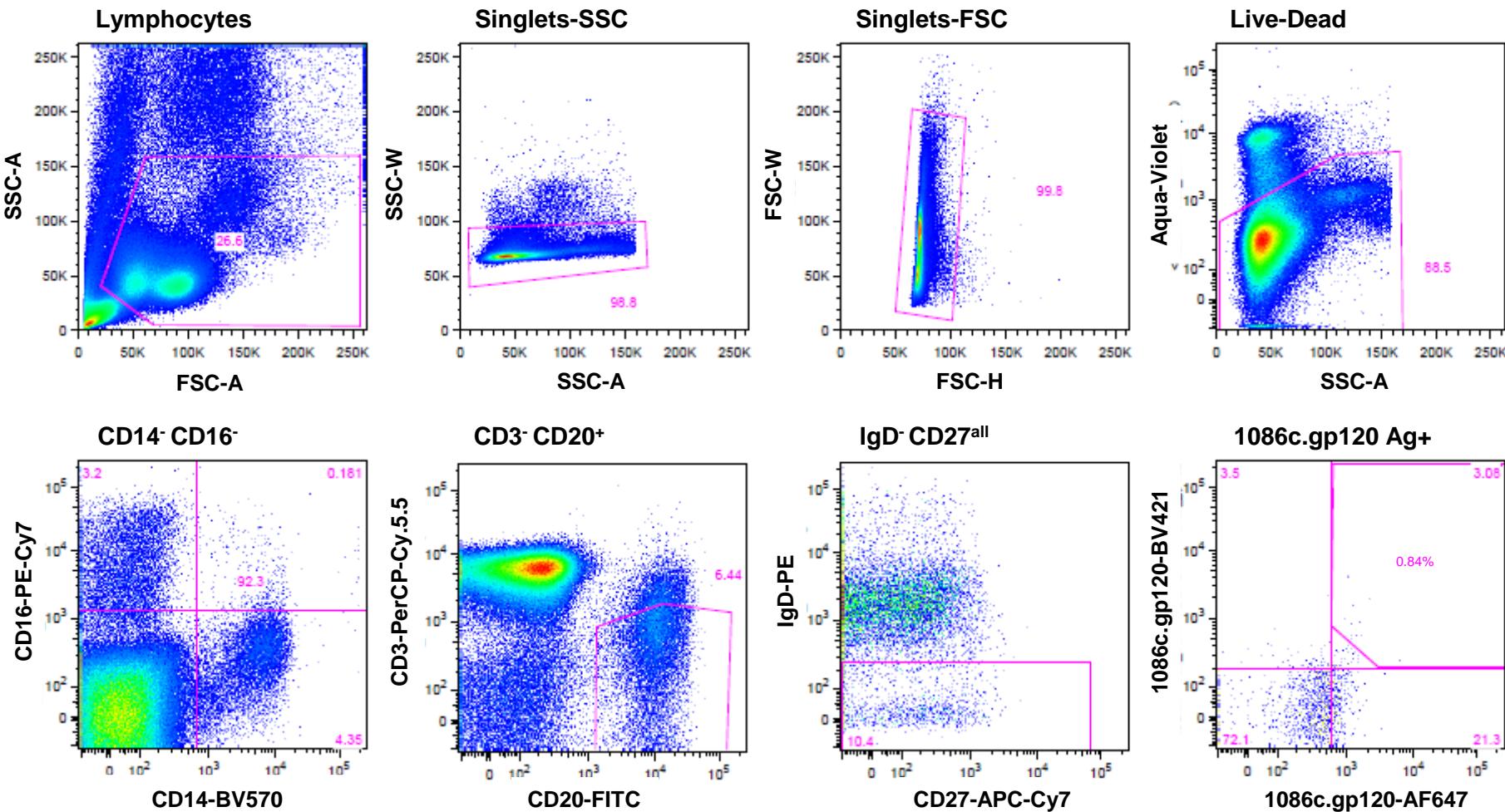
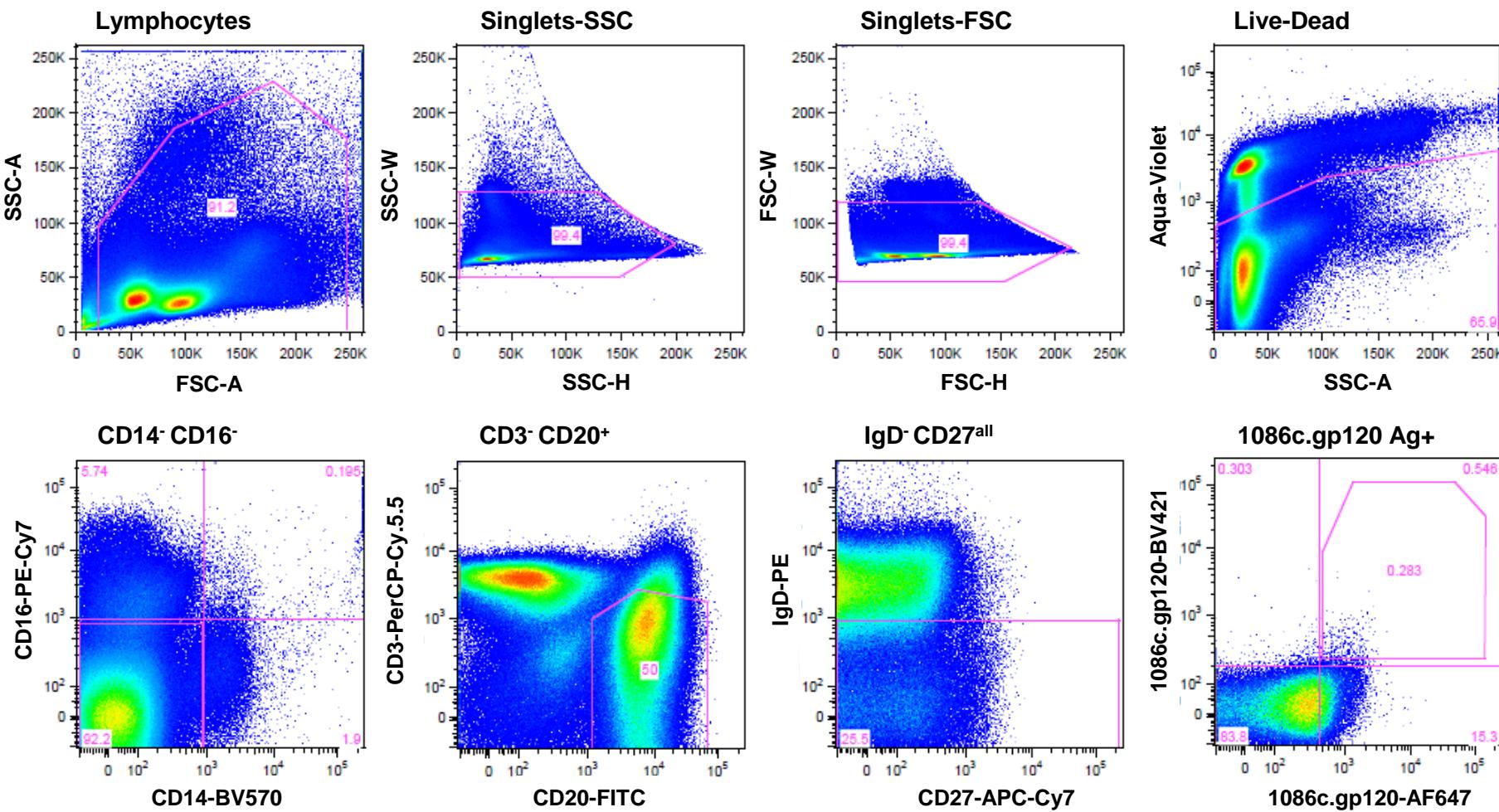


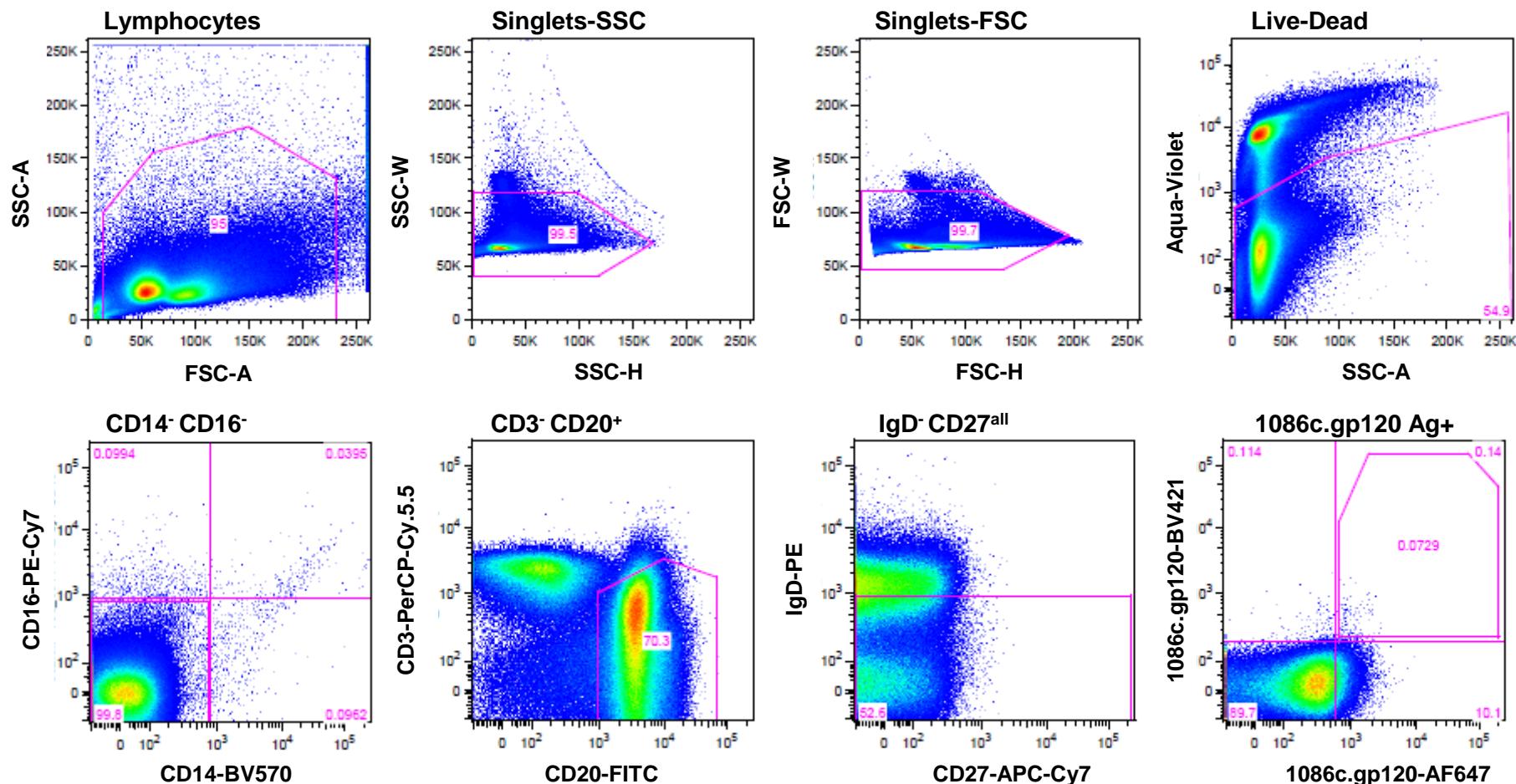
A. HIV Env+adjuvant group, animal 45519, PBMC (W10), gating strategy: lymphocytes → Singlets (SSC or FSC) → Live-dead → CD14-CD16- → CD3-CD20+ → IgD-CD27^{all} → 1086c(gp120) double positive



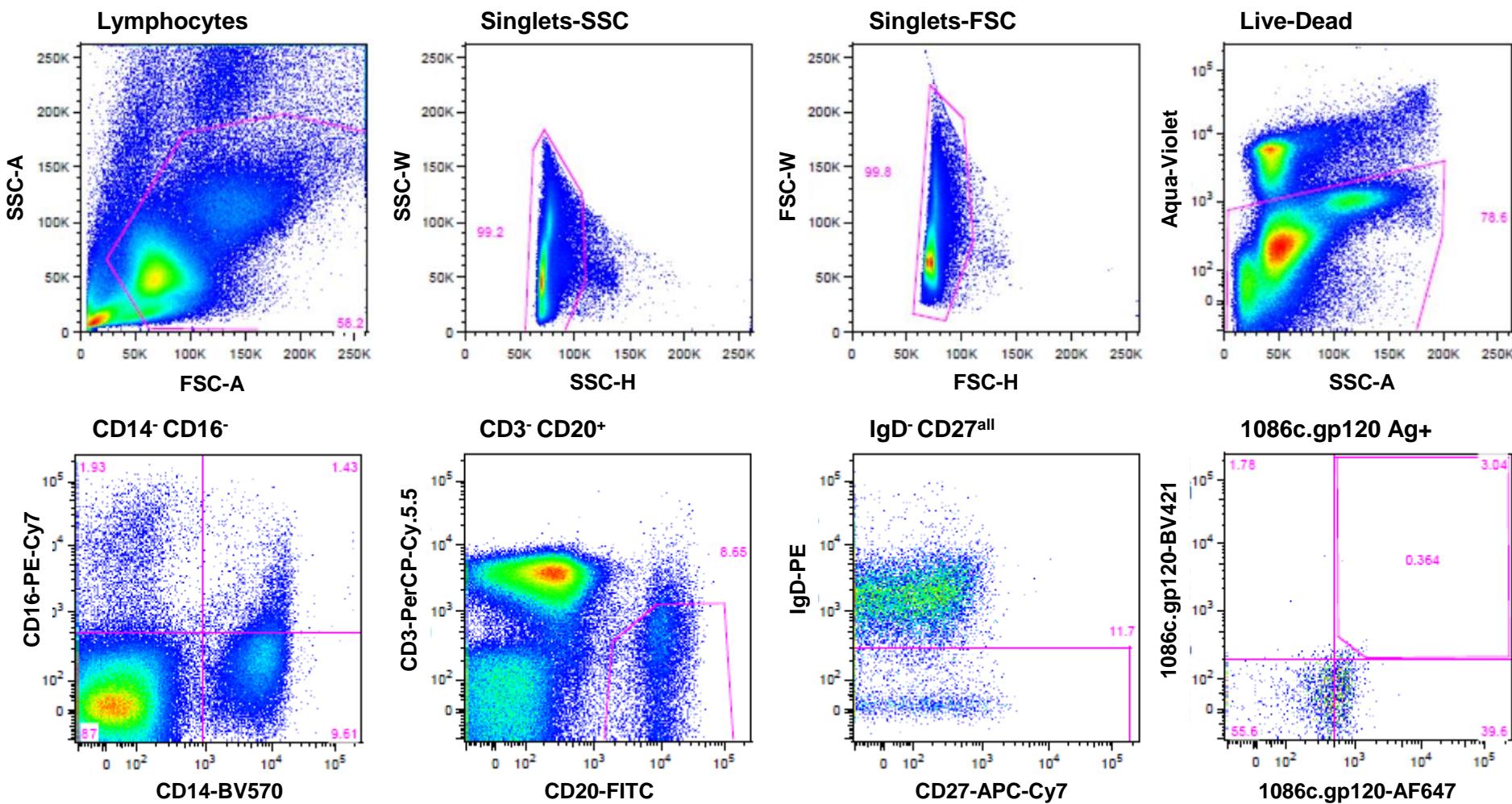
B. HIV Env+adjuvant group, animal 45521, Spleen (necropsy), representative data of 2 independent sort, gating strategy: lymphocytes → Singlets (SSC or FSC) → Live-dead → CD14-CD16- → CD3-CD20+ → IgD-CD27all → 1086c.gp120 double positive



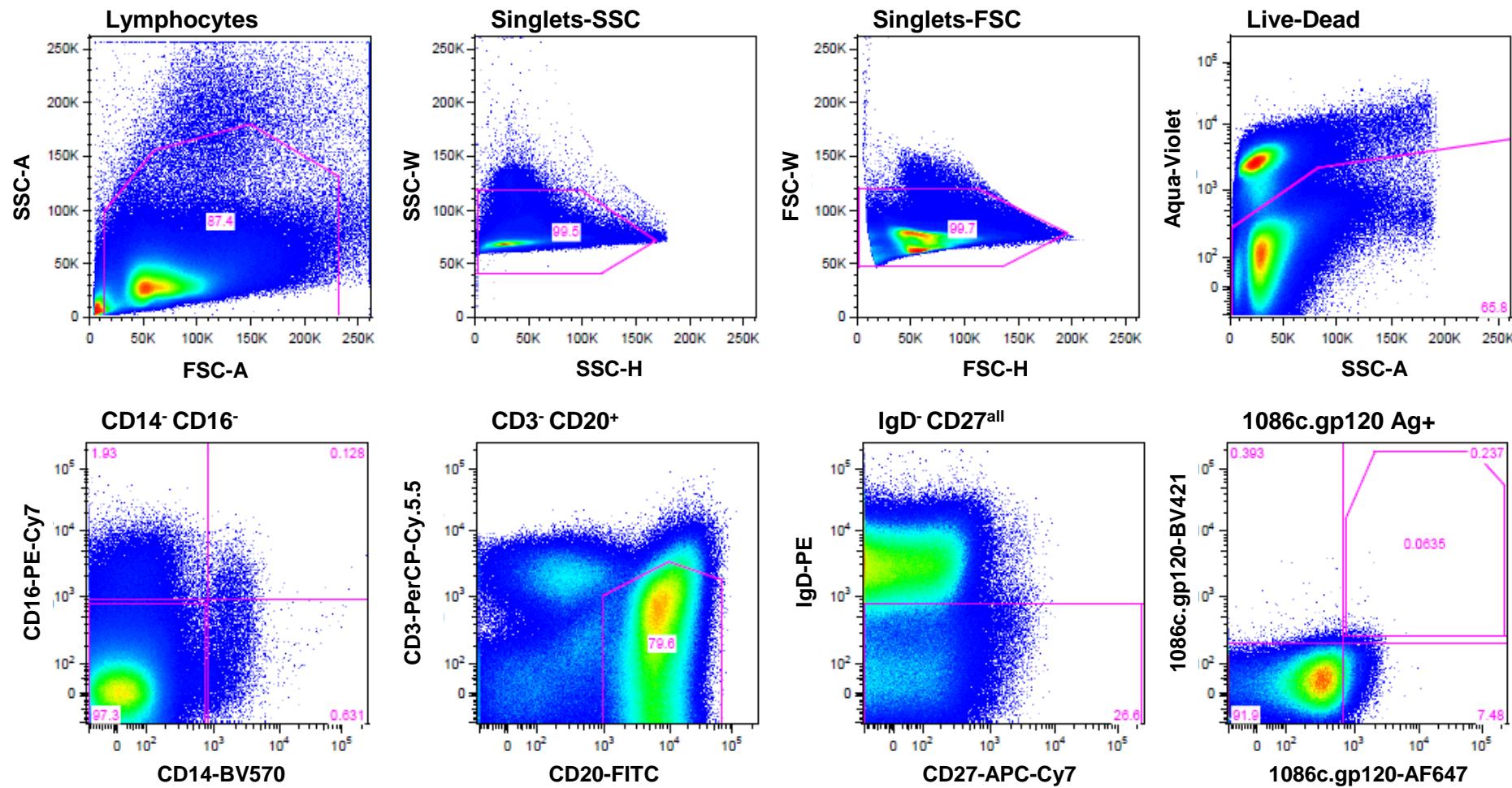
C. HIV Env+adjuvant group, animal 45522, Oral LN (retropharyngeal, necropsy), representative data of 3 independent sort, gating strategy: lymphocytes → Singlets (SSC or FSC) → Live-dead → CD14-CD16- → CD3-CD20+ → IgD-CD27all → 1086c.gp120 double positive



D. Co-administration group, animal 45038, PBMC (W12), gating strategy: lymphocytes → Singlets (SSC or FSC) → Live-dead → CD14-CD16- → CD3-CD20+ → IgD-CD27^{all} → 1086c.gp120 double positive

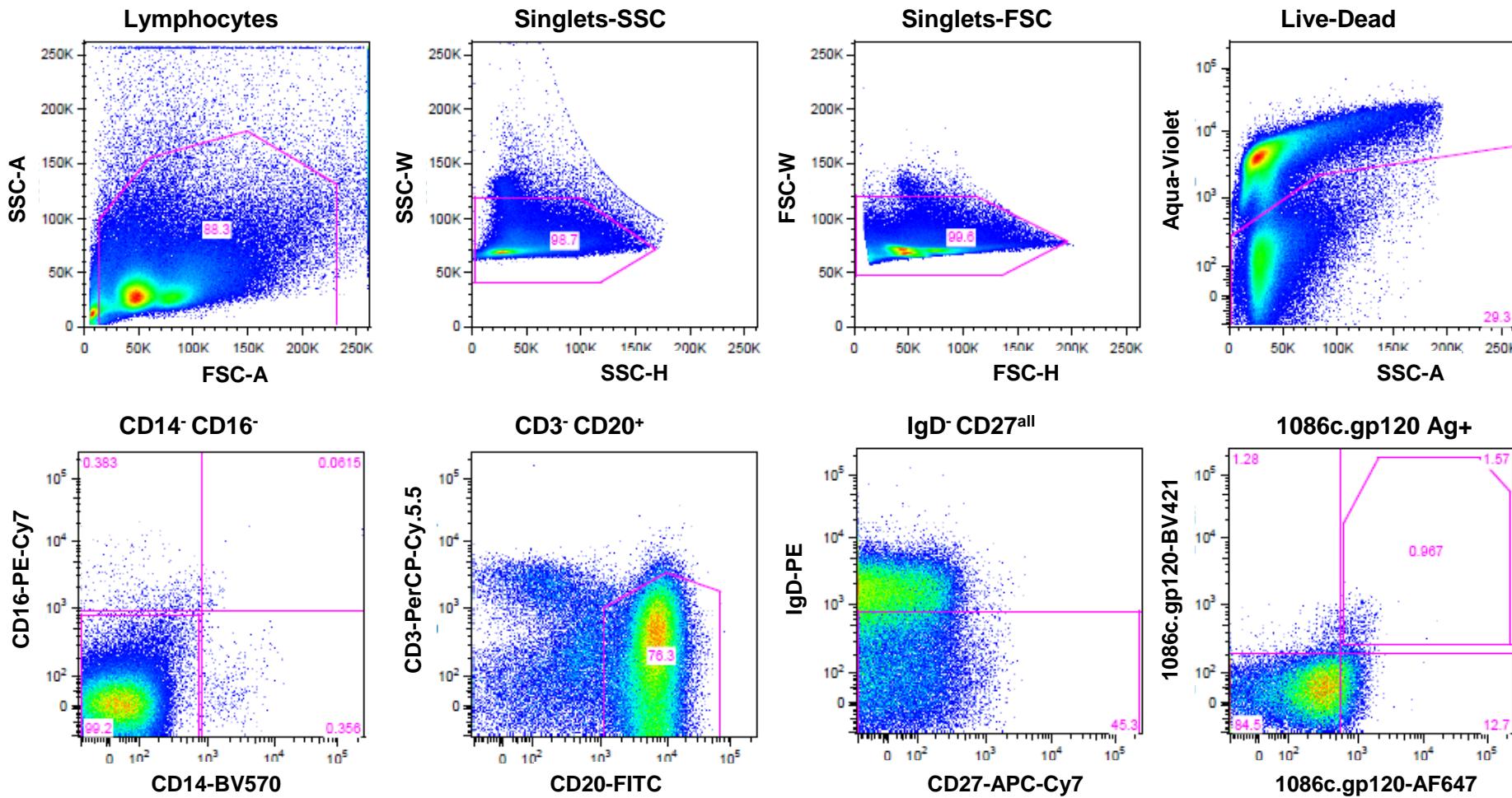


E. Co-administration group, animal 45083, Spleen (necropsy), gating strategy: lymphocytes → Singlets (SSC or FSC) → Live-dead → CD14-CD16- → CD3-CD20+ → IgD-CD27^{all} → 1086c.gp120 double positive

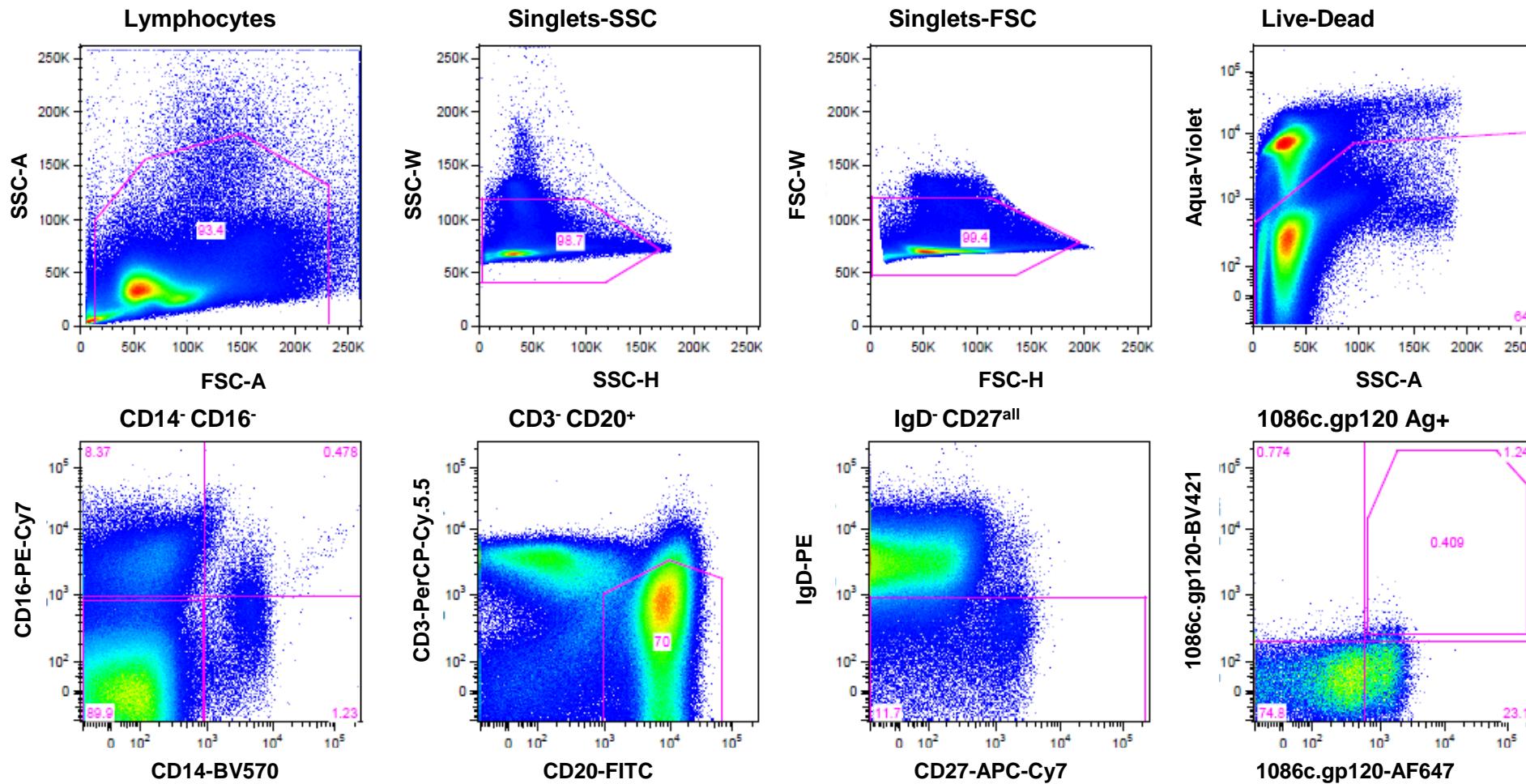


S1 Fig

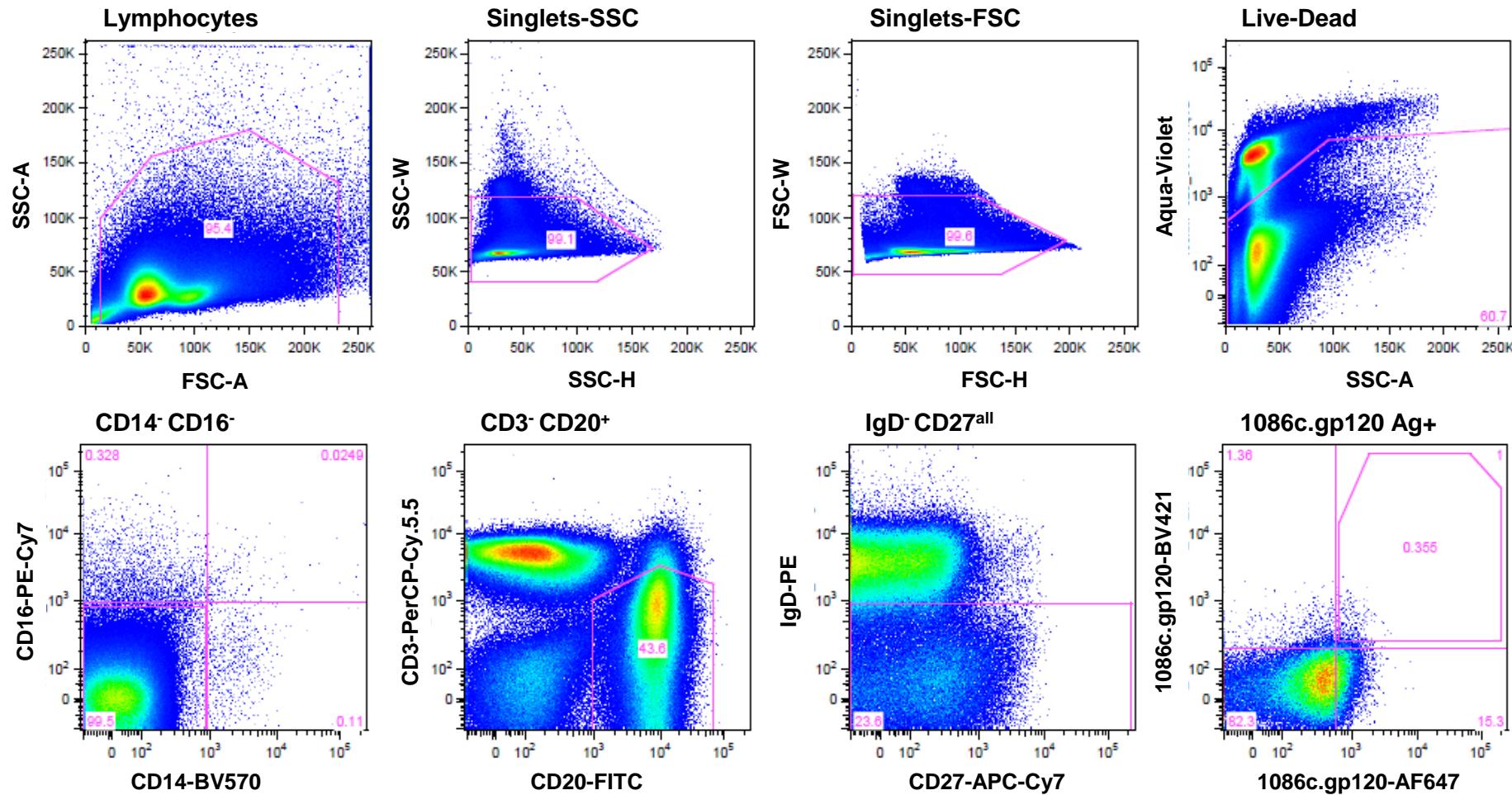
F. Co-administration group, animal 45091, Axillary LN (necropsy), gating strategy: lymphocytes → Singlets (SSC or FSC) → Live-dead → CD14-CD16- → CD3-CD20+ → IgD-CD27^{all} → 1086c.gp120 double positive



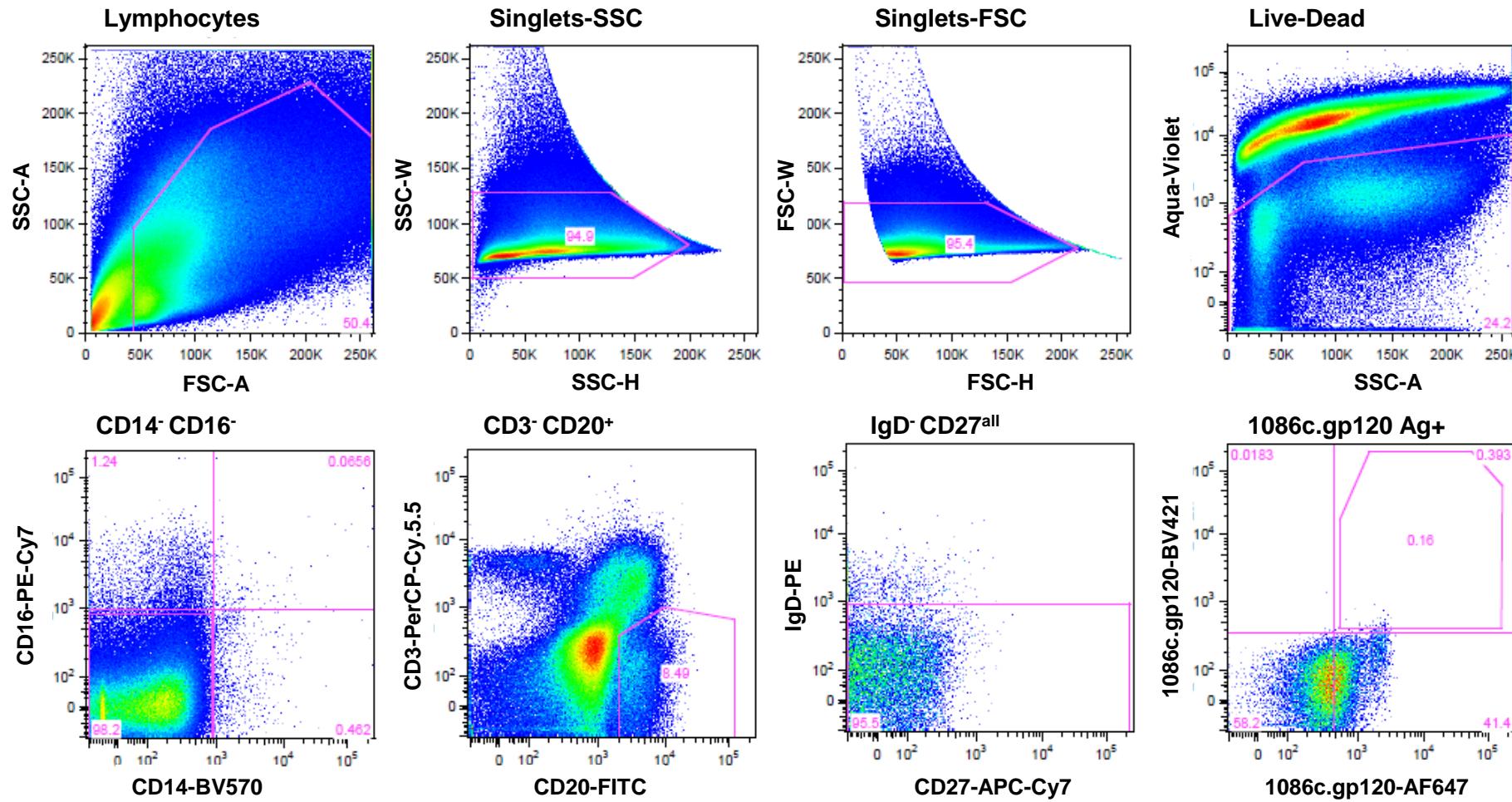
G. Extended Interval group, animal 45435, Spleen (necropsy), representative data of 3 independent sort, gating strategy: lymphocytes → Singlets (SSC or FSC) → Live-dead → CD14-CD16- → CD3-CD20+ → IgD-CD27all → 1086c(gp120) double positive



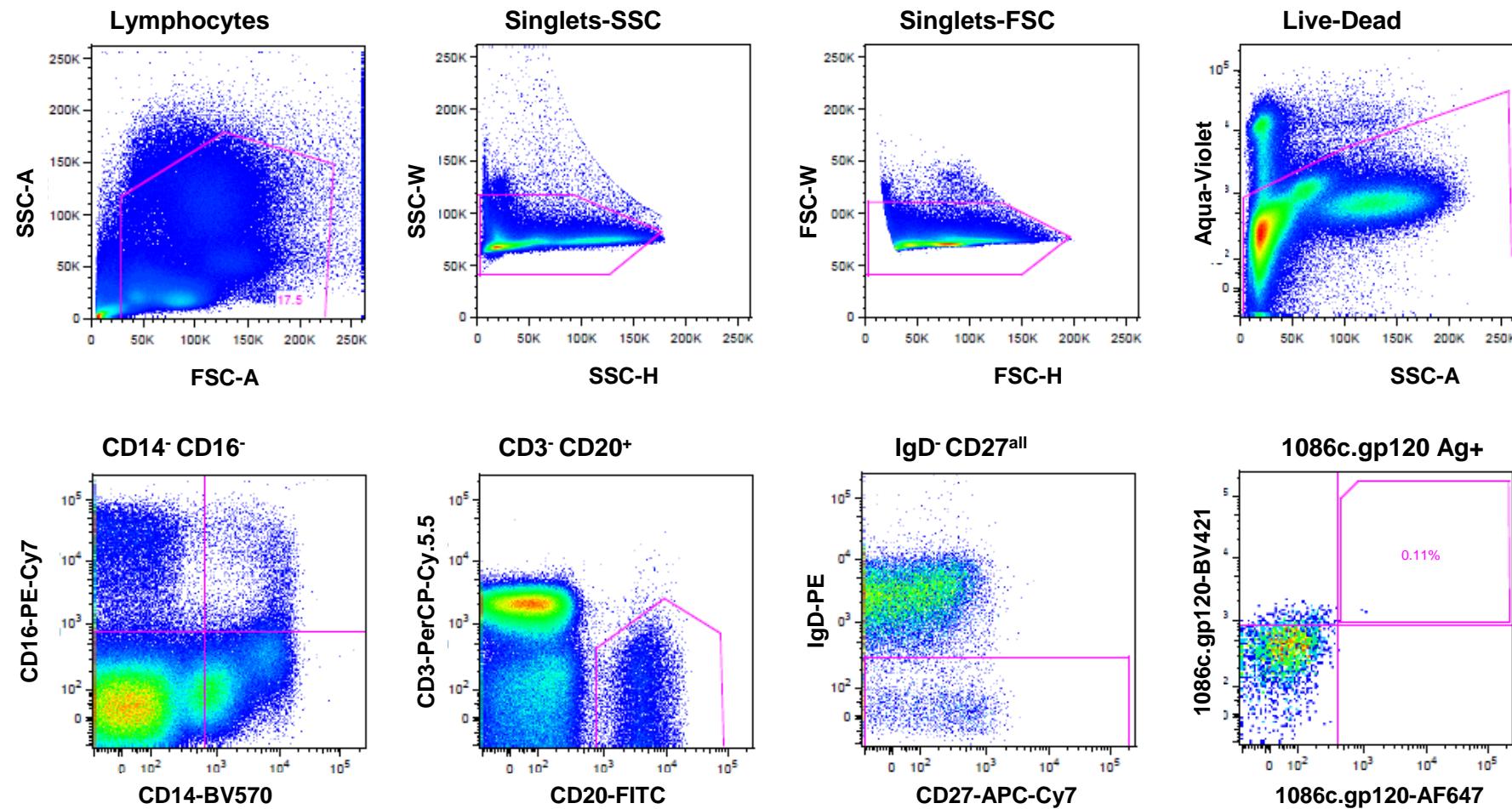
H. Extended Interval group, animal 45441, Oral LN (submandibular, necropsy), representative data of 4 independent sort, gating strategy: lymphocytes → Singlets (SSC or FSC) → Live-dead → CD14-CD16- → CD3-CD20+ → IgD-CD27all → 1086c.gp120 double positive



I. Extended Interval group, animal 45448, Colon (necropsy), representative data of 3 independent sort, gating strategy: lymphocytes → Singlets (SSC or FSC) → Live-dead → CD14-CD16- → CD3-CD20+ → IgD-CD27all → 1086c.gp120 double positive



J. 3M-052-SE group, animal 45838, PBMC (W12), gating strategy: lymphocytes → Singlets (SSC or FSC) → Live-dead → CD14-CD16- → CD3-CD20+ → IgD-CD27all → 1086c(gp120 double positive)



S1 Fig 1A-J. Representative flow cytometry analyses for single cell HIV Env-specific memory B cell sorting from PBMC and tissues of infants by vaccination groups. Percentage of memory B cells (CD20+CD27+IgD-), which were reactive to HIV 1086c gp120 K160N tagged with two colors-BV421 and AF647 (double positive cells) indicated in the upper right quadrant. Gates were drawn based on isotype controls and fluorescent minus one (FMO) controls.