

A single dose, BCG-adjuvanted COVID-19 vaccine provides sterilizing immunity against SARS-CoV-2 infection

SUPPLEMENTARY INFORMATION

Claudio Counoupas^{1,2}, Matt D. Johansen³, Alberto O. Stella⁴, Duc H. Nguyen³, Angela L. Ferguson^{1,2}, Anupriya Aggarwal⁴, Nayan D. Bhattacharyya², Alice Grey⁵, Owen Hutchings⁶, Karishma Patel⁷, Rezwan Siddiquee⁷, Erica L. Stewart¹, Carl G. Feng², Nicole G. Hansbro³, Umaimainthan Palendira¹, Megan C. Steain², Bernadette M. Saunders³, Jason K. K. Low⁷, Joel P. Mackay⁷, Anthony D. Kelleher⁴, Warwick J. Britton^{2,5}, Stuart G Turville⁴, Philip M. Hansbro^{3*}, James A. Triccas^{1,8*}

¹School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Camperdown, NSW, Australia.

²Tuberculosis Research Program at the Centenary Institute, The University of Sydney, Sydney, NSW, Australia.

³Centre for Inflammation, Centenary Institute and University of Technology Sydney, Faculty of Science, School of Life Sciences, Sydney, NSW, Australia.

⁴Kirby Institute, University of New South Wales, Sydney, NSW, Australia.

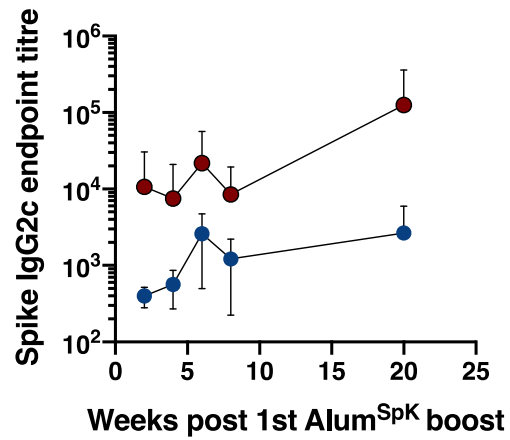
⁵Department of Clinical Immunology, Royal Prince Alfred Hospital, Sydney, NSW, Australia.

⁶RPA Virtual Hospital, Sydney Local Health District, Sydney, NSW, Australia.

⁷School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW 2006.

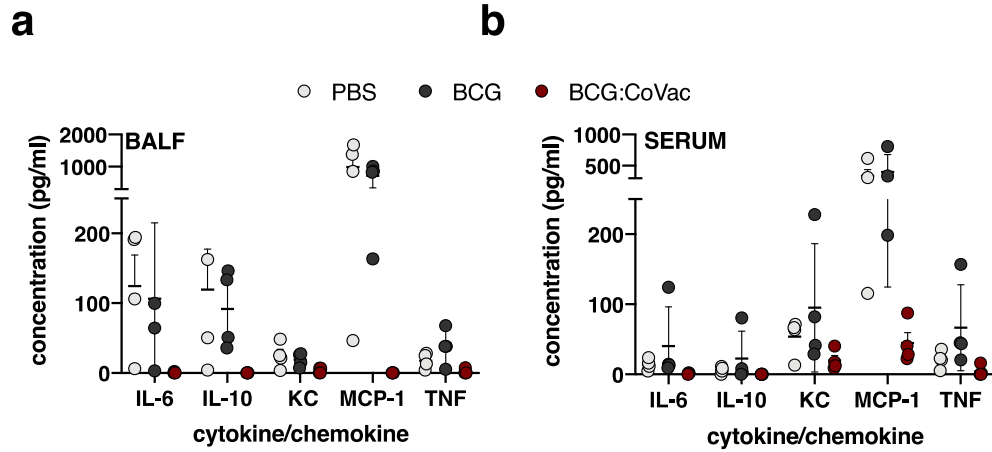
⁸Charles Perkins Centre and Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Camperdown, NSW, Australia.

*Correspondence to James A. Triccas (jamie.triccas@sydney.edu.au); Philip Hansbro (Philip.Hansbro@uts.edu.au)



Supplementary Figure 1. BCG promotes spike-specific antibody responses. Mice were vaccinated s.c with BCG (5×10^5 CFU) and 12 week later were vaccinated twice, 3 weeks apart with s.c with SARS-CoV-2 spike protein ($5 \mu\text{g}$) formulated in alum ($100 \mu\text{g}$; Alum^{SpK}). At the indicated timepoints the titre of spike-specific IgG2c in sera was determined by ELISA.

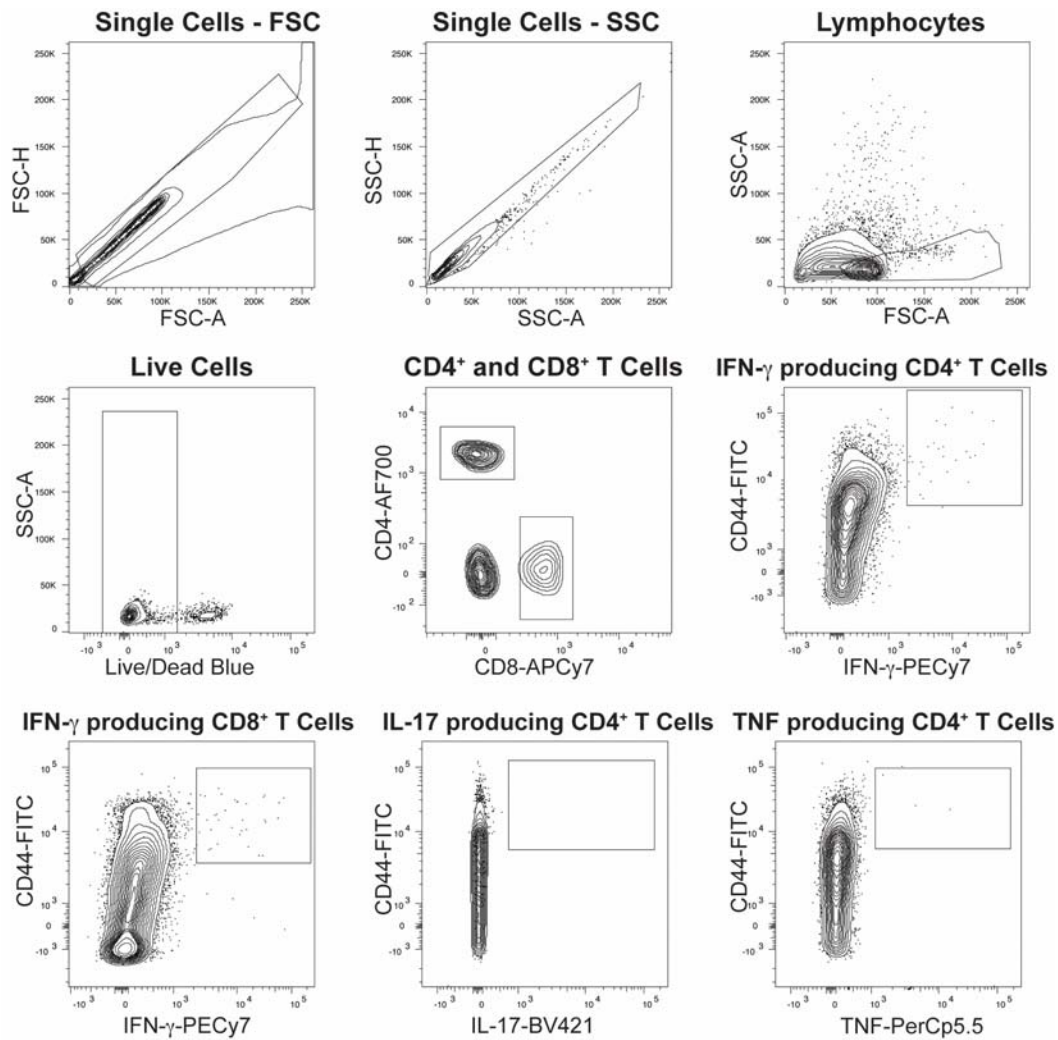
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48



Supplementary Figure 2. Single dose BCG:CoVac prevents the development of clinical disease in SARS-CoV-2 infected K18-hACE2 mice. **a**, Mice were immunised with sham (PBS), BCG or BCG:CoVac 21 days prior to challenge with 10^3 PFU SARS-CoV-2. Cytokine/chemokine levels were determined in BALF (**a**) and serum (**b**) by cytometric bead array 6 days after challenge.

49

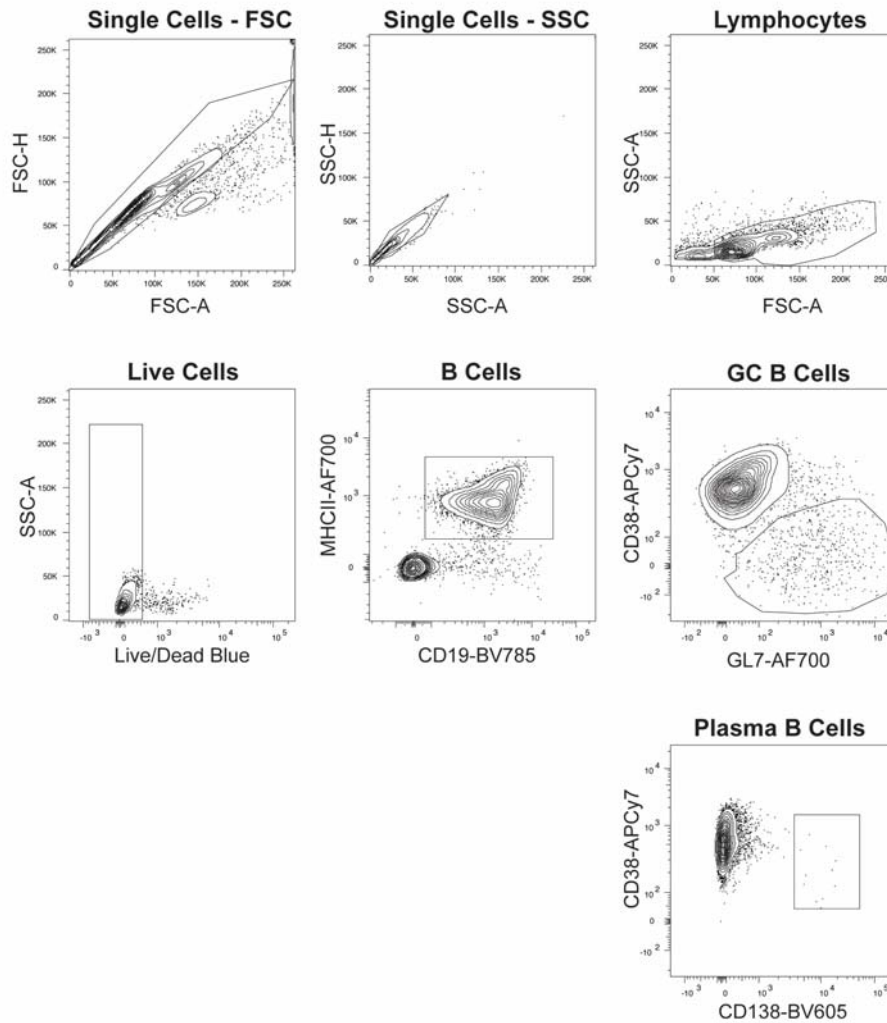
50



Supplementary Figure 3. Gating strategy for cytokine producing CD4⁺ and CD8⁺ T cells from PBMCs. Single events were selected using FSC-H/FSC-A and SSC-A/SSC-H. Lymphocytes populations were gated by SSC-A and FSC-A plots. Dead cells were excluded and viable CD4⁺ and CD8⁺ T cells were gated for further analysis of expression of different cytokines (IFN- γ , IL-17, TNF).

51

52



Supplementary Figure 4. Gating strategy for Germinal Center B cells and Plasma B Cells.

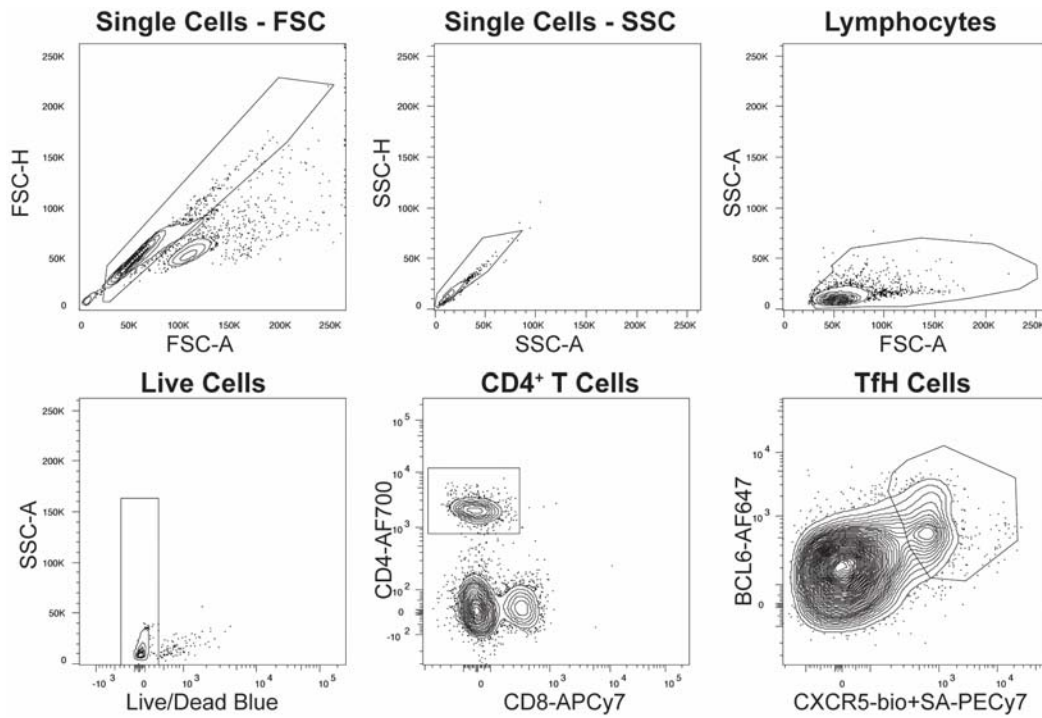
Single events were selected using FSC-H/FSC-A and SSC-A/SSC-H. Lymphocytes populations were gated by SSC-A and FSC-A plots. Dead cells were excluded and viable B cells were gated on the double expression of MHCII and CD19. GC B cells were gated based on their expression of GL7 and lacking expression of CD38. Plasma B cells were gated based on their expression of CD138.

53

54

55

56



Supplementary Figure 5. Gating strategy for T follicular helper cells in the lymph node.

Single events were selected using FSC-H/FSC-A and SSC-A/SSC-H. Lymphocytes populations were gated by SSC-A and FSC-A plots. Dead cells were excluded and viable CD4⁺ T cells were gated for further analysis of expression BCL6 and CXCR5, double positive cells for this markers were considered Tfh cells.

57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74

Supplementary Table 1: Flow cytometry antibodies used in this study

T cell panel

Marker	Fluorophore	Location	Clone	Dilution	Company	Cat. #
Live/Dead	Blue	Surface		1:300	ThermoFisher	L23105
Fc Block	purified	Surface	2462	1:300	BD	553141
CD4	AF700	Surface	RM414	1:200	BD	557956
CD44	BV605	Surface	IM7	1:300	BD	563058
CD8	APC-Cy7	Surface	53-6.7	1:200	BD	557654
CXCR5	Biotin	Surface	2G8	1:100	BD	551960
PD-1	BV711	Surface	29F.1A12	1:200	Biolegend	135231
Streptavidin	PE-Cy7	Surface		1:200	BD	557598
BCL-6	AF647	Intracellular	K112-91	1:200	BD	561525
CD19	BV785	Surface	1D3	1:200	BD	563333
IFN- γ	PECy7	Intracellular	XMG1-2	1:200	BD	557649
IL-17	PB	Intracellular	TC11-	1:200	BioLegend	506918
IL-2	PE	Intracellular	JES6-5H4	1:200	BD	554428
TNF	PerCP-Cy5.5	Intracellular	MP6-	1:200	BD	560659

B cell panel

Marker	Fluorophore	Location	Clone	Dilution	Company	Cat. #
Live/Dead	Blue	Surface		1:300	ThermoFisher	L23105
Fc Block	purified	Surface	2462	1:300	BD	553141
CD19	BV785	Surface	1D3	1:200	BD	563333
Spike	AF647	Surface		1:50		
GL7	AF488	Surface	GL7	1:200	Biolegend	144612
MHCII	AF700	Surface	M5/114.15.2	1:200	Biolegend	107622
CD138	BV605	Surface	281-2	1:200	Biolegend	142516
CD38	APC-Cy7	Surface	90	1:200	Biolegend	102728
IgM	BV421	Surface	RMM-1	1:200	Biolegend	406518
IgD	PerCP5.5	Surface	11-26c.2a	1:200	BD	564273