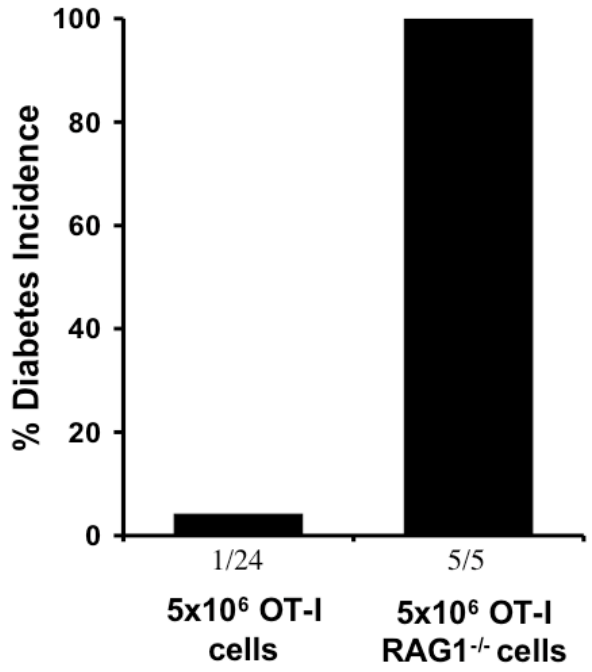
Supplemental Figure 1



Supplemental Figure 2

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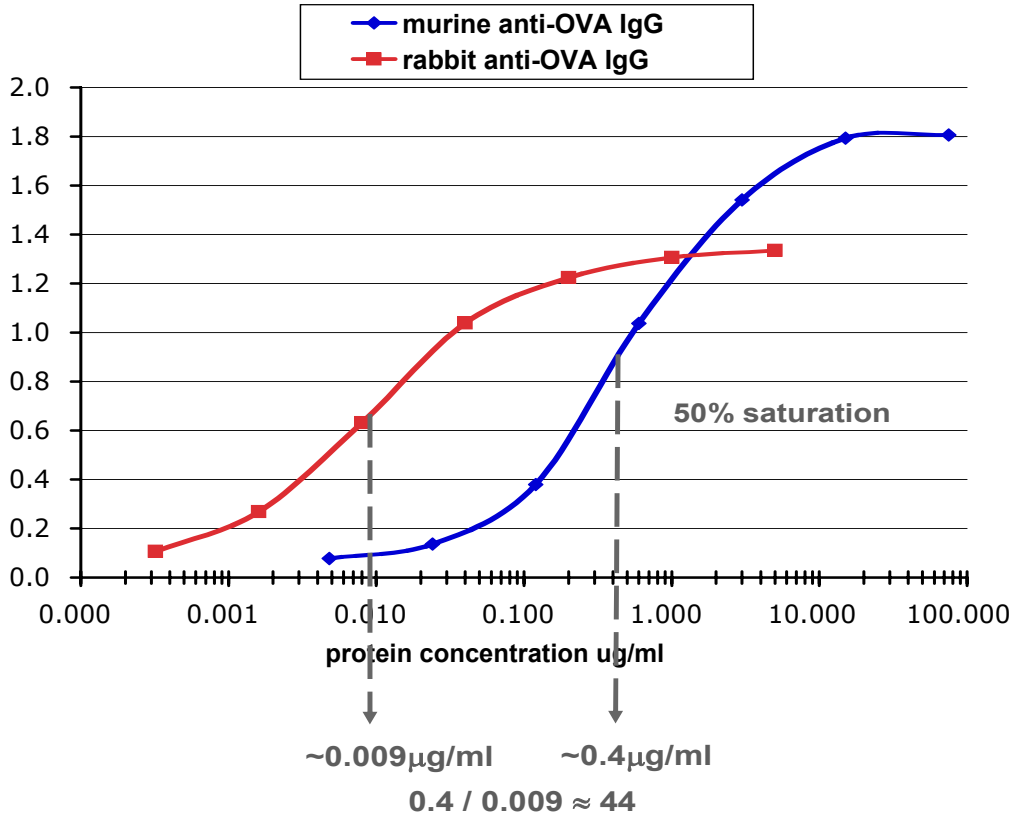

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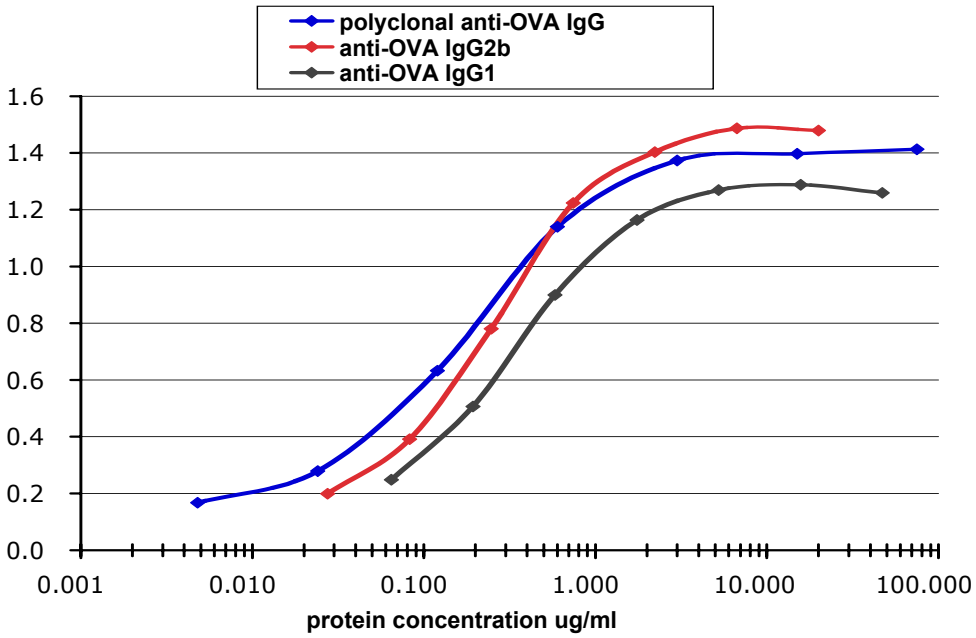
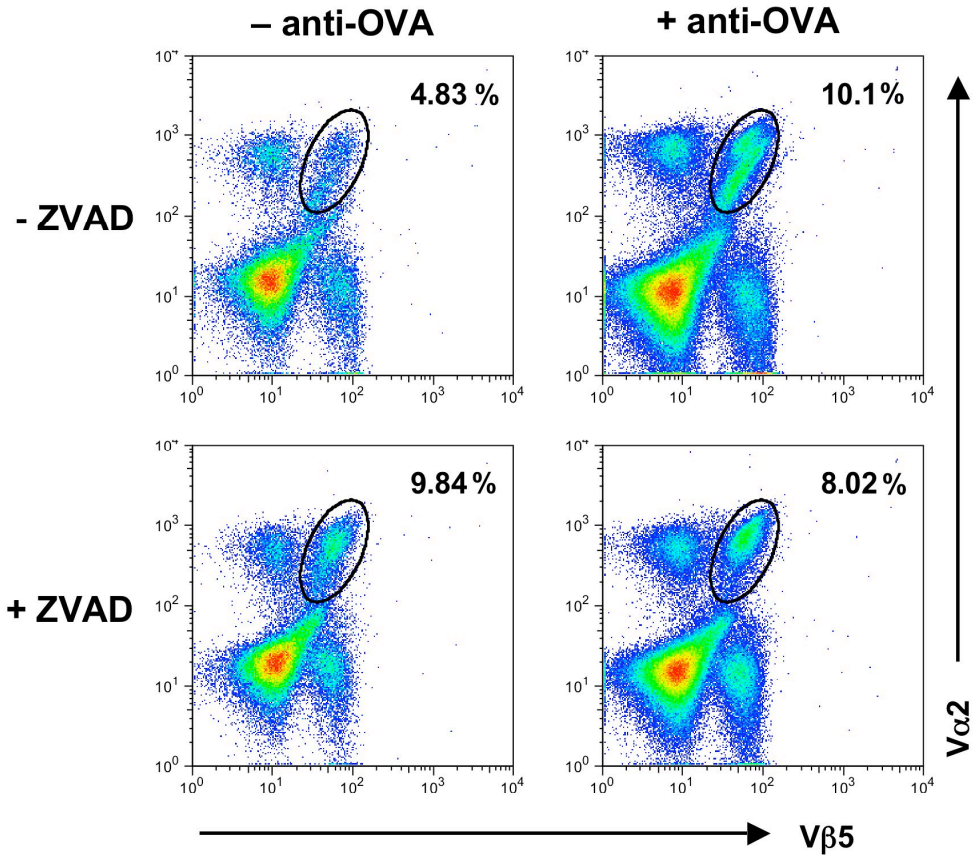


Supplemental Figure 3



Supplemental Figure 4

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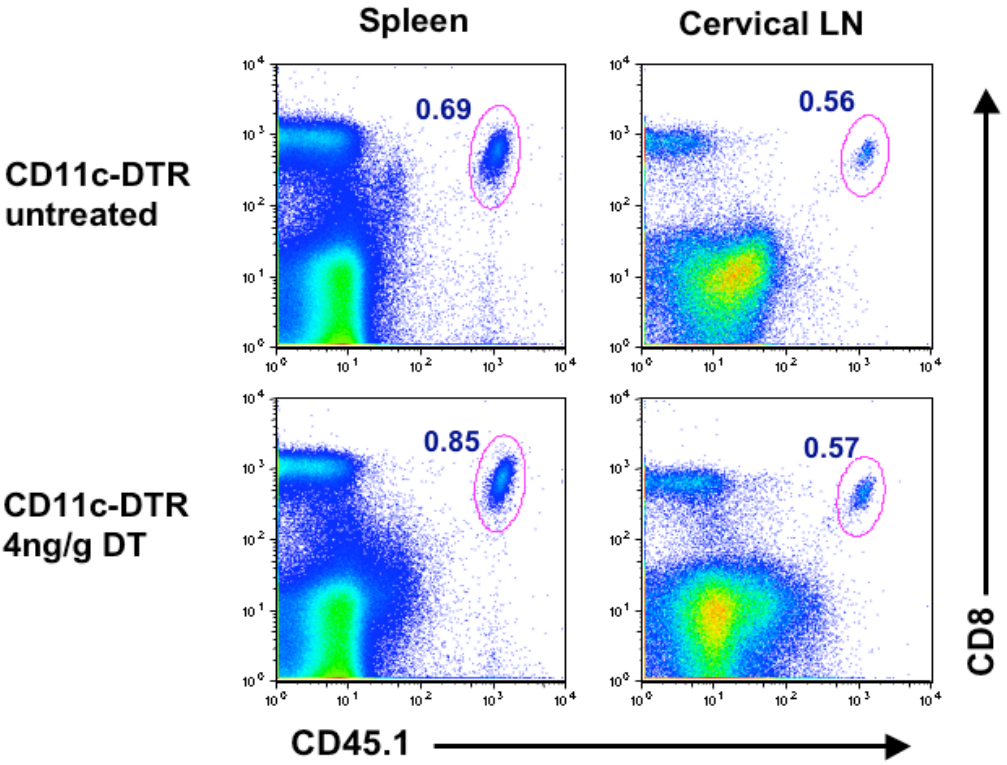

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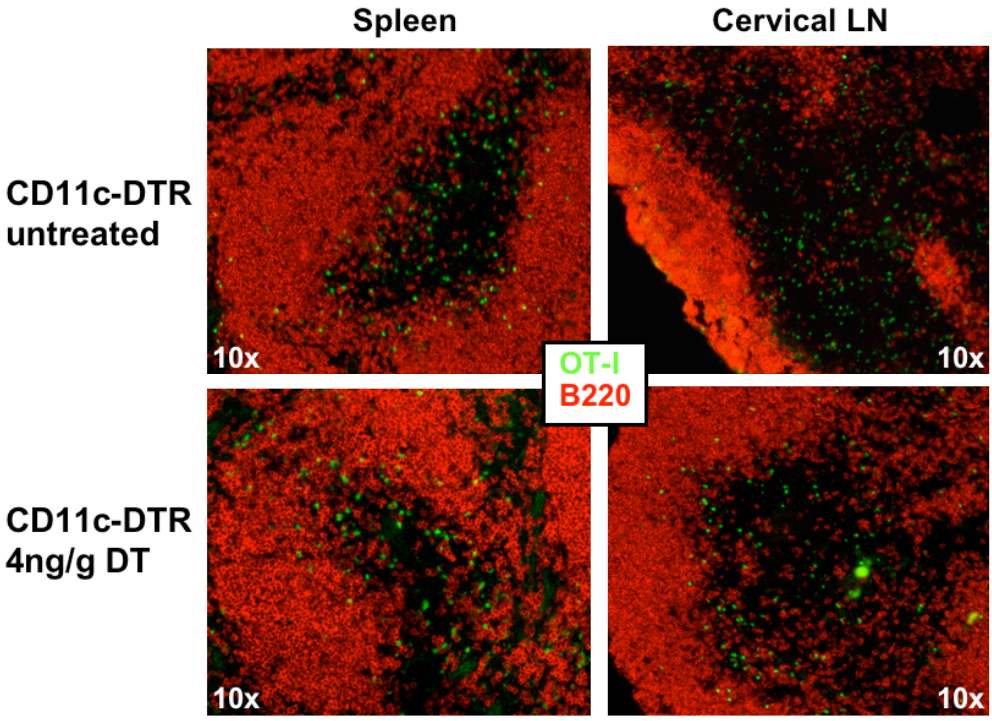

Supplemental Figure 5

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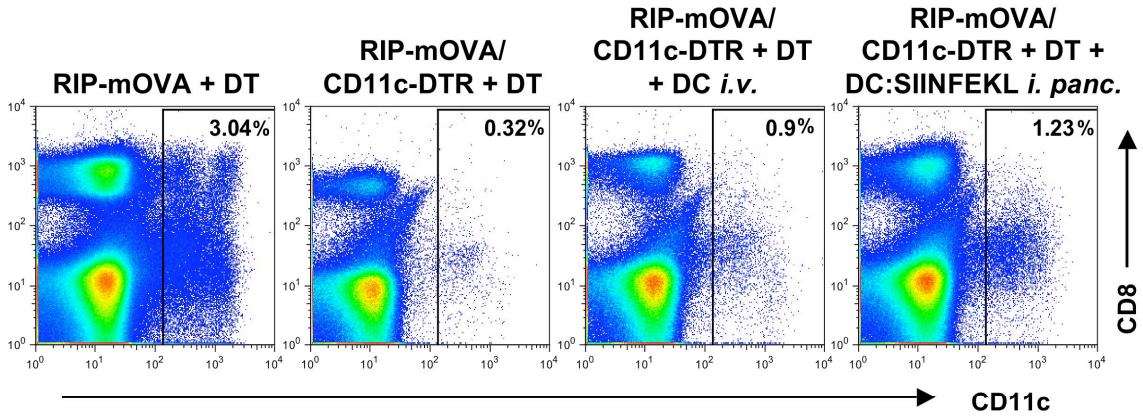

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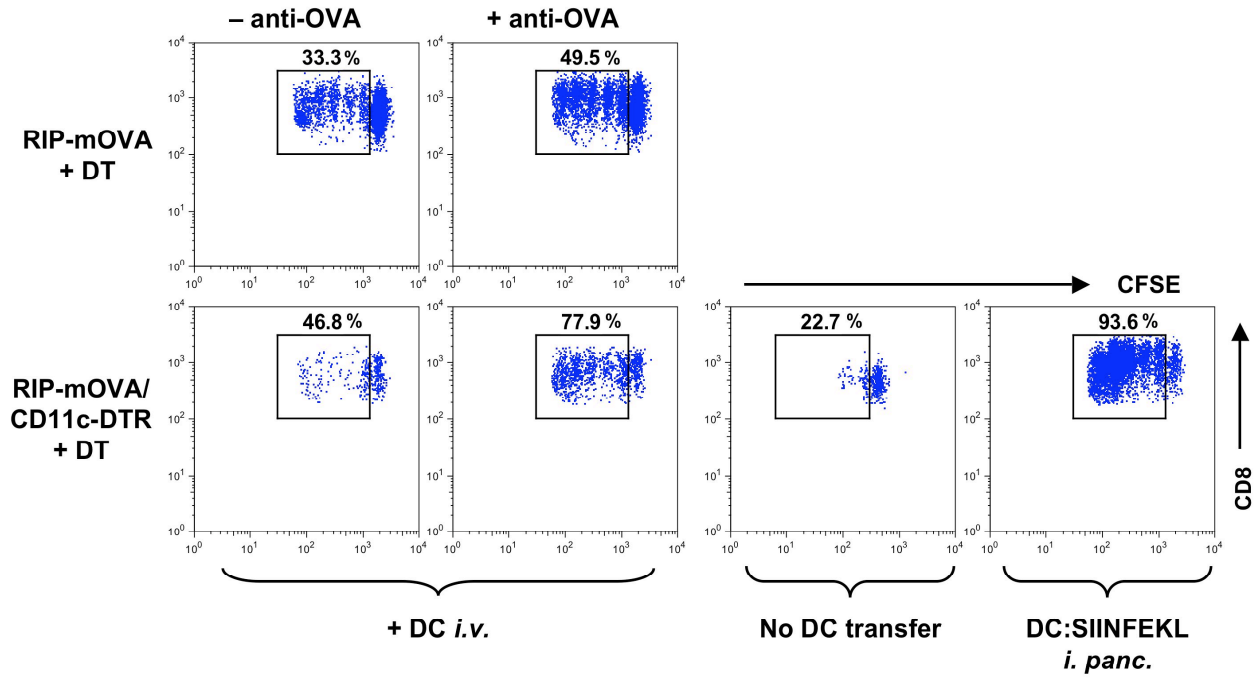

SUPPLEMENTAL FIGURE 1. OT-I cells from RAG1$^{-/-}$ mice but not conventional mice cause diabetes when transferred alone into RIP-mOVA recipients.
RIP-mOVA mice were injected with 5x10^6 CD8$^+$ cells isolated from OT-I TCR transgenic mice from either RAG1$^{-/-}$ (Taconic) or conventional C57Bl/6 backgrounds. Fractions below bars indicate the number of diabetic mice over the total number treated.

SUPPLEMENTAL FIGURE 2. Comparison of the OVA-binding ability of the rabbit and murine anti-OVA IgG preparations. The antibody preparations were compared in their ability to bind plate-bound OVA by ELISA. (A) Polyclonal murine anti-OVA IgG binds OVA 44 times worse than polyclonal rabbit anti-OVA IgG. The activity of the two preparations was compared at their respective 50% saturation points. (B) Murine polyclonal anti-OVA IgG has a similar OVA-binding capacity to the murine monoclonal anti-OVA IgG1 and IgG2b antibodies.
For ELISA, MaxiSorp Immuno plates (Nunc) were coated with 5μg/ml OVA in PBS and blocked in 0.5% porcine skin gelatin (Sigma, G-1890). Antibody preparations were serially diluted in 0.25% porcine skin gelatin and detected with anti-mouse IgG-HRP (Sigma, A-3682) and anti-rabbit IgG-HRP (BD, 554021), followed by OptEIA substrates A and B (BD) and quenching with 2N H_2SO4.

SUPPLEMENTAL FIGURE 3. ZVAD enhances accumulation of OT-I cells in the pancreatic lymph node five days post treatment. RIP-mOVA mice were treated with ZVAD 18 hours prior to and 24 and 72 hours after the transfer of OT-I cells and anti-OVA IgG. OT-I cell accumulation in the draining pancreatic lymph node was assessed by Vα2$^+$Vβ5$^+$ staining on CD8$^+$-gated FACS plots. Despite inhibition of early proliferation in the presence of ZVAD (Figure 4), by day 5 inhibition of T cell expansion was no longer apparent with normal or increased numbers of OT-I cells found in the pancreatic lymph nodes of ZVAD-treated mice.

SUPPLEMENTAL FIGURE 4. Absence of CD11c$^+$ cells does not affect OT-I cell localization to the spleen and lymph nodes.
CD11c-DTR transgenic mice were treated with DT 12 hours prior to transfer of 10^7 CFSE-labeled CD45.1$^+$ OT-I cells. 24 hours later, transferred cells were identified in the spleen and cervical lymph nodes by (A) flow cytometry, and (B) immunofluorescence staining of frozen sections using anti-B220-PE (RA3-6B2). FACS plots are gated on live cells. Comparable numbers of OT-I cells were present in the spleen and lymph nodes and were found localized in the T cell areas in both DT-treated and untreated animals.

SUPPLEMENTAL FIGURE 5. Transfer of dendritic cells into DT-treated RIP-mOVA/CD11c-DTR mice allows reconstitution of antibody-enhanced proliferative responses.
(A) DC reconstitution: 5x10^6 dendritic cells isolated from the spleens of WT mice by CD11c$^+$ MACS bead separation were intravenously (i.v.) injected into RIP-mOVA/CD11c-DTR mice. One mouse was given an intrapancreatic (i. panc.) injection of 5x10^6 DC pulsed with SIINFEKL peptide ($OVA_{257-264}$, New England Peptide) for 2

hours at 37°C. 18 hours later RIP-mOVA and RIP-mOVA/CD11c-DTR mice were treated with 4ng/g DT. After 24 hours all mice were given CFSE-labeled OT-I cells in the presence or absence of anti-OVA IgG. Mice were sacrificed 2 days later for analysis. FACS plots of pancreatic LN cells are shown, gated on live cells and stained with $CD8^+$ and $CD11c^+$. Intravenous DC transfer was able to only partially reconstitute $CD11c^+$ cells in the pancreatic lymph node (30% of that in wt RIP-mOVA mice), while intrapancreatic injection was slightly more efficient (40% of wt RIP-mOVAmice). (B) OT-I cell proliferative responses: Pancreatic lymph node dot plots are gated on $CD8^+CFSE^+$ cells. % divided amongst total $CD8^+CFSE^+$ cells are provided. Antibody-enhanced OT-I cell proliferative responses were partially restored in DT-treated RIP-mOVA/CD11c-DTR mice by intravenous reconstitution with DC (77.9% divided) as compared to reconstituted mice that received OT-I cells alone (46.8%) and those that received no DC (22.7%). After intrapancreatic injection of SIINFEKL-pulsed DC, OT-I proliferative responss were robust (93.6% divided), demonstrating the viability and potency of the transferred DC population.