OMTN, Volume *30*

Supplemental information

Design and lyophilization of lipid nanoparticles for mRNA vaccine and its robust immune response in mice and nonhuman primates

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Table S1 Ionizable lipid screening for mRNA vaccine. CoV-mRNA was encapsulated into LNPs containing nine ionizable lipids. LNP formulations were assessed based on quality aspects, including pKa of ionizable lipids, encapsulation efficiency of mRNA, size, and polydispersity index (PDI).

Ionizable lipid	**L163**	**L165**	**L166**	**L168**	**L172**	**L173**	**L175**	**L177**	**L202**
pKa of ionizable lipid	6.41	6.08	6.29	6.13	6.97	6.59	6.46	6.10	6.04
Encapsulation (%)	93%	91%	95%	98%	95%	92%	52%	86%	97%
Size (nm)	100	136	111	99	94	102	181	183	112
PDI	0.11	0.04	0.06	0.02	0.20	0.11	0.10	0.09	0.07

LNP Composition; ionizable lipid : DSPC : Cholesterol : DMG-PEG = 50 : 10 : 38.5 : 1.5 (mol%)

Table S2 LNP-CoV mRNA for pharmacokinetic studies

Ionizable lipid in LNP	Z-Average (nm)	PDI	mRNA Encapsulation (%)
L202	103	0.04	97%
MC3	92	0.10	91%

Table S3 (Materials used in this study) is provided in a separate Excel file

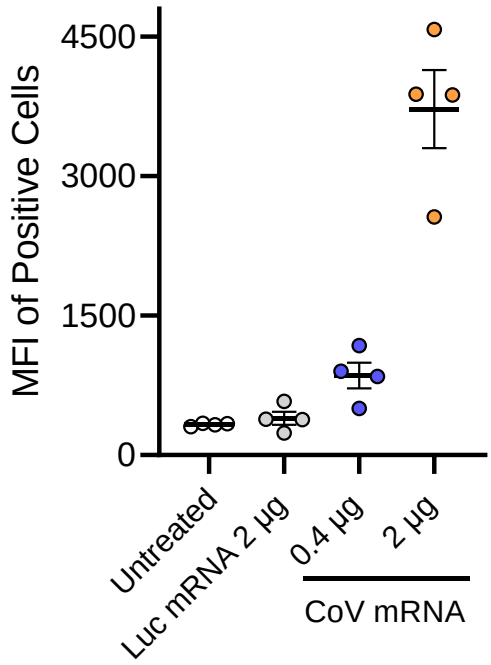


Figure S1 In vitro expression of SARS-CoV-2 S-2P. HEK-293 cells were transfected with Luciferase mRNA (control) or CoV mRNA at 0.4–2 µg of mRNA dose per well. After 24 h, cells were fixed, permeabilized, and stained with Anti-SARS-CoV-2 Spike (S1) antibody (CR3022). Spike expression was evaluated by flow cytometry. Data are presented as mean +/− SEM.

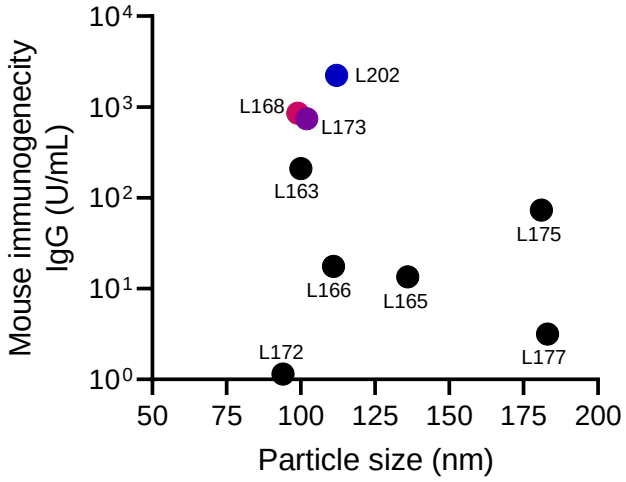


Figure S2 Particle size and mouse immunogenicity. X-axis is particle size derived from Table S1, and Y-axis is mouse immunogenicity derived from Figure 1e. The top three lipids are highlighted as L202 (blue), L168 (magenta), and L173 (purple).

a

Ionizable lipid	L163	L165	L166	L168	L172	L173	L175	L177	L202
Encapsulation (%)	93%	93%	95%	95%	91%	95%	77%	91%	96%
Size (nm)	97	146	100	88	84	92	163	145	84
PDI	0.18	0.02	0.06	0.04	0.18	0.17	0.11	0.07	0.03

LNP Composition: ionizable lipid : DSPC : Cholesterol : DMG-PEG = 50 : 10 : 38.5 : 1.5 (mol%)

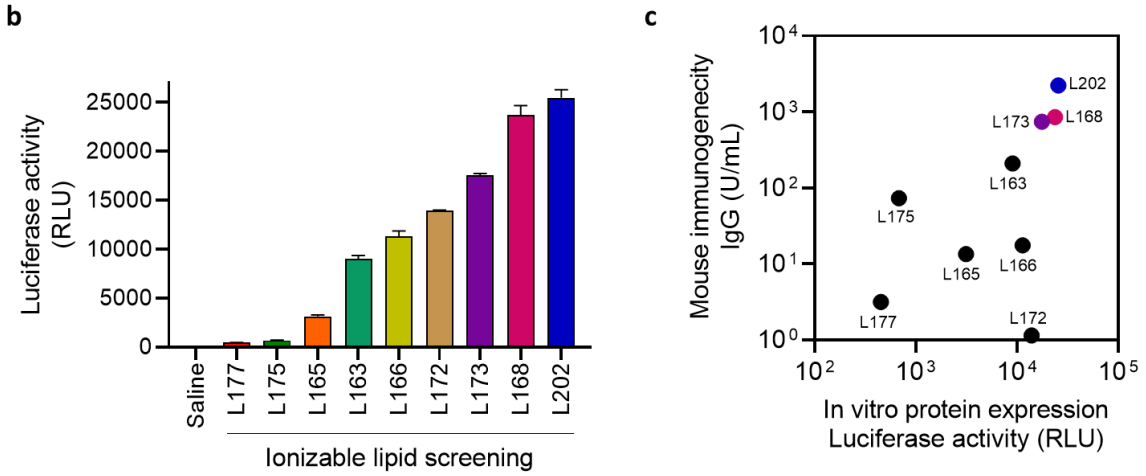
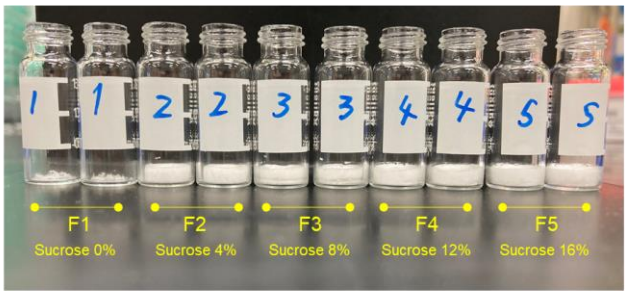
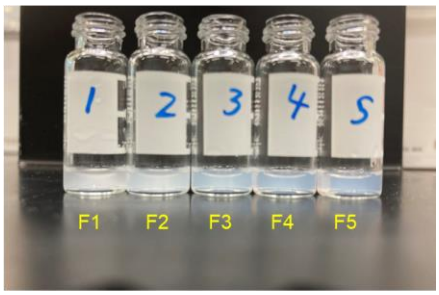


Figure S3 In vitro luciferase expression and mouse immunogenicity. (a) Luciferase-mRNA was encapsulated into LNPs containing nine ionizable lipids. LNP formulations were assessed for encapsulation efficiency of mRNA, size, and polydispersity index (PDI). (b) In vitro protein expression. Hep3B cells were treated with nine LNPs containing luciferase-mRNA at 10 ng of mRNA dose per well (n=3). After 24 h, luciferase activity was evaluated. Data are presented as mean +/− SEM. (c) Expression and immunogenicity. The X-axis is in vitro protein expression derived from (b), and Y-axis is mouse immunogenicity derived from Figure 1e. The top three lipids are highlighted as L202 (blue), L168 (magenta), and L173 (purple). RLU; relative light unit.

a After lyophilization



b After reconstitution with water



c

Formulation ID		**F1**	**F2**	**F3**	**F4**	**F5**
Sucrose in formulation (wt%)		**0%**	**4%**	**8%**	**12%**	**16%**
mRNA encapsulation (%)	After dialysis	90%	93%	92%	91%	93%
	After freeze and thaw test	86%	92%	92%	91%	92%
	After lyophilized test	28%	88%	91%	92%	92%
Z-Average (nm)	After dialysis	116	111	113	109	109
	After freeze and thaw test	119	111	111	110	109
	After lyophilized test	429	198	160	143	136
PDI	After dialysis	0.10	0.11	0.08	0.12	0.10
	After freeze and thaw test	0.13	0.09	0.09	0.10	0.11
	After lyophilized test	0.41	0.06	0.07	0.02	0.07

Figure S4 Buffer selection for lyophilized formulation. (a) Appearance after lyophilization process, (b) Appearance after reconstitution with water, (c) Physicochemical characterization. The image of F5 is used in Figure 2d.

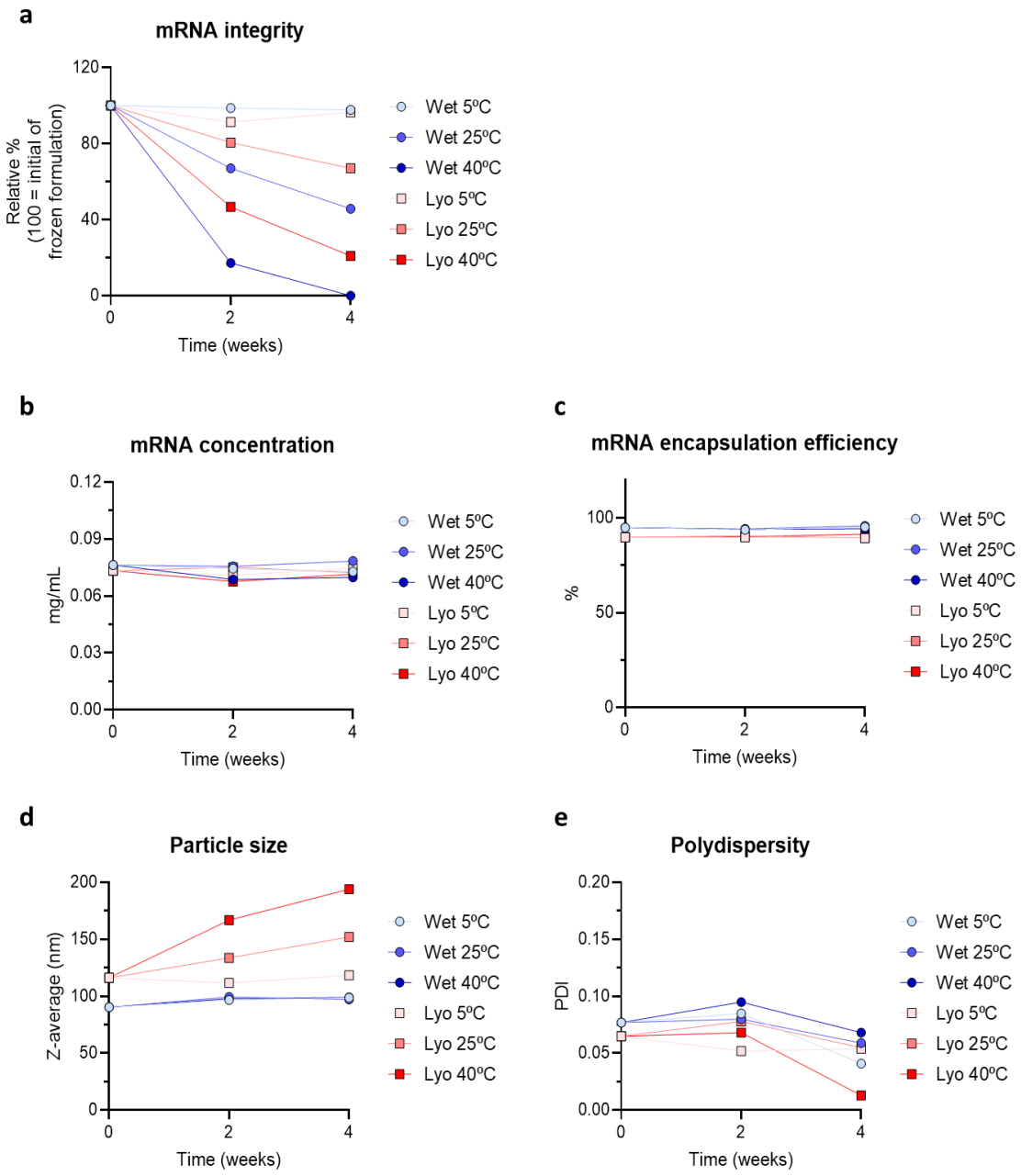


Figure S5 Physicochemical characterization of stored samples. Wet or lyophilized formulation of LNP-COV mRNA stored at 5ºC, 25ºC, or 40ºC for 2 weeks and 1 month. mRNA integrity (a) was assayed by electrophoresis. mRNA concentration (b) and mRNA encapsulation efficiency (c) were determined by RiboGreen assay. Particle size (d) and polydispersity (e) were measured by DLS (dynamic light scattering). For mRNA integrity analysis, mRNA integrity of the frozen formulation without the lyophilization process was set as 100%, and that of each sample was expressed as a relative percentage.

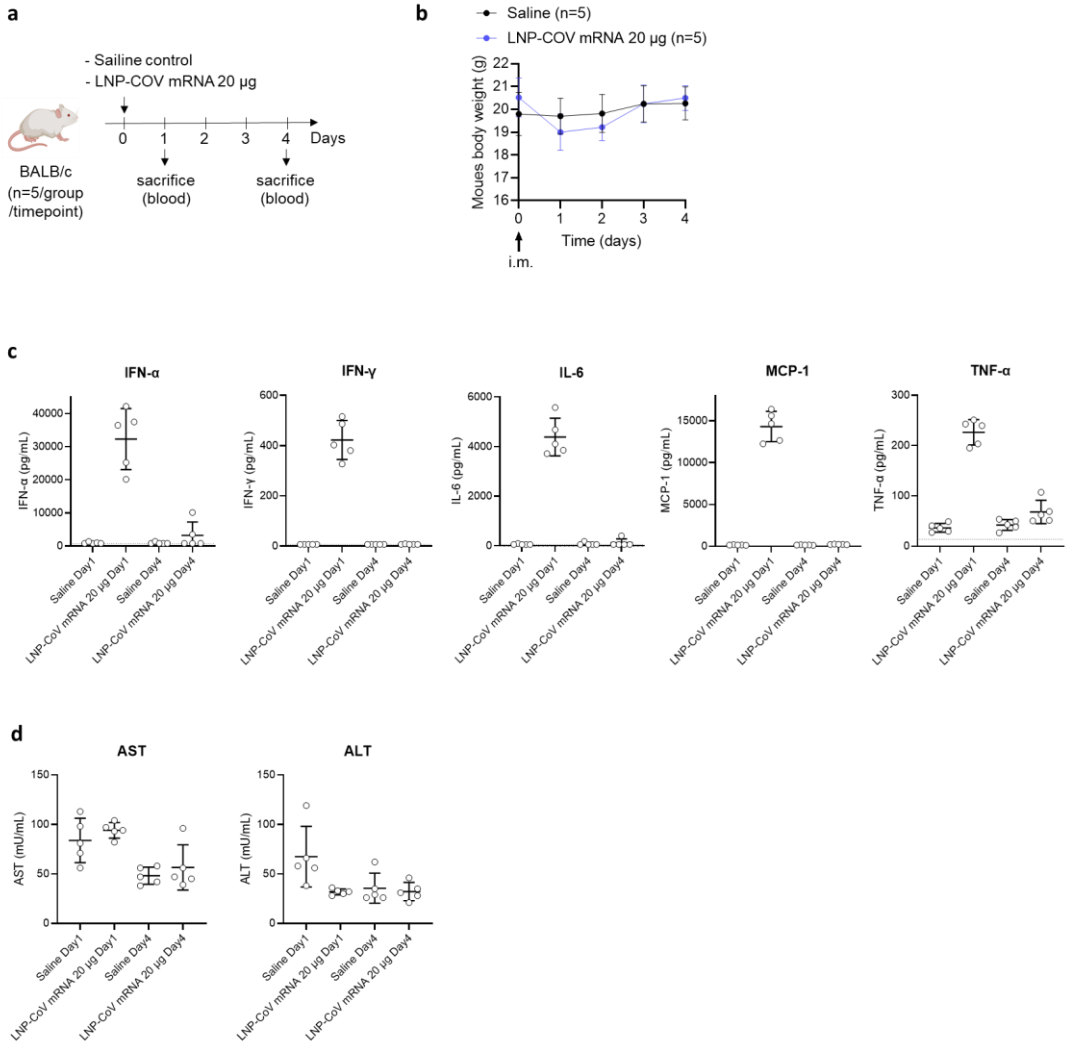


Figure S6 Single-dose tolerability test. (a) BALB/c mice were immunized intramuscularly at day 0 with either PBS or LNP-CoV mRNA containing L202 at 20 μg of mRNA dose. BALB/c mice (n=5/group/time point) were sacrificed on Day 1 and Day 4. (b) Body weight change. (c) Cytokines and chemokines in plasma. (d) Clinical chemistry parameters in plasma. All data are presented as mean +/− SD. The dotted line represents the limit of detection (c).

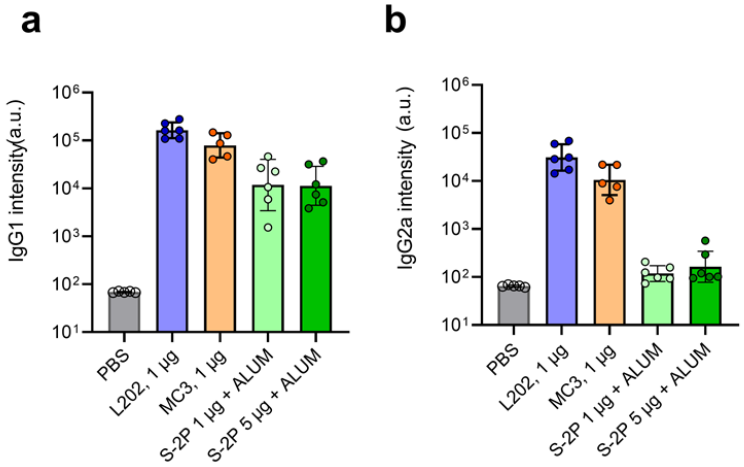


Figure S7 LNP-mRNA elicits Th1-biased responses compared to ALUM-adjuvanted S-2P. BALB/c mice (n=5–6) were immunized on Day 0 and Day 14. Plasma on day 35 was assessed by ELISA for SARS-CoV-2 S1-specific binding IgG1 (a) and IgG2a (b). Data are presented as mean +/− SEM.

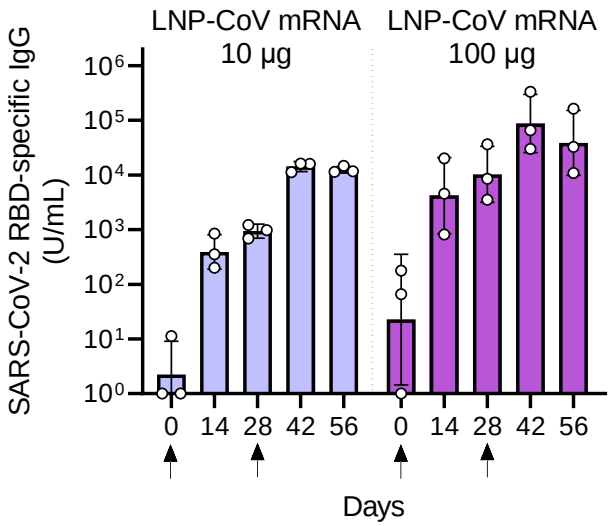


Figure S8 SARS-CoV-2 RBD-specific binding IgG Cynomolgus monkeys (n=3/group) were i.m. injected on Day 0 and Day 28 (arrows below the x-axes indicate the day of injection) with LNP-CoV mRNA containing L202 at 10 µg (blue) or 100 µg (purple) mRNA dose. Serum was assessed by ELISA for SARS-CoV-2 RBD-specific binding IgG. On the day of immunization, serum was collected before administration. Data are presented as geometric mean titer (GMT) +/- geometric SD.

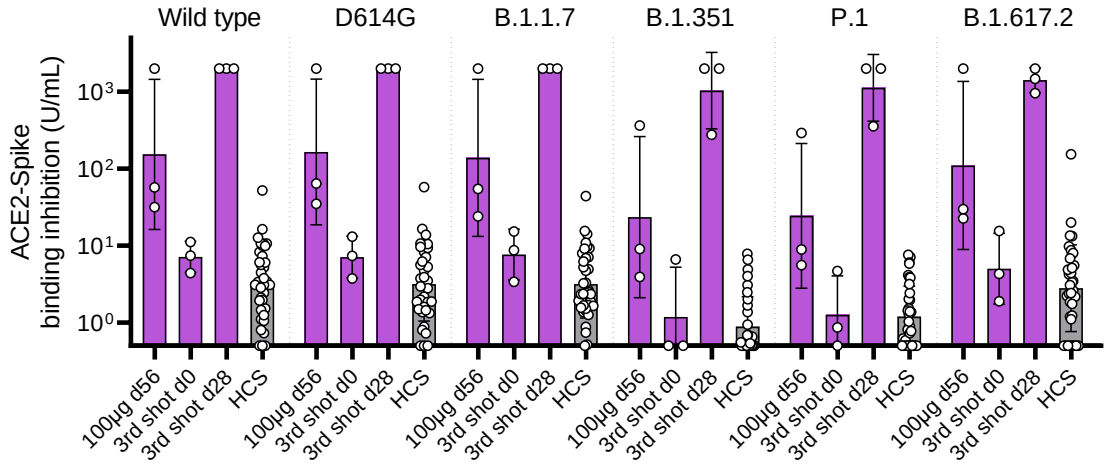


Figure S9 ACE2-Spike binding inhibition. Cynomolgus monkeys (n=3) immunized with 100 μg of two doses were used in a third dose study. After 6 months of Dose 2, 50 μg of third dose (Dose 3) were injected i.m. to animals. Results were compared with the antibody responses in a panel of HCS. Plasma was assessed by ELISA for inhibition of ACE2 binding to Spike of 6 strains, including Wild-type, D614G, B.1.1.7(Alpha), B.1.351(Beta), P.1(Gamma), and B.1.617.2(Delta). On the day of immunization, plasma was collected before administration. Data are presented as geometric mean titer (GMT) +/- geometric SD.

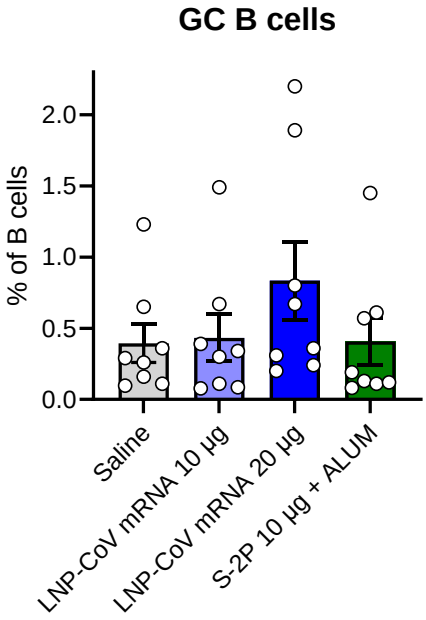


Figure S10 GC B cells. Mice (n=8/group) were immunized i.m. with either saline, LNP-CoV mRNA at 10–20 µg mRNA dose, or ALUM-adjuvanted SARS-CoV-2 spike protein (S-2P) at 10 µg protein dose. GC B cells in inguinal LNs were measured 7 days post-immunization. Frequency of GC B cells at 7 days post-immunization. LNPs in this study are formulated with L202. Data are presented as mean +/− SEM (a, c).