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Rocuronium-specific antibodies drive perioperative anaphylaxis but can also function as reversal agents in preclinical models

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1 **Title: Rocuronium-specific antibodies drive perioperative anaphylaxis but can**
2 **also function as reversal agents in preclinical models**

3 **Overline:** IMMUNOLOGY

4 **One Sentence Summary:** Reversal agents for rocuronium were identified from rocuronium-
5 specific antibody repertoires.

6
7 **Editor's Summary:**

8 Roc Out. Neuromuscular blocking agents (NMBAs) such as rocuronium are used during
9 anesthesia to assist with surgeries and intubations. Although these agents are effective, they are
10 not without risk. Patients can develop postoperative residual neuromuscular blockade that can be
11 treated with an NMBA reversal agent, and life-threatening anaphylaxis can occur in rare cases.
12 Here, Dejoux *et al.* isolated and characterized memory B cells from three individuals who
13 developed reactions to NMBA treatment and had serum antibodies to rocuronium. The authors
14 found that IgE antibodies generated from these memory B cells were sufficient to induce
15 anaphylaxis in mice treated with rocuronium, although they were too low affinity to be used as
16 reversal agents. This led the authors to immunize mice with rocuronium to generate antibodies of
17 higher affinity, which were able to reverse neuromuscular blockade in rocuronium-treated
18 nonhuman primates. Together, these data both explain why these three patients had a reaction
19 and offer a therapeutic approach to reversing neuromuscular blockade. –Courtney Malo

20
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81

82 **Abstract:**

83

84 Neuromuscular blocking agents (NMBA) relax skeletal muscles to facilitate surgeries and
85 ease intubation, but can lead to adverse reactions, including complications due to postoperative
86 residual neuromuscular blockade (rNMB) and, in rare cases, anaphylaxis. Both adverse reactions
87 vary between types of NMBAs with rocuronium, a widely used non-depolarizing NMBA, inducing
88 among the longest rNMB durations and highest anaphylaxis incidence. rNMB induced by
89 rocuronium can be reversed by the synthetic γ -cyclodextrin, sugammadex. However in rare cases,
90 sugammadex can provoke anaphylaxis. Thus, additional therapeutic options are needed.
91 Rocuronium-induced anaphylaxis is proposed to rely on preexisting rocuronium-binding
92 antibodies. To understand the pathogenesis of rocuronium-induced anaphylaxis and to identify
93 potential therapeutics, we investigated the memory B cell antibody repertoire of patients with
94 suspected hypersensitivity to rocuronium. We identified polyclonal antibody repertoires with a
95 high diversity among V(D)J genes without evidence of clonal groups. When recombinantly
96 expressed, these antibodies demonstrated specificity and low affinity for rocuronium without

97 cross-reactivity for other NMBAs. Moreover, when these antibodies were expressed as human IgE,
98 they triggered human mast cell activation and passive systemic anaphylaxis in transgenic mice,
99 although their affinities were insufficient to serve as reversal agents. Rocuronium-specific, high-
100 affinity antibodies were thus isolated from rocuronium-immunized mice. The highest affinity
101 antibody was able to reverse rocuronium-induced neuromuscular blockade in nonhuman primates
102 with kinetics similar to sugammadex. Together, these data support the hypothesis that antibodies
103 cause anaphylactic reactions to rocuronium and pave the way for improved diagnostics and
104 neuromuscular blockade reversal agents.

105

106 **Main text:**

107

INTRODUCTION

108

109 Neuromuscular blocking agents (NMBAs) are used in the clinic to paralyze skeletal
110 muscles during surgery conducted under general anesthesia (1, 2). They help to assist airway
111 management in programmed or emergency anesthesia by facilitating endotracheal intubation.
112 NMBAs operate at the neuromuscular junction by competing with acetylcholine to bind nicotinic
113 receptors. All NMBAs possess two tertiary or quaternary substituted ammonium groups at
114 physiological pH that enable NMBAs to compete with the quaternary ammonium group of
115 acetylcholine for binding to nicotinic receptors. NMBAs have two major side effects, acute
116 hypersensitivity reactions and potentially life-threatening anaphylaxis (3, 4). Moreover, in the
117 absence of appropriate monitoring that, despite international recommendations (1, 2), is not
118 applied to more than 20% of the patients., residual neuromuscular blockade (rNMB), where
119 NMBA remains present after the end of infusion, can lead to tracheal damage and infections (5).

120 rNMB can lead to postoperative pulmonary events, pharyngeal dysfunction, urgent tracheal
121 reintubation, and prolonged stay in post-anesthesia care units and hospitals that lead to a high
122 economic burden (6). The first option for pharmacological neuromuscular blockade reversal,
123 neostigmine (Prostigmine; used for atracurium reversal), is restricted to situations where half of
124 the spontaneous reversal is effective, making it inefficient for deep neuromuscular blockade (7).
125 The second option is the synthetic γ -cyclodextrin sugammadex (Bridion) (8), which encapsulates
126 the aminosteroid NMBAs rocuronium and vecuronium in vivo within minutes following injection,
127 and is efficient at reversing deep neuromuscular blockade. Instant blockade reversal might also be
128 necessary, as in difficult intubation scenarios, explaining that it has been considered economically
129 advantageous to administer sugammadex despite a cost of \$100-200 per patient, leading to a \$400-
130 3,000 economy per patient (6). Yet, multiple cases of anaphylaxis induced by sugammadex or by
131 the sugammadex-rocuronium inclusion complex have been reported (9-12) leading to restrictions
132 of its use in several countries (11). Rocuronium is the most used NMBA worldwide for full-
133 stomach rapid sequence induction, and has become almost exclusive in countries like Japan, due
134 to the existence of sugammadex for deep neuromuscular reversal (13).

135 The most dramatic adverse event following NMBA exposure is anaphylaxis, a severe
136 allergic reaction that can lead to death, even after rapid recognition and correct management. In

137 some countries, half of perioperative anaphylaxis events are caused by NMBAs, with a global
138 mortality rate of 4.1% (14). The current dogma proposes that IgE antibodies with reactivity
139 towards NMBAs lead to mast cell and basophil activation in NMBA-allergic patients (15), and we
140 reported earlier that, indeed, circulating anti-NMBA IgE concentrations, but also anti-NMBA IgG
141 concentrations, correlate with the severity of anaphylaxis (16). In our view, two pathways are
142 potentially involved in NMBA-induced anaphylaxis separately or concomitantly depending on the
143 patient: the IgE pathway, leading to mast cell and basophil activation with histamine release as a
144 major mediator, and the IgG pathway, leading to neutrophil and monocyte activation with platelet-
145 activating factor (PAF) release as a major mediator (15, 17). Both histamine and PAF induce
146 vasodilation, bronchoconstriction, cardiac and pulmonary failure, with probable cumulative or
147 potentiating effects when both mediators are released (18). Routinely, only anti-NMBA IgEs are
148 quantified in clinical practice, using a morphine group (termed Quaternary Ammonium Molecule;
149 QAM) as a surrogate for NMBA in ImmunoCAP detection, as its molecular structure mimics
150 ammonium groups present in some aminosteroid NMBAs (19, 20). The morphine ImmunoCAP
151 demonstrates acceptable specificity and sensitivity for aminosteroids (e.g., rocuronium) and
152 suxamethonium, but not for benzyliisoquinolines (e.g., atracurium) (21).

153 The tertiary and quaternary substituted ammonium groups present in NMBAs have been
154 proposed to be part of the epitope recognized by anti-NMBA antibodies in patients, as patient sera
155 reacting with one or several NMBAs (22) cross-react with unrelated molecules possessing a
156 quaternary ammonium (23, 24). Since anti-NMBA monoclonal antibodies (mAbs) have not been
157 identified from patients or animals, molecular or structural evidence lacks to ascertain these
158 hypotheses. Importantly, anaphylaxis occurs at the first exposure to aminosteroid NMBAs in half
159 of the patients (25), excluding NMBA ammonium groups as primary sensitizing epitopes in the
160 patient's history. NMBAs are small compounds (molecular weight \approx 300-1,000 Da) with unknown
161 properties in terms of immunogenicity; several families of compounds are however considered to
162 sensitize towards NMBAs, including cosmetics, cleaning and bleaching products, as well as cough
163 syrups containing morphine-based compounds (22, 26). Among the latter, studies proposed
164 pholcodine, an opioid cough suppressant with two tertiary ammonium groups at physiological pH,
165 to be a culprit molecule responsible for NMBA hypersensitivity (27, 28). Large variations in serum
166 reactivity profile to NMBAs are found between individuals, from a single NMBA to NMBAs
167 belonging to the same chemical family, and even to all NMBAs in 4% of the cases (22), suggesting

168 that B cell responses develop with one or multiple monospecificities each toward a particular
169 compound, or that cross-reactive B cell responses develop and react towards different NMBA.

170 To investigate the antibody specificity and affinity for NMBA in patients who experienced
171 NMBA-induced anaphylaxis, we analyzed single memory B cells with rocuronium reactivity.
172 Memory B cell antibody repertoires were polyclonal with high V(D)J diversity, exclusive for
173 rocuronium among NMBA and anaphylactogenic when expressed as human IgE in vitro and in
174 vivo in mice. Because patient-derived anti-rocuronium mAbs were of low-to-medium affinity and
175 incapable to capture clinical doses of rocuronium in vivo, we generated high affinity rocuronium-
176 specific mAbs from immunized mice, which could reverse rocuronium-induced neuromuscular
177 blockade in nonhuman primates with similar kinetics as sugammadex. When co-crystallized, these
178 latter mAbs captured rocuronium in a hydrophobic cleft in which the quaternary ammonium group
179 is completely buried and involved in the binding. High affinity anti-NMBA mAbs thus represent
180 a potential therapeutic avenue for the reversal of neuromuscular blockade.

181

RESULTS

Rocuronium-specific memory B cells were isolated from patients with NMBA allergy

Patients with presumed anaphylaxis to NMBAs during general anesthesia were included in two clinical studies (France, registered under NCT05420935, and Belgium) to obtain large volumes of blood for peripheral blood mononuclear cell (PBMC) isolation. Among these, three patients experienced fast occurring symptoms (< 5 min) of anaphylaxis and were admitted to the intensive care unit (ICU) afterwards (Table 1). Patient #1 developed a mild Ring & Messmer grade 2 anaphylaxis to atracurium in 2012 and was sampled for PBMCs in 2014 and 2023. Patient #2 and Patient #3 developed a severe grade 3 and 4 anaphylaxis, respectively, to rocuronium (Fig. 1A) in 2020, and were sampled a few weeks later. QAM IgE ImmunoCAP assays were positive only for Patient #2, although Patient #1 and Patient #3 had been previously exposed to NMBAs (Table 1). The anti-rocuronium IgE ImmunoCAP was positive only for Patient #3 (Fig. 1B). Skin prick tests and basophil activation tests with rocuronium were negative for Patient #1 but positive for Patients #2 and #3 (Table 1). Anti-rocuronium IgG concentrations were, however, elevated compared to controls in all three patients, and in both serum samples of Patient #1 at 9 years difference (Patient #1_2014 and Patient #1_2023) using enzyme-linked immunosorbent assay (ELISA; Fig. 1C), LuLISA and ImmunoCAP (fig. S1). We have previously reported that both IgE and IgG pathways exist with potentially additive or synergistic effects in human NMBA-induced anaphylaxis, and that Patient #1 had elevated serum anti-rocuronium IgG concentrations even while presenting with a grade 2 atracurium-induced anaphylaxis (16). In agreement with these findings, Patient #3 that developed a grade 4 anaphylaxis upon rocuronium infusion presents with elevated concentrations of both anti-rocuronium IgE and IgG (Fig. 1B and C).

IgE memory B cells remain elusive and their existence is still disputed (29), as their survival may be extremely limited (30-33). As it has been proposed that IgE plasmablasts (and plasma cells) are continuously derived from IgG memory B cells (34), we decided to explore the IgG memory B cell compartment as a surrogate for the IgE B cell compartment with the goal of defining the rocuronium-specific antibody repertoire in these patients. We single-sorted CD27⁺ memory B cells from frozen PBMCs from these three patients based on expression of memory B cell markers (IgD⁻ CD19⁺ CD27⁺). We further used fluorescently-labeled monomeric human serum albumin-rocuronium conjugate (HSA-roc) to label B cell receptors (BCRs) with affinity for

213 rocuronium. We also excluded cells expressing markers of other immune cells (CD3⁻, CD14⁻,
214 CD35⁻) and B cells with affinity for HSA alone (Fig.1D). The percentage of HSA-roc⁺ HSA⁻
215 among CD27⁺ IgD⁻ memory B cells varied between patients with a mean of 4.6% (range from 2.75
216 to 6.5%) for both sorts of Patient #1_2014, 1% for Patient #1_2023, 2% for Patient #2 and 3.6%
217 for Patient #3 (fig. S2A and B). The percentage of CD27⁺ cells among antigen-specific CD3⁻
218 CD14⁻ CD19⁺ CD38⁻ IgD⁻ cells varied from 58 to 89% (fig. S2C).

219

220 **Characteristics of anti-rocuronium memory B cell repertoires from patients with NMBA** 221 **allergy**

222 In order to test the specificity towards rocuronium of the antibodies corresponding to the
223 BCRs expressed by these memory B cells before V(D)J sequencing of their heavy chain variable
224 region (V_H) and light chain variable region (V_L), we cultured these single cells in an in vitro culture
225 assay allowing their differentiation in antibody-secreting cells (35, 36). After 25 days of culture,
226 supernatants with detectable amounts of IgG were further tested for rocuronium specificity by
227 ELISA using HSA-rocuronium as an antigen in the absence or presence of free rocuronium
228 (competitive inhibition ELISA). We obtained 57 supernatants with rocuronium specificity from
229 the CD27⁺ memory B cell cultures of Patient #1_2014, 128 for Patient #1_2023, 45 for Patient #2
230 and 23 for Patient #3 (table S1). These represented only a minor fraction of the monoclonal
231 cultures, from 1.3% to 16.7%, indicating poor selectivity of the allophycocyanin (APC)-labeled
232 HSA-roc to label antigen-specific BCRs for B cell sorting. This selectivity did not increase with
233 sorts using antigen labeled with two different fluorophores (HSA-rocuronium-APC versus HSA-
234 rocuronium-AlexaFluor488), or when selecting CD45RB⁺ memory B cells (37) instead of CD27⁺
235 memory B cells (fig. S3). As expected from serum reactivity to rocuronium in humans (16),
236 rocuronium-binding CD27⁺ memory B cells and CD45RB⁺ memory B cells could also be
237 identified and sorted from PBMCs of a healthy donor, but with lower numbers of strong
238 rocuronium binders among the monoclonal cultures from these sorted PBMCs compared with
239 monoclonal cultures from PBMCs sorted from Patient #1_2023 (fig. S4).

240 RNA of the 240 monoclonal cell cultures (data file S1) was extracted to perform V(D)J
241 sequencing of their V_H-V_L domains. Using the repertoire-profiler bioinformatic pipeline (38), we
242 established the anti-rocuronium antibody repertoire profile of the three patients. We obtained a
243 total of 211 V_H and 191 V_L sequences (Fig. 1E) with an average of approximately 14 nucleotide

244 mutations in the V_H and approximately 7 in the V_L compared to germline for Patient #1 and Patient
245 #2 (fig. S5). The V_H and V_L sequences of Patient #3 were less mutated with a majority of non- or
246 poorly-mutated sequences.

247 Analyses of V-J gene usage identified highly diverse and polyclonal repertoires, both
248 within and between patients, for both the V_H and V_L sequences. Indeed, the proportion of B cells
249 with V_H sequences coded by identical V-J genes was lower than 11% for all patients. The V_L
250 sequences were similarly diverse, with identical V-J genes lower than 9% except for Patient #3 for
251 whom 23% of the sequences (five out of 22) were coded by KV3-20*01_J1*01 (Fig. 1E). The
252 alignment of the 211 V_H amino acid sequences revealed 22 groups of at least three members with
253 identical V-J genes but dissimilarities in their sequences and complementarity determining regions
254 (CDRs; fig. S6A). Because of this high diversity, attempts to identify clonal groups inside a patient
255 defined by at least 3 distinct complementary determining region 3 (CDR3) of identical length and
256 identical V-J gene usage failed (fig. S6B). We found, however, two to four identical V_H sequences
257 in seven instances in Patient #1_2023, once in Patient #2 and once in Patient #3 (Table 2). As we
258 obtained PBMC samples 9 years apart from Patient #1, we compared their antibody repertoires
259 over time in the absence of any exposure to NMBAs between the samplings. The repertoires of
260 Patient #1_2014 and Patient #1_2023 were distinct, with only rare similarities of V-J gene usage.
261 V4-39*01_J4*02 was the most predominant for the V_H representing 4 out of 49 (approximately
262 10%) and 8 out of 98 (approximately 8%) of the rearrangements, respectively (Fig. 1E). Six clonal
263 groups for the V_L were identified with sequences present in samples from both 2014 and 2023
264 (KL2-11_KJ1, KV3-20_KJ2, KV1-39_KJ4 and KV2-28_KJ4); however, the amino acid sequence
265 alignment of the corresponding 39 V_H sequences represented in 9 different V(D)J recombinations
266 revealed dissimilarities in their sequences and CDRs, with no V_H clonal groups identified (fig.
267 S7A).

268 Among the 211 V_H and 191 V_L sequences identified (Fig. 1E), 152 were paired V_H - V_L
269 sequences, including 25 V_H - V_L pairs from Patient #1_2014, 76 from Patient #1_2023, 32 from
270 Patient #2 and 19 from Patient #3 (tables S1 and 2). Amino acid alignments led to the identification
271 of five B cells clones identified at least twice with identical V_H - V_L amino acid sequences. The
272 sequences of the shared mAbs had a large diversity in mutation load compared with the germline
273 sequence (up to 41 amino acid mutations in the V_H and 20 in the V_L) and some possessed long
274 CDR3 motifs (Table 2). Only one paired V_H - V_L with identical V-J usage (V3-7*01-J4*02 and

275 KV4-1*01_KJ1*01) was identified from the two repertoires of Patient #1 (2014 and 2023). The
276 amino acid alignment of those two mAbs revealed greater than 80% homology in the V_H and in
277 the V_L with mutations in the frameworks and CDRs (fig. S7B). Altogether, these data show that
278 the four anti-rocuronium repertoires we characterized are diverse, with neither predominant gene
279 usage nor evidence of clonal groups-

280

281 **Anti-rocuronium antibodies from patients are specific, of low affinity, and anaphylactogenic**

282 To further characterize the V_H - V_L pairs identified from single-cell sorting of rocuronium-
283 binding memory B cells, we recombinantly expressed those corresponding to monoclonal cell
284 cultures with the highest anti-rocuronium signal as aglycosylated effectorless (Fc engineered
285 heavy chain mutation N₂₉₇A) human IgG1 mAbs. Anti-rocuronium ELISA using HSA-rocuronium
286 as an antigen enabled a first ranking of the antibodies with mostly poor binding, except mAb h1F10
287 (Fig. 2A, fig. S8A, and Table 3). Competitive inhibition ELISA demonstrated their specificity for
288 rocuronium as only free rocuronium, but not free molecules with the same aminosteroid scaffold,
289 such as the closely related homologue vecuronium, the 5 α -dihydrotestosterone (5 α -DHT) that
290 lacks quaternary ammonium groups, or pholcodine (Fig. 2B and fig. S8B). The mAbs were also
291 not reactive towards irrelevant antigens such as ovalbumin, amoxicillin, and peanut extract (fig.
292 S8C). From the memory B cells sorted initially based on their binding to HSA-rocuronium-APC,
293 only 1.3 to 16% expressed antibodies that bound free rocuronium, suggesting a frequency of
294 antigen-specific B cells ranging from 0.04 to 0.2% in PBMC samples from the three patients
295 (tables S1 and 2). The avidity of the mAbs towards HSA-rocuronium (molar ratio
296 rocuronium:HSA of 15:1) was measured using Bio-layer interferometry (BLI) with the conjugate
297 covalently bound to the sensors. All antibodies had similar profiles with a fast association and a
298 biphasic dissociation. The dissociation constants (K_D) of these mAbs ranged from 21 to 922 nM,
299 with half the antibodies in the 21 to 50 nM range (Table 3 and fig. S9A), and mAb h1F10
300 displaying the best avidity (Fig. 2C). The very small size of rocuronium (530 Da) is incompatible
301 with the relatively poor sensitivity of BLI, but at the lower limit of detection for Surface Plasmon
302 Resonance (SPR). Using SPR with the mAbs captured onto the chip, we confirmed that among the
303 mAbs with the highest avidity for HSA-rocuronium, five mAbs bound free rocuronium in solution,
304 in a transitory mode with a very fast association and dissociation (Table 3 and fig. S9B), with mAb
305 1F10 displaying the best affinity for rocuronium with a K_D of 140 μ M (Fig. 2D).

306 Next, we produced h1F10 as a human IgE to investigate if this V_H-V_L rearrangement
307 represents an allergy-related B cell clone in Patient #1_2014 by promoting human mast cell
308 activation or inducing anaphylaxis in mice in the presence of rocuronium. Human mast cells and
309 basophils express the high-affinity IgE receptor (FcεRI) that allows them to be sensitized (pre-
310 armed) with IgE in vivo for immediate cell activation following IgE engagement with allergens,
311 leading to rapid anaphylactogenic mediator release. Human mast cells derived from PBMCs of
312 healthy donors sensitized with h1F10 IgE dose-dependently degranulated in the presence of HSA-
313 rocuronium but, as expected (39), unsensitized mast cells did not (Fig. 2E). To test the
314 anaphylactogenic potential of IgE h1F10, we used mice expressing human FcεRI, sensitized them
315 with h1F10 and challenged them with HSA-rocuronium in a classical model of passive systemic
316 anaphylaxis. IgE h1F10-sensitized mice, but not unsensitized mice, displayed a pronounced drop
317 in central body temperature (Fig. 2F), a hallmark of anaphylaxis in mice (40). Thus, although
318 identified from an IgG-expressing memory B cell, the h1F10 V_H-V_L rearrangement generates a
319 rocuronium-specific antibody of medium-low affinity that, when produced as an IgE, demonstrates
320 potential for human mast cell activation and induction of anaphylaxis in mice humanized for
321 FcεRI.

322 As h1F10 was of sufficient affinity to measure its interaction with free rocuronium by SPR,
323 and capable of inducing mast cell activation and passive systemic anaphylaxis when expressed as
324 an IgE, we wondered if it could also capture circulating rocuronium and promote reversal of
325 neuromuscular blockade. This possibility would represent an alternative to sugammadex for cases
326 of sugammadex- or sugammadex-rocuronium hypersensitivity (9-12). We first defined the lethal
327 dose of rocuronium in wild-type C57BL/6 mice at 160 µg/kg (Fig. 2G). We then established a
328 prophylactic model to prevent neuromuscular blockade in mice, which consisted of pretreatment
329 with a rocuronium capture molecule, followed by intravenous injection of a lethal dose of
330 rocuronium and vitality assessment. In this model, only sugammadex protected mice from
331 rocuronium-induced neuromuscular blockade. h1F10 and the other sixteen anti-rocuronium mAbs
332 (Table 3) that we produced as fully human IgG1 (N₂₉₇A) antibodies were unable to confer
333 protection (Fig. 2G). We hypothesized that the affinity of these antibodies was too weak to exert
334 capture functions in vivo that would be necessary to cause reversal of neuromuscular blockade.

335

336 **Generation of high-affinity anti-rocuronium mAbs**

337 To investigate the potential use of mAbs as capture reagents for rocuronium, we decided
338 to immunize mice against rocuronium and identify high-affinity mAbs. As we failed to induce
339 anti-rocuronium antibody responses in mice using free rocuronium in adjuvant, we immunized
340 mice with rocuronium coupled to keyhole limpet hemocyanin (KLH), as a large size carrier protein
341 that favors bystander activation. Hybridomas were generated from the mice displaying the highest
342 anti-rocuronium titers (Fig. 3A), and their variable domains were sequenced, leading to the
343 identification of only two different V_H - V_L pairs. Both used KV2-112*KJ2 V_{LS} , but different V_{HS}
344 (mAb m1B6, HV1-63*J4; mAb m2B1, HV1-81*J2). Using ELISA and competitive inhibition
345 ELISA, we confirmed that mAb m1B6 (IgG1, κ) and mAb m2B1 (IgG1, κ) bound specifically
346 rocuronium but not any other NMBA, including analogues vecuronium and pancuronium,
347 suxamethonium, 5 α -DHT, diisopentyl succinate, and pholcodine (Fig. 3B and C). Both mAbs had
348 a very high avidity for HSA-rocuronium measured by BLI (fig. S10A), 800- and 57-times higher,
349 respectively, than mAb 1F10. Whereas mAb m2B1 had only a 140-times better affinity for free
350 rocuronium measured by SPR than mAb 1F10 (K_D =1 μ M versus 140 μ M), mAb m1B6 displayed
351 a 25,000-times better affinity (K_D =5.5 nM versus 140 μ M) (fig. S10B and Table 3).

352 To understand how anti-rocuronium mAbs m1B6 and m2B1 bind rocuronium, we
353 produced monovalent formats of mAb m2B1 and m1B6 as Fab fragment and single-chain variable
354 fragment (scFv), respectively, and generated their co-crystals with rocuronium. We solved the X-
355 ray structures of Fab-m2B1 and scFV-m1B6 in complex with rocuronium at a 1.65 Å and 1.70 Å
356 resolution, respectively, with electron density for rocuronium observed in the binding sites in the
357 two solved structures (Fig. 4A, fig. S11, and table S2). Both antibodies make extensive
358 hydrophobic interactions (van der Waals bonds) with rocuronium involving the V_H and V_L in each
359 antibody. The Fab-m2B1-rocuronium contacts involved residues Glu50, Tyr52, Ser54, Tyr56,
360 Gly96, Val97, Ans98, and His100 of the antibody heavy chain and Tyr27, Tyr32, Leu91, Val92,
361 Glu93, Tyr94, and Tyr96 of the light chain (Fig. 4B). The scFv-m1B6-rocuronium contacts
362 involved residues Trp33, Try52, Ser54, Tyr56, Trp99, Ser100, Tyr100A and Tyr100B of the heavy
363 chain and Tyr27, Tyr32, Leu91, Val92, Glu93, Tyr94, Tyr96 of the light chain (Fig. 4B). The ring
364 A of rocuronium has been reported to exist in twist-boat and chair conformations (41). In the
365 structure of the scFv-m1B6-rocuronium complex, rocuronium ring A adopts the chair
366 conformation; however, in the Fab-m2B1-rocuronium complex, the rocuronium ring A was not
367 sufficiently well-defined within the electron density maps to be determined. The rocuronium

368 hydroxyl group was fully exposed to solvent, in agreement with the use of this group as an
369 attachment point to the rocuronium-carrier proteins used herein for immunization and screening,
370 whereas the acetoxyl group was buried inside the binding pocket of both antibodies. Finally, both
371 co-crystal structures revealed that the allylpyrrolidine substituent containing the quaternary
372 ammonium is completely buried and makes numerous van der Waals contacts with the antibody
373 light chain (Fig. 4), emphasizing the important role of this group in the rocuronium binding mode,
374 in agreement with hypotheses extrapolated from serum cross-reactivity in patients (22, 42). The
375 specificity for rocuronium and not vecuronium or pancuronium could be explained by the fact that
376 no space remained in the hydrophobic pocket formed by the V_H and the V_L of either antibody to
377 accommodate the methylpiperidine substituent of vecuronium and pancuronium, and by numerous
378 hydrophobic contacts made by the allyl group of rocuronium.

379

380 **High-affinity anti-rocuronium antibodies protect from and reverse neuromuscular blockade** 381 **in mice and nonhuman primates**

382 Rocuronium capture may serve two purposes: (i) rocuronium binding and sequestration to
383 prevent interaction with IgE on mast cell and basophil to reduce or stop anaphylaxis, as suggested
384 by some case reports (43-45), and (ii) reversion of neuromuscular blockade to shorten patient's
385 recovery time and associated complications (6). To test the efficacy of the high affinity anti-
386 rocuronium mAbs isolated from mice on rocuronium capture, we first used the prophylactic model
387 of rocuronium-induced neuromuscular blockade (Fig. 2G) with a fixed dose of rocuronium of 4
388 µg per mouse, inducing lethality in less than 2 min. Both mAb 1B6 and mAb 2B1 protected mice
389 for neuromuscular blockade in a dose-dependent manner. mAb 1B6 was more efficient at lower
390 antibody doses (Fig. 5A), as expected when considering the affinities of mAb 1B6 and mAb 2B1
391 for free rocuronium.

392 Neuromuscular blockade is monitored in anesthetized and intubated patients in clinical
393 practice using the Train-Of-Four (TOF) method that compares the twitch response (T1, T2, T3,
394 T4) to four consecutive electric impulses applied by a TOF apparatus on the ulnar nerve through
395 electrodes. Deep neuromuscular blockade corresponds to the absence of response at any of the
396 stimulus, and recovery from neuromuscular blockade to a TOF ratio (T4/T1) greater than 90%.
397 Loss and recovery of spontaneous ventilation is also recorded to calculate the time to retrieve
398 spontaneous ventilation. Because of the small size of mice, TOF devices were not adaptable even

399 using pediatric equipment, and we moved to cynomolgus macaques to evaluate the efficacy of
400 mAb 1B6 to reverse deep neuromuscular blockade induced by rocuronium in a model that closely
401 reflects the clinical setting. We first established the lowest dose of rocuronium to induce a profound
402 myorelaxation (200 μ g/kg) in macaques and the lowest sugammadex dose for fast recovery (<1
403 minute; 2 mg/kg) as a control. Animals were anesthetized, placed under mechanically assisted
404 ventilation, and the myorelaxation status was assessed by TOF monitoring (measured using two
405 TOF instruments, each connected to one upper-limb) and time to retrieval of spontaneous
406 ventilation (Fig. 5B) (46). At 200 μ g/kg rocuronium injected as a bolus, the spontaneous reversal
407 of neuromuscular blockade happened at an average of 28.5 min when monitoring TOF ratio and
408 spontaneous ventilation at 15.5 min (Fig. 5C to F). Injection of mAb 1B6 to macaques under deep
409 neuromuscular blockade induced by rocuronium dose-dependently decreased the time to complete
410 neuromuscular blockade reversal. 2.5 mg/kg of mAb 1B6 were sufficient to reduce from 28.5 min
411 to 16 min the recovery of TOF ratio>90% and to reduce from 15.5 min to 5 min recovery of
412 spontaneous ventilation (Fig. 5D). To obtain the same speed of TOF ratio and spontaneous
413 ventilation recovery than obtained with 1 mg/kg sugammadex, a minimum of 10 mg/kg of mAb
414 1B6 were required (Fig. 5E and fig. S12). Physiological parameters and blood counts remained
415 unaltered during the experiments (fig. S13), indicating that mAb 1B6 does not induce obvious
416 adverse events, even at high doses. These results validate the therapeutic effect of rocuronium-
417 specific mAb 1B6 on rocuronium-induced neuromuscular blockade. They serve as a proof of
418 concept that antibodies may be potential drugs for the reversal of neuromuscular blockade of
419 NMBA, and, as it has been suggested for sugammadex (43-45), may be able to mitigate or even
420 reverse anaphylaxis.

DISCUSSION

This study reports anti-NMBA antibody repertoires from NMBA-allergic patients. Memory B cell repertoires of patients with suspected hypersensitivity to NMBA and evidence of anti-rocuronium antibodies in their serum demonstrated high polyclonality, with no evidence of clonal groups, suggestive of diverse sources of immunization against compounds chemically or structurally related to rocuronium. However, no crossreactivity for other NMBAs or structurally-related molecules could be detected, and the affinities for rocuronium were rather poor, with K_D around hundred micromolar for free rocuronium. These affinities were sufficient to trigger in vitro mast cell activation and in vivo anaphylaxis in mice, indicating that some of these antibodies may have contributed to rocuronium-induced anaphylaxis in patients. Two high-affinity anti-rocuronium mAbs generated from rocuronium-immunized mice also demonstrated selectivity for rocuronium, explained by the structure of their paratope, and demonstrated the ability to capture rocuronium in vivo. The highest affinity mAb provided a proof of concept for antibodies as reversal agents for rocuronium-induced deep neuromuscular blockade that may provide an option for patients with allergy to sugammadex.

Rocuronium represents an advantageous model of NMBA, as it is widely used for standard endotracheal intubation and for rapid sequence induction, although it has one of the longest residual neuromuscular blockade durations and has among the highest anaphylaxis incidences (4, 47-49). This aminosteroid non-depolarizing NMBA was designed as a mono-quaternary ammonium compound (allylated pyrrolidinium group attached to the D ring), unlike its bis-quaternary closely related analog pancuronium (50). It is therefore a weaker antagonist at the neuromuscular junction than pancuronium, making rocuronium easier to outcompete for reversal of neuromuscular blockade. With vecuronium, rocuronium is the only NMBA on the market possessing a reversal reagent for deep neuromuscular blockade (8), and has been adopted as sole NMBA in countries like Japan due to this drug-antidote pair, despite the cost of the antidote. How rocuronium induces anaphylaxis remains unclear, as IgE receptors on mast cells and basophils require aggregation to enable cell activation and degranulation; the most probable hypothesis proposes that rocuronium haptens onto a large molecule upon infusion (51). A previous study using IgE-stripped human basophils sensitized with patient serum containing anti-rocuronium IgE indeed reported that only haptens rocuronium, but not free rocuronium, induced basophil

452 activation in vitro (52). To evaluate IgE-mediated anaphylaxis, allergy work-up mostly includes
453 degranulated biomarkers from mast cells and basophils, specific IgE measurements (during the
454 anaphylaxis and later work-up), and skin tests and basophil activation tests (BAT) to all molecules
455 suspected as the culprit agent (six to eight weeks after the anaphylaxis) (53). Rocuronium-specific
456 IgEs are still quantified with the morphine antigen surrogate in clinical practice (QAM
457 ImmunoCAP). An anti-rocuronium IgE ImmunoCAP is commercially available, but is not yet
458 validated for clinical practice. Exploration of anti-rocuronium IgE antibodies in patients' sera
459 relies on the hypothesis that its quaternary ammonium is part of the epitope bound by anti-QAM
460 antibodies with, however, poor structural and chemical resemblance between rocuronium and the
461 morphine antigen. Anti-QAM ImmunoCAP are also used for screening for hypersensitivity against
462 widely different chemical structures as suxamethonium and atracurium, although with poor
463 sensitivity and specificity (21, 24, 54-58). To avoid this caveat in our study, we used rocuronium
464 derivatives by extending the OH group with a functionalized linker for grafting on carrier proteins
465 such as KLH or HSA, which were used for memory B cell sorting, ELISA, avidity measurements,
466 vaccination, and functional assays. This strategy allowed for identification of anti-rocuronium
467 antibodies with no crossreactivity for other NMBAs. Validated by other groups, some of these
468 rocuronium-based assays could become diagnostic tools in NMBA-suspected allergies.

469 B cells with rocuronium reactivity were sorted from two patients with a history of
470 rocuronium-induced anaphylaxis and from one with atracurium-induced anaphylaxis. For several
471 reasons, we chose to sort IgG-expressing memory B cells and not IgE-expressing B cells to identify
472 anti-rocuronium antibody repertoires. First, all three patients had evidence of anti-rocuronium IgG
473 in their sera but only one had anti-rocuronium IgE; second, IgE-expressing memory B cells elude
474 identification in blood samples (30, 32); and third, IgE antibody-expressing cells have been
475 reported to result from an intermediary switch to IgG rather than directly from IgM-expressing B
476 cells (59). Very recently, type 2-polarized memory B cells defined either as CD23⁺ IgG1⁺ (60) or
477 as IgG1 or IgG4-expressing CD23^{hi}, interleukin (IL)-4R α ^{hi}, and CD32^{low} (61) have been identified
478 from allergic patients to hold the allergen-specific IgE memory. Rocuronium haptenized on HSA
479 allowed for the sorting and culture of single memory B cells from all three patients into IgG-
480 secreting cells coupled to screening for both rocuronium reactivity and specificity by competitive
481 ELISA in their culture supernatant. The latter consideration is fundamental, as greater than 80%
482 of the cultures were excluded because of low reactivity and specificity; this curated the repertoire

483 data by eliminating false positives. The false positive fraction was similar when sorting CD45RB⁺
484 memory B cells (37), and when sorting memory B cells binding HSA-rocuronium labeled with
485 two different fluorochromes. Thus, the highly polyclonal anti-rocuronium antibody repertoires we
486 describe herein, with a high diversity among V(D)J gene usage, corresponds only to bona fide anti-
487 rocuronium antibodies. It remains unclear however if the memory B cell sorting method, using
488 monomeric carrier proteins coupled to approximately 15 rocuronium molecules each, might have
489 biased B cell selection towards higher avidity B cell clones.

490 No evidence of common gene usage, nor of clonal groups, could be found within and
491 between these patients, even between the repertoires of the same patient sampled 9 years apart
492 with no exposure to any NMBA during this period. The underlying reason for the high
493 polyclonality of anti-rocuronium antibody repertoires might be the absence of exposure to
494 rocuronium before the initial anaphylactic episode in the perioperative setting in all these patients,
495 with exposure to other compounds present in cough syrup, cosmetics, hair dyes, household
496 cleaning products or drugs at the probable initiation of the immune reaction towards rocuronium
497 (12). One of the most incriminated culprits is pholcodine, an opioid cough suppressant containing
498 two tertiary ammonium groups, which is now considered a risk factor for anaphylaxis to NMBA
499 (28) and was withdrawn from the European market in December 2022. These difficult to quantify
500 exposures may have led to a diverse repertoire of memory B cells with cross-reactivity to
501 rocuronium; if Patient #1, who demonstrated different V(D)J recombination 9 years apart, is
502 representative of rocuronium-allergic individuals, one could hypothesize that their repertoires
503 would not to be stable over time. However, none of the mAbs corresponding to these V(D)J
504 rearrangements demonstrated cross-reactivity for any other NMBA, including vecuronium and
505 pancuronium, which differ only slightly from rocuronium. This result agrees with our previous
506 data showing that polyclonal IgG from Patient #1 purified on a rocuronium-bound column showed
507 exquisite specificity for rocuronium, with no binding to atracurium, pancuronium or vecuronium,
508 although partial cross-reactivity with suxamethonium was detected (16). This preference may be
509 the result of (i) a particular exposure of these patients to rocuronium-resembling molecules, (ii)
510 the stringency of the cell sort using rocuronium-haptenized HSA, or (iii) the modification
511 (functionalized linker) made to rocuronium to allow protein engraftment. As a comparison in the
512 rocuronium-immunized mouse, from all hybridomas analyzed, only two different V_H-V_L
513 combinations could be identified (mAb m1B6, IGVH1-63*J4 x KV2-112*KJ2; mAb m2B1,

514 IGVH1-81*J2 x KV2-112*KJ2), suggestive of a very restricted oligoclonal repertoire. These
515 results agree with the affinity for rocuronium determined for these human or mouse antibodies:
516 poor affinity when antibodies were isolated from polyclonal repertoires of cross-reactive BCRs to
517 rocuronium (as none of the patients had been pre-exposed to rocuronium) and high affinity from
518 oligoclonal repertoires of “antigen-trained” B cells.

519 The mAb 1F10 isolated from Patient #1_2014 showed the best affinity towards rocuronium
520 from all the antibodies identified from humans we studied herein, although this patient had been
521 included after presenting with a grade 2 hypersensitivity reaction to atracurium (16), with no
522 previous exposure to rocuronium. This mAb expressed as a human IgE gave positive results in a
523 mast cell activation test, which has recently been proposed as an alternative for basophil activation
524 tests (62), and was able to induce severe hypothermia in a mouse model of rocuronium-induced
525 anaphylaxis. Even though this mAb was isolated from an IgG-expressing B cell, it may represent
526 an IgE that is expressed and secreted by a class-switched activated B cell, as recently reported (60,
527 61), or expressed by a plasmablast or plasma cell involved in hypersensitivity to rocuronium.
528 Allergy-related IgE plasma cells have indeed recently been identified in the bone marrow of
529 chronically exposed mice and allergic individuals (63, 64). From our study, it remains unclear,
530 however, if such IgE plasma cells exist in patients exposed to a variety of compounds and not
531 chronically to a given antigen, and will merit further investigation if bone marrow samples from
532 NMBA-allergic individuals become available.

533 The specificity for rocuronium of all the above-mentioned antibodies, with no
534 crossreactivity for other NMBAs, can be explained for the antibodies raised from immunized mice
535 by their co-crystal structures with rocuronium. These indicated a similar binding between the two
536 pairs of V_H-V_L corresponding to mAbs 1B6 and 2B1, which possess different V-J rearrangements
537 of the heavy chain but an identical V-J rearrangement of the light chain with only two amino acid
538 differences. Both antibodies bound rocuronium through a hydrophobic pocket formed by residues
539 from the V_H and V_L chains, that do directly interact with the ammonium group that had been
540 hypothesized to be the main epitope involved in NMBA hypersensitivity (22). The quaternary
541 ammonium group and the uncharged 5 α -dihydrotestosterone counterpart were required for
542 binding. The closely related NMBAs vecuronium and pancuronium were also unable to bind, as
543 their side chains differ and cannot fit into the tight hydrophobic binding cleft of these two
544 antibodies. Indeed, the ring D substituent in vecuronium/pancuronium is a 6-atom piperidinium

545 cycle instead of a 5-atom pyrrolidinium cycle in rocuronium (resulting in a lack of space). In
546 addition, the hydrogen bond between rocuronium and the serine residue at position 54 in the
547 antibody heavy chain (at least in the chair conformation) cannot be established with vecuronium
548 or with pancuronium, as the rocuronium morpholino heterocycle involved is replaced by a
549 piperidin and piperidinium (devoid of an oxygen atom) in these compounds, respectively. One
550 could speculate that these specificities are the result of high selective pressure in rocuronium-
551 immunized mice and that these may not be present in antibodies generated in humans by exposure
552 to various ammonium-containing compounds (3).

553 Ten years ago, almost 40% to 60% of patients in post anesthesia care suffered from residual
554 neuromuscular blockade (5, 30, 65-68). The increased use of neuromuscular monitoring in some
555 countries allowed for this incidence to drop drastically, for example to 3 to 5% in recent years in
556 France and Portugal (69, 70). However, it remains an issue and even today it can have major
557 adverse outcomes by prolonging postoperative recovery, affecting respiratory function, and
558 causing muscle weakness with sometimes fatal outcomes (5, 71). Similarly to the rocuronium-
559 reversal agent sugammadex, both anti-rocuronium mAbs identified from immunized mice with
560 high-affinity for rocuronium demonstrated ability to capture rocuronium in vivo dose-dependently
561 in a prophylactic model of rocuronium-induced paralysis in mice. This rodent model, however,
562 does not recapitulate the state of deep neuromuscular blockade that is difficult to reverse in
563 patients. Currently, only rocuronium- and vecuronium-induced deep neuromuscular blockade can
564 be reversed almost immediately by sugammadex, with no solution available for deep blockade
565 induced by atracurium, the most used NMBA worldwide, nor pancuronium or others. This
566 possibility is of major importance for clinicians, especially for patients with unexpected impossible
567 mechanical ventilation or tracheal intubation. Importantly, anti-rocuronium mAb m1B6
568 demonstrated efficiency at reversing deep neuromuscular blockade in cynomolgus macaques in
569 this study, with similar kinetics at 5.3×10^{-8} M (10 mg/kg) as sugammadex at 4.5×10^{-7} M (1 mg/kg).

570 This study has limitations. We included only three patients in this proof-of-concept study,
571 with heterologous phenotypes in terms of NMBA hypersensitivity as the main inclusion criteria
572 was high serum concentrations of anti-rocuronium IgG. In addition, we established antibody
573 repertoires from IgG⁺ memory B cells as surrogates for IgE⁺ memory B cells, which may not be
574 representative of the IgE antibody repertoire. Further studies focusing on a larger cohort and on
575 anti-NMBA IgE producing cells, in particular long-lived bone marrow IgE plasma cells (63, 64),

576 should shed light on this question. In addition, we used the MT4 antibody clone rather than the
577 MEM55 antibody clone to stain CD45RB on memory B cells which may lead to identification of
578 different subpopulations (72). Finally, the costs of production of sugammadex and a monoclonal
579 antibody are incomparably in favor of sugammadex (73), but we believe this proof-of-concept
580 study provides an alternative for reversal of deep neuromuscular blockade in patients with
581 hypersensitivity to sugammadex (12, 48) or sugammadex-rocuronium inclusion complexes (11).

582 In conclusion, this work reports on the underlying causes of anaphylactic reactions to
583 NMBAs. By characterizing the memory B cell anti-rocuronium antibody repertoires from three
584 patients and producing high-affinity anti-rocuronium antibodies in mice, we have developed
585 potential diagnostic tools for rocuronium hypersensitivity. Moreover, the murine and nonhuman
586 primate studies provide proof-of-concept for antibodies as drugs for the reversal of neuromuscular
587 blockade.

588

589

MATERIALS AND METHODS

590

591 **Study Design**

592 Three patients that suffered previously from anaphylactic reactions to NMBA in the were selected
593 based on the concentration of anti-rocuronium IgG in their serum. The design of the study and
594 detailed clinical characteristics of Patient #1 have been described elsewhere (16, 74). The study
595 “Anesthesia-associated allergy: from new pathogenic insights to reliable diagnoses” was approved
596 by the Belgian ethical committee under #B300202042710 and is sponsored by the University of
597 Antwerp, registered under #8300201316408 in Belgium, and enabled the collection of blood from
598 Patient #2 and Patient #3.

599 BALB/c mice, C57BL/6J mice and Human FcεRI^{tg} mouse FcεRI^{-/-} mice on the C57BL/6
600 background (78) aged 7-12 weeks were randomly assigned to the study for rocuronium
601 immunizations, reversal agents experiments and anaphylaxis experiments, respectively. Sample
602 sizes were determined to be appropriate for determining the effects of each procedure. No power
603 calculation was performed. The experimenters were not blinded to the study.

604 Eighteen adult cynomolgus macaques (*Macaca fascicularis*), aged 4 to 7 years, were randomly
605 assigned to each of the six groups in the study. Sample sizes were determined to be appropriate for
606 determining rocuronium and reversal agents effects. No power calculation was performed. The
607 experimenters were not blinded to the study.

608

609 **Clinical study**

610 Three clinical studies were approved for the recruitment of NMBA-allergic patients. The French
611 multicentric NASA (“Neutrophil Activation in Systemic Anaphylaxis”) study involved 11
612 Anesthesia and Intensive Care departments in the Ile-de-France region in France. The study was
613 approved for all centers by an Institutional Review Board (ethical committee “Comité de
614 Protection des Personnes Ile-de-France I”, reference 2012-avril-12880), and registered before the
615 first inclusion at ClinicalTrials.gov (Identifier: NCT0163722). The design of the study and detailed
616 clinical characteristics of the patients have been described elsewhere (16, 74), and its ancillary
617 study NASAmAbs enabled the collection of 190 mL of blood from Patient #1_2014. The second
618 French multicentric study was MEDIREP (“Repertoire and Properties of Anti-drug Antibodies

619 Involved in Immediate Hypersensitivity in the Operating Room”) was approved by an Institutional
620 Review Board (ethical committee “*Comité de Protection des Personnes Ile-de-France IV*”,
621 reference 2022-A00709-34) on May 2022 and registered before the first inclusion at
622 ClinicalTrials.gov (Identifier: NCT05420935), allowing for the inclusion of Patient #1_2023. The
623 study “Anesthesia-associated allergy: from new pathogenic insights to reliable diagnoses” was
624 approved by the Belgian ethical committee under #B300202042710 and is sponsored by the
625 University of Antwerp, registered under #8300201316408 in Belgium, and enabled the collection
626 of blood from Patient #2 and Patient #3. Written informed consent was obtained from the patients
627 or their legal representative before study inclusion. Patient characteristics are described in Table
628 1.

629

630 **Classical anaphylaxis parameters**

631 As part of standard care procedures, the following circulating parameters were assessed as
632 previously described (55): histamine (EIA, Immunotech, Beckman Coulter) and tryptase (FEIA,
633 ImmunoCAP 250 Phadia, Thermo Fisher) in plasma and anti-quaternary ammonium-specific IgE
634 (FEIA, ImmunoCAP 250 Phadia, Thermo Fisher) in serum. A value less than 0.35 kU/L of specific
635 IgE was considered negative, as recommended (55). Tryptase concentrations two hours following
636 acute hypersensitivity reaction (AHR) were considered elevated when greater than $(1.2 \times [\text{baseline}$
637 $\text{tryptase}] + 2 \mu\text{g/L})$ as recommended (75). A histamine concentration above 20 nmol/L 30 minutes
638 following AHR was considered elevated. Data are summarized in Table 1. In addition, specific
639 IgE concentrations for rocuronium were assessed using the non-clinically-validated rocuronium
640 ImmunoCAP (Phadia AB; ImmunCAP#c202) (76), obtained as experimental prototypes made for
641 research use, and with the LuLISA technique using an anti-IgE nanobody-luciferase tandem (1:50)
642 as previously described (77).

643

644 **Mice**

645 C57BL/6J mice (JAX #000664) were purchased from Charles River, and used for experiments
646 after maintaining the mice for at least one week in SPF conditions after arrival in Institut Pasteur’s
647 animal facility. Human FcεRI^{tg} mouse FcεRI^{-/-} mice on the C57BL/6 background were described
648 previously (78). Mice were bred at Institut Pasteur and used for experiments at 7-12 weeks of age.

649 All animal care and experimentation were conducted in compliance with the guidelines and
650 specific approval of the Animal Ethics committee CETEA number 89 (Institut Pasteur) registered
651 under #170043, #2013-0103 and #27465 and by the French Ministry of Research under agreement
652 #00513.02.

653

654 **Production and characterization of rocuronium bioconjugates**

655 Hapten-protein couplings were performed using active ester derivatives of rocuronium and KLH
656 or monomeric HSA as the carrier protein. A carboxylate function was first introduced by adding
657 succinic anhydride to rocuronium bromide in the presence of 4-(dimethylamino)-pyridine in
658 pyridine. After stirring overnight at room temperature under argon, the solvent was removed under
659 vacuum. The crude compound was purified by reverse-phase flash chromatography, resulting in a
660 rocuronium derivative with a carboxylic acid (Roc-COOH) at the 3-position of the steroid scaffold.
661 The compound was characterized by mass spectrometry and by nuclear magnetic resonance
662 analysis. All quantities of products were calculated relative to the carrier protein. The
663 functionalized rocuronium (200 equivalent) was dissolved in 0.05 M MES + 0.5 M NaCl buffer
664 (pH 5.6) and combined with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (400 equivalent) in
665 the presence of N-hydroxysulfosuccinimide (200 equivalent). After stirring at room temperature
666 for 5 min, the pH was adjusted to 7.2 with 2 M NaOH. This solution was progressively added to
667 the carrier protein dissolved in 100mM phosphate-buffered saline (PBS) at pH 7.2. After 3 hours
668 of gentle agitation at room temperature, samples were buffer exchanged into 20mM HEPES and
669 300mM NaCl with minitrap desalting columns. The exact measurement of the concentration of the
670 bioconjugates was achieved by quantitative amino acid analysis. The average density of
671 conjugated rocuronium derivatives was evaluated by matrix-assisted laser desorption/ionization
672 time-of-flight mass spectrometry. The HSA-rocuronium conjugates used for all experiments were
673 monomeric with an average engraftment of 15 rocuronium molecules per HSA molecule,
674 determined with the mean of the gaussian distribution of the m/z (mass-to-charge ratio) as
675 previously described by matrix-assisted laser desorption/ionization time-of-flight mass
676 spectrometry (16).

677

678 **Human B cell single cell culture**

679 Culture supernatants from single cell cultured memory B cells were generated according to the
680 protocol previously described by Sokal *et al.* (36). After thawing, PBMCs were counted and
681 stained with antibodies directed against surface markers: CD27-brilliant violet (BV) 421⁺ (Clone
682 M-T2711, BD Biosciences) or CD45RB-BV421⁺ (Clone MT4 BD Biosciences), CD3/CD14-
683 allophycocyanin (APC)-cyanine (Cy)-7⁻ CD19-phycoerythrin (PE)-Texas Red⁺ (anti-CD3, Clone
684 SK7, Fischer Scientific; anti-CD14, Clone M ϕ P9 BD Biosciences; anti-CD19, clone HIB19 BD
685 Biosciences), CD38-Peridinin-chlorophyll-protein (PerCP)-Cy5.5⁺ (Clone HIT2 BD Biosciences),
686 IgD-PE⁻ (Clone W18340F BioLegend), HSA-AlexaFluor488⁻ or HSA-Roc-AlexaFluor488⁺,
687 HSA-Roc-APC⁺). HSA and HSA-rocuronium were conjugated to fluorescent probes using Alexa
688 Fluor 488 and 647 microscale labeling kits (Invitrogen) following manufacturer's instructions.
689 Conjugates with a mean of 15 rocuronium molecules grafted on a HSA carrier were used for the
690 sort. Cells were then sorted using the ultra-purity mode (single cell) on Aria III in 96-well plates
691 containing MS40Lo cells expressing CD40L (a kind gift from Garnett Kelsoe) (79). Cells were
692 cultured for 25 days at 37°C with 5% CO₂ in RPMI-1640 (Invitrogen) supplemented with 10%
693 HyClone fetal bovine serum (FBS, Thermo Scientific), 10 mM HEPES, 1 mM sodium pyruvate,
694 100 units/mL penicillin, 100 mg/mL streptomycin, and MEM non-essential amino acids (all
695 Invitrogen) with the addition of recombinant human B-cell activating factor (BAFF, 10 ng/ml),
696 IL-2 (50 ng/ml), IL-4 (10 ng/ml), and IL-21 (10 ng/ml; all Peprotech). The supernatant was
697 removed and replaced every 3 to 4 days of the culture and harvested on day 18, 21 and 25 of cell
698 culture. Clones corresponding to supernatants with the highest ratio of optical density (OD) values
699 from anti-rocuronium ELISA divided by OD value of the anti-IgG ELISA were considered to
700 possess reactivity towards rocuronium (at least to HSA-rocuronium) and were selected to be
701 recombinantly expressed as mAbs.

702

703 **V_H-V_L sequencing from monoclonal cell cultures**

704 Clones of interest were sequenced first by RNA extraction using NucleoSpin96 RNA extraction
705 kit (Macherey-Nagel) according to the manufacturer's instruction. A reverse transcription step was
706 then performed using the SuperScript IV enzyme (Thermo Fisher) in a 14 ml final volume (42°C
707 10 min, 25°C 10 min, 50°C 60 min, 94°C 5 min) with 4 ml of RNA and random hexamers (Thermo
708 Fisher scientific). A polymerase chain reaction (PCR) was further performed based on the protocol
709 established by Tiller *et al.* (80). Briefly, 3.5 ml of cDNA was used as template and amplified in a

710 total volume of 40 ml with a mix of primers and using the HotStar_Taq DNA polymerase (Qiagen)
711 and 50 cycles of PCR (94°C 30 s, 58°C for V_H and kappa/60°C for lambda 30 s, 72°C 60 s). Sanger
712 sequencing was performed by Eurofins.

713

714 **Gene synthesis, cloning, and mAb production**

715 V_H and V_L synthesis in pUC19-Ig γ 1.N297A or pUC-kappachain/pUC-lambdachain-expressing
716 vectors were performed by Synbio Technologies. Antibodies were produced by transient co-
717 transfection of V_H-C_H and V_L-C_L expression plasmids into exponentially growing Freestyle HEK
718 293-F that were cultured in serum-free Freestyle 293 Expression Medium (Life Technologies) in
719 suspension at 37°C in a humidified 8% CO₂ incubator on a shaker platform rotating at 110 rpm.
720 Twenty-four hours before transfection, cells were harvested by centrifugation at 300 x g for 5 min
721 and resuspended in expression medium at a density of 1 x 10⁶ cells/ml, and cultured overnight in
722 the same conditions as mentioned above. To produce mAbs, 40 μ g of each V_H and V_L expressing
723 plasmids were diluted in 80 μ l of FectoPRO reagent (Polyplus) at a final DNA concentration of
724 0.8 μ g/ml, incubated for 10 minutes at room temperature before addition to the cells. Twenty-four
725 hours post-transfection, cells were diluted 1:1 with expression medium. Cells were cultured for 6
726 days after transfection, and then supernatants were harvested, centrifuged at 1800 g for 40 min,
727 and filtered (0.2 μ m). Antibodies were purified by affinity chromatography using an AKTA pure
728 FPLC instrument (GE Healthcare) on a HiTrap Protein A Column (GE Healthcare) and desalted
729 on a HiTrap Desalting Column (GE Healthcare).

730

731 **Production of mouse anti-rocuronium hybridomas**

732 Female C57Bl/6J were immunized subcutaneously with 10 μ g KLH-Rocuronium combined with
733 adjuvant alum 1:1 (v:v) and 20 ng pertussis toxin in physiological saline 0.9% NaCl three times at
734 3-week intervals. Mice were boosted with 10 μ g KLH-Rocuronium without adjuvant three weeks
735 after the last immunization. Four days later the cervical lymph nodes were removed and fused with
736 myeloma cells P3X63Ag8.653 for hybridoma generation using the ClonaCell-HY Hybridoma kit
737 (StemCell Technologies). After screening, the positive clones were expanded in T250cm² flasks,
738 cells were introduced in 2L rollers and incubated in rotating rollers at 37°C for 10 days. The
739 supernatant was filtered and purified with Protein A-affinity chromatography columns.

740

741 **Anti-rocuronium ELISA**

742 96-well plates (Costar) were coated with HSA-rocuronium (mean of 15 rocuronium molecules
743 grafted on a HSA carrier molecule) or HSA at 1 µg/mL in PBS 1X (Gibco) overnight at 4°C
744 overnight, washed 3 times with PBS Tween 20 0.5% (PBST), blocked with PBS-3% bovine serum
745 albumin (BSA) for mAb detection or PBS + FBS low IgG to screen human serum, and washed
746 three times with PBST. Murine sera (diluted 1:250,000-1:5,000-1:100) or pure cell culture
747 supernatant or monoclonal antibodies were incubated for 2 hours at room temperature and bound
748 antibodies were detected with horseradish peroxidase (HRP)-conjugated goat anti mouse or human
749 IgG (Bethyl Laboratories) at 1:10,000. The secondary was revealed using OPD substrate (Sigma-
750 Aldrich) and the reaction stopped with 2M H₂SO₄ and absorbance was subsequently recorded at
751 490 nm and corrected at 620 nm using a spectrophotometer (Biophotometer, Eppendorff). The
752 final OD reported in all graphs and tables corresponds to the subtraction of the OD value of the
753 HSA-rocuronium ELISA minus the OD value of the HSA ELISA. OD values were considered
754 positive if they were at least three times higher than the OD value of the negative control.
755 Cross-reactivity with classical allergens (e.g., peanut extract, amoxicillin and ovalbumin) was
756 assayed using 1 µg/mL of allergens for coating, using afterwards the same protocol as for the HSA-
757 rocuronium ELISA.

758

759 **Competition ELISA**

760 To ascertain antibody reactivity to rocuronium, and not towards the linker employed to attach
761 rocuronium to HSA, or a neo-epitope formed between rocuronium, the linker and HSA, a
762 competitive inhibition ELISA was developed. This inhibition ELISA also enabled to detect cross-
763 binding of antibodies to a panel of different NMBAs. 96-well plates (Costar) were coated with
764 1µg/ml HSA-rocuronium at 4°C overnight, washed 3 times with PBST, blocked with PBST
765 containing 3% BSA at room temperature for 2 hours.

766

767 For inhibition ELISAs of single B cell cultures, supernatants were incubated with 3,000 µM of free
768 rocuronium at room temperature for 20 min. HSA-rocuronium was coated at 1 µg/mL (0.227 µM
769 of rocuronium). 3,000 µM of free rocuronium thus corresponds to an excess of approximately
770 13,000-fold. For purified antibodies, 300 ng/mL (for murine mAbs) or 1,500 ng/mL (for human
771 mAbs) were pre-incubated with 5-fold serial dilution of free rocuronium (Kalcex), vecuronium

772 (Sigma-Aldrich), pancuronium (Euromedex), suxamethonium (Fischer Scientific), or NMBA
773 control devoid of the quaternary ammonium including 5 α -DHT (Merck), diisopentyl succinate
774 (AK Scientific), and pholcodine (Sigma-Aldrich) at concentrations ranging from 15,000 μ M to
775 0.96 μ M.

776

777 The antibody-NMBA mixture was added to the ELISA plates and incubated at room temperature
778 for 2 hours, washed 3 times and revealed with HRP-conjugated goat anti-mouse/human IgG
779 (Bethyl Laboratories) at 1:10,000 dilution. Plates were revealed using an OPD substrate (Sigma-
780 Aldrich). The reaction was stopped with 2M H₂SO₄ and absorbance was subsequently recorded
781 at 490 nm and corrected at 620 nm using a spectrophotometer (Biophotometer, Eppendorff).
782 Antibodies in the supernatant of B cell culture were considered rocuronium specific if more than
783 50% of the signal was inhibited in presence of free rocuronium. For purified mAbs, the half-
784 maximal inhibitory concentration (IC₅₀) was measured using a non-linear fit of [Inhibitor] versus
785 response with a variable slope (four parameters) using GraphPad Prism.

786

787 **Affinity measurements using Bio-Layer Interferometry (BLI)**

788 Affinity of mAbs for rocuronium was tested using Bio-layer interferometry with the OctetRED
789 384 system. Amine Reactive Second-Generation (AR2G) biosensors were covalently immobilized
790 with HSA-rocuronium (mean of 15 rocuronium molecules per HSA carrier molecule), then
791 inserted in wells containing different concentrations of antibodies for 1,800 seconds (association
792 phase). The antigen-antibody complex was dissociated by introducing the biosensor in the
793 reference buffer (PBS+BSA). Curves were retrieved as raw data from the Octet Data Analysis
794 software and processed in the Scrubber2 software prior to analyses with the Biaevaluation
795 software. IgG-free buffer was used as reference and for background subtraction. Fitting curves and
796 KD values were obtained using the 1:1 Langmuir model.

797

798 **Affinity measurements using Surface Plasmon Resonance (SPR)**

799 CM5 chips (Cytiva) were primed using PBS, washed 3 times with NaOH (50 mM) and SDS (0.1%)
800 180seconds each time (5 μ L/min). At least 5,000 RU of antibody was covalently bound to CM5
801 chip by activating using 1:1 NHS:EDC (6,000 seconds), injecting the antibody (900 seconds) in
802 acetate buffer pH5.5 and quenching with EtNH₂ (600 seconds). Free rocuronium was injected (30

803 $\mu\text{L}/\text{min}$) in PBS-BSA (1 mg/mL) at different concentrations. Signal was subtracted to the reference
804 canal bound with a non-specific antibody. K_D was determined using a kinetic analysis (RI fixed to
805 0, model 1:1 binding) with Biaeval software.

806

807 **Antibody repertoire analysis and representative mAb selection**

808 Docker (81) / Apptainer (82), Nextflow (83) and Git (84) were used to better control
809 reproducibility aspects (85). The Nextflow pipeline developed to analyze batches of nucleotide
810 sequence fasta files, representing the raw single cell Sanger sequencing of the V_H - V_L IgG domains,
811 is available at <https://zenodo.org/records/13221082>, and is detailed below. Most of the process is
812 based on tools from the *immcantation portal* (86), specifically from the change-O toolset (38). V_H
813 and V_L sequences, although single cell paired, could only be analyzed independently (V_H
814 sequences, then V_L sequences). First, the *AssignGenes.py igblast* tool was run to detect and
815 annotate the V, D, J, CDR and FWR domains in each sequence, using the *imgt* database (87). Then,
816 *MakeDb.py igblast* converted the result into a readable tsv file. The *ParseDb.py select* tool kept
817 productive sequences, that is those for which: (1) the coding region had an open reading frame, (2)
818 no defects were present in the start codon, splicing sites, or regulatory elements, (3) no internal
819 stop codons were found, and (4) junction regions were in frame. The clustering of sequences into
820 clonal groups was performed as follows: (1) grouping of the sequences according to same V and J
821 genes (allelic variation not considered) and same length of the CDR3, (2) for each group,
822 computation of the distance score $D = (\text{Hamming distance}) / (\text{CDR3 length})$ between each 2 x 2
823 sequences, (3) determination of the D threshold, that determine whether 2 sequences derive from
824 the same germline cell sequence or not, using the *distToNearest()* function of the *shazam* R
825 package, (4) clone assignment for each sequence, with the same ID for sequences belonging to a
826 same clonal group, using the *DefineClones.py* tool. Of note, a few sequences may have been lost
827 at that stage, as they did not fit the criteria required by this tool. Then, the putative germline
828 sequence of each clonal group was inferred using the *CreateGermlines.py* tool. Finally, a single
829 *all_passed_seq.tsv* file was obtained for each batch of V_H or V_L initial fasta files, containing, for
830 each sequence, the V, D, J gene and allelic names returned by the *AssignGenes.py igblast* tool, the
831 clone ID, the putative germline sequence before somatic hypermutations, and the putative V, D, J
832 gene and allelic names of the germline sequence returned by the *CreateGermlines.py* tool.

833

834 Donut plots in Fig. 1E and fig. S6A were drawn after gathering the sequences of the
835 all_passed_seq.tsv file according to the V and J gene and allelic names of their putative germline
836 sequence. Amino acid sequence alignments and percentage identity calculations were performed
837 with Jalview software using MUltiple Sequence Comparison by Log- Expectation (Muscle) and
838 colored by percentage identity. Number of mutations were obtained with IMGT database by
839 comparing sequences with germlines and plotted as histograms using GraphPad Prism.

840

841 **Human mast cell activation test**

842 CD34⁺ precursor cells were isolated from peripheral blood mononuclear cells of healthy donors
843 (provided by the French Blood Bank EFS). CD34⁺ cells were maintained for 1 week under serum-
844 free conditions using StemSpan medium (9655 Stemcell Technologies) supplemented with
845 recombinant human IL-6 (50 ng/ml; 200-06 Peprotech), human IL-3 (10 ng/ml; 200-03 Peprotech),
846 3% supernatant of CHO transfectants secreting murine stem cell factor (SCF; a gift from P.
847 Dubreuil, corresponding to approximately 50 ng/ml SCF) and ciprofloxacin (10 ng/mL; 17850-
848 5G-F Sigma Aldrich). Thereafter, the cells were maintained in IMDM Glutamax I (31980048), 2-
849 mercaptoethanol (31-350-010), insulin-transferrin selenium (2506865) (all from Gibco), sodium
850 pyruvate (S8636 Sigma Aldrich), 0.5% BSA (A2153 Sigma Aldrich), Penicillin/Streptomycin
851 (100 Units/mL / 100µg/mL; Life Technologies), IL-6 (50 ng/ml 200-06 Peprotech) and 3%
852 supernatant of CHO transfectants secreting mouse SCF. Mast cells were considered ready for
853 experiments after 10 to 12 weeks of culture, when >95% of the cells stained positive for both
854 CD117 (KIT, APC-labeled clone YB5.B8; BD Biosciences) and human FcεRI (using PE/Cy7-
855 labeled clone AER37; BioLegend) by flow cytometry. Cells were ready for experiments after
856 approximately 10 weeks in culture (at which time >95% of all cells were CD117⁺ FcεRI⁺).

857

858 Human mast cells were sensitized overnight with the anti-rocuronium antibody h1F10 produced
859 in a human IgE format at 1 µg/mL. Cells were then washed and stimulated with increasing doses
860 of HSA-rocuronium in Tyrode's buffer. Mast cell degranulation was measured by flow cytometry
861 using fluorescent avidin (5 µg/mL; A2170 Invitrogen) which binds to heparin contained in mast
862 cell granules. Data were acquired using a MACSQuant MQ10 flow cytometer (Miltenyi) and
863 analyzed with FlowJo v10.8.1 software (TreeStar).

864

865 **Passive Systemic anaphylaxis in mice**

866 hFcεRI^{tg} mFcεRI^{-/-} mice (78) were injected intravenously with 20 μg of anti-rocuronium human
867 IgE h1F10 at 0h and 24h and challenged at 48h with 267 μg of HSA-roc. Control mice were
868 injected with irrelevant human IgE and physiological serum. Central temperature was monitored
869 using a digital thermometer with rectal probe (YSI), and time of death was recorded.

870

871 **Prophylaxis of neuromuscular blockade in mice**

872 C57BL/6 mice were pre-injected intravenously with roc-specific antibodies in a human IgG1
873 N₂₉₇A format at different doses followed 10 minutes later by a lethal dose of free rocuronium (160
874 μg/kg). A vitality score was defined based on visual cues: 5, no symptom of neuromuscular
875 blockade; 4, transient (<30s) symptoms of flattening, slowing down, difficulty to stand on legs; 3,
876 longer symptoms (<1min) with respiratory distress; 2, inactivity, impossibility to stand on legs,
877 severe respiratory difficulties; 1, death.

878

879 **scFv production, purification and characterization**

880 Rocuronium-specific V_H and V_L sequences were built into scFvs with a polyglycine-serine linker
881 (GGGS)₄ between the V_H and V_L sequences. The V_H-linker-V_L sequences were synthesized at
882 Genscript with codon optimization for expression in Drosophila S2 cells (ATCC CRL-1963),
883 cloned into pT-350 vector using BglIII and BstBI restriction sites (the vector was a gift from F.
884 Rey, Institut Pasteur). For transfection, 5x10⁶ Drosophila S2 cells were seeded in a T25 conical
885 flask containing 5 ml Schneider's Drosophila medium (Gibco) complemented with 10% FBS and
886 incubated at 28°C overnight. Twenty-four hours later, 2 μg of pMT-Roc-scFv-Strep plasmid were
887 co-transfected with 0.1 μg (ratio 20:1) of selection plasmid pCoBlast carrying a gene that confers
888 resistance to puromycin. Effectene transfection kit was used (Qiagen) according to the vendor's
889 protocol. Forty-eight hours after transfection, the selection process was started by addition of
890 puromycin (Invivogen) to the cells at a final concentration of 7 μg/ml. Transfected cells were
891 collected every 4 to 5 days by centrifugation at 90g for 5 min, and cell pellets were resuspended
892 in fresh medium containing 7 μg/ml puromycin. Cell propagation was initiated approximately 2
893 weeks after transfection. Puromycin-selected cells were adapted to serum-free HyClone Insect cell
894 culture media (GE healthcare life science) and were amplified in large volume flasks as needed.

895 Protein expression was induced with 0.5 mM CuSO₄ when the cell density reached approximately
896 7.5x10⁶ cells/ml. Seven days after induction, S2 cell suspension was centrifuged for 30 min at
897 15,000g to remove the cells, and the supernatant was collected. Avidin was added at 15 mg/L to
898 sequester any biotin present in the medium. The supernatant, cleared by centrifugation at 20,000g
899 for 30 min and filtration, was loaded onto a Strep-Tactin Superflow high-capacity 5 mL column
900 (IBA GmbH) using a peristaltic pump or ÄKTA high-pressure liquid chromatography (HPLC)
901 system (Cytiva). The column was washed with 15 mL of 0.1 M Tris pH 8, 0.15 M NaCl, 1 mM
902 EDTA, and the protein was eluted with 8 mL of the same buffer containing 2.5 mM desthiobiotin.
903

904 **Fab preparation**

905 Fabs were prepared following Pierce Fab Preparation Kit (Thermo Fisher Scientific) instructions.
906 Samples were digested for 4h with papain in the presence of 4 mM cysteine. After purification on
907 protein A columns, a gel filtration was performed with Tris 50mM pH, 7.4; 100 mM NaCl.
908

909 **Crystallization and X-ray data collection**

910 Crystallization screening trials were carried out by the vapor diffusion method using a Mosquito
911 nanodispensing system (STPLabtech) following established protocols (88). Briefly, we set up
912 crystallization sitting drops of 400 nL containing a 1:1 mixture of protein sample (Fab-m2B1 or
913 scFv of Fabm1B6) in complex with 10 mM of rocuronium and commercially available
914 crystallization solutions (88) equilibrated against 150 µL of reservoir solution in multiwell plates
915 (Greiner Bio-One). The crystallization plates were stored at 18°C in a RockImager (Formulatrix)
916 automated imaging system to monitor crystal growth. Manual optimization was performed in
917 Linbro plates with the hanging-drop method by mixing 2 µl of protein samples with 2 µl of
918 reservoir solution. The best crystals were obtained with the conditions shown in table S2. The
919 crystals were flash cooled in liquid nitrogen for data collection using the crystallization solution
920 as cryoprotectant. X-ray diffraction data were collected on beamlines PROXIMA-1 and
921 PROXIMA-2A at the synchrotron SOLEIL. Diffraction images were integrated with autoPROC
922 (89) and XDS (90), and crystallographic calculations were carried out with programs from the
923 CCP4 program suite (91).

924

925 **Structure determination and model refinement.**

926 The structures of Fab-m2B1 and scFv-m1B6 in complex with rocuronium were solved by
927 molecular replacement with Phaser (92) using the structures of the catalytic antibody Fab7A1 (pdb
928 2AJU) as search model. The final models of the complexes were obtained through interactive
929 cycles of manual model building with Coot (93) and reciprocal space refinement with Refmac5
930 (94). X-ray data collection and model refinement statistics are summarized in table S2. Figures
931 showing the crystallographic models were generated with Pymol (Schrodinger, LLC).

932

933 **Anesthesia and reversal of neuromuscular blockade in cynomolgus macaques**

934 Adult cynomolgus macaques (*Macaca fascicularis*) were randomly assigned to this study. All
935 animals were housed within IDMIT animal facilities at CEA, Fontenay-aux-Roses in Bio Safety
936 Level (BSL)-2 facilities (Animal facility authorization #D92-032-02, Préfecture des Hauts de
937 Seine, France) and in compliance with the European Directive 2010/63/EU, the French regulations
938 and the Standards for Human Care and Use of Laboratory Animals of the Office for Laboratory
939 Animal Welfare (OLAW, insurance number #A5826-01, US). Animals tested negative for
940 *Campylobacter*, *Yersinia*, *Shigella*, and *Salmonella* before being used in the study. Experiments
941 using macaques were approved by the local ethical committee (CEtEA #44) and the French
942 Research, Innovation, and Education Ministry under registration number APAFIS#36723-
943 2022041910357437 v1.

944

945 Anesthesia and monitoring of neuromuscular blockade in cynomolgus macaques was performed
946 as described in (46). All animals were anaesthetized according to institutional guidelines. For all
947 procedures, the animals were first sedated with ketamine (Imalgene 1000, 5 mg/kg) and
948 medetomidine (Domitor, 0.5 mg/kg). An intravenous (IV) line was placed, followed by tracheal
949 intubation allowing mechanical ventilation (Hallowell EMC Matrix 3002Pro veterinary
950 ventilator), and anesthesia was maintained with isoflurane (Isoflu-vet 1,000 mg/g, 0.5-1.5%).
951 Once complete anesthesia was assessed, macaques received rocuronium bromide (Esmeron, MSD
952 50 mg/5mL) by IV bolus infusion. When indicated, animals also received sugammadex (Bridion,
953 MSD, 1 mg/kg) or antibody m1B6 (2.5-5-10-15 mg/kg) for rocuronium-induced neuromuscular
954 blockade reversal. Animals were monitored using a Nihon Kohden PVM-2703 monitor during all
955 the anesthesia procedures. Physiological parameters (capnography, heart rate, respiratory rate,
956 oxygen saturation, arterial blood pressure, and temperature) were followed in real-time and

957 recorded in a chart every 10 minutes. Neuromuscular blockade monitoring devices (TOF-Watch
958 and ToFscan) measuring the T4/T1 ratio were installed before NMBA infusion. Mechanical
959 ventilation was maintained for the whole procedure until complete recovery of the neuromuscular
960 function, assessed with a TOF ratio > 90%. All experiments were performed under the supervision
961 of a trained anesthesiologist and a veterinarian. After the procedures, anesthesia was reversed with
962 atipamezole (Antisedan, 0.5 mg/kg), and animals were resumed to their cage under supervision
963 until complete recovery.

964

965 Animals were clinically followed for three days post-infusion. Clinical examination, body weight,
966 and rectal temperature were recorded at each bleeding time, including a complete blood count on
967 each day and a blood biochemistry evaluation on days 0 and 1. Blood sampling did not exceed
968 7.5% of the total blood volume per week, following ethical recommendations.

969

970 **Statistical analyses**

971 Individual-level data are presented in data file S2. The R environment v4.3.1 was used for all the
972 analyses (R foundation, <https://www.r-project.org/>). Data were neither averaged nor normalized
973 prior to analyses. Response variables were log₂ converted when required for better adjustment to
974 linear models. For Fig. 2F and Fig. 3A, a mixed models using the lmer() function of the lme4
975 package was used in order to consider the repeated measures on each mouse. For the other figures,
976 data were fitted to a simple linear model. Resulting effects of interest of the linear model were
977 two-by-two compared (contrast comparisons) using the emmeans() function of the emmeans
978 package. Statistical significance was set to a P value of 0.05 or less. In each panel, type I error was
979 controlled by correcting the P values according to the Benjamini & Hochberg method (“BH”
980 option in the p.adjust() function of R).

981

982 Supplemental Materials
983 Figs. S1 to S
984 Tables S1 and S2
985 MDAR Reproducibility Checklist
986 Data files S1 and S2
987

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1266

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1286

1287

AUTHOR CONTRIBUTIONS

1288 SB, SCM, AGC and PB designed experiments. AD, QZ, C Ganneau, C Pecalvel, LH, TD, BI, TR,
1289 CM, PNR, and C Pissis conducted in vitro experiments. AD, QZ, ORL, OG, JL, FR, C Gillis and
1290 YW conducted in vitro experiments. AD, FH, AS, AV, C Pecalvel, LH and TD conducted flow
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1292 AH, LLR, PC, FJ, DGE, GAM, SB, SCM, AG, and PB analyzed and interpreted data. PB acquired
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1294 the manuscript.

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COMPETING INTERESTS

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1301

1302

DATA AND MATERIALS AVAILABILITY

1303 All data associated with this study are in the paper or supplementary materials. The code of the
1304 Nextflow pipeline developed to analyze batches of nucleotide sequence fasta files is deposited
1305 under DOI: 10.5281/zenodo.13221082. Coordinates and structure factors of crystallographic data
1306 have been deposited in the Protein Data Bank under the accession codes 8S4K (Fab2B1 in complex
1307 with rocuronium) and 8S4H (scFv-1B6 in complex with rocuronium). Further information and

1308 requests under MTA for resources, reagents, and materials that are not commercially available
1309 should be directed to and will be fulfilled by the corresponding author (bruhns@pasteur.fr).

1310

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1341 **Figure 1. Identification of rocuronium-specific IgG repertoires from NMBA-allergic**
1342 **patients. (A)** Structural formula of rocuronium with its major groups circled and named. **(B)** IgE
1343 anti-rocuronium ImmunoCAP results from the serum of indicated patients (each value is a
1344 replicate, $n \geq 2$), or serum from five blood bank donors (each value represents one donor). The cut-
1345 off was set at 0.13 kUA/L and is indicated by the dotted line. **(C)** IgG anti-rocuronium ELISA
1346 results ($OD_{\text{HSA-roc}}$ minus OD_{HSA}) from the serum of indicated patients (each value is a replicate,
1347 $n=3$), or serum from five blood bank donors (each value represents one sample from one donor).
1348 The cut-off was set at 0.28 OD and is indicated by the dotted line. **(D)** Example of gating strategy
1349 for the single-cell sort of rocuronium-binding memory B cells ($CD3^- CD14^- CD19^+ CD38^- CD27^+$
1350 $IgD^- HSA\text{-roc}^+ HSA^-$) from the PBMCs of Patient #1_2014. Numbers in the panels indicate the
1351 percentage of cells gated among the represented cells in each panel. BV, brilliant violet; PE,
1352 phycoerythrin; AF, Alexa Fluor. **(E)** Donut plot of V_H and V_L gene usage among the monoclonal
1353 B cell cultures secreting anti-rocuronium IgG from the indicated patients. The total number of cells
1354 is indicated in the center of the donut. The major V-J recombination and their frequency (in
1355 parentheses) are indicated in color. Means \pm standard deviations are indicated in (B) and (C).
1356 Statistical comparisons in (B) and (C) were obtained using contrast analysis after simple linear
1357 modeling and multiple-testing P-value adjustment. ****, $P \leq 0.0001$; ***, $P \leq 0.001$; **, $P \leq 0.01$;
1358 ns, not significant.

1359

1360 **Figure 2: Characterization of recombinant roc-specific mAbs from patients. (A)** IgG anti-
1361 rocuronium ELISA results are shown for mAbs isolated from indicated patients. A non-specific
1362 human IgG1 was used as a control. **(B)** Shown are results of an anti-IgG rocuronium competitive
1363 ELISA using fixed concentration of mAb 1F10 but increasing concentrations of indicated
1364 molecules free in solution. $5\alpha\text{-DHT}$, $5\alpha\text{-dihydrotestosterone}$; IC_{50} , half-maximal inhibitory
1365 concentration. **(C)** Bio-layer interferometry avidity measurement of the interaction between
1366 immobilized HSA-rocuronium and varying concentrations of mAb 1F10 in solution. **(D)** Surface
1367 Plasmon Resonance affinity measurement of the interaction between immobilized 1F10 and
1368 varying concentrations of free rocuronium in solution. In (C) and (D), K_D values were obtained by
1369 fitting the curves using a heterogenous ligand model. **(E)** Human mast cell activation test using
1370 mAb 1F10 in a human IgE format for sensitization, followed by challenge with HSA or HSA-roc,

1371 with degranulation measured using fluorescent avidin binding by flow cytometry (n=3). Unsti.,
1372 unstimulated. Means \pm standard deviations are indicated. Statistical comparisons were obtained
1373 using contrast analysis after linear modeling and multiple-testing P-value adjustment. ***, $P \leq$
1374 0.001. (F) Passive systemic anaphylaxis was measured by changes in body temperature ($\Delta^\circ\text{C}$) in
1375 hFc ϵ RI-transgenic mice sensitized twice with hIgE 1F10 and challenged with HSA-roc
1376 intravenously. Mean \pm standard deviation of 3 mice per group are indicated. Each of the three post
1377 injection effects were analyzed separately, comparing the contrast between 1F10 and isotype
1378 control groups after mixed linear modeling and multiple-testing P-value adjustment. P values are
1379 indicated at the end of each time period analyzed. **, $P \leq 0.01$; ns, not significant. (G)
1380 Experimental timeline (left panel), establishment of the lethal dose of rocuronium and time to
1381 death (middle panel; $n \geq 2$), and prophylaxis of neuromuscular blockade (right, $n=2$) in wild-type
1382 mice. Prophylaxis was evaluated by vitality scores of mice injected first with sugammadex
1383 (sugam.) or 400 μg /mouse of indicated anti-rocuronium mAbs, followed by rocuronium injection.
1384 Vitality is scored as follows: 5 (no behavior changes), 4 (transient inactivity; ≤ 30 sec), 3
1385 (respiratory distress and intermediate inactivity; >30 sec), 2 (flipping or inactivity; >1 min), 1
1386 (death).

1387

1388 **Figure 3. mAbs isolated from immunized mice exclusively bind rocuronium.** (A) IgG anti-
1389 rocuronium ELISA results from sera of mice immunized with KLH or KLH-roc. Means are
1390 indicated by horizontal bars. Each dot represents a mouse. Statistical comparisons were obtained
1391 using contrast analysis after mixed linear modeling and multiple-testing P-value adjustment. ****,
1392 $P \leq 0.0001$; *, $P \leq 0.05$; ns, not significant. (B) IgG anti-rocuronium ELISA results from indicated
1393 mouse mAbs or a non-specific mouse IgG as isotype control. (C) Anti-IgG rocuronium
1394 competition ELISA results are shown, using fixed concentrations of mouse mAbs but increasing
1395 concentrations of indicated molecules free in solution. 5 α -DHT, 5-alpha-Di-Hydro-Testosterone;
1396 QAM, Quaternary Ammonium Molecule.

1397

1398 **Figure 4: X-ray crystal structures of mAbs in complex with rocuronium.** (A) Surface
1399 representation of co-crystal structures of rocuronium-specific mouse Fab-m2B1 (top) and scFv-
1400 m1B6 (bottom) in complex with rocuronium. (B) Superposition of the rocuronium and key

1401 residues (indicated by the one-letter code of the amino acid, and their position in the chain) of the
1402 binding site for the Fab-m2B1-rocuronium complex (top) and scFv-m1B6-rocuronium (bottom).
1403 The antibody heavy chain is colored in green and light chain in cyan. In both panels, the different
1404 atoms of the rocuronium molecule are colored as: C atoms in yellow, O atoms in red, and N atoms
1405 in dark blue.

1406

1407 **Figure 5: mAb m1B6 demonstrates therapeutic capacity to reverse neuromuscular blockade.**

1408 (A) Prophylactic effect of indicated mAbs on rocuronium-induced neuromuscular blockade with
1409 vitality scores of wild-type mice ($n \geq 3$) as in Fig. 2E. Means \pm standard deviations are indicated.
1410 Each dot represents a mouse. Statistical comparisons were obtained using contrast analysis after
1411 linear modeling and multiple-testing P-value adjustment. ***, $P \leq 0.001$, **, $P \leq 0.01$. Results for
1412 m2B1 1 to 8 mg/kg are not significant. (B) Scheme of the experimental protocol in macaques. (C
1413 and D) TOF measurement are shown for one representative macaque following rocuronium
1414 injection to monitor neuromuscular reversal without capture molecule (C) or following mAb m1B6
1415 injection (D). The rocuronium injection time point and the time point of resumption of spontaneous
1416 ventilation (SV) and of TOF ratio $T4/T_{ref} > 0.9$ are indicated. (E and F) Dose-dependent
1417 therapeutic effect of mAb m1B6 in macaques was assessed by time to spontaneous ventilation (E)
1418 or time for the TOF ratio $T4/T_{ref}$ to reach 90% (F). $n=3$ for all groups except $n=4$ for sugammadex
1419 (Suga., in mg/kg). Statistical comparisons were obtained using contrast analysis after linear
1420 modeling and multiple-testing P-value adjustment. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

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TABLES

Table 1: Clinical signs and therapeutic interventions of the allergic patients included in the study. ICU, intensive care unit; IV, intravenous; N.A., not available; ACEI, Angiotensin-Converting-Enzyme Inhibitor; ARA, Angiotensin II Receptor Antagonist; BB, Beta-blocker; CI: Calcium channel inhibitor. *includes drugs, latex, food, Hymenoptera venom, pollen/moth, and animals/mold.

Variable	Patient #1	Patient #2	Patient #3
Gender	Female	Male	Female
Age (years)	43	47	69
Previous general anesthesia	Yes	No	N.A.
Medication (ACEI/ARA/BB/CI)	No	N.A.	N.A.
History of allergy			
Allergy*/Asthma/Atopy	No	No	No
Mastocytosis	N.A.	No	No
Type of surgery			
Scheduled surgery	Visceral	Neurosurgery	Orthopedic
Surgery with a context of infection	Yes	Yes	Yes
Surgery with a context of infection	No	No	No
NMBA used during surgery	Atracurium	Rocuronium	Rocuronium
Clinical signs			
Erythema	No	Yes	Yes
Tachycardia	Yes	No	Yes
Highest heart rate value (/min)	100	N.A.	115
Bradycardia	No	No	No
Arrhythmia	No	No	No
Arterial hypotension	Yes	Yes	Yes
Bronchospasm	No	Yes	Yes
Hypoxemia	No	No	No
Cardiac arrest	No	No	No
Ring and Messmer severity scale			
Ring and Messmer severity scale	2	3	4
Delay between anesthesia induction and first signs of the reaction (min)			
Delay between anesthesia induction and first signs of the reaction (min)	5	5	5
Delay between first signs of the reaction and treatment initiation (min)			
Delay between first signs of the reaction and treatment initiation (min)	15	10	10
Therapeutic interventions			
Epinephrine IV alone	No	Yes	Yes
Phenylephrine IV alone	Yes	No	No
Glucocorticoids	No	Yes	No
Histamine receptor antagonists	No	Yes	No
Fluid resuscitation with crystalloids	No	Yes	Yes
Evolution			
Surgery cancelled	No	rescheduled	rescheduled
Admission to ICU	Yes	Yes	Yes
Delay between anaphylaxis and PBMC collection (months)	24	3.5	3
Allergy exploration			
QAM IgE ImmunoCAP (threshold 0.35)	0.1	2.58	0.28
Rocuronium Basophil Activation Test (BAT)	Negative	Positive	Positive
Rocuronium Skin Prick Test (SPT)	Negative	Positive	Positive
Allergen Exposure			
Pholcodine consumption in the past 12 months prior AHR	No	No	N.A.
Previous exposure to NMBA	Yes(1993, 1996, 1999)	N.A.	Yes, once
Occupational exposure to QAM (Patients reporting current or past cleaning profession or hairdressers)	No	N.A.	N.A.
Hair coloring frequency	Regular	No	N.A.

1429

1430 **Table 2: Characteristics of anti-rocuronium antibodies with identical heavy chain**
 1431 **rearrangements and CDR3 amino acid sequence.** Antibodies produced recombinantly with
 1432 demonstrated binding to rocuronium (roc) by ELISA are indicated in bold and their ELISA values
 1433 indicated for a 1 µg/mL antibody concentration. nd, not determined. Number of amino acid (AA)
 1434 mutations compared to the imgt database are indicated.

Patient	mAb name	V _H	CDR3 V _H	AA mutation # in V _H	V _L	CDR3 V _L	AA mutation # in V _L	roc ELISA (OD)
#1_2023	I8F7	V3-7*01 J4*02	CARGYYGSGTYTASVFDYW	7	nd	nd	nd	nd
	I8D1				nd	nd	nd	nd
	5GI5				LV2-11*01 LJ3*02	CCSFAGTKTWMF	8	nd
	I2G11				KV4-1*02 KJ1*01	CQQYYNTPWTF	9	0.16
	I1G9	V4-59*01 J4*02	CARIWGSSGNYFDFW	7	IGLV2-11*01 LJ1*01	CCSYAGSFYVF	6	nd
	I2H6				LV2-11*01 LJ1*01	CCSYAGSFYVF	8	0.21
	I4D10							
	I7A2	V4-59*01 J4*02	CARGTGYSSDWHVEYW	5	LV3-1*01 LJ2*01	CQAWDSSTFVMF	20	nd
	I7C8				LV1-47*01 LJ1*01	CASWDDSLRGYVF	7	nd
	I1C10	V3-7*01 J6*02	CARTDYGYYYYYGVDVW	6	LV1-51*02 LJ3*02	CGTWDSLSLAEVF	6	1.13
	I1D8							
	I7B8							
	I2H10	V4-39*01 J4*02	CVAMVRGVPAYYW	6	LV2-8*01 LJ1*01	CSSYAGSNNPYVF	4	nd
	I5G8				KV3-20*01 KJ2*01	CQQYGSSPYTF	7	nd
	I7D10	V3-53*01	CARAQTRNLYDGSGHYKGFDLW	11	KV1-5*03 F KJ1*01	CQQYNSYWTF	2	nd
	I7BI7	IGHJ3*01						
I7F5	V4-61*02 J6*02	CARERRYSNGWGSYYYYGLDVW	15	LV2-18*02 LJ3*02	CSSYTNKTNWVF	10	0.23	
I2G10								
#2	G4E12	V4-38*02	AMYCARAPCAGECRTLNWFDPW	41	KV3-20*01 KJ2*02	CQQYGTSPSTF	5	nd
	G5H9	J5*02						nd
#3	B1G2	V3-21*01	CARGHSSFDDFWSGYSPNWFDPW	1	KV4-1*01	CQQYYSTPCSF	1	0.96
	B5G9	J5*02			KJ2*04			

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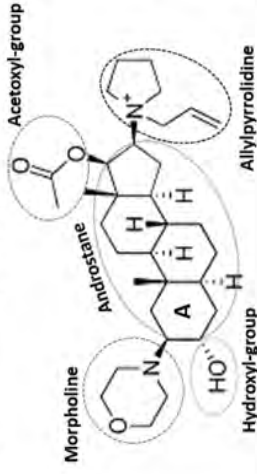
1440 **Table 3: Characteristics of human anti-rocuronium antibodies.** Antibodies were produced
 1441 recombinantly and demonstrated binding to rocuronium by ELISA. They are ranked by their K_D
 1442 value to HSA-rocuronium. roc, rocuronium; K_D , dissociation constant determined using biolayer
 1443 interferometry for HSA-rocuronium and using surface plasmon resonance for free roc; nd, not
 1444 determined; IC_{50} , half-maximal inhibitory concentration.
 1445

Patient	human mAb	Heavy chain			Light chain		K_D to HSA-roc (nM)	IC_{50} free roc (μ M)	K_D to free roc (μ M)
		V _H gene	D _H gene	J _H gene	V _L gene	J _L gene			
#1_2014	h1F10	V3-48	D3-10	J4*02	KV2-30	KJ2*01	21	351	140
	A4E5	V4-61*07	D2-21*01	J6*02	KV3-11*01	KJ4*01	22	917	760
	h1G12	V4-61*02	D3-3*01	J3*02	LV2-8*01	LJ1*01	28	735	>1,000
	h6E11	V3-15*07	D3-22*01	J6*02	KV1-5*03	KJ2*01	30	nd	nd
	A6H7	V4-39*01	D2-15*01	J6*02	KV3-11*01	KJ4*01	35	917	>2,000
#2	D3F8	V4-39*08	D2-21*01	J6*03	KV3-15*01	J4*01	40	464	>2,000
#1_2014	hA3E12	V3-30*18	D6-13*01	J6*02	KV1-9*01	KJ2*01	49	539	nd
	h1B8	V4-39*01	D3-3*01	J3*02	KV4-1*01	KJ1*01	50	375	nd
	hA5H5	V4-61*02	D6-19*01	J3*02	GKV3-11*01	KJ1*01	63	684	nd
	h6A12	V1-2	D3-16	J4*02	KV1-5	KJ3*01	74	2,100	nd
	hA10A9	V1-18	D2-8	J1*01	LV2-14	LV2-14*03	249	2,718	nd
	hA8D8	V1-69*06	D3-22*01	J6*02	KV1-5*03	KJ2*01	257	473	nd
#2	E9B10	V4-34*01	D3-22*01	J3*01	KV1-39*01	KJ4*01	275	2,105	nd
#1_2014	h2D6	V3-30-3	D2-15	J5*02	KV2-28	KJ2*01	922	612	nd
#2	E11B8	V4-30-4*09	D3-10*02	J6*03	KV3-20*01	KJ1*01	nd	2,191	nd
	E11G7	V4-61*01	D6-6*01	J4*02	KV1-39*01	KJ4*01	nd	nd	nd

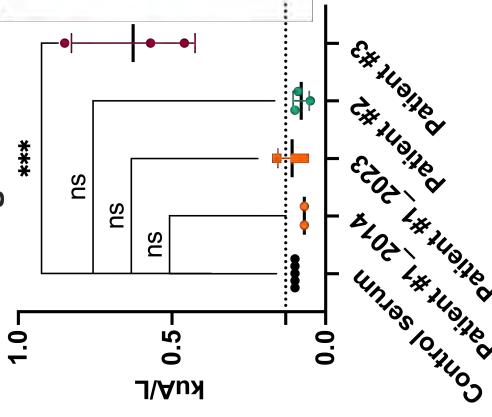
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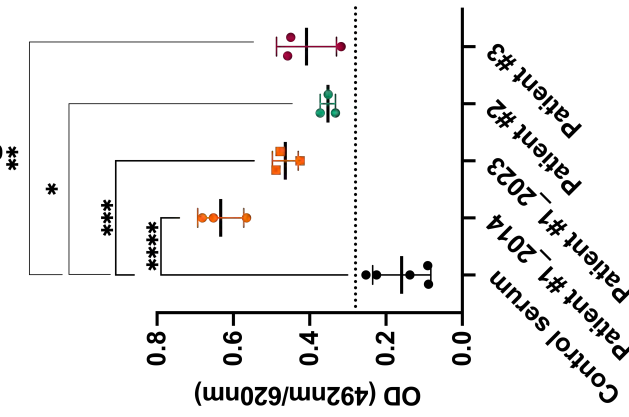
A rocuronium



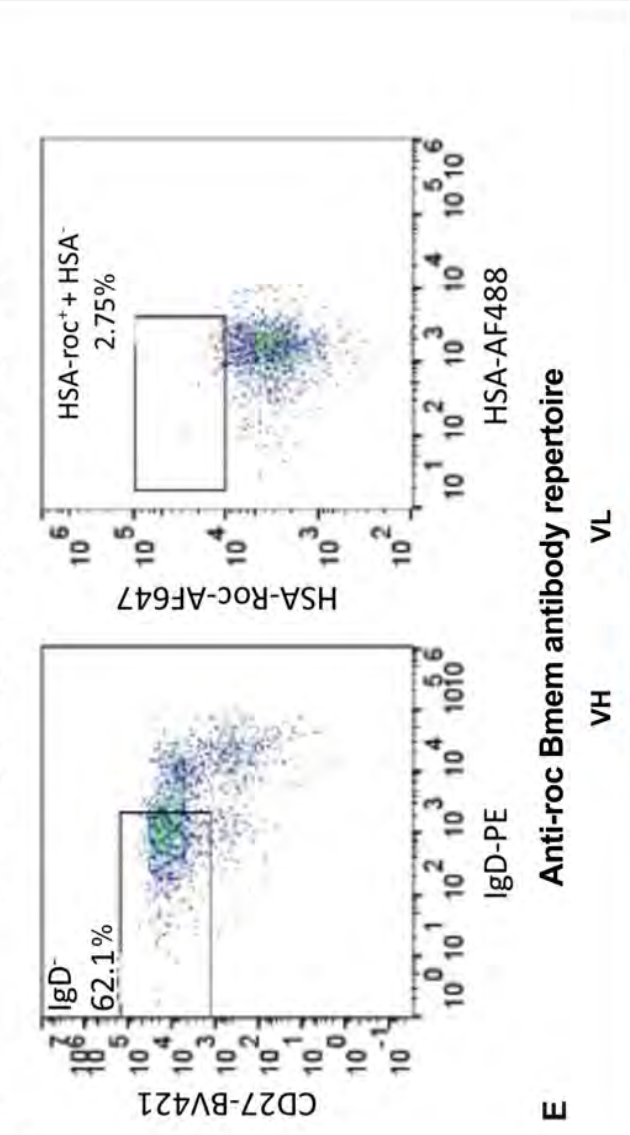
B Anti-roc IgE ImmunoCAP



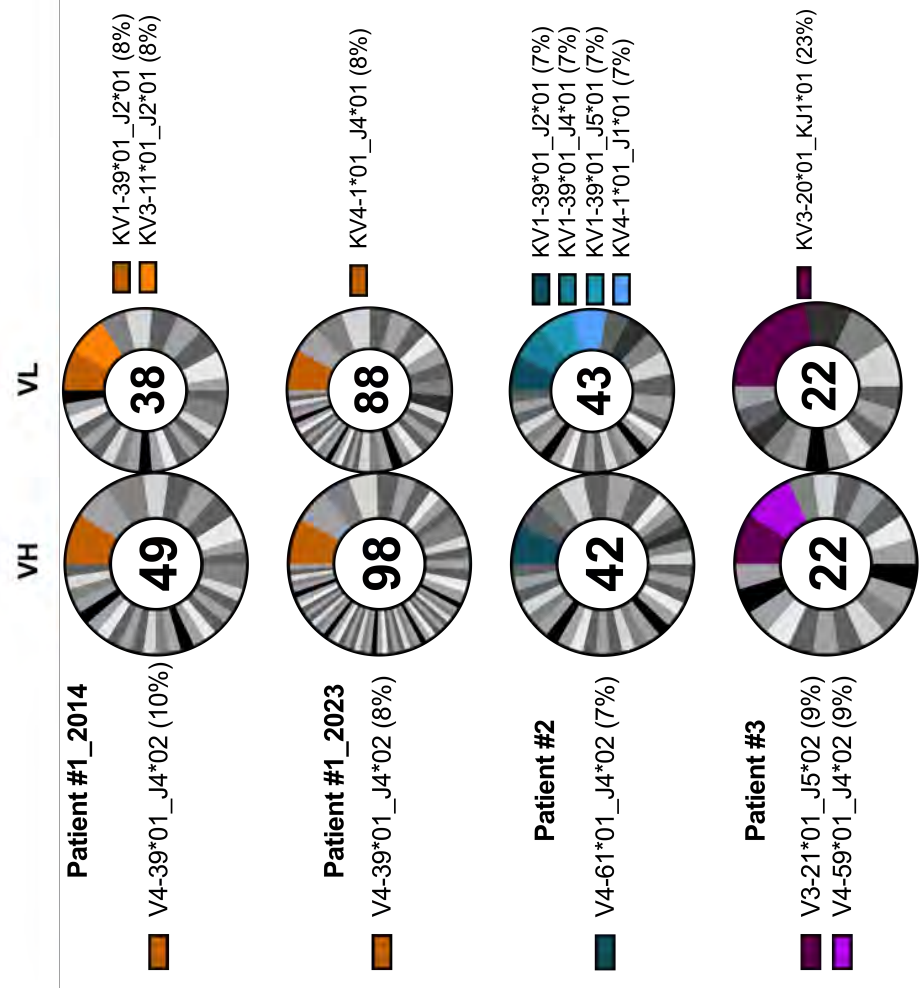
C Anti-roc IgG ELISA



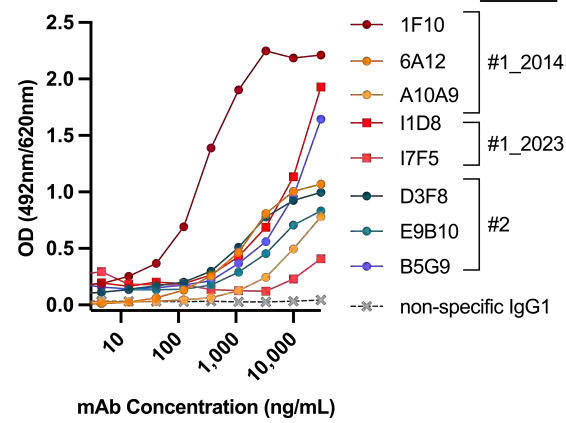
D Bmem sorting gating strategy (Patient #1_2014)



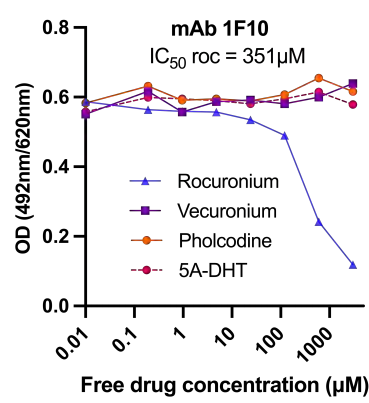
E Anti-roc Bmem antibody repertoire



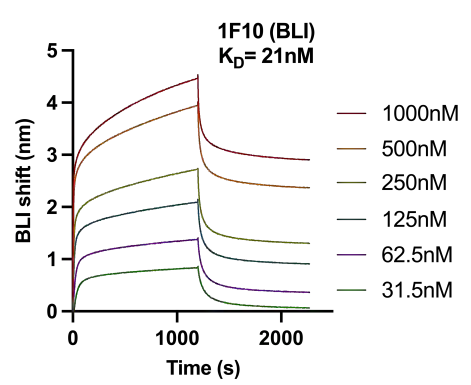
A Anti-Roc IgG



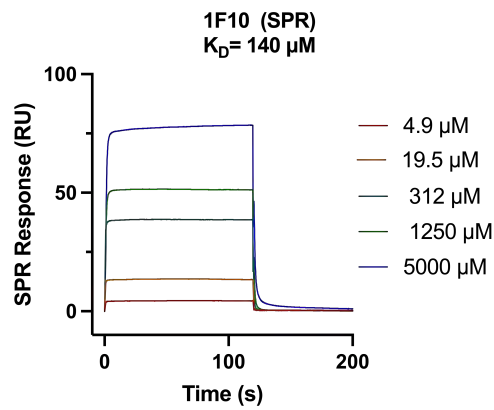
B IgG crossreactivity



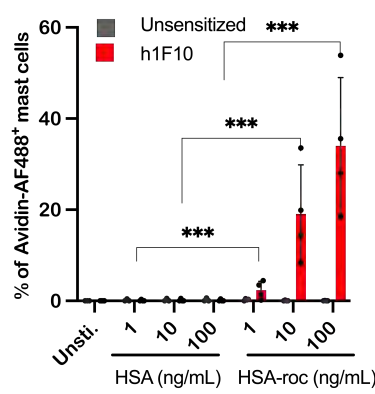
C Avidity for rocuronium



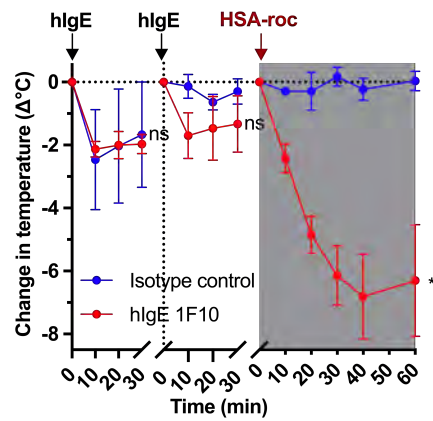
D Affinity for rocuronium



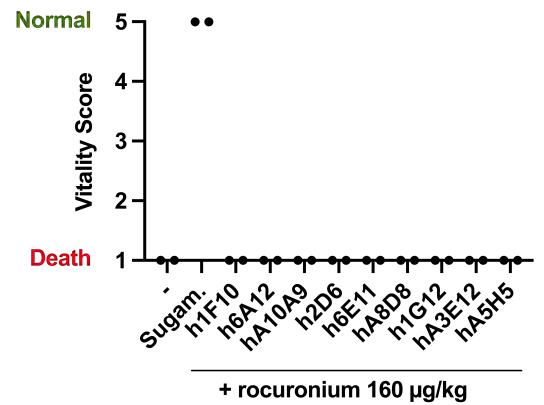
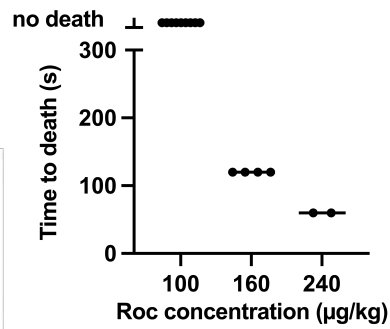
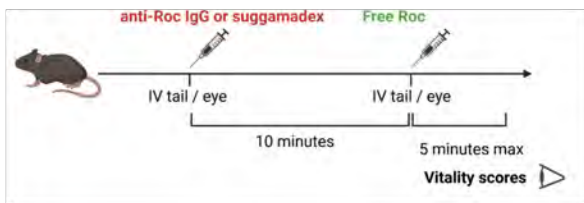
E Mast cell activation test

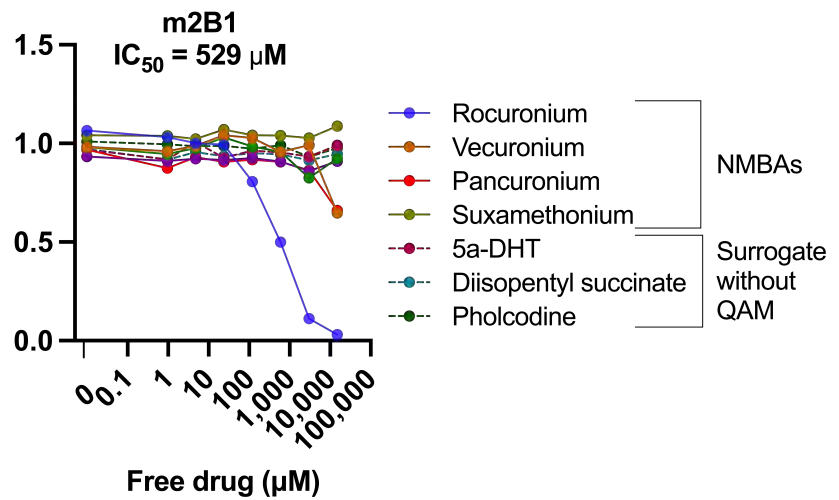
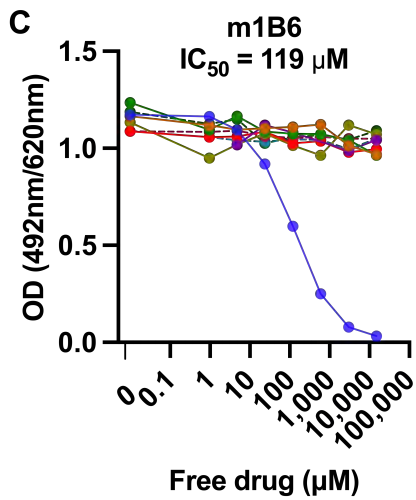
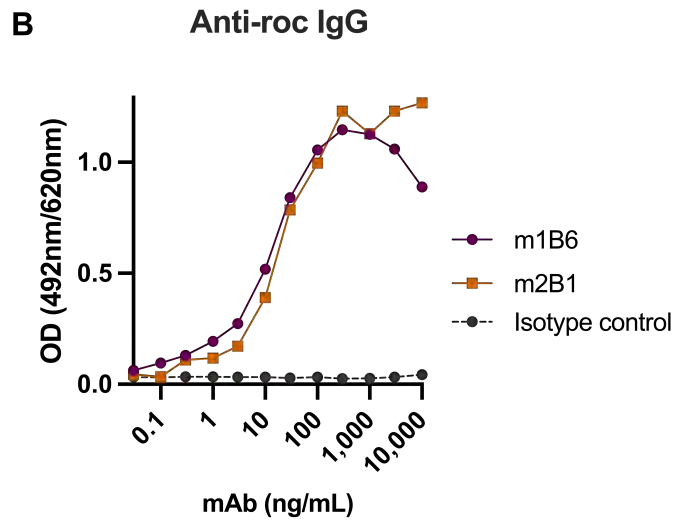
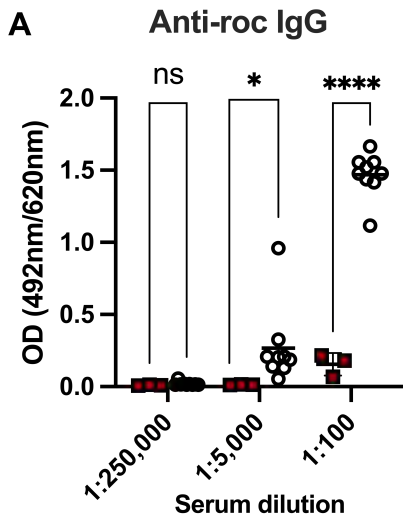


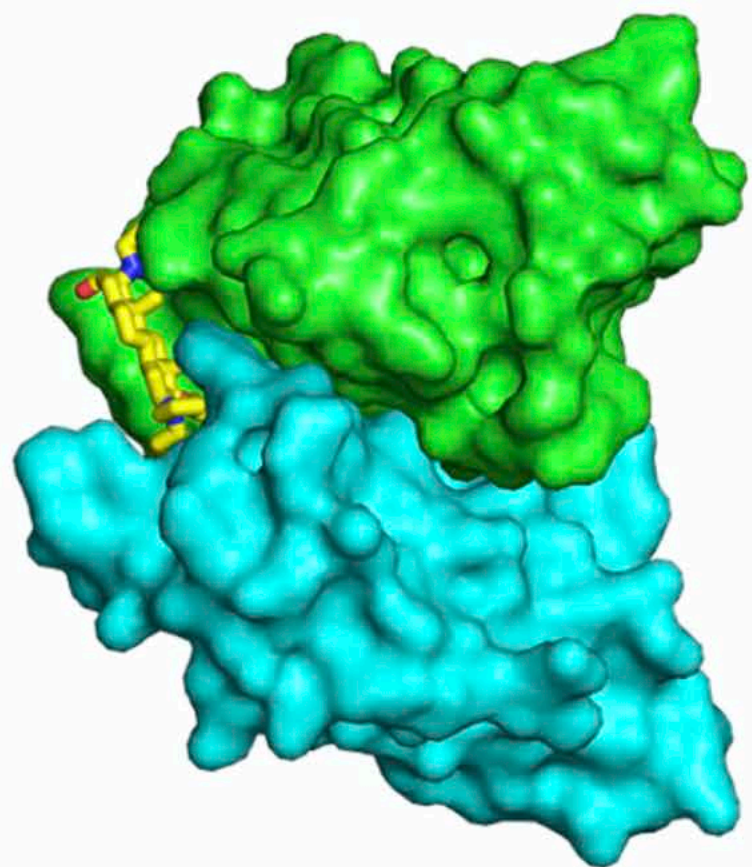
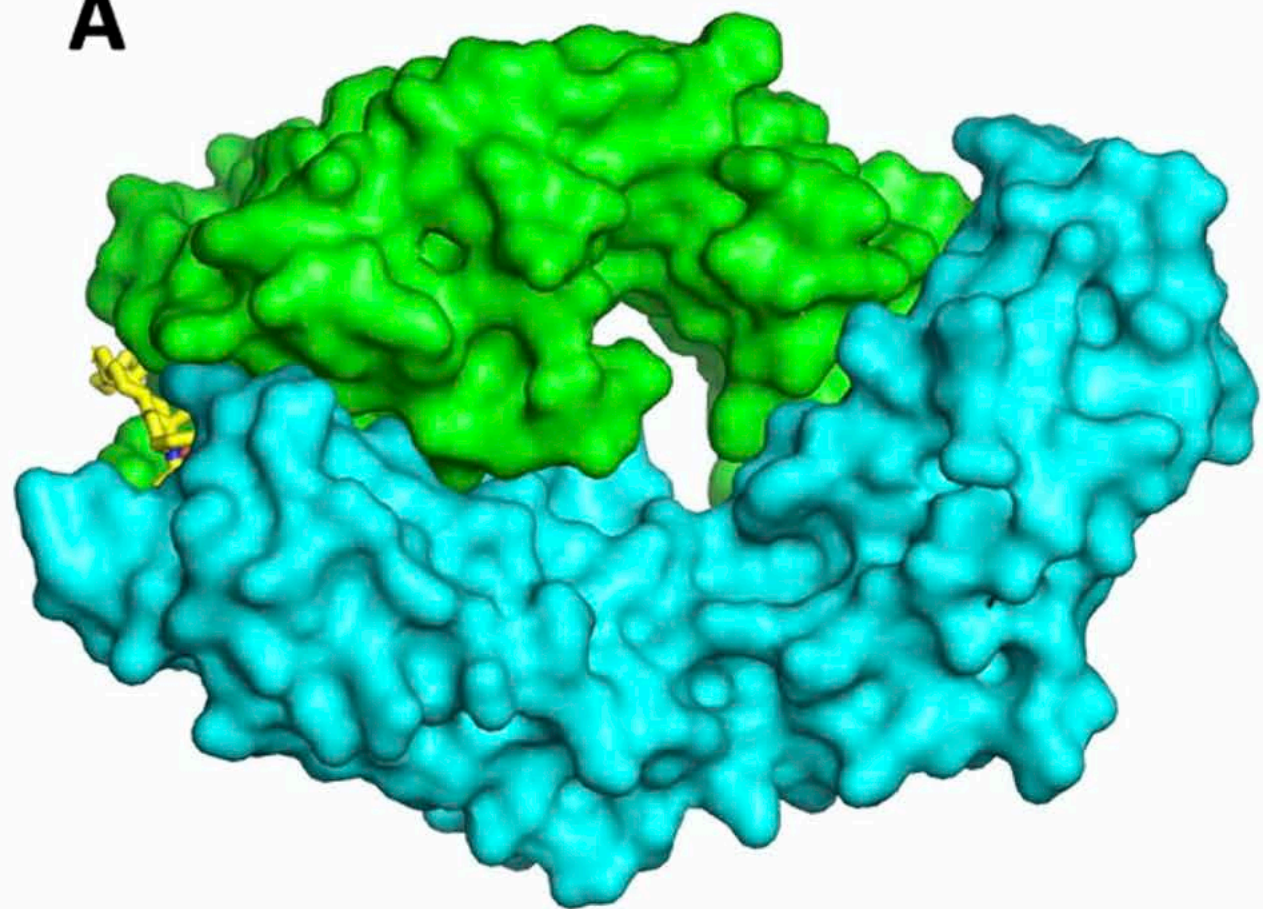
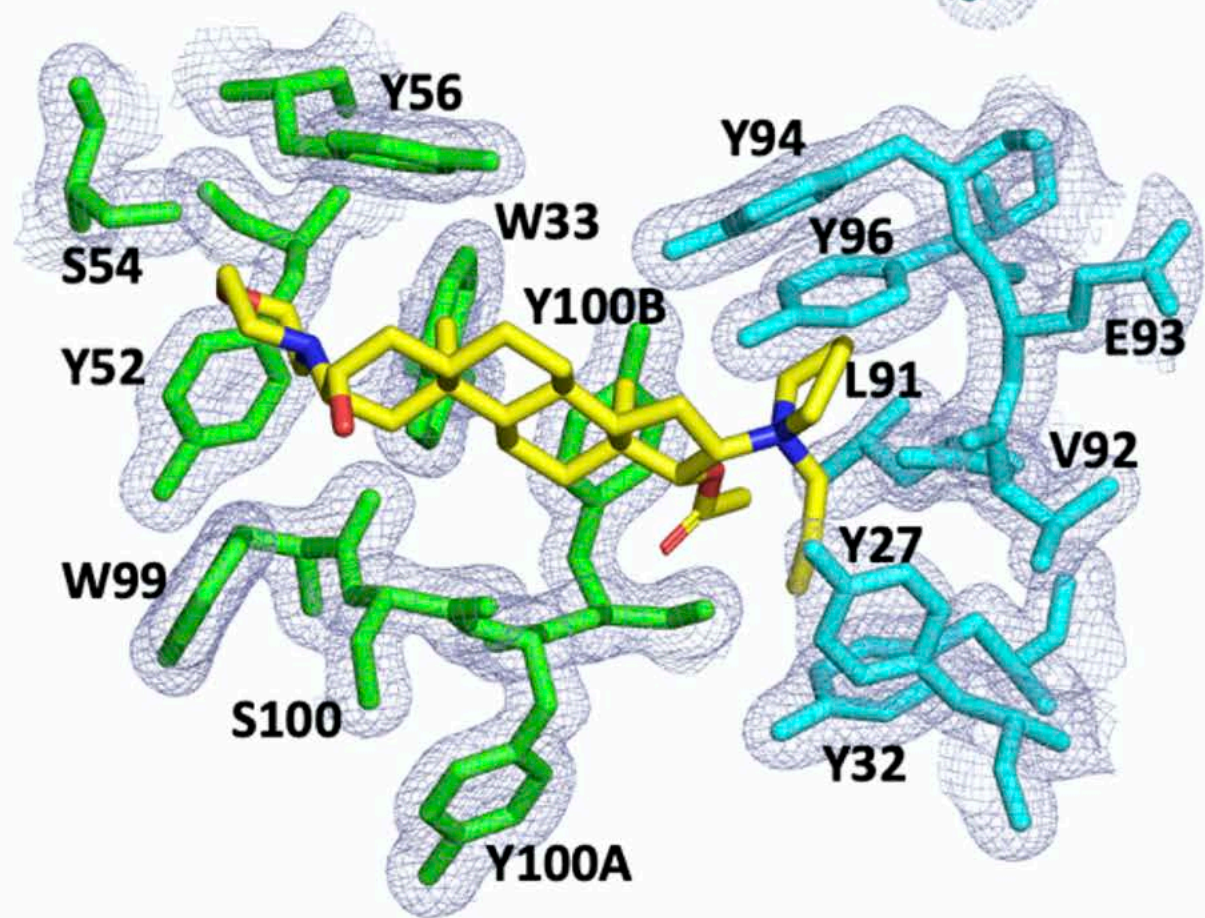
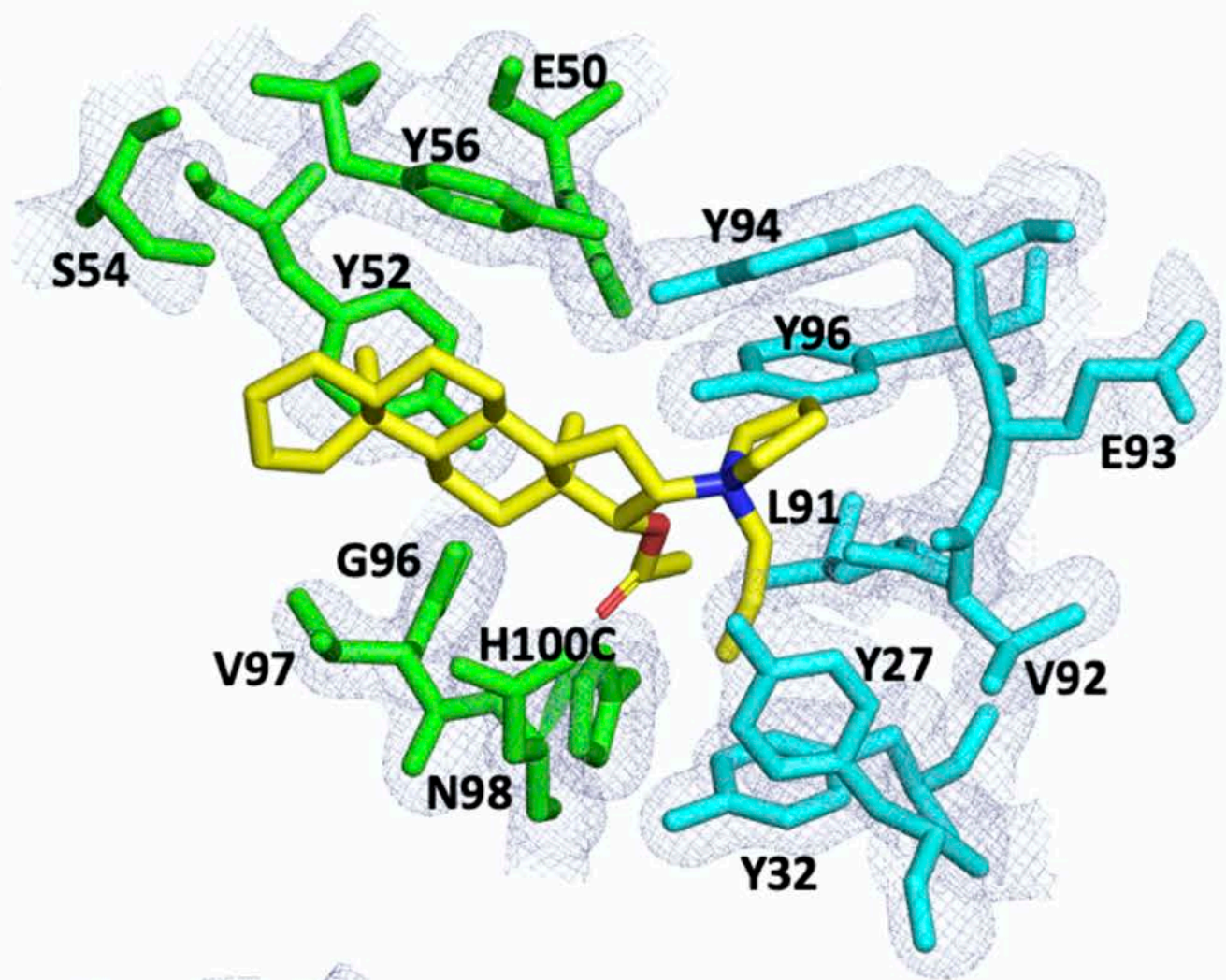
F Passive Systemic Anaphylaxis



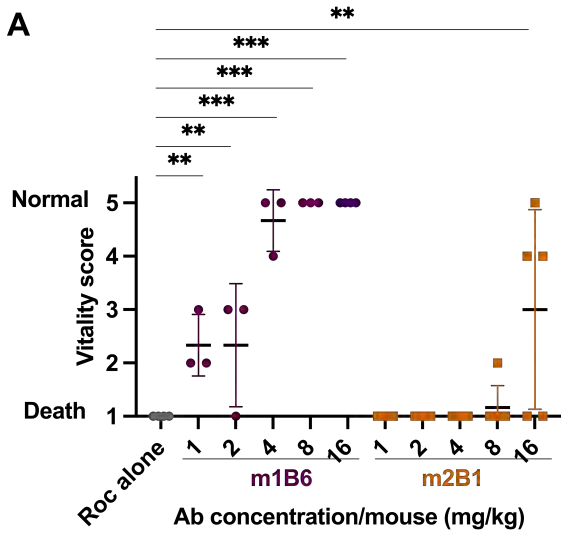
G Neuromuscular blockade prophylaxis



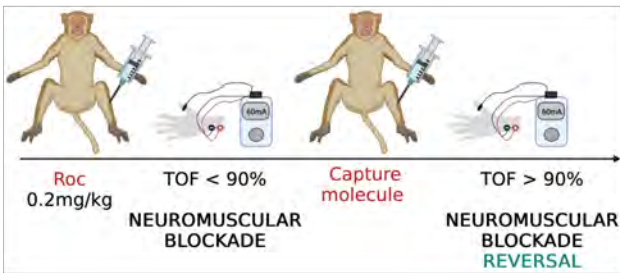


A**B**

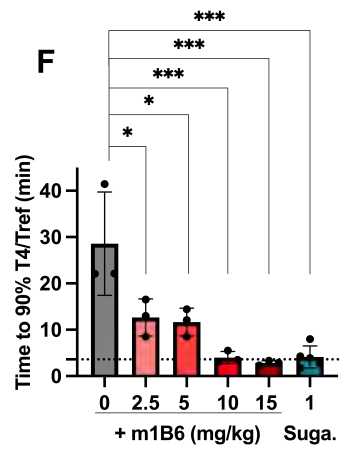
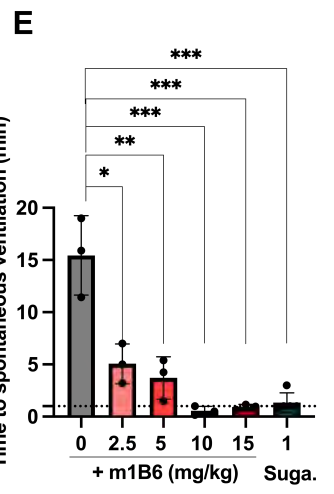
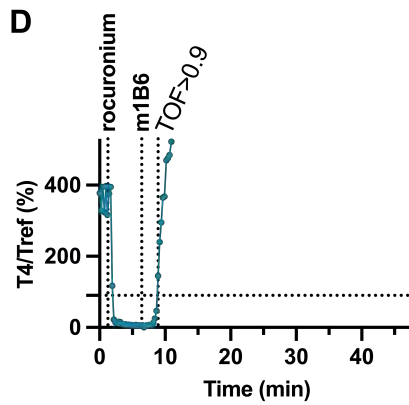
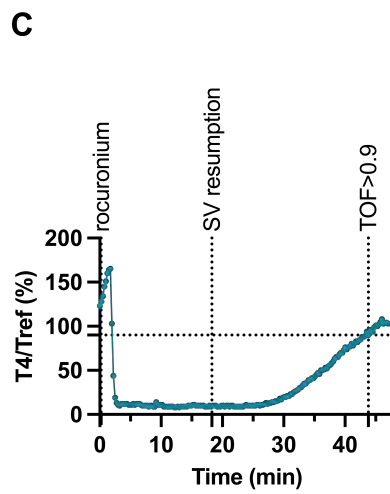
Neuromuscular blockade prophylaxis



B Experimental setup in NHPs



Neuromuscular blockade reversal



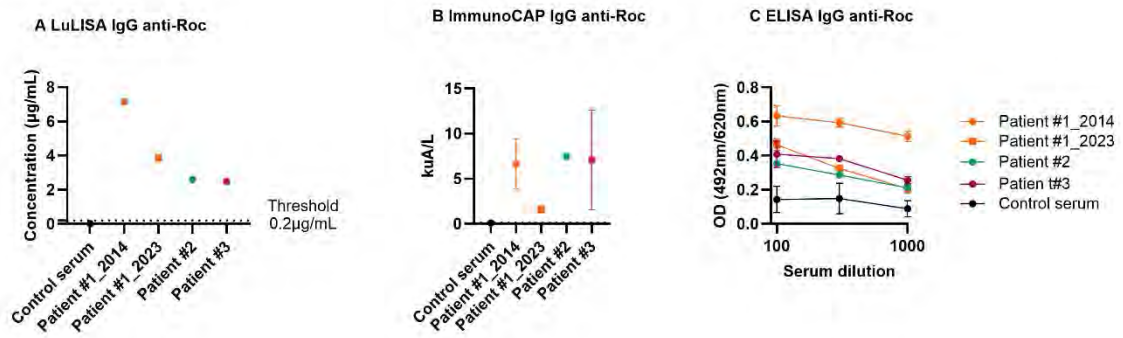


Fig. S1. Screening for anti-rocuronium (roc) IgG in patients' serum samples. (A to C) IgG anti-rocuronium detection by Luciferase-linked immunosorbent assay (LuLISA) with a cut-off at 0.2 µg/mL (n=1 replicate) (A), ImmunoCAP with a cut-off at 0.13 kUA/L (n≥1 replicates) (B), and ELISA performed in triplicate (C), in the serum of indicated patients or serum from five blood bank donors. Data are presented as Mean± Standard Error of Mean (SEM).

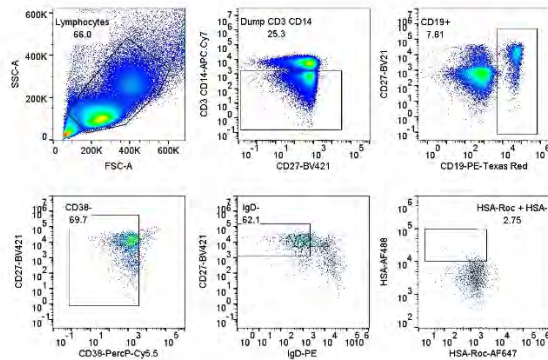
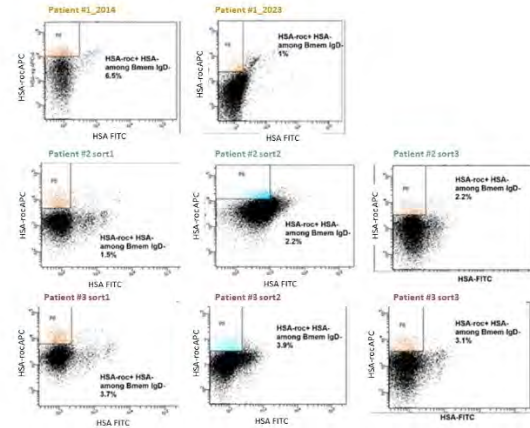
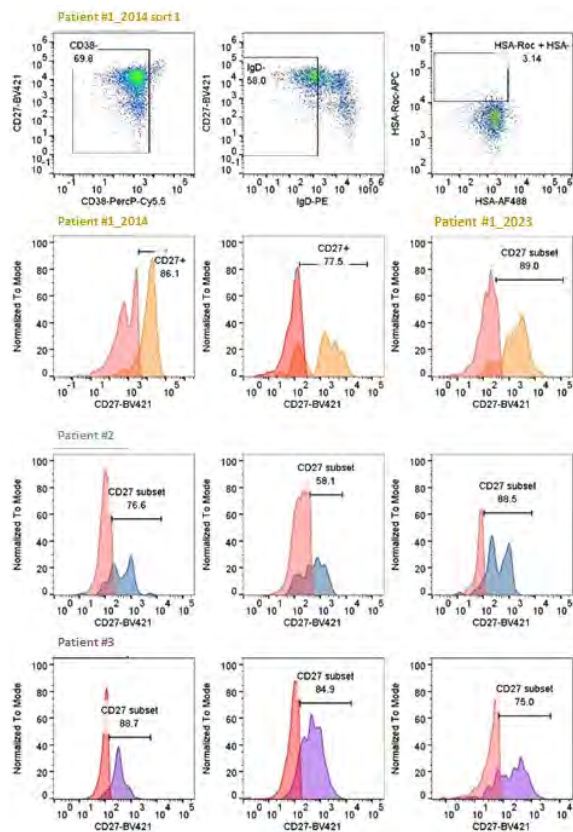
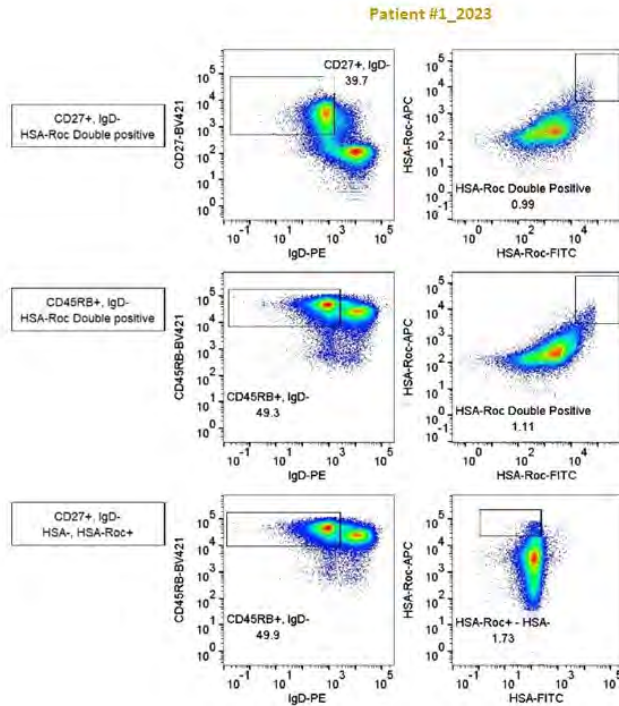
A**B****C**

Fig. S2. Gating strategy for the sorting of rocuronium-binding human memory B cells from human peripheral blood mononuclear cells (PBMCs). (A) Full gating strategy for the single-cell sort of rocuronium-binding memory B cells (CD3⁻ CD14⁻ CD19⁺ CD38⁻ CD27⁺ IgD⁻ Human serum albumin (HSA)-roc⁺ HSA⁻) from the PBMCs of Patient #1_2014, as in Fig. 1D. (B) Dot plots showing the final sorting gate for all the sorts with percentages of cells gated among the represented cells in each panel. (C) Back gating of the CD27⁺ population among CD3⁻, CD14⁻, CD38⁻, CD19⁺, HSA-Roc⁺, HSA⁻, IgD⁻ cell populations. Histogram of the CD27⁺ population for Patient #1 (orange), Patient #2 (blue) and Patient #3 (purple) are superimposed on the histogram of the CD3⁻, CD14⁻, CD38⁻, and CD27⁻ population. Percentage of CD27⁺ among antigen-specific cells are calculated compared to the CD27⁻ population.

A

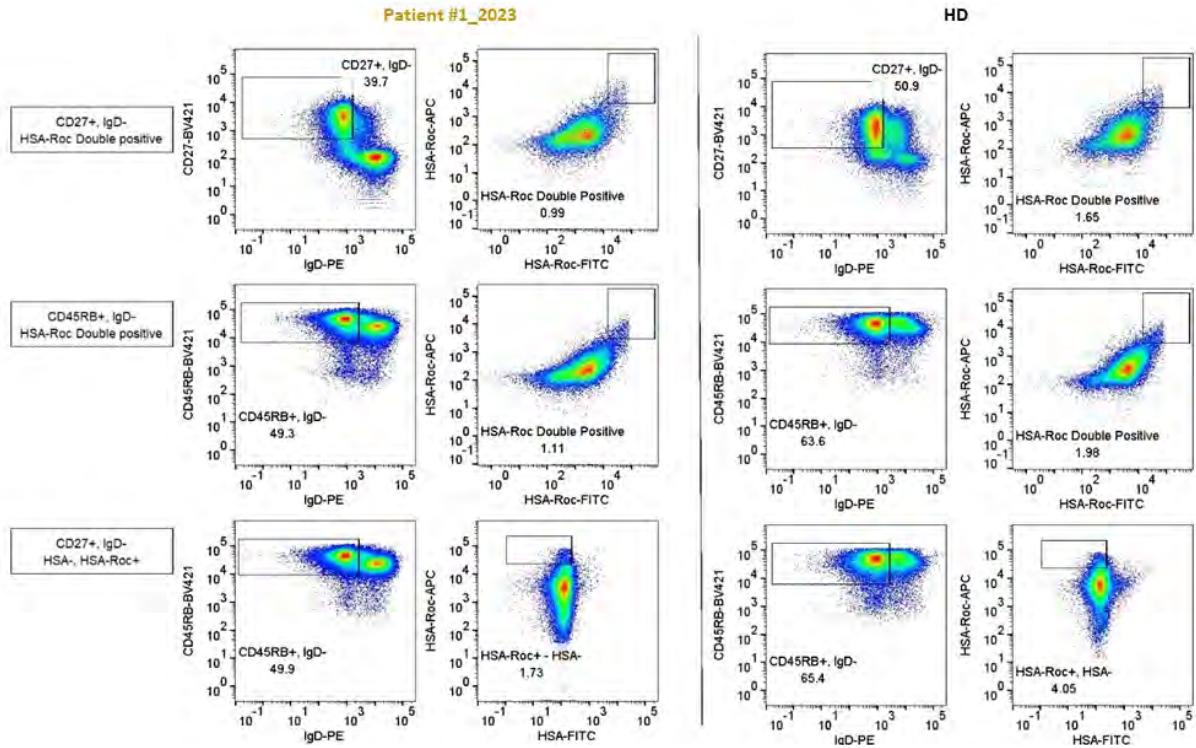


B

		Sorting data			Monoclonal cultures				
		IgD ⁻ among Bmem (%)	Antigen screen	Sorted cells among IgD ⁻ Bmem (%)	(i) cultured cells #	Positive HSA-roc ELISA # (% of i)	Not inhibited by free rocuronium # (% of i)	(ii) Total rocuronium-binding cultures # (% of i)	Strong binders # (% of ii)
CD27 ⁺ memory B cells	Patient #1_2023	39.7%	HSA-rocuronium-APC versus HSA-rocuronium-AF488	0.99%	768	78 (10.2%)	9 (1.2%)	69 (9%)	57 (81%)
CD45RB ⁺ memory B cells		49.3%	HSA-rocuronium-APC versus HSA-rocuronium-AF488	1.11%	672	34 (5.0%)	2 (0.3%)	32 (4.7%)	18 (56%)
CD45RB ⁺ memory B cells		49.9%	HSA-rocuronium-APC versus HSA-AF488	1.72%	672	38 (5.7%)	5 (0.7%)	33 (4.9%)	16 (48%)

Fig. S3. Analysis and quantification of single-cell sorts of rocuronium-binding memory B cells (Bmem). (A) Analysis of single-cell sorts of rocuronium-binding CD27⁺ memory B cells and CD45RB⁺ memory B cells. CD3⁻ CD14⁻ CD19⁺ CD38⁻ PBMCs from Patient #1_2023 were gated for (top, left) CD27⁺ IgD⁻ cells, or (middle, left and bottom, left) CD45RB⁺ IgD⁻ cells, and immediately on their right for (top and middle) HSA-rocuronium-APC⁺/HSA-rocuronium-Alexa Fluor (AF)488⁺ (“HSA-roc double positive”) or HSA-rocuronium-APC⁺/HSA-AF488⁻ (“HSA-roc⁺ HSA⁻”). Numbers in the panels indicate the percentage of cells gated among the represented cells in each panel. The gates in the right column were used for single cell sorting. (B) Summary of sorting data and monoclonal cultures. The middle and bottom row correspond to data of sorts performed with antigen (HSA-rocuronium) labeled with two different fluorophores and gated on double-positive cell populations, or with antigen labeled with a single fluorophore and gated on the corresponding positive cell population, respectively. Rocuronium specificity was assessed using competition ELISA for which free rocuronium inhibited a minimum of 50% of the optical density (OD) value in the absence of free rocuronium. Strong binding to HSA-roc (extreme right column) was determined by the ratio of the OD of the HSA-rocuronium ELISA on the OD of the IgG ELISA, with a ratio >1 being considered a strong binder.

A



B

		Sorting data		Monoclonal cultures				
Patient	Sort	CD27+ IgD- or CD45RB+ IgD- among Bmem cells %	Sorted cells %	(A) cultured cells #	Positive HSA-roc ELISA #	Not inhibited by free rocuronium #	(B) Total rocuronium-binding cultures # (% of A)	Strong binders # (% of B)
#1_2023	CD27+ double positive	39.7	0.99	768	78	9	69 (9.0%)	56 (81%)
HD		50.9	1.65	768	36	0	36 (4.7%)	22 (61%)
#1_2023	CD45RB+ double positive	49.3	1.11	672	34	6	28 (4.2%)	19 (67%)
HD		63.6	1.98	288	15	2	13 (4.5%)	5 (38%)
#1_2023	CD45RB+ HSA-roc+ HSA-	49.9	1.73	672	38	13	25 (3.7%)	21 (84%)
HD		65.4	4.05	288	14	0	14 (4.8%)	4 (29%)

Fig. S4. Analysis of single-cell sorts of rocuronium-binding CD27⁺ memory B cells and CD45RB⁺ memory B cells from Patient #1_2023 and a healthy donor (HD). (A) CD3⁻ CD14⁻ CD19⁺ CD38⁻ PBMCs from indicated individuals were gated for (top) CD27⁺ IgD⁻ cells, or (middle and bottom) CD45RB⁺ IgD⁻ cells, and immediately on their right for (top & middle) HSA-roc-APC⁺/HSA-roc-AF488⁺ (“HSA-roc double positive”) or HSA-roc-APC⁺/HSA-AF488⁻ (“HSA-roc⁺ HSA⁻”). Numbers in the panels indicate the percentage of cells gated among the represented cells in each panel. The gates in the right column were used for single cell sorting. (B) Summary of sorting data and monoclonal cultures. Rocuronium specificity was assessed using competition ELISA for which free rocuronium inhibited a minimum of 50% of the OD value in the absence of free rocuronium. Strong binding to HSA-roc (extreme right column) was determined by the ratio of the OD of the HSA-rocuronium ELISA on the OD of the IgG ELISA, with a ratio >1 being considered a strong binder.

A

Patient	VH mutations			VL mutations		
	mean	median	standard deviation	mean	median	standard deviation
#1_2014	16.9	14	12.9	9.2	10	4.9
#1_2023	11.5	11	6.5	5.0	4	3.6
#2	15.0	15	8.4	7.8	8	4.8
#3	6.0	2	8.7	2.9	1	3.7
All	13.0	12	9.6	6.3	5.5	4.7

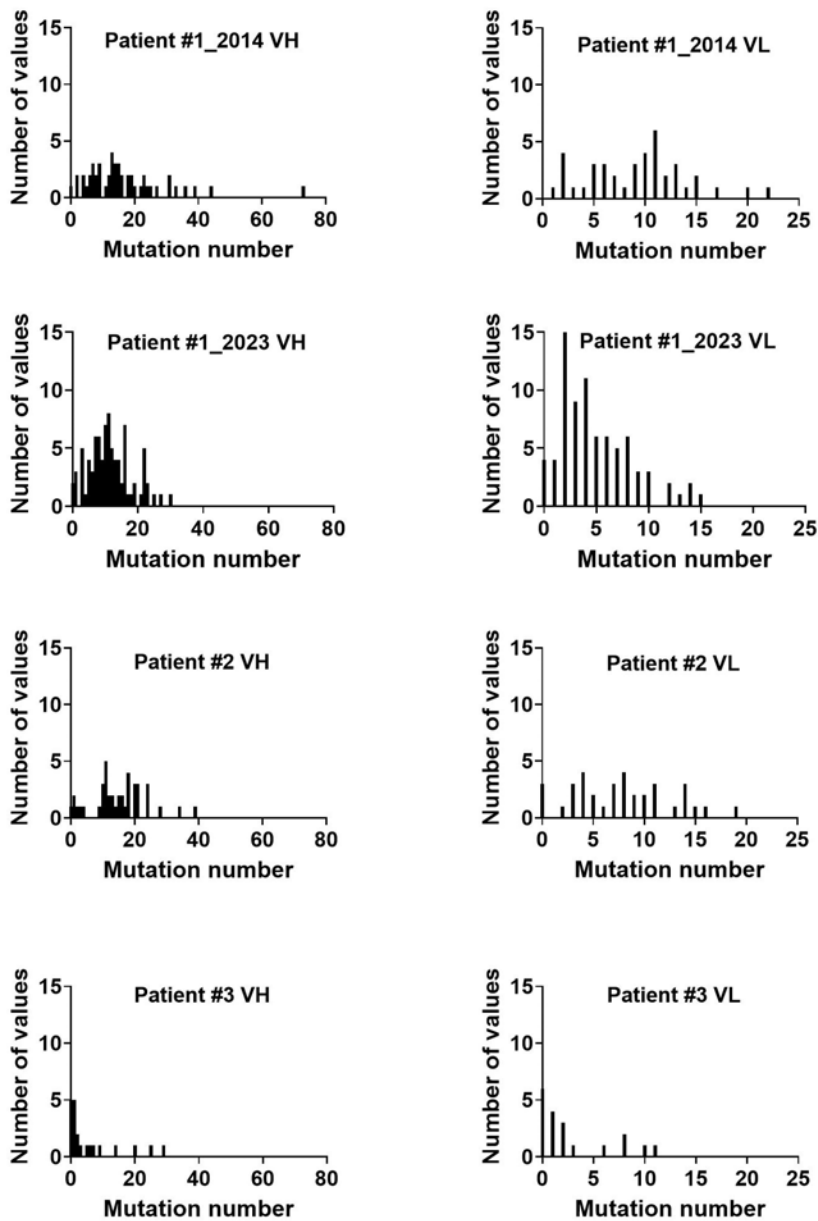
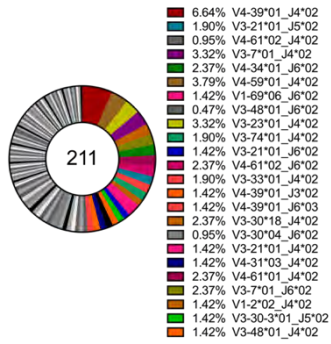
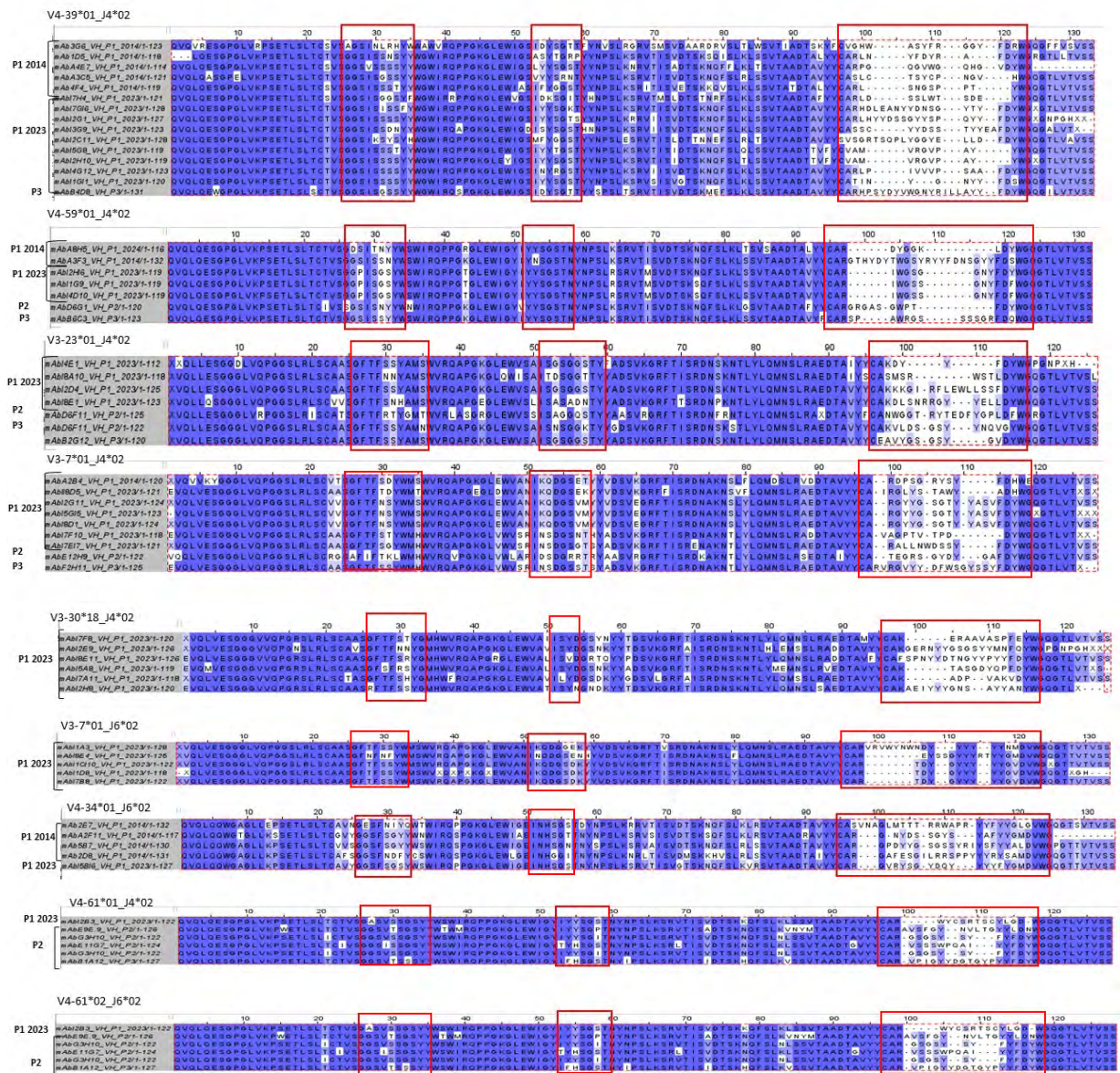
B

Fig. S5. Anti-rocuronium human VH and VL nucleotide mutation frequency. (A) VH and VL mutation frequencies compared to germline for each patient. (B) Distribution of mutation frequency among (left) VH sequences and (right) VL sequences compared to germline in indicated patient samples.

A



B



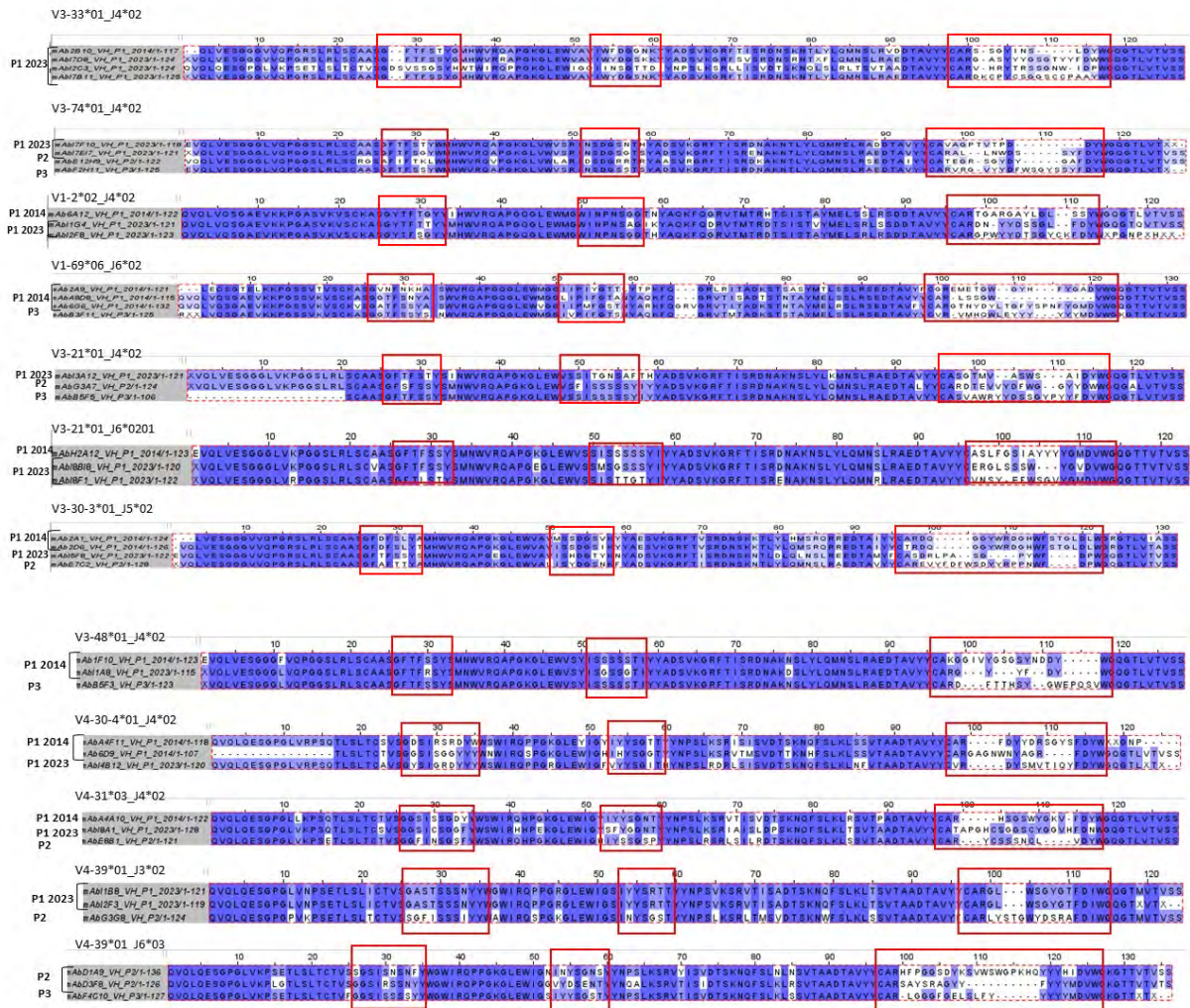
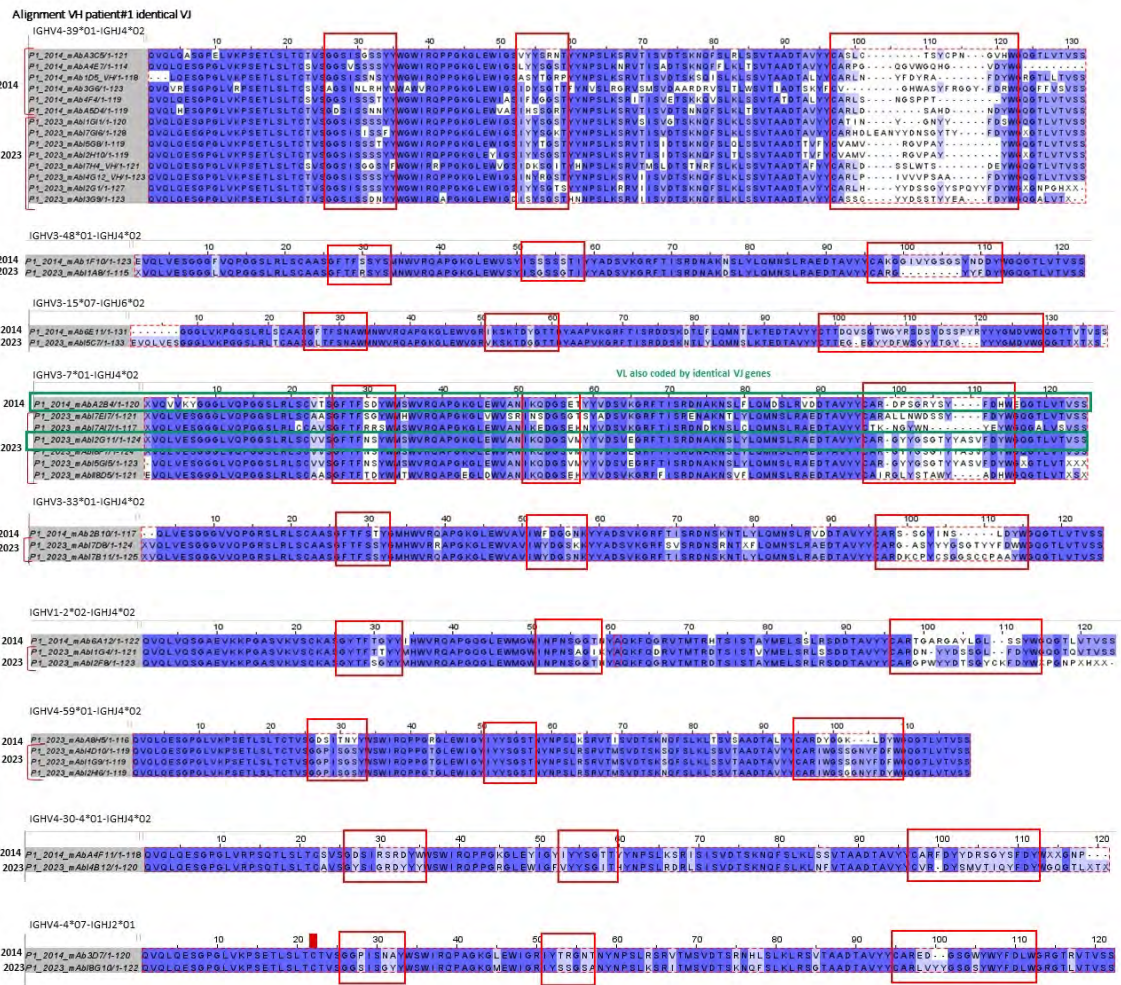


Fig. S6. Antibody repertoire analysis of the different patients. (A) Donut plot of V-J gene usage among all the VH (n=211, indicated in the center of the donut) sequenced from monoclonal B cell cultures of the 3 patients (P1 2014, Patient #1_2023; P2, Patient #2; P3, Patient #3). The major V-J recombinations and their percentage are indicated in color. **(B)** Sequence alignment of the amino acid VH sequences from the 22 V-J families composed of at least 3 members using MULTiple Sequence Comparison by Log-Expectation (Muscle) and colored by percentage identity. Complementary determining regions (CDRs) are framed in red. Names of the V-J families are indicated over the alignments.

A



B

	VH CDR3	% ID VH	VL CDR3	% ID VL
mAb A2B4 Patient #1_2014	CARDPSGRYSYFDHW	81	CQQYYSIPPGTF	86
mAb I2G11 Patient #1_2023	CARGYYSYASVFDYW		CQQYINTPWTF	

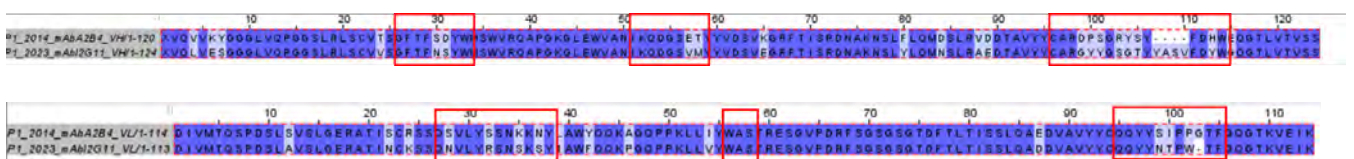


Fig. S7. Anti-rocuronium antibody repertoire comparison between Patient #1_2014 and Patient #1_2023. (A) Sequence alignment of the amino acid VH sequences coded by identical V-J genes using MULTiple Sequence Comparison by Log- Expectation (Muscle) and colored by percentage identity. CDRs are framed in red. Among those, two VH sequences (A2B4 and I2G11) also share the same light chain rearrangement and are highlighted in green. (B) Comparison of monoclonal antibodies (mAbs) A2B4 and I2G11 with percentage similarities of pairwise alignment of the complete VH amino acid sequence. (P1_2014, Patient #1_2014; P1_2023, Patient #1_2023).

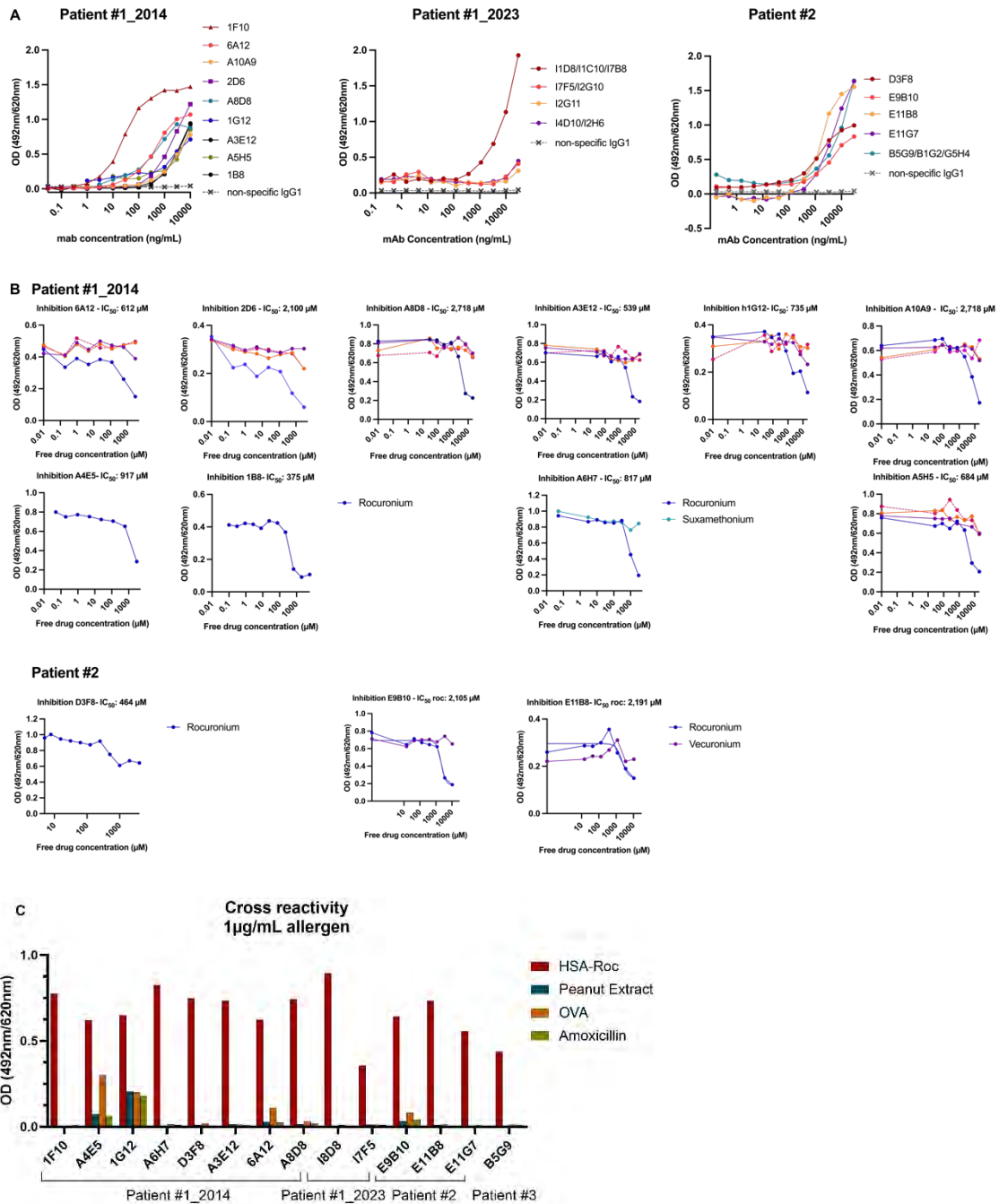


Fig. S8. Characterization of human anti-rocuronium mAbs. (A) Binding of human mAbs to HSA-rocuronium by ELISA. (B) “Competitive ELISA” of anti-IgG rocuronium ELISA from Patient #1_2014 and Patient #2 at fixed mAb concentration (1.5 $\mu\text{g/mL}$) but increasing concentrations of indicated molecules free in solution. 5 α DHT: 5-alpha-Di-Hydro-Testosterone; IC₅₀, Half-maximal inhibitory concentration. (C) Cross-reactivity test by ELISA of representative mAbs. Various allergens [peanut extract, amoxicillin, and ovalbumin (OVA)] were coated at 1 $\mu\text{g/mL}$ with mAb concentrations (2 $\mu\text{g/mL}$ to 0.03 $\mu\text{g/mL}$). For a fixed mAb concentration giving a signal of approximately OD 0.7, the signal given for the representative allergen is represented.

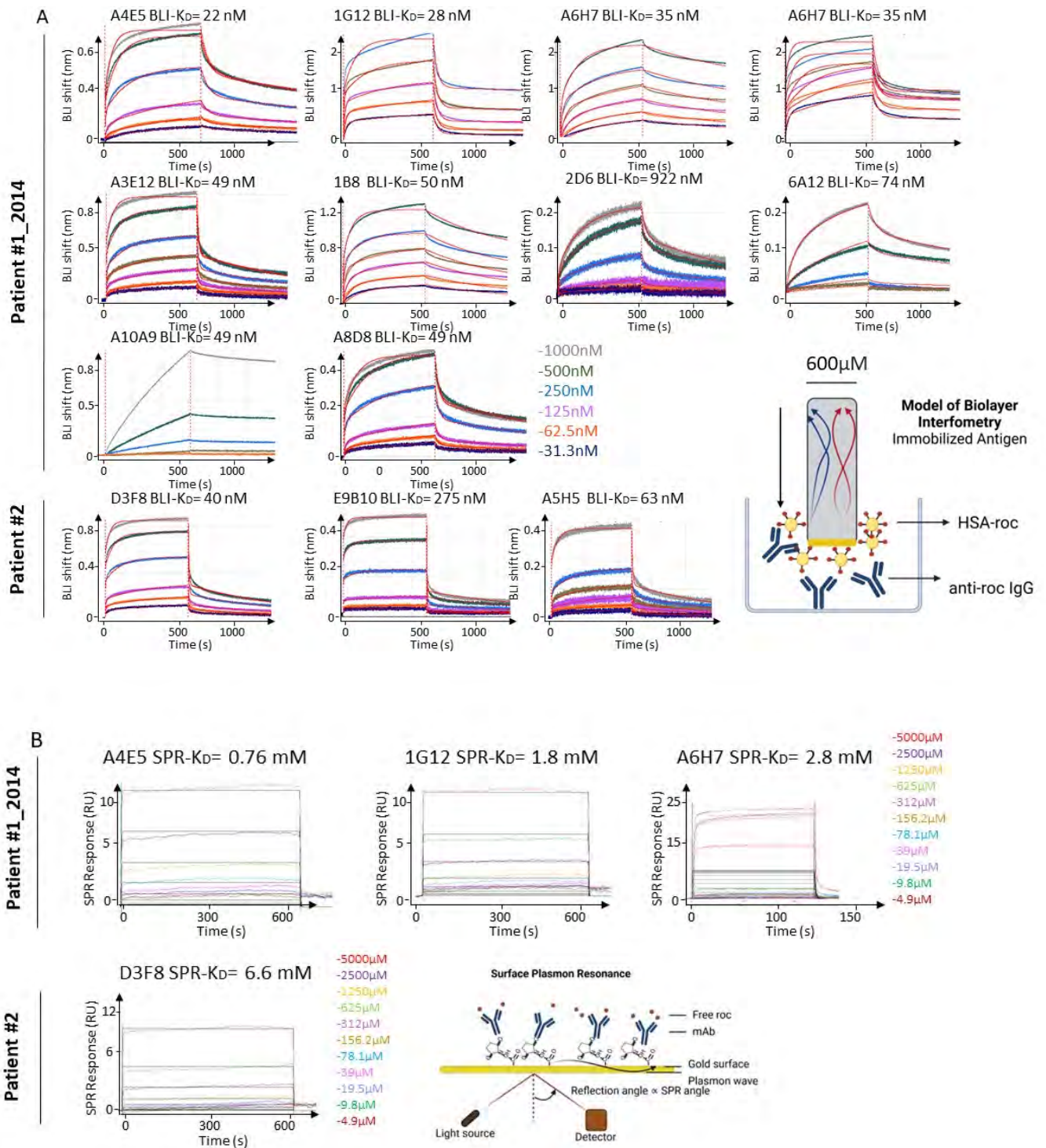
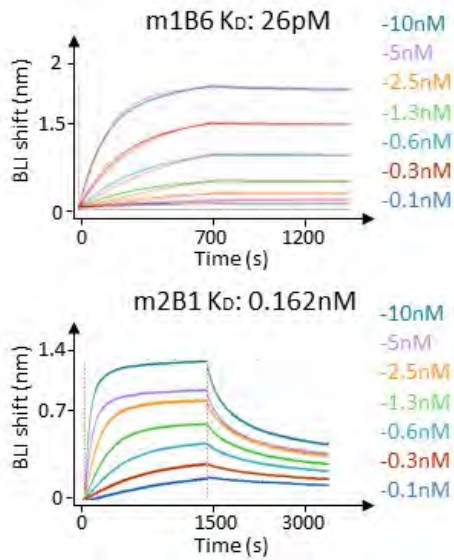


Fig. S9. Avidity and affinity of human anti-rocuronium mAbs for haptenized or free rocuronium. (A) Biolayer interferometry (BLI) avidity measurement of the interaction between immobilized HSA-roc and varying concentrations of human mAbs in solution. A scheme of the setup is provided. (B) Surface Plasmon Resonance affinity measurement of the interaction between immobilized human mAbs captured by an anti-Fc and varying concentrations of free rocuronium in solution. A scheme of the setup is provided. For (A and B), the dissociation constant (K_D) was obtained by fitting the curves using a heterogenous ligand model.

A Avidity measurement

using Biolayer Interferometry



B Affinity measurement

using Surface Plasmon Resonance

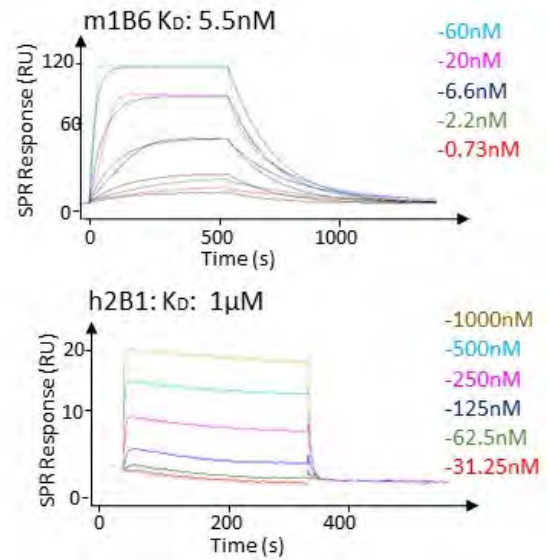
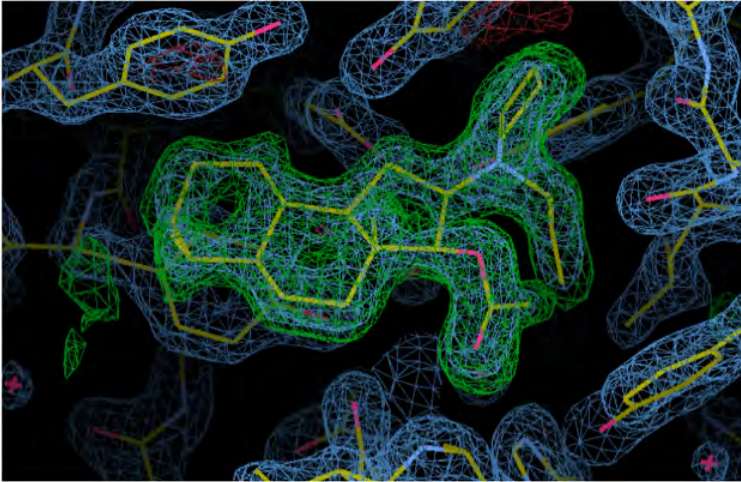


Fig. S10. Avidity and Affinity of mouse anti-rocuronium mAbs for haptenized or free rocuronium. (A) Biolayer interferometry avidity measurement of the interaction between immobilized HSA-roc and varying concentrations of mouse mAbs in solution. (B) Surface plasmon resonance (SPR) affinity measurement of the interaction between immobilized mouse mAbs captured by an anti-Fc and varying concentrations of free rocuronium in solution. RU, response units. In (A and B), fitting was performed using kinetic analysis and 1:1 binding. The dissociation constant (K_D) values are indicated.

A



B

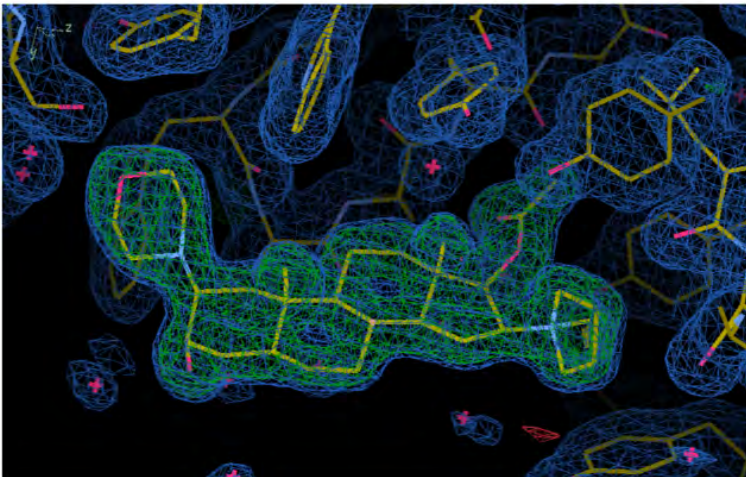


Fig. S11. Density map of rocuronium within the binding cleft of mAb m2B1 and mAb m1B6. (A and B) 2Fo-Fc electron density omitmap contoured at 3.5σ of Fab-m2B1-rocuronium (A) and scFv-m1B6-rocuronium (B), showing the rocuronium molecule in the binding site of both antibodies.

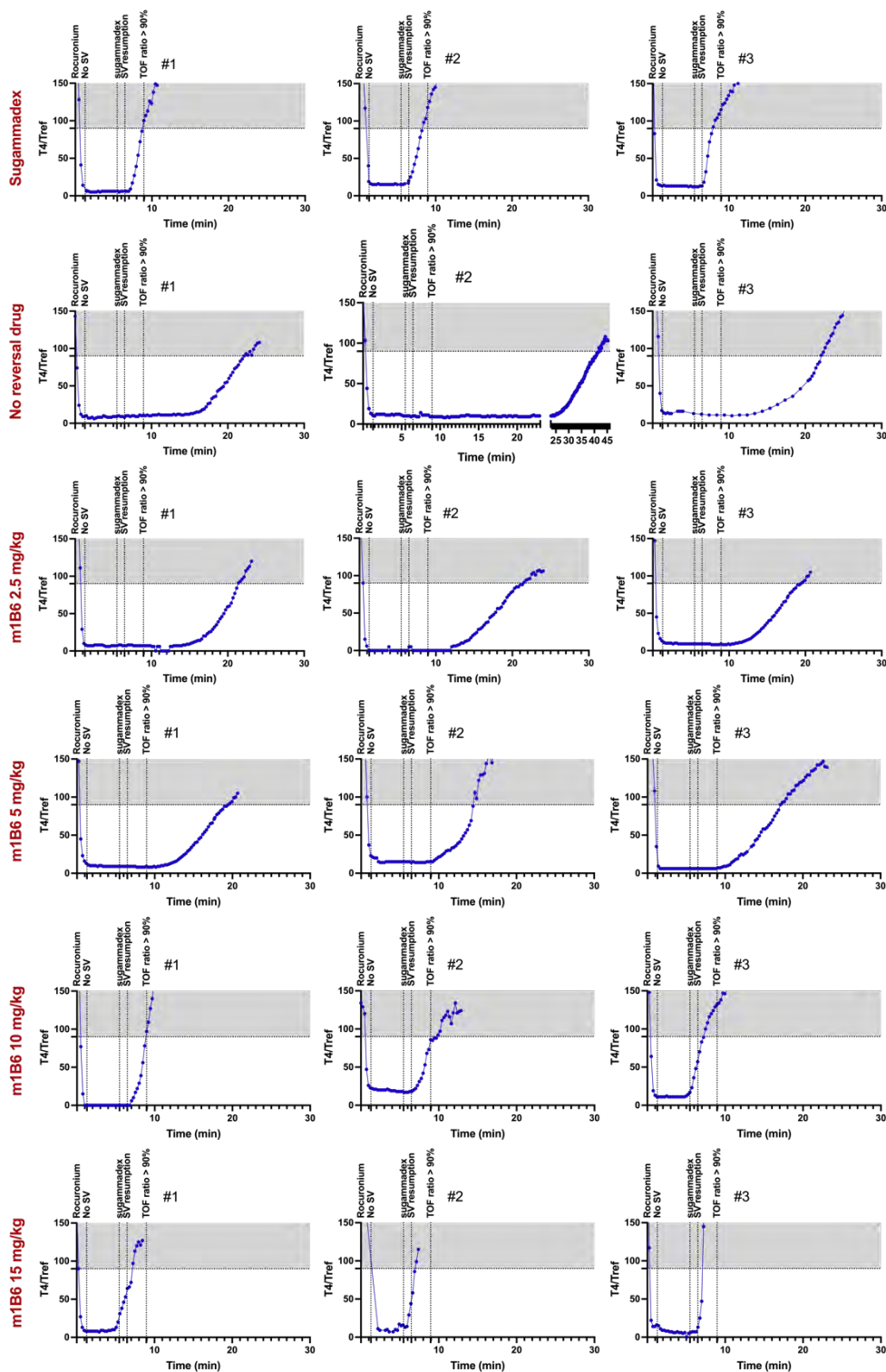


Fig. S12. Train of four (TOF) measurement in the non-human primate cohort. TOF measurement in macaques following rocuronium injection (200 $\mu\text{g}/\text{kg}$) to monitor neuromuscular reversal following injection of indicated compounds. The time points of rocuronium injection, compound injection, resumption of spontaneous ventilation (SV) and TOF ratio $T4/Tref > 0.9$ are indicated in shaded gray. Once deep neuromuscular blockade was established (absence of T1 response), indicated concentrations of (first row) sugammadex, (second row) vehicle, (third row) m1B6 at 2.5 mg/mL, (fourth row) m1B6 at 5 mg/mL, (fifth row) m1B6 at 10 mg/mL, or (sixth row) m1B6 at 15 mg/mL were injected. The ID of each macaque is indicated. The red box indicates an involuntary interruption of monitoring for the 15 mg/mL condition of non-human primate #2.

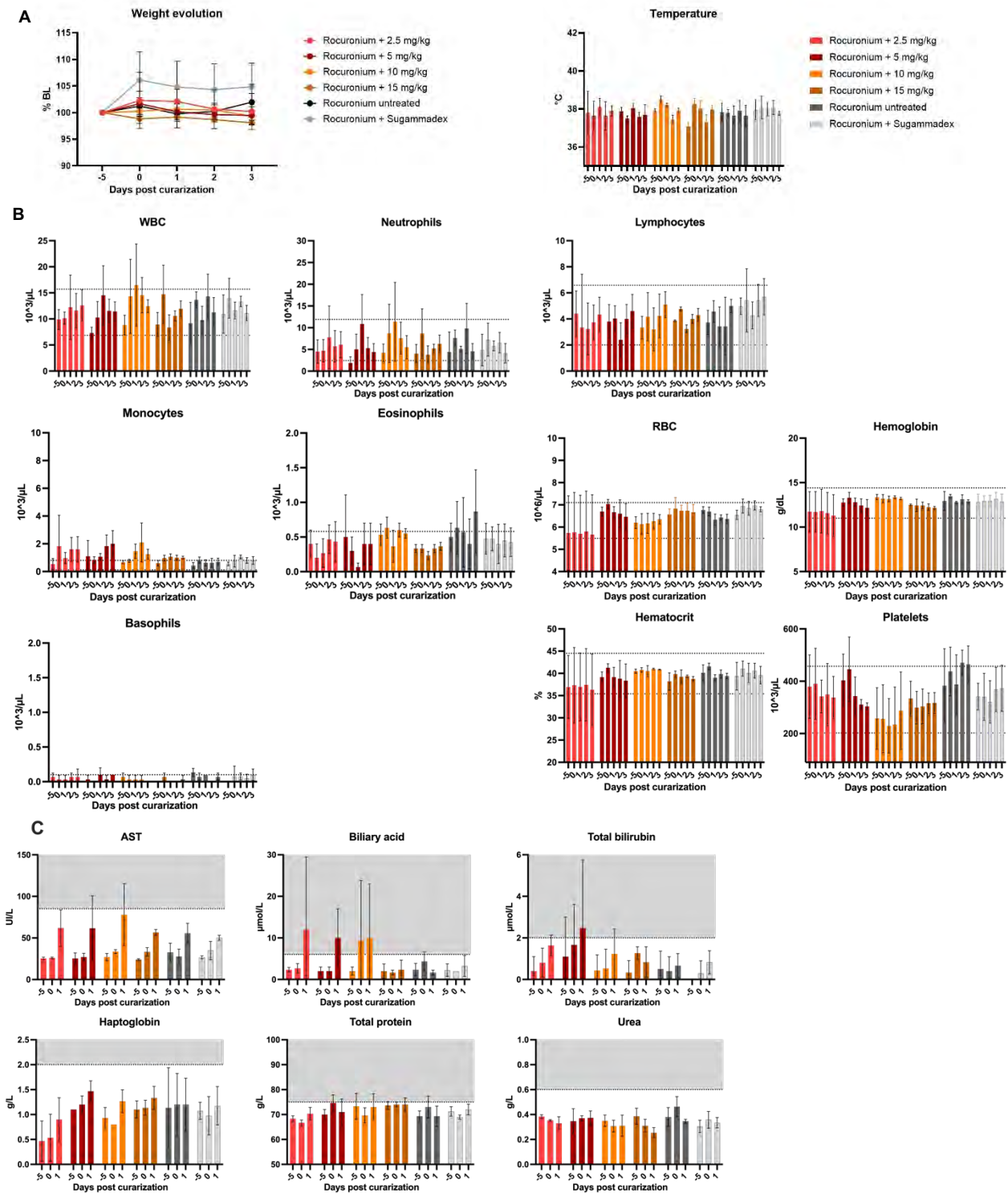


Fig. S13. Follow-up of physiological parameters in macaques. (A) Mean and standard deviation of the macaques' body weight and body temperature modification considering day 0 as the day of curare-enhanced anesthesia (n=5 per group). BL, baseline. (B) Mean and standard deviation of indicated cell populations and hematocrit in circulating blood reported considering day 0 as the day of curare enhanced anesthesia for each macaque (n=5 per group). Upper and lower normal values are indicated in dotted lines. (C) Mean and standard deviation of the macaques' blood biochemistry results for indicated parameters of the macaques (n=3 per group). Normal values reside in the white background, and abnormal values in the gray background. For (A to C), blood draws on day 0 were performed before curare injection. No statistical significance was found for any measurement or cell population count using non parametric Mann-Whitney and Kruskal-Wallis tests.

Table S1. Summary of sorting data and monoclonal culture data of rocuronium-binding human CD27⁺ memory B cells. (Left) For each sort of each patient, the percentage of memory B cells sorted (IgD⁻) is indicated. IgD⁻ memory B cells stained with HSA-rocuronium but not with HSA (HSA-rocuronium⁺ HSA⁻) are considered to bind rocuronium. (Right) The number of rocuronium-specific monoclonal B cell cultures (identified by positive anti-rocuronium ELISA and at least 50% signal inhibition using competitive ELISA with free rocuronium) are shown, as well as the number of paired VH/VL sequences retrieved.

Patient	Sorting data			Monoclonal cultures				
	Sort	IgD- among CD27 ⁺ Bmem and among total cells %	HSA-rocuronium+ HSA- among IgD- Bmem %	(i) cultured cells #	(ii) Positive HSA-rocuronium ELISA # (% among i)	(iii) Not inhibited by free rocuronium # (% among ii)	Total rocuronium-specific cultures # (% among i)	Total of sequenced and paired VH + VL (#)
#1_2014	1	62.1 – 2.7	2.7	576	49 (8.5%)	10 (20%)	57 (3.9%)	25
	2	46.7 – 1	6.5	864	19 (2.1%)	1 (5%)		
#1_2023	1	31.3 – 1	1	768	142 (18%)	14 (10%)	128 (16.7%)	76
#2	1	42.7 – 1	1.5	576	23 (4%)	1 (4%)	45 (2.6%)	32
	2	25.8 – 1	2.2	576	20 (3.5%)	5 (25%)		
	3	34.9 – 1.2	2.2	576	3 (0.5%)	0 (0%)		
#3	1	29 – 0.7	3.7	576	5 (0.8%)	0 (0%)	23 (1.3%)	19
	2	20.5 – 0.9	3.9	576	16 (2.8%)	2 (13%)		
	3	45.3 – 1.1	3.1	576	4 (0.7%)	0 (0%)		

Table S2 Crystallization conditions, data collection, and refinement statistics of the m1B6-rocuronium and m2B1-rocuronium co-crystal structures. Crystallization conditions, data collection and refinement statistics. Values in parentheses are for the highest resolution shell. MPD, 2-methyl-2,4-pentanediol. HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. PEG400, polyethylene glycol 400. MES, 2-(N-morpholino)ethanesulfonic acid.

Crystal	Fab m2B1+rocuronium	ScFv m1B6+rocuronium
Reservoir solution	70% (v/v) MPD 0.1M HEPES pH7.5	30%(v/v) PEG400 0.1M MES pH6.5 0.1 M Na Acetate
Protein concentration, mg/ml	7.1	15
Data collection		
Synchrotron beamline	PROXIMA 1	PROXIMA 2
Space group	C2	C2221
Unit cell dimensions		
a,b,c (Å)	161.57, 40.52, 69.78	135.47, 165.34, 127.52
a,b,g (°)	90, 100.40, 90	90, 90, 90
Resolution, Å	47.8-1.65 (1.68-1.65)	128-1.70 (1.73-1.70)
Rmerge	0.092 (1.372)	0.079 (1.707)
Rpim	0.038 (0.561)	0.027 (0.594)
Unique reflections	53958 (2663)	93815 (4304)
<I/sigma(I)>	10.7 (1.4)	16.4 (1.4)
Mn(I) half-set correlation	0.998 (0.603)	0.999 (0.567)
Completeness, %	100.0 (100.0)	100.0 (100.0)
Multiplicity	6.9 (6.9)	9.6 (9.2)
Refinement		
Resolution, Å	37.5-1.65 (1.67-1.65)	50.0-1.70 (1.72-1.70)
No. of reflections	52843 (1948)	153886 (5647)
Rvalue, working set	0.173 (0.200)	0.167 (0.299)
Rfree	0.301 (0.344)	0.189 (0.288)
Non-hydrogen protein atoms	3644	6409
atoms	340	818
No. of Waters		
RMS deviations from ideal	0.012	0.015
bond length, Å	1.86	1.99
bond angles, °		
Ramachandran plot, %	98.07	96.15
preferred regions	1.45	3.11
allowed regions	0.48	0.74
outliers		

Data file S1. ELISA results of all monoclonal cell cultures. Supernatant of monoclonal cultures with an ELISA signal of HSA-rocuronium minus the ELISA signal of HSA ($OD_{\text{HSA-roc}} - OD_{\text{HSA}} = \text{HSA-roc ELISA}$) superior to 3 times the background ($OD > 0.2$) are shown. Competition was performed with 3,000 μM of free rocuronium, with a cut-off of percentage of inhibition $\geq 50\%$. Non-specific cultures with a percentage of inhibition $< 50\%$ are indicated in gray. Cultures from which mAbs were recombinantly produced are indicated in blue.

Data file S2. Individual-level data for experiments where $n < 20$.