SARS-Cov-2-, HIV-1-, Ebola-neutralizing and anti-PD1 clones are predisposed

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Abstract

Antibody repertoire refers to the totality of the superbly diversified antibodies within an individual to cope with the vast array of possible pathogens. Despite this extreme diversity, antibodies of the same clonotype, namely public clones, have been discovered among individuals. Although some public clones could be explained by antibody convergence, public clones in naïve repertoire or virus-neutralizing clones from not infected people were also discovered. All these findings indicated that public clones might not occur by random and they might exert essential functions. However, the frequencies and functions of public clones in a population have never been studied. Here, we integrated 2,449 Rep-seq datasets from 767 donors and discovered 5.07 million public clones – ~10% of the repertoire are public in population. We found 38 therapeutic clones out of 3,390 annotated public clones including anti-PD1 clones in healthy people. Moreover, we also revealed clones neutralizing SARS-CoV-2, Ebola, and HIV-1 viruses in healthy individuals. Our result demonstrated that these clones are predisposed in the human antibody repertoire and may exert critical functions during particular immunological stimuli and consequently benefit the donors. We also implemented RAPID – a **R**ep-seq **A**nalysis **P**latform with **I**ntegrated **D**atabases, which may serve as a useful tool for others in the field.

Keywords: antibody repertoire, public clone, neutralizing antibody, therapeutic antibody, analysis platform

Background

Antibody is a critical immunoglobulin complex consisting of two identical heavy and two identical light chains. Each chain is encoded by selectively recombining one of the various germline gene fragments, namely variable (V), diversity (D, for heavy chain only), and joint (J) genes. The sequence between V gene end and J gene start is called complementarity determining region 3 (CDR3) which is extremely diverse because of the random

65 nucleotide insertion and deletion in the junctions and by large defines the binding specificity of an antibody[1].
66 This binding specificity makes antibodies favorable for therapeutic purposes.

67 With tremendous efforts and various techniques, many monoclonal antibodies (mAbs) targeting distinct
68 viruses and proteins were discovered in the past decades[2,3]. However, the primary barrier to studying
69 antibodies is their immense diversity. The total number of antibodies in an adult, termed antibody repertoire, is
70 estimated to be around 10^{12} – a number far out of reach for these traditional methods[4].

71 Fortunately, antibody repertoire sequencing (Rep-seq) was invented to acquire millions of antibody variable
72 regions in DNA or RNA form in a single experiment, a great advance thanks to the advent of high-throughput
73 sequencing (HTS) technology. With the aid of this technique, our understanding of the humoral immunity was
74 markedly advanced and many valuable mAbs were identified. For instance, we and others have used Rep-seq
75 method to discover HIV-1 broad neutralizing antibodies[5-7]. It also helped researchers in identifying
76 neutralizing antibodies in the recent SARS-CoV-2 outbreak[8-10]. Thus far, Rep-seq has been proved to be
77 productive in studying cancer immunology[11], virus infection[12,13], vaccination[14,15], etc.

78 Besides the achievement aforementioned, this data-rich method also led to the finding of public clone –
79 antibodies in different individuals but share the same or similar CDR3 which implies the same binding
80 specificity. The fraction of public clones between two individuals is estimated to be ~0.95% to 6%[16,17] in
81 circulating repertoire, or linearly correlate with the product of total clones of the sample pair[18]. They were
82 found in individuals infected with the same virus and thus implicated the antibody convergence, a phenomenon
83 in which antibodies are assimilated to each other[19]. Later, they were also present in B1 and marginal zone B
84 cells in the naïve state[20]. For instance, Soto *et al*. revealed public clones in cord blood[16]. Intriguingly, Jardine *et*
85 *al.* found VRC01-class HIV-1 neutralizing antibody clones in naïve B cells from healthy individuals[21]. Kreer *et*
86 *al.* discovered SARS-CoV-2-neutralizing clones in uninfected healthy people[8]. All these studies were
87 conducted on a limited number of samples, the answers to some of the key questions about public clone
88 remained unsolved. What proportion of an antibody repertoire is public at a population level? What are the
89 other public clones existing in the human repertoire? Have they undergone maturation process? What are their
90 functions? Do they influence our health during disease onset or virus infection?

91 Bearing these questions in mind, we collected 88,059 known antigen-binding or disease-associated antibodies
92 published before, 521 therapeutic antibodies recorded by the World Health Organization (WHO)[22], and 2,449
93 high-quality Rep-seq datasets (767 donors, 306 million clones, and 7.12 billion raw reads) published by others
94 as well as generated in our lab. Integrative and systematic analysis revealed that there are around ~ 10% or
95 more public clones for each individual in a population level. Three thousand three hundred and ninety of these
96 public clones can be annotated indicating they are functionally important for humoral immune response. More
97 importantly, we found public clones that binding to PD1, neutralizing SARS-CoV-2, Ebola, and HIV-1 viruses
98 in healthy individuals. These results demonstrated that public clones in the population are predisposed in the
99 repertoires of particular individuals who may later benefit from their existence upon virus infections and
100 disease onset. All datasets in this study were integrated and implemented in RAPID – **R**ep-seq **A**nalysis
101 **P**latform with **I**ntegrated **D**atabase, a knowledge-rich platform for others to analyze and annotate their own
102 repertoire data.

103

104 # Result

105 **RAPID: a powerful platform for Rep-seq data analysis**

106 Currently, a substantial number of tools or web servers have been proposed to address the issues of Rep-seq
107 data analysis or characterization for repertoires[23-40]. However, these platforms focus on analyzing Rep-seq
108 dataset individually and ignoring exploration of discriminating repertoire features within or among groups.
109 Apart from that, antibody databases are also specialized for antigen annotation, such as bNAber which just
110 documents HIV broadly neutralizing antibodies[41]. Thus, our platform named RAPID which compensates for
111 shortages above was built. As shown in Fig. 1a, the data repositories comprised three different data modules,
112 namely Rep-seq data collection, therapeutic antibody collection, and known antibody collection. The Rep-seq
113 data integrated 2,449 high-quality datasets (see Yang *et al.* for method[18]) from 767 donors either downloaded

114 from published data repository or generated in our lab. These datasets contain samples from different genders,
115 various tissues, immune status, and age spans, and were generated via different amplification strategies. Thus,
116 it provided a rich source of reference for analyzing and comparing antibody repertoires. There are 7.12 billion
117 reads and 306 million clones yielded from a systematic analysis pipeline using exactly the same criteria, thus
118 making them comparable to each other[18]. The therapeutic monoclonal antibodies (mAbs) were downloaded
119 from the Thera-SAbDab database which contains 521 therapeutic mAbs of different types at various stages.
120 The 88,059 known antibodies were downloaded from multiple data repositories and carefully annotated via
121 natural language processing method as well as manual check (Supp. Fig. 1 and Materials and Methods). These
122 annotations included antibody sequences of different chain types as well as the antibodies that binding to and
123 neutralizing virus, associating to particular diseases, etc. The raw sequences, CDR3s, descriptions, and sample
124 metadata information were systematically extracted and stored in a rational database as well as FASTA format
125 files when necessary. A user-friendly interface for searching particular terms, antibodies, and CDR3s (Supp.
126 Fig 2) was also provided (https://rapid.zzhlab.org/).

127 The data analysis module allows users to streamline their own data through a versatile pipeline. Apart from the
128 general low- and high-level analyses, the RAPID also provides some helpful features as described below (Fig.
129 1b). 1) Customizing germline reference. 2) Customizing reference datasets; the users can freely select one or
130 more datasets in the platform as reference for cross-comparisons purpose. 3) Automatic antibody annotation;
131 the CDR3s from the input dataset will be automatically compared to the CDR3s in the data repository on
132 RAPID and annotated where applicable. 4) Downloadable figures and analysis result. Thus, any researcher can
133 upload their datasets and cross compare them to 2,449 datasets with 306 million clones, all therapeutic
134 antibodies, and 88,059 known antibodies and retrieve the relevant information. With the thorough antibody
135 collections and a versatile analysis platform, we believe the RAPID will be helpful for the large cadre of
136 scientists who demand analyzing antibody repertoire data as we demonstrated in this study.

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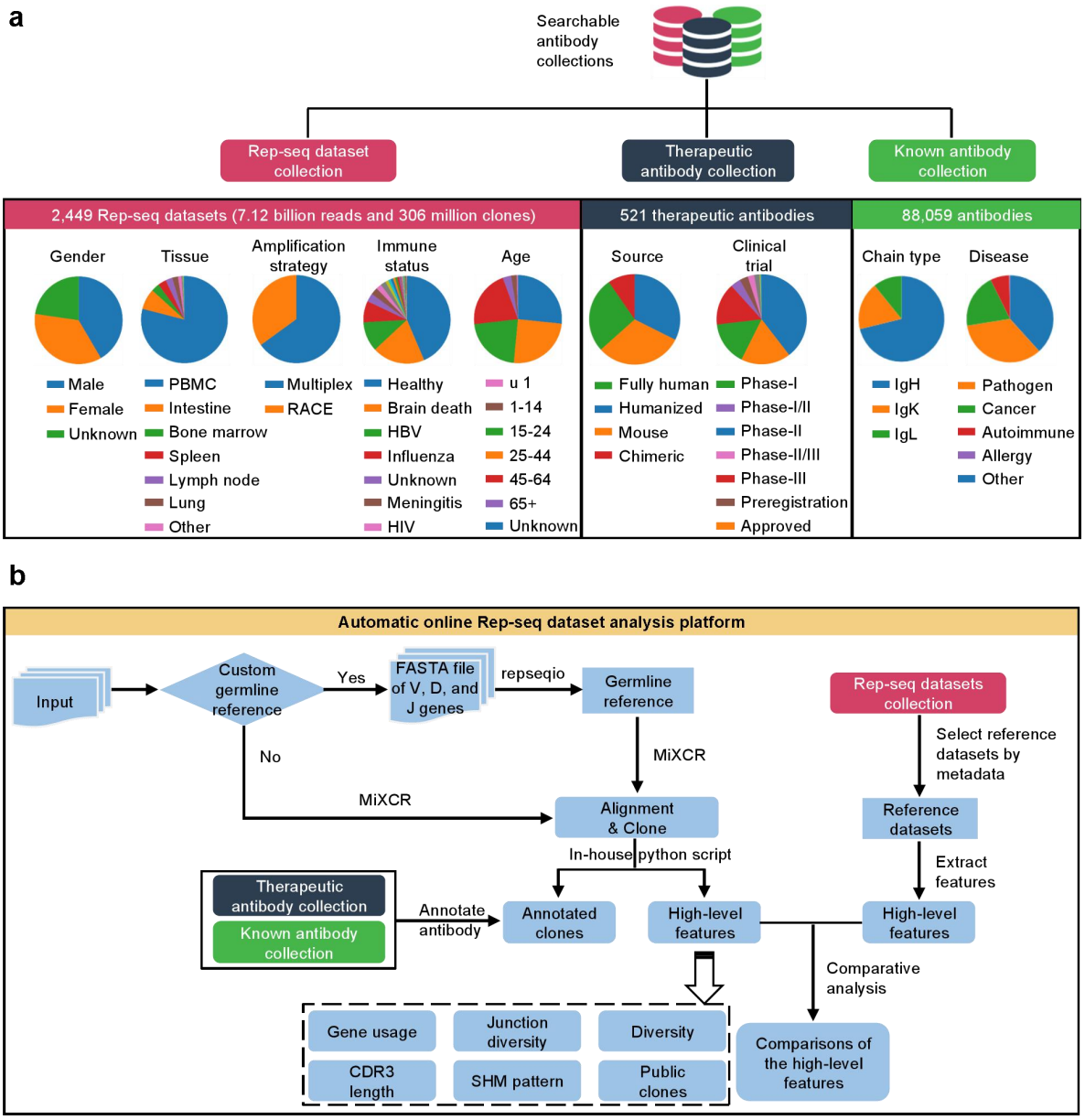

Figure 1 Data composition and automatic online Rep-seq dataset analysis pipeline of the RAPID. (a) Composition and detailed information of the three types of antibody datasets: Rep-seq datasets, therapeutic antibodies and known antibodies. The pie charts in the lower panel showed detailed composition of each type of antibodies. **(b)** The analysis platform for Rep-seq dataset.

Public clones are prevalent in the population

With this unprecedented dataset, we started in-depth inspection of the public clones. Here, we defined public clones as antibodies with the same CDR3 amino acid sequence that present in more than one donor. Even with

149 this stringent criterion, we discovered 5,077,372 public clones. Typically, the public clones represent ~1.23 to
150 100 percent of each individual repertoire with a peak value of 10.46 percent (Fig. 2a). Moreover, 65 public
151 clones occur in more than 100 individuals with one clone shared by 196 individuals (25.55% of the total
152 donors) (Supp. Fig. 3 a and b). Thus, population level study helped us find more public clones and highly
153 frequent ones. As 96.86% of the public clones are from PBMCs (Supp. Fig. 3c), we compared their SHMs and
154 clone fractions of the clones in different groups. The SHMs for naïve, memory, and plasma groups were
155 comparable between public clones and total clones. The public clones of PBMCs and unknown samples
156 displayed mediocre SHMs between naïve and non-naïve clones (Fig. 2b, upper panel). For clone fractions, the
157 public clones from PBMCs and unknown samples were lower than the other counterparts (Fig. 2b, lower panel)
158 indicating they are inactivated. On the other hand, about half of the public clones were from IgM isotype (Fig.
159 2c, lower panel). Therefore, it's reasonable to speculate that majority of these public clones were acquired
160 from naïve and lowly-mutated memory B cells.

161 We also observed that different V and J gene combinations can yield the same CDR3s. As expected, the
162 diversity of V gene usage for public clones increased when clones are shared by more donors (Supp. Fig. 4).
163 However, when normalized to the maximum theoretical diversity with particular number of V genes (see
164 Materials and Methods), this diversity slightly decreased with the increment of sharing donors indicating
165 recombination preference of V genes (Fig. 2c, upper panel). Careful examinations of the V and J genes that
166 formed the same CDR3s showed that same J gene was always preferred while V gene was more replaceable
167 among individuals (Fig. 2d and Supp. Fig. 5). Nevertheless, the substitution rates of V genes were not
168 completely influenced by their sequence similarity (Fig. 2d). This result suggested that J genes might affect the
169 CDR3s more than V genes do.

170 Taken advantage of the rich antibody information integrated in this study, we tried to annotate these public
171 clones. Totally, 3,390 public clones have been annotated by three antibody databases including known
172 antibody, Thera-SAbDab, and Coronavirus-neutralizing antibody incorporated with 459 mAbs from CoV-
173 AbDab[42], 28 mAbs from Kreer *et al.*[8], and 19 mAbs from Liu *et al.*[10] (Fig. 2e). We found that 3,349 out 3,390
174 clones shared the same CDR3s amino acid sequences with known antibodies targeting specific antigens or

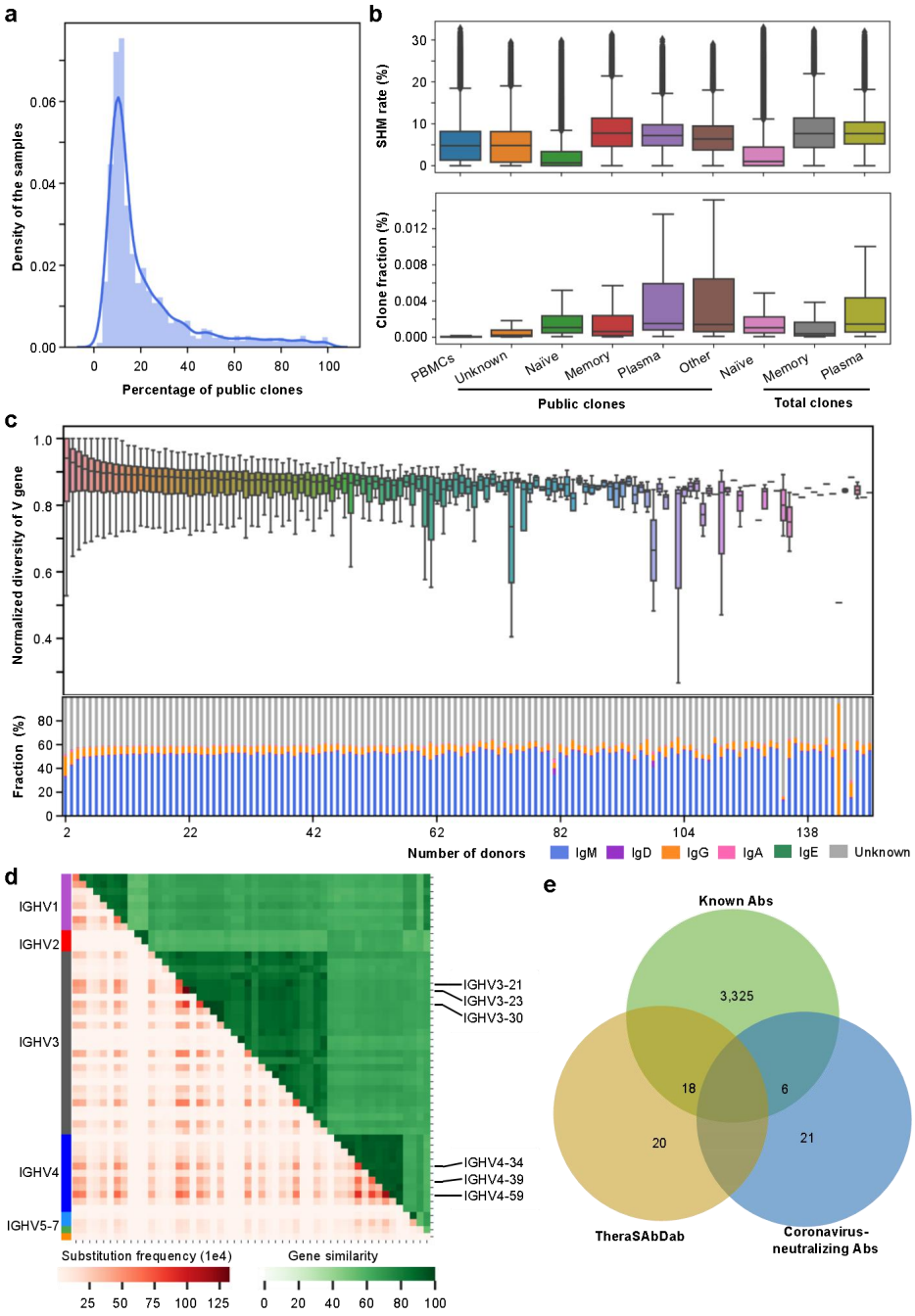

Figure 2 The characteristics of public clones. (a) The sample density distribution with regard to the fractions of public clones. X-axis represents the percent of public clones in a sample. Y-axis shows the sample density. **(b)** Comparisons of the distribution of somatic hypermutation rates (SHMs, upper panel) and clone fractions (lower panel) among different groups of clones. **(c)** Upper panel: The normalized Shannon index of V gene usage (Y-axis) within each CDR3 aa group sorted by the respective numbers of shared donors (X-axis). Lower panel: The stacked bar plot showed the composition of public clones for different antibody isotypes. **(d)** The substitution frequencies (lower left panel) and percent identities of V genes (upper right panel). **(e)** The overlap of 3,390 annotated public clones with known antibodies, thera-SAbDab, and Coronavirus-neutralizing antibodies.

186 associating with diseases. Surprisingly, we found 38 and 27 CDR3s overlapping with Thera-SAbDab and
187 Coronavirus-neutralizing antibodies. Among 27 Coronavirus-binding clones, 8 of them target SARS-CoV-2
188 (CARGDSSGYYYYFDYW binds both SARS-CoV-1 and SARS-CoV-2). As all these Rep-seq datasets were
189 generated before the outbreak of the COVID-19, these SARS-CoV-2-neutralizing clones and therapeutic
190 clones were deposited in the antibody repertoire. These provide evidence that those therapeutic and antigen-
191 specific antibodies exist widely in populations and can be a powerful source for mAb discovery with clinical
192 purposes.

193 **Therapeutic mAb clones are prevalent in healthy people's repertoire**

194 We went on to look into the details of the 3,390 annotated public clones. Among them, 3,354 (98.94%) were
195 found in at least one healthy sample (Fig. 3a, upper panel). The therapeutic antibodies found in this data
196 collection compromise 41 therapeutic mAbs with 38 unique CDR3s. Of these, six are under phase III clinical
197 trial, two are under preregistration and nine are approved for clinical usage (Fig. 3a, lower panel). Interestingly,
198 only 14 (34.15%) mAbs are fully human while the others include 14 (34.15%) humanized, 12 (29.27%) from
199 mouse, and one chimeric. This indicated that therapeutic antibodies generated by mouse model can be
200 generated by our own immune system. Thus, public clone might be a better source for mAb discovery for
201 clinical usage.

202 Also, many of the therapeutic antibody clones found in healthy people which are used for treatment of diseases
203 with top causes of death in the world (Table 1) prevailed in the population (Fig. 3, a and b). For instance,
204 Evolocumab targeting PCSK9 is used to treat Coronary disorders, Stroke, and Hypercholesterolaemia. Stroke
205 alone caused 5.78 million deaths worldwide in 2016[43] and this clone was found in 108 (14.1% of the total of
206 767) donors' repertoire. The CDR3 of anti-PD1 (Camrelizumab), the treatment to various cancers, was found
207 in 14 donors' repertoire. Ramucirumab targeting KDR and Enfortumab targeting PVRL4 were also found in 23
208 and 49 donors, respectively. According to the percent identities to therapeutic antibodies, most of the
209 antibodies from the same clonotype separated into at least two groups (Fig. 3b and Supp. Fig. 6). Detailed
210 inspection revealed that multiple V genes involved in the recombination, again supported the diversity of V
211 genes within clones (Fig. 2d). These antibodies might serve as therapeutic alternatives for the same disease.

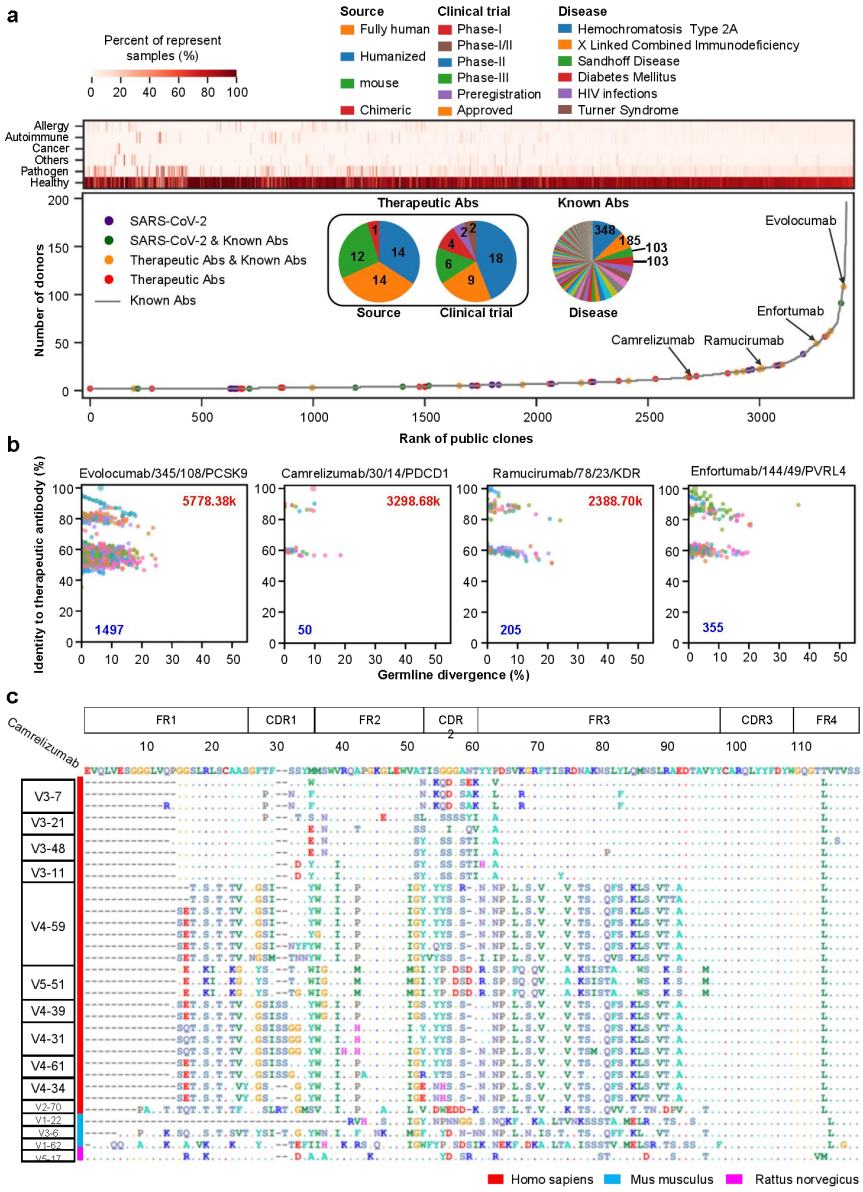

Figure 3 The characteristics of annotated public clones. (a) Overview of 3,390 annotated public clones. The upper heatmap stand for the composition of samples for these annotated public clones. Samples were divided into 6 groups including allergy, autoimmune, cancer, pathogen, healthy, and others. The bottom line chart means the number of donors. Public clones annotated by known antibody database were shown by gray line, while those annotated by other databases were shown by scatters filled in particular colors. The center top pie charts show distribution of source and clinical trial for therapeutic antibodies and associated disease for known antibodies. **(b)** Identity of variable region sequences from FR1 to FR3 of antibodies whose CDR3aa are same as Evolocumab, Camerelizumab, Ramucirumab, and Enfortumab. The X-axis means the germline divergence and different colors of scatters mean different V genes. Numbers filled in red stand for the death caused by diseases treated by such antibody and those filled in blue stand for the number of variable regions identified from Rep-seq datasets. Titles for sub-figures separated by forward slash include inn id of therapeutic antibody, the number of samples and donors with such CDR3aa, and target of therapeutic antibody. Dots for therapeutic antibodies are larger than that of clones identified from Rep-seq datasets. **(c)** Multiple sequences alignment of variable region for antibodies with the same CDR3aa as Camerelizumab from homo sapiens, mus musculus, and rattus norvegicus. The amino acid sequence of Camerelizumab is listed in the top and regions (FR1-FR4) for variable region are marked by boxes above. V gene used in each sequences are labeled in left boxes.

Table 1 Detailed information of therapeutic antibodies whose CDR3aa were same as those identified from Rep-seq datasets

INN	CDR3aa	# Donor	# Sample	Source	Target	Disease	Death_rate	Total_death_rate	# Total_death (k)
Evolocumab	CARGYGMDVW	108	345	fully human	PCSK9	Coronary disorders,Hypercholesterolaemia,Hyperlipidaemia,Hyperlipoproteinaemia type IIa,Myocardial infarction,Stroke	-,-,-,-,-,10.16	10.16	5778.38
Azintuxizumab	CARDRGYYFDYW	62	248	chimeric	SLAMF7	-	-	-	-
Crenezumab	CASGDYW	58	181	humanized	APP	Alzheimer's disease	3.5	3.5	1990.58
Solanezumab	CASGDYW	58	181	humanized	APP	Alzheimer's disease	3.5	3.5	1990.58
Landogrozumab	CARLPDYW	56	189	humanized	MSTN	Cachexia,Muscular atrophy,Pancreatic cancer	-,-,0.65	0.65	369.68
Enfortumab	CARAYYYGMDVW	49	144	fully human	PVRL4	Urogenital cancer	-	-	-
Dusigitumab	CARDPYYYYYGMDVW	27	89	fully human	IGF1&IGF2	-	-	-	-
Eptinezumab	CARGDIW	26	67	humanized	CALCA&CALCB	Migraine	0	0.00	0.00
Ramucirumab	CARVTDAFDIW	23	78	fully human	KDR	Biliary cancer,Carcinoid tumour,Colorectal cancer,Gastric cancer,Liver cancer,Non-small cell lung cancer,Oesophageal cancer,Pancreatic cancer,Solid tumours,Urogenital cancer	-,-,-,1.34,1.46,-,0.75,0.65,-,-	4.2	2388.70
Daclizumab	CARGGGVFDYW	22	70	humanized	IL2RA	Multiple sclerosis,Renal transplant rejection	0.04,-	0.04	22.75
Bimagrumab	CARGGWFDYW	20	47	fully human	ACVR2B	Cachexia,Muscular atrophy,Type 2 diabetes mellitus	-,-,2.81	2.81	1598.15
Glembatumumab	CARGYNWNYFDYW	19	67	fully human	GPNMB	-	-	-	-
Zanolimumab	CARVINWFDPW	18	67	fully human	CD4	-	-	-	-
Valanafusp	CAREWAYW	15	54	mouse	INSR	Mucopolysaccharidosis I	-	-	-
Camrelizumab	CARQLYYFDYW	14	30	humanized	PDCD1	Biliary cancer,Breast cancer,Cervical cancer,Cholangiocarcinoma,Colorectal cancer,Diffuse large B cell lymphoma,Endometrial cancer,Fallopian tube cancer,Gastric cancer,Hodgkin's disease,Liver cancer,Malignant melanoma,Nasopharyngeal cancer,Non small cell lung cancer,Oesophageal cancer,Osteosarcoma,Ovarian cancer,Pancreatic cancer,Peritoneal cancer,Renal cell carcinoma,Solid tumours,Urogenital cancer	-,1.03,-,-,-,-,-,-,1.34,-,1.46,-,-,-,0.75,-,0.29,0.65,-,0.28,-,-	5.8	3298.68
Ascrinvacumab	CARESVAGFDYW	14	37	fully human	ACVRL1	Age-related macular degeneration,Cancer,Liver Cancer	-,-,1.46	1.46	830.36
Cobolimab	CASMDYW	12	23	mouse	HAVCR2	Non-small cell lung cancer, Solid tumours	-,-	-	-
Reslizumab	CAREYYGYFDYW	10	17	humanized	IL5	Asthma,Churg-Strauss syndrome,Sinusitis	0.73,-,-	0.73	415.18
Tesidolumab	CARDTPYFDYW	10	15	fully human	C5	Paroxysmal nocturnal haemoglobinuria	-	-	-
Prasinezumab	CARGGAGIDYW	9	39	humanized	SNCA	Parkinson's disease	0.38	0.38	216.12
Gatralimab	CTPIDYW	9	15	mouse	CD52	-	-	-	-
Utomilumab	CARGYGIFDYW	8	18	fully human	TNFRSF9	B-cell lymphoma,Breast cancer,Colorectal cancer,Diffuse large B cell lymphoma,Follicular lymphoma,Oropharyngeal cancer,Ovarian cancer,Solid tumours	-,1.03,-,-,-,-,0.29,-	1.32	750.73
Bemarituzumab	CARGDFAYW	7	18	humanized	FGFR2	Gastric cancer,Oesophageal cancer,Solid tumours	1.34,0.75,-	2.09	1188.66
Bersanlimab	CARYSGWYFDYW	6	14	mouse	ICAM1	-	-	-	-

Cont. Table 1

INN	CDR3aa	# Donor	# Sample	Source	Target	Disease	Death_rate	Total_death_rate	# Total_death (k)
Iratumumab	CASLTAYW	5	6	fully human	TNFRSF8	-	-	-	-
Mosunetuzumab	CARDSYSNYYFDYW	5	5	humanized	CD3E, MS4A1	Chronic lymphocytic leukaemia,Diffuse large B cell lymphoma,Non-Hodgkin's lymphoma	-,-,-	-	-
Emibetuzumab	CARANWLDYW	4	4	humanized	MET	Cancer,Gastric cancer,Non-small cell lung cancer	-,1.34,-	1.34	762.11
Otilimab	CARGFGTDFW	4	7	mouse	CSF2	Rheumatoid arthritis	0.1	0.10	56.87
Vorsetuzumab	CARDYGDYGMDYW	4	6	humanized	CD70	-	-	-	-
Gatipotuzumab	CTRHYYFDYW	3	9	humanized	MUC1	Ovarian cancer,Solid tumours	0.29,-	0.29	164.93
Pankomab	CTRHYYFDYW	3	9	mouse	MUC1	Ovarian cancer,Solid tumours	0.29,-	0.29	164.93
Brodalumab	CARRQLYFDYW	3	5	fully human	IL17RA	Erythrodermic psoriasis,Plaque psoriasis,Psoriasis,Psoriatic arthritis,Pustular psoriasis,Spondylarthritis,Systemic scleroderma	-,-,-,-,-,-,-	-	-
Clervonafusp	CARRGLLLDYW	3	3	mouse	SLC29A2	Glycogen storage disease type II	-	-	-
Dectrekumab	CARLWFGDLDAFDIW	3	3	fully human	IL13	-	-	-	-
Ivuxolimab	CARESGWYLFDYW	2	3	mouse	TNFRSF4	Acute myeloid leukaemia,Breast cancer,Follicular lymphoma,Renal cell carcinoma,Solid tumours,Squamous cell cancer	-,1.03,-,0.28,-,-	1.31	745.05
Cinpanemab	CTSAHW	2	2	mouse	SNCA	Parkinson's disease	0.38	0.38	216.12
Atoltivimab	CARNWNLFDYW	2	2	mouse	Zaire Ebolavirus GP	-	-	-	-
Dinutuximab	CVSGMEYW	2	2	mouse	Ganglioside GD2	Neuroblastoma,Small cell lung cancer	-,-	-	-
Lorukafusp	CVSGMEYW	2	2	mouse	Ganglioside GD2	Malignant melanoma,Neuroblastoma	-,-	-	-
Lupartumab	CAREGLWAFDYW	2	4	fully human	LYPD3	Solid tumours	-	-	-
Tildrakizumab	CARGGGGFAYW	2	3	humanized	IL23A	Ankylosing spondylitis,Intervertebral disc degeneration,Non-radiographic axial spondyloarthritis,Plaque psoriasis,Psoriatic arthritis	-,-,-,-,-	-	-

Note: Cells filled in gary mean that there are no applied disease for these therapeutic antibodies.

234 Moreover, for therapeutic clones, there were always Rep-seq captured antibodies showing higher SHM rates
235 (Fig. 3b), possibly indicated the engagement of maturation process under the selective pressure of their
236 antigens.

237 Interestingly, we also found antibodies with the same CDR3s as anti-PD1 antibody in the repertoires of mouse
238 and rat. The variations in the other regions of variable sequences (Fig. 3c) indicated that they were purposely
239 selected and retained in the repertoire. Moreover, the differences in their structure might be the result of
240 structural variations of PDCD1 proteins in different species (Supp. Fig. 7). Finding anti-PD1 antibody clones
241 in healthy individuals and other species was exciting. On the contrary, no anti-PD1 clone was found in any of
242 the 56 samples of various cancers – 2 breast cancer, 49 colorectal cancer, and 5 liver cancer samples. Why
243 cancer patients do not possess anti-PD1 antibody clones is an intriguing question. Understanding the
244 mechanism behind this fact may lead to a better understanding of cancer immunology and more effective
245 immunological therapy. Besides, further studies illustrating the causes and consequences of the anti-PD1
246 clones in particular individuals would also benefit the field.

247 **SARS-CoV-2-, Ebola-, and HIV-1-neutralizing antibody clones are predisposed**

248 On a par with the results in finding therapeutic clones, multiple clones neutralizing SARS-CoV-2, Ebola, and
249 HIV-1 were also uncovered from virus-naïve individuals. For SARS-CoV-2-neutralizing clones MT658807,
250 MT658819, and 1-20, there were 20, 506, and 222 heavy chain variable regions extracted from repertoires of 5,
251 17, and 6 healthy donors, respectively. Twenty-five variable regions from 2 healthy donors sharing the same
252 CDR3 sequence with HIV-1-neutralizing class VRC01 were also extracted. In addition, 2,663 variable regions
253 from 4 donors injected with influenza vaccine contained the CDR3 of Ebola-neutralizing antibody of
254 MK901823[44]. Although neutralizing clones to HIV-1 and SARS-CoV-2 were reported to exist in the repertoire
255 of the naïve B cell previously[8,21], this is the first time to identify these neutralizing clones in multiple people.
256 Thus, we concluded that they are predisposed in a population.

257 We then set off to explore the maturation pathways of these neutralizing clones by analyzing the phylogenetic
258 trees of each clone built via DNAMLK (Fig. 4a-d). The Ebola-neutralizing clones exhibited high maturation
259 rates with IgG. Interestingly, the maturation rates of three SARS-CoV-2 clones demonstrated various level of

260 SHMs. While the overall SHM rate for MT658807 clone is lower than 2.5%, some of the antibodies in
261 MT658819 and 1-20 clones displayed more than 5% mutations. Previous studies reported the general lower
262 SHM for SARS-CoV-2-neutralizing antibodies but still some clones with more mutations were identified and
263 verified[8-10]. Therefore, different clones might subject to different selective pressure and consequently manifest
264 various SHM rates. Although the diversity of V gene usage in neutralizing clones by large defined the
265 topology of the phylogenetic trees, oftentimes antibodies from different individuals aggregating in the same
266 branch was observed. This indicated antibody convergence in the same maturation pathway.

267 Apart from the MK901823 clone, which was from a sample after influenza vaccine trivalent, inactivated
268 seasonal influenza (TIV), all the donors of the HIV-1 and SARS-CoV-2 clones are virus-free healthy
269 individuals. Furthermore, we found the same antibodies of MT658807 in donor 1776. This again confirmed the
270 predisposition of this neutralizing clone. However, what triggers their maturation would be an important
271 question to answer for future studies.

272 To further explore their possibility of virus binding, we compared the structures of these Rep-seq retrieved
273 antibodies to their corresponding verified neutralizing antibodies. As shown in Fig. 4a-d, we found antibodies
274 were very similar to MT658819[8] (RMSD: 0.165) and 1-20[10] (RMSD: < 0.001) that neutralizing SARS-CoV-2,
275 MK901823[44] (RMSD: 0.802) that neutralizing Ebola, and KU760937[21] (RMSD: 0.459) that neutralizing HIV.

276 To validate this similarity, we performed pair-wise structure comparison among antibodies the neutralizing
277 these three viruses. As shown in Fig. 4e, the RMSD scores of clone targeting the same antigen were much
278 lower than those targeting different antigens. Thus, the high similarity of Rep-seq retrieved antibodies to
279 neutralizing antibodies are reliable.

280 **SARS-CoV-1-neutralizing and therapeutic antibody clones exist in animals**

281 Inspired by the existence of anti-PD1 clones in mouse and rat, we scrutinized the Rep-seq datasets with four
282 different species, namely Macaca fascicularis, Macaca mulatta, Mus musculus, and Rattus norvegicus. We
283 found 4 SARS-CoV-1-neutralizing and 18 therapeutic clones in at least one species. Taken together, we
284 believe these clones are not randomly generated but purposely selected and disposed in vertebrates' repertoire.

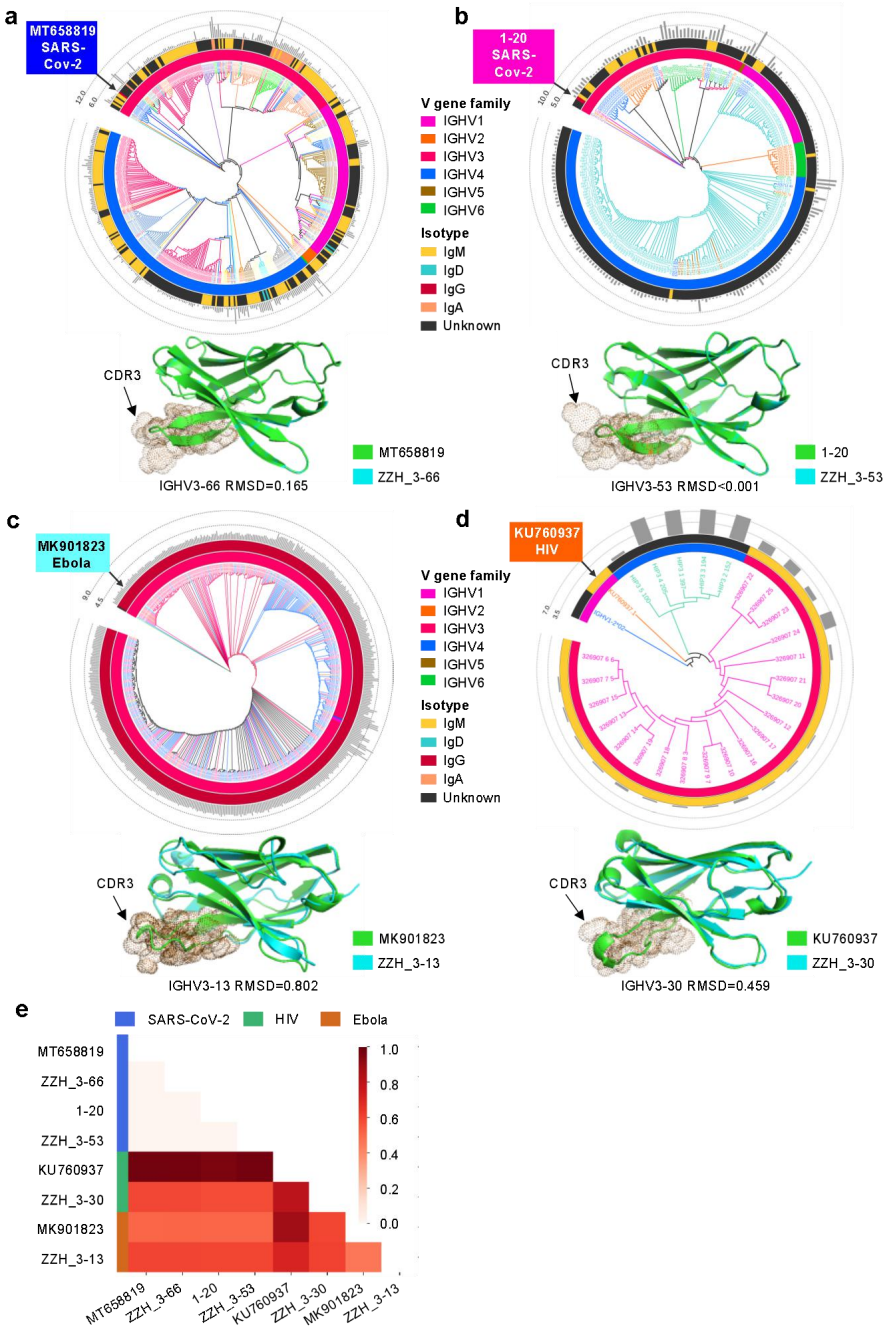

Figure 4 Maturation pathway and structure of antibodies potentially targeting SARS-CoV-2, Ebola, and HIV. Variable region sequences with the same CDR3aa as MT658819 **(a)**, 1-20 **(b)**, MK901823 **(c)**, and KU760937 **(d)** were extracted and compared with those validated neutralizing antibodies and their germline reference. The germline reference was chosen as root of phylogenetic tree and the validated antibodies were marked by arrow. The cluster map contains four layers including similarity of sequences (the sequences extracted from the same donor were marked with the same color), V gene family, isotype, and somatic hypermutation rate from inner to outer. Under each phylogenetic tree, similarity of structures for validated antibody and variable region identified from Rep-seq datasets were shown. **(e)** Pair-wise structure comparison of antibodies targeting SARS-CoV-2, HIV, and Ebola.

Discussions

Public clones are a specific fraction of antibodies among individuals that we know little about. By integrating the largest antibody data to date, population level analyses discovered millions of public clones which represent ~10% or higher fraction of each individual's repertoire. However, compared to the superb diversity of the antibody repertoire, the current dataset might still be smaller than demand. We believe that when more datasets will be integrated, more public clones would be revealed. This is understandable since although the somatic recombination may generate numerous antibodies, majority of them are eliminated during the negative selection process in the bone marrow. Consequently, the once private repertoire might be public[45].

How often can we find these public clones with critical functions in an individual? Are they predisposed in everyone's repertoire? The current data seems to support that only some people possess them. However, we found that sequencing depth is critical for public clone identification as many more public clones were observed in datasets with very high depth. Currently, only a few hundred thousand to a few million reads were captured in general. Compared to the theoretical number of B cells in the sample and the depth needed to identify a clone confidently, much more sequencing reads are demanded. As most of the therapeutic mAbs target proteins of conserved genes such as PDCD1, another helpful practice in finding functional public clones might be comparing antibody repertoires between human and other vertebrates.

The finding of clones that can bind to PDCD1 or neutralize SARS-CoV-2, Ebola, and HIV-1 viruses demonstrated that public clones might be important for the donor's health. Then discovering the functionalities of the vast majority of other public clones would be critical for a deep understanding of the humoral immune system. The major challenge in this regard is the lack of the light chain pair. The techniques of paired heavy and light chain sequencing invented in Georgiou lab[46] and the single cell repertoire sequencing[47] showed great potential in solving this problem.

We'll update RAPID along with the accumulation of Rep-seq datasets generated by others and our lab. We believe more public clones will be identified and their functions will be illustrated along this path.

Methods

Rep-seq datasets enrollment

Method to enroll published and in-house Rep-seq datasets were described in Yang *et al*[18], please refer to it for detailed information. The re-analysis pipeline of these Rep-seq datasets was also included in that paper.

Resources of known antibody

Five open access antibody databases, named abYsis (http://abysis.org/)[48], bNAber (http://bnaber.org/)[41], EMBLIG (http://acrmwww.biochem.ucl.ac.uk/abs/abybank/emblig/), HIV Molecular Immunology Database (HIV-DB: https://www.hiv.lanl.gov/content/immunology/neutralizing_ab_resources.html)[49] and IMGT/LIGM-DB (http://www.imgt.org/ligmdb/)[50] were enrolled. In addition, another two nucleotide sequence databases, including European Nucleotide Archive (ENA) of EMBL-EBI (https://www.ebi.ac.uk/ena/data/view/Taxon:9606&result=coding_release)[51] and National Center for Biotechnology Information (NCBI) Nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide/), were also incorporated. In a word, 7 databases were finally included. The search strategy and download date for them were listed in Supplementary Table 1.

Construction of known antibody database

Although the species was restricted for sequences downloading, some sequences from other species, like Mus musculus, were also included. Thus, we firstly discarded non-human sequences according to descriptions. After that, sequences were aligned to V, D, and J germline reference (downloaded from IMGT: http://www.imgt.org/ and listed in Supplementary Table 2) by IgBLAST[24] (version 1.8.0), as its great performance for error-corrected reads[23]. Based on results of IgBLAST, sequences which meet criteria were reserved, including in-frame, productive, with V, J, and CDR3, without either stop codon or out-of-frame in variable region, and without ambiguous base (N) in CDR3. Sequences with the same nucleotide sequences of variable region within the same database were de-duplicated. To remove non-antibodies from NCBI and ENA, we aligned these sequences to NCBI Nt database (downloaded from NCBI:

343 ftp://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/nt.gz) and discarded the sequences whose descriptions contain no
344 keywords we defined (Listed in Supplementary Table 3). These keywords were selected from descriptions of
345 the antibody sequences are stored in the database of abYsis, bNAber, HIV-DB, EMBLIG, and IMGT/LIGM-
346 DB. Furthermore, antibodies from 7 databases were pooled together and de-duplicated according to the
347 nucleotide sequence of variable region. In the end, disease information for antibodies from EMBLIG, ENA,
348 IMGT/LIGM-DB, and NCBI was annotated by TaggerOne[52] based on description, title, and abstract of
349 sequences. The sequences from abYsis were annotated as "NA", as no annotation information can be
350 downloaded. The sequences from HIV-DB and bNAber were annotated as HIV infections.

351 **Implementation of RAPID**

352 The web interface is implemented by Hyper Text Markup Language (HTML), Cascading Style Sheets (CSS),
353 and JavaScript (JS). It is a single page application based on the JS framework React.js, while using the React
354 component library Ant Design to unify the design style. The back end of the website uses Nginx as the HTTP
355 and reverse proxy server, develops business logic based on Node.js, uses MySQL to manage data, and uses
356 RabbitMQ to process the analysis task queues. Furthermore, the real-time notification of task progress depends
357 on the WebSocket technology.

358 **Extraction of variable region identified from Rep-seq dataset**

359 Firstly, if regions from FR1 to FR4 were reported by MiXCR, we would simply join them together as variable
360 region. For sequences whose FR1 to FR4 regions were not completely reported by MiXCR, we extracted them
361 using our algorithm: I) Reads which can not be merged by MiXCR were discarded; II) The beginning of
362 variable region was acquired by pairwise alignment between germline reference of V genes and the column
363 named "targetSequence" reported by MiXCR(The function *pairwise2.align.localms* from Python *Bio module*
364 was used with parameters 2, -3, -5, and -2); III) If the column named "refPoints" in MiXCR recorded the
365 region of FR4, we would use it instead of aligning "targetSequence" to J gene to find the end of FR4..

366

367

368 **Calculation of gene usage diversity**

369 The Shannon index was used to show the diversity of gene usage of public clones. However, as the number of
370 V genes influences the diversity largely, we used the maximum of diversity with particular number of V genes
371 to normalized the diversity. The function to calculate the normalized diversity is shown below. When different
372 donors use totally different V genes, the normalized diversity equals one.

373

$$Normalized\ V\ gene\ diversity = \frac{\sum_{i=1}^{N} (P_i * \ \ln P_i)}{\ln N}$$

374 P_i means the frequency of specific V gene, i means the order of V gene, and N means the total number of V
375 gene.

376 **Calculation of sequences identity**

377 Both nucleotide and amino acid sequences were aligned by Clustal W 2.1[53] from the Python module named
378 Bio. Gaps at the beginning and ending of aligned sequences were removed and the percentage of matched
379 bases was defined as identity.

380 **Multiple sequence alignment for anti PD-1 antibodies**

381 Variable region sequences with the same CDR3aa of Camrelizumab were extracted from each sample and
382 grouped according to the VJ recombination and CDR3nt. Amino acid sequences of groups with most reads in
383 each individual were used for multiple sequence alignment by Clustal W 2.1 with default parameters and
384 visualized by BioEdit.

385 **Construction of phylogenetic tree**

386 Each phylogenetic tree was generated by the nucleotide sequences of variable regions for antibodies sharing
387 the same CDR3 sequence with MT658807, MT658819, 1-20, MK901823, and KU760937. In addition, the
388 germline V allele of validated neutralizing antibody which was set as the root and validated antibody were also

389 enrolled. Alignments were performed using Clustal W 2.1, and the maximum parsimony trees fitted using
390 DNAMLK by PHYLIP 3.698[54]. Lastly, these phylogenetic trees were displayed and annotated by iTOL[55].

391 **Comparison of antibody structure**

392 As some Rep-seq datasets were amplified by Multiplex PCR, variable regions for these sequences were not
393 complete. Thus, sequences lost several bases at the beginning of the FR1 due to the design of primer set were
394 padded by germline sequences from IMGT. Sequences for validated antibodies were downloaded from NCBI.
395 Variable regions without out-frame were used to predict their structures by Repertoire Builder[56]. Then PyMOL
396 was used to calculate RMSD to compare the similarity of antibody structures.

397

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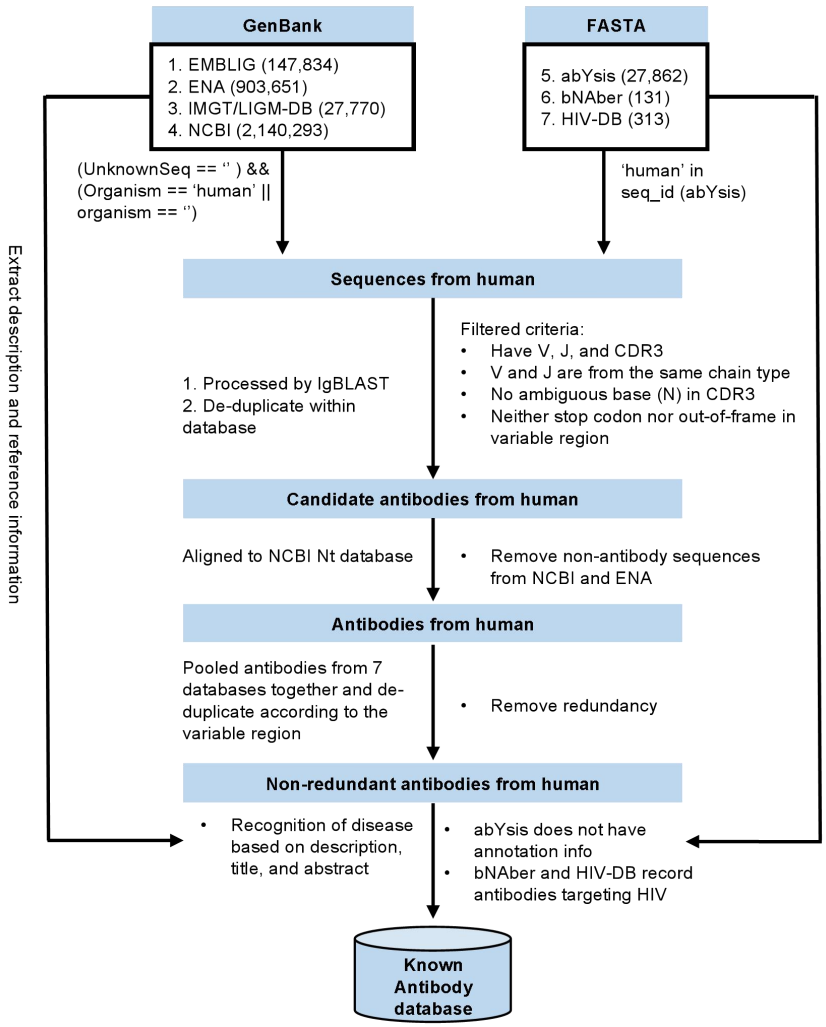
Author Contributions

Y. Z., H. Z., Y. Z., C. L., X. Y., Y. Z., Y. C., Y. Z., J. W., C. W., C. M., and S. C. collected the datasets and performed the bioinformatics analyses. Y. Z. and Q. X. developed and implemented the RAPID platform. M. W., Q. W., H. Tang., W. X., and J. G. collected the samples and conducted the biological experiments. M. W. and S. G. prepared the libraries and ran the Illumina sequencing. C. L. coordinated the project. X. Y. and Z. Z. conceived the project. All authors were involved in the manuscript writing.

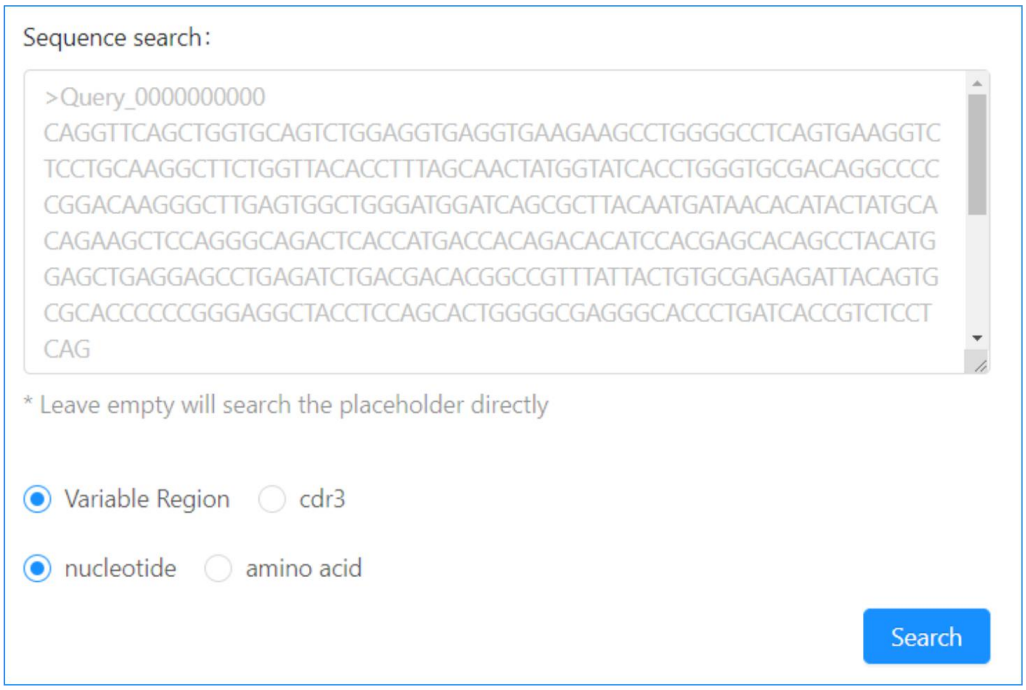
Competing of interests

The authors declared no competing financial interests.



Supp. Fig. 1 Workflow of known antibody database construction. The first two boxes record the total number of sequences downloaded from 7 databases with Genbank and FASTA formats. Each procession on sequences is marked near arrow between intermediate results.

a



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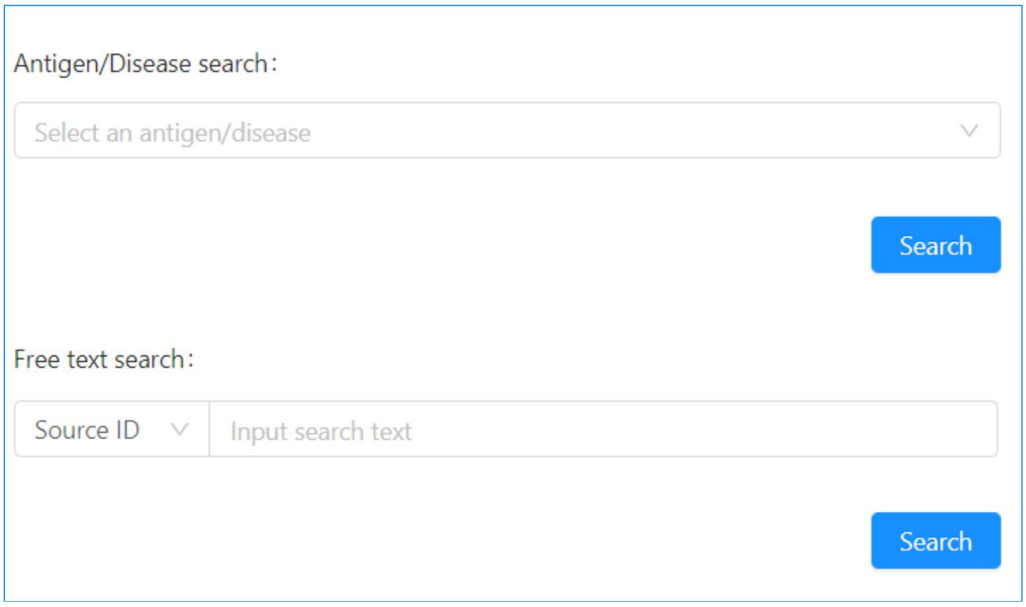
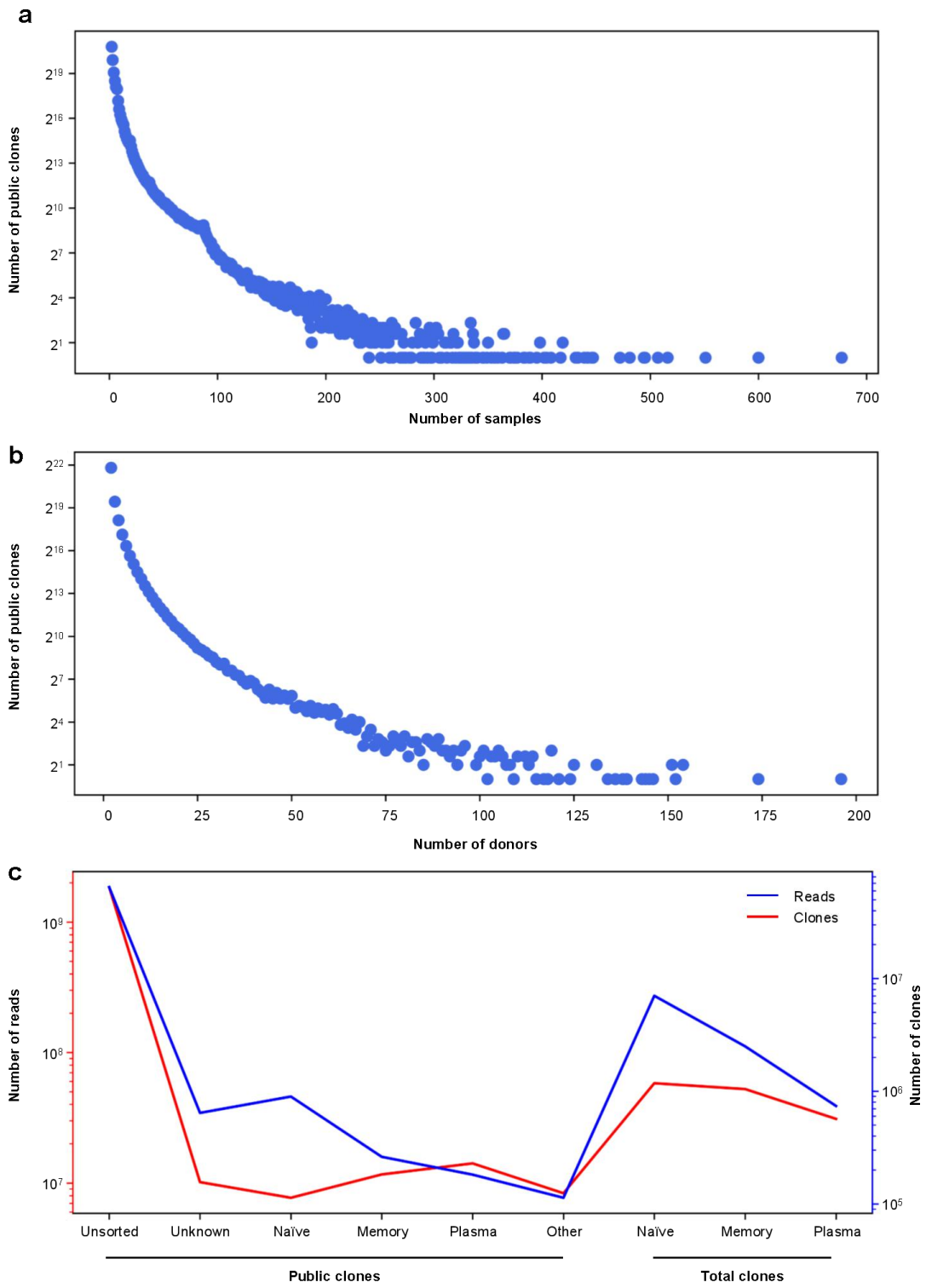
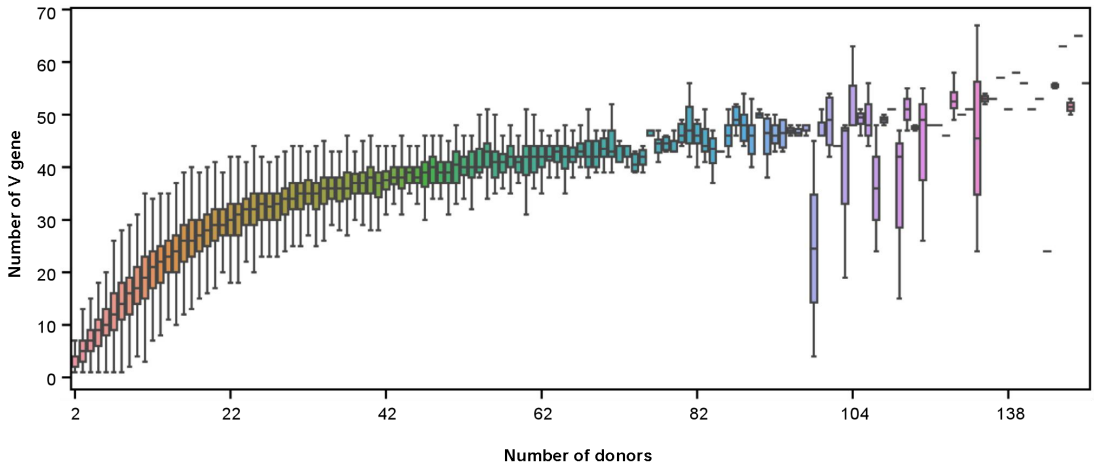


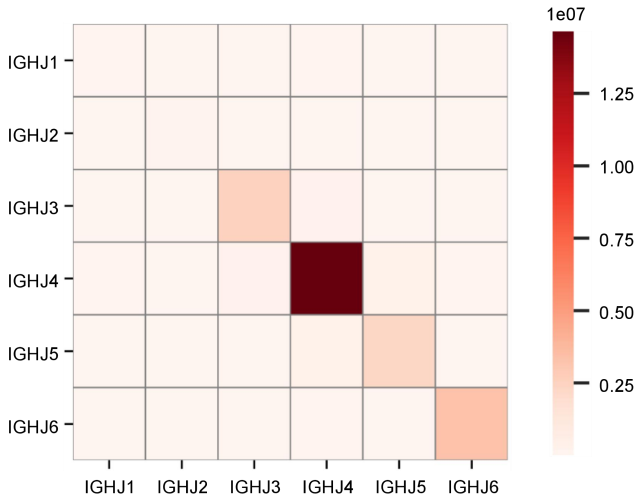
Supp. Fig. 2 Sequence and text search functions of RAPID. (a) The function of nucleotide and amino acid sequences search for both variable region and CDR3. **(b)** Known antibody search based on text such as antigen/disease and source id.



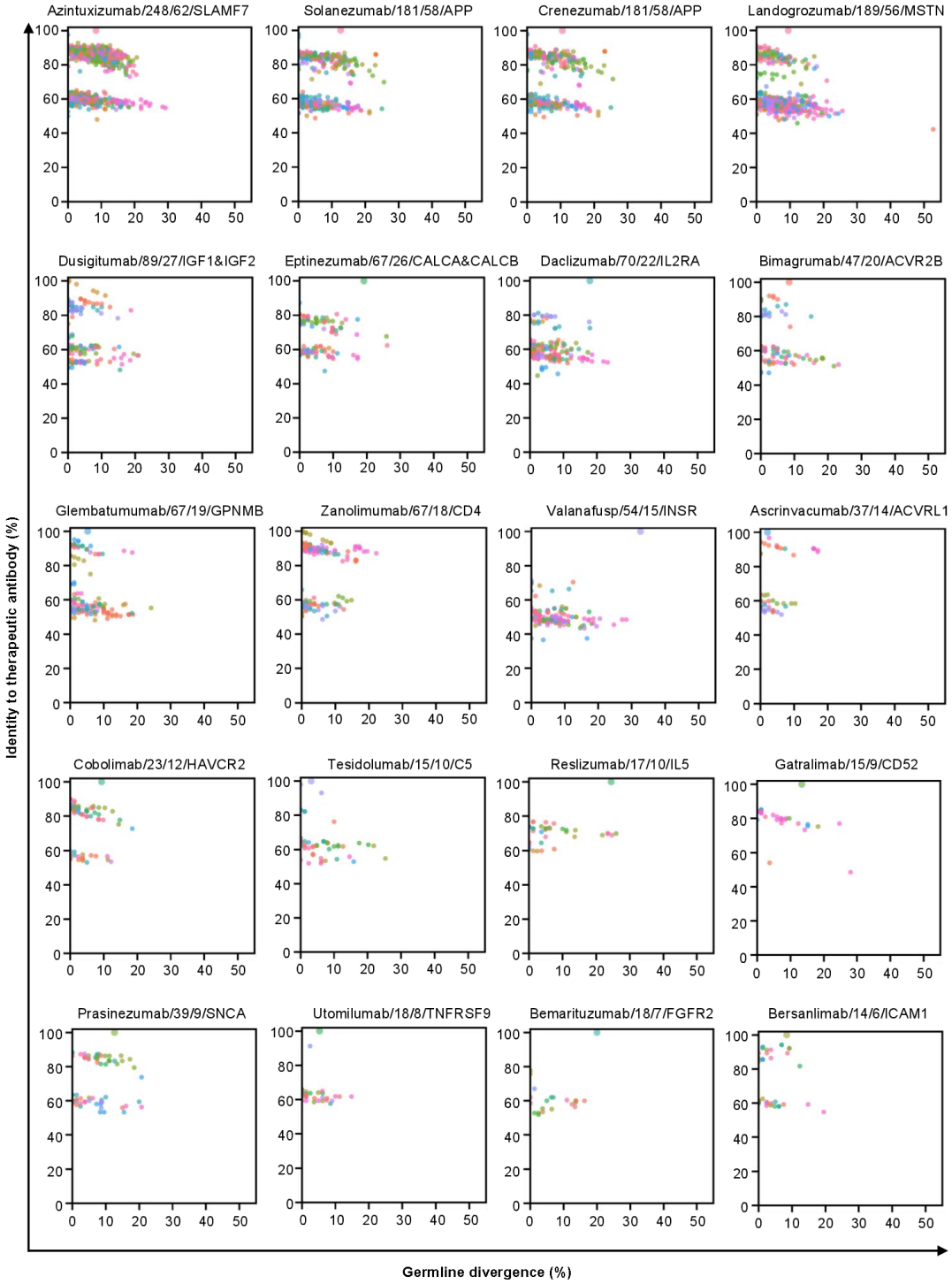
Supp. Fig. 3 Basic information of public clones. The number of public clone changed with the number of samples **(a)** and donors **(b)**. The Y-axis were logarithmically converted with base 2. **(c)** The number of reads and clones from different sources. Sources of clones were defined based on types of B cell including naïve, memory, plasma, PBMCs, other (particular antigen-specific B cells), and unknown. The Y-axis were logarithmically converted with base 10.



Supp. Fig. 4 The number of V genes for public clones shared by different number of donors. The number of donors of public clones is discrete.

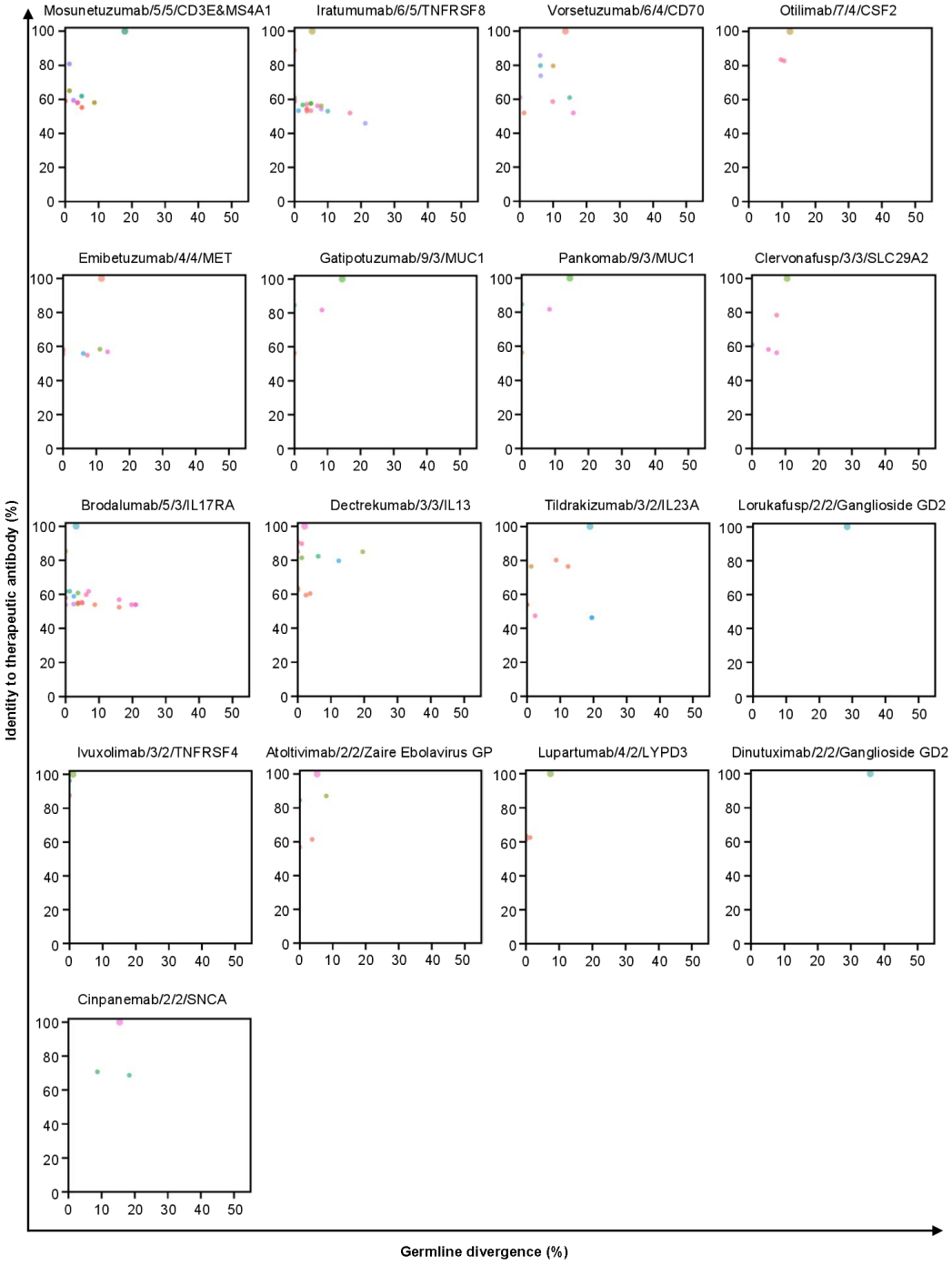


Supp. Fig. 5 The substitution frequencies of J genes with the same CDR3aa among different donors. The darker the color, the higher the substitution frequency.

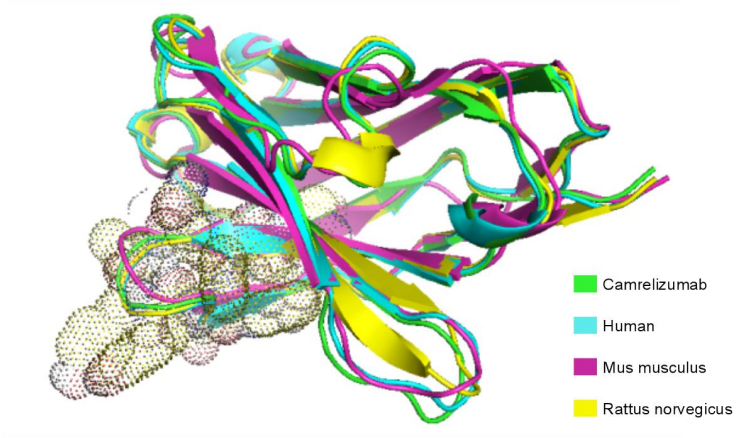


Supp. Fig. 6 Identity of variable regions from FR1 to FR3 between therapeutic antibody and public clones. The X-axis means the divergence to germline reference and the Y-axis means the sequences identity. Different V genes are filled in different colors. Titles for subfigures separated by forward slash include inn id of therapeutic antibody, the number of samples and donors with such CDR3aa, and target of therapeutic antibody. Dots of therapeutic antibodies are larger than that of clones identified from Rep-seq datasets. Sub-figures are sorted according to the number of donors.

Supplementary Figure 6 cont.



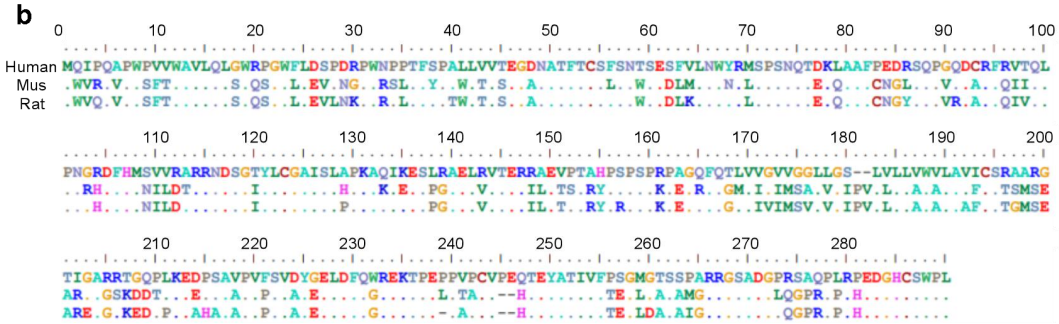
a



RMSD of clones with the same CDR3aa as Camrelizumab between species

Species	Camrelizumab	Human	Mus musculus	Rattus norvegicus
Camrelizumab				
Human	0.496			
Mus musculus	1.036	0.972		
Rattus norvegicus	0.545	0.490	0.872	

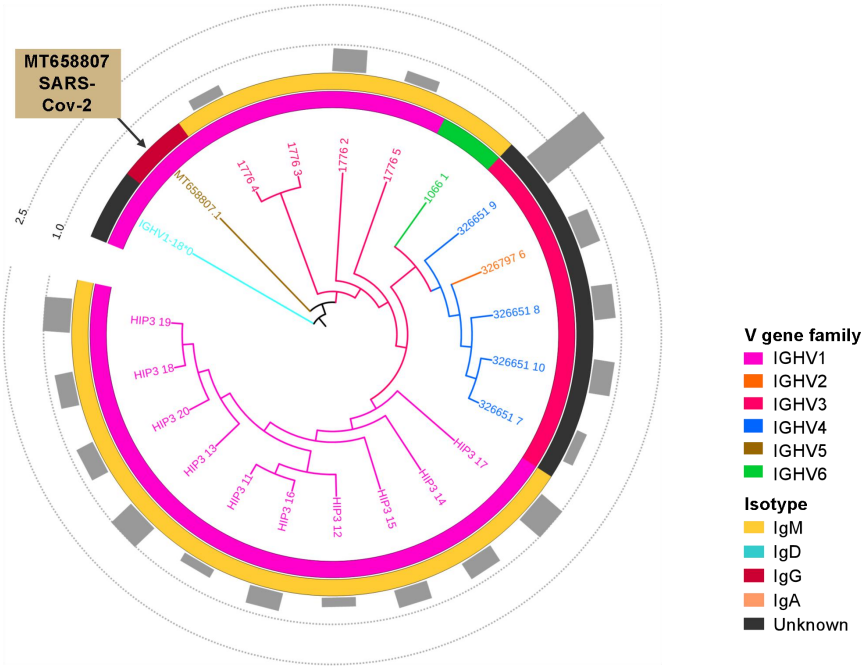
b



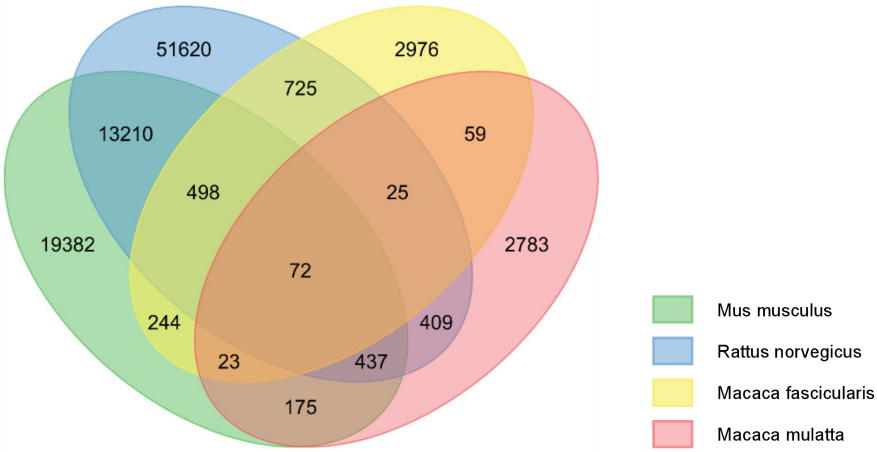
Identity of protein sequences for PDCD1 between species

Species	Human	Mus musculus	Rattus norvegicus
Human			
Mus musculus	59.31%		
Rattus norvegicus	60.69%	86.46%	

Supp. Fig. 7 Structure and sequence similarity of anti-PD1 clones and PDCD1 from different species. (a) Structure similarity of anti-PD1 clones. The upper panel stands for structures of Camrelizumab and clones from Human, Mus musculus, and Rattus norvegicus. Table in the lower panel records the RMSD of structures between paired species. **(b)** Identity of protein sequences for PDCD1 from human, Mus musculus, and Rattus norvegicus. The upper panel shows the multiple sequences alignment for them and the lower panel shows the sequences' identity.



Supp. Fig. 8 Maturation pathway of clones with the same CDR3aa of MT658807. Variable region sequences with the same CDR3aa as MT658807 were extracted and compared with MT658807 and its' germline reference. The germline reference was chosen as root of phylogenetic tree and MT658807 is marked by arrow. The cluster map contains four layers including similarity of sequences (the sequences extracted from the same donor were marked with the same color), V gene family, isotype, and somatic hypermutation rate from inner to outer.



Supp. Fig. 9 Overlap of public clones shared by other species.