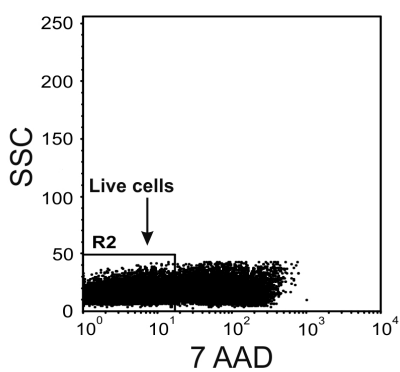
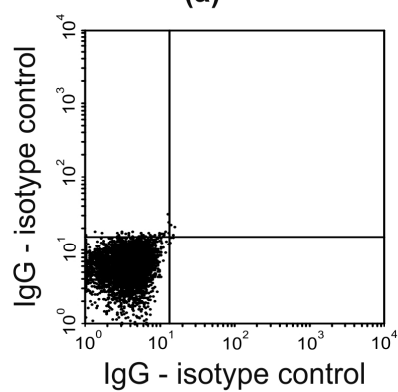


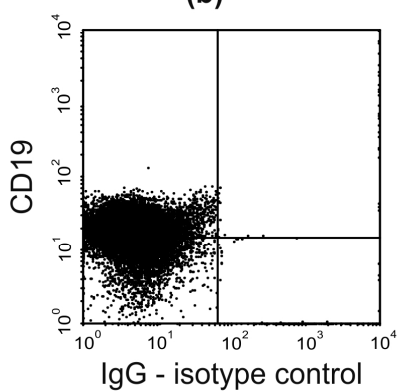
(a)



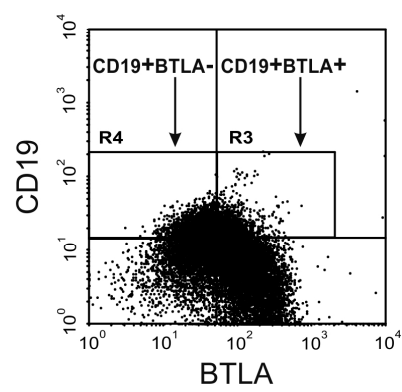
(b)



(c)

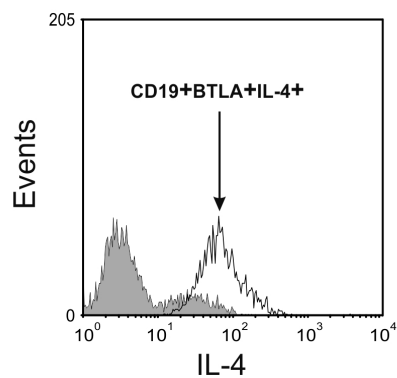


(d)

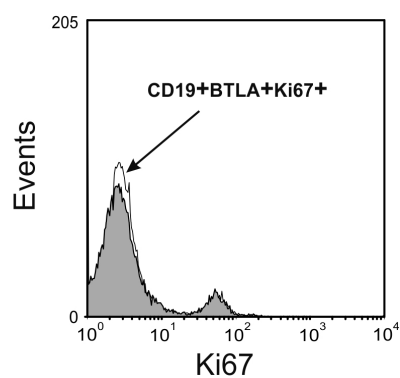


(e)

Gate R3

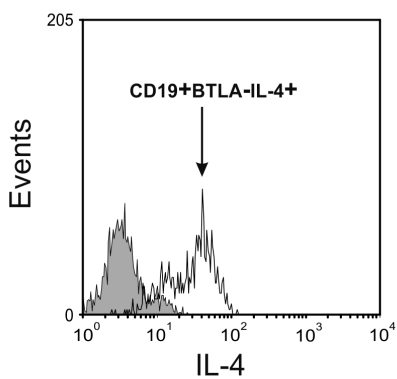


(f)

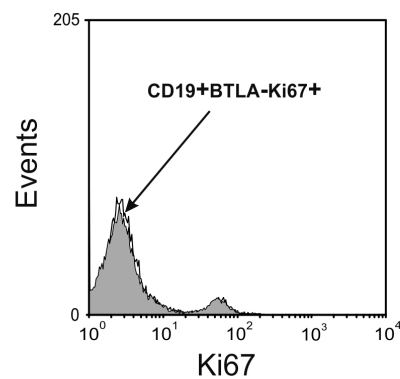


(g)

Gate R4



(i)



(j)

Figure S1. Gating strategy for the evaluation of proportion of BTLA positive IL-4 or Ki67 expressing cells and BTLA negative IL-4 or Ki67 expressing cells in B cell compartment in CLL patients and healthy controls. Representative plots from a CLL patient demonstrating the analytic method for the identification of BTLA-positive and BTLA-negative B cells expressing IL-4 and ki67. (a) PBMCs were gated (R1) based on their FSC/SSC properties. (b) Gated PBMCs were then analyzed on SSC/7AAD profile for live and dead cells' discrimination; hence live cells (7-AAD-negative) were gated as region R2. (c-e) Final dot plots illustrate identification of BTLA-positive and BTLA-negative B cells. Gated live PBMCs were analyzed for CD19 and BTLA staining (dot plots: (c) cells stained with isotype control IgG for CD19; (d) cells stained with CD19 MoAb and isotype control IgG for BTLA; (e) double stained cells (for CD19 and BTLA); CD19+BTLA+ cells were gated as R3, while CD19+BTLA- cells were analyzed as R4). (f-i) Final histograms show IL-4 and ki67 fluorescence in the proportion of BTLA-positive (R3) and BTLA-negative (R4) B cells. Data were analyzed using Cell Quest software (BD Bioscience). FSC, forward scatter; SSC, side scatter; PBMC, peripheral blood mononuclear cell; MoAb, monoclonal antibody; BTLA, B and T lymphocyte attenuator; 7-AAD, (7-amino-actinomycin D) - Viability Staining Solution.