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Supporting Information

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Hemagglutinin Functionalized Liposomal Vaccines Enhance Germinal Center and Follicular Helper T Cell Immunity

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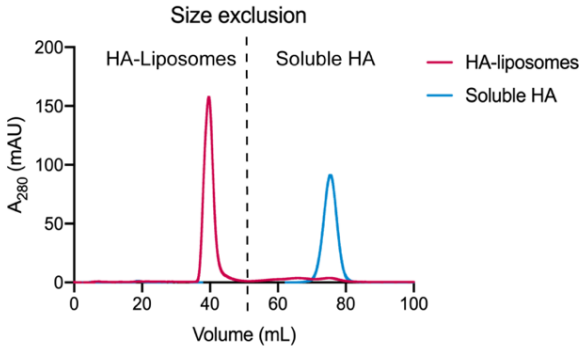


Figure S1: Size exclusion fraction showing separation of HA-liposomes from soluble HA proteins and fraction of soluble HA as control.

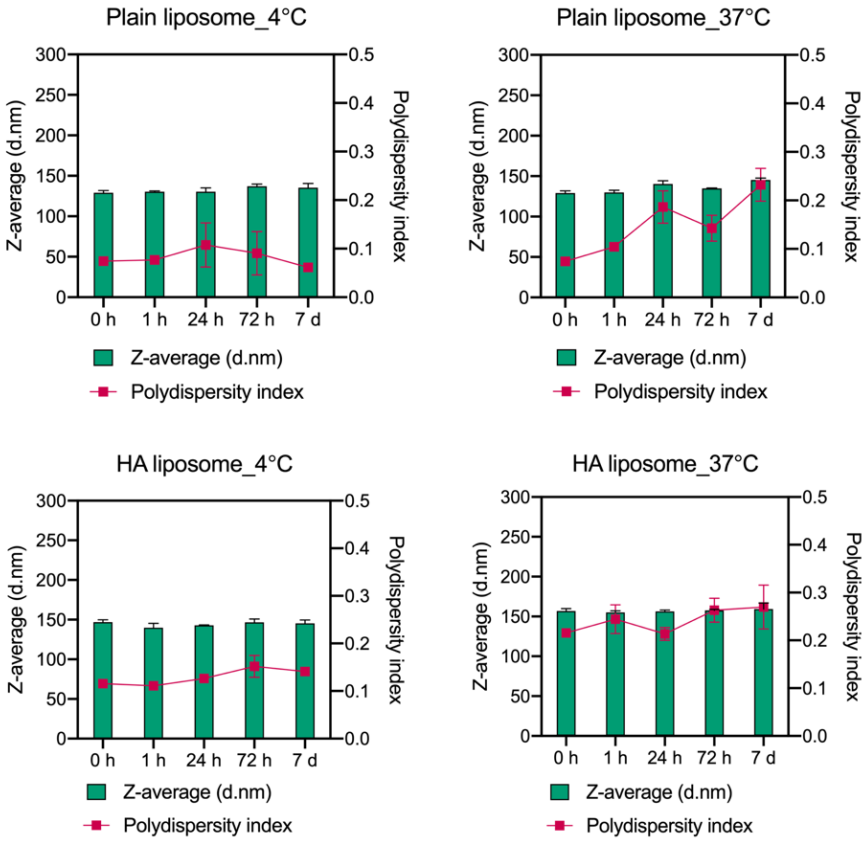


Figure S2: Size and polydispersity index of plain and HA-liposomes in PBS at 4 °C (left) and 37 °C (right) after 0h, 1h, 24h, 72h and 7 days. Data are presented as mean ± SD.

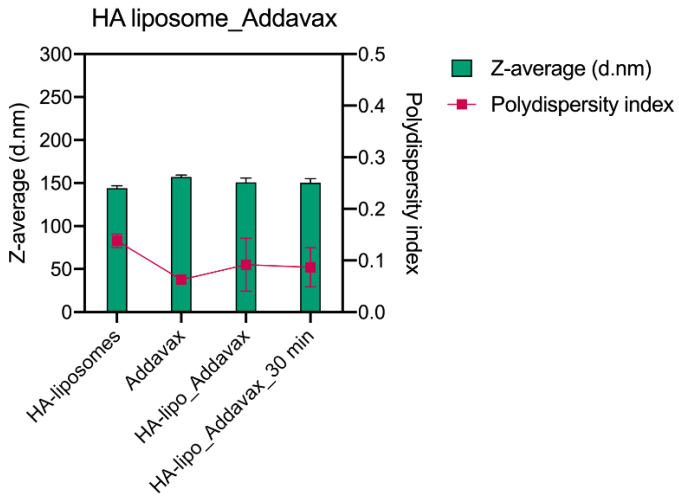


Figure S3: Size and polydispersity index of HA-liposomes, Addavax and their mixture at 0 and 30 minutes. Data are presented as mean ± SD.

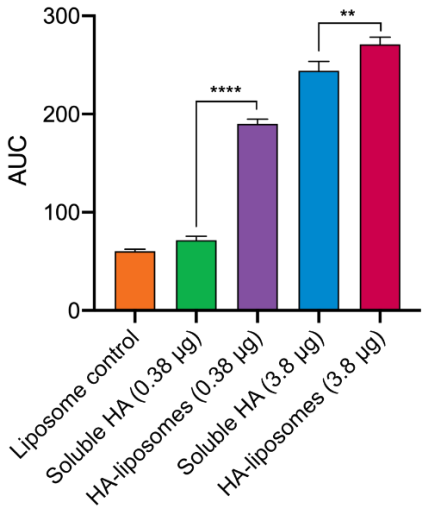


Figure S4: Protective immunity induced in mice analyzed by area under the curve (AUC). Five groups of C57BL/6 mice (n = 5) were immunized with liposome control, HA-liposomes or soluble HA mixed with Co-free liposomes at a low dose (0.38 µg) or a high dose (3.8 µg) of HA proteins. Relative protection of immunized mice from A/PR8/34 virus challenge at a viral dose of 2000 $TCID_{50}$ in day 28 post immunization, assessed by AUC analysis. Data are presented as mean ± SD and representative of one of two independent experiments.

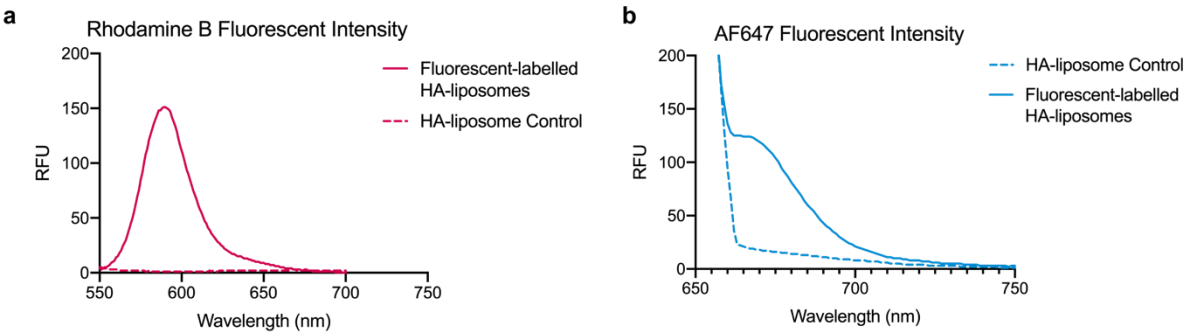
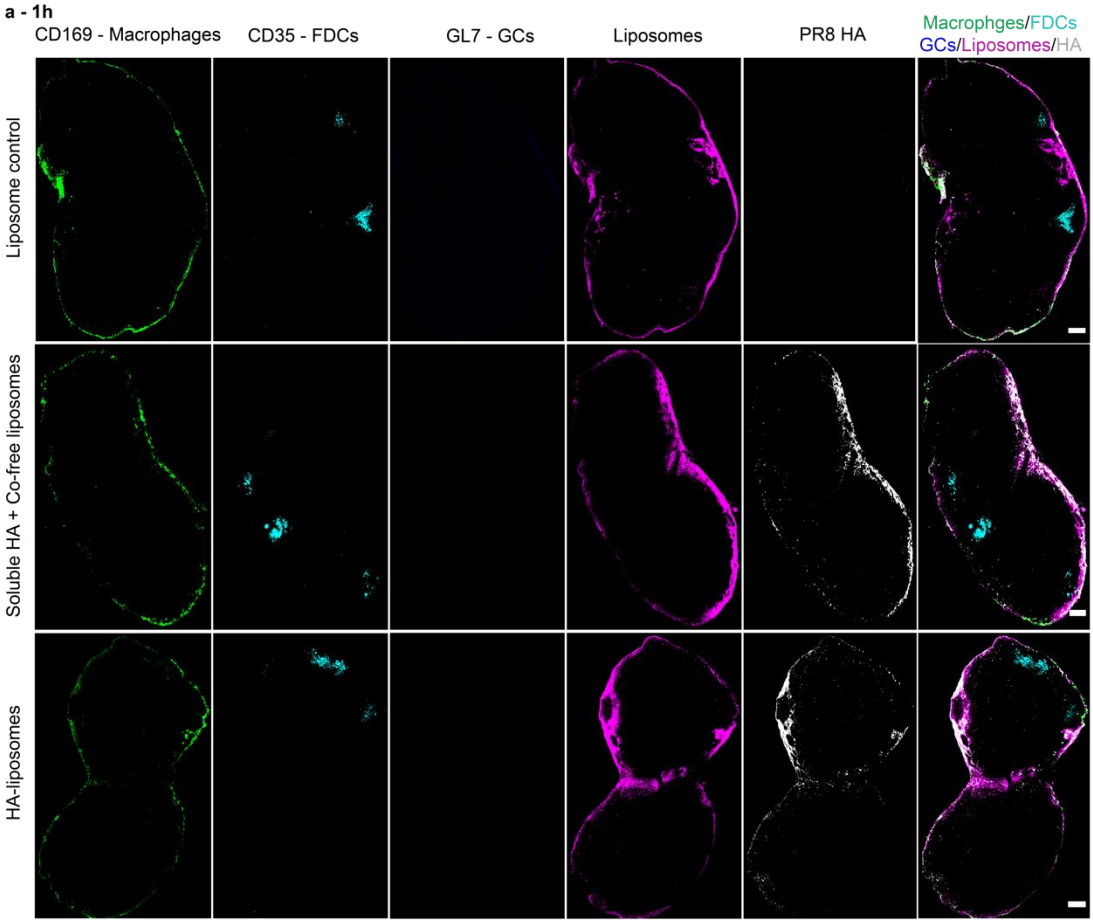
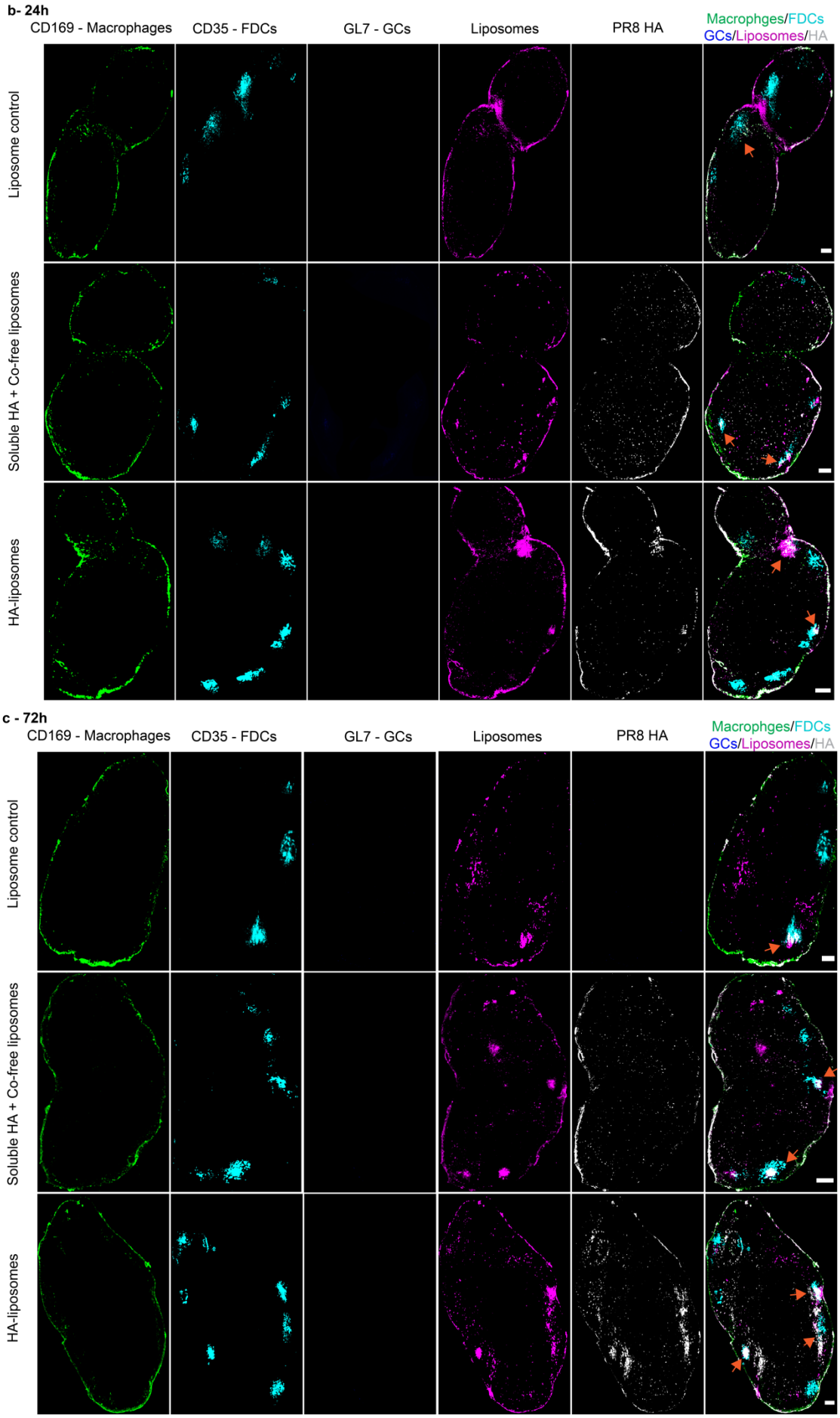


Figure S5: Fluorescence measurement of fluorescent-labelled HA-liposomes (solid lines) using a fluorescence spectrophotometer with an excitation of (**a**) 535 nm for Rhodamine B-contained liposomes and (**b**) 635 nm for AF647-labelled HA. Unlabeled HA-liposomes (dashed lines) were used as control.




b- 24h
CD169 - Macrophages
CD35 - FDCs
GL7 - GCs
Liposomes
PR8 HA
Macrophges/FDCs
GCs/Liposomes/HA
Liposome control
Soluble HA + Co-free liposomes
HA-liposomes
c - 72h
CD169 - Macrophages
CD35 - FDCs
GL7 - GCs
Liposomes
PR8 HA
Macrophges/FDCs
GCs/Liposomes/HA
Liposome control
Soluble HA + Co-free liposomes
HA-liposomes

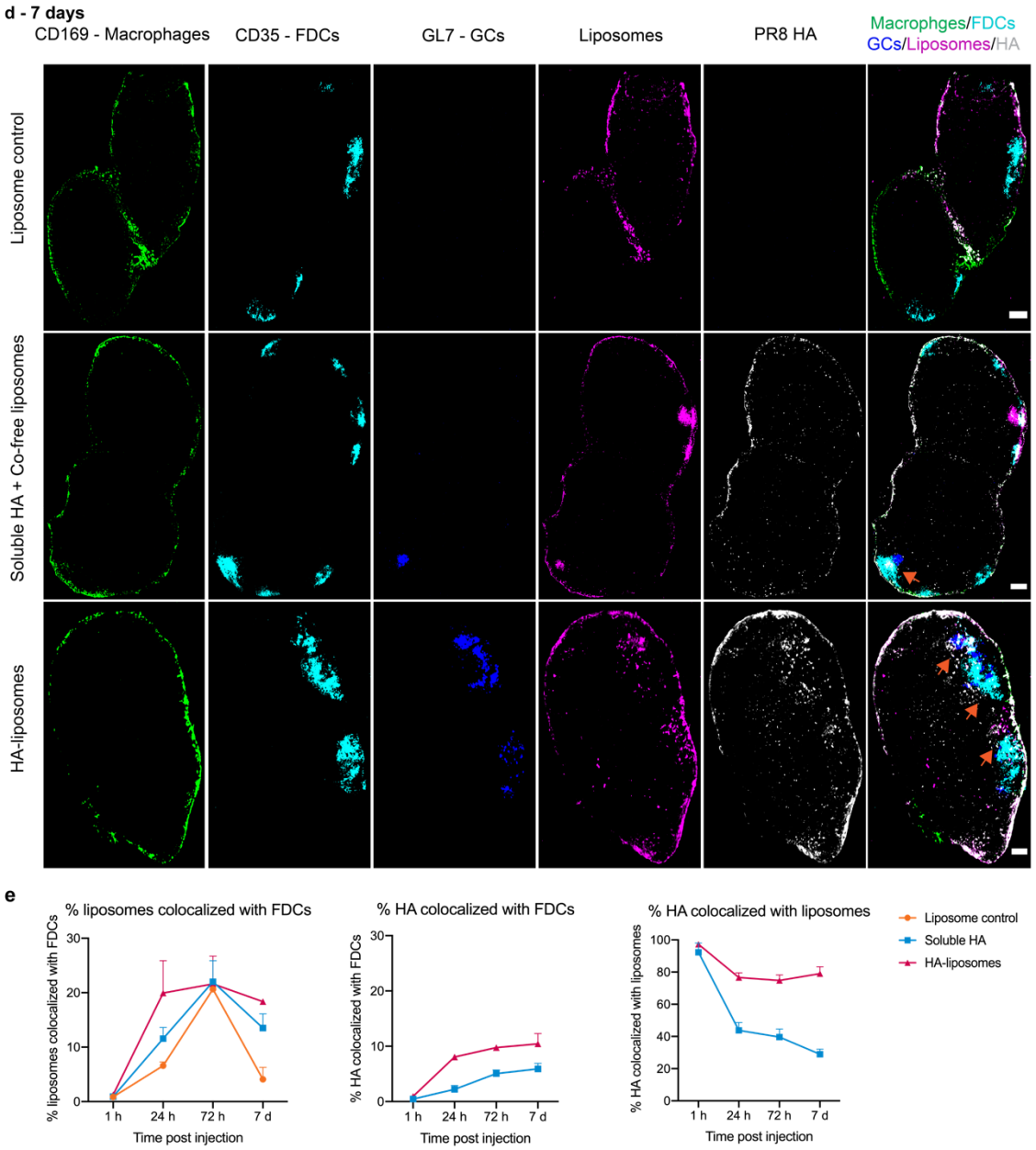
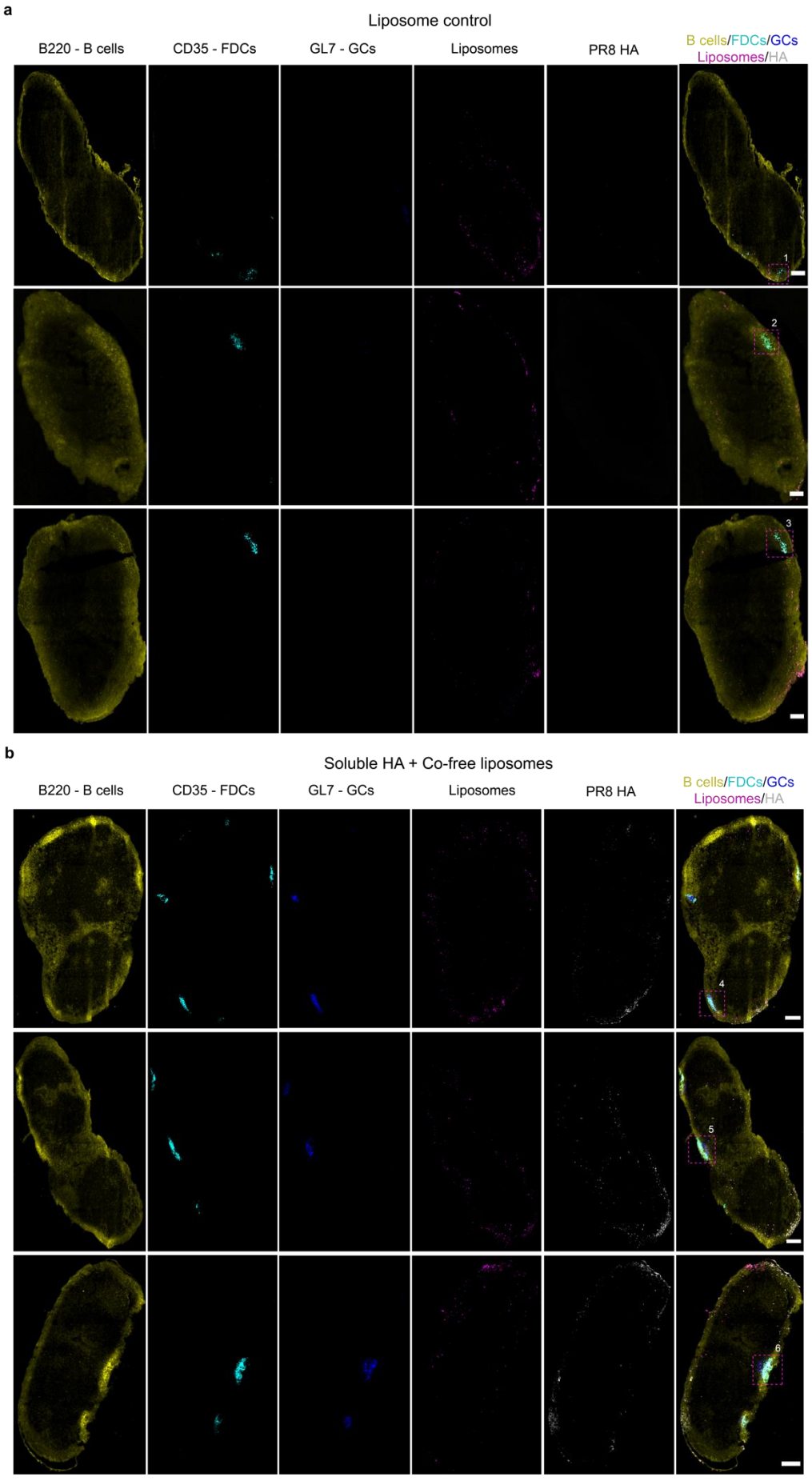


Figure S6: Confocal images of lymph nodes of mice at 1 h (**a**), 24 h (**b**), 72 h (**c**), and 7 days (**d**) post vaccination with plain liposome control, soluble HA mixed with cobalt-free liposomes, and HA-liposomes. HA proteins were labelled with AF647 (gray) while 16:0 Liss Rhod PE (magenta) were stained for liposomes. Cells were labelled with CD169 BV605 (macrophages – green), CD35 BV421 (FDCs – cyan), and GL7 AF488 (GCs – blue). Scale bars = 150 μm. **e**, Percentage of percentage of liposomes colocalized with FDCs (left), HA colocalized with FDCs (middle), and HA colocalized with liposomes (right) analyzed using colocalization tool in FIJI.


a
Liposome control
B220 - B cells
CD35 - FDCs
GL7 - GCs
Liposomes
PR8 HA
B cells/FDCs/GCs
Liposomes/HA
1
2
3
b
Soluble HA + Co-free liposomes
B220 - B cells
CD35 - FDCs
GL7 - GCs
Liposomes
PR8 HA
B cells/FDCs/GCs
Liposomes/HA
4
5
6

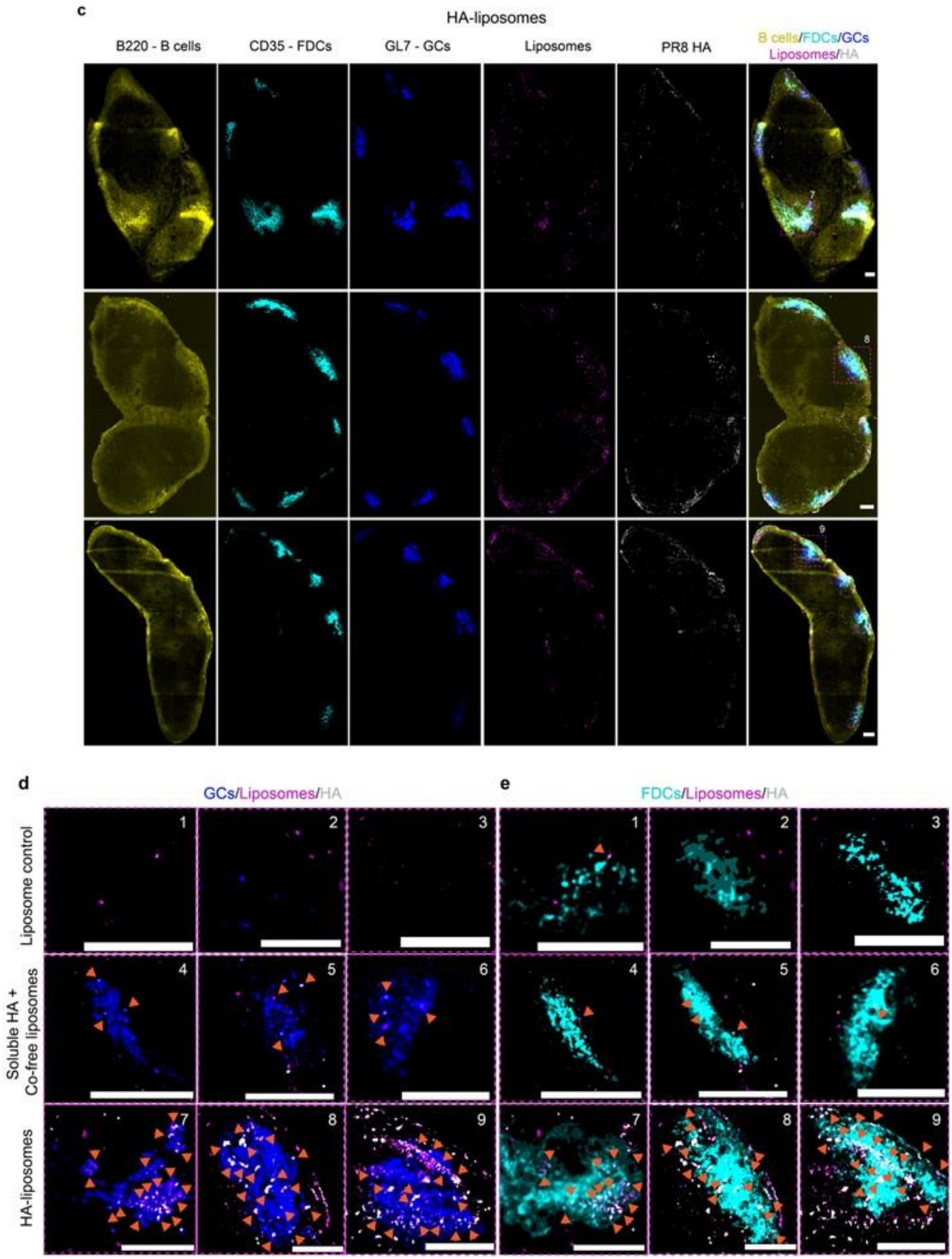


Figure S7: Confocal images of lymph nodes of mice at day 14 post vaccination with plain liposome control (**a**), soluble HA mixed with cobalt-free liposomes (**b**), and HA-liposomes (**c**). HA proteins were labelled with AF647 (gray) while 16:0 Liss Rhod PE (magenta) were stained for liposomes. Cells were labelled with B220 BV510 (B cells – yellow), CD35 BV421 (FDCs – cyan), and GL7 AF488 (GCs – blue). Inset images of follicle regions were magnified

to allow visualization of HA and liposomes colocalized with either GC (**d**) or FDC (**e**) areas. Scale bars = 150 μm.

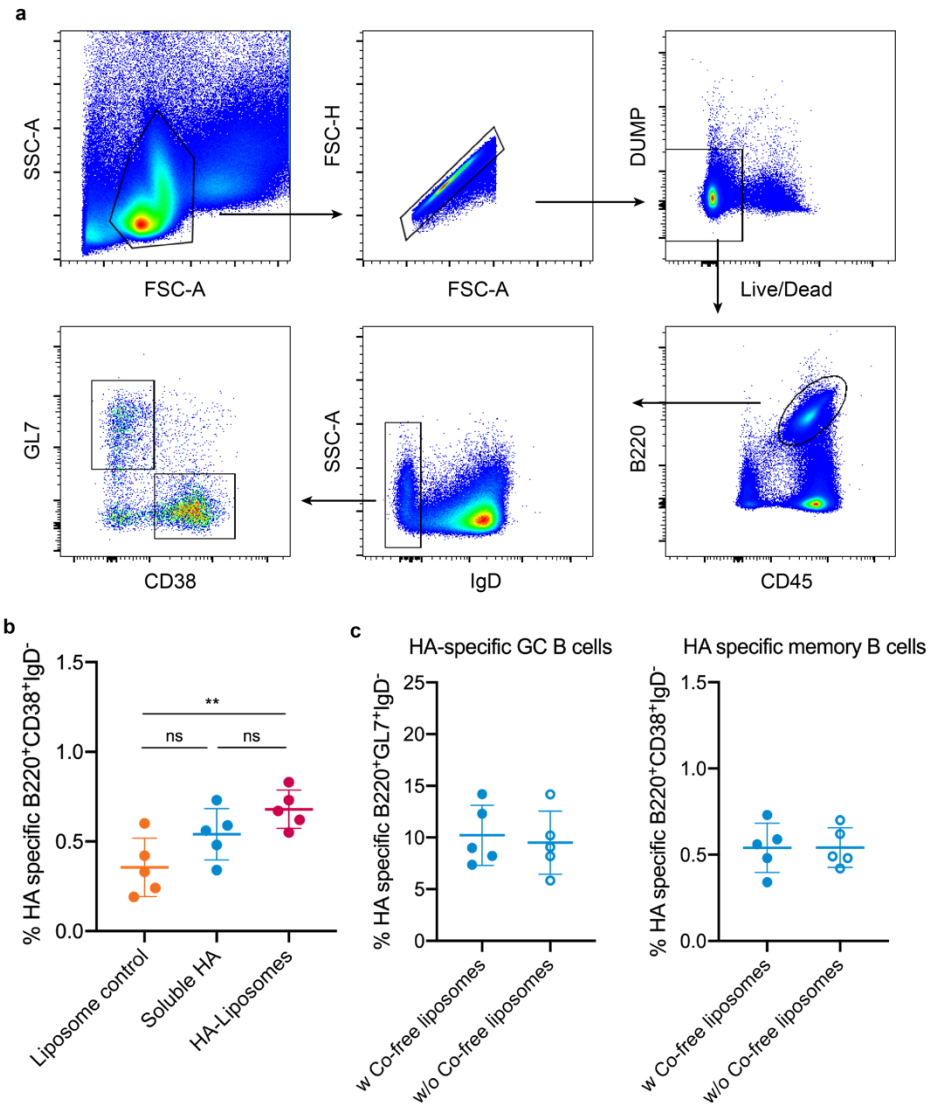


Figure S8: B cell responses in lymph nodes of mice immunized with soluble HA and HA-liposomes. a, Gating strategy to identify germinal central B cells ($IgD^-B220^+GL7^+CD38^{lo}$) and memory B cells ($IgD^-B220^+GL7^{lo}CD38^+$). **b**, Proportion of HA-specific memory B cells in total memory B cells. **c**, Frequencies of HA-specific GC B cells in total GC B cells (right) and HA-specific memory B cells in total memory B cells (left) in mice vaccinated with soluble HA with (w) or without (w/o) presence of Co-free liposomes. Data are presented as mean ± SD and representative of one of two independent experiments. Each dot in plots represent one animal. One-way ANOVA with Tukey's pairwise comparisons post-hoc test was used to assess statistical significance between three group data; ns $p > 0.05$, ** $p < 0.01$.

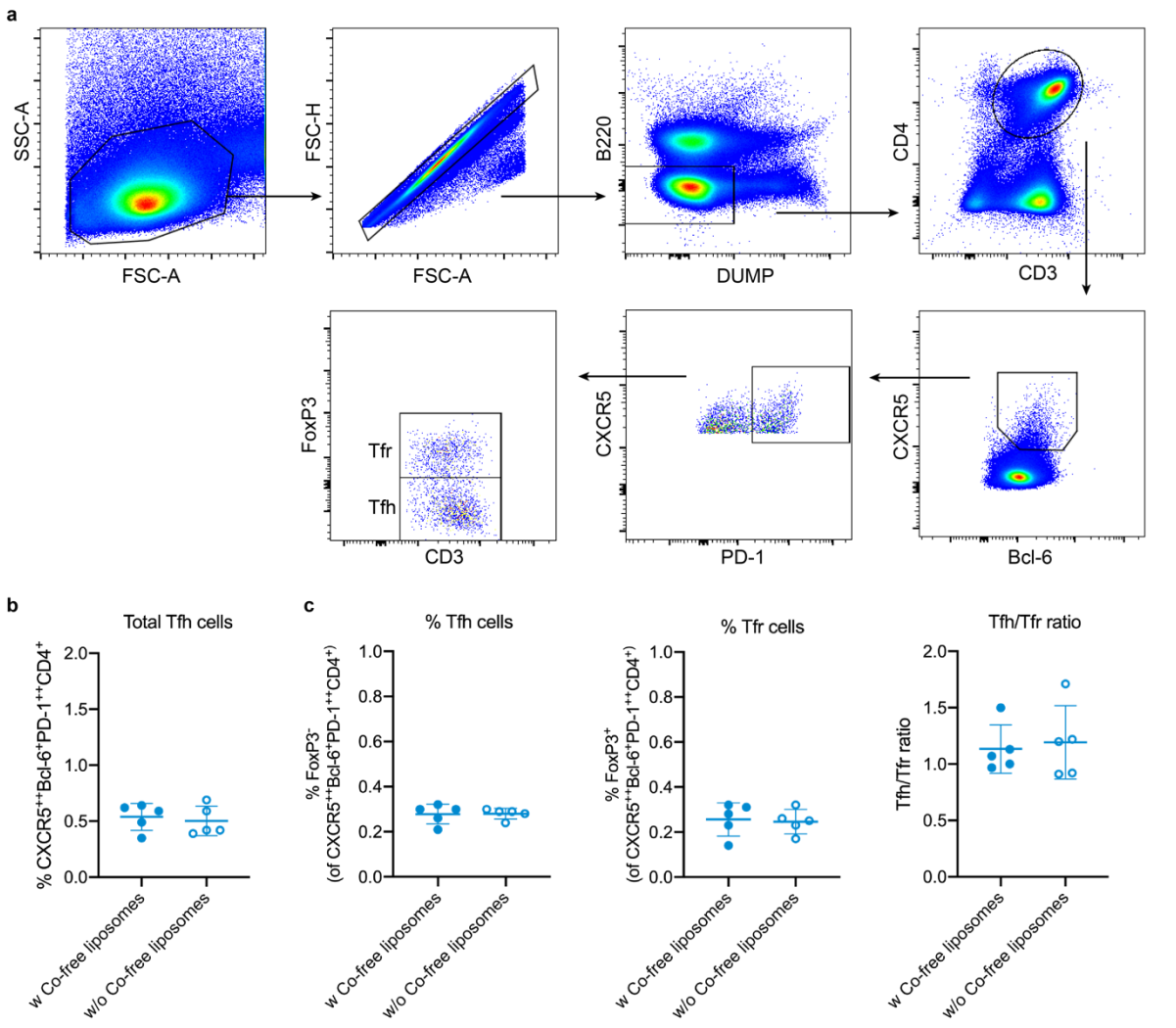


Figure S9: a, Gating strategy to identify follicular helper T (Tfh) cells ($CD4^{+}CXCR5^{hi}Bcl\text{-}6^{hi}PD\text{-}1^{hi}Foxp3^{-}$) and follicular regulatory T (Tfr) cells ($CD4^{+}CXCR5^{hi}Bcl\text{-}6^{hi}PD\text{-}1^{hi}Foxp3^{+}$). **b** and **c**, Frequencies of total Tfh cells (b), $Foxp3^{-}$ Tfh cells (c, left), $Foxp3^{+}$ Tfr cells (c, middle), and ratio Tfh to Tfr cells (c, right) in mice vaccinated with soluble HA with (w) and without (w/o) presence of Co-free liposomes.