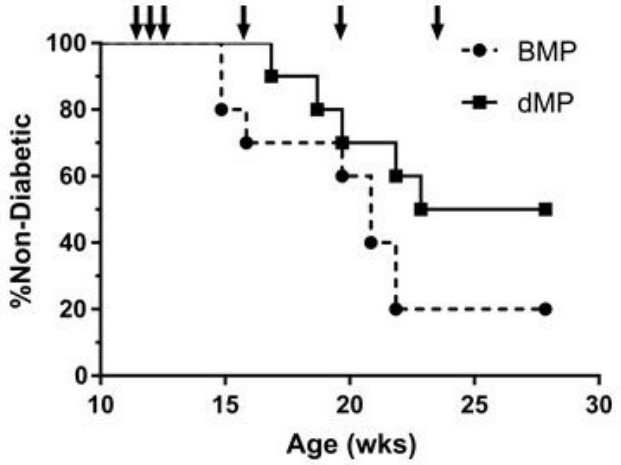
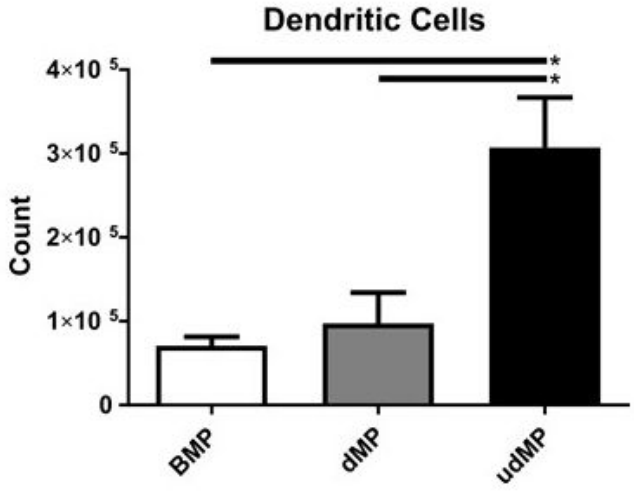
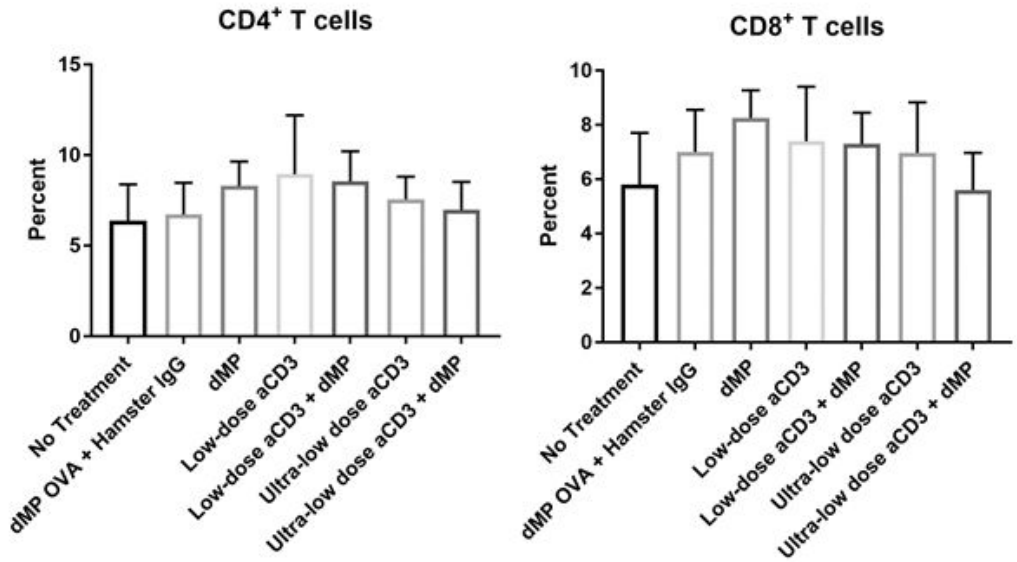
Supplemental Material



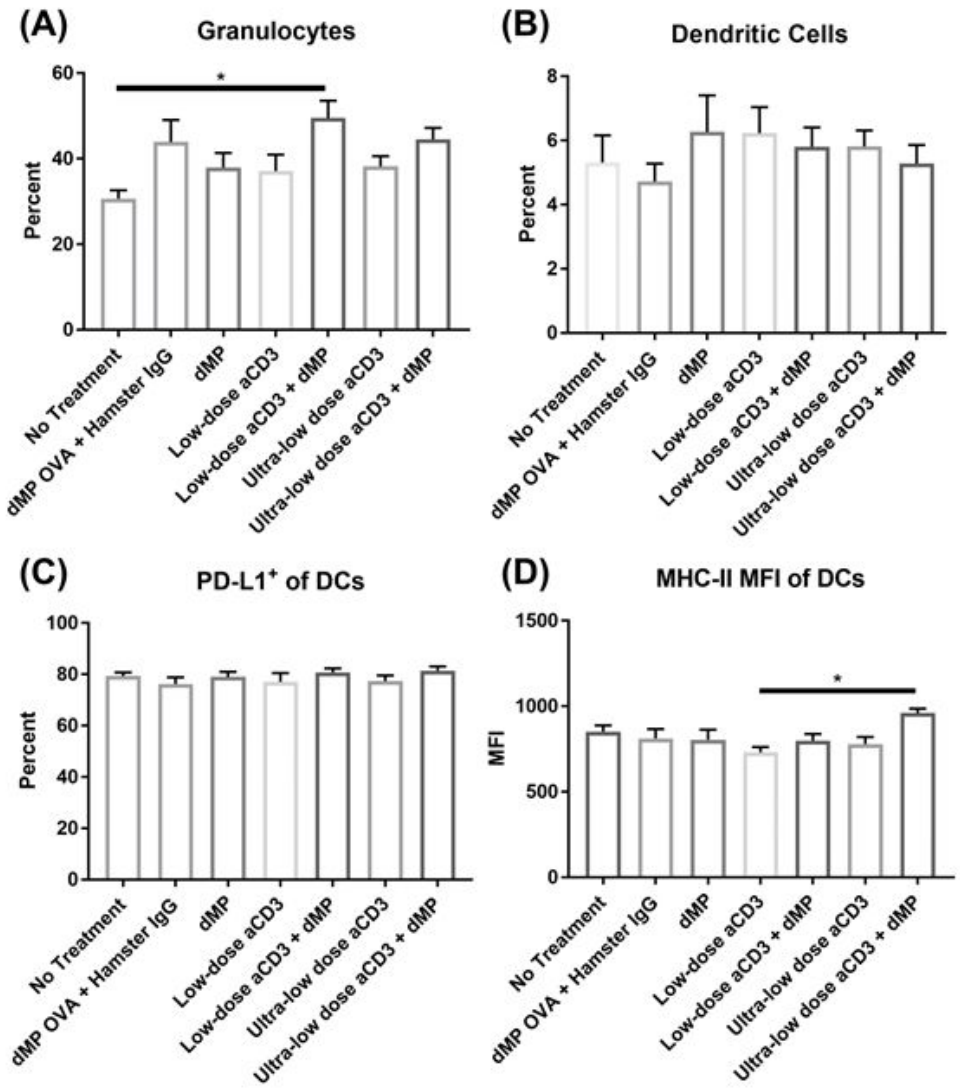
Supplemental Figure 1. The dMP does not prevent diabetes in 12-week-old NOD mice when compared to blank MPs. A cohort of 12-week-old NOD mice (n = 10/group) were injected at a subcutaneous site anatomically proximal to the pancreas with the described MP formulations over 12 weeks. Animals received MP injections (arrows) three times a week for the first week (12 weeks of age) and a monthly booster injection thereafter for three months (16, 20, and 24 weeks of age). Blank MPs were included as a control treatment group. Animals were monitored weekly until week 28, after which remaining non-diabetic mice were euthanized, and mice were considered diabetic when blood glucose levels were ≥ 240 mg/dL on two consecutive days. Survival data is fit using the Kaplan–Meier non-parametric survival analysis model and statistical analysis (p-value = 0.14) performed via log-rank test (Mantel-Cox method).



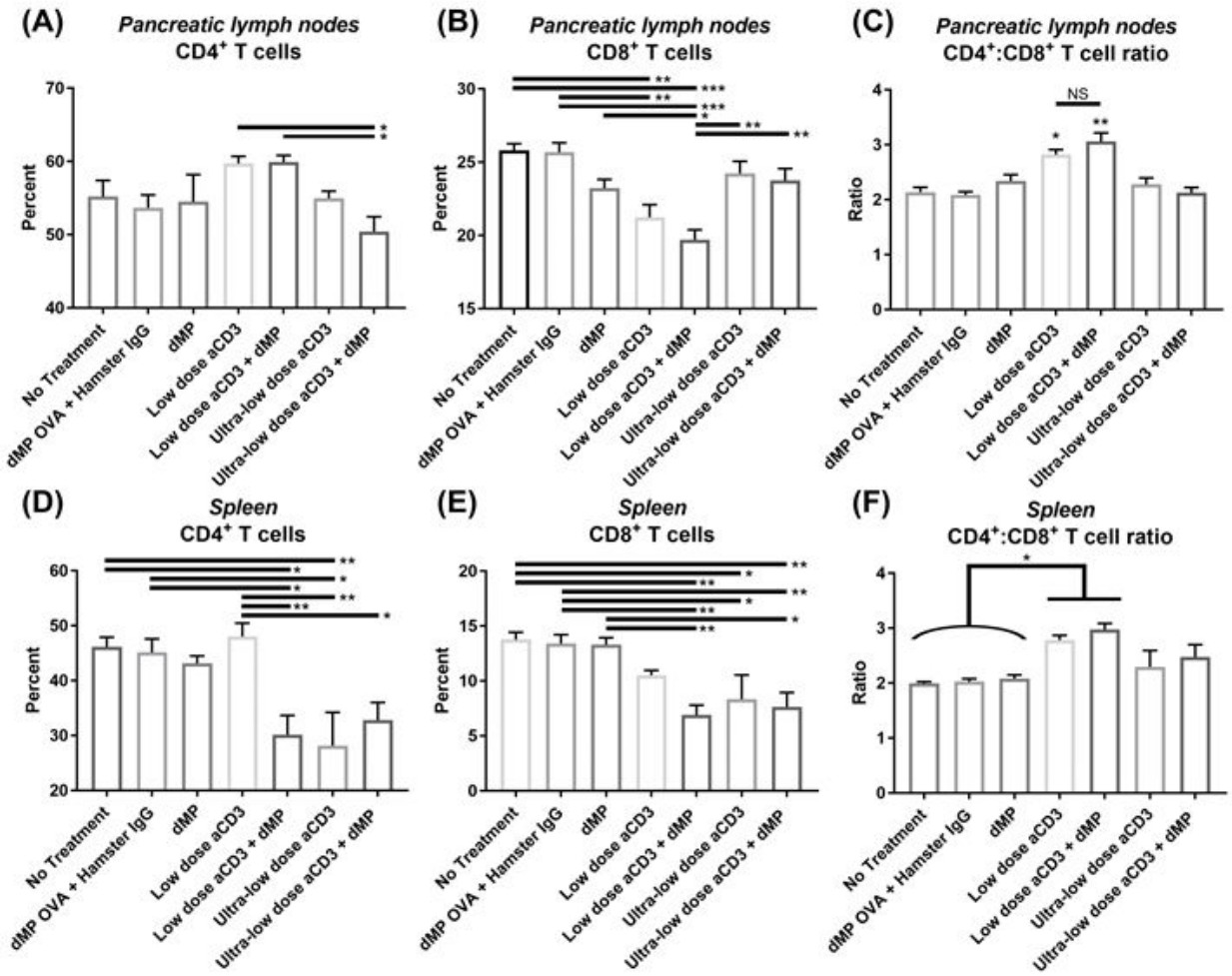
Supplemental Figure 2. Increased GM-CSF and TGF-β1 loading in the udMP enhanced DC recruitment. C57BL/6 mice were injected subcutaneously in the abdominal region with either blank PLGA MPs (BMP), the dMP, or the udMP (n = 4-5). Injection site nodules were excised seven days later, enzymatically digested, and leukocytes analyzed by flow cytometry. Dendritic cell (live $CD45^{+}Ly6G^{-}CD11b^{+}CD11c^{+}$) total numbers were characterized. P-values (* = ≤ 0.05) were obtained by one-way ANOVA with Tukey's significance test. Data is represented by mean ± SEM.



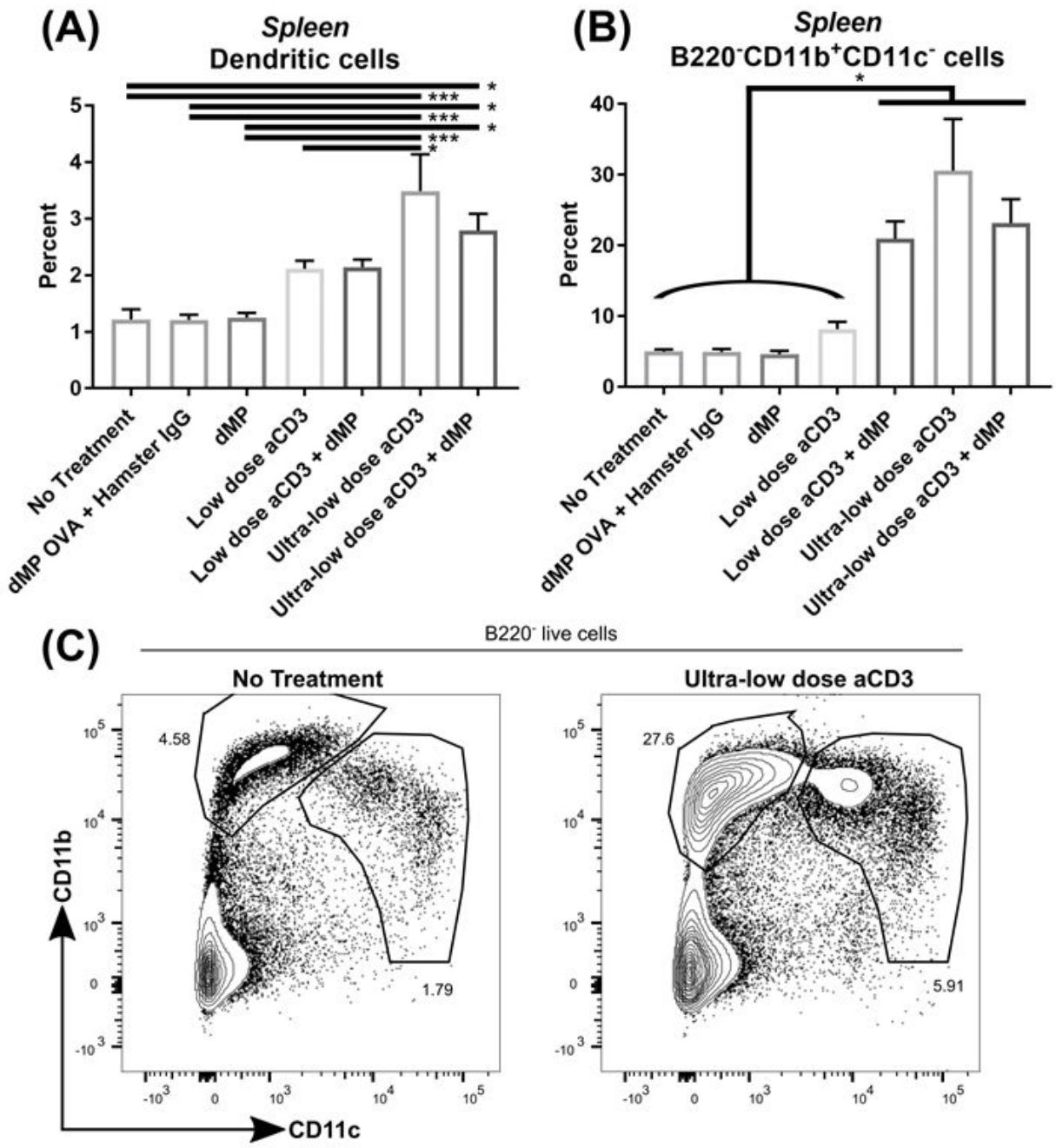
Supplemental Figure 3. Circulating blood lymphocyte frequencies in NOD mice before aCD3 or dMP treatment were comparable. Female NOD mice were randomized into seven treatment groups. At 11 weeks of age, one week before aCD3 and dMP treatment began, four mice were randomly selected from each group and bled to assess circulating blood lymphocyte frequencies as a percentage of total live $CD45^+$ leukocytes in blood ($P > 0.05$, all). Data is represented by mean ± SEM.



Supplemental Figure 4. Anti-CD3 and dMP treatment induced minimal changes on circulating innate immune cells. At ~13 weeks of age, three days after completing a five-day aCD3 treatment regimen, a selection of mice from each group was bled to assess circulating blood leukocyte frequency and phenotype (n = 9-10/group). The frequency of granulocytes ($Ly6G^+CD11b^+CD11c^-$) (A) and DCs ($Ly6G^-CD11b^+CD11c^+$) (B) as a percentage of total live $CD45^+$ leukocytes in blood. Percentage of DCs expressing PD-L1 (C) or MHC-II (D) in blood. P-values (* ≤ 0.05) were obtained by one-way ANOVA with Tukey's significance test. Data is represented by mean ± SEM.

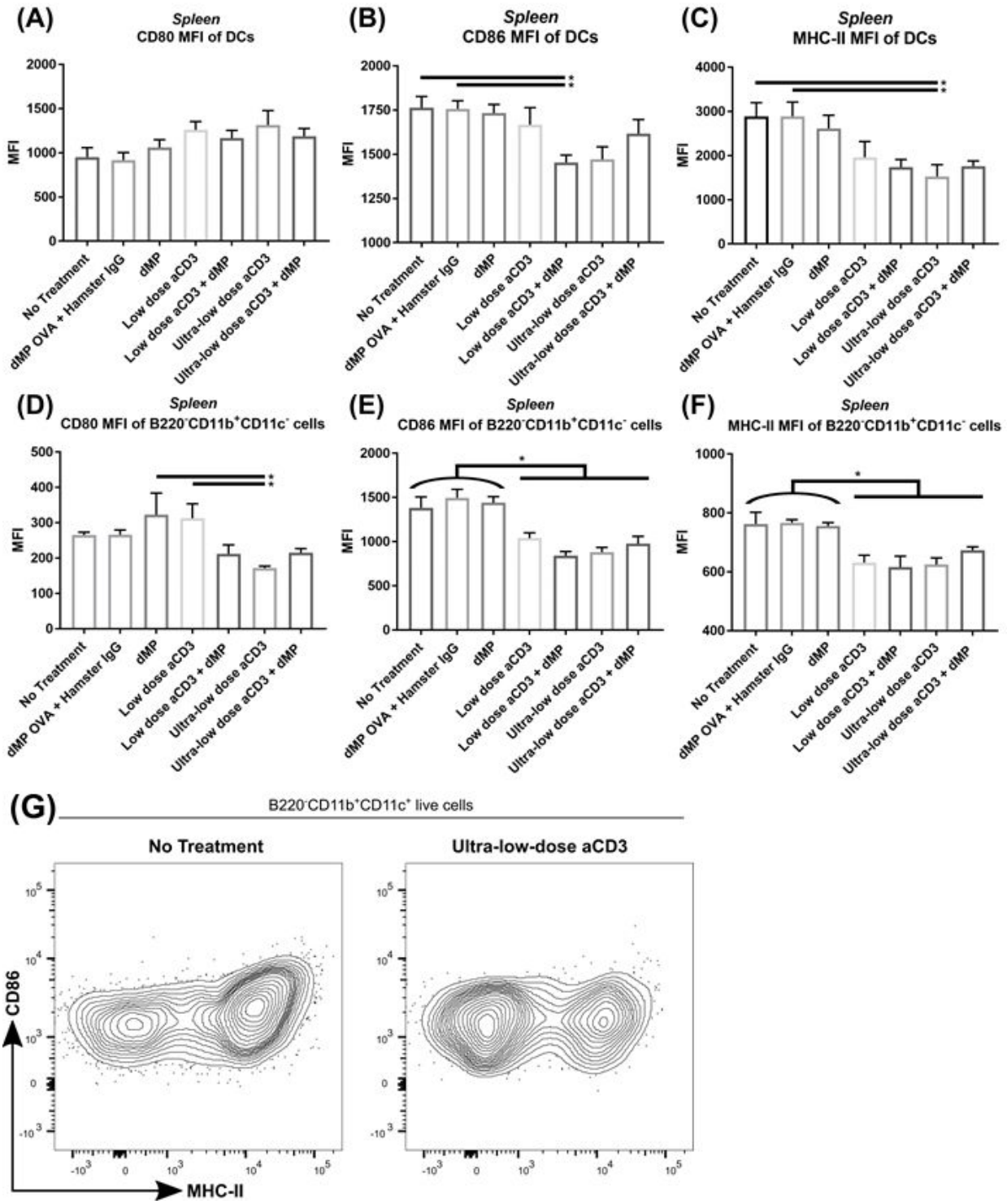


Supplemental Figure 5. Low-dose aCD3 induced long-lasting changes in the CD4$^+$:CD8$^+$ T cell ratio and ultra-low-dose aCD3 reduced both CD4$^+$ and CD8$^+$ T cell frequencies in the spleen. Twelve-week-old pre-diabetic NOD mice received aCD3 and dMP treatment at identical time points as in the prevention study and were euthanized at 20 weeks of age, prior to the sixth dMP injection. As before, MP injections were administered subcutaneously on the right side of the abdomen, proximal to the pancreas. Lymphoid organs were excised and stained for flow cytometry (n = 4-5/group). Frequency of CD4$^+$ (A, D) and CD8$^+$ (B, E) T cells as a percentage of total live CD45$^+$ leukocytes was characterized in pancreatic lymph nodes (A-C) and spleen (D-F). The ratio of CD4$^+$ to CD8$^+$ T cells in pancreatic lymph nodes (C) and spleen (F). P-values (* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001) were obtained by one-way ANOVA with Tukey's significance test. Significance in (C) reflects low-dose aCD3 and low-dose aCD3 + dMP significance against all groups except each other (NS; non-significant). Significance in (F) reflects individual comparisons between no treatment, dMP OVA + control IgG, and dMP groups (curved bars) with low-dose aCD3 and low-dose aCD3 + dMP groups (flat bars; six pairwise comparisons total). Data is represented by mean ± SEM.



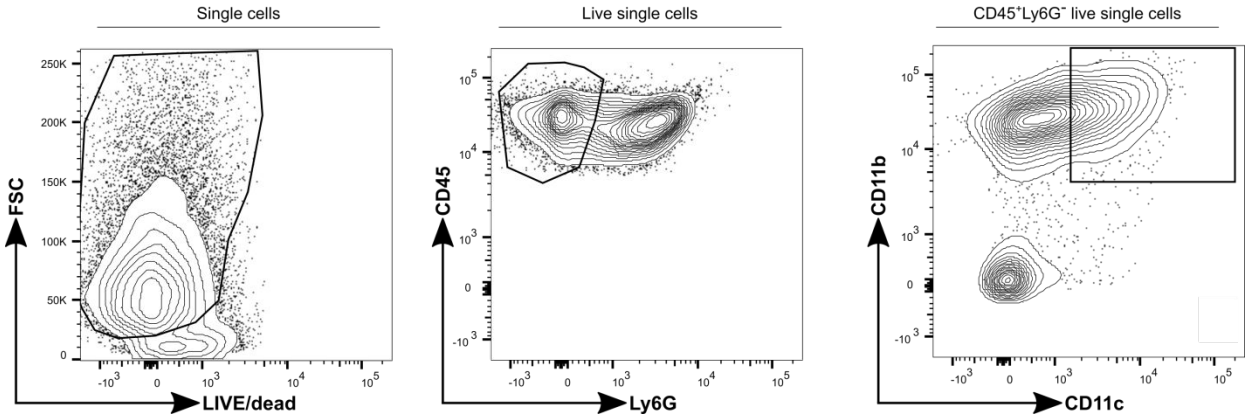
Supplemental Figure 6. Ultra-low-dose aCD3 treatment increased DC and granulocyte frequencies in the spleen. Twelve-week-old pre-diabetic NOD mice received aCD3 and dMP treatment at identical time points as in the prevention study and were euthanized at 20 weeks of age, prior to the sixth dMP injection. As before, MP injections were administered subcutaneously on the right side of the abdomen, proximal to the pancreas. Lymphoid organs were excised and stained for flow cytometry (n = 4-5/group). Frequency of DCs ($B220^{-}CD11b^{+}CD11c^{+}$) (A) and $B220^{-}CD11b^{+}CD11c^{-}$ cells ($\geq$ 70% of these cells were SSC^{hi} granulocytes) (B) as a percentage of total live $CD45^{+}$ leukocytes was characterized in the spleen. (C) Representative flow analysis. P-values (* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001) were obtained by one-way ANOVA with Tukey's

significance test. Significance in (B) reflects individual comparisons between no treatment, dMP OVA + control IgG, dMP, and low-dose aCD3 groups (curved bars) with low-dose aCD3 + dMP, ultra-low-dose aCD3, and ultra-low-dose aCD3 + dMP groups (flat bars; 12 pairwise comparisons total). Data is represented by mean ± SEM.

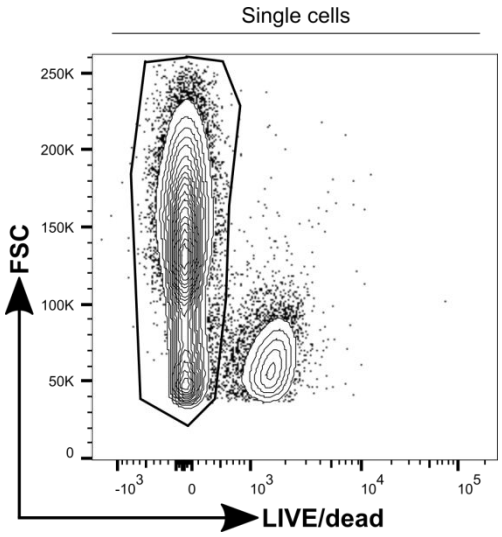


Supplemental Figure 7. Anti-CD3 treatment reduced markers of maturation on DCs and granulocytes in the spleen. Twelve-week-old pre-diabetic NOD mice received aCD3 and dMP treatment at identical time points as in the prevention study and were euthanized at 20 weeks of age, prior to the sixth dMP injection. As before, MP injections were administered subcutaneously on the right side of the abdomen, proximal to the pancreas. Lymphoid organs were excised and stained for flow cytometry (n = 4-5/group). Mean fluorescent intensity (MFI) of activation markers

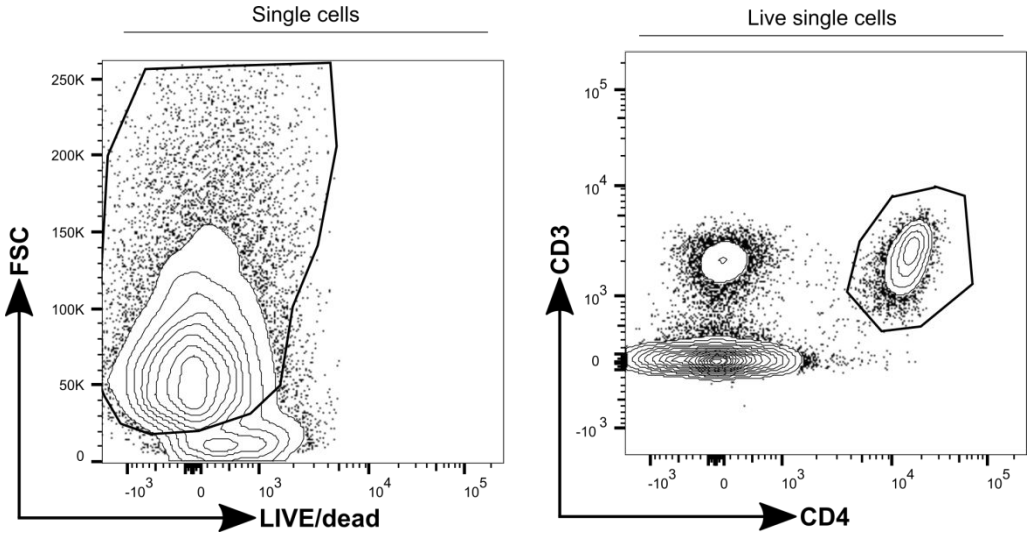
CD80, CD86, and MHC-II were analyzed on DCs (A-C) and $B220^{-}CD11b^{+}CD11c^{-}$ cells (≥ 70% of these cells were SSC^{hi} granulocytes) (D-F) in the spleen. (G) Representative flow analysis. P-values (* ≤ 0.05) were obtained by one-way ANOVA with Tukey's significance test. Significance in (E-F) reflects individual comparisons between no treatment, dMP OVA + control IgG, and dMP groups (curved bars) with low-dose aCD3, low-dose aCD3 + dMP, ultra-low-dose aCD3, and ultra-low-dose aCD3 + dMP groups (flat bars; 12 pairwise comparisons total). Data is represented by mean ± SEM.



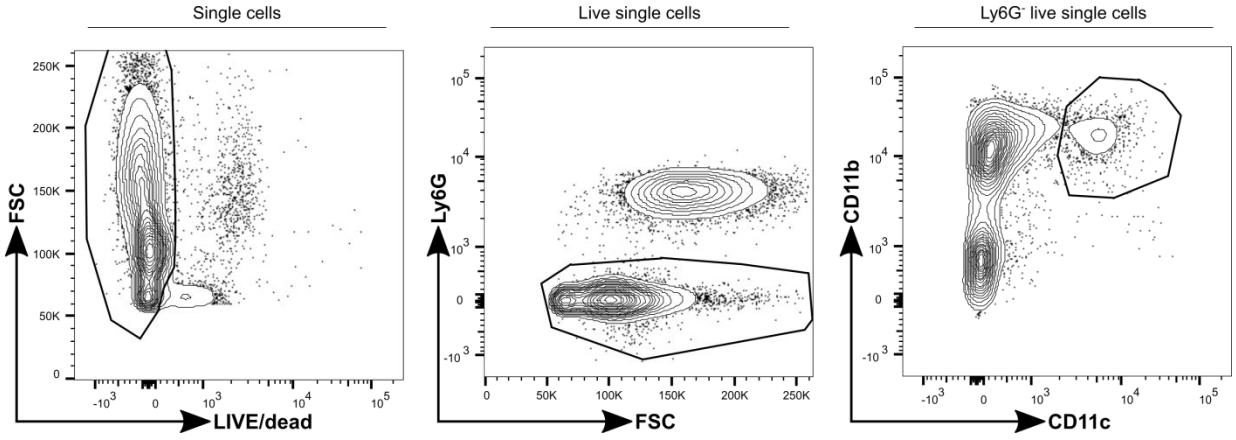
Supplemental Figure 8. Flow cytometry gating scheme for Figures 1 and 2. Dendritic cells (CD45+Ly6G-CD11b+CD11c+ live single cells) were delineated by first gating on single cells and live cells to remove doublets and dead cells. Subsequently, CD45+Ly6G- cells were gated to isolate leukocytes and remove granulocytes, respectively. Lastly, a CD11b+CD11c+gate was used to identify dendritic cells.



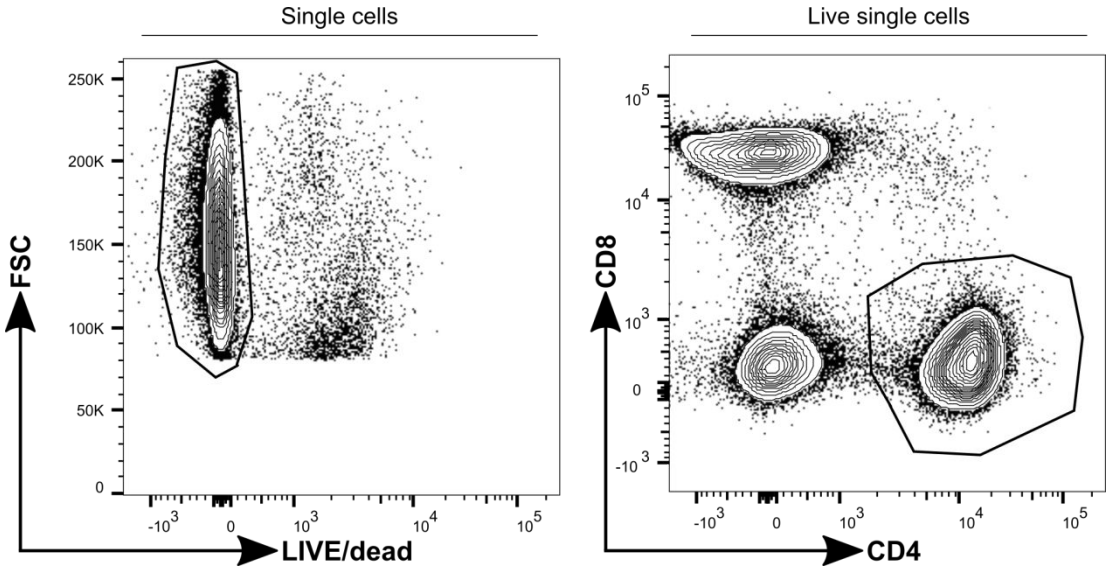
Supplemental Figure 9. Flow cytometry gating scheme for Figure 5. Live cells were delineated by gating on single cells and live cells to remove doublets and dead cells.



Supplemental Figure 10. Flow cytometry gating scheme for Figures 6 and 7. CD3+CD4+ T cells were delineated by first gating on single cells and live cells to remove doublets and dead cells. Subsequently, a CD3+CD4+ gate isolated the final population of T cells to be evaluated.



Supplemental Figure 11. Flow cytometry gating scheme for Figure 8. Dendritic cells (Ly6G-CD11b+CD11c+ live single cells) were delineated by first gating on single cells and live cells to remove doublets and dead cells. Subsequently, Ly6G- granulocytes were removed. Lastly, a CD11b+CD11c+gate was used to identify dendritic cells.



Supplemental Figure 12. Flow cytometry gating scheme for Figures 9-11. CD4+ T cells were delineated by first gating on single cells and live cells to remove doublets and dead cells. Subsequently, a CD4+ gate isolated the final population of T cells to be evaluated.