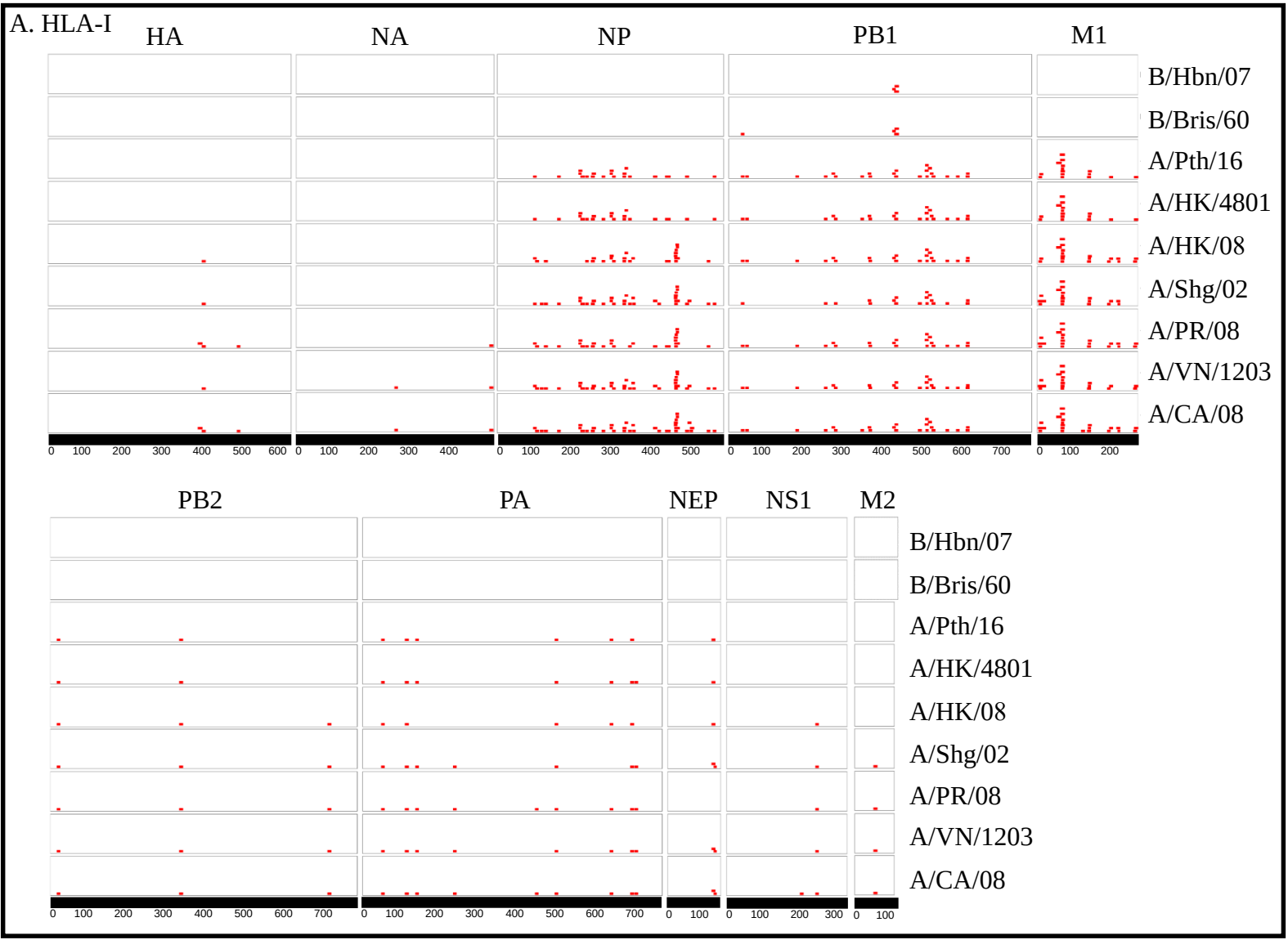
Extensive T cell cross-reactivity between diverse seasonal influenza strains in the ferret model

Adrian J Reber, Nedzad Music, Jin Hyang Kim, Shane Gansebom, Jufu Chen, Ian York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NA
NP
PB1
M1
B/Hbn/07
B/Bris/60
A/Pth/16
A/HK/4801
A/HK/08
A/Shg/02
A/PR/08
A/VN/1203
A/CA/08
0 100 200 300 400 500 600
0 100 200 300 400
0 100 200 300 400 500
0 100 200 300 400 500 600 700
0 100 200
PB2
PA
NEP
NS1
M2
B/Hbn/07
B/Bris/60
A/Pth/16
A/HK/4801
A/HK/08
A/Shg/02
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A/CA/08
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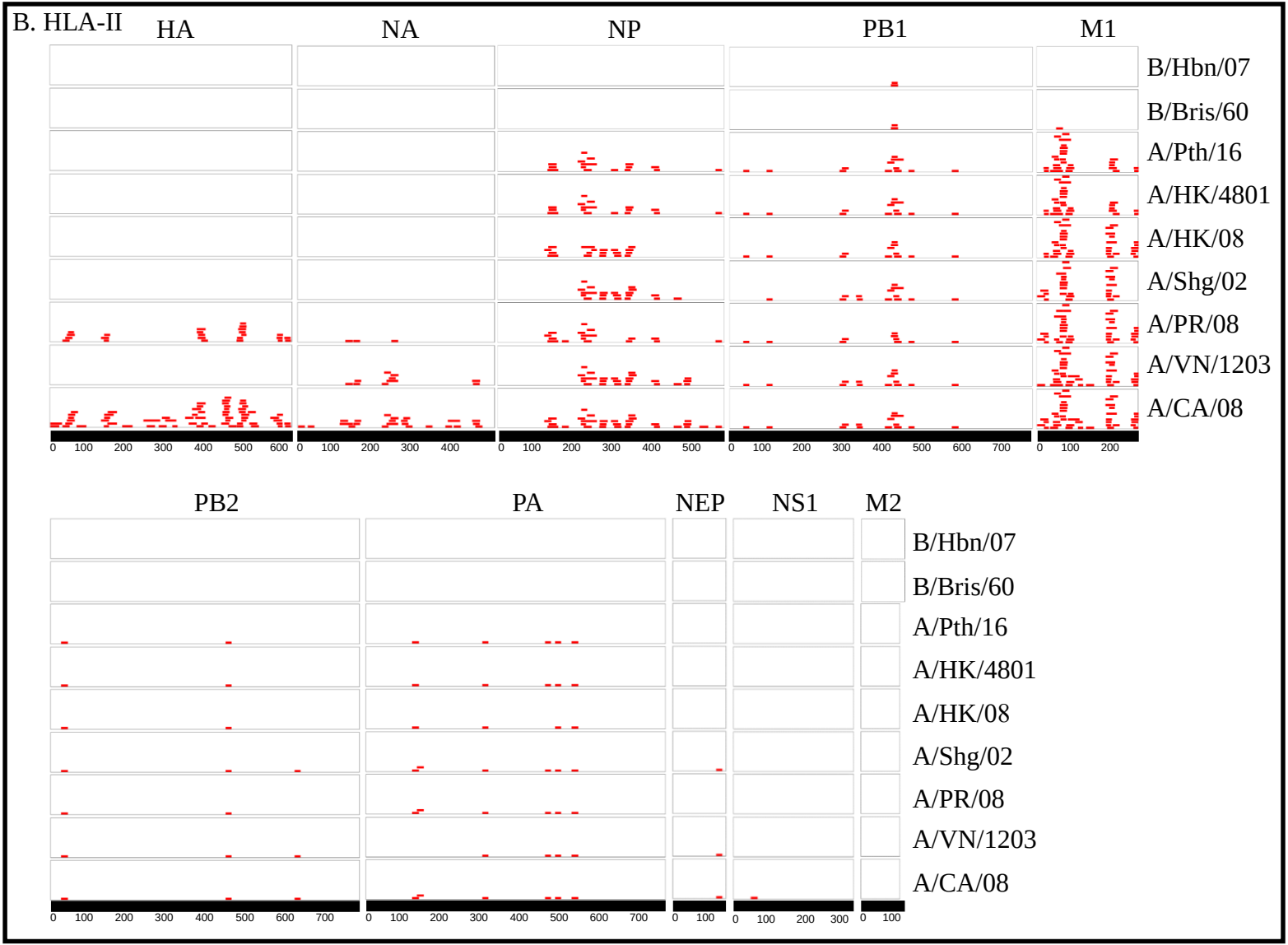
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NP
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M1
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A/Shg/02
A/PR/08
A/VN/1203
A/CA/08
0 100 200 300 400 500 600
0 100 200 300 400
0 100 200 300 400 500
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0 100 200
PB2
PA
NEP
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M2
B/Hbn/07
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A/Shg/02
A/PR/08
A/VN/1203
A/CA/08
0 100 200 300 400 500 600 700
0 100 200 300 400 500 600 700
0 100
0 100 200 300
0 100

Figure. S1. A/California/08/2009 Human Leukocyte Antigen class I and II binding epitopes cross-reactive with a diverse selection of influenza strains. Known HLA class I (A) and HLA class II (B) binding epitopes derived from a diverse group of influenza viruses spanning both human and animal strains were downloaded from the Internet Epitope Database (IEDB: http://www.iedb.org/). The epitopes from the 2009 pandemic H1N1 strain, A/California/08/2009 (A/CA/08), which bound either human HLA-I or HLA-II were first determined, then cross-reactivity of those epitopes with other influenza strains assessed. For a broader comparison of potential cross-reactivity, in addition to influenza strains used within the study, comparisons to the avian influenza strains A/Shanghai/02/2013 (A/Shg/02; H7N9) and A/Vietnam/1203/2004 (A/VN/1203; H5N1) were performed as well as to the currently circulating human H3N2 strain A/Hong Kong/4801/2014 (A/HK/4801). HLA binding epitopes from each protein are depicted in red. Epitopes from the entire influenza proteome are depicted. The y-axis represents the amino acid sequence designation.

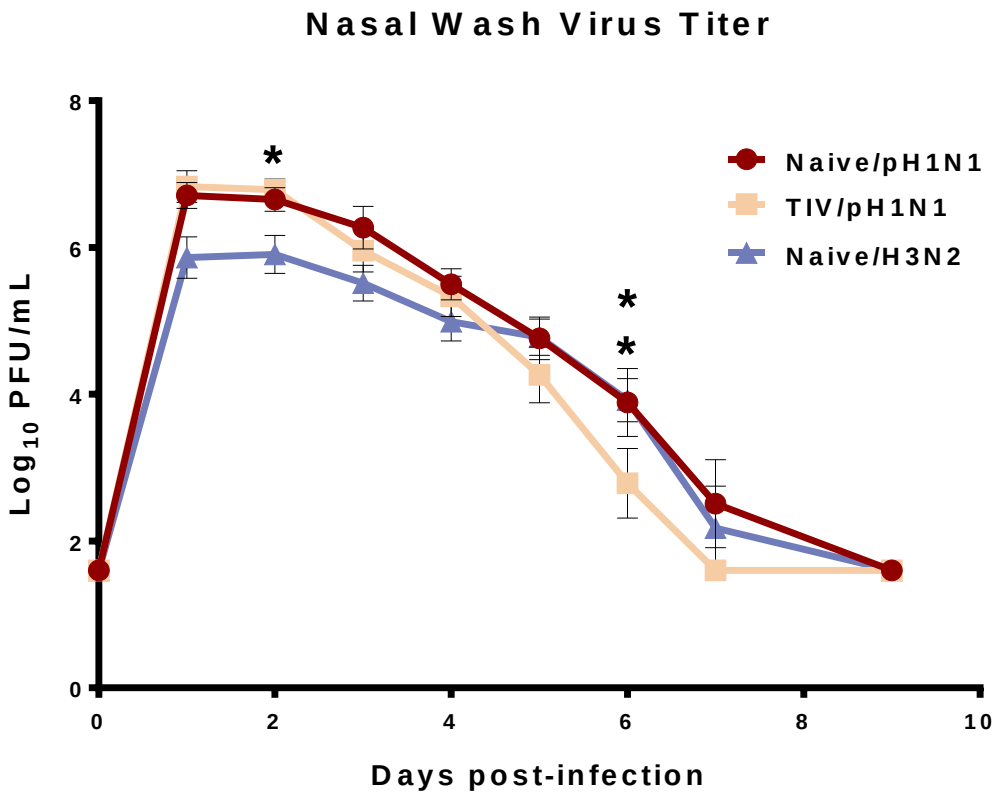
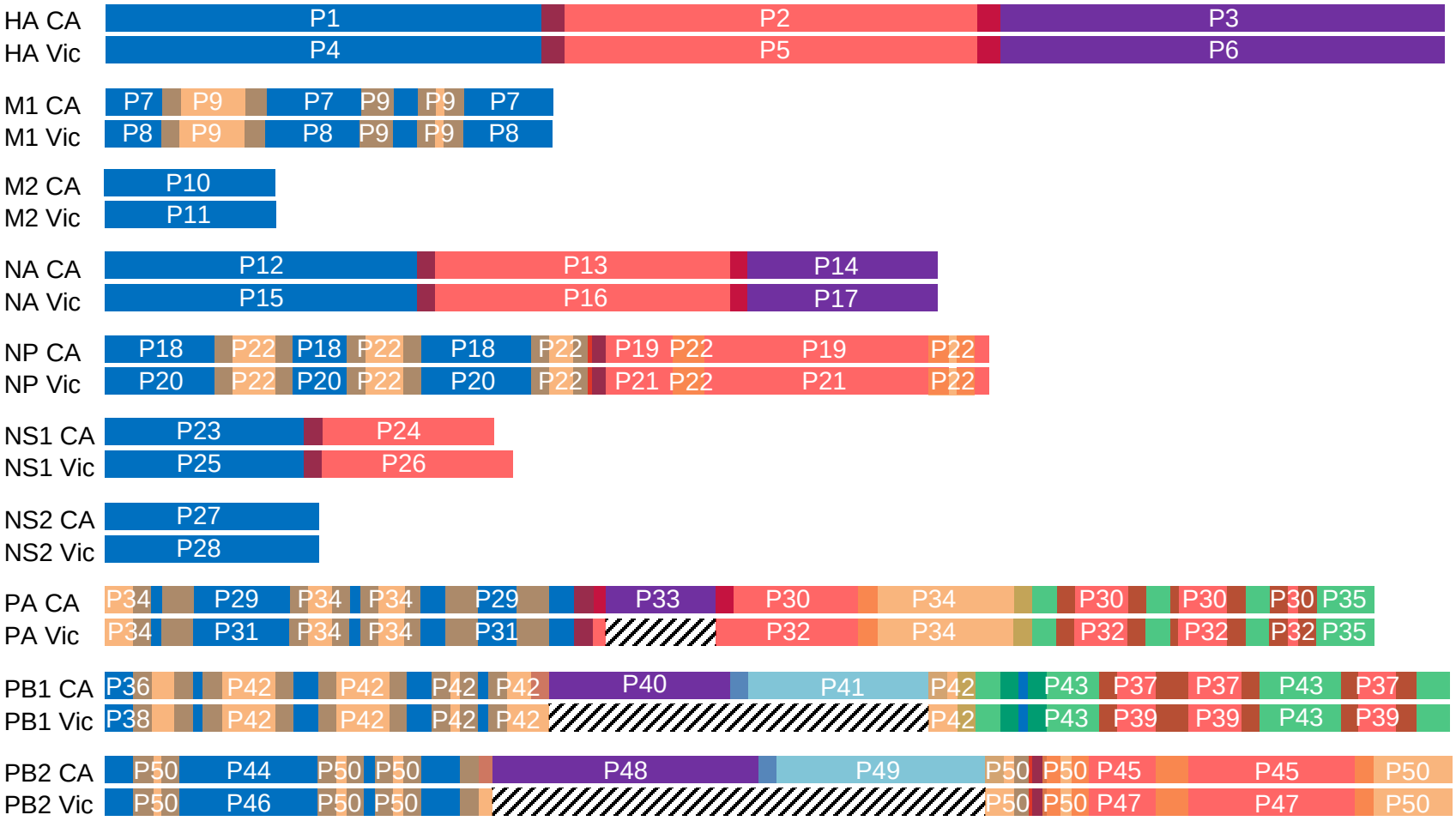


Figure S2. Nasal Wash Titer. Two weeks after booster vaccination, ferrets were challenged intranasally with 10^6 PFU of H1N1pdm09 (TIV/pH1N1). Unvaccinated ferrets were challenged intranasally with 10^6 PFU of H1N1pdm09 (Naive/pH1N1) or with the seasonal H3N2 strain, A/Perth/16/2009 (Naïve/H3N2). Nasal washes were obtained on days 0–9 and virus titers in the nasal washes were measured. Error bars standard error of the mean (SEM). Significance is indicated by * $p < 0.05$.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P1
P2
P3
HA Vic
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P6
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P14
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P50

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VIC210	PB2	KNPSLRMKWMMAMKYPIT	P46
VIC210	PB2	WMMAMKYPITADKRITEM	P46
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CA07	PB2	VMGMIGILPDMTPSTEMS	P49
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CA07	PB2	SKMGVDEYSSTERVVVSI	P49
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CA07/VIC210	PB2	VDHMAIIKKYTSGRQEKN	P50
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CA07/VIC210	PB2	TFGPVHFRNQVKIRRRVD	P50
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CA07/VIC210	PB2	DVIMEVVFPNEVGARILT	P50
CA07/VIC210	PB2	LMVAYMLERELVRKTRFL	P50
CA07/VIC210	PB2	SIDRFLRVRDQRGNVLLS	P50
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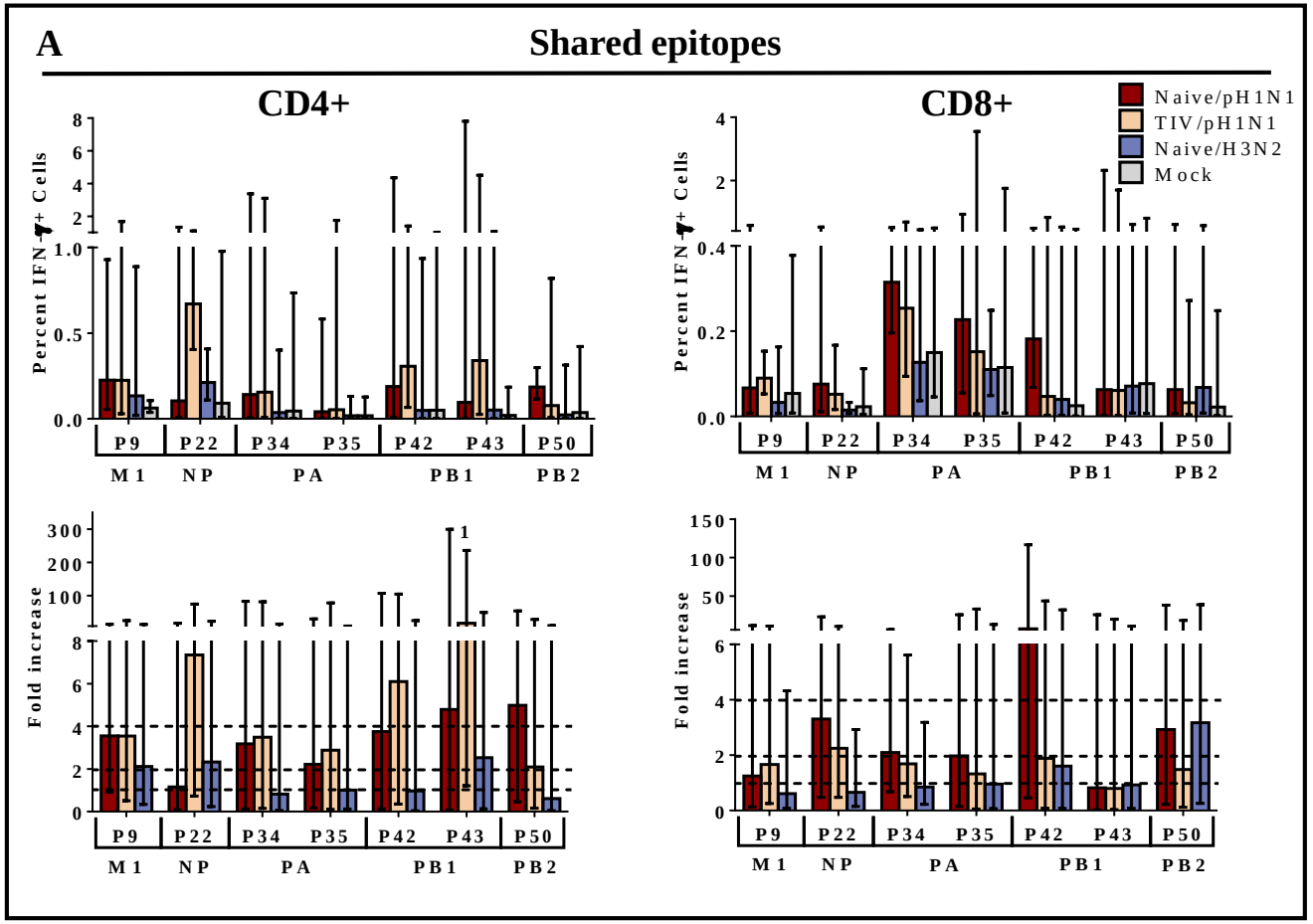
CA07/VIC210	PB2	RKRDSSILTDSQTATKRI	P50
CA07/VIC210	PB2	SILTDSQTATKRIRMAIN	P50

Figure. S3. Influenza peptide pools. Peptides were derived from the proteome of the H1N1pdm09 strain, A/California/07/2009 (CA), or the seasonal A/Perth/16/2009-like H3N2 strain, A/Victoria/210/2009 (Vic). Peptide sequences were composed of 18 amino acids with a 10 amino acid overlap with each subsequent peptide, spanning the entire length of each viral protein. Individual peptides from each viral strain were combined into peptide pools for each protein based on the region of the protein from which the peptide was derived. Homologous peptide sequences between the two strains were combined into their own peptide pools for each viral protein. Regions of each protein which compose each of the 50 peptide pools (P) are depicted. Blue, red, and purple regions represent unique sequences for each viral strain. Tan and green regions represent portions of each protein in which both viruses share 100% homology. The regions depicted in a horizontal line represent portions of the A/Victoria/210/2009 proteome for which no amino acid sequence was available.


A
Shared epitopes
CD4+
CD8+
Naive/pH1N1
TIV/pH1N1
Naive/H3N2
Mock
Percent IFN-γ+ Cells
Fold increase
P9
P22
P34
P35
P42
P43
P50
M1
NP
PA
PB1
PB2

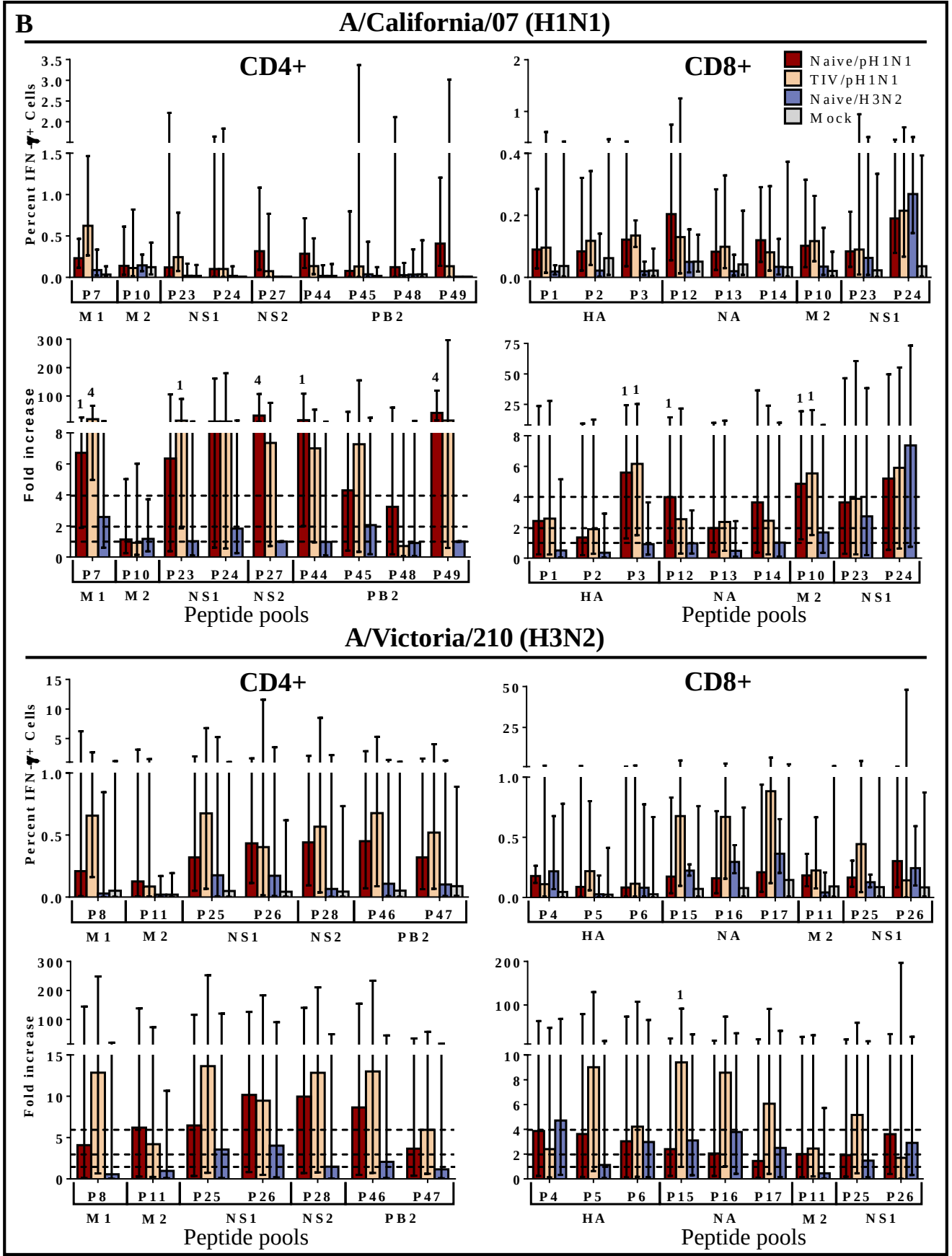
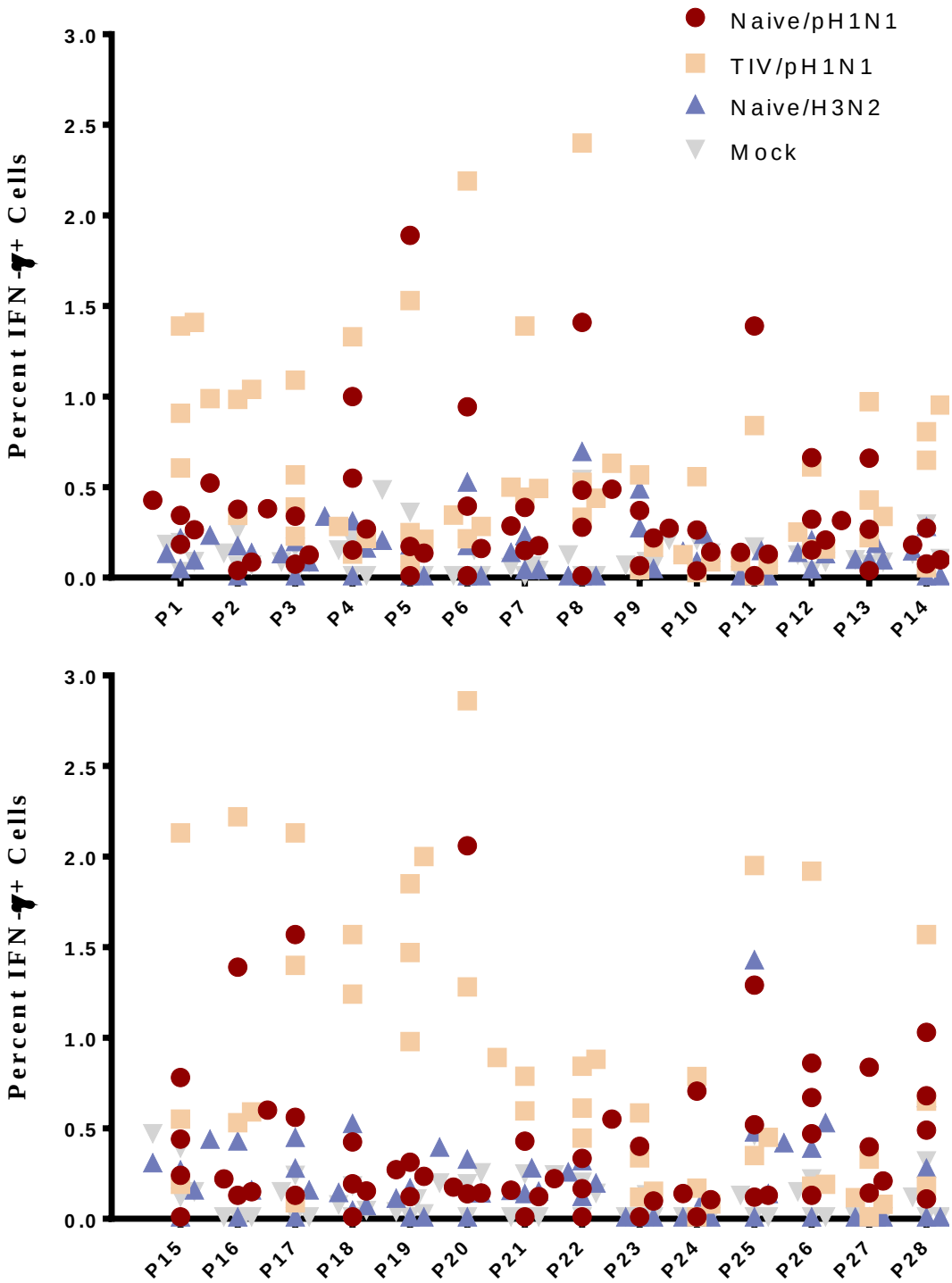

B
A/California/07 (H1N1)
CD4+
CD8+
Naive/pH1N1
TIV/pH1N1
Naive/H3N2
Mock
Percent IFN-γ+ Cells
Fold increase
P7 P10 P23 P24 P27 P44 P45 P48 P49
M1 M2 NS1 NS2 PB2
P1 P2 P3 P12 P13 P14 P10 P23 P24
HA NA M2 NS1
Peptide pools
A/Victoria/210 (H3N2)
P8 P11 P25 P26 P28 P46 P47
P4 P5 P6 P15 P16 P17 P11 P25 P26

Figure. S4. T cell responses to additional influenza peptide pools. Splenocytes were collected from naïve ferrets infected intranasally with A/NY/21/2009 (Naïve/H1N1pdm09, n=4), A/Perth/16/2009 (Naïve/H3N2, n=4), or mock infected ferrets (Naïve/Mock, n=4) 14d post infection. Splenocytes were also collected from ferrets vaccinated with commercial 2011-2012 TIV, challenged with A/NY/21/2009 (TIV/H1N1pdm09, n=4). Splenocytes were stimulated with peptide pools (p) derived from regions of each influenza protein from A/California/07/2009 (H1N1pdm09; A) or the A/Perth/16/2009-like seasonal H3N2 strain, A/Victoria/210/2009 (B). T cells producing IFN-γ in response to peptide stimulation were assessed by flow cytometry. The percent IFN-γ+ cells and fold-increase over mock infected animals are depicted. Responses to peptide sequences sharing 100% homology between both viruses were determined (A). Peptide pools to which lower percentages of CD4+ and CD8+ T cells responded are also represented.
Responses significantly [1]greater ($p < 0.05$) than Mock infected ferrets were considered modest responses, [2]2-fold greater were considered moderate, and [4]4-fold greater were considered substantial. Dotted lines indicate 1-, 2-, and 4-fold increases over mock infected controls. Error bars represent SEM.

CD4 IFN-γ+ Cells – All peptides



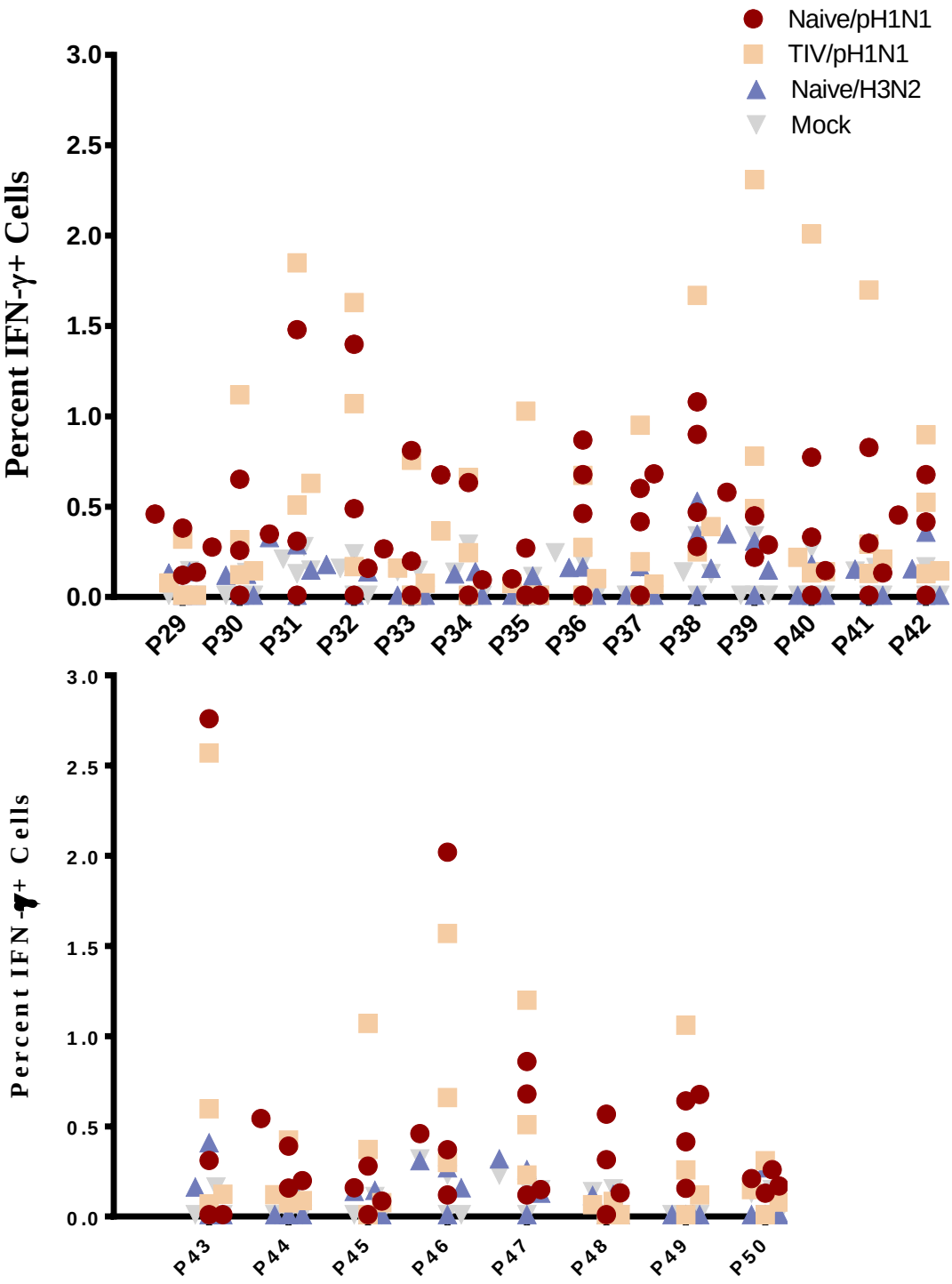
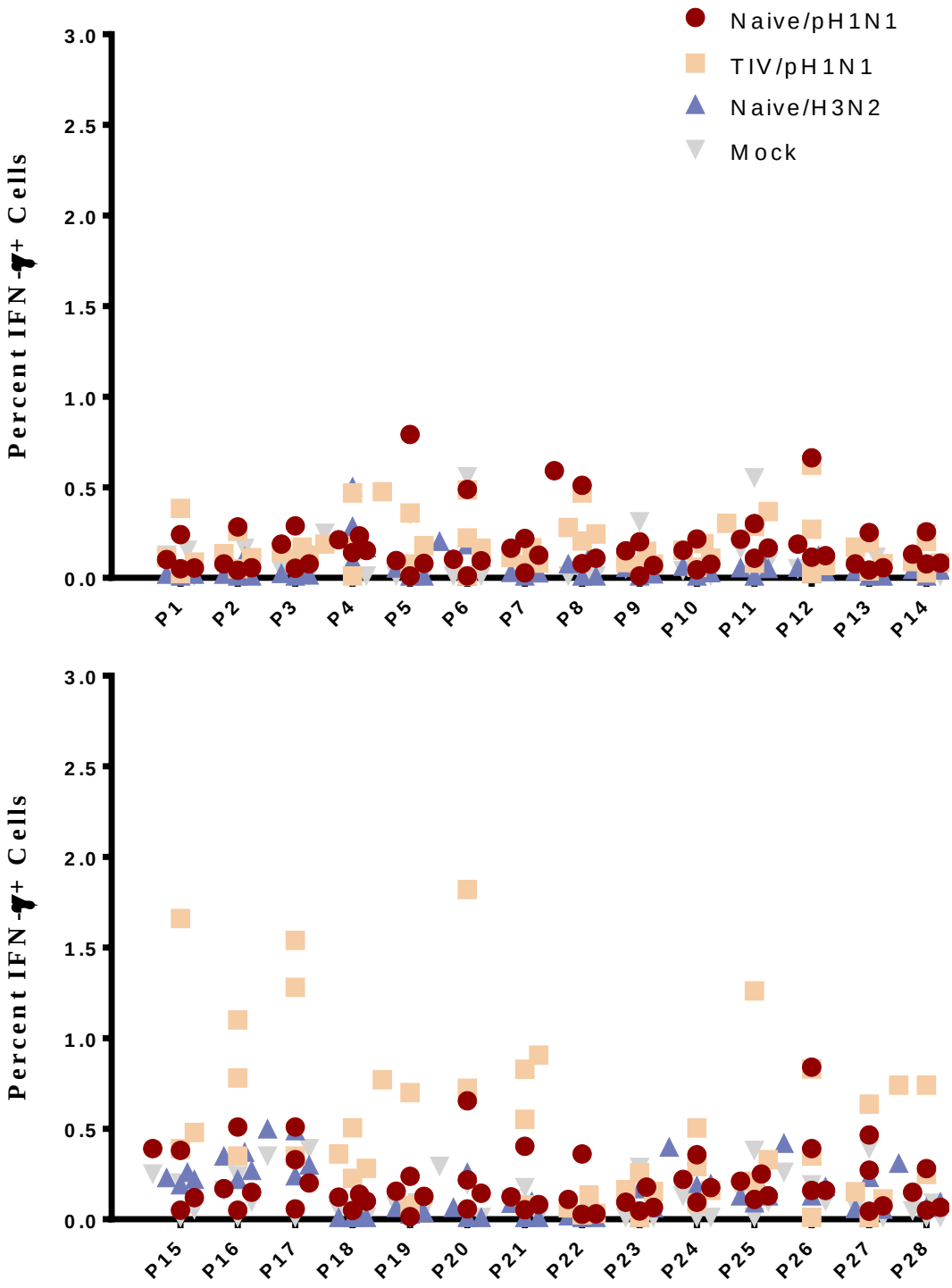

Naive/pH1N1
TIV/pH1N1
Naive/H3N2
Mock
Percent IFN-γ+ Cells
3.0
2.5
2.0
1.5
1.0
0.5
0.0
P29
P30
P31
P32
P33
P34
P35
P36
P37
P38
P39
P40
P41
P42
Percent IFN-γ+ Cells
3.0
2.5
2.0
1.5
1.0
0.5
0.0
P43
P44
P45
P46
P47
P48
P49
P50

Figure. S5. Individual ferret CD4+ T cell responses to influenza peptide pools. Splenocytes collected were collected from naïve ferrets infected with A/NY/21/2009 (Naïve/H1N1pdm09), A/Perth/16/2009 (Naïve/H3N2), or mock infected (Naïve/Mock) 14d post-infection. Splenocytes were also collected from ferrets vaccinated with commercial 2011-2012 TIV, challenged with A/NY/21/2009 (TIV/H1N1pdm09). Splenocytes were stimulated with peptide pools (p) derived from influenza proteins of A/California/07/2009 and A/Victoria/210/2009. The frequency of CD4+ T cells producing IFN-γ in response to peptide stimulation were assessed by flow cytometry.

CD8 IFN-γ+ Cells – All peptides



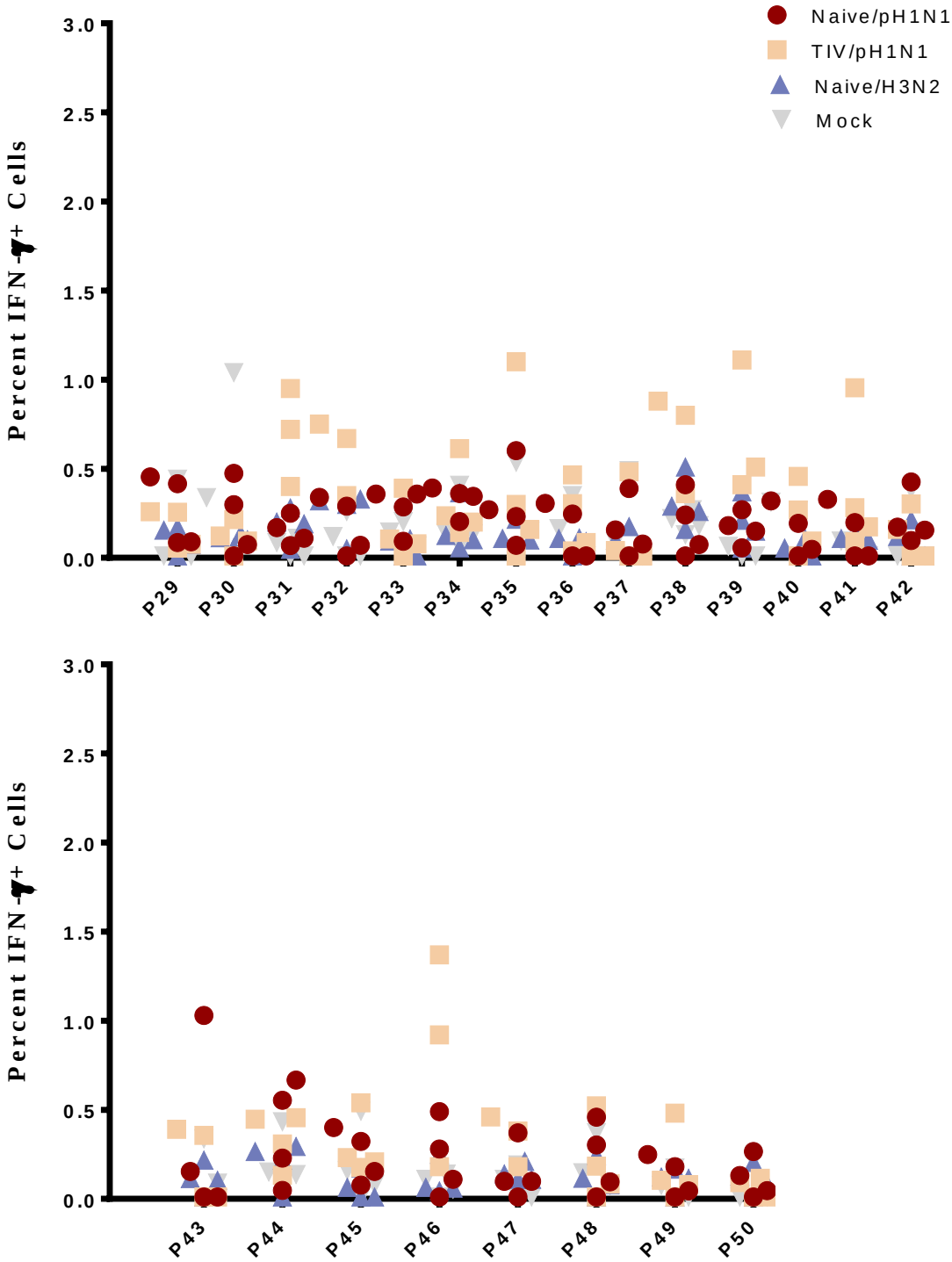
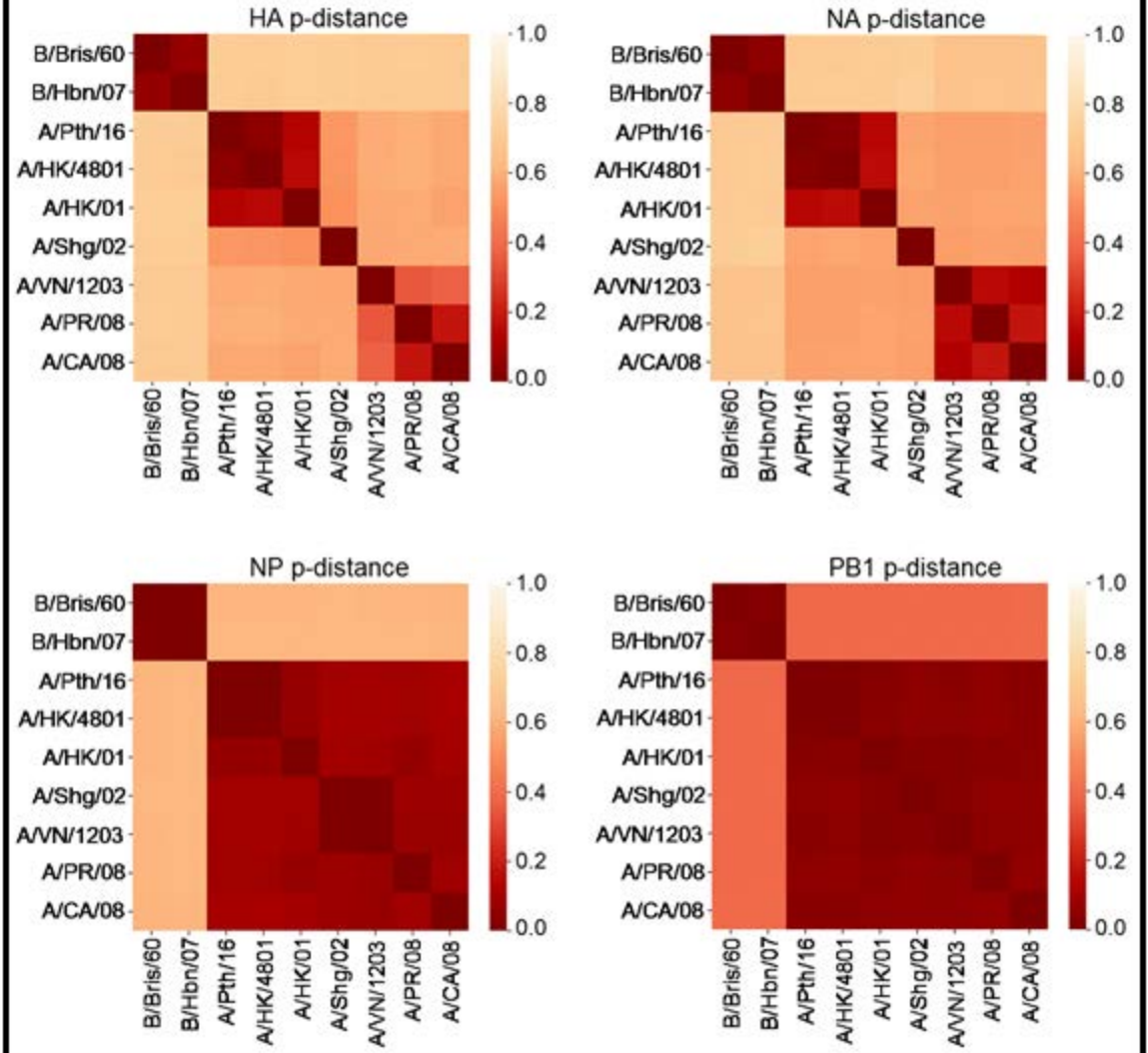

Naive/pH1N1
TIV/pH1N1
Naive/H3N2
Mock
Percent IFN-γ+ Cells
3.0
2.5
2.0
1.5
1.0
0.5
0.0
P29
P30
P31
P32
P33
P34
P35
P36
P37
P38
P39
P40
P41
P42
Percent IFN-γ+ Cells
3.0
2.5
2.0
1.5
1.0
0.5
0.0
P43
P44
P45
P46
P47
P48
P49
P50

Figure. S6. Individual ferret CD8+ T cell responses to influenza peptide pools. Splenocytes collected were collected from naïve ferrets infected with A/NY/21/2009 (Naïve/H1N1pdm09), A/Perth/16/2009 (Naïve/H3N2), or mock infected (Naïve/Mock) 14d post-infection. Splenocytes were also collected from ferrets vaccinated with commercial 2011-2012 TIV, challenged with A/NY/21/2009 (TIV/H1N1pdm09). Splenocytes were stimulated with peptide pools (p) derived from influenza proteins of A/California/07/2009 and A/Victoria/210/2009. The frequency of CD8+ T cells producing IFN-γ in response to peptide stimulation were assessed by flow cytometry.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NA p-distance
NP p-distance
PB1 p-distance
B/Bris/60
B/Hbn/07
A/Pth/16
A/HK/4801
A/HK/01
A/Shg/02
A/VN/1203
A/PR/08
A/CA/08
1.0
0.8
0.6
0.4
0.2
0.0

Figure. S7. Assessment of protein homology of viral proteins dominating the influenza response. Amino acid homology of the viral proteins which tended to dominate the responses to the influenza peptide pools were analyzed against a selection of contemporary and historical influenza strains, constructing p-distance heat maps to determine protein conservation.