

Supplementary information

**Structural principles of B cell antigen
receptor assembly**

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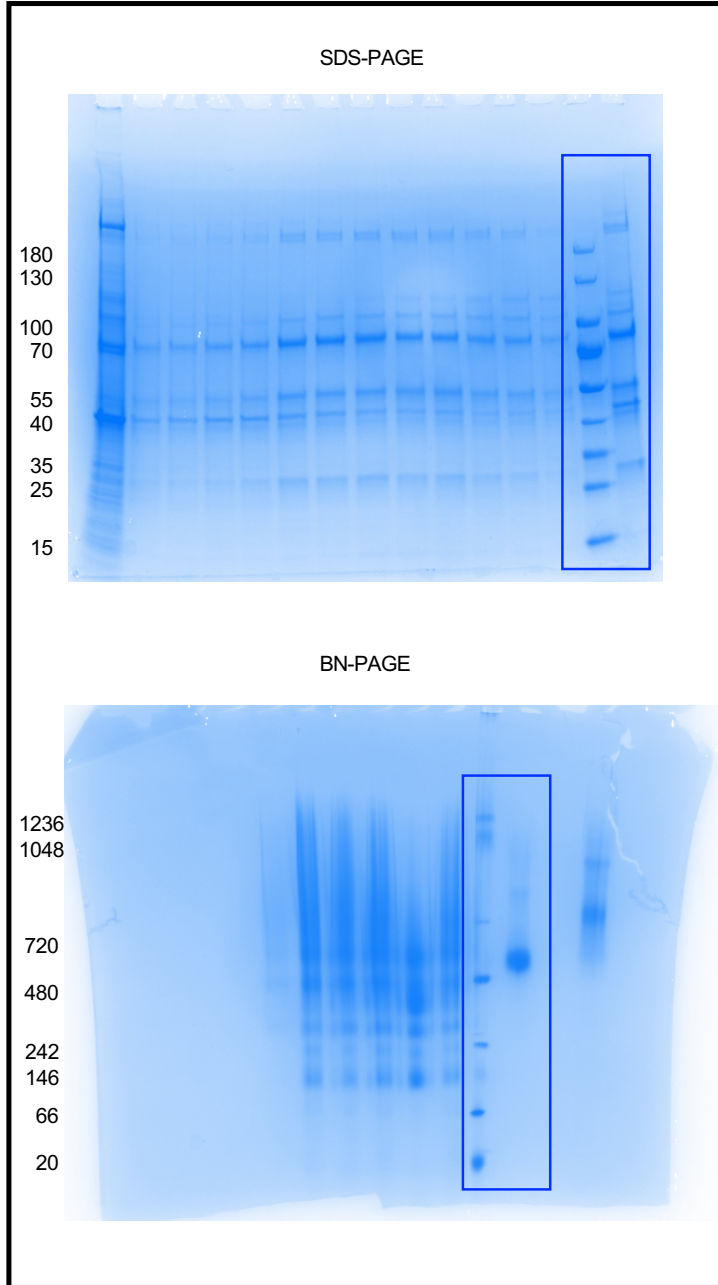
Supplementary Fig 1. Uncropped SDS-PAGE gels and immunoblots.

Supplementary Fig 2. Uncropped SDS-PAGE gels and immunoblots.

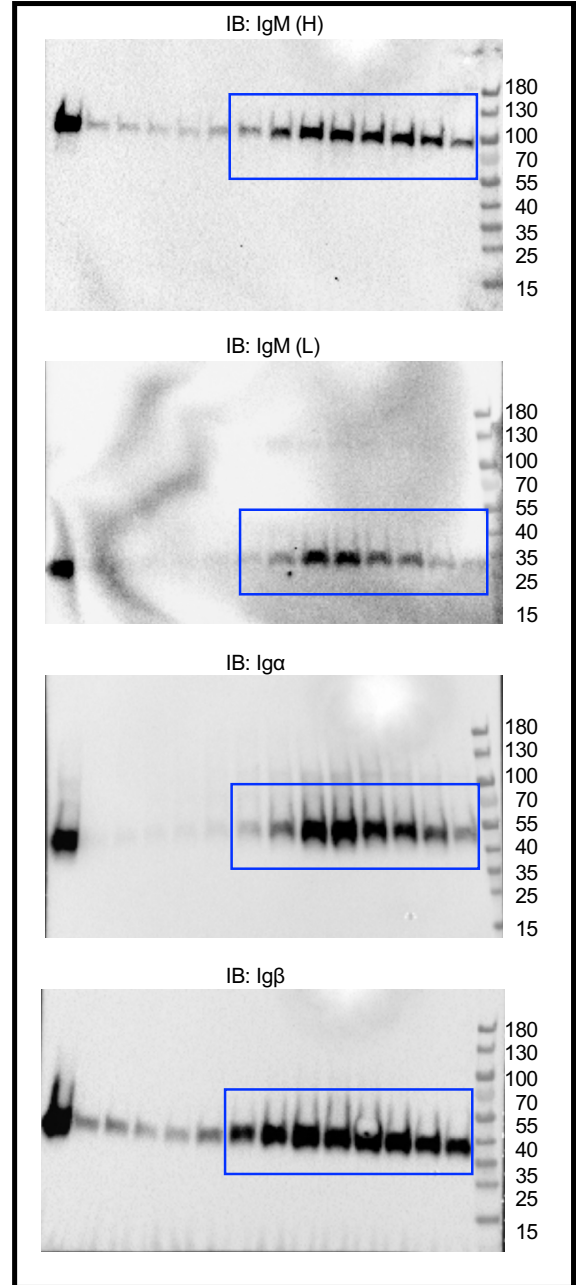
Supplementary Table 1. Summary of mutagenesis data.

Supplementary Fig 1. Uncropped SDS-PAGE gels and immunoblots. Relative positions of the scans within a panel (black box) follow those in the Extended Data Fig. 1b-c. Blue boxes indicate cropped areas. Molecular weight markers are shown in kDa.

Extended Data Fig. 1b

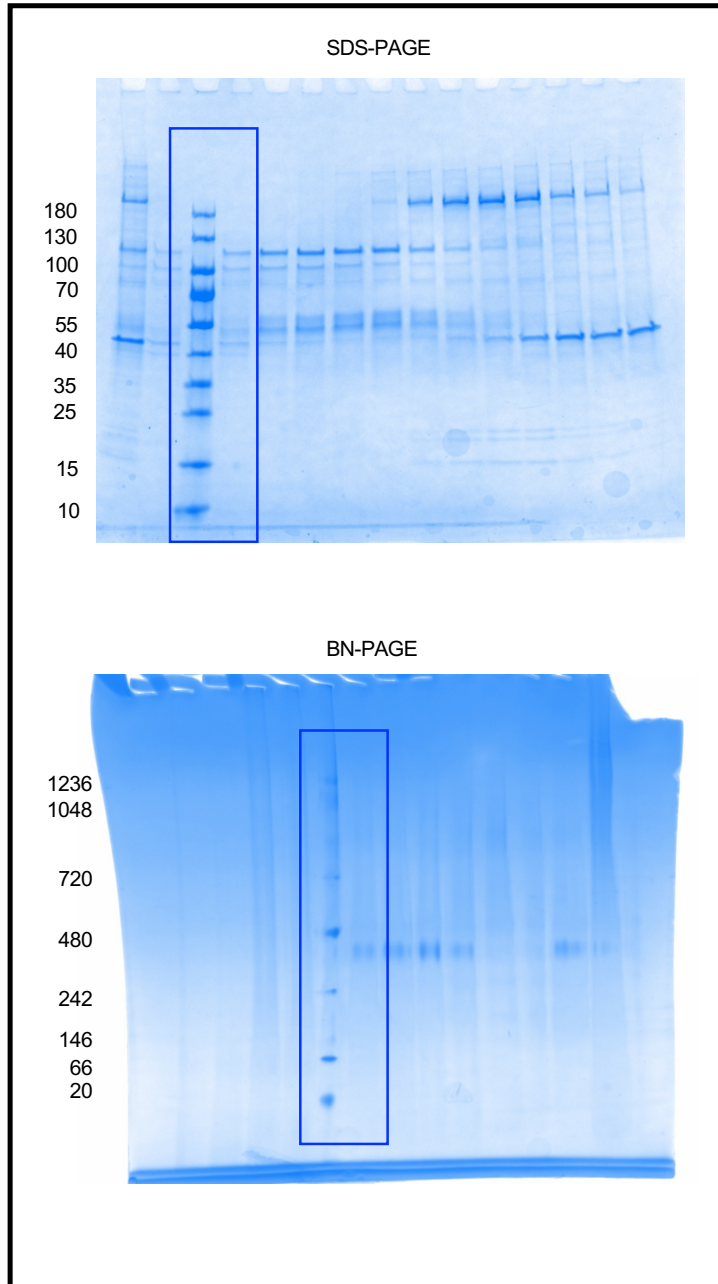


Extended Data Fig. 1c

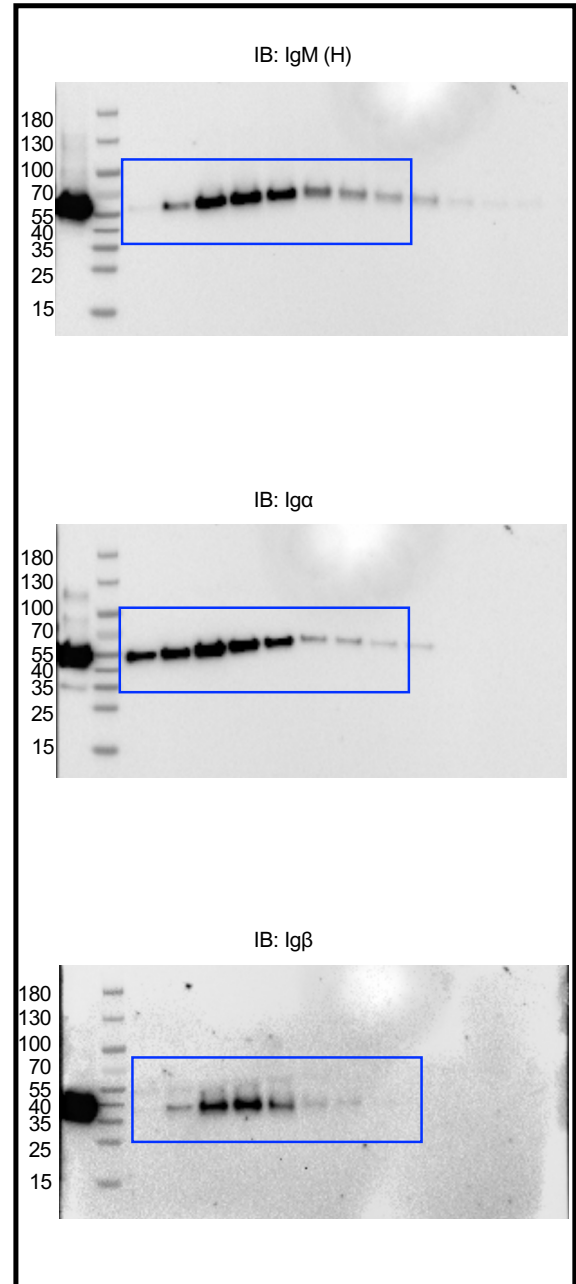


Supplementary Fig 2. Uncropped SDS-PAGE gels and immunoblots. Relative positions of the scans within a panel (black box) follow those in the Extended Data Fig. 2b-c. Blue boxes indicate cropped areas. Molecular weight markers are shown in kDa.

Extended Data Fig. 2b



Extended Data Fig. 2c



Supplementary Table 1. Summary of mutagenesis data.

Region	Mutations in other studies	Residues in mouse IgM BCR
IgM	R479A/E480	R340/E341
	R485A/E486A	R346/E347
	Q487A/Q493A	Q366/Q372
	T530A/T533A	S409/T412
	D553A/E565A	D432/E443
	E567A/E568A	E438/R439
	T573A/Y587A	T452/Y466
	F577W	F456
	F581W	F460
	Y587V/S588V	Y466/S467
Ig α	W76R	W71
	P77W	P72
	R124A/V125A/R126A	R118/V119/R120
	E138H	E132
	E142A/K	E142
	L152W	L147
	L153W	L148
	P153A (mouse)	P153
	A156W	A150
Ig β	W47A	W47
	F52W	F52
	R55A/K56A/R57A	K55/K56/R57
	C135S (mouse)	C135
	K158H	K157
	I161W	I161
	Q164A/K	Q164
	F172A	F171
	P175A (mouse)	P175
IgG	E459A	N440
	E464A	G445
	W469A	W450
	F475A	F456
	F479A	F460
	S482H	S463

All mutations¹⁻⁵ are with human BCR unless labelled as mouse. The mutations are at the interface between IgM and Ig α/β , blocking BCR assembly. The key residues involved in salt bridge and hydrogen bond formation identified in our study are labelled in red.

1. Radaev, S. *et al.* Structural and functional studies of Ig $\alpha\beta$ and its assembly with the B Cell antigen receptor. *Structure* **18**, 934–943 (2010).
2. Gottwick, C. *et al.* A symmetric geometry of transmembrane domains inside the B cell antigen receptor complex. *Proc. Natl. Acad. Sci.* **116**, 13468–13473 (2019).

3. Shaw, C., Mitchell, N., Weaver, Y. K. & Abbas, A. K. Mutations of immunoglobulin transmembrane and cytoplasmic domains: effects on intracellular signaling and antigen presentation. *Cell* **63**, 381–392 (1990).
4. Su, Q. *et al.* Cryo-EM structure of the human IgM B cell receptor. *Science* **337**, 875–880 (2022).
5. Ma, X. *et al.* Cryo-EM structures of two human B cell receptor isotypes. *Science* **377**, 880–885 (2022).