

## Supplemental Information For

### Detergent wash improves vaccinated lymph node handling ex vivo

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**Supplementary Video 1:** Movie of washing lymph nodes in digitonin solution. Harvested lymph nodes were quickly dipped into a 100 µg/mL digitonin solution (< 1 sec) before being rinsed in phosphate-buffered saline (3 – 5 sec) and placed into complete RPMI. Movie plays in real time.

Supplementary Figures:

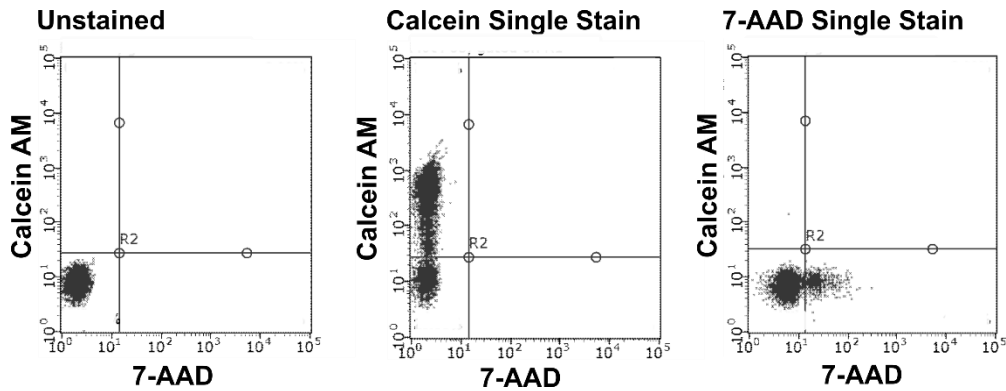


Figure S1: Single stain controls for flow cytometry, as described in Methods.

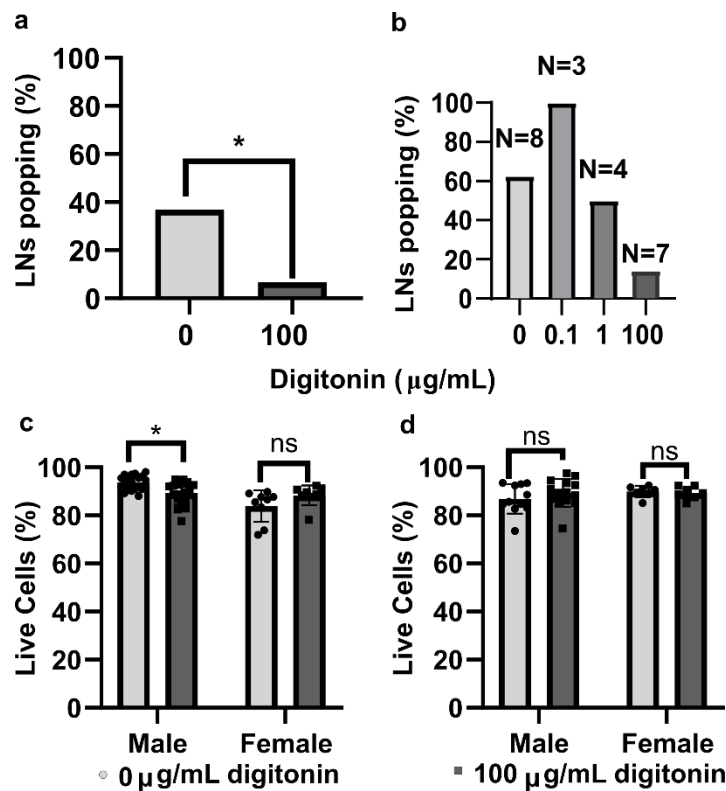


Figure S2: Optimization of digitonin wash and analysis of sex differences in digitonin washing. (a) Popping frequency of LNs from male vaccinated mice with or without digitonin wash.  $n = 16$  for  $0 \mu\text{g/mL}$ ,  $n = 15$  for  $100 \mu\text{g/mL}$ . \* indicates  $p < 0.05$  by unpaired t test. (b) Popping frequencies of LNs from vaccinated animals (male) in preliminary tests with varied concentration of digitonin. (c,d) Overnight viability of lymph node slices with or without a digitonin wash, broken out by sex, for vaccinated mice (c) or naïve mice (d), as assessed by flow cytometry using 7-AAD and Calcein staining. Each dot represents one slice. Bars show mean and standard deviation. \* indicates  $p < 0.05$  by Two-way ANOVA Multiple Comparisons.