Supplementary Materials for

**Balanced cellular and humoral immune responses targeting multiple antigens in adults receiving a quadrivalent inactivated influenza vaccine**

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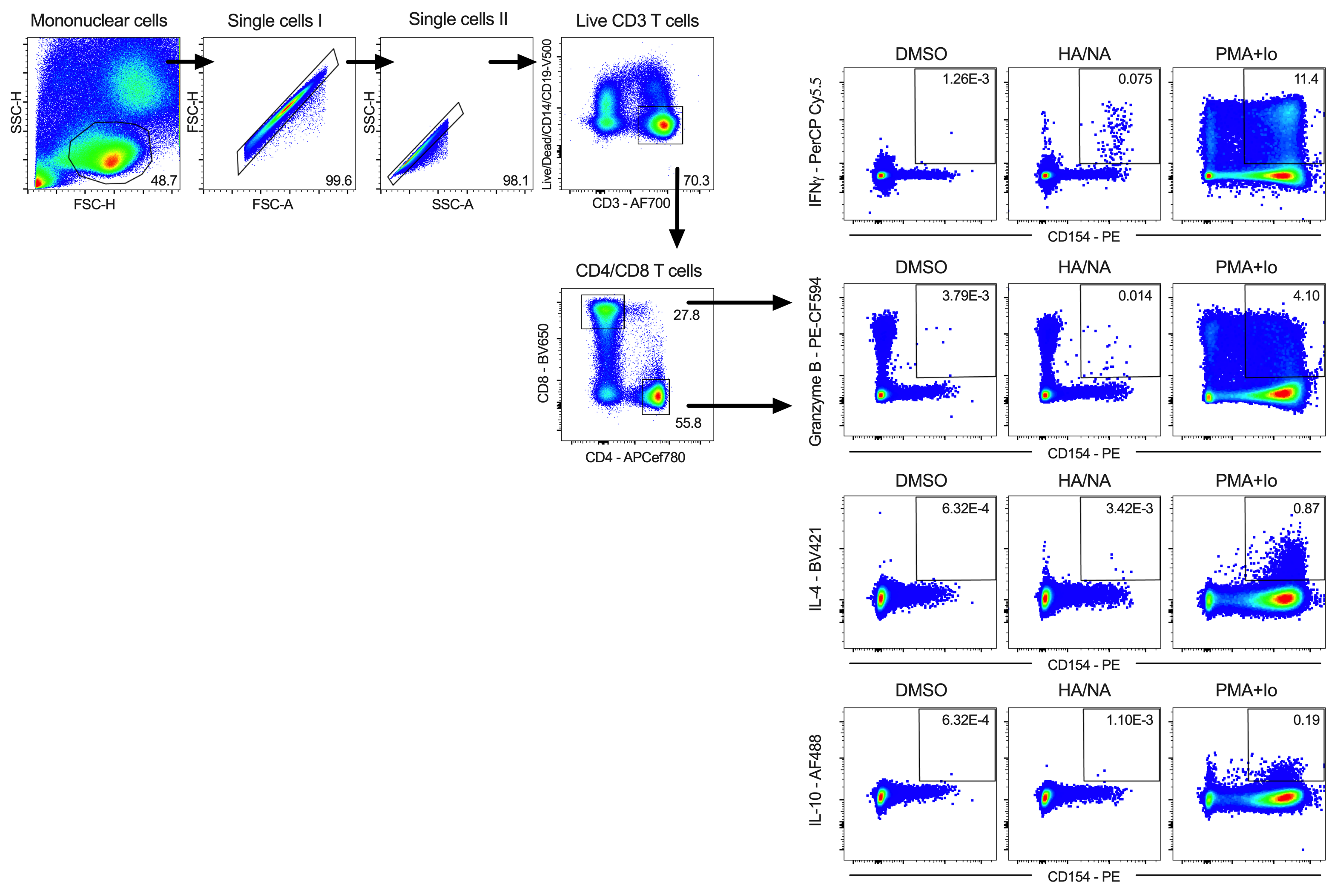
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Conflict of interest statement: Stacey Wooden is employed and receives salaries from Merck & Co., Inc. The rest of the authors declare no conflict of interest.

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**Supplementary Figure S1**

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**Supplementary Figure S1.** Gating strategy and representative cytokine responses plots of the intracellular staining (ICS) assay. Representative gating of live CD3+ T cells, CD4+ T cells, CD8+ T cells and cytokine producing CD154+ CD4 or CD8 T cells from donor PBMCs is shown. Briefly, mononuclear cells were gated out of all events followed by subsequent singlet gating. Live CD3+ cells were gated as Live/Dead-CD14-CD19-CD3+. Cells were then gated as CD4+CD8- or CD4-CD8+ T cells. CD4+ or CD8+ T cells were further subdivided into different cytokines (INF, Granzyme B, IL-4, IL-10) producing CD154+ populations. Representative cytokines responses plots after stimulating with positive (PMA+Io) or negative (DMSO) controls and flu MegaPools were presented on the right side.

**Supplementary Figure S2**



**Supplementary Figure S2.** Cellular responses to CMV and PT control MPs do not change after influenza vaccination. Cellular responses were measured at baseline (D1, pre-immunization), D15 (14 days post vaccination), and D91 (90 days post vaccination). (**A**, **C**) The individual trends of changes in CD4 T cell responses to CMV and PT control MPs before and after vaccination were shown. (**B**) Th1 polarization shown in subjects with positive CMV response at each visit. (**D**) Th2 polarization shown in subjects with positive acellular vaccine (aP) response at each visit, and Th1 polarization in subjects with positive whole-cell vaccine (wP) response at each visit. Childhood vaccination with aP and wP were inferred by date of birth.

**Supplementary Figure S3**



**Supplementary Figure S3.** Total MN titers and HAI titers corelated with each other.For this analysis, we considered each time point for each individual separately. Data from all 3 visits of 10 study subjects were included and correlation were calculated by Spearman correlation test. P values < 0.05 were considered statistically significant.

**Supplementary Figure S4**



**Supplementary Figure S4.** Longitudinal analysis of cellular and humoral immune responses after vaccination by strains. Cellular and humoral immune responses were measured at baseline (D1, pre-immunization), D15 (14 days post vaccination), and D91 (90 days post vaccination). (**A**-**H**) The general trends of changes in CD4 and CD8 T cell responses before and after vaccination for each influenza virus strain (A/H1N1, A/H3/N2, B/IOWA, B/SING) were shown for 10 participants at each visit. CD4 and CD8 T cell responses were represented by number of influenza viruses reactive cytokine producing CD154+ CD4 or CD8 T cells per million of total CD4 or CD8 T cells. (**I**-**P**) Humoral immune responses were measured by both microneutralization (MN) assay and hemagglutination inhibition (HAI) assay. The general trends of changes in MN or HAI antibody titers before and after vaccination for each influenza virus strain were shown for 10 participants at each visit. (**A**-**P**) Normality of data distribution was accessed by Shapiro-Wilks test. Parametric data at D15 or D91 were compared to baseline (D1) by paired Student’s t test (two-tailed). Non-parametric data at D15 or D91 were compared to baseline (D1) by Wilcoxon test (two-tailed). Data were plotted as median with interquartile range for non-parametric data and mean with SEM for parametric data. P values < 0.05 were considered statistically significant.

**Supplementary Table S5.** List of antibodies used in the intracellular cytokine staining (ICS) assay.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Antibody | Fluorochrome | Clone | Vendor | Catalog number |
| CD3 | AF700 | UCHT1 | Invitrogen | 56-0038-42 |
| CD4 | APCef780 | RPA-T4 | Invitrogen | 47-0049-42 |
| CD8 | BV650 | RPA-T8 | Biolegend | 301042 |
| CD14 | V500 | M5E2 | BD Biosciences | 561391 |
| CD19 | V500 | HIB19 | BD Biosciences | 561121 |
| CD154 | PE | TRAP-1 | BD Biosciences | 555700 |
| Live/Dead Viability | eF506/Aqua | - | Invitrogen | 65-0866-18 |
| IFN | PerCP Cy5.5 | 4S.B3 | Invitrogen | 45-7319-42 |
| Granzyme B | PE-CF594 | GB11 | BD Biosciences | 562462 |
| IL-10 | AF488 | JES3-9D7 | Invitrogen | 53-7108-42 |
| IL-4 | BV421 | MP4-25D2 | Biolegend | 500826 |

**Supplementary Table S6.** Sequence similarity analysis of viral protein MPs: calculated as number (and percentage) of common peptides shared between four different strains.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **HA/NA** | A/H1N1 | A/H3N2 | B/IOWA | B/SING |  | **Others** | A/H1N1 | A/H3N2 | B/IOWA | B/SING |
| A/H1N1 | 204 (100%) | 0 (0%) | 0 (0%) | 0 (0%) |  | A/H1N1 | 232 (100%) | 63 (27%) | 0 (0%) | 0 (0%) |
| A/H3N2 |  | 204 (100%) | 0 (0%) | 0 (0%) |  | A/H3N2 |  | 232 (100%) | 0 (0%) | 0 (0%) |
| B/IOWA |  |  | 207 (100%) | 45 (22%) |  | B/IOWA |  |  | 256 (100%) | 145 (57%) |
| B/SING |  |  |  | 207 (100%) |  | B/SING |  |  |  | 256 (100%) |
|  |  |  |  |  |  |  |  |  |  |  |
| **PA/PB1** | A/H1N1 | A/H3N2 | B/IOWA | B/SING |  | **PB1/PB2** | A/H1N1 | A/H3N2 | B/IOWA | B/SING |
| A/H1N1 | 216 (100%) | 120 (56%) | 2 (1%) | 2 (1%) |  | A/H1N1 | 213 (100%) | 133 (62%) | 0 (0%) | 0 (0%) |
| A/H3N2 |  | 216 (100%) | 2 (1%) | 2 (1%) |  | A/H3N2 |  | 213 (100%) | 0 (0%) | 0 (0%) |
| B/IOWA |  |  | 218 (100%) | 170 (78%) |  | B/IOWA |  |  | 227 (100%) | 184 (81%) |
| B/SING |  |  |  | 218 (100%) |  | B/SING |  |  |  | 227 (100%) |

HA/NA MPs include overlapping peptides spanning the hemagglutinin (HA) and neuraminidase (NA) protein sequences; PA/PB1 and PB1/PB2 MPs include overlapping peptides spanning the viral polymerases (PA, PB1 and PB2) protein sequences; and “other” MPs include overlapping peptides spanning the rest of the viral proteins sequences such as the nucleoprotein (NP), matrix protein (M1and M2), non-structural protein (NS1), and nuclear export protein (NEP).