Efficient recall of SARS-CoV-2 variant-reactive B cells and T responses in the elderly upon heterologous mRNA vaccines as boosters

Supplementary Figure 1

Supplementary Figure 2

Supplementary Figure 3

Supplementary Figure 4

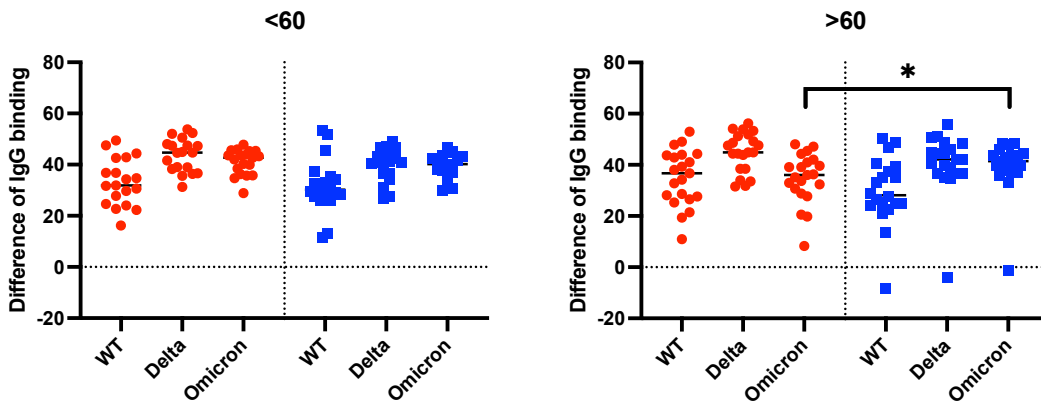Supplementary Figure 5

Supplementary Figure 6

Supplementary Figure 7

Supplementary Figure 8

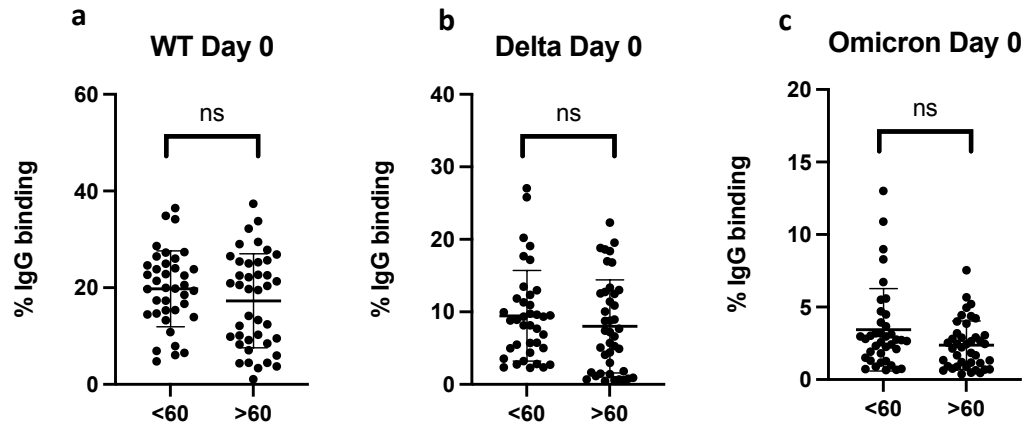Supplementary Figure 9

Supplementary Figure 10

Supplementary Figure 11
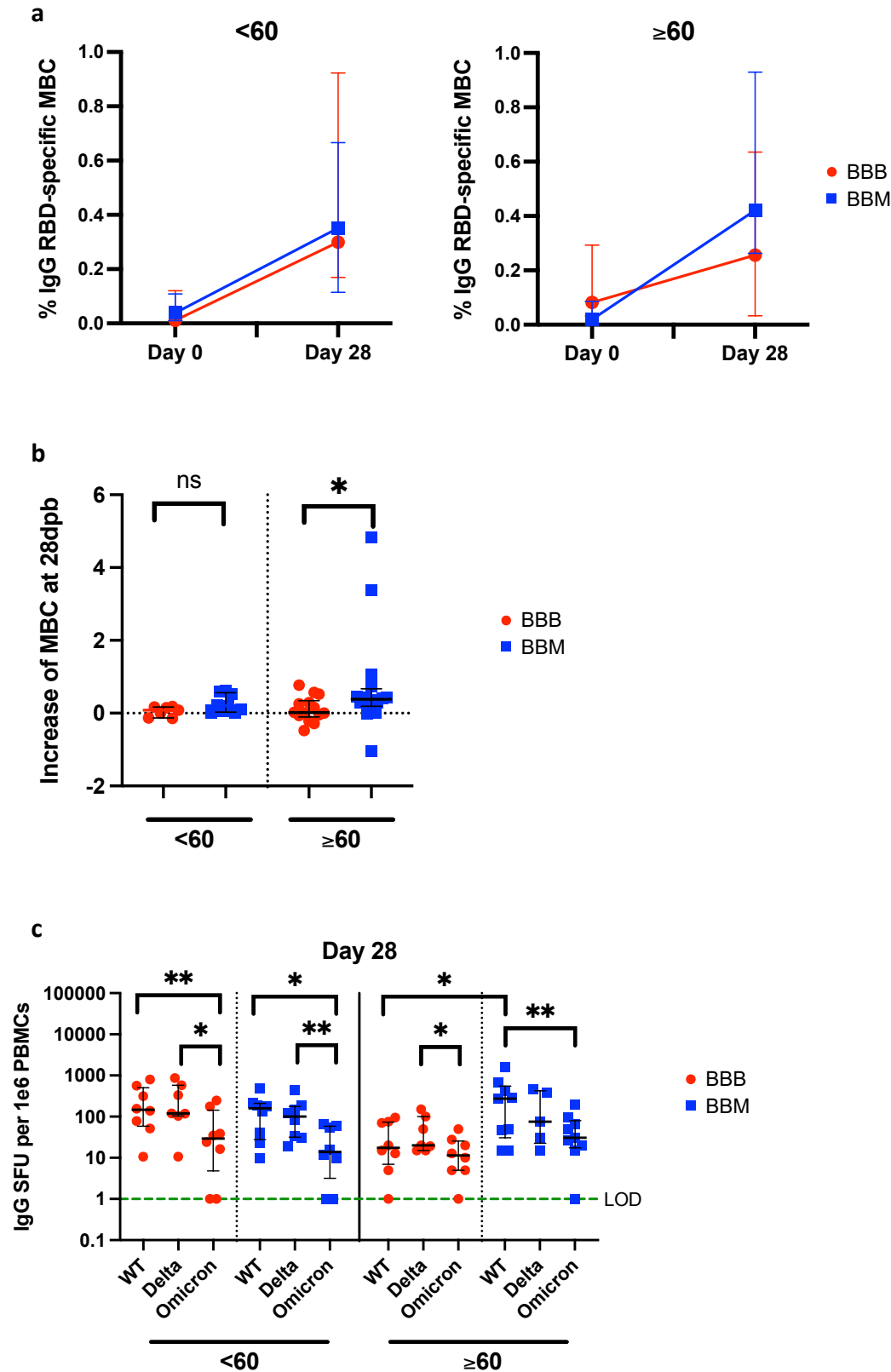
Supplementary Figure 1

Supplementary Figure 1: Higher increase in Omicron-binding plasma IgG with heterologous booster vaccination in the elderly. Difference of IgG binding to WT, Delta and Omicron between day 0 and day 28 in BBB (red circle) or BBM (blue square) groups in <60 (left panel) or ≥60 (right panel). Statistics used Mass-Whitney test.
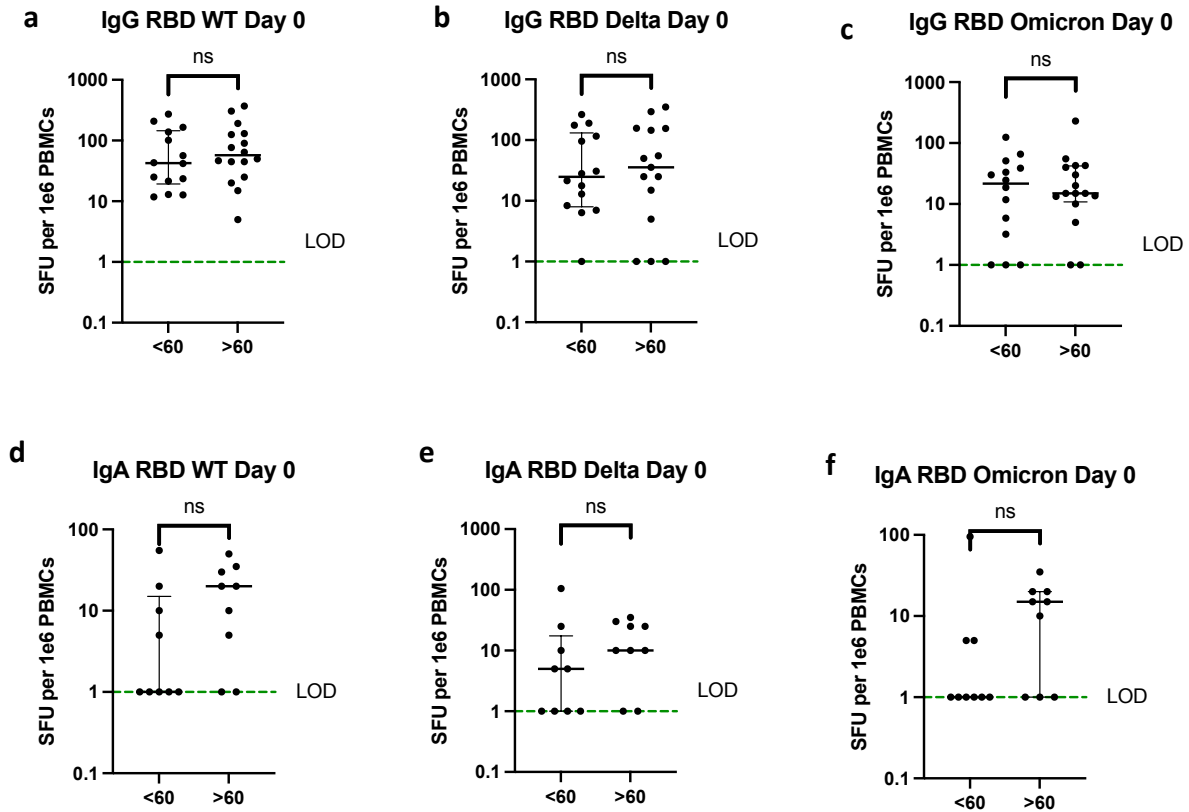
Supplementary Figure 2

Supplementary Figure 2: **Similar levels of plasma IgG against WT, Delta and Omicron variants at Day 0 between <60 and >60 individuals**. Plasma IgG binding against WT (a), Delta (b) and Omicron (c) in individuals below 60 years old (<60) or above 60 years old (≥60). Statistics used Mass-Whitney test.
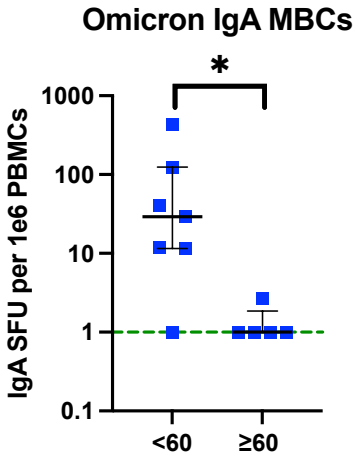
Supplementary Figure 3

Supplementary Figure 3: Higher increase in MBCs with heterologous booster vaccination in the elderly. (a) Frequency of IgG RBD-specific memory B cells at day 0 and 28 in BBB (red circle) or BBM (blue square) groups in <60 (left panel) or ≥60 (right panel). **(b)** Difference of frequency of RBD-specific MBCs between individuals below 60 years old (<60) or above 60 years old (≥60) from BBB (red circle) or BBM (blue square) groups. **(c)** IgG memory B cell response against RBD from WT, Delta and Omicron strains in individuals below 60 years old (<60) or above 60 years old (≥60) from BBB (red circle) or BBM (blue square) groups. Data are presented in Spot Forming Unit (SFU) per 1E6 cells in log axis. The green dotted line represents the limit of detection (LOD=1).
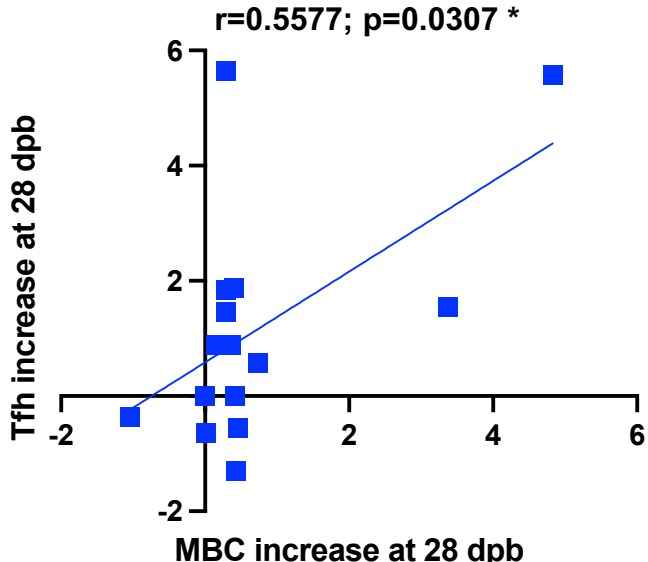
Supplementary Figure 4

a **IgG RBD WT Day 0**

b **IgG RBD Delta Day 0**

c **IgG RBD Omicron Day 0**

d **IgA RBD WT Day 0**

e **IgA RBD Delta Day 0**

f **IgA RBD Omicron Day 0**

Supplementary Figure 4: **Similar levels of IgG and IgA MBCs against WT, Delta and Omicron variants at Day 0 between <60 and >60 individuals**. IgG (a-c) or IgA (d-f) memory B cell response against RBD from WT (a and d), Delta (b and e) and Omicron (c and f) in individuals below 60 years old (<60) or above 60 years old (≥60). Data are presented in Spot Forming Unit (SFU) per 1E6 cells in log axis. The green dotted line represents the limit of detection (LOD=1). Statistics used Mass-Whitney test.

Supplementary Figure 5

Supplementary Figure 5: **Individuals above 60 y.0. in the BBM group have lower level of IgA MBCs against Omicron RBD**. IgA memory B cell response against RBD from Omicron in individuals below 60 years old (<60) or above 60 years old (≥60) from BBM (blue square) groups. Data are presented in Spot Forming Unit (SFU) per 1E6 cells in log axis. The green dotted line represents the limit of detection (LOD=1).
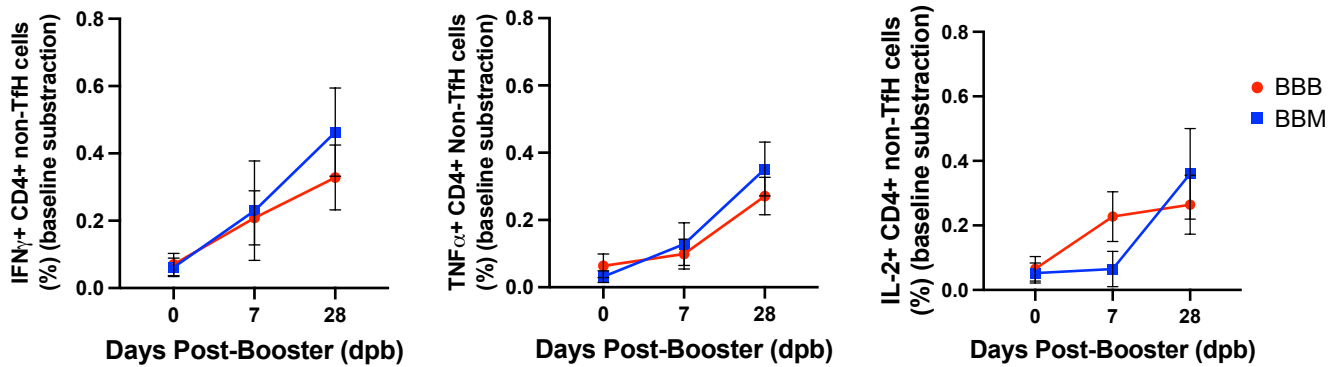
Supplementary Figure 6

Supplementary Figure 6: **Correlation between Tfh and MBC increase.** Correlation between the increase of CD154+ CXCR5+ Tfh cells at 28 dpb and the increase of RBD-specific MBCs at 28 dpb in individuals above 60 y.o. who received the BBM regime. Correlation using Spearman test.
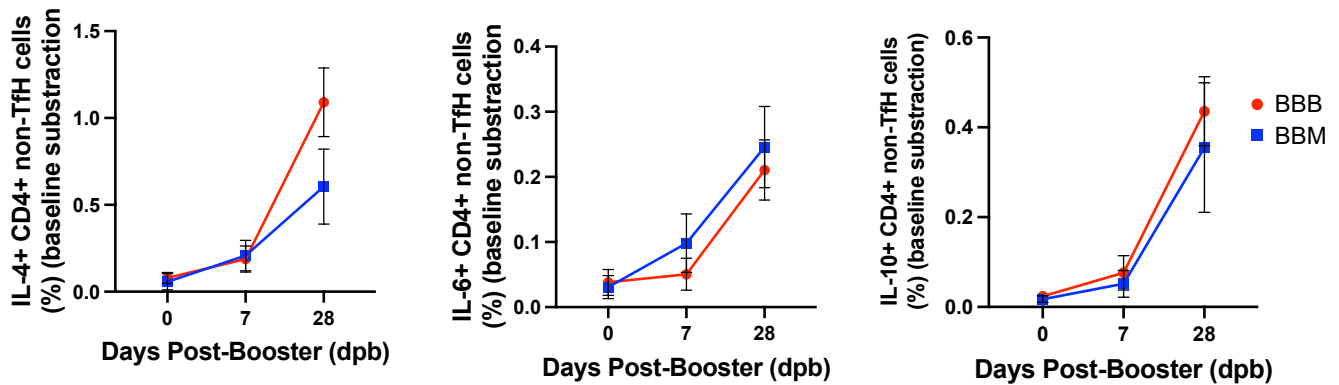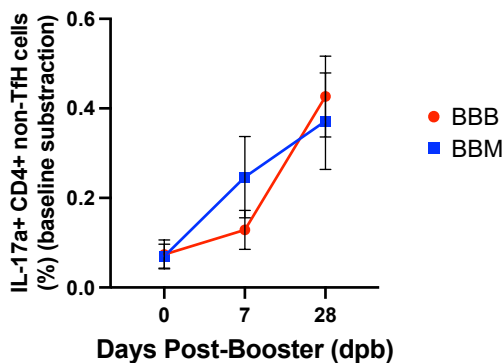
Supplementary Figure 7

Supplementary Figure 7: BBB and BBM increases Th1, Th2 and Th17 responses in the young. (a) Frequency of IFNγ **(left)**, TNFα **(middle)**, and IL-2 **(right)** at 0, 7 and 28 dpb in participants under 60 years old from BBB (red circle) and BBM (blue square) groups. **(b)** Frequency of IL-4 **(left)**, IL-6 **(middle)**, and IL-10 **(right)** at 0, 7 and 28 dpb in participants in participants under 60 years old from BBB (red circle) and BBM (blue square) groups. **(c)** Frequency of IL-17a at 0, 7 and 28 dpb in participants under 60 years old from BBB (red circle) and BBM (blue square) groups
Data are presented after baseline subtraction in log axis. The green dotted line represents the limit of detection (LOD=0.01).
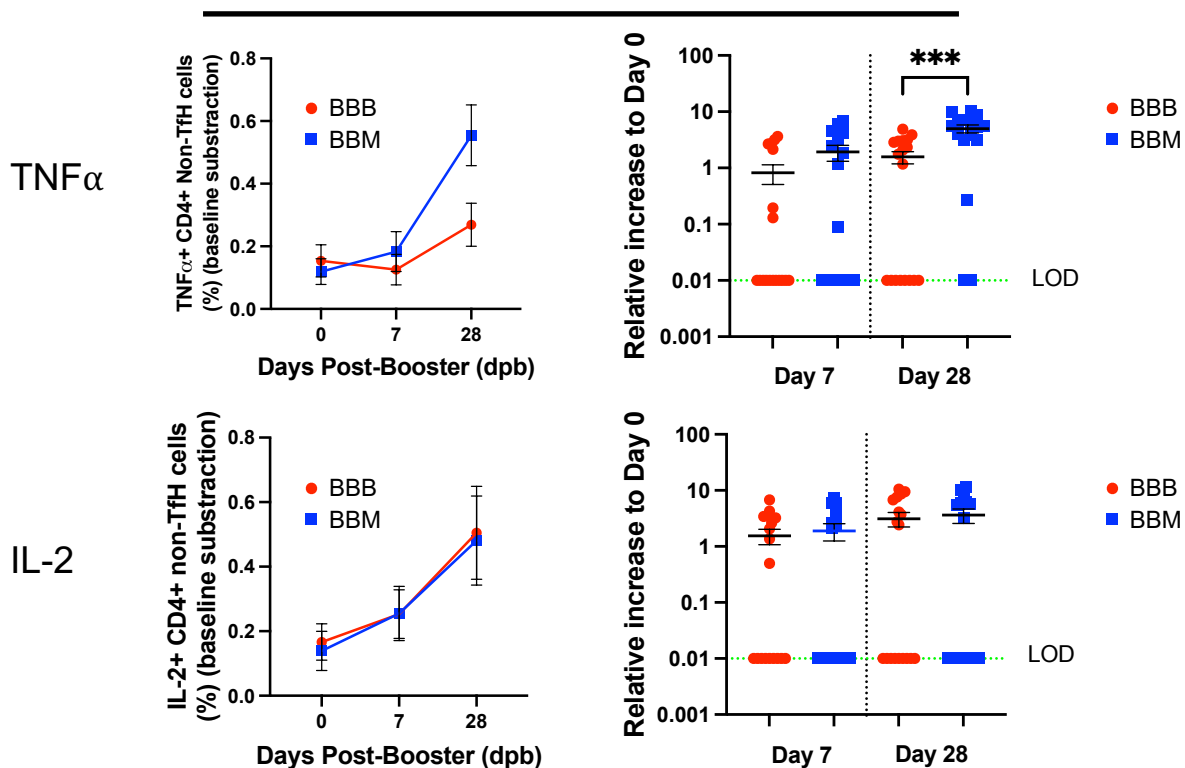Data are presented with mean and SEM.
Mann-Whitney test between BBB and BBM groups. Wilcoxon matched-pairs test for paired samples.
* p<0.05; ** p<0.01; *** p<0.001

Supplementary Figure 8

Supplementary Figure 8: BBM regimen elicits higher Th1 responses in the elderly.
Frequency of TNFα and IL-2 at 0, 7 and 28 dpb in elderly participants in BBB (red circle) or BBM (blue square) groups **(Left panels)**
Differences in relative increases of TNFα and IL-2 at 7 and 28 dpb between elderly participants from BBB (red circle) or BBM (blue square) groups. **(Right panels)**
Data is presented after baseline subtraction in log axis. The green dotted line represents the limit of detection (LOD=0.01).
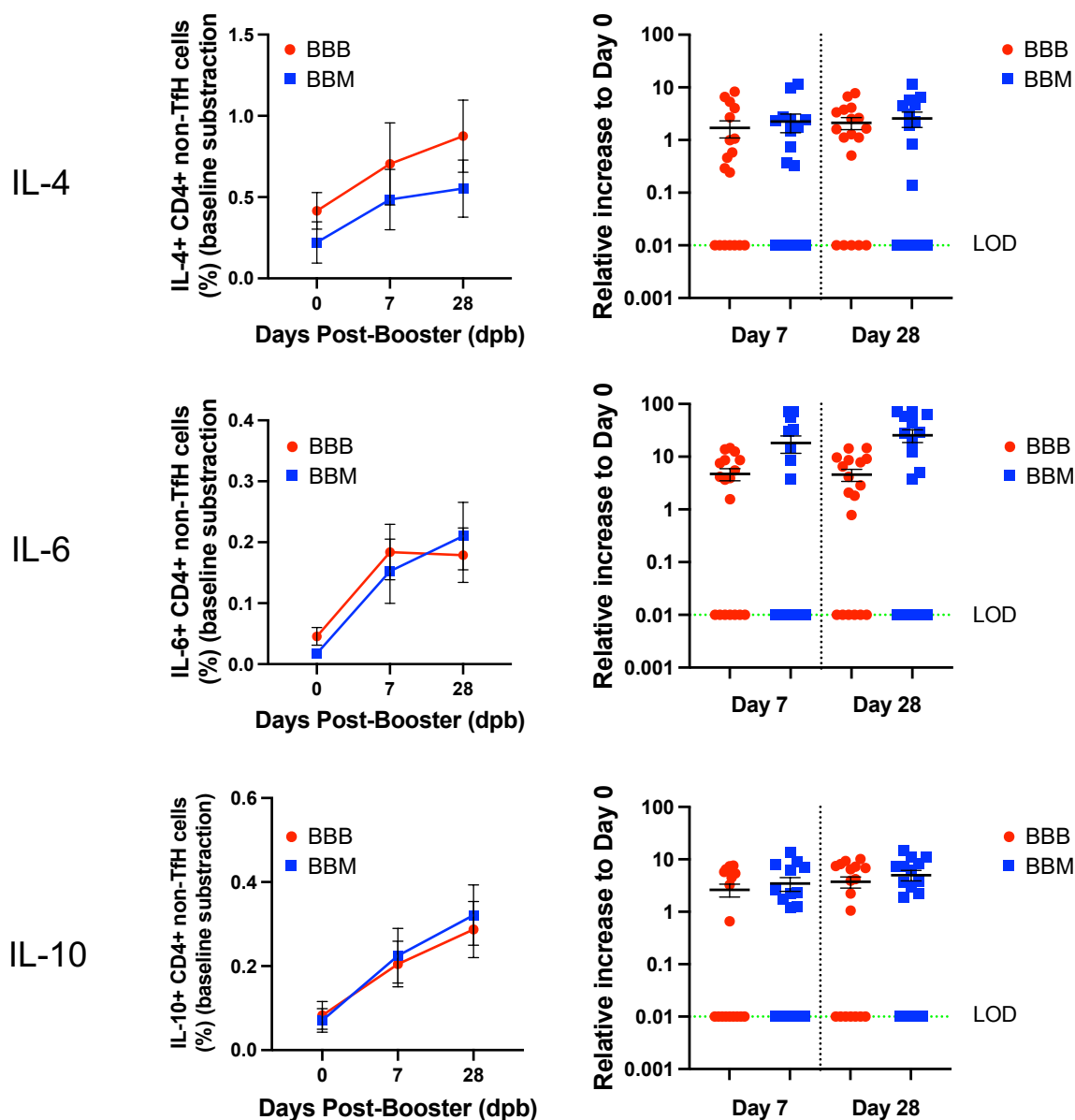Data is presented with mean and SEM.
Mann-Whitney test between BBB and BBM groups. Wilcoxon matched-pairs test for paired samples.
* p<0.05; ** p<0.01; *** p<0.001

Supplementary Figure 9: BBB and BBM elicits comparable increases in levels of Th2 responses in the elderly.
Frequency of IL-4, IL-6 or IL-10 producing CD4$^+$ T cells at 0, 7 and 28 dpb in elderly participants in BBB (red circle) or BBM (blue square) groups **(Left panels)** Differences in relative increases of IL-4, IL-6 and IL-10 producing CD4$^+$ T cells at 7 and 28 dpb between elderly participants from BBB (red circle) or BBM (blue square) groups. **(Right panels)**
Data is presented after baseline subtraction in log axis. The green dotted line represents the limit of detection (LOD=0.01).
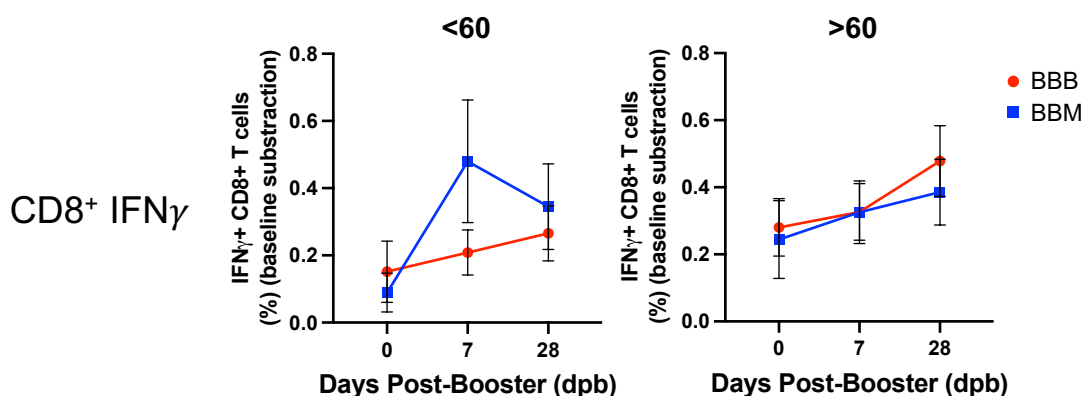Data is presented with mean and SEM.
Mann-Whitney test between BBB and BBM groups. Wilcoxon matched-pairs test for paired samples.
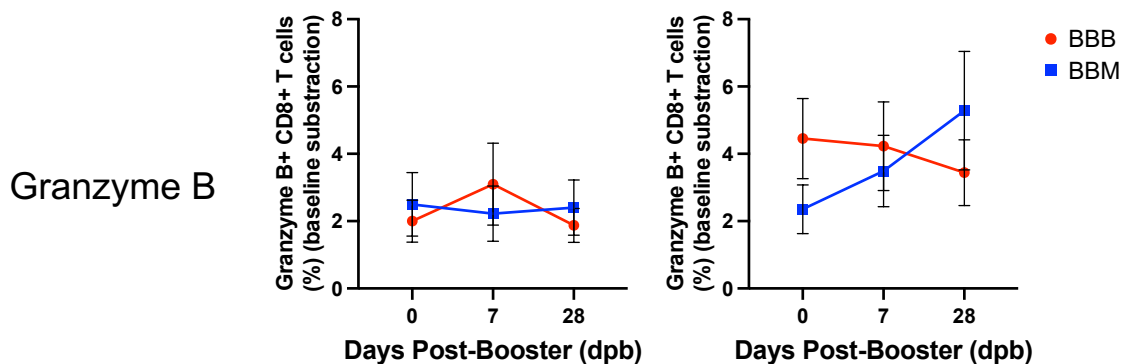* p<0.05; ** p<0.01; *** p<0.001

Supplementary Figure 10

Supplementary Figure 10: Comparable cytotoxic CD8⁺ T cell responses upon heterologous and homologous booster regimes.
(a) Frequency of IFNγ-producing CD8⁺ T cells at 0, 7 and 28 dpb in BBB (red circle) or BBM (blue square) groups in <60 **(Left)** or ≥60 **(Right)**.
(b) Frequency of Granzyme B-producing CD8⁺ T cells at day 0 and 28 in BBB (red circle) or BBM (blue square) groups in <60 **(Left)** or ≥60 **(Right)**.
Data is presented after baseline subtraction in log axis. The green dotted line represents the limit of detection (LOD=0.01).
Data is presented with mean and SEM.
Mann-Whitney test between BBB and BBM groups. Wilcoxon matched-pairs test for paired samples. * p<0.05; ** p<0.01; *** p<0.001

Supplementary Figure 11
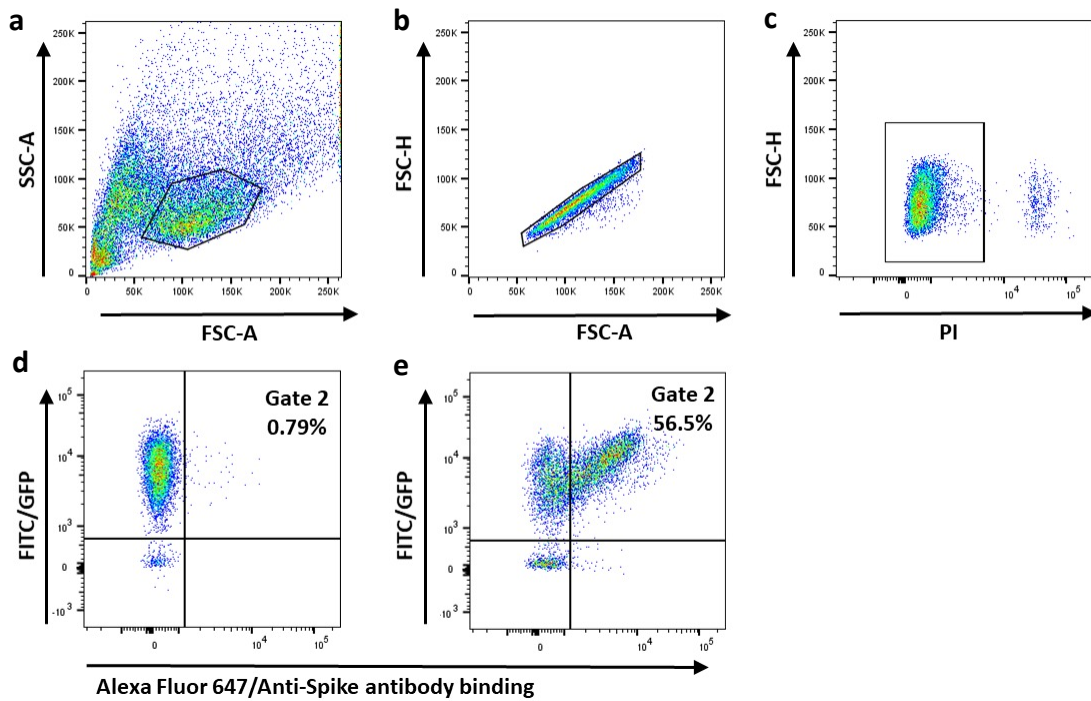
Supplementary Figure 11: Representative gating strategy for SFB assay to determine spike-specific antibody response. Cells were gated on: (a) FSC-A/SSC-A to exclude cell debris, (b) FSC-A/FSC-H to select for single cells, (c) FSC-A/PI to select for live cells (PI-negative population), (d, e) FITC/Alexa Fluor 647. Binding is determined by the percentage of GFP-positive S protein-expressing cells that are bound by specific antibody, indicated by the events that are Alexa Fluor 647- and FITC-positive (Gate 2). (d) FBS control; (e) COVID-19 patient plasma, 1:100 diluted.