A. HIV Env+adjuvant group, animal 45519, PBMC (W10), gating strategy: lymphocytes  $\rightarrow$  Singlets (SSC or FSC)  $\rightarrow$  Live-dead  $\rightarrow$  CD14-CD16-  $\rightarrow$  CD3-CD20+  $\rightarrow$  IgD-CD27all  $\rightarrow$  1086c.gp120 double positive



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



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



D. Co-administration group, animal 45038, PBMC (W12), gating strategy: lymphocytes  $\rightarrow$  Singlets (SSC or FSC)  $\rightarrow$  Live-dead  $\rightarrow$  CD14-CD16-  $\rightarrow$  CD3-CD20+  $\rightarrow$  IgD-CD27all  $\rightarrow$  1086c.gp120 double positive



Singlets-SSC Singlets-FSC Live-Dead Lymphocytes 250K 250K-250K 10<sup>5</sup> 200K 200K 200K Aqua-Violet 10 SSC-W 150K FSC-W 150K SSC-A 150K  $10^{3}$ 100K 1001 10 50K 50K 50K 0-0 100K 150K 200K 250K 50K 100K 150K 250K 50K 50K 100K 150K 200K 250K 50K 100K 150K 200K 250K 0 200K 0 0 0 SSC-H FSC-H FSC-A SSC-A IgD<sup>-</sup> CD27<sup>all</sup> CD14<sup>-</sup> CD16<sup>-</sup> CD3<sup>-</sup> CD20<sup>+</sup> 1086c.gp120 Ag+ 0.128 10<sup>5</sup> 10<sup>5</sup> 10<sup>5</sup> CD3-PerCP-Cy.5.5 CD16-PE-Cy7 104 104 lgD-PE 0.0635 103 10<sup>3</sup> 10<sup>2</sup> 10<sup>2</sup> 10<sup>2</sup> 10<sup>3</sup> 104 10<sup>3</sup> 10<sup>4</sup> 10<sup>5</sup> 10<sup>3</sup> 104 10<sup>3</sup> 10<sup>2</sup> 10<sup>5</sup> 10<sup>2</sup> 0 10<sup>2</sup> 10<sup>5</sup> 10<sup>2</sup> 104 0 0 0 1086c.gp120-AF647 CD14-BV570 CD20-FITC CD27-APC-Cy7

E. Co-administration group, animal 45083, Spleen (necropsy), gating strategy: lymphocytes  $\rightarrow$  Singlets (SSC or FSC)  $\rightarrow$  Live-dead  $\rightarrow$  CD14-CD16-  $\rightarrow$  CD3-CD20+  $\rightarrow$  IgD-

S1 Fig

CD27all  $\rightarrow$  1086c.gp120 double positive

F. Co-administration group, animal 45091, Axillary LN (necropsy), gating strategy: lymphocytes  $\rightarrow$  Singlets (SSC or FSC)  $\rightarrow$  Live-dead  $\rightarrow$  CD14-CD16-  $\rightarrow$  CD3-CD20+  $\rightarrow$  IgD-CD27all  $\rightarrow$  1086c.gp120 double positive



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



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



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



J. 3M-052-SE group, animal 45838, PBMC (W12), gating strategy: lymphocytes  $\rightarrow$  Singlets (SSC or FSC)  $\rightarrow$  Live-dead  $\rightarrow$  CD14-CD16-  $\rightarrow$  CD3-CD20+  $\rightarrow$  IgD-CD27all  $\rightarrow$  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.