

Supplemental Material

Detection of naturally infected bats

The first rabid bat from cage U (unvaccinated controls) was found dead in its cage on day 5 pi. Three additional bats in the cage subsequently developed signs of clinical rabies and were euthanized on days 50, 61, and 91 pi. The only bat in cage U which survived both the natural outbreak and experimental RABV challenge demonstrated a moderate level of RVNA (1.0 IU/mL; Table 4) during baseline sampling and remained seropositive at least until day 13 pi and was DFA negative upon censoring on day 76 pi. Although all cage U bats were challenged experimentally with RABV, the four rabid bats in cage U were infected with a big brown bat RABV that typed as an eastern *E. fuscus* lineage (e1) along with the experimental RABV challenge inoculum, but the inoculum and outbreak viruses consistently differed by 1.2% across 1350bp of nucleoprotein sequence. Following demonstration of a different RABV variant infecting bats from this cage, we determined that bat #65 had a minimum incubation period of 60 days, considering the date of intake from the wild.

Table S1. Cage assignments and treatment details for 57 big brown bats (*Eptesicus fuscus*) used to evaluate the safety, immunogenicity and efficacy of ERA-g333 recombinant rabies virus vaccine.

Experiment	Cage ID	RABV		Bat ID	Comment
		Vaccination	Infection		
		Route	Dose (log 10 MICLD50)		
1	L	IM	2.9	39	
1	L	IM	2.9	496	
1	L	IM	2.9	47	
1	L	IM	2.9	48	
1	L	IM	NI	45	
1	M	IM	2.9	51	
1	M	IM	2.9	55	
1	M	IM	2.9	38	
1	M	IM	2.9	41	
1	M	IM	NI	50	
1	N	Oral	2.9	500	
1	N	Oral	2.9	52	
1	N	Oral	2.9	53	
1	N	Oral	2.9	59	
1	N	NV	NI	31	Contact control
1	N	Oral	NI	463	

1	O	Oral	2.9	44	
1	O	Oral	2.9	43	
1	O	Oral	2.9	40	
1	O	Oral	NI	60	
1	O	NV	NI	33	Contact control
1	O	Oral	NI	61	
1	P	NV	2.9	49	
1	P	NV	2.9	58	
1,2	P	NV	NI	42	Contact control (experiment 1)
1,2	P	NV	NI	72	Contact control (experiment 1)
1,2	P	NV	NI	54	Contact control (experiment 1)
1	Q	NV	2.9	32	
1	Q	NV	2.9	34	
1	Q	NV	2.9	35	
1	Q	NV	2.9	36	
1	Q	NV	2.9	37	
2	R	IM	2.9	66	
2	R	IM	2.9	12	
2	R	IM	2.9	19	
2	R	IM	2.9	26	
2	R	IM	NI	28	
2	S	IM	2.9	69	
2	S	IM	2.9	10	

2	S	IM	2.9	20
2	S	IM	2.9	22
2	S	IM	NI	29
2	V	IM	2.9	67
2	V	IM	2.9	14
2	V	IM	2.9	15
2	V	IM	2.9	23
2	V	IM	NI	25
2	T	NV	2.9	465
2	T	NV	2.9	16
2	T	NV	2.9	18
2	T	NV	2.9	24
2	T	NV	2.9	27
2	U	NV	2.9	65
2	U	NV	2.9	11
2	U	NV	2.9	17
2	U	NV	2.9	21
2	U	NV	2.9	30

IM=intramuscular, NV=not vaccinated, NI=not infected

Table S2 Survival outcomes among five unvaccinated control big brown bats that were experimentally challenged with $10^{2.9}$ MICLD₅₀ of a big brown bat rabies virus. An outbreak of a naturally-acquired big brown bat rabies virus variant was detected post-hoc.

		Final
Cage	Bat ID	Disposition ^a
U	65	D(5)
U	11	D(50)
U	17	D(91)
U	21	N
U	30	D(61)

a D=died from infection with non-inoculum big brown bat rabies virus, with post-infection day in parentheses; N=tested negative for rabies virus, censored on day 76 pi