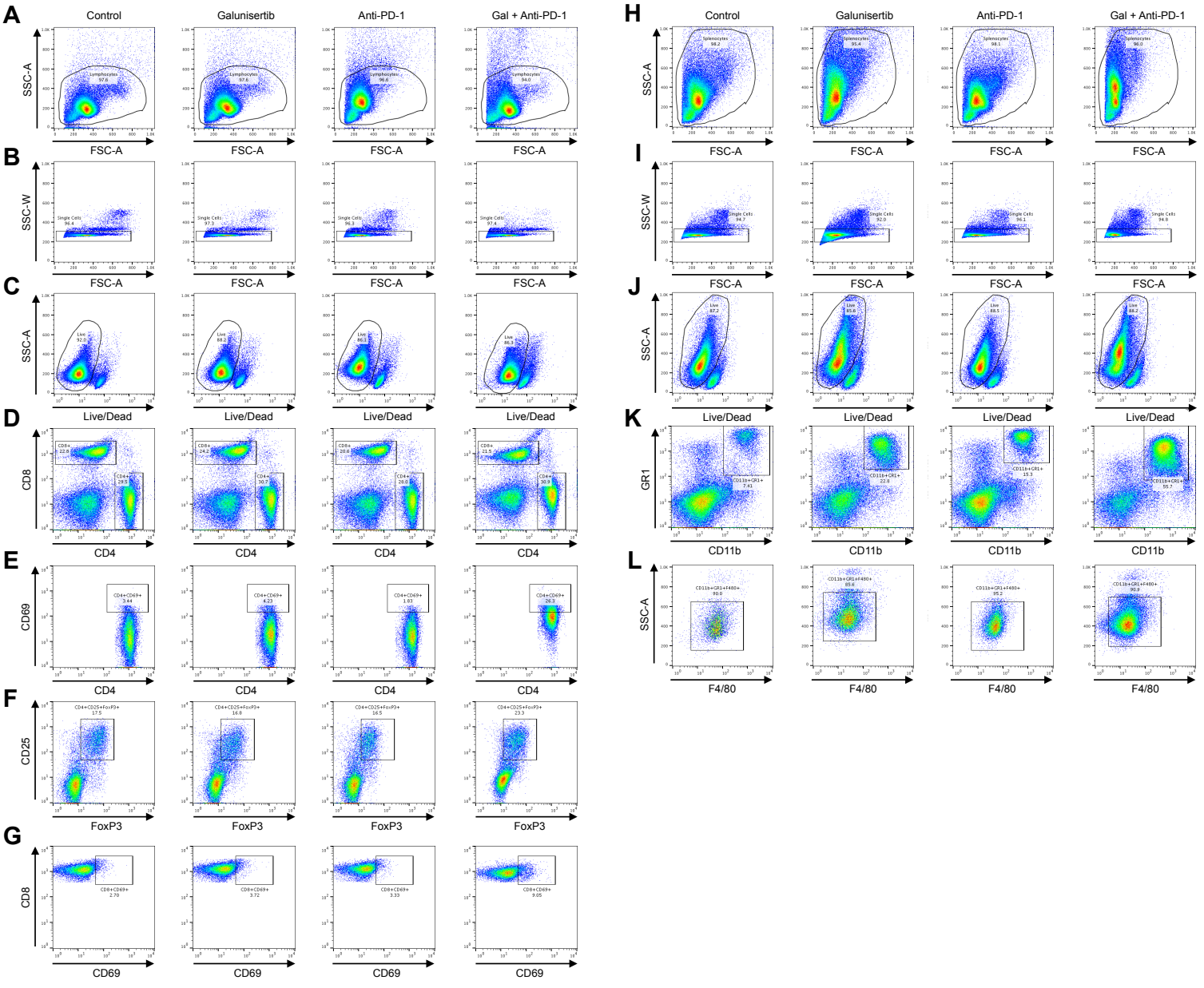

Figure S8. Galunisertib and anti-PD-1 dual treatment leads to systemic alterations in immune function

Mesenteric lymph nodes were collected and evaluated by flow cytometry for lymphocyte activation. Cells were first gated by **(A)** forward scatter versus side scatter to eliminate debris, then **(B)** doublets eliminated by SSC-W gating. **(C)** Non-viable cells were identified and excluded using Live/Dead assay. **(D)** Helper and cytotoxic T cells were identified by staining for CD4 and CD8 respectively. **(E,F)** CD4 populations were evaluated for activation by CD69 expression, and regulatory T-cells by for FoxP3 and CD25. **(G)** CD8 populations were evaluated for activation by CD69 expression. Spleens were also collected and evaluated by flow cytometry. Cells were first gated by **(H)** forward scatter versus side scatter to eliminate debris, then **(I)** doublets eliminated by SSC-W gating. **(J)** Non-viable cells were identified and excluded using Live/Dead assay. **(K)** Granulocytes were identified by staining for the CD11b and GR1. Given the substantial size differences in these populations, manual gating was required **(L)** Macrophages were quantified by gating to CD11b/GR1 dual positive cells and evaluating F4/80 expression. Given the substantial size differences in these populations, manual gating was required.