Supporting Information

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SI Materials and Methods

Mice. C57BL/6 mice (B6, H-2^b) were purchased from Harlan. *Lang-EGFP* mice expressing EGFP under the control of the *langerin* gene were a kind gift from B. Malissen (Centre d'Immunologie Marseille-Luminy, Marseille, France) (1). *CD11c-DTR-EGFP*, OT-1 Rag$^{-/-}$and *Langerin-DTR-EGFP* mice (1–3) were kindly provided through G. Lombardi (King's College London) and C. Bennett (University College London, United Kingdom). All transgenic mice were stably crossed onto the B6 background for more than 10 generations and bred at Kings College London. Mice were used between 6 and 8 wk of age and were maintained in pathogen-free conditions. All animal husbandry and experimentation was approved under a personal project license (to L.S.K.), granted by the United Kingdom Home Office.

Construction of recombinant human adenovirus type 5 (rAdHu5)-CN54gag.opt. The group specific antigen (gag) gene from HIV-1 strain CN54 was codon optimized and synthesized by GeneArt. A Gly to Ala (G2A) substitution was introduced to eliminate myristoylation of the gag protein. The stop codon was replaced by a four amino acid linker to a HA tag (nine amino acids) for screening and detection purposes. The gag-HA sequence was cloned into the pShuttle-CMV (Stratagene) by using KpnI/HindIII restriction sites. The ORF was then placed within an expression cassette comprising the intermediate/early gene of human cytomegalovirus (CMV) and the simian virus 40 (SV40) late polyadenylation signal as upstream and downstream regulatory elements, respectively (Fig. S9). This shuttle plasmid was linearized and the expression cassette was inserted into an E1$^-$E3$^-$deleted Ad5 genomic DNA (pAdEasy-1; Stratagene) by means of homologous recombination in the BJ5183 strain of *Escherichia coli*. Genomic viral rAdHu5-CN54gag.opt was isolated as suggested by the manufacturer. All rAdHu5 viruses were propagated in low passage human embryo kidney 293 (293AD) cells (Cambridge Biosciences), purified by double CsCl gradient ultracentrifugation (4), and then dialyzed against PBS containing 50 mM Hepes at pH 7.8 and 7.5% (wt/vol) LPS-free sucrose (Sigma-Aldrich) and stored at −80 °C until use. Virus particle (vp) titres were determined by the DNA Pico-Green assay (Invitrogen) (5).

Recombinant Adenovirus Desiccation, Recovery, and Immunogenicity. Purified virus [rAdHu5-ovalbumin (OVA)] formulated 1:1 with an unbuffered solution containing 30% (wt/vol) sucrose (Sigma-Aldrich) and 8% (wt/vol) sodium carboxymethylcellulose (Na-CMC; Sigma-Aldrich) was pipetted onto a 24-well plate (100 μL per well, corresponding to 6×10^9 vp). These were air-dried at 25 °C for 24 h, then removed to a desiccator with silica gel bed for storage at 25 °C for up to 1 mo and then reconstituted to 600 μL with PBS. Control samples from the identical stock and titer of virus, but stored at −80 °C, were diluted to 600 μL with PBS. Immunogenicity was determined by s.c. injection of B6 mice with 100 μL of "control" or recovered "dried" virus, then CD8$^+$ T-cell responses was determined by K^b/SIINFEKL tetramer staining.

Fabrication of Microneedle Arrays (MAs). Arrays were fabricated by a two-step centrifugation casting method using a 1 cm^2 inverted cone shaped silicone template comprising 44 needles, each 1,500 μm in height and 670 μm in base diameter. A first layer gel was applied containing 8% (wt/vol) Na-CMC, (Sigma-Aldrich) and 30% (wt/vol) sucrose (Sigma-Aldrich) formulated at a 1:1 ratio with either a rAdHu5 vector or a fluorochrome-labeled tetramethyl rhodamine dextran (M_r 70 kDa) (TR) (10 mg/mL; Invitrogen) or fluorescein isothiocyanate (FITC, 25 mg/mL; Invitrogen) and then centrifuged at 1780 × *g* for 1 min to draw the solution into the template cavity. A second gel (without vaccine vector/fluorochrome-linked dextran) containing 4.8% (wt/vol) lactose (Sigma-Aldrich) in a 12% (wt/vol) Na-CMC solution (to provide mechanical strength) was overlaid, and the template was re-centrifuged at 1780 × *g* for 1 min. Finally, a premade Na-CMC membrane disk, prepared by drying 60 μL of gel (0.8% (wt/vol) lactose and 8% (wt/vol) Na-CMC) on a 1 cm^2 circular template was then applied to form the base of the MA. After air drying overnight at room temperature, the MAs were carefully removed from the template and stored in a desiccator at room temperature. For dendritic cell (DC) tracking experiments, MAs contained both rAdHu5-OVA and TR or FITC-labeled dextran.

Immunization of Mice. MAs were applied manually with gentle pressure for 5 min to either the dorsal surface of the foot, to the ear, or to the back skin of mice, shaved of hair and depilated with Nair cream (Church and Dwight) 2 d earlier. After removal, skin was rinsed with water and then dried with tissue to avoid oral immunization by self-grooming. Additional groups of mice received the equivalent rAdHu5-vector dose, by the i.m., i.d., or s.c. route. All in vivo procedures were performed in accordance with United Kingdom Home Office regulations for animal experimentation.

Conditional Depletion of CD11c$^+$ and Langerin$^+$ Cells. *CD11c*-diphtheria toxin receptor (*DTR*)-*EGFP* mice received an i.p. injection of 4 ng per kg of body weight diphtheria toxin (DT; Sigma-Aldrich) 1 d before immunization as described (2). To deplete all Lang$^+$ cells, *Lang-DTR-EGFP* mice were treated with 1 μg of DT at 4 and 1 d before immunization and at 3 d after immunization (6).

Intravital Microscopy. Langerin-EGFP or CD11c-DTR-EGFP mice were anesthetized by using 50 mg/kg ketamine, 10 mg/kg xylazine, and 1.7 mg/kg acepromazine injected i.p. to induce anesthesia as described (7). "Empty" or TR-dextran containing microneedle arrays were applied on the shaved skin of the back and removed as described in *Materials and Methods*. Mice were then immediately placed (microneedle array exposed surface down) on a custom-made tray-stage insert with a circular 2.5-cm hole covered with a coverslip (50 mm, no. 1.5). Mice were maintained at 37 °C with oxygen (0.5 L/min) with 0.5% isoflurane within a thermostated chamber housing an inverted confocal microscope (SP5; Leica) equipped with 10× 0.4 numerical aperture HCX PL APO and 20× 0.5 N.A. PL Fluotar air objectives. The 488 nm argon ion and 561 nm DPSS laser lines were used to excite GFP and TR, respectively. GFP and TR fluorescence were detected sequentially to minimize cross-talk. Surface volume rendering was performed in Imaris (Bitplane). "Surface clipping layers" were added to reveal TR-dextran colocalized with CD11c-DTR-GFP–positive cells. Imaging and image presentation were performed identically for mice treated with "empty" or TR-Dextran–treated MA.

Immunofluorescence Microscopy of Frozen Skin Sections. Skin was carefully dissected and snap frozen in OCT tissue freezing medium (TissueTek). Six-micrometer frozen sections were cut by using a cryostat microtome; air dried and stored at −80 °C until use. Before staining, slides were allowed to return to room temperature for 20 min, then fixed in cold acetone (10 min) followed by washing in PBS. Sections were blocked with 10% normal goat serum (Vector Laboratories) in PBS for 30 min before incubation (1 h at room temperature) with purified rat anti-Langerin antibody (clone eBioRMUL.2; eBioscience) in PBS containing 1% BSA. Purified rat IgG2a was used as an isotype control. Washed

sections were incubated 30 min with TRITC-conjugated goat anti-rat IgG (Jackson ImmunoResearch Laboratories), then rewashed and mounted in Vectashield mounting medium containing DAPI for nuclei staining (Vector Laboratories). Sections were analyzed with an Axiophot microscope (Carl Zeiss) equipped with a 20× objective (final 200× magnification). Images were acquired by using Nikon Nis-elements software.

Preparation and Immunostaining of Epidermal Sheets. Ears were split into dorsal and ventral halves and each placed dermal side down in PBS plus 0.2% Trypsin (Invitrogen) at 37 °C for 25 min. Epidermis was separated from the underlying dermis by using forceps and washed several times in PBS. The epidermal sheets were then fixed with PBS 3% paraformaldehyde for 15 min, washed, and permeabilized in PBS plus 0.5% Triton X-100 (Sigma-Aldrich) for 30 min. Sheets were blocked by normal goat IgG (Caltag) in PBS plus 0.5% Triton X-100 (staining buffer) for 30 min before being labeled overnight with rat anti-Langerin antibody (clone 929F3.01; Dendritics) and rabbit anti-GFP antibody (Invitrogen). Samples were washed several times in PBS 0.1% Triton X-100 and incubated for an additional 1 h at room temperature with Alexa Fluor 488-conjugated goat-anti-rabbit antibody (Invitrogen) and Cy3-conjugated goat-anti-rat antibody (Jackson Immunoresearch). Sheets were mounted in mounting medium (Vector Labs) complemented with DAPI (Invitrogen) for nuclei staining and analyzed as above.

DC Isolation and Analysis. Popliteal LN were digested with Collagenase A (1 mg/mL; Roche Diagnostics) in RPMI medium 1640 (Invitrogen) supplemented with DNase I (0.1 mg/mL; Roche Diagnostics) and 3% FBS (Biosera), then filtered through a stainless-steel sieve and washed twice in PBS supplemented with DNase I and FBS. Cell suspensions were enriched in low-density cells by centrifugation in cold iso-osmotic Optiprep solution (EUROBIO-ABCYS, pH 7.2; density, 1.065 g/cm^3). For the preparation of dermal DC, the epidermis and dermis were mechanically separated after an incubation overnight at 4 °C in Dispase II (1.2 U/mL; Roche Diagnostics) diluted in PBS. Dermal sheets were then rinsed with cold PBS and digested at 37 °C for 1 h with Collagenase A (1 mg/mL; Roche Diagnostics) in RPMI medium 1640 (Invitrogen) supplemented with DNase I (0.5 mg/mL) and 3% FBS. Cell suspensions were then washed once in RPMI medium 1640 supplemented with 5 mM EDTA (Invitrogen) and 3% FBS. Enriched LN DCs and dermal DCs were analyzed by flow cytometry.

Reagents and Antibodies for Flow Cytometry. The following antibodies were purchased from BD Biosciences: purified anti-FcγRII/III mAb (CD16/32, 2.4G2), anti-CD28 (37.51), FITC, PerCP or Allophycocyanin-Cy7-anti-CD8 (53-6.7), FITC-anti-CD4 (RM4-5), PE-anti-I-A/I-E (M5/114.15.2), PerCp-anti-CD11b (M1/70), PE-Cy7-anti-CD11c (HL3), Allophycocyanin-Cy7-anti-CD45 (30-F11), Allophycocyanin-anti-IFN-γ (XMG1.2), biotin-conjugated anti-CD103 (M290), and PerCP-streptavidin. PE-anti-TNF-α (MP6-XT22), FITC-anti-IL-2 (JES6-5H4), and Allophycocyanin-anti-H2-K^b/SIINFEKL (25-D1.16) were purchased from eBiosciences and Alexa 647-anti-Langerin (929F3.01) was purchased from Dendritics. Samples were acquired by using a FACSCanto II flow cytometer (BD Biosciences) provided by the UK National Institute for Health Research Biomedical Research Centre based at Guy's and St. Thomas' National Health Service Foundation Trust and King's College London, and analyzed using FlowJo software version 9.5.2 (Tree Star).

DC Sorting. Low density enriched cells isolated from inguinal LNs (as detailed above) were incubated with anti-FCγRII/III mAb (CD16/32, clone 2.4G2) for 10 min at 4 °C. Cells were then stained with PE-anti-I-A/I-E (MHC II, clone M5/114.15.2), PerCP-anti-CD8α (clone 53–6.7), PE-Cy7-anti-CD11c (clone HL3), and PerCP-Cy5.5-anti-CD11b (clone M1/70) from BD Biosciences and with Pacific Blue-anti-CD49b (pan-NK, clone DX5) and Allophycocyanin-Cy7-anti-CD326 (EpCAM, clone G8.8) from BioLegend. Cells were sorted with a purity of >98% by using a FACS ARIA II cytometer equipped with a 100-μm nozzle and DIVA 6.1.1 software (BD Biosciences). The sorting strategy allowed us to define LN-resident DCs as CD11c^{hi} MHCII$^{+/int}$ CD49b^{neg} and migratory DCs that were purified based on CD11c$^+$ MHCII^{hi} CD49b^{neg}, then further separated based on CD8α^{neg} EpCAM^{neg} expression from which the CD11b^{hi} cells were separated that contained >95% of Lang^{neg} DCs. CD11c^{neg} MHCII$^+$ CD49^{neg} cells were sorted to serve as a negative control in the ex vivo presentation assay.

Ex Vivo Antigen Presentation Assay. Sorted DC subsets were cultured at a concentration of 2×10^4 DCs with 10^5 CFSE-labeled CD8$^+$ OVA-specific naive T cells (OT-I) for 5 d in DMEM with 10% heat inactivated FBS, 4.5 g/L D-Glc, 2 mM L-Gln, Pyruvate, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 50 μM 2-Mercaptoethanol in U-bottom 96-well plates. CFSE dilution was measured by flow cytometry. Controls in which T cells alone or with DC preincubated with SIINFEKL peptide (2 μg/mL) in vitro were included to check DC functionality after isolation. OVA-specific T cells were purified from RBC-depleted splenocytes isolated from OT-1 Rag$^{-/-}$ mice and then depleted with rat mAbs to CD45R/B220 (clone RA3-6B2), I-A/I-E (MHC II (clone 2G9), CD4 (clone GK1.5), Ly-6G and Ly-6C (clone RB6-8C5), and CD25 (clone 7D4) followed by incubation with sheep anti-rat IgG-coated Dynabeads (Dynal Biotech). The purity of responder cells was ~95%. T cells were finally labeled with 4 μM CFSE-SA (Invitrogen), according to the manufacturer's instructions, before coculture with DCs.

Intravaginal Booster Immunization. Mice were injected s.c. with 3 mg of Depo-Provera (Pfizer), a long-lasting progestin, in 100 μL of sterile PBS 5 d before intravaginal immunization with 20 μg of HIV-1 CN54 gag$_{309–318}$ peptide plus 30 μg of CpG ODN 1826 (Biosource) as described (8). Frequency of CD8$^+$ T cells to the immunodominant D^b restricted HIV-1 CN54 gag (GL10) epitope was detected by D^b/GL10 tetramer 5 d after the booster immunization.

Cell Preparation and Tetramer/Pentamer Staining. Single-cell suspensions were prepared from the spleen and mucosal associated lymphoid tissue (mesenteric LNs and Peyer's Patches) in complete RPMI [RPMI medium 1640 supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine (Invitrogen), and 10% heat inactivated FBS (Biosera)]. Blood and spleen suspensions were further treated with red blood cell lysis buffer (Sigma-Aldrich). After washing, cells were stained 5 min at room temperature with K^b/SIINFEKL pentamer or D^b/GVKNWMTDTL tetramer followed by the addition of anti-CD8 antibody (20 min) and rewashed. Cells were analyzed by flow cytometry.

Analysis of Polyfunctional T-Cell Responses. Spleens were harvested from individual mice at various times after immunization (according to the peak response predetermined for each rAd vector). Splenocytes at 1×10^6 cells per mL were incubated 6 h at 37 °C with anti-CD28 (2 μg/mL) either alone (unstimulated control) or with OVA$_{257–264}$ (5 μg/mL), or HIV-1 CN54 gag$_{309–318}$ (2 μg/mL) peptides for detection of CD8$^+$ T-cell responses. Brefeldin A (10 μg/mL) (Sigma-Aldrich) was added for the last 5 h of culture. After washing, cells were stained with anti-FcγRII/III (10 min), followed by anti-CD8 for 20 min, then fixed and permeabilized with the BD Cytofix/Cytoperm Kit according to the manufacturers' instructions, and then stained 30 min with anti–IFN-γ, anti–IL-2, and anti–TNF-α Abs. For CD107 staining, Abs were added during incubation with the peptide as described by ref. 9. Cells were analyzed by flow cytometry.

In Vivo Killing Assay. Naive syngeneic splenocytes were divided into two populations and labeled with CFSE at 5 μM ($CFSE^{hi}$) or 0.5 μM ($CFSE^{lo}$). $CFSE^{hi}$ cells were pulsed 1 h at 37 °C with HIV-1 CN54 $gag_{309–318}$ peptide (5 μg/mL), whereas $CFSE^{lo}$ remained unpulsed. The two splenocyte populations were then washed and mixed at a 1:1 ratio before i.v. injection into immunized mice. Inguinal, mesenteric LN, and spleen from recipient mice were harvested 15 h later to measure the in vivo cytolysis of target cells by the loss of the $CFSE^{hi}$ peptide-pulsed population relative to the control $CFSE^{lo}$ population by flow cytometry.

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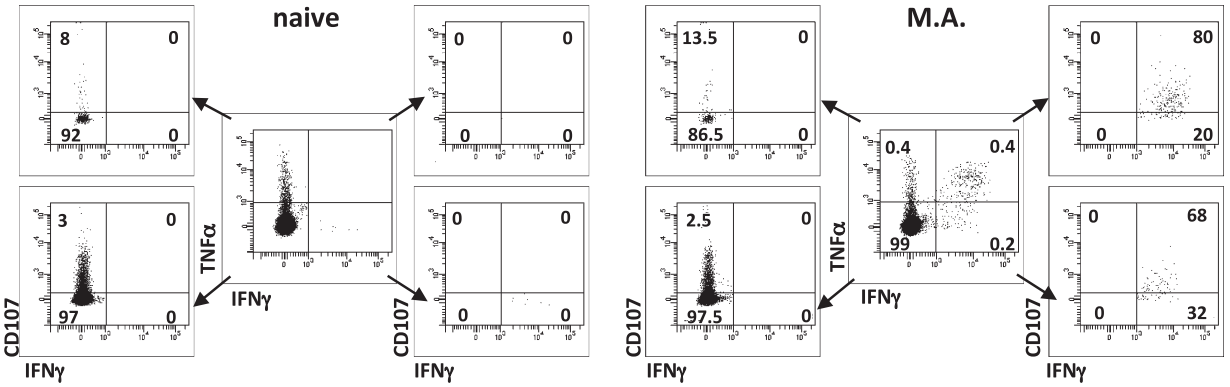


Fig. S1. Dried rAdHu5 vectored vaccines retain thermostability and induce functional $CD8^+$ T cells when fabricated into dissolvable MAs. Frequency of HIV-1 CN54 $gag_{309–318}$-specific CD107, TNF-α, and IFN-γ–expressing $CD8^+$ T cells in the spleen of naïve mice and 14 d after immunization with rAdHu5-HIV-1 CN54 gag MAs was detected by intracellular cytokine staining and surface CD107a and CD107b staining after 6-h stimulation ex vivo with HIV-1 CN54 $gag_{309–318}$ peptide (5 μg/mL) or media only and assessed by flow cytometry. Values represent percentage of cells in each gate. Data are representative of two naïve and four immunized mice.

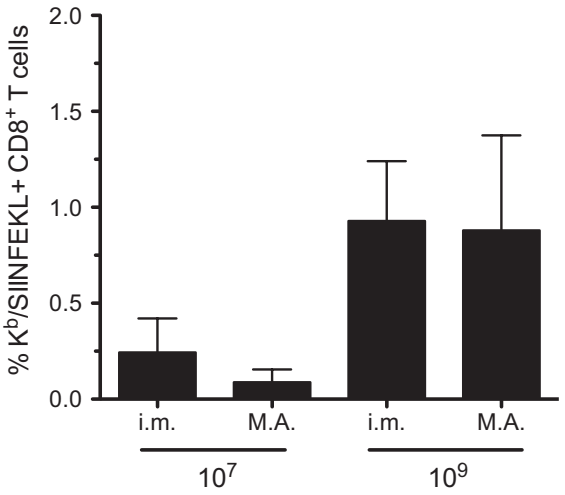


Fig. S2. Titration of rAdHu5-OVA vector dose delivered by MA and the i.m. route. Frequency of K^b/SIINFEKL-specific $CD8^+$ T cells in spleen on day 14 after immunization with either 10^7 or 10^9 vp of rAdHu5-OVA delivered by MA or i.m. injection. Data indicate the mean ± SEM of three to five mice per group.

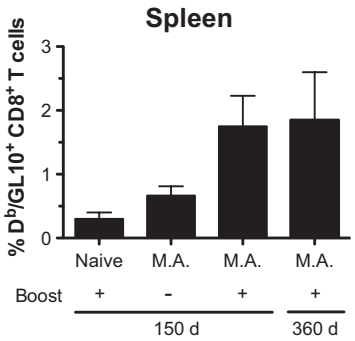


Fig. S3. Vaccination with rAdHu5 HIV-1 CN54 gag by dried MA induces long-lived $CD8^+$ T-cell responses. Frequency of $CD8^+$ T cells to the immunodominant D^b restricted HIV-1 CN54 gag (GL10) epitope detected by D^b/GL10 tetramer in the spleen of mice, either naïve or 150 or 360 d after immunization with rAdHu5 HIV-1 CN54 gag MA, treated with Depo-Provera and boosted 5 d (with or without) 20 μg of HIV-1 CN5 $gag_{309–318}$ peptide plus 30 μg of CpG ODN 1826 by the intravaginal route. Data indicate the mean ± SEM.

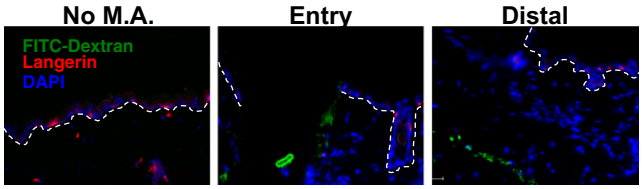


Fig. S4. Entry and penetration of MAs in skin. Frozen sections of back skin from B6 mice under control basal conditions (*Left*) and after removal of FITC-dextran MAs: entry site after 5 min (*Center*) and distal to entry point after 30 min (*Right*). LCs (red) labeled by anti-mouse Langerin Ab and revealed with TR-conjugated Ab, FITC-dextran (green), and DAPI (blue).

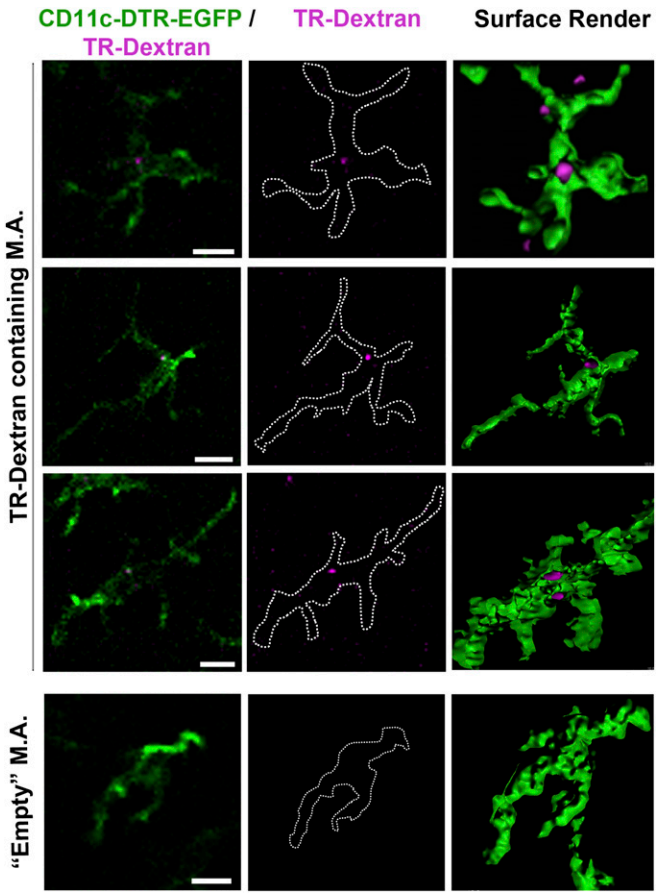


Fig. S5. Acquisition of MA.-derived TR-dextran by CD11c$^+$ EGFP DCs. (*Top*, *Center Upper*, and *Center Lower*) Intravital confocal images show single optical sections (3 μm thick) of CD11c$^+$ cells (green) and TR-dextran (magenta) (*Left*), TR-dextran alone with outline of CD11c$^+$ expressing cell (*Center*), and surface rendered z-stacks with "clipping layers" applied to the GFP fluorescence (green) to reveal areas of TR-dextran (magenta) (*Right*) in CD11c$^+$ DC in back skin of CD11c-DTR-EGFP mouse 1–3 h after TR-dextran MA application. *Lower* shows intravital confocal images taken after application of "Empty" MAs: single optical sections (3 μm thick) CD11c$^+$ cells (green) (*Left*), outline of CD11c expressing cell (*Center*), and surface rendered z-stacks with "clipping layers" applied to the GFP fluorescence (green) (*Right*). (Scale bars: 10 μm.)

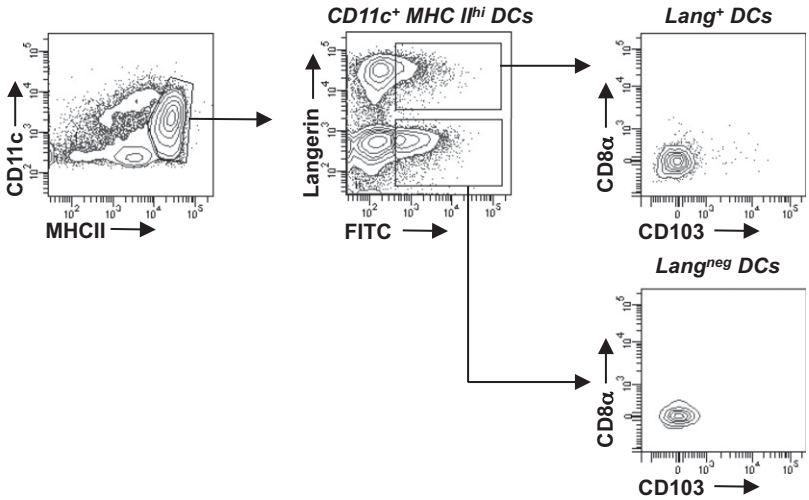


Fig. S6. Gating strategy for the phenotypic characterization of the Lang$^+$ FITC$^+$ DCs and Lang^{neg} FITC$^+$ DC subset accumulating in LNs after rAdHu5 MA application to skin. MAs fabricated with AdHu5-OVA and FITC dextran were applied to dorsal skin of the foot as described in *Materials and Methods*. After 48 h, low density enriched popliteal LNs were analyzed by FACS. The gated CD11c$^+$MHCII^{hi} cells (*Left*) were further analyzed and subdivided into Lang$^+$ and Lang^{neg} cells (*Center*) and then the gated Lang$^+$ (*Upper Right*) and Lang^{neg} (*Lower Right*) populations analyzed for expression of CD8α and CD103. These histograms are representative of three independent experiments.

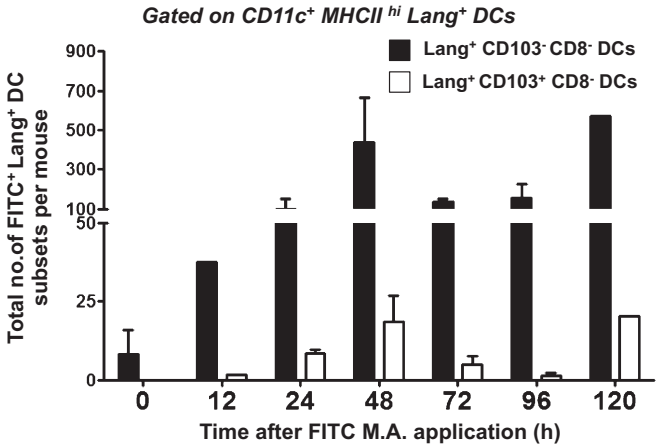


Fig. S7. Relative numbers of Lang$^+$ FITC$^+$ DC subsets in skin-draining lymph nodes after MA application to skin. MAs fabricated with AdHu5-OVA and FITC-dextran were applied to dorsal skin of the foot. At indicated times; low density enriched popliteal LN cells were analyzed by FACS. Total mean numbers of Lang$^+$CD8$^-$ CD103$^-$ FITC$^+$ DCs (LCs) and Lang$^+$CD8$^-$ CD103$^+$FITC$^+$ DCs (dermal Lang$^+$ DCs) analyzed from a CD11c$^+$ MHCII^{hi} gated population) is shown. Histograms indicate total mean number ± SEM of each subset from three independent experiments.

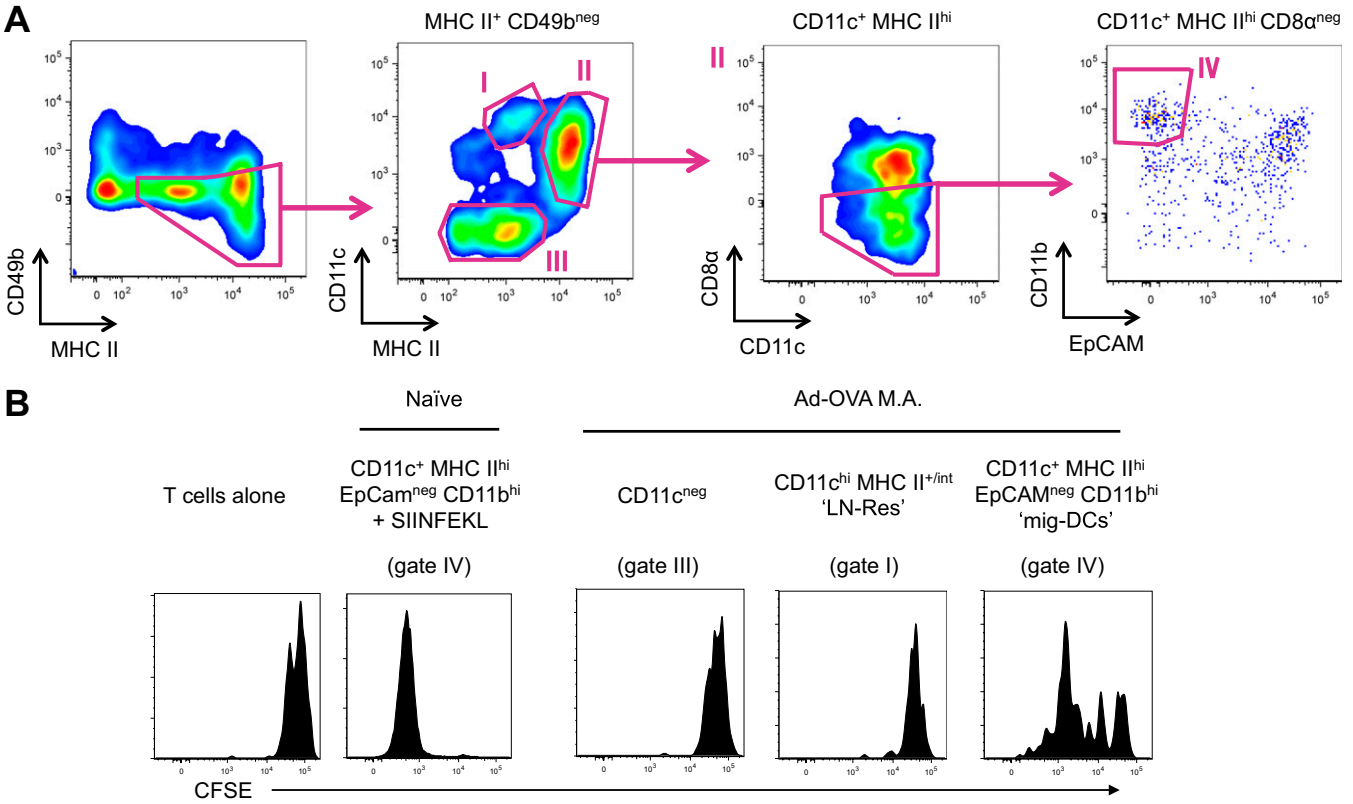
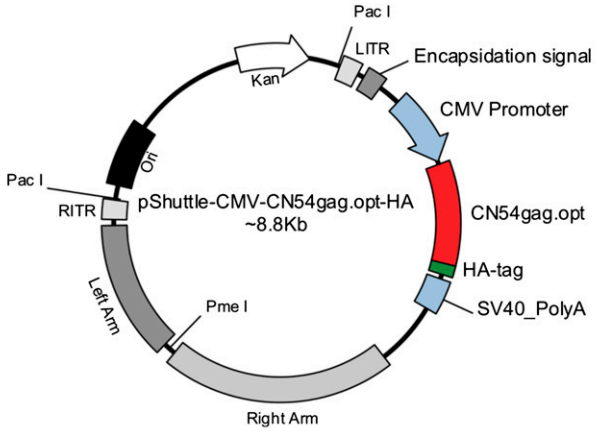


Fig. S8. CD11c$^+$ MHCII^{hi} EpCAM^{neg} CD11b^{hi} "migratory" DCs stimulate naïve CD8$^+$ T cells ex vivo. Inguinal LNs were collected from mice: naïve and 40 h after AdHu5-OVA MA immunization. DCs were enriched from a low-density fraction of LN cells and were highly purified by cell sorting. Sorting strategy (*A*) and coculture of CFSE-labeled CD8$^+$ OVA-specific T cells (OT-I) with either sorted DC subsets or CD11c^{neg} cells for 5 d (*B*). As a further control, OT-I CD8$^+$ T cells were cultured alone or with naïve DC pulsed with SIINFEKL peptide before coculture. Flow cytometry histograms show specific proliferation of gated CD8$^+$ (OT-I) T cells, as measured by CFSE dilution.



Nucleotide and amino acid sequences of the HIV-1-CN54gag-HA insert with the flanking sequence of the pShuttle-CMV

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                                                                >CMV Promoter
                                                                |
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                                                    KpnI        >CN54gag.opt
                                                    |           |
aga gct ggt tta gtg aac cgt cag atc cgc tag aga tct ggt acc atg gcc gcc agg gcc agc atc ctg agg ggc ggc aag ctg gac aag  < 990
R   A   G   L   V   N   R   Q   I   R   *   R   S   G   T   M   A   A   R   A   S   I   L   R   G   G   K   L   D   K

tgg gag aag atc agg ctg agg ccc ggc ggc aag aag cac tac atg ctg aag cac ctg gtg tgg gcc agc agg gag ctg gag agg ttc gcc  < 1080
W   E   K   I   R   L   R   P   G   G   K   K   H   Y   M   L   K   H   L   V   W   A   S   R   E   L   E   R   F   A

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E   E   Q   N   K   I   Q   Q   K   T   Q   Q   A   K   E   A   D   G   K   V   S   Q   N   Y   P   I   V   Q   N   L

cag ggc cag atg gtg cac cag ccc atc agc ccc agg acc ctg aat gca tgg gtg aag gtg gtg gag gag aag gcc ttc agc ccc gag gtg  < 1440
Q   G   Q   M   V   H   Q   P   I   S   P   R   T   L   N   A   W   V   K   V   V   E   E   K   A   F   S   P   E   V

atc ccc atg ttc agc gcc ctg agc gag ggc gcc acc cct cag gac ctg aac acc atg ctg aac acc gtg ggc ggc cac cag gcc gcc atg  < 1530
I   P   M   F   S   A   L   S   E   G   A   T   P   Q   D   L   N   T   M   L   N   T   V   G   G   H   Q   A   A   M

cag atc ctg aag gac acc atc aac gag gag gcc gcc gag tgg gac agg ctg cac ccc gtg cac gcc ggc ccc atc gcc ccc ggc cag atg  < 1620
Q   I   L   K   D   T   I   N   E   E   A   A   E   W   D   R   L   H   P   V   H   A   G   P   I   A   P   G   Q   M

agg gag ccc agg ggc agc gac atc gcc ggc acc acc agc aac ctg cag gag cag atc gcc tgg atg acc agc aac cca ccc gtg ccc gtg  < 1710
R   E   P   R   G   S   D   I   A   G   T   T   S   N   L   Q   E   Q   I   A   W   M   T   S   N   P   P   V   P   V

ggc gac atc tac aag agg tgg atc atc ctg gga ttg aac aag atc gtg agg atg tac agc ccc acc agc atc ctg gac atc aag cag ggc  < 1800
G   D   I   Y   K   R   W   I   I   L   G   L   N   K   I   V   R   M   Y   S   P   T   S   I   L   D   I   K   Q   G

ccc aag gag ccc ttc agg gac tac gtg gac agg ttc ttc aag acc ctg agg gcc gag cag gcc acc cag ggc gtg aag aac tgg atg acc  < 1890
P   K   E   P   F   R   D   Y   V   D   R   F   F   K   T   L   R   A   E   Q   A   T   Q   G   V   K   N   W   M   T

gac acc ctg ctg gtg cag aac gcc aac ccc gac tgc aag acc atc ctg agg gcc ctg ggc ccc ggc gcc agc atc gag gag atg atg acc  < 1980
D   T   L   L   V   Q   N   A   N   P   D   C   K   T   I   L   R   A   L   G   P   G   A   S   I   E   E   M   M   T

gcc tgc cag ggc gtg ggc ggc ccc agc cac aag gcc aag gtg ctg gcc gag gcc atg agc cag acc aac agc gcc atc ctg atg cag agg  < 2070
A   C   Q   G   V   G   G   P   S   H   K   A   K   V   L   A   E   A   M   S   Q   T   N   S   A   I   L   M   Q   R

agc aac ttc aag ggc agc aag agg atc gtg aag tgc ttc aac tgc ggc aag gag ggc cac atc gcc agg aac tgc agg gcc ccc agg aag  < 2160
S   N   F   K   G   S   K   R   I   V   K   C   F   N   C   G   K   E   G   H   I   A   R   N   C   R   A   P   R   K

aag ggc tgc tgg aag tgc ggc aag gag ggc cac cag atg aag gac tgc acc gag agg cag gcc aac ttc ctg ggc aag atc tgg ccc agc  < 2250
K   G   C   W   K   C   G   K   E   G   H   Q   M   K   D   C   T   E   R   Q   A   N   F   L   G   K   I   W   P   S

cac aag ggc ggc ccc ggc aac ttc ctg cag aac agg ccc gag ccc acc gcc ccc ccc gag gag agc ttc agg ttc gag gag gag acc acc  < 2340
H   K   G   G   P   G   N   F   L   Q   N   R   P   E   P   T   A   P   P   E   E   S   F   R   F   E   E   E   T   T

acc ccc agc cag aag cag gag ccc atc gac aag gag ctg tac ccc ctg acc agc ctg aag agc ctg ttc ggc aac gac ccc agc agc cag  < 2430
T   P   S   Q   K   Q   E   P   I   D   K   E   L   Y   P   L   T   S   L   K   S   L   F   G   N   D   P   S   S   Q

            >HA tag                                                                 HindIII
            |                                                                       |
gaa ttc gga tcc tac cca tac gat gtt cca gat tac gct taa taa gga tcc tag tga aag ctt cta gat aag ata tcc gat cca ccg gat  < 2520
E   F   G   S   Y   P   Y   D   V   P   D   Y   A   *   *   G   S   *   *   K   L   L   D   K   I   S   D   P   P   D

            >SV40 PA termination
            |
cta gat aac tga tca taa tca gcc ata cca cat ttg tag agg ttt tac ttg ctt taa aaa acc tcc cac acc tcc ccc tga acc tga aac  < 2610

ata aaa tga atg caa ttg ttg ttg tta act tgt tta ttg cag ctt ata atg gtt aca aat aaa gca ata gca tca caa att tca caa ata  < 2700

aag cat ttt ttt cac tgc att cta gtt gtg gtt tgt cca aac tca tca atg tat ctt aac gct aag ggt ggg aaa gaa tat ata agg tgg  < 2790
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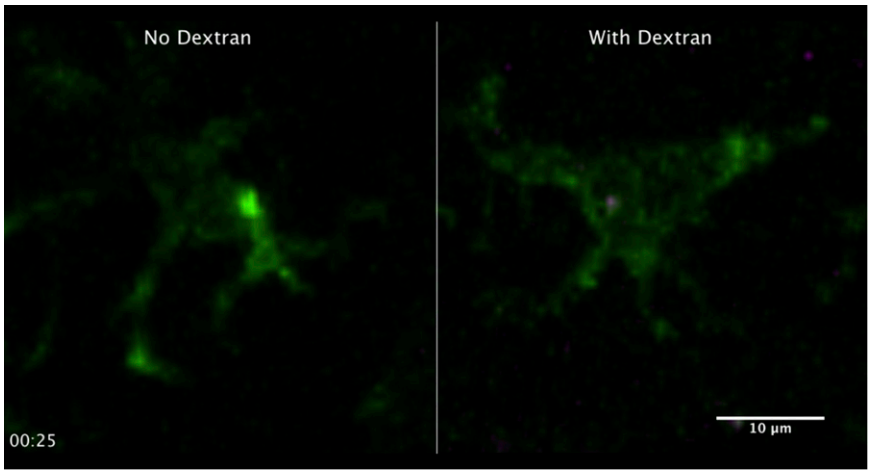
Fig. S9. rAdHu5-CN54gag.opt construct. (*Upper*) Schematic of pShuttle-CMV-CN54gag.opt-HA plasmid used for construction of rAdHu5 HIV-1 CN54gag.opt-HA. (*Lower*) Nucleotide and amino acid sequences of the CN54 gag-HA insert with the flanking sequence of the pShuttle-CMV plasmid.



Movie S1. Time-lapse intravital imaging of representative CD11c-DTR-GFP–expressing cells in the dermis of mice 1–3 h after application of an "empty" (*Left*) or TR-dextran–containing MA. (*Right*) Cell contains TR-dextran (magenta). Time in bottom left corner is hr:min. Movie is sped up to ~500× real time.

Movie S1