



S14 Fig. Gating strategy for Env-specific memory B cells, plasmablasts and plasma cells in rectal tissue. (A) Example of flow cytometry staining for Env-specific memory B cells: Live CD2⁻CD14⁻ cells from rectal pinches were gated for CD19⁺CD20⁺ B cells and then IgD⁺ B cells were excluded. The far right-hand plot shows Env-specific memory B cells in a vaccinated macaque and a control macaque using biotinylated gp120. (B) Example of flow cytometry staining for PB and PC. Live CD2⁻CD14⁻ cells from rectal pinches were gated for CD19⁺CD20⁺ B cells and then IgD⁺ B cells were excluded. IgD⁻ B cells were further gated for IRF4⁺CD138⁻ and IRF4⁺CD138⁺. PB (upper-right quadrant highlighted by the red box) are identified as CD19⁺CD20⁺/IgD⁻IRF4⁺CD138⁻HLA-DR⁺Ki67⁺. PC (lower-left quadrant highlighted by the red box) are identified as CD19⁺CD20⁺/IgD⁻IRF4⁺CD138⁺HLA-DR⁺Ki67⁻. (C) gp120-specific memory B cells quantified by flow cytometry correlate with frequency of Env-specific memory B cells secreting IgG + IgA by ELISpot in rectal biopsies from chronically SIV infected macaques.