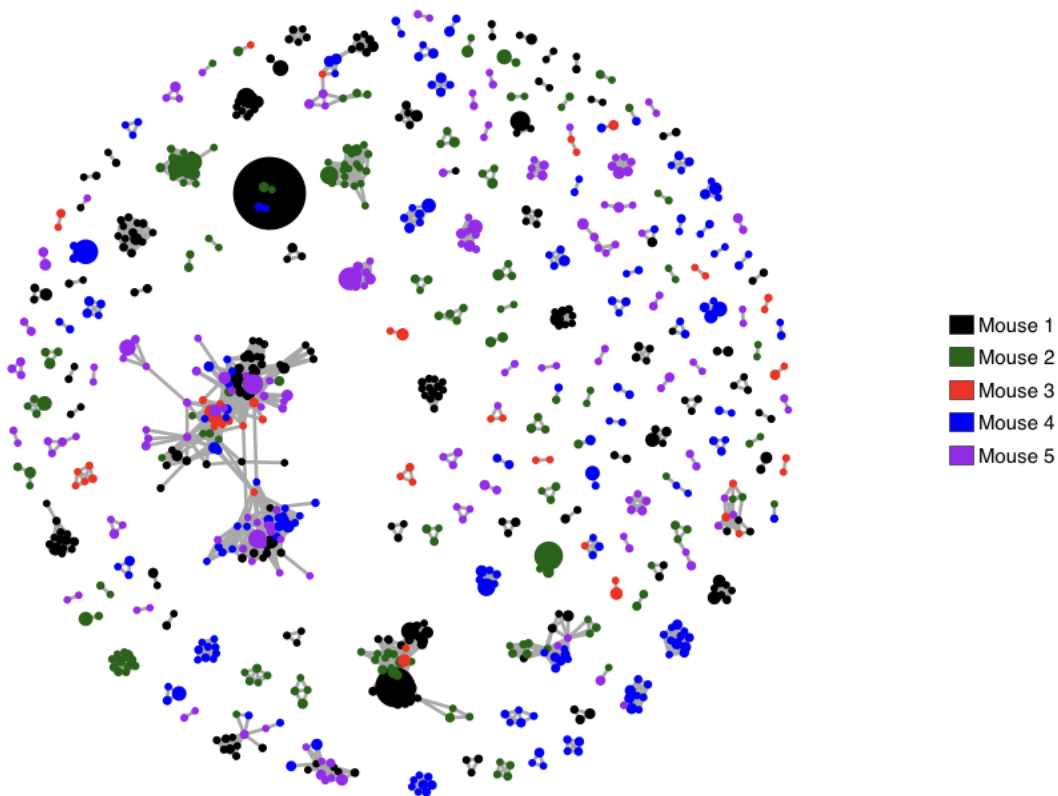
Extended Data Figure 9. Profiling of top and low expanded IgG clones per mouse. **a.** ELISA profiling results of all top expanded clones tested per mouse. Clones were denoted binders if ELISA signal was >0.2 (3-fold above background; red dotted line). Binders are shown in orange, whereas clones that did not bind OVA or could not be expressed are shown in green and grey respectively. **b.** Overview of tested top expanded clones of MS-3 and -4. + denotes ELISA signal >0.2 . **c.** ELISA profiling results of all low expanded MS-1 clones tested. **d.** Table indicating binding of low expanded clones as well as number of cells and corresponding IgG and total clone ranks within MS-1 repertoire.



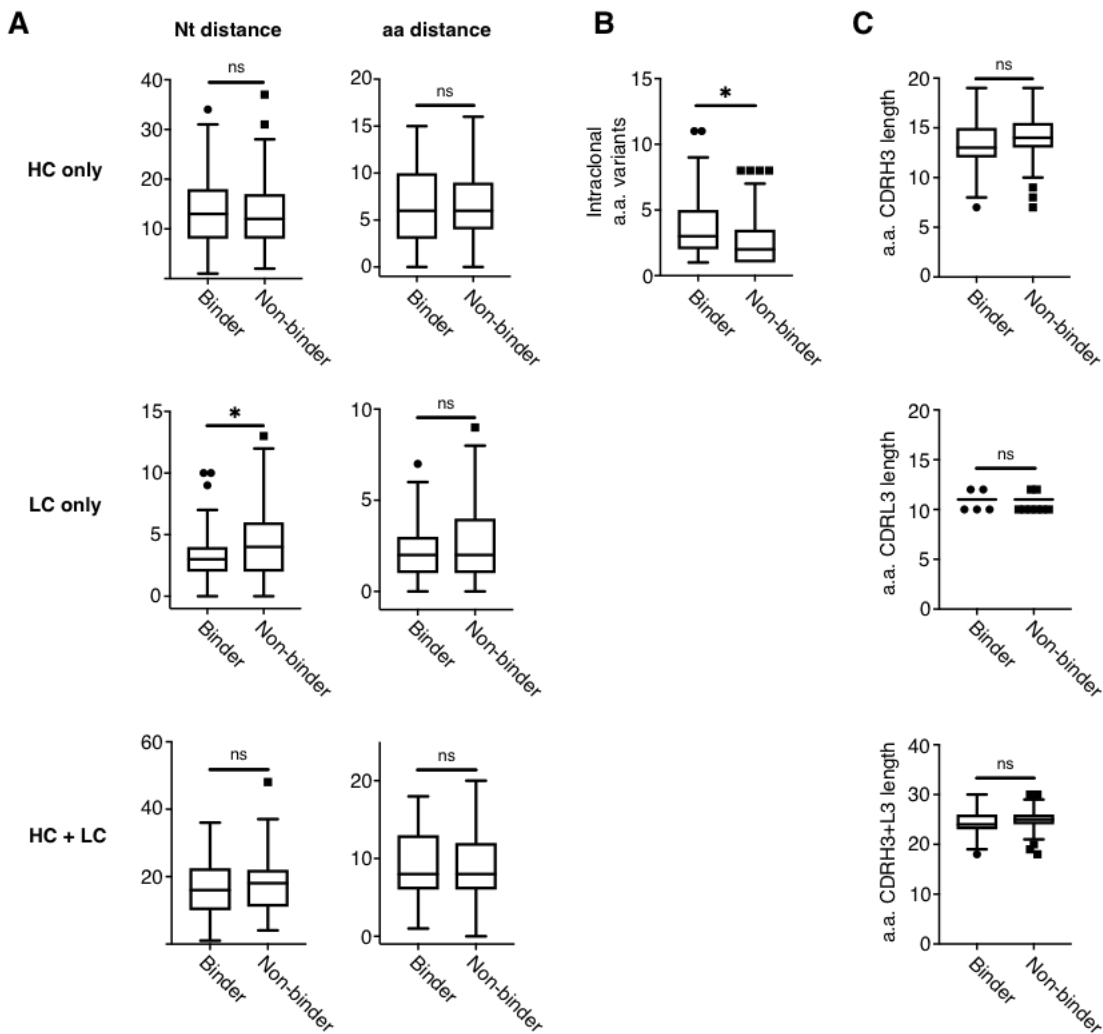
Extended Data Figure 10. Profiling of antibodies with known specificity against OVA. ELISA profiling results of ten antibodies with known specificity to human TNFR2 compared to ten antibodies determined as OVA-specific.



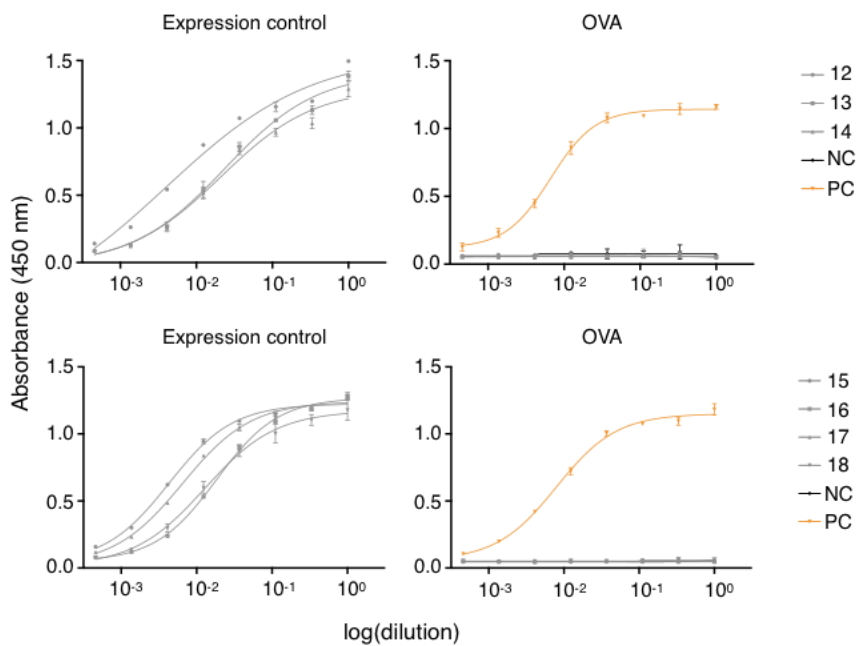
Extended Data Figure 11. Correlation between apparent dissociation constant (K_D^{App}) and nucleotide distance to germline of clones shown in Fig. 2a, b.



Extended Data Figure 12. IgG similarity network. Global IgG similarity network plot for all IgG clones across all mice. Edges represent sequence nodes separated by edit distance of less than four a.a. Only those nodes with at least one edge are plotted for visualization purposes. Clones from different mice are indicated in different colors respectively. Extent of clonal expansion per clone is reflected by the size of the nodes.



Extended Data Figure 13. Selected metrics for binder- and non-binder pools of clones. a. Left: nucleotide distance to germline for heavy- and light-chain only as well as across heavy- and light-chain combined. Top: ns, not significant ($P=0.70$); middle: $*P=0.02$; bottom: ns, not significant ($P=0.64$); unpaired Student's t -test. Right: amino acid distance to germline for heavy- and light-chain only as well as across heavy- and light-chain combined. Top: ns, not significant ($P=0.77$); middle: ns, not significant ($P=0.11$); bottom: ns, not significant ($P=0.69$); unpaired Student's t -test. Analysis encompassed 174 experimentally verified sequences (79 binder and 95 non-binder) provided in **Extended Data Table 1** and **2** respectively. **b.** Number of intracлонаl amino acid sequence variants. $*P=0.02$, unpaired Student's t -test. Analysis contained all sequences provided in **Extended Data Table 1** and **2** except for singlet clones (76 binder and 87 non-binder). **c.** CDR3 amino acid length for CDRH3 and CDRL3 as well as CDRH3/L3 combined. Top: ns, not significant ($P=0.44$); middle: ns, not significant ($P=0.49$); bottom: ns, not significant ($P=0.49$); unpaired Student's t -test. Analysis encompassed experimentally verified sequences (79 binder and 95 non-binder) provided in **Extended Data Table 1** and **2**.

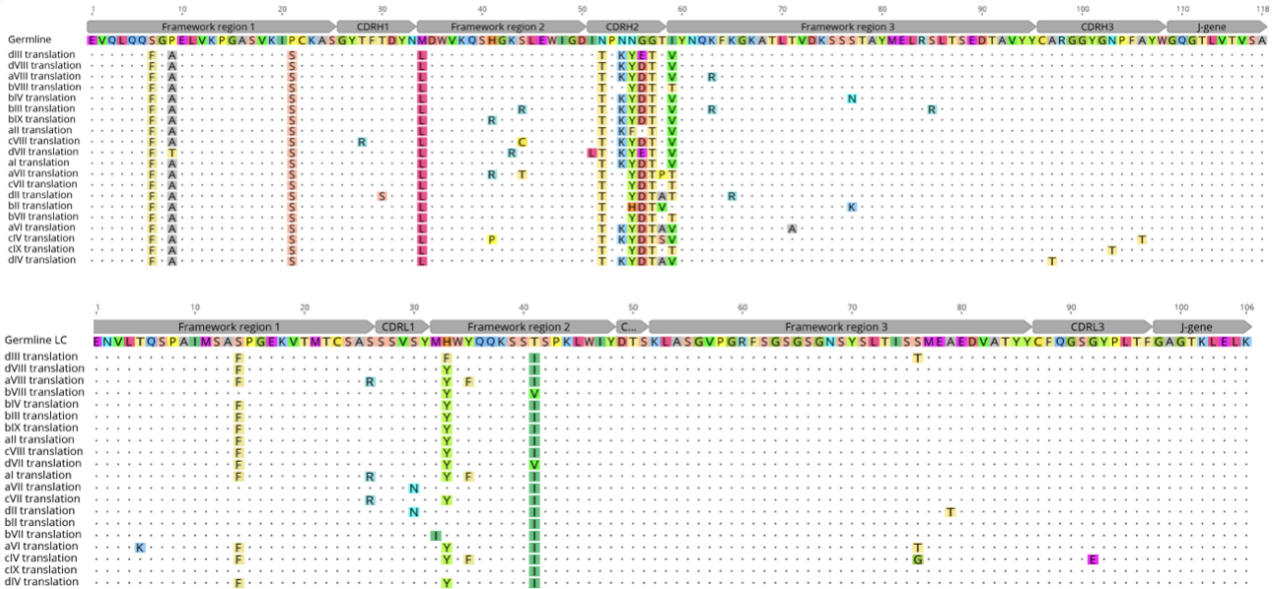


Extended Data Figure 14. ELISA screening of multi-isotype clones shown in Fig. 2g. Sandwich ELISA results of 3-fold serially diluted stable hybridoma cell culture supernatant of engineered cell lines expressing selected clones as IgGs. Plots on the left indicate IgG expression levels and plot on the right shows binding to OVA. For each sample, two technical replicates were analysed and a four-parameter logistical curve was fitted to the data by nonlinear regression. Data are presented as the mean ($n = 2$ measurements) and error bars indicate standard deviation. Supernatant of a HEL specific hybridoma cell line served as negative control (NC, black) and supernatant of an OVA specific inhouse cell line was used as positive control (PC, orange).

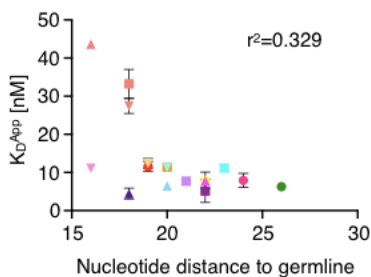
A

ID.	# cells	K_D^{App} [nM]	nt dist. (aa)	VH/VL	CDRH3/L3	Mouse
Germline		n.d.	0 nt (0)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	
aIX	1	315.6 ± 93.9	8 nt (6)	IGHV1-18/IGKV4-63	CARGGYGVPFAYW/CFQGNFYPLTF	2
aIII	4	8.9 ± 1.0	20 nt (10)	IGHV1-18/IGKV4-61	CTRGGYGTFFAYW/CQQYHSYPLTF	1
bX	3	4.0 ± 1.2	11 nt (6)	IGHV1-18/IGKV4-61	CARGGYGC-FAYW/CQQYHSYPLTF	5
bVIII	10	43.7 ± 0.6	16 nt (11)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
bVII	4	11.1 ± 0.2	16 nt (11)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
cVII	5	27.4 ± 2.0	18 nt (12)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
dII	5	11.3 ± 1.1	20 nt (15)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
aVII	7	33.2 ± 3.8	18 nt (14)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
cIX	1	23.3 ± 1.0	15 nt (11)	IGHV1-18/IGKV4-63	CARGGYGTPFAYW/CFQGSYPLTF	1
bII	3	12.4 ± 1.3	19 nt (11)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
aII	13	4.5 ± 1.4	18 nt (12)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
dVII	7	6.3 ± 0.9	26 nt (15)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
dIII	11	5.2 ± 3.0	22 nt (14)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
bIX	25	7.7 ± 0.4	21 nt (14)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
cIV	2	451.1 ± 36.4	24 nt (19)	IGHV1-18/IGKV4-63	CARGGYGNPFYTW/CFQGSYPLTF	1
aI	7	6.5 ± 0.7	20 nt (15)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
aVIII	14	11.1 ± 0.7	23 nt (16)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
aVI	1	7.9 ± 1.8	24 nt (17)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
dIV	1	6.4 ± 0.9	19 nt (15)	IGHV1-18/IGKV4-63	CTRGGYGNPFAYW/CFQGSYPLTF	1
bIII	9	7.9 ± 2.2	22 nt (16)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
cVIII	10	7.8 ± 0.3	22 nt (15)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
bIV	14	11.6 ± 1.3	19 nt (14)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1
dVIII	35	11.4 ± 0.6	20 nt (13)	IGHV1-18/IGKV4-63	CARGGYGNPFAYW/CFQGSYPLTF	1

B



Extended Data Figure 15. Characteristics of tested intraclonal sequence variants shown in Fig. 3b. a. Characteristics of tested variants. Differences in V_L gene usage and CDR3 amino acid sequence as well as sequences coming from different mice are indicated in red. **b.** Heavy (top) and light-chain (bottom) amino acid sequence alignment of clones with shared V/J genes. Sequence disagreements to germline are highlighted.

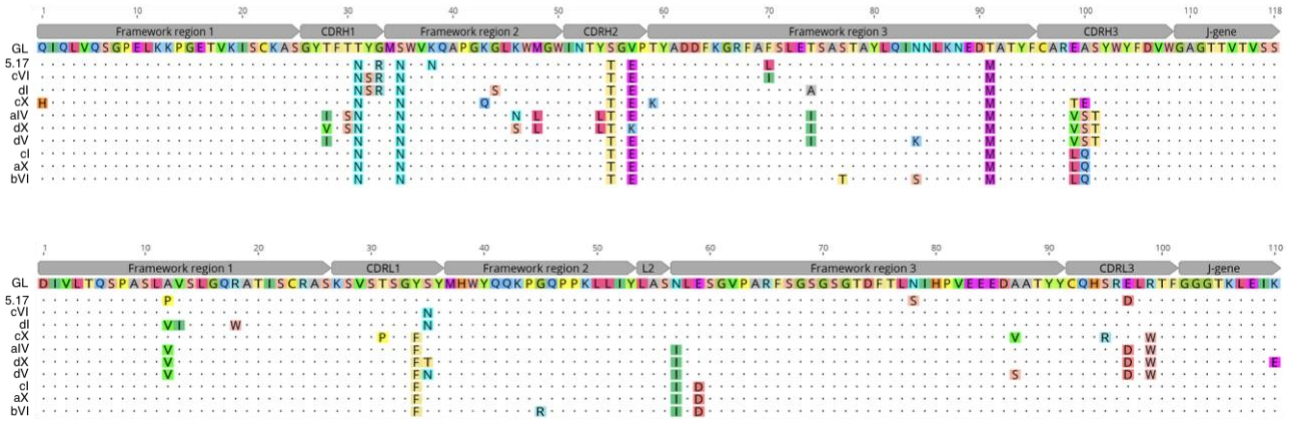


Extended Data Figure 16. Correlation between apparent dissociation constant (K_D^{App}) and nucleotide distance to germline. Error bars indicate standard deviation ($n = 3-5$ measurements of K_D).

A

ID	Mouse	# cells	V _H	CDRH3	V _L	CDRL3	nt dist. (aa-changes)	K _D ^{APP} [nM]
Germline			IGHV9-1	CAREASYWYFDVW	IGKV3-12	CQHSRELRTF	0 nt (0)	
cVI	5	3	IGHV9-1	CAREASYWYFDVW	IGKV3-12	CQHSRELRTF	15 nt (10)	n.d.
dl	5	2	IGHV9-1	CAREASYWYFDVW	IGKV3-12	CQHSRELRTF	21 nt (14)	n.d.
cX	2	3	IGHV9-1	CAR T ESYWYFDVW	IGKV3-12	CQH R EL W TF	25 nt (16)	7.7 ± 3.8
alV	4	2	IGHV9-1	CAR V STYWYFDVW	IGKV3-12	CQHS R DL W TF	41 nt (20)	3.4 ± 0.2
dX	4	2	IGHV9-1	CAR V STYWYFDVW	IGKV3-12	CQHS R DL W TF	46 nt (22)	3.8 ± 1.0
dV	4	2	IGHV9-1	CAR V STYWYFDVW	IGKV3-12	CQHS R DL W TF	29 nt (19)	26.0 ± 4.9
cl	5	5	IGHV9-1	CAR L QSYWYFDVW	IGKV3-12	CQHSRELRTF	21 nt (11)	11.9 ± 0.3
aX	5	8	IGHV9-1	CAR L QSYWYFDVW	IGKV3-12	CQHSRELRTF	21 nt (11)	14.4 ± 1.5
bVI	5	5	IGHV9-1	CAR L QSYWYFDVW	IGKV3-12	CQHSRELRTF	25 nt (14)	10.3 ± 1.6

B

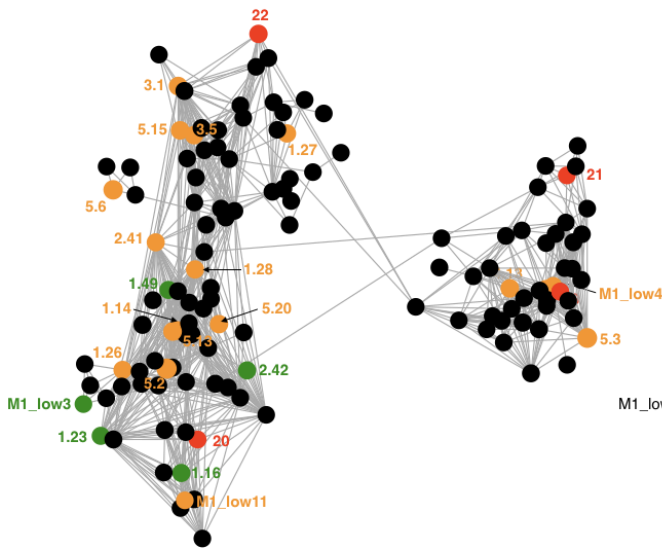


Extended Data Figure 17. Characteristics of tested clones shown in Fig. 3e-g. a. Characteristics of tested clones. Differences in CDR3 amino acid sequence are indicated in red. **b.** Heavy (top) and light-chain (bottom) amino acid sequence alignment of tested clones. Sequence disagreements to germline are highlighted.

A

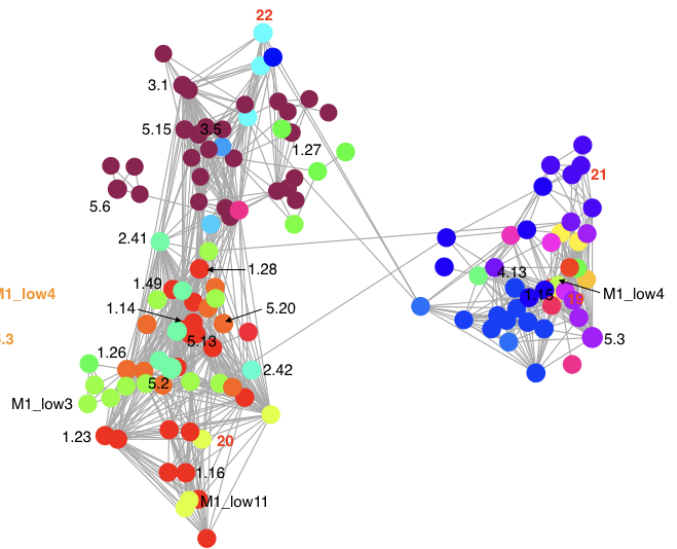
ID	Mouse	# cells	V _H	CDRH3	V _L	CDRL3	nt dist. (aa-changes)	K _D ^{APP} [nM]
19	5	1	IGHV1-15	CARGGYGSFAYW	IGKV4-57-1	CQQFTSSPPTF	22 nt (14)	
20	1	3	IGHV1-82	CARSGYGTGFAYW	IGKV4-55	CQQWNSYPWTF	19 nt (6)	n.d.
21	4	3	IGHV5-12	CARHGYGALAYW	IGKV4-50	CQQFTSLPPTF	14 nt (7)	8.9 ± 0.6
22	4	10	IGHV1-53	CTRGGYGGFAYW	IGKV4-61	CQQYHSYPWTF	29 nt (14)	11.7 ± 1.3

B



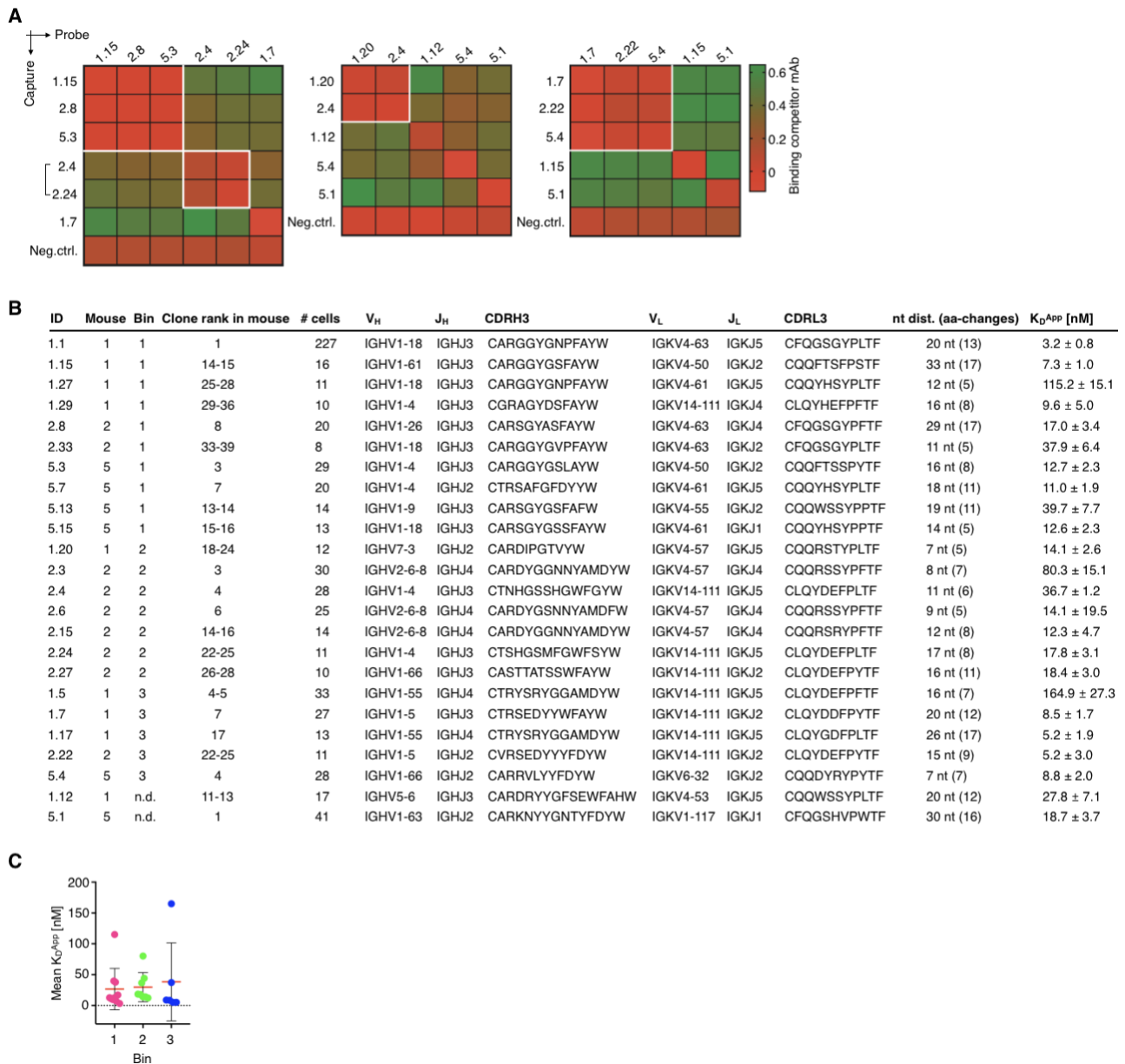
■ Not tested
 ■ Binder
 ■ No binder
 ■ Chosen clones

C

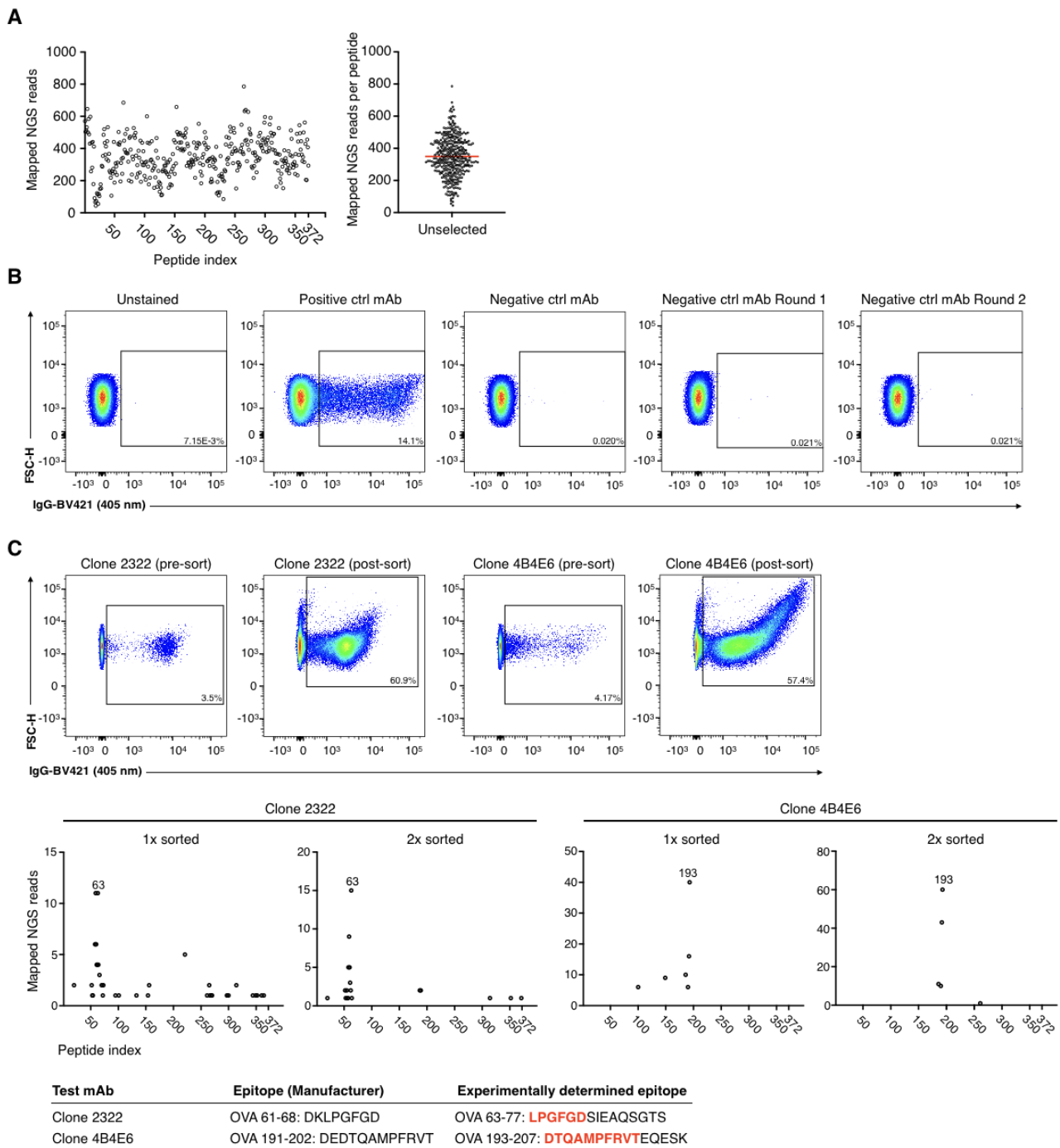


■ IGHV1-4/IGKV4-50 ■ IGHV1-15/IGKV6-32 ■ IGHV1-39/IGKV4-50 ■ IGHV1-55/IGKV4-61
 ■ IGHV1-4/IGKV4-59 ■ IGHV1-18/IGKV3-2 ■ IGHV1-42/IGKV4-50 ■ IGHV1-61/IGKV4-50
 ■ IGHV1-5/IGKV4-50 ■ IGHV1-18/IGKV4-50 ■ IGHV1-52/IGKV4-50 ■ IGHV1-64/IGKV4-61
 ■ IGHV1-9/IGKV4-50 ■ IGHV1-18/IGKV4-55 ■ IGHV1-53/IGKV4-50 ■ IGHV1-80/IGKV4-50
 ■ IGHV1-9/IGKV4-55 ■ IGHV1-18/IGKV4-61 ■ IGHV1-53/IGKV4-61 ■ IGHV1-80/IGKV4-55
 ■ IGHV1-9/IGKV4-61 ■ IGHV1-19/IGKV4-55 ■ IGHV1-54/IGKV4-50 ■ IGHV1-82/IGKV4-55
 ■ IGHV1-9/IGKV6-13 ■ IGHV1-26/IGKV4-55 ■ IGHV1-54/IGKV4-61 ■ IGHV1-84/IGKV4-50
 ■ IGHV1-15/IGKV4-57-1 ■ IGHV1-34/IGKV4-50 ■ IGHV1-54/IGKV10-94 ■ IGHV5-12/IGKV4-50

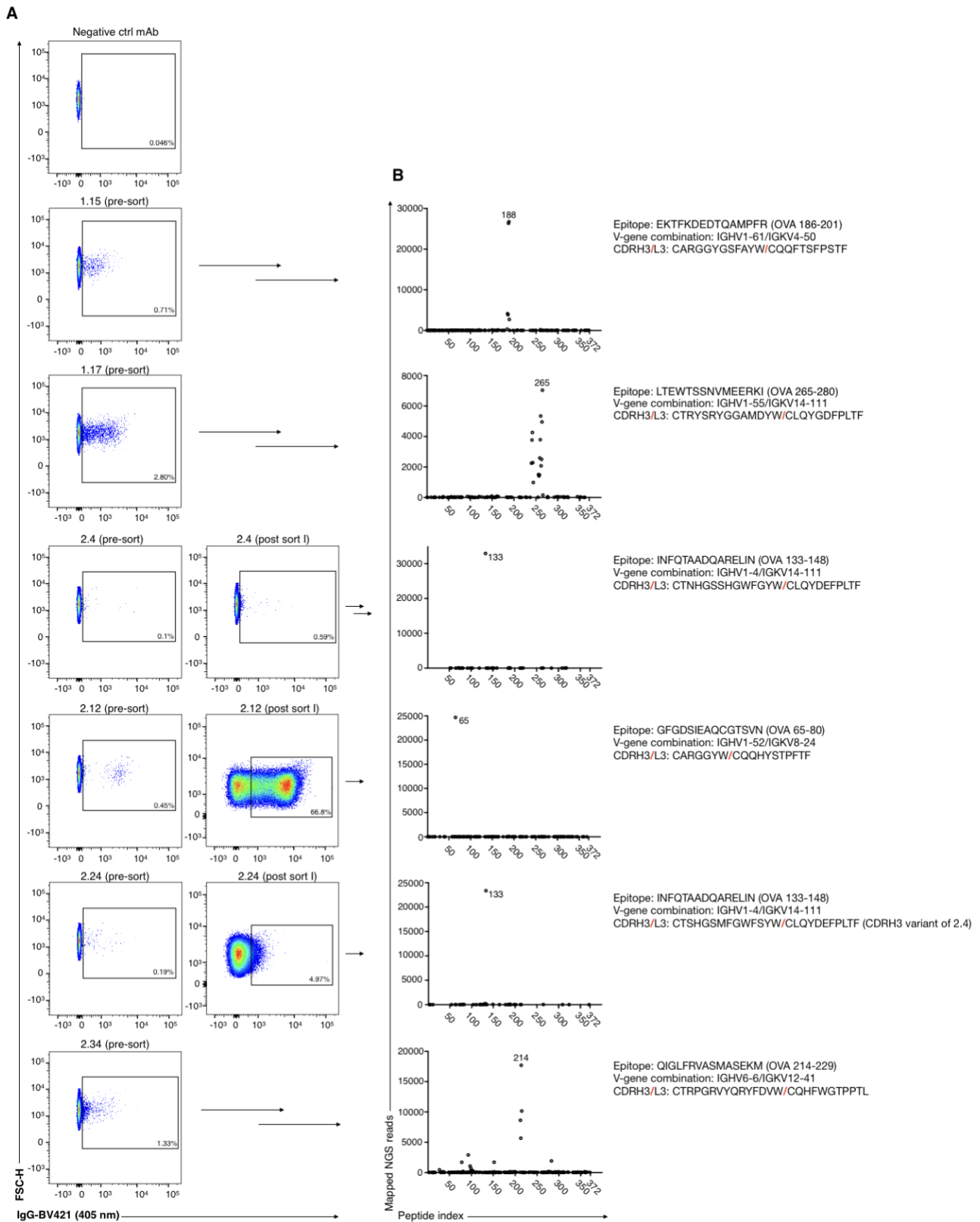
Extended Data Figure 18. Characteristics of tested clones shown in Fig. 3h-j. a. Characteristics of tested clones. **b.** Subnetwork plot of connected IgG clones from all mice shown in **Extended Data Fig. 9**. Edges represent clones separated by edit distance of three or less based on the concatenated CDR3 aa sequence. Binders, non-binders, not tested clones as well as newly chosen clones are shown in orange, green, black and red respectively. Indicated clone ID according to **Extended Data Table 1** and **2**. **c.** Identical network plot as in **b**. Color code indicates differential V_H-V_L gene usage as indicated.



Extended Data Figure 19. Cross-competition epitope binning between clones from different mice. a. Heatmaps show competitive antigen binding between top expanded, bin specific (according to **Fig. 4b**) clones from different mice. Antibodies indicated on the left were captured and probe antibodies on top were used to determine cross-competition for epitope access. Red indicates no binding of the probe antibody as a consequence of epitope blocking by the capture antibody, whereas green denotes binding of the competitor antibody. Groups of antibodies that target the same epitope (epitope bins) are highlighted in white squares. Brackets indicate clonal variants that share the same V_H/V_L as well as CDR3 length and only differ in their CDR3 amino acid sequence. An anti-RSVF capture antibody, which does not bind the antigen was used as negative control for all experiments. Clone ID according to **Extended Data Table 1**. **b.** Characteristics of bin-specific clones. **c** Apparent dissociation constant (K_D^{APP}) for binders separated by epitope bin.



Extended Data Figure 20. Establishment of a bacterial peptide display workflow for linear epitope mapping. **a.** Quality control of the unselected cloned OVA epitope library by NGS. All 372 peptide 15-mer windows (one amino acid offset) were observed (left) at comparable frequencies (right). Mean window occurrence for the respective NGS run is indicated in red. **b.** Flow cytometry dot plots show that secondary FACS antibody does not lead to unspecific enrichment after two rounds of enrichment. Unstained denotes no primary antibody. Positive and negative control primary mAb used were OVA specific mAb Clone 2322 (Chondrex, 7094) and an inhouse RSVF specific mouse IgG. **c.** Assay establishment with two OVA specific antibodies with known epitope specificity. Top: FACS dot plots show FACS enrichment of cells binding to commercial antibodies Clone 2322 (Chondrex, 7094) (left) and Clone 4B4E6 (Chondrex, 7096) (right). Middle: NGS results after one and two rounds of FACS enrichment. Bottom: experimentally determined epitope identity compared to epitope information provided by the manufacturer. Epitope overlap is indicated in bold red.



Extended Data Figure 21. Epitope mapping results of clones with linear epitope specificity as shown in Fig. 4e. a. Flow cytometry dot plots indicate FACS enrichment of positive clones for each antibody. **b.** Deep sequencing results of positive FACS output and epitope assignment for each antibody. Numbers correspond to the peptide index that was enriched the most. Only peptides with one or more occurrences are shown.

ID	Mouse	IgG clone rank in mouse	# cells	V _H	CDRH3	V _L	CDRL3	nt dist. (aa-changes)	K _D ^{APP} [nM]
1.1	1	1	227	IGHV1-18	CARGGYGNPFAYW	IGKV4-63	CFQGSYPLTF	20 nt (13)	3.2 ± 0.8
1.5	1	4-5	33	IGHV1-55	CTRYSTRYGGAMDYW	IGKV14-111	CLQYDEFFPFTF	16 nt (7)	164.9 ± 27.3
1.7	1	7	27	IGHV1-5	CTRSEDIYWFAYW	IGKV14-111	CLQYDDFPYTF	20 nt (12)	8.5 ± 1.7
1.11	1	11-13	17	IGHV1-74	CVLRLFYAMDYW	IGKV6-17	CQQFYNTPWTF	27 nt (15)	n.d.
1.12	1	11-13	17	IGHV5-6	CARDRYYGFSEWFAHW	IGKV4-53	CQQWSSYPLTF	20 nt (12)	27.8 ± 7.1
1.14	1	14-15	16	IGHV1-80	CARSGYGVFAFW	IGKV4-55	CQQWSSYPPTF	21 nt (8)	179.6 ± 2.6
1.15	1	14-15	16	IGHV1-61	CARGGYGSFAYW	IGKV4-50	CQQFTSFPSTF	33 nt (17)	7.3 ± 1.0
1.17	1	17	13	IGHV1-55	CTRYSTRYGGAMDYW	IGKV14-111	CLQYGDFFLTF	26 nt (17)	5.2 ± 1.9
1.20	1	18-24	12	IGHV7-3	CARDIPGTVYW	IGKV4-57	CQQRSTYPLTF	7 nt (5)	14.1 ± 2.6
1.26	1	25-28	11	IGHV1-26	CARSGYATAFAYW	IGKV4-55	CQQWSSYPLTF	23 nt (15)	1.4 ± 1.6
1.27	1	25-28	11	IGHV1-18	CARGGYGNPFAYW	IGKV4-61	CQQYHSYPLTF	12 nt (5)	115.2 ± 15.1
1.28	1	25-28	11	IGHV1-80	CARSGYGAFAYW	IGKV4-55	CQQWSSYPPTF	25 nt (10)	148.4 ± 34.2
1.29	1	29-36	10	IGHV1-4	CGRAGYDSFAYW	IGKV14-111	CLQYHEFFPFTF	16 nt (8)	9.6 ± 5.0
1.32	1	29-36	10	IGHV1-4	CARPFYDYYRRGAMDYW	IGKV3-10	CQQNEDPPTF	16 nt (8)	n.d.
1.40	1	40-46	8	IGHV1-76	CARSELQNFVMDYW	IGKV3-7	CQHSWEIPWTF	36 nt (17)	15.2 ± 2.3
1.42	1	40-46	8	IGHV8-12	CARRDGIITAWYFDVW	IGKV3-5	CQQSNEDPLTF	9 nt (4)	n.d.
1.43	1	40-46	8	IGHV1-64	CARNEYDGVFAYW	IGKV3-12	CQHSRELPWTF	22 nt (12)	n.d.
1.48	1	47-52	7	IGHV2-3	CAKEDYYGSSWGLDYW	IGKV12-98	CQQLYSTPYTF	2 nt (2)	n.d.
1.52	1	47-52	7	IGHV3-6	CARGGYVMDYW	IGKV10-94	CQQYSKIPYTF	8 nt (5)	71.2 ± 70.2
1.53	1	53-63	6	IGHV9-3	CTRRGGNYVYVYAMDYW	IGKV1-110	CSQSTHVPPTF	2 nt (3)	61.8 ± 9.0
1.55	1	53-63	6	IGHV1-64	CARLGVYW	IGKV15-103	CQQGSSYPPTF	16 nt (6)	10.0 ± 10.6
M1_low1	1	68-96	5	IGHV8-12	CARADWITTPDYW	IGKV6-23	CQQYSTYPWTF	14 nt (4)	n.d.
M1_low4	1	138-189	3	IGHV1-39	CARSGYGTFAFW	IGKV4-50	CQQFTSSPSTF	25 nt (13)	n.d.
M1_low8	1	249-404	1	IGHV5-9-1	CAGDRYDGYAMDYW	IGKV4-57	CQQRSSYPWTF	12 nt (11)	183.6 ± 11.0
M1_low11	1	249-404	1	IGHV7-3	CARDKGDSEWFDYW	IGKV12-98	CQQLYNTPYTF	19 nt (7)	363.0 ± 8.0
M1_low13	1	249-404	1	IGHV9-3	CARSNGNYLFAFW	IGKV4-55	CQQWSSYPPITF	6 nt (4)	167.5 ± 3.4
2.1	2	1	55	IGHV5-6	CARRGGYGFYFDYW	IGLV1	CALWYNSHLVF	7 nt (4)	n.d.
2.3	2	3	30	IGHV2-6-8	CARDYGGNNYAMDYW	IGKV4-57	CQQRSSYPPTF	8 nt (7)	80.3 ± 15.1
2.4	2	4	28	IGHV1-4	CTNHGSSHGWFYW	IGKV14-111	CLQYDEFPLTF	11 nt (6)	36.7 ± 1.2
2.6	2	6	25	IGHV2-6-8	CARDYGSNNYAMDFW	IGKV4-57	CQQRSSYPPTF	9 nt (5)	14.1 ± 19.5
2.7	2	7	24	IGHV1-76	CARYGYEYGFYDW	IGKV3-7	CQHSWEIPYTF	29 nt (15)	n.d.
2.8	2	8	20	IGHV1-26	CARSGYASFAFW	IGKV4-63	CFQGSYPPFTF	29 nt (17)	17.0 ± 3.4
2.9	2	9	19	IGHV1-69	CARCLYYSSSCAMDYW	IGKV5-43	CQQSNSWPLTF	36 nt (15)	n.d.
2.12	2	12-13	15	IGHV1-52	CARGGYW	IGKV8-24	CQQHYSTPPTF	14 nt (5)	77.2 ± 27.9
2.15	2	14-16	14	IGHV2-6-8	CARDYGGNNYAMDYW	IGKV4-57	CQQRSSYPPTF	12 nt (8)	12.3 ± 4.7
2.18	2	17-18	13	IGHV1-55	CTRSRQYFYLVDW	IGKV14-111	CLQYDEFFPYTF	15 nt (8)	180.2 ± 121.2
2.22	2	22-25	11	IGHV1-5	CVRSEDIYFFDYW	IGKV14-111	CLQYDEFFPYTF	15 nt (9)	5.2 ± 3.0
2.24	2	22-25	11	IGHV1-4	CTSHGSMFGWFSYW	IGKV14-111	CLQYDEFPLTF	17 nt (8)	17.8 ± 3.1
2.27	2	26-28	10	IGHV1-66	CASITATSSWFAFW	IGKV14-111	CLQYDEFFPYTF	16 nt (11)	18.4 ± 3.0
2.33	2	33-39	8	IGHV1-18	CARGGYGVPFAYW	IGKV4-63	CFQGSYPLTF	11 nt (5)	37.9 ± 6.4
2.34	2	33-39	8	IGHV6-6	CTRPGRVYQRYFDVW	IGKV12-41	CQHFVGTPTPL	17 nt (7)	37.3 ± 10.6
2.39	2	33-39	8	IGHV1-55	CTRSRQYFVFDVW	IGKV14-111	CLQYDEFFPYTF	11 nt (6)	80.3 ± 17.0
2.40	2	40-48	7	IGHV1-4	CTSHASMYGWFSYW	IGKV14-111	CLQYDEFPLTF	23 nt (14)	9.9 ± 2.1
2.41	2	40-48	7	IGHV1-9	CARSGYGSFAFW	IGKV4-55	CQQWSSYPPTF	22 nt (15)	10.9 ± 1.9
2.45	2	40-48	7	IGHV1-34	CAREYEAFFAYW	IGKV10-96	CQQGNTLPWTF	1 nt (1)	n.d.
2.49	2	49-56	6	IGHV1-5	CTRSEDIYFFDYW	IGKV14-111	CLQYDEFFPYTF	13 nt (11)	7.4 ± 1.5
2.51	2	49-56	6	IGHV1-5	CTRSEGYFFDYW	IGKV14-111	CQQYDEFPYTF	18 nt (12)	12.1 ± 2.1
2.55	2	49-56	6	IGHV2-6-8	CARDYGSNDYALDFW	IGKV4-57	CQQRSSYPPTF	14 nt (7)	3.6 ± 6.0
5.1	5	1	41	IGHV1-63	CARKNYGNTYFDYW	IGKV1-117	CFQGSHPWTF	30 nt (16)	18.7 ± 3.7
5.2	5	2	34	IGHV1-9	CARSGYGTFAFW	IGKV4-55	CQQWSSYPPPTF	21 nt (12)	451.8 ± 161.9
5.3	5	3	29	IGHV1-4	CARGGYGSLAYW	IGKV4-50	CQQFTSSPYTF	16 nt (8)	12.7 ± 2.3
5.4	5	4	28	IGHV1-66	CARRVLYFFDYW	IGKV6-32	CQQDYRYPYTF	7 nt (7)	8.8 ± 2.0
5.5	5	5-6	24	IGHV1-18	CARTNWDYFFDYW	IGKV14-111	CLQYDEFFWTF	22 nt (13)	n.d.
5.6	5	5-6	24	IGHV1-18	CARSGYDFFAFW	IGKV4-61	CQQCHSFPLTF	29 nt (18)	61.1 ± 30.5
5.7	5	7	20	IGHV1-4	CTRSAGFDYW	IGKV4-61	CQQYHSYPLTF	18 nt (11)	11.0 ± 1.9
5.12	5	12	15	IGHV8-12	CVRRPLGISDGYFFYW	IGKV6-23	CQQYSSFPLTF	16 nt (8)	n.d.
5.13	5	13-14	14	IGHV1-9	CARSGYGSFAFW	IGKV4-55	CQQWSSYPPPTF	19 nt (11)	39.7 ± 7.7
5.14	5	13-14	14	IGHV1-63	CARKIYYGTYFDYW	IGKV1-117	CFQGSHPWTF	18 nt (8)	44.2 ± 5.9
5.15	5	15-16	13	IGHV1-18	CARSGYGSFAFW	IGKV4-61	CQQYHSYPPPTF	14 nt (5)	12.6 ± 2.3
5.16	5	15-16	13	IGHV9-3	CARLQSYWYFDVW	IGKV3-12	CQHSRELRFT	7 nt (6)	5.0 ± 1.3
5.17	5	17-18	12	IGHV9-3	CAREASYWYFDVW	IGKV3-12	CQHSRDLRFT	8 nt (6)	20.2 ± 2.0
5.18	5	17-18	12	IGHV1-4	CTRSAYGYDYW	IGKV4-61	CQQYHSYPLTF	11 nt (4)	11.8 ± 2.0
5.20	5	19-21	11	IGHV1-26	CARSLGLAFAYW	IGKV4-55	CQQWSSYPPITF	27 nt (15)	35.2 ± 6.2
5.25	5	25-28	9	IGHV5-6	CARDPGLYGKAYFSYW	IGKV3-12	CQHSRELPFTF	8 nt (2)	5.9 ± 5.4
5.29	5	29-38	8	IGHV1-22	CAREGLRENYAMDYW	IGKV6-23	CQQFTNYPYTF	23 nt (15)	n.d.
5.33	5	29-38	8	IGHV2-3	CAKLDYGSLLYAVDLW	IGKV13-85	CQQYWSPTYTF	11 nt (4)	27.7 ± 4.2
5.37	5	29-38	8	IGHV1-4	CTRSAYGFDYW	IGKV4-61	CQQYYSYPLTF	14 nt (6)	4.6 ± 0.9
5.38	5	29-38	8	IGHV1-76	CARWGDSSGTGAMDYW	IGKV3-7	CQHSWEFPLTY	36 nt (15)	20.0 ± 3.7
5.41	5	39-48	7	IGHV2-3	CAKFSYGSYYAIDYW	IGKV12-41	CQHFWRTPYTF	16 nt (11)	34.0 ± 3.1
5.44	5	39-48	7	IGHV5-9-1	CSRYQGYGSSPYFDYW	IGKV3-12	CQHSRELPFTF	9 nt (7)	n.d.
5.50	5	49-54	6	IGHV14-1	CTRSYAAWFPYW	IGKV4-74	CHQYHRSPLTF	10 nt (7)	n.d.
5.51	5	49-54	6	IGHV5-9-1	CAREGLYGNVAMDYW	IGKV13-84	CQQYWSPTPLTF	4 nt (3)	n.d.
3.1	3	1	21	IGHV1-18	CTRSYGSFAFW	IGKV4-61	CQQYHSYPPPTF	23 nt (15)	n.d.
3.2	3	2-3	14	IGHV1-5	CTRSDDYFFDYW	IGKV14-111	CLQYDEFFPYTF	12 nt (9)	n.d.
3.5	3	5	12	IGHV1-18	CVRSGYGSFAFW	IGKV4-61	CQQYHSYPPPTF	15 nt (7)	n.d.
4.1	4	1	45	IGHV1-76	CARAIYNDTTGAFYEW	IGKV3-7	CHHSWEIPYTF	35 nt (13)	n.d.
4.2	4	2	43	IGHV1-19	CARIYRGKAMDYW	IGKV3-2	CQQSKEVPYTF	14 nt (8)	n.d.
4.8	4	8	18	IGHV1-53	CTRFYDYGGDAYW	IGKV4-57	CHQRSYPRFT	21 nt (11)	n.d.
4.11	4	11	14	IGHV1-64	CTRTGGTWGAMDYW	IGKV8-24	CQQHYSTPPTF	15 nt (6)	n.d.
4.13	4	13	13	IGHV1-5	CIRSGYGSFAFW	IGKV4-50	CQQFTSFPYTF	16 nt (11)	n.d.

Extended Data Table 1. Overview and characteristics of OVA binders. Clone ID corresponds to mouse number followed by clone index number corresponding to Fig. 1g.

ID	Mouse	IgG clone rank in mouse	# cells	V _H	CDRH3	V _L	CDRL3	nt dist. (aa-changes)
1.6	1	6	31	IGHV5-9-1	CTGDRYDGYAMDYD	IGKV4-57	CQQRSSYPWTF	6 nt (4)
1.8	1	8	22	IGHV10-1	CVRQGGKIYDYDYFDYD	IGKV17-127	CLQSDNMPYTF	4 nt (2)
1.9	1	9-10	18	IGHV9-3	CTRGGNYYYVYAMDYD	IGKV14-111	CLQYDELYTF	4 nt (4)
1.10	1	9-10	18	IGHV9-3	CARSDGNLYFAYW	IGKV4-55	CQWSSYPPTF	4 nt (1)
1.13	1	11-13	17	IGHV1-34	CARSLYDGYPHFDYD	IGKV4-50	CQQTSSPPTF	11 nt (8)
1.16	1	16	14	IGHV1-80	CARSGYGTGFAYW	IGKV4-55	CQWNSYPWTF	19 nt (6)
1.18	1	18-24	12	IGHV1-5	CTRSDSNFYFDYD	IGKV14-111	CLQYDEFPYTF	11 nt (7)
1.19	1	18-24	12	IGHV5-6	CARYDNYVRVAMDYD	IGKV4-74	CHQYHRSPYTF	18 nt (7)
1.21	1	18-24	12	IGHV13-2	CSRAFHYDYDYGMDYD	IGKV9-124	CLQYASYPWTF	13 nt (9)
1.23	1	18-24	12	IGHV1-80	CARSGYGTGFAYW	IGKV4-55	CQWSSYPWTF	18 nt (6)
1.25	1	25-28	11	IGHV5-6	CARQGGYGYSPYFDYD	IGKV6-15	CQQYNSYPLTF	11 nt (6)
1.30	1	29-36	10	IGHV1-4	CAVRRENYAMEYD	IGKV4-79	CHQWSSFPPTF	24 nt (15)
1.31	1	29-36	10	IGHV7-3	CARDIPYAMDYD	IGKV1-135	CWQGTTHFPPTF	5 nt (0)
1.33	1	29-36	10	IGHV1-4	CARGFGLYFDYD	IGKV4-57	CHQRSSYPPTF	28 nt (19)
1.37	1	37-39	9	IGHV1-5	CARTGTHWYFDVW	IGKV3-5	CQSNEDPWTF	19 nt (10)
1.38	1	37-39	9	IGHV14-3	CVRFGAVPRFSYD	IGKV1-117	CFQGSHPVPTF	18 nt (9)
1.39	1	37-39	9	IGHV5-6	CAKDIGYGYGWFYD	IGKV4-61	CQQYHSPPTF	14 nt (10)
1.41	1	40-46	8	IGHV14-1	CCFFYGYGAWFGYD	IGKV6-17	CQQHYTSPRTF	19 nt (11)
1.44	1	40-46	8	IGHV9-3	CARRGGNYYYVAVDYD	IGKV14-111	CLQYDELYTF	8 nt (5)
1.47	1	47-52	7	IGHV5-17	CARQNDYD	IGKV12-44	CQHHYGTPLTF	18 nt (8)
1.49	1	47-52	7	IGHV1-80	CARSGYGVFAYD	IGKV4-55	CQWSSYPPTF	32 nt (13)
1.50	1	47-52	7	IGHV1-18	CGRGGYGNPFAYD	IGKV4-63	CFQGSYPLTF	23 nt (16)
1.51	1	47-52	7	IGHV9-3	CARSPTWFAYD	IGKV15-103	CQQGQSFYTF	12 nt (9)
1.54	1	53-63	6	IGHV1-4	CARKSNLFPYD	IGKV6-20	CQGTYSYPPTF	16 nt (6)
1.56	1	53-63	6	IGHV1-26	CGRSYGYSYAMDYD	IGKV16-104	CQHNHEYPTF	25 nt (15)
1.57	1	53-63	6	IGHV5-17	CARSYGSSLDYD	IGKV12-41	CQHFWSPTPTF	19 nt (8)
M1_low2	1	97-137	4	IGHV1-4	CARDYDGFAYD	IGKV1-110	CQQTSSPSTF	21 nt (13)
M1_low3	1	97-137	4	IGHV1-18	CARSGYATAFAYD	IGKV4-55	CQQGNTLLMYTF	26 nt (13)
M1_low5	1	190-248	2	IGHV1-26	CARYGNYYFDYD	IGKV10-96	CLQYASSPLTF	19 nt (12)
M1_low6	1	249-404	1	IGHV1-19	CARRGDSYSQFPYAMDYD	IGKV1-110	CQQRSSYPWTF	9 nt (6)
M1_low7	1	249-404	1	IGHV1-26	CARSTAIYGMIDYD	IGKV9-120	CHQRSSYPSTF	30 nt (15)
M1_low9	1	249-404	1	IGHV8-11	CARIEGLLAWFGYD	IGKV1-110	CSQSTHVPWTF	22 nt (8)
M1_low10	1	249-404	1	IGHV1-82	CARAGYGTGFAYD	IGKV4-55	CQQWSTYPWTF	14 nt (8)
M1_low12	1	249-404	1	IGHV1-4	CVRSDDGYGYVPRWFGYD	IGKV8-27	CHQYLSSYTF	29 nt (17)
M1_low14	1	249-404	1	IGHV5-9-1	CQRLRGYFDVW	IGKV1-110	CSQSTHVPYTF	20 nt (9)
M1_low15	1	249-404	1	IGHV13-2	CSRSFYDYDYAMDYD	IGKV9-124	CLQYASYPWTF	5 nt (2)
2.2	2	2	32	IGHV2-6-8	CARDYGANNYAMDYD	IGKV4-57	CQQRSSYPPTF	14 nt (8)
2.5	2	5	27	IGHV7-3	CARDMNNGSYWLVDVW	IGKV12-89	CQNVLYSPWTF	16 nt (8)
2.10	2	10	18	IGHV1-26	CARNPYD	IGKV1-117	CFQGSHPVPLTF	26 nt (8)
2.11	2	11	16	IGHV1-19	CASQLGSWFAYD	IGKV14-111	CLQYDEFPYTF	14 nt (5)
2.13	2	12-13	15	IGHV1-9	CARSGGFYDPGRGYAMDYD	IGKV4-74	CHQYYRSPRTF	32 nt (17)
2.14	2	14-16	14	IGHV8-12	CARSYADFYD	IGKV4-80	CHQWSSYPPTF	9 nt (4)
2.16	2	14-16	14	IGHV1-55	CTRSLRYWYLDVW	IGKV14-111	CLQYDEFPYTF	19 nt (10)
2.17	2	17-18	13	IGHV9-3	CATPDYFAMDYD	IGKV8-21	CKQSYLPLWTF	9 nt (6)
2.19	2	19-21	12	IGHV1-5	CTRSEGYDYFDYD	IGKV14-111	CLQYDEFPYTF	19 nt (14)
2.20	2	19-21	12	IGHV5-6	CTRRDFYGYAMDYD	IGKV8-28	CQNDHSPYPTF	13 nt (5)
2.23	2	22-25	11	IGHV6-6	CTRRDFWYD	IGKV1-135	CWQGTTHFPQTF	13 nt (11)
2.26	2	26-28	10	IGHV5-6	CARRGGYGYGYFDYD	IGLV1	CALWYSNHLVTF	11 nt (6)
2.28	2	26-28	10	IGHV8-8	CARDLYDDGTAIFYD	IGKV8-27	CHQYLSSRSF	23 nt (8)
2.29	2	29-32	9	IGHV14-1	CTPYDYDVSFAFYD	IGKV12-44	CQHHGSPRTF	13 nt (10)
2.30	2	29-32	9	IGHV1-39	CARWGEIYPYAMDYD	IGKV10-96	CQQGNTFPWTF	25 nt (13)
2.31	2	29-32	9	IGHV1-5	CTRCYGNPFYD	IGKV4-74	CHQHRSPPPTF	28 nt (16)
2.32	2	29-32	9	IGHV2-2	CARKGPQLVFDYD	IGKV6-23	CQQYSSYPPTF	19 nt (6)
2.35	2	33-39	8	IGHV1-81	CARLLYGYGYLDYD	IGKV14-111	CLQYDEFWTF	21 nt (12)
2.36	2	33-39	8	IGHV1-39	CARWGEIYPYAMDYD	IGKV10-96	CQQGNTLPWTF	29 nt (17)
2.37	2	33-39	8	IGHV1-54	CARSDGNEDYD	IGKV4-55	CQWSSYPFAT	13 nt (3)
2.38	2	33-39	8	IGHV8-11	CARIYYSRHRFDYD	IGKV3-4	CQQSYEDPTF	18 nt (10)
2.42	2	40-48	7	IGHV1-4	CARSNDGGFAYD	IGKV4-59	CQWSSNPPTF	9 nt (4)
2.43	2	40-48	7	IGHV1-61	CARSDDGYWFAYD	IGKV3-7	CQHSWEIPPTF	25 nt (13)
2.44	2	40-48	7	IGHV1-22	CARGLPYHGLDNW	IGKV4-61	CQQYHSSPPTF	27 nt (20)
2.50	2	49-56	6	IGHV2-2	CAKSPYYGAMDYD	IGKV5-43	CQSNNSWPPTF	12 nt (7)
2.52	2	49-56	6	IGHV1-69	CTRDYHGTSSMDYD	IGKV1-110	CSQSTHVPWTF	48 nt (19)
2.53	2	49-56	6	IGHV2-3	CAKTYGYAMDYD	IGKV4-63	CLQGSYPTF	22 nt (12)
2.54	2	49-56	6	IGHV1-26	CARGITVVPYAMDYD	IGKV10-96	CQQGNTLPWTF	22 nt (12)
5.8	5	8-9	17	IGHV1-15	CTREGYFYDVRVWFAYD	IGKV2-109	CAQNLLEPTF	12 nt (9)
5.9	5	8-9	17	IGHV14-3	CASAATWAYWYFDVW	IGKV4-91	CQQGSSILPTF	3 nt (2)
5.10	5	10-11	16	IGHV9-3	CARGDYGNERYVAWHAYD	IGKV10-96	CQQGDTVPPTF	24 nt (14)
5.11	5	10-11	16	IGHV14-3	CARFGNYPYWYFDVW	IGKV4-70	CHQRSSYPYTF	10 nt (8)
5.19	5	19-21	11	IGHV4-1	CARPGFRYGYAMDYD	IGKV6-25	CQQHYSTPYTF	10 nt (5)
5.21	5	19-21	11	IGHV5-9-1	CSRDSYGSYGYFDVW	IGKV13-85	CQQYSSPPTF	5 nt (5)
5.22	5	22-24	10	IGHV2-3	CAKFSYGSYGYAIDYD	IGKV6-17	CQQHYSTPLTF	19 nt (12)
5.23	5	22-24	10	IGHV5-17	CAREFAYD	IGKV12-44	CQHHYGSPTF	7 nt (5)
5.24	5	22-24	10	IGHV1-64	CARFSGYFDVW	IGKV4-50	CQQTSSPPTF	20 nt (8)
5.26	5	25-28	9	IGHV1-18	CARSGYDFYD	IGKV9-124	CLQYSTYPWTF	33 nt (18)
5.27	5	25-28	9	IGHV3-1	CARFYRSTGIAYD	IGKV4-70	CHQRHYPWTF	13 nt (7)
5.28	5	25-28	9	IGHV9-1	CTQLGLLAWFAYD	IGKV10-94	CQQYSKLPPTF	13 nt (6)
5.30	5	29-38	8	IGHV1-63	CARKNYGSTYFDYD	IGKV1-117	CFQGSHPWTF	15 nt (7)
5.31	5	29-38	8	IGHV1-9	CVRRSPYDYDYD	IGKV17-127	CFQSDNMPYTF	19 nt (12)
5.32	5	29-38	8	IGHV1-66	CAKNYGIHYGMDYD	IGKV13-85	CQQYWTIPYTF	18 nt (10)
5.34	5	29-38	8	IGHV5-6	CARQVYDYGTMIDYD	IGKV10-96	CQQGNTLPRTF	18 nt (7)
5.35	5	29-38	8	IGHV8-8	CARKAYFYDYFDYD	IGKV6-23	CQQYSSYPLTF	9 nt (6)
5.36	5	29-38	8	IGHV9-3	CARGWGYFDYD	IGKV6-17	CQQHYSTPLTF	19 nt (12)
5.39	5	39-48	7	IGHV1-4	CARRVGEYFYD	IGKV4-79	CHQWNSYPPTF	13 nt (8)
5.40	5	39-48	7	IGHV9-3	CARAPLFYAMDYD	IGLV1	CALWYSNHWVTF	17 nt (12)
5.42	5	39-48	7	IGHV1-76	CARWGDSSGTAMDYD	IGKV3-7	CQHSWEIPLTF	37 nt (12)
5.43	5	39-48	7	IGHV4-1	CARPGFRYGYAMDYD	IGKV12-44	CQHHYGTPLTF	10 nt (5)
5.45	5	39-48	7	IGHV8-12	CARLYDGSYALDYD	IGKV12-44	CQHHYGTPTF	14 nt (8)
5.46	5	39-48	7	IGHV1-85	CTTGDYLVHFDVW	IGKV15-103	CQQGQSYPTF	30 nt (17)
5.47	5	39-48	7	IGHV2-9-1	CVRRGGYGNFYWFFDVW	IGKV1-117	CFQGSHPVPTF	11 nt (7)
5.49	5	49-54	6	IGHV1-26	CARRGSRFYD	IGKV6-32	CQQDYNPWTF	30 nt (16)

Extended Data Table 2. Overview and characteristics of non-binders. Clone ID corresponds to mouse number followed by clone index number corresponding to Fig. 1g.

ID	Mouse	IgG clone rank in mouse	# cells	V _H	CDRH3	V _L	CDRL3	nt dist. (aa-changes)
3.3	3	2-3	14	IGHV1-5	CTRSDDYYFDYW	IGKV4-70	CHQRSNYPLTF	13 nt (11)
3.4	3	4	13	IGHV8-12	CARTRWEKYAMDYW	IGKV19-93	CLQYDNLTYF	9 nt (7)
4.3	4	3	30	IGHV1-54	CARSDSSGDYW	IGKV4-55	CQQWSSYPFTF	12 nt (5)
4.5	4	5-6	19	IGHV5-6	CSRRGLVDGVYPMYD	IGKV6-23	CQQYRTYPTF	21 nt (11)
4.6	4	5-6	19	IGHV8-12	CVRRRLWLDYAMDND	IGKV10-96	CQQGNTLPYTF	16 nt (9)
4.14	4	14	12	IGHV1-19	CAITRLDYW	IGKV4-80	CHQWSSYPFTF	14 nt (4)

Extended Data Table 2 (continued). Overview and characteristics of non-binders. Clone ID corresponds to mouse number followed by clone index number corresponding to Fig. 1g.

Primer	Description	Sequence [5' -> 3']
EpMap_1	Reverse primer for pB33eCPX plasmid linearisation (step 1)	CCAGTGCTGAAAGACATGCAA
EpMap_2	Forward primer for pB33eCPX plasmid linearisation (step 1)	ACAACAAAAACCAGTACTACGGC
EpMap_3	Reverse primer for plasmid linearisation (step 2; library generation)	TTGTCCAGATTGTCCAGCTACAGAAG
EpMap_4	Forward primer for plasmid linearisation (step 2; library generation)	GGAGGGCAGTCTGGGCAG
EpMap_5	Forward primer Sanger sequencing	GTGGTTATGAAAAAATTGCATGTC
EpMap_7	Forward primer NGS PCR 1	CCCTCCTTAATCCCCTGGTTATGAAAAAATTGCATGTC
EpMap_8	Reverse primer NGS PCR 1	GAGGAGAGAGAGAGATGCTTGCCAGTCGTTA
EpMap_9	Forward primer NGS PCR 2	AATGATACGGCGACCACCGAGATCTACACTCTTCCCTACAC GACGCTCTCCGATCTNNNNCCCTCTTAATCCC
EpMap_idx	Reverse primer NGS PCR 2 encoding TruSeq IDX1	CAAGCAGAAGACGGCATAAGAT CGTGAT GTGACTGGAGTT CAGACGTGTGCTCTCCGATCTNNNNGAGGAGAGAGAGAGA G

Extended Data Table 3. Primers used for bacterial epitope mapping and NGS library generation.

ssODN	Description	Sequence [5' -> 3']
EpMap1_ssODN	Generation of epitope mapping starting plasmid	TGAAAAAATTGCATGCTTTTCAGCACTGGCCGAGTCTGGCTTTCACAGCAGGAACCTCTGTAGCTGGA CAATCTGGACAAGGAGGGCAGTCTGGGCAGTCAGGTGATT ACAACAAAAACCAGTACTACGGCATCACTG
EpMapW1_ssODN	Exemplary library ssODN for OVA index1	GGAACTTCTGTAGCTGGACAATCTGGACAA ATGGGGTCCATTGGTGCAGCGAGTATGGAGTTCTGTTTTGA TGTTGGAGGGCAGTCTGGGCAGTCAGGTGATTAC

Extended Data Table 4. ssODNs for epitope mapping library generation. Cloning overhang sequences are indicated in bold. Insert sequence shown in red for exemplary library ssODN encodes for the first 15 a.a. of OVA.