

Supplemental Figure 1. **IFC gating strategy for FOXO1 nuclear translocation analysis in primary B cell subsets within human PBMCs.** To assess FOXO1 nuclear translocation in B cell subsets within a total population of PBMCs, each file was analyzed in IDEAS™ v6.2 using the nuclear translocation wizard. First, focused cells are gated based on the gradient RMS of the nuclear dye, DAPI (A). In the focused events, single cells are gated based on area and aspect ratio (B). In the focused, single cells, Live/Dead Dye+ cells are excluded (C) and then live cells are gated for CD19+ (D), CD19+CD20+ cells (E), then further gated on IgD and CD27 (F). In each B cell subset, mean similarity of FOXO1 and DAPI in the FOXO1+DAPI+ population (G) is calculated using a log-transformed Pearson's correlation coefficient (H). Cells in the gate R1 have a similarity ≥ 1 between DAPI and FOXO1, indicating nuclear FOXO1. Representative dot plots and histograms taken from PBMCs from a healthy donor cultured in complete RPMI for 30 minutes.

