

Article

# Sequential Immunization with Universal Live Attenuated Influenza Vaccine Candidates Protects Ferrets against a High-Dose Heterologous Virus Challenge

Irina Isakova-Sivak, Victoria Matyushenko, Tatiana Kotomina, Irina Kiseleva, Elena Krutikova, Svetlana Donina, Andrey Rekstin, Natalia Larionova, Daria Mezhenkaya, Konstantin Sivak, Arman Muzhikyan, Anastasia Katelnikova and Larisa Rudenko

Supplementary Materials

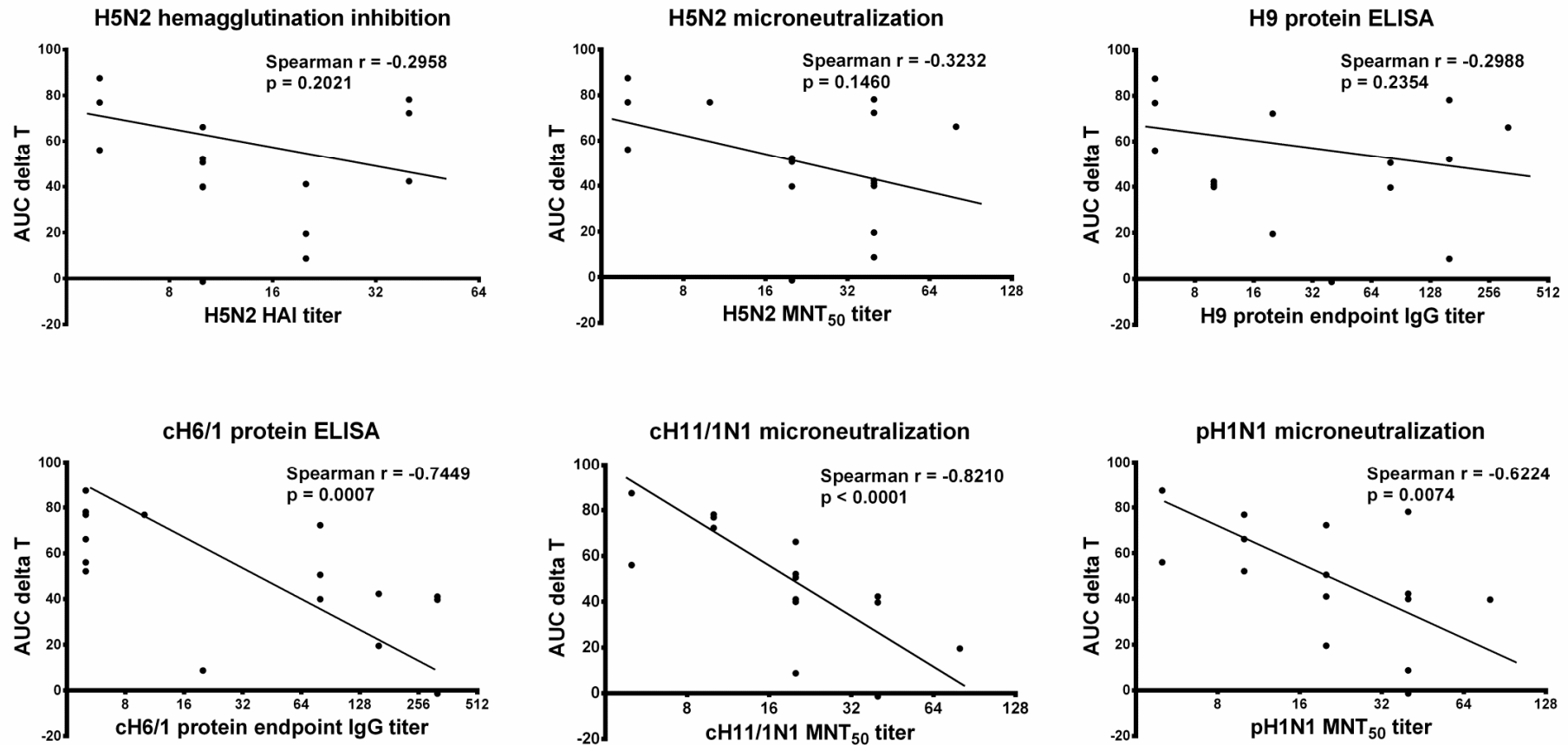
**Table S1.** Individual nasal turbinates pathology scores for immunized ferrets after challenge with H1N1pdm09 virus.

Vaccine group	Animal ID	Affected tissue (%)	NT pathology parameters					Total NT pathology score
			Epithelium damage	Hyperemia	Lymphocytic infiltrate	Exudate	Edema	
cHA LAIVs (NP-MDV)	1.1	70	3	1	3	4	3	14
	1.2	5	0	2	1	0	0	3
	1.3	50	2	2	2	3	2	11
	1.4	60	3	3	3	4	4	17
cHA LAIVs (NP-WT)	2.1	5	1	1	1	1	0	4
	2.2	20	2	3	2	1	2	10
	2.3	20	2	2	3	2	1	10
	2.4	10	1	1	2	1	1	6
LAIVs (NP-MDV)	3.1	65	3	4	3	4	4	18
	3.2	20	1	3	1	1	2	8
	3.3	30	2	3	1	2	2	10
	3.4	75	2	3	3	1	4	13
Mock (PBS)	4.1	70	4	3	4	5	5	21
	4.2	80	4	5	4	5	4	22
	4.3	60	4	3	3	4	3	17
	4.4	50	4	4	4	4	4	20

**Table S2.** Individual lung pathology scores for immunized ferrets after challenge with H1N1pdm09 virus.

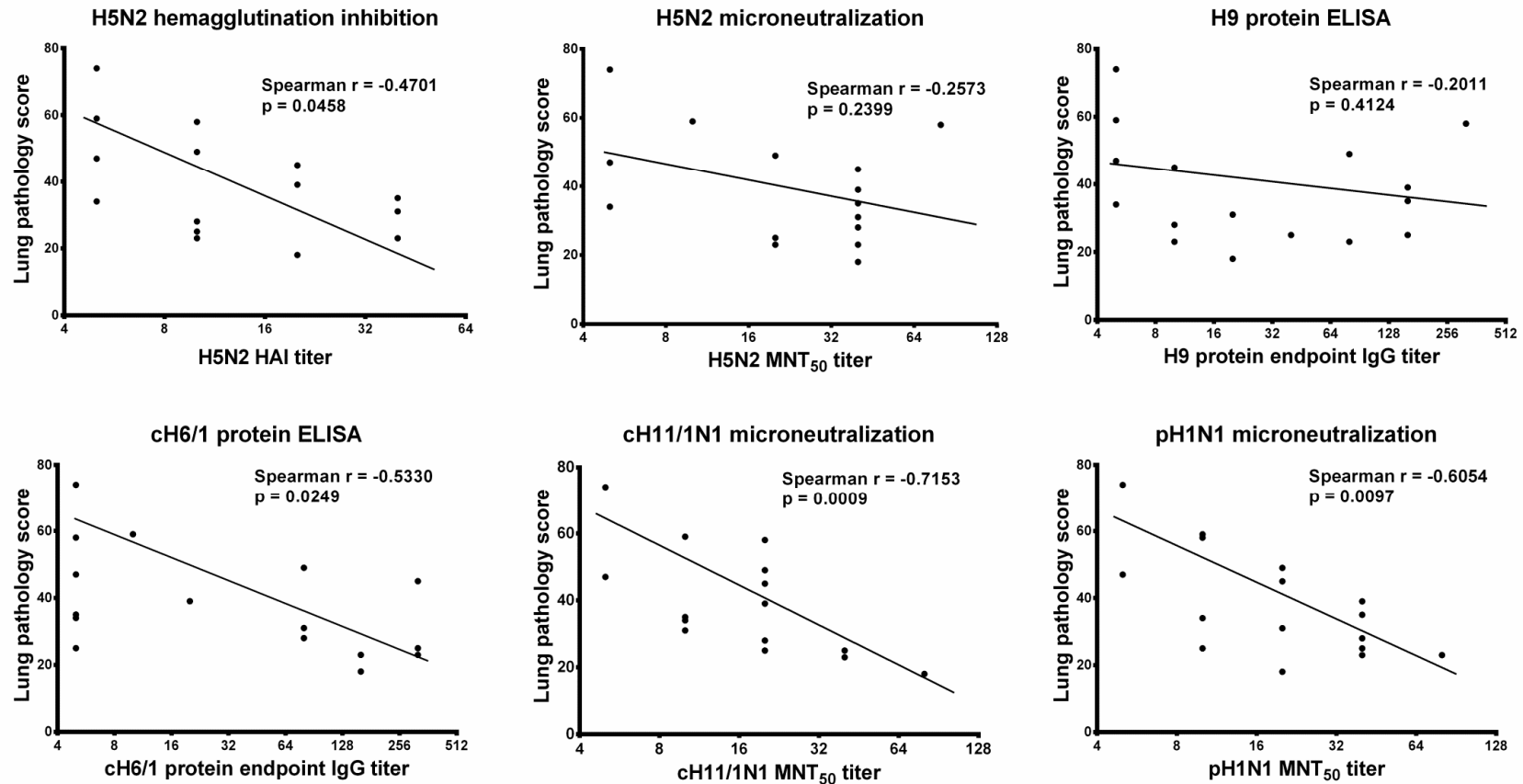
Vaccine group	Animal ID	Damage (0-5)								Inflammation (0-5)										Total lung pathology score		
		Epithelium hypertrophy		Epithelium hyperplasia		Pseudo squamous epithelium		Epithelium necrosis		Hypertrophy pneumocyte type2	Hyperemia septa	Alveolar emphysema	Alveolar hemorrhages	Bronchitis	Bronchiolitis	Peribronchitis	Peribronchiolitis	Interstitial infiltrate	Alveolitis		Vasculitis	Perivascularitis
		bronchi	bronchioli	bronchi	bronchioli	bronchi	bronchioli	bronchi	bronchioli													
cHA LAIVs (NP-MDV)	1.1	3	2	2	3	0	1	2	3	3	4	2	1	3	2	4	3	3	3	2	3	49
	1.2	2	1	1	2	0	0	0	3	3	2	1	1	1	2	2	4	3	1	2	31	
	1.3	0	1	1	2	0	0	0	1	2	2	3	0	0	2	1	2	3	2	0	1	23
	1.4	3	2	2	1	0	0	3	3	3	1	4	1	4	4	3	3	2	2	1	2	45
cHA LAIVs (NP-WT)	2.1	0	0	2	1	0	1	0	2	2	2	2	0	1	1	0	2	2	0	2	2	18
	2.2	1	0	2	1	0	0	0	2	2	1	2	1	1	2	1	2	2	1	2	23	
	2.3	0	1	1	1	0	1	0	3	1	3	0	1	1	2	2	3	2	1	2	25	
	2.4	1	1	1	0	0	0	0	2	3	2	0	0	1	3	2	2	3	3	2	2	28
LAIVs (NP-MDV)	3.1	0	2	0	1	0	0	0	3	2	2	0	1	1	1	2	4	3	1	2	25	
	3.2	2	3	2	2	0	1	1	3	2	3	0	2	2	2	1	3	3	2	3	39	
	3.3	1	2	1	3	0	2	0	1	3	2	1	3	2	1	2	2	3	3	1	2	35
	3.4	2	2	2	2	0	0	3	4	4	2	4	3	4	5	3	4	4	3	3	4	58
Mock (PBS)	4.1	3	3	2	3	0	2	2	4	4	4	3	1	2	4	3	4	5	4	3	3	59
	4.2	2	2	3	4	0	2	0	0	4	3	2	2	1	1	2	0	3	2	0	1	34
	4.3	2	2	3	3	1	1	0	2	3	3	3	4	2	2	2	2	4	3	2	3	47
	4.4	3	3	4	3	2	2	3	3	4	4	4	2	4	5	5	5	5	5	4	4	74

## Correlation of assay results and body temperature increase



**Figure S1.** Correlation of some representative antibody immune responses of ferrets after immunization with body temperature increase measured by the area under the curve during the challenge phase.

## Correlation of assay results and lung pathology score



**Figure S2.** Correlation of some representative antibody immune responses of ferrets after immunization with lung pathology score defined during histopathological analysis of lung tissue sections.

## Correlation of assay results and pH1N1 virus titer in lung

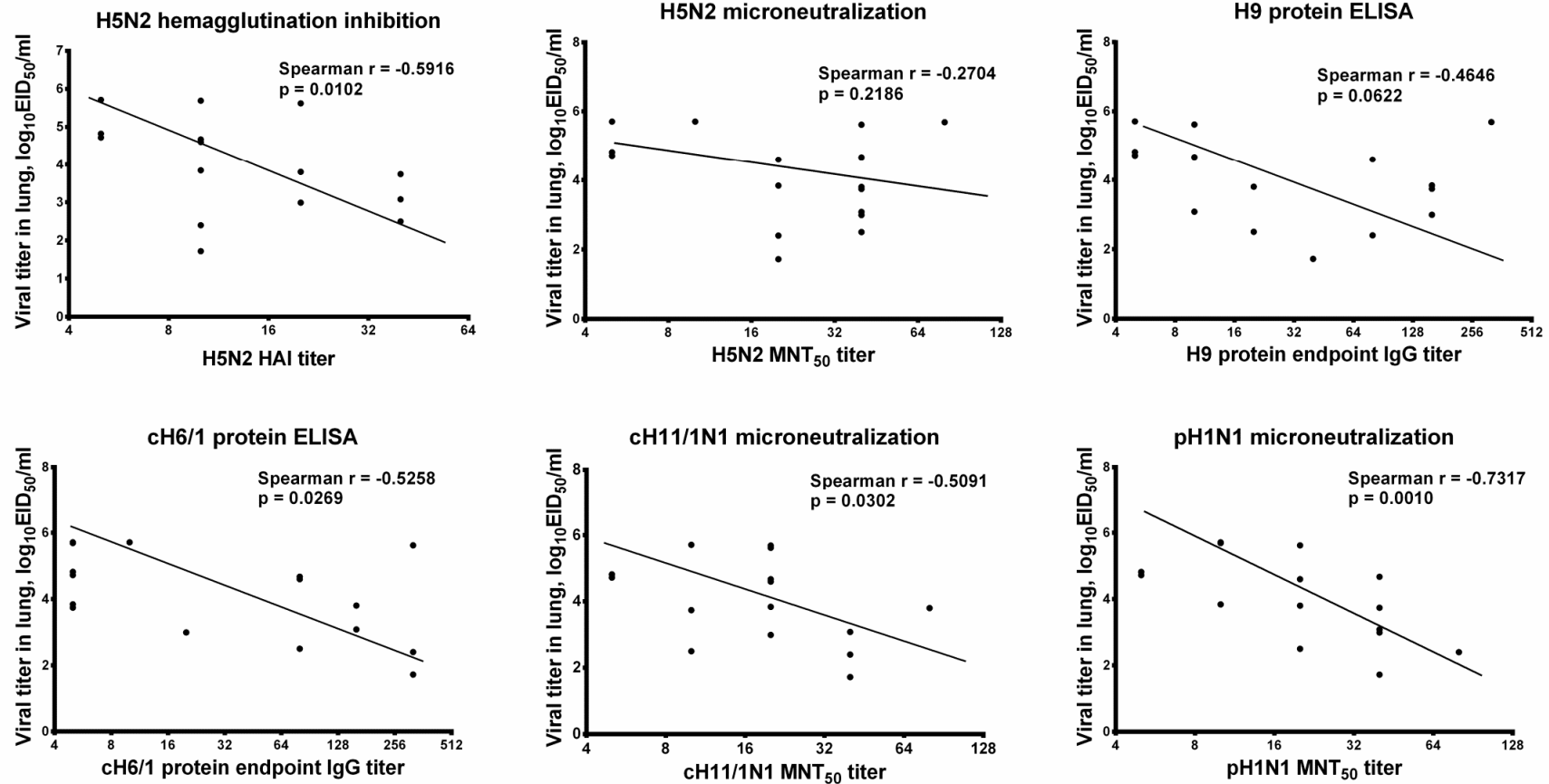


Figure S3. Correlation of some representative antibody immune responses of ferrets after immunization with viral titer in lung on day 4 post-challenge.



© 2019 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).