Supplementary Materials

Immunization Group	Animal ID	MHC Class I A*001	Age (years)
HVV	RSb13	+	6
	RCa11	+	10
	RVw8	+	13
	RRm7	+	15
	ROq10	-	11
	140-10	-	5
	174-10	-	5
	RRv9	+	12
	RUt12	-	7
	110-11	+	3
	287-11	+	3
	REn10	+	11
	RTw8	-	13
	RMt10	-	11
	RCq10	-	11
	RLk10	+	11
	178-10	-	5
	266-10	-	5
	RFl9	-	13
	RDu7*	-	15
	RBe7*	-	16
	RJq7*	+	15
	RZz9*	+	12

Immunization Group	Animal ID	MHC Class I A*001	Age (years)
Env+NP	191-11	-	4
	294-11	-	4
	220-11	-	4
	RWm15	-	3
	19-12	-	3
	147-12	-	3
	261-11	-	4
	217-11	-	4
	322-11	-	4
	207-12	-	3
	323-11	-	4
	203-12	-	3
	97-12	-	3
	239-11	-	4
	427-02	-	13
	411-10	-	5
	390-01	-	14
	501-03	-	11
	RCp8*	-	14
	RSr8*	-	14
	RCk7*	-	15
	RTi8*	-	14

Immunization Group	Animal ID	MHC Class I A*001	Age (years)
HVV, Env+NP	RCw7	-	5
	RJo7	-	15
	RDk8	+	14
	133-10	-	5
	340-10	-	5
	ROj7	-	15
	RRb9	-	13
	RUy8	-	13
	RDe8	+	14
	ROn8	-	14
	RSw8	+	13
	RSk10	+	11
	RHl8	+	14
	RMa12	+	8
	202-10	-	5
	200-10	-	5
	RGc8	+	14
	167-12*	+	3
	RCm15*	+	3
	RBv12*	-	7
	RKa10*	-	12

Immunization Group	Animal ID	MHC Class I A*001	Age (years)
Controls	RRa15	+	4
	RGr14	+	5
	RQl14	-	5
	RVm14	-	5
	RZz14	-	5
	RTt14	-	4
	RMp14	+	4
	RUo14	-	4
	RBw14	-	4
	RYj14	-	4
	RDq13	+	7
	Rlg14	+	6
	Rlc15	-	4
	RBg15	-	4
	RTy11	+	9

Supplemental Table 1. Animal group assignments, MamuA*01 status and age
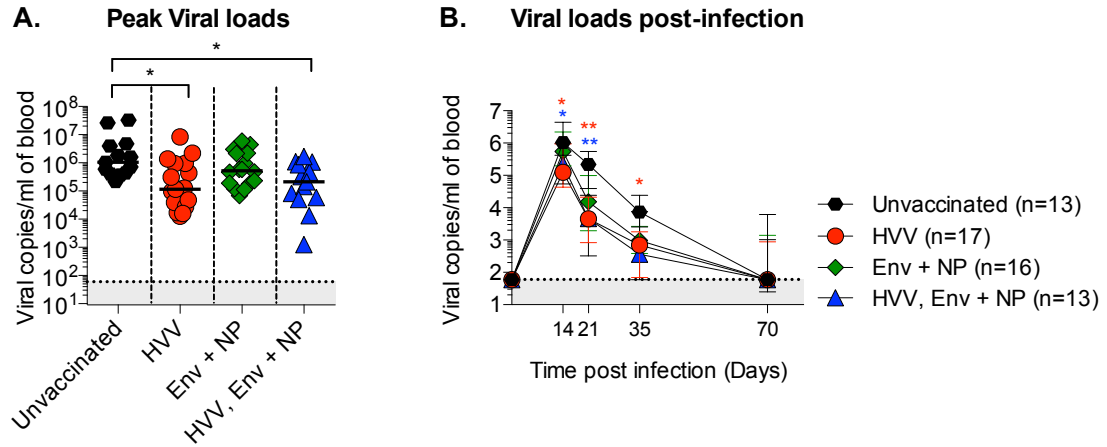
**Supplemental Figure 1. Decreased plasma viral loads in animals vaccinated with HVV.**

(A) Median viral loads measured in plasma at 2 weeks post-infection (peak) by real time PCR. PCR was performed in duplicate with a limit of detection of 60 copies per reaction. Statistical differences between unvaccinated and vaccinated groups were evaluated using the Kruskal-Wallis test with Dunn's multiple comparisons. **(B)** Kinetics of viremia after infection. Significant differences between the unvaccinated group and each of the vaccine groups were determined using the two-tailed Mann-Whitney test. $*p \leq 0.05$, $**p \leq 0.01$.
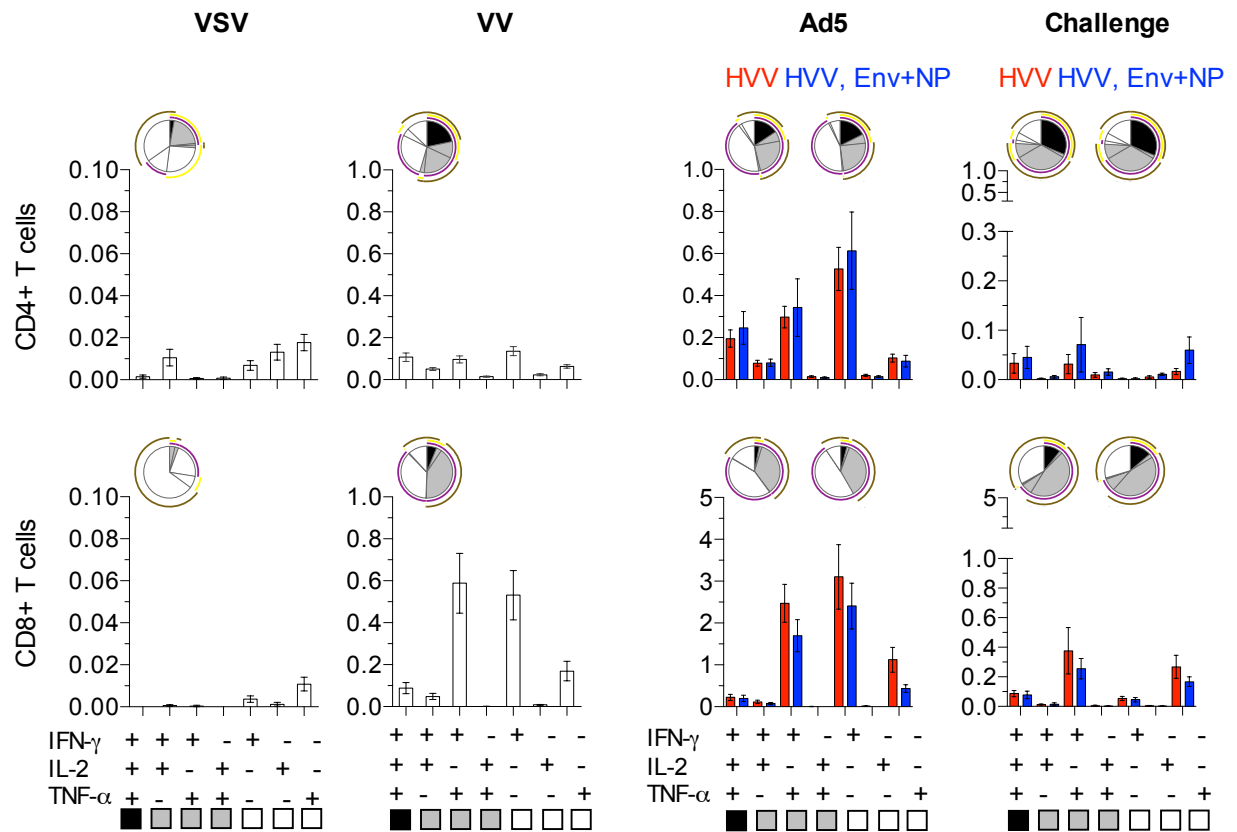
Supplemental Figure 2. Polyfunctional SIV Gag-specific CD4+ and CD8+ T cell responses induced by immunization with HVV
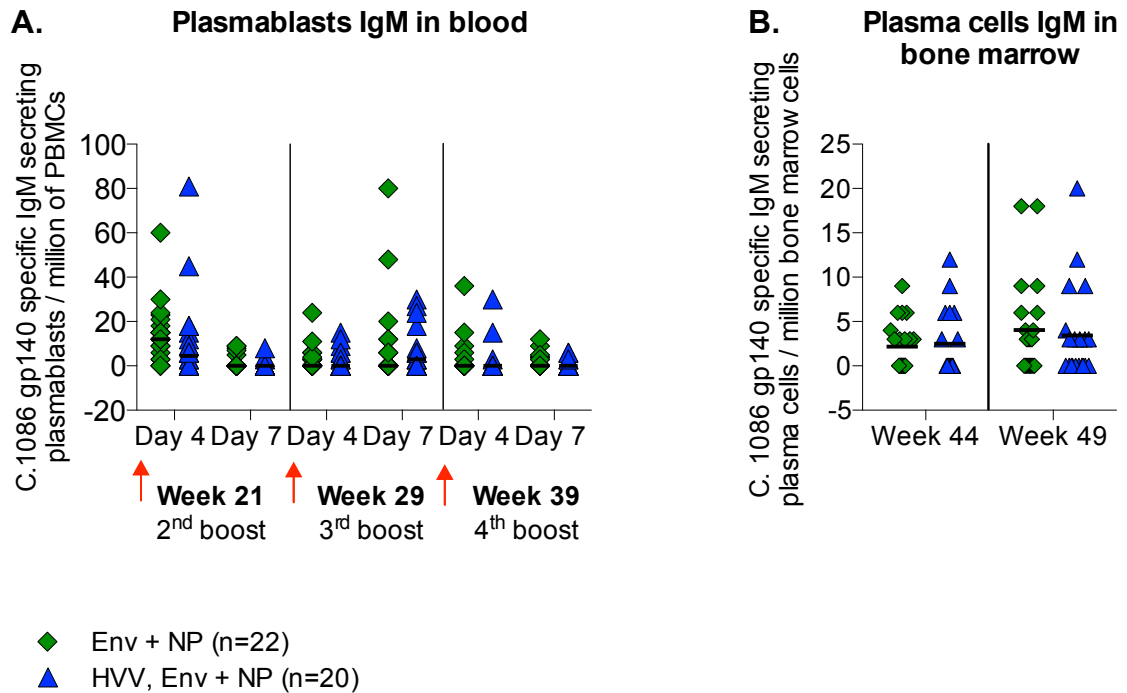
Polyfunctional SIV Gag-specific CD4+ and CD8+ T cell responses measured by ICS for IFN-γ, IL-2 and TNF-α after stimulation of PBMC from HVV (red) or HVV, Env + NP (blue) vaccinated animals with SIVmac239 Gag peptides. Polyfunctionality was determined using SPICE and PESTLE software (NIH). Single, double or triple cytokine producing cells are color coded in pies as indicated in the Pie slice legend. Pie chart arcs represent overlapping production of cytokines and are color coded as indicated in the corresponding legend. Every possible combination of responses is shown on the x axis. Bars represent the mean percentage of a given functional response within the total

CD4+ or CD8+ T cell population. Background was subtracted using an unstimulated control. Mean ±

SEM are shown.

A. Serum IgG

B. Vaginal IgG (Week 54)

C. Serum IgM (Week 54)
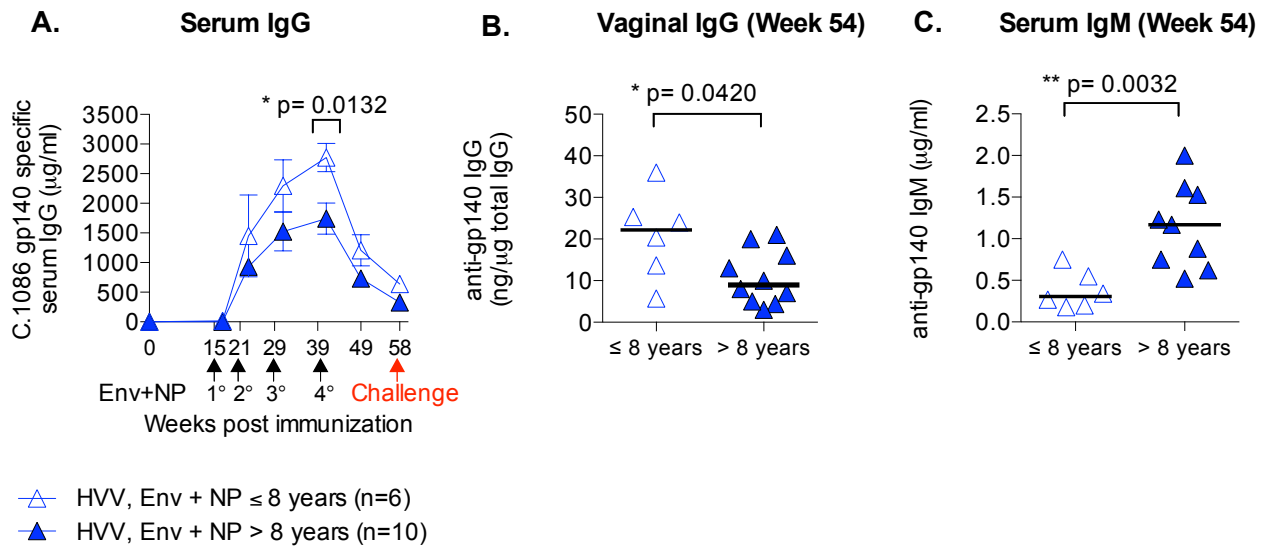
Supplemental Figure 3. Enhanced vaccine-elicited antibody responses in younger animals

(A) Levels of C. 1086 K160N gp140-specific serum IgG antibodies in macaques 8 years old (open triangles) and >8 years (closed triangles). **(B)** Levels of C. 1086 K160N gp140-specific vaginal IgG antibodies in macaques younger than 8 years old (open triangles) and > 8 years (closed triangles) one month before challenge (week 54) Bars represent medians. **(C)** Levels of C. 1086 K160N gp140-specific serum IgM antibodies in animals < 8 years old (open triangles) and > 8 years old (closed triangles) one month before challenge (week 54) Bars represent medians. Significant differences between groups were assessed using the two-tailed Mann Whitney test.

A. Plasmablasts IgM in blood

B. Plasma cells IgM in bone marrow

Legend:
- Env + NP (n=22) (green diamond)
- HVV, Env + NP (n=20) (blue triangle)

Supplemental Figure 4. Env-specific IgM plasmablasts in blood and long-lived plasma cells in bone marrow by adjuvanted Env protein immunizations

(A) C. 1086 K160N gp140-specific IgM plasmablasts measured by ELISPOT in blood collected on day 4 and day 7 after the 2nd (week 21), 3rd (week 29) and 4th (week 39) Env protein immunization in animals vaccinated with Env + NP (green) or HVV, Env + NP (blue) Bars represent medians. **(B)** C.1086 K160N gp140-specific IgM plasma cells measured 5 weeks (week 44) and 8 weeks (week 49) after the last Env protein immunization. Bars represent medians.
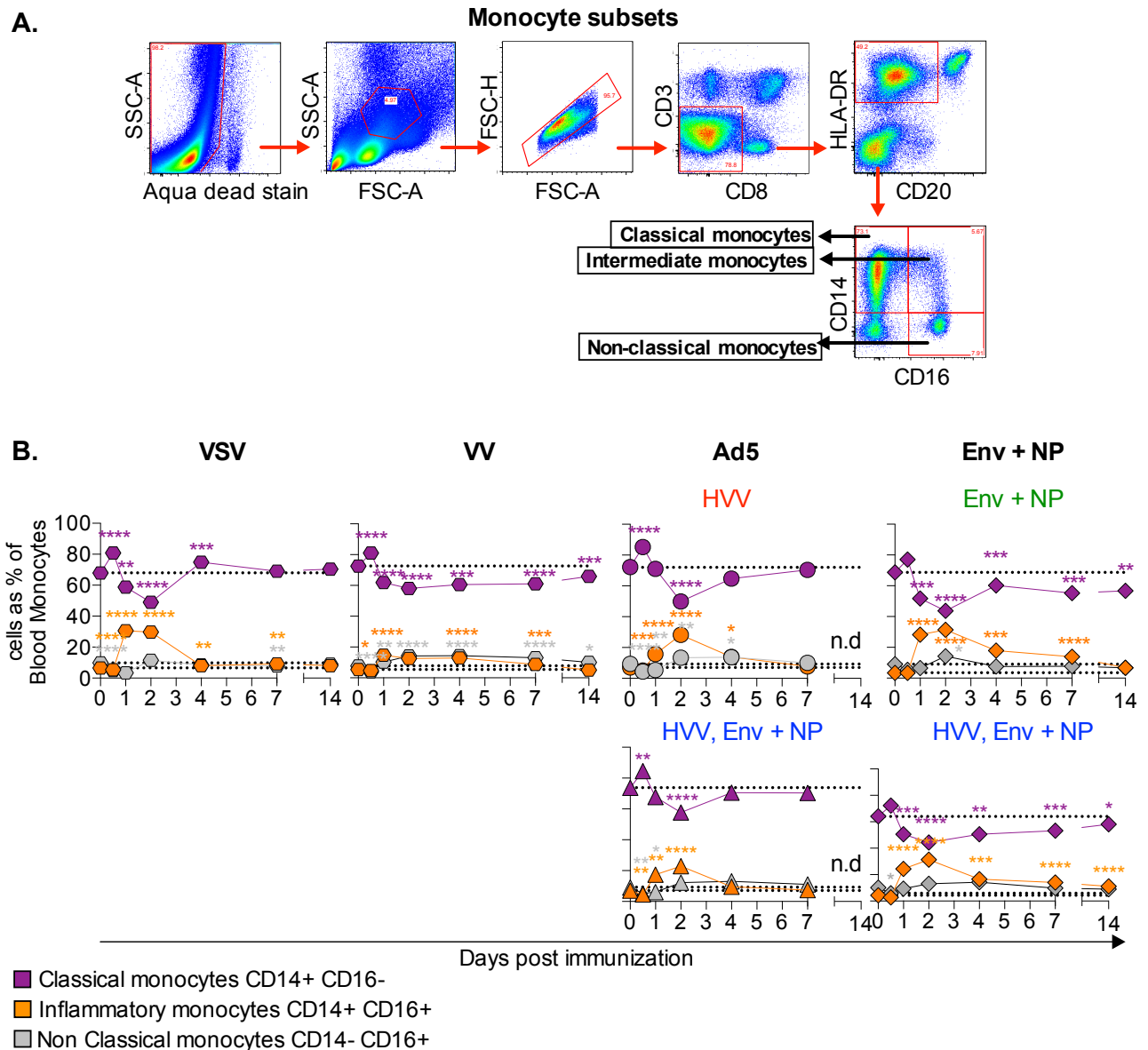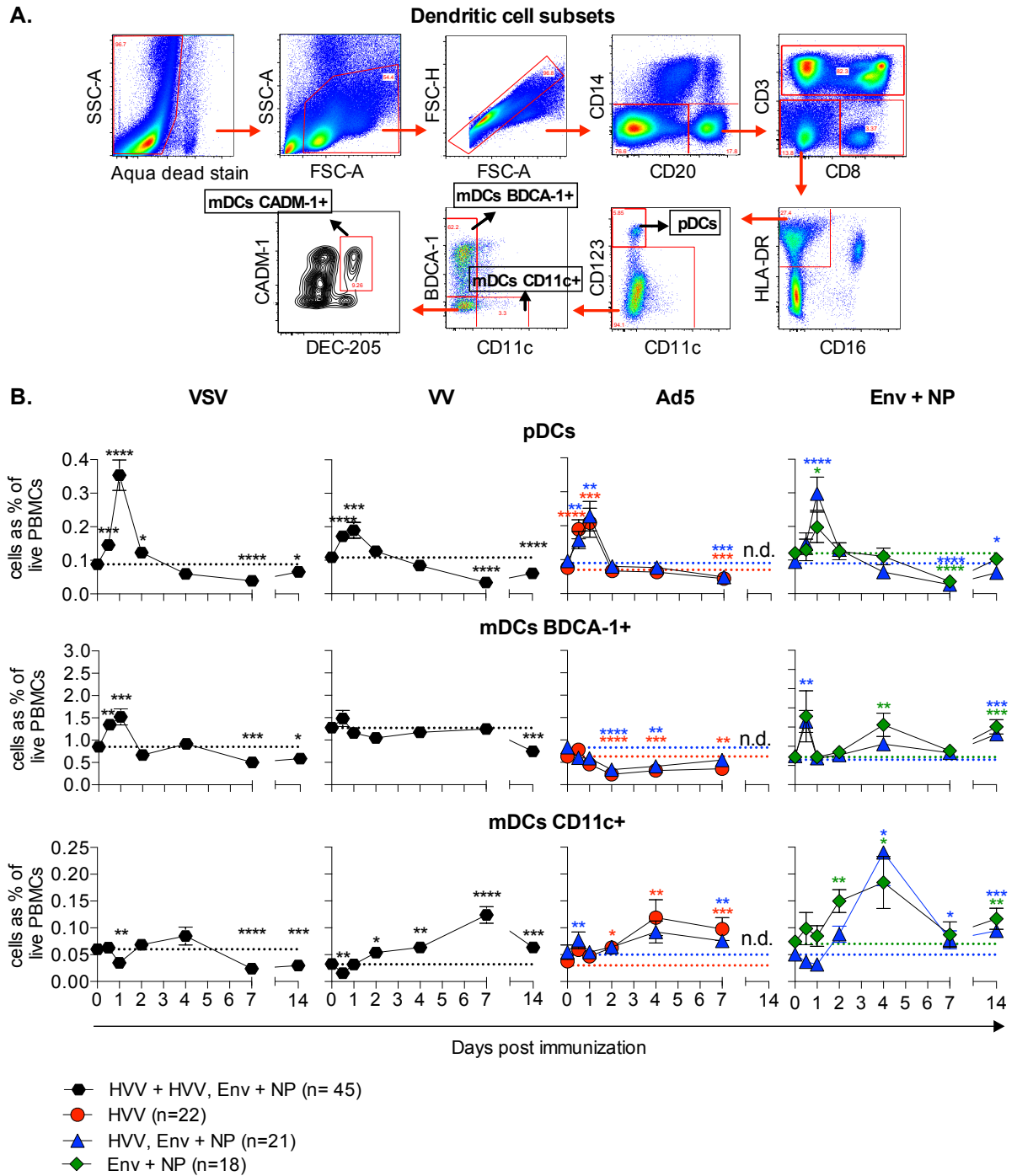
Supplemental Figure 5. Early innate monocyte subsets induced by vaccination

(A) Flow cytometry gating strategy to delineate monocyte subsets. Monocytes were identified after exclusion of dead cells by viability dye staining. Doublets were excluded and the three major monocyte subsets were defined by their expression of CD14 and CD16: CD14+CD16- classical monocytes, CD14+CD16+ inflammatory monocytes and CD14-CD16+ non-classical monocytes.

(B) Kinetics of monocyte subset frequencies as a fraction of the total monocytes in PBMCs after VSV-gag, VV-gag, Ad5-gag and Env + NP immunizations. The Wilcoxon signed rank matched pairs test was used to determine if post-vaccination percentages of cells were significantly greater than those measured at baseline. n.d.: not determined. *p ≤ 0.05, **p ≤ 0.01, *** p ≤ 0.001, ****p ≤

0.0001

Supplemental Figure 6. Early innate DC subsets induced by vaccination

(A) Gating strategy used to identify pDCs and three distinct mDC populations: BDCA1+ mDCs, CD11c+ mDCs and CADM1+mDCs. After exclusion of dead cells and doublets, pDCs were defined as CD14- CD20- CD3- CD8α- CD16- HLADR+ CD11c- CD123+ cells. After gating on the CD123-

population, the three mDC subsets were identified a follow: CD14- CD20- CD3- CD8α- CD16- HLADR+ CD123- CD11c$^{lo/-ve}$ BDCA1+ mDCs; CD14- CD20- CD3- CD8α- CD16- HLADR+ CD123- BDCA1- CD11c+ mDCs and CD14- CD20- CD3- CD8α- CD16- HLADR+ CD123- CD11c- BDCA1- DEC205+CADM1+ mDCs. (**B**) Kinetics of DC subset frequencies as a fraction of the PBMCs after VSV-gag, VV-gag, Ad5-gag and Env + NP immunizations. Data represents the mean ± SEM. Significant changes relative to the baseline were determined using the Wilcoxon matched-pairs signed rank test. *p ≤ 0.05, **p ≤ 0.01, *** p ≤ 0.001, ****p ≤ 0.0001.

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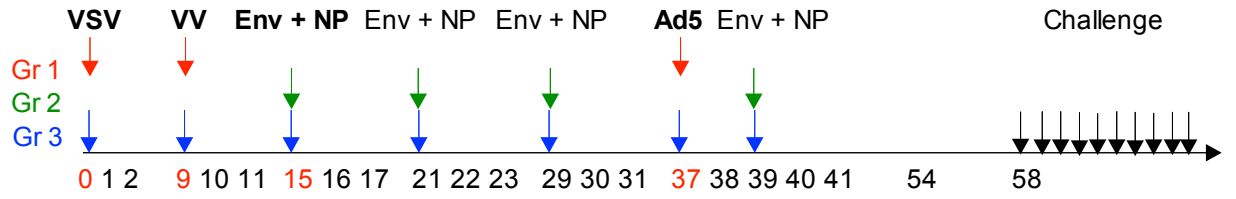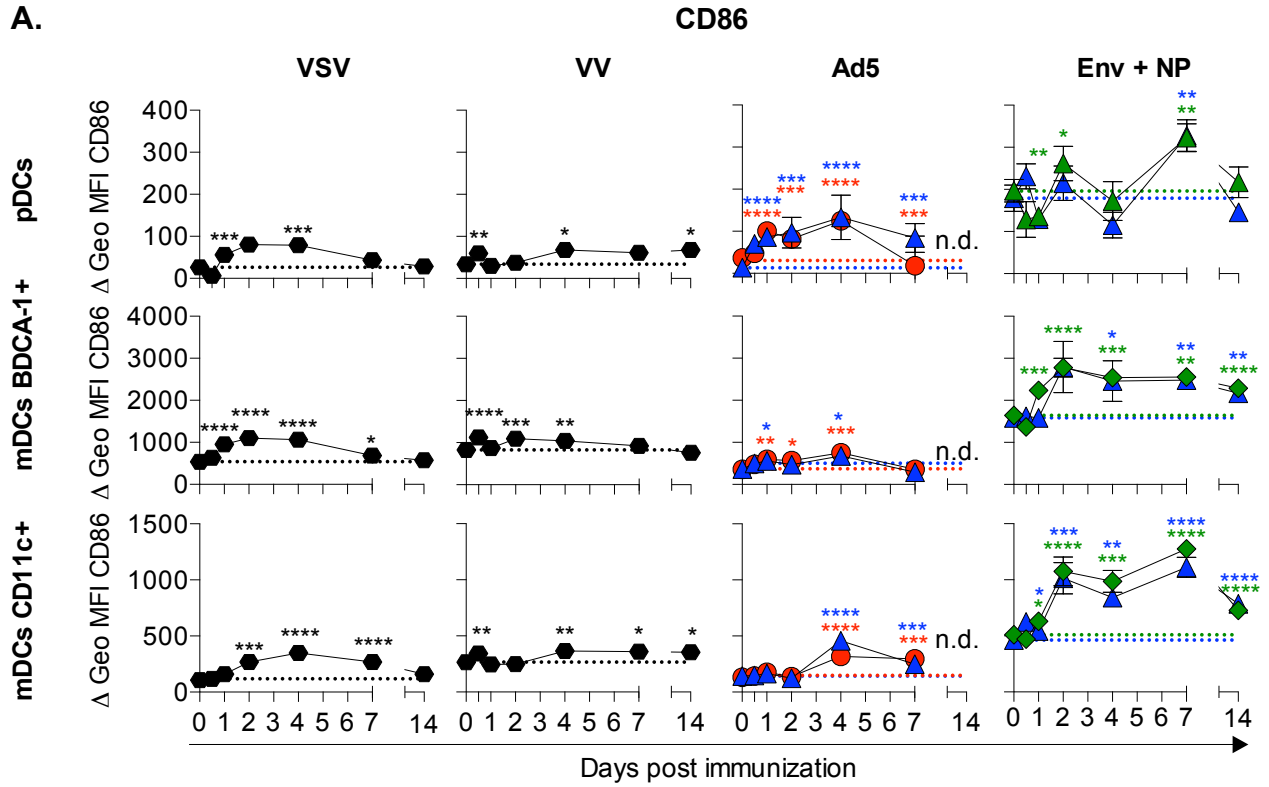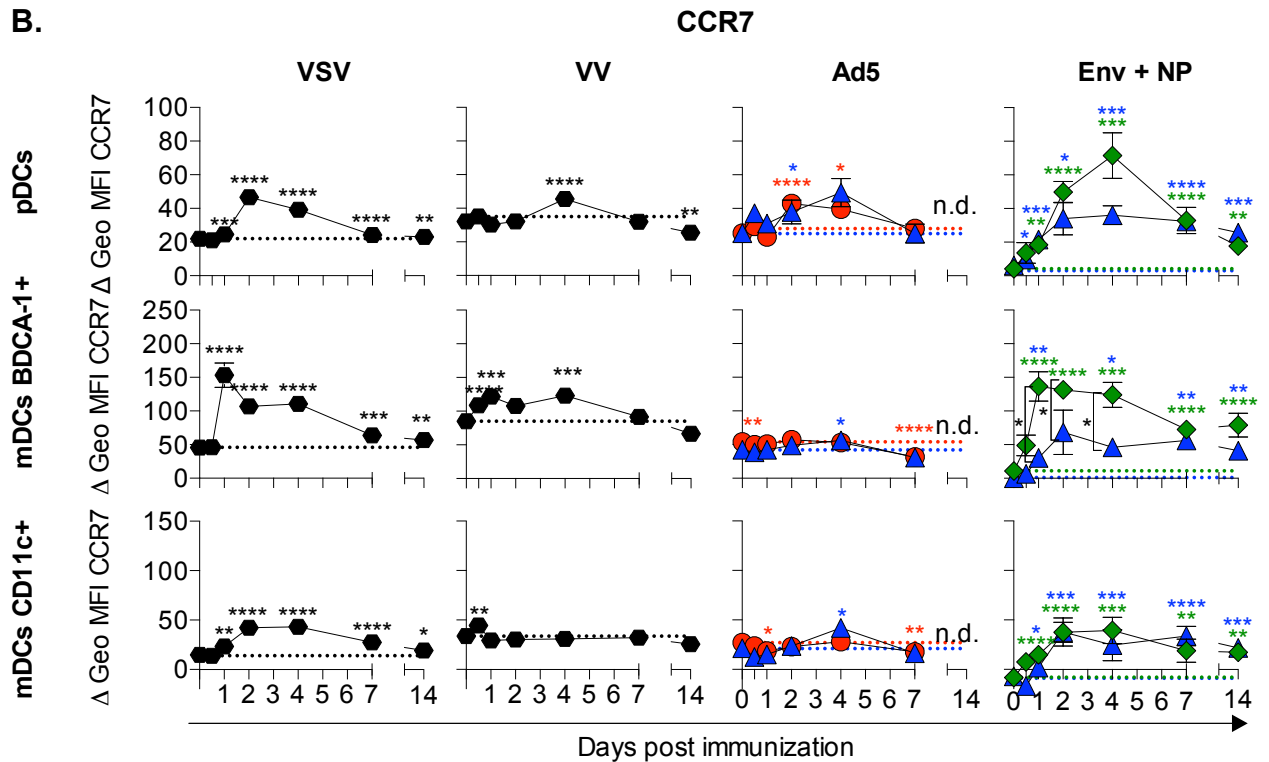
Supplemental Figure 7. Sustained activation of DC subsets following Env + NP immunization

(A) Kinetics of CD86 activation marker expression on the surface of pDCs, BDCA-1+ mDCs and CD11c+ mDCs after vaccination. **(B)** Kinetics of CCR7 chemokine receptor expression on the surface of pDCs, BDCA-1+ mDCs and CD11c+ mDCs (blue) for homing to lymphoid organs. Data represent the mean ± SEM of the geometric mean difference (Δ) between isotype control and antibody staining values. Significant changes relative to the baseline were determined using the Wilcoxon matched-pairs signed rank test and are denoted by asterisks. Statistical differences between vaccine groups were analyzed using the Mann-Whitney test and are indicated by a square bracket.
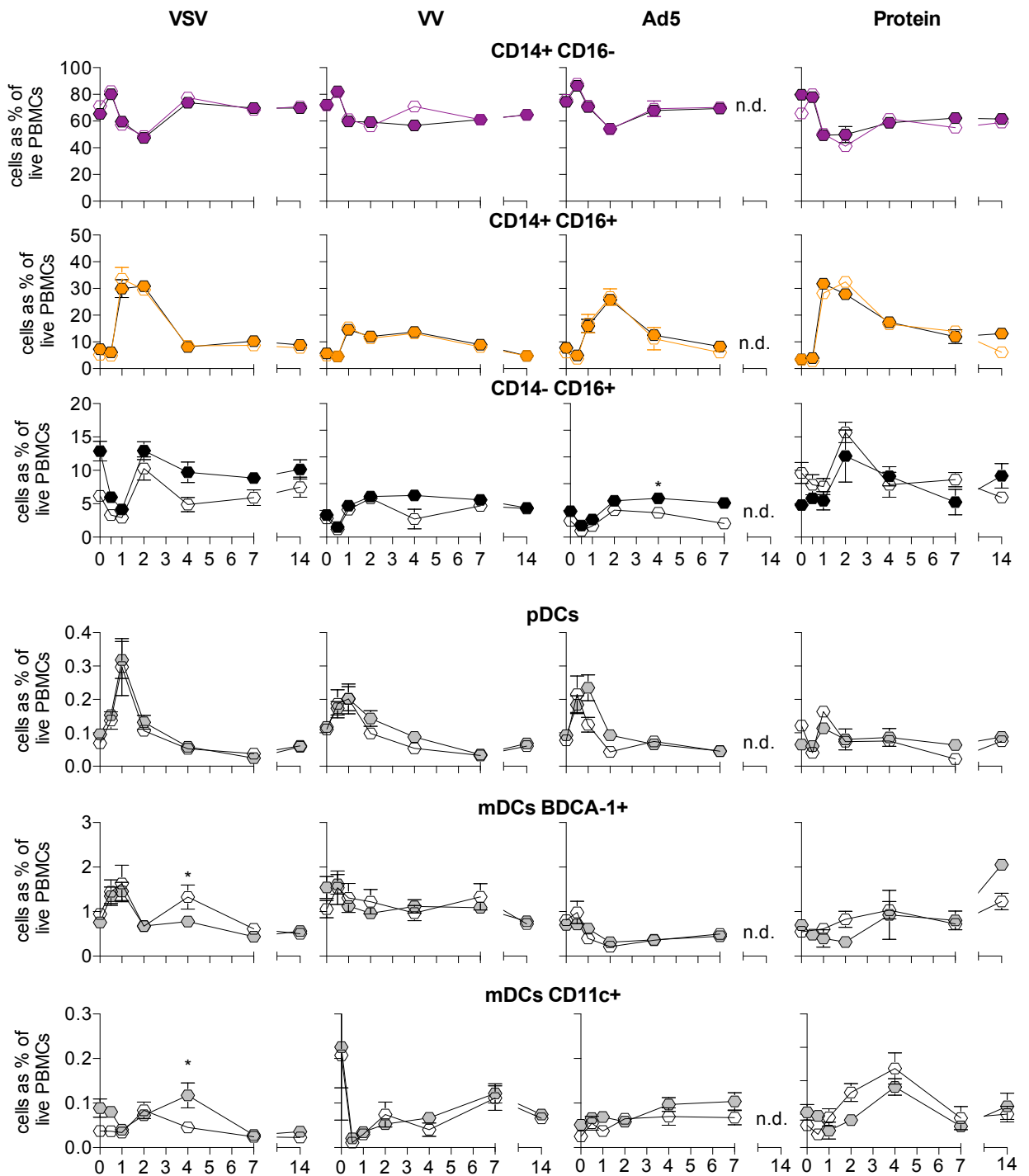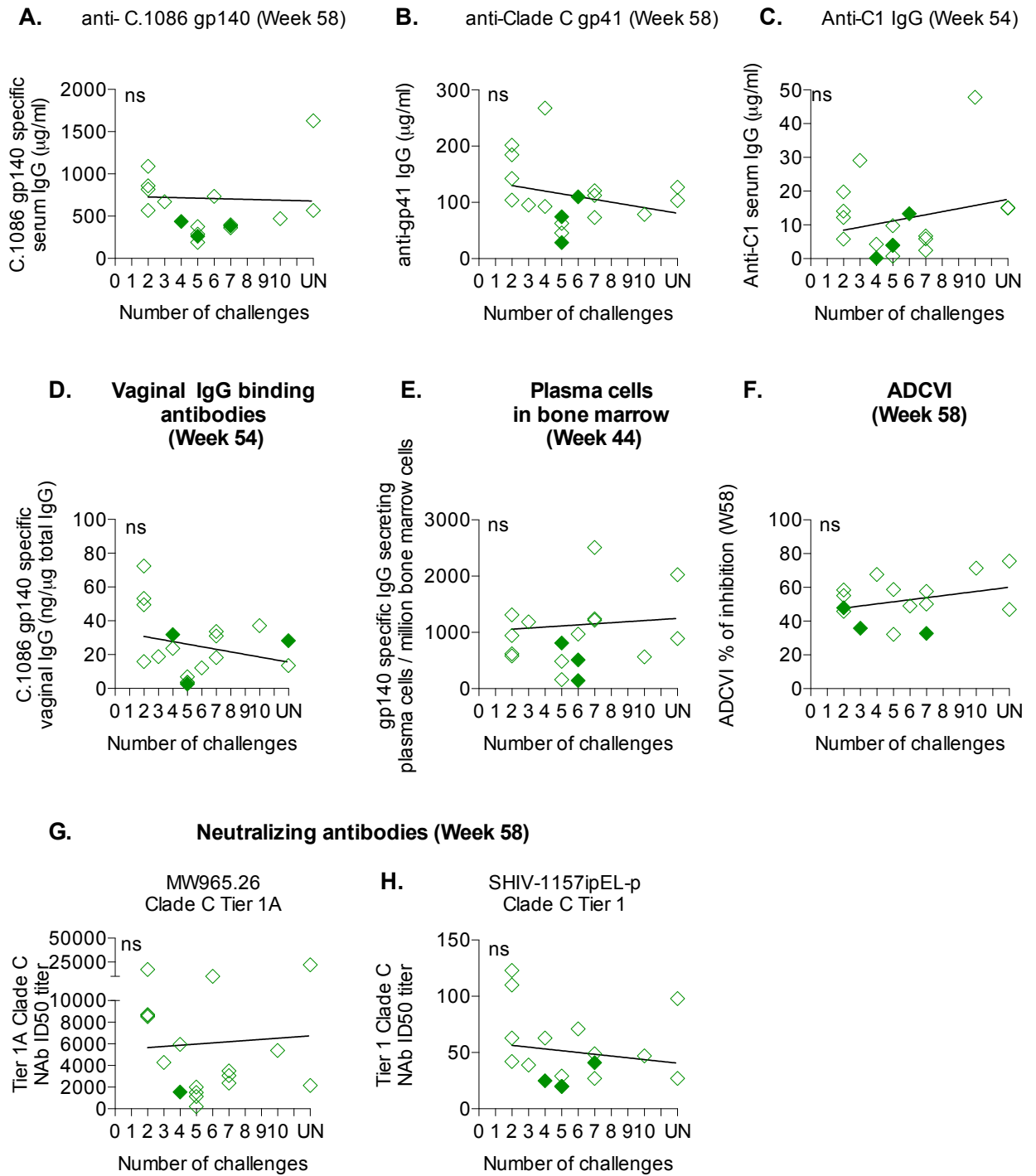
Supplemental Figure 8. Innate monocyte and DC subsets by group of age

Kinetics of monocytes (upper panel) and DCs (lower panel) frequencies as a fraction of the

PBMCs after VSV-gag, VV-gag, Ad5-gag and Env + NP immunizations. Open symbols

represent animals < 8 years old (n= 14, HVV + HVV, Env + NP animals for VSV, VV and Ad5

immunizations and n=21 Env + NP + HVV, Env + NP for Env + NP immunization) and full symbols represent animals > 8 years old (n= 20, HVV + HVV, Env + NP animals for VSV, VV and Ad5 immunizations and n=13 Env + NP + HVV, Env + NP for Env + NP immunization). Data represents the mean ± SEM. Significant changes relative to the baseline were determined using the Wilcoxon matched-pairs signed rank test. *p ≤ 0.05, **p ≤ 0.01, *** p ≤ 0.001, ****p ≤ 0.0001.

Serum IgG binding antibodies

A. anti- C.1086 gp140 (Week 58)

B. anti-Clade C gp41 (Week 58)

C. Anti-C1 IgG (Week 54)

D. Vaginal IgG binding antibodies (Week 54)

E. Plasma cells in bone marrow (Week 44)

F. ADCVI (Week 58)

G. Neutralizing antibodies (Week 58)

MW965.26 Clade C Tier 1A

H. SHIV-1157ipEL-p Clade C Tier 1

Supplemental Figure 9. Associations between pre-challenge antibody responses and rate of infection acquisition in Env + NP immunized animals

Using the two-tailed Spearman rank test, we measured the rate of infection acquisition in Env + P vaccinated animals (**A-C**) concentrations of serum IgG antibodies against the gp140 immunogen, gp41 protein, and C1 peptide, **(D)**

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levels of C.1086 gp140-specific vaginal IgG antibodies, (**E**) numbers of anti-gp140 IgG- secreting plasma cells in bone marrow, (**F**) ADCVI activity against SHIV-1157ipd3N4 in serum, and (**G**) titers of serum neutralizing antibodies to Tier 1 Clade C viruses MW965.26 and SHIV-1157ipEL-p on the weeks indicated. In all graphs, open and closed diamonds denote animals < 8 and > 8 years old, respectively. Correlation tests were performed using results for all animals in the Env + P vaccination group.