

Supplementary Appendix

Supplemental to: Gu, C. *et. al.*, Development of broadly protective influenza B vaccines.

NPJ vaccines

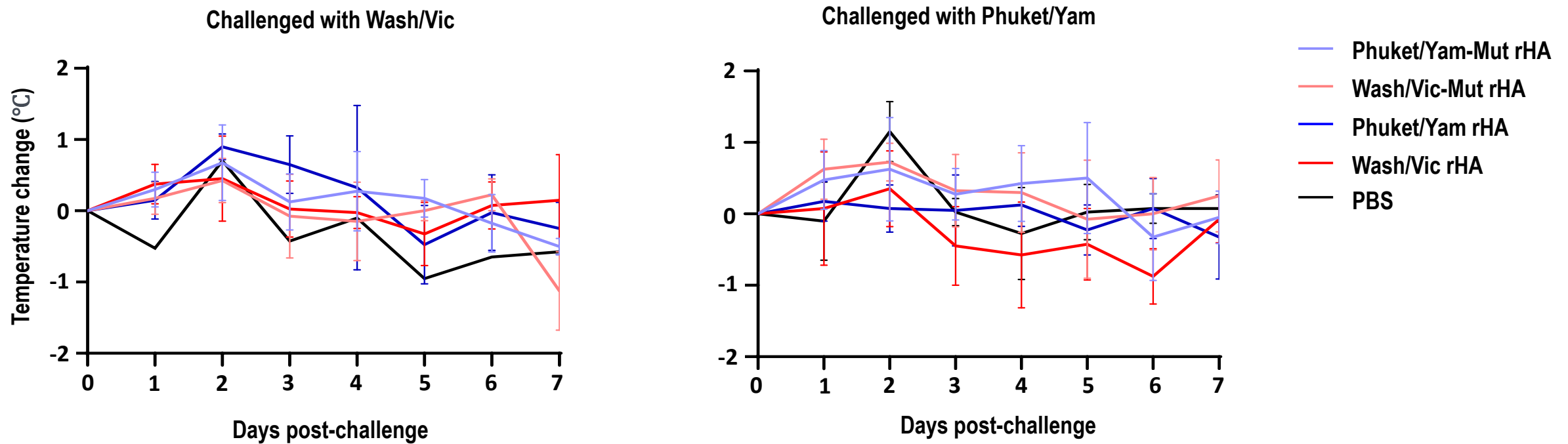
This appendix has been provided by the authors to give the readers additional information about the work.

Development of broadly protective influenza B vaccines

Supplementary Appendix

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Supplementary Figure 1. Body temperature changes of immunized ferrets after challenge with wild-type Phuket/Yam and Wash/Vic viruses. Ferrets were immunized and challenged as shown in Figure 3a. Body temperatures were measured daily until Day 7 post-infection.

Supplementary Data 1. Overview of Wash/Vic and Phuket/Yam mutants

			HI titer		Amino acid differences between Wash/Vic and Phuket/Yam*																														Additional mutations																
			anti-Phuket/Yam serum	anti-Wash/Vic serum	40	48	56	71	73	75	76	80	81	116	117	122	126	129	133	136	137	146	148	149	150	163	164	165	166	168	175	198	202	203	209	230	233	252	255	262	267	299	313	66	157	189	190	207	238	276	282
Control viruses	B/Phuket/3073/2013	160	20	Y	R	D	M	V	T	T	K	A	K	I	Q	D	K	G	R	L	A	S	K	I	K	D	N	Y	N	V	K	K	S	N	D	D	M	P	V	V	E	K	A	A	T	V	D	G	A	V	A
	B/Wash/Vic/02/2019	<20	10240	H	E	K	K	T	K	I	R	V	H	V	H	N	D	R	E	I	I	N	G	N	Δ	Δ	K	N	T	I	E	A	K	K	G	N	V	S	T	I	K	E	.	.	.	.	.	.	.	.	.
	Phuket/YamHA1-Wash/VicHA2-Wash/VicNA**	160	40	Y	R	D	M	V	T	T	K	A	K	I	Q	D	K	G	R	L	A	S	K	I	K	D	N	Y	N	V	K	K	S	N	D	D	M	P	V	V	E	K	A	A	T	D	G	A	A	V	A
	Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA	160	20	Y	R	D	M	V	T	T	K	A	K	I	Q	D	K	G	R	L	A	S	K	I	K	D	N	Y	N	V	K	K	S	N	D	D	M	P	V	V	E	K	A	A	T	D	G	A	A	V	A
	Wash/VicHA1-Phuket/YamHA2-Wash/VicNA	20	10240	H	E	K	K	T	K	I	R	V	H	V	H	N	D	R	E	I	I	N	G	N	Δ	Δ	K	N	T	I	E	A	K	K	G	N	V	S	T	I	K	E	.	.	.	.	.	.	.	.	.
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA	20	10240	H	E	K	K	T	K	I	R	V	H	V	H	N	D	R	E	I	I	N	G	N	Δ	Δ	K	N	T	I	E	A	K	K	G	N	V	S	T	I	K	E	.	.	.	.	.	.	.	.	.
HI titers ≥80 against anti-Phuket/Yam and anti-Wash/Vic serum	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-32	160	320	H	.	K	.	.	K	I	R	V	.	.	H	N	.	.	E	.	I	N	.	.	.	.	.	.	T	I	E	A	.	.	G	.	V	S	.	.	.	.	.	.	.	.	.	.	.	.	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-51	160	160	H	E	.	.	.	K	.	.	V	H	V	.	N	D	.	E	.	I	N	.	.	.	.	.	K	.	I	.	A	K	.	G	.	V	.	T	.	E	.	.	.	.	.	.	.			
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-93	160	80	H	.	K	.	T	.	I	.	V	.	.	.	N	D	.	E	.	I	.	.	.	.	.	.	K	N	.	.	E	.	.	.	N	V	.	T	I	K	.	.	.	.	.	.	.	.		
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-7	160	640	.	.	.	.	T	K	I	R	.	H	.	H	N	.	.	E	.	I	N	.	.	.	.	.	.	K	N	.	I	E	A	K	.	.	N	V	S	T	.	K	.	.	.	.	.	.		
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-25	160	80	.	.	E	K	K	T	K	.	.	V	H	.	H	.	D	.	E	.	.	.	.	.	.	.	.	T	I	.	E	A	K	K	.	.	V	S	T	.	.	.	.	.	.	.	.			
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-32	160	640	.	.	.	.	.	.	K	.	.	V	H	V	.	.	.	E	.	.	N	.	N	.	.	.	K	N	T	I	E	.	.	K	.	N	V	S	.	I	.	E	.	.	.	Y	.	.		
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-33	160	160	H	.	.	.	T	.	.	R	.	.	.	.	H	N	D	.	E	.	.	.	.	.	.	.	.	K	.	I	E	A	K	.	.	.	S	.	I	.	E	.	.	.	.	.				
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-46	80	320	H	E	K	.	T	K	.	.	.	H	.	.	.	.	.	E	.	I	.	.	.	.	N	.	K	.	T	.	I	E	A	K	.	G	.	V	.	T	I	K	E	.	.	.	.			
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-49	80	80	.	E	.	K	.	.	.	I	.	V	H	.	.	.	.	E	.	I	.	G	N	.	.	.	N	.	I	E	A	K	.	.	.	.	V	.	.	.	.	.	.	.	.	.				
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-60	80	1280	H	.	K	.	.	.	I	R	V	H	.	H	N	D	.	E	.	I	.	.	.	.	N	.	K	N	.	.	E	A	K	.	.	N	V	S	T	.	E	.	.	.	.	.	.	.		
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-76***	80	320	H	E	.	K	.	K	.	R	V	H	V	H	.	.	.	E	.	I	.	N	G	.	.	.	K	N	.	I	.	A	K	K	.	.	V	.	T	.	.	.	.	.	.	.	.	.		
	Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-7	80	80	H	.	K	.	.	.	I	R	V	H	V	.	N	D	R	E	.	I	.	.	.	.	.	.	N	.	.	.	E	.	.	K	.	.	V	S	.	K	E	.	.	.	.	.	.			
	Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-12	80	80	.	E	K	.	.	.	I	R	.	H	V	.	N	D	R	E	.	I	.	.	.	.	.	.	.	.	N	T	I	E	A	K	K	G	.	.	.	.	.	.	.	.	.	.	.	.		
	Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-13	80	80	H	E	K	.	T	K	.	.	V	H	.	H	N	.	.	E	.	I	.	.	.	.	.	.	.	N	.	E	.	K	.	G	N	.	S	.	I	K	E	.	.	.	.	.	.			
	Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-32	160	320	.	E	.	.	.	.	.	R	V	H	.	H	.	.	.	E	.	I	.	N	.	.	.	.	.	K	N	.	I	E	.	K	.	G	N	V	S	.	K	E	.	.	.	.	.	.		
	Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-34	80	80	H	.	K	K	T	.	.	R	V	.	.	.	H	.	D	.	E	.	I	.	.	.	.	.	.	N	.	I	E	.	.	.	.	N	.	.	T	.	E	.	.	.	.	.	.			
	Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-40	160	80	.	E	.	K	T	.	.	R	V	.	.	.	N	D	.	E	.	I	.	.	.	.	.	.	.	.	K	.	.	.	E	.	K	K	G	N	V	S	T	I	K	E	.	.	.	.		
	Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-64	80	80	.	E	K	.	T	K	.	R	.	H	.	.	N	.	.	E	.	.	N	.	N	.	.	.	.	K	.	I	.	A	.	K	G	.	.	T	.	.	.	.	.	.	.	.	.			
	Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-73	160	160	H	.	.	.	.	.	K	I	.	V	H	.	H	N	D	.	E	.	I	.	G	.	.	.	.	.	.	T	.	A	.	K	.	.	V	.	I	K	E	.	.	.	.	.	.			
	Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-76	160	160	.	E	K	K	.	K	I	.	.	V	.	.	N	.	.	E	.	.	N	.	.	.	.	.	.	.	.	.	E	.	K	.	G	N	V	.	.	K	E	.	.	.	.	.	.			
	Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-4	160	80	H	E	.	.	K	T	K	.	.	V	.	V	.	N	D	.	E	.	.	.	.	.	.	.	.	.	T	I	E	A	K	K	.	.	V	S	.	I	K	.	.	.	.	.	.			
	Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-11	320	320	H	.	.	.	.	.	.	R	V	.	.	V	H	.	.	E	.	I	.	.	.	.	.	.	.	K	N	.	I	.	A	K	K	.	N	V	S	T	I	.	.	.	.	.	.			
	Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-18	160	160	H	.	K	.	T	K	I	R	.	H	.	.	.	N	D	.	E	.	I	.	.	.	.	.	N	.	.	I	E	A	K	.	G	N	.	.	.	.	.	.	.	.	.	.	.	.		
	Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-29	320	2560	H	E	.	.	T	.	I	R	.	H	.	.	.	.	.	R	E	.	.	.	.	.	.	.	.	K	N	T	.	A	K	.	.	.	V	S	.	I	.	E	.	.	.	.	.			
	Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-92	160	80	H	E	.	.	.	.	.	.	V	H	.	H	N	D	R	E	.	.	.	.	.	.	.	.	.	K	.	T	.	A	.	.	.	.	.	.	T	I	K	E	.	T	.	.	.			
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-5	80	20480	H	.	K	.	T	.	I	R	V	H	V	.	.	.	D	R	E	.	.	N	.	N	Δ	Δ	K	N	T	.	E	A	.	.	.	N	.	.	T	I	K	E	.	.	.	.	.			
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-21	80	1280	H	.	K	K	T	.	.	.	V	H	.	.	.	N	D	R	.	.	I	.	.	.	.	.	Δ	Δ	.	.	.	I	E	A	.	K	.	N	V	.	T	I	.	E	.	.	.	.		
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-29***	80	2560	H	E	K	K	T	K	.	.	V	H	.	.	N	.	.	.	.	I	.	.	G	.	.	Δ	Δ	K	N	.	I	E	.	K	K	.	N	.	T	I	.	E	.	.	.	.	.			
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-87	160	10240	.	E	K	K	T	.	.	.	H	V	.	.	.	.	D	.	E	.	I	.	.	.	.	.	Δ	Δ	K	.	T	I	.	E	A	K	K	.	N	V	.	T	I	K	E	.	.	.	.	
	Wash/VicHA1-Phuket/YamHA2-Wash/VicNA-7	80	5120	.	E	K	K	T	K	I	R	.	.	.	.	.	.	.	D	.	E	.	I	.	.	.	.	Δ	Δ	.	N	T	.	E	A	K	K	.	N	V	.	I	.	.	.	.	.				
	Wash/VicHA1-Phuket/YamHA2-Wash/VicNA-9	80	160	.	E	.	.	.	.	I	R	.	H	.	.	.	.	.	E	.	I	.	.	.	.	.	N	Δ	Δ	K	.	.	E	.	K	K	.	N	.	.	T	.	K	E	.	.	.	.			
	Wash/VicHA1-Phuket/YamHA2-Wash/VicNA-10	320	640	H	.	.	.	.	.	.	I	.	.	V	.	N	.	.	E	.	I	.	.	.	.	.	Δ	Δ	K	.	.	I	.	A	.	K	.	N	V	.	.	.	.	.	.	.	.	.	.		
	Wash/VicHA1-Wash/VicHA2-Wash/VicNA-7	160	5120	H	.	.	.	.	.	I	R	.	.	V	H	.	D	.	E	.	I	.	.	.	.	.	Δ	Δ	.	N	T	I	.	A	K	K	.	N	V	.	.	K	E	.	.	.	.	.	.		
	HI titers ≥80 against anti-Phuket/Yam serum and ≤40 against anti-Wash/Vic serum	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-1	80	40																																															

HI titers ≤40 against anti-Wash/Vic and anti-Phuket/Yam serum	Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-74	40	80	. . . K . K . . V H V H N D . . . N G T . E A . K . . V S T I K E	
	Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-76	<20	640	. E K K . . . V H V . . R E I . N G N . . . K N T I E A K K G N . S T . K E	
	Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-90	40	320	. E K K T K . R V . . H N . R E I I . G N . . . I E A K K G . S . I . K E	
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-4	20	10240	H K . . V . . H N . R E . . . G . Δ Δ K . . . I E . K K . N . . I . . E	
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-15	40	10240	H E K . T K I R V V H V H N D . . . I N G . Δ Δ . N T I E . A K K . N . . T I . K E	
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-16	<20	2560	H . . K . K . . R V . V H N D . . . I N G N Δ Δ . N T I E . A K K . N . . T I K E	
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-19	20	2560	H . . K . T . I R V H V H . . . E I . N G N Δ Δ . N T I E . K K . N . . T I . .	
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-26	<20	20480	H . . . T K I R V . . N D R E I . N G N Δ Δ K N T . . A . K . N V . . I . . V	
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-28	20	2560	H . . . K T . . . H V H . D . . I . N G N Δ Δ . N . . . A K K . N . . I . . I	
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-41	40	2560	. . . K . T . . . H . . N D R . . I . G . Δ Δ . N T . . . A K K . N V . . I . E	
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-53	20	2560	H . . . T K I R V . . . D G N Δ Δ K N T . E A . K . N V . . I . E	
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-79	20	10240	H . . . K . K . R V H . . D . . I . G . Δ Δ K N T I E A K K . N V . . I . .	
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-83	<20	640	H E K . T . I R V H . H . . R . I . . G N Δ Δ . . . I E A . . N V . T . K E	
	Wash/VicHA1-Phuket/YamHA2-Phuket/YamNA-84	<20	2560	H . . . T K I R V . . . D G N Δ Δ K N T . E A . K . N V . . I . E	
	Wash/VicHA1-Phuket/YamHA2-Wash/VicNA-1	40	5120	. E K . T K . R V H V H N . R E . . . G . Δ Δ K . . . I E . K K . N V . . I K .	
	Wash/VicHA1-Phuket/YamHA2-Wash/VicNA-6	40	2560	H E . . . K . R . H . . D . . I . N . N Δ Δ K N T I N V . . I . .	
	Wash/VicHA1-Phuket/YamHA2-Wash/VicNA-16	<20	80	H . . K . T K . R . H . H N D . E . I . N G N Δ Δ . . . I . . . N V . . I . .	
	Wash/VicHA1-Phuket/YamHA2-Wash/VicNA-17	<20	80	. E . . T K . R V H . H . D . E I I . G N Δ Δ . N . . . A . . N V . . K E	
	Wash/VicHA1-Wash/VicHA2-Phuket/YamNA-1	<20	10240	H E . K . K . . V . V H . D . E . . . N Δ Δ K N T . E . K K . N V . . I . .	
	Wash/VicHA1-Wash/VicHA2-Phuket/YamNA-38	20	10240	H E . K T K R E . . . N G . Δ Δ K N T . I E . K K . N V . T . K E	
	Wash/VicHA1-Wash/VicHA2-Phuket/YamNA-44	40	2560	H R V . . . H . D . . I . . N Δ Δ K N T N . . I . .	
	Wash/VicHA1-Wash/VicHA2-Wash/VicNA-2	<20	2560	. E K K T . I R V . . N D . E I I . N . N Δ Δ . . . I E A K . N V . T . .	
	Wash/VicHA1-Wash/VicHA2-Wash/VicNA-3	20	5120	H E K K T K . R V H V . N D R E N Δ Δ . N T I E . K K . N . . I K E	
	Wash/VicHA1-Wash/VicHA2-Wash/VicNA-6	<20	320	H . . K K T K I R V H I N . N Δ Δ . N . . I E . . . N V . T I K	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-3	20	40	. E . K T K . R V . V H . . . E . I . . G N . . . K N . . . E . K K G . . V . . I . .	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-6	<20	40	 K . R V . V H N . . . E . . . N G N . . . N T I E A K . . N V S T . . .	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-10	20	20	 T K . R . . . N D . E I . . N G N . . . K . . . I E . K K G N V . T I . .	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-12	40	20	. . . K K T . I R V H V H . . D . . . I N G I E . K K G . V S . . .	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-16	<20	20	 T N . . . E I . . N G K . T I E . . K G N V S T . K E	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-34	<20	40	H . . . K . K I R D R E I . . G N . . . E . K K . N V S T . .	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-36	40	40	H . . K K T . I R . H V . N . . E . . . N . . . N T . E A K . G . V . T I . .	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-37	20	<20	. E . K T K . R V H V . N . . . I I N G N . . . N . . . E A . K G N V . T . .	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-43	20	20	H K . R V H V I . . G N . . . K N . . . E A K K . N V S . . K	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-49	40	20	 I . V . V . . D R E I I . . N N . . . A K K G N . . I K .	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-54	40	<20	 I . V . V H . D . E . . . G T . . . K . G . . S T . .	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-57	20	<20	 K T . . . R . . . H N G N T I E . K . G . . . E	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-58	20	40	 V . . H . D . E I I N G N E . K . . . S . . K E	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-59	40	40	H E . . . T K I . V H V H N D . E I I . G N T . E . K . G . . S . .	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-61	20	20	. E . K T K . R . H . H N D . E . I . G T . E . K I . .	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-64	20	20	. E K K T . R V H D . . . I N G N I E . K K . . V S . .	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-72	<20	<20	. E . . . K . . . H . H N D . E . . . G N T . E . K K G N . . T . K E	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-75	<20	40	H . . K K . K I . V . . H . . . E I . . G N . . . N T I G N . S . I K E	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-77	40	40	. . . K . K . . . H N D . E . I K E . . . N V S T . K .	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-78	20	20	H E K K . . R V . . . N . . . E . I . G N . . . N T I E . . . G . V . T I K .	
	Phuket/YamHA1-Phuket/YamHA2-Phuket/YamNA-87	40	20	H . . . K . . I . V . V H . D . E . I T . E . K K G . V . . K E	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-2	<20	40	H . . K T . I R . H . . D . . I . . G N . . . K . . . E . . . V S T I . E	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-3	<20	40	. . . K . T H . D . E . . . N G N . . . K N T I . A . . G N V . .	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-4	40	40	H . . . K T . R V H . . N . . . E . . . G N I E A K . . V S . . K	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-13	40	20	. E . K T K I R V H V E I . . G I . . K . . V S T . .	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-18	<20	40	 T . . R V H V . . D . . . I . G N . . . K . . . E . . K G . . I K .	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-20	<20	20	. E . K . K I . . H . H . . R E . . . G N N . . . E . . G N . . T . K E	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-22	<20	40	H . . K K T . I R . H . . N G N . . . K N T I E A K T I K E	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-24	<20	20	H . . . T K I . V H . H N . . R E . . . N G N T . E . K . . V . T I .	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-27	40	40	. E K . . K . . . H . . N . . . I I . . N . . . K N . . . E A . K G N . . I K E	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-31	40	40	H . . K T . I . . H V . N . . . E . . . N . . . N . . I E A K K . . V S T . K E	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-40	<20	40	H . . . K T K I R V . V H . D . E I . . G . . . K . . I . A . . G N V . T I K E	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-50	<20	40	 K I . . H . . N . . . E I I . G N N T . . A K K . . N . S T I K E	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-53	40	40	 T K . . . H . H . . E I I . N N . . . A . . G . . S . I K E	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-62	20	40	H . . . T . I . V H V . . D . . . I . G N N . . E A . K . . S . K E	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-65	20	40	H E . . . K . R V H . . D R E N . . I E . . . G N V S T . K .	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-66	40	40	. . . K K . . R V H . . N D R G . . . K N . . E A . K . N V . I .	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-75	20	20	H . . . T K I R V . V . N D . E . I . N G N N . . I . . . V S T I .	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-79	<20	20	H E . K . . I R V H . . . D R E . . . N N . . . E A K K . . . I K .	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-81	<20	20	H E . K T . . I . . H E . I . G N E A K . G N V . T I K .	
	Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-83	<20	20	H V H . . . D N G N . . . K N T I E A K K . N V . T I K E	

Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-88	<20	40	H . K K T K . . . H G N . . . K N . I E A K . . . S . I K E
Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-90	<20	40	H . K . T K . R . H . . . D . E . I N G N . . . K . T . E . K K . N V S
Phuket/YamHA1-Phuket/YamHA2-Wash/VicNA-92	20	40	. . K . T . . I R V . . . N D . . . I I N . N . . . K N T . E A . . G N V S . I
Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-6	<20	40	. E . K V H . . . N . . . E . I N G N . . . N . . . E . K . G . . . T I
Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-9	40	20	H E . K T K . R V . . . H N . . . I I . N . . . K N T . . A . K . N V . T I . E
Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-21	40	40	H . . K . . . V H . H . D . . . I . N G N . . . K . . I E A K . . . V S . I K E
Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-22	20	40	H E K K . . I . V H V H . D R . . I I N G N . . . N T I . . A K . . . V S
Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-35	40	20	H . . K T K . R V H V H N D G . . . K . . I . A . K G N V S
Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-51	<20	20	. E K K T . . I . V H V H N . . . E . I N G N . . . K . . I E . K K G N V S
Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-54	40	40	. E K K . . R V E . I . . N E . K S T I
Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-63	40	40	. E . . . K . . V . V H . D N I E A K K G . V . T
Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-79	<20	40	. E . . T . . I . V . . . N D R . . I . N G N . . . K N T I E A K . . . T I
Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-84	20	40	H E . K . K . . . H . H N D . E I . N . N . . . K N . . E A . . . N V S . . . E S
Phuket/YamHA1-Wash/VicHA2-Phuket/YamNA-95	40	<20	H . K V H V . N D . E I . . G N T I . . K K . . V S
Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-3	20	40	 T K . R V H . . N . . E I I . G N T . E A K K . . V . A . K
Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-12	20	40	. E . K T K . R V H . H . . R E . I N . N T . E A . K G . V . T I
Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-14	<20	40	H . K . T . . I . . H . H N D . E . I N G N . . . K N . . E A . . G . V . T
Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-16	<20	40	H R V H . . N D R . . . I N G N . . . N . . E . K . . . V S T . K
Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-19	40	40	 T K . R V H . . N . . E I I . G N T . E A K K . . V . T . K
Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-21	<20	40	H . K K . . I . . H . . N D . E I I . . N N T I . . K . G N . . T
Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-26	<20	40	H . . . T . . . V H V H N D . . I I . G N E . K K . N
Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-33	<20	40	. . . K . . . I . . H . H N D . E . . . G N . . . N . . E A K . . N . . T I K E
Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-41	20	20	H . . . T . . I R V H V H N D D . G N E A . . . N . S . . K E
Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-42	40	20	H . . K T K I . . V . . H N D R N N T I E . . . V H
Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-44	<20	40	H . K . . K H E . . . N G N T I E . K K G N V . I
Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-81	20	20	. E . K T K I R . H V H . . . E I . N G . . . K N . I E . . K . N . S T I
Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-84	40	40	. . K K T . . R V H V E I . N . N . . . N T I . A . K . . V . T I K
Phuket/YamHA1-Wash/VicHA2-Wash/VicNA-88	40	20	H E . K . . . I R V H . H N . . . E I I N G N . . . K N . I . A . . . G . V . T I K E

*Dots denote the same amino acid as Phuket/Yam

**Virus names indicate the origin of HA1, HA2, and NA

(dots indicate the same amino acid as Phuket/Yam)

***Used for ferret vaccination study

Supplementary Data 2. HI titers used for antigenic cartography. Hemagglutination inhibition assays were performed to establish the antigenic cartography of influenza B viruses. Refer to excel file “Gu et al – Supplementary Data 2” for data.