Table E1: Comparison of observational controls and active arm before PNOIT

	Geometric Mean (95% CI) Observational controls	Geometric Mean (95% CI) Baseline in active arm	p-value
Number of multimer-positive memory cells per million B cells	3.1 (1.6,6)	4 (2.2,7.2)	0.37
Number of multimer-positive naive cells per million B cells	4.5 (2.4,8.5)	6.8 (3.9,12)	0.19
Number of multimer-positive plasmablasts per million B cells	1.5 (1,2.2)	1.3 (0.9,2)	0.60
Number of multimer-positive memory cells per million memory B cells	8.2 (4.1,16.5)	11.1 (6.1,20.5)	0.32
Number of multimer-positive naive cells per million naive B cells	8.2 (4.9,13.9)	13.1 (7.7,22.1)	0.14
Number of multimer-positive plasmablasts per million plasmablasts	11.8 (6.8,20.6)	9.7 (5.5,16.8)	0.47

Table E2: Categorization of time in longitudinal model

Group	N	Median (weeks)	Mean (weeks)	Minimum (weeks)	Maximum (weeks)
1	16	0	0	0	0
2	14	2	3	2	6
3	16	7	8	6	11
4	16	14	13	11	15
5	15	18	18	15	20
6	14	25	36	20	50
7	16	59	58	51	65
8	15	71	76	66	100

Table E3: NGS sequences per sample

Patient	Time	IgA	IgD	IgE	IgG	IgM	Total
1	2 weeks	2513	1243	666	3579	18842	26843
2	2 weeks	65241	26235	73	161465	361123	614137
3	2 weeks	93312	41716	771	133762	384236	653797
1	14 weeks	28388	14876	582	51793	250996	346635
2	14 weeks	94994	35160	809	224421	497492	852876
3	14 weeks	116127	31213	479	181498	388318	717635

Table E4: Multimer selected sequence similarity to NGS repertoires

		NGS repertoire multimer-similar sequences												
		Subject 1				Subject 2				Subject 3				
Subject of multimer seqeunce	Isotype of multimer sequences	IgA	IgD	IgG	IgM	IgA	IgD	IgE	IgG	IgM	IgA	IgD	IgG	IgM
1	IgM	1	0	1	14	4	6	0	17	49	5	2	24	52
1	IgA	15	0	4	0	0	0	0	0	0	15	0	4	0
1	IgM	0	0	0	0	0	0	0	0	0	0	2	0	0
1	IgM	0	0	0	0	0	0	0	0	5	0	0	0	0
1	IgG	0	0	0	0	1	0	0	27	5	0	0	54	16
2	IgA	1	0	0	20	18	2	0	4	25	4	0	3	22
2	IgM	1	0	0	1	0	0	0	1	18	1	0	0	3
2	IgM	0	0	0	0	0	0	0	0	4	0	0	0	0
2	IgM	0	0	0	0	0	0	0	2	8	0	0	0	1
2	IgA	0	0	0	0	0	3	0	0	2	0	0	0	0
2	IgG	0	0	0	0	0	0	1	4	0	0	0	0	0
2	IgG	0	0	0	0	0	0	0	1	0	0	0	0	0
3	IgM	0	0	0	0	0	0	0	0	0	0	0	0	0
3	IgG	0	0	0	0	0	0	0	0	0	1	0	41	2
3	IgA	0	0	0	0	2	0	0	0	0	0	0	0	0
3	IgG	0	0	0	0	0	0	0	1	0	0	0	6	1
3	IgA	0	0	0	0	21	0	0	21	2	3	0	47	1
3	IgG	0	0	0	0	0	0	0	0	1	0	0	0	0
3	IgG	0	0	0	0	0	0	0	1	0	0	0	1	0
3	IgA	0	0	0	0	0	0	0	31	1	0	0	5	0
3	IgA	0	0	0	0	0	0	0	0	0	0	0	1	0
3	IgG	0	0	0	0	16	3	0	1	1	1	0	17	1
3	IgM	0	0	0	0	0	0	0	0	7	0	0	0	0
3	IgM	0	1	0	1	0	0	0	0	1	0	1	0	1
3	IgG	0	0	0	0	2	1	0	51	2	0	0	0	0
3	IgG	0	0	0	0	0	0	0	16	3	0	0	32	0
3	IgG	0	0	0	0	6	0	0	2	0	0	0	0	0
3	IgG	0	0	0	0	0	0	0	0	0	0	0	3	0
3	IgG	0	0	0	0	0	0	0	0	0	1	0	35	0
3	IgG	0	0	0	0	0	0	0	0	0	1	0	0	0
3	IgM	0	0	0	0	0	0	0	0	1	0	0	0	0
3	IgG	0	0	0	0	0	0	0	0	0	1	0	13	0

		NGS repertoire multimer-similar sequences												
		Subject 1				**Subject 1**					**Subject 1**			
Subject of multimer sequence	Isotype of multimer sequences	IgA	IgD	IgG	IgM	IgA	IgD	IgE	IgG	IgM	IgA	IgD	IgG	IgM
3	IgG	0	0	0	0	0	1	0	6	0	0	0	0	0
3	IgM	1	0	0	17	0	1	0	0	4	1	0	0	19
3	IgA	0	0	0	0	1	0	0	19	3	3	1	17	2
3	IgA	0	0	0	0	9	0	0	49	0	18	0	25	0
3	IgG	0	0	0	0	0	0	0	7	0	0	0	1	0
3	IgA	0	0	0	0	3	0	0	2	0	0	0	11	2
3	IgG	0	0	0	0	0	0	0	3	0	0	0	1	0
3	IgG	1	0	1	5	0	0	0	0	2	1	1	1	8
3	IgA	0	0	0	0	0	1	0	9	0	3	0	12	0
3	IgG	0	0	0	0	1	0	0	0	0	0	0	0	0
3	IgM	1	4	0	10	0	1	0	0	19	1	6	0	13
3	IgM	0	0	0	1	0	2	0	6	15	5	2	0	4
3	IgM	0	0	0	0	0	0	0	0	7	0	1	1	9
3	IgA	0	0	0	0	0	0	0	15	0	0	0	0	0

Table E1: Comparison of observational controls and active arm before PNOIT

The frequency of multimer-positive B cells both within each B cell subset and within the entire B cell population were compared between subjects in the observational arm and the active arm before onset of PNOIT. No significant differences are found.

Table E2: Categorization of time in longitudinal model

Time period groups used in the longitudinal analysis are noted here with the number of observations (N) as well as the median, mean, minimum, and maximum number of weeks characterizing each time period.

Table E3: NGS sequences per sample

Within each of the six samples, with 2 time points during PNOIT of each of the three patients, the number of IgH sequences within each isotype are shown.

Table E4: Multimer selected sequence similarity to NGS repertoires

Each individual multimer-positive sequence with similar sequences in the NGS repertoire are listed, with both their originating subject and isotype, in each row. Columns reflect the NGS repertoires from different subjects by isotype compartment.

Figure E1: Frequency of multimer+ B cell subsets in allergic and non-allergic subjects

The frequency of multimer-positive cells was normalized to the subset (naïve, memory, or plasmablast) population and described as the number of cells per million subset cells or per million $CD19^+$ B cells in either peanut allergic pateints from the PNOIT trial before the start of PNOIT compared to non-allergic control patients. Basophil staining of the non-allergic control patients is shown in Figure1c.

Figure E2: Single cell immunoglobulin amplification

Immunoglobulin amplification of heavy and light chains was performed using single cell, nested RT-PCR. In this example, gel electrophoresis demonstrates recovery of 17 out of 24 paired heavy and light chains.

Figure E3: Antibody cloning vectors

Recombinant antibodies were produced after insertion of the multimer-positive single cell amplified sequences into heavy or light (either kappa or lambda, respectively) vectors, as previously published in Tiller, et.al.

Figure E4: NGS analysis pipeline

The next generation sequencing (NGS) analysis pipeline for processing of raw Illumina MiSeq 2x250 reads is used to generate the heavy chain immunoglobulin sequences used for downstream analysis.

Figure E5: Representative results from NGS data analysis

The numbers of reads retained at each step of the analysis pipeline for one of the subjects' samples is shown.

Figure E6: Ara h 2 specific heavy chain clonal groups

The distribution of clonal groups (same V-J gene usage and similar CDR3) demonstrates that a subset (20%) have more than one sequence.

Figure E7: Inference of antigen selection of Arah2-positive B cell IgH

Heavy chain immunoglobulin sequences of Ara h 2 affinity selected B cells have increased frequency of CDR replacement mutations (R_{CDR}) compared to the total number of mutations in the V region (M_V), suggestive of antigen selection. The gray area represents the 90-95% confidence interval for the probability of random mutations.

Figure E8: Recombinant antibody affinity for Ara h 2

Ara h 2 affinity of the recombinant IgG1 antibodies produced from multimer-positive single cells was characterized using biolayer inferferometry (Octet). The colors of the bars reflect the original multimer-positive single cell isotype from which the recombinant antibody was produced.

Figure E1

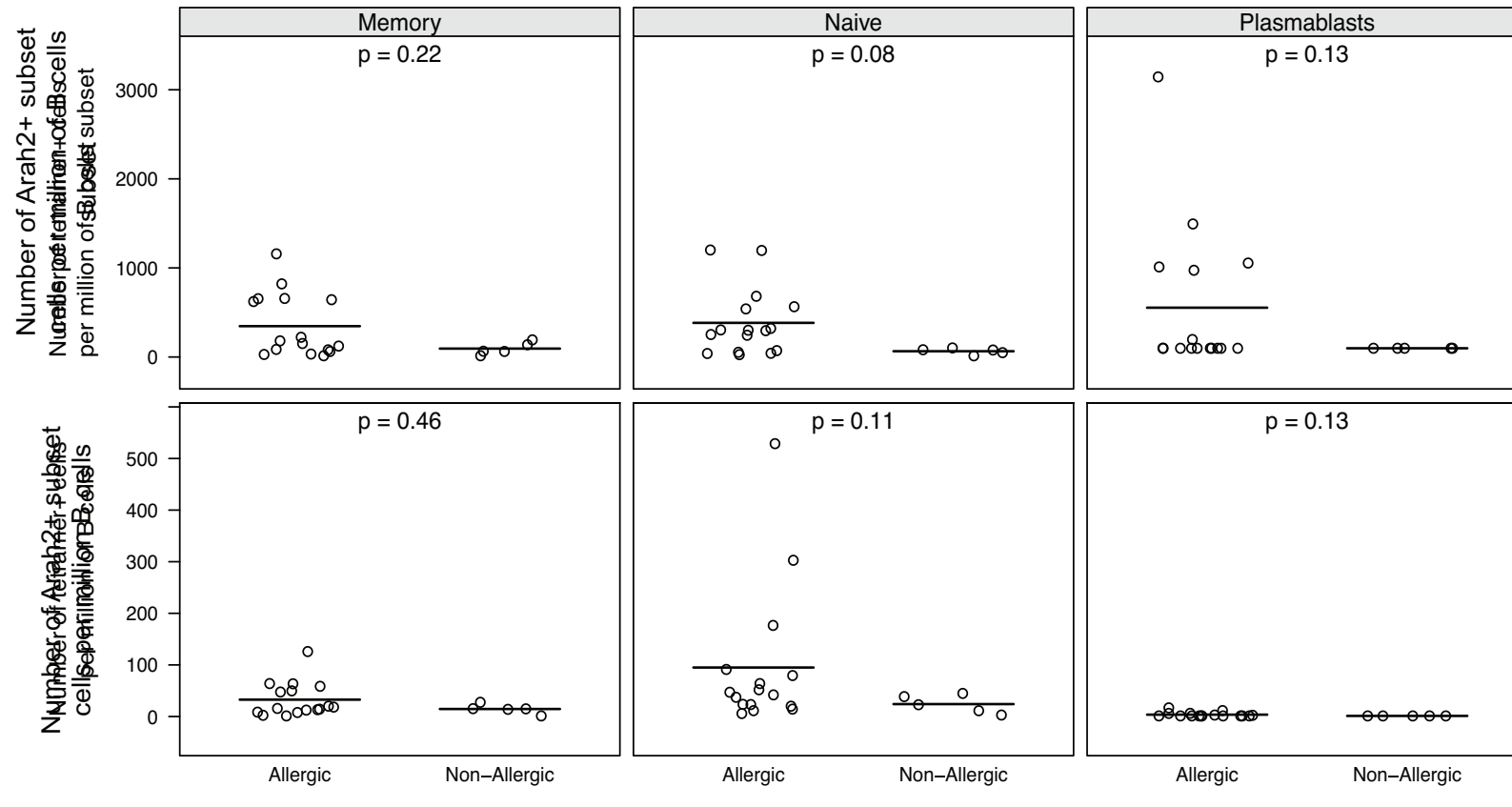

Memory
Naive
Plasmablasts
p = 0.22
p = 0.08
p = 0.13
p = 0.46
p = 0.11
p = 0.13
Number of Arah2+ subset cells per million of B cells
Number of Arah2+ subset cells per million of B cells
0
1000
2000
3000
0
100
200
300
400
500
Allergic
Non−Allergic

Figure E2

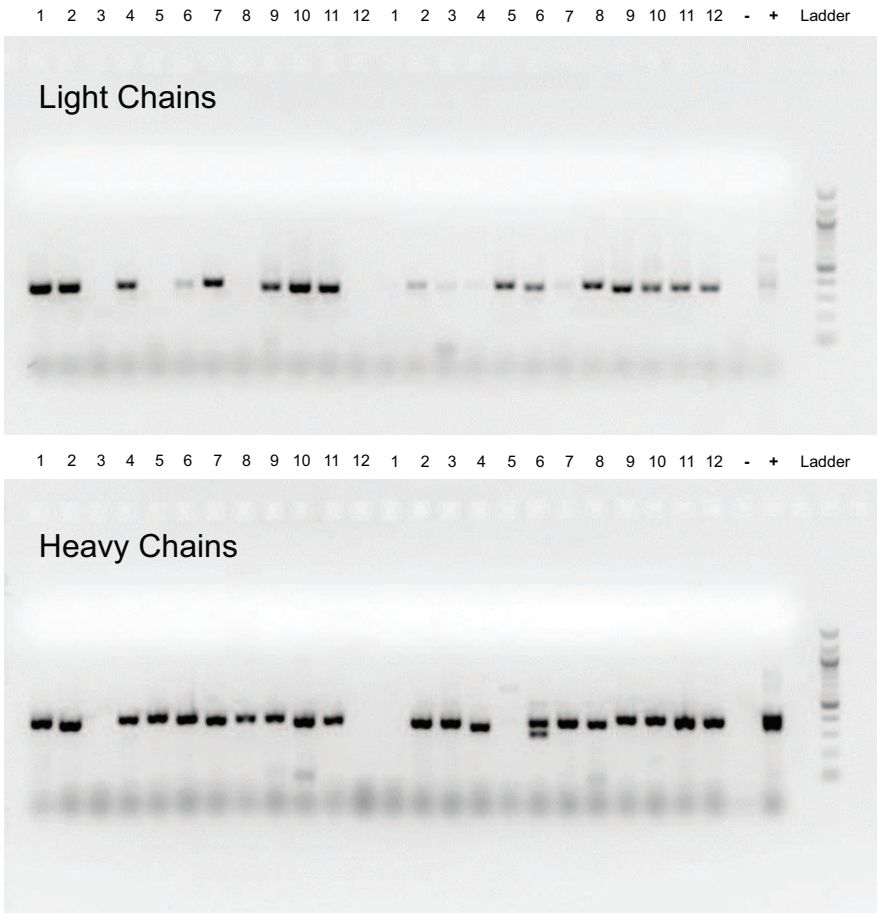
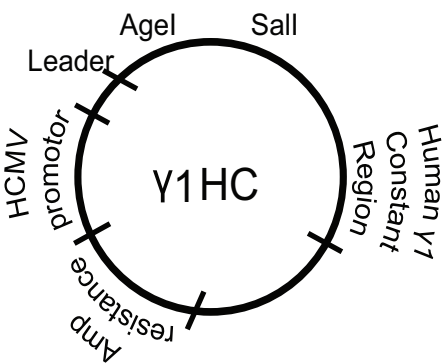
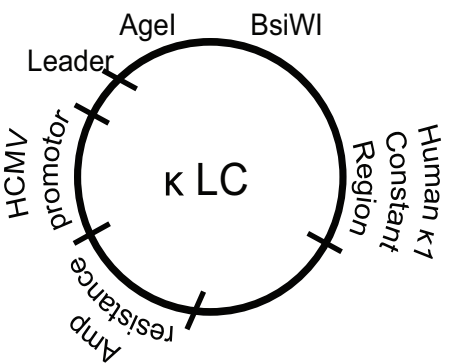

1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 - + Ladder
Light Chains
1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 - + Ladder
Heavy Chains

Figure E3


AgeI
SalI
Leader
HCMV promotor
γ1HC
Human γ1 Constant Region
Amp resistance


AgeI
BsiWI
Leader
HCMV promotor
κ LC
Human κ1 Constant Region
Amp resistance

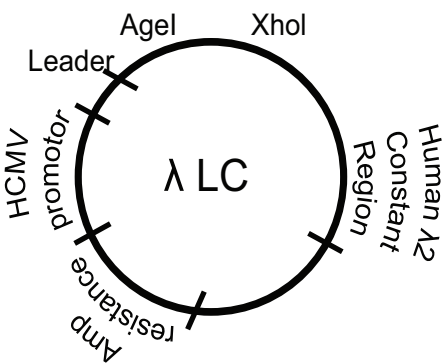

AgeI
XhoI
Leader
HCMV promotor
λ LC
Human λ2 Constant Region
Amp resistance

Figure E4

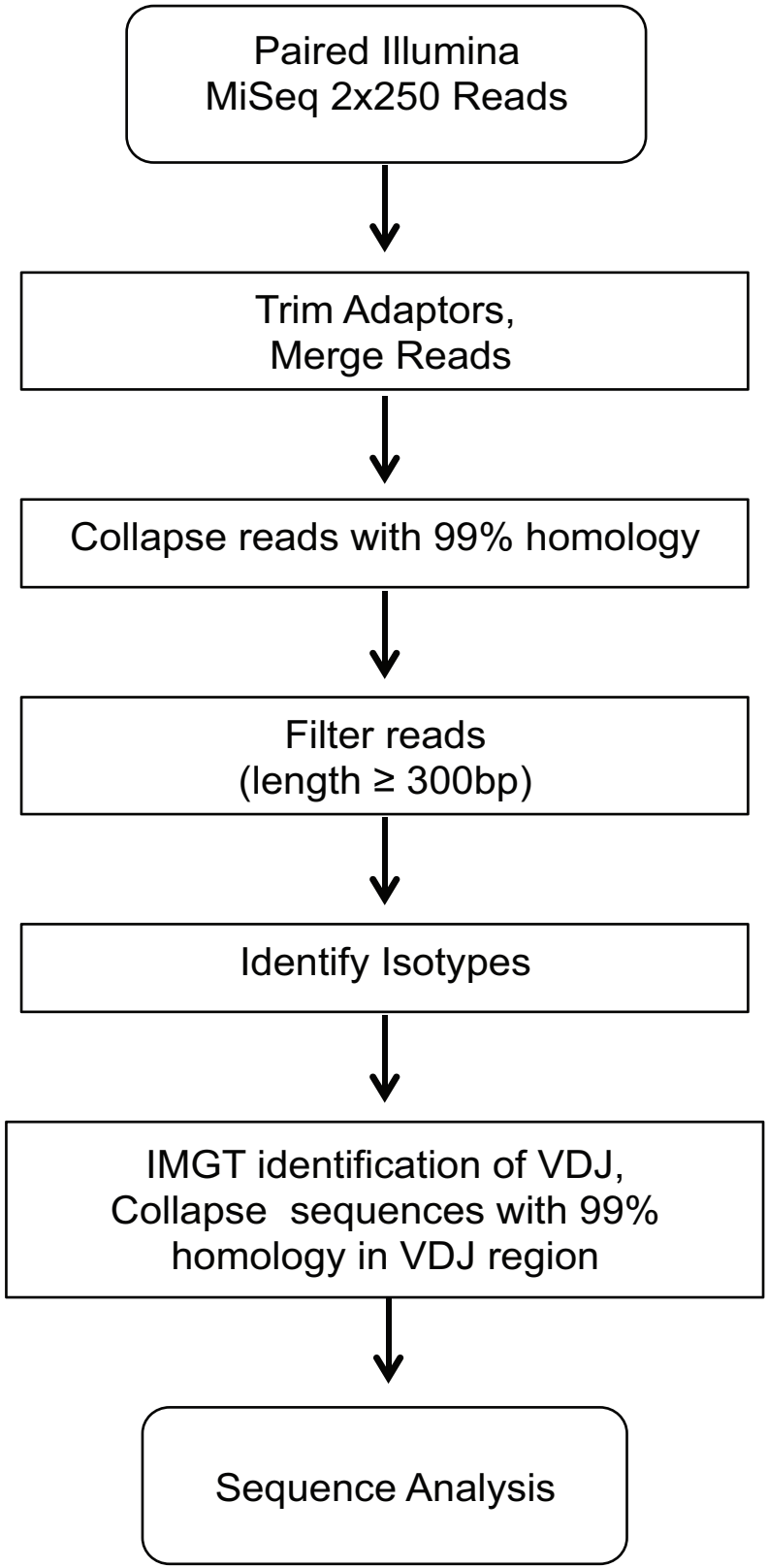

Paired Illumina
MiSeq 2x250 Reads
Trim Adaptors,
Merge Reads
Collapse reads with 99% homology
Filter reads
(length ≥ 300bp)
Identify Isotypes
IMGT identification of VDJ,
Collapse sequences with 99%
homology in VDJ region
Sequence Analysis

Figure E5

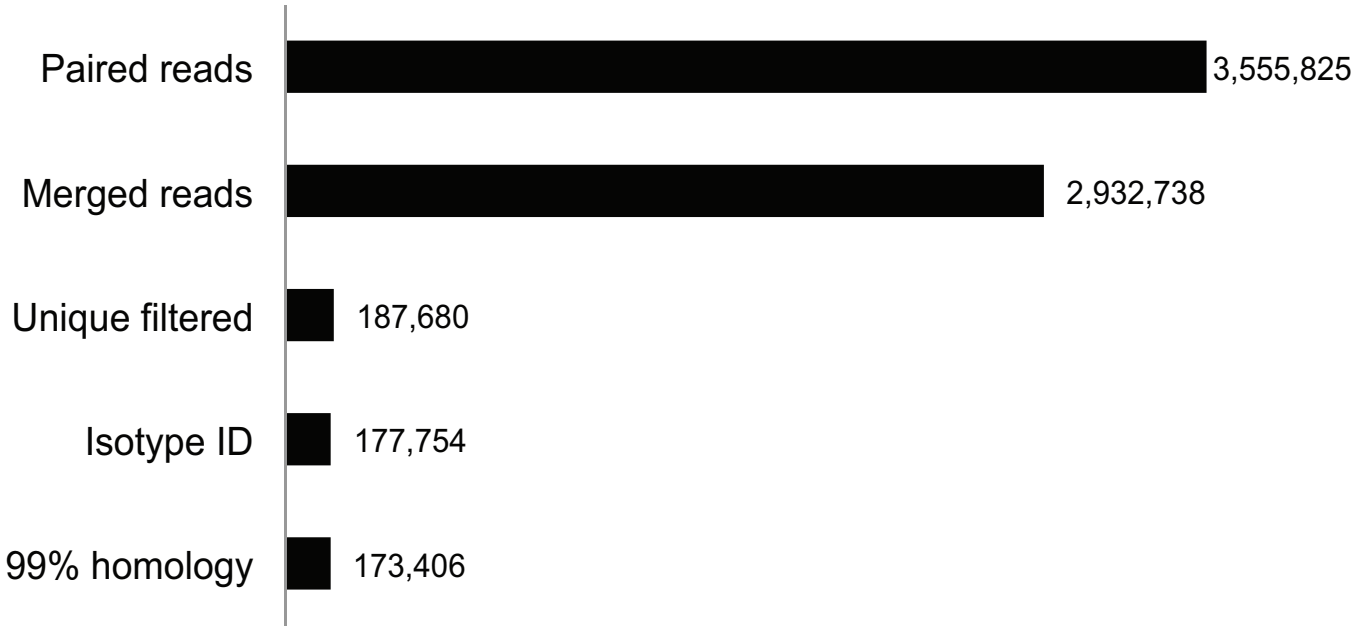

Paired reads
3,555,825
Merged reads
2,932,738
Unique filtered
187,680
Isotype ID
177,754
99% homology
173,406

Figure E6

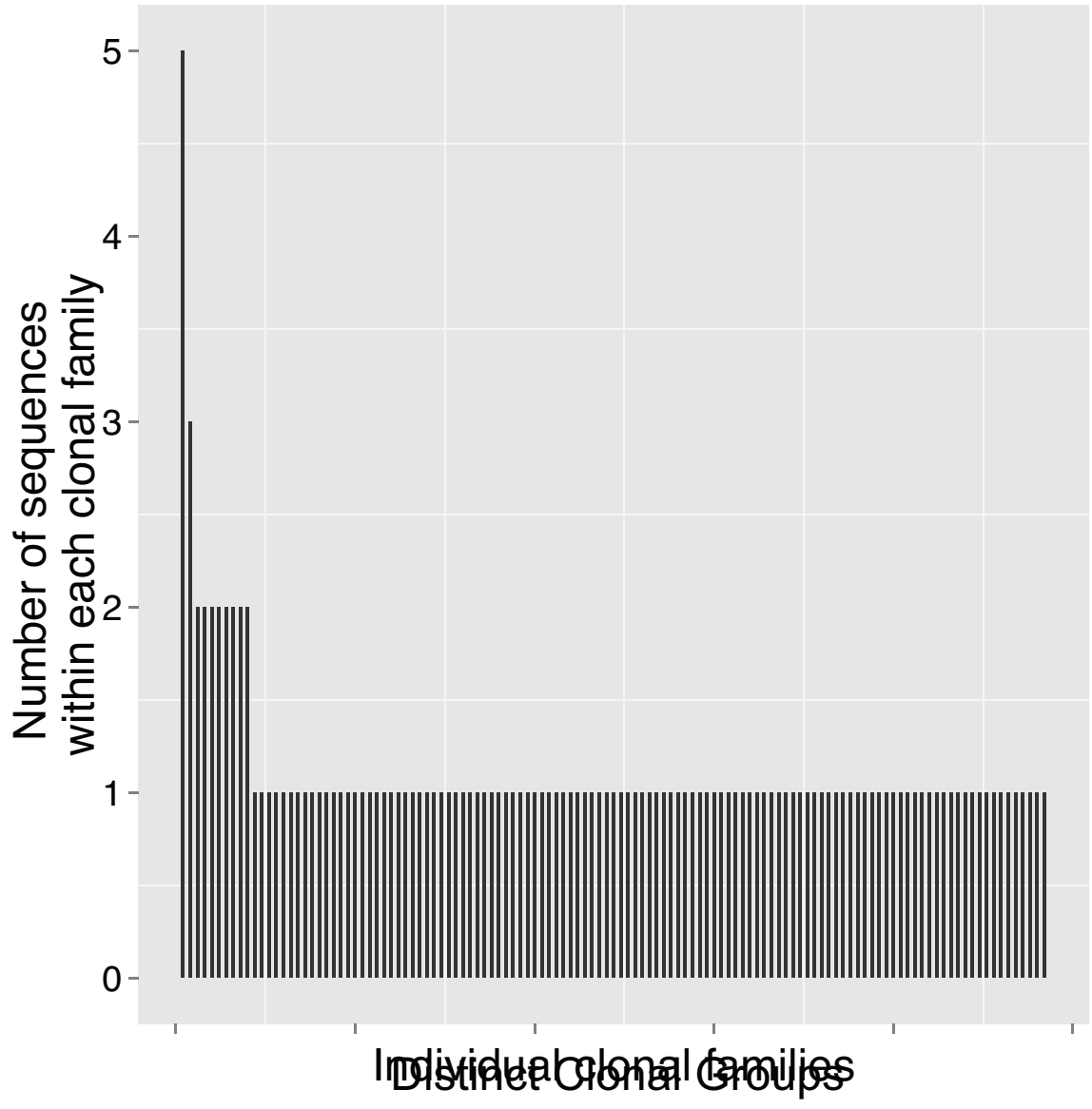

5
4
3
2
1
0
Number of sequences within each clonal family
Individual clonal families
Distinct Clonal Groups

Figure E7

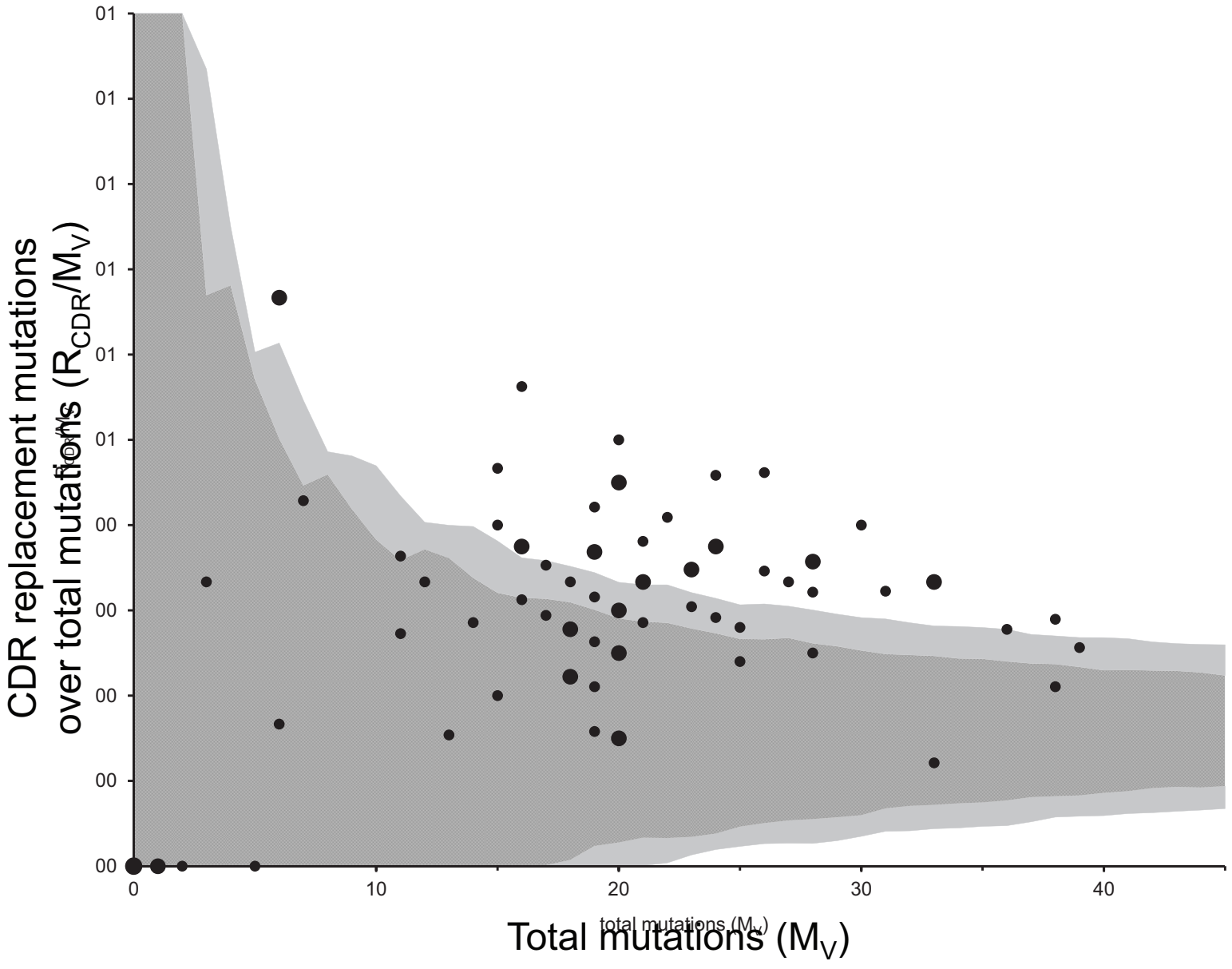
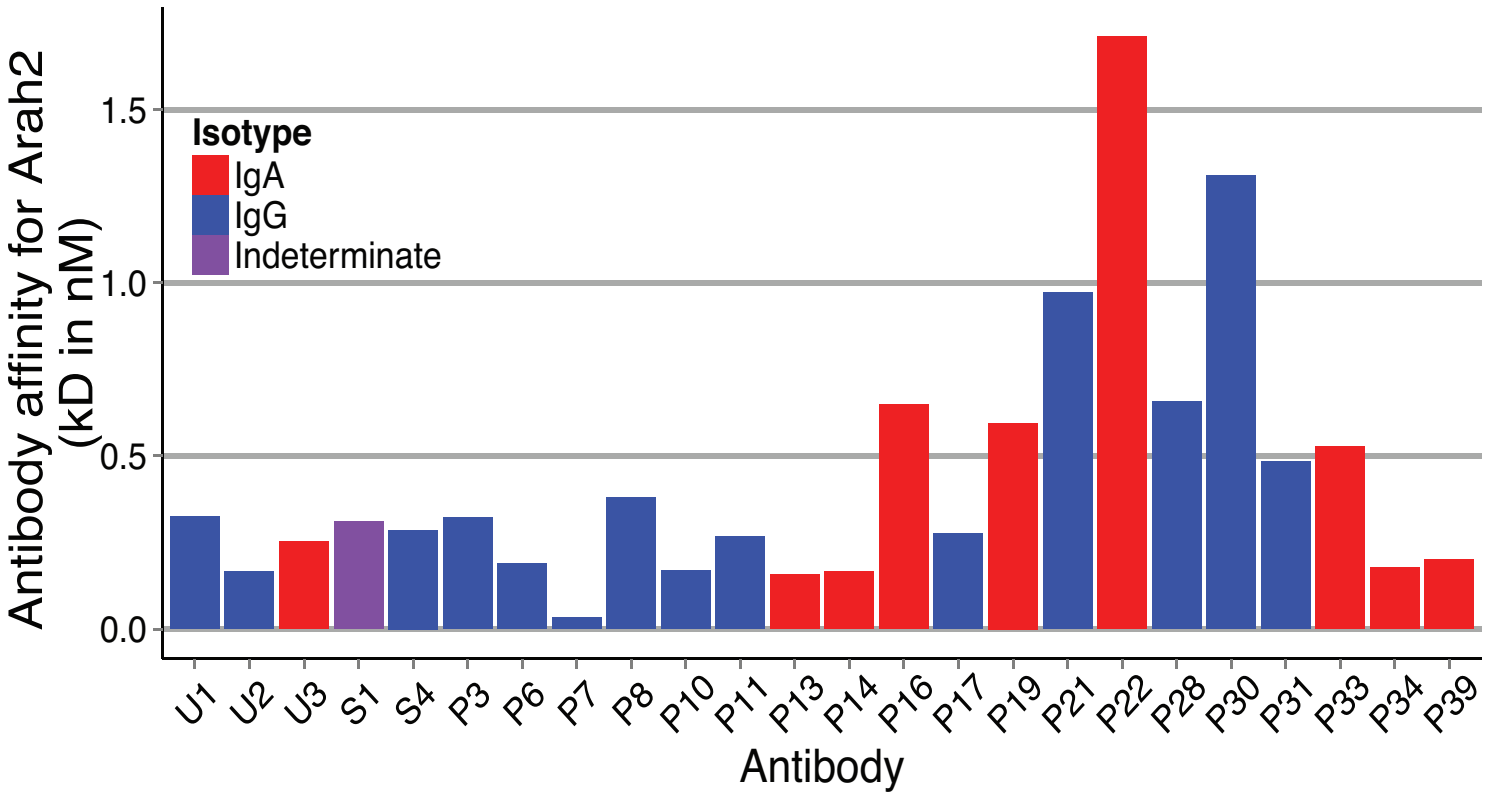

CDR replacement mutations over total mutations (R_{CDR}/M_V)
Total mutations (M_V)
0
10
20
30
40

Figure E8


Antibody affinity for Arah2
(kD in nM)
1.5
1.0
0.5
0.0
Isotype
IgA
IgG
Indeterminate
U1
U2
U3
S1
S4
P3
P6
P7
P8
P10
P11
P13
P14
P16
P17
P19
P21
P22
P28
P30
P31
P33
P34
P39
Antibody