SUPPLEMENTAL MATERIAL

Jenkins et al., http://www.jem.org/cgi/content/full/jem.20121999/DC1

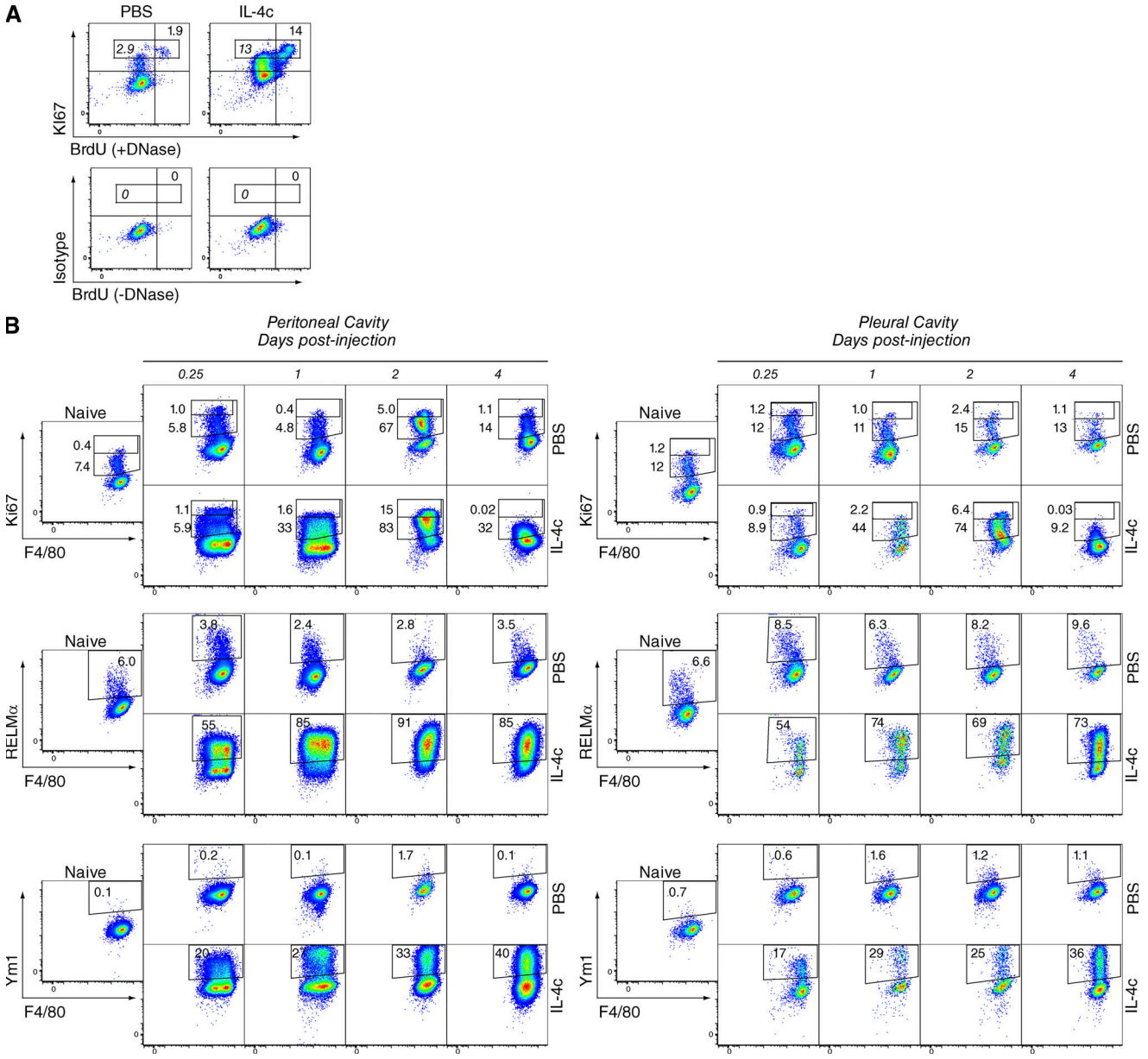

Figure S1. Ex vivo S-phase MΦs are distinguished by high levels of Ki67 expression. (A) BL/6 mice were injected with PBS or IL-4c on days 0 and 2, and peritoneal cavity F4/80^{High} MΦs were analyzed on day 4 for Ki67 expression versus BrdU incorporation. Frequency of cells expressing high levels of Ki67 (italics) is approximate to those positive for BrdU (Roman text). Gates distinguishing positive events were set using isotype control staining (Ki67) or the absence of DNase treatment (BrdU). Data are representative of pleural and peritoneal MΦs from four separate experiments. (B) BL/6 mice were given a single i.p. injection of IL-4c or PBS, and single F4/80^{High}CD19^{-} MΦs from peritoneal and pleural cavities were assessed over the following 96-h period for proliferation and alternative activation. Representative flow cytograms depict the gates used to determine the frequency of Ki67^{+}, Ki67^{High}, RELMα^{+}, and Ym1^{+} cells, with the proportion of F4/80^{High} MΦs in cell cycle or S phase determined as Ki67^{+} or Ki67^{High}, respectively. Of note, control injection of PBS led to a small but significant increase in proliferation of MΦs in the peritoneal cavity injection site but not in the pleural cavity, suggesting that 27-G needle stick injury drives MΦs into cycle, with ramifications for methodologies used to directly manipulate tissue sites and study MΦ functions. Data are from the same experiment as that shown in Fig. 3 C.

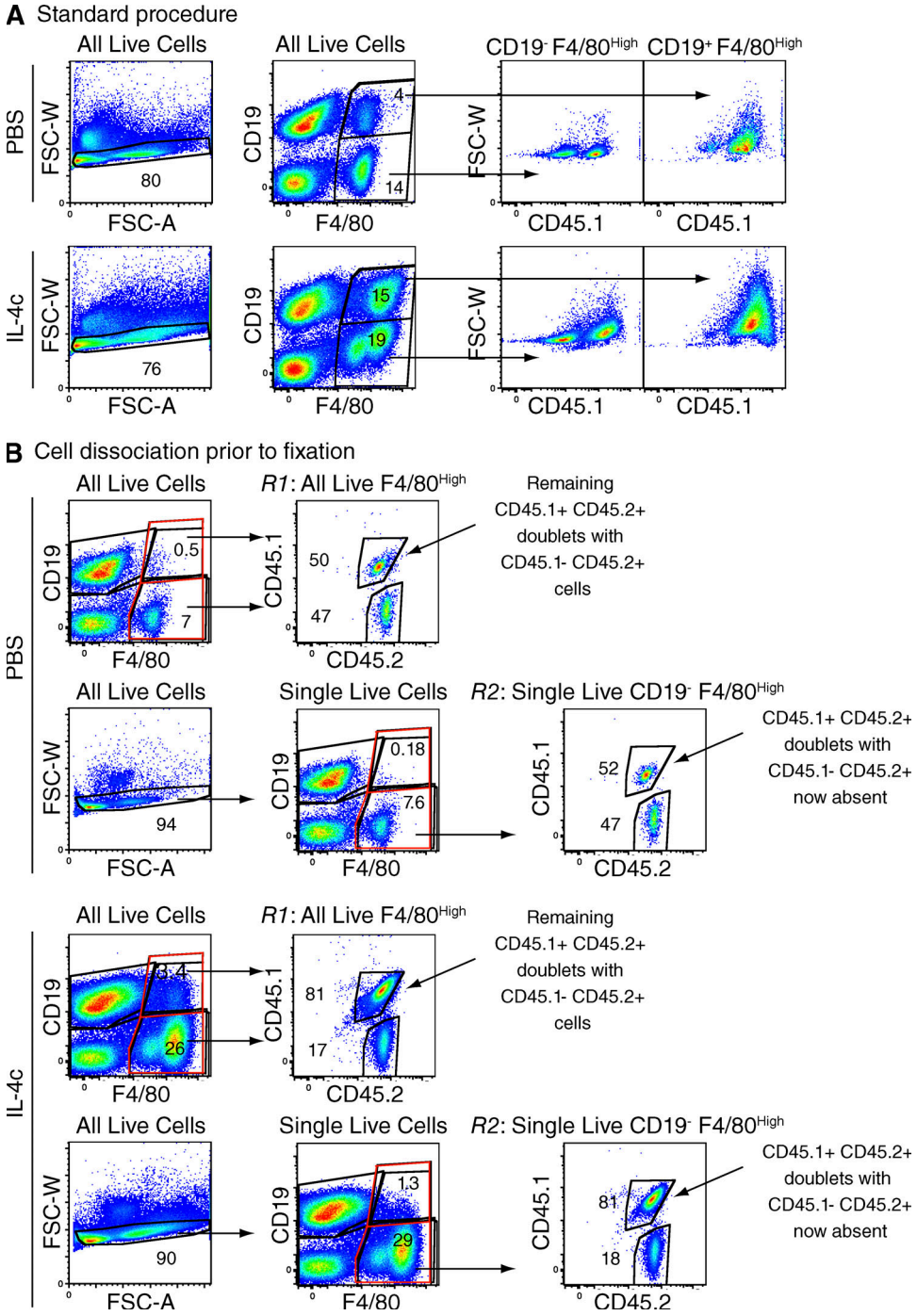

Figure S2. Dissociation of cell aggregates for analysis of F4/80^{High} MΦ composition. (A) FSC-A versus FSC-W plots of pleural cavity cells from PBS- or IL-4c–treated animals showed that between 20 and 25% of events are doublets. The majority of these were B cell–B cell events (not depicted), but the second most common were B cell–MΦ doublets, identified as F4/80^{High}CD19^{+}. The proportion of MΦs in doublets with B cells increased greatly to almost 50% upon treatment with IL-4c and appeared mainly formed of cells expressing the highest levels F4/80, suggesting a WT CD45.1^{+} phenotype. (B) Less than 10% of pleural cavity events were doublets when Accumax cell aggregate dissociation medium was used before fixation. Almost all remaining doublets were B cell–B cell interactions (not depicted), with B cell–MΦ doublets reduced to ≤10% of all F4/80^{High} events. However, to further prevent bias induced by these remaining doublets, the CD45.1^{+}CD45.2^{+} cell gate was set to exclude events that deviated from the expected linear relationship between CD45.1 and CD45.2 on this population. The absence of such events on dot plots from which doublets had been excluded using a FSC-A versus FSC-W gate confirmed them to be doublets of CD45.1^{-}CD45.1^{+} and CD45.1^{+}CD45.2^{+} cells. Analysis of MΦ CD45.1/CD45.2 composition was performed on all live F4/80^{High} cells (R1), whereas analysis of proliferation and alternative activation was performed on CD19^{-}F4/80^{High} cells gated on single events (R2).