Supplemental information

Controllable self-replicating RNA vaccine

delivered intradermally elicits

predominantly cellular immunity

Tomokazu Amano, Hong Yu, Misa Amano, Erica Leyder, Maria Badiola, Priyanka Ray, Jiyoung Kim, Akihiro C. Ko, Achouak Achour, Nan-ping Weng, Efrat Kochba, Yotam Levin, and Minoru S.H. Ko

Supplemental Data

FIG. S1. In vitro expression of c-srRNA1-GFP at 33°C and at 37°C, Related to Figure 1.

mRNAs, either synthetic modified mRNA encoding GFP (mRNA-GFP [5mC, ψ]), T7-VEE-GFP-ts, or c-srRNA1-GFP, were tested for their GFP expression using human adult dermal fibroblast cells (HDFa). 50,000 cells in each well of 24-well plate were transfected with 0.5 µg of mRNA with Lipofectamine MessengerMAX (Day 0) and cultured in the DMEM medium containing 500 ng/ml B18R at 33°C or 37°C.
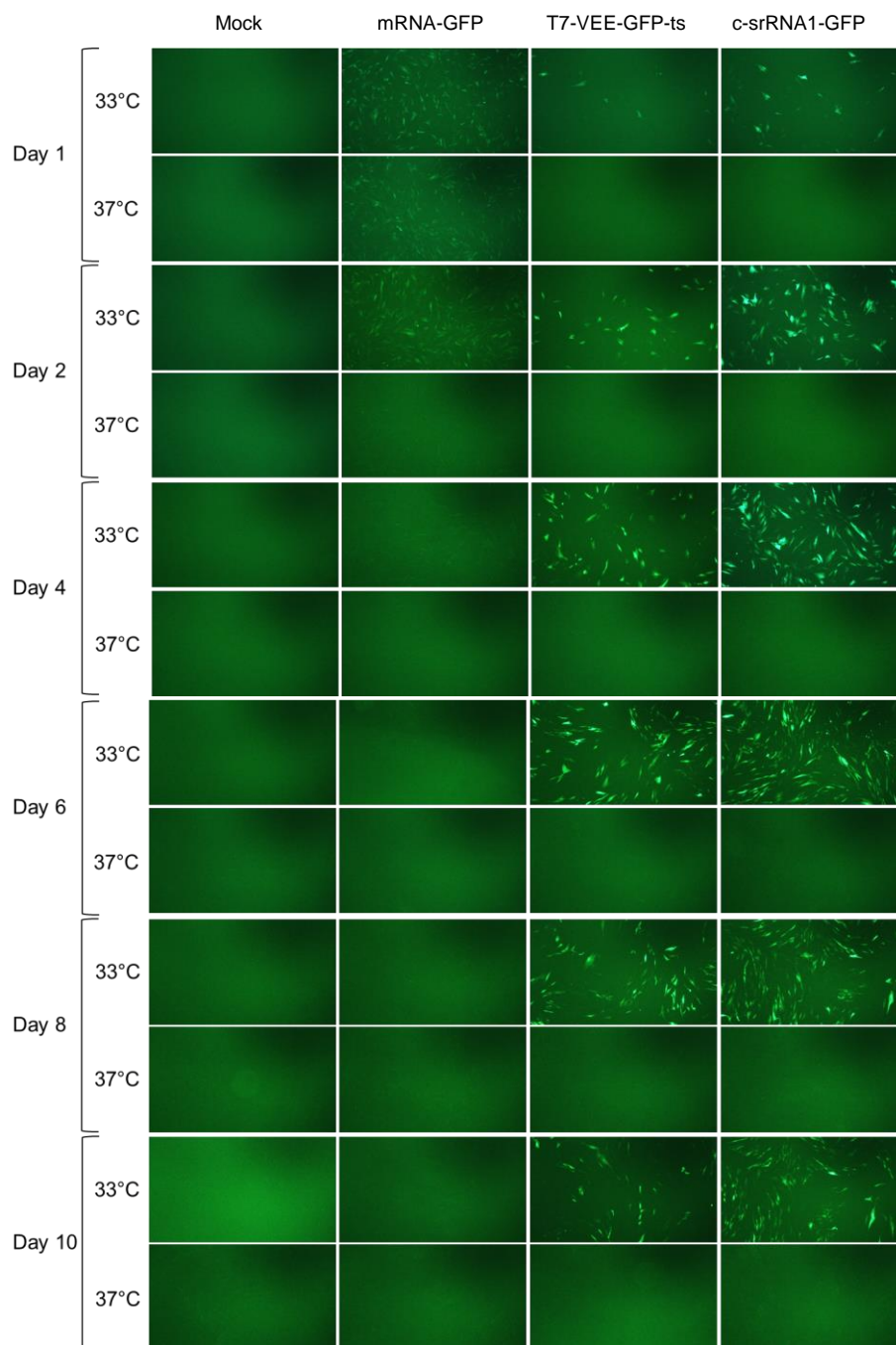

1

FIG. S2. c-srRNA functions in skin by intradermal injection, but not in muscle by intramuscular injection, Related to Figure 1.
(**A**) 5 µg of c-srRNA3-LUC as naked RNAs without LNP was injected intradermally onto the shaved skin or intramuscularly into BALB/c mice (Day 0). Luciferase activity was visualized and quantitated by using a bioluminescent imaging system from Day 1 through Day 9 post-injection. Images of Day 1 and Day 9 are shown. (**B**) Luciferase activities are plotted from Day 1 through Day 9. Left: Results from intradermal injection. Right: Results from intramuscular injection.
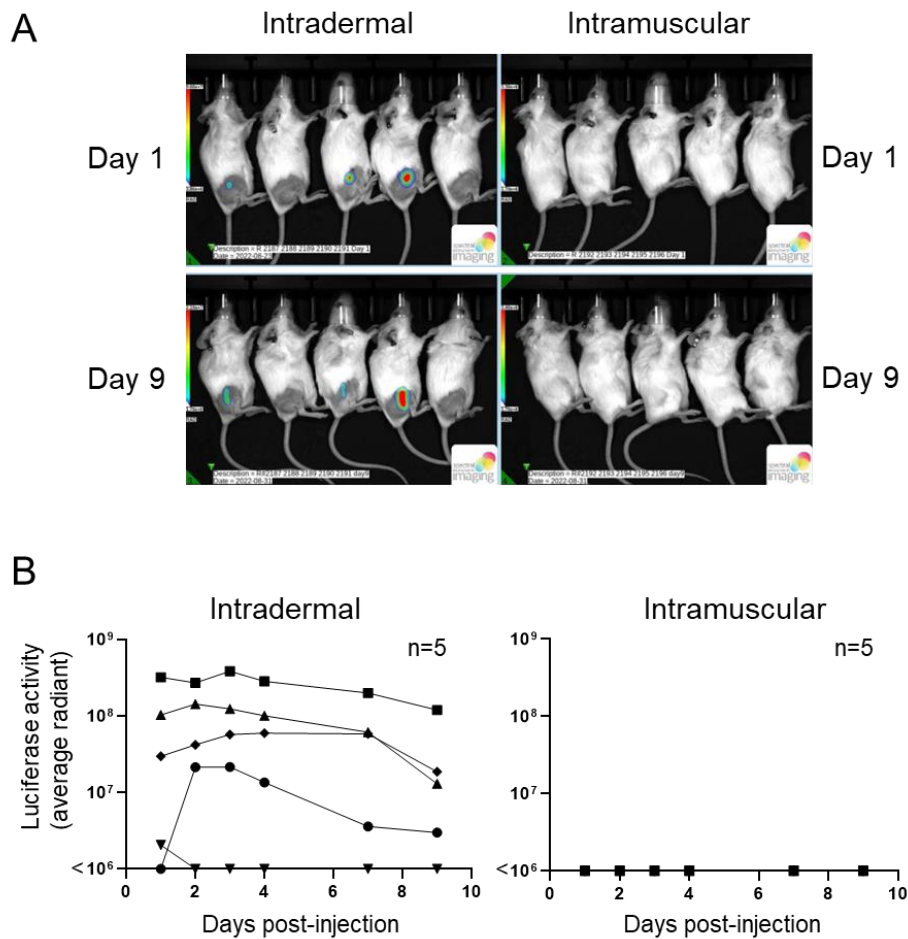
FIG. S3. Comparison of cellular immunity induced by EXG-5006a and EXG-5006b, Related to Figure 6.
(**A**) A schematic diagram of experimental procedures. BALB/c mice were immunized by a single intradermal injection of 100 μL solution containing either a placebo (PBO: buffer only: n=4 or 5), 5 μg (n=5), 25 μg (n=5) of EXG-5006a (non-codon optimized) or EXG-5006b (codon-optimized). After 14 days, mice were sacrificed and splenocytes were collected for ELISpot assays. (**B**) Results of ELISpot assays performed by restimulating splenocytes in the presence or absence of a pool of SARS-CoV-2 nucleoprotein peptides. The frequency of IFN-γ- or IL-4-secreting cells obtained in the presence of peptides is plotted in the graph after subtracting the frequency obtained in the absence of peptides (background). The average and standard deviation (error bars) are shown for each group.

A

B

C
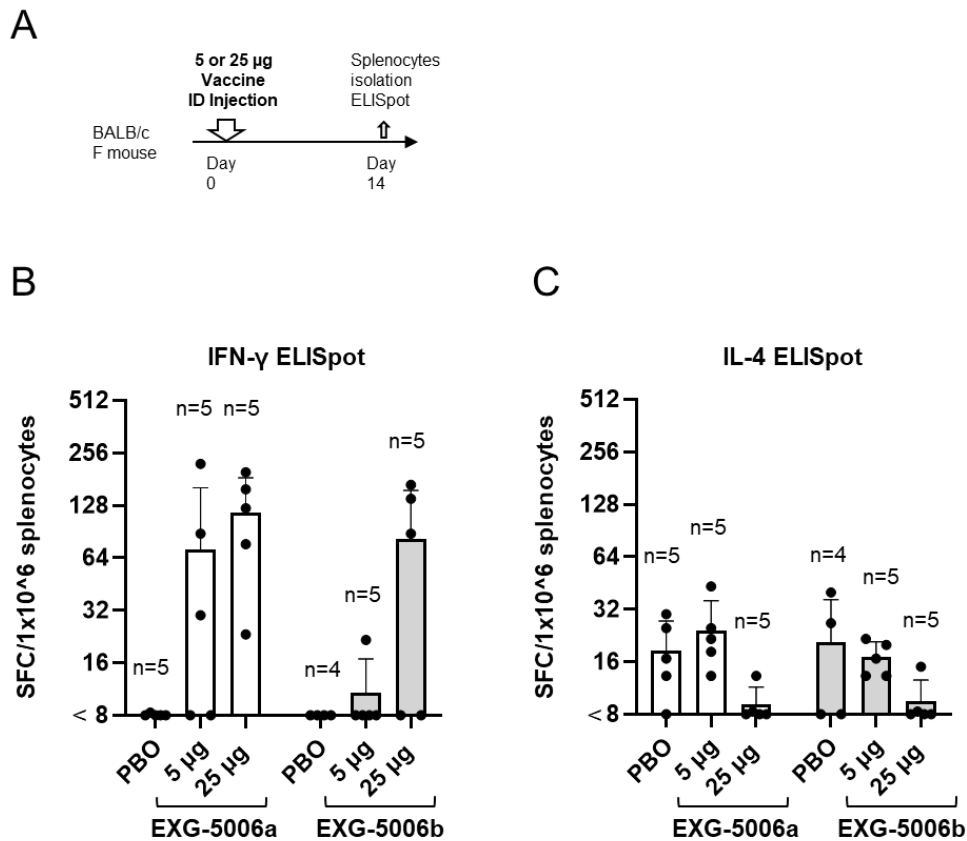
Supplemental Table S1. srRNAs and c-srRNAs developed and used in this study, Related to Figures 1, 2, 3, 4, 5, 6, and 7.

Name	Vector	Gene of interests or antigens
c-srRNA1-LUC	c-srRNA1	LUC
c-srRNA3-LUC	c-srRNA3	LUC
c-srRNA1-GFP	c-srRNA1	GFP
T7-VEE-GFP-ts	T7-VEE-GFP-ts	GFP
c-srRNA1-RBD (EXG-5003)	c-srRNA1	RBD
srRNA0-RBD	srRNA0	RBD
c-srRNA3-RBD	c-srRNA3	RBD
EXG-5003o	c-srRNA3	RBD (omicron)
EXG-5004	c-srRNA3	SARS2-N (no CD5sp)
EXG-5005	c-srRNA3	SARS2-N
EXG-5006a	c-srRNA3	SARS2-N, MERS-N
EXG-5006b	c-srRNA3	SARS2-N, MERS-N (codon optimized)
EXG-5008	c-srRNA3	SARS2-RBD, SARS2-N, MERS-N, MERS-RBD

LUC, luciferase; GFP, Green Fluorescent Protein; RBD, Spike Receptor Binding Domain of SARS-CoV-2; SARS2-N, nucleoprotein of SARS-COV-2; MERS-N, nucleoprotein of MERS-CoV; SARS2-RBD, Spike Receptor Binding Domain of SARS-CoV-2 (also noted just RBD); MERS-RBD, Spike Receptor Domain of MERS-CoV. CD5sp, Signal peptide of human CD5 protein.