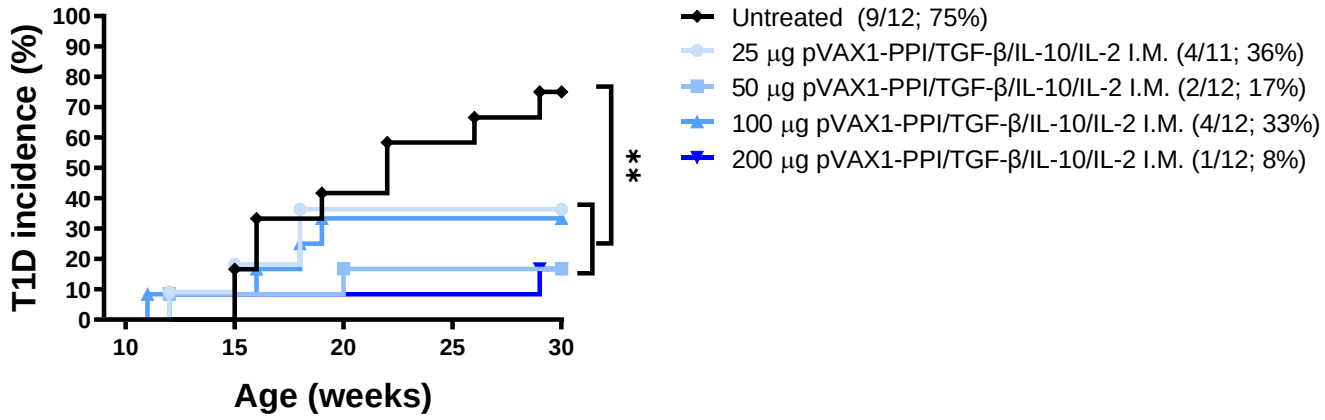
Supplemental Figure 1: T1D incidence in mice dosed with 25-200 µg plasmid and further analysed for plasma cytokines and IL-10R expression in circulating innate immune cells.

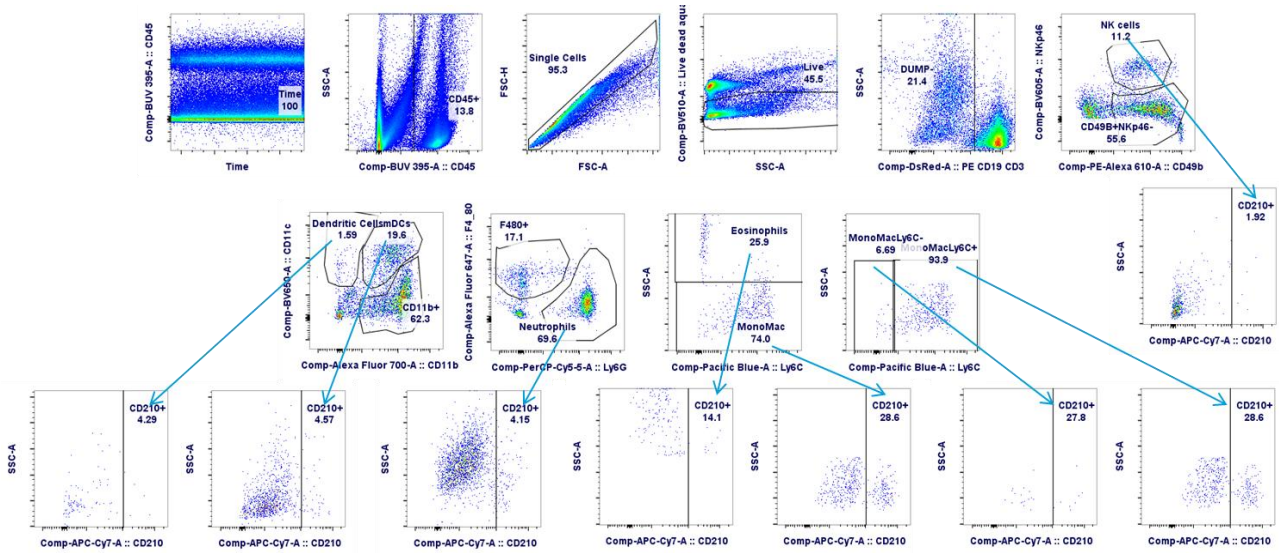


Supplemental Table 1: Application summary for the flow cytometry analysis of innate immune cell subsets and their corresponding CD210 expression in the blood at 13 weeks of age.

Purpose	Phenotype basic immune cell subsets ex vivo		
Species	NOD mice		
Cell types	Dendritic cells, NK cells, Neutrophils, Eosinophils, Monocyte/Macrophage, and Macrophage subsets		
Cross reference	None		
Instrument	LSR Fortessa	**HTS or Tube?**	Either

Antibody (Clone)	**Fluorochrome**	**Vendor**	**Laser**	**Filters**
Ly6C (HK1.4)	Pacific Blue	Biolegend	Violet	450/50 (no LP)
LIVE/DEAD AQUA	AmCyan	Life Technologies	Violet	515/20 (495 LP)
NKp46 (29A1.4)	BV605	BD	Violet	610/20 (595 LP)
CD11c (N418)	BV650	Biolegend	Violet	660/20 (635 LP)
CD19 (1D3)	PE	BD	Yellow-Green	582/12 (no LP)
CD3e (145-2C11)	PE	eBioscience	Yellow-Green	582/12 (no LP)
CD49b (DX5)	PE-CF594	BD	Yellow-Green	620/10 (600 LP)
Ly6G (1A8)	PerCP-Cy5.5	BioLegend	Blue	695/40 (690LP)
F4/80 (BM8)	Alexa Fluor 647	Biolegend	Red	670/30 (no LP)
CD11b (M1/70)	Alexa Fluor 700	Biolegend	Red	730/45 (690 LP)
CD210 (1B1.3a)	Biotin	Biolegend	NA	NA
Streptavidin	APC-Cy7	Biolegend	Red	780/60 (750 LP)

Supplemental Figure 2: Gating strategy to assess percentages of innate immune cell subsets and their corresponding CD210 expression in the blood at 13 weeks of age.



A subset of NOD mice in each treatment group were bled at 13 weeks of age, and leukocytes were stained with mouse antibodies as described in section 2. The exclusion parameter (DUMP) includes antibodies against CD3 (T cells) and CD19 (B cells).

Supplemental Table 2: Percentages of innate immune cell subsets and their corresponding CD210 expression in the blood at 13 weeks of age

Cell population analysed (% parent population)	**pVAX1-PPI/TGF-β/ IL-10/IL-2 25 µg**	**pVAX1-PPI/TGF-β/ IL-10/IL-2 50 µg**	**pVAX1-PPI/TGF-β/ IL-10/IL-2 100 µg**	**pVAX1-PPI/TGF-β/ IL-10/IL-2 200 µg**	**Untreated**
NK (% Dump$^-$)	0.32 ± 0.06	0.32 ± 0.12	0.46 ± 0.15	0.36 ± 0.09	1.13 ± 0.35
mDC (% Dump$^-$)	0.16 ± 0.05	0.22 ± 0.04	0.47 ± 0.09	0.37 ± 0.06	0.48 ± 0.13
Neutrophils (% Dump$^-$)	0.16 ± 0.12	1.77 ± 2.52	0.87 ± 0.87	1.49 ± 1.75	0.45 ± 0.61
Eosinophils (% Dump$^-$)	0.00005 ± 0.00008	0.00251 ± 0.00775	0.00019 ± 0.00033	0.00048 ± 0.00118	0.00038 ± 0.00089
Monocyte (% Dump$^-$)	0.00002 ± 0.00003	0.0007 ± 0.00208	0.00014 ± 0.00025	0.00026 ± 0.0006	0.00053 ± 0.00141
Ly6C$^-$ Monocyte (% Monocyte)	0.000003 ± 0.000004	0.000022 ± 0.000053	0.00002 ± 0.000028	0.000021 ± 0.000034	0.000108 ± 0.000227
Ly6C$^+$ Monocyte (% Monocyte)	0.00002 ± 0.00003	0.00067 ± 0.002	0.00012 ± 0.00022	0.00023 ± 0.00054	0.00042 ± 0.00118
CD210$^+$ NK (% NK)	0.97 ± 0.24	1.01 ± 0.26	1.19 ± 0.22	1.02 ± 0.24	0.96 ± 0.2
CD210$^+$ mDC (% mDC)	7.88 ± 1.46 (p=0.0017)	10.74 ± 2.11 (p<0.0001)	15.68 ± 2.83 (p<0.0001)	17.26 ± 2.78 (p<0.0001)	3.18 ± 0.67
CD210$^+$ Neutrophils (% Neutrophils)	3.92 ± 1.34	2.65 ± 0.86 (p=0.0064)	4.5 ± 1.29	3.21 ± 1.11	1.88 ± 0.28
CD210$^+$ Eosinophils (% Eosinophils)	7.65 ± 4.86	5.58 ± 3.59 (p=0.016)	15.83 ± 6.43	12.48 ± 8.52	12.27 ± 5.16
CD210$^+$ Monocyte (% Monocyte)	41.97 ± 7.5 (p<0.0001)	58.38 ± 9.21 (p<0.0001)	66.21 ± 7.52 (p<0.0001)	79.37 ± 4.23 (p<0.0001)	19.95 ± 3.96
CD210$^+$ Ly6C$^-$ Monocyte (% Ly6C$^-$Monocyte)	22.34 ± 2.95	29.36 ± 8.9 (p=0.0028)	52.19 ± 6.68 (p<0.0001)	56.05 ± 6.77 (p<0.0001)	13.89 ± 3.43
CD210$^+$ Ly6C$^+$ Monocyte (% Ly6C$^+$ Monocyte)	47.88 ± 7.17 (p<0.0001)	62.76 ± 9.03 (p<0.0001)	71.82 ± 7.74 (p<0.0001)	86.99 ± 3.2 (p<0.0001)	24.74 ± 5.02

The percentage of NK cells, mDCs, neutrophils, eosinophils, and (total, Ly6C- and Ly6C+) monocytes was determined. The mean ± 95% confidence interval for each group are listed (13 weeks of age time-point), given either as the percentage of non-T and B cells (Dump$^-$), or as a percentage of monocytes, as indicated. All treatment groups have been compared to the untreated group for statistical analyses, using ordinary one-way analysis of variance (ANOVA) with Tukey's multiple comparisons.

Supplementary Methods

Detection of IL10 in Mouse Serum by Mass Spectrometry

Mouse serum samples were concentrated via immuno-precipitation using a commercial anti-IL-10 antibody (Affymetrix), and an in-house anti-IL-10 antibody detecting plasmid-derived tagged IL-10. Immunoprecipitated samples were analyzed using a targeted mass spectrometry method (parallel reaction monitoring) to detect for peptides corresponding to either tagged IL-10 or endogenous IL-10. Mass spectrometry analysis was performed using a nanoLC1200 chromatography system in line with a QExactive Plus Mass Spectrometer (Both Thermo Electron).