

Figure S1: Comparison between NBF fixation and Zinc-based fixation. A) H&E staining for SCCVII squamous cell tumor fixed with NBF. B) H&E staining for SCCVII squamous cell tumor fixed with zinc-based buffer. C) Immunostaining of CD3 (white), CD4 (magenta), and CD8 (yellow) in SCCVII tumor fixed with formalin. D) Same staining done in the same tumor fixed with zinc-based buffer.

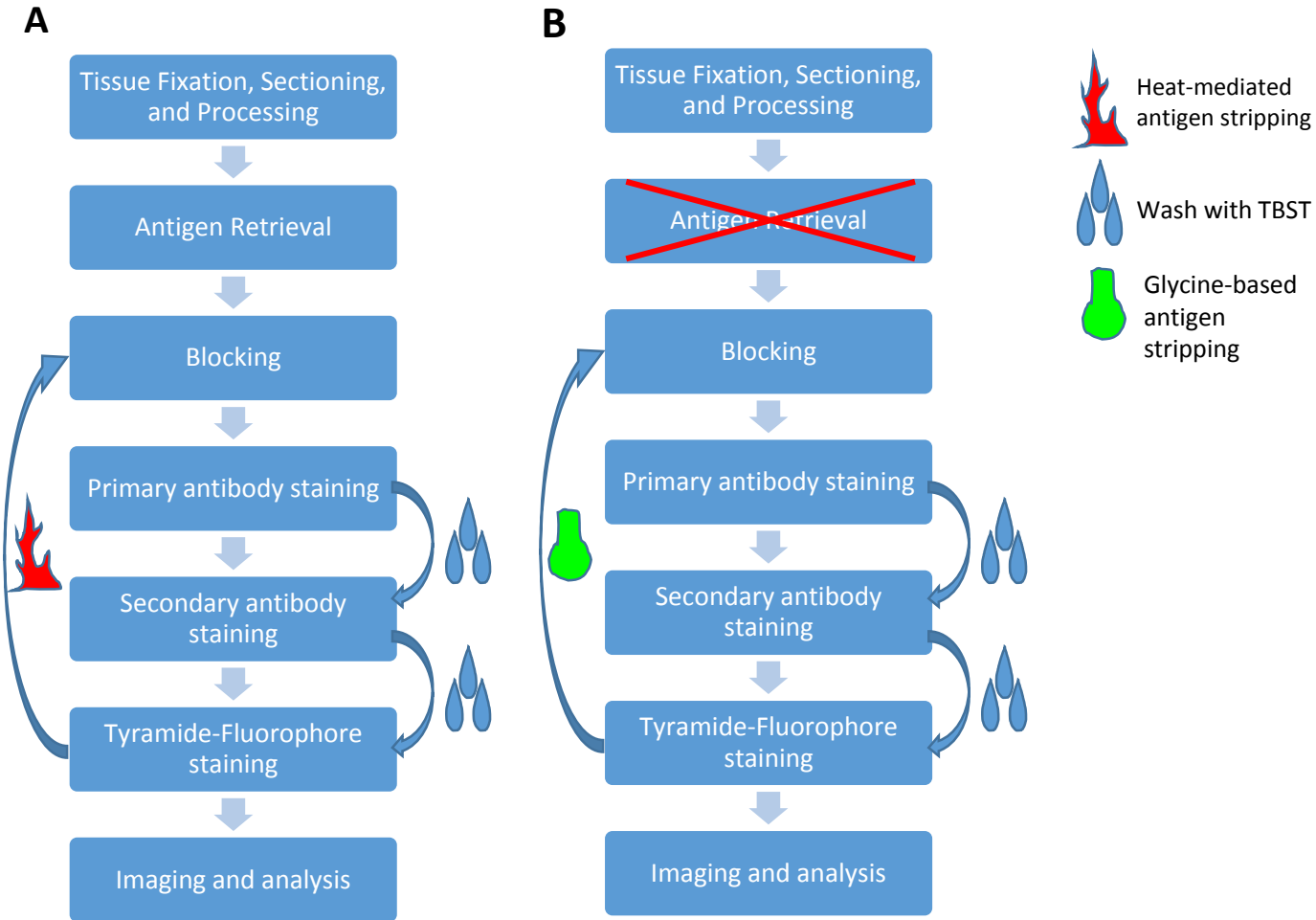


Figure S2: Schematic of multiplex IHC. A) Existing system optimized for use on robust epitopes. B) Modified system for use of multiplex IHC on sensitive epitopes.

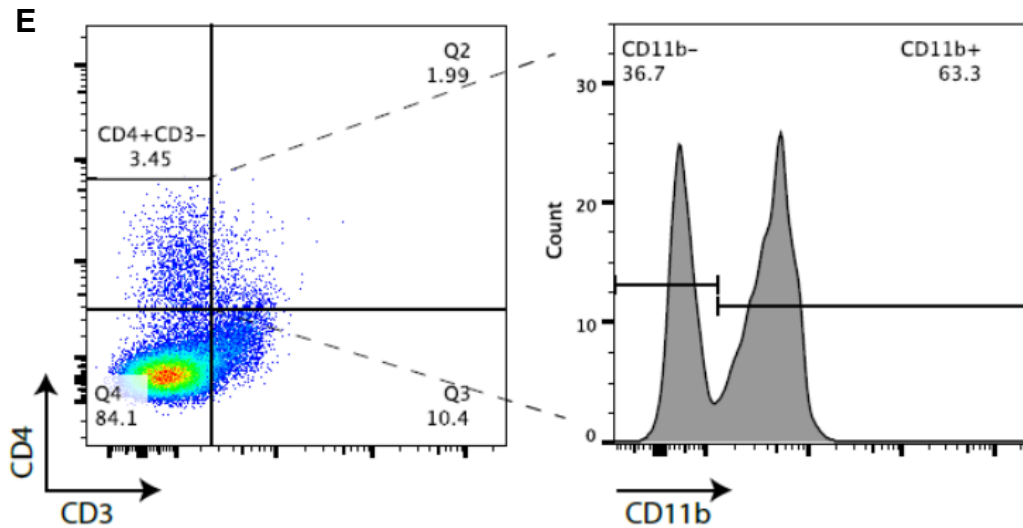
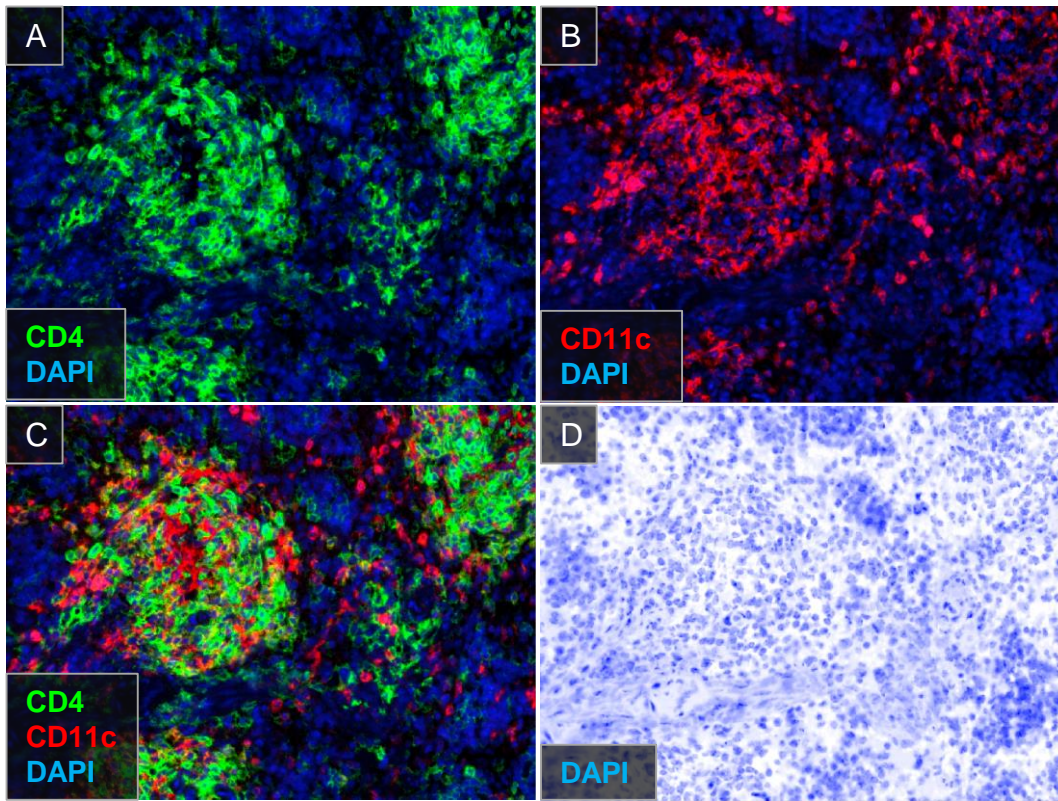


Figure S3: CD3⁺CD4⁺ cells co-express CD11b and CD11c. Spleen sections from Rag1^{-/-} animal were stained with CD4 and CD11c by IHC (A-C). D) Pseudo H&E of the section demonstrating spleen architecture. E) Spleen sections from Rag1^{-/-} were collected and stained for CD3, CD4 and CD11b by flow cytometry.

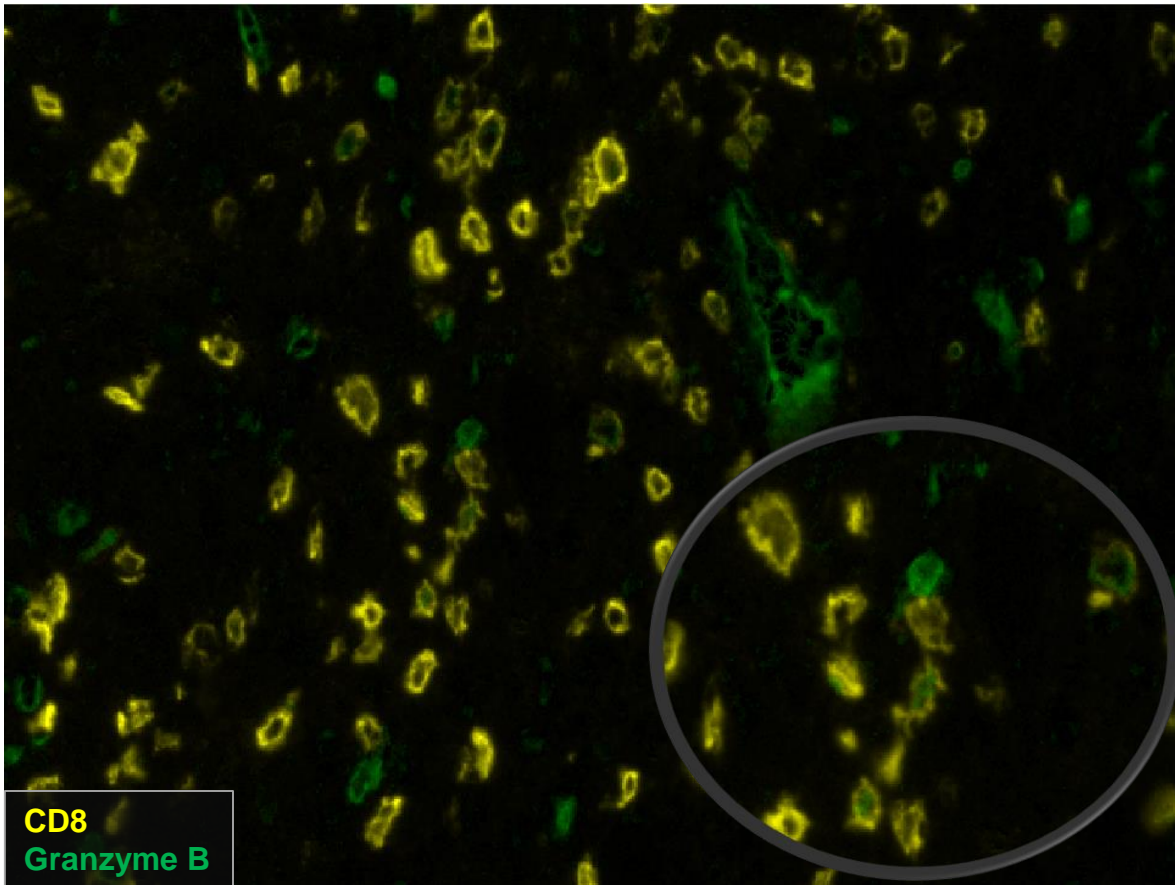


Figure S4: A subset of CD8⁺ T cells express granzyme B. Immunostaining of CD3 (not shown), CD8 (yellow), granzyme B (green) was done in SCCVII tumor sections fixed with zinc-based buffer. Heat mediated antigen-retrieval was performed before granzyme B staining.