Supplementary Data

Figure legends

Supplementary Figure 1. IL-12 serum levels and frequency of subsets in FL patients. (A) IL-12 serum levels measured by multiplex ELISA (Luminex) in FL patients before (median: 0.50 ng/ml, n=30) or after (median: 0.36 ng/ml, n=30) treatment with rituximab. (B) Frequency of subsets of CD4, CD8, CD19, and CD11c cells in biopsy specimens from FL patients measured by flow cytometry. Frequency of subsets was expressed as a percentage of total mononuclear cells in biopsy specimens.

Supplementary Figure 2. Biological functions of IL-12 in FL. (A) Representative histograms showing proliferation of intratumoral $CD4^+$ or $CD8^+$ T cells treated with or without IL-12. Proliferation was measured by CFSE staining and expressed as the number of $CFSE^{dim}$ cells. (B) Representative dot plots showing cytokine production by intratumoral $CD4^+$ T cells. T cells were cultured in an anti-CD3 Ab-coated plate with addition of anti-CD28 Ab in the presence or absence of IL-12. On day 3, cells were restimulated with PMA/Ion for 5 hrs and IFN-γ, IL-17, or IL-2 expression in $CD4^+$ T cells was measured by intracellular staining. (C, D) Histograms from representative patient tumor specimens (C, n=5) and peripheral blood (D, n=2) showing expression of IL-12 receptor β1 or β2 (shaded) over isotype IgG control (line) on resting (upper panel) and TCR-activated (lower panel) $CD4^+$ T cells. (E) Graphs from a representative sample showing the viability of T cells treated with IL-12/IL-2 or IL-2 alone at indicated time points. Cells were cultured in anti-CD3 Ab-coated plate and cell viability was measured by annexin V (AnV) and Propidium Iodide (PI) staining. Dead cells were defined as AnV^+PI^+. Apoptotic cells were AnV^+PI^-. Viable cells were AnV^-PI^-. (F) Dot plots from a representative sample showing forward and side scatters of T cells treated with IL-12/IL-2 or IL-2 alone at indicated time points. The gated cells were analyzed for their ability to produce cytokines in Figure 1E.

Supplementary Figure 3. Co-expression of TIM-3 and T-bet, RORγt, GATA-3 or Foxp3 on cell subsets in FL. Expression of T-bet, ROR-γt, GATA-3 or Foxp3 in $TIM\text{-}3^+$ or $TIM\text{-}3^-$ cells from different

cell subsets from 3 FL patients and 1 representative sample of 3 normal individuals. Freshly-isolated mononuclear cells were fixed, permeabilized and stained for T-bet, ROR-γt, GATA-3 or Foxp3 plus TIM-3, CD3, CD4 and CD8. Plots shown are gated on $CD3^+$ cells.

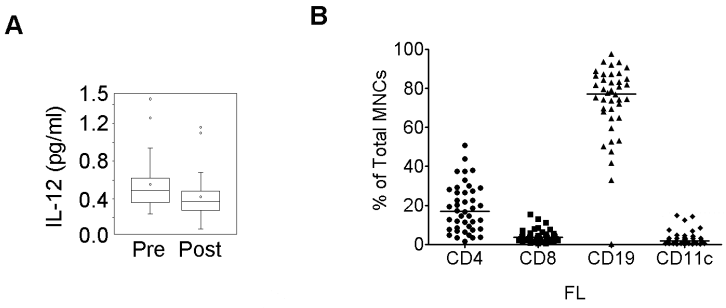
Supplementary Figure 4. Effect of IL-12 on TIM-3 expression on T cells in FL. (A) A summary showing TIM-3 expression on $CD4^+$ T cells treated with or without cytokines IL-1β, IL-6, IL-4, TGF-β, IL-23, IL-12p35, IL-12p40 or IL-12p70. TIM-3 expression on $CD4^+$ T cells was measured by flow cytometry and expressed as fold induction over untreated group (n=3). (B) A summary showing TIM-3 expression on $CD4^+$ T cells treated with or without IL-12, IFN-γ or a neutralizing antibody against IL-12, IFN-γ or isotype IgG control. TIM-3 expression on $CD4^+$ T cells was measured by flow cytometry and expressed as fold induction over the untreated group (n=3).

Supplementary Figure 5. Effect of TCR activation on TIM-3 expression on T cells. (A, B) Representative dot plots showing TIM-3 expression on $CD4^+$ (A) or $CD8^+$ (B) T cells (n=3). $CD3^+$ T cells were cultured in plates coated with a series of doses of anti-CD3 Ab (OKT3) in the presence or absence of IL-12 and TIM-3 expression was determined by flow cytometry.

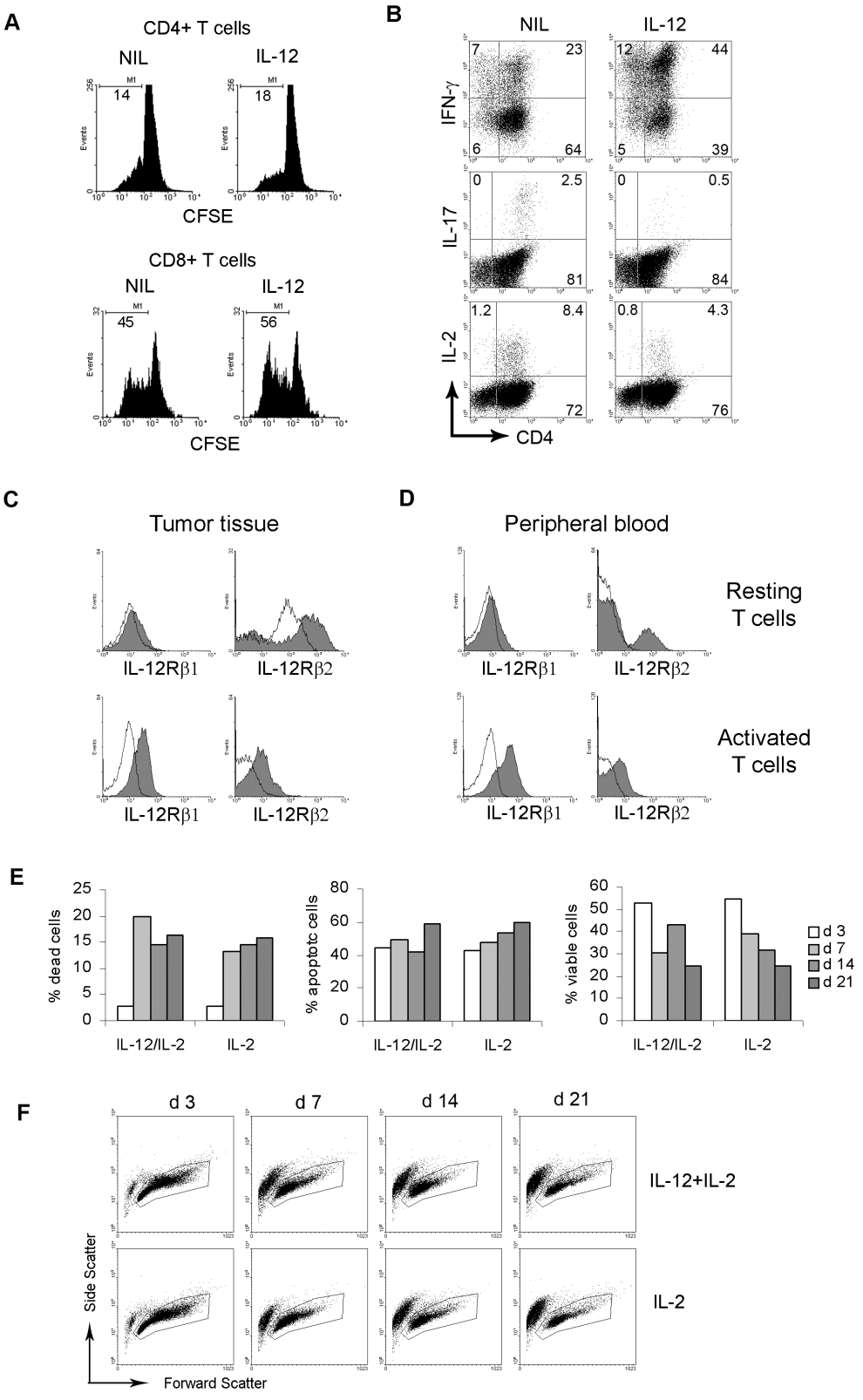
Supplementary Figure 6. Effect of STAT4 inhibition and IFN-γ on TIM-3 expression on T cells. (A) Representative dot plots showing TIM-3 expression on $CD4^+$ T cells treated with either IL-12 or Lisofylline alone or in combination (n=2). (B) Effect of IFN-γ on TIM-3 expression in $CD4^+$ (upper panel) or $CD8^+$ (lower panel) T cells. T cells were cultured in OKT3 (0.2μg/ml)-coated plates with anti-CD28 antibody in the presence or absence of a series of doses of IFN-γ for 3 days. TIM-3 expression was measured by flow cytometry (n=3).

Supplementary Figure 7. TIM-3 frequency and correlation of $PD\text{-}1^+$ cells with survival in FL patients. (A, B, C) Frequency of $CD3^+$, $CD4^+$, or $CD8^+$, TIM-3^+, $CD4^+$TIM-3^+, or $CD8^+$TIM-3^+, $CD25^+$ T cells in a cohort of samples from FL patients. The numbers of subsets were measured by flow cytometry and expressed as a percentage of total mononuclear cells in biopsy specimens.

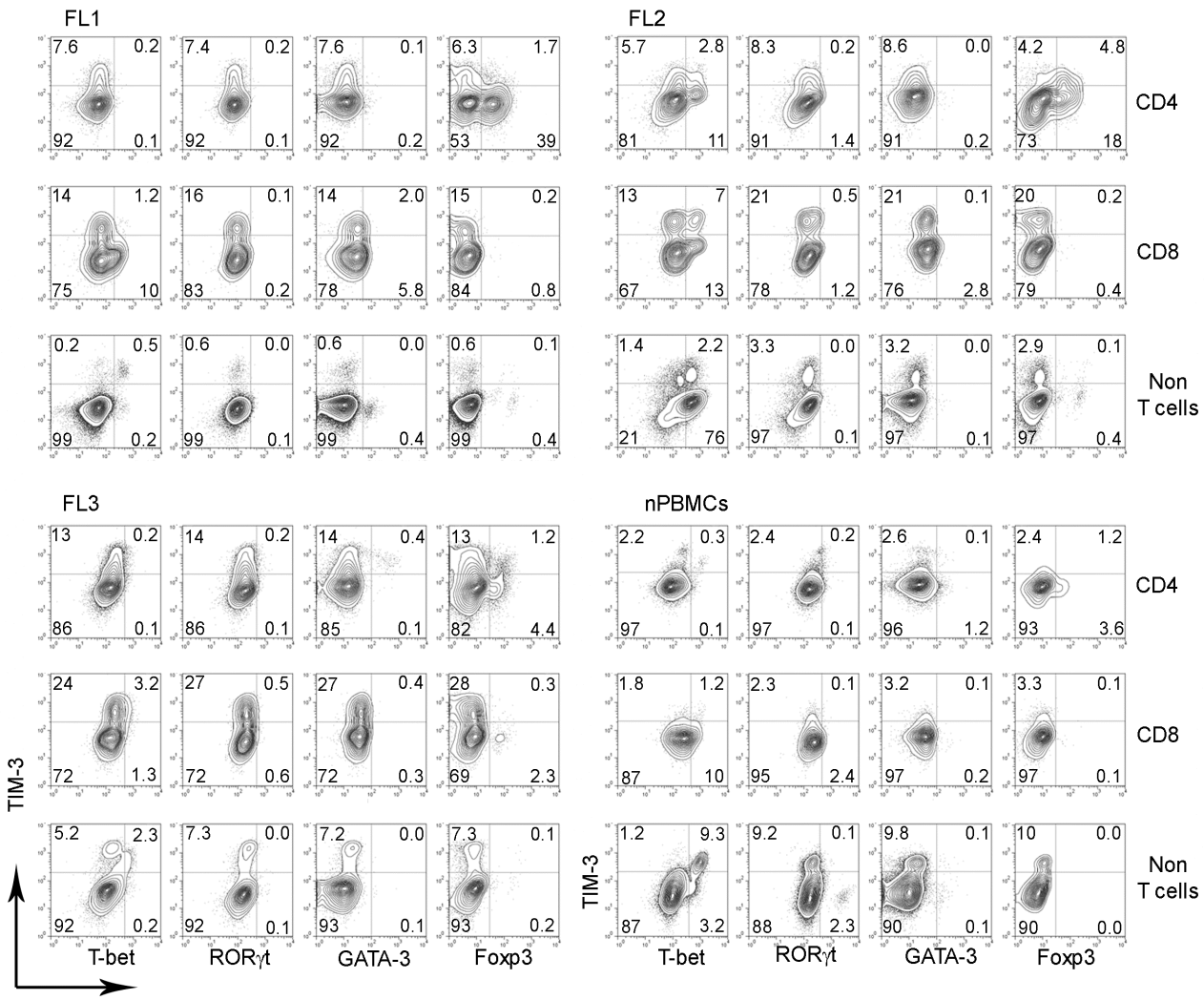
Supplementary Figure 1



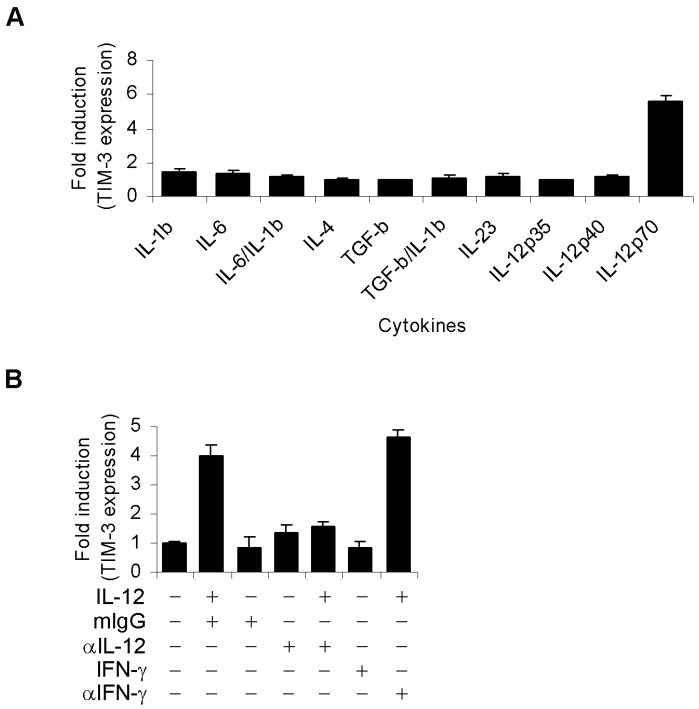
Supplementary Figure 2



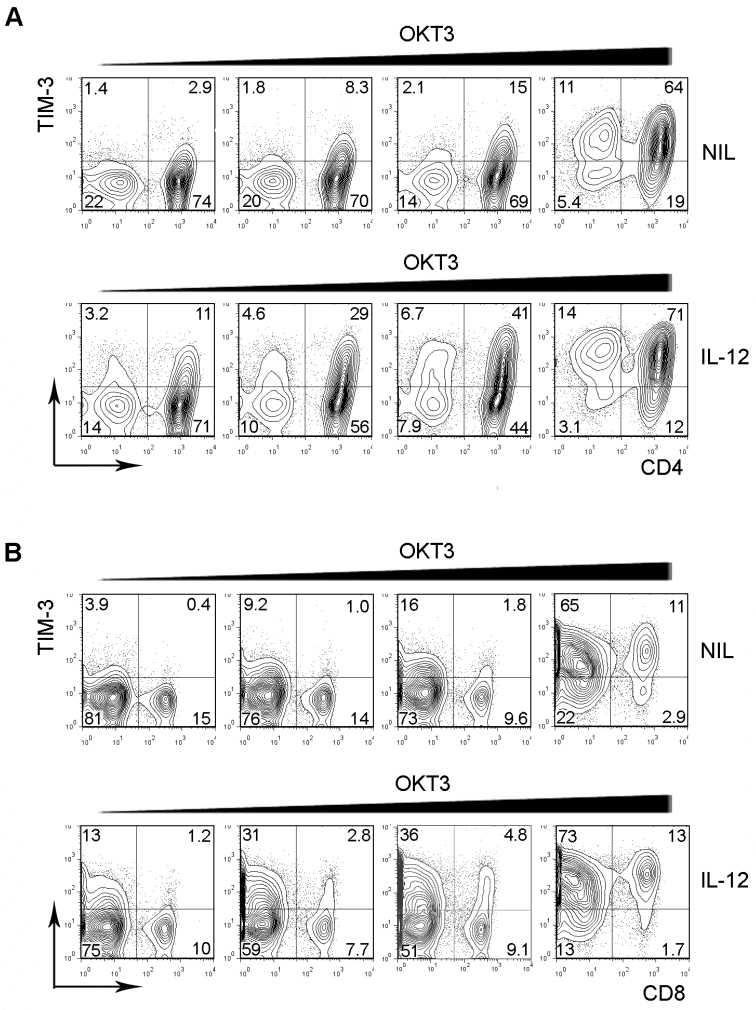
Supplementary Figure 3



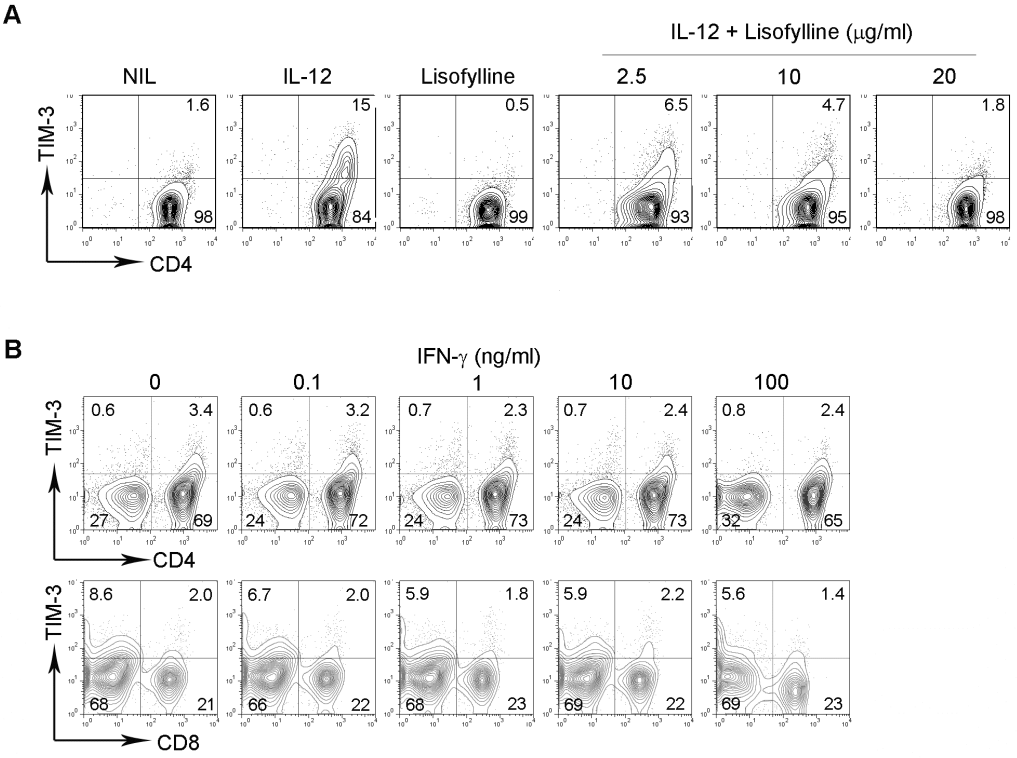
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7