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Supplemental Information

Preclinical and Clinical Demonstration of Immunogenicity by mRNA Vaccines against H10N8 and H7N9 Influenza Viruses

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SUPPLEMENTAL MATERIALS

Figure S1

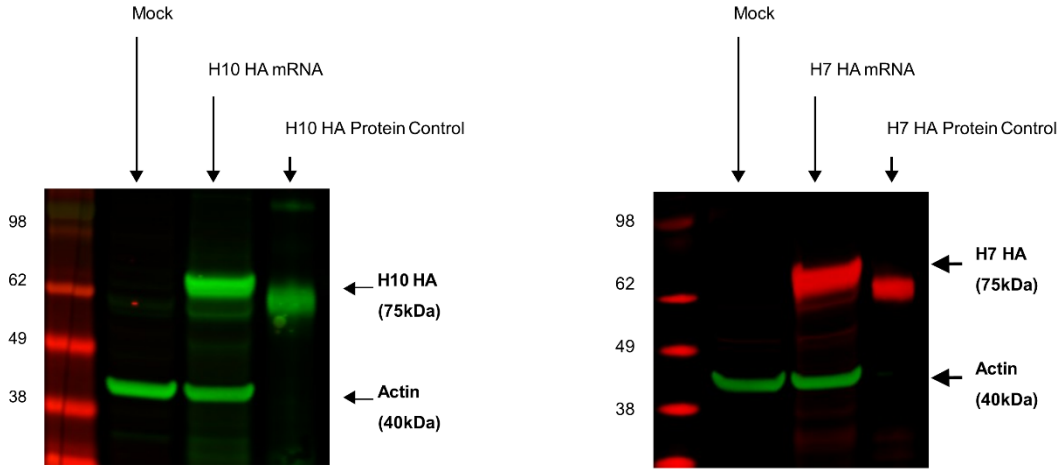

Figure S1. Western blot of resulting cell lysates demonstrated a 75 kDa band for both constructs using the H10 and H7 HA-specific antibodies. H10 and H7 HA protein expression following transfection *in vitro.* HeLa cells were transfected with 2.5 ug of H10 or H7 mRNA for 18–20 h. Lysates were collected and analyzed via Western blot using the corresponding antibodies for detection. H7 and H10 HA protein, along with actin, were included as positive controls.

Figure S2

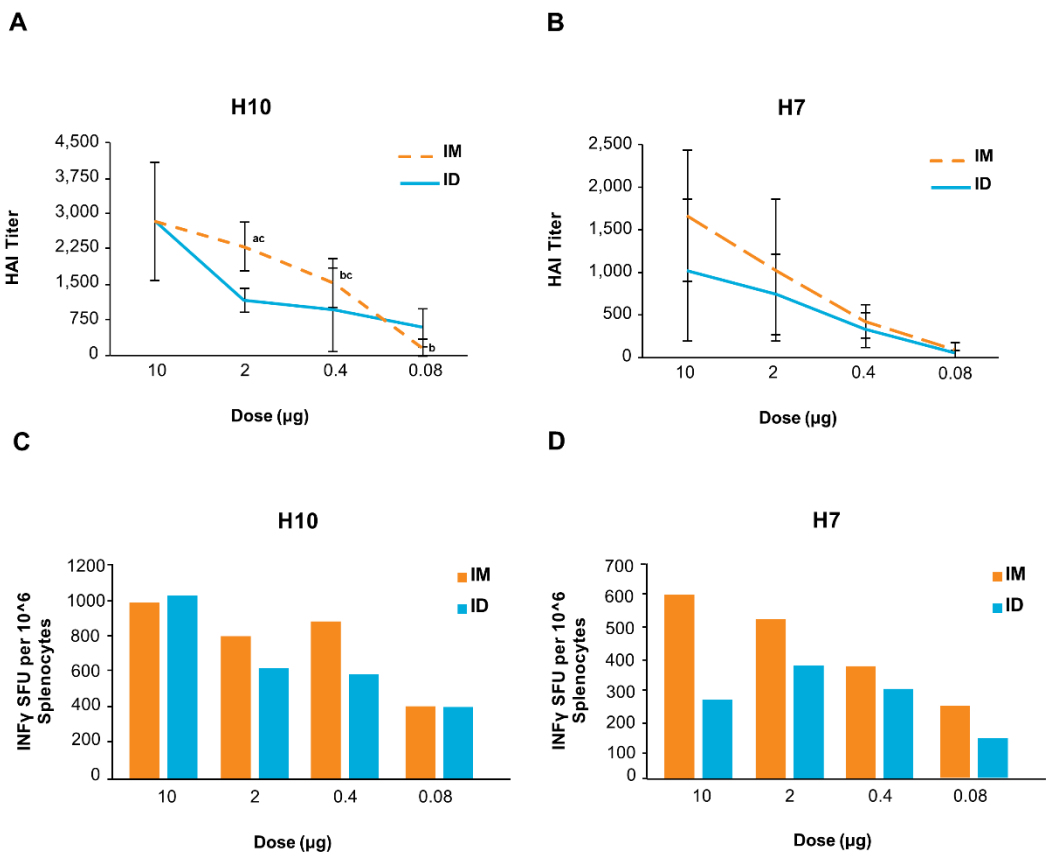

Figure S2. Mice immunized with H10 or H7 mRNA have comparable immune responses following ID and IM immunization at multiple dose levels. BALB/c mice were immunized either ID or IM with doses of 10, 2, 0.4, or 0.08 µg of formulated H10 mRNA or formulated H7 mRNA on days 0 and 21. Serum (individual) and spleens (pooled by group) were collected 28 days post-boost (day 49) to determine HAI titers and T cell responses (IFNγ ELISpot) for H10 (A, C) and H7 (B, D), respectively. [a]$p = 0.0038$ IM versus ID administration; [b]$p < 0.05$ versus 10 µg IM administration and [c]$p < 0.05$ versus 0.08 µg IM administration. (n = 5/group). Error bars indicate standard mean error.

Figure S3

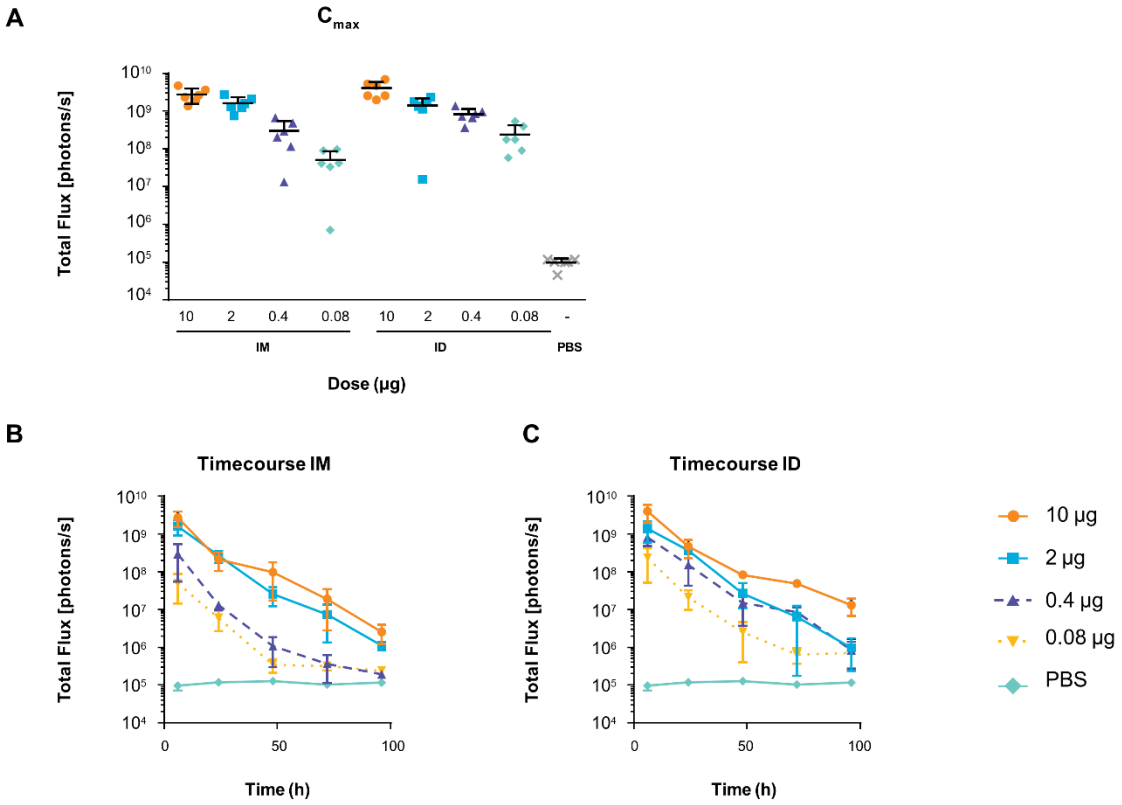

Figure S3. Luciferase expression following IM and ID administration of formulated mRNA. BALB/c mice (*n* = 6/group) were immunized IM or ID with formulated luciferase mRNA with the following doses on day 0: 10 µg, 2 µg, 0.4 µg, or 0.08 µg. At the time of imaging, all mice were injected with 3 mg of luciferin and imaged on an *in vivo* imaging system (IVIS Spectrum, Perkin Elmer). (A) Peak flux (photons/s) after IM and ID administration. (B) Time course of expression following IM administration measured at 6, 24, 48, 72, and 96 hours. (C) Time course of expression following ID administration measured at 6, 24, 48, 72, and 96 hours. Error bars indicate standard mean error.

Figure S4

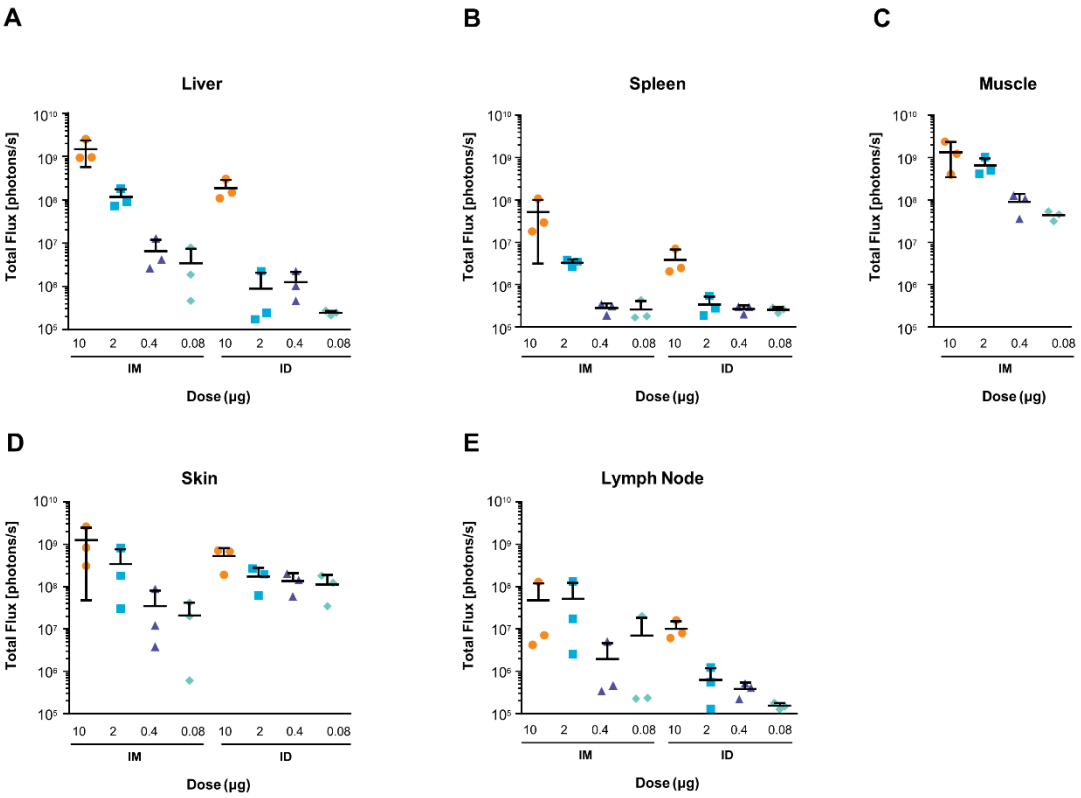

Figure S4. Luciferase expression following IM and ID administration of formulated mRNA. BALB/c mice (*n* = 6/group) were immunized IM or ID with formulated luciferase mRNA with the following doses on day 0: 10 µg, 2 µg, 0.4 µg, or 0.08 µg. At the time of imaging, all mice were injected with 3 mg of luciferin and imaged on an *in vivo* imaging system (IVIS Spectrum, Perkin Elmer). At 6 hours, 3 mice from each group were sacrificed and autopsied, and organs were imaged *ex vivo*. (A) *Ex vivo* liver flux after IM and ID administration. (B) *Ex vivo* spleen flux after IM and ID administration. (C) *Ex vivo* muscle flux after IM administration. (D) *Ex vivo* skin flux after IM and ID administration. (E) *Ex vivo* draining lymph-node flux after IM and ID administration. Error bars indicate standard mean error.

Figure S5

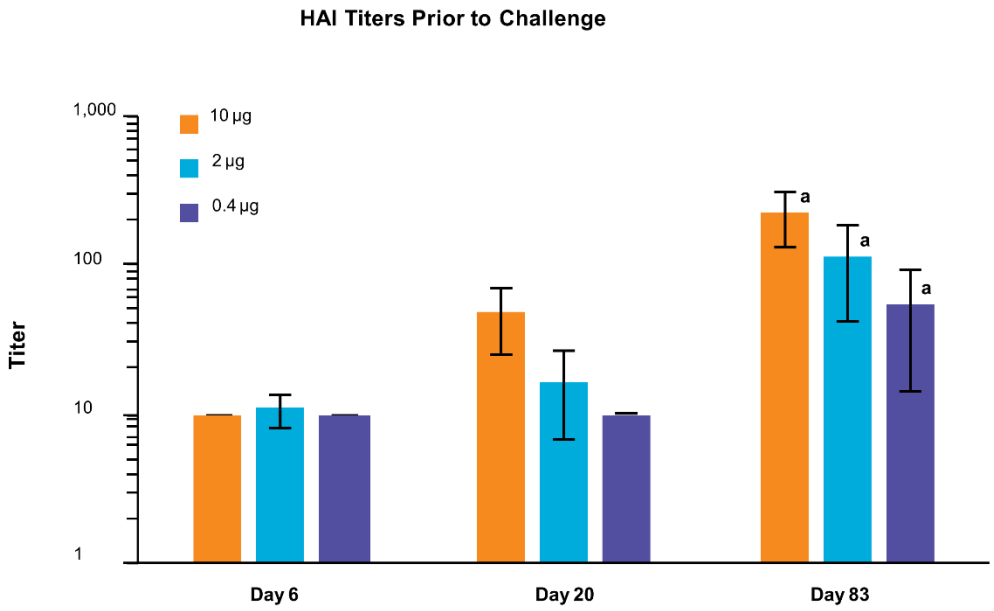

Figure S5. A single injection of an H7 mRNA vaccine achieves high HAI titers in mice. BALB/c mice were vaccinated ID with 10 µg, 2 µg, or 0.4 µg of formulated H7 mRNA. Serum was collected prior to challenge (days 6, 20, and 83) to determine H7 HAI titers. [a]$P < 0.0001$ versus day 6 and day 20 between equivalent dose groups. Error bars indicate standard mean error (n = 15/group).

Figure S6

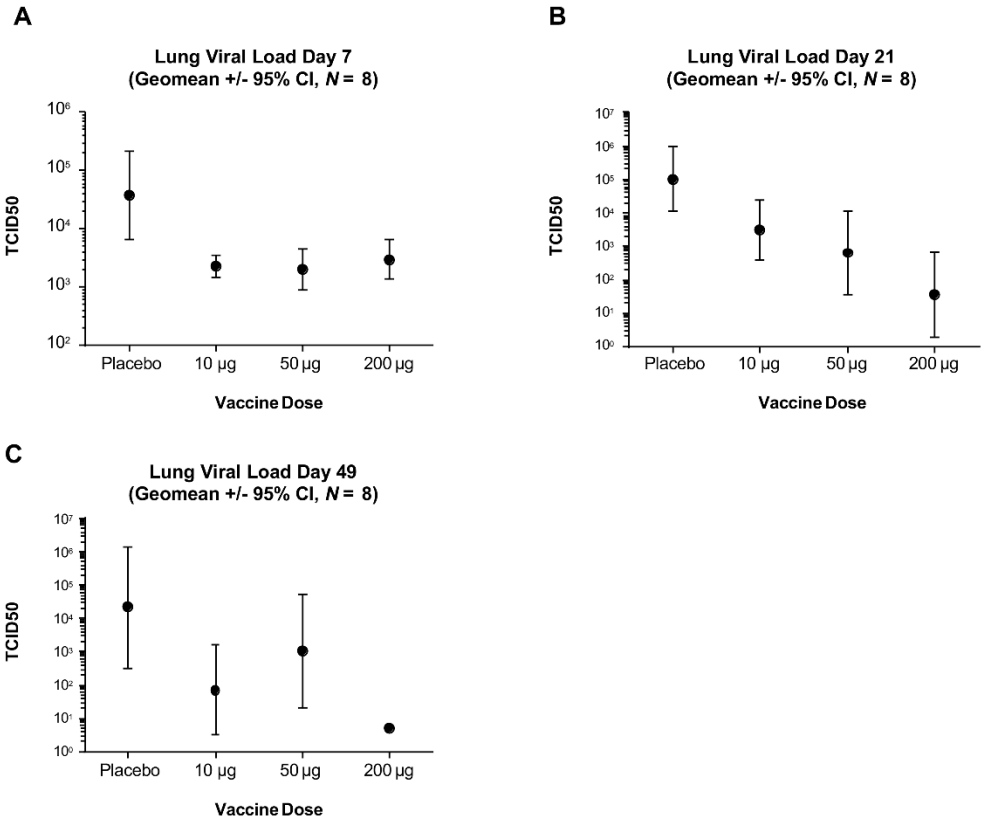

Figure S6. A single dose of H7 mRNA vaccine reduces H7N9 viral loads by 2 logs in ferrets. Ferrets were vaccinated ID with 200 µg, 50 µg, or 10 µg of formulated H7 mRNA. Placebo and 200 µg of formulated H7 mRNA with a reduced 5' cap structure (-15 Da cap) were included as negative controls. A subset of immunized ferrets received a boosting ID vaccination on Day 21 with the indicated doses. (A,B,C) On Day 7 (A), 21 (B), or 49 (C) post-immunization, ferrets were challenged ID with a target dose of 1×10^6 $TCID_{50}$ of influenza A/Anhui/1/2013 (H7N9). Viral burden in the lung was determined by $TCID_{50}$ 3 days post-challenge at the indicated doses. Error bars indicate standard mean error.

Figure S7

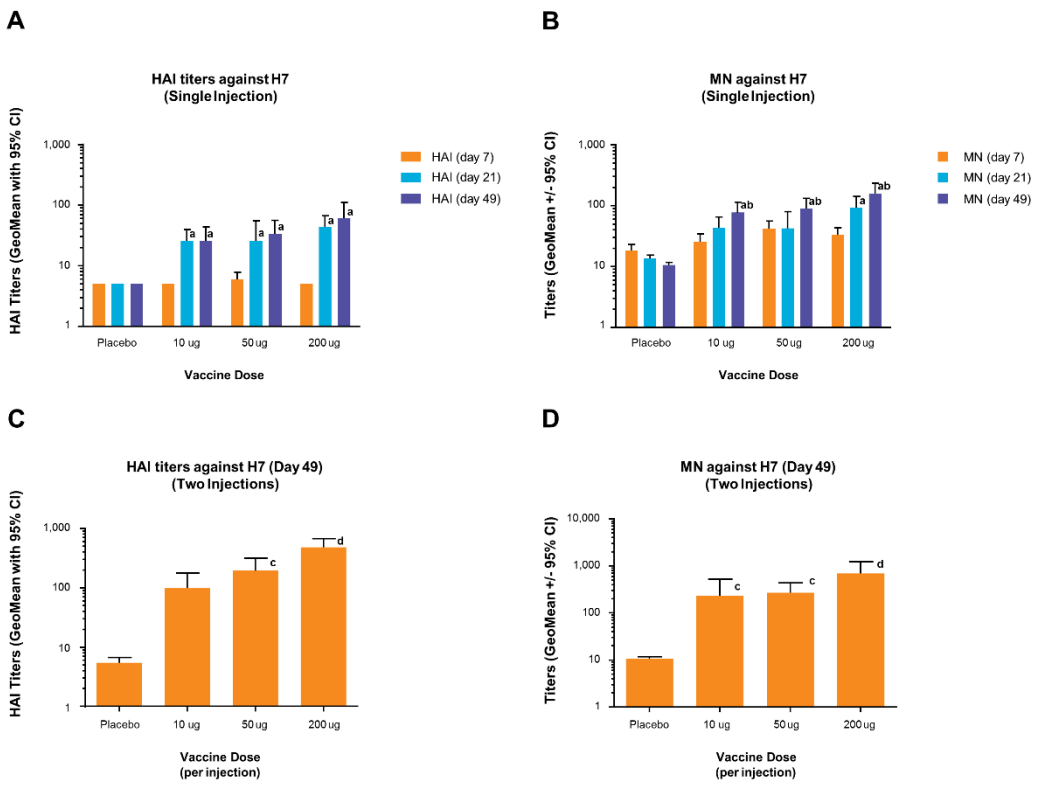

Figure S7. A single dose of H7 mRNA vaccine generates robust HAI titers in ferrets. Ferrets were vaccinated ID with 200 µg, 50 µg, or 10 µg of formulated H7 mRNA. Placebo and 200 µg of formulated H7 mRNA with a reduced 5' cap structure (-15 Da cap) were included as negative controls. A subset of ferrets received a bosting ID vaccination on Day 21 with the indicated doses. Serum was collected from all groups immediately prior to challenge to measure antibody titers via HAI (A) and MN (B) for ferrets that received a single immunization; $^{a}p < 0.05$ versus day 7 and $^{b}p < 0.05$ versus day 21 between equivalent dose groups. Day 49 (28 days post-boost) antibody titers were also measured by HAI (C) and MN (D) for ferrets that received a boosting immunization (n = 8/group). $^{c}P < 0.05$ versus placebo; $^{d}p < 0.05$ versus all others. Error bars indicate standard mean error.

Table S1

Biodistribution of H10 mRNA in plasma and tissue after ID administration in mice. Male CD-1 mice received 300 μg/kg (6 μg) of formulated H10 mRNA via ID immunization. Blood and tissue samples, including heart, lung, spleen, kidney, liver, and skin-injection site, were collected at predose and 2, 4, 8, 24, 48, 72, and 96 hours following dosing. Plasma and tissue sample mRNA levels were quantified using a branched DNA (bDNA) assay (n = 4 mice/time point).

	$t_{1/2}$ (h)	t_{max} (h)	C_{max} (pg/mL)	$AUC_{(0\text{-}96)}$ (h.pg/g or mL)	$AUC_{(0\text{-}inf)}$ (h.pg/g or mL)	T/P
Heart	38.81	24	5.19	226.19	270.16	0.022
Kidney	22.98	24	23.75	612.84	624.76	0.059
Liver	17.98	24	108.62	2957.06	3024.73	0.284
Lung	13.49	24	41.85	1405.46	1433.05	0.134
Spleen	65.74	24	1663.52	114252.46	195225.6	18.3
Skin	23.4	4	18248000	520046043	551134018	50190
Plasma	18.31	24	360.44	10361.63	10660.86	

Table S2

Solicited local and systemic reactogenicity events by severity in subjects who received 100 µg H10N8 mRNA IM or placebo following the toxicity grading scale for heathy adult and adolescent volunteers enrolled in preventative vaccine clinical trials; Tables for laboratory abnormalities (CBER 2007). Adverse events were defined as any unfavorable and unintended medical occurrence. Mild adverse events were defined as those having no limitations in normal daily activities, moderate adverse events as causing some limitations, and severe adverse events were defined as events causing inability to perform normal daily activities. AE = adverse event. [a]Based on the total number of patients who received at least one dose of treatment. [b]Percentages based total number of adverse events after treatment.

	100 µg IM H10N8 mRNA (*N* = 23)					**Placebo (*N* = 8)**				
		Number of Adverse Events[b]					Number of Adverse Events[b]			
	Number of Subjects[a] *n* (%)	Total *n* (%)	Mild *n* (%)	Moderate *n* (%)	Severe *n* (%)	Number of Subjects[a] *n* (%)	Total *n* (%)	Mild *n* (%)	Moderate *n* (%)	Severe *n* (%)
Any solicited adverse events	23 (100)	163 (100)	107 (65.6)	52 (31.9)	4 (2.5)	5 (62.5)	18 (100)	12 (66.7)	3 (16.7)	3 (16.7)
Any solicited local adverse events	21 (91.3)	52 (31.9)	33 (20.2)	16 (9.8)	3 (1.8)	2 (25.0)	2 (11.1)	2 (11.1)	0	0
Injection site ecchymosis	0	0	0	0	0	0	0	0	0	0
Injection site erythema	5 (21.7)	7 (4.3)	2 (1.2)	3 (1.8)	2 (1.2)	0	0	0	0	0
Injection site induration	5 (21.7)	6 (3.7)	2 (1.2)	3 (1.8)	1 (0.6)	0	0	0	0	0
Injection site pain	21 (91.3)	39 (23.9)	29 (17.8)	10 (6.1)	0	2 (25.0)	2 (11.1)	2 (11.1)	0	0
Any solicited systemic adverse events	21 (91.3)	111 (68.1)	74 (45.4)	36 (22.1)	1 (0.6)	5 (62.5)	16 (88.9)	10 (55.6)	3 (16.7)	3 (16.7)
Appetite loss/decrease	4 (17.4)	4 (2.5)	3 (1.8)	1 (0.6)	0	0	0	0	0	0
Arthralgia, generalized	0	0	0	0	0	0	0	0	0	0
Arthralgia, others	7 (30.4)	8 (4.9)	6 (3.7)	2 (1.2)	0	1 (12.5)	1 (5.6)	1 (5.6)	0	0
Chills, common cold, feeling cold	11 (47.8)	11 (6.7)	4 (2.5)	6 (3.7)	1 (0.6)	1 (12.5)	1 (5.6)	0	1 (5.6)	0
Diarrhea	1 (4.3)	1 (0.6)	1 (0.6)	0	0	1 (12.5)	1 (5.6)	1 (5.6)	0	0
Fatigue	12 (52.2)	20 (12.3)	16 (9.8)	4 (2.5)	0	5 (50.0)	4 (22.2)	3 (16.7)	0	1 (5.6)
Fever	4 (17.4)	4 (2.5)	2 (1.2)	2 (1.2)	0	1 (12.5)	1 (5.6)	1 (5.6)	0	0
Headache	18 (78.3)	21 (12.9)	14 (8.6)	7 (4.3)	0	3 (37.5)	3 (16.7)	2 (11.1)	0	1 (5.6)

Malaise	10 (43.5)	14 (8.6)	9 (5.5)	5 (3.1)	0	2 (25.0)	2 (11.1)	1 (5.6)	0	1 (5.6)
Myalgia, generalized	0	0	0	0	0	0	0	0	0	0
Myalgia, others	12 (52.2)	23 (14.1)	17 (10.4)	6 (3.7)	0	2 (25.0)	2 (11.1)	1 (5.6)	1 (5.6)	0
Nausea, vomiting	3 (13.0)	3 (1.8)	2 (1.2)	1 (0.6)	0	0	0	0	0	0
Systemic others (palpitation, night sweats, throat pain)	2 (8.7)	2 (1.2)	0	2 (1.2)	0	1 (2.5)	1 (5.6)	0	1 (5.6)	0