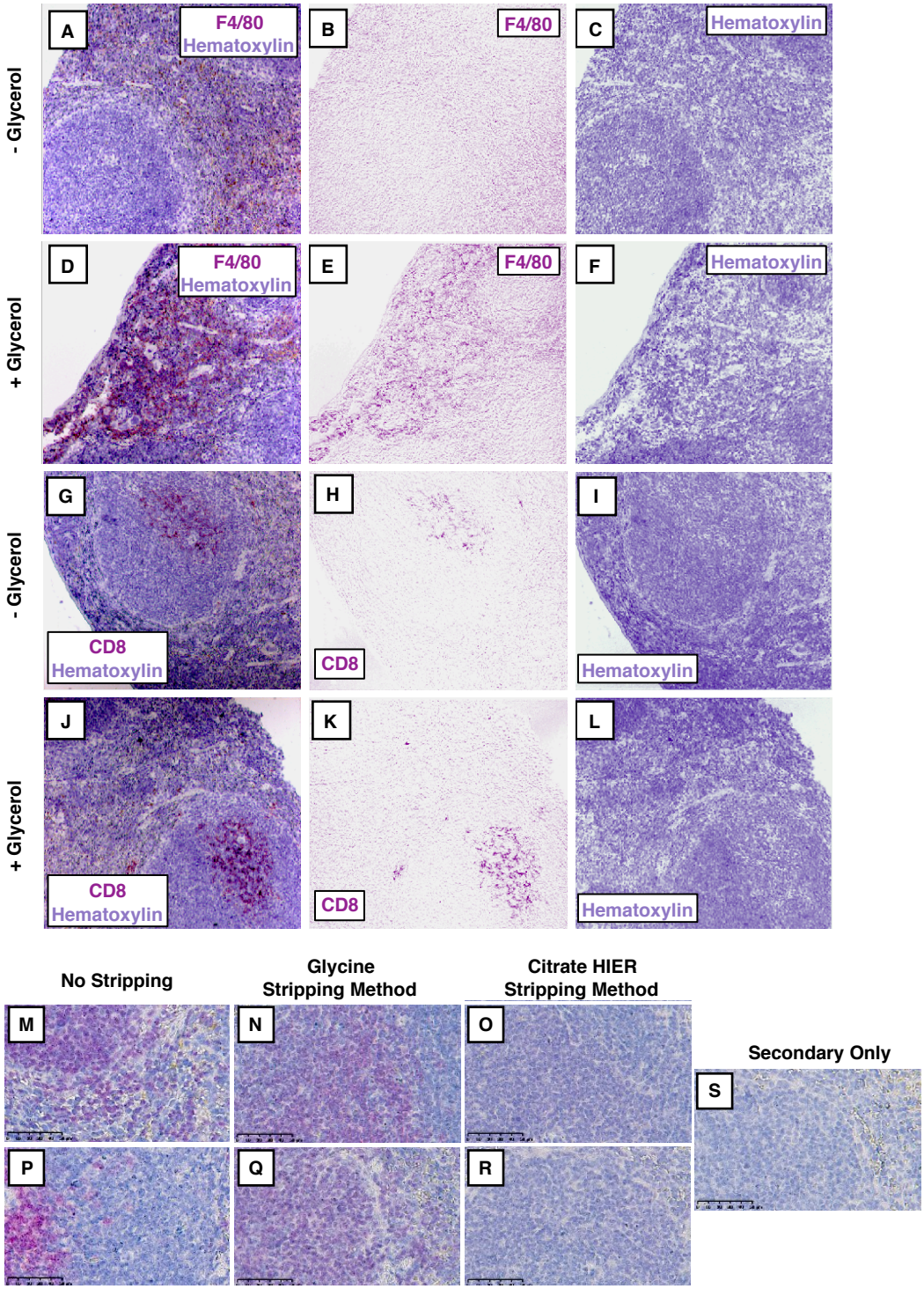
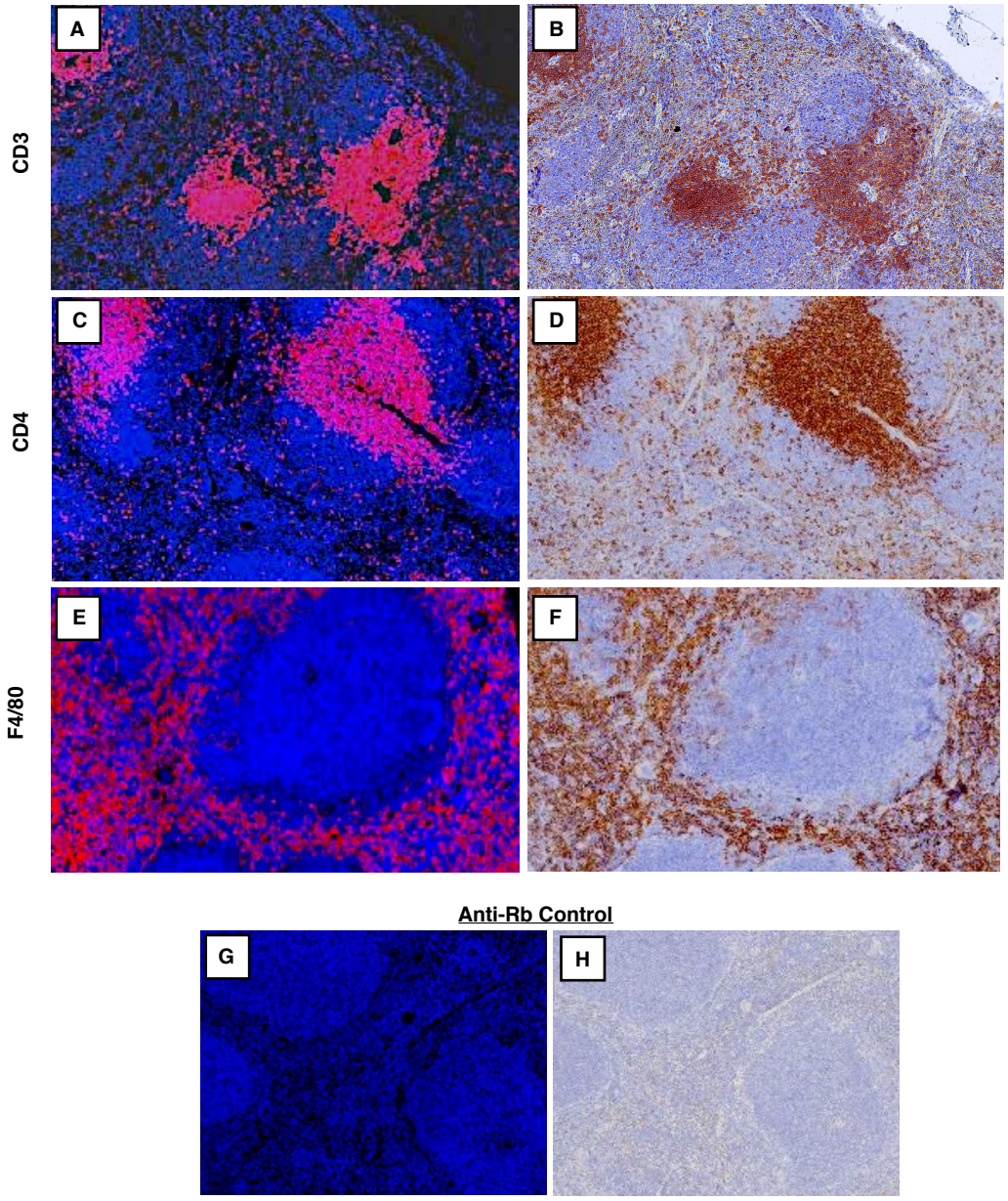
Supplemental Figure 1



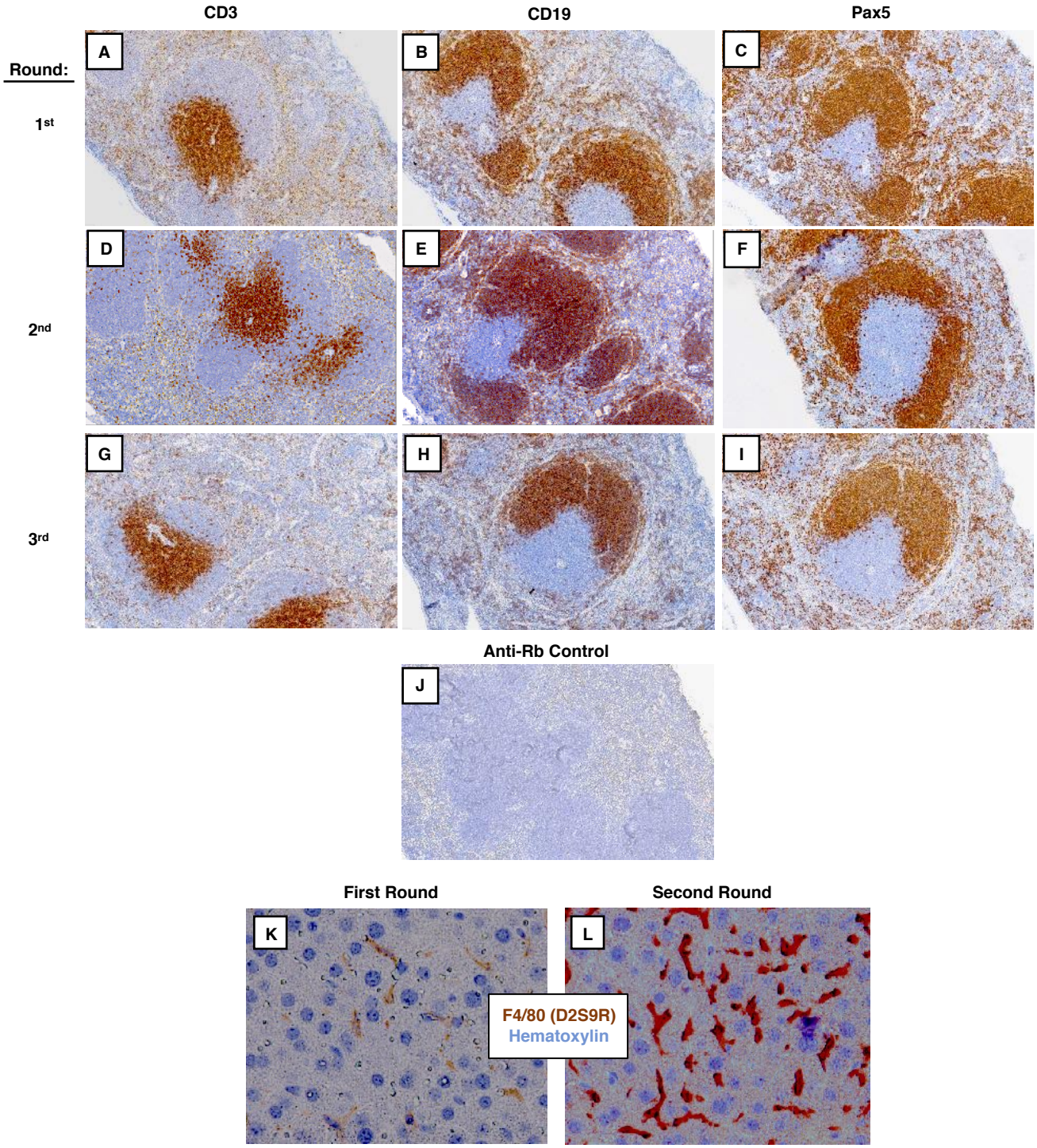
Supplementary Figure 1. Comparison of antigen retrieval buffers and antibody stripping methods. (**A-L**) BALB/c spleens were stained with antibodies specific for F4/80 (SP115; A-F) or CD8 (G-L). Spleen sections were treated with antigen retrieval buffer with (D-F, J-L) or without (A-C, G-I) glycerol. For detection, Bajoran Purple was used as the chromogenic substrate, followed by a hematoxylin counterstain. Images (A, D, G, J) were deconvoluted into their respective hematoxylin (C, F, I, L) and Bajoran Purple (B, E, H, K) components using ImageJ software. Slides were scanned at 20X objective using the Hamamatsu NanoZoomer 2.0-HT. Images represent a 20X field-of-view. (**M-S**) Comparison of different antibody stripping methods. BALB/c spleens were probed for antibodies. First, tissues were treated with Pax5 (M-O), CD3 (PA1-29547; P-R), or secondary-only (S). Next, tissue was developed with chromogen before (M, P) or after stripping and reprobing with secondary antibody (N, Q: glycine stripping; Q, R: citrate HIER stripping). Slides were scanned at 20X objective using the Hamamatsu NanoZoomer 2.0-HT. Images represent a 40X field-of-view. Scale bar = 50 μm.

Supplementary Figure 2



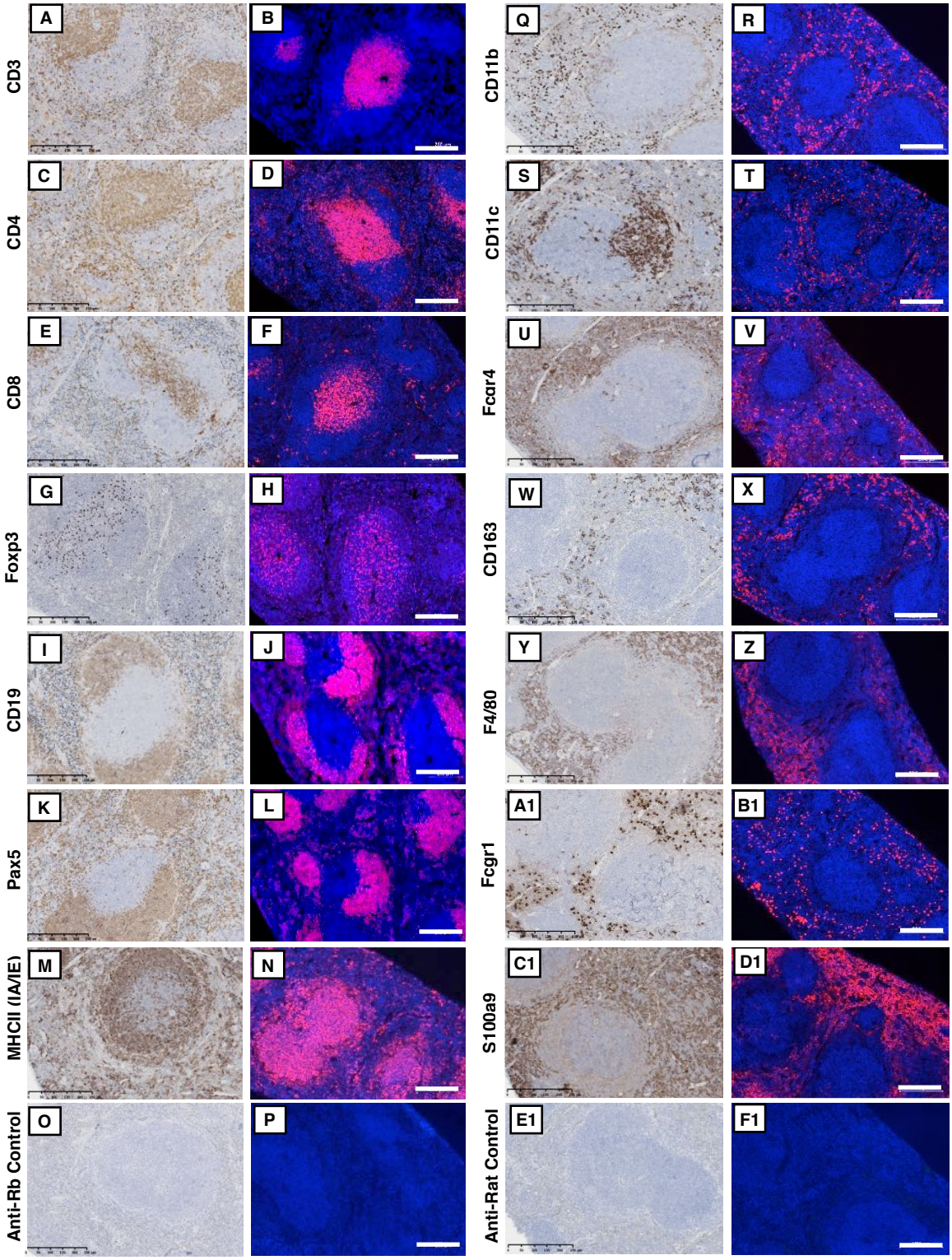
Supplementary Figure 2. Comparison between chromogen and fluorescence detection methods. BALB/c spleens were stained for CD3 (PA1-29547; A, B), CD4 (EPR19514; C, D) and F4/80 (SP115; E, F). Sections were first probed with antibodies, incubated with fluorescence detection reagent (Opal 570; B, D, F) and DAPI, and were subsequently scanned at 20X using the Zeiss Axioscan.Z1. After scanning, cover-slips were removed by incubating the slides in water for 1 hr. Then, the sections underwent antibody stripping, were reprobed with the same respective antibodies, and DAB was used for detection. After, the slides underwent a hematoxylin counterstain and were scanned at 20X using the Hamamatsu NanoZoomer 2.0-HT. All images represent a 10X field-of-view.

Supplementary Figure 3



Supplementary Figure 3. Sensitivity of antibodies to multiple rounds of antigen retrieval. (**A-J**) BALB/c spleens were stained for CD3 (PA1-29547; A, D, G), CD19 (B, E, H), or Pax5 (C, F, I) after antigen retrieval (1st round; A, B, C) or antibody stripping (2nd round; D, E, F) or a second round of antibody stripping (3rd round; G, H, I, J). (**K, L**) BALB/c livers were stained for F4/80 (D2S9R) after antigen retrieval minus (K) or plus (L) one round of antibody stripping. Betazoid DAB was used as the chromogenic substrate for detection. Slides were scanned at 20X using the Hamamatsu NanoZoomer 2.0-HT. (**A-J**) Spleen images represent a 10X field-of-view. (**K, L**) Liver images represent a 40X field-of-view.

Supplemental Figure 4



Supplementary Figure 4. Chromogen and fluorescent staining of lymphoid lineage and myeloid lineage markers. BALB/c spleens were stained with antibodies specific for CD3 (PA1-29547; A, B), CD4 (EPR19514; C, D), CD8 (E, F), Foxp3 (G, H), CD19 (I, J), and Pax5 (K, L), MHCII (M, N), CD11b (Q, R), CD11c (S, T), Fcgr4 (U, V), CD163 (W, X), F4/80 (Y, Z), Fcgr1 (A1, B1) S100a9 (C1, D1), or secondary-only (O, P, anti-rabbit; E1, F1, anti-rat). Betazoid DAB and Opal 570 were used as the chromogenic and fluorescent substrates, respectively. Chromogen-stained sections were counterstained with hematoxylin, and fluorescence images were counterstained with DAPI. Chromogen-stained slides were scanned at 20X using the Hamamatsu NanoZoomer 2.0-HT. Slides Slides with fluorescent detection were scanned at 20X using the Zeiss Axioscan.Z1. Images represent a 10X field-of-view. Scale bar = 250 μm for chromogen images. Scale bar = 200 μm for 10X fluorescent images.