

Supplementary Material

Supplementary Figures

Victoria Lineage (B-V)		
Use	Strain	Sequence
Vaccine	B/Maryland/15/2016	MKAIIVLLMVVTSNADRICTGITSNSPHVVKATQGEVNVTVGVIPLTTPTKSHFANLKGTRGKLCPC KCLNCTDLVALGRPKCTGKIPARSVILHEVRPVTSGCFPIIMHRTKIRQLPNLLRGYEHVRLSTHNV NAEADAGPGPYKIGTSGSCPNIITNGNGFFATMAWAVPDKNKATNPLTIEVPYCTEGEDQITVWGFHSDN EIQMAKLYGDSKPKQFTSSANGVTTHYVSIQIGFNPQTEDGGLPQSGRIVVDYMQKSGKTGTITYQRG LLPQKVVWCASGRSKVIKGSPLIGEADCLHEKYGGLNKSPPYTGHEHAKAIGNCPIWVKTPKLANGTKY RPPAKLLKERGGFAGIAGFLEGGWEGMIAGWGHYTSHGAGHVAVAADLKSTQEAINKITKNLSLELE VKNLQRLSGAMDELHNEILEDEKVDLDRADTSSQIELAVLLSNEGIINSEDEHLLALERLKKMLGSPA VEIGNGCFETKHKCNQTCLDRIAAGTFDAGEFSLPTFDSLNTAASLNDGDLNHTILLYSTAASSLAV VTLMIAIFVVMVSRDINVSCISCL
	B/Iowa/06/2017	MKAIIVLLMVVTSNADRICTGITSNSPHVVKATQGEVNVTVGVIPLTTPTKSHFANLKGTRGKLCPC KCLNCTDLVALGRPKCTGKIPARSVILHEVRPVTSGCFPIIMHRTKIRQLPNLLRGYEHVRLSTHNV NAEAGAPGGPYKIGTSGSCPNIITNGNGFFATMAWAVPDKNKATNPLTIEVPYCTEGEDQITVWGFHSDN ETQMAKLYGDSKPKQFTSSANGVTTHYVSIQIGFNPQTEDGGLPQSGRIVVDYMQKSGKTGTITYQRG LLPQKVVWCASGRSKVIKGSPLIGEADCLHEKYGGLNKSPPYTGHEHAKAIGNCPIWVKTPKLANGTKY RPPAKLLKERGGFAGIAGFLEGGWEGMIAGWGHYTSHGAGHVAVAADLKSTQEAINKITKNLSLELE VKNLQRLSGAMDELHNEILEDEKVDLDRADTSSQIELAVLLSNEGIINSEDEHLLALERLKKMLGSPA VEIGNGCFETKHKCNQTCLDRIAAGTFDAGEFSLPTFDSLNTAASLNDGDLNHTILLYSTAASSLAV VTLMIAIFVVMVSRDINVSCISCL
ELISA	B/Brisbane/60/2008	MKAIIVLLMVVTSNADRICTGITSNSPHVVKATQGEVNVTVGVIPLTTPTKSHFANLKGTRGKLCPC KCLNCTDLVALGRPKCTGKIPARSVILHEVRPVTSGCFPIIMHRTKIRQLPNLLRGYEHVRLSTHNV NAENAGPGPYKIGTSGSCPNIITNGNGFFATMAWAVPDKNKATNPLTIEVPYCTEGEDQITVWGFHS DNEAQMAYLYGDSKPKQFTSSANGVTTHYVSIQIGFNPQTEDGGLPQSGRIVVDYMQKSGKTGTITYQR GILLPQKVVWCASGRSKVIKGSPLIGEADCLHEKYGGLNKSPPYTGHEHAKAIGNCPIWVKTPKLANGTK YRPPAKLLKERGGFAGIAGFLEGGWEGMIAGWGHYTSHGAGHVAVAADLKSTQEAINKITKNLSLELE VKNLQRLSGAMDELHNEILEDEKVDLDRADTSSQIELAVLLSNEGIINSEDEHLLALERLKKMLGSPA SAVEIGNGCFETKHKCNQTCLDRIAAGTFDAGEFSLPTFDSLNTAASLNDGDLNHTILLYSTAASSL AVTLMIAIFVVMVSRDINVSCISCL
Neutralization	B/Colorado/06/2017	MKAIIVLLMVVTSNADRICTGITSNSPHVVKATQGEVNVTVGVIPLTTPTKSHFANLKGTRGKLCPC KCLNCTDLVALGRPKCTGKIPARSVILHEVRPVTSGCFPIIMHRTKIRQLPNLLRGYEHVRLSTHNV NAEAGAPGGPYKIGTSGSCPNIITNGNGFFATMAWAVPDKNKATNPLTIEVPYCTEGEDQITVWGFHSDN ETQMAKLYGDSKPKQFTSSANGVTTHYVSIQIGFNPQTEDGGLPQSGRIVVDYMQKSGKTGTITYQRG LLPQKVVWCASGRSKVIKGSPLIGEADCLHEKYGGLNKSPPYTGHEHAKAIGNCPIWVKTPKLANGTKY RPPAKLLKERGGFAGIAGFLEGGWEGMIAGWGHYTSHGAGHVAVAADLKSTQEAINKITKNLSLELE VKNLQRLSGAMDELHNEILEDEKVDLDRADTSSQIELAVLLSNEGIINSEDEHLLALERLKKMLGSPA VEIGNGCFETKHKCNQTCLDRIAAGTFDAGEFSLPTFDSLNTAASLNDGDLNHTILLYSTAASSLAV VTLMIAIFVVMVSRDINVSCISCL
Yamagata Lineage (B-Y)		
Use	Strain	Sequence
Vaccine	B/Singapore/INFIT-16-0610/2016	MVVTSNADRICTGITSNSPHVVKATQGEVNVTVGVIPLTTPTKSYFANLKGTRTRGKLCPCD CLNCTDLVALGRPMCVGTTSPAKASILHEVRPVTSGCFPIIMHRTKIRQLPNLLRGYEKIRLSTQNV DAEKAPGGPYRLGTSGSCP NATSKIGFFATMAWAVPDKNYKNATNPQTEVEVPYCTEGEDQITVWGFHSDN KTQMKSLYGDSNPKQFTSSANGVTTHYVSIQIGFNPQTEDGGLPQSGRIVVDYMQKSGKTGTITYQRG VLLPQKVVWCASGRSKVIKGSPLIGEADCLHEEYGLNKSPPYTGHEHAKAIGNCPIWVKTPKLANGTKY YRPPAKLLKERGGFAGIAGFLEGGWEGMIAGWGHYTSHGAGHVAVAADLKSTQEAINKITKNLSLELE VKNLQRLSGAMDELHNEILEDEKVDLDRADTSSQIELAVLLSNEGIINSEDEHLLALERLKKMLGSPA AVDIGNCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNTAASLNDGDLNHTILLYSTAASSLA VTLMIAIFVVMVSRDINVSCISCL
ELISA/ Neutralization	B/Phuket/3073/2013	MKAIIVLLMVVTSNADRICTGITSNSPHVVKATQGEVNVTVGVIPLTTPTKSYFANLKGTRTRGKLCPC DCLNCTDLVALGRPMCVGTTSPAKASILHEVRPVTSGCFPIIMHRTKIRQLPNLLRGYEKIRLSTQNV DAEKAPGGPYRLGTSGSCP NATSKIGFFATMAWAVPDKNYKNATNPQTEVEVPYCTEGEDQITVWGFHSDN KTQMKSLYGDSNPKQFTSSANGVTTHYVSIQIGFNPQTEDGGLPQSGRIVVDYMQKSGKTGTITYQRG VLLPQKVVWCASGRSKVIKGSPLIGEADCLHEEYGLNKSPPYTGHEHAKAIGNCPIWVKTPKLANGTKY YRPPAKLLKERGGFAGIAGFLEGGWEGMIAGWGHYTSHGAGHVAVAADLKSTQEAINKITKNLSLELE VKNLQRLSGAMDELHNEILEDEKVDLDRADTSSQIELAVLLSNEGIINSEDEHLLALERLKKMLGSPA AVDIGNCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNTAASLNDGDLNHTILLYSTAASSLA VTLMIAIFVVMVSRDINVSCISCL
Challenge	B/Florida/04/2006	MKAIIVLLMVVTSNADRICTGITSNSPHVVKATQGEVNVTVGVIPLTTPTKSYFANLKGTRTRGKLCPC DCLNCTDLVALGRPMCVGTTSPAKASILHEVVPVTSGCFPIIMHRTKIRQLPNLLRGYENIRLSTQNV DAEKAPGGPYRLGTSGSCP NATSKIGFFATMAWAVPDKNYKNATNPQTEVEVPYCTEGEDQITVWGFHSD DKTQMKNLYGDSNPKQFTSSANGVTTHYVSIQIGFNPQTEDGGLPQSGRIVVDYMQKSGKTGTITYQRG VLLPQKVVWCASGRSKVIKGSPLIGEADCLHEKYGGLNKSPPYTGHEHAKAIGNCPIWVKTPKLANGTKY YRPPAKLLKERGGFAGIAGFLEGGWEGMIAGWGHYTSHGAGHVAVAADLKSTQEAINKITKNLSLELE VKNLQRLSGAMDELHNEILEDEKVDLDRADTSSQIELAVLLSNEGIINSEDEHLLALERLKKMLGSPA AVEIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNTAASLNDGDLNHTILLYSTAASSLA VTLMIAIFVVMVSRDINVSCISCL

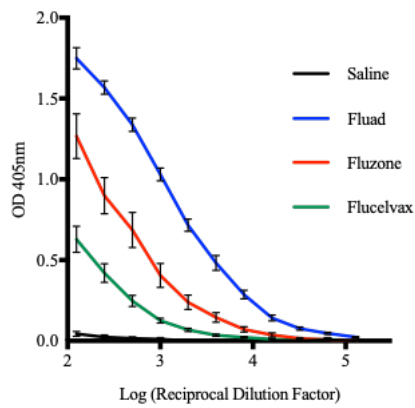
Supplementary Fig. S1. Sequences of influenza B viral HA proteins used in this study. Sequences are for influenza B HA in commercial vaccines, and proteins/viruses used in ELISA and neutralization assays and challenge experiments. Sequences were downloaded from different databases such the NCBI GenBank. Influenza B HA of Victoria lineage is denoted as B-V, while Influenza B HA of Yamagata-lineage is denoted as B-Y.

These are all the pairwise sequence identity percentages (from chimera), and also attached is a graphic of the multiple sequence alignment.

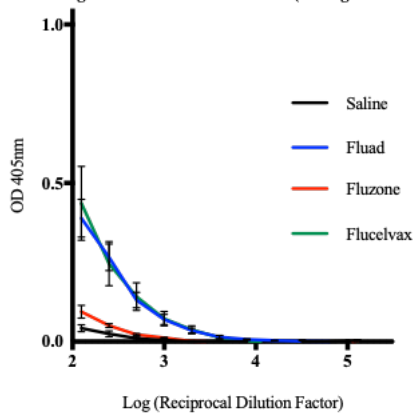
AGM53847.1 hemagglutinin [Influenza B virus (B/Florida/04/2006)] vs. AGM53847.1 hemagglutinin [Influenza B virus (B/Florida/04/2006)]: 100.00% identity
 AGM53847.1 hemagglutinin [Influenza B virus (B/Florida/04/2006)] vs. ANC28539.1 hemagglutinin [Influenza B virus (B/Brisbane/60/2008)]: 93.49% identity
 AGM53847.1 hemagglutinin [Influenza B virus (B/Florida/04/2006)] vs. ASW32353.1 hemagglutinin [Influenza B virus (B/Maryland/15/2016)]: 92.97% identity
 AGM53847.1 hemagglutinin [Influenza B virus (B/Florida/04/2006)] vs. AQY15026.1 hemagglutinin [Influenza B virus (B/Iowa/06/2017)]: 93.14% identity
 AGM53847.1 hemagglutinin [Influenza B virus (B/Florida/04/2006)] vs. ASK81305.1 hemagglutinin [Influenza B virus (B/Colorado/06/2017)]: 92.97% identity
 ANC28539.1 hemagglutinin [Influenza B virus (B/Brisbane/60/2008)] vs. AGM53847.1 hemagglutinin [Influenza B virus (B/Florida/04/2006)]: 93.49% identity
 ANC28539.1 hemagglutinin [Influenza B virus (B/Brisbane/60/2008)] vs. ANC28539.1 hemagglutinin [Influenza B virus (B/Brisbane/60/2008)]: 100.00% identity
 ANC28539.1 hemagglutinin [Influenza B virus (B/Brisbane/60/2008)] vs. ASW32353.1 hemagglutinin [Influenza B virus (B/Maryland/15/2016)]: 99.14% identity
 ANC28539.1 hemagglutinin [Influenza B virus (B/Brisbane/60/2008)] vs. AQY15026.1 hemagglutinin [Influenza B virus (B/Iowa/06/2017)]: 99.14% identity
 ANC28539.1 hemagglutinin [Influenza B virus (B/Brisbane/60/2008)] vs. ASK81305.1 hemagglutinin [Influenza B virus (B/Colorado/06/2017)]: 98.97% identity
 ASW32353.1 hemagglutinin [Influenza B virus (B/Maryland/15/2016)] vs. AGM53847.1 hemagglutinin [Influenza B virus (B/Florida/04/2006)]: 92.97% identity
 ASW32353.1 hemagglutinin [Influenza B virus (B/Maryland/15/2016)] vs. ANC28539.1 hemagglutinin [Influenza B virus (B/Brisbane/60/2008)]: 99.14% identity
 ASW32353.1 hemagglutinin [Influenza B virus (B/Maryland/15/2016)] vs. ASW32353.1 hemagglutinin [Influenza B virus (B/Maryland/15/2016)]: 100.00% identity
 ASW32353.1 hemagglutinin [Influenza B virus (B/Maryland/15/2016)] vs. AQY15026.1 hemagglutinin [Influenza B virus (B/Iowa/06/2017)]: 99.66% identity
 ASW32353.1 hemagglutinin [Influenza B virus (B/Maryland/15/2016)] vs. ASK81305.1 hemagglutinin [Influenza B virus (B/Colorado/06/2017)]: 99.49% identity
 AQY15026.1 hemagglutinin [Influenza B virus (B/Iowa/06/2017)] vs. AGM53847.1 hemagglutinin [Influenza B virus (B/Florida/04/2006)]: 93.14% identity
 AQY15026.1 hemagglutinin [Influenza B virus (B/Iowa/06/2017)] vs. ANC28539.1 hemagglutinin [Influenza B virus (B/Brisbane/60/2008)]: 99.14% identity
 AQY15026.1 hemagglutinin [Influenza B virus (B/Iowa/06/2017)] vs. ASW32353.1 hemagglutinin [Influenza B virus (B/Maryland/15/2016)]: 99.66% identity
 AQY15026.1 hemagglutinin [Influenza B virus (B/Iowa/06/2017)] vs. AQY15026.1 hemagglutinin [Influenza B virus (B/Iowa/06/2017)]: 100.00% identity
 AQY15026.1 hemagglutinin [Influenza B virus (B/Iowa/06/2017)] vs. ASK81305.1 hemagglutinin [Influenza B virus (B/Colorado/06/2017)]: 99.83% identity
 ASK81305.1 hemagglutinin [Influenza B virus (B/Colorado/06/2017)] vs. AGM53847.1 hemagglutinin [Influenza B virus (B/Florida/04/2006)]: 92.97% identity
 ASK81305.1 hemagglutinin [Influenza B virus (B/Colorado/06/2017)] vs. ANC28539.1 hemagglutinin [Influenza B virus (B/Brisbane/60/2008)]: 98.97% identity
 ASK81305.1 hemagglutinin [Influenza B virus (B/Colorado/06/2017)] vs. ASW32353.1 hemagglutinin [Influenza B virus (B/Maryland/15/2016)]: 99.49% identity
 ASK81305.1 hemagglutinin [Influenza B virus (B/Colorado/06/2017)] vs. AQY15026.1 hemagglutinin [Influenza B virus (B/Iowa/06/2017)]: 99.83% identity
 ASK81305.1 hemagglutinin [Influenza B virus (B/Colorado/06/2017)] vs. ASK81305.1 hemagglutinin [Influenza B virus (B/Colorado/06/2017)]: 100.00% identity

Supplementary Fig. S2. Sequence comparison and sequence accession numbers of influenza B viral HA proteins used in this study. Accession numbers and strain names are given with the percent identity at the end of each line for the sequence comparison.

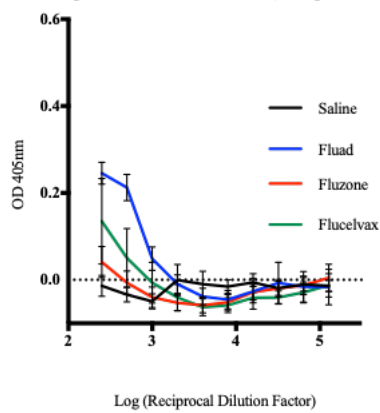
(A) Binding to HA1 B/Brisbane/60/2008 (Victoria Lineage)



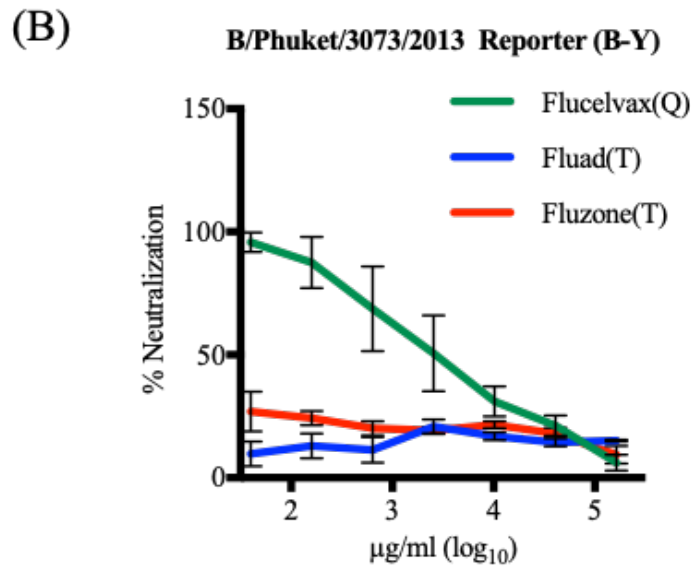
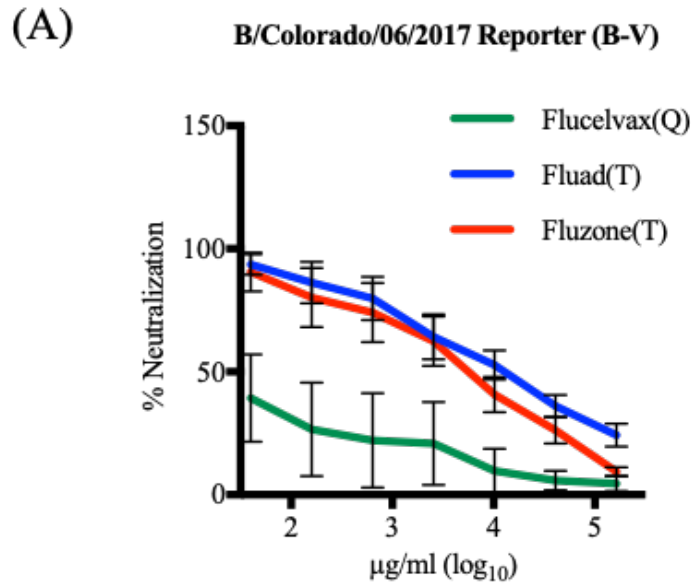
(B) Binding to HA1 B/Phuket/3073/2013 (Yamagata Lineage)



(C) Binding to HA0 B/Phuket/3073/2013 (Yamagata Lineage)



Supplementary Fig. S3. Dilution curves of the antibody binding from vaccinated mouse sera to influenza B HA1 proteins. Serially diluted sera from mice immunized with commercial vaccines Flud (T), Flucelvax (Q), and Fluzone (T) were analyzed by ELISA for binding to antigenically matched and mismatch influenza HA1 proteins representing (A) B/Brisbane/60/08 (Victoria-lineage), (B) B/Phuket/307/13 (Yamagata lineage) viruses.



Supplementary Fig. S4. Titration curves for neutralization of influenza B reporter viruses by sera from mice immunized with commercial influenza vaccines. Reporter influenza B viruses were (A) B/Colorado/17 (B-V) and (B) B/Phuket/307/13 (B-Y). Influenza B HA of Victoria lineage is denoted as B-V, while Influenza B HA of Yamagata-lineage is denoted as B-Y.

(A)

Histologic Score	
Bronchiole lumina	Exudate in bronchiole lumina. None (score 0 points), Exudate present (score 1 point); moderate (score 2 points); Severe-partial to complete occlusion of 1 or more central bronchioles (score 3 points)
Terminal bronchiole lumina	Exudate in terminal bronchiolar lumina. None (score 0 points), mild (score 1 point), moderate (score 2 points), Severe-partial to complete occlusion of 1 or more central bronchioles (score 3 points)
Bronchiolar/TB epithelial necrosis/degeneration	None (score 0 points); Mild (score 1 point); Moderate (score 2 points); Severe (score 3 points)
Perivascular inflammation/fibrin	None (score 0 points); Mild (score 1 point); Moderate (score 2 points); Severe (score 3 points)
Alveoli/interstitium edema	None (score 0 points); <25% (score 1 point); 25-50% (score 2 points); >50% (score 3 points)
Alveoli inflammatory cells	None-few (score 0 points); mild increase (score 1 point); moderate (score 2 points); Marked (score 3 points)
Interstitial inflammatory cells	None-few (score 0 points); mild increase (score 1 point); moderate (score 2 points); Marked (score 3 points)
Anywhere: hemorrhage/necrosis/fibrin	None (score 0 points); up to 5% of section (score 1 point); 5-50% of section (score 2 points); >50% of section (score 3 points)

(B)

Influenza B Immunohistochemical Score	
Trachea and bronchiolar epithelium	None (score 0 points), Up to 5% (score 1 point), 5-50% (score 2 points), >50% (score 3 points)
Terminal bronchiolar epithelium	None (score 0 points), Up to 5% (score 1 point), 5-50% (score 2 points), >50% (score 3 points)
Alveolar septa (with immunoreactive interstitial cells) immunoreactivity	None (score 0 points); up to 5% (score 1 point), 5-50% (score 2 points), >50 % (score 3 points)
Bronchiolar exudate	Immunostaining absent (score 0 points); Immunostaining present score 1 point)
Alveolar exudate (+/- immunoreactive alveolar macrophages)	Immunostaining absent (score 0 points); Immunostaining present score 1 point)

Supplementary Fig. S5. Scoring rubrics for pathology of lungs in mice challenged with influenza. (A) Histologic scoring. (B) Influenza B immunohistochemical scoring. Yamagata-lineage challenge virus was mouse-adapted B/Florida/04/2006.