

SUPPLEMENTARY MATERIALS:

Supplementary Tables (n=3) and Supplementary Figures (n=3)

Supplementary Table 1. RBD-specific Bmem responses to 4th dose COVID-19 booster

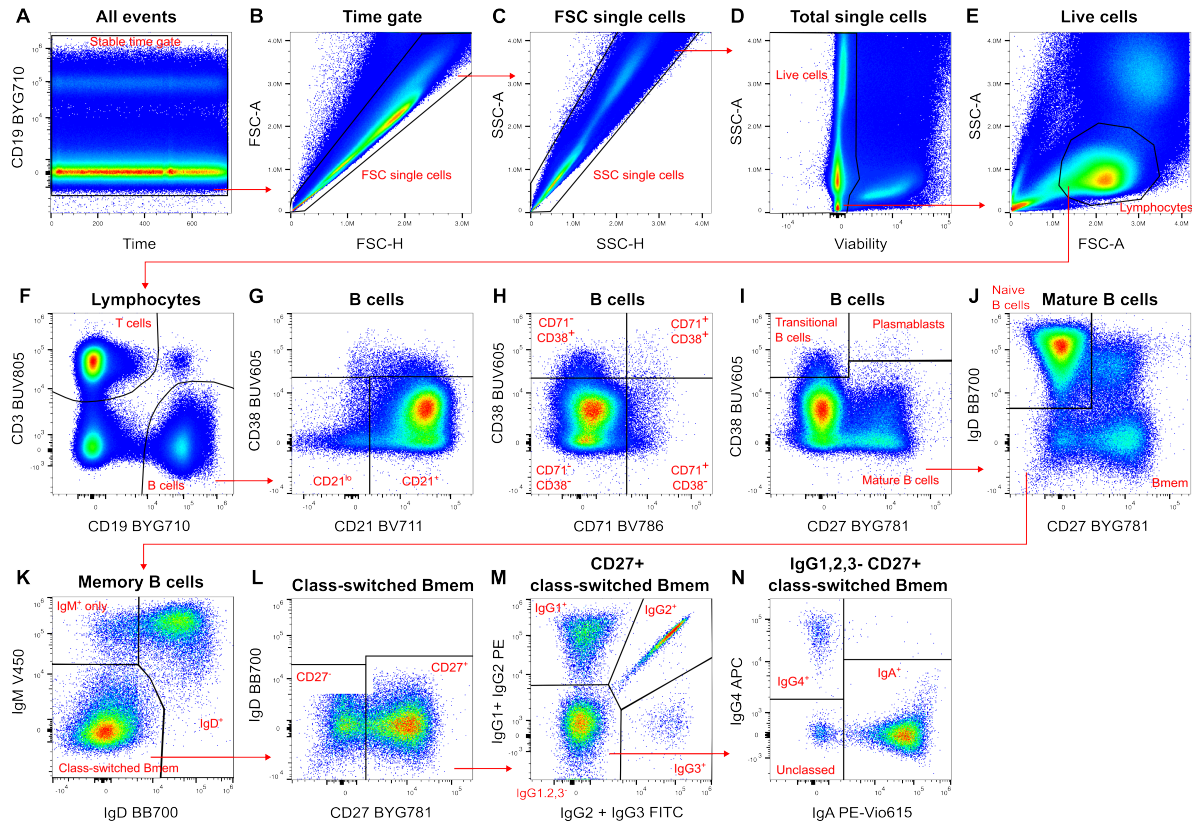
	Bmem numbers (Fold change post-dose 4)		
Booster type	WH1-specific	BA.1-specific	BA.5-specific
Monovalent (n=18)	1.35	1.50	Not measured
BA.1 bivalent (n=33)	1.48	1.38	Not measured
BA.5 bivalent (n=21)	1.46	Not measured	1.42

Supplementary Table 2. Antibody panel compositions

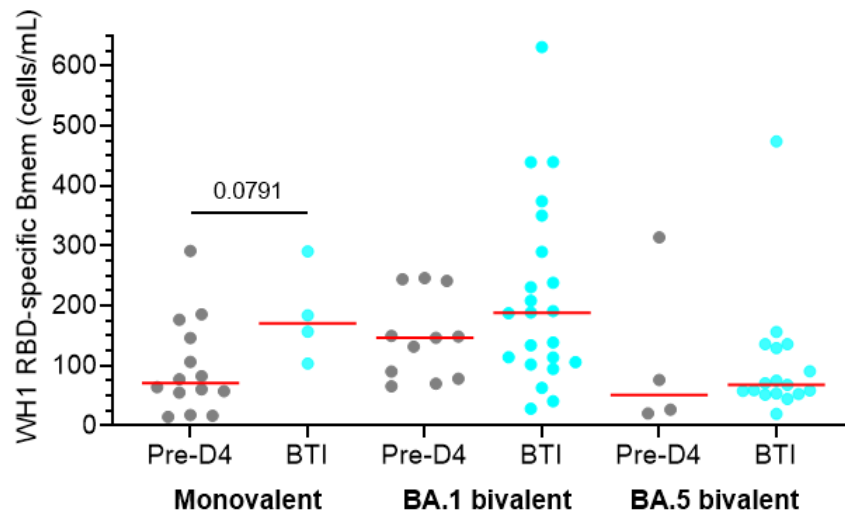
	Fluorochrome																			
Tube	BUV 395	BUV 496	BUV 615	BUV 737	BUV 805	BV421	cFluor V450	BV 480	BV605	BV650	BV711	BV786	FITC	BB700/ PerCP Cy-5.5	PE	PE- Vio615	cFluor BYG710	PE-Cy7/ BYG781	APC	APC-Cy7/ ViaDye Red
1. Trucount	—	—	—	—	—	—	—	—	—	—	—	—	CD3	CD45	CD16 + CD56	—	—	CD4	CD19	CD8a
2a. Bmem (mono- and BA.1 bivalent recipients)	WH1 RBD	BA.5 RBD	—	BA.1 RBD	CD3	WH1 RBD	IgM	BA.1 RBD	CD38	BQ.1.1 RBD	CD21	CD71	IgG2 + IgG3	IgD	IgG1 + IgG2	IgA	CD19	CD27	IgG4	Fixable viability
2b. Bmem (BA.5 bivalent recipients)	WH1 RBD	BA.1 RBD	XBB.1.5 RBD	BA.5 RBD	CD3	WH1 RBD	IgM	BA.5 RBD	CD38	BQ.1.1 RBD	CD21	CD71	IgG2 + IgG3	IgD	IgG1 + IgG2	IgA	CD19	CD27	IgG4	Fixable viability
3a. Strep control (mono- and BA.1 bivalent recipients)	Strep	Strep	—	Strep	CD3	Strep	—	Strep	—	Strep	—	—	—	IgD	—	—	CD19	CD27	—	Fixable viability
3b. Strep control (BA.5 bivalent recipients)	Strep	Strep	Strep	Strep	CD3	Strep	—	Strep	—	Strep	—	—	—	IgD	—	—	CD19	CD27	—	Fixable viability

Supplementary Table 3. Antibody details

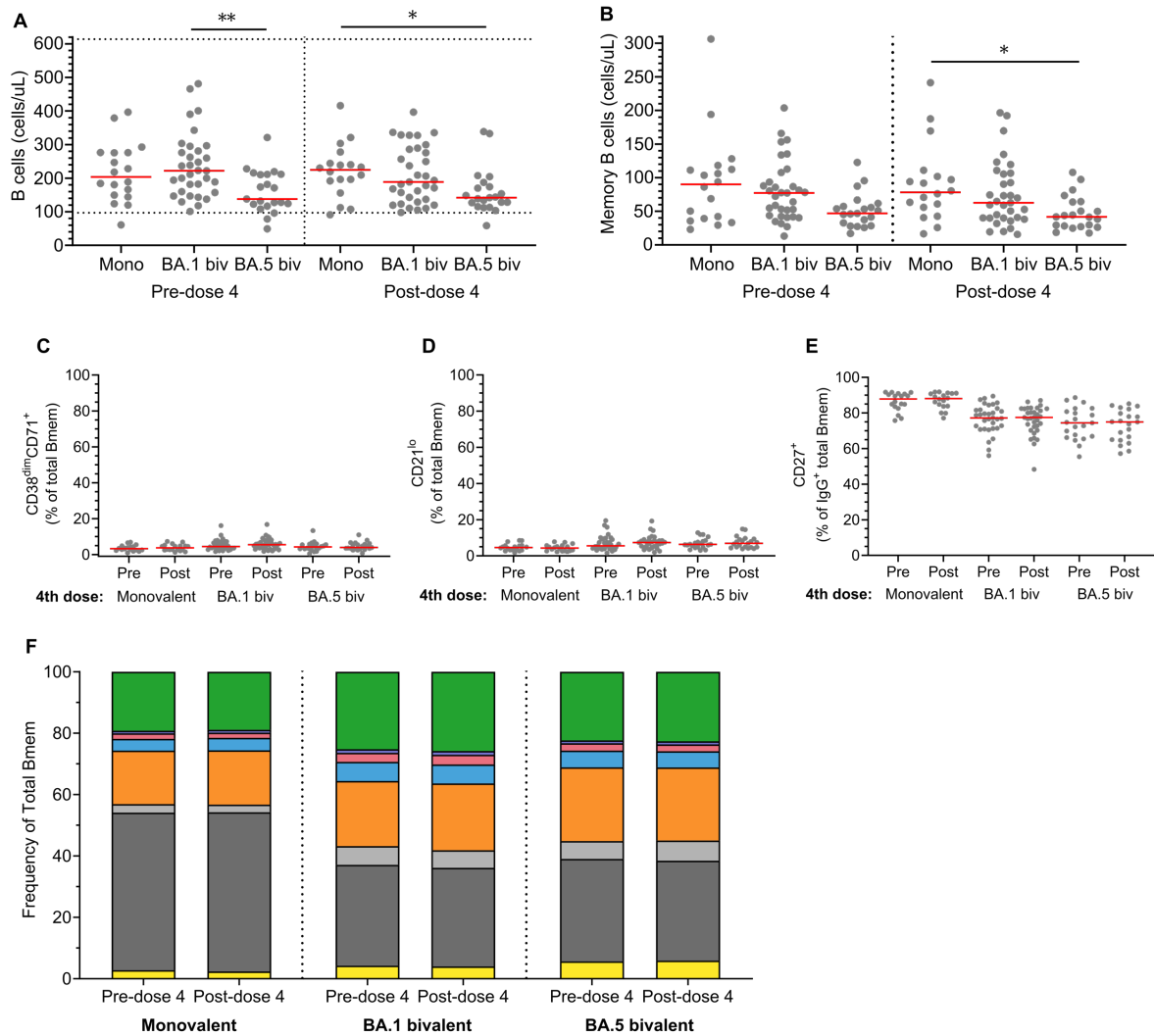
Marker	Fluoro-chrome	Clone	Vendor	Cat. number	Volume (μL)/ 100μL test	Tube
CD3	BUV805	UCHT1	BD Biosciences	612896	2.5	2, 3
CD3	FITC	SK7	BD Biosciences	662995*	46ng	1
CD4	PE-Cy7	SK3	BD Biosciences	662995*	30ng	1
CD8a	APC-Cy7	SK1	BD Biosciences	662995*	126ng	1
CD16	PE	B73.1	BD Biosciences	662995*	33ng	1
CD19	APC	SJ25C1	BD Biosciences	662995*	46ng	1
CD19	cFluor BYG710	HIB19	Cytek Biosciences	SKU-R7- 20010	1	2, 3
CD21	BV711	B-ly4	BD Biosciences	563163	5	2
CD27	cFluor BYG781	O323	Cytek Biosciences	Custom	0.5	2, 3
CD38	BV605	HB7	BD Biosciences	562665	0.2	2
CD45	PerCP Cy-5.5	2D1	BD Biosciences	662995*	120ng	1
CD56	PE	NCAM16.2	BD Biosciences	662995*	22ng	1
CD71	BV786	M-A712	BD Biosciences	563768	1	2
Fixable Viability	ViaDye Red	-	Cytek Biosciences	SKU-R7- 60008	0.025	2, 3
IgA	PE-Vio615	REA1014	Miltenyi Biotec	130-116-882	1.5	2
IgD	BB700	IA6-2	BD Biosciences	566538	1	2, 3
IgG1	PE	G17-1	BD Biosciences	624049	0.1	2
IgG2	FITC	HP6002	BD Biosciences	624045	0.5	2
IgG2	PE	HP6002	BD Biosciences	624049	1	2
IgG3	FITC	HP6047	BD Biosciences	624045	0.5	2
IgG4	APC	SAG4	Cytognos	CYT-IGG4AP	2	2
IgM	cFluor V450	MHM88	Cytek Biosciences	Custom	0.25	2
Streptavidin	BUV395	-	BD Biosciences	564176	0.67	2, 3
Streptavidin	BUV496	-	BD Biosciences	612961	0.67	2, 3
Streptavidin	BUV615	-	BD Biosciences	613013	0.67	2b, 3b only
Streptavidin	BUV737	-	BD Biosciences	612775	0.67	2, 3
Streptavidin	BV421	-	BioLegend	405225	0.13	2, 3
Streptavidin	BV480	-	BD Biosciences	564876	0.67	2, 3
Streptavidin	BV650	-	BioLegend	563855	0.13	2, 3
*Antibodies part of the Multitest™ 6-color TBNK kit (BD Biosciences, Cat. number 662967)						



Supplementary Figure 1. B-cell gating strategy. (A) All events were gated on CD19 vs time to ensure a steady signal across acquisition time and to exclude aggregates. (B-C) Doublets were excluded on FSC-A vs FSC-H, and subsequently SSC-A vs SSC-H. (D) Dead cells were excluded on SSC-A vs viability dye. (E) Lymphocytes were gated as $\text{SSC}^{\text{lo}}\text{FSC}^{\text{mid}}$. (F) B cells were gated as $\text{CD19}^+\text{CD3}^-$. (G) CD21^{lo} and CD21^+ B cells were gated vs CD38. (H) CD38 and CD71 expression on B cells was gated in quadrants. (I) Mature and transitional B cells and plasmablasts were gated on CD38 vs CD27. (J) Memory B cells (Bmem) and naive B cells were gated on IgD vs CD27 within mature B cells. (K) Class-switched, IgD^+ , and IgM^+ only mature Bmem were gated on IgM vs IgD. (L) CD27^- and CD27^+ class-switched mature Bmem were gated vs IgD. (M) IgG1^+ , IgG2^+ , IgG3^+ , and IgG1,2,3^- class-switched mature Bmem were gated within CD27^+ cells (and CD27^- , plot not shown). (N) IgG4^+ , IgA^+ , and unclassified mature Bmem were gated within IgG1,2,3-CD27^+ cells (and CD27^- , plot not shown). Representative plots from BA.1 bivalent booster donor, post-dose 4.



Supplementary Figure 2. The effect of breakthrough infections on the RBD-specific Bmem response. Absolute numbers of WH1 RBD-specific Bmem pre-dose 4, divided by booster type group and breakthrough infection (BTI) status. Blue dots denote any confirmed SARS-CoV-2 BTI before pre-dose 4 sampling. Monovalent, n=18; BA.1 bivalent, n=33; BA.5 bivalent, n=21. Solid lines depict median values. Mann-Whitney test for unpaired data.



Supplementary Figure 3. Total memory B cell numbers and Ig isotypes before and after monovalent or bivalent dose 4. (A) Absolute numbers of total B cells and (B) memory B cells (Bmem) before and after a monovalent, BA.1 bivalent, or BA.5 bivalent dose 4. Dotted lines in (A) denote 5th and 95th percentiles of B-cell numbers from healthy controls previously published (60). (C) Frequencies of CD38^{dim}CD71⁺, (D) CD21^{lo}CD38^{dim}, and (E) CD27⁺IgG⁺ Bmem pre- and 4-weeks post-monovalent, BA.1 bivalent, or BA.5 bivalent dose 4. (F) Frequencies of IgG1⁺, IgG2⁺, IgG3⁺, IgG4⁺, IgA⁺, IgD⁺, and IgM⁺-only within total Bmem pre- and 4-weeks post-monovalent, BA.1 bivalent, or BA.5 bivalent dose 4. Monovalent, n=18; BA.1 bivalent, n=33; BA.5 bivalent, n=21. Solid lines depict median values. Kruskal-Wallis test with Dunn's multiple comparisons for multiple unpaired groups, and Wilcoxon matched-pairs signed rank test for paired data. Only significant differences shown. *p<0.05, **p<0.01.