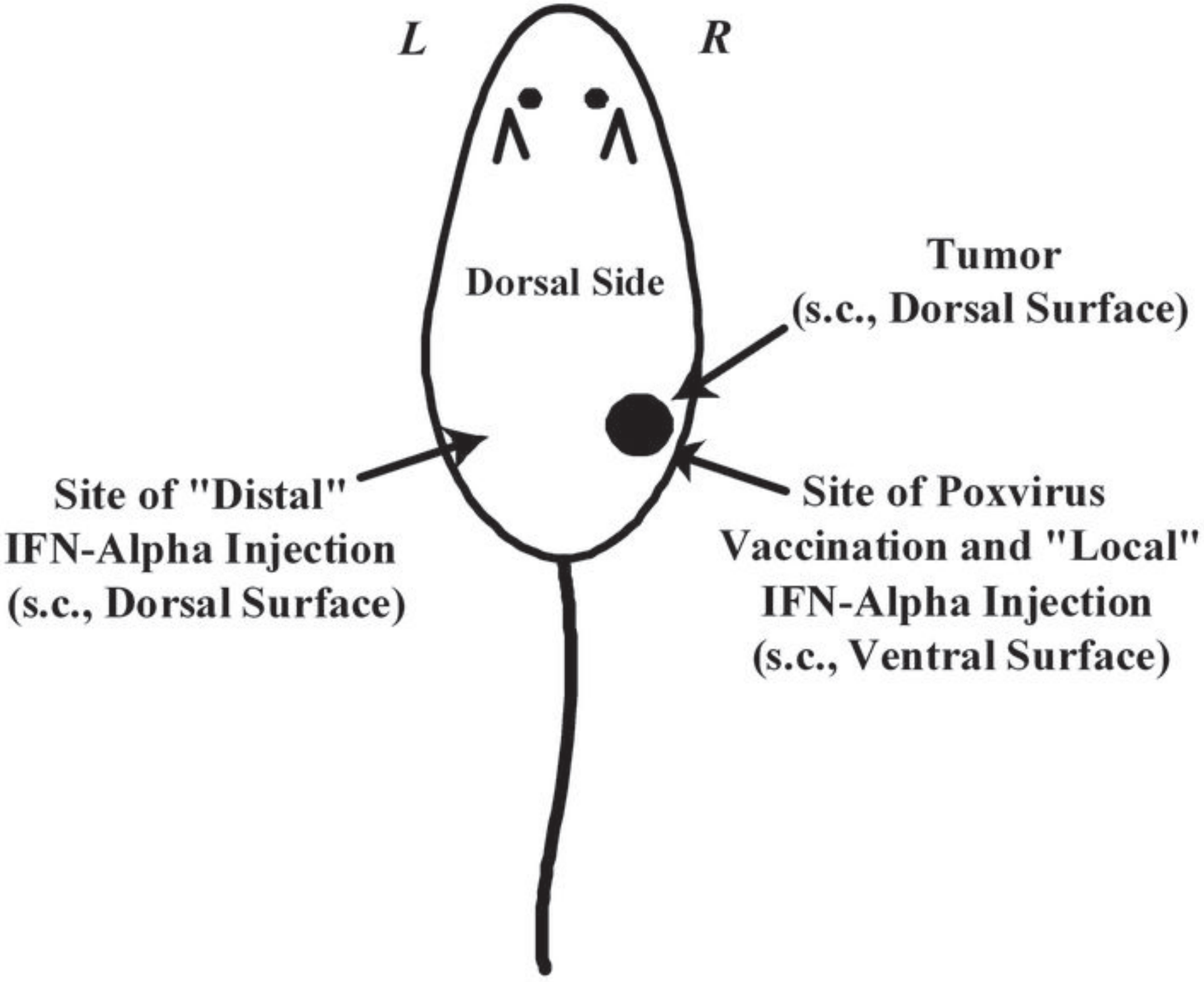
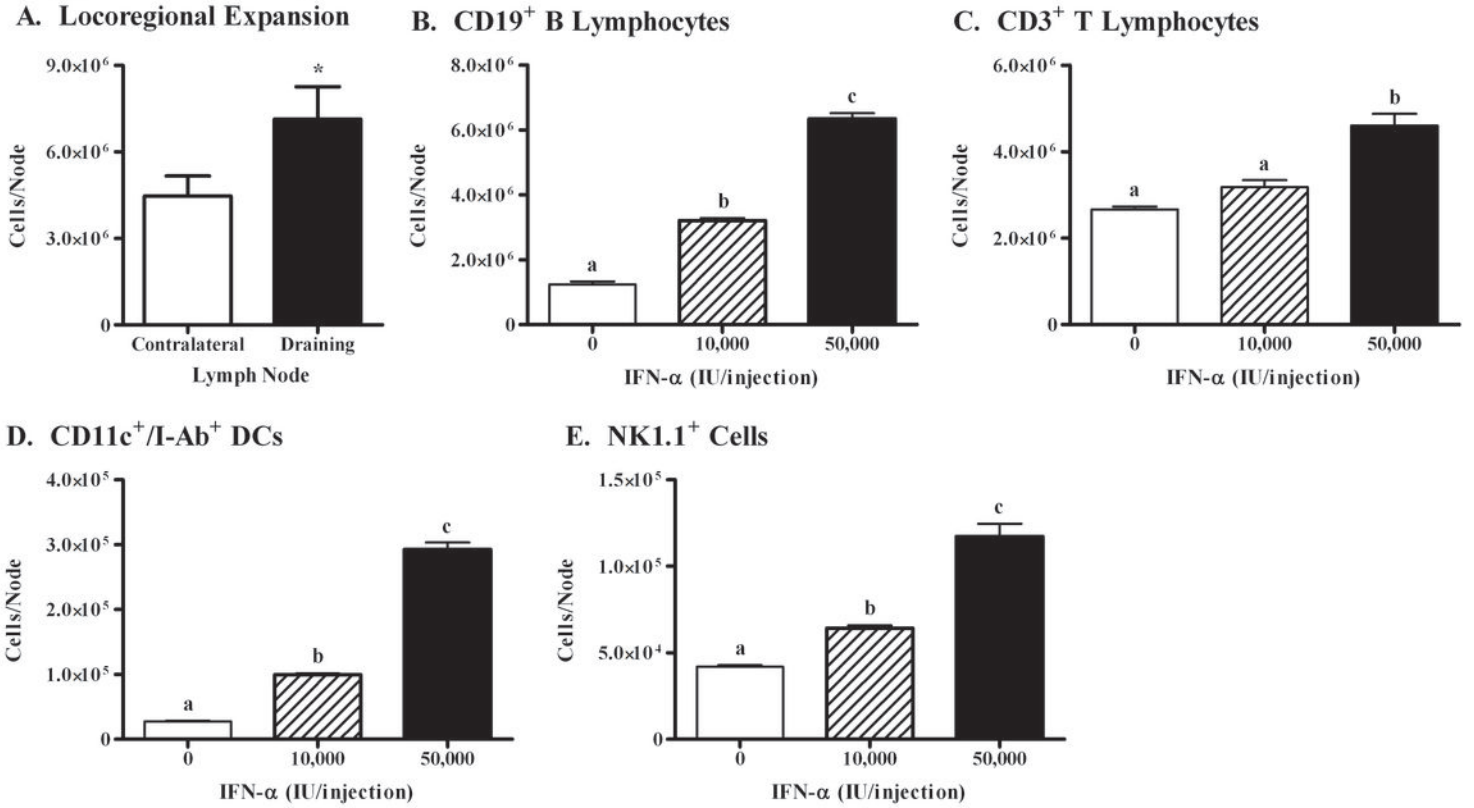
Supplemental Figure 1

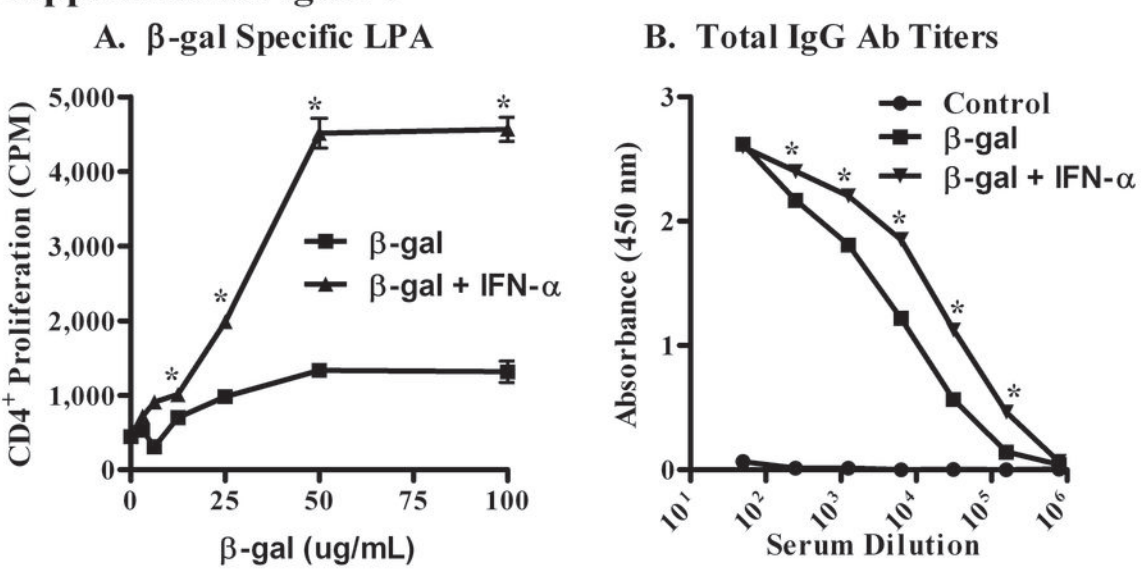
IFN-Alpha and Vaccine Administration in Immune and Antitumor Studies



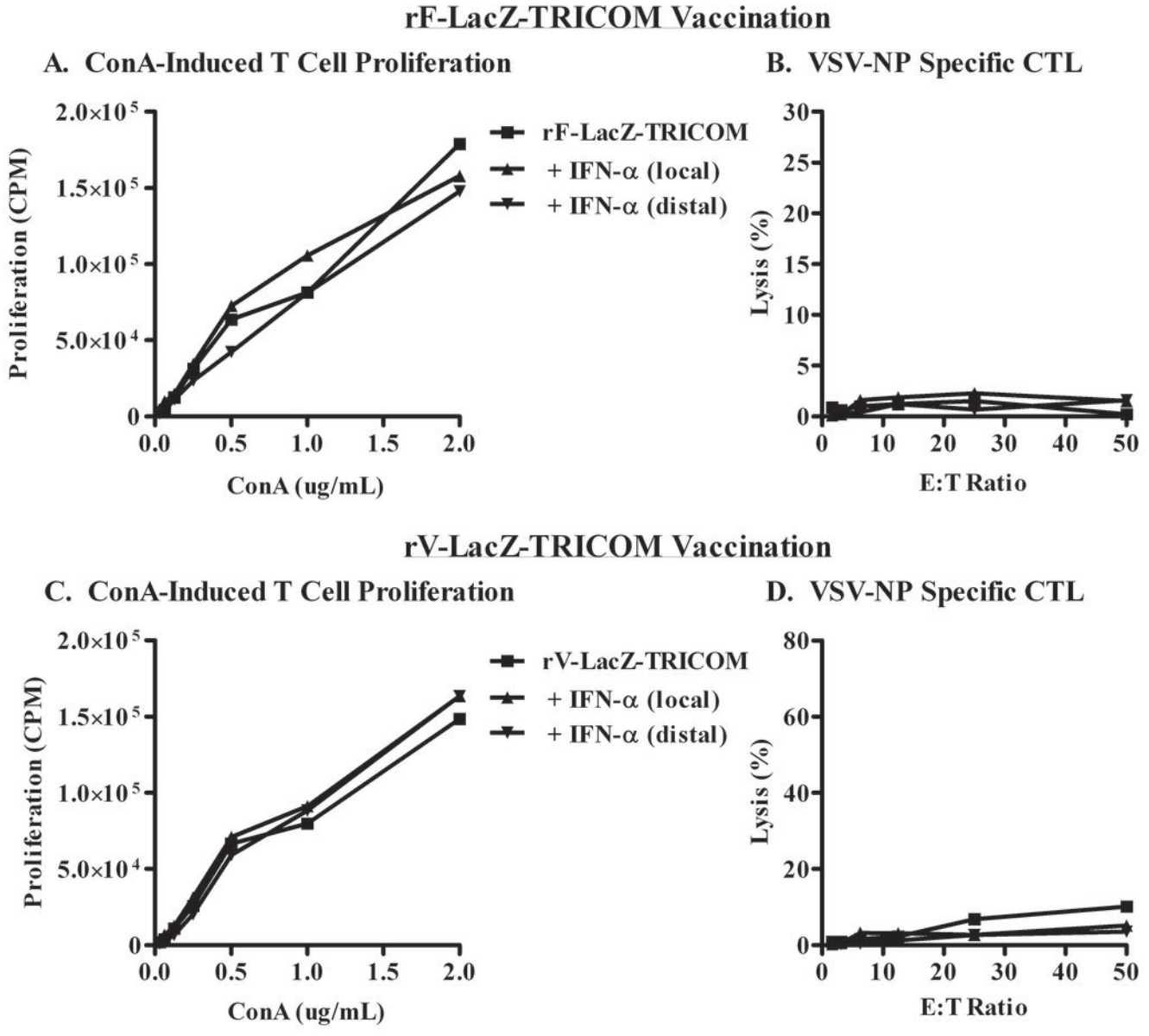
Supplemental Figure 2



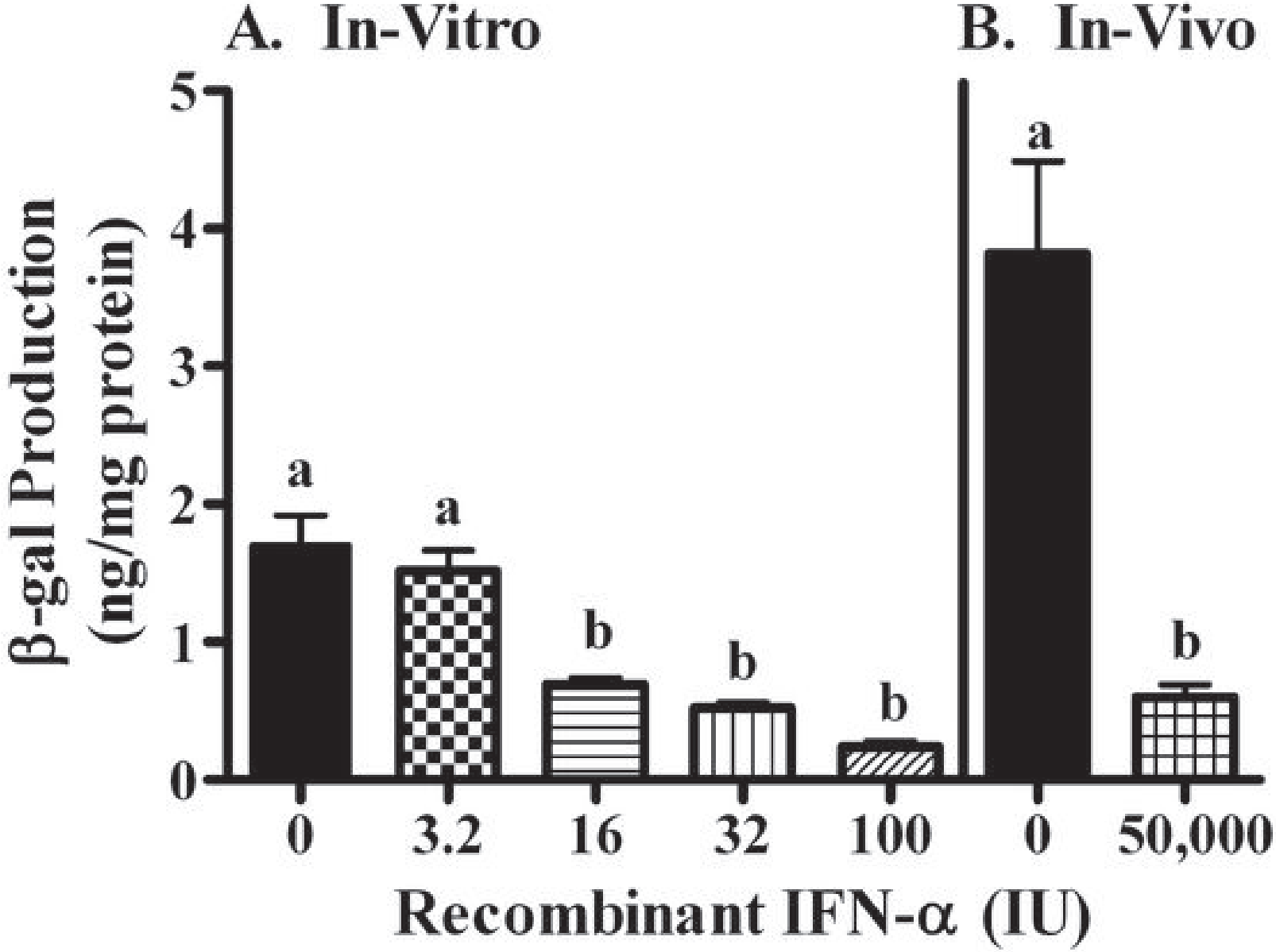
Supplemental Figure 3



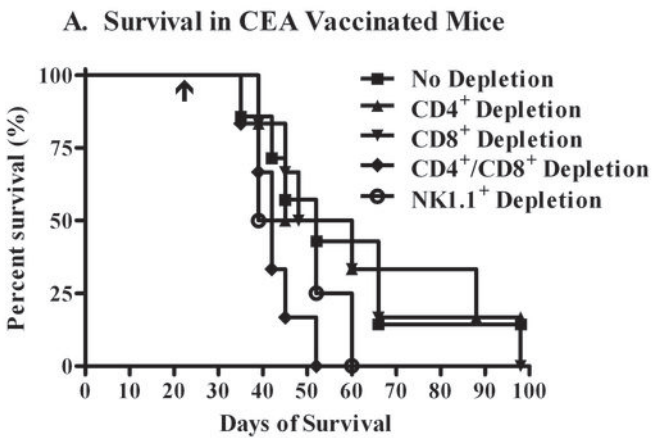
Supplemental Figure 4

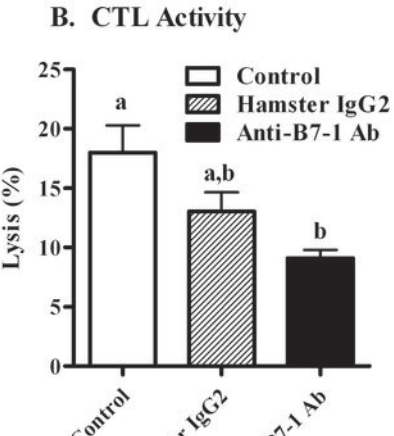


Supplemental Figure 5

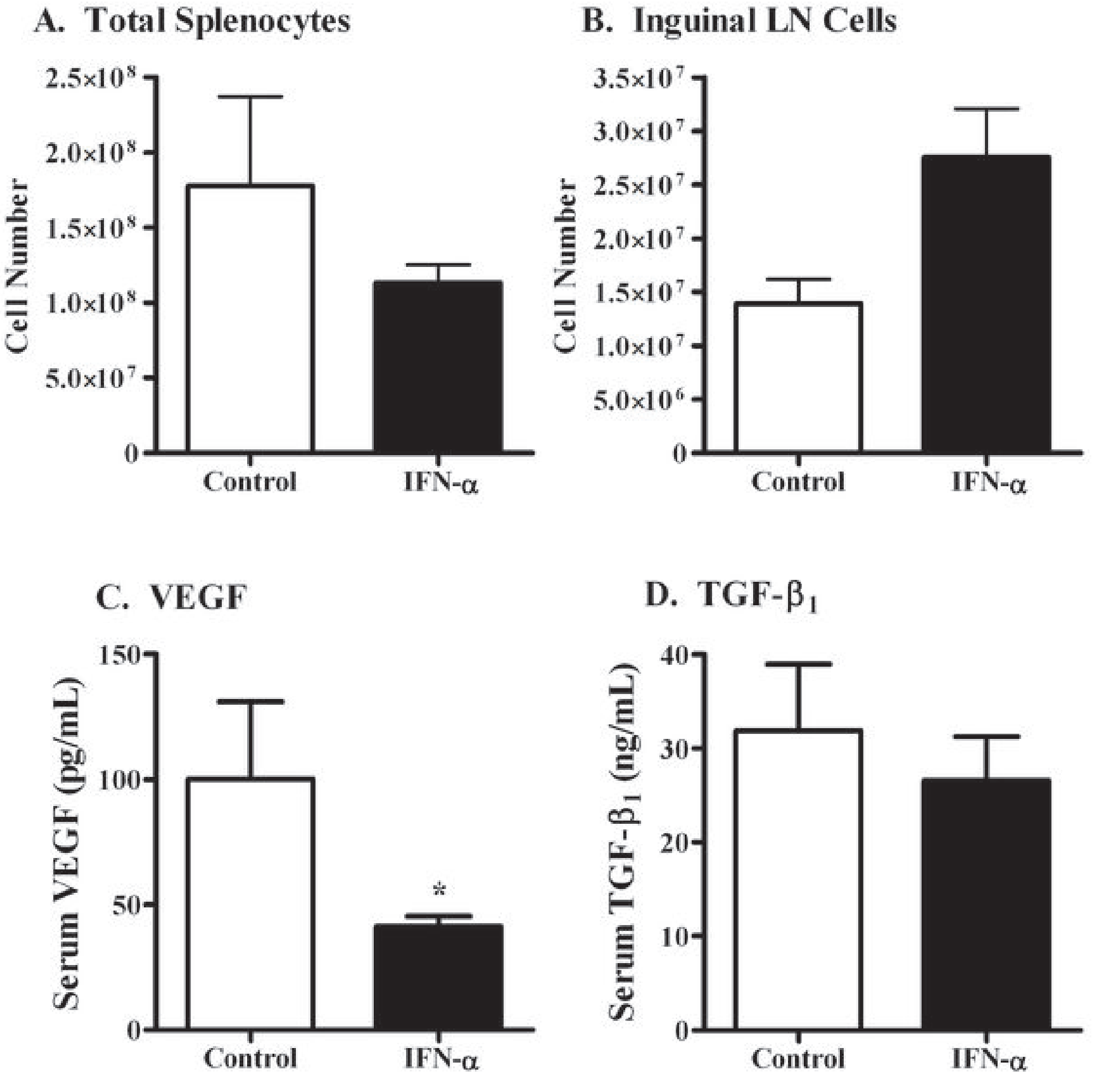


Supplemental Figure 6





Supplemental Figure 7



Supplemental Figure Legends

Supplemental Figure 1: Schema depicting the administration of IFN-α and vaccine in immune and antitumor studies. In all immune and antitumor studies in which IFN-α was combined with the poxvirus vaccines, locally administered IFN-α was given at the site of vaccination (s.c., right, ventral surface) while distally administered IFN-α was given on the left side of the lower back (s.c., left, dorsal surface). A 1-week course of IFN-α (50,000 IU/injections, 3x/wk, MWF) was initiated on the same day as vaccination with either vaccinia or fowlpox (Monday). Recombinant vaccinia (rV) and recombinant fowlpox (rF) vectors expressing the transgenes for CEA, B7-1, ICAM-1, and LFA-3 (designated rV- or rF-CEA-TRICOM) were administered on the right inner thigh (s.c., ventral surface, 10^8 pfu) admixed with recombinant fowlpox virus expressing murine GM-CSF (rF-GM-CSF, 10^7 pfu). In antitumor studies, CEA.Tg mice were challenged on the right side of the lower back (s.c., right, dorsal surface) with either $3x10^5$ MC38.CEA or $1x10^6$ Panc02.CEA tumor cells.

Supplemental Figure 2: Effect of IFN-α administration on LN phenotype, antigen presentation, and cytotoxicity. Twenty-four hours following the last of three injections of IFN-α (0 IU, 10,000 IU, or 50,000 IU), phenotypic changes in the cells of the inguinal lymph nodes were evaluated by flow cytometry (N=3 mice/group). In Figure 2A, the highest dose IFN-α (50,000 IU x 3 injections) was shown to induce a 60% increase in the cellularity of the draining vs. contralateral inguinal lymph nodes (P=0.03). IFN-α administered at 0 IU (open bars), 10,000 IU (hatched bars), and 50,000 IU (solid bars) dose-dependently increased the average number of $CD19^+$ B lymphocytes (Figure 2B), $CD3^+$ T lymphocytes (Figure 2C), $CD11c^+$/I-Ab^+ DCs (Figure 2D), and $NK1.1^+$ cells (Figure 2E) per lymph node.

Supplemental Figure 3: IFN-α as an adjuvant with protein based vaccination. To evaluate the efficacy of IFN-α as an adjuvant to protein-based vaccination, we measured immune responses in mice vaccinated with β-gal protein (■, 100 μg) or β-gal protein and IFN-α (▲, 50,000 IU/mouse, 3x/wk) at the site of vaccination (s.c., right lower back). In these experiments, mice were vaccinated with β-gal protein on Monday and IFN-α was administered at the site of vaccination on Monday, Wednesday, and Friday. In Figure 3A, co-administration of β-gal and IFN-α increased $CD4^+$ proliferative responses by approximately 3-fold (1,500 vs. 4,500 cpm/$2x10^5$ $CD4^+$ cells, $P<0.0001$). In Figure 3B, total IgG serum antibody titers to β-gal protein were also increased 3- to 4-fold in mice vaccinated with β-gal and IFN-α compared to those vaccinated with β-gal alone ($P<0.001$). The β-gal specific antibody titers of unvaccinated control mice are shown in Figure 3B (●). Analysis of variance was used to assess mean differences between treatment groups. The asterisks indicate statistically significant differences between mice receiving β-gal protein alone versus those receiving β-gal protein and IFN-α. Statistical significance was accepted at $P<0.05$.

Supplemental Figure 4: Control samples for lymphoproliferation and CTL activity in mice receiving IFN-α in combination with either recombinant vaccinia or fowlpox vaccinations. The route of IFN-α administration and its effect on poxvirus vaccine responses were evaluated in mice receiving vaccine alone (■) or vaccine in combination with IFN-α given either locally (▲, right ventral surface) or distally (▼, left dorsal surface) to the site of vaccination. The administration of IFN-α (50,000 IU/mouse, 3x/wk), whether local or distal to the site of vaccination, did not significantly alter ConA-induced $CD4^+$ T cell proliferation in mice vaccinated with either rF-LacZ-TRICOM (Figure 4A) or rV-LacZ-TRICOM (Figure 4C). In Figure 4B and 4D, negligible CTL activity was noted in all treatment groups against MC38 tumor cells pulsed with the VSV-NP_{52-59} (RGYVYQGL) control peptide. The immune data presented here were generated from the pooled splenocytes of 3 mice per group and the experiments were conducted three times with consistent findings.

Supplemental Figure 5: IFN-α-mediated inhibition of replication and/or infectivity by the vaccinia virus in vitro and in vivo. Titrating doses of recombinant IFN-α (0 – 100 IU) were shown to dose-dependently inhibit the production of β-gal protein in MC38 cells infected with 0.01 MOI of rV-LacZ (Figure 5A, in vitro). The co-administration of rV-LacZ (10^8 pfu, i.m.) and IFN-α (50,000 IU, 3x/wk, i.m.) in the quadriceps muscle of C57BL/6 mice resulted in significant inhibition of β-gal protein production at the site of vaccination (Figure 5B, in vivo). In the in vivo experiments, mice were injected with rV-LacZ on Monday and IFN-α was administered at the same site on Monday, Wednesday, and Friday. Analysis of variance was used to assess mean differences in β-gal production in each group. Statistical significance was accepted at $P<0.05$ and are indicated by the superscript letters above each bar.

Supplemental Figure 6: The antitumor efficacy of rV/F-CEA-TRICOM vaccination and the role of CD80 in mediating CTL lysis of murine pancreatic adenocarcinoma cells. Antibody-based immune cell depletion studies were carried out in tumor-bearing mice to deplete either $CD4^+$ (rat antibody GK1.5), $CD8^+$ (rat antibody 2.43), or $NK1.1^+$ (PK136) cells prior to receiving rV/F-CEA-TRICOM vaccination. Mice were challenged with Panc02.CEA tumors ($1x10^6$ cells/mouse) on day 0. Antibodies were injected intraperitoneally on days 24-27, 34, and 41. Mice received a primary vaccination with rV-CEA-TRICOM and rF-GM-CSF on day 28 and a booster vaccination with rF-CEA-TRICOM and rF-GM-CSF on day 42 post tumor challenge. The mice were randomized into one of five treatment groups (N=10 mice/group): 1) control (■, no immune cell depletion), 2) $CD4^+$ T cell depletion (▲), 3) $CD8^+$ T cell depletion (▼), 4) $CD4^+$ and $CD8^+$ T cell depletion (♦), and 5) $NK1.1^+$ cell depletion (○). Tumor growth and survival were monitored for up to 100 days following tumor challenge and the initiation of treatment is indicated by the arrow. In Figure 6A, the median survival of mice receiving the CEA vaccine was 52 days post tumor challenge while the median survival in vaccinated mice depleted of $CD4^+$/$CD8^+$ T cells or $NK1.1^+$ cells was 42 (P=0.07) and 45.5 (P=0.27) days, respectively. In Figure 6B, in vitro experiments were conducted to assess the role of CD80 (B7-1) in mediating antigen specific recognition and lysis of Panc02.CEA tumor cells by $CEA_{526-533}$-specific $CD8^+$ T cells. Antibody blockade of the CD80 antigen (hamster antibody 16-10A1; 10 ug/mL) significantly inhibited Panc02.CEA tumor cell lysis by a $CEA_{526-533}$-specific $CD8^+$ T cell line in a 4-hour ^{51}Cr-release assay. Statistically significant differences, determined by analysis of variance, are indicated by letters above each bar ($P<0.05$).

Supplemental Figure 7: The antitumor activity of IFN-α in mice with established murine pancreatic adenocarcinomas. Mice with established Panc02.CEA tumors (N=6 mice/group) were treated for two weeks with either vehicle (open bars) or IFN-α (50,000 IU/mouse, 3x/wk, solid bars). The mice were sacrificed 24 hours after the last injection of IFN-α. The spleens, tumor-draining inguinal lymph nodes, tumors, and sera were isolated from each mouse. Single cell suspensions of each tissue were prepared and 6-color flow cytometry was used for the phenotypic analysis. Non-significant trends were noted in which IFN-α therapy delayed the development of splenomegaly (Figure 7A) and enriched the draining inguinal lymph nodes (Figure 7B) of tumor-bearing mice. Tumor-bearing mice treated with IFN-α had significantly reduced serum VEGF concentrations (Figure 7C, $P=0.03$) while serum TGF-β_1 levels were unchanged (Figure 7D). Asterisks indicate statistically significant differences from the control group ($P<0.05$).