Supplemental Material

Immunologic Mechanisms of Seasonal Influenza Vaccination Administered by Microneedle Patch from a Randomized Phase I Trial

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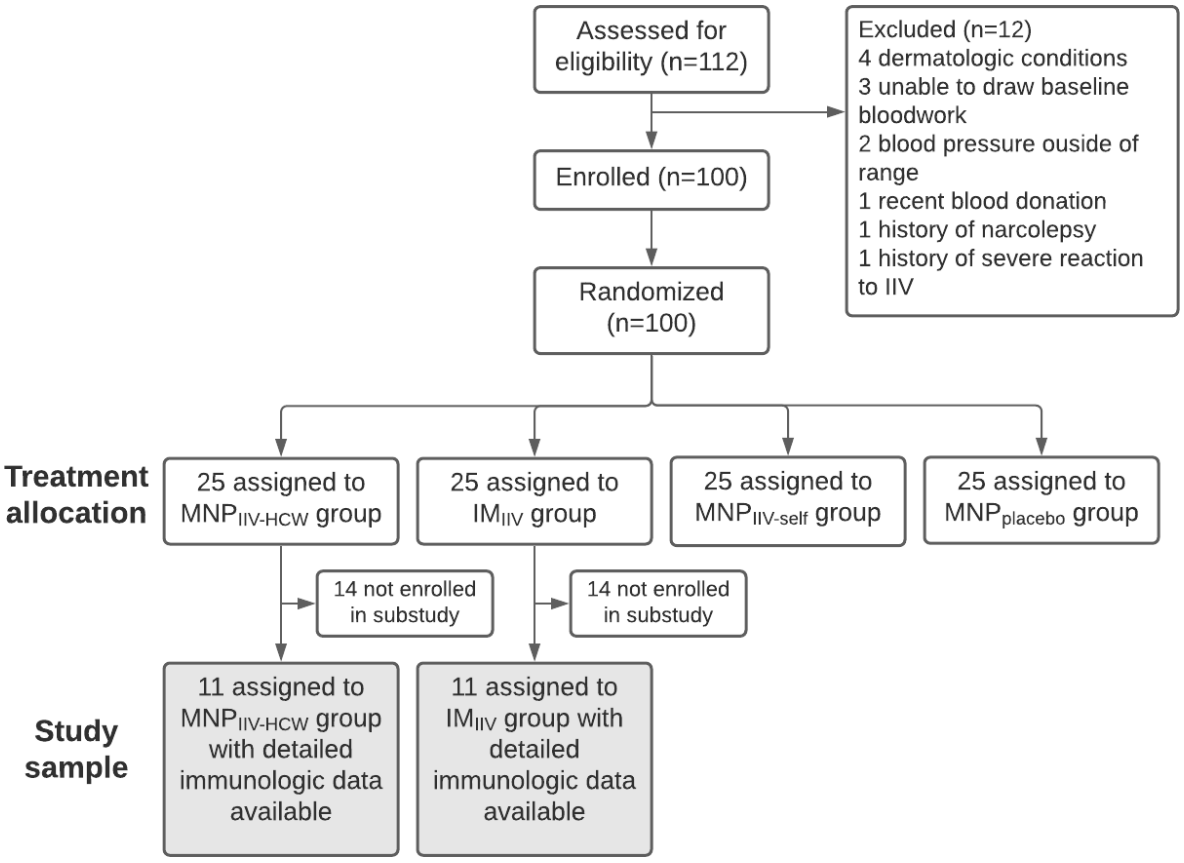
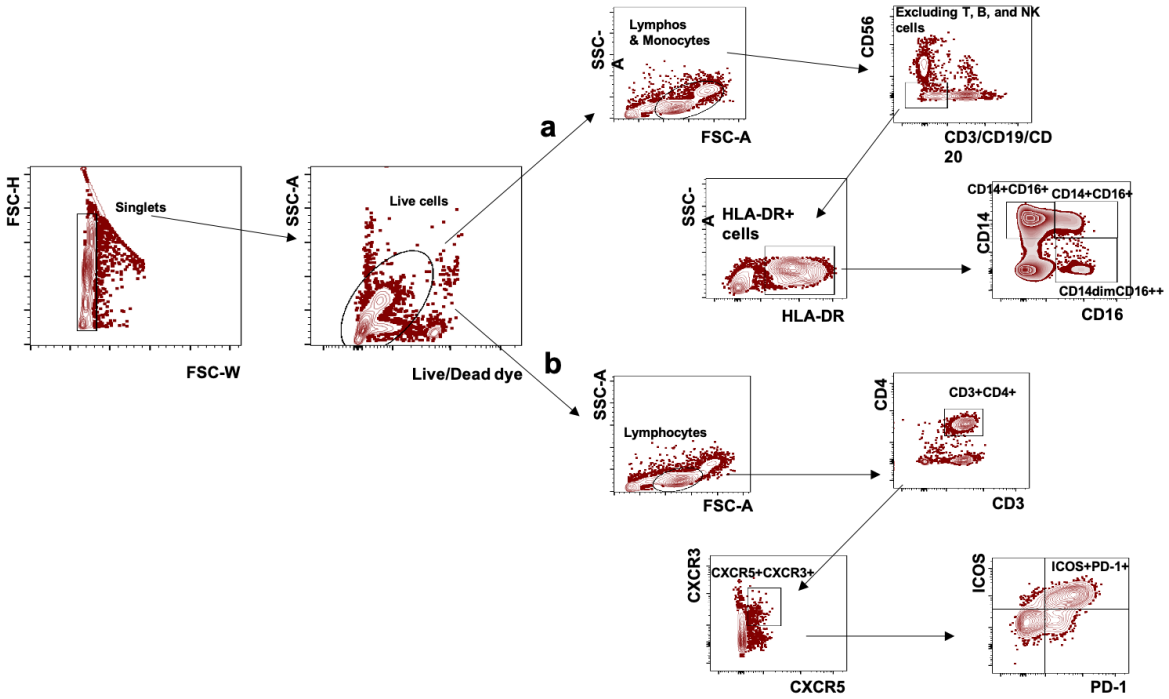


Supplementary Figure 1. Selection of study sample for detailed immunologic analysis. $MNP_{IIV\text{-}HCW}$, IIV by MNP; IM_{IIV}, IIV by IM injection; MNP_{IIV}-self, IIV by MNP self-administered by study participants; $MNP_{placebo}$, placebo by MNP.



Supplementary Figure 2. Gating strategy for monocytes and cT_{FH} cells. Single cells were selected on the basis of forward scatter width and height characteristics (FSC-W and FSC-H). a) Monocytes were identified within the SSC-A-hi FSC-A-hi cells as the CD3-CD19-CD20-CD56-HLA-DR+ population and gated for the CD14+CD16-, CD14+CD16+, and CD14dimCD16++ subsets. b) ICOS+PD1+cT_{FH} cells in PBMCs was identified by ICOS expression on CXCR3+CXCR5+CD4+ T cells.

Supplementary Table 1. Hemagglutination inhibition (HAI) geometric mean titers (GMT), seroprotection, and seroconversion at Day 28 and Day 180 following vaccination.

	A/Christchurch (H1N1)			A/Texas (H3N2)			B/Massachusetts		
	MNP	**IM**	***p***	**MNP**	**IM**	***p***	**MNP**	**IM**	***p***
GMT Day 0	124.4 (63.8, 242.4)	150.2 (92.4, 244.3)	*0.78*	54.81 (28.9, 103.7)	58.4 (28.9, 117.8)	*0.81*	42.6 (25.1, 72.3)	51.5 (35.3, 75.0)	*0.53*
GMT Day 28	1201.8 (612.9, 2356.4)	934.1 (530.9, 1643.6)	*0.59*	248.71 (131.9, 468.9)	219.3 (129.7, 370.8)	*0.66*	132.4 (79.2, 221.4)	80 (59.6, 107.4)	*0.08*
GMT Day 180	411.7 (165.0, 1,027.2)	362.9 (210.7, 625.2)	*0.64*	90.8 (45.8, 180.0)	70.5 (36.7, 135.4)	*0.59*	58.4 (30.9, 110.4)	80 (59.6, 107.4)	*0.99*
GMT Day 28/Day 0	9.7 (3.9, 23.4)	6.2 (3.2, 12.1)	*0.42*	4.5 (2.2, 9.3)	3.8 (1.92, 7.36)	*0.84*	**3.1 (2.3, 4.3)**	**1.6 (1.1, 2.3)**	***0.009***
GMT Day 180/Day 0	3.3 (1.2, 9.4)	2.4 (1.14,5.13)	*0.33*	1.7 (1.2, 2.4)	1.2 (0.8, 1.7)	*0.29*	1.37 (1.0, 1.9)	1.1 (0.9, 1.5)	*0.29*
Seroprotection (Day 28)	100% (71.5, 100)	100% (71.5, 100)	*1.00*	100% (71.5, 100)	100% (71.5, 100)	*1.00*	100% (71.5, 100)	100% (71.5, 100)	*1.00*
Seroprotection (Day 180)	100% (71.5, 100)	100% (71.5, 100)	*1.00*	90.9% (58.7, 99.8)	81.8% (48.2, 97.7)	*0.99*	81.8% (48.2, 97.7)	90.9% (58.7, 99.8)	*1.00*
Seroconversion (D28/D0)	81.8% (48.2, 97.7)	81.8% (48.2, 97.7)	*1.00*	72.7% (39.0, 93.9)	72.7% (39.0, 93.9)	*1.00*	54.5% (23.4, 83.3)	18.2% (2.3, 51.8)	*0.18*
Seroconversion (D180/D0)	45.5% (16.7, 76.6)	27.3% (6.0, 60.9)	*0.66*	18.2% (2.3, 51.8)	0% (0.0, 28.5)	*0.48*	0% (0.0, 28.5)	0% (0.0, 28.5)	*1.00*

Values are GMTs with 95% CIs based on normal distribution of log-transformed values. Mann-Whitney Wilcoxon tests were used to compare MNP (n=11) and IM (n=11) groups. For seroprotection and seroconversion, p-values were calculated using chi-square test and 95% CIs are calculated using Clopper-Pearson exact CIs. A two-sided p-value of < 0.05 was considered statistically significant.

HAI, Hemagglutination inhibition; GMT, geometric mean titer, GMT; microneedle patch; IM, intramuscular injection; CI, confidence interval.

Supplementary Table 2. Neuraminidase inhibition (NAI) geometric mean titers (GMT) at Day 28 and Day 180 following vaccination.

	H6N1			H6N2			H6NB		
	MNP	**IM**	***p***	**MNP**	**IM**	***p***	**MNP**	**IM**	***p***
GMT Day 0	**57.1 (32.2, 101.4)**	**111.9 (88.1, 142.1)**	***0.04***	42.4 (24.3, 74.1)	51.4 (31.6, 83.4)	*0.54*	137.7 (93.2, 203.5)	165.5 (83.2, 329.3)	*0.40*
GMT Day 28	306.2 (170.3, 550.3)	169.5 (132.8, 216.2)	*0.15*	166.7 (100.3, 276.9)	99.8 (64.9, 153.4)	*0.18*	230.6 (150.4, 353.6)	196.3(108.3, 355.6)	*0.90*
GMT Day 180	106.6 (58.8, 193.4)	133.1 (103.8, 170.6)	*0.72*	84.9 (48.8, 148.1)	68.5 (40.1, 117.1)	*0.40*	161.8 (103.4, 253.4)	157.2 (78.2, 315.8)	*0.99*
GMT Day 28/Day 0	**5.4 (2.9, 9.7)**	**1.5 (1.2, 1.9)**	***0.002***	**3.93 (2.7, 5.8)**	**1.9 (1.6, 2.4)**	***0.003***	**1.7 (1.5, 1.9)**	**1.2 (1.1, 1.3)**	***0.0008***
GMT Day 180/Day 0	**1.9 (1.3, 2.7)**	**1.2 (1.0, 1.4)**	***0.05***	2.0 (1.4, 2.9)	1.3 (1.1, 1.6)	*0.11*	**1.2 (1.0, 1.3)**	**0.9 (0.9, 1.0)**	***0.02***

Values are GMTs with 95% CIs based on normal distribution of log-transformed values. Mann-Whitney Wilcoxon tests were used to compare MNP (n=8) and IM (n=11) groups. A two-sided p-value of < 0.05 was considered statistically significant.

NAI, neuraminidase inhibition; GMT, geometric mean titer; MNP; microneedle patch; IM, intramuscular injection; CI, confidence interval