Supplementary Materials:

Figure S1: Antigenic presentation and T cell activation controls

(a) Antigenic presentation by different populations of APCs was measured *in vitro* by pre-incubating CD11c+ (black circle), CD11b+ (triangle) or CD19+ (square) spleen cells with (+) or without (-) the Dby peptide and adding CFSE-stained Dby-specific CD4+T cells for 3 days. The percentage of dividing CFSE-stained T cells was estimated by FACS using the gating strategy indicated on the left part of the Figure. Data from 6 experiments are represented on the right panel. Dots represent individual mouse data and horizontal bars indicate the average value.

(b) Antigenic presentation of the capsid (left panel) and T cell activation by the capsid (right panel) were tested after IM injections of the rAAV1-DBY vector in different conditions. In the left panel, antigenic presentation from CD11c+, CD11b+, or CD19+ cells was tested in C57BL/6 mice as described in Figure 1b and averaged results of 2 experiments are represented with standard deviation. In the right panel, **t**he role of CD11c+ cells in anti-capsid CD4+ T cell responses was investigated in the CD11c-DTR chimeric model by measuring IFN-γ response as in FIgure 1a but testing the draining lymph node (pool of popliteal and inguinal lymph node) instead of spleen of mice 8 days after IM administration of rAAV1-DBY vector (5.10^9 vg/mice). Each point represent individual mouse data and horizontal bars indicate the average value.

(c) Serotype-specific effects are shown as rAAV8 is capable of inducing anti-transgene T cell immune responses regardless of NA digestion. NA-treated (+) or not (-)CD11c+ cells were incubated with rAAV8-SGCA-HY and were injected into C57BL/6 mice. T cell responses were measured by IFN-γELISPOT 14 days after injection. Data are representative of 2 experiments with 3 mice per group. Dots represent individual mouse data and horizontal bars indicate the average value.

Figure S2: Gating strategies and control of acid sialic removal on CD11c+CD8α+ and CD11b+F4/80+

(a) Representative dot plots and histograms with gating strategies that were used in Figures 3 and 4. Total splenocytes were first incubated with anti-CD11c-magnetic beads and CD11c+ cells were purified by automatic magnetic cell sorting. The residual unselected cells were labeled with anti-CD11b-magnetic beads to purify CD11b+ cells. B lymphocytes were obtained from residual unselected cells from these 2 prior steps (i.e. CD11c- CD11b- cells) using anti-CD19-magnetic beads positive selection. The 3 populations of selected APCs were then stained with various monoclonal antibodies to evaluate other markers and the presence of the rAAV1-alexa488 vector in the cells was measured by FACS.

(b) The removal of sialic acids by NA was controlled by incubating CD11c+CD8α+ **(left panel)** and CD11b+F4/80+ **(right panel)** cells with fluorescent WGA lectin (5µg/mL) for 1 hour. The mean fluorescence intensity (MFI) of WGA lectin-positive cells was measured by FACS. Dots show the mean ± SEM of the MFI in 3 independents experiments.

Figure S3: Activation of APCs by rAAV1 and nanospheres

These histogram plots correspond to the experiments described in Figure 6 and show the multicolor FACS analyses of sorted CD11c+ cells or CD11b+ cells respectively in the left or right panels.

Figure S4: Clustering representation of PCA

Hierarchical clustering of CD8α+, CD11b+ CD11c+ and CD11b+, CD11b+F4/80+ subpopulations. Rectangles on the tree plot represent identified major clusters on the basis of the branching distance. Data are representative of three experiments.

Figure S5: Confocal microscopy analysis showing the presence of rAAV1 in macrophages.

C57BL/6 mice were injected IV with PBS or with 10^{13} particles of rAAV1 marked with Alexa 633 and after 2 hours, spleens were collected, digested with DNAse and CD11b+ cells were enriched by magnetic cell sorting (95% purity), stained with biotinylated anti-mouse F4/80 or isotype control, then Alexa 488-labeled streptavidin and DAPI were added and preparations were analyzed by confocal microscopy (63x). Arrows point to cells containing rAAV1. Inserts magnify representative cells to show F4/80 marking in green, rAAV1 in red and nuclei in blue.

Figure S1a

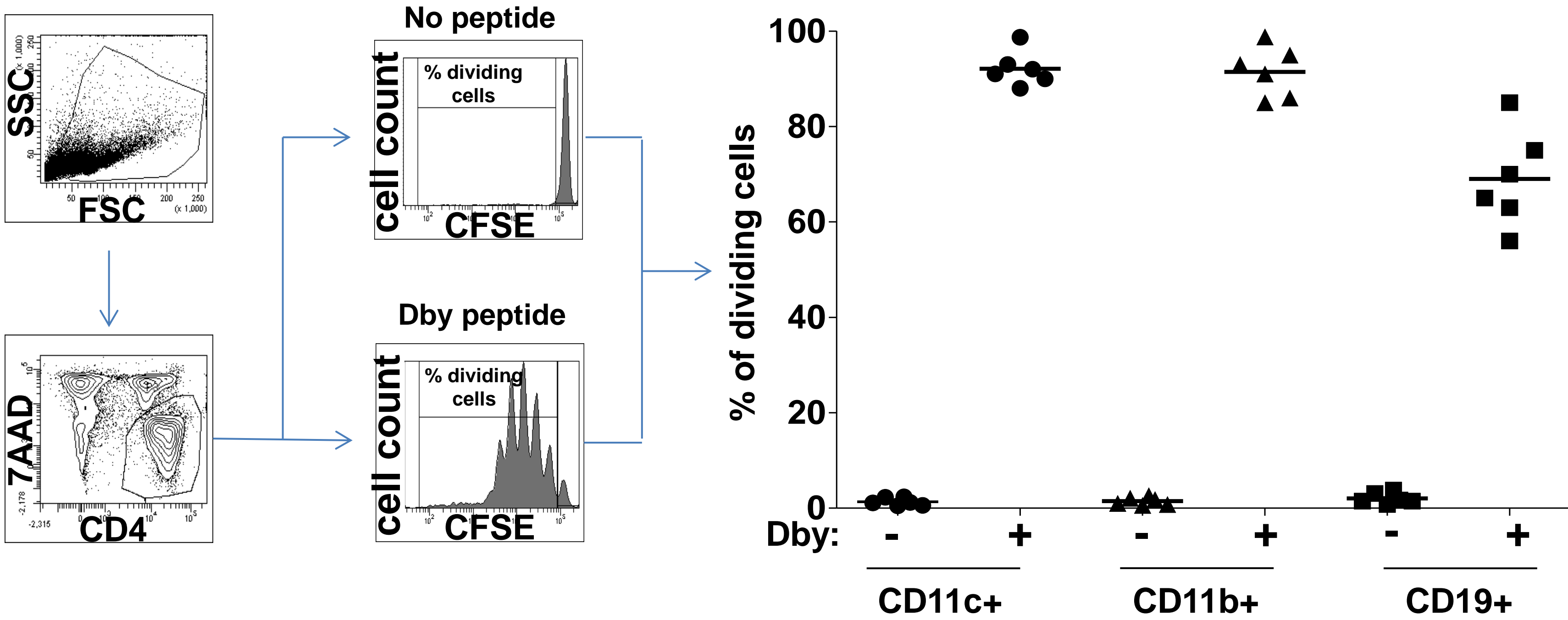
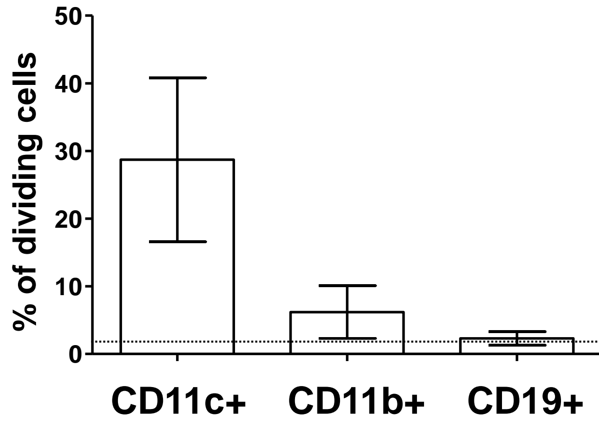


Figure S1b



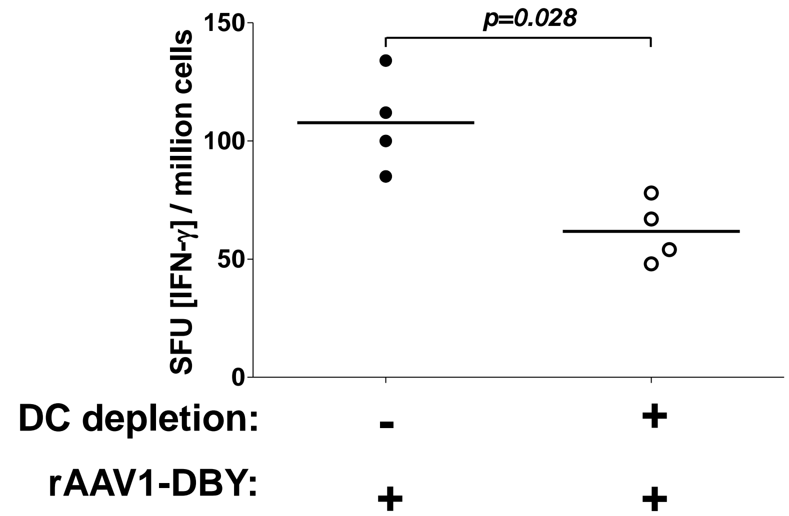


Figure S1c

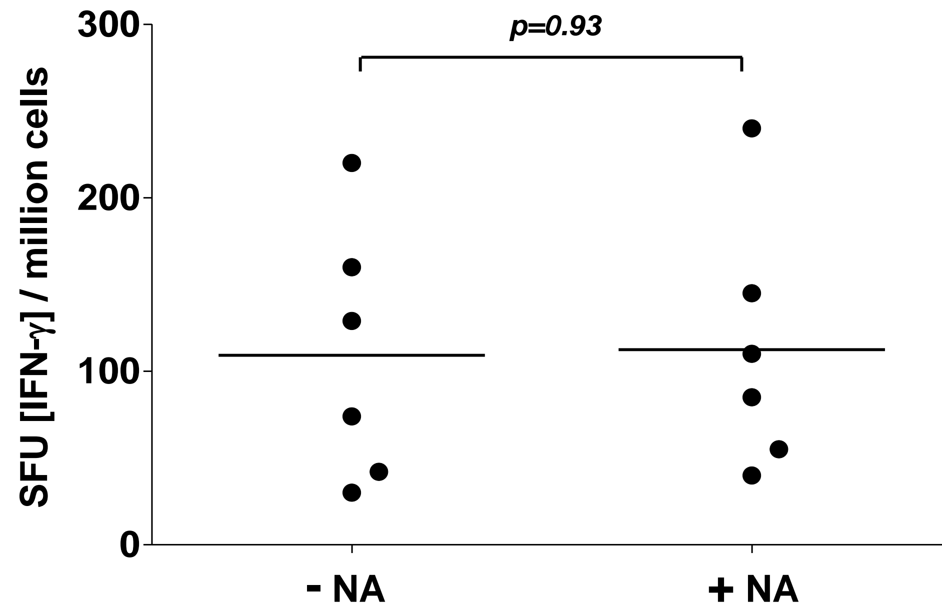
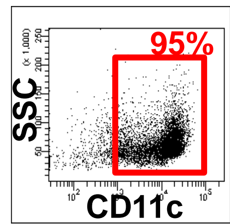
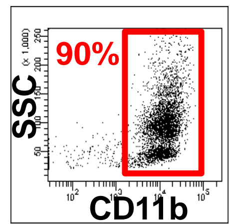


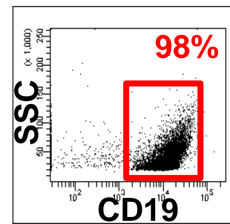
Figure S2a

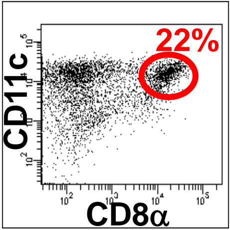
Total spleen cells

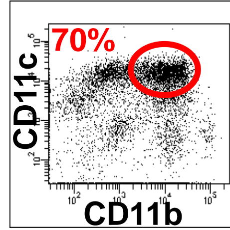
Sequential magnetic cell sorting

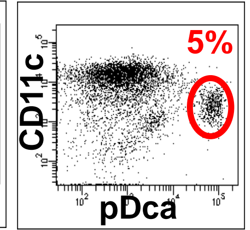


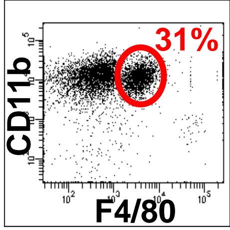


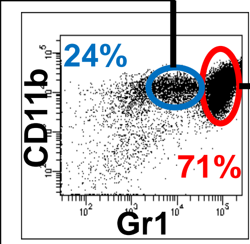


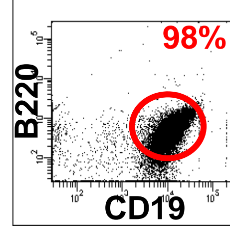


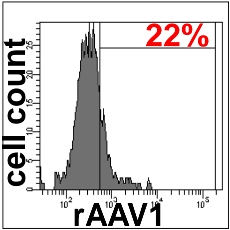


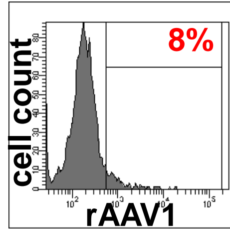


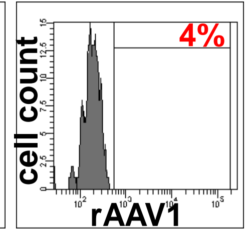


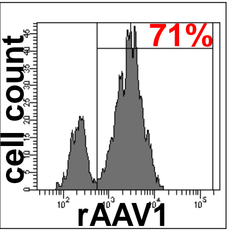


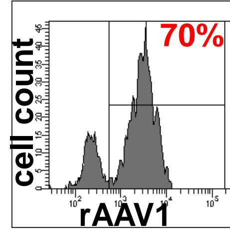


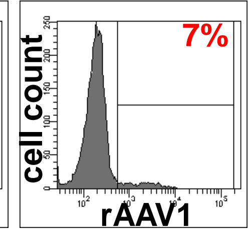












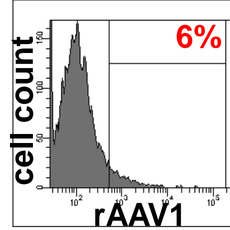
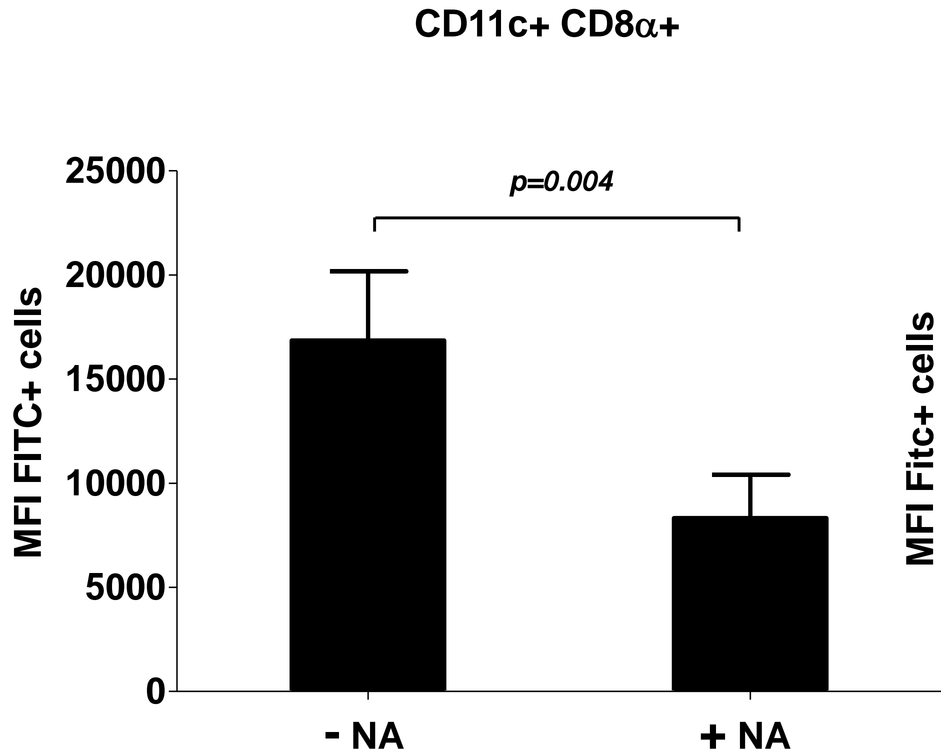


Figure S2b



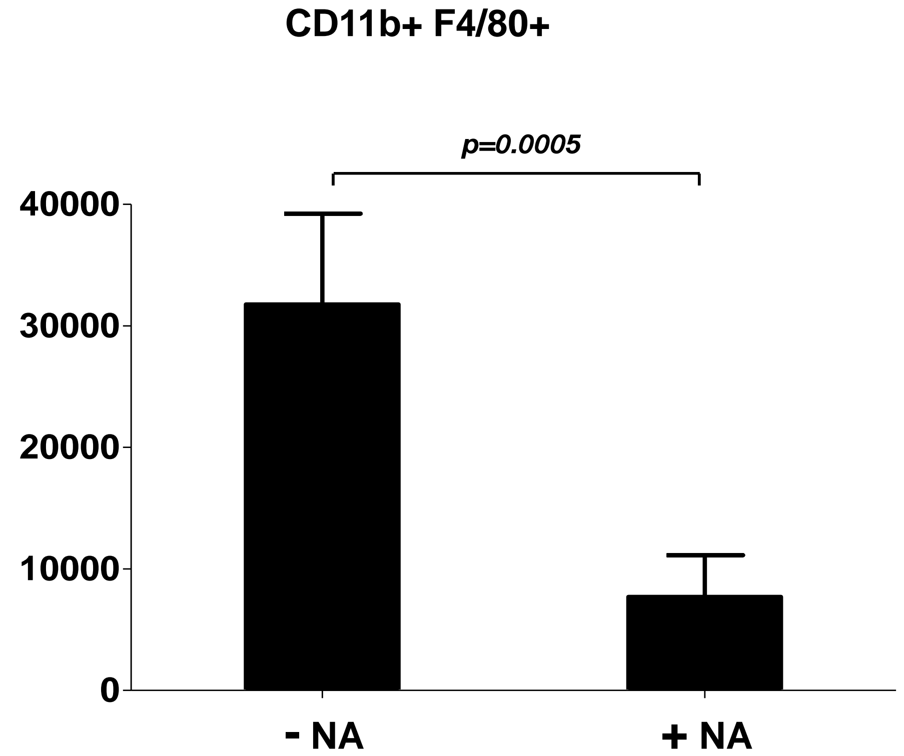


Figure S3

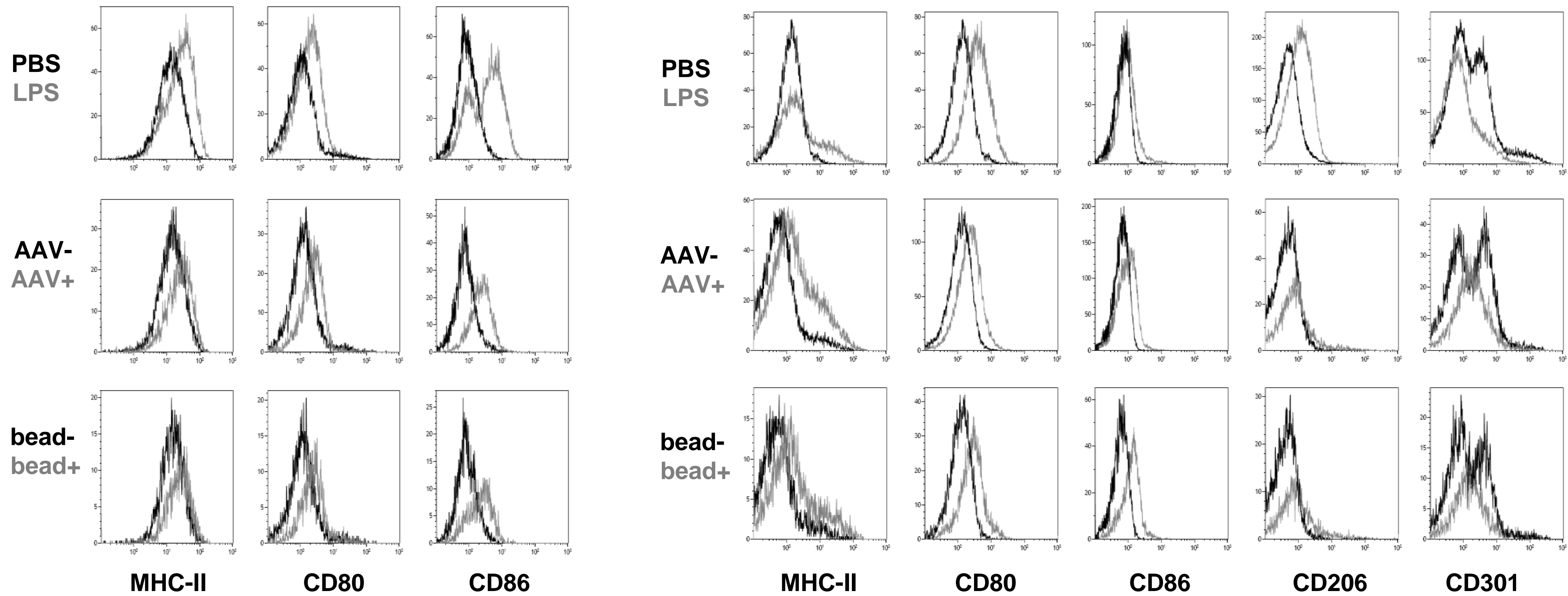
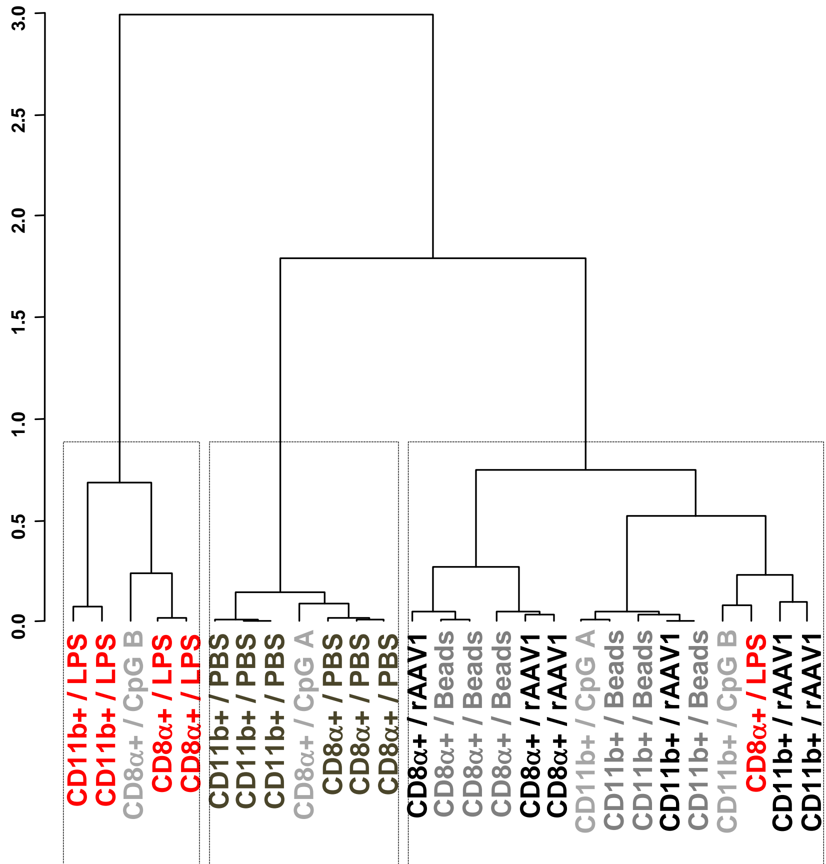
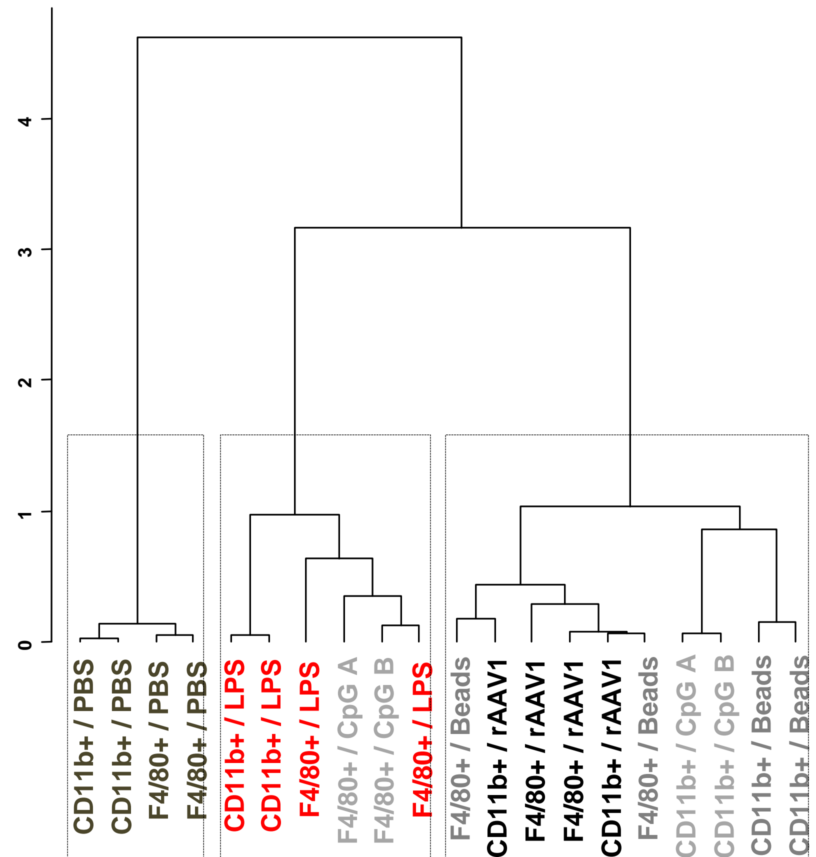


Figure S4





PBS nanospheres rAAV1 CpG A CpG B LPS

Figure S5