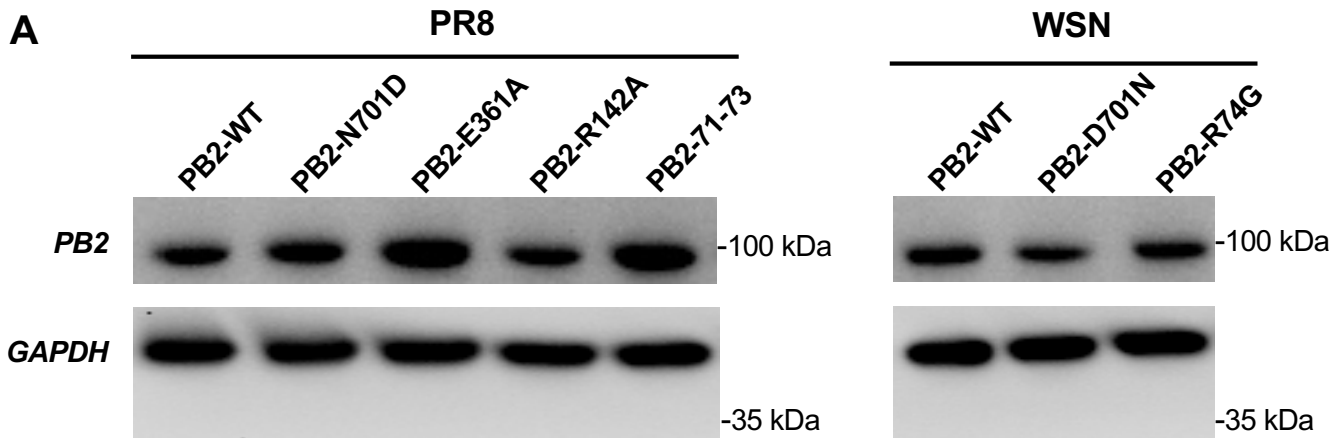
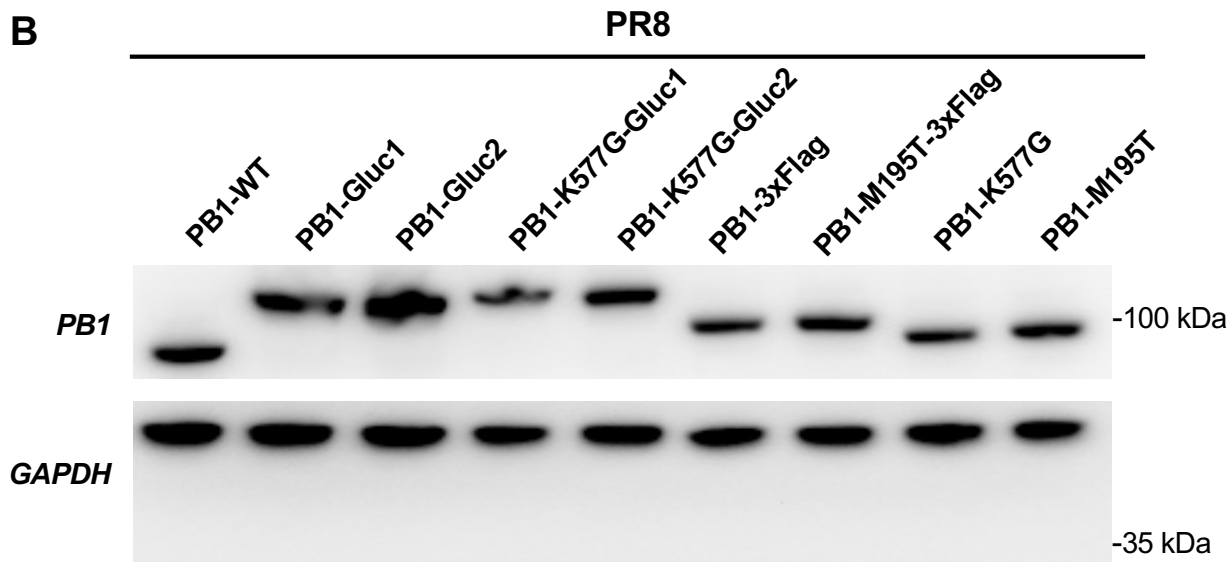


S1 Fig

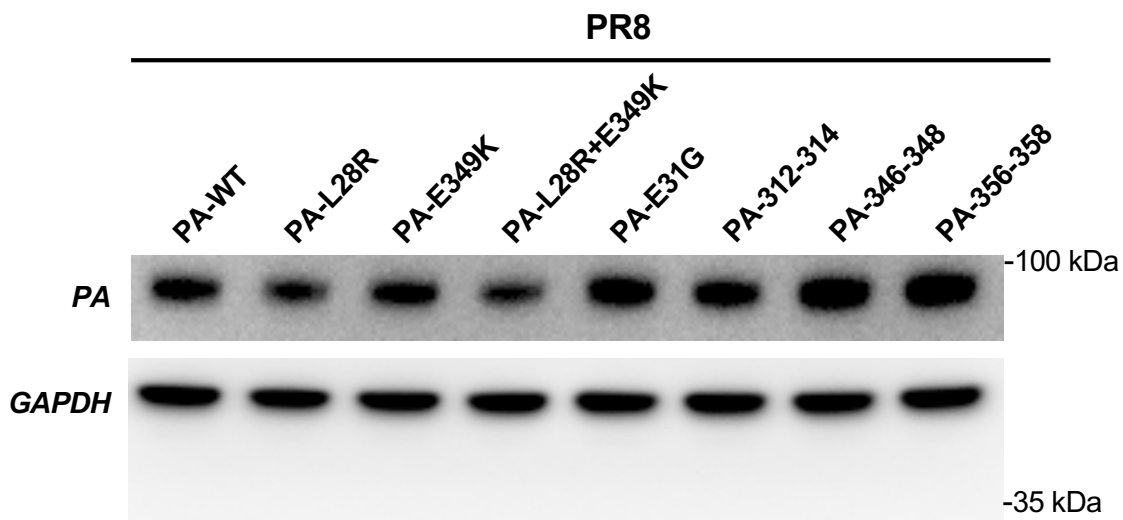
A



B










C

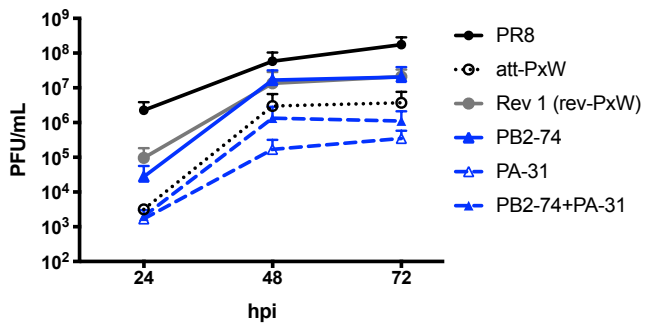


S2 Fig

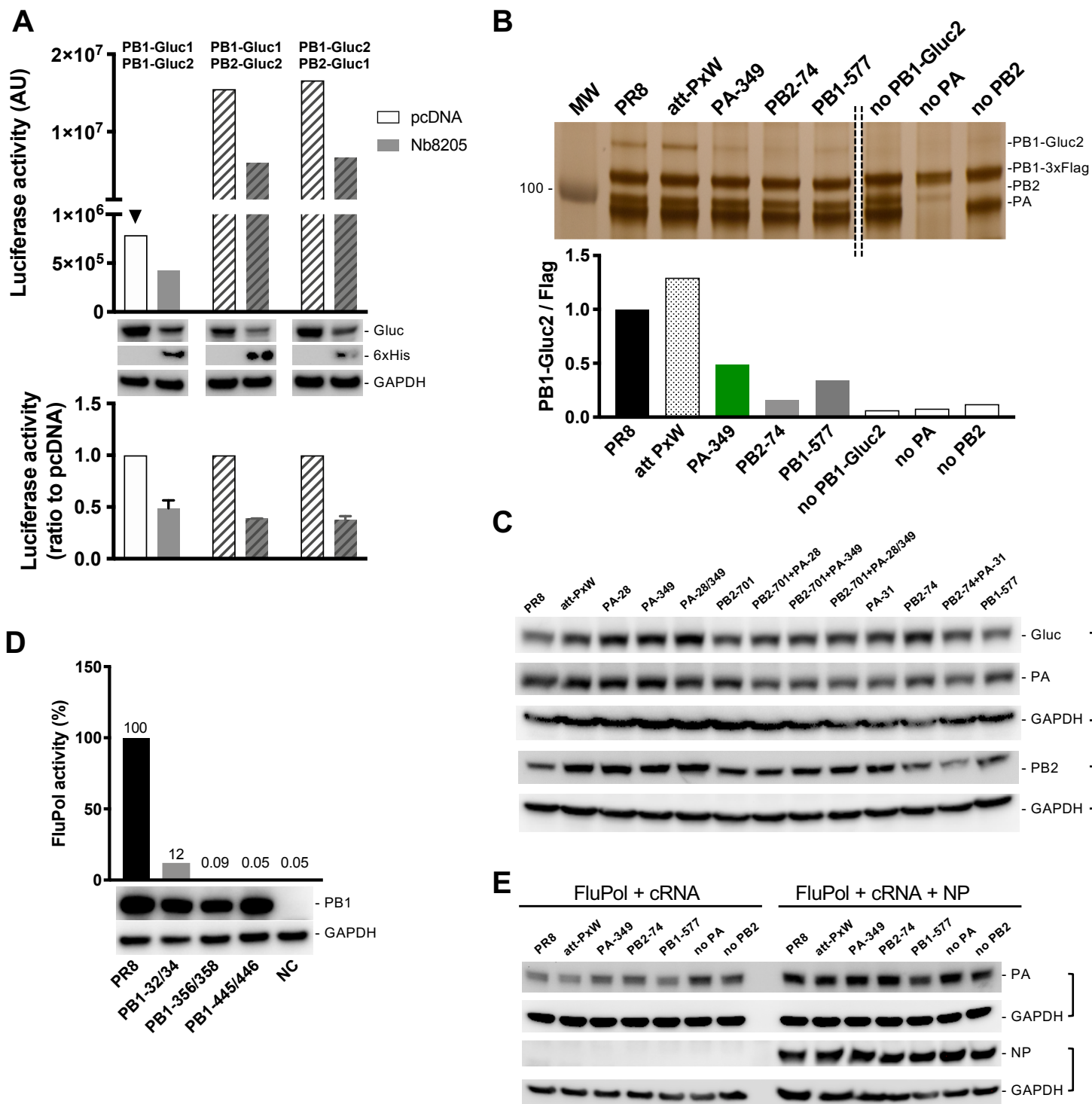
A

Virus	PB2 74	PA 31	PB1 577	Titer (10 ⁶ PFU/mL)	Plaque phenotype
PR8	G	E	K	200	
att-PxW	G	E	K	6.5	
rev-PxW	R	G	G	69	
PB2-74	R	E	K	50	
PA-31	G	G	K	3	
PB2-74+PA-31	R	G	K	5	
PB1-577	G	E	G	50	

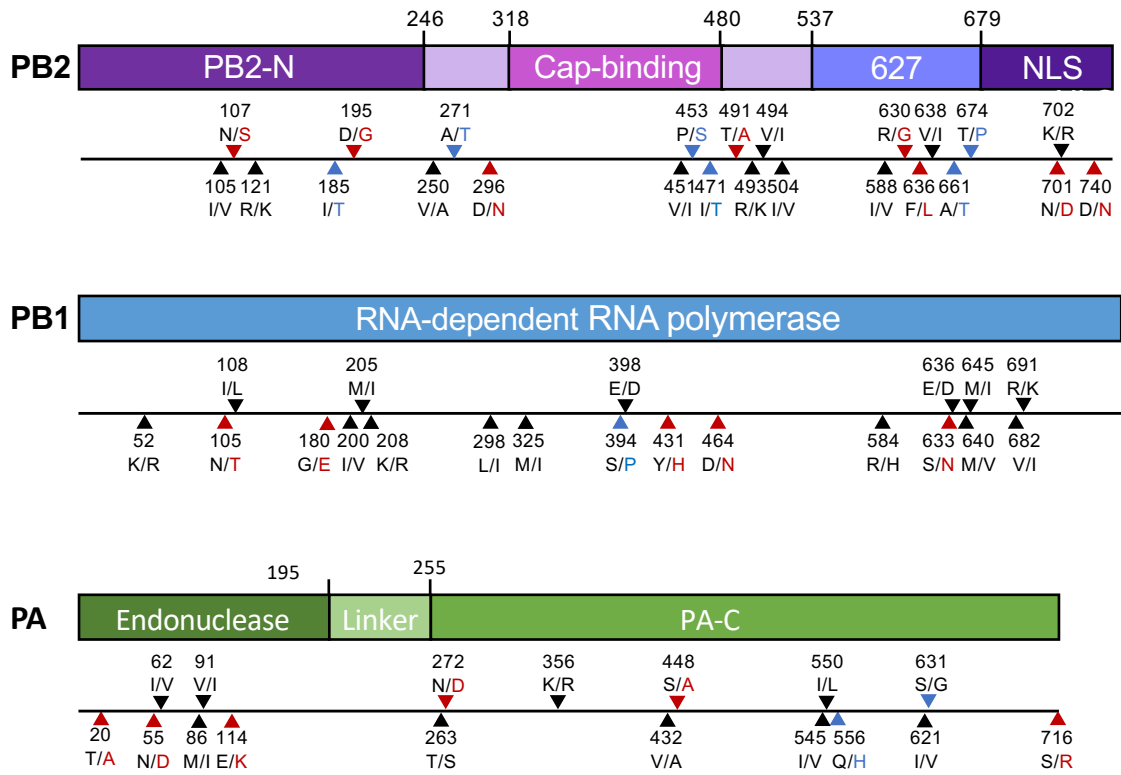
B



S3 Fig


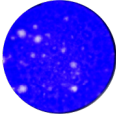
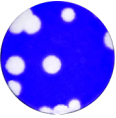
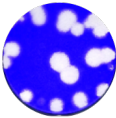


S4 Fig

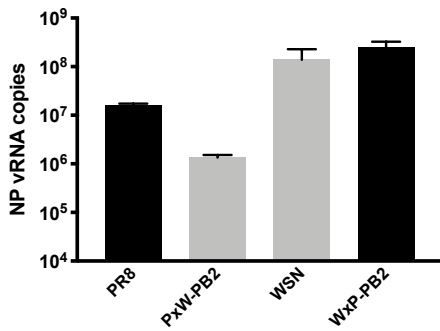


S5 Fig

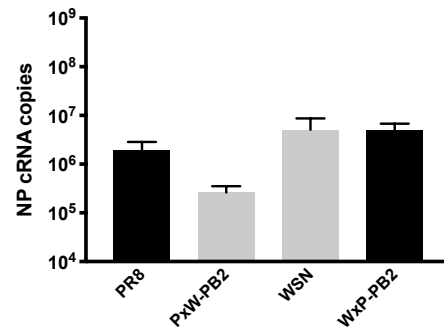
A

Virus	PB2 701	Titer (10 ⁶ PFU/mL)	Plaque phenotype
PR8	N	200	
PxW-PB2	D	6.5	
WSN	D	275	
WxP-PB2	N	33	

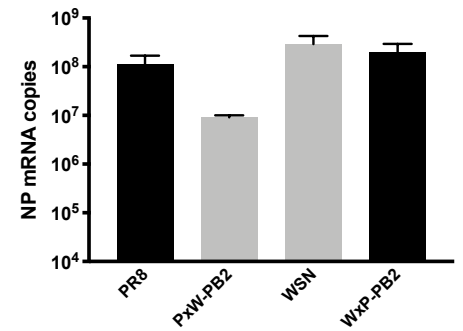
B



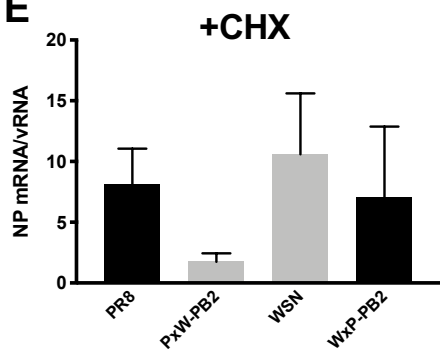
C



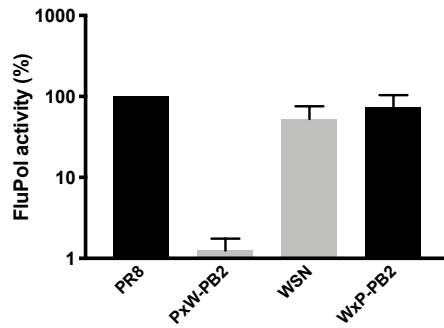
D



E



F



■ PR8-PB2
■ WSN-PB2

1 **S1 Table. Analysis of sequence variation at each mutation site among a subset**
 2 **of human seasonal IAVs.**
 3

Residue	PB2-74	PB2-701	PB1-195	PB1-577	PA-28	PA-349	PA-31
	G: 99.82%	D: 99.25%	M: 90.3%	K: 99.39%	L: 55.11%	E: 99.61%	E: 99.84%
	E: 0.11%	N: 0.66%	V: 8.94%	R: 0.5%	P: 44.36%	K: 0.20%	X: 0.07%
	R: 0.02%	B ^b : 0.07%	I: 0.42%	N: 0.05%	S: 0.39%	G: 0.18%	K: 0.05%
	S: 0.02%	E: 0.02%	T: 0.16%	M: 0.03%	M: 0.07%		G: 0.02%
	X ^a :0.02%		A: 0.08%	Q: 0.03%	Q: 0.05%		V: 0.02%
			L: 0.08%		R: 0.02%		
			R: 0.03%				
Number of Sequences aligned	4394		3782		4391		

4 ^a X : not translated when DNA sequence is NNN.
 5 ^b B : translated to D or N when DNA sequence is RAY.
 6
 7
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1 **S2 Table. Mutations detected upon whole genome sequencing of revertant**
 2 **viruses by NGS, present in >10% of total reads, at a given position.**
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Revertant ^a	Rev 1	Rev 3	Rev 4	Rev 5
Passage	P5 ^b	P6	P8	P8
PB2	D701N (98%) ^c	S286G (81%) P302S (87%)	R251K (67%) R318K (46%)	N153D (38%) C409R (14%) G630R (31%) M645V (13%)
PB1	M195T (100%)	K577G (98%)	K577G (100%) I711V (15%)	K577G (100%)
PA	L28R (98%) E349K (100%)	L425F (56%)		I129L (14%) L226F (49%) D444S (26%) S631G (14%)
HA	K187E (98%)		I230M (37%)	
NP				
NA		V16A (97%) L22P (95%)	V16A (100%) L22P (100%)	L22P (95%) I26T (61%)
M				
NS	L27P (99%)			G45R (11%) S17L (26%)

4 ^a The revertant viruses are numbered according to Fig 5A. For Rev 2, no NGS data
 5 are available; Sanger sequencing was used to sequence the PB2, PB1, PA and NP
 6 segments, and only the PB2-G74R and PA-E31G mutations were detected.

7 ^b Px : x serial passages following plaque purification.

^c In blue : mutations present on a FluPol gene and in $\geq 98\%$ of the reads.

1 **S3 Table. Primers used in this study.**

2

Primer Name	Sequence (5' → 3')
U12	GCCGGAGCTCTGCAGATATCAGCRAAAGCAGG
U12G	GCCGGAGCTCTGCAGATATCAGCGAAAGCAGG
U13	CAGGAAACAGCTATGACAGTAGAAACAAGG
RT-NPvRNA	GGCCGTCATGGTGGCGAATGAATGGACGAAAAACAAGAATTGC
RT-NPcRNA	GCTAGCTTCAGCTAGGCATCAGTAGAAACAAGGGTATTTTTCTTT
RT-NPmRNA	CCAGATCGTTCGAGTCGTTTTTTTTTTTTTTTTCTTTAATTGTC
qPCR_Fw-NPvRNA	CTCAATATGAGTGCAGACCGTGCT
qPCR_Fw-NPcRNA	CGATCGTGCC7TCCTTTG
qPCR_Fw-NPmRNA	CGATCGTGCC7TCCTTTG
Tag_Rv-NPvRNA	GGCCGTCATGGTGGCGAAT
Tag_Rv-NPcRNA	GCTAGCTTCAGCTAGGCATC
Tag_Rv-NPmRNA	CCAGATCGTTCGAGTCGT

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