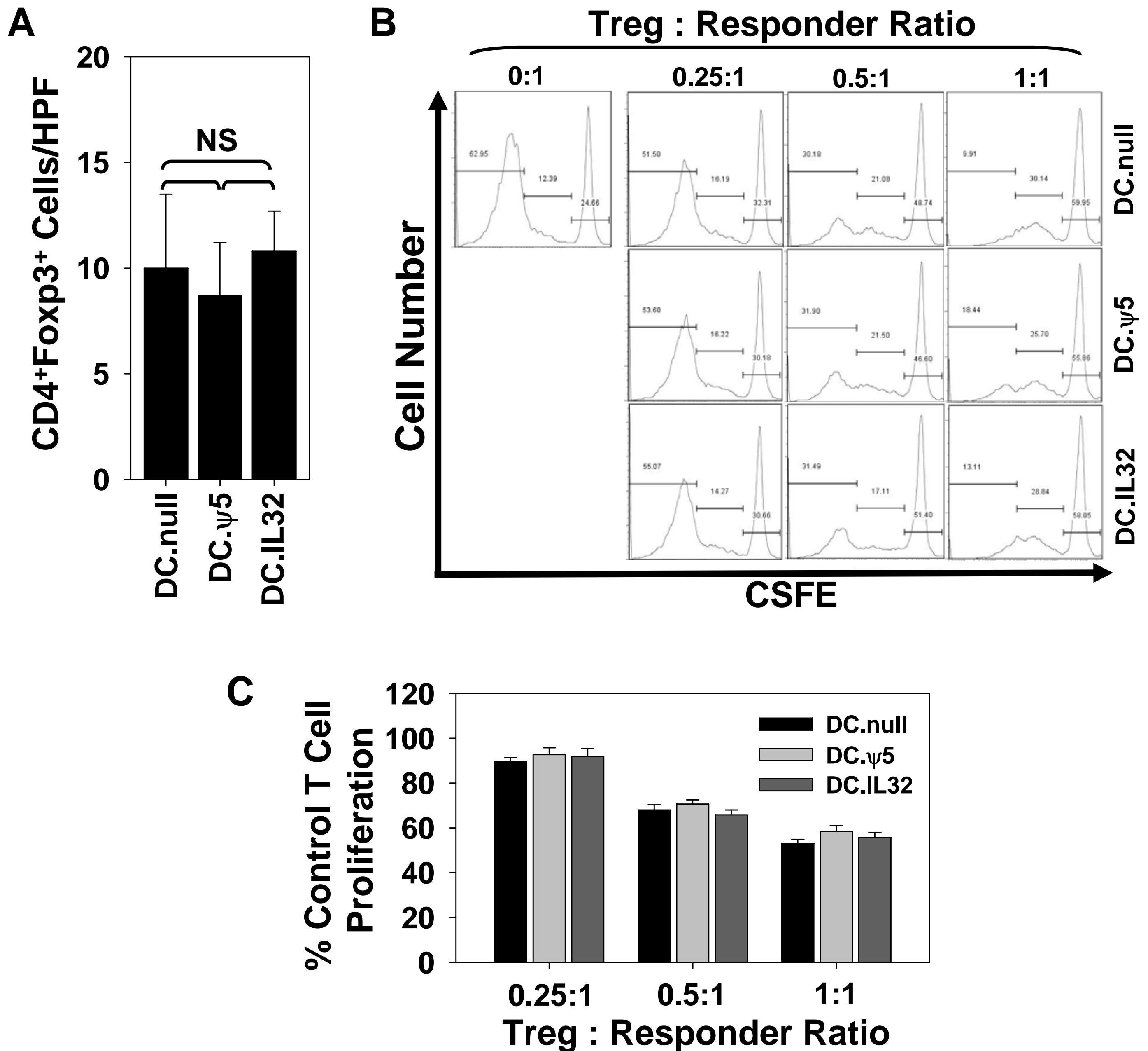

Fig. S4. Intratumoral delivery of DC.IL32β does not significantly alter Treg numbers in the TME or their suppressor function in vitro. **A**.Tumor sections isolated from mice treated as outlined in **Fig. 5A** were stained for co-expression of CD4 and Foxp3 as described in Materials and Methods. The number of double-positive cells was then enumerated for 10 high-power fields (40X), with data reported as the mean $\pm$ SD number of cell/field. NS = not significant. In **B** and **C**, MLR were established using MACS $CD4^{+}CD25^{neg}$ BALB/c splenocytes (pre-labeled with CFSE) and C57BL/6 DC.null stimulator cells as described in Materials and Methods. As indicated, flow-sorted $CD4^{+}CD25^{+}$ Treg isolated from day 21 tumors harvested from mice treated (per **Fig. 5**) with i.t. DC.IL32 or control DC were added at the indicated Treg-to-responder $CD4^{+}$ T cell ratios. After a 72h incubation period, $CD4^{+}$ T cell proliferation was analyzed based on CFSE dilution as monitored by flow cytometry as described in Materials and Methods (panel **B**), with the percentage of control (no Treg added) $CD4^{+}$ T cell proliferation reported in panel **C**. All inter-group differences were not significant. Data are representative of those obtained in 3 independent experiments.