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Supplemental Information

Route of Vaccine Administration Alters Antigen Trafficking but Not Innate or Adaptive Immunity

Sebastian Ols, Lifei Yang, Elizabeth A. Thompson, Pradeepa Pushparaj, Karen Tran, Frank Liang, Ang Lin, Bengt Eriksson, Gunilla B. Karlsson Hedestam, Richard T. Wyatt, and Karin Loré

Figure S1

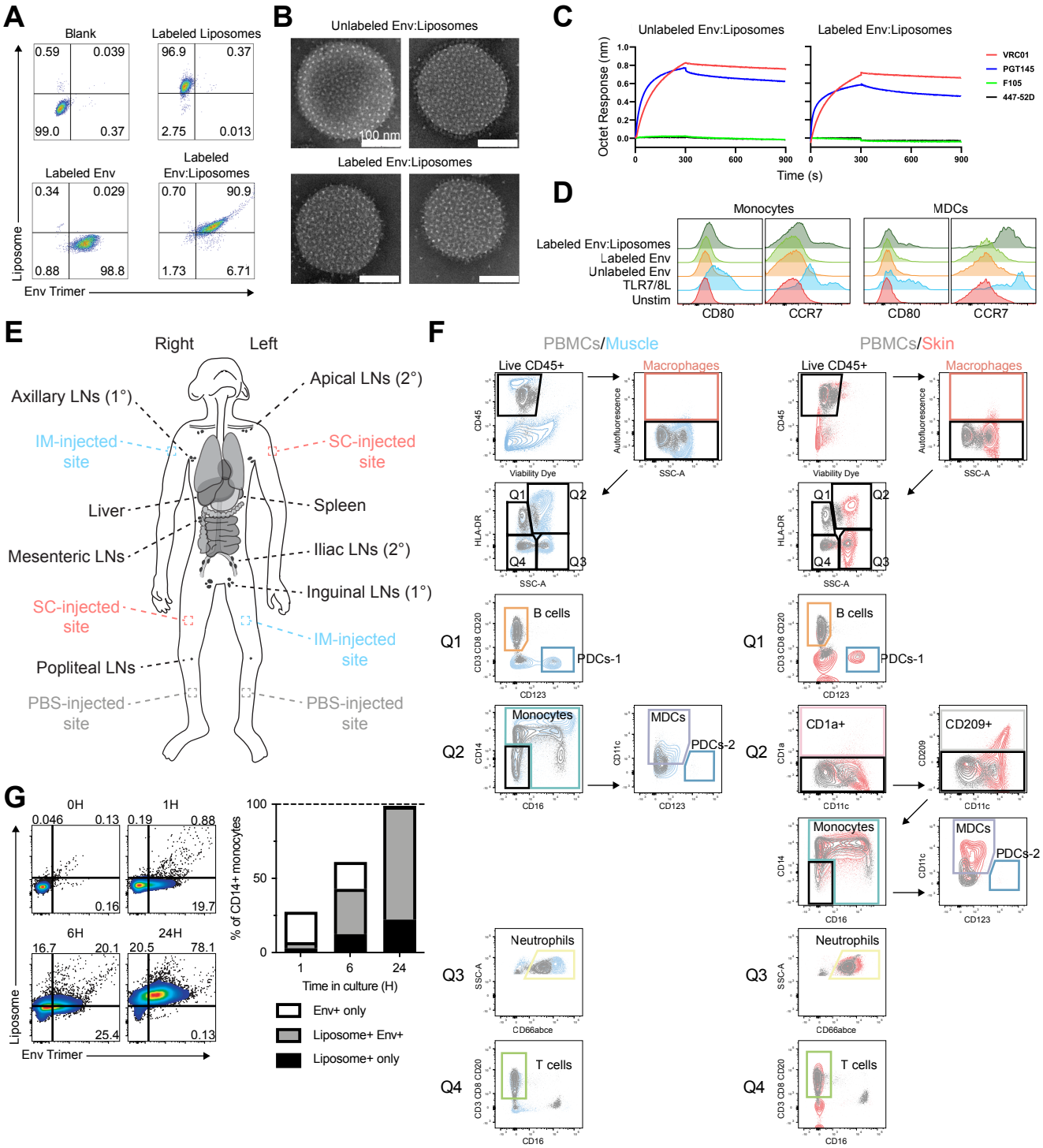

Figure S1. Vaccine formulation and study design. Related to Figure 1 and 2.
(A) Representative flow cytometry plots of labeled vaccine components incubated with a TZM-bl cell line.
(B) Negative stain electron microscopy of unlabeled and labeled Env:liposomes.
(C) Antigenic characterization of unlabeled and labeled Env:liposomes by Octet.
(D) Representative histograms of the activation profile of isolated human monocytes and MDCs after in vitro culture for 24 hours (H) with vaccine components. n = 5 human donors.
(E) Immunization schematic for vaccine tracking experiments with labeled sites of injection and all harvested tissues.
(F) Representative gating strategy for analysis of muscle (blue) and skin (pink) with overlay of PBMC staining (gray).
(G) In vitro time-course of Env:liposome uptake by CD14+ monocytes in human PBMCs. Representative flow cytometry plots of Env:liposome signals from negative control (0), 1, 6, and 24 h culture. Mean displayed. n = 5 human donors.

Figure S2

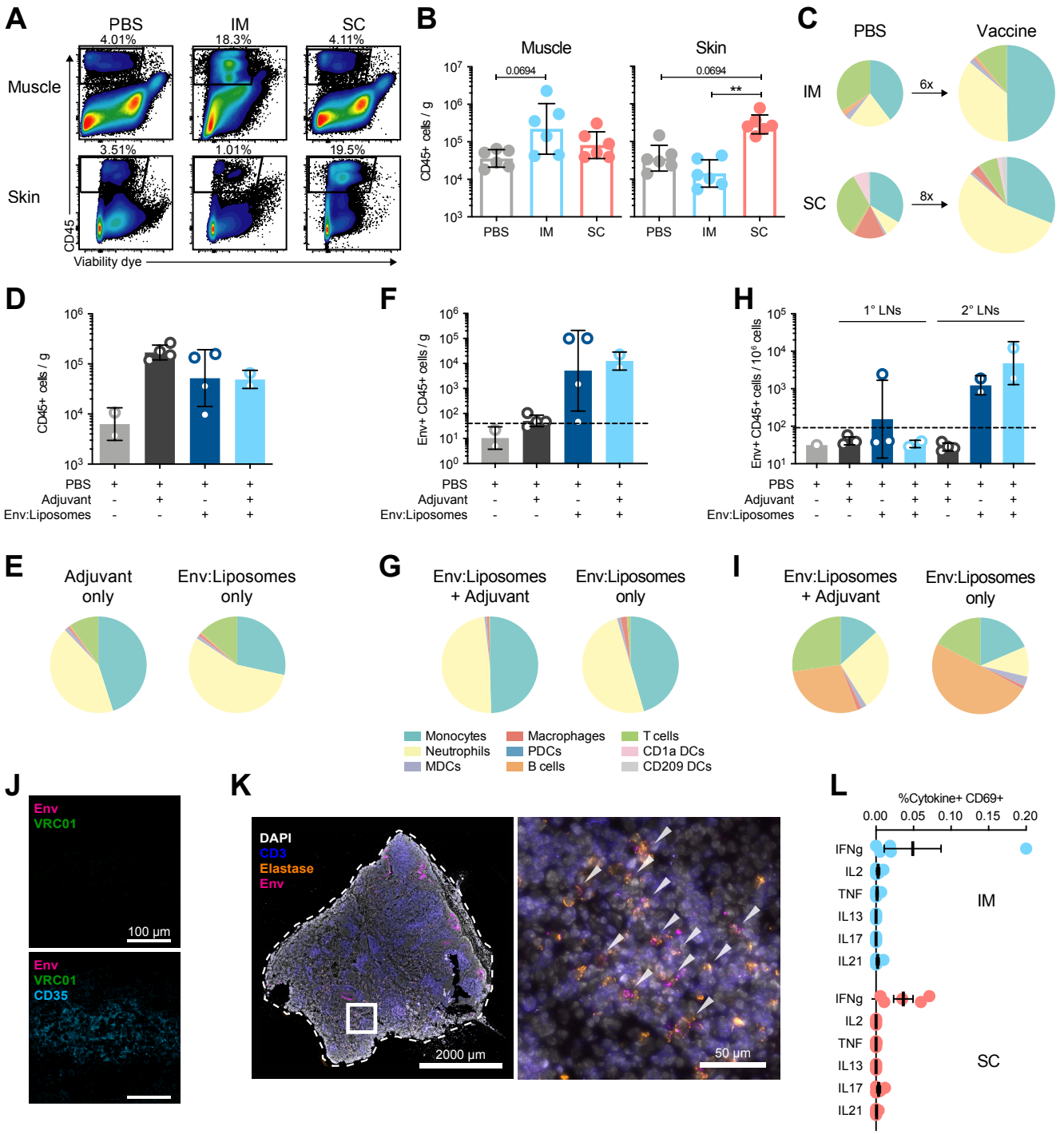

Figure S2. Immune cell infiltration to the site of injection mediated by both adjuvant and liposomes. Related to Figure 1 and 2.
(A) Flow cytometry gating of CD45+ cell infiltration to the site of injection in naive animals.
(B) Quantification of infiltrating CD45+ cells per gram of muscle or skin tissue in naive animals.
(C) Proportions of infiltrating CD45+ cell subsets in the muscle and skin after IM and SC injection, respectively. Fold-change of the mean is shown.
(D) Quantification of CD45+ immune cell subsets per gram of muscle tissue in animals immunized IM with PBS, Matrix-M™ adjuvant only, Env:liposomes only, or Env:liposomes and Matrix-M™ adjuvant.
(E) Proportions of CD45+ immune cell subsets in the muscle after IM injection as in (D).
(F) Env+ CD45+ cells in the muscle represented as in (D).
(G) Env+ CD45+ cell subsets in the muscle represented as in (E).
(H) Quantification of Env+ CD45+ cells in LNs of animals in (D).
(I) Proportions of Env+ CD45+ cell subsets in the draining LNs (sum of 1° and 2° LNs) of animals in (H).
(J) Representative images of a Env- and VRC01- LN follicle with staining for CD35 (cyan), Env-AF680 (magenta), and VRC01 (green). See also Figure 1I.
(K) Representative images of Env+ neutrophils in LNs. LNs stained for DAPI (white), CD3 (blue), Env-AF680 (magenta), and neutrophil elastase (orange). Arrows indicate Env+ neutrophils.
(L) Env-specific CD4+ memory T cell responses in blood measured by intracellular cytokine recall assay at week 22. See also Figure 2H.
(B) Geometric mean ± gSD displayed. Data points represent individual tissue samples; n = 6 per group. *, $p < 0.05$; **, $p < 0.01$.
(D-I) Mean ± SD displayed. Data points represent individual tissue samples; n = 2-4 per group. Dashed line represents the limit of detection, see methods for calculation. (J-K) Image brightness was increased to allow for visualization. (L) Mean ± SEM displayed. Data points represent individual animals; n = 5 per group.

Figure S3

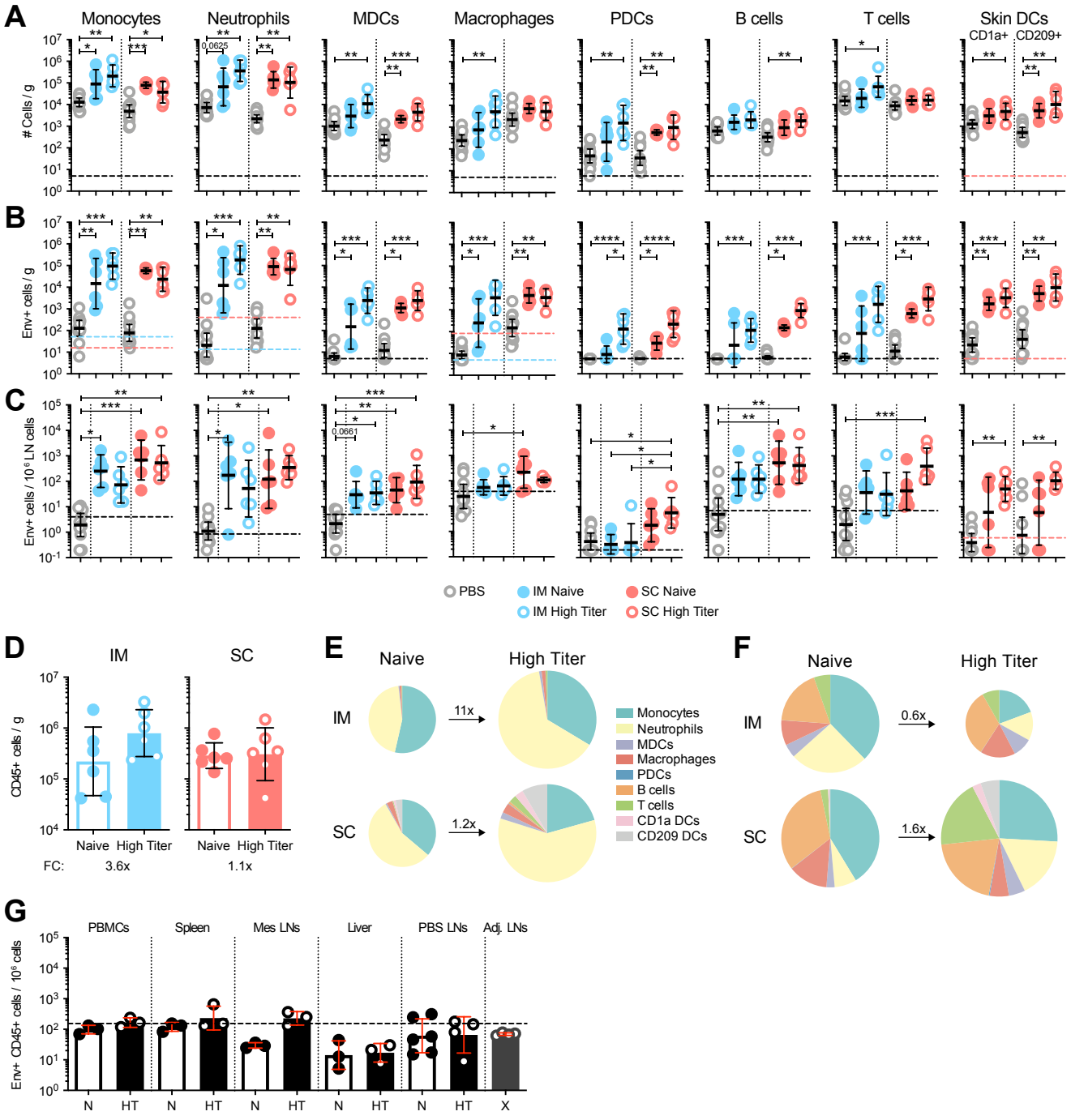

Figure S3. Env uptake is affected by pre-existing antibodies but does not disseminate systemically. Related to Figure 3.
(A) Quantification of infiltrating CD45+ immune cell subsets per gram of muscle or skin tissue after IM or SC immunization, respectively.
(B) Quantification of Env+ CD45+ immune cell subsets per gram of muscle or skin tissue after IM or SC immunization, respectively.
(C) Quantification of Env+ CD45+ immune cell subsets in the draining LNs after IM or SC immunization, respectively. Sum of 1° and 2° LNs displayed.
(D) Quantification of CD45+ cells per gram of muscle or skin tissue of naive and high titer animals.
(E) Proportions of Env+ immune cell subsets in the muscle after IM injection and in the skin after SC injection of naive and high titer animals. Fold change (FC) of the mean is shown.
(F) Same as in (E), but draining LNs displayed.
(G) Quantification of Env+ CD45+ immune cells in peripheral tissues of naive (N), high titer (HT), or adjuvant-only draining LNs (X).
(A-G) Geometric mean ± gSD displayed. Data points represent individual tissue samples; n = 3-12 per group. Dashed line represents the limit of detection, see methods for calculation. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Figure S4

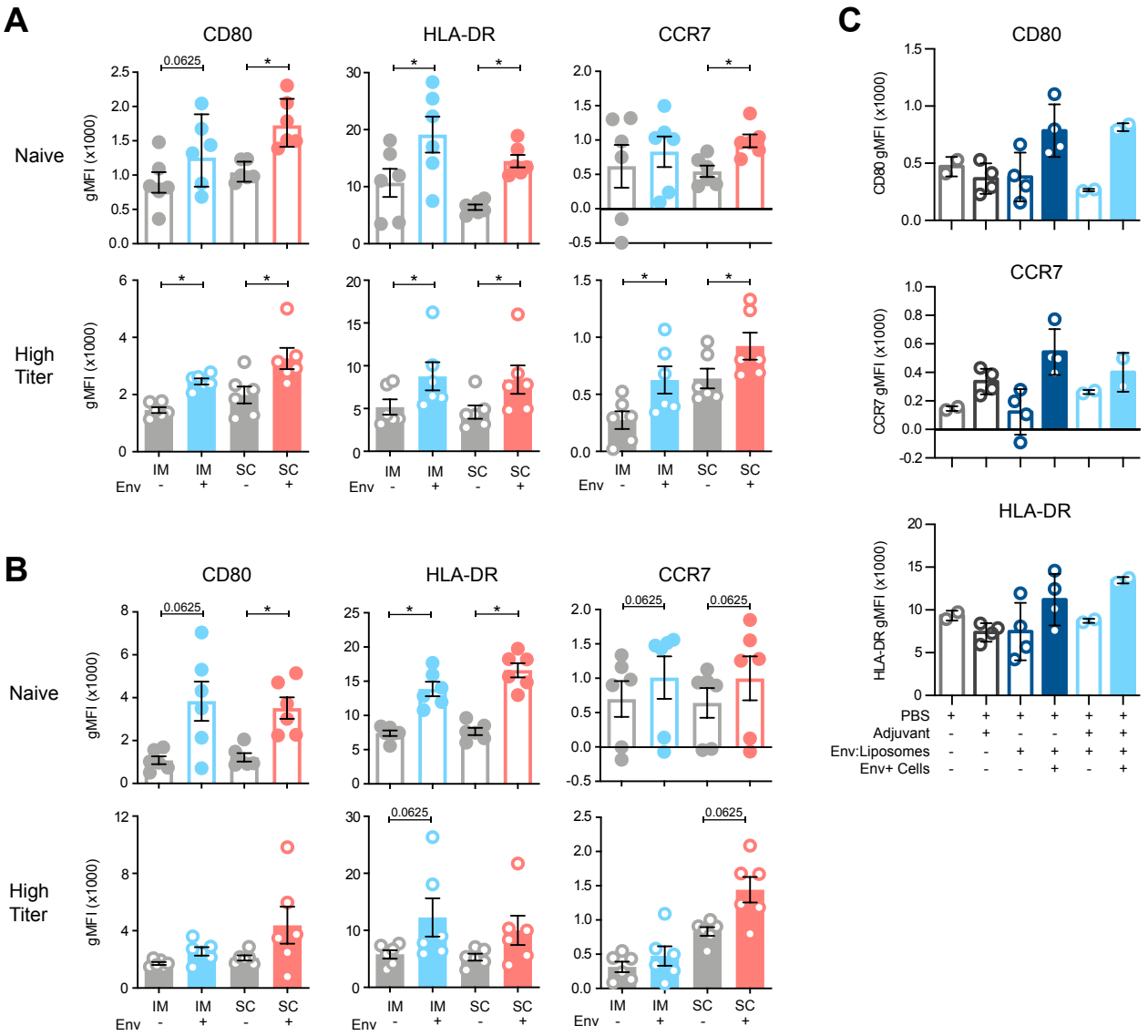

Figure S4. APC activation is coupled to vaccine uptake at the site of injection and the draining LNs. Related to Figure 4.
(A) Geometric mean fluorescence intensity (gMFI) of CD80, HLA-DR, and CCR7 in Env- or Env+ APCs at the site of injection.
(B) Same as in (A), but draining LNs displayed.
(C) gMFI of CD80, CCR7, and HLA-DR of APCs in animals receiving PBS, adjuvant only, Env:liposomes only, or Env:liposomes with adjuvant by IM injection.
(A-C) Mean ± SEM displayed. Data points represent individual tissue samples; (A-B) n = 6 per group; (C) n = 2-4 per group. *, $p < 0.05$.