Supplementary data

Nanoparticular CpG-adjuvanted SARS-CoV-2 S1 protein elicits broadly neutralizing and Th1-biased immunoreactivity in mice

Characterization of TMC and FUC-TMC NPs

To determine the degree of quaternization of the TMC polymers, ^{1}H nuclear magnetic resonance (NMR) spectra with an NMR spectrometer (AV-500, Bruker, Switzerland) was obtained by dissolving samples of the polymers in deuterium oxide at 80°C with suppression of the water peak.

The degree of quaternization was calculated with ^{1}H-NMR data according to the following equation.

$$\%DQ = ([N\text{-}(CH_3)_3]/[COCH_3]) \times 1/3 \times (1-\%DD)$$

where %DQ is the degree of quaternization as a percentage, %DD is the degree of deacetylation, $[N\text{-}(CH_3)_3]$ is the integral of the singlet peak at 3.2 ppm from the methy proton (9H) of the trimethylammonium chloride, and $[COCH_3]$ is the integral of the singlet peak at 2.0 ppm from the acetyl group of the N-acetyl-glucosamine residue. The %DQ obtained from ^{1}H-NMR spectra of TMC was 52%, (as shown in **Fig. S1A**).

FTIR spectrum of FUC (**Fig. S1B**) contained characteristic peaks at 3468 cm^{-1} (O–H stretch), 2943 cm^{-1} (C–H stretch), 1259 cm^{-1} and 1232 cm^{-1} (doublet, asymmetric stretch of the SO_2 group of secondary alkyl sulfate salt), 1051 cm^{-1} (symmetric stretch of the SO_2 group), 1026 cm^{-1} (C–O stretch), and 843 cm^{-1} (asymmetric stretch of the S–O–C). The presence of glucuronic acid in the side chain of FUC yielded a characteristic band at 1638 cm^{-1} (asymmetric stretch of CO_2–). In the (+)- and (–)-FUC-TMC NPs, the 1259 cm^{-1} peak of asymmetric SO_2 stretch shifted to 1256 and 1255 cm^{-1}, respectively, and the 1051 cm^{-1} peak of symmetric SO_2 stretch shifted to 1054 cm^{-1}. These differences indicated that an intermolecular PEC occurred between the quaternary ammonium group of TMC and the sulfate groups of FUC.

(A)

(B)

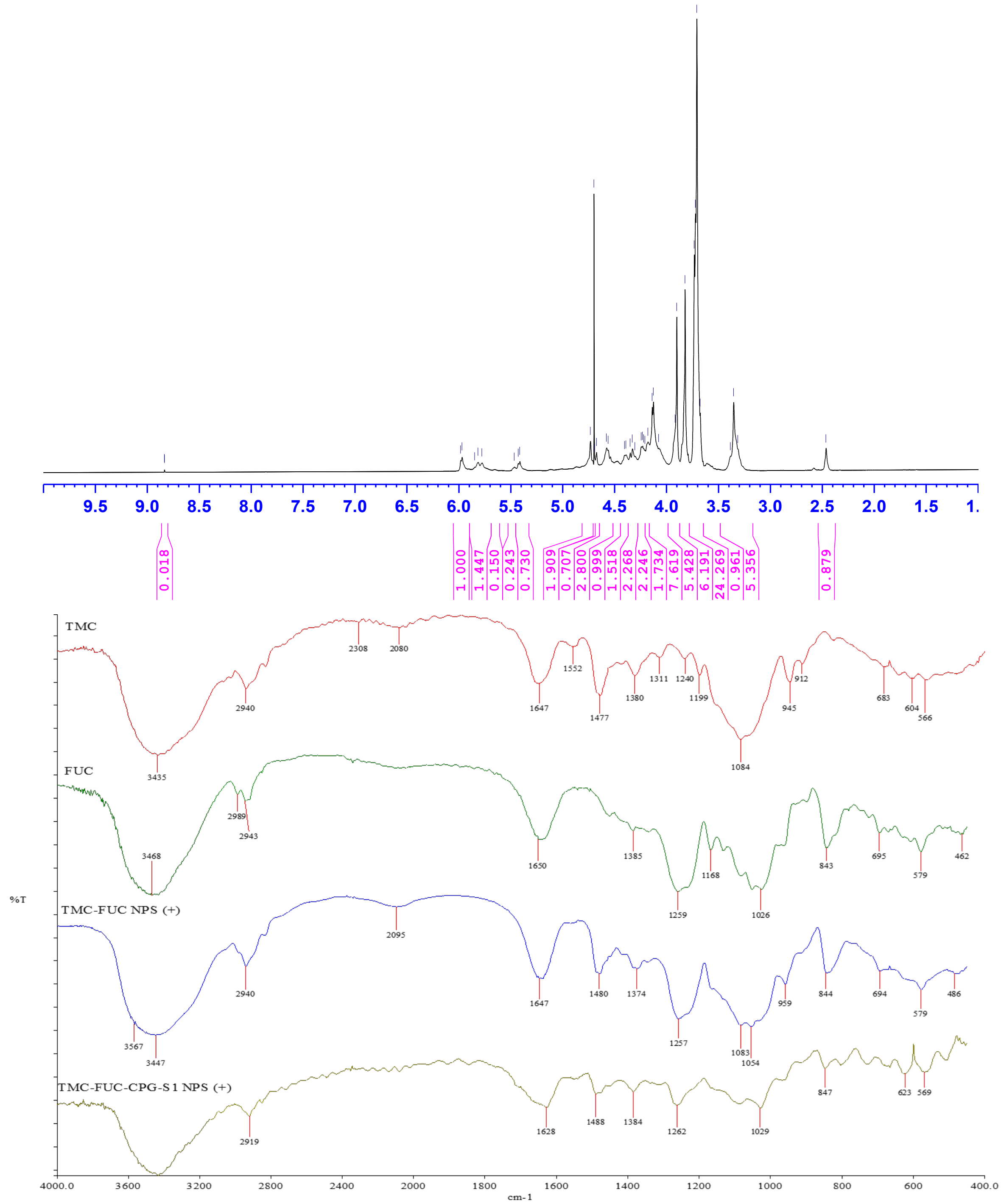

Fig. S1. (A) NMR spectra of TMC. (B) FTIR spectra of TMC, FUC, FUC-TMC NPs and rS1/FUC-TMC NPs/CpG.

Table S1. The particle size and zeta potential of FUC-TMC NPs, FUC-TMC NPs + rS1, FUC-TMC NPs + CpG and FUC-TMC NPs + rS1 + CpG used in immunization.

Nanoparticles	Mass ratio FUC/TMC	Size* nm	PDI*	ζ** mV
FUC-TMC NPs	0.6	357.4	0.435	30.7
FUC-TMC NPs + rS1	0.6	246.1	0.232	15.6
FUC-TMC NPs + CpG	0.6	288.5	0.244	16.6
FUC-TMC NPs + rS1+ CpG	0.6	394.0	0.343	14.1

* Measured by DLS method

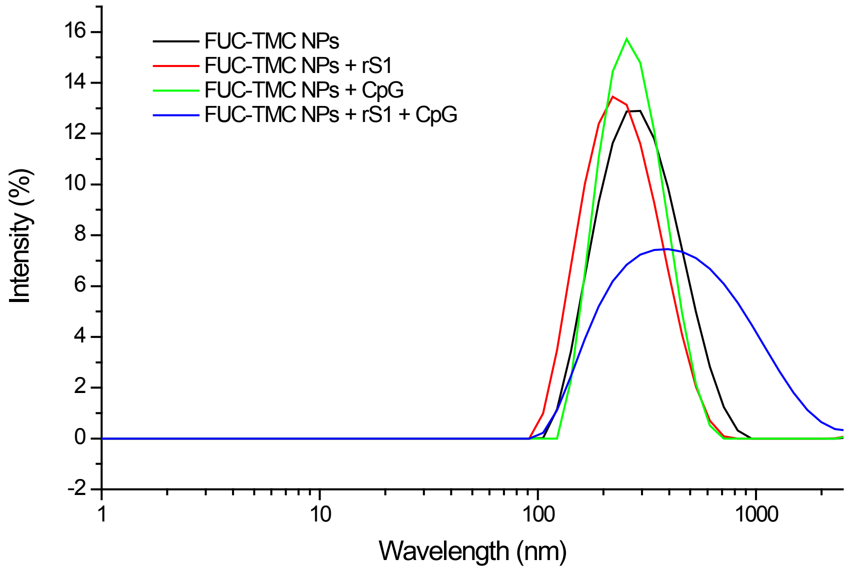

Fig. S2. Intensity size distribution of FUC-TMC NPs, FUC-TMC NPs+rS1, FUC-TMC NPs+CpG, and FUC-TMC NPs+rS1+CpG obtained by DLS.

Table S2. The cell viability, cytotoxicity and cytokines of FUC, TMC, (+)-FUC-TMC NPs and (–)-FUC-TMC NPs at the concentration (or TPC) of 200 μg/mL

Cell or cytokines	FUC*	TMC*	(+)-FUC-TMC NPs**	(–)-FUC-TMC NPs**
Cell viability		MTT assay		
L929	103.5±1.3%	75.8±1.0%	99.4±2.9%	98.8±2.4%
A549	101.9±3.0%	65.5±1.3%	101.1±3.9%	94.2±8.6%
JAW II DCs	89.4±4.6%	46.0±5.6%	92.4±4.5%	84.3±3.4%
Cytotoxicity		LDH release assay		
L929	-8.7±0.3%	27.0±2.2%	-3.9±1.6%	-7.8±0.5%
A549	-32.0±2.4%	25.5±2.5%	-2.2±0.9%	-3.6±1.0%
JAW II DCs	3.0±0.7%	27.2±0.5%	1.2±0.6%	2.7±1.1%
Cytokines		Relative MFI value		
IFN-γ	5.75±0.25	8.64±0.29	3.32±0.14	1.00±0.03
IL-4	4.83±0.11	3.28±0.12	2.86±0.15	1.24±0.01
IL-12p40	6.47±0.31	11.77±0.34	5.18±0.26	1.26±0.03
IL-10	4.30±0.22	4.78±0.14	0.84±0.03	1.10±0.08

* Concentration

** Total polysaccharide concentration (TPC)

Table S3. Peptides included in the SARS-CoV-2 S1 scanning pool

Peptide	Sequence	Peptide	Sequence	Peptide	Sequence
1	SQCVNLTTRTQLPPA	57	RFQTLLALHRSYLTP	113	LKPFERDISTEIYQA
2	NLTTRTQLPPAYTNS	58	LLALHRSYLTPGDSS	114	ERDISTEIYQAGSTP
3	RTQLPPAYTNSFTRG	59	HRSYLTPGDSSSGWT	115	STEIYQAGSTPCNGV
4	PPAYTNSFTRGVYYP	60	LTPGDSSSGWTAGAA	116	YQAGSTPCNGVEGFN
5	TNSFTRGVYYPDKVF	61	DSSSGWTAGAAAYYV	117	STPCNGVEGFNCYFP
6	TRGVYYPDKVFRSSV	62	GWTAGAAAYYVGYLQ	118	NGVEGFNCYFPLQSY
7	YYPDKVFRSSVLHST	63	GAAAYYVGYLQPRTF	119	GFNCYFPLQSYGFQP
8	KVFRSSVLHSTQDLF	64	YYVGYLQPRTFLLKY	120	YFPLQSYGFQPTNGV
9	SSVLHSTQDLFLPFF	65	YLQPRTFLLKYNENG	121	QSYGFQPTNGVGYQP
10	HSTQDLFLPFFSNVT	66	RTFLLKYNENGTITD	122	FQPTNGVGYQPYRVV
11	DLFLPFFSNVTWFHA	67	LKYNENGTITDAVDC	123	NGVGYQPYRVVVLSF
12	PFFSNVTWFHAIHVS	68	ENGTITDAVDCALDP	124	YQPYRVVVLSFELLH
13	NVTWFHAIHVSGTNG	69	ITDAVDCALDPLSET	125	RVVVLSFELLHAPAT
14	FHAIHVSGTNGTKRF	70	VDCALDPLSETKCTL	126	LSFELLHAPATVCGP
15	HVSGTNGTKRFDNPV	71	LDPLSETKCTLKSFT	127	LLHAPATVCGPKKST
16	TNGTKRFDNPVLPFN	72	SETKCTLKSFTVEKG	128	PATVCGPKKSTNLVK
17	KRFDNPVLPFNDGVY	73	CTLKSFTVEKGIYQT	129	CGPKKSTNLVKNKCV
18	NPVLPFNDGVYFAST	74	SFTVEKGIYQTSNFR	130	KSTNLVKNKCVNFNF
19	PFNDGVYFASTEKSN	75	EKGIYQTSNFRVQPT	131	LVKNKCVNFNFNGLT
20	GVYFASTEKSNIIRG	76	YQTSNFRVQPTESIV	132	KCVNFNFNGLTGTGV
21	ASTEKSNIIRGWIFG	77	NFRVQPTESIVRFPN	133	FNFNGLTGTGVLTES
22	KSNIIRGWIFGTTLD	78	QPTESIVRFPNITNL	134	GLTGTGVLTESNKKF
23	IRGWIFGTTLDSKTQ	79	SIVRFPNITNLCPFG	135	TGVLTESNKKFLPFQ
24	IFGTTLDSKTQSLLI	80	FPNITNLCPFGEVFN	136	TESNKKFLPFQQFGR
25	TLDSKTQSLLIVNNA	81	TNLCPFGEVFNATRF	137	KKFLPFQQFGRDIAD
26	KTQSLLIVNNATNVV	82	PFGEVFNATRFASVY	138	PFQQFGRDIADTTDA
27	LLIVNNATNVVIKVC	83	VFNATRFASVYAWNR	139	FGRDIADTTDAVRDP
28	NNATNVVIKVCEFQF	84	TRFASVYAWNRKRIS	140	IADTTDAVRDPQTLE
29	NVVIKVCEFQFCNDP	85	SVYAWNRKRISNCVA	141	TDAVRDPQTLEILDI
30	KVCEFQFCNDPFLGV	86	WNRKRISNCVADYSV	142	RDPQTLEILDITPCS
31	FQFCNDPFLGVYYHK	87	RISNCVADYSVLYNS	143	TLEILDITPCSFGGV
32	NDPFLGVYYHKNNKS	88	CVADYSVLYNSASFS	144	LDITPCSFGGVSVIT
33	LGVYYHKNNKSWMES	89	YSVLYNSASFSTFKC	145	PCSFGGVSVITPGTN
34	YHKNNKSWMESEFRV	90	YNSASFSTFKCYGVS	146	GGVSVITPGTNTSNQ
35	NKSWMESEFRVYSSA	91	SFSTFKCYGVSPTKL	147	VITPGTNTSNQVAVL
36	MESEFRVYSSANNCT	92	FKCYGVSPTKLNDLC	148	GTNTSNQVAVLYQDV
37	FRVYSSANNCTFEYV	93	GVSPTKLNDLCFTNV	149	SNQVAVLYQDVNCTE
38	SSANNCTFEYVSQPF	94	TKLNDLCFTNVYADS	150	AVLYQDVNCTEVPVA
39	NCTFEYVSQPFLMDL	95	DLCFTNVYADSFVIR	151	QDVNCTEVPVAIHAD
40	EYVSQPFLMDLEGKQ	96	TNVYADSFVIRGDEV	152	CTEVPVAIHADQLTP
41	QPFLMDLEGKQGNFK	97	ADSFVIRGDEVRQIA	153	PVAIHADQLTPTWRV
42	MDLEGKQGNFKNLRE	98	VIRGDEVRQIAPGQT	154	HADQLTPTWRVYSTG
43	GKQGNFKNLREFVFK	99	DEVRQIAPGQTGKIA	155	LTPTWRVYSTGSNVF
44	NFKNLREFVFKNIDG	100	QIAPGQTGKIADYNY	156	WRVYSTGSNVFQTRA
45	LREFVFKNIDGYFKI	101	GQTGKIADYNYKLPD	157	STGSNVFQTRAGCLI
46	VFKNIDGYFKIYSKH	102	KIADYNYKLPDDFTG	158	NVFQTRAGCLIGAEH
47	IDGYFKIYSKHTPIN	103	YNYKLPDDFTGCVIA	159	TRAGCLIGAEHVNNS
48	FKIYSKHTPINLVRD	104	LPDDFTGCVIAWNSN	160	CLIGAEHVNNSYECD
49	SKHTPINLVRDLPQG	105	FTGCVIAWNSNNLDS	161	AEHVNNSYECDIPIG
50	PINLVRDLPQGFSAL	106	VIAWNSNNLDSKVGG	162	NNSYECDIPIGAGIC
51	VRDLPQGFSALEPLV	107	NSNNLDSKVGGNYNY	163	ECDIPIGAGICASYQ
52	PQGFSALEPLVDLPI	108	LDSKVGGNYNYLYRL	164	PIGAGICASYQTQTN
53	SALEPLVDLPIGINI	109	VGGNYNYLYRLFRKS	165	GICASYQTQTNSPRR
54	PLVDLPIGINITRFQ	110	YNYLYRLFRKSNLKP	166	SYQTQTNSPRRAR
55	LPIGINITRFQTLLA	111	YRLFRKSNLKPFERD		
56	INITRFQTLLALHRS	112	RKSNLKPFERDISTE		