Supplementary Information for

Induction of innate and adaptive immunity by delivery of poly dA:dT to dendritic cells

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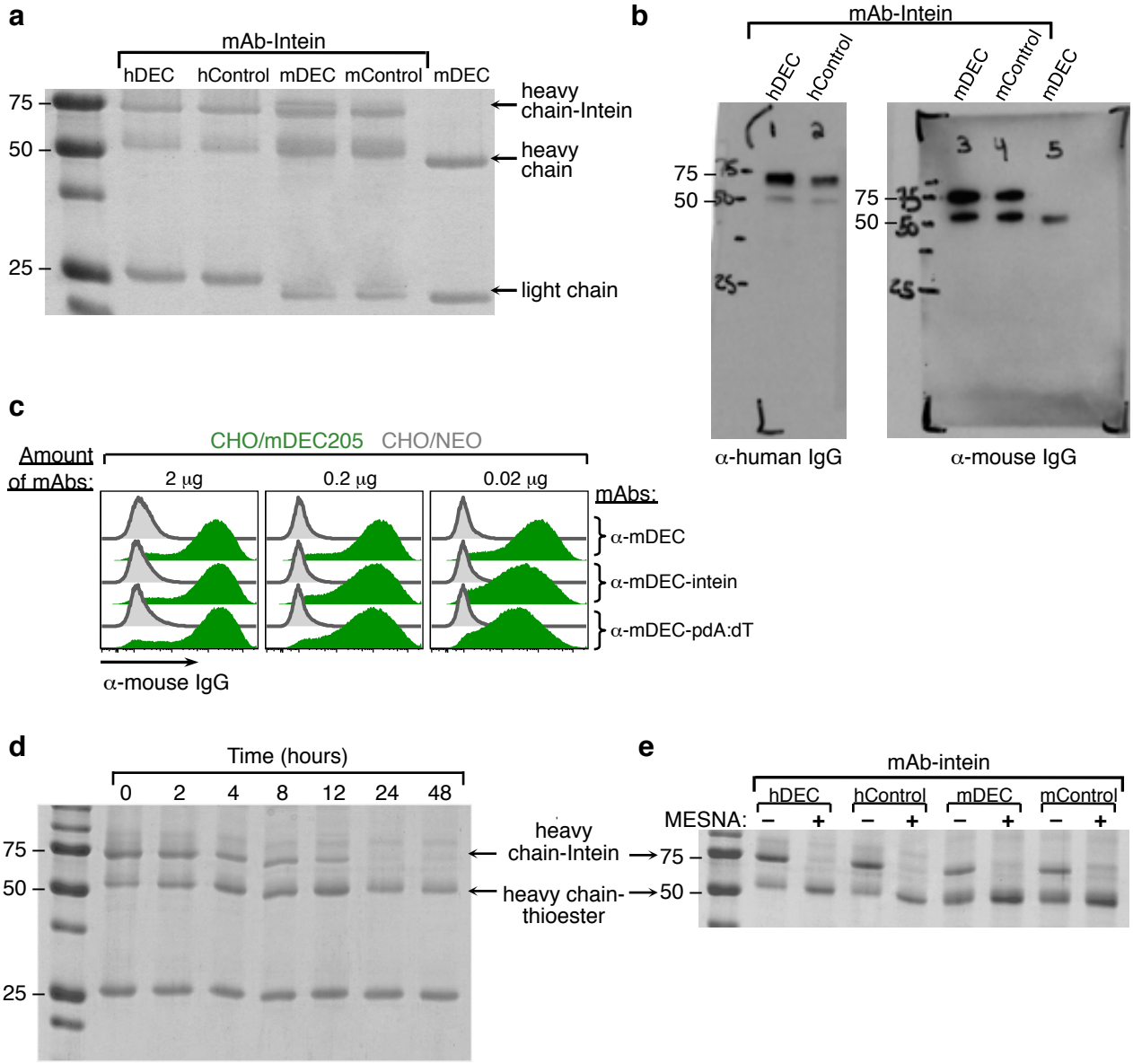
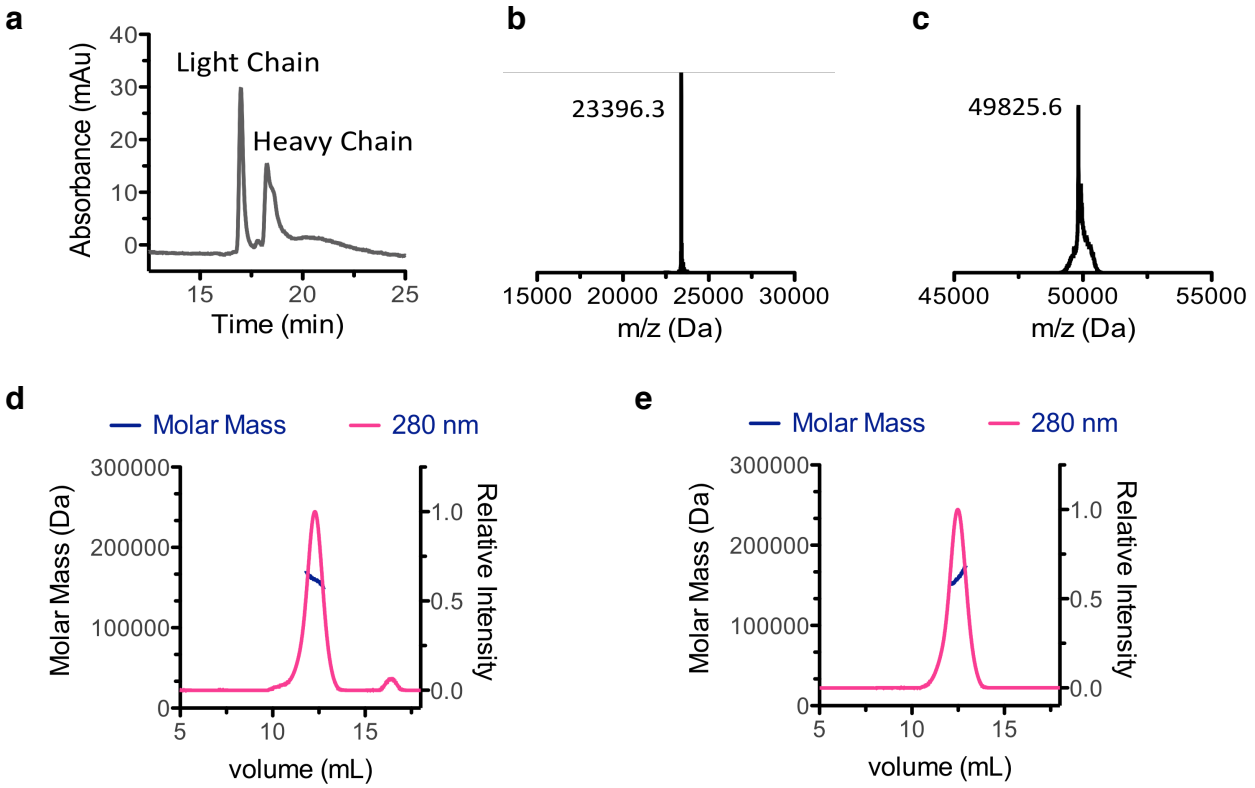
Supplementary Results

Supplementary Figure 1



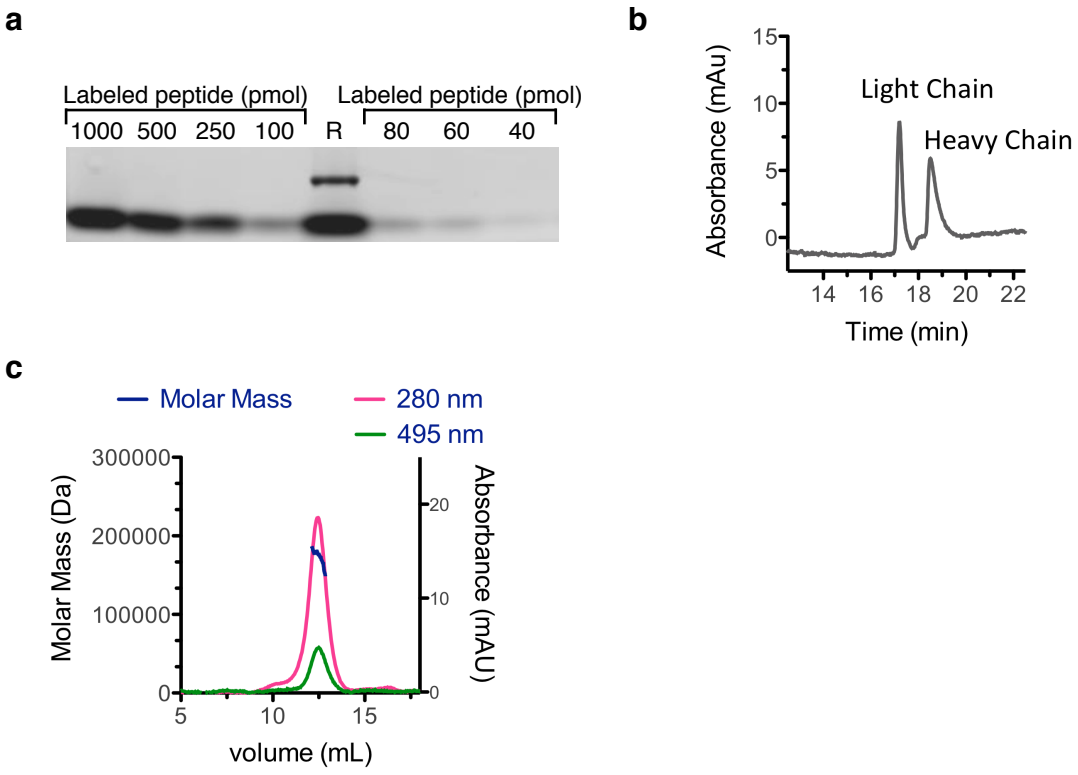
Supplementary Figure 1. Production of mAb-intein conjugates, and MESNA treatment. (a) Production of mAb-intein fusions. 10% SDS-PAGE gel stained with Coomassie blue of the purified mAb-intein constructs used in this study (h corresponds to human and m to mouse constructs). The purified uncoupled α-mDEC mAb is shown for comparison. Numbers are expressed in kDa. **(b)** Western blot analysis of the various mAb-intein constructs used in the study employing either α-human or α-mouse secondary Ab conjugated with HRP. Numbers are expressed in kDa. **(c)** Binding of different doses (2, 0.2 and 0.02 μg) of unconjugated α-mDEC, α-mDEC-intein, and α-mDEC-pdA:dT mAbs to CHO cells expressing mouse DEC205 (CHO/mDEC; green) and control CHO/NEO (gray) cells as monitored by flow cytometry after staining with PE-conjugated α-mouse IgG mAbs. **(d)** Time course of the MESNA thiolysis reaction of the α-hDEC205-intein mAbs. The reaction was complete by 24 hrs yielding the α-thioester derivative of the mAb heavy chain (expected mass = ~50 kDa). Numbers are expressed in kDa. **(e)** The mAb-intein fusions were treated with MESNA for 18 hrs to give α-thioester derivatives. Thiolysis of the intein from the heavy chain was monitored by 10% SDS-PAGE stained with Coomassie blue. Numbers are expressed in kDa.

Supplementary Figure 2



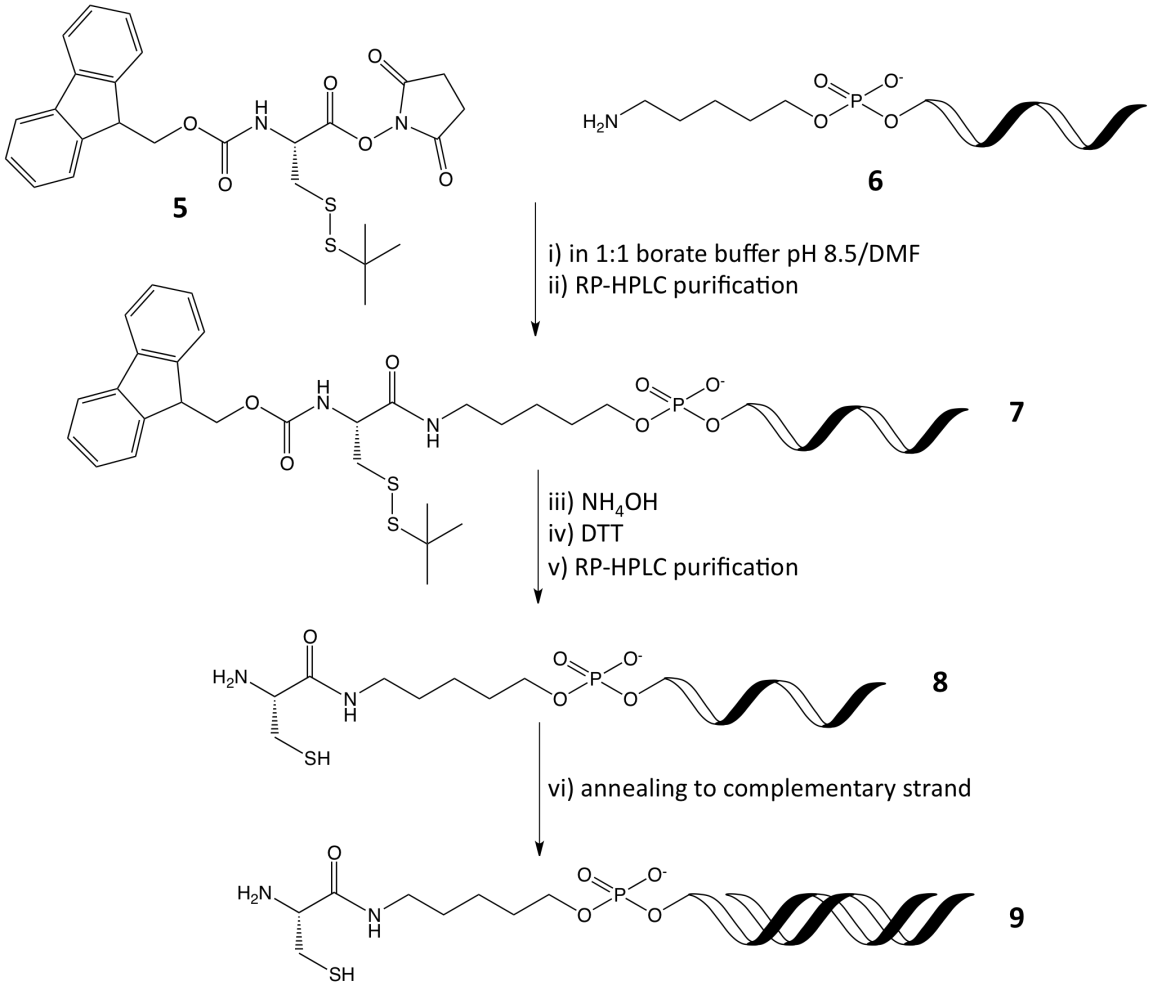
Supplementary Figure 2. RP-HPLC, MS, and SEC-MALS analysis of α-hDEC mAbs. **(a)** RP-HPLC of deglycosylated and reduced MESNA-treated α-hDEC mAbs. Sample was analyzed on a C4 column using a linear gradient of 5-75% solvent D in solvent C over 30 min at 55 ºC and monitored at 280 nm. **(b & c)** Representative MS for the light *(b)* and heavy chains *(c)* are shown. For the light chain, the detected MW was in good agreement with its calculated value of 23398.09 Da. For the heavy chain a MW corresponding to the C-terminal carboxylate was detected (MW_{calcd} = 49823.2 Da). Hydrolysis of the C-terminal MESNA thioester was expected due to sample preparation prior to RP-HPLC and MS analysis, which included deglycosylation (18 h at 37 ºC, pH 7.5 in the absence of thiols) and reduction (6 M Gn·HCl, 10 mM DTT, pH 7.5 for 1-2 h at 37 ºC). **(d & e)** MESNA treated α-hDEC mAbs was analyzed by SEC-MALS 24 h after ligation *(d)* as well as after removal of intein and storage at 4ºC for 2 weeks *(e)*. Both samples show a mono-disperse peak (poly-dispersity index = 1.002) with a measured MW of 155-159 kDa, in agreement with the calculated size for the tetrameric IgG (148 kDa).

Supplementary Figure 3



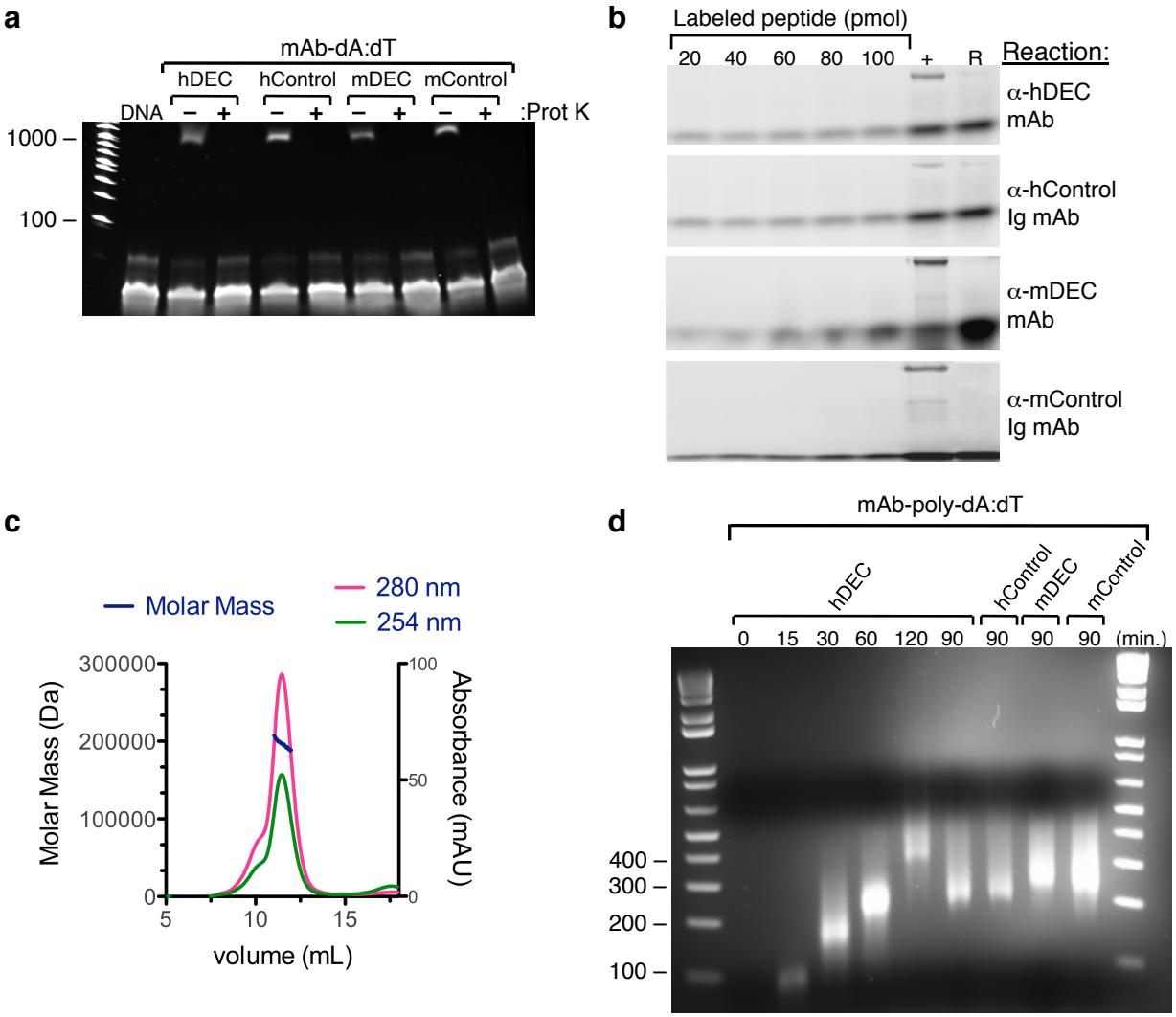
Supplementary Figure 3. Model EPL of α-DEC mAbs and a fluorescent peptide. (a) 100 pmoles of MESNA-treated α-hDEC mAbs were reacted for 18 hrs in ligation buffer (1x PBS, 400 mM NaCl, 6 mM TCEP, 1 mM EDTA, 100 mM MESNA, pH 7.5) with 1 nmol of a short fluorescent-labeled peptide (H-Cys-Gly-Lys[$^{\varepsilon}$*N*-Fluorescein]-Gly-NH_2). The mixture was then resolved by 10% SDS PAGE, imaged using a fluorescent scanner, and analyzed using ImageJ software to quantify the band intensities. To quantify the amount of fluorescent-labeled mAbs, a standard curve was generated by running the fluorescent peptide at varying concentrations on the same gel. Quantification indicated that the ligation efficiency was >90% (based on the amount of reactive mAb starting material). R is mAb ligation reaction. **(b)** RP-HPLC analysis of deglycosylated and reduced α-hDEC mAbs ligated to short fluorescent peptide. Sample was analyzed on a C4 column using a linear gradient of 5-75% solvent D in solvent C over 30 min at 55 ºC and monitored at 280 nm. MS analysis of the heavy chain peak confirmed formation of the desired ligation product (MW = 50529.4 Da, MW_{calcd} = 50526.3) as well as the presence of unmodified heavy chain (MW = 49825.6 Da, MW_{calcd} = 49823.2 Da), which had originated from hydrolysis of the intein fusion during expression and purification (Supplementary Fig. 1 a-b). **(c)** SEC-MALS analysis of α-hDEC mAb ligated to short fluorescent peptide confirms that the antibody remains mono-disperse (poly-dispersity index = 1.007) after thiolysis and ligation. Measured MW was 164 KDa (MW_{caldc} = 150 kDa).

Supplementary Figure 4



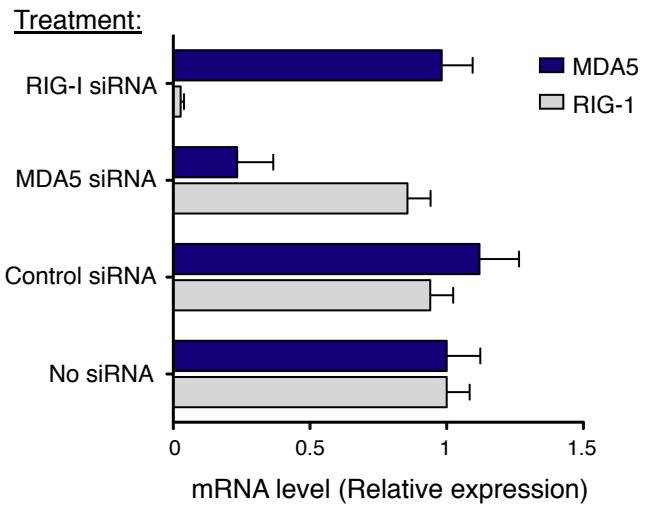
Supplementary Figure 4. Synthesis of Cys-ds-DNA: Fmoc-Cys(StBu)-OSu (5) was coupled to a poly-A oligo (Fisher Scientific) bearing the Amino-C6 Modifier at its 5' end (6), in a 50% DMF/borate buffer pH 8.5 (i). After RP-HPLC purification (ii) the Fmoc and StBu protecting groups of 7 were removed by treatment with 30% aqueous NH_4OH (iii) and 1 M DTT (iv), respectively. Following complete deprotection, the Cys-poly-A (8) was annealed to its complementary poly-T strand by mixing them at a 1:1 ratio and heating them up at 95 ºC followed by slow cooling to room temperature over 1 h. The Cys-ds-polyA-T (9) was directly used in the ligation reactions.

Supplementary Figure 5



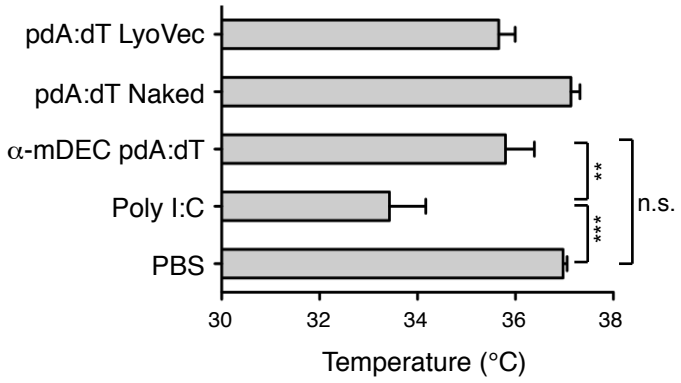
Supplementary Figure 5. Production of mAb-ds-DNA conjugates. (a) The mAb-thioester derivatives were mixed with 20 bp DNA oligo (dA:dT) containing a cysteine residue at the 5' end of the dA oligo. Production of mAb-dA:dT conjugates was monitored on a 4-20% TBE native gel stained with ethidium bromide. Samples were left untreated (-) or treated with Proteinase K (+) to confirm the presence of mAb-DNA conjugates. Numbers are expressed in bp based on markers. Cys-DNA alone (DNA) is shown for comparison. **(b)** Indirect estimate of mAb-DNA ligation efficiency using a chase reaction. The indicated mAb-thioester was reacted with Cys-DNA for 18 hrs after which the fluorescent peptide (1,000 pmoles, 20 fold excess) was added and allowed to react for a further 18 hrs. The reaction mixtures were then resolved on a 10% SDS PAGE. Numbers at the top are pmoles of fluorescent peptide used to generate a standard curve. + indicates direct fluorescent labeling of the mAb, i.e., no chase. R corresponds to the actual mAb-DNA reaction followed by the chase. **(c)** Ligated α-hDEC-dA:dT mAbs was analyzed by SEC-MALS. The conjugate runs as a single peak with a polydispersity index of 1.005 and a measured MW of 185 KDa (MW_{calcd} = 172 KDa). No aggregates could be detected after 24 hrs incubation at room temperature. **(d)** Purified mAb-dA:dT conjugates were reacted with Klenow Pol-I fragment in the presence of dATP and dTTP for the indicated times in minutes. Aliquots of elongation reaction were treated with proteinase K and run on a 2% agarose gel stained with EtBr. Numbers are expressed in bp based on markers.

Supplementary Figure 6



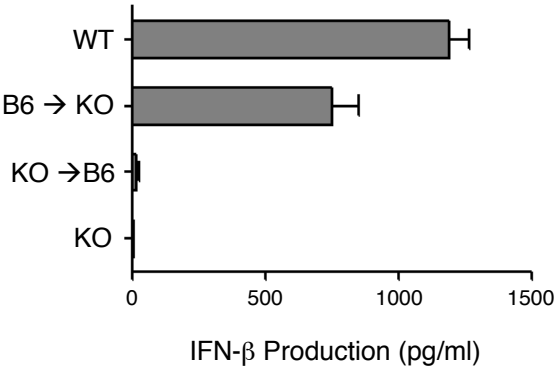
Supplementary Figure 6. Blocking of RIG-I in MoDCs using siRNA. Quantitative PCR was performed to determine the relative mRNA levels of RIG-I (gray) and MDA5 (blue) in MoDCs treated with RIG-I, MDA5, and control siRNA (treatment). Histograms show the mean +/- SD of 2 experiments with a total of 3 samples each.

Supplementary Figure 7



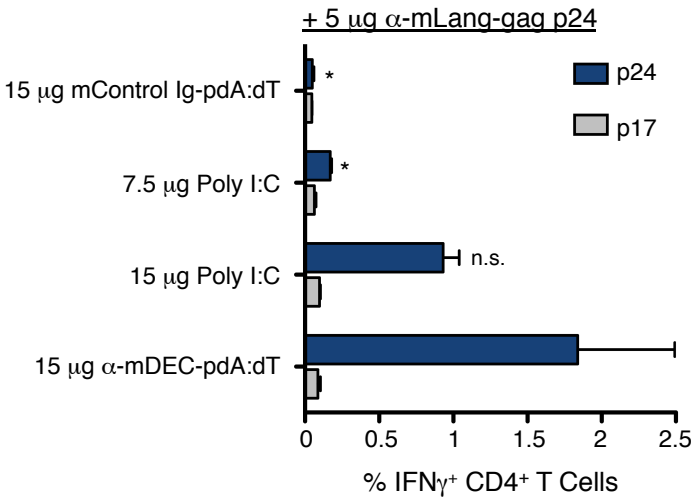
Supplementary Figure 7. α-mDEC-pdA:dT mAbs induce less hypothermia than Poly I:C. B6 mice were inoculated i.p. with indicated immune stimulants (10 μg), and the temperature of each mouse was determined 5-6 hrs after. Shown is the mean +/- SD of 2 experiments with 6 animals total in each group. In all cases, statistical differences were determined between all groups. p values of ≤ 0.01 (**) and 0.001 (***) are indicated; n.s. is non-significant.

Supplementary Figure 8



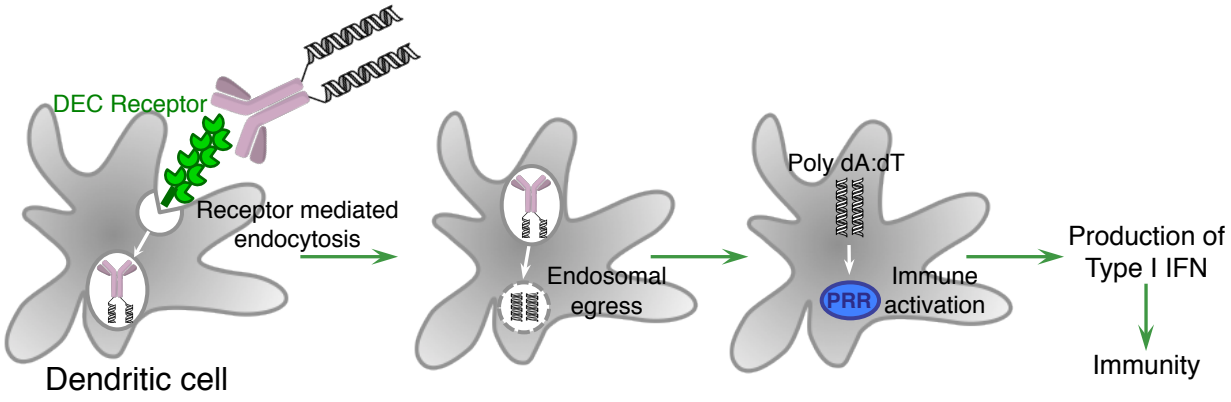
Supplementary Figure 8. α-mDEC-pdA:dT mAbs triggers type I IFN production *in vivo*. B6 or DEC KO mice were lethally irradiated and injected with bone marrow cells from DEC KO mice (KO → B6) or B6 mice (B6 → KO), respectively. 12-14 weeks after bone marrow transplant, chimeras were inoculated with 10 μg of α-mDEC-pdA:dT mAbs. As controls, we also inoculated B6 mice and DEC KO mice. 12 hrs after inoculation serum was collected and production of IFN-β was evaluated by ELISA. Shown is the mean +/- SD of two experiments with a total of 4 mice per group.

Supplementary Figure 9



Supplementary Figure 9. Targeting adjuvant and antigen to DCs induces CD4 T cell immunity. B6 mice were primed and boosted 4 wks apart with 5 μg of HIV Gag-p24 antigen fused to α-mLangerin mAbs. 15 μg of α-mDEC-pdA:dT mAbs, 7.5-15 μg of poly I:C, or 15 μg of mControl Ig-pdA:dT mAbs was used as the adjuvant. Seven days after boost, splenocytes were restimulated *in vitro* with the reactive HIV Gag-p24 peptide mix (p24) or a non-reactive HIV Gag-p17 peptide pool (p17, negative control), in the presence of Brefeldine A (BFA) for 6 hrs. Intracellular staining was performed to detect IFNγ in $CD3^+$ $CD4^+$ gated T cells. The % of $IFN\gamma^+$ $CD4^+$ T cells is shown as the mean +/- SD of 3 independent experiments with a total of 6 mice.

Supplementary Figure 10



Supplementary Figure 10. Proposed mechanism of DC activation by α-DEC-pdA:dT mAbs. Targeted delivery of ds-DNA adjuvant to DEC expressing DCs using α-DEC-pdA:dT mAbs. Upon binding to the cognate receptor and endocytosis of the α-DEC-ds-DNA complexes, the DNA is released into the cytoplasm, transcribed into ds-RNA, which then activates pattern recognition receptors (PRR) such as RIG-I, leading to immune activation.