Title: Activation of T cell checkpoint pathways during β-cell antigen presentation by engineered dendritic cells promotes protection from type 1 diabetes

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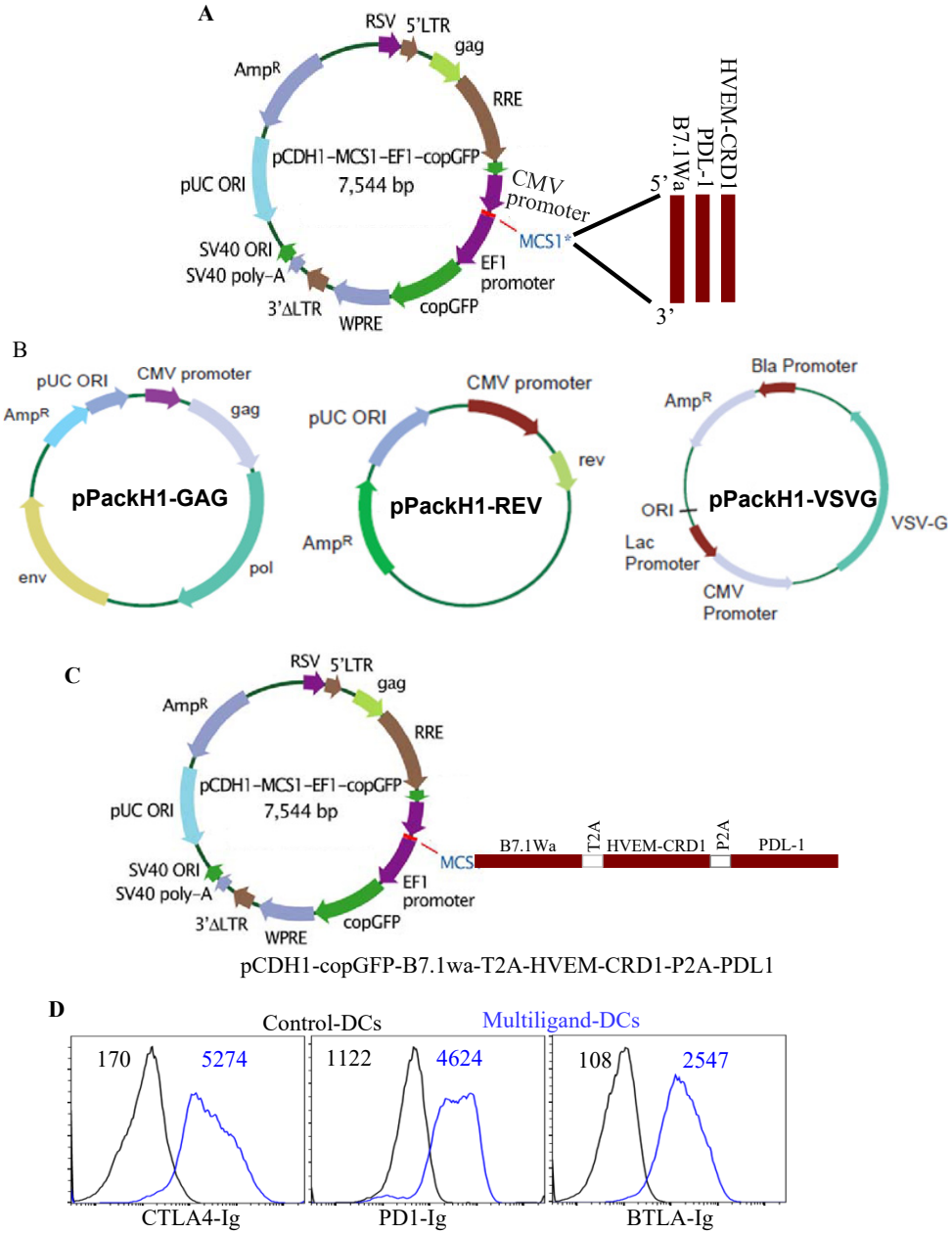
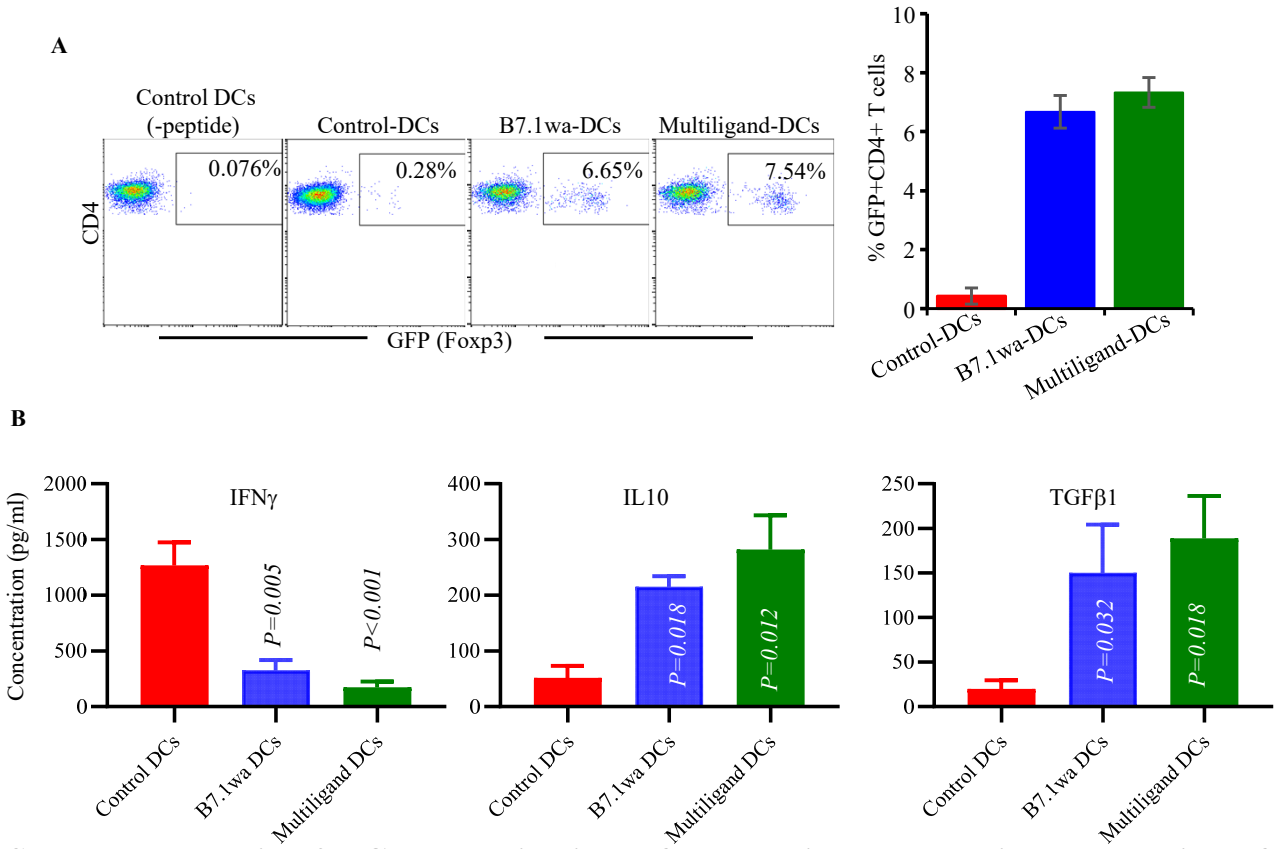
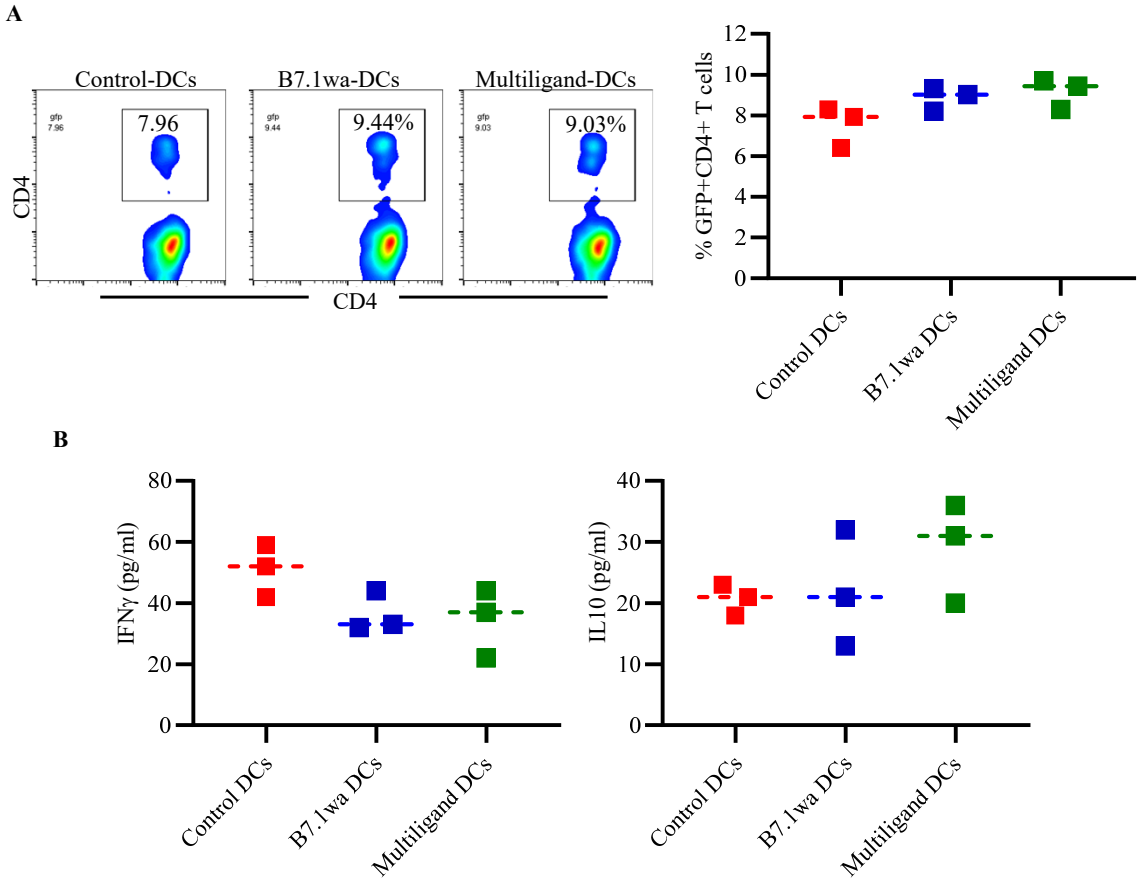


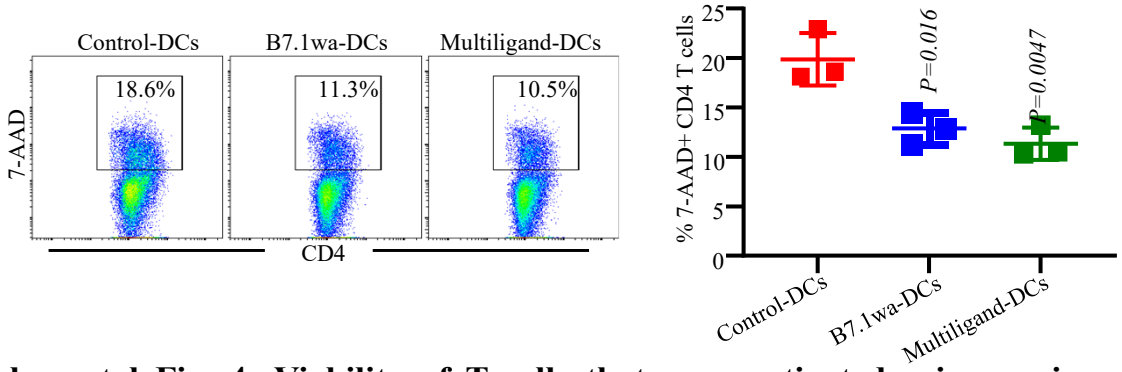
Supplemental Fig. 1: Lentiviral system and the constructs used in this study. A) T cell negative regulatory ligands were cloned individually without fluorescent tags under the CMV promoter of a 3rd generation lentiviral vector from SBI Inc. In these vectors, GFP is expressed under a different (EF1) promoter. Unmodified vector was used as control. **B)** Shows packaging vectors originally purchased as a pool from SBI were separated, propagated and used for generating lentivirus in HEK293T cells or GFPG cells. **C)** In some experiments, a multiligand-cDNA construct was used for expressing multiple ligands from the same vector. Individual cDNAs were separated by self-cleaving peptides as indicated. **D)** Functional ligand expression levels on control- and multiligand-DCs (generated using the vector constructs shown in panel C) were determined after incubating with soluble receptors (CTLA4-Ig, PD1-Ig or BTLA-Ig), followed by PE-labeled Fab(2) fragment of anti-IgG (Fc specific) Ab and testing by FACS. Histogram overlay graphs (representative of 3 independent experiments) showing ligand specific staining of control virus transduced and multiligand virus transduced DCs along with MFI values are shown.



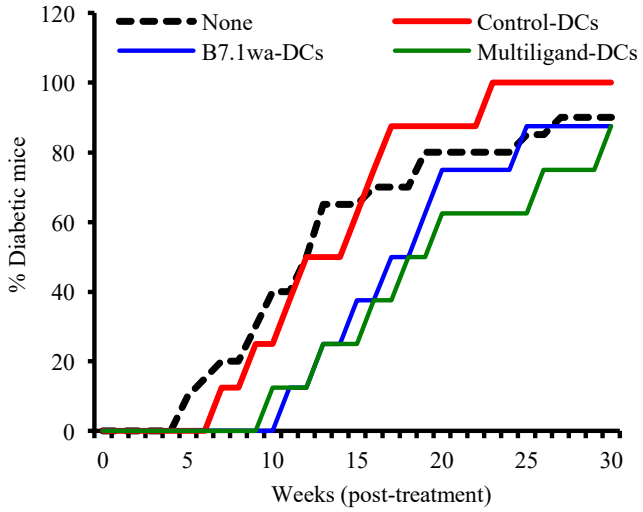
Supplemental Fig. 2: Characterization of the antigen presenting properties of engineered NOD mouse DCs. NOD mouse BM DCs were transduced with lentiviral vectors as described in Figure 1. **A)** GFP- CD4+ T cells were sorted from BDC2.5-Foxp3-GFP mouse spleens and used in primary cultures for 4 days in the presence of BDC2.5-peptide pulsed or non-pulsed DCs and subjected to FACS to determine GFP+ CD4+ cell frequencies by FACS. CD4+ cells were gated and representative FACS plots (left) and Mean±SD values from an experiment in triplicate (representative of two independent experiments) (right) are shown. Since the assay was performed only twice, statistical analysis was not performed. **B)** Equal number of live BDC2.5 T cells isolated from primary cultures by negative selection magnetic sorting were cultured with BDC2.5-peptide pulsed fresh splenic DCs for 16h, and supernatants were subjected to Luminex multiplex assay or ELISA. Mean±SD values of 3 independent experiments, each done in triplicate, are shown. *P*-value by paired *t*-test. All *P*-values are in comparison with control-DC group.



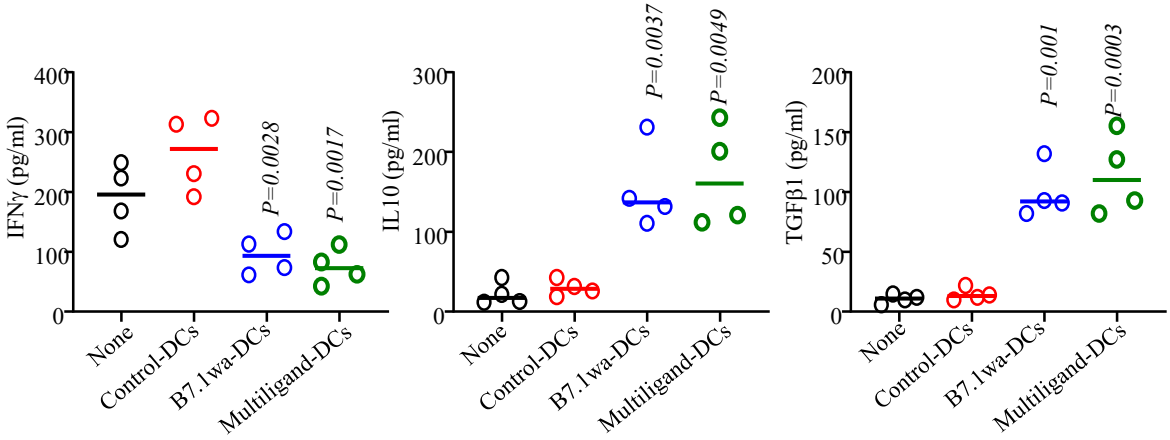
Supplemental Fig. 3: Characterization of the effect of DCs that are not pulsed with antigen on T cells. NOD mouse BM DCs were transduced with lentiviral vectors as described in Figure 1. **A)** CD4+ T cells were sorted from BDC2.5-Foxp3-GFP mouse spleens and used in primary cultures for 4 days in the presence of DCs that are not pulsed with peptide and subjected to FACS to determine GFP+ CD4+ cell frequencies by FACS. CD4+ cells were gated and representative FACS plots (left) and Mean±SD values of three independent experiments, each in triplicate (right) are shown. **B)** Supernatants from the primary cultures (of panel A) were subjected to Luminex multiplex assay. Mean±SD values of 3 independent experiments, each done in triplicate, are shown. *P*-value were not significant by paired *t*-test. Note: since lentiviral transduced/activated DCs were used for the culture, cytokine levels of respective DC alone cultures were subtracted for panel B.



Supplemental Fig. 4: Viability of T cells that were activated using engineered DCs. NOD mouse BM DCs were transduced with lentiviral vectors as described in Figure 1. CD4+ T cells were sorted from BDC2.5 mouse spleens and used in primary cultures in the presence of BDC2.5-peptide pulsed engineered DCs. Cells harvested after 4 days were subjected to staining for dead cells using 7-AAD and subjected to FACS. CD4+ cells were gated and representative FACS plots (left) and Mean±SD values of three independent experiments, each in triplicate, (right) are shown. *P*-values by paired *t*-test.



Supplemental Fig. 5: Treatment with ligand-DCs that are not pulsed with BcAg did not induce significant delay in hyperglycemia. Ten-week-old pre-diabetic female NOD mice were left untreated (none) or injected i.v. with engineered-DCs, that are not pulsed with BcAg, twice at a 15-day interval ($2x10^6$ cells/mouse/injection). Cohorts of mice (8 mice/group for treated groups and 20 mice for "none" group) were monitored for hyperglycemia by testing for blood glucose levels every week as described for Fig. 4. This experiment was conducted along with the experiments of Fig. 4 in two small batches of 4 DC-treated mice/group and the cumulative results (8 mice per each DC-treated group) are shown. Same values were used for "None" group in Fig. 4 and this supplemental figure. *P*-values were not statistically significant by log-rank test.



Supplemental Fig. 6: Short-term cytokine response of PnLN cells from ligand-DC treated NOD mice upon BcAg challenge ex vivo. The assay design was described under Fig. 4. PnLN cells were cultured as described for Fig. 4D. Culture supernatants (4 mice/group) collected at 24h time-point were tested for cytokine levels. *P*-values by Mann-Whitney test. Culture supernatants of these and additional mice collected at 72h time-point were tested for Fig. 4D and the cytokine levels were much higher at this later time-point compared to 24h time-point.