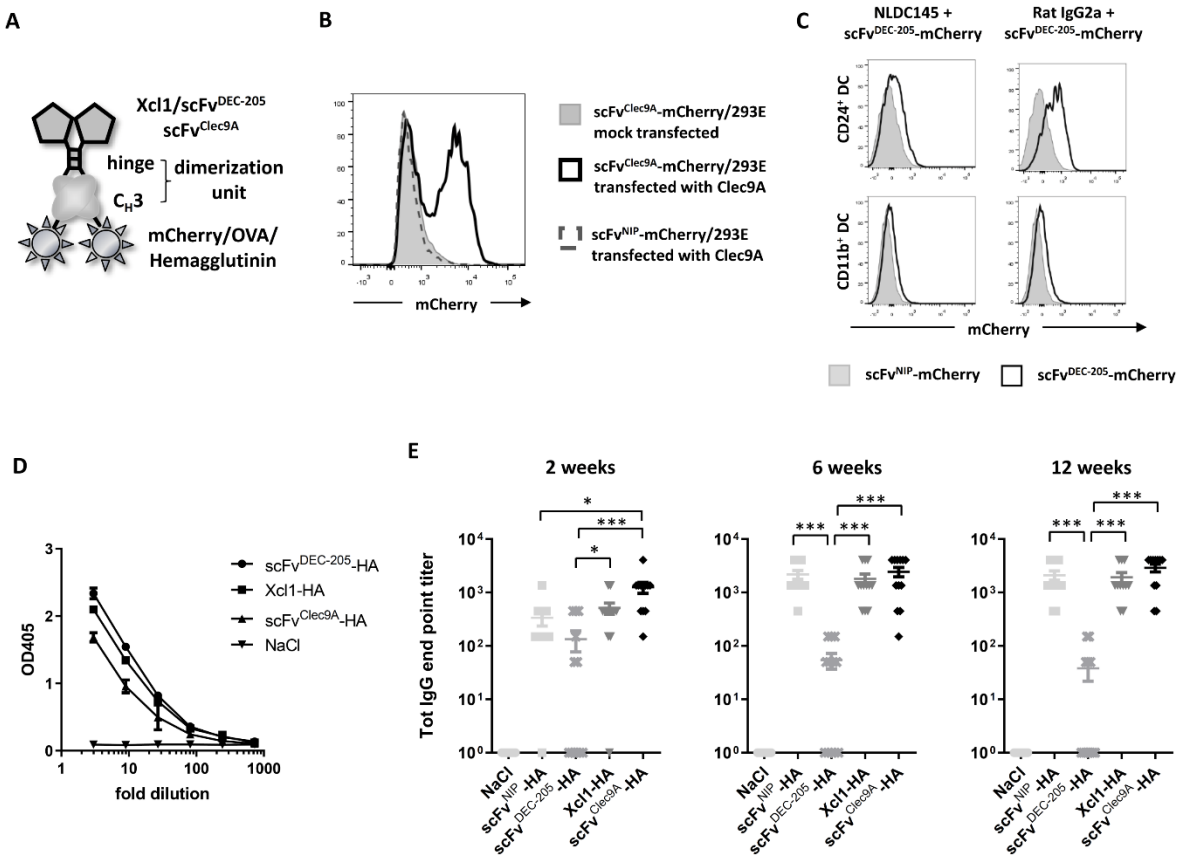
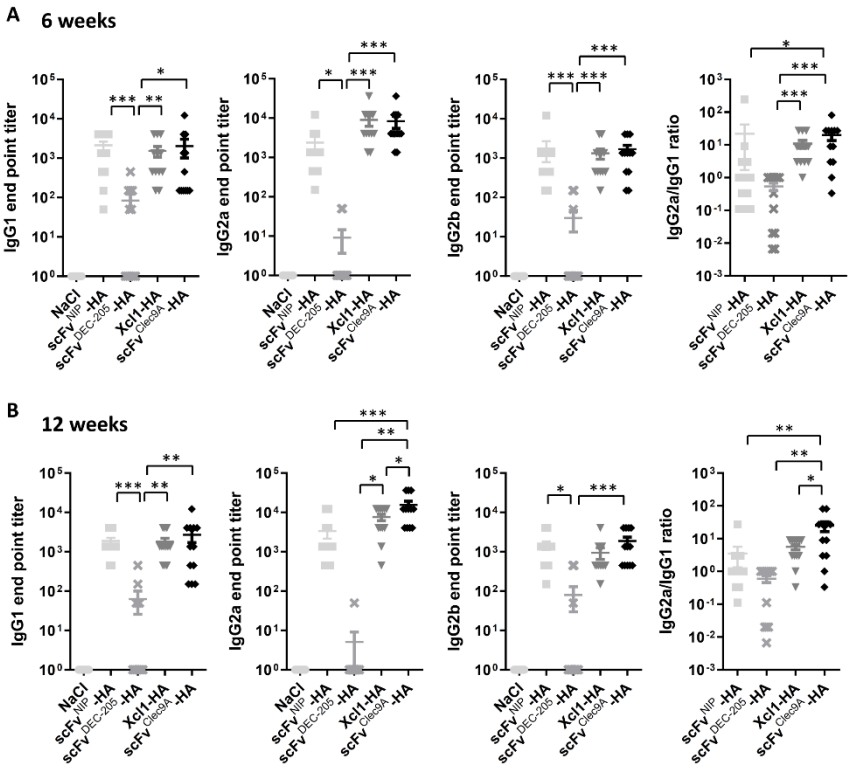
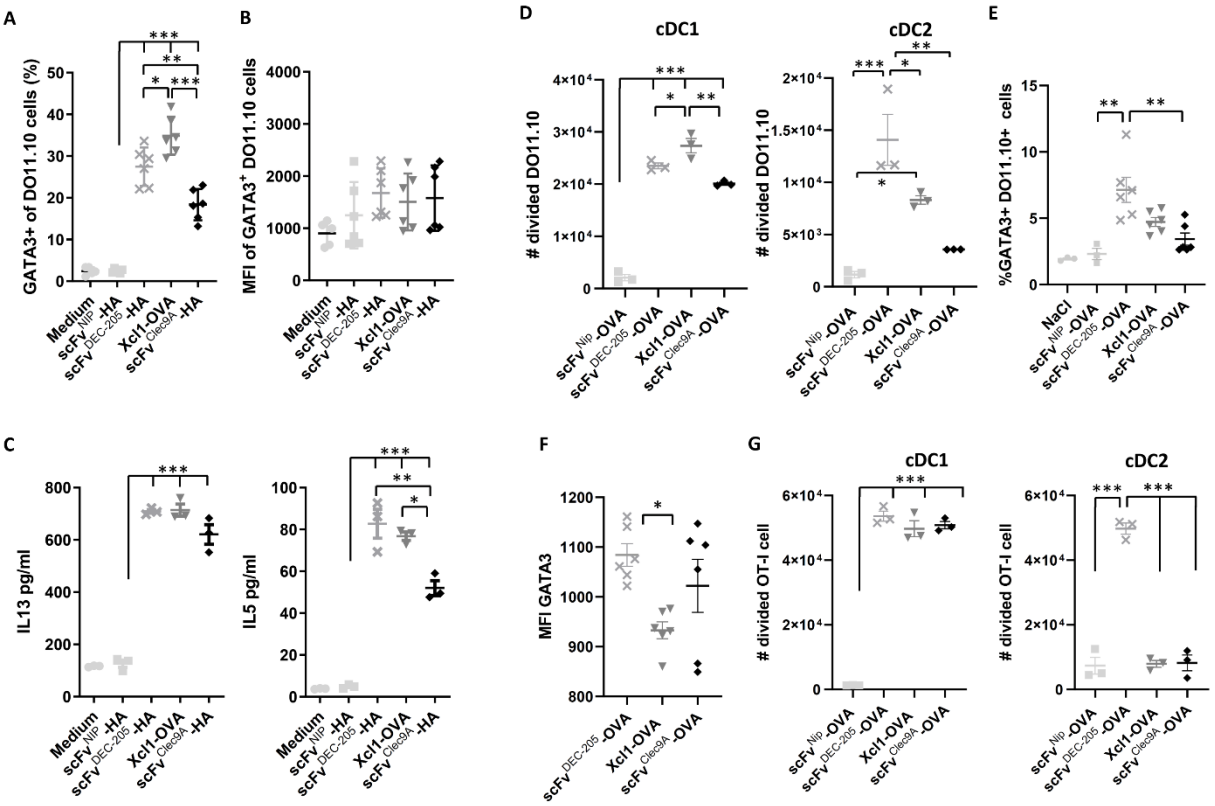


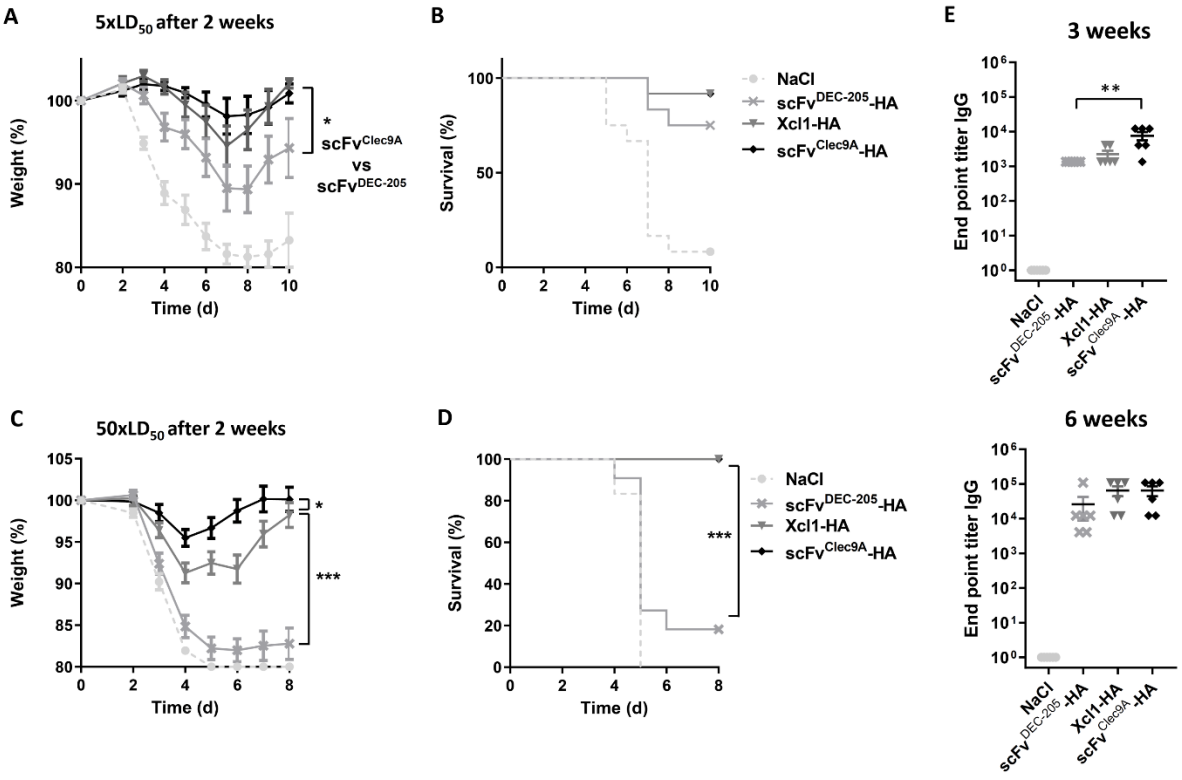
Supplemental Figure 1: Characterization of cDC1 targeted fusion vaccines. (A) Structure of the bivalent fusion vaccine (Vaccibody) consisting of i) a targeting unit (Xcl1, $scFv^{DEC-205}$ or $scFv^{Clec9A}$), ii) a dimerization domain containing the hinge and CH_3 domain of human IgG3 and iii) an antigenic unit (mCherry, OVA or HA). (B) Specificity of $scFv^{Clec9A}$-mCherry was evaluated by flow cytometry on HEK293E cells transfected with plasmids encoding the Clec9A receptor. As controls, binding of $scFv^{NIP}$-mCherry to Clec9A transfected HEK293E cells and binding of $scFv^{Clec9A}$-mCherry to mock transfected HEK293E cells were included. (C) Specificity of the $scFv^{DEC-205}$-mCherry fusion protein was tested by incubation with splenocyttes pre-incubated with the NLDC145 antibody or a non-specific rat IgG2a antibody. Binding of $scFv^{DEC-205}$-mCherry to $CD24^+$ cDC1 was evaluated by flow cytometry. (D) Expression and secretion of $scFv^{DEC-205}$-, Xcl1- and $scFv^{Clec9A}$-HA was evaluated by transient transfection into HEK293E cells, and ELISAs performed on supernatants 48h after transfection. (E) Total IgG in serum samples from BALB/c mice harvested 2, 6 or 12 weeks after intradermal immunization with $scFv^{NIP}$-, $scFv^{DEC-205}$-, Xcl1- or $scFv^{Clec9A}$-HA. Data presented are mean ± SEM. (B-D) Representative data of 2 independent experiments. (E) Results pooled from 2 independent experiments with n = 12 mice per group. Statistical analysis performed using non-parametric one-way ANOVA with Dunn's multiple comparison test, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.



Supplementary Figure 2: Polarization of antibody responses when targeting cDC1 by intradermal DNA immunization. (A-B) BALB/C mice were DNA immunized as in Figure 1, and serum samples analyzed for HA specific antibodies of the subclass IgG1, IgG2a and IgG2b after (A) 6 or (B) 12 weeks. Right graphs present the IgG2a/IgG1 ratio based on the serum titers presented in A) and B). Data presented are mean ± SEM. Results pooled from 2 independent experiments with n = 12 mice pr group. Statistical analysis performed using non-parametric one-way ANOVA with Dunn's multiple comparison test, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.



Supplementary Figure 3: T cell responses when targeting cDC1. (A-C) OVA specific DO11.10 cells were incubated with BM DC and 0.5 μg scFv^{NIP}-, scFv$^{DEC\text{-}205}$- Xcl1- or scFv^{Clec9A}-OVA for 72h. (A) Percentages of GATA3^{+} D011.10 cells analyzed by flow cytometry after gating as indicated in Figure 3A. (B) GATA3 MFI of GATA3^{+} cells presented in (A). (C) Concentration of IL5 and IL13 in supernatants of DO11.10 incubated with BM DCs and OVA containing fusion vaccines as in (A). (D) Cell Trace Violet stained CD4^{+} T cells (DO11.10) were incubated with sorted BM derived cDC1 or cDC2 in the presence of 1 μg scFv^{NIP}-, scFv$^{DEC\text{-}205}$-, Xcl1- or scFv^{Clec9A}-OVA protein for 4 days. Proliferation was determined by flow cytometry. (E-F) 1x10^{6} DO11.10 cells were transferred to naïve BALB/c, and inguinal LN harvested 5 days after immunization with 25 μg DNA encoding scFv^{NIP}-, scFv$^{DEC\text{-}205}$-, Xcl1- or scFv^{Clec9A}-OVA. (E) Percentages of GATA3^{+} D011.10 cells. (F) GATA3 MFI of GATA3^{+} D011.10 cells presented in (E). (G) Cell Trace Violet stained CD8^{+} T cells (OT-I) were incubated with sorted BM derived cDC1 or cDC2 from C57BL/6 in the presence of 1 μg scFv^{NIP}-, scFv$^{DEC\text{-}205}$-, Xcl1- or scFv^{Clec9A}-OVA protein for 4 days. Proliferation was determined by flow cytometry. Data presented are mean ± SEM. (A and B) Results pooled from 2 independent experiments with n = 6 samples pr group. (C, D and G) Data presented is representative of 2 independent experiments with n = 3 samples pr group. (E and F) Data from 1 experiment with n = 3-6 mice per group. Statistical analysis performed using one-way ANOVA with Tukey`s multiple comparison corrections, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.



Supplementary Figure 4: Xcl1-HA and scFv^{Clec9A}-HA immunization protect against influenza in C57BL/6 mice. (A) C57BL/6 mice were immunized with 25μg DNA encoding scFv$^{DEC-205}$-, Xcl1- or scFv^{Clec9A}-HA and challenged two weeks later with 5xLD$_{50}$ influenza A (PR8). Weight loss was monitored as a sign of disease progression and morbidity. (B) Survival of mice presented in (A). (C) BALB/C mice were immunized as in (A) and challenged with 50xLD$_{50}$ 2 weeks late. (D) Survival of mice presented in (C). (E) BALB/C mice were immunized with 25μg DNA encoding scFv$^{DEC-205}$-, Xcl1- or scFv^{Clec9A}-HA, and boosted after 3 weeks. Serum IgG levels were determined 3 weeks after first immunization (3 weeks, upper panel), and 3 weeks after boost (6 weeks, lower panel). (A-D) Data presented are pooled from 2 independent experiments with 12 mice per group, or (E) from one single experiment with 6 mice per group. Statistical analysis was performed using two-way ANOVA (A and C), Mantel Cox (B and D), or Non parametric one-way ANOVA with Dunn`s multiple comparison test. * = $p < 0.05$, ** = $p < 0.01$.