

1 **Supplemental figure legends**

2

3 **Supplemental Fig. S1. Plasma concentrations of BXA in ferrets after subcutaneous**

4 **injection of 8 mg/kg BXA.** BXA was suspended in 0.5% methylcellulose (5.32 mg/mL)

5 and injected subcutaneously into four sites on the dorsal side of the ferret (0.5 mL/site).

6 Blood samples were collected at various time points, and the plasma concentration of

7 BXA was determined using a qualified LC-MS/MS assay. The EC<sub>50</sub>s of the IBVs were

8 plotted based on calculated values derived from plaque reduction assays in MDCK cells

9 (1). Dotted line indicates the estimated target of effectiveness (6.85 ng/mL) (2).

10

11 **Supplemental Fig. S2. Comparison of AUCs for virus shedding in the vehicle-**

12 **treated and BXA-treated groups of ferrets inoculated with a single virus or with a**

13 **competitive-mixture of viruses.** The AUCs for virus shedding in vehicle-treated (A)

14 and BXA-treated (B) ferrets inoculated with BR/08-I38, BR/08-I38T, or the BR/08

15 competitive-mixture were compared using two-way ANOVA with Sidak's multiple

16 comparison post-hoc test. The associated bars are color coded to indicate the groups

17 being compared. \*\* $P < 0.01$ ; \*\*\*\* $P < 0.0001$ .

18

19 **Supplemental Fig. S3. Mean proportions of BXA-Sen and BXA-Res virus**

20 **subpopulations in the URTs of ferrets inoculated with the BR/08 competitive-**

21 **mixture.** Shown are the mean proportions of the BR/08-I38 and I38T virus populations

22 in the ferrets on their first and last days of virus shedding.

23

24 **Supplemental Fig. S4. Comparison of AUCs for virus shedding in vehicle-treated**  
25 **and BXA-treated ferrets inoculated with the BR/08 or PH/13 competitive-mixture.**

26 The AUCs for virus shedding in vehicle-treated (A) and BXA-treated (B) ferrets  
27 inoculated with the BR/08 or PH/13 competitive-mixture were compared using two-way  
28 ANOVA with Sidak's multiple comparison post-hoc test. The associated bars are color  
29 coded to indicate the groups being compared. \* $P < 0.051$ ; \*\* $P < 0.01$ .

30

31 **Supplemental Fig. S5. Mean proportions of BXA-Sen and BXA-Res virus**  
32 **subpopulations in the URTs of ferrets inoculated with the PH/13 competitive-**  
33 **mixture.** Shown are the mean proportions of PH/13-I38 and I38T virus populations in  
34 the ferrets on their first and last days of virus shedding.

35

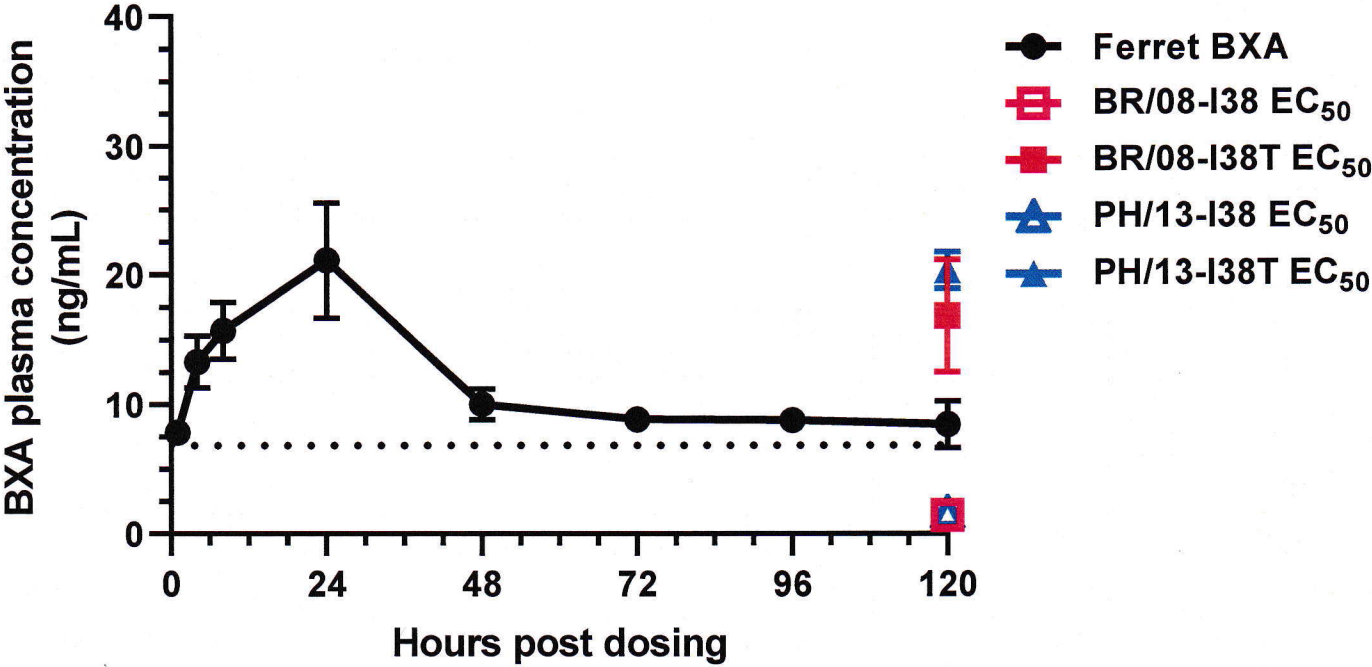
36

## References

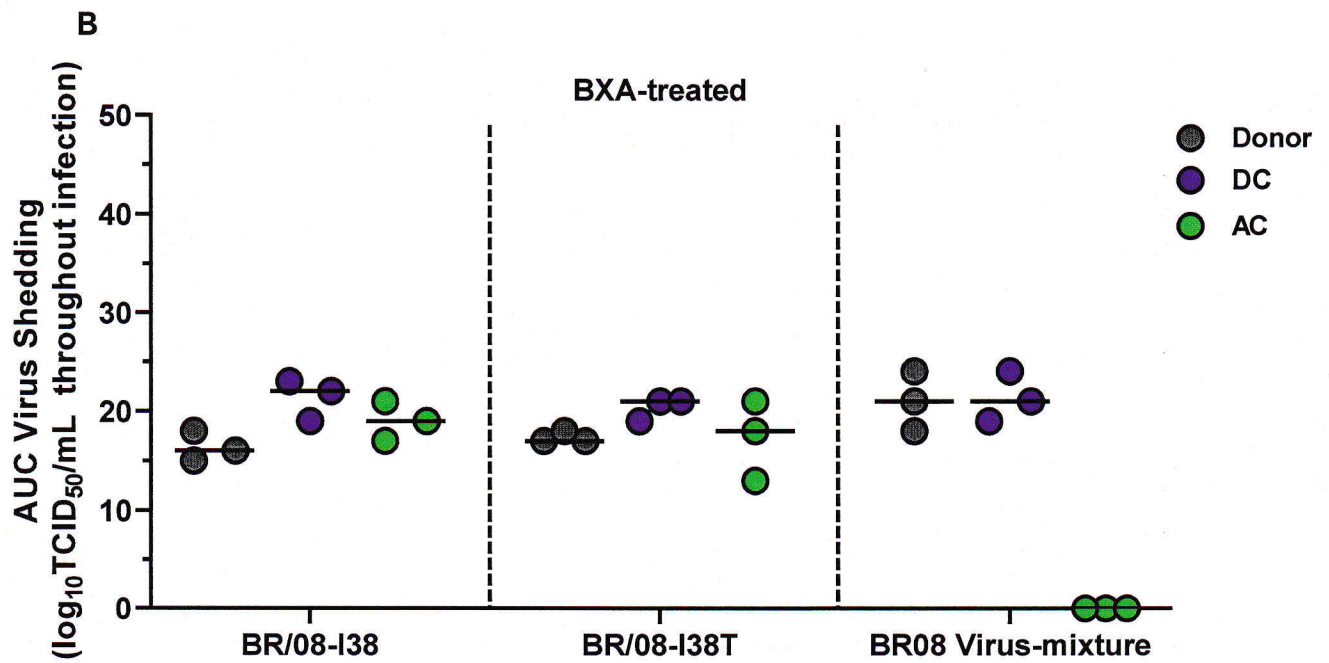
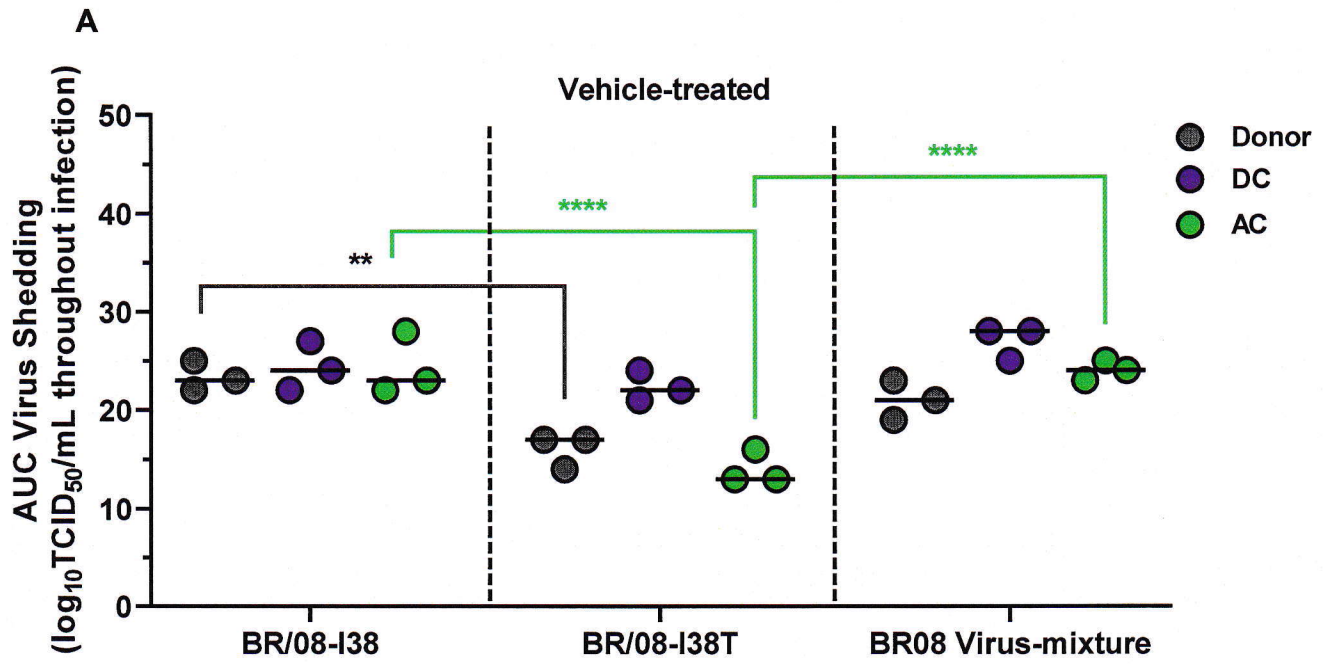
37

- 38 1. Jones JC, Pascua PNQ, Fabrizio TP, Marathe BM, Seiler P, Barman S, Webby RJ,  
39 Webster RG, Govorkova EA. 2020. Influenza A and B viruses with reduced baloxavir  
40 susceptibility display attenuated in vitro fitness but retain ferret transmissibility. Proc Natl  
41 Acad Sci U S A 117:8593–8601. doi: 10.1073/pnas.1916825117
- 42 2. Koshimichi H, Ishibashi T, Kawaguchi N, Sato C, Kawasaki A, Wajima T. 2018.  
43 Safety, tolerability, and pharmacokinetics of the novel anti-influenza agent baloxavir  
44 marboxil in healthy adults: phase I study findings. Clin Drug Investig 38:1189–1196. doi:  
45 [10.1007/s40261-018-0710-9](https://doi.org/10.1007/s40261-018-0710-9)

Supplemental Figure S1

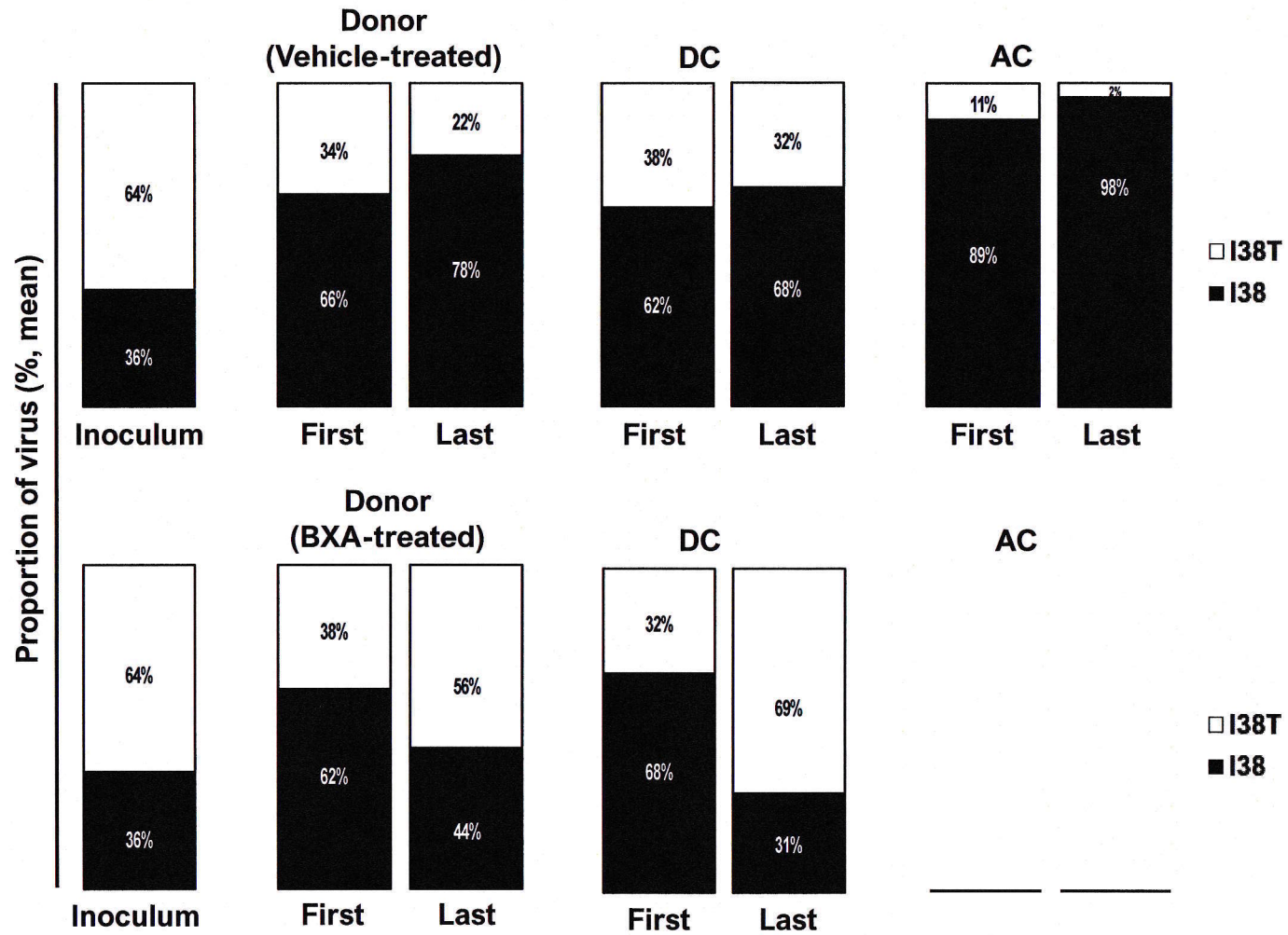


Supplemental Figure S2

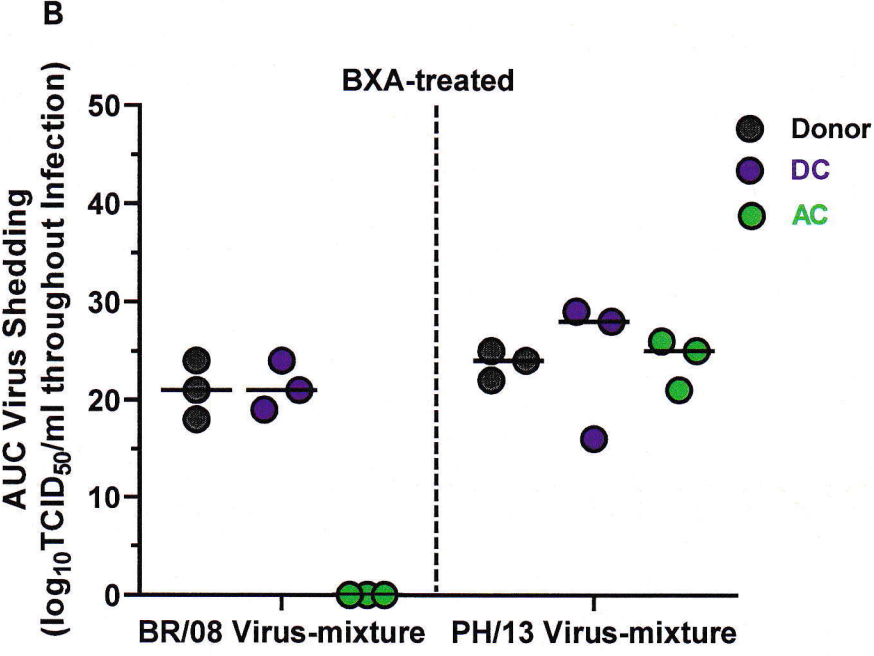
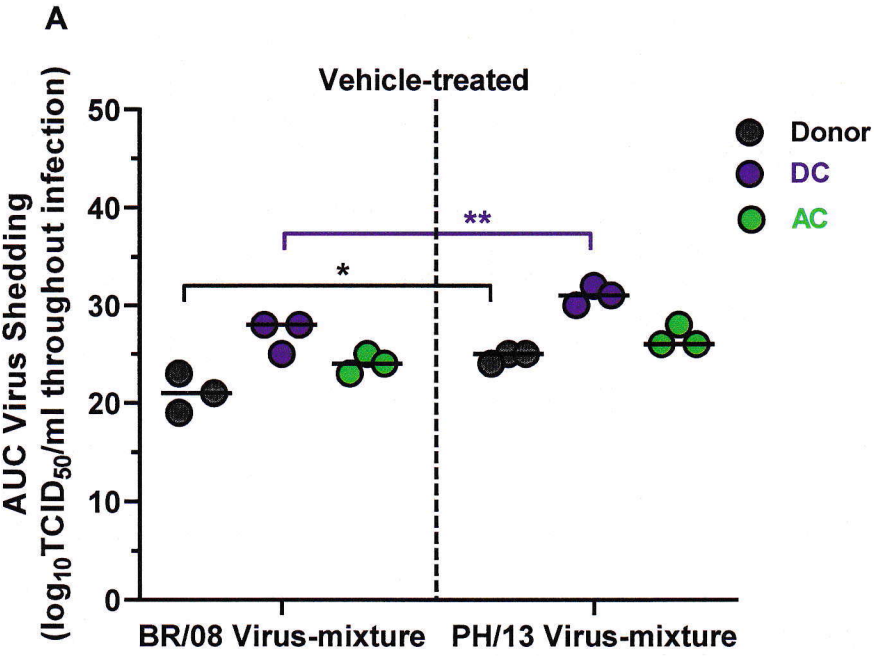




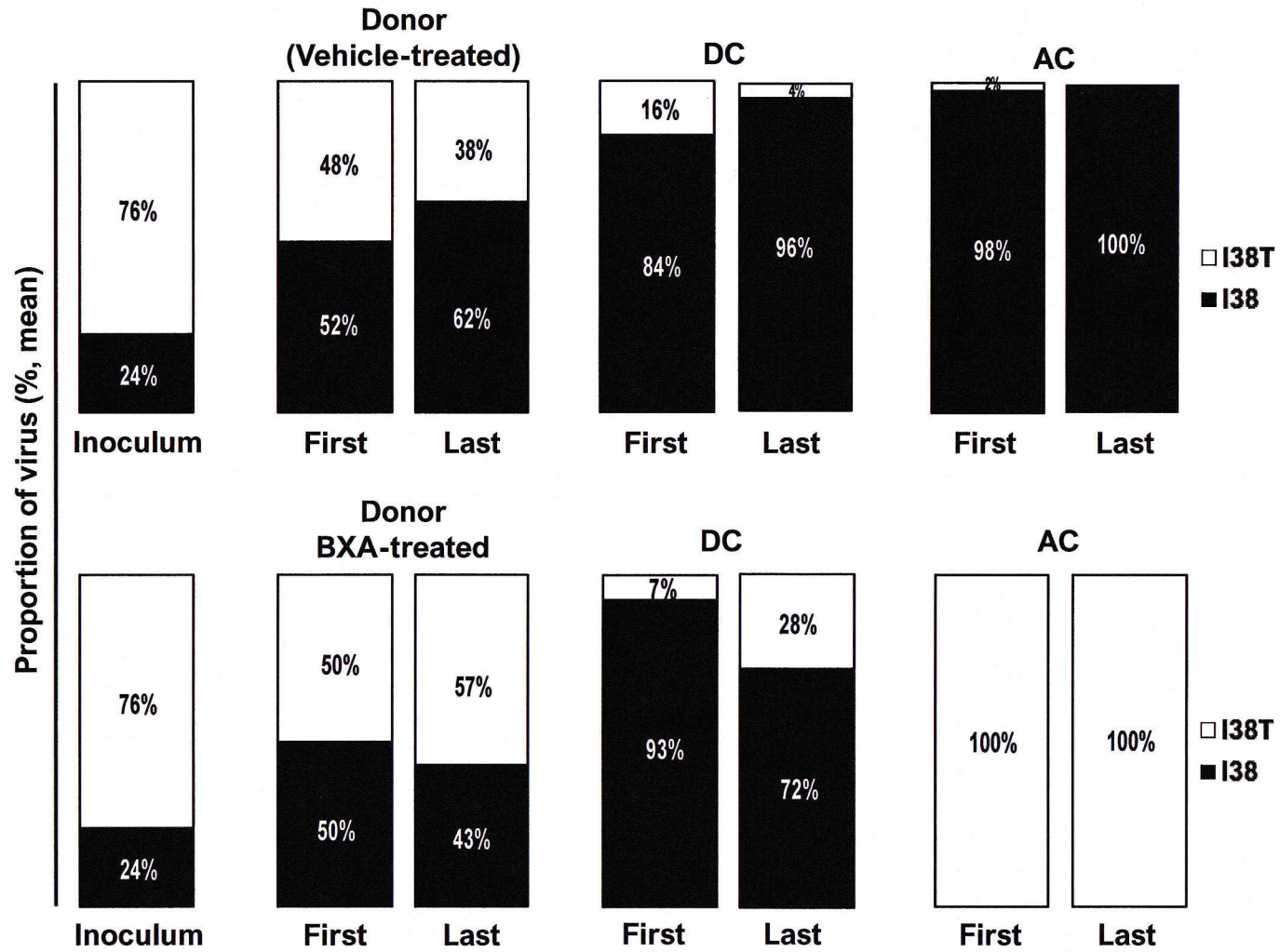
Supplemental Figure S3



Supplemental Figure S4



Supplemental Figure S5





**Supplemental Table S1.** Virus pathogenicity, transmission, and seroconversion in ferrets inoculated with BXA-Sen or BXA-Res IBV.

Experimental group	Antiviral treatment <sup>a</sup>	Status	Maximum weight loss (%) <sup>b</sup>	Body temp. elevation (°C) <sup>c</sup>	Virus detection	Day of onset of virus shedding <sup>d</sup>	Day of highest virus shedding <sup>e</sup>	Serology <sup>f</sup>
<b>BR/08-I38 (BXA-Sen)</b>	Control	Donor	3.4 (0.8)	0.9 (0.3)	3/3	2	2	3/3 (160–640)
		DC	0.6 (1.1)	0.3 (0.3)	3/3	4	6	3/3 (160–640)
		AC	1 (1.0)	0.3 (0.1)	3/3	4 to 6	6 to 10	3/3 (160–320)
	BXA	Donor	7.8 (4.1)	1 (0.8)	3/3	2	2	3/3 (160–640)
		DC	0 (0)	0.4 (0.2)	3/3	4 to 8	4 to 6	3/3 (160–640)
		AC	2.6 (4.4)	0.7 (0.5)	3/3	8 to 12	6 to 10	3/3 (160–320)
<b>BR/08-I38T (BXA-Res)</b>	Control	Donor	7.9 (2)	0.9 (0.2)	3/3	2	2	3/3 (160–320)
		DC	2.0 (1.5)	0.5 (0.2)	3/3	4 to 6	6 to 8	3/3 (160–320)
		AC	0 (0)	0.5 (0.3)	3/3	8 to 12	10 to 14	3/3 (320–640)
	BXA	Donor	3.3 (0.6)	1.7 (0.2)	3/3	2	2 to 4	3/3 (320–640)
		DC	0 (0)	0.7 (0.3)	3/3	4 to 8	6 to 10	3/3 (160–640)
		AC	0 (0)	0.9 (0.5)	3/3	8 to 12	8 to 14	3/3 (80–320)

<sup>a</sup>Subcutaneous administration of a single dose of BXA at 8 mg/kg or 0.5% methylcellulose to donor ferrets. Methylcellulose recipients were designated as untreated controls.

<sup>b</sup>Mean, relative to initial body weight at 0 dpi (standard deviation from mean is shown in parenthesis).

<sup>c</sup>Mean, relative to initial body temperature at 0 dpi (standard deviation from mean is shown in parenthesis).

<sup>d</sup>Days post donor inoculation with a positive nasal wash in at least one ferret.

<sup>e</sup>Days post donor inoculation.

<sup>f</sup>Number of ferrets exhibiting serum neutralizing titers of  $\geq 1:40$  reciprocal endpoint dilution to homologous virus/total number of ferrets in the group. HI titers are indicated in parentheses.

**Supplemental Table S2.** Virus pathogenicity, transmission, and seroconversion in ferrets co-inoculated with mixtures of BXA-Sen and BXA-Res IBVs

Experimental group	Antiviral treatment <sup>a</sup>	Status	Maximum weight Loss (%) <sup>b</sup>	Body temp. elevation (°C) <sup>c</sup>	Virus detection	Day of onset of virus shedding <sup>d</sup>	Day of highest virus shedding <sup>e</sup>	Serology <sup>f</sup>
<b>BR/08-I38:I38T (BXA-Sen:BXA-Res)</b>	Control	Donor	0 (0)	1.8 (0.3)	3/3	2 (3/3)	2	3/3 (80–160)
		DC	0.7 (0.6)	0.6 (0.4)	3/3	4 (3/3)	4 to 6	3/3 (80–160)
		AC	0 (0)	0.3 (0.3)	3/3	4 (3/3)	5	3/3 (80–160)
	BXA	Donor	2.3 (3)	0.7 (0.3)	3/3	2 (3/3)	2	3/3 (160)
		DC	1.0 (0.6)	0.7 (0.5)	3/3	4 (2/3)	4 to 6	3/3 (80–160)
		AC	0.3 (0.6)	0.5 (0.1)	0/3	0 (0/3)	— <sup>g</sup>	0/3 (<20)
<b>PH/13-I38:I38T (BXA-Sen:BXA-Res)</b>	Control	Donor	0 (0)	0.9 (0.7)	3/3	2 (3/3)	2 to 4	3/3 (80–160)
		DC	0 (0)	0.5 (0.2)	3/3	4 (3/3)	4 to 6	3/3 (80–160)
		AC	0 (0)	0.4 (0.1)	3/3	6 (3/3)	6 to 10	3/3 (160)
	BXA	Donor	0 (0)	1.2 (0.6)	3/3	2 (3/3)	2	3/3 (80)
		DC	0 (0)	0.5 (0.2)	3/3	4 (2/3)	6 to 12	3/3 (80–160)
		AC	0 (0)	0.7 (1.1)	3/3	8 (1/3)	10 to 16	3/3 (80–320)

<sup>a</sup>Subcutaneous administration of a single dose of BXA at 8 mg/kg or 0.5% methylcellulose to donor ferrets. Methylcellulose recipients were designated as untreated controls.

<sup>b</sup>Mean, relative to initial body weight at 0 dpi (standard deviation from mean is shown in parenthesis).

<sup>c</sup>Mean, relative to initial body temperature at 0 dpi (standard deviation from mean is shown in parenthesis).

<sup>d</sup>Days post donor inoculation with a positive nasal wash in at least one ferret (number of ferrets that met the criterion/total number of ferrets in the group).

<sup>e</sup>Days post donor inoculation.

<sup>f</sup>Number of ferrets exhibiting serum neutralizing titers of  $\geq 1:40$  reciprocal endpoint dilution to homologous virus/total number of ferrets in the group. HI titers are indicated in parentheses.

<sup>g</sup>No virus detected.