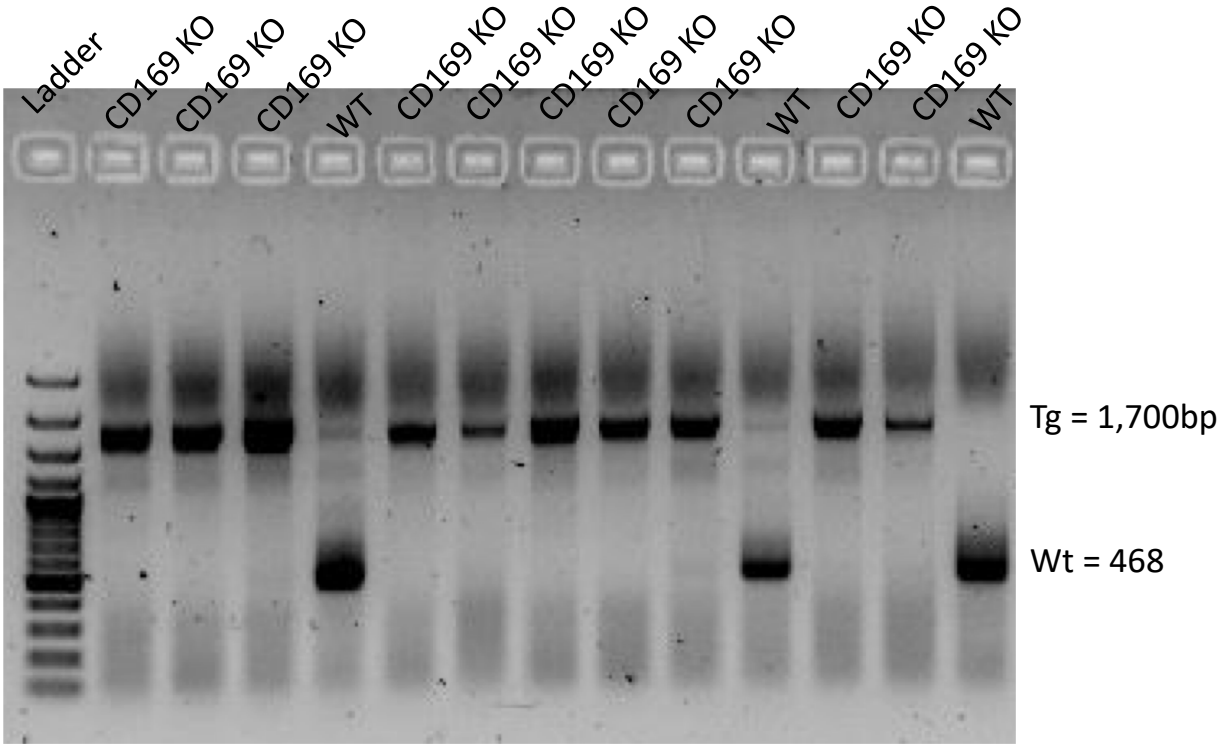
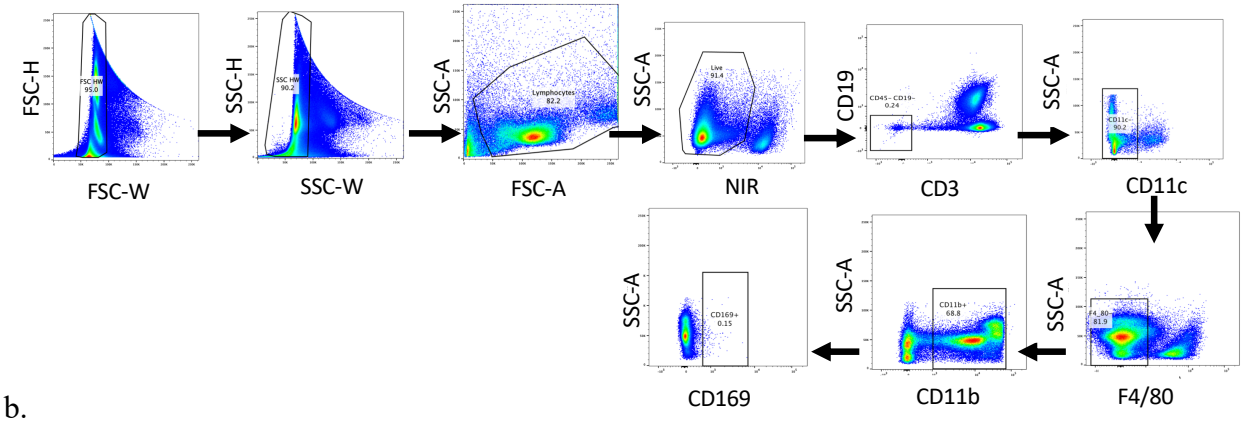
Supplemental Figure 1: CD169 Knockout PCR gel

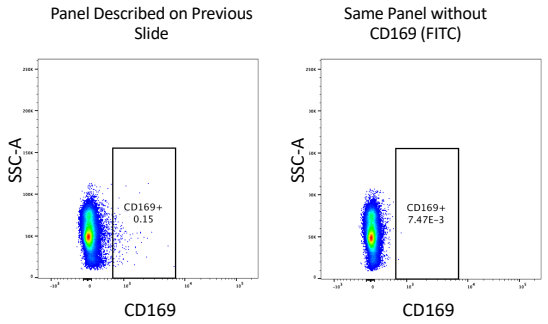


Supplemental Figure 1: CD169 PCR gel. Transgene (Tg) for CD169 is shown at 1,700 bp. Wildtype (Wt) is shown at 468 bp.

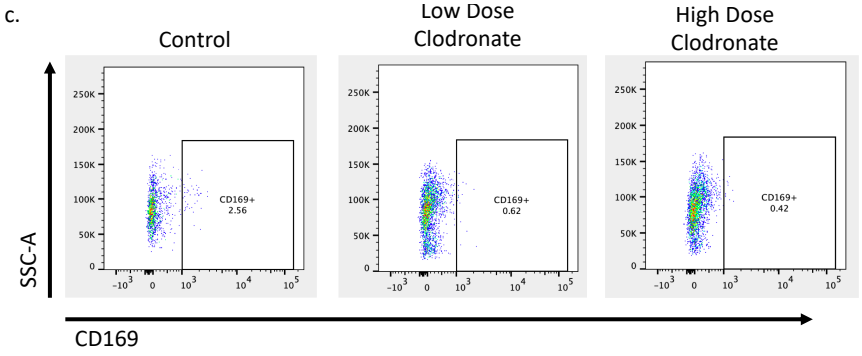
a.


FSC-H
FSC-W
SSC-H
SSC-W
SSC-A
FSC-A
SSC-A
NIR
CD19
CD3
SSC-A
CD11c
SSC-A
F4/80
SSC-A
CD11b
SSC-A
CD169

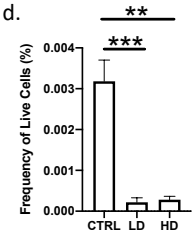
b.


Panel Described on Previous Slide
Same Panel without CD169 (FITC)
SSC-A
CD169+
0.15
CD169
SSC-A
CD169+
7.47E-3
CD169

c.


Control
Low Dose Clodronate
High Dose Clodronate
SSC-A
CD169+
2.56
CD169+
0.62
CD169+
0.42
CD169

d.


**
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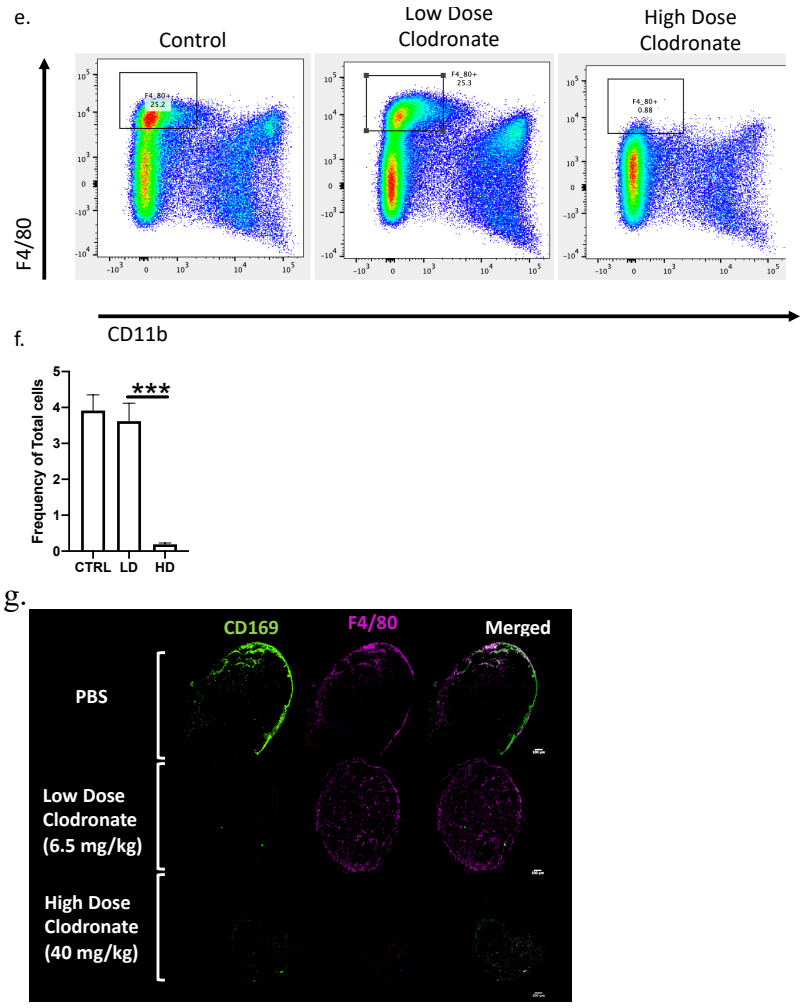
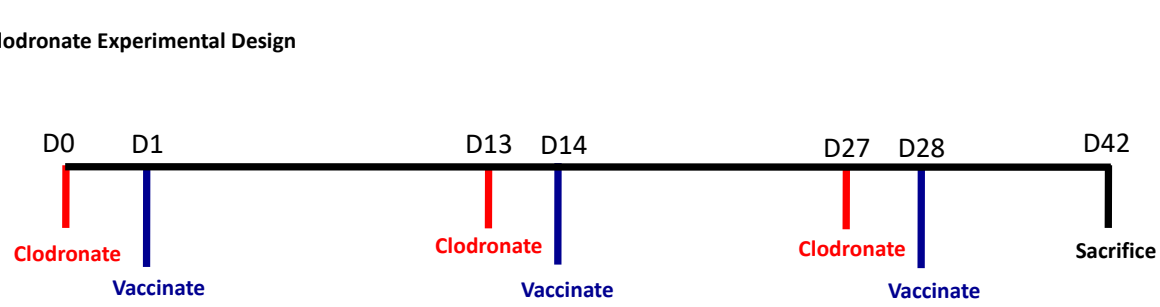
Frequency of Live Cells (%)
0.004
0.003
0.002
0.001
0.000
CTRL
LD
HD

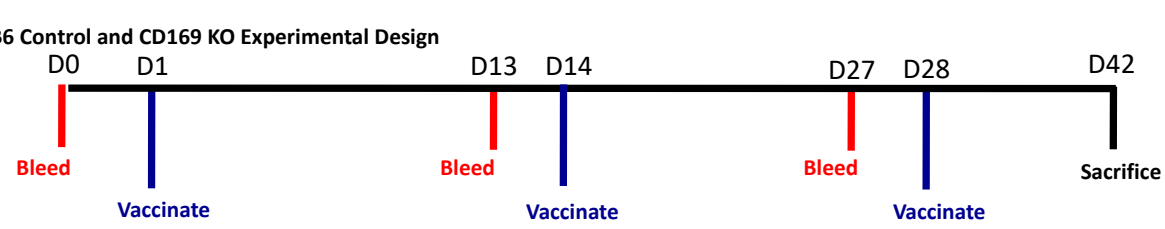


Supplemental Figure 2a. Gating strategy for CD169^{+} macrophages in lymph nodes. Supplemental Figure 2b: Fluorescence minus one (FMO) for CD169. Supplemental Figure 2c and d: (c) Final gate from strategy shown in Figure 4.2a of vehicle control, low dose clodronate treated (6.5mg/kg) , or high dose clodronate (40mg/kg) treated animals. Y-axis shows side scatter. X-axis shows CD169. (d) Quantification from inguinal lymph nodes of animals treated with vehicle control, low dose clodronate treated (6.5mg/kg) , or high dose clodronate (40mg/kg). n = 5. **p<0.01 ***p<0.001Supplemental Figure 2e and f: (e) Gating for F4/80^{+}CD11b^{-} events in inguinal lymph nodes 24 hours post vehicle control, low dose clodronate treated (6.5mg/kg) , or high dose clodronate (40mg/kg) treated animals. Y-axis shows F4/80. X-axis shows CD11b. (f) Quantification from inguinal lymph nodes of animals treated with vehicle control, low dose clodronate treated (6.5mg/kg) , or high dose clodronate (40mg/kg). n = 5. ***p<0.001Supplemental Figure 2g: Effect of clodronate on macrophages populations. Iliac lymph nodes were stain for CD169 (green) and F4/80 (purple) 24 hours post intravenous injection with either PBS, low dose clodronate (6.5 mg/kg), or high dose clodronate (40 mg/kg). Images were taken at 10x objective on a Leica SP5 microscope. Images shown are representative. Scale bar = 100um

a.



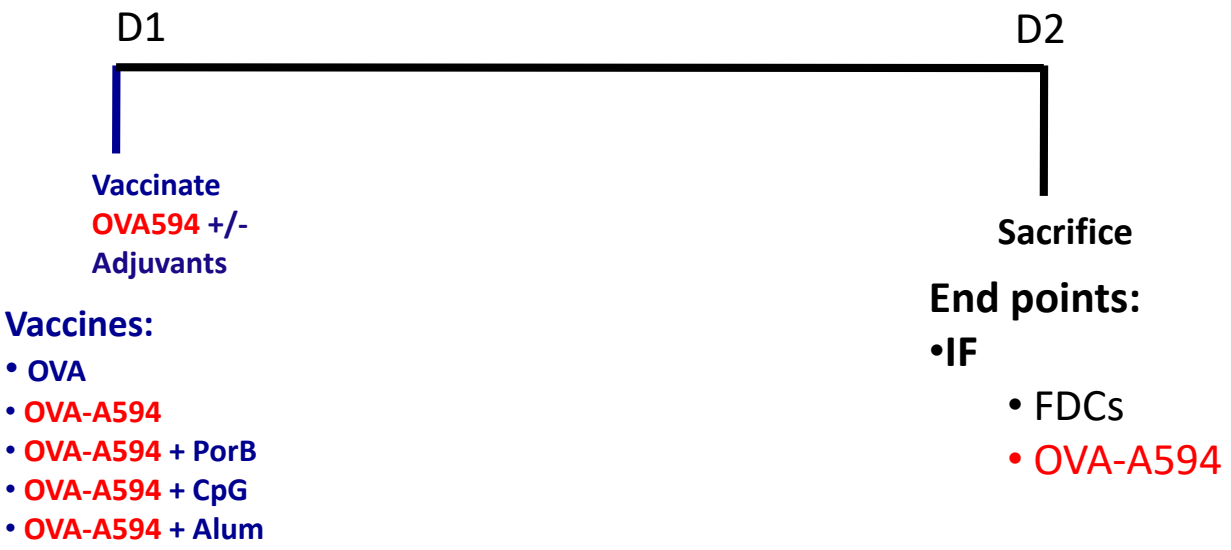
b.



Vaccines:
- **PBS**
- **OVA**
- **OVA + PorB**
- **OVA + CpG**
- **OVA + Alum**

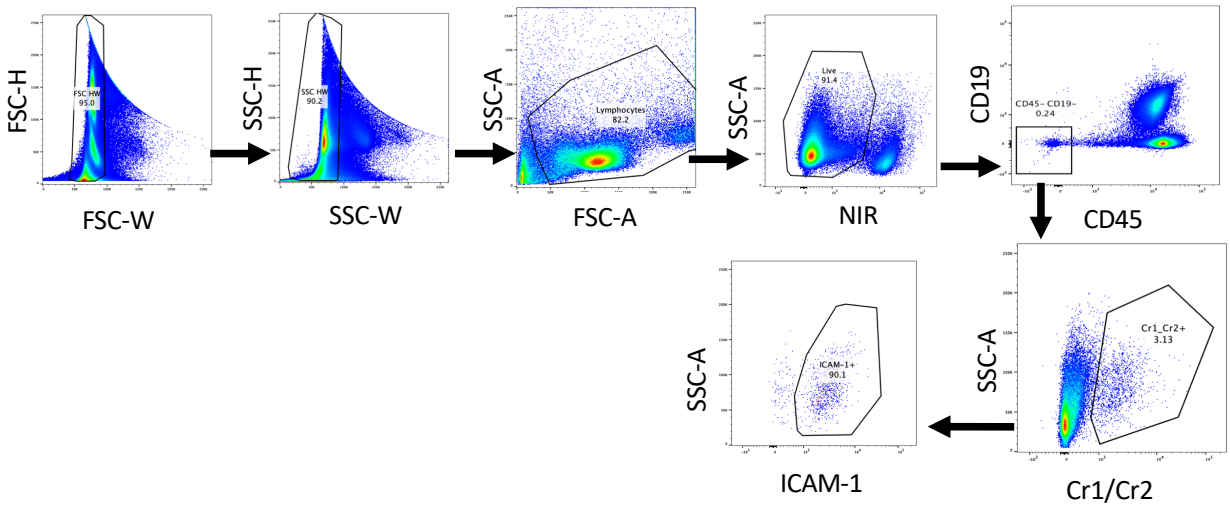
End points:
- **IgG ELISA**
- **IgG subtypes**

Supplemental Figure 3. Low dose clodronate treatments included in vaccine regime decreases antibody production. (a) Low dose clodronate and immunization schedule. This schedule was modified from a previously published immunization schedule from our lab. Mice were divided into 2 groups – one group receiving low dose clodronate treatment one day prior to immunization and the other group receiving encapasomes control. These groups were further divided into PBS, OVA, OVA+ PorB, OVA+ CpG, or OVA+Alum. Mice received subcutaneous immunizations 3 times, with 2 weeks in between immunizations.

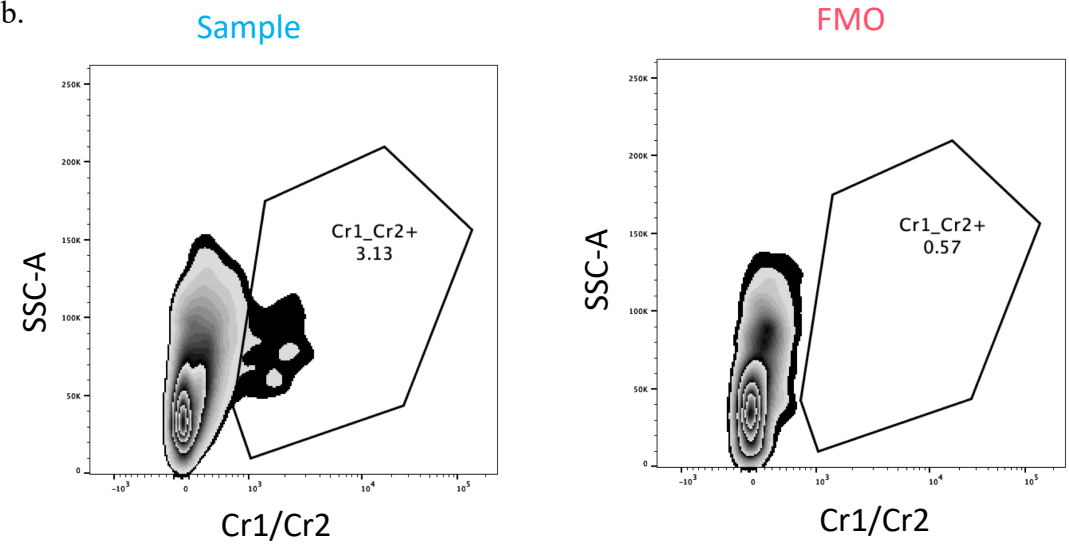


Supplemental Figure 4. Experimental design for antigen trafficking within the lymph nodes. mice were divided into OVA, OVA-A594, OVA-A594 + PorB, OVA-A594+ CpG, or OVA-A594+Alum. Mice received subcutaneous injection.

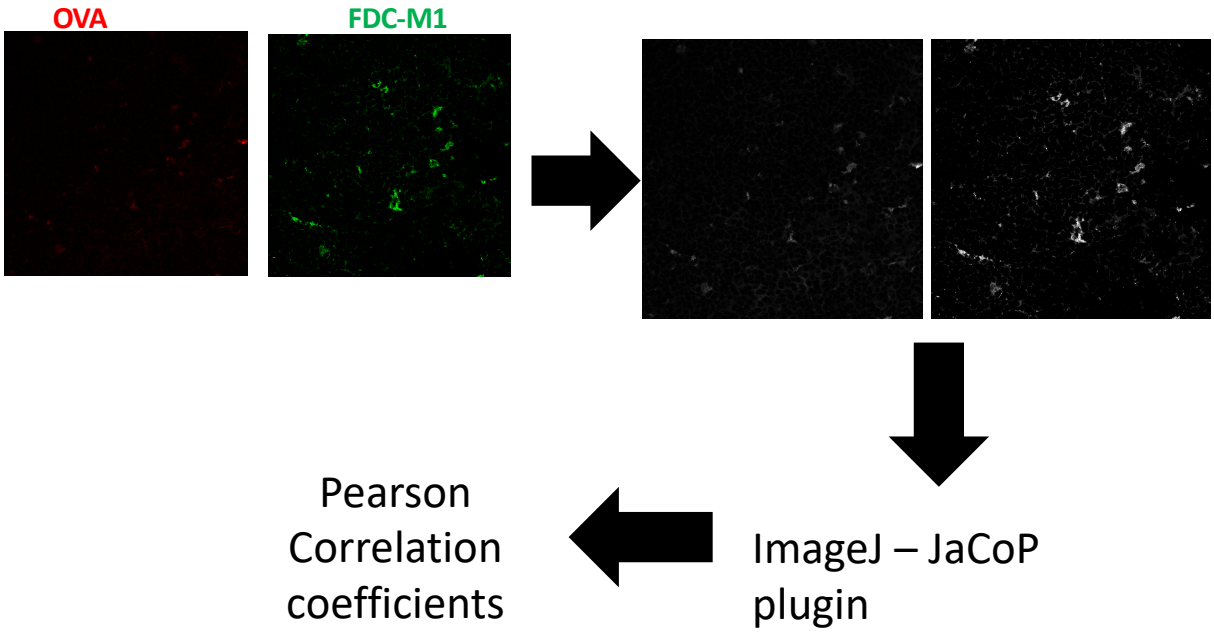
a.



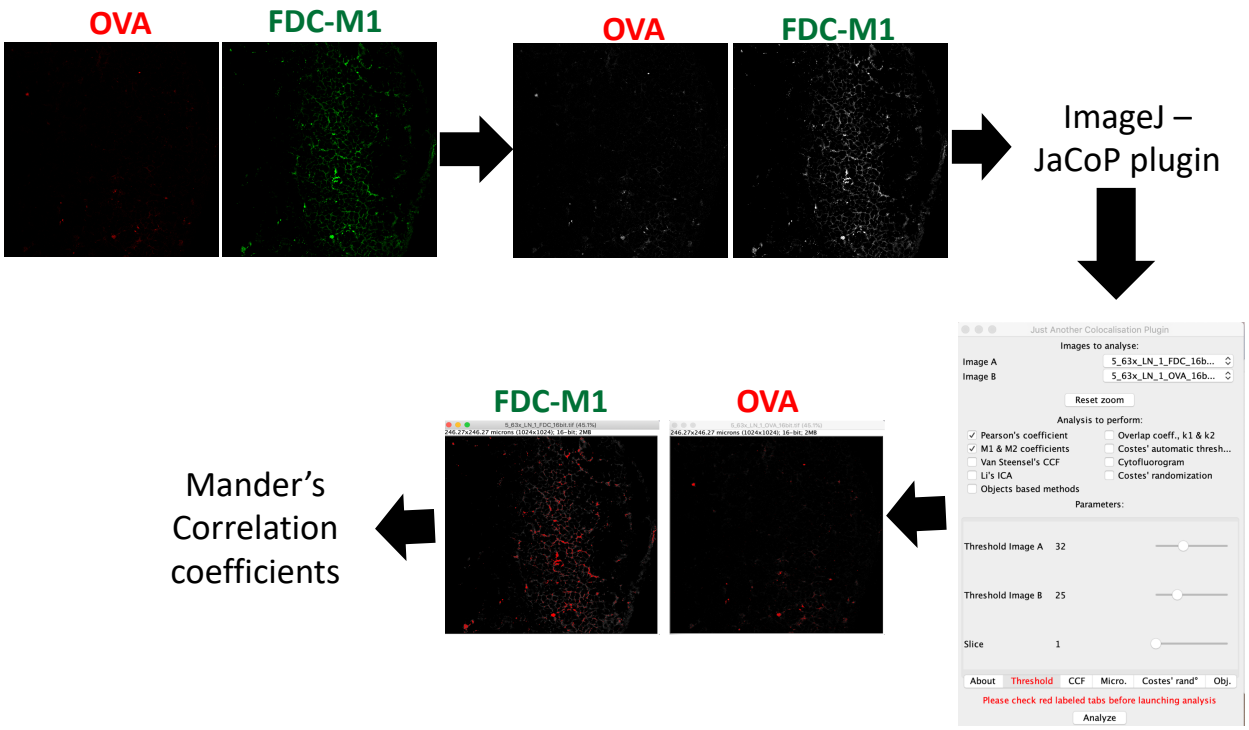
b.



Supplemental Figure 5: (a) Gating strategy for follicular dendritic cells (FDCs). (b) Fluorescence minus one (FMO) for Cr1/Cr2. Two samples were analyzed based on the gating strategy in Supplemental figure 5 a. One sample contained Cr1/Cr2 antibody (pictured on the left). The other did not (pictured on the right). These are the results of the flow cytometry.



Supplemental Figure 5: Pearson correlation coefficient analysis. Images were imported into ImageJ and then changed to a 16-bit image. The plugin JaCoP was then utilized to determine Pearson correlation coefficients for OVA and FDCs.



Supplemental Figure 6: Pearson correlation coefficient analysis. Images were imported into ImageJ and then changed to a 16-bit image. The plugin JaCoP was then utilized to determine Manders correlation coefficient to determine what percent of OVA was associated with either DCs or FDCs.