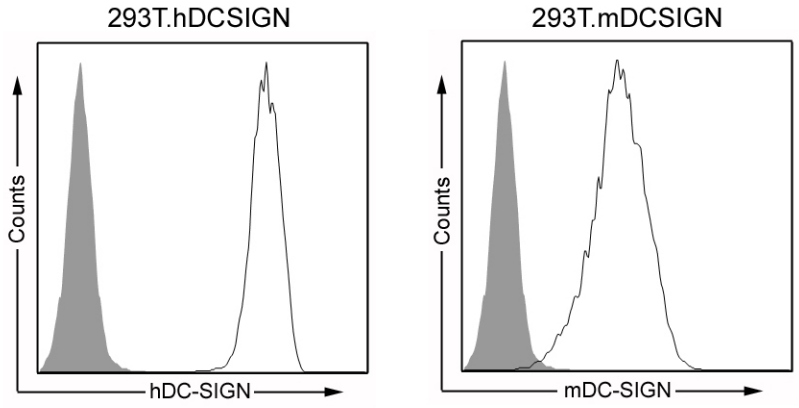
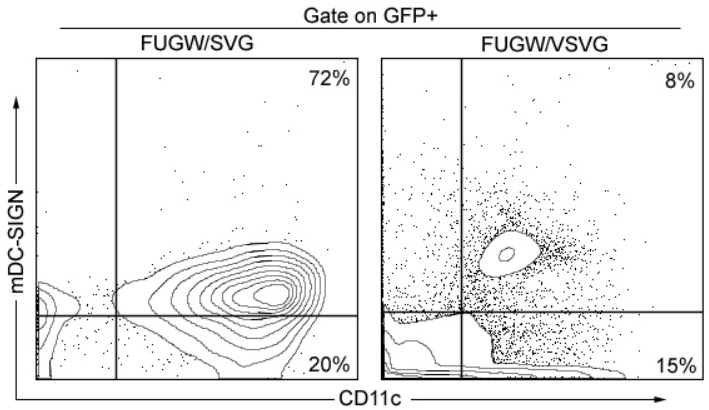
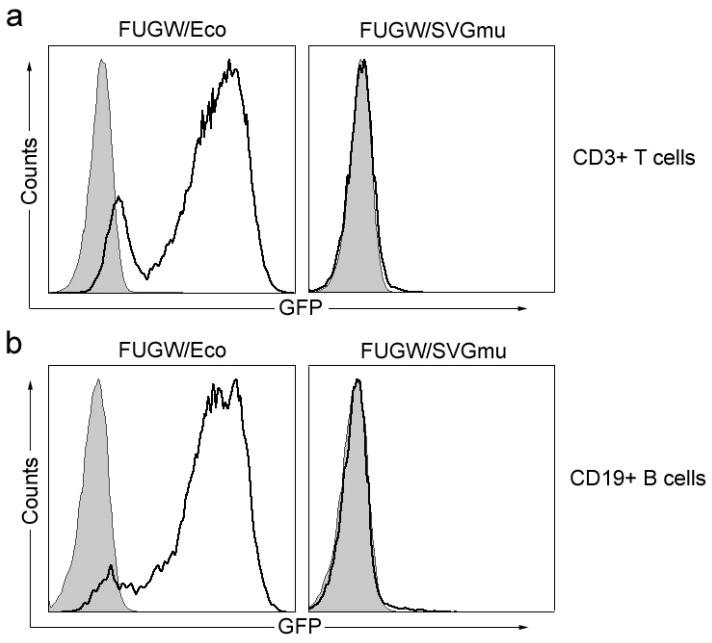
Supplementary Figures



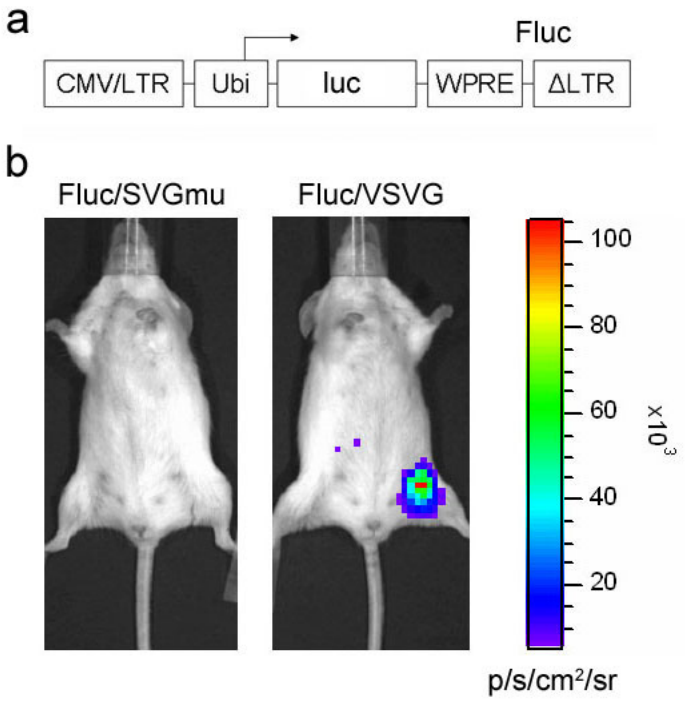
Supplementary Figure 1: Flow cytometry analysis of constructed target cell lines 293T.hDCSIGN expressing human DC-SIGN, and 293T.mDCSIGN expressing murine DC-SIGN. DC-SIGN expression was detected with either anti-human DC-SIGN or anti-murine DC-SIGN antibodies. Solid line, expression of DC-SIGN in target cell lines; shaded area, background staining in parental 293T cells.



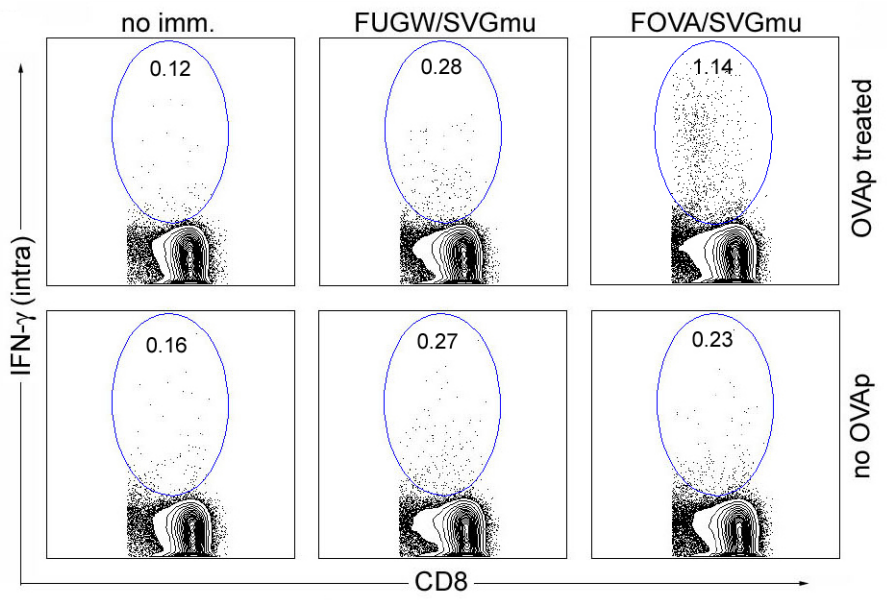
Supplementary Figure 2: Whole bone marrow cells isolated from B6 mice were exposed to the fresh viral supernatant of FUGW/SVG or FUGW/VSVG. Three days post-transduction, the cells were collected for flow cytometric analysis of GFP expression. Surface antigens of the GFP-positive cells were assessed by staining with anti-CD11c and anti-DC-SIGN antibodies.



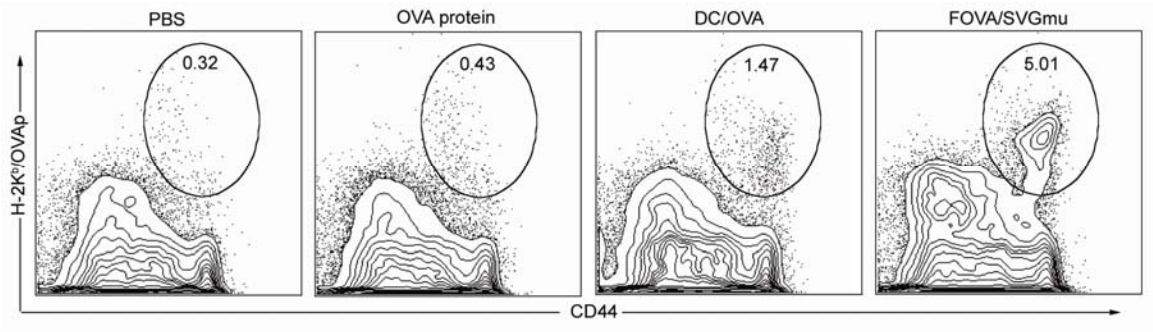
Supplementary Figure 3: Primary T cells ($CD3^+$, **a**) and B cells ($CD19^+$, **b**) isolated from the mouse spleen were transduced with the fresh viral supernatant of either the targeting FUGW/SVGmu or non-targeting FUGW/Eco vector. GFP expression was analyzed by flow cytometry. Solid line, cells exposed to indicated lentiviral vector; shaded area, cells without transduction (as a negative control).



Supplementary Figure 4: (**a**) A schematic representation of the lentivector (Fluc) encoding an imaging gene (firefly luciferase). (**b**) Bioluminescence imaging of mice injected subcutaneously with $50x10^6$ TU of either the DC-specific Fluc/SVGmu lentivector or the non-specific Fluc/VSVG lentivector. The representative image was obtained at day 30 post-injection using IVIS200 (Xenogen).



Supplementary Figure 5: Wild-type B6 mice were immunized by subcutaneous injection of $50x10^6$ TU of the DC-specific lentivector FOVA/SVGmu. Mice injected with the same dose of FUGW/SVGmu were included as a control. Two weeks later, the spleen cells were harvested and analyzed by intracellular IFN-γ staining with or without OVAp peptide restimulation. Indicated percentages are the percent of IFN-γ^+CD8^+ T cells.



Supplementary Figure 6: Naïve B6 mice were immunized by subcutaneous injection of OVA protein (100 μg), or $1x10^6$ DCs that were transduced by FOVA/Eco, or $50x10^6$ TU of FOVA/SVGmu. Mice injected with PBS were included as a control. Two weeks later, spleen cells were harvested and analyzed for the presence of OVA-specific T cells measured by H-2K^b-SIINFEKL-PE tetramer and CD44 staining. Indicated percentages are a percent of total CD8$^+$ T cells.

Supplementary Methods

Live animal imaging. We anesthetized mice and injected them intraperitoneally with D-luciferin (3 mg/mouse, Xenogen). The mice were placed in the chamber of the IVIS200 imaging instrument (Xenogen). The images were obtained using a cooled IVIS CCD camera and analyzed with Living Imaging 2.50 Software (Xenogen). We performed data acquisition after 10 min of D-luciferin injection.

Antibodies and FACS Analysis. Fluorochrome-conjugated antibodies specific for mouse CD11c, CD4, CD8, CD25, CD69, CD62L, CD44, TCRVα2, and TCRVβ5.1,5.2 were purchased from BD Biosciences. Human DC-SIGN antibody was purchased from BD Bioscience. Mouse DC-SIGN antibody was purchased from eBioscience. H-2K^b/OVAp tetramer was purchased from Beckman Coulter. Surface staining was performed by blocking with anti-CD16/CD32 (mouse Fc receptor, BD Biosciences) followed by staining with fluorochrome-conjugated antibodies. Intracellular staining of IFN-γ was performed using the Cytofix/Cytoperm Kit from BD Pharmingen following the protocol provided by the manufacturer. FACScan and FACSort (BD Bioscience) were used for all the flow cytometry analysis.

Biodistribution analysis of mice injected with the targeting lentivector. Mice received subcutaneous injection of the recombinant lentivector Fluc/SVGmu (50x10^6 TU). One month later mice were sacrificed by CO_2 inhalation and various organs were isolated for genomic DNA exaction using a DNeasy kit (Qiagen) following the manufacture's

protocol. Detection of vector integration was performed by using SYBRgreen real-time PCR kit (Qiagen) and a Bio-Rad MyiQ real-time PCR detection system. The firefly luciferase-specific primers for the analysis were Flucfw (5'-CCACGCTGGGCTACTTGATC-3') and Flucbw (5'-GCAAGAATAGCTCCTCCTCGAA-3').

Serum Anti-OVA IgG ELISA. Mouse sera at serial 10-fold dilutions, starting from 1:100, were added to ELISA plates (96-well C bottom, Nunc) pre-coated with 10μg/ml of chicken egg albumin (Sigma). The plates were then washed and the bound IgG were detected with biotinylated goat anti-mouse IgG (Vector), streptavidin conjugated HRP (R&D), and TMB peroxidase substrate (Kirkegaard and Perry). The TMB reaction was stopped by the addition of 2M H_2SO_4 at a uniform time point for each set of the assay, and the absorbance at 450nm was assayed using a VERSAmax plate reader (Molecular Devices). Titer values were assigned as the highest dilution at which the optical density was 2 SDs higher than the optical density of the control serum at equivalent dilutions.

IFN-γ ELISA. Cell culture supernatant was added to ELISA plates (96-well C bottom, Nunc) pre-coated with 1μg/ml of anti-mouse IFN-γ antibody (Clone R4-6A2, BD Biosciences). The plates were then washed and the captured mouse IFN-γ was detected with biotinylated anti-mouse IFN-γ antibody (Clone XMG1.2, BD Biosciences), streptavidin conjugated AKP and SIGMA FAST^{TM} p-Nitrophenyl Phosphate Tablets (Sigma). The absorbance at 405nm was assayed using a VERSAmax plate reader (Molecular Devices) with the maximal value not exceeding 1.500.

Intracellular IFNγ Staining. Mouse spleen or lymph node cells were cultured in 24-well plate ($2x10^6$ cells per well) in RPMI containing 10% FBS and 0.1μg/ml OVAp in the presence of 0.67μg/ml of GolgiStop™ (BD Biosciences) for 6 hours. The cells were then collected and stained for surface markers, followed by intracellular staining of IFN-γ using the Cytofix/Cytoperm Kit from BD Biosciences following the protocol provided by the manufacturer, and analyzed using flow cytometry.

T Cell Proliferation Assay. Mouse T cells were stimulated for 3 days. In the last 12 hours, [^{3}H]thymidine was added at 0.01mCi/ml (1Ci= 37 GBq). The proliferation was measured by [^{3}H]thymidine incorporation using a Wallac (Gaithersburg, MD) ^{3}H counter.