

# Selective targeting of Toll-like receptors and OX40 inhibit regulatory T cell function in follicular lymphoma

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## SUPPLEMENTARY FIGURE LEGENDS

**Supplementary Figure 1. Peripheral blood Tregs from FL patients suppress the function of antitumor T cells.** A, B) CFSE-labeled or unlabeled intratumoral CD4<sup>+</sup>CD25<sup>-</sup> effector T cells were stimulated with anti-CD3 and anti-CD28 antibodies or autologous CD3-depleted FL tumor-derived antigen-presenting cells in the presence or absence of autologous peripheral blood Tregs obtained at initial diagnosis (A) or after induction of clinical remission (B). Proliferation was assessed by CFSE dilution or <sup>3</sup>H thymidine incorporation assay. Percentages of CFSE-labeled proliferating cells are shown in the upper left quadrants.

**Supplementary Figure 2. Intratumoral Tregs from FL patients inhibit cytokine production by tumor-specific T cell clone.** CD4<sup>+</sup> tumor-specific T cell clone derived from FL tumor-infiltrating lymphocytes was incubated with autologous tumor cells for 6 days in the presence or absence of autologous intratumoral Tregs at the indicated ratios. Production of cytokines in supernatants was measured by multiplex cytokine assay. The percent inhibition of cytokine production in the presence of Tregs is shown in each panel. *P* values were calculated by paired *t*-test.

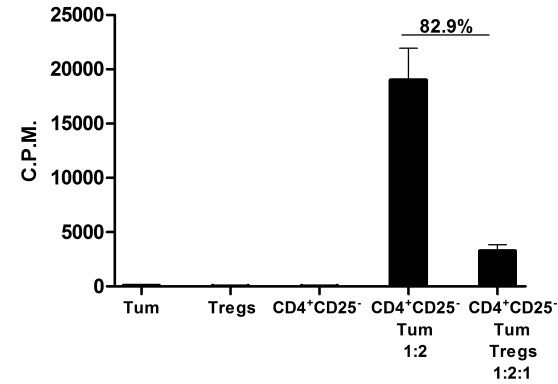
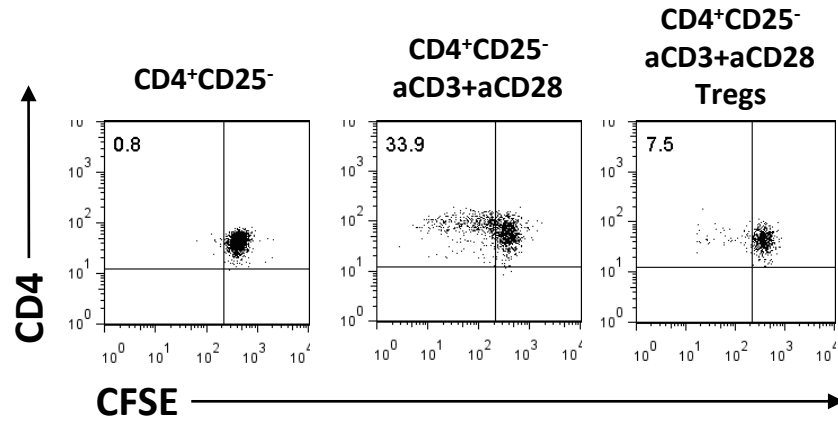
**Supplementary Figure 3. Pam<sub>3</sub>CSK4, Flagellin (FLA-ST ultrapure grade), and CpGs do not induce IL-10 from human antigen-presenting cells.** CD3-depleted FL tumor-derived antigen-presenting cells (FL-APC) (A) and healthy donor PBMCs (B) myeloid DCs (mDCs) (C) and plasmacytoid DCs (pDCs) (D) were cultured in triplicate with the indicated concentrations of TLRs. IL-10 and IFN- $\alpha$  levels were measured in the culture supernatants after 24 hours of incubation.

**Supplementary Table 1. Patient characteristics at diagnosis (n = 72)**

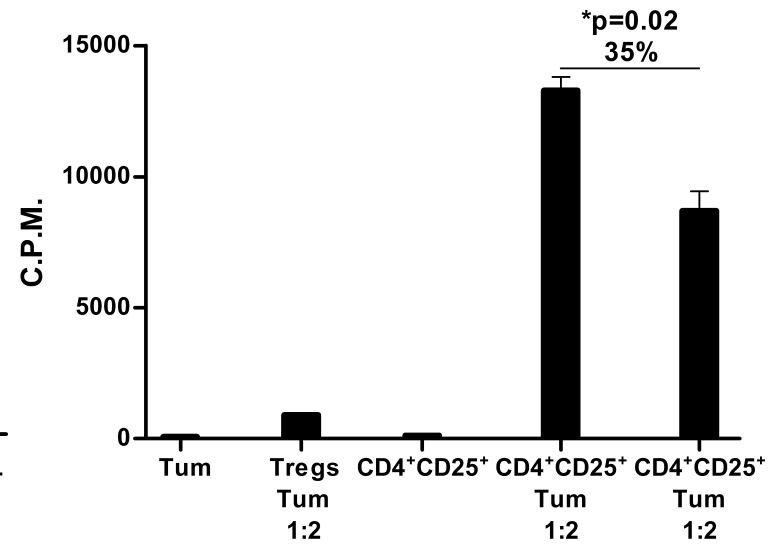
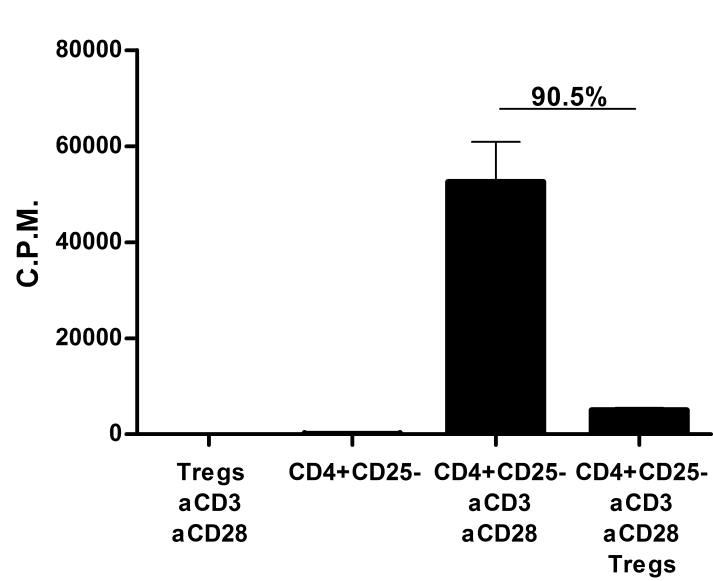
<b>Characteristic</b>	<b>No. (%)</b>
Age	
Median	49
Range	20-71
Sex	
Males	47 (65)
Females	25 (35)
Histology	
FL, Grade 1	31 (43)
FL, Grade 2	41 (57)
Stage	
Stage II	2 (3)
Stage III	15 (21)
Stage IV	55 (76)
FLIPI	
Low	36 (50)
Intermediate	18 (25)
High	14 (19)
Not available	4 (6)
CR/CRu after PACE chemotherapy	51 (71)

# Voo and Foglietta Suppl. Fig. 1.

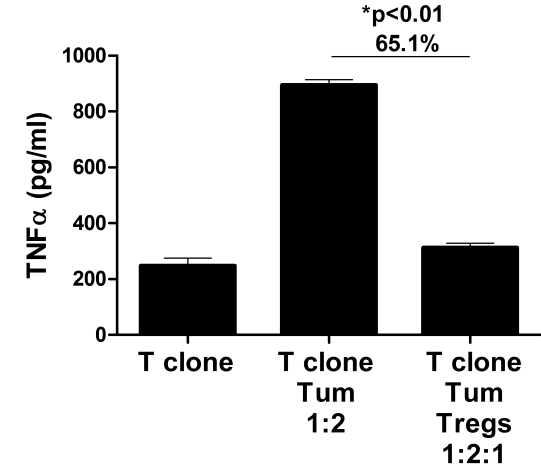
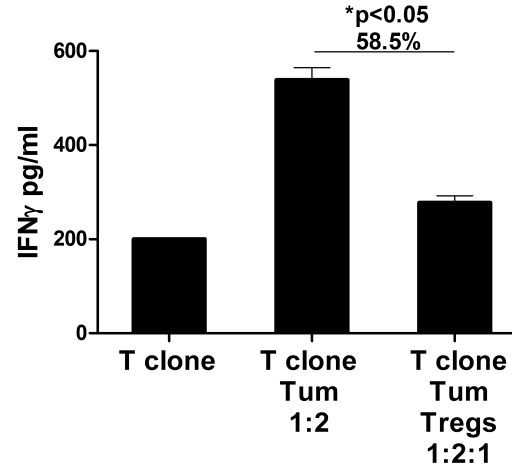
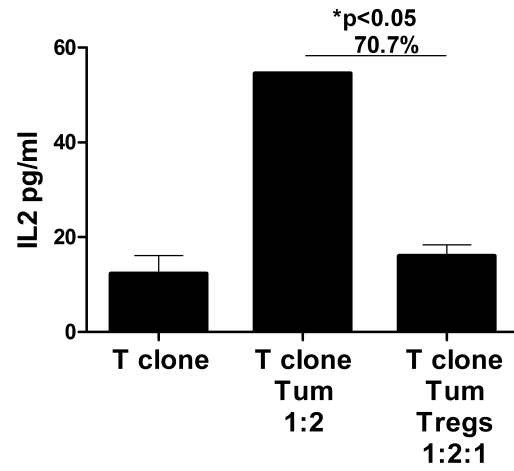
**A**



**B**



## Voo and Foglietta Suppl. Fig. 2



Voo and Foglietta Suppl. Fig. 3.

