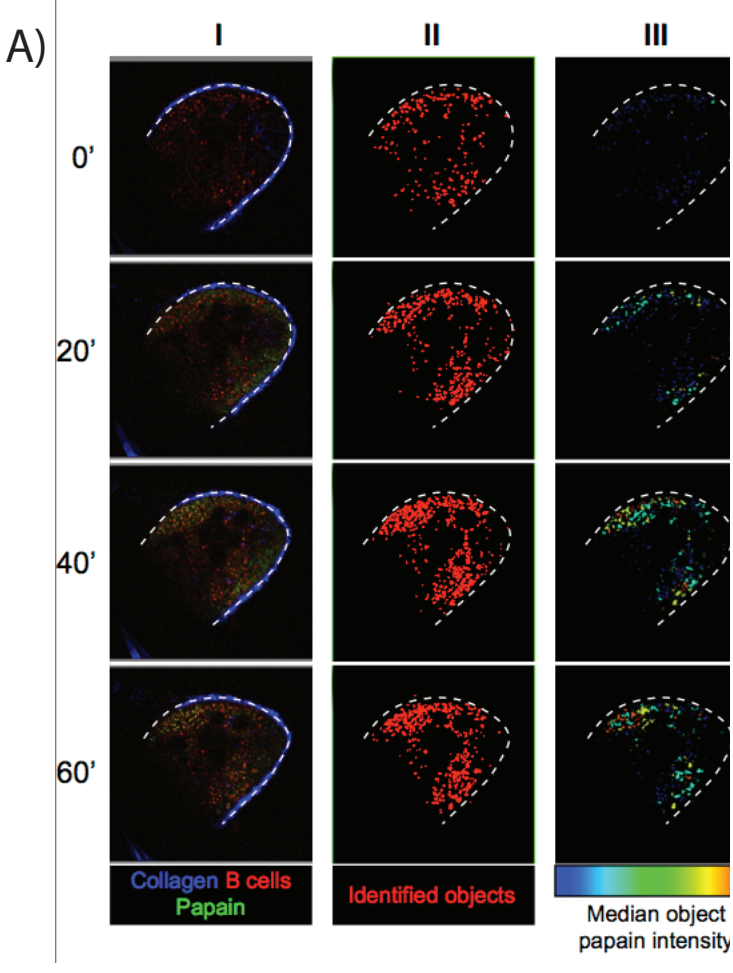
Supplemental Movie 1 Live imaging of PLN B cell uptake of labeled papain following footpad injection. A C57BL/6 mouse was adoptively transferred with $5x10^6$ CD21-cre _tdTomato B cells and 24 h later, the recipient was anesthetized and the PLN was exposed for live imaging. AF488-papain was injected into the footpad at t=0, and serial 150 μm image stacks were collected every 2 min for 60 min. Movie displayed is visualized as a 3 dimensional rendering, and corrected for movement in Volocity image analysis software. Blue indicates collagen, green indicates papain, and red indicates B cells



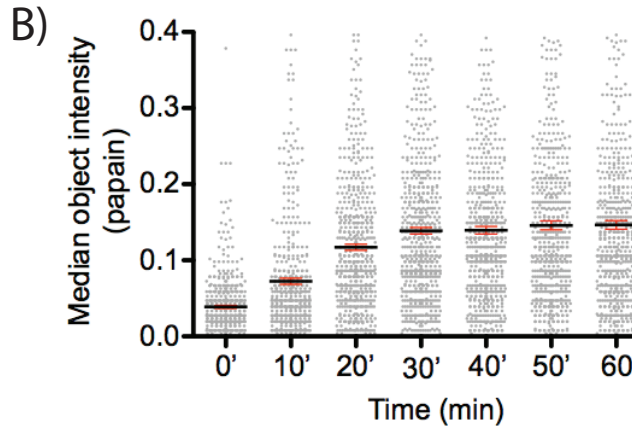


FIGURE S1. Intravital microscopy analysis of PLN B cell uptake of labeled papain following footpad injection. (A) B cells from CD21-cre_tdTomato mice were adoptively transferred into C57BL/6 recipients, and the PLNs were live-imaged using IV-MPM. AF488-papain was injected into the footpad at t=0 and 150 µm image stacks (3 µm steps) were captured every 2 min for 60 min. Example of CD21 positive object identification at the indicated time points at a selected z depth (60 µm). Raw images (I) were collected at each z-depth, and then processed by CellProfiler to identify CD21 positive objects (II). Identified objects were then assessed for papain colocalization, and median papain intensity was converted into visual heat-mapping data (III). (B) Median papain intensity from objects identified from (A) at the 60 µm depth. Heavy lines indicate the median intensity of papain staining at each given time point and red bars indicate SD. ANOVA $p<0.0001$.

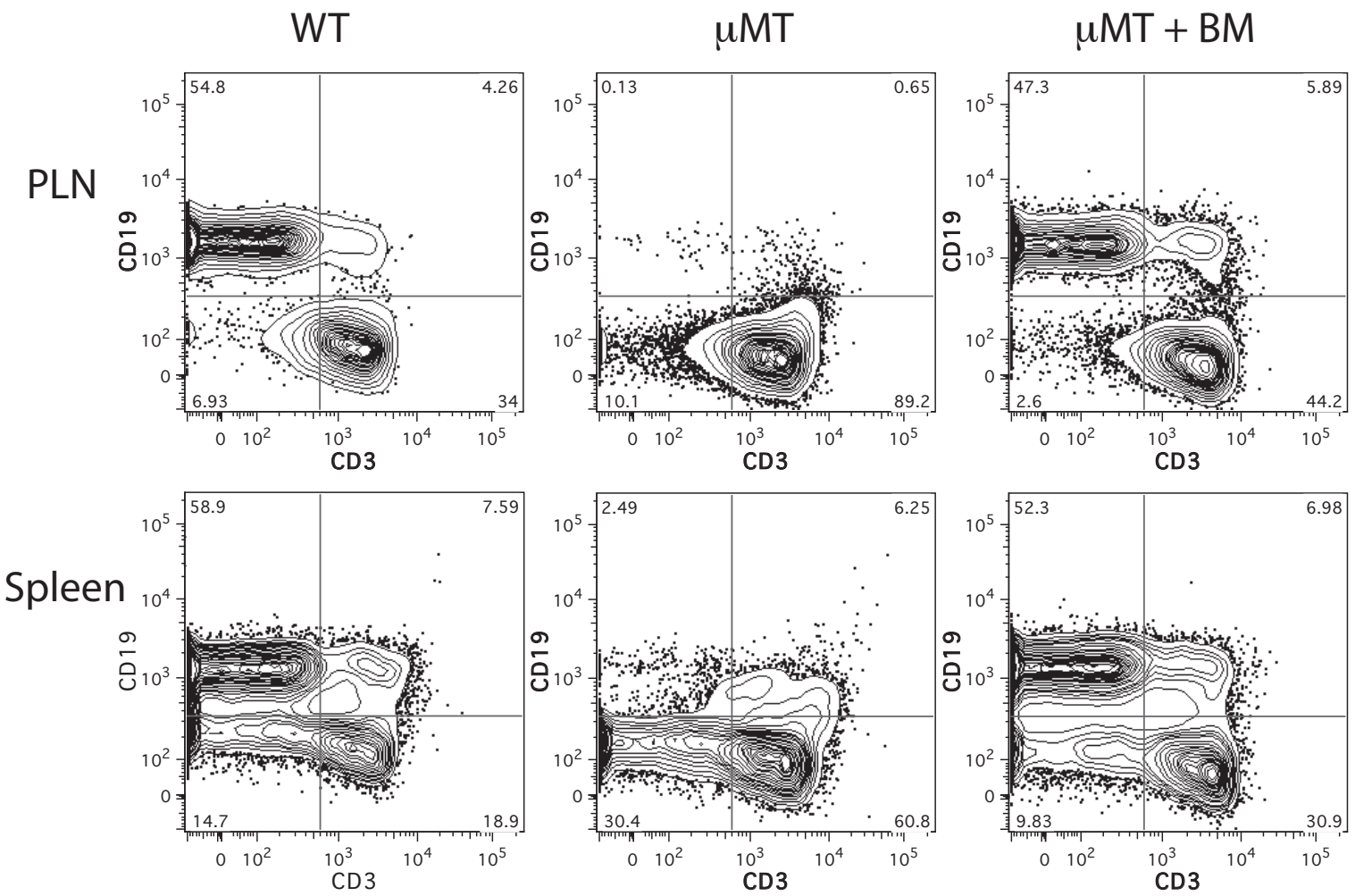


FIGURE S2. Assessment of B cell reconstitution following sublethal irradiation. muMT mice were sublethally irradiated and reconstituted with 10^6 WT BM cells. After 8 weeks mice were injected in the rear footpad with papain and the numbers of CD3+ and CD19+ were evaluated in the PLN and spleen. Reconstituted μMT mice were compared to age-matched WT and non-irradiated, non-reconstituted μMT mice. Representative FACS plots are shown. Sublethal irradiation followed by WT BM adoptive transfer was able to restore the B cell compartment in the LN and spleen to near-WT levels.

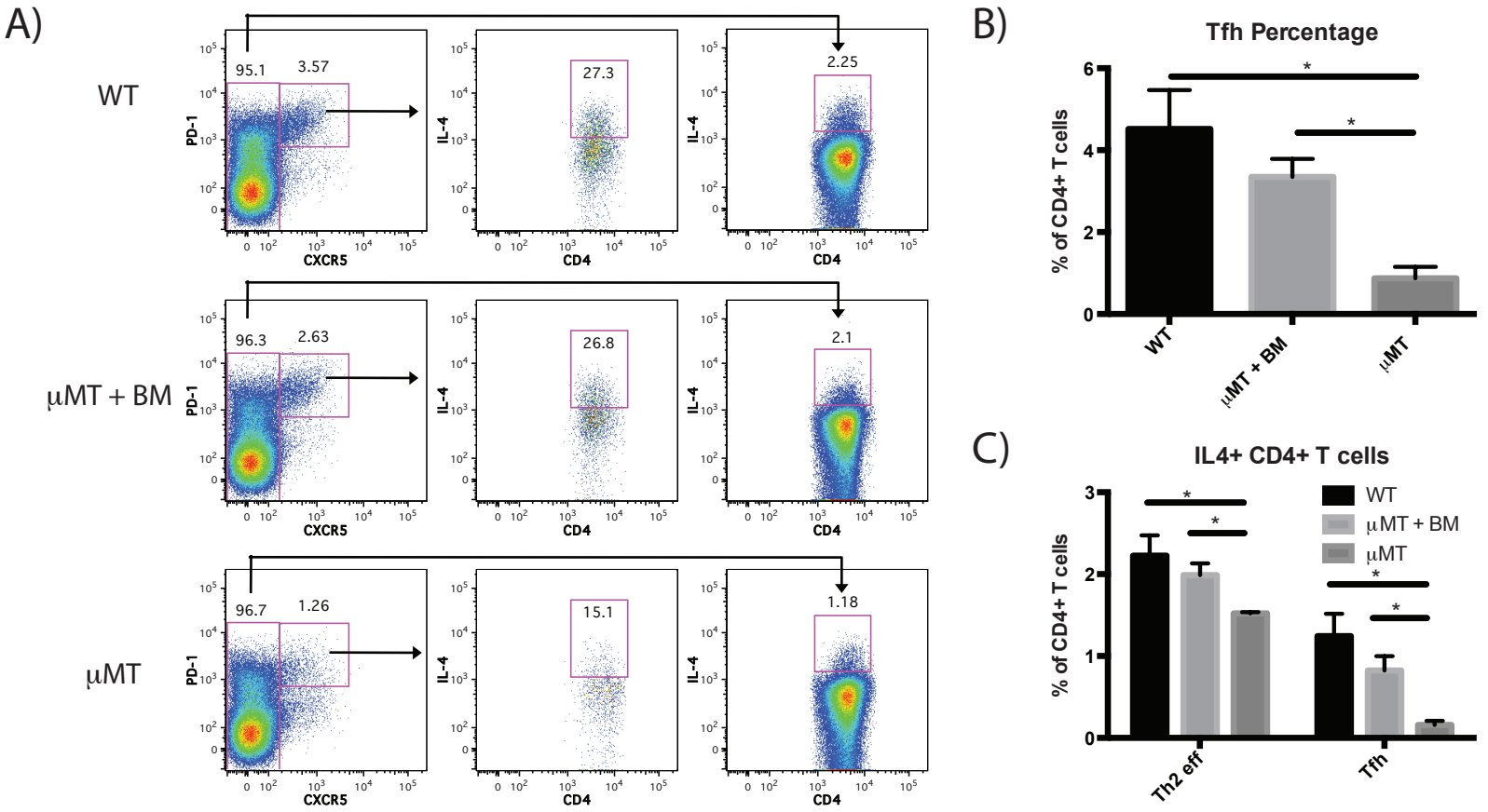


FIGURE S3. Analysis of the impact of B cell reconstitution on Tfh and Th2 responses. μMT mice were sublethally irradiated and reconstituted with 10^6 WT BM cells. After 8 weeks mice were injected in the rear footpad with papain and their immune responses were assessed after 5 d. Reconstituted μMT mice were compared to age matched WT and non-irradiated, non-reconstituted μMT mice. (A) Representative FACS plots showing the induction of Tfh ($CXCR5^{hi}$ $PD1^{hi}$) and IL-4 expression by Tfh and conventional T cells ($CXCR5^{lo}$) in WT, sublethally irradiated and reconstituted μMT, or non-irradiated or reconstituted μMT mice analyzed on d 5 post-immunization. (B) Quantification of total Tfh as a percentage of all CD4+ T cells in WT, sublethally irradiated and BM-reconstituted μMT, or non-irradiated, non-reconstituted μMT mice. Bars indicate mean ± SD of n=3 mice per group. (C) Comparison of effector Th2 cells ($IL\text{-}4^{+}$ $CXCR5^{lo}$) vs IL-4+ Tfh cells ($CXCR5^{hi}PD\text{-}1^{hi}$ $IL\text{-}4^{+}$) as a mean percentage of all CD4+ T cells in WT, sublethally irradiated and BM reconstituted μMT, or non-irradiated, non-reconstituted μMT mice. Bars indicate mean ± SD of n=3 mice per group