# Supplemental Material to:

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Immunization with cross-conserved H1N1 influenza
CD4+ T-cell epitopes lowers viral burden in HLA DR3
transgenic mice

**Table S1.** Cultured human IFNγ ELISpot responses elicited by cross-conserved epitopes before and after 2010-2011 TIV vaccination.

| Stimulation | 182 | | | | | | 311 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-vaccination | | | Post-Vaccination | | | Pre-vaccination | | | Post-Vaccination | | |
| | SFC | SI | p-value | SFC | SI | p-value | SFC | SI | p-value | SFC | SI | p-value |
| 38-61 | 40 | 1.17 | 0.218 | 80 | 1.17 | 0.327 | -5 | 0.57 | 0.516 | 3 | 0.33 | 0.170 |
| 112-129 | -103 | 0.81 | 0.093 | -100 | 0.74 | 0.037 | -8 | 0.29 | 0.366 | 0 | 0.00 | 0.516 |
| 129-140 | - | - | - | - | - | - | **118** | **11.14** | **0.000** | 3 | 0.33 | 0.626 |
| 319-345 | -30 | 0.90 | 0.463 | 3 | 1.01 | 0.960 | **435** | **38.29** | **0.000** | **177** | **17.67** | **0.000** |
| 321-341 | 90 | 1.29 | 0.033 | 187 | 1.39 | 0.049 | **78** | **7.71** | **0.000** | 40 | 4.00 | 0.000 |
| 396-417 | **580** | **2.09** | **0.003** | 220 | 1.74 | 0.019 | 18 | 2.57 | 0.068 | 20 | 2.00 | 0.034 |
| 432-453 | 53 | 1.18 | 0.183 | 77 | 1.16 | 0.521 | **162** | **14.86** | **0.001** | 27 | 2.67 | 0.005 |
| 456-480 | 90 | 1.25 | 0.177 | 167 | 1.52 | 0.006 | 48 | 5.14 | 0.002 | **70** | **7.00** | **0.000** |
| 516-540 | 27 | 1.19 | 0.250 | 80 | 1.22 | 0.118 | -8 | 0.29 | 0.366 | 0 | 0.00 | 0.516 |
| 548-563 | 217 | 1.49 | 0.019 | 63 | 1.15 | 0.160 | -12 | 0.00 | 0.139 | 0 | 0.00 | 1.000 |
| Pool | 773 | 0.90 | 0.523 | 423 | 1.16 | 0.169 | **255** | **22.86** | **0.000** | 440 | 44.00 | 0.000 |
| % Peptides Positive | 11% | | | 0% | | | 40% | | | 20% | | |

**Table S1. continued**

| Stimulation | 720 | | | | | | 844 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-vaccination | | | Post-Vaccination | | | Pre-vaccination | | | Post-Vaccination | | |
| | SFC | SI | p-value | SFC | SI | p-value | SFC | SI | p-value | SFC | SI | p-value |
| 38-61 | -35 | 0.70 | 0.262 | -108 | 0.34 | 0.000 | 0 | 1.00 | 1.000 | - | - | - |
| 112-129 | -82 | 0.29 | 0.058 | -38 | 0.77 | 0.731 | -10 | 0.92 | 0.923 | - | - | - |
| 129-140 | **168** | **2.46** | **0.004** | 98 | 1.60 | 0.006 | 20 | 1.17 | 0.432 | - | - | - |
| 319-345 | **732** | **7.36** | **0.000** | 782 | 5.74 | 0.000 | 53 | 1.44 | 0.102 | 37 | 2.38 | 0.111 |
| 321-341 | **162** | **2.41** | **0.001** | 108 | 1.66 | 0.027 | 47 | 1.39 | 0.133 | 37 | 2.38 | 0.173 |
| 396-417 | **218** | **2.90** | **0.000** | 98 | 1.60 | 0.005 | 37 | 1.31 | 0.243 | 47 | 2.75 | 0.027 |
| 432-453 | **402** | **4.49** | **0.000** | 438 | 3.66 | 0.000 | 33 | 1.28 | 0.304 | 47 | 2.75 | 0.094 |
| 456-480 | -15 | 0.87 | 0.799 | -35 | 0.79 | 0.636 | 70 | 1.58 | 0.168 | 230 | 9.63 | 0.257 |
| 516-540 | **505** | **5.39** | **0.000** | 518 | 4.14 | 0.000 | 163 | 2.36 | 0.001 | 323 | 13.13 | 0.131 |
| 548-563 | 178 | 29.33 | 0.001 | 92 | 11.00 | 0.011 | 3 | 1.54 | 0.877 | - | - | - |
| Pool | **1222** | **11.62** | **0.000** | 855 | 6.18 | 0.000 | 93 | 1.78 | 0.016 | 50 | 2.88 | 0.028 |
| % Peptides Positive | 70% | | | 40% | | | 10% | | | 0% | | |

**Table S1. continued**

| Stimulation | 923 Pre-vaccination | | | 923 Post-Vaccination | | | 1003 Pre-vaccination | | | 1003 Post-Vaccination | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SFC | SI | p-value | SFC | SI | p-value | SFC | SI | p-value | SFC | SI | p-value |
| 38-61 | 17 | 1.07 | 0.706 | -40 | 0.89 | 0.451 | 13 | 2.18 | 0.043 | 2 | 1.04 | 0.934 |
| 112-129 | 90 | 1.39 | 0.236 | 37 | 1.10 | 0.009 | 13 | 1.82 | 0.336 | -18 | 0.59 | 0.365 |
| 129-140 | -3 | 0.99 | 0.721 | **460** | **2.31** | **0.000** | **1630** | **81.45** | **0.000** | **2425** | **54.89** | **0.000** |
| 319-345 | **510** | **3.22** | **0.000** | **1777** | **6.08** | **0.000** | **107** | **8.91** | **0.000** | 38 | 1.85 | 0.140 |
| 321-341 | 147 | 1.64 | 0.020 | **377** | **2.08** | **0.001** | **90** | **6.73** | **0.000** | **52** | **2.15** | **0.023** |
| 396-417 | 123 | 1.54 | 0.070 | **460** | **2.31** | **0.000** | **500** | **29.45** | **0.000** | **462** | **11.26** | **0.000** |
| 432-453 | **417** | **2.81** | **0.000** | **677** | **2.93** | **0.000** | **197** | **13.45** | **0.000** | **195** | **5.33** | **0.000** |
| 456-480 | -10 | 0.96 | 0.620 | 133 | 1.38 | 0.081 | **177** | **13.27** | **0.000** | **202** | **5.48** | **0.000** |
| 516-540 | 110 | 1.48 | 0.035 | **787** | **3.25** | **0.000** | 33 | 4.00 | 0.001 | 45 | 2.00 | 0.045 |
| 548-563 | 63 | 17.60 | 0.161 | **300** | **5.13** | **0.004** | 7 | 1.45 | 0.556 | -15 | 0.67 | 0.476 |
| Pool | **830** | **4.61** | **0.000** | **3033** | **9.67** | **0.000** | **1885** | **103.82** | **0.000** | **2135** | **48.44** | **0.000** |
| % Peptides Positive | 20% | | | 70% | | | 60% | | | 50% | | |

**Table S1. continued**

| Stimulation | 1434 Pre-vaccination | | | 1434 Post-Vaccination | | | 1438 Pre-vaccination | | | 1438 Post-Vaccination | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SFC | SI | p-value | SFC | SI | p-value | SFC | SI | p-value | SFC | SI | p-value |
| 38-61 | 7 | 1.06 | 0.761 | -23 | 0.85 | 0.262 | 8 | 0.91 | 0.892 | 0 | 1.11 | 0.863 |
| 112-129 | 23 | 1.22 | 0.423 | -10 | 0.93 | 0.892 | -2 | 0.55 | 0.199 | 7 | 1.33 | 0.445 |
| 129-140 | **133** | **2.25** | **0.000** | 60 | 1.39 | 0.048 | 18 | 1.27 | 0.626 | 20 | 2.22 | 0.078 |
| 319-345 | 77 | 1.72 | 0.003 | 133 | 1.87 | 0.000 | **88** | **7.09** | **0.000** | **260** | **15.11** | **0.000** |
| 321-341 | 47 | 1.44 | 0.086 | 3 | 1.02 | 0.772 | **68** | **4.36** | **0.001** | **130** | **10.89** | **0.001** |
| 396-417 | **223** | **3.09** | **0.002** | 53 | 1.35 | 0.122 | -2 | 1.09 | 0.722 | **57** | **6.22** | **0.002** |
| 432-453 | **117** | **2.09** | **0.001** | **230** | **2.50** | **0.000** | **168** | **11.27** | **0.000** | **620** | **40.44** | **0.000** |
| 456-480 | **133** | **2.25** | **0.015** | -20 | 0.87 | 0.534 | 32 | 3.27 | 0.000 | **53** | **4.89** | **0.000** |
| 516-540 | **410** | **4.84** | **0.000** | -30 | 0.80 | 0.356 | 5 | 2.00 | 0.125 | 7 | 1.33 | 0.329 |
| 548-563 | 60 | 10.00 | 0.098 | 7 | 2.67 | 0.782 | 45 | 24.00 | 0.018 | 33 | 0.73 | 0.422 |
| Pool | **480** | **5.50** | **0.000** | **477** | **4.11** | **0.000** | **658** | **36.91** | **0.000** | **392** | **27.11** | **0.000** |
| % Peptides Positive | 50% | | | 10% | | | 30% | | | 50% | | |

Immunoreactivity data for influenza cross-conserved peptides are presented from cultured IFNγ ELISpot assays performed for sixteen human subjects. An ELISpot response was considered positive if three criteria were met: (1) spot-forming cells (SFC) per million PBMC were at least 50, (2) SFC per million PBMC were at least two-fold over background, and (3) SFC per million PBMC were statistically different from "no stimulus" measurements by the Student's t-test ($p < 0.05$). Results that meet individual criteria are highlighted in gray. Results that meet all three criteria are denoted in bold. Column headers: human subject ID code; numbers of spot forming cells per million PBMCs that secrete IFNγ in response to individual and pooled cross-conserved peptides (SFC); stimulation indices (SI); and Student's t-test results (p-value); and percent of subjects responding. Row labels: peptide ID. Epitopes are grouped according to their source antigen, either HA or NA.