

Supplementary Fig. 1

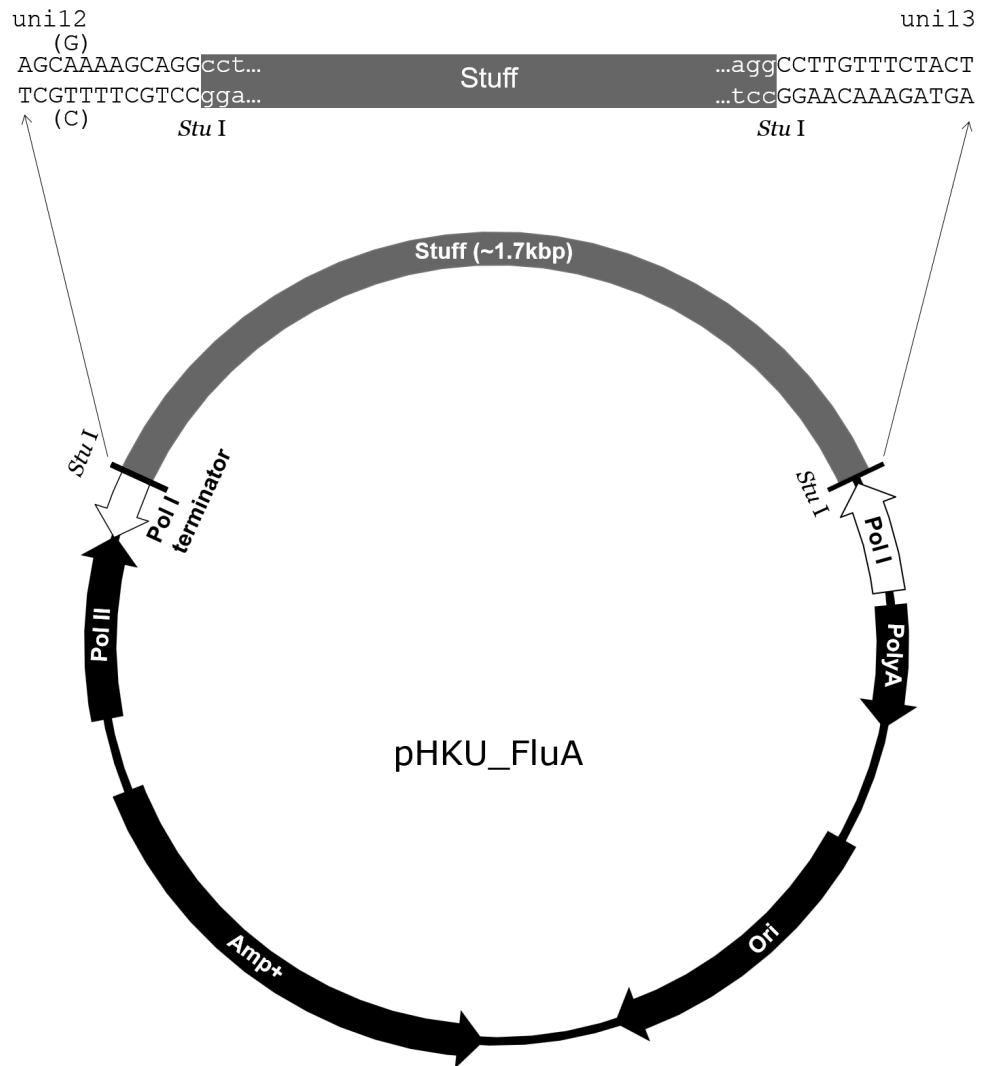
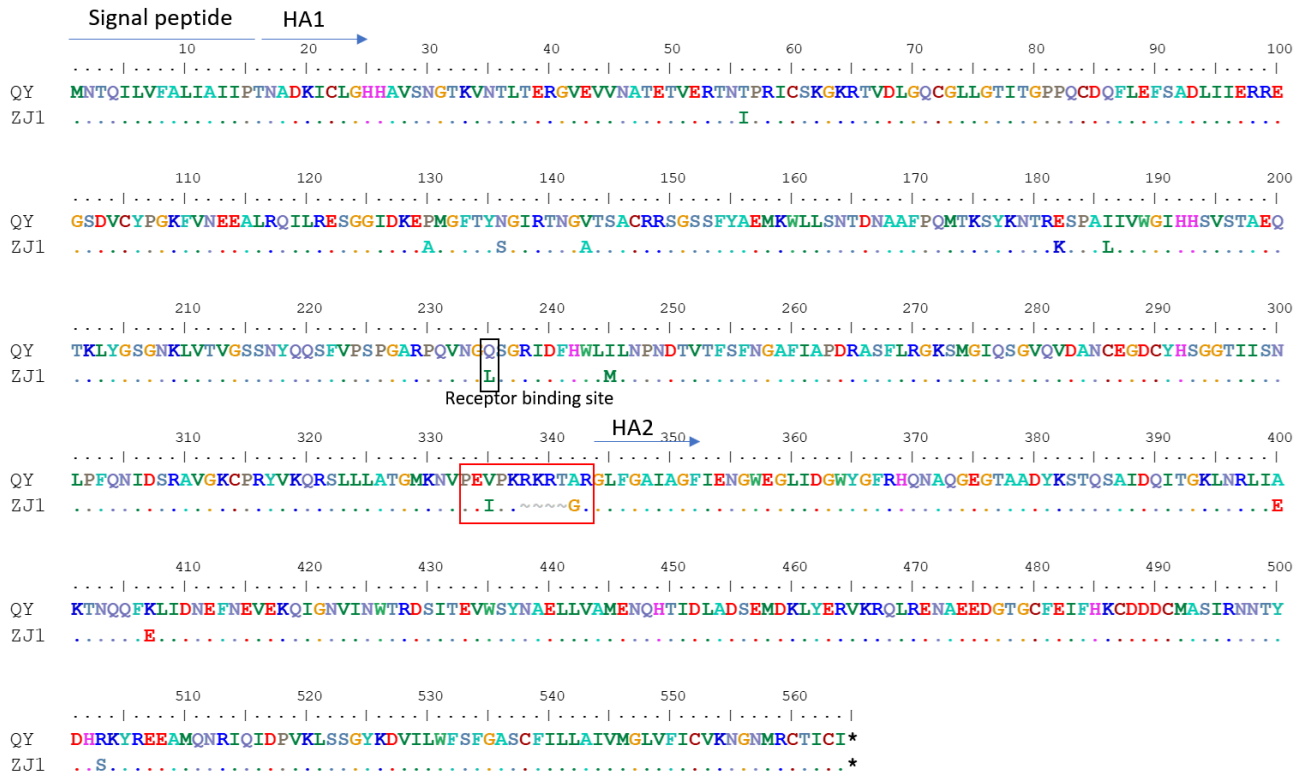


Figure S1 Physical map of the pHKU_FluA plasmid used as a backbone for construction of reverse genetic influenza A viruses in this study.

Supplementary Fig. 2A HA alignment



Supplementary Fig. 2B Reverse genetic viruses

Virus Sequence at HA cleavage site	
QY WT	PEVPKRKRRTAR/G
QY HAA	PEI PK ----GR/G
/, HA cleavage site	

Figure S2 HA sequence analysis and reverse genetics. (A) HA sequences from A/Qingyuan/GIRD1/2017 (H7N9) (QY) and A/Zhejiang/DTID-ZJU01/2013 (H7N9) (ZJ1) viruses are aligned. The black box indicates the HA receptor binding site (H3 numbering), and the red frame indicates the area that was engineered in the reverse genetic QY HAA virus. (B) Amino acid differences between QY WT and QY HAA.

Subtype	Phenotype	Location	Year	Strain name	Cleavage site	Accession No.
H7	LP				PEIPKGR/GLF	
					PELPKGR/GLF	
					PEPPKGR/GLF	
					PENPKTR/GLF	
					PESPKTR/GLF	
H7N9	HP	China	2017	A/Qingyuan/GIRD1/2017	PEVVKRKRRTAR/GLF	KY621542
				A/chicken/Guangdong/GD15/2016		EPI960361
				A/chicken/Guangdong/GD4/2017	PEVVKGRKTAR/GLF	KY855518
				A/Environment/Guangdong/C16283222/2016		EPI919615
				A/Guangdong/Th005/2017	PEVVKGRKIAR/GLF	EPI926825
H7N3	HP	Japan	2018	A/duck/Japan/AQ-HE30-1/2018	PEVVKRRTAR/GLF	LC416566
H7N4	LP	China	2018	A/chicken/Jiangsu/1/2018	PELPKGR/GLF	EPI_ISL_332358
H7N9	HP	USA	2017	A/chicken/Tennessee/17-007147-2/2017	PENPKTRKSRHRRTR/GLF	KY818811
H7N7	HP	Italy	2016	A/chicken/Italy/16VIR-1873/2016	PELPKGRKRR/GLF	EPI220955
H7N8	HP	USA	2016	A/turkey/Indiana/16-001403-1/2016	PENPKRKRTR/GLF	KU558906.1
H7N7	HP	UK	2015	A/chicken/England/26352/2015	PEIPRHRKGR/GLF	EPI623939
H7N7	HP	Germany	2015	A/chicken/Germany/AR1385/2015	PEIPKRRRR/GLF	FLI
H7N7	HP	Italy	2013	A/chicken/Italy/13VIR4527_11/2013	PETPKRRRR/GLF	KF569186.1
H7N3	HP	Mexico	2012-2018	A/chicken/Jalisco/CPA1/2012	PENPKDRKSRHRRTR/GLF	JX397993.1
				A/chicken/Puebla/CPA-04451/2016	PENPKDRKSRHRRTR/GLF	KX351916.1
				A/chicken/Jalisco/CPA-01859/2016	PENPKGRKSRHRKTR/GLF	KX351892.1
H7N7	HP	Spain	2009	A/chicken/Spain/6279-2/2009	PELPKGTKPRRR/GLF	GU121458.1
H7N7	HP	UK	2008	A/chicken/England/1158-11406/2008	PEIPKRRKR/GLF	FJ476173.1
H7N3	HP	Canada	2007	A/chicken/Saskatchewan/HR-00011/2007	PENPKTTKPRRR/GLF	EU500860.1
H7N7	HP	North Korea	2005	A/chicken/North Korea/1/2005	PEIPKGRHRRPKR/GLF	
H7N3	HP	Canada	2004	A/chicken/Canada/rv504/2004	PENPKQAYRKRMT/GLF	CY015006.1
					PENPKQAYQKRMT/GLF	
					PENPKQAYKKRMT/GLF	
					PENPKQAYHKRMT/GLF	
					PENPKQAHQKRMT/GLF	
					PENPKQAYRKRMT/GLF	
					PENPKQACQKRMT/GLF	
H7N1	LP	UAE	2004	A/houbara bustard/UAE/2004	PELPKRR/GLF	

Figure S3 Sequence at HA cleavage site determines pathogenicity.