

Spike alignment

SARS_CoV.2	OC43	HKU1	<p> -MFVFLVLLP-----LVSSQCVNLITRITQLPPAYNSFTGCVYYPDKVFRRSSVLHSTQDLFLP--F-SNVTVWEHAIHVSQTN--GTRKNDNPVDFNDGVYFASTEKSNIRIRGWIFGTTLDSDK MFLLISLITAFVIGDLKCTSDNINDKDPPTPTDVTGNTGTYVLDVRYLNTLITFNGYVLLTSGSYRMAKSGVLLSRLEWKPPLFSDFIGAFKAKNTKVKIKDRVMYSEPAI -MLLIIFLITTLAVIGDNTFALNDKNTVPRISEYVGVSYGLQTYILDRVYLTITLITGYFKAGANERDLSLKTGTYLSTLWYQKPFUSDENFGIFSRVNTKLVYVNTKLYLSESTI TQSLILVNNAATNVILKVCFOFCNDPFLGVYHKNNKSMWSEFRVYSSANCTFEYVSQPFIMDLLEGQGNFKNLREFVFKNDIGYFKLYSKHTINLVRDLTPQGSALERLVDPLGINITRF TIGSTFVNITSYVQPRNTNSTQDGN-KLQGLLEVSCQNMCEVRPTICKS-KGSRNKEWHLDTVSCVCKRNFYVDNADYLFHYHQEGGTFYAYTDGVVTKLFNVLGMALSHY VIGSVFINNSYITVQVP-----HNGVLDTACQITMCEPHTICKS-KGSRNKEWHLDTVSCVCKRNFYVDNADYLFHYHQEGGTFYAYTDGVVTKLFNVLGMALSHY QTLALHRSYVLTGSSSGWTAGAAAYVGVYQPRTEFLKYENGTTDAVGLADLPSETKCLTKSFVEKGVYQTSNERVQPTTESIVFPNNITLCPFEVFNATRFASVYAWNRKIRISNOVA YVMP-----LTCN-----SKLTLEWVTPITSROYLLAFKQDGIIFNAVOMSDSEIKCTQSIAPPTGYELNGYVQVADYRKRPNLNCNTI EAWLNOKSVSPSLNWERKTFSCNF YVLP-----LTCNAISSNTDNETLQVWVTLKSRQYLKFNENRGVITNAVDSSESFEIQLCTSKSLPNTQVYDLSGTVKVPATVPAHRIPLDQCDIOKWLNFVNVPSSLNWERKIFSCNF DYSLVYNASFTFKCYGVSPTKLNDLCFFITNVYADSVIRGEVVRQVAPGQTKIADYVNYKLDPTDTGVIAMNNLDSKVG--GNVNYLHFRXSNL NMSSIMSFQADSFICNNIDAAKIFCMCFSTIDKFAIPNGKVKQLQGLNLYLFQNFYRIDDITATSQLYVRLPAANVSRRFPSTWNKRFGTIEDSVFKPRAGVLTTHDNYVQAHCCKAP NLSTILRLVATDSFNENFDESKIYSCFKSILDKFAIPNRSRDLQGLSSVFLQSSVYKIDITSSSKQLYSLPAINVITNNPSSWRRYGENN--FN-----LSSHSVVVSRVYCFSVN </p>
SARS_CoV.2	OC43	HKU1	<p> QTLALHRSYVLTGSSSGWTAGAAAYVGVYQPRTEFLKYENGTTDAVGLADLPSETKCLTKSFVEKGVYQTSNERVQPTTESIVFPNNITLCPFEVFNATRFASVYAWNRKIRISNOVA YVMP-----LTCN-----SKLTLEWVTPITSROYLLAFKQDGIIFNAVOMSDSEIKCTQSIAPPTGYELNGYVQVADYRKRPNLNCNTI EAWLNOKSVSPSLNWERKTFSCNF YVLP-----LTCNAISSNTDNETLQVWVTLKSRQYLKFNENRGVITNAVDSSESFEIQLCTSKSLPNTQVYDLSGTVKVPATVPAHRIPLDQCDIOKWLNFVNVPSSLNWERKIFSCNF DYSLVYNASFTFKCYGVSPTKLNDLCFFITNVYADSVIRGEVVRQVAPGQTKIADYVNYKLDPTDTGVIAMNNLDSKVG--GNVNYLHFRXSNL NMSSIMSFQADSFICNNIDAAKIFCMCFSTIDKFAIPNGKVKQLQGLNLYLFQNFYRIDDITATSQLYVRLPAANVSRRFPSTWNKRFGTIEDSVFKPRAGVLTTHDNYVQAHCCKAP NLSTILRLVATDSFNENFDESKIYSCFKSILDKFAIPNRSRDLQGLSSVFLQSSVYKIDITSSSKQLYSLPAINVITNNPSSWRRYGENN--FN-----LSSHSVVVSRVYCFSVN </p>
SARS_CoV.2	OC43	HKU1	<p> DYSLVYNASFTFKCYGVSPTKLNDLCFFITNVYADSVIRGEVVRQVAPGQTKIADYVNYKLDPTDTGVIAMNNLDSKVG--GNVNYLHFRXSNL NMSSIMSFQADSFICNNIDAAKIFCMCFSTIDKFAIPNGKVKQLQGLNLYLFQNFYRIDDITATSQLYVRLPAANVSRRFPSTWNKRFGTIEDSVFKPRAGVLTTHDNYVQAHCCKAP NLSTILRLVATDSFNENFDESKIYSCFKSILDKFAIPNRSRDLQGLSSVFLQSSVYKIDITSSSKQLYSLPAINVITNNPSSWRRYGENN--FN-----LSSHSVVVSRVYCFSVN </p>
SARS_CoV.2	OC43	HKU1	<p> DYSLVYNASFTFKCYGVSPTKLNDLCFFITNVYADSVIRGEVVRQVAPGQTKIADYVNYKLDPTDTGVIAMNNLDSKVG--GNVNYLHFRXSNL NMSSIMSFQADSFICNNIDAAKIFCMCFSTIDKFAIPNGKVKQLQGLNLYLFQNFYRIDDITATSQLYVRLPAANVSRRFPSTWNKRFGTIEDSVFKPRAGVLTTHDNYVQAHCCKAP NLSTILRLVATDSFNENFDESKIYSCFKSILDKFAIPNRSRDLQGLSSVFLQSSVYKIDITSSSKQLYSLPAINVITNNPSSWRRYGENN--FN-----LSSHSVVVSRVYCFSVN </p>
SARS_CoV.2	OC43	HKU1	<p> DYSLVYNASFTFKCYGVSPTKLNDLCFFITNVYADSVIRGEVVRQVAPGQTKIADYVNYKLDPTDTGVIAMNNLDSKVG--GNVNYLHFRXSNL NMSSIMSFQADSFICNNIDAAKIFCMCFSTIDKFAIPNGKVKQLQGLNLYLFQNFYRIDDITATSQLYVRLPAANVSRRFPSTWNKRFGTIEDSVFKPRAGVLTTHDNYVQAHCCKAP NLSTILRLVATDSFNENFDESKIYSCFKSILDKFAIPNRSRDLQGLSSVFLQSSVYKIDITSSSKQLYSLPAINVITNNPSSWRRYGENN--FN-----LSSHSVVVSRVYCFSVN </p>
SARS_CoV.2	OC43	HKU1	<p> DYSLVYNASFTFKCYGVSPTKLNDLCFFITNVYADSVIRGEVVRQVAPGQTKIADYVNYKLDPTDTGVIAMNNLDSKVG--GNVNYLHFRXSNL NMSSIMSFQADSFICNNIDAAKIFCMCFSTIDKFAIPNGKVKQLQGLNLYLFQNFYRIDDITATSQLYVRLPAANVSRRFPSTWNKRFGTIEDSVFKPRAGVLTTHDNYVQAHCCKAP NLSTILRLVATDSFNENFDESKIYSCFKSILDKFAIPNRSRDLQGLSSVFLQSSVYKIDITSSSKQLYSLPAINVITNNPSSWRRYGENN--FN-----LSSHSVVVSRVYCFSVN </p>
SARS_CoV.2	OC43	HKU1	<p> DYSLVYNASFTFKCYGVSPTKLNDLCFFITNVYADSVIRGEVVRQVAPGQTKIADYVNYKLDPTDTGVIAMNNLDSKVG--GNVNYLHFRXSNL NMSSIMSFQADSFICNNIDAAKIFCMCFSTIDKFAIPNGKVKQLQGLNLYLFQNFYRIDDITATSQLYVRLPAANVSRRFPSTWNKRFGTIEDSVFKPRAGVLTTHDNYVQAHCCKAP NLSTILRLVATDSFNENFDESKIYSCFKSILDKFAIPNRSRDLQGLSSVFLQSSVYKIDITSSSKQLYSLPAINVITNNPSSWRRYGENN--FN-----LSSHSVVVSRVYCFSVN </p>
SARS_CoV.2	OC43	HKU1	<p> DYSLVYNASFTFKCYGVSPTKLNDLCFFITNVYADSVIRGEVVRQVAPGQTKIADYVNYKLDPTDTGVIAMNNLDSKVG--GNVNYLHFRXSNL NMSSIMSFQADSFICNNIDAAKIFCMCFSTIDKFAIPNGKVKQLQGLNLYLFQNFYRIDDITATSQLYVRLPAANVSRRFPSTWNKRFGTIEDSVFKPRAGVLTTHDNYVQAHCCKAP NLSTILRLVATDSFNENFDESKIYSCFKSILDKFAIPNRSRDLQGLSSVFLQSSVYKIDITSSSKQLYSLPAINVITNNPSSWRRYGENN--FN-----LSSHSVVVSRVYCFSVN </p>
SARS_CoV.2	OC43	HKU1	<p> DYSLVYNASFTFKCYGVSPTKLNDLCFFITNVYADSVIRGEVVRQVAPGQTKIADYVNYKLDPTDTGVIAMNNLDSKVG--GNVNYLHFRXSNL NMSSIMSFQADSFICNNIDAAKIFCMCFSTIDKFAIPNGKVKQLQGLNLYLFQNFYRIDDITATSQLYVRLPAANVSRRFPSTWNKRFGTIEDSVFKPRAGVLTTHDNYVQAHCCKAP NLSTILRLVATDSFNENFDESKIYSCFKSILDKFAIPNRSRDLQGLSSVFLQSSVYKIDITSSSKQLYSLPAINVITNNPSSWRRYGENN--FN-----LSSHSVVVSRVYCFSVN </p>
SARS_CoV.2	OC43	HKU1	<p> DYSLVYNASFTFKCYGVSPTKLNDLCFFITNVYADSVIRGEVVRQVAPGQTKIADYVNYKLDPTDTGVIAMNNLDSKVG--GNVNYLHFRXSNL NMSSIMSFQADSFICNNIDAAKIFCMCFSTIDKFAIPNGKVKQLQGLNLYLFQNFYRIDDITATSQLYVRLPAANVSRRFPSTWNKRFGTIEDSVFKPRAGVLTTHDNYVQAHCCKAP NLSTILRLVATDSFNENFDESKIYSCFKSILDKFAIPNRSRDLQGLSSVFLQSSVYKIDITSSSKQLYSLPAINVITNNPSSWRRYGENN--FN-----LSSHSVVVSRVYCFSVN </p>
SARS_CoV.2	OC43	HKU1	<p> DYSLVYNASFTFKCYGVSPTKLNDLCFFITNVYADSVIRGEVVRQVAPGQTKIADYVNYKLDPTDTGVIAMNNLDSKVG--GNVNYLHFRXSNL NMSSIMSFQADSFICNNIDAAKIFCMCFSTIDKFAIPNGKVKQLQGLNLYLFQNFYRIDDITATSQLYVRLPAANVSRRFPSTWNKRFGTIEDSVFKPRAGVLTTHDNYVQAHCCKAP NLSTILRLVATDSFNENFDESKIYSCFKSILDKFAIPNRSRDLQGLSSVFLQSSVYKIDITSSSKQLYSLPAINVITNNPSSWRRYGENN--FN-----LSSHSVVVSRVYCFSVN </p>
SARS_CoV.2	OC43	HKU1	<p> DYSLVYNASFTFKCYGVSPTKLNDLCFFITNVYADSVIRGEVVRQVAPGQTKIADYVNYKLDPTDTGVIAMNNLDSKVG--GNVNYLHFRXSNL NMSSIMSFQADSFICNNIDAAKIFCMCFSTIDKFAIPNGKVKQLQGLNLYLFQNFYRIDDITATSQLYVRLPAANVSRRFPSTWNKRFGTIEDSVFKPRAGVLTTHDNYVQAHCCKAP NLSTILRLVATDSFNENFDESKIYSCFKSILDKFAIPNRSRDLQGLSSVFLQSSVYKIDITSSSKQLYSLPAINVITNNPSSWRRYGENN--FN-----LSSHSVVVSRVYCFSVN </p>
SARS_CoV.2	OC43	HKU1	<p> DYSLVYNASFTFKCYGVSPTKLNDLCFFITNVYADSVIRGEVVRQVAPGQTKIADYVNYKLDPTDTGVIAMNNLDSKVG--GNVNYLHFRXSNL NMSSIMSFQADSFICNNIDAAKIFCMCFSTIDKFAIPNGKVKQLQGLNLYLFQNFYRIDDITATSQLYVRLPAANVSRRFPSTWNKRFGTIEDSVFKPRAGVLTTHDNYVQAHCCKAP NLSTILRLVATDSFNENFDESKIYSCFKSILDKFAIPNRSRDLQGLSSVFLQSSVYKIDITSSSKQLYSLPAINVITNNPSSWRRYGENN--FN-----LSSHSVVVSRVYCFSVN </p>
SARS_CoV.2	OC43	HKU1	<p> DYSLVYNASFTFKCYGVSPTKLNDLCFFITNVYADSVIRGEVVRQV</p>

Membrane alignment

SARS_CoV_2TVEELKKLLEQWNLVIGFLFLTLTWICLLLQQAIVANRNRFLVITIKLTFEWLWPVTTLACQVLAAYVRIHWITGGIAITAMACLVGMWLSYFIASFRFLFARTSMWSFNPETNILL
OC43	MSKTTAPAPVIVITADEAIAKFLKEWNFSLGIIILFTIILQFGYTSKSMFVAVIKMIILWLWMLPTIITLTFNCVYALNNVVLGSLISIVFTIVAHIMWIVFVNSIRLFIKRTGSAFWSFNPETNML
HKU1	MNKSFFPQ----FTSQAVTFUKENWFLGVIIILFTIILQFGYTSKSMFVEIKMIILWLWMLPTIITLTFNCFYALNNAFLAFSIVFTIISIVWILYEVNSIRLFIKRTGSAFWSFNPETNML
SARS_CoV_2
SARS_CoV_2	NVPLHGTILTRALLESELVIGAVILRGHRIAGHHLEGR-CDIKDLPKETIVATSRITLSYKLGASQRVAGDSGFAASRYRIRGNKLNTHDSSSSSDNIALLVQ--
OC43	CIDMKGTMYVRRIIEDYHTLTVTIIRGHVYIQGIKLGTYGWADLPAMTMVAKVTHLCITYKRGFLDRIISDTSGFAVYKSKVGYRLLPSTQKSGMDTALLRNNI
HKU1	CIDMKGKMFVRVRIEDYHTLTATVIRGHVYIQGVKLGTYTILSLDLPVAVTVAKVQVLCITYKRAFLDKLDVNSGFAFVYKSKVGYRLLPSS-KPSGMDTALLRA--

Nucleocapsid alignment

OC43	MSFTPKSSSRASSGNSGNIGLK--WADQSQFRNQTRGRAQPKQTATSQPSGQNVVPHYSWFSGITQFKGKELEFAEGGVPIAPGVPTAEKGYWYRNRSRFATDGQRQLLPR
HKU1	MSYTPGHVAGSRSSGNSG--ILKKTSWADQSERNYQTFNRGRKTPKPTVSTDPQ--ENTIPHYSWFSGITQFKGGRDKFSDQGVPIAFGIPSEAKGYWYRNRSRSEKATDGQKQLLPR
SARS_CoV_2	-MSDNGPQNRNPRITFGP-----DSTGSGNQNGERSKQRRPQGLPNN-----TASWFTALTQHCQ-EDLKFPREGQVPIINTSSDDQIGYRRATRR-IRGGDGKMKDLSPR
OC43	WYFYVLGTGPHAKDQYGTDINGVYHVASNQADVNPADIVDRDSSDEATPTRFPPGTVLPQGYVEGSGRSAPIRSRTPT-SRASSACSRRSANGNPTSGVTPDMADQIASLVLAELG
HKU1	WYFYVLGTGPYANASYGESLEGVFWANHQADTSPSDVSSRDPITTEQAIPTRFPGTILLPGYVEGS-GRSASMSRPPGRS-QRSGPNKSLSRNSNFHSDSIVKPDMADEIANLVLAELG
SARS_CoV_2	WYFYVLGTGPEAGLPYGANKDGIIVVATEGALNTPKDHITGTRNANNAAIVLQLMQGTTLPKGFVAEGSRGSGASRSRSRNSRSTPGSSRTSPAMAGNGGDAIALLLDLRLNQLES
OC43	KDATKPPQVTKHFAKEVQKILNK-PRQXRSNNKQCTVQQCFGKRGPNQ--NFGGEMLKLGTSDPQFPILAEAPTAGAFFFGSKLELAKVQNLGSGNPDEPKQDVYELRYNGAIRFDSTLSGF
HKU1	KDS-KPQQVTKQMAKEIKHILTK-PRXRTPNMHCNVQQCFGKRGPSQ--NFGNAEMLKLGTDNPQFPILAEAPTGPAGFFGSKLELVRE---SEADSPVKDVFELRYNGSIRFDSTLPGF
SARS_CoV_2	KMSGKQQQQQQGVTKKSAAEASKPRQKRTATAYNVITQAFGRGPEQTQGNFQDQELIRQGTDYKHWPPQIAQFAPSASAFFGMRIGMEVTP-----SGTWLTTCAIKLDDKDNF
OC43	ETIMKVLSENINAYQ--QQGMMNMSPKPQRGRGHKGGQGENDNISVAVPKSRVQQNKSIELTAEDI SLKKMDEPFTEDTSEI
HKU1	ETIMKVLKENLDAYNSNQNTVSGLSLPKQKRKGKQSPPELFDLSNLSADT---QHISNFTPEDHSLLATLDDPYVEDSVA-
SARS_CoV_2	KDQVILLNKHIDAYKTFPTTEPKDKKKKADETALPQKQKQTVTLLPAA-----DLDDFSQQLQQSMSSADSTQA---

Figure S1: Alignment of peptide sequences from spike, nucleocapsid and membrane proteins shows areas of overlap for each protein. Alignments performed using clustal omega and visualised by UGENE.

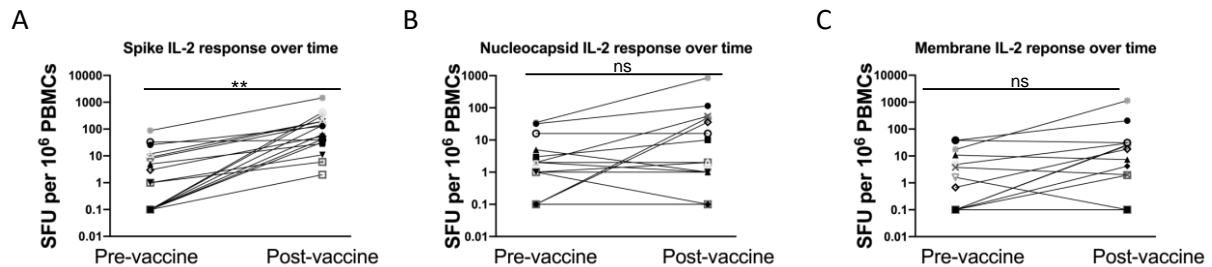


Figure S2: Vaccination increases IL-2 responses to Spike but not nucleocapsid or membrane peptides. PBMCs were isolated from 17 donors with diagnosed long COVID pre- and post-vaccination. These PBMCs were stimulated with spike, nucleocapsid or membrane peptides and IL-2 responses were measured by fluorospot assay as spot forming units per million PBMCs. Each condition was run in duplicate and an unstimulated control was subtracted to remove background cytokine production. $P < 0.01$ and ns = not significant by Wilcoxon Signed-Rank Test.

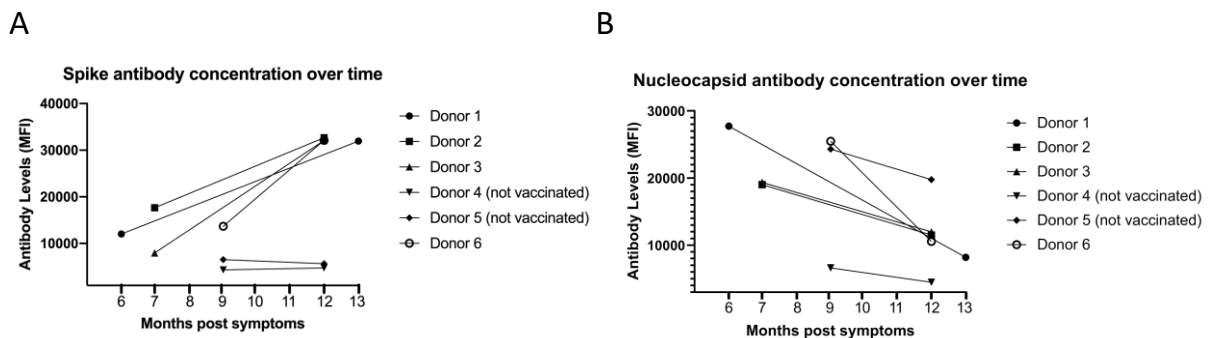


Figure S3: Spike antibodies are confounded by vaccination while nucleocapsid antibodies wane quickly. Antibody levels were quantified from 6 donors before and after vaccination using multiplex particle-based flow cytometry for A) spike (not significant by Wilcoxon Signed-Rank) or B) nucleocapsid antibodies ($p < 0.05$ by Wilcoxon Signed-Rank).