

Supplementary Information

Engineering genome-free bacterial cells for effective SARS-COV-2 neutralisation

Yutong Yin¹, Chang Liu^{2,3}, Xianglin Ji⁴, Yun Wang⁴, Juthathip Mongkolsapaya^{2,3,5}, Gavin R. Screaton^{2,3}, Zhanfeng Cui^{1,6}, Wei E. Huang^{1*}.

1. Department of Engineering Science, University of Oxford, Parks Road, OX1 3PJ, Oxford, UK.
2. Wellcome Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, Oxford OX3 7BN, UK.
3. Chinese Academy of Medical Science (CAMS) Oxford Institute (COI), University of Oxford, Oxford OX3 7FZ, UK.
4. Oxford Suzhou Centre for Advanced Research (OSCAR), University of Oxford, Suzhou Industrial Park, Jiangsu, China
5. Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, Department of Medicine, University of Oxford, Oxford, UK
6. Institute of Biomedical Engineering, Department of Engineering Science, University of Oxford, OX3 7DQ, UK.

*Corresponding author

Wei E. Huang

Tel.: +44 1865 283786

Email: wei.huang@eng.ox.ac.uk

Keywords: synthetic biology, SimCell, mini-SimCell, bacterial therapy, chromosome-free, synthetic cell, live virus neutralisation

Table S1. Bacterial strains and plasmids in this study.

Strains	Genotype description	Source
<i>Escherichia coli</i> DH5 α	<i>fhuA2 lac(del)U169 phoA glnV44 Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17</i>	Lab collection
<i>Escherichia coli</i> ClearColi BL21(DE3)	<i>F- ompT hsdSB (rB- mB-) gal dcm lon λ(DE3 [<i>lacI lacUV5-T7 gene 1 ind1 sam7 nin5</i>]) msbA148 ΔgutQΔkdsD ΔlpxLΔlpxMΔpagPΔlpxPΔeptA</i>	Purchased from Lucigen ¹
ClearColi <i>AminD</i>	<i>F- ompT hsdSB (rB- mB-) gal dcm lon λ(DE3 [<i>lacI lacUV5-T7 gene 1 ind1 sam7 nin5</i>]) msbA148 ΔgutQΔkdsD ΔlpxLΔlpxMΔpagPΔlpxPΔeptA <i>AminD</i></i>	This study
ClearColi Negative control (pNV_J23100)	ClearColi: for FACS negative control (high expression of nanobody displaying system without VHH)	This study
ClearColi Negative control (pNV_J23100)	ClearColi: for FACS negative control (high expression of nanobody displaying system without VHH)	This study
ClearColi pNV_J23100_TY1 VHH_	ClearColi high expression of TY1 VHH ² display	This study
ClearColi pNV_J23100_NB6 VHH	ClearColi high expression of NB6 VHH ³ display	This study
ClearColi pNV_J23100_NIH112 VHH	ClearColi high expression of NIH112 VHH ⁴ display	This study
ClearColi pNV_J23100_VE VHH	ClearColi high expression of VE VHH ³ display	This study
ClearColi pNV_J23100_NB6 VHH_sfGFP + pRH12X(I-CeuI)	ClearColi: for SimCell production (high expression of VHH display with sfGFP downstream)	This study
ClearColi <i>AminD</i> pNV_J23100_NB6 VHH_sfGFP	ClearColi: for mini-SimCell production (high expression of NB6 VHH display with sfGFP downstream)	This study
ClearColi <i>AminD</i> pNV_J23100_VE VHH_sfGFP	ClearColi: for mini-SimCell production (high expression of VE VHH display with sfGFP downstream)	This study
Plasmids		
Negative control (pNV_J23100)	Plasmid with constitutively promoter BBa_J23100 and an RBS 12K, expressing β -intimin as the outer. CmR.	This study
pNV_J23100 non-binding VHH	Plasmid with constitutively promoter BBa_J23100 and an RBS 12K, expressing β -intimin as the outer membrane with anti-HER2 (VHH) fused to it. CmR.	This study
pNV_J23100 TY1 VHH	Plasmid with constitutively promoter BBa_J23100 and an RBS 12K, expressing β -intimin as the outer membrane with anti-spike RBD nanobody TY1(VHH) fused to it. CmR.	This study

pNV_J23100 NIH112 VHH	Plasmid with constitutively promoter BBa_J23100 and an RBS 12K, expressing β -intimin as the outer membrane with anti-spike RBD nanobody NIH112(VHH) fused to it. CmR.	This study
pNV_J23100_NB6 VHH	Plasmid with constitutively promoter BBa_J23100 and an RBS 12K, expressing β -intimin as the outer membrane with anti-spike RBD nanobody NB6(VHH) fused to it. CmR.	This study
pNV_J23100_VE VHH	Plasmid with constitutively promoter BBa_J23100 and an RBS 12K, expressing β -intimin as the outer membrane with anti-spike RBD bivalent nanobody VE(VHH) fused to it. CmR.	This study
pNV_J23100 non-binding VHH_sfGFP	Plasmid with constitutively promoter BBa_J23100 and an RBS 12K, expressing β -intimin as the outer membrane with anti-HER2 (VHH) fused to it and sfGFP controlled by RBS BBa_B0034. CmR.	This study
pNV_J23100_TY1 VHH_sfGFP	Plasmid with constitutively promoter BBa_J23100 and an RBS 12K, expressing β -intimin as the outer membrane with anti-spike RBD TY1 (VHH) fused to it and sfGFP controlled by RBS BBa_B0034. CmR.	This study
pNV_J23100_NIH112 VHH_sfGFP	Plasmid with constitutively promoter BBa_J23100 and an RBS 12K, expressing β -intimin as the outer membrane with anti-spike RBD NIH112 (VHH) fused to it and sfGFP controlled by RBS BBa_B0034. CmR.	This study
pNV_J23100_NB6 VHH_sfGFP	Plasmid with constitutively promoter BBa_J23100 and an RBS 12K, expressing β -intimin as the outer membrane with anti-spike RBD NB6 (VHH) fused to it and sfGFP controlled by RBS BBa_B0034. CmR.	This study
pNV_J23100_VE VHH_sfGFP	Plasmid with constitutively promoter BBa_J23100 and an RBS 12K, expressing β -intimin as the outer membrane with anti-spike RBD VE (VHH) fused to it and sfGFP controlled by RBS BBa_B0034. CmR.	This study
pRH12x	Plasmid with ICEuI endonuclease controlled by a crystal violet inducible promoter. KanR.	This study

Table S2. Primers and DNA gblocks in this study.

Primers	Sequence (5' -> 3')	Purpose	Plasmids
Nanobody FWD	gtaaggcaaccgtaacgtgaagtcgagtagc	Nanobody VHH sequence into the nanobody display system	pNV_J23100 TY1VHH_sfGFP; pNV_J23100 NIH112
Nanobody REV	tctcctctttTTATGCAGCTGCATCCTC		
Backbone FWD	agctgcataaAAAGAGGAGAAAGGTACC ATG		
Backbone FWD	caacgttacgggtgccttacgtagcatc	pNV_J23100_sfGFP backbone	VHH_sfGFP; pNV_J23100 NB6VHH_sfGFP; pNV_J23100 NB6VHH_sfGFP; pNV_J23100 non-binding VHH_sfGFP
gblocks			
Anti-Spike RBD VHH: TY1	gtaaggcaaccgtaacgtgaagtcgagtagcgcaggac aggtcgtcgtgtctgctaaaaccgcggagatgacttcagc actaatgccagtgcggttatattttgatgggtgcgccggt gccgtatccggatccgctggaaccggcccagccggcc CAGGTGCAGCTCGTGGAGACGGGGG GGAGGCTTGGTGCAGCCTGGGGGG TCTCTGAGACTCTCCTGTGCAGCCT CTGGATTCACCTTCAGTAGCGTCTA CATGAACTGGGTCCGCCAGGCTCC AGGGAAGGGGCCCCGAGTGGGTCTC GCGTATTAGTCCGAATAGTGGTAAT ATTGGGTATACAGACTCCGTGAAG GGCCGATTCACCATCTCCAGAGAC AACGCCAAGAACACACTGTATCTG CAAATGAATAACCTGAAACCTGAG GACACGGCCCTGTATTACTGTGCGA TTGGTTTGAATTTGAGTAGTAGCTC CGTTAGGGGCCAGGGGACCCAGGT CACCGTCTCCTCAgctcgagccgaacaaaaa ctccttcagaaGAGGATGCAGCTGCATA Aaaagaggaga	anti-spike RBD TY1 (VHH) (highlighted in yellow)	
Anti-Spike RBD VHH: NIH112	gtaaggcaaccgtaacgtgaagtcgagtagcgcaggac aggtcgtcgtgtctgctaaaaccgcggagatgacttcagc actaatgccagtgcggttatattttgatgggtgcgccggt gccgtatccggatccgctggaaccggcccagccggcc GACGTACAGTTGCAGGAGAGTGGT GGCGGCCTGGTCCAGCCCCGGCGGT TCTCTCCGGCTGTCATGTGCAGCGA GTGGTCTTACCTTAGATTACTACGC CATTGGCTGGTTTCGTCAAGCGCCT GGAAAAGAACGCGAAGGGGTGTCT TGCATCAGTTCGAGCGATGGAAGC ACATACTATGCTGACTCCGTGAAAG GCCGCTTCACGACTAGCCGTGACA ACGCTAAAAATACGGTTTATCTGCA GATGAACTCCCTGAAACCGGAAGA TACAGCCGTCTATTACTGCGCGGCA	anti-spike RBD NIH112 (VHH) (highlighted in yellow)	

	GTTCCATCGACCTATTACAGCGGGA CTTACTATTATACGTGTCATCCGGG TGGGATGGATTATTGGGGCAAGGG TACCCAAGTGACCGTATCGTCA	
Anti-Spike RBD VHH: NB6	gtaaggcaaccgtaacgttgaagtcgagtagccaggac aggtcgtcgtgtctgctaaaaccgcggagatgacttcagc actaatgccagtgcggttatattttgatgggtgcgccgt gccgtatccggatccgctggaaccggcccagccggcc CAGGTGCAACTTGTGCAATCGGGA GGCGGCTTAGTTCAGGCGGGTGGGA TCTCTCCGCTTGTCTGCGCAGCTA GCGGTTATATCTTTGGGCGAAATGC CATGGGTTGGTATCGTCAGGCTCCA GGGAAGGAGCGCGAACTGGTCGCC GGCATCACGCGGCGTGGCTCAATT ACTTACTACGCGGACAGCGTAAAA GGCCGTTTCACGATTAGTCGCGATA ATGCAAAAAACACAGTATATCTGC AAATGAACTCGCTGAAACCGGAAG ATACCGCCGTGTATTACTGTGCAGC GGACCCGGCGTCCCCTGCGTACGGT GATTATTGGGGCCAGGGTACCCAG GTTACCGTGAGCAGTCATCATCATC ACCACCACgctcgagccgaacaaaactcatctca gaaGAGGATGCAGCTGCATAAaaagag gaga	anti-spike RBD NB6 (VHH) (highlighted in yellow)
Anti-Spike RBD VHH: VE	gtaaggcaaccgtaacgttgaagtcgagtagccaggac aggtcgtcgtgtctgctaaaaccgcggagatgacttcagc actaatgccagtgcggttatattttgatgggtgcgccgt gccgtatccggatccgctggaaccggcccagccggcc CAGGTCCAGCTGGTTGAGACCGGG GGCGGTCTTGTTACGCCAGGCGGTA GCCTGCGCCTGTCTTGCGCCGCGTC AGGGTTTACCTTCTCAAGCTACGCA ATGGGATGGGCCCCGACAAGTACCA GGTAAAGGTCTCGAATGGGTGTCCT ATATTTATTTCGGACGGCAGTACGGA GTATCAAGATAGTGTGAAAGGTCG CTTCACAATCAGCCGTGATAACGCT AAAAGCACCGTGTACCTGCAAATG AACTCCCTGAAACCGGAAGATACT GCAGTTTATTATTGTGCTACCGAAG GGAGTCTGGGAGGGTGGGGCCGGG ATTTTGGTTCGTGGGGACAGGGCAC ACAAGTAACGGTCTCTTCAGGCGG CGGTGGCTCGGGTGGTGGGGGTTT CGGGGGCGGCGGATCACAGGTCCA GTTAGTCGAAACGGGCGGTGGTTTC GTGCAACCCGGAGGCTCGCTGCGT CTTTCTTGCGCGGCGTCGGGCGTTA CGTTAGATTATTACGCTATTGGCTG GTTTCGCCAGGCACCGGGCAAAGA AAGAGAGGGCGTGTCTTGCAATTGG CTCCAGCGACGGCCGTACATACTAT AGTGACAGCGTAAAGGGTTCGTTTT	anti-spike bivalent RBD VE (VHH) (highlighted in yellow)

	ACCATCTCCCGCGACAATGCGAAA AACACCGTTTACTTGCAGATGAATA GCCTAAAGCCGGAAGACACCGCCG TCTATTATTGTGCCTTGACCGTTGG TACTTATTACTCTGGCAATTATCAC TATACTTGTAGTGATGACATGGATT ATTGGGGAAAAGGGACGCAGGTGA CGGTGAGCAGCGGTGGGTACCCTT ACGATGTGCCGGATTACGCGGGTC ATCACCACCATCATCATgctcgagccgaa caaaaactcatctcagaaGAGGATGCAGCTG CATAAaaagaggaga	
Non-binding VHH (anti-Spike RBD)	gtaaggcaaccgtaacgttgaagtcgagtagccaggac aggtcgtcgtgtctgctaaaaccgcgagatgacttcagc actaatgccagtcggttatattttgatggtgcgccggt gccgtatccgatccgctggaaccggcccagccggcc CAGGTGCAGCTGCAGGAAAGCGGT GGTGGTTCAGTGCAAGCAGGAGGC AGCCTGAAACTCACCTGCGCGGCCT CTGGCTACATTTTAACTCGTGTGG AATGGGGTGGTACCGCCAGAGCCC GGGCCGCGAGCGTGAATTAGTCTC GCGTATTTCTGGCGATGGCGATACG TGGCATAAAGAAAGTGTCAAAGGT CGGTTTACAATCTCACAGGATAACG TAAAAAAGACTCTGTATCTTCAAAT GAATAGTCTGAAACCAGAGGACAC CGCTGTTTATTTCTGTGCGGTGTGC TACAATTTGGAAACCTATTGGGGGC AAGGTACTCAGGTAACGGTTTCCTC CgctcgagccgaacaaaaactcatctcagaaGAGG ATGCAGCTGCATAAaaagaggaga	Anti-HER2 nanobody (highlighted in yellow)

Table S3

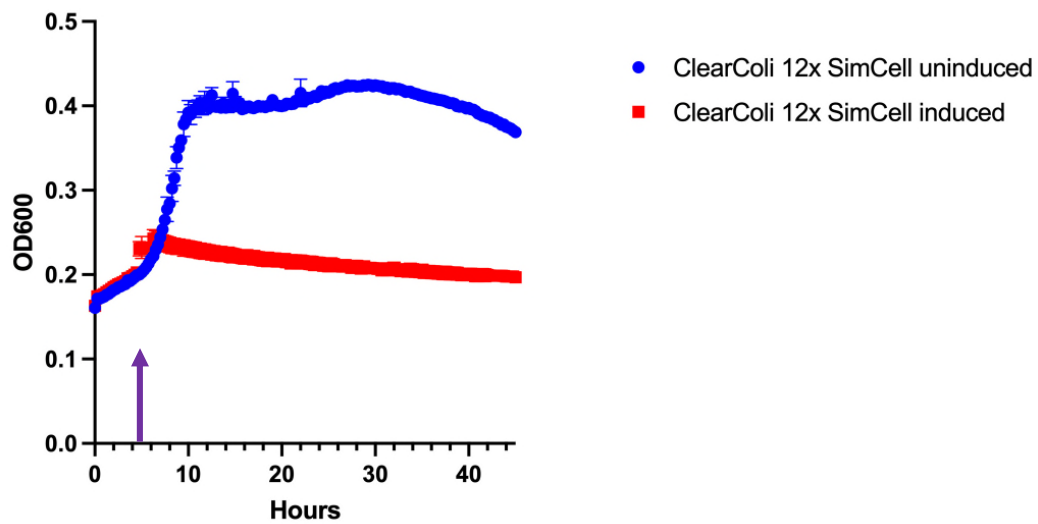
This table presents the half-maximal inhibitory concentration (IC₅₀) values for each experimental group against the Victoria viral variants.

IC ₅₀ (Number of Cells per mL)	
NB6 SimCell	4.78E+08
NB6 mini-SimCell	3.68E+09

Supplementary Figure S1

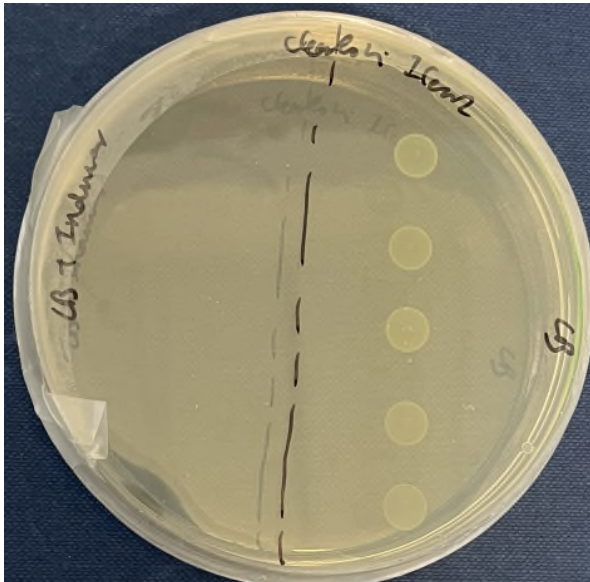
a

SimCell conversion of ClearColi 12x



b

ClearColi 12x + Inducer ClearColi 12x - Inducer



Supplementary Figure S1. (a) OD600 growth curve of the ClearColi strain containing ICEuI, referred to as ClearColi 12x, for SimCell conversion. The purple arrow marks the addition of

an inducer for ICeul expression at the 3-hour time point. The blue line represents the growth curve of the uninduced ICeul, while the red line depicts the growth curve following ICeul induction and ClearColi SimCell conversion. Error bars indicate the standard deviation from three biological replicates. **(b)** 50 μ L of either induced or uninduced ClearColi 12x culture from the growth curve experiment was spotted onto an LB-only agar plate and incubated overnight at 37°C in a static incubator to assess SimCell purity.

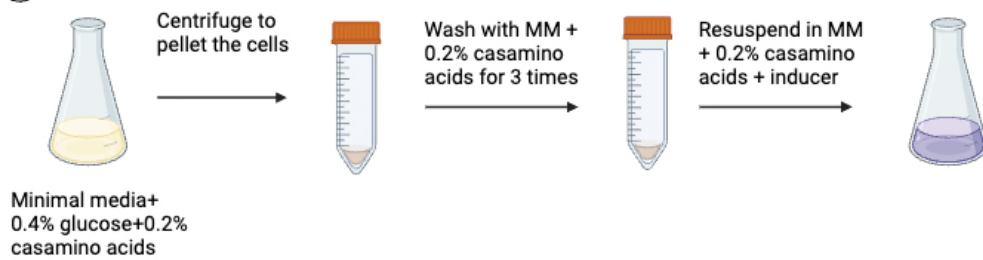
Supplementary Figure S2

a

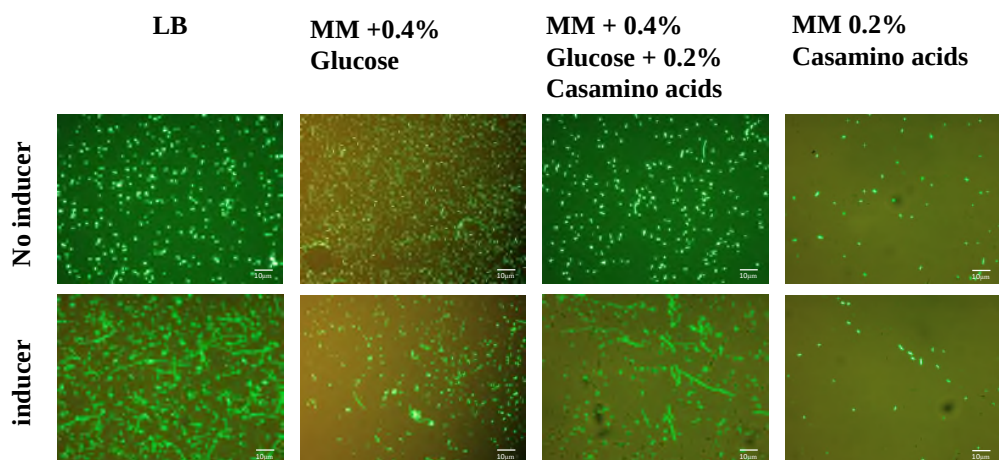
①



②



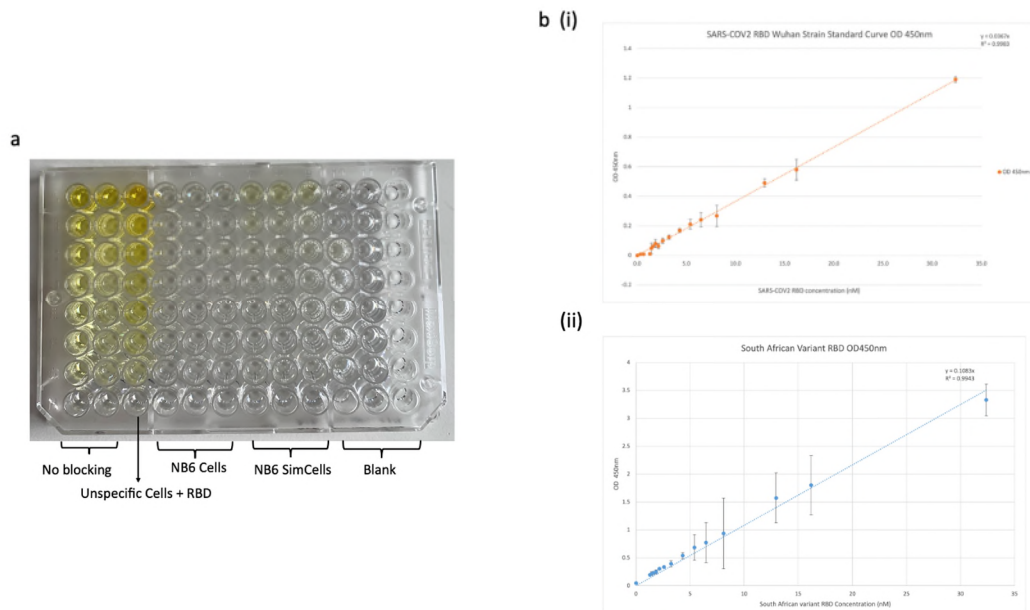
b



Supplementary Figure S2. Purification procedure and characterisation for ClearColi SimCells (a) Schematics of the purification workflow for ClearColi SimCell conversion utilizing either LB media or minimal media exchange to yield SimCells of high purity and uniform sizes. (b) Fluorescence microscopic images of the ClearColi cells before and after

induction into SimCells, using different media types. The magnification is 20x, with a scale bar of 10 μ m presented for reference.

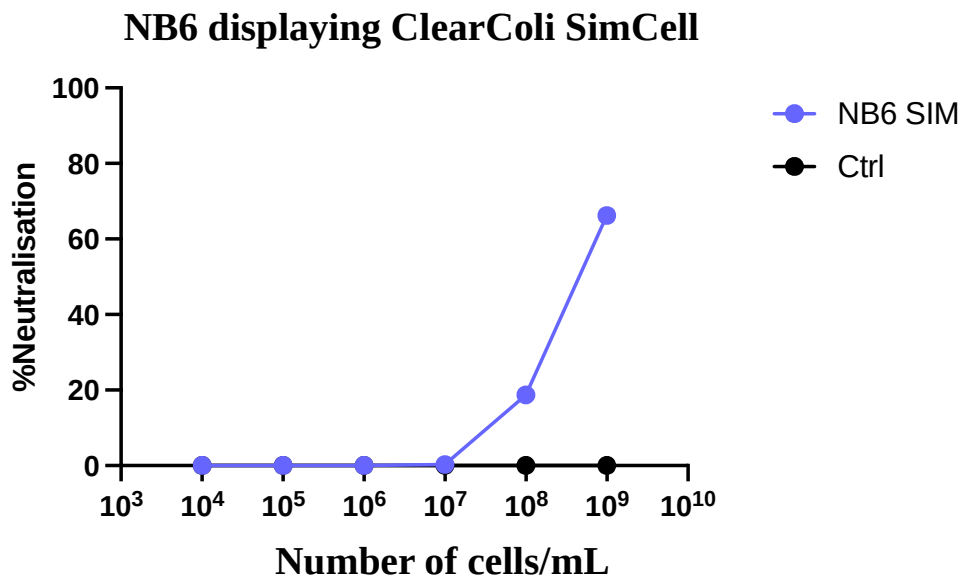
Supplementary Figure S3



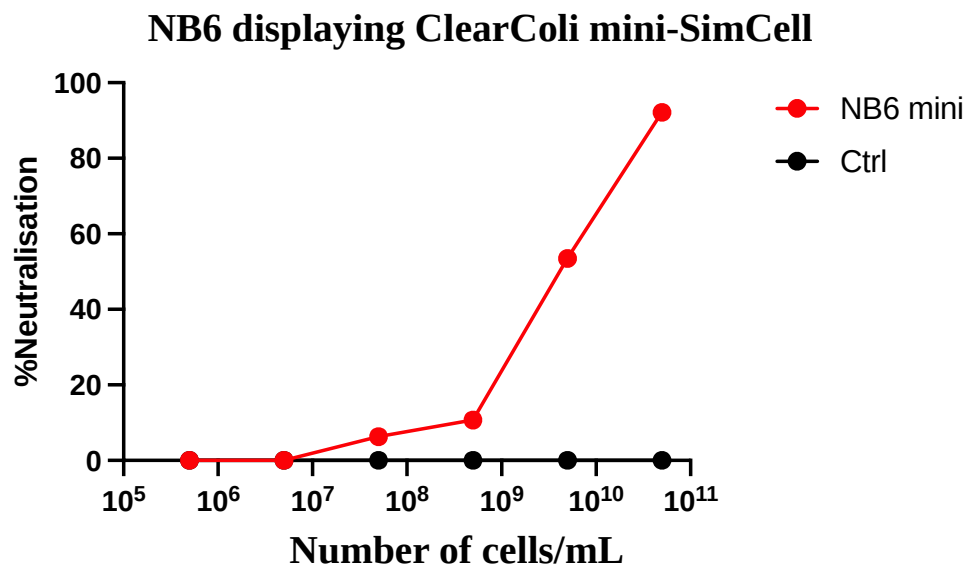
Supplementary Figure S3. (a) Top-down image of pNV_Nb6 sfGFP whole-cell and pNV_Nb6 sfGFP SimCell neutralization assay plate. The neutralization assay was repeated for three biological replicates. **(b) (i)** Calibration standard curve for Wuhan HRP-RBD. The equation of the standard curve is displayed in the top-right corner of the graph: y (OD450nm) = $0.0367 \times$ (HRP-RBD concentration). **(ii)** Calibration standard curve for the South African variant HRP-RBD. The equation of the standard curve is displayed in the top-right corner of the graph: y (OD450nm) = $0.1083 \times$ (HRP-RBD concentration).

Supplementary Figure S4

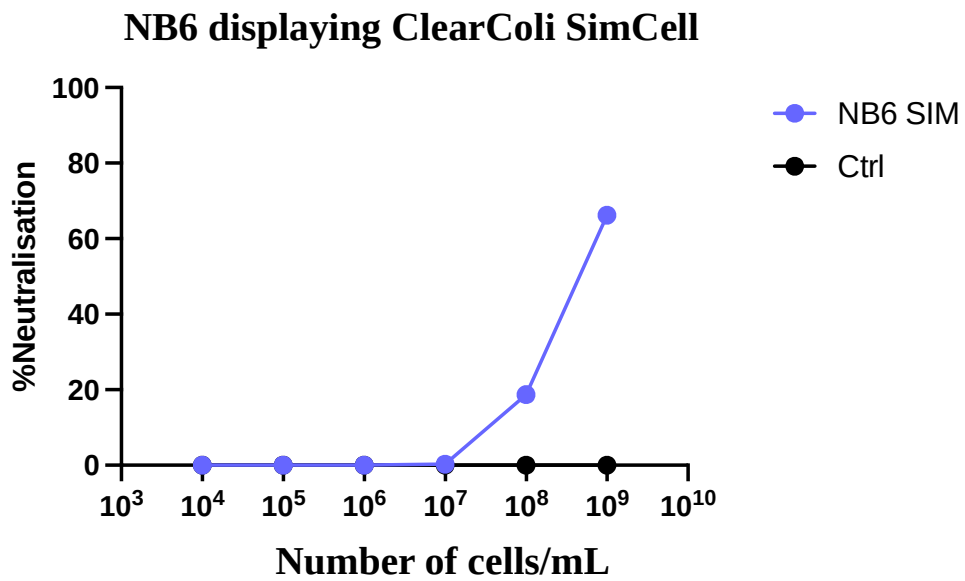
a



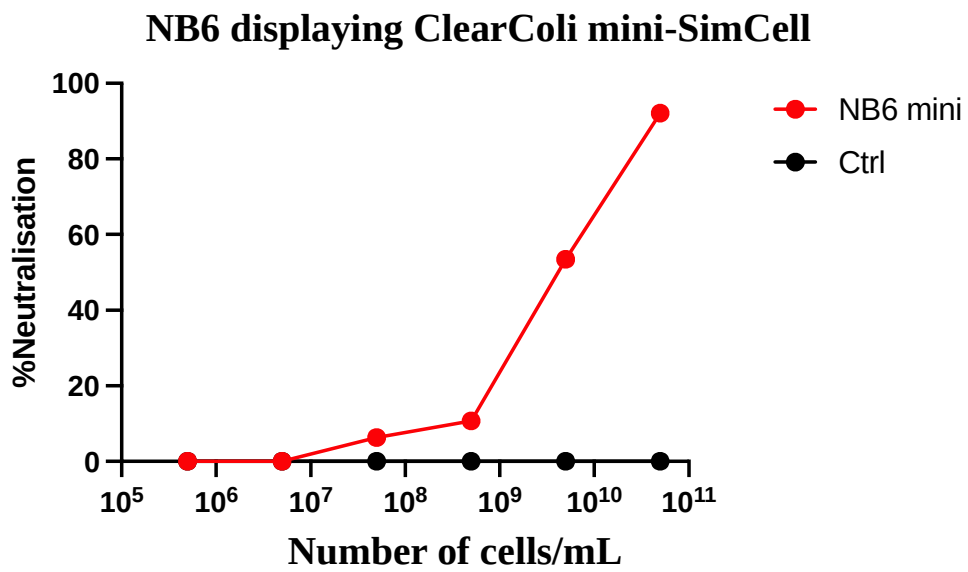
b



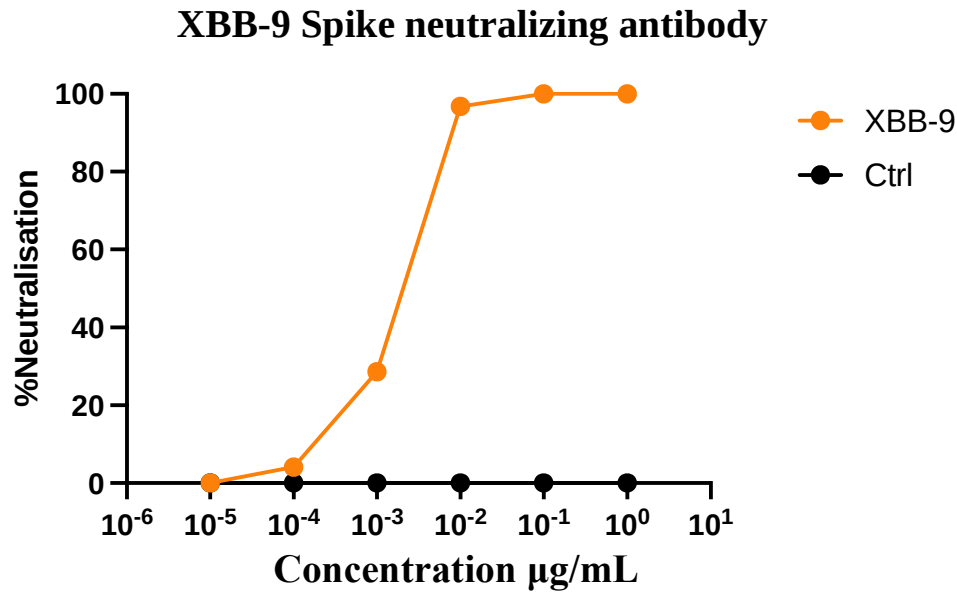
a



b



c



Supplementary Figure S4. Neutralization curves of anti-S RBD nanobody NB6 displaying SimCell and mini-SimCell. This panel illustrates the neutralization curves of **(a)** NB6 anti-Spike RBD monomeric nanobody-expressing ClearColi SimCell and **(b)** mini-SimCells, and with non-binding (anti-HER2) nanobody-expressing SimCell/mini-SimCells serving as a negative control, against the Victoria and B.1.351 (Beta) variants. SimCells were serially diluted from $10^9/\text{mL}$ to $10^4/\text{mL}$. Mini-SimCells underwent a 10-fold serial dilution five times, starting from $5 \times 10^{10}/\text{mL}$ to $5 \times 10^5/\text{mL}$. For the assay, $50 \mu\text{L}$ of SimCell/mini-SimCell samples were mixed with 200 viral foci in $25 \mu\text{L}$ per well. **(c)** neutralization curves of the XBB-9 antibody, known for its neutralization capability against both the Victoria and Beta variants, was used as a positive control.

Reference:

1. Mamat, U. *et al.* Endotoxin-free protein production-ClearColi™ technology. (2013) doi:10.1038/nmeth.f.367.
2. Hanke, L. *et al.* An alpaca nanobody neutralizes SARS-CoV-2 by blocking receptor interaction. *Nat Commun* **11**, 1–9 (2020).
3. Schoof, M. *et al.* An ultrapotent synthetic nanobody neutralizes SARS-CoV-2 by stabilizing inactive Spike. *Science (1979)* **370**, 1473–1479 (2020).
4. Esparza, T. J., Martin, N. P., Anderson, G. P., Goldman, E. R. & Brody, D. L. High affinity nanobodies block SARS-CoV-2 spike receptor binding domain interaction with human angiotensin converting enzyme. *Sci Rep* **10**, 1–13 (2020).